Theoretical Study of the Alkaline Hydrolysis of Tricyclic Carbapenem

by **Hector J. Fasoli**[a]) and **Juan Frau***[b])

[a]) Facultad de Ciencias Fisicomatemáticas e Ingeniería, Universidad Católica Argentina,
Alicia M. de Justo 1500, C1107AAZ, Argentina
[b]) Departament de Química (Química-Física) and Institut Universitari de les Ciències de la Salut,
Universitat de les Illes Balears, Palma de Mallorca 07122, Illes Balears, Spain
(e-mail: juan.frau@uib.es)

Alkaline hydrolysis is a good test to evaluate the properties of β-lactam compounds and derivatives. In this work, a RHF/6-31 + G*//RHF/6-31 + G* theoretical study of the mechanism controlling the alkaline hydrolysis of sanfetrinem was conducted. The geometric properties of this compound are consistent with an intrinsic reactivity similar to that of other β-lactams including penicillins and cephalosporins. Also, similarly to cephalosporins, the MeO group provides an alternative route for the hydrolysis mechanism.

Introduction. – One of the most-common ways that bacteria become resistant to β-lactam antibiotics (*viz.* penicillins, cephalosporins, and related compounds) is by producing β-lactamases. These enzymes catalyze the cleavage of the amide bond in the β-lactam ring of the antibiotic and give rise to the formation of bacterially inactive products [1][2]. β-Lactamases can be of four different classes according to the amino acid sequence at the active site, *viz.*, A, B, C, and D. In class-A, -C, and -D β-lactamases, a serine residue at the active site acts as the hydrolyzing agent; on the other hand, class-B β-lactamases are Zn^{2+}-dependent enzymes [3–5].

Attempts at solving the problems derived from bacterial resistance are currently aimed in two directions. One is the development of antibiotics spanning a broad antibacterial spectrum and capable of withstanding the action of enzymes such as β-lactamases [6]. The other is the development of compounds capable of inactivating β-lactamases for use in combination with provenly efficient antibiotics (*e.g.*, clavulanic acid in conjunction with amoxicillin [7]).

In 1996, scientists at *Glaxo Wellcome* reported a novel antibiotic class, *viz.*, the tricyclic β-lactam antibiotics known as 'trinems' or tricyclic carbapenem compounds [8]. The following year, *Hanessian* and co-workers [9] developed an efficient synthesis of 4α-methoxytrinem (sanfetrinem, *Fig. 1*) and some structural variants. Recently, *Vilar et al.* reported a comprehensive kinetic study of the interaction between two ethylidene derivatives of tricyclic carbapenems (*Fig. 1*) and representative β-lactamases [10]. The results showed both compounds to be highly efficient inactivators for class-C β-lactamases (*E. cloacae* 908R), as well as highly stable against class-A (TEM-1) and class-D β-lactamases (OXA-10). However, these compounds are readily hydrolyzed by carbapenem-hydrolyzing β-lactamases of classes A (NmcA) and B (BcII). This is especially interesting, because other inhibitors of this site (*viz.*, clavulanic acid and sulbactam) usually exhibit very low activity against class-C enzymes [11].

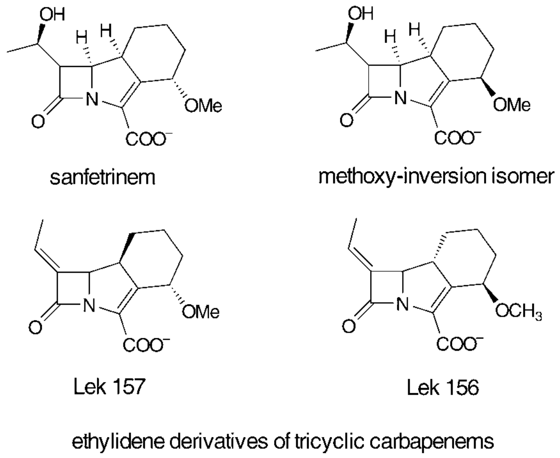


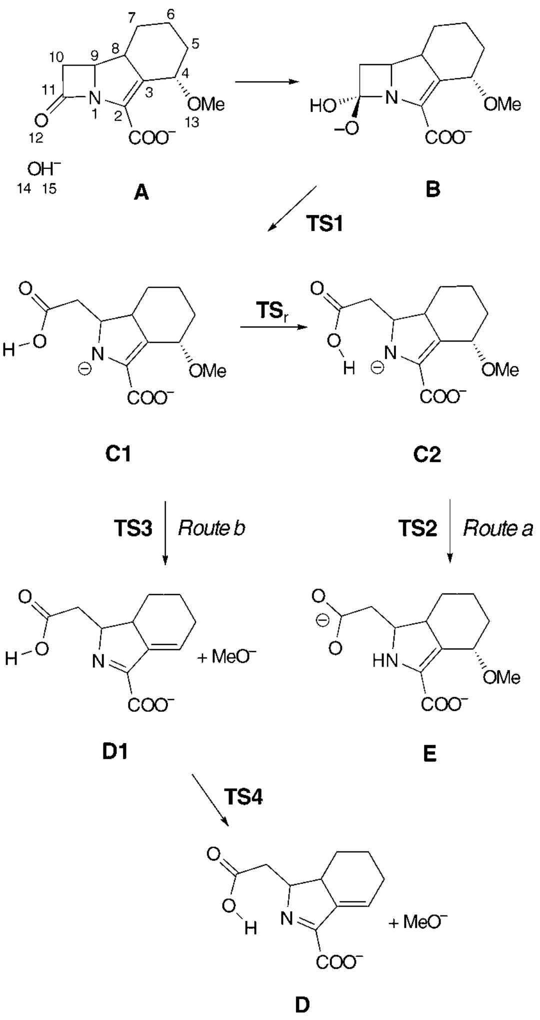
Fig. 1. *Sanfetrinem structure and derivatives*

Computational chemistry has proved extremely useful for the accurate interpretation of the structural and kinetic information available for a wide variety of β-lactam antibiotics and predicting the behavior of related substances, as well as for designing tailor-made antibiotics. In this context, alkaline hydrolysis has been widely used to rationalize the intrinsic reactivity of β-lactam compounds and their derivatives [12–15], one of the major requisites for adopting a substance as potential drug or inactivator. However, no theoretical study on the hydrolysis of tricyclic antibiotics such as those of *Fig. 1* appears to have been reported to date. The only exception is seemingly a recent study by *Kim et al.* [16], who used *ab initio* calculations to analyze the geometric and conformational properties of sanfetrinem and some of its isomers.

In this work, a comprehensive theoretical study of various potential mechanisms for the alkaline hydrolysis of the trinem model compound **A** was conducted. Two different routes were considered, namely: *a*) proton transfer to the β-lactam ring and *b*) cleavage of the β-lactam ring and elimination of the MeO group by electron rearrangement. Both processes started from the tetrahedral intermediate **B** (see the *Scheme*).

Experimental. – The studied structure **A** (see *Scheme*) was essentially identical with sanfetrinem (*Fig. 1*), except that the substituent at C(10) was replaced with a proton to simplify the computations. Geometric and energetic *ab initio* calculations on both the initial structure and those resulting from the reactions examined were performed at the RHF/6-31 + G* level, which includes polarized and diffuse functions on heavy atoms. Diffuse functions are especially relevant in calculations on anionic systems [17]. Solvent effects were taken into account by using the *Onsager* model to optimize structures in terms of geometry and energy. The resulting structures were then energy-relaxed by using the isodensity polarizable continuum model (IPCM) [18], in which the solute is placed inside a cavity surrounded by a continuous medium of dielectric constant 78.39 (H_2O). The cavity volume is computed from an isosurface the electron density of which is obtained by using standard RHF calculations. IPCM Computations were performed without geometry optimization. The energies of the compounds in solution were computed at the RHF/6-31 + G* level. All characterized transition states exhibited a single imaginary frequency above $100i$ cm^{-1} [19]. IRC Calculations of such states were performed to confirm the intermediates examined. Computations were made on *SGI-Origin-2000* and *-200* computers running the software Gaussian 98 [20].

Scheme. *Reaction Pathway of Alkaline Hydrolysis of the Trinem Model* **A** (arbitrary numbering) *with Structures Corresponding to the Different Intermediate and Final States*



Results and Discussion. – As in other β-lactams, the four-membered ring of **A** is essentially planar (4.3^0); also, the N(1)–C(11) distance is similar to the *ab initio* calculated values for other β-lactams and slightly greater than that for azetidin-2-one (1.356 Å) [21]. The *Scheme* shows the transition states and energy minima involved in the gas-phase hydrolysis of the model trinem **A** studied. *Table 1* lists the most-salient geometric and energetic parameters for each structure.

The alkaline hydrolysis of a β-lactam involves a nucleophilic attack of the hydroxy ion at the carbonyl group of the β-lactam ring. Only the attack from the α side of the ring was examined here as this is the target of the attack of the Ser residue in class-A and class-C β-lactamases [22][23]. As can be seen in the *Scheme*, the attack produces a

Table 1. *Main Geometric Parameters and Energy at HF/6-31 + G*//HF/6-31 + G* of the Reactants, Intermediates, Transition States, and Final Products of the Alkaline Hydrolysis of the Trinem Model A*. Bond lengths in Å, dihedral angles in °. Arbitrary numbering.

	A	**TS_{AB}**	**B**	**C1**	**C2**	**D**	**D1**	**E**	**TS1**	**TS_r**	**TS2**	**TS3**	**TS4**
N_1-C_{11}	1.379	1.461	1.563	2.971	2.932	3.017	2.934	3.317	1.876	2.735	2.865	2.966	2.782
N_1-H_{15}	–	2.801	2.712	3.971	1.609	1.861	4.044	0.999	2.737	2.918	1.444	1.916	2.714
C_4-O_{13}	1.416	1.434	1.440	1.453	1.446	–	–	1.428	1.448	1.453	1.445	1.916	–
$N_1C_9-C_8C_3$	18.8	– 10.9	– 10.4	31.3	29.2	– 21.3	– 23.0	28.2	– 13.9	31.4	28.9	21.3	– 22.9
$N_1C_2-C_3C_8$	6.0	2.4	2.6	15.0	13.0	– 13.0	– 13.9	3.1	0.4	14.6	12.3	8.4	– 14.0
$N_1C_2-C_3C_4$	178.6	179.5	179.5	160.7	163.1	158.7	158.9	166.5	177.0	161.0	163.7	166.8	158.4
$C_4C_3-C_8C_7$	– 63.4	– 45.4	– 45.8	– 53.8	– 55.6	– 21.56	– 21.2	– 61.8	– 42.3	– 54.6	– 56.3	– 55.5	– 20.9
$C_3C_8-C_7C_6$	29.8	– 1.6	– 0.8	30.3	29.7	50.6	50.2	30.9	– 34.1	31.8	30.0	40.7	49.9
$O_{12}C_{11}-O_{14}H_{15}$	–	– 61.2	– 41.5	– 10.1	176.5	177.8	6.3	–	– 45.9	– 57.8	176.6	9.8	74.7
Energy a)	0.0	25.6	12.2	– 4.8	– 26.2	– 70.6 b)	– 63.6 b)	– 59.4	14.5	– 3.1	– 24.2	6.5	– 57.5 b)

a) Relative energies (0.0 implies **A** plus OH^- at 8.25 Å) in kcal/mol. These values involve ZPE corrections. b) Energy of **D**, **D1**, and **TS4** structures plus MeO^-.

tetrahedral intermediate **B** that evolves through compound **C** (with a broken amide bond) to end-products **D** and **E**.

The nucleophilic attack in the gas-phase alkaline hydrolysis of most β-lactams involves no activation energy [15][24]. However, the resulting tetrahedral intermediate exhibits a higher energy than the reactants at 8.25 Å (12.2 kcal/mol), and a transition state between **A** and **B** is found at a C(11)–O(14) distance of 2.067 Å and 25.6 kcal/mol higher in energy than the reactants at 8.25 Å. This differential behavior has also been observed in the gas-phase reaction of a derivative of clavulanic acid and cephalothin [25]. Therefore, the absence of a side chain at position 6 or 7 seemingly makes the reactants more stable than the tetrahedral intermediate in HF calculations involving the 6-31 + G* basis set. Introducing electron correlation has little effect on the results: based on B3LYP/6-31 + G* calculations, the reactants at 8.25 Å are 8.0 kcal/mol more stable than the tetrahedral intermediate. An analysis of charges revealed that the atom bound to C(3) in carbapenems is highly charge deficient, which is consistent with the results of *Kim et al.* [16]. No comparable effect has been observed in penams, cephams, or their derivatives. In the tetrahedral intermediate **B**, the charge is distributed throughout the tricyclic system.

The tetrahedral intermediate **B** evolves to the transition state **TS1** by cleavage of its N(1)–C(11) bond, with an energy barrier of 2.3 kcal/mol (*Fig. 2*); this is much lower than the barriers for other β-lactams as a result of the low stability of the tetrahedral intermediate. Such a low barrier is comparable only to that for the cleavage of the C–O bond in oxa-β-lactam [14]. IRC Calculations on this transition state led to the tetrahedral intermediate **B** on the one hand and to product **C1** on the other. **C1** is only 17 kcal/mol more stable than the tetrahedral intermediate; also, it exhibits a broken C–N bond.

Two alternative routes were examined beyond this point in the mechanism, namely: *a*) proton transfer to the β-lactam N-atom *via* transition state **TS2** to give compound **E**; and *b*) electron displacement from the N-atom through the C=C bond to give compound **D1** with elimination of the MeO group *via* transition state **TS3**. For the former route (*Route a*) to be possible, the proton to be transferred (H_{15}) should be properly located. **C1** has an O(12)–C(11)–O(14)–H(15) dihedral angle of – 10.1°;

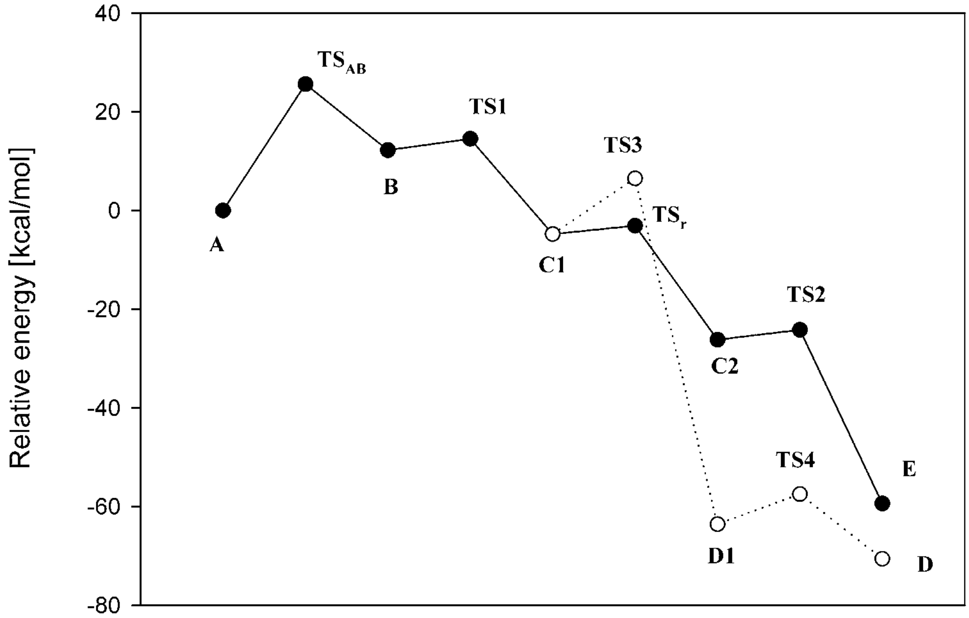


Fig. 2. *Reaction profile for the alkaline hydrolysis of trinem* **A** *in the gas phase at the RHF/6-31 + G*//RHF/6-31 + G* level*

therefore, the N(1)–H(15) distance is too long (4 Å) for any transfer to occur. Also, the transfer requires the previous dihedral to rotate *via* a transition state **TS$_r$** to give product **C2**. The energy barrier involved is 1.7 kcal/mol, and product **C2** possesses an O(12)–C(11)–O(14)–H(15) angle of 176.5°, so the N(1)–H(15) distance is only 1.609 Å. This structure evolves to product **E** *via* transition state **TS2**. The energy barrier for this process is very low (*ca.* 2 kcal/mol), and the final structure is much more stable (71.6 kcal/mol) than the tetrahedral intermediate. As in cephalosporins [26], the MeO group in sanfetrinem is a good leaving group. Therefore, **C1** can evolve to **D1** *via* transition state **TS3** (*Route b*). Similarly to the O(12)–C(11)–O(14)–H(15) dihedral in **C1**, the one in **D1** can rotate to give compound **D** *via* transition state **TS4**. Based on RHF calculations, the energy barrier is 11.3 kcal/mol, and structure **D** (plus the MeOH group removed) is the most stable. It should be noted that both the proton transfer and the elimination of the MeO group (*Routes a* and *b*, resp.) occur without the need for major rotational changes in the COOH group bonded to C(11); also, the N(1)–C(11) distance remains virtually unchanged from **C** to **D** and increases to the expected extent in the *N*-protonated compound **E**.

Some authors [16] have pointed out that the conformation of the MeO group in sanfetrinem influences its activity. Biological data [27] have shown sanfetrinem to be more active than the isomer with an inverted configuration at the MeO-substituted C(4). To examine the differences between the two structures, we studied the process by which the MeO group leaves the structure, *i.e.*, **C1** → **TS3** (see *Table 2*). Based on the energy barrier for the elimination of the MeO group (7.8 kcal/mol in the MeO-inverted isomer and 11.3 kcal/mol in sanfetrinem model **A**), the intrinsic reactivity of both compounds is similar, so the differences between both compounds must be ascribed to differences in the orientation into the active site, which can play a prominent role in the molecular recognition process.

Table 2. *Main Geometric and Energetic Parameters for the* **C1** → **TS3** *Process* (see *Scheme*) *for Sanfetrinem Model* **A** *and the Methoxy-Inverted Isomer.* Bond lengths in Å, dihedral angles in °. Arbitrary numbering.

	$\mathbf{C1}_{inv}$	$\mathbf{TS3}_{inv}$	**C1**	**TS3**
N_1-C_{11}	2.938	2.931	2.971	2.966
N_1-H_{15}	3.976	3.982	3.971	1.916
C_4-O_{13}	1.458	1.854	1.453	1.916
$N_1C_9-C_8C_3$	− 27.5	− 21.7	31.3	21.3
$N_1C_2-C_3C_8$	− 12.4	− 8.2	15.0	8.4
$N_1C_2-C_3C_4$	− 172.2	171.8	160.7	166.8
$C_4C_3-C_8C_7$	− 42.1	− 32.4	− 53.8	− 55.5
$C_3C_8-C_7C_6$	56.2	57.4	30.3	40.7
$O_{12}C_{11}-O_{14}H_{15}$	10.5	9.5	− 10.1	9.8
Energy[a])	− 14.2	− 6.4	− 17.0	− 5.7

[a]) Relative energies (0.0 implies **B**) in kcal/mol; these values involve ZPE corrections.

The potential-energy surfaces for a number of reactions – particularly those involving charged species – can be altered by the presence of a solvent. The method used in this work was based on *Onsager*'s reaction-field model, which assumes the solvent to be a continuum with a specific dielectric constant and the solute to occupy a cavity within the continuum. *Fig. 3* shows the reaction profile obtained by assuming the solvent to be a continuum. The primary effects of a solvent are stabilization of the reactants and the decrease of the energy barrier for the initial nucleophilic attack. This has been observed both in β-lactams [13][28] and in other carbonyl compounds [29][30]. The free-energy barrier due to the desolvation of the hydroxy group on approaching the carbonyl group is 19.5 kcal/mol ($\mathbf{A} \rightarrow \mathbf{TS_{AB}}$). This value is similar to those obtained for other β-lactam systems, which can be estimated to be *ca.* 16 kcal/mol irrespective of the particular system.

As a whole, the rate-limiting process is the nucleophilic attack. On the other hand, **E** is the most stable structure, and **C2** cannot be stabilized as it evolves to end-product **E**. The presence of a hydrolysis product where the MeO group at C(4) is gone (see **D** and **D1**) was experimentally confirmed by *Vilar et al.* in a kinetic study of the interactions between two ethylidene derivatives of tricyclic carbapenems (*Fig. 1*) and representative β-lactamases [10].

These results show that the geometry of sanfetrinem is consistent with an intrinsic reactivity similar to that of other β-lactams such as penicillins and cephalosporins. Also, similarly to cephalosporins, the MeO group provides an alternative route for the hydrolysis mechanism. Therefore, this compound meets one of the requirements for use as a β-lactam antibiotic and possesses the reactivity needed to form the tetrahedral intermediate, which plays an essential role in both the alkaline and the enzymatic hydrolysis process.

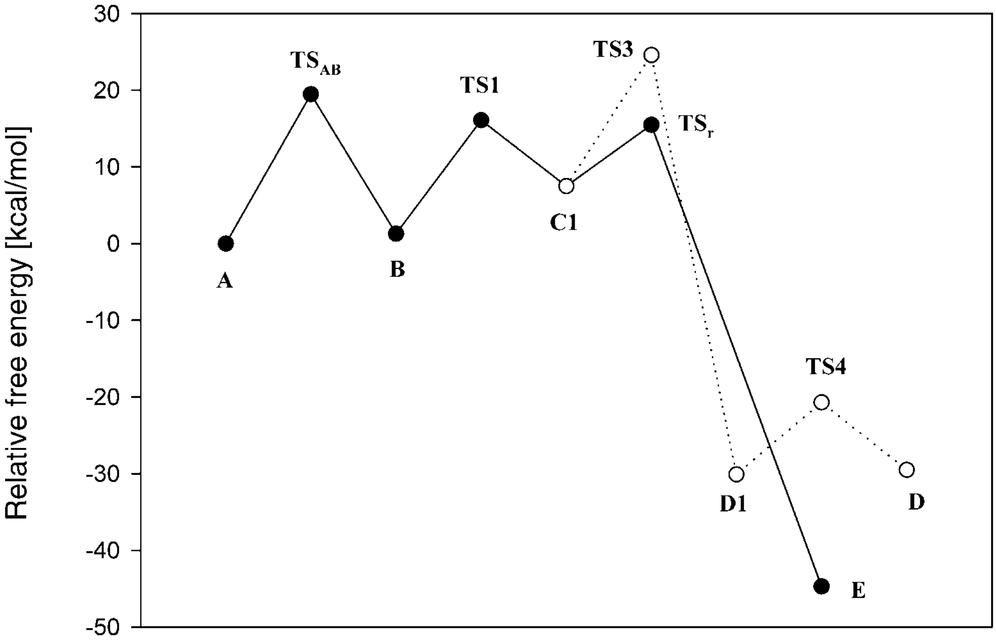


Fig. 3. *Reaction profile for the alkaline hydrolysis of trinem* **A** *in the aqueous phase at the IPCM/6-31 + G*// RHF/6-31 + G* level*

We thank the *Supercomputational Center of Catalunya* (CESCA) and the *European Center for Parallelism of Barcelona* (CEPBA) for access to their computer facilities. The support of the Spanish *DGICYT* is gratefully acknowledged (BQU2003-02592).

REFERENCES

[1] J. M. Frère, *Mol. Microbiol.* **1995**, *16*, 385.
[2] S. G. Waley, in 'The Chemistry of β-Lactams', Ed. M. I. Page, Chapman & Hall, Glasgow, 1992.
[3] A. Matagne, J. Lamotte-Brasseur, J. M. Frère, *Biochem. J.* **1998**, *330*, 581.
[4] J. Knox, P. C. Moews, J. M. Frère, *Chem. Biol.* **1996**, *11*, 937.
[5] R. P. Ambler, *Phylos. Trans. R. Soc. London B* **1980**, *289*, 321.
[6] J. E. Baldwin, G. P. Lynch, J. Pitlik, *J. Antibiotics* **1999**, *44*, 1.
[7] C. Reading, M. Cole, *Antimicrob. Agents Chemother.* **1997**, *11*, 852.
[8] S. Géhanne, E. Piga, D. Andreotti, S. Biondi, D. Pizzi, *Bioorg. Med. Chem. Lett.* **1996**, *6,* 2791
[9] S. Hanessian, A. M. Griffin, M. J. Rozema, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1857, and ref. cit. therein.
[10] M. Vilar, M. Galleni, T. Solmajer, B. Turk, J. M. Frère, A. Matagne, *Antimicrob. Agents Chemother.* **2001**, *45*, 2215.
[11] A. Matagne, A. Dabus, M. Galleni, J. M. Frère, *Nat. Prod. Rep.* **1999**, *16*, 1.
[12] J. Frau, J. Donoso, F. Muñoz, F. García Blanco, *J. Comput. Chem.* **1992**, *13*, 681.
[13] J. Frau, J. Donoso, F. Muñoz, B. Vilanova, *Helv. Chim. Acta* **1996**, *79*, 353.
[14] M. Coll, J. Frau, B. Vilanova, J. Donoso, F. Muñoz, F. García Blanco, *J. Phys. Chem A* **1999**, *103*, 8879.
[15] J. Pitarch, M. Ruiz-López, J. L. Pascual-Ahuir, E. Silla, J. Tuñon, *J. Phys. Chem. B* **1997**, *101*, 3581.
[16] M. S. Kim, W. K. Park, S. C. Park, *J. Mol. Struct. (Theochem)* **2003**, *630*, 265.
[17] J. D. Madura, W. L. Jorgensen, *J. Am. Chem. Soc.* **1986**, *108*, 2517.
[18] J. B. Foresman, T. A. Keith, K. B. Wiberg, J. Snoonian, M. J. Frisch, *J. Phys. Chem* **1996**, *15*, 269.
[19] J. W. McIver, J. Komornicki, *J. Am. Chem. Soc.* **1972**, *94*, 2625.

[20] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. V. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, 'Gaussian98', Gaussian, Inc., Pittsburgh, PA, 1998.
[21] J. Frau, J. Donoso, F. Muñoz, B. Vilanova, *Helv. Chim. Acta* **1997**, *80*, 739.
[22] B. Vilanova, J. Donoso, J. Frau, F. Muñoz, *Helv. Chim. Acta* **1999**, *82*, 1274.
[23] C. Fenollar-Ferrer, J. Frau, J. Donoso, F. Muñoz, *Proteins: Struct., Func. and Genet.*, **2003**, *51*, 442.
[24] J. Frau, J. Donoso, F. Muñoz, F. García Blanco, *Helv. Chim. Acta* **1994**, *77*, 1557.
[25] H. J. Fasoli, J. Frau, unpublished data
[26] J. Frau, J. Donoso, F. Muñoz, F. García Blanco, *J. Comput. Chem.* **1993**, *12*, 1545.
[27] D. Andreotti, T. Rossi, G. Gaviraghi, D. Donati, C. Marchiori, E. D. Modugno, A. Perboni, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 491.
[28] M. Coll, J. Frau, B. Vilanova, J. Donoso, F. Muñoz, F. García Blanco, *J. Phys. Chem. B* **2000**, *104*, 11389.
[29] S. J. Weiner, U. C. Sing, P. A. Kollman, *J. Am. Chem. Soc.* **1985**, *107*, 2219.
[30] H. Yu, M. Karplus, *J. Am. Chem. Soc.* **1987**, *109*, 6299.

Received September 27, 2004