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Bioactive glycoglycerolipid analogues: an expeditious enzymatic approach to mono- and diesters of 2-*O*-β-D-galactosylglycerol

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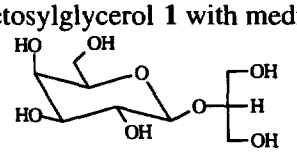
Abstract

2-*O*-β-D-Galactosylglycerol **1** was submitted to acylation using *Pseudomonas cepacia* or *Candida antarctica* lipases as catalysts and 2,2,2-trifluoroethyl esters of butanoic, hexanoic, octanoic or decanoic acid as acyl carriers. Taking advantage of the high diastereoselectivity and regioselectivity of the two enzymes, the 1-*O*-, 3-*O*-, 6′-*O*-acyl and the 1,6′-di-*O*-acylderivatives of **1** were obtained pure or in an appreciably enriched form.

1. Introduction

Glycoglycerolipids from natural sources exhibit several interesting activities; in particular, some of them have recently shown both *in vitro* and *in vivo* tumour inhibitory activity.[1–3] In this context, in order to ascertain the structural features responsible for the activity, we have performed several syntheses of bioactive gluco- and galactosylglycerols through chemoenzymatic approaches;[4–6] we found that non-natural analogues of the glucose series bearing medium length fatty acid acyl chains, shorter than the chains present in the natural compounds, exhibited a more pronounced activity.[3]

Moreover, the nature of the carbohydrate moiety of glycosylglycerols also has an influence on the cancer chemoprevention activity; in particular the presence of galactose makes, in some cases,[1] the compounds more active than the presence of glucose; thus, we have turned our efforts to the preparation of mono- and diesters of 2-*O*-β-D-galactosylglycerol **1** with medium length fatty acid acyl chains.



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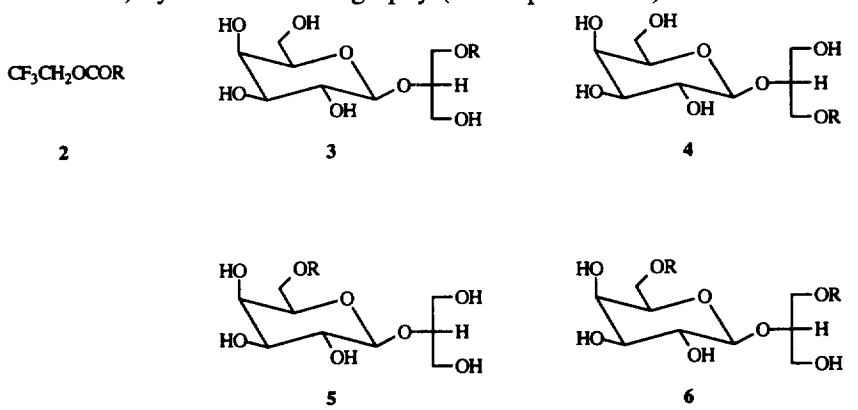
As enzymatic approaches are known to give high selectivity in the regio- and stereodifferentiation of polyhydroxylated substrates,[7] *Pseudomonas cepacia* (LPS) and *Candida antarctica* (LCA) lipases[4,6] have been used as catalysts for the acylation of **1**.

2. Results and discussion

With the aim of obtaining from **1** the various monoesters deriving from acylation at one of the primary hydroxyl functions and the diesters deriving from double acylation at both the glycerol and the sugar moieties we chose two lipases which, in our hands, had shown opposite stereoselectivities, in order to facilitate the complementary production of diastereoisomeric compounds. The transesterification reactions were performed as usual:[6] LPS or LCA as the enzyme, pyridine as solvent and 2,2,2-trifluoroethyl *n*-alkanoates **2a–d** as acyl carriers.

In the case of LPS, after short reaction times (40–60 mins) significant amounts of diesters were obtained as well as the monoesters (Table 1). The monoester and diester fractions were then separated by silica gel flash chromatography and the composition of each fraction was determined by ^{1}H NMR analyses. While three monoesters could be identified, a single compound was present in the diester fraction, namely the 1,6′-di-*O*-acylderivative **6** (see below for the determination of the configuration at C-2). Moreover, the 6′-*O*-acylderivative **5** was separated from the corresponding 1-*O*-isomer **3** (containing traces of the 3-*O*-isomer **4**) by flash chromatography (see Experimental).



a: R= butanoyl; b: R= hexanoyl; c: R= octanoyl; d: R= decanoyl.

The configuration at C-2 of compound **3** was determined by chemical correlation to commercial 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerol. Compounds **3a–d** were hydrolyzed with β-galactosidase from *Aspergillus oryzae* and the monoacyglycerols present in the reaction mixtures were transformed into the corresponding isopropylidene derivatives.[8,9] A comparison by chiral GLC[8] with authentic samples, easily obtained through acylation of 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerol, allowed the configuration of 1-*O*-acyl-2-*O*-β-D-galactosylglycerol to be assigned to the monoester **3**. The same configuration at C-2 for the diester **6** was established by conversion, through selective LPS acylation, of the 1-monoester **3** into the corresponding 1,6′-*O*-diacylderivative which gave diester **6** identical to that obtained in the direct enzymatic procedure from **1**.

The relative monoester ratios reported in Table 1 evidence the high reactivity of both C-1 and C-6′ primary hydroxyl groups of **1** in LPS catalyzed transesterifications. In fact LPS acylated with comparable

Table 1
Enzymatic transesterification of **1**[a]

enzyme	acyl carrier	time (min)	conversion[b] (%)	monoester[b] yield (%)	diester[b] yield (%)	monoester ratio[c] **3:4:5**
LPS	**2a**	40	87	39	47	68:t[d]:30
LPS	**2b**	60	69	40	29	47:t[d]:51
LPS	**2c**	60	78	49	29	38:t[d]:61
LPS	**2d**	60	85	49	34	36:t[d]:62
LCA	**2a**	45	85	67	18	7:83:10
LCA	**2b**	45	80	69	11	17:65:18
LCA	**2c**	45	83	72	11	15:58:27
LCA	**2d**	60	80	73	7	14:52:34

[a] No acylation took place in the absence of the enzyme.

[b] Determined through ^{1}H NMR analysis of the crude reaction mixtures.

[c] Determined through ^{1}H NMR analysis of the monoester fractions at 323 K.

[d] t = traces.

rates the glycerol and galactose moieties: the 1-monoester **3** was the main product when short chain acyl donors (C_4) were used, while the 6′-monoester **5** prevailed with longer chains (C_8 and C_{10}). Moreover, the 1-monoester **3** was the predominant stereoisomer at the glycerol moiety, while only traces of the 3-monoester **4** were found.

The high reactivity of the C-6′ primary hydroxyl group of galactose was unexpected if compared to the known behaviour of 2-*O*-β-D-glucosylglycerol under the same reaction conditions, where a strong preference toward the C-1 primary hydroxyl group was observed.[6] Moreover, the elongation of the acyl donor chain influenced the regioselectivity of the enzymatic reaction, as the yields of 6′-monoester **5** were increased with the chain length. In contrast, the diastereoselectivity was not influenced, as the 1-isomer **3** always prevailed over the 3-isomer **4**.

It is worthy of note that in a single experiment performed with LPS we succeeded in the simultaneous production of 1-, 6′-monoesters and 1,6′-diesters; in fact, while the three monoesters of 2-*O*-β-D-glucosylglycerol are inseparable,[6] the 6′-*O*-acylgalactoside **5** could be separated from the 1-isomer (containing traces of the 3-isomer) by flash chromatography (see Experimental), as was also the case for the monoacylderivatives of 3-*O*-β-D-galactosyl-*sn*-glycerol recently described.[10]

We then turned to the acylation of **1** catalyzed by LCA, performed under the same conditions described above for LPS, exploiting the same procedure for the separation and identification of the compounds and for the assignment of the configuration at C-2 to the 3-monoesters. As expected,[4] LCA showed (Table 1) a diastereoselectivity opposite to LPS in the acylation of substrate **1**. In fact the major product in all the LCA catalyzed transesterifications was the 3-monoester **4** obtained, after chromatographic separation from the 6′-monoester **5**, with a diastereoisomeric purity of about 80–90%. In these experiments the amount of the 6′-monoester varied from 10% to a maximum of 30%, revealing that LCA acylates the C-6′ primary hydroxyl group of **1** less easily than LPS.

Moreover LCA afforded only low yields of diesters, which were complex isomeric mixtures as indicated by preliminary ^{1}H NMR analyses, and were not investigated further.

In conclusion, it was possible to obtain in a single reaction from LPS a moderate yield of pure 1,6′-diesters **6a–d**, and 6′-monoesters **5a–d** and 1-monoesters **3a–d**, containing traces of their 3-diastereoisomer, separated after repeated chromatography. At the same time, taking advantage of the

opposite diastereoselectivity of LCA toward **1**, the 3-monoesters **4a–d** were obtained in good yield and appreciable diastereoisomeric purity.[†]

3. Experimental

3.1. General procedures

^{1}H NMR spectra were recorded with a Bruker AM-500 spectrometer, on 0.05 M pyridine-d_5 solutions at 303 K, unless otherwise stated; chemical shifts are reported as δ (ppm) relative to tetramethylsilane as an internal standard. Mass experiments were performed as described by Colombo et al.[11] Melting points were recorded on a Büchi 510 capillary melting point apparatus and were uncorrected. Optical rotations were determined on a Perkin–Elmer 241 polarimeter in methanol solutions (c=1.0) in a 1 dm cell at 20°C. Analytical thin layer chromatography (TLC) was carried out on Merck 60 F_{254} silica gel plates (0.25 mm thickness) and the spots were detected by spraying with 50% aqueous H_2SO_4 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;[4] *Candida antarctica* lipase SP 435 L, immobilized on a macroporous acrylic resin (Novozym® 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. β-Galactosidase from *Aspergillus oryzae* (specific activity with lactose 5 units/mg solid) was purchased from Sigma. Compound **1** was synthesized according to literature procedures.[12] The trifluoroethyl esters were synthesized according to Steglich et al.;[13] 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerol were purchased from Aldrich. Pyridine was distilled from calcium hydride. Evaporation under reduced pressure was always effected with the bath temperature kept below 40°C. All the new compounds were characterized through ^{1}H NMR analysis and chemical ionization mass spectrometry (CI-MS). Their elemental analyses were consistent with the theoretical ones.

3.2. General procedure for LPS-catalyzed transesterification of 1

2-*O*-β-D-Galactosylglycerol (**1**) (2.0 mmol) was dissolved in 10 ml of pyridine; the appropriate trifluoroethyl ester (6.0 mmol) and LPS (2.5 g) were added sequentially 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 and the diesters were separated from the monoester fraction by silica gel flash chromatography (methylene chloride:methanol from 10:1 to 8:2, v:v). In this way pure 1,6′-*O*-diesters **6a–d** were obtained. For the ^{1}H NMR signals see Table 2.

6a (47% yield): oil. MS *m/z* 412 $[M+NH_4]^+$. $[\alpha]_D$: −4.0. **6b** (29% yield): oil. MS *m/z* 468 $[M+NH_4]^+$. $[\alpha]_D$: −3.0. **6c** (29% yield): mp: 55–57°C (isopropyl ether). MS *m/z* 524 $[M+NH_4]^+$. $[\alpha]_D$: −3.0. **6d** (34% yield): mp: 74–76°C (isopropyl ether). MS *m/z* 580 $[M+NH_4]^+$. $[\alpha]_D$: −3.0.

Further chromatographic purification (methylene chloride:methanol 9:1, v:v) of the monoester fractions afforded after three runs 6′-monoesters **5a–d** and 1-monoesters **3a–d**. For the ^{1}H NMR signals see Table 2.

[†] The antitumour promoting effects of the synthesized compounds on Epstein–Barr virus early antigen (EBV-EA) will be reported elsewere.

Table 2
Significant ^{1}H-NMR signals of **1** and its 1-*O*-, 3-*O*-, 6′-*O*-acyl (**3**, **4**, **5**) and 1,6′-di-*O*-acyl derivatives (**6**)

	chemical shifts, δ#							
	H-1′	H-1a	H-1b	H-3a	H-3b	H-6′a	H-6′b	H-4′
1	5.07	4.12 - 4.19				4.39	4.42	4.52
3a	4.98	4.60	4.64	4.09 - 4.20		4.38 - 4.51		4.54
4a	5.04	4.05 - 4.16		4.60	4.66	4.35 - 4.52		4.55
5a	5.04	4.11 - 4.24				4.72	4.86	4.34
6a	4.94	4.61	4.65	4.08 - 4.18		4.70	4.85	4.34
3b	5.00	4.63	4.66	4.09 - 4.20		4.38 - 4.51		4.54
4b	5.05	4.07 - 4.15		4.62	4.69	4.38 - 4.51		4.55
5b	5.05	4.11 - 4.24				4.74	4.88	4.36
6b	4.96	4.64	4.67	4.10 - 4.21		4.74	4.87	4.36
3c	5.00	4.64	4.67	4.09 - 4.20		4.38 - 4.51		4.54
4c	5.05	4.07 - 4.15		4.62	4.70	4.38 - 4.51		4.55
5c	5.06	4.11 - 4.24				4.75	4.89	4.36
6c	4.97	4.65	4.69	4.10 - 4.21		4.75	4.89	4.36
3d	5.00	4.64	4.68	4.09 - 4.20		4.39 - 4.52		4.54
4d	5.05	4.07 - 4.15		4.63	4.70	4.38 - 4.52		4.55
5d	5.05	4.12 - 4.23				4.75	4.89	4.36
6d	4.98	4.65	4.69	4.10 - 4.21		4.75	4.88	4.36

when measurable, the coupling constants in Hz resulted: $J_{1',2'}$=8.0, $J_{1a,2}$=5.0, $J_{1b,2}$=6.0, $J_{1a,1b}$=12.0, $J_{3a,2}$=5.0, $J_{3b,2}$=5.5, $J_{3a,3b}$=12.0, $J_{6'a,5'}$=5.0, $J_{6'b,5'}$=7.0, $J_{6'a,6'b}$=12.0, $J_{3',4'}$=3.0.

5a (8% yield): oil. MS *m/z* 342 $[M+NH_4]^+$. $[\alpha]_D$: +4.0. **5b** (16% yield): mp: 101–102°C (isopropyl ether). MS *m/z* 370 $[M+NH_4]^+$. $[\alpha]_D$: +4.0. **5c** (23% yield): mp: 107–108°C (methanol). MS *m/z* 398 $[M+NH_4]^+$. $[\alpha]_D$: +4.0. **5d** (25% yield): mp: 114–116°C (methanol). MS *m/z* 426 $[M+NH_4]^+$. $[\alpha]_D$: +3.0.

3a (20% yield): MS *m/z* 342 $[M+NH_4]^+$. **3b** (14% yield): MS *m/z* 370 $[M+NH_4]^+$. **3c** (15% yield): MS *m/z* 398 $[M+NH_4]^+$. **3d** (14% yield): MS *m/z* 426 $[M+NH_4]^+$.

3.3. General procedure for LCA-catalyzed transesterification of 1

2-*O*-β-D-Galactosylglycerol (**1**) (1.18 mmol) was treated with LCA (900 mg) as described for LPS-catalyzed transesterification yielding, after chromatographic separation from **5a–d**, the enriched 3-monoesters **4a–d**. For the ^{1}H NMR signals see Table 2.

4a (40% yield): MS *m/z* 342 $[M+NH_4]^+$. **4b** (33% yield): MS *m/z* 370 $[M+NH_4]^+$. **4c** (30% yield): MS *m/z* 398 $[M+NH_4]^+$. **4d** (30% yield): MS *m/z* 426 $[M+NH_4]^+$.

3.4. Assignment of the configuration of 3a–d and 4a–d

3.4.1. Preparation of the standard compounds

2,3-*O*-Isopropylidene-*sn*-glycerol (0.1 ml, 0.79 mmol), 4-*N*,*N*-dimethylaminopyridine (4 mg, 0.033 mmol) and triethylamine (0.12 ml, 0.86 mmol) were dissolved in dichloromethane (1 ml). To this solution the proper acyl chloride (0.81 mmol) was added at 0°C. After 30 min at room temperature

dichloromethane (1.0 ml) was added and the mixture was washed twice with water (0.4 ml) and dried with sodium sulfate: the filtered solution was diluted with dichloromethane (1.6 ml) and the obtained 1-*O*-acyl-2,3-*O*-isopropylidene-*sn*-glycerols analyzed by GLC.

The same procedure was repeated on 1,2-*O*-isopropylidene-*sn*-glycerol to obtain 3-*O*-acyl-1,2-*O*-isopropylidene-*sn*-glycerols.

3.4.2. Preparation of the analytical samples

A quantity (0.014 mmol) of **3** (from LPS-catalyzed acylation) or **4** (from LCA-catalyzed acylation) was dissolved in water (0.5 ml) and 5 mg of β-galactosidase from *Aspergillus oryzae* was added. After 0.5 h at 30°C the mixture was extracted with 5 ml of dichloromethane and the organic phase was evaporated under vacuum yielding 2 mg of crude reaction mixture. The residue was dissolved in acetone (0.1 ml), 2,2-dimethoxypropane (0.1 ml, 0.80 mmol) and *p*-toluenesulfonic acid (1 mg) were added. After 30 min at room temperature the reaction was stopped by adding solid sodium hydrogen carbonate and diluting with dichloromethane (2.5 ml). The mixture was filtered, washed twice with water (0.5 ml), dried with sodium sulfate and evaporated under vacuum. The crude mixture (3 mg) was diluted with dichloromethane (0.1 ml) and directly analyzed by GLC.

3.4.3. Chromatographic conditions

Standard and analytical samples were analyzed on a chiral GLC-capillary column (dimethylpentyl-β-cyclodextrin, 25 m, ID 0.25 mm, from MEGA-Italy) under the following conditions: oven temperature, from 120 to 200°C (for the butanoyl and hexanoyl derivatives) or from 140 to 200°C (for the octanoyl and decanoyl derivatives), 0.2°C/min; injector temperature, 250°C; detector temperature, 295°C; helium flow, 1 ml/min; split ratio 80:1.

In the chromatogram, the lower retention time peak corresponded to the 1-*O*-acyl-2,3-*O*-isopropylidene-*sn*-glycerols and the higher retention time peak to the 3-*O*-acyl-isomers.

3.5. Assignment of the configuration of ***6a–d***

The 1-butanoate **3a** (0.05 mmol) was dissolved in 0.4 ml of pyridine; trifluoroethyl butanoate **2a** (0.25 mmol) and LPS (100 mg) were added in that order and the suspension was stirred at 45°C for 3 h. The reaction was worked-up as described in the LPS-catalyzed transesterification general procedure affording, after flash chromatography (methylene chloride:methanol 9:1, v:v), a single product (88% yield), identical to the 1,6′-dibutanoate **6a**. In the same way from the 1-monoesters **3b–d** the corresponding 1,6′-diesters **6b–d** were obtained.

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