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## A Facile Lipase Catalyzed Access to Fatty Acid Monoesters of 2-*O*- $\beta$ -D-Glucosylglycerol

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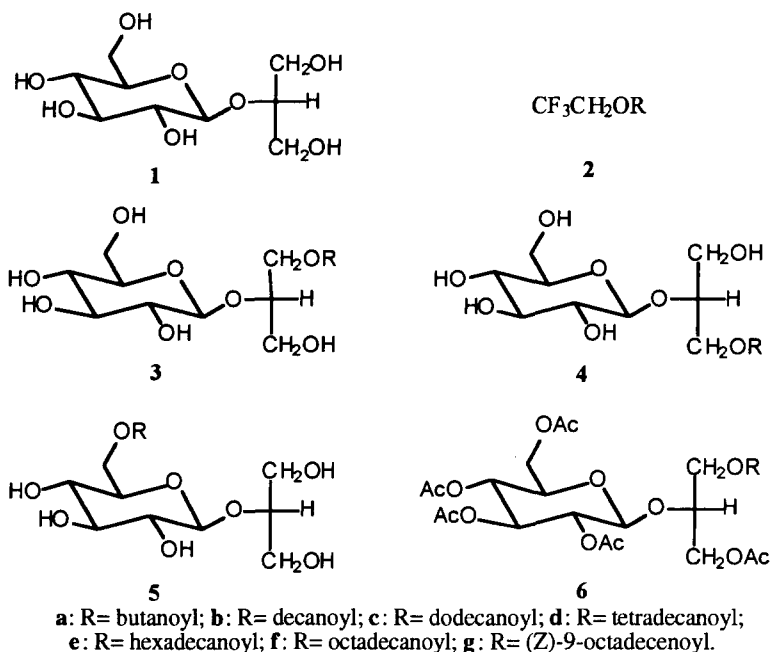
**Abstract:** 2-*O*- $\beta$ -D-glucopyranosylglycerol was submitted to acylation using *Pseudomonas cepacia* lipase as catalyst and 2,2,2-trifluoroethyl esters of various long chain carboxylic acids as acyl carriers. Monoacylation was easily accomplished with preferred formation of the (2*S*)-1-*O*-acyl derivatives in reactions showing high regio- and diastereoselectivity.

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There is a growing demand for molecules with amphiphilic properties and/or able to form liquid crystalline phases.<sup>1,2</sup> Among them, non-ionic surfactants derived from carbohydrates deserve special attention for new biological and biomedical applications as they are required, e.g., for the solubilization of integral membrane proteins.<sup>3</sup>

Recently we described an enzymatic approach to (2*R*)- and (2*S*)-1-*O*-acetyl-2-*O*- $\beta$ -D-glucopyranosylglycerols, which could be easily obtained from glucosylglycerol **1** via transesterification mediated by lipases using vinyl acetate as the acyl donor.<sup>4</sup> In the same way, the introduction of chains longer than the acetyl could afford compounds with a hydrophobic chain linked to the polar hydrophilic carbohydrate moiety, so able to exhibit an amphiphilic behaviour. We describe here the enzymatic acylation of 2-*O*- $\beta$ -D-glucopyranosylglycerol **1** performed in an organic solvent using several acyl carriers with different chain length which has allowed the monoesters to be obtained in good yield and to ascertain the influence of the size of the aliphatic chain on the regioselectivity, the diastereoselectivity and the yield of the reaction.

Our previous results have shown that 2-*O*- $\beta$ -D-glucopyranosylglycerol **1** can be easily acetylated in pyridine by two enzymes, *Pseudomonas cepacia* (LPS) and *Candida antarctica* (LCA) lipases; both the catalysts proved highly diastereoselective: while the former yielded the (2*S*)-1-*O*-acetyl derivative, the latter yielded its (2*R*)-diastereoisomer.<sup>4</sup>



So a preliminary screening of the best experimental conditions using the same two enzymes was performed, with the aim of introducing the decanoyl moiety on **1**: neither the vinyl<sup>5</sup> nor the oxime<sup>6</sup> ester of decanoic acid or decanoic acid itself<sup>7</sup> exhibited any advantage compared to the trifluoroester **2b**, which can be prepared by an easier procedure. LPS afforded, as expected, the 2S-monoester in good yields and purity, whereas LCA yielded a mixture of the 2R-, 2S- and 6'-monoesters in which the expected 2R-isomer was scarcely prevailing.

Thus the conditions chosen and used throughout this work were: LPS as enzyme, pyridine as solvent and 2,2,2-trifluoroethyl-*n*-alkanoates or alkenoates as acyl carriers. The reactions were carried out at 45 °C and stopped when the maximum yield of monoesters was obtained. The monoester and diester fractions were isolated by silica gel flash chromatography and the former ones were characterized by <sup>1</sup>H NMR analysis (see Experimental). In Table 1 the results obtained with seven acyl carriers of chain length ranging from C<sub>4</sub> **2a** to C<sub>18</sub> **2f**, **2g** are reported. In all the cases the 2S-1-*O*-alkanoyl derivative **3** was the predominant compound of the monoester fraction in which small amounts of the 2R-isomer **4** and of the 6'-isomer **5** were also identified. To the monoesters **3** the 2S configuration was assigned on the basis of chemical correlation with (2R)-1-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)glycerol.<sup>4</sup> This compound was transformed by appropriate acyl chloride into the derivatives **6**, alternatively obtained as main products by chemical peracetylation of the monoester fractions derived from the enzymatic acylation. So, the enzymatic acylation occurred with the same regiochemistry and the same steric preference of the corresponding acetylation reaction.<sup>4</sup> However, the elongation of the chain increased the contamination due to the 6'-isomer (present in negligible amount in the acetylation reaction), but did not influence the diastereoselectivity. Moreover, the yields seemed to be insensitive to the length of the aliphatic chain: more or less the same monoester yields were obtained with the acetate.<sup>4</sup>

Table 1. Enzymic Transesterification of **1**.<sup>†</sup>

ACYL CARRIER	ENZYME	TIME (hours)	DIESTER YIELD (%)	MONOESTER YIELD (%)	MONOESTER RATIO <sup>††</sup> 3 : 4 : 5
<b>2a</b>	LPS	3.5	26	73	94:4:2
<b>2b</b>	LPS	7.0	26	65	81:6:13
<b>2b</b>	LCA	4.0	18	73	17:51:32
<b>2c</b>	LPS	7.0	27	62	85:4:11
<b>2d</b>	LPS	7.0	31	63	83:4:12
<b>2e</b>	LPS	7.0	21	61	81:6:13
<b>2f</b>	LPS	7.0	7	77	80:5:15
<b>2g</b>	LPS	7.0	13	60	83:5:12

<sup>†</sup>No acylation took place in the absence of the enzyme.

<sup>††</sup>The relative ratio of the isomers was determined through <sup>1</sup>H NMR analysis.

In conclusion, this study shows that the selectivity of LPS in the enzymatic transesterification of glucosylglycerol **1** is independent from the nature of the acyl carrier and from the length of its chain. On the contrary LCA exhibits a marked decrease of the selectivity going from acetate to decanoate.

The procedure here described is a simple enzymatic methodology which facilitates the highly regio- and diastereoselective monoesterification of 2-*O*- $\beta$ -D-glucopyranosylglycerol **1** with long chain fatty acids. Work is in progress to achieve the synthesis of glyceroglycolipids of biological significance.

## EXPERIMENTAL

### General procedures

<sup>1</sup>H NMR spectra were recorded with a Bruker AM-500 spectrometer, on 0.05 M pyridine-*d*<sub>5</sub> solutions at 303 K, unless otherwise stated; chemical shifts are reported as (ppm) relative to tetramethylsilane as internal standard. Mass experiments were performed as described in ref. 8. Melting points were recorded on a Büchi 510 capillary melting point apparatus and were uncorrected. Analytical thin layer chromatography (TLC) was carried out on Merck 60 F<sub>254</sub> silica gel plates (0.25 mm thickness) and the spots were detected by spraying with 50% aqueous H<sub>2</sub>SO<sub>4</sub> or with concentrated H<sub>2</sub>SO<sub>4</sub> diluted to 70 % with a saturated aqueous solution of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and heating at 110 °C. Flash chromatography was performed with Merck 60 silica gel (230-400 mesh). *Pseudomonas cepacia* lipase (lipase PS, LPS, specific activity 30.5 triacetin units/mg solid), a generous gift from Amano Pharmaceutical Co (Mitsubishi Italia), was supported on celite according to Bovara *et al.*;<sup>9</sup> *Candida antarctica* lipase SP 435 L, immobilized on a macroporous acrylic resin, (Novozym<sup>®</sup> 435, LCA, specific activity 9.5 PL units/mg solid), was a generous gift from Novo Nordisk A/S. LPS and LCA were kept under vacuum overnight prior to use. Pyridine was distilled from calcium hydride. Compound **1** was synthesized according to literature procedures.<sup>10</sup> The trifluoroethyl esters were synthesized from the corresponding acyl chloride and 2,2,2-trifluoroethanol according to a general methodology;<sup>11</sup> for **2a-c** and **2e** see ref. 12; **2d**: b.p. 160 °C (12 mmHg); **2f**: m.p. 42-43 °C (from diethyl ether); **2g**: b.p. 190 °C (5 mmHg). Evaporation under reduced pressure was always effected with the bath temperature kept below 40 °C. The elemental analyses of the new compounds were consistent with the theoretical ones.

*General procedure for LPS-catalyzed transesterification of 1*

2-O--D-glucopyranosylglycerol (**1**) (0.39 mmol) was dissolved in 2 mL of pyridine; the proper trifluoroethyl ester (1.17 mmol) and LPS (500 mg) were added in the order and the suspension was stirred at 45 °C (see Table 1 for reaction times). The reaction was stopped by filtering off the enzyme which was washed with pyridine. The solvent was removed under reduced pressure, the residue submitted to flash chromatography (methylene chloride-methanol from 10:1 to 8:2, v/v) and the monoester fraction characterized through NMR analysis and chemical ionization MS. The <sup>1</sup>H NMR signals of the predominant isomer **3** and significant selected signals for the minor isomers **4** and **5** are here reported:

**3a** 0.80 (t, 3H,  $J = 7.0$  Hz, CH<sub>3</sub>), 1.58 (m, 2H, CH<sub>2</sub>), 2.26 (m, 2H, CH<sub>2</sub>), 3.96 (ddd, 1H,  $J_{5',4'} = 9.0$  Hz,  $J_{5',6'a} = 2.5$  Hz,  $J_{5',6'b} = 5.5$  Hz, H-5'), 3.99 (dd, 1H,  $J_{2',1'} = 8.0$  Hz,  $J_{2',3'} = 9.0$  Hz, H-2'), 4.08 (dd, 1H,  $J_{3b,3a} = 11.5$  Hz,  $J_{3b,2} = 5.5$  Hz, H-3b), 4.15 (dd, 1H,  $J_{3a,2} = 5.5$  Hz, H-3a), 4.17-4.25 (m, 2H, H-3' and H-4'), 4.33 (dd, 1H,  $J_{6'b,6'a} = 11.5$  Hz, H-6'b), 4.46 (dddd, 1H,  $J_{2,1a} = 5.5$  Hz,  $J_{2,1b} = 4.5$  Hz, H-2), 4.52 (dd, 1H, H-6'a), 4.63 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz, H-1b), 4.67 (dd, 1H, H-1a), 5.07 (d, 1H, H-1'); **4a** 4.60 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz,  $J_{1b,2} = 4.5$  Hz, H-1b), 5.13 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); **5a** 4.96 (dd, 1H,  $J_{6'a,6'b} = 11.5$  Hz,  $J_{6'a,5'} = 2.5$  Hz, H-6'a), 5.11 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); MS  $m/z$ : 342 [M+NH<sub>4</sub>]<sup>+</sup>.

**3b** 0.83 (t, 3H,  $J = 7.0$  Hz, CH<sub>3</sub>), 1.10-1.30 (m, 12H, 6 CH<sub>2</sub>), 1.62 (m, 2H, CH<sub>2</sub>), 2.35 (m, 2H, CH<sub>2</sub>), 3.96 (ddd, 1H,  $J_{5',4'} = 9.0$  Hz,  $J_{5',6'a} = 2.5$  Hz,  $J_{5',6'b} = 5.5$  Hz, H-5'), 4.00 (dd, 1H,  $J_{2',1'} = 8.0$  Hz,  $J_{2',3'} = 9.0$  Hz, H-2'), 4.10 (dd, 1H,  $J_{3b,3a} = 11.5$  Hz,  $J_{3b,2} = 5.5$  Hz, H-3b), 4.17 (dd, 1H,  $J_{3a,2} = 5.5$  Hz, H-3a), 4.17-4.25 (m, 2H, H-3' and H-4'), 4.33 (dd, 1H,  $J_{6'b,6'a} = 11.5$  Hz, H-6'b), 4.48 (dddd, 1H,  $J_{2,1a} = 5.5$  Hz,  $J_{2,1b} = 4.5$  Hz, H-2), 4.52 (dd, 1H, H-6'a), 4.67 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz, H-1b), 4.70 (dd, 1H, H-1a), 5.09 (d, 1H, H-1'); **4b** 4.64 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz,  $J_{1b,2} = 4.5$  Hz, H-1b), 5.15 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); **5b** 4.99 (dd, 1H,  $J_{6'a,6'b} = 11.5$  Hz,  $J_{6'a,5'} = 2.5$  Hz, H-6'a), 5.13 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); MS  $m/z$ : 426 [M+NH<sub>4</sub>]<sup>+</sup>.

**3c** 0.84 (t, 3H,  $J = 7.0$  Hz, CH<sub>3</sub>), 1.13-1.30 (m, 16H, 8 CH<sub>2</sub>), 1.63 (m, 2H, CH<sub>2</sub>), 2.35 (m, 2H, CH<sub>2</sub>), 3.95 (ddd, 1H,  $J_{5',4'} = 9.0$  Hz,  $J_{5',6'a} = 2.5$  Hz,  $J_{5',6'b} = 5.5$  Hz, H-5'), 3.99 (dd, 1H,  $J_{2',1'} = 8.0$  Hz,  $J_{2',3'} = 9.0$  Hz, H-2'), 4.09 (dd, 1H,  $J_{3b,3a} = 11.5$  Hz,  $J_{3b,2} = 5.5$  Hz, H-3b), 4.16 (dd, 1H,  $J_{3a,2} = 5.5$  Hz, H-3a), 4.16-4.24 (m, 2H, H-3' and H-4'), 4.31 (dd, 1H,  $J_{6'b,6'a} = 11.5$  Hz, H-6'b), 4.48 (dddd, 1H,  $J_{2,1a} = 5.5$  Hz,  $J_{2,1b} = 4.5$  Hz, H-2), 4.51 (dd, 1H, H-6'a), 4.66 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz, H-1b), 4.69 (dd, 1H, H-1a), 5.08 (d, 1H, H-1'); **4c** 4.63 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz,  $J_{1b,2} = 4.5$  Hz, H-1b), 5.13 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); **5c** 4.98 (dd, 1H,  $J_{6'a,6'b} = 11.5$  Hz,  $J_{6'a,5'} = 2.5$  Hz, H-6'a), 5.11 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); MS  $m/z$  454 [M+NH<sub>4</sub>]<sup>+</sup>.

**3d** 0.86 (t, 3H,  $J = 7.0$  Hz, CH<sub>3</sub>), 1.16-1.34 (m, 20H, 10 CH<sub>2</sub>), 1.64 (m, 2H, CH<sub>2</sub>), 2.36 (m, 2H, CH<sub>2</sub>), 3.96 (ddd, 1H,  $J_{5',4'} = 9.0$  Hz,  $J_{5',6'a} = 2.5$  Hz,  $J_{5',6'b} = 5.5$  Hz, H-5'), 4.00 (dd, 1H,  $J_{2',1'} = 8.0$  Hz,  $J_{2',3'} = 9.0$  Hz, H-2'), 4.10 (dd, 1H,  $J_{3b,3a} = 11.5$  Hz,  $J_{3b,2} = 5.5$  Hz, H-3b), 4.17 (dd, 1H,  $J_{3a,2} = 5.5$  Hz, H-3a), 4.18-4.25 (m, 2H, H-3' and H-4'), 4.33 (dd, 1H,  $J_{6'b,6'a} = 11.5$  Hz, H-6'b), 4.48 (dddd, 1H,  $J_{2,1a} = 5.5$  Hz,  $J_{2,1b} = 4.5$  Hz, H-2), 4.52 (dd, 1H, H-6'a), 4.66 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz, H-1b), 4.70 (dd, 1H, H-1a), 5.09 (d, 1H, H-1'); **4d** 4.63 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz,  $J_{1b,2} = 4.5$  Hz, H-1b), 5.15 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); **5d** 4.99 (dd, 1H,  $J_{6'a,6'b} = 11.5$  Hz,  $J_{6'a,5'} = 2.5$  Hz, H-6'a), 5.12 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); MS  $m/z$  482 [M+NH<sub>4</sub>]<sup>+</sup>.

**3e** 0.86 (t, 3H,  $J = 7.0$  Hz, CH<sub>3</sub>), 1.16-1.34 (m, 24H, 12 CH<sub>2</sub>), 1.63 (m, 2H, CH<sub>2</sub>), 2.35 (m, 2H, CH<sub>2</sub>), 3.95 (ddd, 1H,  $J_{5',4'} = 9.0$  Hz,  $J_{5',6'a} = 2.5$  Hz,  $J_{5',6'b} = 5.5$  Hz, H-5'), 3.99 (dd, 1H,  $J_{2',1'} = 8.0$  Hz,  $J_{2',3'} = 9.0$  Hz, H-2'), 4.10 (dd, 1H,  $J_{3b,3a} = 11.5$  Hz,  $J_{3b,2} = 5.5$  Hz, H-3b), 4.16 (dd, 1H,  $J_{3a,2} = 5.5$  Hz, H-3a), 4.16-4.24 (m, 2H, H-3' and H-4'), 4.32 (dd, 1H,  $J_{6'b,6'a} = 11.5$  Hz, H-6'b), 4.48 (dddd, 1H,  $J_{2,1a} = 5.5$  Hz,  $J_{2,1b} = 4.5$  Hz, H-2), 4.51 (dd, 1H, H-6'a), 4.66 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz, H-1b), 4.69 (dd, 1H, H-1a), 5.08 (d, 1H, H-1'); **4e** 4.63 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz,  $J_{1b,2} = 4.5$  Hz, H-1b), 5.14 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); **5e** 4.98 (dd, 1H,  $J_{6'a,6'b} = 11.5$  Hz,  $J_{6'a,5'} = 2.5$  Hz, H-6'a), 5.11 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); MS  $m/z$  510 [M+NH<sub>4</sub>]<sup>+</sup>.

**3f** 0.86 (t, 3H,  $J = 7.0$  Hz, CH<sub>3</sub>), 1.15-1.34 (m, 28H, 14 CH<sub>2</sub>), 1.63 (m, 2H, CH<sub>2</sub>), 2.36 (m, 2H, CH<sub>2</sub>), 3.96 (ddd, 1H,  $J_{5',4'} = 9.0$  Hz,  $J_{5',6'a} = 2.5$  Hz,  $J_{5',6'b} = 5.5$  Hz, H-5'), 4.00 (dd, 1H,  $J_{2',1'} = 8.0$  Hz,  $J_{2',3'} = 9.0$  Hz, H-2'), 4.10 (dd, 1H,  $J_{3b,3a} = 11.5$  Hz,  $J_{3b,2} = 5.5$  Hz, H-3b), 4.17 (dd, 1H,  $J_{3a,2} = 5.5$  Hz, H-3a), 4.17-4.25 (m, 2H, H-3' and H-4'), 4.32 (dd, 1H,  $J_{6'b,6'a} = 11.5$  Hz, H-6'b), 4.48 (dddd, 1H,  $J_{2,1a} = 5.5$  Hz,  $J_{2,1b} = 4.5$  Hz, H-2), 4.52 (dd, 1H, H-6'a), 4.66 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz, H-1b), 4.69 (dd, 1H, H-1a), 5.09 (d, 1H, H-1'); **4f** 4.63 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz,  $J_{1b,2} = 4.5$  Hz, H-1b), 5.14 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); **5f** 4.99 (dd, 1H,  $J_{6'a,6'b} = 11.5$  Hz,  $J_{6'a,5'} = 2.5$  Hz, H-6'a), 5.12 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); MS  $m/z$  538 [M+NH<sub>4</sub>]<sup>+</sup>.

**3g** 0.86 (t, 3H,  $J = 7.0$  Hz, CH<sub>3</sub>), 1.15-1.40 (m, 20H, 10 CH<sub>2</sub>), 1.62 (m, 2H, CH<sub>2</sub>), 2.07 (m, 4H, 2 CH<sub>2</sub>), 2.37 (m, 2H, CH<sub>2</sub>), 3.84 (ddd, 1H,  $J_{5',4'} = 9.0$  Hz,  $J_{5',6'a} = 2.5$  Hz,  $J_{5',6'b} = 5.5$  Hz, H-5'), 3.96 (dd, 1H,  $J_{2',1'} = 8.0$  Hz,  $J_{2',3'} = 9.0$  Hz, H-2'), 4.07 (dd, 1H,  $J_{3b,3a} = 11.5$  Hz,  $J_{3b,2} = 5.5$  Hz, H-3b), 4.13 (dd, 1H,  $J_{3a,2} = 5.5$  Hz, H-3a), 4.13-4.21 (m, 2H, H-3' and H-4'), 4.28 (dd, 1H,  $J_{6'b,6'a} = 11.5$  Hz, H-6'b), 4.45 (dddd, 1H,  $J_{2,1a} = 5.5$  Hz,  $J_{2,1b} = 4.5$  Hz, H-2), 4.48 (dd, 1H, H-6'a), 4.62 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz, H-1b), 4.66 (dd, 1H, H-1a), 5.04 (d, 1H, H-1'), 5.40-5.50 (m, 2H, HC=CH); **4g** 4.60 (dd, 1H,  $J_{1b,1a} = 11.5$  Hz,  $J_{1b,2} = 4.5$  Hz, H-1b), 5.09 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); **5g** 4.95 (dd, 1H,  $J_{6'a,6'b} = 11.5$  Hz,  $J_{6'a,5'} = 2.5$  Hz, H-6'a), 5.07 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'); MS  $m/z$  536 [M+NH<sub>4</sub>]<sup>+</sup>.

#### LCA-catalyzed transesterification of **1**

**1** (0.39 mmol) was dissolved in 2 mL of pyridine; **2b** (1.17 mmol) and LCA (500 mg) were added in the order and the suspension was stirred at 45 °C for 4 h. The reaction was stopped by filtering off the enzyme and processed as above, to yield the monoester fraction (see Table 1).

#### Assignment of the configuration of **3**

a) To a solution of (2R)-1-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)glycerol<sup>4</sup> (0.06 mmol) in dry pyridine (1.5 mL) acyl chloride [butanoyl, decanoyl, dodecanoyl, tetradecanoyl, hexadecanoyl, octadecanoyl or (Z)-9-octadecenoyl] (0.32 mmol) was added at room temperature under N<sub>2</sub> atmosphere. After 4 hours the solvent was removed under reduced pressure and the residue was purified by flash chromatography (ethyl acetate-petroleum ether 1:1) affording **6** (85-90% yield), which was submitted to <sup>1</sup>H NMR analysis (CDCl<sub>3</sub>) and chemical ionization MS:

**6a** 3.67 (ddd, 1H,  $J_{5',4'} = 9.5$  Hz,  $J_{5',6'a} = 5.0$  Hz,  $J_{5',6'b} = 2.5$  Hz, H-5'), 4.05 (m, 1H, H-2), 4.06-4.20 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-6'b), 4.22 (dd, 1H,  $J_{6'a,6'b} = 12.0$  Hz, H-6'a), 4.62 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.96 (dd, 1H,  $J_{2',3'} = 9.8$  Hz, H-2'), 5.04 (dd, 1H,  $J_{4',3'} = 9.8$  Hz, H-4'), 5.17 (dd, 1H, H-3'); MS  $m/z$  552 [M+NH<sub>4</sub>]<sup>+</sup>.

**6b** 3.67 (ddd, 1H,  $J_{5',4'} = 9.5$  Hz,  $J_{5',6'a} = 5.0$  Hz,  $J_{5',6'b} = 2.5$  Hz, H-5'), 4.04 (m, 1H, H-2), 4.06-4.20 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-6'b), 4.22 (dd, 1H,  $J_{6'a,6'b} = 12.0$  Hz, H-6'a), 4.61 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.96 (dd, 1H,  $J_{2',3'} = 9.8$  Hz, H-2'), 5.04 (dd, 1H,  $J_{4',3'} = 9.8$  Hz, H-4'), 5.16 (dd, 1H, H-3');

MS  $m/z$  636  $[M+NH_4]^+$ .

**6c** 3.67 (ddd, 1H,  $J_{5',4'} = 9.5$  Hz,  $J_{5',6'a} = 5.0$  Hz,  $J_{5',6'b} = 2.5$  Hz, H-5'), 4.03 (m, 1H, H-2), 4.06-4.20 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-6'b), 4.21 (dd, 1H,  $J_{6'a,6'b} = 12.0$  Hz, H-6'a), 4.61 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.95 (dd, 1H,  $J_{2',3'} = 9.8$  Hz, H-2'), 5.03 (dd, 1H,  $J_{4',3'} = 9.8$  Hz, H-4'), 5.16 (dd, 1H, H-3');

MS  $m/z$  664  $[M+NH_4]^+$ .

**6d** 3.67 (ddd, 1H,  $J_{5',4'} = 9.5$  Hz,  $J_{5',6'a} = 5.0$  Hz,  $J_{5',6'b} = 2.5$  Hz, H-5'), 4.04 (m, 1H, H-2), 4.06-4.20 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-6'b), 4.22 (dd, 1H,  $J_{6'a,6'b} = 12.0$  Hz, H-6'a), 4.61 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.96 (dd, 1H,  $J_{2',3'} = 9.8$  Hz, H-2'), 5.04 (dd, 1H,  $J_{4',3'} = 9.8$  Hz, H-4'), 5.17 (dd, 1H, H-3');

MS  $m/z$  692  $[M+NH_4]^+$ .

**6e** 3.67 (ddd, 1H,  $J_{5',4'} = 9.5$  Hz,  $J_{5',6'a} = 5.0$  Hz,  $J_{5',6'b} = 2.5$  Hz, H-5'), 4.04 (m, 1H, H-2), 4.06-4.20 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-6'b), 4.22 (dd, 1H,  $J_{6'a,6'b} = 12.0$  Hz, H-6'a), 4.61 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.96 (dd, 1H,  $J_{2',3'} = 9.8$  Hz, H-2'), 5.04 (dd, 1H,  $J_{4',3'} = 9.8$  Hz, H-4'), 5.17 (dd, 1H, H-3');

MS  $m/z$  720  $[M+NH_4]^+$ .

**6f** 3.67 (ddd, 1H,  $J_{5',4'} = 9.5$  Hz,  $J_{5',6'a} = 5.0$  Hz,  $J_{5',6'b} = 2.5$  Hz, H-5'), 4.04 (m, 1H, H-2), 4.06-4.20 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-6'b), 4.22 (dd, 1H,  $J_{6'a,6'b} = 12.0$  Hz, H-6'a), 4.61 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.96 (dd, 1H,  $J_{2',3'} = 9.8$  Hz, H-2'), 5.04 (dd, 1H,  $J_{4',3'} = 9.8$  Hz, H-4'), 5.17 (dd, 1H, H-3');

MS  $m/z$  748  $[M+NH_4]^+$ .

**6g** 3.67 (ddd, 1H,  $J_{5',4'} = 9.5$  Hz,  $J_{5',6'a} = 5.0$  Hz,  $J_{5',6'b} = 2.5$  Hz, H-5'), 4.04 (m, 1H, H-2), 4.06-4.20 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-6'b), 4.22 (dd, 1H,  $J_{6'a,6'b} = 12.0$  Hz, H-6'a), 4.61 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.96 (dd, 1H,  $J_{2',3'} = 9.8$  Hz, H-2'), 5.04 (dd, 1H,  $J_{4',3'} = 9.8$  Hz, H-4'), 5.17 (dd, 1H, H-3'), 5.32 (m, 2H, HC=CH);

MS  $m/z$  746  $[M+NH_4]^+$ .

b) Each monoester fraction (0.02 mmol) from LPS-catalyzed transesterification of **1** was dissolved in dry pyridine (1.5 mL) and treated overnight at room temperature with 1.5 mL of acetic anhydride. The solvent was then removed under reduced pressure, the crude product was purified as described above and submitted to  $^1H$  NMR analysis, which proved the identity of the major isomer with the corresponding compound **6**.

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