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Note

Regiospecific enzymic acylation of butyl α-D-glucopyranoside

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Acylated sugars are important sources of surfactants and pharmaceutical materials.

Regioselective acylation is necessary for the synthesis of carbohydrate derivatives where selective protection and deprotection of hydroxyl groups is critical[1-2]. Hydrolytic enzymes such as lipases[3-7] or proteases[8,9] can effect novel regioselective acylation of sugars in non-aqueous solvents.

Therisod and Klibanov[6] showed that porcine pancreatic lipase (PPL) in anhydrous pyridine catalyses the esterification of primary hydroxyl groups in monosaccharides by trichloroethyl carboxylates.

Proteases[8,9] also catalyse the transesterification of sugars in anhydrous *N*,*N*-dimethylformamide or pyridine with the same regioselectivity. Successive acylations of sugars using PPL or lipases from *Chromobacterium viscosum* (CVL), *Candida cylindracea* (CCL), and *Aspergillus niger* (ANL) have been reported[10]. Acylation initially occurs at position 6, followed by positions 2 or 3. The mixtures of 2,6- and 3,6-diesters formed from 6-*O*-acyl derivatives of D-glucose, D-galactose, or D-mannose reflect the enhancement of the regioselectivity when the size of the substituent at C-6 is increased. *n*-Alkyl β-D-glucopyranosides can also be used for the selective formation of diesters.

An attempt to rationalise the regioselective transesterification reaction for the diacylation of sugars by activated butyric esters has been published[11]. The importance of a specific sequence of hydroxyl groups in the substrate which determines the orientation at the active site was pointed out. Starting from methyl 6-*O*-butyryl-

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α-D-galacto- or -manno-pyranoside, with PPL, CCL, or *Pseudomonas fluorescens* lipase (PFL) in pyridine, mixtures of 2,6-, 3,6, and 4,6-diesters were obtained.

Other reactions have been developed in solvent-free media for the enzymic production of n-alkyl 6-*O*-acyl-α-D-glucopyranosides. Although high conversions were obtained, contamination by 2–14% of diesters was observed[12,13].

We now report reaction conditions that lead to high yields of diesters in a regiospecific manner.

The esters were obtained in high yield by enzymic reverse hydrolysis which involved the azeotropic removal of water from mixtures of the acid and substrate in hexane. The use of an apolar solvent such as hexane limits acyl-group migration. The purity of the products was determined by GLC after silylation[14] and characterised on the basis of ^{13}C NMR data (Table I) that accorded with those of Yoshimoto et al.[15].

Butyl α-D-glucopyranoside (**1**) was obtained by treatment of a commercial α,β-mixture with almond-meal β-D-glucosidase.

The 6-*O*-lauroyl (**2**), 6-*O*-palmitoyl (**3**), 6-*O*-stearoyl (**4**), and 6-*O*-oleoyl (**5**) derivatives of **1** were prepared by reverse hydrolysis, using two equivalents of the appropriate fatty acid and Lipozyme^R. The progress of each reaction was monitored by TLC.

Lipozyme^R catalysed the esterification of **1** at position 6 as indicated by the ^{13}C NMR data of the products (Table I: downfield shifts of 2 and 0.3 ppm for the resonances of C-6 and C-4, respectively; upfield shift of 1.4 ppm for that of C-5).

When monoester synthesis was performed with **1** and stearic acid by a solvent-free process[12,13], a mixture of the 6- (**4**), 2,6-di- (**6**), and 3-*O*-stearoyl (**7**) derivatives was obtained, the composition of which was determined by calculation of the ^{13}C chemical shifts according to the literature[15] (Table I).

Thus, with hexane as the solvent, the regiospecificity of the Lipozyme^R-mediated esterifications was enhanced, as was the yield of monoesters uncontaminated by diesters, in contrast to solid-phase synthesis[12,13].

TABLE I

^{13}C NMR data ($CDCl_3$) for the carbohydrate moiety in derivatives of butyl α-D-glucopyranoside (**1**): **2**, 6-*O*-lauroyl; **3**, 6-*O*-palmitoyl; **4**, 6-*O*-stearoyl; **5**, 6-*O*-oleoyl; **6**, 2,6-di-*O*-stearoyl; **7**, 3-*O*-stearoyl; and **8**, 2-*O*-lauroyl-6-*O*-stearoyl

Compound	C-1	C-2	C-3	C-4	C-5	C-6
1	98.65	71.96	74.40	69.49	71.64	61.15
2	98.26	72.13	74.41	69.88	70.24	63.42
3	98.29	72.05	74.32	69.86	70.36	63.57
4	98.27	72.08	74.36	69.87	70.31	63.40
5	98.28	72.09	74.38	69.87	70.30	63.49
6	96.09	73.25	71.33	70.55	69.53	62.98
7	98.46	72.00	74.25	69.46	71.67	61.34
8	96.01	73.24	71.32	70.56	69.53	63.03

The reaction of **1** and 2 equiv of fatty acid, with Lipozyme^R in hexane for 5 days, gave 15% of the diesters. In contrast, the reaction of the 6-*O*-stearoyl derivative **4**, which is highly soluble in boiling hexane, with 1 equiv of stearic acid gave > 95% of the 2,6-diester **6** after 5 days. Likewise, **4** reacted with 1 equiv of lauric acid to give 96% of the 2-*O*-lauroyl-6-*O*-stearoyl derivative **8**.

EXPERIMENTAL

General methods.—The ^1H and ^{13}C NMR spectra were recorded for solutions in $CDCl_3$ with a Bruker AC 250 spectrometer. Optical rotations were measured at 20°C with a Perkin–Elmer 241 polarimeter. IR spectra were recorded variously on thin films between NaCl plates or in KBr discs with a Perkin–Elmer 1600 Sencs FTIR spectrometer. GLC was performed on a 5890 Series II gas chromatograph, with a 12-m OV1 capillary fused-silica gel column coated with methylsilicone gum (0.22 mm i.d., 0.33 μm film thickness, N_2 carrier gas at 30 mL/min, 80 to 240°C at 10°C/min, injector and detector port at 250°C). TLC was performed on Silica Gel 60 WF 254 (Merck) with detection by 0.2% anthrone in H_2SO_4 at 110°C for 5 min. Flash-column chromatography was performed on Matrex Amicon Silica Gel (35–70 μm). Lipozyme^R (Novo Industry) was used without modification. Experiments were carried out either in sealed flasks at 75°C without solvent or with a Dean and Stark aparatus.

The proton of butyl, and 6-*O*- and 2-*O*-acyl substituents are designated H′, H″, and H‴, respectively.

Butyl α-D-glucopyranoside (**1**).—A solution of a commercial α,β-mixture (50 g dry weight; 60% of the α form; a gift from Cerestar Holding) in 10 mM acetate buffer (277 mL, pH 5) was incubated with almond meal (27 g) for 18 h at 50°C when the β anomer had disappeared completely (HPLC). The mixture was filtered, then concentrated to dryness, and **1** (25 g, 83%), mp 64°C (lit.[16] 86–87°C), was extracted from the residue with hot CH_2Cl_2 and used without further purification. ^1H NMR data: δ 0.91 (t, 3 H, *J* 7.1 Hz, H-4′), 1.38 (m, 2 H, H-3′), 1.59 (m, 2 H, H-2′), 3.65 (c, 7 H, H-1′,2,4,5,6), 4.11 (s, 4 H, OH), 4.84 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1). For the ^{13}C NMR data, see Table I.

6-O-Acyl derivatives of butyl α-D-glucopyranoside.—(*a*) Lauric acid (1.7 g, 8.5 mmol) and **1** (1 g , 4.23 mmol) were added to boiling hexane (a gummy mixture was obtained when the reactants were mixed at room temperature) in a Dean and Stark apparatus. Lipozyme^R (0.15 g) was added and the suspension was stirred at 70°C for 3 days, after which time the formation of diester was detected. The mixture was filtered and the solvent was evaporated under reduced pressure. Flash-column chromatography (94:6 CH_2Cl_2–MeOH) of the residue (2.60 g) gave the 6-*O*-lauroyl derivative **2** (1.4 g, 80%); mp 25°C; $[\alpha]_D^{20}$ +29° (*c* 1. 3, $CHCl_3$); ν_{max}^{film} 3490 (OH), 2980 (CH), and 1730 cm^{-1} (C=O). ^1H NMR data: δ 0.89 (m, 6 H, H-4′, 12″), 1.42 (m, 22 H, H-2′,3′ and H-3″/11″), 2.33 (t, 2 H, *J* 7.8 Hz, H-2″), 3.70 (m, 9 H, H-1′,2,3,4,5 and OH), 4.31 (m, 2 H, H-6), 4.81 (d, 1 H, $J_{1,2}$ 3.78 Hz, H-1). For

TABLE II

Data on the 6-*O*-acyl derivatives (**3**–**5**) of butyl α-D-glucopyranoside

6-*O*-Acyl group	Mp (°C)	Yield (%)	$[\alpha]_D^{20}$ (*c* 1.3, $CHCl_3$)	Formula	Calcd	Found
Palmitoyl (**3**)	30	78	+43°	$C_{28}H_{50}O_7$	C, 64.56 H, 10.62	64.38 10.65
Stearoyl (**4**)	37	78	+36°	$C_{30}H_{54}O_7$	C, 66.89 H, 10.82	66.60 11.08
Oleoyl (**5**)	oil	80.5	+32°	$C_{30}H_{52}O_7$	C, 65.98 H, 10.48	65.85 10.39

the ^{13}C NMR data, see Table I. Anal. Calcd for $C_{22}H_{42}O_7$: C, 63.10; H, 10.04. Found: C, 62.72; H, 10.04.

(*b*) Using the procedure in (*a*) the 6-*O*-palmitoyl (**3**), 6-*O*-stearoyl (**4**), and 6-*O*-oleoyl (**5**) derivatives of **1** were prepared, data for which are recorded in Table II.

(*c*) To a solution of **1** (1 g, 4.23 mmol) in stearic acid (2.41 g, 8.46 mmol) at 75°C was added Lipozyme^R (0.15 g). After 72 h, ^{13}C NMR spectroscopy was used to identify the products as a mixture of the 6- (**4**), 2,6-di- (**6**), and 3-*O*-acyl (**7**) derivatives (see Table I).

Butyl 2,6-di-O-stearoyl-α-D-glucopyranoside (**6**).—The synthesis of **6** from **4** and 1 equiv of stearic acid was performed as in (*a*). Flash-column chromatography (98:2 CH_2Cl_2–MeOH) of the crude product gave **6** (96%); mp 42°C; $[\alpha]_D^{20}$ +34° (*c* 1.3, $CHCl_3$); ν_{max}^{KBr} 3480 (OH), 2970 (CH), and 1730 cm^{-1} (C=O). ^1H NMR data: δ 0.86 (t, 9 H, H-4′,16″,16‴), 1.51 (m, 66 H, H-3″/15″ and H-3‴/15‴), 2.36 (t, 4 H, H-2″,2‴), 2.88 (s, 2 H, OH), 3.78 (m, 4 H, H-2′,4,5), 3.97 (d, 1 H, *J* 9.88 Hz, H-3), 4.22 (dd, 1 H, *J* 2.19 and 12.25 Hz, H-6), 4.50 (dd, 9 H, *J* 4.21 and 12.25 Hz, H-6), 4.63 (dd, 1 H, *J* 3.75 and 10.02 Hz, H-2), 5.01 (d, 1 H, $J_{1,2}$ 3.72 Hz, H-1). For the ^{13}C NMR data, see Table I. Anal. Calcd for $C_{46}H_{88}O_8$: C, 71.82; H, 11.53. Found: C, 71.74; H, 11.52.

No esters were formed in the absence of the enzyme.

Butyl 2-O-lauroyl-6-O-stearoyl-α-D-glucopyranoside (**8**)—Reaction of **4** (1 g, 1.99 mmol) and lauric acid (0.4 g, 1.99 mmol), as described above for **6**, yielded **8** (1.30 g, 96%); mp 39°C; $[\alpha]_D^{20}$ +58° (*c* 1.16, $CHCl_3$). Mass spectrum (DCI, NH_3^+): *m/z* 618 (M^++17), 601 (M^+). For the ^{13}C NMR data, see Table I. Anal. Calcd for $C_{34}H_{64}O_8$: C, 67.96; H, 10.73. Found: C, 67.92; H, 11.10.

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