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Lipase-catalysed resolution of cyclic *cis*- and *trans*-β-hydroxy esters

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Abstract—Lipases A and B from *Candida antarctica* are shown to be highly efficient and complementary biocatalysts for the resolution of five- to seven-membered cyclic β-hydroxy esters by *O*-acylation. Using this procedure, all four stereoisomers of each one are obtained in enantiopure form and very high yields.

1. Introduction

The importance of optically active β-hydroxy acid derivatives as versatile building blocks for the synthesis of biologically active compounds is well established.[1] Amongst them, those bearing a substituent on the α-position are especially interesting, since they are bifunctionalised molecules with two contiguous stereogenic centres. However, until now they have remained relatively unavailable, and new general methodologies for their preparation are still required.

The biocatalytic approach to these compounds has been mainly via enantio- and diastereoselective reduction of the corresponding β-keto esters,[2] amides[3] or nitriles[4] using whole cells. Although in many cases the desired products are obtained in high enantiomeric and/or diastereomeric excesses, only one of the stereoisomers is usually (a few exceptions have been reported)[5] available by this procedure.

Another a priori appealing possibility to prepare the target molecules is the kinetic resolution of the racemic mixtures with hydrolases (lipases or esterases).[6] Although in recent years a number of elegant strategies has been developed to overcome its limitations,[7] it is still one of the major methods for the production of enantiopure compounds on an industrial scale.[8] Furthermore, it can make both enantiomers available.

Encouraged by the excellent results obtained in our research group over the last few years in the enzymatic kinetic resolution of cyclic amino alcohols[9] and diamines,[10] we decided to examine the lipase-catalysed enantioselective acylation of the cyclic β-hydroxy esters (±)-**1**–**6** (Fig. 1).

These compounds are very interesting building blocks for organic synthesis. For instance, (−)-**3** was used as starting material in the synthesis of biologically active 10-ethyl-trinem.[11] On the other hand, Gellman et al. employed **1** for the preparation of *trans*-2-aminocyclopentanecarboxylic acid, whose oligomer adopts an helical conformation.[12] However, as pointed out by these authors, a general approach to all stereoisomers would be highly desirable in terms of further biological studies.[13]

Xie et al. reported some years ago the enzymatic hydrolysis of the structurally related (±)-2-acetoxycy-

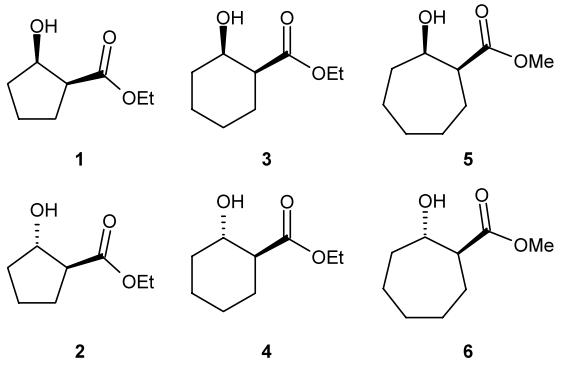

Figure 1.

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cloalkanecarboxylates with *Pseudomonas fluorenscens* lipase (PFL). However, in this study only the corresponding alcohols were obtained in high enantiomeric excess, making only half of the enantiomers available.[14]

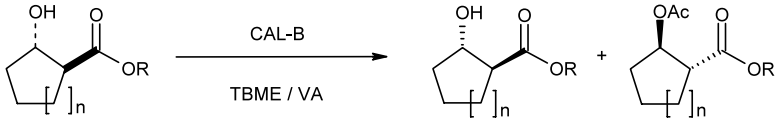
2. Results and discussion

The resolution of racemic alcohols (±)-**1**–**6** was carried out by CALB-catalysed enantioselective acylation using vinyl acetate (VA) as the acyl donor and *tert*-butyl methyl ether (TBME) as the organic solvent. In all cases, the acyl donor was used in moderate excess (molar ratio VA: alcohol 3:1), and the temperature was kept at 30°C.

Under these reaction conditions, the *O*-acylation of the *trans* isomers (±)-**2**, (±)-**4** and (±)-**6** took place smoothly, and after 1–4 h 50% conversion was reached, yielding both the substrates and the products in their enantiopure forms (Table 1). From these values, excellent enantioselectivities (*E*>200 in all cases) could be calculated.[15] With respect to the ring size, no difference in the enantioselectivity was observed, although a lower activity of CALB towards the seven-membered ring substrate (±)-**6** was noticed.

The same methodology was then applied to the *cis*-configured β-hydroxy esters, (±)-**1**, (±)-**3** and (±)-**5**. In the case of the five- and seven-membered ring compounds, very similar results were observed (Table 2): again, after short reaction times (2–6 h), both substrates and products were obtained in enantiomerically pure in high yields. Thus, an *E* value of >200 was calculated for these two processes. Quite surprisingly, CALB showed very low activity towards substrate (±)-**3** (and therefore was unsuitable for preparative purposes): after 2 days, a conversion value of only 5% was reached, although it

Table 1. CALB catalysed enantioselective acylation of (±)-*trans*-β-hydroxy esters



(±)-**2** R = Et
(±)-**4** R = Et
(±)-**6** R = Me

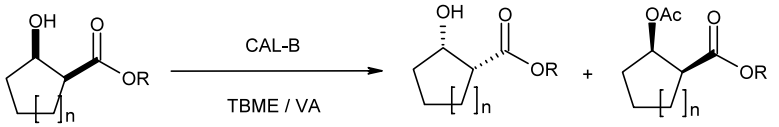
(1*S*,2*S*)-**2**
(1*S*,2*S*)-**4**
(1*S*,2*S*)-**6**

(1*R*,2*R*)-**8**
(1*R*,2*R*)-**10**
(1*R*,2*R*)-**12**

n	Substrate	*t* (min)	(1*S*,2*S*)-**2**, **4** or **6**		(1*R*,2*R*)-**8**, **10** or **12**		*c* (%)[a]	*E*[a]
			Yield (%)[b]	E.e. (%)[c]	Yield (%)[b]	E.e. (%)[c]		
1	(±)-**2**	80	70	>99	81	>99	50	>200
2	(±)-**4**	100	93	>99	95	>99	50	>200
3	(±)-**6**	240	90	>99	90	>99	50	>200

[a] Calculated from the e.e. of the substrate and the product. See Ref. 15.
[b] After purification by flash column chromatography and calculated taking into account the conversion.
[c] Determined by chiral GC.

Table 2. CALB catalysed enantioselective acylation of (±)-*cis*-β-hydroxy esters



(±)-**1** R = Et
(±)-**3** R = Et
(±)-**5** R = Me

(1*R*,2*S*)-**1**
(1*R*,2*S*)-**3**
(1*R*,2*S*)-**5**

(1*S*,2*R*)-**7**
(1*S*,2*R*)-**9**
(1*S*,2*R*)-**11**

n	Substrate	*t* (min)	(1*R*,2*S*)-**1**, **3** or **5**		(1*S*,2*R*)-**7**, **9** or **11**		*c* (%)[a]	*E*[a]
			Yield (%)[b]	E.e. (%)[c]	Yield (%)[b]	E.e. (%)[c]		
1	(±)-**1**	160	92	>99	70	>99	50	>200
2	(±)-**3**	2880	n.i.[d]	3	n.i.[d]	>99	5	>200
3	(±)-**5**	390	92	>99	80	99	50	>200

[a] Calculated from the e.e. of the substrate and the product. See Ref. 15.
[b] After purification by flash column chromatography and calculated taking into account the conversion.
[c] Determined by chiral GC.
[d] n.i.: not isolated

should be pointed out that this enzyme exhibited again complete enantiodiscrimination, since the product had >99% e.e. A similar result (high enantioselectivity but poor conversion) had been already observed in the kinetic resolution of the structurally related *cis*-2-amino cyclohexanol.[9b]

Our first attempt to increase the conversion value was the use of higher temperature. However, the reaction rate remained almost constant. The use of other organic solvents also failed to improve the rate of acylation. Disappointingly, the activity was again very low when acetonitrile, dichloromethane, toluene or 1,4-dioxane were used as solvents.

Next, we considered the possibility of using a different biocatalyst whose binding pocket can accept more sterically hindered substrates. Bornscheuer et al. have recently shown that lipases and esterases bearing a GGGX amino acid motif are highly active towards tertiary alcohols.[16] Another isozyme from *Candida antarctica* (lipase A, CALA) belongs to this group of enzymes, and so we decided to test it for our sterically demanding substrate.

Very satisfyingly, under the reaction conditions described above for the resolution of the (±)-**1**, **2**, **4**, **5** and **6**, a 34% conversion could be reached after just 30 min (Table 3, entry 2). However, longer reaction times only led to lower e.e.s of the product. We therefore, increased the amount of enzyme, which led to a higher conversion value after just 1 h while maintaining a good enantiomeric excess of the product. However, the substrate could not be isolated in an enantiopure form. Therefore, the reaction was left for longer time (90 min), and the substrate could be isolated in >99% e.e. at 51% of conversion (Table 3, entries 3 and 4).

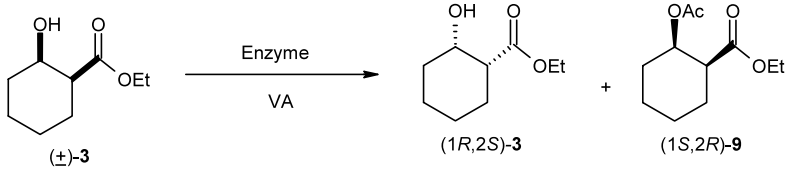
The absolute configuration of all compounds described herein has been determined as follows: for the substrates **1**–**4**, by comparison of the sign of the specific rotation of substrates **1**–**4** with the literature values (see Section 4) assuming that no epimerisation occurred during the transesterification. Substrates (1*R*,2*S*)-**5** and (1*S*,2*S*)-**6**, were transformed into the previously described ethyl esters (1*R*,2*S*)-**13** and (1*S*,2*S*)-**14**, respectively, according to a procedure developed by Baumhof et al.[17] which avoids inversion of the estereogenic centre bearing the hydroxyl group. In all cases, the enzymatic acylation took place only at the hydroxyl groups of *R* configuration, independently of the enzyme used (CALA or CALB) and of the configuration of the adjacent stereocentre. This is in accordance with Kazlauskas' rule,[18] which predicts the stereochemical outcome of the acylation of secondary alcohols, and hydrolysis of carboxylic esters (Fig. 2).

Figure 2. Preferred stereoisomer by lipases according to Kazlauskas's rule.

3. Conclusion

In summary, we have demonstrated that both lipases from *C. antarctica* are excellent biocatalysts for the enantioselective transesterification of five to seven-membered ring cyclic β-hydroxy esters. This allows, for the first time, a direct access to all four stereoisomers of these interesting building blocks in enantiopure form and high yield.

Table 3. Enantioselective acylation of (±)-**3** by CALB and CALA



Entry	Lipase	*t* (min)	(1*R*,2*S*)-**3**		(1*S*,2*R*)-**9**		*c* (%)[a]	*E*[a]
			Yield (%)[b]	E.e. (%)[c]	Yield (%)[b]	E.e. (%)[c]		
1	CALB[e]	2880	n.i.[d]	3	n.i.[d]	>99	5	>200
2	CALA[e]	30	n.i.[d]	51	n.i.[d]	>99	34	>200
3	CALA[f]	60	64	85	75	99	46	>200
4	CALA[f]	90	85	>99	83	94	51	>200

[a] Calculated from the e.e. of the substrate and the product. See Ref. 15.
[b] After purification by flash column chromatography and calculated taking into account the conversion..
[c] Determined by chiral GC.
[d] n.i.: not isolated
[e] For 0.58 mmol of substrate, 80 mg of CALA were used.
[f] For 0.58 mmol of substrate, 123 mg of CALA were used.

4. Experimental

4.1. General

Lipase B from *C. antarctica* (CALB) 'Novozym 435' was donated by Novo Nordisk Co., and lipase A from *C. antarctica* (CALA) 'Chiralzyme L-5' was obtained from Roche. Both were employed without any further treatment. Compounds (±)-**1**, (±)-**3** and (±)-**4** are commercially available from Aldrich and were used without further purification. Solvents were of spectrophotometric grade and were stored over 4 Å molecular sieves under nitrogen prior to use. IR spectra were recorded on a Perkin–Elmer 1720-X FT IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker DPX 300 (^{1}H 300 MHz and ^{13}C 75.5 MHz) spectrometer, using $CDCl_3$ as solvent. GC analyses were performed on a Hewlett–Packard 5890 Series II chromatograph equipped with an FID detector using the capillary column RtβDEXse (30 mm×0.25 mm; Restek) as the stationary phase and nitrogen as the carrier gas (110 kPa). HPLC analyses were carried out on a Shimadzu LC liquid chromatograph at 20°C using a Chiralcel OD column. Optical rotations were measured by means of a Perkin–Elmer 241 polarimeter. Mass spectra were recorded using electrospray (40 V) as ionisation source.

4.2. Syntheses of racemic substrates

4.2.1. Synthesis of (±)-2. A procedure similar to that reported by Nakata and Oishi was used.[19] Ethyl 2-oxocyclopentanecarboxylate (3 mmol) was treated under N_2 with an excess of a solution of $Zn(BH_4)_2$ in dry diethyl ether (10 mL) for 30 min at 0°C. Then, the reaction was quenched with a saturated solution of NH_4OAc (2 mL) and extracted with dichloromethane (3×50 mL). Evaporation of the organic phase yielded the corresponding β-hydroxy ester as a mixture of diastereomers (*trans*:*cis* 9:1), which was separated by flash column chromatography (eluent: Et_2O/CH_2Cl_2 1:2), to obtain (±)-**2** in 71% yield.

4.2.2. Synthesis of (±)-5 and (±)-6. The corresponding β-keto ester (2 mmol) was treated with $NaBH_4$ (1.4 mmol) in ethanol (6 mL) at 0°C until disappearance of the starting material (TLC monitoring). Then, the reaction was quenched with a saturated solution of NH_4OAc (10 mL) and extracted with dichloromethane (3×30 mL). Evaporation of the organic phase yielded the corresponding diastereomeric mixture of hydroxy esters. Further purification was carried out by flash column chromatography (eluent: Et_2O/CH_2Cl_2 3:7) to obtain the hydroxy esters (±)-**5** (56% yield) and (±)-**6** (40% yield).

4.3. Typical procedure for the enzymatic acetylation of the cyclic β-hydroxy esters (±)-1–6

To a mixture of the corresponding racemic hydroxy ester (0.58 mmol) and CAL-B (80 mg) under nitrogen atmosphere, the organic solvent (7 mL) and vinyl acetate (1.74 mmol) were added. The resulting mixture was shaken at 30°C and 200 rpm. Aliquots were taken regularly and analysed through chiral GC until 50% of conversion was achieved. The enzyme was then filtered off and washed with dichloromethane and the solvent evaporated under reduced pressure. The products were further purified by flash column chromatography of the residue (eluent: Et_2O/CH_2Cl_2 2:8).

4.3.1. Ethyl (1*R*,2*S*)-2-hydroxycyclopentanecarboxylate, (1*R*,2*S*)-1. Colourless oil. Yield 92%. $[\alpha]_D^{20}$ +22.1 (*c* 1.0, MeOH) >99% e.e. Lit.[20] $[\alpha]_D^{20}$ +25.0 (*c* 1.3, MeOH) 95% e.e. GC conditions for its *O*-propanoyl derivative: 100°C 15 min, 100–170°C, 1°C/min, t_R (1*R*,2*S*) 71.8 min; t_R (1*S*,2*R*) 73.2 min.

4.3.2. Ethyl (1*S*,2*S*)-2-hydroxycyclopentanecarboxylate, (1*S*,2*S*)-2. Colourless oil. Yield 70%. $[\alpha]_D^{20}$ +50.3 (*c* 1.3, Et_2O) >99% e.e. Lit.[20] $[\alpha]_D^{20}$ +52.0 (*c* 0.9, Et_2O) 99% e.e. GC conditions: 100°C 15 min, 100–170°C, 1°C/min, t_R (1*S*,2*S*) 32.3 min and t_R (1*R*,2*R*) 33.6 min. ^{1}H NMR δ 1.26 (t, 3H, $J=7.0$ Hz), 1.5-2.1 (m, 6H), 2.2 (br s, 1H), 2.60 (dd, 1H, $J=8.3$ and 15.3 Hz), 4.25 (q, 2H, $J=7.0$ Hz), 4.4 (m, 1H) ppm. ^{13}C NMR δ 14.7 (CH_3), 22.4 (CH_2), 27.4 (CH_2), 34.5 (CH_2), 53.1 (CH), 61.0 (CH_2), 76.7 (CH), 175.3 (CO) ppm. ESIMS m/z 159 $(M+H)^+$. IR (film) 1731 (CO) and 3436 (OH) cm^{-1}.

4.3.3. Ethyl (1*R*,2*S*)-2-hydroxycyclohexanecarboxylate, (1*R*,2*S*)-3. Colourless oil. Yield 85%. $[\alpha]_D^{20}$ +18.2 (*c* 2.3, $CHCl_3$) >99% e.e. Lit.[21] $[\alpha]_D^{20}$ +16.0 (*c* 1.0, $CHCl_3$) 68% e.e. GC conditions for its *O*-propanoyl derivative: 100°C 15 min, 100–170°C, 1°C/min, t_R (1*R*,2*S*) 53.9 min and t_R (1*S*,2*R*) 54.4 min.

4.3.4. Ethyl (1*S*,2*S*)-2-hydroxycyclohexanecarboxylate, (1*S*,2*S*)-4. Colourless oil. Yield 93%. $[\alpha]_D^{20}$ +40.1 (*c* 1.0, $CHCl_3$) >99% e.e. Lit.[22] $[\alpha]_D^{20}$ +43 (*c* 1.0, $CHCl_3$) 94% e.e. GC conditions: 100°C 15 min, 100–170°C, 1°C/min, t_R (1*S*,2*S*) 35.1 min and t_R (1*R*,2*R*) 35.6 min.

4.3.5. Methyl (1*R*,2*S*)-2-hydroxycycloheptanecarboxylate, (1*R*,2*S*)-5. Colourless oil. Yield 92%. $[\alpha]_D^{20}$ +43.2 (*c* 1.2, $CHCl_3$) >99% e.e. HPLC conditions: hexane/propan-2-ol, 95:5, 0.6 mL/min, t_R (1*R*,2*S*) 5.2 min and t_R (1*S*,2*R*) 13.3 min. ^{1}H NMR δ 1.3–1.98 (m, 10H), 2.3 (br s, 1H), 2.57 (dt, 1H, $J=2.7$ and 10.0 Hz), 3.70 (s, 3H), 4.2 (m, 1H) ppm. ^{13}C NMR δ 21.8 (CH_2), 24.0 (CH_2), 25.5 (CH_2), 27.7 (CH_2), 34.8 (CH_2), 49.6 (CH_3 or CH), 51.6 (CH_3 or CH), 70.1 (CH), 176.6 (CO) ppm. ESIMS m/z 195 $(M+Na)^+$, 173 $(M+H)^+$. IR (film) 1726 (CO) and 3493 (OH) cm^{-1}.

4.3.6. Methyl (1*S*,2*S*)-2-hydroxycycloheptanecarboxylate, (1*S*,2*S*)-6. Colourless oil. Yield 90%. $[\alpha]_D^{20}$ +8.1 (*c* 1.9, $CHCl_3$) >99% e.e. GC conditions: 100°C isothermic, t_R (1*S*,2*S*) 82.7 min and t_R (1*R*,2*R*) 85.1 min. ^{1}H NMR δ 1.5–1.9 (m, 10H), 2.5 (ddd, 1H, $J=3$, 9.1 and 9.1 Hz), 3.7 (s, 3H), 4.0 (dt, 1H, $J=3.7$ and 9.0 Hz). ^{13}C NMR δ 22.3 (CH_2), 26.4 (CH_2), 27.0 (CH_2), 27.8 (CH_2), 35.6 (CH_2), 51.8 (CH_3 or CH), 53.5 (CH_3 or CH), 73.6 (CH), 176.5 (CO). ESIMS m/z 195 $(M+Na)^+$, 173 $(M+H)^+$. IR (film) 1736 (CO) and 3452 (OH) cm^{-1}.

4.3.7. Ethyl (1*S*,2*R*)-2-acetoxycyclopentanecarboxylate, (1*S*,2*R*)-7. Colourless oil. Yield 70%. $[\alpha]_D^{20}$ −8.0 (*c* 0.8, $CHCl_3$) >99% e.e. GC conditions: 100°C 15 min, 100–170°C, 1°C/min, t_R (1*R*,2*S*) 36.7 min and t_R (*S*,*R*) 37.5 min. ^{1}H NMR δ 1.23 (t, 3H, J=7.2 Hz), 1.5–2.4 (m, 9H), 2.9 (ddd, 1H, J=5.8, 8.2 and 9.4 Hz), 4.1 (m, 2H), 5.42 (ddd, H, J=2.6, 5.4 and 5.3 Hz) ppm. ^{13}C NMR δ 14.3 (CH_3), 21.1 (CH_3), 22.0 (CH_2), 25.7 (CH_2), 32.3 (CH_2), 48.6 (CH), 60.4 (CH_2), 76.6 (CH), 170.3 (CO), 171.9 (CO) ppm. ESIMS m/z 223 [(M+Na)$^+$, 100%], 201 [(M+H)$^+$, 20%]. IR (film) 1739 (CO) cm^{-1}.

4.3.8. Ethyl (1*R*,2*R*)-2-acetoxycyclopentanecarboxylate, (1*R*,2*R*)-8. Colourless oil. Yield 81%. $[\alpha]_D^{20}$ −53.0 (*c* 1.3, $CHCl_3$) >99% e.e. HPLC conditions: hexane/propan-2-ol, 96.5:3.5, 0.6 mL/min, t_R (1*S*,2*S*) 5.0 min and t_R (1*R*,2*R*) 5.6 min. ^{1}H NMR δ 1.25 (t, 3H, J=7.2 Hz), 1.7–2.1 (m, 9H), 2.8 (m, 1H), 4.35 (q, 2H, J=7.2 Hz), 5.3 (m, 1H) ppm. ^{13}C NMR δ 14.1 (CH_3), 21.1 (CH_3), 23.5 (CH_2), 28.8 (CH_2), 32.5 (CH_2), 50.4 (CH), 60.6 (CH_2), 78.3 (CH), 170.5 (CO), 174.2 (CO) ppm. ESIMS m/z 223 [(M+Na)$^+$, 100%], 201 [(M+H)$^+$, 15%]. IR (film) 1739 (CO) cm^{-1}.

4.3.9. Ethyl (1*S*,2*R*)-2-acetoxycyclohexanecarboxylate, (1*S*,2*R*)-9. Colourless oil. Yield 75%. $[\alpha]_D^{20}$ −24.2 (*c* 1.1, $CHCl_3$) 99% e.e. GC conditions: RtβDEXse, 100°C 15 min, 100–170°C, 1°C/min, t_R (1*R*,2*S*) 44.6 min and t_R (1*S*,2*R*) 45.4 min. ^{1}H NMR δ 1.21 (t, 3H, J=7 Hz), 1.25–2.2 (m, 11H), 2.50 (ddd, 1H, J=3, 8.8 and 8.8 Hz), 4.1 (m, 2H), 5.4 (m, 1H) ppm. ^{13}C NMR δ 14.1 (CH_3), 20.3 (CH_2), 21.1 (CH_3), 23.1 (CH_2), 24.1 (CH_2), 29.5 (CH_2), 45.4 (CH), 60.9 (CH_2), 69.9 (CH), 170.2 (CO), 172.6 (CO) ppm. ESIMS m/z 237 [(M+Na)$^+$, 18%], 215 [(M+H)$^+$, 1%]. IR (film) 1740 (CO) cm^{-1}.

4.3.10. Ethyl (1*R*,2*R*)-2-acetoxycyclohexanecarboxylate, (1*R*,2*R*)-10. Colourless oil. Yield 95%. $[\alpha]_D^{20}$ −53.0 (*c* 1.3, $CHCl_3$) >99% e.e. GC conditions: 100°C 15 min, 100–170°C, 1°C/min, t_R (1*S*,2*S*) 46.7 min and t_R (1*R*,2*R*) 47.1 min. ^{1}H NMR δ 1.2 (t, 3H, J=7.0 Hz), 1.25–2.4 (m, 11H), 2.46 (ddd, 1H, J=3.8, 10.2 and 12.0 Hz), 4.12 (m, 2H), 5.0 (ddd, 1H, J=4.4, 10.3, 14.5 Hz) ppm. ^{13}C NMR δ 14.2 (CH_3), 21.1 (CH_3), 23.8 (CH_2), 24.4 (CH_2), 28.4 (CH_2), 30.8 (CH_2), 48.8 (CH), 60.5 (CH_2), 73.1 (CH), 170.0 (CO), 173.4 (CO) ppm. ESIMS m/z 273 [(M+K)$^+$, 20%], 237 [(M+Na)$^+$, 20%], 215 [(M+H)$^+$, 100%]. IR (film) 1740 (CO) cm^{-1}.

4.3.11. Methyl (1*S*,2*R*)-2-acetoxycycloheptanecarboxylate, (1*S*,2*R*)-11. Colourless oil. Yield 80%. $[\alpha]_D^{20}$ +10.5 (*c* 1.4, $CHCl_3$) >99% e.e. HPLC conditions: hexane/propan-2-ol, 99:1, 0.6 mL/min, t_R (1*R*,2*S*) 9.3 min and t_R (1*S*,2*R*) 10.4 min. ^{1}H NMR δ 1.4–2.2 (m, 13H), 2.7 (dt, 1H, J=3.7 and 10.0 Hz), 3.70 (s, 3H), 5.4 (dt, 1H, J=4.0, 7.3 Hz) ppm. ^{13}C NMR δ 21.1 (CH_3), 23.0 (CH_2), 24.9 (CH_2), 26.3 (CH_2), 28.3 (CH_2), 31.8 (CH_2), 48.3 (CH or CH_3), 51.7 (CH or CH_3), 73.5 (CH), 170.2 (CO), 174.0 (CO) ppm. ESIMS m/z 237 [(M+Na)$^+$, 40%], 215 [(M+H)$^+$, 100]. IR (film) 1739 (CO) cm^{-1}.

4.3.12. Methyl (1*R*,2*R*)-2-acetoxycycloheptanecarboxylate, (1*R*,2*R*)-12. Colourless oil. Yield 90%. $[\alpha]_D^{20}$ −13.4 (*c* 2.4, $CHCl_3$) >99% e.e. GC conditions: 100°C isothermic, t_R (1*S*,2*S*) 134.0 min and t_R (1*R*,2*R*) 137.6 min. ^{1}H NMR δ 1.4–1.8 (m, 13H), 2.6 (dt, 1H, J=3.5 and 9.0 Hz), 3.6 (s, 3H), 5.10 (ddd, 1H, J=3.8, 9.1 and 10.8 Hz) ppm. ^{13}C NMR δ 21.2 (CH_3), 22.4 (CH_2), 26.4 (CH_2), 27.5 (CH_2), 28.2 (CH_2), 32.6 (CH_2), 50.8 (CH or CH_3), 51.8 (CH or CH_3), 75.6 (CH), 170.0 (CO), 174.8 (CO) ppm. ESIMS m/z 253 [(M+K)$^+$, 5%], [(M+Na)$^+$, 20%], 201 [(M+H)$^+$, 100%]. IR (film) 1739 (CO) cm^{-1}.

4.3.13. Ethyl (1*R*,2*S*)-2-hydroxycycloheptanecarboxylate, (1*R*,2*S*)-13. $[\alpha]_D^{20}$ +48.7 (*c* 0.9, $CHCl_3$) >99% e.e. Lit.[2b] $[\alpha]_D^{20}$ +37.0 (*c* 1.0, $CHCl_3$) 94% e.e. The product exhibited physical properties fully in agreement with the published data. ^{1}H NMR δ 1.27 (t, J=7.1 Hz, 3H), 1.3–2.1 (m, 10H), 2.59 (dt, 1H, J=2.7 and 9.8 Hz), 3.0 (m, 1H), 4.16 (m, 1H), 4.16 (q, J=7.1, 2H) ppm. ^{13}C NMR δ 14.2 (CH_3), 21.8 (CH_2), 24.2 (CH_2), 26.5 (CH_2), 27.7 (CH_2), 34.9 (CH_2), 49.6 (CH), 60.6 (CH_2), 70.2 (CH), 176.5 (CO) ppm.

4.3.14. Ethyl (1*S*,2*S*)-2-hydroxycycloheptanecarboxylate, (1*S*,2*S*)-14. $[\alpha]_D^{20}$ +17.3 (*c* 0.7, $CHCl_3$) >99% e.e. Lit.[2b] $[\alpha]_D^{20}$ +15.0 (*c* 1.0, $CHCl_3$) 93% e.e. The product exhibited physical properties fully in agreement with the published data. ^{1}H NMR δ 1.27 (t, 3H, J=7.2), 1.4–2.0 (m, 10H), 2.5 (dt, 1H, J=3.1 and 9.2 Hz), 2.52 (d, 1H, J=3.6 Hz), 4.00 (ddd, 1H, J=3.7, 8.0 and 13.0 Hz), 4.20 (q, 2H, J= 7.2 Hz) ppm. ^{13}C NMR δ 14.2 (CH_3), 22.4 (CH_2), 26.5 (CH_2), 27.0 (CH_2), 27.8 (CH_2), 35.5 (CH_2), 53.5 (CH), 60.7 (CH_2), 73.6 (CH), 175.1 (CO) ppm.

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References

1. Müller, H. M.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 477–502.
2. (a) Buisson, D.; Azerad, R. *Tetrahedron Lett.* **1986**, *27*, 2631–2634; (b) Danchet, S.; Bigot, C.; Buisson, D.; Azerad, R. *Tetrahedron: Asymmetry* **1997**, *8*, 1735–1739; (c) Rodríguez, S.; Schroeder, K. T.; Kayser, M. M.; Stewart, J. D. *J. Org. Chem.* **2000**, *65*, 2586–2587; (d) Bertau, M.; Bürli, M.; Hungerbühler, E.; Wagner, P. *Tetrahedron: Asymmetry* **2001**, *12*, 2103–2107.
3. Quirós, M.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymmetry* **1999**, *10*, 473–486.
4. (a) Mehmandoust, M.; Buisson, D.; Azerad, R. *Tetrahedron Lett.* **1995**, *36*, 6461–6462; (b) Dehli, J. R.; Gotor,

V. J. Org. Chem. **2002**, *67*, 1716–1718; (c) Dehli, J. R.; Gotor, V. J. Org. Chem. **2002**, *67*, 6816–6819.
5. (a) Buisson, D.; Azerad, R. *Tetrahedron: Asymmetry* **1991**, *2*, 987–988; (b) Jannet, H. B.; Al Mourabit, A.; Gateau-Elesker, A.; Marazano, C.; Mighri, Z. *Tetrahedron: Asymmetry* **1999**, *10*, 2381–2386; (c) Cha, J. H.; Pae, A. N.; Choi, K.; Cho, Y. S.; Kim, W. H.; Han, Y. S.; Yun, H.-C.; Lee, J.; Koh, H. Y.; Lee, E. *Biotechnol. Lett.* **2002**, *24*, 1695–1698; (d) Chartrain, M.; Armstrong, J.; Katz, L.; Kellr, J.; Mathre, D.; Greasham, R. *J. Fermentation Bioeng.* **1995**, *80*, 176–179.
6. (a) Sih, C. J.; Wu, S.-H. *Top. Stereochem.* **1989**, *19*, 63–125; (b) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*: *Regio- and Stereoselective Biotransformations*; Wiley-VCH: Weinheim, 1999.
7. (a) Faber, K. *Chem. Eur. J.* **2001**, *7*, 5004–5010; (b) Huerta, F. F.; Minidis, A. B. E.; Bäckvall, J.-E. *Chem. Soc. Rev.* **2001**, *30*, 321–331; (c) Dehli, J. R.; Gotor, V. *Chem. Soc. Rev.* **2002**, *31*, 365–370.
8. Liese, A.; Seelbach, K.; Wandrey, C. *Industrial Biotransformations*; Wiley-VCH: Weinheim, 2000.
9. (a) Maestro, A.; Astorga, C.; Gotor, V. *Tetrahedron: Asymmetry* **1997**, *8*, 3153–3154; (b) Luna, A.; Astorga, C.; Fülöp, F.; Gotor, V. *Tetrahedron: Asymmetry* **1998**, *9*, 4483–4487; (c) Luna, A.; Maestro, A.; Astorga, C.; Gotor, V. *Tetrahedron: Asymmetry* **1999**, *10*, 1969–1977.
10. (a) Alfonso, I.; Astorga, C.; Rebolledo, F.; Gotor, V. *Chem. Commun.* **1996**, 2471–2472; (b) Luna, A.; Alfonso, I.; Gotor, V. *Org. Lett.* **2002**, *4*, 3627–3629.
11. Panunzio, M.; Camerini, R.; Mazzoni, A.; Donati, D.; Marchioro, C.; Pachera, R. *Tetrahedron: Asymmetry* **1997**, *8*, 15–17.
12. (a) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Richards, M. R.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 7574–7581; (b) Apella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J., Jr.; Gellman, S. H. *Nature (London)* **1997**, *387*, 381–384.
13. LePlae, P. R.; Umezawa, N.; Lee, H.-S.; Gellman, S. H. *J. Org. Chem.* **2001**, *66*, 5629–5632.
14. (a) Xie, Z.-F.; Nakamura, I.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1988**, 966–967; (b) Xie, Z.-F.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1987**, 838–839.
15. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
16. (a) Henke, E.; Pleiss, J.; Bornscheuer, U. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 3211–3213; (b) Krishna, S. H.; Persson, M.; Bornscheuer, U. T. *Tetrahedron: Asymmetry* **2002**, *13*, 2693–2696.
17. Baumhof, P.; Matzitschek, R.; Giannis, A. *Angew. Chem., Int. Ed.* **2001**, *40*, 372–374.
18. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665.
19. Nakata, T.; Oishi, T. *Tetrahedron Lett.* **1980**, *21*, 1641–1644.
20. Gensler, W. J.; Johnson, F.; Sloan, A. D. B. *J. Am. Chem. Soc.* **1960**, *82*, 6074–6081.
21. Buisson, D.; Azerad, R. *Tetrahedron: Asymmetry* **1996**, *7*, 9–12.
22. Kanerva, L. T.; Sundholm, O. *Acta Chem. Scand.* **1993**, *47*, 823–825.