Chemo-Enzymatic Synthesis and Determination of the Absolute Configuration of Both Enantiomers of Methyl \textit{trans}-5-Oxo-2-pentylpyrrolidine-3-carboxylate Precursors of the Aza Analougues of (\texttt{+})- and (\texttt{-})-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 (\texttt{+})(2R,3S)-(2R,3S)- and (\texttt{-})(2S,3R)-5-oxo-2-pentylpyrrolidine-3-carboxylic acid with 99% and 98% ee were obtained by enzymatic resolution of the corresponding racemic mixture using \texttt{c97}-chymotrypsin and pig-liver acetone powder, respectively. Their absolute configurations were established by chemical methods, \textit{i.e.}, conversion of the \textit{trans}-\textit{\gamma}-lactam moiety to the corresponding \textit{\gamma}-lactone of known configuration. The favorable interactions between the \textit{trans}-\textit{\gamma}-lactam and \texttt{c97}-chymotrypsin were rationalized by molecular-mechanics calculations, which suggest a different situation for the \textit{cis}-diastereoisomer.

1. Introduction. – \textit{\gamma}-Lactam and \textit{\gamma}-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.

\begin{center}
\textbf{1} \hspace{1cm} \textbf{2} \hspace{1cm} \textbf{3}
\end{center}

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 ‘enantipreference’ 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 AcONH₄ and NaBH₃CN, followed by thermally-induced cyclization (Scheme 1). The initially-formed 1:1 diastereoisomeric mixture was equilibrated with DBU in CHCl₃ 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-CH₃ group of the aliphatic chain linked to C(2).

The amination-reaction mechanism deserves some comment. The kinetically controlled enamindiestester 10, formed from 9 by addition of AcONH₄, was isolated and characterized as the (Z)-diastereoisomer, as shown by a 10% NOE enhancement (cf. Scheme 1). However, weakly acidic CDCl₃ 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-1H-pyrrole-3-carboxylate (12). All attempts to reduce the unsaturated lactam
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 AcONH₄.

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, hydrolys 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 methanalysis [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
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 (2S,3S) and (2R,3S), 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 CH2N2. 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 (2R,3S), and that of (−)-14 is (2S,3R).

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).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>E-value</th>
<th>Conversion [%]</th>
<th>Substrate recovered</th>
<th>Isolated Product</th>
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<tr>
<td></td>
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<td>Yield [%]</td>
<td>ee [%]</td>
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<tr>
<td>α-CT&lt;sup&gt;)&lt;/sup&gt;</td>
<td>3</td>
<td>27</td>
<td>(+)-14: 83</td>
<td>17</td>
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<tr>
<td></td>
<td></td>
<td>80</td>
<td>(+)-14: 23</td>
<td>99</td>
</tr>
<tr>
<td>PLAP&lt;sup&gt;)&lt;/sup&gt;</td>
<td>3</td>
<td>38</td>
<td>(−)-14: 51</td>
<td>41</td>
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<tr>
<td></td>
<td></td>
<td>88</td>
<td>(−)-14: 20</td>
<td>98</td>
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<sup>a)</sup> Isolated yields. <sup>b)</sup> Enantiomeric excess, determined by chiral HR-GC. <sup>c)</sup> Conditions: substrate (500 mg), enzyme (50 mg), 0.1 M phosphate buffer, pH 7.4 (20 ml), r.t. <sup>d)</sup> Conditions: substrate (500 mg), enzyme (500 mg), 0.1 M phosphate buffer, pH 7.4 (30 ml), r.t.

![Scheme 2](image)

i) (Boc)2O, DMAP, Et3N, CH2Cl2; ii) 2N MeONa, MeOH; iii) anh. HCl, MeOH; iv) 1N NaNO2.
Nonetheless, the complexation energies $E_{\text{complex}}$ for (2$R$,3$R$)-13 and (2$S$,3$S$)-13 are in accordance with the experimental evidence (Table 2). In fact, $E_{\text{complex}}$ for (2$R$,3$R$)-13 and (2$S$,3$S$)-13 are practically identical, which is in line with the observation that $\alpha$-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>
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<tr>
<th></th>
<th>(2$R$,3$R$)-13</th>
<th>(2$S$,3$S$)-13</th>
<th>(2$S$,3$R$)-14</th>
<th>(2$R$,3$S$)-14</th>
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<tr>
<td>$E_{\text{complex}}$</td>
<td>–3.2</td>
<td>–3.0</td>
<td>–4.8</td>
<td>+2.6</td>
</tr>
</tbody>
</table>

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 $\alpha$-CT, where it favorably interacts with the hydrophobic side chain of Met 192.

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

We gratefully acknowledge the financial support of MIUR (Rome), CNR (Rome), and the University of Trieste.
Experimental Part

1. General. Abbreviations: DBU: 1,8-diazabicyclo[5.5.0]undec-7-ene, DMAP: 4-(dimethylamino)pyridine, FC: flash chromatography, HR: high-resolution gas chromatography. M.p.: Büchi SHP-20 apparatus, uncorrected. TLC: Polygram Sil G/UV254 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. ¹H- and ¹³C-NMR (CDCl₃, unless otherwise stated): Perkin-Elmer 241 polarimeter. CD: Jasco J-715A 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 solutions. GC: OV1701 column (25 m × 0.25 mm, carrier gas He, 180 kPa, split 1:50): Thermo Nicolet instrument, temp. program.: 150°C (2 min), then 3°C/min up to 200°C. Chiral GC: Chiralcel™ column, type G-TA, trifluoroacetyl-cyclodextrin (40 m × 0.25 mm, He carrier gas, 180°C, split 1:100). Shimadzu 14B apparatus, temp. 150°C, isothermal. Enzymatic hydrolses: pH-stat Controller PHM290 Radiometer. Porcine-liver acetone powder (FLAP) 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 solution of 9 [21] (2.50 g, 10.0 mmol) in 30 ml of anh. MeOH, AcONH₄ (7.70 g, 100 mmol), and NaBH₃CN (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 reflushed in toluene (20 ml) for 30 min. After removal of the solvent, the residue was dissolved in sat. NaCl soln., extracted with Et₂O (3 × 20 ml), the combined org. extracts were washed with sat. NaHCO₃ soln. (2 × 10 ml), and dried (Na₂SO₄). 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 DIBU in CHCl₃ at r.t. for 72 h gave a 1:9 mixture of 13/14. When the reaction was carried out in the absence of NaBH₃CN, 10 was formed, which cyclized to 11 and 12 in the presence of acid traces from CDCl₃.

3. Enzymatic Hydrolysis. Compounds (+)-13 and (−)-14 (0.50 g, 2.3 mmol) were suspended in a 0.1 M KH₂PO₄/Na₂PO₄ buffer at pH 7.4 (40 ml), and the appropriate enzyme was added under vigorous stirring. The pH was continuously adjusted to pH 7.4 with 1m 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 1m 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 with the COOH function with CH₃N₃.

(+)-(2R,3S)-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. [α]D² = +23 (c = 0.75, MeOH). CD (MeOH): Δτ = −2.2 (213 nm).
H₂₈.1 (C₉₇ (13), 98 (13), 82 (10), 57 (88), 41 (61), 28 (32). ESI-MS: 336.1 (m/z to give (film): 3372 (NH); 1740, 1716, 1696 (COOMe, CONH); 1520 (NHCO). 1H-NMR: 4.57 (br. t, 1 H, C(4)); 1.64±1.43 (m, (CH₂)₄S); 1.39±1.33 (m, (CH₂)₄C). ESI-MS: 198 (M⁺), 171 (33), 157 (10), 142 (10), 129 (100), 116 (80), 101 (25), 100 (30), 82 (35), 57 (98), 56 (80). Analog. calc. for C₉H₁₈NO (199.3): C 60.28, H 8.60, N 703; found: C 60.32, H 8.64, N 6.94.

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

4. Conversion of (+)-(2R,3S)-14 to (+)-(2S,3S)-20 and (−)-(2S,3S)-21. 1. J-(tert-Butyl) 3-Methyl S-5-oxo-2-pentptyrylridine-1,3-dicarboxylate (17). To a soln. of (+)-14 (0.200 g, 0.94 mmol) in 5 ml of CH₂Cl₂, Boc₂O (0.35 g, 1.9 mmol), DMAP (0.170 g, 0.94 mmol), and Et₃N (0.11 ml, 0.64 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 (CHCl₃) to give 0.26 g of (−)-17 (95%, [α]D₂⁰ = +35.0 (c = 1.0, MeOH). IR (film): 1788, 1742 (COOBU, COOMe), 1716 (NCO). ¹H-NMR: 4.25 (br. d, H – C(2)); 3.7 (s, COOMe); 2.82–2.67 (m, H – C(1)); 1.76, 1.49 (2m, 1 H, C₈H₄(CH₂)Me); 1.48 (s, Bu); 1.24 (br. m, C₈H₄(CH₂)Me); 0.81 (br. t, CH₃Me). ¹³C-NMR: 172.9 (q); 171.6 (s); 149.3 (s); 83.0 (d); 65.4 (d); 52.5 (q); 34.0 (q); 33.8 (q); 31.2 (q); 27.8 (q); 24.6 (t); 22.3 (t); 13.7 (q). CD: 214 (24), 196 (48), 129 (13), 114 (13), 98 (13), 82 (10), 57 (88), 41 (61), 38 (23). ESI-MS: 356.1 ([M + Na]⁺), 352 ([M + K]⁺).

4.3. Dimethyl (+)-(2S)-2-(1R)-2-Aminohexylbutane-1,4-dioate Hydrochloride (19). A soln. of (+)-18 (0.210 g, 0.60 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. [α]D₂⁰ = +13 (c = 0.5, MeOH). ¹H-NMR: 8.52 (br. s, NH⁺); 3.79 (s, MeO); 3.68 (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 CH₂CH₂(CH₂)Me); 1.22 (m, CH₃CH₂(CH₂)Me); 0.84 (br. t, CH₃Me). ¹³C-NMR: 173.5 (s); 172.4 (s); 155.4 (s); 79.4 (q); 52.0 (q); 51.9 (q); 51.8 (q); 46.2 (d); 32.7 (t); 32.6 (t); 28.3 (q); 25.6 (t); 22.4 (t); 13.9 (q). CD: 346.1 (M⁺).

4.4. Methyl (−)-(2S)- and (+)-(2R,3S)-5-Oxo-2-pentetylhydrofururan-3-carboxylate 20 and 21. A litq. soln. of NaNO₂ (0.09 ml, 0.9 mmol) was added dropwise to a soln. of (+)-19 (0.60 mmol) in H₂O 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 EtO. Evaporation of the solvent furnished a solid 1:1 mixture of (−)-20 and (+)-21 (44%), both with 87% ee. (−)-20 [α]D₂⁰ = −65 (c = 0.25, MeOH); (+)-21 [α]D₂⁰ = +29 (c = 0.5, MeOH). The remaining 54% of material was identified as dimethyl hexylidenbutane-1,4-dioate (22). ESI-MS: 246.3 ([M + H]⁺).

4.5. Methyl (−)-(2R,3S)- and (−)-(2S,3S)-5-Oxo-2-pentetylhydrofururan-3-carboxylate 20 and 21. A litq. soln. of NaNO₂ (0.09 ml, 0.9 mmol) was added dropwise to a soln. of (+)-19 (0.60 mmol) in H₂O 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 EtO. Evaporation of the solvent furnished a solid 1:1 mixture of (−)-20 and (+)-21 (44%), both with 87% ee. (−)-20 [α]D₂⁰ = −65 (c = 0.25, MeOH); (+)-21 [α]D₂⁰ = +29 (c = 0.5, MeOH). The remaining 54% of material was identified as dimethyl hexylidenbutane-1,4-dioate (22).
5. Molecular-Mechanics/Dynamics Calculations. The starting model of α-CT was based on its X-ray crystallographic structure [22]. H₂O 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 [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 was carried out with the SANDER module energy minimization using parameters by residues were considered in the standard ionization state at neutral pH. The all-atom force field (FF) the protein backbone and side chains with the PARSE module of the AMBER 6.0 package [23]. All ionizable 

The model structures of all enantiomers of the 2,3-disubstituted lactams were generated with the 3-D sketcher tool of Discover (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 10⁻⁶ kcal mol⁻¹ Å⁻³. 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 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 × 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 Ecomplex were calculated from the equilibrium MD energy components of the non-bonded interactions for the α-CT/lactam complex (Eα-CT/lactam), the α-CT (Eα-CT), and the lactam (Elactam) according to [14]:

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

REFERENCES


