Dedicated to Professor Dumitru Oancea
on the occasion on his 75th anniversary

ENTROPIC EFFECT AND THERMODYNAMIC PROPERTIES FOR THE CONFORMERS OF (3R,5S,6R)-6-ACETYLAMIDOPENICILLANIC ACID CALCULATED WITH THE PM3, PM6 AND PM7 SEMIEMPIRICAL MO METHODS[**]

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Thirty conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid have been obtained by using the PM3 Conformational Search implemented in the HyperChem 7.5 software. After performing the energy minimization with the PM3, PM6 and PM7 semiempirical MO methods, which are included in the MOPAC12 software, thirty, eight, and, respectively, nine conformers were obtained. The following cubic interpolation relations of functional dependence of enthalpy (ΔH), entropy (ΔS) and C_P on temperature (T$\in$[100K,1000K]) were established:

- for PM3:

$\Delta H(T)=0.0613(\pm0.0241)+0.0079(\pm0.0004)\cdot T+1.1621(\pm0.0055)\cdot10^{-4}T^2-3.4367(\pm0.0270)\cdot10^{-8}T^3$
$\Delta S(T)=60.4738(\pm1.2434)+0.2981(\pm0.0029)\cdot T-1.2594(\pm0.0411)\cdot10^{-4}T^2+3.2563(\pm0.1969)\cdot10^{-8}T^3$
$C_P(T)=7.8283(\pm0.6499)+0.2364(\pm0.0029)\cdot T-1.0955(\pm0.4350)\cdot10^{-4}T^2+1.2023(\pm0.2133)\cdot10^{-8}T^3$

- for PM6:

$\Delta H(T)=0.1015(\pm0.0188)+0.0084(\pm0.0005)\cdot T+1.1741(\pm0.0069)\cdot10^{-4}T^2-3.3992(\pm0.0324)\cdot10^{-8}T^3$
$\Delta S(T)=61.4428(\pm1.4661)+0.3043(\pm0.0033)\cdot T-1.3004(\pm0.0524)\cdot10^{-4}T^2+3.4746(\pm0.2617)\cdot10^{-8}T^3$
$C_P(T)=9.3563(\pm0.7470)+0.2312(\pm0.0033)\cdot T-10.0693(\pm0.4757)\cdot10^{-7}T^2+24.3696(\pm22.4091)\cdot10^{10}T^3$

- for PM7:

$\Delta H(T)=0.0400(\pm0.0218)+0.0089(\pm0.0006)\cdot T+1.1498(\pm0.0082)\cdot10^{-4}T^2-3.2727(\pm0.0367)\cdot10^{-8}T^3$
$\Delta S(T)=61.3072(\pm1.2728)+0.3049(\pm0.0039)\cdot T-1.3469(\pm0.0589)\cdot10^{-4}T^2+3.7687(\pm0.2888)\cdot10^{-8}T^3$
$C_P(T)=9.4670(\pm0.8905)+0.2286(\pm0.0036)\cdot T-10.0442(\pm0.5069)\cdot10^{-5}T^2+39.8522(\pm23.6941)\cdot10^{10}T^3$

Considering the $\Delta G = \Delta H - T\cdot\Delta S$ relation, we can state that the thermodynamic factor, which determines the variation of the conformers' biological activity, is the entropic factor, which represents the quantity of bound energy. Based on this observation, the following theorem can be stated: *The dispersion of biological activities of all conformers of a flexible molecule at a temperature* T *is determined by their entropy.*
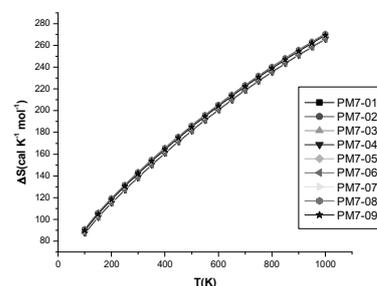
INTRODUCTION

About sixty years ago one hypothesis was developed which stated that between the thermodynamic potentials, namely the ΔF free energy, but especially the ΔG Gibbs free enthalpy (Gibbs free energy) and various descriptors of the chemical structure should exist some relations of linear dependence.[1-16]

The structural descriptors of molecules have two sources: experimentally obtained descriptors, and

calculated descriptors obtained by using different methods of computational chemistry.[17-22] Starting from old ideas (Wiener-Hansch-Charlton),[1-16] today's field of quantitative chemical structure – properties relationships (QSPR) or, in particular, the quantitative chemical structure – biological activity relationships (QSAR) were developed.[17,18]

QSPR/QSAR relationships rely on the fact that for ΔG_i (Gibbs free energies) of a $\mathbf{M} = \{m_i\}$ set of molecules with similar structures a functional relation can exist. This can be expressed by a functional dependence of the type $\mathbf{f(D)} \rightarrow \mathbf{A}$, where:

– **f** represents the different analytical functions of the Gibbs free energy;
– **D** is the set of real values of the $\{x_i\}$ descriptors, which is a subset of the set of real numbers $\mathbf{D} \subset \mathfrak{R}$;
– $\mathbf{A} \subset \mathfrak{R}$ for the molecules in the $\mathbf{M}$ set, is a set of real values of Gibbs free energies, or equivalent forms: equilibrium or complexation constants, biological activities, etc.

Because usually the **f** function is not known, it is replaced by an interpolation function of type $f(\mathbf{D_1}) \rightarrow \mathbf{A_1}$, where $\mathbf{D_1} \subset \mathbf{D} \subset \mathfrak{R}$, and $\mathbf{A_1} \subset \mathbf{A} \subset \mathfrak{R}$. It is considered that the f interpolation function on the $\mathbf{D_1}$ domain is a good estimate for the known $\mathbf{A_1}$ values if the root-mean-square deviation between the values calculated through f and the experimental values is minimal. From analytical point of view, any f interpolation function is strictly true only on the $\mathbf{D_1}$ definition domain and any extrapolation of its use outside of this domain can lead to serious interpretation errors. Because it is admitted that the biological activities or different chemical properties of the molecules from the $\mathbf{M}$ set ($\mathbf{A_1}$ set) are linear functions of free energy, then the f function is proposed as being a linear or linearizable dependence (but not only) of the $\mathbf{D} = \{x_i\}$ set of descriptor variables.[23-28] The determination of the analytical expression of this f function constitutes the whole strategy of QSPR/QSAR relationships or of molecular modeling, and applied mathematical method is the least squares method, with a series of validation statistical methods.[23-38] In the case of compounds of biological interest (QSAR) the majority of the molecules from the $\mathbf{M}$ set are not rigid molecules and have a multitude of $\{c_{ij}\} = \mathbf{C}$ conformers, and each of them could be characterized through its own ΔG_{ij} Gibbs free energy. If the $m_i \in \mathbf{M}$ molecule has j conformers (the $\mathbf{C_i} \subset \mathbf{C}$ set of

conformers of the m_i molecule), then a certain conformer can be characterized through a $\mathbf{P_i}$ set of its structural and energetic properties, one of them being its Gibbs free energy, or ΔG_{ij}. At a temperature T at which the $m_i \in \mathbf{M}$ molecule exists in solution or in gaseous state, the x_{ij} molar fraction of the c_{ij} conformer is proportional with the partition function: Z_{ij} or $Q_{ij} = g_{ij} \cdot \exp(-E_{ij}/kT)/\Sigma_i g_{ij} \cdot \exp(-E_{ij}/kT)$, where g_{ij} is the degeneration degree of the E_{ij} energy state of the c_{ij} conformer, and $E_{ij} = E_{ij,rot} + E_{ij,vib} + E_{ij,el} + E_{ij,nuc}$ represents the total energy of the c_{ij} conformer located in the E_{ij} energy state.[39-40] The experimental determination of the $\mathbf{P_i}$ set of properties of a certain conformer is virtually impossible, because the separation of a certain conformer from the mixture is in most of the cases impossible. In principle only properties in the fundamental state, when the geometry is frozen, can be known. When a molecule of biological interest binds to a protein, then through the protein's isolation and crystallization just some properties of the bound conformer can be known. The estimation of the $\mathbf{P_i}$ properties for $\mathbf{C_i}$ conformers of the m_i molecule can be done mainly through computational chemistry.

The knowledge of conformers for a molecule which presents a biological interest (ligand, substrate, effector etc.) is important because, during the interaction with proteins the involved conformer is not the global minimum conformer. This specific conformer determines the biological activity, a fact that was proven through the different docking techniques.[41-49] For now, the only experimental method for showing the existence of conformers is the high resolution nuclear magnetic resonance (NMR), and ^{1}H NMR for the organic compounds, through the jj coupling constants.[50]

The objectives of this paper are by using different quantochemical methods (the PM3, PM6 and PM7 MO semiempirical methods), the computation of their ΔH, ΔS and C_P thermodynamic properties, and also their temperature dependence on the $T \in [100K, 1000K]$ interval.

METHOD

The PM3 method from the HyperChem7.52 – Hy[51] software was used, and also the PM3, PM6 and PM7 from the MOPAC12 Version 13.004W – M12 software.[52] Because the PM6 and PM7 methods were not implemented in programs that

search for conformers, we have used the Conformational Search module from the Hy package and the PM3 method for the optimization of the conformers' geometries.[53] For the computation of the thermodynamic properties: ΔH, C_P, and ΔS with the M12 program it was necessary to work in two stages.[52] In the first stage the geometries of the conformers were optimized with one of the PM3, PM6 and PM7 methods, and in the second stage the thermodynamic properties were calculated.[52] For the conversion of the "hin" files[53] from the Hy software in "mop" files, the AVOGADRO[54] program was used, applying the "MOPAC" and "Geometry Optimization" keywords.

For the geometry optimization with the M12 program,[52] in the first stage the following sets of keywords were used: PM3, (PM6 or PM7), SCFCRT=1.D-10, GEO-OK, PRECISE, GNORM=0.001, CYCLES=5000, T=345600, LET. The obtained geometries were converted in M12 input files for the computing of the thermodynamic properties. For computing the thermodynamic properties $\Delta H(T)$, $C_p(T)$ and $\Delta S(T)$ in the second stage the following keywords were used: PM3, (PM6 or PM7), SCFCRT=1.D-10, GEO-OK, PRECISE, GNORM=0.001, CYCLES=5000, T=345600, AUX, LARGE, CHARGE=0, SINGLET, SYMMETRY, FORCE,

THERMO(100,1000,50), LET. The atom numbering in the (3R,5S,6R)-6-acetylamidopenicillanic acid is given in Fig. 1.

RESULTS AND DISCUSSION

Thirty conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid were obtained by using the PM3 Conformational Search module from the Hy software.[51] The standard formation enthalpies ($\Delta_f H^0$ – PM3, PM6 and PM7 calculations) were used for ordering the conformers.[53]

For each conformer of (3R,5S,6R)-6-acetylamidopenicillanic acid obtained with the PM3, PM6 and PM7 M12 Hamiltonians, we computed the functional dependencies of the molar enthalpies, the molar entropies and the molar heats at constant pressure as polynomial interpolation functions depending on temperature until the third degree (cubic interpolation functions $y = a_0 + a_1 T + a_2 T^2 + a_3 T^3$, where y is ΔH, ΔS or C_p). The chosen temperature range was $T \in [100K, 1000K]$ at a step of 50º. The primary data are presented in the Supplementary material: Tables 1a, 1b, 3a, 3b, 5a, 5b for PM3, Tables 7, 9, 11 for PM6 and Tables 13, 15, 17 for PM7.
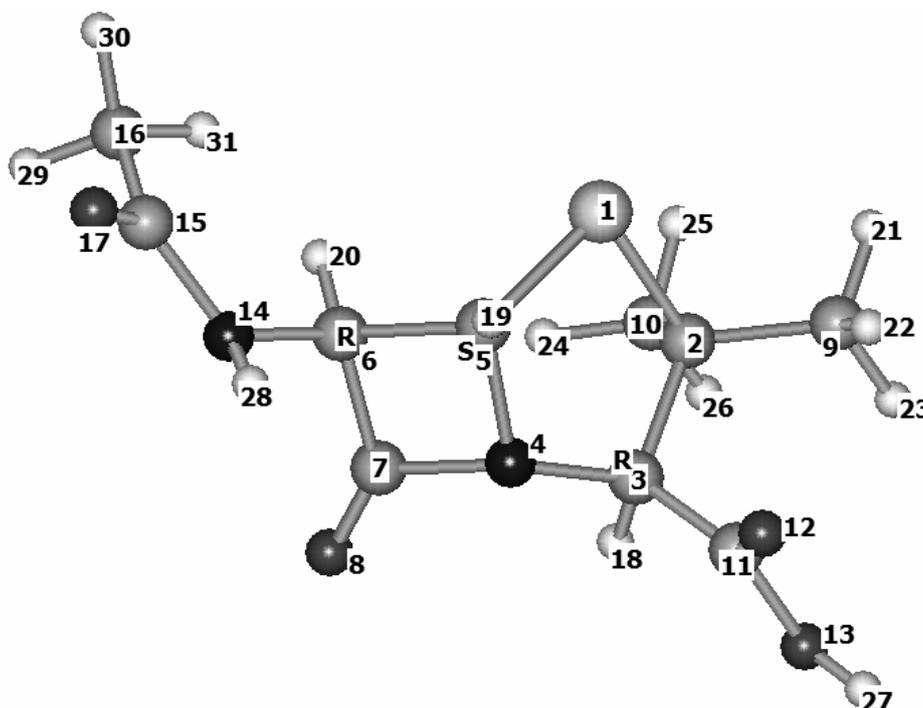

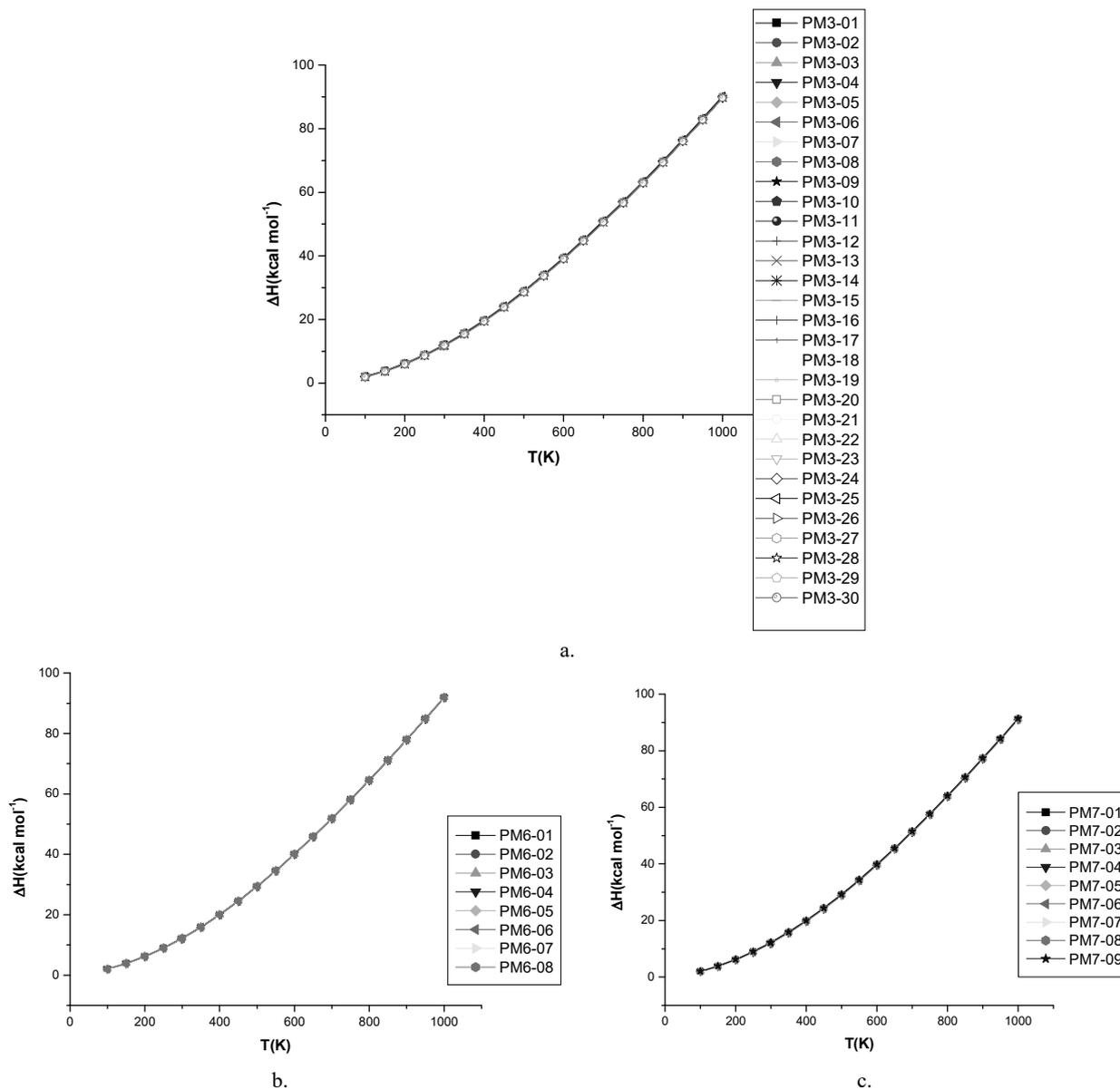Fig. 1 – Atom numbering in (3R,5S,6R)-6-acetylamidopenicillanic acid.

b.

c.

Fig. 2 – Functional dependence $\Delta H(T)$ plots in the range $T \in [100K, 1000K]$ resulted for: a. PM3; b.PM6; and c. PM7 - M12 conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid.[55]

Enthalpy. With the primary data (Supplementary material: Tables 1a and 1b) for $\Delta H(T)$ of the thirty PM3 conformers of (3R,5S,6R)-6-acetylami-dopenicillanic acid, we have calculated the cubic functions, $\Delta H_{PM3}(T) = a_0 + a_1 T + a_2 T^2 + a_3 T^3$, for the $T \in [100K, 1000K]$ (Supplementary material, Table 2). The plots for these functions are shown in Fig. 2a.[55]

Similarly, we have calculated the cubic functions of correlation for the $T \in [100K, 1000K]$, $\Delta H_{PM6orPM7}(T) = a_0 + a_1 T + a_2 T^2 + a_3 T^3$, for the eight PM6 conformers, and, respectively, for the nine PM7 conformers. The diagrams for these functions, resulted from the primary data (Supplementary material: Table 7 and, respectively, Table 13) are

shown in Fig. 2b for the PM6 conformers, and, respectively, in Fig. 2c for the PM7 conformers.[55]

The analysis from Supplementary material data (Table 2 and Figs. 2a – 2c for the PM3 conformers, Table 8 and Figs. 8a – 8c for the PM6 conformers, and, respectively, Table 14 and Figs. 14a – 14c for the PM7 conformers) shows that, between the a_0 (or a_1 or a_2 or a_3) values of the interpolation functions and the $\Delta_f H^0$ or ΔH^0 values there is no functional dependency (correlation).[55] For the PM3 conformers, the difference between $min(a_0)$ and $Max(a_0)$ is only 0.0956 kcal·mol^{-1}, the mean value being 0.0613±0.0241. For the PM6 conformers, the difference between $min(a_0)$ and $Max(a_0)$ is only 0.0475 kcal·mol^{-1} and the mean

value is 0.1015±0.00188, while for the PM7 conformers this difference is only 0.0609 kcal·mol^{-1}, the mean value being 0.0400±0.0218. The grouping on a tight interval of interpolation functions proves that, in the case of the same molecule's conformers, the ΔH enthalpy (the internal energy content of the system) is relatively unchanged and it does not depend significantly on the conformers' geometry.

Through the computation of the a_1/a_2 ratio between the mean values from the interpola-

tion mean functions we obtain the following values: 680 for the PM3 conformers, 716 for the PM6 conformers, and, respectively, 774 for the PM7 conformers. This means that, through interpolation, we obtain three packages of parallel functions which are almost overlapped, and which have the same concavity (Fig. 2a – Fig. 2c), differing only by the a_0, free term.[55] Owing to this observation, the following three interpolation mean relations can be introduced:

$$\Delta H_{PM3}(T) = 0.0613(\pm 0.0241) + 0.0079(\pm 0.0004)T + 1.162(\pm 0.006)\cdot 10^{-4}T^2 - 3.437(\pm 0.027)\cdot 10^{-8}T^3$$
$$T \in [100K, 1000K] \qquad (SD = 0.0347(\pm 0.0010); F = 4.2154(\pm 0.2232)\cdot 10^6)$$

$$\Delta H_{PM6}(T) = 0.1015(\pm 0.0188) + 0.0084(\pm 0.0005)T + 1.174(\pm 0.007)\cdot 10^{-4}T^2 - 3.399(\pm 0.032)\cdot 10^{-8}T^3$$
$$T \in [100K, 1000K] \qquad (SD = 0.0298(\pm 0.0003); F = 5.9632(\pm 0.1224)\cdot 10^6)$$

$$\Delta H_{PM7}(T) = 0.0400(\pm 0.0218) + 0.0089(\pm 0.0006)T + 1.150(\pm 0.008)\cdot 10^{-4}T^2 - 3.273(\pm 0.037)\cdot 10^{-8}T^3$$
$$T \in [100K, 1000K] \qquad (SD = 0.0251(\pm 0.0008); F = 8.0891(\pm 0.2542)\cdot 10^6)$$

These mean relations can constitute a way of computing the enthalpies for every single conformer, without any significant error, keeping in mind that the statistic parameters (SD, F) correspond to some relations with a confidence degree of over 99.9%. The interpolation relations from Supplementary material (Tables 2, 8 and 14) allow the enthalpy computation for every single conformer in the $T \in [100K, 1000K]$ temperature domain. The thus calculated enthalpy can be used for the establishment of some QSAR/QSPR relationships. With the same confidence degree, the interpolation mean relation can also be used, which allows the estimation of an enthalpy with a high confidence degree.

Entropy. The primary data for the ΔS(T) entropies of the thirty PM3 conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid, resulted through the use of the PM3 M12 Hamiltonian, are presented in Supplementary material (Tables 3a and 3b). With these primary data of each PM3 conformer, the $\Delta S_{PM3}(T) = a_0 + a_1T + a_2T^2 + a_3T^3$ cubic interpolation function for the $T \in [100K, 1000K]$ temperature domain were calculated (Supplementary material, Table 4). The diagrams for all functions are plotted in Fig. 3a.

The analysis of data from Supplementary material (Table 4, and Figs. 4a and 4c) shows that, between the a_0 (or a_1 or a_2 or a_3) values of the interpolation functions and the $\Delta_f H^0$ or ΔH^0 values there is no correlation. From Supplementary material (Fig. 4b) it results that between the free term, a_0, of the polynomial and ΔS_0, there is a somewhat good correlation, and this suggests that the entropies in standard conditions might be used as a criterion for ordering the conformers. The difference between $min(a_0)$ and $Max(a_0)$ is of only 3.8031 cal·K^{-1}·mol^{-1}, and the mean value is 60.4738±1.2434. The dispersion on the 3.8031 cal·K^{-1}·mol^{-1} interval of the interpolation functions (Suplementary material, Fig. 4a and 4b) proves that, in the case of conformers of the same molecule, the ΔS(T) entropy is not a constant and it depends on the conformer geometry.[55]

The a_1/a_2 ration between the mean values from the interpolation mean functions is -2367. This means that, through interpolation, a parallel polynomial package results, which have the same concavity (Fig. 3a), and they only differ through the free term, a_0.[55] Owing to these observations, the interpolation mean relation:

$$\Delta S_{PM3}(T) = 60.4738(\pm 1.2434) + 0.2981(\pm 0.0029)T - 1.2594(\pm 0.0411)\cdot 10^{-4}T^2 + 3.256(\pm 0.197)\cdot 10^{-8}T^3$$
$$T \in [100K, 1000K] \qquad (SD = 60.4738(\pm 1.2434); F = 2.17517(0.33015)\cdot 10^5)$$

cannot be used for computing the entropy of each conformer, although the statistic parameters (SD,

F) correspond to a relation with a confidence degree of over 99.9%. This statement is based on

the high error degree resulted from the significant dispersion of the interpolation function, and that can be observed in Fig. 3a.[55] The interpolation relations from Supplementary material (Table 4) allow the computation of entropy in the $T \in$ [100K,1000K] temperature domain.

The interpolation relations from Supplementary material (Tables 2, and 4) can be used for computing the free enthalpy, $\Delta G(T) = \Delta H(T) - T \Delta S(T)$, at any temperature on the $T \in$ [100K, 1000K] domain. According to the data obtained for

the enthalpy in this temperature domain we can state that only the entropy depends on the conformers' geometry. In conclusion, the Gibbs free energies will depend on the conformers' geometries for the same molecule through the entropy. As the Gibbs free energy is the measure that determines the biological activity, we can conclude that, in reality, the thermodynamic factor that influences the variation of the biological activity with the conformer's nature is the entropic factor.
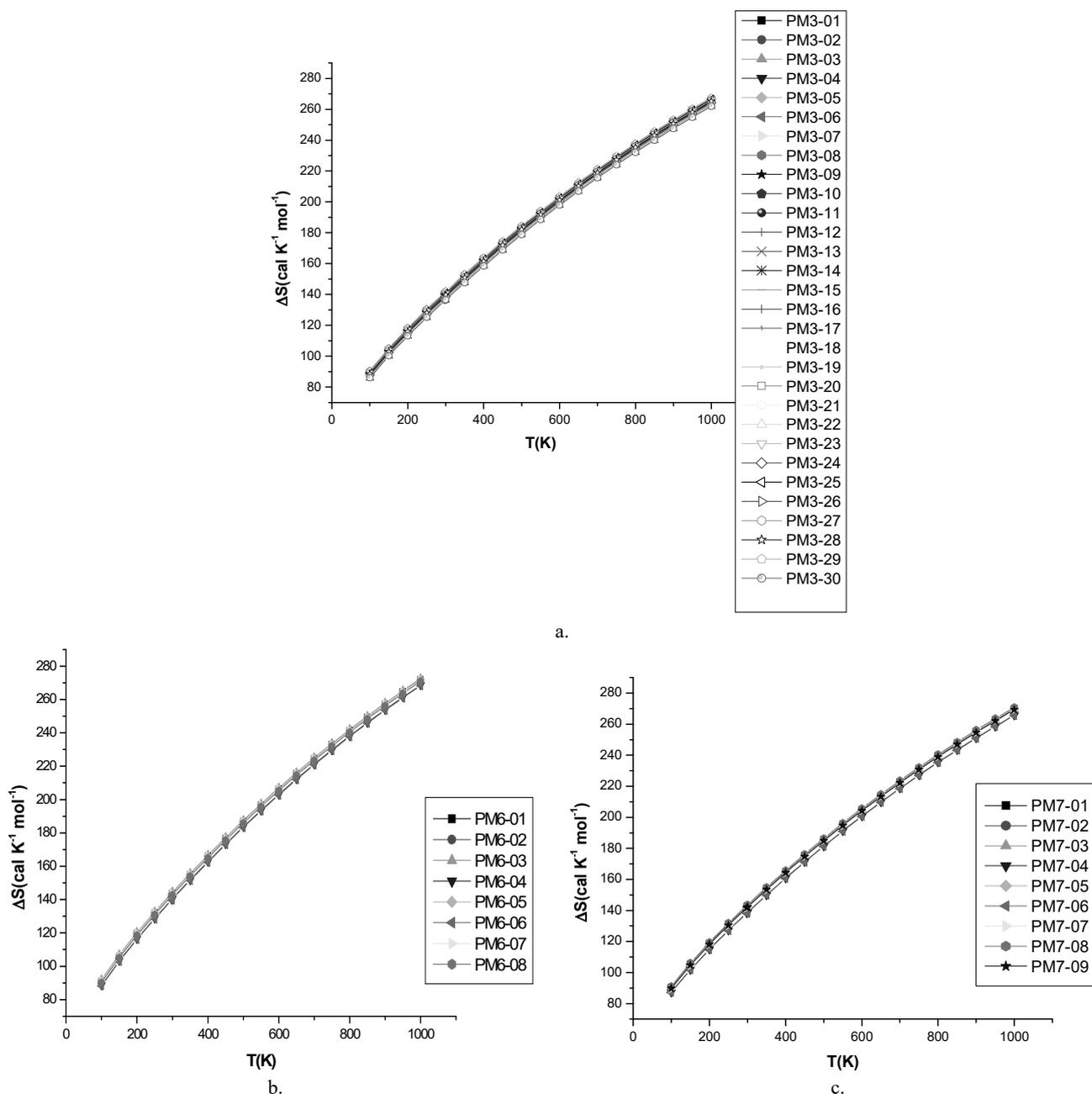

Fig. 3 – Functional dependence $\Delta S(T)$ plots in the range $T \in$ [100K,1000K] resulted for: a. PM3; b. PM6; and c. PM7 - M12 conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid.[55]

The primary data for the $\Delta S(T)$ entropies of the eight conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid, resulted from the PM6 M12 Hamiltonian, are given in Supplementary material (Table 9). With this primary data for each PM6 conformer the $\Delta S(T) = a_0 + a_1T + a_2T^2 + a_3T^3$, interpolation cubic functions for the $T \in [100K,1000K]$ temperature domain were computed (Supplementary material, Table 10). The plots for these functions are given in Fig. 3b. The analysis of data in Supplementary material (Table 10, and Fig.s 10a and 10c) shows that, between the a_0 (or a_1 or a_2 or a_3) values of the interpolation functions and the Δ_fH^0 or ΔH^0 values there is no correlation. The difference between $min(a_0)$ and $Max(a_0)$ is 3.5487 cal·K^{-1}·mol^{-1}, while the mean

value is 61.4428±1.4661 cal·K^{-1}·mol^{-1}. The dispersion of the a_0 values (3.5487 cal·K^{-1}·mol^{-1} from Supplementary material, Figs. 10a and 10c) and of interpolation functions (Fig. 3b) prove that, in the case of the same molecule the $\Delta S(T)$ entropies of its conformers are not constant and they depend of the conformer' geometry.[55] The computed a_1/a_2 ratio between the mean values becomes negative, having the value of -2333.59. This means that through interpolation a package of parallel functions result, which have the same concavity (Fig. 3b), and differ through the free term, a_0. Owing to the dispersion (±1.4661) and to the fact that $\Delta a_0 = 3.5487$ cal·K^{-1}·mol^{-1}, the interpolation mean relation:

$$\Delta S_{PM6}(T) = 61.443(\pm 1.4661) + 0.304\,(\pm 0.0033)T - 1.300(\pm 0.0524)\cdot 10^{-4}T^2 + 3.475(\pm 0.2617)\cdot 10^{-8}T^3$$
$$T \in [100K,1000K] \qquad (SD = 0.3344 \pm 0.0291;\ F = 1.7692(\pm 1.4057)\cdot 10^5$$

cannot be used for computing the entropy for each conformer, because of the high dispersion degree of the interpolation curves (Fig. 3b).[55]

The interpolation relations from Supplementary material (Table 10) allow to calculate the entropy for each conformer in the $T \in [100K,1000K]$ temperature domain. The interpolation relations from Supplementary material, Table 10) can also be used for computing the free enthalpy, $\Delta G(T) = \Delta H(T) - T \cdot \Delta S(T)$, at any temperature in $T \in [100K, 1000K]$ interval. In this temperature domain ($T \in [100K,1000K]$) we can also state that only the entropy depends on the conformer geometry.

Based on this observation we can also conclude that the Gibbs free energy depends on conformer geometries by means of their entropy.

The primary data for the entropies of the nine conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid calculated with the PM7 M12 Hamiltonian are shown in Supplementary material (Table 15). With this primary data, for each PM7 conformer of (3R,5S,6R)-6-acetylamidopenicillanic acid, we computed the $\Delta S(T) = a_0 + a_1T + a_2T^2 + a_3T^3$, cubic interpolation functions for the

$T \in [100K,1000K]$ temperature domain (Supplementary material, Table 16). The diagrams of these functions are shown in Fig. 3c. The analysis of data from Supplementary material (Table 16, Fig.s. 16a and 16c) shows that,[55] between the a_0 (or a_1 or a_2 or a_3) values of the interpolation functions and the Δ_fH^0 or ΔH^0 values there is no correlation. The difference between $min(a_0)$ and $Max(a_0)$ is 3.3011 cal·K^{-1}·mol^{-1}, and the mean value is 61.3072±1.2728. The dispersion of the a_0 parameter 3.3011 cal·K^{-1}·mol^{-1} from the interpolation functions, (see Fig. 3c, and Supplementary material (Figs. 16a and 16c),[55] proves that in the case of the conformers of a molecule the entropy is not a constant and depends of the conformer geometry. The computation of the a_1/a_2 ratio between the mean values gives a negative value of -2263.72. This means that through interpolation a pack of parallel functions results, which have the same concavity (Fig. 3c), and differ through the free term, a_0. Owing to these observations, the interpolation mean relation:

$$\Delta S_{PM7}(T) = 61.307(\pm 1.278) + 0.305(\pm 0.004)T - 1.347(\pm 0.059)\cdot 10^{-4}T^2 + 3.769\,(\pm 0.289)\cdot 10^{-8}T^3$$
$$T \in [100K,1000K] \qquad (SD = 0.3448 \pm 0.0298;\ F = 1.6426(\pm 0.3056)\cdot 10^5$$

cannot be used for computing the entropy for each conformer, because of the high error degree (the dispersion of the interpolation curves). The

interpolation relations from Supplementary material (Table 16) allow the computation of the entropy in the $T \in [100K,1000K]$ temperature

domain for each conformer of a molecule. The interpolation relations from Supplementary material (Table 16) can be used for the calculation of $\Delta G(T)$, Gibbs free energy for each conformer of a molecule at any temperature in the $T \in [100K, 1000K]$ domain.

Because this analysis proves that the conformer entropies depend on their geometries, the following theorem can be stated: *The dispersion of the biological activities of conformers of a flexible molecule at a* T *temperature is determined by the conformer entropy.*

Molar heat at constant pressure. The primary data for the $C_P(T)$ molar heat at constant pressure, for the thirty conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid, obtained with the PM3 M12 Hamiltonian, are presented in Supplementary material (Tables 5a and 5b). With this primary data, for each PM3 conformer we have computed the $C_P(T) = a_0 + a_1T + a_2T^2 + a_3T^3$ cubic interpolation functions for the $T \in [100K,$ 1000K] temperature interval (Supplementary material, Table 6). The plots of these functions are presented in Fig. 4a.[55] The analysis of the data from Supplementary material (Table 6, and Figs 6a and 6c) shows that, between the a_0 (or a_1 or a_2 or a_3) values of the interpolation functions and the Δ_fH^0 or ΔH^0 values there is no correlation. The difference between *min*(a_0) and *Max*(a_0) is only 2.1923 cal·K^{-1}·mol^{-1}, and the mean value is 7.8283±0.6499. The grouping in a relatively tight interval of the interpolation functions (Fig. 4a) proves that,[55] for the conformers of a molecule, the $C_P(T)$ molar heat at constant pressure is relatively constant, and does not significantly depend on the conformer geometries. Through the computation of the a_1/a_2 ratio between the mean values we obtain a negative value of -2157.92. This means that through interpolation, a package of overlapping parallel functions results, which have the same concavity (Fig. 4a),[55] and do not significantly differ through the free term, a_0. Owing to these observations, the interpolation mean relation:

$$C_{P,PM3}(T) = 7.828(\pm 0.650) + 0.236(\pm 0.003)T - 1.096(\pm 0.435)\cdot 10^{-4}T^2 + 1.202(\pm 0.213)\cdot 10^{-8}T^3$$
$$T \in [100K, 1000K] \qquad (SD = 0.5360 \pm 0.0191; F = 25549.35 \pm 2042.21)$$

can be used for computing the molar heats at constant pressure for each conformer. The statistic parameters (SD, F) show that the mean cubic function of $C_P(T)$ has a correlation with a confidence degree of over 99.99%.

The interpolation relations from Supplementary material (Table 6) allow the computation of the molar heats at constant pressure in the $T \in [100K, 1000K]$ temperature domain. With the same confidence degree the interpolation mean relation can be used, which allows the estimation of an molar heat at constant pressure with a good confidence degree. The interpolation relations from Supplementary material (Table 6) and the interpolation mean relation can be used in the computation of the enthalpy, $\Delta H = \int_T C_P(T)dT$. The tight interval in which $C_P(T)$ varies as a function of the conformer geometries justifies the tight interval of enthalpy variation.

The primary data for the $C_P(T)$ molar heat at constant pressure of the eight conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid, obtained with the PM6 M12 Hamiltonian, are presented in Supplementary material (Table 11). With these primary data for every PM6 conformer of (3R,5S,6R)-6-acetylamidopenicillanic acid, we have calculated the $C_P(T) = a_0 + a_1T + a_2T^2 + a_3T^3$ interpolation cubic functions for the $T \in [100K, 1000K]$ temperature domain (Supplementary material, Table 12). The plots of these functions are shown in Fig. 4b.

The analysis of data from Supplementary material (Table 12, Figs. 12a and 12c) shows that, between the a_0 (or a_1 or a_2 or a_3) values of the interpolation functions and the Δ_fH^0 or ΔH^0 values there is no correlation. The difference between *min*(a_0) and *Max*(a_0) is only $\Delta a_0 = 1.9245$ cal·K^{-1}·mol^{-1}, while the mean value is 9.3563±0.7470. The grouping on a relatively tight interval of the interpolation functions (Fig. 4b) proves that, in the case of the conformers of a certain molecule, the $C_P(T)$ molar heat at constant pressure is relatively constant and does not significantly depend on the conformer geometries. Through the computing of the a_1/a_2 ratio between the mean values a negative value of -2296.09 was obtained.

This means that, through interpolation, a package of parallel functions results, which have the same concavity (Fig. 4b), and do not significantly differ through the free term, a_0. Thus, the interpolation mean relation:

$$C_{P,PM6}(T) = 9.356(\pm 0.747) + 0.231(\pm 0.003)T - 10.070(\pm 0.476)\cdot 10^{-5}T^2 + 24.370(\pm 22.409)\cdot 10^{-10}T^3$$
$$T \in [100K, 1000K] \qquad (SD = 0.5388 \pm 0.0138; F = 26654.93 \pm 1570.81)$$

can be used for computation of the molar heats at constant pressure for each conformer, with very small errors. The interpolation relations from Supplementary material (Table 12) allow the computation of molar heats at constant pressure in the $T \in [100K, 1000K]$ temperature domain. With the same confidence degree, the interpolation mean

relation can be used for the estimation of an enthalpy, $\Delta H = \int_T C_P(T)dT$. The tight interval in which $C_P(T)$ varies, as a function of the conformer geometries, justifies the tight interval of enthalpy variation.
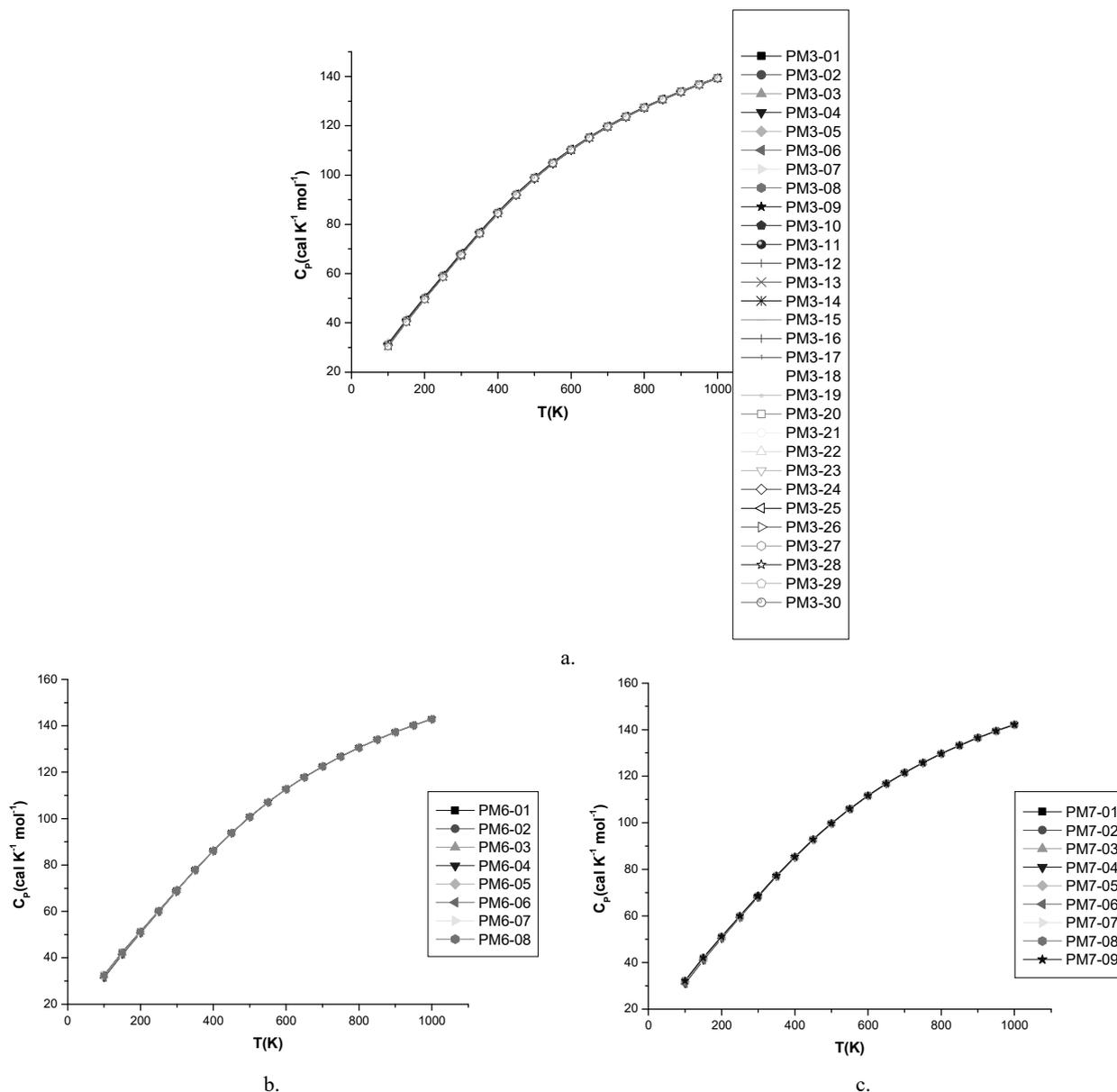

a.

b.

c.

Fig. 4 – Functional dependence $C_P(T)$ plots in the range $T \in [100K, 1000K]$ resulted for: a. PM3; b.PM6; and c. PM7 - M12 conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid.[55]

The primary data for the $C_P(T)$ molar heat at constant pressure for the nine PM7 M12 conformers of (3R,5S,6R)-6-acetylamidopenicillanic

acid are presented in Supplementary material (Table 17). With this primary data, for each PM7 conformer we have calculated the $C_P(T) = a_0 + a_1T$

+ $a_2T^2 + a_3T^3$ cubic interpolation functions for the $T \in [100K, 1000K]$ temperature interval (Supplementary material, Table 18). The plots for these functions are presented in Fig. 4c. The analysis of the data from Supplementary material (Table 18, and Figs 18a and 18c) shows that, between the a_0 (or a_1 or a_2 or a_3) values of the interpolation functions and the $\Delta_f H^0$ or ΔH^0 values there is no correlation. The difference between $min(a_0)$ and $Max(a_0)$ is only 2.3109 cal·K^{-1}·mol^{-1}, and the mean value is 9.4670±0.8905. The grouping on a relatively tight interval of the interpolation functions (Fig. 4c) proves that for the conformers of a molecule, the $C_P(T)$ molar heat at constant pressure is relatively constant and does not depend on the conformer geometries. The calculated a_1/a_2 ratio between the mean values is -2275.96. This means that, through interpolation, a package of parallel functions results, which have the same concavity (Fig. 4c), and do not significantly differ through the free term, a_0. Owing to these observations, the interpolation mean relation:

$$C_{P,PM7}(T) = 9.467(\pm 0.891) + 0.229(\pm 0.004)T - 10.044(\pm 0.507) \cdot 10^{-5}T^2 + 39.852(\pm 23.694) \cdot 10^{-10}T^3$$
$$T \in [100K, 1000K] \qquad (r^2 = 0.9998; \; SD = 0.4453 \pm 0.0149; \; F = 38621.9 \pm 2969.1)$$

can be used in the computation of molar heats at constant pressure for each conformer, without having significant errors, especially because the statistic parameters (SD, F) present it as a trustworthy relation. The interpolation relations from Supplementary material (Table 18) allow the computation of the molar heats at constant pressure in the $T \in [100K, 1000K]$ temperature interval.

With the same confidence degree, we can also use de interpolation mean relation. The interpolation relations from Supplementary material (Table 18) and the mean values relation can be used for computing the enthalpy, $\Delta H = \int_T C_P(T)dT$. The tight interval in which $C_P(T)$ varies as a function of the conformers' geometries justifies the tight variation interval of enthalpy.

The interpolation relations as temperature functions for the $\Delta H(T)$, $\Delta S(T)$ and $C_P(T)$, thermodynamic properties of the (3R,5S,6R)-6-acetylamidopenicillanic acid's conformers prove that the entropy, through its dispersion, is the factor that determines the variation of biological activity of the conformers, because the biological activity is dependent on the $\Delta G(T)$ free enthalpy. Because the free energy, ΔG, can be calculated as $\Delta G = \Delta H - T \cdot \Delta S$, it means that ΔG has a dispersion that is dependent on the entropy dispersion, namely conformer geometries. Thus, the following theorem can be stated: ***The dispersion of both the chemical reactivities and the biological activities of conformers of a flexible molecule at a* T *temperature is determined by the conformer entropy.***

CONCLUSIONS

We have obtained the interpolation cubic relations of the temperature dependence of enthalpy, entropy and molar heat at constant pressure ($Y = a_0 + a_1T + a_2T^2 + a_3T^3$, $Y = \Delta H(T)$, $\Delta S(T)$ and $C_P(T)$) for the PM3, PM6 and PM7 conformers of (3R,5S,6R)-6-acetylamidopenicillanic acid. The a_1 a_2 and a_3 values do not depend on the computational method and the conformer geometries. For the enthalpy and the molar heat at constant pressure, the a_0, free term depends relatively little on the conformer geometries in a tight interval, while the interpolation functions form a pack of nearly identical parallel curves that can be replaced with a mean interpolation function on the $T \in [100K, 1000K]$ temperature domain. For the entropy, we have noticed that the interpolation functions depend on the conformer geometries.

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