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Enzymic Acylation of Methyl D- and L-Glycopyranosides: Influence of the 3-Hydroxyl Group†

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Abstract—Porcine pancreatic (PPL), *Candida cylindracea* (CCL) and *Pseudomonas cepacia* (LPS) lipases, suspended in organic solvents, were used to regioselectively acylate methyl 6-*O*-butyryl-α-D- and L-allopyranosides and methyl 6-*O*-butyryl-3-deoxy-α-D- and L-*ribo*-hexopyranosides. Both the D- and the L-3-deoxy sugars showed a complete regioselectivity, while the reactions of the allosides proved to be less regioselective. This indicates that the presence of the hydroxyl group at C-3 is an unfavourable factor for the action of the lipases.

Introduction

In preceding papers we have studied[1-4] the lipase catalyzed transesterification of the secondary hydroxyl groups of methyl α-D- and L-glycopyranosides of the mannose, galactose and glucose series in which the primary hydroxyl group at C-6 was either protected as acyl ester or was absent. The results obtained with porcine pancreatic lipase (PPL) and *Pseudomonas cepacia* lipase (LPS) have been rationalised on the basis of the orientations of the three contiguous free hydroxyl groups, which constitute a so called "triplet". In all the cases studied the sugars having an equatorial–equatorial–equatorial (EEE "triplet", Figure 1) or axial–equatorial–equatorial (AEE "triplet") sequence seemed to be the best substrates. There is a general tendency of lipases to acylate at the right-hand terminus of the "triplet" (i.e. 2-OH in the D series and 4-OH in the L series), and the orientation of this hydroxyl can heavily influence the outcome of the reaction, while the orientation of the left hydroxyl group has minor influence. For the best results, the hydroxyl group at the right-hand terminus should be equatorial: in fact, sugars having an equatorial–equatorial–axial sequence (EEA "triplet") of the secondary hydroxyl groups usually give lower yields and poor regioselectivity. Moreover, the other substituents on the pyranoside ring such as 5-methyl or 5-acyloxymethyl and 1-methoxyl exert their influence mainly on the yields but do not significantly influence the regiochemical outcome of the reactions.[4]

More puzzling results were obtained with *Candida cylindracea* lipase (CCL): this enzyme resulted sensitive not only to the orientation of the hydroxyl groups but also to the steric series (D or L) and to the substitution on the C-6 carbon atom of the sugar. In several cases a preferred acylation of the 3-position was obtained, contrary to PPL and LPS which always furnished the 2- or 4-butyrate as the main product.

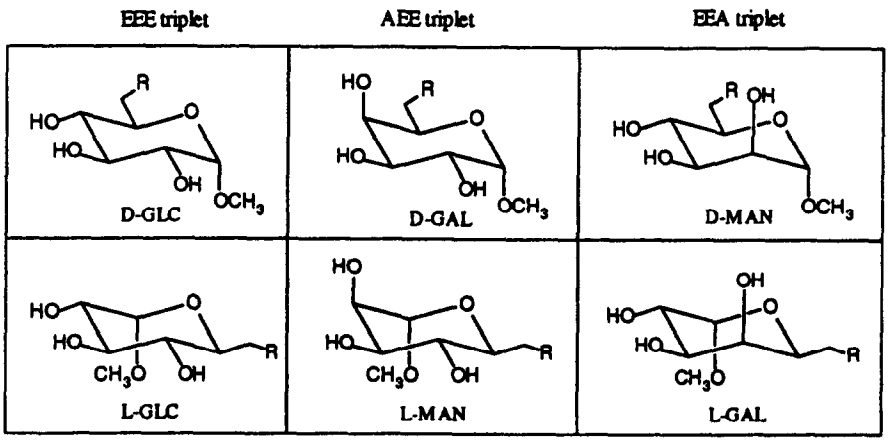


Figure 1. R=$OCOC_3H_7$ or R=H

Presented at the *XVIth Int. Carbohyd. Symp.*, Paris, 5–10 July, 1992.

As all the glycopyranosides till now studied have the 3-hydroxyl group equatorially oriented, we have decided to submit to lipase catalyzed transesterification sugars which have the 3-OH axially oriented (i.e. methyl 6-*O*-butyryl-α-D- and L-allopyranosides **1b** and **2b**, Figure 2) and 3-deoxysugars (i.e. methyl 6-*O*-butyryl-3-deoxy-α-D- and L-*ribo*-hexopyranosides **3b** and **4b**, Figure 2) in order to study the influence of the 3-hydroxyl group on the regiochemical outcome of this enzymic reaction.

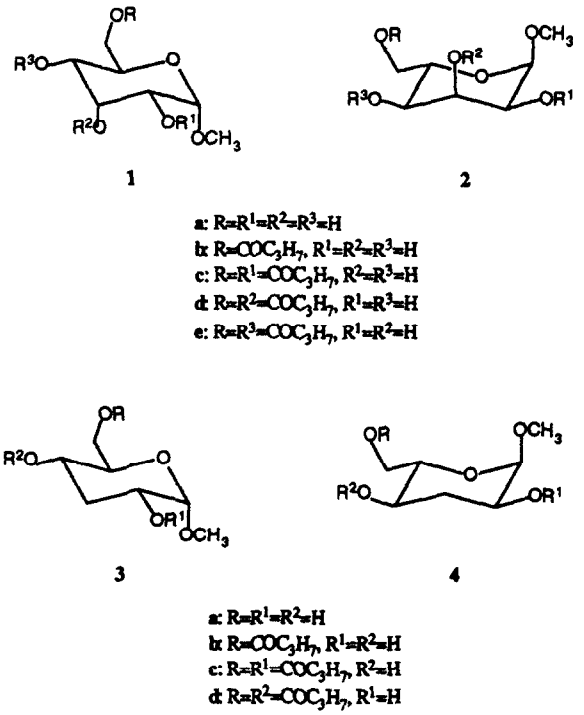


Figure 2.

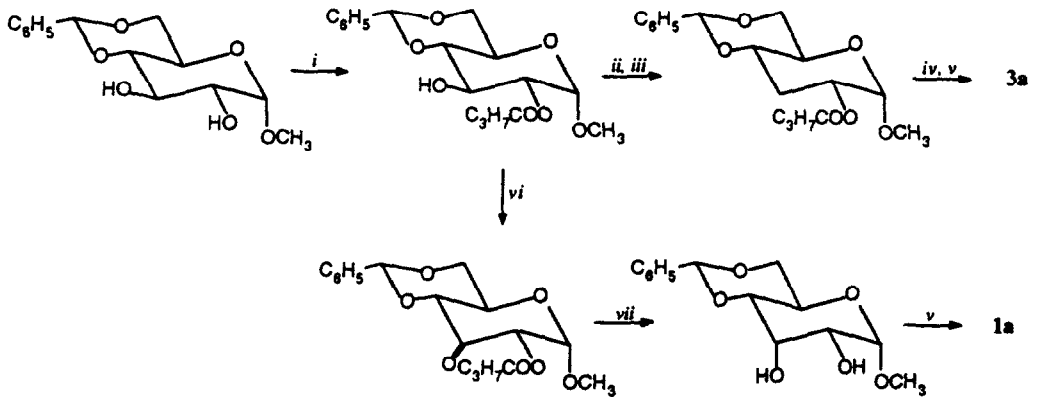
Results

In order to make available the 6-butyrates **1b–4b**, substrates of the enzymic reactions under study, it was first necessary to synthesize the corresponding methyl α-glycosides **1a–4a**, which are not commercially available. Methyl α-D-allopyranoside (**1a**) could be obtained by direct glycosidation of the commercial but expensive β-D-allose, but the yield is low.[5] So, the synthesis of **1a–4a** from methyl α-D- or L-glucopyranoside, elaborating the 3-equatorial hydroxyl by inversion to get **1a** and **2a**, or by removal to get **3a** and **4a**, is more convenient.

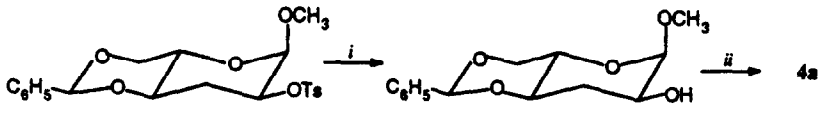
The compounds belonging to the D-series, **1a** and **3a**, were obtained (Scheme I) in good yields through a new chemoenzymatic procedure,[6] starting from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, which improves the published methods.[7–9]

On the contrary the L-pyranosides **2a** and **4a** could not be synthesized with the same chemoenzymatic procedure: in fact, preliminary enzymatic experiments carried out on methyl 4,6-*O*-benzylidene-α-L-glucopyranoside showed very low yields in the 2-*O*-butyryl derivative, confirming the low reactivity of the 2-position in the L-series also in the case in which only two free hydroxyl groups are present.

So, methyl α-L-allopyranoside (**2a**) was synthesized starting from methyl 4,6-*O*-benzylidene-α-L-glucopyranoside according to the procedure[7,8] published for its D-enantiomer, whereas methyl 3-deoxy-α-L-*ribo*-hexopyranoside (**4a**) was prepared (Scheme II) from methyl 4,6-*O*-benzylidene-2-*O*-tosyl-3-deoxy-α-L-*ribo*-hexopyranoside, obtained according to the procedure applied by Rasmussen *et al.*[9] for the D-enantiomer, on treatment[7] with sodium methoxide, followed by acidic hydrolysis.



Scheme I.
i. LPS/celite, TFEB, toluene-THF 4:1, 45 °C, 7 h
ii. *N,N'*-Thiocarbonyldiimidazole, 1,2-dichloroethane, reflux, 3 h
iii. tri-*n*-butylstannane, toluene, reflux, 1 h
iv. sodium methoxide, methanol, 1 h
v. Dowex-50-X8 (H^+), water, 60 °C, 1 h
vi. dimethyl sulfoxide, dicyclohexylcarbodiimide, orthophosphoric acid, 18 h
vii. sodium borohydride, *N,N*-dimethylformamide, methanol, 0.5 h



Scheme II.
i. Sodium methoxide, methanol, reflux, 4 h
ii. Dowex-50-X8 (H^+), water, 60 °C, 1 h

The 6-*O*-butyryl derivatives **1b–4b**, substrates for the enzymic acylation under study, were prepared by enzymic butyrylation[10] of the methyl α-D and L-glycosides **1a–4a**, using porcine pancreatic lipase (PPL)/trifluoroethyl butyrate (TFEB) as the acylating agent in pyridine. Reactions were fast and gave good yields in the monobutyryl derivatives only for the allopyranosides **1a** and **2a** and for the L-hexopyranoside **4a**. On the contrary, the D-hexopyranoside **3a** gave, in the same reaction conditions, an almost equimolar mixture of the 6-monoester **3b** and of the 2,6-diester **3c**. Compound **3b** was, however, obtained pure by column chromatography.

The enzymic butyrylation of compounds **1b–4b** (Table 1) was performed through the lipase/TFEB system using crude porcine pancreatic lipase (PPL), *Pseudomonas cepacia* lipase (LPS) and *Candida cylindracea* lipase (CCL) at 45 °C in organic solvents according to the procedures already described.[1–4]

The dibutyrate mixtures were obtained by flash chromatography and the relative ratios of the diesters were determined by ^{1}H-NMR analysis. The signals used for the integration were assigned on the ^{1}H-NMR spectra (Table 2) of each D- or L-dibutyrate obtained through the procedures described below.

Table 1. Enzymic butyrylation of compounds **1b–4b**

entry	substr.	enzyme	time (days)	yield (%)	% dibutyrates (C-2) c	(C-3) d	(C-4) e
1	**1b**	PPL	2	81	98	1	1
2	**1b**	LPS	2	84	99	1	[a]
3	**1b**	CCL	2	82	98	1	1
4	**2b**	PPL	7	40	2	29	69
5	**2b**	LPS	7	23	19	23	58
6	**2b**	CCL	7	26	4	16	80
					c		d
7	**3b**	PPL	6[b]	98	100		[a]
8	**3b**	LPS	6[b]	92	100		[a]
9	**3b**	CCL	6[b]	90	100		[a]
10	**4b**	PPL	7	60	1		99
11	**4b**	LPS	7	70	1		99
12	**4b**	CCL	7	21	1		99

[a]Traces (by ^{1}H-NMR).
[b]Hours.

Table 2. ^{1}H-NMR data of compounds **1b**, **1c**, **2d**, **1e**, **3b**, **3c** and **4d**

chemical shifts, δ

	H-1	H-2	H-3	H-3a	H-3b	H-4	H-5	H-6a	H-6b	MeO
1b	4.81	3.68	4.14	-	-	3.50	3.79	4.47	4.39	3.48
1c	4.91	4.81	4.19	-	-	3.55	3.85	4.51	4.36	3.46
2d	4.74	3.81	5.48	-	-	3.69	3.94	4.43	4.33	3.46
1e	4.82	3.73	4.27	-	-	4.82	4.12	4.30	4.24	3.50
3b	4.68	3.71	-	2.25	1.71	3.44	3.59	4.62	4.18	3.46
3c	4.80	4.82	-	2.18	1.95	3.54	3.65	4.59	4.20	3.43
4d	4.69	3.76	-	2.32	1.70	4.76	3.83	4.20	4.16	3.46

coupling constants, Hz

	$J_{1,2}$	$J_{2,3}$	$J_{2,3a}$	$J_{2,3b}$	$J_{3a,3b}$	$J_{3,4}$	$J_{3a,4}$	$J_{3b,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
1b	3.5	3.5	-	-	-	3.5	-	-	10.0	2.5	5.0	12.0
1c	3.5	3.5	-	-	-	3.5	-	-	10.0	2.0	5.5	12.0
2d	4.5	3.5	-	-	-	3.5	-	-	10.0	5.0	2.5	12.0
1e	3.5	3.5	-	-	-	3.5	-	-	10.5	5.0	2.0	12.0
3b	3.5	-	4.5	11.0	12.0	-	4.5	11.0	10.0	4.0	2.0	12.0
3c	3.5	-	4.5	10.0	12.0	-	5.0	11.0	10.0	4.0	2.5	12.0
4d	3.5	-	3.5	11.5	11.5	-	4.5	11.0	10.0	4.5	2.5	12.0

Pure 2,6-Di-*O*-butyryl-α-D-allopyranoside (**1c**) was obtained by flash chromatography of the mixture deriving from the enzymatic butyrylation of **1b**.

Pure methyl 2,6-di-*O*-butyryl-3-deoxy-α-D-*ribo*-hexopyranoside (**3c**) was obtained by flash chromatography of the PPL/TFEB butyrylation mixture of **3a**. Methyl 4,6-di-*O*-butyryl-3-deoxy-α-D-*ribo*-hexopyranoside (**3d**) was never obtained in the enzymatic butyrylation mixtures from **3b**, whereas from reaction mixtures of lipase catalyzed butyrylations of **4b** was obtained its enantiomer **4d**, purified in small amounts by flash chromatography.

In order to obtain reasonable amounts of the 3,6- and 4,6-di-*O*-butyryl-α-D-allopyranosides (**1d**) and (**1e**), compound **1b** was submitted to chemical butyrylation with butyric anhydride in pyridine which furnished **1c** and **1e** in a 43:57 ratio. From this mixture compound **1e** was obtained pure by flash chromatography.

As neither in the chemical nor in the enzymatic acylation was it possible to obtain compound **1d**, only the ^{1}H NMR data of its enantiomer **2d**, obtained in the enzymatic butyrylation of **2b** are described here. No attempt to purify **2d** was made owing to the small amount of this product obtained in the reactions.

Discussion

The results obtained with the two allopyranosides under investigation once more confirm the general tendency of methyl glycosides to be butyrylated at the right-hand terminus of the hydroxyl "triplet". However, some main differences are observed between the D-sugar **1b** and the L-sugar **2b**. The butyrylation of the 2-OH group of **1b** proceeds with excellent regioselectivity in good yields, whereas very poor yields are obtained with **2b**, though a preference for the 4-OH group can be observed. A comparison of these data with those observed with other methyl α-D- and L-pyranosides[1-4] shows that the axial orientation of the central OH group has only a minor influence on the outcome of the reaction in the case of PPL and LPS. On the contrary, differently from most of the other substrates already studied in which CCL butyrylates the 3-OH of the L-sugars, L-allopyranoside **2b** was butyrylated by this enzyme at 4-OH. So, the 3-position is butyrylated only if the hydroxyl group is equatorial: when a 3-OH with axial orientation is present, it cannot be butyrylated.

The 3-deoxysugar **3b** shows completely regioselective reactions in very short reaction times and excellent yields in the 2,6-dibutyrate **3c** are obtained. Similarly, one

dibutyrate, the 4,6-L-dibutyrate **4d**, is obtained from **4b**, with only traces of the other one, though in longer reaction times and lower yields. The absence of the 3-OH group "destroys" the "triplet" and makes more efficient the acylation of the adjacent 2- or 4-hydroxyl group.

In conclusion, this study shows that the orientation of the central hydroxyl group in the three contiguous secondary functions of the sugars has an influence ranging from minor (PPL, LPS, D-series) to major (CCL, L-series) on the outcome of the enzymic butyrylation reaction. However, the results of the butyrylation of the 3-deoxysugars show that the lack of the 3-hydroxyl group can make the reaction more regioselective (**3b** and **4b**) and faster (**3b**), indicating that, whichever is its orientation, it has an unfavourable influence on the reaction, as demonstrated by the complete regioselectivity observed for sugars lacking this functional group.

Experimental

General methods

^{1}H-NMR spectra were recorded with a Bruker AC-200 or AM-500 spectrometer in deuteriochloroform solutions. Optical rotations were measured with a Perkin Elmer 241 polarimeter at 25 °C, as chloroform solutions, unless otherwise stated. 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).

Methyl α-D-glucopyranoside and L-glucopyranose were purchased from Fluka, methyl 4,6-*O*-benzylidene-α-L-glucopyranoside was prepared according to Evans.[11]

Porcine pancreatic lipase (type II) (PPL) (specific activity 13.1 units/mg solid) and *Candida cylindracea* lipase (type VII) (CCL) (specific activity 665 units/mg solid) were purchased from Sigma; *Pseudomonas cepacia* lipase (lipase PS) (LPS) (specific activity 30.5 units/mg solid) was a generous gift from Amano Pharmaceutical Co. CCL was used as received; PPL and LPS were kept under vacuum prior to use in order to lower the water content to 0.5%. Tetrahydrofuran and pyridine were distilled just prior to use from, respectively, sodium/benzophenone and calcium hydride. Methylene chloride and acetone were dried over 3Å molecular sieves. Evaporation under reduced pressure was always effected with the bath temperature kept below 40 °C.

Methyl 6-O-butyryl-α-D-allopyranoside (1b)

Methyl α-D-allopyranoside[6] (**1a**) (1 g, 5.15 mmol) was dissolved in anhydrous pyridine (10 mL), then 2,2,2-trifluoroethyl butyrate (TFEB, 2.5 mL) and PPL (3 g) were added in this order and the suspension was stirred overnight at 45 °C. The enzyme was then filtered-off, the solvent was removed under reduced pressure and the crude product (1.52 g) was purified by flash chromatography (methylene chloride–methanol 9:1) to yield pure **1b** (1.09 g,); oil, $[\alpha]_D$ +77.5° (c 1.0). Anal. calcd for $C_{11}H_{20}O_7$: C, 49.99; H, 7.63. Found: C, 49.89; H, 7.51. For ^{1}H-NMR data see Table 2.

Methyl α-L-allopyranoside (2a)

2a (0.380 g) was obtained[7,8] from methyl 4,6-*O*-benzylidene-α-L-glucopyranoside (1.25 g, 4.43 mmol); amorphous solid, $[\alpha]_D$ -152.1° (c 1.0, water). Anal. calcd for $C_7H_{14}O_6$: C, 43.30; H, 7.27. Found: C, 42.91; H, 7.14.

Methyl 6-O-butyryl-α-L-allopyranoside (2b)

With the same procedure used for the synthesis of **1b**, **2a** (0.380 g, 1.96 mmol) afforded **2b** (0.425 g); oil, $[\alpha]_D$ -78.2° (c 1.0). Anal. calcd for $C_{11}H_{20}O_7$: C, 49.99; H, 7.63. Found: C, 50.11; H, 7.49. The ^{1}H-NMR spectrum was identical to the spectrum of **1b**.

*Methyl 6-O-butyryl-3-deoxy-α-D-*ribo*-hexopyranoside (3b) and methyl 2,6-di-O-butyryl-3-deoxy-α-D-*ribo*-hexopyranoside (3c)*

Methyl 3-deoxy-α-D-*ribo*-hexopyranoside[6] **3a** (1 g, 5.62 mmol) was submitted to the same reaction conditions used for the synthesis of **1b**. The reaction was monitored by TLC (ethyl acetate–isopropanol–water 3:3:1) which showed the formation of two less polar compounds. On disappearance of **3a** (18 h), usual work-up yielded a crude product (1.7 g), which was purified by flash chromatography (ethyl acetate–methanol 95:5) first affording **3c** (0.655 g); oil, $[\alpha]_D$ +54.5° (c 1.0). Anal. calcd for $C_{15}H_{26}O_7$: C, 56.59; H, 8.23. Found: C, 56.42; H, 8.25. For ^{1}H-NMR data see Table 2. Then **3b** (0.651 g) was eluted; oil, $[\alpha]_D$ +72.1° (c 1.0). Anal. calcd for $C_{11}H_{20}O_6$: C, 53.22: H, 8.12. Found: C, 53.07; H, 7.93. For ^{1}H-NMR data see Table 2.

*Methyl 3-deoxy-α-L-*ribo*-hexopyranoside (4a)*

Methyl 4,6-*O*-benzylidene-2-*O*-tosyl-3-deoxy-α-L-*ribo*-hexopyranoside,[9] (1.18 g, 2.81 mmol) was dissolved in 170 mL of sodium methoxide 1.7 M. The reaction mixture was refluxed for 4 h yielding, after flash chromatography (ethyl acetate–hexane 1:1), methyl 4,6-*O*-benzylidene-3-deoxy-α-L-*ribo*-hexopyranoside (0.508 g, for the D-enantiomer see Ref. 12), which was treated with Dowex-50-X8 (H^+) (1.4 g) in water (10 mL) at 60 °C for 1 h, affording **4a** (0.290 g): oil, $[\alpha]_D$ -124.2° (c 1.0, water). Anal. calcd for $C_7H_{14}O_5$: C, 47.19; H, 7.92. Found: C, 47.28; H, 7.79.

*Methyl 6-O-butyryl-3-deoxy-α-L-*ribo*-hexopyranoside (4b)*

With the same procedure used for the synthesis of **1b**, compound **4a** (0.290 g, 1.63 mmol) afforded, after flash chromatography (ethyl acetate–methanol 95:5), **4b** (0.364 g); oil, $[\alpha]_D$-73.8° (c 1.0). Anal. calcd for $C_{11}H_{20}O_6$: C, 53.22; H, 8.12. Found: C, 53.38; H, 8.11. The ^{1}H-NMR spectrum was identical to the spectrum of **3b**.

General procedure for lipase catalyzed transesterification of compounds 1b–4b

1b–4b (0.05 g) was dissolved in 1 mL of THF for PPL or LPS and methylene chloride–acetone 4:1 for CCL catalyzed reactions. TFEB (0.25 mL) and then lipase (0.250 g) were added and the suspension was stirred at 45 °C. The reactions, monitored by TLC using toluene–acetone 4:1 for **1b** and **2b** and ethyl acetate–hexane 3:2 for **3b** and **4b**, were stopped by filtering-off the enzyme which was then washed with hot acetone. After evaporation of the solvent, the dibutyrate mixtures were purified by flash chromatography (ethyl acetate–hexane 3:2) and analyzed by ^{1}H-NMR for the determination of the product ratios (see Table 1).

Methyl 2,6-di-O-butyryl-α-D-allopyranoside (1c)

From the LPS butyrylation mixture of **1b** described above, compound **1c** was obtained by flash chromatography (toluene–acetone 4:1); oil, $[\alpha]_D$ +62.2° (c 1.0). Anal. calcd for $C_{15}H_{26}O_8$: C, 53.88; H, 7.84. Found: C, 53.97; H, 7.25. For ^{1}H-NMR data see Table 2.

Methyl 4,6-di-O-butyryl-α-D-allopyranoside (1e)

To a solution of **1b** (0.500 g, 1.89 mmol) in pyridine (3 mL) butyric anhydride (0.42 mL, 2.6 mmol) was added and the mixture was stirred overnight at room temperature. Evaporation with toluene under vacuum afforded 0.850 g of a crude mixture of products. Flash chromatography (toluene–acetone 4:1) afforded in the order a tributyrate fraction (0.412 g), **1c** (0.092 g), and **1e** (0.120 g); oil, $[\alpha]_D$ +127.7° (c 1.0). Anal. calcd for $C_{15}H_{26}O_8$: C, 53.88; H, 7.84. Found: C, 53.78; H, 7.81. For ^{1}H-NMR data see Table 2.

Methyl 4,6-di-O-butyryl-3-deoxy-α-L-ribo-hexopyranoside (4d)

From the LPS butyrylation mixture of **4b** described above compound **4d** was obtained by flash chromatography (ethyl acetate–hexane 3:2); oil, $[\alpha]_D$ -124.7° (c 1.0). Anal. calcd for $C_{15}H_{26}O_7$: C, 56.59; H, 8.23. Found: C, 56.51; H, 8.01. For ^{1}H-NMR data see Table 2.

Acknowledgements

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