Parasite glycoconjugates. Part 6.[1] Chemical synthesis of phosphorylated penta- and hepta-saccharide fragments of *Leishmania major* antigenic lipophosphoglycan

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The phosphorylated branched oligosaccharides 1 and 2, fragments of the phosphoglycan portion of *Leishmania major* lipophosphoglycan, have been synthesized using the trichloroacetimidate method for the glycosylation reactions and the phosphoramidite and hydrogenphosphonate methods for phosphorylation.

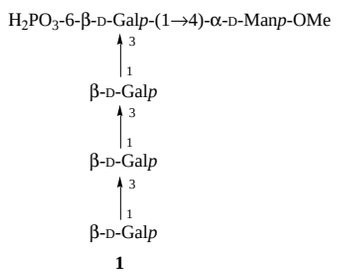
Introduction

We have recently described chemical syntheses of phosphorylated oligosaccharide fragments[1,2] and the phosphoglycan chain[3] of the antigenic lipophosphoglycan (LPG) of *Leishmania donovani*, a parasitic protozoan organism that causes visceral leishmaniasis. *L. major*, another species of *Leishmania* parasites, generally causes a self-limiting skin lesion called oriental sore. The phosphoglycan portion of *L. major* LPG is an irregular polymer (see Table 1)[4,5] consisting of β-D-Gal*p*-(1→4)-α-D-Man*p*-phosphate repeating units, where the 3-OH group of D-galactose is in the main randomly substituted with β-D-Gal*p*, β-D-Ara*p*-(1→2)-β-D-Gal*p* and β-(1→3)-linked D-galactobiose. In addition, a small proportion of the repeats is substituted with β-D-Glc*p*-(1→3)-β-D-Gal*p*, β-D-Ara*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Gal*p* and β-(1→3)-linked D-galactotriose. Both the proportion of these substituents and the average chain-length of the phosphoglycan in *L. major* vary during differentiation from the non-infectious procyclic promastigote form (existing in the sand-fly mid-gut) into the highly infectious metacyclic promastigote form that can successfully invade the macrophages of the mammalian host (see Table 1). The β-(1→3)-linked D-galactotriose side-chain was found to be responsible for binding procyclic parasites to the gut wall of the sand-fly vector[6,7] and metacyclic parasites to macrophage cell-surface receptors.[8] We now report the chemical synthesis of phospho-oligosaccharides **1** and **2** containing the galactotriose side-chain linked to phosphodisaccharide (in **1**) and phosphotetrasaccharide (in **2**) fragments of the LPG backbone.

Results and discussion

A retrosynthetic analysis of the phosphoheptasaccharide **2** showed that it might be prepared from the glycobiosyl H-phosphonate **3** (as a donor of the Gal^{e}-Man^{b}-phosphate fragment) and the corresponding pentasaccharide (Gal^{d}-Gal^{c}-Gal^{b}-Gal^{a}-Man^{a}) monohydroxylic compound, which would also give the phosphopentasaccharide **1** on phosphorylation. The linear pentasaccharide (Gal^{d}-Gal^{c}-Gal^{b}-Gal^{a}-Man^{a}) can be synthesized by stepwise chain-elongation from the mannoside acceptor **4** (for the Man^{a} residue) using the compounds **5**, **6** and **7** or **8** as galactobiosyl and galactosyl donors in the trichloroacetimidate procedure.[9,10]

The disaccharide trichloroacetimidate **5**, modelling the terminal Gal^{d}-Gal^{c} fragment, could be prepared from the known[11] disaccharide **17** (see below). As with previous syntheses of β-(1→3)-linked D-galacto-oligosaccharides,[12,13] the benzyl group



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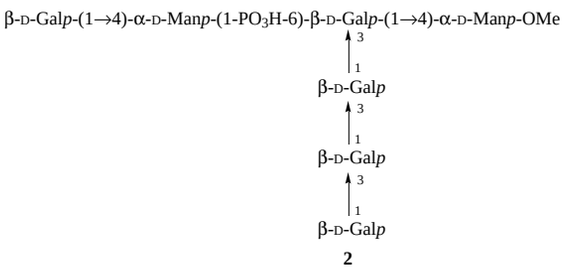


2

was chosen for the temporary protection of O-3 of the trichloroacetimidates **6** (donor of the Gal^{b} residue) and **7** and **8** (donors of the Gal^{a} residue). The chloroacetyl (CA) or *tert*-butyldiphenylsilyl (TBDPS) group served for the temporary protection of O-6 of the donors **7** and **8**; this position is phosphorylated in the oligosaccharides **1** and **2**.

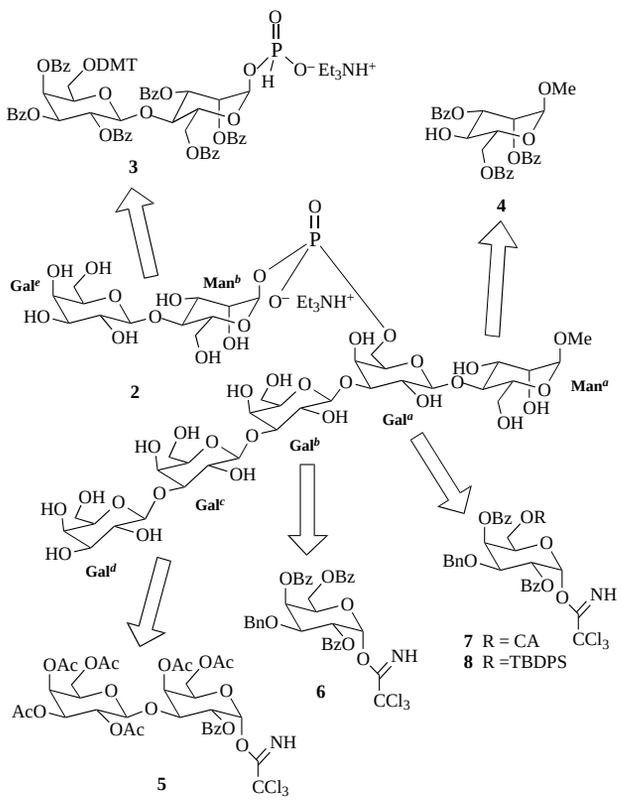
The glycobiosyl H-phosphonate **3** has been described[2] by us recently. Methyl 2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside **4** was prepared in 77% yield by selective benzoylation[14] of methyl α-D-mannopyranoside. The disaccharide trichloroacetimidate **5** was synthesized in 88% yield from the galactose derivative **17**[11] (Scheme 1) by anomeric O-deacetylation,[1,2,15] with Me_2NH in acetonitrile, followed by treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[16]

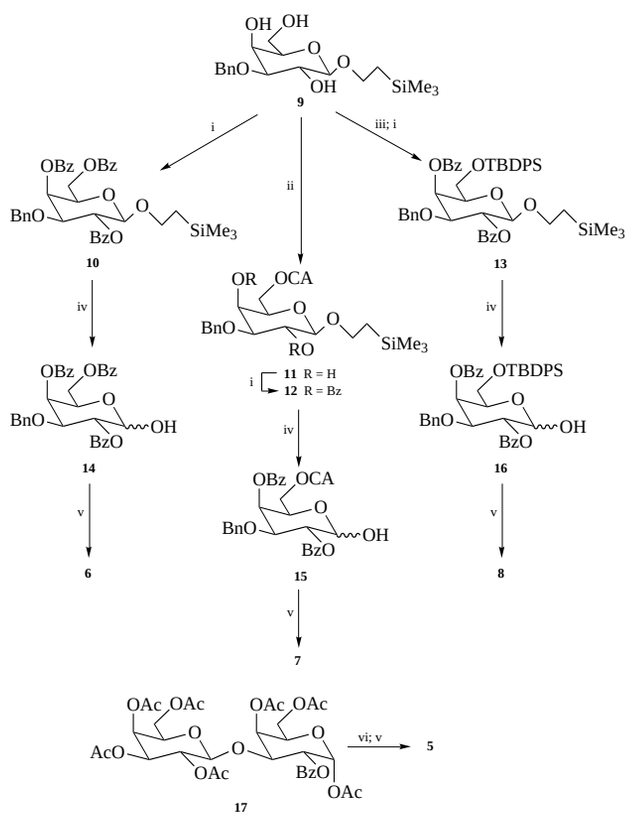
The galactosyl trichloroacetimidates **6**, **7** and **8** were all prepared from 2-(trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside **9**, which in turn was synthesized by way of stannylidenation[17,18] of 2-(trimethylsilyl)ethyl β-D-galactopyranoside[19] with dibutyltin oxide (DBTO) in methanol, followed by selective benzylation of the 3,4-stannylidene acetal with benzyl bromide in the presence of tetrabutylammonium iodide (TBAI). The galactoside **10** was obtained on conventional benzoylation of compound **9**. Subsequent cleavage[19] of the 2-(trimethylsilyl)-ethyl (TMS-ethyl) aglycone from the galactoside **10** with CF_3CO_2H (TFA) in CH_2Cl_2 and treatment of the hemiacetal **14**

Table 1 Primary structure of *L. major* LPG (procyclic and metacyclic promastigote forms)[4,5]

R
↓
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-]$_n$-glycosyl phosphatidylinositol anchor

R	Procyclic promastigote mole%	Metacyclic promastigote mole%
H	7	15
β-D-Gal*p*-(1→3)	52	31
β-D-Ara*p*-(1→2)-β-D-Gal*p*-(1→3)	9	45
β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)	25	6
β-D-Glc*p*-(1→3)-β-D-Gal*p*-(1→3)	1	1
β-D-Ara*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)	2	3
β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)	4	2
n, average number of repeating units per molecule	14	30



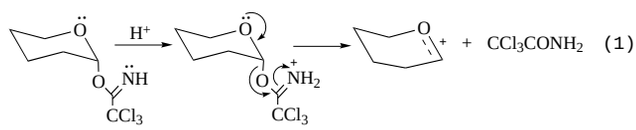


Scheme 1 *Reagents:* i, PhCOCl, pyridine; ii, (*a*) BTBTO, toluene; (*b*) $ClCH_2COCl$, CH_2Cl_2; iii, TBDPSCl, imidazole, pyridine; iv, TFA–CH_2Cl_2 (2:1); v, CCl_3CN, DBU, CH_2Cl_2; vi, Me_2NH, acetonitrile

with CCl_3CN in the presence of DBU produced the galactosyl trichloroacetimidate **6** in 95% yield.

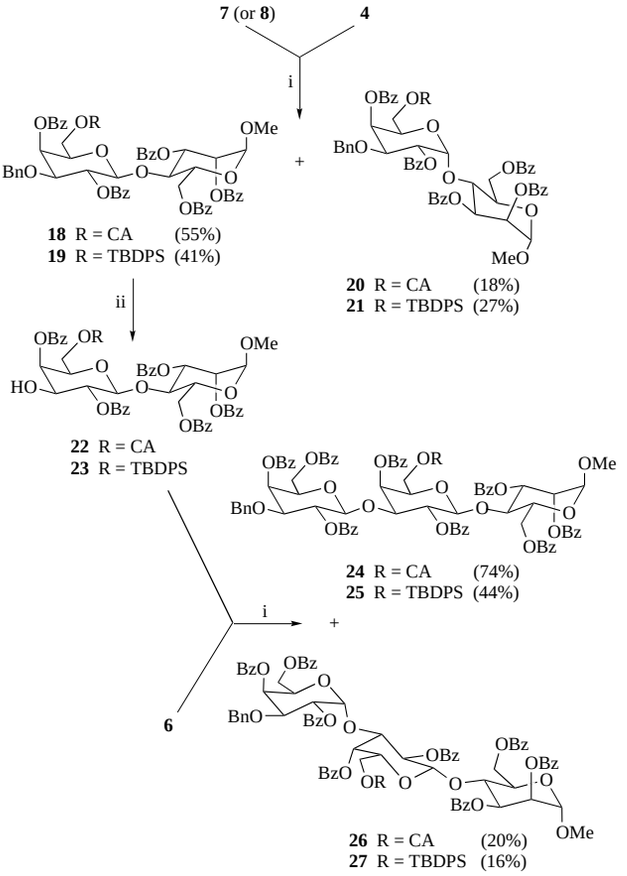
To prepare the 6-*O*-chloroacetyl derivative **7**, compound **9** was first 6-*O*-stannylated by reaction with bis(tributyltin) oxide (BTBTO), followed in turn by selective acylation with chloroacetyl chloride (⟶ **11**, 90%) and benzoylation (⟶ **12**, 97%). The dibenzoate **12** was then deprotected at the anomeric position (⟶ **15**) and converted into the trichloroacetimidate **7** in 69% yield as described for the TMS-ethyl galactoside **10**. Consecutive treatments of compound **9** in pyridine with TBDPS chloride (TBDPSCl) and benzoyl chloride gave the 6-TBDPS ether **13** (84%), which was similarly transformed into the trichloroacetimidate **8** (87%) *via* the hemiacetal derivative **16**.

The structures of the galactosyl α-trichloroacetimidates **5**–**8** were confirmed by their ^{1}H and ^{13}C NMR data, which revealed signals characteristic of the trichloroacetimidate group at δ_H 8.50–8.60 (NH) and δ_C 90.66–91.26 (CCl_3) and 160.12–160.50 (C=NH). The α-configuration of these derivatives was evident from the characteristic value (3.0–3.6 Hz) of the $J_{H1,H2}$-coupling constants. Electrospray mass spectra in the positive mode [ESMS(+)] of the trichloroacetimidates **5** and **6** revealed the presence of signals for the corresponding glycosyl cations (*m*/*z* 681.1, $[M - CCl_3CONH]^+$ for **5** and *m*/*z* 565.0, $[M - CCl_3CONH]^+$ for **6**, which are likely to arise by the ES-ionization as shown in equation (1).

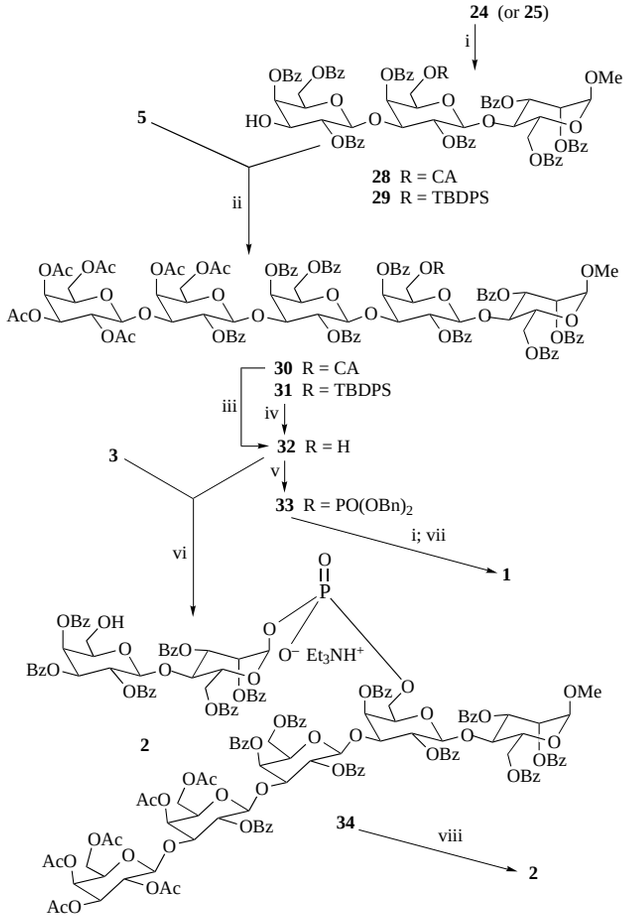


(1)

The linear pentasaccharide monohydroxylic derivative **32** (shown later in Scheme 3) was assembled by two parallel pathways, one using the 6-*O*-chloroacetyl derivative **7** and the other the 6-TBDPS ether **8** as the Gal^a donor. Glycosylation of the mannoside **4** with the trichloroacetimidate **7** (Scheme 2) was accomplished in CH_2Cl_2 at −60 °C in the presence of trimethylsilyl trifluoromethanesulfonate (TMS triflate) to give the β-



Scheme 2 *Reagents:* i, TMS triflate, CH_2Cl_2; ii, $Pd(OH)_2$/C, 2-methoxyethanol (or MeOH–ethyl acetate)



Scheme 3 *Reagents:* i, $Pd(OH)_2$/C, 2-methoxyethanol (or MeOH–ethyl acetate); ii, TMS triflate (or TES triflate), CH_2Cl_2; iii, $(NH_2)_2CS$, MeOH; iv, TBAF, THF; v, (*a*) $(BnO)_2PN(Pr^i)_2$, tetrazole, CH_2Cl_2; (*b*) MCPBA, CH_2Cl_2; vi, (*a*) Me_3CCOCl, pyridine; (*b*) I_2, pyridine–water; (*c*) 1% TFA–CH_2Cl_2; vii, NaOMe, MeOH; viii, (*a*) NaOMe, MeOH; (*b*) NaOH, MeOH–water

(1→4)-linked disaccharide **18** (55%) together with some (18%) of the α-linked isomer **20**. A similar coupling of the mannoside **4** with the trichloroacetimidate **8** was less stereoselective, giving the β- and α-linked disaccharides **19** and **21** in yields of 41 and 27%, respectively.

Hydrogenolysis of the disaccharides **18** and **19** over $Pd(OH)_2$/C afforded the disaccharide acceptors **22** (96%) and **23** (86%), respectively, which were each glycosylated with the galactosyl trichloroacetimidate **6**. Galactosylation of the 6′-*O*-chloroacetyl derivative **22** in the presence of TMS triflate furnished the β,β-linked trisaccharide **24** in 74% yield and a small proportion (20%) of the α,β-linked isomer **26**. Analogous coupling of the 6′-*O*-TBDPS derivative **23** gave a lower yield (44%) of the β,β-linked trisaccharide **25**, as well as the α,β-linked trisaccharide **27** (16%) and recovered acceptor **23** (18%).

Hydrogenolysis (as above) of compounds **24** and **25** (Scheme 3) afforded the trisaccharide monohydroxylic acceptors **28** and **29**, respectively, in reasonable yields. Glycosylation of the trisaccharide **28** with the galactobiose trichloroacetimidate **5** in the presence of TMS triflate resulted in a highly stereoselective formation of the pentasaccharide **30** (68%). The 6′-*O*-TBDPS-protected pentasaccharide **31** was prepared in 57% yield by coupling the same donor and the acceptor **29** in the presence of triethylsilyl triflate; 25% of the trisaccharide **29** was recovered from this reaction. Dechloroacetylation[20] of compound **30** with thiourea in MeOH gave the monohydroxylic pentasaccharide block **32** (60%), which could also be obtained (64% yield) by desilylation[21] of the pentasaccharide **31** with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF).

The ^1H and ^{13}C NMR spectra of the pentasaccharide derivatives **30**–**32** revealed characteristic signals for all five monosaccharide residues (see Experimental section and Table 2). The β-configuration of each of the D-galactosyl units followed from the characteristic value (7.5–8.0 Hz) of the $J_{H1,H2}$-coupling constants. The molecular mass of compound **31** was ascertained from its ES(+) mass spectrum, wherein the main signal corresponded to the pseudo-molecular ion (*m*/*z* 2269.4, $[M + H]^+$).

The preparation of the phosphorylated pentasaccharide **1** was accomplished using the phosphoramidite method.[22,23] Phosphitylation of the monohydroxylic compound **32** with dibenzyl *N*,*N*-diisopropylphosphoramidite[24,25] in the presence of 1*H*-tetrazole, followed by *in situ* oxidation with 3-chloroperbenzoic acid (MCPBA), afforded the phosphotriester **33** in 89% yield. Hydrogenolysis of the protected phosphopentasaccharide **33** over $Pd(OH)_2$/C and O-deacylation of the product with 0.2 mol dm^{-3} NaOMe in methanol–1,4-dioxane at 40 °C gave the deprotected pentasaccharide 6′-phosphate **1** (70%).

The phosphorylated heptasaccharide **2** was prepared using the glycosyl hydrogenphosphonate method.[1,2,15] Coupling of the disaccharide H-phosphonate **3** and the pentasaccharide acceptor **32** in the presence of trimethylacetyl chloride, followed by oxidation with iodine and dedimethoxytritylation (1% TFA–CH_2Cl_2; 0 °C), gave the protected heptasaccharide phosphodiester **34** (71%). Conventional O-deacylation of the latter compound with 0.05 mol dm^{-3} methanolic sodium methoxide at 1 °C afforded three UV-active products, which on TLC migrated slightly faster than the pentasaccharide **1** and which were presumably partially benzoylated derivatives. It has been observed[26,27] that the 2-*O*-acetyl and 2-*O*-benzoyl groups in 3-*O*-glycosylated hexopyranosides (as in Gal^a, Gal^b and Gal^c) are more stable towards deacylation by mild transesterification due to the absence of vicinal hydroxy groups. Further treatment

Table 2 ^{13}C NMR data [δ_C in ppm; $J_{C,P}$ in Hz (in parentheses)] for oligosaccharides **1** and **2** (in D_2O) and **30**–**34** (in $CDCl_3$)

Resid.	Atom	**1**[a]	**2**	**30**[b]	**31**[c]	**32**	**33**[d]	**34**[a]
Man[a]	C-1	101.74	101.71	98.55	98.43	98.36	98.33	98.07
	C-2	70.62	70.54	70.49	70.70	70.54	70.65	70.89
	C-3	70.62	70.54	69.50	69.40	69.57	69.32	69.38
	C-4	78.42	78.89	73.17	72.85	72.85	73.08	74.32
	C-5	72.26	72.22	69.34	69.13	69.17	68.94	69.12
	C-6	61.68	61.63	62.40	62.67	62.37	62.41	62.38
Gal[a]	C-1	104.06	104.12	101.10	101.15	101.42	100.82	100.78
	C-2	71.30	71.24	71.71	72.10	71.66	71.82	72.26
	C-3	82.86	82.84	76.33	75.86	77.60	75.81	76.51
	C-4	69.32	69.32	69.60	69.57	70.43	69.46	70.09
	C-5	75.07d (8.5)	74.71d (8.3)	71.29	73.84	73.67	71.96d (9.0)	72.26d (7.5)
	C-6	64.27d (5.1)	65.92d (4.6)	63.15	60.12	59.39	63.83br	61.36br
Gal[b]	C-1	105.14	105.18	100.89	100.88	100.78	100.77	100.70
	C-2	71.43	71.40	71.99	72.61	71.87	72.06	72.49
	C-3	83.16	83.21	75.63	75.81	75.59	75.65	75.60
	C-4	69.60	69.52	69.69	69.57	69.72	69.46	69.95
	C-5	75.89	75.88	71.64	71.51	71.59	71.55	72.15
	C-6	62.07	62.07	62.40	62.11	62.37	62.09	62.53
Gal[c]	C-1	105.14	105.28	100.99	101.06	100.91	100.96	100.87
	C-2	71.43	71.40	71.05	71.18	70.98	71.04	70.89
	C-3	83.16	83.21	74.73	74.37	74.73	74.70	75.60
	C-4	69.60	69.52	68.77	68.96	68.72	68.77	68.69
	C-5	75.89	75.88	71.37	71.51	71.33	71.36	71.23
	C-6	62.07	62.07	61.91	62.11	61.84	61.91	61.73
Gal[d]	C-1	105.42	105.52	100.12	99.95	100.08	100.05	99.78
	C-2	72.26	72.15	68.00	68.09	67.98	67.99	67.92
	C-3	73.74	73.68	70.40	70.60	70.43	70.47	70.41
	C-4	69.77	69.75	66.43	66.55	66.40	66.43	66.35
	C-5	76.24	76.25	70.34	70.44	70.31	70.34	70.23
	C-6	62.17	62.14	60.73	60.87	60.68	60.74	60.44
Man[b]	C-1		97.16d (4.6)					93.42br
	C-2		71.13d (8.3)					70.80d (8.9)
	C-3		69.82					69.21
	C-4		76.99					72.72
	C-5		73.68					69.63
	C-6		61.34					62.61
Gal[e]	C-1		104.24					100.12
	C-2		72.15					70.23
	C-3		73.68					71.86
	C-4		69.82					68.43
	C-5		76.56					74.54
	C-6		62.34					60.16
CH_3CO	CH_3			19.84–20.70	20.03–20.70	19.73–20.61	19.83–20.66	19.76–20.47
	C=O			169.02–170.24	169.13–170.32	168.90–170.12	169.03–170.25	168.93–170.24
C_6H_5CO	C=O			163.92–165.59	163.65–165.89	164.03–166.04	163.56–165.33	163.45–166.19
C_6H_5				128.00–129.98, 132.60–133.42	127.61–130.44, 132.58–133.31, 135.63, 135.69	127.92–130.15, 132.44–133.27	127.74–130.02, 132.72–133.19, 135.39	127.90–129.84, 132.19–133.44
CH_3O		56.0	56.0	55.33	55.31	55.20	55.29	55.07

[a] Additional signals of Et_3NH^+ [δ_C 9.25–9.45 (CH_3) and δ_C 45.45–47.91 (CH_2)] were present. [b] Additional signals of $ClCH_2CO$ [δ_C 40.20 ($ClCH_2$) and δ_C 166.69 (CO)] were present. [c] Additional signals of Me_3CSi [δ_C 18.82 (C) and δ_C 26.60 (CH_3)] were present. [d] Additional signals of two PhCH_2 (δ_C 69.11 and 69.32, 2 d, $J_{C,P}$ = 4.5 Hz) were present.

of these products with 1% NaOH in aq. methanol gave the deprotected heptasaccharide **2** in 75% yield and a small proportion (22%) of the pentasaccharide **1**, which were isolated by ion-exchange chromatography. Similar partial cleavage of glycosyl phosphodiester linkages under basic conditions has been reported recently.[28]

NMR spectroscopic and mass spectrometric data were used to confirm the structures of compounds **1**, **2**, **33** and **34**. Signals characteristic of all five (for the pentasaccharides **1** and **33**) and all seven (for the heptasaccharides **2** and **34**) monosaccharide residues are present in the ^{13}C and ^{1}H NMR spectra (see Table 2 and Experimental section). The ^{31}P NMR data are characteristic of the deprotected phosphomonoester **1** (δ_P 1.78) and phosphodiester **2** (δ_P −1.28) and of the protected phosphotriester **33** (δ_P −3.69).

The presence of the (1→6)-phosphodiester linkage in the heptasaccharide **2** and the C-6′ position of the phosphate group in the pentasaccharide **1** were confirmed by ^{13}C NMR spectroscopy. The C-5 and C-6 signals of the Gal^{a} unit (in both spectra) and C-1 and C-2 signals of the Man^{b} unit (in the spectrum of the heptasaccharide **2**) were shifted as a result of the α- and β-effects of phosphorylation and were coupled with phosphorus. The molecular masses of the oligosaccharides **1** and **2** were confirmed by ES(−) mass spectrometry. The main signals in the spectra corresponded to the pseudo-molecular ions for the pentasaccharide phosphomonoester **1** (*m*/*z* 921.0, [M − 2 Et_3N − H]$^-$) and the heptasaccharide phosphodiester **2** (*m*/*z* 1244.9, [M − NH_3 − H]$^-$).

Experimental

General procedures

Mps 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}. NMR spectra (^{1}H at 200 and 500 MHz, ^{13}C-{^{1}H} at 50.3 and 125 MHz, and ^{31}P-{^{1}H} 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 ^{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). TLC was performed on Polygram Sil G/UV$_{254}$ (Macherey-Nagel, Germany) with *A*, toluene–ethyl acetate (19:1); *B*, toluene–ethyl acetate (9:1); *C*, toluene–ethyl acetate (3:1); *D*, toluene–ethyl acetate (1:1); *E*, chloroform–methanol (19:1); and *F*, chloroform–methanol–water (10:10:3) as developers and detection under UV light or by charring with sulfuric acid–water–ethanol (15:85:5). Flash-column chromatography (FCC) was performed on Kieselgel 60 (0.040–0.063 mm) (Merck). Dichloromethane, acetonitrile and pyridine (for the H-phosphonate condensation) were freshly distilled from CaH_2. Solutions worked up were concentrated under reduced pressure at <40 °C.

Methyl 2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 4

To a stirred and cooled (−40 °C) solution of methyl α-D-mannopyranoside (3.88 g, 20 mmol) in pyridine (160 cm^3) was added dropwise benzoyl chloride (7.2 cm^3, 62 mmol) over a period of 20 min. The temperature was increased to 20 °C for 3 h, and the mixture was stirred overnight, whereafter most of the pyridine was evaporated off under reduced pressure and chloroform (200 cm^3) was added to the residue. The resulting solution was washed successively with saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. FCC (solvent *B*) of the residue gave the tribenzoate **4** (7.77 g, 77%) as a syrup; $[\alpha]_D^{22}$ +1.4 (*c* 1, $CHCl_3$) [lit.,[14] −6.5 (*c* 0.93, $CHCl_3$)]; R_f 0.12 (solvent *A*); δ_H 3.10 (1 H, d, $J_{OH,4}$ 4.7, 4-OH), 3.51 (3 H, s, OCH_3), 4.09 (1 H, ddd, $J_{5,6a}$ 2.5, 5-H), 4.29 (1 H, dt, $J_{3,4} = J_{4,5} = 9.5$, 4-H), 4.66 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H^a), 4.90 (1 H, dd, $J_{5,6b}$ 4.0, 6-H^b), 4.93 (1 H, d, $J_{1,2}$ 1.5, 1-H), 5.61 (1 H, dd, $J_{2,3}$ 3.0, 2-H), 5.64 (1 H, dd, 3-H) and 7.28–8.14 (15 H, m, 3 × Ph); δ_C 55.2 (OCH_3), 63.4 (C-6), 66.1 (C-4), 70.4 (C-2), 71.0 (C-5), 72.5 (C-3), 98.6 (C-1), 128.29–129.87 and 133.12–133.28 (Ph) and 165.31–166.80 (Ph*C*O$_2$).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate 5

To a cooled (−15 °C) and stirred solution of the disaccharide derivative **17**[11] (300 mg, 0.41 mmol) in dry acetonitrile (4 cm^3) was added dimethylamine (0.16 cm^3, 2.43 mmol), and the reaction was allowed to proceed at room temperature for 1–2 h, whereupon TLC (solvent *D*) showed the formation of one product (R_f 0.43; corresponding hemiacetal derivative). The mixture was concentrated and toluene was evaporated off from the residue, which was taken up in dichloromethane (4 cm^3). The solution was stirred under nitrogen and treated with CCl_3CN (1.5 cm^3, 15 mmol) and DBU (0.068 cm^3, 0.45 mmol) at 0 °C for 2 h and then concentrated. FCC of the residue (solvent *D*) gave the galactobiosyl trichloroacetimidate **5** (303 mg, 88%) as an amorphous solid; $[\alpha]_D^{20}$ +71 (*c* 1.25, $CHCl_3$); R_f 0.61 (solvent *D*); δ_H 1.51, 1.85, 1.99, 2.02, 2.10 and 2.13 (18 H, 6 × s, 6 × Ac), 3.79–4.26 (5 H, m, 5′-H and 6- and 6′-H$_2$), 4.30–4.45 (2 H, m, 3- and 5-H), 4.62 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.75 (1 H, dd, $J_{2',3'}$ 10.5, 3′-H), 5.01 (1 H, dd, 2′-H), 5.26 (1 H, d, $J_{3',4'}$ 2.9, 4′-H), 5.58 (1 H, d, $J_{3,4}$ 3.2, 4-H), 5.61 (1 H, dd, $J_{2,3}$ 10.5, 2-H), 6.58 (1 H, d, $J_{1,2}$ 3.6, 1-H), 7.35–8.00 (5 H, m, Ph) and 8.60 (1 H, s, NH); δ_C 20.06, 20.52 and 20.71 (*Me*CO), 61.24 (C-6′), 62.24 (C-6), 65.81 (C-4), 66.74 (C-4′), 68.57 (C-2′), 69.23 (C-2), 70.04 (C-5), 70.67 (C-3′), 70.85 (C-5′), 73.10 (C-3), 90.97 (CCl_3), 93.88 (C-1), 101.48 (C-1′), 128.27–129.73 and 133.82 (Ph), 160.36 (C=NH), 165.25 (Ph*C*O$_2$) and 168.02–171.50 (Me*C*O$_2$); ESMS(+) data: *m*/*z* 681.1 (100%, $[M - CCl_3CONH]^+$) ($C_{33}H_{38}Cl_3NO_{18}$ requires M, 841.12).

2-(Trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside 9

A mixture of 2-(trimethylsilyl)ethyl β-D-galactopyranoside[19] (6.62 g, 23.8 mmol) and DBTO (6.22 g, 25 mmol) in anhydrous methanol (250 cm^3) was heated under reflux for 2 h, after which time the reactants had completely dissolved. The methanol was then evaporated off. The resulting syrup was dissolved in anhydrous toluene (250 cm^3), TBAI (9.23 g, 25 mmol) and benzyl bromide (3 cm^3, 25 mmol) were added, and the mixture was boiled for 3 h; TLC (solvent *C*) then showed that only traces of starting material remained. The mixture was concentrated and the major product was isolated by column chromatography (4:1 toluene–ethyl acetate) as a syrup (6.36 g, 72%), which crystallized on storage. After recrystallization from diethyl ether–hexane, the *monobenzylated derivative* **9** had mp 74 °C; $[\alpha]_D^{20}$ −11 (*c* 1, $CHCl_3$) (Found: C, 58.2; H, 8.0. $C_{18}H_{30}O_6Si$ requires C, 58.35; H, 8.2%); δ_H 0.00 (9 H, s, Me_3Si), 1.00 (2 H, m, C*H*$_2$SiMe$_3$), 3.42 (1 H, dd, $J_{3,4}$ 3.5, 3-H), 3.50 (1 H, m, C*H*CH$_2$SiMe$_3$), 3.59 (1 H, dd, $J_{2,3}$ 10.5, 2-H), 3.71–4.07 (5 H, m, 4- and 5-H, 6-H$_2$ and C*H*CH$_2$SiMe$_3$), 4.25 (1 H, d, $J_{1,2}$ 7.8, 1-H), 4.72 (2 H, s, C*H*$_2$Ph) and 7.22–7.43 (5 H, m, Ph); δ_C −1.30 (Me_3Si), 18.34 (*C*H$_2$SiMe$_3$), 62.40 (C-6), 67.11 (C-4), 67.51 (*C*H$_2$CH$_2$SiMe$_3$), 71.08 (*C*H$_2$Ph), 72.17 (C-2), 74.30 (C-5), 80.24 (C-3), 102.69 (C-1) and 128.03–128.71 and 137.76 (Ph).

2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranoside 10

To a cooled (0 °C) and stirred solution of compound **9** (2.81 g, 7.58 mmol) in pyridine (30 cm^3) was added benzoyl chloride (5.3 cm^3, 45.5 mmol), whereafter the reaction mixture was allowed to attain room temperature and was stirred for a further 2 h; TLC (solvent *B*) then showed the absence of any starting material. The mixture was diluted with $CHCl_3$ (100 cm^3) and washed in turn with saturated aq. $NaHCO_3$ and water. After drying ($MgSO_4$), the solvents were evaporated off and the residue was purified by FCC (15:1 toluene–ethyl acetate) to give the *benzoylated galactoside* **10** (4.2 g, 81%) as an amorphous solid; $[\alpha]_D^{20}$ +76 (*c* 1, $CHCl_3$); R_f 0.43 (solvent *A*) (Found: C, 68.75; H, 6.0. $C_{39}H_{42}O_9Si$ requires C, 68.6; H, 6.2%); δ_H 0.00 (9 H, s, Me_3Si), 1.00 (2 H, m, C*H*$_2$SiMe$_3$), 3.67 and 4.10 (2 H, 2 × m, C*H*$_2$CH$_2$SiMe$_3$), 3.90 (1 H, dd, $J_{2,3}$ 10.0, 3-H), 4.19 (1 H, br t, $J_{5,6}$ 7.0, 5-H), 4.49–4.86 (5 H, m, 1-H, 6-H$_2$ and C*H*$_2$Ph), 5.63 (1 H, dd, $J_{1,2}$ 7.5, 2-H), 6.02 (1 H, d, $J_{3,4}$ 3.0, 4-H) and 7.10–8.32 (20 H, m, 4 × Ph); δ_C −1.44 (Me_3Si), 18.17 (*C*H$_2$SiMe$_3$), 62.75 (C-6), 66.77 (C-4), 67.54 (*C*H$_2$CH$_2$SiMe$_3$), 71.00 (*C*H$_2$Ph), 71.38 (2 C, C-2 + -5), 77.2 (C-3), 101.06 (C-1), 127.75–130.66, 133.08, 133.52 and 137.35 (Ph) and 165.12–166.38 (Ph*C*O$_2$).

2-(Trimethylsilyl)ethyl 3-*O*-benzyl-6-*O*-chloroacetyl-β-D-galactopyranoside 11

The 3-*O*-benzyl derivative **9** (1.85 g, 5.0 mmol) and BTBTO (1.27 cm^3, 2.5 mmol) in anhydrous toluene (200 cm^3) were heated under reflux in a Dean–Stark apparatus (to remove water) for 1.5 h before the reaction mixture was concentrated. The residue was dissolved in a minimal amount of dichloromethane, and chloroacetyl chloride (0.44 cm^3, 5.5 mmol) was added to the stirred solution at 0 °C; TLC (solvent *C*) after 40 min showed no trace of the starting material. The reaction mixture was concentrated and then coevaporated with toluene. FCC (4:1 toluene–ethyl acetate) of the residue gave the chloroacetyl derivative **11** (2.01 g, 90%) as a syrup; $[\alpha]_D^{20}$ 0 (*c* 1, $CHCl_3$); δ_H 0.00 (9 H, s, Me_3Si), 1.00 (2 H, m, C*H*$_2$SiMe$_3$), 2.73 and 2.83 (2 H, 2 × br s, 2 × OH), 3.42 (1 H, dd, $J_{3,4}$ 3.0, 3-H), 3.60 (2 H, m, 5-H + C*H*CH$_2$SiMe$_3$), 3.76 (1 H, dd, $J_{2,3}$ 9.5, 2-H), 3.90 (1 H, d, 4-H), 3.98 (1 H, m, C*H*CH$_2$SiMe$_3$), 4.05 (2 H, s, CH_2Cl), 4.22 (1 H, d, $J_{1,2}$ 7.5, 1-H), 4.41 (2 H, m, 6-H$_2$), 4.74 (2 H, s, C*H*$_2$Ph) and 7.22–7.43 (5 H, m, Ph); δ_C −1.61 (Me_3Si), 17.97 (*C*H$_2$SiMe$_3$), 40.52 (CH_2Cl), 64.54 (C-6), 66.18 (C-4), 67.09 (*C*H$_2$CH$_2$SiMe$_3$), 70.64 (C-5), 71.63 (*C*H$_2$Ph), 72.07

(C-2), 79.84 (C-3), 102.21 (C-1), 127.76–128.39 and 137.50 (Ph) and 166.93 (CO_2CH_2Cl).

2-(Trimethylsilyl)ethyl 2,4-di-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-β-D-galactopyranoside 12

To a cooled (0 °C) and stirred solution of the chloroacetate **11** (0.12 g, 0.28 mmol) dissolved in a minimal amount of dichloromethane were added pyridine (1 cm^3) and benzoyl chloride (0.39 cm^3, 3.36 mmol), and the reaction mixture was stirred at room temperature for 1 h; TLC (solvent *B*) then showed no trace of the starting material. The reaction mixture was diluted with chloroform (50 cm^3) and the resulting solution was washed in turn with ice–water, saturated aq. $NaHCO_3$, and water. After drying ($MgSO_4$), the solvents were evaporated off and the residue was subjected to FCC (15:1 toluene–ethyl acetate) to give the *dibenzoate* **12** (0.18 g, 97%) as an amorphous solid; $[\alpha]_D^{20}$ +74 (*c* 1.6, $CHCl_3$) (Found: C, 62.8; H, 6.1. $C_{34}H_{39}ClO_9Si$ requires C, 62.3; H, 6.0%); δ_H 0.00 (9 H, s, Me_3Si), 0.97 (2 H, m, C*H*$_2$SiMe$_3$), 3.66 (1 H, m, C*H*CH$_2$SiMe$_3$), 3.89 (1 H, dd, $J_{3,4}$ 3.0, 3-H), 4.09 (2 H, m, 5-H + CHCH$_2$SiMe$_3$), 4.14 (2 H, s, CH_2Cl), 4.46 (2 H, m, 6-H_2), 4.57 and 4.78 (2 H, AB q, *J* 12.8, C*H*$_2$Ph), 4.71 (1 H, d, $J_{1,2}$ 7.8, 1-H), 5.62 (1 H, dd, $J_{2,3}$ 10.0, 2-H), 5.90 (1 H, d, 4-H) and 7.10–8.28 (15 H, m, 3 × Ph); δ_C −1.61 (Me_3Si), 17.82 (*C*H$_2$SiMe$_3$), 40.50 (CH_2Cl), 63.76 (C-6), 66.42 (C-4), 67.36 (*C*H$_2$CH$_2$SiMe$_3$), 70.81 (2 C, C-2 + *C*H$_2$Ph), 71.04 (C-5), 76.04 (C-3), 100.82 (C-1), 127.59–130.42, 132.93–134.46 and 137.05 (Ph), 165.02 and 165.81 (CO_2Ph) and 166.82 (CO_2CH_2Cl). Compound **12** can be made by a one-pot procedure from the TMS-ethyl galactoside **9** using toluene as the solvent for all three steps.

2-(Trimethylsilyl)ethyl 2,4-di-*O*-benzoyl-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside 13

To a solution of the galactoside **9** (2.51 g, 6.7 mmol) in pyridine (20 cm^3) were added imidazole (1.0 g, 14.7 mmol) and TBDPSCl (2.0 cm^3, 7.7 mmol), and the mixture was set aside at room temperature for 18 h; TLC (solvent *C*) then revealed that only traces of the starting material remained. The reaction mixture was stirred, cooled (0 °C), and treated with benzoyl chloride (3.5 cm^3, 30 mmol), whereafter stirring was continued at room temperature for 16 h. Work-up as described for the tribenzoate **10** gave the 6-O-*silylated derivative* **13** (4.66 g, 84%); mp 131–132 °C (from diethyl ether–hexane); $[\alpha]_D^{22}$ +78 (*c* 1, $CHCl_3$) (Found: C, 70.85; H, 6.9. $C_{48}H_{56}O_8Si_2$ requires C, 70.55; H, 6.9%); δ_H 0.00 (9 H, s, Me_3Si), 0.97 (2 H, m, C*H*$_2$SiMe$_3$), 1.15 (9 H, s, Me_3C), 3.61 and 4.09 (2 H, 2 × m, C*H*$_2$CH$_2$SiMe$_3$), 3.90 (4 H, m, 3-, 5-H and 6-H_2), 4.67 (1 H, d, $J_{1,2}$ 7.8, 1-H), 4.69 and 4.88 (2 H, AB q, *J* 12.8, C*H*$_2$Ph), 5.59 (1 H, dd, $J_{2,3}$10.0, 2-H), 6.09 (1 H, d, $J_{3,4}$ 3.0, 4-H) and 7.18–8.30 (25 H, m, 5 × Ph); δ_C −1.39 (Me_3Si), 18.11 (*C*H$_2$SiMe$_3$), 19.19 (Me_3C), 26.83 (*Me*$_3$C), 61.82 (C-6), 66.39 (C-4), 67.37 (*C*H$_2$CH$_2$SiMe$_3$), 70.86 (*C*H$_2$Ph), 71.66 (C-2), 74.03 (C-5), 76.52 (C-3), 101.12 (C-1), 127.75–130.22, 133.01, 133.21, 135.61, 135.69 and 137.68 (Ph), 165.29 and 165.87 (CO_2Ph).

2,4,6-Tri-*O*-benzoyl-3-*O*-benzyl-α,β-D-galactopyranose 14

To a cooled (0 °C) and stirred solution of compound **10** (550 mg, 0.81 mmol) in CH_2Cl_2 (3 cm^3) was added TFA (6 cm^3) and the reaction mixture was left for 25 min at 0 °C, whereafter a mixture of toluene (30 cm^3) and ethyl acetate (15 cm^3) was added. The solvents were then removed under reduced pressure, and toluene was evaporated off twice from the residue (to remove traces of TFA) to give the hemiacetal derivative **14** (455 mg, 97%) as an amorphous solid, which ^{1}H NMR spectroscopy revealed to contain the α- and β-anomer in the ratio of 2.5:1, respectively; $[\alpha]_D^{25}$ +109.5 (*c* 1, $CHCl_3$); R_f 0.14 (solvent *A*); δ_H(*inter alia*) 3.88 (dd, $J_{3,4}$ 3.0, 3-Hβ), 4.12 (t, $J_{5,6}$ 6.0, 5-Hβ), 4.28 (dd, $J_{3,4}$ 3.0, 3-Hα), 4.30–4.78 (m, 5-Hα and 6-H_2), 4.69 (2 H, m, *J* 12.0, C*H*$_2$Ph), 4.82 (d, $J_{1,2}$ 8.0, 1-Hβ), 5.43 (dd, $J_{2,3}$ 10.3, 2-Hβ), 5.48 (dd, $J_{2,3}$10.3, 2-Hα), 5.75 (d, $J_{1,2}$ 3.5, 1-Hα), 5.93 (d, 4-Hβ) and 5.99 (d, 4-Hα); δ_C(*inter alia*) 62.8 (C-6β), 63.0 (C-6α), 68.2 (C-4α), 70.0 (C-4β + C-5α), 71.1 (C-2α), 71.4 (C-5β), 71.5 (*C*H$_2$Ph), 72.7 (C-3α), 74.8 (C-2β), 76.0 (C-3β), 91.0 (C-1α) and 96.7 (C-1β).

2,4-Di-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-α,β-D-galactopyranose 15

To a stirred solution of compound **12** (2.0 g, 3.0 mmol) in dichloromethane (10 cm^3) at room temperature was added TFA (20 cm^3) and the reaction mixture left for 30 min, whereafter a mixture of toluene (120 cm^3) and ethyl acetate (60 cm^3) was added. After processing as described for compound **14**, the resulting syrup (1.7 g, 99.9%) was shown by ^{1}H NMR spectroscopy to contain the α- and β-anomer of the chloroacetyl derivative **15** in the ratio of 2.5:1, respectively; $[\alpha]_D^{22}$ +7 (*c* 1, $CHCl_3$); δ_H(*inter alia*) 4.00 (s, CH_2Cl, α-anomer), 4.04 (s, CH_2Cl, β-anomer), 4.57 (d, $J_{1,2}$ 8.4, 1-Hβ), 5.46 (dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.4, 2-Hα), 5.74 (br d, 1-Hα), 5.85 (br d, 4-Hβ) and 5.90 (br d, 4-Hα); δ_C(*inter alia*) 40.54 (CH_2Cl), 63.91 (C-6β), 64.17 (C-6α), 66.60 (C-4β + -5α), 67.92 (C-4α), 70.77 (C-2α), 71.21 (C-5β), 71.40 (*C*H$_2$Ph), 72.27 (C-3α), 73.50 (C-2β), 75.66 (C-3β), 90.81 (C-1α) and 96.08 (C-1β).

2,4-Di-*O*-benzoyl-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-α,β-D-galactopyranose 16

A cooled and stirred solution of the TMS-ethyl glycoside **13** (500 mg, 0.61 mmol) in CH_2Cl_2 (2.5 cm^3) was treated with TFA (5 cm^3) for 20 min at 0 °C before a mixture of toluene (30 cm^3) and ethyl acetate (15 cm^3) was added. The solvents were then removed under reduced pressure and toluene was evaporated off from the residue several times to give the hemiacetal derivative **16** (438 mg, 99.9%) as an amorphous solid, which ^{1}H NMR spectroscopy showed to contain the α- and β-anomer in the ratio of 2.5:1, respectively; $[\alpha]_D^{22}$ +99 (*c* 0.9, $CHCl_3$); δ_H(*inter alia*) 1.03 (9 H, Me_3C), 3.70–3.85 (2 H, m, 6-H_2), 3.85 (dd, $J_{3,4}$ 3.0, 3-Hβ), 4.26 (dd, $J_{3,4}$ 3.0, 3-Hα), 4.38 (1 H, t, $J_{5,6}$ 7.0, 5-H), 4.66 (d, $J_{1,2}$ 8.2, 1-Hβ), 4.74 (2 H, m, *J* 12.5, C*H*$_2$Ph), 5.28 (dd, $J_{2,3}$ 10.0, 2-Hβ), 5.38 (dd, $J_{2,3}$ 10.0, 2-Hα), 5.59 (d, $J_{1,2}$ 3.5, 1-Hα) and 6.06 (1 H, d, 4-H); δ_C(*inter alia*) 18.1 (Me_3C), 26.3 (*Me*$_3$C), 60.7 (C-6β), 61.3 (C-6α), 65.5 (C-4β), 67.0 (C-4α), 68.7 (C-5α), 70.6 (C-2α), 70.8 (*C*H$_2$Ph), 72.2 (C-3α), 73.4 (C-5β), 73.6 (C-2β), 77.8 (C-3β), 90.0 (C-1α) and 96.6 (C-1β).

2,4,6-Tri-*O*-benzoyl-3-*O*-benzyl-α-D-galactopyranosyl trichloroacetimidate 6

To a stirred solution of the hemiacetal **14** (500 mg, 0.86 mmol) and CCl_3CN (3 cm^3, 30 mmol) in CH_2Cl_2 (10 cm^3) cooled to 0 °C was added DBU (0.l28 cm^3, 0.86 mmol) under nitrogen. The mixture was stirred for 2 h at 0 °C and then was concentrated. FCC of the residue (solvent *B*) gave the trichloroacetimidate **6** (620 mg, 98%) as a syrup; $[\alpha]_D^{20}$ +121 (*c* 2, $CHCl_3$); R_f 0.43 (solvent *A*); δ_H 4.38 (1 H, dd, $J_{3,4}$ 3.0, 3-H), 4.50 (1 H, dd, $J_{6a,6b}$ 12.2, 6-H[a]), 4.60 (1 H, t, $J_{5,6a} = J_{5,6b} = 6.0$, 5-H), 4.64 and 4.84 (2 H, AB q, *J* 12.0, C*H*$_2$Ph), 4.70 (1 H, dd, 6-H[b]), 5.85 (1 H, dd, $J_{2,3}$ 10.5, 2-H), 6.22 (1 H, d, 4-H), 6.93 (1 H, d, $J_{1,2}$ 3.5, 1-H), 7.20–8.30 (20 H, m, 4 × Ph) and 8.60 (1 H, s, NH); δ_C 62.90 (C-6), 67.46 (C-4), 69.33 (C-2), 70.18 (C-5), 71.41 (*C*H$_2$Ph), 72.45 (C-3), 90.98 (CCl_3), 94.17 (C-1), 127.97–130.19, 133.40–133.70 and 137.32 (Ph), 160.48 (C=NH), 165.05, 165.10 and 165.24 (CO_2Ph); ESMS(+) data: *m/z* 565.0 (100%, $[M - CCl_3CONH]^+$) ($C_{36}H_{30}Cl_3NO_9$ requires M, 725.10).

2,4-Di-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-α-D-galactopyranosyl trichloroacetimidate 7

A cooled and stirred solution of the hemiacetal derivative **15** (430 mg, 0.77 mmol) and trichloroacetonitrile (2.5 cm^3, 25 mmol) in dichloromethane (10 cm^3) was treated with

DBU (0.113 cm^3, 0.76 mmol) for 1.5 h; TLC (solvent *B*) then revealed the formation of two major components (presumably the α- and β-trichloroacetimidate). Stirring was continued at room temperature for 1 h, whereafter TLC showed the presence of a single major product. The solvents were evaporated off under reduced pressure and the residue was subjected to FCC (solvent *B*) to give the trichloroacetimidate **7** (370 mg, 69%) as an amorphous solid; $[\alpha]_D^{21}$ +143 (*c* 1, $CHCl_3$); δ_H 4.02 (2 H, s, CH_2Cl), 4.22–4.45 (3 H, m, 3-H and 6-H_2), 4.56 (1 H, m, 5-H), 4.60 and 4.77 (2 H, AB q, *J* 12.0, CH_2Ph), 5.71 (1 H, dd, $J_{2,3}$ 10.5, 2-H), 6.00 (1 H, br s, 4-H), 6.79 (1 H, d, $J_{1,2}$ 3.0, 1-H), 7.10–8.20 (15 H, m, 3 × Ph) and 8.57 (1 H, s, NH); δ_C 40.41 (CH_2Cl), 63.73 (C-6), 67.03 (C-4), 68.98 (C-2), 69.57 (C-5), 71.16 (*C*H_2Ph), 71.92 (C-3), 90.66 (CCl_3), 93.76 (C-1), 127.73–129.87, 133.27, 133.52 and 136.91 (Ph), 160.14 (C=NH), 165.43 and 165.66 (*C*O_2Ph) and 166.75 (*C*O_2CH_2Cl).

2,4-Di-*O*-benzoyl-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-galactopyranosyl trichloroacetimidate 8

This compound was prepared from compound **16** (438 mg, 0.61 mmol) and CCl_3CN (2 cm^3, 20 mmol) in the presence of DBU (0.09 cm^3, 0.6 mmol) as described for the derivative **6**. FCC (toluene) gave the trichloroacetimidate **8** (460 mg, 87%) as a syrup; δ_H 1.05 (3 H, s, Me_3C), 3.83 (2 H, d, $J_{5,6}$ 7.2, 6-H_2), 4.33 (1 H, dd, $J_{3,4}$ 3.0, 3-H), 4.41 (1 H, t, 5-H), 4.78 and 4.98 (2 H, AB q, *J* 12.3, C*H*$_2$Ph), 5.79 (1 H, dd, $J_{2,3}$ 10.2, 2-H), 6.30 (1 H, d, 4-H), 6.88 (1 H, d, $J_{1,2}$ 3.6, 1-H), 7.11–8.20 (25 H, m, 5 × Ph) and 8.50 (1 H, s, NH); δ_C 18.98 (Me_3C), 26.61 (*Me*$_3$C), 61.52 (C-6), 66.75 (C-4), 69.35 (C-2), 71.06 (*C*H_2Ph), 72.25 (C-5), 72.37 (C-3), 90.89 (CCl_3), 94.03 (C-1), 127.91–130.64, 132.94–133.46, 135.69, 135.76 and 137.67 (Ph), 160.52 (C=NH) and 166.61 (*C*O_2Ph).

Methyl 2,4-di-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 18

To a stirred and cooled (−60 °C) solution of the trichloroacetimidate **7** (1.40 g, 2.0 mmol) and the D-mannopyranoside acceptor **4** (1.20 g, 2.4 mmol) in dry dichloromethane (5 cm^3) under nitrogen was added TMS triflate (0.11 cm^3, 0.56 mmol), whereafter the temperature was allowed to rise to −30 °C and stirring was continued for 80 min. *N*,*N*-Diisopropylethylamine (0.12 cm^3, 0.7 mmol) was then added and the solvent was removed under reduced pressure. FCC (solvent *B*) of the residue provided first methyl 2,4-di-*O*-benzoyl-3-*O*-benzyl-6-*O*-chloroacetyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside **20** (368 mg, 18%) as an amorphous solid; $[\alpha]_D^{20}$ +50 (*c* 1.43, $CHCl_3$); δ_H 3.51 (3 H, s, OCH_3), 4.02 (2 H, s, CH_2Cl), 4.12 (1 H, dd, $J_{2',3'}$ 10.5, 3′-H), 4.24 (2 H, d, $J_{5',6'}$ 6.0, 6′-H_2), 4.28 (1 H, m, 5-H), 4.40–4.70 (6 H, m, 4- and 5′-H, 6-H_2 and C*H*$_2$Ph), 4.89 (1 H, d, $J_{1,2}$ 1.5, 1-H), 5.53–5.68 (3 H, m, $J_{1',2'}$ 4.0, 1′-, 2′- and 3-H), 5.73 (1 H, d, $J_{3',4'}$ 3.5, 4′-H), 5.88 (1 H, dd, $J_{2,3}$ 3.2, 2-H) and 7.00–8.20 (30 H, m, 6 × Ph). Continued elution gave the β-*linked disaccharide derivative* **18** (1.135 g, 55%) as an amorphous solid; $[\alpha]_D^{20}$ +59 (*c* 1.3, $CHCl_3$) (Found: C, 65.4; H, 5.0. $C_{57}H_{51}ClO_{17}$ requires C, 65.6; H, 4.9%); δ_H 3.43 (3 H, s, OCH_3), 3.53 (1 H, dd, $J_{5',6a'}$ 4.3, $J_{6a',6b'}$ 13.0, 6′-H^a), 3.58 (1 H, m, 5′-H), 3.65 (1 H, dd, 3′-H), 3.71 (2 H, s, CH_2Cl), 3.82 (1 H, dd, $J_{5',6b'}$ 9.0, 6′-H^b), 4.07 (1 H, ddd, $J_{5,6b}$ 2.0, 5-H), 4.38 and 4.61 (2 H, AB q, *J* 12.8, C*H*$_2$Ph), 4.40 (1 H, t, $J_{3,4} = J_{4,5} = 9.5$, 4-H), 4.47 (1 H, dd, $J_{5,6a}$ 3.5, $J_{6a,6b}$ 12.0, 6-H^a), 4.62 (1 H, dd, 6-H^b), 4.74 (1 H, d, $J_{1',2'}$ 8.0, 1′-H), 4.83 (1 H, d, $J_{1,2}$ 1.9, 1-H), 5.46 (1 H, dd, $J_{2',3'}$ 10.0, 2′-H), 5.58 (1 H, d, $J_{3',4'}$ 3.0, 4′-H), 5.64 (1 H, dd, $J_{2,3}$ 3.5, 2-H), 5.81 (1 H, dd, 3-H) and 6.90–8.05 (30 H, m, 6 × Ph); δ_C 40.22 (CH_2Cl), 55.35 (OCH_3), 62.50 (C-6), 62.94 (C-6′), 65.72 (C-4′), 69.32 (C-5), 70.02 (C-3), 70.44 (C-2), 70.76 (2 C, C-5′ + *C*H$_2$Ph), 71.36 (C-2′), 73.69 (C-4), 76.03 (C-3′), 98.50 (C-1), 101.21 (C-1′), 127.74–129.80, 133.08 and 137.51 (Ph), 164.07, 165.03 and 165.92 (*C*O_2Ph) and 166.81 (*C*O_2CH_2Cl).

Methyl 2,4-di-*O*-benzoyl-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 19

The reaction of compounds **8** (180 mg, 0.21 mmol) and **4** (132 mg, 0.26 mmol) in CH_2Cl_2 (2 cm^3) in the presence of TMS triflate (0.01 cm^3, 0.052 mmol) was accomplished under nitrogen at −70 to −10 °C, as described for the preparation of the disaccharide **18**. FCC (toluene ⟶ solvent *A*) provided first *methyl* 2,4-*di*-O-*benzoyl*-3-O-*benzyl*-6-O-(tert-*butyldiphenylsilyl*)-α-D-*galactopyranosyl*-(1→4)-2,3,6-*tri*-O-*benzoyl*-α-D-*mannopyranoside* **21** (68 mg, 27%) as an amorphous solid; $[\alpha]_D^{24}$ +41 (*c* 1, $CHCl_3$); R_f 0.40 (solvent *A*) (Found: C, 70.45; H, 6.0. $C_{71}H_{68}O_{16}Si$ requires C, 70.75; H, 5.7%); δ_H 1.00 (9 H, s, Me_3C), 3.48 (3 H, s, OCH_3), 3.75 (1 H, t, $J_{5',6a'} = J_{6a',6b'} = 9.0$, 6′-H^a), 3.83 (1 H, dd, $J_{5',6b'}$ 6.0, 6′-H^b), 4.20 (1 H, dd, $J_{3',4'}$ 3.0, 3′-H), 4.28 (1 H, m, 5-H), 4.40 (1 H, dd, $J_{5,6a}$ 4.2, 6-H^a), 4.52 (1 H, t, $J_{3,4} = J_{4,5} = 9.5$, 4-H), 4.55 (1 H, m, 5′-H), 4.60 and 4.83 (2 H, AB q, *J* 12.8, C*H*$_2$Ph), 4.85 (1 H, d, $J_{1,2}$ 1.5, 1-H), 5.19 (1 H, dd, $J_{5,6b}$ 1.5, $J_{6a,6b}$ 11.9, 6-H^b), 5.58–5.72 (4 H, m, 1′-, 2-, 2′- and 3-H), 6.23 (1 H, d, 4′-H) and 7.00–8.10 (40 H, m, 8 × Ph); δ_C 19.11 (Me_3C), 26.78 (*Me*$_3$C), 55.53 (OCH_3), 61.33 (C-6′), 63.31 (C-6), 67.31 (C-4′), 69.33 (C-5), 69.78 (C-2′), 69.86 (C-5′), 70.50 (C-2), 71.25 (*C*H_2Ph), 72.81 (C-4), 73.11 (C-3′), 73.27 (C-3), 98.43 (C-1), 99.07 (C-1′), 127.45–129.96, 133.05–133.32, 135.50 and 137.96 (Ph) and 165.02–166.39 (*C*O_2Ph). Continued elution gave the β-*linked disaccharide derivative* **19** (104 mg, 41%) as an amorphous solid; $[\alpha]_D^{24}$ +39 (*c* 1, $CHCl_3$); R_f 0.26 (solvent *A*), 0.57 (solvent *B*) (Found: C, 70.45; H, 5.9%); δ_H 0.95 (9 H, s, Me_3C), 3.38 (2 H, m, 6′-H_2), 3.41 (3 H, s, OCH_3), 3.55 (1 H, dd, $J_{5',6a'}$ 5.0, $J_{5',6b'}$ 9.0, 5′-H), 3.72 (1 H, dd, $J_{3',4'}$ 3.0, 3′-H), 4.09 (1 H, dt, $J_{5,6}$ 3.0, 5-H), 4.42 (1 H, t, $J_{3,4} = J_{4,5} = 10.0$, 4-H), 4.59 (2 H, m, 6-H_2), 4.60 and 4.79 (2 H, AB q, *J* 13.0, C*H*$_2$Ph), 4.75 (1 H, d, $J_{1',2'}$ 8.0, 1′-H), 4.86 (1 H, d, $J_{1,2}$ 2.0, 1-H), 5.50 (1 H, dd, $J_{2',3'}$ 10.1, 2′-H), 5.61 (1 H, dd, $J_{2,3}$ 3.5, 2-H), 5.78 (1 H, dd, 3-H), 5.97 (1 H, d, 4′-H) and 6.90–8.01 (40 H, m, 8 × Ph); δ_C 19.01 (Me_3C), 26.79 (*Me*$_3$C), 55.44 (OCH_3), 60.20 (C-6′), 62.92 (C-6), 65.45 (C-4′), 69.65 (C-5), 69.95 (C-3), 70.85 (2 C, C-2 + *C*H$_2$Ph), 71.93 (C-2′), 73.48 (2 C, C-4 + -5′), 76.64 (C-3′), 98.53 (C-1), 101.45 (C-1′), 127.73–130.22, 132.42–133.43, 135.68, 137.60 and 138.00 (Ph) and 165.04–166.02 (*C*O_2Ph). The condensation reaction at −12 °C (instead of at −70 to −10 °C) afforded compounds **19** and **21** in 47 and 37% yield, respectively.

Methyl 2,4-di-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 22

A solution of the disaccharide derivative **18** (1.0 g, 0.96 mmol) in 2-methoxyethanol (30 cm^3) containing 20% $Pd(OH)_2$/C (240 mg) was shaken under a slight overpressure of hydrogen at room temperature for 2.5 h; TLC (solvent *C*) then showed that no trace of the starting material remained. The spent catalyst was filtered off with the aid of a Celite pad and the filtrate was concentrated. FCC (4:1 toluene–ethyl acetate) of the residue gave the *debenzylated disaccharide* **22** (878 mg, 96%) as an amorphous solid; $[\alpha]_D^{20}$ −11 (*c* 1, $CHCl_3$) (Found: C, 63.0; H, 4.8. $C_{50}H_{45}ClO_{17}$ requires C, 63.0; H, 4.8%); δ_H 2.86 (1 H, br s, OH), 3.43 (3 H, s, OCH_3), 3.49 (1 H, t, $J_{5',6a'} = J_{5',6b'} = 6.0$, 5′-H), 3.62 (1 H, dd, 6′-H^a), 3.65 (2 H, s, CH_2Cl), 3.77 (1 H, dd, $J_{6a',6b'}$ 10.5, 6′-H^b), 3.95 (1 H, m, 3′-H), 4.10 (1 H, dt, $J_{5,6}$ 2.5, 5-H), 4.44 (1 H, t, $J_{3,4} = J_{4,5} = 9.5$, 4-H), 4.63 (2 H, d, 6-H_2), 4.79 (1 H, d, $J_{1',2'}$ 7.8, 1′-H), 4.85 (1 H, d, $J_{1,2}$ 2.0, 1-H), 5.30 (1 H, dd, $J_{2',3'}$ 9.9, 2′-H), 5.39 (1 H, d, $J_{3',4'}$ 3.2, 4′-H), 5.64 (1 H, dd, $J_{2,3}$ 3.5, 2-H), 5.81 (1 H, dd, 3-H) and 7.03–8.11 (25 H, m, 5 × Ph); δ_C 40.30 (CH_2Cl), 55.49 (OCH_3), 62.73 (C-6), 63.00 (C-6′), 69.49 (C-5), 69.84 (2 C, C-3 + -4′), 70.64 (C-2), 71.06 (C-5′), 71.66 (C-3′), 73.67 (2 C, C-2′ + -4), 98.66 (C-1), 100.86 (C-1′), 128.24–130.06 and 133.22–133.59 (Ph), 165.06–166.04 (*C*O_2Ph) and 166.58 (*C*O_2CH_2Cl).

Methyl 2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 23

This compound was prepared by hydrogenolysis of the disaccharide **19** (530 mg) in the presence of 20% $Pd(OH)_2$/C (180 mg) in 1:1 methanol–ethyl acetate (10 cm^3) during 10 h as described for the derivative **22**. FCC (solvent *B*) gave the *monohydroxylic disaccharide derivative* **23** (422 mg, 86%) as an amorphous solid; $[\alpha]_D^{25}$ −19.5 (*c* 1, $CHCl_3$); R_f 0.40 (solvent *B*) (Found: C, 68.7; H, 5.6. $C_{64}H_{62}O_{16}Si$ requires C, 68.9; H, 5.6%); δ_H 0.93 (9 H, s, Me_3C), 2.85 (1 H, d, $J_{3',OH}$ 4.9, OH), 3.35 (2 H, m, 6′-H_2), 3.40 (3 H, s, OCH_3), 3.62 (1 H, dd, $J_{5',6a'}$ 5.0, $J_{5',6b'}$ 9.0, 5′-H), 4.08 (2 H, m, 3′- and 5-H), 4.43 (1 H, t, $J_{3,4} = J_{4,5} = 10.0$, 4-H), 4.62 (2 H, m, 6-H_2), 4.78 (1 H, d, $J_{1',2'}$ 7.6, 1′-H), 4.84 (1 H, d, $J_{1,2}$ 2.0, 1-H), 5.30 (1 H, dd, $J_{2',3'}$ 10.0, 2′-H), 5.55 (1 H, dd, $J_{2,3}$ 3.4, 2-H), 5.72 (1 H, dd, 3-H), 5.75 (1 H, d, $J_{3',4'}$ 3.2, 4′-H) and 6.65–8.10 (35 H, m, 7 × Ph); δ_C 18.93 (Me_3C), 26.68 (Me_3C), 55.46 (OCH_3), 60.09 (C-6′), 62.92 (C-6), 69.60 (C-5), 69.78 (2 C, C-3 + -4′), 70.89 (C-2), 72.36 (C-3′), 73.31 (C-2′), 73.42 (C-5′), 73.83 (C-4), 98.54 (C-1), 101.12 (C-1′), 127.67–130.24, 132.39–133.41 and 135.61 (Ph) and 165.02–166.61 (CO_2Ph).

Methyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 24

The reaction of compounds **6** (535 mg, 0.74 mmol) and **22** (467 mg, 0.49 mmol) in CH_2Cl_2 (7 cm^3) in the presence of TMS triflate (0.029 cm^3, 0.15 mmol) was accomplished under nitrogen at −42 to −15 °C, as described for the preparation of the disaccharide **18**. FCC (solvent *C*) provided first methyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside **26** (148 mg, 20%) as an amorphous solid; $[\alpha]_D^{20}$ + 103 (*c* 1, $CHCl_3$); δ_H(*inter alia*) 3.44 (3 H, s, OCH_3), 3.68 and 3.69 (2 H, AB q, *J* 15.0, CH_2Cl), 4.83 (1 H, d, $J_{1',2'}$ 8.0, 1′-H), 4.85 (1 H, d, $J_{1,2}$ 1.5, 1-H) and 5.59 (1 H, d, $J_{1'',2''}$ 3.5, 1″-H); δ_C 40.16 (CH_2Cl), 55.38 (OCH_3), 62.10 (C-6″), 62.27 (C-6), 62.61 (C-6′), 64.23 (C-4′), 67.25 (C-5″), 67.60 (C-4″), 69.14 (2 C, C-2″ + -5), 70.00 (C-3), 70.50 (C-2), 70.70 (2 C, C-5′ + CH_2Ph), 71.49 (C-2′), 72.25 (C-3′), 72.83 (C-3″), 74.36 (C-4), 92.55 (C-1″), 98.53 (C-1), 101.52 (C-1′), 127.39–129.50, 133.21 and 137.15 (Ph) and 164.51–166.06 (CO_2Ph and CO_2CH_2Cl). Continued elution gave the β,β-linked trisaccharide **24** (541 mg, 74%) as an amorphous solid; $[\alpha]_D^{20}$ +43 (*c* 1, $CHCl_3$); δ_H 3.25 (1 H, dd, $J_{5',6a'}$ 7.5, $J_{6a',6b'}$ 11.7, 6′-H[a]), 3.38 (3 H, s, OCH_3), 3.55 and 3.56 (2 H, AB q, *J* 15.0, CH_2Cl), 3.60 (2 H, m, 3′- and 5′-H), 3.82 (1 H, dd, $J_{5',6b'}$ 5.5, 6′-H[b]), 3.95 (2 H, m, 5- and 5″-H), 4.03 (1 H, dd, $J_{2'',3''}$ 10.0, 3″-H), 4.18 (1 H, dd, $J_{5'',6a''}$ 6.5, $J_{6a'',6b''}$ 11.5, 6″-H[a]), 4.30 and 4.53 (2 H, AB q, *J* 12.5, CH_2Ph), 4.33 (1 H, t, $J_{3,4} = J_{4,5} = 10.0$, 4-H), 4.44 (2 H, m, 6-H_2), 4.60 (1 H, dd, $J_{5'',6b''}$ 6.5, 6″-H[b]), 4.70 (1 H, d, $J_{1',2'}$ 8.0, 1′-H), 4.74 (1 H, d, $J_{1'',2''}$ 8.0, 1″-H), 4.79 (1 H, d, $J_{1,2}$ 1.8, 1-H), 5.22 (1 H, dd, $J_{2',3'}$ 10.5, 2′-H), 5.46 (1 H, dd, 2″-H), 5.59 (1 H, dd, $J_{2,3}$ 3.5, 2-H), 5.63 (1 H, d, $J_{3'',4''}$ 3.5, 4″-H), 5.72 (1 H, dd, 3-H), 5.75 (1 H, d, $J_{3',4'}$ 3.4, 4′-H) and 6.88–8.05 (45 H, m, 9 × Ph); δ_C 40.15 (CH_2Cl), 55.46 (OCH_3), 62.19 (C-6″), 62.61 (C-6), 63.43 (C-6′), 66.05 (C-4″), 69.41 (C-5), 69.70 (2 C, C-3 + -4′), 70.55 (C-2), 70.80 (CH_2Ph), 70.93 (C-5′), 71.13 (C-2″), 71.39 (2 C, C-2′ + -5″), 73.31 (C-4), 75.76 (C-3″), 77.40 (C-3′), 98.49 (C-1), 100.78 (C-1′), 101.47 (C-1″), 127.69–129.86, 132.46–133.20 and 136.83 (Ph), 163.60–165.60 (CO_2Ph) and 166.44 (CO_2CH_2Cl).

Methyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 25

The reaction of compounds **6** (353 mg, 0.49 mmol) and **23** (417 mg, 0.37 mmol) in CH_2Cl_2 (5 cm^3) in the presence of TMS triflate (0.02 cm^3, 0.1 mmol) was accomplished under nitrogen at 0 °C, as described for the preparation of the disaccharide **18**. FCC [toluene–ethyl acetate (99.2:0.8 ⟶ 91:9)] provided first *methyl* 2,4,6-*tri*-O-*benzoyl*-3-O-*benzyl*-α-D-*galactopyranosyl*-(1→3)-2,4-*di*-O-*benzoyl*-6-O-(tert-*butyldiphenylsilyl*)-β-D-*galactopyranosyl*-(1→4)-2,3,6-*tri*-O-*benzoyl*-α-D-*mannopyranoside* **27** (100 mg, 16%) as an amorphous solid; $[\alpha]_D^{20}$ +82 (*c* 1, $CHCl_3$); R_f 0.52 (solvent *B*) (Found: C, 70.0; H, 5.7. $C_{98}H_{90}O_{24}Si$ requires C, 70.1; H, 5.4%); δ_H(*inter alia*) 0.85 (9 H, s, Me_3C), 3.40 (3 H, s, OCH_3), 4.76 (1 H, d, $J_{1',2'}$ 8.0, 1′-H), 4.82 (1 H, d, $J_{1,2}$ 1.5, 1-H) and 5.55 (1 H, d, $J_{1'',2''}$ 3.5, 1″-H); δ_C 18.68 (Me_3C), 26.49 (Me_3C), 55.28 (OCH_3), 59.90 (C-6′), 62.59 (2 C, C-6 + -6″), 64.03 (C-4′), 67.11 (C-5″), 67.66 (C-4″), 68.66 (C-2″), 69.47 (C-5), 69.85 (C-3), 70.50 (C-2), 71.12 (C-2′), 71.34 (CH_2Ph), 72.51 (C-3″), 73.14 (C-3′), 73.36 (C-5′), 73.77 (C-4), 92.55 (C-1″), 98.39 (C-1), 101.28 (C-1′), 127.32–129.87, 132.09–133.57, 135.35, 135.47 and 137.32 (Ph) and 165.01–166.15 (CO_2Ph); ESMS(+) data: *m*/*z* 1679.8 (32%, $[M + H]^+$) and 1702.3 (60%, $[M + Na]^+$) ($C_{98}H_{90}O_{24}Si$ requires M, 1678.56). Continued elution gave the β,β-*linked trisaccharide* **25** (275 mg, 44%) as an amorphous solid; $[\alpha]_D^{25}$ +42 (*c* 1, $CHCl_3$); R_f 0.43 (solvent *B*) (Found: C, 70.1; H, 5.7%); δ_H 0.90 (9 H, s, Me_3C), 3.02 (1 H, m, 5′-H), 3.17–3.40 (2 H, m, 6′-H_2), 3.31 (3 H, s, OCH_3), 3.83 (2 H, dd, $J_{3',4'} = J_{3'',4''} = 3.0$, 3′- and 3″-H), 3.94 (1 H, dt, $J_{4,5}$ 9.6, $J_{5,6}$ 3.0, 5-H), 4.25 (1 H, t, $J_{5'',6''}$ 7.3, 5″-H), 4.37 and 4.63 (2 H, AB q, *J* 12.0, CH_2Ph), 4.39–4.57 (5 H, m, 4-H, 6- and 6″-H_2), 4.79 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.90 (1 H, d, $J_{1'',2''}$ 7.7, 1″-H), 4.97 (1 H, d, $J_{1',2'}$ 7.6, 1′-H), 5.36 (1 H, dd, $J_{2',3'}$ 10.0, 2′-H), 5.46 (1 H, dd, $J_{2'',3''}$ 10.0, 2″-H), 5.53 (1 H, dd, $J_{2,3}$ 3.2, 2-H), 5.65 (1 H, dd, $J_{3,4}$ 9.6, 3-H), 5.98 (1 H, d, 4″-H), 6.18 (1 H, d, 4′-H) and 6.60–8.60 (55 H, m, 11 × Ph); δ_C 18.81 (Me_3C), 26.54 (Me_3C), 55.21 (OCH_3), 60.14 (C-6′), 62.27 (C-6″), 62.85 (C-6), 66.52 (C-4″), 69.71 (3 C, C-3, -4′ and -5), 70.77 (CH_2Ph), 71.01 (2 C, C-2 + -2″), 71.52 (C-5″), 71.78 (C-2′), 73.02 (C-5′), 73.80 (C-4), 76.58 (C-3″), 77.07 (C-3′), 98.45 (C-1), 101.31 (C-1′), 101.50 (C-1″), 127.49–130.46, 132.53–133.36, 135.56, 135.65 and 137.39 (Ph) and 165.10–166.91 (CO_2Ph). Also isolated was the disaccharide acceptor **23** (75 mg, 18% recovery).

Methyl 2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 28

This compound was prepared by hydrogenolysis of the trisaccharide **24** (200 mg) in the presence of 20% $Pd(OH)_2$/C (50 mg) in 2-methoxyethanol (5 cm^3) during 5 h, as described for the derivative **22**. FCC (2:1 toluene–ethyl acetate) gave the trisaccharide acceptor **28** (128 mg, 73%) as an amorphous solid; $[\alpha]_D^{20}$ +10 (*c* 1, $CHCl_3$); δ_H 2.89 (1 H, br s, OH), 3.30 (1 H, dd, $J_{6a',6b'}$ 11.5, 6′-H[a]), 3.37 (3 H, s, OCH_3), 3.55 and 3.58 (2 H, AB q, *J* 15.0, CH_2Cl), 3.59 (1 H, t, $J_{5',6a'} = J_{5',6b'} = 6.0$, 5′-H), 3.83 (2 H, m, 3′-H and dd, 6′-H[b]), 3.96 (1 H, br d, 5-H), 3.97 (1 H, t, $J_{5'',6a''} = J_{5'',6b''} = 6.5$, 5″-H), 4.12 (1 H, dd, $J_{3'',4''}$ 3.5, 3″-H), 4.18 (1 H, dd, $J_{6a'',6b''}$ 11.5, 6″-H[a]), 4.36 (1 H, t, $J_{3,4} = J_{4,5} = 9.5$, 4-H), 4.47 (2 H, m, 6-H_2), 4.58 (1 H, dd, 6″-H[b]), 4.74 (1 H, d, $J_{1',2'}$ 8.2, 1′-H), 4.79 (2 H, d, $J_{1,2}$ 1.8, 1-H and d, $J_{1'',2''}$ 7.5, 1″-H), 5.09 (1 H, dd, $J_{2'',3''}$ 10.0, 2″-H), 5.52 (1 H, dd, $J_{2',3'}$ 9.9, 2′-H), 5.60 (1 H, dd, $J_{2,3}$ 3.5, 2-H), 5.63 (1 H, d, $J_{3',4'}$ 3.0, 4′-H), 5.74 (1 H, dd, 3-H), 5.75 (1 H, d, 4″-H) and 7.00–8.05 (40 H, m, 8 × Ph); δ_C 40.32 (CH_2Cl), 55.45 (OCH_3), 62.16 (C-6″), 62.63 (C-6), 63.39 (C-6′), 69.45 (C-5), 69.81 (2 C, C-3 + -4′), 70.11 (C-4″), 70.50 (C-2), 71.39 (3 C, C-2′, -5′ and -5″), 71.58 (C-3″), 73.24 (C-2″), 73.34 (C-4), 76.81 (C-3′), 98.63 (C-1), 101.02 (2 C, C-1′ + -1″), 128.13–130.06 and 132.94–133.59 (Ph), 164.41–166.08 (CO_2Ph) and 166.57 (CO_2CH_2Cl).

Methyl 2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 29

This compound was prepared by hydrogenolysis of the trisaccharide **25** (245 mg) in the presence of 20% $Pd(OH)_2$/C (200 mg) in 3:2 methanol–ethyl acetate (5 cm^3) during 4.5 h, as

described for the derivative **22**. FCC (solvent *B*) gave the *trisaccharide derivative* **29** (180 mg, 78%) as an amorphous solid; $[\alpha]_D^{25}$ +24 (*c* 1, $CHCl_3$); R_f 0.24 (solvent *B*) (Found: C, 69.0; H, 5.6. $C_{91}H_{84}O_{24}Si$ requires C, 68.8; H, 5.3%); δ_H 0.93 (9 H, s, Me_3C), 2.91 (1 H, br s, OH), 3.35 (2 H, m, 6′-H_2), 3.37 (3 H, s, OCH_3), 3.67 (1 H, dd, $J_{5',6a'}$ 5.0, $J_{5',6b'}$ 8.8, 5′-H), 3.92 (1 H, br d, 5-H), 4.00 (1 H, dd, $J_{3',4'}$ 3.0, 3′-H), 4.08 (1 H, t, $J_{5'',6a''} = J_{5'',6b''} = 6.5$, 5″-H), 4.29 (1 H, $J_{3'',4''}$ 3.2, 3″-H), 4.39 (1 H, dd, $J_{6a'',6b''}$ 11.0, 6″-H[a]), 4.44 (1 H, t, $J_{3,4} = J_{4,5} = 9.9$, 4-H), 4.55 (1 H, d, $J_{5,6}$ 2.5, 6-H_2), 4.74 (1 H, dd, 6″-H[b]), 4.80 (1 H, d, $J_{1',2'}$ 8.0, 1′-H), 4.83 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.90 (1 H, d, $J_{1'',2''}$ 7.6, 1″-H), 5.18 (1 H, dd, $J_{2'',3''}$ 10.0, 2″-H), 5.53 (1 H, dd, $J_{2',3'}$ 10.1, 2′-H), 5.58 (1 H, dd, $J_{2,3}$ 3.4, 2-H), 5.74 (2 H, dd, 3-H and dd, 4″-H), 6.10 (1 H, d, 4′-H) and 6.60–8.38 (50 H, m, 10 × Ph); ESMS(+) data: *m*/*z* 1588.9 (100%, $[M + H]^+$) and 1611.0 (45%, $[M + Na]^+$) ($C_{91}H_{84}O_{24}Si$ requires M, 1588.51).

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 30

The reaction of compounds **5** (275 mg, 0.33 mmol) and **28** (288 mg, 0.204 mmol) in CH_2Cl_2 (2.5 cm^3) in the presence of TMS triflate (0.016 cm^3, 0.083 mmol) was accomplished under nitrogen at −45 to −15 °C, as described for the preparation of the disaccharide **18**. FCC (2:1 toluene–ethyl acetate) gave the pentasaccharide derivative **30** (291 mg, 68%) as an amorphous solid; $[\alpha]_D^{20}$ +16 (*c* 0.9, $CHCl_3$); δ_H 1.49, 1.81, 1.98, 2.00, 2.03 and 2.08 (18 H, 6 × s, 6 × Ac), 3.32 (1 H, dd, $J_{5,6a}$ 6.8, $J_{6a,6b}$ 11.5, 6-H[a], Gal[a]), 3.37 (3 H, s, OCH_3), 3.47–3.56 (3 H, m, 3-H, Gal[b]; 5-H, Gal[c]; 5-H, Gal[d]), 3.54 and 3.58 (2 H, AB q, *J* 15.0, CH_2Cl), 3.70 (1 H, m, 5-H, Gal[a]), 3.77 (1 H, dd, $J_{5,6b}$ 5.7, 6-H[b], Gal[a]), 3.89 (1 H, ddd, $J_{5,6a}$ 4.0, 5-H, Man[a]), 3.91–4.05 (6 H, m, 3-H, Gal[a]; 5-H, Gal[b]; 6-H_2, Gal[c]; 6-H_2, Gal[d]), 4.07 (1 H, dd, $J_{3,4}$ 3.5, 3-H, Gal[c]), 4.19 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal[c]), 4.28 (1 H, dd, $J_{5,6a}$ 6.0, $J_{6a,6b}$ 11.5, 6-H[a], Gal[b]), 4.29 (1 H, t, $J_{3,4} = J_{4,5} = 10.0$, 4-H, Man[a]), 4.34 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H[a], Man[a]), 4.43 (1 H, dd, $J_{5,6b}$ 2.0, 6-H[b], Man[a]), 4.44 (1 H, dd, $J_{5,6b}$ 5.0, 6-H[b], Gal[b]), 4.47 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal[d]), 4.48 (1 H, dd, $J_{3,4}$ 3.0, 3-H, Gal[d]), 4.63 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal[b]), 4.72 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal[a]), 4.76 (1 H, d, $J_{1,2}$ 1.5, 1-H, Man[a]), 4.81 (1 H, dd, $J_{2,3}$ 10.5, 2-H, Gal[d]), 5.05 (1 H, dd, $J_{2,3}$ 10.0, 2-H, Gal[c]), 5.16 (1 H, d, 4-H, Gal[d]), 5.18 (1 H, d, 4-H, Gal[c]), 5.20 (1 H, dd, $J_{2,3}$ 9.8, 2-H, Gal[b]), 5.39 (1 H, dd, $J_{2,3}$ 10.0, 2-H, Gal[a]), 5.53 (1 H, d, $J_{3,4}$ 3.8, 4-H, Gal[a]), 5.57 (1 H, dd, $J_{2,3}$ 3.5, 2-H, Man[a]), 5.68 (1 H, dd, 3-H, Man[a]), 5.69 (1 H, d, $J_{3,4}$ 3.2, 4-H, Gal[b]) and 7.00–8.05 (45 H, m, 9 × Ph); δ_C see Table 2.

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 31

To a stirred and cooled (−40 °C) solution of the trichloroacetimidate **5** (111 mg, 0.132 mmol) and the trisaccharide acceptor **29** (172 mg, 0.108 mmol) in CH_2Cl_2 (3 cm^3) under nitrogen was added triethylsilyl trifluoromethanesulfonate (0.01 cm^3, 0.045 mmol), whereafter the temperature was allowed to rise to −20 °C during 2 h and a second portion of the imidate **5** (90 mg, 0.107 mmol) in CH_2Cl_2 (0.5 cm^3) was added. Stirring was continued for a further 1 h at −10 °C, whereafter the reaction was quenched with *N*,*N*-diisopropylethylamine (0.02 cm^3, 0.11 mmol) and the mixture was concentrated. FCC (7:3 toluene–ethyl acetate) of the residue gave the *protected pentasaccharide* **31** (140 mg, 57%) as an amorphous solid; $[\alpha]_D^{20}$ +22 (*c* 1, $CHCl_3$); R_f 0.52 (solvent *D*); δ_H(*inter alia*) 0.85 (9 H, s, Me_3C), 1.50, 1.82, 1.98, 2.00, 2.03 and 2.10 (18 H, 6 × s, 6 × Ac), 3.33 (3 H, s, OCH_3), 4.20 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal[c]), 4.30 (1 H, t, $J_{3,4} = J_{4,5} = 9.7$, 4-H, Man[a]), 4.40 (2 H, m, 6-H_2, Man[a]), 4.50 (2 H, d, $J_{1,2}$ 7.7, 1-H and dd, $J_{3,4}$ 3.2, 3-H, Gal[d]), 4.63 (1 H, d, $J_{1,2}$ 7.8, 1-H, Gal[b]), 4.75 (1 H, d, $J_{1,2}$ 1.6, 1-H, Man[a]), 4.76 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal[a]), 4.84 (1 H, dd, $J_{2,3}$ 10.5, 2-H, Gal[d]), 5.11 (1 H, dd, $J_{2,3}$10.1, 2-H, Gal[c]), 5.16 (1 H, d, 4-H, Gal[d]), 5.20 (1 H, d, $J_{3,4}$ 3.0, 4-H, Gal[c]), 5.25 (1 H, dd, $J_{2,3}$ 10.5, 2-H, Gal[b]), 5.34 (1 H, dd, $J_{2,3}$ 10.1, 2-H, Gal[a]), 5.49 (1 H, dd, $J_{2,3}$ 3.2, 2-H, Man[a]), 5.60 (1 H, dd, 3-H, Man[a]), 5.78 (1 H, d, $J_{3,4}$ 3.2, 4-H, Gal[b]), 5.93 (1 H, d, $J_{3,4}$ 2.9, 4-H, Gal[a]) and 6.60–8.20 (55 H, m, 11 × Ph); δ_C see Table 2; ESMS(+) data: *m*/*z* 2269.4 (100%, $[M + H]^+$) ($C_{122}H_{120}O_{41}Si$ requires M, 2268.71). Also isolated was the trisaccharide acceptor **29** (43 mg, 25% recovery).

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*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-mannopyranoside 32

(A) A solution of the pentasaccharide derivative **30** (291 mg, 0.139 mmol) and thiourea (42 mg, 0.566 mmol) in methanol (4 cm^3) and 1,4-dioxane (1 cm^3) was heated at 50 °C for 2 h. Chloroform (40 cm^3) was added to the reaction mixture and the resulting solution was washed in turn with 0.01 mol dm^{-3} hydrochloric acid, water, saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. FCC (2:1 toluene–ethyl acetate) gave unchanged substrate **30** (86 mg, 30% recovery) and the monohydroxylic pentasaccharide derivative **32** (168 mg, 60%) as an amorphous solid; $[\alpha]_D^{21}$ +18 (*c* 0.9, $CHCl_3$); R_f 0.29 (solvent *D*); δ_H 1.46, 1.82, 1.98, 2.01, 2.04 and 2.10 (18 H, 6 × s, 6 × Ac), 2.86 (2 H, m, 6-OH and 6-H[a], Gal[a]), 3.05 (1 H, dt, $J_{5,6b} = J_{6b,OH} = 6.0$, $J_{6a,6b}$ 11.0, 6-H[b], Gal[a]), 3.31 (1 H, dd, $J_{5,6a}$ 7.5, 5-H, Gal[a]), 3.35 (3 H, s, OCH_3), 3.51 (1 H, dd, $J_{3,4}$ 3.0, 3-H, Gal[b]), 3.52 (1 H, t, $J_{5,6}$ 6.8, 5-H, Gal[d]), 3.56 (1 H, t, $J_{5,6}$ 6.7, 5-H, Gal[c]), 3.89 (1 H, ddd, $J_{5,6a}$ 4.0, 5-H, Man[a]), 3.91–4.06 (4 H, m, 6-H_2, Gal[c]; 6-H_2, Gal[d]), 3.95 (1 H, dd, $J_{5,6a}$ 5.8, 5-H, Gal[b]), 3.98 (1 H, dd, $J_{3,4}$ 3.5, 3-H, Gal[a]), 4.08 (1 H, dd, $J_{3,4}$ 3.0, 3-H, Gal[c]), 4.18 (1 H, d, $J_{1,2}$ 7.7, 1-H, Gal[c]), 4.23 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H[a], Gal[b]), 4.28 (1 H, dd, $J_{5,6b}$ 4.0, 6-H[b], Gal[b]), 4.34 (1 H, t, $J_{3,4} = J_{4,5} = 9.5$, 4-H, Man[a]), 4.35 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H[a], Man[a]), 4.44 (1 H, dd, $J_{5,6b}$ 1.1, 6-H[b], Man[a]), 4.48 (2 H, d, $J_{1,2}$ 7.5, 1-H and dd, $J_{3,4}$ 3.0, 3-H, Gal[d]), 4.63 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal[b]), 4.73 (1 H, d, $J_{1,2}$ 7.9, 1-H, Gal[a]), 4.78 (1 H, d, $J_{1,2}$ 1.5, 1-H, Man[a]), 4.81 (1 H, dd, $J_{2,3}$ 10.3, 2-H, Gal[d]), 5.03 (1 H, dd, $J_{2,3}$ 9.8, 2-H, Gal[c]), 5.15 (1 H, d, 4-H, Gal[d]), 5.18 (1 H, d, 4-H, Gal[c]), 5.23 (1 H, dd, $J_{2,3}$ 10.0, 2-H, Gal[b]), 5.44 (1 H, dd, $J_{2,3}$10.0, 2-H, Gal[a]), 5.47 (1 H, d, 4-H, Gal[a]), 5.51 (1 H, dd, $J_{2,3}$ 3.5, 2-H, Man[a]), 5.66 (1 H, d, 4-H, Gal[b]), 5.68 (1 H, dd, 3-H, Man[a]) and 7.00–8.05 (45 H, m, 9 × Ph); δ_C see Table 2.

(B) 1 Mol dm^{-3} TBAF in THF (Aldrich) (0.07 cm^3, 0.07 mmol of Bu_4NF) was added to a solution of the TBDPS pentasaccharide derivative **31** (140 mg, 0.062 mmol) in THF (1 cm^3) and the mixture was kept at 20 °C for 16 h with monitoring by TLC (solvent *D*). FCC [toluene–ethyl acetate (2:1→1:1)] of the mixture gave the monohydroxylic compound **32** (80 mg, 64%).

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-(2,4-di-*O*-benzoyl-β-D-galactopyranosyl 6-dibenzylphosphate)-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-mannopyranoside 33

To a solution of the pentasaccharide derivative **32** (150 mg, 0.074 mmol) and dibenzyl *N*,*N*-diisopropylphosphoramidite (103 mg, 0.30 mmol) in CH_2Cl_2 (5 cm^3) was added 1*H*-tetrazole (26 mg, 0.37 mmol), and the reaction mixture was stirred under nitrogen at room temperature for 3 h; TLC (solvent *D*) then revealed the formation of a single major product. The reaction mixture was cooled (−18 °C), treated with 3-chloroperbenzoic acid (MCPBA) (55% purity; 77 mg, 0.45 mmol) in CH_2Cl_2 (2 cm^3) and stirred for 90 min. The solvent was removed under reduced pressure and the residue was subjected to FCC (2:1

toluene–ethyl acetate) to give the phosphorylated pentasaccharide derivative **33** (150 mg, 89%) as an amorphous solid; $[\alpha]_D^{20}$ +10.5 (*c* 1, $CHCl_3$); δ_H 1.40, 1.75, 1.89, 1.90, 1.91 and 2.00 (18 H, 6 × s, 6 × Ac), 3.27 (3 H, s, OCH_3), 3.35–3.52 (6 H, m, 3-H, Gal[b]; 5-H, Gal[c]; 5-H, Gal[d]; 5-H and 6-H_2, Gal[a]), 3.80 (1 H, ddd, $J_{5,6a}$ 4.0, 5-H, Man[a]), 3.82–4.00 (7 H, m, 3-H, Gal[a]; 5-H, Gal[b]; 6-H_2, Gal[d]; 3-H and 6-H_2, Gal[c]), 4.12 (1 H, d, $J_{1,2}$ 7.9, 1-H, Gal[c]), 4.16 (1 H, dd, $J_{5,6a}$ 7.0, $J_{6a,6b}$ 11.0, 6-H[a], Gal[b]), 4.23 (1 H, dd, $J_{6a,6b}$ 11.8, 6-H[a], Man[a]), 4.29 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.5, 4-H, Man[a]), 4.35 (1 H, dd, $J_{5,6b}$ 2.0, 6-H[b], Man[a]), 4.36 (1 H, dd, $J_{5,6b}$ 6.0, 6-H[b], Gal[b]), 4.40 (1 H, d, $J_{1,2}$ 7.5, 1-H, Gal[d]), 4.41 (1 H, dd, $J_{3,4}$ 3.5, 3-H, Gal[d]), 4.53 (1 H, d, $J_{1,2}$ 7.5, 1-H, Gal[b]), 4.61 (1 H, d, $J_{1,2}$ 7.5, 1-H, Gal[a]), 4.69 and 4.72 (2 H, 2 × dd, $J_{H,H}$ 11.5, $J_{H,P}$ 8.0, CH_2Ph), 4.71 (1 H, d, $J_{1,2}$ 1.5, 1-H, Man[a]), 4.74 (1 H, dd, $J_{2,3}$ 10.0, 2-H, Gal[d]), 4.80 and 4.82 (2 H, 2 × dd, $J_{H,H}$ 11.5, $J_{H,P}$ 8.0, CH_2Ph), 4.98 (1 H, dd, $J_{2,3}$10.0, 2-H, Gal[c]), 5.08 (1 H, d, 4-H, Gal[d]), 5.11 (1 H, d, $J_{3,4}$ 3.2, 4-H, Gal[c]), 5.13 (1 H, dd, $J_{2,3}$ 9.8, 2-H, Gal[b]), 5.30 (1 H, dd, $J_{2,3}$ 9.8, 2-H, Gal[a]), 5.44 (1 H, dd, $J_{2,3}$ 3.5, 2-H, Man[a]), 5.53 (1 H, d, $J_{3,4}$ 3.5, 4-H, Gal[a]), 5.62 (1 H, dd, 3-H, Man[a]), 5.63 (1 H, d, $J_{3,4}$ 3.0, 4-H, Gal[b]) and 6.80–8.01 (55 H, m, 11 × Ph); δ_P −3.69; δ_C see Table 2.

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-{2,4-di-*O*-benzoyl-β-D-galactopyranosyl 6-[2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzoyl-α-D-manno-pyranosyl phosphate]}-(1→4)-2,3,6-tri-O-benzoyl-α-D-manno-pyranoside, triethylammonium salt 34

A mixture of compounds **3** (55 mg, 0.038 mmol) and **32** (65 mg, 0.032 mmol) was dried by evaporation of pyridine (3 × 1 cm^3) therefrom. The residue was dissolved in 9:1 pyridine–triethylamine (1 cm^3), trimethylacetyl chloride (0.011 cm^3, 0.086 mmol) was added, and the mixture was stirred at 22 °C for 1 h, whereafter a freshly prepared solution of iodine (20 mg, 0.08 mmol) in 95% aq. pyridine (2 cm^3) was added. After 10 min, $CHCl_3$ was added and the solution was washed successively with cold 1 mol dm^{-3} $Na_2S_2O_3$ and cold 0.5 mol dm^{-3} triethylammonium (TEA) hydrogen carbonate, dried by filtration through cotton wool, and concentrated. The residue was dissolved in CH_2Cl_2 (5 cm^3), and 2% TFA in CH_2Cl_2 (5 cm^3) was added at 0 °C. After 1 min, the solution was diluted with $CHCl_3$ and washed successively with ice-cold saturated aq. $NaHCO_3$ and 0.5 mol dm^{-3} TEA hydrogen carbonate, dried by filtration through cotton wool, and concentrated. FCC [CH_2Cl_2–MeOH–Et_3N (98.9:0.1:1 ⟶ 96:3:1)] of the residue gave the heptasaccharide phosphate derivative **34** (72 mg, 71%) as an amorphous solid; $[\alpha]_D^{20}$ +32 (*c* 1, $CHCl_3$); R_f 0.21 (solvent *E*); δ_H(*inter alia*) 1.09 (9 H, t, 3 × *Me*CH_2), 1.41, 1.76, 1.86, 1.90, 1.92 and 2.00 (18 H, 6 × s, 6 × Ac), 2.75 (6 H, q, 3 × MeCH_2), 3.26 (3 H, s, OCH_3), 4.11 (1 H, d, $J_{1,2}$ 8.0, 1-H, Gal[c]), 4.39 (1 H, d, $J_{1,2}$ 7.7, 1-H, Gal[d]), 4.68 (1 H, d, $J_{1,2}$ 1.5, 1-H, Man[a]), 4.72 (2 H, d, $J_{1,2}$ 7.5, 1-H, Gal[a]; 1-H, Gal[b]), 4.92 (1 H, d, $J_{1,2}$ 7.9, 1-H, Gal[e]), 5.55 (1 H, dd, $J_{1,2}$ 1.5, $J_{1,P}$ 8.0, 1-H, Man[b]) and 6.95–7.95 (75 H, m, 15 × Ph); δ_C see Table 2.

Methyl β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-(β-D-galactopyranosyl 6-phosphate)-(1→4)-α-D-mannopyranoside, bistriethylammonium salt 1

A solution of the protected phosphopentasaccharide derivative **33** (120 mg) in 1:1 methanol–ethyl acetate (6 cm^3) containing 20% $Pd(OH)_2$/C (73 mg) was stirred vigorously under a slight overpressure of hydrogen at room temperature for 3 h; TLC (solvent *D*) then revealed that the starting material was completely consumed. The spent catalyst was filtered off with the aid of a Celite pad and the filtrate was concentrated. The residue was taken up in 1:1 methanol–1,4-dioxane (5 cm^3) and treated with 0.5 mol dm^{-3} NaOMe in MeOH (3 cm^3) at 40 °C for 24 h; TLC (solvent *F*) then revealed the formation of a single major product which was UV-inactive. The reaction mixture was deionized by passage through a short column of Dowex 50W-X4 (H^+) resin, the eluate was neutralized with triethylamine (1.5 cm^3), and the solvents were removed under reduced pressure. The resulting residue was suspended in water (10 cm^3) and extracted with toluene (3 × 10 cm^3) to remove methyl benzoate and then the water was evaporated off to give the pentasaccharide phosphate **1** (41 mg, 70%) as an amorphous solid; $[\alpha]_D^{21}$ +23 (*c* 1.6, H_2O); R_f 0.10 (solvent *F*); $\delta_H(D_2O)$ (*inter alia*) 1.25 (18 H, t, 6 × *Me*CH_2), 3.20 (12 H, q, 6 × MeCH_2), 3.42 (3 H, s, OCH_3), 3.61 (1 H, dd, $J_{2,3}$ 10.0, 2-H, Gal[d]), 3.67 (1 H, m, 3-H, Gal[a]), 3.72 (1 H, m, 2-H, Gal[a]), 3.80 (2 H, m, 2-H, Gal[b]; 2-H, Gal[c]), 3.87 (2 H, dd, $J_{2,3}$ 10.0, 3-H, Gal[b]; 3-H, Gal[c]), 3.89 (1 H, m, 3-H, Gal[d]), 3.93 (1 H, d, $J_{3,4}$ 3.0, 4-H, Gal[a]), 4.00 (1 H, dd, $J_{2,3}$ 3.2, 2-H, Man[a]), 4.20 (2 H, d, $J_{3,4}$ 3.0, 4-H, Gal[b]; 4-H, Gal[c]), 4.28 (1 H, d, $J_{3,4}$ 3.0, 4-H, Gal[d]), 4.52 (1 H, d, $J_{1,2}$ 7.9, 1-H, Gal[a]), 4.62 (1 H, d, $J_{1,2}$ 7.1, 1-H, Gal[d]), 4.69 (2 H, d, $J_{1,2}$ 7.8, 1-H, Gal[b]; 1-H, Gal[c]) and 4.77 (1 H, d, $J_{1,2}$ 1.5, 1-H, Man[a]); $\delta_P(D_2O)$ 1.78; δ_C see Table 2; ESMS(−) data: *m/z* 460.3 (80%, [M − 2 Et_3N − 2 H]$^{2-}$), 921.0 (100, [M − 2 Et_3N − H]$^-$) and 942.9 (3, [M − 2 Et_3N − 2 H + Na]$^-$) ($C_{43}H_{85}N_2O_{29}P$ requires M, 1124.49).

Methyl β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-{β-D-galactopyranosyl 6-[β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate]}-(1→4)-α-D-mannopyranoside, ammonium salt 2

To a solution of compound **34** (60 mg) in MeOH (20 cm^3) was added 4.6 mol dm^{-3} NaOMe in MeOH (0.22 cm^3). The mixture was kept for 16 h at 1 °C and was then deionized with Dowex 50W-X4 (H^+) resin, filtered, and neutralized with Et_3N. After concentration, water (5 × 10 cm^3) was evaporated off from residue to remove methyl benzoate; TLC (solvent *F*) then revealed the formation of three UV-active products (R_f 0.30–0.45). The residue was taken up in 1% NaOH in 4:1 MeOH–water (7 cm^3), the resulting solution was kept at 20 °C for 7 h and then was deionized as described above. Ion-exchange chromatography of the residue on a column (24 × 1 cm) of Fractogel TSK DEAE-650 (S) (HCO_3^- form) (Merck) eluted with a linear gradient of aq. NH_4HCO_3 (0 ⟶ 0.3 mol dm^{-3}) at 1 cm^3 min^{-1} gave the heptasaccharide phosphate **2** (18 mg, 75%) as an amorphous solid; $[\alpha]_D^{20}$ +37 (*c* 1.5, H_2O); R_f 0.23 (solvent *F*); $\delta_H(D_2O)$ (*inter alia*) 3.40 (3 H, s, OCH_3), 3.90 (2 H, d, $J_{3,4}$ 3.0, 4-H, Gal[a]; 4-H, Gal[e]), 3.99 (1 H, m, 2-H, Man[a]), 4.02 (1-H, m, 2-H, Man[b]), 4.19 (2 H, d, $J_{3,4}$ 3.0, 4-H, Gal[b]; 4-H, Gal[c]), 4.23 (1 H, d, $J_{3,4}$ 3.0, 4-H, Gal[d]), 4.38 (1 H, d, $J_{1,2}$ 7.3, 1-H, Gal[e]), 4.50 (1 H, d, $J_{1,2}$ 7.3, 1-H, Gal[a]), 4.60 (1 H, d, $J_{1,2}$ 7.0, 1-H, Gal[d]), 4.67 (2 H, d, $J_{1,2}$ 7.3, 1-H, Gal[b]; 1-H, Gal[c]), 4.77 (1 H, d, $J_{1,2}$ 1.5, 1-H, Man[a]) and 5.42 (1 H, dd, $J_{1,2}$ 1.5, $J_{1,P}$ 7.8, 1-H, Man[b]); $\delta_P(D_2O)$ −1.28; δ_C see Table 2; ESMS(−) data: *m/z* 622.3 (96%, [M − NH_3 − 2 H]$^{2-}$), 640.2 (100, [M − NH_3 − 3 H + K]$^{2-}$) and 1244.9 (93, [M − NH_3 − H]$^-$) ($C_{43}H_{78}NO_{39}P$ requires M, 1263.39). Also isolated was the pentasaccharide phosphate **1** (bisammonium salt; 4 mg, 22%).

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