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Further probing of the substrate specificities and inhibition of enzymes involved at an early stage of glycosylphosphatidylinositol (GPI) biosynthesis☆

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Dedicated to Professor Derek Horton on the occasion of his 70th birthday

Abstract

1-D-6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-1-*O*-hexadecyl-*myo*-inositol (**14**), 1-D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-(octadecyl phosphate) (**18**), 1-D-6-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (**24**), 1-D-6-*O*-(2-amino-2-deoxy-α-D-mannopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (**30**) and the corresponding 2-amino-2-deoxy-α-D-galactopyranosyl analogue **36** have been prepared and tested in cell-free assays as substrate analogues/inhibitors of α-(1→4)-D-mannosyltransferases that are active early on in the glycosylphosphatidylinositol (GPI) biosynthetic pathways of *Trypanosoma brucei* and HeLa (human) cells. The corresponding *N*-acetyl derivatives of these compounds were similarly tested as candidate substrate analogues/inhibitors of the *N*-deacetylases present in both systems. Following on from an early study, 1-L-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-*O*-methyl-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (**44**) was prepared and tested as an inhibitor of the trypanosomal α-(1→4)-D-mannosyltransferase. A brief summary of the biological evaluation of the various analogues is provided.

Keywords: Glycosylphosphatidylinositol (GPI) membrane anchors; Substrate analogues of 1-D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate); GPI biosynthesis in *Trypanosoma brucei* and HeLa (human) cell-free systems

1. Introduction

Over the past decade or so we have used various analogues of 1-D-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (**1**) and its N-deacetylated form **2** to probe the substrate specificities or inhibition of two enzymes that exert their activities early on in the glycosylphosphatidylinositol (GPI) biosynthetic pathway of the bloodstream form of the protozoan parasite *Trypanosoma brucei*.[2] This work is predicated on the belief that disruption of GPI-anchor biosynthesis would seriously impair the parasite's ability to survive in a mammalian host. The truncated glycosylphosphatidylinositol anchors **1** and **2**,[3] hereafter denoted by α-D-Glc*p*NAc-PI and α-D-Glc*p*N-PI, respectively, differ from early intermediates of the GPI biosynthetic pathway only in acylation of positions-1 and -2 of the *sn*-glycerol moiety by hexadecanoyl (palmitoyl) rather than by octadecanoyl (stearoyl) at position-1 and other long-chain fatty acids at position-2.[4] Since this exchange has no discernible effect on the ability of the glycosylphosphatidylinositols **1** and **2** to act as substrates for their respective enzymes (see be-

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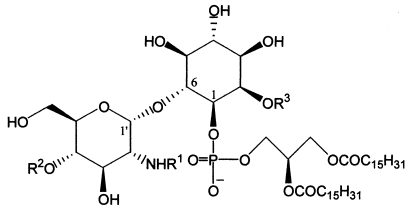
low), they function as ideal surrogates for the natural substrates.[5,6]

One of the enzymes of interest is a *N*-deacetylase that N-deacetylates α-D-Glc*p*NAc-PI (**1**) in both *T. brucei* and HeLa (human) cell-free systems[7] to form α-D-Glc*p*N-PI (**2**). The latter compound is then acted upon by the other enzyme of interest, an α-(1→4)-D-mannosyltransferase (MT-1), which transfers an α-D-Man*p* residue from dolichol phosphate D-mannose to form α-D-Man*p*-(1→4)-α-D-Glc*p*N-PI (**3**). In the trypanosomal system, this step is followed by the addition of a fatty acyl group (most often hexadecanoyl) to 2-OH of the D-*myo*-inositol residue, thus differing from the mammalian system wherein inositol 2-acylation occurs *before* the first α-D-Man*p* residue is attached.[8]

Extensive probing of the substrate specificities/inhibition of these enzymes has elicited the following information. The fatty acid esters assist in the presentation of the α-D-Glc*p*NAc-PI substrate **1** to the trypanosomal *N*-deacetylase but are not essential for substrate recognition.[5] Another study[9] examined the substrate specificities of *T. brucei* and HeLa *N*-deacetylases with respect to the size of the *N*-acyl (R) group that can be cleaved from a series of α-D-Glc*p*NR-PI substrates. It was concluded that the trypanosomal and HeLa enzymes are active on α-D-Glc*p*NR-PI substrates where R is acetyl or propionyl but are much less active on substrates where R is butyryl, isobutyryl, pentanoyl or hexanoyl. A further study[10] examined the abilities of trypanosomal and HeLa *N*-deacetylases to act on substrates with alkyl substituents at 2-OH of the D-*myo*-inositol residue and their specificities towards the configuration (D or L) of the *myo*-inositol residue. The mammalian enzyme is more fastidious than the trypanosomal enzyme on both counts. Thus, whereas the trypanosomal enzyme turned over α-D-Glc*p*NAc-2-*O*-methyl-PI (**4**, $R^3 = CH_3$), α-D-Glc*p*NAc-2-*O*-octyl-PI (**4**, $R^3 = C_8H_{17}$), and α-D-Glc*p*NAc-[L]-PI, albeit at lower rates than that for α-D-Glc*p*NAc-PI (**1**), the mammalian enzyme could not act on any of these analogues at a detectable rate. Equally significant is the discovery that the analogue α-D-Glc*p*$NCONH_2$-PI (**5**) is a suicide substrate inhibitor of both trypanosomal and human *N*-deacetylases (Fig. 1).[11]



	R1	R2	R3
1	Ac	H	H
2	H	H	H
3	H	α-D-Man*p*	H
4	Ac	H	CH_3 or C_8H_{17}
5	$CONH_2$	H	H
6	H	H	CH_3, C_8H_{17} or $C_{16}H_{33}$

Fig. 1. Some previously prepared analogues of α-D-Glc*p*NAc-PI (**1**) and α-D-Glc*p*N-PI (**2**).

The apparent difference in the substrate specificities of trypanosomal and HeLa (human) MT-1 enzymes, requiring α-D-Glc*p*N-PI (**2**) and α-D-Glc*p*N-2-*O*-acyl-PI, respectively, as substrates, has been investigated[12,13] using cell-free systems and a series of synthetic α-D-Glc*p*N-2-*O*-alkyl-PIs.[14,15] Of these, α-D-Glc*p*N-2-*O*-methyl-PI (**6**, $R^3 = CH_3$) was shown to be a good substrate for trypanosomal MT-1, but neither a substrate for nor an inhibitor of HeLa cell MT-1.[12] Moreover, α-D-Glc*p*N-2-*O*-octyl-PI (**6**, $R^3 = C_8H_{17}$) and the 2-*O*-hexadecyl compound **6** ($R^3 = C_{16}H_{33}$) turned out to be parasite-specific GPI pathway inhibitors with different modes of action: the latter inhibits MT-1 whereas the former inhibits the succeeding inositol acyltransferase.[13]

The 3-deoxy analogues of the substrates **1** and **2**, denoted by α-D-3dGlc*p*N(Ac)-PI,[16] are not recognised by either the trypanosomal *N*-deacetylase or MT-1, whereas the 6′-OH group, as in α-D-6dGlc*p*NAc-PI,[16] is dispensable in both the trypanosomal and HeLa *N*-deacetylase systems.[11] The 4′-OH group too is not essential for substrate recognition by the trypanosomal *N*-deacetylase, although methylation of this group reduces substrate turnover.[11] Because the 4′-OH is no longer present in α-D-4dGlc*p*N-PI,[16] it cannot function as a substrate for trypanosomal MT-1, which transfers an α-D-Man*p* residue to this position. But it might and, indeed, does act as an inhibitor.[17]

In continuing to identify essential and nonessential structural features recognised by the *N*-deacetylase and MT-1 enzymes, we have fashioned certain analogues of the substrates **1** and **2** in order to answer specific questions. The phosphate-free analogue **14**, for example, would indicate whether or not a charged phosphoric diester unit is an essential feature recognised by one or both enzymic activities. A charged phosphoric diester unit is restored in the analogue **18**, but a long-chain alkyl group is introduced instead of the more complex and synthetically less-accessible 1,2-di-*O*-hexadecanoyl-*sn*-glycerol in the hope that the simpler analogue **18** might act as a substrate. Three of the analogues, **24**, **30**, and **36**, have a different stereochemistry at positions-1, -2 and -4, respectively, from those of the natural α-D-glucosaminyl component of GPIs and should indicate the essentialness of the stereochemistry at these positions. Equally, though less likely, they might act to inhibit the enzymes. The diastereoisomeric analogue **44**, containing 1-L-2-*O*-methyl-*myo*-inositol, was prepared in order to ascertain whether the 2-OH group of the previously tested α-D-Glc*p*N-[L]-PI engaged in hydrogen bonding in the active site of try-

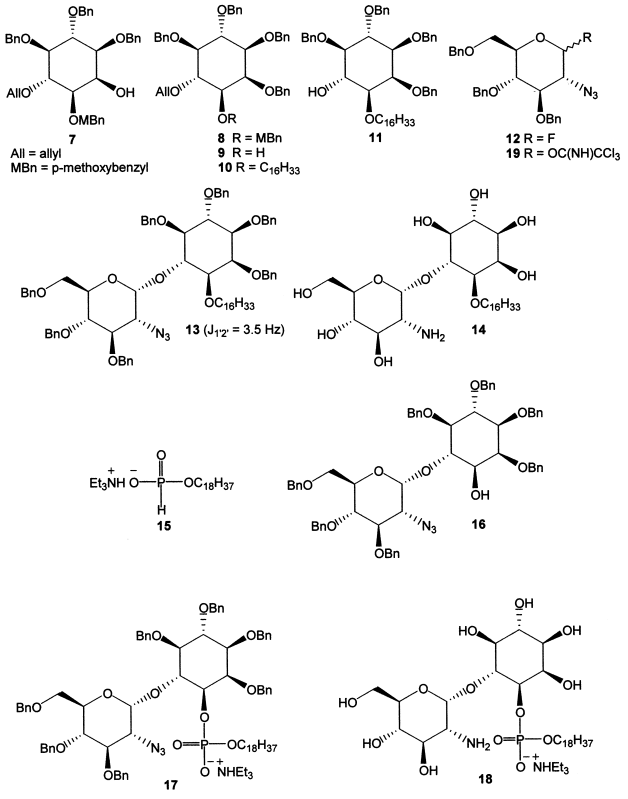


Fig. 2. Synthesis of 1-D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-1-*O*-hexadecyl-*myo*-inositol (**14**) and of triethylammonium 1-D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-(octadecyl phosphate) (**18**).

panosomal MT-1. This might then explain the somewhat unexpected finding that this unnatural analogue is a selective inhibitor of trypanosomal MT-1.[18]

2. Results and discussion

A synthesis of 1-D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-1-*O*-hexadecyl-*myo*-inositol (**14**) was accomplished straightforwardly from the known D-*myo*-inositol derivative **7**,[3] which on conventional benzylation gave the fully protected compound **8**. The latter compound, unlike previously,[3] was obtained in crystalline form and was fully characterised. Removal of the 1-*O*-methoxybenzyl group from the compound **8** with trifluoroacetic acid (TFA) in CH_2Cl_2 provided the alcohol **9**, which on etherification with hexadecyl bromide in DMF in the presence of NaH gave the compound **10**. A two-step removal of the 6-*O*-allyl group, via acidic hydrolysis of the vinylic ether,[19] then furnished the glycosyl acceptor **11**, which was coupled with the glycosyl fluoride **12**[3] in diethyl ether in the presence of zirconocene dichloride and silver perchlorate[20] to give the α-linked pseudodisaccharide **13** ($J_{1',2'}$ 3.5 Hz) after radial-band chromatography. Catalytic debenzylation, with concomitant reduction of the 2-azido group, then produced the 1-*O*-hexadecyl analogue **14** (Fig. 2).

The phosphoric diester **18** was accessible from the known pseudodisaccharide **16**[3] by means of the H-phosphonate route.[21] Thus, condensation of the H-phosphonate salt **15** (prepared from 1-octadecanol) with the pseudodisaccharide **16** furnished a mixture of diastereoisomeric phosphonic diesters that was converted into the phosphoric diester **17** on oxidation in situ with iodine in wet pyridine.[21] The final transformation (**17** → **18**) was accomplished by hydrogenolysis over 20% $Pd(OH)_2$ on carbon.

The synthesis of the other analogues followed a similar and familiar approach.[3,14,15,22] In this, a suitable glycosyl donor is coupled with the 6-OH group of an appropriately protected *myo*-inositol derivative (usually **20**[3]), whereafter the 1-OH group of the coupled product is exposed for further coupling with the H-phosphonate **22**.[14,23] Thereafter in situ oxidation[21] provides the related phosphoric diester, which is transformed into the fully deprotected analogue on hydrogenolysis.

A synthesis of the unnatural β-linked analogue β-D-Glc*p*N-PI (**24**) emerged from a solvent-assisted coupling[24] between the trichloroacetimidate **19**[25] and the glycosyl acceptor **20**[3] in propionitrile at −80 °C with TMSOTf as the promoter. The formation of an increased proportion of the β-linked pseudodisaccharide **21** ($J_{1',2'}$ 9.0 Hz) in the presence of the non-participating 2-azido group presumably results from an S_N2 displacement[26] on an α-nitrilium ion intermediate.[27] Note too that demethoxybenzylation also occurs at some stage during the coupling process, thereby freeing the 1-OH group for subsequent coupling with the H-phosphonate **22**.[14,23] Although the 30% yield of the β-coupled product **21** is rather low and not optimised, this approach was appealing in the short term insofar as both the glycosyl donor and acceptor were in hand. Progression by the H-phosphonate route[21] provided the fully protected compound **23**, which on hydrogenolysis gave β-D-Glc*p*N-PI (**24**) (Fig. 3).

Similar approaches to the one described above were used to prepare α-D-Man*p*N-PI (**30**) and α-D-Gal*p*N-PI (**36**), based on couplings between the glycosyl fluorides **26** or **32** and the D-*myo*-inositol derivative **20**,[3] respectively, promoted by zirconocene dichloride–silver perchlorate.[20] The starting glycosyl fluorides **26** and **32** were obtained on treatment of the hemiacetal **25**[28] and the phenyl selenogalactopyranoside **31**,[28] respectively, with DAST. In these cases, treatment of the coupled products with TFA was necessary to effect demethoxybenzylation (**27** → **28**; **33** → **34**). The closing sequences **28** → **29** → **30** and **34** → **35** → **36** were then conducted without incident (Figs. 4 and 5).

The L-*myo*-inositol acceptor **39** needed for the preparation of α-D-Glc*p*N-[L]-2-*O*-methyl-PI (**44**) was ob-

tained by a conventional methylation of the alcohol **37**[3] (→**38**), followed by deallylation of the methylated product (→**39**). Coupling between the glycosyl fluoride **12** and the acceptor **39** in the customary manner[20] gave

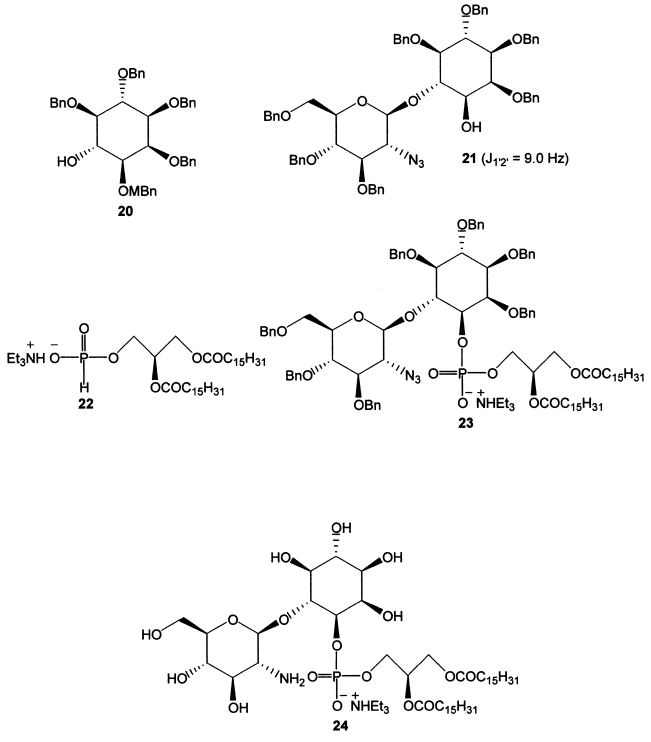


Fig. 3. Synthesis of β-D-Glc*p*N-PI (**24**).

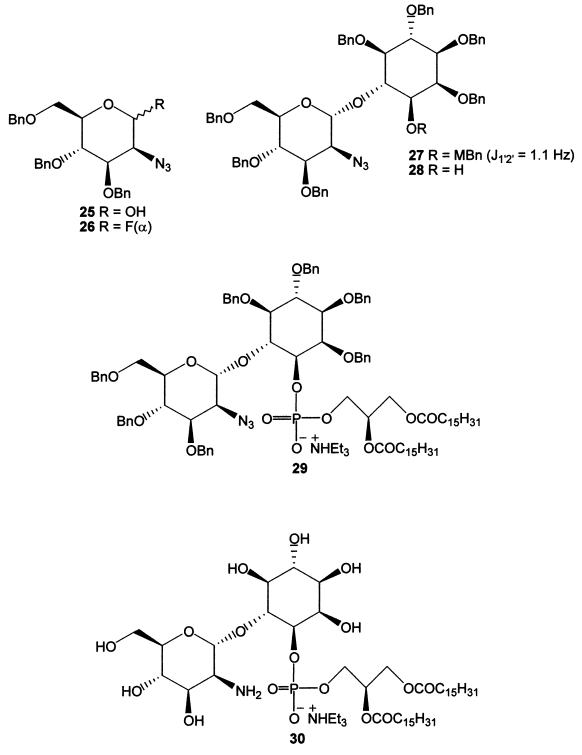


Fig. 4. Synthesis of α-D-Man*p*N-PI (**30**).

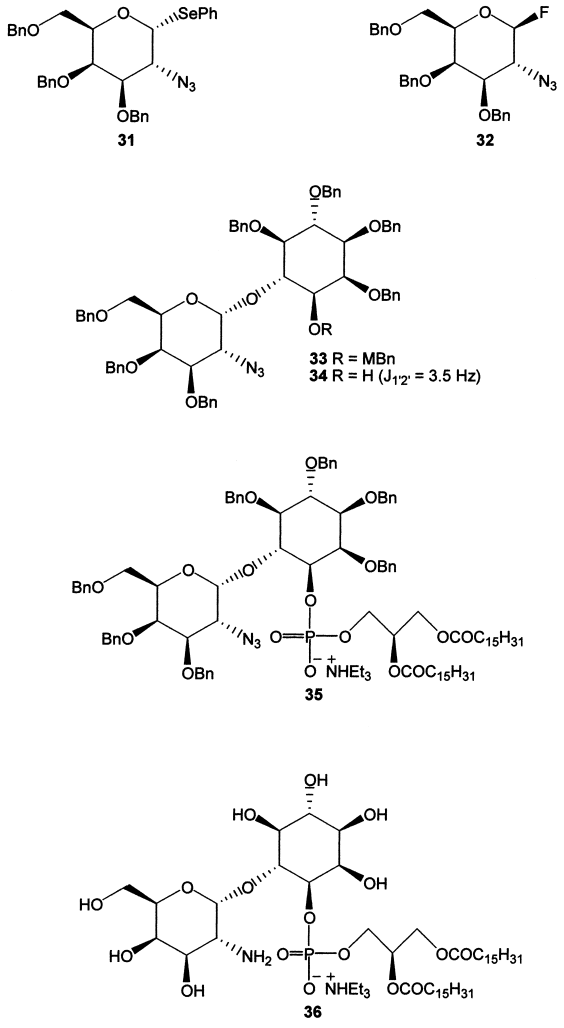


Fig. 5. Synthesis of α-D-Gal*p*N-PI (**36**).

the α-linked pseudodisaccharide **40** in ~64% yield. Thereafter, the sequence of reactions from the coupled product **40** to the deprotected analogue **44** was executed along the lines previously described. In this instance, the sodium salt **43** (readily prepared from the TEA salt **42**) was used for the final hydrogenolysis (Fig. 6).

N-Acetylated derivatives of the foregoing analogues, required for biological studies with the *N*-deacetylases, were prepared by standard procedures.[6]

Details of the results of enzymic studies with the above analogues are or will be reported elsewhere. In brief, the phosphate-free analogue **14** and its *N*-acetyl derivative are neither substrates for nor inhibitors of GPI biosynthesis in either trypanosomal or HeLa cell-free systems, indicating the importance of a charged phosphodiester unit for substrate recognition. This returns on restoration of the phosphodiester linkage, as in α-D-Glc*p*N-IP-C18 (**18**), which is a substrate for both trypanosomal and (after 2-O-acylation in situ) HeLa MT-1 enzymes.[17] This lipid-modified analogue of α-D-Glc*p*N-PI (**2**) is a perfectly good substrate in the

trypanosomal system but is a poorer substrate in the HeLa system, as is its *N*-acetyl derivative for the HeLa *N*-deacetylase.[11] The unnatural β-linked analogue **24** and its *N*-acetyl derivative are substrates for trypanosomal MT-1[17] and *N*-deacetylase,[11] respectively, but are not recognised as substrates/inhibitors of the corresponding HeLa enzymes.[11,17] Thus is revealed a fundamental difference between the two systems. This contrasts with the behaviour of α-D-Man*p*N-PI (**30**), α-D-Gal*p*N-PI (**36**) and their *N*-acetyl derivatives which, perhaps not unexpectedly, are neither substrates for nor inhibitors of any of the enzymes of interest.[11,17] Whereas the unnatural α-D-Glc*p*N-[L]-PI is a selective inhibitor of trypanosomal MT-1, 2-O-methylation of the L-*myo*-inositol residue (→**44**) abolishes any inhibition.[18]

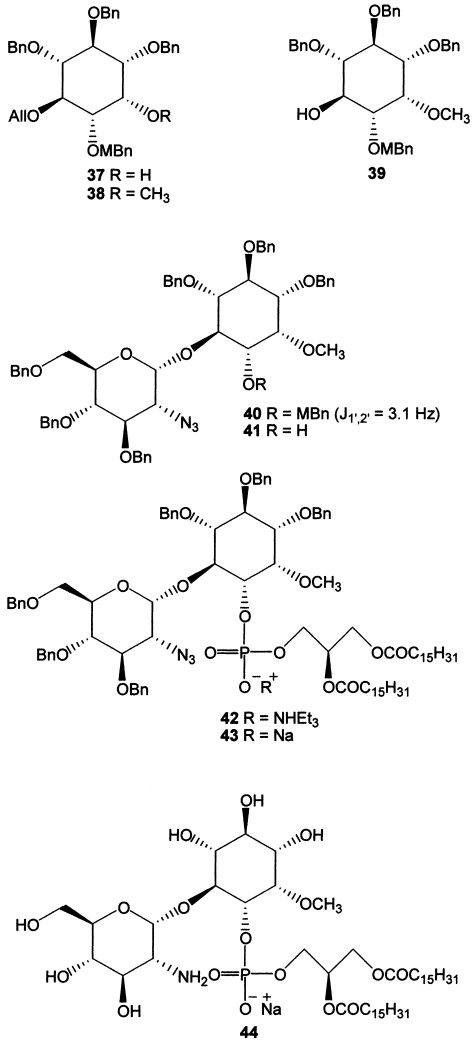


Fig. 6. Synthesis of α-D-Glc*p*N-[L]-2-*O*-methyl-PI (**44**).

3. Experimental

General methods.—^{1}H NMR and COSY spectra were recorded on either a Bruker AM 300 MHz or AC 500 MHz spectrometer using deuteriochloroform as the solvent and tetramethylsilane as the internal standard, unless otherwise indicated. ^{31}P NMR spectra used 85% phosphoric acid in D_2O as the external standard. Optical rotations were measured using a Perkin–Elmer 141 or 343 polarimeter. Electrospray mass spectra (ESMS) were recorded with a VG Quattro system (VG Biotech, UK). Melting points were determined on a Reichert hot-plate apparatus and are uncorrected. TLC was performed on Kieselgel 60 F_{254} (E. Merck) with detection under UV light or by charring with 3:17:1 H_2SO_4–water–ethanol. Radial-band chromatography (RBC) was performed using a Chromatotron (model 7924T, TC Research, UK) with Adsorbosil Plus-P (6–15 μm) (Alltech) as the adsorbent. Light petroleum refers to the fraction having a boiling point 60–80 °C. All anhydrous solvents were purchased from Aldrich Chemical Co. Ltd.

1-D-*6*-O-*Allyl*-*2,3,4,5*-*tetra*-O-*benzyl*-*1*-O-*(4-methoxybenzyl)*-myo-*inositol* (**8**).—To a stirred and cooled (0 °C) solution of the alcohol **7**[3] (0.5 g, 0.82 mmol) in DMF (20 mL) under argon was added NaH (84 mg, 3.5 mmol) and the solution was stirred for 15 min before benzyl bromide (0.16 mL, 1.31 mmol) was added dropwise. The reaction mixture was stirred at rt for 2 h, quenched with MeOH and diluted with Et_2O (50 mL). The ethereal solution was washed with water (3 × 25 mL), dried ($MgSO_4$) and concentrated under reduced pressure. RBC (2:1 cyclohexane–Et_2O) of the residue yielded the fully protected compound **8** (0.29 g, 54%); mp 78–79 °C; $[\alpha]_D^{25}$ −2° (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.42–6.84 (24 H, C_6H_4, 4 × Ph), 6.04–5.96 (m, 1 H, $CH_2CH{=}CH_2$), 5.27–5.11 (m, 2 H, $CH_2CH{=}CH_2$), 4.92–4.51 (10 H, 5 × CH_2Ar), 4.40–4.34 (m, 2 H, $CH_2CH{=}CH_2$), 4.04 (t, 1 H, H-6), 3.98 (t, 1 H, H-2), 3.93 (t, 1 H, H-4), 3.82 (s, 3 H, OCH_3), 3.42 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.2 Hz, H-5), 3.33 (dd, 1 H, $J_{1,2}$ 2.2, $J_{1,6}$ 9.2 Hz, H-1), 3.25 (dd, 1 H, $J_{2,3}$ 2.2, $J_{3,4}$ 9.2 Hz, H-3); Anal. Calcd for $C_{45}H_{48}O_7$: C, 77.12; H, 6.90. Found: C, 77.06; H, 6.90.

1-D-*6*-O-*Allyl*-*2,3,4,5*-*tetra*-O-*benzyl*-myo-*inositol* (**9**).—A solution of the methoxybenzyl derivative **8** (83 mg, 0.12 mmol) in CH_2Cl_2 (10 mL) containing TFA (92 μL, 1.2 mmol) was set aside at rt for 1 h, whereafter it was neutralised with Et_3N, washed successively with water and brine, dried (Na_2SO_4) and concentrated under reduced pressure. RBC (20:1 CH_2Cl_2–Et_2O) of the residue gave the alcohol **9** (52 mg, 75%), mp 67–68 °C; $[\alpha]_D^{25}$ −10° (*c* 1.2, $CHCl_3$); ^{1}H NMR; δ 7.50–7.20 (20 H, 4 × Ph), 6.00–5.90 (m, 1 H, $CH_2CH{=}CH_2$), 5.30–5.13 (m, 2 H, $CH_2CH{=}CH_2$), 5.10–4.60 (8 H, 4 × CH_2Ph), 4.40–4.20 (m, 2 H, $CH_2CH{=}CH_2$), 4.04 (t, 1

H, $J_{1,2} = J_{2,3}$ 2.4 Hz, H-2), 4.02 (t, 1 H, $J_{1,6} = J_{5,6}$ 9.6 Hz, H-6), 3.67 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.50–3.40 (3 H, H-1, 3, 5), 2.31 (d, 1 H, $J_{1,OH}$ 6.5 Hz, OH); Anal. Calcd for $C_{37}H_{40}O_6$: C, 76.53; H, 6.94. Found: C, 76.42; H, 6.96.

1-D-*6*-O-*Allyl*-*2,3,4,5*-*tetra*-O-*benzyl*-*1*-O-*hexadecyl*-myo-*inositol* (***10***).—To a stirred solution of the alcohol **9** (533 mg, 0.92 mmol) in DMF (15 mL) at 0 °C were added NaH (88 mg, 3.7 mmol) and hexadecyl bromide (550 μL, 1.8 mmol). The reaction mixture was stirred at rt overnight, whereafter MeOH was added to destroy the excess of NaH and the resulting solution was extracted with Et_2O (3 × 25 mL). The ethereal extracts were combined and washed successively with water (25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated under reduced pressure. RBC (1:5 Et_2O–cyclohexane) of the residue gave the hexadecyl derivative **10** (653 mg, 88%) as an amorphous solid; $[\alpha]_D^{25}$ −4° (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 7.50–7.20 (20 H, 4 × Ph), 6.05–5.95 (m, 1 H, $CH_2CH{=}CH_2$), 5.30–5.10 (m, 2 H, $CH_2CH{=}CH_2$), 4.95–4.60 (8 H, 4 × CH_2Ph), 4.40–4.35 (m, 2 H, $CH_2CH{=}CH_2$), 4.00 (2 H, H-2, 4), 3.85 (t, 1 H, H-6), 3.55–3.45 (m, 2 H, OCH_2), 3.40 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.3 Hz, H-5), 3.35 (dd, 1 H, $J_{2,3}$ 2.3, $J_{3,4}$ 9.9 Hz, H-3), 3.15 (dd, 1 H, $J_{1,2}$ 2.2, $J_{1,6}$ 9.8 Hz, H-1), 1.56 (m, 2 H, OCH_2CH_2), ~1.25 (26 H, $[CH_2]_{13}$), 0.89 (t, 3 H, *J* 7.1 Hz, CH_2CH_3).

1-D-*2,3,4,5*-*Tetra*-O-*benzyl*-*1*-O-*hexadecyl*-myo-*inositol* (**11**).—A solution of the allyl derivative **10** (491 mg, 0.61 mmol) in anhyd Me_2SO (20 mL) containing potassium *tert*-butoxide (595 mg, 5.3 mmol) was heated and stirred at 100 °C for 1 h, cooled and then poured into ice water (100 mL). The resulting aqueous solution was extracted with EtOAc (3 × 25 mL), and the organic extracts were combined and washed successively with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The resulting propenyl derivative in 1 M 1:9 HCl–acetone (20 mL) was boiled under reflux for 10 min, whereafter the solvents were removed under reduced pressure. A solution of the residue in Et_2O was percolated through a short column of silica gel and the eluent concentrated under reduced pressure. RBC (1:5 Et_2O–cyclohexane) of the residue gave the deallylated compound **11** (380 mg, 81%); $[\alpha]_D^{25}$ −5° (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 7.50–7.20 (20 H, 4 × Ph), 5.00–4.60 (8 H, 4 × CH_2Ph), 4.15–4.03 (3 H, H-2, 4, 6), 3.50–3.30 (4 H, OCH_2, H-3, 5), 3.02 (dd, 1 H, $J_{1,2}$ 2.1, $J_{1,6}$ 9.8 Hz, H-1), 2.55 (s, 1 H, 6-OH), 1.57 (m, 2 H, OCH_2CH_2), ~1.28 (26 H, $[CH_2]_{13}$), 0.87 (t, 3 H, *J* 7.0 Hz, CH_2CH_3).

1-D-*6*-O-*(2-Azido*-*3,4,6*-*tri*-O-*benzyl*-*2*-*deoxy*-α-D-*glucopyranosyl)*-*2,3,4,5*-*tetra*-O-*benzyl*-*1*-O-*hexadecyl*-myo-*inositol* (**13**).—After drying overnight over P_2O_5 in a vacuum desiccator, the glycosyl donor **12**[3] (100 mg, 0.21 mmol) and the acceptor **11** (115 mg, 0.15 mmol) were dissolved in anhyd Et_2O (15 mL) containing zirconocene dichloride (242 mg, 0.83 mmol) and 4 Å molecular sieves (500 mg). After stirring of the mixture at rt for 15 min under argon, it was cooled to 0 °C and pre-dried silver perchlorate (169 mg, 0.75 mmol) and 1,1,3,3-tetramethylurea (21.5 μL, 180 μmol) were added. Stirring of the mixture under argon at 0 °C was continued in the dark overnight, whereafter it was percolated through a short column of silica gel (further elution with Et_2O) and the eluent was concentrated under reduced pressure. RBC (1:10 EtOAc–hexane) of the residue gave the α-linked pseudodisaccharide **13** (100 mg, 55%); $[\alpha]_D^{25}$ +34° (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.40–6.90 (35 H, 7 × Ph), 5.68 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.00–4.20 (14 H, 7 × CH_2Ph), 4.15 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 4.05 (t, 1 H, $J_{1,6} = J_{5,6}$ 9.8 Hz, H-6), 4.00 (t, 1 H, $J_{1,2} = J_{2,3}$ 2.0 Hz, H-2), 3.95 (m, 1 H, H-5′), 3.85 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 9.0 Hz, H-3′), 3.65 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9.0 Hz, H-4′), 3.40–3.35 (4 H, OCH_2, H-1, 5), 3.25 (2 H, H-2′, 3), 3.20 (dd, 1 H, $J_{5',6'a}$ 1.8, $J_{6'a,6'b}$ 11.0 Hz, H-6′a), 3.10 (dd, 1 H, $J_{5',6'b}$ 2.0 Hz, H-6′b), 1.52 (m, 2 H, OCH_2CH_2), ~1.25 (26 H, $[CH_2]_{13}$), 0.80 (t, 3 H, *J* 7.0 Hz, CH_2CH_3); ESMS(+): *m*/*z* 1239.0 $[M + NH_4]^+$.

1-D-*6*-O-*(2*-*Amino*-*2*-*deoxy*-α-D-*glucopyranosyl)*-*1*-O-*hexadecyl*-myo-*inositol* (**14**).—A solution of the protected compound **13** (88 mg, 0.072 mmol) in 1:2 THF–MeOH (9 mL) containing 20% $Pd(OH)_2$ on carbon (100 mg) was stirred under 3 atm of hydrogen for 2 h. It was then percolated through a short column of Chelex 100 on a bed of Celite (further elution with 1:2 THF–MeOH) and the eluent was concentrated under reduced pressure to give the hexadecyl derivative **14** (40 mg, 98%); $[\alpha]_D^{25}$ +17° (*c* 0.4, 10:10:3 $CHCl_3$–MeOH–water); ^{1}H NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 5.39 (d, 1 H, $J_{1',2'}$ 3.0 Hz, H-1′), 4.26 (bs, 1 H, H-2), 3.97 (m, 1 H, H-5′), 3.87 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 9.7 Hz, H-3′), 3.81 (2 H, H-4, 6′a), 3.74 (dd, 1 H, $J_{5',6'b}$ 4.0, $J_{6'a,6'b}$ 12.1 Hz, H-6′b), 3.65–3.30 (7 H, OCH_2, H-1, 3, 4′, 5, 6), 3.22 (dd, 1 H, H-2′), 1.63 (m, 2 H, OCH_2CH_2), ~1.27 (26 H, $[CH_2]_{13}$), 0.88 (t, 3 H, *J* 7.0 Hz, CH_2CH_3); ESMS(+): *m*/*z* 566.6 $[M + H]^+$.

Triethylammonium octadecyl hydrogenphosphonate (**15**).—1-Octadecanol (200 mg, 0.74 mmol) was dried by evaporation of pyridine therefrom and was afterwards dissolved in dry 10:1 THF–pyridine (11 mL). This solution was added dropwise over 30 min to a stirred solution of salicylchlorophosphite (180 mg, 0.89 mmol) in dry THF (5 mL) under argon at rt. After 2 h, TLC (19:1 $CHCl_3$–MeOH) indicated complete conversion of the starting material into a product of lower mobility (R_f 0.30). The reaction mixture was quenched with 1 M triethylammonium hydrogen carbonate (TEAB) buffer solution (10 mL) and the resulting aqueous solution was stirred for 30 min. Chloroform (30 mL) was then added and the organic layer was separated and washed with 1 M TEAB buffer solution

(2×10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The crude hydrogenphosphonate TEA salt **15** (300 mg, 93%) was used in the next experiment without further purification; ^{31}P NMR: δ_p 2.00 (with heteronuclear decoupling), J_{PH} 635 Hz; ESMS(−): m/z 333.4 $[M - NEt_3 - H]^-$.

*Triethylammonium 1-*D*-6-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-*D*-glucopyranosyl)-2,3,4,5-tetra-*O*-benzyl*-myo*-inositol 1-(octadecyl phosphate)* (**17**).—Each of the compounds **15** (148 mg, 0.34 mmol) and **16**[3] (100 mg, 0.10 mmol) were dried overnight over P_2O_5 in a vacuum desiccator, whereafter anhyd pyridine (5 mL) was evaporated therefrom. They were then dissolved in dry pyridine (10 mL), pivaloyl chloride (258 μL, 2.1 mmol) was added and the resulting solution was stirred under argon at rt for 1 h. A freshly prepared solution of iodine (173 mg, 0.68 mmol) in 19:1 pyridine–water (10 mL) was then added and stirring of the reaction mixture was continued for 45 min. After the addition of $CHCl_3$ (30 mL), the organic solution was washed successively with 5% aq $NaHSO_3$ (25 mL), water (25 mL), and 1 M TEAB buffer solution (3×15 mL), dried ($MgSO_4$), and concentrated under reduced pressure. RBC of the residue (elution first with $CHCl_3$ and then with 20:1 $CHCl_3$–MeOH) afforded the TEA phosphate derivative **17** (110 mg, 77%); $[\alpha]_D^{25}$ +53° (*c* 1.3, $CHCl_3$); ^{1}H NMR: δ 7.50–6.90 (35 H, $7 \times$ Ph), 5.87 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.05–4.25 (15 H, H-2 and $7 \times C\mathit{H}_2$Ph), 4.34 (2 H, H-1, 6), 4.14 (m, 1 H, H-5′), 4.09 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4), 4.02 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 10.0 Hz, H-3′), 3.97 (t, 2 H, *J* 6.9 Hz, OCH_2), 3.71 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 10.0 Hz, H-4′), 3.54 (bd, 1 H, $J_{3,4}$ 9.4 Hz, H-3), 3.47 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.4 Hz, H-5), 3.38 (m, 2 H, H-6′a,b), 3.20 (dd, 1 H, H-2′), 2.92 (q, 6 H, *J* 6.0 Hz, $3 \times C\mathit{H}_2CH_3$), 1.60 (m, 2 H, $OCH_2C\mathit{H}_2$), ~1.25 (30 H, $[CH_2]_{15}$), 1.18 (t, 9 H, *J* 7.2 Hz, $3 \times CH_2C\mathit{H}_3$), 0.88 (t, 3 H, *J* 6.9 Hz, $CH_2C\mathit{H}_3$); ^{31}P NMR: δ_p–4.27 (with heteronuclear decoupling); ESMS(−): m/z 1328.3 $[M - NEt_3 - H]^-$.

*Triethylammonium 1-*D*-6-*O*-(2-amino-2-deoxy-α-*D*-glucopyranosyl)-myo-inositol 1-(octadecyl phosphate)* (**18**).—A solution of the TEA salt **17** (60 mg, 0.042 mmol) in 2:2:1 *n*-propanol–THF–water (10 mL) containing 20% $Pd(OH)_2$ on carbon (50 mg) was stirred under 3 atm of hydrogen for 1 h before it was percolated through a short column of Chelex 100 on a bed of Celite (further elution with 1:1 *n*-propanol–water). The eluent was concentrated under reduced pressure to give the deprotected product **18** (30 mg, 94%); $[\alpha]_D^{25}$ +14° (*c* 0.1, 10:10:3 $CHCl_3$–MeOH–water); ^{1}H NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 5.22 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1′), 3.82 (dd, 1 H, $J_{1,2}$ 2.5, $J_{1,6}$ 9.6 Hz, H-1), 3.76 (t, 1 H, $J_{1,2} = J_{2,3}$ 2.5 Hz, H-2), 3.73 (m, 1 H, H-5′), 3.62 (t, 1 H, $J_{1,6} = J_{5,6}$ 9.6 Hz, H-6), 3.58–3.46 (4 H, OCH_2, H-3′, 6′a), 3.40 (dd, 1 H, $J_{5',6'b}$ 4.0, $J_{6'a,6'b}$ 12.3 Hz, H-6′b), 3.33 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 3.12 (2 H, H-3, 4′), 3.00 (t, 1 H, H-5), 2.85 (dd, 1 H, H-2′), 2.80 (m, 6 H, $3 \times C\mathit{H}_2CH_3$), 1.25 (m, 2 H, $OCH_2C\mathit{H}_2$), ~0.95 (39 H, $3 \times CH_2C\mathit{H}_3$, $[CH_2]_{15}$), 0.56 (t, 3 H, *J* 7.1 Hz, $CH_2C\mathit{H}_3$); ^{31}P NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ_p 1.80 (with heteronuclear decoupling); ESMS(−): m/z 672.4 $[M - NEt_3 - H]^-$.

*1-*D*-6-*O*-(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2,3,4,5-tetra-*O*-benzyl-myo-inositol* (**21**).—To a stirred solution of the donor **19**[25] (117 mg, 0.19 mmol) and the acceptor **20**[3] (139 mg, 0.21 mmol) in dry propionitrile (10 mL) at −80 °C was added TMSOTf (34.5 μL, 0.19 mmol). Stirring of the reaction mixture was continued at −80 °C for 2 h, whereafter it was diluted with satd aq $NaHCO_3$ (25 mL) and extracted with Et_2O (3×15 mL). The ethereal extract was washed with brine (25 mL), dried ($MgSO_4$), and the solvent removed under reduced pressure. RBC (elution first with hexane and then with 4:1 hexane–EtOAc) of the residue gave the alcohol **21** (57 mg, 30%); mp 157–159 °C; $[\alpha]_D^{25}$ −9° (*c* 1.1, $CHCl_3$); ^{1}H NMR: δ 7.50–7.00 (35 H, $7 \times$ Ph), 5.00–4.75 (10 H, $5 \times C\mathit{H}_2$Ph), 4.67–4.60 (ABq, 2 H, J_{AB} 12.2 Hz, $C\mathit{H}_2$Ph), 4.56–4.43 (d + ABq, 3 H, $J_{1',2'}$ 9.0, J_{AB} 12.2 Hz, H-1′, $C\mathit{H}_2$Ph), 4.15–4.03 (3 H, H-2, 4, 6), 3.71–3.63 (2 H, H-4′, 5′), 3.61–3.45 (6 H, H-1, 2′, 3, 5, 6′a, OH), 3.42 (2 H, H-3′,6′b); Anal. Calcd for $C_{61}H_{63}N_3O_{10}$: C, 73.40; H, 6.36; N, 4.21. Found: C, 73.10; H, 6.55; N, 4.20.

*Triethylammonium 1-*D*-6-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-2,3,4,5-tetra-*O*-benzyl*-myo*-inositol 1-(1,2-di-*O*-hexadecanoyl*-sn*-glycerol 3-phosphate)* (**23**).—This compound was obtained from the alcohol **21** (84.4 mg, 0.085 mmol) and 1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-hydrogenphosphonate TEA salt (**22**)[14,23] (124 mg, 0.17 mmol) essentially as described for the 1-(octadecyl phosphate) **17**. RBC (elution first with $CHCl_3$ and then with 19:1 $CHCl_3$–MeOH) gave the TEA salt **23** (67 mg, 46%); $[\alpha]_D^{25}$ −5° (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.50–7.10 (35 H, $7 \times$ Ph), 5.30 (m, 1 H, H-2 glycerol), 5.10–4.90 (5 H, H-1′, $2 \times C\mathit{H}_2$Ph), 4.85–4.50 (11 H, H-2, $5 \times C\mathit{H}_2$Ph), 4.45–4.23 (t + m, 3 H, $J_{1,6} = J_{5,6}$ 9.9 Hz, H-6, 1- or 3-CH_2 glycerol), 4.15–4.05 (4 H, H-1, 4, 1- or 3-CH_2 glycerol), 3.75–3.50 (5 H, H-3, 4′, 5′, 5, 6′a), 3.37 (dd, 1 H, $J_{1',2'}$ 8.4, $J_{2',3'}$ 9.9 Hz, H-2′), 3.28–3.20 (2 H, H-3′, 6′b), 2.88 (6 H, $3 \times C\mathit{H}_2CH_3$), 2.25 (m, 4 H, $2 \times COCH_2$), 1.50 (m, 4 H, $2 \times COCH_2C\mathit{H}_2$), ~1.20 (57 H, $3 \times CH_2C\mathit{H}_3$, $2 \times [CH_2]_{12}$), 0.85 (t, 6 H, *J* 6.7 Hz, $2 \times CH_2C\mathit{H}_3$); ^{31}P NMR: δ_p −3.50 (with heteronuclear decoupling); ESMS(−): m/z 1626.2 $[M - NEt_3 - H]^-$.

*Triethylammonium 1-*D*-6-*O*-(2-amino-2-deoxy-β-*D*-glucopyranosyl)-myo-inositol 1-(1,2-di-*O*-hexadecanoyl-sn-glycerol 3-phosphate)* (**24**).—The TEA salt **23** (45 mg, 0.026 mmol) in 2:2:1 *n*-propanol–THF–water (5 mL) containing 20% $Pd(OH)_2$ on carbon (90 mg) was stirred under 3 atm of hydrogen for 2 h. Work-up as described for the compound **18** gave β-D-Glc*p*N-PI (**24**)

(27 mg, 96%); $[\alpha]_D^{25}$ +7° (*c* 0.3, 10:10:3 $CHCl_3$–MeOH–water); ^{1}H NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 5.15 (m, 1 H, H-2 glycerol), 4.75 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.35–4.05 (3 H, H-2, 1- or 3-CH_2 glycerol), 4.00–3.90 (m, 2 H, 1- or 3-CH_2 glycerol), 3.77 (dd, 1 H, $J_{1,2}$ 1.9, $J_{1,6}$ 9.9 Hz, H-1), 3.64 (2 H, H-6, 6′a), 3.54 (2 H, H-4, 6′b), 3.42 (1 H, H-3′), 3.35 (dd, 1 H, $J_{2,3}$ 2.7, $J_{3,4}$ 9.1 Hz, H-3), 3.30–3.20 (8 H, H-4′, 5′, 3 × CH_2CH$_3$), 3.17 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.4 Hz, H-5), 2.77 (t, 1 H, $J_{1',2'} = J_{2',3'}$ 8.3 Hz, H-2′), 2.25 (m, 4 H, 2 × COCH_2), 1.50 (m, 4 H, 2 × COCH$_2$CH_2), ~1.20 (57 H, 3 × CH$_2$CH_3, 2 × [CH$_2$]$_{12}$), 0.80 (t, 6 H, *J* 7.0 Hz, 2 × CH$_2$CH_3); ^{31}P NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ_p 0.70 (with heteronuclear decoupling); ESMS(−): *m*/*z* 970.2 $[M - NEt_3 - H]^-$.

2-Azido-3,4,6-tri-O-*benzyl-2-deoxy*-α-D-*mannopyranosyl fluoride* (**26**).—To a cooled (−30 °C) and stirred solution of the hemiacetal **25**[28] (158 mg, 0.33 mmol) in anhyd 1,2-dichloroethane (15 mL) was added DAST (183 μL, 1.38 mmol). The reaction mixture was allowed to attain rt over 30 min and it was then partitioned between ice-water (20 mL) and Et_2O (20 mL). The aqueous phase was separated and further extracted with Et_2O (20 mL). The ethereal extracts were combined and washed with brine (20 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue so obtained was percolated through a short silica-gel column (further elution with Et_2O) and the eluent was concentrated under reduced pressure. RBC (elution first with cyclohexane and then with 4:1 cyclohexane–Et_2O) of the residue gave the α-glycosyl fluoride **26** (106 mg, 67%); $[\alpha]_D^{25}$ +33° (*c* 1.4, $CHCl_3$); ^{1}H NMR: δ 7.40–7.05 (15 H, 3 × Ph), 5.55 (dd, 1 H, $J_{1,2}$ 1.4, $J_{1,F}$ 50.2 Hz, H-1), 4.90–4.45 (6 H, 3 × CH_2Ph), 4.05–3.95 (3 H, H-2, 3, 4), 3.88 (m, 1 H, H-5), 3.74 (dd, 1 H, $J_{5,6a}$ 3.9 Hz, H-6a), 3.63 (dd, 1 H, $J_{5,6b}$ 1.9, $J_{6a,6b}$ 11.0 Hz, H-6b).

1-D-*6*-O-*(2-Azido-3,4,6-tri*-O-*benzyl-2-deoxy*-α-D-*mannopyranosyl)-2,3,4,5-tetra*-O-*benzyl-1*-O-*(4-methoxybenzyl)*-myo-*inositol* (**27**).—The coupling between the acceptor **20**[3] (99 mg, 0.15 mmol) and the glycosyl fluoride **26** (100 mg, 0.21 mmol) was conducted essentially as described for the preparation of the pseudodisaccharide **13**. RBC (elution first with cyclohexane and then with 4:1 cyclohexane–Et_2O) afforded the α-linked pseudodisaccharide **27** (102 mg, 61%) as an oil; $[\alpha]_D^{25}$ +13° (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.50–6.80 (39 H, C_6H_4, 7 × Ph), 5.39 (d, 1 H, $J_{1',2'}$ 1.1 Hz, H-1′), 5.10–4.20 (16 H, 8 × CH_2Ar), 4.19 (t, 1 H, H-6), 4.11 (t, 1 H, H-4), 4.03 (t, 1 H, $J_{1,2} = J_{2,3}$ 2.0 Hz, H-2), 3.96–3.92 (2 H, H-3′, 4′), 3.84 (m, 1 H, H-5′), 3.75 (s, 3 H, ArOCH_3), 3.72 (dd, 1 H, H-2′), 3.37 (dd, 1 H, $J_{2,3}$ 2.0, $J_{3,4}$ 10.0 Hz, H-3), 3.30 (t, 1 H, $J_{4,5} = J_{5,6}$ 10.0 Hz, H-5), 3.25 (dd, 1 H, $J_{1,2}$ 2.0, $J_{1,6}$ 10.0 Hz, H-1), 3.19–3.13 (2 H, H-6′a,b); ESMS(+): *m*/*z* 1140.5 $[M + Na]^+$.

1-D-*6*-O-*(2-Azido-3,4,6-tri*-O-*benzyl-2-deoxy*-α-D-*mannopyranosyl)-2,3,4,5-tetra*-O-*benzyl*-myo-*inositol* (**28**).—Demethoxybenzylation of the compound **27** (227 mg, 0.20 mmol) in CH_2Cl_2 (10 mL) containing TFA (216 μL, 2.8 mmol) was achieved as in the preparation of the compound **9**. RBC (elution first with hexane and then with 3:1 hexane–EtOAc) furnished the alcohol **28** (123 mg, 62%) as an oil; $[\alpha]_D^{25}$ +30° (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 7.50–7.00 (35 H, 7 × Ph), 5.45 (d, 1 H, $J_{1',2'}$ 1.1 Hz, H-1′), 5.10–4.20 (14 H, 7 × CH_2Ph), 4.05–4.00 (3 H, H-2′, 4′, 6), 3.95–3.91 (3 H, H-2, 3′, 5′), 3.88 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.48–3.41 (2 H, H-1, 3), 3.37 (dd, 1 H, $J_{5',6'a}$ 1.9, $J_{6'a,6'b}$ 11.3 Hz, H-6′a), 3.33–3.25 (2 H, H-5, 6′b), 2.25 (d, 1 H, $J_{1,OH}$ 7.0 Hz, OH); ESMS(+): *m*/*z* 1020.6 $[M + Na]^+$.

Triethylammonium 1-D-*6*-O-*(2-azido-3,4,6-tri*-O-*benzyl-2-deoxy*-α-D-*mannopyranosyl)-2,3,4,5-tetra*-O-*benzyl*-myo-*inositol 1-(1,2-di*-O-*hexadecanoyl*-sn-*glycerol 3-phosphate)* (**29**).—This phosphoric diester was obtained from the H-phosphonate **22**[14,23] (227 mg, 0.31 mmol) and the alcohol **28** (120 mg, 0.12 mmol) essentially as described for the 1-(octadecyl phosphate) **17**. RBC (elution first with $CHCl_3$ and then with 12:1 $CHCl_3$–MeOH) provided the phosphoric diester as the TEA salt **29** (145 mg, 70%); $[\alpha]_D^{25}$ +24° (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 7.50–6.90 (35 H, 7 × Ph), 5.46 (d, 1 H, $J_{1',2'}$ 1.2 Hz, H-1′), 5.16 (m, 1 H, H-2 glycerol), 4.96–4.47 (14 H, 7 × CH_2Ph), 4.42 (2 H, H-2, 2′), 4.30 (m, 2 H, 1- or 3-CH_2 glycerol), 4.13 (2 H, H-1, 6), 4.05–3.97 (2 H, H-3′, 4), 3.96–3.82 (4 H, H-4′, 5′, 1- or 3-CH_2 glycerol), 3.46 (dd, 1 H, $J_{2,3}$ 1.7, $J_{3,4}$ 9.6 Hz, H-3), 3.29 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.0 Hz, H-5), 3.21 (dd, 1 H, $J_{5',6'a}$ 1.8 Hz, H-6′a), 3.12 (d, 1 H, $J_{6'a,6'b}$ 10.5 Hz, H-6′b), 2.73 (q, 6 H, *J* 7.0 Hz, 3 × CH_2CH$_3$), 2.15 (m, 4 H, 2 × COCH_2), 1.48 (m, 4 H, 2 × COCH$_2$CH_2), ~1.20 (48 H, 2 × [CH$_2$]$_{12}$), 1.08 (t, 9 H, *J* 7.2 Hz, 3 × CH$_2$CH_3), 0.85 (t, 6 H, *J* 7.0 Hz, 2 × CH$_2$CH_3); ^{31}P NMR: δ_p −3.41 (with heteronuclear decoupling): ESMS(−): *m*/*z* 1626.8 $[M - NEt_3 - H]^-$.

Triethylammonium 1-D-*6*-O-*(2-amino-2-deoxy*-α-D-*mannopyranosyl)*-myo-*inositol 1-(1,2-di*-O-*hexadecanoyl*-sn-*glycerol 3-phosphate)* (**30**).—A solution of the TEA salt **29** (105 mg, 0.061 mmol) in 2:2:1 *n*-propanol–THF–water (5 mL) containing 20% $Pd(OH)_2$ on carbon (50 mg) was stirred under 3 atm of hydrogen for 1 h. Work-up as described for the compound **18** gave α-D-Man*p*N-PI (**30**) (60 mg, 92%); $[\alpha]_D^{25}$ +13° (*c* 0.2, 10:10:3 $CHCl_3$–MeOH–water); ^{1}H NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 5.03 (s, 1 H, H-1′), 4.98 (m, 1 H, H-2 glycerol), 4.13 (m, 2 H, 1- or 3-CH_2 glycerol), 3.90–3.70 (6 H, H-1, 2, 3′, 5′, 1- or 3-CH_2 glycerol), 3.58 (t, 1 H, $J_{1,6} = J_{5,6}$ 9.5 Hz, H-6), 3.53 (2 H, H-6′a,b), 3.47 (m, 1 H, H-2′), 3.33 (2 H, H-4, 4′), 3.14 (dd, 1 H, $J_{2,3}$ 2.6, $J_{3,4}$ 9.8 Hz, H-3), 3.04 (m, 1 H, H-5), 2.89 (q, 6 H, *J* 7.3 Hz, 3 × CH_2CH$_3$), 2.05 (dt, 4 H, *J* 7.3 Hz, 2 × COCH_2), 1.31 (m, 4 H, 2 ×

$COCH_2CH_2$), 1.13 (t, 9 H, J 7.3 Hz, $3 \times CH_2CH_3$), ~0.98 (48 H, $2 \times [CH_2]_{12}$), 0.60 (t, 6 H, J 7.1 Hz, $2 \times CH_2CH_3$); ^{31}P NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ_p −1.04 (with heteronuclear decoupling); ESMS(−): m/z 970.5 $[M - NEt_3 - H]^-$.

2-Azido-3,4,6-tri-O-*benzyl-2-deoxy-β*-D-*galactopyranosyl fluoride* (**32**).—To a stirred solution of the selenogalactopyranoside **31**[28] (0.1 g, 0.16 mmol) in freshly distilled CH_2Cl_2 (10 mL) at −5 °C under argon was added dropwise DAST (0.034 mL, 0.26 mmol). Stirring was continued for 1 min, whereafter *N*-iodosuccinimide (0.047 g, 0.21 mmol) was added. The resulting mixture was then stirred for 40 min before it was diluted with CH_2Cl_2 (30 mL) and washed with cold water (30 mL), satd $NaHCO_3$ solution (25 mL) and brine (25 mL), dried ($MgSO_4$), and concentrated under reduced pressure. RBC (12:1 → 10:1 hexane–EtOAc) of the residue, with sacrificial cuts, provided the pure β-anomer **32** (0.034 g, 44%); $[\alpha]_D^{25}$ −21° (*c* 1.9, $CHCl_3$); IR (film); ν 2100 cm^{-1} (azide); ^1H NMR: δ 7.38–7.17 (15 H, $3 \times$ Ph), 5.08–4.82 (dd + d, 2 H, $J_{1,2}$ 7.5, $J_{1,F}$ 52.5 Hz, H-1, C*Ha*Ph), 4.68 (ABq, 2 H, J_{AB} 11.7 Hz, CH_2Ph), 4.55 (d, 1 H, J 11.5 Hz, C*Hb*Ph), 4.45 (ABq, 2 H, J_{AB} 11.7 Hz, CH_2Ph), 4.00–3.88 (2 H, H-2, 4), 3.65–3.55 (3 H, H-5, 6a,b), 3.39 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 1.9 Hz, H-3).

1-D-*6*-O-*(2-Azido-3,4,6-tri*-O-*benzyl-2-deoxy-α*-D-*galactopyranosyl)-2,3,4,5-tetra*-O-*benzyl*-myo-*inositol* (**34**).—The coupling between the β-galactosyl fluoride **32** (0.178 g, 0.37 mmol) and the D-*myo*-inositol derivative **20**[3] (0.176 g, 0.27 mmol) in anhyd 1,4-dioxane (15 mL) was carried out essentially as described for the preparation of the pseudodisaccharide **13**. RBC (10:1 → 4:1 hexane–EtOAc) gave the α-linked pseudodisaccharide **33** (0.154 g, 38%), which was contaminated with a trace of impurity; ESMS(+): m/z 1140.1 $[M + Na]^+$ and m/z 1156.1 $[M + K]^+$. This material was used in the next step without further purification.

A solution of the pseudodisaccharide **33** (0.104 g, 0.092 mmol) in anhyd CH_2Cl_2 (15 mL) containing TFA (0.1 mL, 1.3 mmol) was kept at rt for 2 h before it was diluted with $CHCl_3$ (50 mL), neutralised with Et_3N, washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. RBC (6:1 → 5:1 hexane–EtOAc) gave the alcohol **34** (0.068 g, 73%); $[\alpha]_D^{25}$ +37° (*c* 0.7, $CHCl_3$); ^1H NMR: δ 7.60–6.80 (35 H, $7 \times$ Ph), 5.38 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.01–4.58 (13 H, $6 \times CH_2$Ph, C*Ha*Ph), 4.45 (d, 1 H, J 11.2 Hz, C*Hb*Ph), 4.18–4.03 (3 H, H-4, 4′, 5′), 4.03–3.90 (3 H, H-2, 2′, 6), 3.85 (1 H, H-3′), 3.60 (m, 1 H, H-1), 3.49–3.35 (3 H, H-3, 5, 6′a), 3.25 (dd, 1 H, $J_{5',6'b}$ 5.7, $J_{6'a,6'b}$ 8.7 Hz, H-6′b); ESMS(+): m/z 1020.2 $[M + Na]^+$.

Triethylammonium 1-D-*6*-O-*(2-azido-3,4,6-tri*-O-*benzyl-2-deoxy-α*-D-*galactopyranosyl)-2,3,4,5-tetra*-O-*benzyl*-myo-*inositol 1-(1,2-di*-O-*hexadecanoyl*-sn-*glycerol 3-phosphate)* (**35**).—This phosphoric diester was obtained from the pseudodisaccharide derivative **34** (0.068 g, 0.068 mmol) and the H-phosphonate **22**[14,23] (0.150 g, 0.2 mmol) essentially as described in the preparation of the 1-(octadecyl phosphate) **17**. RBC (30:1 → 15:1 $CHCl_3$–MeOH) furnished the phosphoric diester as the TEA salt **35** (0.079 g, 67%); $[\alpha]_D^{25}$ +46° (*c* 0.8, $CHCl_3$); ^1H NMR: δ 7.50–7.10 (35 H, $7 \times$ Ph), 5.83 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1′), 5.24 (m, 1 H, H-2 glycerol), 5.10–4.58 (15 H, H-2, $7 \times CH_2$Ph), 4.50–4.20 (m, 5 H, 1- or 3-CH_2 glycerol, H-1, 5′, 6), 4.18–4.00 (t + m, 3 H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4, 1- or 3-CH_2 glycerol), 3.78 (dd, $J_{3',4'}$ 2.4 Hz, H-3′), 3.68–3.60 ($2 \times$ dd, 2 H, $J_{1',2'}$ 3.3, $J_{2',3'}$ 10.8 Hz, H-2′, 4′), 3.57 (dd, $J_{2,3}$ 2.0, $J_{3,4}$ 9.7 Hz, H-3), 3.49 (dd, 1 H, $J_{5',6'a}$ 5.9, $J_{6'a,6'b}$ 9.3 Hz, H-6′a), 3.48–3.42 (2 H, $J_{5,6}$ 10.2 Hz, H-5, 6′b), 2.95 (m, 6 H, $3 \times CH_2CH_3$), 2.25 ($2 \times$ t, 4 H, $2 \times COCH_2CH_2$), 1.55 (m, 4 H, $2 \times COCH_2CH_2$), ~1.20 (57 H, $3 \times CH_2CH_3$, $2 \times [CH_2]_{12}$), 0.90 (m, 6 H, J 7.0 Hz, $2 \times CH_2CH_3$); ^{31}P NMR: δ_p −0.27 (with ^1H heteronuclear decoupling); ESMS(−): m/z 1626.3 $[M - NEt_3 - H]^-$.

Triethylammonium 1-D-*6*-O-*(2-amino-2-deoxy-α*-D-*galactopyranosyl)*-myo-*inositol 1-(1,2-di*-O-*hexadecanoyl*-sn-*glycerol 3-phosphate)* (**36**).—A solution of the benzylated compound **35** (79 mg, 0.045 mmol) in 1:1 THF–propanol (4 mL) containing 20% $Pd(OH)_2$ on carbon (100 mg) was stirred under 3 atm of hydrogen for 1 h. Work-up as described for the compound **18** gave α-D-Gal*p*N-PI (**36**) (38 mg) in essentially quantitative yield; $[\alpha]_D^{25}$ +25° (*c* 0.3, 10:10:3 $CHCl_3$–MeOH–water); ^1H NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 5.55 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 5.27 (m, 1 H, H-2 glycerol), 4.42 (dd, 1 H, 1- or 3-CHa glycerol), 4.30 (t, 1 H, $J_{5',6'a} = J_{5',6'b}$ 6.0 Hz, H-5′), 4.23–4.12 (2 H, H-1, 1- or 3-CHb glycerol), 4.10–3.85 (6 H, H-2, 3′, 4′, 6, 1- or 3-CH_2 glycerol), 3.80–3.64 (t + m, 3 H, $J_{3,4} = J_{4,5}$ 9.6, $J_{6'a,6'b}$ 10.6 Hz, H-4, 6′a,b), 3.45 (2 H, H-2′, 3), 3.35 (m, 1 H, H-5), 3.15 (q, 6 H, J 7.3 Hz, $3 \times CH_2CH_3$), 2.30 (m, 4 H, $2 \times COCH_2$), 1.60 (m, 4 H, $2 \times COCH_2CH_2$), 1.50–1.10 (57 H, $3 \times CH_2CH_3$, $2 \times [CH_2]_{12}$), 0.80 (t, 6 H, J 7.1 Hz, $2 \times CH_2CH_3$); ^{31}P NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ_p 1.06 (with ^1H heteronuclear decoupling); ESMS(−): m/z 970.5 $[M - NEt_3 - H]^-$.

1-L-*6*-O-*Allyl-3,4,5-tri*-O-*benzyl-1*-O-*(4-methoxybenzyl)-2*-O-*methyl*-myo-*inositol* (**38**).—This compound was prepared from the alcohol **37**[3] as described for the D-enantiomer.[14] It had mp 88–89 °C, lit. (D-enantiomer)[14] mp 88–90 °C; $[\alpha]_D^{25}$ +10° (*c* 1.2, $CHCl_3$), lit. (D-enantiomer)[14] $[\alpha]_D$ −11° (*c* 1.0, $CHCl_3$).

1-L-*3,4,5-Tri*-O-*benzyl-1*-O-*(4-methoxybenzyl)-2*-O-*methyl*-myo-*inositol* (**39**).—This compound was prepared from the allylated derivative **38** as described for the D-enantiomer.[14] It had mp 43–45 °C, lit. (D-enantiomer)[14] mp 43–45 °C; $[\alpha]_D^{25}$ +20° (*c* 1.1, $CHCl_3$), lit. (D-enantiomer)[14] $[\alpha]_D$ −24° (*c* 1.0, $CHCl_3$).

1-L-6-O-(2-Azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl)-3,4,5-tri-O-benzyl-2-O-methyl-myo-inositol (**41**).—The coupling between the L-*myo*-inositol derivative **39** (0.189 g, 0.323 mmol) and the glycosyl fluoride **12**[3] (0.216 g, 0.453 mmol) was carried out essentially as described for the preparation of the pseudodisaccharide **13**. RBC (10:1→4:1 hexane–EtOAc) afforded the α-linked pseudodisaccharide **40** (0.214 g, ~64%) admixed with an inseparable impurity; ^{1}H NMR: δ 7.60–6.60 (34 H, C_6H_4, 6 × Ph), 5.61 (d, 1 H, $J_{1',2'}$ 3.1 Hz, H-1′), 5.15–4.35 (14 H, 7 × CH_2Ar), 4.28 (m, 1 H, H-5′), 4.18 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.99 (t, 1 H, $J_{1,6} = J_{5,6}$ 9.5 Hz, H-6), 3.93 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 9.5 Hz, H-3′), 3.73 (bs, 1 H, H-2), 3.71–3.68 (m, 1 H, H-4′), 3.65 (s, 3 H, ArOCH_3), 3.60 (s, 3 H, OCH_3), 3.55 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.5 Hz, H-5), 3.40–3.30 (3 H, H-1, 6′a,b), 3.28–3.20 (2 H, H-2′, 3); ESMS(+): m/z 1063.9 $[M + Na]^+$. This slightly impure material was used in the next step.

The pseudodisaccharide **40** (0.188 g, 0.18 mmol) in anhyd CH_2Cl_2 (10 mL) was treated with TFA (0.19 mL, 2.5 mmol) at rt for 2 h and the reaction mixture was then worked-up as described for the compound **9**. RBC (6:1→2:1 cyclohexane–Et_2O) furnished the alcohol **41** (0.157 g, 72%); $[\alpha]_D^{25}$ +39° (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.45–7.10 (30 H, 6 × Ph), 5.39 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1′), 5.20–4.61 (8 H, 4 × CH_2Ph), 4.60–4.40 (5 H, 2 × CH_2Ph, H-5′), 4.02–3.92 (2 H, H-3′, 4), 3.88 (t, 1 H, $J_{1,6} = J_{5,6}$ 9.4 Hz, H-6), 3.71 (t, 1 H, $J_{1,2} = J_{2,3}$ 2.5 Hz, H-2), 3.66 (dd, 1 H, $J_{5',6'a}$ 1.6, $J_{6'a,6'b}$ 10.4 Hz, H-6′a), 3.61–3.45 (3 H, H-4′, 5, 6′b), 3.56 (s, 3 H, OCH_3), 3.44–3.38 (2 H, H-1, 3), 3.36 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-2′), 2.90 (d, 1 H, $J_{1,OH}$ 9.5 Hz, OH); ESMS(+): m/z 944.0 $[M + Na]^+$.

Triethylammonium 1-L-6-O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl)-3,4,5-tri-O-benzyl-2-O-methyl-myo-inositol 1-(1,2-di-O-hexadecanoyl-sn-glycerol 3-phosphate) (**42**).—This compound was obtained from the inositol derivative **41** (0.105 g, 0.11 mmol) and the H-phosphonate **22**[14,23] (0.207 g, 0.28 mmol) essentially as described for the 1-(octadecyl phosphate) **17**. RBC (30:1→19:1 $CHCl_3$–MeOH) provided the TEA salt **42** (0.13 g, 70%); $[\alpha]_D^{25}$ +16° (*c* 1.5, $CHCl_3$); ^{1}H NMR: δ 7.40–7.00 (30 H, 6 × Ph), 5.62 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1′), 5.19 (m, 1 H, H-2 glycerol), 5.12–4.51 (12 H, 6 × CH_2Ph), 4.47–4.39 (1 H, H-2), 4.31–4.23 (3 H, H-5′, 1- or 3-CH_2 glycerol), 4.19 (t, 1 H, $J_{1,6} = J_{5,6}$ 9.3 Hz, H-6), 4.15–3.99 (3 H, H-4, 1- or 3-CH_2 glycerol), 3.96 (1 H, H-1), 3.89 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 9.8 Hz, H-3′), 3.83–3.75 (2 H, H-4′, 6′a), 3.70–3.60 (m, 1 H, H-6′b), 3.66 (s, 3 H, OCH_3), 3.56 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.3 Hz, H-5), 3.51–3.44 (7 H, H-3, 3 × CH_2CH$_3$), 3.28 (dd, 1 H, H-2′), 2.25 (m, 4 H, 2 × COCH_2), 1.65 (bt, 4 H, 2 × COCH$_2$CH_2), ~1.25 (57 H, 3 × CH$_2$CH_3, 2 × [CH$_2$]$_{12}$), ~0.90 (2 × t, 6 H, 2 × CH$_2$CH_3); ^{31}P NMR: δ_p −1.10 (with ^{1}H heteronuclear decoupling); ESMS(−): m/z 1550.1 $[M - Et_3N - H]^-$.

Sodium 1-L-6-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-O-methyl-myo-inositol 1-(1,2-di-O-hexadecanoyl-sn-glycerol 3-phosphate) (**44**).—A solution of the protected sodium salt **43** (52 mg, 0.031 mmol; prepared from the TEA salt **42**) in 4:4:1 THF–propanol–water (9 mL) containing 20% $Pd(OH)_2$ on carbon (130 mg) was stirred under 3 atm of hydrogen for 1.5 h. Work-up as described for the compound **18** gave α-D-Glc*p*N-[L]-2-*O*-methyl-PI (**44**) (32 mg) in essentially quantitative yield; $[\alpha]_D^{23}$ +2° (*c* 0.3, 10:10:3 $CHCl_3$–MeOH–water); ^{1}H NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 5.35 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′), 3.74 (s, 3 H, OCH_3), 3.30 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9.7 Hz, H-4′), 2.21 (m, 4 H, 2 × $COCH_2$), 1.58 (m, 4 H, 2 × COCH$_2$CH_2), ~1.30 (48 H, 2 × [CH$_2$]$_{12}$), 0.77 (6 H, 2 × CH$_2$CH_3); ^{31}P NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ_p 3.70 (with ^{1}H heteronuclear decoupling); ESMS(−): m/z 984.3 $[M - Na]^-$.

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