Synthetic Fragments of Antigenic Lipophosphoglycans from *Leishmania major* and *Leishmania mexicana* and Their Use for Characterisation of the *Leishmania* Elongating α-D-Mannopyranosylphosphate Transferase**

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Abstract: The phosphorylated branched heptasaccharides **7** and **8**, the octasaccharide **9** and the phosphorylated trisaccharides **5** and **6**, which are fragments of the phosphoglycan portion of the surface lipophosphoglycans from *Leishmania mexicana* (**5**) or *L. major* (**6**–**9**), were synthesised by using the glycosyl hydrogenphosphonate method for the preparation of phosphodiester bridges. The compounds were tested as acceptor substrates/putative inhibitors for the *Leishmania* elongating α-D-mannosylphosphate transferase.

Keywords: carbohydrates · glycosides · glycosyl phosphates · inhibitors · lipophosphoglycans

Introduction

Leishmania are sandfly-transmitted protozoan parasites that cause a variety of debilitating and often fatal diseases throughout the tropics and subtropics. The life cycle of the parasites involves an insect vector (the sandfly) and mammalian hosts. The parasites' survival and infectivity are due to the glycocalyx and in particular the lipophosphoglycan (LPG), which is one of its major components.[2] LPGs are common to *L. major*, *L. donovani* and *L. mexicana* promastigotes[3–8] and *L. major* amastigotes,[9] but they are absent in *L. donovani* and *L. mexicana* amastigotes.[10,11] The LPG contains a polymeric phosphoglycan region consisting of [-6)-(R→3)-β-D-Gal*p*-(1→4)-(R′→2)-α-D-Man*p*-(1-PO_3H-]$_n$ repeating units where the nature of the R and R′ constituents varies according to the species. For example, in *L. donovani*[2] R = R′ = H, whereas in *L. major*[2,12] R′ = H and R is mostly a mono-, di- or trisaccharide made up of β-D-Gal*p* and β-D-Ara*p* residues. In *L. aethiopica*[7] 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 produced by *L. mexicana*[6,13] R′ is H (100%) and about 25% of the D-galactose residues are substituted at O-3 with β-D-glucopyranose (that is, R is β-D-Glc*p* (25%) or H (75%)). The importance of the LPG for parasite infectivity and survival[14,15] makes the enzymes responsible for its biosynthesis of great interest.

The LPG β-D-Gal*p*-(1→4)-α-D-Man*p* phosphate backbone has been shown[16,17] to be assembled by sequential action of the *Leishmania* α-D-mannopyranosylphosphate transferase and β-D-galactopyranosyl transferase. The elongating α-D-mannopyranosylphosphate transferase (eMPT) transfers a α-D-Man*p* phosphate moiety en bloc from GDP-Man to O-6 of the terminal β-D-Gal*p* in the growing phosphoglycan chain (GDP = guanosine diphosphate). We previously reported[18] that the synthetic phosphodisaccharide **1**[19,20] (Figure 1), representing one repeating unit of the phosphoglycan, acts as an acceptor substrate for the eMPT activity in *Leishmania* promastigote membranes. Biosynthetic transfer of α-D-Man*p* phosphate from GDP-Man to compound **1** produced the expected trisaccharide diphosphate

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L. donovani

β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **1**

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-]$_n$-O-$[CH_2]_8CH{=}CH_2$ **2** $n=1$; **3** $n=2$

L. mexicana

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-6)-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-$[CH_2]_8CH{=}CH_2$ **4**
(β-D-Glc*p* on 3)

β-D-Glc*p*-(1→3)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **5**

L. major

β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **6**

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-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **7**
(β-D-Gal*p* on 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-6)-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-$[CH_2]_8CH{=}CH_2$ **8**
(β-D-Gal*p* on 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-6)-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-$[CH_2]_8CH{=}CH_2$ **9**
(β-D-Gal*p* on 3; β-D-Gal*p* on 3)

Figure 1. Synthetic fragments of the lipophosphoglycan from different species of *Leishmania*.

α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$. In Parts 9,[20] 11[21] and 12[22] of this series, we disclosed our interest in the design and synthesis of various structural analogues of phosphodisaccharide **1** to test the fine acceptor-substrate specificity of the eMPT and to gain more information about enzyme–substrate recognition. We have also synthesised compounds **2** and **3**,[23] representing longer fragments of the LPG from *L. donovani*, and shown[18] they were also effective as acceptor substrates for the eMPT but with slightly reduced kinetics relative to those of compound **1**.

It has been already mentioned that *L. major* and *L. mexicana* substitute the β-D-Gal*p*-(1→4)-α-D-Man*p* phosphate repeating units of the LPG with either β-D-Gal*p* or β-D-Glc*p*, respectively, at O-3 of the β-D-Gal*p* residue. The timing of the biosynthetic addition of the side chains is not known, that is, it is not clear whether this process can occur concurrently with phosphosaccharide backbone assembly or whether these modifications can only occur distal to the site of the assembly. To address these questions, we now report the chemical synthesis of tri-, hepta- and octasaccharide fragments **6**–**9** (Figure 1) of the LPG from *L. major* and the trisaccharide fragment **5** of the LPG from *L. mexicana*. Compounds **5**–**9**, as well as the earlier described[24] heptasaccharide fragment **4** of the LPG from *L. mexicana*, have been tested as acceptor substrates for the eMPT. By determining the effect of the β-D-Gal*p* or β-D-Glc*p* side-chain substitution on the enzyme activity, it is possible to determine whether the side chains are attached concurrently with or after the assembly of the phosphosaccharide backbone. For example, if all of the compounds **4**–**9** work as acceptor substrates for the eMPT then it may be concluded that phosphosaccharide backbone assembly may occur concurrently with side-chain addition. Alternatively, if none of them can act as the α-D-Man*p* phosphate acceptor then we may conclude that the assembly of the backbone necessarily precedes side-chain addition. All of the synthetic phosphosaccharides **5**–**9** contain a dec-9-enyl moiety to 1) assist biochemical assays[18] and 2) make the compounds available for the preparation of neoglycoconjugates by consecutive ozonolysis of the double bond and coupling to a protein carrier by reductive amination.[25,26]

Results and Discussion

The preparation of compounds **5**–**9** was based on the principles of glycosyl hydrogenphosphonate chemistry, which was developed in this laboratory[19,23,24] for the synthesis of the phosphosaccharides **1**–**4** and was recently applied by the New Delhi group[27–30] for the synthesis of more *Leishmania* LPG synthetic fragments. The first examples of the hydrogenphosphonate method used for the preparation of glycoside phosphodiesters of biological origin were described by van Boom and co-workers[31] and by one of us (A.V.N.)[32] in the late 1980s. The phosphosaccharides **6**–**9**, representing

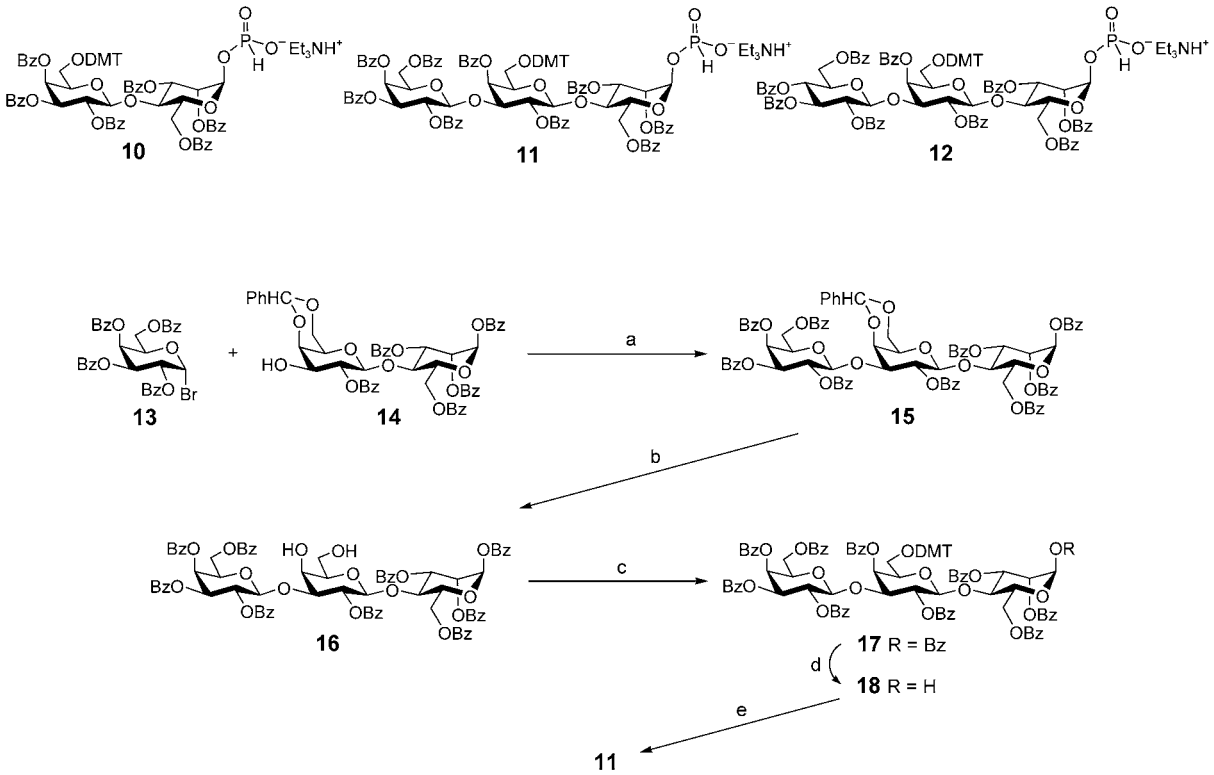
fragments of the *L. major* LPG, were synthesised by the use of the protected di- and trisaccharide hydrogenphosphonate blocks **10**[23] and **11** (Scheme 1) for the consecutive introduction of di- and trisaccharide phosphate moieties, respectively. Both the hydrogenphosphonates **10** and **11** contain a temporary *p,p′*-dimethoxytrityl (DMT) protecting group at O-6′ to allow selective deprotection and condensation to extend the oligomeric chain. The hydrogenphosphonate derivative **12**[24] containing D-glucose was used for the preparation of the trisaccharide phosphate fragment **5** of the *L. mexicana* LPG.

The preparation of the trisaccharide hydrogenphosphonate **11** (Scheme 1) was started from 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **13** and the disaccharide glycosyl acceptor **14**.[24] Silver triflate assisted base-deficient[33] glycosylation reaction gave the β,β-linked trisaccharide **15** (73%) and 14% of the corresponding α,β-linked isomer (see the Experimental Section). The β configuration of the newly created D-galactoside bond was confirmed by the characteristic value (8.0 Hz) for $J_{1'',2''}$ coupling constant in the ^{1}H NMR spectrum of **15**. The protecting-group pattern was then modified by initial hydrolysis with 80% aq. acetic acid to give the diol **16** in 91% yield. Trisaccharide **16** was treated with DMTCl in pyridine and then benzoyl chloride in pyridine to produce derivative **17** (86%). Successive anomeric deprotection[19–24] with dimethylamine in acetonitrile/THF gave the α-hemiacetal **18** (83%), which upon phosphitylation[19–24] with triimidazolylphosphine (prepared in situ from PCl_3, imidazole and Et_3N) and mild hydrolysis produced the hydrogenphosphonate **11** in 88% yield. Signals characteristic to the hydrogenphosphonate group (δ_H = 5.68 (dd, 1H, $J_{1,2}$ = 1.4, $J_{1,P}$ = 9.0 Hz; H-1), 6.96 (d, 1H, $J_{H,P}$ = 635.4 Hz; HP) ppm; δ_P = −0.37 ppm) were present in the ^{1}H and ^{31}P NMR spectra of derivative **11**.

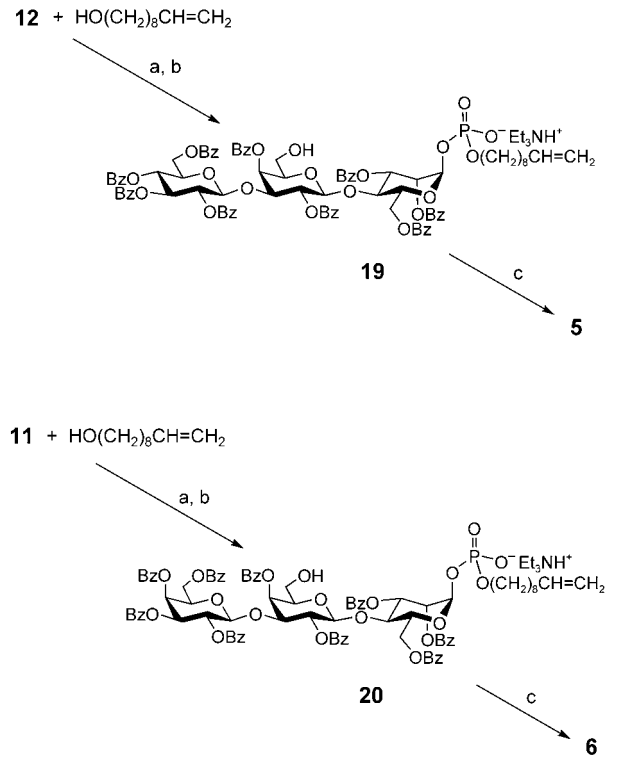
The preparation of the trisaccharide phosphates **5** and **6** progressed (Scheme 2) directly from the hydrogenphosphonate derivatives **12** and **11**, respectively. The condensation of each of them with dec-9-en-1-ol in the presence of pivaloyl chloride followed by oxidation (in situ) with iodine in aqueous pyridine and mild detritylation (1% TFA/DCM, 0 °C) formed the protected phosphosaccharides **19** (90% from **12**) or **20** (88% from **11**). The *O*-benzoyl protecting groups were then removed by using 0.25 M NaOMe in methanol to give the required trisaccharide phosphodiesters **5** (95%) and **6** (88%).

The synthesis of the oligophosphosaccharides **7–9** is outlined in Schemes 3 and 4. All three progressed through a common intermediate **21**, which was prepared[19] by condensation of the disaccharide hydrogenphosphonate **10** with dec-9-en-1-ol, followed by oxidation and detritylation as described above for the trisaccharides **19** and **20**.

Firstly, the chain elongation was continued with another coupling with hydrogenphosphonate **10** to give the tetrasaccharide **23** (72%), which was further coupled with trisaccharide hydrogenphosphonate **11** (in the presence of adamantane-1-carbonyl chloride followed by oxidation and de-



Scheme 1. Reagents: a) $AgOSO_2CF_3$, 2,6-di-*tert*-butylpyridine, MS4A, DCM; b) 80% aq. AcOH; c) 1. *p,p′*-dimethoxytrityl chloride, pyridine; 2. PhCOCl, pyridine; d) Me_2NH, MeCN/THF; e) 1. triimidazolylphosphine, MeCN; 2. Et_3NHHCO_3, water (pH 7). Bz = benzoyl, DMT = 4,4′-dimethoxytrityl, MS4A = 4 Å molecular sieves, DCM = dichloromethane, THF = tetrahydrofuran.

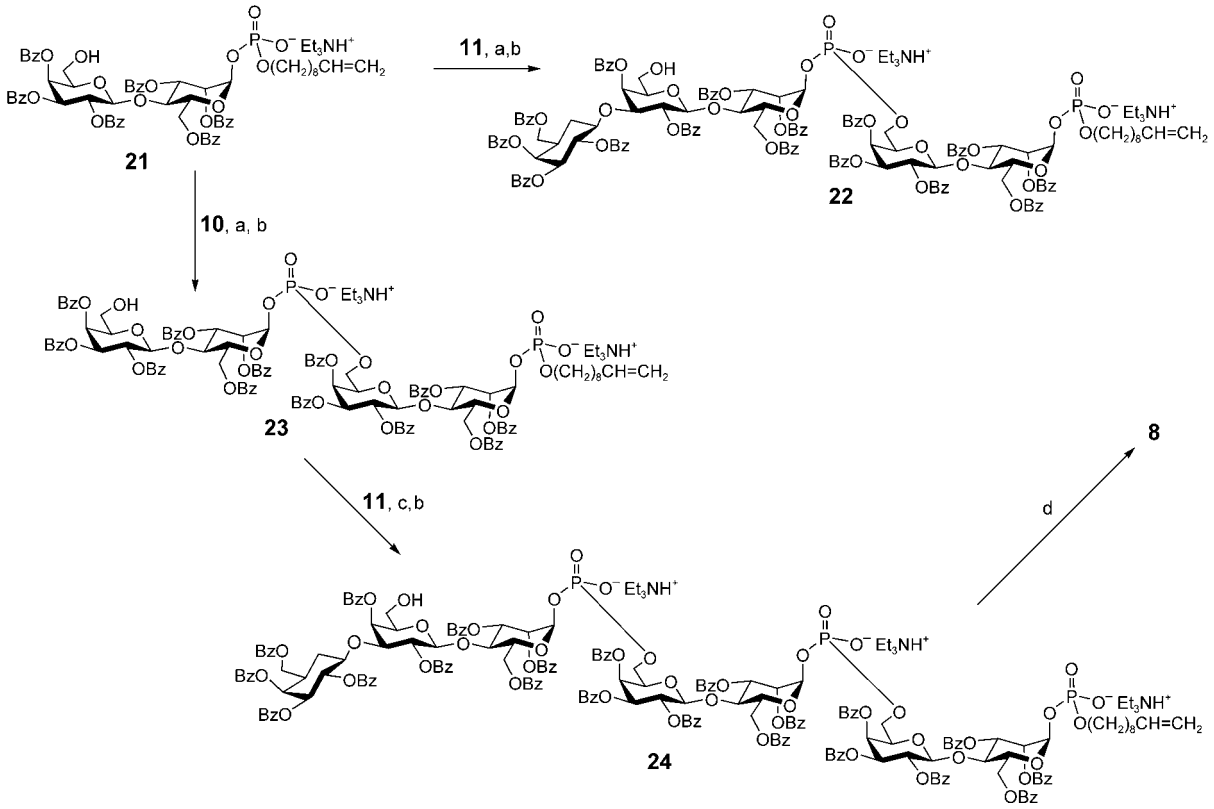


Scheme 2. Reagents: a) 1. pivaloyl chloride, pyridine; 2. I_2, pyridine/water; b) TFA, DCM; c) NaOMe, MeOH. TFA = trifluoroacetic acid.

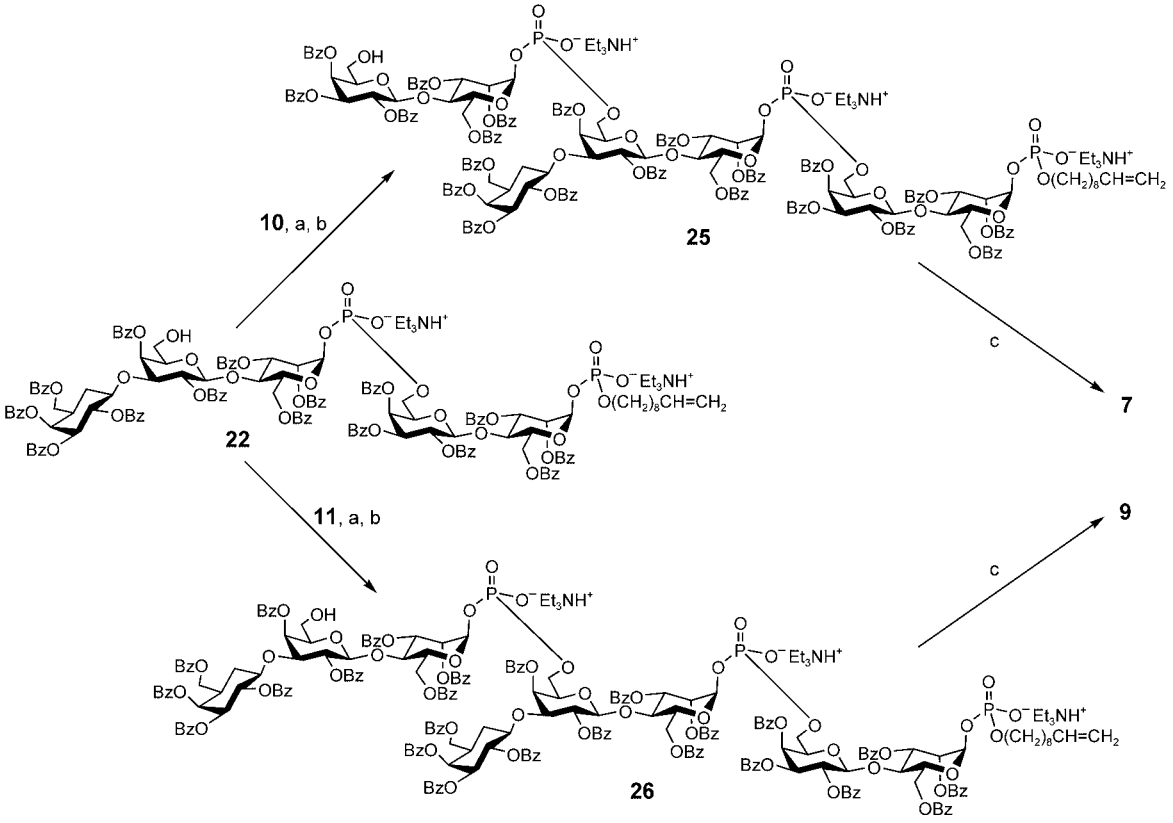
tritylation) to provide the protected heptasaccharide **24** in 84% yield. This gave the heptasaccharide triphosphate **8** (88%) upon debenzoylation with 0.25 M NaOMe in methanol/THF.

Secondly, the disaccharide phosphate **21** was coupled with the trisaccharide hydrogenphosphonate **11** (in the conditions described above for the trisaccharides **19** and **20**) to form the pentasaccharide block **22** (68%). Compound **22** was then extended (Scheme 4) with either the disaccharide hydrogenphosphonate **10** or the trisaccharide hydrogenphosphonate **11** to provide the protected heptasaccharide **25** (65%) or the protected octasaccharide **26** (76%), respectively. Successive standard debenzoylation then produced the final targeted phosphosaccharides **7** and **9** in 58% and 82%, respectively. All the deprotected phosphosaccharides were purified by anion-exchange chromatography on diethylaminoethyl TSK (DEAE TSK) gel (Merck).

The structures of compounds **5**–**9** were supported by NMR spectroscopy and electrospray mass spectrometry data. The presence of the (1→1)- and (1→6)-phosphodiester linkages were confirmed by the C-1 and C-2 signals in the corresponding D-mannosyl and dec-9-en-1-yl units and the C-5 and C-6 signals of the corresponding D-galactosyl units in the ^{13}C NMR spectra (see Table 1). The signals were shifted as a result of the α and β effects of phosphorylation and coupled with the P nucleus. The ^{31}P NMR data (see the Experimental Section) are characteristic of glycoside-linked



Scheme 3. Reagents: a) 1. pivaloyl chloride, pyridine; 2. I_2, pyridine/water; b) TFA, DCM; c) 1. adamantane-1-carbonyl chloride, pyridine; 2. I_2, pyridine/water; d) NaOMe, MeOH/THF.



Scheme 4. Reagents: a) 1. pivaloyl chloride, pyridine; 2. I_2, pyridine/water; b) TFA, DCM; c) NaOMe, MeOH.

phosphoric diesters[19–24] and contain either one (for the monophosphates **5** and **6**) or two signals (in the ratio 1:2; for the triphosphates **7**–**9**), thus indicating the nonequivalence of the phosphate groups in the latter oligomers (see refs. [19] and [24]). The molecular masses for the oligophosphosaccharides **5**–**9** were confirmed by electrospray (ES) mass spectrometry. The signals in the ES(−) mass spectra corresponded to the pseudo molecular ions for the targeted compounds (see the Experimental Section).

Compounds **1**, **3** and **4**–**9** were tested in a biochemical assay in a cell-free system[34] as acceptor substrates for the eMPT in *Leishmania*. Promastigote membranes from *L. major*, *L. mexicana* and *L. donovani* were used, with the substrate activity of compound **1** normalised to 100% and the activities of the other compounds compared relative to this value. In all cases, compounds **5**, **6**, **8** and **9**, containing a substituent (β-D-Glc*p* or β-D-Gal*p*) at O-3′ of the terminal (at the nonreducing end of the chain) β-D-Gal*p*-(1→4)-α-D-Man*p* phosphate repeating unit, were not acceptor substrates for the enzyme with no mannopyranosyl phosphate transfer observed. In contrast, a monosaccharide substitution at O-3′ of the penultimate disaccharide phosphate repeating unit did not have such a dramatic effect, with compound **4** (containing a β-D-glucopyranosyl side chain) and compound **7** (containing a β-D-galactopyranosyl side chain) both acting as acceptor substrates for the enzyme with no statistically significant difference in activity.

There was some difference between phosphoheptasaccharides with internal side chains (**4** and **7**) and the linear phosphohexasaccharide **3** containing no side chains. In the *L. major* assay there was a preference for the unsubstituted acceptor, with compound **3** showing 30% more activity than compounds **4** and **7**. In *L. mexicana* this was reversed, with a slight preference for the substituted acceptors of about 10–15%. The *L. donovani* assay did not show any significant difference in the acceptor activity of compounds **3**, **4** and **7**. These results indicate that 1) substitution of the terminal β-D-Gal*p* 3-hydroxy group with a monosaccharide abrogates substrate recognition by the eMPT and 2) during the LPG biosynthesis in *L. major* and *L. mexicana* the side-chain addition is likely to occur after the phosphosaccharide backbone has been prepared or may proceed distal to its assembly site. A comprehensive characterisation of the eMPT in *Leishmania* by using synthetic acceptor substrate analogues (a set of 25 phosphosaccharides) was recently published.[34]

Compounds **5**, **6**, **8** and **9**, which were not substrates for the enzyme, were tested for inhibitory activity by using *L. major* procyclic promastigote membranes (for inhibition assay conditions, see ref. [34]). The long-chain phosphosaccharides **8** and **9** showed either no (**9**) or low (≈9%; **8**) inhibition when used at an equimolar concentration (600 μM) to the acceptor substrate **1**. The trisaccharide phosphates **5** and **6** were better inhibitors for the eMPT and, when taken at the same concentration, inhibited the Man*p* phosphate transfer to substrate **1** by ≈19 and ≈22%, respectively. A

Table 1. ^{13}C NMR (75 MHz, D_2O) data (δ_C in ppm, with multiplicity (where applicable) and the $J_{C,P}$ coupling constant in Hz (in parenthesis)) for the deprotected phosphosaccharides **4**–**9**.

Residue	Atom	**4**[24][a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[a]
dec-9-enyl	C-1	67.83, d (5.2)	67.79, d (6.2)	67.84, d (5.5)	67.86, d (5.3)	67.87, d (5.1)	67.80, br
	C-2	30.84, d (5.9)	30.86, d (6.9)	30.87, d (6.7)	30.97, d (6.7)	30.87, d (6.6)	30.91, d (6.7)
	C-9	141.53	141.56	141.58	141.59	141.59	141.50
	C-10	115.00	115.00	115.00	115.00	115.00	115.00
Man	C-1	96.94, d (6.1)	96.83, d (6.1)	96.84, d (6.1)	96.95, d (5.4)	96.93, d (5.9)	96.92, d (6.1)
	C-2	71.04, br	71.15, d (7.9)	71.15, d (7.9)	71.08, d (8.2)	71.03, d (8.3)	71.10, d (8.9)
	C-3	69.84	69.80	69.82	69.88	69.87	69.87
	C-4	76.94	76.72	76.76	76.96	76.78	76.80
	C-5	73.59	73.36	73.40	73.60	73.58	73.58
	C-6	61.33	61.12	61.14	61.39	61.37	61.36
Gal	C-1	104.38	103.69	103.72	104.39	104.39	104.38
	C-2	71.89	71.20	71.28	71.91	71.91	71.89
	C-3	73.23	83.03	82.91	73.27	73.29	73.24
	C-4	69.17	69.41	69.58	69.19	69.19	69.18
	C-5	74.80, d (7.4)	76.06	76.13	74.79, d (8.2)	74.72, d (7.1)	74.80, d (7.5)
	C-6	65.39, br	62.14	62.15	65.48, d (5.0)	65.42, d (5.0)	65.40, d (5.6)
Man′	C-1	96.70, d (6.1)			96.73, d (5.4)	96.93, d (5.9)	96.73, d (6.1)
	C-2	71.04, br			71.08, d (8.2)	71.03, d (8.3)	71.10, d (8.9)
	C-3	69.72[b]			69.75[b]	69.77[b]	69.72[b]
	C-4	77.98			78.05	77.98[c]	78.00
	C-5	73.40			73.42	73.45	73.42
	C-6	61.23			61.28	61.22	61.26
Gal′	C-1	104.04		105.40	104.11	104.39	104.07
	C-2	72.03		72.11	71.14	71.91	71.12
	C-3	82.87		73.60	82.78	73.45	82.80
	C-4	68.94		69.66	69.11	69.19	69.09
	C-5	74.46, d (7.4)		76.13	74.57, d (8.2)	74.72, d (7.1)	74.51, d (7.5)
	C-6	65.39, br		62.05	65.48, d (5.0)	65.42, d (5.0)	65.40, d (5.6)
Glc	C-1	104.89	104.82				
	C-2	74.35	74.34				
	C-3	76.57	76.56				
	C-4	70.46	70.46				
	C-5	76.80	76.81				
	C-6	61.54	61.53				
Gal(1→3)Gal′	C-1				105.49		105.49
	C-2				72.09		72.11
	C-3				73.60		73.58
	C-4				69.68		69.65
	C-5				76.12		76.07
	C-6				62.02		62.01
Man″	C-1	96.94, d (6.1)			96.95, d (5.4)	96.93, d (5.9)	96.99, d (6.1)
	C-2	71.04, br			71.08, d (8.2)	71.03, d (8.3)	71.10, d (8.3)
	C-3	69.90[b]			69.93[b]	69.94[b]	69.93[b]
	C-4	77.98			78.05	78.10[c]	78.00
	C-5	73.40			73.42	73.45	73.42
	C-6	61.23			61.28	61.28	61.26
Gal″	C-1	104.10			104.11	103.73	103.76
	C-2	71.89			72.09	71.29	71.29
	C-3	73.55			73.60	82.94	82.91
	C-4	69.72			69.75	69.60	69.58
	C-5	76.42			76.45	76.13	76.07
	C-6	62.14			62.22	62.17	62.15
Gal(1→3)Gal″	C-1					105.44	105.44
	C-2					72.11	72.11
	C-3					73.58	73.58
	C-4					69.67	69.65
	C-5					76.13	76.07
	C-6					62.03	62.01

[a] Additional signals of CCH_2C (δ_C = 25.91–25.95, 29.15–29.56 and 34.12–34.31) were present. [b] The signals of C-3 in Man′ and C-3 in Man″ might be reversed. [c] The signals of C-4 in Man′ and C-4 in Man″ might be reversed.

detailed discussion of inhibitory experiments for the enzyme with a set of compounds prepared in this laboratory will be published elsewhere in due course.

Experimental Section

General procedures: M.p. values were determined on a Reichert hot-plate apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter; $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H and ^{13}C NMR spectra (^{1}H at 300 and 500 MHz, ^{13}C at 75 and 125 MHz) were recorded with Bruker AM-300 and Bruker AM-500 spectrometers, while ^{31}P NMR spectra were recorded with Bruker AM-200 (at 81 MHz) and Bruker AM-300 (at 121 MHz) instruments. Chemical shifts (δ in ppm) are given relative to those for Me_4Si (for ^{1}H 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 Quattro system (VG Biotech, UK) and with a Mariner system (Applied Biosystems). MALDI-TOF mass spectra were recorded with a PE Biosystems Voyager system 4029 (Applied Biosystems). 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, pyridine and toluene were freshly distilled from CaH_2. Solutions that were worked up were concentrated under reduced pressure at <40 °C.

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose (15): Benzobromogalactose **13** was prepared from 1,2,3,4,6-penta-*O*-benzoyl-α-D-galactopyranose[35] as described in ref. [20]. A solution of benzobromogalactose **13** (1.12 g, 1.70 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a stirred and cooled (0 °C) mixture of the disaccharide **14**[24] (800 mg, 0.84 mmol), silver triflate (630 mg, 2.44 mmol), 2,6-di-*tert*-butylpyridine (0.30 mL, 1.34 mmol) and 4 Å molecular sieves (900 mg) in CH_2Cl_2 (12 mL) and toluene (3 mL). After the addition was complete, the temperature was allowed to rise to 20 °C and stirring was continued for 2 h. The solids were filtered off and washed with chloroform, then the filtrate was washed successively with aq. sodium thiosulfate and water, dried ($MgSO_4$) and concentrated. FCC (benzene/ethyl acetate, 93:7) of the residue provided, first, 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose (180 mg, 15%); m.p. 227–228 °C (from ethyl acetate/hexane); $[\alpha]_D^{23} = +171$ ($c = 1.70$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.64$ (br, 1H; H-5′), 3.41 (dd, $J_{5',6a'} = 1.3$ Hz, 1H; H-6a′), 3.74 (br d, $J_{6a',6b'} = 12.4$ Hz, 1H; H-6b′), 3.95 (dd, $J_{5'',6a''} = 6.0$, $J_{6a'',6b''} = 10.8$ Hz, 1H; H-6a″), 4.03 (dd, $J_{3',4'} = 3.3$ Hz, 1H; H-3′; and d, 1H; H-4′), 4.30 (dd, $J_{5'',6b''} = 5.7$ Hz, 1H; H-6b″), 4.32–4.41 (m, 3H; H-5, H-5″ and H-6a), 4.78 (t, $J_{3,4} = J_{4,5} = 9.4$ Hz, 1H; H-4), 4.82 (dd, $J_{5,6b} = 2.0$, $J_{6a,6b} = 12.2$ Hz, 1H; H-6b), 4.97 (d, $J_{1',2'} = 8.1$ Hz, 1H; H-1′), 5.10 (s, 1H; C*H*Ph), 5.45 (dd, $J_{2'',3''} = 10.7$ Hz, 1H; H-2″), 5.54 (dd, $J_{3'',4''} = 3.3$ Hz, 1H; H-4″), 5.69 (d, $J_{1'',2''} = 3.5$ Hz, 1H; H-1″), 5.72 (dd, 1H; H-3″), 5.79 (dd, $J_{2',3'} = 10.3$ Hz, 1H; H-2′), 5.91 (dd, $J_{2,3} = 3.5$ Hz, 1H; H-2), 6.04 (dd, 1H; H-3), 6.49 (d, $J_{1,2} = 2.2$ Hz, 1H; H-1), 6.90–8.25 (m, 50H; 10×Ph) ppm; ^{13}C NMR (75 MHz, $CDCl_3$; partial): $\delta = 61.83$ (C-6), 62.10 (C-6″), 66.47 (C-5′), 66.97 (C-2″), 67.80 (C-4″), 68.07 (C-3″), 68.62 (C-5″), 68.90 (C-6′), 69.28 (C-2), 70.47 (C-3), 71.18 (2C; C-2′ and C-4′), 71.81 (C-5), 73.73 (C-4), 74.99 (C-3′), 91.24 (C-1), 93.42 (C-1″), 100.09 (C-1′), 101.84 (*C*HPh) ppm; elemental analysis calcd (%) for $C_{88}H_{72}O_{25}$ (1529.5): C 69.10, H 4.74; found: C 68.86, H 4.85. Continued elution gave the β,β-linked trisaccharide derivative **15** (930 mg, 73%) as an amorphous solid; $[\alpha]_D^{22} = +96.6$ ($c = 1.11$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.78$ (br, 1H; H-5′), 3.43 (br d, $J_{6a',6b'} = 12.0$ Hz, 1H; H-6a′), 3.85 (br d, 1H; H-6b′), 4.09 (dd, $J_{3',4'} = 3.5$ Hz, 1H; H-3′), 4.20 (dt, $J_{5,6a} = J_{5,6b} = 2.2$ Hz, 1H; H-5), 4.22 (d, 1H; H-4′), 4.23–4.35 (m, 3H; H-5″, H-6a and H-6a″), 4.61 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, 1H; H-4), 4.62–4.72 (m, 2H; H-6b and H-6b″), 4.83 (d, $J_{1',2'} = 8.0$ Hz, 1H; H-1′), 5.12 (d, $J_{1'',2''} = 8.0$ Hz, 1H; H-1″), 5.35 (s, 1H; C*H*Ph), 5.40 (dd, $J_{3'',4''} = 3.3$ Hz, 1H; H-3″), 5.60 (dd, $J_{2',3'} = 10.0$ Hz, 1H; H-2′), 5.75 (dd, $J_{2'',3''} = 10.3$ Hz, 1H; H-2″), 5.86 (dd, $J_{2,3} = 3.5$ Hz, 1H; H-2), 5.92 (dd, 1H; H-4″), 5.97 (dd, 1H; H-3), 6.45 (d, $J_{1,2} = 2.1$ Hz, 1H; H-1), 6.90–8.20 (m, 50H; 10×Ph) ppm; ^{13}C NMR (75 MHz, $CDCl_3$; partial): $\delta = 61.83$ (2C; C-6 and C-6″), 66.80 (C-5′), 67.93 (2C; C-4′ and C-4″), 68.17 (C-6′), 69.02 (C-2″), 69.65 (C-2), 71.09 (C-3), 71.26 (C-2′), 71.37 (2C; C-3″ and C-5″), 71.67 (C-5), 73.11 (C-4), 75.73 (C-3′), 91.18 (C-1), 100.51 (C-1″), 101.60 (2C; C-1′ and *C*HPh) ppm; elemental analysis calcd (%) for $C_{88}H_{72}O_{25}$ (1529.50): C 69.10, H 4.74; found: C 68.86, H 4.71.

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose (16): A solution of the trisaccharide **15** (340 mg, 0.222 mmol) in 80% aq. acetic acid was heated at 70 °C for 3 h, whereafter the mixture was concentrated and the residue coevaporated twice with toluene. FCC (toluene/ethyl acetate, 91:9→65:35) of the residue gave the trisaccharide diol **16** (290 mg, 91%) as an amorphous solid; $[\alpha]_D^{28} = +113$ ($c = 0.97$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.90$ (br, 1H; 6′-OH), 2.94 (br, 1H; 4′-OH), 3.24 (dd, $J_{5',6a'} = 4.6$, $J_{5',6b'} = 5.3$ Hz, 1H; H-5′), 3.33 (ddd, $J_{6a',6b'} = 11.8$, $J_{6a',OH} = 9.1$ Hz, 1H; H-6a′), 3.47 (m, 1H; H-6b′), 3.85 (dd, $J_{3',4'} = 3.0$ Hz, 1H; H-3′), 4.03 (br, 1H; H-4′), 4.08 (br d, $J_{4,5} = 9.5$ Hz, 1H; H-5), 4.23 (dd, $J_{5,6a} = 2.9$ Hz, 1H; H-6a), 4.26 (dd, $J_{5'',6a''} = 5.5$ Hz, 1H; H-5″), 4.42 (br d, $J_{6a,6b} = 12.1$ Hz, 1H; H-6b), 4.45 (dd, $J_{6a'',6b''} = 11.6$ Hz, 1H; H-6a″), 4.49 (t, $J_{3,4} = 9.5$ Hz, 1H; H-4), 4.53 (dd, $J_{5'',6b''} = 7.3$ Hz, 1H; H-6b″), 4.71 (d, $J_{1',2'} = 8.0$ Hz, 1H; H-1′), 4.88 (d, $J_{1'',2''} = 7.9$ Hz, 1H; H-1″), 5.41 (dd, $J_{3'',4''} = 3.6$ Hz, 1H; H-3″), 5.49 (dd, $J_{2',3'} = 10.4$ Hz, 1H; H-2′), 5.69 (dd, $J_{2'',3''} = 10.4$ Hz, 1H; H-2″), 5.80 (dd, $J_{2,3} = 3.3$ Hz, 1H; H-2), 5.88 (m, 2H; H-3 and H-4″), 6.45 (d, $J_{1,2} = 2.7$ Hz, 1H; H-1), 6.80–8.00 (m, 45H; 9×Ph) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 61.59$ (C-6′), 62.05 (C-6″), 62.17 (C-6), 67.90 (C-4″), 68.12 (C-4′), 69.21 (C-2), 69.38 (C-2″), 70.64 (C-3), 70.79 (C-2′), 71.30 (C-3″), 71.44 (C-5″), 71.76 (C-5), 73.02 (C-4), 74.62 (C-5′), 81.32 (C-3′), 91.39 (C-1), 101.20 (C-1″), 101.68 (C-1′), 127.98–130.10 and 132.70–133.83 (Ph), 165.44–167.70 (C=O); elemental analysis calcd (%) for $C_{81}H_{68}O_{25}$ (1441.39): C 67.49, H 4.76; found: C 67.38, H 4.77.

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(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 (17): The trisaccharide diol **16** (700 mg, 0.486 mmol) was dried by coevaporation of pyridine (2×5 mL). The residue was dissolved in pyridine (15 mL) and *p*,*p*′-dimethoxytrityl chloride (247 mg, 0.729 mmol) added. The solution was stirred for 14 h at room temperature, then it was cooled to 0 °C and benzoyl chloride (0.17 mL, 1.46 mmol) was added. After 24 h at room temperature, the reaction mixture was diluted with CH_2Cl_2, washed successively with saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. FCC (toluene/ethyl acetate, 99:1→85:15) of the residue gave the dimethoxytrityl derivative **17** (768 mg, 86%) as an amorphous solid; $[\alpha]_D^{25} = +74.5$ ($c = 1.03$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.11$ (t, $J_{5',6a'} = J_{6a',6b'} = 8.8$ Hz, 1H; H-6a′), 3.24 (dd, $J_{5',6b'} = 5.3$ Hz, 1H; H-6b′), 3.62 (s, 3H; CH_3O), 3.65 (s, 3H; CH_3O), 3.76 (dd, 1H; H-5′), 4.07 (br d, 1H; H-5), 4.25 (dd, $J_{3',4'} = 3.3$ Hz, 1H; H-3′), 4.30 (dd, $J_{5'',6a''} = 6.7$ Hz, 1H; H-5″), 4.42–4.53 (m, 3H; H-6a, H-6b and H-6a″), 4.60 (t, $J_{3,4} = J_{4,5} = 9.4$ Hz, 1H; H-4), 4.83 (d, $J_{1',2'} = 7.9$ Hz, 1H; H-1′), 4.90 (dd, $J_{5'',6b''} = 5.9$, $J_{6a'',6b''} = 11.2$ Hz, 1H; H-6b″), 4.98 (d, $J_{1'',2''} = 7.6$ Hz, 1H; H-1″), 5.39 (dd, $J_{3'',4''} = 3.5$ Hz, 1H; H-3″), 5.50 (dd, $J_{2',3'} = 9.6$ Hz, 1H; H-2′), 5.60 (dd, $J_{2'',3''} = 10.4$ Hz, 1H; H-2″), 5.77–5.83 (m, 2H; H-2 and H-3), 5.94 (d, 1H; H-4″), 6.19 (d, 1H; H-4′), 6.48 (d, $J_{1,2} = 1.6$ Hz, 1H; H-1), 6.62–8.10 (m, 63H; 11×Ph and 2×C_6H_4) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 54.96$ (2C; 2×OCH_3), 59.92 (C-6′), 61.40 (C-6″), 62.13 (C-6), 67.49 (C-4″), 69.58 (2C; C-2 and C-4′), 69.62 (C-2″), 69.96 (C-3), 70.91 (C-3″), 71.39 (C-5″), 71.76 (C-2′), 71.93 (C-5), 72.00 (C-5′), 72.95 (C-4), 76.90 (C-3′), 86.31 (Ar_3C), 91.12 (C-1), 101.25 (2C; C-1′ and C-1″), 112.95, 126.69, 127.65–130.27, 132.36–135.87, 144.10 and 159.25 (C_6H_4 and Ph), 163.95–165.94 (C=O) ppm; elemental analysis calcd (%) for $C_{109}H_{90}O_{28}$ (1847.86): C 70.85, H 4.91; found: C 70.76, H 4.93.

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(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 (18): The trisaccharide derivative **17** (310 mg, 0.17 mmol) was dried by coevaporation of acetonitrile (3 mL). The residue was dissolved in THF (4 mL), 2 M Me_2NH in THF (0.76 mL,

1.51 mmol) was added and the mixture was stirred at room temperature with monitoring by TLC (toluene/ethyl acetate, 9:1). After 24 h, a second portion of 2 M Me_2NH in THF (0.38 mL, 0.76 mmol) was added to the solution. After a further 24 h, the mixture was concentrated and the residue was coevaporated with acetonitrile. FCC (toluene/ethyl acetate, 98:2→80:20) of the residue gave the 1-hydroxy derivative **18** (242 mg, 83%) as an amorphous solid; $[\alpha]_D^{27}=+42$ ($c=0.96$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=3.19$ (dd, $J_{5',6a'}=J_{6a',6b'}=8.8$ Hz, 1H; H-6a′), 3.29 (dd, $J_{5',6b'}=5.5$ Hz, 1H; H-6b′), 3.42 (d, $J_{1,OH}=3.7$ Hz, 1H; OH-1), 3.62 (dd, 1H; H-5′), 3.65 (s, 3H; CH_3O), 3.69 (s, 3H; CH_3O), 4.21 (dd, $J_{3',4'}=3.3$ Hz, 1H; H-3′), 4.24 (ddd, $J_{5,6a}=4.0$ Hz, 1H; H-5), 4.28 (dd, $J_{5'',6a''}=6.6$ Hz, 1H; H-5″), 4.46 (dd, $J_{6a,6b}=12.3$ Hz, 1H; H-6a), 4.47 (dd, $J_{6a'',6b''}=11.1$ Hz, 1H; H-6a″), 4.51 (dd, $J_{3,4}=J_{4,5}=9.8$ Hz, 1H; H-4), 4.56 (dd, $J_{5,6b}=1.6$ Hz, 1H; H-6b), 4.79 (d, $J_{1',2'}=7.9$ Hz, 1H; H-1′), 4.87 (dd, $J_{5'',6b''}=5.8$ Hz, 1H; H-6b″), 4.94 (d, $J_{1'',2''}=7.6$ Hz, 1H; H-1″), 5.92 (dd, $J_{1,2}=1.7$ Hz, 1H; H-1), 5.37 (dd, $J_{3'',4''}=3.3$ Hz, 1H; H-3″), 5.46 (dd, $J_{2',3'}=9.9$ Hz, 1H; H-2′), 5.57 (dd, $J_{2'',3''}=10.5$ Hz, 1H; H-2″), 5.61 (dd, $J_{2,3}=3.3$ Hz, 1H; H-2), 5.75 (dd, 1H; H-3), 5.93 (d, 1H; H-4″), 6.09 (d, 1H; H-4′), 6.60–8.23 (m, 58H; 10× Ph and 2×C_6H_4) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=55.01$ (2C; 2×OCH_3), 60.25 (C-6′), 61.42 (C-6″), 62.68 (C-6), 67.49 (C-4″), 69.15 (C-2″), 69.55 (C-5), 69.62 (C-4′), 69.67 (C-3), 70.86 (C-3″), 71.15 (C-2), 71.42 (C-5″), 71.81 (C-2′), 72.22 (C-5′), 72.85 (C-4), 76.74 (C-3′), 86.21 (Ar_3C), 91.99 (C-1), 100.46 (C-1″), 101.19 (C-1′), 112.98, 126.69, 127.73–130.42 and 132.40–133.42, 135.33, 136.11, 144.31, 158.31 (C_6H_4 and Ph), 164.20–166.08 (C=O) ppm; elemental analysis calcd (%) for $C_{102}H_{86}O_{27}$ (1743.76): C 70.26, H 4.97; found: C 70.14, H 4.93.

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(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 (11): The trisaccharide derivative **18** (220 mg, 0.126 mmol) was dried by coevaporation of acetonitrile (2×3 mL). Phosphorus trichloride (0.051 mL, 0.58 mmol) was added to a stirred solution of imidazole (133 mg, 1.95 mmol) in acetonitrile (5 mL) at 0°C and this was followed by addition of triethylamine (0.29 mL, 2.07 mmol). The mixture was stirred for 15 min, after which time a solution of **18** in acetonitrile (10 mL) was added dropwise over a period of 20 min at 0°C. The mixture was stirred at room temperature for 15 min and quenched with 1 M aq. triethylammonium (TEA) hydrogen carbonate (pH 7; 3 mL). The clear solution was stirred for 15 min and CH_2Cl_2 was added, then the organic layer was washed in turn with ice-water (twice) and cold 0.5 M TEA hydrogen carbonate (twice), dried by filtration through cotton wool and concentrated. FCC (CH_2Cl_2/MeOH/Et_3N, 98.5:0.5:1→92:7:1) of the residue gave the trisaccharide hydrogenphosphonate **11** (211 mg, 88%) as an amorphous solid; $[\alpha]_D^{26}=+48.5$ ($c=1$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=$ 1.30 (t, 9H, $J=7.1$ Hz; 3×$MeCH_2$), 3.00–3.09 (m, 7H; 3×MeCH_2 and H-6a′), 3.19 (dd, $J_{5',6b'}=5.3$, $J_{6a',6b'}=8.4$ Hz, 1H; H-6b′), 3.60 (s, 3H; CH_3O), 3.62 (s, 3H; CH_3O), 3.68 (dd, $J_{5',6a'}=8.5$ Hz, 1H; H-5′), 4.16 (brd, 1H; H-5), 4.20 (dd, $J_{3',4'}=3.3$ Hz, 1H; H-3′), 4.29 (dd, $J_{5'',6a''}=7.6$ Hz, 1H; H-5″), 4.42 (t, $J_{3,4}=J_{4,5}=9.9$ Hz, 1H; H-4), 4.46 (br, 2H; H-6a and H-6b), 4.49 (dd, $J_{6a'',6b''}=11.3$ Hz, 1H; H-6a″), 4.72 (d, $J_{1',2'}=7.8$ Hz, 1H; H-1′), 4.87 (dd, $J_{5'',6b''}=5.4$ Hz, 1H; H-6b″), 4.96 (d, $J_{1'',2''}=7.9$ Hz, 1H; H-1″), 5.37 (dd, $J_{3'',4''}=2.4$ Hz, 1H; H-3″), 5.38 (dd, $J_{2',3'}=10.7$ Hz, 1H; H-2′), 5.58 (dd, $J_{2'',3''}=10.5$ Hz, 1H; H-2″), 5.61 (dd, $J_{2,3}=2.9$ Hz, 1H; H-3), 5.63 (dd, $J_{1,2}=1.4$ Hz, 1H; H-2), 5.68 (dd, $J_{1,P}=9.0$ Hz, 1H; H-1), 5.92 (d, 1H; H-4″), 6.18 (d, 1H; H-4′), 6.96 (d, $J_{H,P}=635.4$ Hz, 1H; HP) and 6.55–8.25 (m, 58H; 10×Ph and 2×C_6H_4) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=$ 8.67 ($MeCH_2N$), 45.70 (MeCH_2N), 55.00 (2C; 2×OCH_3), 59.76 (C-6′), 61.49 (C-6″), 62.61 (C-6), 67.54 (C-4″), 69.58 (2C; C-4′ and C-2″), 69.92 (C-3), 70.70 (C-5), 70.93 (C-3″), 71.00 (d, $J_{C,P}=6.9$ Hz; C-2), 71.47 (C-5″), 71.67 (C-2′), 71.79 (C-5′), 72.86 (C-4), 76.90 (C-3′), 86.20 (Ar_3C), 92.64 (br; C-1), 101.05 (C-1″), 101.34 (C-1′), 112.99, 127.88–133.08, 133.23, 144.12 and 158.32 (C_6H_4 and Ph), 164.08–165.96 (C=O) ppm; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta=-0.39$ ppm; ES MS (+): m/z (%): 1808.15 (20) $[M-Et_3N+H]^+$, 2010.40 (100) $[M+Et_3N+H]^+$; calcd for $C_{108}H_{102}NO_{29}P$: $[M]=1907.63$.

Dec-9-enyl 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, triethylammonium salt (19): The trisaccharide hydrogenphosphonate **12**[24] (40 mg, 0.021 mmol) was dried by coevaporation of pyridine (2×2 mL). The residue was dissolved in pyridine (1 mL) and dec-9-en-1-ol (0.011 mL, 0.063 mmol) was added to the solution followed by pivaloyl chloride (0.008 mL, 0.063 mmol). The mixture was stirred at room temperature for 15 min, whereafter a freshly prepared solution of iodine (11 mg, 0.042 mmol) in 95% aq. pyridine (1 mL) was added. After 10–15 min, CH_2Cl_2 was added and the solution was washed successively with cold 1 M aq. $Na_2S_2O_3$ and cold 0.5 M aq. TEA hydrogen carbonate, dried by filtration through cotton wool and concentrated. The residue was dissolved in CH_2Cl_2 (1.5 mL) and then TFA (0.015 mL) was added at 0°C. After 1 min, the solution was diluted with CH_2Cl_2, washed successively with ice-cold saturated aq. $NaHCO_3$ and 0.5 M aq. TEA hydrogen carbonate, dried by filtration through cotton wool and concentrated. FCC (CH_2Cl_2/MeOH/Et_3N, 99:0:1→96:3:1) of the residue gave the trisaccharide phosphate derivative **19** (34 mg, 90%) as an amorphous solid; $[\alpha]_D^{22}=+34.5$ ($c=0.8$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=1.20$ (m, 10H; and 5×CH_2), 1.30 (t, 9H; 3×$MeCH_2$), 1.52 (quintet, $J=7.0$ Hz, 2H; OCH_2CH_2), 1.99 (m, 2H; CH_2CH_2CH=), 2.96 (dd, $J_{5',6a'}=8.3$, $J_{6a',6b'}=$ 12.1 Hz, 1H; H-6a′), 3.00–3.12 (m, 7H; 3×MeCH_2N and H-6b′), 3.38 (dd, $J_{5',6b'}=6.7$ Hz, 1H; H-5′), 3.78–3.88 (m, 2H; $POCH_2$), 4.00 (dt, $J_{5'',6a''}=$ $J_{5'',6b''}=3.5$ Hz, 1H; H-5″), 4.05 (dd, $J_{3',4'}=3.2$ Hz, 1H; H-3′), 4.25 (brd, 1H; H-5), 4.35 (dd, $J_{6a'',6b''}=12.4$ Hz, 1H; H-6a″), 4.38 (dd, $J_{5,6a}=2.2$, $J_{6a,6b}=11.8$ Hz, 1H; H-6a), 4.43 (t, $J_{3,4}=J_{4,5}=10.1$ Hz, 1H; H-4), 4.47 (m, 2H; H-6b and H-6b″), 4.70 (d, $J_{1'',2''}=8.2$ Hz, 1H; H-1″), 4.90 (brd, $^3J_{cis}=$ 11.5 Hz, 1H; CH=HCH), 4.92 (d, $J_{1',2'}=8.1$ Hz, 1H; H-1′), 4.97 (brd, $^3J_{trans}=17.1$ Hz, 1H; CH=HCH), 5.29 (dd, $J_{2'',3''}=9.7$ Hz, 1H; H-2″), 5.47 (t, $J_{3'',4''}=J_{4'',5''}=9.7$ Hz, 1H; H-4″), 5.50 (d, 1H; H-4′), 5.52 (dd, $J_{2',3'}=$ 10.2 Hz, 1H; H-2′), 5.60 (dd, $J_{1,P}=8.2$ Hz, 1H; H-1), 5.64 (dd, $J_{1,2}=$ 1.2 Hz, 1H; H-2), 5.67 (dd, 1H; H-3″), 5.76 (dd, $J_{2,3}=3.3$ Hz, 1H; H-3), 5.78 (ddt, $J_{H,CH2}=7.0$ Hz, 1H; CH=CH_2), 6.95–8.00 (m, 45H; 9× Ph) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=12.62$ ($MeCH_2N$), 25.87, 28.81–29.76 and 33.89 (CH_2), 31.03 (d, $J_{C,P}=8.2$ Hz; $POCH_2CH_2$), 45.71 (MeCH_2N), 59.36 (C-6′), 62.22 (C-6″), 62.39 (C-6), 66.06 (d, $J_{C,P}=6.1$ Hz; $POCH_2$), 68.80 (C-4″), 69.85 (C-4′), 69.94 (2C; C-3 and C-5), 70.75 (d, $J_{C,P}=8.7$ Hz; C-2), 71.13 (C-2″), 71.59 (C-2′), 72.07 (C-5″), 72.34 (C-3″), 72.63 (C-4), 73.70 (C-5′), 78.65 (C-3′), 93.43 (br; C-1), 100.49 (C-1″), 101.56 (C-1′), 113.96 (CH=CH_2), 127.22–130.56 and 132.14–133.29 (Ph), 139.05 (CH=CH_2), 164.21–167.46 (C=O) ppm; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta=-3.02$ ppm; ES MS (−): m/z (%): 1657.2 (100) $[M-Et_3N-H]^-$; calcd for $C_{97}H_{102}NO_{28}P$: $[M]=1759.63$.

Dec-9-enyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate, triethylammonium salt (20): This compound was prepared by condensation of the trisaccharide hydrogenphosphonate **11** (40 mg, 0.021 mmol) and dec-9-en-1-ol (0.011 mL, 0.063 mmol) in the presence of pivaloyl chloride (0.008 mL, 0.063 mmol), followed by oxidation with iodine (11 mg, 0.042 mmol) and detritylation with 1% TFA in CH_2CH_2, as described for the preparation of compound **19**. FCC (CH_2Cl_2/MeOH/Et_3N, 99:0:1→96:3:1) of the residue gave the trisaccharide phosphate derivative **20** (33 mg, 88%) as an amorphous solid; $[\alpha]_D^{19}=$ +47 ($c=0.85$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=1.21$ (m, 10H; 5×CH_2), 1.32 (t, 9H; 3×$MeCH_2$), 1.52 (quintet, $J=7.0$ Hz, 2H; OCH_2CH_2), 1.99 (m, 2H; CH_2CH_2CH=), 2.96 (dd, 1H, $J_{5',6a'}=8.1$ Hz; H-6a′), 3.06 (quartet, 6H; 3×MeCH_2), 3.12 (dd, $J_{6a',6b'}=12.1$ Hz, 1H; H-6b′), 3.41 (dd, $J_{5',6b'}=6.0$ Hz, 1H; H-5′), 3.85 (m, 2H; $POCH_2$), 4.10 (dd, $J_{3',4'}=3.2$ Hz, 1H; H-3′), 4.13 (dd, $J_{5'',6a''}=7.0$ Hz, 1H; H-6a″), 4.19 (dd, $J_{5'',6b''}=5.7$ Hz, 1H; H-5″), 4.27 (brd, 1H; H-5), 4.39 (brd, $J_{6a,6b}=12.0$ Hz, 1H; H-6a), 4.45 (t, $J_{3,4}=J_{4,5}=10.0$ Hz, 1H; H-4), 4.48 (brd, 1H; H-6b), 4.56 (dd, $J_{6a'',6b''}=10.9$ Hz, 1H; H-6b″), 4.72 (d, $J_{1'',2''}=7.9$ Hz, 1H; H-1″), 4.90 (brd, $^3J_{cis}=11.4$ Hz, 1H; CH=HCH), 4.91 (d, $J_{1',2'}=7.5$ Hz, 1H; H-1′), 4.97 (brd, $^3J_{trans}=17.1$ Hz, 1H; CH=HCH), 5.35 (dd, $J_{3'',4''}=3.5$ Hz, 1H; H-3″), 5.52 (dd, $J_{2',3'}=11.0$ Hz, 1H; H-2′), 5.54 (dd, $J_{2'',3''}=10.3$ Hz, 1H; H-2″), 5.59 (d, 1H; H-4′), 5.62 (brd, $J_{1,P}=7.5$ Hz, 1H; H-1), 5.67 (br, 1H; H-2), 5.74–5.84 (m, 3H; H-3, H-4″ and CH=CH_2) and 6.95–8.15 (m, 45H; 9×Ph) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=13.15$ ($MeCH_2N$), 25.81, 28.88–29.34 and 33.75 (CH_2), 30.61 (d, $J_{C,P}=8.4$ Hz; $POCH_2CH_2$), 45.65 (MeCH_2N), 59.55 (C-6′), 61.52 (C-6″), 62.41 (C-6), 66.13 (d, $J_{C,P}=$ 5.9 Hz; $POCH_2$), 67.49 (C-4″), 69.38 (C-2″), 69.77 (C-4′), 69.98 (C-3), 70.47 (C-5), 70.75 (d, $J_{C,P}=8.7$ Hz; C-2), 71.08 (C-3″), 71.34 (2C; C-2′ and C-5″), 72.39 (C-4), 73.75 (C-5′), 78.31 (C-3′), 93.51 (br; C-1), 100.52

(C-1″), 101.71 (C-1′), 113.98 (CH=*C*H$_2$), 128.64–134.91 (Ph), 139.04 (*C*H=CH$_2$), 164.43–166.88 and 168.85 (C=O) ppm; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = -3.04$ ppm; ES MS (−): *m*/*z* (%): 1657.3 (100) $[M-Et_3N-H]^-$; calcd for $C_{97}H_{102}NO_{28}P$: $[M] = 1759.63$.

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-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate], bis-triethylammonium salt (22): A mixture of compounds **11** (120 mg, 0.063 mmol) and **21**[19] (98 mg, 0.076 mmol) was dried by coevaporation of pyridine (2× 2 mL). The residue was dissolved in pyridine (2 mL), pivaloyl chloride (0.023 mL, 0.19 mmol) was added and the mixture was stirred at room temperature for 20–30 min, whereafter a freshly prepared solution of iodine (32 mg, 0.126 mmol) in 95% aq. pyridine (1.5 mL) was added. After 20 min, CH_2Cl_2 was added and the solution was washed successively with cold 1 M aq. $Na_2S_2O_3$ and cold 0.5 M aq. TEA hydrogen carbonate, dried by filtration through cotton wool and concentrated. The residue was dissolved in CH_2Cl_2 (5 mL) and 2% TFA in CH_2Cl_2 (5 mL) was added at 0 °C. After 1 min, the solution was diluted with CH_2Cl_2, washed successively with ice-cold saturated aq. $NaHCO_3$ and 0.5 M aq. TEA hydrogen carbonate, dried by filtration through cotton wool and concentrated. FCC (CH_2Cl_2/MeOH/Et_3N, 98:1:1→96:3:1) of the residue gave the pentasaccharide diphosphate derivative **22** (124 mg, 68%) as an amorphous solid; $[\alpha]_D^{21} = +49$ ($c = 0.99$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.90$–1.32 (m, 28H; 5×CH_2 and 6×*Me*CH_2), 1.44 (quintet, $J = 7.0$ Hz, 2H; OCH_2CH_2), 1.91 (m, 2H; CH_2CH_2CH=), 2.70–3.00 (m, 14H; 6×MeCH_2, H-6a‴ and H-6b‴), 3.15–3.23 (m, 1H; H-6a′), 3.25 (t, $J_{5''',6a'''} = J_{5''',6b'''} = 5.8$ Hz, 1H; H-5‴), 3.73–3.84 (m, 3H; $POCH_2$ and H-6b′), 3.96 (dd, $J_{3''',4'''} = 3.7$ Hz, 1H; H-3‴), 3.99 (m, 1H; H-5′), 4.05 (br d, 1H; H-5″), 4.08 (dd, $J_{5''',6a'''} = 4.8$, $J_{6a''',6b'''} = 12.4$ Hz, 1H; H-6a‴), 4.19–4.27 (m, 4H; H-5, H-5‴, H-6a and H-6b‴), 4.29 (t, $J_{3'',4''} = J_{4'',5''} = 9.8$ Hz, 1H; H-4″), 4.36–4.50 (m, 4H; H-4, H-6b, H-6a″ and H-6b″), 4.66 (d, $J_{1''',2'''} = 7.9$ Hz, 1H; H-1‴), 4.80 (d, $J_{1',2'} = 7.5$ Hz, 1H; H-1′), 4.82 (br d, $^3J_{cis} = 11.0$ Hz, 1H; CH=HC*H*), 4.85 (d, $J_{1''',2'''} = 7.7$ Hz, 1H; H-1‴), 4.88 (br d, $^3J_{trans} = 17.0$ Hz, 1H; CH=*H*CH), 5.24 (dd, $J_{3''',4'''} = 3.3$ Hz, 1H; H-3‴), 5.27 (dd, $J_{3',4'} = 3.2$ Hz, 1H; H-3′), 5.29 (br d, $J_{1,P} = 8.3$ Hz, 1H; H-1), 5.41 (dd, $J_{2''',3'''} = 10.8$ Hz, 1H; H-2‴), 5.42 (dd, $J_{2''',3'''} = 9.6$ Hz, 1H; H-2‴), 5.43–5.48 (m, 2H; H-2″ and H-4‴), 5.55 (dd, $J_{1'',2''} = 1.5$, $J_{1'',P} = 7.9$ Hz, 1H; H-1″; and dd, $J_{2',3'} = 10.1$ Hz, 1H; H-2′), 5.61 (dd, $J_{2'',3''} = 3.2$ Hz, 1H; H-3″; and br d, $J_{2,3} = 3.2$ Hz, 1H; H-2), 5.70 (coinciding doublets, $J_{3',4'} = J_{3''',4'''} = 3.3$ Hz, 2H; H-4′ and H-4‴), 5.71 (ddt, $J_{H,CH2} = 7.1$ Hz, 1H; C*H*=CH_2), 5.78 (dd, $J_{3,4} = 9.5$ Hz, 1H; H-3), 7.10–8.05 (m, 75H; 15×Ph) ppm; ^{13}C NMR: see Table 2; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = -2.76$ (P), −4.58 (P′) ppm; ES MS (−): *m*/*z* (%): 1342.5 (100) $[M-2Et_3N-2H]^{2-}$; calcd for $C_{157}H_{162}N_2O_{47}P_2$: $[M] = 2888.98$.

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-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate], bis-triethylammonium salt (23): This compound was prepared by condensation of the disaccharide hydrogenphosphonate **10**[23] (93 mg, 0.065 mmol) and the disaccharide phosphate **21**[19] (70 mg, 0.054 mmol) in the presence of pivaloyl chloride (0.025 mL, 0.195 mmol), followed by oxidation with iodine (24 mg, 0.108 mmol) and detritylation with 1% TFA in CH_2CH_2 as described for the preparation of compound **22**. FCC (CH_2Cl_2/MeOH/Et_3N, 98.5:1:0.5→93.5:6:0.5) gave the tetrasaccharide diphosphate derivative **23** (94 mg, 72%) as an amorphous solid; $[\alpha]_D^{20} = +62$ ($c = 0.94$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.21$ (m, 10H; 5×CH_2), 1.24 (t, 18H; 6×*Me*CH_2), 1.53 (quintet, $J = 7.0$ Hz, 2H; OCH_2CH_2), 1.99 (m, 2H; CH_2CH_2CH=), 2.96 (quartet, 12H; 6×MeCH_2), 3.08 (dd, 1H, $J_{6a'',6''} = 12.0$ Hz; H-6a‴), 3.21 (dd, 1H; H-6b‴), 3.34 (q, 1H, $J_{5',6a'} = J_{6a',6b'} = J_{6a',P} = 9.8$ Hz; H-6a′), 3.55 (t, $J_{5''',6a'''} = J_{5''',6b'''} = 6.4$ Hz, 1H; H-5‴), 3.87 (m, 2H; $POCH_2$), 3.95 (m, 1H; H-6b′), 4.06 (dd, $J_{5',6b'} = 5.4$ Hz, 1H; H-5′), 4.32–4.38 (m, 3H; H-5, H-5″ and H-6a), 4.43 (br d, $J_{6a,6b} = 11.0$ Hz, 1H; H-6b), 4.50 (t, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H; H-4), 4.52 (t, $J_{3'',4''} = J_{4'',5''} = 8.8$ Hz, 1H; H-4″), 4.53 (br d, $J_{6a'',6b''} = 11.6$ Hz, 1H; H-6a″), 4.59 (dd, $J_{5'',6b''} = 1.7$ Hz, 1H; H-6b″), 4.90 (d, $J_{1',2'} = 8.1$ Hz, 1H; H-1′), 4.90 (br d, $^3J_{cis} = 10.0$ Hz, 1H; CH=HC*H*), 4.96 (d, $J_{1''',2'''} = 8.1$ Hz, 1H; H-1‴), 4.97 (br d, $^3J_{trans} = 17.1$ Hz, 1H; CH=*H*CH), 5.35 (dd, $J_{3''',4'''} = 3.1$ Hz, 1H; H-3‴), 5.39 (dd, $J_{3',4'} = 3.0$ Hz, 1H; H-3′), 5.44 (br d, $J_{1,P} = 6.7$ Hz, 1H; H-1), 5.58 (d, 1H; H-4′), 5.64–5.68 (m, 2H; H-1″ and H-2), 5.67 (dd, $J_{2''',3'''} = 10.2$ Hz, 1H; H-2‴), 5.71 (br, 1H, H-2″), 5.73 (dd, $J_{2',3'} = 10.1$ Hz, 1H; H-2′), 5.78 (ddt, $J_{H,CH_2} = 7.1$ Hz, 1H; C*H*=CH_2), 5.84 (d, 1H; H-4‴), 5.87 (dd, $J_{2'',3''} = 3.5$ Hz, 1H; H-3″), 5.88 (dd, $J_{2,3} = 3.2$ Hz, 1H; H-3), 7.10–8.05 (m, 60H; 12×Ph) ppm; ^{13}C NMR: see Table 2; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = -2.51$ (P), −4.29 (P′) ppm; MALDI-TOF MS (−): *m*/*z* (%): 2211.47 (100) $[M-2Et_3N-H]^-$, 2233.47 (25) $[M-2Et_3N-2H+Na]^-$, 2249.45 (5) $[M-2Et_3N-2H+K]^-$; calcd for $C_{130}H_{140}N_2O_{39}P_2$: $[M] = 2414.85$.

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-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-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate]}, tris-triethylammonium salt (24): This compound was prepared by condensation of the trisaccharide hydrogenphosphonate **11** (99 mg, 0.053 mmol) and the tetrasaccharide diphosphate **23** (90 mg, 0.037 mmol) in the presence of adamantane-1-carbonyl chloride (31 mg, 0.15 mmol; used as a condensing reagent instead of pivaloyl chloride), followed by oxidation with iodine (19 mg, 0.074 mmol) and detritylation with 1% TFA in CH_2Cl_2 as described for the preparation of compound **22.** FCC (CH_2Cl_2/MeOH/Et_3N, 98.8:0.2:1→93:6:1) gave the heptasaccharide triphosphate derivative **24** (125 mg, 84%) as an amorphous solid; $[\alpha]_D^{19} = +50.5$ ($c = 0.92$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; partial): $\delta = 1.00$–1.29 (m, 37H; 5×CH_2 and 9×*Me*CH_2), 1.47 (quintet, $J = 7.0$ Hz, 2H; OCH_2CH_2), 1.93 (m, 2H; CH_2CH_2CH=), 2.79 (dd, $J_{6a'''',6''''} = 12.0$ Hz, 1H; H-6a‴″), 2.90 (quartet, 18H; 9×MeCH_2), 2.95 (m, 1H; H-6b‴″), 3.27 (t, $J_{5'''',6a''''} = J_{5'''',6b''''} = 6.4$ Hz, 1H; H-5‴″), 3.73–3.81 (m, 2H; $POCH_2$), 4.60 (d, $J_{1'''',2''''} = 7.8$ Hz, 1H; H-1‴″), 4.82 (d, $J_{1',2'} = 7.2$ Hz, 1H; H-1′), 4.83 (br d, $^3J_{cis} = 11.3$ Hz, 1H; CH=HC*H*), 4.85 (d, $J_{1'''',2''''} = 8.3$ Hz, 1H; H-1‴″), 4.89 (br d, $^3J_{trans} = 17.0$ Hz, 1H; CH=*H*CH), 4.92 (d, $J_{1''',2'''} = 8.0$ Hz, 1H; H-1‴), 5.35 (br d, $J_{1,P} = 6.5$ Hz, 1H; H-1), 5.43 (dd, $J_{2'''',3''''} = 10.2$ Hz, 1H; H-2‴″), 5.44 (dd, $J_{2'''',3''''} = 10.1$ Hz, 1H; H-2‴″), 5.57 (br d, $J_{1''',P} = 7.0$ Hz, 1H; H-1‴), 5.59 (br d, $J_{1'',P} = 7.8$, 1H; H-1″), 5.65 (br d, $J_{2,3} = 2.8$ Hz, 1H; H-2), 5.66–5.77 (m, 5H; H-4′, H-4‴, H-4‴″, H-4‴″ and C*H*=CH_2), 5.82 (dd, $J_{3,4} = 9.5$ Hz, 1H; H-3), 6.85–8.05 (m, 105H; 21×Ph) ppm; ^{13}C NMR: see Table 2; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = -2.79$ (P), −4.52 (P′ and P″; ratio 1:2) ppm; ES MS (−): *m*/*z* (%): 1237.2 (100) $[M-3Et_3N-3H]^{3-}$, 1855.7 (25) $[M-3Et_3N-2H]^{2-}$; calcd for $C_{217}H_{222}N_3O_{66}P_3$: $[M] = 4018.33$.

Dec-9-enyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate 6-{2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(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]}, tris-triethylammonium salt (25): This compound was prepared by condensation of the disaccharide hydrogenphosphonate **10**[23] (56 mg, 0.039 mmol) and the pentasaccharide diphosphate **22** (66 mg, 0.023 mmol) in the presence of pivaloyl chloride (0.014 mL, 0.117 mmol), followed by oxidation with iodine (20 mg, 0.078 mmol) and detritylation with 1% TFA in CH_2Cl_2 as described for the preparation of compound **22.** FCC (CH_2Cl_2/MeOH/Et_3N, 98.5:1:0.5→96.5:3:0.5) gave the heptasaccharide triphosphate derivative **25** (59 mg, 65%) as an amorphous solid; $[\alpha]_D^{21} = +54$ ($c = 1.04$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; partial): $\delta = 1.00$–1.30 (m, 37H; 5×CH_2 and 9×*Me*CH_2), 1.48 (quintet, $J = 7.0$ Hz, 2H; OCH_2CH_2), 1.92 (m, 2H; CH_2CH_2CH=), 2.88 (quartet, 18H; 9×MeCH_2), 3.16 (dd, $J_{6a'''',6''''} = 11.4$ Hz; 1H; H-6a‴″), 3.27 (dd, 1H; H-6b‴″), 3.54 (t, $J_{5'''',6a''''} = J_{5'''',6b''''} = 6.0$ Hz, 1H; H-5‴″), 3.82 (m, 2H; $POCH_2$), 4.50 (d, $J_{1'''',2''''} = 8.3$ Hz, 1H; H-1‴″ (side chain)), 4.83 (d, $J_{1',2'} = 8.0$ Hz, and br d, $^3J_{cis} = 10.1$ Hz, 2H; H-1′ and CH=HC*H*, respectively), 4.90 (br d, $^3J_{trans} = 17.2$ Hz, 1H; CH=*H*CH), 4.92 (d, $J_{1''',2'''} = 7.6$ Hz, 1H; H-1‴), 5.00 (d, $J_{1'''',2''''} = 7.8$ Hz, 1H; H-1‴″), 5.20 (dd, $J_{2'''',3''''} = 9.5$ Hz, 1H; H-2‴″), 5.34 (br d, $J_{1,P} = 7.3$ Hz, 1H; H-1), 5.53–5.62 (m, 4H; H-1″, H-1‴″, H-2‴ and H-2‴″ (side chain)), 5.65 (br d, $J_{2,3} = 2.8$ Hz, 1H; H-2), 5.66–5.76 (m, 5H; H-4′, H-4‴, H-4‴″, H-4‴″ (side chain) and C*H*=CH_2), 6.80–8.00 (m, 105H; 21×Ph) ppm; ^{13}C NMR: see Table 2; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = -2.86$ (P), −3.57 (P″), −4.40 (P′) ppm; ES MS (−): *m*/*z* (%): 1237.2 (100) $[M-3Et_3N-3H]^{3-}$, 1855.9 (40) $[M-3Et_3N-2H]^{2-}$; calcd for $C_{217}H_{222}N_3O_{66}P_3$: $[M] = 4018.33$.

Table 2. ^{13}C NMR (125 MHz, $CDCl_3$) data (δ_C in ppm with multiplicity (where applicable) and the $J_{C,P}$ coupling constant in Hz (in parenthesis)) for the protected oligophosphosaccharide derivatives **22**–**26**.

Residue	Atom	**23**[a]	**22**[a]	**24**[a]	**25**[a]	**26**[a]
dec-9-enyl	C-1	65.87, br	66.01, br	65.92, br	66.16, d (5.0)	66.09, d (5.4)
	C-2	30.58, br	30.68, d (5.4)	30.38, br	30.71, d (7.6)	30.84, br
	C-9	139.18	139.14	139.03	139.24	139.31
	C-10	113.91	113.98	113.72	113.96	113.99
Man	C-1	93.44, br	93.52, br	93.36, d (5.1)	93.56, br	93.53, br
	C-2	70.90, d (7.5)	70.91, d (5.8)	70.86, d (7.1)	70.86, br	70.88, br
	C-3	69.73	69.87	69.76	69.63	70.14
	C-4	73.50	73.50	73.39	73.39	72.55
	C-5	69.78	70.19	70.17	70.26	70.41
	C-6	62.36	62.42	62.26	62.44	62.50
Gal	C-1	101.29	101.70	101.20	100.93	101.76
	C-2	70.26	70.42	70.25	70.41	70.59
	C-3	72.24	72.15	72.18	72.16	72.22
	C-4	67.09	67.17	67.04	67.36	67.34
	C-5	71.92, d (9.0)	71.90, d (9.0)	71.83, d (7.1)	71.79, d (9.0)	71.81, d (7.0)
	C-6	61.44, br	61.50, br	61.30, br	61.69, br	60.78, br
Man′	C-1	93.18, br	93.36, br	93.21, br	93.35, br	93.53, br
	C-2	70.52, d (7.5)	70.67, d (7.8)	70.55, d (7.5)	70.59, d (6.9)	70.64, br
	C-3	69.63	69.65	69.59	69.63	70.09
	C-4	72.77	72.29	73.39	73.23	72.55
	C-5	70.12	70.33	70.17	70.35	70.41
	C-6	62.36	62.34	62.11	62.38	62.43
Gal′	C-1	100.33	101.70	101.07	100.93	100.71
	C-2	70.12	71.34	70.29	71.76	71.51
	C-3	71.85	78.41	71.99	78.94	77.90
	C-4	68.38	69.58	67.04	68.57	68.60
	C-5	74.10	73.70	71.72, d (8.5)	72.00, d (9.0)	71.81, d (7.0)
	C-6	59.96	59.50	61.30, br	63.33, br	60.78, br
Gal(1→3)Gal′	C-1		100.39		100.39	100.44
	C-2		69.36		69.16	69.38
	C-3		71.06		71.03	71.08
	C-4		67.46		67.44	67.44
	C-5		71.34		71.76	71.34
	C-6		61.50		60.74	61.50
Man″	C-1			93.21, br	93.56, br	93.53, br
	C-2			70.69, d (7.5)	70.88, br	70.88, br
	C-3			69.50	69.16	69.60
	C-4			72.28	72.29	72.22
	C-5			70.17	70.13	70.33
	C-6			62.01	62.38	62.37
Gal″	C-1			101.55	100.39	101.76
	C-2			71.23	70.41	71.34
	C-3			78.28	72.16	78.49
	C-4			69.32	68.57	69.60
	C-5			73.59	74.36	73.96
	C-6			59.35	60.30	59.77
Gal(1→3)Gal″	C-1			100.21		100.44
	C-2			69.27		69.38
	C-3			70.95		71.08
	C-4			67.38		67.44
	C-5			71.23		71.34
	C-6			61.39		61.50
C=O		164.62–166.33	165.19–165.70	164.00–167.43	164.28–166.41	164.13–166.08
C_6H_4 and C_6H_5		127.99–130.00	127.93–128.72	127.66–130.00	128.00–129.62	127.73–130.39
		132.71–133.58	132.56–133.44	132.28–133.14	132.38–133.63	132.33–133.55

[a] Additional signals of Et_3NH^+ (δ_C = 8.41–12.94 (CH_3) and 45.24–45.54 (CH_2)) and CCH_2C (δ_C = 25.24–25.65, 28.55–29.82 and 33.31–34.76) 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-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate 6-[2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate]}, tris-triethylammonium salt (26): This compound was prepared by condensation of the trisaccharide hydrogenphosphonate **11** (50 mg, 0.026 mmol) and the pentasaccharide diphosphate **22** (50 mg, 0.017 mmol) in the presence of pivaloyl chloride (0.0096 mL, 0.078 mmol), followed by oxidation with iodine (13 mg, 0.052 mmol) and detritylation with 1% TFA in CH_2Cl_2 as described for the preparation of compound **22.** FCC (CH_2Cl_2/MeOH/

Et_3N, 99.5:0:0.5→96.5:3:0.5) gave the octasaccharide triphosphate derivative **26** (57 mg, 76%) as an amorphous solid; $[\alpha]_D^{19} = +50.5$ ($c = 0.96$ in $CHCl_3$); ^{13}C NMR: see Table 2; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = -2.80$ (P), -3.76 (P″), -4.59 (P′) ppm; ES MS (−): *m/z* (%): 1395.0 (100) $[M-3Et_3N-3H]^{3-}$, 2093.1 (65) $[M-3Et_3N-2H]^{2-}$; calcd for $C_{244}H_{244}N_3O_{74}P_3$: $[M] = 4492.46$.

Dec-9-enyl β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate, ammonium salt (5): The protected trisaccharide **19** (33 mg, 0.019 mmol) was dissolved in 0.25 M NaOMe in MeOH (3 mL) and the mixture was stirred at room temperature. After 47 h, the solution was deionised with Dowex 50W-X4 (H^+) resin, filtered and immediately neutralised with Et_3N. After the solution had been concentrated, water (5×5 mL) was evaporated off from the residue to remove methyl benzoate. ^1H NMR spectroscopy of the prepared compound then revealed the presence of residual aromatic signals. After additional treatment with 0.25 M NaOMe in MeOH (72 h, room temperature) followed by a 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) and was eluted with a linear gradient of aq. NH_4HCO_3 (0→0.2 M) in water/propan-2-ol (3:2) at 1 mL min^{-1} to afford the trisaccharide phosphate **5** (14 mg, 95%) as an amorphous solid; $[\alpha]_D^{25} = +17.9$ ($c = 0.8$ in MeOH); ^1H NMR (selected data): see Table 3; ^{13}C NMR: see Table 1; ^{31}P NMR (121 MHz, D_2O): $\delta = -1.68$ ppm; ES MS (−): *m/z* (%): 721.1 (100) $[M-NH_3-H]^-$; calcd for $C_{28}H_{54}NO_{19}P$: $[M] = 739.30$.

Dec-9-enyl β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate, ammonium salt (6): Debenzoylation of the protected trisaccharide **20** (30 mg, 0.017 mmol) with 0.25 M NaOMe in MeOH (47 h and then additional treatment for 48 h), followed by work-up and isolation by anion-exchange chromatography as described for the preparation of **5**, gave the trisaccharide phosphate **6** (11 mg, 88%) as an amorphous solid; $[\alpha]_D^{27} = +23$ ($c = 1.1$ in MeOH); ^1H NMR (selected data): see Table 3; ^{13}C NMR: see Table 1; ^{31}P NMR (121 MHz, D_2O): $\delta = -1.68$ ppm; ES MS (−): *m/z* (%): 720.9 (100) $[M-NH_3-H]^-$; calcd for $C_{28}H_{54}NO_{19}P$: $[M] = 739.30$.

Dec-9-enyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate 6^{Gal}-{β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate 6^{Gal}-[β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate]}, tris-ammonium salt (7): This compound was prepared from the protected heptasaccharide **25** (50 mg, 0.012 mmol) by debenzoylation with 0.25 M NaOMe in MeOH (24 h), followed by additional treatment with 0.25 M NaOMe in MeOH/THF (1:1; 48 h) and work-up as described for the preparation of **5**. The crude product was applied to a column (18×1.5 cm) of Fractogel TSK DEAE-650 (S; HCO_3^- form; Merck) and was eluted with a linear gradient of aq. NH_4HCO_3 (0→0.3 M) in water/propan-2-ol (3:2) at 1 mL min^{-1} to provide the heptasaccharide triphosphate **7** (11 mg, 58%) as an amorphous solid; $[\alpha]_D^{22} = +31$ ($c = 0.45$ in MeOH/water, 1:1); ^1H NMR (selected data): see Table 3; ^{13}C NMR: see Table 1; ^{31}P NMR (121 MHz, D_2O): $\delta = -1.61$ (P), -2.04 (P′ and P″; ratio 1:2) ppm; ES MS (−): *m/z* (%): 509.1 (100) $[M-3NH_3-3H]^{3-}$, 764.0 (20) $[M-3NH_3-2H]^{2-}$, 775.0 (10) $[M-3NH_3-3H+Na]^{2-}$; calcd for $C_{52}H_{102}N_3O_{45}P_3$: $[M] = 1581.50$.

Dec-9-enyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate 6^{Gal}-{β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate 6^{Gal}-[β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate]}, tris-ammonium salt (8): 4.6 M Methanolic NaOMe (0.5 mL) was added to a solution of the protected heptasaccharide **24** (58 mg, 0.0144 mmol) in MeOH/THF (1:1; 8.7 mL). The mixture stirred at room temperature for 24 h, whereafter it was deionised with Dowex 50W-X4 (H^+) resin, filtered and immediately neutralised with Et_3N. After the solution had been concentrated, water (5×5 mL) was evaporated off from the residue to remove methyl benzoate. Anion-exchange chromatography (as described for the preparation of **7**) gave the heptasaccharide triphosphate **8** (20 mg, 88%) as an amorphous solid; $[\alpha]_D^{24} = +27$ (c = 1 in MeOH/water, 1:1); ^1H NMR (selected data): see Table 3; ^{13}C NMR: see Table 1; ^{31}P NMR (121 MHz, D_2O): $\delta = -1.66$ (P), -2.00 (P′ and P″; ratio 1:2); ES MS (−): *m/z* (%): 509.4 (100) $[M-3NH_3-3H]^{3-}$, 763.9 (20) $[M-3NH_3-2H]^{2-}$, 775.1 (13) $[M-3NH_3-3H+Na]^{2-}$; calcd for $C_{52}H_{102}N_3O_{45}P_3$: $[M] = 1581.50$.

Dec-9-enyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate 6^{Gal}-{β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-α-D-man-

Table 3. Selected ^1H NMR (300 MHz, D_2O) data (δ_H in ppm with multiplicities (where applicable) and *J* coupling constants in Hz (in parentheses)) for the deprotected phosphosaccharides **5**–**9**.

Residue	Atom(s)	**5**	**6**	**7**	**8**	**9**
dec-9-enyl	$CH_2\times5$	1.25, m	1.27, m	1.27, m	1.25, m	1.26, m
	$OCH_2CH_2CH_2$	1.56, quintet	1.58, quintet	1.59, quintet	1.56, quintet	1.57, quintet
	CH_2CH_2CH=	1.98, m	2.00, m	2.00, m	1.98, m	2.00, m
	CH=HC*H*	4.88, br d ($^3J_{cis}$ = 10.2)	4.93, br d ($^3J_{cis}$ = 10.3)	4.90, br d ($^3J_{cis}$ = 11.0)	4.89, br d ($^3J_{cis}$ = 10.3)	4.91, br d ($^3J_{cis}$ = 10.3)
	CH=*H*CH	4.97, br d ($^3J_{trans}$ = 17.0)	5.00, br d ($^3J_{trans}$ = 17.0)	4.98, br d ($^3J_{trans}$ = 17.3)	4.97, br d ($^3J_{trans}$ = 17.0)	4.99, br d ($^3J_{trans}$ = 17.1)
	CH_2CH=CH_2	5.85, ddt ($^3J_{H,CH2}$ = 6.6)	5.87, ddt ($^3J_{H,CH_2}$ = 6.6)	5.86, ddt ($^3J_{H,CH_2}$ = 6.9)	5.84, ddt ($^3J_{H,CH_2}$ = 6.6)	5.87, ddt ($^3J_{H,CH_2}$ = 7.0)
Man	H-1	5.33, br d ($J_{1,P}$ = 8.8)	5.35, br d ($J_{1,P}$ = 8.8)	5.34, br d ($J_{1,P}$ = 8.5)	5.33, br d ($J_{1,P}$ = 10.1)	5.36, br d ($J_{1,P}$ = 8.8)
Gal	H-1	4.44, d ($J_{1,2}$ = 7.7)	4.47, d ($J_{1,2}$ = 7.8)	4.41, d ($J_{1,2}$ = 7.4)	4.40, d ($J_{1,2}$ = 7.8)	4.42, d ($J_{1,2}$ = 7.8)
Glc	H-1	4.60, d ($J_{1,2}$ = 7.7)				
Man′	H-1			5.38, br d ($J_{1,P}$ = 8.3)	5.37, br d ($J_{1,P}$ = 8.6)	5.38, br d ($J_{1,P}$ = 8.4)
Gal′	H-1		4.57, d ($J_{1,2}$ = 7.3)	4.46, d ($J_{1,2}$ = 7.8)	4.40, d ($J_{1,2}$ = 7.8)	4.47, d ($J_{1,2}$ = 7.6)
Gal(1→3)Gal′	H-1			4.55, d ($J_{1,2}$ = 7.2)		4.55, d ($J_{1,2}$ = 7.4)
Man″	H-1			5.38, br d ($J_{1,P}$ = 8.3)	5.37, br d ($J_{1,P}$ = 8.6)	5.38, br d ($J_{1,P}$ = 8.4)
Gal″	H-1			4.38, d ($J_{1,2}$ = 7.5)	4.44, d ($J_{1,2}$ = 7.9)	4.45, d ($J_{1,2}$ = 7.5)
Gal(1→3)Gal″	H-1				4.54, d ($J_{1,2}$ = 7.3)	4.55, d ($J_{1,2}$ = 7.4)

nopyranosyl phosphate 6$^{Gal'}$-[β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate]}, tris-ammonium salt (9): Debenzoylation of the protected octasaccharide **26** (53 mg, 0.0118 mmol) with 0.25 M NaOMe in MeOH (24 h), followed by work-up and isolation by anion-exchange chromatography as described for the preparation of **8**, gave the octasaccharide triphosphate **9** (17 mg, 82%) as an amorphous solid; $[\alpha]_D^{24} = +35$ ($c = 0.4$ in MeOH/water, 1:1); ^{1}H NMR (selected data): see Table 3; ^{13}C NMR: see Table 1; ^{31}P NMR (121 MHz, D_2O): $\delta = -1.54$ (P), -1.91 (P′ and P″; ratio 1:2) ppm; ES MS (−): *m*/*z* (%): 563.0 (100) $[M-3NH_3-3H]^{3-}$, 856.0 (25) $[M-3NH_3-3H+Na]^{2-}$, 864.0 (18) $[M-3NH_3-3H+K]^{2-}$; calcd for $C_{58}H_{112}N_3O_{50}P_3$: $[M] = 1743.55$.

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