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Synthesis of Fucosidase Substrates using Propane-1,3-diyl phosphate as the Anomeric Leaving Group

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Abstract: Activation of the anomeric centre of suitably protected L-fucopyranose and D-galactopyranose with propane-1,3-diyl phosphate allowed the synthesis of the disaccharide α-L-Fuc*p*-(1,2)-β-D-Gal*p*(1)-4-methylumbelliferone as a fucosidase substrate.

Over the last twenty-five years there has been recognition of the role that carbohydrates play in the maintenance of health and the onset of disease. 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, and oxidation or reduction states, and points of attachment give them a wide range of biological functions. An understanding of how and why these structures are recognised by enzymes, antibodies and lectins, have become important areas of research in organic chemistry and molecular biology.

A number of biologically important glycoconjugates contain the fucosyl moiety at their reducing ends. The α-L-fucopyranosyl group has been found linked α1-2 to galactopyranose residue(s) or to Glc*p*NAc with an α1-3, α1-4 or α1-6 linkage. The occurrence of oligosaccharides containing fucose has been established in human milk[1] and the disaccharide Fuc*p*α1-2Gal*p* occurs as part of the carbohydrate motif of the blood group specific glycoproteins and glycolipid.[2] Furthermore the α-L-fucopyranosyl group in sialyl Lewis X plays a pivotal role in binding to E-, L- and P-selectins.[3] In this note we describe the synthesis of both α1-2 and β1-2 linked L-fucogalactopyranoses and also of the corresponding disaccharides having 4-methylumbelliferyl attached at the anomeric centre of D-galactose.

At the outset 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 this to obtain β-glycosides.[4] Furthermore 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 hydrolase activity[5] and as ligands in carbohydrate-protein interaction studies.[6]

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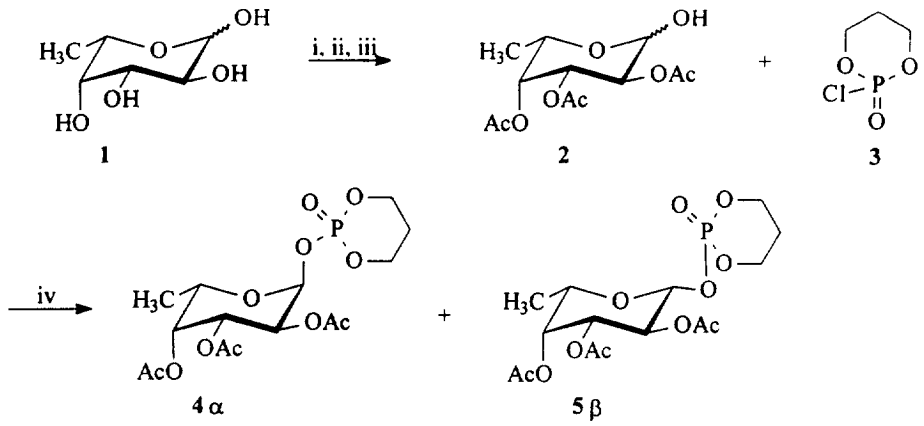
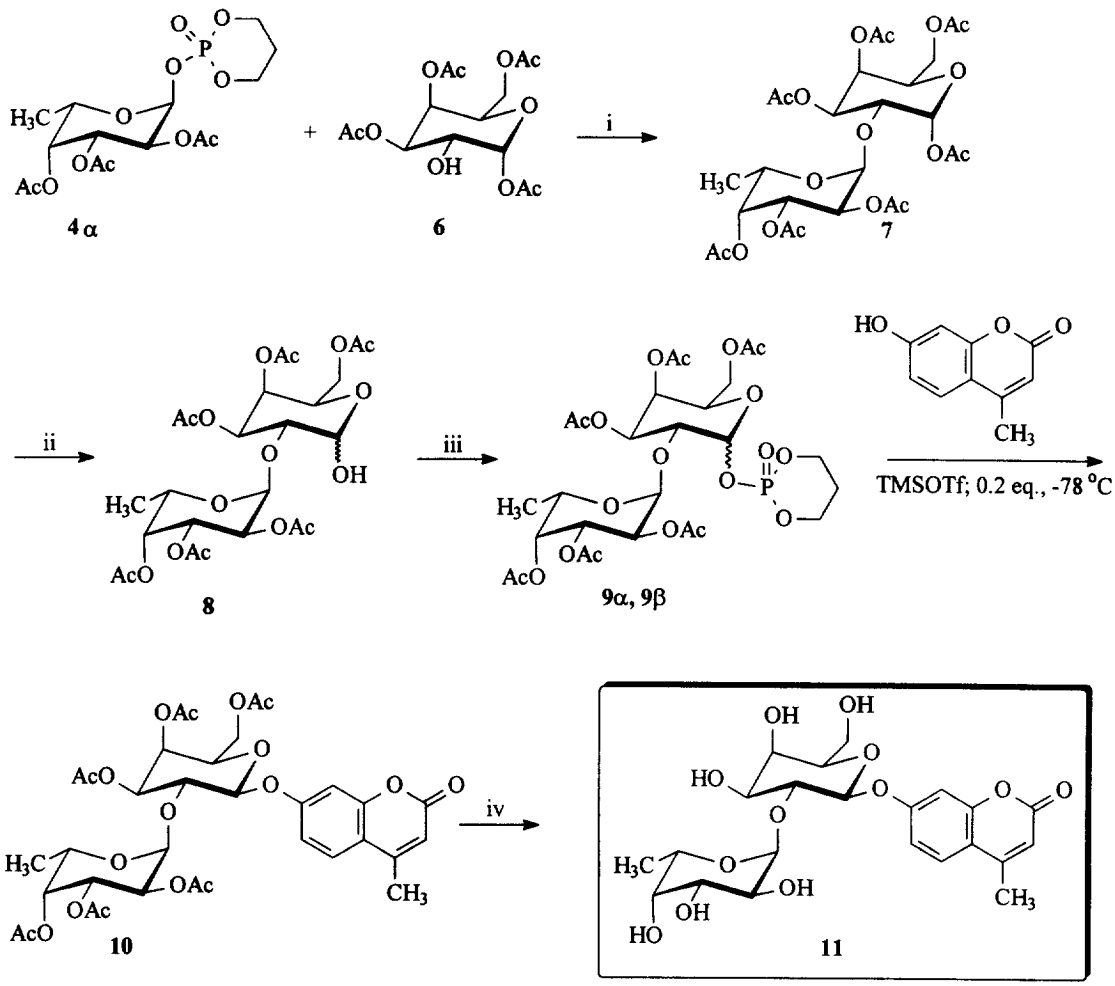


Scheme 1 *Reagents and conditions: i, Ac_2O, Py, RT 12h; ii, 33% HBr-AcOH, RT, 15 mins.; iii, Ag_2CO_3, acetone; iv, N-MeIm, DCM, RT, 16h.*

The required 2,3,4-tri-*O*-acetyl-α,β-L-fucopyranose **2** was obtained by literature methodology[7] in 90% overall yield in three steps. Phosphorylation at the anomeric centre with cyclic phosphoryl chloride **3** proceeded uneventfully and afforded the phosphates[8] **4α** and **5β** in 65 % yield in a ratio of 9:1 as determined by ^{1}H nmr. 1,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose **6** was prepared as reported by Chittenden[9] in a yield of 70%. With both the sugars available we investigated their coupling reactions and to our delight the coupling of **4α** with **6** proceed smoothly in the presence of trimethylsilyl triflate and gave the disaccharide α-L-Fuc*p*-(1,2)-α-D-Gal*p* **7** in 63% yield after chromatography. The α-stereochemistry at the fucose anomeric centre was assigned on the basis of the ^{13}C-^{1}H, coupling constant 165.4 Hz, for the newly formed bond.[10] The removal of the acetate protection of the disaccharide **7** was effected with base and the resulting disaccharide was identical in all respects with that reported in the literature.[11] Interestingly if the coupling of the phosphate **4α** to **6** was conducted using a catalytic amount of TMSOTf, it resulted in the formation of the β-isomer of **7** 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 findings suggest that the isomer **7** is the thermodynamic product and is the result of the coupling reaction proceeding *via* an oxonium ion intermediate whilst the β-isomer is formed by an S_N2 like process or *via* participation of the 2-acetoxy group.

Selective removal of the anomeric acetate function of the disaccharide **7** was accomplished by treatment with ammonia gas in acetonitrile[12] to afford **8** in 69% yield. The anomeric hydroxyl function was coupled with the cyclic phosphoryl chloride **3** and gave the mixture **9α** (δ_P -10.54) and **9β** (δ_P -11.50) anomers in 60 % yield in a ratio of 20:1, on the basis of ^{1}H nmr.



Scheme 2 *Reagents and conditions:* i,1.5 eq TMSOTf, CH_2Cl_2, -78 °C; ii, NH_3(g), MeCN, 0 °C; iii, **3**, MeNIm; iv, NaOMe, MeOH, RT, 30 mins.

At this juncture we decided to study the displacement reaction of the phosphates 9α,β with 4-methylumbelliferone, as this function has been widely used in the fluorimetric assay of glycosides. Displacement of the propane-1,3-diyl-phosphate by 4-methylumbelliferone was achieved by using 0.2eq of TMSOTf as the activator and afforded the β-linked disaccharide **10** in 56% yield after chromatography. This result is an improvement on reported methods for the introduction of the 4-methylumbelliferone group which routinely proceed in 14% yields. Removal of the acetate function[5] of the disaccharide **10** afforded the fully deprotected **11**. The stereochemistry of the anomeric linkage was assigned on the basis of ^{13}C-^{1}H coupling constant, 159.2 Hz.[10]

In summary we have demonstrated that activation of anomeric centres of mono- and disaccharides using propane-1,3-diylphosphate proceeds readily in good yields and provides access to fluorimetrically labelled compounds.

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8. All new compounds gave analytical and spectral data consistent with their structures. Selected data:**4α** m.p. 151-153 °C; $[\alpha]_D$ -100.6 (c 3.2, $CHCl_3$); δ_H (270 MHz, $CDCl_3$) 1.24 (3H, d, *J* 6.6); 1.85-1.89 (1H, m, J_{P-H} 15.17); 2.02 (3H, s); 2.10 (3H, s); 2.19 (3H, s); 2.30-2.36 (1H, m, J_{P-H} 15.17); 3.90 (1H, dq, *J* 7.3, 6.6); 4.33-4.59 (4H, m); 5.01 (1H, dd, *J* 3.30, 3.29); 5.22 (1H, dd, *J* 3.30, 2.64); 5.31-5.33 (1H, m); 5.92 (1H, dd, *J* 3.30, 2.64); δ_C (67.8 MHz, $CDCl_3$) 15.82, 20.52, 20.60 (2C), 25.63, 68.80, 68.86(2C), 66.92, 67.35, 69.75, 70.36, 94.63 (C-1, d, J_{C-P} 4.93), 170.14 (2C), 170.46; δ_P (109.25 MHz, $CDCl_3$) -10.36; m/z (CI, NH_3) Found 410.0978 M^+ $C_{15}H_{23}O_{11}P$ requires 410.0978. **5β** δ_H (270 MHz, $CDCl_3$) 1.19 (3H, d, *J* 6.6); 1.78-1.83 (1H, m, J_{P-H} 15.19); 2.02 (3H, s); 2.10 (3H, s); 2.19 (3H, s); 2.23-2.28 (1H, m, J_{P-H} 15.17); 3.67 (1H, dq, *J* 5.94, 5.95); 4.07-4.31 (2H, m); 4.33-4.59 (4H, m); 5.31-5.33 (1H,m); 5.35 (1H, dd, *J* 5.90, 7.92); δ_C (67.8 MHz, $CDCl_3$) 15.85, 20.49, 20.65, 20.74, 25.79, 66.83, 68.91, 68.97, 69.31, 70.27, 70.74, 96.71 (C-1, d, J_{C-P} 4.41), 170.41, 170.43, 170.49; δ_P (109.25 MHz, $CDCl_3$) -10.80. **11** m.p. 251-253 °C; $[\alpha]_D$ +24.7 (c 0.7, H_2O); ν_{max} (KBr) 3480; 1709; 1615; 834 cm^{-1}, $\lambda_{max}(H_2O)$ 318, 294, 252nm, δ_H (270 MHz, DMSO-d_6) 1.09 (3H, d, *J* 6.6); 2.39 (3H, s); 3.46-3.69 (3H, m); 3.53 (1H, dd, *J* 6.0, 6.0); 3.76 (1H, *J* 7.3, 10.0); 3.86 (1H, brd, *J* 4.0); 3.92 (1H, brd, *J* 2.7); 4.06 (1H, dd, *J* 3.3, 9.6); 4.24 (1H, q, *J* 7.3); 4.34 (1H, dd, *J* 3.3, 5.3); 5.09 (1H, appt, *J* 8.0); 5.70 (1H, d, *J* 3.3); 6.23 (1H, s); 7.03 (1H, q, *J* 2.0, 7.3); 7.09 (1H, d, *J* 2.7); 7.67 (1H, d, *J* 9.3); δ_C (67.8 MHz, 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_{C-H} 173.5), 101.99 (J_{C-H} 159.2), 104.16, 111.73, 114.15, 114.21, 126.36, 153.33, 154.32, 159.99, 160.11; m/z (CI, NH_3) Found 501.1619 $(M+H)^+$ $C_{22}H_{28}O_{12}$ requires 484.1580 $(M-OH)^+$.
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