Carbohydrate Research, 235 (1992) 95–114
Elsevier Science Publishers B.V., Amsterdam

Stereoselective total synthesis of the glycosyl phosphatidylinositol (GPI) anchor of *Trypanosoma brucei* *

Chikara Murakata [a] and Tomoya Ogawa [a,b]

[a] *RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama, 351-01 (Japan)*
[b] *Faculty of Agriculture, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113 (Japan)*

(Received February 25th, 1992; accepted March 30th, 1992)

ABSTRACT

The total synthesis of *O*-{*O*-[6-*O*-(2-aminoethylphosphono)-α-D-mannopyranosyl]-(1 → 2)-*O*-α-D-mannopyranosyl-(1 → 6)-*O*-[*O*-α-D-galactopyranosyl-(1 → 6)-α-D-galactopyranosyl-(1 → 3)]-*O*-α-D-mannopyranosyl-(1 → 4)-2-amino-2-deoxy-α-D-glucopyranosyl}-(1 → 6)-{1-*O*-(1,2-dimyristoyl-*sn*-glycero-3-phosphono)-1D-*myo*-inositol}, the GPI anchor of *Trypanosoma brucei* was achieved for the first time. The core structure of the GPI molecule, the glycoheptaosyl part, was constructed in a highly stereocontrolled manner from *O*-[*O*-(2,4-di-*O*-benzyl-α-D-mannopyranosyl-(1 → 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranosyl]-(1 → 6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-D-*myo*-inositol, *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-D-galactopyranosyl fluoride, 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride, and 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl fluoride. The introduction of two phosphodiester functions was efficiently achieved using the H-phosphonate method.

INTRODUCTION

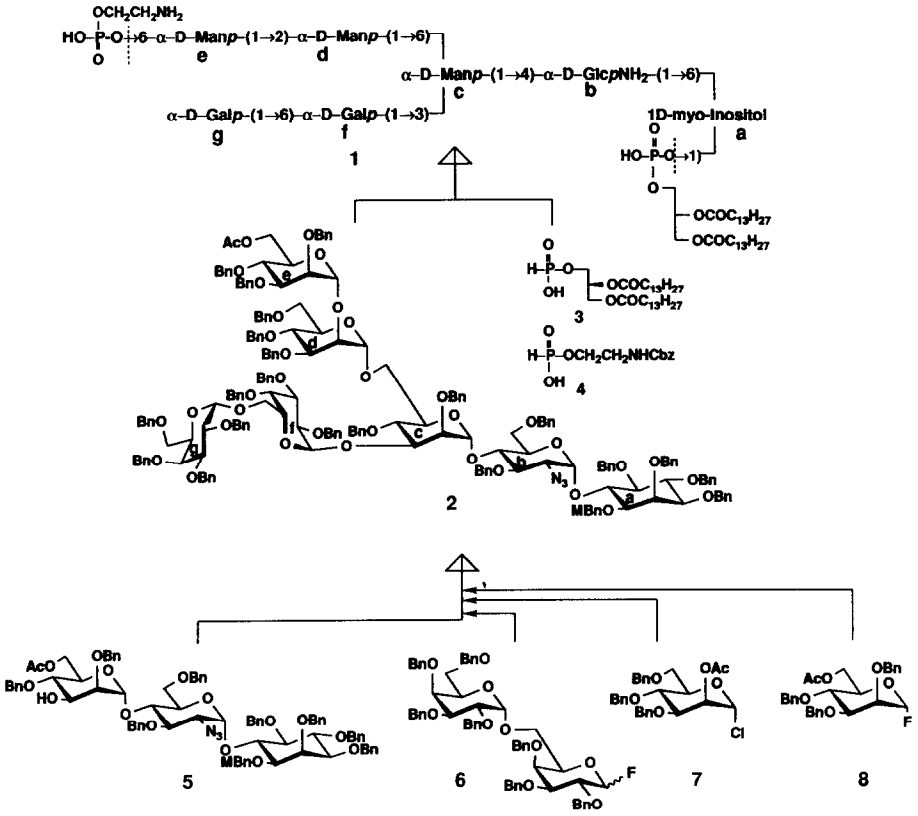
Recently, it was reported that the glycosyl phosphatidylinositol (GPI) anchor[2] was involved in the signal transduction of insulin[3], IL-2 (ref. 4), and nerve growth factor (NGF)[5]. In a previous paper[6], we reported in detail the synthesis of glycobiosyl phosphatidylinositol, a part of the structure of the GPI anchor of *Trypanosoma brucei*. Relevant synthetic studies[7] on this topic have recently appeared from other research groups. In a continuation of our synthetic studies[8] on this type of GPI anchor, we now describe a total synthesis of **1**.

RESULTS AND DISCUSSION

Retrosynthetic analysis of the GPI anchor (**1**) led us to design the glycoheptaosyl core **2** to which two different kinds of phosphodiester functions could be

Correspondence to: Dr. T. Ogawa, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama, 351-01, Japan.

* Part 85 in the series "Synthetic Studies on Cell-Surface Glycans". For part 84, see ref. 1.

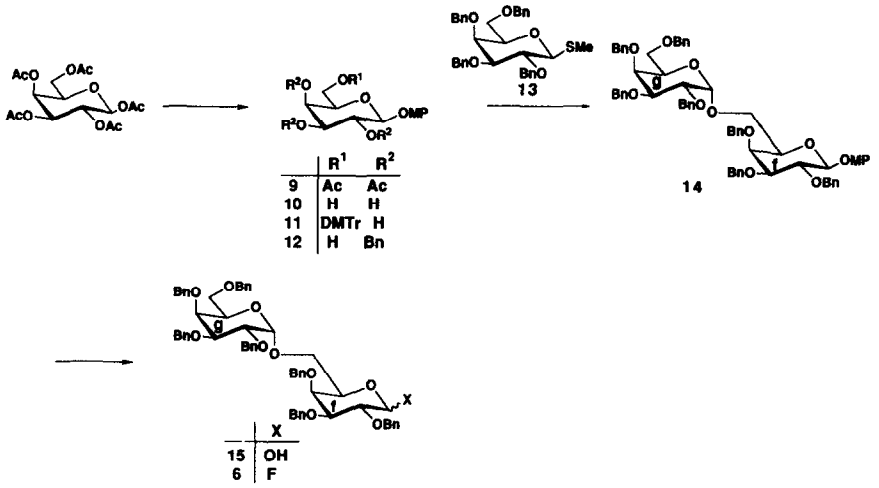


Scheme 1.

regioselectively introduced by use of two types of H-phosphonate compounds, **3** and **4**. The glycoheptaosyl core **2** may be disconnected into four key synthetic blocks; the glycotriosyl acceptor **5**, the galactobiosyl donor **6**, and two mannosyl donors **7** and **8** (Scheme 1).

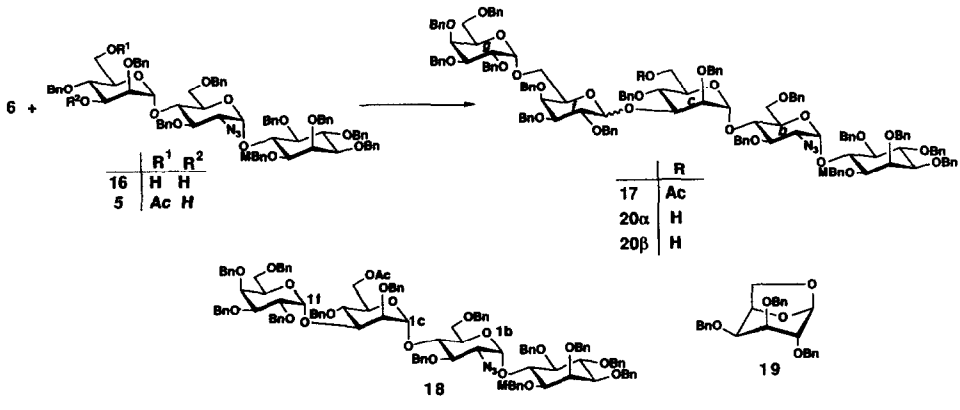
The synthon **6** was prepared as follows. Treatment of penta-*O*-acetyl-β-D-galactopyranose with 4-methoxyphenol in the presence of trimethylsilyl trifluoromethanesulfonate gave 88% of **9**. Deacetylation of **9**, followed by selective protection of O-6 with the 4,4′-dimethoxytrityl group, afforded 84% of **11**. Conversion of **11** into **12** was achieved by benzylation, followed by acid treatment in 66% yield. Glycosylation of **12** with the known donor **13** (ref. 9) in the presence of copper(II) bromide and tetrabutylammonium bromide[10] gave 67% of the disaccharide **14** and 10% of its β isomer. Reaction of **14** with ammonium cerium(IV) nitrate[11] in 1.6:2:1 toluene–acetonitrile–water, followed by treatment with DAST[12], afforded the galactobiosyl donor **6** in 62% yield as a 2:3 $\alpha:\beta$ mixture of anomers (Scheme 2).

The crucial coupling of **6** with the key acceptor **5**, which was readily obtainable from the reported compound **16** (ref. 6), by regioselective acetylation was achieved

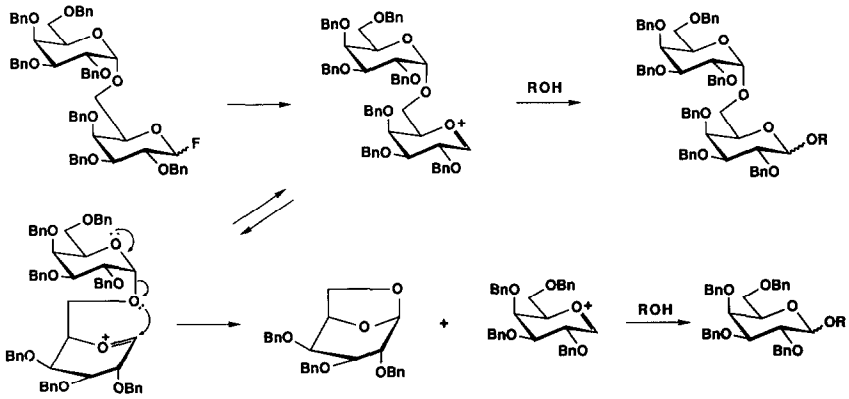


Scheme 2.

in the presence of zirconocene dichloride and silver perchlorate[13] in dry ether to give 76% of the pentasaccharide **17**, along with 18% of the tetrasaccharide **18** and 41% (based on excess **6**) of the 1,6-anhydro compound **19** (Scheme 3). The structure of **18** was confirmed by its ^{13}C NMR spectrum, which contained signals for the anomeric carbons at 97.7 ppm with $^{1}J_{C,H}$ 178.5 Hz for C-1b and at 98.7 ppm with $^{1}J_{C,H}$ 170.9 Hz and 102.0 ppm with $^{1}J_{C,H}$ 172.4 Hz for C-1cf. Deacetylation of the α,β mixture **17** and subsequent purification by preparative TLC gave 89% of the α-linked derivative **20**α and 11% of its β isomer **20**β, which, on acetylation, were converted back into the α-linked compound **17**α and its β isomer **17**β, respectively, in order to confirm their structures. The ^{13}C NMR spectrum of **17**α contained signals for the anomeric carbons at 97.6, 98.9, 99.4, and 99.9 ppm with



Scheme 3.

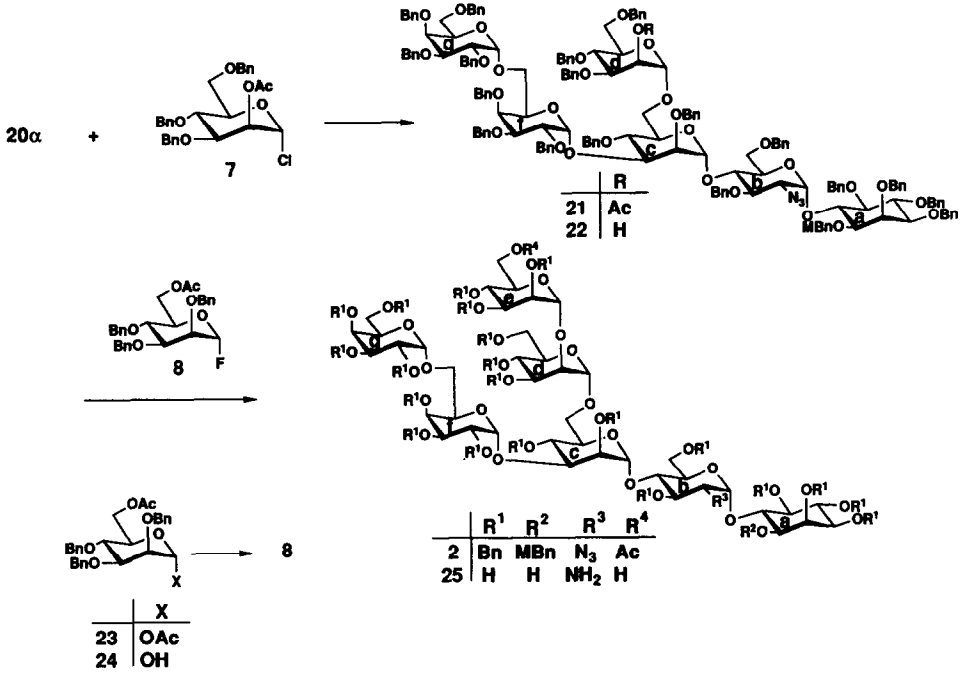


Scheme 4.

$^1J_{C,H}$ values of 166.0 to 175.8 Hz for C-1bcfg. Therefore the configuration at C-1f was assigned α-D. Similarly, the structure of **17**β was confirmed by its ^{13}C NMR spectrum, which contained signals for the anomeric carbons at 97.6, 99.2, and 99.7 ppm with $^1J_{C,H}$ values of 167.4 to 174.4 Hz for C-1bcg and at 101.8 ppm with $^1J_{C,H}$ 158.9 Hz for C-1f. The formation of compounds **18** and **19** could be rationalized by an unexpected intramolecular nucleophilic attack by interglycosidic oxygen (O-6) on the oxocarbonium ion at C-1 formed in situ as shown in Scheme 4.

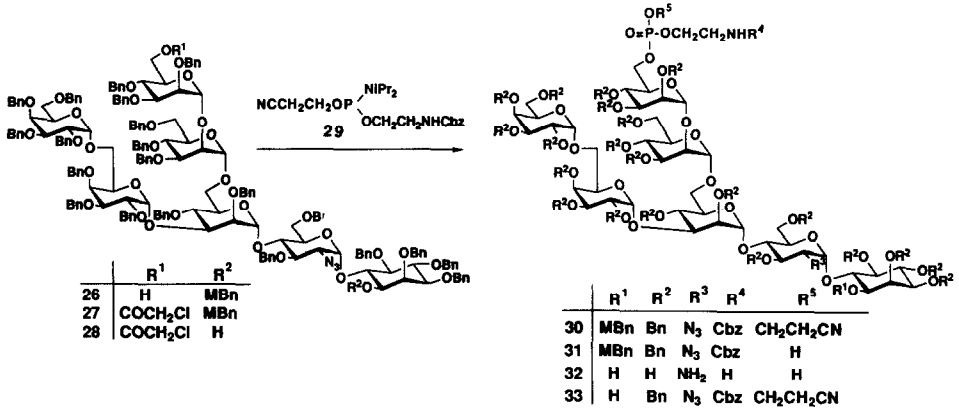
Glycosylation of **20**α with the known mannosyl donor **7** (ref. 14) having a participating group at O-2 was promoted by mercury(II) bromide and mercury(II) cyanide and proceeded with remarkable stereocontrol as expected giving 89% of the hexasaccharide **21**. Deacetylation of **21** afforded 94% of **22**, which was then coupled according to the method of Suzuki[13] with the mannosyl donor **8** obtained from the known compound **23** (ref. 15) in 2 steps (1, $NH_2NH_2 \cdot AcOH$ in DMF[16]; 2, DAST in dichloroethane), yielding 93% of the heptaosyl core **2** and 6% of its β isomer. In order to confirm the structure of **2** from the stereocontrolled synthetic sequence, deprotection of **2** into the free hydroxy, free amino compound **25** was carried out by hydrogenation, followed by *O*-deacetylation (Scheme 5). The ^1H NMR spectrum of **25** showed clearly the signals for six anomeric protons, and the configurations of six glycosidic linkages were confirmed to be α-D.

Subsequently we proceeded to introduce a phosphodiester function at O-6e of **26**, which was obtained from **2** by deacetylation. Transformation of **26** into **30** was successfully carried out by phosphitylation[17]. Coupling between **26** and **29**, which was readily prepared from 2-[(*N*-benzyloxycarbonyl)amino]ethanol and the bifunctional phosphitylating reagent, chloro-(2-cyanoethoxy)-*N*,*N*-bis(2-propylamino)phosphine[18] in the presence of 2-Pr_2NEt in dichloromethane, gave the intermediate phosphite triester that was oxidized without isolation with *m*-chloroperoxybenzoic acid[19] to afford **30** in 97% yield. The structure of **30** was firmly confirmed by conversion into **32** by treatment with 1,8-diazabicyclo[5,4,0]undec-7-



Scheme 5.

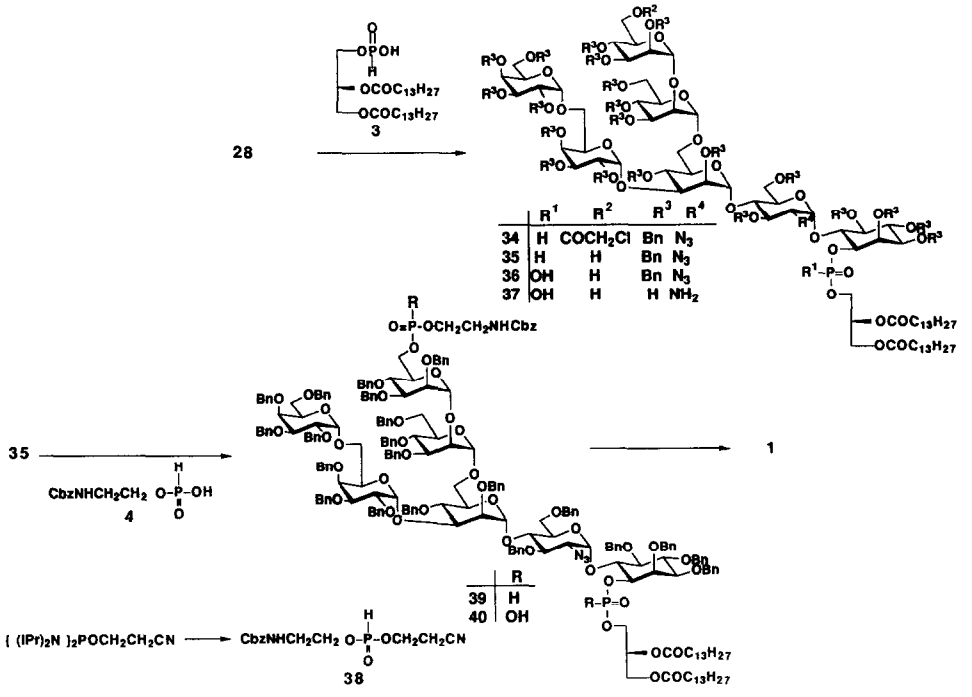
ene (DBU), followed by hydrogenation with 20% $Pd(OH)_2/C$. The ^{31}P NMR spectrum of **32** contained a signal at 1.09 ppm, and the ^1H NMR spectrum (see Experimental) was also in agreement with the assigned structure. However, removal of methoxybenzyl group at O-1a of **30** by either ammonium cerium(IV) nitrate or trimethylsilyl trifluoromethanesulfonate unexpectedly failed to give the desired compound **33**. Therefore, an alternative strategy was devised to circumvent this difficulty as follows. Compound **2** was first transformed into **27** through replacement of the acetyl group at O-6e with a chloroacetyl group, in agreement with a scenario that the chemoselective removal of the O-6e protective group should be achieved in the presence of the diacylglycerol moiety in a compound such as **34**. Treatment of **26** with chloroacetic anhydride[20] in pyridine quantitatively afforded **27**, which was smoothly converted to **28** as expected in 90% yield by removal of the 4-methoxybenzyl group at O-1a with trimethylsilyl trifluoromethanesulfonate in dichloroethane. The introduction of the phosphodiester function into **28** was successfully performed by the H-phosphonate method[21]. First, condensation of **28** with **3** was performed smoothly by using pivaloyl chloride in pyridine to give a 64% yield of **34** (Scheme 6). In order to confirm the structure, compound **34** was transformed into the free hydroxy phosphodiester **37** in 3 steps: (1) regioselective *O*-dechloroacetylation with thiourea[22] in 1:1 ethanol–THF (75%); (2) oxidation with iodine in 50:1 pyridine–H_2O (quant.); and (3) hy-



Scheme 6.

drogenolysis (78%). The ^{1}H NMR (see Experimental) and ^{31}P NMR spectra of **37** were in agreement with the structure assigned. FABMS gave the $[M^+ + H]$ ion with m/z 1726 as expected for **37** (Scheme 7).

Having prepared the desired key intermediate **35**, the introduction of the phosphodiester function to O-6e of **35** was next examined. The phosphitylating



Scheme 7.

reagent **29**, which was found to be so efficient in the transformation of **26** into **30**, was examined first for its reaction with **35**, but it gave none of the desired phosphite intermediate. However, the H-phosphonate approach proved to be highly efficient by use of compound **4** that was prepared as follows. Reaction of 2-[(*N*-benzyloxycarbonyl)amino]ethanol with a bifunctional phosphitylating reagent $NCCH_2CH_2OP[N(2\text{-}Pr_2)_2]$[23] in the presence of 1H-tetrazole in acetonitrile, and subsequent treatment of the product with H_2O and 1*H*-tetrazole afforded **38**, which, upon decyanoethylation with DBU in dichloromethane gave the unstable H-phosphonate **4** that was used immediately for the next step. The crucial coupling between **35** and **4** was executed in the presence of pivaloyl chloride to afford 40% of the desired **39** as a mixture of four diastereomers. Oxidation of **39** with iodine gave a 68% yield of **40**, the ^{31}P NMR spectrum of which contained two signals at −0.65 and 0.31 ppm.

Complete deprotection of **40** by hydrogenolysis in the presence of 20% $Pd(OH)_2/C$ in 45:35:1 chloroform–methanol–H_2O afforded a 23% yield of the target molecule **1**, the structure of which was confirmed by ^1H and ^{31}P NMR spectroscopy (see Experimental).

In summary, the first total synthesis of the GPI anchor **1** of *Trypanosoma brucei* was accomplished by using recently available, stereocontrolled glycosylation technologies, as well as a highly efficient H-phosphonate approach to construct the two different phosphodiester linkages.

EXPERIMENTAL

General.—Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter for solutions in $CHCl_3$ at 25°, unless noted otherwise. Column chromatography was performed on Silica Gel-6O (E. Merck 70–230 mesh). Flash chromatography was performed on columns of Wako-gel C-300 (200–300 mesh). TLC and HPTLC were performed on Silica Gel-60 F_{254} (E. Merck). Molecular sieves were purchased from Nakarai Chemicals. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded with either a GNM-GSX-500, a JEOL GX400, or a FX90Q spectrometer. The values of δ_H and δ_C are expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$, unless noted otherwise. Values of δ_H (D_2O) are expressed in ppm downfield from the signal for Me_4Si by reference to internal Me_3COH (1.230 ppm). Values of δ_P are expressed in ppm downfield from the signal for external 85% H_3PO_4, for solutions in $CDCl_3$, unless noted otherwise.

4-Methoxyphenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**9**).—To a stirred mixture of penta-*O*-acetyl-β-D-galactopyranose (19.5 g, 50 mmol) and 4-methoxyphenol (9.3 g, 75 mmol) in dry dichloroethane (500 mL) was added $CF_3SO_3SiMe_3$ (2.9 mL, 15 mmol) at −15° under Ar. The mixture was stirred for 3 h at 20°, then

poured into aq $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (1:2 EtOAc–hexane) of the residue gave **9** (19.9 g, 88%); R_F 0.24 (1:2 EtOAc–hexane); $[\alpha]_D$ +9.6° (*c* 0.54). NMR data: ^1H, δ 2.010 (s, 3 H, Ac), 2.054 (s, 3 H, Ac), 2.084 (s, 3 H, Ac), 2.183 (s, 3 H, Ac), 3.777 (s, 3 H, OMe), 4.006 (dt, 1 H, *J* 1.2 and 6.7 Hz, H-5), 4.160 (dd, 1 H, *J* 6.7 and 11.3 Hz, H-6), 4.232 (dd, 1 H, *J* 7.0 and 11.3 Hz, H-6′), 4.916 (d, 1 H, *J* 8.2 Hz, H-1), 5.090 (dd, 1 H, *J* 3.5 and 10.5 Hz, H-3), 5.441 (dd, 1 H, *J* 0.9 and 3.4 Hz, H-4), 5.454 (dd, 1 H, *J* 7.9 and 10.4 Hz, H-2), 6.802–6.972 (m, 4 H, ArH); ^{13}C, δ 100.9 ($^1J_{C,H}$ 162.4 Hz).

Anal. Calcd for $C_{21}H_{26}O_{11} \cdot 0.25\ H_2O$: C, 54.95; H, 5.82. Found: C, 54.69; H, 5.81.

4-Methoxyphenyl β-D-galactopyranoside (**10**).—A solution of **9** (19.8 g, 43.6 mmol) in MeOH (200 mL) and a 28% solution of NaOMe in MeOH (2 mL) was mixed and stirred for 1 h at 20°. The mixture was neutralized with Amberlyst-15 (H^+) resin and filtered through Celite. The filtrate was concentrated in vacuo, and the residue was chromatographed on silica gel (1:4 MeOH–$CHCl_3$) to give **10** (11.6 g, 93%); mp 160–161° (from MeOH), R_F 0.42 (1:4 MeOH–$CHCl_3$); $[\alpha]_D$ −41.8° (*c* 0.28, MeOH). ^1H NMR data (10:1 $CDCl_3$–CD_3OD): δ 3.774 (s, 3 H, OMe), 3.974 (d, 1 H, *J* 1.8 Hz, H-4), 4.748 (d, 1 H, *J* 7.9 Hz, H-1), 6.808-7.050 (m, 4 H, Ar*H*).

Anal. Calcd for $C_{13}H_{18}O_7 \cdot 0.33\ H_2O$: C, 53.42; H, 6.43. Found: C, 53.54; H, 6.31.

4-Methoxyphenyl 6-O-(4,4′-dimethoxytrityl)-β-D-galactopyranoside (**11**).—A solution of **10** (11.6 g, 40.6 mmol) and 4,4′-dimethoxytrityl chloride (19.5 g, 57.5 mmol) in pyridine (250 mL) was stirred overnight at 20° under Ar. After addition of MeOH, the solvent was evaporated in vacuo. The residue was extracted with EtOAc, washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (1:2:98 Et_3N–MeOH–$CHCl_3$) of the residue gave **11** (21.6 g, 90%): R_F 0.51 (MeOH–$CHCl_3$, 1:4); $[\alpha]_D$ −22.3° (*c* 0.56). ^1H NMR data: δ 3.378 (dd, 1 H, *J* 5.0 and 9.9 Hz, H-6), 3.503 (dd, 1 H, *J* 6.1 and 10.1 Hz, H-6′), 3.630 (br t, 1 H, *J* 5.8 Hz, H-5), 3.756 (s, 3 H, OMe), 3.774 (s, 6 H, OMe), 3.884 (dd, 1 H, *J* 7.6 and 9.5 Hz, H-2), 3.994 (br s, 1 H, H-4), 4.729 (d, 1 H, *J* 7.6 Hz, H-1), 6.792–7.447 (m, 17 H, ArH).

Anal. Calcd for $C_{34}H_{36}O_9$: C, 69.37; H, 6.16. Found: C, 68.99; H, 6.51.

4-Methoxyphenyl 2,3,4-tri-O-benzyl-β-D-galactopyranoside (**12**).—To a stirred solution of **11** (21.5 g, 36.5 mmol) in DMF (500 mL) was added NaH (60% in mineral oil; 5.9 g, 146 mmol) at 0° under Ar. Stirring was continued for 1 h at 0°, benzyl bromide (15.6 mL, 128 mmol) was added at 0°, and the mixture was stirred at 20° for 2 days. The reaction mixture was poured into ice–water and extracted with ether. The extract was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. To a stirred solution of the residue in $CHCl_3$ (420 mL) and MeOH (180 mL) was added *p*-toluenesulfonic acid (300 mg), and the mixture was stirred at 20° for 0.5 h. The reaction mixture was poured into aq $NaHCO_3$ and extracted with

$CHCl_3$. The extract was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. Chromatography (1:9 EtOAc–toluene) of the residue gave **12** (13.5 g, 66%); R_F 0.25 (1:4 EtOAc–toluene); $[\alpha]_D$ −25.8° (*c* 0.21). ^1H NMR data: δ 3.476 (br t, 1 H, *J* 5.5 Hz, H-5), 3.509 (ddd, 1 H, *J* 5.1, 8.8, and 11.0 Hz, H-6), 3.610 (dd, 1 H, *J* 2.9 and 9.5 Hz, H-3), 3.765 (s, 3 H, OMe), 3.826 (d, 1 H, *J* 2.6 Hz, H-4), 4.100 (dd, 1 H, *J* 7.7 and 9.9 Hz, H-2), 4.675–4.875 (m, 4 H, CH_2Ph), 4.882 (d, 1 H, *J* 8.1 Hz, H-1), 4.994 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 5.018 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 6.789–7.006 (m, 4 H, ArH), 7.258–7.397 (m, 15 H, ArH).

Anal. Calcd for $C_{34}H_{36}O_7$: C, 73.36; H, 6.52. Found: C, 73.23; H, 6.51.

4-Methoxyphenyl O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**14**).—To a mixture of 4A molecular sieves (15 g), $CuBr_2$ (4.5 g, 20 mmol), and Bu_4NBr (6.4 g, 20 mmol) was added a solution of **12** (5.6 g, 10 mmol) and **13** (6.8 g, 12 mmol) in dichloroethane (83 mL) under Ar. The mixture was stirred overnight at 20°, neutralized with Et_3N, and filtered through Celite. The filtrate was washed with aq $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated in vacuo. Chromatography (1:39 EtOAc–toluene) of the residue gave **14** (7.3 g, 67%) and its β isomer (1.1 g, 10%).

Compound **14** had R_F 0.48 (1:9 EtOAc–toluene); mp 126–127°; $[\alpha]_D$ +17.7° (*c* 0.35). NMR data: ^1H, δ 3.466 (dd, 1 H, *J* 5.9 and 9.9 Hz), 3.500 (d, 2 H, *J* 7.0 Hz), 3.570 (dd, 1 H, *J* 2.9 and 9.9 Hz, H-3f), 3.666 (s, 3 H, OMe), 3.870 (dd, 1 H, *J* 2.7 and 10.1 Hz, H-3g), 4.014 (dd, 1 H, *J* 3.5 and 10.1 Hz, H-2g), 4.053 (dd, 1 H, *J* 7.9 and 9.7 Hz, H-2f), 4.322–4.998 (m, 14 H, CH_2Ph), 4.735 (d, 1 H, *J* 3.3 Hz, H-1b), 4.823 (d, 1 H, *J* 7.7 Hz, H-1f), 6.720–7.011 (m, 4 H, ArH), 7.204–7.383 (m, 35 H); ^{13}C, δ 98.2 ($^1J_{C,H}$ 169.7 Hz, C-1g), 103.1 ($^1J_{C,H}$ 161.1 Hz, C-1f).

Anal. Calcd for $C_{68}H_{70}O_{12}$: C, 75.67; H, 6.54. Found: C, 75.31; H, 6.53.

The β isomer had R_F 0.24 (CH_2Cl_2); $[\alpha]_D$ −9.1° (*c* 0.33). NMR data: ^1H, δ 3.518 (dd, 1 H, *J* 2.9 and 9.5 Hz), 3.562 (dd, 1 H, *J* 7.5 and 8.6 Hz), 3.604 (s, 3 H, OMe), 4.058 (dd, 1 H, *J* 7.7 and 9.9 Hz), 4.377 (d, 1 H, *J* 7.3 Hz, H-1g), 4.830 (d, 1 H, *J* 7.7 Hz, H-1f), 6.666–6.982 (m, 4 H, ArH), 7.179–7.358 (m, 35 H, ArH); ^{13}C, δ 102.7 ($^1J_{C,H}$ 153.8 Hz), 103.7 ($^1J_{C,H}$ 156.3 Hz).

Anal. Calcd for $C_{68}H_{70}O_{12}$: C, 75.67; H, 6.54. Found: C, 75.41; H, 6.48.

O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-D-galactopyranoside (**15**).—To a stirred solution of **14** (2.3 g, 2 mmol) in CH_3CN (30 mL) and H_2O (15 mL) was added ammonium cerium(IV) nitrate (5.5 g, 10 mmol) at 0°, and the mixture was stirred at 0° for 10 min. The mixture was then diluted with EtOAc, and the organic layer was washed with H_2O and brine, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (1:9 EtOAc–toluene) of the residue gave **15** (1.3 g, 65%); R_F 0.15 (1:9 EtOAc–toluene).

Anal. Calcd for $C_{61}H_{64}O_{11} \cdot 0.5\ H_2O$: C, 74.59; H, 6.57. Found: C, 74.36; H, 6.62.

O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-D-galactopyranosyl fluoride (**6**).—To a stirred solution of **15** (250 mg, 0.26 mmol) in dichloroethane (5 mL) was added DAST (0.04 mL, 0.3 mmol) at −23° under Ar,

and the mixture was stirred at 20° for 0.5 h. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed with aq $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated in vacuo. Chromatography (1:19 EtOAc–toluene) of the residue gave **6** (241 mg, 96%); R_F 0.66 (1:9 EtOAc–toluene). ^1H NMR data: δ 5.097 (dd, 0.6 H, J 7.0 and 53.1 Hz, H-1fβ), 5.551 (dd, 0.4 Hz, J 2.6 and 54.2 Hz, H-1fα).

Anal. Calcd for $C_{61}H_{63}FO_{10}$: C, 75.13; H, 6.51. Found: C, 75.18; H, 6.49.

O-*(6-O-Acetyl-2,4-di-O-benzyl-α-D-mannosyl)-(1 → 4)*-O-*(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-2,3,4,6-tetra-O-benzyl-1*-O-*(4-methoxybenzyl)-1D*-myo-*inositol* (**5**).—To a solution of **16** (66 mg, 48 mmol) in dry pyridine (2 mL) was added acetyl chloride (14 μL, 0.2 mmol) at 0°, and the mixture stirred at 0° for 7 h. After addition of MeOH, the solvents were evaporated in vacuo, and the residue was purified by preparative TLC with 1:9 EtOAc–toluene to give **5** (64 mg, 95%); R_F 0.24 (1:9 EtOAc–toluene); $[\alpha]_D$ +69.3° (c 0.61). ^1H NMR data: δ 1.922 (s, 3 H, Ac), 3.226 (dd, 1 H, J 3.7 and 10.1 Hz, H-2b), 3.284 (dd, 1 H, J 2.1 and 10.1 Hz), 3.372 (dd, 1 H, J 2.1 and 9.8 Hz), 3.434 (d, 2 H, J 2.1 Hz, H-6 and H-6′b), 3.585 (t, 2 H, J 9.3 Hz), 3.666 (s, 3 H, OMe), 3.836 (t, 1 H, J 9.3 Hz), 3.884 (t, 1 H, J 9.9 Hz), 3.947 (d, 1 H, J 11.6 Hz, CH_2Ph), 3.979 (br s, 1 H, H-2a), 4.091 (t, 1 H, J 9.5 Hz), 4.142 (d, 2 H, J 3.4 Hz, H-6 and H-6′c), 4.152 (d, 1 H, J 11.0 Hz, CH_2Ph), 4.288 (br d, 1 H, J 9.8 Hz, H-5c), 4.331 (t, 1 H, J 9.6 Hz), 4.387–5.078 (m, 16 H, CH_2Ph), 5.274 (d, 1 H, J 1.2 Hz, H-1c), 5.635 (d, 1 H, J 3.7 Hz, H-1b), 6.816 (d, 2 H, J 8.5 Hz, ArH), 7.054–7.395 (m, 42 H, ArH).

Anal. Calcd for $C_{84}H_{89}N_3O_{17}$: C, 71.42; H, 6.35; N, 2.97. Found: C, 71.42; H, 6.37; N, 2.98.

O-*[O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri-O-benzyl-α-D-galactopyranosyl)-(1 → 3)*-O-*(6-O-acetyl-2,4-di-O-benzyl-α-D-mannopyranosyl)-(1 → 4)*-O-*(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)]-(1 → 6)-2,3,4,5-tetra-O-benzyl-1*-O-*(4-methoxybenzyl)-1D*-myo-*inositol* (**17**).—To a mixture of 4A molecular sieves (200 mg), Cp_2ZrCl_2 (12.4 mg, 0.43 mmol) and $AgClO_4$ (88 mg, 0.43 mmol) was added **5** (60 mg, 43 μmol) and **6** (83 mg, 85 μmol) in dry ether (5 mL), and the mixture was stirred for 4 h at 20°. The mixture was neutralized with Et_3N, diluted with EtOAc, and filtered through Celite. The filtrate was washed with aq $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by preparative TLC with 1:3 EtOAc–hexane to afford **17** (76 mg, 76%) as an anomeric mixture, **18** (18 mg, 18%), and **19** (15 mg, 41% based on **6**). Although **17**$\alpha\beta$ could not be directly separated, they were obtained after reacetylation of **20**α and **20**β, respectively.

Compound **17**α had R_F 0.35 (1:3 EtOAc–hexane); $[\alpha]_D$ +72.2° (c 0.45). NMR data: ^1H, δ 1.824 (s, 3 H, Ac), 3.179 (dd, 1 H, J 3.7 and 10.1 Hz, H-2b), 3.222 (dd, 1 H, J 2.0 and 9.9 Hz), 3.481 (s, 3 H, OMe), 5.235 (br s, 1 H, H-1c), 5.626 (d, 1 H, J 3.7 Hz, H-1b), 6.768 (d, 2 H, J 8.5 Hz, ArH), 7.130–7.359 (m, 77 H, ArH); ^{13}C, δ 97.6 ($^1J_{C,H}$ 175.8 Hz, C-1b), 98.9 ($^1J_{C,H}$ 169.7 Hz), 99.4 ($^1J_{C,H}$ 169.7 Hz), 99.9 ($^1J_{C,H}$ 169.0 Hz).

Anal. Calcd for $C_{145}H_{151}N_3O_{27} \cdot 0.5\ H_2O$: C, 73.27; H, 6.44; N, 1.77. Found: C, 73.00; H, 6.40; N, 1.60.

Compound **17β** had R_F 0.42 (1:3 EtOAc–hexane); $[\alpha]_D$ +43.4° (*c* 0.75). NMR data: ^1H, δ 1.907 (s, 3 H, Ac), 3.161 (dd, 1 H, *J* 3.9 and 10.1 Hz, H-2b), 3.209 (dd, 1 H, *J* 2.0 and 10.1 Hz), 3.432 (s, 3 H, OMe), 5.323 (d, 1 H, *J* 2.2 Hz, H-1c), 5.576 (d, 1 H, *J* 3.7 Hz, H-1b), 6.801 (d, 2 H, *J* 8.4 Hz, ArH), 6.966–7.361 (m, 77 H, ArH); ^{13}C, δ 97.6 ($^1J_{C,H}$ 174.4 Hz, C-1b), 99.2 ($^1J_{C,H}$ 167.4 Hz), 99.7 ($^1J_{C,H}$ 170.2 Hz), 101.8 ($^1J_{C,H}$ 158.9 Hz, C-1f).

Compound **18** had R_F 0.4 (1:3 EtOAc–hexane); $[\alpha]_D$ +56.7° (*c* 0.33). NMR data: ^1H, δ 1.845 (s, 3 H, Ac), 3.504 (s, 3 H, OMe), 5.136 (d, 1 H, *J* 3.1 Hz, H-1f), 5.392 (d, 1 H, *J* 1.8 Hz, H-1c), 5.621 (d, 1 H, *J* 3.7 Hz, H-1b), 6.814 (d, 2 H, *J* 8.5 Hz, ArH), 7.085–7.367 (m, 62 H, ArH), ^{13}C, δ 97.7 ($^1J_{C,H}$ 178.5 Hz, C-1b), 98.7 ($^1J_{C,H}$ 170.9 Hz), 102.0 ($^1J_{C,H}$ 172.4 Hz).

Anal. Calcd for $C_{118}H_{123}N_3O_{22}$: C, 73.23; H, 6.41; N, 2.17. Found: C, 73.21; H, 6.48; N, 1.95.

Compound **19** had R_F 0.42 (1:3 EtOAc–hexane); $[\alpha]_D$ +43.5° (*c* 0.85). NMR data: ^1H, δ 3.526 (t, 1 H, *J* 1.5 Hz), 3.622 (t, 1 H, *J* 5.9 Hz), 3.878 (t, 1 H, *J* 4.2 Hz), 4.415 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 4.443 (t, 1 H, *J* 4.6 Hz), 4.478 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 4.498 (d, 1 H, *J* 7.3 Hz), 4.523 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 4.553 (s, 2 H, CH_2Ph), 4.597 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 5.356 (t, 1 H, *J* 1.5 Hz), 7.242–7.355 (m, 15 H, ArH).

Anal. Calcd for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53. Found: C, 74.63; H, 6.52.

O-*(2,3,4,6-Tetra*-O-*benzyl-α-*D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzyl-α-*D-*galactopyranosyl)-(1 → 3)*-O-*(2,4-di*-O-*benzyl-α-*D-*mannopyranosyl)-(1 → 4)*-O-*(2-azido-3,6-di*-O-*benzyl-2-deoxy-α-*D-*glucopyranosyl)-(1 → 6)-2,3,4,5-tetra*-O-*benzyl-1*-O-*(4-methoxybenzyl)-1*D-myo-*inositol* (**20α**).—Deacetylation of **17** (136 mg, 57 μmol) was carried out as described above, followed by purification of the product by preparative TLC (1:9 EtOAc–toluene) to give **20α** (118 mg, 89%) and **20β** (14 mg, 11%).

Compound **20α** had R_F 0.46 (1:9 EtOAc–hexane); $[\alpha]_D$ +63.0° (*c* 0.36). ^1H NMR data: δ 3.473 (s, 3 H, OMe), 5.289 (br s, 1 H, H-1c), 5.585 (d, 1 H, *J* 3.7 Hz, H-1b), 6.740 (d, 2 H, *J* 8.1 Hz, ArH), 7.095–7.401 (m, 77 H, ArH).

Anal. Calcd for $C_{143}H_{149}N_3O_{26}$: C, 73.85; H, 6.45; N, 1.81. Found: C, 73.71; H, 6.47; N, 1.70.

O-*(2*-O-*Acetyl-3,4,6-tri*-O-*benzyl-α-*D-*mannopyranosyl)-(1 → 6)*-O-*[*O-*(2,3,4,6-tetra*-O-*benzyl-α-*D-*galactopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzyl-α-*D-*galactopyranosyl-(1 → 3)]*-O-*(2,4-di*-O-*benzyl-α-*D-*mannopyranosyl)-(1 → 4)*-O-*(2-azido-3,6-di*-O-*benzyl-2-deoxy-α-*D-*glucopyranosyl)-(1 → 6)-2,3,4,5-tetra*-O-*benzyl-1*-O-*(4-methoxybenzyl)-1*D-myo-*inositol* (**21**).—To a mixture of 4A molecular sieves (500 mg), $HgBr_2$ (61 mg, 0.17 mmol), and $HgCN_2$ (128 mg, 0.5 mmol) was added successively **20α** (98 mg, 42 μmol) in dichloroethane (5 mL) and **7** (86 mg, 0.17 mmol) in dichloroethane (3 mL) at 0°. The mixture was allowed to warm to ambient temperature, and stirring was continued overnight. Workup as described above for

14, followed by purification by preparative TLC (1:9 EtOAc–toluene, then extraction with $CHCl_3$) gave **21** (105 mg, 89%); R_F 0.54 (1:9 EtOAc–toluene); $[\alpha]_D$ +73.2° (*c* 0.57). NMR data: ^1H, δ 2.052 (s, 3 H, Ac), 3.176 (dd, 1 H, *J* 4.0 and 10.3 Hz, H-2b), 3.206 (dd, 1 H, *J* 1.8 and 9.9 Hz), 3.457 (s, 3 H, OMe), 5.246 (d, 1 H, *J* 1.1 Hz, H-1c), 5.322 (d, 1 H, *J* 1.5 Hz, H-1d), 5.384 (d, 1 H, *J* 1.8 Hz, H-1f), 5.391 (dd, 1 H, *J* 1.8 and 3.3 Hz, H-2d), 5.616 (d, 1 H, *J* 3.7 Hz, H-1b), 6.782 (d, 2 H, *J* 8.4 Hz, ArH), 7.026–7.349 (m, 92 H, ArH); ^{13}C, δ 97.7 ($^1J_{C,H}$ 176.1 Hz, C-1b), 98.2 ($^1J_{C,H}$ 170.7 Hz), 98.5 ($^1J_{C,H}$ 172.1 Hz), 99.4 ($^1J_{C,H}$ 168.5 Hz), 100.3.

Anal. Calcd for $C_{172}H_{179}N_3O_{32} \cdot 0.2\ CHCl_3$: C, 73.23; H, 6.40; N, 1.49. Found: C, 73.32; H, 6.68; N, 1.43.

O-*(3,4,6-Tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 6)-*O*-[*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-*D*-galactopyranosyl-(1 → 3)]-*O*-(2,4-di-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-1*D*-myo-inositol* (**22**).—As described above for **10**, treatment of **21** (225 mg, 80 μmol) with a 28% solution of NaOMe in MeOH (48 μL) in THF (4 mL) and MeOH (6 mL), followed by purification by preparative TLC (1:9 EtOAc–toluene), gave **22** (207 mg, 94%); R_F 0.18 (1:9 EtOAc–hexane); $[\alpha]_D$ +72.9° (*c* 0.55). NMR data: ^1H, δ 3.177 (dd, 1 H, *J* 3.7 and 10.3 Hz, H-2b), 3.209 (dd, 1 H, *J* 1.8 and 9.9 Hz), 3.451 (s, 3 H, OMe), 5.315 (d, 1 H, *J* 1.8 Hz, H-1f), 5.627 (d, 1 H, *J* 3.7 Hz, H-1b), 6.769 (d, 2 H, *J* 8.4 Hz, ArH), 7.046–7.355 (m, 92 H, ArH); ^{13}C, 97.7 ($^1J_{C,H}$ 177.2 Hz, C-1b), 98.5 ($^1J_{C,H}$ 168.8 Hz), 99.3 ($^1J_{C,H}$ 168.8 Hz), 99.8 ($^1J_{C,H}$ 167.4 Hz), 100.1 ($^1J_{C,H}$ 163.1 Hz).

Anal. Calcd for $C_{170}H_{177}N_3O_{31}$: C, 74.02; H, 6.47; N, 1.52. Found: C, 73.38; H, 6.53; N, 1.48.

*6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-*D*-mannopyranose* (**25**).—A mixture of **23** (3.2 g, 6 mmol) and $NH_2NH_2 \cdot AcOH$ (1.1 g, 12 mmol) in DMF (40 mL) was stirred at 20° for 2 h, then diluted with ether. The mixture was washed with H_2O and brine, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (1:2 EtOAc–hexane) of the residue gave **24** (2.2 g, 75%); R_F 0.30 (1:2 EtOAc–hexane). ^1H NMR data: δ 2.036 (s, 0.81 H, Ac), 2.043 (s, 2.19 H, Ac), 3.504 (ddd, 0.27 H, *J* 2.2, 5.9, and 9.5 Hz, H-5β), 3.644 (dd, 0.27 H, *J* 3.1 and 9.7 Hz, H-3β), 3.815 (dd, 0.73 H, *J* 1.9 and 2.3 Hz, H-2α), 3.921 (t, 0.73 H, *J* 9.3 Hz, H-4α), 3.985 (dd, 0.73 H, *J* 2.9 and 9.2 Hz, H-3α), 4.018 (ddd, 0.73 H, *J* 1.8, 5.1, and 9.5 Hz, H-5α), 4.229 (dd, 0.27 H, *J* 5.7 and 11.9 Hz, H-6β), 4.256 (dd, 0.73 H, *J* 5.1 and 12.1 Hz, H-6α), 4.366 (dd, 0.27 H, *J* 2.2 and 11.7 Hz, H-6′β), 4.378 (dd, 0.73 H, *J* 2.0 and 11.9 Hz, H-6′α), 4.584–5.116 (m, 6 H, C*H*$_2$Ph), 5.244 (dd, 0.73 H, *J* 1.8 an 3.3 Hz, H-1α), 7.252–7.370 (m, 15 H, Ar*H*).

Anal. Calcd for $C_{29}H_{32}O_7 \cdot 0.25\ H_2O$: C, 70.07; H, 6.59. Found: C, 70.03; H, 6.61.

*6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-*D*-mannopyranosyl fluoride* (**8**).—Treatment of **24** (492 mg, 1 mmol) with DAST, as described for **6**, and chromatography (1:5 EtOAc–hexane) of the product afforded **8** (449 mg, 91%); R_F 0.42 (1:4 EtOAc–

hexane); $[\alpha]_D$ +29.9° (*c* 1.42). NMR data: ^1H, δ 2.052 (s, 3 H, Ac), 4.276 (dd, 1 H, *J* 4.9 and 12.9 Hz, H-4), 4.378 (dd, 1 H, *J* 1.8 and 11.9 Hz, H-3), 4.591–4.936 (m, 6 H, CH_2Ph), 5.549 (dd, 1 H, *J* 1.8 and 50.6 Hz, H-1), 7.153–7.352 (m, 15 H, ArH); ^{13}C, δ 106.3 ($^1J_{C,F}$ 223.4 Hz, $^1J_{C,H}$ 182.0 Hz).

Anal. Calcd for $C_{29}H_{31}FO_6$: C, 70.43; H, 6.32. Found: C, 70.31; H, 6.39.

O-*(6-O-Acetyl-2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-O-[O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-α-D-galactopyranosyl-(1 → 3)]-O-(2,4-di-O-benzyl-α-D-mannopyranosyl)-(1 → 4)-O-(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-O-benzyl-1-O-(4-methoxybenzyl)-1D-myo-inositol* (**2**).—Glycosylation of **22** (27.6 mg, 0.01 mmol) and **8** (19.8 mg, 0.04 mmol) was carried out with Cp_2ZrCl_2 (58.5 mg, 9.2 mmol), $AgClO_4$ (41.5 mg, 0.2 mmol), and 4A molecular sieves (200 mg) in dry ether (5 mL) at 20° overnight, and the mixture was worked up as described for **17**. The crude product was purified by preparative TLC (1:9 EtOAc–toluene, then extracted with $CHCl_3$) to give **2** (30 mg, 93%) and its β isomer (2 mg, 6%).

Compound **2** had R_F 0.73 (1:9 EtOAc–hexane); $[\alpha]_D$ +51.0° (*c* 0.31). NMR data (C_6D_6, 60°): ^1H, δ 1.767 (s, 3 H, Ac), 3.154 (dd, 1 H, *J* 3.7 and 10.4 Hz, H-2b), 3.222 (dd, 1 H, *J* 2.3 and 9.9 Hz), 3.268 (dd, 1 H, *J* 2.1 and 10.1 Hz), 3.404 (s, 3 H, OMe), 3.586 (br d, 1 H, *J* 9.8 Hz), 3.639 (dd, 1 H, *J* 5.8 and 9.2 Hz), 3.668 (t, 1 H, *J* 9.0 Hz), 5.031 (d, 1 H, *J* 3.7 Hz, H-1g), 5.191 (d, 1 H, *J* 1.8 Hz), 5.229 (d, 1 H, *J* 1.8 Hz), 5.382 (d, 1 H, *J* 3.4 Hz, H-1f), 5.783 (d, 1 H, *J* 1.8 Hz), 5.868 (d, 1 H, *J* 3.7 Hz, H-1b), 6.986–7.535 (m, 109 H, ArH); ^{13}C, δ 97.6 ($^1J_{C,H}$ 175.0 Hz, C-1b), 98.5 ($^1J_{C,H}$ 174.0 Hz), 99.2 ($^1J_{C,H}$ 170.4 Hz), 99.4 ($^1J_{C,H}$ 170.4 Hz), 99.5 ($^1J_{C,H}$ 168.9 Hz), 100.1 ($^1J_{C,H}$ 175.5 Hz).

Anal. Calcd for $C_{199}H_{207}N_3O_{37} \cdot 0.2\ CHCl_3$: C, 73.46; H, 6.41; N, 1.29. Found: C, 73.08; H, 6.40; N, 1.23.

The β isomer had R_F 0.63 (1:9 EtOAc–hexane); $[\alpha]_D$ +32.6° (*c* 0.19). NMR data (C_6D_6, 60°): ^1H, δ 1.603 (s, 3 H, Ac), 2.971 (dd, 1 H, *J* 3.2 and 10.8 Hz, H-2b), 3.391 (s, 3 H, OMe), 5.377 (d, 1 H, *J* 3.1 Hz, H-1f), 5.732 (d, 1 H, *J* 1.2 Hz), 5.815 (d, 1 H, *J* 3.4 Hz, H-1b), 6.984–7.621 (m, 109 H, ArH); ^{13}C, δ 97.6 ($^1J_{C,H}$ 175.3 Hz, C-1b), 97.8 ($^1J_{C,H}$ 169.5 Hz), 98.9 ($^1J_{C,H}$ 173.3 Hz), 99.5 ($^1J_{C,H}$ 173.3 Hz), 99.6 ($^1J_{C,H}$ 153.8 Hz, C-1e), 100.1 ($^1J_{C,H}$ 164.7 Hz).

O-*α-D-Mannopyranosyl-(1 → 2)-O-α-D-mannopyranosyl-(1 → 6)-O-[O-α-D-galactopyranosyl-(1 → 6)-α-D-galactopyranosyl-(1 → 3)]-O-α-D-mannopyranosyl-(1 → 4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1 → 6)-1D-myo-inositol* (**25**).—A mixture of **2** (3 mg, 0.93 μmol) and 10% Pd/C (3 mg) in THF (0.2 mL) and MeOH (0.5 mL) was stirred in the presence of 5% aq HCl (trace amounts) overnight at 20°, then diluted with MeOH, filtered through Celite, and concentrated in vacuo. To a solution of the residue in MeOH (0.1 mL) was added a 28% solution of NaOMe in MeOH (large excess), stirred at 20° for 2 h, and the solvent was evaporated in vacuo. Chromatography (Sephadex G-25, H_2O) of the residue gave **25** (1 mg, 93%): R_F 0.10 (1:1:2 BuOH–EtOH–H_2O); $[\alpha]_D$ +2.9° (*c* 0.07, H_2O). ^1H NMR

data (D_2O, 60°): δ 4.976 (d, 1 H, *J* 3.7 Hz, H-1g), 5.035 (d, 1 H, *J* 1.6 Hz, H-1e), 5.115 (d, 1 H, *J* 1.2 Hz, H-1d), 5.197 (d, 1 H, *J* 3.7 Hz, H-1f), 5.278 (d, 1 H, *J* 2.0 Hz, H-1c), 5.478 (d, 1 H, *J* 3.7 Hz, H-1b). Mass spectrum: *m/z* 1152 ($M^+ + H$).

*O-(2,3,4-Tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 6)-*O*-[*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-*D*-galactopyranosyl-(1 → 3)]-*O*-(2,4-di-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-1*D*-myo-inositol* (**26**).—Deacetylation of **2** (26 mg, 8 μmol) as described above for **10** followed by chromatography (1:9 EtOAc–toluene) gave **26** (25.6 mg, quantitative): R_F 0.47 (1:9 EtOAc–hexane); $[\alpha]_D$ +56.7° (*c* 1.54). NMR data: ^1H, δ 3.198 (dd, 1 H, *J* 3.5 and 10.2 Hz, H-2b), 3.450 (s, 3 H, OMe), 5.369 (d, 1 H, *J* 2.1 Hz), 5.615 (d, 1 H, *J* 3.7 Hz, H-1b), 6.781 (d, 2 H, *J* 8.5 Hz, ArH), 6.962–7.466 (m, 107 H, ArH).

Anal. Calcd for $C_{197}H_{205}N_3O_{36} \cdot 2$ toluene: C, 75.08; H, 6.60; N, 1.25. Found: C, 75.41; H, 6.82; N, 1.24.

*O-(2,3,4-Tri-*O*-benzyl-6-*O*-chloroacetyl-α-*D*-mannopyranosyl)-(1 → 2)-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 6)-*O*-[*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-*D*-galactopyranosyl-(1 → 3)]-*O*-(2,4-di-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-1*D*-myo-inositol* (**27**).—A mixture of **26** (94 mg, 29 μmol), chloroacetic anhydride (26 mg, 0.15 mmol), and pyridine (24 μL, 0.3 mmol) in dichloroethane (3 mL) was stirred at 20° for 0.5 h. After addition of MeOH, the mixture was diluted with $CHCl_3$ washed with aq $NaHCO_3$, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (1:19 EtOAc–toluene) of the residue gave **27** (96 mg, quantitative); R_F 0.62 (1:9 EtOAc–hexane); $[\alpha]_D$ +63.8° (*c* 3.82). ^1H NMR data (C_6D_6, 60°): δ 3.198 (dd, 1 H, *J* 3.7 and 10.4 Hz, H-2b), 3.268 (dd, 1 H, *J* 2.1 and 10.1 Hz), 3.402 (s, 3 H, OMe), 3.576 (dd, 1 H, *J* 1.5 and 11.5 Hz), 3.626 (s, 2 H, $COCH_2Cl$), 5.033 (d, 1 H, *J* 3.7 Hz, H-1g), 5.163 (d, 1 H, *J* 1.5 Hz), 5.182 (d, 1 H, *J* 1.5 Hz), 5.388 (d, 1 H, *J* 3.4 Hz, H-1f), 5.781 (d, 1 H, *J* 1.8 Hz), 5.874 (d, 1 H, *J* 3.7 Hz, H-1b).

Anal. Calcd for $C_{199}H_{206}ClN_3O_{37}$: C, 73.15; H, 6.36; N, 1.29. Found: C, 73.38; H, 6.43; N, 1.32.

*O-(2,3,4-Tri-*O*-benzyl-6-*O*-chloroacetyl-α-*D*-mannopyranosyl)-(1 → 2)-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 6)-*O*-[*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-*D*-galactopyranosyl-(1 → 3)]-*O*-(2,4-di-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-*O*-benzyl-1*D*-myo-inositol* (**28**).—A mixture of **27** (94 mg, 29 μmol), 0.84 M solution of $CF_3SO_3SiMe_3$ in dichloroethane (51 μL, 43 μmol) and AW300 molecular sieves (100 mg) in dichloroethane (5 mL) was stirred at 0° for 20 min and poured into aq $NaHCO_3$. The mixture was extracted with EtOAc, washed with H_2O and brine, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (1:9 EtOAc–toluene) of the residue gave **28** (82 mg, 90%): R_F 0.54 (1:9 EtOAc–hexane); $[\alpha]_D$ +56.1° (*c* 2.20). ^1H NMR data (C_6D_6, 60°): δ

3.237 (dd, 1 H, *J* 4.0 and 10.1 Hz, H-2b), 3.252 (dd, 1 H, *J* 2.1 and 9.8 Hz), 3.503 (td, 1 H, *J* 9.2 and 2.4 Hz), 3.605 (s, 2 H, $COCH_2Cl$), 3.643 (dd, 1 H, *J* 3.4 and 5.8 Hz), 3.747 (dd, 1 H, *J* 7.2 and 9.0 Hz), 3.777 (dd, 1 H, *J* 3.8 and 11.1 Hz), 5.037 (d, 1 H, *J* 3.7 Hz), 5.137 (d, 1 H, *J* 4.6 Hz, H-1f), 5.160 (d, 1 H, *J* 1.2 Hz), 5.201 (d, 1 H, *J* 1.8 Hz), 5.440 (d, 1 H, *J* 4.3 Hz, H-1b), 5.574 (d, 1 H, *J* 2.4 Hz), 7.006–7.487 (m, 105 H, ArH).

Anal. Calcd for $C_{191}H_{198}ClN_3O_{36}$: C, 72.89; H, 6.34; N, 1.34. Found: C, 72.72; H, 6.36; N, 1.30.

*2-[(*N*-Benzyloxycarbonyl)amino]ethyl 2-(cyanoethyl)* N,N-*bis(2-propyl)phosphoramidite* (**29**).—A mixture of 2-[(*N*-benzyloxycarbonyl)amino]ethanol (39 mg, 0.2 mmol), 2-Pr_2NEt (87 μL, 0.5 mmol), and 2-(cyanoethyl) *N,N*-bis(2-propyl)chlorophosphoramidite (71 μL, 0.3 mmol) in dichloroethane (1 mL) was stirred at 20° for 0.5 h. The mixture was quenched with aq $NaHCO_3$ (0.5 mL) and extracted with CH_2Cl_2. The extract was washed with aq NaCl, dried (Na_2SO_4), and concentrated in vacuo. Chromatography (3:6:1 EtOAc–hexane–Et_3N) of the residue gave **29** (69.8 mg, 88%): R_F 0.69 (3:6:1 EtOAc–hexane–Et_3N). ^1H NMR data: δ 1.17 (d, 6 H, *J* 6.8 Hz, $CHMe_2$), 1.19 (d, 6 H, *J* 6.8 Hz, $CHMe_2$), 2.59 (t, 2 H, *J* 6.5 Hz, CH_2CN), 5.11 (s, 2 H, CH_2Ph), 7.34 (s, 5 H, ArH).

*O-[2,3,4-Tri-*O*-benzyl-6-*O*-[[2-[(*N*-benzyloxycarbonyl)amino]ethyl] 2-cyanoethyl phosphono]-α-*D*-mannopyranosyl]-(1 → 2)-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 6)-*O*-[*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-*D*-galactopyranosyl-(1 → 3)]-*O*-(2,4-di-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-1*D*-myo-inositol* (**30**).—To a solution of **28** (32 mg, 0.01 mmol) and **29** (28 mg, 0.07 mmol) in dichloroethane (1 mL) was added 1*H*-tetrazole (5 mg, 0.07 mmol) in CH_3CN (0.2 mL) at 20°. Stirring was continued for 40 min, then *m*-chloroperoxybenzoic acid (37 mg, 0.21 mmol) was added at 0° and stirred for 20 min. The mixture was diluted with CH_2Cl_2, washed with aq $NaHCO_3$ and aq NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on Bio-beads SX-8 (toluene), followed by purification by preparative TLC (1:99 MeOH–$CHCl_3$) to give a 1:1 diastereomeric mixture of **30** (34 mg, 97%): R_F 0.42 (1:99 MeOH–$CHCl_3$). NMR data: ^1H, δ 3.220 (dd, 2 H, *J* 2.3 and 9.9 Hz), 3.281 (dd, 1 H, *J* 1.8 and 10.1 Hz), 3.416 and 3.417 (2 s, 3 H, OMe), 5.379 (d, 1 H, *J* 3.4 Hz), 5.818 and 5.829 (d, 1 H, *J* 2.0 Hz), 5.902 (d, 1 H, *J* 3.7 Hz, H-1b), 6.995–7.565 (m, 114 H, ArH); ^{31}P (C_6D_6), δ −0.054, −0.136.

Anal. Calcd for $C_{210}H_{220}N_5O_{41}P$: C, 72.04; H, 6.33; N, 2.00. Found: C, 71.67; H, 6.34; N, 1.84.

*O-[2,3,4-Tri-*O*-benzyl-6-[[O-2-[(*N*-benzyloxycarbonyl)amino]ethyl] phosphono]-α-*D*-mannopyranosyl]-(1 → 2)-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 6)-*O*-[*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-*D*-galactopyranosyl-(1 → 3)]-*O*-(2,4-di-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-1*D*-myo-inositol* (**31**).—A solution of **30** (20 mg, 5.7 μmol) and

DBU (2.6 μL, 17 μmol) in CH_2Cl_2 (0.6 mL) was stirred at 20° for 2 h. The mixture was diluted with EtOAc, washed with 5% aq HCl, H_2O, and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by preparative TLC (1:9 MeOH–$CHCl_3$). The product in $CHCl_3$ was washed with 5% aq HCl and brine, dried (Na_2SO_4), and concentrated in vacuo to give **31** (16.7 mg, 85%): R_F 0.53 (1:9 MeOH–$CHCl_3$); $[\alpha]_D$ +53.1° (*c* 1.40). NMR data (C_6D_6): ^1H, δ 3.217 (dd, 1 H, *J* 2.0 and 9.9 Hz), 3.271 (dd, 1 H, *J* 2.1 and 10.1 Hz), 3.409 (s, 3 H, OMe), 5.223 (br s, 1 H), 5.388 (d, *J* 3.2 Hz, 1 H), 5.902 (d, 1 H, *J* 1.3 Hz), 6.984–7.600 (m, 114 H, ArH); ^{31}P (C_6D_6), δ 0.531.

Anal. Calcd for $C_{207}H_{217}N_4O_{41}P$: C, 72.11; H, 6.34; N, 1.63. Found: C, 71.79; H, 6.43; N, 1.53.

*O-[6-O-(2-Aminoethylphosphono)]-α-*D*-mannopyranosyl-(1 → 2)-O-α-*D*-mannopyranosyl-(1 → 6)-O-[O-α-*D*-galactopyranosyl-(1 → 6)-α-*D*-galactopyranosyl-(1 → 3)]-O-α-*D*-mannopyranosyl-(1 → 4)-O-2-amino-2-deoxy-α-*D*-glucopyranosyl-1*D*-myo-inositol* (**32**).—A mixture of **31** (11 mg, 3 μmol) and 20% $Pd(OH)_2/C$ (20 mg) in MeOH (1 mL), THF (1 mL), and H_2O (0.2 mL) was stirred in an atmosphere of H_2 at 20° for 6 h, filtered through Celite, and concentrated in vacuo. The residue was chromatographed on Sephadex G-25 (H_2O) to give **32** (3.9 mg, quant.); R_F 0.27 (2:2:1:3 BuOH–EtOH–28% NH_4OH–H_2O); $[\alpha]_D$ +90.3° (*c* 0.30). NMR data (D_2O, 60°): ^1H, δ 3.190 (dd, 1 H, *J* 3.2 and 10.2 Hz, H-2b), 3.288 (t, 2 H, *J* 4.9 Hz, CH_2NH_2), 3.483 (t, 1 H, *J* 9.3 Hz), 3.506 (dd, 1 H, *J* 2.6 and 9.6 Hz), 4.973 (d, 1 H, *J* 3.7 Hz, H-1g), 5.039 (d, 1 H, *J* 1.5 Hz, H-1e), 5.094 (d, 1 H, *J* 1.3 Hz, H-1d), 5.199 (d, 1 H, *J* 4.0, H-1f), 5.300 (d, 1 H, *J* 2.3 Hz, H-1c), 5.385 (d, 1 H, *J* 3.7 Hz, H-1b); ^{31}P (D_2O), δ 1.092. FABMS: *m/z* 1275 ($M^+ + H$).

*O-(2,3,4-Tri-O-benzyl-6-O-chloroacetyl-α-*D*-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-benzyl-α-*D*-mannopyranosyl)-(1 → 6)-O-[O-(2,3,4,6-tetra-O-benzyl-α-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-α-*D*-galactopyranosyl-(1 → 3)]-O-(2,4-di-O-benzyl-α-*D*-mannopyranosyl)-(1 → 4)-O-(2-azido-3,6-di-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-O-benzyl-1-O-(1,2-di-O-myristoyl-*sn*-glycero-3-yl H-phosphono)-1*D*-myo-inositol* (**34**).—To a stirred solution of **28** (72 mg, 23 μmol) and **3** (26.4 mg, 46 μmol) in pyridine (3 mL) was added pivaloyl chloride (17 μL, 0.14 mmol) at 20° and stirred for 1.5 h. After addition of H_2O (0.1 mL), the mixture was diluted with $CHCl_3$, washed with H_2O and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by preparative TLC (1:4 EtOAc–hexane, then extracted with $CHCl_3$) to afford **34** (53.7 mg, 64%); R_F 0.44, 0.50 (1:9 EtOAc–toluene). NMR data: ^1H, δ 0.876 (m, 6 H), 1.244 (m, 40 H), 7.004–7.574 (m, 105 H, ArH); ^{31}P, δ 8.629 ($^1J_{P,H}$ 708 Hz, $^3J_{P,H}$ 9.8 Hz).

Anal. Calcd for $C_{222}H_{257}ClN_3O_{42}P \cdot 0.2\ CHCl_3$: C, 71.55; H, 6.95; N, 1.13. Found: C, 71.51; H, 6.93; N, 1.14.

*O-(2,3,4-Tri-O-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-benzyl-α-*D*-mannopyranosyl)-(1 → 6)-O-[O-(2,3,4,6-tetra-O-benzyl-α-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-α-*D*-galactopyranosyl-(1 → 3)]-O-(2,4-di-O-benzyl-α-*D*-mannopyranosyl)-(1 → 4)-O-(2-azido-3,6-di-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1*

→ 6)-2,3,4,5-tetra-O-benzyl-1-O-(1,2-di-O-myristoyl-sn-glycero-3-yl H-phosphono)-1D-myo-inositol (**35**).—A mixture of **34** (47 mg, 12.9 μmol) and thiourea (22 mg) in THF (1 mL) and EtOH (1 mL) was heated under reflux for 5 h, then diluted with EtOAc, washed with H_2O and brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by preparative TLC (2:7 EtOAc–hexane) to afford **35** (35 mg, 75%): R_F 0.60, 0.63 (1:3 EtOAc–hexane). NMR data: ^1H, δ 0.875 (m, 6 H), 1.242 (m, 40 H), 5.541 and 5.597 (d, 1 H, *J* 3.4 Hz), 6.971–7.484 (m, 105 H, ArH); ^{31}P, δ 8.575 ($^1J_{P,H}$ 709.0 Hz), 8.910 ($^1J_{P,H}$ 727.5 Hz).

Anal. Calcd for $C_{220}H_{256}N_3O_{41}P$: C, 72.80; H, 7.11; N, 1.16. Found: C, 72.80; H, 7.12; N, 1.24.

O-(2,3,4-Tri-O-benzyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-O-[O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-α-D-galactopyranosyl-(1 → 3)]-O-(2,4-di-O-benzyl-α-D-mannopyranosyl)-(1 → 4)-O-(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-O-benzyl-1-O-(1,2-di-O-myristoyl-sn-glycero-3-yl phosphono)-1D-myo-inositol (**36**).—A mixture of **35** (11 mg, 3.0 μmol) and iodine (10 mg) in 1:49 H_2O–pyridine (0.4 mL) was stirred at 20° for 2 h. The mixture was diluted with $CHCl_3$, washed with 5% aq $Na_2S_2O_3$, 5% aq HCl, and brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was purified by preparative TLC (3:97 MeOH–$CHCl_3$). The extract in $CHCl_3$ was then washed 5% aq HCl, dried (Na_2SO_4), and evaporated in vacuo to give **36** (10.9 mg, quant.); R_F 0.38 (3:97 MeOH–$CHCl_3$); $[\alpha]_D$ +44.5° (*c* 1.09). NMR data (20:1 C_6D_6–D_2O, 60°): ^1H, δ 0.911 (t, 6 H, *J* 6.7 Hz, 2 × CH_2CH_3), 3.047 (dd, 1 H, *J* 3.4 and 10.0 Hz, H-2b), 5.316 (br s, 1 H), 5.363 (br s, 1 H), 5.828 (d, 1 H, *J* 3.4 Hz); ^{31}P (C_6D_6), δ 0.178.

O-α-D-Mannopyranosyl-(1 → 2)-O-α-D-mannopyranosyl-(1 → 6)-O-[O-α-D-galactopyranosyl-(1 → 6)-α-D-galactopyranosyl-(1 → 3)]-O-α-D-mannopyranosyl-(1 → 4)-O-2-amino-2-deoxy-α-D-glucopyranosyl-(1 → 6)-1-O-(1,2-di-O-myristoyl-sn-glycero-3-yl phosphono)-1D-myo-inositol (**37**).—As described for **32** above, **36** (7.5 mg, 2.1 μmol) was hydrogenated with 20% $Pd(OH)_2/C$ (15 mg) in $CHCl_3$ (0.45 mL), MeOH (0.35 mL), and H_2O (0.1 mL), filtered through Celite, and concentrated in vacuo. Amberlite IRC-50 (Na^+) was added to the solution of the residue in 9:7:2 $CHCl_3$–MeOH–H_2O (1 mL). The mixture was stirred for 3 h, filtered, and the filtrate was concentrated in vacuo. The residue was chromatographed on LH-20 (9:7:2 $CHCl_3$–MeOH–H_2O) to give **37** (2.8 mg, 78%); R_F 0.26 (9:7:2 $CHCl_3$–MeOH–H_2O). NMR data (Me_2SO-d_6–D_2O, 49:1, 60°): ^1H, δ 0.856 (t, 6 H, *J* 7.0 Hz, Me), 1.244 (m, 40 H), 1.508 (m, 4 H), 2.257 (m, 4 H), 4.007 (br s, 1 H, H-2a), 4.096 (dd, 1 H, *J* 7.3 and 12.1 Hz, H-1gly), 4.313 (dd, 1 H, *J* 3.1 and 11.9 Hz, H-1'gly), 4.646 (br s, 1 H), 4.855 (br s, 1 H), 4.893 (d, 1 H, *J* 1.5 Hz), 4.942 (d, 1 H, *J* 4.0 Hz), 5.086 (m, 1 H, H-2gly), 5.235 (br s, 1 H); ^{31}P(Me_2SO-d_6–D_2O, 49:1), δ-0.169. FABMS: *m/z* 1726 ($M^+ + H$).

2-[(N-Benzyloxycarbonyl)amino]ethyl 2-cyanoethyl H-phosphonate (**38**).—A mixture of 2-[(*N*-benzyloxycarbonyl)amino]ethanol (293 mg, 1.5 mmol), 2-cyanoethyl *N,N,N',N'*-tetrakis(2-propyl)phosphorodiamidite (0.48 mL, 1.5 mmol), and 1*H*-te-

trazole (105 mg, 1.5 mmol) was stirred at 20° for 0.5 h, then H_2O (0.5 mL) and 1*H*-tetrazole (105 mg, 1.5 mmol) were added to the mixture and stirred for 0.5 h. The mixture was diluted with EtOAc, washed with H_2O, aq $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (3:97 MeOH–$CHCl_3$) of the residue gave **38** (184 mg, 59%): R_F 0.31 (3:97 MeOH–$CHCl_3$). NMR data: ^{1}H, δ 2.596 (t, 0.68 H, *J* 6.3 Hz, C*H*$_2$CN), 2.703 (t, 1.32 H, *J* 6.1 Hz, C*H*$_2$CN), 3.363 (m, 0.68 H), 3.511 (m, 1.32 H), 3.726 (t, 0.68 H, *J* 4.8 Hz), 3.884 (t, 0.68 H, *J* 6.1 Hz), 4.201 (m, 1.32 H), 4.249 (m, 1.32 H), 5.119 (s, 2 H, C*H*$_2$Ph), 6.894 (d, 0.66 H, *J* 718.7 Hz, P*H*), 7.306–7.382 (m, 5 H, ArH); ^{31}P, δ 13.149 ($^1J_{P,H}$ 719.2 Hz, $^3J_{P,H}$ 9.3 Hz).

*2-[(*N*-Benzyloxycarbonyl)amino]ethyl H-phosphonate* (**4**).—A mixture of **38** (150 mg, 0.48 mmol) and DBU (0.22 mL, 1.44 mmol) was stirred at 20° overnight. The mixture was acidified with Amberlyst-15 (H^+) resin, filtered, and the filtrate was evaporated in vacuo. The residue was chromatographed using 3:7 MeOH–$CHCl_3$, and the product was treated with Amberlyst-15 (H^+) resin to give **4** (47 mg, 38%); R_F 0.56 (3:7 MeOH–$CHCl_3$). ^{1}H NMR data: δ 3.43 (t, 2 H, *J* 5.0 Hz, C*H*$_2$NH), 4.08 (quint., 2 H, *J* 4.8 Hz, OC*H*$_2$), 5.10 (s, 2 H, C*H*$_2$Ph), 7.33 (s, 5 H, ArH).

O-*[2,3,4-Tri*-O-*benzyl-6*-O-*[[2-(*N*-benzyloxycarbonyl)amino]ethyl H-phosphono]-α*-D-*mannopyranosyl]-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 6)*-O-*[*O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*galactopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzyl-α*-D-*galactopyranosyl-(1 → 3)]*-O-*(2,4-di*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 4)*-O-*(2-azido-3,6-di*-O-*benzyl-2-deoxy-α*-D-*glucopyranosyl)-(1 → 6)-2,3,4,5-tetra*-O-*benzyl-1-*O-*(1,2-di*-O-*myristoyl*-sn-glycero-*3-yl H-phosphono)-1*D-myo-*inositol* (**39**).—To a stirred solution of **35** (12 mg, 3.3 mmol) and **4** (4.2 mg, 16.5 μmol) in pyridine (0.5 mL) was added pivaloyl chloride (2 μL, 16.5 mol) at 20°, and the mixture was stirred for 1.5 h. The mixture was diluted with $CHCl_3$, washed with H_2O and brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was purified by successive chromatography on Bio-Beads SX-2 (toluene) and preparative TLC (1:9 acetone–toluene) to give **39** (5.1 mg, 40%); R_F 0.33 (1:19 acetone–toluene). ^{1}H NMR data (20:1 C_6D_6–D_2O, 60°): δ 0.909 (t, 6 H, *J* 7.0 Hz, 2 × C*H*$_2$C*H*$_3$), 7.016–7.577 (m, 110 H, ArH); ^{31}P (20:1 C_6D_6–D_2O): δ 8.747 (d, $^1J_{P,H}$ 708.0 Hz), 8.780 (d, $^1J_{P,H}$ 708.0 Hz), 9.115 (d, $^1J_{P,H}$ 713.8 Hz), 10.524 (d, $^1J_{P,H}$ 706.1 Hz).

O-*[2,3,4-Tri*-O-*benzyl-6*-O-*[[2-(*N*-benzyloxycarbonyl)amino]ethyl phosphono]-α*-D-*mannopyranosyl]-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 6)*-O-*[*O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*galactopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzyl-α*-D-*galactopyranosyl-(1 → 3)]*-O-*(2,4-di*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 4)*-O-*(2-azido-3,6-di*-O-*benzyl-2-deoxy-α*-D-*glucopyranosyl)-(1 → 6)-2,3,4,5-tetra*-O-*benzyl-1-*O-*(1,2-di*-O-*myristoyl*-sn-glycero-*3-yl phosphono)-1*D-myo-*inositol* (**40**).—Oxidation of **39** (4 mg, 1.0 mmol) with iodine (5.2 mg) was carried out as described for **36**. The product was purified by preparative TLC (1:19 MeOH–$CHCl_3$) to give **40** (2.7 mg, 68%): R_F 0.53 (1:19 MeOH–$CHCl_3$); $[\alpha]_D$ +42.6° (*c* 0.27). NMR data (20:1 Me_2SO-d_6–D_2O): ^{1}H, δ 0.810 (t, 6 H, *J* 7.3 Hz, 2 × C*H*$_2$C*H*$_3$), 7.080–7.818 (m, 110 H, ArH); ^{31}P ($CDCl_3$), δ −0.646, 0.305.

O-[6-O-(2-Aminoethyl phosphono)-α-D-mannopyranosyl]-(1 → 6)-O-[O-α-D-galactopyranosyl-(1 → 6)-α-D-galactopyranosyl-(1 → 3)]-O-α-D-mannopyranosyl-(1 → 4)-O-2-amino-2-deoxy-α-D-glucopyranosyl-(1 → 6)-1-O-(1,2-dimyristoyl-sn-glycero-3-yl phosphono)-1D-myo-inositol disodium salt (**1**).—Hydrogenation of **40** (2.7 mg, 0.69 mmol) with 20% $Pd(OH)_2/C$ (10 mg) was carried out as described for **37**. The product was purified by chromatography on Sephadex G-25 (H_2O) to give **1** (0.3 mg, 23%); R_F 0.68 (2:2:1:3 EtOH–BuOH–28% NH_4OH–H_2O). NMR data (49:1 Me_2SO-d_6–D_2O): ^1H, δ 0.856 (t, 6 H, *J* 7.0 Hz, $2 \times CH_2CH_3$), 1.245 (m, 40 H), 1.516 (m, 4 H), 4.095 (dd, 1 H, *J* 7.1 and 11.9 Hz, H-1′gly), 4.312 (dd, 1 H, *J* 3.1 and 12.3 Hz, H-1gly), 4.648 (br s, 1 H, H-1g), 4.826 (br s, 1 H, H-1e), 4.902 (d, 1 H, *J* 3.3 Hz, H-1f), 4.987 (br s, 1 H, H-1d), 5.019 (br s, 1 H, H-1b), 5.091 (m, 1 H, H-2gly), 5.266 (br s, 1 H, H-1c); ^{31}P (Me_2SO-d_6–D_2O, 49:1), δ −0.818, −0.018.

ACKNOWLEDGMENTS

We thank Professor Anne Dell (Imperial College, London) for kindly providing the FABMS data. We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the NMR spectra, Mrs. Suzuki and her staff for the elemental analyses, and Ms. A. Takahashi, and Ms. K. Moriwaki for technical assistance.

REFERENCES

1 Y. Matsuzaki, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, in press.
2 M.A.J. Ferguson and A.F. Williams, *Annu. Rev. Biochem.*, 57 (1988) 285–320.
3 A.R. Saltiel, J.A. Fox, P. Sherline, and P. Cuatrecasas, *Science*, 233 (1986) 967–972.
4 D.D. Eardley and M.E. Koshland, *Science*, 251 (1991) 78–81.
5 B.L. Chan, M.V. Chao, and A.R. Saltiel, *Proc. Natl. Acad. Sci. U.S.A.*, 86 (1989) 1756–1760.
6 C. Murakata and T. Ogawa, *Carbohydr. Res.*, 234 (1992) 75–91.
7 D.R. Mootoo, P. Konradsson, and B. Fraser-Reid, *J. Am. Chem. Soc.*, 111 (1989) 8540–8542; R. Verduyn, C.J.J. Elie, C.E. Dreef, G.A. van der Marel, and J.H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 109 (1990) 591–593.
8 C. Murakata and T. Ogawa, *Tetrahedron Lett.*, 31 (1990) 2439–2442; 32 (1991) 101–104, 671–674.
9 K. Koike, M. Sugimoto, S. Sato, Y. Ito, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, 163 (1987) 189–208.
10 S. Sato, M. Mori, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 155 (1986) c6-c10.
11 R. Johansson and B. Samuelsson, *J. Chem. Soc., Chem. Commun.*, (1984) 201–202; T. Fukuyama, A.A. Laird and L.M. Hotchkiss, *Tetrahedron Lett.*, 26 (1985) 6291–6292.
12 W. Rosenbrook, Jr., D.A. Riley, and P.A. Lartey, *Tetrahedron Lett.*, 26 (1985) 3–4; G.H. Posner and S.R. Haines, *ibid.*, 26 (1985) 5–8.
13 T. Matsumoto, H. Maeta, K. Suzuki, and G. Tsuchihashi, *Tetrahedron Lett.*, 29 (1988) 3567–3570.
14 T. Ogawa, K. Katano, and M. Matsui, *Carbohydr. Res.*, 64 (1978) C3–C9; P.J. Garegg and L. Maron, *Acta. Chem. Scand., Ser. B*, 33 (1979) 39–41.
15 T. Ogawa and K. Sasajima, *Carbohydr. Res.*, 93 (1981) 67–81.
16 G. Excoffier, D. Gagnaire, and J.-P. Utille, *Carbohydr. Res.*, 39 (1975) 368–373.
17 R.L. Letsinger, J.L. Finnan, G.A. Heavner, and W.B. Lunsford, *J. Am. Chem. Soc.*, 97 (1975) 3278–3279; M.A. Dorman, S.A. Noble, L.J. McBride, and M.H. Caruthers, *Tetrahedron*, 40 (1984) 95–102.
18 N.D. Sinha, J. Biernat, J. McManus, and H. Köster, *Nucl. Acids Res.*, 12 (1984) 4539–4557.

19 J.W. Perich and R.B. Johns, *Tetrahedron Lett.*, 28 (1987) 101–102; W. Bannwarth and A. Trzeciak, *Helv. Chim. Acta.*, 70 (1987) 175–186; K.-L. Yu and B. Fraiser-Reid, *Tetrahedron Lett.*, 29 (1988) 979–982.
20 M. Bertolini and C.P.J. Glaudemans, *Carbohydr. Res.*, 15 (1970) 263–270.
21 I. Londh and J. Stawinski, *J. Org. Chem.*, 54 (1989) 1338–1342; T.M. Slaghek, A.A.M. Maas, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 211 (1991) 25–39.
22 F.A. Cook and D.T. Maichuk, *J. Org. Chem.*, 35 (1970) 1940–1943.
23 J. Nielsen, J.E. Marugg, M. Taagaard, J.H. van Boom, and O. Dahl, *Recl. Trav. Chim. Pays-Bas*, 105 (1986) 33–34; J. Nielsen, J.E. Marugg, J.H. van Boom, J. Honnens, M. Taagaard, and O. Dahl, *J. Chem. Res. Synop.*, (1986) 26–27.