SCSA Code: Applications on the Cyclopeptide Renieramide

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SCSA is an algorithm designed to get information on molecular conformational properties. The most stable conformers are determined by the homemade *SCSA* code, performing a multistep systematic conformational search, which involves energy and structure quantum chemical optimizations at low-level and high-level. The *SCSA* method was employed to analyze the conformational space of the in vacuo cyclopeptide renieramide at AM1 and B3LYP/6-31G(d) levels. Calculations at B3LYP level of the GIAO ^{13}C NMR chemical shifts were also performed on the final conformers. In fact, to validate the conformational search results experimental and calculated ^{13}C NMR spectra of renieramide were compared. Slight disagreements observed between experimental and calculated spectra could be attributed to solute−solvent interactions, which were not taken into account in the algorithm proposed here.

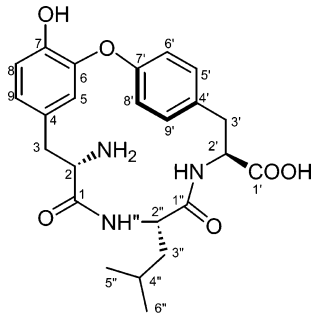
INTRODUCTION

The structural and energetic description of the conformer set characterizing a given species is a fundamental step in the study of the energetics and kinetics of organic-,[1−3] organometallic-,[4] and bio-chemistry[5−7] as well as of homogeneous,[8] enzymatic,[9] and heterogeneous catalysis.[10] With regards to the biological activity,[11,12] the conformational studies become also more relevant when the bioactive conformer is not the most stable one. This occurrence, which is often caused by competition between thermodynamic and kinetic contributions, implies that sets of conformers have to be known in order to investigate such systems.[13] The latter statement, following the Hammond−Leffler postulate,[14] has to be generally considered each time an equilibrium among conformers having small energy differences occurs. Accordingly, the corresponding chemical properties should be represented by averaged properties.[15,16] The considerations above become very important in modeling spectral properties,[17] namely in mimicking ^{13}C NMR chemical shift (*cs*) values.[15]

We have shown that a Boltzmann-like average on the GIAO ^{13}C NMR *cs* values of the most stable conformers of flexible low polar organic molecules fitted the experimental ^{13}C NMR *cs* values of the same molecules if solvent effects were negligible.[15] Moreover, it was shown that strong solute−solvent interactions were able to modify both geometries and energies of conformers found in vacuo or in low polar media.[18,19]

Indeed, the involvement of the solvent in quantum chemistry (**qc**) calculations is still a computational challenge even for medium-sized solutes.[16] Progress has been done by introducing the Polarized Continuum Model (PCM) [20] and/or by including a limited number of coordinated solvent molecules.[21,22]

Scheme 1



Conformational search algorithms[13,23−32] are characterized by systematic and/or stochastic rotations of dihedral angles of given molecular fragments. However, the time-consuming systematic search methods are at present appropriate to study just small-sized molecules.[23,24] Moreover, the search protocols followed so far reveal consistence limitations since they usually employ energy minimizations performed via molecular mechanics, which are based on empirical force fields. So, to obtain reliable results, the involvement of experimental constraints[25] and/or structure energy refinements by high-level **qc** methods are needed.[28−32]

In this work we illustrate a Systematic Conformational Search Analysis (*SCSA*) algorithm, designed to investigate local minima by low- and high-level **qc** calculations in a given conformer space. The consistence of the conformational investigation by *SCSA* was checked, comparing[15,33] the experimental and the GIAO ^{13}C NMR *cs* values (see **Methods**) of renieramide.[34] This is a marine tripeptide related to the OF4949 anticancer family compounds, showing immunomodulating activity.[34] Renieramide, found in the polar extract of the Vanuatu sponge *Reniera* sp., is a 17-atom cyclic ring system, containing a side-chain-linked biphenyl ether skeleton, showing on the whole 62 atoms, see Scheme 1.

The present atom-numbering of renieramide is consistent with that previously introduced in a work of restrained NMR

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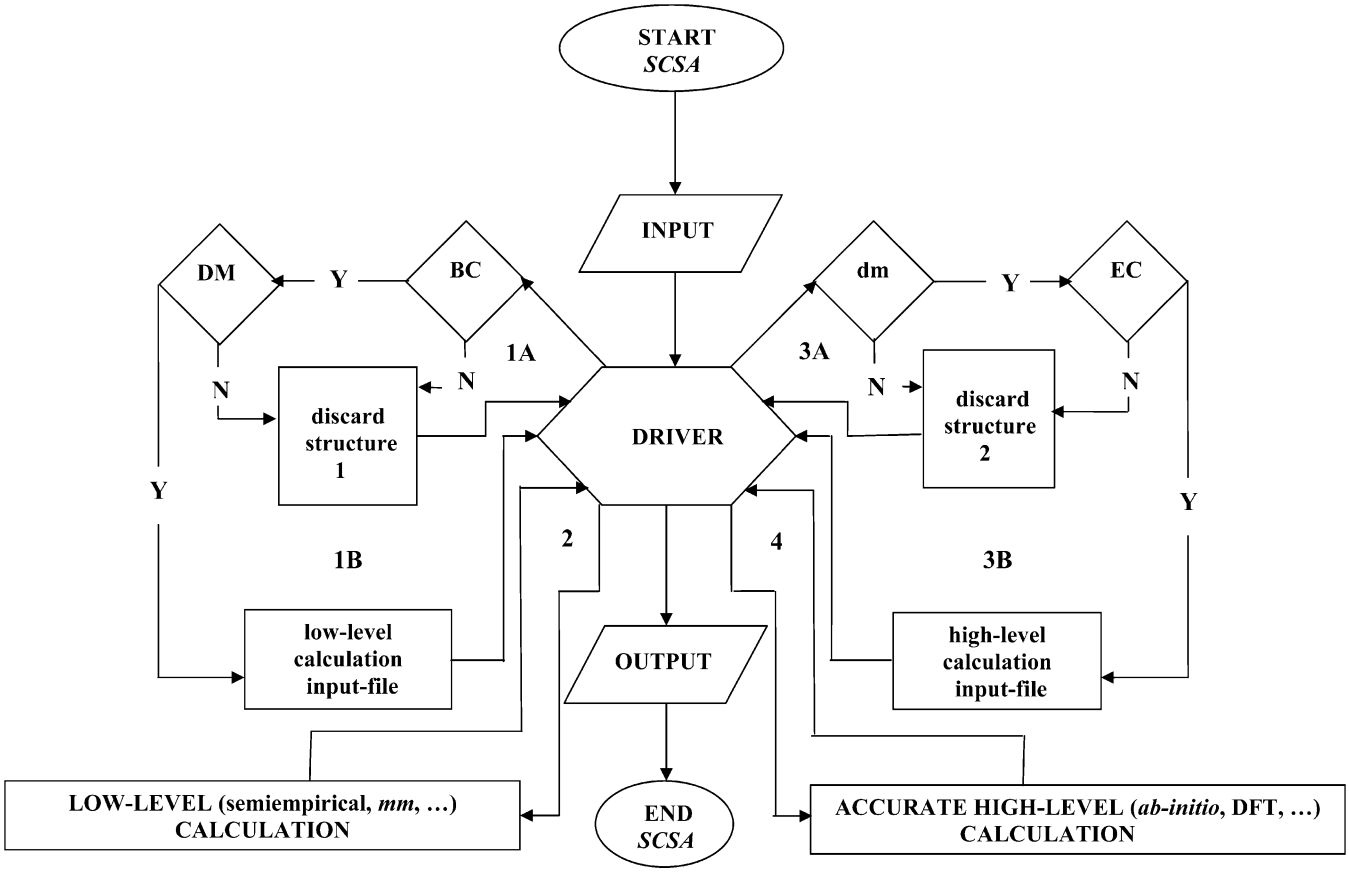
Scheme 2



molecular mechanics and dynamics characterization, carrying out a three-dimensional structure of the title molecule in d6-DMSO.[34] Note that the carbon atom labels in Schemes 1 and 3 are reported without C.

Information on the algorithm and code and details on the GIAO ^{13}C NMR calculations on renieramide are given in the following **Methods** section, whereas the *SCSA* treatment on renieramide as well as the discussion on the results concerning the mimicking of the experimental NMR findings are presented in the **Application** section. Two development lines of the *SCSA* code are finally indicated in the **Conclusion and Future Direction** last section.

METHODS

***SCSA* Code.** *SCSA*, based on a systematic search algorithm,[35] is implemented as a F90 code to run under several operating systems. Scheme 2 shows a flowchart of the code.

In the **INPUT** box the Cartesian coordinates of the parent structure, i.e., the molecular structure from where the conformers originated, and the needed information on the atomic connectivity are introduced. Along the systematic search procedure, the connectivity rules of the parent structure are unchanged, every dihedral angle is incremented by given amounts, and all the possible combinations of each dihedral angle with every other one are taken into consideration.

In the **DRIVER** section, $n = \Pi_{i=1}^{\nu}\rho_i$ scratch files per parent structure are created, being ν and ρ the number of dihedral angles and of the allowed rotations per single dihedral angle, respectively. Each scratch file, containing information on one conformational structure, arises from and produces another scratch file. The maximum number of conformational isomers is $N = ps\Pi_{i=1}^{\nu}\rho_i$, being ps the number of different parent structures. The interatomic distances of every conformer are tested by a bump check procedure,[35] **BC** box of Scheme 2, and a distance matrix analysis, **DM** box. The first and the second action on the structures are performed (i) to verify that the atoms of the corresponding conformer are not falling in nonphysical neighboring spots and (ii) to impose structural constraints.

The nondiscarded structures, following the cycle **1B** of Scheme 2, become the starting structures. These are encoded by *SCSA* in G98W[36] low-level input files, **low-level calculation input-file** box, automatically batched and optimized by a link to the Gaussian software package,[36] cycle **2** in Scheme 2. The resulting structures undergo a new distance matrix analysis and a new energy check, **dm** and **EC** boxes, which eliminate, cycle **3A**, similar conformers and eventually sort out the remaining ones in a selected energy range.

The corresponding geometries, are encoded, cycle **3B** of Scheme 2, in high-level optimization files to be run under G98W,[36] cycle **4**. After the optimization, the resultant structures go through a final distance matrix analysis and energy check, which isolate the final conformers to be employed in studying the average properties of the molecule.

Two relevant metrics[37] of the *SCSA* procedure, concerning (i) time to generate conformers and (ii) ability to cover conformational space, are shortly to be discussed. Points (i) and (ii) are strictly related to the computer architecture employed and to the system analyzed. In the following, the two points above are referred to the title case, analyzing $\sim 4 \cdot 10^7$ rotamers and fixing 11 final species, when a single P−IV

processor of 2.8 GHz in a IBM-compatible PC architecture, having 1Gbyte of RAM, is employed under Windows2000. Concerning point (i), and referring to the flowchart of Scheme 2, cycles **1A** + **1B** last ~8 h, cycle **2** about ~6 h, cycles **3A** + **3B** few seconds, and cycle **4** ~600 h. Concerning point (ii), it has to be stressed that just computational time affects the density of the dihedral angle grid to be set in the conformational space. So, the choice of the grid density is just fixed by the spanning speed discussed in point (i) for renieramide.

Some further final considerations regard the distinctive features of the *SCSA* code in the outline of the commercially available software. The first important feature regards the possibility to combine and to filter the results of the conformational search by **qc** methods. Indeed, the usually available computing capabilities have limited the conformational study of species having medium-large molecular weight to the use of molecular mechanics and dynamics calculations, employing empirical force fields. For this reason, systematic conformational search protocols, which are based on empirical methods, are widely employed in all the most advanced commercial software. On the other hand, commercial **qc** programs, even the most user-friendly, usually offer a large number of optimization methods at a different calculation level, but they lack any conformational search protocol.

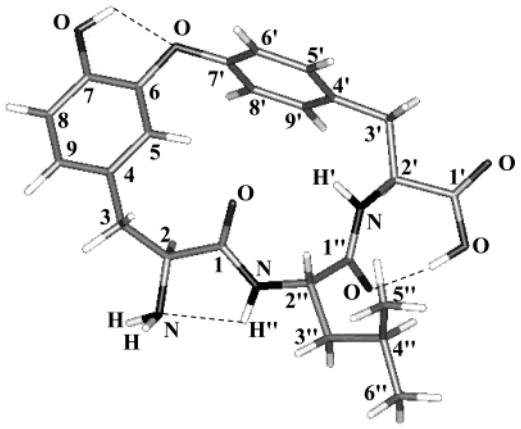
Another *SCSA* feature, which is very important in the general view of the stereostructural exploration protocols, concerns the possibility to systematically modify the configuration of uncertain stereocenters. This feature, coupled with the possibility to consider in the search protocol any experimental structural constraints, e.g. NMR scalar and/or dipolar coupling constraints,[38,39] allows one to get information on the unknown stereocenters of given investigated molecules. Finally, a third noteworthy feature concerns the applicability of the code to the study of noncovalent interactions. Indeed, the application of the *SCSA* code is not meant to be restricted to a single molecule or a single species, and the systematic conformational search of complexes and in general of any aggregate containing more than two chemical species is only limited by the available computational power.

GIAO ^{13}C NMR Chemical Shift Calculations on Renieramide. The geometries of tetramethylsilane, TMS, and of the final renieramide conformers, were fully optimized at B3LYP level, using the 6-31G(d) basis set. The ^{13}C *cs* values of each carbon atom *X* in a given final conformer *i* (cs_{Xi}) were obtained by subtracting its calculated ^{13}C isotropic magnetic shielding (I.M.S.$_{Xi}$) from the averaged ^{13}C I.M.S. of TMS (I.M.S.$_{TMS}$): cs_{Xi} = I.M.S.$_{TMS}$ − I.M.S.$_{Xi}$.[15,33] The averaged ^{13}C *cs* value, $\langle\delta\rangle$, of a given carbon atom *X*, was obtained by a Boltzmann-like distribution working on the *i* considered conformers[15]

$$\langle\delta\rangle = \frac{\sum_{i=1}^{nf} cs_{Xi} \exp(-G_i^\circ/RT)}{\sum_{i=1}^{nf} \exp(-G_i^\circ/RT)}$$

being that *nf* is the number of the final conformers, *R* is the ideal gas constant, *T* is the temperature of the mimicked system, and G_i° is the standard Gibbs free energy value of the *i*th conformer at *T*, calculated in the harmonic approximation of the vibrational potential in the framework of the G98W package.[40]

Scheme 3



APPLICATION

By the *SCSA* code ~4·10^7 conformational renieramide rotamers were scanned, allowing three rotations of 120°, irrespective of the dihedral angle under investigation. Just one parent structure had to be considered. This was obtained (i) opening the renieramide molecule by cutting the NH″−C2″ bond, (ii) optimizing at AM1 semiempirical level the resulting structure saturated by hydrogen atoms on the NH″ and C2″ fragments and, finally, (iii) eliminating the hydrogen atoms introduced. Along the cycle **1A** of Scheme 2, the minimum distance allowed to avoid bumping between two atoms was fixed to 75 pm, and the distance condition NH″−C2″ less than 175 pm was fixed to enable the ring closure.

By this procedure, 1040 starting cyclic structures were isolated and optimized at the AM1 level. After this and following the cycles **3A**, **3B**, and **4** of Scheme 2, 11 final conformers among the 43 B3LYP/6-31G(d) optimized geometries were taken into consideration for determining the averaged GIAO ^{13}C NMR chemical shifts of renieramide. The DFT energy values calculated for the set of the 43 and 11 conformers were within 40 and 13 kJ mol^{-1}, respectively.

The set of the final conformers consisted of the most stable species **R1**, detailed in Scheme 3, and the ones, **R2**−**R11**, closest in energy to the latter.[15,33] These show the carbonyl group in C1 pointing inside the ring and the amine group in C2 outside. Their largest structural relative changes concern the spatial orientation of the isopropyl fragment identified by the C4″, C5″, and C6″ centers, see Scheme 1.

In Table 1 the calculated standard Gibbs free energy differences, ΔG°, as well as the DFT energy differences, ΔE, of the conformers above, are reported. The structure of the conformer **R12** and its energy values, whose differences are also reported in Table 1, were determined by the geometrical optimization starting from the experimental NMR[34] three-dimensional structure. The resulting optimized **R12** geometry was closer to the experimental one than those referable to the more stable **R1**−**R11** conformers.

It has to be noticed that energy and free energy values of the renieramide conformers show a different behavior in the

Table 1. Values of the Calculated Standard Gibbs Free Energy Difference, $\Delta G°$/kJ mol^{-1}, and of the DFT Energy Difference, ΔE/kJ mol^{-1}, Concerning the Different Renieramide Conformers Employed in the NMR Analysis

conformer	$\Delta G°$	ΔE	conformer	$\Delta G°$	ΔE
R1[a]	0.0	0.0	**R7**	7.2	13.4
R2	0.6	1.0	**R8**	7.5	12.5
R3	4.2	11.6	**R9**	9.6	10.8
R4	5.0	4.6	**R10**	12.4	13.1
R5	6.2	8.5	**R11**	12.8	12.8
R6	7.1	10.8	**R12**[b]	16.3	22.0

[a] **R1** is the minimum energy conformer hence its energy is set as zero in the energy scales here reported; the standard Gibbs free energy at 298.15 K, $G°$/au, and the DFT energy, E_h/au, of this conformer were equal to −1547.2126 and −1547.6637, respectively. [b] **R12** conformer structure and energy were calculated at B3LYP/6-31G(d) level, optimizing the experimental three-dimensional NMR geometry.[34]

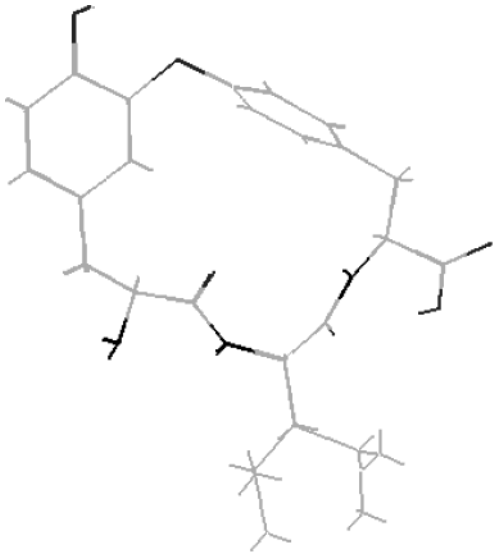


Figure 1. Overlapped structures of the conformers **R1** and **R2**: the spatial position of the isopropyl (C4″, C5″, C6″) fragment differentiates them.

conformer set. This could be related to the shape of the molecule hence to the molecular volume and to the formation of intramolecular bonds.

The $\Delta G°$ values of Table 1 show that the largest contribution to the average properties of the vapor phase renieramide at 298.15 K and in particular to its average GIAO ^{13}C chemical shifts should arise from **R1** and **R2** conformers. These, having a relative free energy gap of 0.6 kJ mol^{-1}, would contribute to given renieramide vapor phase properties by about 75%.

Figure 1 shows the overlapped **R1** and **R2** conformer structures. The main structural difference between these conformers concern, as already mentioned, the spatial arrangement of their isopropyl fragment at C3″. Both structures are characterized by intramolecular H-bonds involving the carboxyl group on C1′ and the carbonyl group on C1″, with H−O distance, **d_C1′OH−OC1″** see Table 2, of 171 pm. Two other weaker H-bond interactions ought to be pointed out for **R1** and **R2** conformers, involving the hydroxyl group at C7 and the ether oxygen bridging the centers C6 and C7′, and the amino group NH_2 linked to the NH″ fragment. The OH−O and the H″−NH_2 distances, **d_C7OH−OC7′** and **d_NH″-NH2,** as reported into Table 2 are 214 pm and ∼217 pm, respectively. The whole analysis of Table 2 shows that at least one among the H-bond interactions occurs in each conformer isolated by the *SCSA* procedure. Therefore, the intramolecular interactions should rather contribute to influence the in vacuo stabilization effects of the isolated conformers, **R1**−**R11**.

Table 2. Relevant Intramolecular Distances, d_X−Y[a]/pm, Characterizing the **R1**−**R12** Conformer Structures, Which Can Involve H-Bonds

conformer	d_NH″−NH2	d_C7OH−OC7′	d_C1′OH−OC1″
R1	216	214	171
R2	218	214	171
R3	354	215	553
R4	215	214	170
R5	215	213	364
R6	218	214	171
R7	346	215	530
R8	363	215	562
R9	218	214	171
R10	327	216	179
R11	324	216	180
R12	404	361	567

[a] X and Y, are fragments containing hydroxyl or amino groups and oxygen or nitrogen atoms, respectively. Molecule fragments follow the atom-numbering of Scheme 1.

Table 3. Intercept, Slope, and Correlation Coefficient (*a*, *b*, and *r*) Obtained by Linear Regressions Performed on the GIAO vs the Experimental ^{13}C Chemical Shift Plots of Renieramide

conformer	*a*	*b*	*r*	conformer	*a*	*b*	*r*
R1	(5)	0.9(2)	0.997(6)	**R7**	(4)	0.9(3)	0.997(6)
R2	(4)	0.9(3)	0.997(3)	**R8**	(4)	0.9(3)	0.997(7)
R3	(4)	0.9(3)	0.997(8)	**R9**	(4)	0.9(2)	0.997(1)
R4	(6)	0.9(1)	0.997(2)	**R10**	(4)	0.9(3)	0.997(1)
R5	(5)	0.9(2)	0.998(2)	**R11**	(4)	0.9(3)	0.997(0)
R6	(4)	0.9(2)	0.997(1)	**R12**[a]	(4)	0.9(3)	0.998(9)

[a] GIAO ^{13}C NMR chemical shifts of **R12**, as for the other conformers, were calculated at the B3LYP level. The slightly higher value of the **R12** correlation coefficient could be related to the genesis of this calculated conformer, which is obtained by optimizing the experimental renieramide three-dimensional NMR structure.[34]

The values of intercept, **a**, slope, **b**, and correlation coefficient, ***r***, reported in Table 3, were obtained by linear regression analyses separately performed on the calculated vs the experimental chemical shift plots, relating to each of the **R1**−**R12** conformers. Figure 2 shows the correlation plot reporting the **R1**−**R11** average GIAO ^{13}C NMR *cs* values, $\langle\delta\rangle$, vs the experimental[34] *cs* values, δ. The corresponding **a**, **b**, and ***r***, obtained by a linear regression carried out on the points of Figure 2 are (4), 0.9(2), and 0.997(8). The findings above suggest a good agreement among the theoretical structures and between these and the experimental one.[33]

Figure 2 shows that the largest deviation from the linearity in the GIAO vs the experimental *cs* plot concerns the chemical shift of the carboxylic C1′ carbon atom, being the experimental and the corresponding average value at 177.7 and 159.4 ppm, respectively. This, in our opinion, could be an evidence of interactions, occurring between renieramide and DMSO solvent molecules, which induce shielding effects on the carbon nucleus of this polar fragment. Figure 3 shows the overlapped structures of **R1** and **R12** species, the first being the most stable conformer and having the second the

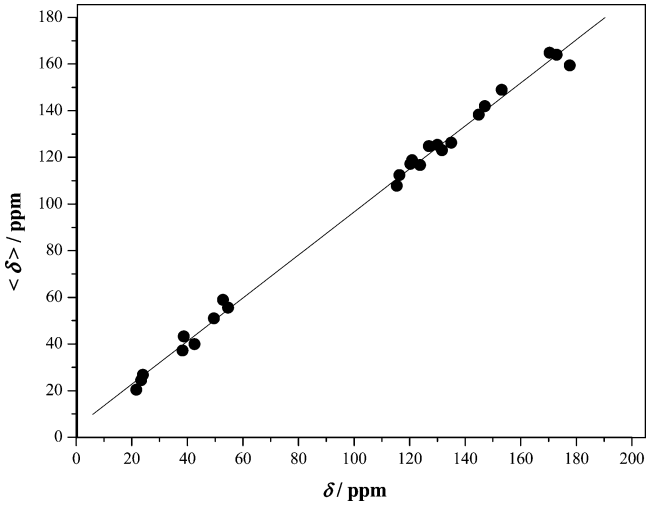


Figure 2. Correlation plot of the averaged GIAO ^{13}C NMR chemical shifts, $\langle\delta\rangle$, versus the corresponding experimental ^{13}C NMR chemical shifts, δ.[34]

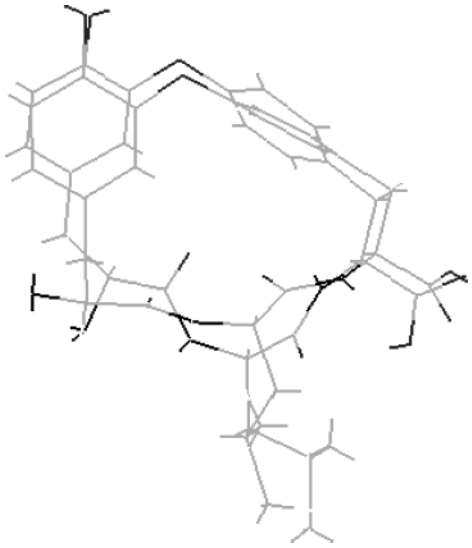


Figure 3. Overlapped structures of the conformers **R1** and **R12**: the latter differs with respect to the earlier for the absence of the intramolecular H-bond interactions individually involved in the fragments NH″−NH2, C7OH−OC7′, C1′OH−OC1″; the corresponding H-bond distances, **d_NH″-NH2**, **d_C7OH−OC7′**, **d_C1′OH−OC1″**, are reported in Table 2.

structure closest to that of the experimental one. The structure of **R12** mainly differs from that of **R1** for the absence of the H-bond interactions, involving the NH″−NH_2, C7OH−OC7′, and C1′OH−OC1″ fragments. This could induce structure stress in **R12** and hence could justify the increase of 16 kJ mol^{-1} of its in vacuo $\Delta G°$ value at 298.15 K with respect to that of the **R1** conformer.

If we hypothesize that the main conformational geometry of the renieramide in DMSO solution is close to that of the **R12** species, stabilizing solvent effects have to be supposed to justify its formation. In fact, a single point calculation performed on the **R1** and **R12** optimized structures, employing the COSMO polarized continuum model (CPCM)[41,42] of DMSO, inverted at 298.15 K their relative energy difference, which, referring to the usual zero, resulted in −7 kJ mol^{-1}.

However, in the present case, concerning the calculation of the renieramide chemical shift values, the structural differences between the in vacuo and in solution conformers do not affect the final results, as pointed out by the similar values of correlation coefficients of the different **R1**−**R12** species. Hence, the in vacuo chemical-model taken into account here for mimicking the renieramide ^{13}C NMR spectrum in DMSO may be considered consistent, whereas it seems that eventual improvements could be also obtained without the direct involvements of coordinated solvent molecules.[43]

CONCLUSION AND FUTURE DIRECTION

The homemade *SCSA* is, in the present implementation, able to capture at low- as well as at high-level of **qc** theory the conformational characteristics of very complex in vacuo systems, showing to be a good candidate for shape similarity chemical applications.[44] Indeed, slight problems regarding lack of reproducing solute−solvent interactions (such as renieramide−DMSO interactions) cannot concern the *SCSA* search engine, which, exploring a large conformational space and isolating a significant number of conformers, appears to be robust and to work properly also in the present application.

Actually, the *SCSA* and experimental renieramide NMR findings mainly differ for the absence in the latter of intramolecular H-bonds. The absence of the renieramide intramolecular H-bonds in DMSO solution could be caused by extrastabilization effects attributable to interactions between the polar renieramide fragments with DMSO molecules.

Two current development ways of the *SCSA* algorithm deserve closing remarks. The first development line concerns the automatic involvement in the conformational analysis of the medium molecules, e.g. solvent molecules, even enclosed into a given dielectric model. It is possible to consider this first approach as the "natural evolution" of the SCSA model in the direction of a more physical consistency. However, this methodology defines superimposed coordination environments raising the conformational space dimensions thus raising the already nonslight *SCSA* computational time.

The second development line involves a statistical approach, nondelineating any a priori distribution weights hence nonfixing very limited energy ranges in which the conformers, found in vacuo or in a dielectric medium, have to be singled out and their characteristics averaged. At the moment, statistics referring to the Maximum Entropy Principle[45] are taken into account. In this case, a larger conformer energy range, which has to include the "experimental" energy of the studied species, is considered. Of course, a larger number of final conformers are isolated by this approach, and, among these, there are some very distorted structures with respect to that of the most stable conformer.

It is possible to get the physical idea inherent in this second line of work, hypothesizing that the molecule-medium, e.g. solute−solvent interactions, induce molecular distortions analogous to those which are possible to find by a deep systematic in vacuo conformational search analysis. Hence, the molecular flexibility, although soundly affected by the medium presence, should not be strongly influenced by the occurrence of discrete and long-term contacts, e.g. there is no formation of lasting intermolecular solute−solvent bonds. Indeed, the preliminary results give confidence on the advantage to also develop further this second way, which,

although less physically meaning, appears very useful and much more time-saving than the first one, when peculiar local effects due to specific interactions are not present.

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