Chemo-Enzymatic Synthesis and Determination of the Absolute Configuration of Both Enantiomers of Methyl *trans*-5-Oxo-2-pentylpyrrolidine-3-carboxylate Precursors of the Aza Analogues of (+)- and (−)-Methylenolactocin

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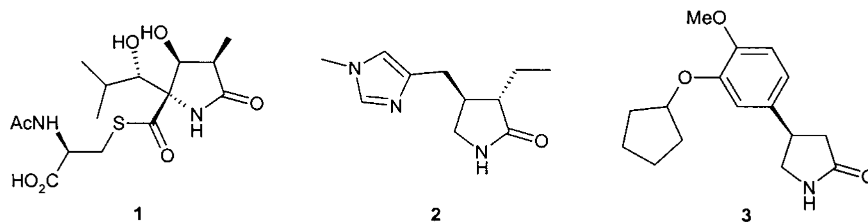
Dedicated with warm regards to Professor *Dieter Seebach* on the occasion of his 65th birthday

Enantiomerically pure methyl esters of (+)-(2*R*,3*S*)- and (−)-(2*S*,3*R*)-5-oxo-2-pentylpyrrolidine-3-carboxylic acid with 99% and 98% ee were obtained by enzymatic resolution of the corresponding racemic mixture using α-chymotrypsin and pig-liver acetone powder, respectively. Their absolute configurations were established by chemical methods, *i.e.*, conversion of the *trans*-γ-lactam moiety to the corresponding γ-lactone of known configuration. The favorable interactions between the *trans*-γ-lactam and α-chymotrypsin were rationalized by molecular-mechanics calculations, which suggest a different situation for the *cis*-diastereoisomer.

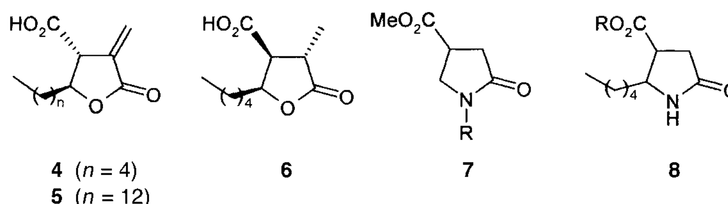
1. Introduction. – γ-Lactam and γ-lactone nuclei (pyrrolidin-2-ones and tetrahydrofuran-2-ones, respectively) are present in many compounds possessing biological and pharmaceutical activities [1]. Among the compounds containing the lactam ring, lactacystin (**1**) [1e,i] occupies a prominent position, since it is a potent 20S proteasome peptidase inhibitor and a challenge for researchers owing to the presence of four contiguous stereocenters [2]. Further examples are pilolactam (**2**) [3], recently patented by *Garst* and co-workers [4], which is important as a drug of muscarinic activity, and Rolipram (**3**), an antidepressant and phosphodiesterase inhibitor synthesized by *Meyers* and *Snyder* [5] and by *Mulzer et al.* [6] and manufactured by *Schering*.



Among the huge number of naturally occurring compounds containing the lactone ring, paraconic acids constitute an interesting small class of biologically active

trisubstituted γ-butyrolactones [7], which are characterized by the presence of a COOH group in β-position. Examples are (−)-methylenolactocin (**4**) [8], which possesses antitumor and antibiotic activity, (−)-protolichesterinic acid (**5**) [8a,c] [9], an antitumor, antibacterial, and growth-regulating compound, and (−)-phaseolinic acid (**6**) [8b][10], a metabolite of the fungus, *Macrophomina phaseolina*. Several asymmetric syntheses of these polyfunctionalized lactones have been reported in the literature [11], a few based on a chemo-enzymatic approach, *e.g.*, the synthesis of a precursor of (−)-**4** [12] and both enantiomers of **6** [10].

We started our studies on the synthesis of enantiomerically pure aza analogues of paraconic acids, in which the O-atom of the lactone ring is replaced by N, to determine their biological activity and toxicity [13]. Recently, we described [14] the optical resolution of the methyl esters of 1-alkyl-5-oxopyrrolidine-3-carboxylic acids (**7**) by chemo-enzymatic hydrolysis of the ester function. Among the commercially available lipases, proteases, and esterases tested [15], α-chymotrypsin (α-CT) turned out to be the choice for resolving most of the lactams studied, since it allows the isolation of the corresponding acids and esters with high enantiomeric excess (ee). The specificity of the enzyme and the high 'enantiopreference' observed were fully rationalized by means of molecular-mechanics calculations of the corresponding enzyme–substrate complex.

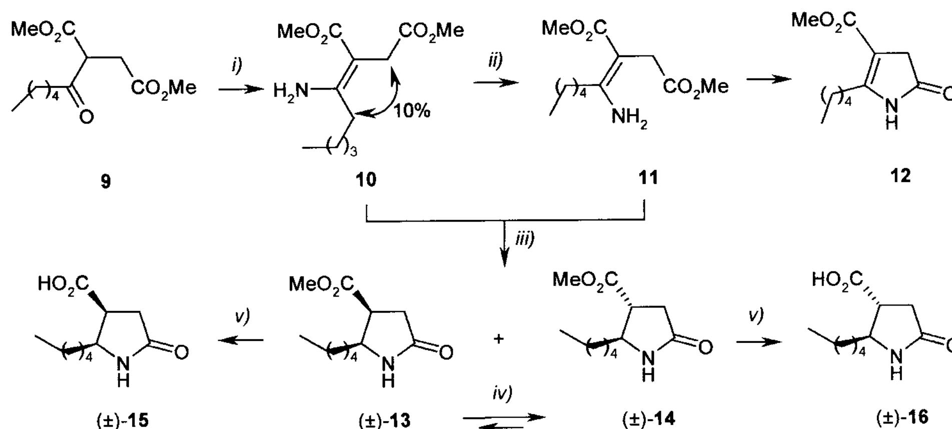


Here, we report our first results regarding the synthesis of 2-pentyl-5-oxopyrrolidine-3-carboxylic acids (and their methyl esters) of type **8** in pure enantiomeric form, with the aim of obtaining aza analogues of related paraconic acids.

2. Synthesis of Racemic Substrates. – The *cis*- and *trans*-configured target compounds **13** and **14**, respectively, were prepared by reductive amination [16] of dimethyl 2-hexanoylbutanedioate (**9**) with $AcONH_4$ and $NaBH_3CN$, followed by thermally-induced cyclization (*Scheme 1*). The initially-formed 1:1 diastereoisomeric mixture was equilibrated with DBU in $CHCl_3$ at room temperature for 72 h to afford a 1:4 mixture of the isomers *cis*-**13** and *trans*-**14**. The geometry of the thermodynamically more stable *trans*-isomer was confirmed by DIFNOE measurements. Irradiation of the H−C(3) *multiplet* at 2.84 ppm caused an enhancement (9%) of the signal at 1.60 ppm relative to the α-CH_2 group of the aliphatic chain linked to C(2).

The amination-reaction mechanism deserves some comment. The kinetically controlled enaminodiester **10**, formed from **9** by addition of $AcONH_4$, was isolated and characterized as the (*Z*)-diastereoisomer, as shown by a 10% NOE enhancement (*cf. Scheme 1*). However, weakly acidic $CDCl_3$ is sufficient to induce isomerization of **10** to **11**, which has the correct geometry for cyclization to methyl 4,5-dihydro-5-oxo-2-pentyl-1*H*-pyrrole-3-carboxylate (**12**). All attempts to reduce the unsaturated lactam

Scheme 1



i) $AcONH_4$, MeOH; *ii*) $CDCl_3$; *iii*) $NaBH_3CN$, MeOH; *iv*) DBU, $CHCl_3$, r.t.; *v*) H_3O^+, r.t.

ring of **12** failed. However, formation of the undesired by-product **12** could be avoided by immediately adding the reducing agent after treatment of **9** with $AcONH_4$.

3. Kinetic Resolution of (±)-13 and (±)-14. – Several enzymes were tested separately for their potential of resolving the racemic lactams **13** and **14**. Unfortunately, no commercially available hydrolytic enzyme led to a satisfactory resolution of the *cis*-diastereoisomer **13**. In fact, hydrolyses of **13** with α-chymotrypsin (α-CT) and pig-liver acetone powder (PLAP) proceeded with complete lack of stereoselectivity, leading to the racemic lactamic acid (±)-**15** (*Scheme 1*). Porcine pancreatic lipase (PPL) and *Candida Rugosa* lipase (CRL) were even completely inactive. However, the *trans*-diastereoisomer **14** was successfully resolved by both α-CT and PLAP (*Table 1*), although not very efficiently (the *E*-values [17] were low for both enzymes). Interestingly, however, the two enzymes showed an opposite preference towards the substrate, allowing the isolation of (+)-**14** with 99% ee (18% yield) and that of (−)-**14** with 98% ee (20% yield), respectively. Owing to the fact that the *E*-values were low, the corresponding acids (+)- and (−)-**16** were obtained with moderate ee at low conversion values (*Table 1*).

Interestingly, PPL, which was totally inactive with respect to the resolution of the *trans*-configured lactam (±)-**14**, had proved before to be most efficient in the hydrolysis of the analogous *trans*-oriented γ-lactone [12].

4. Determination of the Absolute Configuration of (+)- and (−)-14. – The absolute configuration of the optically active esters (+)- and (−)-**14**, obtained with 99% and 98% ee, respectively, was assigned by chemical methods (*Scheme 2*). The *trans*-compound (+)-**14** was *N*-Boc-protected [18]. Subsequent methanolysis [18] under basic conditions of the resulting lactam (−)-**17** furnished the corresponding dimethyl aminomethylsuccinate derivative (+)-**18** as a single stereoisomer. Deprotection of the amino group with HCl-saturated MeOH gave (+)-**19**, which was isolated and

Table 1. *Enzymatic Resolution of Lactam* (±)-**14**

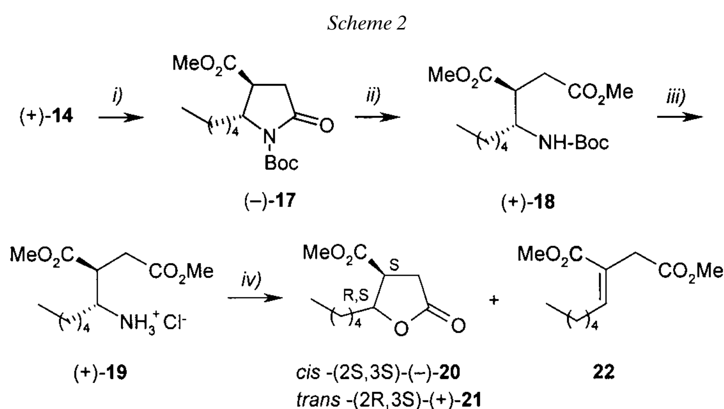
Enzyme	*E*-value	Conversion [%]	Substrate recovered		Isolated Product	
			Yield[a]) [%]	ee[b]) [%]	Yield[a]) [%]	ee[b]) [%]
α-CT[c])	3	27	(+)-**14**: 83	17	(−)-**16**: 23	45
		80	(+)-**14**: 23	99	(−)-**16**: 78	25
PLAP[d])	3	38	(−)-**14**: 51	41	(+)-**16**: 18	20
		88	(−)-**14**: 20	98	(+)-**16**: 15	13

[a]) Isolated yields. [b]) Enantiomeric excess, determined by chiral HR-GC. [c]) Conditions: substrate (500 mg), enzyme (50 mg), 0.1M phosphate buffer, pH 7.4 (20 ml), r.t. [d]) Conditions: substrate (500 mg), enzyme (500 mg), 0.1M phosphate buffer, pH 7.4 (30 ml), r.t.

characterized. Unfortunately, nitrosation of (+)-**19** according to [19] was by no means stereoselective and furnished, among the elimination product **22**, a 1:1 mixture of the corresponding *cis*- and *trans*-oriented γ-lactonic esters (−)-**20** and (+)-**21**. Their absolute configurations are known [12] [20] to be (2*S*,3*S*) and (2*R*,3*S*), respectively, for the respective ethyl esters. Since these experiments were originally made by high-resolution gas chromatography (HR-GC), separate samples of (−)-**20** and (+)-**21** were prepared for comparison starting from the available optically active *cis*- and *trans*-configured ethyl esters, which were first hydrolyzed and then esterified with CH_2N_2. Since the whole process did not involve C(3) in any step, the same absolute configuration, which turned out to be (*S*), could be attributed to C(3) in the parent lactam (+)-**14**. Therefore, the absolute configuration of (+)-**14** is (2*R*,3*S*), and that of (−)-**14** is (2*S*,3*R*).

5. Molecular modelling. – Although the *E*-value did not indicate a strong preference of α-CT for either enantiomer of **14**, the fact that optical resolution of the antipodes was achieved induced us to analyze the interactions of both diastereoisomers **13** and **14** with the enzyme. The analysis of the molecular models of the two α-CT/lactam complexes revealed that, in all cases, the lactams occupy the entire aryl binding site (a pocket comprising residues 189–194 on one side, and 214–220 on the other).

Scheme 2



i) $(Boc)_2O$, DMAP, Et_3N, CH_2Cl_2; *ii*) 2N MeONa, MeOH; *iii*) anh. HCl, MeOH; *iv*) 1N $NaNO_2$.

Nonetheless, the complexation energies $E_{complex}$ for (2*R*,3*R*)-**13** and (2*S*,3*S*)-**13** are in accordance with the experimental evidence (*Table 2*). In fact, $E_{complex}$ for (2*R*,3*R*)-**13** and (2*S*,3*S*)-**13** are practically identical, which is in line with the observation that α-CT does not discriminate the enantiomers with respect to hydrolysis. On the contrary, the calculated complexation energies for (2*R*,3*S*)-**14** and (2*S*,3*R*)-**14** agree with the observed preference of the enzyme towards the (2*S*,3*R*)-enantiomer (higher negative value).

Table 2. *Complexation Energies* ($E_{complex}$) *in kcal mol*$^{-1}$ *Calculated for* α*-CT/***13** *and* α*-CT/***14**

	(2*R*,3*R*)-**13**	(2*S*,3*S*)-**13**	(2*S*,3*R*)-**14**	(2*R*,3*S*)-**14**
$E_{complex}$	− 3.2	− 3.0	− 4.8	+ 2.6

In the case of (2*S*,3*R*)-**14**, which was hydrolyzed with the highest rate constant, the COOMe group adopts an orientation that should be favorable for the interaction with the catalytic triad His 57, Asp 102, and Ser 195. In contrast, it is impossible for the (2*R*,3*S*)-enantiomer to achieve a similar spatial arrangement in the enzyme active site because of the different orientation of the pentyl chain at C(2). Any attempt to eliminate this unfavorable situation by considering alternative conformers caused the chain to collide with the peptide backbone. Furthermore, although the above catalytic triad allows for a stable H-bonding pattern between the N(E2)-atom of His 57 and the OH group of Ser 195 and between the carboxylate oxygen O(D1) of Asp 102 and the NH of the peptide bond of Ala 56 and His 57, for the (2*S*,3*R*)-enantiomer, the analysis of the corresponding molecular-dynamics (MD) trajectory indicates that the ester C=O group appears to form an additional H-bond with the peptide NH bond between Asp 194 and Ser 195. The same group is also involved in another, alternative H-bond with the peptide backbone NH between Met 192 and Gly 193, characterized by an average dynamic length of 2.24 Å. Such interactions are not detected in the MD trajectory of the corresponding opposite enantiomer (2*R*,3*S*)-**14**. The aliphatic linear chain at the lactam ring nicely points into the aryl binding site of α-CT, where it favorably interacts with the hydrophobic side chain of Met 192.

6. Conclusions. – The kinetic resolution of the *trans* β,γ-disubstituted γ-lactam **14**, performed with two different enzymes, was achieved in high enantiomeric excess. Since (+)- and (−)-**14** are precursors of the aza analogues of optically active methylenolactocin, the synthesis of this target molecule in both enantiomeric forms is under study. An interesting observation regards the different behavior observed for these γ-lactams relative to the corresponding lactone analogues. While both diastereoisomeric, disubstituted γ-lactones can be enzymatically resolved, in the case of the corresponding γ-lactams, this was possible only for the *trans*-isomer, at least with the commercially available enzymes tested.

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Experimental Part

1. *General.* Abbreviations: DBU: 1,8-diazabicyclo[5.5.0]undec-7-ene, DMAP: 4-(dimethylamino)pyridine, FC: flash chromatography, HR-GC: high-resolution gas chromatography. M.p.: *Büchi SHP-20* apparatus, uncorrected. TLC: *Polygram® Sil G/UV$_{254}$* silica-gel coated plastic sheets, AcOEt/petroleum ether. FC: *Merck* silica-gel *60* (230–400 mesh), AcOEt/petroleum ether, unless otherwise stated. IR: *Avatar 320 FT-IR* spectrophotometer (*Thermo Nicolet*), in cm^{-1}. ^{1}H- and ^{13}C-NMR in $CDCl_3$ (unless otherwise stated): *Jeol EX-400* spectrometer (400 and 100 MHz, resp.), δ in ppm rel. to $SiMe_4$, J in Hz. Optical rotations: *Perkin-Elmer 241* polarimeter. CD: *Jasco J-700A* spectropolarimeter (0.1 cm cell). EI-MS: *VG 7070* spectrometer at 70 eV. ESI-MS: *PE-API* spectrometer at 5600 V by infusion of MeOH solns. GC: *OV 1701* column (25 m × 0.32 mm, carrier gas He, 180 kPa, split 1:50): *Carlo Erba GC-8000* instrument, temp. program.: 150° (2 min), then 3°/min up to 200°. Chiral GC: *Chiraldex*™ column, type *G-TA*, trifluoroacetyl-γ-cyclodextrin (40 m × 0.25 mm, He carrier gas, 180 kPa, split 1:100): *Shimadzu 14B* apparatus, temp. 150°, isothermal. Enzymatic hydrolyses: pH-stat *Controller PHM290 Radiometer*, Copenhagen. Porcine-liver acetone powder (PLAP) was supplied by *Sigma*, α-Chymotrypsin (α-CT; 53.1 U/mg) was purchased from *Fluka*.

2. *Synthesis of Racemic Lactams* **13** *and* **14**. To a soln. of **9** [21] (2.50 g, 10.0 mmol) in 30 ml of anh. MeOH, $AcONH_4$ (7.70 g, 100 mmol), and $NaBH_3CN$ (0.40 g, 6.3 mmol) were added. The mixture was stirred at r.t. for 24 h, the solvent was removed *in vacuo*, and the residue was refluxed in toluene (20 ml) for 30 min. After removal of the solvent, the residue was dissolved in sat. NaCl soln., extracted with Et_2O (3 × 20 ml), the combined org. extracts were washed with sat. $NaHCO_3$ soln. (2 × 10 ml), and dried (Na_2SO_4). The residue obtained after removal of the solvent was purified by FC (petroleum ether/AcOEt 90:10–60:40) to give the diastereoisomers **13** and **14** in a ratio of 55:45 in 70% overall yield. Treatment of this mixture with DBU in $CHCl_3$ at r.t. for 72 h gave a 1:9 mixture of **13/14**. When the reaction was carried out in the absence of $NaBH_3CN$, **10** was formed, which cyclized to **11** and **12** in the presence of acid traces from $CDCl_3$.

Dimethyl (Z)*-(1-Aminohexylidene)butanedioate* (**10**). IR (film): 3440, 3320 (NH), 1732 (CO_2Me), 1668 (NHCO), 1619 (C=C). ^{1}H-NMR: 3.65 (*s*, MeO); 3.63 (*s*, MeO); 3.21 (*s*, CH_2CO_2Me); 2.18 (*t*, $CH_2(CH_2)_3Me$); 1.51 (*m*, $CH_2CH_2(CH_2)_2Me$); 1.29–1.33 (*m*, $CH_2CH_2(CH_2)_2Me$); 1.19 (*t*, $(CH_2)_4Me$). ^{13}C-NMR: 173.7 (*s*); 170.4 (*s*); 162.6 (*s*); 87.1 (*s*); 51.7 (*q*); 50.6 (*q*); 34.1 (*t*); 32.4 (*t*); 31.5 (*t*); 27.5 (*t*); 22.4 (*t*); 13.9 (*q*). ESI-MS: 244.3 ($[M+H]^+$).

*Methyl 4,5-Dihydro-5-oxo-2-pentyl-1*H*-pyrrole-3-carboxylate* (**12**). IR (film): 3241 (NH), 1727 (CO_2Me), 1695 (NHCO), 1619 (C=C). ^{1}H-NMR: 9.13 (*s*, NH); 3.73 (*s*, MeO); 3.31 (*s*, 2 H–C(4)); 2.82 (*t*, $CH_2(CH_2)_3Me$); 1.60 (*m*, $CH_2CH_2(CH_2)_2Me$); 1.35 (*m*, $CH_2CH_2(CH_2)_2Me$); 0.90 (*t*, $(CH_2)_4Me$); ^{13}C-NMR: 177.3 (*s*); 164.3 (*s*); 155.9 (*s*); 103.6 (*s*); 51.0 (*q*); 37.3 (*t*); 31.3 (*t*); 27.0 (*t*); 26.9 (*t*); 22.3 (*t*); 13.9 (*q*). ESI-MS: 212.0 ($[M+H]^+$).

*Methyl (±)-*cis*-5-Oxo-2-pentylpyrrolidine-3-carboxylate* (**13**). M.p. 100–103°. IR (nujol): 3225 (NH), 1730 (CO_2Me); 1690 (NHCO). ^{1}H-NMR: 7.36 (br. *s*, NH); 3.87 (*m*, H–C(2)); 3.73 (*s*, MeO); 3.44 (*m*, H–C(3)); 2.77 (*dd*, J = 8.4, 16.8, H–C(4)); 2.41 (*dd*, J = 8.8, 16.8, H–C(4)); 1.27–1.38 (*m*, $CH_2(CH_2)_3Me$); 0.88 (*t*, $(CH_2)_4Me$). ^{13}C-NMR: 176.6 (*s*); 171.8 (*s*); 55.7 (*q*); 52.1 (*d*); 43.7 (*d*); 32.6 (*t*); 32.0 (*t*); 31.7 (*t*); 25.9 (*t*); 22.5 (*t*); 14.1 (*q*). EI-MS: 213 (4, M^+), 170 (10), 156 (15), 142 (100), 114 (17), 98 (13), 84 (16), 56 (18), 55 (35). Anal. calc. for $C_{11}H_{19}NO_3$ (213.3): C 61.95, H 8.98, N 6.57; found: C 61.78, H 8.92, N 6.70.

*Methyl (±)-*trans*-5-Oxo-2-pentylpyrrolidine-3-carboxylate* (**14**). M.p. 65–68°. IR (nujol): 3225 (NH), 1730 (CO_2Me), 1690 (NHCO). ^{1}H-NMR: 6.62 (br. *s*, NH); 3.82 (*m*, H–C(2)); 3.72 (*s*, MeO); 2.84 (*m*, H–C(3)); 2.62 (*m*, 2 H–C(4)); 1.60 (*m*, $CH_2(CH_2)_3Me$); 1.35–1.22 (*m*, $CH_2(CH_2)_3Me$); 0.85 (*m*, $(CH_2)_4Me$). ^{13}C-NMR: 175.6 (*s*); 173.4 (*s*); 57.3 (*q*); 52.5 (*d*); 45.2 (*d*); 36.5 (*t*); 33.8 (*t*); 31.6 (*t*); 25.5 (*t*); 22.5 (*t*); 14.1 (*q*). EI-MS: 213 (32, M^+), 185 (12), 184 (20), 143 (10), 142 (100), 129 (22), 114 (21), 98 (18), 82 (25), 55 (46). Anal. calc. for $C_{11}H_{19}NO_3$ (213.3): C 61.95, H 8.98, N 6.57; found: C 62.07, H 8.92, N 6.70.

3. *Enzymatic Hydrolysis.* Compounds (±)-**13** and (±)-**14** (0.50 g, 2.3 mmol) were suspended in a 0.1M KH_2PO_4/Na_2PO_4 buffer at pH 7.4 (40 ml), and the appropriate enzyme was added under vigorous stirring. The pH was continously adjusted to pH 7.4 with 1N NaOH soln. with a pH-stat. At the desired conversion value, the unreacted esters were extracted from the suspension with AcOEt (5 ×), using a centrifuge for the separation of the layers. The aqueous phase was acidified to pH 2 with 1N aq. HCl soln., evaporated *in vacuo*, and the corresponding acid (**15** or **16**) was extracted from the solid residue with MeCN. The enantiomeric excess (ee) of the product was determined by chiral HR-GC after re-esterification of the COOH function with CH_2N_2.

(+)-(2*R*,3*S*)-**14**, after esterification, was isolated from hydrolysis with α-CT (0.050 g/0.500 g substrate) at 80% conversion (30 h) with 99% ee in 23% yield. $[\alpha]_D^{25} = +23$ (c = 0.75, MeOH). CD (MeOH): $\Delta\varepsilon = -2.2$ (213 nm).

(−)-(2*S*,3*R*)-**14**, was isolated from hydrolysis with PLAP (0.500 g/0.500 g substrate) at 88% conversion (8 h) in 20% yield and 98% ee. $[\alpha]_D^{25} = -22$ ($c = 0.6$, MeOH). CD (MeOH): $\Delta\varepsilon = +2.2$ (213 nm).

*(±)-*cis*-5-Oxo-2-pentylpyrrolidine-3-carboxylic Acid* (**15**). Hydrolysis of (±)-**13** with α-CT and PLAP, quenched at 20% conversion, led, after the usual workup, to (±)-**13** (70% yield) and (±)-**15** (18% yield). Data of (±)-**15**: m.p. 138–140°. IR (nujol): 3238 (COOH), 1715 (COOH), 1650 (NCO). ¹H-NMR: 10.80 (br. *s*, COOH); 7.73 (*s*, NH); 3.90 (*m*, H−C(2)); 3.44 (*m*, H−C(3)); 2.83 (*dd*, *J* = 17.6, 8.3, H−C(4)); 2.48 (*dd*, *J* = 17.6, 8.8, H−C(4)); 1.30–1.59 (*m*, $(CH_2)_4$Me); 0.88 (br. *t*, $(CH_2)_4$*Me*). ¹³C-NMR: 178.1 (*s*); 175.1 (*s*); 55.9 (*d*); 43.4 (*d*); 32.5 (*t*); 31.5 (*t*); 31.4 (*t*); 25.8 (*t*); 22.4 (*t*); 13.9 (*q*). EI-MS: 199 (4, M^+), 171 (3), 142 (4), 128 (100), 115 (10), 100 (15), 100 (30), 56 (82). Anal. calc. for $C_{10}H_{17}NO_3$ (199.3): C 60.28, H 8.60, N 7.03; found: C 60.32, H 8.64, N 6.94.

*(−)-(2*S*,3*R*)-5-Oxo-2-pentylpyrrolidine-3-carboxylic Acid* (**16**). Isolated from the enzymatic hydrolysis with α-CT at 27% conversion (8 h) in 19% yield and 45% ee. M.p. 141–143°. $[\alpha]_D^{25} = -14.0$ ($c = 0.5$, MeOH). CD (MeOH): $\Delta\varepsilon = -0.73$ (214 nm). IR (nujol): 3238 (COOH), 1715 (COOH), 1650 (NCO). ¹H-NMR: 10.02 (br. *s*, COOH); 7.59 (*s*, NH); 3.83 (*m*, H−C(2)); 2.85 (*m*, H−C(3)); 2.72 (*dd*, *J* = 17.1, 7.3, H−C(4)); 2.59 (*dd*, *J* = 17.1, 9.8, H−C(4)); 1.64–1.43 (*m*, $CH_2(CH_2)_3$Me), 1.30–1.20 (*m*, $CH_2(CH_2)_3$Me); 0.80 (br. *t*, $(CH_2)_4$*Me*). ¹³C-NMR: 177.4 (*s*); 176.6 (*s*); 57.9 (*d*); 44.8 (*d*); 36.3 (*t*); 33.5 (*t*); 31.4 (*t*); 25.4 (*t*); 22.4 (*t*); 13.9 (*q*). EI-MS: 199 (18, M^+), 171 (33), 157 (10), 142 (10), 129 (100), 116 (80), 101 (25), 100 (30), 82 (35), 57 (98), 56 (80). Anal. calc. for $C_{10}H_{17}NO_3$ (199.3): C 60.28, H 8.60, N 7.03; found: C 60.42, H 8.56, N 6.98.

The enantiomer (2*R*,3*S*)-(+)-**16** was isolated from the hydrolysis with PLAP at 38% conversion (70 min) in 20% yield and 41% ee.

4. *Conversion of (+)-(2*R*,3*S*)-**14** to (+)-(2*S*,3*S*)-**20** and (−)-(2*S*,3*S*)-**21***. 4.1. *1-(*tert*-Butyl) 3-Methyl 5-oxo-2-pentylpyrrolidine-1,3-dicarboxylate* (**17**). To a soln. of (+)-(**14**) (0.200 g, 0.94 mmol) in 5 ml of CH_2Cl_2, Boc_2O (0.35 g, 1.9 mmol), DMAP (0.170 g, 0.94 mmol), and Et_3N (0.11 ml, 0.94 mmol) were added, and the resulting soln. was stirred at r.t. until the substrate was consumed (TLC, AcOEt). The solvent was removed *in vacuo* and the residue was purified by FC ($CHCl_3$) to give 0.26 g of (−)-**17** (90%). $[\alpha]_D^{25} = -35.0$ ($c = 1.0$, MeOH). IR (film): 1788, 1742 (COO*ᵗ*Bu, COOMe), 1716 (NCO). ¹H-NMR: 4.25 (br. *d*, H−C(2)); 3.7 (*s*, COOMe); 2.82–2.67 (*m*, H−C(3), 2 H−C(4)); 1.76, 1.49 (2*m*, 1 H, $CH_2(CH_2)_2$Me); 1.48 (*s*, *ᵗ*Bu); 1.24 (br. *m*, $CH_2(CH_2)_3$Me); 0.81 (br. *t*, $(CH_2)_4$*Me*). ¹³C-NMR: 172.9 (*s*); 171.6 (*s*); 149.3 (*s*); 83.0 (*s*); 60.4 (*d*); 52.5 (*q*); 39.6 (*d*); 34.0 (*q*); 33.8 (*q*); 31.2 (*t*); 27.8 (*q*); 24.6 (*t*); 22.3 (*t*); 13.7 (*q*). EI-MS: 214 (2), 198 (5), 154 (8), 142 (100), 129 (13), 114 (13), 98 (13), 82 (10), 57 (88), 41 (61), 28 (32). ESI-MS: 336.1 ([M + Na]⁺), 352 ([M + K]⁺).

4.2 *Dimethyl (+)-(2*S*)-2-{(1*R*)-1-[*N*-(*tert*-butoxycarbonyl)amino]hexyl}butane-1,4-dioate* (**18**). To a soln. of (−)-**17** (0.250 g, 0.8 mmol) in MeOH, a 2.0N soln. of MeONa (0.4 ml) was added. After stirring at r.t. for 30 min, the solvent was removed *in vacuo*, brine was added, and the aq. phase was extracted with Et_2O. Evaporation of the solvent and FC (CH_2Cl_2) gave 0.257 g of (+)-**18** (93%). $[\alpha]_D^{25} = +25$ ($c = 0.5$, MeOH). IR (film): 3372 (NH); 1740, 1716, 1696 (COOMe, CONH); 1520 (NHCO). ¹H-NMR: 4.57 (br. *d*, NH); 3.86 (*m*, CHNH); 3.68 (*s*, MeO); 3.64 (*s*, MeO); 2.96 (*m*, H−C(2)); 2.74, 2.70 (*dd*, *J* = 16.5, 10.2, H−C(3)); 2.42 (*dd*, *J* = 16.5, 4.4, H−C(3)); 1.39–1.33 (*s* and *m*, *t*-Bu and $CH_2CH_2(CH_2)_2$Me); 1.22 (*m*, $CH_2CH_2(CH_2)_2$Me)); 0.84 (br. *t*, $(CH_2)_4$*Me*). ¹³C-NMR: 173.35 (*s*); 172.4 (*s*); 155.4 (*s*); 79.4 (*s*); 52.0 (*q*); 51.9 (*q*); 51.6 (*d*); 46.2 (*d*); 32.7 (*t*); 32.6 (*t*); 28.3 (*q*); 25.6 (*t*); 22.4 (*t*); 13.9 (*q*). ESI-MS: 346 (M^+).

4.3. *Dimethyl (+)-(2*S*)-2-[(1*R*)-1-Aminohexyl]butane-1,4-dioate Hydrochloride* (**19**). A soln. of (+)-**18** (0.210 g, 0.6 mmol) in MeOH was saturated with gaseous HCl. After 30 min stirring, the solvent was evaporated to give (+)-**19** as a semisolid material that was used in the next step without purification. $[\alpha]_D^{25} = +13$ ($c = 0.5$, MeOH). ¹H-NMR: 8.52 (br. *s*, NH_3^+); 3.79 (*s*, MeO); 3.68 (*s*, MeO); 3.64 (br. *m*, *CH*NH_3^+); 3.40 (*m*, H−C(2)); 2.96 (*dd*, H−C(3)); 2.64 (*dd*, H−C(3)); 1.85–1.38 (*m*, 8 H); 0.87 (*t*, $(CH_2)_4$*Me*). ¹³C-NMR: 171.8 (*s*); 171.7 (*s*); 53.0 (*d*); 52.5 (*q*); 52.2 (*q*); 42.8 (*d*); 31.8 (*t*); 31.2 (*t*); 30.3 (*t*); 25.3 (*t*); 22.3 (*t*); 14.00 (*q*). ESI-MS: 246.3 ([M + H]⁺).

4.4. *Methyl (−)-(2*S*,3*S*)- and (+)-(2*R*,3*S*)-5-Oxo-2-pentyltetrahydrofuran-3-carboxylate* **20** *and* **21**. A 1M aq. soln. of $NaNO_2$ (0.9 ml, 0.9 mmol) was added dropwise to a soln. of (+)-**19** (0.6 mmol) in H_2O at 0–5° under vigorous stirring. The soln. was then stirred at r.t. overnight. Evaporation to dryness gave a residue, which was extracted with Et_2O. Evaporation of the solvent furnished a solid 1:1 mixture of (−)-**20** and (+)-**21** (44%), both with 87% ee. (−)-**20**: $[\alpha]_D^{25} = -65$ ($c = 0.25$, MeOH); (+)-**21**: $[\alpha]_D^{25} = +29$ ($c = 0.5$, MeOH). The remaining 54% of material was identified as *dimethyl hexylidenbutane-1,4-dioate* (**22**). *Data of* **22**: IR (film): 1739, 1712 (COOMe), 1654 (C=C). ¹H-NMR: 6.98 (*t*, *J* = 7.7, *CH*=C); 3.75 (*s*, MeO); 3.69 (*s*, MeO); 3.36 (*s*, CH_2COOMe); 2.18 (*q*, *J* = 7.3, CH_2−C=C); 1.46 (*quint.*, $CH_2(CH_2)_3$Me); 1.30 (*m*, $CH_2(CH_2)_3$Me); 0.89 (*t*, $(CH_2)_4$*Me*). ¹³C-NMR: 171.2 (*s*); 167.4 (*s*); 146.1 (*d*); 125.2 (*s*); 51.91 (*q*); 51.88 (*q*); 32.1 (*t*); 31.4 (*t*); 28.9 (*t*); 28.1 (*t*); 22.4 (*t*); 13.9 (*q*). ESI-MS: 229.2 (M^+).

5. *Molecular-Mechanics/Dynamics Calculations.* The starting model of α-CT was based on its X-ray crystallographic structure [22]. H_2O Molecules in the coordinate file were removed, and H-atoms were added to the protein backbone and side chains with the PARSE module of the AMBER 6.0 package [23]. All ionizable residues were considered in the standard ionization state at neutral pH. The all-atom force field (FF) parameters by *Cornell et al.* [24] (*parm94.dat* file of the AMBER 6.0 code) was applied for protein relaxation. The GB/SA continuum solvation model [25] was used to mimic an aqueous environment. Geometry refinement was carried out with the SANDER module *via* a combined steepest descent/conjugate gradient algorithm. As a convergence criterion for the energy gradient, the root-mean-square of the Cartesian elements of the gradient were set equal to 0.01 kcal mol^{-1} $Å^{-1}$. As expected, no relevant structural changes were observed between the active site of the α-CT relaxed structure and the original three-dimensional structure.

The model structures of all enantiomers of the 2,3-disubstituted lactams were generated with the 3-D sketcher tool of *Cerius²* (vers. 4.2, *Accelrys*, San Diego, CA, USA). All the molecules were subjected to an initial energy minimization using *Discover*. In this case, the convergence criterion was set to 10^{-4} kcal mol^{-1} $Å^{-1}$. The conformational search was carried out by means of a combined molecular-mechanics/molecular-dynamics-simulated annealing (MDSA) protocol [26]. The docking of the lactams to the protein was performed by the program *AutoDock* (vers. 3.0) [27].

The energetic and conformational details of the free and bound substrates and α-CT structures, at 298 K were obtained by performing molecular-dynamics (MD) simulations under isochoric/isothermal (*NVT*) conditions. Each MD run was started by assigning an initial velocity to the atoms according to a *Boltzmann* distribution at $2 \times T$. The temp. T was maintained constant by the *Berendsen* coupling algorithm [28]. The *Newton* molecular equation of motion was solved by the *Verlet* leapfrog algorithm [29] with an integration step of 1 fs for a total simulation time of 200 ps. In all cases, the complexation energies $E_{complex}$ were calculated from the equilibrium MD energy components of the *non-bonded* interactions for the α-CT/lactam complex ($E_{\alpha\text{-CT/lactam}}$), the α-CT ($E_{\alpha\text{-CT}}$), and the lactam (E_{lactam}) according to [14]:

$$E_{complex} = E_{\alpha\text{-CT/lactam}} - E_{\alpha\text{-CT}} - E_{lactam}.$$

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