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The synthesis of some deoxygenated analogues of early intermediates in the biosynthesis of glycosylphosphatidylinositol (GPI) membrane anchors ☆

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Abstract—Syntheses are described of 2-azido-4,6-di-*O*-benzyl-2,3-dideoxy-D-*ribo*-hexopyranosyl fluoride, 6-*O*-acetyl-2-azido-3-*O*-benzyl-2,4-dideoxy-D-*xylo*-hexopyranosyl fluoride and 2-azido-3,4-di-*O*-benzyl-2,6-dideoxy-D-glucopyranosyl fluoride. These glycosyl donors were coupled with the acceptor 1D-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol and the α-coupled products were transformed into α-D-3dGlc*p*N-PI, α-D-4dGlc*p*N-PI and α-D-6dGlc*p*N-PI by way of the H-phosphonate route. Brief mention is made of the biological evaluation of these deoxy-sugar analogues and their N-acetylated forms as candidate substrate/inhibitors of the N-deacetylase and α-(1→4)-D-mannosyltransferase activities present in trypanosomal and HeLa (human) cell-free system.

Keywords: Glycosylphosphatidylinositol (GPI) membrane anchors; Deoxy-sugar 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

Our efforts over the past decade or so to produce analogues of 1-D-6-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (**1**) and the N-deacetylated form **2** have been summarised previously.[1] The various analogues described therein were used to probe the substrate specificities or inhibition of two enzymes, a N-deacetylase and an α-(1→4)-D-mannosyltransferase (MT-1), that exert their activities on substrates identical in all essential features to the synthetic glycosylphosphatidylinositols **1** and **2**, respectively, in GPI membrane-anchor biosynthesis in 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 (human) host. It is equally important to test these analogues against the related enzymes present in a HeLa (human) system, whose GPI-anchor biosynthesis must survive the assault on that of the parasite.

The N-deacetylase activities of interest transform α-D-Glc*p*NAc-PI (**1**) into α-D-Glc*p*N-PI (**2**) in both *T. brucei* and HeLa cell-free systems.[3] The N-deacetylated compound **2** is then acted upon by MT-1, which in the trypanosome 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**). This step is followed by the addition of a fatty acyl group (most often hexadecanoyl) to 2-OH of the D-*myo*-inositol residue. This sequence is reversed in the mammalian system wherein inositol 2-acylation

☆ Parasite glycoconjugates, Part 14. For Part 13, see Ref. 1.

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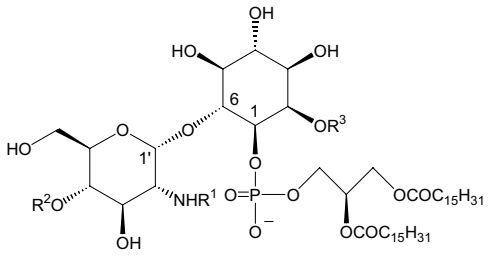
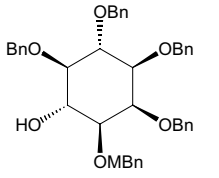


	R^1	R^2	R^3
1	Ac	H	H
2	H	H	H
3	H	α-D-Man*p*	H
4	H	H	$CH_2C_{15}H_{31}$
5	H	H	$COC_{15}H_{31}$



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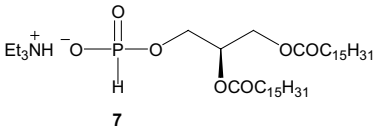
MBn = p-methoxybenzyl



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occurs *before* the first α-D-Man*p* residue is attached.[4] Thus there is a fundamental difference between the trypanosomal and mammalian biosynthetic pathways with respect to the timing of inositol 2-acylation that might be exploited in the development of parasite-specific therapeutic agents. A synthetic analogue displaying parasite-specific inhibition is α-D-Glc*p*N-2-*O*-hexadecyl-PI (**4**), which inhibits trypanosomal MT-1 in a cell-free system but is not a substrate for HeLa MT-1 despite being a close analogue of the proven substrate α-D-Glc*p*N-2-*O*-hexadecanoyl-PI (**5**).[5]

To date we have described the synthesis of substrate analogues of the developing GPI anchors **1** and **2** having structural modifications to the phospholipid, α-D-glucosamine and D-*myo*-inositol components.[6–9] Attention herein focuses on further modifications to the α-D-glucosamine residue of compounds **1** and **2**, which has been deoxygenated at each of positions 3, 4 and 6 with the intent of establishing which of these OH groups are involved in essential hydrogen bonding in the active site of the respective enzymes. Preliminary communications on this work have appeared.[10]

2. Results and discussion

The synthesis of the targeted deoxy analogues followed a familiar approach.[1,6–9] In this, a suitable glycosyl donor is coupled with the protected D-*myo*-inositol acceptor **6**,[6] whereafter at the next or a later stage the 1-OH group of the α-coupled product is exposed for attachment of the H-phosphonate **7**.[8,11] Thereafter in situ oxidation provides the related phosphoric diester, which is transformed into the fully deprotected analogue (e.g., **28**) on hydrogenolysis. In keeping with previous experience,[1,6–9] we opted for the glycosyl fluorides **16**, **37** and **56** as the glycosyl donors (see Figs. 1, 2, 4 and 7), thereby ensuring that the requisite deoxy function was already in place in the coupled product. The nonparticipating 2-azido group favours the formation of an α-glycosidic linkage in the coupling reaction and is reduced to a 2-amino group during the final deprotection step.

Two essentially traditional approaches to the synthesis of 2-azido-4,6-di-*O*-benzyl-2,3-dideoxy-D-*ribo*-hexopyranosyl fluoride (**16**) are illustrated in Figures 1 and 2. In the first approach, ring opening of the β-D-*manno*-epoxide **9** with $LiAlH_4$ in boiling Et_2O furnished the 3-deoxy compound **10**,[12] which was straightforwardly transformed into the axial tosylate **11** and thereafter into the 2-azide **12**, with inversion of configuration at the reaction centre. Removal of the 4,6-*O*-benzylidene group with acid (→**13**) and a conventional benzylation (→**14**) was followed by glycoside hydrolysis (→**15**) and subsequent treatment with DAST (→**16**).

An alternative approach (Fig. 2) was based on conjugate addition of hydrazoic acid to ethyl 2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside (**18**), which is available in three steps from 3,4,6-tri-*O*-acetyl-D-glucal (**17**).[13] This 1,4-addition yields the *threo*-adduct **19** as the kinetically favoured product, which slowly equilibrates with the thermodynamically more-stable *erythro*-adduct **20**.[14] Reduction of a mixture of the azido-ketones **19** and **20** with $NaBH_4$ in MeOH gave ethyl 2-azido-2,3-dideoxy-α-D-*ribo*-hexopyranoside (**22**)[14] as one of the products. Radial-band chromatography (RBC), with sacrificial cuts, permitted the separation of the pure 2-azido-2,3-dideoxy compound **22** in a lowly 18% yield from the other dominant product, which on the evidence of previous work[14] was assumed to be the α-D-*lyxo* isomer **21**. Because no physical data were recorded for the targeted 2-azido-2,3-dideoxy compound **22**, a sample of this compound in MeOH was hydrogenated over 20% $Pd(OH)_2$ on carbon and the resulting amine was N-acetylated in situ to give ethyl 2-acetamido-2,3-dideoxy-α-D-*ribo*-hexopyranoside, also known as ethyl *N*-acetyl-lividosaminide, whose physical data matched those reported.[14] Conventional benzylation of the diol **22** (→**23**), followed by successive acidolysis (→**15**) and DAST treatment provided the glycosyl fluoride **16**.

There is little to choose between the two routes to the glycosyl fluoride **16** since, besides the penultimate glycoside hydrolysis (**14**→**15**, **23**→**15**, see Experimental for details), each of them has one other low-yielding step that reduces the overall efficiency of the synthesis; for the approach outlined in Figure 1, the β-D-*manno*-epoxide **9** was obtained in only 22% yield, along with the isomeric β-D-*allo*-epoxide (31%), in the second of the

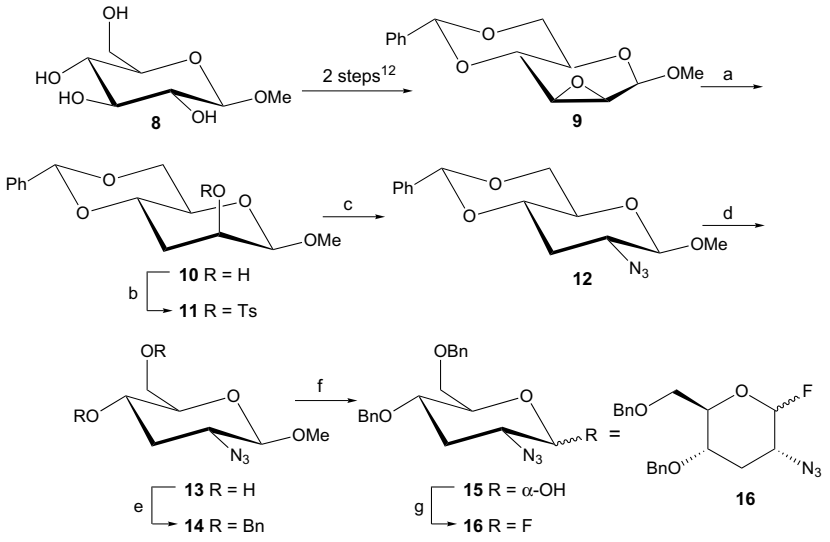


Figure 1. Synthesis of the 3-deoxyglycosyl fluoride **16**: (a) $LiAlH_4$, Et_2O; (b) TsCl, DMAP, py, CH_2Cl_2; (c) NaN_3, DMF, 18-crown-6; (d) 70% AcOH; (e) BnBr, NaH, DMF; (f) 1 M HCl, 80% AcOH; (g) DAST, $ClCH_2CH_2Cl$.

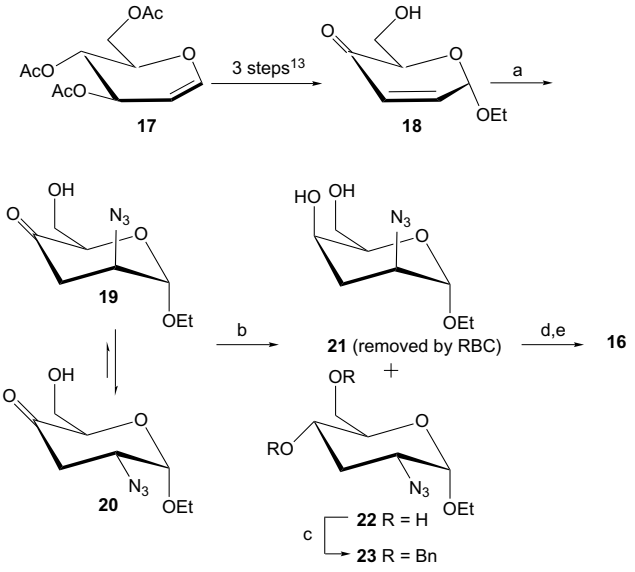


Figure 2. Alternative synthesis of the 3-deoxyglycosyl fluoride **16**: (a) NaN_3, AcOH, H_2O; (b) $NaBH_4$, MeOH; (c) BnBr, NaH, DMF; (d) 1 M HCl, 80% AcOH; (e) DAST, $ClCH_2CH_2Cl$.

two steps proceeding from methyl β-D-glucopyranoside (**8**).[12] In mitigation, both approaches are rapidly developed from readily available starting materials and, despite the deficiencies already noted, yielded ample quantities of the glycosyl fluoride **16**.

The route depicted in Figure 3 for the synthesis of α-D-3dGlc*p*N-PI (**28**) was, with minor adjustments, also followed for those of the corresponding 4′- and 6′-deoxy analogues (see later). Thus the 3-deoxyglycosyl fluoride **16** was coupled with the D-*myo*-inositol acceptor **6**[6] in 4:1 1,4-dioxane–toluene in the presence of zirconocene dichloride and silver perchlorate[15] to give a 3:1 mixture of the α-linked pseudodisaccharide **24** ($J_{1'2'}$ 3.0 Hz) and the β-anomer in 35% yield. Removal of the 1-*O*-methoxybenzyl group from the pseudodisaccharide **24** and coupling of the resulting alcohol **25** with the H-phosphonate **7**[8,11] provided a mixture of two diastereoisomeric phosphonic diesters **26** that was oxidised in situ to the phosphoric diester **27** with iodine in wet pyridine.[16] The final transformation (**27**→**28**) was accomplished by hydrogenolysis over 20% $Pd(OH)_2$ on carbon.

Our approach to the 2-azido-2,4-dideoxyglycosyl fluoride **37** (Fig. 4) was somewhat speculative insofar as it required the radical-mediated removal of a thiocarbonyl group[17] in the presence of a reducible azide.[18] The selective reduction of alkyl iodides brought about by Bu_3Sn radicals in the presence of an azido group is not unknown;[19] most notably, a recent review[20] cites the conversion of the disaccharide 2′-iodide **29** (X = I) into the 2′-deoxy compound **29** (X = H) with Bu_3SnH in boiling benzene in the presence of 2,2′-azobisisobutyronitrile (AIBN) as the radical initiator. Whilst the introduction of a 4-iodo group along the lines of a recent synthesis[21] of the 4-fluoro compound **30** suggested itself, we decided first to test whether the related alcohol **32** could be deoxygenated by way of Bu_3SnH treatment of the 4-*O*-(thiocarbonylimidazolyl) derivative **33**. The overriding attraction of this route is that it is readily developed from D-glucal (**31**) by established procedures.[22]

Gratifyingly, radical-mediated deoxygenation of the imidazolide **33** with Bu_3SnH and AIBN in boiling benzene occurred without significant reduction of the 2-azido group to give the 2-azido-2,4-dideoxy compound **34** in a satisfactory 55% yield. The presence of the

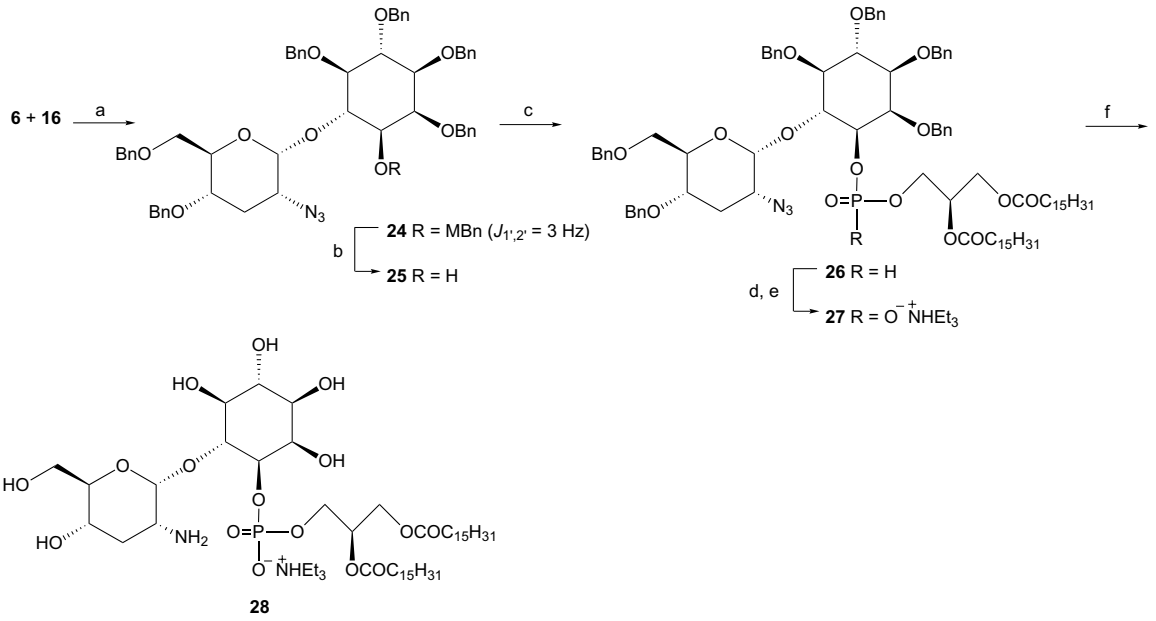


Figure 3. Synthesis of α-D-3dGlc*p*N-PI (**28**): (a) Cp_2ZrCl_2, $AgClO_4$, tetramethylurea, 1,4-dioxane–toluene; (b) TFA, CH_2Cl_2; (c) **7**, Me_3CCOCl, py; (d) I_2, py, H_2O; (e) TEAB buffer; (f) 20% $Pd(OH)_2$/C.

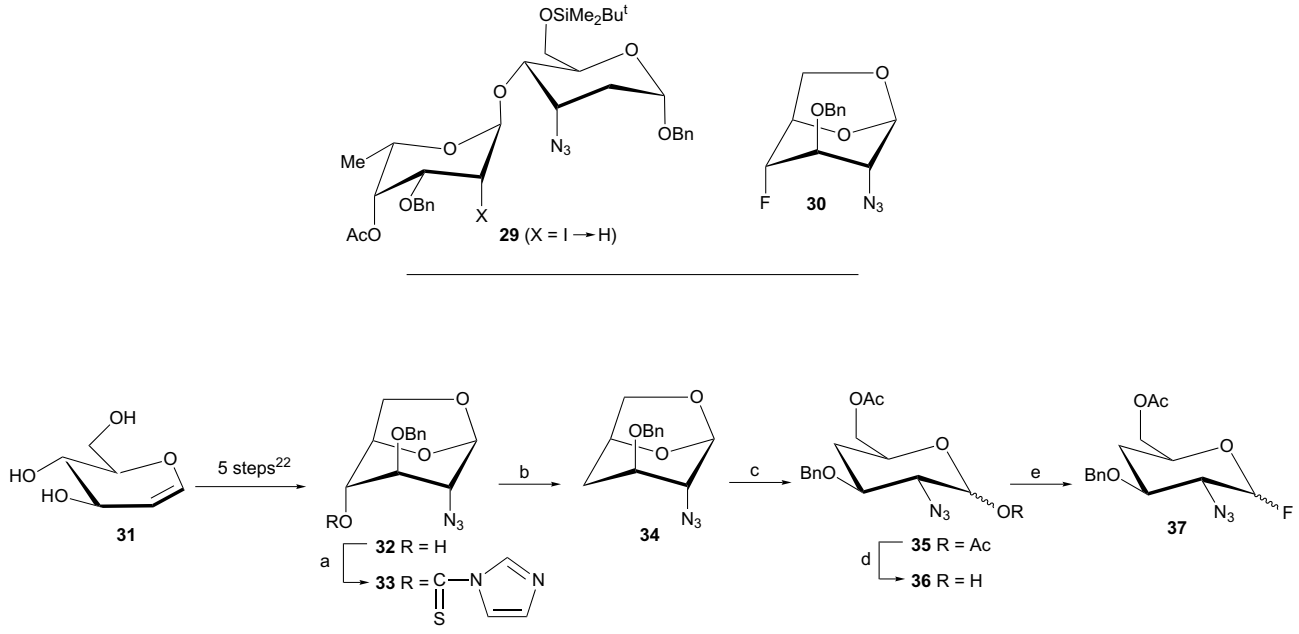


Figure 4. Synthesis of the 4-deoxyglycosyl fluoride **37**: (a) 1,1′-thiocarbonyldiimidazole, toluene; (b) Bu_3SnH, AIBN, C_6H_6; (c) TFA, Ac_2O; (d) Me_2NH, MeCN; (e) DAST, $ClCH_2CH_2Cl$.

4-deoxy group was indicated by distinctive signals for two spin-coupled protons at δ 2.17 and 1.79 (J_{gem} 15.1 Hz), well upfield from those of the other protons, while the retention of the 2-azido group was signalled by an IR absorption at 2100 cm^{-1}. Thereafter, acetolysis (→**35**), selective removal of the anomeric *O*-acetyl group (→**36**), and DAST treatment provided the 4-deoxyglycosyl fluoride **37**.

Coupling of the glycosyl fluoride **37** with the D-*myo*-inositol acceptor **6**[6] in the customary manner[1,6–9] furnished the α-linked pseudodisaccharide **38** ($J_{1',2'}$ 3.6 Hz) in 35.5% yield following RBC (Fig. 5). In this particular case, it was necessary to replace the 6′-*O*-acetyl group with an *O*-benzyl group (**38**→**39**→**40**) before completing the remaining steps leading to α-D-4dGlc*p*N-PI (**41**→**42**→**43**).

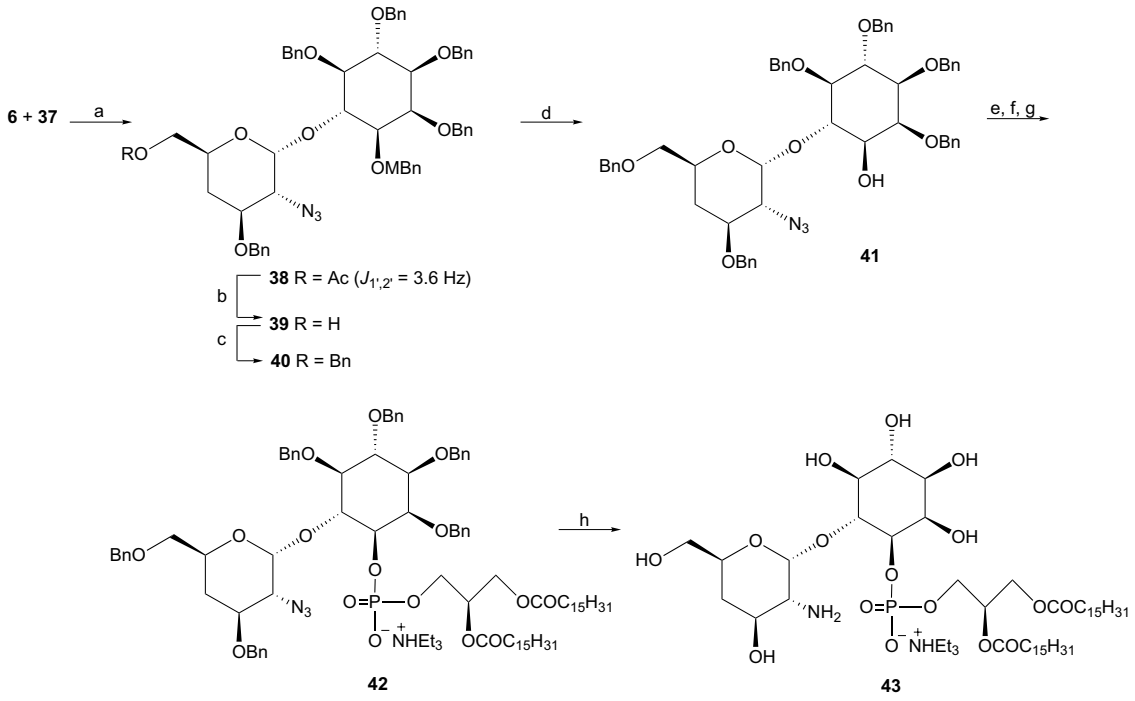


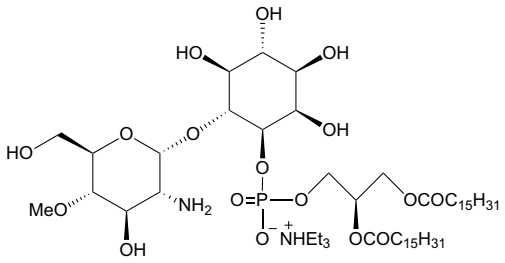
Figure 5. Synthesis of α-D-4dGlc*p*N-PI (**43**): (a) Cp_2ZrCl_2, $AgClO_4$, tetramethylurea, Et_2O; (b) MeONa, MeOH; (c) BnBr, NaH, DMF; (d) TFA, CH_2Cl_2; (e) **7**, Me_3CCOCl, py; (f) I_2, py, H_2O; (g) TEAB buffer; (h) 20% $Pd(OH)_2$/C.

As a safeguard against failure of the key deoxygenation sequence (**32**→**33**→**34**), we had previously taken the 4-*O*-methyl derivative of the alcohol **32** through an identical series of reactions to those depicted in Figures 4 and 5 to give α-D-Glc*p*N4Me-PI (**44**) (no experimental details are provided). Although less satisfactory in some respects, this analogue might provide much the same information as α-D-4dGlc*p*N-PI (**43**) in the enzymic evaluations.

The route devised to α-D-6dGlc*p*N-PI (**60**) required an S_N2 displacement with azide ion on an α-D-rhamnopyranoside 2-sulfonate in the preparation of the glycosyl fluoride **56**. Such displacements are notoriously difficult, sometimes impossible, to effect with charged nucleophiles because of unfavourable dipolar interactions in the transition state.[23] Even with the weakly basic azide ion, β-elimination leading to a 2,3-unsaturated sugar is often dominant.[24] An S_N2 displacement on a β-D-rhamnopyranoside 2-sulfonate provides a more promising option,[25] since the unfavourable dipolar interactions are minimised,[23] but entails the more involved preparation of a β-D-rhamnopyranoside. Since the completion of this work, azide displacements have been achieved[26] on α-linked pseudodisaccharide 2′-triflates, using a hypervalent silicon azide as the nucleophile, in yields comparable to that recorded below but not without an accompanying β-elimination.

We were confronted with this situation in attempting an azide displacement on methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-trifluoromethylsulfonyl-α-D-mannopyranoside (**45**) (Fig. 6). The 2-azide **46** (26%) was the minor product, the major product being the keto sugar **47** (64%).[27] The latter compound most likely results from an initial β-elimination to give an enol benzoate, which either simply loses the 3-*O*-benzoyl group during heating in situ (cf. Ref. 24b) or undergoes attack by azide ion at the ester carbonyl group to furnish the keto sugar **47** by way of the enolate anion. In light of this rationale, we reasoned that an S_N2 displacement on the butane-3,4-diacetal (BDA)-protected triflate **50** (Fig. 7) was more likely to succeed because of the torsional strain imposed on formation of the unsaturated linkage at the ring-junction in the β-elimination reaction. This might be regarded as torsional suppression of the elimination process. Although the reactions outlined in Figure 7 refer to those carried out in the D-series, the practicability of the key displacement reaction (**50**→**51**) was established[28] with the enantiomeric BDA-protected α-L-rhamnopyranoside 2-triflate (details not included). The route to the latter compound from methyl α-L-rhamnopyranoside is somewhat shorter since the 6-deoxy group is already in place.

The BDA-protected α-D-rhamnopyranoside 2-triflate **50** (Fig. 7), readily obtained from the known[29] alcohol **49**, reacted with sodium azide in DMF at 75 °C to produce the crystalline 2-azide **51** in 57% yield (over the two steps) and a minor product (21%) tentatively assigned as the 2,3-unsaturated sugar **52**, based on the presence of a single olefinic proton (H-2) as a doublet ($J_{1,2}$ 2.7 Hz) at δ 5.05 in its ^{1}H NMR spectrum. The



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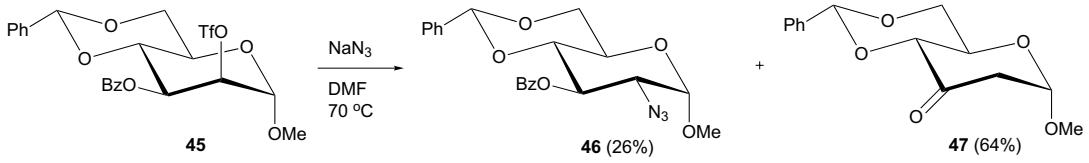

Figure 6. The products of an azide displacement on the α-D-mannopyranoside 2-triflate **45**.

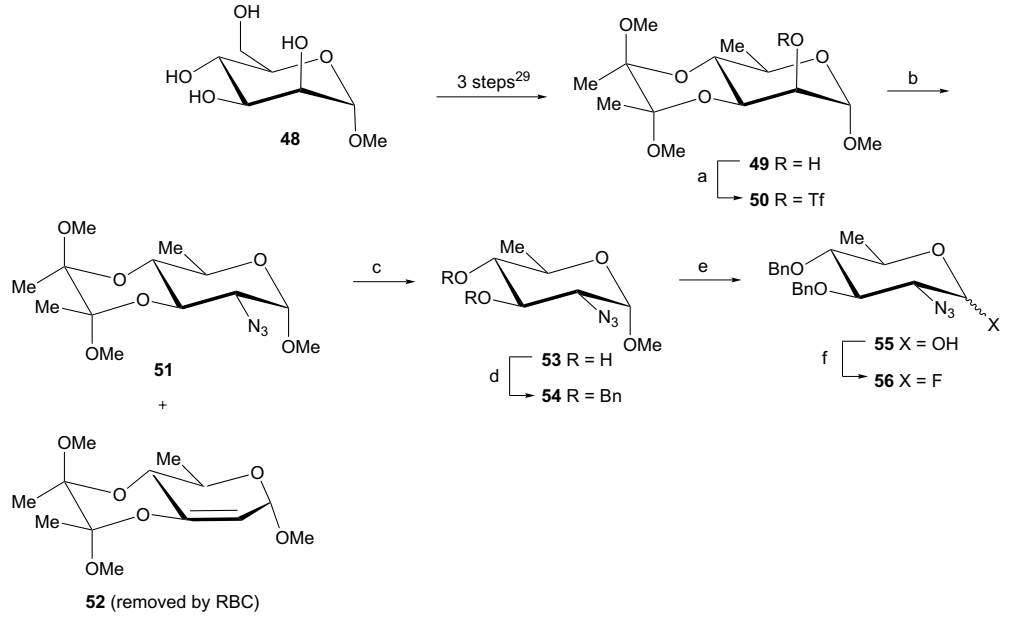

Figure 7. Synthesis of the 6-deoxyglycosyl fluoride **56**: (a) Tf_2O, py, CH_2Cl_2; (b) NaN_3, DMF, 75 °C; (c) TFA, H_2O; (d) BnBr, NaH, DMF; (e) 1 M HCl, 80% AcOH; (f) DAST, $ClCH_2CH_2Cl$.

unsaturated sugar **52** proved to be extremely unstable and decomposed on standing or in CD_3COCD_3 or $CDCl_3$ solution, thereby precluding a more detailed characterisation. Whilst elemental analyses and spectroscopic data served to identify the azido sugar **51**, an X-ray crystal structure of its L-enantiomer confirmed that no untoward changes had occurred during the displacement reaction.[28]

The remaining steps yielding the 6-deoxyglycosyl fluoride **56** were conducted without incident and entailed removal of the BDA protecting group (→**53**), benzylation of the liberated diol (→**54**), glycoside hydrolysis (→**55**) and treatment of the resulting hemiacetal with DAST (→**56**). Progression to α-D-6dGlc*p*N-PI (**60**) was equally incident-free, the α-coupled product **57** ($J_{1',2'}$ 3.7 Hz) initiating a standard series of transforma-

tions (**57→58→59→60**) to complete the synthesis (Fig. 8).

N-acetylated derivatives of the foregoing analogues (denoted, for example, as α-D-6dGlc*p*NAc-PI) required for biological studies with the N-deacetylase activities were prepared by existing procedures.[30]

The details of biological tests with the various deoxygenated analogues are published elsewhere.[31] These showed that only α-D-3dGlcpNAc-PI was *not* accepted as a substrate by the N-deacetylase activities in both trypanosomal (*T. brucei*) and HeLa (human) cell-free systems, unlike α-D-4dGlc*p*NAc-PI, α-D-6dGlc*p*NAc-PI and α-D-Glc*p*NAc4Me-PI. This indicates that neither the 4′- nor 6′-OH group is required for substrate recognition by the N-deacetylases, whereas the 3′-OH group is essential.

Only α-D-6dGlc*p*N-PI (**60**) was accepted as a substrate by both the trypanosomal and HeLa MT-1 activities, requiring prior inositol 2-acylation in situ in the latter instance. Since the 6′-OH group is not required for recognition by either the N-deacetylase or MT-1 activities, it raises the possibility of introducing fluorescent or other probes at this position for further in-depth studies of GPI-anchor biosynthesis. As already noted, α-D-3dGlc*p*N-PI (**28**) was not recognised by either of the MT-1 activities, once again demonstrating the essentialness of the 3′-OH group. Obviously neither α-D-4dGlc*p*N-PI (**43**) nor α-D-Glc*p*4Me-PI (**44**) can act as a substrate for the MT-1 activities because of the removal or blocking of the 4′-OH group required for D-mannosylation. On the other hand, these analogues inhibited both MT-1 activities in cell-free systems, with α-D-4dGlc*p*N-PI (**43**) exhibiting a more pronounced effect.

3. Experimental

3.1. General methods

^{1}H NMR and COSY spectra were routinely 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) and FAB mass spectra (FABS) with a VG 70-250 SE mass spectrometer using an Ion-tech xenon gun. IR spectra were recorded on a Nicolet 205 FT-IR spectrometer either as liquid films or Nujol mulls. Melting points were determined on a Reichert hot-plate apparatus and are uncorrected. TLC was performed on Kieselgel 60 F_{254} (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. Flash-column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck). Light

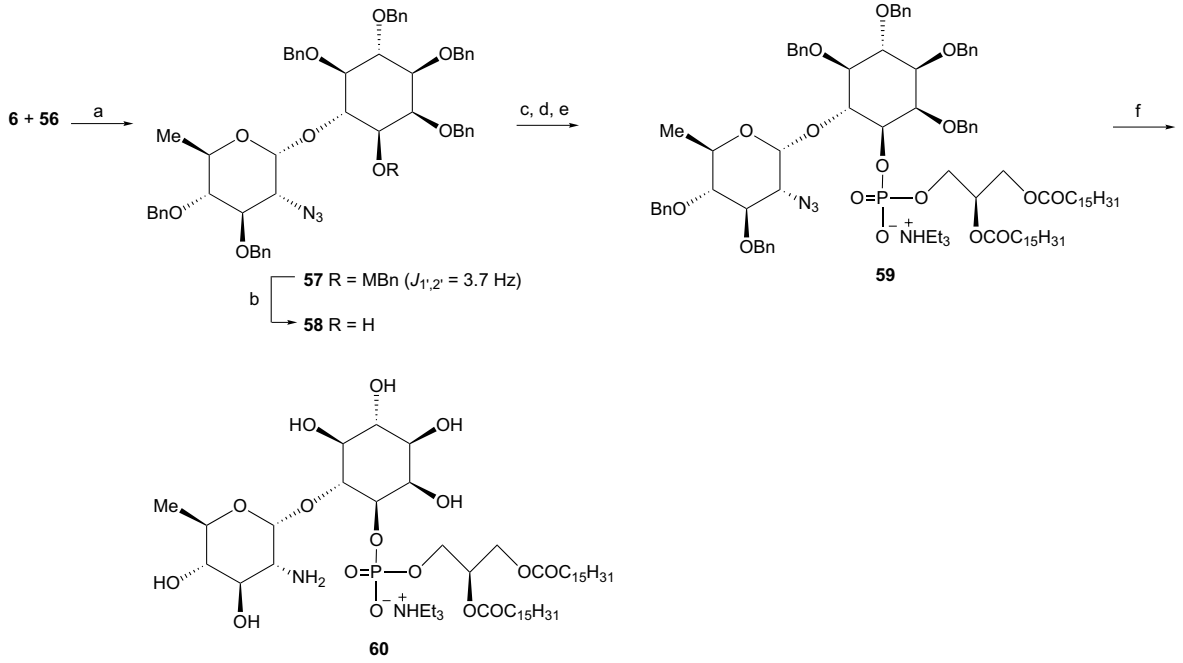

Figure 8. Synthesis of α-D-6dGlc*p*N-PI (**60**): see legend to Figure 3 for reagents (a)–(f).

petroleum refers to the fraction having a boiling point 60–80 °C. All anhydrous solvents were purchased from Aldrich Chemical Company Ltd.

3.2. Methyl 4,6-*O*-benzylidene-3-deoxy-2-*O*-*p*-toluenesulfonyl-β-D-*arabino*-hexopyranoside (11)

A solution of the alcohol **10**[12] (2.45 g, 9.2 mmol) in CH_2Cl_2 (100 mL) containing *p*-toluenesulfonyl chloride (2.62 g, 13.74 mmol), DMAP (0.11 g, 0.90 mmol) and pyridine (3.71 mL. 46.3 mmol) was heated under reflux overnight, whereafter it was cooled and poured into ice–water (200 mL), and the aqueous solution was extracted with CH_2Cl_2 (4×100 mL). The organic extracts were combined, washed with 1 M HCl (3×50 mL), satd $NaHCO_3$ solution (2×50 mL) and water (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the tosylate **11** (2.26 g, 58%); mp 198–199 °C (from hexane–EtOAc); $[\alpha]_D^{25}$ −42 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ ~7.50 (9H, C_6H_4, C_6H_5), 5.55 (s, 1H, PhC*H*), 4.78 (br s, 1H, H-2), 4.44 (s, 1H, H-1), 3.35 (s, 3H, OCH_3), 2.43 (s+m, 4H, CH_3Ar, H-3eq), 1.89 (t, 1H, $J_{gem} \approx J_{3ax,4}$ 12.0, $J_{2,3ax} < 1$ Hz, H-3ax); Anal. Calcd for $C_{21}H_{24}O_7S$: C, 60.0; H, 5.75; S, 7.6. Found: C, 60.1; H, 5.7; S, 7.5.

3.3. Methyl 2-azido-4,6-*O*-benzylidene-2,3-dideoxy-β-D-*ribo*-hexopyranoside (12)

A mixture of the tosylate **11** (0.12 g, 0.29 mmol), sodium azide (90 mg, 1.38 mmol) and 18-crown-6 (20 mg, 0.076 mmol) in DMF (5 mL) was stirred at 100 °C overnight and then the solvent was removed under reduced pressure (oil pump). A solution of the residue in CH_2Cl_2 (50 mL) was washed with water (10 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the azide **12** (70 mg, 84%); mp 112–114 °C (from hexane); $[\alpha]_D^{25}$ −51 (*c* 1.0, $CHCl_3$); *v* 2120 cm^{-1} (N_3); ^{1}H NMR: δ ~7.40 (5H, C_6H_5), 5.53 (s, 1H, PhC*H*), 4.33 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.60 (s, 3H, OCH_3), 4.35, 3.86–3.35 (5H, H-2, 4, 5, 6a,b), 2.37 (dt, 1H, $J_{2,3eq} = J_{3eq,4}$ 4.8 Hz, H-3eq), 1.65 (q, 1H, $J_{gem} \approx J_{2,3ax} \approx J_{3ax,4}$ 12.0 Hz, H-3ax); Anal. Calcd for $C_{14}H_{17}N_3O_4$: C, 57.7; H, 5.9; N, 14.4. Found: C, 57.75; H, 5.9; N, 14.2.

3.4. Methyl 2-azido-4,6-di-*O*-benzyl-2,3-dideoxy-β-D-*ribo*-hexopyranoside (14)

A solution of the azide **12** (1.28 g, 4.39 mmol) in 70% AcOH (13 mL) was heated at 80 °C for 30 min and then concentrated under reduced pressure with co-evaporation with toluene. The diol **13**, obtained in virtually quantitative yield, was taken on to the next stage without further purification.

To a cooled (0 °C) and stirred solution of the diol **13** (0.89 g, 4.38 mmol) in DMF (25 mL) was added NaH (0.42 g, 17.5 mmol). The mixture was allowed to warm to room temperature and stirring was continued for 30 min. Benzyl bromide (1.56 mL, 13.12 mmol) was then added dropwise and stirring of the mixture was continued overnight, whereupon MeOH was added to destroy the excess of NaH. The resulting mixture was partitioned between EtOAc (25 mL) and water (25 mL), and the aqueous phase was separated and extracted with EtOAc (4×25 mL). The organic extracts were combined, washed with brine (25 mL), dried ($MgSO_4$) and concentrated under reduced pressure. An ethereal solution of the residue was passed down a short silica-gel column (further elution with Et_2O) and the eluent was concentrated under reduced pressure. RBC (first with hexane and then with 16:1 hexane–EtOAc) of the residue gave the 4,6-dibenzylated compound **14** (1.1 g, 65%); mp 43–44 °C (without recrystallisation); $[\alpha]_D^{25}$ +4 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ ~7.10 (10H, 2×C_6H_5), 4.36, 4.25 (2×ABq, 4H, J_{AB} 12.0 Hz, 2×PhCH_2), 3.97 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 3.33 (s, 3H, OCH_3), 3.60–2.95 (5H, H-2, 4, 5, 6a,b), 2.19 (m, 1H, H-3eq), 1.17 (q, 1H, $J_{gem} \approx J_{2,3ax} \approx J_{3ax,4}$ 11.0 Hz, H-3ax); Anal. Calcd for $C_{21}H_{25}N_3O_4$: C, 65.8; H, 6.6; N, 11.0. Found: C, 65.6; H, 6.5; N, 10.9.

3.5. 2-Azido-4,6-di-*O*-benzyl-2,3-dideoxy-α-D-*ribo*-hexopyranose (15)

A mixture of the methyl β-glycoside **14** (100 mg, 0.26 mmol), 80% AcOH (3.2 mL) and 1 M HCl (1 mL) was heated at 95 °C for 20 h, whereafter the reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (50 mL) and satd $NaHCO_3$ solution (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure. RBC (5:1→1:1 light petroleum–Et_2O) of the residue and recrystallisation from hexane–Et_2O gave the α-hemiacetal **15** (30 mg, 31%); mp 96–100 °C; $[\alpha]_D^{25}$ +102 (*c* 0.96, $CHCl_3$); ^{1}H NMR: δ 7.54–7.12 (10H, 2×C_6H_5), 5.25 (t, 1H, $J_{1,OH} = J_{1,2}$ 3.2 Hz, H-1), 4.63–4.34 (4H, 2×CH_2Ph), 4.04 (m, 1H, H-5), 3.76–3.41 (4H, OH, H-4, 6a,b), 3.20 (m, 1H, H-2), 2.33, 2.05 (2×m, 2H, H-3eq, ax); Anal. Calcd for $C_{20}H_{23}N_3O_4$: C, 65.0; H, 6.3; N, 11.4. Found: C, 64.9; H, 6.3; N, 11.2.

Hydrolysis of the ethyl α-glycoside **23** (see later) by an identical procedure also provided the α-hemiacetal **15** in 29% yield.

3.6. 2-Azido-4,6-di-*O*-benzyl-2,3-dideoxy-D-*ribo*-hexopyranosyl fluoride (16)

To a cooled (−30 °C) and stirred solution of the hemiacetal **15** (0.104 g, 0.28 mmol) in anhyd 1,2-dichloroethane (10 mL) under argon was added DAST (0.15 mL, 1.14 mmol). The reaction mixture was allowed to attain room temperature and was then partitioned between water (40 mL) and $CHCl_3$ (20 mL). The aqueous phase

was separated and further extracted with $CHCl_3$ (25 mL), and the organic extracts were combined, washed with brine (45 mL), dried ($MgSO_4$) and concentrated under reduced pressure. An ethereal solution of the residue was percolated through a short silica-gel column (further elution with Et_2O) and the eluent was concentrated under reduced pressure. RBC (first with hexane and then with 8:1 hexane–Et_2O) gave the glycosyl fluoride **16** (81 mg, 77%) as a 1:2 mixture of the α- and β-anomers; ^{1}HNMR (α-anomer): δ 7.39–7.15 (10H, 2×C_6H_5), 5.59 (dd, 1H, $J_{1,2}$ 2.8, $J_{1,F}$ 53.6 Hz, H-1), 4.64–4.49 (4 H, 2×CH_2Ph), 4.43 (m, 1H, H-5), 3.77, 3.71 (2×m, 2H, >H-6a,b), 3.62 (m, 1H, H-4), 3.20 (m, 1H, H-2), 2.41, 1.97 (2×m, 2H, H-3eq, ax); β-anomer: δ 5.10 (dd, 1H, $J_{1,2}$ 7.0, $J_{1,F}$ 52.7 Hz, H-1); ESMS(+): m/z 394.2 $[M+Na]^+$.

3.7. Ethyl 2-azido-2,3-dideoxy-α-D-*ribo*-hexopyranoside (22)

To a stirred solution of the enone **18**[13] (1.3 g, 7.55 mmol) in glacial AcOH (10.4 mL) was added a solution of sodium azide (1.3 g, 20 mmol) in water (6.5 mL). Stirring of the reaction mixture was continued for 5 h before it was diluted with ice–water (10 mL) and extracted with $CHCl_3$ (4×15 mL). The organic extracts were combined, washed with satd $NaHCO_3$ solution (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give a ~1:2 mixture of the azido ketones **19** and **20** (1.3 g, 80%); ν 2100 (N_3), 1720 cm^{-1} (C=O); ^{1}H NMR: δ 5.00 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1) and 4.95 (s, 1H, H-1) for the azido ketones **20** and **19**, respectively.

To a stirred and cooled (0 °C) solution of the foregoing mixture of compounds **19** and **20** (1.26 g, 5.85 mmol) in MeOH (40 mL) was added $NaBH_4$ (1.26 g, 33.31 mmol) over a period of 30 min, whereafter stirring of the mixture was continued for 2 h. It was then neutralised with Amberlite IR-120 (H^+) ion-exchange resin, filtered and concentrated under reduced pressure. RBC (99:1→96:4 $CHCl_3$–MeOH) of the residue, with sacrificial cuts, furnished the *ribo*-diol **22** (0.23 g, 18%); $[\alpha]_D^{25}$ +126 (c 1.0, $CHCl_3$); ν 2100 cm^{-1} (N_3); ^{1}H NMR: δ 4.80 (d, 1H, H-1), 3.56–3.50 (6H, CH_2CH$_3$, H-4, 5, 6a,b), 3.10 (dt, 1H, $J_{1,2} = J_{2,3eq}$ 3.5 Hz, H-2), 2.90 (br s, 1H, OH), 2.16 (dt, 1H, $J_{2,3eq} = J_{3eq,4}$ 3.5 Hz, H-3eq), 2.03 (q, 1H, $J_{gem} \approx J_{2,3ax} \approx J_{3ax,4}$ 11.0 Hz, H-3ax), 1.26 (t, 3H, J 7.0 Hz, CH_2CH_3); Anal. Calcd for $C_8H_{15}N_3O_4$: C, 44.2; H, 7.0; N, 19.3. Found: C, 44.0; H, 7.2; N, 19.1. Also obtained was a small amount of the pure *lyxo*-isomer **21** (0.12 g, 9%).

The compound **22** was characterised by its transformation[14] into ethyl 2-acetamido-2,3-dideoxy-α-D-*ribo*-hexopyranoside; mp 184–187 °C (from EtOAc), lit.[14] mp 185–187 °C; $[\alpha]_D^{25}$ +158 (c 1.0, MeOH), lit.[14] $[\alpha]_D^{25}$ +149 (c 1.05, MeOH).

3.8. Ethyl 2-azido-4,6-di-*O*-benzyl-2,3-dideoxy-α-D-*ribo*-hexopyranoside (23)

To a stirred and cooled (0 °C) solution of the diol **22** (0.65 g, 2.99 mmol) in DMF (18 mL) was added NaH (0.29 g, 12.08 mmol). Stirring of the mixture was continued at rt for 30 min before benzyl bromide (1.06 mL, 8.91 mmol) was added dropwise. After stirring of the reaction mixture for a further 3 h, the excess of NaH was destroyed by the addition of cold MeOH. Water (20 mL) was then added and the aqueous solution was extracted with EtOAc (4×25 mL). The organic extracts were combined, washed with brine (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. An ethereal solution of the residue was percolated through a short silica-gel column (further elution with Et_2O) and the eluent was concentrated under reduced pressure. RBC (first with hexane and then with 32:1 hexane–EtOAc) of the residue gave the dibenzylated compound **23** (0.76 g, 64%); $[\alpha]_D^{25}$ +71 (c 1.0, $CHCl_3$); ν 2100 cm^{-1} (N_3); ^{1}H NMR: δ ~7.30 (10H, 2×C_6H_5), 4.88 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), ~4.57 (2×ABq, 4H, J_{AB} 11.3 Hz, 2×CH_2Ph), 3.12 (m, 1H, $J_{1,2} = J_{2,3eq}$ 3.2 Hz, H-2), 2.35, 2.07 (2×m, 2H, H-3eq, ax), 1.23 (t, 3H, J 7.0 Hz, CH_2CH_3); Anal. Calcd for $C_{22}H_{27}N_3O_4$: C, 66.5; H, 6.85; N, 10.6. Found: C, 66.5; H, 6.9; N, 10.5.

3.9. 1D-6-*O*-(2-Azido-4,6-di-*O*-benzyl-2,3-dideoxy-α-D-*ribo*-hexopyranosyl)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol (24)

To a stirred solution of the D-*myo*-inositol derivative **6**[6] (92 mg, 0.14 mmol) and the glycosyl fluoride **16** (70 mg, 0.19 mmol) in anhyd 1,4-dioxane (8 mL) under argon were added powdered 4 Å molecular sieves (0.5 g) and a mixture of zirconocene dichloride (0.225 g, 0.77 mmol) and pre-dried silver perchlorate (0.145 g, 0.7 mmol) in toluene (2 mL). 1,1,3,3-Tetramethylurea (23 μL, 0.19 mmol) was added after 5 min and stirring of the reaction mixture was continued in the dark for 22 h. It was then filtered and concentrated under reduced pressure. An ethereal solution of the residue was passed through a short silica-gel column (further elution with Et_2O) and concentrated under reduced pressure. RBC (first with hexane and then with 1:2 hexane–Et_2O) yielded a 3:1 mixture (50 mg, 35%) of the α-pseudodisaccharide **24** and the β-anomer; ^{1}H NMR (α-anomer): δ 7.35–6.69 (34H, C_6H_4, 6×C_6H_5), 5.63 (d, 1H, $J_{1',2'}$ 3.0 Hz, H-1′), 4.96–4.36 (14H, 7×CH_2Ar), 4.23 (t, 1H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 4.05 (t, 1H, $J_{1,6} = J_{5,6}$ 9.5 Hz, H-6), 3.95 (m, 1H, H-2), 3.88 (m, 1H, H-5′), 3.71 (s, 3H, OCH_3), 3.57 (m, 1H, H-4′), 3.37 (m, 1H, H-3), 3.32 (t, 1H, $J_{4,5} = J_{5,6}$ 9.5 Hz, H-5), 3.30 (m, 1H, H-1), 3.20, 3.09 (2×m, 2H, H-6′a,b), 2.95 (m, 1H, H-2′), 2.24, 1.98 (2×m, 2H, H-3′eq, ax); ESMS(+): m/z 1033.5 $[M+Na]^+$.

3.10. 1D-6-*O*-(2-Azido-4,6-di-*O*-benzyl-2,3-dideoxy-α-D-*ribo*-hexopyranosyl)-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol (25)

A 3:1 mixture of the α-pseudodisaccharide **24** and the β-anomer (50 mg, 0.05 mmol) in anhyd CH_2Cl_2 (10 mL) containing TFA (54 μL, 0.70 mmol) was set aside at rt for 1 h, whereafter it was neutralised with Et_3N and diluted with CH_2Cl_2 (40 mL). The organic solution was washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. An ethereal solution of the residue was percolated through a short silica-gel column (further elution with Et_2O) and the eluent was concentrated under reduced pressure. Flash-column chromatography (2 → 25% EtOAc in toluene) of the residue gave the pure α-pseudodisaccharide **25** (28.5 mg, 65%); $[\alpha]_D^{25}$ +54 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.37–6.99 (30H, 6×C_6H_5), 5.53 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 5.00–4.05 (12H, 6×CH_2Ph), 4.01 (t, 1H, $J_{1,6} = J_{5,6}$ 9.3 Hz, H-6), 3.92 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4), 3.90 (t, 1H, $J_{1,2} = J_{2,3}$ 2.1 Hz, H-2), 3.84 (m, 1H, H-5′), 3.57 (2H, H-1, 4′), 3.42 (dd, 1H, H-3), 3.33 (t, 1H, H-5), 3.29 (2×m, 2H, H-2′, 6′a), 3.04 (dd, 1H, $J_{5',6'b}$ 2.1, J_{gem} 10.7 Hz, H-6′b), 2.67 (d, 1H, $J_{1,OH}$ 8.0 Hz, OH), 2.24, 1.93 (2×m, 2H, H-3′eq, ax); ESMS(+): *m*/*z* 914.0 $[M+Na]^+$.

3.11. Triethylammonium 1D-6-*O*-(2-azido-4,6-di-*O*-benzyl-2,3-dideoxy-α-D-*ribo*-hexopyranosyl)-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (27)

A mixture of the compounds **7**[8] (104 mg, 0.14 mmol), **25** (55 mg, 0.061 mmol) and pivaloyl chloride (81 μL, 0.66 mmol) in anhyd pyridine (7 mL) was stirred at rt for 2 h. The diastereoisomeric phosphonic diesters **26** thereby formed in solution were oxidised by the addition of a freshly prepared solution of iodine (72 mg, 0.28 mmol) in 19:1 pyridine–water (9.6 mL). After 45 min, the reaction mixture was dispersed between $CHCl_3$ (30 mL) and 5% aq $NaHSO_3$ (30 mL). The aqueous phase was separated and extracted with $CHCl_3$ (25 mL), and the organic extracts were combined, washed with 1 M triethylammonium hydrogen carbonate (TEAB) buffer solution (3×10 mL), dried ($MgSO_4$) and concentrated under reduced pressure. RBC (elution first with $CHCl_3$ and then with 15:1 $CHCl_3$–MeOH) of the residue provided the TEA salt, which in $CHCl_3$ solution (20 mL) was washed again with 1 M TEAB buffer solution (3×10 mL), dried ($MgSO_4$) and concentrated to give finally the TEA phosphate salt **27** (75.8 mg, 76%); $[\alpha]_D^{25}$ +48 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.46–6.98 (30H, 6×C_6H_5), 5.76 (d, 1H, $J_{1',2'}$ 3.1 Hz, H-1′), 5.25 (m, 1H, H-2 glycerol), 5.03–4.24 (12H, 6×CH_2Ph), 4.75 (m, 1H, H-2), 4.39 (t, 1H, $J_{1,6} = J_{5,6}$ 9.3 Hz, H-6), 4.34 (m, 2H, 1- or 3-CH_2 glycerol), 4.28 (m, 1H, H-1), 4.16–4.02 (4H, H-4, 5′, 1- or 3-CH_2 glycerol), 3.65 (m, 1H, H-4′), 3.58 (dd, 1H, $J_{2,3}$ 2.4, $J_{3,4}$ 9.8 Hz, H-3), 3.49 (t, 1H, $J_{4,5} = J_{5,6}$ 9.3 Hz, H-5), ~3.35 (m, 2H, H-6′a,b), 2.97 (7H, H-2′, 3×CH_2CH$_3$), 2.33 (m, 1H, H-3′eq), 2.25 (m, 4H, 2×COCH_2CH$_2$), 2.09 (m, 1H, H-3′ax), 1.56 (m, 4H, 2×COCH$_2$CH_2), 1.25 (48H, 2×[CH$_2$]$_{12}$), 1.22 (t, 9H, *J* 7.4 Hz, 3×CH$_2$CH_3), 0.88 (t, 6H, *J* 6.8 Hz, 2×CH$_2$CH_3); ^{31}P NMR: δ −4.57 (with heteronuclear decoupling); ESMS(+): *m*/*z* 1544.4 $[M-Et_3NH+Na]^+$.

3.12. Triethylammonium 1D-6-*O*-(2-amino-2,3-dideoxy-α-D-*ribo*-hexopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (28)

A solution of the protected compound **27** (70 mg, 0.043 mmol) in 2:2:1 THF–propanol–water (5 mL) containing 20% $Pd(OH)_2$ on carbon (328 mg) was stirred under 3 atm of hydrogen for 6 h. It was then percolated through a short column of Chelex 100 on a bed of Celite (further elution with 2:2:1 THF–propanol–water) and the eluent was concentrated under reduced pressure to give α-D-3dGlc*p*N-PI (**28**) (16 mg, 35%); $[\alpha]_D^{25}$ +12 (*c* 1.0, 10:10:3 $CHCl_3$–MeOH–water); ^{1}H NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 5.42 (d, 1H, $J_{1',2'}$ 3.6 Hz, H-1′), 5.29–5.25 (m, 1H, 2-H glycerol), 4.43 (dd, 2H, 1- or 3-CH_2 glycerol), 4.23–4.18 (m, 2H, 1- or 3-CH_2 glycerol), 4.16 (dd, 1H, $J_{1,2}$ 2.6, $J_{1,6}$ 9.6 Hz, H-1), 4.09 (t, 1H, $J_{1,2} = J_{2,3}$ 2.6 Hz, H-2), 4.04–4.00 (m, 1H, H-5′), 3.97 (t, 1H, $J_{1,6} = J_{5,6}$ 9.6 Hz, H-6), 3.62 (dd, 1H, $J_{5',6'a}$ 2.2 J_{gem} 12.0 Hz, H-6′a), 3.72 (m, 1H, H-6′b), 3.69 (t, 1H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4), 3.65–3.57 (m, 1H, H-4′), 3.47 (dd, 1H, H-3), 3.41–3.37 (m, 1H, H-2′), 3.36 (dd, 1H, H-5), 2.32 (t, 4H, *J* 7.2 Hz, 2×COCH_2CH$_2$), 2.21–2.14, ~1.90 (2×m, 2H, H-3′eq, ax), 1.60 (m, 4H, 2×COCH$_2$CH_2), 1.27 (48H, 2×[CH$_2$]$_{12}$), 0.89 (t, 6H, *J* 7.1 Hz, 2×CH$_2$CH_3); ^{31}P NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ −0.98 (with heteronuclear decoupling); ESMS(−): *m*/*z* 954.3 $[M-Et_3N-H]^-$.

3.13. 1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(thiocarbonylimidazol-1-yl)-β-D-glucopyranose (33)

A solution of the monobenzylated derivative **32**[22] (0.2 g, 0.72 mmol) and 1,1-thiocarbonyldiimidazole (0.19 g, 1.07 mmol) in toluene (7 mL) was heated under reflux for 2.5 h, whereafter it was decanted from any solids and concentrated under reduced pressure. An ethereal solution of the residue was passed down a short silica-gel column (further elution with Et_2O) and the eluent was concentrated under reduced pressure. RBC (first with hexane and then with 9:1 hexane–EtOAc) of the residue gave the thiocarbonyl derivative **33** (0.22 g, 79%); mp 103–104 °C; $[\alpha]_D^{25}$ −23 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ ~7.30 (5H, C_6H_5), 8.39, 7.67, 7.06 (3H, imidazole), 5.60, 5.50 (2×br s, 2H, H-1, 4), 4.74 (s, 2H, CH_2Ph), 4.79, 4.39, 3.87 (3×m, 3H, H-5, 6a,b), 3.75 (br s, 1H, H-3), 3.45 (br

s, 1H, H-2); Anal. Calcd for $C_{17}H_{17}N_5O_4S$: C, 52.7; H, 4.4; N, 18.1; S, 8.3. Found: C, 52.7; H, 4.4; N, 18.0; S, 7.7.

3.14. 1,6-Anhydro-2-azido-3-*O*-benzyl-2,4-dideoxy-β-D-*xylo*-hexopyranose (34)

To a solution of the thiocarbonyl derivative **33** (0.84 g, 2.17 mmol) in refluxing benzene (60 mL) was added dropwise over 1 h a solution of Bu_3SnH (0.7 mL, 2.6 mmol) and AIBN (71 mg, 0.43 mmol) in benzene (20 mL). The reaction mixture was heated under reflux for a further 30 min and then concentrated under reduced pressure. An ethereal solution of the residue was passed down a short silica-gel column (further elution with Et_2O) and the eluent was concentrated under reduced pressure. RBC (first with hexane and then with 4:1 hexane–EtOAc) of the residue provided the 4-deoxy compound **34** (0.31 g, 55%); $[\alpha]_D^{25}$ +47 (*c* 1.0, $CHCl_3$); *v* 2100 cm^{-1} (N_3); ^1H NMR: δ ~7.30 (5H, C_6H_5), 5.41 (br s, 1H, H-1), ~4.48 (m+ABq, 3H, J_{AB} 12.0 Hz, H-5, CH_2Ph), 4.15 (d, 1H, H-6a), 3.65 (t, 1H, $J_{5,6b} = J_{gem}$ 6.6 Hz, H-6b), 3.60 (bd, 1H, H-3), 3.27 (br s, 1H, H-2), 2.17, 1.79 (m+bd, 2H, $J_{3,4ax} = J_{4ax,5}$ 4.9, J_{gem} 15.1 Hz, H-4ax, eq); Anal. Calcd for $C_{13}H_{15}N_3O_3$: C, 59.8; H, 5.8; N, 16.1. Found: C, 59.7; H, 5.7; N, 16.0.

3.15. 6-*O*-Acetyl-2-azido-3-*O*-benzyl-2,4-dideoxy-D-*xylo*-hexopyranose (36)

A solution of the compound **34** (0.64 g, 2.45 mmol) in acetic anhydride (10.8 mL) containing TFA (1.2 mL) was set aside at rt overnight, whereafter it was dispersed in satd $NaHCO_3$ solution (100 mL). Nitromethane (100 mL) was then added and the organic phase was separated, dried ($MgSO_4$) and concentrated under reduced pressure. A solution of the residue in $CHCl_3$ (50 mL) was washed with water (25 mL), dried ($MgSO_4$) and concentrated under reduced pressure. An ethereal solution of the residue was passed down a short silica-gel column (further elution with Et_2O) and the eluent was concentrated to give the diacetate **35** (0.66 g, 74%) as a ~3:1 mixture of the α- and β-anomers; ^1H NMR (α-anomer): δ ~7.35 (5H, C_6H_5), 6.25 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.65 (ABq, 2H, J_{AB} 11.3 Hz, CH_2Ph), ~4.10 (3H, H-5, 6a,b), 3.94 (m, 1H, H-3), 3.52 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2), ~2.20 (m, 1H, H-4eq), 2.14, 2.08 (2×s, 6H, 2×OAc), 1.55 (q, 1H, $J_{3,4ax} \approx J_{4ax,5} \approx J_{gem}$ 11.7 Hz, H-4-ax); β-anomer: δ 5.41 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1).

A solution of the diacetate **35** (0.457 g, 1.26 mmol) in acetonitrile (10 mL) containing 2 M Me_2NH in THF (4.25 mL, 8.50 mmol) was set aside at rt for 6 h and then concentrated under reduced pressure, with co-evaporation with acetonitrile, to furnish an α,β mixture of the hemiacetal **36** (0.327 g, 81%); *v* 2100 cm^{-1} (N_3); ^1H NMR (α-anomer): δ ~7.35 (5H, C_6H_5), 5.33 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 4.67 (ABq, 2H, J_{AB} 11.4 Hz, CH_2Ph), 4.30–3.95 (4H, H-3, 5, 6a,b), 3.37 (dd, 1H, $J_{2,3}$ 10 Hz, H-2), 2.09 (s, 3H, OAc), 2.15, 1.50 (2×m, 2H, H-4eq, ax); β-anomer: δ 4.50 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1); Anal. Calcd for $C_{15}H_{19}N_3O_5$: C, 56.1; H, 6.0; N, 13.1. Found: C, 56.1; H, 6.1; N, 12.6.

3.16. 6-*O*-Acetyl-2-azido-3-*O*-benzyl-2,4-dideoxy-D-*xylo*-hexopyranosyl fluoride (37)

The hemiacetal **36** (0.103 g, 0.32 mmol) in anhyd 1,2-dichloroethane (10 mL) under argon at −30 °C was treated with DAST (0.17 mL, 1.29 mmol) as previously described for the preparation of the glycosyl fluoride **16**. RBC (first with hexane and then with 2:1 hexane–Et_2O) gave the fluoride α-**37** (0.039 g, 38%); $[\alpha]_D^{25}$ +55 (*c* 2.2, $CHCl_3$); followed by the fluoride β-**37** (0.058 g, 56%); $[\alpha]_D^{25}$ −28 (*c* 3.3, $CHCl_3$); ^1H NMR (α-anomer): δ 5.65 (dd, 1H, $J_{1,2}$ 2.4, $J_{1,F}$ 52.4 Hz, H-1); β-anomer: δ ~7.38 (5H, C_6H_5), 4.98 (dd, 1H, $J_{1,2}$ 7.2, $J_{1,F}$ 52.1 Hz, H-1), 4.67 (2H, CH_2Ph), ~4.18 (2H, H-6a,b), 3.75 (m, 1H, H-5), 3.47 (2H, H-2, 3), 2.23 (dt, 1H, $J_{3,4eq} = J_{4eq,5}$ 2.0, J_{gem} 12.5 Hz, H-4eq), 2.10 (s, 3H, OAc), 1.58 (q, 1H, H-4ax). The α- and β-glycosyl fluorides were combined for the next step.

3.17. 1D-6-*O*-(6-*O*-Acetyl-2-azido-3-*O*-benzyl-2,4-dideoxy-α-D-*xylo*-hexopyranosyl)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol (38)

The coupling between the D-*myo*-inositol acceptor **6**[6] (0.129 g, 0.195 mmol) and an α,β-mixture of the glycosyl fluoride **37** (0.087 g, 0.27 mmol) was conducted essentially as described for the preparation of the pseudodisaccharide **24**. RBC (10:1→4:1 hexane–Et_2O) yielded the α-pseudodisaccharide **38** (0.065 g, 35%); mp 109–110 °C (from Et_2O–hexane); $[\alpha]_D^{25}$ +38 (*c* 6.5, $CHCl_3$); as well as a small proportion of the unrecovered β-anomer; ^1H NMR: δ 7.40–6.77 (29H, C_6H_4, 5×C_6H_5), 5.72 (d, 1H, $J_{1',2'}$ 3.6 Hz, H-1′), 5.08–4.44 (12H, 6×CH_2Ar), 4.24 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 4.12–4.05 (2H, H-5′,6), 4.01 (t, 1H, $J_{1,2} = J_{2,3}$ 2.1 Hz, H-2), 3.84–3.77 (s+m, 4H, OCH_3, H-3′), 3.69 (dd, 1H, $J_{5',6'a}$ 2.9, J_{gem} 11.9 Hz, H-6′a), 3.49 (dd, 1H, $J_{5',6'b}$ 4.3 Hz, H-6′b), 3.44 (dd, 1H, H-3), 3.42–3.34 (2H, H-1,5), 3.14 (dd, 1H, $J_{2',3'}$ 10.1 Hz, H-2′), 1.94 (s, 3H, OAc), 1.54 (dt, 1H, $J_{3',4'eq} = J_{4'eq,5'}$ 2.1, J_{gem} 12.2 Hz, H-4′eq), 1.33 (m, 1H, H-4′ax); Anal. Calcd for $C_{57}H_{61}N_3O_{11}$: C, 71.0; H, 6.4; N, 4.4. Found: C, 71.0; H, 6.4; N, 4.3.

3.18. 1D-6-*O*-(2-Azido-3,6-di-*O*-benzyl-2,4-dideoxy-α-D-*xylo*-hexopyranosyl)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol (40)

A solution of the acetylated compound **38** (0.145 g, 0.15 mmol) in 5:4 THF–MeOH (18 mL) containing 0.5 M sodium methoxide in MeOH (2.5 mL, 1.25 mmol)

was kept at rt for 1.5 h and then neutralised with Amberlite IR-120 (H^+) ion-exchange resin. Filtration and concentration of the filtrate under reduced pressure gave the alcohol **39** (0.135 g, 97%), which was used in the next step without further purification.

A cooled (0 °C) mixture of the alcohol **39** (0.13 g, 0.14 mmol) and NaH (0.02 g, 0.83 mmol) in DMF (5 mL) was stirred for 30 min, whereafter benzyl bromide (76 μL, 0.64 mmol) was added dropwise. Stirring of the reaction mixture for 2 h, followed by a conventional work-up and RBC (first with hexane and then with 2:1 hexane–Et_2O) gave the etherified derivative **40** (0.096 g, 67%); $[\alpha]_D^{25}$ +44 (*c* 1.1, $CHCl_3$); ^{1}H NMR: δ 7.42–6.79 (34H, C_6H_4, 6×C_6H_5), 5.78 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.35–4.41 (14H, 7×CH_2Ar), 4.28 (t, 1H, $J_{3,4} = J_{4,5}$ 10.6 Hz, H-4), 4.12–4.04 (2H, H-5′, 6), 4.00 (m, 1H, H-2), 3.83 (m, 1H, $J_{2',3'} = J_{3'4'ax}$ 10.1, $J_{3'4'eq}$ 2.7 Hz, H-3′), 3.51 (s, 3H, OCH_3), 3.45 (dd, 1H, H-3), 3.42–3.34 (2H, H-1, 5), 3.18 (dd, 1H, $J_{5'6'a}$ 3.0, J_{gem} 10.8 Hz, H-6′a), 3.14 (dd, 1H, H-2′), 2.62 (dd, 1H, $J_{5',6'b}$ 3.9 Hz, H-6′b), 1.65 (dt, 1H, $J_{3',4'eq} = J_{4'eq,5'}$ 2.7, J_{gem} 12.0 Hz, H-4′eq), 1.52 (m, 1H, H-4′ax); ESMS(+): *m*/*z* 1034.2 $[M+Na]^+$.

3.19. 1D-6-*O*-(2-Azido-3,6-di-*O*-benzyl-2,4-dideoxy-α-D-*xylo*-hexopyranosyl)-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol (41)

A solution of the methoxybenzyl compound **40** (0.096 g, 0.095 mmol) in anhyd CH_2Cl_2 (10 mL) containing TFA (0.1 mL, 1.3 mmol) was set aside at rt for 1.5 h and then neutralised with Et_3N. Work-up as described for the isomeric compound **25** and RBC (elution first with hexane and then with 2:1 hexane–Et_2O) gave the demethoxybenzylated derivative **41** (0.071 g, 84%); $[\alpha]_D^{25}$ +29 (*c* 0.85, $CHCl_3$); ^{1}H NMR: δ 7.52–7.10 (30H, 6×C_6H_5), 5.49 (d, 1H, $J_{1'2'}$ 2.6 Hz, H-1′), 5.03–4.16 (12H, 6×CH_2Ph), 4.08 (t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 4.05–3.96 (3H, H-2, 5′, 6), 3.89 (m, 1H, H-3′), 3.60 (m, 1H, $J_{1,6}$ 9.6 Hz, H-1), 3.47–3.35 (3H, H-2′, 3, 5), 3.11 (dd, 1H, $J_{5',6'a}$ 3.7, J_{gem} 10.1 Hz, H-6′a), 2.96 (dd, 1H, $J_{5'6'b}$ 3.7 Hz, H-6′b), 1.89, 1.52 (2×m, 2H, J_{gem} 12.0 Hz, H-4′eq, ax); ESMS(+): *m*/*z* 914.3 $[M+Na]^+$.

3.20. Triethylammonium 1D-6-*O*-(2-azido-3,6-di-*O*-benzyl-2,4-dideoxy-α-D-*xylo*-hexopyranosyl)-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (42)

This phosphoric diester was obtained from the pseudodisaccharide derivative **41** (0.07 g, 0.078 mmol) and the H-phosphonate **7**[8] (0.115 g, 0.16 mmol) essentially as described for the preparation of the isomeric diester **27**. RBC (elution first with $CHCl_3$ and then with 20:1 $CHCl_3$–MeOH) and washing as before with 1 M TEAB buffer solution gave the TEA phosphate salt **42** (0.102 g, 80%); $[\alpha]_D^{25}$ +35 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ ~7.17 (30H, 6×C_6H_5), 5.69 (br s, 1H, H-1′), 5.19 (m, 1H, H-2 glycerol), 4.95–4.42 (12H, 6×CH_2Ph), 4.64 (1H, H-2), 4.35–4.19 (4H, H-1, 6, 1- or 3-CH_2 glycerol), 4.15 (m, 1H, H-5′), 4.04 (m, 2H, 1- or 3-CH_2 glycerol), 3.99 (t, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 3.77 (m, 1H, $J_{2',3'} = J_{3',4'ax}$ 10.0 Hz, H-3′), 3.47 (m, 1H, H-3), 3.35 (t, 1H, $J_{4,5} = J_{5,6}$ 9.7 Hz, H-5), 3.16 (m, 1H, J_{gem} 10.7 Hz, H-6′a), 2.99 (m, 1H, H-2′), 2.86 (7H, H-6′b, 3×CH_2CH_3), 2.18 (m, 4H, 2×COCH_2CH_2), 1.59, 1.35 (2×m, 2H, J_{gem} 11.9 Hz, H-4′eq, ax), 1.48 (m, 4H, 2×COCH$_2$CH_2), ~1.19 (48H, 2×$[CH_2]_{12}$), ~1.06 (9H, 3×CH_2CH_3), 0.82 (m, 6H, 2×CH_2CH_3); ^{31}P NMR: δ −3.59 (with heteronuclear decoupling); ESMS(−): *m*/*z* 1520.1 $[M-Et_3N-H]^-$.

3.21. Triethylammonium 1D-6-*O*-(2-amino-2,4-dideoxy-α-D-*xylo*-hexopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (43)

A solution of the benzylated compound **42** (38.7 mg, 0.018 mmol) in 1:1 THF–MeOH (6 mL) containing 20% $Pd(OH)_2$ on carbon (206 mg) was stirred under 3 atm of hydrogen for 4 h. Work-up as described for the isomeric compound **28** gave α-D-4dGlc*p*N-PI (**43**) (20.1 mg, 80%); $[\alpha]_D^{25}$ +29 (*c* 0.18, 10:10:3 $CHCl_3$–MeOH–water); ^{1}H NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 5.55 (d, 1H, $J_{1'2'}$ 2.7 Hz, H-1′), 5.28 (m, 1H, H-2 glycerol), 4.43 (m, 2H, 1- or 3-CH_2 glycerol), 4.32 (m, 1H, H-5′), 4.25–4.22 (m, 2H, 1- or 3-CH_2 glycerol), 4.15 (dd, 1H, $J_{1,2}$ 2.7, $J_{1,6}$ 9.5 Hz, H-1), 4.09 (t, 1H, H-2), 4.08–4.00 (m, 1H, H-3′), 3.93 (t, 1H, H-6), 3.69–3.65 (m, 1H, H-4), 3.64–3.58 (m, 2H, H-6′a,b), 3.46 (dd, 1H, $J_{2,3}$ 2.7, $J_{3,4}$ 9.9 Hz, H-3), 3.35–3.31, 3.06 (2H, H-5,2′), ~2.83 (6H, 3×CH_2CH_3), 2.37 (t, 4H, 2×COCH_2CH_2), 2.06–1.98, 1.56 (2×m, J_{gem} 11.1 Hz, H-4′eq, ax), 1.61 (m, 4H, 2×COCH$_2$CH_2), 1.40–1.20 (57H, 2×$[CH_2]_{12}$, 3×CH_2CH_3), 0.94–0.63 (6H, 2×CH_2CH_3); ^{31}P NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 1.49 (with heteronuclear decoupling); ESMS(−): *m*/*z* 954.5 $[M-Et_3N-H]^-$.

3.22. (2′*S*,3′*S*)-Methyl 2-azido-2,6-dideoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-α-D-glucopyranoside (51)

To a cooled (−40 °C) and stirred solution of the BDA derivative **49**[29] (0.55 g, 1.88 mmol) and pyridine (0.73 mL) in CH_2Cl_2 (40 mL) was added Tf_2O (0.4 mL, 2.38 mmol). The temperature of the mixture was then allowed to rise to 10 °C over 2 h, whereafter it was poured into cold satd $NaHCO_3$ solution (20 mL) and the resulting dispersion was stirred for 10 min. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The organic extracts were combined, washed with cold 2 M HCl (10 mL), cold satd $NaHCO_3$ solution (10 mL) and water (10 mL),

dried ($MgSO_4$) and concentrated under reduced pressure to give the crude 2-triflate **50** (0.82 g), which was used in the next experiment without further purification.

A mixture of the 2-triflate **50** (0.82 g) and sodium azide (0.68 g, 10.46 mmol) in DMF (25 mL) was heated at 75 °C overnight and then concentrated under reduced pressure (oil pump). A solution of the residue in CH_2Cl_2 (50 mL) was washed with water (10 mL) and the aqueous washing was further extracted with CH_2Cl_2 (3×20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. An ethereal solution of the residue was passed down a short silica-gel column (further elution with Et_2O) and the eluent was concentrated under reduced pressure. RBC (19:1 toluene–EtOAc) gave the 2-azide **51** (0.34 g, 57% over the two steps); mp 89 °C (from MeOH); $[\alpha]_D^{25}$ +298 (*c* 1.0, $CHCl_3$); *v* 2100 cm^{-1} (N_3); ^1H NMR: δ 4.66 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.14 (t, 1H, $J_{2,3} = J_{3,4}$ 10.0 Hz, H-3), 3.78 (m, 1H, H-5), 3.33 (dd, 1H, H-2), 3.28 (t, 1H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 3.35, 3.30, 3.21 (3×s, 9H, 3×OCH_3), 1.29, 1.26 (2×s, 6H, 2′, 3′-CH_3), 1.20 (d, 3H, $J_{5,6}$ 6.2 Hz, 5-CH_3); Anal. Calcd for $C_{13}H_{23}N_3O_6$: C, 49.2; H, 7.3; N, 13.2. Found: C, 49.3; H, 7.6; N, 13.2. Also isolated was the 2,3-unsaturated compound **52** (0.11 g, 21% over the two steps); ^1H NMR (CD_3COCD_3): δ 5.05 (d, 1H, $J_{1,2}$ 2.7 Hz, H-2), 4.89 (d, 1H, H-1), 3.90 (m, 1H, H-5), 3.70 (d, 1H, $J_{4,5}$ 9.0 Hz, H-4), 3.37, 3.29, 3.27 (3×s, 9H, 3×OCH_3), 1.33, 1.26 (2×s, 6H, 2′,3′-CH_3), 1.23 (d, 3H, $J_{5,6}$ 5.2 Hz, 5-CH_3).

3.23. Methyl 2-azido-3,4-di-*O*-benzyl-2,6-dideoxy-α-D-glucopyranoside (54)

A solution of the BDA-protected 2-azide **51** (1.31 g, 4.13 mmol) in 9:1 TFA–water (26 mL) was stirred at rt for 2 min and was then concentrated under reduced pressure to give the diol **53** (0.79 g), which was used in the next experiment without further purification.

To a stirred and cooled (0 °C) solution of the diol **53** (0.79 g, 3.9 mmol) in DMF (40 mL) was added NaH (0.37 g, 15.4 mmol) and stirring of the mixture was continued at rt for 30 min, whereafter benzyl bromide (1.4 mL, 11.8 mmol) was added dropwise. Work-up as described for the preparation of compound **14** and RBC (first with hexane and then with 19:1 hexane–EtOAc) gave the 3,4-dibenzylated compound **54** (1.2 g, 76% over the two steps); mp 77–78 °C (from aqueous ethanol); $[\alpha]_D^{25}$ +63 (*c* 1.0, $CHCl_3$); *v* 2100 cm^{-1} (N_3); ^1H NMR: δ ~7.30 (10H, 2×C_6H_5), 4.72 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 4.89–4.63 (s+ABq, 4H, J_{AB} 11.0 Hz, 2×CH_2Ph), 3.93 (t, 1H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3), 3.77 (m, 1H, H-5), 3.40 (dd+s, 4H, H-2, OCH_3), 3.19 (t, 1H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4), 1.27 (d, 3H, $J_{5,6}$ 6.2 Hz, 5-CH_3); Anal. Calcd for $C_{21}H_{25}N_3O_4$: C, 65.8; H, 6.6; N, 11.0. Found: C, 65.5; H, 6.5; N, 10.8.

3.24. 2-Azido-3,4-di-*O*-benzyl-2,6-dideoxy-D-glucopyranose (55)

A mixture of the glycoside **54** (0.2 g, 0.52 mmol), 80% AcOH (3.2 mL) and 1 M HCl (1 mL) was heated at 90–100 °C for 48 h, whereafter the resulting solution was concentrated under reduced pressure. The residue was dissolved in EtOAc and the solution was washed with satd $NaHCO_3$ solution (3×5 mL) and water (10 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the hemiacetal **55** (80 mg, 42%); mp 81–86 °C (from EtOAc–hexane); *v* 2100 cm^{-1} (N_3); as a mixture of anomers; ^1H NMR: δ 5.23 (br s, H-1 α-anomer), 4.55 (d, $J_{1,2}$ 8.9 Hz, H-1 β-anomer); Anal. Calcd for $C_{20}H_{23}N_3O_4$: C, 65.0; H, 6.3; N, 11.4. Found: C, 64.9; H, 6.2; N, 11.2.

3.25. 2-Azido-3,4-di-*O*-benzyl-2,6-dideoxy-D-glucopyranosyl fluoride (56)

To a cooled (−30 °C) and stirred solution of the hemiacetal **55** (0.107 g, 0.29 mmol) in anhyd 1,2-dichloroethane (10 mL) under argon was added DAST (0.16 mL, 1.21 mmol), whereafter work-up and RBC as described for the isomeric compound **16** furnished the glycosyl fluoride **56** (86.9 mg, 81%) as a 1:3 mixture of the α- and β-anomers; ^1H NMR: δ 5.46 (dd, $J_{1,2}$ 2.6, $J_{1,F}$ 52.6 Hz, H-1 α-anomer), 4.91 (dd, $J_{1,2}$ 7.3, $J_{1,F}$ 52.6 Hz, H-1 β-anomer); FABMS (+): *m*/*z* 394.0 $[M+Na]^+$.

3.26. 1D-6-*O*-(2-Azido-3,4-di-*O*-benzyl-2,6-dideoxy-α-D-glucopyranosyl)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol (57)

The coupling between the D-*myo*-inositol acceptor **6**[6] (100 mg, 0.15 mmol) and the glycosyl fluoride **56** (79 mg, 0.21 mmol) was conducted essentially as described for the preparation of the pseudodisaccharide **24**. RBC (first with hexane and then with 4:1 hexane–Et_2O) gave the α-pseudodisaccharide **57** (76.9 mg, 50%); $[\alpha]_D^{25}$ +37 (*c* 2.4, $CHCl_3$); as well as a small proportion of the unrecovered β-anomer; ^1H NMR: δ 7.40–6.81 (34H, C_6H_4, 6×C_6H_5), 5.67 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1′), 5.02–4.46 (14H, 7×CH_2Ar), 4.27 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4), 4.14 (t, 1H, $J_{1,6} = J_{5,6}$ 9.3 Hz, H-6), 4.09 (m, 1H, H-5′), 4.01 (dd, 1H, $J_{1,2}$ 2.1, $J_{2,3}$ 3.6 Hz, H-2), 3.94 (t, 1H, $J_{2',3'} = J_{3',4'}$ 9.6 Hz, H-3′), 3.78 (s, 3H, OCH_3), 3.47 (dd, 1H, H-3), 3.45 (t, 1H, H-5), 3.39 (dd, 1H, H-1), 3.20 (dd, 1H, H-2′), 3.08 (t, 1H, $J_{3',4'} = J_{4',5'}$ 9.6 Hz, H-4′), 0.92 (d, 3H, $J_{5',6'}$ 6.2 Hz, 5′-CH_3); FABMS(+): *m*/*z* 1034.7 $[M+Na]^+$.

3.27. Triethylammonium 1D-6-*O*-(2-azido-3,4-di-*O*-benzyl-2,6-dideoxy-α-D-glucopyranosyl)-2,3,4,5-tetra-*O*-benzyl-*myo*-*inositol* 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (59)

Demethoxybenzylation of the α-pseudodisaccharide derivative **57** (69 mg, 0.068 mmol) essentially as described for the preparation of the isomeric compound **25** gave, after RBC (elution first with hexane and then with 2:1 hexane–Et_2O), the alcohol **58** (57 mg, 94%); $[\alpha]_D^{25}$ +31 (*c* 0.4, $CHCl_3$); ESMS(+): *m*/*z* 914.1 $[M+Na]^+$.

The phosphoric diester **59** was obtained from the pseudodisaccharide derivative **58** (42 mg, 0.047 mmol) and the H-phosphonate **7**[8] (94 mg, 0.128 mmol) essentially as described for the preparation of the isomeric diester **27**. RBC (elution first with $CHCl_3$ and then with 15:1 $CHCl_3$–MeOH) and washing as before with 1 M TEAB buffer solution gave the TEA phosphate salt **59** (63 mg, 82%); $[\alpha]_D^{25}$ +57 (*c* 0.7, $CHCl_3$); ^{1}H NMR: δ 7.40–6.97 (30H, 6×C_6H_5), 5.61 (d, 1H, $J_{1',2'}$ 3.1 Hz, H-1′), 5.18 (m, 1H, H-2 glycerol), 4.94–4.46 (12H, 6×CH_2Ph), 4.58 (m, 1H, H-2), 4.32–4.19 (4H, H-1, 6, 1- or 3-CH_2 glycerol), 4.09–4.03 (3H, H-5′, 1- or 3-CH_2 glycerol), 4.02 (t, 1H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 3.90 (dd, 1H, $J_{2',3'}$ 10.2, $J_{3',4'}$ 9.2 Hz, H-3′), 3.50 (m, 1H, H-3), 3.42–3.38 (m, 1H, H-5), 3.13 (dd, 1H, H-2′), 3.02 (t, 1H, $J_{3',4'} = J_{4',5'}$ 9.2 Hz, H-4′), 2.89 (6H, 3×C$H_2$$CH_3$), 2.18 (4H, 2×COC$H_2$$CH_2$), 1.46 (m, 4H, 2×$COCH_2CH_2$), ~1.17 (57H, 2×$[CH_2]_{12}$, 3×$CH_2CH_3$), 0.95 (d, 3H, $J_{5',6'}$ 6.0 Hz, 5′-CH_3), 0.80 (t, 6H, *J* 7.0 Hz, 2×CH_2CH_3); ^{31}P NMR: δ −6.06 (with heteronuclear decoupling); ESMS(−): *m*/*z* 1520.4 $[M-Et_3N-H]^-$.

3.28. Triethylammonium 1D-6-*O*-(2-amino-2,6-dideoxy-α-D-glucopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (60)

A solution of the benzylated compound **59** (85 mg, 0.052 mmol) in 2:2:1 THF–propanol–water (5 mL) containing 20% $Pd(OH)_2$ on carbon (240 mg) was stirred under 3 atm of hydrogen for 5 h. Work-up as described for the isomeric compound **28** gave α-D-6dGlc*p*N-PI (**60**) (53.4 mg, 96%); $[\alpha]_D^{25}$ +17 (*c* 0.4, 10:10:3 $CHCl_3$–MeOH–water); ^{1}H NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 5.37 (d, 1H, $J_{1'2'}$ 4.1 Hz, H-1′), 5.15 (m, 1H, H-2 glycerol), 4.30, 4.11 (2×m, 4H, 1- and 3-CH_2 glycerol), 4.06–4.00 (2H, H-1, 5′), 3.97 (t, 1H, $J_{1,2} = J_{2,3}$ 2.5 Hz, H-2), 3.82 (t, 1H, $J_{1,6} = J_{5,6}$ 9.4 Hz, H-6), 3.68 (t, 1H, $J_{2',3'} = J_{3',4'}$ 9.8 Hz, H-3′), 3.56, 3.36 (2 H, H-4, 3), 3.25 (t, 1H, $J_{4,5} = J_{5,6}$ 9.4 Hz, H-5), 3.07 (7H, H-2′, 3×C$H_2$$CH_3$), 2.98 (t, 1H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4′), 2.22 (4H, 2×COC$H_2$$CH_2$), 1.49 (m, 4H, 2×$COCH_2CH_2$), 1.35–1.09 (60H, 2×$[CH_2]_{12}$, 3×$CH_2CH_3$, 5′-$CH_3$), 0.79 (6H, 2×$CH_2CH_3$); ^{31}P NMR (10:10:3 $CDCl_3$–CD_3OD–D_2O): δ 1.45 (with heteronuclear decoupling); ESMS(−): *m*/*z* 954.1 $[M-Et_3N-H]^-$.

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