Searching Conformational Space in Flexible Molecules Using NOEs and Molecular Modeling

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A routine method for searching the conformational space of small flexible chain molecules in solution is presented. This involves the quantitative use of NOEs aided by molecular modeling. The *l* diastereomer of model compound (±)-*N*-(1-phenylethyl)-2-(ethylsulfinyl)-1-propenylamine (**1**) was studied in $CDCl_3$ and pyridine-d_5 solutions using ^1H NMR. It was shown to undergo self-association in $CDCl_3$. Comparison of calculated and observed steady-state NOE values and the kinetics of NOEs shows that the compound has the *E* geometry around the double bond. The conformational space accessible to the sulfinyl enamine was searched using the program PCMODEL with the help of ancillary programs and programs to calculate theoretical NOEs. These conformations could be limited substantially by using coupling constants and by comparing observed NOEs to those calculated by assuming conformational averaging about a small number of energy minima.

Introduction

Assignment of *E* or *Z* geometry to highly substituted ethylenes is not a trivial exercise. This was the case for earlier studies on β-sulfinyl enamines, for which an erroneous *Z* configuration was assigned,[1] a point of controversy.[2] Most of the published β-sulfinyl enamines exist as only one isomer in solution, in equilibrium with an imine form.[1,3–5] Recently, two compounds that exist in solution in both enamine forms have been synthesized.[6,7] One of these, sulfoxide **2**, *t*-BuSOCH=CHNH-*t*-Bu, was used as a model compound for establishing the influence of solvent, temperature, concentration, and water content on the amine proton chemical shift values, δ(NH), in both *Z* and *E* enamine isomers.[7] Furthermore, intermolecular NOEs between the *Z* and *E* isomers indicated self-association in $CDCl_3$ solution.

Bearing in mind the uncertainties of coupling constants[8–10] and δ(NH)[7] as probes for assigning *Z*/*E* stereochemistry, we have turned our attention to the ^1H NOE to aid in the stereochemical assignments of di-[7] and trisubstituted ethylenes of this type.

With respect to the remaining conformational details in this class of compounds, there are several contributions in the recent literature concerning the search for quantitative rotamer populations in flexible small molecules using NMR observables[11–14] as pioneered in a classic work by Schirmer *et al.*[15] This is a key problem in all stereochemical elucidations. The combined use of spin–spin coupling constants and nuclear Overhauser effects seems to give the most reliable results,[11] providing that enough spin–spin coupling constants are available, which can usually be achieved for biological molecules like amino acids[11] or sugars.[14] However, if spin–spin coupling constant data are lacking or ambiguous, as in substituted β-sulfinyl enamines, NOE measurements, aided by molecular modeling, seem to be the method of choice. Such an approach is presented in the present study.

The aim of this work was to establish the usefulness of ^1H NOE measurements for stereochemical elucidations in this class of compounds, in view of the large intermolecular NOEs observed in the analogue **2**.[7] This is attempted by means of comparisons of experimental and theoretical NOEs with the aid of molecular modeling using PCMODEL.[16] Sulfoxide **1**, $C_2H_5SOC(CH_3)$=CH-

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(1) (a) Kozerski, L.; Kawęcki, R.; Bednarek, E. *Magn. Reson. Chem.* **1987**, *25*, 712. (b) Kozerski, L.; Kawęcki, R. *Phosphorus, Sulfur Silicon Relat. Elem.* **1991**, *59*, 201.

(2) The *E* configuration was postulated by: Kamieńska-Trela, K. *Magn. Reson. Chem.* **1990**, *28*, 1070.

(3) (a) Annunziata, R.; Cinquini, M.; Restelli, A.; Cozzi, F. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1183. (b) McMullen, C. H.; Stirling, C. J. M. *J. Chem. Soc. B* **1966**, 1217.

(4) Katano, K.; Atsumi, K.; Nishihata, K.; Kai, F.; Akita, E.; Niida, T. *Chem. Pharm. Bull.* **1982**, *30*, 3054.

(5) Knorr, R.; Weiss, A.; Löw, P.; Rapple, E. *Chem. Ber.* **1980**, *113*, 2462.

(6) Baldenius, K.-U.; Kagan, H. B. *Tetrahedron: Asymmetry* **1990**, *1*, 597.

(7) Kozerski, L.; Kawęcki, R.; Hansen, P. E. *Magn. Reson. Chem.* **1994**, *32*, 517.

(8) Diz, A. C.; Giribet, C. G.; Ruiz de Azua, M. C.; Contreras, R. H.; Aucar, G. A. *Magn. Reson. Chem.* **1987**, *25*, 883.

(9) Mitchell, T. N.; Heesche-Wagner, K.; Belt, H.-J. *Magn. Reson. Chem.* **1991**, *29*, 78.

(10) Koole, N. J.; de Bie, M. J. A.; Hansen, P. E. *Org. Magn. Reson.* **1984**, *22*, 146.

(11) Džakula, Ž.; Edison, A. S.; Westler, W. M.; Markley, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 6200.

(12) Reggelin, M.; Hoffmann, H.; Köck, M.; Mierke, D. F. *J. Am. Chem. Soc.* **1992**, *114*, 3272.

(13) Martin, J. T.; Norrby, P.-O.; Åkermark, B. *J. Org. Chem.* **1993**, *58*, 1400.

(14) Poppe, L. *J. Am. Chem. Soc.* **1993**, *115*, 8421.

(15) Schirmer, R. E.; Davis, J. P.; Noggle, J. H.; Hart, P. A. *J. Am. Chem. Soc.* **1972**, *94*, 2561.

(16) *PCMODEL, Molecular-modeling software*; Serena software: P.O. Box 3076, Bloomington, IN 47402-3076.

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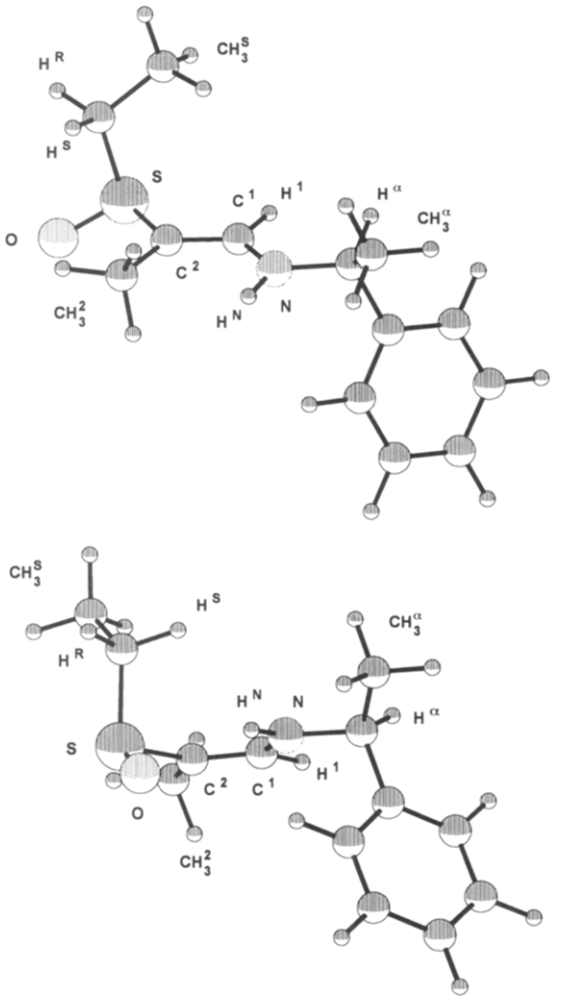

Figure 1. Lowest energy conformations of the (top) *E* and (bottom) *Z* isomers calculated by PCMODEL.

NHCH(CH_3)Ph, was chosen for a study; its low-energy conformations in the *Z* and *E* forms are shown in Figure 1.

Methods

The method of stereochemical elucidation used in this work is based on searching conformational space around the global minimum using force field calculations. The program MMXCOMP[17] is designed for analyzing this conformational space, *i.e.* finding the unique conformations and grouping them into families of conformations having populations according to the Boltzmann distribution. It is clear that PCMODEL calculations are done for the molecules in a vacuum and that solvent interactions may perturb the energetics to the extent of making conformations unfavorable in the gas phase favorable in solution. That is the reason why we use coupling constraints as an additional filter where possible. The averaging of conformations is checked by comparison of experimentally available vicinal H,H spin−spin coupling constants with calculated average values using a Karplus type equation developed recently by Imai and Osawa.[18] The "averaged" molecule is used to calculate theoretical steady-state NOE values using the program NOE[19] by taking $\langle r^{-6} \rangle$ averages of all internuclear distances. These values are compared with the experimental ones using an RMS factor, defined as

$$\mathrm{RMS} = \{\sum(\mathrm{NOE}_{\mathrm{calc}} - \mathrm{NOE}_{\mathrm{obs}})^2 / \sum(\mathrm{NOE}_{\mathrm{obs}})^2\}^{1/2}$$

in a manner similar to its use in crystallography.[20] The scaling of NOE values is accomplished by introducing an external relaxation parameter ϱ^*.[15] This parameter is adjusted so that theoretical and experimental values agree for the strongest NOEs, usually found in geminal protons used as a reference distance. Steady-state NOEs and the buildup of NOEs for single conformations were calculated by the program BUILDUP.[21] Both programs use input geometry defined by Cartesian coordinates. Twenty-five spins in 24 conformations can be treated by the program NOE which also calculates any torsion angle in a molecule and vicinal proton−proton or proton−carbon spin−spin coupling constants along a required pathway. The average molecule is constructed using a Boltzmann distribution of computed conformers (the internuclear distances r_{ij}^{-6} are weighted). Both BUILDUP and NOE calculate NOEs based on the Solomon equations; BUILDUP uses a numerical solution, while NOE uses a relaxation matrix approach. Both programs can use explicitly defined correlation times, spectrometer frequencies, ϱ^*, and NH exchange rates. NOEs involving magnetically equivalent protons are obtained by summing the NOE to each equivalent group of protons. The program BUILDUP calculates relaxation rates involving methyl groups using the equations given in refs 22 and 23. The two programs give very similar results. They are available by application to the authors.

Results and Discussion

Relaxation Time Measurements. ^{1}H T_1 values in $CDCl_3$ solution, cited in Table 1, served as a guide to set the timing of the steady-state and TOE experiments.

Table 1 presents ^{13}C T_1 data for **1** with the calculated values of the correlation times τ_c in $CDCl_3$ and pyridine-d_5 solutions. The relaxation times (compared as NT_1 values) are similar for the protonated carbons $\mathbf{C}H_2S$, $\mathbf{C}^1$, $\mathbf{C}^\alpha$, and $C^\alpha\mathbf{C}H_3$ but are much longer for the $SCH_2\mathbf{C}H_3$ and $C^2\mathbf{C}H_3$ methyl groups. The differences in the two solvents can be attributed to the change in temperature of the measurements and the solvent viscosity. It seems reasonable to assume that the backbone of the molecule is characterized by a single correlation time of about 0.3×10^{-10} s in $CDCl_3$ and 0.16×10^{-10} s in pyridine-d_5 solutions. This means that, even at −20 °C, **1** still tumbles fast enough that its relaxation is described by the extreme narrowing limit formalism, with $\omega\tau_c \approx 0.01$. Furthermore, the correlation time of the CH_2 carbon is similar to the values for the other carbon atoms in the skeleton. This allows us to use the nonequivalent H^R and H^S protons as a reference geminal methylene group in

(17) Pupek, K.; Kozerski, L.; Krajewski, P. *Program MMXCOMP*; Institute of Organic Chemistry, Polish Academy of Sciences: Warszawa, Poland, 1993.
(18) Imai, K.; Osawa, E. *Magn. Reson. Chem.* **1990**, *28*, 668.
(19) Kozerski, L.; Gluziński, P.; Krajewski, P. Program NOE; Institute of Organic Chemistry, Polish Academy of Sciences: Warszawa, Poland, 1993.
(20) (a) Edmondson, S. P. *J. Magn. Reson.* **1992**, *98*, 283. (b) Gonzales, C.; Rullmann, J. A. C.; Bonvin, A. M. J. J.; Boelens, R.; Kaptein, R. *J. Magn. Reson.* **1991**, *91*, 655.
(21) Williamson, M. P. *Magn. Reson. Chem.* **1987**, *25*, 356.
(22) Tropp, J. *J. Chem. Phys.* **1980**, *72*, 6035.
(23) Koning, T. M. G.; Boelens, R.; Kaptein, R. *J. Magn. Reson.* **1990**, *90*, 111.

Table 1. ^1H and ^{13}C T_1 Relaxation Times (NT_1) and Correlation Times for 1

	$SCCH_3$	SCH_2	C^2CH_3	C^1H	NH	$C^\alpha H$	$C^\alpha CH_3$	solvent
^1H								
T_1 (s)	0.8	0.6	1.0	1.7	0.8	1.2	0.4	$CDCl_3$[a]
^{13}C								
NT_1 (s)	12.5	1.1	4.0	0.9		1.3	1.1	
τ_c ($\times 10^{-10}$ s)	0.023	0.296	0.077	0.314		0.253	0.270	
NT_1 (s)	13.5	2.4	9.6	1.4		1.9	3.6	C_5D_5N[b]
τ_c ($\times 10^{-10}$ s)	0.021	0.120	0.030	0.208		0.153	0.081	

[a] Temperature: −20 °C; 3 mg/0.6 mL of $CDCl_3$. [b] Temperature: 22 °C; 10 mg/0.6 mL of pyridine-d_5.

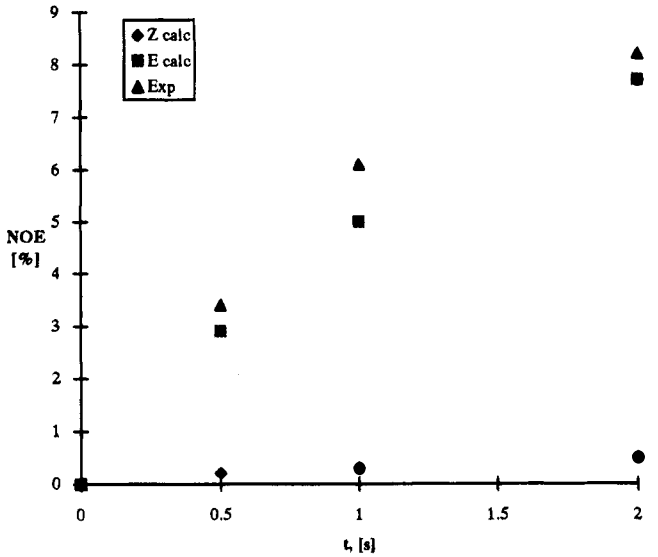

Figure 2. Experimental (in $CDCl_3$) and theoretical NOE buildup for E and Z isomers for NH{C^2CH_3}. Note that the 0,0 point was also included as a valid calculated data point.

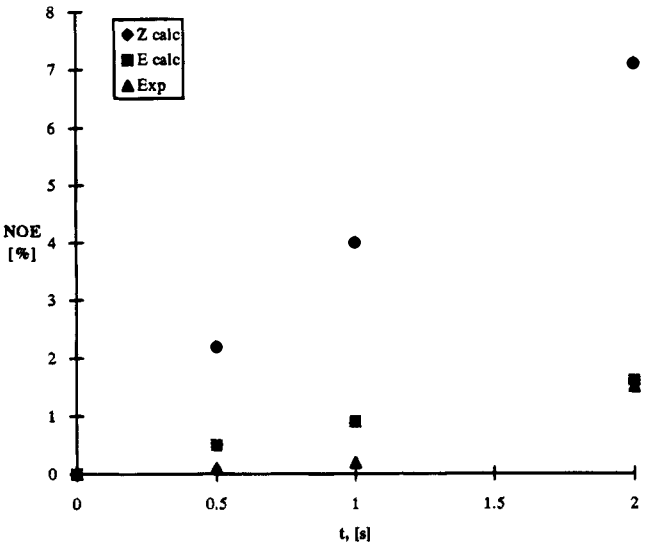

Figure 3. Experimental (in $CDCl_3$) and theoretical NOE buildup for E and Z isomers for H^1{C^2CH_3}. Note that the 0,0 point was included as a valid calculated data point.

NOE-based calculations of the intramolecular distances (*vide infra*). The correlation time for SCH_2CH_3 and C^2CH_3 methyl groups is 1 order of magnitude shorter (*ca.* 0.2 and 0.8 × 10^{-11} s in $CDCl_3$) due to a contribution from the internal rotation correlation time specific for each methyl group.

In our earlier study of **2**,[7] we observed a correlation time for the E isomer, 1 order of magnitude longer than the Z isomer (10^{-9} and 10^{-10} s, respectively). Taken together with temperature-dependent δ(NH) changes and large intermolecular NOEs, it was suggested that these compounds can associate in $CDCl_3$ solution under our experimental conditions, with the Z isomer of **2** forming dimers but the E isomer forming larger aggregates. The correlation times seen for **1**, and its relatively low δ(NH) temperature dependence (+2.3 ppb/deg), imply possible dimerization of **1** in $CDCl_3$ solution. Intermolecular NOEs (*vide infra*) support this suggestion.

Kinetic NOE Measurements. Kinetic NOEs (also called truncated driven NOEs or TOEs)[24] are less influenced by intermolecular self-association and by distant protons than are steady-state NOEs because, at short NOE buildup times, three-spin effects (also known as spin diffusion) are less significant. We therefore measured kinetic effects to determine the E/Z geometry of **1**, as shown in Figures 2–3. The most diagnostic NOEs are H^1{C^2CH_3} and NH{C^2CH_3} which are documented in Table 2. The CH^RH^S geminal distance was used as a reference distance in calculating the cross-relaxation, and the buildup curves were calculated for both isomers using the lowest energy MMX conformer for each isomer. The results clearly demonstrate that **1** is E.

Table 2. Experimental TOEs (Exp) and TOEs Calculated for E and Z Isomers

	NH{C^2CH_3}			H^1{C^2CH_3}		
t (s)	Exp	E	Z	Exp	E	Z
0.5	3.4	2.9	0.2	0.1	0.5	2.2
1.0	6.1	5.0	0.3	0.2	0.9	4.0
2.0	8.2	7.7	0.5	1.5	1.6	7.1
15.0	10.3	10.4	0.5	2.8	3.3	10.0
a	1.94	2.03	3.15	2.81	2.78	2.09

[a] Apparent internuclear distance from a best fit to the two-spin exponential equation (4.5) of ref 24. The fit was calibrated using a measured cross-relaxation rate of 0.155 s^{-1} for the methylene pair, which was assumed to have a separation of 1.75 Å. Experimental TOE values refer to a $CDCl_3$ solution (5 mg/0.6 mL) measured at −20 °C. Steady-state experimental NOEs (15 s irradiation) are the averaged values from two independent experiments.

General Strategy for Conformational Analysis of 1. The conformation of **1** (hereafter assumed to be E) can be described using four torsional angles (Figure 4). Limitations to angles ϕ and χ can be obtained from three-bond coupling constants. Further limits to these angles, and limits to the other angles ω and ψ, can be obtained using NOEs. However, NOEs and coupling constants do not provide enough parameters to fully define the conformations. We have therefore used PCMODEL calculations[16] to help to determine the energetically accessible conformations.

(a) Molecular Modeling by PCMODEL. The energies of the nine lowest energy E families are shown in Table 3. These are the lowest energy members from each

(24) Neuhaus, D.; Williamson, M. P. *The Nuclear Overhauser Effect in Structural and Conformational Analysis;* VCH: New York, 1989.

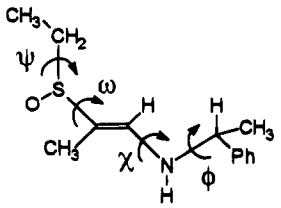

Figure 4. *E* isomer of **1** showing the freely rotatable backbone torsion angles.

Table 3. Families of Low-Energy Conformations of 1 Generated by the Global Minimization Procedure of PCMODEL[a] Using MMXCOMP[b]

family	lowest energy (kcal/mol)	relative population (%)	RMS factor[c] $CDCl_3$	RMS factor[c] pyridine-d_5[d]
1	9.48	12.60	0.263	0.253
2	9.56	10.99	0.269	0.259
3	9.59	10.43	0.281	0.269
4	9.71	8.49	0.288	0.251
5	9.77	7.66	0.370	0.414
6	9.77	7.66	0.288	0.231
7	9.85	6.68	0.361	0.366
8	9.86	6.56	0.298	0.254
9	10.01	5.01	0.350	0.330
Boltzmann[e]			0.275	0.199

[a] All single bonds were rotated in a conformational search around the global energy minimum. [b] Only the lowest energy member of each family is cited. Its energy was used for the calculation of the relative population of the conformer according to the Boltzmann distribution. [c] This is a measure of the fit of theoretical to experimental NOEs (see text). [d] The NOEs between the C^1H and NH protons were excluded from the calculation of the RMS factor due to the presence of scalar relaxation of the second kind between the two spins (see Table 4A in the Supplementary Material). [e] The RMS factor is for a conformational mixture of all families.

family which contributes more than 5% to the total conformation. There were 19 families within 4.5 kcal mol^{-1} of the lowest energy structure. The torsion angles of the nine lowest energy families are shown in Figure 5. The torsion angles seen for angle ϕ (as defined in Figure 4) are limited, and those for angle χ are extremely limited. By contrast, angle ω is more variable (adopting two orientations 180° apart with equal likelihood), and angle Ψ is extremely variable. The energy calculations therefore predict considerable conformational freedom in the sulfinyl part of the molecule. MNDO calculations[25] give similar results.

(b) Three-Bond Spin–Spin Coupling Constants. The value of $^3J(H^1, NH) = 13$ Hz is extreme and indicates a rather rigid transoid disposition of the coupled protons. This is in agreement with the energy calculations (Figure 5), and is also confirmed by the X-ray structure of **1**[26] and other β-sulfinyl enamines,[27,28] which have a short C^1–N bond length of 1.35 Å, equal in length to a double bond. The rigidity of the bond is also confirmed by the high barrier to internal rotation around C^1–N of 12.5 kcal mol^{-1} (52.4 kJ mol^{-1}) established in $CH_3SOC^2H{=}C^1HN(CH_3)_2$[29] and comparable with the same barrier found in enaminones.

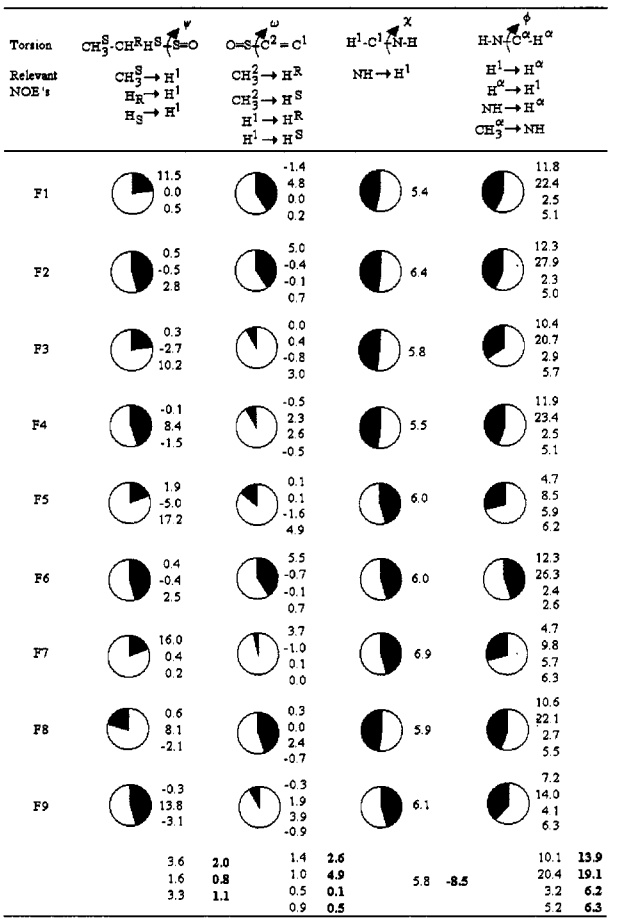

Figure 5. Torsion angles of the nine lowest energy families (1–9) shown as four circles for the four torsion angles shown in Figure 4. The angles run from 0° at the top clockwise to +180° at the bottom and then from −180 to 0°. The figure also shows the theoretical NOEs relevant to each torsion angle calculated by program NOE using external relaxation ϱ^* to fit the experimental values in pyridine-d_5 solution. At the bottom, calculated values, averaged for nine families, and experimental values in pyridine-d_5 solution (in bold) are shown. See Table 4A in the supplementary material for the complete set of NOEs.

Torsion angle ϕ gives rise to a spin–spin coupling constant between NH and $C^\alpha H$ of 5.5 Hz. Using a Karplus equation for the peptide $HN{-}C^\alpha H$,[30,31] this corresponds to angles in the range ±45 and ±160°. MMX calculations show that conformations characterized by a small torsion angle (*ca.* −30 to −60° or +30 to +60°) have an energy higher by at least 1 kcal mol^{-1} than the ones cited in Table 3. Therefore, it is likely that only conformations in the region of ±160° contribute significantly. Although conformations around −160° predominate (see Figure 5), conformations around +160° have comparable energy. More detailed analysis of this angle requires NOE constraints, which are presented next.

(c) Steady-State NOE Results. Table 4 presents experimental steady-state NOE results for **1**. Because the conformation of **1** is defined by only four torsion angles, it is possible to calculate expected NOEs for all likely accessible conformers and compare these with

(25) Barański, A.; Kozerski, L.; Kawęcki, R. Unpublished results.
(26) Urbańczyk-Lipkowska, Z.; Krajewski, J. W.; Gluziński, P.; Kawęcki, R.; Kozerski, L.; Andreetti, G. D.; Bocelli, G. *J. Mol. Struct.* **1988**, *172*, 309.
(27) Kozerski, L.; Kawęcki, R.; Urbańczyk-Lipkowska, Z.; Krajewski, J. W.; Gluziński, P. *J. Crystallogr. Spectrosc. Res.* **1991**, *21*, 1.
(28) Urbańczyk-Lipkowska, Z.; Krajewski, J. W.; Gluziński, P.; Kawęcki, R.; Kozerski, L. *J. Crystallogr. Spectrosc. Res.* **1988**, *18*, 609.
(29) Kawęcki, R. Ph.D. Thesis, Institute of Organic Chemistry, Polish Academy of Sciences, Warszawa, Poland, 1990.
(30) Govil, G.; Hosur, R. V. In *Conformations of Biological Molecules, NMR, Basic Principles and Progress*; Diehl, P., Fluck, E., Kosfeld, R., Eds.; Springer Verlag: Berlin, 1982; Vol. 20.
(31) Bystrov, V. F.; Arseniev, A. S.; Gavrilov Yu. D. *J. Magn. Reson.* **1978**, *30*, 151.

Table 4. Experimental Steady-State NOE Values (in brackets) and NOEs Calculated for the Boltzmann Distribution of Nine Families Using Program NOE[a]

	calculated and experimental NOEs (%)							
irrdn	H^1	H^α	NH	H^R	H^S	$CH_3{}^2$	$CH_3{}^\alpha$	$CH_3{}^S$
H^1	–	6.0	1.1	0.4	0.7	0.1	0.0	0.1
		(7.5)	(1.2)	(−0.8)	(0.1)	(0.2)	(0.0)	(0.0)
H^α	8.6	–	1.3	0.0	0.0	0.0	1.4	0.0
	(13.4)		(−1.7)	(−2.6)	(−1.7)	(−1.1)	(0.5)	(−1.4)
NH	2.6	2.1	–	0.0	0.0	1.9	0.9	0.0
	(3.6)	(3.8)		(−0.6)	(1.0)	(5.0)	(3.6)	(1.3)
H^R	0.8	0.0	0.0	–	26.2	0.2	0.0	1.0
	(−2.3)	(−0.5)	(−0.4)		(25.7)	(−0.6)	(−0.7)	(0.8)
H^S	1.5	−0.6	0.0	26.3	–	0.1	0.0	1.0
	(0.6)	(0.2)	(2.4)	(28.6)		(2.4)	(1.0)	(1.9)
$CH_3{}^2$	1.2	0.0	10.4	1.1	0.9	–	0.0	0.3
	(2.8)	(1.9)	(10.3)	(2.6)	(5.9)		(2.2)	(2.2)
$CH_3{}^\alpha$	−0.4	12.2	5.0	0.0	0.0	0.0	–	0.0
	(1.6)	(14.3)	(8.3)	(−0.9)	(0.3)	(0.5)		(0.4)
$CH_3{}^S$	1.6	0.0	0.1	6.1	6.0	0.3	0.0	–
	(0.6)	(0.8)	(0.6)	(4.6)	(4.4)	(0.7)	(0.7)	

[a] The values are cited for the $CDCl_3$ (3 mg of 1/0.6 mL, −20 °C) solution. For the details of the sample composition, see the Experimental Section. The experimental values are the average of two measurements on two different samples of the same concentration of **1** and added sulfoxide. The mean standard deviation is 0.8% as calculated for 39 experimental points.

experimental results. This is a more flexible approach than that typically adopted for biological macromolecules, where, because of the greater complexity of the problem, it is normally assumed that all NOEs are consistent with a single conformer and can therefore be used as distance constraints on the structure. In the present case, it is very likely that the structure is flexible, and such an approach is therefore not appropriate. Some of the expected NOEs for each of the low-energy families are shown in Figure 5. In Tables 4 and 4A (4A in the Supplementary Material), the experimental NOEs found in the two studied solvents are compared with the theoretical NOEs expected from a Boltzmann distribution of the nine lowest energy conformations. RMS factors,[20] describing the level of agreement between experimental and theoretical NOEs, are presented in Table 3. In pyridine-d_5 solution, the agreement is improved slightly by using the complete Boltzmann distribution rather than only the lowest energy structure, but not in $CDCl_3$.

From Figure 5, it can be seen that a conformation of angle ϕ of around +160° rotates the $C^\alpha CH_3$ away from the NH proton and produces a theoretical NOE between them much lower than that observed. It is therefore possible to conclude that the +160° orientation is not populated to a great extent, as also suggested by the MMX calculations.

An inspection of Figure 5 permits some conclusions to be made about the sulfinyl part of the molecule. Changes in the torsion angles ψ and ω produce different theoretical NOEs between the ethyl protons and C^2-Me and C^1-H. The experimental NOE values are much better reproduced by a Boltzmann average of nine conformations than by any single discrete conformation. It is therefore likely that the sulfinyl part of the molecule can access several conformers with high probability, as implied by the energy calculations.

In order to obtain agreement between experimental and theoretical NOEs, the value for the external relaxation rate ϱ^* (*i.e.* the relaxation rate caused by relaxation processes other than intramolecular dipole−dipole) was adjusted. Best agreement was found using a rather large value for ϱ^* of 0.1−0.2 s^{-1} in $CDCl_3$ solution. This is

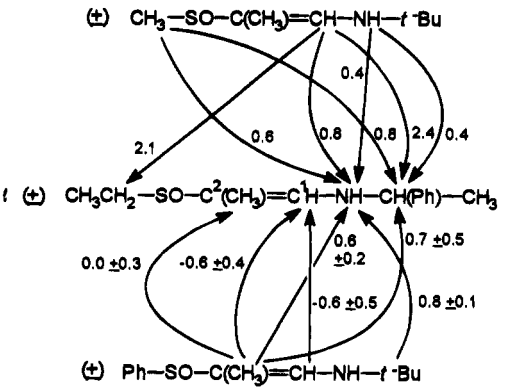

Figure 6. Intermolecular steady-state NOEs for 1:1 molar ratio mixtures of **1** with the β-sulfinyl enamines shown, in $CDCl_3$ at −20 °C. For the sample composition and concentration, see the Experimental Section. The standard deviations of NOE measurements in $CDCl_3$ solution are also shown.

larger than the value of about 0.05 s^{-1} expected for a degassed sample. The value of 0.05 s^{-1} was used to fit the experimental and theoretical NOEs in pyridine-d_5 solution. We note that an increase in ϱ^* in $CDCl_3$ can be caused by dimerization since this provides increased intermolecular relaxation. Some aggregation process was anticipated, on the basis of the correlation time measurements described above. The nature of the aggregation was investigated further by conducting NOE measurements of **1** in the presence of equimolar amounts of the structurally similar sulfinyl enamines $CH_3SOC(CH_3)$=CHNH-*t*-Bu and $PhSOC(CH_3)$=CHNH-*t*-Bu (Figure 6). These NOEs provide an indication of the extent and specificity of intermolecular relaxation in **1** and therefore also of the possible errors in measured NOE intensities in **1** due to intermolecular effects. In agreement with the correlation time results, the interaction appears to be less significant than that previously observed for **2**,[7] but some are comparable to values found by Pirkle and Pochapsky in a strong charge-transfer complex.[32] In summary, the smaller external relaxation, shorter correlation time, and larger NOEs found for the pyridine-d_5 solution, as compared with the $CDCl_3$ solution, taken together suggest a monomeric structure of **1** in pyridine-d_5 but significant aggregation in $CDCl_3$.

Conclusions

Both kinetic and steady-state NOE results show that the *l* diastereomer of compound (±)-**1** is *E* in $CDCl_3$ and pyridine-d_5 solutions. This revises the erroneous conclusion on its geometry put forward earlier.[26] NOEs, coupling constants, and energy calculations using PCMODEL show that the conformations about the C^1−N bond and N−CH bond in the enamine part of the molecule (C=$C^1HNHCH(CH_3)Ph$) are very restricted, with a single conformational minimum. By contrast, the sulfinyl part is conformationally mobile. In $CDCl_3$, **1** self-associates. This leads to a decrease in intramolecular NOEs, as well as alterations in amine proton chemical shifts. This may be the explanation for previous difficulties in assigning the geometry of single enamine tautomers in solution.[1,3,27,28]

(32) Pirkle, W. H.; Pochapsky, T. C. *J. Am. Chem. Soc.* **1986**, *108*, 5627.

Experimental Section

The synthesis of the studied β-sulfinyl enamines has been published;[33] crystalline compounds were dissolved in appropriate solvents. Compound **1** was studied as a single racemic *l* diastereoisomer of known X-ray structure.[26] All NOE measurements were taken on samples degassed immediately after dissolving in hot $CDCl_3$, by the freeze–thaw technique, and closed under argon. After being degassed, the samples run in $CDCl_3$ were immediately transferred to the NMR probe of an AM-500 Bruker spectrometer, prechilled to −20 °C. This procedure froze out NH exchange and reduced the buildup of imines and the other diastereoisomer (±)-*ul*. The experiments in pyridine-d_5 were run at room temperature. Steady-state NOE measurements were run on a Bruker AM-500 spectrometer using a routine program for multiplet irradiation. The timing of the experiment was as follows: 15 s irradiation time, 6 s acquisition time, and 1 s relaxation time. The irradiating power was low, and good selectivity was achieved. The saturation factor for multiplets was on the order of 0.6–0.8. Line broadening (1 Hz) during FID processing was used, and the limits of integration for each signal were kept constant for all irradiation lists. The fractional NOEs were calculated using absorption spectra scaled against the reference spectrum and were corrected for different saturation factors. Usually eight averaging cycles, each of eight scans preceded by two dummy scans, were performed. The TOE spectra were acquired in the same manner, except that the relaxation delay was set to 15 s and irradiation times were shorter. Calculations on this molecule using the observed correlation times show that at least 98% of the magnetization should have been recovered by this time. The experiments were repeated two or three times on different samples of the same concentration, or on the same sample at room temperature in the pyridine-d_5 solution, to check the reproducibility of the NOE. The samples examined in $CDCl_3$ solution were used as mixtures of 3 mg of the *l* diastereomer, of (±)-**1** and 3 mg of (±)-C_6H_5-$SOC(CH_3)$=CHNH-*t*-Bu (the values cited in Table 4 are averages of two independent runs for that composition, and the mean standard deviation for 37 experimental NOEs in this solution is ±0.8%) or 5 mg of (±)-$CH_3SOC(CH_3)$=CHNH-*t*-Bu in 0.6 mL of solvent. The latter compounds were added to check the intermolecular NOEs shown in Figure 6. The standard deviation for each intermolecular NOE in $CDCl_3$ solution cited in Figure 6 was obtained from four measurements. The measurements in pyridine were run on neat compound (10 mg) dissolved in 0.5 mL of pyridine-d_5. The experimental NOEs cited in Table 4A are the averages of three independent experiments. The mean standard deviation for 37 experimental values is ±0.5%.

The samples used for ^1H and ^{13}C T_1 measurements in $CDCl_3$ were not degassed. The concentrations of the samples were as stated above. The ^{13}C T_1 measurements in pyridine-d_5 were run on the same degassed sample as the NOE experiment. These experiments were run using the standard Bruker inversion–recovery program. A relaxation delay of 20 s was used, and the delays after the inversion pulse were set in a geometrical progression starting from 0.1 s and increasing to 15 s. The calculations of the T_1 values were accomplished using a routine Bruker program. The correlation times were calculated using the formulas quoted in ref 24.

Force field calculations[16] were performed to determine the low-energy conformations of **1**. The quoted positive energies in each conformer include the terms due to stretching, bending, stretch-bending, torsion, van der Waals, and dipole–dipole potentials. To find the global minimum, a multiple rotation of all single bonds incremented by 15° was executed, followed by energy minimization, which yielded 37 conformations for the *E* isomer. No hydrogen bond potential was included for the intramolecular hydrogen bond. This only contributes *ca.* 0.5 kcal mol^{-1} in energy. Using the program MMXCOMP,[17] the structures were arranged into families of conformations. A conformation family is defined as a group of conformations in which all given torsion angles differ simultaneously by less than a preset value (in the present case, this was 15°).

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Supplementary Material Available: The experimental and calculated NOE values in pyridine-d_5 solution of **1**, at 22 °C, for the eight-spin system, as in Table 4 (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(33) Kawęcki, R.; Kozerski, L. *Tetrahedron* **1986**, *42*, 1469.