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NMR and computer modeling conformational study of *N*-benzyl,*N*-*n*-propyl (2-methyl-3-nitrophenyl)acetamide

Edwige Nicolle[1], Pascale Maldivi[2], Martine Benoit-Guyod[1], Abdel Namil[1], Max Cussac[1], Gérard Leclerc[1]*

[1] *Groupe de pharmacochimie moléculaire, Université Joseph-Fourier, Grenoble 1, UFR de pharmacie, BP 138, 38243 Meylan Cedex*
[2] *CEA, Département de recherche fondamentale sur la matière condensée, CNRS/Laboratoire de chimie de coordination (URA 1194) SESAM/CC, Centre d'études nucléaires de Grenoble, 17 Rue des Martyrs, 38054 Grenoble Cedex 9, France*

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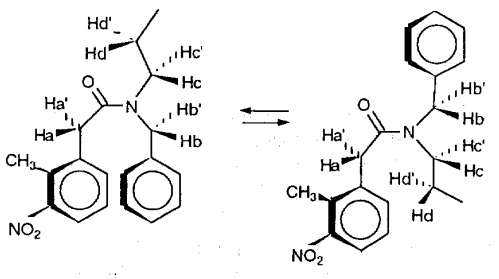
Summary – The conformation of *N*-benzyl-*N*-*n*-propyl (2-methyl-3-nitrophenyl)acetamide **1** in dimethyl sulfoxide (DMSO-d_6) or chloroform ($CDCl_3$) solution was studied using ^1H and ^{13}C NMR analysis. In solution, **1** existed as two distinct *Z* and *E* isomers, which could not be separated at laboratory temperature. Both conformations were in equivalent proportions in chloroform whereas in a polar solvant (DMSO), the conformation *Z* was more usual with the aromatic rings in a *trans* position. Major and minor rotation isomers were assigned from the ^1H and ^{13}C NMR chemical shifts determined at 293 K. Separate treatment of signals displayed by two different methylene groups gave comparable activation parameters ($\Delta G \approx 16$ kcal/mol). Conformational analysis and measurement of the rotational barrier between the *E* and *Z* conformers by molecular modeling (Sybyl program) were performed.

acetamide derivative / NMR / NOE / molecular modeling

Introduction

In the course of our studies [1] on DA_2 dopaminergic compounds designed for ophthalmologic use [2], we have synthesized *N*-benzyl-*N*-*n*-propyl (2-methyl-3-nitrophenyl)acetamide **1** (fig 1). This compound, which is a precursor for the synthesis of a phenylethylamine derivative, was obtained by acylation of *N*-benzyl-*n*-propylamine [3] with 2-methyl-3-nitrophenylacetyl chloride [4]. Amide **1** can exist as *Z* and *E* conformers, and thus we thought it would be interesting to perform conformational analysis and measure the rotational barriers between the *E* and *Z* forms by NMR and molecular modeling.



1E 1Z

Fig 1. Conformers of amide **1** (1*E* : aromatic ring in *cis* position and 1*Z* : aromatic rings in *trans* position).

Results and discussion

IR Study

Amide **1** could exist as both tautomeric forms **I** and **II** (fig 2). Tautomerism can be envisaged due to the stabilization of structure **II** by conjugation of the double bond with the 2-methyl-3-nitrophenyl ring. Infrared spectra showed $\nu C{=}O$ absorptions at 1 640 cm^{-1} (2%

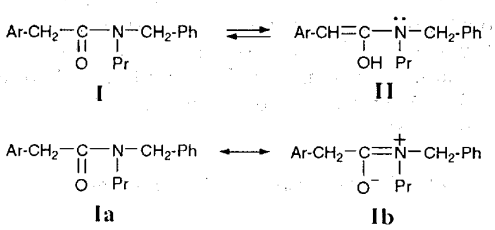


Fig 2. Tautomeric and canonical forms of amide **1**.

* Correspondence and reprints

Table I. ^{1}H NMR data for compound **1** (400 MHz) in DMSO-d_6 at 193 K (δppm).

	CH_3-CH_2	CH_3-CH_2 (Hd-Hd′)	CH_3-Ar	-CH_2-CH_2-N (Hc-Hc′)	COCH_2-Ar (Ha-Ha′)	N-CH_2-Ph (Hb-Hb′)
Min	0.81(t)	1.52(m)	2.15(s)	3.28(m)	3.90(s)	4.76(s)
Maj	0.89(t)	1.66(m)	2.24(s)	3.34(m)	4.01(s)	4.56(s)

Table II. ^{13}C NMR data for compound **1** (400 MHz) in DMSO-d_6 at 193 K (δppm).

	CH_3-CH_2	CH_3-CH_2 (Cd)	CH_3-Ar	-CH_2-CH_2-N (Cc)	COCH_2-Ar (Ca)	N-CH_2-Ph (Cb)
Min	10.02	19.24	13.24	46.31	36.67	49.40
Maj	9.98	20.02	13.26	47.50	36.43	46.67

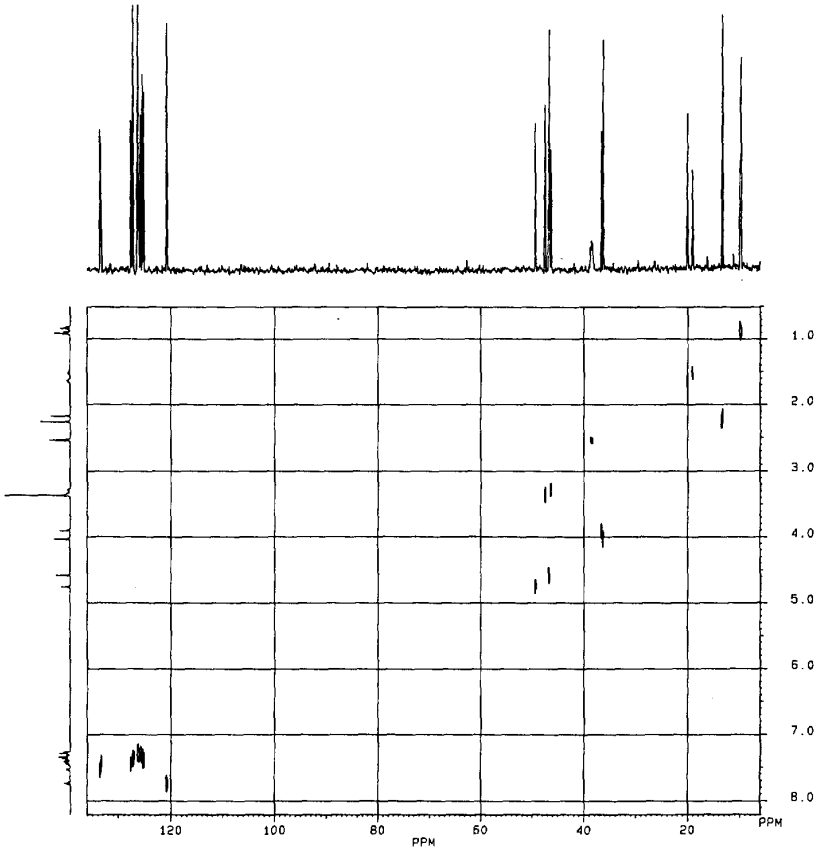


Fig 3. Inverse H-C correlation spectrum of amide **1**.

KBr) and 1 650 cm^{-1} (10% in $CHCl_3$) fitting with the carbonyl group of an amide function. However, no O-H bond was detected which would indicate the presence of the conjugated isomer **II**. We were thus able to conclude that **1** existed predominantly, if not exclusively, as **I** (**Ia** + **Ib**).

NMR study. Conformation of the amide group

^{1}H and ^{13}C NMR spectra of **1** at 400 MHz in DMSO-d_6 and $CDCl_3$ showed a splitting of each signal, which allowed us to conclude that both torsion isomers **1***E* and **1***Z* existed (or their canonical forms) (fig 2). The populations of the conformers was measured at 293 K from peak heights of the CH_3-Ar singlet and were 1:1 in $CDCl_3$ and 3:2 in DMSO-d_6.

^{1}H NMR data of **1** at 400 MHz in DMSO-d_6 are shown in table I. The comparison of chemical shifts of the two conformers revealed that the main differences occurred in the absorption of Hb-Hb′ protons ($\Delta\delta$ = 0.2 ppm), Ha-Ha′ protons ($\Delta\delta$ = 0.11 ppm), and Hc-Hc′ protons ($\Delta\delta$ = 0.06 ppm). However, none of these anisotropic effects could be used for the safe assignment of *Z* or *E* conformers, as was emphasized

by Combrisson and Roques [5]. Indeed, in addition to anisotropic phenomena, solvent effect and structural changes induced by the solvent rendered comparisons hazardous. Nevertheless, the shielding effect ($\Delta\delta$ = 0.09 ppm) observed on the CH_3-Ar group by the phenyl ring lying above the CH_3-Ar protons in the minor conformer was in agreement with the 1*E* conformation.

The interpretation of ^{1}H NMR spectra in the field of tertiary amides is not obvious [5-7]. The assignment of major and minor conformers was therefore completed by ^{13}C NMR experiments. ^{13}C-^{1}H correlations (X-H CORR), based on ^{1}H spectra, confirmed the assignments of the carbon atoms, particularly for Ca, Cb and Cc (fig 3) [8].

Chemical shifts for ^{13}C NMR are presented in table II. The main differences were observed for Cb and Cc signals which were shifted downfield of 2.73 ppm in the minor conformer for Cb, and upfield of 1.2 ppm for Cc. It is generally admitted [5-7] that the carbon *syn* to the carbonyl oxygen of an amide (Cc in 1*E* and Cb in 1*Z*) is shielded compared with the corresponding carbon in the conformational *trans* isomer. Thus, at this stage, the 1*E* conformation could be tentatively assigned to the minor isomer in DMSO. However, in certain cases opposite ^{13}C NMR results were obtained [5]. In view of the conflicting reported data, 1D and 2D NOE experiments [9] were performed to validate our proposal.

In 1D spectra, saturation of Ha-Ha′ protons pertaining to the minor conformational isomer resulted in a 12% NOE on Hb-Hb′ protons, whereas no Overhauser effect was observed between Ha-Ha′ and Hb-Hb′ protons of the major isomer.

In 2D experiments, a row in the phased mode of the Ha-Ha′ signal in both conformers (3.90 and 4.01 ppm for the minor and major conformers respectively) resulted in a strong NOE on CH_3-Ar signal. More interestingly, an Overhauser effect was observed between the Ha-Ha′ signal of the minor conformer and the Hb-Hb′ signal of the same conformer at 4.76 ppm (fig 4). Conversely, no effect was detected between these groups of protons in the major conformer. Furthermore, a row in the magnitude mode on the aromatic protons induced a dipolar interaction on the Hd-Hd′ protons of the major isomer at 1.66 ppm and no effect in the minor one.

However, 1*E* and 1*Z* are in conformational equilibrium and so we checked that the Overhauser effects observed were actually due to a saturation transfer and not to an exchange phenomenon. The mean-life time τ of each conformer was calculated from Eyring equation [10] and was estimated at 0.38 s whereas the relaxation times T_1 of methylenic protons are between 0.26 and 0.30 s. Therefore, these values ($\tau > T_1$) tally with an intermediate rate of exchange that validates the Overhauser effect observed. Consequently, NOE results that are only in agreement with the *Z* structure for the major product (and with the *E* structure for the minor one) confirmed unambiguously our previous NMR assignments.

The ^{1}H NMR study of amide **1** at various temperatures at 200 MHz showed (fig 5) a splitting of each signal corresponding to a slow exchange between 1*E* and 1*Z* at low temperature (< 343 K); a coalescence

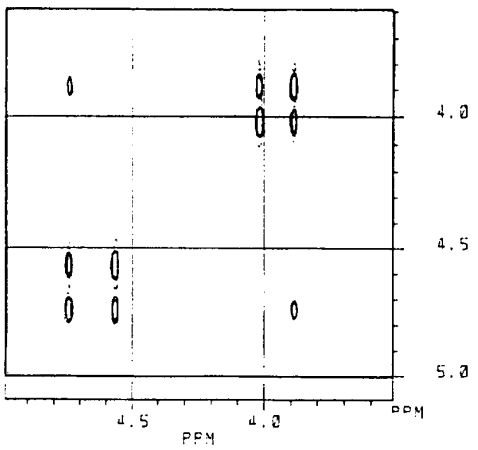


Fig 4. Partial representation of 2D NOESY spectrum of amide **1** at 193 K, in DMSO-d_6.

of the signals at 343 K; and narrow and single signals corresponding to a fast exchange at high temperature (> 343 K).

Free activation energy around the C(O)-N bond in **1** at the coalescence temperature could be calculated by the Eyring equation (table III).

Table III. Activation parameters for the restricted rotation around the amide bond obtained from ^{1}H NMR data.

Parameters	N-CH_2-Ph 1*Z*/1*E*	CO-CH_2-Ar 1*Z*/1*E*
Tc (K)	343	343
ΔS (kcal/mol/deg)	−13.38	−19
ΔH (kcal/mol)	12.31	10.55
ΔG (kcal/mol)	16.30	16.22

Separate treatment of two different signals (N-CH_2-Ph and Ar-CH_2-CO) gave close results ($\Delta G \approx$ 16 kcal/mol). The ΔG values obtained for **1** were comparable to those of most amides [11-12], but they were not high enough to allow the separation of the two conformers 1*E* and 1*Z* at room temperature.

Molecular modeling. Results and discussion

In order to explain our experimental results, we performed a detailed computer simulation of the structural properties of both conformers 1*E* and 1*Z* of the amide as well as an estimate of the rotational barrier between them. The minimized structures obtained in the two cases are represented in figure 6.

Several structural features are noteworthy, such as the quasi-parallel ($\Delta\Theta \approx 4°$) phenyl rings in the 1*E* conformation, the angle between the plane containing the nitro group and the aromatic plane ($\Delta\Theta \approx 40°$), and the slight deviation from flatness of the aromatic carbon bearing the methyl group. These two last features are most likely due to a steric constraint between the two adjacent NO_2 and methyl groups.

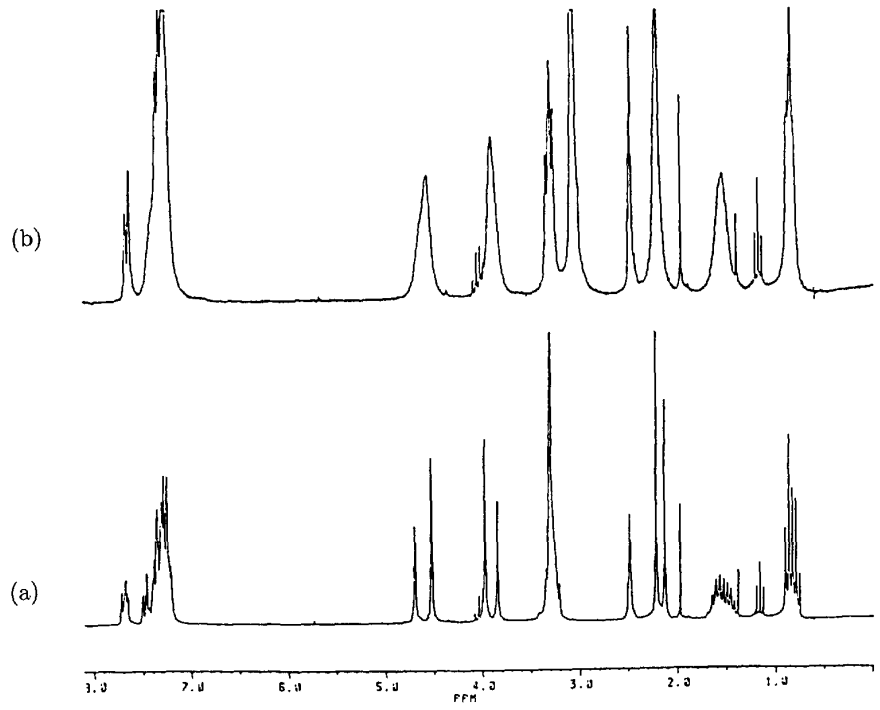


Fig 5. ^{1}H NMR spectra of amide **1** at 200 MHz in DMSO-d_6 at (a) 303 K and (b) 343 K.

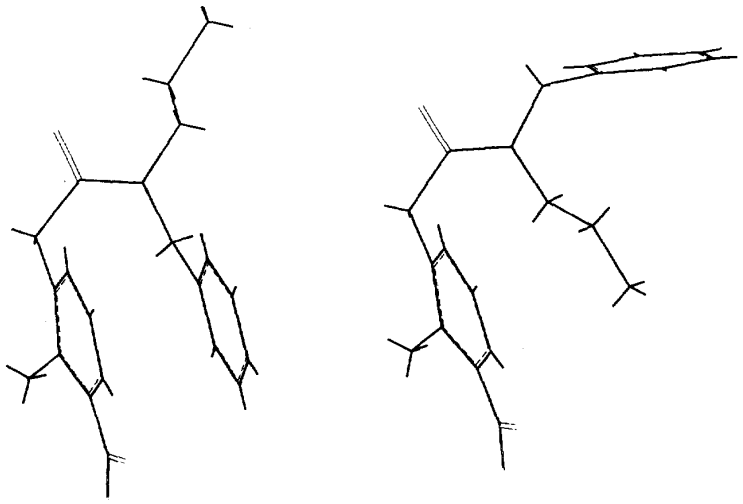


Fig 6. Optimized structures of the **1***E* (*cis*) and **1***Z* (*trans*) stereomers obtained after building and minimizing with MAXIMIN2.

The total energies found are reported in table IV, along with three important components : the deformation energy (due to bond stretching, angle bending, bond torsion and out-of-plane bending), the van der Waals interaction and the electrostatic energy. The *E* form was found to be more stable than the *Z* form, with an energy difference of 3.6 kcal/mol.

Table IV. Total energies and the three main energetic contributions found after minimization of both conformers. All values are in kcal/mol.

Isomers	Total energy	Deformation energy	Van der Waals energy	Electrostatic energy
1*E*	4.14	10.14	−9.28	0.00
1*Z*	7.72	10.70	−6.28	−0.03

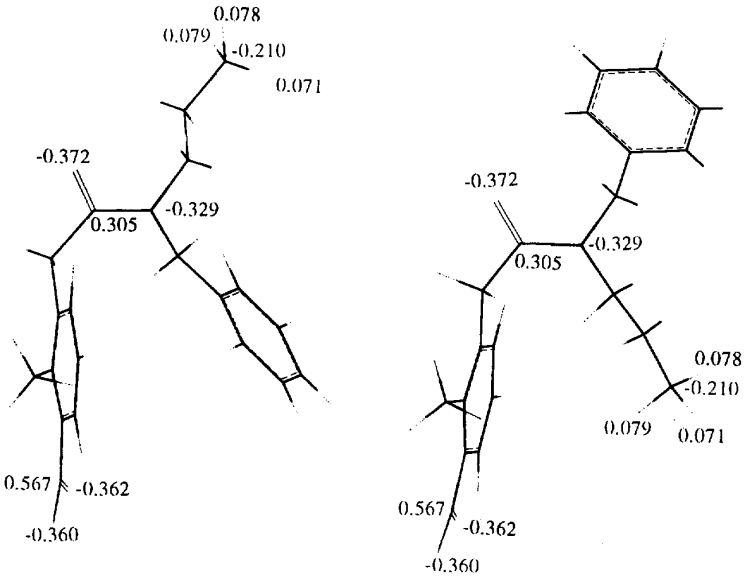


Fig 7. Partial charge distributions of the 1*E* (*cis*) and 1*Z* (*trans*). For the sake of clarity, only relevant charges calculated by AM1 method (MOPAC) are indicated. Distances between the *CO* and the CH_3 of the *N*-propyl chain were 4.5 and 5.9 Å for the *E* and *Z* conformers respectively.

One key factor to explain the better stability of the *E* form, at least in a vacuum, is certainly the existence of an interaction between the two aromatic rings. As we have seen from the minimized geometry of this conformer (fig 6), the phenyl rings are nearly parallel and in close proximity (mean distance around 3 Å). Such an arrangement may favor interactions between the two rings.

This assumption finds support in the comparison of the different energy components for each conformation (see table IV). The deformation energy and the electrostatic energy do not vary significantly between both forms, whereas the van der Waals interaction term is more important for the *E* than for the *Z* species. The discrepancy in these contributions (3 kcal/mol in favor of the *E* conformation) is nearly entirely responsible for the total energy difference (3.6 kcal/mol) between both species. Nevertheless, this interpretation is no longer valid when the compound is in solution.

However, the dipole moment of the *Z* conformer (3.8 D) calculated by the AM1 method (MOPAC) is greater than that of the *E* conformer (3.4 D) (fig 7). We can thus assume that the *Z* conformer is favored in a polar solvent such as DMSO as was observed in NMR.

Our second objective was to give a theoretical estimate of the rotational barrier between *E* and *Z* species. A systematic conformational analysis with the SEARCH procedure, taking into account six rotatable bonds, *ie* the amide bond, the three closest bonds (one connected to the carbonyl and two to the nitrogen) and the two adjacent bonds to the phenyl rings, gave no satisfactory results. This is due to the SEARCH algorithm which eliminates all conformers with bad contacts (*ie* interatomic distances < sum of the van der Waals radii). The molecule described here has three bulky substituents and presents a sterically hindered structure. Therefore a simple systematic conformation search resulted in too many conformers being discarded. For each conformation found in the search, it was necessary to relax the steric constraints by further minimization. This procedure is available in SYBYL through the GRID SEARCH module.

We have therefore performed a more detailed conformational analysis, using GRID SEARCH. Nevertheless, this algorithm is much more time- and memory-consuming than the simple SEARCH, due to the subsequent minimization of each conformer, and this precludes any conformational analysis on six rotatable bonds between 0 and 359°. Thus we have carried out a preliminary GRID SEARCH calculation by using only one rotatable bond, *ie* the amide bond, starting from the minimized structure of 1*E* described above.

The dependence of the total energy as a function of the torsion angle of the amide bond is represented in figure 8. We observe two minima, corresponding to the 1*E* and 1*Z* conformations, and two barriers, with slightly different heights, separating these two forms.

The maximum energy is 31 kcal/mol, which allows us to give a rough estimate of the rotational barrier of 27 kcal/mol. This value is a little high compared with the experimental value of 16 kcal/mol. However we must bear in mind that this value was only obtained with a minimization, without any systematic exploration of the conformational space around the maximum region. It was therefore possible to find a slightly more stable maximum conformation by rotating adjacent bonds.

To check this hypothesis, we carried out a second GRID SEARCH around the maximum region, with three possible values of the torsion angle of the amide bond (80°, 90° and 100°) and by varying the torsion angles of the other five adjacent bonds (described above).

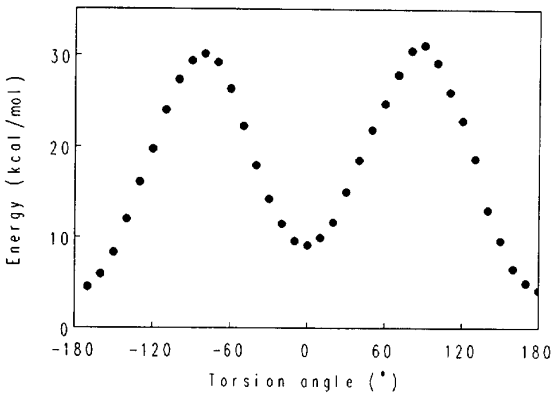


Fig 8. Conformational energy diagram for the torsion angle of the amide bond, as determined with the GRID SEARCH procedure.

This procedure gave a more significant value of the maximum energy, *eg*, 25.5 kcal/mol. The rotational barrier of 21.4 kcal/mol obtained is in fair agreement with the experimental value (16 kcal/mol).

Conclusion

In conclusion, we can say that the amide **1** exists preferentially as the **1***Z* conformer (*Z*/*E* = 3:2) in DMSO. This observation could probably be explained both by the solvent effect and electrostatic interactions. Conversely, by computer modeling the **1***E* conformer appears more stable than the **1***Z* conformer *in vacuo*. The van der Waals interactions between the two parallel aromatic rings can probably account for this result.

Experimental section

IR were recorded on a Philips Pye Unicam SP3-100 and NMR spectra were performed on a Bruker AC 200 and a Bruker AM 400. Chemical shifts are given in ppm with internal reference. Standard parameters were used : ^{1}H (200 MHz), ^{13}C (75 MHz).

Molecular modeling

All molecular modeling procedures were performed with the SYBYL 6.0 software package [13], running on a Silicon Graphics Iris 4D-20 workstation.

Conformers **1***E* and **1***Z* were first built independently and then minimized. All energy calculations were based on the Tripos force field [14] and included the electrostatic potential. Charges were calculated by the Gasteiger-Hückel method available in SYBYL. The dielectric constant was taken as 40 (approximate value for DMSO).

Geometry minimizations were carried out with the MAXIMIN2 module of SYBYL, and the Powell minimizer. We used the SEARCH module for systematic conformational analysis, as well as the GRID SEARCH module which combines a conformational analysis and a geometry optimization by MAXIMIN2 of each conformer found.

In the SEARCH procedure described in the text, six rotatable bonds were varied, all between 0° and 359°, with an increment of 10° for the amide bond and 30° for the other bonds. The other parameters for the SEARCH algorithm (such as scaling factors) were the default values provided by SYBYL.

The first GRID SEARCH was run by rotating the amide bond with an increment of 10° between 0 and 359°. The minimization was performed with a maximum of 400 iterations, with the Powell minimizer, taking into account the electrostatic energy (see above for details on this last term). In the second GRID SEARCH, torsion angles of the five adjacent bonds were varied in increments of 30°, in the usual 0-359° angle range. The minimization was carried out as previously.

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