Parasite glycoconjugates. Part 5.[1] Blockwise approach to oligo(glycosyl phosphates): chemical synthesis of a terminal tris(glycobiosyl phosphate) fragment of *Leishmania donovani* antigenic lipophosphoglycan

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The model tetramannosyl triphosphate 7 and the hexaglycosyl triphosphate 8, which is a terminal fragment of the phosphoglycan portion of *Leishmania donovani* lipophosphoglycan, have been synthesized. Elongation of the chain was performed using the glycosyl hydrogenphosphonate method to couple together two phosphodiester blocks.

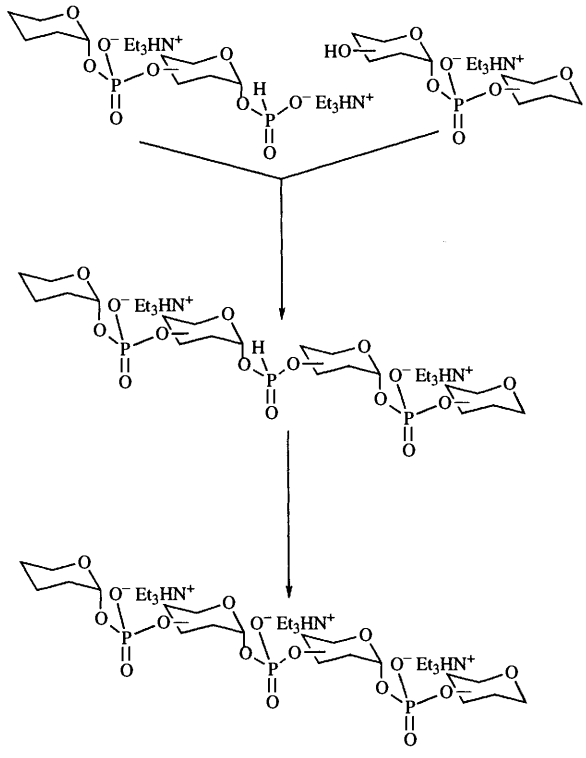
Introduction

In Part 4 [1] of this series, we disclosed our interest in the synthesis of phosphorylated oligosaccharide fragments of the structure of the antigenic lipophosphoglycan (LPG) of *Leishmania donovani*, a protozoan parasite that causes visceral leishmaniasis. This, often fatal, disease is one of a variety of diseases in the tropics and subtropics caused by the *Leishmania*. We resume our exploration of the chemical synthesis of oligo(glycosyl phosphate) fragments of *L. donovani* LPG by reporting a different approach to those used previously (see below). The natural occurrence of phosphoglycans composed of glycosyl phosphate (or oligosyl phosphate) repeat units as surface antigens of some protozoa [2] and yeasts,[3] as well as being the immunologically active components of the cell wall or capsule of numerous bacteria,[4] upholds the need for the development of potential routes to these biopolymers.

Since the glycosyl hydrogenphosphonate method [5–7] provides an efficient means of constructing a phosphodiester linkage between the anomeric (*i.e.*, hemiacetal) and other hydroxy groups, three major strategic approaches to oligo- and poly(glycosyl phosphates) can be contemplated. They are: (1) the polycondensation of a partially protected glycosyl (or oligosyl) H-phosphonate derivative; (2) the stepwise chain elongation using protected glycosyl (or oligosyl) H-phosphonates for the successive introduction of the sugar phosphate residues; and (3) the block synthesis, involving the H-phosphonate condensation of two phosphodiester units (or blocks). Application of the polycondensation [8] and stepwise [1,9] approaches to phosphoglycan synthesis has been described recently. We now report how the blockwise approach, involving *the coupling of a glycosyl H-phosphonate phosphodiester block and a monohydroxylic phosphodiester block, followed by oxidation of the newly formed H-phosphonic diester to the phosphate* (Scheme 1), can be equally effective in phosphoglycan synthesis.

Results and discussion

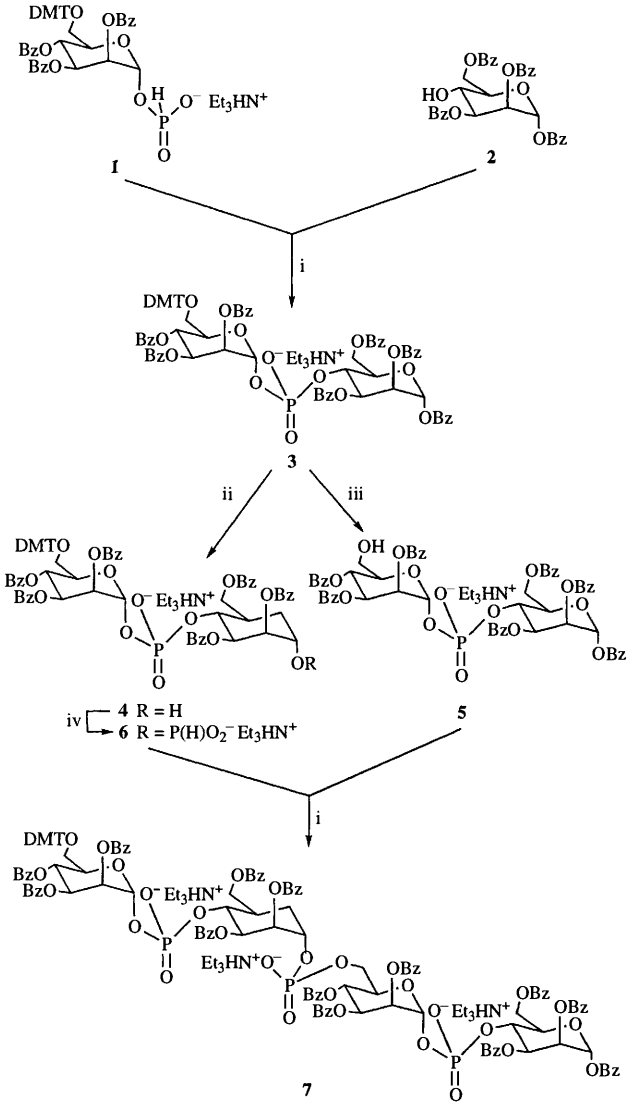
The model protected tetramannosyl triphosphate **7** (Scheme 2), containing alternate (1→4)- and (1→6)-phosphodiester linkages, was synthesized using 2,3,4-tri-*O*-benzoyl-6-*O*-dimethoxytrityl-α-D-mannosyl H-phosphonate **1**[9] and 1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose **2**[1] as the starting materials. Condensation of these compounds in pyridine in the presence of pivaloyl chloride, followed by *in situ* oxidation of the resulting H-phosphonic diester with iodine in aq. pyridine,



Scheme 1

gave the key (1→4)-linked dimannosyl phosphoric diester **3**, which could be used to prepare both the monohydroxylic and H-phosphonate blocks **5** and **6**, respectively.

Brief treatment of the synthon **3** with 1% CF_3CO_2H (TFA) in CH_2Cl_2 removed the dimethoxytrityl (DMT) group and gave the 6′-monohydroxylic phosphodiester derivative **5** in 86% overall yield (based on the tetrabenzoate **2**). Selective 1-*O*-debenzoylation of the phosphate **3** with dimethylamine [1,5–7,9] furnished the α-hydroxy compound **4** (78%, based on the tetrabenzoate **2**), which on phosphitylation with triimidazolylphosphine (prepared from PCl_3, imidazole and Et_3N) and subsequent mild hydrolysis gave the mannosyl H-phosphonate 4-(mannosyl phosphate) block **6** in 75% yield. It is notable that phosphitylation of the hemiacetal **4** (⟶ **6**) required a much longer time (27 h) than that (35 min) of unphosphorylated mono- and di-saccharide derivatives.[1,6–9] When the reaction



Scheme 2 *Reagents:* i, (*a*) Me_3CCOCl, pyridine; (*b*) I_2, pyridine–water; ii, Me_2NH, MeCN–THF–toluene; iii, TFA, CH_2Cl_2; iv, (*a*) triimidazolylphosphine, MeCN–pyridine; (*b*) Et_3NHHCO_3, water (pH 7)

with phosphodiester **4** was stopped after 1.5 h, the H-phosphonate **6** and the starting material were isolated in 43 and 33% yield, respectively.

The structure of compound **6** was supported by electrospray [ES(−)] mass spectrometry (*m/z* 705.3, [M − 2 Et_3N − 2 H]$^{2-}$) and NMR data. The presence of the H-phosphonic monoester moiety and the (1→4)-phosphodiester linkage was evident from the ^{31}P and ^1H NMR spectra: δ_P 0.88 (dd, $^1J_{P,H}$ 637.7, $^3J_{P,H}$ 8.7 Hz, P), −3.37 (dd, $^3J_{P,H}$ 7.0 and 10.5 Hz, P′); δ_H 5.32 (q, $J_{3,4} = J_{4,5} = J_{4,P'} = 10.5$ Hz, 4-H), 5.80 (dd, $J_{1,2}$ 2.0, $J_{1,P}$ 8.7 Hz, 1-H), 5.87 (br d, $J_{1',P'}$ 7.0 Hz, 1′-H), 7.14 (d, $^1J_{H,P}$ 637.7 Hz, HP). The α-configuration of each of the D-mannosyl residues followed from the characteristic positions of the 1-, 3- and 5-H resonances of Man and Man′ (see Experimental section). The structure of compound **4** was established in a similar manner, while that of compound **5** was confirmed by ^{13}C and ^{31}P NMR and ES(−) mass spectrometric data (see Experimental section).

Coupling of the H-phosphonate block **6** with the hydroxylic acceptor **5** and *in situ* oxidation, as described in the preparation of phosphodiester **3**, gave the protected tetramannosyl triphosphate **7** in 67% yield. The structure of this compound was confirmed by ES-MS(−) (*m/z* 852.9, [M − 3 Et_3N − 3 H]$^{3-}$) and NMR data. The ^{31}P NMR spectrum contained two characteristic signals, in the ratio 1:2, at δ_P −2.36 (q, $^3J_{P,H}$ 6.8 Hz, P′) and −3.20 (dd, $^3J_{P,H}$ 7.7 and 10.0 Hz, P + P″). The presence of one (1→6)- and two (1→4)-phosphodiester linkages followed from the position and spin-coupling of the C-1′ and C-4; C-1″ and C-6′; and C-1‴ and C-4″ signals in the ^{13}C NMR spectrum (see Experimental section). These signals were shifted as a result of the α-effect of phosphorylation and were coupled with phosphorus as well as were the signals of the neighbouring carbon atoms (C-3 and -5; C-2′ and -5′; C-2″, -3″ and -5″; and C-2‴). Analogous couplings of the coincident 4- and 4″-H signals [δ_H 5.35 (2 H, q, $J_{3,4} = J_{4,5} = J_{4,P} = 10.0$ Hz)] in the ^1H NMR spectrum clearly indicated that both Man and Man″ are phosphorylated at position-4.

As already demonstrated with the dimannosyl phosphate **3**, it should also be possible to convert the tetramannosyl triphosphate **7** into both monohydroxylic and H-phosphonate tetrasaccharide blocks for subsequent coupling to form a linear octamannosyl heptaphosphate. A block synthesis would be particularly advantageous in the preparation of the regular phosphoglycans, since fewer coupling steps are required than in stepwise elongation of the chain.

α-D-Man*p*-(1→2)-α-D-Man*p*-(1-PO_3H-[-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-]$_2$-O[CH_2]$_8$CH=CH_2

8

β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O[CH_2]$_8$CH=CH_2

9

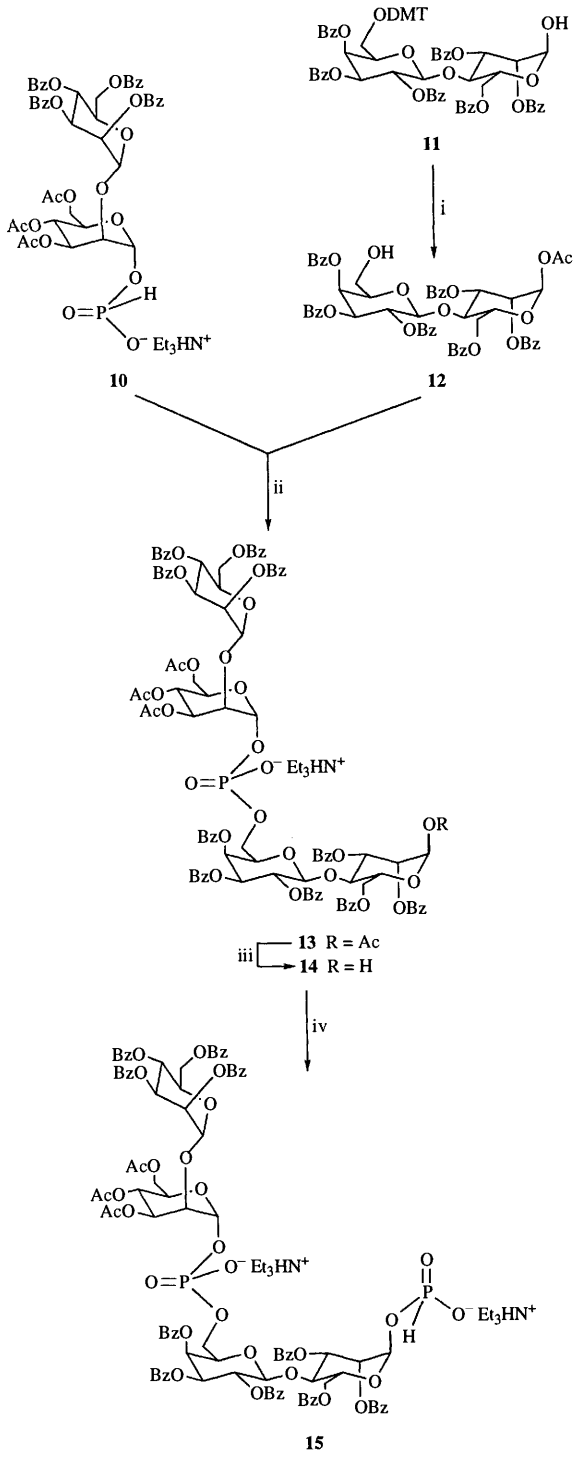
The tris(glycobiosyl phosphate) **8**, representing a fragment of the structure at the non-reducing end of the phosphoglycan portion of *Leishmania donovani* LPG, was prepared using the blockwise approach as well. It consists of two galactosyl-mannosyl phosphate repeat units capped by a D-mannobiosyl phosphate unit. In this context, a shorter fragment **9**, representing just one glycobiosyl phosphate repeat unit of the phosphoglycan, was synthesized. Both compounds contain a dec-9-enyl moiety and are designed to be used for both biosynthetic studies and the preparation of artificial antigens. The chemical synthesis of four related fragments of *Leishmania donovani* phosphoglycan has been described recently.[1]

The synthesis of the hexaglycosyl triphosphate **8** was accomplished from recently described[1] disaccharide derivatives **10** and **11**, which served for the preparation of both the tetrasaccharide H-phosphonate block **15** (Scheme 3) and the disaccharide monohydroxylic block **17** (Scheme 4).

Standard acetylation of the hemiacetal **11** (Scheme 3), followed by cleavage of the dimethoxytrityl group from the product under acidic conditions, resulted in the 6′-monohydroxylic compound **12** (73%). Condensation of the mannobiosyl H-phosphonate **10** with the disaccharide **12** in the presence of adamantane-1-carbonyl chloride, followed by *in situ* oxidation with iodine in aq. pyridine (⟶ **13**) and subsequent selective anomeric deacetylation with dimethylamine, gave the tetrasaccharide α-hemiacetal derivative **14** in 78% overall yield. This compound was converted into the glycobiosyl H-phosphonate 6′-(mannobiosyl phosphate) block **15** (65%) by phosphonylation using the procedure already described.

The structure of compound **15** was confirmed by fast-atom bombardment [FAB(+)] mass spectrometry (*m/z* 2080.47, [M − Et_3N + H]$^+$) and ^{31}P and ^1H NMR data [δ_P 1.68 (dd, $^1J_{P,H}$ 640.0, $^3J_{P,H}$ 8.7 Hz, P), −2.54 (dt, $^3J_{P,H}$ 8.5 and 10.5 Hz, P′); δ_H 5.67 (dd, $J_{1,2}$ 1.5, $J_{1,P'}$ 8.5 Hz, 1-H Man′), 5.72 (br d, $J_{1,P}$ 8.7 Hz, 1-H, Man), 7.01 (d, $^1J_{H,P}$ 640.0 Hz, HP)]. The α-configuration of the H-phosphonylated and phosphorylated D-mannose residues was evident from the characteristic values (176.1 and 170.2 Hz, respectively) of $^1J_{C,H}$ for the C-1 signals of Man and Man′ in the ^{13}C NMR spectrum (see Experimental section).

To prepare the monohydroxylic block **17** (Scheme 4), the hemiacetal **11** was first H-phosphonylated to give the



Scheme 3 *Reagents:* i, (*a*) Ac_2O, pyridine; (*b*) TFA, CH_2Cl_2; ii, (*a*) adamantane-1-carbonyl chloride, pyridine; (*b*) I_2, pyridine–water; iii, Me_2NH, MeCN–THF; iv, (*a*) triimidazolylphosphine, MeCN; (*b*) Et_3NHHCO_3, water (pH 7)

glycobiosyl H-phosphonate **16** (92%), as described in ref. 1. Condensation of the derivative **16** with dec-9-en-1-ol, followed by oxidation, as described for the preparation of the phosphodiester **13**, and subsequent dedimethoxytritylation with 1% TFA in CH_2Cl_2 gave the disaccharide phosphate derivative **17** in 90% overall yield.

Coupling of the H-phosphonate phosphodiester block **15** and the monohydroxylic phosphodiester block **17** was accomplished in the prescribed manner (with adamantane-1-carbonyl chloride), after which *in situ* oxidation with iodine afforded the protected hexasaccharide triphosphate **18** in 62% yield. The deprotected tris(glycobiosyl phosphate) **8** and dec-9-enyl glycobiosyl phosphate **9** were readily obtained from the derivatives **18** and **17**, respectively, by *O*-deacylation with 0.05 mol dm^{-3} methanolic sodium methoxide.

The structures of the compounds **8**, **9**, **17** and **18** were confirmed by NMR and mass spectrometric data (see Experimental section). For the monophosphates **17** and **9** the ^{31}P NMR spectra exhibited just single signals at δ_P −1.40 and −1.04, respectively. The ^{31}P NMR spectra of the triphosphates **18** and **8** consisted of three and two signals, respectively: δ_P −1.40 (P), −2.56 (P″) and −3.05 (P′) for the protected derivative **18**, and −0.91 (P) and −1.24 (P′ + P″) in the ratio 1:2 for the deprotected oligomer **8**.

The presence of the (1→6)-phosphodiester linkages in compound **8** was established from the C-1 and C-2 signals of the corresponding D-mannose units and the C-5 and C-6 signals of the corresponding D-galactose units in the ^{13}C NMR spectrum, while the presence of the (1→1)-phosphodiester linkage at the reducing terminus of each of the compounds **8** and **9** was likewise confirmed by the C-1 and C-2 signals of the D-mannose and the dec-9-enyl units (see Table 1). These signals were shifted as a result of the α- and β-effects of phosphorylation and were coupled with phosphorus. The α-configuration of the D-mannosyl phosphate fragments followed from the positions of the C-3 and C-5 resonances of Man, Man′ and Man″. The chemical shifts of these signals are close to those of C-3 and C-5 of α-D-mannopyranosyl phosphate,[10] taking into account the influence of the glycosyl substituents at position-4 (of Man and Man′) and position-2 (of Man″).

The molecular masses of the oligomers **8**, **9**, **17** and **18**, were confirmed by ES(−) and FAB(+) mass spectrometry. The dominant signals in the spectra corresponded to the pseudo-molecular ions for the monophosphates **9** (*m/z* 559.3, $[M - Et_3N - H]^-$) and **17** (*m/z* 1287.0, $[M + H]^+$) and for the triphosphates **8** (*m/z* 455.3, $[M - 3\ Et_3N - 3\ H]^{3-}$) and **18** (*m/z* 1052.3, $[M - 3\ Et_3N - 3\ H]^{3-}$).

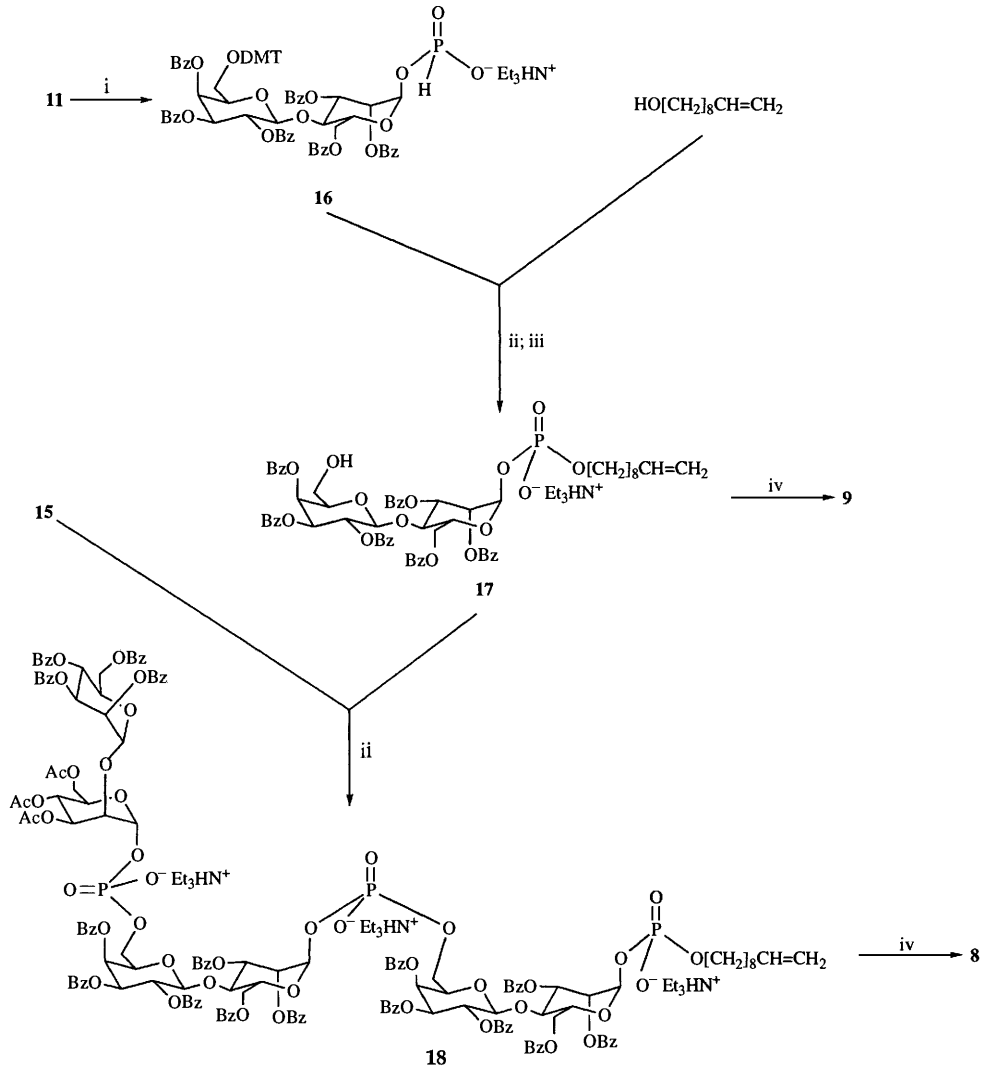
The reasonably high yields of the oligomers **7** (67%) and **18** (62%) achieved in coupling of the H-phosphonate and the monohydroxylic phosphodiester blocks [(**6** + **5**) and (**15** + **17**), respectively] indicate that side-reactions, possibly involving the reaction of the activated H-phosphonate with intersaccharide phosphate groups, did not intervene to a significant extent.

It has been demonstrated earlier[11] that the mixed anhydride **19** is the main reactive intermediate in the glycosyl phosphosugar synthesis from glycosyl H-phosphonates in the presence of pivaloyl chloride. The analogous H-phosphono(adamantane-1-carbonic) anhydride **20** fulfils the same role when the H-phosphonate condensation is accomplished in the presence of adamantane-1-carbonyl chloride. We presume that the activated anhydrides **19** and **20** can react, in principle, with both the intersaccharide phosphate and the alcoholic hydroxy group. The first reaction results in an unstable glycosyl H-phosphono(phosphoric) anhydride and seems to be reversible,† whereas the second reaction results in the irreversible formation of the hydrogenphosphonic diester (see Scheme 1), which has to be oxidized further to the target oligo(glycosyl phosphate).

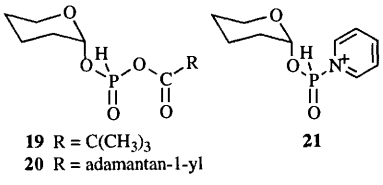
In accord with data[13] describing the mechanism of the H-phosphonate condensation in pyridine, the solvent can participate in the reaction as a nucleophilic catalyst to assist the formation of the H-phosphonic diester *via* the activated pyridinium intermediate **21**.

To summarize, a new blockwise approach has been introduced into the synthetic chemistry of the phosphoglycans composed of glycosyl phosphate residues. A biological

† This could be supported by data[12] describing the presence of equilibrium in the system: ethyl H-phosphono(pivalic) anhydride ⇌ diethyl H-pyrophosphonate, which is to the left.



Scheme 4 *Reagents:* i, (*a*) triimidazolylphosphine, MeCN; (*b*) Et_3NHHCO_3, water (pH 7); ii, (*a*) adamantane-1-carbonyl chloride, pyridine; (*b*) I_2, pyridine–water; iii, TFA, CH_2Cl_2; iv, NaOMe, MeOH



19 R = $C(CH_3)_3$
20 R = adamantan-1-yl

21

evaluation of compounds **8** and **9** and related synthetic fragments of *L. donovani* LPG will be published elsewhere[14] in due course.

Experimental

General procedures

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}. NMR spectra (^1H at 200 and 500 MHz, ^{13}C-{^1H} at 50.3 and 125 MHz, and ^{31}P 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. FAB mass spectra were recorded with a VG 70-250 SE mass spectrometer using an Ion-tech xenon gun. ES mass spectra were recorded with a VG Quattro system (VG Biotech, UK). TLC was performed on Polygram Sil G/UV_{254} (Macherey-Nagel, Germany) with *A*, chloroform–methanol (95:5); *B*, chloroform–methanol (92:8); *C*, chloroform–methanol (9:1); *D*, chloroform–methanol–water (10:10:3); and *E*, toluene–ethyl acetate (8:2) as developers and detection by charring with sulfuric acid–water–ethanol (15:85:5). Column chromatography was performed on Kieselgel 60 (0.040–0.063 mm) (Merck). Pivaloyl chloride and adamantane-1-carbonyl chloride were purchased from Aldrich. Pyridine, acetonitrile and tetrahydrofuran (THF) were freshly distilled from CaH_2. Solutions worked up were concentrated under reduced pressure at < 40 °C.

2,3,6-Tri-*O*-benzoyl-α-D-mannopyranose 4-[2,3,4-tri-*O*-benzoyl-6-*O*-(*p*,*p*′-dimethoxytrityl)-α-D-mannopyranosyl phosphate], triethylammonium salt 4

A mixture of compounds **1**[9] (471 mg, 0.49 mmol) and **2**[1] (250 mg, 0.42 mmol) was dried by evaporation of pyridine (3 × 3 cm^3) therefrom. The residue was dissolved in pyridine (3 cm^3), pivaloyl chloride (0.151 cm^3, 1.23 mmol) was added, and the mixture was stirred at 20 °C for 10 min, whereafter a freshly prepared solution of iodine (254 mg, 1 mmol) in pyridine–water (95:5; 4 cm^3) was added. After 10 min, $CHCl_3$ was added, and the solution was washed successively with ice-cold 1 mol dm^{-3} aq. $Na_2S_2O_3$ and cold 0.5 mol dm^{-3} aq. triethylammonium (TEA) hydrogen carbonate (pH 7.0–7.5), dried by filtration through cotton wool, and concentrated. TLC (solvent *A*) of the residue showed the formation of one major product (R_f 0.54; presumed to be the phosphodiester **3**). After drying *in vacuo*, the residue (800 mg; amorphous solid) then was divided into two

Table 1 ^{13}C NMR data (D_2O; δ_C in ppm; J in Hz) for phosphooligosaccharides **9** and **8** ($J_{C,P}$-values in parentheses)

Residue	Atom	**9**[a]	**8**[a]
Dec-9-enyl	OCH_2	67.81d (5.5)	67.84br
	OCH_2CH_2	30.95d (5.5)	31.02d (5.5)
	CCH_2C	25.96, 29.22, 29.32, 29.41, 29.57, 34.19	26.03, 29.27, 29.37, 29.48, 29.62, 34.19
	–CH=	141.64	141.67
	$=CH_2$	115.04	115.13
Man	C-1	96.84d (5.6)	96.90d (5.6)
	C-2	71.29d (7.4)	71.18d (7.4)
	C-3	69.73	70.00
	C-4	76.99	78.22
	C-5	73.43	73.52
	C-6	61.20	61.44
Gal	C-1	104.15	104.51
	C-2	72.07	71.98
	C-3	73.64	73.52
	C-4	69.85	69.25
	C-5	76.48	74.56d (7.4)
	C-6	62.23	65.42d (5.6)
Man′	C-1		96.90d (5.6)
	C-2		71.18d (7.4)
	C-3		70.00
	C-4		78.12
	C-5		73.52
	C-6		61.44
Gal′	C-1		104.51
	C-2		71.98
	C-3		73.52
	C-4		69.25
	C-5		74.56d (7.4)
	C-6		65.42d (5.6)
Man″	C-1		95.85d (3.7)
	C-2		80.16d (8.1)
	C-3		70.67
	C-4		67.84
	C-5		75.05
	C-6		62.04
Man‴	C-1		103.38
	C-2		71.14
	C-3		71.48
	C-4		67.84
	C-5		74.41
	C-6		62.04

[a] Additional signals for Et_3NH^+ (δ_C 9.34–9.43 and δ_C 47.81–47.85) were present.

portions, one of which (300 mg) was used to prepare the detritylated compound **5** (see below). To the other portion (500 mg) dissolved in MeCN–THF–toluene (4:1:1; 6 cm^3) was added anhydrous dimethylamine (0.112 cm^3, 1.68 mmol) at −20 °C, and the mixture was then kept at 20 °C for 16 h (with monitoring by TLC in solvent *A*), whereafter a second portion of dimethylamine (0.12 cm^3, 1.80 mmol) was added. After a further 22 h, the mixture was concentrated and MeCN was evaporated off from the residue. Column chromatography [CH_2Cl_2–MeOH–Et_3N, (98.8:0.2:1) ⟶ (95:4:1)] gave the *disaccharide monophosphate derivative* **4** (295 mg, 78%) as an amorphous solid; $[\alpha]_D^{22}$ −60 (*c* 1, $CHCl_3$); R_f 0.31 (solvent *A*), 0.63 (solvent *C*); δ_H 1.39 (9 H, t, 3 × *Me*CH_2), 2.82 (6 H, q, 3 × MeCH_2), 2.95 (1 H, dd, $J_{6a',6b'}$ 10.5, 6′-H[a]), 3.21 (1 H, dd, $J_{5',6b'}$ 1.4, 6′-H[b]), 3.62 and 3.63 (6 H, 2 s, 2 × MeO), 4.29 (1 H, ddd, $J_{5',6a'}$ 2.4, 5′-H), 4.35 (1 H, ddd, $J_{5,6a}$ 4.0, 5-H), 4.78 (1 H, dd, $J_{6a,6b}$ 12.5, 6-H[a]), 4.99 (1 H, dd, $J_{5,6b}$ 1.5, 6-H[b]), 5.31 (1 H, q, $J_{3,4} = J_{4,5} = J_{4,P}$ = 10.0, 4-H), 5.38 (1 H, d, $J_{1,2}$ 1.5, 1-H), 5.64 (2 H, m, $J_{2,3}$ 3.3, 2- and 3-H), 5.82 (1 H, dd, $J_{2',3'}$ 3.5, 3′-H), 5.83 (1 H, dd, 2′-H), 5.86 (1 H, dd, $J_{1',2'}$ 2.0, $J_{1',P}$ 8.0, 1′-H), 6.27 (1 H, dd, $J_{3',4'} = J_{4',5'}$ = 10.5, 4′-H), 6.60 and 6.67 (4 H, 2 d, *o*-protons of 2 × C_6H_4) and 7.05–8.15 (39 H, m, *m*-protons of 2 × C_6H_4, 7 × Ph); δ_C 8.75 and 45.55 (Et), 55.01 and 55.06 (MeO), 60.88 (C-6′), 63.18 (C-6), 66.22 (C-4′), 69.58 (d, $J_{C,P}$ 5.4, C-4), 70.33 (d, $J_{C,P}$ 5.4, C-5), 70.75 (C-3′), 70.88 (d, $J_{C,P}$ 5.2, C-2′), 70.90 (C-2), 71.18 (C-5′), 71.22 (br, C-3), 85.55 (Ar_3C), 92.13 (C-1), 94.12 (d, $J_{C,P}$ 4.1, C-1′), 112.91, 112.99, 126.32–133.17, 135.71, 136.07, 144.83, 158.04 and 158.10 (C_6H_4 and Ph) and 166.03–166.62 (Ph*C*O_2); δ_P −2.50 (dd, $J_{P,H}$ 8.0 and 10.0); ES-MS(−): *m*/*z* 941.3 (18%, [M − Et_3N − $(MeOC_6H_4)_2PhC$ − PhCO + H]$^-$), 1045.2 (17, [M − Et_3N − $(MeOC_6H_4)_2PhC$]$^-$), 1243.2 (7, [M − Et_3N − PhCO]$^-$) and 1347.3 (100, [M − Et_3N − H]$^-$) ($C_{81}H_{80}NO_{22}P$ requires M, 1449.49).

1,2,3,6-Tetra-*O*-benzoyl-α-D-mannopyranose 4-[2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate], triethylammonium salt 5

To a stirred and cooled (0 °C) solution of the crude phosphodiester **3** (300 mg, prepared in the preceding experiment) in CH_2Cl_2 (15 cm^3) was added TFA (0.15 cm^3). After 1 min, the solution was 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. Column chromatography [CH_2Cl_2–MeOH–Et_3N, (98.8:0.2:1) ⟶ (96:3:1)] of the residue gave the *monohydroxylic derivative* **5** (168 mg, 85.5% based on compound **2**) as an amorphous solid; $[\alpha]_D^{22}$ −33.5 (*c* 1, $CHCl_3$); R_f 0.40 (solvent *A*), 0.70 (solvent *C*); δ_C 9.61 and 45.70 (Et), 61.30 (C-6′), 63.40 (C-6), 66.91 (C-4′), 69.13 (d, $J_{C,P}$ 5.6, C-4), 69.51 (C-2), 69.95 (C-3′), 70.61 (d, $J_{C,P}$ 8.3, C-2′), 71.39 (d, $J_{C,P}$ 2.8, C-3), 72.07 (C-5′), 72.41 (d, $J_{C,P}$ 4.9, C-5), 91.32 (C-1), 94.10 (d, $J_{C,P}$ 4.9, C-1′), 128.10–129.70 and 132.90–133.20 (Ph) and 165.12–165.91 (Ph*C*O_2); δ_P −3.04 (dd, $J_{P,H}$ 7.0 and 10.0); ES-MS(−): *m*/*z* 1149.1 (100%, [M − Et_3N − H]$^-$) ($C_{67}H_{66}NO_{21}P$ requires M, 1251.39).

2,3,6-Tri-*O*-benzoyl-α-D-mannopyranosyl hydrogenphosphonate 4-[2,3,4-tri-*O*-benzoyl-6-*O*-(*p*,*p*′-dimethoxytrityl)-α-D-mannopyranosyl phosphate], bistriethylammonium salt 6

To a stirred solution of imidazole (432 mg, 6.32 mmol) in MeCN (4 cm^3) at 0 °C were added phosphorus trichloride (0.166 cm^3, 1.90 mmol) and then triethylamine (0.95 cm^3, 6.65 mmol). The mixture was stirred for 15 min, after which a solution of compound **4** (270 mg, 0.186 mmol) in MeCN–pyridine (2:1; 7 cm^3) was added dropwise during 20 min at 0 °C. The mixture was stirred at 24 °C for 27 h and quenched with 1 mol dm^{-3} aq. TEA hydrogen carbonate (3 cm^3). The clear solution was stirred for 15 min, $CHCl_3$ (150 cm^3) was added, and the organic layer was washed in turn with ice–water (2 × 80 cm^3) and cold 0.5 mol dm^{-3} aq. TEA hydrogen carbonate (2 × 80 cm^3), dried by filtration through cotton wool, and concentrated. Column chromatography [CH_2Cl_2–MeOH–Et_3N, (98.8:0.2:1) ⟶ (95.1:3.9:1)] gave the *H-phosphonate phosphodiester derivative* **6** (224 mg, 74.6%) as an amorphous solid; R_f 0.29 (solvent C); δ_H 1.20 (18 H, t, 6 × *Me*CH_2), 2.94 (12 H, q, 6 × MeCH_2), 3.25 (2 H, m, 6′-H_2), 3.62 and 3.63 (6 H, 2 s, 2 × MeO), 4.32 (1 H, br d, 5′-H), 4.57 (1 H, ddd, $J_{5,6b}$ 1.1, 5-H), 4.81 (1 H, dd, $J_{5,6a}$ 4.0, 6-H[a]), 5.05 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H[b]), 5.32 (1 H, q, $J_{3,4} = J_{4,5} = J_{4,P'}$ = 10.5, 4-H), 5.69 (1 H, dd, $J_{2,3}$ 2.5, 3-H), 5.71 (1 H, dd, 2-H), 5.80 (1 H, dd, $J_{1,2}$ 2.0, $J_{1,P}$ 8.7, 1-H), 5.84 (1 H, dd, $J_{2',3'}$ 3.0, 3′-H), 5.86 (1 H, br, 2′-H), 5.87 (1 H, br d, $J_{1',P'}$ 7.0, 1′-H), 6.31 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 10.0, 4′-H), 6.61 and 6.67 (4 H, 2 d, *o*-protons of 2 × C_6H_4), 7.14 (1 H, d, $J_{H,P}$ 637.7, HP) and 7.00–8.11 (39 H, m, *m*-protons of 2 × C_6H_4, 7 × Ph); δ_C 8.71 and 45.50 (Et), 54.89 and 54.91 (MeO), 60.75 (C-6′), 63.28 (C-6), 65.94 (C-4′), 69.61 (d, $J_{C,P}$ 4.7, C-4), 70.87 (C-3′), 70.98 (2 C, br, C-2 and -2′), 71.01 (d, $J_{C,P}$ ~3.0, C-5), 71.20 (C-5′), 71.31 (d, $J_{C,P}$ 5.0, C-3), 85.43 (Ar_3C), 92.92 (d, $J_{C,P}$ 3.4, C-1), 94.12 (d, $J_{C,P}$ 4.1, C-1′), 112.90, 112.95, 126.32–133.17, 135.70, 136.15, 144.80, 158.00 and 158.09 (C_6H_4 and Ph) and 166.02–166.57 (Ph*C*O_2); δ_P 0.88 (dd, $^1J_{P,H}$ 637.7, $^3J_{P,H}$ 8.7, P) and −3.37 (dd, $^3J_{P,H}$ 7.0 and 10.5, P′); ES-MS(−): *m*/*z* 705.3 (100%, [M − 2 Et_3N − 2 H]$^{2-}$) and 1411.3 (20, [M − 2 Et_3N − H]$^-$) ($C_{87}H_{96}N_2O_{24}P_2$ requires M, 1614.58).

1,2,3,6-Tetra-*O*-benzoyl-α-D-mannopyranose 4-[2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate 6-{2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate 4-[2,3,4-tri-*O*-benzoyl-6-*O*-(*p*,*p*′-dimethoxytrityl)-α-D-mannopyranosyl phosphate]}], tristriethylammonium salt 7

This compound was prepared by condensation of the H-phosphonate block **6** (130 mg, 0.08 mmol) and the monohydroxylic block **5** (75 mg, 0.06 mmol) in the presence of pivaloyl chloride (0.02 cm^3, 0.185 mmol) in pyridine (0.5 cm^3) during 30 min, followed by oxidation with iodine (40 mg, 0.157 mmol), as described in the synthesis of compound **4**. Column chromatography [CH_2Cl_2–MeOH–Et_3N, (98.5:0.5:1) ⟶ (92:7:1)] gave the *tetrasaccharide trisphosphate derivative* **7** (115 mg, 67%) as an amorphous solid; $[\alpha]_D^{24}$ −57 (*c* 1, $CHCl_3$); R_f 0.43 (solvent *C*); δ_H 1.15 (27 H, t, 9 × *Me*CH_2), 2.75 (1 H, d, $J_{6a''',6b'''}$ 11.0, 6‴-H^a), 2.89 (18 H, q, 9 × MeCH_2), 3.10 (1 H, d, 6‴-H^b), 3.63 and 3.64 (6 H, 2 s, 2 × MeO), 4.17 (3 H, m, 5′-H and 6′-H_2), 4.44 (1 H, d, $J_{4''',5'''}$ 10.0, 5‴-H), 4.59 (2 H, m, 5- and 5″-H), 4.73 (1 H, br d, $J_{6a,6b}$ 12.0, 6-H^a), 4.90 (1 H, d, $J_{6a'',6b''}$ 11.5, 6″-H^a), 4.92 (1 H, dd, $J_{5,6b}$ 4.0, 6-H^b), 5.12 (1 H, d, 6″-H^b), 5.35 (2 H, q, $J_{3,4}$ = $J_{3'',4''}$ = $J_{4,5}$ = $J_{4'',5''}$ = $J_{4,P}$ = $J_{4'',P''}$ = 10.0, 4- and 4″-H), 5.59–5.89 (11 H, m, 1′-, 1″-, 1‴-, 2-, 2′-, 2″-, 2‴-, 3′-, 3″-, 3‴- and 4′-H), 5.97 (1 H, dd, $J_{2,3}$ 3.0, 3-H), 6.25 (1 H, t, $J_{3''',4'''}$ 10.0, 4‴-H), 6.59 (5 H, m, 1-H and *o*-protons of 2 × C_6H_4) and 7.00–8.22 (74 H, m, *m*-protons of 2 × C_6H_4, 14 × Ph); δ_C 8.90 and 45.39 (Et), 54.94 and 54.96 (MeO), 60.68 (C-6‴), 62.98 and 63.40 (C-6 and -6″), 64.95 (d, $J_{C,P}$ 4.2, C-6′), 65.97 (C-4‴), 67.15 (C-4′), 68.97 and 69.23 (2 d, $J_{C,P}$ 5.2 and 5.5, C-4 and -4″), 69.60 (C-2), 70.28 (C-3′), 70.57 (d, $J_{C,P}$ 7.6, C-2′), 70.80 (C-3‴), 70.85, 70.95 and 71.01 (3 d, $J_{C,P}$ 4.5, 4.5 and 4.9, C-2″, -2‴ and - 5″), 71.10 (C-5‴), 71.25 (d, $J_{C,P}$ 7.6, C-5′), 71.34 (br, C-3″), 71.39 (d, $J_{C,P}$ 2.7, C-3), 72.94 (d, $J_{C,P}$ 4.2, C-5), 85.36 (Ar_3C), 91.35 (C-1), 93.72 (d, $J_{C,P}$ 4.2, C-1″), 94.00 and 94.08 (2 d, $J_{C,P}$ 4.5 and 4.4, C-1′ and -1‴), 112.80, 112.87, 126.28, 127.56–133.56, 135.55, 136.23, 144.86, 158.02 and 158.06 (C_6H_4 and Ph) and 164.10–166.14 (Ph*C*O_2); δ_P −2.36 (q, $J_{P,H}$ 6.8, P′) and −3.20 (dd, $J_{P,H}$ 7.7 and 10.0, P + P″) (ratio 1:2); ES-MS(−): *m*/*z* 852.9 (85%, [M − 3 Et_3N − 3 H]$^{3-}$) and 1279.7 (100, [M − 3 Et_3N − 2 H]$^{2-}$) ($C_{154}H_{160}N_3O_{45}P_3$ requires M, 2863.95).

2,3,4-Tri-*O*-benzoyl-β-D-galactopyranosyl-(1⟶4)-1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-α-D-mannopyranose 12

A solution of compound **11**[1] (200 mg) in pyridine (4 cm^3) containing acetic anhydride (2 cm^3) was kept overnight at room temperature and then concentrated to dryness. Toluene was twice evaporated off from the residue, which was then treated with 1% TFA in CH_2Cl_2 (1–2 min; 0 °C) as described for the synthesis of compound **5**. Column chromatography [toluene–ethyl acetate, (85:15)] gave the *disaccharide derivative* **12** (116 mg, 73%) as an amorphous solid; $[\alpha]_D^{24}$ +101 (*c* 1, $CHCl_3$); R_f 0.26 (solvent *E*); δ_H 2.19 (3 H, s, Ac), 3.01 (1 H, dd, $J_{6a',6b'}$ 11.5, 6′-H^a), 3.16 (1 H, dd, 6′-H^b), 3.58 (1 H, t, $J_{5',6a'}$ = $J_{5',6b'}$ = 6.4, 5′-H), 4.18 (1 H, ddd, $J_{5,6a}$ 3.0, 5-H), 4.49 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H^a), 4.60 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.4, 4-H), 4.69 (1 H, dd, $J_{5,6b}$ 2.0, 6-H^b), 4.96 (1 H, d, $J_{1',2'}$ 7.9, 1′-H), 5.41 (1 H, dd, $J_{2',3'}$ 10.5, 3′-H), 5.59 (1 H, d, $J_{3',4'}$ 3.5, 4′-H), 5.65 (1 H, dd, $J_{2,3}$ 3.4, 2-H), 5.80 (1 H, dd, 2′-H), 5.89 (1 H, dd, 3-H), 6.26 (1 H, d, $J_{1,2}$ 2.0, 1-H) and 7.13–8.05 (30 H, m, 6 × Ph); δ_C 21.05 (*Me*CO_2), 59.87 (C-6′), 61.93 (C-6), 68.45 (C-4′), 69.43 (C-2), 69.90 (C-3), 70.15 (C-2′), 71.39 (C-5), 71.75 (C-3′), 73.31 (C-4), 74.19 (C-5′), 90.67 (C-1), 101.30 (C-1′), 128.20–133.93 (Ph), 165.21–166.68 (Ph*C*O_2) and 168.47 (Me*C*O_2); ES-MS(+): *m*/*z* 1031.3 (100%, [M + Na]$^+$); ES-MS(−): *m*/*z* 903.2 (38%, [M − PhCO]$^-$), 965.1 (40, [M − MeCO]$^-$), 1007.3 (100, [M − H]$^-$), 1051.4 (25, [M + 2 Na − 3 H]$^-$) and 1083.1 (45, [M + 2 K − 3 H]$^-$) ($C_{56}H_{48}O_{18}$ requires M, 1008.29).

2,3,4-Tri-*O*-benzoyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranose 6-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1⟶2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl phosphate], triethylammonium salt 14

A mixture of compounds **10**[1] (167 mg, 0.159 mmol) and **12** (116 mg, 0.115 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 (80 mg, 0.40 mmol) was added, and the mixture was stirred at 22 °C for 20 min, whereafter a freshly prepared solution of iodine (80 mg, 0.32 mmol) in pyridine–water (95:5; 2 cm^3) was added and stirring was continued for 10 min. Conventional work-up, as described in the preparation of phosphodiester **4**, then gave the crude product **13** (R_f 0.40, solvent *A*), which was dissolved in MeCN–THF (1:1; 4 cm^3), and the solution was cooled to −20 °C and treated with anhydrous dimethylamine (0.05 cm^3, 0.75 mmol). The mixture was kept at 22 °C for 26 h, with monitoring by TLC (solvent *B*), whereafter a second portion of the reagent (0.05 cm^3) was added. After a further 24 h, the mixture was concentrated and MeCN was evaporated off from the residue. Column chromatography [CH_2Cl_2–MeOH–Et_3N, (98.8:0.2:1) ⟶ (95.1:3.9:1)] gave the *tetrasaccharide monophosphate derivative* **14** (180 mg, 77.7%) as an amorphous solid; $[\alpha]_D^{24}$ +13 (*c* 1, $CHCl_3$); R_f 0.56 (solvent *B*); δ_C 8.57 and 45.50 (Et), 20.71 (*Me*CO_2), 62.03 (C-6, Man′), 62.33 (C-6, Man″), 62.49 (d, $J_{C,P}$ 5.4, C-6, Gal), 62.87 (C-6, Man), 65.90 (C-4, Man′), 66.58 (C-4, Man″), 67.67 (C-4, Gal), 68.48 (C-5, Man), 69.09 (C-5, Man′), 69.36 (C-3, Man″), 69.39 (C-3, Man), 69.74 (C-5, Man″), 70.24 (2 C, C-2, Gal + C-3, Man′), 70.63 (C-2, Man″), 71.54 (C-2, Man), 72.22 (d, $J_{C,P}$ 7.2, C-5, Gal), 72.36 (C-3, Gal), 73.31 (C-4, Man), 77.07 (d, $J_{C,P}$ 7.6, C-2, Man′), 91.92 (C-1, Man), 94.39 (br, C-1, Man′), 99.04 (C-1, Man″), 99.48 (C-1, Gal), 127.04–130.01 and 132.40–133.31 (Ph), 164.95–166.43 (Ph*C*O_2) and 169.29, 170.71 and 171.42 (Me*C*O_2); δ_P −2.49 (q, $J_{P,H}$ 8.8); FAB-MS(+): *m*/*z* 2016.04 (100%, [M + H]$^+$) ($C_{106}H_{104}NO_{37}P$ requires M, 2014.95).

2,3,4-Tri-*O*-benzoyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl hydrogenphosphonate 6-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1⟶2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl phosphate], bistriethylammonium salt 15

To a stirred solution of imidazole (91 mg, 1.33 mmol) in MeCN (2 cm^3) at 0 °C was added phosphorus trichloride (0.035 cm^3, 0.40 mmol), followed by triethylamine (0.20 cm^3, 1.40 mmol). The mixture was stirred for 15 min, after which a solution of compound **14** (133 mg, 0.066 mmol) in MeCN (3 cm^3) was added dropwise at 0 °C. The mixture was stirred overnight at room temperature and then quenched with 1 mol dm^{-3} TEA hydrogen carbonate (1 cm^3). Work-up as described in the preparation of the compound **6** followed by column chromatography [CH_2Cl_2–MeOH–Et_3N, (98.7:0.8:0.5)-⟶ (93.5:6:0.5)], gave the *tetrasaccharide H-phosphonate derivative* **15** (93 mg, 64.6%) as an amorphous solid; $[\alpha]_D^{24}$ +15 (*c* 1, $CHCl_3$); R_f 0.40 (solvent *B*); δ_H 1.26 (18 H, t, 6 × *Me*CH_2), 2.02, 2.05 and 2.10 (9 H, 3 s, 3 × Ac), 3.02 (12 H, q, 6 × MeCH_2), 3.43–3.52 (2 H, m, 6-H_2, Gal), 4.00–4.11 (2 H, m, 5-H and 6-H^a, Man′), 4.16 (1 H, dd, $J_{5,6}$ 5.5 and 9.3, 5-H, Gal), 4.28–4.34 (3 H, m, 5-H, Man; 2-H and 6-H^b, Man′), 4.54 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.6, 4-H, Man), 4.57 (1 H, dd, $J_{5,6a}$ 3.5, $J_{6a,6b}$ 12.0, 6-H^a, Man), 4.64 (1 H, dd, $J_{5,6b}$ 1.5, 6-H^b, Man), 4.66–4.72 (3 H, m, 5-H and 6-H_2, Man″), 5.12 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal), 5.35 (1 H, d, $J_{1,2}$ 1.7, Man″), 5.40 (2 H, m, 3- and 4-H, Man′), 5.45 (1 H, dd, $J_{2,3}$ 10.6, 3-H, Gal), 5.67 (1 H, dd, $J_{1,2}$ 1.5, $J_{1,P}$ 8.5, 1-H, Man′), 5.68 (1 H, br, 2-H, Man), 5.69 (1 H, dd, 2-H, Gal), 5.72 (1 H, br d, $J_{1,P}$ 8.7, 1-H, Man), 5.77 (1 H, dd, $J_{2,3}$ 3.0, 2-H, Man″), 5.83 (1 H, dd, $J_{2,3}$ 3.5, 3-H, Man), 5.87 (1 H, d, $J_{3,4}$ 3.2, 4-H, Gal), 5.99 (1 H, dd, 3-H, Man″), 6.23 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 10.0, 4-H, Man″), 7.01 (1 H, d, $J_{H,P}$ 640.0, HP) and 6.95–8.10 (50 H, m, 10 × Ph); δ_C 8.61 and 45.72 (Et), 20.62 (*Me*CO_2), 61.60 (d, $J_{C,P}$ 5.4, C-6, Gal), 62.03 (C-6, Man′),

62.40 (2 C, C-6, Man + C-6, Man″), 66.00 (C-4, Man′), 66.59 (C-4, Man″), 67.29 (C-4, Gal), 69.23 (C-5, Man′), 69.36 (C-3, Man″), 69.78 (C-5, Man″), 69.92 (C-5, Man), 70.18 (C-3, Man), 70.24 (C-2, Gal), 70.60 (C-3, Man′), 70.67 (C-2, Man″), 70.81 (d, $J_{C,P}$ 6.3, C-2, Man), 71.74 (d, $J_{C,P}$ 7.0, C-5, Gal), 72.27 (C-3, Gal), 73.41 (C-4, Man), 77.13 (d, C-2, Man′), 92.68 (br, $^1J_{C,H}$ 176.1, C-1, Man), 94.34 (d, $J_{C,P}$ ~5.0, $^1J_{C,H}$ 170.2, C-1, Man′), 99.01 ($^1J_{C,H}$ 175.8, C-1, Man″), 101.18 ($^1J_{C,H}$ 161.5, C-1, Gal), 127.80–130.05 and 132.42–133.29 (Ph), 164.78–166.15 (Ph*C*O$_2$), 169.10, 170.41 and 170.95 (Me*C*O$_2$); δ_P 1.68 (dd, $^1J_{P,H}$ 640.0, $^3J_{P,H}$ 8.7, P) and −2.54 (dt, $^3J_{P,H}$ 8.5 and 10.5, P′); FAB-MS(+): *m/z* 2017.45 (50%, [M − 2 Et_3N + K]$^+$) and 2080.47 (95, [M − Et_3N + H]$^+$) ($C_{112}H_{120}N_2O_{39}P_2$ requires M, 2180.12). Also isolated was the starting material **14** (15 mg, 11.3% recovery).

Dec-9-enyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranosyl phosphate, triethylammonium salt 17

The preparation of H-phosphonate **16** from compound **11** has been described recently.[1] Compound **17** was prepared by condensation of the glycobiosyl H-phosphonate **16** (57 mg, 0.04 mmol) and dec-9-en-1-ol (0.021 cm^3, 0.12 mmol) in the presence of adamantane-1-carbonyl chloride (20 mg, 0.10 mmol), followed by oxidation with iodine (20 mg, 0.08 mmol) (as described for the synthesis of compound **14**) and subsequent dedimethoxytritylation with 1% TFA in CH_2Cl_2 (1 min; 0 °C; as described in the preparation of compound **5**). Column chromatography [CH_2Cl_2–MeOH–Et_3N, (99:0:1) ⟶ (97:2:1)] gave the *biosyl phosphate derivative* **17** (46 mg, 90%) as an amorphous solid; $[\alpha]_D^{22}$ +64 (*c* 1, $CHCl_3$); R_f 0.23 (solvent *A*); δ_C 8.64 and 45.66 (Et), 25.78, 28.99, 29.14, 29.39, 29.75 and 33.85 (CH_2), 30.79 (d, $J_{C,P}$ 7.5, OCH_2CH_2), 60.23 (C-6′), 62.61 (C-6), 66.20 (d, $J_{C,P}$ 5.0, OCH_2CH_2), 68.60 (C-4′), 70.00 (2 C, C-3 and -5), 70.23 (C-2′), 70.81 (d, $J_{C,P}$ 7.4, C-2), 71.88 (C-3′), 73.09 (C-4), 74.31 (C-5′), 93.62 (d, $J_{C,P}$ 5.0, C-1), 100.63 (C-1′), 114.12 (CH=*C*H$_2$), 128.35–131.13 and 133.11–133.77 (Ph), 139.39 (*C*H=CH$_2$) and 165.17–165.90 (Ph*C*O$_2$); δ_P −1.40 (q, $J_{P,H}$ 7.0); FAB-MS(+): *m/z* 1287.0 (90%, [M + H]$^+$) ($C_{70}H_{80}NO_{20}P$ requires M, 1286.37).

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-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl phosphate]}, tristriethylammonium salt 18

This compound was prepared by condensation of the tetrasaccharide H-phosphonate block **15** (72 mg, 0.033 mmol) and the monohydroxylic disaccharide block **17** (30 mg, 0.023 mmol) in the presence of adamantane-1-carbonyl chloride (20 mg, 0.10 mmol) in pyridine (0.5 cm^3) during 1 h, followed by oxidation with iodine (17 mg, 0.067 mmol), as described in the preparation of compound **14**. Column chromatography [CH_2Cl_2–MeOH–Et_3N, (98.8:0.2:1) ⟶ (95:4:1)] gave the *protected hexasaccharide triphosphate* **18** (50 mg, 62%) as an amorphous solid; $[\alpha]_D^{22}$ +20.2 (*c* 1, $CHCl_3$); R_f 0.27 (solvent *A*); δ_C 8.40 and 45.50 (Et), 20.66 (*Me*CO$_2$), 25.90, 28.62–29.65 and 33.62 (CH_2), 61.53 (2 C, br, C-6, Gal + C-6, Gal′), 61.96 (C-6, Man″), 62.21 (C-6, Man′), 62.40 (2 C, C-6, Man + C-6, Man‴), 66.00 (C-4, Man″), 66.04 (d, $J_{C,P}$ 6.1, OCH_2CH_2), 66.60 (C-4, Man‴), 67.25 (2 C, C-4, Gal + C-4, Gal′), 69.19 (C-5, Man″), 69.30 (C-3, Man‴), 69.78 (2 C, C-3, Man′ + C-5, Man‴), 70.15, 70.23 and 70.28 (C-3, Man; C-5, Man; C-5, Man′), 70.38 (2 C, C-2, Gal + C-2, Gal′), 70.59 (d, $J_{C,P}$ ~7.0, C-2, Man′), 70.66 (2 C, C-2, Man‴ + C-3, Man″), 70.94 (d, $J_{C,P}$ ~7.0, C-2, Man), 71.71 (d, $J_{C,P}$ ~7.0, C-5, Gal′), 71.91 (d, $J_{C,P}$ ~7.0, C-5, Gal), 72.31 and 72.38 (C-3, Gal; C-3, Gal′), 73.28 and 73.48 (C-4, Man; C-4, Man′), 77.19 (d, $J_{C,P}$ ~7.0, C-2, Man″), 93.39 (br, C-1, Man′) 93.52 (br, C-1, Man), 94.30 (br, C-1, Man″), 98.96 (C-1, Man‴), 101.11 (C-1 Gal′), 101.20 (C-1, Gal), 113.94 (CH=*C*H$_2$), 127.80–130.02 and 132.35–133.25 (Ph), 139.35 (*C*H=CH$_2$), 164.75–166.12 (Ph*C*O$_2$) and 169.02, 170.30 and 170.87 (Me*C*O$_2$); δ_P −1.40 (q, $J_{P,H}$ 6.5, P), −2.56 (dt, $J_{P,H}$ 8.0 and 10.0, P″) and −3.05 (dt, $J_{P,H}$ 7.1 and $J_{P,H}$ 9.0, P′); ES-MS(−): *m/z* 1052.4 (100%, [M − Et_3N − 3 H]$^{3-}$) and 1579.1 (20, [M − 3 Et_3N − 2 H]$^{2-}$) ($C_{182}H_{198}N_3O_{59}P_3$ requires M, 3462.18).

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}-[α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl phosphate]}, tristriethylammonium salt 8

To a solution of compound **18** (50 mg) in MeOH–THF (4:1; 10 cm^3) was added NaOMe in MeOH (4.6 mol dm^{-3}; 0.11 cm^3). The mixture was kept at 24 °C for 1.5 h and at 1 °C for 16 h, whereafter it 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 *hexasaccharide triphosphate* **8** (23 mg, 95.3%) was thereby obtained as an amorphous solid; $[\alpha]_D^{22}$ +35 (*c* 1, MeOH); R_f 0.30 (solvent *D*); $\delta_H(D_2O)$ (*inter alia*) 1.29 (10 H, m, 5 × CH_2) 1.62 (2 H, quintet, *J* 6.5, $OCH_2CH_2CH_2$), 2.05 (2 H, quartet, *J* 6.5, CH_2CH_2CH=), 4.46 (1 H, d, $J_{1,2}$ 7.4, 1-H, Gal′), 4.47 (1 H, d, $J_{1,2}$ 7.4, 1-H, Gal), 4.96 (1 H, br d, *J* 10.1, CH=CH_2), 5.04 (1 H, br d, *J* 17.0, CH=CH_2), 5.06 (1 H, d, $J_{1,2}$ 1.2, 1-H, Man‴), 5.40 (1 H, dd, $J_{1,2}$ 1.2, $J_{1,P}$ 7.2, 1-H, Man), 5.43 (1 H, dd, $J_{1,2}$ 1.2, $J_{1,P}$ 7.2, 1-H, Man′), 5.65 (1 H, dd, $J_{1,2}$ 1.2, $J_{1,P}$ 7.2, 1-H, Man″) and 5.92 [1 H, ddt, *J*(H,CH_2) 6.5, CH_2CH=CH_2]; $\delta_P(D_2O)$ −0.91 (P) and −1.24 (P′ + P″) (ratio 1:2); δ_C, see Table 1; ES-MS(−): *m/z* 455.3 (100%, [M − 3 Et_3N − 3 H]$^{3-}$), 683.3 (50, [M − 3 Et_3N − 2 H]$^{2-}$), 694.4 (65, [M − 3 Et_3N − 3 H + Na]$^{2-}$), 1367.4 (1, [M − 3 Et_3N − H]$^-$), 1389.5 (2, [M − 3 Et_3N − 2 H + Na]$^-$) and 1411.5 (3, [M − 3 Et_3N − 3 H + 2 Na]$^-$) ($C_{64}H_{128}N_3O_{40}P_3$ requires M, 1671.73).

Dec-9-enyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate, triethylammonium salt 9

O-Debenzoylation of compound **17** (14 mg) with NaOMe in MeOH (0.05 mol dm^{-3}) (1 h; 24 °C), followed by work-up as in the preceding experiment gave the *dec-9-enyl glycobiosyl phosphate* **9** (7 mg, 97.2%) as an amorphous solid; $[\alpha]_D^{22}$ +21.5 (*c* 0.7, MeOH); R_f 0.65 (solvent *D*); $\delta_H(D_2O)$ 1.31 (19 H, m, 3 × *Me*CH_2 and 5 × CH_2), 1.63 (2 H, quintet, *J* 6.5, $OCH_2CH_2CH_2$), 2.05 (2 H, quartet, *J* 6.5, CH_2CH_2CH=), 3.21 (6 H, quartet, 3 × MeCH_2), 3.56 (1 H, dd, $J_{2',3'}$ 10.0, 2′-H), 3.68 (1 H, dd, $J_{3',4'}$ 3.0, 3′-H), 3.76 (2 H, m, 5′- and 6′-H^a), 3.82 (1 H, dd, $J_{5',6b'}$ 9.0, $J_{6a',6b'}$ 12.0, 6′-H^b), 3.86–3.96 (7 H, m, 4-, 4′- and 5-H, 6-H_2 and OCH_2CH_2), 4.02 (1 H, dd, $J_{3,4}$ 9.0, 3-H), 4.04 (1 H, dd, $J_{2,3}$ 3.0, 2-H), 4.46 (1 H, d, $J_{1',2'}$ 7.5, 1′-H), 4.98 (1 H, dd, $^2J_{H,H}$ 2.0, $^3J_{H,H}$ 10.0, CH=CH_2), 5.06 (1 H, dd, $^3J_{H,H}$ 17.5, CH=CH_2), 5.41 (1 H, dd, $J_{1,2}$ 1.5, $J_{1,P}$ 7.5, 1-H) and 5.93 [1 H, ddt, *J*(H,CH_2) 6.5, CH_2CH=CH_2]; $\delta_P(D_2O)$ −1.04; δ_C, see Table 1; ES-MS(−): *m/z* 559.3 (100%, [M − Et_3N − H]$^-$), 663.4 (3, [M − Et_3N − 5 H + 3 Na + K]$^-$), 1119.5 (5, [2 M − 2 Et_3N − H]$^-$) and 1141.5 (4, [2 M − 2 Et_3N − 2 H + Na]$^-$) ($C_{28}H_{56}N\,O_{14}P$ requires M, 661.34).

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