Chemo-Enzymic Synthesis of Optically Active α,α-Disubstituted α-Amino Acids

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A series of α,α-disubstituted α-amino esters was chemically synthesized and then resolved through enantioselective hydrolysis catalysed by a new enzyme isolated from crude *Humicola langinosa* lipase. This enzyme only accepts free amino esters as substrates with neither lipase activity toward olive oil nor esterase activity toward *o*-nitrophenyl butyrate. It is unique in that it successfully catalyses the resolution of amino esters with two large α-alkyl groups including aliphatic, aromatic and cyclic amino esters. Examples of resolutions where the alkyl groups differ in size by as little as a single carbon atom have been demonstrated. For determination of absolute configuration, some of the optically active α,α-disubstituted amino acids were also prepared through Schöllkopf's asymmetric synthesis and the structures were verified by X-ray crystallography. A model depicting the substrate binding site of the enzyme is proposed.

α,α-Disubstituted α-amino acids have attracted increasing attention in recent years. This group of nonproteinogenic amino acids induces dramatic conformational change when incorporated into peptides,[1] and renders them more resistant to protease hydrolysis. They are found in natural peptide antibiotics,[2] and some are potent inhibitors of amino acid decarboxylases.[3] Chiral α,α-disubstituted α-amino acids are also important building blocks for the synthesis of pharmaceuticals and other biological agents.[4]

A variety of methods exists for the synthesis of optically active α,α-disubstituted amino acids including asymmetric synthesis[5] and classic resolution. Enzymatic resolution of amino acids has many advantages[6] and is being applied on an industrial scale, but, general applications are often limited due to the narrow substrate specificities of the enzymes. For example: whole cell systems containing hydantoinase[7] were reported to produce only α-monosubstituted amino acids; the acylase catalysed resolution of *N*-acyl amino acids[8] has an extremely low rate (often zero) of catalysis toward α-dialkylated amino acids; and the nitrilase system[9] requires the relatively difficult to prepare amino amide as substrate. During the course of our work, Yee *et al.*[10] reported a new esterase which catalysed enantioselective hydrolysis of a series of α-methyl amino esters. Although α-methyl α-amino acids are studied the most for biological activity, α,α-dialkyl amino acids with both side-chains larger than methyl are also of significant interest because of their distinct steric properties and chemical activities. Some of them were found to induce more dramatic structure changes in peptides than do α-methyl amino acids.[11] There has been no previous report on enzymic resolution of this class of amino acids. For the synthesis of a drug candidate currently under development in our laboratories, a method is needed for the large scale preparation of optically active 2-amino-2-ethylhexanoic acid and its analogues. In this report, we describe a practical resolution of α,α-disubstituted α-amino esters through enantioselective hydrolysis catalysed by a minor protein component isolated from crude *Humicola langinosa* lipase. A model depicting the substrate binding site of the new enzyme is proposed for predicting enantioselectivity.

Racemic α,α-disubstituted α-amino esters were synthesized by standard chemistry through alkylation of the Schiff's base of the corresponding natural amino esters[12] or through formation of hydantoins.[13] Initial enzyme screening was aimed at obtaining optically active 2-amino-2-ethylhexanoic acid **8** or the corresponding amino alcohol. Enzymes reported for resolving α-H or α-Me analogues of amino acids† failed to catalyse the corresponding reaction of this substrate, primarily due to the presence of the α-ethyl group which causes a critical increase in steric hindrance at the α-carbon. Out of 50 different enzymes and microorganisms screened in our laboratories, pig liver esterase and *Humicola langinosa* lipase (Lipase CE, Amano) were the only ones found to catalyse the hydrolysis of the substrate.

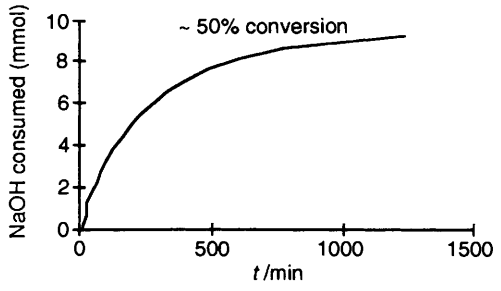


Fig. 1 Profile of the enantioselective hydrolysis of ethyl 2-amino-2-ethylhexanoate catalysed by *Humicola* amino esterase

Both enzymes catalyse the hydrolysis of the amino ester **8** enantioselectively. At about 60% substrate conversion, the enantiomeric excess (ee) of recovered ester **8b** from both reactions exceeds 98%. In addition, the acid product **8a** with 96–98% ee was obtained by carrying the hydrolysis of the ester to 40%. The rates of hydrolysis become significantly slower when conversion approaches 50%, allowing a wide window for kinetic control of the resolution process (Fig. 1). Both enzymes function well in a concentrated water–substrate (oil) two-phase system while maintaining high enantioselectivity, making this system very attractive for industrial processes. The enantiomerically pure 2-amino-2-ethylhexanoic acid is currently being produced on a 100 kg scale in our laboratories using this method.

Although pig liver esterase catalyses the hydrolysis of all amino esters tested in this work, it was only enantioselective toward esters **4** and **8**. This lack of correlation between enantioselectivity and substrate structure has been reported for

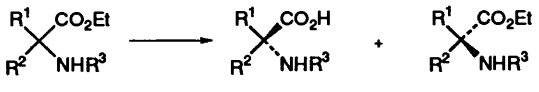
† Enzymes used in initial screening include acylase I and II for the *N*-chloroacetylamino acid, nitrilase for the corresponding amino nitrile, 35 lipases, esterases and proteases, and 12 microorganisms for the amino ester, *O*-acetylamino alcohol, and transesterification of the amino alcohol in organic solvents.

Table 1 Enantioselective hydrolysis of α-amino esters catalysed by *Humicola* amino esterase

Compd.	R^1	R^2	R^3	% Conv.	% ee Acid **a**	% ee Ester **b**	*E* Value[g]
1	$Me(CH_2)_8$	Et	H	50	91 (*R*)[d]	—	58
2	$Me(CH_2)_5$	Et	H	50	90 (*R*)[d]	99 (*S*)	58
3	$Me(CH_2)_5$	Pr	H	37	92 (*R*)[c]	60 (*S*)	41
4	$PhCH_2$	Et	H	50	85 (*R*)[a]	—	35
5	$PhCH_2$	Me	H	74	32 (*R*)[a]	88 (*S*)	4.4
6	$PhCH_2$	H	H	68	—	72 (*S*)[b]	4.0
7	Ph	H	H	62	—	78 (*S*)[b]	6.3
8	Bu	Et	H	46	92 (*R*)[d]	—	58
				58	—	98 (*S*)[e]	
9	Bu	Et	OCOMe		NR		
10	MeCH=CH	Et	H	42	94 (*R*)[d]	66 (*S*)	66
11	Pr	Et	H	65	26 (*R*)[d]	53 (*S*)	2.6
12	Me	Et	H	72	36 (*S*)[a]	100 (*R*)	20
13	H	Pr	H	40	72 (*S*)[b]	42 (*R*)	9.6
14	H	Et	H	55	53 (*S*)[b]	42 (*R*)	6.1
15	H	Et	OCOMe		NR		
16					NR		
17				64	56[f]	99	19

Absolute configurations were assigned by: [a] comparison of optical rotation with literature data; [b] comparison of HPLC retention time with authentic samples; [c] analogy to the results obtained from [d]; [d] comparison of HPLC retention time of its diastereoisomeric derivative with those obtained through Schöllkopf's asymmetric synthesis; [e] X-ray crystallographic analysis. [f] Absolute configuration undetermined. [g] Calculated from equations in reference 15.

many PLE catalysed reactions.[14] The lipase CE was obtained as a crude extract containing about 10% of total protein. The active enzyme catalysing the amino ester hydrolysis was isolated from the mixture and partially purified. This new enzyme which we term *Humicola* amino esterase is present as a minor protein component, has a molecular weight of about 35 000 Da, and shows neither esterase activity toward *o*-nitrophenyl butyrate nor lipase activity to olive oil. It is however highly effective in catalysing the amino ester hydrolysis with very broad substrate specificity and high enantioselectivity (Scheme 1). Various substrates including aliphatic, aromatic

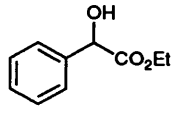


1–15 **1a–15a** **1b–15b**

Scheme 1

and cyclic amino esters were resolved into optically active esters and acids (Table 1) with good *E* values.[15] Aliphatic amino esters with alkyl or alkenyl side chains as long as ten carbon atoms were well accepted by the enzyme. Substrates with longer chain length were limited by the solubility of the compounds rather than their binding with the enzyme. Resolutions were also extended to α,α-disubstituted amino esters in which the two alkyl groups differ in length by as little as a single carbon atom. The fact that the enzyme successfully catalyses the resolution of straight chain aliphatic amino esters with two α-alkyl groups both larger than a methyl group is unique. These amino esters and their acids have been difficult to resolve by chemical and biochemical means due to the increased flexibility of the two large alkyl groups which become indistinguishable to most resolving agents. Unsubstituted amino esters **6**, **7**, **13** and **14** underwent significant chemical hydrolysis under the experimental conditions, resulting in relatively lower *E* values.

It was found that when some amino esters are protected by *N*-acetylation, they become resistant to hydrolysis by the enzyme. *N*-Acetyl amino esters **9** and **15** are not substrates of the amino esterase, but the corresponding unacetylated amino esters **8** and **14** are. Replacement of the α-amino group of the amino ester with a hydroxy group also changes it from substrate to nonsubstrate. The enzyme showed high catalytic activity toward hydrolysis of phenylglycine ethyl ester **7**, but was found incapable of catalysing the hydrolysis of mandelic ethyl ester **16**



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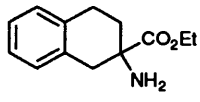

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in which the amino group had been replaced with an α-hydroxy group. No inhibitions were detected when compounds **9**, **15** and mandelic ester **16** were tested as inhibitors, indicating that the free amino group is necessary for binding between the enzyme and substrates. It is most likely that the substrate is protonated at the amino group. The ammonium cation then binds to an anion on the enzyme's active site forming a strong ionic bonding to facilitate the catalysis. This mechanism was further supported by the strong pH dependence of the enzymatic activity toward amino ester hydrolysis. Stability and relative activity of the amino esterase were measured over a range of pHs with ethyl 2-amino-2-ethylhexanoate **8** as substrate and the data were compared as shown in Fig. 2. The enzyme has an optimum pH of 7.5 and is most stable at pH 8. At low pHs, both the stability and activity of the enzyme suffered a gradual loss and this is consistent with the effect of gradual protein denaturation. At high pHs however, the enzyme remained relatively stable but its activity decreased sharply. The most significant drop of activity occurs when the medium's pH is greater than 9.6 which coincides with the substrate's pK_a. Clearly when the substrate exists mostly in the form of a free base, its affinity with the enzyme dramatically diminishes at the active site where a charged ammonium cation is required for binding.

The enantiomeric excesses of both the amino acids and their esters were measured through derivatization with Marfey's reagent[16] followed by HPLC separation. Absolute configurations of the known amino acids were determined either by comparison of their optical rotations with published data or through diastereoisomeric derivatization followed by comparison of HPLC retention time with authentic samples. Optically active *R*-amino acids **1a**, **2a**, **8a**, **10a** and **11a** were prepared by Schöllkopf's asymmetric synthesis[17] starting from (*S*)-(+)-2-aminobutyric acid (Scheme 2). Formation of

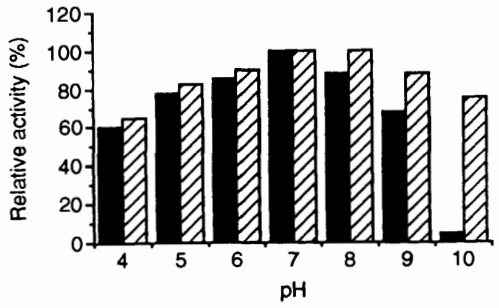
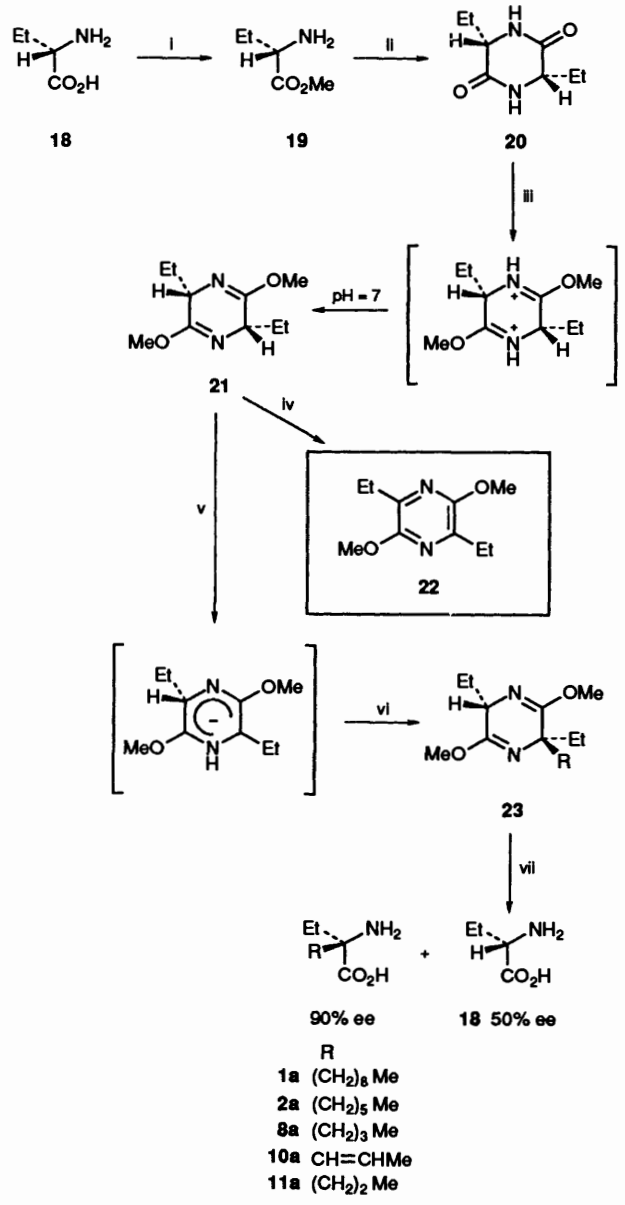


Fig. 2 Activity (■) and stability (⟋) of *Humicola* amino esterase at different pHs. Activity was determined as % conversion of the amino ester to the amino acid in 0.1 mol dm^{-3} phosphate buffer at 25 °C for 4 h. Stability was measured by incubating the enzyme at the given pH for 24 h and then assaying for activity at pH 7.5.



Scheme 2 *Reagents and conditions:* i, $SOCl_2$, MeOH; ii, 110 °C; iii, $[Me_3O]BF_4$, CH_2Cl_2; iv, O_2; v, BuLi–THF; vi, RBr; vii, HCl (6 mol dm^{-3}), 100 °C

piperazinedione **20** through dimerization of methyl (*S*)-(+)-2-aminobutyrate **19** followed by enolization and methylation gave (3*S*,6*S*)-3,6-diethyl-2,5-dimethoxy-3,6-dihydropyrazine **21**. This dihydropyrazine intermediate is unstable in air, and can be oxidized by O_2 to pyrazine **22** which has been isolated as a major impurity. Operating carefully under N_2, compound **21** was used as a template for diastereoselective alkylation with a series of alkyl bromides to produce derivatives **23** which were then hydrolysed by strong acid to afford the dialkylated (*R*)-amino acids of *ca.* 90% ee, together with partially racemized 2-aminobutyric acid. These amino acid products were compared by HPLC with those obtained from enzymatic hydrolysis and were found to have same chirality. The assignments of absolute configuration were further confirmed by X-ray crystallographic analysis (Fig. 3) of the (*S*)-2-(*N*-chloroacetylamino)-2-ethylhexanoic acid ammonium salt derived from the recovered amino ester from the enzymatic hydrolysis.

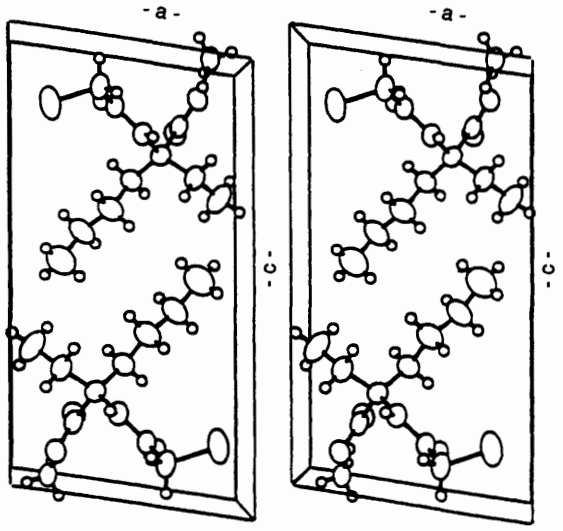


Fig. 3 Stereoscopic view of the crystal packing of (*S*)-2-*N*-chloroacetylamino)-2-ethylhexanoic acid ammonium salt derived from **8b**

The varying enantioselectivity of *Humicola* amino esterase toward different amino esters should be noted. Most of the amino esters in this work were resolved into (*R*)-amino acids and (*S*)-amino esters, with the opposite enantioselectivity to most of the enzymatic resolutions reported in the literature. Amino esters **12**–**14** were however resolved into (*S*)-amino acids and (*R*)-amino esters by the same enzyme. The quest for explanation of this inversion of enantioselectivity prompted us to draw a working model of the enzyme's active site (Fig. 4). It is assumed that the substrate arranges itself on the enzyme's active site with the ester group aligned for hydrolysis, the charged amino group binding with an anion on the peptide chain, and the dialkyl groups occupying, respectively, the two hydrophobic pockets S_1 and S_2. The large pocket S_1 can also be an open pocket which accommodates all the long chain amino esters tested in this work. The small pocket S_2 is approximately 80–100 Å in diameter with a hydrophobic environment best suited for an ethyl group. Most of the dialkylated amino esters bind to the enzyme with the longer chains in S_1 and smaller chains in S_2 [Fig. 4(*a*)], which gives the *R* selectivity. However, for amino esters **12**–**14**, the larger group (Et or Bu) of the two α-substituents fits well into the smaller pocket S_2 leaving the smaller methyl or hydrogen in the larger pocket S_1. This arrangement provides maximum contacts for hydrophobic binding between the substrates and the enzyme resulting in the reversed enantioselectivity [Fig. 4(*b*)].

The optically active amino acids and esters obtained in this work have been converted into chiral amino alcohols, aziridines, hydantoins, and oxazolidines to be used as intermediates for synthesis of drug candidates and their analogues.

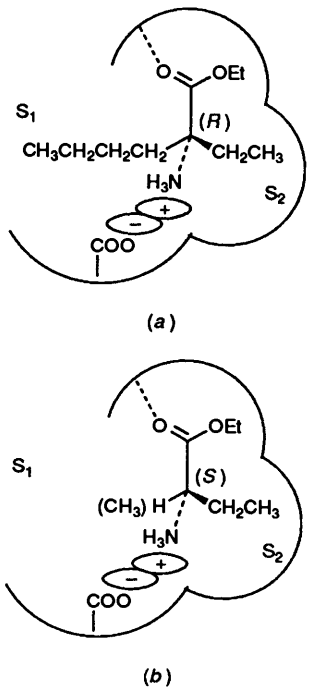


Fig. 4 Simple working models for the active site of *Humicola* amino esterase

Experimental

M.p.s were determined using a Thomas Hoover apparatus and are uncorrected. B.p.s were determined in a Normag Micro-micro short-path distillation apparatus. Gas chromatography was obtained on an HP-1 (methyl silicon gum) capillary column (10 m × 0.53 mm × 2.65 μm) using a 5890A Hewlett Packard (HP) gas chromatograph with carrier gas (helium) flow rate of 15 cm^3 min^{-1}, initial temperature of 80 °C for 1.0 min, final temperature of 150 °C for 10 min, and heating rate of 10 °C min^{-1}. NMR Spectra were obtained on a Varian VXR 300 Sun spectrometer with $CDCl_3$ as solvent unless otherwise noted. *J*-Values are given in Hz. Mass spectra were recorded on a VG70SQ spectrometer. HPLC was performed on a Hewlett Packard Model 1050 pump with variable wavelength detector and an autosampler. A Zorbax C_{18} column, 80 × 4.6 mm was used for all separations with an elution profile of methanol: buffer (20 mmol dm^{-3} aqueous KH_2PO_4) 2:8 to 8:2 at 1.5% of methanol min^{-1} flowing at 1 cm^3 min^{-1}. A wavelength of 340 nm was used to monitor the column eluent. $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}. Pig liver esterase was purchased from Sigma. Lipase CE was obtained from Amano. α-Methylphenylalanine, phenylalanine, phenylglycine, norleucine and 2-aminobutyric acid were purchased from Aldrich, and their corresponding ethyl esters **5**, **6**, **7**, **13** and **14** were prepared by standard chemistry. Other chemicals were purchased from Aldrich. Ether refers to diethyl ether.

General Synthesis of Racemic α,α-Disubstituted Amino Esters.—A mixture of 2-aminobutyric acid (50 g, 0.46 mol) in absolute ethanol was stirred with $SOCl_2$ (70 g, 60 mmol) at 0 °C for 1 h and then refluxed for 4 h. The mixture was cooled to room temperature and concentrated to obtain 75 g (0.45 mol, 97%) of ethyl 2-aminobutyrate hydrochloride as a crystalline solid, m.p. 103–108 °C (Found: C, 43.0; H, 8.35; N, 8.3; Cl, 21.2. $C_6H_{14}O_2NCl$ requires C, 43.0; H, 8.4; N, 8.4; Cl, 21.15%); δ_H 1.10 (3 H, t, *J* 7.4), 1.30 (3 H, t, *J* 7.0), 2.09 (2 H, br s), 4.09 (1 H, t, *J* 6.0), 4.25 (2 H, q, *J* 7.0) and 8.79 (2 H, br s). To the solution of ethyl 2-aminobutyrate hydrochloride (60 g, 0.36 mol) in toluene (600 cm^3) were added triethylamine (72 g, 0.72 mol) and benzaldehyde (38 g, 0.36 mol). The mixture was refluxed until 6.4 cm^3 (0.36 mol) of water had been removed, and then cooled and filtered. The filtrate was concentrated and distilled to obtain ethyl 2-(benzylideneamino)butyrate as a clear oil (78 g, 0.35 mol, 98%), b.p. 120–122 °C/12 mmHg (Found: C, 71.2; H, 7.8; N, 6.4. $C_{13}H_{17}O_2N$ requires C, 71.2; H, 7.8; N, 6.4%); δ_H 0.93 (3 H, t, *J* 7.5), 1.28 (3 H, t, *J* 7.3), 2.04 (2 H, m), 3.88 (1 H, t, *J* 5.3), 4.22 (3 H, t, *J* 7.3), 7.43 (3 H, br s), 7.78 (2 H, br s) and 8.28 (1 H, s). To a solution of lithium diisopropylamine (40 mmol) in THF (20 cm^3) cooled to −50 °C was added ethyl 2-(benzylideneamino)butyrate (8.3 g, 38 mmol) followed by iodobutane (8.4 g, 34 mmol). The mixture was stirred at room temperature for 10 h, treated with saturated NH_4Cl, and then extracted with ether. The organic extracts were concentrated to obtain ethyl 2-(benzylideneamino)-2-ethylhexanoate as a pale yellow oil (9 g, 32 mmol, 85%), b.p. 122–125 °C/0.1 mmHg (Found: C, 74.2; H, 9.2; N, 5.1. $C_{17}H_{25}O_2N$ requires C, 74.1; H, 9.15; N, 5.1%); δ_H 0.89 (3 H, t, *J* 7.0), 0.92 (3 H, t, *J* 7.2), 1.28 (3 H, t, *J* 7.0), 4.22 (2 H, q, *J* 7.0), 7.41 (3 H, m), 7.77 (2 H, m) and 8.32 (1 H, s).

Ethyl 2-Amino-2-ethylhexanoate **8**.—A mixture of ethyl 2-(benzylideneamino)-2-ethylhexanoate (8.0 g, 29 mmol) in HCl (1 mol dm^{-3}; 40 cm^3) was stirred at room temperature for 1 h and then extracted with ether, neutralized with NaOH (1 mol dm^{-3}), and extracted with ether again. The latter ether extract was concentrated and distilled to obtain a clear oil (5.0 g, 27 mmol, 94%), b.p. 95 °C/15 mmHg (Found: C, 64.25; H, 11.3; N, 7.5. $C_{10}H_{21}O_2N$ requires C, 64.1; H, 11.3; N, 7.5%); δ_H 0.78 (3 H, t, *J* 7.6), 0.81 (3 H, t, *J* 7.3), 1.19 (3 H, t, *J* 7.0), 1.75 (4 H, m), 1.45 (2 H, m) and 4.10 (2 H, q, *J* 6.0); δ_C 177.0, 61.2, 60.6, 39.3, 32.7, 25.9, 22.8, 14.2, 13.8 and 8.1.

The following compounds were obtained by the above procedure. Since they are oils and were obtained in small quantities, only spectral data are given.

Ethyl 2-amino-2-ethylundecanoate **1**. δ_H 0.76 (3 H, t, *J* 7.2), 1.16 (6 H, t, *J* 7.2), 4.06 (2 H, q, *J* 7.2), 1.45 (2 H, m) and 1.61 (2 H, m); δ_C 177.1, 61.3, 60.7, 39.7, 32.8, 31.8, 29.8, 29.5, 29.3, 29.2, 23.8, 22.6, 14.2, 14.0 and 8.1 [Found: $(M + H)^+$, 258.2411. $C_{15}H_{31}NO_2$ + H requires *M*, 258.2433].

Ethyl 2-amino-2-ethyloctanoate **2**. δ_H 0.77 (3 H, t, *J* 7.2), 1.64 (2 H, m), 1.43 (2 H, m), 1.19 (6 H, t, *J* 7.3) and 4.09 (2 H, q, *J* 7.3); δ_C 177.0, 61.2, 60.6, 39.7, 32.7, 31.5, 29.4, 23.7, 22.4, 14.2, 13.9 and 8.0 [Found: $(M + H)^+$, 216.1691. $C_{12}H_{25}NO_2$ + H requires *M*, 216.1963].

Ethyl 2-amino-2-propyloctanoate **3**. δ_H 0.86 (3 H, t, *J* 7.0), 1.19 (3 H, t, *J* 6.0), 1.23 (3 H, t, *J* 7.0) and 3.65 (2 H, q, *J* 6.0); δ_C 176.1, 59.6, 56.8, 41.1, 38.8, 30.4, 28.2, 22.5, 21.3, 17.1, 15.9, 13.1 and 12.8; *m/z* 230 [$(M + H)^+$, 52%].

Ethyl 2-amino-2-ethyl-3-phenylpropionate **4**. δ_H 0.88 (3 H, t, *J* 7.5), 1.26 (3 H, t, *J* 7.2), 1.62 (1 H, m), 1.94 (1 H, m), 2.74 (1 H, d, *J* 13.2), 3.17 (1 H, d, *J* 13.2), 4.15 (2 H, q, *J* 7.2), 7.14 (2 H, d, *J* 6.0) and 7.24 (3 H, m); δ_C 176.4, 136.4, 129.8, 128.2, 126.8, 62.3, 60.8, 45.7, 33.2, 14.2 and 8.3 [Found: $(M + H)^+$, 222.1490. $C_{13}H_{19}NO_2$ + H requires *M*, 222.1494].

Ethyl 2-amino-2-ethylpent-3-enoate **10**. δ_H 0.82 (3 H, t, *J* 7.4), 1.23 (3 H, t, *J* 7.1), 1.53 (1 H, dq, *J* 14.8, 7.3), 1.75 (1 H, dq, *J* 14.8, 7.3), 2.19 (1 H, dd, *J* 8.6, 13.7), 2.51 (1 H, dd, *J* 6.4, 13.4), 4.13 (2 H, q, *J* 7.0), 5.06 (1 H, s), 5.11 (1 H, d, *J* 5.6) and 5.64 (1 H, m); δ_C 176.5, 132.7, 119.2, 60.8, 51.9, 43.8, 32.6, 14.2 and 8.1; *m/z* 172 [$(M + H)^+$, 76%].

Ethyl 2-amino-2-ethylpentanoate **11**. δ_H 0.76 (3 H, t, *J* 7.4), 0.79 (3 H, t, *J* 7.2), 1.56 (3 H, t, *J* 6.4), 4.15 (2 H, q, *J* 7.3) and 1.65–1.13 (6 H, m); δ_C 177.0, 61.1, 60.6, 41.9, 32.7, 17.1, 14.2, 14.1 and 8.0; *m/z* 174 [$(M + H)^+$, 100%].

Ethyl 2-amino-2-methylbutyrate **12**. δ_H 0.82 (3 H, t, *J* 7.5), 1.22 (3 H, t, *J* 7.2), 1.26 (3 H, s), 1.55 (1 H, m), 1.68 (1 H, m) and 4.12 (2 H, q, *J* 7.2); δ_C 177.5, 60.7, 57.9, 33.7, 25.8, 14.1 and 8.3; *m/z* 146 [$(M + H)^+$, 100%].

Ethyl 2-*Amino*-1,2,3,4-*tetrahydronaphthalene*-2-*carboxylate* **17**.—A mixture of 7,8-benzo-1,3-diazaspiro[4.5]decane-2,4-dione (10.8 g, 50 mmol, Aldrich) and $Ba(OH)_2 \cdot 8H_2O$ (78.9 g, 250 mmol) in H_2O (100 cm^3) was heated in a 500 cm^3 steel autoclave at 160 °C for 24 h. The mixture was cooled to room temp., acidified with H_2SO_4 (8 mol dm^{-3}) and then filtered. The aqueous solution was evaporated to *ca.* 20 cm^3 and neutralized with concentrated NH_3 solution. The precipitate was collected through filtration, washed with H_2O and dried to obtain a light yellow powder (5.4 g, 28 mmol, 56%) which was esterified with EtOH–$SOCl_2$ to afford a yellow oil (4.2 g, 20 mmol, 71%); δ_H 1.27 (3 H, t, *J* 7.2), 1.92 (1 H, m), 2.17 (1 H, m), 2.75 (1 H, d, *J* 16.9), 3.30 (1 H, d, *J* 16.4), 2.85 (1 H, m), 2.96 (1 H, m), 4.19 (2 H, q, *J* 7.2) and 7.11 (4 H, m); δ_C 176.5, 134.6, 133.5, 129.3, 128.6, 126.0, 125.9, 61.1, 56.3, 39.2, 31.8, 25.3 and 14.1; *m/z* 220 [$(M + H)^+$, 87%].

General Procedure for the Determination of the Enantiomeric Excess of the Amino Acids and Esters.—Approximately 5 mg of amino ester or amino acid was mixed in an autosampler vial with aqueous $NaHCO_3$ (1 mol dm^{-3}; 0.1 cm^3) and an acetone solution (0.1 cm^3) containing 5 mg cm^{-3} of Marfey's reagent (Pierce Chemical Co.) The vial was sealed, heated for 2 h at 50 °C, and then cooled to room temperature. HCl (1 mol dm^{-3}; 0.1 cm^3) was added, and a 5 μl (1 μl ≡ 1 mm^3) portion of the solution was injected into the HPLC.

Partial Purification of Humicola langinosa *Amino Esterase Activity.*—A freeze-dried preparation of Lipase CE was obtained from Amano Enzyme USA Co., Ltd (Troy, VA). The dry powder (200 g) was suspended in Tris-Cl (50 mmol dm^{-3}; 1.1 dm^3), pH 8.0, stirred overnight at 4 °C, and then centrifuged at 50 000 *g* for 1 h. The supernatant containing 22 g of protein and 95% of the enzyme activity was concentrated to 450 cm^3 using a 20 000 molecular mass cut-off filter (Sartorius Easy Flow, 0.18 m^2) and diafiltered against Tris-Cl (20 mmol dm^{-3}; 2 dm^3), pH 8.0. The protein solution was loaded onto a 500 cm^3 radial flow column (Sepragen Corp.), containing Q-Sepharose, and washed with Tris-TFA (20 mmol dm^{-3}), pH 8.0 until the base-line at 280 nm was zero. The enzyme activity was eluted with NaCl (0.3 mol dm^{-3}). The material was concentrated to 15 cm^3 using the above molecular mass cut-off filter, followed by further concentration with Amicon Centriprep 10 filter units. The concentrated protein sample was loaded onto a Superdex 75 sieving column and washed with Tris-TFA (20 mmol dm^{-3}), pH 8.0, containing NaCl (150 mmol dm^{-3}). Analysis of the resulting fractions by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis indicated that the enzyme activity coincided with fractions containing a visible stained protein band at 35 kDa. Fractions containing the amino esterase activity were pooled and stored at 4 °C for use in the following reactions.

General Procedure for Enzymatic Hydrolysis of Amino Esters.—For a typical enzymatic hydrolysis, amino ester (200 mg) was stirred at room temperature in phosphate buffer (50 mmol dm^{-3}; 20 cm^3) with pig liver esterase (20 units) at pH 8.0 or partially purified *Humicola* amino esterase solution (2.4 mg protein cm^{-3}; 2 cm^3) at pH 7.5. The progress of the reaction was followed by monitoring the disappearance of the amino ester on an HP-1 methyl silicon gum GC column with 1,4-dichlorobenzene as an internal standard. At an appropriate conversion, the enantiomerically enriched amino ester was recovered by extraction with ethyl ether and the acid product was obtained by concentrating the aqueous phase and recrystallizing the residue from methanol.

The following compounds were obtained by the above procedure.

(R)-2-*Amino*-2-*ethylundecanoic acid* **1a**. Polymorphous; m.p. 275–277 °C (Found: C, 66.6; H, 11.7; N, 6.0. $C_{13}H_{27}NO_2 \cdot 0.34H_2O$ requires C, 66.5; H, 11.9; N, 6.1%); $\delta_H(D_2O)$ 0.45 (3 H, t, *J* 6.8), 0.59 (3 H, t, *J* 7.1), 0.86 (14 H, m) and 1.56 (4 H, m); δ_C 173.9, 64.9, 36.0, 32.7, 30.4, 30.3, 30.1, 29.9, 29.3, 23.8, 23.4, 14.6 and 8.2.

(R)-2-*Amino*-2-*ethyloctanoic acid* **2a**. Polymorphous; m.p. 295–300 °C (decomp.) (Found: C, 63.8; H, 11.1; N, 7.4. $C_{10}H_{21}NO_2$ requires C, 64.1; H, 11.3; N, 7.5%); $\delta_H(D_2O)$ 0.71 (3 H, t, *J* 7.0), 0.83 (3 H, t, *J* 7.5), 1.15 (8 H, m) and 1.79 (4 H, m); δ_C 174.8, 65.5, 36.3, 31.7, 29.9, 29.4, 23.7, 23.0, 14.5 and 8.3.

(R)-2-*Amino*-2-*propyloctanoic acid* **3a**. Polymorphous; m.p. 279–283 °C (Found: C, 65.6; H, 11.5; N, 7.0. $C_{11}H_{23}NO_2$ requires C, 65.6; H, 11.5; N, 7.0%); $\delta_H(D_2O)$ 0.66 (3 H, t, *J* 7.0), 0.75 (3 H, t, *J* 7.3), 1.10 (10 H, m) and 1.74 (4 H, m); δ_C 174.9, 64.9, 38.7, 36.5, 31.6, 29.2, 23.6, 22.8, 17.4, 14.4 and 14.3.

(R)-2-*Amino*-2-*ethyl*-3-*phenylpropionic acid* **4a**. Plates; m.p. 254–256 °C (Found: C, 65.7; H, 7.7; N, 7.0. $C_{11}H_{15}NO_2 \cdot 0.4H_2O$ requires C, 65.9; H, 7.9; N, 7.0%); $[\alpha]_D^{25}$ −24 (*c* 1 in H_2O) [lit.,[18] −22.8 (*c* 2 in H_2O)]; $\delta_H(D_2O)$ 0.74 (3 H, t, *J* 7.5), 1.58 (1 H, m), 1.81 (1 H, m), 2.72 (1 H, d, *J* 14.3), 3.08 (1 H, d, *J* 14.3), 7.06 (2 H, m) and 7.18 (3 H, m); δ_C 177.0, 135.4, 131.0, 130.0, 128.9, 67.2, 43.0, 30.6 and 8.5.

(R)-2-*Amino*-2-*methyl*-3-*phenylpropionic acid* **5a**. Plates; m.p. 291–294 °C (decomp.) (Found: C, 67.2; H, 7.5; N, 7.6. $C_{10}H_{13}NO_2$ requires C, 67.0; H, 7.3; N, 7.8%); $[\alpha]_D^{25}$ +5 (*c* 0.5 in H_2O) [lit.,[19] +19.3 (*c* 1.0 in H_2O)]; $\delta_H(D_2O)$ 1.28 (3 H, s), 2.70 (1 H, d, *J* 14), 3.04 (1 H, d, *J* 14), 7.00 (2 H, m) and 7.12 (3 H, m).

(R)-2-*Amino*-2-*ethylhexanoic acid* **8a**. Needles; m.p. > 300 °C (decomp.) (Found: C, 59.1; H, 10.5; N, 8.6. $C_8H_{17}NO_2 \cdot 0.2H_2O$ requires C, 59.0; H, 10.8; N, 8.6%); $\delta_H(D_2O)$ 0.68 (3 H, t, *J* 6.9), 0.71 (3 H, t, *J* 7.7), 0.99 (2 H, m), 1.13 (4 H, m) and 1.52 (4 H, m); δ_C 177.7, 66.8, 36.8, 30.5, 26.3, 23.1, 14.1 and 8.4.

(R)-2-*Amino*-2-*ethylpent*-3-*enoic acid* **10a**. Needles; m.p. 264–265 °C (Found: C, 57.3; H, 8.9; N, 9.5. $C_7H_{13}NO_2 \cdot 0.15H_2O$ requires C, 57.5; H, 9.2; N, 9.6%); $\delta_H(D_2O)$ 0.74 (3 H, t, *J* 7.5), 1.60 (1 H, m), 1.72 (1 H, m), 2.27 (1 H, dd, *J* 8.6, 14.5), 2.47 (1 H, dd, *J* 6.4, 14.5), 5.08 (2 H, d, *J* 12.4) and 5.55 (1 H, m); δ_C 176.7, 131.6, 122.3, 66.0, 62.9, 41.4, 30.0 and 8.4.

(R)-2-*Amino*-2-*ethylpentanoic acid* **11a**. Needles; m.p. > 310 °C (decomp.) (Found: C, 56.2; H, 10.2; N, 9.55. $C_7H_{15}NO_2 \cdot 0.2H_2O$ requires C, 56.5; H, 10.4; N, 9.4%); $\delta_H(D_2O)$ 0.75 (6 H, t, *J* 7.2), 1.06 (1 H, m), 1.21 (1 H, m) and 1.61 (4 H, m); δ_C 177.0, 66.8, 39.0, 30.3, 17.7, 14.4 and 8.3.

(S)-2-*Amino*-2-*methylbutyric acid* **12a**. Needles; m.p. > 320 °C (decomp.) (Found: C, 49.8; H, 9.3; N, 11.6. $C_5H_{11}NO_2 \cdot 0.2H_2O$ requires C, 49.7; H, 9.5; N, 11.6%); $[\alpha]_D^{25}$ +4.5 (*c* 2 in H_2O) [lit.,[20] +11.1 (*c* 5 in H_2O)]; $\delta_H(D_2O)$ 0.72 (3 H, t, *J* 7.2), 1.27 (3 H, s), 1.57 (1 H, m) and 1.70 (1 H, m); δ_C 178.0, 63.1, 31.4, 23.2 and 8.6.

2-*Amino*-1,2,3,4-*tetrahydronaphthalene*-2-*carboxylic acid* **17a**. Plates; m.p. 301–304 °C (Found: C, 61.4; H, 7.15; N, 6.5. $C_{11}H_{13}NO_2 \cdot 1.3H_2O$ requires C, 61.45; H, 7.3; N, 6.5%); $\delta_H(D_2O)$ 1.67 (1 H, m), 1.81 (1 H, m), 2.42 (2 H, m), 2.54, 2.89 (2 H, AB, *J* 11) and 6.68 (4 H, br s); δ_C 174.2, 134.6, 131.3, 129.8, 129.5, 127.9, 127.4, 59.3, 35.4, 29.4 and 24.7.

Large Scale Preparation of (R)-2-*Amino*-2-*ethylhexanoic Acid* **8a**.—In a typical reaction, ethyl 2-amino-2-ethylhexanoate **8** (100 g) distilled water (400 cm^3) were stirred in a 1 dm^3 Morton flask at room temperature and then the suspension was adjusted to pH 8 with HCl (2 mol dm^{-3}). The reaction was started with the addition of pig liver esterase (2300 units) or the supernatant (80 cm^3) (with 20 000 molecular mass cut-off filtration) from crude lipase CE (20 g). The mixture was maintained at pH 8 by the addition of aqueous NaOH (1 mol dm^{-3}) by the use of the pH meter. The progress of the reaction was monitored by

derivatizing the unchanged amino ester with Marfey's reagent and measuring the diastereoisomeric ratio of the product on HPLC. At about 40% conversion, the mixture was adjusted to pH 9.6 and extracted with hexane (3 × 300 cm^3). The aqueous phase was concentrated to about 60 cm^3 and the precipitate was collected by filtration to obtain the product (*ca.* 35 g, 40–45%) with typical enantiomeric purity of 95–98% ee.

Typical Procedure for the Asymmetric Synthesis of α,α-Disubstituted Amino Acids **1a**, **2a**, **8a**, **10a** *and* **11a**.—(3S,6S)-3,6-*Diethylpiperazine*-2,5-*dione* **20**. Freshly distilled methyl (*S*)-(+)-2-aminobutyrate **19** (5 g, 42 mmol) was heated at 100–110 °C under N_2 for 20 h. The solid was washed with ether to obtain the title compound **20** as white crystalline flakes, m.p. 258–260 °C (Found: C, 56.3; H, 8.3; N, 16.4. $C_8H_{14}N_2O_2$ requires C, 56.45; H, 8.3; N, 16.5%); δ_H([$^2H_6$]DMSO) 0.82 (6 H, t, *J* 7.5), 1.67 (4 H, m), 3.78 (2 H, t, *J* 5.0) and 8.09 (2 H, br s); δ_C 168.7, 56.0, 26.9 and 10.0.

(3S,6S)-3,6-*Diethyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **21**.—A suspension of (3*S*,6*S*)-3,6-Diethylpiperazine-2,5-dione **20** (0.7 g, 4.1 mmol) and trimethyloxonium tetrafluoroborate (2.5 g, 16.9 mmol) in CH_2Cl_2 (100 cm^3) was vigorously stirred at room temperature under N_2 for 72 h. The mixture was treated with phosphate buffer (1 mol dm^{-3}; 40 cm^3, pH 7), and extracted with CH_2Cl_2. The organic extract was concentrated and then purified by flash chromatography on SiO_2 eluted with CH_2Cl_2 to obtain the title compound **21** as a clear oil (0.6 g, 3.0 mmol, 74%); b.p. 82 °C/15 mmHg; δ_H 0.90 (6 H, t, *J* 7.5), 1.63 (2 H, m), 1.82 (2 H, m), 3.66 (6 H, s) and 3.97 (2 H, t, *J* 4.5); δ_C 163.9, 56.9, 52.2, 28.1 and 10.0 (Found: M^+, 198.1299. $C_{10}H_{18}O_2N_2$ requires *M*, 198.1368).

(3S,6S)-3-*Butyl*-3,6-*diethyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **23** (R = Bu).—To a solution of (3*S*,6*S*)-3,6-diethyl-2,5-dimethoxy-3,6-dihydropyrazine **21** (0.45 g, 2.3 mmol) in THF (8 cm^3) cooled to −70 °C under N_2, was added butyllithium in hexane (1.6 mol dm^{-3}; 1.8 cm^3, 3.0 mmol) and iodobutane (0.42 g, 2.3 mmol) in THF (5 cm^3). The mixture was stirred at −70 °C for 8 h, then at room temperature overnight, treated with water (20 cm^3), and then extracted with ether. The ether extract was concentrated and then purified by flash chromatography on SiO_2 eluted with $CHCl_3$ to obtain the title compound **23** as a pale yellow oil (0.4 g, 1.5 mmol, 67%); b.p. 60–64 °C/0.5 mmHg (Found: C, 66.3; H, 10.05; N, 10.8. $C_{14}H_{26}O_2N_2$ requires C, 66.11; H, 10.3; N, 11.01%); δ_H 0.66 (3 H, t, *J* 7.5), 0.83 (3 H, t, *J* 7.5), 0.87 (3 H, t, *J* 7.3), 3.64 (3 H, s), 3.66 (3 H, s) and 3.96 (1 H, t, *J* 4.3); δ_C 164.2, 162.6, 62.8, 56.7, 52.1, 52.0, 40.5, 32.8, 27.6, 26.3, 22.6, 14.0, 9.6 and 9.0.

(R)-2-*Amino*-2-*ethylhexanoic Acid* **8a**.—A mixture of dihydropyrazine **23** (R = Bu) (0.1 g, 0.4 mmol) in HCl (6 mol dm^{-3}; 5 cm^3) was stirred at 100 °C for 24 h. The solution was neutralized with aqueous NaOH (5 mol dm^{-3}) and then concentrated. The residue was subjected to column chromatography on SiO_2 eluted with $CHCl_3$–MeOH (4:1) to obtain the 2-amino acid **8a** (32 mg, 50%) which was derivatized with Marfey's reagent and separated by HPLC. The compound was found to be the same enantiomer (92% ee) as the corresponding amino acids obtained from enzymatic resolution.

X-Ray Crystallographic Analysis of (S)-(+)-2-(N-*Chloroacetylamino*)-2-*ethylhexanoic Acid Ammonium Salt Derived from Compound* **8b**.—*Crystal data.* $C_{10}H_{21}ClN_2O_3$, M = 252.74. Monoclinic, a = 8.059(2), b = 5.750(4), c = 18.194(5) Å, β = 98.73(2)°, V = 700.6(6) Å^3 (by least-squares refinements on diffractometer angles for 25 carefully centred reflections, λ = 1.541 78 Å), space group $P2_1/\#4$, Z = 2, D_x = 1.198 g cm^{-3}. Colourless needle, crystal dimensions (distance to faces from centre): 0.450 × 0.100 × 0.080 mm, μ(Cu-Kα) = 24.25 cm^{-1}.

Data collection and processing. Rigaku AFC5R diffractometer, ω–2θ mode with ω scan width = 1.37 + 0.30 tan θ, scan speed 8.0 deg min^{-1}, graphite-monochromated Cu-Kα radiation; 2534 reflections measured (64.75 < 2θ < 94.80°), 1165 unique [merging R_{int} = 0.109 (max; min. transmission factors = 0.87–1.10)], giving 1855 with $I > 3.00\sigma(I)$. No decay correction was applied.

Structure analysis and refinement. Direct method (Cl atoms) followed by normal heavy-atom procedures. Full-matrix least-squares refinement with all non-hydrogen atoms anisotropic. The hydrogens were either refined isotropically or included in the structure factor calculation in idealized positions (d_{C-H} = 0.95 Å). The weighting scheme was based on counting statistics and included a factor (p = 0.01) to downweight the intense reflections. Plots of $\Sigma w(|F_o| - |F_c|)^2$ *versus* $|F_o|$ gave satisfactory agreement analyses. Final R and R_w values are 0.053, 0.054. Neutral atom scattering factors were included in F_c; the values for $\Delta f'$ and $\Delta f''$ were those of Cromer. All calculations were performed using the TEXSAN Crystallographic Software package of Molecular Structure Corporation.

Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.*

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* For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc., Perkin Trans. 1*, 1995, Issue 1.

References

1 P. K. C. Paul, M. Sukumar, B. Di Blasio, V. Pavone, C. Pedone and P. Balaram, *J. Am. Chem. Soc.*, 1986, **108**, 6363; R. Bardi, A. M. Piazzesi, C. Toniolo, M. Sukumar and P. Balaram, *Biopolymers*, 1986, **25**, 1635; M. Mutter, *Angew. Chem.*, 1985, **97**, 639; V. Barone, F. Lelj, A. Bavoso, B. Di Blasio, P. Grimaldi, V. Pavone and C. Pedone, *Biopolymers*, 1985, **24**, 1759.

2 M. C. Khosla, K. Stackiwiak, R. R. Smeby, F. M. Bumpus, F. Piriou, K. Lintner and S. Fermandijan, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 757; J. Turk, G. T. Panse and G. R. Marshall, *J. Org. Chem.*, 1975, **40**, 953; T. Deeks, T. P. A. Crooks and R. D. Waigh, *J. Med. Chem.*, 1983, **26**, 762; H. N. Christensen, M. E. Handlogten, J. V. Vadgama, E. de la Cuesta, P. Ballesteros, G. C. Trigo and C. Avendano, *J. Med. Chem.*, 1983, **26**, 1374.

3 D. E. Zembower, J. A. Gilbert and M. M. Ames, *J. Med. Chem.*, 1993, **36**, 305; J. Kollonitsch, A. A. Patchett, S. Marburg, A. L. Maycock, L. M. Perkins, G. A. Doldouras, D. E. Duggan and S. D. Aster, *Nature*, 1978, **274**, 906; M. K. Bhattacharjee and E. E. Snell, *J. Biol. Chem.*, 1990, **265**, 6664; D. Schirlin, F. Gerhart, J. M. Hornsperger, M. Hamon, J. Wagner and M. J. Jung, *J. Med. Chem.*, 1988, **31**, 30.

4 G. M. Coppola and H. F. Schuster, *Asymmetric Synthesis: Construction of Chiral Molecules Using Amino Acids*, Wiley, New York, 1987; D. A. Evans, in *Asymmetric Synthesis*, ed. J. D. Morrison, Academic, Orlando, FL, 1984, vol. 3, pp. 2–10; S. Mzengeza, C. M. Yang and R. A. Whitney, *J. Am. Chem. Soc.*, 1987, **109**, 276; C. G. Knudson, A. D. Palkowitz and H. Rapoport, *J. Org. Chem.*, 1985, **50**, 325; W. D. Lubell and H. Rapoport, *J. Am. Chem. Soc.*, 1987, **109**, 236.

5 R. M. Williams and M.-N. Im, *J. Am. Chem. Soc.*, 1991, **113**, 9276; U. Schöllkopf, *Pure Appl. Chem.*, 1983, **55**, 1799; U. Schöllkopf, U. Busse, R. Lonsky and R. Hinrichs, *Liebigs Ann. Chem.*, 1986, 2150; D. Seebach, M. Boes, R. Naef and W. B. Schweizer, *J. Am. Chem. Soc.*, 1983, **105**, 5390; D. Seebach, J. D. Aebi, R. Naef and Th. Weber, *Helv. Chim. Acta*, 1985, **68**, 144; M. J. O'Donnell, W. D. Bennett, W. A. Bruder, W. N. Jacobson, K. Knuth, B. LeClef, R. L. Polt,

F. G. Bordwell, S. R. Mrozack and T. A. Cripe, *J. Am. Chem. Soc.*, 1988, **110**, 8520; M. Kolb and J. Barth, *Angew Chem.*, 1980, **92**, 753; G. I. Goerg, X. Guan and J. Kant, *Tetrahedron Lett.*, 1988, **29**, 403; D. Obrecht, C. Spiegler, P. Schönholzer, K. Müller, H. Heimgartner and F. Stierli, *Helv. Chim. Acta*, 1992, **75**, 1666.

6 R. Bosch, H. Brückner, G. Jung and W. Winter, *Tetrahedron*, 1982, **38**, 3579; G, M. Anantharamaiah and R. W. Roeske, *Tetrahedron Lett.*, 1982, **23**, 3335; A. Berger, M. Smolarsky, N. Kurn and H. R. Bosshard, *J. Org. Chem.*, 1973, **38**, 457; J. Kamphuis, W. H. J. Boesten, Q. B. Broxterman, H. F. M. Hermes, E. M. Meijer and H. E. Schoemaker, in *Advances in Biochemical Engineering Biotechnology*, ed. A. Flechter, Springer-Verlag, Berlin Heidelberg, 1990, vol. 42.

7 C. Gross, C. Syldatk, V. Mackowiak and F. J. Wagner, *Biotechnology*, 1990, **14**, 363; P. Chevalier, D. Roy and A. Morin, *Appl. Microbiol. Biotechnol.*, 1989, **30**, 482; T. P. West, *Arch Microbiol.*, 1991, **156**, 513; S. Shimizu, H. Shimada, S. Takahashi, T. Ohashi, Y. Tani and H. Yamada, *Agric. Biol. Chem.*, 1980, **44**, 2233; C. Syldatk and F. Wagner, *Food Biotechnol.*, 1990, **4**, 87; S. Runser, N. Chinski and E. Ohleyer, *Appl. Microbiol. Biotechnol.*, 1990, **33**, 382; Y. Nishida, K. Nakamichi, K. Nabe and T. Tosa, *Enzyme Micro. Technol.*, 1987, **9**, 721.

8 H. K. Chenault, J. Dahmer and G. M. Whitesides, *J. Am. Chem. Soc.*, 1989, **111**, 6354; J. W. Keller and B. J. Hamilton, *Tetrahedron Lett.*, 1986, **27**, 1249.

9 W. H. Kruizinga, J. Bolster, R. M. Kellogg, J. Kamphuis, W. H. J. Boesten, E. M. Meijer and H. E. Schoemaker, *J. Org. Chem.*, 1988, **53**, 1826; H. E. Schoemaker, W. H. J. Boesten, B. Kaptein, H. F. M. Hermes, T. Sonke, Q. B. Broxterman, W. J. J. van den Tweel and J. Kamphuis, *Pure Appl. Chem.*, 1992, **64**, 1171.

10 C. Yee, T. A. Blythe, T. J. McNabb and A. E. Walts, *J. Org. Chem.*, 1992, **57**, 3525.

11 E. Benedetti, C. Toniolo, P. Hardy, V. Barone, B. Bavoso, B. di Blasio, P. Grimaldi, F. Lelj, V. Pavone, C. Pedone, M. Bonora and I. Lingham, *J. Am. Chem. Soc.*, (*a*) 1984, **106**, 8146; (*b*) 1984, **106**, 8152; M. Crisma, G. Valle, G. M. Bonora, C. Toniolo, F. Lelj, V. Barone, F. Fraternali, P. M. Hardy and H. L. S. Maia, *Biopolymers*, 1991, **31**, 637.

12 G. A. Stein, H. A. Bronner and K. Pfister, *J. Am. Chem. Soc.*, 1955, **77**, 700.

13 E. Ware, *Chem. Rev.*, 1950, **46**, 403.

14 E. J. Toone and J. B. Jones, *Tetrahedron: Asymmetry*, 1991, **2**, 207; L. K. P. Lam, C. M. Brown, B. D. Jeso, L. Lym, E. J. Toone and J. B. Jones, *J. Am. Chem. Soc.*, 1988, **110**, 4409.

15 C. S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294.

16 P. Marfey, *Carlsberg Res. Commun.*, 1984, **49**, 591.

17 U. Schöllkopf, W. Hartwig, U. Groth and K.-O. Westphalen, *Liebigs Ann. Chem.*, 1981, 696.

18 W. H. Kruizinga, J. Bolster, R. M. Kellogg, J. Kamphuis, W. H. J. Boesten, E. M. Meijer and H. E. Schoemaker, *J. Org. Chem.*, 1988, **53**, 1826.

19 G. M. Anantharamaiah and R. W. Roeske, *Tetrahedron Lett.*, 1982, **23**, 3335.

20 C. G. Baker, S.-C. J. Fu, S. M. Birnbaum, H. A. Sober and J. P. Greenstein, *J. Am. Chem. Soc.*, 1952, **74**, 4701.

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