Enantiomerically pure beta,gamma-disubstituted gamma-lactams: Enzyme-mediated synthesis and computational studies

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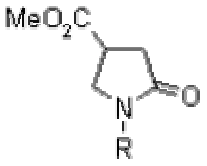
In a recent paper,[1] we described the asymmetric synthesis of some β-alkoxycarbonyl-γ-lactams **1**, based on the enzyme-mediated hydrolysis of their racemates. In the case of α-chymotrypsin (α-CT), the hydrolysis of three N-substituted lactams proved to be highly enantioselective, whereas the unsubstituted one was recovered in the corresponding racemic form. This experimental evidence prompted us to apply several molecular modeling protocols to explain the substrate specificity and the enantioselectivity of this enzyme.[2] The adopted procedures involved accurate docking experiments of both enantiomers of each lactam to the protein active site, whose 3-D structure was obtained from X-ray crystallographic data, followed by extensive conformational and energetic analysis of the computer-generated complexes. The results obtained fully accounted for the experimental evidences on the enantioselective hydrolysis of these compounds by α-chymotrypsin.

As an extension of the work concerning the production of β-alkoxycarbonyl-γ-lactams in enantiomerically pure form, we devoted our attention to the β,γ-disubstituted derivatives **2** and **3.**



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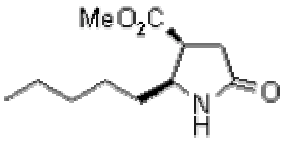

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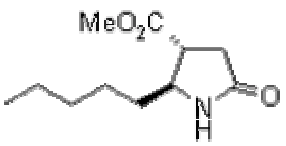

3

A number of substances based on the γ-lactam (2-pyrrolidinone) structure, besides being key intermediates in the synthesis of many biologically important compounds,[3-5] exhibit themselves interesting biological and pharmacological properties, such as psychotropic, antihypertensive and antimuscarinic activity.[6-8] Nonetheless, despite their intrinsic potentiality for biological activity, both the racemic form and the enantiomerically pure derivatives of the γ-lactam bearing

the carboxylic group at the β-position have received little attention. Our interest in the enantiomerically pure form of such compounds stems from the fact that their naturally occurring oxygen analogs (i.e. paraconic acid derivatives) possess topical biological properties,[9] but these aza analogs could be potentially more effective, due to the lower toxicity of the lactam ring with respect to the lactone one.[10]

Accordingly, compounds **2** and **3**, obtained in their racemic form by a literature method[11], were treated for kinetic resolution with several enzymes. At present no commmercially available hydrolytic enzyme was found to be active on the *cis* diastereomer **2**, whereas for the *trans* derivative **3**, Pig Liver Acetone Powder (PLAP) and α-CT proved to be active. Although without high efficiency (E was low in both cases), the two enzymes showed an opposite enantiopreference towards the substrate, providing (–)-**3** with 98% e.e. and 11% yield, and (+)-**3** with 82% e.e. and 18 % yield (see Table 1).

Table 1

Enzymatic resolution of lactam **3**

		Low conversion				High conversion		
Substrate	Enzyme	E	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]
3	PLAP[c]	3	38	(–)-**14** 25 (62)	(+)-**16** 41	88	(–)-**14** 98 (11)	(+)-**16** 13 (15)
	a-CT[d]	4	24	(+)-**14** 17 (83)	(–)-**16** 54 (19)	82	(+)-**14** 82 (18)	(–)-**16** 18 (18)

a)Enantiomeric excesses were determined by chiral HRCG. b) Yields in isolated products. c) Reaction conditions: 300 mg substrate, 300 mg enzyme, 0.1 phosphate buffer at pH 7.4 (20 ml), room temperature. d) Reaction conditions: 300 mg substrate, 30 mg enzyme, 0.1 phosphate buffer at pH 7.4 (20 ml), room temperature.

To rationalize these results, the interaction of (R,R)-2, (S,S)-2, (R,S)-3 and (S,R)-3 with the α-chymotrypsin active site has been simulated using a fine-tuned automated docking procedure, subsequently refined by quenched molecular dynamics[2]. Further, to investigate the molecular basis of α-CT towards the experimentally verified enantioselective hydrolysis of this series of compounds,

with a combination of molecular mechanics energy derived from MD simulations in explicit solvent, and solvation free energy derived from a continuum solvation model, we have calculated reasonable absolute free energies of binding for all α-CT/enantiomer complexes formation.

In general, the energetic analysis reveals that the van der Waals interactions and the nonpolar contributions to solvation always provide the basis for the favorable absolute free energy of binding. On the other hand, a delicate balance exists between the always favorable gas-phase electrostatics term and the unfavorable change in electrostatic contribution to the solvation. Indeed, by counteracting the favorable electrostatic interactions that form between the lactam and the protein binding site, the desolvation of the protein residues plays an important role in determining the effect of the electrostatics, as a whole, on the formation of the α-CT/lactam enantiomer complex.

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