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Versatile Enzymatic Diacid Ester Synthesis of Butyl α-D-Glucopyranoside.

Jean FABRE, Didier BETBEDER, François PAUL*, Pierre MONSAN, Jacques PERIE✧

BioEurope, 4 impasse Didier Daurat BP 4196, 31031 Toulouse, FRANCE

✧Laboratoire de Chimie Organique Biologique, U R.A. C.N.R.S. 470, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse cedex, FRANCE

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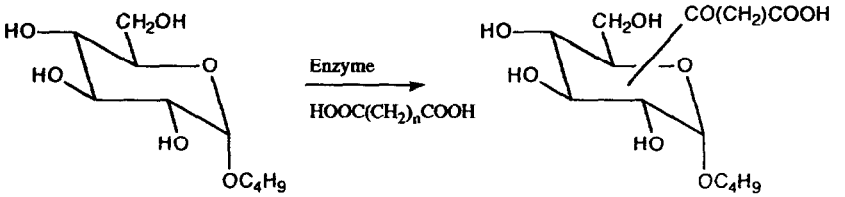
Abstract: The enzymatic esterification of butyl α-D-glucopyranoside by diacids was investigated with pig pancreatic lipase in acetone. The nature of the acylating agent influenced the regiospecificity of the reaction. A chemoenzymatic reaction scheme was developed to obtain diacid esters using an activated diacid as acyl donor, in a solvent-free process with the immobilized lipase of *Mucor miehei* (Lipozyme®).

INTRODUCTION

The chemical reactivity of alkyl glucopyranoside fatty acid esters is restricted to the free hydroxyl groups of the alkyl glucopyranoside moiety. Introducing a carboxylic acid function on these compounds by an enzymatic process (Scheme 1) may be of interest either as such or as intermediates for further synthesis.



Scheme 1. Enzymatic synthesis of alkyl glucopyranoside diacid esters bearing a carboxylic function.

The use of a diacid in an enzymatic or chemoenzymatic approach has been recently reviewed in the field of polymers[1]. Diacid esters are easily obtainable with anhydride, as recently demonstrated by Aranda and Blanco[2] in the enzymatic synthesis of 3-O-succinyl glycerol from succinic anhydride, glycerol and *Pseudomonas cepacia* lipase in diisopropylether. Under the same conditions, no reaction was observed with porcine pancreatic lipase (PPL).

The aim of this paper is to report an enzymatic alkyl glucopyranoside diacid synthesis, using either free diacids or anhydride with PPL or di-(2,2,2 trichloroethyl) adipate as the acylating agent as well as the solvent using Lipozyme®

RESULTS AND DISCUSSION

Esterification of 1 using PPL

Compound **1** was submitted to enzymatic esterification with a diacid (succinic, adipic, and hexadecanedioic acid), under the optimum reaction conditions determined for lauric acid (see experimental part). Table 1 summarizes the yields and the nature of the products characterized as previously reported [3,4].

Table 1: Esterification of **1** by various Diacids with PPL compared to Results obtained with Lauric Acid.

Acylating agent	Yield (a)	Monoester derivative of **1**
lauric acid	60 %	6-O-lauroyl
succinic acid	n.d.	-
adipic acid	n.d.	-
hexadecanedioic acid	47 %	6-O-hexadecanedioyl

(a) after 192 hours incubation

n.d. no reaction detected by t.l.c.

PPL was found to be active in acetone, although Gao *et al.*[5] recently reported negative results under similar conditions. Esterification of **1** by lauric or hexadecanedioic acid with PPL was regiospecific since the 6-O-acyl derivative of **1** was the only reaction product unseen in the transesterification reaction between glucose and 2, 2, 2-trichloroethyl butyrate with PPL[6]. The esterification of **1** by reverse hydrolysis with short- to medium-chain diacids (succinic and adipic acids) was not possible to any extent using PPL in acetone.

Table 2 : Esterification of Butyl α-D-glucopyranoside **1** with Acetic and Succinic Anhydrides.

Acylating agent	Overall yield	Monoester (a)	Diester
acetic anhydride	34 %	29 % 6-O-acetoyl 5 % 3-O-acetoyl	-
succinic anhydride	28 %	14 % 3-O-succinyl 7 % 6-O-succinyl	7 % 3-6-di-O succinyl

(a) The proportions of the different monoesters were determined on the basis of ^{13}C NMR spectra.

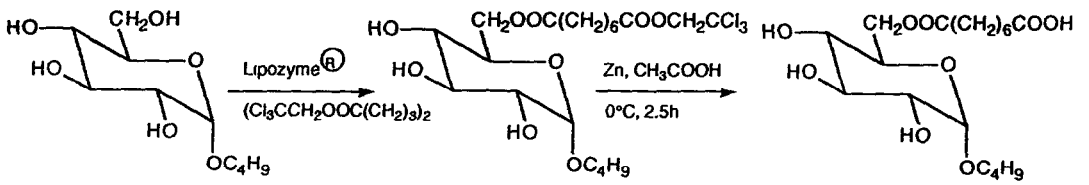
In order to achieve acylation with short-chain diacids, an activated form of the acylating agent was chosen, namely succinic anhydride. Table 2 summarizes the results obtained with succinic and acetic anhydrides.

The enzyme regiospecificity was lost when succinic or acetic anhydrides were used, since two monoester derivatives of **1** were obtained (Table 2). Moreover, a diester was obtained, namely the 3,6-di-O-succinyl derivative of **1**. No reaction occurred without the enzyme under the same conditions.

The acylating agent (succinic or acetic anhydrides) induces a change in enzyme regioselectivity, as is the case for the solvent[7], the alkyl glucopyranoside hydroxyl sequence[8], and the steric hindrance generated around the alkyl glucopyranoside primary hydroxyl group[9]. The modification of the regiospecificity of a given enzyme by an acyl donor has thus been demonstrated.

In order to rationalize enzymatic diacid ester synthesis in a regiospecific manner, the following synthetic pathway was adopted.

Transesterification using Lipozyme®



7 **8**

Scheme 2: Chemoenzymatic pathway of diacid ester synthesis.

Enzymatic production of *n*-alkyl 6-O-acyl-α-D-glucopyranoside has been developed in solvent-free media (molten fatty acid) with Lipozyme®[10,11]. High monoester conversion yields are obtained. However, the diacid melting point is too high (adipic acid m.p.: 152-154°C) to allow such a reaction.

In contrast, esterifying the diacid with 2 equivalents of 2,2,2-trichloroethanol resulted in a lower m.p. (< 45°C), and increased reactivity of the acyl moiety[6,9].

Under these conditions, the reaction was successfully performed in molten di-(2 ,2 ,2-trichloroethyl) adipate with **1** and Lipozyme® to yield (**7**) (38 %) and (**8**) (52 %) after 48 hours. Moreover, (**7**) was selectively hydrolyzed using Zn/AcOH, 2.5 hours leading to (**8**), according to Woodward *et al* [12], using the reactivity of the chlorinated ester towards the Lewis acid. No diester nor polymerization was observed in these highly concentrated media.

It is interesting to note that the use of an activated diester such as di-(2, 2, 2-trichloroethyl) adipate allowed the rapid transesterification of butyl α-D-glucopyranoside in a regiospecific manner and paved the way for the subsequent chemical hydrolysis of the unreacted activated ester part. This means of activation is thus the cornerstone of the chemoenzymatic synthesis scheme which couples an enzymatically regiospecific esterification with the chemically selective removal of the unreacted activated esters, leading to sugar derivatives bearing a carboxylic function separated by a spacer from the 6-carbon position.

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra were recorded for solutions in CD_3OD or $CDCl_3$ with a Bruker AC 250 spectrometer. Optical rotations were measured at 20°C with a Perkin-Elmer 241 polarimeter. Mass spectrometry analyses were performed using a MERMAF R 10.10 apparatus (DCI, NH_4^+). The water content was measured with a Melter DL 18 Karl Fischer titrator. T.l.c. was performed on silica gel 60 WF 254 plates (Merck) with detection by a reaction with a 0.2 % anthrone solution in H_2SO_4 at 110°C for 5 min. Flash column chromatography was performed on Matrex Amicon silica gel (35-70 mesh). Lipozyme® (NOVO Industry, lipase from *Mucor meihie* adsorbed on anionic resin duolite 568, 48 BUI/g, batch LUC 0004-4) was used without modification, as was PPL (Sigma, Type II, crude, 39 U/mg of protein (triacetin), batch 70H0688). Experiments were carried out in sealed flasks at 45°C with acetone for PPL and without solvent for Lipozyme®. The protons of butyl, 6-O-, and 3-O-acyl substituents are designated H', H" and H"' respectively.

Substrate Synthesis

Di-(2 ,2 ,2-trichloroethyl) adipate was synthesized according to the procedure developed by Patil *et al.*[13]. Butyl α-D-glucopyranoside **1** and butyl 6-O-lauroyl-α-D-glucopyranoside **2** were prepared as previously reported[14].

Diacid solubility is a critical problem which was overcome by screening various solvents, in which the starting alkyl glucopyranoside is also freely soluble[15]. Acetone and t-amyl alcohol were selected. PPL was tested to acylate **1** with lauric acid at 45°C. Conversion was followed by t.l.c. for 72 hours. PPL was found to be active in both solvents (especially in acetone). Lipozyme® was only used in solvent-free processes during transesterification reactions with an activated acylating agent.

Thus, the reaction with PPL was performed in acetone for 192 hours with lauric acid as model. The optimum water content was determined as being 1 % (v/v) (Karl-Fischer titration). The final product **2** was separated by flash column chromatography on silica gel. The acylation position was determined by calculation of ^{13}C chemical shifts according to data taken from the literature[3,4] and is in accordance with previously published results[14].

General procedure for PPL-catalyzed esterification of compound 1

1 (1 g, 4.24 mmoles) was dissolved in 15 ml acetone with one equivalent of the acyl donor and 0.5 g of PPL in a sealed vial. The suspension was stirred (250 rpm) and the reaction progress followed by t.l.c. analysis. After 72 h, the enzyme is filtered out, the solvent evaporated under reduced pressure and the resulting powder purified on a flash chromatographic column.

Butyl 6-O-lauroyl-α-D-glucopyranoside (2). **1** was esterified with lauric acid and PPL to yield butyl 6-O-lauroyl-α-D-glucopyranoside (**2**) in acetone. The best yield (60 %) was obtained with 1 % water content, determined with a Karl Fischer titrator, m.p. 25°C, $[\alpha]_D$ + 29° (C1.3, chloroform). Anal. Calcd. for $C_{22}H_{42}O_7$: C, 63.10; H, 10.04. Found: C, 62.72; H, 10.04. ^1H NMR ($CDCl_3$): 0.89 (m, 6H) H_4', $H_{12}"$; 1.42 (m, 22H) H_2', H_3', $H_3"$ to $H_{11}"$; 2.33 (t, J = 7,8 Hz, 2H) $H_2"$; 3.70 (m, 9H) H_2', H_3', H_4', H_5', H_1' and 2 OH; 4.31 (m, 2H) H_6; 4.87 (d, J = 3.78 Hz, 1H) H_1. For ^{13}C NMR ($CDCl_3$) data, see Table 3.

Butyl 6-O-acetoyl- and 3-O-acetoyl-α-D-glucopyranoside mixture (3). PPL catalyzed the esterification of compound **1** (0.5 g, 2.12 mmoles) with acetic anhydride (0.2 ml, 2.12 mmoles) followed by flash chromatography (CH_2Cl_2/MeOH 95/5) of the reaction mixture, yielding mixture (**3**), the composition of which was determined by calculation of the ^{13}C chemical shifts according to the literature[3,4] (29 % 6-O-acetoyl and 5 % 3-O-acetoyl derivative of **1** (0.2 g, 0.719 mmoles)), oil, $[\alpha]_D$ +15° (C 0.34, methanol), Anal. Calcd for $C_{12}H_{22}O_7$: C, 51.80; H, 7.91. Found: C, 51.80; H 8.01. For ^{13}C-NMR (CD_3OD) data, see Table 3.

Butyl 3,6-di-O-succinyl-α-D-glucopyranoside (4). As above, butyl α-D-glucopyranoside (**1**) (1 g, 4.24 mmoles) and succinic anhydride (0.42 g, 4.24 mmoles) afforded, after flash chromatography, pure butyl 3,6-di-O-succinyl-α-D-glucopyranoside **4** (0.130 g, 0.3 mmole) together with a mixture **5** of 7 % 6-O-succinyl and 14 % 3-O-succinyl ester derivatives of **1**, the composition of which was determined as reported for **3**.

4. $[\alpha]_D$ +12° (C 0.5, methanol)· ^{1}H NMR (CD_3OD); 0.96 (t, J = 7.28 Hz, 3H) H_4'; 1.38 (m, 2H) H_3'; 1.53 (m, 2H) H_2', 2.66 (m, 4H) H_2", H_3", H_2"', H_3"'; 3.80 (m, 4) H_4, H_5, H_1'; 4.20 (dd, J = 7.0 Hz, J = 16.0 Hz, 1H) H_6; 4.30 (dd, J = 3.75 Hz, J = 16.0 Hz, 1H) H_6; 4.78 (d, J = 7.74 Hz, 1H) H_1; 5.10 (s) OH; 5.20 (dd, J = 10.50 Hz, 1H) H_3. Mass spectrometry (DCl, NH_4^+) m/z 436, (M^+ + 17) 454, 354, 254. For ^{13}C NMR (CD_3OD) data, see Table 3.

Butyl 6-O-hexadecanedioyl-α-D-glucopyranoside (6). According to the same procedure, butyl 6-O-hexadecanedioyl-α-D-glucopyranoside (3 g, 6 mmoles) was obtained from **1** (3 g, 12.72 mmoles), PPL (1.5 g) and hexadecanedioic acid (3.6 g, 12.7 mmoles) at 45°C in 45 ml of acetone with a 47 % yield, $[\alpha]_D$ + 30° (C 1, methanol). Anal. Calcd $C_{26}H_{48}O_9$ 1.5H_2O. C, 58.63; H, 9.09 Found; C, 58.63; H, 9.58. Mass spectrometry (DCI, NH_4^+) m/z 522 (M^+ + 17), 505 (M^+) ^{1}H NMR ($CDCl_3$) (cf. compound **2**). For ^{13}C NMR ($CDCl_3$), see Table 3.

General procedure for Lipozyme® esterification

Esterification of **1** (0.5 g, 2.12 mmoles) was achieved by molten di-(2,2,2-trichloroethyl) adipate (3.46 g, 8.47 mmoles) / Lipozyme® (0.5 g) in sealed vials. The reaction progress was followed for 72 hours by t.l.c.. Then the reaction medium was dissolved in dichloromethane, and the enzyme removed by filtration. The solvent was eliminated under reduced pressure to give a yellowish syrup (3.9 g) which was purified by flash chromatography to give pure butyl 6-O-(2, 2, 2-trichloroethyl adipate)-α-D-glucopyranoside (**7**) (0.4 g, 0.8 mmole) and butyl 6-O-(adipyl)-α-D-glucopyranoside (**8**) (0.4 g, 1.10 mmoles).

Butyl 6-O-(2,2,2-trichloroethyl adipate)-α-D-glucopyranoside (7). Oil, $[\alpha]_D$ + 45° (C 0.55, methanol). Anal. Calcd for $C_{18}H_{29}O_9Cl_3$: C, 43.54; H, 5.89. Found: C, 43.61; H, 6.06. ^{1}H-NMR (CD_3OD) (cf. compound **2**). For ^{13}C-NMR (CD_3OD) data, see Table 3.

Butyl 6-O-adipyl-α-D-glucopyranoside (8). Oil, $[\alpha]_D$ + 1° (C 1, methanol). Anal. Calcd for $C_{16}H_{28}O_9$, 1 H_2O: C, 50.25; H, 7.90. Found: C, 50.47; H, 7.87. ^{1}H-NMR (CD_3OD) (cf. compound **2**), For ^{13}C-NMR (CD_3OD) data, see Table 3.

Chemical hydrolysis of 7 into 8. 0.100 g (0.202 10^{-3} mole) of **7** was dissolved in 10 ml of acetic acid at 0°C. 50 mg of zinc dust was added and the suspension stirred for 2.5 h, until the total disappearance of the starting product followed by t.l.c. (CH_2Cl_2/MeOH 9/1) (Rf. values **7**: 0.30; **8**: 0.20).

Table 3: ^{13}C-NMR data, $CDCl_3$ or CD_3OD* for the Butyl α-D-glucopyranoside Moiety in **1-8**

Compound		C-1	C-2	C-3	C-4	C-5	C-6
1		98.65 (100.10)	71.96 (73.66)	74.40 (75.17)	69.49 (71.87)	71.64 (73.66)	61.15 (62.74)
2		98.26	72.13	74.41	69.88	70.24	63.42
3	3-O 6-O	(99.99) (100.20)	(71.65) (73.52)	(77.25) (75.10)	(69.80) (72.01)	(73.87) (71.15)	(62.33) (65.10)
4		(99.99)	(71.64)	(77.30)	(70.05)	(71.05)	(64.82)
5	3-O 6-O	(99.96) (100.13)	(71.75) (73.24)	(77.59) (75.10)	(69.67) (71.95)	(73.47) (71.17)	(62.33) (65.18)
6		98.20	72.23	74.58	69.91	69.91	63.06
7		(100.17)	(73.52)	(75.10)	(72.10)	(71.20)	(64.96)
8		(100.17)	(73.53)	(75.06)	(72.55)	(71.19)	(64.90)

* numbers in parentheses correspond to data obtained in CD_3OD.

1 = butyl-α-D glucopyranoside

2-8 = derivatives of **1**: **2** 6-O-lauroyl ; **3** mixture of 3-O-acetoyl and 6-O-acetoyl ; **4** 3,6-di-O-succinyl ; **5** mixture of 3-O-succinyl and 6-O-succinyl ; **6** 6-O-hexadecanedioyl ; **7** 6-O-(2,2,2-trichloroethyl adipate) ; **8** 6-O-adipyl

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