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Lipase-catalyzed stereoresolution of long-chain 1,2-alkanediols: A screening of preferable reaction conditions

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ABSTRACT

Scalable lipase-catalytic method for the kinetic resolution of long-chain 1,2-alkanediol enantiomers via stereoselective cleavage of esters was developed. The influence of lipase, reaction medium, nucleophile, temperature and the structure of the acyl group on the reaction velocity, the stereopreference and the stereoselectivity of the deacylation was studied. In addition, the rate of the spontaneous intramolecular migration of different acyl groups was determined for the intermediate 2-monoesters. The acyl group migration may diminish the apparent stereoselectivity of the two-step process if fast migrating acyl groups are used. It was found that the migration rate of different acyl groups differs by up to two orders of magnitude, being faster for acetyl and isobutyryl and much slower for butyryl and benzoyl groups.

The best results were obtained by the sequential methanolysis of *bis*-butyryl-1,2-alkanediols in an acetonitrile/methanol mixture catalyzed by *Candida antarctica* lipase B (CALB) at 20 °C, affording (*S*)-1,2-alkanediols. Stereo- and chemoselective crystallization of the deacylated (*S*)-1,2-alkanediols from the reaction mixture complements the enzymatic process improving the stereochemical purity to up to *ee* > 99.8%. (*R*)-1,2-Alkanediol 2-monoesters were separated from the mother liquor and enriched stereochemically by repeated incubation with CALB, then separated, hydrolyzed with alkali and crystallized to afford (*R*)-alkanediols of *ee* > 99.8%.

1. Introduction

Enantiomers of long-chain 1,2-alkanediols are highly valuable chiral building blocks for the synthesis of biologically active natural products like insect sex pheromones, acetogenins, etc [1]. Enantiomeric alcohols of this type have been previously prepared by hydrolytic kinetic resolution of terminal epoxides using asymmetric chemical catalysis [2–7] as well as enzymatically, using epoxide hydrolases [8,9]. Asymmetric hydroxylation of olefins [10] and diboration–oxidation of olefins and alkynes [11,12] have given good results as well.

In lipase-catalytic separations, enantiomeric diols with protected primary OH-groups have been obtained [13–16]. A chemical catalytic resolution [17] as well as an enzymatic oxidation of β-hydroxy ketones [18] have afforded enantiomeric 1,2-alkanediols with protected secondary hydroxyl groups. Enzymatic hydrolysis of diol diesters has afforded diols with high stereochemical purity [19–22]. However, sequential alcoholysis [23–25] has given even better results. A stereoselective lipase-catalyzed transesterification process has been used quite successfully for the resolution of 1,2-alkanediol enantiomers as well [26,27].

The analysis of the highly enantiomerically pure [28] 1,2-alkanediols necessitates derivatization for the introduction of a suitable chromophore prior to analyzing in HPLC. For HPLC using chiral stationary phase, derivatization of alcohols with non-chiral agents is the usual practice, while for NMR, on the contrary, the use of chiral derivatizing agents (CDAs) and studying the diastereomeric derivatives is preferred. 1,2-Alkanediols may be selectively derivatized as acetals [29] or boronates [30,31] involving both of the hydroxyl groups. Conventional chiral derivatizing agents [32]

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like methoxyphenylacetic acid [33,34] or mandelic acid [35] can be used to derivatize all hydroxyl groups of a diol or polyol compound. Named derivatization would allow NMR spectroscopic determination of stereoisomeric homogeneity and absolute configuration of the stereogenic centres by the differential shielding effects in NMR spectra [35,36]. A regioselective tosylation of the primary OH group of 1,2-diols for HPLC analysis over chiral stationary phase has been proposed [37].

Regardless of the above-mentioned separation results, long-chain enantiomeric 1,2-alkanediols remain too expensive for several synthetic applications. The objective of our research was to develop a scalable lipase-catalytic method for the separation of pure stereoisomers, preferably without resorting to chromatography. Our recent work involved a molecular dynamics modeling, mass spectrometric and ^{1}H and diffusion NMR (DOSY) studies of the aggregation of enantiomeric 1,2-alkanediols with the aim to develop a method for the stereo- and chemoselective crystallization of the target alcohol enantiomers from the reaction mixtures. These results offered a detailed and novel insight into the aggregation of such diols and provided an efficient protocol for stereo- and chemoselective crystallization of (*S*)-1,2-dodecanediol and related compounds from the crude bioconversion mixtures [38].

The current article presents detailed results of the screening for more preferable reaction conditions for the lipase-catalyzed stereoresolution by varying reaction components such as the solvent, the nucleophile, the lipase and the acyl groups. The semipreparative separation of both enantiomers of 1,2-dodecanediol with high stereochemical purity has been demonstrated.

2. Experimental

2.1. Materials and general methods

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on 800 or 400 MHz Bruker Avance spectrometers. All signals were referenced relative to the residual solvent signal. 2D FT methods were used for the full assignment of NMR spectra. Column chromatography was performed on Merck silica gel 60 (230–400 mesh). Thin layer chromatography (TLC) was performed using DC-Alufolien Kieselgel 60 F_{254} (Merck) silica gel plates and stains were visualized by heating with anisaldehyde solution (3 mL of anisaldehyde, 10 mL of conc. H_2SO_4 in 90 mL of EtOH). HPLC determination of the enantiomeric ratio (*er*) was performed using an IA column (Daicel Chiralpak® IA; 0.46 cm Ø/25 cm); eluent – 10/90 iPrOH/n-hexane; flow rate – 1.0 ml/min; detection – UV 254 nm. Commercial racemic 1,2-octanediol and 1,2-dodecanediol were used without additional purification. Immobilized enzymes were donated by the producer: *Candida antarctica* lipase B (Novozym® 435; Batch no.: LC200210; Novozymes®); *Rhizomucor miehei* lipase (RML) (Lipozyme® RM IM; Batch no.: LUX00205; Novozymes®).

2.2. General synthetic protocols

2.2.1. General protocol A. The acylation of 1,2-alkanediols with acid chloride

1,2-Alkanediol was dissolved in pyridine on slight heating; petroleum ether (PE) was added and the mixture was shaken until homogenization. Acid chloride was added dropwise on efficient magnetic stirring. The mixture was stirred at RT for 0.25–16 h depending on the target compound. Reaction was monitored by TLC. Methanol (0.5 ml) was added to the mixture when excess of acid chloride was used and stirring was continued for additional 10 min. The reaction mixture was diluted with ethyl acetate (EtOAc) and aqueous $NaHCO_3$ (10%) was added. Following the neutralization the water layer was separated, and the organic layer washed twice with brine, dried over anhydrous $MgSO_4$, filtered, concentrated and purified by flash chromatography over silica gel. (NB! 1,2-Alkanediol bisbutyrates were used in preparative-scale lipase-catalyzed cleavage without previous purification.) The target products were gained in 91–96% yields.

2.2.2. General protocol B. The lipase-catalyzed stereoselective cleavage of the diesters

A diester (0.35 mmol) was dissolved in 4.8 ml of solvent followed by the addition of the nucleophile and the immobilized enzyme preparation and the mixture was shaken until a suitable conversion was identified by TLC. Then the enzyme was filtered off and the reaction mixture was concentrated and analyzed quantitatively by NMR. After the analysis the relevant components were separated from the crude product by column chromatography and the esters hydrolyzed with NaOH in EtOH. Finally, the resulting diols were 1-tosylated and analyzed by HPLC over a chiral stationary phase. (The analytical results are presented in Table 1).

2.2.3. General protocol C. The alkaline hydrolysis of the 1,2-alkanediol esters

The mono- or diesters of 1,2-alkanediols were dissolved in EtOH, 1 M NaOH solution in EtOH (2.5 eq.) was added and the mixture was stirred for 30 min. The completion of the reaction was verified by TLC. EtOH was removed on a rotary evaporator, aqueous $NaHCO_3$ was added and the product was extracted with 1/1 mixture of PE and EtOAc. The extract was washed with an additional portion of aqueous $NaHCO_3$ and twice with brine; dried over anhydrous $MgSO_4$, filtered, evaporated and tosylated for the HPLC analysis or refined by recrystallizing from $CHCl_3$.

2.2.4. General protocol D. The synthesis of 1-tosylates of the 1,2-alkanediols

An 1,2-alkanediol (0.5 mmol) was dissolved in dichloromethane (10 mL) and triethylamine (0.55 mmol) was added while stirring at RT. Then, the tosyl chloride (0.55 mmol) was added, followed by a catalytic amount of dibutyltin oxide (5 mg) and the mixture was stirred at RT for 16 h. Subsequently, EtOAc (60 ml) was added and the resulting solution was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated on a rotary evaporator. The crude product was purified by chromatography over silica gel to afford target 1-tosylate in 75–85% yield.

2.3. The synthesis of the diester substrates

2.3.1. Synthesis of (rac)-1,2-dodecanediol bisacetate (**1**)

The synthesis was performed following the General protocol A (Section 2.2.1). Racemic 1,2-dodecanediol (**3**) (1.01 g; 5 mmol; 1 eq.) in pyridine (6 ml) and PE (14 ml) was acylated with acetyl chloride (0.85 ml; 12 mmol; 2.4 eq.). The target product (**1**) was gained in 94% yield (1.346 g).

1: ^{1}H NMR (800 MHz, $CDCl_3$) δ 5.06 (1H, dddd, J = 3.3, 5.9, 6.6, 7.4 Hz, H-2), 4.21 (1H, dd, J = 3.3, 11.9 Hz, H-1), 4.02 (1H, dd, J = 6.6, 11.9 Hz, H-1), 2.06 and 2.05 (2 × 3H, 2s, Ac-1,2), 1.56 (2H, m, H-3), 1.30–1,23 (16H, m, H-4–H-11), 0.87 (3H, t, J = 7.1 Hz, H-12). ^{13}C NMR (201 MHz, $CDCl_3$) δ 170.76 (Ac-1), 170.56 (Ac-2), 71.55 (C-2), 65.09 (C-1), 31.85 (C-10), 30.65 (C-3), 29.52, 29.47, 29.37, 29.32, 29.26 (C-5–C-9), 25.06 (C-4), 22.63 (C-11), 21.03 (Ac-2), 20.73 (Ac-1), 14.05 (C-12). MS (*m*/*z*): 287, 227, 166, 153, 138, 128, 124, 117, 116, 111, 110, 109, 101, 100, 97, 96, 95, 86, 83, 82, 81, 73, 72, 71, 68, 67, 58, 57, 43. IR (neat, cm^{-1}): 606, 961, 1048, 1226, 1371, 1466, 1746, 2856, 2926. Anal. Calcd. for $C_{16}H_{30}O_4$ (286.46): C, 67.08; H,

Table 1
Lipase-catalyzed kinetic resolution of enantiomers of 1,2-alkanediols. Reaction conditions: 0.35 mmol of the substrate and 0.1 g of the immobilized lipase preparation were incubated with 0.2 ml of nucleophile in 4.8 ml of solvent (see General protocol A, Section 2.2.1).

Run no.	Substrate	Lipase	Solvent	Nucleophile	Temp. (°C)	Incubation time (h)	Conversion of substrate (%)	Components in crude product			
								Product no.	Configuration	Amount (%)	*ee*[a]
1	**1**	CALB	CH_3CN	CH_3OH	20	48	85.2	**1**	***S***	14.8	66.4
								2	***R***	50.5	86.2[b]
								4		0.6	
								3	***S***	34.1	98.3
2	**1**	CALB	CH_3CN	CH_3OH	55	18	93.4	**1**		6.6	nd[c]
								2		45.4	nd
								4		10.9	nd
								3	***S***	37.1	93.4
3	**1**	CALB	CH_3CN	H_2O	20	44	41.5	**1**	***S***	58.5	51.2
								2	***R***	26.3	86.3[b]
								4		8.2	
								3		7	nd
4	**1**	CALB	CH_3CN	H_2O	55	48	76.6	**1**	***S***	23.4	46.4
								2	***R***	40.9	70.6[b]
								4		9.1	
								3	***S***	26.6	83.7
5	**1**	RML	CH_3CN	CH_3OH	20	168	2	**1**		98.0	nd
								2		2.0	nd
								3		Not detected	
6	**1**	RML	CH_3CN	H_2O	20	120	30	**1**	***R***	70.9	31.6
								2	***S***	29.1	76.5
								4		<1	nd
								3		Not detected	
7	**1**	RML	CH_3CN	H_2O	55	168	28	**1**	***R***	72.0	30.9
								2	***S***	27.3	76.0
								4		0.7	nd
								3		Not detected	
8	**1**	CALB	C_6H_6	CH_3OH	20	264	89.2	**1**		8.7	nd
								2		44.2	nd
								4		6.4	nd
								3	***S***	40.7	93.6
9	**1**	CALB	C_6H_6	CH_3OH	55	24	87	**1**		13.0	nd
								2		37.5	nd
								4		20.5	nd
								3	***S***	29.0	91.4
10	**5**	CALB	CH_3CN	CH_3OH	20	16	85.0	**5**		15.0	nd
								8		52.3	nd
								11		Not detected	
								3	***S***	32.7	99.7
11	**5**	CALB	CH_3OH	CH_3OH	20	120 days	95[d]	**3**	***S***	37.0	85.1
12	**6**	CALB	CH_3CN	H_2O	20	528	29.1	**6**		70.9	nd
								9	***R***	24.1	89.4[b]
								12		3.5	
								3		1.5	nd
13	**6**	RML	CH_3CN	H_2O	20	168	40.8	**6**	***R***	59.2	54.0
								9	***S***	36.1	79.0[b]
								12		4.7	
								3		Not detected	
14	**15**	CALB	CH_3CN	CH_3OH	20	72	32.8	**15**	***S***	67.2	nd
								16	***R***	32.8	90.5
15	**15**	CALB	CH_3CN	H_2O	20	60 days	17.4	**15**	***S***	82.6	20.2
								16	***R***	17.4	91.5
16	**7**	CALB	CH_3CN	CH_3OH	20	16	85.5	**7**		14.5	nd
								10		50.4	nd
								13		3.3	nd
								14	***S***	31.8	>99.8

[a] The enantiomeric excess for 1,2-alkanediols was determined in the form of 1-monotosylates by HPLC over chiral stationary phase [38].
[b] *ee* was determined for the mixture of regioisomeric monoesters (which was isolated, hydrolyzed and the diol 1-tosylated).
[c] nd, not determined.
[d] Estimated by isolated unreacted diester.

10.58. Found: C, 67.02; H, 10.54. TLC: R_f = 0.20 (EtOAc/PE: 0.2/10). Flash chromatography eluent: EtOAc/PE: 0.5/10.

2.3.2. Synthesis of (rac)-1,2-dodecanediol bisbutyrate (**5**)

The synthesis was performed following the General protocol A (Section 2.2.1). Racemic 1,2-dodecanediol (**3**) (1.01 g; 5 mmol; 1 eq.) in pyridine (4 ml) and PE (12 ml) was acylated with butyryl chloride (1.25 ml; 12 mmol; 2.4 eq.). The target product (**5**) was gained in 96% yield (1.641 g). The characterization of the product **5** has been reported [38].

2.3.3. Synthesis of (rac)-1,2-dodecanediol bisisobutyrate (**6**)

The synthesis was performed following the General protocol A (Section 2.2.1). Racemic 1,2-dodecanediol (**3**) (0.404 g; 2 mmol; 1 eq.) in pyridine (5 ml) and PE (15 ml) was acylated with isobutyryl chloride (0.52 ml; 5 mmol; 2.5 eq.). The target product (**6**) was gained in 96% yield (0.655 g).

6: ^{1}H NMR (800 MHz, $CDCl_3$) δ 5.09 (1H, dddd, J = 3.3, 6.2, 6.9, 6.9 Hz, H-2), 4.22 (1H, dd, J = 3.3, 11.8 Hz, H-1), 4.03 (1H, dd, J = 6.9, 11.8 Hz, H-1), 2.54 (2 × 1H, hept, J = 7.0 Hz, Bu-2), 1.56 (2H, m, H-3), 1.29–1.24 (16H, m, H-4–H-11), 1.162, 1.158, 1.152, 1.150 (4 × 3H, d, J = 7.0 Hz, Bu-3), 0.81 (3H, t, J = 6.9 Hz, H-12). ^{13}C NMR (201 MHz, $CDCl_3$) δ 176.71 (1-Bu-1), 176.54 (2-Bu-1), 71.13 (C-2), 64.98 (C-1), 34.10 and 33.91 (Bu-2), 31.86 (C-10), 30.73 (C-3), 29.53, 29.47, 29.38, 29.32, 29.28 (C-5–C-9), 25.00 (C-4), 22.64 (C-11), 18.94, 18.94, 18.88 and 18.85 (Bu-3), 14.07 (C-12). MS (m/z): 343, 255, 254,241, 211, 184, 183, 166, 153, 132, 138, 129, 128, 124, 111, 110, 109, 97, 96, 95, 83, 82, 71. IR (neat, cm^{-1}): 1155, 1192, 1257, 1387, 1470, 1740, 2856, 2927. Anal. Calcd. for $C_{20}H_{38}O_4$ (342.58): C, 70.12; H, 11.20. Found: C, 70.16; H, 11.23. TLC: R_f = 0.36 (EtOAc/PE: 0.3/10). Flash chromatography eluent: EtOAc/PE: 0.2/10.

2.3.4. Synthesis of (rac)-1,2-dodecanediol bisbenzoate (**15**)

The synthesis was performed following the General protocol A (Section 2.2.1). Racemic 1,2-dodecanediol (**3**) (1.01 g; 5 mmol; 1 eq.) in pyridine (5 ml) and PE (15 ml) was acylated with benzoyl chloride (1.13 ml, 9.8 mmol, 2 eq.). The target product (**15**) was gained in 91% yield (1.867 g).

15: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (2H, dm, J = 8,3 Hz, H-o''), 8.03 (2H, dm, J = 8,3 Hz, H-o'), 7.57 (1H, tm, J = 7.4 Hz, H-p''), 7.55 (1H, tm, J = 7.4 Hz, H-p'), 7.45 (2H, m, H-m''), 7.42 (2H, m, H-m'), 5.53 (1H, m, H-2), 4.58 (1H, dd, J = 3.3, 11.9 Hz, H-1), 4.39 (1H, dd, J = 6.8, 11.9, Hz, H-1), 1.87 and 1.80 (2H, m, H-3), 1.46 (2H, m, H-4), 1.38–1.23 (14H, m, H-5–H-11), 0.89 (3H, t, J = 7.2 Hz, H-12). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.28 (C-1′), 166.08 (C-1″) 133.02 (C-p'), 132.96 (C-p''), 130.01 (C-s'), 129.62 (C-s''), 129.58 (C-o', C-o''), 128.31 (C-m'), 128.30 (C-m''), 72.10 (C-2), 65.68 (C-1), 31.81 (C-10), 30.84 (C-3), 29.50, 29.46, 29.36, 29.35, 29.26 (C-5–C-9), 25.12 (C-4), 22.62 (C-11), 14.10 (C-12). MS (m/z): 410, 288, 241, 190, 166, 138, 123, 118, 106, 105, 96. IR (neat, cm^{-1}): 687, 711, 1027, 1070, 1109, 1176, 1265, 1315, 1452, 1603, 1723, 2855, 2926. Anal. Calcd. for $C_{26}H_{34}O_4$ (410.60): C, 76.05; H, 8.36. Found: C, 76.10; H, 8.32. TLC: R_f = 0.18 (EtOAc/PE: 0.2/10).

Flash chromatography eluent: EtOAc/PE: 0.4/10.

2.3.5. Synthesis of (rac)-1,2-octanediol bisbutyrate (**7**)

The synthesis was performed following the General protocol A (Section 2.2.1) and the synthesis described in Section 2.3.2 [38]. Starting from racemic 1,2-octanediol (**14**) (1.46 g, 10 mmol) the target bisbutyrate **7** was obtained in 96% yield (2.756 g). The characterization of the product **7** has been reported [38].

2.4. The lipase-catalyzed stereoselective cleavage of the racemic diesters

2.4.1. Methanolysis of rac-1,2-dodecanediol bisacetate (**1**) catalyzed by CALB (Run 1, Table 3)

The substrate – bisacetate of racemic 1,2-dodecanediol (1.432 g; 5 mmol) was dissolved in acetonitrile (38.4 ml), methanol (1.6 ml) and 3 g of the immobilized CALB preparation Novozym 435 was added. The mixture was shaken at RT for 16 h. The enzyme was filtered off, the solution was evaporated and the residual crude product was recrystallized from 2 ml of $CHCl_3$. Recrystallization needs some heating for the complete dissolution of the material and stirring of the solution on an ice bath for a couple of minutes in order to allow (*S*)-1,2-dodecanediol to crystallize. The target **(*S*)-3** was gained in 26% yield (266 mg). (See Run 1, Table 3 for conversion and *ee* data.)

Solvent was evaporated from the mother liquor (residual crude product) and the residue chromatographed over silica gel. The components were eluted with a EtOAc/PE gradient (from 0.5/10 to 1/2 ratio) to afford 64 mg (5%) of the starting diester, 1.627 g (yield 51%) of the monoacetate **(*R*)-2** contaminated with a small quantity of 1-monoacetate **4** and also an additional portion of **(*S*)-3** – 110 mg (11%).

The mixture of the monoesters was incubated additionally with CALB under methanolytic conditions (in CH_3CN/CH_3OH 96/4 mixture; with 1.0 g of Novozym 435). The process was complete in 48 h; the additional 48 h of incubation improved the already high *ee* of the product only slightly (Run 2, Table 3).

After 96 h of incubation the residual **(*R*)-2** was isolated from the mixture by column chromatography to afford 524 mg (2.15 mmol) of the target compound that was dissolved in 10 ml of EtOH and hydrolyzed promptly (in accordance with the General protocol C; in Section 2.2.3.) by adding 1.5 eq. of NaOH (3.2 ml of 1 M NaOH in EtOH). The crude target **(*R*)-3** obtained was recrystallized from 1.5 ml of $CHCl_3$ to afford 273 mg of the refined product.

(S)-1,2-dodecanediol ((S)-3) [38,39]: ^{1}H NMR (800 MHz, $CDCl_3$, 30 °C) δ 3.71 (1H, m, H-2), 3.66 (1H, dd, J = 2.9, 11.2 Hz, H-1), 3.44 (1H, dd, J = 7.8, 11.2 Hz, H-1), 2.30 (2H, bs, OH), 1.43 (3H, m, 2 × H-3, H-4), 1.33–1.25 (15H, m, H-4–H-11), 0.88 (3H, t, J = 7.2 Hz, H-12). ^{13}C NMR (201 MHz, $CDCl_3$, 30 °C) δ 72.33 (C-2), 66.79 (C-1), 33.10 (C-3), 31.88 (C-10), 29.64 (C-5), 29.59 (C-8), 29.58 (C-7), 29.55 (C-6), 29.32 (C-9), 25.55 (C-4), 22.67 (C-11), 14.11 (C-12). $[\alpha]_D^{20}$ −14 (1.0; EtOH); IR (KBr; cm^{-1}): 530, 582, 662, 720, 838, 871, 972, 992, 1006, 1032, 1053, 1073, 1105, 1135, 1311, 1335, 1470, 2850, 2919, 3240, 3323, 3478. Anal. Calcd. for $C_{12}H_{26}O_2$ (202.38): C, 71.21; H, 12.98. Found: C, 71.20; H, 13.01. TLC: R_f = 0.14 (3/5 EtOAc/PE). Flash chromatography eluent: EtOAc/PE 1/2. Mp = 68–70 °C.

(R)-2-Acetyl-1,2-dodecanediol ((R)-2): ^{1}H NMR (800 MHz, $CDCl_3$) δ 4.89 (1H, dddd, J = 3.1, 6.2, 6.3, 7.5 Hz, H-2), 3.70 (1H, dd, J = 3.3, 11.2 Hz, H-1), 3.60 (1H, dd, J = 6.3, 11.2 Hz, H-1), 2.39 (1H, bs, OH), 2.09 (3H, s, Ac-2), 1.55 (2H, m, H-3), 1.27–1.22 (16H, m, H-4–H-11), 0.86 (3H, t, J = 7.1 Hz, H-12). ^{13}C NMR (201 MHz, $CDCl_3$) δ 171.50 (Ac-2), 75.64 (C-2), 64.66 (C-1), 31.85 (C-10), 30.44 (C-3), 29.53, 29.50, 29.41, 29.40, 29.27 (C-5–C-9), 25.26 (C-4), 22.63 (C-11), 21.16 (Ac-2), 14.06 (C-12). MS (m/z): 245, 244, 215, 214, 158, 157, 156, 155, 139, 108, 93, 92, 91, 59. $[\alpha]^{20}_D$ +1.53 (c 5.9; EtOAc); *ee* > 99.4%. IR (neat, cm^{-1}): 713, 1053, 1242, 1375, 1466, 1739, 2855, 2926, 3436. Anal. Calcd. for $C_{14}H_{28}O_3$ (244.42): C, 68.79; H, 11.57. Found: C, 68.75; H, 11.60. TLC: R_f = 0.13 (EtOAc/C_6H_6 1/5). Flash chromatography eluent: EtOAc/PE 3/10.

(R)-1,2-Dodecanediol ((R)-3): For the NMR see the data presented for **(*S*)-3**. $[\alpha]_D^{20}$ +13.6 (c 1.0; EtOH). Anal. Calcd. for $C_{12}H_{26}O_2$ (202.38): C, 71.21; H, 12.98. Found: C, 71.23; H, 13.02. Mp = 69–71 °C.

2.4.2. Semipreparative methanolysis of rac-1,2-dodecanediol bisbutyrate (**5**) catalyzed by CALB (Run 3, Table 3)

The substrate – bisbutyrate of racemic 1,2-dodecanediol (**5**) (17.12 g; 50 mmol) – was dissolved in 96 ml of CH_3CN, MeOH (4 ml) was added followed by 4 g of Novozym 435. The mixture was shaken for 96 h at RT. The enzyme was filtered off, the solution was evaporated and the residual crude product recrystallized from $CHCl_3$ (20 ml). The target **(*S*)-3** (2.33 g) was gained in 23% yield. The components in mother liquor were separated by column chromatography over silica gel to afford 8.35 g (49%) of (*R*)-2-butyryl-1,2-dodecanediol **(*R*)-8**, which was incubated again with Novozym 435 (3 g) at RT for 96 h. The product was chromatographed, hydrolyzed with NaOH in EtOH and the target **(*R*)-3** was recrystallized from 15 ml of $CHCl_3$ to afford 2.43 g of the refined product with very high enantiomeric purity.

(S)-1,2-dodecanediol ((S)-3) characterization of the compound is presented in Section 2.4.1.

(R)-2-Butyryl-1,2-dodecanediol ((R)-8): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 (1H, dtd, J = 3.3, 2 × 6.3, 7.1 Hz, H-2), 3.73 (1H, ddd, J = 3.3, 5.3,12.0 Hz, H-1), 3.63 (1H, ddd, J = 5.3, 6.3, 12.0 Hz, H-1), 2.34 (2H, t, J = 7.4 Hz, Bu-2), 2.12 (1H, t, J = 5.3 Hz, OH), 1.69 (2H,

hex, J = 7.4 Hz, Bu-3), 1.60 (2H, m, H-3), 1.35–1.25 (16H, m, H-4–H-11), 0.98 (3H, t, J = 7.4 Hz, Bu-4), 0.88 (3H, t, J = 6.9 Hz, H-12). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.17 (Bu-1), 75.39 (C-2), 64.86 (C-1), 36.38 (Bu-2), 31.86 (C-10), 30.50 (C-3), 29.54, 29.50, 29.42, 29.40, 29.28 (C-5–C-9), 25.26 (C-4), 22.64 (C-11), 18.50 (Bu-3), 14.07 (C-12), 13.60 (Bu-4). MS (m/z): 274, 273, 241, 201, 172, 171, 153, 131, 124, 111, 110, 109, 103, 102, 97, 96, 95, 89, 88, 87, 83, 82, 74, 73, 72, 71, 70, 69, 58, 57. $[\alpha]_D^{20}$ +4.0 (c 12, EtOAc), ee > 99%. IR (neat, cm^{-1}): 1090, 1185, 1260, 1381, 1465, 1736, 2855, 2926, 3447. Anal. Calcd. for $C_{16}H_{32}O_3$ (272.48): C, 70.52; H, 11.86. Found: C, 70.47; H, 11.89. TLC: R_f = 0.34 (EtOAc/PE 1/5). Flash chromatography eluent: EtOAc/PE 1/5.

2.4.3. Hydrolysis of rac-1,2-dodecanediol bisisobutyrate (**6**) catalyzed by RML (Run 13, Table 1)

The substrate, bisisobutyrate of racemic 1,2-dodecanediol (**6**) (343 mg, 1 mmol) was dissolved in acetonitrile (9.6 ml), H_2O (0.4 ml) was added followed by Lipozyme RM IM (0.5 g). The mixture was shaken at RT for 120 h. Subsequently, the enzyme was filtered off, the solvent evaporated and separated by column chromatography over silica gel to afford (*S*)-2-isobutyryl-1,2-dodecanediol **(*S*)-9** (106 mg; 39%, ee = 78.4%).

(S)-2-Isobutyryl-1,2-dodecanediol ((S)-9): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.89 (1H, dtd, J = 3.3, 6.3, 6.3, 7.1 Hz, H-2), 3.70 (1H, dd, J = 3.3, 12.0 Hz, H-1), 3.61 (1H, dd, J = 6.3, 12.0 Hz, H-1), 2.58 (1H, hep, J = 7.0 Hz, Bu-2), 2.30 (1H, bs, OH), 1.57 (2H, m, H-3), 1.31–1.25 (16H, m, H-4–H-11), 1.18 1.17 (2 × 3H, d, J = 7.0 Hz, Bu-3), 0.87 (3H, t, J = 6.9 Hz, H-12). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.66 (Bu-1), 75.33 (C-2), 64.93 (C-1), 34.17 (Bu-2), 31.90 (C-10), 30.53 (C-3), 29.58, 29.53, 29.46, 29.42, 29.32 (C-5–C-9), 25.26 (C-4), 22.68 (C-11), 19.12, 18.95 (Bu-3), 14.10 (C-12). MS (m/z): 273, 171, 132, 131, 111, 110, 103, 102, 97, 87, 83, 82, 74, 71, 58, 43. $[\alpha]_D^{20}$ −1.6 (c 6.0, EtOAc), ee = 79%. IR (neat, cm^{-1}): 1075, 1161, 1198, 1388, 1469, 1735, 2856, 2926, 3447. Anal. Calcd. for $C_{16}H_{32}O_3$ (272.48): C, 70.52; H 11.86. Found: C, 70.48; H, 11.82. TLC: R_f = 0.28 (EtOAc/C_6H_6 1/5). Flash chromatography eluent: EtOAc/PE 1/5.

2.4.4. Methanolysis of rac-1,2-dodecanediol bisbenzoate (**15**) catalyzed by CALB (Run 14, Table 1)

The substrate – racemic bisbenzoate **15** – was dissolved in acetonitrile (9.6 ml); methanol (0.4 ml) was added followed by Novozym 435 (0.3 g). The mixture was shaken at RT for 64 h. The enzyme was filtered off, the solvent evaporated and the product separated by chromatography to afford 111 mg of the (R)-2-benzoyl-1,2-dodecanediol **(*R*)-16** (*y*.: 36%; ee = 90.2%).

(R)-2-benzoyl-1,2-dodecanediol ((R)-16): ^{1}H NMR (800 MHz, $CDCl_3$) δ 8.07 (2H, m, Ph-*o*), 7.58 (1H, m, Ph-*p*), 7.46 (2H, m, Ph-*m*), 5.17 (1H, dddd, J = 3.2, 5.4, 6.3 7.9 Hz H-2), 3.84 (1H, ddd, J = 3.2, 5.4, 12.0 Hz, H-1), 3.77 (1H, dt, J = 2 × 5.4, 12.0 Hz, H-1,), 2.19 (1H, bt, J = 2 × 5.4 Hz, OH), 1.76 and 1.71 (2H, m, H-3), 1.36–1.22 (16H, m, H-4–H11), 0.88 (3H, t, J = 6.9 Hz, H-12). ^{13}C NMR (201 MHz, $CDCl_3$) δ 166.95 (C-1′), 133.08 (Ph-*p*), 130.10 (Ph-*s*), 129.65 (Ph-*o*), 128.37 (Ph-*m*), 76.43 (C-2), 64.99 (C-1), 31.86 (C-10), 30.64 (C-3), 29.54, 29.51, 29.44, 29.42, 29.29 (C-5–C-9), 25.33 (C-4), 22.65 (C-11), 14.11 (C-12). MS (m/z): 309, 308, 307, 184, 169, 166, 165, 137, 136, 135, 123, 118, 106, 105, 93, 92, 91. $[\alpha]_D^{20}$ +23.3 (c 5.8; EtOAc). IR (neat, cm^{-1}): 712, 1027, 1070, 1116, 1177, 1275, 1315, 1452, 1603, 1719, 2855, 2926, 3435. Anal. Calcd for $C_{19}H_{30}O_3$ (306.49): C, 74.45; H, 9.89. Found: C, 74.40; H, 9.93. TLC: R_f = 0.42 (EtOAc/PE 3/10). Flash chromatography eluent: EtOAc/PE 1/5.

2.4.5. Methanolysis of rac-1,2-octanediol bisbutyrate (**7**) catalyzed by CALB

The semi-preparative separation of (*S*)-1,2-octanediol **(*S*)-14** [40] from racemic mixture was performed starting from 0.730 g (5 mmol) of the racemic bisbutyrate (**7**); details of the separation of **(*S*)-14** were recently reported [38]; in this trial the residual (*R*)-2-monobutyrate **(*R*)-10** was separated from the mother liquor by column chromatography and incubated additionally under methanolytic conditions (analogously to the process described in Section 2.4.1.). Stereochemically enriched **(*R*)-10** (470 mg; 2.17 mmol, ee = 99.6%) was gained after column chromatography over silica gel. The monoester was hydrolyzed in 10 ml of EtOH with NaOH (1.5 eq.). The target **(*R*)-14** was recrystallized from 1.5 ml of $CHCl_3$ by storing at −15 °C overnight, 196 mg of **(*R*)-14** (*y*.: 27%; ee = 99.8%) was gained.

(S)-1,2-Octanediol ((S)-14): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (1H, dddd, J = 2.9, 5.3, 7.0, 7.9 Hz, H-2), 3.63(1H, dd, J = 2.9, 11.2 Hz, H-1), 3.40 (1H, dd, J = 7.9, 11.2, Hz, H-1), 3.26 (1H, bs, OH), 1.41 (3H, m, 2 × H-3, H-4), 1.32–1.25 (7H, m, H-4, H-5–H-7), 0.88 (3H, t, J = 6.8 Hz, H-8). ^{13}C NMR (101 MHz, $CDCl_3$) δ 72.35 (C-2), 66.74 (C-1), 33.08 (C-3), 31.73 (C-6), 29.30 (C-5), 25.52 (C-4), 22.56 (C-7), 14.02 (C-8). $[\alpha]_D^{20}$ −14.8 (c 1.0; MeOH), ee > 99.8%. mp = 44–46 °C. IR (KBr, cm^{-1}): 492, 537, 581, 653, 861, 881, 988, 1018, 1044, 1092, 1135, 1215, 1334, 1469, 2859, 2929, 3315, 3477. Anal. Calcd. for $C_8H_{18}O_2$ (146.26): C, 65.69; H, 12.43. Found: C, 65.65; H, 12.39. TLC: R_f = 0.08 (EtOAc/PE 6/10). Flash chrom. eluent: EtOAc/PE 7/10.

(R)-2-Butyryl-1,2-octanediol ((R)-10): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (1H, dddd, J = 3.4, 2 × 6.3, 7.0 Hz, H-2), 3.68 (1H, dd, J = 3.4 12.0 Hz, H-1), 3.60 (1H, dd, J = 6.3, 12.0 Hz, H-1), 2.31 (2H, t, J = 7.4 Hz, Bu-2), 1.65 (2H, hex, J = 5 × 7.4 Hz, Bu-3), 1.59–1.54 (2H, m, H-3), 1.33–1.23 (8H, m, H-4–H-7), 0.94 (3H, t, J = 7.4 Hz, Bu-4), 0.86 (3H, t, J = 6.9 Hz, H-8). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.12 (Bu-1), 75.34 (C-2), 64.75 (C-1), 36.36 (Bu-2), 31.60 (C-6), 30.49 (C-3), 29.04 (C-5), 25.19 (C-4), 22.47 (C-7), 18.47 (Bu-3), 13.95 (C-8), 13.56 (Bu-4). MS (m/z): 217, 185, 145, 131, 128, 115, 114, 103, 102, 97, 87, 74, 71, 58, 55, 43. $[\alpha]_D^{20}$ +4.85 (c 7.2; EtOAc), ee > 99%. IR (neat, cm^{-1}): 1089, 1187, 1260, 1384, 1465, 1735, 2852, 2929, 3445. Anal. Calcd. for $C_{12}H_{24}O_3$ (216.36): C, 66.61; H, 11.20. Found: C, 66.65; H, 11.16. TLC: R_f = 0.18 (EtOAc/PE 1/5). Flash chromatography eluent: EtOAc/PE 2/5.

(R)-1,2-Octanediol ((R)-14): $[\alpha]_D^{20}$ +14.6 (c 1.0; MeOH); mp = 44–45 °C. Anal. Calcd. for $C_8H_{18}O_2$ (146.26): C, 65.69; H, 12.43. Found: C, 65.66; H, 12.38. Other characteristics were identical to those determined for ***(S)-14***.

2.5. The synthesis of the reference 1-monoesters

2.5.1. The synthesis of (rac)-1-acetyl-1,2-dodecanediol (**4**)

Racemic 1,2-dodecanediol (**3**) (202 mg; 1 mmol) was dissolved in EtOAc (4 ml), vinyl acetate (2 ml) and Novozym 435 (200 mg) were added and the mixture was shaken at RT for 12 h, until the non-stereoselective acetylation of the primary hydroxyl group was complete by TLC. Then the enzyme was filtered off, the solution concentrated and the product purified by flash column chromatography over silica gel to afford 225 mg (*y*.: 92%) of the target 1-monoacetate (**4**) that was used as a standard compound in quantitative analysis of bioconversion crude products.

4: ^{1}H NMR (800 MHz, $CDCl_3$) δ 4.15 (1H, dd, J = 2.8, 11.4 Hz, H-1), 3.94 (1H, dd, J = 7.7, 11.4 Hz, H-1), 3.85 (1H, m, H-2), 2.11 (3H, s, Ac), 2.09 (1H, bs, OH), 1.46 (3H, m, 2 × H-3, H-4), 1.33 (1H, m, H-4), 1.28–1.23 (14H, m, H-5–H-11), 0.87 (3H, t, J = 7.1 Hz, H-12). ^{13}C NMR (201 MHz, $CDCl_3$) δ 171.21 (Ac), 69.96 (C-2), 68.78 (C-1), 33.30 (C-3), 31.88 (C-10), 29.57, 29.55, 29.53, 29.50, 29.30 (C-5–C-9), 25.34 (C-4), 22.66 (C-11), 20.89 (Ac), 14.09 (C-12). MS (m/z): 245, 227, 172, 171, 152, 138, 127, 126, 125, 124, 123, 111, 103, 97, 96, 95, 85, 83, 82, 81, 75, 74, 71, 69, 58, 57, 55, 43. IR (neat, cm^{-1}): 1240, 1463, 1738, 2852, 2925, 3440. Anal. Calcd. for $C_{14}H_{28}O_3$ (244.42): C, 68.79; H, 11.57. Found: C, 68.74; H, 11.62. TLC: R_f = 0.44 (EtOAc/PE 1/2). Flash chromatography eluent: EtOAc/PE 1/5.

*2.5.2. The synthesis of (R)-1-butyryl-1,2-dodecanediol (**(R)-11**)*

The synthesis was performed following the General protocol A (Section 2.2.1). (R)-1,2-dodecanediol (**(R)-3**) (202 mg, 1 mmol; 1 eq.) in pyridine (2 ml) and PE (6 ml) was acylated with butyryl chloride (0.11 ml, 1 mmol, 1 eq.). The reaction time was 15 minutes and the target product (**(R)-11**) was gained in 93% yield (253 mg).

(R)-11: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 (1H, dd, J = 3.2, 11.4 Hz, H-1), 3.90 (1H, dd, J = 7.3, 11.4 Hz, H-1), 3.76 (1H, m, H-2), 2.26 (2H, t, J = 7.4 Hz, Bu-2), 2.11 (1H, bs, OH), 1.60 (2H, hex, J = 7.4 Hz, Bu-3), 1.40 (2H, m, H-3), 1.30–1.22 (16H, m, H-4–H-11), 0.88 (3H, t, J = 7.4 Hz, Bu-4), 0.81 (3H, t, J = 6.9 Hz, H-12). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.85 (Bu-1), 70.01 (C-2), 68.49 (C-1), 36.39 (Bu-2), 33.35 (C-3), 31.85 (C-10), 29.53, 29.50, 29.42, 29.40, 29.27 (C-5–C-9), 25.26 (C-4), 22.63 (C-11), 18.50 (Bu-3), 14.05 (C-12), 13.59 (Bu-4). MS (m/z): 273, 255, 241, 225, 211, 201, 184, 171, 166, 153, 131, 124, 102, 87, 71,69, 43,41. $[\alpha]_D^{20}$ +2.64 (c 3.8; EtOAc), *ee* > 99%. IR (neat, cm^{-1}): 410, 457, 475, 558, 718, 749, 840, 876, 946, 991, 1019, 1078, 1109, 1143, 1213, 1269, 1312, 1377, 1413, 1426, 1449, 1471, 1705, 2849, 2873, 2921, 2960, 3507. Anal. Calcd. for $C_{16}H_{32}O_3$ (272.48): C, 70.52; H, 11.86. Found: C, 70.45; H, 11.91. TLC: R_f = 0.49 (EtOAc/PE 3/10). Flash chromatography eluent: EtOAc/PE 1.5/10.

*2.5.3. The synthesis of (R)-1-iso-butyryl-1,2-dodecanediol (**(R)-12**)*

The synthesis was performed following the General protocol A (Section 2.2.1). (*R*)-1,2-dodecanediol (**(R)-3**) (202 mg, 1 mmol; 1 eq.) in pyridine (2 ml) and PE (6 ml) was acylated with iso-butyryl chloride (0.11 ml, 1 mmol, 1 eq.). The reaction time was 15 min and the target product (**(R)-12**) was gained in 91% yield (248 mg).

(R)-12: ^{1}H NMR (800 MHz, $CDCl_3$) δ 4.13 (1H, dd, J = 3.2, 11.4 Hz, H-1), 3.96 (1H, dd, J = 7.2 and 11.4 Hz, H-1), 3.83 (1H, m, H-2), 2.59 (1H, hep, J = 7.0 Hz, Bu-2), 1.44 (2H, m, H-3), 1.29–1.23 (16H, m, H-4–H-11), 1.15 and 1.14 (2 × 3H, d, J = 7.0 Hz, Bu-3), 0.84 (3H, t, J = 7.2 Hz, H-12). ^{13}C NMR (201 MHz, $CDCl_3$) δ 177.33 (Bu-1), 69.84 (C-2), 68.38 (C-1), 33.62 (Bu-2), 33.18 (C-3), 31.77 (C-10), 29.48, 29.44, 29.41, 29.29, 29.22 (C-5–C-9), 25.27 (C-4), 22.56 (C-11), 18.85 and 18.77 (Bu-3), 14.02 (C-12). MS (m/z): 273, 255, 211, 201, 184, 171, 166, 153, 131, 124, 102, 87, 71, 69, 43, 41. $[\alpha]_D^{20}$ +5.16 (c 4.0; EtOAc), *ee* > 99%. IR (neat, cm^{-1}): 457, 479, 506, 564, 722, 762, 840, 880, 916, 965, 984, 998, 1050, 1085, 1108, 1135, 1170, 1218, 1247, 1295, 1322, 1355, 1391, 1453, 1470, 1709, 2849, 2920, 2957, 3514. Anal. Calcd. for $C_{16}H_{32}O_3$ (272.48): C, 70.52; H 11.86. Found: C, 70.46; H, 11.83. TLC: R_f = 0.52 (EtOAc/PE 3/10). Flash chromatography eluent: EtOAc/PE 1.5/10.

*2.5.4. The synthesis of (R)-1-benzoyl-1,2-dodecanediol (**(R)-17**)*

The synthesis was performed following the General protocol A (Section 2.2.1). (R)-1,2-dodecanediol (**(R)-3**) (202 mg, 1 mmol; 1 eq.) in pyridine (2 ml) and PE (6 ml) was acylated with benzoyl chloride (0.12 ml, 1 mmol, 1 eq.). The reaction time was 15 minutes and the target product (**(R)-17**) was gained in 91% yield (279 mg).

(R)-17: ^{1}H NMR (800 MHz, $CDCl_3$) δ 8.06 (2H, m, Ph-*o*), 7.59 (1H, m, Ph-*p*), 7.46 (2H, m, Ph-*m*), 4.40 (1H, dd, J = 3.2 and 11.6 Hz, H-1), 4.23 (1H, dd, J = 7.2 and 11.6 Hz, H-1), 3.99 (1H, m, H-2), 2.18 (1H, bs, OH), 1.57 (2H, m, H-3), 1.51 and 1.40 (2H, m, H-4), 1.35–1.23 (14H, m, H-5–H-11), 0.89 (3H, t, J = 6.9 Hz, H-12). ^{13}C NMR (201 MHz, $CDCl_3$) δ 166.73 (CO), 133.16 (Ph-*p*), 129.81 (Ph-*s*), 129.62 (Ph-*o*), 128.41 (Ph-*m*), 70.11 (C-2), 69.22 (C-1), 33.40 (C-3), 31.88 (C-10), 29.58, 29.56, 29.55, 29.52, 29.31 (C-5–C-9), 25.40 (C-4), 22.67 (C-11), 14.12 (C-12). MS (m/z): 307, 289, 275, 259, 245, 231, 218, 204, 184, 169,165, 141, 136, 123, 105, 92, 71, 55, 41. $[\alpha]_D^{20}$ + 0.90 (c 14; EtOAc). IR (neat, cm^{-1}): 534, 689, 709, 918, 960, 1028, 1074, 1106, 1131, 1147, 1182, 1250, 1304, 1326, 1410, 1450, 1471, 1586, 1603, 1696, 2849, 2915, 3493. Anal. Calcd. for $C_{19}H_{30}O_3$ (306.49): C, 74.45; H, 9.89. Found: C, 74.38; H, 9.92. TLC: R_f = 0.54 (EtOAc/PE 3/10). Flash chromatography eluent: EtOAc/PE 1.5/10.

*2.5.5. The synthesis of (R)-1-butyryl-1,2-octanediol (**(R)-13**)*

The synthesis was performed following the General protocol A (Section 2.2.1). (*R*)-1,2-octanediol (**(R)-14**) (146 mg, 1 mmol; 1 eq.) in pyridine (2 ml) and PE (6 ml) was acylated with butyryl chloride (0.11 ml, 1 mmol, 1 eq.). The reaction time was 15 min and the target product (**(R)-13**) was gained in 94% yield (203 mg).

(R)-13: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (1H, dd, J = 3.2, 11.4 Hz, H-1), 3.96 (1H, dd, J = 7.2, 11.4 Hz, H-1), 3.83 (1H, m, H-2), 2.34 (2H, t, J = 7.4 Hz, Bu-2), 2.26 (1H, bs, OH), 1.67 (2H, hex, J = 5 × 7.4 Hz, Bu-3), 1.46 (2H, m, H-3), 1.33–1.25 (8H, m, H-4–H-7), 0.97 (3H, t, J = 7.4 Hz, Bu-4), 0.87 (3H, t, J = 6.9 Hz, H-8). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.91 (Bu-1), 69.98 (C-2), 68.51 (C-1), 36.07 (Bu-2), 33.36 (C-3), 31.71 (C-6), 29.22 (C-5), 25.33 (C-4), 22.57 (C-7), 18.42 (Bu-3), 14.04(C-8), 13.64 (Bu-4). MS (m/z): 217, 199, 185, 173, 155, 145, 131, 115, 102, 97, 87, 74, 71, 69, 55, 43. $[\alpha]_D^{20}$ +2.85 (c 4.4; EtOAc). IR (neat, cm^{-1}): 725, 998, 1097, 1183, 1261, 1381, 1460, 1740, 2859, 2932, 2959, 3454. Anal. Calcd. for $C_{12}H_{24}O_3$ (216.36): C, 66.61; H, 11.20. Found: C, 66.67; H, 11.15. TLC: R_f = 0.44 (EtOAc/PE 3/10). Flash chromatography eluent: EtOAc/PE 2/5.

2.6. The synthesis of 1-tosyl-1,2-alkanediols

The synthesis of (*S*)-1-tosyl-1,2-dodecanediol **(*S*)-18** and (*S*)-1-tosyl-1,2-octanediol **(*S*)-19** was performed in accordance with the General protocol D; it has recently been described along with characterization of the products [38]. The corresponding racemic standards of these tosylates for use as HPLC references as well as corresponding (*R*)-configured 1-tosylates to be analyzed for stereochemical purity were synthesized in an analogous manner.

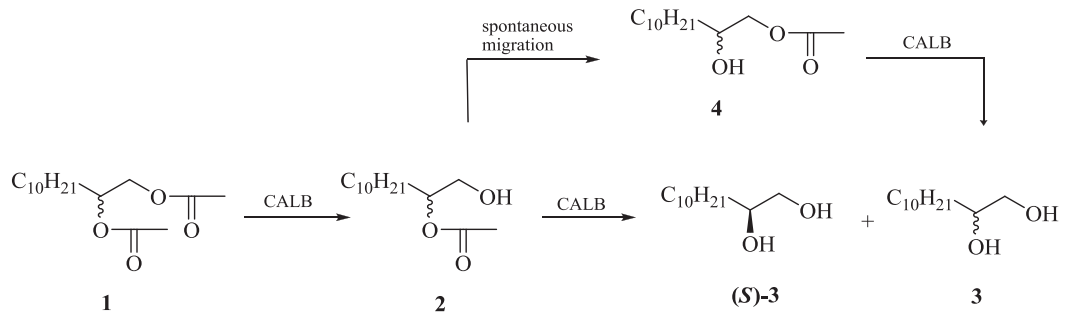
HPLC determination of the *ee* of 1,2-dodecanediols **(*S*)-3** and **(*R*)-3** as well as **(*S*)-14** and **(*R*)-14** gave the retention times of the 1-tosylates as follows: **(*R*)-18**: 8.69 min; **(*S*)-18**: 10.50 min; **(*R*)-19**: 10.33 min; **(*S*)-19**: 12.50 min [38] (eluent – 10/90 iPrOH/n-hexane; flow rate – 1.0 ml/min).

3. Results and discussion

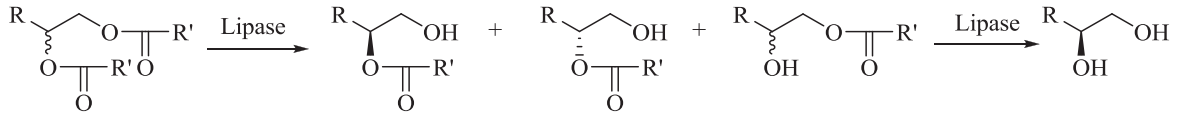
The screening for the preferable reaction conditions for the stereoselective lipase-catalysed cleavage of long-chain 1,2-alkanediol diesters was based on previous empirical results. An efficient lipase-catalytic process based on the use of CALB in CH_3CN/CH_3OH (96/4, v/v) mixture at RT has been used for the kinetic resolution of enantiomers of 1-phenyl-1,2-ethanediol, involving sequential cleavage of both of the acetyl groups from the corresponding bisacetate [23]. This was chosen as a lead process because our preliminary trials showed the ability of this methodology to produce deacetylated (*S*)-1,2-dodecanediol **(*S*)-3** from racemic bisacetate **1** at a satisfactory rate and considerably high stereoselectivity (Scheme 1). Examples of the use of acetonitrile (which is an atypical reaction medium for lipases) for the regioselective hydrolysis of some deoxysugar diesters catalyzed by CALB have been previously reported [41,42].

The points to be addressed by the screening trials herein were: (i) possibility to enhance the reaction rate while retaining an acceptable stereoselectivity; (ii) clarification of the influence the spontaneous intramolecular migration of the acyl group (in the initially formed intermediate 2-monoester **2** and related intermediates; Scheme 1) has on the "apparent" stereoselectivity of the overall process and how to optimize it in this regard; (iii) development of a method allowing to prepare both of the alkanediol enantiomers in high stereochemical purity.

Screening of the reaction conditions along with the corresponding results are listed in Table 1. The tested modifications of the



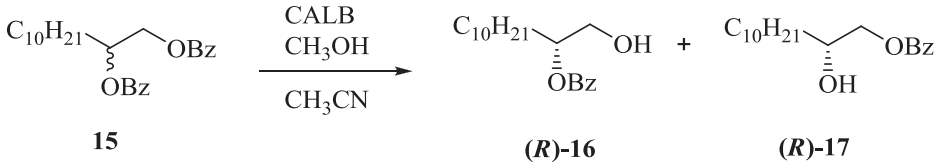
Scheme 1. CALB catalyzed sequential methanolysis of the diester **1** for the kinetic resolution of 1,2-dodecanediol enantiomers involves a minor spontaneous acetyl migration reaction which diminishes the apparent stereoselectivity of the process.



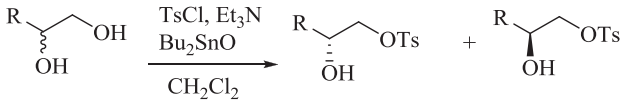
5: R= $C_{10}H_{21}$, R'= C_3H_7 **(*S*)-8**: R= $C_{10}H_{21}$, R'= C_3H_7 **(*R*)-8**
6: R= $C_{10}H_{21}$, R'= $CH(CH_3)_2$ **(*S*)-9**: R= $C_{10}H_{21}$, R'= $CH(CH_3)_2$ **(*R*)-9**
7: R= C_6H_{13}, R'= C_3H_7 **(*S*)-10**: R= C_6H_{13}, R'= C_3H_7 **(*R*)-10**

11: R= $C_{10}H_{21}$, R'= C_3H_7 **(*S*)-3**: R= $C_{10}H_{21}$
12: R= $C_{10}H_{21}$, R'= $CH(CH_3)_2$
13: R= C_6H_{13}, R'= C_3H_7 **(*S*)-14**: R= C_6H_{13}

Scheme 2. Lipase-catalysed stereoselective deacylation of 1,2-alkanediol diesters.



Scheme 3. Stereoselective methanolysis of 1,2-dodecanediol bisbenzoate **15** catalysed by CALB; **(*R*)-17** is produced by the acyl migration.



3: R= $C_{10}H_{21}$ **(*R*)-18**: R= $C_{10}H_{21}$ **(*S*)-18**: R= $C_{10}H_{21}$
14: R= C_6H_{13} **(*R*)-19**: R= C_6H_{13} **(*S*)-19**: R= C_6H_{13}

Scheme 4. Synthesis of 1,2-alkanediol 1-tosylates.

process were: (i) for lipase – RML vs. CALB; (ii) for nucleophile – H_2O vs. MeOH, (iii) for temperature – 55 °C vs. 20 °C; (iv) for the reaction medium – C_6H_6 or MeOH vs. CH_3CN and (v) for the structure of the acyl group of the substrate – butyryl or iso-butyryl or benzoyl vs. acetyl (Schemes 2 and 3).

The crude products were analyzed by ^1H NMR to afford exact percentages of all of the four possible components in the mixtures (Fig. 1). The *ee* was determined by HPLC analysis of the 1,2-alkanediol 1-tosylates over a chiral stationary phase (Scheme 4), derived from relevant components of the crude products (the ester components were separated, hydrolyzed and tosylated).

Comparing the lipases' catalytic performance, CALB first cleaves the ester of the primary hydroxyl group of the bisacetate **1** with a slight preference for *R*-enantiomer of 2-monoacetate, leaving remaining diester **1** mainly in *S* configuration (Table 1, Run 1). Similar selectivity has been described by Virsu et al. in the methanolysis of 1-phenylethan-1,2-diol bisacetate [23]. The second step of the sequential methanolysis catalyzed by CALB is clearly the *S*-specific one – affording **(*S*)-3** in high enantiomeric purity (*ee* > 98%). We attribute the formation of up to one percent of opposite (*R*)-enantiomer in product **3** to the side-reaction of intramolecular spontaneous acetyl migration in the non-racemic mixture of enantiomers of 2-monoester **2** occurring after the first step (Scheme 1).

RML in acetonitrile/methanol (96/4) system was found to be almost inactive towards bisacetate **1** (Table 1, Run 7). In contrast, RML in acetonitrile/water (96/4) cleaves the primary ester group with modest stereoselectivity preferring molecules with *S* configuration (Table 1, Runs 5 and 6). In conclusion, our screening results have shown CALB to be preferable for the stereoselective cleavage of the diols' diesters.

Two nucleophiles, water and methanol were compared in acetonitrile for CALB and, somewhat unexpectedly, methanol is clearly preferable. (Runs 1–4; Table 1)

Regarding reaction temperature, in most cases performing reactions at higher temperatures (e.g. at 55 °C vs. 20 °C) leads to a higher rate, but lower stereoselectivity of the process (Runs 1–5, Table 1). However, there were two examples where stereoselectivity of the process was independent from temperature, one concerned CALB in benzene/methanol (Runs 8, 9; Table 1) and the other RML in acetonitrile/water (Runs 6 and 7, Table 1).

As for the solvent, CALB in acetonitrile/methanol utilizing 1,2-dodecanediol bisbutyrate **5** as a substrate afforded **(*S*)-3** with exceptional stereoselectivity (*ee* = 99.7% determined for the diol

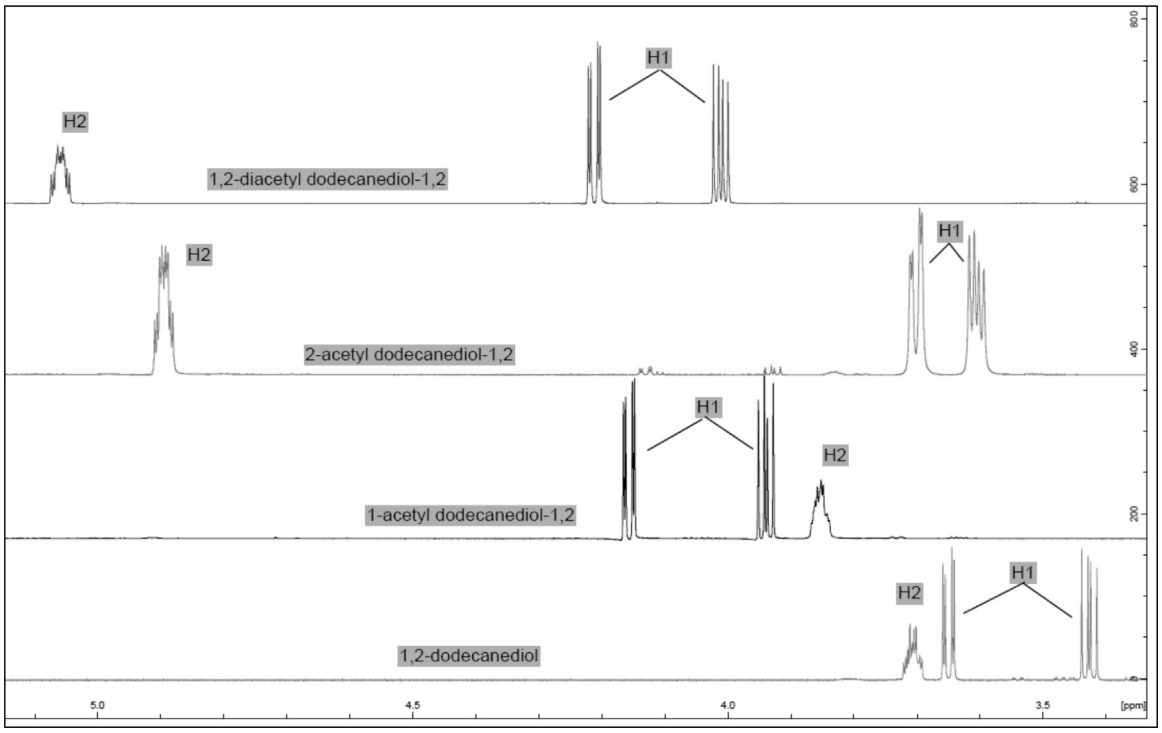


Fig. 1. The NMR signals of the hydrogen atoms attached to C-1 and C-2 atoms of 1,2-dodecanediol and its acetates which were used for the quantification of the components in the crude products of enzymatic cleavage of the diester **1**.

Table 2
Migration rate (at RT in acetonitrile) of the acyl groups within 1,2-alkanediol 2-monoesters, estimated by NMR.

Starting 2-monester	Product, 1-monoester	Migrating acyl group	Time (hours or days)	Conversion (%)	Initial rate (% × 24 h^{-1})
2	**4**	Acetyl	72 h	10.9	3.6
8	**11**	Butyryl	70 days	5.4	0.08
9	**12**	*Iso*-butyryl	72 h	6.1	2.0
10	**13**	Butyryl	70 days	1.8	0.03
16	**17**	Benzoyl	108 days	1.2	0.02

Table 3
The lipase-catalyzed kinetic resolution of 1,2-dodecanediol enantiomers in semi-preparative scale. (The reactions were carried out in CH_3CN/CH_3OH at RT with CALB, other details are given in Experimental: for Runs 1–2 in Section 2.4.1 and for Run 3 in Section 2.4.2).

Run no.	Substrate	Incubation time (h)	Conversion of substrate (%)	Components in crude product			
				Product no	Configuration	Amount (%)	*ee*[a]
1	**1** (5.0 mmol)	16	94.6	**1**	*S*	5.2	nd[b]
				2	*R*	48.7	82.6
				4		5.7	nd
				3	*S*	40.4	98.1[d]
							99.4[e]
							92.8[f]
2	**(*R*)-2** (2.5 mmol)	48[g]		**2**	*R*		99.3
		96[g]		**2**	*R*		99.5
3	**5** (50 mmol)	96	90[c]	**3**	*S*	23[i]	99.8[e]
						nd	69.4[f]
				3	*R*	24[i]	99.9[h]

[a] The *ee* were determined for the corresponding 1-monotosylates by HPLC over chiral stationary phase [38].
[b] nd, not determined.
[c] Estimated by isolated unreacted diester.
[d] *ee* of **(*S*)-3** in the crude product prior to crystallization.
[e] *ee* of **(*S*)-3** crystallized from the crude product.
[f] *ee* of **(*S*)-3** remaining in the mother liquor.
[g] **(*R*)-2** was gained from Run 1; in the Run 2 it was further incubated with CALB under methanolytic conditions in order to enrich the sample stereochemically; ee was determined after 48 h and 96 h of incubation.
[h] The separation of **(*R*)-3** was performed analogously to the one described in Run 1/Run 2 followed by alkaline hydrolysis of **(*R*)-2** and recrystallization of resulting **(*R*)-3**.
[i] The yield of the recrystallized product.

as it formed, prior to crystallization; Run 10, Table 1). The use of CALB in benzene/methanol affords (*S*)-1,2-dodecanediol with lower stereoselectivity (*ee* = 90%). Diester cleavage catalyzed by CALB in neat methanol occurs at a very low rate with modest stereoselectivity (Run 11, Table 1).

The influence of the acyl group on the stereoselectivity of the process emerges via two basic mechanisms: (i) firstly, the degree of the steric fit of the structure of the acyl group to the enzyme, and (ii) secondly, the rate of the spontaneous intramolecular migration of a certain acyl group in 2-monoesters (which is an equilibrium process). As mentioned above, the acyl migration diminishes apparent stereoselectivity of the process (Scheme 1). A special study – the estimation of the spontaneous acyl group migration rate was performed and the rates were found to differ up to two orders of magnitude under identical conditions (Table 2).

Acetyl and iso-butyryl were found to be fast migrating acyl groups while butyryl and benzoyl migrate very slowly (Schemes 2 and 3). The use of slowly migrating acyl groups (e.g. butyryl or benzoyl) is exceptionally important if stereoisomers of a polyol compound (triols, tetrols) are to be sterically resolved, as random acyl migration produces unwanted ester regioisomers that may be cleaved with "wrong" stereopreference. Thus, neither acetyl nor iso-butyryl groups should be the first option in this situation. In conclusion, butyryl group is clearly preferable compared to acetyl due to being cleaved by CALB with the same stereopreference, albeit at a higher rate and higher (apparent) stereoselectivity.

The simultaneous stereoselectivity of the chemoselective crystallization of **(*S*)-3** from the crude product (Run 17, Table 1) has been demonstrated by determining the *ee* for three samples of **(*S*)-3**: (i) as it formed, (ii) for **(*S*)-3** which crystallized from the crude product and (iii) for the **(*S*)-3** from the mother liquor. In order to gain the opposite enantiomer **(*R*)-3** in high stereoisomeric purity, the corresponding (*R*)-2-monoacetate **(*R*)-2** was separated from the mother liquor by column chromatography over silica gel and subsequently allowed to incubate again with CALB under methanolytic conditions in order to stereochemically enrich the product (Run 2, Table 3). Then the **(*R*)-2** was separated by column chromatography and hydrolyzed with NaOH in EtOH to afford **(*R*)-3** which was recrystallized to afford a product in high *ee*.

A similar trial for the semi-preparative stereoresolution via the methanolysis of the bisbutyrate **5** gave somewhat better results (Run 3, Table 3).

4. Conclusions

A screening of preferable reaction conditions for the stereoselective lipase-catalysed deacylation of long-chain 1,2-alkanediol diesters with the aim of elaborating a scalable method for the resolution of enantiomers was performed. A process involving the use of CALB for the sequential stereoselective methanolysis of 1,2-octanediol bisbutyrate or 1,2-dodecanediol bisbutyrate in acetonitrile at room temperature gave the best results – this process was found to be highly stereoselective. The use of butyric esters was found to be preferable compared to acetyl groups because of the much slower rate of the acyl group migration in the 2-monoester intermediate, allowing the process to have higher "apparent" stereoselectivity. An approach for separating the (*R*)-enantiomers of the 1,2-alkanediols was elaborated. The use of benzoyl group afforded (*R*)-2-monoesters of high stereoisomeric purity upon the methanolysis catalyzed by CALB in acetonitrile while the RML-catalyzed hydrolysis of bisisobutyrate afforded the corresponding 2-monoester of (*S*)-configuration.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2015.03.006.

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