Investigation of the Regioselectivity of Some Esterifications Involving Methyl 4,6-*O*-Benzylidene D-Pyranosides and *Pseudomonas fluorescens* Lipase

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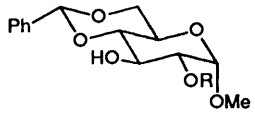
Using *Pseudomonas fluorescens* lipase in vinyl acetate, the methyl 4,6-*O*-benzylidene-α-D-pyranosides **1** and **6–8** have been esterified with very high selectivity to afford the C-2 acetates **3** and **10–12**, respectively. In contrast the methyl 4,6-*O*-benzylidene-β-D-pyranosides **2** and **9** are acetylated under the same conditions to give the C-3 esters **5** and **13** with high/exquisite regioselectivity.

The enzyme-catalysed regioselective protection of polyhydroxy compounds is a topic of current interest.[1] In a contribution from the Exeter laboratories we have recently shown[2] that the regioselectivity of acetylation reactions catalysed by *Pseudomonas fluorescens* lipase (PFL) on methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **1** and methyl 4,6-*O*-benzylidene-β-D-glucopyranoside **2** are dependent on the configuration of the anomeric carbon atom. With the α anomer, the C-2 monoester **3** was the only product of the reaction but when using the β anomer as the substrate, the C-3 monoester **5** was formed predominantly. This difference in selectivity could be of interest to the synthetic organic chemist as a means of controlling the selective protection of sugar derivatives. Hence, we decided to investigate the reactivity of different methyl 4,6-*O*-benzylidene-α- and β-D-pyranosides towards PFL. In this connection the following sugar derivatives were synthesised:[3] methyl 4,6-*O*-benzylidene-α-D-mannopyranoside **6**, methyl 4,6-*O*-benzylidene-α-D-altropyranoside **7**, methyl 4,6-*O*-benzylidene-α-D-galactopyranoside **8** and -β-D-galactopyranoside **9**.[4] Their reactivity towards vinyl acetate/PFL using our standard experimental procedure[2] was explored.



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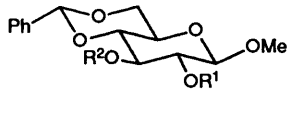
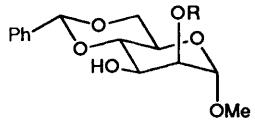
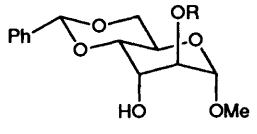
3 R = Ac



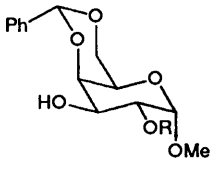
2 $R^1 = R^2 = H$
4 R^1 = Ac, R^2 = H
5 R^1 = H, R^2 = Ac



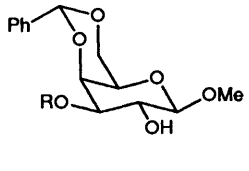
6 R = H
10 R = Ac



7 R = H
11 R = Ac



8 R = H
12 R = Ac



9 R = H
13 R = Ac

Results and Discussion

As shown in Table 1, when substrates **6–8**, possessing an α-configuration at the anomeric centre, were acetylated using PFL as the catalyst, C-2 monoesters **10–12**, respectively, were formed predominantly or exclusively, whatever the configuration of the 2-, 3- or 4-hydroxy substituent. Indeed the introduction of an axial hydroxy group at C-2 (compounds **6**, **7**) only affected the speed of the reaction but not the regioselectivity of the process. In the case of compound **6** the acetyl group migrated from the 2-hydroxy group to the 3-hydroxy group during the purification of the reaction products over silica. This phenomenon prompted us to determine the ratio of the two monoesters in the crude reaction mixture using ^{1}H NMR spectroscopy and by integration of the two distinct $COCH_3$ signals. Compounds **6** and **7** gave good yields of monoesters after 10 days of reaction. On the contrary, galactopyranoside derivatives (**8** and **9**) were poor substrates for PFL-catalysed esterification reactions giving a 19% yield of monoesters after 10 days. The lack of reactivity of these compounds is probably due to steric factors preventing access of the sugar derivatives to the active site of the enzyme. However, it is noteworthy that for compound **9** the C-3 monoester is formed exclusively. Thus, the switch in regioselectivity observed for compounds **1** and **2** is seen again for compounds **8** and **9**.

The structures of the acetylated products were elucidated by comparison of physical data (m.p., $[\alpha]_D$ values) with authentic samples and/or ^{1}H NMR spectroscopy. The lowfield shift of the proton C*H*OAc is particularly useful in this connection.

Conclusions

Ronchetti *et al.*[5] have shown that methyl 6-*O*-butyrylpyranosides and methyl 6-deoxypyranosides can be acylated with some regioselectivity using PFL as the catalyst in almost non-aqueous media. Our results complement the Italian work and show that, for the 4,6-*O*-benzylidene derivatives of various pyranosides, highly regioselective acylation reactions at C-2 or C-3 can be achieved. Indeed the position of acetylation is dictated by the configuration at the anomeric centre. The products are differentially protected pyranosides that are obviously ripe for further modification.

Experimental

IR spectra were recorded on a Perkin-Elmer 881 spectrometer; the absorption peaks are quoted in reciprocal centi-

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Table 1 Reaction of vinyl acetate with some methyl 4,6-*O*-benzylidene-α-and β-pyranosides catalysed by PFL

Substrate	Time (days)	Yield (%)	Product(s) % monoesters resulting from acetylation C-2 ester	C-3 ester	Recovered substrate (%)
1	3	94	**3** 100	—	—
2	1	93	**4** 8	**5** 92	—
6	10	81	**10** >95	<5*	14
7	10	92	**11** 100	—	4
8	10	19	**12** 100	—	74
9	10	19	—	**13** 100	75

* Determined by ^{1}H NMR spectroscopy on the crude reaction mixture.

meters. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 250 spectrometer at 250 and 62.8 MHz respectively, using deuteriochloroform as the solvent. All chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (J) are quoted in Hz. The m.p.s were determined on a capillary apparatus and reported in °C. Vinyl acetate was from Aldrich and used without purification. Lipase from *Pseudomonas fluorescens* (PFL) was purchased from Biocatalysts. The various methyl 4,6-*O*-benzylidene-D-gluco- **1** and **2** and -galacto-pyranosides **8** and **9** were prepared from the corresponding methyl pyranoside and benzaldehyde.[3] Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside **6** was prepared as for the former compounds but in low yield (10%) and was separated from methyl 2,3-*O*-4,6-*O*-dibenzylidene-α-D-mannopyranoside (50%). Methyl 4,6-*O*-benzylidene-α-D-altropyranoside **7** was prepared from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (Fluka).[3] Physical data of these compounds were in full accordance with those reported in literature;[4] thus ^{1}H and ^{13}C NMR, IR and mass spectra were consistent with the purported structures.

General Procedure for the Enzymatic Reaction.—The methyl 4,6-*O*-benzylidene-D-pyranoside (1 g) was dissolved in vinyl acetate (200 cm^3) at 35 °C. The reaction was initiated by adding PFL (1 g) with gentle stirring. The progress of the reaction was followed by TLC (eluent toluene–ethyl acetate, 50:50 except with methyl 4,6-*O*-benzylidene-α- and -β-D-galactopyranoside when 100% ethyl acetate was employed). If the reaction was not complete within 10 days it was stopped. After removal of the enzyme by filtration and evaporation of the solvent, the product(s) was (were) purified by chromatography over silica with the same solvent system as described above.

Methyl 4,6-O-*Benzylidene*-α-D-*glucopyranoside* **1**.—The reaction was stopped after 3 days and the crude reaction mixture chromatographed to afford methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside **3** (1.08 g, 94%) as a white solid, m.p. 128–130 °C (lit.,[4] 129–131 °C); $[\alpha]_D^{27}$ +106.2 (*c* 1, $CHCl_3$) (lit.,[4] $[\alpha]_D$ +115, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3607, 3013, 1740 (C=O), 1373, 1199 and 1057; δ_H(250 MHz) 7.51 and 7.37 (2 H, and 3 H respectively, 2 × m, ArH), 5.52 (1 H, s, ArCH), 4.94 (1 H, d, *J* 3.7, 1-H), 4.78 (1 H, dd, *J* 9.7, 3.7, 2-H), 4.28 (1 H, dd, *J* 9.3, 4.0, 6-H_{eq}), 4.14 (1 H, dd, *J* 9.7, 9.7, 3-H), 3.88–3.68 (2 H, m, 5-H, 6-H_{ax}), 3.51 (1 H, dd, *J* 9.7, 9.7, 4-H), 3.38 (3-H, s, OCH_3), 2.70 (1 H, br s, OH) and 2.13 (3 H, s, $COCH_3$); δ_C(62.8 MHz) 170.73 (CO_2), 137.08 (PhC), 129.28, 128.34 and 126.35 (PhCH), 102.01 (PhCH), 97.61, 81.45, 73.65, 66.87 (C-6), 66.61, 62.07, 55.38 (OCH_3) and 20.89 ($COCH_3$); *m/z* (CI) 325 ($[M + H]^+$, 100%) and 293 ($[M - OCH_3]^+$, 34) (Found: $M^+ + H$, 325.1286. $C_{16}H_{20}O_7$ requires *M* + H, 325.1287).

Methyl 4,6-O-*Benzylidene*-β-D-*glucopyranoside* **2**.—The reaction was stopped after 1 day and the crude reaction mixture chromatographed to afford methyl 3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside **5** (0.99 g, 86%) as a white solid, m.p. 159–161 °C (lit.,[4] 162–163 °C); $[\alpha]_D^{25}$ −56.9 (*c* 1, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3603, 3012, 1738 (C=O), 1368, 1201, 1102 and 1029; δ_H(250 Hz) 7.45 and 7.37 (2 H, and 3 H respectively, 2 × m, ArH), 5.50 (1 H, s, ArCH), 5.23 (1 H, dd, *J* 9.3, 9.3, 3-H), 4.37 (1 H, d, *J* 7.7, 1-H), 4.36 (1 H, dd, *J* 10.3, 5.0, 6-H_{eq}), 3.78 (1 H, dd, *J* 10.3, 10.3, 6-H_{ax}), 3.63 (1 H, dd, *J* 9.3, 9.3, 4-H), 3.60–3.48 (2 H, m, 2-H, 5-H), 3.57 (3 H, s, OCH_3), 2.83 (1 H, br s, OH) and 2.12 (3 H, s, $COCH_3$); δ_C(62.8 MHz) 170.99 (CO_2), 137.01 (PhC), 129.08, 128.24 and 126.17 (PhCH), 104.59 (PhCH), 101.50, 78.58, 73.68, 73.39, 66.55 (6-C), 66.5, 57.56 (OCH_3) and 20.95 ($COCH_3$); *m/z* (CI) 325 ($[M + H]^+$, 100%) and 293 ($[M - OCH_3]^+$, 20) (Found: $M^+ + H$, 325.1286. $C_{16}H_{20}O_7$ requires M + H, 325.1287) and methyl 2-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside **4** (0.08 g, 7%) as a white solid, m.p. 173–176 °C (lit.,[4] 174–177 °C); $[\alpha]_D^{25}$ −75.8 (*c* 1, $CHCl_3$) (lit.,[4] $[\alpha]_D$ −74.4); $\nu_{max}(CHCl_3)/cm^{-1}$ 3615, 3012, 1737 (C=O), 1372, 1200, 1057 and 993; δ_H(250 MHz) 7.49 and 7.36 (2 H, and 3 H respectively, 2 × m, ArH), 5.51 (1 H, s, ArCH), 4.90 (1 H, dd, *J* 9.0, 8.0, 2-H), 4.40 (1 H, d, *J* 8.0, 1-H), 4.35 (1 H, dd, *J* 10.0, 5.0, 6-H_{eq}), 3.83 (1 H, dd, *J* 9.0, 9.0, 3-H), 3.77 (1 H, dd, *J* 10.0, 10.0, 6-H_{ax}), 3.54 (1 H, dd, *J* 9.0, 9.0, 4-H), 3.50 (3 H, s, OCH_3), 3.50–3.36 (1 H, m, 5-H), 2.87 (1 H, br s, OH) and 2.12 (3 H, s, $COCH_3$); δ_C(62.8 MHz) 170.33 (CO_2), 137.03 (PhC), 129.28, 128.34 and 126.34 (PhCH), 102.25, 101.84, 80.92, 74.04, 72.22, 68.59 (C-6), 66.19, 57.01 (OCH_3) and 20.92 ($COCH_3$).

Methyl 4,6-O-*Benzylidene*-α-D-*mannopyranoside* **6**.—The reaction was stopped after 10 days and the crude reaction mixture chromatographed to afford methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside **10** (0.21 g, 18%) as a colourless oil, $[\alpha]_D^{23}$ +22.4 (*c* 1, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3590, 2920, 1740 (C=O), 1376, 1134 and 1103; δ_H(250 MHz) 7.51 and 7.41 (2 H and 3 H respectively, 2 × m, ArH), 5.59 (1 H, s, ArCH), 5.18 (1 H, dd, *J* 3.8, 1.5, 2-H), 4.69 (1 H, d, *J* 1.5, 1-H), 4.28 (1 H, br m, 6-H_{eq}), 4.17 (1 H, dd, *J* 9.3, 3.8, 3-H), 3.94–3.76 (3 H, br m, 4-H, 5-H, 6-H_{ax}), 3.38 (3 H, s, OCH_3), 2.71 (1 H, br s, OH) and 2.16 (3 H, s, $COCH_3$); δ_C(62.8 MHz) 170.56 (CO_2), 137.24 (PhC), 129.23, 128.32 and 126.30 (PhCH), 102.22 (PhCH), 99.61, 79.05, 72.16, 68.76 (C-6), 67.10, 63.34, 55.18 (OCH_3) and 20.94 ($COCH_3$); *m/z* CI 325 ($[M + H]^+$, 76%), 293 ($[M - OCH_3]^+$, 20) and 219 ($[M - PhCO]^+$, 100) (Found: $M^+ + H$, 325.1286. $C_{16}H_{20}O_7$ requires *M* + H, 325.1287) and a mixture of methyl 2-*O*-acetyl and 3-*O*-acetyl 4,6-*O*-benzylidene-α-D-mannopyranoside (0.63 g, 55%).

Methyl 4,6-O-*Benzylidene*-α-D-*altropyranoside* **7**.—The reaction was stopped after 10 days and the crude reaction mixture chromatographed to afford methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-altropyranoside **11** (1.06 g, 92%) as a colourless oil, $[\alpha]_D^{23}$ +54.1 (*c* 1, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3588, 2988, 1732 (C=O), 1372, 1199, 1036 and 907; δ_H(250 MHz) 7.49 and 7.34 (2 H, and 3 H respectively, 2 × m, ArH), 5.58 (1 H, s, ArCH), 4.97 (1 H, dd, *J* 3.2, 0.7, 2-H), 4.61 (1 H, d, *J* 0.7, 1 H), 4.30 (1 H, dd, *J* 9.6, 5.2, 6-H_{eq}), 4.28–4.16 (1 H, br m, *J* 9.6, 9.5, 5.2, 5-H), 4.10 (1 H, dd, *J* 3.2, 3.1, H-3), 3.81 (1 H, dd, *J* 9.5, 3.1, 4-H), 3.77 (1 H, dd, *J* 9.6, 9.6, 6-H_{ax}), 3.36 (3 H, s, OCH_3), 3.16 (1 H, br s, OH) and 2.06 (3 H, s, $COCH_3$); δ_C(62.8 MHz) 169.31 (CO_2), 137.42 (PhC), 129.08, 128.21 and 126.34 (PhCH), 102.17, 99.24, 76.59, 71.28, 69.08 (6-C), 66.83, 57.99, 55.69 (OCH_3) and 20.81 ($COCH_3$); *m/z* (CI) 325 ($[M + H]^+$, 44%), 293 ($[M - OCH_3]^+$, 100) (Found: $M^+ + H$, 325.1286. $C_{16}H_{20}O_7$ requires *M* + H, 325.1287).

Methyl 4,6-O-*Benzylidene*-α-D-*galactopyranoside* **8**.—The reaction was stopped after 10 days and the crude reaction mixture chromatographed to afford methyl-2-*O*-acetyl-4,6-*O*-benzylidene-α-D-galactopyranoside **12** (0.22 g, 19%) as a white solid: $[\alpha]_D^{25}$ +456 (*c* 1, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3607, 3013, 1740 (C=O), 1373, 1199, 1057 and 992; δ_H(250 MHz) 7.49 and 7.36 (2 H, and 3 H respectively, 2 × m, ArH), 5.50 (1 H, s, ArCH), 5.11 (1 H, dd, *J* 10.5, 3.5, 2-H), 4.93 (1 H, d, *J* 3.5, 1-H), 4.36 (1 H, dd, *J* 3.5, 1, 4-H), 4.25 (1 H, dd, *J* 12.5, 1.5, 6-H_{eq}), 4.16 (1 H, dd, *J* 10.5, 3.5, 3-H), 4.04 (1 H, dd, *J* 12.5, 1.5, 6-H_{ax}), 3.69 (1 H, bm, 5-H), 3.45 (3 H, s, $COCH_3$) and 2.12 (3 H, s, OCH_3); δ_C(62.8 MHz) 171.36 (CO_2), 137.70 (Ph*C*), 128.97, 128.15 and 126.17 (Ph*C*H), 100.79, 100.33, 74.27, 71.64, 69.18 (6-C), 66.72, 62.52, 55.67 (OCH_3) and 21.07 ($COCH_3$); *m*/*z* (CI) 325 ($[M + H]^+$, 35%) and ($[M - OCH_3]^+$, 100) (Found: $M^+ + H$, 325.1287. $C_{16}H_{20}O_7$ requires *M* + H, 325.1286).

Methyl 4,6-O-*Benzylidene*-β-D-*glucopyranoside* **9**.—The reaction was stopped after 10 days and the crude reaction mixture chromatographed to afford methyl 3-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside **13** (0.22 g, 19%) as a white solid, m.p. 74–76 °C (lit.,[4] 75–78 °C), $[\alpha]_D^{31}$ +84.7 (*c* 1, $CHCl_3$) {lit.,[4] $[\alpha]_D$ +87 (*c* 1, $CHCl_3$)}; $\nu_{max}(CHCl_3)/cm^{-1}$ 3603, 3014, 1727 (C=O), 1368, 1199 and 1044; δ_H(250 MHz) 7.50 and 7.36 (2 H, and 3 H respectively, 2 × m, ArH), 5.48 (1 H, s, ArCH), 4.84 (1 H, dd, *J* 10.5, 3.5, 3-H), 4.38–4.26 (3 H, m, 1-H, 4-H), 6-H_{eq}), 4.08–3.94 (2 H, m, 2-H, 6-H_{ax}), 3.57 (3 H, s, OCH_3), 3.47 (1 H, m, 5-H), 2.55 (1 H, br s, OH) and 2.12 (3 H, s, $COCH_3$); δ_C(62.8 MHz) 171.10 (CO_2), 137.67 (PhC), 128.97, 128.12 and 126.28 (Ph*C*H), 104.04, 100.92, 73.83, 73.42, 69.00 (6-C), 68.44, 66.42, 57.15 (OCH_3) and 21.01 ($COCH_3$); *m*/*z* (CI) 325 ($[M + H]^+$, 50%), ($[M - OCH_3]^+$, 100% (Found: $M^+ + H$, 325.1286. $C_{16}H_{20}O_7$ requires *M* + H, 325.1286).

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References

1 K. Faber, *Biotransformations in Organic Chemistry*, Springer-Verlag, Heidelberg, 1992, p. 269–272.
2 M. J. Chinn, G. Iacazio, D. G. Spackman, N. J. Turner and S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1992, 661.
3 *Textbook of Practical Organic Chemistry*, R. I. Vogel, Longmans 4th edn.
4 *Carbohydrates*, ed. P. M. Collins, Chapman and Hall, London, 1987.
5 D. Colombo, F. Ronchetti and L. Toma, *Tetrahedron*, 1991, **47**, 103; P. Ciuffreda, D. Colombo, F. Ronchetti and L. Toma, *J. Org. Chem.*, 1990, **55**, 4187.

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