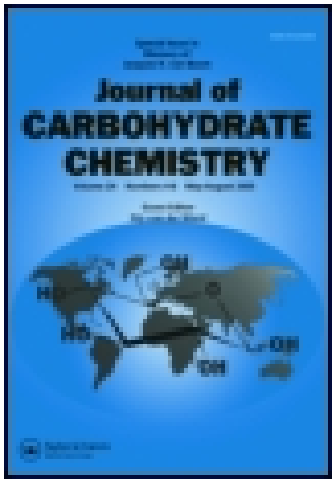

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Efficient Differentiation of the Hydroxyl Groups of 3,4-O-Isopropylidene-D-Galactopyranosides by Lipase Catalyzed Esterification and De - Esterification

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EFFICIENT DIFFERENTIATION OF THE HYDROXYL GROUPS OF 3,4-*O*-ISOPROPYLIDENE-D-GALACTOPYRANOSIDES BY LIPASE CATALYZED ESTERIFICATION AND DE-ESTERIFICATION

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Dedicated to the memory of Professor Giuseppe Bellucci

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ABSTRACT

The *Pseudomonas sp.* (LPS) promoted acyl transfer from vinyl acetate to selected 3,4-*O*-isopropylidene-D-galactopyranosides takes place in a completely selective manner giving in high yield the corresponding 6-*O*-acetates. The acetylation rate is strongly dependent on the type and the orientation of the aglycon, varying from a maximum of reactivity for the 1-deoxy derivative, 1,5-anhydro-3,4-*O*-isopropylidene-D-galactitol (**1d**), to a minimum for β configurated alkyl glycosides and showing a complete loss of reactivity for 3',4':2,3:5,6-tri-*O*-isopropylidenelactose dimethyl acetal (**1e**). The latter compound is, however, selectively 6'-*O*-esterified in good yield by lipase from *Candida Antarctica* and vinyl acetate. Also the course of the enzymatic hydrolysis of 2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-D-galactopyranosides **2** is dependent on the type of the aglycon, both for the reaction rate and the selectivity. The 2-*O*-acetates **4** are selectively

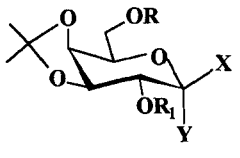
obtained in good yields with porcine pancreatic lipase (PPL) promoted hydrolysis in the case of β- and α-methyl, and 1-deoxy derivatives (**2a**, **2b** and **2d**), while for β-benzyl (**2c**) and lactose (**2e**) analogues satisfactory results are obtained with lipase from *Mucor miehei* (IM20).

INTRODUCTION

During our investigations[1] on the synthesis of rare and complex sugars from D-galactose and other milk derived carbohydrates, we used extensively as key intermediates several 3,4-*O*-isopropylidene-D-galactopyranoside derivatives such as **1a-e**. In order to use them for further elaboration, efficient methods for the selective protection of each of the two free OH groups were needed, but this turned out not to be as simple as one would have expected from their respectively primary and secondary nature. For instance, the selective tritylation under standard conditions,[2] gives disappointingly low yields (< 50%) of the 6-*O*-trityl ethers in the case of the β derivatives **1a**,[3] **1c**[4] and **1e**,[5] whereas for the α-methyl derivative **1b**[6] and the 1-deoxy-anaogue **1d**[7] a "normal" behaviour (quasi-quantitative 6-*O*-tritylation) was observed.

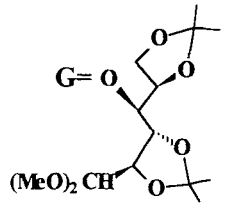
A prevalent formation of 6-*O*-acyl derivatives of **1a**,[8] **1c**[4] and **1e**[10] was obtained with conventional chemical esterification under carefully controlled conditions, but in all cases substantial amounts of 2,6-di-*O*-acyl derivatives were present and chromatographic procedures for the separation of 6-*O*-monoesters were needed. The selective formation of 6-*O*-acyl derivatives was achieved only with special reagents. A highly regioselective 6-*O*-acetylation of **1a** and **1e** was obtained in moderate yields (63 and 46 %) by Matta and coworkers[10] through transesterification with ethyl acetate and neutral alumina, and a regiospecific 6-*O*-benzoylation of **1b** was reported by Mocerino and Stick[11] through a two-step procedure involving the selective formation of a 6-*O*-phenyldithioorthoester followed by controlled hydrolysis.

Furthermore multistep sequences are needed to obtain selectively 2-*O*-protected compounds **4**, as previously exemplified only in the case of the derivative **4d**.[7]



1 R = R_1 =H
2 R = R_1 =Ac
3 R = Ac, R_1 =H
4 R = H, R_1 = Ac

a X =OMe, Y =H
b X =H, Y = OMe
c X =OBn, Y =H
d X = Y =H
e X =G, Y =H



We have thus turned our attention to the use of enzymes as biocatalysts for the selective acetylation of **1** or deacylation of **3**. The hyperbolic increase of applications of lipases in the carbohydrate area as been excellently revieved[12] showing that unprotected or partially protected pyranoses, furanoses and their glycosides, in the presence of lipases, often give acylation products with total or very high regioselectivities.

RESULTS AND DISCUSSION

Preliminary experiments for obtaining the 6-*O*-acetates **3** were conducted with a supported lipase from *Pseudomonas sp.* (LPS), with vinyl acetate as the acyl donor and solvent, and tetrahydrofuran as a cosolvent at 45 °C.

With the exception of **1e**, which was completely unreactive under these conditions and underwent only a very slow acylation in acetonitrile (<20% conversion after 15 days), in all other cases smooth reactions took place with formation of a single less polar product. When the reactions were observed to be complete (TLC) simple removal of the catalyst, followed by a filtration on silica gel, led to pure samples of 6-*O*-acetyl derivatives **3a-d**, characterized by NMR analysis and, in the case of the previously reported ones **3a**[6] and **3b**,[13] by comparison of their physico-chemical constants.

Although these results agree with previous ones,[12] some interesting observations can be made on the influence of the type and orientation of the anomeric substituent on the rates of enzymatic acetylation, deduced by NMR analyses of the mixtures obtained in incomplete esterifications performed under standard conditions (18 h at 45 °C) (Table 1).

If the fastest reacting 1,5-anhydro-D-galactitol derivative **1d** is taken as a reference, it can be seen that the percentage of conversion is little affected by the introduction of an α-methoxy anomeric substituent (**1b**), whereas a β-methoxy one (**1a**) considerably reduces the esterification rate. On the other hand a limited change in the steric hindrance of the β anomeric substituent (from β-OMe to β-OBn, **1c**) does not influence the rate appreciably, whereas in the case of the protected lactose derivative **1e**, a complete absence of reactivity was found, probably because of a failure of the very bulky anomeric group to fit in the enzyme active site.

A point of particular practical interest is the complete site selectivity of these LPS catalyzed reactions. In all examined cases exclusive acetylation of the primary hydroxyl occurred to give **3a-d**, even when reaction times were doubled and more biocatalyst was added after completion of the reaction. This is particularly striking for substrate **1d** in which OH-2 is free from steric interactions with an anomeric group, and points to a peculiar inhibitory effect of the 3,4-*O*-isopropylidene group in the *galacto* series. Data on

Table 1. Relative rates of LPS catalyzed acetylations of **1a-e**[a].

Compound	Product	Conversion (%)
1a	**3a**	34
1b	**3b**	75
1c	**3c**	34
1d	**3d**	86
1e[b]	--	0

a. All reactions were conducted under the following standardized conditions: 1 mmol of **1** in 7 mL of vinyl acetate and 3 mL of THF stirred for 18 h at 45 °C in the presence of 1 g of supported LPS. The conversions were determinated by NMR on the crude products obtained after filtration of lipase, followed by concentration to dryness.
b. A small conversion into **3e** (< 20 %) was found for the reaction (15 days) of **1e** (1 mmol) and LPS (1.0 g) in vinyl acetate (1.0 mL) and CH_3CN (10 mL).

the enzymatic acylation of this class of compounds were almost completely absent from the literature. The observed complete site specificity of the reaction under discussion, allowing the preparation of esters of type **3a-d** in high chemical yields (Table 2), without the need of a precise control of reaction times and with no purification problems, makes the procedure highly competitive with other methods.

In view of our interest, as an intermediate for the synthesis of more complex milk oligosaccharides,[14] of the lactose derivative **3e**, other types of supported lipases were screened as catalysts for its transacetylation with vinyl acetate. *Subtilisin Carlsberg*, porcine pancreatic lipase (PPL) and supported *Mucor miehei* lipase (Lipozyme IM20) gave completely negative results under our conditions. This is surprising, since the latter enzyme in its non-supported version (Lipozyme IM60) has recently been reported[15] to convert **1e** and free long chain fatty acids into the corresponding 6'-*O*-acyl derivatives in good yields. An efficient and regiospecific acetylation of **1e** was finally achieved with an enzymatic preparation from *Candida Antarctica* (Novozym 435), which with vinyl acetate in *t*-butyl methyl ether (TBME) gave **3e** in 90% isolated yield. Also when used on **1d**, this enzyme significantly reduced reaction times with respect to LPS.

The selective enzymatic conversion of the 2,6-di-*O*-acetates **2** into the corresponding 2-*O*-acetates **4** was also investigated, since these reactions are often highly site selective.[12] PPL in acetone/phosphate buffer (pH 7) catalyzed the hydrolysis of all substrates of type **2**, but with a wide range of conversion times and of selectivities. In the

Table 2. Lipase catalyzed acetylation of **1a-e**[a].

Compd	Lipase (g/mmol)	Acetylating agent/ solvent (ml/mmol)	Temp (°C)	Time (h)	Product	Yield (%)
1a	LPS (1.0)	VA (7) THF (3)	45	48	**3a**	90
1b	LPS (1.0)	VA (7) THF (3)	45	24	**3b**	93
1c	LPS (1.0)	VA (7) THF (3)	45	48	**3c**	87
1d	LPS (1.0)	VA (7) THF (3)	45	24	**3d**	94
1d	N435 (1.8)	VA (1) TBME (30)	45	5	**3d**	90
1e	N435 (1.8)	VA (1) TBME (30)	45	12	**3e**	90

a. All reactions were stopped after complete disappearance of the starting materials (TLC); LPS refers to the supported preparation described in the experimental; N435 refers to an immobilized lipase from *Candida Antarctica* (see experimental); VA= vinyl acetate and TBME=*t*-butyl methyl ether.

case of **2a**, **2b** and **2d** specific hydrolysis at the primary ester function gave **4a**, **4b** and **4d** in satisfactory yields, but at significantly different rates, the α-methyl glycoside reacting again much faster than the β-methyl anomer, and, in this case, also of the 1-deoxy derivative (Table 3). However, the substrate carrying bulkier anomeric substituents (**2c** and **2e**) gave, at complete conversion, mixtures of monoacetates of type **3** and **4** and products of complete hydrolysis of type **1**. In these two cases IM20 lipase solved the problem, since complete discrimination in favour of 6-OAc was observed, even if in the case of **2e** at very long reaction times (15 days), and compounds **4c** and **4e** were isolated in at least 80% yields. To the best of our knowledge, of this series of compounds only **4d** had been previously prepared through a somewhat laborious chemical sequence.[7]

In conclusion, our results show that the enzymic approach to selectively protected glycosides may offer promising developments if applied to the as yet little explored *O*-isopropylidene derivatives, and that further investigations on long range effects of the anomeric, and possibly other, substituents on reaction rates, a more extensive screening of lipases, and extension to derivatives of other stereochemical series of carbohydrates may well be worthwhile. We hope that in particular the easy preparation of intermediates **3e** and **4e** from the now easily accessible **1e**,[5b] may be of great help in our planned syntheses of the immunologically important milk oligosaccharides on a medium to large scale.

Table 3. Lipase catalyzed deacetylation of **2a-e**[a].

Compd	Lipase (g/mmol)	Time (h)	Product	Yields (%)
2a	PPL (1.0)	48	**4a**	82
2b	PPL (6.0)	5	**4b**	90
2c[b]	IM20 (6.0)	48	**4c**	84
2d	PPL (6.0)	40	**4d**	75
2e[b]	IM20 (10.0)	360	**4e**	80

a. All reactions were conducted in a 1:10 mixture of acetone and 0.1*M* phosphate buffer (pH 7.0) (22 ml for mmol of **2**) by shaking at 29 °C until **2** was completely reacted (TLC).
b. Mixtures of mono-acetates and completely deacetylated products **1** were obtained with PPL (see discussion).

EXPERIMENTAL

General Methods. Melting points were determined with a Kofler apparatus and are uncorrected. Optical rotations were measured at 20-22 °C with a Perkin-Elmer 241 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker 200 AC instrument in $CDCl_3$ (TMS internal standard). COSY or J-RES experiments assisted by simulation of the spectra with the PANIC (Bruker) computer program, and DEPT and HETCOR experiments, allowed a complete analysis of the ^{1}H and ^{13}C NMR spectral data to be made. Analytical TLC was carried out on silica plates (Merck, PSC Fertigplatten, Kieselgel 60 F_{254}) with detection by charring with 10% ethanolic phosphomolybdic acid. Column chromatography was performed on silica gel (Merck, 70-230 mesh) without pretreatment. Elemental analyses were performed with a Carlo Erba Elemental Analyzer Model 1106.

Lipase Preparations and Products. Porcine pancreatic lipase (PPL) and *Subtilisin Carlsberg*, were purchased from Sigma and where used after drying in vacuo for three days. Free lipase from *Pseudomonas species* (LPS) was a gift of Amano Mitsubishi Italia s.p.a. and was supported before use on Hyflo Supercell (1:3 w/w) in 0.1M phosphate buffer (pH 7.0, 10 mL) and dried in vacuo (0.1 mm Hg) for 48 h. Lipozyme IM20 (a lipase from *Mucor miehei* immobilized on a macroporous polyphenolic resin) and

Novozym 435 (a lipase from *Candida Antarctica* immobilized on a macroporous polypropylenic resin) were gifts of Novo Nordisk Bioindustriale S.r.l. Italia.

Compounds **1a**, **1b**, **1c**, **1d** and **1e** were obtained from previous work.[16,7,5b] Their 2,6-di-*O*-acetyl derivatives were obtained by routine acetylation at room temperature (24 h) with a mixture of acetic anhydride and pyridine (1:2 ratio, 5 mL/0.3 mmol of diol) followed by coevaporation of the solvent with toluene under reduced pressure and, finally, by flash chromatography, giving, in all cases, pure compounds (≈95% isolated yields) with chemico-physical data in close accordance with the previously reported ones: **2a** and **2b**,[17] **2c**,[4] **2d**[7] and **2e**.[5]

General Procedure for Lipase Catalysed Trans-esterifications. In a typical experiment 1.0 mmol of the sugar (**1a-e**) is introduced into a reaction vessel with 7 mL of vinyl acetate, 3 mL of appropriate solvent (see Tables 1 and 2) and the lipase preparation in the amount specified in Tables 1 and 2. The mixture is shaken on an orbit shaker at 250 rpm at 45 °C. The course of reaction is monitored by TLC and NMR spectroscopy and is quenched by filtering off the enzyme. The solution is dried under vacuum and the crude product is purified through silica gel column chromatography. The following products were obtained.

Methyl 6-*O*-Acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (3a), as a solid; R_f 0.65 (1:1 hexane-EtOAc), mp 123-124 °C (from EtOAc-hexane); $[\alpha]_D$ +18.7° (*c* 1.1, $CHCl_3$); lit.[6]: mp 125-126 °C, $[\alpha]_D$ +19.2°.

Methyl 6-*O*-Acetyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (3b), as a solid; R_f 0.48 (3:7 hexane-EtOAc), mp 102-103 °C (from EtOAc-hexane); $[\alpha]_D$ +123.2° (*c* 1.2, $CHCl_3$); lit.[7]: mp 101-102 °C; $[\alpha]_D$ +126°.

Benzyl 6-*O*-Acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (3c), as a solid; R_f 0.52 (3:7 hexane-EtOAc), mp 81-83 °C (from EtOAc/hexane); $[\alpha]_D$ -5.9° (*c* 0.9, $CHCl_3$); ^1H NMR (CD_3CN) δ: 1.28 and 1.43 (2 s, 6 H, 3 dioxolane CH_3); 2.04 (s, 3 H, CH_3CO); 3.45 (dd, 1 H, $J_{2,3}$ = 7.08 Hz, H-2); 3.99 (ddd, 1 H, $J_{5,6}$ = 7.80 Hz, $J_{5,6'}$ = 4.40 Hz, H-5); 4.00 (dd, 1 H, $J_{3,4}$ = 5.58 Hz, H-3); 4.15 (dd, 1 H, $J_{4,5}$ = 2.19 Hz, H-4); 4.21 (dd, 1 H, $J_{6,6'}$ = 11.55 Hz, H-6'); 4.26 (d, 1 H, $J_{1,2}$ = 8.12 Hz, H-1); 4.30 (dd, 1 H, H-6); 4.60 and 4.81 (AB system, 2 H, $J_{A,B}$ = 11.93 Hz, benzylic CH_2); from 7.31 to 7.38 (5 aromatic H); ^{13}C NMR (CD_3CN) δ: 20.97 (CH_3CO); 26.49 and 28.29 (2 dioxolane CH_3); 63.98 (C-6); 71.13 (benzylic CH_2); 74.00 (C-2); 74.46 (C-4); 71.56 (C-5); 80.10 (C-3); 102.22 (C-1); 110.48 (dioxolane $C(CH_3)_2$); 128.64, 129.02 and 129.24 (aromatic CH); 138.68 (aromatic C); 171.45 (C=O).

Anal. Calcd for $C_{18}H_{24}O_7$ (352.39): C, 61.35; H, 6.86. Found: C, 61.25; H, 7.10.

6-*O*-Acetyl-1,5-anhydro-3,4-*O*-isopropylidene-D-galactitol (3d), as a solid; R_f 0.26 (1:1 hexane-EtOAc), mp 76-77 °C (from AcOEt/petroleum ether 80-100°); $[\alpha]_D$ +63.3° (*c*

0.75, $CHCl_3$); ^1H NMR (CD_3CN) δ: 1.29 and 1.44 (2 s, 6 H, 3 dioxolane CH_3); 2.01 (s, 3 H, CH_3CO); 3.06 (dd, 1 H, $J_{1,2}$ = 9.73 Hz, H-1); 3.63 (ddd, 1 H, $J_{2,3}$ = 6.94 Hz, $J_{1',2}$ = 5.25 Hz, H-2); 3.79 (dd, 1 H, $J_{1',1}$ = 11.05 Hz, H-1'); 3.87 (ddd, 1 H, $J_{5,6}$ = 4.84 Hz, $J_{5,6'}$ = 7.08 Hz, H-5); 3.93 (dd, 1 H, $J_{3,4}$ = 5.80 Hz, H-3); 4.11 (dd, 1 H, $J_{6,6'}$ = 11.64 Hz, H-6'); 4.16 (dd, 1 H, $J_{4,5}$ = 2.22 Hz, H-4); 4.17 (dd, 1 H, H-6); ^{13}C NMR (CD_3CN) δ: 20.98 (CH_3CO); 26.44 and 28.25 (2 dioxolane CH_3); 64.83 (C-6); 68.73 (C-1); 69.42 (C-2); 74.44 (C-5 and C-4); 79.61 (C-3); 110.21 (dioxolane $C(CH_3)_2$); 171.63 (C=O).

Anal. Calcd for $C_{11}H_{18}O_6$ (246.26): C, 53.65; H, 7.37. Found: C, 53.31; H, 7.09.

6'-*O*-Acetyl-2,3:5,6:3',4'-tri-*O*-isopropylidenelactose dimethyl acetal (3e), as a syrup; R_f 0.51 (1:1 hexane-EtOAc), $[\alpha]_D$ +40.3° (*c* 1.1, $CHCl_3$); lit[6]: $[\alpha]_D$ +39.8°; lit[8]: $[\alpha]_D$ +41°.

The preparation of **3e** was performed also on a 10.0 mmol scale without apparent change of the reaction time and isolated yield.

General Procedure for Lipase Catalysed Hydrolyses of Diacetates. In a typical experiment 1.0 mmol of the sugar diacetate (**2a-e**) is introduced into a reaction vessel with a mixture of acetone (2 mL), 0.1M phosphate buffer (pH 7.0, 20 mL) and 1.0 g of the lipase preparation. The mixture is shaken at 29 °C on an orbit shaker at 250 rpm. The course of reaction is monitored by TLC and NMR spectroscopy and is quenched by removal of the enzyme by filtration. After extraction with EtOAc (2 x 20 mL) the organic layer is dried ($MgSO_4$), concentrated under vacuum and the crude product is purified through silica gel column chromatography. The following products were obtained.

Methyl 2-*O*-Acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (4a), as a solid; R_f 0.55 (1:1 hexane-EtOAc), mp 102-105 °C (from EtOAc/hexane); $[\alpha]_D$ + 20.1° (*c* 0.9, $CHCl_3$); ^1H NMR (C_6D_6) δ: 1.21 and 1.54 (2 s, 6 H, 3 dioxolane CH_3); 1.80 (s, 3 H, CH_3CO); 3.32 (s, 3 H, OCH_3); 3.59 (ddd, 1 H, $J_{5,6}$ = 7.16 Hz, $J_{5,6'}$ = 4.93 Hz, H-5); 3.83 (dd, 1 H, $J_{6,6'}$ = 11.39 Hz, H-6'); 3.89 (dd, 1 H, $J_{4,5}$ = 2.19 Hz, H-4); 3.99 (dd, 1 H, H-6); 4.01 (dd, 1 H, $J_{3,4}$ = 5.45 Hz, H-3); 4.14 (d, 1 H, $J_{1,2}$ = 8.10 Hz, H-1); 5.31 (dd, 1 H, $J_{2,3}$ = 7.46 Hz, H-2); ^{13}C NMR (C_6D_6) δ: 20.57 (CH_3CO); 26.52 and 27.80 (2 dioxolane CH_3); 55.83 (OCH_3); 62.37 (C-6); 74.02 (C-5); 73.25 (C-2); 74.33 (C-4); 77.76 (C-3); 101.66 (C-1); 110.58 (dioxolane $C(CH_3)_2$); 169.15 (C=O).

Anal. Calcd for $C_{12}H_{20}O_7$ (276.29): C, 52.17; H, 7.30. Found: C, 52.27; H, 7.30.

Methyl 2-*O*-Acetyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (4b), as a solid; R_f 0.42 (3:7 hexane-EtOAc), mp 98-100 °C (from EtOAc/hexane); $[\alpha]_D$ + 157.4° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 1.35 and 1.52 (2 s, 6 H, 3 dioxolane CH_3); 2.14 (s, 3 H, CH_3CO); 3.40 (s, 3 H, OCH_3); parameters determined by computer simulation (PANIC computer program, Bruker): 3.849 (H-6', $J_{6,6'}$ = 11.49 Hz); 3.950 (H-6); 4.067 (H-5, $J_{5,6}$ = 6.55 Hz, $J_{5,6'}$ = 4.59 Hz); 4.270 (H-4, $J_{4,5}$ = 2.48 Hz); 4.355 (H-3, $J_{3,4}$ = 5.41 Hz);

4.909 (H-2, $J_{2,3}$ = 8.30 Hz); 4.867 (H-1, $J_{1,2}$ = 3.51 Hz); ^{13}C NMR ($CDCl_3$) δ: 21.48 (*CH_3*CO); 26.88 and 28.39 (2 dioxolane CH_3); 55.95 (OCH_3); 62.77 (C-6); 68.13 (C-5); 72.43 (C-2); 74.01 (C-3); 74.55 (C-4); 97.61 (C-1); 110.44 (dioxolane *C*$(CH_3)_2$); 171.15 (C=O).

Anal. Calcd for $C_{12}H_{20}O_7$ (276.29): C, 52.17; H, 7.30. Found: C, 52.02; H, 7.20.

Benzyl 2-*O*-Acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (4c), as a solid; R_f 0.42 (3:7 hexane-EtOAc), mp 105-107 °C (from EtOAc/hexane); $[\alpha]_D$ -6.6° (*c* 0.9, $CHCl_3$); ^1H NMR (CD_3CN/ D_2O) δ: 1.29 and 1.45 (2 s, 6 H, 3 dioxolane CH_3); 2.02 (s, 3 H, CH_3CO); 4.59 and 4.83 (AB system, 2H, $J_{A,B}$ = 12.12 Hz, benzylic CH_2); from 7.30 to 7.38 (5 aromatic H); parameters determined by computer simulation (PANIC computer program, Bruker): 3.690 (H-6', $J_{6,6'}$ = 11.24 Hz); 3.731 (H-6); 3.852 (H-5, $J_{5,6}$ = 5.59 Hz, $J_{5,6'}$ = 6.63 Hz); 4.131 (H-3, $J_{3,4}$ = 5.35 Hz); 4.187 (H-4, $J_{4,5}$ = 2.02 Hz); 4.413 (H-1, $J_{1,2}$ = 8.42 Hz); 4.831 (H-2, $J_{2,3}$ = 7.56 Hz); ^{13}C NMR (CD_3CN) δ: 21.12 (CH_3CO); 26.57 and 28.12 (2 dioxolane CH_3); 62.20 (C-6); 71.13 (benzylic CH_2); 74.23 (C-2); 74.61 (C-5); 74.85 (C-4); 77.96 (C-3); 100.16 (C-1); 110.79 (dioxolane *C*$(CH_3)_2$); 128.83, 128.74 and 129.35 (aromatic CH); 139.71 (aromatic C); 170.62 (C=O).

Anal. Calcd for $C_{18}H_{24}O_7$ (352.39): C, 61.35; H, 6.86. Found: C, 61.40; H, 6.61.

2-*O*-Acetyl-1,5-anhydro-3,4-*O*-isopropylidene-D-galactitol (4d), as a solid; R_f 0.21 (1:1 hexane-EtOAc), mp 83-85 °C (from EtOAc/hexane); lit.[7]: mp 84-85 °C (from EtOAc/hexane); $[\alpha]_D$ +82.8°

2'-*O*-Acetyl-2,3:5,6:3',4'-tri-*O*-isopropylidenelactose dimethyl acetal (4e), as a syrup; R_f 0.68 (EtOAc); $[\alpha]_D$ +31.5° (*c* 1.0, $CHCl_3$); ^1H NMR (CD_3CN) δ: 1.29, 1.29, 1.30, 1.32, 1.44, 1.46 (6 s, 18 H, 6 dioxolane CH_3); 2.07 (s, 3 H, CH_3CO); 3.41 and 3.42 (2s, 6 H, 2 OCH_3); 3.61 (dd, 1 H, H-6a'); 3.68 (dd, 1 H, H-6b'); 3.85 (m, 1 H, H-4); 3.93 (m, 3 H, H-5' and H-6a and H-6b); 3.98 (dd, 1 H, H-3); 4.17 (m, 2 H, H-3' and H-4'); 4.25 (ddd, 1 H, H-5) 4.36 (m, 2 H, H-1 and H-2); 4.67 (d, 1 H, H-1'); 4.89 (m, 1 H, H-2'). ^{13}C NMR (CD_3CN) δ: 21.17 (CH_3CO); 28.41, 26.54, 26.54, 26.54, 27.50 and 28.16 (6 dioxolane CH_3); 54.74 and 57.49 (2 OCH_3); 62.40 (C-6'); 65.42 (C-6); 74.20 (C-2'); 74.83 (C-4'); 75.02 (C-4); 75.84 (C-5'); 76.46 (C-2); 78.21 (C-3'); 78.30 (C-5); 78.90 (C-3); 101.53 (C-1'); 107.66 (C-1); 108.85, 110.72 and 110.95 (3 dioxolane *C*$(CH_3)_2$); 170.53 (C=O).

Anal. Calcd for $C_{25}H_{42}O_{13}$ (550.61): C, 54.54; H, 7.69. Found: C, 54.66; H, 7.93.

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REFERENCES

1. P.L. Barili, G. Berti, G. Catelani, F. D'Andrea, and L. Miarelli, *Carbohydr. Res.*, **274**, 197 (1995), and references therein.
2. a) T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, J. Wiley & Sons, New York, 2° ed., 1991, p 60; b) G.R. Barker, *Methods Carbohydr. Chem.*, **2**, 168 (1963).
3. A. Stoffyn and P. Stoffyn, *J. Org. Chem.*, **32**, 4001 (1967).
4. A. Levy, H.M. Flowers, and N. Sharon, *Carbohydr. Res.*, **4**, 305 (1967).
5. a) T. Yoshino, G. Reuter, S. Kelm, and R. Schauer, *Glycoconjugate J.*, **3**, 7 (1986); b) P.L. Barili, G. Catelani, F. D'Andrea, F. De Rensis, and P. Falcini *Carbohydr. Res.*, **298**, 75 (1997).
6. a) J. Schneider and Y.C. Lee, *Carbohydr. Res.*, **36**, 159 (1974); b) Y. Shibata, Y. Kosuge, and S. Ogawa, *Carbohydr. Res.*, **199**, 37 (1990).
7. P.L. Barili, G. Berti, F. D'Andrea, V. Di Bussolo, and I. Granucci, *Tetrahedron*, **48**, 6273 (1992).
8. T. Lin, P. Kovác, and C.P.J. Glaudemans, *Carbohydr. Res.*, **188**, 228 (1989).
9. S.A. Abbas, J.J. Barlow, and K.L. Matta, *Carbohydr. Res.*, **88**, 51 (1981).
10. S.S. Rana, J.J. Barlow, and K.L. Matta, *Tetrahedron Lett.*, **22**, 5007 (1981).
11. M. Mocerino and R.V. Stick, *Tetrahedron Lett.*, **31**, 3051 (1990).
12. a) D.G. Drueckhammer, W.J. Hennen, R.L. Pederson, C.F. Barbas, C.M. Gautheron, T. Krach, and C.H. Wong, *Synthesis*, 499 (1991); b) H. Waldmann and D. Sebastian, *Chem. Rev.*, **94**, 911 (1994); c) N.B. Bashir, S.J. Phythian, A.J. Reason, and S.M. Roberts, *J. Chem. Soc. Perkin 1*, 2203 (1995).
13. J.G. Buchanan, *J. Chem. Soc.*, 995 (1958).
14. a) K.L. Matta, R.K. Jain, and D.R. Locke, US 5,438,124 (CA, **124** 30256j, 1996); b) G. Catelani, F. D'Andrea, unpublished results.
15. D.B. Sarney, H. Kapeller, G. Fregapane, and E.N. Vulfson, *J. Am. Oil. Chem. Soc.*, **71**, 711 (1994).
16. G. Catelani, F. Colonna, and A. Marra, *Carbohydr. Res.*, **182**, 299 (1989).
17. P.L. Barili, G. Catelani, G. Fabrizi, and D. Lamba, *Carbohydr. Res.*, **243**, 165 (1993).