Stereoselective synthesis of α-L-Fuc*p*-(1,2)- and -(1,3)-β-D-Gal*p*(1)-4-methylumbelliferone using glycosyl donor substituted by propane-1,3-diyl phosphate as leaving group

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Received (in Cambridge, UK) 6th April 2000, Accepted 2nd June 2000
Published on the Web 27th June 2000

The synthesis of the disaccharides α-L-Fuc*p*-(1,2)- and α-L-Fuc*p*-(1,3)-β-D-Gal*p*(1)-4-methylumbelliferone as fucosidase substrates was accomplished by activation of the anomeric centre of 2,3,4-tri-*O*-acetyl- and 2,3,4,6-tetra-*O*-acetyl-α,β-L-fuco- and -D-galactopyranosyl propane-1,3-diyl phosphates with TMSOTf and with minimal protection of 4-methylumbelliferyl β-D-galactopyranoside.

Introduction

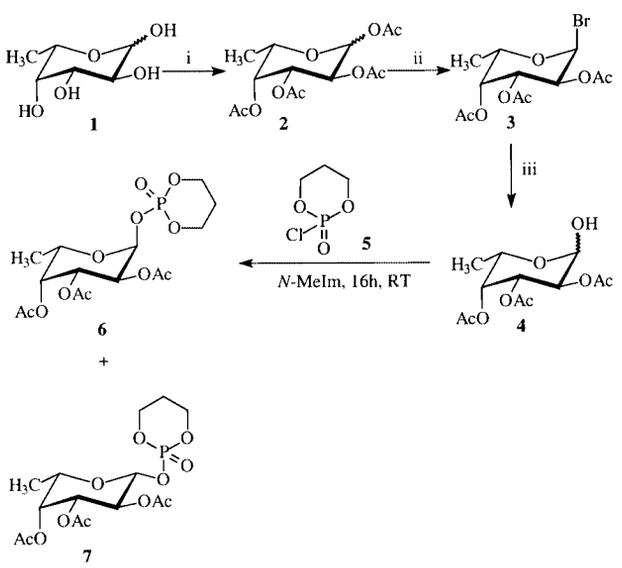
The importance of the role that carbohydrates play in the maintenance of health and the onset of disease has received due recognition during the last two decades. The diversity of these carbohydrate structures can be found in oligosaccharides that are covalently attached to lipids and proteins both at cell surfaces and in biological fluids. The length of these oligosaccharides is normally less than twenty sugar residues, yet the possible changes in configuration, oxidation and/or reduction states, and points of attachment give them a wide range of biological functions. Research in organic chemistry and molecular biology has been stimulated by the desire to attain an understanding of how and why these structures are recognised by enzymes, antibodies and lectins.

The fucose-containing disaccharides, *e.g.* L-Fuc-α-1,3-β-D-Gal*p* and L-Fuc-α-1,2-β-D-Gal*p*, are usually found at the non-reducing terminus of oligosaccharides of the blood-group-specific glycoproteins and glycolipid[1] with the structures L-Fuc-α-1,2-Gal-β-1,4-GlcNAc-β- and the grouping L-Fuc-α-1,3-Gal-β-1,4-Glu both occurring among human milk oligosaccharides.[2] Furthermore in sialyl Lewis X the α-L-fucopyranosyl group plays a pivotal role in binding to E-, L- and P-selectins.[3] As part of our ongoing programme of synthesis of oligosaccharides that occur in human milk and of their analogues as fucosidase substrates, we describe the synthesis of both α-L-Fuc-1,3-β-galactopyranoses, α-L-Fuc-1,2-β-galactopyranoses and also the corresponding disaccharides having a 4-methylumbelliferyl moiety attached at the anomeric centre of D-galactopyranose.

Results and discussion

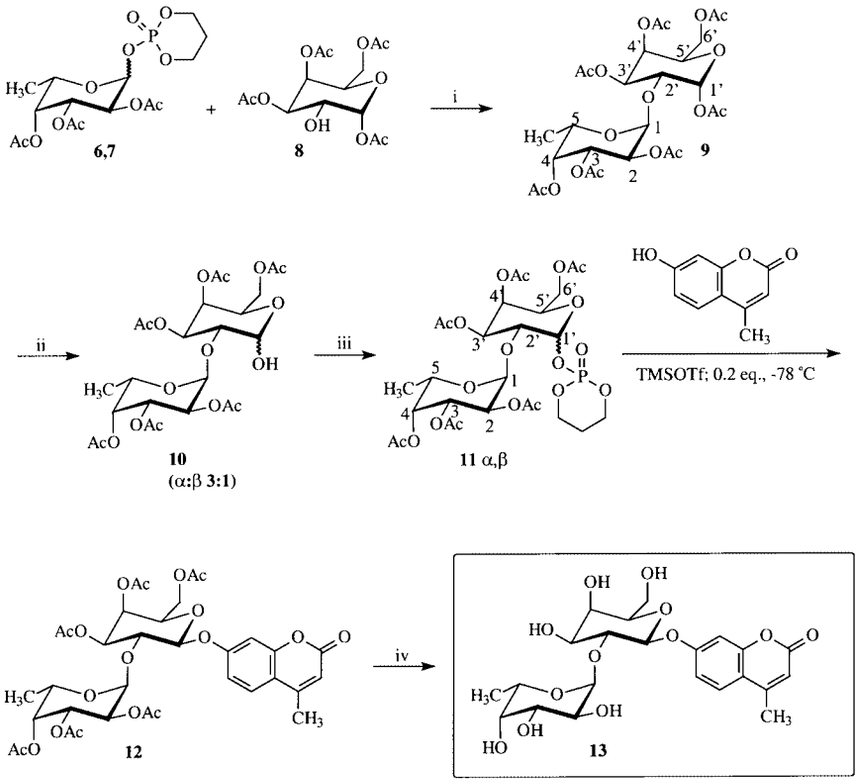
We chose to employ propane-1,3-diyl phosphate as the anomeric activating group for the synthesis of these compounds as we had successfully used it to obtain β-*O*-glycosides.[4] In addition we reasoned that the displacement of this function with 4-methylumbelliferone would provide access to glycosides that are used as convenient substrates for the fluorimetric assay of glycoside hydrolyase activity[5] and as ligands in carbohydrate–protein interaction studies.[6]

Acetylation of L-fucopyranose **1** with acetic anhydride and pyridine, Scheme 1, afforded 1,2,3,4-tetra-*O*-acetyl-α,β-L-fucopyranose **2**, which was converted to the α-L-fucopyranosyl bromide **3**. Oxidation of the latter with silver carbonate in acetone gave the required 2,3,4-tri-*O*-acetyl-α,β-L-fucopyranose **4** in 90% overall yield.[7] Treatment of **4** with propane-1,3-diyldioxyphosphoryl chloride **5**[4d] afforded the phosphates **6** and **7**, as an inseparable crystalline mixture, in 65% yield, in the ratio 9:1 as determined by ^{1}H NMR. For the major isomer **6** a resonance at δ 5.92 was observed as a doublet of doublets (dd) for the anomeric proton and exhibited couplings of 3.3 and 2.6 Hz, whilst for the isomer **7** this resonance occurred at δ 5.35 with couplings of 5.9 and 7.9 Hz. The ^{13}C NMR data were most informative for assignment of the stereochemistry at C-1 of **6** and **7**; in the case for the former the resonance was observed at δ_C 94.63 with a $J_{C\text{-}P}$ of 4.9 Hz whilst for the latter anomer this was found at δ_C 96.71 with $J_{C\text{-}P}$ of 4.4 Hz.



Scheme 1 *Reagents and conditions*: i, Ac_2O, Py, RT, 12 h; ii, 33% HBr–AcOH, RT, 15 min; iii, Ag_2CO_3, acetone, 0.5 h.

1,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose **8** was prepared as reported by Chittenden[8] in a yield of 70%. Having both saccharides available we investigated the coupling of the phosphate **6** and **7** with **8**, Scheme 2, and were gratified that this proceeded uneventfully when we employed trimethylsilyl triflate (TMSOTf) as the activating agent, and gave the peracetylated derivative of disaccharide α-L-Fuc*p*-(1,2)-α-D-Gal*p*, compound **9**, in 63% yield after chromatographic purification on silica gel. The α-stereochemistry for the anomeric centre was assigned on



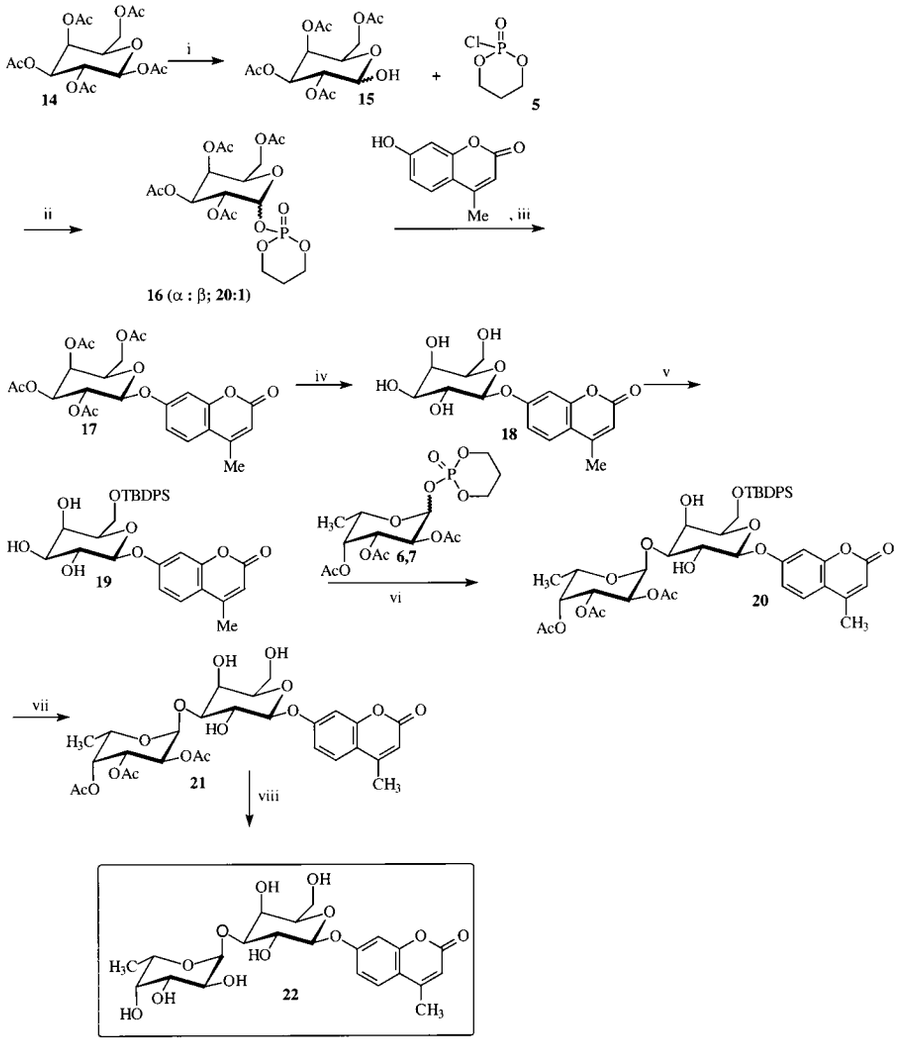
Scheme 2 *Reagents and conditions*: i, 1.5 equiv. TMSOTf, CH_2Cl_2, −78 °C; ii, NH_3(g), MeCN, 0 °C; iii, **5**, *N*-MeIm, 16 h; iv, NaOMe, MeOH, RT, 30 min.

the basis of the observed ^{13}C–^{1}H coupling constant of 165.4 Hz for the newly formed bond.[9] To further establish that the coupling reaction had proceeded with the desired stereochemical outcome we undertook the removal of the acetate protecting groups in **9** with NaOMe in methanol and obtained the corresponding disaccharide which displayed spectral and physical properties that were in agreement with those reported by Lemieux.[10] However, if the coupling of **6** and **7** with **8** was conducted with a catalytic amount of TMSOTf, the formation of the β-isomer of **9** was observed in 46% yield along with recovered starting material (39%). In this case a coupling of 159.6 Hz was observed for the 1,2-linkage. These observations suggest that the disaccharide **9** is the thermodynamic product and is the result of the bond-forming reaction proceeding *via* an oxonium ion intermediate whilst the corresponding β-isomer is formed *via* participation of the C-2 acetoxy function or by an S_N2-like process.

The anomeric acetate group of **9** was selectively removed by employing ammonia gas in acetonitrile[11] and gave the disaccharide **10** in 69% yield after chromatographic purification. Treatment of **10** with propane-1,3-diyldioxyphosphoryl chloride **5** afforded the mixture of anomeric phosphates **11** in 60% yield, in the ratio 20:1 (**11α** major) as determined by ^{1}H NMR. In the ^{31}P NMR spectrum the resonance for **11α** occurred at δ_P −10.5 whilst that for **11β** was found at δ_P −11.5. At this juncture we embarked upon a study of the displacement reaction of the mixture of phosphates **11** with 4-methylumbelliferone, as this function has been used extensively in the fluorimetric assay of glycosides. The propane-1,3-diyl phosphate group was successfully displaced by 4-methylumbelliferone using 0.2 mol equiv. of TMSOTf as the activator and afforded the β-linked disaccharide **12** in 56% yield following chromatography. This result is an improvement on the reported methods for the introduction of the 4-methylumbelliferone group which normally proceed in yields in the order of 14%. Removal of the acetate function of **12** yielded the fully deprotected disaccharide **13**, Scheme 2. The stereochemical integrity of the anomeric centre of **13** was established on the basis of the ^{13}C–^{1}H coupling constant which was determined as 159.2 Hz.

Having had success in the introduction of the 4-methylumbelliferone function we decided to investigate its introduction at the anomeric centre of galactose at an early stage and to study the subsequent coupling reaction with the fucopyranosyl phosphates **6** and **7**, Scheme 3. Hence, selective removal of the anomeric acetate group of **14**[8b] afforded 2,3,4,6-tetra-*O*-acetyl-α,β-D-galactopyranose **15** in 96% yield. Treatment of **15** with propane-1,3-diyldioxyphosphoryl chloride **5** afforded the phosphates **16** as a mixture of separable crystalline α (mp 143–145 °C) and β isomers in the ratio of 20:1, respectively, in a combined yield of 63%. The displacement of the phosphate **16α** was accomplished with 4-methylumbelliferone and gave 4-methylumbelliferyl† 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **17** in 68% yield; the same outcome was observed if the mixture of **16α**,**β** was used. The stereochemistry of the anomeric linkage was assigned on the basis of a ^{13}C–^{1}H coupling constant of 160.2 Hz. Subsequent deacetylation of the galactose derivative **17** with sodium methoxide in methanol cleanly afforded the saccharide **18** in 83% yield. The primary hydroxy group of the latter was protected as the silyl ether on treatment with *tert*-butyldiphenylsilyl chloride (TBDPSCl) and afforded the partially protected 4-methylumbelliferyl β-D-galactopyranoside **19** in 79% yield after chromatographic purification. At this juncture we investigated the chemistry of **19** with the fucopyranosyl phosphates **6** and **7**, employing one mole equivalent of TMSOTf as the activator. This treatment resulted in the formation of the disaccharide **20** in 52% isolated yield along with a trace amount of the corresponding 1,2-linked disaccharide (**23**; see below) as evidenced by TLC; all of our attempts to isolate the 1,2-linked product by chromatography proved unfruitful. That the coupling reaction had proceeded regiospecifically at the C-3 OH function was tentatively assigned on the basis of ^{1}H and ^{13}C NMR data. Removal of the primary silyl ether group afforded **21**, which on treatment gave the fully

† '4-Methylumbelliferyl' refers to 4-methylcoumarin-7-yl.



Scheme 3 *Reagents and conditions*: i, NH_3(gas), MeCN, 0 °C; ii, *N*-MeIm, CH_2Cl_2, 0 °C, 16 h; iii, TMSOTf, 0.2 equiv., −78 °C, 0.5 h; iv, NaOMe, MeOH, RT, 0.5 h; v, TBDPSCl, Im, DMF, RT, 8 h; vi, TMSOTf, 1 equiv., −78 °C, 1 h; vii, Bu_4NF, THF; viii, NaOMe, MeOH.

deprotected α-L-Fuc*p*-1,3-β-D-Gal(1)-4-methylumbelliferone **22** in 59% yield for the two steps, Scheme 3. That the newly formed bond had a 1,3-linkage was strongly supported by the fact that the spectral and physical properties of **22** were different from those of the 1,2-linked isomer **13** that we had prepared earlier. In order to further support these conclusions we undertook the synthesis of both **13** and **22** by employing classical coupling chemistry, Scheme 4. Thus coupling of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide **3** with the partially protected derivative **19** using tetraethylammonium bromide afforded the disaccharides **20** and **23** in yields of 27% and 51%, respectively. Removal of the silyl and acetate functions resulted in the formation of the isomeric fully deprotected disaccharides **22** and **13** which had spectral and physical properties in agreement with the compounds prepared using the propane-1,3-diyl phosphate coupling protocol, Schemes 2 and 3.

Conclusions

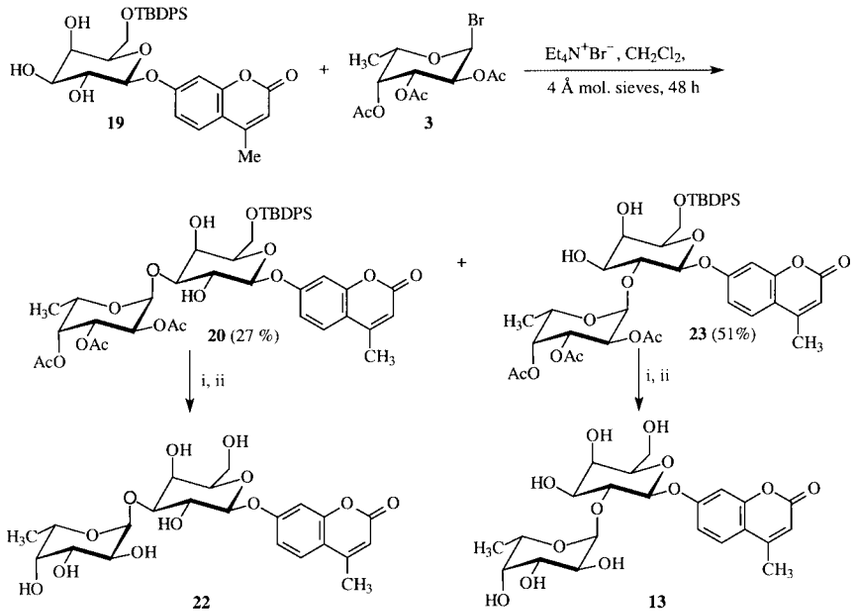
In summary we have established that fucose-containing disaccharides can be prepared using propane-1,3-diyl phosphate activation of the anomeric centre of glycosides in a regio/stereoselective manner.

Experimental

General methods

^{1}H NMR spectra were recorded in $CDCl_3$ (unless stated otherwise) on a JEOL GSX 270 NMR spectrometer and are reported as follows: chemical shifts δ (ppm), [number of protons, multiplicity, coupling constants J (Hz), and assignment]. Residual protic solvent $CHCl_3$ (δ_H 7.26) was used as the internal reference. ^{13}C NMR spectra were recorded in $CDCl_3$, at 67.8 MHz on the JEOL GSX 270 NMR spectrometer, using the central resonance of $CDCl_3$ (δ_C 77.0) as the internal reference. ^{13}P NMR spectra were recorded in $CDCl_3$, at 109.25 MHz on a JEOL GSX 270 NMR spectrometer, using trimethyl phosphate as the external reference. IR spectra were recorded on a UNICAM series FTIR spectrometer. Mass spectra were obtained on an AEI MS 902 or a VG ZAB-E spectrometer. UV-visible spectra were measured on a UNICAM UV/VIS spectrophotometer. Microanalysis were performed by MEDAC Ltd, Surrey. Mps were determined on a GallenKamp capillary melting-point apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solution using a Bellingham & Stanley ADP 220 polarimeter. $[\alpha]_D$-Values are in units of 10^{-1} deg cm^2 g^{-1}. Flash chromatography was carried out using Fluka silica gel 60 (230–400 mesh) unless otherwise indicated. Analytical TLC was performed using precoated aluminium plates (Merck Kiselgel 60 F_{254}) and visualised by UV, phosphomolybdic acid or basic aq. potassium permanganate solutions. 'Petrol' refers to petroleum spirit (distillation range 40–60 °C) which was distilled prior to use, and ether refers to diethyl ether.

All reactions were carried out under an argon or nitrogen atmosphere in oven-dried glassware unless otherwise stated. CH_2Cl_2 and DMF were distilled from calcium hydride and stored over 4 Å molecular sieves. Aqueous solutions are saturated unless otherwise specified.



Scheme 4 *Reagents and conditions*: ii, Bu_4NF, THF; iii, NaOMe, MeOH, RT, 0.5 h.

1,2,3,4-Tetra-*O*-acetyl-α,β-L-fucopyranose 2[7]

A solution of L-fucose **1** (5 g, 30.45 mmol) in dry pyridine (50 ml) containing acetic anhydride (35 ml) was stored at 4 °C for 12 h. Following this water (10 ml) was added and, after 1 h, the solution was extracted with CH_2Cl_2 (100 ml). The combined CH_2Cl_2 layers were back-extracted with 100 ml of cold 1 M H_2SO_4 and then with cold aq. $NaHCO_3$ (100 ml), and finally washed with water (100 ml). The organic phase was dried (Na_2SO_4) and concentrated to give α,β-L-fucopyranosyl tetraacetate **2** as a thick syrup (11.00 g, 100%).

For isomer **2α**, IR (film) ν_{max} 1751, 1371 cm^{-1}; δ_H 1.29 (3H, d, *J* 6.6), 2.00 (3H, s), 2.05 (3H, s), 2.10 (3H, s), 2.18 (3H, s), 4.08 (1H, appt d, *J* 7.3), 4.39 (1H, appt d, *J* 6.6), 5.10–5.16 (1H, m), 5.39 (1H, d, *J* 3.3), 6.29 (1H, d, $J_{1,2}$ 4.6, H-1); δ_C 15.97, 20.44, 20.56, 20.60, 20.64, 67.65, 69.04, 70.19, 83.22, 90.29 (C-1), 169.88, 170.12 (2C), 170.47.

For isomer **2β**, δ_H 1.13 (3H, d, *J* 6.6), 2.09 (3H, s), 2.12 (3H, s), 2.13 (3H, s), 2.19 (3H, s), 3.86–3.92 (1H, m), 4.20–4.34 (1H, m), 5.01–5.07 (1H, m), 5.30 (1H, dd, *J* 3.3, 1.3), 5.43 (1H, d, $J_{1,2}$ 7.3, H-1); δ_C 15.91, 20.41, 20.49, 20.57, 20.90, 64.02, 68.39, 70.84, 82.07, 95.45 (C-1), 170.01, 170.32, 170.65, 170.82.

2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl bromide 3[7]

The mixture of α,β-fucopyranosyl tetraacetates **2** (10 g, 30.1 mmol) was dissolved in glacial acetic acid (20 ml) and 30 ml of 33% HBr–HOAc was added slowly at 20 °C. Stirring was continued for 15 min and the yellow coloured solution was dissolved in CH_2Cl_2 (200 ml), and back-extracted with cold water (2 × 200 ml), cold 1 M aq. $NaHCO_3$ (200 ml) and finally with 200 ml of water. The CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated *in vacuo* to give the α-L-fucopyranosyl bromide **3** as a thick syrup (10.32 g, 97%), δ_H 1.21 (3H, d, *J* 7.3), 2.05 (3H, s), 2.11 (3H, s), 2.18 (3H, s), 4.08 (1H, appt d, *J* 7.3), 4.37 (1H, appt d, *J* 6.6), 5.00 (1H, appt d, *J* 4.0), 5.36 (1H, dd, *J* 3.3, 2.6), 6.68 (1H, d, $J_{1,2}$ 4.0, H-1); δ_C 15.50, 20.49, 20.56, 20.72, 67.84, 68.40, 69.79, 69.98, 89.27, 169.79, 170.12, 170.25.

2,3,4-Tri-*O*-acetyl-α,β-L-fucopyranose 4[7]

The α-L-fucopyranosyl bromide **3** (9.5 g, 26.9 mmol) was dissolved in acetone (50 ml) containing 1.4 ml of water at 0 °C. To the resulting solution was added Ag_2CO_3 (7.4 g, 26.9 mmol) and the mixture was stirred for 0.5 h. The reaction mixture was filtered through a pad of Celite and the filtrate was evaporated to give a thick syrup, which was purified by column chromatography (1 : 1 EtOAc–petrol) and afforded 2,3,4-tri-*O*-acetyl-α,β-L-fucopyranose **4** (7.35 g, 94%).

For isomer **4α**, IR (film) ν_{max} 3357, 1745, 1369 cm^{-1}; δ_H 1.24 (3H, d, *J* 6.6), 2.00 (3H, s), 2.10 (3H, s), 2.17 (3H, s), 4.39 (1H, appt d, *J* 6.6), 5.12 (1H, dd, *J* 4.0, 3.3), 5.31 (1H, dd, *J* 4.0, 1.3), 5.43 (1H, dd, *J* 4.0, 3.3), 5.46 (1H, d, $J_{1,2}$ 3.3, H-1); δ_C 15.86, 20.55, 20.60, 20.75, 64.33, 67.72, 68.45, 71.26, 90.57 (C-1), 170.13, 170.43, 170.66.

For isomer **4β**, δ_H 1.14 (3H, d, *J* 6.6), 2.05 (3H, s), 2.10 (3H, s), 2.18 (3H, s), 3.83 (1H, appt d, *J* 6.6), 4.64 (1H, d, $J_{1,2}$ 7.9, H-1), 5.01 (1H, dd, *J* 5.3, 2.6), 5.25 (1H, d, *J* 1.3), 5.39 (1H, d, *J* 4.0); δ_C 14.09, 20.50, 20.53, 20.93, 60.38, 69.42, 70.26, 70.78, 95.75 (C-1), 170.10, 170.47, 170.57.

2′,3′,4′-Tri-*O*-acetyl-α,β-L-fucopyranosyloxy-1,3,2-dioxaphosphinane 2-oxide 6 and 7

2,3,4-Tri-*O*-acetyl-α,β-L-fucopyranose **4** (5.00 g, 17.2 mmol) was dissolved in CH_2Cl_2 (50 ml) and the solution was cooled to 0 °C. To this solution were added 2-chloro-1,3,2-dioxaphosphinane 2-oxide (5.4 g, 34.39 mmol) and 1-methylimidazole (2.8 g, 34.48 mmol) dropwise over a period of 15 min. Stirring was continued for 16 h, with gradual warming to room temperature. The solvent was removed, the residue was re-dissolved in CH_2Cl_2 (50 ml), and the solution was evaporated to remove traces of 1-methylimidazole. Addition of CH_2Cl_2 (50 ml) to the residue gave a pale yellow solution, which was washed successively with ice–water (50 ml), aq. $NaHCO_3$ (2 × 50 ml) and water (50 ml). The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The oily residue was purified by column chromatography (EtOAc–petrol 1 : 1) to afford the phosphates **6** and **7** (ratio 9 : 1) as an inseparable crystalline mixture (4.68 g, 65%), mp_{mix} 151–153 °C; $[\alpha]_D^{22}$ −100.6 (*c* 3.2, $CHCl_3$).

For isomer **6**, IR (KBr) ν_{max} 1745, 1373, 1216 cm^{-1}; δ_H 1.24 (3H, d, *J* 6.6, CH_3), 1.85–1.89 (1H, m, $J_{P\text{-}H}$ 15.17, H^{ax}-5), 2.02 (3H, s), 2.10 (3H, s), 2.19 (3H, s), 2.30–2.36 (1H, m, $J_{P\text{-}H}$ 15.2, H^{eq}-5), 3.90 (1H, dq, *J* 7.3, 6.6), 4.33–4.59 (4H, m), 5.01 (1H, appt d, *J* 3.3, 3.3), 5.22 (1H, dd, *J* 3.3, 2.6), 5.31–5.33 (1H, m), 5.92 (1H, dd, $J_{1,2}$ 3.3, 2.6, H-1); δ_C 15.82, 20.52, 20.60 (2C), 25.63, 66.92, 67.35, 68.80, 68.86 (2C), 69.75, 70.36, 94.63 (d, $J_{C\text{-}P}$ 4.93, C-1), 170.14 (2C), 170.46; δ_P −10.36.

For isomer **7** δ_H 1.19 (3H, d, *J* 6.6), 1.78–1.83 (1H, m, $J_{P\text{-}H}$ 15.2, H^{ax}-5), 2.02 (3H, s), 2.10 (3H, s), 2.19 (3H, s), 2.23–2.28 (1H, m, $J_{P\text{-}H}$ 15.2, H^{eq}-5), 3.67 (1H, dq, *J* 6.0, 5.9), 4.07–4.31 (2H, m), 4.33–4.59 (4H, m), 5.31–5.33 (1H, m), 5.35 (1H, dd,

$J_{1,2}$ 5.9, 7.9, H-1); δ_C 15.85, 20.49, 20.65, 20.74, 25.79, 66.83, 68.91, 68.97, 69.31, 70.27, 70.74, 96.71 (d, $J_{C\text{-}P}$ 4.41, C-1), 170.41, 170.43, 170.49; δ_P −10.80; *m/z* (CI, NH_3) (Found: M^+, 410.0978. $C_{15}H_{23}O_{11}P$ requires *M*, 410.0978) (Found: C, 43.68; H, 5.58; P, 7.58. $C_{15}H_{23}O_{11}P$ requires C, 43.91; H, 5.65; P, 7.55%).

1,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose 8

The title compound was prepared as detailed by Chittenden.[8] Thus β-D-galactose pentaacetate (5 g, 12.8 mmol) in TFA (17.5 ml) containing water (1.75 ml) was stirred at RT for 5 h and concentrated *in vacuo*. The residue was dissolved in toluene (20 ml), which was subsequently removed *in vacuo*. The residual product was crystallised from Pr^i_2O to give **8** (6.23 g, 70%), mp 149–151 °C; $[\alpha]_D^{20}$ +125.3 (*c* 1.4, $CHCl_3$); IR (film) ν_{max} 3444, 1747, 1373 cm^{-1}; δ_H 2.04 (3H, s), 2.06 (3H, s), 2.15 (3H, s), 2.18 (3H, s), 4.07–4.09 (2H, m), 4.14 (1H, dd, *J* 10.6, 4.0), 4.26 (1H, t, *J* 6.6), 5.15 (1H, appt d, *J* 3.3), 5.44 (1H, dd, *J* 3.3, 1.3), 6.29 (1H, d, $J_{1,2}$ 4.0, H-1); δ_C 20.49, 20.55, 20.67, 20.86, 61.23, 65.87, 67.48, 68.60, 70.27, 91.87 (C-1), 169.49, 170.08, 170.44, 170.88.

1′,3′,4′,6′-Tetra-*O*-acetyl-2′-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-α-D-galactopyranose 9

Propane-1,3-diyl 2,3,4-tri-*O*-acetyl-α,β-L-fucopyranosyl phosphate **6/7** (2.0 g, 4.9 mmol) was dissolved in dry CH_2Cl_2 (20 ml) and the solution was cooled to −78 °C. To the resultant solution was added TMSOTf (1.62 g, 7.3 mmol) and, after 5 min stirring, a solution of glycosyl acceptor **8** (1.69 g, 4.85 mmol) in CH_2Cl_2 (5 ml) was added. The reaction mixture was stirred at −78 °C for 30 min and was then allowed to warm up to 0 °C. The reaction mixture was quenched with aq. $NaHCO_3$ (20 ml) and extracted into CH_2Cl_2 (20 ml). The organic layer was dried over anhydrous Na_2CO_3, concentrated, and the residue was purified by column chromatography (EtOAc–'petrol' 1:1) to give compound **9** (1.93 g, 63%), mp 113–117 °C; $[\alpha]_D^{22}$ +32.85 (*c* 2.8, $CHCl_3$); IR (film) ν_{max} 1751, 1373 cm^{-1}; δ_H 1.19 (3H, d, *J* 6.6), 1.97 (3H, s), 2.00 (3H, s), 2.02 (3H, s), 2.03 (3H, s), 2.04 (3H, s), 2.14 (3H, s), 2.17 (3H, s), 3.78 (1H, dd, *J* 6.6, 5.9), 4.06–4.16 (3H, m), 4.25 (1H, t, *J* 6.6), 4.49 (1H, d, *J* 7.9), 4.94 (1H, dd, *J* 9.9, 3.3), 5.07 (1H, dd, *J* 10.6, 7.9), 5.20 (1H, d, *J* 2.6), 5.28 (1H, dd, *J* 9.2, 3.3), 5.47 (1H, d, $J_{1,2}$ 2.6, H-1), 6.26 (1H, d, $J_{1',2'}$ 4.0, H-1); δ_C 16.12 (CH_3), 20.49, 20.57 (3C), 20.65 (2C), 20.70, 61.09, 67.73, 68.09, 68.38, 68.69, 69.22, 69.90, 71.34, 73.76, 89.77 (C-1′), 101.67 (C-1), 169.22, 169.33, 169.84, 169.94, 170.12, 170.35, 170.58; *m/z* (CI, NH_3) [Found: (M + NH_4), 638.2294. $C_{26}H_{40}NO_{17}$ requires *m/z*, 638.2296].

3,4,6-Tri-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-α,β-D-galactopyranose 10

To solution of ammonia in CH_3CN (25 ml), prepared by bubbling ammonia gas through the solvent at 0 °C (10 min), was added compound **9** (1.5 g, 2.4 mmol) and the mixture was stirred at RT for 24 h. The solvent was then removed *in vacuo* and the residue was purified by column chromatography (EtOAc–'petrol' 1:1) to give a mixture of **10α** and **10β** anomers in the ratio 3:1 (960 mg, 69%), mp 74–78 °C; $[\alpha]_D^{19}$ +24.6 (*c* 2.4, $CHCl_3$).

For isomer **10α** IR (KBr) ν_{max} 3352, 1753 cm^{-1}; δ_H 1.24 (3H, t, *J* 7.3), 1.99 (3H, s), 2.01 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 2.07 (3H, s), 2.18 (3H, s), 3.69 (1H, dd, *J* 7.9, 2.0), 3.79 (1H, t, *J* 7.9), 3.88 (1H, m), 3.98–4.20 (2H, m), 4.66 (1H, d, *J* 7.9), 4.72 (1H, dd, *J* 7.9, 4.6), 5.00 (1H, dd, *J* 7.3, 3.3), 5.08–5.18 (2H, m), 5.21 (1H, d, $J_{1',2'}$ 3.3), 5.30–5.37 (1H, m), 5.39 (1H, d, $J_{1,2}$ 3.3); δ_C 16.02 (CH_3), 20.51, 20.57 (2C), 20.66, 20.70, 20.94, 60.34, 61.46, 67.38, 68.14, 69.05, 69.45, 70.00, 71.03, 71.46, 91.53 (C-1′), 96.22 (C-1), 169.70, 169.81, 170.09, 170.14, 170.61, 170.52.

For isomer **10β**, (selected features) δ_H 1.18 (3H, t, *J* 5.94, CH_3), 4.44 (1H, d, $J_{1,2}$ 6.6, H-1); δ_C 101.86 (C-1).

2-[3′,4′,6′-Tri-*O*-acetyl-2′-*O*-(2″,3″,4″-tri-*O*-acetyl-α-L-fucopyranosyl)-α,β-D-galactopyranosyloxy]-,1,3,2-dioxaphosphinane 2-oxide 11α and 11β

The title compounds were prepared using a similar procedure to that employed for compounds **6** and **7**, by treating compound **10** (1.2 g, 2.1 mmol) with propane-1,3-diyldioxyphosphoryl chloride **5** (0.64 g, 4.0 mmol) in CH_2Cl_2 (15 ml) and 1-methylimidazole (0.34 g, 4.15 mmol) at RT for 16 h. Chromatographic purification of the resulting residue (EtOAc–'petrol' 1:1) gave an inseparable crystalline mixture of **11α** and **11β** (ratio 9:1) (861 mg, 60%), mp 109–112 °C; $[\alpha]_D^{20}$ +36.23 (*c* 2.8, $CHCl_3$).

For isomer **11α**, IR (KBr) ν_{max} 1749, 1214 cm^{-1}; δ_H 1.23 (3H, d, *J* 7.3), 1.78–1.84 (1H, m, $J_{P\text{-}H}$ 15.2, H^{ax}-5), 2.24–2.30 (1H, m, $J_{P\text{-}H}$ 15.2, H^{eq}-5), 1.97 (3H, s), 2.04 (3H, s), 2.05 (6H, s), 2.08 (3H, s), 2.17 (3H, s), 3.80 (1H, appt d, *J* 8.6), 3.92–4.04 (2H, m), 4.07 (1H, dd, *J* 7.3, 3.3), 4.15 (1H, t, *J* 5.3), 4.43–4.54 (4H, m), 4.62 (1H, d, *J* 7.9), 4.96 (1H, dd, *J* 9.2, 2.0), 5.07 (1H, d, *J* 7.3), 5.21–5.38 (2H, m), 5.43 (1H, d, $J_{1'',2''}$ 2.6, H-1″), 5.90 (1H, dd, $J_{1',2'}$ 3.3, H-1′); δ_C 16.00 (CH_3), 20.33, 20.38, 20.42, 20.51, 20.60, 20.79, 25.62, 61.67, 66.64, 67.39, 67.65, 68.65, 68.45 (d, *J* 4.67), 68.79, 68.94, 69.26, 69.97, 71.58, 95.14 (d, *J* 5.46, C-1′), 95.39 (d, *J* 6.23, C-1″), 169.15, 169.68, 169.87, 169.98, 170.21, 171.41; δ_P −10.54.

For isomer **11β**, (selected features) δ_H 1.20 (3H, d, *J* 6.6), 5.82 (1H, dd, $J_{1',2'}$ 6.6, H-1′); δ_C 97.88 (d, *J* 5.71, C-1″); δ_P −11.50; *m/z* (CI, NH_3) [Found: (M + NH_4), 716.1266. $C_{27}H_{43}NO_{19}P$ requires *m/z*, 716.1267] (Found: C, 46.46; H, 5.52; P, 4.65. $C_{27}H_{39}O_{19}P$ requires C, 46.42; H, 5.58; P, 4.44%).

4-Methylumbelliferyl 3′,4′,6′-tri-*O*-acetyl-2′-(2″,3″,4″-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside 12

The title compound was prepared using a similar method to that employed for **9**, by activating phosphates **11αβ** (1 g, 1.4 mmol) with TMSOTf (0.08 g, 0.35 mmol) at −78 °C in CH_2Cl_2 (10 ml). Following the addition of 4-methylumbelliferone (0.25 g, 1.43 mmol) at the same temperature the reaction mixture was stirred for 0.5 h and allowed to warm to 0 °C. The solution was quenched with aq. $NaHCO_3$ (10 ml) and the solvent was removed *in vacuo*. The resultant residue was dissolved in CH_2Cl_2 (10 ml), and the extract was dried over Na_2CO_3, concentrated, and purified by column chromatography (EtOAc–'petrol' 1:1) to afford **12** as a white solid (560 mg, 56%), mp 121–123 °C; $[\alpha]_D^{18}$ +37.2 (*c* 0.8, $CHCl_3$); IR (KBr) ν_{max} 1745, 1614, 1371 cm^{-1}; δ_H 1.15 (3H, d, *J* 6.6), 1.96 (3H, s), 1.98 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 2.07 (3H, s), 2.13 (3H, s), 2.43 (3H, s, CH_3), 3.93 (1H, dd, *J* 7.3, 6.6), 4.07–4.16 (3H, m), 4.24 (1H, dd, *J* 5.3, 4.6), 4.56 (1H, appt d, *J* 7.9), 4.95 (1H, dd, *J* 6.6, 4.0), 5.15 (1H, d, $J_{1'',2''}$ 7.9, H-1″), 5.19 (1H, dd, *J* 7.3, 4.0), 5.34 (1H, d, *J* 3.3), 5.47–5.52 (1H, m), 5.67 (1H, dd, $J_{1',2'}$ 4.0, 3.3, H-1′), 6.20 (1H, s), 7.02 (1H, dd, *J* 7.9, 2.0), 7.12 (1H, d, *J* 4.0), 7.53 (1H, d, *J* 9.2); δ_C 16.00 (CH_3), 18.59 (CH_3), 20.47, 20.47, 20.50, 20.57, 20.60, 20.62, 20.96, 61.25, 67.58, 67.86, 68.44, 69.46, 69.91, 71.20, 72.95, 83.26, 96.04 (C-1′), 103.85 (C-1″), 104.24, 113.04, 114.14, 115.20, 125.74, 152.18, 154.84, 159.12, 168.83, 169.86, 170.04, 170.08, 170.28, 170.52, 170.54; *m/z* (CI, NH_3) [Found: (M + NH_4), 754.2560. $C_{34}H_{44}NO_{18}$ requires *m/z*, 754.2558].

4-Methylumbelliferyl 2′-*O*-(α-L-fucopyranosyl)-β-D-galactopyranoside 13

To a solution of the disaccharide **12** (500 mg, 0.68 mmol) in methanol (50 ml) was added NaOMe (0.055 g, 1 mmol) and the resultant solution was stirred at RT for 0.5 h. The solution was neutralised by passage down a Dowex 50 (H^+) ion-exchange resin. Evaporation of the combined fractions gave the title compound as a white solid (205 mg, 62%), mp 251–253 °C; $[\alpha]_D^{22}$ +24.7 (*c* 0.7, H_2O); IR (KBr) ν_{max} 3480, 1709, 1615, 834 cm^{-1};

λ_{max} (H_2O) 318, 294, 252 nm; δ_H (DMSO-d_6) 1.09 (3H, d, *J* 6.6), 2.39 (3H, s), 3.46–3.69 (3H, m), 3.53 (1H, appt d, *J* 6.0), 3.76 (1H, dd, *J* 10.0, 7.3), 3.86 (1H, br d, *J* 4.0), 3.92 (1H, br d, *J* 2.7), 4.06 (1H, dd, *J* 9.6, 3.3), 4.24 (1H, q, *J* 7.3), 4.34 (1H, dd, *J* 5.3, 3.3), 5.09 (1H, appt d, $J_{1'',2''}$ 8.0, H-1″), 5.70 (1H, d, $J_{1',2'}$ 3.30, H-1′), 6.23 (1H, s), 7.03 (1H, dd, *J* 7.3, 2.0), 7.09 (1H, d, *J* 2.7), 7.67 (1H, d, *J* 9.3); δ_C (DMSO-d_6) 16.52, 18.10, 60.21, 67.82, 68.18, 69.06, 70.62, 71.00, 72.53, 73.12, 73.21, 96.91 (J_{CH} 173.5, C-1″), 101.99 (J_{CH} 159.2, C-1′), 104.16, 111.73, 114.15, 114.21, 126.36, 153.33, 154.32, 159.99, 160.11; *m*/*z* (CI, NH_3) [Found: (M + NH), 502.1925. $C_{22}H_{37}NO_{12}$ requires 502.1924].

2,3,4,6-Tetra-*O*-acetyl-α,β-D-galactopyranose 15

β-D-Galactose pentaacetate **14** (5 g, 12.8 mmol) was added to a solution of ammonia in CH_3CN (200 ml), prepared by bubbling ammonia gas through the solvent at 0 °C (20 min). The mixture was stirred at RT for 24 h. The solvent was removed *in vacuo* and the residue was purified by column chromatography (EtOAc–'petrol' 3:2) to afford tetraacetate **15** (4.32 g, 96%), $[\alpha]_D^{19}$ +31.66 (*c* 1.2, $CHCl_3$).

For α isomer, IR (film) ν_{max} 3417, 1747, 1371 cm^{-1}; δ_H 2.07 (3H, s), 2.16 (6H, s), 2.18 (3H, s), 4.13–4.18 (2H, m), 4.21 (1H, dd, *J* 7.9, 6.6), 5.07 (1H, d, *J* 7.9), 5.34 (1H, d, *J* 4.0), 5.50 (1H, d, *J* 3.3), 6.32 (1H, d, $J_{1,2}$ 3.3, H-1); δ_C 20.52, 20.55, 20.59, 20.70, 61.73, 66.06, 67.23, 68.17, 68.35, 90.55 (C-1), 170.10, 170.28, 170.43, 170.60.

For β isomer, δ_H 1.98 (3H, s), 2.00 (3H, s), 2.03 (3H, s), 2.04 (3H, s), 3.90 (1H, dd, *J* 7.3, 6.6), 4.05–4.11 (2H, m), 4.57 (1H, d, $J_{1,2}$ 7.9, H-1), 4.93 (1H, q, *J* 3.3), 5.29 (1H, d, *J* 3.3), 5.43 (1H, d, *J* 2.64); δ_C 20.44, 20.49, 20.57, 20.65, 60.40, 61.41, 67.12, 70.41, 70.91, 95.85 (C-1), 170.06, 170.20, 170.55, 170.95.

2-(2′,3′,4′,6′-Tetra-*O*-acetyl-α,β-D-galactopyranosyloxy)-1,3,2-dioxaphosphinane 2-oxide 16α and 16β

Treatment of 2,3,4,6-tetra-*O*-acetyl-α,β-D-galactopyranose **15** (5 g, 14.4 mmol) with propane-1,3-diyldioxyphosphoryl chloride (4.5 g, 29.0 mmol) in CH_2Cl_2 (50 ml) and 1-methylimidazole (2.35 g, 28.7 mmol), at RT for 16 h as for the preparation of **6** above, afforded the title compounds in the crude state. Chromatographic separation of the resulting residue using (EtOAc–'petrol' 1:1) gave crystalline oxides **16α**, **16β** in a combined yield of 4.22 g (63%), mp 143–145 °C; $[\alpha]_D^{21}$ +55.5 (*c* 4.0, $CHCl_3$).

For *isomer* **16α**, IR (KBr) ν_{max} 1751, 1214 cm^{-1}; δ_H 1.81–1.90 (1H, m, $J_{P\text{-}H}$ 15.2, H^{ax}-5), 2.02 (3H, s), 2.05 (3H, s), 2.11 (3H, s), 2.17 (3H, s), 2.25–2.38 (1H, m, $J_{P\text{-}H}$ 15.2, H^{eq}-5), 4.04–4.21 (2H, m), 4.37–4.57 (4H, m), 5.26 (1H, t, *J* 4.6), 5.30 (1H, dd, *J* 7.9, 3.3), 5.37 (1H, appt d, *J* 3.3), 5.51 (1H, appt d, *J* 3.3), 5.95 (1H, appt d, $J_{1',2'}$ 3.3 H-1′); δ_C 20.46, 20.52 (2C), 20.64, 25.69 (d, $J_{C\text{-}P}$ 7.27 C-5), 61.24, 66.80 (d, $J_{C\text{-}P}$ 7.79, C-4), 67.21 (C-6), 68.23 (2C), 69.00 (2C), 94.09 (d, $J_{C\text{-}P}$ 4.67, C-1′), 170.02 (2C), 170.05, 170.32; δ_P −10.55 (Found: C, 43.45; H, 5.32; P, 6.88. $C_{17}H_{25}O_{13}P$ requires C, 43.60; H, 5.38; P, 6.61%).

For isomer **16β**, δ_H 5.22 (1H, dd, $J_{1,2}$ 5.3, 3.3, H-1′); δ_C 96.56 (d, $J_{C\text{-}P}$ 4.4, C-1′); δ_P −10.90.

4-Methylumbelliferyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside 17[6d]

Propane-1,3-diyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl phosphate **16α** (3 g, 6.4 mmol) was dissolved in CH_2Cl_2 (30 ml), and the solution was cooled to −78 °C and treated with TMSOTf (0.35 g, 1.6 mmol). To the resultant mixture was added 7-hydroxy-4-methylcoumarin (1.13 g, 6.41 mmol) whilst the temperature was maintained at −78 °C, and the mixture was stirred for an additional 0.5 h. Following this the reaction mixture was warmed to 0 °C, neutralised with aq. $NaHCO_3$ (30 ml), and extracted into CH_2Cl_2 (20 ml), and the extract was dried over Na_2CO_3 and concentrated *in vacuo*. The resultant residue was purified by column chromatography (EtOAc–'petrol' 1:1) to afford **17** (2.25 g, 68%), mp 142–143 °C; $[\alpha]_D^{21}$ −10.0 (*c* 1.3, $CHCl_3$); IR (KBr) ν_{max} 1750, 1614; δ_H 2.03 (3H, s), 2.09 (3H, s), 2.11 (3H, s), 2.20 (3H, s), 2.42 (3H, d, *J* 1.3), 4.08–4.24 (3H, m), 5.16 (1H, d, *J* 6.0), 5.17 (1H, dd, *J* 10.6, 4.0), 5.49 (1H, d, *J* 3.3), 5.51 (1H, dd, *J* 10.6, 7.3), 6.19 (1H, d, *J* 1.3), 6.93 (1H, dd, *J* 8.6, 2.6), 6.99 (1H, d, *J* 2.6), 7.52 (1H, d, *J* 8.6); δ_C 18.48, 20.39, 20.46 (2C), 20.50, 61.31, 66.72, 68.26, 70.53, 71.32, 98.72 ($J_{C\text{-}H}$ 160.18), 103.86, 113.00, 113.72, 115.34, 125.57, 152.08, 154.69, 159.15, 160.60, 169.21, 169.89, 170.04, 170.30; *m*/*z* (CI, NH_3) [Found: (M + Na), 529.1325. $C_{24}H_{26}NaO_{12}$ requires *m*/*z*, 529.1322].

4-Methylumbelliferyl β-D-galactopyranoside 18[6d]

Compound **17** (2 g, 3.95 mmol) was *O*-deacetylated as described for the preparation of **13** above. Recrystallisation from ethanol gave the title compound **18** as a white solid (1.17 g, 83%), mp 261–263 °C; IR (KBr) ν_{max} 3521, 1721, 1614 cm^{-1}; δ_H 2.38 (3H, s), 3.52–3.72 (2H, m), 4.54 (1H, d, *J* 4.6), 4.66 (1H, t, *J* 5.3), 4.90 (1H, d, *J* 6.0), 4.97 (1H, d, *J* 7.9), 5.24 (1H, d, *J* 5.3), 6.21 (1H, s), 6.99–7.02 (2H, m), 7.65 (1H, d, *J* 9.2); δ_C 18.11, 60.44, 68.12, 70.15, 73.24, 75.74, 100.62, 103.17, 111.67, 113.46, 114.03, 126.41, 153.37, 154.44, 160.17, 160.29; *m*/*z* (CI, NH_3) [Found: (M + H), 339.1080. $C_{16}H_{19}O_8$ requires *m*/*z*, 339.1080].

4-Methylumbelliferyl 6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside 19

4-Methylumbelliferyl β-D-galactopyranoside **18** (1 g, 2.9 mmol) was dissolved in DMF (10 ml) and the solution was cooled to 0 °C. To the resultant solution was added, with stirring, *tert*-butyldiphenylsilyl chloride (0.8 g, 2.9 mmol) and imidazole (0.4 g, 5.9 mmol) over a period of 5 min. Stirring was continued for 8 h at RT. The solvent was removed *in vacuo*, the residue was dissolved in dichloromethane (50 ml) and this solution was washed with water (20 ml). The organic layer was dried (Na_2SO_4), and the solvent removed *in vacuo*. Column chromatography (EtOAc–'petrol', 5:1) gave **19** (1.34 g, 79%), $[\alpha]_D^{22}$ −30.4 (*c* 3.0, $CHCl_3$); IR (film) ν_{max} 3413, 1727, 1614 cm^{-1}; δ_H 1.03 (9H, s), 2.30 (3H, s), 3.67–3.76 (2H, m), 3.90 (1H, d, *J* 6.0), 3.94 (2H, s), 4.01 (1H, dd, *J* 7.9, 3.3), 4.91 (1H, d, *J* 7.9), 6.09 (1H, d, *J* 1.3), 6.84 (1H, d, *J* 2.6), 6.95 (1H, dd, *J* 8.6, 2.6), 7.24–7.37 (7H, m), 7.60–7.65 (3H, m), 7.99 (1H, s); δ_C 18.48, 19.02, 26.67 (2C), 31.40, 63.13, 68.76, 71.10, 73.67, 75.21, 100.56 ($J_{C\text{-}H}$ 160.43), 104.24, 112.52, 113.42, 114.77, 125.20, 127.67 (3C), 129.73, 132.76, 132.89, 135.45 (2C), 135.50 (2C), 152.36, 159.83, 160.96, 162.65; *m*/*z* (CI, NH_3) [Found: (M + H), 577.2263. $C_{32}H_{37}O_8Si$ requires *m*/*z*, 577.2257].

4-Methylumbelliferyl 6′-*O*-(*tert*-butyldiphenylsilyl)-3′-*O*-(2″,3″,4″-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside 20

To a solution of 2,3,4-tri-*O*-acetyl-α,β-L-fucopyranosyl propane-1,3-diyl phosphate **6/7** (1 g, 2.4 mmol) in dichloromethane (10 ml) at −78 °C was added TMSOTf (0.54 g, 2,44 mmol). After 2 min, a solution of compound **19** (1.40 g, 2.44 mmol) in dichloromethane (10 ml) was added to the reaction flask. The reaction mixture was stirred at −78 °C for 1 h and was then allowed to warm up to 0 °C before quenching with aq. $NaHCO_3$ (10 ml). The organic layer was dried over (Na_2SO_4), and concentrated *in vacuo*. Column chromatography (EtOAc – 'petrol', 6:4) gave **20** as a white crystalline solid (1.01 g, 52%), mp 109–111 °C; $[\alpha]_D^{22}$ −38.7 (*c* 3.0, $CHCl_3$); IR (KBr) ν_{max} 3457, 1747, 1614 cm^{-1}; δ_H 1.05 (9H, s), 1.17 (3H, d, *J* 6.6), 1.98 (3H, s), 2.04 (3H, s), 2.19 (3H, s), 2.37 (3H, s), 3.70 (1H, dd, *J* 5.9, 3.3), 3.90–3.96 (3H, m), 4.06–4.16 (3H, m), 4.70 (1H, d, $J_{1,2}$ 7.3, H-1′), 5.02 (1H, br d, *J* 7.0), 5.08 (1H, d, $J_{1'',2''}$ 3.3, H-1″), 5.13–5.22 (2H, m), 5.27 (1H, dt, *J* 7.3, 2.6), 6.17 (1H, s), 6.94 (1H, d, *J* 2.0), 6.97 (1H, dd, *J* 8.6, 2.0), 7.28–7.44 (7H, m), 7.62–7.67

(4H, m); δ_C 14.04, 15.78, 18.50, 19.04, 20.29, 20.42, 20.47, 20.51, 26.67, 60.29, 62.90, 67.70, 68.59, 69.63, 70.89, 71.87, 75.05, 80.81, 98.79 (C-1″), 101.63 (C-1′), 104.13, 112.95, 113.17, 115.01, 125.59, 127.58, 127.65 (2C), 127.69, 129.72, 129.74, 132.78, 132.95, 134.73, 135.46, 135.52, 152.02, 159.46, 160.73, 169.44, 169.97, 170.43; *m*/*z* (CI, NH_3) [Found: (M + NH_4), 866.3480. $C_{44}H_{56}NO_{15}Si$ requires *m*/*z*, 866.3419].

4-Methylumbelliferyl 3′-*O*-(2″,3″,4″-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside 21

To a THF solution (5 ml) of silyl compound **20** (0.5 g, 0.58 mmol) was added tetrabutylammonium fluoride (0.1 M solution in THF; 15 ml) and the mixture was stirred for 12 h at RT. The solvent was removed *in vacuo*, the residue was dissolved in CH_2Cl_2 (10 ml), and the solution was washed with water (10 ml), dried over Na_2SO_4, and concentrated. The resultant material was purified by column chromatography (EtOAc–'petrol' 3 : 2) and gave thiol **21** (250 mg, 69%), $[\alpha]_D^{23}$ −28.4 (*c* 1.8, $CHCl_3$); IR (film) ν_{max} 3437, 1745, 1612 cm^{-1}; δ_H 1.26 (3H, d, *J* 7.3), 1.98 (3H, s), 2.04 (3H, s), 2.19 (3H, s), 2.40 (3H, s), 3.72–3.78 (2H, m), 3.86–4.03 (2H, m), 4.08 (1H, d, *J* 7.3), 4.13 (1H, dd, *J* 7.3, 2.6), 4.28 (1H, dd, *J* 7.3, 6.6), 4.71 (1H, d, $J_{1',2'}$ 7.9, H-1′), 5.05–5.10 (2H, m), 5.14 (1H, appt d, *J* 7.9), 5.27 (1H, d, $J_{1'',2''}$ 2.6, H-1″), 6.18 (1H, s), 6.95–7.00 (2H, m), 7.51 (1H, d, *J* 9.9); δ_C 15.84, 18.56, 20.30, 20.46, 20.55, 62.17, 67.90, 68.32, 68.65, 69.72, 69.85, 71.72, 74.76, 80.63, 98.86 ($J_{C\text{-}H}$ 167.70, C-1″), 101.64 ($J_{C\text{-}H}$ 161.99, C-1′), 104.10, 113.04, 113.23, 115.16, 125.70, 152.17, 154.84, 159.45, 160.81, 169.45, 170.02, 170.46; *m*/*z* (CI, NH_3) [Found: (M + NH_4), 628.1928. $C_{28}H_{38}NO_{15}$ requires *m*/*z*, 628.1928].

4-Methylumbelliferyl 1-*O*-(α-L-fucopyranosyl)-β-D-galactopyranoside 22

The disaccharide **21** (0.2 g, 0.32 mmol) was *O*-deacetylated as described for the preparation of compound **13** above. Recrystallisation from EtOH afforded the title compound **22** as a white solid (138 mg, 87%), mp 287–289 °C; $[\alpha]_D^{21}$ −27.3 (*c* 0.2, H_2O); IR (KBr) ν_{max} 3440, 1698, 1619 cm^{-1}; λ_{max} (H_2O) 320, 292, 252 nm; δ_H (DMSO-d_6) 1.06 (3H, d, *J* 6.6), 2.40 (3H, s), 3.53–3.59 (2H, m), 3.63 (1H, dd, *J* 9.9, 3.3), 3.71 (1H, m), 3.78 (1H, dd, *J* 9.9, 7.3), 4.40 (1H, d, *J* 7.3), 4.45 (1H, q, *J* 3.3), 4.51 (1H, d, *J* 1.3), 4.69–4.72 (2H, m), 4.94 (1H, d, *J* 4.0), 5.08 (1H, d, *J* 6.6), 6.24 (1H, s), 7.00–7.10 (2H, m), 7.67 (1H, d, *J* 8.6); δ_C (DMSO-d_6) 16.40, 18.08, 60.34, 67.33, 70.21 (2C), 70.97, 71.49, 73.32, 75.57, 78.31, 100.08 (J_{CH} 173.7), 103.04 (J_{CH} 159.4), 103.96, 111.71, 113.96, 114.27, 126.23, 153.31, 154.24, 160.07, 160.25; *m*/*z* (CI, NH_3) [Found: (M + NH_4), 502.1920. $C_{22}H_{32}NO_{12}$ requires *m*/*z*, 502.1924].

4-Methylumbelliferyl 6′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-(2″,3″,4″-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside 23

To a solution of the galactopyranoside **19** (1.01 g, 1.74 mmol) in CH_2Cl_2 (10 ml), containing $Et_4N^+Br^-$ (0.36 g, 1.74 mmol) and molecular sieves (4 g), was added 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide **3** (0.62 g, 1.74 mmol). The resulting mixture was stirred at RT for 48 h. The solid residue was removed by passage through a pad of Celite and the filtrate was washed with water (10 ml). The CH_2Cl_2 layer was dried over Na_2SO_4, and evaporated *in vacuo*. Column chromatography (EtOAc–'petrol' 1 : 4) gave compound **23** as a major (750 mg, 51%) and compound **20** as a minor product (390 mg, 27%), mp 79–81 °C; $[\alpha]_D^{20}$ −27.32 (*c* 1.8, $CHCl_3$); IR (KBr) ν_{max} 3452, 1745, 1614 cm^{-1}; δ_H 1.05 (9H, s), 1.16 (3H, d, *J* 6.6), 2.06 (3H, s), 2.17 (6H, s), 2.37 (3H, s), 3.59 (1H, dd, *J* 4.0, 2.6), 3.68–3.72 (1H, m), 3.90–3.99 (2H, m), 4.10–4.22 (3H, m), 5.04 (1H, d, *J* 7.26, H-1′), 5.17–5.22 (2H, m), 5.27 (1H, dd, *J* 7.3, 2.6), 5.46 (1H, dd, *J* 4.0, 3.3, H-1″), 6.16 (1H, s), 6.93 (1H, d, *J* 1.98), 6.97 (1H, dd, *J* 8.5, 2.6), 7.28–7.46 (7H, m), 7.62–7.65 (4H, m); δ_C 14.09, 15.87, 18.53, 19.07, 20.31, 20.50, 20.54, 20.72, 20.93, 26.70, 60.32, 62.92, 67.74, 68.44, 69.67, 70.79, 71.91, 80.83, 98.83 (C-1″), 101.67 (C-1′), 104.22, 112.98, 113.22, 115.05, 125.61, 127.68 (2C), 127.73, 127.75, 127.81, 129.75, 129.77, 132.82, 135.47, 135.49, 135.55, 152.05, 152.23, 154.75, 159.50, 169.01, 170.01, 170.42; *m*/*z* (CI, NH_3) [Found: (M + NH_4), 866.5243. $C_{44}H_{56}NO_{15}Si$ requires *m*/*z*, 866.3075].

Acknowledgements

We thank the EPSRC for access to the mass spectrometry service at the University of Wales, Swansea (Director, Prof. D. E. Games).

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