

*Dedicated to Professor Alexandru T. Balaban
on the occasion of his 85th anniversary*

CONFORMATIONAL ANALYSIS AND FORMATION ENTHALPY OF (3R,5S,6R)-6-ACETYLAMIDOPENICILLANIC ACID CALCULATED BY THE PM3, PM6 AND PM7 SEMIEMPIRICAL MO METHODS**

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Thirty conformers of the (3R,5S,6R)-6-acetylamidopenicillanic acid were obtained by PM3 Conformational Search performed with HyperChem7.52 software. By energy minimization with the PM3, PM6 and PM7 semiempirical MO methods included in the MOPAC12 software, thirty, eight, and nine conformers respectively were obtained. The difference $\Delta\Delta_f H^0$ between the lowest and the highest standard formation enthalpy of conformers is 7.6934 kcal·mol⁻¹, 3.6541 kcal·mol⁻¹ and 4.8064 kcal·mol⁻¹ for PM3, PM6, and PM7 semiempirical MO methods, respectively. For all the conformers cubic interpolation relations of functional dependence of formation enthalpy on temperature ($\Delta_f H(T) = a_0 + a_1 T + a_2 T^2 + a_3 T^3$, $T \in [100K, 1000K]$) were established: for PM3

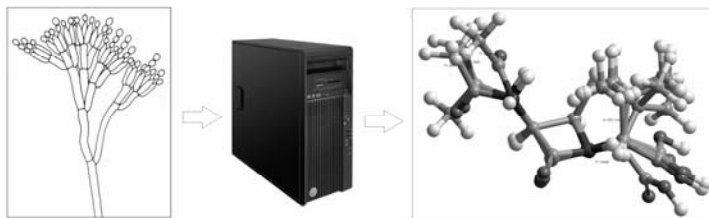
$\Delta_f H(T) = -153.681(\pm 2.132) + 0.0079(\pm 0.0004)T + 1.1622(\pm 0.006)T^2 \cdot 10^{-4} - 3.437(\pm 0.027) T^3 \cdot 10^{-8}$; for PM6

$\Delta_f H(T) = -179.965(\pm 1.233) + 0.008(\pm 0.001)T + 1.174(\pm 0.007)T^2 \cdot 10^{-4} - 3.399(\pm 0.033) T^3 \cdot 10^{-8}$

and PM7 respectively

$\Delta_f H(T) = -165.517(\pm 1.589) + 0.009(\pm 0.001)T + 1.150(\pm 0.008)T^2 \cdot 10^{-4} - 3.273(\pm 0.037) T^3 \cdot 10^{-8}$.

The a_1 , a_2 and a_3 values are neither dependent on the method of calculation, or on the conformer geometry. The a_0 free term is the only one which depends on the conformer geometry. It increases with the decreasing of the thermodynamic stability of conformers. These interpolation relations allow the calculation of molar fraction of each conformer in the mixture at a certain temperature in the interpolation range of $T \in [100K, 1000K]$.



INTRODUCTION

The 6-acetylamidopenicillanic acid is one of the simplest antibacterial compounds of the penicillin class. Its molecular structure, as the one of all

penicillins, contains three chiral centers,¹ marked in Fig. 1. These chiral centers generate $2^3=8$ diastereoisomers: 3R,5R,6R; 3S,5R,6R; 3R,5S,6R; 3R,5R,6S; 3S,5S,6R; 3S,5R,6S; 3R,5S,6S; 3S,5S,6S.^{1,2} Regarding the action mechanism of

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penicillins, the experimental data confirmed the hypothesis proposed by Strominger stating that the D-Ala-D-Ala sequence in the general structure of penicillins is responsible for their antibacterial activity.^{3,4} Although all eight classes of diastereoisomers have been synthesized,⁵ geometric and electronic structures of (3S,5R,6R)-natural isomers of penicillins have been exclusively and extensively studied by different quantum MO methods.⁶⁻⁸

The interest for the thermal behavior of drugs is given by the necessity to know which is their thermal stability, especially for the establishment of storage and transport conditions.⁹ Even if chemical synthesis of drugs had a similar evolution as chemical thermodynamics,¹⁰ only some few information on drug thermodynamic properties are reported. Generally thermodynamic properties are seldom referred to in the introduction sections of organic chemistry books and the content of this information is rarely used.¹¹ Most of the time it is considered that thermodynamic properties have only technological interest.¹⁰ Drugs and biological products are obtained by certain technological processes in which the main product (drug) is in a mixture with a series of secondary products. For example, the synthesis of antibacterial products – antibiotics,^{3,11c} especially penicillins and cephalosporins are described in literature as a stereospecific one. From this reaction different conformers, unstable intermediates, intermediates being in equilibrium or in transition states result. For such a complex system a series of thermodynamic data should be taken into account. The thermal analysis solves partially some of the thermodynamic problems related to the thermal stability, phase transitions, thermal oxidative decompositions and kinetics of thermal decompositions.⁹ Under these circumstances, the data obtained by thermal analysis are only partially useful. If from synthesis result many conformers, an important problem is to know the thermodynamic properties and the percentage of each conformer in the mixture, especially when a single conformer is active. There are many ways to calculate such thermodynamic properties. One is the conformational analysis followed by the obtaining of certain quantitative structure – property relationships (QSPR). QSPR/QSAR

methods are known also as free energy relationships, because they are based on Gibbs free energy (ΔG), a thermodynamic property.^{12,13}

The conformational analysis of molecules of biological interest is performed using thermodynamic properties, too.¹⁴ This implies the knowledge of geometry and energy of distinct conformers. M. J. S. Dewar et al. developed some semiempirical quantum mechanical methods, which can simulate chemical reactions.¹⁵ These approaches allow a good estimation of molecular chemical (thermodynamic) stability and an acceptable assessment of molecular geometry. The standard formation enthalpy ($\Delta_f H^0$) was considered to be the basic experimental property for the optimization of quantum chemical parameters. J. J. P. Stewart^{15c} developed the MOPAC software and continued to improve the optimization of AM1 and PM3 hamiltonians.¹⁶ In the last 10 years J. J. P. Stewart built up the MOPAC package, with versions MOPAC07 (M07), MOPAC09 (M09) and MOPAC12 (M12), by improving the AM1 to RM1 parametrization, the PM3 parametrization and by developing the PM6 and PM7 hamiltonians in the NDDO approximation.^{17,18} In the PM3 parametrization (in MOPAC (M), HyperChem (Hy), SPARTAN softwares)^{15c,16,18-20} the estimation of standard formation enthalpy ($\Delta_f H^0$), ionization energies and electron affinities are satisfactorily calculated, relative to the experimental data. Starting with M07 fundamental thermodynamic properties like: ($\Delta H(T)$) (enthalpy at the T temperature), ($\Delta_f H^0$), ($\Delta_f H(T)$), $C_p(T)$ (heat capacity), $\Delta S(T)$ (entropy) and implicitly $\Delta G(T)$ (Gibbs free energy; $\Delta G(T) = \Delta H(T) - T \Delta S(T)$), outside the phase transformations can be satisfactorily estimated.¹⁸ This way the quantum mechanics offers a tool for the evaluation of certain thermodynamic properties, for which experimental thermodynamic data do not exist.

In this study the geometries of conformers of the (3R,5S,6R)-6-acetylamidopenicillanic acid previously obtained by PM3 Conformational Search performed with HyperChem7.5 (Hy)¹⁹ software were reoptimized by the PM3, PM6 and PM7 hamiltonians, respectively from M12.¹⁸ The formation enthalpies ($\Delta_f H(T)$) obtained by these semiempirical MO methods were calculated.

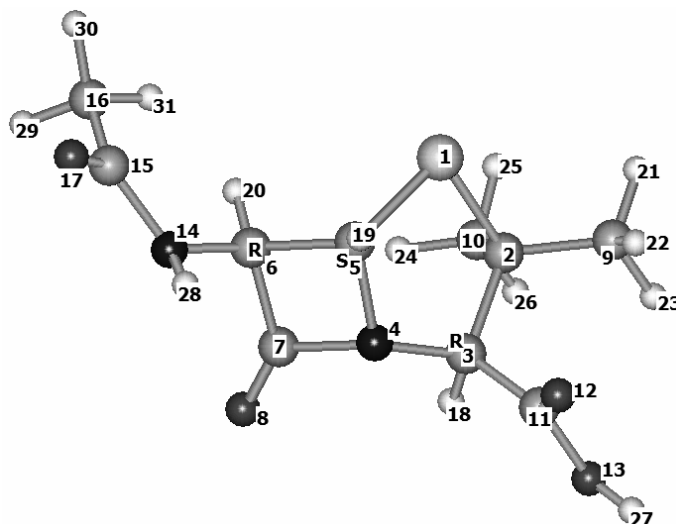


Fig. 1 – Atom numbering in (3R,5S,6R)-6-acetylamidopenicillanic acid.

COMPUTATIONAL DETAILS

Quantum chemical calculations were carried out by using the PM3, PM6 and PM7 hamiltonians included in the M12 Version 13.004W software.¹⁸ The conformer geometries of the (3R,5R,6S)-6-acetylamidopenicillanic acid, obtained by using PM3 Conformational Search performed with Hy7.52¹⁹ were converted into M12 type files by the AVOGADRO software,²¹ by using the "MOPAC" and "Geometry Optimization" keywords. For geometry reoptimization using M12 software in the first step the same keywords were employed: PM3, (PM6 or PM7), SCFCRT=1.D-10, GEO-OK, PRECISE, GNORM=0.01, CYCLES= 5000, T=345600, LET. The resulted optimized geometries thus derived by M12 were transformed into input "mop" files by the AVOGADRO software,²¹ by using the "MOPAC" and "Frequencies" keywords. In the second step, last created MOP files with the following keywords: AUX, LARGE, CHARGE=0, SINGLET, FORCE, THERMO, PM3, (PM6 or PM7), LET were used for the calculation of $\Delta_f H(T)$ with the M12 software.

Same atom numbering used in the previous studies for the SRR enantiomer⁸ was employed also for the (3R,5S,6R)-6-acetylamido-penicillanic acid (see Fig. 1). The standard formation enthalpy ($\Delta_f H^0$) – PM3, PM6 and PM7 calculations) was considered for the ordering of conformers. In addition, the E_{HOMO} and E_{LUMO} energies, the zero vibration point energy (E_{ZVP}) and the total dipole moment (μ_{TOT}) were collected in Tables 1, 2a,2b, 3, 4a,4b, 5a,5b, (Supplementary material) and in

Tables 1 and 2 in this paper. The wave number of the minimum vibration ν_0 was used to demonstrate that the optimized geometry is thermodynamically stable and not a transition state. The calculated formation enthalpy includes all conformer vibrations being thus accurately calculated.¹⁸

The dihedral angles: 5-1-2-3 and 5-4-3-2 were measured in order to appreciate the puckering of the thiazolidinic ring and the dihedral angle 28-14-15-17 for the *anti/syn* conformation of the exocyclic amidic group. A value of $\pm 180^\circ$, respectively $\pm 0^\circ$ of 28-14-15-17, indicates an *anti*, respectively *syn* conformation of the exocyclic amido group. The most thermodynamically stable is the *anti* conformer. In agreement with the

partition function $Q_i = \exp(-E_i/kT) / \sum_{i=1}^n \exp(-E_i/kT)$,

at a given T temperature, all conformers are possible because this function takes into account the molecular conformation, not molecular energy.^{10a}

In addition two other dihedral angles were considered. The 4-5-6-7 angle was taken into account in order to estimate the non-planarity of the β -lactamic ring and the improper dihedral angle 6.15.28.14 for the pseudochirality at the N14 atom belonging the exocyclic amidic group. This pseudochirality was experimentally proved, being generated by the lone pair of the amidic nitrogen atom.²² In the title structure S, respectively R pseudochirality at the N14 atom can be present if the 6.15.28.14 angle has a positive, respectively negative value.

RESULTS AND DISCUSSION

Thirty conformers of the (3R,5S,6R)-6-acetylamidopenicillanic acid were obtained by PM3 Conformational Search performed with Hy software.¹⁹ All conformers have v_0 positive values, confirming that the optimized geometries were in fundamental states. A summary of conformer energy and structure features are presented in Supplementary material (Table 1). The conformers were noted in increasing order of their standard formation enthalpy ($\Delta_f H^0$). The energy difference between the most stable (Hy-01) and unstable (Hy-30) conformer was of 7.6939 kcal·mol⁻¹. From Table 1 (Supplementary material) it can be seen that 12 conformers have negative value of the 6.15.28.14 improper dihedral angle (meaning an R pseudochirality at the N14 atom) and 18 conformers have a positive value of the same improper dihedral angle (meaning an S pseudochirality at the N14 atom). The range of the positive (S) values is between 20.10° and 32.69° and the range of negative (R) ones: -21.82° and -23.40° Supplementary material (Table 1). Among all 30 conformers 14 have *anti* and 16 *syn* amido conformation Supplementary material (Table 1). The minimum energy conformer has *syn* amido conformation and S pseudochirality at the N14 atom. Puckering classes were not observed through the superposition of the thirty PM3 Hy conformers, in which N4, C5 and C6 atoms were considered in the superposition procedure (Fig. 2 a). By geometry optimization with the PM3-M12 hamiltonian of the thirty conformers of the (3R,5S,6R)-6-acetylamido-penicillanic acid given by PM3 Conformational Search (Hy7.5 softwares) the same conformers resulted. Energy and structural features of these conformers are included in Table 3 Supplementary material, in which conformers were numbered in increasing order of their standard enthalpy of formation $\Delta_f H^0$.

The difference $\Delta(\Delta_f H^0)$ between the standard enthalpy of formation of the conformers having the lowest and highest standard enthalpy of formation was of 7.6934 kcal·mol⁻¹, almost identical to the one resulted from the PM3 method implemented in Hy software. From Supplementary material (Table 2) it can be seen that 12 conformers have R pseudochirality and 18 conformers have S pseudochirality at the N14 atom, similar to those resulted from the PM3 method implemented in Hy

software. The range of the positive (S) values is between 20.12° and 32.69°, and the range of negative (R) ones: -21.84° and -23.40° (Supplementary material, Table 2), almost identical to those resulted from the PM3 method implemented in Hy software. Among all thirty conformers 14 have *anti* and 16 *syn* exocyclic amido conformation Supplementary material (Table 2). The minimum energy conformer has *syn* amido conformation and S pseudochirality at the N14 atom. According to these data we reach the conclusion that by conformers's reoptimization by PM3-M12, the values of standard formation enthalpy ($\Delta_f H^0$), the orders of the conformers, the pseudochirality at N14 and *anti-syn* arrangement remain unchanged. Puckering classes were not observed by the superposition of the thirty PM3 M12 conformers, in which N4, C5 and C6 atoms were considered in the superposition procedure (Fig. 2 b).

By optimizing the geometry of all thirty conformers by the PM6-M12 hamiltonian eight distinct conformers resulted (Supplementary material, Table 4a,4b). All conformers have v_0 positive values, confirming that the conformer geometries were accurately optimized and they are not pseudostationary states (Table I). Energy and structural features of these conformers are included in Table I, in which conformers were numbered in increasing order of their standard formation enthalpy of ($\Delta_f H^0$). The difference $\Delta(\Delta_f H^0)$ between the standard formation enthalpy of the conformers having the lowest and highest standard formation enthalpy was of only 3.6541 kcal/mol. During the optimization process a smaller number of conformers was obtained: Hy-03 and Hy-05 generate the PM6-01 conformer; Hy-09 and Hy-10 generate the PM6-02 conformer; Hy-01, Hy-02, Hy-06, Hy-08, and Hy-14 generate the PM6-03 conformer; Hy-15, Hy-16, and Hy-13 generate the PM6-04 conformer; Hy-04, Hy-12, Hy-11, Hy-18, and Hy-07 generate the PM6-05 conformer; Hy-20, Hy-17. and Hy-19 generate the PM6-06 conformer; Hy-29, Hy-24, Hy-22, Hy-27, and Hy-21 generate the PM6-07 conformer; and Hy-25, Hy-30, Hy-26, Hy-23, and Hy-28 generate the PM6-08 conformer (Supplementary material, Table 4a). Puckering classes were not observed by the superposition of the eight PM6 conformers, in which N4, C5 and C6 atoms were used in the superposition procedure (Fig. 2c).

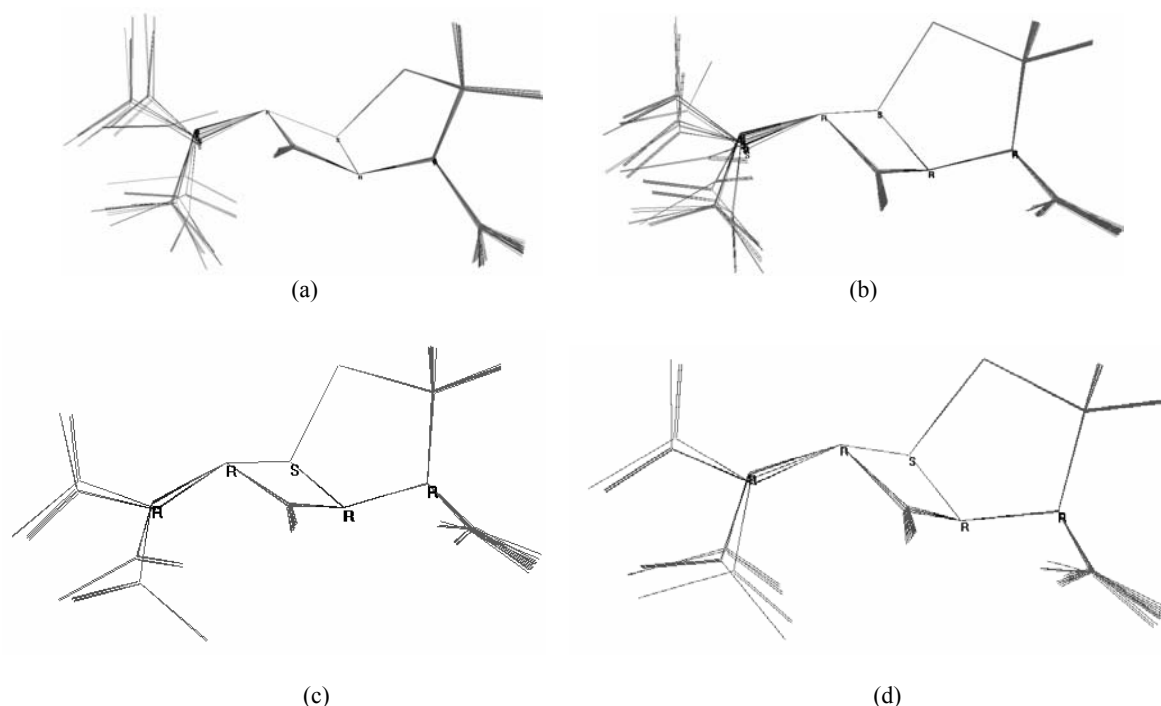


Fig. 2 – Superposition of the 30 (a), 30 (b), 8 (c), and 9 (d) distinct conformers of the (3R,5S,6R)-6-acetylamidopenicillanic acid obtained by the PM3-Hy, PM3-M12, PM6-M12 and PM7-M12 hamiltonians, respectively.

Four out of the eight PM6 conformers have *anti* amido conformation (01, 02, 07 and 08); the other four conformers have *syn* amido conformation (Table 1). Unlike the conformers PM3 HyperChem and PM3 M12, the minimal energy conformer PM6 M12 has an *anti*-conformation. There are some few *anti* conformers which do not have the most low energies. The *syn* conformers have tendency to form intramolecular hydrogen bonds. A result of the PM6 method is that all conformers keep their *anti* or *syn* conformation of the amido group with respect to the starting geometries. Six PM6 conformers have S pseudochirality at the N14 atom; the other two conformers have R pseudochirality. The range of the positive (S) values is between 1.937° and 4.669° and the range of negative (R) ones: -7.518° and -7.678° (Table 1). This fact supports the PM6 tendency to planarize amido group in agreement with experimental data (Supplementary material, Table 6). Data suggest that the N14 atom chirality can be changed, but the *anti-syn* conformation of the amido group remains unchanged by geometry optimization with the PM6 hamiltonian. Thus the interconversion between the *anti/syn* conformations of the amido exocyclic group implies a higher energy barrier than does a pseudochirality change at the N14 atom.

By geometry optimization of the thirty PM3-Hy conformers of the (3R,5S,6R)-6-acetylamidopenicillanic acid by using the PM7-M12 hamiltonian nine

distinct conformers resulted (Supplementary material, Table 5a,5b). All conformers have v_0 positive values (Table 2). Energy and structural features of these conformers are included in Table 2, in which conformers are numbered in increasing order of their standard enthalpy of formation ($\Delta_f H^0$). The difference $\Delta(\Delta_f H^0)$ between the standard enthalpy of formation of conformers having the lowest and highest standard enthalpy of formation, $\Delta_f H^0$, is of only $4.8064 \text{ kcal}\cdot\text{mol}^{-1}$. During the optimization process a smaller number of conformers was obtained. The same PM7 conformer has been obtained from many PM3-Hy conformers. Hy-03, Hy-05, Hy-24, and Hy-27 generate the PM7-01 conformer; Hy-09, Hy-10, Hy-26, and Hy-28 generate the PM7-02 conformer; Hy-01, Hy-02, Hy-06, Hy-08, and Hy-14 generate the PM7-03 conformer; Hy-13, Hy-15, and Hy-16 generate the PM7-04 conformer; Hy-04, Hy-07, Hy-11, Hy-12, and Hy-18 generate the PM7-05 conformer; Hy-17, Hy-19, and Hy-20 generate the PM7-06 conformer; Hy-29 generate the PM7-07 conformer; Hy-21, and Hy-22 generate the PM7-08 conformer; Hy-25, and Hy-30 generate the PM7-08 conformer; Hy-23 generate the PM7-09 conformer (Supplementary material, Table 5a). Puckering classes were not observed by the superposition of the nine PM7 conformers, in which N4, C5 and C6 atoms were used in the superposition procedure (Fig. 2 d).

Table 1

PM6-ID for conformers obtained by M12 hamiltonian, standard formation enthalpy ($\Delta_f H^0$), E_{HOMO} , E_{LUMO} , μ_{TOT} , ν_0 , E_{VZP} , dihedral angles 5-1-2-3, 5-4-3-2, 28-14-15-17, 4-5-6-7, and improper dihedral angle 6.15.28.14

ID	$\Delta_f H^0$ kcal·mol ⁻¹	E_{HOMO} eV	E_{LUMO} eV	μ_{TOT} D	5-1-2-3	5-4-3-2	28-14-15-17 (°)	4-5-6-7	6.15.28.14	N14 Chir.	17, 28 Conf.	ν_0 cm ⁻¹	E_{VZP} kcal·mol ⁻¹
PM6-01	-170.0039	-9.3585	-0.2894	2.136	-8.989	-30.377	-178.217	-5.319	2.289	S	<i>anti</i>	30.88	138.477
PM6-02	-168.8314	-9.3553	-0.2947	2.796	-10.203	-31.174	-177.586	-5.407	3.125	S	<i>anti</i>	31.64	138.514
PM6-03	-168.6504	-9.5596	-0.4934	5.872	-7.706	-30.665	2.466	-5.601	1.937	S	<i>syn</i>	14.95	138.617
PM6-04	-168.1605	-9.5659	-0.5008	5.732	-9.219	-29.638	0.669	-3.938	4.594	S	<i>syn</i>	31.34	138.741
PM6-05	-167.6045	-9.5642	-0.5004	5.327	-9.130	-31.817	4.079	-5.766	3.405	S	<i>syn</i>	15.60	138.643
PM6-06	-167.0480	-9.5670	-0.5078	4.723	-10.654	-30.697	0.353	-4.119	4.669	S	<i>syn</i>	33.31	138.791
PM6-07	-166.9291	-9.2995	-0.1766	3.761	-8.379	-29.859	176.784	-3.321	-7.518	R	<i>anti</i>	22.28	138.509
PM6-08	-166.3517	-9.3048	-0.1802	3.990	-9.323	-31.893	176.726	-3.869	-7.678	R	<i>anti</i>	25.86	138.519
Mean²³		-9.4469	-0.3679	4.292	-9.200	-30.765		-4.668				25.73	138.601
SD²³		0.1272	0.1483	1.367	0.933	0.828		0.951				7.38	0.117

Table 2

PM7-ID for conformers obtained by MOPAC12, standard formation enthalpy ($\Delta_f H^0$), E_{HOMO} , E_{LUMO} , μ_{TOT} , ν_0 , E_{VZP} , dihedral angles 5-1-2-3, 5-4-3-2, 28-14-15-17, 4-5-6-7, and improper dihedral angle 6.15.28.14

ID	$\Delta_f H^0$ kcal·mol ⁻¹	E_{HOMO} eV	E_{LUMO} eV	μ_{TOT} D	5-1-2-3	5-4-3-2	28-14-15-17 (°)	4-5-6-7	6.15.28.14	N14 Chir.	17, 28 Conf.	ν_0 cm ⁻¹	E_{VZP} kcal·mol ⁻¹
PM7-01	-156.1798	-9.1131	-0.6459	2.377	-11.147	-30.356	-177.605	-5.680	3.087	S	<i>anti</i>	30.62	140.215
PM7-02	-155.2229	-9.1175	-0.6503	2.828	-11.108	-30.753	-177.913	-5.864	2.984	S	<i>anti</i>	30.21	140.258
PM7-03	-154.4311	-9.3184	-0.8654	5.760	-10.561	-30.902	3.680	-5.796	3.716	S	<i>syn</i>	15.70	140.393
PM7-04	-153.9480	-9.3129	-0.8678	5.620	-11.180	-29.512	-0.516	-4.298	3.187	S	<i>syn</i>	35.64	140.499
PM7-05	-153.6369	-9.3273	-0.8737	4.856	-10.326	-31.357	3.784	-6.017	3.467	S	<i>syn</i>	15.64	140.440
PM7-06	-153.0885	-9.3225	-0.8763	4.324	-11.097	-30.268	-0.304	-4.587	4.590	S	<i>syn</i>	35.94	140.566
PM7-07	-152.0008	-9.0584	-0.5892	3.630	-11.392	-30.047	179.862	-4.386	2.993	S	<i>anti</i>	34.36	140.067
PM7-08	-151.8152	-9.0191	-0.5529	3.830	-11.315	-30.028	176.755	-2.340	-7.323	R	<i>anti</i>	24.95	140.139
PM7-09	-151.3734	-9.0354	-0.5622	4.577	-9.961	-31.035	175.860	-3.057	-8.400	R	<i>anti</i>	28.01	140.220
Mean²³		-9.1805	-0.7204	4.200	-10.899	-30.473		-4.669				27.90	140.311
SD²³		0.1364	0.1463	1.157	0.495	0.583		1.313				7.80	0.171

Five out of the nine PM7 conformers have *anti* amido conformation (01, 02, 07, 08 and 09), the other four conformers have *syn* amido conformation (Table 2). Unlike according to PM3 Hy and PM3 M12, the global minimum PM7 conformer has the *anti* conformation. The *syn* conformers have tendency to form intramolecular hydrogen bonds. A result of the PM7 geometry optimization is that all conformers keep their *anti* or *syn* conformation of the amido group with respect to the starting geometries.

Six PM7 conformers have S pseudochirality at the N14 atom, the other three conformers have R pseudochirality. The range of the positive S values is between 2.984° and 4.590° and the range of negative R ones is between -7.323° and -8.400° (Table 2). This proves that by optimising the geometry with the PM7 hamiltonian the amido group has a tendency to be planar, much closer to the experimental data (Table 6, Supplementary material). Data suggest that the N14 atom chirality can be interchanged, but the *anti-syn* conformation of the exocyclic amido group remains unchanged by geometry optimization with the PM7 hamiltonian. Thus the interconversion between the *anti/syn* conformations of the amido group implies a higher energy barrier than does a pseudochirality change at the N14 atom.

Obviously, there are not significant differences between calculated average values for E_{HOMO} , and E_{LUMO} given by PM3 HyCh or PM3 M12 ($E_{\text{HOMO}}(\text{Hy})$, and $E_{\text{LUMO}}(\text{Hy})$ are -9.6066±0.1255eV, and -0.3770±0.1278eV, respectively, while $E_{\text{HOMO}}(\text{M12})$, and $E_{\text{LUMO}}(\text{M12})$ are -9.6066e±0.1255eV, and -0.3772±0.1277eV, Supplementary material). There are significant differences between calculated average values for E_{HOMO} , and E_{LUMO} given by PM6 (E_{HOMO} , and E_{LUMO} are -9.4469±0.1272eV, and -0.3679 ±0.1483eV, Table 1), respectively, and PM7 (E_{HOMO} , and E_{LUMO} are -9.1805±0.1364eV, and -0.7204 ±0.1463eV, Table 2).²³

Similarly the mean values of the calculated total dipole moment: $\mu_{\text{TOT}}(\text{PM3,Hy})$, and $\mu_{\text{TOT}}(\text{PM3,M12})$ (3.731±0.926D, and 3.731±0.924D respectively, Supplementary material) are similar, and different from $\mu_{\text{TOT}}(\text{PM6})$ (4.292±1.367D, Table 1), and $\mu_{\text{TOT}}(\text{PM7})$ (4.200±1.157D, Table 2).²³ Both in the case of PM3 (Hy and M12, Supplementary material) methods, as well in the case of PM6 (Table 1) and PM7 methods (Table 2) respectively, we can remark a difference between dipole moments of *syn*, respectively *anti* conformers.

The mean puckering of the β -lactamic ring 4-5-6-7 estimated by the PM3(Hy), PM3(M12) (Supplementary material, -2.294°±1.004; respectively -2.135°±1.329) is slightly different from that

estimated by the PM6 (-4.668°±0.951, Table 1), respectively PM7 (-4.669°±1.313, Table 2) methods.²³ The values of the dihedral angle 4-5-6-7 calculated by PM3, PM6, and PM7 methods suggest that the puckering of β -lactamic cycle exists, and the values of PM6 and PM7 are slightly different from the values of PM3.

The mean puckering of the five-member thiazolidinic ring, namely the 5-1-2-3 and 5-4-3-2 dihedral angles, resulted from the PM3(Hy), PM3(M12) methods have the following values: -10.208° ±0.697, and -18.680°±0.768, respectively, -10.222°±0.672, and -18.696°±0.775, Supplementary material). The values given by the PM6 and PM7 methods are: -9.200°±0.933, and -30.765°±0.828, Table 1), respectively (-10.899°±0.495, and -30.473°±0.583, Table 2).²³ Indifferently of the calculation method the average values of angle 5-1-2-3 are similar, while the average values of angle 5-4-3-2 given by the PM3 method are different from those given by PM6 or PM7 methods.

The average values of ν_0 vibration (cm^{-1}) are almost the same for PM3(Hy) (30.74±4.33), and PM3(M12) (30.77±4.38, respectively, Supplementary material). The calculated average values of the zero vibration energy $E_{\text{ZVP}}(\text{R}_0)$ ($\text{kcal}\cdot\text{mol}^{-1}$) are: PM3(Hy) (149.837±0.237), PM3(M12) (Supplementary material, 149.821±0.236), respectively, PM6 (138.601±0.117) (Table 1), and PM7 (140.311±0.171) (Table 2).²³ They have small dispersion and therefore the obtained values are different with respect to the used methods. One can observe that the calculated PM6 values are comparable to the PM7 ones.

By superposition of global minimum conformers, optimized by PM3(Hy), PM3(M12), PM6(M12) and PM7(M12) one can remark as follows: a) the conformers obtained with PM3(Hy) and PM3(M12) (Fig.3) are perfectly overlaying and they have *syn* conformation, whereas the ones obtained with PM6, and PM7 methods have a different spatial arrangement and *anti*-conformation (Fig. 3).

Formation enthalpy. The functional dependence of the formation enthalpy $\Delta_f H$ for each PM3, PM6 or PM7 conformer has been calculated as cubic function of temperature: $a_0 + a_1 T + a_2 T^2 + a_3 T^3$.²⁴ The chosen temperature range was 100K-1000K, varied by a step of 50°. The elementary data obtained for each conformer are included in Supplementary material (Table 7a, and Table 7b (PM3), Table 8 (PM6), and Table 9 (PM7)). Formation enthalpies are calculated relative to the elements in their standard state at 298K, according to MOPAC manual.^{18b}

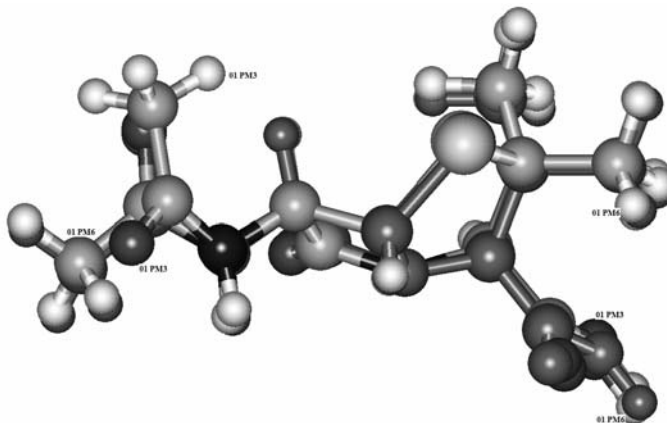


Fig. 3 – Superposition of the PM3(Hy), and PM3(M12) over the PM6, respectively PM7 (violet) minimum energy conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid.

Graphical representation of the formation enthalpy resulted from PM3(M12) calculations against temperature variation is presented in Fig. 4a.²⁴

By analysis of the data from Table 3 it can be noted that the parameter a_0 increases with the degree of instability of the conformer. From Fig. 4a a nearly parallel pack of interpolation functions can be observed. The resulted curves have almost the same concavity and differ only by the free term, i.e. differ only by the coordinate ($\Delta_f H$, $T=0$ K). The a_0 value is proportional to the standard formation enthalpy, and can be considered a measure of the conformer's thermodynamic stability. Between the lowest and the highest value of the free term, a_0 , there is an energy difference of $7.7916 \text{ kcal}\cdot\text{mol}^{-1}$. The linear correlation between the $\Delta_f H^0(\text{PM3})$ and $a_0(\text{PM3})$ is:

$$\Delta_f H^0(\text{PM3}) = 9.7023 + 0.9867a_0(\text{PM3}) \quad (1)$$

$(r^2 = 0.9981; \text{SD} = 0.0929; F = 14868)$

with a very good correlation coefficient of 99.81% and a very high statistical confidence. The graphical representation of this correlation is presented in Fig. 5a.²⁴

From correlation $\Delta_f H^0(\text{PM3}) = f(a_0(\text{PM3}))$ one observes an energetic grouping of the conformers: a first group is formed by conformers starting with PM3-01 to PM3-12, a second group is formed by conformers from PM3-13 to PM3-28 and a third group is formed by the last two – the most unstable – conformers.

Between parameters a_1 , a_2 , and a_3 of the interpolation function it cannot be established a correlation. In order to compare these functions between themselves (PM3, PM6, and PM7) an average interpolation function has been computed:

$$\Delta_f H^0(T) = -153.6810(\pm 2.1322) + 0.0079(\pm 0.0004)T + 1.1622(\pm 0.0055)T^2 \cdot 10^{-4} - 3.4368(\pm 0.0269)T^3 \cdot 10^{-8} \quad (2)$$

$T \in [100\text{K}, 1000\text{K}]; \quad (\text{SD} = 0.0347(\pm 0.0010); F = 4.2185(\pm 0.2289) \cdot 10^6)$

The interpolation coefficients in Table 3 allow the formation enthalpy calculation in the temperature range $T \in [100\text{K}, 1000\text{K}]$ using the PM3(M12) method.^{23,24} This data cannot be obtained experimentally.

Formation enthalpy resulted from PM6 method was plotted against the temperature variation (Fig. 4b). By analysis of the data from Table 4 it can also be noted that the parameter a_0 increases with the degree of instability of the conformer.

Table 3

Polynomial interpolation relations ($y = a_0 + a_1T + a_2T^2 + a_3T^3$) of the functional dependence of $\Delta_f H(T)$ for conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid given by PM3-M12 method^{23,24}

Conformerul	a_0	a_1	$a_2 \cdot 10^4$	$a_3 \cdot 10^8$	r^2	SD	$F \cdot 10^{-6}$
PM3-01	-156.5227	0.0078	1.1585	-3.4107	1.0000	0.0337	4.4361
PM3-02	-156.3397	0.0080	1.1563	-3.4024	1.0000	0.0334	4.5140
PM3-03	-156.2600	0.0085	1.1559	-3.4121	1.0000	0.0344	4.3008
PM3-04	-156.0241	0.0078	1.1592	-3.4146	1.0000	0.0336	4.4649
PM3-05	-156.1691	0.0084	1.1569	-3.4160	1.0000	0.0343	4.3031
PM3-06	-155.9493	0.0080	1.1613	-3.4333	1.0000	0.0347	4.2152
PM3-07	-155.8557	0.0080	1.1568	-3.4050	1.0000	0.0335	4.4976
PM3-08	-155.8255	0.0081	1.1600	-3.4282	1.0000	0.0343	4.3116

Table 3 (continued)

PM3-09	-155.6904	0.0085	1.1565	-3.4153	1.0000	0.0342	4.3308
PM3-10	-155.5469	0.0084	1.1574	-3.4198	1.0000	0.0345	4.2761
PM3-11	-155.4477	0.0080	1.1621	-3.4382	1.0000	0.0347	4.2122
PM3-12	-155.3385	0.0081	1.1605	-3.4315	1.0000	0.0345	4.2518
PM3-13	-153.6861	0.0072	1.1716	-3.4799	1.0000	0.0360	3.9104
PM3-14	-153.5352	0.0076	1.1639	-3.4368	1.0000	0.0342	4.3198
PM3-15	-153.3728	0.0074	1.1716	-3.4857	1.0000	0.0363	3.8433
PM3-16	-153.2856	0.0079	1.1662	-3.4631	1.0000	0.0359	3.9401
PM3-17	-153.0534	0.0072	1.1723	-3.4845	1.0000	0.0362	3.8667
PM3-18	-153.0160	0.0075	1.1646	-3.4412	1.0000	0.0343	4.3070
PM3-19	-152.7469	0.0074	1.1722	-3.4893	1.0000	0.0365	3.8186
PM3-20	-152.7019	0.0079	1.1670	-3.4670	1.0000	0.0360	3.9189
PM3-21	-152.6090	0.0084	1.1598	-3.4346	1.0000	0.0352	4.0949
PM3-22	-152.4778	0.0085	1.1595	-3.4335	1.0000	0.0352	4.1238
PM3-23	-152.2643	0.0084	1.1603	-3.4371	1.0000	0.0353	4.0780
PM3-24	-152.0513	0.0079	1.1599	-3.4172	1.0000	0.0337	4.4443
PM3-25	-152.2098	0.0084	1.1604	-3.4391	1.0000	0.0354	4.0645
PM3-26	-151.8683	0.0078	1.1603	-3.4197	1.0000	0.0337	4.4672
PM3-27	-151.3209	0.0083	1.1560	-3.4034	1.0000	0.0333	4.5847
PM3-28	-151.1292	0.0082	1.1567	-3.4071	1.0000	0.0333	4.5712
PM3-29	-149.4057	0.0072	1.1702	-3.4669	1.0000	0.0352	4.0626
PM3-30	-148.7311	0.0072	1.1710	-3.4714	1.0000	0.0354	4.0236
Mean²³	-153.6810	0.0079	1.1622	-3.4368	1.0000	0.0347	4.2185
SD²³	2.1322	0.004	0.0055	0.0269	0.0000	0.0010	0.2289

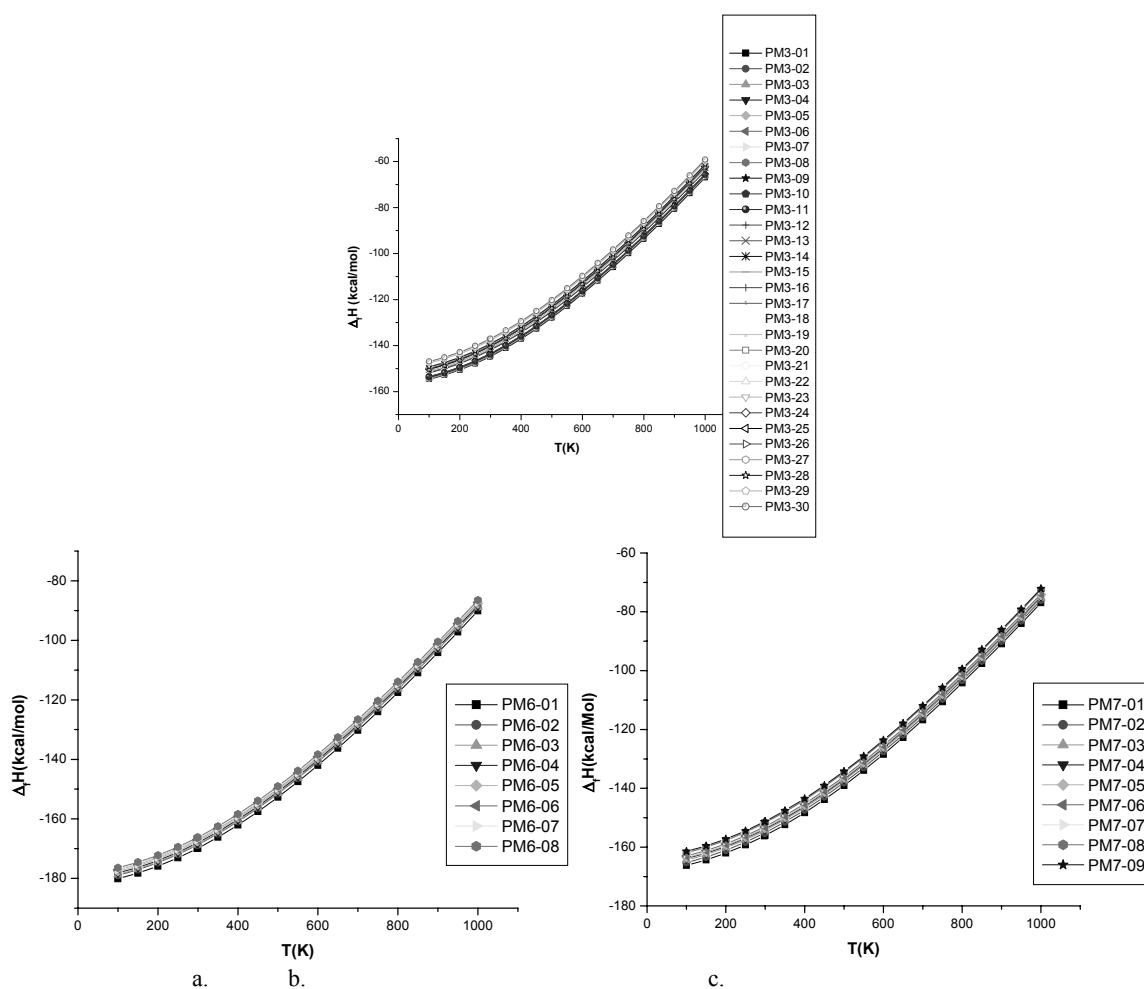
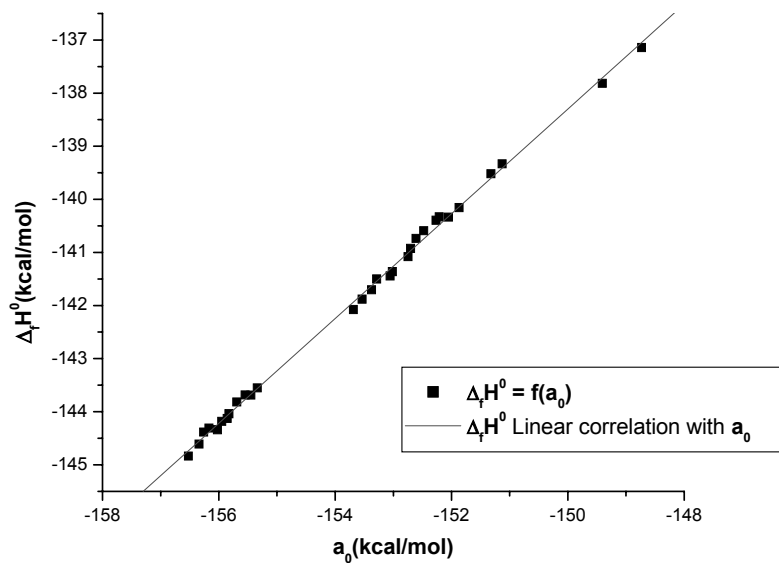
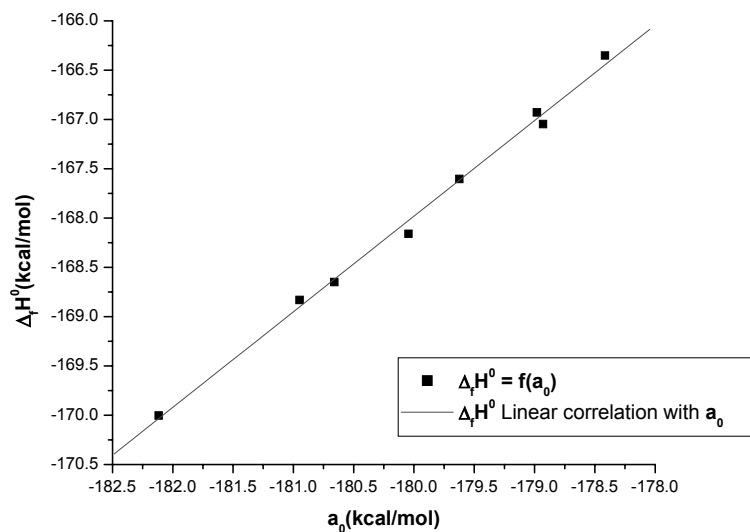


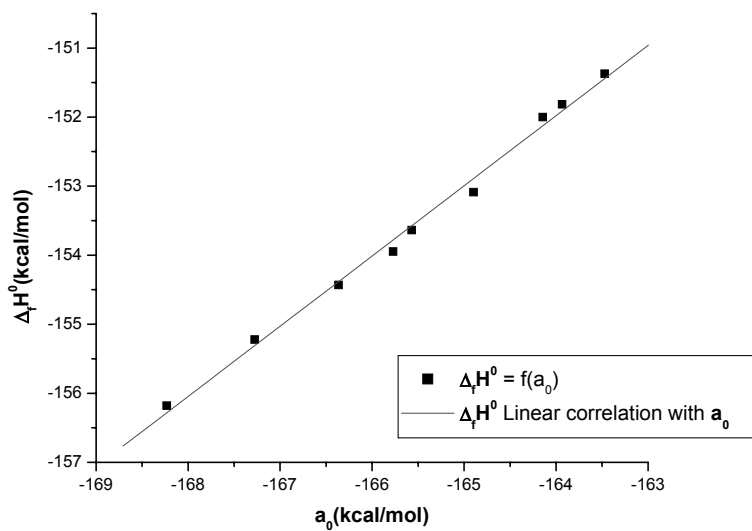
Fig. 4 – Functional dependence $\Delta_r H(T)$ plots in the range of 100K-1000K resulted for: a. PM3; b. PM6; and c. PM7 - M12 conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid.^{18b,24}



a.



b.



c.

Fig. 5 – Linear correlation of: a. $\Delta_f H^\circ(\text{PM3}) = f(a_0(\text{PM3}))$; b. $\Delta_f H^\circ(\text{PM6}) = f(a_0(\text{PM6}))$; c. $\Delta_f H^\circ(\text{PM7}) = f(a_0(\text{PM7}))$.²⁴

Fig. 4b shows a pack of interpolation functions nearly parallel. Curves have almost the same concavity and differ only by the free term, a_0 . The difference between the highest and the lowest a_0

values is $2.7015 \text{ kcal}\cdot\text{mol}^{-1}$. The free term a_0 can be correlated with the standard formation enthalpies $\Delta_f H^0$. The linear correlation between $\Delta_f H^0(\text{PM6})$ and $a_0(\text{PM6})$ is:

$$\Delta_f H^0(\text{PM6}) = 6.5606 + 0.9697 a_0(\text{PM6}) \quad (r^2 = 0.9944; \text{SD} = 0.0899; F = 1238.9) \quad (3)$$

with a very good correlation coefficient of 99.44% and a very high statistical confidence.²³ The

graphical representation of this correlation is presented in Fig. 5b.

Table 4

Polynomial interpolation relations ($y = a_0 + a_1 T + a_2 T^2 + a_3 T^3$) of the functional dependence of $\Delta_f H(T)$ for the 8 PM6 conformers^{23,24}

Conformer	a_0	a_1	$a_2 \cdot 10^4$	$a_3 \cdot 10^8$	r^2	SD	$F \cdot 10^{-6}$
PM3-01	-182.1165	0.0089	1.1674	-3.3680	1.0000	0.0297	5.9922
PM3-02	-180.9480	0.0089	1.1666	-3.3638	1.0000	0.0295	6.1048
PM3-03	-180.6600	0.0084	1.1741	-3.3988	1.0000	0.0300	5.8796
PM3-04	-180.0451	0.0077	1.1845	-3.4487	1.0000	0.0304	5.7432
PM3-05	-179.6231	0.0084	1.1733	-3.3953	1.0000	0.0297	5.9832
PM3-06	-178.9295	0.0077	1.1842	-3.4470	1.0000	0.0300	5.8872
PM3-07	-178.9796	0.0086	1.1715	-3.3864	1.0000	0.0299	5.9438
PM3-08	-178.4150	0.0086	1.1708	-3.3838	1.0000	0.0295	6.1098
Mean ²³	-179.9650	0.0084	1.1741	-3.3990	1.0000	0.0298	5.9555
SD ²³	1.2333	0.0005	0.0069	0.0325	0.0000	0.0003	0.0218

Between parameters a_1 , a_2 and a_3 of the interpolation function it cannot be established a

correlation. An average interpolation function for the PM6 calculations has been computed:

$$\Delta_f H^0(T) = -179.9650 (\pm 1.2333) + 0.0084 (\pm 0.0005) T + 1.1741 (\pm 0.0069) T^2 \cdot 10^{-4} - 3.3990 (\pm 0.0325) T^3 \cdot 10^{-8} \quad (4)$$

$T \in [100\text{K}, 1000\text{K}] \quad (\text{SD} = 0.0298 (\pm 0.0003); 5.9555 (\pm 0.0218) \cdot 10^6)$

The interpolation parameters in Table 4 allow the formation enthalpy calculation in the temperature range $T \in [100\text{K}, 1000\text{K}]$ with the PM6(M12) method.²³

Terms of the interpolation functions (a_0 , a_1 , a_2 and a_3) and the statistical indices (r^2 , SD and F) are presented in Fig. 5c, and Table 5 for PM7(M12) calculations.²⁴

Table 5

Polynomial interpolation relations ($y = a_0 + a_1 T + a_2 T^2 + a_3 T^3$) of the functional dependence of $\Delta_f H(T)$ for the PM7 conformers^{23,24}

Conformer	a_0	a_1	$a_2 \cdot 10^4$	$a_3 \cdot 10^8$	r^2	SD	$F \cdot 10^{-6}$
PM3-01	-168.2308	0.0092	1.1454	-3.2510	1.0000	0.0255	8.0108
PM3-02	-167.2752	0.0093	1.1444	-3.2457	1.0000	0.0250	8.3339
PM3-03	-166.3647	0.0087	1.1527	-3.2840	1.0000	0.0257	7.8913
PM3-04	-165.7704	0.0080	1.1624	-3.3312	1.0000	0.0261	7.6299
PM3-05	-165.5688	0.0087	1.1523	-3.2817	1.0000	0.0254	8.0765
PM3-06	-164.8964	0.0080	1.1626	-3.3311	1.0000	0.0258	7.8364
PM3-07	-164.1434	0.0096	1.1429	-3.2457	1.0000	0.0255	8.0715
PM3-08	-163.9335	0.0095	1.1424	-3.2412	1.0000	0.0252	8.2177
PM3-09	-163.4713	0.0094	1.1431	-3.2437	1.0000	0.0246	8.5897
Mean	-165.5170	0.0089	1.1498	-3.2728	1.0000	0.0254	8.0731
SD	1.5893	0.0006	0.0082	0.0367	0.0000	0.0004	0.2840

Data from Table 5 show that the parameter a_0 increases with the degree of instability of the conformer. Fig. 5c presents a pack of interpolation functions similar to the ones resulted for the PM6 conformers. For the nine interpolation functions, the free term, a_0 , has an almost identical range

$\Delta a_0 = 2.7138 \text{ kcal}\cdot\text{mol}^{-1}$. The free term, a_0 , has been correlated with the standard formation enthalpies $\Delta_f H^0$. The linear correlation between the $\Delta_f H^0(\text{PM7})$, and $a_0(\text{PM7})$ is:

$$\Delta_f H^0(\text{PM7}) = 14.7699 + 1.0167 a_0(\text{PM7}) \quad (r^2 = 0.9934; \text{SD} = 0.1314; F = 1209.8) \quad (5)$$

with a very good correlation coefficient of 99.34% and a very high statistical confidence.²³ The graphical representation of this correlation is presented in Fig. 5c.

Between parameters a_1 , a_2 and a_3 of the

$$\Delta_f H^0(\text{T}) = -165.5170(\pm 1.5893) + 0.0089(\pm 0.0006)\text{T} + 1.1498(\pm 0.0082)\text{T}^2 \cdot 10^{-4} - 3.2728(\pm 0.0367)\text{T}^3 \cdot 10^{-8} \\ \text{T} \in [100\text{K}, 1000\text{K}] \quad (\text{SD} = 0.0254(\pm 0.0004); 8.0731(\pm 0.2840) \cdot 10^6) \quad (4)$$

The interpolation relations in Table 5 allow the formation enthalpy calculation in the temperature range $\text{T} \in [100\text{K}, 1000\text{K}]$ using the PM7(M12) method.²³

By analyzing the average interpolation relationships (2), (4), and (8) one can note that each semiempirical method PM3, PM6 or PM7 leads to similar interpolation relationships. Small observed differences are due to the free term, a_0 . Each of the three used methods allows the estimation of the temperature dependency of the formation enthalpies with a high degree of precision and within the error limits presented by the authors of the MOPAC12 software.¹⁸

CONCLUSIONS

Through geometry reoptimization of thirty PM3 (HyperChem) conformers of the (3R,5S,6R)-6-acetylamidopenicillanic acid by using the PM3, PM6, respectively PM7 hamiltonians included in the MOPAC12 software thirty, eight, and nine conformers were obtained. From thirty conformers PM3 M12, 12 have R pseudochirality, and 18 have S pseudochirality at N14 atom, 14 have *anti*, and 16 *syn* exocyclic amido conformation. The most stable *syn*-conformer PM3(M12) is in total disagreement with the experimental data. The difference between the PM3-01, and PM3-30 enthalpies, $\Delta(\Delta_f H^0)$ is 7.6934 kcal·mol⁻¹. From eight PM6(M12) conformers, six have S pseudochirality, and the other two have R pseudochirality at N14 atom. Four PM6 conformers have *anti* exocyclic amido conformation, and other four have *syn* amido conformation. The most stable PM6(M12) conformer has *anti* amido conformation in agreement with experimental data. The difference between PM6-01, and PM6-08 formation enthalpies, $\Delta(\Delta_f H^0)$, is 3.6541 kcal·mol⁻¹. From the

interpolation function it cannot be established a correlation. To compare the functions PM3, PM6, and PM7 an average interpolation function also for the PM7 calculations has been computed:

nine PM7(M12) conformers, six have S pseudochirality, and other two have R pseudochirality at N14 atom, five out of the nine PM7 conformers have *anti* amido conformation, and other four have *syn* amido conformation. The most stable PM7 conformer has an *anti* conformation of the exocyclic amido group, in agreement with experimental data. The difference between the PM7-01, and PM7-09 formation enthalpies, $\Delta(\Delta_f H^0)$ is 4.8064 kcal·mol⁻¹.

Puckering classes were not observed for the thiazolidinic ring.

By geometry reoptimizing using the PM6 or PM7 hamiltonians the number of conformers is less than the one resulted from the PM3 method, the most stable conformer has *anti*-conformation of the exocyclic amido group, and the difference between the formation enthalpy of most stable and most unstable conformer drops almost three times.

For the PM3, PM6, and PM7 conformers of the (3R,5R,6S)-6-acetylamidopenicillanic acid cubic interpolations relations of the functional dependence of the formation enthalpy as function of temperature ($\Delta H_{\text{form}} = a_0 + a_1\text{T} + a_2\text{T}^2 + a_3\text{T}^3$) were obtained. The a_1 , a_2 , and a_3 values do not depend on the method of calculation and on conformer geometry. The free term a_0 is the only one which depends on the geometry and which increases with the decreasing of the thermodynamic stability of conformers. Three average interpolation relationships have been established. These allow the calculation of molar fraction of the conformers of the title compound in the interpolation range (100K-1000K).

Such a relation affords to estimate the temperature at which a 1H-NMR signal could appear for one conformer, if the $\Delta_f H$ dependence on temperature is known.

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