Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemical Physics Letters 372 (2003) 275–281
CHEMICAL PHYSICS LETTERS
www.elsevier.com/locate/cplett

Theoretical study of the alkaline hydrolysis of an aza-β-lactam derivative of clavulanic acid

Rafael C. García s, Miguel Coll, Josefa Donoso, Francisco Muñoz *

Department de Química, Universitat de les Illes Balears, Ctra. Valldemossa, Km. 7.5 07071 Palma de Mallorca, Illes Balears, Spain

Received 30 January 2003; in final form 24 February 2003

Abstract

DFT calculations based on the hybrid functional B3LYP/6-31+G* were used to study the alkaline hydrolysis of an aza-clavulanic acid, which results from the substitution of the carbon atom at position 6 in clavulanic acid by a nitrogen atom. The presence of the nitrogen atom endows the compound with special properties; in fact, once formed, the tetrahedral intermediate can evolve with cleavage of the N_4–C_7 or N_6–C_7 bond, which obviously leads to different reaction products. These differential bond cleavages may play a central role in the inactivation of β-lactamases, so the compound may be a powerful inactivator of these enzymes.

1. Introduction

β-Lactam antibiotics are currently the most widely used antimicrobial agents by virtue of their high efficiency, broad spectrum and low toxicity. Their antibacterial action relies on the acylation of so-called 'penicillin binding proteins' (PBPs), which constitute an enzyme group involved in the formation of the bacterial cell wall [1].

Although bacteria resistant to this type of antibiotic have been known to exist since shortly after its inception in clinical practice, it is in recent years that, possibly through their massive misuse [2], the number of bacterial strains resistant to the action of β-lactams has grown to an alarming extent [3].

The primary defensive mechanism of the bacteria involves the production of β-lactamases [4,5]; these enzymes use an acylation–deacylation mechanism to hydrolyse the β-lactam ring before the antibiotic can reach its target and render it inactive against PBPs [6].

A number of approaches have been developed to overcome the effects of these enzymes that include the use of compounds such as tazobactam, sulbactam or clavulanic acid, which are powerful inhibitors of β-lactamases despite their low – if any – antibacterial activity [7]. These compounds, which act by irreversibly inhibiting the enzymes, are always administered in combination with other, effective, compatible antibiotics. Specifically, the inhibitory mechanism of clavulanic acid involves the cleavage of the C_5–O_1 bond after the

* Corresponding author. Fax: 34-971-17-34-26.
E-mail address: dqufmi0@clust.uib.es (F. Muñoz).

0009-2614/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0009-2614(03)00322-1

acyl–enzyme complex is formed; this hinders the deacylation reaction, which would develop to completion in the presence of an appropriate substrate [8].

Based on the values of structural parameters obtained from semi-empirical calculations, Nangia et al. [9–11] inferred that aza-β-lactam structures could be potential antibacterial agents, which was subsequently confirmed experimentally [12]. The antibacterial properties of aza-β-lactams originate from the formation of carbamoyl–enzyme complexes, which are more stable than penicilloyl–enzyme complexes and were originally reported by Ghosez et al. [13–15] in their studies on bicyclic imidazolidinones (aza-γ-lactams).

In recent work on β-lactam structures where the carbon atom next to the β-lactam carbonyl group was substituted by an oxygen, nitrogen or sulphur atom, we found the nucleophilic attack of the hydroxyl group on the β-lactam carbonyl to yield products possessing the chemical reactivity required from effective antibacterial agents capable of inhibiting β-lactamases. In these compounds, the formation of the tetrahedral intermediate allows the system to evolve via the cleavage of the lactam C_7–N_4 bond or the C_7–X_6 bond (with X = O, N or S), which produces especially stable carbamoyl structures. In most cases, the C_5–S_1 bond is also broken, similarly as in clavulanic acid [16–18].

Based on structural (particularly electrostatic) parameters for these compounds calculated by distributed multipole analysis (DMA) and their comparison with those for other penicillins, cephalosporins and clavulanic acid, these substituted β-lactams must behave more similarly to an inhibitor than to an effective antibacterial agent [19].

In this work we studied the alkaline hydrolysis of a compound (**a**), in Scheme 1, with a molecular structure similar to that of clavulanic acid which retained the oxygen atom at position 1 in the oxazolidine ring but had the carbon at position 6 substituted by a nitrogen atom. The study was based on theoretical calculations at the B3LYP/6-31+G* level, which allowed the effects of the substitution on the chemical reactivity of the compound to be exposed. The structural and DMA parameters for the compound were compared with those for penicillin G and clavulanic acid. Based on the electrostatic parameters, the compound possesses an enzyme affinity similar to that of clavulanic acid; however, its increased number of potential cleavage sites results in also increased chemical reactivity and inhibitory power.

2. Methodology

The ab initio calculations were carried out at the B3LYP/6-31+G*//B3LYP/6-31+G* levels. The incorporation of diffuse functions is especially relevant in the calculation of anionic system [20]. All the energies in the text include the ZPE correction. The transition states were characterized by exhibiting just one imaginary frequency. IRC calculations of the former transition states were performed to confirm all the transition states proposed in this study.

The calculations were performed on IBM SP2 and SGI Origin 200 computers running the GAUSSIAN 98 program [21].

We compared the structural similarity between the molecule studied with penicillin G and clavulanic acid by minimizing the root-mean-square (rms) distances between specified pairs of atoms that are expected to be in the same position in the active site. The sequence that we used for the structural overlays was the O_8–C_7–N_4–C_3–C_{11}–($O_{12}O_{13}$), present in all the structures studied. The rms separations of the atoms of the studied sequence has been calculated by the Chem3D software package [22].

The electrostatic similarity is likely to be more important that the finer details of steric overlap. The electrostatic models were derived from the SCF wavefunction of the molecules (RHF). Each wavefunction was represented by sets of multipoles up to the hexadecapole at each atomic site, obtained by a DMA. These calculations were carried out running the CADPAC ab initio program suite [23]. The electrostatic potential was examined at fixed distances outside the van der Waals surfaces of the molecules, as defined by the Pauling radii, and the minima of electrostatic potential were determined by minimizing the interaction energy of a single positive point charge

(radius 0.5 Å) with each molecule, using hard-sphere repulsion between sites with nonzero van der Waals radii, using the ORIENT software package [24]. We compared, through the use of rms separations of the positions of the minima calculated by the Chem3D software package, the electrostatic potential of the structure studied with that the penicillin and the clavulanic acid.

3. Results and discussion

Scheme 1 shows the overall mechanism for the hydrolysis of aza-clavulanic acid (**a**), as well as the traditional numbering convention for these compounds. Fig. 1 shows the corresponding reaction profile.

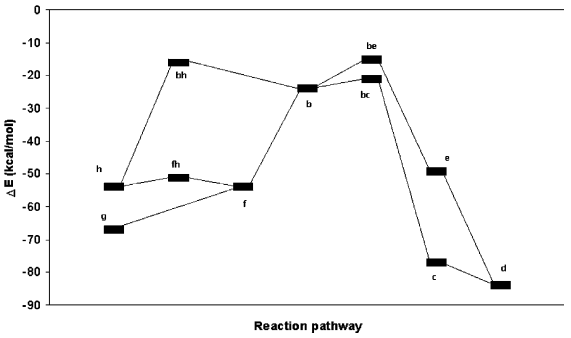
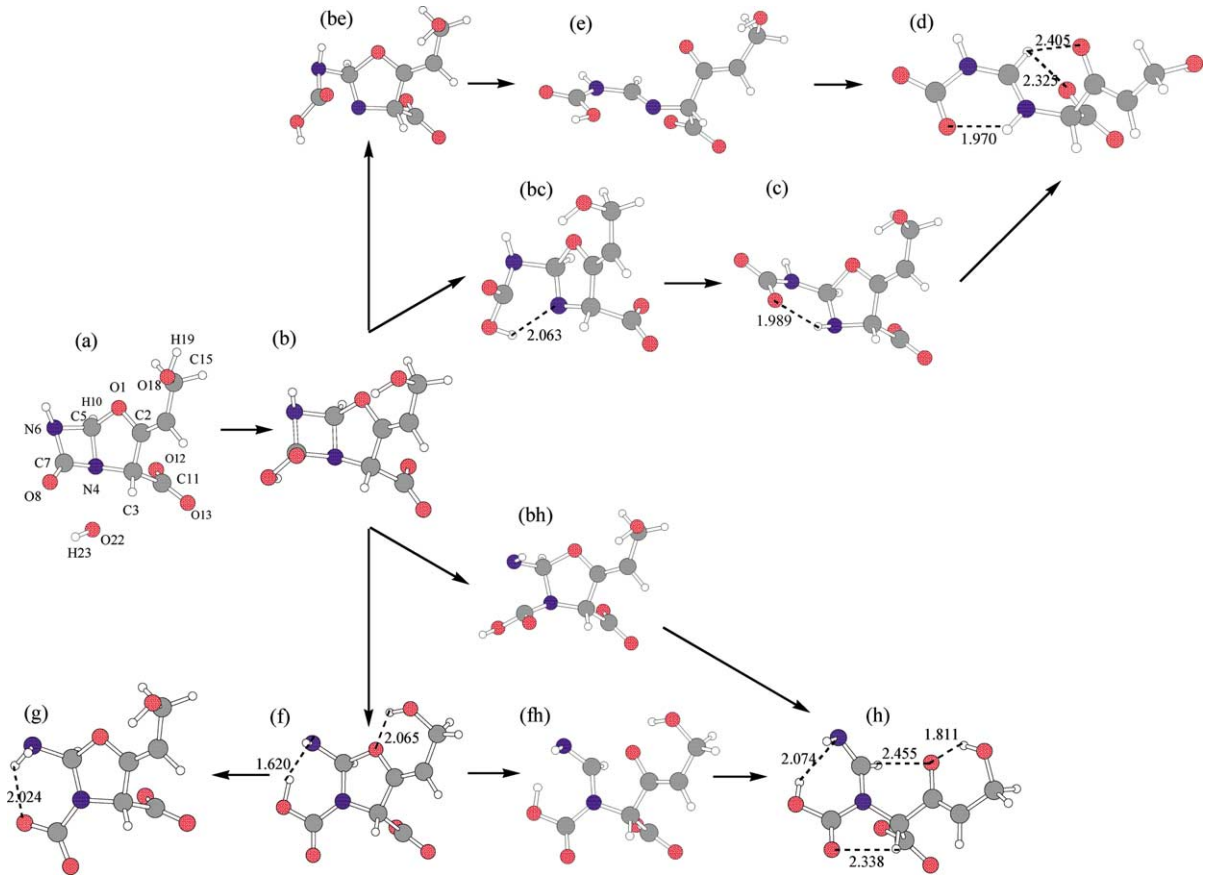


Fig. 1. Reaction profile of the alkaline hydrolysis of the aza-clavulanic acid (molecular energies are relatives to the reactants).

The presence of the two nitrogen atoms next to the carbon in the β-lactam carbonyl group increases the amount of positive charge on the



Scheme 1. Structures corresponding to the reactants, intermediates, transition states and final products of the reaction of alkaline hydrolysis of the aza-clavulanic compound at B3LYP/6-31+G* level.

carbon and results in a slightly favoured attack of the hydroxyl group relative to clavulanic acid [17,25]. As shown in a number of theoretical studies on the hydrolysis of carbonyl groups [20,26], the attack of the hydroxyl group in the gas phase is subject to no potential barrier until the tetrahedral intermediate (**b**) is formed. This intermediate can evolve with cleavage of the N_4–C_7 bond (as in clavulanic acid and most penicillins and cephalosporins) to form compound **c**, where the β-lactam cycle is open and the β-lactam nitrogen protonated. This process exhibits a low activation energy (3.14 kcal/mol, Table 1).

The transition state that connects these two compounds, **bc**, clearly exhibits a hydrogen bond between N_4 and H_{23} (2.063 Å) that facilitates the subsequent transfer to the nitrogen atom after the β-lactam cycle is opened.

Compound **c** possesses a substantial amount of negative charge on O_1, N_4, C_5 and N_6, consistent with its tendency to evolve spontaneously, with no energy barrier, to compound **d**, which has both cycles open and is the most stable of all compounds in the reaction scheme. The presence of N_6 results in much easier cleavage of the O_1–C_5 bond than in clavulanic acid [17,25]. Because such a cleavage is involved in the permanent inactivation of β-lactamases by clavulanic acid [8], aza-clavulanic acid can be expected to be a much more powerful inactivator than clavulanic acid. The end product **d** is stabilized by the formation of three intramolecular hydrogen bonds, viz. a strong one between O_{22} and H_{23} (1.970 Å), and two weak ones of H_{10} with O_1 (2.405 Å) and O_{12} (2.325 Å).

Compound **d** can be obtained via a different reaction pathway. Thus, if the proton cannot be transferred to the β-lactam nitrogen during opening of the ring owing to an inappropriate orientation, the five-membered ring breaks in order to neutralize the resulting negative charge on the nitrogen atom (**e**). This process is subject to an activation energy of 9.48 kcal/mol. If the hydroxyl of the acid group of **e** is rotated, the proton, H_{23}, is transferred to the β-lactam nitrogen. As can clearly be seen from Fig. 1, the former pathway is the more energetically favourable.

The presence of a heteroatom at position 6 allows the tetrahedral intermediate to also evolve with cleavage of the N_6–C_7 bond, a process that is subject to no activation energy (see Fig. 1). The resulting product, **f**, has a weak hydrogen bond (H_{19}–O_1, 2.065 Å) and a very strong one (H_{23}–N_6, 1.620 Å). The formation of the latter facilitates the conversion of **f** into **g** via the transfer of H_{23} to N_6, which neutralizes the charge on this nitrogen atom. Again, the process is subject to no activation energy.

Table 1
Principal bond distances (Å), energies, charges, and imaginary frequencies of all compounds of the alkaline hydrolysis of the aza-β-lactam acid

Distances	**a**	**b**	**be**	**e**	**bc**	**c**	**d**	**bh**	**h**	**f**	**fh**	**g**
C_7–N_4	1.4224	1.6126	2.2153	3.0815	2.0195	3.0744	3.0123	1.4502	1.3818	1.3713	1.3721	1.4434
N_6–C_7	1.4212	1.5770	1.4470	1.3515	1.4964	1.4575	1.4785	2.1158	3.0208	2.9794	3.0199	3.0758
N_6–C_5	1.4758	1.4486	1.4637	1.4410	1.4498	1.4026	1.3486	1.4138	1.2972	1.3475	1.3247	1.4504
C_5–O_1	1.4102	1.4875	1.5679	2.7707	1.5146	1.5865	2.7682	1.5264	2.553	1.5421	1.8790	1.4576
C_2–O_1	1.3936	1.3581	1.3425	1.2674	1.3519	1.3382	1.2709	1.3434	1.3029	1.3596	1.3277	1.3636
C_5–N_4	1.4656	1.4318	1.3759	1.2631	1.4045	1.4212	1.3059	1.4516	1.4092	1.4930	1.4575	1.4409
O_{22}–H_{23}	–	0.9716	0.9714	0.9731	0.9781	1.9886	1.9704	0.9715	0.9798	1.0550	0.9978	2.0237
H_{23}–N_6	–	2.5766	3.1321	3.0641	2.5317	2.4317	2.4971	3.1637	2.0745	1.6201	1.8650	1.0298
H_{23}–N_4	–	2.2691	3.1613	3.8359	2.0627	1.0264	1.0222	3.1319	2.3358	2.2445	2.2932	2.5605
H_{19}–O_1	4.6164	3.4324	3.4651	3.5848	3.4343	3.4826	3.5497	3.5490	1.8108	2.0646	1.9428	3.5993
ΔE (kcal/mol)	0	−24.269	−14.787	−49.098	−21.127	−77.828	−84.237	−15.462	−53.653	−53.493	−51.813	−67.365
$Q(O_1)$	−0.27	−0.32	−0.35	−0.64	−0.32	−0.31	−0.65	−0.36	−0.70	−0.46	−0.54	−0.37
$Q(N_4)$	−0.06	−0.15	−0.15	−0.20	−0.32	−0.39	−0.5	0.14	0.02	−0.15	−0.11	−0.23
$Q(C_5)$	0.08	−0.09	−0.24	0.15	−0.05	−0.33	0.23	−0.24	0.05	0.10	−0.05	−0.10
$Q(N_6)$	−0.63	−0.63	−0.48	−0.60	−0.53	−0.61	−0.69	−0.79	−0.68	−0.93	−0.79	−0.79
$Q(C_7)$	0.79	0.44	0.47	0.70	0.49	0.69	0.68	0.58	0.81	0.84	0.85	0.80
Imaginary frequencies (cm^{-1})	–	–	138.7 i	–	156.9 i	–	–	354.2 i	–	–	101.4 i	–

Compound **f** can also evolve with cleavage of the O_1–C_5 bond, which yields **h**; this is similar to **d**: it has both rings open and the negative charge, which is localized primarily on O_1, is partially stabilized by formation of a hydrogen bond with H_{10} – with a low energy barrier (1.68 kcal/mol).

The tetrahedral intermediate could also yield **h** via a TS (**bh**) involving the simultaneous opening of the two fused rings. However, as can be seen from Fig. 1, this process is energetically unfavourable.

The nucleophilic attack of the hydroxyl group (Fig. 1) on the β-lactam carbonyl yields two main end products, namely: **g**, which is subject to no energy barrier, and **d**, which exhibits a low barrier (3.14 kcal/mol).

Chemical reactivity is not the sole factor that dictates the antibacterial or β-lactamase inhibitory properties of β-lactams. The three-dimensional structure and, especially, the distribution of the electrostatic potential, are also significant to the enzymatic recognition of the compound. Thus, molecules with a similar electrostatic potential distribution are known to be able to interact with the same receptor [27], so such a distribution can be used to predict the suitability of a given compound as substrate for a specific enzyme – even if the active site for the enzyme is unknown. The DMA, the most widely used method for examining electrostatic similarity, has previously been applied to β-lactams [19,28,29].

Aza-clavulanic acid exhibits two minima on the carboxyl group (**a**, **b**), three on oxygen atoms [viz. one each on O_1 (**g**), O_8 (**d**) and O_{18} (**f**)] and two others on N_4 (**c**) and N_6 (**e**) (see Fig. 2 and Table 2). The electrostatic potential distributions for penicillin G and clavulanic acid are substantially different. The former exhibits two minima at position 4 and minima **f** and **g** are not associated to oxygen atoms. Similarly, the latter does not exhibit minimum **e** as it lacks the side chain or N_6 in clavulanic acid. It should be noted that the minima on the carboxyl group are much stronger in aza-clavulanic acid and clavulanic acid than they are in penicillin G.

Direct inspection of the structures and electrostatic potential minima only provides qualitative indications of similarity between molecules. In order to derive quantitative information, the structures of aza-clavulanic acid, penicillin and clavulanic acid were superimposed and the rms deviation for the distances in the atom sequence O_8–C_7–N_4–C_3–C_{11}–($O_{12}O_{13}$) in both structures was calculated. The structural rms values obtained

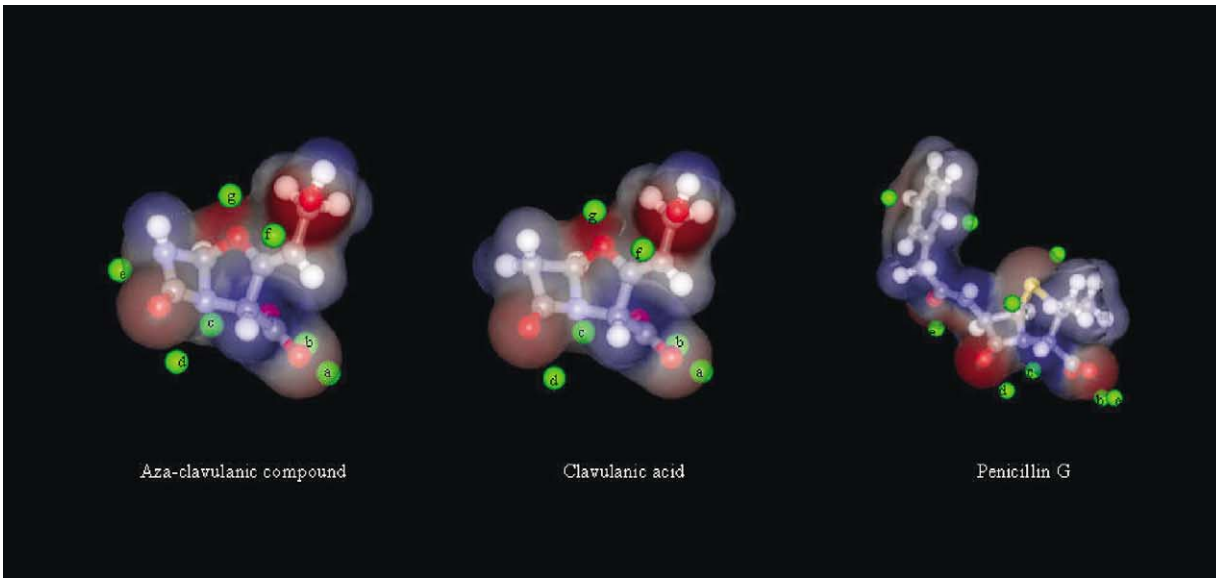


Fig. 2. Different minima of the electrostatic potential in the van der Waals surface for the aza-clavulanic acid, clavulanic acid and penicillin G.

Table 2
Values of the minima electrostatic potential (kcal/mol) in the van der Waals surface

	Aza-clavulanic acid	Clavulanic acid	Penicillin G
COO^- (a/b)	−573.59/−574.15	−576.66/−575.61	−568.69/−567.97
N_4 (c)	−522.85	−516.26	−541.00
O_8 (d)	−400.27	−407.65	−419.30
(e)	−338.07	–	−335.31
O_{18} (f)	−383.93	−395.02	–
O_1 (g)	−301.69	−331.77	–
Structural clavulanic acid rms	0.089	0	0.270
Structural Penicillin G rms	0.347	0.270	0
Electrostatic clavulanic acid rms	0.118	0	0.551
Electrostatic Penicillin G rms	0.725	0.551	0

(Table 2) suggest a significant similarity of the aza-clavulanic compound to clavulanic acid, and less so to penicillin G. These results are consistent with those obtained in previous work by comparing various aza-β-lactam structures with clavulanic acid and different penicillins [19].

The overlap between the potential minima for the aza-clavulanic compound, penicillin G and clavulanic acid therefore provides significant information about the ability of the compound to bind to the active site of bacterial enzymes. The calculated rms values between the potential minima related to the carbonyl group (**a** and **b**), the β-lactam nitrogen (**c**) and the carbonyl group (**d**), shown in Table 2, confirm a substantial electrostatic similarity with clavulanic acid, and less so with penicillin G.

In summary, the presence of the nitrogen atom at position 6 endows aza-clavulanic acid with special properties. On the one hand, following cleavage of the N_4–C_7, it substantially facilitates that of the O_1–C_5 bond (leading to **d**), which causes the permanent inhibition of β-lactamases [8]; on the other, it enables the cleavage of the N_6–C_7 bond, which does not occur in classical antibiotics and allows the enzymes to be inactivated via a rather different pathway. Based on the foregoing, aza-clavulanic acid and its derivatives can be powerful inactivators of β-lactamases.

Acknowledgements

The support of the Spanish DGICYT is gratefully acknowledged (Project BQU2000-0242).

References

[1] D.M. Livermore, J.D. Williams, in: William and Wilkins (Eds.), Antibiotics in Laboratory Medicine, fourth ed., V. Lorian (Ed.), Baltimore, 1996, p. 502.
[2] K. Kristinsson, Microb. Drug Res. 3 (1997) 117.
[3] F.C. Tenover, Clin. Infect. Dis. 33 (Suppl. 3) (2001) 108.
[4] S.G. Waley, Sci. Prog. Oxf. 72 (1998) 579.
[5] M.H. Nicolas-Chanoine, Int. J. Antimicrob. Agents 7 (1996) 21.
[6] R.F. Pratt, J. Chem. Soc. Perkin Trans. 2 (2002) 851.
[7] R.F. Pratt, in: M.I. Page (Ed.), The Chemistry of β-Lactams, Blackie, Glasgow, 1992, p. 229.
[8] J.R. Knowles, Acc. Chem. Res. 18 (1985) 97.
[9] A. Nangia, J. Mol. Struct. (THEOCHEM) 251 (1991) 237.
[10] A. Nangia, Proc. Indian Acad. Sci., Chem. Sci. 105 (1993) 131.
[11] A. Nangia, P.S. Chandrakala, P.V. Balaramakrishma, T.V. Latta, J. Mol. Struct. (THEOCHEM) 343 (1995) 157.
[12] P.S. Chandrakala, A.K. Katz, H.L. Carrell, P.R. Sailaja, A.R. Podile, A. Nangia, G.R. Desinaju, J. Chem. Soc. Perkin Trans. 1 (1998) 2597.
[13] J. Merchant-Brynaert, L. Ghosez, Recent Progress in the Chemical Synthesis of Antibiotics, Springer, Berlin, 1990.
[14] J. Merchant-Brynaer, L. Ghosez, Bull. Soc. Chim. Belg. 94 (1985) 1021.
[15] J. Merchant-Brynaert, Z. Buunkhala-Khrouz, J.C. Carretero, J. Davies, D. Ferroud, B.J. Van Keulen, B. Serckx-Poncin, L. Ghosez, Recent Advances in the Chemistry of β-Lactam Antibiotics, Special Publication No. 70, Royal Society of Chemistry, London, 1989.
[16] M. Coll, J. Frau, B. Vilanova, J. Donoso, F. Muñoz, F. García Blanco, J. Phys. Chem. A 103 (1999) 8879.
[17] M. Coll, J. Frau, B. Vilanova, J. Donoso, F. Muñoz, F. García Blanco, J. Phys. Chem. B 104 (2000) 11389.
[18] M. Coll, J. Frau, B. Vilanova, J. Donoso, F. Muñoz, Chem. Phys. Lett. 326 (2000) 304.
[19] M. Coll, J. Frau, B. Vilanova, J. Donoso, F. Muñoz, J. Comput. Aid.-Mol. Des. 15 (2001) 819.

[20] J.D. Madura, W.L. Joergensen, J. Am. Chem. Soc. 108 (1986) 2517.
[21] M.J. Frisch et al., GAUSSIAN 98, Gaussian, Inc, Pittsburgh, PA, 1998.
[22] CS Chem3D Pro. Cambridge Soft Corporation, Cambridge, MA, USA.
[23] CADPAC5: The Cambridge Analytical Derivates Package, Issue 5.0, 1992.
[24] A.J. Stone, ORIENT v.2., University of Cambridge, Cambridge, UK, 1990.
[25] J. Frau, J. Donoso, F. Muñoz, F. García Blanco, Helv. Chim. Acta 77 (1994) 1555.
[26] J. Frau, J. Donoso, F. Muñoz, B. Vilanova, F. García Blanco, Helv. Chim. Acta 80 (1997) 739.
[27] M. Orozco, E.I. Canela, R. Franco, Mol. Pharmacol. 35 (1998) 257.
[28] J. Frau, S.L. Price, J. Comput. Aid.-Mol. Des. 10 (1996) 107.
[29] J. Frau, S.L. Price, Theor. Chim. Acta 95 (1997) 151.