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Tetrahedron 60 (2004) 927–932
Tetrahedron

Regioselective acylation of carbohydrate derivatives using lipases leading to a facile two-step procedure for the separation of some α- and β-glucopyranosides and galactopyranosides

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Received 12 September 2003; revised 23 October 2003; accepted 13 November 2003

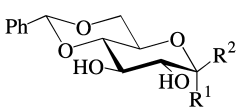
Abstract—The resolution of α- and β-anomers of glucopyranosides and galactopyranosides was achieved via enzyme-catalysed regioselective acylation. This two-step procedure to prepare pure α- and β-anomers of glycopyranosides would be most useful for the cases where glycosidases are not available or expensive to purchase. From a synthetic viewpoint, the regioselective acylation of glycopyranosides provides access to mono- and di-esters with well-defined substitution patterns.

1. Introduction and background information

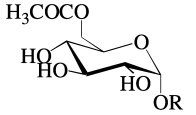
Enzyme-catalysed esterification of carbohydrates and their simple derivatives has been studied extensively.[1] For hexapyranoses, the primary hydroxyl group is esterified preferentially and the preparation of 6-*O*-acylated carbohydrates and glycopyranosides has been a subject of continued interest,[2] especially in connection with the synthesis of novel surfactants[3] and the manufacture of antibacterial agents of potential interest to the food industry.[4]

Following acylation at the 6-position of glycopyranosides, one or more of the secondary hydroxyl groups may be esterified on prolonged reaction.[5] It has also been shown that the configuration of the substituent at the anomeric position can influence the regioselectivity of the acylation of the secondary hydroxyl groups. For example, in a study of the acylation of 4,6-benzylidene glucopyranosides using *Pseudomonas cepacia* lipase as catalyst, acetylation of the α-methoxy compound **1** occurred exclusively at C(2)–OH while the isomer **2** was acetylated with very high selectivity at C(3)–OH.[6] The ethylthio derivative **3** behaves in a similar manner and this regioselective reaction has been used as a key step in the synthesis of the core motif of asparagine-linked glycoprotein oligosaccharides.[7]

Of greatest relevance to the present study is the earlier work by Riva and co-workers.[8] The Italian team showed that α-methyl- and α-benzyl-D-glucopyranoside gave only the 6-acetates **4** and **5** (98% yield) on reaction with vinyl acetate and *Candida antarctica* lipase B in THF/pyridine over 20 h., while β-methyl-D-glucopyranoside furnished the 3,6-di-acetate **6** in 97% yield. Under the same conditions α-methyl-D-galactopyranoside gave a mixture of the 6-acetylated product **7** (74%) and a small amount of the 2,6-diester **8** (24%). In contrast β-methyl-(D)-galacto-pyranoside afforded the 3,6-diacetate **9** as the major product (44%) with lesser amounts of the 2,6-diacetate **10** (31%) and the 6-monoester **11** (24%) after 48 h. Later, it was shown that the benzyl compound **12** was acylated using trifluoro-ethyl butanoate in acetone and in the presence of subtilisin to give the 3,6-diester **13** in 89% yield.[9]



(1) R^1 = OMe, R^2 = H
(2) R^1 = H, R^2 = OMe
(3) R^1 = H, R^2 = SEt



(4) R = Me
(5) R = Bn

Keywords: Regioselective acylation; α- and β-Anomers; Glycopyranosides.
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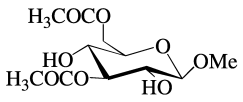
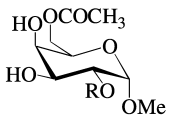
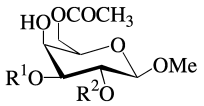
doi:10.1016/j.tet.2003.11.035



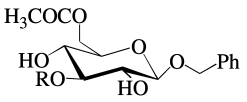
(6)



(7) R = H
(8) R = $COCH_3$



(9) $R^1 = COCH_3$, R^2 = H
(10) R^1 = H, $R^2 = COCH_3$
(11) $R^1 = R^2$ = H

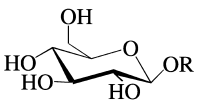


(12) R = H
(13) R = COC_3H_7

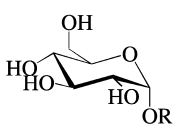
In this paper, we extend the studies of Riva et al.[8] to show that the methodology can lead to the separation of α- and β-glycopyranosides.

2. Results and discussion

Benzyl β-D-glucopyranoside **14** and ethyl β-D-glucopyranoside **15** were synthesised from D-glucose in 43 and 85% yield, respectively, using β-glucosidase from almonds.[10]



(14) R = CH_2Ph
(15) R = Et



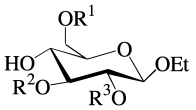
(16) R = CH_2Ph
(17) R = Et

.

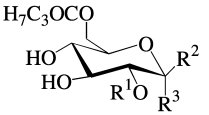
The same enzyme was used selectively to hydrolyse an anomeric mixture of α- and β-benzyl glucoside to afford the unreacted α-anomer **16** in 67% yield. The α-ethoxy compound **17** was prepared from glucose in a similar manner, but in a more moderate yield of 36% over the two steps.

A battery of lipases were inspected for their ability to catalyse the acylation of ethyl β-glucopyranoside **15**. Immobilised *Mucor miehei* lipase (Lipozyme®) and immobilised *Candida cylindracea* B lipase (Novozyme®) were the most efficient catalysts for esterification using vinyl butyrate in THF. Both enzymes gave the 6-*O*-butyrate **18** as the first formed product. Further esterification of the mono-ester **18** with Novozyme® in THF at 60 °C gave the 3,6-diester (50%) **19** and the 2,6-diester (30%) **20**. In contrast, prolonged reaction of the β-benzyl compound **14** with vinyl butyrate in THF containing Novozyme® afforded only the 6-*O*-butanoate **21** (98% isolated yield after a 3 h reaction), in accord with the earlier results documented by Riva et al.[8]

The α-anomers **16** and **17** showed an interesting difference in reactivity, inasmuch as the α-benzyl anomer **16** reacted ca. 5×faster than the β-isomer **14** while the α-ethyl compound **17** reacted ca. 5×slower than the corresponding β-anomer **15** under our standard esterification conditions. In fact, similar patterns of α-/β-reactivity for large[11] and small[12] substituents at the anomeric position have been documented previously. The products from the reactions of **16** and **17** were the appropriate 6-*O*-butanoates **22** and **23**. No further reaction of the latter compounds with vinyl butanoate and Novozyme® could be detected even after prolonged periods of time. Only when a different enzyme was employed (namely *Pseudomonas cepacia* lipase PS-CII) could further acylation of monoester **22** be achieved. By employing this enzyme in vinyl butyrate as solvent, an 86% yield of diester **24** was obtained after 3 days. It was noted that *Ps cepacia* lipase PS CII did not catalyse esterification of monoester **21** under these conditions.



(18) $R^1 = COC_3H_7$, $R^2 = R^3$ = H
(19) $R^1 = R^2 = COC_3H_7$, R^3 = H
(20) $R^1 = R^3 = COC_3H_7$, R^2 = H



(21) $R^1 = R^3$ = H, $R^2 = OCH_2Ph$
(22) $R^1 = R^2$ = H, $R^3 = OCH_2Ph$
(23) $R^1 = R^2$ = H, R^3 = OEt
(24) $R^1 = COC_3H_7$, R^2 = H, $R^3 = OCH_2Ph$
(25) $R^1 = COC_3H_7$, $R^2 = R^3$ = H

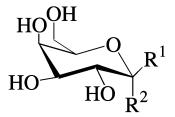
.

From this initial study, it was clear that the different reactivity patterns could be used to separate mixtures of α- and β-anomers of glycopyranosides. Thus the mixture of α- and β-ethyl glucopyranoside, simply prepared from ethanol and glucose under acidic conditions, was reacted with vinyl

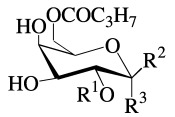
butyrate in THF at 60 °C in the presence of Novozyme® for a period of 4 days. The diesters **19** and **20** (31%) were separated from the monoester **23** (48%). Methanolysis of the diesters gave ethyl β-D-glucopyranoside **15** while similar treatment of the mono-ester gave the α-anomer **17** in quantitative yield.

A combination of Novozyme® and *Ps. cepacia* lipase PS CII could be used to effect a separation of α- and β-benzyl (D)-glucopyranosides. A mixture of the α- and β-benzyl anomers was reacted with the two enzymes in vinyl butyrate for 4 days. The monoester **21** (36%) was readily separated from the diester **24** (28%) by column chromatography. Base-catalysed methanolysis of these esters gave pure β-benzyl-(D)-glucopyranoside and pure α-benzyl-D-glucopyranoside, respectively, in quantitative yields.

It was also clear that this three-step procedure to prepare glycopyranosides would be more useful for the cases where glycosidases are not available, or are expensive to purchase. Thus, we extended the study to galactosides because, while β-benzyl-D-galactoside **26** is available by a modified Königs–Knorr glycosidation procedure,[13] the corresponding α-anomer **27** is not easy to access. Standard Fischer derivatisation of galactose gives a mixture of **26** and **27**. Incubation of this mixture with Novozyme® in THF containing vinyl butyrate over 7.5 h gave the mono ester **28** (46%) and the diester **29** (23%) which were readily separated by chromatography over silica. Methanolysis of the mono ester **28** gave benzyl α-D-galactoside **27** in 91% yield.



(26) $R^1 = OCH_2Ph$, $R^2 = H$
(27) $R^1 = H$, $R^2 = OCH_2Ph$



(28) $R^1 = R^2 = H$, $R^3 = OCH_2Ph$
(29) $R^1 = COC_3H_7$, $R^2 = OCH_2Ph$, $R^3 = H$

.

It is also obvious, but noteworthy from a synthetic viewpoint, that selectively esterified glucose or galactose derivatives are available from some of the compounds in the above portfolio by hydrogenation to remove the anomeric benzyl group. For example, the ester **24** afforded the 2,6-diester **25** (75% yield) on reduction, using palladium on charcoal (10%) as the catalyst.

This work provides further evidence that the regioselective acylation of glycopyranosides can provide access to mono- and di-esters with well-defined substitution patterns. We have shown that the different degrees of reactivity of α- and β-glycosides potentially allows access to a wide range of pure α-anomers which may not be readily accessed by other methodology.

3. Experimental

3.1. General

All reactions were monitored by thin layer chromatography, which was performed on 200–250 μm thickness Merck silica gel plates (60_{F254}). Compounds were detected by ultraviolet light or by staining with ceric ammonium molybdate solution followed by heating. Column chromatography was performed on Merck-60 silica gel (230–240 mesh). Melting points were recorded on a Reichert instrument and are uncorrected. Elemental analyses were performed on a Carlo Erba Elemental Analyser model 1106. IR spectra were recorded on a Perkin–Elmer 883 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded as solutions in deuteriated solvents (Aldrich, Fluorochem) on AVANCE 400 MHz or Varian Gemini300 instruments. ^1H- and ^{13}C-spectra were referenced using TMS as internal standard. Chemical shifts (δ) are quoted in parts per million (ppm) and the coupling constants (J) in Hertz (Hz). The following abbreviations are used to describe the multiplicity: s, singlet; d, doublet; t, triplet; tt, triplet of triplets; q, quartet; dd, doublet of doublets; ddd, doublet of doublets of doublets; dt, doublet of triplets; m, multiplet; br, broad. Optical rotations $[\alpha]_D^T$ (concentration in g/100 cm^3, solvent) were recorded on an Optical Activity A1000 polarimeter at 589 nm, where T is the temperature in °C. Low resolution CI mass spectra were measured on a Fisons TRIO 1000 spectrometer. Accurate mass spectra were obtained on a VG Analytical 7070E double focussing magnetic mass spectrometer. β-Glucosidase from almonds (EC number 3.2.1.21) and Novozyme® 435 (EC number 3.1.1.3) were purchased from Sigma-Aldrich. Lipase PS-C II was purchased from Amano Pharmaceuticals. All solvents and reagents were purchased and used directly from commercial suppliers without purification.

3.1.1. Ethyl β-D-glucopyranoside (15). D-Glucose (0.81 g, 4.48 mmol) was added to a solution of water (2 cm^3) in ethanol (18 cm^3), and heated to 50 °C, with stirring. After 10 min β-glucosidase from almonds (0.11 g) was added to the reaction mixture which was stirred for 3 days. The solution was filtered through Celite® and washed with ethanol (20 cm^3). The excess of solvent was removed under reduced pressure yielding a yellow crude product, which was chromatographed over silica with chloroform–methanol (9:1) yielding compound **15** as a white solid (0.79 g, 85%); mp 82–84 °C (lit.[14] 98–100 °C). (Found: C, 45.88; H, 7.80. $C_8H_{16}O_6$ requires C, 46.15; H, 7.75%); $[\alpha]_D^{23}$ −28.5 (*c* 1.0 in MeOH); ^1H NMR (D_2O): δ 1.11 (3H, t, J=7.1 Hz, CH_3), 3.12 (1H, t, J=9.1 Hz), 3.25 (1H, t, J=9.1 Hz), 3.31–3.38 (2H, m, CH_2), 3.56–3.64 (2H, m), 3.79 (1H, d, J=12.1 Hz), 3.84 (1H, t, J=8.1 Hz,), 4.34 (1H, d, J=8.1 Hz,); ^{13}C NMR (D_2O) δ 14.7, 61.2, 66.6, 70.1, 73.6, 76.3, 76.3, 102.3; *m/z* (CI) $[M+NH_4]^+$226 (100%).

3.1.2. Ethyl 6-*O*-butyryl β-D-glucopyranoside (18). Novozyme® 435 (0.42 g) was added to a solution of ethyl β-D-glucopyranoside **15** (0.42 g, 2.01 mmol) in vinyl butyrate (0.52 cm^3, 4.1 mmol) and THF (10 cm^3). The reaction mixture was stirred for 1 h at 60 °C, after which time the enzyme was filtered off and washed with THF (10 cm^3). The excess THF was evaporated under reduced

pressure to afford a crude residue which was purified by flash chromatography over silica with ethyl acetate–ethanol (95:5) as eluent, yielding compound **18** (0.45 g, 81%) as a white solid; mp 80 °C. (Found: C, 51.92; H, 8.06. $C_{12}H_{22}O_7$ requires C, 51.79; H, 7.97%); $[\alpha]_D^{26}$ −51 (*c* 1.0 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1730 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=8.1 Hz, C*H*$_3$), 1.26 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.63–1.71 (2H, m, C*H*$_2$CH$_3$), 2.36 (2H, t, *J*=8.1 Hz, C*H*$_2$CO), 3.35–3.41 (2H, m), 3.46 (1H, ddd, *J*=2.0, 5.1, 9.1 Hz), 3.55–3.65 (2H, m), 3.96 (1H, qd, *J*=3.0, 14.2 Hz, C*H*$_2$) 4.29 (1H, d, *J*=7.1 Hz), 4.30 (1H, dd, *J*=12.1, 2.0 Hz), 4.49 (1H, dd, *J*=12.1, 5.1 Hz); ^{13}C NMR ($CDCl_3$), δ 13.9 (C*H*$_3$), 15.4 (C*H*$_3$), 18.7 (C*H*$_2$CH$_3$), 36.4 (C*H*$_2$CO), 63.4, 66.0, 70.4, 74.0, 74.4, 76.3, 102.8, 174.9 (CO); *m*/*z* (CI) $[M+NH_4]^+$ 296 (100%).

3.1.3. Ethyl 3,6-*O*-butyryl β-D-glucopyranoside (19) and ethyl 2,6-*O*-butyryl β-D-glucopyranoside (20). Novozyme® (0.21 g) was added to a solution of ethyl 6-*O*-butyryl β-D-glucopyranoside **18** (0.44, 1.59 mmol) and vinyl butyrate (0.4 cm^3, 3.16 mmol) in THF (10 cm^3). The reaction mixture was heated to 60 °C and stirred for 7 days, after which time the enzyme was filtered off and washed with THF (10 cm^3). The solvent was evaporated under reduced pressure to afford a crude residue which was chromatographed over silica with ethyl acetate–ethanol (95:5) as eluent and re-chromatographed over silica gel with ethyl acetate–petroleum ether (2:1) as eluent affording compound **19** (0.28 g, 50%) and compound **20** (0.17 g, 30%). Compound **19**. (Found: C, 54.97; H, 8.17. $C_{16}H_{28}O_8$ requires C, 55.16; H, 8.10%); $[\alpha]_D^{27}$ −17.6 (*c* 1.1 in $CHCl_3$); mp 36–38 °C; ν_{max} ($CHCl_3$)/cm^{-1} 1730 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.94–0.99 (6H, m, C*H*$_3$), 1.26 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.62–1.74 (4H, m, 2×C*H*$_2$CH$_3$), 2.35 (2H, t, *J*=8.1 Hz, C*H*$_2$CO), 2.40 (2H, t, *J*=7.1 Hz, $'$C*H*$_2$CO), 3.46–3.55 (3H, m), 3.63 (1H, qd, *J*=2.0, 16.2 Hz), 3.96 (1H, qd, *J*=3.0, 14.2 Hz, C*H*$_2$), 4.34 (1H, d, *J*=8.1 Hz), 4.35–4.44 (2H, m), 4.93 (1H, t, *J*=9.1 Hz); ^{13}C NMR ($CDCl_3$) δ 13.5 (*C*H$_3$), 13.6 (*C*H$_3$), 15.1 (*C*H$_3$), 18.4 (*C*H$_2$CH$_3$), 18.4 (*C*H$_2$CH$_3$), 36.0 (*C*H$_2$CO), 36.2 (*C*H$_2$CO), 63.0 (*C*H$_2$), 65.7 (*C*H$_2$), 69.4, 72.13, 74.4, 77.6, 102.6, 174.0 (C═O), 175.1 (C═O); *m*/*z* (CI) $[M+NH_4]^+$ 366 (88%). Compound **20**. (Found: C, 54.96; H, 8.15. $C_{16}H_{28}O_8$ requires C, 55.16; H, 8.10%); $[\alpha]_D^{26}$ −48.8 (*c* 1.1 in $CHCl_3$); mp 48 °C; ν_{max} $CHCl_3$/cm^{-1} 1760 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.94–0.99 (6H, m, 2×C*H*$_3$), 1.19 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.63–1.73 (4H, m, 2×C*H*$_2$CH$_3$), 2.36 (4H, t, *J*=7.1 Hz, 2×C*H*$_2$CO), 3.41–3.48 (2H, m), 3.51–3.62 (2H, m), 3.89 (1H, qd, *J*=2.0, 16.2 Hz), 4.32 (1H dd, *J*=2.0, 12.1 Hz), 4.43 (1H, d, *J*=8.1 Hz), 4.44 (1H dd, *J*=2.0, 12.1 Hz), 4.76 (1H, dd, *J*=9.1, 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 13.8 (*C*H$_3$), 13.9 (*C*H$_3$), 15.4 (*C*H$_3$), 18.7 (*C*H$_2$CH$_3$), 18.8 (*C*H$_2$CH$_3$), 36.4 (*C*H$_2$CO), 36.6 (*C*H$_2$CO), 63.3 (*C*H$_2$), 65.7 (*C*H$_2$), 71.1, 74.1, 74.1, 75.7, 101.0, 174.0 and 174.8 (C═O); *m*/*z* (CI) $[M+NH_4]^+$ 366 (37%).

3.1.4. Ethyl α-D-glucopyranoside (17). A mixture of Amberlite IR-120 (H^+) (3.5 g) and anhydrous D-glucose (3.02 g, 16.76 mmol) in ethanol (25 cm^3) was refluxed for 20 h. After cooling and filtration of the resin, excess ethanol was evaporated under reduced pressure affording a yellow syrup which was dissolved in pH 5.0 buffer (25 cm^3) and heated to 35 °C. β-Glucosidase from almonds (0.047 g) was added to the reaction mixture. After 3 days the solution was filtered through Celite® and the buffer evaporated under reduced pressure. The crude product was chromatographed over silica with chloroform–methanol (9:1) as eluent affording **17** as a syrup (1.02 g, 30%). (Found: C, 45.98; H, 7.88. $C_8H_{16}O_6$ required C, 46.15; H, 7.75%); $[\alpha]_D^{22}$ +147.9 (*c* 0.57 in MeOH) [lit.[15] +150 (*c* 1.0 in MeOH)]; ^{1}H NMR (DMSO) δ 1.12 (3H, t, *J*=7.2 Hz, C*H*$_3$), 3.01–3.09 (1H, m), 3.14–3.21 (1H, m), 3.32–3.48 (5H, m), 3.56–3.65 (2H, m), 4.63 (1H, d, *J*=3.9 Hz); ^{13}C NMR (DMSO) δ 15.2 (*C*H$_3$), 61.1, 62.7, 70.4, 70.5, 72.0, 72.0, 98.4; *m*/*z* (CI) $[M+NH_4]^+$ 226 (100%).

3.1.5. Ethyl 6-*O*-butyryl α-D-glucopyranoside (23). A solution of ethyl α-D-glucopyranoside **17** (0.22 g, 1.04 mmol) and vinyl butyrate (0.2 cm^3, 1.58 mmol) in THF (10 cm^3) was prepared and immersed in a oil bath at 60 °C. After 10 min Novozyme® (0.13 g) was added. The mixture was stirred for 6 h, after which time the enzyme was filtered off and washed with THF (5 cm^3). Excess solvent was evaporated under reduced pressure and the crude product was chromatographed over silica with ethyl acetate–ethanol (95:5) as eluent yielding compound **23** as a white solid (0.18 g, 60%). (Found: C, 51.80; H, 7.99. $C_{12}H_{22}O_7$ required C, 51.79; H, 7.97%); mp 70–72 °C; $[\alpha]_D^{21}$ +81.9 (*c* 1.05 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1730 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.25 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.61–1.73 (2H, m, C*H*$_2$CH$_3$), 2.36 (2H, t, *J*=8.1 Hz, C*H*$_2$CO), 3.45 (1H, t, *J*=9.1 Hz), 3.47–3.60 (2H, m), 3.67 (1H, br s, O*H*), 3.72–3.85 (3H, m), 4.26 (1H, dd, *J*=12.1, 2.0 Hz), 4.47 (1H, dd, *J*=12.1, 4.0 Hz), 4.89 (1H, d, *J*=4.0 Hz); ^{13}C NMR ($CDCl_3$) δ 13.6 (*C*H$_3$), 15.0 (*C*H$_3$), 18.4 (*C*H$_2$CH$_3$), 36.0 (*C*H$_2$CO), 63.1 (*C*H$_2$), 63.9, 69.8, 70.0, 72.0, 74.4, 98.0, 174.4 (*C*═O); *m*/*z* (CI) $[M+NH_4]^+$ 296 (100%).

3.2. Separation of anomers of ethyl D-glucopyranoside

Novozyme 435® (0.53 g) was added to a solution of ethyl D-glucopyranoside (0.88 g, 4.24 mmol) and vinyl butyrate (1.1 cm^3, 8.66 mmol) in THF (20 cm^3). The reaction mixture was immersed in a oil bath at 60 °C and stirred for 4 days, after which time the enzyme was filtered off and washed with THF (10 cm^3). The excess solvent was evaporated under reduced pressure and the crude product was chromatographed over silica gel using a gradient of ethyl acetate–petroleum ether (2:1) to ethyl acetate (100%) as eluent, affording compound **19** (0.27 g, 18%), compound **20** (0.19 g, 13%), and compound **23** (0.57 g, 48%).

3.2.1. Benzyl β-D-glucopyranoside (14). β-Glucosidase from almonds (120 mg) was added to a solution of D-glucose (0.81 g, 4.48 mmol) in distilled water (2 cm^3) and benzyl alcohol (18 cm^3). The solution was stirred for 30 h at 50 °C, after which time the enzyme was filtered off and washed with distilled water (5 cm^3). The excess of benzyl alcohol was removed under reduced pressure at 90 °C and 1 mbar. Flash chromatography of the residue over silica with chloroform–methanol (9:1) as the eluent afforded compound **14** (0.52 g, 43%) as a white solid. (Found: C, 57.73; H, 6.77. $C_{13}H_{18}O_6$ requires C, 57.77; H, 6.71%); mp 104 °C (lit.[16] 120–121 °C); $[\alpha]_D^{27}$ −52 (*c* 0.5 in MeOH) [lit.[16] −55.1 (*c* 1.0 in MeOH)]; ν_{max} ($CHCl_3$)/cm^{-1} 1140 (C═O); ^{1}H NMR (DMSO) δ 3.02–3.19 (3H, m),

3.44–3.52 (2H, m), 3.71 (1H, dd, J=11.6, 4.0 Hz), 4.24 (1H, d, J=7.8 Hz), 4.49 (1H, t, J=6.1 Hz), 4.59 (1H, d, J=12.4 Hz, CH_2), 4.83 (1H, d, J=12.4 Hz, $^{'}$CH_2), 4.88 (2H, dd, J=11.6, 4.8 Hz), 7.26–7.41 (5H, m, Ar); ^{13}C NMR (DMSO) δ 48.9, 61.4, 69.7, 70.4, 73.8, 77.0, 77.3, 102.4, 127.6, 127.9, 128.4, 138.4; *m/z* (CI) $[M+NH_4]^+$ 288 (100%).

3.2.2. Benzyl 6-*O*-butyryl β-D-glucopyranoside (21). Novozyme 435® (0.10 g) was added to a solution of β-benzyl D-glucopyranoside **14** (0.31 g, 1.14 mmol) and vinyl butyrate (0.3 cm^3, 2.36 mmol) in dry THF (10 cm^3) and immersed in a oil bath at 60 °C with stirring. After 3 h the reaction was complete by TLC and was cooled to room temperature; the excess solvent was evaporated under reduced pressure. Flash chromatography of the residue over silica with EtOAc–EtOH (95:5) as eluent gave compound **21** as a white solid (0.38 g, 98%). (Found: C, 59.77; H, 7.17. $C_{17}H_{24}O_7$ requires C, 59.99; H, 7.11%); mp 65 °C; $[\alpha]_D^{23}$ −55.7 (*c* 1.1 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1720 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, J=8.1 Hz, CH_3), 1.62–1.71 (2H, m, CH_2CH$_3$), 2.36 (2H, t, J=7.1 Hz, CH_2CO), 3.35–3.43 (2H, m), 3.51 (1H, t, J=8.1 Hz), 4.30–4.32 (1H, m), 4.33 (1H, d, J=7.1 Hz), 4.40 (1H, d, J=4.0 Hz), 4.43 (1H, d, J=4.0 Hz), 4.59 (1H, d, J=12.1 Hz, CH_2Ph) 4.89 (1H, d, J=12.1 Hz, CH_2Ph), 7.27–7.36 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) δ 13.6 (*C*H$_3$), 18.4 (*C*H$_2$CH$_3$), 36.0 (*C*H$_2$CO), 63.1, 70.0, 71.0, 73.5, 73.9, 75.9, 101.4, 128.1, 128.2, 128.5, 136.8, 174.4 (*C*═O); *m/z* (CI) $[M+NH_4]^+$ 358 (100%).

3.2.3. Benzyl α-D-glucopyranoside (16). β-Glucosidase from almonds (0.06 g) was added to a solution of benzyl-D-glucopyranoside (0.42 g, 1.56 mmol) in a citric acid buffer pH 5.0 (20 cm^3). The reaction mixture was stirred for 3 days at 50 °C. The enzyme was filtered off and the buffer was evaporated under reduced pressure. The crude product was chromatographed over silica with chloroform–methanol (9:1) as eluent yielding compound **16** (0.28 g, 67%). (Found: C, 57.49; H, 6.77. $C_{13}H_{18}O_6$ required C, 57.77; H, 6.71%); mp 102 °C (lit.[17] 122 °C); $[\alpha]_D^{22}$ +134.7 (*c* 1.01 in MeOH) [lit.[17]+133.5 (*c* 2.5 in H_2O)]; ^{1}H NMR (DMSO) δ 3.04–3.10 (1H, m), 3.20–3.25 (1H, m), 3.41–3.50 (3H, m), 3.63 (1H, dd, J=6.1, 9.1 Hz), 4.41–4.48 (2H, m), 4.66 (1H, s, O*H*), 4.70 (2H, d, J=6.1 Hz), 4.73 (1H, d, J=3.0 Hz), 4.83 (1H, d, J=5.1 Hz), 7.25–7.38 (5H, m); ^{13}C NMR (DMSO) δ 61.3, 68.2, 70.7, 72.3, 73.4, 73.7, 98.2, 127.6, 127.8, 128.5, 138.5; *m/z* (CI) $[M+NH_4]^+$, 288 (100%).

3.2.4. Benzyl 6-*O*-butyryl α-D-glucopyranoside (22). Vinyl butyrate (0.2 cm^3, 1.58 mmol) was added to a solution of benzyl α-D-glucopyranoside **16** (0.25 g, 0.91 mmol) in THF (20 cm^3) and stirred for 5 min at room temperature. Novozyme 435® (0.10 g) was added and the reaction mixture was heated to 60 °C and stirred for 1 h. The enzyme was filtered and washed with THF (10 cm^3). The solvent was evaporated under reduced pressure and the crude product chromatographed over silica with ethyl acetate–ethanol (95:5) as eluent yielding compound **22** (0.30 g, 97%). (Found: C, 60.20; H, 7.15. $C_{17}H_{24}O_7$ requires C, 59.99; H, 7.11%); mp 72 °C; $[\alpha]_D^{21}$ +77.8 (*c* 1.0 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1729 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t, J=7.1 Hz, CH_3), 1.62–1.71 (2H, m, CH_2CH$_3$), 2.35 (2H, t, J=7.1 Hz, CH_2CO), 3.33 (1H, t, J=9.1 Hz), 3.5 (1H, dd, J=3.0, 9.1 Hz), 3.74–3.78 (2H, m), 4.19 (1H, dd, J=2.0, 12.1 Hz), 4.42 (1H, dd, J=4.0, 12.1 Hz), 4.53 (1H, d, J=11.1 Hz), 4.72 (1H, d, J=11.1 Hz), 4.95 (1H, d, J=4.0 Hz), 7.28–7.37 (5H, m); ^{13}C NMR ($CDCl_3$) δ 14.0 (*C*H$_3$), 14.5 (*C*H$_2$CH$_3$), 18.7 (*C*H$_2$CO), 36.4, 60.7, 63.3, 70.3, 70.4, 70.5, 72.5, 74.7, 98.0, 128.5, 128.9, 137.2, 174.7 (*C*═O). (Found: $[M+NH_4]^+$, 358.186 $C_{17}H_{28}O_7N$ requires $[M+NH_4]^+$, 358.187); *m/z* (CI) $[M+NH_4]^+$ 358 (100%).

3.2.5. Benzyl 2,6-*O*-butyryl α-D-glucopyranoside (24). Lipase PS-C II (0.054 g) was added to a slurry of benzyl 6-*O*-butyryl α-D-glucopyranoside **22** (0.03 g, 0.09 mmol) in vinyl butyrate (5 cm^3) and immersed in a oil bath at 30 °C and stirred for 3 days. The enzyme was filtered off and washed with THF (5 cm^3), before the solvent was evaporated under reduced pressure. Flash chromatography of the crude residue over silica with ethyl acetate–petroleum ether (2:1) as eluent afforded compound **24** as a yellow syrup (0.032 g, 86%); $[\alpha]_D^{24}$ +46 (*c* 0.5 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1720 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, J=8.1 Hz, CH_3), 0.97 (3H, t, J=7.1 Hz, CH_3), 1.59–1.73 (4H, m, 2×CH_2CH_3), 2.39–2.39 (4H, m, 2×CH_2CO), 3.41 (1H, t, J=10.1 Hz), 3.82 (1H, ddd, J=10.1, 4.0, 2.0 Hz), 4.04 (1H, t, J=10.1 Hz), 4.17 (1H, dd, J=12.1, 2.0 Hz), 4.48–4.53 (2H, m), 4.67–4.71 (2H, m), 5.11 (1H, d, J=4.0 Hz), 7.27–7.36 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.0, 18.5, 18.7, 36.3, 36.4, 63.1, 70.2, 70.2, 70.9, 71.7, 73.3, 95.9, 128.2, 128.3, 128.8, 137.4, 173.8, 174.9. (Found: $[M]^+$411.203 $C_{21}H_{31}O_8$ requires $[M]^+$, 411.202); *m/z* (CI) $[M]^+$ 411 (6%).

3.2.6. 2,6-*O*-Butyryl-D-glucopyranoside (25). Benzyl 2,6-butyryl α-D-glucopyranoside **24** (0.35 g, 0.86 mmol) was dissolved in ethyl acetate, followed by the addition of Pd/C (10%) (0.11 g). The reaction mixture was purged with hydrogen and then placed under hydrogen pressure (0.2 bar), with stirring, for 7 days at room temperature. After completion of the reaction, the catalyst was filtered off and washed with ethyl acetate. The solvent was evaporated under reduced pressure and the crude product was chromatographed over silica using ethyl acetate–petroleum ether (2:1) as eluent affording compound **25** (0.22 g, 75%), as a mixture of α- and β-anomers. (Found: C, 52.63; H, 7.62. $C_{14}H_{24}O_8$ requires C, 52.49; H, 7.55%); $[\alpha]_D^{22}$ +49.6 (*c* 0.6 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1740 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.93–0.99 (12H, m), 1.62–1.72 (8H, m) 2.33–2.41 (8H, m), 3.23 (1H, bs), 3.43 (2H, t, J=9.2 Hz), 3.52 (1H, ddd, J=9.9, 4.8, 2.2 Hz), 3.63–3.71 (3H, m), 3.78 (1H, bs) 4.00–4.05 (3H, m), 4.33 (2H, m), 4.43–4.49 (2H, m), 4.64 (1H, t, J=7.3 Hz), 4.67–4.72 (2H, m), 5.41 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 13.9, 13.9 and 14.0, 18.7, 18.7, 36.3, 36.3, 60.8, 63.3, 63.4, 69.9, 70.9, 71.2 73.5, 74.5, 74.7, 75.8, 90.9, 95.9, 175.2 and 175.2 (*C*═O); *m/z* (CI) $[M+NH_4]^+$ 338 (64%).

3.3. Separation of anomers from benzyl D-glucopyranoside

Novozyme 435® (0.054 g) and lipase PS-C II (0.22 g) was added to benzyl D-glucopyranoside (0.31 g, 1.15 mmol) in vinyl butyrate (5 cm^3). The reaction was stirred for 4 days at

40 °C, after which time the enzymes were filtered off and washed with THF (10 cm^3). Excess solvent was evaporated under reduced pressure and the crude product chromatographed over silica with ethyl acetate–petroleum ether (2:1) as eluent affording compound **21** (0.141 g, 36%) and compound **24** (0.13 g, 28%).

3.3.1. Benzyl 6-*O*-butyryl α-D-galactopyranoside (28) and benzyl 2,6-*O*-butyryl β-D-galactopyranoside (29). Novozyme 435® (0.12 g) was added to a solution of benzyl D-galactopyranoside (0.12 g, 0.43 mmol) and vinyl butyrate (0.1 cm^3, 0.79 mmol) in THF (10 cm^3). The reaction mixture was heated to 60 °C and stirred for 7.5 h; the enzyme was filtered off and washed with THF (10 cm^3). Excess solvent was evaporated under reduced pressure, and the crude product was chromatographed over silica with ethyl acetate–ethanol (95:5) as eluent yielding compound **28** (0.067 g, 46%) and compound **29** (0.041 g, 23%). Compound **28**. (Found: C, 59.94; H, 7.12. $C_{17}H_{24}O_7$ requires C, 59.99; H, 7.11%); $[\alpha]_D^{21}$ +92.5 (*c* 0.56 in $CHCl_3$); mp 106–108 °C; ν_{max} ($CHCl_3$)/cm^{-1} 1735 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=7.6 Hz, C*H*$_3$), 1.62–1.72 (2H, m, C*H*$_2$CH$_3$), 2.34 (2H, t, *J*=7.6 Hz, C*H*$_2$CO), 3.79–3.87 (2H, m), 3.97 (1H, d, *J*=2.0 Hz), 4.01 (1H, t, *J*=6.6 Hz), 4.23 (1H, dd, *J*=11.6, 6.5 Hz), 4.41 (1H, dd, *J*=11.6, 6.1 Hz), 4.54 (1H, d, *J*=11.6 Hz, C*H*$_2$Ph), 4.76 (1H, d, *J*=11.6 Hz), 5.05 (1H, d, *J*=3.5 Hz), 7.31–7.40 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) δ 13.7 (*C*H$_3$), 18.3 (*C*H$_2$), 36.0 (*C*H$_2$), 63.0, 68.3, 68.8, 69.4, 69.8, 70.8, 97.6, 128.1, 128.6, 136.7, 173.7(*C*═O; *m/z* (CI) 358 $[M+NH_4]^+$. Compound **29**. (Found: C, 61.33; H, 7.37. $C_{21}H_{30}O_8$ require C, 61.45; H, 7.37%); $[\alpha]_D^{22}$ −24.2 (*c* 0.8 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1738 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, *J*=7.6 Hz, CH$_3$), 0.97 (3H, t, *J*=7.6 Hz, $'$CH$_3$), 1.60–1.72 (4H, m, C*H*$_2$CH$_3$), 2.29–2.36 (4H, m, C*H*$_2$CO), 3.60–3.67 (2H, m), 3.87 (1H, d, *J*=2.9 Hz), 4.32 (1H, dd, *J*=11.4, 6.7 Hz), 4.43 (1H, dd, *J*=11.4, 6.4 Hz), 4.45 (1H, d, *J*=7.9 Hz), 4.62 (1H, d, *J*=12.1 Hz), 4.88 (1H, d, *J*=12.1 Hz), 5.01 (1H, dd, *J*=7.9, 9.5 Hz), 7.26–7.36 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.0, 18.7, 18.7, 36.4, 36.5, 62.7, 69.1, 70.7, 72.5, 73.1, 73.6, 99.8, 128.2, 128.2, 128.7, 137.3, 174.0 and 174.6 (*C*═O); *m/z* (CI) 428 $[M+NH_4]^+$.

3.3.2. Benzyl α-D-galactopyranoside (27). Benzyl 6-*O*-butyryl α-D-galactopyranoside **28** (0.065 g, 0.19 mmol) was added to a solution of sodium methoxide in methanol (0.08 M 10 cm^3). The reaction mixture was stirred for 3 min after which time the solvent was evaporated under reduced pressure affording a crude product, which was chromatographed over silica with chloroform–methanol (9:1) as eluent, yielding compound **27** as a syrup (0.047 g, 91%); $[\alpha]_D^{24}$ +96.1 (*c* 1.55 in MeOH); ^{1}H NMR (DMSO) δ 3.41–3.56 (2H, m), 3.59–3.69 (3H, m) 3.73 (1H, br s, *O*H), 4.31–4.34 (1H, br s, O*H*), 4.23 (1H, d, *J*=12.1 Hz), 4.49–4.55 (3H, m), 4.68 (1H, d, *J*=12.1 Hz), 4.76 (1H, d, *J*=3.2 Hz), 7.26–7.69 (5H, m, Ar); ^{13}C NMR (DMSO) δ 61.0, 68.2, 68.7, 69.2, 70.0, 71.8, 98.6, 127.6, 127.8, 128.4, 138.5. (Found: $[M+NH_4]^+$, 288.145 $C_{13}H_{18}O_6N$ requires $[M+NH_4]^+$, 288.145); *m/z* (CI) $[M+NH_4]^+$ 288 (100%).

Acknowledgements

The generosity of BAT and FCT (Fundação para a Ciência e Tecnologia) is acknowledged (studentship to P.G.).

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