Conformational Analysis of Reverse-Turn Constraints by N-Methylation and N-Hydroxylation of Amide Bonds in Peptides and Non-Peptide Mimetics

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Abstract: Several non-peptide systems have been designed to mimic different types of reverse turns. The incorporation of some of these mimetics into biologically active peptides has led to peptidomimetics with enhanced activity or metabolic stability. This paper reports the conformational analysis of tetrapeptides containing several bicyclic mimetics, sequences containing proline, other *N*-methyl and *N*-hydroxy amino acids, and pipecolic acid at residue $i + 2$ of the turn, and control peptide sequences using the Monte Carlo/stochastic dynamics simulation with the new set of AMBER* parameters for proline-containing peptides in water as implicitly represented by the GB/SA solvation model. Simple N-methylation (Pro-D-NMeAA and D-Pro-NMeAA) and N-hydroxylation of the amide bond between residues $i + 1$ and $i + 2$ or inclusion of the larger ring homolog pipecolic acid (D-Pro-Pip) in the third position ($i + 2$) causes significant nucleation of reverse-turn structures. Spirotricycle analogs restrict three of the four torsion angles that characterize the type II β-turn. Spirolactam analogs also restrict two of the four torsion angles as effective β-turn constraints. However, the geometry of a turn induced by indolizidinone and BTD differs significantly from that of an ideal β-turn and (*S*)-indolizidinone is more effective as a reverse turn than as a β-turn mimetic. These systems provide useful conformational constraints when incorporated into the structure of selected bioactive peptides. Such analogs can scan receptors for biological recognition of β-turn scaffolds with oriented side chains through combinatorial libraries to efficiently develop three-dimensional structure−activity relationships.

Introduction

Reverse turns play an important structural role in the compact globular architecture of native folded proteins[1−3] and have often been implicated as recognition elements in intermolecular interactions.[3−5] High-resolution examples of turns as recognition motifs can be found in crystal structures of antibody−peptide complexes.[4,6,7] These complexes are entirely consistent with the receptor recognition of turn motifs deduced from structure−activity studies of the peptide hormones, angiotensin II,[8,9] bradykinin,[10−12] GnRH (gonadotrophin releasing hormone),[13,14] somatostatin,[15,16] RGD (Arg-Gly-Asp) sequence,[17,18] repeated NPNA (Asn-Pro-Asn-Ala) tetrapeptide,[19] and many others. One of the most common reverse turns is the β-turn. A β-turn consists of four residues, which are designated as i, $i + 1$, $i + 2$, and $i + 3$, where the chain changes direction by almost 180°. Several different types of β-turns are possible depending upon the Φ and Ψ torsion angles of the $i + 1$ and $i + 2$ residues.[2,3] In addition, these turns may (classic β-turn) or may not (open β-turn) be stabilized by an intramolecular hydrogen bond between the carbonyl oxygen of the first residue (i) and the amide hydrogen of the fourth residue ($i + 3$),[3] although the classical and more stringent definition of a β-turn requires the hydrogen bond. An alternative method of characterizing reverse turns, which focuses on the topography of the side chains, has been suggested by Ball et al.[20]

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Several non-peptide systems have been designed to mimic the different types of β-turns.[21−29] The incorporation of some of these mimics into biologically active peptides has led to peptidomimetics with enhanced activity or metabolic stability.[30−34] Examples of modifications which enhance reverse-turn propensity are the dipeptide lactam,[35] the bicyclic dipeptide BTD[25] and similar proline derivatives,[36] spirolactam bicyclic and tricyclic systems based on proline,[28,37−39] substitution by α,α-dialkyl amino acids,[28,40−42] *N*-aminoproline,[43] functionalized dibenzofurans,[44−46] and substitution by dehydroamino acids.[47−50] Other efforts have focused on stabilizing Type VI β-turns by stabilizing a *cis*-amide bond through disulfide bonds,[16,51] incorporating of tetrazole rings as *cis*-amide bond surrogates,[52−54] or incorporating certain sequences into cyclic peptides.[55] In other turn mimetics, hydrogen bonding groups stabilizing the turn are replaced by covalent bonds.[56−63] Benzodiazepines have also been used as turn mimetics.[64−66]

Chalmers et al.[67] reported the conformational analysis of tetrapeptides containing several bicyclic mimetics, sequences containing proline and other *N*-methyl amino acids in the residues $i + 1$ and $i + 2$ of the turn, and control peptide sequences using a Monte Carlo conformational search followed by molecular dynamics simulation in water as implicitly represented by the GB/SA solvation model. Stimulated by the discrepancy in the calculated and experimentally observed conformation of c[Pro-D-Pro-Pro-D-Pro] reported by Chalmers et al.,[67] McDonald et al.[68] recently reported a reparametrization of the AMBER* force field in MacroModel for proline-containing peptides based on the results of high-level *ab initio* calculations for *N*-acetylproline methylamide. In addition, the conformational search−molecular dynamics protocol[67] used previously does not give a true Boltzmann sample of conformers which would be desirable to make the statistical comparisons equitably, while the newer combined Monte Carlo/stochastic dynamics protocol of Guarnieri and Still[69] produces a true Boltzmann distribution.

In this paper we report the conformational analysis of model blocked tetrapeptides of the type Ac-Ala-Pro-Pro-Ala-NHMe using Monte Carlo searches[70] in water as implicitly represented by the GB/SA solvation model.[71] We compare the results on the differences in free energy between minima on the potential

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surface of the original AMBER* force field parameters[72,73] in MacroModel 4.5[74] with those of the modified AMBER* force field parameters[68] in MacroModel 5.5 and other force fields. Improved conformational analysis was also performed of tetrapeptides containing several bicyclic mimetics, sequences containing proline, other *N*-methyl and *N*-hydroxy amino acids, and pipecolic acid (Pip) at residue $i + 2$ of the turn, and control peptide sequences using the Monte Carlo/stochastic dynamics simulation with the new set of AMBER* parameters for proline-containing peptides in the GB/SA solvation water model.

Methods

Conformational searches and molecular dynamics were performed with MacroModel[74] version 4.5 and 5.5 on Silicon Graphics Iris Indigo R4000 and Indigo Impact R10000 workstations. The MacroModel implementations of either the AMBER all-atom force field,[72] MM2,[75] MM3,[76] the Merck Molecular Force Field (MMFF),[77−81] or the AMBER/OPLS united-atom force fields[82] were used (respectively denoted AMBER*, MM2*, MM3*, MMFF*, and AMBER/OPLS*). For solution-phase calculations, the GB/SA continuum models for water or chloroform were used.[71]

Amide bonds were required to be *trans*, i.e. structures containing non-proline *cis*-amide bonds were discarded as energetically improbable except in the case of *N*,*N*-dialkyl amino acids (Pro, Pip, NMeAla, etc.) whose imide bonds were purposefully sampled and accepted with either *cis* or *trans* geometry in the conformational searches.

Conformational Searches. Conformational searches were performed with use of the systematic Monte Carlo method of Goodman and Still.[70] For each search, 5000 starting structures were generated and minimized until the gradient was less than 0.05 (kJ/mol)/Å^{-1}, using the truncated Newton-Raphson method implemented in MacroModel. Duplicate conformations and those with an energy greater than 50 kJ/mol above the global minimum were discarded.

Monte Carlo/Stochastic Dynamics. All simulations were performed at 300 K with use of the recently described Monte Carlo/stochastic dynamics (MC/SD) hybrid simulation algorithm[69] with the new AMBER* all-atom force field in MacroModel 5.5. A time step of 1.5 fs was used for the stochastic dynamics (SD) part of the algorithm. The MC part of the algorithm used random torsional rotations between ±60° and ±180° that were applied to all rotatable bonds except the proline amide C−N bond where the random rotations were between ±90° and ±180°. No torsion rotations were applied to bonds in the pyrrolidine ring of proline as the barriers are low enough to permit adequate sampling from the SD part of the simulation. The total simulation time was 1000 ps and samples were taken at 1 ps intervals, yielding 1000 conformations for analysis.

Results and Discussion

Conformational Searches. Monte Carlo searches were performed on model blocked tetrapeptides of the type Ac-Ala-Pro-Pro-Ala-NHMe with use of the GB/SA solvation water model. The results of the comparison with the original AMBER* force field parameters[72,73] in MacroModel 4.5 and the new AMBER* force field parameters[68] in MacroModel 5.5 are summarized in Table 1.

Table 1. Energy Differences between the Minimum Energy Proline ω_{12} and ω_{23} *trans*- and *cis*-Conformers for Tetrapeptides of the Type Ac-Ala-ω_{12}-Xxx-ω_{23}-Yyy-Ala-NHMe Using the Systematic Monte Carlo Searches with Original AMBER/OPLS*, Original AMBER* All-Atom, New AMBER/OPLS*, New AMBER* All-Atom, and MMFF* All-Atom

	original		v5.5 new		v5.5
Xxx-Yyy	AMBER/ OPLS*	AMBER* all-atom	AMBER/ OPLS*	AMBER* all-atom	MMFF* all-atom
			$\Delta E(\omega_{12}$ *cis-trans*) (kJ/mol)		
Pro-Pro	8.5	5.0	6.7	2.6	9.6
Pro-D-Pro	12.5	6.2	10.9	1.5	9.1
D-Pro-Pro	15.2	16.5	12.9	10.4	9.8
D-Pro-D-Pro	17.7	4.7	14.1	7.1	10. 1
			$\Delta E(\omega_{23}$ *cis-trans*) (kJ/mol)		
Pro-Pro	−6.3[a]	−0.1[a]	−6.7[a]	−3.6[a]	5.8
Pro-D-Pro	25.2	8.5	22.4	1.7	10.5
D-Pro-Pro	12.8	13.0	11.1	5.8	16.3
D-Pro-D-Pro	3.7	−0.9[a]	1.5	5.8	5.6

[a] ω_{23} is *cis* in the minimum energy structure.

To increase the speed of calculation, the normal amide bonds such as those of alanine were required to be *trans* due to the improbable occurrence of *cis*-amides in low energy structures. The united-atom force field, AMBER/OPLS*, the AMBER* all-atom force field, and the MMFF* all-atom force field were also investigated to make sure that the differences observed were not due to parametrization.

As can be seen from Table 1, both the united-atom force field, AMBER/OPLS* ΔE, and the AMBER* all-atom ΔE for relative *cis*−*trans* stability of all tetrapeptides with the new AMBER* parameters were lower than those with the original AMBER* parameters. The AMBER* all-atom ΔE for relative ω_{12} *cis*−*trans* stability of all tetrapeptides with the new AMBER* parameters were lower than the united-atom force field, AMBER/OPLS* ΔE with the new AMBER* parameters. The united-atom force field, AMBER/OPLS* provides more rapid conformational searching. However, the energetic results, particularly for imide *cis*−*trans* isomerism in Ac-Ala-Pro-D-Pro-Ala-NHMe, were substantially different (9−21 kJ/mol) from those of the AMBER* all-atom force field. The energetic results with the MMFF* all-atom force field, particularly for imide *cis*−*trans* isomerism in Ac-Ala-Pro-Pro-Ala-NHMe and Ac-Ala-Pro-D-Pro-Ala-NHMe, were substantially different (7−9 kJ/mol) from those of the AMBER* all-atom force field. The stabilization of the *cis* conformer (−4 kJ/mol) for ω_{23} in Ac-Ala-Pro-Pro-Ala-NHMe was found with the new AMBER* all-atom parameters. Upon inversion of chirality of the first proline α-carbons, the stabilization of the *trans* conformation (10 kJ/mol) of the imide (ω_{12}) in Ac-Ala-D-Pro-Pro-Ala-NHMe was found with the new AMBER* all-atom parameters. The hydrogen bonding between the carbonyl oxygen of residue 1 (i) and the NH of residue 4 ($i + 3$) plays an important part in stabilizing the β-turn in the model tetrapeptides.[67]

Monte Carlo/Stochastic Dynamics. A summary of the results from the Monte Carlo/stochastic dynamics simulations is given in Table 2. Three parameters were used as measures of reverse-turn forming ability: (1) Reverse turns can be identified by using the criterion that the Cα1−Cα4 distance is less than 7 Å.[3,20] (2) The virtual torsion angle, β, is defined by the atoms Cα1, Cα2, Cα3, and N4 (Figure 1).[20,21] The range

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Table 2. Percentage of Tetrapeptide Conformers of Blocked Tetrapeptides Ac-Ala-Xxx-Yyy-Ala-NHMe and BILD1263 which Exhibit Characteristics of a Reverse Turn from 1000 ps, 300 K MC/SD Simulations Using New All-Atom AMBER* Parameters and GB/SA Water in MacroModel 5.5

			% *d*(C═O···H−N)	
Xxx-Yyy	% $\|\beta\| < 30°$	% $d < 7$ Å	<4 Å	<2.5 Å
Pro-Pro	32	40	35	4
Pro-D-Pro	18	34	23	4
D-Pro-Pro	26	35	31	9
D-Pro-D-Pro	13	18	16	2
Pro-D-NMeAla	44	64	34	13
D-Pro-NMeAla	49	61	41	6
D-Pro-Pip	62	74	47	7
Proψ[CN$_4$]-Ala	26	40	15	5
Pro-NOHAla	41	78	48	23
Pro-D-NOHAla	0	7	4	1
D-Pro-NOHAla	2	2	2	0
D-Pro-D-NOHAla	25	72	40	14
BTD	69	33	0	0
(*S*)-spirolactam	48	48	47	16
spirotricycle	76	71	57	27
(*R*)-indolizidinone	78	58	1	0
(*S*)-indolizidinone	77	85	0	0
BILD1263	9	6	0	0

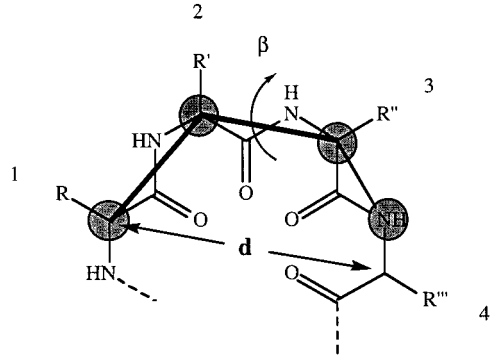

Figure 1. Schematic of tetrapeptide showing the virtual torsion β[82] and the distance *d* from Cα1 to Cα4 used to characterize reverse turns.

$0 \pm 30°$ was taken to indicate a tight reverse turn. (3) The distance between the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ indicates an appropriate hydrogen bond characteristic of a β-turn. A distance of less than 4 Å was taken to indicate significant interaction between these groups.[67] A distance less than 2.5 Å may imply a hydrogen bond between residues *i* and $i + 3$, which characterizes some types of β-turn.[84]

Ac-Ala-Pro-D-Pro-Ala-NHMe and Ac-Ala-D-Pro-Pro-Ala-NHMe. A plot of the distances between the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ against ω_{23} (Figure 2a) of the tetrapeptide Ac-Ala-Pro-D-Pro-Ala-NHMe shows that ω_{23} of most conformers where distances are less than 4 Å is *trans*. Table 2 shows that $|\beta|$ is less than 30° in 18% of the structures, *d* is less than 7 Å in 34%, the distance between the amide hydrogen and carbonyl oxygen is less than 4 Å for 23%, and the distance is less than 2.5 Å for 4% of the time. These percentages of conformers which can be classified as turns with use of the new AMBER* force field parameters in MacroModel 5.5 were lower than those[67] with use of the original AMBER* force field parameters in MacroModel 4.5 due to the smaller ΔE (Table 1) for relative *cis*−*trans* stability with the new AMBER* force field parameters.

(84) Constantine, K. L.; Mueller, L.; Andersen, N. H.; Tong, H.; Wandler, C. F.; Friedrichs, M. S.; Bruccoleri, R. E. *J. Am. Chem. Soc.* **1995**, *117*, 10841−10854.

As a result of swapping the chirality of residues $i + 2$ and $i + 3$ to give Ac-Ala-D-Pro-Pro-NMe, ω_{23} of all conformers where distances between the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ are less than 4 Å is also *trans* (Figure 2b). A plot of the distances between the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ against ω_{12} (Figure 2c) shows that both ω_{12} and ω_{23} of all conformers where distances are less than 4 Å are *trans*. ω_{12} and ω_{23} of most conformers where distances between the carbonyl oxygen and the amide hydrogen are between 4 and 6.5 Å are *trans* and *cis*, respectively. Both ω_{12} and ω_{23} of most conformers where distances between the carbonyl oxygen and the amide hydrogen are between 6.5 and 8 Å are *cis*. Table 2 shows that the percentage of conformers with $|\beta| < 30°$ and $d < 7$ Å has risen to 26% and 35%, respectively. The carbonyl oxygen and amide hydrogen of residues *i* and $i + 3$ are less than 4 and 2.5 Å apart in 31% and 9% of the sampled structures, respectively.

Ac-Ala-Pro-Pro-Ala-NHMe and Ac-Ala-D-Pro-D-Pro-Ala-NHMe. A plot of the distances between the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ against ω_{23} (Figure 2d) of the tetrapeptide Ac-Ala-Pro-Pro-Ala-NHMe shows that ω_{23} of most conformers where distances are less than 4 Å is *cis*. Table 2 shows that $|\beta|$ is less than 30° in 32% of the structures, *d* is less than 7 Å in 40%, the distance between the amide hydrogen and carbonyl oxygen is less than 4 Å for 35%, and the distance is less than 2.5 Å for 4% of the time. These percentages of conformers which can be classified as type VIa β-turns by using the new AMBER* force field parameters in MacroModel 5.5 were considerably higher than those[67] with use of the original AMBER* force field parameters in MacroModel 4.5 due to the stabilization of the *cis* conformer (−4 kJ/mol) for ω_{23} (Table 1) with the new AMBER* force field parameters.

The corresponding sequence with D-amino acids in positions $i + 2$ and $i + 3$ had lower values for the percentage of hydrogen bonded conformers (16%, 2%) and with *d* under 7 Å (18%) and $|\beta|$ under 30° (13%) (Table 2). Some reduction of turn induction was due to the stabilization of the *trans* conformer (6 kJ/mol) for ω_{23} (Table 1) with use of the new AMBER* force field parameters.

Ac-Ala-Pro-D-NMeAla-Ala-NHMe and Ac-Ala-D-Pro-NMeAla-Ala-NHMe. Because the torsion angle (Φ_3) associated with the proline ring was not ideal for a β-turn and whether an *N*-methyl-substituted amino acid was sufficient for turn induction, we investigated tetrapeptides containing NMeAla at position $i + 2$. Ac-Ala-Pro-D-NMeAla-Ala-NHMe proved to be an extremely efficient turn promoter. During the MC/SD simulation the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ were less than 4 and 2.5 Å apart in 34% and 13% of the sampled conformers, respectively (Table 2). $|\beta|$ is less than 30° in 44% of the MC/SD structures and 64% have a Cα1−Cα4 distance of less than 7 Å, higher values than the two proline compounds. Figure 2e shows that ω_{23} of all conformers where distances between the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ are less than 4 Å is *trans*.

The corresponding sequence with a D-amino acid in position $i + 1$ and and L-amino acid in position $i + 2$ also had higher values for the percentage of hydrogen bonded conformers (41%, 6%) and with *d* under 7 Å (61%) and $|\beta|$ under 30° (49%). Figure 2f shows that ω_{23} of all conformers where distances between the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ are less than 4 Å is *trans*. The

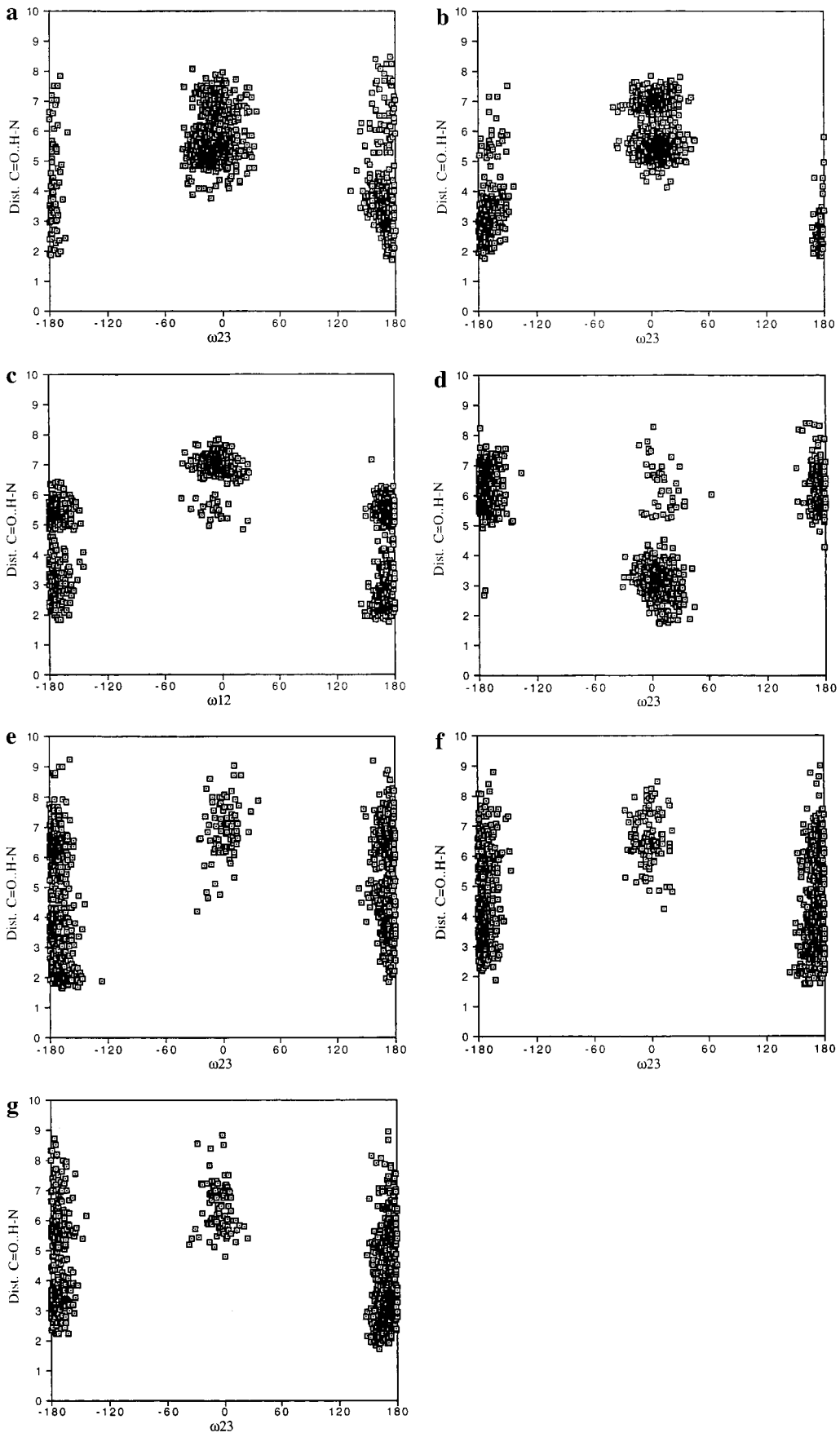

Figure 2. Distance d (Å) between the carbonyl oxygen of residue 1 and the amide hydrogen of residue 4 versus ω_{23} or ω_{12} for selected tetrapeptides: Ac-Ala-Pro-D-Pro-Ala-NMe (a), Ac-Ala-D-Pro-Pro-Ala-NMe (b, c), Ac-Ala-Pro-Pro-Ala-NMe (d), Ac-Ala-Pro-D-NMeAla-Ala-NMe (e), Ac-Ala-D-Pro-NMeAla-Ala-NMe (f), Ac-Ala-D-Pro-Pip-Ala-NMe (g). In each case samples were taken at 1 ps intervals during 1000 ps of the Monte Carlo/stochastic dynamics simulations in GB/SA water.

conformational restrictions caused by this sequence are clear in plots of Φ versus Ψ for residues $i + 1$ and $i + 2$ (Figure 3, a and b, respectively), indicating the enhanced propensity of this sequence for the type II′ β-turn. Figure 3b shows the decreased restrictions for Φ_3 versus Ψ_3 with the new AMBER* all-atom parameters compared to the result[66] with the original AMBER* all-atom parameters. Figure 4a shows a plot of the distance *d* versus potential energy for this tetrapeptide. The distance *d* is restricted to be under 7 Å. Figure 5a shows the restriction of β in the D-Pro-NMeAla sequence. Figure 6a shows that the hydrogen bond between residues *i* and $i + 3$ of a β-turn is present in the lower energy conformers obtained from the D-Pro-NMeAla simulation.

Clearly from these results, the presence of proline at position $i + 2$ is not necessary for the induction of tight β-turns. In fact, the enhanced β-turn propensity of these sequences reflects the fact that the angles Φ_3 and Ψ_3 are less restricted than in proline and can assume values closer to those of an ideal Type II or II′ turn.

Ac-Ala-D-Pro-Pip-Ala-NHMe. To see if increasing the ring size of residue $i + 2$ would allow conformations more compatible with β-turns by allowing a wider variation in Φ_3 and Ψ_3, we examined the effect of replacing proline by pipecolic acid (Pip, homoproline), which contains a 6-membered ring. Ac-Ala-D-Pro-Pip-Ala-NHMe showed better stabilization by all three criteria compared to the two proline model compounds and D-Pro-NMeAla sequence. Table 2 shows that the percentage of conformers for Ac-Ala-D-Pro-Pip-Ala-NHMe where $|\beta| < 30°$ is 62% and the percentage with *d* less than 7 Å is 74%; the percentage of conformers with hydrogen bonding between residues 1 and 4 has risen to 47% and 7%. Figure 2g shows that ω_{23} of all conformers where distances are less than 4 Å is *trans*. In contrast to Ac-Ala-D-Pro-Pro-Ala-NHMe (Figure 2b), ω_{23} of most conformers where distances are more than 4 Å is also *trans*.

Ac-Ala-Proψ[CN$_4$]-Ala-Ala-NHMe. Marshall *et al.* showed that the tetrazole ring system, when incorporated into peptides, mimics a *cis* amide bond by X-ray crystal structure of Z-Proψ-[CN$_4$]-Ala-OBzl.[52,53] We investigated the sequence Ac-Ala-Proψ[CN$_4$]-Ala-Ala-NHMe containing the tetrazole ring (Figure 8). Table 2 shows that $|\beta|$ is less than 30° in 26% of the MC/SD structures and 40% have a Cα1−Cα4 distance of less than 7 Å. The amide hydrogen and carbonyl oxygen are less than 4 and 2.5 Å apart in 15% and 5% of the sampled structures, respectively. These values for the percentage of three criteria are between the Pro-Pro sequence where the *cis*-amide conformer for ω_{23} is stable and the D-Pro-D-Pro sequence where the *trans*-amide conformer is stable (Table 1).

Ac-Ala-Pro-D-NOHAla-Ala-NHMe and Ac-Ala-D-Pro-NOHAla-Ala-NHMe. To investigate whether an *N*-hydroxy-substituted amino acid (NOHAA) was sufficient for turn induction, we investigated tetrapeptides containing NOHAla. These studies were stimulated by the experimental work of the Marraud group.[85] Ac-Ala-Pro-D-NOHAla-Ala-NHMe had lower values for the percentage of hydrogen-bonded conformers (4%, 1%) and conformers with *d* under 7 Å (7%) and with $|\beta|$ under 30° (0%) compared to Ac-Ala-Pro-D-NMeAla-Ala-NHMe (Table 2). A plot of ω_{23} against ω_{12} (Figure 7) shows that ω_{23} of most conformers is *cis*. Some reduction of turn induction was due to the stabilization of the *cis* conformer for ω_{23}. For Ac-Ala-D-Pro-NOHAla-Ala-NHMe, $|\beta|$ is less than 30° in only 2% of the MC/SD) structures and *d* is less than 7 Å in 2%. The percentages of conformers with the characteristic hydrogen bond are also only 2% and 0%.

Ac-Ala-Pro-NOHAla-Ala-NHMe and Ac-Ala-D-Pro-D-NOHAla-Ala-NHMe. It was found that, in the case of Ac-Ala-Pro-NOHAla-Ala-NHMe and Ac-Ala-D-Pro-D-NOHAla-AlaNHMe, there was significant turn stabilization. Table 2 shows that, for Ac-Ala-Pro-NOHAla-Ala-NHMe, $|\beta|$ is less than 30° in 41% of the MC/SD structures and 78% have a Cα1−Cα4 distance of less than 7 Å. During the MC/SD simulation the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ were less than 4 and 2.5 Å apart in 48% and 23% of the sampled conformers, respectively, the higher value for other tetrapeptides. Swapping the chirality of residues $i + 1$ and $i + 2$ to give Ac-Ala-D-Pro-D-NOHAla-Ala-NHMe had lower values for the percentage of hydrogen-bonded conformers (40%, 14%), conformers with *d* under 7 Å (72%), and conformers with $|\beta| < 30°$ (25%), but still appears to be a highly effective turn inducer.

Piv-Pro-NOHGly-NHiPr. The stabilization of the *cis* conformer for ω_{23} in the tetrapeptides containing NOHAla is not consistent with the X-ray and NMR studies of Dupont et al., who found that N-hydroxylation or N-amination of an amide bond within RCO-Pro-Gly-NHiPr seems to have no tendency to induce a *cis* conformation of the modified peptide bond.[85] The relevant structural data of the *N*-hydroxy amide were obtained by searching the Cambridge Structural Database (CSD).[86,87] Among 23 *N*-hydroxy amide analogues in CSD, there were only two structures[88,89] which have the *cis* amide bond.

We investigated several force fields for the *N*-hydroxy analogue of RCO-Pro-Gly-NHiPr (Me_3CCO-Pro-NOHGly-NHiPr). There are no parameters for *N*-hydroxy amide in either the MM2* force field or the MM3* force field. As shown in Table 3, the results (98% *trans* in water and 91% *trans* in $CHCl_3$) from only the MMFF* force field for *cis*−*trans* isomerism of the amide bond (ω_{23}) are consistent with the X-ray result[85] due to the high-quality parameters for the *N*-hydroxy amide group in the MMFF* force field.[77−81] There are no specific parameters for the *N*-hydroxy amide in either the AMBER* all-atom force field or the AMBER/OPLS* united-atom force field. The AMBER* all-atom force field in $CHCl_3$ had higher values for the percentage of hydrogen-bonded conformers (82%) and conformers with *d* under 7 Å (80%) compared to those of the AMBER* all-atom force field in water (Table 3).

Comparison with Other Reverse-Turn Peptidomimetics

BTD dipeptide. The dipeptide mimetic BTD (Figure 8) designed and prepared by Nagai et al.[25] has been incorporated at positions thought to require a reverse turn for recognition in a number of biologically active peptides with varying success. This 6,5-bicyclic ring system is composed of a six-membered ring annulated onto thioproline. The percentage of conformers for Ac-Ala-BTD-Ala-NHMe in which $|\beta|$ is less than 30° is 69%, a higher value than those of other tetrapeptides. The percentage where *d* is less than 7 Å is 33%. Importantly, no

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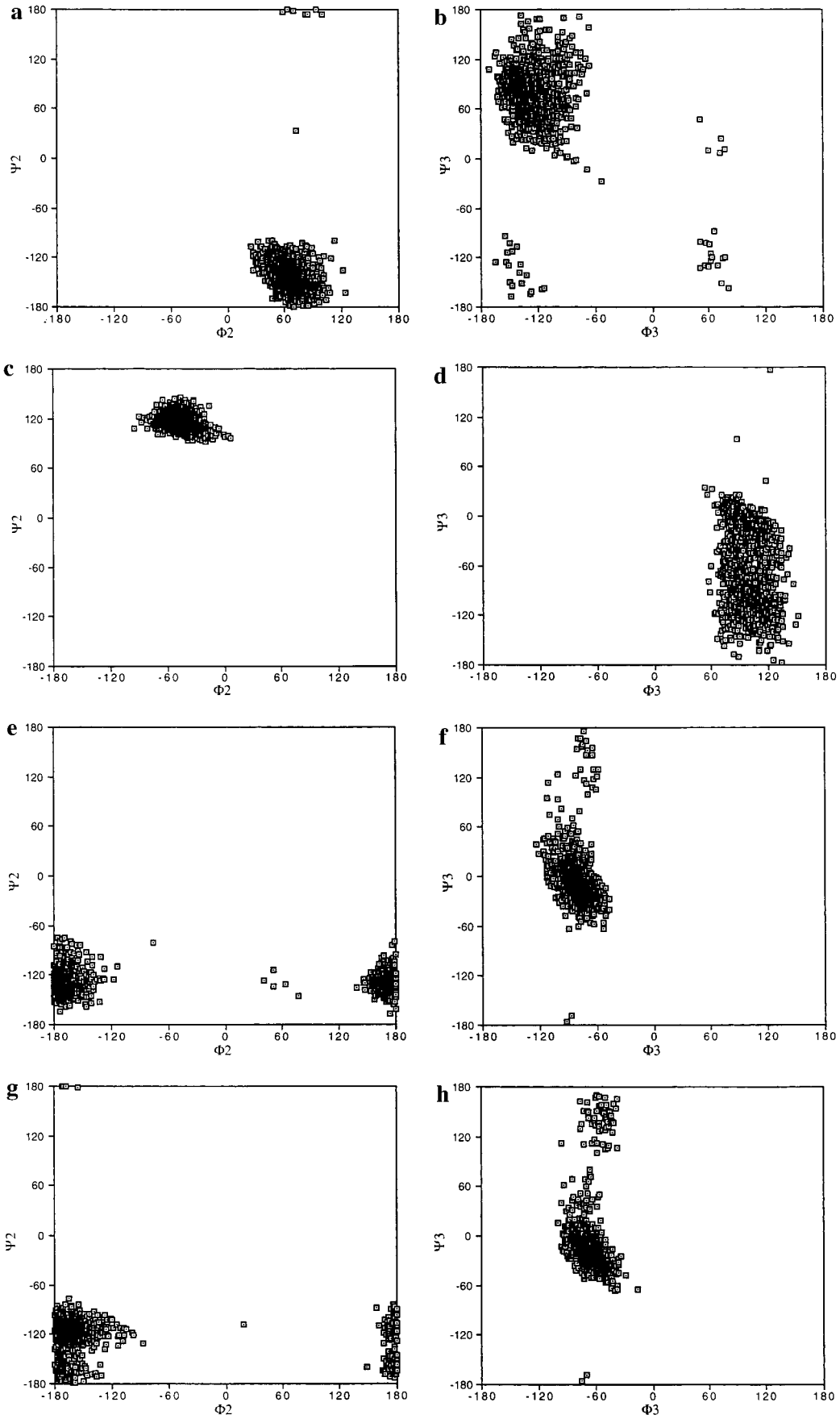

Figure 3. Plots of the backbone torsion angle Φ_2 versus Ψ_2 and Φ_3 versus Ψ_3 during the Monte Carlo/stochastic dynamics simulation for selected tetrapeptides: Ac-Ala-D-Pro-NMeAla-Ala-NMe (a, b), Ac-Ala-spirotricycle-Ala-NMe (c, d), Ac-Ala-(*R*)-indolizidinone-Ala-NMe (e, f), and Ac-Ala-(*S*)-indolizidinone-Ala-NMe (g, h).

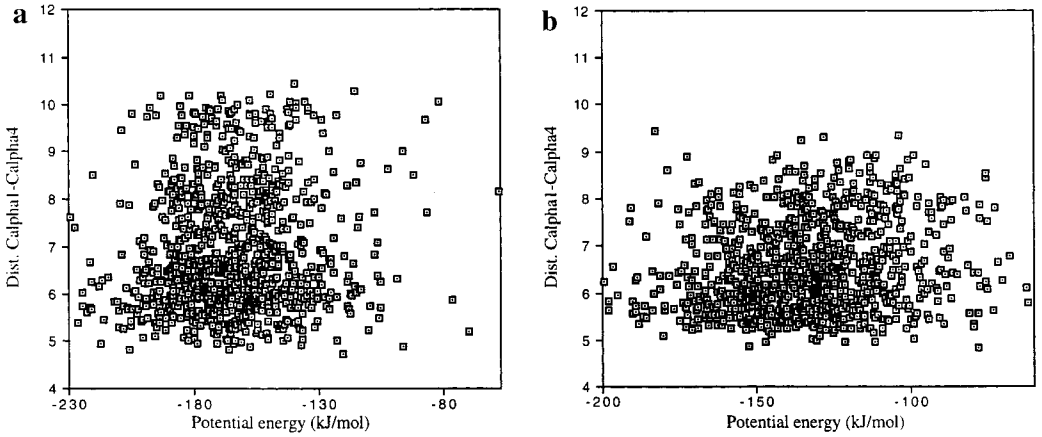

Figure 4. Plots of the distance between Cα1 and Cα4 versus potential energy during the Monte Carlo/stochastic dynamics simulation for (a) the restricted tetrapeptide Ac-Ala-D-Pro-NMeAla-Ala-NMe and (b) Ac-Ala-spirotricycle-Ala-NMe which contains a rigid peptidomimetic.

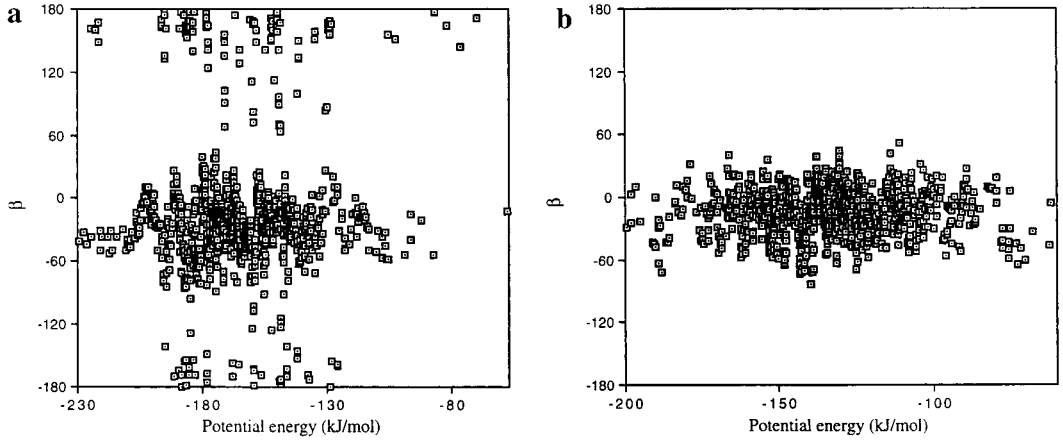

Figure 5. Plots of the β-turn parameter β[82] versus potential energy during the Monte Carlo/stochastic dynamics simulation for (a) Ac-Ala-D-Pro-NMeAla-Ala-NMe and (b) Ac-Ala-spirotricycle-Ala-NMe which are tightly constrained to adopt turn-like conformations.

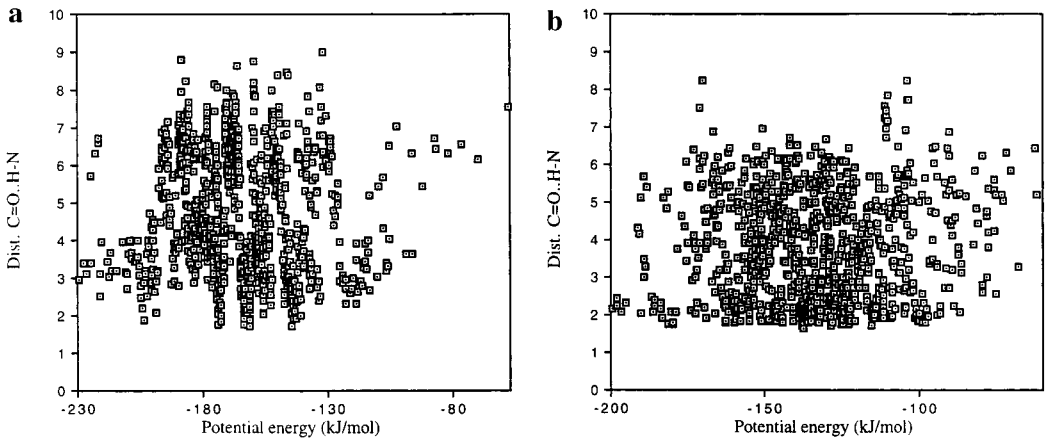

Figure 6. Plots of the distance d (Å) between the carbonyl oxygen of residue 1 and the amide hydrogen of residue 4 versus potential energy during the Monte Carlo/stochastic dynamics simulation for (a) Ac-Ala-D-Pro-NMeAla-Ala-NMe and (b) Ac-Ala-spirotricycle-Ala-NMe.

interaction was observed between the carbonyl oxygen of residue i and the amide hydrogen of residue $i + 3$. The percentage of conformers where d_{O-H} is less than 4 Å is 0% (Table 2). These results suggest that the geometry of a turn induced by BTD differs significantly from that of an ideal β-turn. It would be inappropriate, therefore, to utilize BTD to initiate a β-hairpin peptide conformation.

***S*-Spirolactam Compound.** These spirolactam bicyclic proline derivatives (Figure 8) in which an α-alkyl substituent on the pyrrolidine ring is cyclized to the amide nitrogen of the adjacent amino acid can be considered chimeras of the cyclic lactam of Freidinger et al.[35] and α-methyl-Pro.[37,42] This bicyclic constraint restricts both Φ and Ψ of the spiro derivative. Table 2 shows that for Ac-Ala-*S*-spirolactam-Ala-NHMe, 48% of conformers had $|\beta|$ less than 30° and 48% had values of d lower than 7 Å. The percentages of conformers with an appropriate distance for the hydrogen bond characteristic of the β-turn are 47% and 16%, which are higher values than those for other tetrapeptides. These results are consistent with the NMR studies in DMSO of the cyclic pseudopentapeptide (c(Arg-Gly-Asp-spiro)).[18]

Spirotricyclic Compound. Genin and Johnson[38,90] have combined the spiro lactam constraint with annulation of a

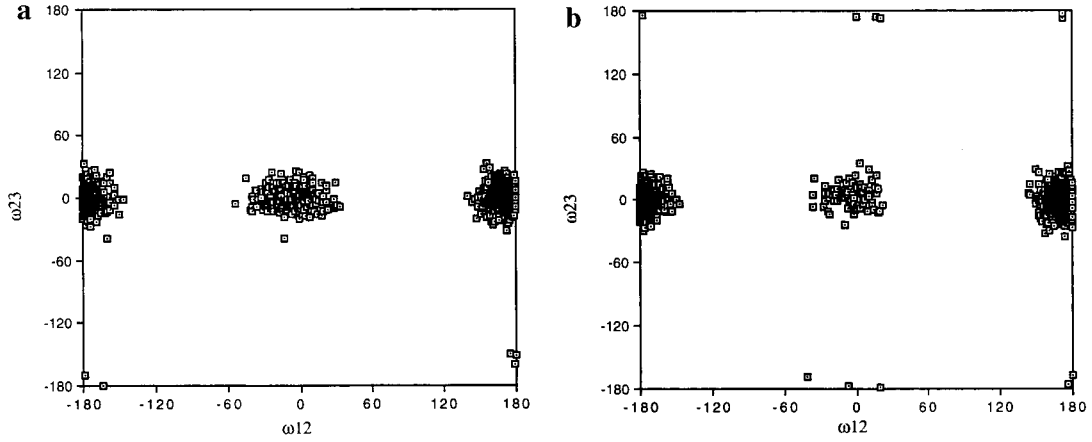

Figure 7. Plot of the backbone torsion angle ω_{12} versus ω_{23} during the Monte Carlo/stochastic dynamics simulation for Ac-Ala-Pro-D-NOHAla-Ala-NMe.

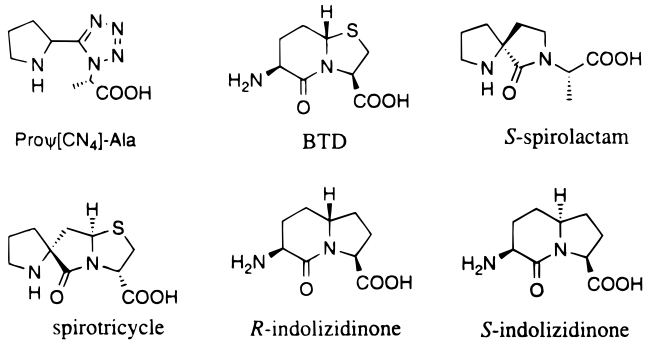

Figure 8. Structures of nonpeptidic reverse turn mimetics.

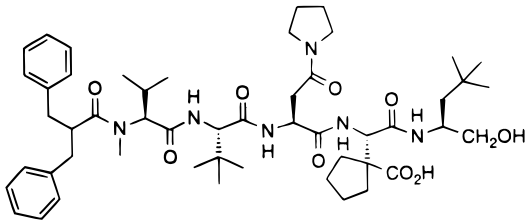

Figure 9. Structure of BILD 1263.

Table 3. Percentage of Conformers of Me_3CCO-Pro-ω-NOHGly-NHiPr Which Exhibit Characteristics of a Reverse Turn and ω *trans* Form During 1000 ps MC/SD Simulations Using MacroModel 5.5

	% $\lvert\beta\rvert$ < 30°	% d < 7 Å	% d (C═O···H−N) < 4 Å	% ω *trans* form
in water				
AMBER* all-atom	19	30	25	0
MMFF*	9	6	8	98
in $CHCl_3$				
AMBER* all-atom	69	80	82	12
AMBER/OPLS*	27	25	21	0
MMFF*	29	34	25	91

thioalkyl ring similar to BTD, generating a spirotricyclic ring system (Figure 8) that restricts Φ_2, Ψ_2, and Φ_3. Plots of Φ_2 versus Ψ_2 and Φ_3 versus Ψ_3 for the MC/SD simulation of Ac-Ala-spirotricycle-Ala-NHMe are shown in Figure 3, plots c and d. These figures show that the spirotricycle tightly constrains all the backbone angles except Ψ_3. A plot of the distance between Cα1 and Cα4 versus potential energy for the MC/SD simulation (Figure 4b) shows that mimetic effectively constrains this parameter (d < 7 Å, 71%). Figure 5 shows the dramatic restriction of β in the spirotricyclic mimetic (plot b, $|\beta|$ < 30°, 76%) compared to the D-Pro-NMeAla tetrapeptide (plot a). These may be due to the highest percentage (Table 2) of hydrogen-bonded conformers (d < 4 Å, 57%, d < 2.5 Å, 27%, Figure 6b) for any of the model compounds, implying an excellent β-turn mimetic. These results are consistent with the modeling studies and NMR studies in $CDCl_3$ of the spirotricyclic analog[38] due to three restrictions (Φ_2, Ψ_2, and Φ_3) of the four torsion angles that characterize the type II β-turn.

Indolizidinone Compounds. Lombart and Lubell[36] prepared bicyclic turn mimetics containing the indolizidinone ring system (Figure 8). Plots e and f in Figure 3 show Ac-Ala-(*R*)-indolizidinone-Ala-NHMe has only rigidified Ψ_2 and Φ_3, while the spirotricycle constrains all the backbone angles except Ψ_3 shown in Figure 3, plots c and d. The percentage (Table 2) of conformers in which $|\beta|$ is less than 30° is 78%, the highest value for any of the model compounds. The percentage of conformers with the distance Cα1−Cα4 less than 7 Å was also high (58%). However, like BTD (Table 3) the percentage of conformers with the i to $i + 3$ hydrogen bond is very low (1%, 0%).

Modifying the chirality of this compound gave more significant results. Plots g and h in Figure 3 show Ac-Ala-(*S*)-indolizidinone-Ala-NHMe has only rigidified Ψ_2 and Φ_3. The percentage (Table 2) of conformers in which $|\beta|$ was less than 30° was 77% and the percentage with d under 7 Å was 85%, which was the highest value for any of the model compounds. No interaction was observed between the carbonyl oxygen of residue i and the amide hydrogen of residue $i + 3$ (0%, 0%).

However, the hydrogen bond between the amide hydrogen of residue $i + 1$ and the carbonyl oxygen of residue $i + 3$ was found in the lowest energy conformation. These results are consistent with the NMR studies in DMSO of the cyclic pseudopentapeptide (c(Arg-Gly-Asp-BTD)).[18] These results suggest that the geometry of a turn induced by BTD and indolizidinone differs significantly from that of an ideal β-turn and that (*S*)-indolizidinone is more effective as a reverse turn than other β-turn mimetics.

BILD1263. Moss et al.[91] have reported a new class of peptidomimetic inhibitor, BILD 1263 (Figure 9), effective against herpes simplex virus (HSV) *in vitro* and *in vivo*. To investigate whether the Monte Carlo/stochastic dynamics simulations roughly represent the conformers present in aqueous

(90) Genin, M. J.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **1993**, *36*, 3481−3483.

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solution, the simulation using the GB/SA water model was performed on BILD 1263. Table 2 shows that, for BILD 1263, $|\beta|$ is less than 30° in only 9% of the MC/SD structures and only 6% have a Cα1−Cα4 distance of less than 7 Å. No interaction was observed between the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ (0%, 0%). These three measures of reverse-turn propensity reflect predominately extended structures. The distance between the amide hydrogen of *tert*-butyl in residue 2 and the α-hydrogen of valine in residue 1 is 1.9 Å in the lowest energy conformation. The *N*-methyl hydrogen of valine in residue 1 is 2.1 Å apart from the β-hydrogen of valine in residue 1 and the α-hydrogen of *N*-terminal dibenzylacetyl. These results are consistent with NOE data of the NMR studies of Moss et al.[90] It was found that the distance between the *N*-methyl hydrogen of valine in residue 1 and the α-hydrogen of *N*-terminal dibenzylacetyl is 4.3 Å due to *cis*-amide in the second lowest minimum energy conformation (13 kJ/mol higher than the lowest energy conformer).

Cyclo(D-Pro-L-Pro-D-Pro-L-Pro). We studied cyclo(D-Pro-L-Pro-D-Pro-L-Pro) where Chalmers and Marshall with the original AMBER* parameters found a poor correlation with experiment.[67] This cyclic tetrapeptide is well characterized in water solution and in the solid state[92] and was found to exist exclusively in a conformation having alternating *cis*- and *trans*-amide bonds: the *cis-trans-cis-trans* (*ctct*) conformation. McDonald and Still reevaluated the conformational preferences of this compound using new AMBER* all-atom parameters in GB/SA water.[68] The authors found that all conformations within 3 kcal/mol of the global minimum have the experimentally observed *ctct* structure. To compare this result with the new AMBER* all-atom parameters, we performed the conformational search of this cyclic tetrapeptide with the MMFF* all-atom force field in GB/SA water. From this search we found that all conformations within 4 kcal/mol of the global minimum have the experimentally observed *ctct* structure.

Conclusions

As noted above, Monte Carlo searches on the model blocked tetrapeptides of the type Ac-Ala-Pro-Pro-Ala-NHMe with the new AMBER* parameters show differences from the results with the original AMBER* parameters. The new set of AMBER* parameters specifically for proline residues generally decreases the *cis*−*trans* energy differences for the amide bond. When Monte Carlo/stochastic dynamics simulations from the GB/SA solvation model were performed, calculated observables such as the reverse-turn forming ratios are found to be generally in good agreement with available experimental data measured in solution.

N-Methylation and N-hydroxylation of the amide bond between residues $i + 1$ and $i + 2$ can be used as effective reverse-turn constraints with D-Pro-NMe-amino acid being a specific example. Constraints by the introduction of two proline residues (Pro-D-Pro or D-Pro-Pro) destabilize the β-turn propensity compared to *N*-methyl and *N*-hydroxy analogues of Pro-Ala, or the larger ring homolog pipecolic acid, which contains a six-membered ring in the third position ($i + 2$) (D-Pro-Pip). Surprisingly, swapping the chirality of only one residue (Pro-Pro) results in the higher percentages of conformers which can be classified as type VIa β-turns than those of Pro-D-Pro or D-Pro-Pro due to the stabilization of the *cis* conformer of the amide bond between residues $i + 1$ and $i + 2$. Simple inclusion of N-methylation and N-hydroxylation of the amide bond between residues $i + 1$ and $i + 2$ and the larger ring homolog pipecolic acid in the third position ($i + 2$) causes significant nucleation of reverse-turn structures.

Spirotricycle restricts three of the four torsion angles that make up a type II β-turn. Spirolactam also restricts two of the four torsion angles as effective β-turn constraints. However, no interaction was observed between the carbonyl oxygen of residue *i* and the amide hydrogen of residue $i + 3$ in (*R*)-indolizidinone, (*S*)-indolizidinone, and BTD, while the hydrogen bond between the amide hydrogen of residue $i + 1$ and the carbonyl oxygen of residue $i + 3$ was found in the lowest energy conformation of (*S*)-indolizidinone. These unexpected results show that the geometry of a turn induced by indolizidinone and BTD differs significantly from that of an ideal β-turn and (S)-indolizidinone is more effective as a reverse turn than as a β-turn mimetic. We believe that these non-peptide systems can serve as a useful conformational constraint that, when incorporated into the structure of selected bioactive peptides, will yield new conformationally constrained peptide analogs for combinatorial libraries and structure−activity relationship studies.

The compounds with inclusion of such constraints, predetermined conformational effects, and accessible synthetic routes to fixed side chain placement provide ideal structural probes for applications in combinatorial libraries. Analysis of activity from screening of such libraries can be used to test for a consistent hypothesis concerning the receptor-bound conformation of the parent peptide. This hypothetical conformation can then be used to select additional classes of modifications (cyclization, new scaffold, etc.) to be included in further optimization of the receptor-bound conformation as well as the pharmacological properties.

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