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A chemoenzymatic approach to the synthesis of enantiomerically pure aza analogues of paraconic acid methyl ester and both enantiomers of methyl β-proline

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Abstract—Enantiopure methyl esters of 1-alkyl-5-oxo-3-pyrrolidinecarboxylic acids were obtained by enzymatic resolution of the corresponding chiral racemic mixtures. A particularly favourable interaction, also supported by molecular mechanics calculations, was observed between the 1-benzyl derivative and α-chymotrypsin, for which the enantiomeric ratio, *E*, exceeded 200. The absolute configurations of the lactams were determined by means of CD spectroscopy. From the resulting enantiomerically pure (99% e.e.) (*S*)-(+)-1-benzyl-3-pyrrolidinecarboxylic acid and methyl (*R*)-(−)-1-benzyl-3-pyrrolidinecarboxylate, the methyl esters of (+) and (−)-β-proline were synthesised in 99% e.e. and 18 and 22% overall yield, respectively.

1. Introduction

A number of compounds containing the γ-lactam (2-pyrrolidinone) moiety exhibit interesting biological and pharmacological activities. For instance, they are used as psychotropic[1] and anti-hypertensive agents,[2] inhibitors of proteolytic catalysis[3] and antimuscarinic agents.[4] They are also key intermediates in the synthesis of other biologically important compounds.[5–7] For instance, removal of the lactamic carbonyl group gives rise to pyrrolidine derivatives[5a,b] while the hydrolysis of the lactam ring leads to analogues of GABA (γ-aminobutyric acid),[8] an inhibitory neurotransmitter in the mammalian central nervous system.[9]

Despite their intrinsic potential for biological activity, both the racemic and enantiomerically pure derivatives of the γ-lactam bearing a carboxylic group at the β-position **1** (aza analogue of paraconic acid **2**[10]) have received little attention.

The racemic 1-benzyl-3-alkyl-4-methoxycarbonyl-2-pyrrolidinones were prepared[11] from β-alkylated itaconates and benzylamine through a conjugate addition–lactamization sequence in moderate to good yields. As to the synthesis of this class of compounds in enantiomerically pure form, few examples, either based on the use of chiral auxiliaries[12,13] or resolution via diastereomer formation,[14] are described in the literature. For example, (*S*)-(−)-4-methoxycarbonyl-2-pyrrolidinone was synthesised by Wyatt et al.[12] starting from itaconic acid and (*S*)-(−)-1-phenylethylamine, with high enantiomeric excess (95%), but in very poor overall chemical yield. Subsequently, Ma and Jiang[13] reported the synthesis of optically active 3-hydroxy-4-methoxycarbonyl-5-alkyl-2-pyrrolidinone, with high diastereomeric and enantiomeric excesses making use of a chiral auxiliary. Resolution by formation of diastereomeric salts with cinchonine was the method used on the racemic 1-benzyl derivative of lactam **1**.[14]

To our knowledge, the preparation of enantiomerically pure β-alkoxycarbonyl substituted γ-lactams by the use of enzymes is unreported in the literature to date. Our interest in such compounds derives from the fact that their naturally occurring oxygen analogues, which belong to a small class of compounds, generally known as paraconic acids, possess important biological properties.[15] Among them, (−)-methylenolactocin **3**,[16] isolated from the culture filtrate of *Penicillium sp*., shows antitu-

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mor and antibiotic activity, protolichesterinic acid **4**,[17] isolated from several species of moss *Cetraria*, is an antitumor, antibacterial and growth regulating compound, while (–)-phaseolinic acid **5**[18] is a metabolite of a fungus, *Macrophomina phaseolina* (Fig. 1). Many asymmetric syntheses of paraconic acid derivatives are reported,[19] and also a few chemoenzymatic syntheses.[20]

As an extension of our work concerning the production of enantiomerically pure paraconic acids, we directed our attention to the synthesis of their aza analogues, which, in view of their potential biological activity, could be of interest due to the lower toxicity of the lactam ring when compared to that of the lactone ring.[21]

2. Results and discussion

2.1. Synthesis of the substrates

The substrates chosen for these preliminary studies were the methyl esters of 5-oxo-3-pyrrolidinecarboxylic acid **8a–f** (Scheme 1) which were easily prepared by a literature method[22] involving the conjugate Michael addition of the appropriate primary amine to itaconic acid dimethyl ester **6**, resulting in spontaneous cyclization and leading to the desired heterocyclic ring. When the amine was *iso*-propylamine, the open chain intermediate **7c** could be detected in a ca. 1:1 ratio with the lactam **8c** by ^{1}H NMR analysis of the crude reaction mixture. The complete cyclization of **7c** was accomplished on heating the reaction mixture in toluene under acidic conditions. For the synthesis of **8a**, a mixture of ammonium chloride and triethylamine was used as a source of ammonia.

2.2. Kinetic resolution of lactams 8a–f

The substrates **8a–f** were treated with a series of commercially available hydrolytic enzymes for kinetic resolution. Among the others, pig liver acetone powder (PLAP), porcine pancreatic lipase (PPL), and α-chymotrypsine (α-CT) gave the most significant results, which are summarised in Table 1. Other enzymes, such as *Candida rugosa* lipase (CRL) and *Mucor miehei* lipase (MML), were unactive or not at all enantioselective. However, even when active, the enzymes listed in Table 1, with the exception of α-chymotrypsin, exhibited enantiomeric ratios[23] which were not satisfactory in most cases. As a consequence, at low conversion values, only the lactamic acid **15f** was isolated in 28% yield in enantiopure form (99% e.e.), while **15c** was isolated in 20% yield with 88% e.e. and the other acids **15a**, **15b**, **15d** and **15e** were obtained with low enantiomeric excesses (from 31 to 75%). As expected, at high conversion values, all of the unreacted esters **8a–f** were recovered with fairly good enantiomeric excess (Table 1). It is noteworthy that, owing to the very high *E* value, the hydrolysis of **8f** did not proceed beyond 54% conversion and that both the unreacted ester (*R*)-(–)-**8f** and the acid (*S*)-(+)-**15f** were recovered with high enantiomeric excesses.

The extraordinarily high *E* value exhibited by α-chymotrypsin in the hydrolysis of the sole lactam **8f**, bearing the benzyl group at nitrogen, prompted us to perform a molecular mechanics study on the enzyme-substrate interaction in order to investigate the reasons for this peculiar behaviour. The analysis of the models of **8a**, **8c**, **8e** and **8f** revealed that in all cases the ester group pointed towards the O-H···N hydrogen bond between Ser 195 and Hys 57 residues in the binding site of the enzyme.[24] The complexation energies, calculated for both enantiomers of the lactams **8a**, **8c**, **8e** and **8f** with the binding site of the protein (Table 2), are in accordance with the experimental evidence. The complexation energy values found for (+)- and (–)-**8a** are practically the same and actually this molecule was hydrolysed by α-CT with no enantioselectivity. On the contrary, the binding energy values found for **8c**, **8e** and **8f** are in accordance with the observed enantiopreference of the enzyme toward the (*S*)-enantiomer for which the calculated values are lower. The energy difference increased from **8c** to **8f**, in accordance with the observed *E* values.

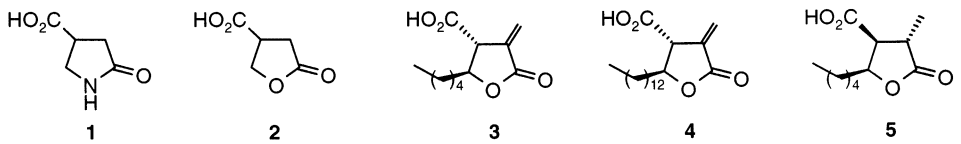
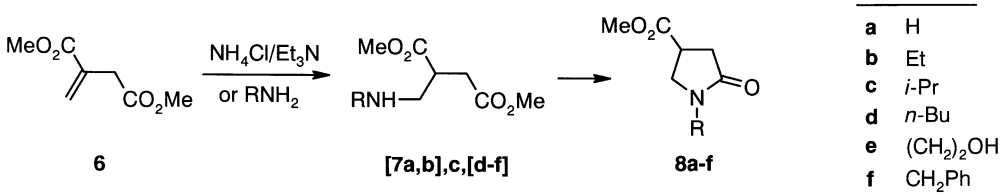
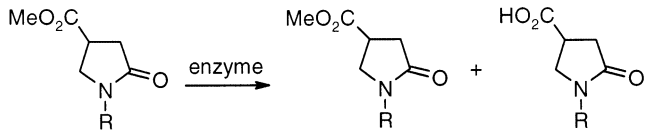


Figure 1.



Scheme 1.

Table 1. Enzymatic resolution of (±)-**8a–f**



(±)-**8a-f** (+) or (−)-**8a-f** (+) or (−)-**15a-f**

Substrate	Enzyme	*E*	Low conversion			High conversion		
			Conv. (%)	Unreacted ester e.e., (%)[a] (Yield, %)[b]	Acid e.e., (%)[a] (Yield, %)[b]	Conv. (%)	Unreacted ester e.e., (%)[a] (Yield, %)[b]	Acid e.e., (%)[a] (Yield, %)[b]
8a	PLAP[c]	2	31	*S*-(−)-**8a** 17 (75)	*R*-(+)-**15a** 34 (15)	85	*S*-(−)-**8a** 76 (23)	*R*-(+)-**15a** 14 (10)
8b	PLAP[c]	4	27	*R*-(+)-**8b** 20 (70)	*S*-(−)-**15b** 54 (17)	77	*R*-(+)-**8b** 95 (25)	*S*-(−)-**15b** 28 (22)
8c	α-CT[d]	19	21	*R*-(+)-**8c** 24 (72)	*S*-(−)-**15c** 88 (20)	65	*R*-(+)-**8c** 95 (20)	*S*-(−)-**15c** 51 (26)
8d	PPL[c]	9	23	*S*-(−)-**8d** 23 (78)	*R*-(+)-**15d** 75 (20)	86	*S*-(−)-**8d** 96 (23)	*R*-(+)-**15d** 16 (20)
8e	α-CT[d]	2	39	*R*-(+)-**8e** 21 (80)	*S*-(−)-**15e** 31 (15)	93	*R*-(+)-**8e** 99 (20)	*S*-(−)-**15e** 7 (16)
8f	α-CT[e]	>200	29	*R*-(−)-**8f** 41 (78)	*S*-(+)-**15f** 99 (28)	54	*R*-(−)-**8f** 99 (36)	*S*-(+)-**15f** 85 (30)

[a] Enantiomeric excesses were determined by chiral HRGC.
[b] Yields in isolated products.
[c] Reaction conditions: substrate (300 mg), enzyme (300 mg), phosphate buffer (0.1 M, pH 7.4, 20 mL), room temperature.
[d] Reaction conditions: substrate (300 mg), enzyme (30 mg), phosphate buffer (0.1 M, pH 7.4, 20 mL), room temperature.
[e] Reaction conditions: substrate (300 mg), enzyme (100 mg), phosphate buffer (0.1 M, pH 7.4, 20 mL), room temperature.

Table 2. Energy values (Kcal mol^{-1}) for the complexes Enz-*R* and Enz-*S* of **8a**, **8c**, **8e** and **8f**

	8a	**8c**	**8e**	**8f**
Enz-*S*	−6.2	−4.4	−11.3	−13.6
Enz-*R*	−6.5	+5.2	+6.2	+6.8

As to the particular case of **8f**, which was hydrolysed with the highest (*S*) enantioselectivity, a favourable hydrophobic interaction between the phenyl group and the binding pocket defined by the residues Ser 189–Asp 194 and Ser 214–Cys 220 was observed for both enantiomers. Both enantiomers also benefit from hydrogen bond stabilisation between the methoxy group and the NH group of the peptide bonds Ser 195-Asp 194 and Gly 193-Met 192, while for the more reactive (*S*)-enantiomer an additional hydrogen bond is present between the lactam carbonyl group and the NH of the peptide bond of the residues Gly 126-Trp 125, respectively, as a consequence of the better fit of the benzylic substituent into the aryl binding site.

2.3. Determination of the absolute configuration of (+)-8b, (+)-8c, (−)-8d and (+)-8e

The absolute configuration of the lactamic esters (−)-**8a** and (−)-**8f**, obtained by enzymatic resolution with PLAP and α-CT, respectively, was assigned as (*S*) and (*R*), respectively, by comparison of their specific rotation values with those reported in the literature.[12,14] The absolute configuration of the other enantiomerically pure lactamic esters (+)-**8b**, (+)-**8c**, (−)-**8d** and (+)-**8e** was assigned by a comparison of their CD spectra with that of (−)-**8a**, having determined by conformational analysis (carried out by VT molecular dynamics) that the most populated conformer was the same for all of them. Lactam (*S*)-(−)-**8a** showed a negative Cotton effect ($\Delta\varepsilon_{209.8}$ −0.9) associated with the $n\rightarrow\pi^*$ transition of the lactam group, as did (−)-**8d** ($\Delta\varepsilon_{214}$ −1.9) and therefore, (−)-**8d** was assigned the (*S*)-configuration. On the contrary, the lactams (+)-**8b**, (+)-**8c** and (+)-**8e** showed a positive Cotton effect at the same wavelength ((+)-**8b**: $\Delta\varepsilon_{214}$ +1.50; (+)-**8c**: $\Delta\varepsilon_{213.4}$ +1.83 and (+)-**8e**: $\Delta\varepsilon_{213}$ +1.7) and therefore, they were all assigned the (*R*)-configuration. Since lactams **8a–e** only differ in the nature of the substituent at the nitrogen atom, evidently the sign of the Cotton effect is not influenced by the substituent at nitrogen.

2.4. Synthesis of methyl (*S*)-(+)- and (*R*)-(−)-β-proline

An important corollary of these enzymatic resolutions is that compounds (*R*)-(−)-**8f** and (*S*)-(+)-**15f**, which can be obtained as pure enantiomers in good yield, are precursors of both enantiomers of methyl β-proline.[25] In fact, when reduction of the lactam carbonyl function in (*R*)-(−)-**8f** (99% e.e.) was carried out with borane–dimethylsulfide, the corresponding methyl 1-phenylmethyl-3-pyrrolidinecarboxylate (*R*)-(−)-**17**[25a] was obtained. Subsequent debenzylation of the nitrogen atom afforded methyl 3-pyrrolidinecarboxylate (*R*)-(−)-**18** having 99% e.e. The same sequence of reactions carried out on (*S*)-(+)-**8f**, prepared by esterification of (*S*)-(+)-**15f** (99% e.e.) with diazomethane, allowed the preparation of (*S*)-(+)-**18**.[25a]

Interestingly, when the reduction of the carbonyl group of (*R*)-(−)-**8f** was performed with borane–tetrahydrofuran (2 equiv.) the corresponding amino alcohol (*R*)-(+)-1-phenylmethyl-3-hydroxymethyl pyrrolidine **19** was formed, in about 1:1 ratio with (*R*)-(−)-**17**, whereas the same reduction carried out on the racemate was claimed to leave the ester group unaffected.[5e] Hydrogenolysis of (*R*)-(+)-**19**, performed with H_2 with 10% $Pd(OH)_2$ as the catalyst, gave (*R*)-(+)-**20**.

The e.e. of all the compounds derived from (*R*)-(−)-**8f**, having 99% e.e. (as determined by HRGC), namely (*R*)-(−)-**17**, (*R*)-(−)-**18**, (*R*)-(+)-**19** and (*R*)-(+)-**20** (Scheme 2), were also established by comparison of their specific rotations with the respective literature values.[14,25a]

3. Conclusions

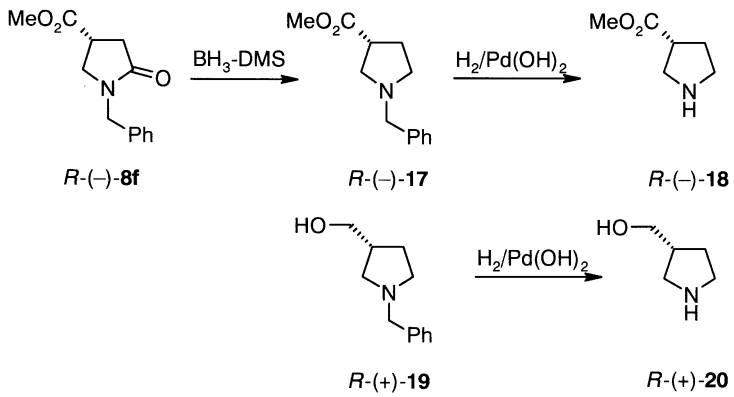
In conclusion, the enzymatic resolution of these β-methoxycarbonyl-γ-lactams is not an easy process, at least when commercially available enzymes are used, and therefore a search for the best strategy may be needed. When the lactam nitrogen atom was unsubstituted or substituted with alkyl groups the enzymatic resolution was unsatisfactory because of the low enantioselectivity exhibited by all enzymes checked. The enantiodifferentiation was excellent only when the nitrogen atom was substituted with a benzyl group and only when α-chymotrypsin was applied as the enzyme. This is attributed to the particularly favourable non-bonding interactions established in the enzyme–substrate complex.

Finally, it should be underlined that this method constitutes a facile and efficient synthesis of (+)- and (−)-β-proline, potent agonists at the strychnine-sensitive glycine receptor.[25a] Both enantiomers might also be used as conformationally restricted γ-amino acids and as building blocks for the preparation of inhibitors of bacterial DNA gyrase.[25b]

4. Experimental

4.1. General

IR spectra were recorded on a Jasco FT-IR 200 spectrophotometer. ^1H and ^{13}C NMR spectra were run on a Jeol EX-400 (400 MHz for proton), using deuterochloroform as a solvent and tetramethylsilane as internal standard, unless otherwise stated. Coupling constants are given in Hz. Optical rotations were determined on a Perkin–Elmer model 241 polarimeter, at 25°C. CD spectra were obtained on a Jasco J-700A spectropolarimeter (0.1 cm cell) for methanol solutions. Mass spectra (EI, positive ions) were run on a VG 7070 spectrometer at 70 eV. ESI-MS spectra were obtained on a PE-API spectrometer at 5600 volts by infusion of methanolic solutions. High resolution GLC analyses were obtained on a Carlo Erba GC 8000 instrument using an OV 1701 (25 m×0.32 mm) column (carrier gas He, 180 kPa, split 1:50), and on a Shimadzu 14B apparatus, using a Chiraldex™ column, type G-TA, trifluoroacetyl-γ-cyclodextrin (40 m×0.25 mm) (carrier gas He, 180 kPa, split 1:100). Enzymatic hydrolyses were performed using a pH-stat Controller PHM290 Radiometer, Copenhagen. Mps were determined on a Büchi SHP-20 apparatus and are uncorrected. TLC's were performed on Polygram® Sil G/UV_{254} silica gel pre-coated plastic sheets (eluant: light petroleum–ethyl acetate). Flash chromatography was run on silica gel, 230–400 mesh ASTM (Kieselgel 60, Merck), using mixtures of light petroleum 40–70°C and ethyl acetate as the eluant.



Scheme 2.

Porcine pancreatic lipase type II (PPL), esterase from pig liver in 3.2 M $(NH_4)_2SO_4$ suspension (PLE), and porcine liver acetone powder (PLAP) were supplied by Sigma, while α-chymotrypsin (α-CT; 53.1 U/mg) was purchased from Fluka.

4.2. Synthesis of substrate 8a

4.2.1. Methyl 5-oxo-3-pyrrolidinecarboxylate 8a[12]. A suspension of ammonium chloride (10.7 g, 0.2 mol) at room temperature was treated by dropwise addition of a solution of dimethyl itaconate (4.0 g, 25 mmol) in methanol (100 mL) and triethylamine (3.3 mL, 0.2 mol). The mixture was stirred overnight, then further ammonium chloride (0.2 mol) and triethylamine (0.2 mol) was added. Stirring was continued for further 24 h, then the solid was filtered off, the solution evaporated and the residue distilled (bp 135–138°C) (1.0 torr) to give pure **8a** as a solid (2.2 g, 60%), mp 61–62°C (lit.[12] 61–3°C); IR, cm^{-1} (Nujol): 3360, 3242 (NH), 1735 (COO), 1685 (CON); ^1H NMR, δ, ppm: 6.57 (1H, bs, NH), 3.75 (3H, s, OCH_3), 3.63 (2H, m, H-4), 3.36 (1H, m, H-3), 2.57, 2.65 (2H, part AB of an ABX system, J 17.1, 9.8, 7.8, H-2); ^{13}C NMR, δ, ppm: 176.5 (s), 172.9 (s), 52.1 (q), 44.1 (t), 38.2 (d), 32.8 (t); EI–MS, m/z: 143 (3, $M^{+\bullet}$), 115 (57), 112 (21), 101 (84), 87 (13), 83 (40), 70 (21), 59 (19), 56 (53), 55 (100).

4.3. Synthesis of lactams 8b–f

To a solution of dimethyl itaconate (0.16 g, 1 mmol) in methanol, an equimolar amount of the appropriate primary amine was added dropwise under stirring at room temperature (0°C in the case of $EtNH_2$). After stirring the mixture for 12 h at room temperature, the solvent was removed in vacuo and the residue was distilled under reduced pressure. When the amine was *iso*-propylamine, the residue was stirred under reflux in toluene for 1 h in the presence of a catalytic amount of *p*-toluenesulphonic acid before distillation. When the amine was benzylamine, the product was obtained by trituration of the crude reaction mixture with light petroleum.

4.3.1. Methyl 1-ethyl-5-oxo-3-pyrrolidinecarboxylate 8b. The title compound was obtained in 85% yield as an oil, bp 89–92°C (0.05 torr); IR, cm^{-1} (film): 1735 (COO), 1688 (CON); ^1H NMR, δ, ppm: 3.69 (3H, s, OCH_3), 3.54 (2H, m, H-2), 3.32–3.17 (3H, m, H-3 and NCH_2CH_3), 2.62 (2H, m, 2 H-4), 1.07 (3H, t, J 8.0, NCH_2CH_3); ^{13}C NMR, δ, ppm: 173.2 (s), 171.7 (s), 52.3 (q), 48.2 (t), 36.9 (t), 35.7 (d), 34.1 (t), 12.2 (q); EI-MS, m/z: 171 (41, $M^{+\bullet}$), 156 (71), 143 (54), 140 (18), 128 (26), 127 (58), 112 (56), 96 (25), 84 (75), 68 (42), 58 (55), 56 (46), 55 (52), 42 (87), 41 (100).

4.3.2. Methyl 1-(methylethyl)-5-oxo-3-pyrrolidinecarboxylate 8c. The title compound was formed in admixture (1:1) with the open chain adduct dimethyl [(methylethyl)aminomethyl]-butanedioate **7c** which was detected in the NMR spectrum of the crude reaction mixture: ^1H NMR, δ, ppm: 3.71 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 2.99 (1H, m, $CH(CH_3)_2$), 2.90 (1H, dd), 2.80–2.60 (3H, m), 2.60 (1H, dd), 1.55 (1H, br, NH), 1.02 (6H, d, J 7.7, $CH(CH_3)_2$); ^{13}C NMR, δ, ppm: 174.3 (s), 172.3 (s), 51.8 (q), 51.6 (q), 48.3 (d), 48.(t), 42.0 (d), 34.05 (t), 22.8 (q). Treatment of the crude reaction mixture in refluxing toluene in the presence of *p*-toluenesulphonic acid allowed compound **8c** to be obtained in 81% yield as an oil, bp 85–87°C (0.05 torr); IR, cm^{-1} (film): 1738 (COO), 1690 (CON); ^1H NMR, δ, ppm: 4.37 (1H, septet, J 7.0, $CH(CH_3)_2$), 3.75 (3H, s, OCH_3), 3.51 (2H, d, H-2), 3.22 (1H, m, H-3), 2.66, 2.62 (2H, part AB of an ABX system, J 17.1, 8.3, 7.3, H-4), 1.15 (6 H, d, J 7.0, $CH(CH_3)_2$); ^{13}C NMR, δ, ppm: 173.2 (s), 171.3 (s), 52.3 (q), 43.7 (t), 42.5 (d), 35.9 (d), 34.5 (t), 19.6 (q), 19.4 (q); EI-MS, m/z: 185 (9, $M^{+\bullet}$), 171 (9), 170 (100), 127 (14), 110 (7), 99 (8), 84 (16), 82 (8), 56 (67), 55 (15).

4.3.3. Methyl 1-(1-butyl)-5-oxo-3-pyrrolidinecarboxylate 8d. The title compound was obtained in 88% yield as an oil, bp 132–134°C (3.0 torr); IR, cm^{-1} (film): 1733 (COO), 1689 (CON); ^1H NMR, δ, ppm: 3.74 (3H, s, OCH_3), 3.59 (2H, m, H-2), 3.34–3.21 (3H, m, H-3, NCH_2), 2.68 (2H, m, H-4), 1.51 (2H, m, $NCH_2CH_2CH_2CH_3$), 1.32 (2H, m, $N(CH_2)_2CH_2CH_3$), 0.93 (3H, t, $N(CH_2)_3CH_3$); ^{13}C NMR, δ, ppm: 173.2 (s), 172.0 (s), 52.3 (q), 48.8 (t), 42.1 (t), 35.9 (d), 34.2 (t), 29.1 (t), 19.9 (t), 13.6 (q); EI–MS, m/z: 199 (18, $M^{+\bullet}$), 170 (14), 157 (45), 156 (100), 140 (13), 127 (48), 96 (16), 68 (27), 55 (21).

4.3.4. Methyl 1-(2-hydroxyethyl)-5-oxo-3-pyrrolidinecarboxylate 8e. The title compound was obtained in 72% yield as an oil, bp 157–159°C (0.1 torr). IR, cm^{-1} (film): 3379 (OH), 1737 (COO), 1678 (CON); ^1H NMR δ, ppm: 3.74 (1H, s, OH), 3.20 (1H, m, H-3), 3.65 (7H, m and s, $N(CH_2)_2OH$, OCH_3), 3.33 (2H, m, H-2), 2.60 (2H, m, H-4); ^{13}C NMR δ, ppm: 173.4 (s), 173.3 (s), 60.0 (t), 52.3 (q), 50.1 (t), 45.5 (t), 36.1 (d), 34.1 (t); EI–MS, m/z: 187 (5, $M^{+\bullet}$), 169 (8), 157 (16), 156 (100), 144 (29), 128 (18), 127 (43), 96 (9), 68 (20), 42 (30).

4.3.5. Methyl 1-(phenylmethyl)-5-oxo-3-pyrrolidinecarboxylate 8f. The title compound was obtained in 85% yield as an oil, bp 144–147°C (0.06 torr), which crystallised by treatment with petroleum ether, mp 65–67°C; IR, cm^{-1} (film): 1733 (COO), 1689 (CON); ^1H NMR δ, ppm: 7.28–7.35 (3H, m, Ar-H), 7.23 (2H, d, Ar-H), 4.45 (2H, AB system, J 14.7, NCH_2Ph), 3.70 (3H, s, OCH_3), 3.48–3.43 (2H, m, H-2), 3.20 (1H, m, H-3), 2.76, 2.73 (2H, part AB of an ABX system, J 17.2, 9.8, 7.3, H-4); ^{13}C NMR δ, ppm: 173.0 (s), 172.2 (s), 133.7 (s), 128.6 (d), 128.0 (d), 127.6 (d), 52.3 (q), 48.3 (t), 46.4 (t), 35.7 (d), 33.9 (t); EI–MS, m/z: 213 (24, M^+), 198 (10), 170 (100), 142 (14), 141 (19), 113 (14), 86 (9), 68 (7).

4.4. General procedure for the enzymatic hydrolysis of lactams 8a–f

The lactamic esters were reacted with the enzyme in a 0.1 M KH_2PO_4/Na_2PO_4 buffer, at pH 7.4, under vigorous stirring. The pH was continuously adjusted to pH 7.4 with 1N aqueous NaOH using a pH-stat. At the

desired conversion value, the unreacted lactamic ester was extracted from the suspension with ethyl acetate (five times), using a centrifuge for the separation of the layers. The aqueous phase was acidified to pH 2 with 1N HCl, the water was evaporated in vacuo, and the lactamic acid was extracted from the solid residue with acetonitrile. The enantiomeric excess of the acid was determined by chiral HRGC after esterification of the carboxylic function with diazomethane.

4.4.1. Methyl (*S*)-(−)-5-oxo-3-pyrrolidinecarboxylate 8a.[12] The product (*S*)-(−)-**8a** was isolated from the enzymatic hydrolysis with PLAP in 23% yield; 76% e.e.; $[\alpha]_D^{20}$ −7.3 (*c* 0.7, CH_3OH); $[\alpha]_D^{20}$ −14.6 (*c* 0.7, $CHCl_3$); (lit.:[12] 95% e.e., $[\alpha]_D^{35}$ −19 (*c* 0.7, $CHCl_3$)); $\Delta\varepsilon_{209}$ −0.88 (CH_3OH).

The enzymatic hydrolysis of **8a**, carried out with α-CT (300 mg of substrate, 30 mg of enzyme, 0.1 phosphate buffer at pH 7.4 (20 mL), room temperature), afforded the corresponding compound **15a** in racemic form.

4.4.2. Methyl (*R*)-(+)-1-ethyl-5-oxo-3-pyrrolidinecarboxylate 8b. The product (*R*)-(+)-**8b** was isolated from the enzymatic hydrolysis with PLAP in 25% yield; 95% e.e.; $[\alpha]_D^{25}$ +8.7 (*c* 1.1, CH_3OH); $\Delta\varepsilon_{214}$ +1.50 (CH_3OH).

4.4.3. Methyl (*R*)-(+)-1-(methylethyl)-5-oxo-3-pyrrolidinecarboxylate 8c. The product (*R*)-(+)-**8c** was isolated from the enzymatic hydrolysis with α-CT in 20% yield; 95% e.e.; $[\alpha]_D^{25}$ +2.9 (*c* 0.7, CH_3OH); $\Delta\varepsilon_{213.4}$ +1.83 (CH_3OH).

4.4.4. Methyl (*S*)-(−)-1-(1-butyl)-5-oxo-3-pyrrolidinecarboxylate 8d. The product (*S*)-(−)-**8d** was isolated from the enzymatic hydrolysis with PPL in 23% yield; 96% e.e.; $[\alpha]_D^{25}$ −5.0 (*c* 0.9, CH_3OH); $\Delta\varepsilon_{214}$ −1.9 (CH_3OH).

4.4.5. Methyl (*R*)-(+)-(2-hydroxyethyl)-5-oxo-3-pyrrolidinecarboxylate 8e. The product (*R*)-(+)-**8e** was isolated from the enzymatic hydrolysis with α-CT in 20% yield; 99% e.e.; $[\alpha]_D^{25}$ +8.4 (*c* 0.75, CH_3OH), $\Delta\varepsilon_{213}$ +1.7 (CH_3OH).

4.4.6. Methyl (*R*)-(−)-1-(phenymethyl)-5-oxo-3-pyrrolidinecarboxylate 8f.[14] The product (*R*)-(−)-**8f** was isolated from the enzymatic hydrolysis with α-CT in 36% yield; 99% e.e.;.$[\alpha]_D^{25}$ −19.0 (*c* 1.1, CH_3OH), (lit.[14] for *ent*-**8f**: $[\alpha]_D^{22}$ +18.9, c 2.88, CH_3OH), $\Delta\varepsilon_{197}$ −2.7 (CH_3OH).

4.4.7. (*R*)-(+)-5-oxo-3-Pyrrolidinecarboxylic acid 15a.[12] The product (*R*)-(+)-**15a** was isolated from the enzymatic hydrolysis with PLAP in 15% yield as a crystalline solid, mp 147–149°C.[12] IR, cm^{-1} (Nujol): 3380 (broad, COOH, NH), 1740 (COO), 1680 (CON); ^1H NMR, δ, ppm (D_2O): 3.73 (1H, dd, 16.6, 9.8, H-2), 3.62 (1H, dd, *J* 16.6, 7.3, H-2), 3.47 (1H, m, H-3), 2.68, 2.66 (2H, part AB of an ABX system, *J* 18.1, 9.3, 7.1, H-4); ^{13}C NMR, δ, ppm (D_2O): 182.4 (s), 182.2 (s), 48.1 (t), 42.6 (d), 36.4 (t); EI–MS, *m*/*z*: 129 (8, $M^{+\bullet}$), 101 (21), 85 (12), 56 (41), 55 (100); 34% e.e.; $[\alpha]_D^{25}$ +10.2 (*c* 1.0, CH_3OH); $\Delta\varepsilon_{209}$ +0.12 (CH_3OH).

4.4.8. (*S*)-(−)-1-Ethyl-5-oxo-3-pyrrolidinecarboxylic acid 15b. The product (*S*)-(−)-**15b** was isolated from the enzymatic hydrolysis with PLAP in 17% yield as a crystalline solid, mp 98–100°C; IR, cm^{-1} (Nujol): 3430–2500 (broad, OH), 1735 (COO), 1640 (CON); ^1H NMR, δ, ppm: 10.37 (1H, bs, COOH), 3.62 (1H, dd, *J* 5.9, 10.2, H-2), 3.29 (2H, dq, *J* 7.3, NCH_2), 3.19 (1H, m, H-3), 2.70 (2H, m, H-4), 1.07 (3H, t, *J* 7.3, CH_2CH_3); ^{13}C NMR, δ, ppm: 175.9 (s), 173.0 (s), 48.7 (t), 37.3 (t), 35.8 (d), 34.2 (t), 12.3 (q); EI–MS, *m*/*z*: 157 (50, $M^{+\bullet}$), 142 (100, $M-C_2H_5$), 129 (20), 112 (18), 100 (10), 96 (21), 84 (32), 68 (41), 58 (42); 54% e.e., $[\alpha]_D^{25}$ −3.5 (*c* 1.0, CH_3OH), $\Delta\varepsilon_{214}$ −1.2 (CH_3OH).

4.4.9. (*S*)-(−) 1-(1-Methylethyl)-5-oxo-3-pyrrolidinecarboxylic acid 15c. The product (*S*)-(−)-**15c** was isolated from the enzymatic hydrolysis with α-CT in 20% yield as a crystalline solid, mp 105–107°C. IR, cm^{-1} (Nujol): 3430–2500 (broad, COOH), 1730 (COO), 1640 (CON); ^1H NMR, δ, ppm: 10.46 (1H, bs, COOH), 4.32 (1H, sept, *J* 6.6, $CH(CH_3)_2$), 3.60 (2H, m, H-2), 3.22 (1H, m, H-3), 2.77, 2.72 (2H, *J* 17.1, 10.7, 7.3, H-4), 1.11 (6 H, d, *J* 6.6, $CH(CH_3)_2$); ^{13}C-NMR, δ, ppm: 175.9 (s), 172.6 (s), 44.2 (t), 43.0 (d), 36.0 (d), 34.6 (t), 19.7 (q), 19.5 (q); EI–MS, *m*/*z*: 171 (12), 156 (100), 142 (10), 82 (8), 56 (67); 88% e.e., $[\alpha]_D^{25}$ −2.4 (*c* 0.45, CH_3OH), $\Delta\varepsilon_{215}$ −1.15 (CH_3OH).

4.4.10. (*R*)-(+)-1-(1-Butyl)-5-oxo-3-pyrrolidinecarboxylic acid 15d. The product *(R)*-(+)-**15d** was isolated from the enzymatic hydrolysis with PPL in 20% yield, oil, IR, cm^{-1} (film): 3430–2500 (broad, COOH), 1727 (COO), 1642 (CON); ^1H NMR, δ, ppm: 8.96 (1H, bs, COOH), 3.61 (1H, m, H-2), 3.53 (1H, m, H-2), 3.28–3.14 (3H, 2m, H-3, NCH_2), 2.67 (2H, m, H-4), 1.44 (2H, quint., *J* 7.3, $NCH_2CH_2CH_2CH_3$), 1.24 (2H, sext., *J* 7.3, $N(CH_2CH_2CH_2CH_3$), 0.85 (3H, t, *J* 7.3, $N(CH_2)_2CH_3$); ^{13}C NMR, δ, ppm: 175.9 (s), 173.3 (s), 49.3 (t), 42.6 (t), 36.0 (d), 34.3 (t), 29.2 (t), 20.0 (t), 13.7 (q); EI–MS, *m*/*z*: 199 (2, $M^{+\bullet}$), 185 (18), 170 (13), 156 (15), 143 (31), 142 (100), 113 (14), 96 (11), 68 (18), 57 (10); 75% e.e.; $[\alpha]_D^{25}$ +3.7 (*c* 1.0, CH_3OH); $\Delta\varepsilon_{214}$ +0.67 (CH_3OH).

4.4.11. (*S*)-(−)-1-(2-Hydroxyethyl)-5-oxo-3-pyrrolidinecarboxylic acid 15e. The product (*S*)-(−)-**15e** was isolated from the enzymatic hydrolysis with α-CT in 15% yield as a semisolid; IR, cm^{-1} (film): 3705–3550 (broad, COOH, OH), 1728 (COOH), 1680 (CON); ^1H NMR, δ, ppm ($CDCl_3$/MeOD): 3.46 (2H, t, NCH_2CH_2OH), 3.41 (2H, t, NCH_2CH_2OH), 3.18, 3.07 (2H dt, H-2), 2.93 (1H, m H-3), 2.44, 2.37 (2H, part AB of an ABX system, *J* 17.2, 9.7, 7.7, H-4); ^{13}C NMR δ, ppm: 176.4 (s), 174.5 (s), 58.5 (t), 50.1 (t), 44.5 (t), 36.3 (d), 34.1 (t). EI–MS, *m*/*z*: 173 (10, $M^{+\bullet}$), 156 (12), 154 (10), 142 (100), 130 (52), 113 (45), 96 (38), 84 (10), 68 (65), 55 (40); 31% e.e., $[\alpha]_D^{25}$ −4.4 (*c* 1.0, CH_3OH), $\Delta\varepsilon_{214}$ −0.7 (CH_3OH).

4.4.12. (*S*)-(+)-1-(Phenylmethyl)-5-oxo-3-pyrrolidinecarboxylic acid 15f. The product (*S*)-(+)-**15f** was isolated from the enzymatic hydrolysis with α-CT in 28% yield as a crystalline solid, mp 98–100°C; (lit.[14] 99–100°C); IR, cm^{-1} (Nujol): 3650 (broad, COOH), 1733 (COO),

1689 (CON); ^{1}H NMR δ, ppm: 9.50 (1H, bs, COOH), 7.35–7.28 (3H, m, Ar-H), 7.23 (2H, d, Ar-H), 4.45 (2H, AB system, J 14.7, NCH_2Ph), 3.52–3.48 (2H, m, H-2), 3.20 (1H, m, H-3), 2.80, 2.76 (2H, part AB of an ABX system, J 17.2, 9.8, 7.3, H-4); ^{13}C NMR δ, ppm: 175.1 (s), 173.6 (s), 135.4 (s), 128.8 (d), 128.1 (d), 127.8 (d), 48.9 (t), 46.7 (t), 35.8 (d), 34.1 (t); EI–MS, m/z: 213 (24, $M^{+\bullet}$), 198 (10), 170 (100), 142 (14), 141 (19), 113 (14), 86 (9), 68 (7); 99% e.e., $[\alpha]_D^{25}$ +15.5 (c 0.5, abs. EtOH), (lit.:[14] $[\alpha]_D^{22}$ +15.7 (c 2.98, abs. EtOH); $\Delta\varepsilon_{197}=+1.66$ (CH_3OH).

4.5. Reduction of the lactam (*R*)-(−)-8f with BH_3–DMS[5e]

To a solution of (*R*)-(−)-**8f** (2.0 g, 8.6 mmol) having 99% e.e. in dry THF (20 mL), a THF solution of BH_3–DMS (2 M, 9 mL, 18 mmol) were slowly added at −10°C, under stirring under inert atmosphere. After the addition was complete, the temperature was left to rise to room temperature, then 4N HCl in methanol was added (10 mL), and the solution refluxed 1 h. After removal of the solvents in vacuo, water was added and the aqueous phase was extracted with ethyl acetate, the pH was raised to 10 with NaOH added and the mixture extracted with ethyl acetate. Evaporation of the solvent left an oily residue (1.8 g), which was purified on column (eluent: ethyl acetate), to yield (*R*)-(−)-**17**.

4.5.1. Methyl (*R*)-(−)-1-(phenylmethyl)-3-pyrrolidinecarboxylate 17.[25a] Compound (*R*)-(−)-**17** was isolated as an oil (64% yield), IR, cm^{-1} (film): 1731 (COO); ^{1}H NMR δ, ppm: 7.25 (5H, m, ArH), 3.61 (3H, s, OCH_3), 3.56 (2H, s, CH_2Ph), 2.97 (1H, quint., H-3), 2.84 (1H, dd, 9.3, 7.0, H-2), 2.65 (1H, m, H-5), 2.56 (1H, dd, J 9.3, 8.3, H-2), 2.45 (1H, q, H-5), 2.05 (2H, m, H-4); ^{13}C NMR, δ, ppm: 175.5 (s), 138.7 (s), 128.8 (d), 128.2 (d), 127.0 (d), 60.1 (t, Bz), 56.7 (t, C-5), 53.7 (t, C-2), 51.8 (q, OCH_3), 41.9 (d, C-3), 27.6 (t, C-5); ESI–MS, m/z: 220.1 (MH^+); EI–MS, m/z: 219 (15, $M^{+\bullet}$), 218 (12), 188 (10), 142 (20), 128 (45), 92 (10), 91 (100); 99% e.e.; $[\alpha]_D^{25}$ −19.7 (c 1.0, $CHCl_3$) [lit.:[25a] $[\alpha]_D^{23}$ −20 (c 1.0, $CHCl_3$)].

The enantiomer (*S*)-(+)-**17** was prepared starting from (*S*)-(+)-1-benzyl-5-oxo-3-pyrrolidinecarboxylic acid, 99% e.e., after esterification of the carboxylic group with diazomethane; $[\alpha]_D^{25}$ +19.0 (c 1.0, MeOH) [lit.:[14] $[\alpha]_D^{22}$ +18.9 (c 2.88, MeOH)].

4.5.2. (*R*)-(+)-1-(Phenylmethyl)-3-hydroxymethylpyrrolidine 19. When the reduction of (*R*)-(−)-**8f** was carried out with 1 M BH_3–THF, under the conditions described above, a 1:1 mixture of (*R*)-(−)-**17** and (*R*)-(+)-**19** was obtained. Compound (*R*)-(+)-**19** was separated by flash chromatography (eluant: ethyl acetate) as an oil. IR, cm^{-1} (film): 3377 (OH); ^{1}H NMR, δ, ppm: 7.30 (5H, m, Ph), 3.66 (1H, dd, J 9.9, 8.9, CH_2OH), 3.59 (3H, s, OCH_3), 3.50 (1H, dd, J 9.9, 4.8, CH_2OH), 3.0 (1H, bs, OH), 2.79 (1H, m, H-3), 2.61 (1H, dd, J 10.1, 3.3, H-2), 2.52 (1H, dd, J 10.1, 7.0, H-2), 2.33 (2H, m, H-5), 2.00 (1H, m, H-4). 1.69 (1H, m, H-4); ^{13}C NMR, δ, ppm: 138.6 (s), 128.7 (d), 128.3 (d), 127.0 (d), 67.4 (t, CH_2OH), 60.15 (t, CH_2Ph), 58.1 (t, C-2), 53.7 (t, C-5), 38.7 (d, C-3), 26.9 (C-4); ESI-MS, m/z: 192 (MH^+); EI–MS, m/z: 191 (10, $M^{+\bullet}$), 190 (10), 114 (9), 92 (12), 91 (100); 99% e.e.; $[\alpha]_D^{25}$ +2.6 (c 1.0, abs. EtOH).

The enantiomer (*S*)-(−)-**19**[14,25b] was prepared by reduction of (*S*)-(+)-1-(phenylmethyl)-5-oxo-3-pyrrolidinecarboxylic acid **15f** with either BH_3–DMS or BH_3–THF, under the above reported conditions; 99% e.e.; $[\alpha]_D^{25}$ −2.6 (c 1.0, abs. EtOH). [lit.:[14] $[\alpha]_D^{20}$ −2.55 (c 3.98, EtOH); lit.:[25b] $[\alpha]_D^{20}$ −3.1 (c 4.1, EtOH)].

4.6. Hydrogenolysis of (*R*)-(−)-17 and (*S*)-(−)-19

4.6.1. Methyl (*R*)-(−)-3-pyrrolidinecarboxylate 18.[25a] A solution of (*R*)-(−)-**17** (0.13 g, 0.56 mmol) in methanol was hydrogenated overnight at room temperature and atmospheric pressure of H_2, over 20% $Pd(OH)_2$/C (0.05 g). After the catalyst was filtered off on a pad of Celite, the solvent was evaporated in vacuo to give pure (*R*)-(−)-**18** as a colourless oil (0.075 g, 95% yield), IR, cm^{-1} (film): 2951 (NH), 1732 (COOMe); ^{1}H NMR, δ, ppm: 3.70 (3H, s, OCH_3), 3.53, 3.50 (2H, part AB of an ABX system, J_{AB} 11.6, 2 H-2), 3.35 (2H, m, 2 H-5), 3.24 (1H, m, H-3), 3.19 (1H, broad s, NH), 2.26 (2H, m, 2 H-4); ^{13}C NMR, δ, ppm: 172.5 (s), 52.5 (q, OCH_3), 47.3 (t, C-2), 45.2 (t, C-5), 42.2 (d, C-3), 28.4 (t, C-4); ESI–MS, m/z: 129.9 (MH^+); 99% e.e., $[\alpha]_D^{25}$ −7.0 (c 1.0, $CHCl_3$), [lit.:[25a] $[\alpha]_D^{25}$ −7.2 (c 1.0, $CHCl_3$).

4.6.2. (*S*)-(−)-3-Hydroxymethylpyrrolidine 20.[14,25b] A solution of (*S*)-(−)-**19** (0.20 g, 1.05 mmol) in methanol was hydrogenated over 20% $Pd(OH)_2$/C (0.08 g), under the above conditions to give (*S*)-(−)-**20** (98% yield). The compound was obtained by chromatographic purification (eluant: ethyl acetate) of the corresponding *N*-Boc derivative,[26] followed by deprotection with 50% TFA in CH_2Cl_2, and elution of the residue on Amberlite IRA 400 (Cl^-), with CH_3OH. IR, cm^{-1} (film): 3385–3290 (br, OH and NH); ^{1}H NMR, δ, ppm ($CDCl_3$/MeOD): 3.59 (1H, dd, J 10.6, 5.5, CH_2OH), 3.50 (1H, dd, J 10.6, 6.9, CH_2OH), 3.13 (1H, dd, J 11.3, 8.0, H-2), 3.10 (1H, m, H-5), 3.02 (1H, m, H-5), 2.86 (1H, dd, J 11.3, 6.6, H-2), 2.41 (1H, m, H-3), 2.00 (1H, m, H-4), 1.62 (1H, m, H-4); ^{13}C NMR, δ, ppm ($CDCl_3$/MeOD): 63.4 (t), 48.4 (t), 45.5 (t), 40.3 (d), 27.4 (t); ESI–MS, m/z: 101.6 (MH^+); 99% e.e.; $[\alpha]_D^{25}$ −17.8 (c 0.8, abs. EtOH), [lit.:[14] −19.1 (c 4.6, EtOH); lit.:[25b] $[\alpha]_D^{25}$ −26 (c 6, EtOH)].

4.7. Molecular mechanics calculations

Starting from the crystal structure coordinates of α-chymotrypsin,[24] the 3-D structure of the protein was fully optimised by molecular mechanics (MM) with the CVFF force field[27] of *Cerius*^{2} platform (version 4.2, MSI, San Diego, CA, USA) using a combination of conjugate gradient/Newton Raphson method of optimisation. The convergence threshold of 0.01 kcal/mol per Å was used, which required more than 10000 iterations to fully satisfy the optimisation of all geometrical parameters. The solvent effect has been modelled using the polarizable continuum model (PCM),[28] according to which the solvent is represented by a homogeneous dielectric medium with permitivity ε equal to that of

water (80) and includes electrostatic, dispersion–repulsion and cavitation terms.

The docking of the γ-lactams to the protein was performed by the program AutoDock (v. 3.0).[29]

The energetic and conformational details of the isolated esters and α-chymotrypsin structures, and the relevant binding complexes at 298 K, were obtained by performing MD simulations under isochoric/isothermal (NVT) conditions. Each molecular dynamics run was started by assigning initial velocity for the atoms according to a Boltzmann distribution at $2\times T$. Temperature was controlled via weak coupling to a temperature bath[30] with coupling constant $\tau_T = 0.01$ ps. The Newton molecular equations of motion were solved by the Verlet leapfrog algorithm,[31] using an integration step of 1 fs for a total simulation time of 200 ps.

In all cases, the complexation energies were calculated from the equilibrium molecular dynamics energy components of the non-bonded interactions for the α-CT/lactam complex $(E_{\alpha-CT/lactam})_{NB}$, the α-CTBCD $(E_{\alpha-CT})_{NB}$ and the γ-lactam $(E_{lactam})_{NB}$ using the following relationship:

$$(E^{lactam}_{complex})_{NB} = (E_{\alpha-CT/lactam})_{NB} - (E_{\alpha-CT})_{NB} - (E_{lactam})_{NB}$$

where $(E^{lactam}_{complex})_{NB}$ is the complexation energy for a given enantiomer inside the protein active site.

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