

# Relationship between structure and entropy contributions in an anthraquinone mercapto derivative

Maciej Roman · Agnieszka Kaczor ·  
Malgorzata Baranska

Received: 2 November 2009 / Accepted: 22 December 2009 / Published online: 25 February 2010  
© Springer-Verlag 2010

**Abstract** The structural and thermodynamic properties of an anthraquinone derivative were studied by means of quantum-chemical calculations. Conformational analysis using *ab initio* and density functional theory methods revealed 14 low-energy conformers. In order to discuss similarities and differences in entropy of the conformers, the rotational and vibrational contributions to entropy were correlated with changes in conformer structure. The component of the moment of inertia perpendicular to the molecular plane gives significant input to  $\Delta S_{rot}$ , whereas the largest contributions to the  $\Delta S_{vib}$  have vibrations associated with the  $\tau_{S1C20}$  coordinate.

**Keywords** Anthraquinone · *Ab initio* · Density functional theory · Entropy effect · Conformational analysis

## Introduction

Anthraquinones have recently attracted increasing attention due to their various biological activities and wide ranging applications in the pharmaceutical industry [1–4]. Additionally, some anthraquinones have proved to possess anti-cancer and antifungal properties [2, 3]. They are also known as a family of photosensitizers, which photo-generate reactive oxygen

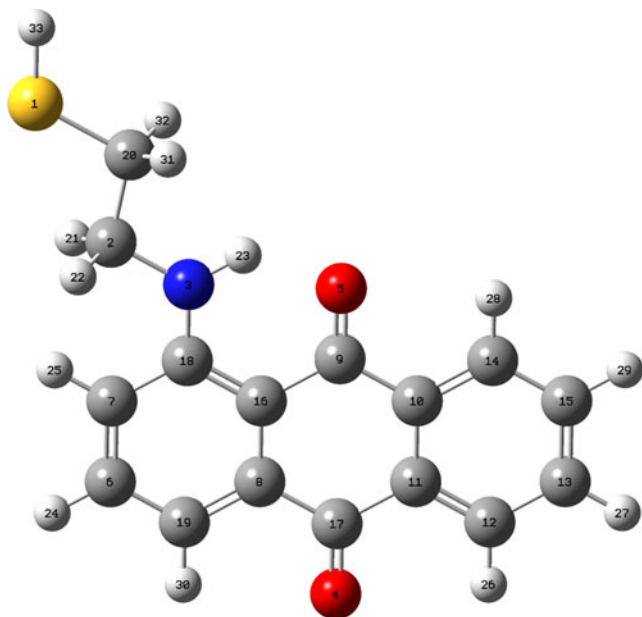
species such as  $^1O_2$  and  $O_2^-$  [5–8]. Thus, anthraquinones have been studied using quantum-chemical methods, mainly TD-DFT (time-dependent density functional theory), in order to discuss their electronic spectra [9–11].

Hydrophobic effects, side-chain conformational entropy, steric factors, and main-chain electrostatic interactions have been indicated as the dominant physical factors determining the conformational preferences of amino acids in proteins [12]. In particular, it has been demonstrated that restriction of amino acids side-chain motion is enthalpically favorable, but unfavorable in terms of Gibbs energy and entropy [13]. The stabilization of some conformations on Gibbs free energy and entropy grounds, manifesting as an increase in their abundance, was previously described for phenylalanine and tryptophan and called “the entropy effect” [14, 15]. Structurally, this effect was related to the strength of the intramolecular H-bond and steric hindrance in amino acid conformers [14, 15].

In our studies on anthraquinone derivatives, we found that 1-[(2-mercaptoethyl)amino]-9,10-anthraquinone (MEAA; Fig. 1) shows a significant entropy effect. Therefore, we undertook an analysis of the structural and thermodynamic properties of MEAA by using quantum-chemical methods in order to shed some light on the reasons for entropic stabilization/destabilization of conformers. The highest emphasis was made on detailed analysis of the entropy effect in order to understand the relationship between structure and entropy contribution (translational, electronic, rotational and vibrational). To simplify the computations, they were performed in the gas phase. Nevertheless, it is hoped that this study can be a starting point for future research concerning modeling of the biological activity of MEAA in more complicated systems mimicking the biological environment.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00894-010-0654-3) contains supplementary material, which is available to authorized users.

M. Roman · A. Kaczor · M. Baranska (✉)  
Faculty of Chemistry, Jagiellonian University,  
3 Ingardena Str.,  
30-060 Kraków, Poland  
e-mail: baranska@chemia.uj.edu.pl



**Fig. 1** Atom numbering for 1-[(2-mercaptoethyl)amino]-9,10-anthraquinone (MEAA)

## Computational methods

MEAA geometries and harmonic vibrational frequencies were calculated using the Gaussian 03 package of programs [16]. The conformational analysis was carried out at the B3LYP/6-31G level of theory in order to find all low-energy conformers of the investigated compound. The obtained geometries of MEAA conformers were optimized using two methods: Hartree-Fock (HF) and density functional theory (DFT), combined with the 6-31G(d,p) basis set. In the case of DFT, four functionals were applied: local SVWN [17, 18], gradient BLYP [19, 20], hybrid B3LYP [20, 21] and B3PW91 [21, 22]. Additionally, the optimization of the low-energy conformers was carried out at the B3LYP/6-311++G(d,p) level.

Vibrational frequencies were calculated for the low-energy conformers at each level of theory, and the nature of minima on the potential energy surface (PES) resulting from optimization were determined by inspection of the corresponding calculated Hessian matrix in order to verify that true minimum energy conformations were considered. The relative abundances of the 14 most stable conformers were then estimated at room temperature using the equation  $\Delta G = RT \ln K_c$ , where  $\Delta G$  is the Gibbs free energy relative to the most stable conformer, and  $K_c$  is the ratio of the concentrations of a pair of conformers.

Potential energy distributions (PED) of the normal modes were computed in terms of natural internal coordinates [23] with the GAR2PED program [24] in order to discuss vibrational entropy contribution to the entropy effect.

## Results and discussion

### Conformational analysis

Fourteen low-energy conformers of MEAA were obtained as a product of the conformational analysis. They were recalculated at the B3LYP/6-311++G(d,p) level (Fig. 2). The selected dihedrals defining the conformers are listed in Table 1.

Since the occurrence of conformers results from the different arrangements of the side-chains of MEAA, their spatial configuration is related to three dihedral angles defined as:  $\angle(\text{C}20\text{-C}2\text{-N}3\text{-C}18)$ ,  $\angle(\text{S}1\text{-C}20\text{-C}2\text{-N}3)$  and  $\angle(\text{H}33\text{-S}1\text{-C}20\text{-C}2)$ . The conformer notation applied in this paper is based on the values of proper dihedral angles and consists of three elements. The first corresponds to the value of the C20–C2–N3–C18 dihedral angle: ca.  $80^\circ$  (synclinal, Sc), ca.  $110^\circ$  (anticlinal, Ac), or ca.  $180^\circ$  (trans, T). The second and third elements are related to the conformation of the S1–C20–C2–N3 and the H33–S1–C20–C2 dihedral angles, respectively (trans, T, gauche, G, and minus gauche, –G).

In most cases differences between MEAA conformer geometries calculated by using ab initio and DFT methods with the same basis set are not significant. The largest discrepancy was found for the structures obtained from HF and SVWN calculations.

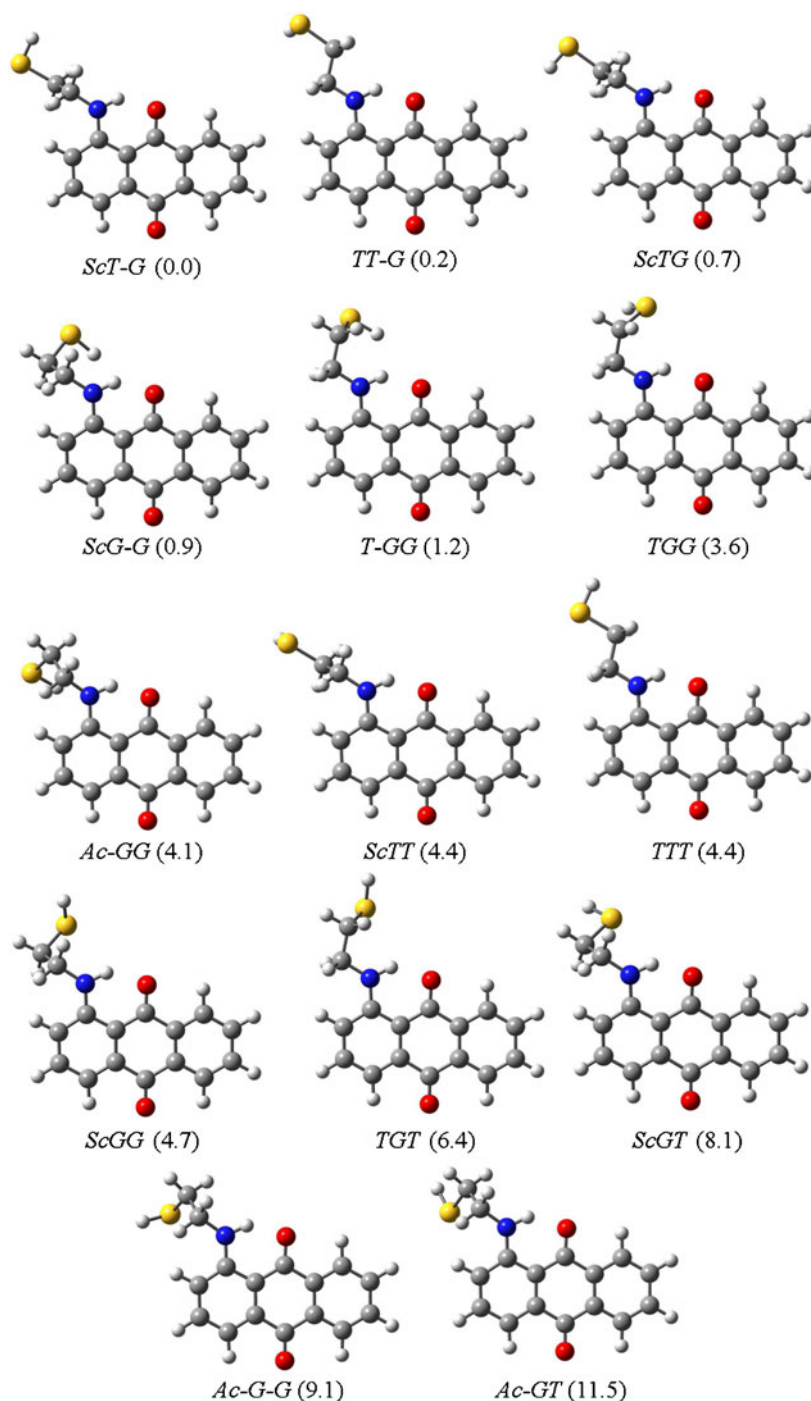
The intramolecular hydrogen bond between the N3H23 group and the O5 oxygen atom is one of the characteristic features of all low-energy MEAA conformers (see [Supplementary Materials](#)). For all considered structures, the B3LYP/6-311++G(d,p) calculations predict the N3···O5 distance in the 2.624–2.637 Å range and the  $\angle(\text{N}3\text{-H}23\cdots\text{O}5)$  angle in the range of  $133.5\text{--}138.1^\circ$ . For most MEAA conformations, the distance between the H23 and O5 atoms varies in the 1.801–1.810 Å range [B3LYP/6-311++G(d,p)] and is predicted to be slightly shorter only for the Ac-GT, Ac-GG and Ac-G-G conformers (ca. 1.78 Å) and somewhat longer for TGT and TGG forms (ca. 1.82 and 1.83 Å, respectively). The obtained distances and angles indicate that the H-bond predicted for all considered conformations is of comparable strength.

The length of the H-bond calculated by using the B3LYP and BLYP functionals combined with the 6-31G(d,p) basis set is very similar to that obtained from calculations with the larger basis set. Shorter H-bonds were obtained from the B3PW91 and SVWN calculations, while the H-bonds obtained from HF calculations are longer. Nevertheless, the qualitative agreement between all applied methods was satisfactory.

### Thermochemistry of MEAA conformers

There are certain principal physical factors that determine the conformational state of a molecule. In general, the role of

**Fig. 2** Optimized B3LYP/6-311++G(d,p) geometries of the most stable MEAA conformers. The values of the zero-point-corrected energy (in  $\text{kJ}\cdot\text{mol}^{-1}$ ) relative to the most stable conformer are given in parentheses



intramolecular interactions of the side chains is crucial; however, in the case of MEAA conformers, side-chain conformational entropy and steric factors are more significant. Since the restriction of internal rotations results from steric effects, it makes an important contribution to conformer stability [25].

The calculated relative zero-point-corrected energies and Gibbs free energies for all stable MEAA conformers are presented in Fig. 3. It can be seen that the relative zero-point-corrected energies do not follow the

order of Gibbs free energies. Thus, it is evident that the entropy effect is important for the thermochemistry of MEAA conformers, and should be taken into account when considering conformer stability. In the case of MEAA, H-bonds do not differentiate the entropy of the structures, as the H-bonds predicted in all conformers are of the same type and similar in strength (Fig. 2). Therefore, MEAA is a convenient example to illustrate the correlation of the entropy effect with the geometry of the side chain.

**Table 1** Relative zero-point-corrected energies ( $\Delta E_{\text{corr}}$ ), dipole moments ( $\mu$ ) and main dihedral angles of all low-energy 1-[(2-mercaptoethyl)amino]-9,10-anthraquinone (MEAA) conformers calculated at the B3LYP/6-311++G(d,p) level

Conformer	$\Delta E_{\text{corr}}$ [KJ-mol <sup>-1</sup> ]	$\mu$ [D]	$\angle(\text{H23-N3-C18-C16})$ [°]	$\angle(\text{C2-N3-C18-C7})$ [°]	$\angle(\text{C20-C2-N3-C18})$ [°]	$\angle(\text{S1-C20-C2-N3})$ [°]	$\angle(\text{H33-S1-C20-C2})$ [°]
ScT-G	0.00 <sup>a</sup>	2.42	-1.5	3.2	81.5	-176.7	-66.3
TT-G	0.24	1.33	0.8	-0.3	178.7	-178.1	-69.0
ScTG	0.65	1.32	-1.0	0.8	82.8	177.5	68.3
ScG-G	0.88	2.13	0.5	-0.8	81.9	64.9	-64.2
T-GG	1.19	2.46	4.8	-2.7	171.5	-70.5	65.5
TGG	3.58	2.81	7.7	-2.8	172.8	62.6	63.5
Ac-GG	4.08	3.23	2.2	-14.1	115.5	-67.8	64.3
ScTT	4.38	1.57	-0.9	0.4	83.2	-177.5	-178.8
TTT	4.44	2.26	0.0	0.0	180.0	180.0	180.0
ScGG	4.70	3.39	-3.9	3.7	80.0	63.9	71.0
TGT	6.41	3.57	1.8	1.4	177.6	66.8	-179.8
ScGT	8.06	3.12	-0.6	1.9	78.9	61.2	166.1
Ac-G-G	9.07	3.95	0.4	-6.6	110.5	-66.6	-75.9
Ac-GT	11.5	4.17	-0.2	-6.2	106.8	-67.4	-171.7

<sup>a</sup>  $E_{\text{corr}} = -3205631.03$  [KJ-mol<sup>-1</sup>]

The most important basic thermodynamic values and abundances of all considered MEAA conformers are shown in Table 2. Compared to ScT-G, four MEAA conformers (Ac-GG, ScGG, ScG-G and TGG) are unfavored by entropy due to their negative entropic contribution. The largest effect is observed for the Ac-GG form ( $T\Delta S_{\text{tot}} = -1.4$  KJ-mol<sup>-1</sup>), and is caused by the presence of steric constraints due to interactions between the hydrogen atom from the mercapto group and the nitrogen atom. Indeed, the SH group is hovering over the nitrogen atom. Thus, the internal rotation of the mercapto group can be considered as internally hindered in this conformer. Analogous behavior is also predicted for the ScG-G conformer, which is also significantly destabilized by entropy ( $T\Delta S_{\text{tot}} = -0.9$  KJ-mol<sup>-1</sup>, Table 2).

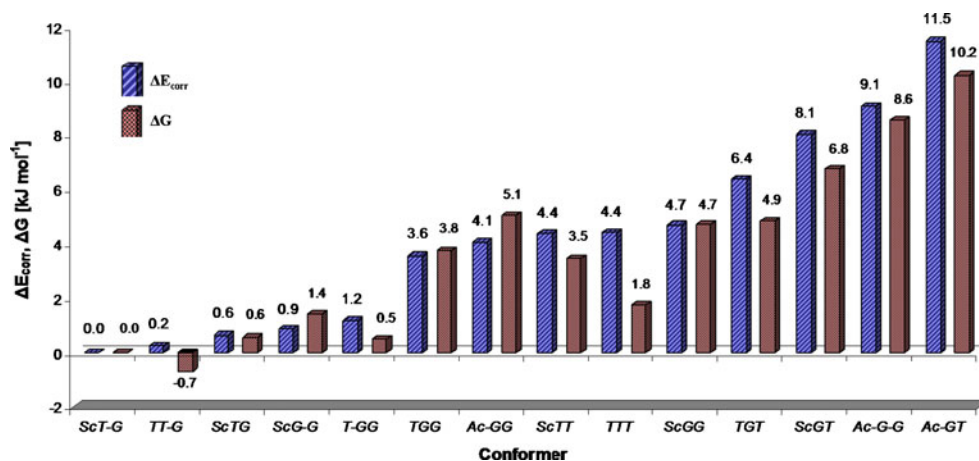
In comparison to ScT-G, the remaining forms are stabilized by entropy, with the largest effect being observed

for conformers with a “straight” side-chain, i.e., with side-chain dihedral angles close to 180°. Close examination of the structures with the highest  $T\Delta S_{\text{tot}}$  values (TTT, TGT, ScGT and Ac-GT) indicates that the key factor is the trans arrangement of the H33-S1-C20-C2 group.

The entropy effect has a strong impact on some relative Gibbs free energies and abundances of the considered conformers (Table 2). TT-G has the lowest  $\Delta G$  and the largest abundance, despite a small enthalpic destabilization. Strong entropy effects are also responsible for the non-negligible abundance of TTT (4.3%). It should be emphasized that only this conformer has a degeneracy value equal to one.

Calculations of thermochemical values and abundances of conformers were also carried out based on the other methods/basis sets applied in this paper. The results were very similar to these obtained from the B3LYP/6-311++G(d,p)

**Fig. 3** Correlation of the calculated B3LYP/6-311++G(d,p) zero-point corrected energy ( $\Delta E_{\text{corr}}$ ) and Gibbs free energy ( $\Delta G$ , both in KJ-mol<sup>-1</sup>, relative to the most stable conformer) for the most stable conformers of MEAA



**Table 2** Basic thermodynamic values and abundances of all low-energy MEAA conformers calculated at the B3LYP/6-311++G(d,p) level at 25°C

Conformer	Degeneracy	$\Delta E_{\text{corr}}$ [KJ-mol <sup>-1</sup> ]	$\Delta G$ [KJ-mol <sup>-1</sup> ]	$\Delta H$ [KJ-mol <sup>-1</sup> ]	$T\Delta S_{\text{tot}}$ [KJ-mol <sup>-1</sup> ]	Abundance [%]
ScT-G	2	0.00 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>	0.00	17.4
TT-G	2	0.24	-0.68	0.82	1.50	22.9
ScTG	2	0.65	0.56	0.69	0.13	13.9
ScG-G	2	0.88	1.41	0.56	-0.86	9.84
T-GG	2	1.19	0.49	1.27	0.78	14.3
TGG	2	3.58	3.76	3.59	-0.17	3.82
Ac-GG	2	4.08	5.05	3.62	-1.44	2.27
ScTT	2	4.38	3.46	4.75	1.29	4.31
TTT	1	4.44	1.75	5.60	3.84	4.29
ScGG	2	4.70	4.74	4.58	-0.15	2.58
TGT	2	6.41	4.85	6.99	2.13	2.46
ScGT	2	8.06	6.77	8.40	1.63	1.13
Ac-G-G	2	9.07	8.57	8.98	0.41	0.55
Ac-GT	2	11.5	10.2	11.8	1.55	0.28

<sup>a</sup>  $E_{\text{corr}} = -3205631.03$  [KJ-mol<sup>-1</sup>]

<sup>b</sup>  $G = -3205752.20$  [KJ-mol<sup>-1</sup>]

<sup>c</sup>  $H = -3205584.33$  [KJ-mol<sup>-1</sup>]

calculations. The most significant differences were noted with SVWN calculations. Despite all observed differences, some general conclusions can be derived from this analysis. The conformers with considerably twisted side-chains are destabilized by entropy because of the presence of steric constraints that restrict internal rotation and lead to an increase in  $\Delta G$  with respect to  $\Delta E_{\text{corr}}$ . The increase in  $\Delta G$  results from the decrease in  $\Delta S_{\text{tot}}$ , but its magnitude is reduced by the decrease in  $\Delta H$ . For instance, for the Ac-GG conformer,  $\Delta G = 5.1$  KJ-mol<sup>-1</sup>, despite the fact that  $\Delta H$  is only 3.6 KJ-mol<sup>-1</sup> (less than  $\Delta E_{\text{corr}}$ , see Table 2). This results from large entropy effects, with  $T\Delta S_{\text{tot}}$  shifted down to -1.4 KJ-mol<sup>-1</sup>. The most extended conformers (e.g., TTT, TT-G, ScTT, Ac-GT, ScGT and TGT) are stabilized by entropy. Moreover, XXT conformers (X denotes any conformation of the relevant dihedral angles) have the mercapto group facing to the outside of MEAA, so this group does not interact with the rest of the molecule.

The essential aim of this work was to determine the relationship between entropy values and the structure of the molecule. The total entropy is defined as:

$$S_{\text{tot}} = R \left( \ln(q) + T \left( \frac{\partial \ln(q)}{\partial T} \right)_V \right) \quad (1)$$

where  $R$  is the gas constant ( $R = 8.31441$  KJ-mol<sup>-1</sup>K<sup>-1</sup>),  $e$  is the Euler constant ( $e = 2.71828$ ),  $q = q(T, V)$  is the total partition function, defined as  $q = q_{\text{trans}} q_{\text{elect}} q_{\text{rot}} q_{\text{vib}}$  (where subscripts denote translational, electronic, rotational, and vibrational contribution to partition function) and  $T$  is the

temperature [25]. The total entropy can be also defined as the sum of the translational, electronic, rotational and vibrational entropy.

The translational entropy depends on several constants as well as temperature, pressure and the mass of a molecule. All these factors are constant when different conformations of the same compound are considered at constant temperature and pressure. The same can be stated for the electronic entropy  $S_{\text{elec}}$ . Therefore, the structure of the conformers is affected only by the rotational and vibrational contributions to entropy, as analyzed below.

#### Rotational entropy of MEAA conformers

Generally, for a nonlinear polyatomic molecule, the rotational partition function  $q_{\text{rot}}$  [25] is given by Eq. 2:

$$q_{\text{rot}} = \frac{\pi^{1/2}}{\sigma_r} \left( \frac{T^{3/2}}{(\Theta_{r,x} \Theta_{r,y} \Theta_{r,z})^{1/2}} \right) \quad (2)$$

where  $\sigma_r$  is the symmetry number for rotation and  $\Theta_{r,i}$  is the function defined in Eq. 3:

$$\Theta_{r,i} = \frac{h^2}{8\pi^2 k_B I_i} \quad (3)$$

where  $I_i$  is the moment of inertia, while the rotational entropy  $S_{\text{rot}}$  is expressed by Eq. 4:

$$S_{\text{rot}} = R \left( \ln(q_{\text{rot}}) + \frac{3}{2} \right) \quad (4)$$

Analysis of Eqs. 2–4 shows that rotational entropy depends on some constants as well as temperature and moments of inertia. Since the moment of inertia changes with structural modification, the rotational entropy is different for different conformers. Therefore,  $S_{\text{rot}}$  has an influence on the observed entropy effect.

The relative rotational entropies of all considered conformers calculated at the B3LYP/6-311++G(d,p) level of theory are presented in Fig. 4a. Two conformers (TTT and TT-G) are stabilized by  $S_{\text{rot}}$  relative to the ScT-G form, but this effect is not significant, since the rotational entropy of both is only ca. 0.5 KJ·mol<sup>-1</sup>K<sup>-1</sup>. This entropy is correlated with the spatial structure that extends perpendicularly to the direction of the z-component of the moment of inertia of these conformers. Particularly, these are the only two forms with both C20–C2–N3–C18 and S1–C20–C2–N3 dihedral angles in trans arrangement, see Fig. 3). As the ScTX conformers have similar spatial structure (differing only by the value of the H33–S1–C20–C2 angle) they have similar  $\Delta S_{\text{rot}}$  values. In comparison to the ScT-G conformer, the remaining conformers are destabilized by rotational entropy. Their  $\Delta S_{\text{rot}}$  values fall within the range –0.2 KJ·mol<sup>-1</sup>K<sup>-1</sup> for T(-)GX conformers to –0.9 KJ·mol<sup>-1</sup>K<sup>-1</sup> for Ac-GT conformers; for forms with the C20–C2–N3–C18 angle in the anticlinal or synclinal arrangement,  $\Delta S_{\text{rot}}$  values are considerably lower than those with the angle in trans configuration. The

results obtained are practically independent of the method applied, thus allowing the conclusion that the arrangement of the C20–C2–N3–C18 chain is a key factor responsible for small rotational stabilization/destabilization.

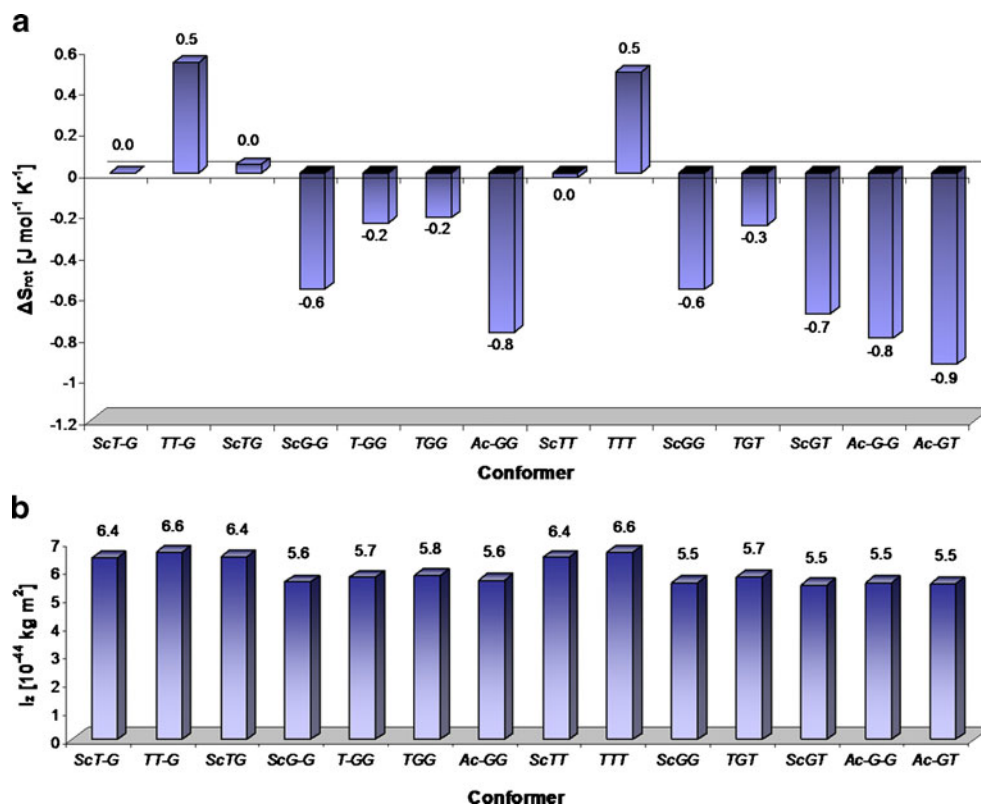
According to Eqs. 2–4 the rotational entropy depends on the product of moments of inertia. The larger the product, the larger  $\Delta S_{\text{rot}}$  contribution. Table S2 shows the  $I_x \cdot I_y \cdot I_z$  of all considered MEAA conformers calculated by all applied methods and basis sets. The results obtained clearly confirm the above considerations. As expected, the highest values are predicted for TTX conformers, they are a bit lower for ScTX, and lowest for ScGX or Ac-GX conformers.

#### Vibrational entropy of MEAA conformers

The relationship between the vibrational partition function  $q_{\text{vib}}$  and the vibrational entropy  $S_{\text{vib}}$  with the molecular structure is more complicated because all vibrations of the molecule must be taken into consideration. The complexity of interpreting  $q_{\text{vib}}$  and  $S_{\text{vib}}$  increases with the size of the studied molecule. If the first vibrational level is defined as zero, the vibrational partition function is given by Eq. 5:

$$q_{\text{vib}} = \prod_{i=1}^{3N-6} \frac{1}{1 - e^{-\frac{h\nu_i}{k_B T}}} \quad (5)$$

**Fig. 4** **a** Rotational contributions to entropy ( $\Delta S_{\text{rot}}$ , in KJ·mol<sup>-1</sup>K<sup>-1</sup> relative to the most stable conformer) and **b** z-components of the moment of inertia ( $I_z$ , in kg m<sup>2</sup>) for most-stable conformers of MEAA calculated at the B3LYP/6-311++G(d,p) level



where  $\nu_i$  is the frequency of the  $i$ -th normal vibration and  $N$  is the number of atoms in the molecule [25], while the vibrational entropy is expressed by Eq. 6:

$$S_{\text{vib}} = R \sum_{i=1}^{3N-6} \left( \frac{\frac{h\nu_i}{k_B T}}{e^{\frac{h\nu_i}{k_B T}} - 1} - \ln \left( 1 - e^{-\frac{h\nu_i}{k_B T}} \right) \right) \quad (6)$$

According to Eqs. 5–6,  $S_{\text{vib}}$  depends on some constants and the frequencies of all molecular vibrations, whereas frequencies are associated with internal coordinates and force constants [25]. Thus,  $S_{\text{vib}}$  is strongly dependent on molecular structure and conformation.

Figure 5a presents the relative vibrational entropies of all considered conformers calculated at the B3LYP/6-311++G(d,p) level. It is clear from this figure that, in comparison to ScT-G form, most conformers are stabilized by the vibrational entropy and only three of them are destabilized (Ac-GG, ScG-G and TGG). Among the stabilized conformers, the highest  $\Delta S_{\text{vib}}$  is seen with the TTT form (12.4 KJ-mol<sup>-1</sup>K<sup>-1</sup>), while the most destabilized is Ac-GG (-4.1 KJ-mol<sup>-1</sup>K<sup>-1</sup>).

Among conformers possessing the same arrangement of the C20–C2–N3–C18 and S1–C20–C2–N3 angles  $\Delta S_{\text{vib}}$  generally decreases in order XXT > XXG > XX-G with the exception of Ac-GX. According to their  $\Delta S_{\text{vib}}$  values, Ac-GX conformers can be ordered as follows: Ac-GT > Ac-G-G > Ac-GG. This observation leads to the

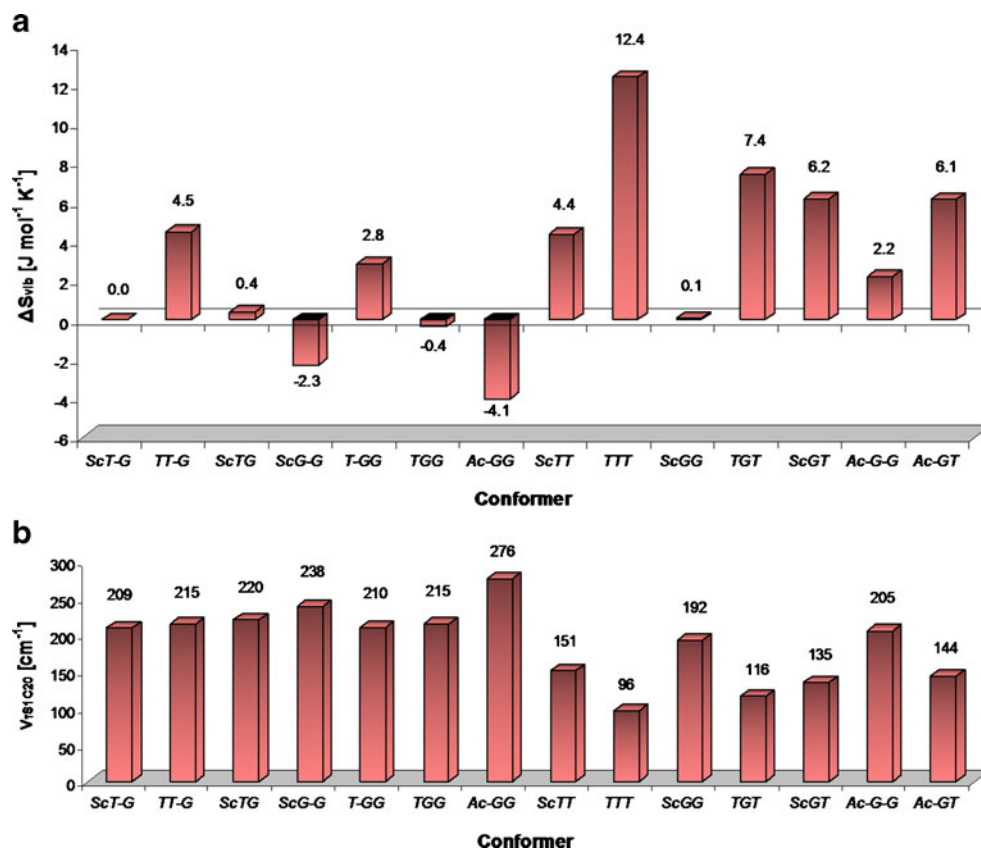
conclusion that the arrangement of the mercapto group with respect to the remaining part of the side-chain is the main factor determining the vibrational contribution to entropy.

Investigation of Eq. 6 leads to a very important finding. The largest contributions to total vibrational entropy have low frequency vibrations. For example, a vibration with a frequency of 50 cm<sup>-1</sup> makes a contribution of 20.2 KJ-mol<sup>-1</sup>K<sup>-1</sup>, while one with frequency of 500 cm<sup>-1</sup> provides an input of only 2.8 KJ-mol<sup>-1</sup>K<sup>-1</sup>. Contributions from stretching vibrations (e.g. C–H, N–H or S–H) are lower than  $4 \times 10^{-4}$  KJ-mol<sup>-1</sup>K<sup>-1</sup>, but bending, torsion and skeletal vibrations, which are low-frequency modes, must all be taken into account.

Results obtained at other levels of theory specified in the **Computational methods** are in slight disagreement with those obtained using B3LYP/6-311++G(d,p) level of theory. As expected, most dissimilarities are produced by the SVWN calculations. Therefore, a further investigation was carried out only with the results calculated with the B3LYP/6-311++G(d,p) basis set. PEDs of all investigated MEAA conformers and definition of internal coordinates are provided in Tables S3–S17.

The  $\Delta S_{\text{vib}}$  values of ScTX conformers relative to ScT-G are 4.4 KJ-mol<sup>-1</sup>K<sup>-1</sup> for ScTT, and 0.4 KJ-mol<sup>-1</sup>K<sup>-1</sup> for ScTG, so  $S_{\text{vib}}$  decreases in the order: ScTT > ScTG > ScT-

**Fig. 5 a** Vibrational contributions to entropy ( $\Delta S_{\text{vib}}$ , in KJ-mol<sup>-1</sup>K<sup>-1</sup> relative to the most stable conformer) and **b** wavenumber associated with the vibration with the highest contribution from the  $\tau_{\text{S1C20}}$  mode ( $\nu_{\tau_{\text{S1C20}}}$ , in cm<sup>-1</sup>) for most stable conformers of MEAA calculated at the B3LYP/6-311++G(d,p) level



G. Nineteen of the lowest-frequency vibrations of these conformers have similar values and to some extent are responsible for the same structural changes (similar PEDs). Therefore these vibrations do not differentiate the vibrational entropy of conformers significantly. The opposite situation exists for vibrations associated with  $\tau_{S1C20}$ —an internal coordinate involving hydrogen atom H33, predicted to absorb at  $151\text{ cm}^{-1}$ ,  $220\text{ cm}^{-1}$  and  $209\text{ cm}^{-1}$  for ScTT, ScTG and ScT-G, respectively (Figs. 5b, S18)—which differ considerably in the above-mentioned forms.

Similar considerations can be carried out for Ac-GX conformers. The  $\Delta S_{\text{vib}}$  values of these conformers are:  $6.1\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  (Ac-GT),  $-4.1\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  (Ac-GG) and  $2.2\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  (Ac-G-G), relative to ScT-G. For Ac-GX conformers, the order in which  $S_{\text{vib}}$  decreases is as follows: Ac-GT > Ac-G-G > Ac-GG. The vibrations of  $144\text{ cm}^{-1}$  (Ac-GT),  $276\text{ cm}^{-1}$  (Ac-GG) and  $205\text{ cm}^{-1}$  (Ac-G-G) reveal the contribution of the internal coordinate  $\tau_{S1C20}$  and have enormous influence on  $\Delta S_{\text{vib}}$  values (Tables S7, S8, S9).

In the case of ScGX conformers,  $\Delta S_{\text{vib}}$  (relative to ScT-G) is  $6.2$ ,  $0.1$  and  $-2.3\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  for ScGT, ScGG and ScG-G, respectively. For this group the following frequencies are related to the internal coordinate  $\tau_{S1C20}$ :  $135\text{ cm}^{-1}$  (ScGT),  $192\text{ cm}^{-1}$  (ScGG) and  $238\text{ cm}^{-1}$  (ScG-G). These vibrational modes, and the fact that there are significant differences in the frequencies of most remaining vibrations involving the mercapto group (described by internal coordinates defined as:  $\delta_{1/C20H31H32}$ ,  $\delta_{2/C20H31H32}$ ,  $\tau_{C2C20}$  and  $\tau_{C2N3}$ ), explain the values of  $\Delta S_{\text{vib}}$ .

TTX conformers have also different  $\Delta S_{\text{vib}}$ . For TTT, the vibration at  $96\text{ cm}^{-1}$  with 31% of  $\tau_{S1C20}$ , and for TT-G, the vibration at  $215\text{ cm}^{-1}$  with 78% of this coordinate can explain the significant difference between  $\Delta S_{\text{vib}}$  values and the stability of TTT. Additionally, other vibrations of TTT involving the S-H group have lower values than the corresponding frequencies for TT-G.

For T(-)GX-type conformers, the following values of  $\Delta S_{\text{vib}}$ , relative to ScT-G, are predicted by calculations:  $7.4$  (TGT),  $2.9$  (T-GG) and  $-0.4\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  (TGG). Contributions to the vibrational entropy from vibrations containing the  $\tau_{S1C20}$  coordinate are different, but sufficient to make TGT the most stabilized conformer.

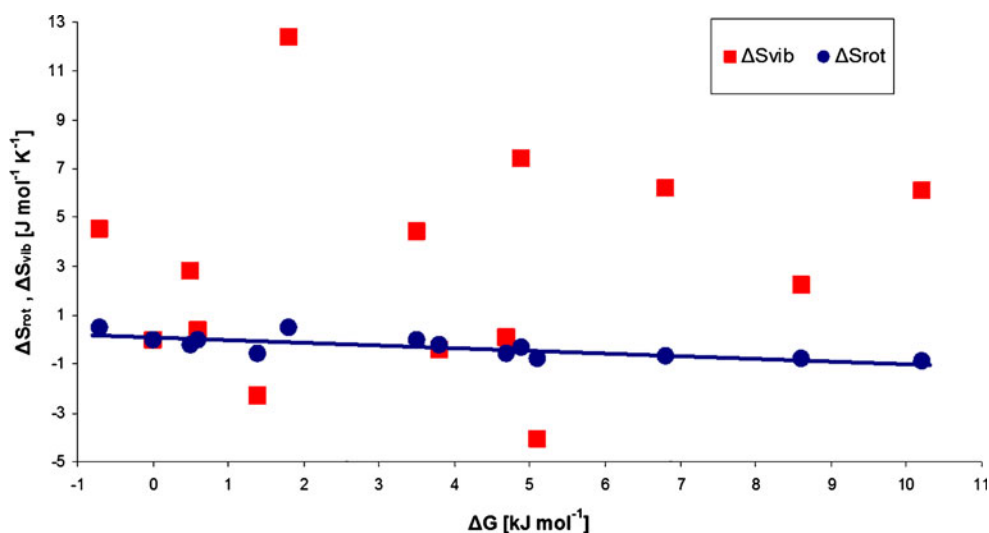
In conclusion, vibrational modes involving the mercapto group have an important influence on vibrational entropy changes. This refers especially to vibrations dominated by the  $\tau_{S1C20}$  internal coordinate. Vibrational frequencies associated with conformers strongly stabilized by vibrational entropy are lowest:  $\Delta S_{\text{osc}}=12.9\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  and  $\nu=96\text{ cm}^{-1}$  (TTT),  $\Delta S_{\text{osc}}=7.4\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  and  $\nu=116\text{ cm}^{-1}$  (TGT),  $\Delta S_{\text{osc}}=6.2\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  and  $\nu=135\text{ cm}^{-1}$  (ScGT) and  $\Delta S_{\text{osc}}=6.2\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  and  $\nu=144\text{ cm}^{-1}$  (Ac-GT). In contrast, vibrational frequencies of conformers strongly entropically destabilized are highest:  $\Delta S_{\text{osc}}=-4.1\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  and  $\nu=276\text{ cm}^{-1}$  for Ac-GG and  $\Delta S_{\text{osc}}=-2.3\text{ KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$  and  $\nu=238\text{ cm}^{-1}$  for ScG-G.

The above analysis confirms the key importance of the H33–S1–C20–C2 arrangement for vibrational entropy. In conformers with the trans arrangement of this dihedral, the S–H group is able to oscillate loosely. As a result, energies of vibrations involving this group (vibrations where the contribution from the equilibrium position of the hydrogen atom H33 is largest) are the lowest. In conformers possessing steric constraints involving the S–H group (conformers destabilized by entropy), mercapto group oscillation is restricted and the energies of the corresponding vibrations are comparatively high.

## Conclusions

The conformational analysis of MEAA allowed prediction of the existence of 14 low-energy conformers. The

**Fig. 6** Rotational  $\Delta S_{\text{rot}}$  and vibrational  $\Delta S_{\text{vib}}$  contributions to entropy (in  $\text{KJ}\cdot\text{mol}^{-1}\text{K}^{-1}$ ) correlated to the Gibbs free energy ( $\Delta G$ , in  $\text{KJ}\cdot\text{mol}^{-1}$  relative to the most stable conformer)





thermochemical values computed for these forms show that the Gibbs free energy order does not follow the zero-point energy order, indicating that entropy affects the abundance of conformers (the entropy effect). Analysis of the structures of MEAA shows that the H-bond is not a factor that can be used to differentiate entropy values between conformers, as intramolecular H-bonds of similar strength are predicted for all considered forms. Therefore, the side-chain arrangements affecting steric conditions are the only factor determining entropy differences between conformers.

A detailed analysis of entropy contributions demonstrates that the entropy effect is associated only with the difference between rotational and vibrational entropy, with the latter factor being predominant. The rotational entropy depends on the product of moments of inertia, and the structural feature most correlated with it is the conformation of the C20–C2–N3–C18 chain, with trans forms being stabilized compared to anticlinal or synclinal forms. Thus, a moment of inertia perpendicular to the molecular plane makes a significant contribution to  $\Delta S_{\text{rot}}$ .

Vibrational entropy depends on vibrational frequencies and is related structurally mainly with the arrangement of the H33–S1–C20–C2 angle, with trans conformers being considerably entropically stabilized as compared to “gauche” or “minus gauche” ones. Accordingly, vibrations associated with the  $\tau_{\text{S1C20}}$  coordinate have the greatest impact on vibrational entropy differences (Fig. 6).

**Acknowledgment** The authors thank the Academic Computer Centre ‘Cyfronet’, Kraków, Poland, for computing time.

## References

- Brown JP, Brown JR (1976) *Mutat Res* 40:203–224
- Huang Q, Lu G, Shen HM, Chung MCM, Choon NO (2007) *Med Res Rev* 27:609–630
- Agarwal K, Singh SS, Verma S, Kumar S (2000) *J Ethnopharmacol* 72:43–46
- Hatano T, Uebayashi H, Ito H, Shiota S, Tsuchiya T, Yoshida T (1999) *Chem Pharmacol Bull* 47:1121–1127
- Montoya SCN, Comini LR, Sarmiento M, Becerra C, Albesa I, Argüello GA, Cabrera J (2005) *J Photochem Photobiol B: Biol* 78:77–83
- Rajendran M, Ramasamy S, Rajamanickam C, Gandhidasan R, Murugesan R (2003) *Biochim Biophys Acta* 1622:65–72
- Inbaraj JJ, Krishna MC, Gandhidasan R, Murugesan R (1999) *Biochim Biophys Acta* 1472:462–470
- Reszka KJ, Bilski P, Chignell CF, Hartley JA, Khan N, Souhami RL, Mendonca AJ, Lown JWJ (1992) *Photochem Photobiol Biol* 15:317–335
- Jacquemin D, Wathelet V, Preat J, Perpète EA (2007) *Spectrochim Acta A* 67:334–341
- Perpète EA, Wathelet V, Preat J, Lambert C, Jacquemin D (2006) *J Chem Theory Comput* 2:434–440
- Guillaumont D, Nakamura S (2000) *Dyes Pigment* 46:85–92
- Aybelj F, Fele L (1998) *J Mol Biol* 279:665–684
- Doig A (1996) *J Biophys Chem* 61:131–141
- Kaczor A, Reva ID, Proniewicz LM, Fausto R (2006) *J Phys Chem A* 110:2360–2370
- Kaczor A, Reva ID, Proniewicz LM, Fausto R (2007) *J Phys Chem A* 111:2957–2965
- Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA, Vreven T Jr, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA (2004) *Gaussian 03, Revision E.01*. Gaussian Inc, Wallingford CT
- Slater JC (1974) *Quantum theory of molecular and solids. Vol 4: the self-consistent field for molecular and solids*. McGraw-Hill, New York
- Vosko SH, Wilk L, Nusair M (1980) *Can J Phys* 58:1200–1211
- Becke AD (1988) *Phys Rev A* 38:3098–3100
- Lee CT, Yang WT, Parr RG (1988) *Phys Rev B* 37:785–789
- Becke AD (1993) *J Chem Phys* 98:5648–5652
- Perdew JP, Wang Y (1992) *Phys Rev B* 45:13244–13249
- Pulay P, Fogarasi G, Pang F, Boggs JE (1979) *J Am Chem Soc* 101:2550–2560
- Martin JML, Van Alsenoy C (1995) *Gar2ped*. University of Antwerp
- McQuarrie DA, Simon JD (1999) *Molecular thermodynamics*. University Science Books, Sausalito