Calculations on the low energy conformers of N-acetyl-D-alanyl-D-alanine

ARTICLE *in* BIOPOLYMERS · FEBRUARY 1998

Impact Factor: 2.39 · DOI: 10.1002/(SICI)1097-0282(199802)45:2<119::AID-BIP3>3.0.CO;2-V

4 AUTHORS, INCLUDING:

Juan Frau
University of the Balearic Islands
157 PUBLICATIONS **883** CITATIONS
SEE PROFILE

Josefa Donoso
University of the Balearic Islands
206 PUBLICATIONS **1,250** CITATIONS
SEE PROFILE

Francisco Munoz
University of the Balearic Islands
229 PUBLICATIONS **1,269** CITATIONS
SEE PROFILE

J. Frau[1]
J. Donoso[1]
F. Muñoz[1]
F. García Blanco[2]
[1] *Departament de Química, Facultat de Ciències, Universitat Illes Balears, E-07071 Palma de Mallorca, Islas Baleares, Spain*

[2] *Departamento de Química Física, Facultad de Farmacia, Instituto Pluridisciplinar, Universidad Complutense de Madrid, E-28040, Madrid, Spain*

Received 18 March 1997; accepted 25 August 1997

Calculations on the Low Energy Conformers of N-Acetyl-D-Alanyl-D-Alanine

Abstract: *In this article a conformational analysis of the D-alanyl-D-alanine dipeptide, both charged and neutral, has been carried out. The preferred conformations were determined by means of ab initio and semiempirical quantum, together with empirical force field calculations. The AMBER* force field and the 6-31 + G** and 6-31G** ab initio levels give rise to a coincident minimum energy structure, which, on the other hand, differs from that determined by AM1, 3-21 + G, and 3-21G. The solvent effect on the different charged and neutral conformations have been considered through the AMSOL semiempirical method. A quantification regarding the structural similarities between the different dipeptide conformations and the ampicillin has been performed. The results show that the best overlay is attained by the minimum structure energy obtained by using the 6-31 + G** methodology, which presents a planar amidic nitrogen.* © *1998 John Wiley & Sons, Inc. Biopoly* ***45:*** *119–133, 1998*

Keywords: *conformations of D-alanyl-D-alanine; β-lactam; structural overlay; AMBER force field; AM1; ab initio*

INTRODUCTION

Both carboxy and transpeptidases are enzymes responsible for catalyzing the cross-linking of peptidoglycans in the cell wall. Peptidoglycan is made up of $\beta(1-4)$ linked repeating units of N-acetylglucosamine (NAG) and N-acetyl muramic acid (NAM). The NAM units are linked to short peptide

Correspondence to: Juan Frau Munar, Departament de Química, Universitat Illes Balears, Crta. Valldemossa, km 7.5, Palma de Mallorca 07071, Islas Baleares, Spain
Contract grant sponsor: Spanish Government, DGICYT
Contract grant number: PB96-0596
Biopolymers, Vol. 45, 119–133 (1998)

CCC 0006-3525/98/020119-15

units that present the general sequence L-alanyl-γ-D-glutamyl-L-R-D-alanyl-D-alanine (where L-R = L-lysine; meso- or LL-diaminopimelic acid). During the cross-linking process the D-alanine terminal residue is liberated.

The β-lactam antibiotics structurally mimic the terminal portion of the peptidoglycans (D-Ala-D-Ala portion where the carboxylic group is free) in such a way that the enzyme mistakes them for its natural substrate, being hydrolyzed and inactivated[1]; hence the development of the cell wall is inhibited and the cell ends up exploding.

At present one of the most common practices for the determination of the chemical and physical properties of the molecular system involves the use of the different types of theoretical calculation methods. The first theoretical studies performed on the above-mentioned structural mimicking[2–4] showed that, in fact, a certain similarity could be found between conformations of β-lactam antibiotics and some deformed conformations regarding the D-Ala-D-Ala portion of the natural substrate, i.e., conformations in which the nitrogen corresponding to the peptidic link possessed a certain pyramidal character. However, recent results[5] show that this comparable structure similarity can be achieved either by models in which the nitrogen is planar, or by models in which the nitrogen has a certain pyramidal character.

Most of the theoretical studies carried out on the terminal portion of the peptidoglycan conformations have employed molecular mechanics methods with different force fields. Barnickel et al.,[6] using the ECEPP force field, calculated the lowest energy conformations of the L-alanyl-γ-D-glutamyl-L-R-D-alanyl-D-alanine pentapeptide. Virudachalam and Rao[7] investigated in a fragmented way the possible conformations of the NAG-NAM disaccharide and the pentapeptide, using the Momany and the Scheraga force field.[8] De Coen et al.,[9] by a force field determined by themselves, performed a conformation analysis of the Ac_2-L-Lys-D-Ala-D-Ala tripeptide; however, only the most stable conformer was reported. Finally, Wolfe et al.[10] carried out a conformational analysis of the phenylacetyl-D-alanyl-D-alanine-O^- system by using the MMPEP parameterization by means of the ECEPP force field.

Although these model compounds consist of different polypeptides, they all contain the same terminal residue and should therefore offer the same conformational description of the former. Nevertheless, there is no agreement between the structures obtained by the different models, either regarding the structure of minimum global energy or that of the local minima. On the other hand, and as previously mentioned, in spite of being the D-Ala-D-Ala residue of great biological interest, studies involving better calculation methods, e.g., ab initio, have not been carried out up to the present moment.

This work aims to cover the lack of studies and presents a complete description of the conformation map of the N-acetyl-D-alanyl-D-alanine dipeptide (Figure 1), both charged and neutral. The study has been carried out by molecular mechanics methods using the AMBER* force field,[11–13] by semiempirical methods (AM1),[14] and by ab initio calculation methods using the 3-21G and 6-31G** bases, in addition to diffuse functions when studying the charged system. The use of polarization functions is due to the fact that they are known to provide a better description of the charge distribution within the molecule, thus improving the accuracy of the electrostatic and polarization contributions to the nonbonded interactions. In order to analyze the solvent effect on the different conformations of the dipeptide system, the 6-31G** and 6-31 + G** minima were fully optimized using the AMSOL method,[15] which considers the solvent as a continuum.

The structural similarity between the minimum energy conformers obtained by the ab initio methodology in the gas phase and by the semiempirical methodology in the aqueous phase, and a modified crystal structure of one β-lactam compound representative of the penicillins, ampicillin, has been analyzed.

The results obtained provide a better and more detailed knowledge of the potential energy surface regarding the terminal portion of the peptidoglycan. The inclusion of high quality bases in the ab initio calculations and the resulting computational effort implied has prevented from working with a more extensive peptidic model. Nevertheless, comparative results regarding the former minimum energy conformations and that of the β-lactam antibiotics show that the selected peptide is perfectly adequate for this kind of study.

Finally, it should be outlined that the use of three different theoretical methodologies in order to obtain the results makes an exhaustive comparison of the former feasible.

METHODOLOGY

By using the following different methodologies—molecular mechanics, semiempirical, and ab initio methods—a complete study of the different conformations of the N-acetyl-D-alanyl-D-alanine, both charged and neutral (D-Ala-D-Ala) has been carried out. These computations

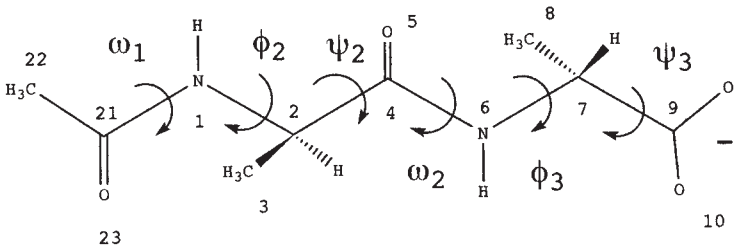


FIGURE 1 Atom numbering scheme for N-acetyl-D-alanyl-D-alanine. The torsion angles discussed in the text are defined as follows: $\omega_1 = C_{23}C_{21}\text{-}N_1C_2$; $\phi_2 = C_{21}N_1\text{-}C_2C_4$; $\Psi_2 = N_1C_2\text{-}C_4N_6$; $\omega_2 = C_2C_4\text{-}N_6C_7$; $\phi_3 = C_4N_6\text{-}C_7C_9$; $\Psi_3 = N_6C_7\text{-}C_9O_{11}$.

have been performed on a Silicon Graphics Iris Indigo XZ4000 and an ALPHA DEC 10620 AXP.

In order to identify the lowest energy structures, a Monte Carlo conformational search with minimization of 30,000 structures was carried out using the phenylacetyl-D-alanyl-D-alanine crystal structure[16] as an input (Cambridge Structural Database[17] code: DIFNAH), with the phenylic chain substituted for an acetyl in the dipeptide. Of all the obtained structures, those in which the convergence was attained and whose energy difference in relation to the global minimum is smaller than 10 kcal/mol were selected.

In the former Monte Carlo search the AMBER* force field[11–13] implemented in the MacroModel 4.5 program[18] was used. The former calculations were performed by means of the electrostatic interactions treatment, used by default, i.e., point charges and a distant-dependent dielectric constant. Besides, an extended cutoff of 8 Å for van der Waals interactions, 12 Å for electrostatic interactions, and 4 Å for hydrogen bonding were carried out.[19]

All selected structures were analyzed by the XCluster 1.1. program,[20] which allowed the classification of these structures and the determination of the clusters and leading structures. The chosen criteria for the determination of the former clusters were atomic rms displacement between pairs of structures following rigid-body overlay (by using the heavy atoms), and rms difference between the corresponding torsion angles in pairs of structures (from the ϕ_2, ψ_2, ϕ_3, and ψ_3 dihedrals, see Figure 1).

Subsequently, the former structures were minimized, with no imposition of geometric restrictions by means of the AM1 semiempirical method,[14] implemented in the AMPAC 5.0 program,[21] and at the SCF level with the 3-21 + G and 6-31 + G** basis sets for the charged structures and with the 3-21G and 6-31G** for the neutral structures, by means of the GAMESS US program[22] as modified by Schmidt et al.[23]

Finally, we considered the influence of the solvent using the AMSOL continuum method (with the AM1-SM2.1 parameterization[24]) as implemented in the program AMPAC 5.0.

In all the obtained 6-31 + G** structures, the values corresponding to both the bond distances and angles underwent minimal variations as shown in the supplementary material.

RESULTS AND DISCUSSION

Charged Dipeptide

Once the conformational search was performed following the methodology described in the previous section a clustering analysis was carried out. The former analysis did not reveal the existence of well-differentiated clusters; however, some structures seemed to possess very similar geometric characteristics. Table I shows the main geometric parameters in addition to the relative energy of the 30 leading structures regarding the D-Ala-D-Ala dipeptide and whose energy does not exceed 10 kcal/mol in relation to the global minimum.

Subsequently, all these structures were minimized with no restrictions by the AM1 semiempirical method and only 12 different conformers obtained (Table II). The remaining structures when being minimized by the AM1 method led to minima that had been previously determined. Thus, as an example, the **1**, **2**, and **25** AMBER* conformers, when being optimized by AM1, give rise to the same structure. Next, the AM1 structures were minimized by the 3-21 + G and 6-31 + G** ab initio methodologies and only 10 structures happened to be real minima (Table II).

Figure 2 shows the four 6-31 + G** conformers whose difference in energy in relation to the global minimum is lower than 3.5 kcal/mol. The lowest energy conformation found (**1**) is characterized by two intramolecular bonds ($N_1H\cdots O_5$ = 2.11 Å and $N_6H\cdots O_{11}$ = 2.01 Å). This structure is different from that found by Wolfe et al.,[10] which presents values for the ψ_3, ϕ_3, ψ_2, and ϕ_2 dihedrals of −120°, 159°, 42°, and 56°, respectively. Wolfe's structure has the side chain almost parallel to the carboxylic group and is very similar to structure **5**. Nevertheless, it is very similar to that of the L-lysyl-D-alanyl-D-alanine model built by Kelly et al.[25] to fit in the binding site of the D-alanyl-D-alanine peptidase

Table I Leading Structures of the *N*-Acetyl-D-Alanyl-D-Alanine Charged Dipeptide Whose Energy Difference Regarding the Global Minimum is Lower than 10.00 kcal/mol, Obtained Through a Conformational Analysis with the AMBER* Force Field

	ψ_3[a]	ϕ_3	ω_2	ψ_2	ϕ_2	ω_1	Relative Energy[b]
1	−171.7	172.3	−180.0	−152.7	166.3	179.6	0.00
2	167.1	160.7	−171.9	−153.7	166.4	179.5	2.28
3	−171.9	172.7	−179.9	−142.2	155.8	−3.8	2.95
4	−172.0	172.6	−180.0	−148.3	164.3	−2.3	3.02
5	−170.8	176.2	177.3	−32.9	154.9	154.2	3.20
6	−170.8	174.4	178.7	−59.2	157.5	−176.6	3.41
7	−166.8	169.2	0.1	−131.8	163.2	179.6	4.05
8	−17.7	118.0	−141.0	42.0	−177.9	176.3	4.52
9	−174.0	171.1	−177.5	42.7	−179.4	176.1	4.72
10	−44.8	97.1	4.1	−61.1	162.9	−171.9	4.78
11	20.6	−125.8	140.5	−29.1	156.9	−175.9	5.01
12	−172.1	172.0	−179.2	71.7	178.8	175.9	5.02
13	−10.0	160.7	−171.6	−142.6	156.0	−3.8	5.23
14	−10.0	160.7	−171.7	−148.2	164.4	−2.3	5.29
15	19.9	−161.0	163.8	−29.5	153.7	−175.7	5.35
16	178.9	115.9	−140.1	39.9	179.8	176.6	5.44
17	−7.2	164.1	−174.4	−34.5	154.9	−176.3	5.83
18	−8.6	163.0	−173.9	−53.6	157.4	−176.5	5.92
19	−9.3	159.7	8.8	−134.7	163.4	179.7	6.08
20	−172.4	−125.9	141.3	−27.1	156.9	−175.7	6.23
21	−166.0	169.0	−0.7	−130.0	154.8	−3.6	7.00
22	−11.6	158.8	−170.6	79.2	178.7	175.5	7.20
23	150.5	94.5	1.8	−60.3	162.0	−171.7	7.31
24	−172.2	172.7	−179.4	−8.4	−71.2	175.2	7.76
25	−171.8	174.8	178.8	131.0	−62.4	176.9	8.52
26	−73.8	108.2	−0.2	−155.1	169.4	−178.9	8.73
27	−172.7	173.4	−179.6	59.8	153.5	−3.7	8.78
28	−8.4	159.8	7.9	−132.7	155.0	−3.7	9.08
29	−8.6	160.0	8.5	−134.5	163.4	−2.2	9.75
30	−160.2	171.5	177.2	75.6	−74.8	178.3	9.96

[a] Dihedral angles are in degrees.
[b] Relative energy is in kcal/mol in relation to the global minimum. Global minimum energy = −20.07 kcal/mol.

from *Streptomyces* R61, and to the global minimum obtained by De Coen et al.[9] with values for the ψ_3, ϕ_3, ψ_2, and ϕ_2 dihedrals of 20°, 160°, −160°, and 160°, respectively.

The next conformer (**6**) possesses two strong hydrogen bonds between the hydrogen atoms bonded to N_1 and N_6 with the O_{11} (1.93 and 2.32 Å, respectively). Conformer **5** is characterized by an intramolecular hydrogen bond ($N_6H\cdots O_{11}$ = 1.94 Å) shorter than that obtained in conformer **1**. Finally, conformer **3** shows a linear chain with two weaker hydrogen bonds. All these structures are influenced by the presence of a deprotonated carboxylic group, which allows the formation of strong hydrogen bonds between one of the oxygen atoms of the carboxylic acid and the hydrogen of the amidic nitrogen.

When comparing the different structures obtained by the four methodologies, the following should be taken into account: the obtained global minimum, energy order of the local minima, and the main geometric characteristics of the obtained minima.

With regard to the global minimum, it can be observed that the AMBER* method and the highest ab initio level determine conformer **1** as a global minimum. The 3-21 + G (**6**) global minimum considerably differs from the former conformer since it consists of a structure that possesses a pyramidal amidic nitrogen, with a dihedral value of ω_2 of −144.1°. The same structure optimized by the 6-31 + G** method is only 0.97 kcal more energetic than the global minimum. The AM1 most stable conformer (**10**) is characterized by a ω_2 dihedral

value of 13.6°, together with the fact that the corresponding AMBER* and ab initio structures possess higher energies in relation to the global minimum (see Table II).

The energy order of the local minima also differs in the four methodologies. The greater similarities are established between AMBER* and 6-31 + G**, which determine the same four conformers as the less energetic. In the ab initio method the stability order is **1** < **6** < **5** < **3**, whereas in AMBER* the conformers **3** and **6** are interchanged.

The equivalent to the ω_2 dihedral in penicillins and cephalosporins is directly related to the pyramidality of the β-lactam nitrogen. In penicillins the former dihedral adopts values of approximately 135°, whereas cephalosporins usually show slightly higher values (155°, approximately).[4] The MM (molecular mechanics) methodology shows that planar structures prevail over pyramidal structures. The most stable pyramidal conformer (**8**, Table I) presents a relative energy of 4.52 kcal/mol. By the AM1 method, pyramidal structures acquire a greater predominance; however, the most stable pyramidal structure does not correspond to the global minimum. On the other hand, the 3-21 + G level shows a pyramidal structure (**6**) as the most stable, 0.3 kcal/mol below the planar structure. With the 6-31 + G** level the results are reversed and the pyramidal structure (**6**) is 0.97 kcal/mol more energetic than the planar global minimum (**1**). The presence of high stability pyramidal structures in these peptidic systems can be attributed to the tendency of the free carboxylic group to establish a hydrogen bond with the two hydrogens of the amidic nitrogen atoms, thus being the peptidic chain distorted (**6**, Figure 2).

The results obtained when we consider the solvent effect (Table II) show that the pyramidal character of the equivalent to the β-lactam nitrogen decreases, as we can see in the structures **6** and **11**. The lowest energy structure, **27**, is characterized by an amidic dihedral angle (ω_1) close to 0°. In the gas phase, this structure is 6.5 kcal/mol more energetic than the global minimum, according to the 6-31 + G** level. The most stable structures (**27**, **5**, **6**, and **1**; Figure 3) correspond to the combination of dihedral angles, (ψ_3, ϕ_3), (ψ_2, ϕ_2), of (−150°, 100°), (50°−70°, 80°−90°), for all structures and (−150°, 100°), (−160°, 100°) for structure **1**. According to the high structural similarity between structures **5** and **6** (see the dihedral angles in Table II), we only consider the most stable of these structures, **5**.

Neutral Dipeptide

An analogous procedure to that previously mentioned and described in the methodology section has allowed us to obtain 41 leading structures with an energy lower than 10 kcal/mol in relation to the global minimum (Table III). Subsequently, these structures were minimized by the semiempirical methodology. Once more the semiempirical optimization leads to analogous structures, thus reducing the number of local minima. These structures were minimized by the ab initio level using the 3-21G basis set, and those structures with a relative energy lower than 5 kcal/mol were minimized at the 6-31G** level. Table IV shows the obtained minimum energy structures.

The main difference with regard to charged conformers lies in the almost negligible pyramidalization of the amidic nitrogen in the minimum energy structures either by the MM, AM1, or ab initio methodologies. The most stable AM1 structure with a certain pyramidal character is **41**, energetically very far from the global minimum. Both the **11** and **25** 3-21G structures, with energies not much higher than that of the global minimum, show a certain pyramidal character; however, values corresponding to the ω_2 dihedral lower than 155°, typical of β-lactam compounds, were not obtained. The lack and/or weakness of hydrogen bonding between the less negative oxygen and the hydrogen of the amidic nitrogen can be the reason for the elimination of this piramidality.

Optimization at the 6-31G** level shows that the global minimum is identical to that obtained by the AMBER* method, structure which, on the other hand, is only 0.03 kcal/mol more energetic than the global minimum obtained by the 3-21G method. The 6-31G** and AMBER* global minimum coincides with that obtained with the same methodology for the charged dipeptide.

The seven lowest energy structures obtained by 6-31G** calculations are **1** < **4** < **25** < **8** < **11** < **26** < **5**. The former structures can be classified into three groups according to the ϕ_2, ψ_2 dihedrals that control the β-lactam ring. The first group consists of structure **1**, which presents an extended conformation and whose values for the ϕ_2, ψ_2 dihedral angles are approximately 160° and −160°, respectively. The second group (**4**, **5**, **8**, **25**, and **26**) consists of a set of structures that present a hydrogen bond between $N_6H\cdots O_{23}$ and the values regarding the ϕ_2, ψ_2 dihedrals range between 80° and 90°, and between −60° and −80°, respectively. The third group consists of structure **11**, also characterized by a hydrogen bond ($N_6H\cdots O_{23}$), and presents ϕ_2, ψ_2 dihedral values of −70° and 70°, respectively. In Figure 4 are depicted the lowest energy structures (**1**, **4**, and **11**) of each of the former groups. Conformer **1** is similar to the C_5 conformation described

Table II Leading Structures of the *N*-Acetyl-D-Alanyl-D-Alanine Charged Dipeptide Whose Energy Difference Regarding the Global Minimum is Lower than 10.00 kcal/mol[a]

	ψ_3	ϕ_3	ω_2	ψ_2	ϕ_2	ω_1	Group[b]	Relative Energy[c]
AMBER*	−171.7	172.3	−180.0	−152.7	166.3	179.6		0.00
AM1	−170.6	131.8	−164.2	−159.3	124.2	−168.2	1, 2, 25	4.27
3-21+G	−173.4	166.6	−176.2	−167.4	165.6	179.8		0.31
6-31+G**	−174.1	157.8	−170.7	−163.4	158.5	−177.3		0.00
AM1-SM2.1	−148.3	106.9	−173.3	−158.5	96.2	−172.5		1.27
AMBER*	−171.9	172.7	−179.9	−142.2	155.8	−3.8		2.95
AM1	−172.6	134.8	−163.4	−150.6	150.8	0.0	3, 4, 13, 14	4.00
3-21+G	−174.1	166.9	−174.3	−159.4	155.5	−4.0		4.10
6-31+G**	−174.7	156.6	−168.2	−150.7	148.6	−2.4		3.52
AM1-SM2.1	−150.4	100.4	−172.4	−151.2	142.9	3.5		1.94
AMBER*	−170.8	176.2	177.3	−32.9	154.9	154.2		3.20
AM1	−163.9	−169.2	170.3	−29.1	113.8	−163.4	5, 15, 17, 18	5.71
3-21+G	−177.4	171.6	−179.3	3.1	94.1	−165.0		2.31
6-31+G**	−177.1	163.8	−174.6	5.8	97.9	−164.7		2.93
AM1-SM2.1	−150.8	101.1	−170.9	54.8	93.9	−179.3		0.42
AMBER*	−170.8	174.4	178.7	−59.2	157.5	−176.6		3.41
AM1	−179.3	91.8	−135.2	63.4	131.0	−170.3	6, 8, 9, 12	0.17
3-21+G	175.9	91.6	−144.1	56.4	164.1	−176.6	16, 22, 24	0.00
6-31+G**	179.9	94.6	−143.4	56.8	159.4	−175.2		0.97
AM1-SM2.1	−149.6	100.0	−169.4	54.6	92.5	−178.6		0.66
AMBER*	−166.8	169.2	0.1	−131.8	163.2	179.6		4.05
AM1	−163.7	159.0	9.1	−135.2	165.0	178.8	7, 19	5.28
3-21+G	−163.7	159.0	8.9	−135.1	165.0	178.9		5.95
6-31+G**	−170.6	152.5	16.7	−142.4	159.8	179.8		5.36
AM1-SM2.1	−123.1	148.4	15.7	−142.3	97.7	−174.7		1.93
AMBER*	−44.8	97.1	4.1	−61.1	162.9	−171.9		4.78
AM1	73.6	91.6	13.6	−67.2	161.7	−168.6	10	0.00
3-21+G	−53.8	99.0	3.3	−52.0	162.4	−180.0		2.28
6-31+G**	−43.7	91.8	12.9	−65.3	161.0	−175.0		3.88
AM1-SM2.1	−25.5	76.1	30.1	−81.1	143.9	178.8		6.93
AMBER*	20.6	−125.8	140.5	−29.1	156.9	−175.9		5.01
AM1	−168.3	−94.1	133.1	−51.4	118.6	−164.1	11, 20	2.04
3-21+G	−2.1	−73.1	142.1	−52.4	163.3	177.6		2.97
6-31+G**	−3.6	−68.9	142.5	−56.7	156.7	−179.1		3.89
AM1-SM2.1	−149.9	−87.2	170.3	−82.2	81.6	179.4		3.75
AMBER*	−166.0	169.0	−0.7	−130.0	154.8	−3.6		7.00
AM1	−162.5	160.1	7.0	−133.0	147.8	−4.6	21, 28	3.75
3-21+G	−171.3	163.5	5.4	−132.6	145.7	−4.0		9.13
6-31+G**	−170.6	155.6	12.4	−135.5	146.0	−5.0		8.27
AM1-SM2.1	−149.6	148.9	13.9	−135.4	141.2	4.4		2.78
AMBER*	150.5	94.5	1.8	−60.3	162.0	−171.7		7.31
AM1	−177.5	75.7	14.8	−65.3	142.6	−164.6	23	0.54
3-21+G	170.4	86.7	4.2	−54.0	154.4	−175.8		2.93
6-31+G**	172.4	80.4	14.2	−67.2	154.8	−170.2		4.01
AM1-SM2.1	−12.9	74.1	27.0	−78.6	161.4	−179.4		6.76
AMBER*	−73.8	108.2	−0.2	−155.1	169.4	−178.9		8.73
AM1	78.6	69.9	20.4	−159.8	174.2	−172.1	26	7.30
3-21+G[d]								
AMBER*	−172.7	173.4	−179.6	59.8	153.5	−3.7		8.78
AM1	−164.9	179.8	175.4	63.9	141.3	−3.0	27	4.74
3-21+G	−173.6	169.1	176.7	23.6	78.5	−10.9		6.09
6-31+G**	−173.6	163.1	−179.0	22.2	83.8	11.7		6.50
AM1-SM2.1	−146.1	107.0	−168.9	72.8	80.3	14.7		0.00

Table II (*Continued from the previous page.*)

	ψ_3	ϕ_3	ω_2	ψ_2	ϕ_2	ω_1	Group[b]	Relative Energy[c]
AMBER*	−8.6	160.0	8.5	−134.5	163.4	−2.2		9.75
AM1	−157.8	160.8	2.9	−130.1	94.9	12.7	29	3.32
3-21+G[e]								

[a] These structures were obtained through a conformational analysis with the AMBER* force field, the AM1 semiempirical method, the 3-21+G and 6-31+G** basis set, and the AM1-SM2.1 solvation model.

[b] AMBER* leading structures that after semiempirical optimization lead to the same structure. Numbering is the same as in Table I.

[c] Relative energy is in kcal/mol in relation to the global minimum for each methodology. Therefore these values should be compared with those obtained with the same methodology. AMBER* energy corresponds to the lowest energy minimum. The total energy of the lowest energy conformation is −20.07 kcal/mol (AMBER*), −213.386 kcal/mol (AM1), −715.0915232 au (3-21+G), −719.0212748 au (6-31+G**), and −281.64 kcal/mol (AM1-SM2.1).

[d] Optimization of this structure with 3-21+G basis set leads to leading structure 7.

[e] Optimization of this structure with 3-21+G basis set leads to leading structure 21.

by Böhm and Brode[26] in the conformational analysis of the N-acetyl-N′-methylalanineamide ($\phi_2 = 156°$ and $\psi_2 = -161°$), which is characterized by two hydrogen bond interactions ($N_1H\cdots O_5 = 2.20$ Å and $N_6H\cdots O_{11} = 2.26$ Å) weaker than those obtained for the most stable charged conformer. Structure **4**, similar to that of C_{7eq} ($\phi_2 = 76°$ and $\psi_2 = -59°$) is characterized by the presence of a hydrogen bond between $N_6H\cdots O_{23} = 2.20$ Å, whereas conformer **11** is similar to C_{7ax} ($\phi_2 = -86°$ and $\psi_2 = 79°$). By means of the AMBER* method, the same stability order is obtained for the former

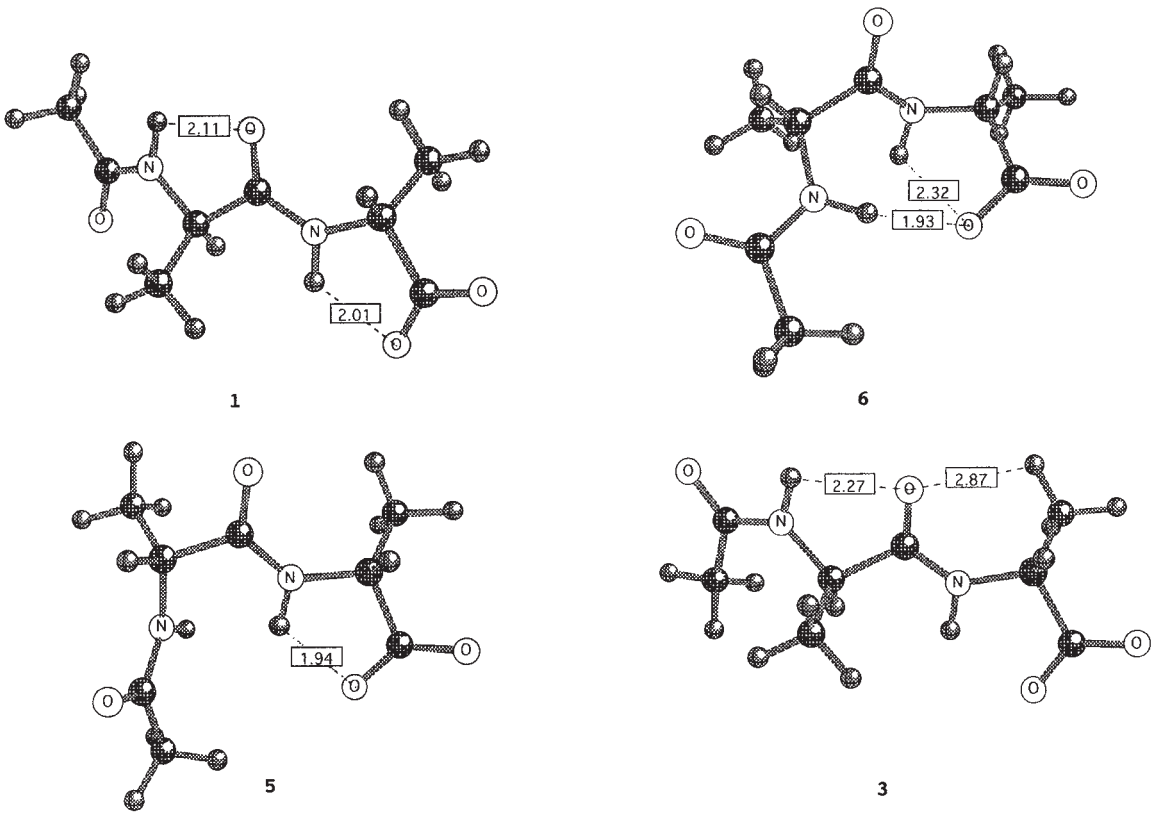


FIGURE 2 Structures of the four low energy conformations of N-acetyl-D-alanyl-D-alanine at 6-31 + G**.

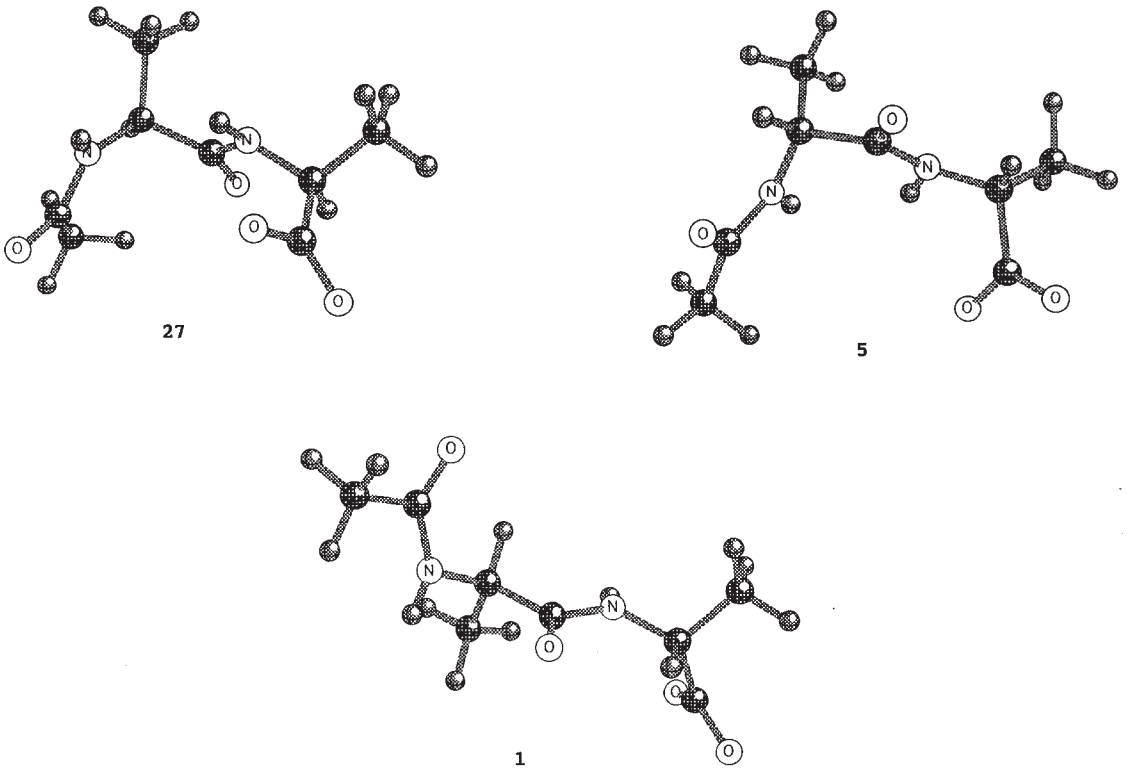


FIGURE 3 Structures of the four AM1-SM2.1 low energy conformations of charged N-acetyl-D-alanyl-D-alanine.

three conformers. Nevertheless, these results differ from those obtained by the AM1 and 3-21G methodologies. These two methods determine the C_{7ax} conformation as the most stable (conformers **4** and **8** in AM1 and 3-21G, respectively).

The seven structures of minima energy when we consider the solvent effect can be grouped according to the values of ϕ_2 and ψ_2 dihedral angles. The first group (structures **1**, **2**, and **18**) is characterized by a values of the former angles of 100° and −160°. The other groups have values of as follows: group 2 (structures **4** and **5**; 90°, −60°), group 3 (structure **25**; 80°, −80°), and group 4 (structure **11**; −80°, 70°). These groups of structures are very similar to those obtained in the gas phase. The main difference is the level of stability; in the aqueous phase structure **4** is calculated as the most stable.

Comparison with Penicillins

We now quantify the structural similarity between the different conformations of the dipeptide and the ampicillin. The conformation of this ampicillin is that obtained for the precatalytic complex with *Staphylococcus aureus* PC1.[27] This structure is similar to the crystallographic ampicillin[28] as the most important difference the orientation of the side chain where the 6-amido N-H group points in a direction parallel to the β-lactam carbonyl oxygen. This orientation is consistent with the obtained by Knox and company in the study of the penicillin docking in different β-lactamases[29–32] and in the D-alanyl-D-alanine peptidase from *Streptomyces* R61.[25]

The 6-31 + G** lowest energy conformers of the charged dipeptide (see Table II) that present a pyramidalization almost negligible for the amidic nitrogen ($\omega_2 \approx 180°$; **1** and **5**) together with those that present a certain pyramidalization with dihedral values $\omega_2 \approx 140°$ (**6** and **11**) were overlaid on ampicillin by minimizing the rms distances between specified pairs of atoms, using the three-dimensional MacroModel molecular graphics software (Table V). The remaining conformers present ω_1 or/and ω_2 dihedrals close to zero degrees, this orientation being unusual for the β-lactam systems. The overlapping sequence includes the O_{23}, C_{21}, N_1, C_2, C_4, O_5, N_6, C_7, and C_9 atoms. The same methodology was used with the AM1-SM2.1 lowest energy conformers (**27**, **5**, and **1**).

A short rms value is indicative of a good overlay;

Table III AMBER* Leading Structures of the *N*-Acetyl-D-Alanyl-D-Alanine Neutral Dipeptide Whose Energy Difference Regarding the Global Minimum is Lower than 10.00 kcal/mol

	ψ_3[a]	ϕ_3	ω_2	ψ_2	ϕ_2	ω_1	O═C_9—O_{10}—H	Relat. Energy[b]
1	−165.9	167.4	179.0	−148.3	164.5	179.2	0.0	0.00
2	−55.3	156.8	−177.3	−148.2	164.4	179.1	1.1	1.12
3	40.0	169.9	176.1	−147.9	164.3	179.1	−1.1	1.98
4	−163.2	166.7	178.9	−67.3	78.6	178.3	−0.2	2.07
5	−62.8	157.7	−177.6	−63.5	78.0	177.6	−1.0	2.10
6	−64.0	79.1	175.3	−147.9	164.0	179.0	−176.0	2.51
7	56.4	−176.3	174.9	−63.1	78.8	178.1	1.8	3.13
8	−62.5	79.2	174.9	−65.4	77.1	177.5	−176.1	3.24
9	−61.3	155.4	−176.8	58.3	−69.8	−178.7	−1.7	3.44
10	61.1	−71.7	−176.7	−145.5	163.8	179.1	174.9	3.57
11	−158.7	165.8	178.5	57.9	−70.8	−178.4	−0.3	3.87
12	59.8	−71.0	−176.8	−86.6	77.5	177.6	175.2	4.04
13	31.3	63.2	−176.1	−148.6	164.5	179.1	2.5	4.57
14	−62.9	78.3	175.3	57.9	−69.5	−178.0	−176.0	4.75
15	−42.2	146.8	−168.4	98.0	−52.5	179.3	−175.3	4.83
16	54.6	179.0	174.5	57.9	−70.1	−178.1	1.0	4.85
17	46.1	−164.2	164.2	−89.0	64.9	179.3	174.5	4.98
18	−42.0	−49.8	176.1	−147.4	164.3	179.2	−3.2	5.29
19	−44.9	146.3	176.0	−147.8	164.2	179.1	−176.8	5.78
20	59.6	−71.9	−176.8	57.6	−69.9	−177.8	175.4	5.85
21	−39.5	148.9	176.4	−63.9	77.4	177.1	−174.0	5.86
22	−41.2	145.9	−168.4	8.6	61.4	172.8	−173.6	5.87
23	34.4	60.1	−176.3	−64.9	77.9	177.7	2.5	5.88
24	50.0	−92.0	178.1	−146.1	164.1	179.3	−0.3	6.04
25	118.8	−56.0	178.8	−146.6	164.3	179.3	3.7	6.07
26	−42.3	−49.1	175.0	−67.5	77.7	178.7	−3.2	6.23
27	−51.5	155.8	−175.4	65.8	−174.5	177.7	1.2	6.38
28	−165.4	167.1	178.9	−148.3	164.4	179.2	−179.8	6.72
29	10.5	−74.9	175.7	−68.2	77.6	178.8	−1.8	6.95
30	117.8	−55.2	177.6	−66.6	78.0	178.4	3.8	6.99
31	−32.4	−57.3	171.0	−100.4	59.9	179.2	−178.0	7.20
32	33.6	61.5	−175.4	59.5	−69.6	−179.2	2.4	7.26
33	50.8	−169.4	−179.9	−146.3	163.5	178.8	179.4	7.89
34	47.4	−161.8	164.8	−6.0	−58.5	−172.3	176.1	7.95
35	−42.2	−48.8	175.2	57.6	−70.3	−178.0	−3.3	8.06
36	13.6	−76.0	175.9	57.3	−70.6	−178.1	−1.7	8.82
37	117.0	−55.2	178.2	58.8	−70.2	−178.1	3.9	8.83
38	−163.0	166.2	178.8	−67.7	78.6	178.4	−179.9	8.89
39	46.7	−172.5	177.4	51.0	−70.8	−176.3	173.8	9.27
40	−18.3	−65.6	−173.7	−142.7	162.9	178.7	−177.3	9.36
41	−33.0	−53.8	168.9	−16.0	−54.1	−174.1	−178.1	9.79

[a] Dihedral angles are in degrees.
[b] Relative energy is in kcal/mol in relation to the global minimum. Global minimum energy = −17.58 kcal/mol.

however, it should be taken into account that distances between the atoms that are involved in the overlapping sequence should not greatly exceed the rms value. In this way overlays that according to the rms value could be considered as valid, although characterized by either very large or very small distances between analogous points, must be eliminated. Both the carbonyl and the carboxylic groups in addition to the nitrogen of the side chain are of great importance since they are responsible for the hydrogen bond interactions between the ligand and the enzyme.[5] In Table V are shown the distances

Table IV Leading Structures of the *N*-Acetyl-D-Alanyl-D-Alanine Neutral Dipeptide Whose Energy Difference Regarding the Global Minimum is Lower than 10.00 kcal/mol[a]

	ψ_3	ϕ_3	ω_2	ψ_2	ϕ_2	ω_1	O═C_9—O—H	Group[b]	Relative Energy[c]
AMBER*	−165.9	167.4	179.0	−148.3	164.5	179.2	0.0		0.00
AM1	−160.1	105.0	−172.3	−148.5	108.4	−171.8	−0.4	1	1.10
3-21G	−175.3	166.4	−177.1	−171.0	167.2	−178.4	0.2		0.03
6-31G**	−169.6	154.4	−174.0	−162.7	157.1	−177.0	0.5		0.00
AM1-SM2.1	−164.0	94.3	−177.2	−157.2	99.1	−173.4	−0.2		0.47
AMBER*	−55.3	156.8	−177.3	−148.2	164.4	179.1	1.1		1.12
AM1	37.8	110.7	−174.4	−147.8	108.4	−171.3	0.5	2, 3, 13	1.67
3-21G	4.9	163.8	−176.7	−170.6	166.8	−178.9	0.9		2.35
6-31G**	28.1	148.8	−178.5	−161.3	155.5	−176.8	0.6		2.55
AM1-SM2.1	50.1	109.5	179.8	−156.8	96.2	−172.9	0.3		1.18
AMBER*	−163.2	166.7	178.9	−67.3	78.6	178.3	−0.2		2.07
AM1	−162.7	97.8	−173.6	−67.4	83.4	−179.7	−0.3	4	0.00
3-21G	−175.8	77.7	−175.5	−65.4	85.1	174.7	−1.2		0.88
6-31G**	−165.6	73.8	−172.0	−63.5	87.2	176.8	−0.8		1.21
AM1-SM2.1	−166.2	87.6	−175.2	−58.3	84.9	178.4	0.1		0.00
AMBER*	−62.8	157.7	−177.6	−63.5	78.0	177.6	−1.0		2.10
AM1	29.0	105.0	−174.9	−68.1	84.5	178.3	1.1	5, 7, 23	0.29
3-21G	39.0	157.5	178.3	−70.8	86.2	175.2	2.6		2.31
6-31G**	33.7	150.3	174.4	−81.2	87.2	175.7	0.8		2.48
AM1-SM2.1	52.6	100.8	−179.6	−67.0	85.1	178.1	0.6		1.21
AMBER*	−64.0	79.1	175.3	−147.9	164.0	179.0	−176.0		2.51
AM1	−74.5	82.7	177.1	−147.3	103.8	−172.9	−178.1	6, 19	4.83
3-21G	−62.2	77.9	174.5	−169.5	165.0	−179.3	−179.5		3.13
6-31G**	−63.2	80.7	176.2	−156.3	154.8	−179.3	−179.9		3.92
AM1-SM2.1	−80.2	80.1	177.0	−154.3	97.7	−174.7	−176.3		4.87
AMBER*	−62.5	79.2	174.9	−65.4	77.1	177.5	−176.1		3.24
AM1	−72.9	82.5	177.0	−68.7	84.3	177.2	−178.4	8, 21	3.03
3-21G	−61.5	77.0	172.3	−68.0	83.9	173.7	−179.0		0.00
6-31G**	−61.5	82.0	170.6	−72.1	86.4	174.9	179.6		2.32
AM1-SM2.1	−76.2	82.8	176.8	−63.3	85.2	176.7	−177.1		4.30
AMBER*	−61.3	155.4	−176.8	58.3	−69.8	−178.7	−1.7		3.44
AM1	42.5	112.0	179.9	60.3	−78.0	−179.9	0.5	9, 16, 32	0.97
3-21G	43.9	161.0	177.5	57.6	−74.7	−175.1	2.9		5.01
AMBER*	61.1	−71.7	−176.7	−145.5	163.8	179.1	174.9		3.57
AM1	70.1	−73.4	−175.4	−136.1	105.0	−172.6	179.2	10	6.24
3-21G	59.2	−69.0	−170.8	−167.0	165.7	−179.0	175.2		5.21
AMBER*	−158.7	165.8	178.5	57.9	−70.8	−178.4	−0.3		3.87
AM1	−168.2	94.3	−172.5	64.3	−75.9	175.7	−0.8	11	0.21
3-21G	−178.2	81.1	−161.6	67.0	−72.8	171.0	−0.6		2.14
6-31G**	−168.8	72.5	−165.2	70.2	−73.0	172.2	−0.7		2.36
AM1-SM2.1	−168.2	89.9	−178.7	68.5	−75.2	−179.3	0.1		0.25
AMBER*	59.8	−71.0	−176.8	−86.6	77.5	177.6	175.2		4.04
AM1	70.2	−72.8	−177.5	−67.2	84.1	179.4	179.8	12, 40	3.61
3-21G	59.3	−66.3	−176.2	−67.8	84.6	174.1	175.1		1.52
6-31G**	56.8	−68.4	−177.9	−72.4	85.9	177.2	179.1		4.09
AM1-SM2.1	71.0	−73.7	−173.3	−72.7	84.5	176.8	179.8		4.86
AMBER*	−62.9	78.3	175.3	57.9	−69.5	−178.0	−176.0		4.75
AM1	−74.9	81.6	176.5	62.3	−76.9	−179.0	−177.8	14	3.19
3-21G	−74.9	81.6	174.5	63.5	−77.8	−178.9	−175.2		1.80
6-31G**	−62.8	79.7	173.1	56.7	−75.5	−175.5	−179.9		4.48
AM1-SM2.1	−76.4	82.7	173.2	64.1	−76.7	−176.2	−177.2		4.41
AMBER*	−42.2	146.8	−168.4	98.0	−52.5	179.3	−175.3		4.83
AM1	96.7	174.2	−167.4	77.3	−69.5	179.8	−178.6	15	5.49
3-21G	−30.8	114.6	−173.5	126.0	−51.4	176.5	177.2		6.31
AMBER*	46.1	−164.2	164.2	−89.0	64.9	179.3	174.5		4.98

Table IV (*Continued from the previous page.*)

	ψ_3	ϕ_3	ω_2	ψ_2	ϕ_2	ω_1	O═C_9—O—H	Group[b]	Relative Energy[c]
AM1	52.4	111.5	−179.4	−73.0	83.8	178.0	−178.3	17, 22, 33	5.65
3-21G	−24.6	129.8	168.9	−74.0	84.4	173.5	−175.7		5.87
AMBER*	−42.0	−49.8	176.1	−147.4	164.3	179.2	−3.2		5.29
AM1	−39.1	−93.9	173.8	−146.8	107.9	−173.5	−2.3	18, 24	4.52
3-21G	−37.5	−51.8	179.9	−170.8	166.9	−178.8	5.5		3.52
6-31G**	−35.2	−54.9	178.2	−163.0	154.6	−175.5	−3.6		3.69
AM1-SM2.1	−66.6	−83.2	179.4	−158.7	94.3	−173.2	−1.3		3.52
AMBER*	59.6	−71.9	−176.8	57.6	−69.9	−177.8	175.4		5.85
AM1	68.9	−73.2	−178.1	65.5	−76.3	−178.3	−179.9	20	4.64
3-21G	58.3	−67.8	−176.1	54.8	−73.6	−172.3	175.9		4.19
6-31G**	54.9	−70.4	−175.5	56.4	−76.2	−172.9	−179.8		6.79
AM1-SM2.1	69.9	−73.9	−175.8	60.1	−77.8	−176.1	179.8		5.32
AMBER*	118.8	−56.0	178.8	−146.6	164.3	179.3	3.7		6.07
AM1	−174.2	−80.0	167.3	−76.2	81.5	−176.0	1.7	25, 30	1.75
3-21G	174.2	−64.2	159.5	−76.1	81.3	−171.4	2.2		0.33
6-31G**	168.9	−60.8	162.1	−81.9	81.8	−171.0	1.2		1.52
AM1-SM2.1	−167.9	−79.0	176.5	−83.9	80.5	179.9	1.5		2.17
AMBER*	−42.3	−49.1	175.0	−67.5	77.7	178.7	−3.2		6.23
AM1	−24.3	−79.6	173.9	−73.7	85.2	179.1	−1.7	26, 29	2.74
3-21G	−37.0	−50.5	175.9	−69.8	83.8	177.3	−5.2		1.30
6-31G**	−34.4	−52.7	171.7	−77.7	83.3	−176.2	−3.3		2.41
AM1-SM2.1	−48.0	−74.0	−177.2	−75.6	85.3	177.5	−1.5		4.21
AMBER*	−51.5	155.8	−175.4	65.8	−174.5	177.7	1.2		6.38
AM1	33.6	110.7	−175.3	40.8	107.2	176.2	1.7	27	2.78
3-21G	4.5	165.8	−175.7	−21.5	124.3	173.4	2.0		4.62
AMBER*	−165.4	167.1	178.9	−148.3	164.4	179.2	−179.8		6.72
AM1	−158.0	108.6	−172.3	−149.3	106.9	−172.2	−179.1	28	6.52
3-21G	−174.0	166.6	−176.8	−170.4	167.2	−179.2	−176.0		8.45
AMBER*	−32.4	−57.3	171.0	−100.4	59.9	179.2	−178.0		7.20
AM1	−38.8	−81.3	165.4	−88.6	73.0	−179.3	178.6	31	7.62
3-21G	−32.4	−59.8	172.4	−106.2	64.5	−177.9	179.9		5.53
AMBER*	47.4	−161.8	164.8	−6.0	−58.5	−172.3	176.1		7.95
AM1	30.6	98.9	−168.7	77.3	−69.5	−179.5	−179.4	34, 39	5.38
3-21G	−31.2	114.8	−173.3	126.1	−51.4	175.9	177.4		6.32
AMBER*	−42.2	−48.8	175.2	57.6	−70.3	−178.0	−3.3		8.06
AM1	−14.7	−80.0	171.9	55.6	−76.3	178.9	−2.2	35, 36	3.34
3-21G	−36.6	−50.4	173.9	56.7	−73.4	−174.2	−5.7		4.03
6-31G**	−35.4	−54.2	175.7	52.0	−76.0	−172.7	−3.7		5.37
AM1-SM2.1	−53.6	−76.7	178.6	58.9	−77.3	−177.7	−1.6		4.16
AMBER*	117.0	−55.2	178.2	58.8	−70.2	−178.1	3.9		8.83
AM1	−172.0	−78.8	168.5	61.1	−75.8	178.7	1.7	37	2.89
3-21G	176.0	−63.1	166.8	59.0	−73.7	−174.0	2.3		4.35
AMBER*	−163.0	166.2	178.8	−67.7	78.6	178.4	−179.9		8.89
AM1	−155.0	101.2	−173.8	−68.7	84.1	178.2	−178.2	38	5.50
3-21G	−173.1	163.8	180.0	−41.2	86.0	175.2	−174.9		9.01
AMBER*	−33.0	−53.8	168.9	−16.0	−54.1	−174.1	−178.1		9.79
AM1	−13.3	−94.3	157.1	32.6	−77.2	−170.4	178.2	41	10.54
3-21G	−28.9	−57.0	179.4	−32.5	−55.5	−168.5	−169.0		6.38

[a] These structures were obtained through a conformational analysis with the AMBER* force field, the AM1 semiempirical method, the 3-21G and 6-31G** basis set, and the semiempirical solvation model AM1-SM2.1.

[b] AMBER* leading structures which after semiempirical optimization lead to the same structure. Numbering is the same as in Table I.

[c] Relative energy is in kcal/mol in relation to the global minimum for each methodology. Therefore these values should be compared with those obtained with the same methodology. AMBER* energy corresponds to the lowest energy minimum. The total energy of the lowest energy conformation is −17.58 kcal/mol (AMBER*), −183.356 kcal/mol (AM1), −715.5076916 au (3-21G), −719.548884 au (6-31G**), and −199.29 kcal/mol (AM1-SM2.1).

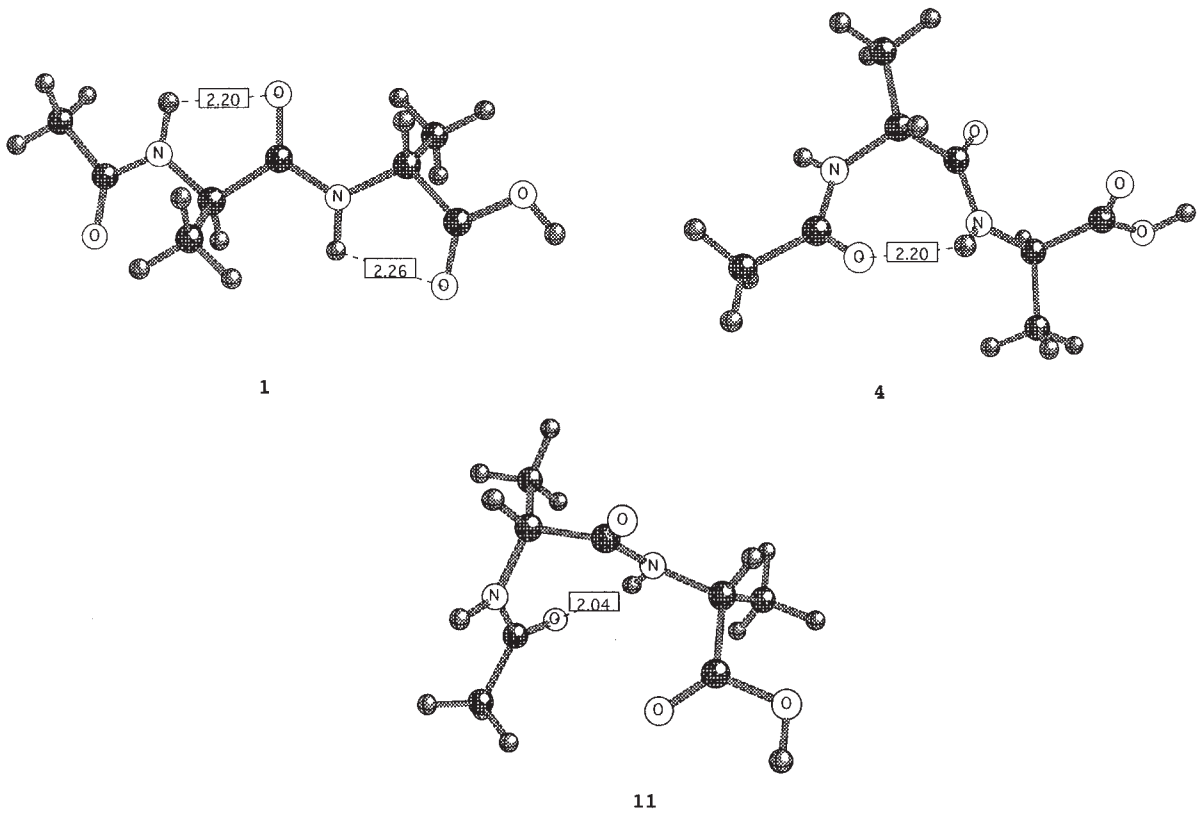


FIGURE 4 Structures of selected conformations of N-acetyl-D-alanyl-D-alanine at 6-31G**: **1** (C_5), **4** (C_{7ax}), and **11** (C_{7eq}).

obtained after the overlay between the side-chain carbonyl oxygen, the side-chain nitrogen, the β-lactam oxygen, and the carbon of the carboxylic group with the equivalent atoms of the substrate. These distances have been assigned as *a*, *b*, *c*, and *d*, and will allow a more detailed overlap analysis.

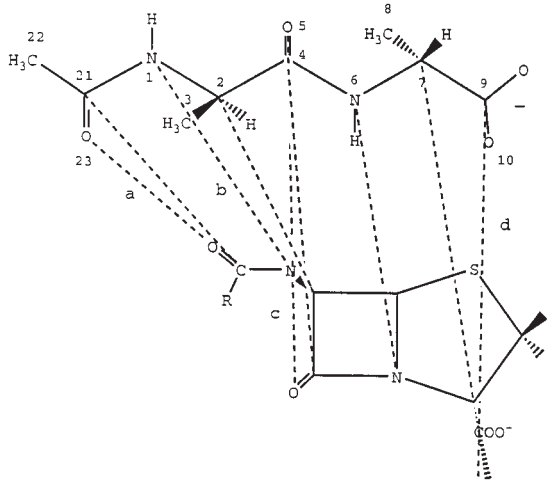
Overlays with conformer **1** are those that give rise to lower rms values (Table V), although the pyramidality regarding both structures is different. Figure 5 shows the overlay of ampicillin with 6-31 + G** conformer **1** and, as we can see, the spatial position of the involved heavy atoms is very similar, showing the structural similarity of these structures. The other overlaps, both with the 6-31 + G** and AM1-SM2.1 conformers, lead to high *d* distance values, which is therefore indicative of a very different carboxylic group orientation. This fact can be once more explained in terms of the existence of a free carboxylic group that has a great capacity for formation of hydrogen bonds without restrictions, in contrast with β-lactam compounds where the rigidity of the bicyclic system hinders this rotational freedom.

From the former results it is clearly revealed that in spite of the β-lactam nitrogen pyramidalization in penicillins, the pyramidal peptidic structure does not show a better overlap with ampicillin than the planar global minimum. These results agree with those obtained by Boyd.[33] Comparing the overlays between the gas phase and the solvated structures, we can see that gas phase ab initio conformers are more suitable to be compared to crystallographic penicillin than solvated conformers.

The same analysis has been carried out on neutral structures. In this case conformers **1**, **4**, and **11**, representative of each of the conformations at 6-31G** level, and conformers **1**, **4**, **25**, and **11** representative of each of the AM1-SM2.1 conformations, were overlaid on the antibiotic by using the same methodology and sequence, as described previously. It becomes clear that, in both methodologies, overlays with conformers **1** and **4** (the lowest energy conformers in 6-31G** and AM1-SM2.1) are those that give rise to the best rms results and critical distances related to the hydrogen bonds in the docking (Table V).

These results indicate that in spite of the large number of obtained structures due to the flexibility

Table V Structural Overlays of Various Conformations of the *N*-Acetyl-D-Alanyl-D-Alanine Charged and Neutral with the Ampicillin Docking Conformation; RMS Distance (Å) Between the Atoms Specifying the Overlay



Charged Structures			
Struct. 1	Struct. 2	RMS	Distances (a/b/c/d)
1 6-31+G**	Ampicilin	0.70	0.69/0.29/0.70/0.47
5 6-31+G**	Ampicilin	0.94	0.78/0.84/1.12/1.39
6 6-31+G**	Ampicilin	1.10	1.01/1.38/0.92/1.38
11 6-31+G**	Ampicillin	0.87	0.72/1.07/0.36/1.70
27 SM2.1	Ampicillin	1.05	0.35/0.65/1.32/1.32
5 SM2.1	Ampicillin	1.10	1.11/0.92/1.63/1.14
1 SM2.1	Ampicillin	0.72	0.61/0.81/1.21/0.79
Neutral Structures			
Struct. 1	Struct. 2	RMS	Distances (a/b/c/d)
1 6-31G**	Ampicilin	0.67	0.64/0.29/0.69/0.46
4 6-31G**	Ampicilin	0.58	0.90/0.26/0.38/0.73
11 6-31G**	Ampicilin	1.11	1.28/1.04/0.52/1.66
1 SM2.1	Ampicillin	0.71	0.58/0.75/1.15/0.88
4 SM2.1	Ampicillin	0.61	1.00/0.25/0.37/0.77
25 SM2.1	Ampicillin	0.83	1.12/0.21/0.23/1.59
11 SM2.1	Ampicillin	1.02	1.25/0.96/0.43/1.39

of this system together with less possibility of pyramidalization of N_6, especially in neutral structures, the most relevant structural mimicking takes place with the minimum potential surface energy, in which no kind of pyramidality is present.

CONCLUSIONS

Once the different methodologies have been analyzed it can be clearly stated that the results regarding molecular mechanics by the AMBER* force field are the most similar to those obtained at ab initio level with relatively high quality bases (6-31G** and 6-31 + G**). It is important to underline that both methods predict the same global minimum. However, AM1 and 3-21 + G (3-21G) methods lead to considerably different results either when predicting the global minimum or with regard to the energy order of the different conformers. Besides, the semiempirical methodology exceedingly stabilizes structures that show a pyramidalization of

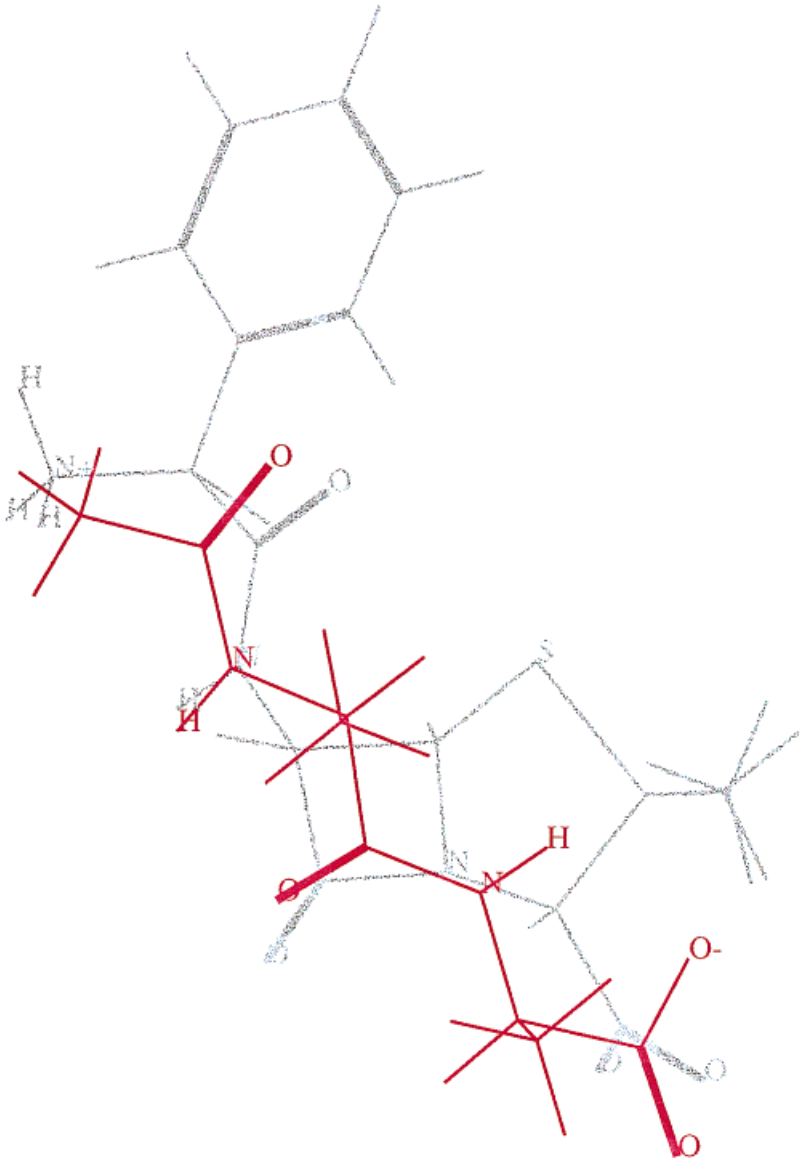


FIGURE 5 Structural overlay (O_{23}, C_{21}, N_1, C_2, C_4, O_5, N_6, C_7, and C_9 atoms sequence) obtained between the 6-31 + G** conformer **1** (red) and ampicillin (grey).

the amidic nitrogen, especially in the case of the charged dipeptide. These results prove that the AM1 semiempirical methodology is not adequate for the analysis of these peptidic systems. Also, comparison between 3-21G and 6-31G** in the conformational study shows the need of using high quality bases in addition to the inclusion of polarization functions. The inclusion of the solvent effect leads to a very different lowest energy conformer in the charged dipeptide, which shows a worse overlay with the docking conformation of ampicillin.

The conformational analysis of the neutral N-acetyl-D-alanyl-D-alanine reveals that conformer **1** is the most stable obtained either by the AMBER* or the 6-31G** methodologies, whereas conformers **4** and **8** (corresponding to conformation C_{7ax}) are the most stable by AM1 and 3-21G. AM1-SM2.1 optimization leads to conformer **4** as the most stable with a geometric parameters very similar to those obtained in the gas phase.

The results obtained prove that for systems with a large number of atoms in which the ab initio methodology with high quality bases (i.e., 6-31G**) is unfeasible, the use of the AMBER force field is more adequate than the AM1 semiempirical methodology.

The large number of analyzed structures in addition to the large number of atoms in the dipeptide

has hindered considering correlation effects that can affect both the geometry and the energy difference between the different conformers.[34] Therefore, in this work a first description of this system of great importance in the study of β-lactam compounds has been carried out. Obviously, a more global description of the process should include molecular dynamic simulations in order not to restrain the conformational study to obtain an static vision of the system. This analysis in vacuum and using a continuum representation of the solvent represents the first relevant step for further consideration of the dynamic effects.

The authors wish to thank Dr. M. Schmidt for kindly supplying the current version of the program GAMESS. We also thank the Centre de Càlcul de la Universitat de les Illes Balears for access to their computer facilities. The support of the Spanish Government is gratefully acknowledged (DGICYT, Project PB96-0596).

REFERENCES

1. Tipper, D. J. & Strominger, J. L. (1965) *Proc. Natl. Acad. Sci. USA* **54,** 1133–1141.
2. Lee, B. (1971) *J. Mol. Biol.* **61,** 463–469.
3. Simon, G. L., Morin, R. B. & Dahl, L. F. (1972) *J. Am. Chem. Soc.* **94,** 8557–8563.
4. Boyd, D. B. (1982) in *Chemistry and Biology of β-Lactams Antibiotics,* Vol. 1, Morin, R. B., & Gorman, M., Eds., Academic Press, London, pp. 437–549.
5. Frau, J. & Price, S. L. (1996) *J. Comp.-Aided Mol. Design* **10,** 107–122.
6. Barnickel, G., Labischinski, H., Bradczek, H. & Giesbrecht, P. (1979) *Eur. J. Biochem.* **95,** 157–165.
7. Virudachalam, R. & Rao, V. S. R. (1979) *Biopolymers* **18,** 571–589.
8. Momany, F. A., Mc Guire, R. F., Burgess, A. W. & Scheraga, H. A. (1975) *J. Phys. Chem.* **79,** 2361.
9. De Coen, J. L., Lamotte-Brasseur, J., Ghuysen, J. M., Frere, J. M. & Perkins, H. R. (1981) *Eur. J. Biochem.* **121,** 221–232.
10. Wolfe, S., Yang, K. & Khalil, M. (1988) *Can. J. Chem.* **66,** 2733–2750.
11. Weiner, S. J., Kollman, P. A., Case, D. A., Singh, U. C., Chio, C., Alagona, G., Profeta, S. & Weiner, P. (1984) *J. Am. Chem. Soc.* **106,** 765–784.
12. Weiner, S. J., Kollman, P. A., Nguyen, D. T. & Case, D. A. (1986) *J. Comput. Chem.* **7,** 230–252.
13. McDonald, D. Q. & Still, W. C. (1992) *Tetrahed. Lett.* **33,** 7743–7746.
14. Dewar, M. J. S., Zoebisch, E. G., Healy, E. F. & Stewart, J. J. P. (1985) *J. Am. Chem. Soc.* **107,** 3902.
15. Cramer, C. J. & Truhlar, D. G. (1991) *J. Am. Chem. Soc.* **113,** 8305–8311.
16. Labischinski, H., Barnickel, G., Naumann, D., Ronspeck, W. & Bradaczek, H. (1985) *Biopolymers* **24,** 2087–2112.
17. Allen, F. H., Davies, J. E., Galloy, J. J., Johnson, O., Kennard, O., Macrae, C. F., Mitchell, E. M., Mitchell, G. F., Smith, J. M. & Watson, D. G. (1991) *J. Chem. Inf. Comput. Sci.* **31,** 187.
18. Mohamadi, F., Richards, N. G. J., Guida, W. C., Liskamp, R., Caufield, C., Chang, G., Hendrickson, T. & Still, W. C. (1990) *J. Comput. Chem.* **11,** 440.
19. *MacroModel 4.5 Manual. Introduction to Molecular Modeling with MacroModel—A Primer* (1994). Dept. of Chemistry, Columbia University, New York.
20. Shenkin, P. S. & McDonald, D. Q. (1994) *J. Comp. Chem.* **15,** 899–916.
21. AMPAC 5.0 (1994) Semichem, 7128 Summit, Shawnee, KS 66216.
22. Dupuis, M., Spangler, D. & Wendoloski, J. J. (1980) *National Resources for Computations in Chemistry, Software Catalog,* Vol. 1, Program QG01, Lawrence Berkeley Laboratory, U.S. Department of Energy, CA.
23. Schmidt, M. W., Baldridge, K. K., Boatz, J. A., Elbert, S. T., Gordon, M. S., Jensen, J. H., Koseki, S., Matsunaga, N., Nguyen, K. A., Su, S. J., Windus, T. L., Dupuis, M. & Montgomery, J. A. (1993) *J. Comput. Chem.* **14,** 1347–1363.
24. Cramer, C. J., Lynch, G. C., Hawkins, G., Truhlar, D. G. & Liotard, D. A. (1993) *QCPE Bull.* **13,** 78.
25. Kelly, J. A., Knox, J. R., Zhao, H., Frere, J. M. & Ghuysen, J. M. (1989) *J. Mol. Biol.* **209,** 281–295.
26. Böhm, H. J. & Brode, S. (1991) *J. Am. Chem. Soc.* **113,** 7129–7135 and references cited therein.
27. Frau, J. & Price, S. L. (1997) *Theor. Chim. Acta* **95,** 151–163.
28. Boles, M. O. & Girven, R. J. (1976) *Acta Crystallog.* **B32,** 2279–2284.
29. Moews, P. C., Knox, J. R., Dideberg, O., Charlier, P. & Frere, J. M. (1990) *Proteins Struct. Funct. Genet.* **7,** 156–171.
30. Juteau, J. M., Billings, E., Knox, J. R. & Levesque, R. C. (1992) *Protein Eng.* **5,** 693–701.
31. Knox, J. R., Moews, P. C., Escobar, W. A. & Fink, A. L. (1993) *Protein Eng.* **6,** 11–18.
32. Lobkovsky, E., Moews, P. C., Liu, H., Zhao, H., Frere, J. M. & Knox, J. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11257–11261.
33. Boyd, D. B. (1979) *J. Med. Chem.* **22,** 533–537.
34. Frey, R. F., Coffin, J., Newton, S. Q., Ramek, M., Cheng, V. K. W., Momany, F. A. & Schäfer, L. (1992) *J. Am. Chem. Soc.* **114,** 5369–5377.