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Note

Parasite glycoconjugates. Part 16: Synthesis of a disaccharide and phosphorylated di- and tri-saccharides from *Leishmania* lipophosphoglycan☆

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Abstract—A neutral disaccharide β-D-Gal*p*-(1→4)-α-D-Man*p* and phosphorylated di- and tri-saccharides β-D-Gal*p*-(1→3)-[H_2PO_3-6]-β-D-Gal*p*-O[CH_2]$_8$CH=CH_2 and β-D-Gal*p*-(1→3)-[H_2PO_3-6]-β-D-Gal*p*-(1→4)-α-D-Man*p*, which are fragments of the phosphoglycan portion of the surface lipophosphoglycan from *Leishmania donovani* (the disaccharide) or *Leishmania major* (all three compounds), were prepared and used as TLC standards to help the identification and differentiation of the elongating and branching β-D-galactosyl transferase activities in *Leishmania*. The phosphosaccharides were synthesised using the H-phosphonate method for phosphorylation.
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Keywords: Carbohydrates; Phosphates; Glycosylation; *Leishmania* enzymes

1. Introduction

The *Leishmania* are sandfly-transmitted protozoan parasites that cause a variety of debilitating and often fatal diseases throughout the tropics and sub-tropics. The life cycle of the parasite involves an insect vector (the sandfly) and mammalian hosts. The parasites' survival and infectivity are due to the glycocalyx and in particular the lipophosphoglycan (LPG), which is one of its major components.[2] LPGs are common to *Leishmania major*, *Leishmania donovani* and *Leishmania mexicana* promastigotes[3–8] and *L. major* amastigotes,[9] but they are absent in *L. donovani* and *L. mexicana* amastigotes.[10,11] The LPG of the above *Leishmania* species contains a polymeric phosphoglycan region consisting of [-6)-(R→3)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-]$_n$ repeating units where the nature of side-chain groups R varies with the species. For example, in *L. donovani*[2] R = H (100%), whereas in *L. major*[2,12] R is predominantly β-D-Gal*p* (52%) or β-D-Gal*p*-(1→3)-β-D-Gal*p* (25%). In the LPG produced by *L. mexicana*,[6,13] R is β-D-Glc*p* (25%) or H (75%). The importance of the LPG for parasite infectivity and survival[14,15] makes the enzymes responsible for its biosynthesis of great interest.

The biosynthesis of the β-D-Gal*p*-(1→4)-α-D-Man*p* phosphate backbone was shown[16,17] to be performed by sequential action of the *Leishmania* elongating α-D-mannopyranosyl phosphate transferase (eMPT) and the elongating β-D-galactopyranosyl transferase (eGT). There is also expected to be a branching β-D-galactopyranosyl transferase (or transferases, bGT), which introduces side-chain β-D-Gal*p* residues (i.e., R = β-D-Gal*p*) in *L. major*,[18] but not in *L. donovani*. In Parts 4,[19] 9,[20] 11,[21] 12[22] and 15[1] of this series, we disclosed our interest in the design and synthesis of various substrates/substrate analogues/inhibitors to study the fine acceptor–substrate specificity of the eMPT[23,24] and to gain more information about enzyme–substrate recognition. We now report the chemical synthesis of disaccharide **4**, phosphotrisaccharide **5** and phosphodisaccharide **6**

☆ See Ref. 1.

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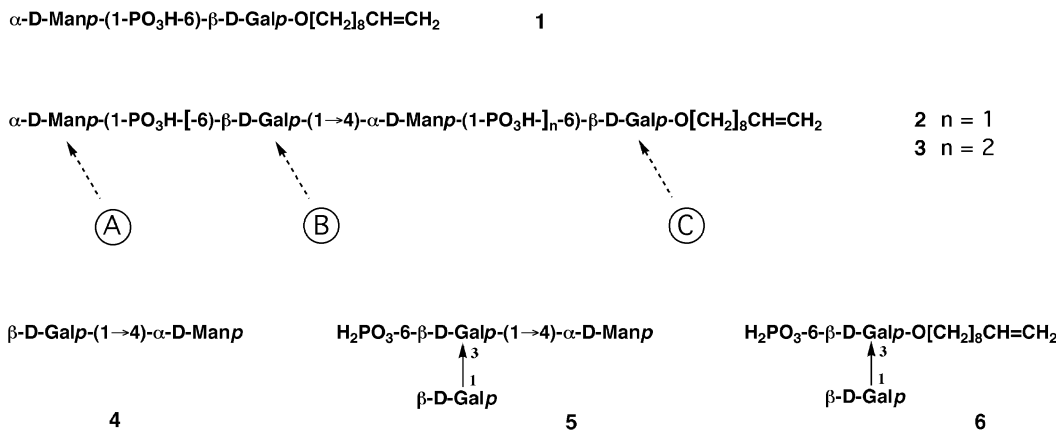


Figure 1.

(Fig. 1), which then were used for the identification and differentiation of the elongating and branching galactosyl transferase activities in *Leishmania*.

First, a biochemical assay was developed for a galactose transfer in crude membrane preparation from either *L. donovani* or *L. major* (a cell free system[24] as enzyme source), using UDP-[^{3}H]Gal (as a β-D-galactopyranose donor substrate) and synthetic LPG fragments **1**, **2** or **3**[25] (as exogenous acceptor substrates). Conditions for the assay and for the isolation of galactosylated products were similar to those used previously for the eMPT.[24] It appeared that no [^{3}H]Gal transfer was detected for disaccharide monophosphate **1**, but the longer phosphoglycans **2** and **3** containing two and three phosphodiester bridges, respectively, each produced [^{3}H]-labelled products of β-D-galactosylation in the presence of both *L. donovani* and *L. major* membrane preparation.

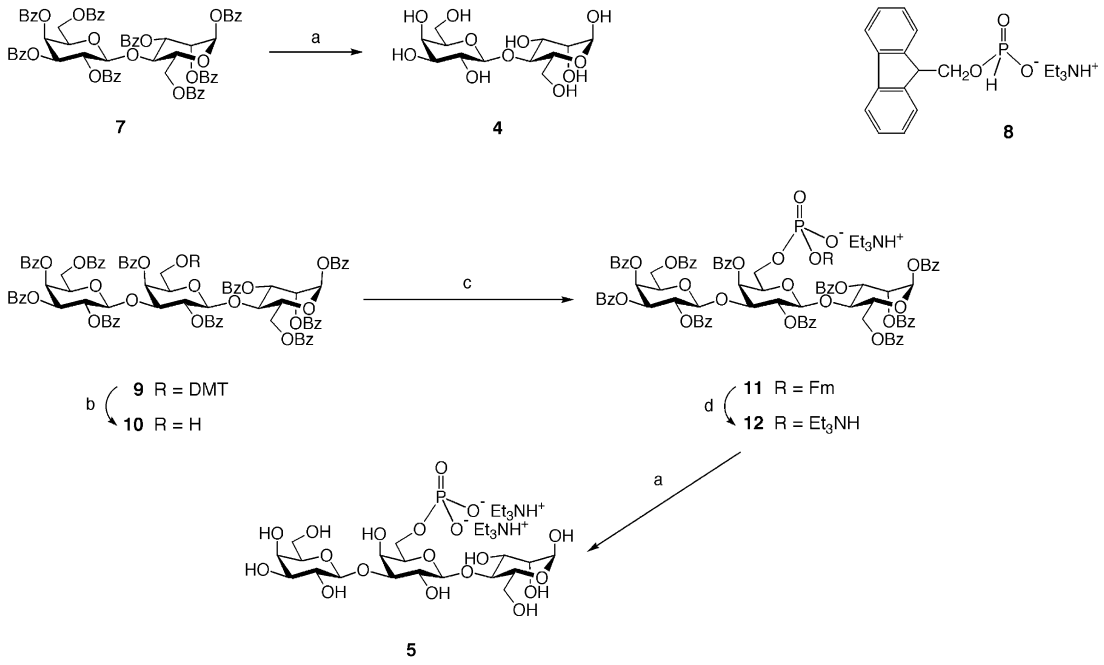
In order to analyse the structures and to identify the galactosylation sites, which were expected to be **A** (corresponding to a putative eGT activity), **B** or **C** (both corresponding to putative bGTs), the products of the biochemical transfer of [^{3}H]Gal to **3** were isolated[24] (C18 Sep-Pak) and then hydrolysed with mild acid (40 mM trifluoroacetic acid, 100 °C). Since the conditions were selective enough to hydrolyse the α-D-mannosyl phosphate bonds only,[4] we expected the products to be [^{3}H]-labelled compounds **4**, **5** or **6** (or mixtures of any two or all three of them) with [^{3}H]Gal residue at the non-reducing terminus of each of them. Thus, the authentic saccharides **4**–**6** were needed as standards to properly analyse the hydrolysis products by TLC.

2. Results and discussion

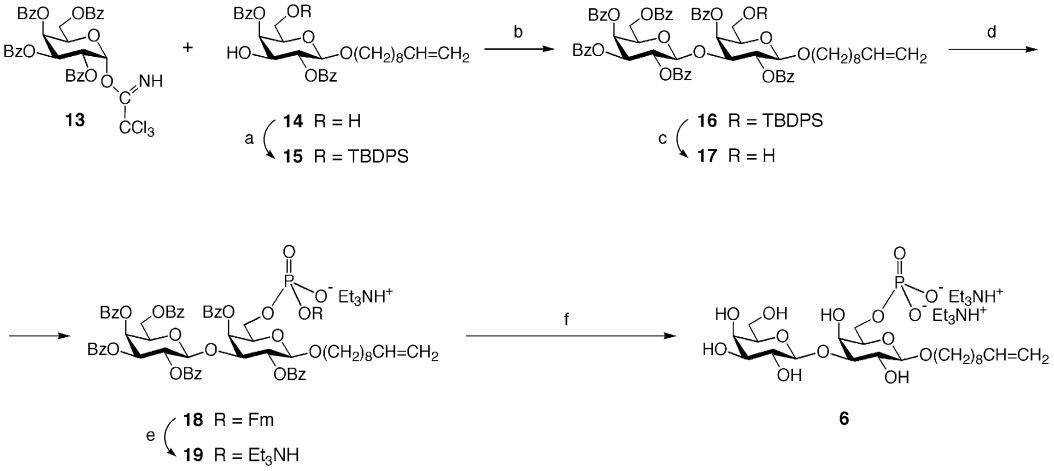
The neutral disaccharide **4** was prepared by conventional debenzoylation of the O-protected derivative **7**[20] (Scheme 1) with 0.06 M MeONa in methanol.

The synthesis of trisaccharide phosphate **5** was performed starting from the recently described trisaccharide compound **9**,[1] which was converted to the monohydroxyl derivative **10** (96%) by detritylation with 3% trifluoroacetic acid (TFA) in dichloromethane. The H-phosphonate approach to phosphomonoesters[26] was then applied for the phosphate formation. As we showed previously,[26] efficacious phosphorylation of primary, secondary and anomeric HO-groups of carbohydrates could be performed via the consecutive preparation of 'sugar' H-phosphonates and corresponding 'sugar' 9-fluorenemethyl phosphodiesters. In that way, the 9-fluorenemethyl ester was used as P-protecting group to facilitate the oxidation step. The protection was then easily removed (with piperidine) to give the desired phosphomonoesters. In the present paper, triethylammonium 9-fluorenemethyl H-phosphonate **8** was used for phosphorylating alcohol **10** in the presence of pivaloyl chloride followed by oxidation with iodine, thus forming trisaccharide 9-fluorenemethyl phosphodiester **11**. Subsequent P-deprotection (20% piperidine in CH_2Cl_2) produced the O-protected phosphotrisaccharide **12** (64%), which gave phosphate **5** upon debenzoylation with 0.3 M MeONa in methanol. The required H-phosphonate **8** was made essentially as described in Ref. 27, that is, by the reaction of 9-fluorenemethanol and PCl_3 followed by hydrolysis (producing crystalline 9-fluorenemethyl H-phosphonic acid) and neutralisation with Et_3N.

The preparation of disaccharide phosphate **6** was started from galactosyl trichloroacetimidate **13**[20] and the glycosyl acceptor **15** (Scheme 2), which in turn, was synthesised from known 3,6-diol **14**[25] by the reaction with *tert*-butyldiphenylsilyl chloride (TBDPS chloride) in pyridine. Trimethylsilyl triflate (TMS triflate) assisted glycosylation reaction gave β-linked disaccharide **16** in 45% yield and the recovered acceptor **15** (24%). The β-configuration of the newly created D-galactoside bond was confirmed by the characteristic value



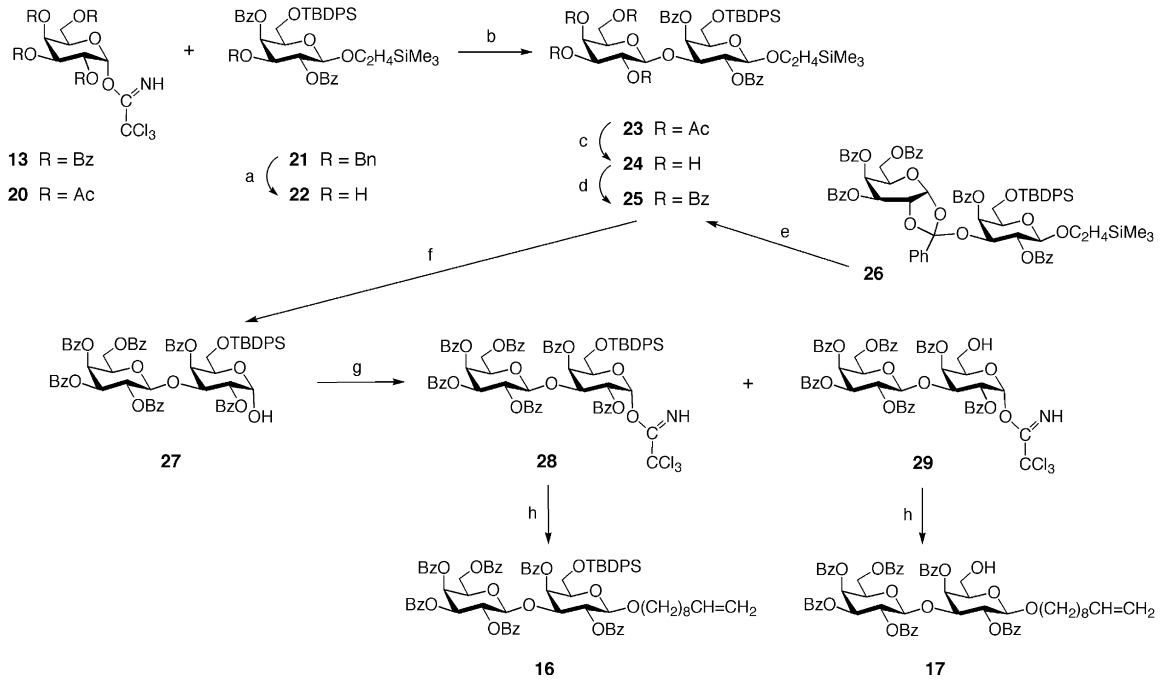
Scheme 1. Reagents: (a) NaOMe, MeOH; (b) TFA–CH_2Cl_2 (3:97); (c) (i) **8**, pivaloyl chloride, C_5H_5N; (ii) I_2, C_5H_5N–water; (d) piperidine, CH_2Cl_2.



Scheme 2. Reagents: (a) TBDPS chloride, imidazole, C_5H_5N; (b) $TMSOSO_2CF_3$, MS 4 Å, CH_2Cl_2; (c) *n*-Bu_4NF, AcOH, THF; (d) (i) **8**, pivaloyl chloride, C_5H_5N; (ii) I_2, C_5H_5N–water; (e) piperidine, CH_2Cl_2; (f) NaOMe, MeOH.

(7.6 Hz) for $J_{1',2'}$-coupling constant in the ^1H NMR spectrum of **16**. It was then desilylated with tetra-*n*-butylammonium fluoride (TBAF)–acetic acid reagent (an equimolar mixture in THF) to give the monohydroxyl derivative **17** (77%). Condensation of **17** with H-phosphonate **8** followed by oxidation (in the same manner as described for the phosphorylation of trisaccharide alcohol **10**) then produced phosphodiester **18**. It then afforded the targeted phosphate **6** (76% for four steps starting from **17**) upon deprotection by consecutive treatment with piperidine in CH_2Cl_2 (→**19**) and MeONa in methanol–THF.

Since disaccharide **16** was synthesised in moderate yield, we also attempted its preparation via the formation of the galactobiose derivative **25**, followed by transformation to disaccharide trichloroacetimidate **28** and glycosylation of dec-9-en-1-ol (Scheme 3). In this way, glycosylation of 2-(trimethylsilyl)ethyl galactoside **22** with the benzoylated glycosyl donor **13** in the presence of TMS triflate proceeded slowly and resulted in galactobiose **25** (29%) together with isomeric disaccharide orthoester **26** (33%) and the recovered acceptor **22** (38%). The structure of the orthoester was confirmed by the characteristic[28] chemical shifts and coupling



Scheme 3. Reagents: (a) H_2, $Pd(OH)_2/C$, AcOH–ethanol; (b) $TMSOSO_2CF_3$, MS 4 Å, CH_2Cl_2; (c) $Mg(OMe)_2$, MeOH; (d) benzoyl chloride, C_5H_5N; (e) **22**, $TMSOSO_2CF_3$, MS 4 Å, CH_2Cl_2; (f) TFA–CH_2Cl_2 (2:1); (g) CCl_3CN, DBU, CH_2Cl_2; (h) dec-9-ene-1-ol, $TMSOSO_2CF_2$, MS 4 Å, CH_2Cl_2.

constants for 1′-H (δ_H 6.43, d, $J_{1',2'}$ 4.6) and 2′-H (δ_H 4.58–4.70, m, $J_{2',3'}$ 5.0) signals in the ^1H NMR spectrum, and by the presence of the diagnostic signal[28] of the quaternary orthoester carbon (δ_C 121.16) in the ^{13}C NMR spectrum. Orthoester **26** was then converted to additional disaccharide **25** (51% from **26**) by the reaction with the recovered compound **22** in the presence of TMS triflate, thus making the total yield of **25** as 46%.

Glycosylation of the same acceptor **22** with the acetylated glycosyl donor **20** appeared to be more efficient and provided the expected β-linked disaccharide **23** ($J_{1',2'} = 7.8$ Hz) in 80% yield. Glycosyl trichloroacetimidate **20**, in turn, was effectively made (72% yield) from penta-*O*-acetyl-β-D-galactopyranose by selective anomeric de-O-acetylation with Me_2NH in acetonitrile followed by the reaction with CCl_3CN in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[29] Disaccharide **23** was then converted to **25** by consecutive de-O-acetylation[30] with $Mg(OMe)_2$ in MeOH (→**24**, 75%) and conventional benzoylation (91%).

To prepare disaccharide trichloroacetimidate **28**, compound **25** was first anomerically deprotected[31] with TFA in CH_2Cl_2 (→**27**) followed by the reaction with CCl_3CN in the presence of DBU. Very much to our surprise, some of the de-O-silylated trichloroacetimidate **29** (31%) was isolated in addition to the main product **28** (69%). Since the structure of the hemiacetal **27** was clearly confirmed by the ^1H NMR spectrum (see Experimental section), we assume that partial de-O-silylation was caused by the excessive DBU treatment. Glycosylation of dec-9-en-1-ol with the trichloroacetimidate **28** in the presence of TMS triflate proceeded smoothly and afforded disaccharide **16** in 72% yield. Similarly, the reaction of dec-9-en-1-ol with trichloroacetimidate **29** provided the de-O-silylated compound **17** (38%) as the main product.

The synthetic saccharides **4**–**6** were used later as TLC standards to identify products of acid hydrolysis of [^{3}H]-galactosylated phosphosaccharide **3** ([^{3}H]Gal–**3**), which was biochemically prepared with the use of crude membrane preparation from *L. donovani* or *L. major* (see Introduction section). Only disaccharide **4** (which corresponds to the galactosylation site **A**, Fig. 1) was detected in the hydrolysate of the [^{3}H]Gal–**3** made with *L. donovani* membranes, but both disaccharide **4** and phosphotrisaccharide **5** (corresponding to the galactosylation sites **A** and **B**, respectively) were found in the hydrolysate of products formed in the *L. major* assay. This shows the presence of the elongating β-D-galactosyl transferase in both species and the presence of the branching β-D-galactosyl transferase in *L. major* only. Phosphodisaccharide **6** was not detected in the *L. major* products hydrolysate. This result (indicating that galactosylation does not take place at site **C**) reflects, probably, a substrate specificity of the bGT enzyme. A detailed and comprehensive discussion of identification and characterisation of the elongating and branching β-D-galactosyl transferases in *Leishmania* using a set of compounds prepared in this laboratory will be published elsewhere in due course.

3. Experimental

3.1. General procedures

Melting points were determined on a Reichert hot-plate apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter; $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H, ^{13}C and ^{31}P NMR spectra (^{1}H at 300 MHz, ^{13}C at 75 MHz, ^{31}P at 121 MHz) were recorded with a Bruker AM-300 spectrometer 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 hertz. ES mass spectra were recorded with a VG Quattro system (VG Biotech, UK) and with a Mariner system (Applied Biosystems). TLC was performed on Kieselgel 60 F_{254} (Merck) with detection under UV light or by charring with sulfuric acid–water–ethanol (15:85:5). Flash column chromatography (FCC) was performed on Kieselgel 60 (0.040–0.063 mm) (Merck). Dichloromethane, acetonitrile, pyridine and toluene were freshly distilled from CaH_2. Solutions worked up were concentrated under reduced pressure at <40 °C.

3.2. 9-Fluorenemethyl H-phosphonic acid

A solution of 9-fluorenemethanol (588 mg, 3 mmol) in acetonitrile (5 mL) was added to a stirred solution of phosphorus trichloride (1.9 mL, 21.8 mmol) in the same solvent (5 mL). After stirring for 15 min, the mixture was concentrated and acetonitrile was evaporated off from the residue. The residue was dissolved in acetonitrile (20 mL), water (5 mL) was added dropwise (over 5 min) and the mixture was stored at room temperature for 3 h, whereafter it was concentrated and acetonitrile was evaporated off from the residue to leave a white solid. The residue was recrystallised from the same solvent (5 mL) to form crystalline 9-fluorenemethyl H-phosphonic acid (663 mg, 85%); mp 114–116 °C {lit.,[27] mp 118–120 °C}; δ_H 4.25 (1H, m, 9-H), 4.35 (2H, m, CH_2), 5.60 and 7.98 (1H, d, $^1J_{H,P}$ 711.6, P-H), 7.27–7.80 (8H, m, $2\times C_6H_4$) and 9.65 (1H, br s, P-OH); δ_P 9.19 (dt, $^1J_{P,H}$ 711.6, $^3J_{P,CH_2}$ 7.0).

3.3. β-D-Galactopyranosyl-(1→4)-α-D-mannopyranose (4)

To a solution of benzoylated disaccharide **7** (42 mg, 0.036 mmol) in MeOH (10 mL) was added 3 M methanolic NaOMe (0.2 mL). The mixture was stored for 3 h, whereafter it was deionised with Dowex 50W-X4 (H^+) resin, filtered and concentrated. Water (5×5 mL) was then evaporated off from the residue (to remove methyl benzoate). The residue was then dissolved in water (20 mL), the solution was extracted with diethyl ether (2×10 mL) and the aqueous layer was concentrated to give compound **4** (11.7 mg, 95%) as an amorphous solid; $[\alpha]_D^{20}$ +16.4 (*c* 1, MeOH); R_f 0.38 [$CHCl_3$–MeOH–water, (10:10:3)]; δ_H (D_2O) (inter alia) 3.44 (dd, $J_{2',3'}$ 10.0, 2′-H), 3.56 (dd, $J_{3',4'}$ 3.3, 3′-H), 3.88 (m, 2- and 4′-H), 4.34 (d, $J_{1',2'}$ 7.7, 1′-H), 5.08 (d, $J_{1,2}$ 1.5, 1-H); β-anomer components: 3.43 (dd, $J_{2',3'}$ 9.9, 2′-H), 4.33 (d, $J_{1',2'}$ 7.7, 1′-H); δ_C (D_2O) 60.70 (C-6), 61.48 (C-6′), 68.97 (C-4′), 69.39 (C-3), 70.54 (C-2), 71.33 (C-2′), 71.39 (C-5), 72.87 (C-3′), 75.75 (C-5′), 76.92 (C-4), 94.16 (C-1) and 103.42 (C-1′); β-anomer components: 71.00 (C-2), 72.18 (C-3), 72.74 (C-3′), 75.32 (C-5), 76.52 (C-4) and 94.00 (C-1); ES-MS (−): *m*/*z* 341.07 (5%) $[M-H]^-$, 377.07 (45%) $[M+^{35}Cl]^-$, 379.10 (12%) $[M+^{37}Cl]^-$, 387.07 (100%) $[M+HCOO]^-$; ES-MS (+): *m*/*z* 343.17 (100%) $[M+H]^+$, 365.06 (70%) $[M+Na]^+$, 381.06 (50%) $[M+K]^+$ (calcd for $C_{12}H_{22}O_{11}$: *M*, 342.12).

3.4. 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-mannopyranose (10)

To a solution of trisaccharide derivative **9** (41 mg, 0.022 mmol) in CH_2Cl_2 (5 mL) was added 6% TFA in CH_2Cl_2 (5 mL). After 30 min, the mixture was neutralised with Et_3N–MeOH–CH_2Cl_2 (1:1:2, 4 mL), concentrated and toluene was evaporated off from the residue. FCC [toluene–ethyl acetate, (9:1)→(8:2)] of the residue produced the 6′-hydroxy derivative **10** (33 mg, 96%) as an amorphous solid; $[\alpha]_D^{22}$ +63.1 (*c* 1.1, $CHCl_3$); δ_H 2.87 (1H, dd, $J_{5',6a'}$ 8.5, 6′-H^a), 3.06 (1H, dd, $J_{6a',6b'}$ 11.8, 6′-H^b), 3.38 (1H, dd, $J_{5',6b'}$ 5.7, 5′-H), 3.97–4.08 (3H, m, 3′-H, 5-H and 6″-H^a), 4.11 (1H, t, $J_{5'',6a''}=J_{5'',6b''}=6.1$, 5″-H), 4.28 (1H, dd, $J_{5,6a}$ 2.5, 6-H^a), 4.38 (1H, br d, $J_{6a,6b}$ 12.1, 6-H^b), 4.43–4.58 (2H, m, 4-H and 6″-H^b), 4.72 (1H, d, $J_{1',2'}$ 7.9, 1′-H), 4.85 (1H, d, $J_{1'',2''}$ 7.7, 1″-H), 5.28 (1H, dd, $J_{3'',4''}$ 3.3, 3″-H), 5.45 (1H, dd, $J_{2'',3''}$ 10.3, 2″-H), 5.53 (1H, d, 4″-H), 5.55 (1H, dd, $J_{2',3'}$ 10.1, 2′-H), 5.69 (1H, dd, $J_{2,3}$ 3.2, 2-H), 5.72 (1H, d, $J_{3',4'}$ 3.0, 4′-H), 5.81 (1H, dd, $J_{3,4}$ 9.1, 3-H), 6.38 (1H, d, $J_{1,2}$ 1.9, 1-H) and 6.88–8.06 (50H, m, $10\times$ Ph); δ_C 59.42 (C-6′), 61.49 (C-6″), 61.91 (C-6), 67.44 (C-4″), 69.42 (C-2″), 69.60 (C-4′), 69.91 (C-2), 70.37 (C-3), 71.07 (C-3″), 71.32 (2C, C-2′ and C-5″), 71.58 (C-5), 72.77 (C-4), 73.69 (C-5′), 78.41 (C-3′), 91.10 (C-1), 101.10 (C-1″), 101.71 (C-1′), 127.94–130.38 and 133.14–133.67 (Ph) and 164.00–171.05 (C=O). Anal. Calcd for $C_{88}H_{72}O_{26}$: C, 68.39; H, 4.70. Found: C, 68.29; H, 4.94.

3.5. β-D-Galactopyranosyl-(1→3)-6-*O*-phosphonato-β-D-galactopyranosyl-(1→4)-α-D-mannopyranose, bis-triethylammonium salt (5)

9-Fluorenemethyl H-phosphonate **8** was prepared by evaporation of pyridine–Et_3N (8:2) (3×3 mL) from 9-fluorenemethyl H-phosphonic acid (5.6 mg, 0.022 mmol). Trisaccharide **10** (30 mg, 0.019 mmol) was then

added to the residue and the mixture was dried by evaporation of pyridine (3 × 3 mL) therefrom. The residue was dissolved in pyridine (1 mL), pivaloyl chloride (0.01 mL, 0.081 mmol) was added and the mixture was stirred at room temperature for 1 h, whereafter a second portion of pivaloyl chloride (0.01 mL, 0.081 mmol) was added. After a further 25 min, a freshly prepared solution of iodine (11 mg, 0.043 mmol) in 95% aq pyridine (1 mL) was added to the mixture. After 30 min, the mixture was diluted with CH_2Cl_2 and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$ and cold 0.5 M triethylammonium (TEA) hydrogen carbonate, dried by filtration through cotton wool and concentrated to produce crude phosphodiester **11** [δ_H (inter alia) 3.92 (1H, br d, $J_{4,5}$ 9.7, 5-H), 4.26–4.44 (5H, m, 1′-H, 6″-Hb and CH_2–CH of 9-fluorenemethyl), 4.50 (1H, t, $J_{3,4} = J_{4,5} = 9.7$, 4-H), 4.60 (2H, br s, 6-H_2), 4.65 (1H, d, $J_{1'',2''}$ 7.8, 1″-H), 5.21 (1H, dd, $J_{3'',4''}$ 3.2, 3″-H), 5.33 (1H, dd, $J_{2'',3''}$ 10.2, 2″-H), 5.44 (1H, dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.8, 2′-H), 5.60 (1H, d, 4″-H), 5.62 (1H, d, $J_{3',4'}$ 3.1, 4′-H), 5.65 (1H, dd, 2-H), 5.81 (1H, dd, $J_{2,3}$ 3.4, 3-H) and 6.38 (1H, d, $J_{1,2}$ 1.6, 1-H); δ_P −1.06]. The residue was dissolved in CH_2Cl_2 (1 mL), piperidine (0.2 mL) was added and the mixture was stirred at room temperature for 40 min, then diluted with CH_2Cl_2, washed successively with cold 0.1 M HCl and cold 1 M TEA hydrogen carbonate, dried by filtration through cotton wool and concentrated. FCC [dichloromethane–MeOH–Et_3N, (99:0:1)→(64:35:1)] of the residue gave O-protected trisaccharide phosphomonoester**12** (22 mg, 64%; δ_P 1.78) as an amorphous solid. Compound **12** (22 mg) was dissolved in 0.3 M methanolic NaOMe (3.3 mL) and the mixture was stirred at room temperature. After 16 h, the solution was deionised with Dowex 50W-X4 (H^+) resin, filtered and neutralised with Et_3N. After concentration, water (5 × 5 mL) was evaporated off from the residue to remove methyl benzoate. ^{1}H NMR of the prepared compound then revealed the presence of residual aromatic signals. After additional treatment with 0.3 M NaOMe in MeON (24 h, room temperature) followed by a work-up as described above, trisaccharide phosphate **5** (9.8 mg, 64% from **10**) was produced as an amorphous solid; $[\alpha]_D^{20}$ +11 (*c* 0.3, water); R_f 0.14 [$CHCl_3$–MeOH–water, (10:10:3)]; δ_H (D_2O) (inter alia) 1.20 (18H, t, $J = 7.3$, 6 × *Me*CH_2), 3.13 (12H, q, 6 × MeCH_2), 4.19 (dd, $J_{3',4'}$ 3.2, 4′-H), 4.46 (d, $J_{1',2'}$ 7.9, 1′-H), 4.55 (d, $J_{1'',2''}$ 7.3, 1″-H) and 5.11 (d, $J_{1,2}$ 1.6, 1-H); β-anomer component: 4.83 (d, $J_{1,2}$ 0.9, 1-H); δ_C (D_2O) 8.60 (*Me*CH_2), 47.05 (Me*C*H_2), 60.88 (C-6), 61.30 (C-6″), 64.40 (d, $^2J_{C,P}$ 4.1, C-6′), 68.47 (C-4′), 68.97 (C-3), 69.44 (C-4″), 70.46 (C-2), 70.53 (C-2′), 71.27 (C-5), 71.40 (C-2″), 72.90 (C-3″), 74.02 (d, $^3J_{C,P}$ 7.9, C-5′), 75.44 (C-5″), 77.84 (C-4), 82.11 (C-3′), 94.06 (C-1), 103.24 (C-1′) and 104.75 (C-1″); β-anomer components: 70.99 (C-2), 72.20 (C-3), 75.22 (C-5) and 77.46 (C-4); δ_P (D_2O) 1.65; ES-MS (−): *m*/*z* 583.08 (100%) $[M-2Et_3N-H]^-$ (calcd for $C_{30}H_{63}N_2O_{19}P$: *M*, 786.38).

3.6. Dec-9-enyl 2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (15)

To a solution of galactoside **14**[25] (104 mg, 0.197 mmol) and imidazole (31 mg, 0.455 mmol) in pyridine (1 mL) was added TBDPS chloride (0.059 mL, 0.227 mmol) and the mixture was stirred at room temperature. After 17 h, the mixture was diluted with CH_2Cl_2, washed successively with $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. FCC [toluene–ethyl acetate, (99:1)→(9:1)] of the residue produced compound **15** (127 mg, 84%) as an amorphous solid; $[\alpha]_D^{20}$ +11.9 (*c* 1, $CHCl_3$); δ_H 1.05 (9H, s, Me_3C), 1.08–1.37 (10H, m, 5 × CH_2), 1.54 (2H, m, OCH_2CH_2), 2.02 (2H, q, *J* 6.7, CH_2CH_2CH=), 2.83 (1H, d, $J_{H,OH} = 3.9$, 3-OH), 3.52 (1H, dt, $^2J_{H,H}$ 9.8, $^3J_{H,H}$ 6.9, O*H*CHCH_2), 3.82–3.98 (4H, m, OHC*H*CH_2, 5-H and 6-H_2), 4.19 (1H, ddd, $J_{2,3}$ 10.0, 3-H), 4.66 (1H, d, $J_{1,2}$ 7.9, 1-H), 4.96 (1H, br d, $^3J_{H,H}$ 10.2, CH=*H*CH), 5.02 (1H, br d, $^3J_{H,H}$ 17.0, CH=HC*H*), 5.35 (1H, dd, 2-H), 5.82 (1H, ddt, J_{H,CH_2} 6.7, C*H*=CH_2), 5.86 (1H, d, $J_{3,4}$ 3.5, 4-H) and 7.14–8.18 (20H, m, 4 × Ph); δ_C 19.02 (Me_3C), 26.65 (*Me*$_3$C), 25.82, 28.82, 28.95, 29.21, 29.24, 29.42 and 33.71 (C*C*H_2C), 61.58 (C-6), 70.10 (OCH_2), 70.38 (C-4), 72.20 (C-3), 73.77 (2C, C-2 and -5), 101.23 (C-1), 114.07 (=CH_2), 127.58–130.11 and 132.69–135.52 (Ph), 139.15 (–CH=) and 166.52 and 166.59 (C=O). Anal. Calcd for $C_{46}H_{56}O_8Si{\cdot}H_2O$: C, 70.56; H, 7.47. Found: C, 70.94; H, 7.70.

3.7. Dec-9-enyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (16)

(A) To a stirred and cooled (−60 °C) solution of galactosyl trichloroacetimidate **13**[20] (119 mg, 0.161 mmol) and acceptor **15** (104 mg, 0.136 mmol) in dry dichloromethane (1.5 mL) containing freshly activated molecular sieves 4 Å (240 mg) under argon was added a 2% (v/v) solution of TMS triflate in the same solvent (0.073 mL, 0.0081 mmol). The temperature was allowed to rise to room temperature for 3 h, and the mixture was stirred overnight. *N*,*N*-Diisopropylethylamine (0.1 mL, 0.58 mmol) was then added, the solids were filtered off through a Celite pad and the solution was concentrated. Two consecutive FCCs of the residue [first, in toluene–ethyl acetate, (99:1)→(90:10) and then in petroleum ether (40–60 °C)–ethyl acetate, (99:1)→(80:20)] provided β-linked disaccharide **16** (83 mg, 45%) as an amorphous solid; $[\alpha]_D^{20}$ +78 (*c* 1.04, $CHCl_3$); δ_H 1.06 (9H, s, Me_3C), 1.10–1.45 (10H, m, 5 × CH_2), 1.65 (2H, m, OCH_2CH_2), 1.95 (2H, q, *J* 6.7, CH_2CH_2CH=), 3.38 (1H, dt, $^2J_{H,H}$ 9.5, $^3J_{H,H}$ 6.5, O*H*CHCH_2), 3.78–3.91 (4H, m, OHC*H*CH_2, 5-H and 6-H_2), 4.24 (1H, m, 5′-H), 4.27 (1H, dd, $J_{3,4}$ 3.5, 3-H), 4.35 (1H, dd, $J_{5',6a'}$ 7.3, $J_{6a',6b'}$ 11.1, 6′-H^a), 4.54 (1H, d, $J_{1,2}$ 8.0, 1-H), 4.77 (1H, dd, $J_{5',6b'}$ 6.1, 6′-H^b), 4.92 (1H, br d, $^3J_{H,H}$ 10.2, CH=*H*CH),

4.97 (1H, br d, $^3J_{H,H}$ 16.9, CH=HC*H*), 4.99 (1H, d, $J_{1',2'}$ 7.6, 1′-H), 5.37 (1H, dd, $J_{3',4'}$ 3.3, 3′-H), 5.52 (1H, dd, $J_{2,3}$ 10.0, 2-H), 5.57 (1H, dd, $J_{2',3'}$ 10.4, 2′-H), 5.78 (1H, ddt, J_{H,CH_2} 6.7, C*H*=CH_2), 5.88 (1H, d, 4′-H), 6.00 (1H, d, 4-H) and 6.98–8.20 (40H, m, 8 × Ph); δ_C 19.10 (Me_3C), 26.67 (Me_3C), 25.72, 28.82, 28.94, 29.14, 29.17, 29.33 and 33.71 (CCH_2C), 61.58 (C-6′), 62.32 (C-6), 67.55 (C-4′), 69.63 (C-2′), 69.73 (OCH_2), 70.21 (C-4), 70.99 (C-5′), 71.54 (C-3′), 71.65 (C-2), 74.67 (C-5), 77.04 (C-3), 101.29 (C-1′), 101.45 (C-1), 114.03 (=CH_2), 127.58–130.18 and 132.51–135.56 (Ph), 139.18 (–CH=) and 164.40–165.97 (C=O). Anal. Calcd for $C_{80}H_{82}O_{17}Si$: C, 71.51; H, 6.15. Found: C, 71.52; H, 6.38. Also isolated was the galactoside **15** (25 mg, 24% recovery).

(B) To a stirred and cooled (−70 °C) solution of trichloroacetimidate **28** (72 mg, 0.053 mmol) and dec-9-en-1-ol (0.1 mL, 0.56 mmol) in CH_2Cl_2 (1 mL) containing freshly activated molecular sieves 4 Å (200 mg) under argon was added a 2% (v/v) solution of TMS triflate in the same solvent (0.027 mL, 0.003 mmol). The stirring was continued, while the temperature was allowed to rise to −30 °C for 1.5 h. A second portion of 2% (v/v) solution of TMS triflate in CH_2Cl_2 (0.027 mL, 0.003 mmol) was then added, the mixture was slowly warmed up to 0 °C, and the stirring was continued at this temperature for a further 19 h. After standard work-up (as above), FCC [toluene–ethyl acetate, (95:5)] gave the disaccharide **16** (52 mg, 73%).

3.8. Dec-9-enyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-galactopyranoside (17)

(A) To a solution of derivative **16** (50 mg, 0.037 mmol) in THF (1 mL) was added a 10% (v/v) solution of acetic acid in THF (0.064 mL, 0.112 mmol) and then 1 M TBAF in THF (0.112 mL, 0.112 mmol). The mixture was stored at room temperature for 23 h, whereafter a second portion of the AcOH/THF solution (0.064 mL, 0.112 mmol) followed by 1 M TBAF/THF (0.112 mL, 0.112 mmol) was added. After 6.5 h, the mixture was applied for a silica column and FCC [toluene–ethyl acetate, (100:0)→(70:30)], then gave the 6-hydroxy derivative **17** (32 mg, 78%) as an amorphous solid; $[\alpha]_D^{22}$ +71 (*c* 0.5, $CHCl_3$); δ_H 0.75–1.40 (12H, m, 6 × CH_2), 1.87 (2H, q, *J* 6.7, CH_2CH_2CH=), 3.32 (1H, dt, $^2J_{H,H}$ 9.4, $^3J_{H,H}$ 6.5, O*H*CHCH_2), 3.51 (1H, dd, $J_{5,6a}$ 8.2, $J_{6a,6b}$ 11.3, 6-H^a), 3.63–3.80 (3H, m, OHC*H*CH_2, 5-H and 6-H^b), 4.11 (1H, dd, $J_{5',6a'}$ 6.8, $J_{6a',6b'}$ 10.8, 6′-H^a), 4.17 (1H, dd, $J_{3,4}$ 3.8, 3-H), 4.19 (1H, m, 5′-H), 4.49 (1H, d, $J_{1,2}$ 8.0, 1-H), 4.57 (1H, dd, $J_{5',6b'}$ 5.7, 6′-H^b), 4.83 (1H, br d, $^3J_{H,H}$ 10.2, CH=*H*CH), 4.89 (1H, br d, $^3J_{H,H}$ 16.9, CH=HC*H*), 4.97 (1H, d, $J_{1',2'}$ 7.8, 1′-H), 5.33 (1H, dd, $J_{3',4'}$ 3.3, 3′-H), 5.52 (1H, dd, $J_{2',3'}$ 10.0, 2′-H), 5.57 (1H, dd, $J_{2,3}$ 9.8, 2-H), 5.70 (1H, ddt, J_{H,CH_2} 6.7, C*H*=CH_2), 5.72 (1H, d, 4-H), 5.77 (1H, d, 4′-H) and 6.91–8.15 (30H, m, 6 × Ph); δ_C 25.66, 28.80, 28.92, 29.10, 29.30, 29.66 and 33.71 (CCH_2C), 60.03 (C-6), 61.65 (C-6′), 67.55 (C-4′), 69.48 (C-2′), 70.08 (OCH_2), 70.91 (C-4), 71.95 (2C, C-2 and -5′), 71.39 (C-3′), 73.63 (C-5), 78.43 (C-3), 101.54 (C-1), 101.79 (C-1′), 114.03 (=CH_2), 127.95–130.43 and 132.59–133.56 (Ph), 139.18 (–CH=) and 164.45–168.33 (C=O). Anal. Calcd for $C_{64}H_{64}O_{17}$: C, 69.55; H, 5.84. Found: C, 69.68; H, 6.21.

(B) To a stirred and cooled (−40 °C) solution of trichloroacetimidate **29** (27 mg, 0.024 mmol) and dec-9-en-1-ol (0.043 mL, 0.241 mmol) in CH_2Cl_2 (1 mL) containing freshly activated molecular sieves 4 Å (200 mg) under argon was added 2% (v/v) solution of TMS triflate in the same solvent (0.022 mL, 0.0024 mmol). The stirring was continued while the temperature was allowed to rise to 0 °C for 2 h, whereafter cooling was discontinued and the mixture was stirred at room temperature for a further 16 h. After standard work-up (as described for the preparation of compound **16**), FCC [toluene–ethyl acetate, (90:10)] gave the disaccharide derivative **17** (10 mg, 38%).

3.9. Dec-9-enyl β-D-galactopyranosyl-(1→3)-6-*O*-phosphonato-β-D-galactopyranoside, bis-triethylammonium salt (6)

9-Fluorenemethyl H-phosphonate **8** was prepared by evaporation of pyridine–Et_3N (8:2) (3 × 3 mL) from 9-fluorenemethyl H-phosphonic acid (6.5 mg, 0.025 mmol). The disaccharide derivative **17** (23 mg, 0.021 mmol) was then added to the residue and the mixture was dried by evaporation of pyridine (3 × 3 mL) therefrom. The residue was dissolved in pyridine (1 mL), pivaloyl chloride (0.011 mL, 0.089 mmol) was added and the mixture was stirred at room temperature for 40 min, whereafter a freshly prepared solution of iodine (12 mg, 0.047 mmol) in 95% aq pyridine (1 mL) was added. After 30 min, the mixture was diluted with CH_2Cl_2 and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$ and cold 0.5 M TEA hydrogen carbonate, dried by filtration through cotton wool and concentrated. A solution of the residue in toluene–ethyl acetate (7:3) was passed through a silica gel pad, using, first, the same solvent and then CH_2Cl_2–MeOH–Et_3N (89:10:1) for elution. The latter fraction was concentrated to produce disaccharide phosphodiester **18** [δ_H (inter alia) 4.05 (1H, dd, $J_{5',6a'}$ 7.8, $J_{6a',6b'}$ 11.1, 6′-H^a), 4.15–4.30 (4H, m, 1-H and CH_2–CH of 9-fluorenemethyl), 4.53 (1H, dd, $J_{5',6b'}$ 5.7, 6′-H^b), 4.74 (1H, d, $J_{1',2'}$ 7.7, 1′-H), 4.85 (1H, br d, $^3J_{H,H}$ 10.2, CH=*H*CH), 4.89 (1H, br d, $^3J_{H,H}$ 17.0, CH=HC*H*), 5.30 (1H, dd, $J_{3',4'}$ 3.3, 3′-H), 5.43 (1H, dd, $J_{2',3'}$ 10.5, 2′-H), 5.45 (1H, dd, $J_{1,2}$ 7.1, $J_{2,3}$ 9.9, 2-H) and 5.63–5.78 (3H, m, H-4, -4′ and C*H*=CH_2); δ_C (inter alia) 8.33 ($MeCH_2$), 25.67, 28.82, 28.94, 29.14, 29.27, 29.66 and 33.71 (CCH_2C), 45.26 (MeCH_2), 48.32 (d, $^3J_{C,P}$ 8.9, OCH_2CH of 9-fluorenemethyl), 61.16 (C-6′), 64.34 (d, $^2J_{C,P}$ 3.7, C-6), 67.20 (br, OCH_2CH of 9-fluorenemethyl),

67.49 (C-4′), 69.54 (C-2′), 69.97 (OCH_2 of dec-9-enyl), 70.24 (C-4), 70.76 (C-5′), 71.40 (C-2), 71.50 (C-3′), 72.86 (d, $^3J_{C,P}$ 7.6, C-5), 77.87 (C-3), 101.22 (2C, C-1 and -1′), 114.0 (=CH_2) and 139.21 (–CH=); δ_P −0.83]. The residue was dissolved in CH_2Cl_2 (1 mL), piperidine (0.2 mL) was added and the mixture was stirred at room temperature for 1 h, prior to being diluted with CH_2Cl_2 and washed successively with cold 0.1 M HCl and cold 1 M TEA hydrogen carbonate. The organic layer was dried by filtration through cotton wool and concentrated, thus providing crude phosphomonoester **19** [δ_C (inter alia) 61.20 (C-6′), 63.42 (br, C-6), 67.53 (C-4′), 69.59 (C-2′), 69.87 (OCH_2), 70.11 (C-4), 70.75 (C-5′), 71.61 (2C, C-2 and -3′), 73.22 (d, $^3J_{C,P}$ 7.6, C-5), 77.40 (C-3), 100.91 (C-1), 101.27 (C-1′), 114.0 (=CH_2) and 139.20 (–CH=); δ_P 2.07]. The residue was dissolved in 0.5 M NaOMe in MeOH–THF (1:1, 1 mL) and the mixture was stirred at room temperature. After 22 h, the solution was deionised with Dowex 50W-X4 (H^+) resin, filtered and neutralised with Et_3N. After concentration, water (5 × 5 mL) was evaporated off from the residue to remove methyl benzoate. The residue was then dissolved in water (20 mL) and the solution was washed successively with CH_2Cl_2 (2 × 10 mL) and diethyl ether (2 × 10 mL) to remove dibenzofulvene and its piperidine adduct (formed at the P-deprotection step). The aqueous layer was concentrated to give the disaccharide phosphate **6** (12 mg, 76% from **17**) as an amorphous solid; $[\alpha]_D^{20}$ +23 (*c* 0.1, water–MeOH, 9:1); R_f 0.65 [$CHCl_3$–MeOH–water, (10:10:3)]; δ_H (D_2O) (inter alia) 1.00–1.30 (28H, m, 5 × CH_2 and 6 × *Me*CH_2), 1.59 (2H, m, OCH_2CH_2), 2.01 (2H, m, CH_2CH_2CH=), 3.16 (12H, q, *J* 7.2, 6 × $MeCH_2$), 4.44 (1H, d, $J_{1,2}$ 7.5, 1-H), 4.58 (1H, d, $J_{1',2'}$ 6.5, 1′-H), 4.93 (1H, br d, $^3J_{H,H}$ 10.2, CH=*H*CH), 5.01 (1H, br d, $^3J_{H,H}$ 16.9, CH=HC*H*) and 5.88 (1H, ddt, J_{H,CH_2} 6.7, C*H*=CH_2); δ_C (D_2O) 9.31 (*Me*CH_2), 26.11, 29.25, 29.36, 29.61, 29.83, 30.91 and 34.23 (CCH_2C), 47.74 (Me*C*H_2), 62.02 (C-6′), 64.53 (d, $^2J_{C,P}$ 4.0, C-6), 68.99 (C-4), 69.68 (C-4′), 70.94 (C-2), 71.76 (OCH_2), 72.13 (C-2′), 73.62 (C-3′), 74.25 (d, $^3J_{C,P}$ 7.9, C-5), 76.15 (C-5′), 83.43 (C-3), 103.47 (C-1), 105.47 (C-1′), 115.00 (=CH_2) and 141.49 (–CH=); δ_P (D_2O) 3.34; ES-MS (−): *m*/*z* 559.21 (100%) $[M-2Et_3N-H]^-$ (calcd for $C_{34}H_{71}N_2O_{14}P$: *M*, 762.46).

3.10. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (20)

Anhydrous dimethylamine (4 mL, 60 mmol) was added to a solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (5 g, 12.81 mmol) in acetonitrile (50 mL) at −20 °C and the mixture was kept at room temperature. After 50–60 min, the mixture was concentrated and toluene was evaporated off from the residue. The residue was dissolved in CH_2Cl_2 (70 mL) and the solution was washed with water (3 × 20 mL), dried ($MgSO_4$) and concentrated (thus forming 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose, 4.5 g). The residue was dissolved in CH_2Cl_2 (40 mL), the solution was cooled (0 °C) and Cl_3CCN (45 mL, 449 mmol) was added under argon followed by DBU (1.9 mL, 12.7 mmol). The mixture was stirred for 2 h at 0 °C and was then concentrated. FCC [toluene–ethyl acetate, (100:0)→(85:15)] of the residue gave trichloroacetimidate **20** (4.57 g, 72%); mp 126–127 °C (from ether–hexane); $[\alpha]_D^{25}$ +124 (*c* 1, $CHCl_3$); {lit.,[32] mp 122–124 °C (from benzene–hexane); $[\alpha]_D$ +115.5}; δ_H 2.02, 2.03, 2.04 and 2.18 (12H, 4 × s, 4 × Ac), 4.09 (1H, dd, $J_{6a,6b}$ 11.3, 6-H^a), 4.18 (1H, dd, $J_{5,6b}$ 6.6, 6-H^b), 4.45 (1H, ddd, $J_{5,6a}$ 6.8, 5-H), 5.37 (1H, dd, $J_{2,3}$ 10.8, 2-H), 5.44 (1H, dd, $J_{3,4}$ 3.0, 3-H), 5.58 (1H, dd, $J_{4,5}$ 1.8, 4-H), 6.61 (1H, d, $J_{1,2}$ 3.3, 1-H) and 8.69 (1H, s, NH); δ_C 20.50 and 20.58 (CH_3), 61.20 (C-6), 66.85 (C-2), 67.32 (C-4), 67.45 (C-3), 68.94 (C-5), 90.72 (CCl_3), 93.48 (C-1), 160.88 (C=NH) and 169.91–170.23 (C=O); ES-MS (+): *m*/*z* 514.00 (100%) $[M+Na]^+$ (calcd for $C_{16}H_{20}Cl_3NO_{10}$: *M*, 491.02).

3.11. 2-(Trimethylsilyl)ethyl 2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (22)

A solution of compound **21**[33] (2.17 g, 2.62 mmol) in a mixture of glacial acetic acid and ethanol (1:1, 60 mL) containing 20% $Pd(OH)_2$/C (3 g) was stirred under a mild overpressure of hydrogen at room temperature for 24 h. More catalyst (0.5 g) was added and stirring was continued for another 4 h. The mixture was filtered through a Celite pad and the filtrate was concentrated. FCC (toluene–ethyl acetate, 95:5) of the residue gave the monohydroxy derivative **22** (1.81 g, 95%); mp 146–148 °C (from petroleum ether, 60–80 °C); $[\alpha]_D^{24}$ +11.5 (*c* 1.02, $CHCl_3$); δ_H 0.00 (9H, s, Me_3Si), 0.97 (2H, m, CH_2SiMe_3), 1.09 (9H, s, Me_3C), 3.65 (1H, dt, $^2J_{H,H}$ 10.0, $^3J_{H,H}$ 6.7, O*H*CHCH_2), 3.85–4.00 (3H, m, 5-H and 6-H_2), 4.08 (1H, dt, $^3J_{H,H}$ 5.7, OHC*H*CH_2), 4.21 (1H, dd, $J_{3,4}$ 3.5, 3-H), 4.74 (1H, d, $J_{1,2}$ 7.9, 1-H), 5.37 (1H, dd, $J_{2,3}$ 10.0, 2-H), 5.88 (1H, d, 4-H) and 7.15–8.22 (20H, m, 4 × Ph); δ_C −1.50 (Me_3Si), 18.05 (CH_2SiMe_3), 19.01 (Me_3C), 26.65 (*Me*$_3$C), 61.62 (C-6), 67.49 (OCH_2), 70.44 (C-4), 72.36 (C-3), 73.85 (2C, C-2 and -5), 100.68 (C-1), 125.27–130.11 and 132.74–135.54 (Ph), and 166.56 and 166.59 (C=O). Anal. Calcd for $C_{41}H_{50}O_8Si_2$: C, 67.74; H, 6.93. Found: C, 67.30; H, 7.04.

3.12. 2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (23)

To a stirred and cooled (−40 °C) solution of galactosyl trichloroacetimidate **20** (763 mg, 1.55 mmol) and acceptor **22** (562 mg, 0.77 mmol) in dry dichloromethane (15 mL) containing freshly activated molecular sieves 4 Å (1 g) under argon was added TMS triflate (0.074 mL,

0.39 mmol), whereafter the stirring was continued at −40 °C for 2 h. The temperature was allowed to rise and the mixture was stirred at room temperature for a further 16 h. *N*,*N*-Diisopropylethylamine (0.12 mL, 0.7 mmol) was then added, the solids were filtered off through a Celite pad and the solution was concentrated. FCC [toluene–ethyl acetate, (100:0)→(80:20)] of the residue provided the β-linked disaccharide **23** (650 mg, 80%) as an amorphous solid; $[\alpha]_D^{25}$ +24.6 (*c* 1.06, $CHCl_3$); δ_H 0.00 (9H, s, Me_3Si), 1.00 (2H, m, CH_2SiMe_3), 1.14 (9H, s, Me_3C), 1.60, 1.94, 2.13 and 2.14 (12H, 4 × s, 4 × Ac), 3.65 (1H, dt, $^2J_{H,H}$ 10.0, $^3J_{H,H}$ 6.8, O*H*CHCH$_2$), 3.83–3.98 (4H, m, 5-, 5′-H and 6-H_2), 4.03–4.18 (2H, m, 6′-H^a and OHC*H*CH$_2$), 4.25 (1H, dd, $J_{3,4}$ 3.5, 3-H), 4.28 (1H, dd, $J_{5',6b'}$ 5.9, $J_{6a',6b'}$ 11.2, 6′-H^b), 4.70 (1H, d, $J_{1',2'}$ 7.8, 1′-H), 4.73 (1H, d, $J_{1,2}$ 8.0, 1-H), 4.80 (1H, dd, $J_{3',4'}$ 3.3, 3′-H), 5.06 (1H, dd, $J_{2',3'}$ 10.4, 2′-H), 5.34 (1H, d, 4′-H), 5.68 (1H, dd, $J_{2,3}$ 9.9, 2-H), 5.83 (1H, d, 4-H), and 7.25–8.20 (20H, m, 4 × Ph). Anal. Calcd for $C_{55}H_{68}O_{17}Si_2$: C, 62.48; H, 6.48. Found: C, 62.20; H, 6.69.

3.13. 2-(Trimethylsilyl)ethyl β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (24)

To a stirred and cooled (0 °C) solution of the disaccharide derivative **23** (400 mg, 0.378 mmol) in MeOH (60 mL) was added 8% methanolic solution of $Mg(OMe)_2$ (3.75 mL). The mixture was stirred at 0 °C for 1.5 h, whereafter it was deionised with Dowex 50W-X4 (H^+) resin, filtered and concentrated. FCC [MeOH–dichloromethane, (100:0)→(90:10)] of the residue gave the deacetylated derivative **24** (253 mg, 75%) as an amorphous solid; $[\alpha]_D^{25}$ +37.1 (*c* 1, $CHCl_3$); δ_H 0.00 (9H, s, Me_3Si), 1.00 (2H, m, CH_2SiMe_3), 1.11 (9H, s, Me_3C), 3.43 (1H, dd, $J_{3',4'}$ 3.2, 3′-H), 3.50 (1H, m, 5′-H), 3.54 (1H, dd, $J_{2',3'}$ 9.6, 2′-H), 3.66 (1H, dt, $^2J_{H,H}$ 10.0, $^3J_{H,H}$ 6.8, O*H*CHCH$_2$), 3.81–3.99 (6H, m, 4′-, 5-H, 6-H_2 and 6′-H_2), 4.10 (1H, m, OHC*H*CH$_2$), 4.17 (1H, dd, $J_{3,4}$ 3.4, 3-H), 4.41 (1H, d, $J_{1',2'}$ 7.4, 1′-H), 4.80 (1H, d, $J_{1,2}$ 8.0, 1-H), 5.54 (1H, dd, $J_{2,3}$ 9.8, 2-H), 6.06 (1H, d, 4-H) and 7.25–8.20 (20H, m, 4 × Ph); δ_C −1.50 (Me_3Si), 18.01 (*C*H$_2$Si), 19.03 (Me_3C), 26.69 (*Me*$_3$C), 61.70 (C-6′), 63.49 (C-6), 67.41 (OCH$_2$), 70.33 (2C, C-4 and -4′), 71.08 (C-2′), 71.98 (C-2), 72.92 (C-3′), 73.69 (C-5′), 74.02 (C-5), 79.33 (C-3), 100.60 (C-1), 104.31 (C-1′), 127.63–130.16 and 132.78–135.52 (Ph) and 166.01 and 166.41 (C═O). Anal. Calcd for $C_{47}H_{60}O_{13}Si_2 \cdot H_2O$: C, 62.23; H, 6.89. Found: C, 62.22; H, 6.86.

3.14. 2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (25)

(A) Benzoyl chloride (0.1 mL, 0.86 mmol) was added to a stirred and cooled (0 °C) solution of the disaccharide derivative **24** (38 mg, 0.043 mmol) in pyridine (1 mL) and the mixture was allowed to warm to room temperature, whereafter it was kept for 5 h. The mixture was then diluted with CH_2Cl_2, and the solution was washed successively with saturated aq $NaHCO_3$ and water, dried by filtration through cotton wool and concentrated. Toluene was evaporated off from the residue to remove traces of pyridine. FCC [toluene–ethyl acetate, (95:5)] of the residue gave benzoylated disaccharide **25** (51 mg, 91%) as an amorphous solid; $[\alpha]_D^{20}$ +84.8 (*c* 1.03, $CHCl_3$); δ_H 0.00 (9H, s, Me_3Si), 0.97 (2H, m, CH_2SiMe_3), 1.18 (9H, s, Me_3C), 3.64 (1H, dt, $^2J_{H,H}$ 10.0, $^3J_{H,H}$ 7.0, O*H*CHCH$_2$), 3.93–4.05 (3H, m, 5-H and 6-H_2), 4.12 (1H, dt, $^3J_{H,H}$ 6.1, OHC*H*CH$_2$), 4.39 (1H, m, 5′-H), 4.41 (1H, dd, $J_{3,4}$ 3.4, 3-H), 4.49 (1H, dd, $J_{5',6a'}$ 7.3, $J_{6a',6b'}$ 11.0, 6′-H^a), 4.73 (1H, d, $J_{1,2}$ 8.0, 1-H), 4.90 (1H, dd, $J_{5',6b'}$ 6.1, 6′-H^b), 5.13 (1H, d, $J_{1',2'}$ 8.0, 1′-H), 5.50 (1H, dd, $J_{3',4'}$ 3.3, 3′-H), 5.67 (1H, dd, $J_{2,3}$ 10.0, 2-H), 5.72 (1H, dd, $J_{2',3'}$ 10.5, 2′-H), 6.02 (1H, d, 4′-H), 6.13 (1H, d, 4-H) and 7.15–8.29 (40H, m, 8 × Ph); δ_C −1.50 (Me_3Si), 18.05 (*C*H$_2$SiMe$_3$), 19.01 (Me_3C), 26.65 (*Me*$_3$C), 61.57 (C-6′), 62.30 (C-6), 67.13 (OCH$_2$), 67.53 (C-4′), 69.62 (C-2′), 70.27 (C-4), 70.97 (C-5′), 71.56 (C-3′), 71.67 (C-2), 74.68 (C-5), 77.15 (C-3), 100.92 (C-1), 101.28 (C-1′), 127.58–130.18 and 132.51–135.56 (Ph) and 164.40–165.97 (C═O). Anal. Calcd for $C_{75}H_{76}O_{17}Si_2$: C, 69.00; H, 5.87. Found: C, 68.73; H, 6.08.

(B) To a stirred and cooled (−40 °C) solution of galactosyl trichloroacetimidate **13**[20] (76 mg, 0.103 mmol) and acceptor **22** (62 mg, 0.085 mmol) in dry dichloromethane (1 mL) containing freshly activated molecular sieves 4 Å (200 mg) under argon was added a 2% (v/v) solution of TMS triflate in the same solvent (0.045 mL, 0.005 mmol). The temperature was allowed to rise to −30 °C and the stirring was continued for 2 h, whereafter the mixture was warmed up to 0 °C and stirred for a further 3 h. *N*,*N*-Diisopropylethylamine (0.1 mL, 0.58 mmol) was then added, the solids were filtered off through a Celite pad and the solution was concentrated. FCC [petroleum ether (40–60 °C)–ethyl acetate, (95:5)→(80:20)] of the residue provided first galactoside **22** (24 mg, 38% recovery). Continued elution gave the disaccharide orthoester **26** (37 mg, 33%) as an amorphous solid; δ_H 0.00 (9H, s, Me_3Si), 1.00 (2H, m, CH_2SiMe_3), 1.20 (9H, s, Me_3C), 3.60 (1H, dt, $^2J_{H,H}$ 10.1, $^3J_{H,H}$ 7.0, O*H*CHCH$_2$), 3.77–3.90 (3H, m, 5-H and 6-H_2), 4.05 (1H, dt, $^3J_{H,H}$ 6.5, OHC*H*CH$_2$), 4.11 (1H, dd, $J_{2,3}$ 10.0, 3-H), 4.41–4.51 (2H, m, 5′-H and 6′-H^a), 4.58–4.70 (3H, m, 1-, 2′-H and 6′-H^b), 5.54 (1H, dd, $J_{2',3'}$ 5.0, 3′-H), 5.60 (1H, dd, $J_{1,2}$ 8.1, 2-H), 5.82 (1H, dd, $J_{3',4'}$ 4.4, $J_{4',5'}$ 2.9, 4′-H), 5.92 (1H, d, $J_{3,4}$ 3.0, 4-H), 6.43 (1H, d, $J_{1',2'}$ 4.6, 1′-H) and 7.20–8.25 (40H, m, 8 × Ph); δ_C −1.59 (Me_3Si), 18.00 (*C*H$_2$SiMe$_3$), 19.09 (Me_3C), 26.71 (*Me*$_3$C), 61.68 (C-6), 62.20 (C-6′), 66.16 (C-4′), 67.27 (OCH$_2$), 68.75

(C-5′), 68.96 (C-4), 69.97 (C-3′), 71.02 (C-2), 72.77 (C-2′), 73.95 (C-3), 74.03 (C-5), 98.32 (C-1′), 100.88 (C-1), 121.16 (orthoester quaternary C), 126.47 and 135.84 (orthoester Ph), 127.65–130.05 and 132.85–135.54 (rest of Ph), and 164.95–166.64 (C=O). Also isolated was the required β-linked disaccharide derivative **25** (32 mg, 29%). The prepared orthoester **26** (37 mg, 0.028 mmol) was combined with compound **22** (24 mg, 0.033 mmol) in dry dichloromethane (1 mL), and freshly activated molecular sieves 4 Å (100 mg) were added to the solution. The mixture was cooled (−10 °C) and a 2% (v/v) solution of TMS triflate in the same solvent (0.027 mL, 0.003 mmol) was added under argon, whereafter the mixture was stirred at room temperature for 72 h. After standard work-up (as above), FCC [toluene–ethyl acetate, (99:1)→(90:10)] produced additional portion of the disaccharide derivative **25** (19 mg; total yield 51 mg, 46% from acceptor **22**).

3.15. 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-galactopyranosyl trichloroacetimidate (28) and 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (29)

To a stirred and cooled (0 °C) solution of compound **25** (102 mg, 0.078 mmol) in CH_2Cl_2 (1 mL) was added TFA (2 mL). The mixture was left for 55 min, whereafter a mixture of toluene (16 mL) and ethyl acetate (8 mL) was added and the solution was concentrated. Toluene was evaporated off from the residue 3 times (to remove traces of TFA) to give the hemiacetal derivative **27** [δ_H (inter alia) 0.93 (9H, s, Me_3C), 3.64 (dd, $J_{5,6a}$ 6.7, $J_{6a,6b}$ 10.3, 6-H^a), 3.75 (dd, $J_{5,6b}$ 6.3, 6-H^b), 4.23–4.40 (m, 5-, 5′-H and 6′-H^a), 4.59 (dd, $J_{3,4}$ 3.3, 3-H), 4.68 (dd, $J_{5',6b'}$ 6.4, $J_{6a',6b'}$ 11.0, 6′-H^b), 5.08 (d, $J_{1',2'}$ 7.7, 1′-H), 5.28 (dd, $J_{2,3}$ 10.4, 2-H), 5.37 (dd, $J_{3',4'}$ 3.2, 3′-H), 5.48 (d, $J_{1,2}$ 3.6, 1-H), 5.53 (dd, $J_{2',3'}$ 10.5, 2′-H), 5.86 (d, 4′-H), 6.02 (d, 4-H) and 6.79–8.11 (40H, m, 8 × Ph); β-anomer components: 5.01 (d, $J_{1',2'}$ 7.7, 1′-H), 6.05 (d, $J_{3,4}$ 4.4, 4-H)]. The residue was then dissolved in CH_2Cl_2 (2 mL), the solution was cooled (0 °C) and trichloroacetonitrile (0.27 mL, 2.69 mmol) was added under argon followed by DBU (0.012 mL, 0.08 mmol). The mixture was stirred for 3 h at 0 °C before a second portion of DBU (0.012 mL, 0.08 mmol) was added. The mixture was kept at room temperature for 16 h and then concentrated. FCC [toluene–ethyl acetate, (95:5)] of the residue produced disaccharide trichloroacetimidate **28** (73 mg, 69%) as an amorphous solid; $[\alpha]_D^{20}$ +90.0 (*c* 1.04, $CHCl_3$); δ_H 0.93 (9H, s, Me_3C), 3.68 (1H, dd, $J_{5,6a}$ 6.7, $J_{6a,6b}$ 10.5, 6-H^a), 3.78 (1H, dd, $J_{5,6b}$ 6.0, 6-H^b), 4.28–4.40 (3H, m, 5-, 5′-H and 6′-H^a), 4.55 (1H, dd, $J_{3,4}$ 3.3, 3-H), 4.71 (1H, dd, $J_{5',6b'}$ 5.6, $J_{6a',6b'}$ 10.3, 6′-H^b), 5.08 (1H, d, $J_{1',2'}$ 7.7, 1′-H), 5.38 (1H, dd, $J_{3',4'}$ 3.2, 3′-H), 5.53 (1H, dd, $J_{2',3'}$ 10.5, 2′-H), 5.56 (1H, dd, $J_{2,3}$ 10.3, 2-H), 5.86 (1H, d, 4′-H), 6.14 (1H, d, 4-H), 6.64 (1H, d, $J_{1,2}$ 3.8, 1-H), 6.81–8.08 (40H, m, 8 × Ph) and 8.41 (1H, s, NH); δ_C 19.10 (Me_3C), 26.63 (Me_3C), 61.91 (C-6), 62.13 (C-6′), 67.68 (C-4′), 69.56 (C-2), 69.75 (C-2′), 70.40 (C-4), 71.17 (C-5′), 71.54 (C-3′), 72.89 (C-5), 73.78 (C-3), 90.97 (CCl_3), 93.79 (C-1), 101.54 (C-1′), 127.58–130.21 and 132.85–135.59 (Ph), 160.42 (C=NH) and 164.46–166.02 (C=O). Further elution gave the 6-OH trichloroacetimidate derivative **29** (27 mg, 31%) as an amorphous solid; δ_H 4.25–4.38 (3H, m, 5′-H, 6-H^a and 6′-H^a), 4.49–4.56 (2H, m, 3-H and 6-H^b), 4.60–4.74 (2H, m, 5-H and 6′-H^b), 5.07 (1H, d, $J_{1',2'}$ 7.7, 1′-H), 5.39 (1H, dd, $J_{3',4'}$ 3.3, 3′-H), 5.52 (1H, dd, $J_{2',3'}$ 10.5, 2′-H), 5.66 (1H, dd, $J_{2,3}$ 10.3, 2-H), 5.84 (1H, d, 4′-H), 6.06 (1H, d, $J_{3,4}$ 3.2, 4-H), 6.66 (1H, d, $J_{1,2}$ 3.8, 1-H), 6.85–8.15 (30H, m, 6 × Ph) and 8.45 (1H, s, NH).

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References

1. Part 15: Higson, A. P.; Ross, A. J.; Tsvetkov, Y. E.; Routier, F. H.; Sizova, O. V.; Ferguson, M. A. J.; Nikolaev, A. V. *Chem. Eur. J.* **2005**, *11*, 2019–2030.
2. McConville, M. J.; Ferguson, M. A. J. *Biochem. J.* **1993**, *294*, 305–324.
3. Turco, S. J.; Orlandi, P. A.; Homans, S. W.; Ferguson, M. A. J.; Dwek, R. A.; Rademacher, T. W. *J. Biol. Chem.* **1989**, *264*, 6711–6715.
4. McConville, M. J.; Thomas-Oates, J. E.; Ferguson, M. A. J.; Homans, S. W. *J. Biol. Chem.* **1990**, *265*, 19611–19623.
5. Thomas, J. R.; McConville, M. J.; Thomas-Oates, J. E.; Homans, S. W.; Ferguson, M. A. J.; Gorin, P. A. J.; Greis, K. D.; Turco, S. J. *J. Biol. Chem.* **1992**, *267*, 6829–6833.
6. Ilg, T.; Etges, R.; Overath, P.; McConville, M. J.; Thomas-Oates, J. E.; Thomas, J. R.; Homans, S. W.; Ferguson, M. A. J. *J. Biol. Chem.* **1992**, *267*, 6834–6840.
7. McConville, M. J.; Schnur, L. F.; Jaffe, C.; Schneider, P. *Biochem. J.* **1995**, *310*, 807–818.
8. Mahoney, A. B.; Sacks, D. L.; Saraiva, E.; Modi, G.; Turco, S. J. *Biochemistry* **1999**, *38*, 9813–9823.
9. McConville, M. J.; Blackwell, J. M. *J. Biol. Chem.* **1991**, *266*, 23670–23675.
10. Bahr, V.; Stierhof, Y.-D.; Ilg, T.; Demar, M.; Quinten, M.; Overath, P. *Mol. Biochem. Parasitol.* **1993**, *58*, 107–122.
11. Moody, S. F.; Handman, E.; McConville, M. J.; Bacic, A. *J. Biol. Chem.* **1993**, *268*, 18457–18466.
12. Ilg, T.; Stierhof, Y. D.; Craik, D.; Simpson, R.; Handman, E.; Bacic, A. *J. Biol. Chem.* **1996**, *271*, 21583–21596.
13. Ilg, T.; Overath, P.; Ferguson, M. A. J.; Rutherford, T.; Campbell, D. G.; McConville, M. J. *J. Biol. Chem.* **1994**, *269*, 24073–24081.

14. Turco, S. J.; Spath, G. F.; Beverly, S. M. *Trends Parasitol.* **2001**, *17*, 223–226.
15. Ilg, T. *Parasitol. Today* **2000**, *16*, 489–497.
16. Carver, M. A.; Turco, S. J. *J. Biol. Chem.* **1991**, *266*, 10974–10981.
17. Carver, M. A.; Turco, S. J. *Arch. Biochem. Biophys.* **1992**, *295*, 309–317.
18. Dobson, D. E.; Scholtes, L. D.; Valdez, K. E.; Sullivan, D. R.; Mengeling, B. J.; Cilmi, S.; Turco, S. J.; Beverley, S. M. *J. Biol. Chem.* **2003**, *278*, 15523–15531.
19. Nikolaev, A. V.; Rutherford, T. J.; Ferguson, M. A. J.; Brimacombe, J. S. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1977–1988.
20. Ivanova, I. A.; Ross, A. J.; Ferguson, M. A. J.; Nikolaev, A. V. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1743–1753.
21. Ross, A. J.; Ivanova, I. A.; Ferguson, M. A. J.; Nikolaev, A. V. *J. Chem. Soc., Perkin Trans. 1* **2001**, 72–81.
22. Yashunsky, D. V.; Tsvetkov, Y. E.; Ferguson, M. A. J.; Nikolaev, A. V. *J. Chem. Soc., Perkin Trans. 1* **2002**, 242–256.
23. Brown, G. M.; Millar, A. R.; Masterson, C.; Brimacombe, J. S.; Nikolaev, A. V.; Ferguson, M. A. J. *Eur. J. Biochem.* **1996**, *242*, 410–416.
24. Routier, F. H.; Higson, A. P.; Ivanova, I. A.; Ross, A. J.; Tsvetkov, Y. E.; Yashunsky, D. V.; Bates, P. A.; Nikolaev, A. V.; Ferguson, M. A. J. *Biochemistry* **2000**, *39*, 8017–8025.
25. Ross, A. J.; Ivanova, I. A.; Higson, A. P.; Nikolaev, A. V. *Tetrahedron Lett.* **2000**, *41*, 2449–2452.
26. Yashunsky, D. V.; Nikolaev, A. V. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1195–1198.
27. Yang, Z. W.; Xu, Z. S.; Shen, N. Z.; Fang, Z. Q. *Nucleosides Nucleotides* **1995**, *14*, 167–173.
28. Kovac, P.; Taylor, R. *Carbohydr. Res.* **1987**, *167*, 153–173.
29. Hasegawa, A.; Adachi, K.; Yoshida, M.; Kiso, M. *Carbohydr. Res.* **1992**, *230*, 273–288.
30. Iversen, T.; Josephson, S.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2379–2385.
31. Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 5629–5647.
32. Amvam-Zollo, P.-H.; Sinay, P. *Carbohydr. Res.* **1986**, *150*, 199–212.
33. Nikolaev, A. V.; Watt, G. M.; Ferguson, M. A. J.; Brimacombe, J. S. *J. Chem. Soc., Perkin Trans. 1* **1997**, 969–979.