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Approach to Highly Enantiopure β-Amino Acid Esters by Using Lipase Catalysis in Organic Media

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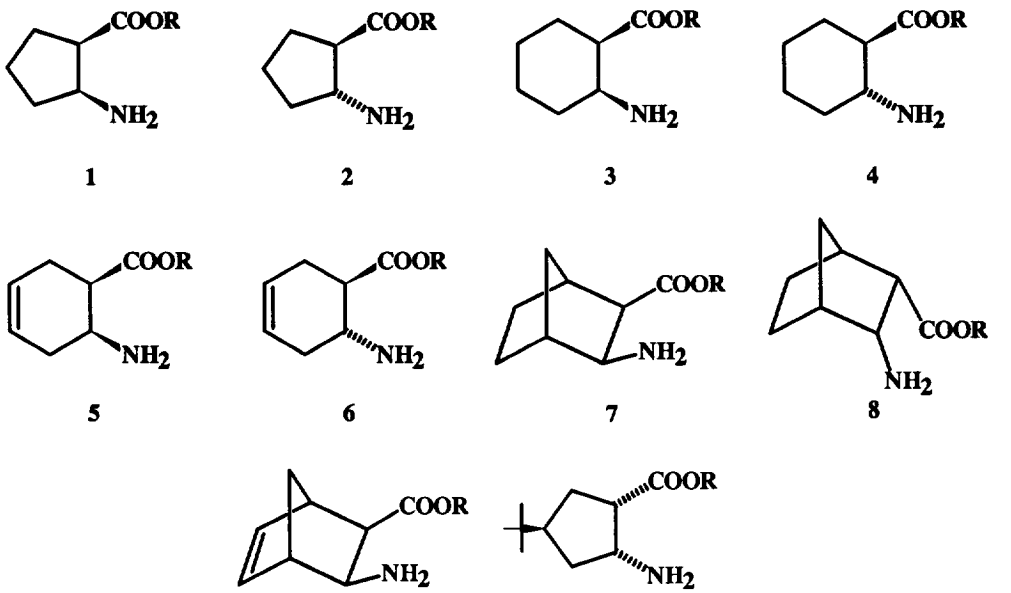
Abstract: Ethyl esters of ten alicyclic β-aminocarboxylic acids were resolved by lipase catalysis in organic solvents. The resolution was based on acylation of the amino group at the *R*-stereogenic centre with various 2,2,2-trifluoroethyl esters. An increase in the hydrophobic nature of the acyl donor enhanced the enantioselectivity and reactivity in the case of lipase SP 526 from *Candida antarctica*, while the opposite effect was observed with lipase PS from *Pseudomonas cepacia*. An unexceptional enantioselectivity enhancement was observed when 2,2,2-trifluoroethyl chloroacetate was used in the case of lipase PS catalysis. Copyright © 1996 Elsevier Science Ltd

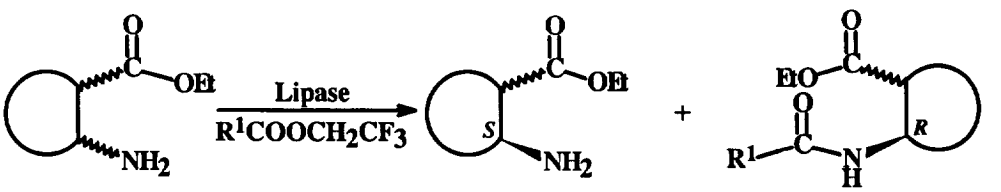
Alicyclic β-aminocarboxylic acids **1-10** (R = H; Scheme 1) are attractive targets for the preparation of various pharmaceutically important 1,3-heterocycles.[1-3] The compounds **1-10** each contain at least two stereogenic centres, and accordingly there are two enantiomers for each compound. Of all the possible stereoisomers of **1-10,** cispentacin (FR 109615), the (1*R*,2*S*) enantiomer of compound **1** (R = H), has received most attention. It is a natural product isolated from the fermentation broths of *Streptomyces setonii* and *Bacillus cereus*, and it possesses effective antifungal properties against *Candida* strains.[4-6] The taste of *L*-aspartyl dipeptides varies from very sweet to bitter, depending on the absolute configuration at position 2 of the isomeric **1** and **2** (R = Me) as second amino acid residue.[7] In consequence of these practical aspects, preparation of the stereoisomers (1*R*,2*S*)-**1**, (1*S*,2*R*)-**1**, (1*R*,2*R*)-**2** and (1*S*,2*S*)-**2** through traditional chemical resolution or asymmetric synthesis has been the goal of several recent studies.[6-10] Biocatalytic routes to cispentacin have included the hydrolysis of racemic building blocks, such as (*E*)-7-(1,3-dithian-2-yl)-3-hepten-2-yl acetate and 6-azabicyclo[3.2.0]hept-3-en-7-one, followed by the necessary chemical transformations to the final product.[11,12] The enantiomers of **5** are highly potential intermediates in the synthesis of various antibiotics.[13]

The aim of the present work was to study lipase-catalysed procedures for the preparation of optically active β-aminocarboxylic acid esters **1-10** (R = Et) in organic solvents. Chemoselectivity towards the formation of an ester bond over that of an amide bond has been achieved with some lipases.[14,15] Thus, asymmetric esterification between a racemic acid **1-10** (R = H) and an achiral alcohol is one possibility for resolution. Free carboxylic acids as



Scheme 1: R= H or Et

substrates were avoided, however, because of the water formed in the esterification, and also in order to prevent possible intermolecular amide formation. Kinetic resolution based on the alcoholysis of ethyl carboxylates **1-10** (R = Et) with an achiral alcohol was not used either, because the two resolution products (the less reactive enantiomer and the new ester produced) are then both esters, separation of which presumes that their structures differ considerably. Moreover, the low enantioselectivity observed for the resolution of the β-hydroxy analogues of amines **3** and **4** (R = Et) with 6-amino-hexan-1-ol was not encouraging.[15] On the other hand, the lipase PS (from *Pseudomonas cepacia*)-catalysed acylation of the hydroxyl function at position 2 of the same analogues tended to stop at 50% conversion, resulting in practically enantiopure products.[16] In the present work, the primary tool of resolution was the lipase-catalysed enantioselective acylation of the amino group at the position 2 or 3 of **1-10** (R = Et) (Scheme 2). Scheme 2



Results and Discussion

Lipase PS is among the most applicable enzymes for the resolution of secondary alcohols.[17] In previous work, butyrylation took place almost exclusively at the 2*R* centres of ethyl *trans*-2-hydroxy-1-cyclohexane-carboxylates.[16]

Table 1. Acetylation of **1-10** (0.05 M; R = Et) in the presence of two lipase preparations (*25-75* mg ml^{-1})[a] and 2,2,2-trifluoroethyl acetate (0.2 M) in Et_2O.

	Lipase PS		SP 526	
Substrate	Conversion[b]/%	E	Conversion[b]/%	E
1	49 (1 h)	»100	24 (1 h)	31
2	28 (0.5 h)	12	-	1
3	24 (8 h)	6	46 (24 h)	51
4	44 (17 h)	33	5[c] (22 h)	1
5	40 (24 h)	6	52 (24 h)	29
6	50 (8 h)	87	4 (24 h)	1
7	44 (24 h)	1	34 (4 h)	30
8	52 (10 h)	1	54 (2 h)	30
9	49 (24 h)	2	30 (4 h)	10
10	28 (24 h)	1	25 (4 h)	30

[a]Contains 20% (w/w) of the lipase adsorbed on Celite in the presence of sucrose; ref. 20.
[b]Reaction time in parentheses. [c]Propionation in Pr^i_2O.

For resolution of the 2-amino analogues **3** and **4** (R = Et) in the present work, the enantioselectivity of lipase PS was considerably lower (Table 1). Accordingly, extensive lipase screening was performed for the asymmetric acetylation of compounds **1-10** (R = Et) with 2,2,2-trifluoroethyl acetate in diethyl ether. Although most of the 18 lipases screened were catalytically active under the reaction conditions, the two enantiomers usually reacted at equal rates. The enantioselectivities of lipases PS and SP 526 (from *Candida antarctica*), on the other hand, displayed nice complementary behaviour, the former usually being the more applicable when the amino and ester functions were situated *trans* to each other [compounds **2**, **4** and **6** (R = Et); Table 1]. The two lipases directed the reaction preferentially to the amino group at the 2*R* centre of **1-6**. The same *R* selectivity of the enzymes is also predictable for the acylation of compounds **7-10**. Enantioselectivities throughout this work are considered in terms of the enantiomeric ratio (*E*).[18] The accuracy of *E* values is often rather low. This is especially true with $E > 100$ because then even minor errors in ee (the enantiomeric excess of the prevailing enantiomer) may cause a significant variation in *E*.[17] Accordingly, when $E \geq 100$, only the signs > or >> indicate the extent of enantioselectivity in Tables 1-3.

The nature of the reaction medium usually affects enzymatic enantioselectivity.[20-23] This provides a useful tool in optimizing kinetic resolution, although no generally valid explanation for the solvent-dependent reactivity and enantioselectivity of an enzyme has yet been proposed. In accordance with the prevailing data, the results in Table 2

clearly underline the lack of a correlation between solvent parameters such as log *P* (*P* is the partition coefficient of the solvent between water and 1-octanol) or ε (the permittivity of the solvent) and the enantioselectivity ratio. Rather, it can be concluded that the nature of the solvent markedly influences the enantioselectivity for a single amino acid ester, the most favourable solvents being found among the simple ethers (rows 2-4) or tertiary alcohols (rows 6 and 7). In the case of a single lipase, the extent of the solvent effects depends on the structural features of the amino acid esters, implying complicated solvent-induced interactions between the constituens of the reaction mixture. In terms of reactivity, lipase PS is catalytically active in a wide variety of organic solvents (Table 2)[23]. For lipase SP 526, the solvent choice seems to be more restricted. In the present work, the enzyme was practically inactive in tetrahydrofuran. On the basis of the present results, diethyl ether, as an economical and readily volatile solvent, was generally used.

Table 2. E[18] for the acetylation of **1-10** (0.05 M; R = Et) in the presence of two lipase preparations (*25-75* mg ml^{-1})[a] and 2,2,2-trifluoroethyl acetate (0.2 M) in various organic solvents.

	Lipase PS				Lipase SP 526					
Solvent (log *P*; ε)[b]	**1**	**3**	**4**	**6**	**1**	**3**	**5**	**7**	**9**	**10**
THF (0.49; 7.6)	>100	-	-	-	-	-	-	3	2	-
Et_2O (0.85; 4.2)	»100	6	33	87	31	51	29	30	10	30
Pr^i_2O (1.9; 3.9)	>100	3	13	51	46	61	21	34	7	>100
$MeOBu^t$ (1.9; 4.5)	>100	7	-	>100	37	57	41	46	26	55
Bu_2O (2.9; 1.1)	19	1	4	8	-	4	2	4	1	-
$Me_2C(OH)Et$ (1.4; 5.8)	»100	8	24	63	40	12	6	23	-	35
$Et_2C(OH)Me$ (2.0; 4.3)	»100	7	-	79	66	80	14	41	18	76
Toluene (2.5; 2.4)	-	1	2	4	-	13	6	2	1	-
Cyclohexane (3.1; 2.0)	-	-	-	7	-	-	-	2	-	-
Hexane (3.5; 1.9)	14	1	3	26	-	7	5	2	1	-

[a]Contains 20% (w/w) of the lipase adsorbed on Celite in the presence of sucrose; ref. 19 [b]Ref. 17.

One of the fundamental criteria of enzymatic kinetic resolutions is the irreversibility of acyl transfer. In order to fulfil this criteria and also to enhance reactivity, alkyl activated esters (halogen-substituted, oxime or vinyl esters) or acid anhydrides often serve as acyl donors.[17] For the lipase-catalysed acylation of an amino group, the irreversibility demand is always met because lipases do not catalyse the breakdown of an amide bond. Thus, the focus in choosing an acyl donor is primarily on reactivity. For the resolution of **1-10** (R = Et), acid anhydrides or acetone oxime esters are not suitable as acyl donors because of fast non-enzymatic acylations, leading to racemic products. Moreover, acid

anhydrides liberate carboxylic acids, which form salts with the free amino group of **1-10**. Schiff base formation between the amino group and acetaldehyde (the tautomerization product of vinyl alcohol) prevents the use of vinyl esters as acylating agents. On the other hand, lipase PS- and SP 526-catalysed acylations with the application of various 2,2,2-trifluoroethyl carboxylates proceeded smoothly in diethyl ether (Table 3).

Table 3. Effects of various acyl donors (0.2 M) on the acylation of alicyclic ethyl β-aminocarboxylates (0.05 M) in the presence of two lipase preparations[a] (*10-75* mg ml^{-1}) in Et_2O.

	$MeCO_2CH_2CF_3$		$EtCO_2CH_2CF_3$		$PrCO_2CH_2CF_3$		$C_5H_{11}CO_2CH_2CF_3$		$ClCH_2CO_2CH_2CF_3$	
Substrate[a]	Conv.[b]/%	*E*	Conv.[b]/%	*E*	Conv.[b]/%	*E*	Conv.[b]/%	*E*	Conv.[b]/%	*E*
3 (PS)	24 (8 h)	6	42 (24 h)	4	18 (24 h)	2			49 (5 min)[c]	53
4 (PS)	44 (17 h)	33	33 (48 h)	8	12 (48 h)	4			50 (4 min)	»100
8 (PS)	52 (10 h)	1							49 (5 min)	48
9 (PS)	49 (24 h)	2							45 (5 min)	64
10 (PS)	28 (24 h)	1							25 (30 min)	2
1 (SP526)	24 (1 h)	31	40 (0.5 h)	30	54 (0.5 h)	47	52 (0.5 h)	64		
3 (SP526)	46 (24 h)	51			40 (30)	»100	32 (30 h)	»100	24 (5 min)[c]	4
7 (SP526)	34 (4 h)	30	49 (2 h)	57	47 (1 h)	»100	50 (1 h)	»100		
8 (SP526)	54 (2 h)	30					41 (0.2 h)	»100		
9 (SP526)	30 (4 h)	10	45 (4 h)	24	44 (2h)	42	47 (1 h)	60		

[a]Contains 20% (w/w) of the lipase (mentioned in parentheses) adsorbed on Celite in the presence of sucrose; ref. 19. [b]Reaction time in parentheses. [c]2,2,2-Trifluoroethyl chloroacetate 0.05 M.

For further optimization of the enantioselectivity, the structure of an achiral acyl donor needs consideration. In theory, both the alkyl and acyl groups of an achiral ester can be changed, but because of the facts mentioned above, 2,2,2-trifluoroethyl carboxylates ($R^1CO_2CH_2CF_3$) were used throughout this work. In terms of the two-step reaction mechanism proceeding through a tetrahedral acyl-enzyme intermediate (R^1CO-enzyme), the nature of the intermediate is a key feature when group R^1 is varied. Enantiodiscrimination occurs when this intermediate reacts with one of amines **1-10** (R = Et). In this work, the acyl donors ranged from 2,2,2-trifluoroethyl acetate to hexanoate and the reactions were conducted in diethyl ether in the presence of lipases PS and SP 526 (Table 3). The opposite behaviour of the enantioselectivity and the reactivity with the two lipases is obvious from the data in Table 3. Thus, for lipase PS catalysis, both the reactivity and the enantioselectivity tend to decrease with increasing carbon chain length of R^1, while the same properties clearly increase when lipase SP 526 is used. In other words, the increasing hydrophobic nature of the acyl-enzyme intermediate seems to be unfavourable for the lipase PS-catalysed reactions,

whereas the opposite is true for lipase SP 526. This reasoning led us to use 2,2,2-trifluoroethyl chloroacetate in place of the acetate. The intermediate ($ClCH_2CO$-enzyme) of an enzymatic reaction is now polar in nature and is of about the same size as the EtCO-enzyme intermediate. As expected, there is a huge increase in *E* for lipase PS. and a decrease for lipase SP 526 catalysis (Table 3, last column). 2,2,2-Trifluoroethyl chloroacetate clearly makes lipase PS a highly applicable enantioselective biocatalyst for the resolution of any of **1-9**. For some reason, this system cannot be used for the resolution of compound **10**. The chloroacetate has been previously used as an acyl donor in at least one case.[24] The exceptional behaviour of a *Pseudomonas* lipase (lipase AK) was also reported in that case: the enantioselectivity became extremely low and the stereochemical preference of the enzyme was inverted from *S* to *R* when vinyl or isopropenyl acetate was changed to the corresponding chloroacetate for the acylation of methyl mandelates in diisopropyl ether.

In 2,2,2-trifluoroethyl chloroacetate, both the alkyl and acyl parts of the ester are activated. This leads to an increase in reactivity in both enzymatic and non-enzymatic acylations. The first requirement for exploitation of the enantioselectivity enhancement caused by chloroacetylation in the presence of lipase PS is that the reactivity difference between the enzymatic and non-enzymatic reactions can be controlled by the amount of the enzyme. For example, the chemical reaction between 2,2,2-trifluoroethyl chloroacetate (0.05 M) and **4** (0.05 M) in diethyl ether proceeds to 4% conversion in 5 minutes, whereas a 44% conversion is reached in this time in the presence of the lipase PS preparation (2.5 mg ml^{-1}). As the second requirement, the non-enzymatic reaction must be suppressed immediately after the enzymatic resolution is completed. The use of only slight excess of the acyl donor compared to the reactive enantiomer seems to work nicely in most cases. Moreover, the addition of some ethanol before removing the enzyme effectively destroys the unreacted 2,2,2-trifluoroethyl chloroacetate in the reaction mixture. The unreacted β-amino carboxylate, on the other hand, can be easily removed as its hydrochloride either by extracting with dilute hydrochloric acid or more conveniently through the crystallization by bubbling dry hydrogen chloride to the resolved mixture. The latter method is also the most useful means of separating the less reactive enantiomer as crystalline product from the reaction mixture. The more reactive enantiomer is then readily obtained as an amide by using column chromatography. The chromatographic separation of resolved counterparts is also possible. Thus, when ethyl acetate is used as an eluent, the amide product (Scheme 2) moves almost together with the solvent front, while the unreacted free 2-amino-1-carboxylate hardly moves at all. Change of the solvent to methanol or a methanolic solution allows this enantiomer to be washed out.

Accordingly, successful gram-scale preparations of the enantiomers of **1-7**, **9** and **10** in diethyl ether can be performed by using lipase PS or SP 526 as catalyst and a suitable 2,2,2-trifluoroethyl ester as an acyl donor. Values of ee > 95% are easily obtained for the products isolated simultaneously from the same resolution. The results obtained for the fast and highly enantioselective lipase PS-catalysed chloroacetylations are mainly to be found in the Experimental part.

Experimental: *Materials and Methods* - Racemic compounds **1-10** were prepared according to known methods.[1-3] 2,2,2-Trifluoroethyl esters were prepared from 2,2,2-trifluoroethanol and an acid anhydride or acid chloride. All the solvents were of the highest analytical grade and were dried over molecular sieves (3 Å) before use. The lipases screened were lipases AP6 (*Aspergillus niger*), AY (*Candida rugosa*), AK (*Pseudomonas sp.*), Y (*Penicillium cyclopium*), L (*Candida lipolytica*), PS (*Pseudomonas cepacia*), M (*Mucor javanicus*), N (*Rhizopus niveus*) and R (*Penicillium roqueforti*) from Amano Pharmaceuticals; *Candida rugosa* (type VII) and porcine pancreatic (type II) lipases from Sigma Chemicals; lipases SP 525, SP 526 and Novozym 435 (*Candida antarctica*) from Novo Nordisk; and *Rhizopus javanicus*, *Candida lypolytica*, *Mucor miehei* and *Geotrichum candidum* lipases from Biocatalysts. Lipases AK, PS and SP 526 (5 g) were dissolved in Tris-HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g) and were then adsorbed on Celite (17 g).[19] The lipase preparations thus contained 20% (w/w) of the lipase. The other lipases were used as received.

In a typical small-scale experiment, a solution (2 ml) of one of the amines **1-10** (R = Et; 0.05 M) in an organic solvent was added to the lipase preparation (25-75 mg ml^{-1}) and an acyl donor (0.2 M in the reaction mixture) was pipetted in. The mixture was shaken at room temperature. Samples (0.1 ml) were withdrawn at intervals, the enzyme was filtered off and the unreacted amine was derivatized by using acetic, propionic, butyric or hexanoic anhydride in the presence of 4-dimethylaminopyridine.[25] The acyl group in the derivatization must differ from that used in the enzymatic acylation. The conversion of the reaction and the ee values of all the constituents were usually determined simultaneously by injecting the derivatized sample into a gas chromatograph equipped with a 25 m Chirasil-L-Val column. However, the enantiomers of chloroacetamides did not always separate completely in the Chirasil-L-Val column. For gram-scale resolutions, this problem was overcomed by performing first the deprotection of the chloroacetamide group with thiourea and then the normal derivatization with an appropriate acid anhydride.[26] The separation of the reaction components using a 25 m NB-30 column was another way to determine the conversion. In this case, octadecane (0.02 M) was used as an internal standard.

$[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

Resolution of **1** *(R = Et)*. Racemic **1** (0.71 g; 4.5 mmol) and 2,2,2-trifluoroethyl chloroacetate (0.44 ml; 3.5 mmol) in diethyl ether (22.5 ml) were added to the lipase PS preparation (0.11 g). The mixture was stirred at room temperature for 5 minutes. The reaction stopped at 51% conversion with 99.7 and 95% ee for unreacted **1** and the chloroacetamide produced, respectively (Scheme 2). Ethanol was added. The enzyme was filtered off and the temperature was lowered to 0 °C. Gaseous hydrogen chloride was bubbled through the solution for 20 minutes. After evaporation, the residue was dissolved in diethyl ether (15 ml) and the less reactive enantiomer was allowed to precipitate as the white hydrochloride of **1** (R = Et; 0.31 g; 1.6 mmol; m.p. 68-69.5 °C; $[\alpha]_D^{20}$ = -10.4 (*c* = 1, EtOH); ee 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.29 (3H, t, J = 7.1, $CH_2\underline{CH_3}$), 1.65-2.25 (6H, 3xCH_2), 3.02 (1H, dt, J = 8.3, 6.2, H-1), 3.90 (1H, q, J = 5.9, H-2), 4.24 (2H, q, J = 7.1, $\underline{CH_2}CH_3$), 8.50 (3H, bs, NH_3^+). Analysis calculated for $C_8H_{16}NO_2Cl$: C, 49.61; H, 8.33; N, 7.23. Found: C, 48.47; H, 8.80; N, 6.98.

The hydrochloride of **1** (R = H; m.p. 155-156 °C; $[\alpha]_D^{20}$ = -4.5 (*c* = 1, H_2O)) was then obtained by acidic hydrolysis.[6] Subsequent desalting with an anion-exchange resin (Amberlite CG-400) and recrystallization (water/acetone) gave the free amino acid with $[\alpha]_D^{20}$ = -9.6 (*c* = 1, H_2O) in accordance with the literature values of -8 - -10.7 for the (1*R*,2*S*) absolute configuration.[5-8,11,12]

The corresponding (1*S*,2*R*)-chloroacetamide was separated from the remaining reaction mixture by column chromatography on silica, eluting with ethyl acetate. Evaporation of the solvent and recrystallization from diisopropyl ether resulted in white crystals (0.49 g; 2.1 mmol; m.p. 53-54 °C; $[\alpha]_D^{20}$ = +108.0 (*c* = 1, EtOH); ee = 96%).^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.27 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.60-2.05 (6H, 3xCH_2), 3.00 (1H, q, J = 7.4, H-1), 4.02 (2H, AB, J = 15.1, CH_2Cl), 4.16 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 4.47 (1H, qui, J = 7.6, H-2), 7.29 (1H, bs, NH). Analysis calculated for $C_{10}H_{16}NO_3Cl$: C, 51.40; H, 6.90; N, 5.99. Found: C, 51.51; H, 7.11; N, 5.97.

Resolution of **2** *(R = Et)*. Racemic **2** (0.71 g; 4.5 mmol) and 2,2,2-trifluoroethyl chloroacetate (0.44 ml; 3.5 mmol) in diethyl ether (90 ml) were added to the lipase PS preparation (0.45 g). The mixture was stirred for 20 minutes. The reaction stopped at 57% conversion with >99 and 74% ee for unreacted **2** and chloracetamide produced, respectively (Scheme 2). The work-up followed the lines given above. Ethyl 2-amino-1-cyclopentane-carboxylate hydrochloride (0.36 g; 1.9 mmol; m.p. 69-71 °C; $[\alpha]_D^{20}$ +61.8 (*c* = 1, EtOH); ee >99%) was transformed to the free amino acid **2** (R = H; 0.11 g; 0.85 mmol; m.p. 218-221 °C; $[\alpha]_D^{20}$ +62.3 (*c* = 1, H_2O) in accordance with the literature values of **2** (R = H; m.p. 221 °C dec.; $[\alpha]_D^{20}$ +51.8 (*c* = 1, H_2O)) for the (1*S*,2*S*) absolute configuration.[7]^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm) for the ethyl ester hydrochloride **2**: 1.29 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.65-2.30 (6H, 3xCH_2), 3.14 (1H, q, J = 7.7, H-1), 3.90 (1H, bs, H-2), 4.20 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 8.15 (3H, bs, NH_3^+). Analysis calculated for $C_8H_{16}NO_2Cl$: C, 49.61; H, 8.33; N, 7.23. Found: C, 49.62; H, 8.35; N, 6.95.

The corresponding (1*R*,2*R*)-chloroacetamide (0.29 g; 1.2 mmol; m.p. 49.5-51 °C; $[\alpha]_D^{20}$ -36.3 (*c* = 1, EtOH); ee 99%) was obtained as white crystals by column chromatography followed by recrystallization from diisopropyl ether. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.26 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.50-2.25 (6H, 3xCH_2), 2.65 (1H, q, J = 8.3, H-1), 4.02 (2H, s, CH_2Cl), 4.15 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 4.30 (1H, qui, J = 7.6, H-2), 6.61 (1H, bs, NH). Analysis calculated for $C_{10}H_{16}NO_3Cl$: C, 51.40; H, 6.90; N, 5.99. Found: C, 51.53; H, 7.07; N, 5.93.

Resolution of **3** *(R = Et)*. Racemic **3** (3.0 g; 17.5 mmol) and 2,2,2-trifluoroethyl chloroacetate (2.2 ml; 17.5 mmol) in diethyl ether (87 ml) were added to the lipase PS preparation (2.2 g). The mixture was stirred for 20 minutes. The reaction stopped at 54% conversion with 98 and 84% ee for unreacted **3** and the chloroacetamide produced, respectively (Scheme 2). The work-up followed the lines given above. Ethyl 2-aminocyclohexane-carboxylate hydrochloride (1.47 g; 7.1 mmol; m.p. 138-140 °C; $[\alpha]_D^{22}$ -15.3 (*c* = 1, EtOH); ee 98%) was obtained as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.29 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.30-2.30 (8H, 4xCH_2), 3.15 (1H, q, J = 4.3, H-1), 3.59 (1H, bs, H-2), 4.19-4.29 (2H, m, $C\underline{H}_2CH_3$), 8.48 (3H, bs, NH_3^+). Analysis calculated for $C_9H_{18}NO_2Cl$: C, 52.05; H, 8.73; N, 6.74. Found: C, 52.00; H, 9.07; N, 6.66. The absolute

configuration (1*R*,2*S*) was determined through epimerization at the 1C stereogenic centre in ethanol in the presence of sodium ethoxide, leading to the (1*S*,2*S*) enantiomer of **4** (R = Et) according to the chiral GLC method.

The corresponding (1*S*,2*R*)-chloroacetamide (2.5 g; 10 mmol; $[\alpha]_D^{22}$ +38.5 (*c* = 1, EtOH); ee 94%) was obtained as a colourless oil after column chromatography, on elution with ethyl acetate:hexane (1:1). ^{1}H NMR (400 MHz, $CDCl_3$) d(ppm): 1.29 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.44-2.14 (8H, 4xCH_2), 2.80 (1H, dt, J = 14, H-1), 4.07 (2H, s, CH_2Cl), 4.11-4.24 (2H, m, $C\underline{H}_2CH_3$), 7.75 (1H, bs, NH). Analysis calculated for $C_{11}H_{18}NO_3Cl$: C, 53.33; H, 7.32; N, 5.65. Found: C, 49.78; H, 7.35; N, 4.80.

Resolution of **4** *(R = Et)*. Racemic **4** (3.0 g; 17.5 mmol) and 2,2,2-trifluoroethyl chloroacetate (1.2 ml; 9.6 mmol) in diethyl ether (88 ml) were added to the lipase PS preparation (2.2 g). The mixture was stirred for 15 minutes. The reaction stopped at 46% conversion with 82% ee for unreacted **4** (R = Et). The work-up followed the above procedure except that hydrogen chloride bubbling was performed at -18 °C. Ethyl 2-amino-1-cyclohexanecarboxylate hydrochloride (1.74 g; 8.4 mmol; m.p. 182-185 °C; $[\alpha]_D^{22}$ +43.7 (*c* = 1, EtOH); ee 88%) was obtained as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.28 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.30-2.36 (8H, 4xCH_2), 2.75 (1H, dt, J = 10.8, 3.8, H-1), 3.40 (1H, dt, J = 11.1, 4.0, H-2), 4.24 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 8.46 (3H, bs, NH_3^+). Analysis calculated for $C_9H_{18}NO_2Cl$: C, 52.05; H, 8.73; N, 6.74. Found: C, 52.31; H, 8.69; N, 6.60. The absolute configuration (1*S*,2*S*) was concluded by comparing the observed value of $[\alpha]_{578}^{20}$ +51.5 (*c* = 0.27, EtOH) to the literature value of +56.3.[27]

The corresponding (1*R*,2*R*)-chloroacetamide (2.0 g; 8.1 mmol; m.p. 51-53 °C; $[\alpha]_D^{22}$ -12.0 (*c* = 1, EtOH); ee 96%) was obtained as a waxy compound by column chromatography, on elution with ethyl acetate:hexane (1:1). ^{1}H NMR (400 MHz, DMSO) d(ppm): 1.17 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.20-1.83 (8H, 4xCH_2), 2.34 (1H, dt, J = 10.8, 3.7, H-1), 3.78 (1H, dq, H-2), 3.96 (2H, s, CH_2Cl), 3.98-4.04 (2H, m, $C\underline{H}_2CH_3$), 8.17 (1H, bs, NH). Analysis calculated for $C_{11}H_{18}NO_3Cl$: C, 53.33; H, 7.32; N, 5.65. Found: C, 52.27; H, 7.25; N, 5.36.

Resolution of **5** *(R = Et)*. Racemic **5** (3.0 g; 17.8 mmol) and 2,2,2-trifluoroethyl chloroacetate (2.1 ml; 16.9 mmol) in diethyl ether (89 ml) were added to the lipase PS preparation (2.2 g). The mixture was stirred for 15 minutes. The reaction stopped at 48% conversion with 78% ee for unreacted **5** (R = Et). The work-up followed the above procedure with bubbling of hydrogen chloride at -18 °C. Recrystallization from diethyl ether:ethanol (1:1) afforded ethyl 2-amino-1-cyclohex-4-enecarboxylate hydrochloride (1.43 g; 7.0 mmol; m.p. 122-123 °C; $[\alpha]_D^{22}$ = -17.6 (*c* = 1, EtOH); ee 93%) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.28 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 2.40-2.75 (4H, 2xCH_2), 3.22 (1H, dt, J = 7.3, 5.5, H-1), 3.83 (1H, ddd, J = 8.3, 5.5, 3.0, H-2), 4.23 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 5.62 (1H, ddd, J = 10.3, 5.5, 2.5, CH), 5.73 (1H, ddd, J = 10.0, 4.5, 2.5, CH), 8.56 (3H, bs, NH_3^+). Analysis calculated for $C_9H_{16}NO_2Cl$: C, 52.56; H, 7.84; N, 6.81. Found: C, 51.89; H, 7.86; N, 6.81. The absolute configuration (1*R*,2*S*) was proven through hydrogenation to (1*R*,2*S*)-**3** in the presence of Pd/C.

The corresponding (1*S*,2*R*)-chloroacetamide (2.1 g; 8.5 mmol; $[\alpha]_D^{22}$ +37.5 (*c* = 1, EtOH); ee 85%) was

obtained as a colourless oil by eluting through silica with ethyl acetate:hexane (1:1). ^{1}H NMR (400 MHz, $CDCl_3$) d(ppm): 1.26 (3H, t, J = 7.0, $CH_2C\underline{H}_3$), 2.18-2.56 (4H, 2xCH_2), 2.80 (1H, m, H-1), 4.04 (2H, s, CH_2Cl), 4.15 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 4.46 (1H, m, H-2), 5.62 (1H, m, CH), 5.68 (2H, m, CH=CH), 7.24 (1H, bd, NH). Analysis calculated for $C_{11}H_{16}NO_3Cl$: C, 53.77; H, 6.56; N, 5.70. Found: C, 50.65; H, 6.52; N, 5.14.

Resolution of **6** *(R = Et)* Racemic **6** (2.0 g; 11.8 mmol) and 2,2,2-trifluoroethyl chloroacetate (0.81 ml; 6.5 mmol) in diethyl ether (59 ml) were added to the lipase PS preparation (1.5 g). The mixture was stirred for 40 minutes. The reaction stopped at 54% conversion with 99.9% ee for unreacted **6** (R = Et). The work-up followed the above procedure. Recrystallization from diethyl ether:ethanol (1:1) afforded ethyl 2-amino-4-cyclohexene-1-carboxylate hydrochloride (1.1 g; 5.4 mmol; m.p. 130-132 °C; $[\alpha]_D^{22}$ +118.3 (*c* = 1, EtOH); ee 99%) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) d(ppm): 1.22 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 2.22-2.70 (4H, 2xCH_2), 3.03 (1H, m, H-1), 3.66 (1H, m, H-2), 4.18 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 5.56 (2H, m, CH=CH), 8.51 (1H, bs, NH_3^+). Analysis calculated for $C_9H_{16}NO_2Cl$: C, 52.56; H, 7.84; N, 6.81. Found: C, 52.57; H, 8.03; N, 6.66. The absolute configuration (1*S*,2*S*) was proven through hydrogenation to (1*S*,2*S*)-**4** in the presence of Pd/C.

The corresponding (1*R*,2*R*)-chloroacetamide (1.4 g; 5.7 mmol; $[\alpha]_D^{22}$ -22.7 (*c* = 1, EtOH); ee 100%) was obtained as an oil by eluting through silica with hexane:ethyl acetate (1:1). The oil turned to a wax at 4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) d(ppm): 1.24 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.95-2.65 (4H, 2xCH_2), 2.73 (1H, m, H-1), 4.03 (2H, AB, J = 15.4, CH_2Cl), 4.16 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 4.34 (1H, ddd, J = 14.6, 9.3, 5.4, H-2), 5.67 (2H, m, CH=CH), 6.67 (1H, bd, J = 3.7, NH). Analysis calculated for $C_{11}H_{16}NO_3Cl$: C, 53.77; H, 6.56; N, 5.70. Found: C, 53.94; H, 6.70; N, 5.73.

Resolution of **7** *(R = Et).* Racemic **7** (0.92 g; 5.0 mmol) and 2,2,2-trifluoroethyl hexanoate (2.0 ml; 10 mmol) in diethyl ether (50 ml) were added to the lipase SP 526 preparation (2.5 g). The reaction was stopped by filtering off the enzyme after 7 h, at 51% conversion, with 98 and 96% ee for unreacted **7** and the amide produced, respectively (Scheme 2). Dry hydrogen chloride was bubbled through the reaction mixture as above, leading to the precipitation of **7** as hydrochloride (0.46 g; 2.1 mmol; m.p. 161.5-163 °C; $[\alpha]_D^{20}$ -6.3 (*c* = 1, EtOH); ee 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.29 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.31 (overlapped) (1H,d, J = 11.1, CH_2), 1.31-1.67 (4H, CH_2-CH_2), 2.08 (1H, d, J = 11.1, CH_2), 2.54, 2.79 (2H, d, 2xCH), 2.73 (1H, d,J = 7.8, H-2), 3.57 (1H, d, J =7.8, H-3), 4.21 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 8.47 (3H, bs, NH_3^+). Analysis calculated for $C_{10}H_{18}NO_2Cl$: C, 54.67; H, 8.26; N, 6.38. Found: C, 554.64; H, 8.62; N, 6.34. The acidic hydrolysis afforded the corresponding amino acid hydrochloride (R = H; m.p. 195-197 °C; $[\alpha]_D^{20}$ +3.9 (*c* = 2, MeOH). In accordance with the general *R* selectivity of the enzyme, the absolute configuration (1*S*,2*R*,3*S*,4*R*) was presumed.

The corresponding (1*R*,2*S*,3*R*,2*S*)-amide (a pale-brown oil) was obtained by column chromatography on elution with ethyl acetate (0.65 g; 2.3 mmol; $[\alpha]_D^{20}$ +84.9(*c* = 1, EtOH); ee 96%).^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 0.86 (3H, t, J = 7.1, $CH_2CH_2C\underline{H}_3$), 1.25 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.20-1.90 (overlapped) (12H, 6xCH_2), 2.10

(2H, dd, J = 7.8, 7.5, C(O)CH_2), 2.13 (overlapped) (1H, bs, CH), 2.42 (1H, bs, CH), 2.69 (1H, bd, J = 8.6, H-2), 4.02-4.15 (2H, m, $C\underline{H}_2CH_3$), 4.23 (1H,dt, J = 8.56, 1.26, H-3), 6.33 (1H, d, J = 8.56, NH). Analysis calculated for $C_{16}H_{27}NO_3$: C, 68.29; H, 9.67; N, 4.98. Found: C, 68.33; H, 9.95; N, 4.82.

Resolution of **9** *(R = Et).* Racemic **9** (1.3 g; 7.2 mmol) and 2,2,2-trifluoroethyl chloroacetate (0.47 ml; 3.8 mmol) in diethyl ether (140 ml) were added to the lipase PS preparation (3.5 g). The mixture was stirred for 30 minutes. The reaction stopped at 51% conversion with 95 and 91% ee for the unreacted **9** (R = Et) and the chloroacetamide produced, respectively (Scheme 2). The normal work-up afforded (1*S*,2*R*,3*S*,4*R*)-**9** as white hydrochloride crystals (0.69 g; 3.2 mmol; m.p. 124-126 °C; $[\alpha]_D^{20}$ -23.9 (*c* = 1, EtOH); ee 99 %).^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.31 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.65 (1H,d, J = 10.7, CH_2), 2.13 (1H, d, J = 10.7, CH_2), 2.65 (1H, dd, J = 7.8, 1.5, H-2), 3.10, 3.42 (2H, bs, 2xCH), 3.50 (1H, dd, J = 7.8, 1.5, H-3), 4.23 (2H, q, J =7.1, $C\underline{H}_2CH_3$), 6.27 (2H, d (AB), J = 5.5, 3.0, CH=CH), 8.65 (3H, bs, NH_3^+). Analysis calculated for $C_{10}H_{16}NO_2Cl$: C, 55.17; H, 7.41; N, 6.43. Found: C, 55.30; H, 7.48; N, 6.44. Hydrogenation in the presence of Pd/C resulted in (1*S*,2*R*,3*S*,4*R*)-**7** with 98% ee, indicating the same selectivity of the two enzymes.

The corresponding (1*R*,2*S*,3*R*,4*S*)-chloroacetamide was obtained by column chromatography on elution with ethyl acetate. Recrystallization from diisopropyl ether afforded white crystals (0.65 g; 2.9 mmol; m.p. 67-68 °C; $[\alpha]_D^{20}$ +116.2 (*c* = 1, EtOH); ee 95%).^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.28 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.60 (1H, quid, J = 9.3, 1.5, CH_2), 2.00 (1H, td, J = 9.4, 1.5, CH_2), 2.63 (1H, dd, J = 8.3, 1.8, CH=CH), 2.78 (1H, bs, CH), 3.01 (1H, bs, CH), 4.02 (2H, AB, J = 15.1, CH_2Cl), 4.15 (1H, ddd, J = 8.3, 7.1 , 1.8, H-3), 4.17 (2H, q, J = 7.1 $C\underline{H}_2CH_3$), 6.22-6.26 (1H, m, CH=CH), 7.74 (1H, d, J = 7.1, NH). Analysis calculated for $C_9H_{16}NO_3Cl$: C, 55.93; H, 6.26; N, 5.43. Found: C, 56.06; H, 6.33; N, 5.36.

Resolution of **10** *(R = Et).* Racemic **10** (1.1 g; 5.1 mmol) and 2,2,2-trifluoroethyl hexanoate (2 ml; 10 mmol) in diethyl ether (50 ml) were added to the lipase SP 526 preparation (2.5 g). The reaction was stopped by filtering off the enzyme at 50% conversion after 3.3 h with 99% ee for unreacted **10** (R = Et) and the amide produced (Scheme 2). In this case, the unreacted amine did not precipitate during the HCl-bubbling. Therefore, the resolved products were separated by column chromatography on elution first with ethyl acetate for the separation of the produced (1*S*,2*R*,4S)-amide (presumed absolute configuration according to the general *R* selectivity). The evaporation of the solvent afforded pale brown oil (0.72 g; 2.3 mmol; $[\alpha]_D^{20}$ +59.9 (*c* = 1, EtOH); ee 99 %).^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 0.84 (9H, s, 3xCH_3), 0.89 (overlapped) (3H, t, J = 7.1 CH_3), 1.26 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.25-1.70 (overlapped) (7H), 1.74 (2H, t, J = 7.1 $CH_2C\underline{H}_2CH_3$), 1.95-2.10 (2H), 2.13 (2H, t, J = 7.8, C(O) CH_2), 2.98 (1H ddd, J = 8.8, 6.2, 4.0, H-1), 4.09-4.17 (2H, m, $C\underline{H}_2CH_3$) 4.46 (1H, td, J = 14.6, 6.8, H-2), 6.15 (1H, d, J = 8.6, NH). Analysis calculated for $C_{18}H_{33}NO_3Cl$: C, 69.41; H, 10.68; N, 4.50. Found: C, 68.74; H, 10.28; N, 4.49.

The unreacted compound, (1*R*,2*S*,4*R*)-**10**, as a hydrochloride (yellow oil) was eluted using

dichloromethane:ethanol (95:5). In hexane at -25°C, the product turned to white crystals (0.59 g; 2.4 mmol; m.p. 96-98 °C; $[\alpha]_D^{20}$ -2.4 (*c* = 1, EtOH); ee 99 %).^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 0.85 (9H, s, 3xCH_3), 1.29 (3H, t, J = 7.1, $CH_2C\underline{H}_3$), 1.78-2.31 (5H, 2xCH_2 + 1xCH), 3.03-3.05 (1H, m, H-1), 3.83-3.87 (1H, m, H-2), 4.24 (2H, q, J = 7.1, $C\underline{H}_2CH_3$), 8.50 (3H, bs, NH_3^+). Analysis calculated for $C_{12}H_{24}NO_2Cl$: C, 57.70; H, 9.68; N, 5.61. Found: C, 55.97; H, 9.85; N, 5.46.

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References:

1. Bernáth, G.; Fülöp, F.; Stájer, G. *Janssen Chimica Acta* **1991**, *9*, 12.
2. Bernáth, G. *Acta Chim. Hung. - Models in Chemistry* **1992**, *129*, 107.
3. Fülöp, F.; Bernáth, G.; Spitzner, R.; Mattinen, J.; Pihlaja, K. *Acta Chim. Hung. - Models in Chemistry* **1994**, *131*, 435.
4. Oki, T.; Hirano, M.; Tomatsu, K.; Numata, K.; Kamei, H. *J. Antibiotics* **1989**, *42*, 1756.
5. Iwamoto, T.; Tsujii, E.; Ezaki, M.; Fujie, A.; Hashimoto, S.; Okuhara, M.; Kohsaka, M.; Imanaka, H.; Kawabata, K.; Inamoto, Y.; Sakane, K. *J. Antibiotics* **1990**, *43*, 1.
6. Kawabata, K.; Inamoto, Y.; Sakane, K.; Iwamoto, T.; Hashimoto, S. *J. Antibiotics* **1990**, *43*, 513.
7. Yamazaki, T.; Zhu, Y.-F.; Probstl, A.; Chadha, R. K.; Goodman, M. *J. Org. Chem.* **1991**, *56*, 6644.
8. Konishi, M.; Nishio, M.; Saitoh, K.; Miyaki, T.; Oki, T.; Kawaguchi, H. *J. Antibiotics* **1989**, *42*, 1749.
9. Davies, S. G.; Ichihara, O.; Walters, I. A. S. *Synlett* **1993**, 461.
10. Cimarelli, C.; Palmieri, G.; Bartoli, G. *Tetrahedron: Asymmetry* **1994**, *5*, 1455.
11. Evans, C.; McCague, R.; Roberts, S. M.; Sutherland, A. G.; Wisdom, R. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2276.
12. Konosu, T.; Oida, S. *Chem. Pharm. Bull.* **1993**, *41*, 1012.
13. Tamura, N.; Kawano, Y.; Matsushita, Y.; Yoshioka, K.; Ochiai, M. *Tetrahedron Lett.* **1986**, *27*, 3749. Kaga, H.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1989**, *30*, 113. Kobayashi, S.; Kamiyama, K.; Ohno, M. *J. Org. Chem.* **1990**, *55*, 1169.
14. Tawaki, S.; Klibanov, A. M. *Biocatalysis* **1993**, *8*, 3.
15. Kanerva, L. T.; Kosonen, M.; Vänttinen, E.; Huuhtanen, T. T.; Dahlqvist, M. *Acta Chem. Scand.* **1992**, *46*, 1101.
16. Kanerva, L. T.; Sundholm, O. *Acta Chem. Scand.* **1993**, *47*, 823.
17. Kanerva, L. T. In *Enzymatic Reactions in Organic Media*, Koskinen, A. M. P. and Klibanov, A. M., Eds. Chapman & Hall, London, **1996**, Ch. 7, p. 170.
18. $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$ or $E = \ln[(1-c)(1+ee_P)]/\ln[(1-c)(1-ee_P)]$; Chen, C.-S.; Sih, C. J. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 695.
19. Kanerva, L. T.; Sundholm, O. *J. Chem. Soc., Perkin Trans 1* **1993**, 2407.
20. Wescott, C. R.; Klibanov, A. M. *Biochim Biophys. Acta.* **1994**, *1206*, 1.
21. Nakamura, K.; Kinoshita, M.; Ohno, A. *Tetrahedron*, **1994**, *50*, 4681.
22. Herradón, B.; Valverde, S. *Tetrahedron: Asymmetry* **1994**, *5*, 1479.
23. Secundo, F.; Riva, S.; Carrea, G. *Tetrahedron: Asymmetry* **1992**, *3*, 267.
24. Miyazawa, T.; Kurita, S.; Ueji, S.; Yamada, T.; Kuwata, S. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2253.
25. Kanerva, L. T.; Rahiala, K.; Vänttinen, E. *J. Chem. Soc., Perkin Trans 1* **1992**, 1759.
26. Baldwin, J. E. ; Otsuka, M.; Wallace, P. M. *Tetrahedron.* **1986**, *42*, 3097.
27. Armarego, W. L. F.; Kobayashi, T. *J. Chem. Soc. (C)* **1969**, 1635.

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