Conformations of Peptidomimetics Formed by S_NAr Macrocyclizations: 13- to 16-Membered Ring Systems[+]

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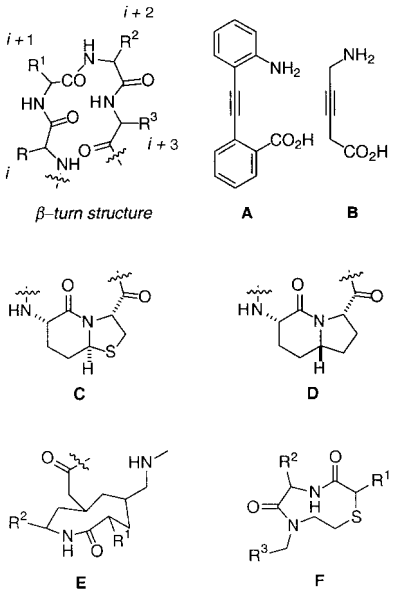
Abstract: The S_NAr macrocyclization products **1a–d** were designed to present two amino acids residues in β-turn conformations. Compound **1b** in the series should give the most ideal fit because it could adopt a "turn–extended-pseudo-turn" orientation. To test this hypothesis, compounds **1a–d** were examined by a combination of CD and NMR spectroscopic techniques, and simulated by a computational approach that did not use constraints from spectroscopic data. Good correspondence between the spectroscopic data and the calculated conformations was obtained. All the compounds appear to be capable of adopting type I β-turns (or closely related states) but the bias towards this structure was most prevalent for the 14-membered macrocycle **1b**, as predicted for the desired turn–extended-pseudo-turn conformation.

Keywords: β-turn · conformation analysis · macrocycles · molecular dynamics · peptidomimetics

The term "β-turn mimic" has been applied to diverse structures designed for different purposes.[1–3] For instance, many β-turn mimics designed for biophysical chemistry serve only to link peptide fragments in the same way as the $i+1/i+2$ residues of a natural β-turn.[4–8] Compounds **A**[7] and **B**[9] fall into that category; they might be more accurately described as "β-turn templates" than β-turn mimics. Another class of β-turn analogues is that consisting of bicyclic and medium ring compounds designed to project side chains in turnlike orientations, such as structures **C** to **F**.[10–19]

Our group is interested in developing efficient solid-phase syntheses of β-turn mimics containing amino acid residues corresponding to key loop regions of target protein structures. Structures **1** evolved from sampling molecular models of organic fragments that, when connected to the C- and N-termini of a dipeptide, appeared capable of inducing β-turn conformations. Ready access to such compounds in high-throughput parallel syntheses would help bridge the gap between discovery of peptide leads and identification of small molecules with the similar pharmacological profiles.[20] Details relating to preparations of compounds **1** are given in the preceding paper, and have been communicated.[20] This manuscript concerns their conformations in solution.



Target molecule **1b** ($n=1$) was identified as a key compound in the series **1a–1d**. It has the correct atom-count to exist in a conformation characterized by fused C^{10}-rings sharing an NH···OC edge. We hypothesized that **1b** might therefore adopt a "turn–extended-pseudo-turn" arrangement similar to cyclic hexapeptides. The latter are well known to favor orientations with two C^{10} rings separated by an amino acid at each side, that is turn–extended-turn conformations.[1–3] Systems **1a**, **1c**, and **1d**, where $n=0$ and 2–3, were predicted to be less able to attain the ideal turn–extended-pseudo-turn conformations.

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[+] Supporting information for this article is available from the author. This includes 1D ^{1}H NMR, phase-sensitive COSY, ROESY, temperature coefficient, and CD data for compounds **1a–d**.

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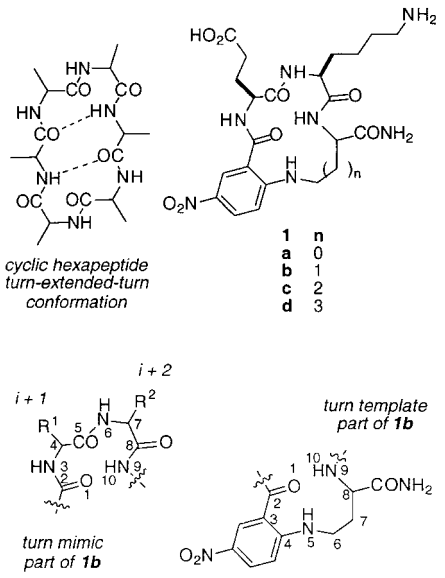

Other considerations also indicate structures **1**, and **1b** in particular, would be well suited to present dipeptides in β-turnlike conformations. Compounds **1** are smaller macrocycles than cyclic hexapeptides (14-membered rings for $n=1$ versus 18-membered cyclic hexapeptides), and the smaller ring size should favor rigidity. Moreover, whereas cyclic hexapeptides can exist in several turn–extended-turn conformations,[21] there should be less conformational ambiguity associated with structures **1** because they have less alternative possible intramolecular NH $\cdots$ OC interactions.

This paper reports data from experiments designed to test for preferred conformations of structures **1**. It provides experimental tests of the hypothesis presented above, that is that the structures **1** have a tendency to adopt turn conformations like those in cyclic hexapeptides.

Results and Discussion

Compounds **1a–d** were studied by a combination of NMR techniques including ROESY spectra, measurement of NH temperature coefficients, determination of NH/ND exchange rates, and determination of coupling constants. DMSO was used as solvent in most of these experiments. Water was not used because of limited solubility of the compounds, and because our goal was to measure the conformation of these turn mimics in an environment that resembles that at a protein–protein interface, that is neither extremely hydrophilic nor extremely hydrophobic. Overall, these NMR studies gave a set of data for comparison with the molecular simulations outlined below.

Molecular simulations of **1a–d** were performed by using the quenched molecular dynamics (QMD) technique.[22] The key feature of this approach is that a set of conformers is generated without any bias from experimentally determined constraints, hence the good correlation between the simulated and the actually observed structure is highly informative. Details of QMD simulations have been published elsewhere, and applied several times in work from these labs.[22–30] Briefly, a large ensemble of structures (600 in this work) is generated under conditions designed to sample a large percentage of the conformational space. These structures are minimized thoroughly, and the lowest energy conformations are isolated then grouped into families. Representative members of these families are then compared with the experimental data generated from NMR experiments.

NMR studies of compounds 1a–d: NMR experiments were performed to probe several parameters that would be characteristic of β-turn conformations, if present. Figure 1 shows conformations of molecule **1b** wherein the dipeptide fragment is artificially constrained in type I and in type II β-turn orientations. Clearly, the NH of the template (i.e., the $i+3$ NH) is somewhat uniquely projected inside the ring in both these structures. Only the most stringent definitions of β-turns require that this proton should be hydrogen–bonded, although it should have unusual properties in the absence of this interaction, even if only because of solvent shielding. These may be lower chemical shift values, small temperature coefficients, and relatively slow rates of exchange in protic deuterated solvents.

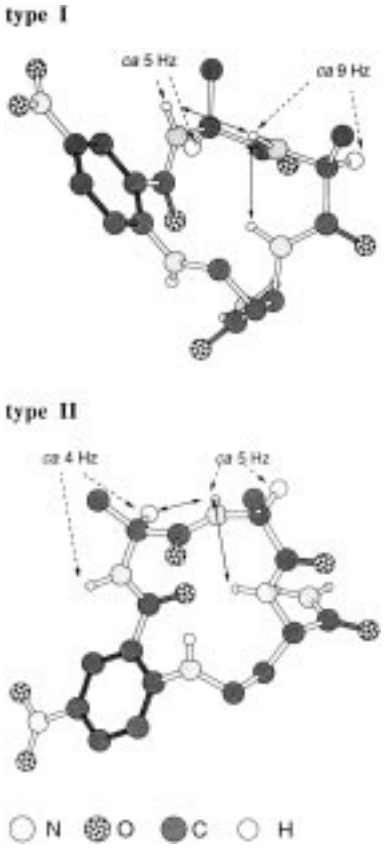

Figure 1. Compound **1b** constrained in type I and type II β-turn conformations. Expected ROE contacts are marked with double–headed arrows, and the dashed arrows indicate couplings.

The critical difference between these two conformations shown in Figure 1 is that the $i+1$ carbonyl and the $i+1/i+2$ amino acid side chains are at opposite faces of the ring in the type I case, but on the same side in the type II orientation.

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This difference has significant implications on the ROE connectivities that are to be expected. Aubé and co-workers clearly summarized these differences in the context of another β-turn system,[31] and their conclusions may be extrapolated to this work. Type I conformations are characterized by ROE crosspeaks between the $i+1$ and $i+2$, and between the $i+2$ and $i+3$ NH protons. Conversely, type II turns feature α_{i+1} to NH_{i+2} connectivities. These differences are also manifest in $^3J_{H,N\alpha}$ coupling constants: values of approximately 4 and 9 Hz are expected for the second and third residues of a type I β-turn, while these values are 4 and 5 Hz for type II β-turns.[32]

Salient NMR data for compounds **1a–d** are shown in Table 1. The NH_{i+3} proton is of particular interest because in the anticipated turn–extended-pseudo-turn conformation of these molecules this proton should be proximal, and possibly hydrogen-bonded to the ArCO carbonyl. Temperature coefficients for the NH_{i+3} of compounds **1b** and **1d** are the lowest in the series, probably[33] indicative of solvent shielding and/or hydrogen bonding,[34, 35] and imply well-populated turn conformers are most likely to be present for compounds **1b** and **1d**. The presence of NH_{i+1} to NH_{i+2} ROE crosspeaks, and the absence of α_{i+1} to NH_{i+2} connectivities in all cases, indicate that these turns are similar to type I structures. This inference is supported by the NH_{i+2} to NH_{i+3} crosspeak observed for compound **1b**, and by the smaller/larger relative $^3J_{N,H\text{-}\alpha}(i+1)$ and $^3J_{N,H\text{-}\alpha}(i+2)$ coupling constants observed for all the compounds in the series.

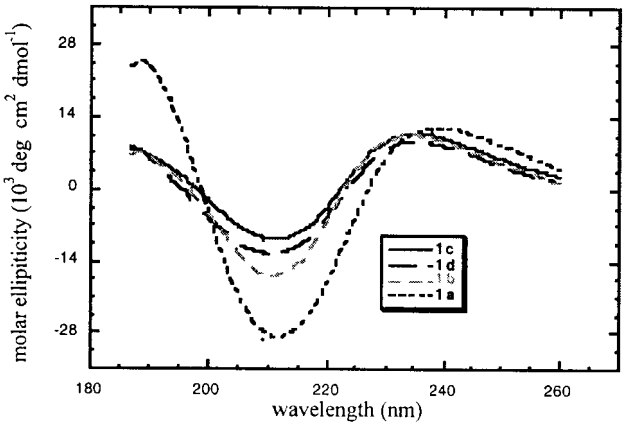

Figure 2. Overlaid CD spectra of compounds **1** in H_2O/MeOH 80:20.

Table 1. Important NMR data for compounds **1**.

1	Ring size	δ NH			NH_{i+3}	ROE values		Coupling constants [Hz]	
		$i+1$ (Glu)	$i+2$ (Lys)	$i+3$ (varies)	$\Delta\delta/\Delta T$ [ppb K^{-1}]	NH_{i+1}/ NH_{i+2}	NH_{i+2}/ NH_{i+3}	$^3J_{N,H\text{-}\alpha}(i+1)$	$^3J_{N,H\text{-}\alpha}(i+2)$
a	13	8.445	7.164	8.300	−3.56	S	–	8.0	9.0
b	14	8.681	7.779	7.677	−2.04	M	M	6.0	9.5
c	15	8.842	7.463	7.821	−4.56	S	–	6.0	8.0
d	16	8.853	7.66–7.70	7.584	−2.40	M	–	6.0	8.5

Circular dichroism studies for compounds 1a–d: Type I β-turns tend to give CD spectra with a positive peak at about 190 nm, and a negative peak at around 208 nm.[36] CD spectra of type II turns give no significant maximum ellipticity below 200 nm, and a maximum at about 203 nm. Figure 2 shows the CD spectra of compounds **1** in H_2O/MeOH 80:20. The inference from these data is that all the compounds have CD spectra similar in shape to those expected for type I turns, but not type II. However, we cannot anticipate the effects of the aryl chromophore in these molecules, as this could also have a bearing on the shapes of the CD spectra observed.

Some practical issues of these CD studies require explanation. First, the conclusion presented above is based on CD spectra shapes but not intensities. No significance can be placed on the relative intensities in Figure 2, because the solutions probably had significantly different concentrations despite our efforts to ensure equimolarity. Difficulties in preparing equimolar solutions arose from weighing errors as a result of the small sample amounts available and the hygroscopic characteristics of these compounds. Second, the choice of solvent for the CD spectra was made upon the following considerations. Optimally, the CD spectra should be recorded in the same medium with the same dipole moment as used for the NMR studies, that is DMSO, $\mu = 45$. However, DMSO is unsuitable for CD studies since it obscures most of the regions of interest. A mixture of H_2O/MeOH 35:65 has the same dipole moment as DMSO.[27] We found that this media gave noisy spectra at 190 nm, because methanol has a significant absorbance in the 190 nm region. Thus the proportion of methanol was reduced until it was found that an H_2O/MeOH 80:20 mixture gave acceptable CD spectra.

Molecular simulations of compounds 1a–d: The QMD technique gives an ensemble of low energy structures that are then grouped into families with a matching routine. In all four compounds **1**, one of the families had many more conformers than the others, and also contained the overall lowest energy structure located. In fact, in each of the simulations the most populated family contained at least 48 structures, while the next most populated contained 20 or less. Moreover, as described below, representatives of the most populated families gave good correspondence with the NMR data. For these reasons, only the most populated conformers are considered in the text (Table 2); complete details of the four simulations for compounds **1** are given in the Supporting Information.

One incidental finding in the QMD studies was interesting in the context of the current study. The overlay process converged well to one major family for compounds **1a** and **1b**, but the allowable conformations were less similar in the case of **1c** and **1d**. This observation is entirely logical. As the ring size is increased the compounds would be expected to be more flexible and to adopt a more diverse set of conformational states.

The next stage in the process was to compare the data from the molecular simulations with ideal bond parameters for type I and type II β-turns. Idealized ϕ,ψ bond angles[37, 38] for the

Table 2. Important details from the QMD simulations.

Compound	Ring size	Parameter	Lowest energy conformer	Average from family containing lowest energy conformer
1a	13	$i+1$ (Glu) ϕ	-68°	-78°
		$i+1$ (Glu) ψ	-47°	-46°
		$i+2$ (Lys) ϕ	-71°	-96°
		$i+2$ (Lys) ψ	-31°	-22°
		CO_i-NH_{i+3} distance [Å]	2.93	3.71
		turn type	βIII	βI
1b	14	$i+1$ (Glu) ϕ	-67°	-78°
		$i+1$ (Glu) ψ	-33°	0°
		$i+2$ (Lys) ϕ	-74°	-160°
		$i+2$ (Lys) ψ	-32°	48°
		CO_i-NH_{i+3} distance [Å]	2.69	3.10
		turn type	βIII	βVIII
1c	15	$i+1$ (Glu) ϕ	-79°	-78°
		$i+1$ (Glu) ψ	-40°	-19°
		$i+2$ (Lys) ϕ	-63°	-93°
		$i+2$ (Lys) ψ	-61°	38°
		CO_i-NH_{i+3} distance [Å]	2.77	3.17
		turn type	βIII	βI
1d	16	$i+1$ (Glu) ϕ	-87°	-77°
		$i+1$ (Glu) ψ	-28°	-47°
		$i+2$ (Lys) ϕ	-68°	-91°
		$i+2$ (Lys) ψ	-35°	101°
		CO_i-NH_{i+3} distance [Å]	3.40	4.97
		turn type	βIII	βIII

$i+1$ and $i+2$ residues of a type I β-turn are -60°, -30° and -90°, 0° respectively. The corresponding ϕ,ψ angles for a type II turn are -60°, $+120^\circ$ and $+80^\circ$, 0°. The comparison of the data presented in Table 2 reveals that ϕ,ψ angles of all the low energy conformers correspond more closely to type I β-turn conformations than type II. In fact, the bond angles indicated may fit other turn types even more closely than a type I. However, the differences between some of the various types are subtle (e.g. the type III $i+1$ and $i+2$ ϕ,ψ angles are -60°, -30° and -60°, -30°, respectively, which is close to the type I situation). Our assessment is that these differences are within the margins of experimental error for molecular simulations. Indeed, if all the structures in the dominant family are averaged, and the resulting structure is minimized to relax bonds that are distorted by the averaging process, another conformation is then generated. Table 2 indicates that these structures (for which ϕ,ψ parameters listed under the heading "average from family containing lowest energy conformer") can differ from the lowest energy conformer. Those differences are greater than those between idealized type I and type III turns; this indicates that the simulations do not distinguish between these. However, the differences are less than those between type I and type II turns hence the latter distinction is valid.

Further support for the inference that compounds **1** can adopt type I turn conformations was obtained by comparison of allowable bond angles calculated from coupling constants with those simulated. Throughout angles measured for the averaged structures from each major family gave a good correspondence with values calculated[32] from the experimentally observed coupling constants (Table 3).

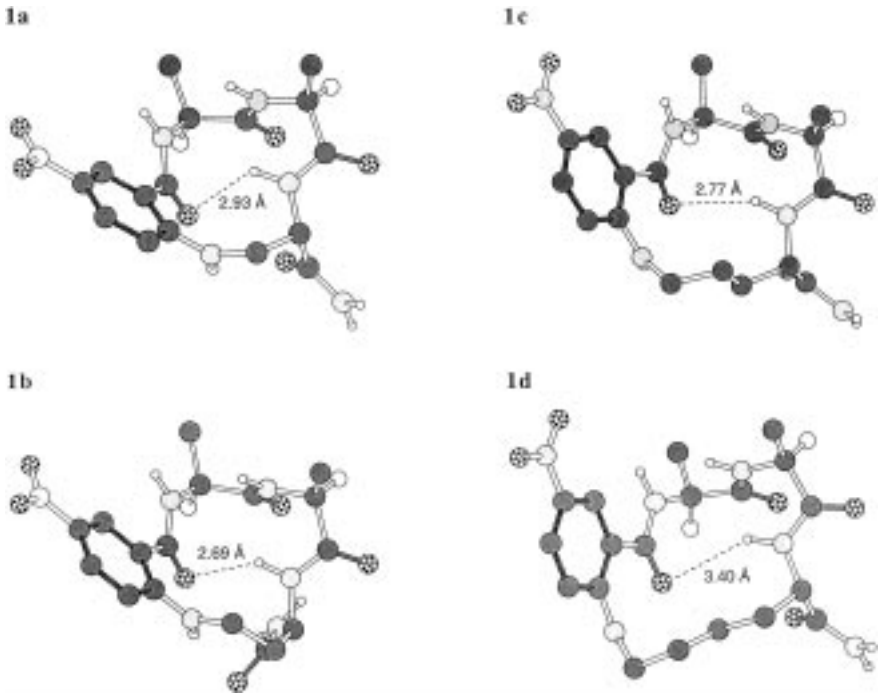

Figure 3. Lowest energy conformers located in the QMD study of compounds **1** (see Figure 1 for the atom key; the dashed lines highlight distances between the ArCO carbonyl and the NH_{i+3} proton which are similar to the proposed turn–extended-pseudo-turn structures).

Conclusion

S_NAr macrocyclizations provide a convenient means to prepare 13- to 16-membered rings by solid-phase syntheses. CD studies (Figure 2) and molecular dynamics simulations indicate all the compounds **1** resemble type I β-turns. This assertion is supported by the ROE data (Table 1) which reveals that all the compounds have the requisite NH_{i+1}/NH_{i+2} crosspeak for such conformations, but do not have the α_{i+1}/NH_{i+2} crosspeaks that would be expected for type II turns and related conformers (Figure 1). The evidence for population of type I turn states is strongest for compound **1b** in the series. This compound has a low chemical shift and temperature coefficient for the NH_i hydrogen, and its simulated structure fits the idealized type I turn parameters relatively well. This finding supports the hypothesis made at the beginning of this study, that the 14-membered ring should resemble closest the turn–extended-turn conformation of cyclic hexapeptides. However, the nature of the heteroatom used in the cyclization (in all cases nitrogen was used here) may also have some

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Table 3. Comparison of coupling constant data for compounds **1**.

Compound	Parameters	$i+1$	$i+2$	$i+3$
1a	observed $^3J_{N,H\text{-}\alpha}$ [Hz]	8.0	9.0	8.0
	calcd allowable ϕ [°]	−91	−102	−91
	simulated in averaged structures [°]	−78	−96	−68
1b	observed $^3J_{N,H\text{-}\alpha}$ [Hz]	6.0	9.5	8.0
	calcd allowable ϕ [°]	+84, +37, or −74	−110	−91
	simulated in averaged structures [°]	−78	−160	−77
1c	observed $^3J_{N,H\text{-}\alpha}$ [Hz]	6.0	8.0	8.0
	calcd allowable ϕ [°]	+84, +37, or −74	−91	−91
	simulated in averaged structures [°]	−78	−93	−138
1d	observed $^3J_{N,H\text{-}\alpha}$ [Hz]	6.0	8.5	8.0
	calcd allowable ϕ [°]	+84, +37, or −74	−95	−91
	simulated in averaged structures [°]	−77	−91	−144

conformational effects. This issue was not explored in this particular study but will be addressed in subsequent work.

Experimental Section

NMR studies: NMR spectra were recorded on a Varian UnityPlus 500 spectrometer (500 MHz). The concentrations of the samples were approximately 5 mM in [D_6]DMSO. One-dimensional (1D) ^{1}H NMR spectra were recorded with a spectral width of 8000 Hz, 32 transients, and a 2.5 s acquisition time. Vicinal coupling constants were measured from 1D spectra at 25 °C. Assignments of ^{1}H NMR resonances in DMSO were performed by using sequential connectivities. Temperature coefficients of the amide protons were measured by several 1D experiments in the temperature range 25–50 °C adjusted in 5 °C increments with an equilibration time of more than 10 min after successive temperature steps.

Two-dimensional (2D) NMR spectra were recorded at 25 °C with a spectral width of 8000 Hz. Through-bond connectivities were identified by COSY,[39] and through-space interactions by ROESY spectra,[40] recorded in 512 t_1 increments and 32 scans per t_1 increment with 2 K data points at t_2. ROESY experiments were performed with mixing times of 100, 200, 300, 400 ms; normally a mixing time of 300 ms was superior. The intensities of the ROESY crosspeaks were assigned as S (strong), M (medium), and W (weak) from the magnitude of their volume integrals.

CD studies: CD measurements were obtained on an Aviv (model 62 DS) spectrometer. For these experiments the cyclic peptidomimetics were dissolved in H_2O/MeOH (80:20 *v/v*, $c = 0.1$ mg mL^{-1}, 0.1 cm path length). The CD spectra were recorded at 25 °C.

Molecular simulations: CHARMm (version 23.2, Molecular Simulations Inc.) was used for the molecular simulations performed in this work. Explicit atom representations were used throughout the study. The residue topology files (RTF) for all the peptidomimetics were built with QUANTA97 (Molecular Simulations Inc.).

Quenched molecular dynamics simulations (QMD) were performed by using the CHARMm standard parameters. All four molecules were modeled as neutral compounds in a dielectric continuum of $\mu = 45$ (simulating DMSO). Thus, the starting conformers were minimized with 200 steps of steepest descent (SD) and 1000 steps of the adopted basis Newton–Raphson method (ABNR), respectively. The minimized structures were then subjected to heating, equilibration, and dynamics simulation. Throughout, the equations of motions were integrated by using the Verlet algorithm with a time step 1 fs. SHAKE was used to constrain all bond lengths containing polar hydrogens. Each peptidomimetic was heated to 1000 K over 10 ps and equilibrated for another 10 ps at 1000 K, then molecular dynamics runs were performed for a total time of 600 ps with trajectories saved every 1 ps. The resulting 600 structures were thoroughly minimized by using 200 steps of SD followed by ABNR until an RMS energy derivative of ≤ 0.001 kcal mol^{-1} Å^{-1} was obtained. Structures with energies less than 3.50 kcal mol^{-1} relative to the global minimum were selected for further analysis.

The QUANTA97 package was used again to display, overlay, and classify the selected structures into conformational groups. The best clustering was obtained by using a grouping method based on calculation of RMS deviation of a subset of atoms; in this study these were the ring backbone atoms. Thus, threshold cutoff values between 0.60 Å and 0.75 Å were selected to obtain families with reasonable homogeneity. The lowest energy from each family was considered as a typical representative of the family as a whole. Additionally, a second approach was also used to obtain a representation of each family. In this alternative protocol, the coordinates of all the heavy atoms in each family were averaged in Cartesian space. The protons were rebuilt on those heavy atoms with standard geometries for each atom type, then the resulting structures were minimized with 50–100 steps of SD to smooth the bond lengths and angles. Finally, interproton distances and dihedral angles from both the lowest energy and the averaged structure were calculated for comparisons with the ROE data.

Acknowledgments

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