Parasite glycoconjugates. Part 8.[1] Chemical synthesis of a heptaglycosyl triphosphate fragment of *Leishmania mexicana* lipo- and proteo-phosphoglycan and of a phosphorylated trisaccharide fragment of *Leishmania donovani* surface lipophosphoglycan

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The phosphorylated branched heptasaccharide β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-[β-D-Glc*p*-(1→3)]-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O$[CH_2]_8CH{=}CH_2$, which is a fragment of the phosphoglycan portion of *Leishmania mexicana* lipophosphoglycan and proteophosphoglycan, has been synthesized using the thioglycoside and Helferich methods for the glycosylations and the glycosyl hydrogenphosphonate method for the successive introduction of the disaccharide phosphate and trisaccharide phosphate blocks.

Introduction

The *Leishmania* are sandfly-transmitted protozoan parasites that cause a variety of debilitating and often fatal diseases throughout the tropics and the sub-tropics. All life-cycle stages of all species of the *Leishmania* synthesize large amounts of glycoconjugate virulence-factors that contain phosphosaccharide repeating units of [6-(R→3)-β-D-Gal*p*-(1→4)-(R′→2)-α-D-Man*p*-(1-PO_3H-]$_n$. These glycoconjugates include the most abundant surface molecule of the infectious metacyclic promastigote stage of the parasite, the lipophosphoglycan (LPG)[2–4] and secreted proteophosphoglycans (PPG) such as acid phosphatase[5] and the amastigote filamentous mucin-like PPG.[6] The nature of the R and R′ groups varies according to the species of *Leishmania*. For example, in *L. donovani*[2] R = R′ = H, whereas in *L. Major*[2,6] R′ = H and R is mostly mono-, di- or tri-saccharide made up of β-D-Gal*p* and β-D-Ara*p* residues. In *L. aethiopica*[3] R is mostly β-D-Gal*p* or β-D-Gal*p*-(1→3)-β-D-Gal*p*, but R′ is α-D-Man*p* (35%) or H (65%). In the LPG and PPG produced by *L. mexicana*[4,5] R′ is H (100%) and about 20–25% of the D-galactose residues are substituted at O-3 with β-D-glucopyranose.

We have recently described chemical syntheses of oligosaccharide fragments (including compounds **1**–**4**) of the LPG of *L. donovani*[7,8] and *L. major*[9] and the polymeric phosphoglycan chain of *L. donovani* LPG.[10] Compounds **1**–**4** were tested *in vitro* as acceptor substrates for the *Leishmania* α-D-mannopyranosyl phosphate transferase (MPT) responsible for the transfer of α-D-Man*p* phosphate from GDP-Man to the growing phosphoglycan chain of the LPG. It has been shown[11] that the phosphorylated oligosaccharides **1**, **3** and **4** are efficient exogenous acceptor substrates for the MPT and that the non-phosphorylated disaccharide **2** is inactive.

We now report the chemical synthesis of the branched heptaglycosyl triphosphate fragment **5** of the phosphoglycan portion of *L. mexicana* LPG and PPG and the linear trisaccharide phosphate fragment **6** of the phosphoglycan chain of *L. donovani* LPG. Both compounds contain a dec-9-enyl moiety that assists biochemical assays, and are designed to be used for further studies of *Leishmania* biosynthetic enzymes.

β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-OR

1

H-[6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-]$_n$-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-OR

2 $n = 0$ **3** $n = 1$ **4** $n = 2$

β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-
↑3
|1
β-D-Glc*p*

α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-OR

5

α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-OR

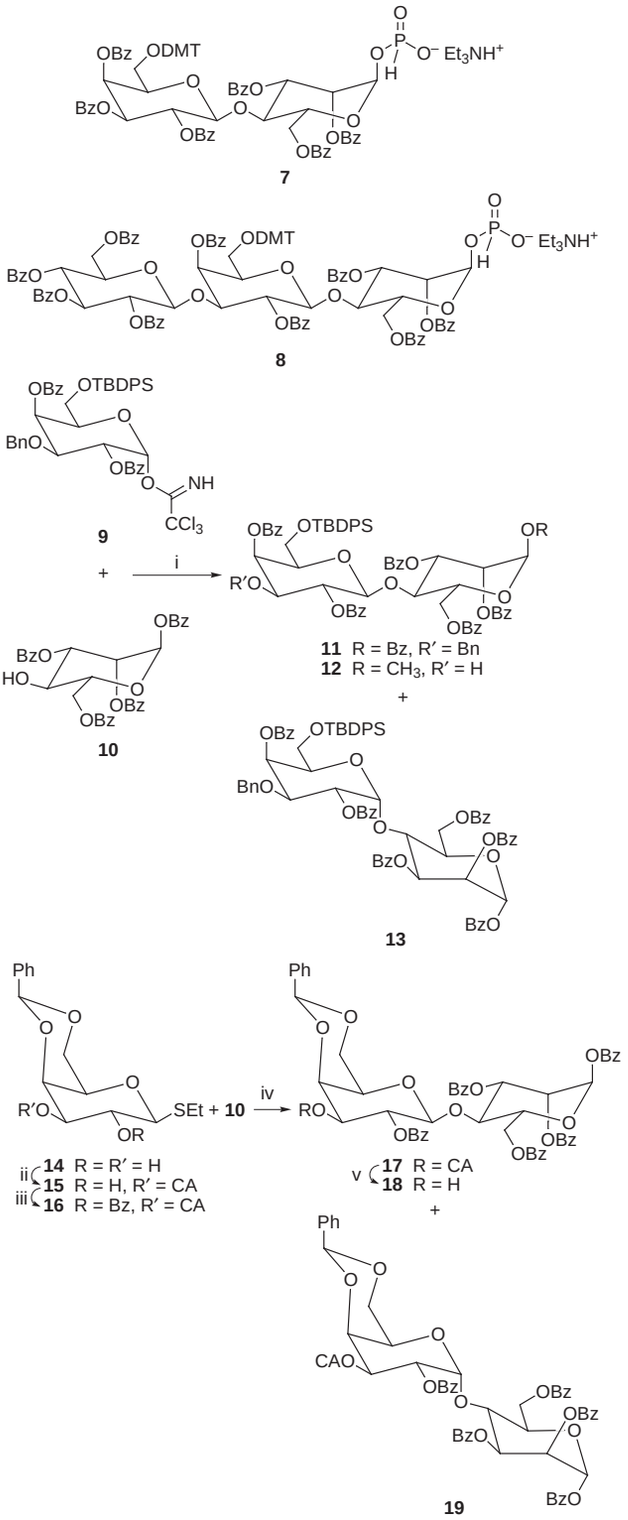
6 R = $[CH_2]_8CH{=}CH_2$

Results and discussion

A retrosynthetic analysis of the heptaglycosyl triphosphate **5** shows that it can be prepared by stepwise chain elongation from dec-9-en-1-ol using the disaccharide H-phosphonate **7** and trisaccharide H-phosphonate **8** (Scheme 1) for the consecutive introduction of the glycobiosyl and glycotriosyl phosphate fragments. The glycosyl hydrogenphosphonate method[7,12] can be used for the construction of the phosphodiester linkages.

The disaccharide H-phosphonate **7** has been described by us recently.[7] In the context of preparation of the trisaccharide H-phosphonate **8**, two galactosylmannose derivatives **11** and **17** (Scheme 1) were synthesized. They differ by the temporary protection on O-3′ (the position to be glycosylated) and O-6′ (the position to be phosphorylated).

Glycosylation of 1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose[7] **10** with 2,4-di-*O*-benzoyl-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-galactopyranosyl trichloroacetimidate[9] **9** in the presence of triethylsilyl triflate gave the β-(1→4)-linked disaccharide **11** (55%) together with some (14%) of the α-linked isomer **13**. The disaccharide **17** was prepared from ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-

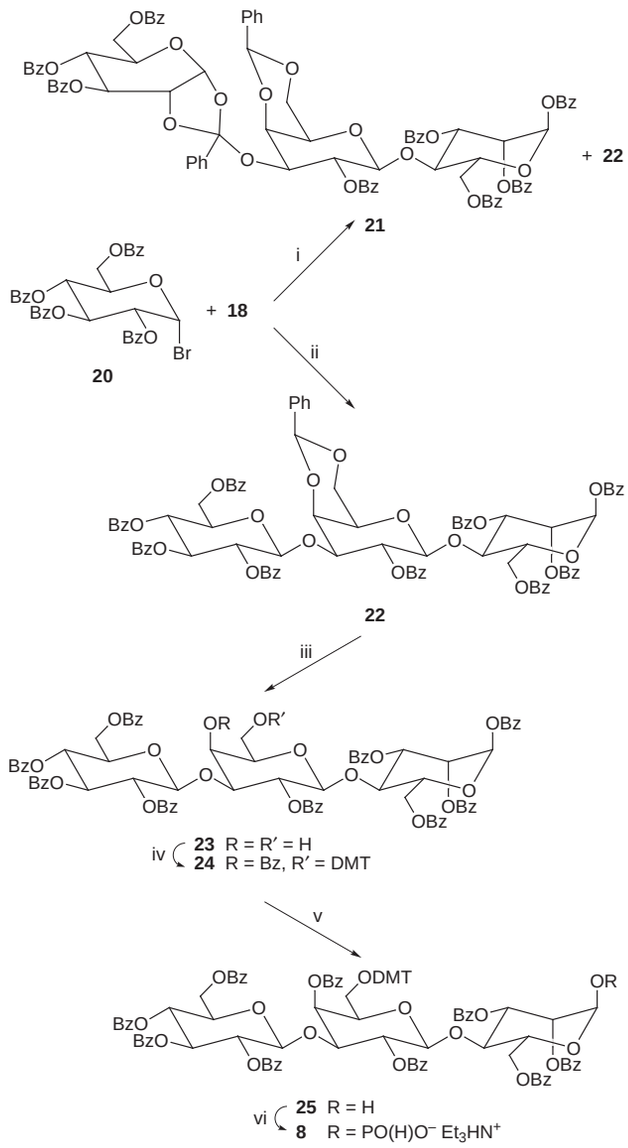


Scheme 1 *Reagents:* i, $Et_3SiOSO_2CF_3$, CH_2Cl_2; ii, $ClCH_2COCl$, pyridine, CH_2Cl_2; iii, PhCOCl, pyridine, CH_2Cl_2; iv, $MeOSO_2CF_3$, CH_2Cl_2, MS 4Å; v, $(NH_2)_2CS$, 2,6-lutidine, MeOH, CH_2Cl_2

galactopyranoside **16**, which in turn was synthesized by way of consecutive 3-*O*-chloroacetylation and 2-*O*-benzoylation of the thiogalactoside **14**.[13] Coupling of the mannose acceptor **10** with the thiogalactoside donor **16** was accomplished in the presence of methyl triflate to give the β- and α-linked disaccharides **17** and **19** in yields of 53 and 23%, respectively.

Only the disaccharide **17** was used further for the preparation of the trisaccharide derivative **22**, since we have shown recently[9] that glycosylation of the disaccharide acceptor **12**, which is a close structural analog of debenzylated compound **11**, was not highly effective and stereoselective.

Dechloroacetylation of compound **17** with thiourea and 2,6-dimethylpyridine (2,6-lutidine) gave the monohydroxylic derivative **18** in excellent yield. Glycosylation of the acceptor **18** with benzobromoglucose **20** in the presence of $Hg(CN)_2$–$HgBr_2$ in acetonitrile (Scheme 2) proceeded smoothly and produced

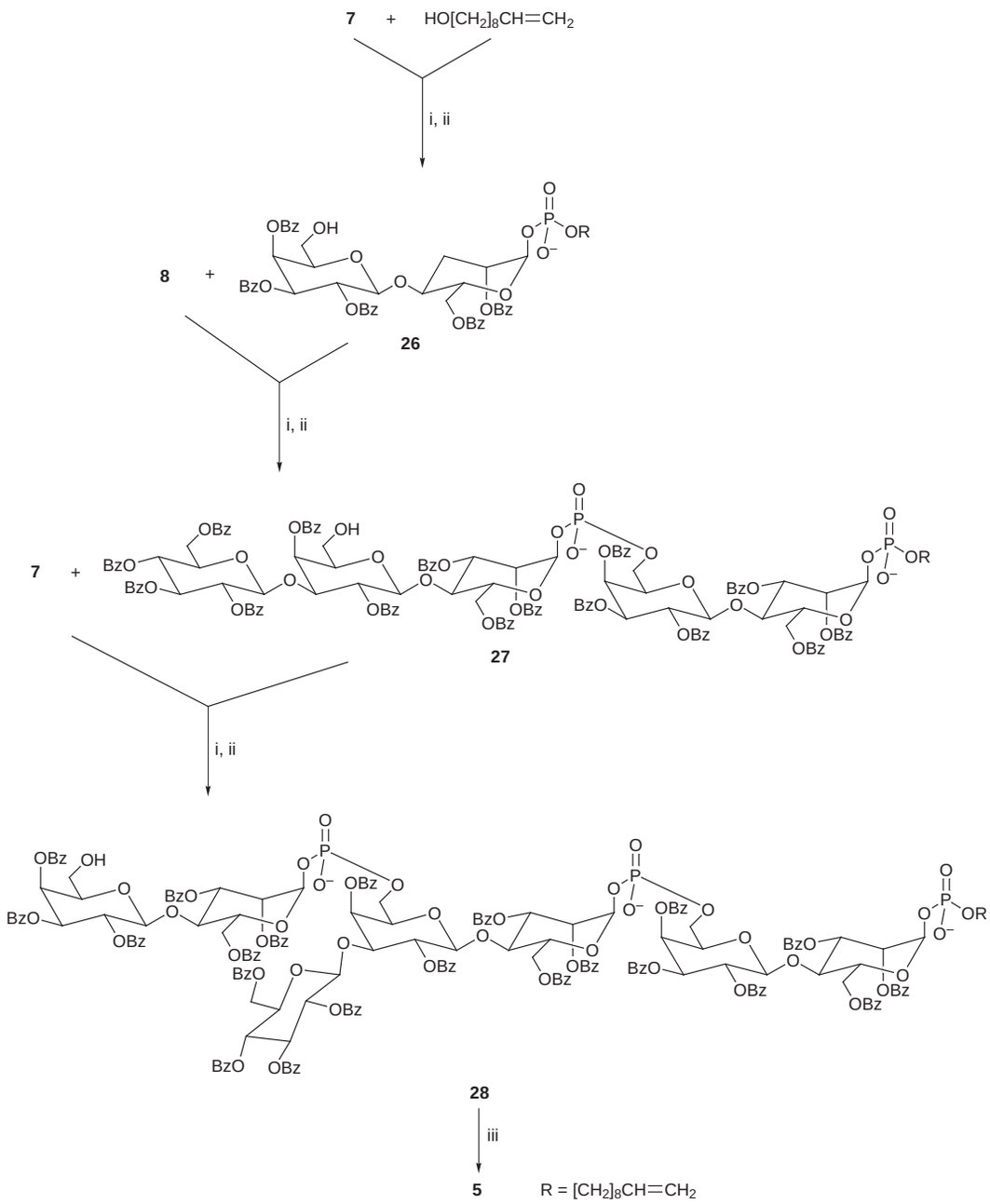


Scheme 2 *Reagents:* i, $AgOSO_2CF_3$, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, MS 4 Å; ii, $Hg(CN)_2$, $HgBr_2$, MeCN; iii, 80% aq. AcOH; iv, (*a*) *p,p′*-dimethoxytrityl chloride, pyridine; (*b*) PhCOCl, pyridine; v, Me_2NH, MeCN–THF; vi, (*a*) triimidazolylphosphine, MeCN; (*b*) Et_3NHHCO_3, water (pH 7)

exclusively the β,β-linked trisaccharide **22** (82%). Similar condensation in the presence of silver triflate and 2,6-di-*tert*-butylpyridine resulted in the trisaccharide orthoester **21** as a major product (80%) together with some (15%) of the glycoside **22**.

The ^1H NMR spectrum of the trisaccharide **22** revealed characteristic signals for all three monosaccharide residues (see Experimental section). The β-configuration of the D-Gal*p* and D-Glc*p* units followed from the characteristic value (7.8 Hz) of the corresponding $J_{1,2}$-coupling constants.

The presence of the orthoester linkage in the derivative **21** was confirmed by the specific hydrolysis test for orthoesters (treatment with 0.05 mol dm^{-3} aq. H_2SO_4 in acetone),[14] which afforded 2,3,4,6-tetra-*O*-benzoyl-D-glucose and the



Scheme 3 *Reagents:* i, (*a*) adamantane-1-carbonyl chloride, pyridine; (*b*) I_2, pyridine–water; ii, TFA, CH_2Cl_2; iii, NaOME, MeOH

disaccharide **18** indicated by TLC. The α-configuration of the D-Glc*p* moiety was evident from the characteristic values of (1) the $J_{1'',2''}$-coupling constant (5 Hz) in the ^{1}H NMR spectrum and (2) the chemical shift of C-1″ (δ_C 97.61) in the ^{13}C NMR spectrum of the trisaccharide orthoester **21**.

The unusual values (for the $^4C_1$-conformation) of the $J_{2'',3''}$- and $J_{3'',4''}$-coupling constants (3.0 and 1.5 Hz, respectively) suggest that the conformation of the D-Glc*p* unit is close to a half-chair $^0H_5$, where both H2″–H3″ and H3″–H4″ are anticlinal.

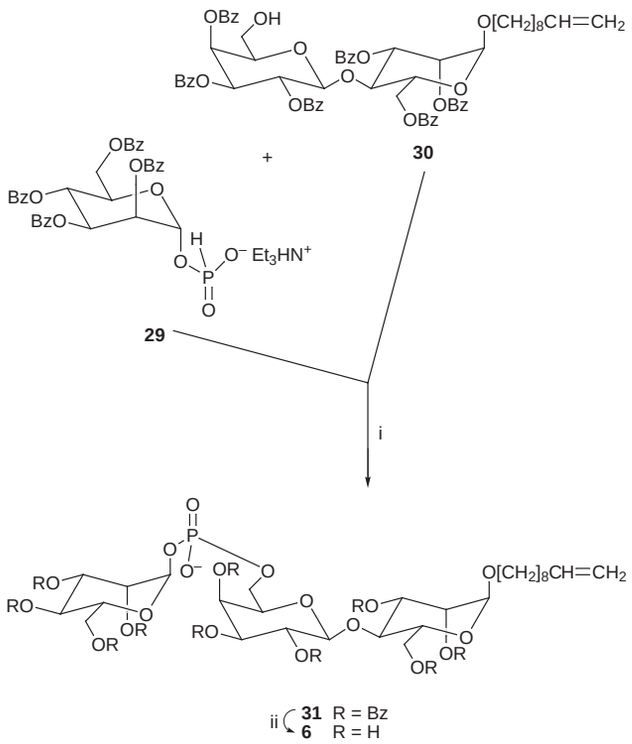
The trisaccharide **22** was converted into the 6′-*O*-dimethoxytrityl (DMT) derivative **24** (86%) by O-debenzylidenation with 80% acetic acid, followed by treatment of the resulting diol **23** first with DMTCl in pyridine and then with benzoyl chloride in pyridine. The trisaccharide **24** was selectively 1-O-debenzoylated with dimethylamine in acetonitrile[7,12] to give the α-hydroxy derivative **25** (88%), which on phosphitylation[7,12] with tri-imidazolylphosphine (prepared *in situ* from PCl_3, imidazole and Et_3N) and mild hydrolysis gave the H-phosphonate block **8** in 87% yield. Signals characteristic of the H-phosphonate group [δ_P 0.56; δ_H 5.71 (dd, $J_{1,2}$ 1.8, $J_{1,P}$ 9.0, 1-H), 7.00 (d, $^1J_{H,P}$ 631.2, HP)] were present in the ^{31}P and ^{1}H NMR spectra of the trisaccharide **8**. The α-configuration of the D-manno-pyranosyl residue followed from the characteristic positions of the 1-, 3- and 5-H resonances (see Experimental section).

The protected heptasaccharide triphosphate **28** (Scheme 3) was assembled starting from the preparation of the phosphodiester **26**, as described in ref. 8. Coupling of the H-phosphonate **7** with dec-9-en-1-ol in pyridine in the presence of adamantane-1-carbonyl chloride, followed by oxidation of the resulting H-phosphonic diester with iodine in pyridine and subsequent dedimethoxytritylation with 1% TFA in CH_2Cl_2 (0 °C) gave the disaccharide phosphate derivative **26** in 90% overall yield. The pentaglycosyl diphosphate derivative **27** was prepared in 71% yield from the trisaccharide H-phosphonate **8** and compound **26** by using a similar sequence of reactions involving condensation, oxidation and detritylation. The last step of the chain-elongation, *i.e.* the coupling of the disaccharide H-phosphonate **7** and compound **27**, followed by oxidation and detritylation gave the heptasaccharide derivative **28** (79%).

The deprotected heptaglycosyl triphosphate **5** was obtained

in 77% yield from compound **28** by O-debenzoylation with 0.25 mol dm^{-3} methanolic sodium methoxide followed by anion-exchange chromatography.

The trisaccharide phosphodiester **6** (Scheme 4) was synthesized from 2,3,4,6-tetra-*O*-benzoyl-D-mannosyl H-phosphonate[15] **29** and the monohydroxylic galactosylmannoside **30**[7] as starting materials. Condensation of these compounds in pyridine in the presence of adamantane-1-carbonyl chloride, followed by *in situ* oxidation with iodine, gave the protected phosphodiester **31** (81%), which then was converted into the phosphorylated trisaccharide **6** by O-debenzoylation with methanolic sodium methoxide.



Scheme 4 *Reagents:* i, (*a*) adamantane-1-carbonyl chloride, pyridine; (*b*) I_2, pyridine–water; ii, NaOMe, MeOH

The structures for compounds **5**, **6**, **27**, **28** and **31** were confirmed by NMR and mass spectrometric data (see Experimental section). For the monophosphonates **31** and **6** the ^{31}P NMR spectra exhibited only single signals, at δ_P −3.48 and −1.11, respectively, which are characteristic of glycoside-linked phosphodiesters (*cf.* refs 7–10, 12, 15). The spectrum of the diphosphate **27** consisted of two signals at −2.28 (P) and −4.03 (P′), whereas the ^{31}P NMR spectra of the triphosphates **28** and **5** consisted of three and two signals, respectively: δ_P −2.33 (P), −3.15 (P″) and −4.02 (P′) for the protected derivative **28**, and −1.13 (P) and −1.50 (P′ + P″) in the ratio 1:2 for the deprotected oligomer **5**.

The presence of the (1→6)-phosphodiester linkages in compounds **5** and **6** was established from the C-1 and C-2 signals of the corresponding D-mannosyl units and the C-5 and C-6 signals of the corresponding D-galactosyl units in the ^{13}C NMR spectra, while the presence of the (1→1)-phosphodiester linkage at the reducing terminus of compound **5** was likewise confirmed by the C-1 and C-2 signals of the D-mannosyl and the dec-9-enyl units (see Experimental section). These signals were shifted as a result of the α- and β-effects of phosphorylation and were coupled with phosphorus (or broadened). The 1-O-phosphorylation of each D-mannose unit in the heptasaccharide **5** and D-Man′ in the trisaccharide **6** was evident also from the characteristic values (7.4–7.5 Hz) of the $J_{1,P}$-coupling constants in the ^1H NMR spectra. The α-configuration of the D-mannosyl phosphate fragments followed from the positions of the C-3 and C-5 resonances of each D-mannose unit in the heptasaccharide **5** and D-Man′ in the trisaccharide **6**. The chemical shifts of these signals are close to those of C-3 and C-5 of α-D-mannopyranosyl phosphate,[16] taking into account the influence of the glycosyl substituents at position-4 (in compound **5**).

The relative molecular masses of the oligomers **5**, **6**, **27**, **28** and **31** were confirmed by ES(−) and ES(+) electrospray mass spectrometry. The dominant signals in the spectra corresponded to the pseudo-molecular ions for the monophosphates **6** (*m/z* 721.1 $[M - Et_3N - H]^-$) and **31** (*m/z* 1761.5 $[M - Et_3N - H]^-$; *m/z* 1864.2 $[M + H]^+$), the diphosphate **27** (*m/z* 1342.1 $[M - 2\ Et_3N - 2\ H]^{2-}$) and the triphosphates **5** (*m/z* 509.4 $[M - 3\ NH_3 - 3\ H]^{3-}$) and **28** (*m/z* 1236.9 $[M - 3\ Et_3N - 3\ H]^{3-}$).

To summarize, the first chemical synthesis of a natural phosphoglycan long-chain fragment containing a glycotriosyl phosphate unit and the first chemical synthesis of a fragment of *Leishmania mexicana* LPG and PPG has been achieved using the glycosyl H-phosphonate method.

Experimental

General procedures

Mps were determined on a Reichert hot-plate apparatus and are uncorrected. Optical rotations were measured with JASCO DIP-360 and Perkin-Elmer 141 polarimeters; $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra (^1H at 200 and 500 MHz, $^{13}C\{^1H\}$ at 50.3 and 125 MHz, and $^{31}P\{^1H\}$ at 81 and 202.5 MHz) were recorded with Bruker AM-200 and AM-500 spectrometers for solutions in $CDCl_3$, unless otherwise indicated. Chemical shifts (δ in ppm) are given relative to those for Me_4Si (for ^1H and ^{13}C) and external aq. 85% H_3PO_4 (for ^{31}P); *J*-values are given in Hz. ES mass spectra were recorded with a VG Quatro system (VG Biotech, UK). TLC was performed on Kieselgel 60 F_{254} (Merck) with detection under UV light or by charring with sulfuric acid–water–ethanol (15:85:5). Flash-column chromatography (FCC) was performed on Kieselgel 60 (0.040–0.063 mm) (Merck). Dichloromethane, acetonitrile and pyridine (for the H-phosphonate condensations) were freshly distilled from CaH_2. Solutions worked up were concentrated under reduced pressure at <40 °C.

2,4-Di-*O*-benzoyl-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose 11

To a stirred and cooled (−30 °C) solution of the trichloroacetimidate **9**[9] (18.6 mg, 0.216 mmol) and the tetrabenzoate **10**[7] (117 mg, 0.196 mmol) in dry dichloromethane (3 cm^3) under nitrogen was added triethylsilyl triflate (0.016 cm^3, 0.07 mmol), whereafter the temperature was allowed to rise to 0 °C and stirring was continued for 1 h. Pyridine (0.2 cm^3) was then added and the solvent was removed under reduced pressure. FCC [toluene–ethyl acetate, (100:0) ⟶ (93:7)] of the residue provided first 2,4-*di*-O-*benzoyl*-3-O-*benzyl*-6-O-(tert-*butyldiphenylsilyl*)-α-D-*galactopyranosyl*-(1→4)-1,2,3,6-*tetra*-O-*benzoyl*-α-D-*mannopyranose* **13** (35 mg, 14%) as an amorphous solid (Found: C, 71.4; H, 5.55. $C_{77}H_{70}O_{17}Si$ requires C, 71.4; H, 5.4%); δ_H 0.95 (9 H, s, Me_3C), 3.72 (2 H, m, 6′-H_2), 4.17 (1 H, dd, $J_{2',3'}$ 10.2, 3′-H), 4.23–4.45 (3 H, m, 5- and 5′-H, 6-H^a), 4.54 and 4.81 (2 H, AB q, *J* 12.0, CH_2Ph), 4.59 (1 H, t, $J_{3,4} = J_{4,5} = 9.0$, 4-H), 5.09 (1 H, dd, $J_{5,6b}$ 1.5, $J_{6a,6b}$ 11.0, 6-H^b), 5.53 (1 H, dd, $J_{1',2'}$ 4.0, 2′-H), 5.71 (3 H, m, 1′-, 2- and 3-H), 6.16 (1 H, br d, $J_{3',4'}$ 3.0, 4′-H), 6.43 (1 H, $J_{1,2}$ 2.0, 1-H) and 6.95–8.15 (45 H, m, 9 × Ph). Continued elution gave the β-*linked disaccharide derivative* **11** (140 mg, 55%) as an amorphous solid; $[\alpha]_D^{20}$ +62 (*c* 1, $CHCl_3$) (Found: C, 71.6; H, 5.3%); δ_H 0.91 (9 H, s, Me_3C), 3.27 (2 H, m, 6′-H_2), 3.49 (1 H, dd, $J_{5',6a'}$ 5.0, $J_{5',6b'}$ 9.0, 5′-H), 3.64 (1 H, dd, $J_{3',4'}$ 3.0, 3′-H), 4.13 (1 H, br d, 5-H), 4.48 (2 H, m, 6-H_2), 4.50 (1 H, t, $J_{3,4} = J_{4,5} = 10.0$, 4-H), 4.51 and 4.82 (2 H, AB q, *J* 13.0, CH_2Ph), 4.69 (1 H, d,

$J_{1',2'}$ 8.0, 1′-H), 5.44 (1 H, dd, $J_{2',3'}$ 10.5, 2′-H), 5.73 (1 H, dd, $J_{2,3}$ 3.3, 2-H), 5.85 (1 H, dd, 3-H), 5.88 (1 H, br d, 4′-H), 6.44 (1 H, $J_{1,2}$ 2.0, 1-H) and 6.62–8.15 (45 H, m, 9 × Ph).

Ethyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-galactopyranoside 15

To a stirred and cooled (0 °C) solution of ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[13] **14** (936 mg, 3 mmol) and pyridine (1 cm^3) in dichloromethane (20 cm^3) was added dropwise a solution of chloroacetyl chloride (0.263 cm^3, 3.3 mmol) in the same solvent (3 cm^3) during a period of 5 min. Stirring was continued for a further 30 min and then chloroform (100 cm^3) was added to the mixture. The resulting solution was washed successively with cold 1 mol dm^{-3} hydrochloric acid, saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. FCC (benzene–ethyl acetate, 4:1) of the residue gave the *chloroacetyl derivative* **15** (687 mg, 59%), mp 183–186 °C, $[a]_D^{20}$ +52 (*c* 1.75, $CHCl_3$) (Found: C, 52.8; H, 5.5. $C_{17}H_{21}ClO_6S$ requires C, 52.5; H, 5.4%); δ_H 1.36 (3 H, t, *J* 7.5, Me), 2.54 (1 H, d, $J_{OH,2}$ 2.1, OH), 2.80 (2 H, q AB q, $J_{A,B}$ 12.8, C*H*$_2$Me), 3.58 (1 H, dt, $J_{5,6}$ 1.8, 5-H), 4.02 (1 H, dd, $J_{6a,6b}$ 12.5, 6-H^a), 4.08 (1 H, dt, $J_{1,2} = J_{2,3} = 9.5$, 2-H), 4.14 and 4.21 (2 H, AB q, *J* 15.1, CH_2Cl), 4.35 (1 H, dd, 6-H^b), 4.42 (1 H, d, 1-H), 4.46 (1 H, dd, $J_{4,5}$ 0.8, 4-H), 4.96 (1 H, dd, $J_{3,4}$ 3.5, 3-H), 5.50 (1 H, s, C*H*Ph) and 7.33–7.55 (5 H, m, Ph). Also isolated was ethyl 4,6-*O*-benzylidene-2,3-di-*O*-chloroacetyl-1-thio-β-D-galactopyranoside (233 mg, 17%).

Ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-galactopyranoside 16

To a stirred solution of the chloroacetate **15** (2.56 mg, 6.59 mmol) in dichloromethane (20 cm^3) were added pyridine (8 cm^3, 100 mmol) and benzoyl chloride (4.6 cm^3, 39.5 mmol), and the reaction mixture was stirred at rt for 1 h. TLC then showed no trace of the starting material and water was added to destroy an excess of benzoyl chloride. The reaction mixture was diluted with chloroform (200 cm^3) and the resulting solution was washed successively with cold 1 mol dm^{-3} hydrochloric acid, water, saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. The residue was redissolved in benzene–ethyl acetate (9:1), the solution was filtered through a Kieselgel pad, and the filtrate was concentrated. After crystallization of the residue from ethyl acetate–hexane, the *thiogalactoside* **16** (2.75 g, 85%) had mp 172–174 °C; $[a]_D^{20}$ +48 (*c* 2.4, $CHCl_3$) (Found: C, 58.4; H, 5.05. $C_{24}H_{25}ClO_7S$ requires C, 58.5; H, 5.1%); δ_H 1.30 (3 H, t, *J* 7.5, Me), 2.85 (2 H, q AB q, $J_{A,B}$ 12.6, C*H*$_2$Me), 3.66 (1 H, dt, $J_{5,6}$ 1.5, 5-H), 3.94 and 4.03 (2 H, AB q, *J* 15.0, CH_2Cl), 4.08 (1 H, dd, $J_{6a,6b}$ 12.5, 6-H^a), 4.40 (1 H, dd, 6-H^b), 4.52 (1 H, dd, $J_{4,5}$ 1.0, 4-H), 4.66 (1 H, d, $J_{1,2}$ 9.9, 1-H), 5.26 (1 H, dd, $J_{3,4}$ 3.5, 3-H), 5.55 (1 H, s, C*H*Ph), 5.78 (1 H, t, $J_{2,3}$ 9.9, 2-H) and 7.35–8.08 (10 H, m, 2 × Ph).

2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galacto-pyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose 17

To a stirred mixture of the thiogalactoside **16** (2.3 g, 4.79 mmol), the tetrabenzoate **10** (3.58 g, 6 mmol) and molecular sieves 4 Å (10 g) in dry dichloromethane (40 cm^3) was added methyl triflate (1.63 cm^3, 14.4 mmol) and the stirring was continued at rt for a further 2.5 h. The reaction was quenched by addition of pyridine (1.5 cm^3). The solids were filtered off and washed with chloroform, and the filtrate was washed with water, dried ($MgSO_4$), and concentrated. FCC (benzene–ethyl acetate, 95:5) of the residue gave a mixture (3.97 g) of the disaccharides **17** and **19**, which was subsequently subjected to FCC (*n*-hexane–ethyl acetate, 3:1) to provide, first, 2-O-*benzoyl*-4,6-O-*benzylidene*-3-O-*chloroacetyl*-α-D-*galactopyr-anosyl*-(1→4)-1,2,3,6-*tetra*-O-*benzoyl*-α-D-*mannopyranose* **19** (1.13 g, 23%) as an amorphous solid; $[a]_D^{20}$ +68 (*c* 2, $CHCl_3$) (Found: C, 65.5; H, 4.6. $C_{56}H_{47}ClO_{17}$ requires C, 65.5; H, 4.6%); δ_H 3.84 and 3.96 (2 H, AB q, *J* 15.0, CH_2Cl), 3.92 (3 H, m, 5′-H and 6′-H$_2$), 4.46 (1 H, dt, $J_{5,6}$ 2.0, 5-H), 4.51 (1 H, br d, $J_{3',4'}$ 3.1, 4′-H), 4.67 (1 H, dd, $J_{6a,6b}$ 11.5, 6-H^a), 4.75 (1 H, dd, 6-H^b), 4.96 (1 H, t, $J_{3,4} = J_{4,5} = 9.4$, 4-H), 5.48 (1 H, s, C*H*Ph), 5.58 (1 H, dd, $J_{2',3'}$ 11.2, 3′-H), 5.72 (1 H, dd, 2′-H), 5.79 (2 H, m, 2- and 3-H), 5.92 (1 H, d, $J_{1',2'}$ 3.8, 1′-H), 6.56 (1 H, d, $J_{1,2}$ 1.7, 1-H) and 7.23–8.22 (30 H, m, 6 × Ph). Continued elution gave the β-*linked disaccharide derivative* **17** (2.59 g, 53%) as an amorphous solid, $[a]_D^{20}$ +99.5 (*c* 2.2, $CHCl_3$) (Found: C, 65.3; H, 4.6%); δ_H 2.87 (1 H, dt, $J_{4',5'} = J_{5',6b'} = 1.0$, 5′-H), 3.55 (1 H, dd, $J_{5',6a'}$ 1.8, 6′-H^a), 3.84 (1 H, dd, $J_{6a',6b'}$ 12.8, 6′-H^b), 3.85 and 3.95 (2 H, AB q, *J* 15.2, CH_2Cl), 4.22 (1 H, dd, $J_{3',4'}$ 3.6, 4′-H), 4.31 (1 H, dt, $J_{5,6}$ 2.2, 5-H), 4.38 (1 H, dd, $J_{6a,6b}$ 12.1, 6-H^a), 4.67 (1 H, t, $J_{3,4} = J_{4,5} = 9.6$, 4-H), 4.73 (1 H, dd, 6-H^b), 4.92 (1 H, d, $J_{1',2'}$ 8.0, 1′-H), 5.11 (1 H, dd, $J_{2',3'}$ 10.3, 3′-H), 5.36 (1 H, s, C*H*Ph), 5.68 (1 H, dd, 2′-H), 5.87 (1 H, dd, $J_{2,3}$ 3.4, 2-H), 6.03 (1 H, dd, 3-H), 6.48 (1 H, d, $J_{1,2}$ 2.0, 1-H) and 7.10–8.20 (30 H, m, 6 × Ph).

2-*O*-Benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose 18

A solution of the disaccharide **17** (2.54 g, 2.47 mmol), thiourea (0.94 g, 12.35 mmol) and 2,6-lutidine (0.29 cm^3, 2.47 mmol) in methanol (15 cm^3)–dichloromethane (10 cm^3) was heated at 65 °C for 3.5 h and then the mixture was concentrated. Chloroform was added to the residue and the resulting solution was washed in turn with 1 mol dm^{-3} hydrochloric acid, water, saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. FCC (benzene–ethyl acetate, 85:15) of the residue gave the *monohydroxylic disaccharide derivative* **18** (2.27 g, 96.5%) as an amorphous solid, $[a]_D^{20}$ +48.6 (*c* 1.6, $CHCl_3$) (Found: C, 67.9; H, 4.8. $C_{54}H_{46}O_{16}$ requires C, 68.2; H, 4.9%); δ_H 2.56 (1 H, br d, $J_{OH,3'}$ 11.1, OH), 2.91 (1 H, br, 5′-H), 3.58 (1 H, dd, $J_{5',6a'}$ 1.8, 6′-H^a), 3.79 (1 H, ddd, $J_{3',4'}$ 3.8, 3′-H), 3.83 (1 H, dd, $J_{5',6b'}$ 1.0, $J_{6a',6b'}$ 12.8, 6′-H^b), 4.03 (1 H, br d, 4′-H), 4.28 (1 H, dt, $J_{5,6}$ 2.2, 5-H), 4.47 (1 H, dd, $J_{6a,6b}$ 12.2, 6-H^a), 4.66 (1 H, t, $J_{3,4} = J_{4,5} = 9.6$, 4-H), 4.69 (1 H, dd, 6-H^b), 4.81 (1 H, d, $J_{1',2'}$ 8.0, 1′-H), 5.37 (1 H, dd, $J_{2',3'}$ 10.0, 2′-H), 5.40 (1 H, s, C*H*Ph), 5.88 (1 H, dd, $J_{2,3}$ 3.4, 2-H), 6.00 (1 H, dd, 3-H), 6.50 (1 H, d, $J_{1,2}$ 2.0, 1-H) and 7.15–8.20 (30 H, m, 6 × Ph).

3-*O*-{2-Phenyldihydro-(3,4,6-tri-*O*-benzoyl-1,2-dideoxy-α-D-glucopyranoso)[2,1-*d*]-1,3-dioxol-2-yl}-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose 21

Benzobromoglucose **20** was prepared from 1,2,3,4,6-penta-*O*-benzoyl-α-D-glucopyranose as described in ref. 17. To a stirred and cooled (0 °C) mixture of the disaccharide **18** (1.3 g, 1.37 mmol), silver triflate (1.02 g, 3.98 mmol), 2,6-di-*tert*-butylpyridine (0.49 cm^3, 2.18 mmol) and molecular sieves 4 Å (1.5 g) in dry dichloromethane (20 cm^3)–toluene (6 cm^3) was added dropwise a solution of benzobromoglucose **20** (1.8 g, 2.74 mmol) in CH_2Cl_2 (8 cm^3). After the addition was complete, the temperature was allowed to rise to 20 °C and stirring was continued for 2.5 h. The solids were filtered off and washed with chloroform, and the filtrate was washed successively with aq. sodium thiosulfate and water, dried ($MgSO_4$), and concentrated. FCC (benzene–ethyl acetate, 95:5) of the residue provided, first, the orthoester **21** (1.68 g, 80%) as an amorphous solid, $[a]_D^{23}$ +63 (*c* 1.13, $CHCl_3$); δ_H 2.67 (1 H, br, 5′-H), 3.40 (1 H, br d, $J_{6a',6b}$ 12.2, 6′-H^a), 3.80 (2 H, m, 3′-H and 6′-H^b), 4.80 (1 H, ddd, $J_{5'',6a''}$ 5.1, $J_{5'',6b''}$ 3.0, 5″-H), 4.20–4.35 (4 H, m, 4′- and 5-H, 6- and 6″-H^a), 4.44 (1 H, dd, $J_{6a'',6b''}$ 12.2, 6″-H^b), 4.60 (1 H, t, $J_{3,4} = J_{4,5} = 10.0$, 4-H), 4.63 (1 H, dd, $J_{2'',3''}$ 3.0, 2″-H), 4.67 (1 H, dd, $J_{5,6b}$ 2.0, $J_{6a,6b}$ 12.0, 6-H^b), 4.79 (1 H, d, $J_{1',2'}$ 8.0, 1′-H), 5.17 (1 H, s, C*H*Ph), 5.32 (1 H, dd, $J_{4'',5''}$ 8.5, 4″-H), 5.51 (1 H, dd, $J_{2',3'}$ 10.2, 2′-H), 5.53 (1 H, dd, $J_{3'',4''}$ 1.5, 3″-H), 5.67 (1 H, d, $J_{1'',2''}$ 5.0, 1″-H), 5.83 (1 H, dd, $J_{2,3}$ 3.5, 2-H), 5.96 (1 H, dd, 3-H), 6.45 (1 H, d, $J_{1,2}$ 2.0, 1-H) and 6.95–8.18 (50 H, m, 10 × Ph); δ_C(*inter alia*) 61.91 (C-6), 63.93 (C-6″), 69.02 (C-2), 71.23 (C-3), 71.56 (C-5), 73.33 (C-4), 73.91 (C-3′), 91.20 (C-1), 97.61 (C-1″),

100.71 (C-1′) and 101.35 (*C*HPh); ESMS (+) data: *m*/*z* 1528.5 $[M]^+$ and 1545.4 $[M + NH_3]^+$ ($C_{88}H_{72}O_{25}$ requires *M*, 1528.44). Continued elution gave the isomeric trisaccharide derivative **22** (310 mg, 15%).

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose 22

A solution of benzobromoglucose **20** (1.32 g, 2 mmol) in dry acetonitrile (10 cm^3) was added to a stirred solution of the disaccharide **18** (950 mg, 1 mmol), $Hg(CN)_2$ (500 mg, 2 mmol) and $HgBr_2$ (360 mg, 1 mmol) in the same solvent (15 cm^3). The mixture was stirred at 20 °C for 18 h, then pyridine was added (0.5 cm^3) and the solvent was removed under reduced pressure. The residue was taken up in chloroform, the suspension was filtered to remove mercury salts, and the filtrate was washed successively with 1 mol dm^{-3} aq. KI and water, dried ($MgSO_4$), and concentrated. FCC (benzene–ethyl acetate, 93:7) of the residue gave the β,β-*linked trisaccharide* **22** (1.26 g, 82%) as an amorphous solid, $[\alpha]_D^{22}$ +78 (*c* 1.07, $CHCl_3$) (Found: C, 69.0; H, 4.7. $C_{88}H_{72}O_{25}$ requires C, 69.1; H, 4.7%); δ_H 2.81 (1 H, br, 5′-H), 3.46 (1 H, dd, $J_{5',6a'}$ 1.7, $J_{6a',6b'}$ 12.2, 6′-H^a), 3.83 (1 H, br d, 6′-H^b), 4.03 (1 H, dd, $J_{3',4'}$ 3.5, 3′-H), 4.07 (1 H, dt, $J_{5'',6a''} = J_{5'',6b''} = 3.5$, 5″-H), 4.17 (1 H, d, 4′-H), 4.19 (1 H, ddd, $J_{5,6a}$ 2.8, 5-H), 4.31 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H^a), 4.41 (1 H, dd, $J_{6a'',6b''}$ 12.1, 6″-H^a), 4.60 (1 H, t, $J_{3,4} = J_{4,5} = 9.2$, 4-H), 4.65 (1 H, dd, $J_{5,6b}$ 2.0, 6-H^b), 4.68 (1 H, dd, 6″-H^b), 4.81 (1 H, d, $J_{1'',2''}$ 7.8, 1″-H), 5.13 (1 H, d, $J_{1',2'}$ 7.8, 1′-H), 5.28 (1 H, s, C*H*Ph), 5.46 (1 H, dd, $J_{2'',3''}$ 9.5, 2″-H), 5.53 (1 H, dd, $J_{2',3'}$ 10.1, 2′-H), 5.64 (1 H, t, $J_{3'',4''} = J_{4'',5''} = 9.5$, 4″-H), 5.75 (1 H, t, 3″-H), 5.85 (1 H, dd, $J_{2,3}$ 2-H) 5.95 (1 H, dd, 3-H), 6.44 (1 H, d, $J_{1,2}$ 2.0, 1-H) and 6.95–8.20 (50 H, m, 10 × Ph).

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose 23

A solution of the trisaccharide derivative **22** (1.1 g) in 80% aq. acetic acid was heated at 70 °C for 2 h, whereafter the mixture was concentrated and toluene was twice evaporated off from the residue. FCC [toluene–ethyl acetate, (95:5) ⟶ (80:20)] of the residue gave the *trisaccharide diol* **23** (0.93 g, 90%) as an amorphous solid, $[\alpha]_D^{24}$ +56 (*c* 1.07, $CHCl_3$) (Found: C, 67.6; H, 4.7. $C_{81}H_{68}O_{25}$ requires C, 67.5; H, 4.75%); δ_H 2.87 (2 H, br, 2 × OH), 3.24 (1 H, t, $J_{5',6'}$ 5.0, 5′-H), 3.43 (2 H, m, 6′-H_2), 3.83 (1 H, dd, $J_{3',4'}$ 3.0, 3′-H), 4.09 (3 H, m, 4′-, 5- and 5″-H), 4.24 (1 H, dd, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 12.1, 6-H^a), 4.35 (1 H, dd, $J_{5'',6a''}$ 5.5, $J_{6a'',6b''}$ 12.5, 6″-H^a), 4.45 (1 H, dd, $J_{5,6b}$ 2.2, 6-H^b), 4.50 (1 H, t, $J_{3,4} = J_{4,5} = 9.5$, 4-H), 4.71 (1 H, d, $J_{1'',2''}$ 7.8, 1″-H), 4.75 (1 H, dd, $J_{5'',6''}$ 2.5, 6″-H^b), 4.93 (1 H, d, $J_{1',2'}$ 7.8, 1′-H), 5.42 (1 H, dd, $J_{2'',3''}$ 9.7, 2″-H), 5.46 (1 H, dd, $J_{2',3'}$ 10.0, 2′-H), 5.55 (1 H, t, $J_{3'',4''} = J_{4'',5''} = 9.7$, 4″-H), 5.76 (1 H, t, 3″-H), 5.81 (1 H, dd, $J_{2,3}$ 3.0, 2-H), 5.87 (1 H, dd, 3-H), 6.45 (1 H, d, $J_{1,2}$ 2.2, 1-H) and 6.85–8.15 (45 H, m, 9 × Ph); δ_C, see Table 1.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*p*,*p*′-dimethoxytrityl)-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose 24

The trisaccharide derivative **23** (400 mg, 0.278 mmol) was dried by evaporation of pyridine (2 × 10 cm^3) therefrom. The residue was dissolved in pyridine (15 cm^3), *p*,*p*′-dimethoxytriphenylmethyl chloride (140 mg, 0.413 mmol) was added, and the solution was kept for 24 h at 20 °C before benzoyl chloride (0.1 cm^3, 0.834 mmol) was also added to the stirred mixture at 0 °C. After 16 h at 20 °C, the reaction mixture was diluted with $CHCl_3$ and washed successively with saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. FCC [toluene–ethyl acetate, (100:00) ⟶ (93:7)] of the residue gave the *dimethoxytrityl trisaccharide derivative* **24** (494 mg, 96%) as an amorphous solid, $[\alpha]_D^{22}$ +36.7 (*c* 1.12, $CHCl_3$) (Found: C, 70.5; H, 4.9. $C_{109}H_{90}O_{28}$ requires C, 70.85; H, 4.9%); δ_H 3.02 (1 H, t, $J_{5',6a'} = J_{6a',6b'} = 8.8$, 6′-H^a), 3.16 (1 H, dd, $J_{5',6b'}$ 5.0, 6′-H^b), 3.63 (6 H, s, 2 × MeO), 3.71 (1 H, dd, 5′-H), 4.04 (2 H, m, 5- and 5″-H), 4.21 (1 H, dd, $J_{3',4'}$ 3.5, 3′-H), 4.42 (2 H, br, 6-H_2), 4.56 (1 H, dd, $J_{3,4}$ 10.0, $J_{4,5}$ 8.5, 4-H), 4.66 (2 H, m, 6″-H_2), 4.77 (1 H, d, $J_{1'',2''}$ 8.0, 1″-H), 4.92 (1 H, d, $J_{1',2'}$ 7.8, 1′-H), 5.31 (1 H, dd, $J_{2'',3''}$ 9.0, 2″-H), 5.46 (1 H, dd, $J_{2',3'}$ 10.0, 2′-H), 5.62 (2 H, m, 3″- and 4″-H), 5.72 (1 H, dd, $J_{2,3}$ 3.3, 3-H), 5.76 (1 H, m, 2-H), 6.03 (1 H, d, 4′-H), 6.42 (1 H, $J_{1,2}$ 1.7, 1-H) and 6.50–8.13 (63 H, 11 × Ph and 2 × C_6H_4); δ_C, see Table 1.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*p*,*p*′-dimethoxytrityl)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranose 25

The trisaccharide derivative **24** (390 mg, 0.211 mmol) was dried by evaporation of acetonitrile (4 cm^3) therefrom. The residue was dissolved in the same solvent (14 cm^3), 2 mol dm^{-3} Me_2NH in THF (0.64 cm^3, 1.27 mmol) was added and the mixture was stirred at 20 °C with monitoring by TLC (toluene–ethyl acetate, 9:1). After 47 h, the mixture was concentrated to dryness and acetonitrile was evaporated off from the residue. FCC [toluene–ethyl acetate, (95:5) ⟶ (75:25)] of the residue gave the 1-*hydroxy derivative* **25** (324 mg, 88%) as an amorphous solid, $[\alpha]_D^{22}$ +16.6 (*c* 0.94, $CHCl_3$) (Found: C, 70.0; H, 5.0. $C_{102}H_{86}O_{27}$ requires C, 70.3; H, 5.0%); δ_H 3.12 (1 H, t, $J_{5',6a'} = J_{6a',6b'} = 8.5$, 6′-H^a), 3.23 (2 H, m, 6′-H^b and OH), 3.55 (1 H, m, 5′-H), 3.67 (6 H, s, 2 × MeO), 4.04 (1 H, t, $J_{5',6''}$ 4.0, 5″-H), 4.17 (1 H, dd, $J_{3',4'}$ 3.1, 3′-H), 4.22 (1 H, ddd, $J_{5,6a}$ 3.0, 5-H), 4.41 (1 H, dd, $J_{6a,6b}$ 12.5, 6-H^a), 4.47 (1 H, t, $J_{3,4} = J_{4,5} = 9.5$, 4-H), 4.55 (1 H, dd, $J_{5,6b}$ 2.0, 6-H^b), 4.64 (2 H, d, 6″-H_2), 4.74 (1 H, d, $J_{1'',2''}$ 7.7, 1″-H), 4.92 (1 H, d, $J_{1',2'}$ 7.8, 1′-H), 5.26 (1 H, br, 1-H), 5.30 (1 H, dd, $J_{2'',3''}$ 9.0, 2″-H), 5.44 (1 H, dd, $J_{2',3'}$ 10.0, 2′-H), 5.60 (3 H, m, 2-, 3″- and 4″-H), 5.71 (1 H, dd, $J_{2,3}$ 3.1, 3-H), 5.96 (1 H, d, 4′-H) and 6.50–8.00 (58 H, m, 10 × Ph and 2 × C_6H_4); δ_C, see Table 1.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*p*,*p*′-dimethoxytrityl)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl hydrogenphosphonate, triethylammonium salt 8

The trisaccharide derivative **25** (220 mg, 0.126 mmol) was dried by evaporation of acetonitrile (2 × 10 cm^3) therefrom. To a stirred solution of imidazole (133 mg, 1.95 mmol) in MeCN (12 cm^3) at 0 °C was added phosphorus trichloride (0.051 cm^3, 0.58 mmol) followed by triethylamine (0.29 cm^3, 2.07 mmol). The mixture was stirred for 15 min, after which a solution of compound **25** in MeCN (12 cm^3) was added dropwise over a period of 20 min at 0 °C. The mixture was stirred at 20 °C for 15 min and quenched with 1 mol dm^{-3} aq. triethylammonium (TEA) hydrogen carbonate (pH 7; 3 cm^3). The clear solution was stirred for 15 min, $CHCl_3$ was added, and the organic layer was washed in turn with ice–water (twice) and cold 0.5 mol dm^{-3} aq. TEA hydrogen carbonate (twice), dried by filtration through cotton wool, and concentrated. FCC [CH_2Cl_2–MeOH–Et_3N, (98:1:1) ⟶ (93:6:1)] of the residue gave the triosyl H-phosphonate **8** (209 mg, 86.5%) as an amorphous solid, $[\alpha]_D^{24}$ +16.8 (*c* 1.09, $CHCl_3$); δ_H 1.30 (9 H, t, 3 × *Me*CH_2), 3.03 (7 H, m, 3 × MeCH_2 and 6′-H^a), 3.20 (1 H, dd, $J_{6a',6b'}$ 8.6, 6′-H^b), 3.65 (6 H, s, 2 × MeO), 3.69 (1 H, dd, $J_{5a',6a'}$ 8.6, $J_{5',6b'}$ 5.2, 5′-H), 4.14 (1 H, ddd, $J_{5'',6a''}$ 3.6, 5″-H), 4.17 (1 H, br d, 5-H), 4.24 (1 H, dd, $J_{3',4'}$ 3.1), 4.45 (1 H, t, $J_{3,4} = J_{4,5} = 9.8$, 4-H), 4.50 (2 H, br, 6-H_2), 4.70 (1 H, dd, $J_{6a'',6b''}$ 12.0, 6″-H^a), 4.74 (1 H, d, $J_{1'',2''}$ 7.5, 1″-H), 4.75 (1 H, dd, $J_{5'',6b''}$ 5.0, 6″-H^b), 5.01 (1 H, d, $J_{1',2'}$ 7.8, 1′-H), 5.35 (1 H, dd, $J_{2'',3''}$ 8.8, 2″-H), 5.43 (1 H, dd, $J_{2',3'}$ 9.7, 2′-H), 5.62 (1 H, dd, $J_{2,3}$ 3.2, 3-H), 5.65 (3 H, m, 2-, 3″- and 4″-H), 5.71 (1 H, dd, $J_{1,2}$ 1.8, $J_{1,P}$ 9.0, 1-H), 6.10 (1 H, d, 4′-H), 7.00 (1 H, d, $J_{H,P}$ 631.2, HP) and 6.55–8.05 (58 H, m, 10 × Ph and 2 × C_6H_4); δ_P 0.56; δ_C, see Table 1; ESMS (−) data: *m*/*z* 1805.5 (100%, $[M - Et_3N - H]^-$) ($C_{108}H_{102}NO_{29}P$ requires *M*, 1907.63).

Table 1 ^{13}C NMR data [δ_C in ppm; $J_{C,P}$ in Hz (in parentheses)] for the protected oligosaccharide derivatives **8** and **23**–**28** (in $CDCl_3$)

Residue	Atom	**8**[a]	**23**	**24**[b]	**25**[b]	**26**[c] (ref. 8)	**27**[c]	**28**[c]
Man	C-1	92.43br	91.21	91.01	91.99	93.62d (5.0)	93.38d (4.9)	93.47br
	C-2	70.72d (9.0)	69.14	69.54	71.49	70.81d (7.4)	70.84d (6.4)	70.92d (7.0)
	C-3	69.70	70.54	69.87	69.82	70.00	69.77	69.99
	C-4	72.65	72.78	72.92	73.04	73.09	73.43	73.42
	C-5	70.38	72.29	71.90	69.63	70.00	69.82	70.23
	C-6	63.00	62.26	63.08	63.30	62.61	62.28	62.34
Gal	C-1	100.77	101.26	100.83	100.75	100.63	101.46	101.20
	C-2	71.55	70.54	71.78	71.95	70.23	70.24	70.41
	C-3	85.94	81.20	86.15	86.43	71.88	72.17	72.42
	C-4	69.45	67.68	69.54	69.63	68.60	67.02	67.24
	C-5	72.41	74.43	72.51	72.83	74.31	71.79d (8.3)	71.80d (7.0)
	C-6	59.40	61.23	59.86	60.24	60.23	61.31d (5.8)	61.46d (5.2)
Man′	C-1						93.22br	93.17br
	C-2						70.59d (6.5)	70.50d (7.0)
	C-3						69.55	69.41
	C-4						72.58	73.15
	C-5						70.11	70.33
	C-6						62.22	62.34
Gal′	C-1						100.28	100.49
	C-2						71.50	71.66
	C-3						78.67	78.41
	C-4						69.40	69.04
	C-5						73.61	71.89d (7.0)
	C-6						59.24	63.15d (5.2)
Glc	C-1	100.77	101.26	101.05	101.08		101.22	100.59
	C-2	71.55	71.38	71.77	72.14		71.05	71.37
	C-3	71.95	72.29	72.10	72.28		72.02	72.19
	C-4	69.02	69.14	69.09	69.40		68.68	68.50
	C-5	71.55	71.38	71.90	72.20		71.98	72.00
	C-6	62.43	61.91	62.07	62.86		62.12	62.25
Man″	C-1							93.47br
	C-2							70.92d (7.0)
	C-3							69.13
	C-4							72.85
	C-5							69.65
	C-6							62.34
Gal″	C-1							100.49
	C-2							70.41
	C-3							71.83
	C-4							68.89
	C-5							73.23
	C-6							60.16
Dec-9-enyl	OCH_2CH_2					66.20d (5.5)	65.86d (5.8)	65.98d (6.0)
	OCH_2CH_2					30.79d (7.5)	30.60d (5.8)	30.65d (6.1)
	CH=					139.39	139.28	139.16
	$=CH_2$					114.12	114.06	113.96
C=O		163.85 to 165.83	163.96 to 165.94	164.10 to 165.89	164.39 to 166.29	165.17 to 165.90	164.24 to 167.51	163.68 to 166.93
C_6H_4 and C_6H_5		126.47 to 129.87, 132.31 to 135.53, 112.72, 143.85, 157.97, 158.04	127.63 to 129.92, 132.57 to 133.69	126.59 to 130.24, 132.59 to 135.94, 113.12, 144.28, 158.22, 158.46	125.43 to 130.28, 132.66 to 135.95, 113.16, 144.39, 158.39, 158.47	128.35 to 131.13, 133.11 to 133.77	127.98 to 130.17, 132.63 to 133.12	127.89 to 129.93, 132.72 to 133.40

[a,b] Additional signals of $MeOC_6H_4Ph_2C$ [δ_C 54.77–55.19 (MeO) and δ_C 86.41–87.18 (Ar_3C)] were present. [a,c] Additional signals of Et_3NH^+ [δ_C 8.53–9.96 (CH_3) and δ_C 45.39–45.93 (CH_2)] were present. [c] Additional signals of CCH_2C (δ_C 25.56–25.78, 28.78–29.98 and 33.66–33.85) were present.

Dec-9-enyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate 6-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate], bistriethylammonium salt 27

The preparation of the disaccharide phosphate **26** from the H-phosphonate **7** has been described previously.[8] A mixture of compounds **8** (120 mg, 0.063 mmol) and **26** (98 mg, 0.076 mmol) was dried by evaporation of pyridine (3 × 1 cm^3) therefrom. The residue was dissolved in pyridine (1 cm^3),

adamantane-1-carbonyl chloride (38 mg, 0.191 mmol) was added, and the mixture was stirred at 20 °C for 20–30 min, whereafter a freshly prepared solution of iodine (32 mg, 0.126 mmol) in 95% aq. pyridine (2 cm^3) was added. After 20 min, $CHCl_3$ was added, and the solution was washed successively with cold 1 mol dm^{-3} aq. $Na_2S_2O_3$ and cold 0.5 mol dm^{-3} aq. TEA hydrogen carbonate, dried by filtration through cotton wool, and concentrated. The residue was dissolved in CH_2Cl_2 (6 cm^3) and 2% TFA in CH_2Cl_2 (6 cm^3) was added at 0 °C. After 1 min, the solution was diluted with $CHCl_3$ and washed successively with ice-cold saturated aq. $NaHCO_3$ and 0.5 mol dm^{-3} aq. TEA hydrogen carbonate, dried by filtration through cotton wool, and concentrated. FCC [CH_2Cl_2–MeOH–Et_3N, (99.4 : 0.1 : 0.5) ⟶ (92.5 : 7 : 0.5)] of the residue gave the pentasaccharide diphosphate derivative **27** (131 mg, 71%) as an amorphous solid; $[\alpha]_D^{23}$ +40.7 (*c* 0.99, $CHCl_3$); δ_P −2.28 (P) and −4.03 (P′); δ_C, see Table 1; ESMS (−) data: *m*/*z* 1342.1 (100%, $[M - 2\ Et_3N - 2\ H]^{2-}$) ($C_{157}H_{162}N_2O_{47}P_2$ requires *M*, 2888.98).

Dec-9-enyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate 6-{2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate 6-[2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate]}, tristriethylammonium salt 28

This compound was prepared by condensation of the disaccharide H-phosphonate **7** (81 mg, 0.057 mmol) and the pentasaccharide diphosphate **27** (110 mg, 0.038 mmol) in the presence of adamantane-1-carbonyl chloride (34 mg, 0.171 mmol), followed by oxidation with iodine (28 mg, 0.11 mmol) and detritylation with 1% TFA in CH_2Cl_2 as described for the preparation of compound **27**. FCC [CH_2Cl_2–MeOH–Et_3N, (98.7 : 0.3 : 1) ⟶ (94 : 5 : 1)] gave the heptasaccharide triphosphate derivative **28** (119 mg, 79%) as an amorphous solid, $[\alpha]_D^{23}$ +54 (*c* 1.1, $CHCl_3$); δ_P −2.33 (P), −3.15 (P″) and −4.02 (P′); δ_C, see Table 1; ESMS (−) data: *m*/*z* 1236.9 (100%, $[M - 3\ Et_3N - 3\ H]^{3-}$) and 1855.9 (10%, $[M - 3\ Et_3N - 2\ H]^{2-}$) ($C_{217}H_{222}O_{66}N_3P_3$ requires *M*, 4018.33).

Dec-9-enyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 6-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl phosphate), triethylammonium salt 31

This compound was prepared by condensation of the mannosyl H-phosphonate **29**[5] (85 mg, 0.112 mmol) and the disaccharide **30**[7] (108 mg, 0.096 mmol) in the presence of adamantane-1-carbonyl chloride (56 mg, 0.282 mmol), followed by oxidation with iodine (48 mg, 0.19 mmol), as described for the synthesis of compound **27**. FCC (CH_2Cl_2–MeOH–Et_3N, (99 : 0 : 1) ⟶ (97.8 : 1.2 : 1) gave the triscaccharide phosphate **31** (147 mg, 81%) as an amorphous solid, $[\alpha]_D^{23}$ +11.3 (*c* 1, $CHCl_3$); δ_H 1.30 (19 H, m, 3 × $MeCH_2$ and 5 × CH_2), 1.63 (2 H, quintet, $OCH_2CH_2CH_2CH_2$), 2.06 (2 H, quartet, CH_2CH_2CH=), 3.00 (6 H, quartet, 3 × $MeCH_2$), 3.42 (1 H, q, $J_{6a',6b'} = J_{5',6a'} = J_{6a',P} = 10.0$, 6′-H[a]), 3.45 and 3.69 (2 H, 2 × dt, $^2J_{H,H}$ 9.5, $^3J_{H,H}$ 6.5, OCH_2CH_2), 4.04 (1 H, ddd, $J_{6b',P}$ 7.8, 6′-H[b]), 4.07 (1 H, dt, $J_{5,6}$ 2.7, 5-H), 4.24 (1 H, dd, $J_{5',6b'}$ 5.3, 5′-H), 4.26 (1 H, dd, $J_{6a,6b}$ 12.2, 6-H[a]), 4.52 (1 H, dd, 6-H[b]), 4.56 (1 H, t, $J_{3,4} = J_{4,5} = 9.6$, 4-H), 4.60 (2 H, br, 6″-H_2), 4.63 (1 H, dt, $J_{5'',6''}$ 2.0, 5″-H), 4.94 (1 H, dd, $^2J_{H,H}$ 1.5, $^3J_{H,H}$ 10.1, CH=CH_2), 4.96 (1 H, d, $J_{1,2}$ 1.8, 1-H), 5.01 (1 H, dd, $^2J_{H,H}$ 1.5, $^3J_{H,H}$ 17.1, CH=CH_2), 5.07 (1 H, d, $J_{1',2'}$ 7.9, 1′-H), 5.48 (1 H, dd, $J_{3',4'}$ 3.5, 3′-H), 5.60 (1 H, dd, $J_{2,3}$ 3.5, 2-H), 5.62 (1 H, dd, $J_{1'',2''}$ 1.8, $J_{1'',P}$ 7.3, 1″-H), 5.73 (1 H, dd, $J_{2',3'}$ 10.3, 2′-H), 5.81 (2 H, m, 2″-H and =CH), 5.86 (1 H, dd, 3-H), 5.93 (1 H, d, 4′-H), 5.97 (1 H, dd, $J_{2'',3''}$ 3.2, 3″-H), 6.14 (1 H, t, $J_{3'',4''} = J_{4'',5''} = 10.2$, 4″-H) and 7.16–8.11 (50 H, m, 10 × Ph); δ_C 8.38 and 45.47 (Et) 25.91, 28.78, 28.96, 29.18, 29.58 and 33.70 (CH_2), 61.60 (d, $J_{C,P}$ 5.2, C-6′), 62.29 (2 C, C-6 and 6″), 66.50 (C-4″), 67.21 (C-4′), 68.43 ($OCH_2CH_2CH_2$), 69.20 (C-3″), 69.70 (2 C, C-3 and -5), 70.06 (C-5″), 70.32 (C-2′), 70.49 (d, $J_{C,P}$ 9.0, C-2″), 71.00 (C-2), 72.00 (d, $J_{C,P}$ 7.7, C-5′), 72.41 (C-3′), 74.98 (C-4), 93.58 (d, $J_{C,P}$ 4.6, C-1″), 97.23 (C-1), 101.44 (C-1′), 114.01 (CH_2CH_2), 128.01–129.88, 132.93 and 133.12 (Ph), 139.11 (CH_2CH_2) and 164.76–165.87 ($PhCO_2$); δ_P −3.48; ESMS (+) data: *m*/*z* 1864.2 (100%, $[M + H]^+$); ESMS (−) data: *m*/*z* 1761.5 (100%, $[M - Et_3N - H]^-$) ($C_{104}H_{106}NO_{29}P$ requires *M*, 1863.66).

Dec-9-enyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranoside 6^{Gal}-(α-D-mannopyranosyl phosphate), triethylammonium salt 6

The trisaccharide phosphodiester **31** (118 mg) was dissolved in 0.05 mol dm^{-3} NaOMe in MeOH (20 cm^3) and the mixture was stirred at 23 °C. After 2 h, the mixture was deionized with Dowex 50W-X4 (H^+) resin, filtered, and immediately neutralized with Et_3N. After concentration, water (5 × 10 cm^3) was evaporated off from the residue to remove methyl benzoate. The trisaccharide monophosphate **6** (52 mg, 99.7%) was thereby obtained as an amorphous solid, $[\alpha]_D^{23}$ +31.5 (*c* 1, 9 : 1 MeOH–$CHCl_3$); $\delta_H(D_2O)$ (*inter alia*) 1.30 (19 H, m, 3 × *Me*CH_2 and 5 × CH_2), 1.60 (2 H, br quintet, $OCH_2CH_2CH_2CH_2$), 2.03 (2 H, quartet, *J* 6.5, CH_2CH_2CH=), 4.48 (1 H, d, $J_{1',2'}$ 7.6, 1′-H), 4.83 (1 H, br, 1-H), 5.43 (1 H, br d, $J_{1'',P}$ 7.5, 1″-H) and 5.83 (1 H, ddt, J_{H,CH_2} 6.5, $J_{H,CH\text{-}cis}$ 10.0, $J_{H,CH\text{-}trans}$ 17.1, CH_2CH=CH_2); $\delta_C(D_2O)$ 9.41 and 48.40 (Et), 27.80, 29.80–30.50 and 35.10 (CH_2), 61.38 (C-6), 62.01 (C-6″), 65.42 (br, C-6′), 67.57 (C-4″), 69.04 (OCH_2CH_2), 69.22 (C-4′), 70.75 (C-3), 70.84 (C-2), 70.96 (C-3″), 71.58 (d, $J_{C,P}$ 9.0, C-2″), 72.00 (C-2′), 72.32 (C-5), 73.73 (C-3′), 74.78 (d, $J_{C,P}$ 8.3, C-5′), 75.01 (C-5″), 78.20 (C-4), 97.27 (d, $J_{C,P}$ 5.9, C-1″), 100.71 (C-1), 104.40 (C-1′), 115.27 (CH_2CH_2) and 141.60 (CH_2CH_2); $\delta_P(D_2O)$ −1.11; ESMS (−): *m*/*z* 721.1 (100%, $[M - Et_3N - H]^-$) ($C_{34}H_{60}NO_{19}P$ requires *M*, 823.39).

Dec-9-enyl β-D-galactopyranosyl-(1-4)-α-D-mannopyranosyl phosphate 6^{Gal}-{β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate 6^{Gal}-[β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate]}, trisammonium salt 5

The protected heptasaccharide **28** (50 mg) was dissolved in 0.25 mol dm^{-3} NaOMe in MeOH (3.5 cm^3) and the mixture was kept for 24 h at 22 °C, followed by work-up as in the preceding experiment. TLC (10 : 10 : 3 $CHCl_3$–MeOH–water) of the residue then revealed the formation of a minor, fast running, UV-active product in addition to the major one, which was UV-inactive. After additional treatment with 0.25 mol dm^{-3} NaOMe in MeOH (19 h; 22 °C) followed by work-up as described above, the residue was applied to a column (18 × 1.5 cm) of Fractogel TSK DEAE-650 (S) (HCO_3^--form) (Merck) eluted with a linear gradient of aq. NH_4HCO_3 (0 ⟶ 0.3 mol dm^{-3}) in 3 : 2 water–propan-2-ol at 1 cm^3 min^{-1} to afford the heptasaccharide trisphosphate **5** (15 mg, 77%) as an amorphous solid, $[\alpha]_D^{22}$ +29.7 (*c* 1, MeOH); $\delta_H(D_2O)$ (*inter alia*) 1.30 (10 H, m, 5 × CH_2), 1.62 (2 H, quintet, *J* 6.8, $OCH_2CH_2CH_2CH_2$), 2.05 (2 H, quartet, *J* 6.8, CH_2CH_2CH=), 4.44 (1 H, d, $J_{1,2}$ 7.9, 1-H, Gal′), 4.47 (1 H, d, $J_{1,2}$ 7.7, 1-H, Gal), 4.52 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal″), 4.67 (1 H, d, $J_{1,2}$ 7.7, 1-H, Glc), 4.96 (1 H, br d, *J* 10.2, CH=CH_2), 5.05 (1 H, br d, *J* 1.70, CH=CH_2), 5.40 (1 H, br d, $J_{1,P}$ 7.5, 1-H, Man), 5.44 (2 H, br d, $J_{1,P}$ 7.4, 1-H, Man′ and 1-H, Man″) and 5.92 (1 H, ddt, J_{H,CH_2} 6.5, CH_2CH=CH_2); $\delta_C(D_2O)$ 25.92, 29.15–29.51 and 34.12 (CH_2), 30.84 (d, $J_{C,P}$ 5.9, OCH_2CH_2), 61.23 (2 C) and 61.33 (C-6, Man, Man′ and Man″), 61.54 (C-6, Glc), 62.14 (C-6, Gal″), 65.39 (2 C, br, C-6, Gal and Gal′), 67.83 (d, $J_{C,P}$ 5.2 OCH_2CH_2), 68.94 (C-4, Gal′), 69.17 (C-4, Gal), 69.72 (3 C, C-3, Man, Man′ and Man″), 69.84 (C-4, Gal″), 70.46 (C-4, Glc), 71.04 (3 C, br, C-2, Man, Man′ and Man″), 71.89 (C-2, Gal and Gal″), 72.03 (C-2, Gal′), 73.23, 73.37, 73.43, 73.55 and 73.59 (C-3, Gal, and Gal″, C-5, Man, Man′ and Man″), 74.35 (C-2, Glc), 74.46 (d, $J_{C,P}$ 7.4, C-5, Gal), 74.80 (d, $J_{C,P}$ 7.4, C-5, Gal′), 76.42 (C-5, Glc), 76.57 (C-3, Glc), 76.80 (C-5, Gal″), 76.94 (C-4, Man″), 77.98 (2 C, C-4, Man and

Man′), 82.87 (C-3, Gal′), 96.70 (d, $J_{C,P}$ 6.1, Man′), 96.94 (2 C, d, $J_{C,P}$ 6.1, C-1, Man and Man″), 104.04 (C-1, Gal), 104.10 (C-1, Gal′), 104.38 (C-1, Gal″), 104.89 (C-1, Glc), 115.00 (CH_2=CH) and 141.53 (CH_2=CH); $\delta_P(D_2O)$ −1.13 (P) and −1.50 (P′ + P″) (ratio 1:2); ESMS (−): *m*/*z* 509.4 (100%, $[M - 3\ NH_3 - 3\ H]^{3-}$), 764.7 (26, $[M - 3\ NH_3 - 2\ H]^{2-}$) and 775.2 (26, $[M - 3\ NH_3 - 3\ H + Na]^{2-}$) ($C_{52}H_{102}N_3O_{45}P_3$ requires *M*, 1581.50).

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