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Enzymatic resolution of analgesics: δ-hydroxytramadol, ε-hydroxytramadol and *O*-desmethyltramadol

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Abstract

Efficient enzymatic resolutions of the analgesic δ-hydroxytramadol *rac*-**3** and ε-hydroxytramadol *rac*-**4** have been achieved through pig liver esterase- and *Candida rugosa* lipase-catalyzed hydrolyses of the corresponding butyrates. The *Candida rugosa* lipase-catalyzed hydrolysis of *O*-desmethyltramadol butyrate *rac*-**8a**, having a remote aromatic acyloxy group as the only functional group amendable to a hydrolase-catalyzed reaction, proceeded with a good selectivity.

1. Introduction

According to the World Health Organization (WHO)[1] the development of new non-addictive analgesics for the combat of strong pain is highly desirable.[2] While searching for new analgesics the structure of morphine **1** has been widely varied.[3–5]

It was found that even strongly modified analogs such as tramadol **2** (Scheme 1) retain at least some of the typical biological activities of morphine.[4] Tramadol, which was introduced in 1976 by Grünenthal as an opiate-agonist, has less side-effects than other typical analgesics.[3,6] It has been recommended by the WHO as an analgesic with a low addiction potential for the treatment of patients with cancer pain. Tramadol is administered as the racemate because both enantiomers exhibit synergistic effects.[3,7–10] In the search for still better analgesics the lead structure of tramadol was systematically varied. It was found that δ-hydroxytramadol **3** and ε-hydroxytramadol **4** exhibit interesting pharmacological properties.[11,12] Furthermore, both compounds have turned out to be versatile intermediates for the synthesis of other derivatives of tramadol with strong local analgesic activities.[11,12] A prerequisite for the further pharmacological evaluation of **3** and **4** and for their use as starting materials for the synthesis of further analogs is the availability of both enantiomers, in each case on a preparative scale. Because

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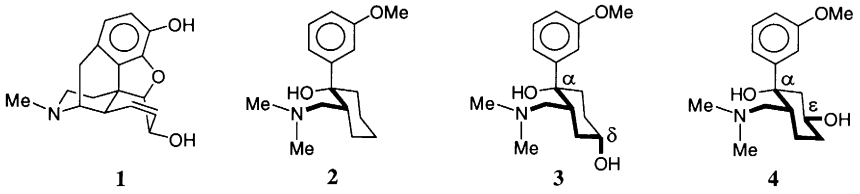
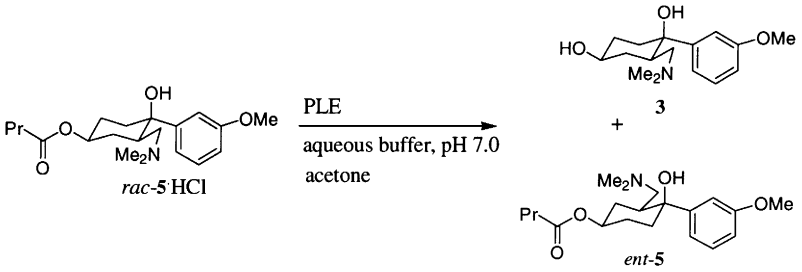
of these requirements and the ready availability of *rac*-**3** and *rac*-**4**[11–13] the attainment of the respective enantiomers by resolution was deemed to be appropriate. To date, the resolution of *rac*-**3** and *rac*-**4** by HPLC on chiral stationary phases or by more classical methods was either successful only on a small scale or failed completely,[11,12] and an asymmetric synthesis of **2**–**4** has not been described. Because of previous efficient resolutions of cyclohexanol derivatives using lipases and pig liver esterase (PLE)[14,15] we considered an enzymatic resolution of *rac*-**3** and *rac*-**4** to be particularly attractive.



Scheme 1.

2. Results and discussion

The PLE-Chirazyme E-1[16] catalyzed hydrolysis of the butyrate *rac*-**5**·HCl at room temperature in aqueous buffer solution at pH 7.0 in the presence of 16% acetone proceeded readily and gave the alcohol **3** with 93% ee in 96% yield[17] and the ester *ent*-**5** with 72% ee in 99% yield (E=59)[18] (Scheme 2). The ee value of **3** could be increased to ≥99% by recrystallization. In this manner 0.46 mol of *rac*-**5**·HCl were resolved in one batch with the same results. Because of sufficiently different log P values[19] (Table 1) the separation of **3** and *ent*-**5** was conveniently achieved simply by extraction with variations of the solvent and the pH value of the solution.



Scheme 2.

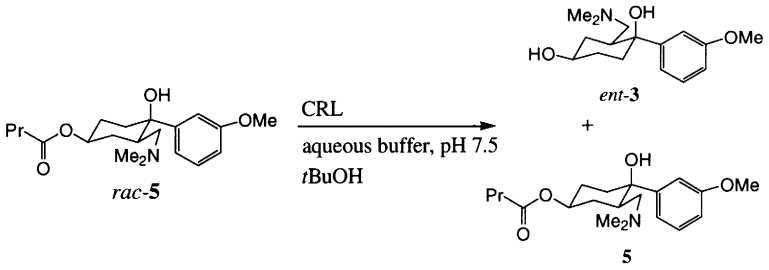
Table 1
Log P (water/cyclohexane) values of *rac*-**3** and *rac*-**5** at different pH values

pH	*rac*-**3**	*rac*-**5**
7.0	−2.691	0.557
7.4	−2.297	0.946
8.0	−1.724	1.498
10.0	−0.680	2.333
12.0	0.633	2.360

In the above hydrolysis the enzyme was discarded. However, we are confident that an economical multi-mol PLE-catalyzed resolution of *rac*-**5**·HCl can be achieved since we have shown previously that in large-scale PLE-catalyzed hydrolyses in water the enzyme can be stabilized by the addition of bovine serum albumin and recovered by membrane filtration with only a minor loss of activity.[20] In the absence

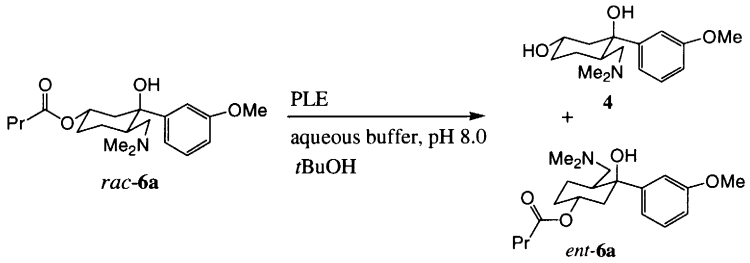
of the cosolvent acetone the enantioselectivity of the hydrolysis of *rac*-**5**·HCl was lower as expressed in an *E* value of 28. The PLE-Chirazyme E-1-catalyzed hydrolysis of the free base *rac*-**5** in the presence of acetone proceeded with a similar enantioselectivity. However, because of the low solubility of *rac*-**5** in water, scale-up of the resolution of the free base posed problems and its hydrochloride, which has a high solubility in water, was used instead.

Interestingly, the *Candida rugosa* lipase (CRL)-catalyzed hydrolysis of *rac*-**5** in water in the presence of 10% *tert*-butanol proceeded with the opposite enantiomeric preference to give, after 28% conversion (24 h), the alcohol *ent*-**3** with 89% ee and the ester **5** with 37% ee (*E*=24) (Scheme 3).



Scheme 3.

The PLE-catalyzed hydrolysis of the butyrate *rac*-**6a** in aqueous buffer solution at pH 8.0 in the presence of *tert*-butanol as cosolvent was as effective as that of its isomer *rac*-**5** and gave, after 40% conversion, the alcohol **4** with 94% ee in 77% yield and the ester *ent*-**6a** with 86% ee in 79% yield (Scheme 4, Table 2).



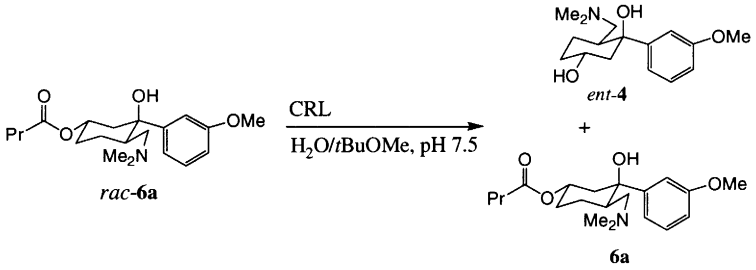
Scheme 4.

Table 2
Enzymatic hydrolysis of *rac*-**6a**

enzyme (U/ml)	conditions	t (h)	conv. (%)	alcohol	ee (%)	ester	ee (%)	E
PLE (2.3)	*t*BuOH (10%), H_2O, pH 8.0	6	40	**4**	94	*ent*-**6a**	86	46
CRL (4.6)	*t*BuOH (10%), H_2O, pH 7.0	5	49	*ent*-**4**	95	**6a**	93	133
CRL (4.3)	*t*BuOMe (50%), H_2O, pH 7.5	6	45	*ent*-**4**	≥98	**6a**	≥98	≥200

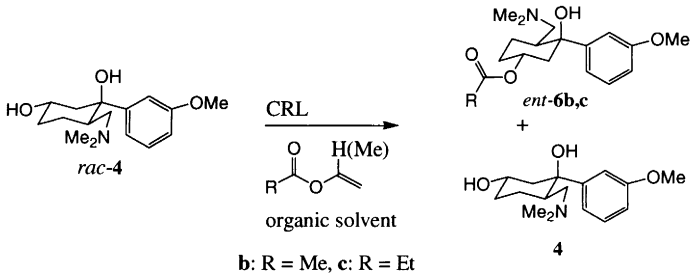
The selectivity of the CRL-catalyzed hydrolysis of *rac*-**6a** was even higher than that with PLE, and the hydrolysis proceeded, as in the case of *rac*-**5**, with the opposite enantiomer preference (Scheme 5, Table 2). Thus, the CRL-catalyzed hydrolysis of *rac*-**6a** in aqueous buffer solution at pH 7.0 with *tert*-butanol as cosolvent gave, after 49% conversion, the alcohol *ent*-**4** with 95% ee in 83% yield and the ester **6a** with 93% ee in 85% yield. An almost optimal resolution of *rac*-**6a** with CRL was achieved by carrying out the hydrolysis in an emulsion of water and *tert*-butyl methyl ether.[14,21] The CRL-catalyzed hydrolysis of *rac*-**6a** under these conditions at room temperature afforded the alcohol *ent*-**4** with ≥99% ee in 79%

yield and the ester **6a** with ≥99% ee in 80% yield. Even the extension of the reaction time to 72 h saw no hydrolysis of **6a**.



Scheme 5.

Because of the above results we also studied the CRL-catalyzed transesterification of *rac*-**4** under formation of *ent*-**6b**,**c** and **4** in organic solvents (Scheme 6, Table 3).



Scheme 6.

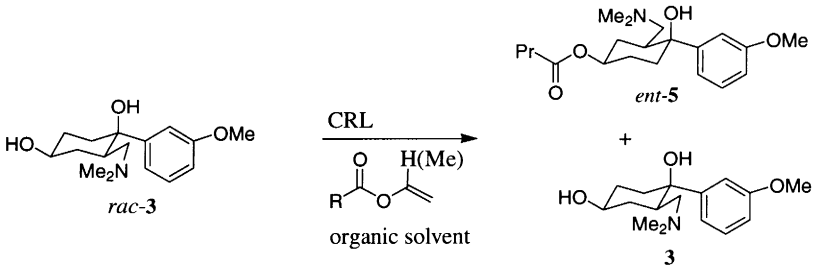
Table 3
CRL-catalyzed (37 U/mg) transesterification of *rac*-**4**

conditions (equiv.)	t (d)	conv. (%)	ester	ee (%)	alcohol	ee (%)	E
vinyl propionate (5), toluene	9	48	*ent*-**6c**	87	**4**	68	30
vinyl acetate (215)	7	56	*ent*-**6b**	91	**4**	97	89
isopropenyl acetate (5.5), toluene	5	34	*ent*-**6b**	99	**4**	60	≥200

A synthetically useful selectivity as expressed in an *E* value of 30 was obtained with vinyl propionate in toluene. The use of vinyl acetate not only as the acyl donor but also as the solvent saw an increase of the selectivity of the transesterification of *rac*-**4** to *E*=89. Since, in the above acylation acetaldehyde was liberated, which may have chemically modified the lipase,[22,23] we used isopropenyl acetate as the acyl donor. The CRL-catalyzed acylation of *rac*-**4** with isopropenyl acetate in toluene proceeded with very high selectivity as expressed in an *E* value ≥200.

Having obtained favorable results in the case of the transesterification of *rac*-**4** we also investigated the CRL-catalyzed transesterification of *rac*-**3**, which gave the ester *ent*-**5** and the alcohol **3** (Scheme 7, Table 4). However, the acylation of *rac*-**3** was not as selective as that of *rac*-**4**. In addition, the rate of the acylation was much lower than that of *rac*-**4**.

The absolute configuration of alcohol **3** was determined by X-ray crystal structure analysis of the hydrochloride of its δ-*O*-*p*-fluorobenyzl ether by using anomalous X-ray scattering and that of **4** was assigned on the basis of the observation that α*R*,β*R*-tramadol and all its derivatives prepared thus far showed a positive sign of optical rotation.[24]



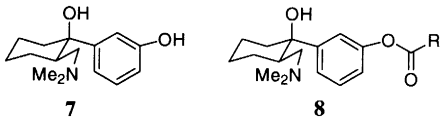
Scheme 7.

Table 4
CRL-catalyzed (37 U/mg) transesterification of *rac*-**3**

conditions (equiv.)	t (d)	conv. (%)	ester	ee (%)	alcohol	ee (%)	E
vinyl acetate (5), toluene	16	18	*ent*-**5**	44	**3**	72	5
vinyl acetate (215)	16	12	*ent*-**5**	47	**3**	48	4
isopropenyl acetate (5.5), toluene	16	25	*ent*-**5**	68	**3**	87	14

Attempts to achieve a PLE-mediated transesterification[25,26] of *rac*-**3** and *rac*-**4** with vinyl acetate in toluene in the presence of methoxypolyethylene glycol met with no success.

For a resolution of tramadol itself the molecule lacks a functional group suitable for a hydrolase-catalyzed transformation since tertiary alcohols are generally only poor substrates for hydrolases.[14] However, we expected *O*-desmethyltramadol (*rac*-**7**)[27] (Scheme 8), which is a biologically active metabolite of **2**,[10] and the corresponding esters *rac*-**8** to be substrates for hydrolases.



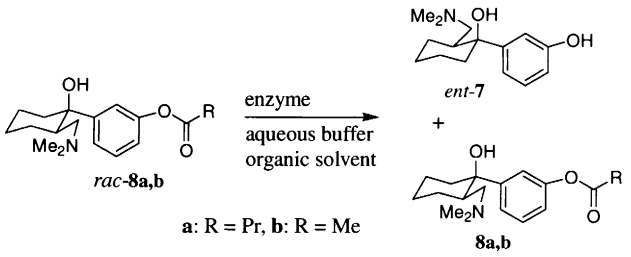
Scheme 8.

The aromatic alcohol **7** and its esters **8** are members of a large group of biologically active chiral compounds which all contain, besides a tertiary amino group, an aromatic hydroxy or acyloxy group but no other functional groups suitable for a hydrolase-catalyzed reaction.[3,4,6,28] Thus, it was hoped that an investigation of the enzymatic transesterification and hydrolysis of *rac*-**7** and *rac*-**8**, respectively, would not only yield enantiomerically enriched **2** and *ent*-**2** but also give information as to the feasibility of using an aromatic hydroxy or acyloxy group for a hydrolase-catalyzed resolution. Although several studies have appeared on the enzymatic hydrolysis of esters of racemic aromatic alcohols[29–32] we are aware of only two reports dealing with the enzymatic hydrolysis of racemic aromatic esters containing a tertiary amino group.[33,34]

Hydrolysis of *rac*-**8a** in aqueous solution in the presence of *tert*-butanol as cosolvent was studied by using PLE, horse liver esterase (HLE), *Pseudomonas cepacia* lipase (PCL), CRL, pig pancreas lipase (PPL) and *Candida antarctica* lipase (CAL) (Scheme 9, Table 5).

All enzymes tested exhibited a preference for *ent*-**8a** and, thus, gave preferentially the alcohol *ent*-**7** and the ester **8a**. Table 5 reveals, however, that the enantioselectivities were rather low and not sufficiently high for synthetic purposes. For example, the PLE-catalyzed hydrolysis of *rac*-**8a** furnished the enantiomerically pure alcohol *ent*-**7** in only 13% yield after 69% conversion.

All attempts to enhance the selectivity of the PLE-catalyzed hydrolysis of *rac*-**8a** and *rac*-**8b** by variation of the cosolvent and the pH were unsuccessful as revealed in Table 6.



Scheme 9.

Table 5
Enzymatic hydrolysis of *rac*-**8a**[a]

enzyme (U/ml)	conditions	t (h)	conv. (%)	alcohol	ee (%)	ester	ee (%)	E
PLE (3.9)	*t*BuOH (10%), H_2O, pH 8.0	2	52	*ent*-**7**	76	**8a**	62	13
HLE (0.3)	*t*BuOH (10%), H_2O, pH 8.0	4	24	*ent*-**7**	50	**8a**	18	4
PCL (15)	*t*BuOH (10%), H_2O, pH 7.0	22	36	*ent*-**7**	67	**8a**	75	10
CRL (2.4)	*t*BuOH (10%), H_2O, pH 7.0	4	56	*ent*-**7**	65	**8a**	58	9
PPL (21)	*t*BuOH (10%), H_2O, pH 7.0	2.5	67	*ent*-**7**	78	**8a**	19	8
CAL (0.8)	*t*BuOH (10%), H_2O, pH 7.0	4	8	*ent*-**7**	[b]	**8a**	13	[b]

[a] All reactions where carried out on a 1 mmol scale. [b] Not determined.

Table 6
PLE-catalyzed hydrolysis of *rac*-**8a** and *rac*-**8b**

substrate	conditions[a]	t (min)	conv. (%)	alcohol	ee (%)	ester	ee (%)	E
rac-**8a**	acetone (20%), H_2O, pH 8.0	5	51	*ent*-**7**	11	**8a**	27	2
rac-**8a**	MeOH (10%), H_2O, pH 8.0	30	19	*ent*-**7**	81	**8a**	17	11
rac-**8b**	*t*BuOH (10%), H_2O, pH 8.0	40	31	*ent*-**7**	74	**8b**	50	10
rac-**8b**	*t*BuOH (10%), H_2O, pH 7.0	120	36	*ent*-**7**	52	**8b**	35	5

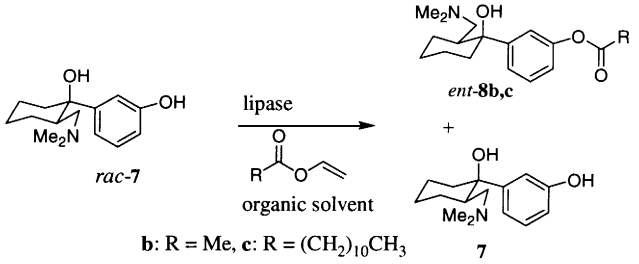
[a] Ester *rac*-**8b** was already hydrolyzed slowly at pH 8.0 in the absence of PLE.

The use of CRL in an emulsion of aqueous phosphate buffer and *tert*-butyl methyl ether, however, resulted in a significantly higher and synthetically useful selectivity of the hydrolysis of *rac*-**8a** to *ent*-**7** and **8a** (Table 7). With purified CRL the selectivity of the hydrolysis of *rac*-**8a** was even higher as expressed in an *E* value of 34. In a preparative experiment the ester **8a** was obtained with ≥99% ee in 35% yield after 60% conversion.

A study of the lipase-catalyzed transesterification of alcohol *rac*-**7** (Scheme 10) showed it to be a substrate for the lipases used. However, the enantioselectivities were low (Table 8). The absolute configurations of alcohol **7** and esters *ent*-**8b**,**c** were assigned by comparison of their specific rotations with those reported in the literature.[10,35–37]

Table 7
CRL-catalyzed hydrolysis of *rac*-**8a**

activity	conditions	t (h)	conv. (%)	alcohol	ee (%)	ester	ee (%)	E
2.4 U/mg	*t*BuOH (10%), H_2O, pH 7.0	5	51	**7**	85	*ent*-**8a**	75	27
37 U/mg	*t*BuOH (10%), H_2O, pH 7.0	3	33	**7**	90	*ent*-**8a**	58	34



Scheme 10.

Table 8
Enzymatic transesterification of *rac*-**7**

enzyme (U/mg)	conditions (equiv.)	t (d)	conv. (%)	ester	ee (%)	alcohol	ee (%)	E
CRL (37)	vinyl laurate (2), Et_2O	14	4	*ent*-**8c**	[a]	**7**	[a]	[a]
CRL (37)	vinyl acetate (5), CH_2Cl_2	21	66	*ent*-**8b**	33	**7**	26	2
CRL (37)	vinyl acetate (250)	21	36	*ent*-**8b**	38	**7**	21	3
PCL (4)	vinyl acetate (5), CH_2Cl_2	21	66	*ent*-**8b**	33	**7**	26	2
PPL (300)	vinyl laurate (5), CH_2Cl_2	28	43	*ent*-**8c**	37	**7**	22	3

[a] Not determined.

3. Conclusion

In summary, enzymatic resolution of the hydroxytramadols *rac*-**3** and *rac*-**4** by using PLE and CRL allows for an efficient attainment of both enantiomers in each case. In these hydrolyses PLE and CRL are complementary in synthetic terms because of the opposite enantiomer preference they exhibit. The significant selectivity recorded in the CRL-catalyzed hydrolysis of the butyrate of *O*-desmethyltramadol is further confirmation that a remote aromatic acyloxy group can be utilized successfully for the resolution of substrates being devoid of other suitable functional groups.

4. Experimental

4.1. General remarks

Chemical shifts are given in ppm relative to Me_4Si: δ=0.00 as the internal standard. Peaks in the ^{13}C NMR spectra are denoted as ‘u’ for carbons with zero or two attached protons or as ‘d’ for carbons with one or three attached protons, as determined from the ATP pulse sequence. Enzymatic reactions were run at room temperature and monitored by GC using a CP-Sil-8 column. Enantiomer compositions were

determined by HPLC analysis with Chiralcel OD/OD-H column (25×0.46 cm) (Baker–Daicel) or by GC analysis with an octakis-(2,3-*O*-dipentyl-6-*O*-methyl)-γ-cyclodextrin column (25 m×0.25 mm) (Lipodex γ-6-Me) (Macherey–Nagel) and a permethylated β-cyclodextrin column (25 m×0.25 mm) (CP-Chirasil-Dex-CB) (Chrompack). The carrier gas was hydrogen at 100 kPa. Retention times are given in minutes. Column chromatography was performed with E. Merck silica gel 60 (230–400 mesh). PLE (150 U/mg, suspension in 3.2 M $(NH_4)_2SO_4$) and PLE-Chirazyme E-1 (lyophilisate, 40 U/mg) were purchased from Roche Diagnostics. CRL (2.4 U/mg), PPL, HLE, PCL and CAL (A and B) were purchased from Fluka. Enzymatic reactions were carried out at room temperature.

4.2. Determination of enantiomer composition

GC: *rac*-**3** and *rac*-**4**: CP-Chirasil-Dex-CB, split 1:40, 140°C (2 min)→160°C (10°C/min) (2 min)→180°C (10°C/min) (2 min)→200°C (10°C/min): t_R (**3**)=24.6, t_R (*ent*-**3**)=25.5, t_R (**4**)=21.1, t_R (*ent*-**4**)=21.6 min; *rac*-**5**, *rac*-**6a**, *rac*-**6b** and *rac*-**6c**: Lipodex γ-6-Me, split 1:40, 100°C (45 min)→130°C (10°C/min) (15 min)→160°C (10°C/min) (5 min)→200°C (10°C/min): t_R (*ent*-**5**)=71.8, t_R (**5**)=76.3, t_R (*ent*-**6a**)=189.9, t_R (**6a**)=191.6, t_R (**6b**)=70.3, t_R (*ent*-**6b**)=74.5, t_R (**6c**)=171.6, t_R (*ent*-**6c**)=175.4; *rac*-**7**, *rac*-**8b** and *rac*-**8c**: CP-Chirasil-Dex-CB, split 1:40, 100°C (45 min)→130°C (10°C/min) (15 min)→160°C (10°C/min) (5 min)→200°C (10°C/min): t_R (*ent*-**7**)=74.0, t_R (**7**)=73.5, t_R (**8b**)=68.0, t_R (*ent*-**8b**)=68.3, t_R (**8c**)=83.2, t_R, (*ent*-**8c**)=84.0.

HPLC: *rac*-**3** and *rac*-**5**: *n*-hexane:*i*PrOH:Et_2NH, 950:50:1, flow rate: 0.75 mL/min, detection at 273 nm: t_R (**3**)=29.9, t_R (*ent*-**3**)=33.8, t_R (*ent*-**5**)=20.9, t_R (**5**)=14.1; *rac*-**7**, *rac*-**8a** and *rac*-**8b**: *n*-hexane:*i*PrOH:Et_2NH, 970:30:1, flow rate: 0.75 mL/min, detection at 254 nm: t_R (*ent*-**7**)=26.3, t_R (**7**)=22.4, t_R (**8a**)=12.0, t_R (*ent*-**8a**)=9.9, t_R (**8b**)=12.4, t_R (*ent*-**8b**)=14.6.

*4.3. Butyric acid (±)-(1*RS,*2*SR,*4*SR*)-3-dimethylaminomethyl-4-hydroxy-4-(3-methoxyphenyl)cyclohexyl ester (*rac-***5***) and butyric acid (±)-(1*RS,*3*SR,*4*SR*)-4-dimethylaminomethyl-3-hydroxy-3-(3-methoxyphenyl)cyclohexyl ester* rac-***6a***

Potassium *tert*-butoxide (20 mmol) was added carefully at room temperature to a suspension of *rac*-**3**·HCl (*rac*-**4**·HCl) (8 mmol) in THF (27 ml) and the mixture was stirred until the reaction was completed. Subsequently, butyryl chloride (12 mmol) in THF (2.5 ml) was added and the mixture was stirred overnight at room temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and stirred for 12 h. The organic layer was separated, dried (Na_2SO_4) and concentrated in vacuo. The ester *rac*-**5** (*rac*-**6a**) was purified by chromatography (diisopropyl ether:methanol, 1:1), whereby eventually the remaining alcohol was recovered.

Compound *rac*-**5**: 79% yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.3 (m, 1H), 7.1 (s, 1H), 7.0 (m, 1H), 6.8 (m, 1H), 4.8 (m, 1H), 3.8 (s, 3H), 2.4 (dd, 1H, *J*=4, *J*=14 Hz), 2.3−1.6 (m, 20H), 1.0 (t, 3H, *J*=7 Hz); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 173.1 (u), 159.4 (u), 150.4 (u), 129.0 (d), 117.0 (d), 111.5 (d), 110.7 (d), 75.7 (u), 72.9 (d), 60.9 (u), 55.1 (d), 47.7 (d), 43.0 (d), 39.0 (u), 36.5 (u), 32.9 (u), 27.5 (u), 18.5 (u), 13.6 (d).

Compound *rac*-**6a**: 81% yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.3 (m, 1H), 7.2 (s, 1H), 7.1 (m, 1H), 6.8 (m, 1H), 5.2 (m, 1H), 3.8 (s, 3H), 2.4 (dd, 1H, *J*=4, *J*=14 Hz), 2.3–1.5 (m, 19H), 1.4 (m, 1H), 0.9 (t, 3H, *J*=8 Hz); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 172.7 (u), 159.4 (u), 150.1 (u), 129.0 (d), 117.0 (d), 111.5 (d), 110.7 (d), 78.0 (u), 70.6 (d), 60.5 (u), 55.1 (d), 47.7 (d), 46.0 (u), 44.1 (d), 36.4 (u), 32.2 (u), 25.9 (u), 18.5 (u), 13.6 (d).

*4.4. Butyric acid (±)-(1*RS,*2*RS*)-3-(2-dimethylaminomethyl-1-hydroxycyclohexyl)phenyl ester* rac-***8a*** *and acetic acid (±)-(1*RS,*2*RS*)-3-(2-dimethylaminomethyl-1-hydroxycyclohexyl)phenyl ester* rac-***8b***

A solution of sodium hydroxide (40%, 2 ml) was added at room temperature to a suspension of *rac*-**7**·HCl (15 mmol) in water (8 ml). The aqueous layer was extracted twice with CH_2Cl_2 (15 ml). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to give *rac*-**7**. Alcohol *rac*-**7** (8 mmol) was mixed at room temperature with a solution of butyric anhydride (acetic anhydride) (9.6 mmol) and pyridine (0.8 mmol) in CH_2Cl_2 (30 ml). After stirring the mixture at room temperature until the completion of the acylation (48 h), it was poured into saturated aqueous $NaHCO_3$ and stirred for 12 h. The organic layer was separated, dried (Na_2SO_4) and concentrated in vacuo to give *rac*-**8a** (*rac*-**8b**).

Compound *rac*-**8a**: 94% yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.3 (m, 1H), 7.2 (s, 1H), 6.9 (m, 2H), 2.5 (t, 2H, *J*=5 Hz), 2.4 (dd, 1H, *J*=4, *J*=13 Hz), 2.1−1.4 (m, 17H), 1.4−1.2 (m, 1H), 1.0 (t, 3H, *J*=5 Hz); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 172.1 (u), 152.2 (u), 150.8 (u), 128.8 (d), 122.2 (d), 119.0 (d), 118.6 (d), 76.9 (u), 61.5 (u), 47.7 (d), 44.7 (d), 41.3 (u), 36.2 (u), 27.9 (u), 26.8 (u), 22.2 (u), 18.4 (u), 13.7 (d).

Compound *rac*-**8b**: 99% yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.3 (m, 3H), 6.9 (m, 1H), 2.4 (dd, 1H, *J*=4, *J*=14 Hz), 2.3 (s, 3H), 2.2–1.5 (m, 16H), 1.4–1.3 (m, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 169.3 (u), 152.0 (u), 150.5 (u), 128.7 (d), 122.2 (d), 118.9 (d), 118.4 (d), 77.0 (u), 61.5 (u), 47.7 (d), 45.0 (d), 41.3 (u), 27.9 (u), 26.8 (u), 22.2 (u), 21.1 (d).

4.5. Hydrolysis of rac-***5***·*HCl with PLE*

Aqueous phosphate buffer solution (pH 7.0, 1.54 L), acetone (0.36 L) and the hydrochloride of the ester *rac*-**5** (178 g, 0.46 mol) were combined and stirred for 10 min. Subsequently, PLE-Chirazyme E-1 (1.54 g) and aqueous $NaHCO_3$ solution (370 mL) were added to the mixture at room temperature. The pH value of the solution at the beginning of the hydrolysis was 7.5. After the reaction stopped (15 h, pH 7.0) the aqueous buffer solution was extracted first with diisopropyl ether (1 L) and subsequently with diethyl ether (1 L). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to give *ent*-**5** (79.4 g, 99%) with 72% ee ($[\alpha]_D^{22}$ −12.0 (*c* 1.02, MeOH)). The aqueous layer was separated from the remaining solvent and adjusted to pH 10 by the addition of aqueous 2 M $NaHCO_3$ solution. Subsequently, the mixture was extracted with ethyl acetate. The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to give **3** (62.0 g, 96%) with 93% ee ($[\alpha]_D^{22}$ +36.5 (*c* 1.06, MeOH)).

4.6. Hydrolysis of rac-***6a*** *with PLE*

The ester *rac*-**6a** (349 mg, 1 mmol) dissolved in *t*BuOH (4 mL) was added to the aqueous phosphate buffer solution (36 mL, pH 8). Subsequently, PLE (1 mg, 183 U) was added and the mixture was efficiently stirred at room temperature while the pH value was held constant at 8.0 by the addition of 1 M sodium hydroxide with a pH-stat autotitrator. After the reaction stopped (6 h) the mixture was extracted continuously with CH_2Cl_2 (50 mL) for 15 h. The organic phase was dried (Na_2SO_4) and concentrated in vacuo. Chromatography (MeOH:diisopropyl ether, 1:1) of the residue gave **4** (107.4 mg, 77%) with 94% ee ($[\alpha]_D^{22}$ +21.7 (*c* 0.80, MeOH)) and *ent*-**6** (137.8 mg, 79%) with 86% ee ($[\alpha]_D^{22}$ −6.0 (*c* 0.81, MeOH)).

4.7. Hydrolysis of rac-***6a*** *with CRL*

A solution of *rac*-**6a** (349 mg, 1 mmol) in *t*BuOMe (26 mL) was added to an aqueous phosphate buffer solution (pH 7.5, 26 mL). Subsequently, CRL (6 mg, 220 U) was added and the mixture was efficiently stirred at room temperature while the pH value was held constant at 7.5 by the addition of 1 M

sodium hydroxide with a pH-stat autotitrator. After the reaction stopped (6 h), the mixture was extracted continuously with CH_2Cl_2 (50 mL) for 15 h. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. Chromatography (MeOH:diisopropyl ether, 1:1) of the residue gave *ent*-**4** (110.2 mg, 79%) with ≥99% ee ($[\alpha]_D^{22}$ −29.5 (*c* 1.01, MeOH)) and **6a** (139.6 mg, 80%) with ≥99% ee ($[\alpha]_D^{22}$ +7.5 (*c* 0.74, MeOH)).

Caution: peroxide free diisopropyl ether was used, and before concentration of the diisopropyl ether solution the absence of peroxides was secured by a test with potassium iodide.

4.8. *Transesterification of* rac-***4***

With vinyl propionate: alcohol *rac*-**4** (70 mg, 0.25 mmol) and vinyl propionate (125 mg, 1.25 mmol) were dissolved in toluene (5 mL). Subsequently, CRL (5 mg, 185 U) was added and the reaction mixture was stirred at room temperature. The reaction was stopped at 48% conversion (9 days) by silica gel filtration. GC analysis of a sample from the mixture showed the presence of *ent*-**6c** with 87% ee and of **4** with 68% ee.

With vinyl acetate: alcohol *rac*-**4** (70 mg, 0.25 mmol) was dissolved in vinyl acetate (5 mL, 54 mmol) and CRL (5 mg, 185 U) was added under the above conditions. The reaction was stopped at 56% conversion (7 days) by silica gel filtration. GC analysis of a sample from the reaction mixture showed the presence of *ent*-**6b** with 91% ee and of **4** with 97% ee.

With isopropenyl acetate: transesterification of alcohol *rac*-**4** (70 mg, 0.25 mmol) with isopropenyl acetate (145 mg, 1.5 mmol) in the presence of CRL (5 mg, 185 U) in toluene (5 mL) under the above conditions was stopped at 34% conversion (5 days) by silica gel filtration. GC analysis of a sample from the reaction mixture showed the presence of *ent*-**6b** with 99% ee and of **4** with 60% ee.

4.9. *Transesterification of* rac-***7***

With CRL: alcohol *rac*-**7** (62 mg, 0.25 mmol) and vinyl acetate (108 mg, 1.25 mmol) were dissolved in CH_2Cl_2 (5 mL). Subsequently, CRL (30 mg, 72 U) was added and the reaction mixture was stirred at room temperature. The reaction was stopped at 66% conversion (21 days) by silica gel filtration. GC analysis of a sample from the reaction mixture showed the presence of **7** with 21% ee and of *ent*-**8b** with 38% ee.

With PCL: reaction of *rac*-**7** (62 mg, 0.25 mmol) with vinyl acetate (108 mg, 1.25 mmol) in the presence of PCL (15 mg, 600 U) in CH_2Cl_2 (5 mL) under the above conditions was stopped at 66% conversion (21 days) by silica gel filtration. GC analysis of a sample from the reaction mixture showed the presence of **7** with 26% ee and of *ent*-**8b** with 33% ee.

With PPL: reaction of *rac*-**7** (62 mg, 0.25 mmol) with vinyl laurate (280 mg, 1.25 mmol) in the presence of PPL (100 mg, 1600 U) in CH_2Cl_2 (5 mL) under the above conditions was stopped at 43% conversion (28 days) by silica gel filtration. GC analysis of a sample from the reaction mixture showed the presence of **7** with 22% ee and of *ent*-**8b** with 37% ee.

With CRL: alcohol *rac*-**7** (62 mg, 0.25 mmol) was dissolved in vinyl acetate (5.8 mL, 63 mmol) and CRL (30 mg, 72 U) was added under the above conditions. The reaction was stopped at 36% conversion (21 days) by silica gel filtration. GC analysis of a sample from the reaction mixture showed the presence of **7** with 21% ee and of *ent*-**8b** with 38% ee.

4.10. *Hydrolysis of* rac-***8a*** *with CRL*

The hydrolysis of *rac*-**8a** (316 mg, 1 mmol) in *t*BuOMe (20 mL) and aqueous phosphate buffer (pH 7.0, 20 mL) in the presence of CRL (3 mg, 111 U) under the above conditions (see Section 4.7) was stopped

at 33% conversion (3 h) by continuous extraction with CH_2Cl_2 (50 mL) for 15 h. Chromatography (ethyl acetate:MeOH, 3:1) of the residue gave *ent*-**7** (76 mg, 90%) with 90% ee ($[\alpha]_D^{22}$ −30.6 (*c* 1.10, MeOH)) and **8a** (203 mg, 92%) with 58% ee ($[\alpha]_D^{22}$ +10.4 (*c* 0.87, MeOH)).

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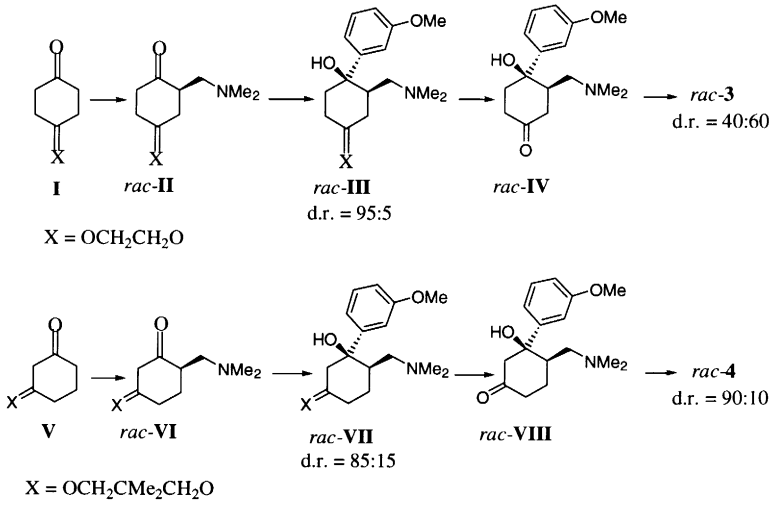

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