Pro-D-NMe-Amino Acid and D-Pro-NMe-Amino Acid: Simple, Efficient Reverse-Turn Constraints

David K. Chalmers[†] and Garland R. Marshall*[,†,‡]

Contribution from the Department of Molecular Biology and Pharmacology and the Center for Molecular Design, Washington University, St. Louis, Missouri 63130

Received January 9, 1995[⊗]

Abstract: The design of mimetics that, when incorporated into a peptide, constrain it to adopt a reverse turn is currently of considerable interest. Numerous bicyclic dipeptide surrogates have been suggested which fix two of the four backbone dihedral angles of residues $i + 1$ and $i + 2$ of the turn. In this paper, we report the conformational analysis of tetrapeptides containing several bicyclic mimetics, sequences containing proline and other *N*-methylamino acids in the $i + 1$ and $i + 2$ positions of the turn, and control peptide sequences using a Monte Carlo conformational search followed by molecular dynamics simulation in water as implicitly represented by a solvation model. These studies indicate that the sequences D-Pro-*N*-methylamino acid (D-Pro-NMe-AA) and Pro-D-NMe-AA are effective at stabilizing the *trans* conformer of the amide bond between residues $i + 1$ and $i + 2$ and constraining three of the four backbone dihedral angles to those associated with structures which satisfy several criteria for reverse turns. In many cases, these sequences were more effective than more complicated polycyclic mimetics. D-Pro-NMe-AA and Pro- D-NMe-AA are compatible with conventional peptide synthesis methods and should provide a simple method to probe receptors for reverse-turn recognition through combinatorial libraries and structure–activity studies.

Introduction

Reverse turns are common motifs in protein structure and have often been implicated as recognition elements.[1–3] High-resolution examples of turns as recognition motifs can be found in crystal structures of antibody–peptide complexes.[2,4,5] These complexes are entirely consistent with the receptor recognition of turn motifs deduced from structure–activity studies of the peptide hormones angiotensin II,[6,7] bradykinin,[8,9] GnRH (gonadotrophin-releasing hormone),[10,11] somatostatin,[12,13] and many others. In this study, we have chosen to focus on β-turns which contain four consecutive residues where the chain changes direction by almost 180° and a hydrogen bond exists between the carbonyl oxygen of the first residue (i) and the amide hydrogen of the fourth residue ($i + 3$).[1]

The prevalence of this motif in recognition has led to the development of numerous cyclic and bicyclic dipeptide analogs[14–16] intended to stabilize the peptide chain in a reverse turn. Examples of modifications which enhance reverse-turn propensity are the dipeptide lactam,[17] the bicyclic dipeptide BTD[18] and similar proline derivatives,[19] spirolactam–bicyclic and tricyclic systems based on proline,[20–23] substitution by α,α-dialkylamino acids,[20,24–26] *N*-aminoproline,[27] functionalized dibenzofurans,[28–30] and substitution by dehydroamino acids.[31–34] Other efforts have focused on stabilizing type VI β-turns by

* To whom correspondence should be addressed at the Center for Molecular Design.
† Department of Molecular Biology and Pharmacology.
‡ Center for Molecular Design.
⊗ Abstract published in *Advance ACS Abstracts*, June 1, 1995.

(1) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1–109.
(2) Stanfield, R. L.; Fieser, T. M.; Lerner, R. A.; Wilson, I. A. *Science* **1990**, *248*, 712–719.
(3) Marshall, G. R. *Curr. Opin. Struct. Biol.* **1992**, *2*, 904–919.
(4) Rini, J. M.; Schulze-Gahmen, U.; Wilson, I. A. *Science* **1992**, *255*, 959–965.
(5) Garcia, K. C.; Ronco, P. M.; Veroust, P. J.; Brunger, A. T.; Amzel, L. M. *Science* **1992**, *257*, 502–507.
(6) Plucinska, K.; Kataoka, T.; Yodo, M.; Cody, W. L.; He, J. X.; Humblet, C.; Lu, G. H.; Lunney, E.; Major, T. C. *J. Med. Chem.* **1993**, *36*, 1902–1913.
(7) Nikiforovich, G. V.; Marshall, G. R. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 222–228.
(8) Kaczmarek, K.; Li, K.-M.; Skeean, R.; Dooley, D.; Humblet, C.; Lunney, E.; Marshall, G. R. In *Peptides: Proceedings of the 13th American Peptide Symposium*; Hodges, R., Smith, J. A., Eds.; ESCOM Scientific Publishers: Leiden, 1994; pp 687–689.
(9) Kyle, D. J.; Blake, P. R.; Smithwick, D.; Green, L. M.; Martin, J. A. *J. Med. Chem.* **1993**, *36*, 1450–1460.
(10) Nikiforovich, G.; Marshall, G. R. *Int. J. Pept. Protein Res.* **1993**, *42*, 171–180.
(11) Nikiforovich, G. V.; Marshall, G. R. *Int. J. Pept. Protein Res.* **1993**, *42*, 181–193.
(12) Nutt, R. F.; Veber, D. F.; Saperstein, R. *J. Am. Chem. Soc.* **1980**, *102*, 6539–6545.
(13) Brady, S. F.; Paleveda, W. J., Jr.; Arison, B. H.; Saperstein, R.; Brady, E. J.; Raynor, K.; Reisine, T.; Veber, D. F.; Freidinger, R. M. *Tetrahedron* **1993**, *49*, 3449–3466.
(14) Ball, J. B.; Alewood, P. F. *J. Mol. Recognit.* **1990**, *3*, 55–64.
(15) Holzemann, G. *Kontakte (Darmstadt)* **1991**, 3–12.
(16) Holzemann, G. *Kontakte (Darmstadt)* **1991**, 55–63.
(17) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104–109.
(18) Nagai, U.; Sato, K.; Nakamura, R.; Kato, R. *Tetrahedron Lett.* **1993**, *49*, 3577–3592.
(19) Lombart, H.-G.; Lumbell, W. D. *J. Org. Chem.* **1994**, *59*, 6147–6149.
(20) Hinds, M. G.; Richards, N. G. J.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1447–1449.
(21) Hinds, M. G.; Welsh, J. H.; Brennand, D. M.; Fisher, J.; Glennie, M. J.; Richards, N. G. J.; Turner, D. L.; Robinson, J. A. *J. Med. Chem.* **1991**, *34*, 1777–1789.
(22) Genin, M. J.; Johnson, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8778–8783.
(23) Ward, P.; Ewan, G. B.; Jordan, C. C.; Ireland, S. J.; Hagan, R. M.; Brown, J. R. *J. Med. Chem.* **1990**, *33*, 1848–1851.
(24) Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; Blasio, B. D.; Pavone, V.; Pedone, C. *Biopolymers* **1983**, *22*, 205–215.
(25) Valle, G.; Crisma, M.; Toniolo, C.; Sudhanand; Rao, R. B.; Sukumar, M.; Balaram, P. *Int. J. Pept. Protein Res.* **1991**, *38*, 511–518.
(26) Welsh, J. H.; Zerbe, O.; von Philipsborn, W.; Robinson, J. A. *FEBS Lett.* **1992**, *297*, 216–220.
(27) Zerkout, S.; Dupont, V.; Aubry, A.; Vidal, J.; Collet, A.; Vicherat, A.; Marraud, M. *Int. J. Peptide Protein Res.* **1994**, *44*, 378–384.
(28) Diaz, H.; Espina, J. R.; Kelly, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 8316–8318.
(29) Diaz, H.; Tsang, K. Y.; Choo, D.; Kelly, J. W. *Tetrahedron* **1993**, *49*, 3533–3545.
(30) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. *J. Am. Chem. Soc.* **1994**, *116*, 3988–4005.

0002-7863/95/1517-5927$09.00/0

stabilizing a *cis*-amide bond through disulfide bonds,[13,35] by incorporation of tetrazole rings as *cis*-amide bond surrogates,[36-38] or by incorporation of certain sequences into cyclic peptides.[39] In other turn mimetics, hydrogen-bonding groups stabilizing the turn are replaced by covalent bonds.[40-47] Benzodiazepines have also been used as turn mimetics.[48-50] The introduction of constrained amino acids, or larger structural units such as bicyclic dipeptides, into peptides is an important technique for the study of conformation and its relation to bioactivity.

Unfortunately, many of methods listed above for the induction of reverse turns, particularly the polycyclic systems, require multistep syntheses for their preparation and severely limit the possibilities for the incorporation of side chain groups into the turn. In this paper, we describe theoretical calculations which suggest that the simple incorporation of Pro-D-NMe-AA or D-Pro-NMe-AA (AA = amino acid other than Gly) into a peptide sequence stabilizes a β-turn as well, or better, than many of the more complicated alternatives. Pro-NMe-AA sequences are readily available using standard peptide synthesis methods with commercially available starting materials. This sequence should allow the simple induction of a β-turn with side chain functionality in the $i + 2$ position. The availability of chimeric proline analogs,[6,8,51-54] where side chain functional groups have been attached to the proline ring, suggests the preparation of analogs of D-Pro-NMe-AA and Pro-D-NMe-AA which contain side chain functional groups at all positions in the turn. The "off the shelf" nature of these reverse turn mimetics should facilitate the determination of the receptor-bound conformation of peptides using automated and peptide library methods for developing bioactive compounds.

Methods

Conformational searches and molecular dynamics were performed using Macromodel[55] version 4.5. The Macromodel implementations of either the AMBER all-atom force field,[56] MM2,[57] MM3,[58] or the AMBER/OPLS united-atom force fields[59] were used (respectively denoted AMBER*, MM2*, MM3*, and AMBER/OPLS*). All calculations were performed using the implicit water GB/SA solvation model of Still et al.[60] Results for ideal β-turns were calculated by setting the conformation of the peptide to the ideal torsional angles associated with that turn and minimizing with the AMBER all-atom potential and the GB/SA solvation model. The sterically allowed maps were calculated with the systematic search module of SYBYL utilizing the calibrated VDW derived for peptides by Iijima et al.[61]

Conformational Searches. Conformational searches were performed using the systematic Monte Carlo method of Goodman and Still.[62] 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*-dialkylamino acids (Pro, Pip, NMeAla, etc.) whose imide bonds were purposefully sampled and accepted with either *cis* or *trans* geometry in the conformational search. For each search, 5000 starting structures were generated and minimized until the gradient was less than 0.05 (kJ/mol)/Å 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. Conformations were tested to confirm that they were true minima.

Molecular Dynamics Simulations. Molecular dynamics simulations were performed at 300 K using the AMBER* all-atom force field with a time step of 1.5 fs. Hydrogen atoms were constrained using the SHAKE algorithm. Two protocols were used to examine the effects of selection of starting conformers on the distribution of turnlike conformers: (protocol A) For each model compound, 20 runs of 50 ps each were performed, commencing with the 20 lowest energy structures found using the conformational search described above. Samples were taken at 1 ps intervals, yielding 1000 conformations for analysis. (protocol B) Conformers less than 15 kJ above the local minimum were used as starting conformations (range of the number of conformers, 8-65, Table 3). At least 25 and up to 125 ps of dynamics was run for each starting conformation to generate approximately 1000 conformations for analysis in each case. The results from both methods were quantitatively similar, and only the data using protocol B are presented.

(31) Bach, A. C., II; Gierasch, L. M. *J. Am. Chem. Soc.* **1985**, *107*, 3349-3350.
(32) Bach, A. C., II; Gierasch, L. M. *Biopolymers* **1986**, *25*, S175-S191.
(33) Chauhan, V. S.; Sharma, A. K.; Uma, K.; Paul, P. K. C.; Balaram, P. *Int. J. Pept. Protein Res.* **1987**, *29*, 126-133.
(34) Palmer, D. E.; Pattaroni, C.; Nunami, K.; Chadha, R. K.; Goodman, M.; Wakamiya, T. *J. Am. Chem. Soc.* **1992**, *114*, 5634-5642.
(35) Sukumaran, D. K.; Prorok, M.; Lawrence, D. S. *J. Am. Chem. Soc.* **1991**, *113*, 706-707.
(36) Zabrocki, J.; Smith, G. D.; Dunbar Jr., J. B.; Iijima, H.; Marshall, G. R. *J. Am. Chem. Soc.* **1988**, *110*, 5875-5880.
(37) Smith, G. D.; Zabrocki, J.; Flak, T. A.; Marshall, G. R. *Int. J. Pept. Protein Res.* **1991**, *37*, 191-197.
(38) Zabrocki, J.; Dunbar Jr., J. B.; Marshall, K. W.; Toth, M. V.; Marshall, G. R. *J. Org. Chem.* **1992**, *57*, 202-209.
(39) Muller, G.; Gurrath, M.; Kurz, M.; Kessler, H. *Proteins: Struct., Funct., Genet.* **1993**, *15*, 235-251.
(40) Kahn, M.; Nakanishi, H.; Chrusciel, R. A.; Fitzpatrick, D.; Johnson, M. E. *J. Med. Chem.* **1991**, *34*, 3395-3399.
(41) Nakanishi, H.; Chrusciel, R. A.; Shen, R.; Bertenshaw, S.; Johnston, M. E.; Rydel, T. J.; Tulinsky, A.; Kahn, M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1705-1709.
(42) Chen, S.; Chrusciel, R. A.; Nakanishi, H.; Raktabutr, A.; Johnson, M. E.; Sato, A.; Weiner, D.; Hoxie, J.; Saragovi, H. U.; Greene, M. I.; Kahn, M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5872-5876.
(43) Gardner, B.; Nakanishi, H.; Kahn, M. *Tetrahedron* **1993**, *49*, 3433-3448.
(44) Arrhenius, T.; Satterthwait, A. C. In *Peptides: Chemistry, Structure and Biology*; Rivier, J. E., Marshall, G. R., Eds.; ESCOM Scientific Publishers: Leiden, 1990; pp 870-872.
(45) Callahan, J. F.; Newlander, K. A.; Burgess, J. L.; Eggleston, D. S.; Nichols, A.; Wong, A.; Huffman, W. F. *Tetrahedron* **1993**, *49*, 3479-3488.
(46) Hermkens, P. H. H.; v. Dinther, T. G.; Joukema, C. W.; Wagenaars, G. N.; Ottenheijm, H. C. J. *Tetrahedron Lett.* **1994**, *35*, 9271-9274.
(47) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580-11581.
(48) Ripka, W. C.; DeLucca, G. V.; Bach, A. C., II; Pottorf, R. S.; Blaney, J. M. *Tetrahedron* **1993**, *49*, 3593-3608.
(49) Ripka, W. C.; Lucca, G. V. D.; Bach, A. C., II; Pottorf, R. S.; Blaney, J. M. *Tetrahedron* **1993**, *49*, 3609-3628.
(50) Ku, T. W.; Ali, F. E.; Barton, L. S.; Bean, J. W.; Bondinell, W. E.; Burgess, J. L.; Callahan, J. F.; Calvo, R. R.; Chen, L.; Eggelston, D. S.; Gleason, J. S.; Huffman, W. F.; Hwang, S. M.; Jakas, D. R.; Karash, C. B.; Keenan, R. M.; Kopple, K. D.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M. F.; Peishoff, C. E.; Samanen, J. M.; Uzinskas, I.; Venslavsky, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 8861-8862.
(51) Marshall, G. R.; Kataoka, T.; Yodo, M.; Kaczmarek, K.; Plucinzska, K.; Kao, J.; Li, K.; Zhang, W.-J.; Nikiforovich, G. In *Peptide Chemistry 1992, Proceedings of the 2nd Japanese Symposium on Peptide Chemistry*; Yanihara, N., Ed.; ESCOM Scientific Publishers: Leiden, 1993; pp 474-478.
(52) Olma, A.; Nikiforovich, G. V.; Nock, B.; Marshall, G. R. *Peptides: Chemistry, Structure and Biology (13th American Peptide Symposium)*; ESCOM Scientific Publishers: Leiden, **1994**; pp 684-686.
(53) Kolodziej, S. A.; Nikiforovich, G. V.; Martinez, J.; Marshall, G. R. *J. Med. Chem.* **1995**, *38*, 137-149.
(54) Kolodziej, S. A.; Marshall, G. R. *J. Org. Chem.*, in press.
(55) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440-467.
(56) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230-252.
(57) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127-8134.
(58) Lii, J.-H.; Allinger, N. L. *J. Comput. Chem.* **1991**, *12*, 186-199.
(59) Tirado-Rives, J.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1990**, *112*, 2773-2781.
(60) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127-6129.
(61) Iijima, H.; Dunbar Jr., J. B.; Marshall, G. R. *Proteins* **1987**, *2*, 330-339.
(62) Goodman, J. M.; Still, W. C. *J. Comput. Chem.* **1991**, *12*, 1110-1117.

Results and Discussion

As part of our continuing investigations in the design of bicyclic peptidomimetics derived from proline[63,64] and the effects of side chain cyclization on peptide conformation,[65] we observed that the incorporation of consecutive proline residues would stabilize a reverse turn. Proline is found in all four positions of β-turns in proteins, particularly in position 2 ($i + 1$),[66] and others had commented on turn stabilization by D-Pro-Pro and even incorporated this sequence into peptides for this purpose.[67,68] However, to date there has been no detailed conformational analysis of the effect of this sequence on peptide conformation, and comparisons with other turn mimetics have not been reported. Inspection of the torsional requirements for ideal β-turns suggested that effective turn constraints might be produced when the sequences D-Pro-Pro or Pro-D-Pro were used. This was supported by preliminary conformational searches and energy minimizations (Figure 1). We, therefore, performed more extensive calculations employing systematic Monte Carlo conformational searches and molecular dynamics simulations using implicit solvation by water of model tetrapeptides designed to mimic minimal reverse-turn segments. Although several attempts have been made to classify the type of β-turn stabilized by the various turn mimetics, little has been done to quantitate the relative stabilization of turn versus extended structure as a basis for comparison.

Conformational Searching. Conformational searches were performed on model blocked tetrapeptides of the type Ac-Ala-Xxx-Yyy-Ala-NHMe. Because *cis*−*trans* isomerism is frequently observed in imide bonds, it was necessary that the Monte Carlo search included these torsion angles. The alkylation of the amide nitrogen reduces the energy difference between the *cis* and *trans* forms to approximately 2.1 kJ/mol in contrast to the "conventional" amino acids where this energy difference is around 10.9 kJ/mol.[69] Class VI β-turns observed in proteins have a *cis*-proline at position 3 ($i + 2$). 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 results of the conformational searches for Xxx = Pro and Yyy = Pro are summarized in Table 1. Several force fields were investigated. Initial investigations were performed using the united-atom force field, AMBER/OPLS*, which provides more rapid conformational searching. However, although the qualitative results obtained (Table 1) were similar to those obtained for MM3* and the AMBER* all-atom force field, the energetic results, particularly for imide *cis*−*trans* isomerism, were substantially different. This is somewhat surprising considering the effort to parameterize the amide bond by the Jorgensen group,[70] and may reflect either insufficient sampling or an implementation error in Macromodel (both are under investigation). As shown in Table 1, the results from the AMBER* all-atom and the MM3* force fields for *cis*−*trans* isomerism of the imide bond (ω_{23}) of Pro-Pro are in reasonable agreement with the overall AMBER all-atom results appearing more intermediate. In order to gain further insight into possible causes for the discrepancies in the results as a function of force field, the minima found for *cis*−*trans* conformers of *N*-methylformamide and *N*-methylacetamide *in vacuo* and using the GB/SA water solvation model were compared (Table 2). In these examples, agreement with limited experimental results would appear to favor the MM3* or AMBER/OPLS* parametrization. However, the availability of parameters for functional

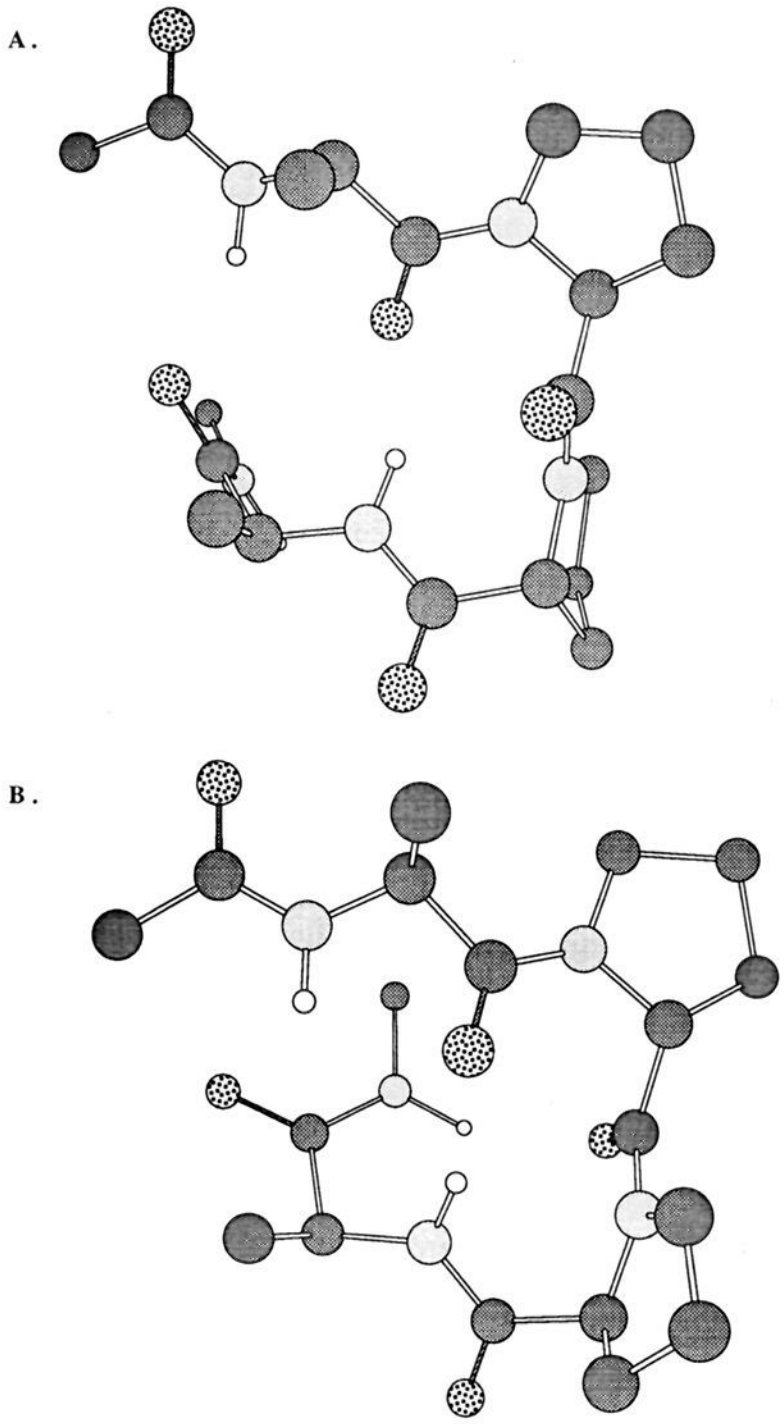

Figure 1. (A) Structure of the global energy minimum found by a Monte Carlo search of Ac-Ala-Pro-D-Pro-Ala-NHMe using the AMBER* all-atom force field and the GB/SA solvation water model. Note the hydrogen bond between the carbonyl oxygen of residue 1 (i) and the amide hydrogen of residue 4 ($i + 3$). (B) Structure of the global energy minimum found by a Monte Carlo search of Ac-Ala-D-Pro-Pro-Ala-NHMe. Note the hydrogen bond between the carbonyl oxygen of residue 1 (i) and the amide hydrogen of residue 4 ($i + 3$).

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

	$\Delta E(\omega_{12}$ *cis*−*trans*) (kJ/mol)			$\Delta E(\omega_{23}$ *cis*−*trans*) (kJ/mol)		
Xxx-Yyy	AMBER/ OPLS*	AMBER* all-atom	MM3*	AMBER/ OPLS*	AMBER* all-atom	MM3*
Pro-Pro	7.4	5.0	1.2	3.8	-0.1[a]	2.4
Pro-D-Pro	8.6	6.2	8.8	25.3	8.5	8.4
D-Pro-Pro	15.2	16.5	5.9	24.6	13.0	14.1
D-Pro-D-Pro	18.1	8.2	6.9	9.0	-0.9[a]	7.7

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

(63) Cornille, F.; Fobian, Y. M.; Slomczynska, U.; Beusen, D. D.; Marshall, G. R.; Moeller, K. D. *Tetrahedron Lett.* **1994**, *35*, 6989−6992.
(64) Cornille, F.; Slomczynska, U.; Smythe, M. L.; Beusen, D. D.; Moeller, K. D.; Marshall, G. R. *J. Am. Chem. Soc.* **1995**, *117*, 909−917.
(65) Kataoka, T.; Beusen, D. D.; Clark, J. D.; Yodo, M.; Marshall, G. R. *Biopolymers* **1992**, *32*, 1519−33.
(66) Chou, P. Y.; Fasman, G. D. *J. Mol. Biol.* **1977**, *115*, 135−175.
(67) Bean, J. W.; Kopple, K. D.; Peishoff, C. E. *J. Am. Chem. Soc.* **1992**, *114*, 5328−5334.
(68) McElroy, A. B.; Clegg, S. P.; Deal, M. J.; Ewan, G. B.; Hagan, R. M.; Ireland, S. J.; Jordan, C. C. *J. Med. Chem.* **1992**, *35*, 2582−2591.
(69) Stewart, D. E.; Sarkar, A.; Wampler, J. E. *J. Mol. Biol* **1990**, *214*, 253−260.
(70) Jorgensen, W. L.; Gao, J. *J. Am. Chem. Soc.* **1988**, *110*, 4212−4216.

Table 2. Energy Differences (kJ/mol, numbers in parentheses are in kcal/mol) for *cis*−*trans* Conformers of *N*-Methylformamide and *N*-Methylacetamide *in Vacuo* and Using the GB/SA Solvation Water Model and Evaluation with Three Different Force Fields

force field	AMBER/OPLS*	AMBER* all-atom	MM3*
	N-Methylformamide		
solvated (H_2O)			
cis	−77.10	−69.67	−58.20
trans	−75.90	−72.27	−68.04
ΔE	−1.20 (−0.29)	5.30 (1.27)	9.84 (2.35)
unsolvated			
cis	−59.76	−45.25	−35.89
trans	−55.82	−49.58	−42.24
ΔE	−3.94 (−0.83)	4.33 (1.03)	6.35 (1.52)
	N-Methylacetamide		
solvated (H_2O)			
cis	−77.56	−85.13	−51.14
trans	−93.91	−92.03	−63.48
ΔE	16.35 (3.91)	6.90 (1.65)	12.34 (2.95)
unsolvated			
cis	−52.78	−60.23	−29.53
trans	−63.34	−74.84	−39.09
ΔE	10.56 (2.52)	14.61 (3.49)	9.56 (2.29)

groups present in several of the polycyclic peptidomimetics led us to use AMBER* for all subsequent calculations.

In order for compounds containing a D-Pro-Pro or Pro-D-Pro sequence at position 2 ($i + 1$) and 3 ($i + 2$) to be good reverse-turn constraints, it is necessary that the imide bonds ω_{12} and ω_{23} of the prolyl residues remain *trans*. This is due to the incompatibility of the dihedral angle requirements for type VI turns[1] (VIa, $\Phi_{i+1} = -60°$, $\Psi_{i+1} = 120°$, $\Phi_{i+2} = -90$, $\Psi_{i+2} = 0°$; VIb, $\Phi_{i+1} = -120°$, $\Psi_{i+1} = 120°$, $\Phi_{i+2} = -60°$, $\Psi_{i+2} = 0°$) with those required by either D-Pro-Pro ($\Phi_{i+1} = 60-70$ by the pyrrolidine ring constraint, no low-energy *cis*-amides found) or Pro-D-Pro ($\Phi_{i+2} = 60-70$ by the pyrrolidine ring constraint). As can be seen from Table 1, the AMBER* all-atom ΔE for relative *cis*−*trans* stability was found to be low or negative for both ω_{12} and ω_{23} in Pro-Pro and D-Pro-D-Pro. Upon inversion of chirality of either one of the proline α-carbons, however, a dramatic destabilization of the *cis* conformers occurs; both Ac-Ala-Pro-D-Pro-Ala-NHMe and Ac-Ala-D-Pro-Pro-Ala-NHMe have a significant energy difference of approximately 8−13 kJ/mol between the *cis* and *trans* conformations of the imide (ω_{12}) of the first proline residue, and dramatic stabilization of the *trans* conformation (22−25 kJ/mol) of the intervening imide bond (ω_{23}) between the two proline residues. The minimum energy structures of Ac-Ala-Pro-D-Pro-Ala-NHMe and Ac-Ala-D-Pro-Pro-Ala-NHMe are shown in Figure 1. It is clear that hydrogen bonding between the carbonyl oxygen of residue 1 and the NH of residue 4 plays an important part in stabilizing the β-turn in both model tetrapeptides.

Molecular Dynamics. The conformational search procedure used above gives an indication of the conformational space accessible to each of the model compounds. Because each of the structures produced by the search is a local minimum energy structure (see Figure 1 for the minimum energy conformations of Ac-Ala-Pro-D-Pro-Ala-NHMe and Ac-Ala-D-Pro-Pro-Ala-NHMe), there is no indication of the size of each energy well, or estimate of the free energy difference. In the case of conformationally unrestricted compounds, with large, shallow energy wells, examination of the minima alone can be particularly misleading. A molecular dynamics procedure was, therefore, used to investigate the conformations available to each of the compounds in the vicinity of their local minima. In other words, the difference in free energy rather than enthalpic differences between the β-turn and other conformations are relevant, and one could hope to approximate free energies through population analysis of adequately sampled conformations.

Table 3. Percentage of Tetrapeptide Conformers of Blocked Tetrapeptides Ac-Ala-Xxx-Yyy-Ala-NHMe which Exhibit Characteristics of a Reverse Turn

Xxx-Yyy	no. of structures < 15 kJ/mol	% $\lvert\beta\rvert$ < 30°	% d < 7 Å	% d(C=O···H−N) < 4 Å
Ala-Ala	11	13	14	1
Pro-Pro	65	15	11	20
Pro-D-Pro	52	45	77	21
D-Pro-Pro	30	59	74	38
D-Pro-D-Pro	56	15	46	25
Ala-D-Pro	29	30	55	18
D-Ala-Pro	55	63	45	4
D-Pro-Ala	17	34	44	20
Pro-D-NMeAla	13	46	81	51
D-Pro-NMeAla	8	55	83	82
NMe-Ala-D-Pro	109	9	45	19
D-NMe-Ala-Pro	22	17	47	27
Pro-D-Pip	45	57	87	48
D-Pro-Pip	28	77	95	73
D-Pip-Pro	43	51	74	34
BTD	14	73	48	17
(*R*)-spirolactam	46	34	47	38
(*S*)-spirolactam	10	78	69	69
spirotricycle	6	91	53	51
Ser-((*R*)-ec)-Pro	28	76	54	3
Ser-((*S*)-ec)-Pro	10	63	85	29

A 1000 ps dynamics run commencing from a single starting structure was found to give inadequate coverage of conformational space; others have noted similar difficulties in systems with numerous minima separated by significant energy barriers.[71] Dynamics trajectories of this time were insufficient to cross the ω_{12} or ω_{23} *cis*−*trans* barrier in Ac-Ala-Pro-Pro-Ala-NHMe or Ac-Ala-D-Pro-D-Pro-Ala-NHMe (data not shown) because of the activation energy barrier (estimated at 20 kcal/mol,[69] 1 cal = 4.184 J) although the energy difference between these two states is low (as shown in Table 1). Therefore, a conformational search−molecular dynamics protocol was adopted to examine the conformational space most accessible to the compounds under investigation. For each tetrapeptide, conformers within 15 kJ/mol of the global minimum were selected from the Monte Carlo search of 5000 starting conformations (range of number of conformers, 8−109, Table 3). At least 25 and up to 125 ps of dynamics was run for each starting conformation of each compound in order to generate at least 1000 conformations for analysis in each case. While this protocol does not give a true Boltzmann sample of conformers which would be desirable to make the statistical comparisons equitably, it provides results that are similar[92] to those obtained using the combined Monte Carlo−stochastic dynamics protocol of Guarnieri and Still,[72] which does produce a true Boltzmann distribution. Our results are also consistent with experimental studies of tetrapeptides in solution[73] (see below). We, therefore, believe the trends seen in this study are significant and will use them as the basis for more extensive quantitative studies with explicit representation of the solvent.

A summary of the results from the molecular dynamics simulations is given in Table 3. Three parameters were used as measures of reverse-turn-forming ability: (1) The distance d between Cα1 and Cα4 of the tetrapeptide (Figure 2). A distance of less than 7 Å for this parameter is often used to define the presence of a reverse turn.[1] (2) The virtual torsion

(71) McDonald, D. Q.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 11550−11553.
(72) Guamieri, F.; Still, W. C. *J. Comput. Chem.* **1994**, *15*, 1302−1310.
(73) Imperiali, B.; Fischer, S. L.; Moats, R. A.; Prins, R. A. *J. Am. Chem. Soc.* **1992**, *114*, 3182−3188.

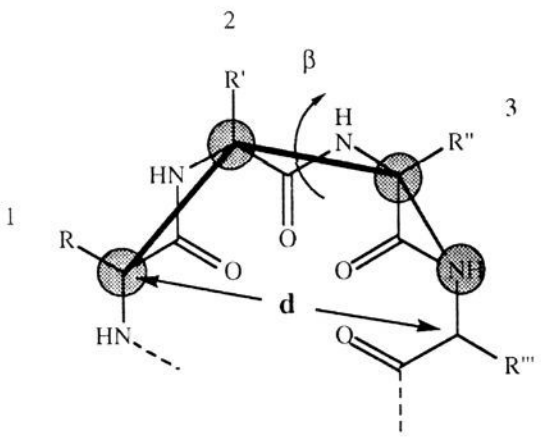

Figure 2. Schematic of a tetrapeptide showing the virtual torsion β[74] and the distance d from Cα1 to Cα4 used to characterize reverse turns.

angle, β, defined by the atoms Cα1, Cα2, Cα3, and N4 (Figure 2).[14,74] The range $0 \pm 30°$ was taken to indicate a tight reverse turn. (3) The distance between the carbonyl oxygen of residue i and the amide hydrogen of residue $i + 3$ which 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.

Ac-Ala-Ala-Ala-Ala-NHMe. As a control, the combined conformational search−molecular dynamics protocol, described above, was performed on Ac-Ala-Ala-Ala-Ala-NHMe which is constrained only by the side chain methyl groups and the restricted rotation around the amide bonds. To visualize the results of the simulations, plots of the backbone angle Φ against angle Ψ for residues 2 ($i + 1$) and 3 ($i + 2$) were found to be particularly useful in understanding the conformational behavior of the model tetrapeptides. Figure 3 shows plots of the backbone angles for selected compounds where each point represents one conformation sampled from the molecular dynamics simulation. Conformations were sampled at 1 ps intervals from approximately 1000 ps of dynamics simulation for each tetrapeptide.

Plots of Φ_2 against Ψ_2 and Φ_3 against Ψ_3 for the alanine tetrapeptide are shown in parts A and B, respectively, of Figure 3. These graphs show clustering around dihedral angles associated with extended structures ($\Phi_2 = -162°$, $\Psi_2 = 140°$, $\Phi_3 = -163°$, $\Psi_3 = 138°$), but with considerable variation (rmsd > 42°). The two measures of reverse-turn propensity, β and d (Table 3), also reflect predominately extended structures. A plot of d against potential energy (Figure 4A) shows that, for most of the structures, and particularly those with lower energies, this distance is greater than 10 Å. Plotting the parameter β against potential energy (Figure 5A) shows a wide scatter of values, with lower energy structures favoring values closer to 180°. Table 3 shows that the percentage of conformers which can be classified as turns is around 14% ($|\beta| < 30°$, 13%; $d <$ 7 Å, 14%). This is further reinforced by plotting the distance between the carbonyl oxygen of residue i and the amide hydrogen of residue $i + 3$ (Figure 6A) which reveals no evidence of hydrogen bonding between these residues.

In order to characterize the effects of the force field and minimization on classical β-turn conformations, the capped tetraalanine peptide was set to the classical torsion angles associated with the different turn types (Table 4) and minimized, both *in vacuo* and using the GB/SA solvation model. In all cases, significant changes in the torsional angles were observed, implying that the classical β-turn torsion angles do not belong to a minima, at least with the force field and minimization techniques used. As shown in Table 5, the three parameters selected for reverse-turn classification were still satisfied for the minimized structures.

Ac-Ala-Pro-D-Pro-Ala-NHMe and Ac-Ala-D-Pro-Pro-Ala-NHMe. In contrast to the case described above, the tetrapeptide Ac-Ala-Pro-D-Pro-Ala-NHMe has two backbone angles constrained by the introduction of two proline residues. Table 4 shows that during the simulation these rings tightly restrict Φ_2 to be near −66° and Φ_3 to be near 70°. Importantly, the angle Ψ_2 between the two proline residues is also tightly restricted near −133°. Angle Ψ_3 is clearly the least restricted backbone dihedral angle with an rms deviation of 60° from the most favored value of −124° (Table 4). Although some starting structures had a *cis*-amide between residues 2 and 3, the predominance of turn structures during the simulation is shown by the fact that $|\beta|$ is less than 30° in 45% of the structures, d is less than 7 Å in 77%, and the distance between the amide hydrogen and carbonyl oxygen is less than 4 Å 21% of the time. Comparison of the backbone angles of Ac-Ala-Pro-D-Pro-Ala-NMe during the dynamics simulation with the ideal values for classical β-turns (Table 4) shows that this compound adopts a structure close to a type II β-turn.

Swapping the chirality of residues $i + 2$ and $i + 3$ to give Ac-Ala-D-Pro-Pro-NMe also results in a tightly constrained structure which, in this case, has backbone angles that are associated with a type II′ β-turn ($\Phi_2 = 62°$, $\Psi_2 = -133°$, $\Phi_3 = -76°$, $\Psi_3 = 0°$). *cis*-Peptide bonds between residues 1 and 2 were present in a small number of starting conformers. As for Ac-Ala-Pro-D-Pro-Ala-NMe, Ψ_3 was the least restricted backbone angle. For tetrapeptides containing either D-Pro-Pro or Pro-D-Pro, tight reverse turns ($d = 5-6$ Å) result when Ψ_3 is in the range $0° \pm 60°$. The sinelike dependence of the Cα1−Cα4 distance upon Ψ_3 is shown in Figure 7 (all *trans* conformers are shown as diamonds; conformers where ω_{12} is *cis* are shown as crosses). This correlation shows that the backbone angles apart from Ψ_3 are relatively fixed. Table 3 shows that the percentage of conformers with $|\beta| < 30°$ and d < 7 Å has risen to 59% and 74%, respectively. The carbonyl oxygen and amide hydrogen of residues i and $i + 3$ are less than 4 Å apart in 38% of the sampled structures. Approximately 50% of the conformers found could be classified as type II′ β-turns depending on the criteria chosen, a dramatic enhancement of turn propensity.

Ac-Ala-Pro-Pro-Ala-NHMe and Ac-Ala-D-Pro-D-Pro-Ala-NHMe. Sequences containing Pro-Pro and D-Pro-D-Pro at the positions $i + 1$ and $i + 2$ were modeled to test that alternating chirality was necessary for good turn induction. It was found that Pro-Pro and D-Pro-D-Pro are poorer constraints due to enhanced *cis−trans* isomerism and the resulting small energy difference between turnlike and extended structures. Nevertheless, 20% of the conformers detected for Ac-Ala-Pro-Pro-Ala-NHMe had hydrogen-bond distances appropriate for β-turns. These arise from type VIa β-turns as well as others. Only 25% of the conformers of Ac-Ala-D-Pro-D-Pro-Ala-NHMe had hydrogen-bond distances appropriate for β-turns (Table 3).

Ac-Ala-D-Pro-Ala-Ala-NHMe and Ac-Ala-D-Ala-Pro-Ala-NHMe. To test that two consecutive proline residues were necessary for good turn induction, we investigated the sequences Ac-Ala-Pro-D-Ala-Ala-NMe, Ac-Ala-D-Pro-Ala-Ala-NHMe, Ac-Ala-Pro-D-Ala-Ala-NMe, and Ac-Ala-D-Ala-Pro-Ala-NHMe which have alternating chirality but only one proline residue in the $i + 1$ or $i + 2$ position. These sequences were found to be of intermediate turn-promoting ability with 18% of Ac-Ala-Pro-D-Ala-Ala-NMe conformers, 20% Ac-Ala-D-Pro-Ala-Ala-

(74) Ball, J. B.; Hughes, R. A.; Alewood, P. F.; Andrews, P. R. *Tetrahedron* **1993**, *49*, 3467−3478.

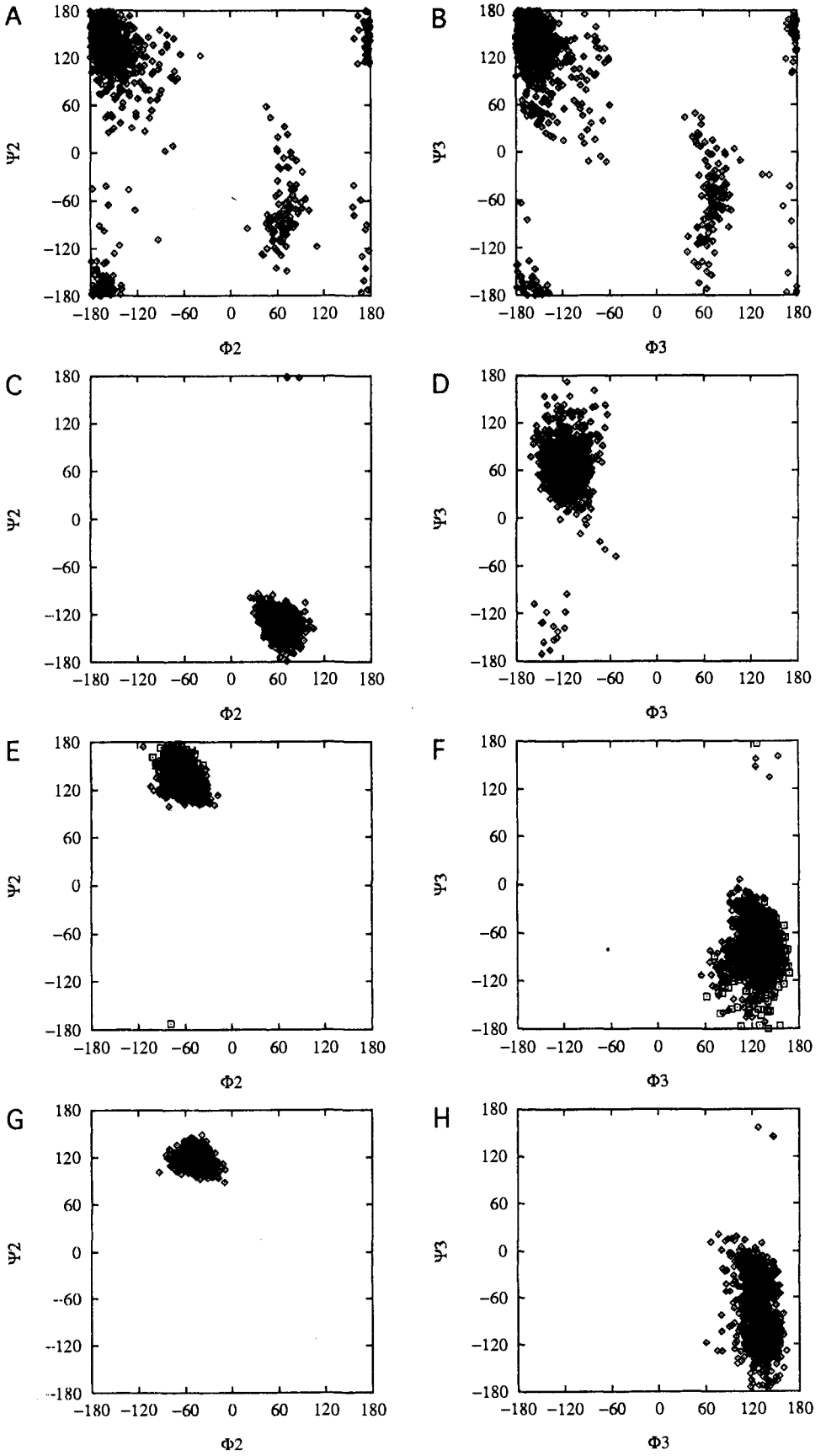

Figure 3. Backbone torsion angle Φ_2 versus Ψ_2 and Φ_3 versus Ψ_3 for selected tetrapeptides: Ac-Ala-Ala-Ala-Ala-NMe (A, B), Ac-Ala-D-Pro-NMeAla-Ala-NMe (C, D), Ac-Ala-Pro-D-NMeAla-Ala-NMe (E, F), and Ac-Ala-spirotricycle-Ala-NMe (G, H). In each case samples were taken at 1 ps intervals during approximately 1000 ps of dynamics in GB/SA water from multiple starting points. Diamonds indicate all conformations where all amides are *trans*. Squares indicate conformations where ω_{12} is *cis*.

NHMe conformers, and 4% Ac-Ala-D-Ala-Pro-Ala-NHMe conformers having hydrogen-bond distances appropriate for reverse turns (Table 3). This finding is consistent with the NMR studies of Imperiali et al. who found that tetrapeptides containing

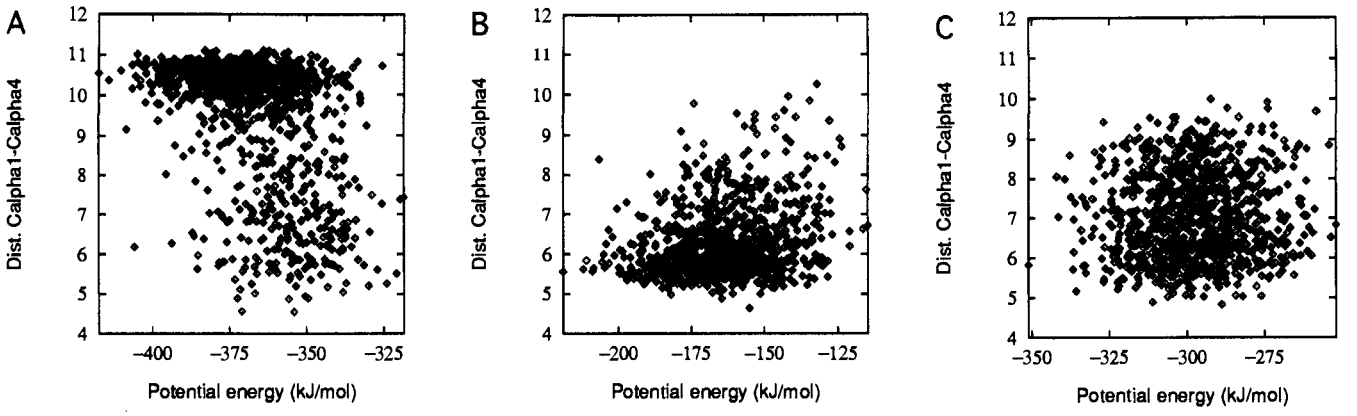

Figure 4. Distance between Cα1 and Cα4 versus potential energy during molecular dynamics simulation for (A) the unconstrained peptide Ac-Ala-Ala-Ala-Ala-NMe, (B) the tightly restricted tetrapeptide Ac-Ala-D-Pro-NMeAla-Ala-NMe, and (C) Ac-Ala-spirotricycle-Ala-NMe which contains a rigid peptidomimetic.

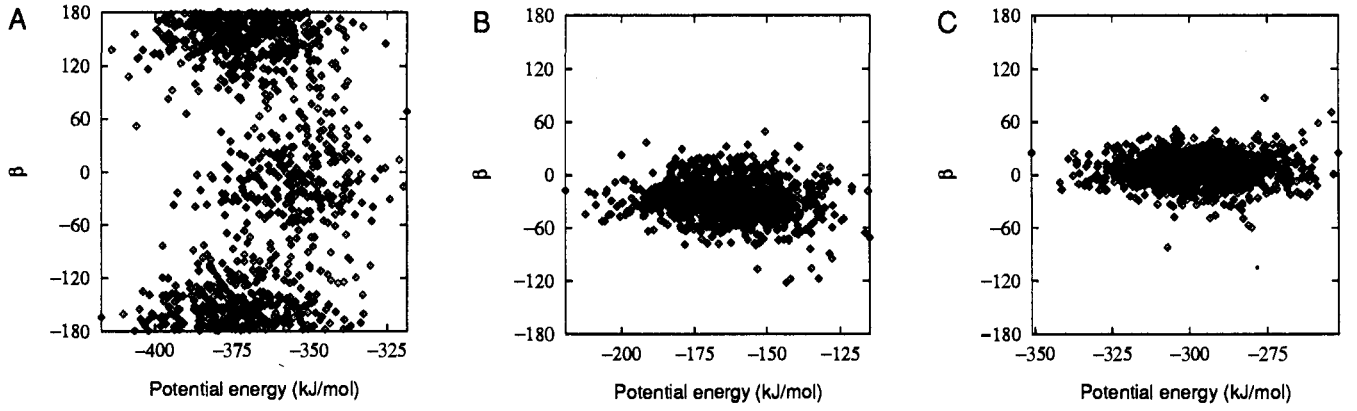

Figure 5. β-turn parameter β^{74} versus potential energy during dynamics simulation for (A) Ac-Ala-Ala-Ala-Ala-NMe which adopts a mostly extended conformation and (B) Ac-Ala-D-Pro-NMeAla-Ala-NMe and (C) Ac-Ala-spirotricycle-Ala-NMe which are tightly constrained to adopt turnlike conformations.

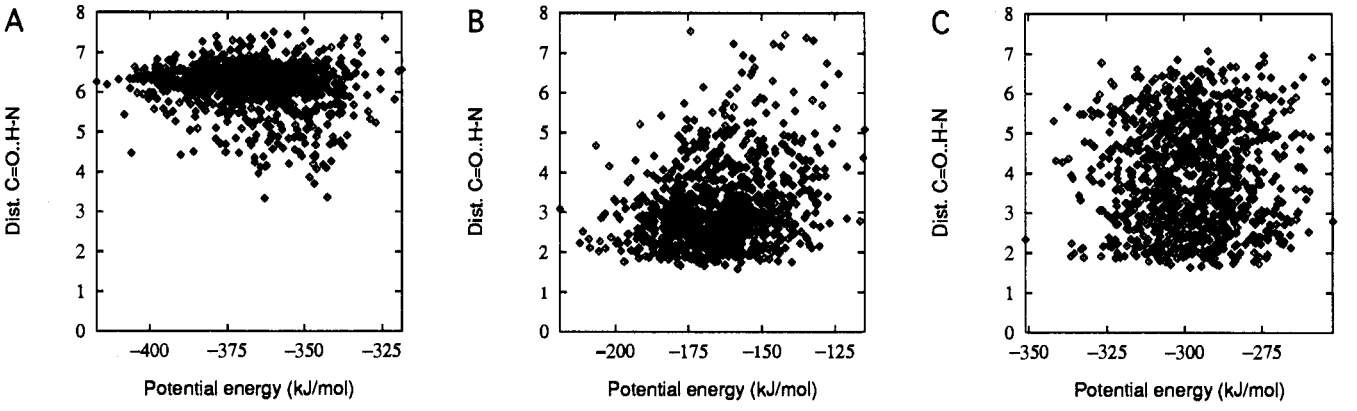

Figure 6. Distance d (Å) between the carbonyl oxygen of residue 1 and the amide hydrogen of residue 4 during dynamics simulation for (A) Ac-Ala-Ala-Ala-Ala-NMe, (B) Ac-Ala-D-Pro-NMeAla-Ala-NMe, and (C) Ac-Ala-spirotricycle-Ala-NMe.

the Pro-D-Xaa motif adopted a type II β-turn in aqueous and DMSO solutions.[73]

Ac-Ala-D-Pro-NMeAla-Ala-NHMe and Ac-Ala-Pro-D-NMeAla-Ala-NHMe. To investigate whether the proline ring itself was necessary or whether an *N*-methyl-substituted amino acid was sufficient for turn induction, we investigated tetrapeptides containing NMeAla. To our surprise, Ac-Ala-D-Pro-NMeAla-Ala-NHMe proved to be an extremely efficient turn promoter. During the molecular dynamics simulation the carbonyl oxygen of residue i and the amide hydrogen of residue $i + 3$ were less than 4 Å apart in 82% of the sampled conformers, 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 (83%). The conformational restrictions caused by this sequence are clear in plots of Φ versus Ψ for residues $i + 2$ and $i + 3$ (Figure 3C,D, respectively). Figure 4B shows a plot of the distance d versus potential energy for this tetrapeptide. The distance d is restricted to be near 6 Å in sharp contrast to the alanine tetrapeptide shown in Figure 4A. Figure 5 shows the dramatic restriction of β in the D-Pro-NMeAla sequence (B) compared to the alanine tetrapeptide (A). Similarly, Figure 6B shows that the hydrogen bond between residues i and $i + 3$ of a β-turn is present in the majority of conformers obtained from the D-Pro-NMeAla simulation, indicating the markedly enhanced propensity of this sequence for the type II′ β-turn.

The corresponding sequence with an L-amino acid in position $i + 2$ and a D-amino acid in position $i + 3$ had lower values for

Table 4. Most Populated Backbone Angles of the Tetrapeptides Ac-Ala-Xxx-Yyy-Ala-NHMe Resulting from the Dynamics Runs[a]

Xxx-Yyy	Φ_2 (deg)	Ψ_2 (deg)	Φ_3 (deg)	Ψ_3 (deg)
type I β-turn	−60	−30	−90	0
type I′ β-turn	60	30	90	0
type II β-turn	−60	120	80	0
type II′ β-turn	60	−120	−80	0
Ala-Ala	−162 (42)	140 (51)	−163 (48)	138 (59)
Pro-Pro	−65 (14)	144 (15)	−77 (16)	131 (81)
Pro-D-Pro	−66 (14)	133 (15)	70 (14)	−124 (60)
D-Pro-Pro	62 (14)	−133 (14)	−76 (15)	127 (74)
D-Pro-D-Pro	68 (15)	−144 (27)	77 (16)	−128 (72)
Ala-D-Pro	−66 (14)	130 (60)	158 (21)	−136 (44)
D-Ala-Pro	157 (25)	−142 (22)	−72 (13)	128 (79)
D-Pro-Ala	69 (13)	−121 (50)	−154 (27)	141 (47)
NMe-Ala-D-Pro	−137 (59)	86 (31)	72 (14)	−124 (58)
D-NMe-Ala-Pro	134 (27)	−91 (27)	−74 (15)	127 (57)
D-Pro-NMeAla	59 (13)	−127 (14)	−119 (17)	57 (34)
Pro-D-NMeAla	−65 (14)	144 (18)	131 (20)	−81 (34)
Pro-D-Pip	−65 (14)	147 (17)	96 (19)	−104 (53)
D-Pro-Pip	64 (13)	−141 (18)	−89 (18)	50 (43)
D-Pip-Pro	77 (20)	−131 (18)	−70 (14)	131 (68)
BTD	−176 (61)	−136 (14)	−102 (11)	37 (50)
(*R*)-spirolactam	−48 (11)	127 (10)	66 (107)	112 (104)
(*S*)-spirolactam	47 (10)	−123 (10)	−116 (26)	100 (47)
spirotricycle	−44 (12)	117 (10)	128 (15)	−102 (48)
Ser-((*R*)-ec)-Pro	−169 (79)	−127 (17)	−99 (21)	123 (81)
Ser-((*S*)-ec)-Pro	−178 (32)	−111 (16)	−66 (20)	−19 (89)

[a] Angles are the center point of the most highly populated 20° range. The rms deviation from this value is given in parentheses.

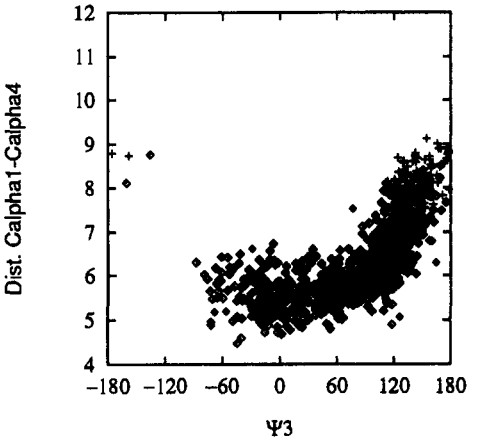

Figure 7. Distance d (Å) between Cα1 and Cα4 versus Ψ_3 for dynamics simulation of Ac-Ala-D-Pro-Pro-Ala-NHMe. The sinelike relationship indicates that other backbone torsional angles are relatively fixed. Diamonds indicate all conformations where all amides are *trans*. Crosses indicate conformations where ω_{23} is *cis*.

the percentage of hydrogen-bonded conformers (51%) and conformers with d under 7 Å (81%), but it still appears to be a highly effective turn inducer. Some reduction of turn induction was due to the presence of a *cis*- amide bond between residues i and $i + 1$ (shown as squares in Figure 3E,F) in some of the low-energy starting conformers and hence in the dynamics simulation. Conformers containing one *cis*-amide are not able to form the characteristic hydrogen bond.

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-NMeAla-D-Pro-Ala-NHMe and Ac-Ala-D-NMeAla-Pro-Ala-NHMe. The success of incorporating NMeAla in position $i + 2$ of the β-turn led us to investigate the effect of incorporating it at the $i + 1$ position. However, it was found that, in the case of Ac-Ala-NMeAla-D-Pro-Ala-NHMe and Ac-Ala-D-NMeAla-Pro-Ala-NHMe, there was significantly less turn stabilization, indicating the importance of having proline at position $i + 1$. Table 3 shows that, for Ac-Ala-NMeAla-D-Pro-Ala-NHMe, $|\beta|$ is less than 30° in only 9% of the dynamics structures and only 45% have a Cα1−Cα4 distance of less than 7 Å. The amide hydrogen and carbonyl oxygen are less than 4 Å apart in only 19% of the sampled structures. For Ac-Ala-D-NMeAla-Pro-Ala-NHMe, $|\beta|$ is less than 30° in 17% of the structures and d is less than 7 Å in 47%. The percentage of conformers with the characteristic hydrogen bond is 27%. The decreased β-turn propensity for these sequences reflects the absence of the pyrrolidine ring constraint at the second ($i + 1$) position of the turn.

Ac-Ala-D-Pro-Pip-Ala-NHMe and Ac-Ala-Pro-D-Pip-Ala-NHMe. To see if increasing the ring size of residue $i + 2$ would allow conformations more compatible with β-turns, we examined the effect of replacing proline by pipecolic acid (Pip, homoproline) which contains a six-membered ring. Indeed, Ac-Ala-D-Pro-Pip-Ala-NHMe showed better stabilization by two of the three criteria (β, d) than its NMeAla counterpart and by all three criteria compared to the D-Pro-Pro sequence. Table 3 shows that the percentage of conformers for Ac-Ala-D-Pro-Pip-Ala-NHMe where $|\beta| < 30°$ is 77% and the percentage with d less than 7 Å is 95%; the percentage of conformers with hydrogen bonding between residues 1 and 4 has risen to 73%. For Ac-Ala-Pro-D-Pip-Ala-NHMe, 57% of the structures satisfy the criterion that $|\beta| < 30°$ and 87% have d less than 7 Å, but only 48% have the characteristic hydrogen bond. To test that the relaxation of the ring constraint was most effective at the $i + 2$ position, the tetrapeptide with pipecolic acid in position $i + 1$ was also studied. For Ac-Ala-D-Pip-Pro-Ala-NHMe, 51% have $|\beta| < 30°$ and 74% have $d < 7$ Å, but again the percentage of conformers with the characteristic hydrogen bond is low (34%). These results are very similar to those for the tetrapeptide containing D-Pro-Pro which indicates that the use of proline

Table 5. Minimum Conformation of Ac-Ala-Ala-Ala-Ala-NHMe Starting with Values for Torsional Angles Corresponding to Classic β-Turns (Table 3)

type	Φ_2	Ψ_2	Φ_3	Ψ_3	ω_2	ω_3	d(α-1−4) (Å)	β (deg)	d(HB) (Å)
I (V)	−70.7	15.2	−149.6	25.2	−173.7	168.4	4.86	33.1	1.81
I (S)	−68.3	−2.7	−145.7	35.3	−177.2	174.05	4.72	29.7	2.09
I′ (V)	54.1	31.0	73.0	12.3	179.1	−178.7	5.27	−56.9	1.79
I′ (S)	54.0	34.8	59.5	33.0	−177.6	−178.7	5.37	−58.4	2.01
II (V)	−70.6	77.4	169.7	−38.1	178.3	−168.8	5.32	41.0	1.83
II (S)	−57.9	125.1	69.8	10.0	178.7	−178.8	4.95	4.3	1.92
II′ (V)	63.5	−77.7	−158.7	38.1	175.0	178.4	5.41	−22.8	2.00
II′ (S)	62.4	−88.4	−153.3	43.9	178.9	177.7	5.50	−26.8	2.09

[a] These structures were generated by unconstrained minimization in vacuo (V) or by constraining the backbone angles in residues $i + 2$ and $i + 3$ to be close to the ideal values and minimizing using the AMBER* all-atom force field with the GB/SA solvation model, followed by relaxation of the constraints and further minimization (S).

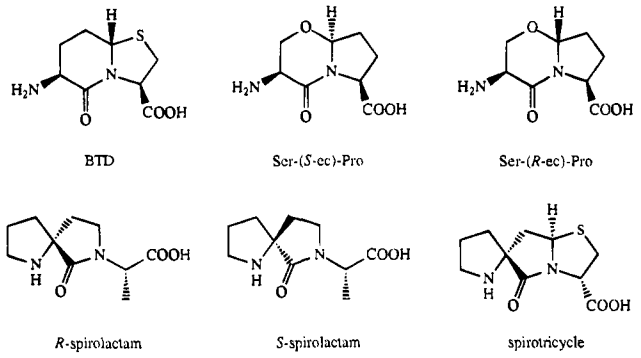

Figure 8. Nonpeptidic reverse-turn mimetics.

in position $i + 1$ constrains this residue to angles appropriate for turn induction and relaxation of this constraint is detrimental.

Comparison with Other Reverse-Turn Peptidomimetics

BTD Dipeptide. The dipeptide mimetic BTD (Figure 8) designed and prepared by Nagai et al.[18] 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 73%, while the percentage where d is less than 7 Å is 48% which is similar to that of Ac-Ala-D-Pro-Pro-Ala-NHMe. Importantly, little interaction was observed between the carbonyl oxygen of residue i and the amide hydrogen of residue $i + 1$. The percentage of conformers where d_{O-H} is less than 4 Å is only 17% (Table 3). These results suggest that the geometry of a turn induced by BTD differs significantly from that of an ideal β-turn and that BTD is more effective as a reverse turn than as a β-turn mimetic.

Spirolactam Compounds. These spirolactam bicyclic proline derivatives 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.[17] and α-methyl-Pro.[21,26] This bicyclic constraint restricts both Φ and Ψ of the spiro derivative. Table 3 shows that, for Ac-Ala-(*R*)-spirolactam-Ala-NHMe, 24% of the conformers had $|\beta|$ less than 30° and 45% had values of d lower than 7 Å. Despite the covalent constraints present in this compound, the percentage of conformers with an appropriate distance for the hydrogen bond characteristic of the β-turn was only 44%. Modifying the chirality of this compound gave substantially improved results. For Ac-Ala-(*S*)-spirolactam-Ala-NHMe, the percentage of conformers in which $|\beta|$ was less than 30° was 78% and the percentage with d under 7 Å was 69%. The percentage of conformers with an appropriate distance for the hydrogen bond characteristic of the β-turn is quite high, 69%, implying an excellent β-turn mimetic.

Spirotricyclic Compounds. Genin and Johnson[22,75] have combined the spirolactam constraint with annulation of a thioalkyl ring similar to BTD, generating a spirotricyclic ring system which restricts Φ_2, Ψ_2, and Φ_3. Conformational analysis in vacuo with MM2 gave a lowest energy conformation with $\Phi_2 = -40.3°$, $\Psi_2 = 108.1°$, $\Phi_3 = 77.7°$, and $\Psi_3 = -16.7°$. The rms fit of nine backbone atoms to an ideal type II β-turn was 0.161 Å for this conformer. The third lowest minimum energy conformation (1.45 kcal/mol higher than the lowest energy conformer) found had $\Phi_2 = -43.9°$, $\Psi_2 = 111.0°$, $\Phi_3 = 111.1°$, and $\Psi_3 = -28.4°$ which more closely resembles the conformation (Φ_2 −44°, $\Psi_2 = 117°$, $\Phi_3 = 128°$, and $\Psi_3 = -102°$) predominating using the solvation model and force field in these studies (Table 4). Plots of Φ_2 versus Ψ_2 and Φ_3 versus Ψ_3 for the dynamics simulation of Ac-Ala-spirotricycle-Ala-NHMe are shown in Figure 3G,H. These figures show that the spirotricycle tighly constrains all the backbone angles except Ψ_3. A plot of β versus potential energy for the simulation (Figure 5C) shows that the mimetic effectively constrains this parameter ($|\beta| < 30°$, 91%). Interestingly despite the very tight constraint on β, the distance between Cα1 and Cα4 was less well constrained (Figure 4C), with only 53% having this distance less than 7 Å. This may be due to the relatively low percentage of hydrogen-bonded conformers (43%, Figure 6C) which was actually lower than for the less constrained (*S*)-bicyclic spirolactam mimetic.

Ser-((*R*)-ec)-Pro and Ser-((*S*)-ec)-Pro. Recently, bicyclic turn mimetics containing 6,5-ring systems have been prepared by electrochemical oxidation of a protected dipeptide of serine-proline,[76] and by a more conventional synthetic approach.[77] Here, we present an analysis of the effect of incorporating the two epimers Ser-((*R*)-ec)-Pro and Ser-((*S*)-ec)-Pro, which differ in the configuration at the cyclization carbon of the 6,5-bicyclic system. The percentage (Table 3) of conformers for Ac-Ala-Ser-((*R*)-ec)-Pro-Ala-NHMe in which $|\beta|$ is less than 30° is 76%, and 54% had values of d less than 7 Å which are similar results to those obtained for Ac-Ala-D-Pro-Pro-Ala-NHMe. However, like BTD the percentage of conformers with the i to $i + 4$ hydrogen bond is very low (3%). In the case of Ac-Ala-Ser-((*S*)-ec)-Pro-Ala-NHMe, 63% had $|\beta|$ less than 30° and 85% had d less than 7 Å. For this epimer, the percentage of conformers with the hydrogen bond characteristic of the β-turn is a more significant 29%.

The reason why the Pro-D-NMeAla and D-Pro-NMeAla sequences are so effective compared with other turn mimetics can be attributed to stabilization of the *trans* conformer of the intervening amide bond (ω_{23}) and fixation of the intervening rotatable bond (Ψ_2) connecting the α-carbon of the first proline residue with its carbonyl due to steric interactions between the pyrrolidine ring in $i + 1$ and the *N*-methyl group of the amino acid in $i + 2$. In effect, five (ω_{23}, Φ_2, Ψ_2, ω_{34}, Φ_3) out of six of the central backbone torsional angles of the reverse turn are relatively fixed (Table 4) with an rms deviation of less than 20° for Φ_2, Ψ_2, and Φ_3. The dihedral angle Ψ_3 remains relatively free (rms = 34°), although more constrained than for any of the other compounds studied (Table 4). A plot of angle ψ_3 against the distance d (Figure 7) shows the tight, sinelike correlation between this angle and the tightness of the turn for Ac-Ala-D-Pro-Pro-Ala-NMe. Bicyclic dipeptide analogs such as BTD and Ser-(ec)-Pro have only rigidified three angles (Ψ_2, ω_{23}, Φ_3), while the spirolactam strongly rigidifies Φ_2 and Ψ_2 through the combination of α,α-dialkyl and spirobicyclic constraints. The amide bond (ω_{12}) entering both BTD and Ser-(ec)-Pro is assumed to remain *trans* since it is not an imide. Even though the spirotricyclic analog gives excellent correspondence to an ideal type II β-turn *in vacuo*,[22] it still maintains sufficient flexibility to allow stabilization in solution (as estimated with the GB/SA solvation model) of an alternative conformation which does not stabilize the characteristic hydrogen bond associated with the β-turn.

Relevant Experimental Data. The realization that a proline dipeptide with alternating configurations would restrict its

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

(76) Slomczynska, U.; Chalmers, D. K.; Cornille, F.; Beusen, D. D.; Moeller, K. D.; Marshall, G. R. *J. Org. Chem.*, submitted for publication.

(77) Baldwin, J. E.; Hulme, C.; Schofield, C. J.; Edwards, A. J. *J. Chem. Soc., Chem. Commun.* **1993**, 935−936.

conformations is not necessarily novel. Rothe and his colleagues[78] have studied both cyclic and linear sequences composed of alternating L- and D-proline residues. The crystal and solution structure of the cyclic tetrapeptide shows alternating *cis*- and *trans*-imide bonds as the *all-trans* cyclic tetrapeptide is precluded on steric grounds. Three (Φ_2, Ψ_2, Φ_3) of the four backbone torsional angles for the dipeptide containing the *trans*-imide bond are consistent with those favored by these calculations. The fourth (Ψ_3) assumes an energetically less favored value in order to allow cyclization. The cyclic hexapeptide cyclo(D-Pro-L-Pro)$_3$ exhibits an *all-trans* structure in water.[78] Bean et al.[67] have incorporated D-Pro-Pro in two cyclic hexapeptides based on steric arguments and Ramachandran plots. The NMR experimental data on both cyclic hexapeptides indicates significant restriction of the peptide backbone to the D-Pro-Pro dipeptide region to the single type II′ β-turn conformation indicated by our calculations. In this experimental system, however, the additional cyclic constraint of the hexapeptide makes it difficult to ascertain the overall impact of the proline dipeptide itself from the additional effects of cyclization. A crystal structure[79] of a linear tripeptide, pivaloyl-D-Pro-Pro-Ala-NMe, clearly shows a conformation consistent with a type II′ β-turn conformation with D-Pro at the $i + 1$ position ($\Phi = 58.9°$, $\Psi = -135.8°$) and Pro at the $i + 2$ position ($\Phi = -58.5°$, $\Psi = -23.7°$). As we were completing this paper, our attention was drawn to a paper on substance P antagonists by McElroy et al.[68] in which D-Pro^9-Pro^{10} and D-DTC^9-Pro^{10} (D-DTC = (*S*)-5,5-dimethylthiazolidine-4-carboxylic acid[80]) was incorporated as a β-turn mimetic. The following statement appears in the paper: "A conformational analysis of the dipeptide Ac-D-Pro-Pro-NHMe (data not shown) indicates a high propensity for a Type II′ β-turn conformation." Clearly, others have been led to similar conclusions about proline dipeptides with alternating chirality. Surprisingly, no details of a conformational analysis of such systems have ever appeared to our knowledge and no assessment of the negative impact of the proline ring constraint in position 3 ($i + 2$) has been given. Relaxation of this constraint by replacement of proline with pipecolic acid or an *N*-methylamino acid is predicted to further stabilize the β-turn conformer.

Kyle et al.[9,81-83] have introduced analogs of D-Pro-Pro sequences in which the first residue is a derivative of D-4-hydroxyproline and the pyrrolidine ring of the second residue has an additional fused hydrocarbon ring (either octahydroindolecarboxylic acid, Oic, or tetrahydroisoquinolinecarboxylic acid, Tic) in positions 7 and 8 of bradykinin antagonists and described restriction to a type II′ β-turn. Their conformational and NMR analyses of these specific systems indicated conformational restrictions similar to those described here, but it was unclear whether the described effects were due to the increased steric requirements of the particular combination of unusual amino acids used.

Haque et al.[84] have recently reported that "the sequence of α-carbon configurations along a four-residue sequence can profoundly affect the backbone's propensity to adopt a β-hairpin folding pattern". This statement summarizes their results from NMR and FT-IR studies on model depsipeptides in organic solvents, and is certainly consistent with the theoretical results reported in these studies. Aubrey and Marraud[85] have studied capped model dipeptides of Pro-NMe-AA crystallographically and spectroscopically in chlorinated organic solvents. A clear stabilization of the type II β-turn conformation is seen with Pro-NMe-D-AA sequences while Pro-NMe-L-AA sequences favor the type VI β-turn conformation with the imide bond in the *cis* conformation. These observations are entirely consistent with the results presented above in which the heterochiral sequence destabilizes the *cis*-imide conformation.

Table 6. Comparative Calculations on *cis*−*cis*−*trans*−*trans* (cctt) and *cis*−*trans*−*cis*−*trans* (ctct) Conformers of Cyclo(D-Pro-L-Pro-D-Pro-L-Pro)[78]

force field	E(cctt) (kJ/mol)	E(ctct) (kJ/mol)	ΔE(ctct) − ΔE(cctt) (kJ/mol)
AMBER* all-atom	157.6	178.3	20.7
AMBER/OPLS*	−125.4	−131.6	−6.2
MM2*	353.1	318.4	−34.8
MM3*	545.3	527.1	−18.2

[a] These numbers result from a Monte Carlo conformational search peformed on cyclo(D-Pro-L-Pro-D-Pro-L-Pro) using the AMBER* all-atom force field and the GB/SA solvation model. All the unique structures (138) obtained from this search were reminimized using each of the other force fields. The energies listed refer to the lowest energy forms of the cctt and ctct conformers in each case. Experimental data suggest that only the ctct conformation is observed by NMR in water.

Caveats. The results presented above are based on the use of Ac-Ala-Ala-Ala-Ala-NMe as a model system, a particular force field (AMBER* all-atom), limited sampling of conformational space using a particular protocol, and a solvation model (GB/SA) to represent water. Recently, the GB/SA model has been shown[86] to accurately reproduce the hydration energy trends seen by dynamics simulations with explicit water for conformers of a crown ether, a cryptand, and a calixerene. The differences (Tables 1 and 2) between results obtained with the three force fields examined for minima of simple model compounds raise concern over any quantitative interpretation of these results. In support of this concern, comparative results (Table 6) with different force fields on cyclo(D-Pro-L-Pro-D-Pro-L-Pro) were obtained at the end of these studies. The discrepancies in the relative energies of the minima found for the *cis*−*trans*−*cis*−*trans* (ctct) and cctt conformations are dramatic, especially as the experimental data (crystal structure and solution NMR indicate exclusively ctct and tctc conformations[78]). Nevertheless, considerable evidence exists in the literature to warrant a certain degree of qualitative confidence in these calculations. Assuming that the simulations roughly represent the populations of conformers present in aqueous solution, one can estimate the free energies of stabilization of β-turns (those conformers with the characteristic hydrogen bond) by application of Boltzmann's equation to the relative populations. Compared with the control tetraalanine peptide, the inclusion of Pro-D-Pro has stabilized the β-turn by −8.0 kJ/mol and the inclusion of D-Pro-Pro has stabilized the β-turn by −9.6 kJ/mol. These numbers are in reasonable agreement with the estimates of Searle and Williams[87,88] on the entropy loss

(78) Mastle, W.; Weber, T.; Thewalt, U.; Rothe, M. *Biopolymers* **1989**, *28*, 161−174.
(79) Nair, C. M.; Vijayan, M.; Venkatachalapathi, Y. V.; Balaram, P. *J. Chem. Soc., Chem. Commun.* **1979**, 1183−1184.
(80) Samanen, J.; Zuber, G.; Bean, J.; Eggleston, D.; Romoff, T.; Kopple, K.; Saunders, M.; Regoli, D. *Int. J. Pept. Protein Res.* **1990**, *35*, 501−509.
(81) Kyle, D. J.; Martin, J. A.; Farmer, S. G.; Burch, R. M. *J. Med. Chem.* **1991**, *34*, 1230−1233.
(82) Kyle, D. J.; Martin, J. A.; Burch, R. M.; Carter, J. P.; Lu, S.; Meeker, S.; Prosser, J. C.; Sullivan, J. P. *J. Med. Chem.* **1991**, *34*, 2649−2653.
(83) Kyle, D. J.; Green, L. M.; Blake, P. R.; Smithwick, D.; Summers, M. F. *Pept. Res.* **1992**, *5*, 206−209.
(84) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4105−4106.
(85) Aubrey, A.; Marraud, M. *Biopolymers* **1989**, *28*, 109−122.
(86) Varnek, A. A.; Wipff, G.; Glebov, A. S.; Feil, D. *J. Comput. Chem.* **1995**, *16*, 1−19.
(87) Searle, M. S.; Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 10690−10697.
(88) Searle, M. S.; Williams, D. H.; Gerhard, U. *J. Am. Chem. Soc.* **1992**, *114*, 10697−10704.

(−1.6 to −3.6 kJ/mol) of freezing out a torsional rotation within a hydrocarbon chain assuming that between 2 and 4 torsional degrees of freedom of the peptide backbone have been limited by substitution of the proline dipeptide unit into the tetraalanine peptide. Nevertheless, confirmation of these trends through the use of longer simulations with explicit representation of water is essential, and is currently underway in our laboratory.

Conclusions

These calculations indicate that Pro-D-NMe-AA and D-Pro-NMe-AA can be used as effective reverse-turn constraints, with Pro-D-Pip and D-Pro-Pip being specific examples. Surprisingly, the presence of the second pyrrolidine ring constraint in either Pro-D-Pro or D-Pro-Pro actually destabilizes the β-turn propensity compared with the parents Pro-D-NMe-AA and D-Pro-NMe-AA, respectively, or the larger ring homolog pipecolic acid in the third position ($i + 2$). These reverse-turn constraints can easily be incorporated into synthetic peptides using off the shelf materials which should prove useful in combinatorial libraries focused on a hierarchical approach[89] to peptidomimetics. D-Pro-Pro, Pro-D-Pro, Pro-D-Pip, D-Pro-Pip, D-Pro-NMe-AA, and D-Pro-NMe-AA offer relatively rigid scaffolds on which to orient sidechains for interaction with receptors which recognize reverse-turn conformations. Several substituted prolines such as 4-hydroxyproline are readily available as chiral synthons. Stereoselective routes to *cis*- and *trans*-3-mercaptoproline[54] and *cis*- and *trans*-4-mercaptoproline[53] have been developed to provide chimeric amino acids for side chain incorporation in CCK analogs[53] as well as convenient cyclization sites in angiotensin,[6] bradykinin,[8] opioid,[90] and CCK[90] studies. Ho et al.[91] have developed an asymmetric synthesis of *cis*-5-alkyl-proline derivatives. The incorporation into peptides of appropriately substituted prolines and *N*-methylamino acids in such dipeptide sequences should prove useful to probe receptors for biological recognition of β-turn scaffolds with oriented side chains.

Acknowledgment. The authors thank the National Institutes of Health for partial support of this work (Grants GM-24883 and GM-48184) as well as Richard Head of the Center for Molecular Design for technical support. We also gratefully acknowledge discussions with Andrea Tafi (Siena) and Mark Smythe (Brisbane) at the Center for Molecular Design on quantitatively characterizing reverse-turn constraints.

Supplementary Material Available: Figures showing plots of the backbone angle Φ versus Ψ for residues 2 and 3 of blocked tetrapeptides (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA950078T

(89) Marshall, G. R. *Tetrahedron* **1993**, *49*, 3547−3558.

(90) Nikiforovich, G. V.; Kolodziej, S. A.; Nock, B.; Bernad, N.; Martinez, J.; Marshall., G. R. *Biopolymers*, in press.

(91) Ho, T. L.; Gopalan, B.; Nestor, J. J., Jr. *J. Org. Chem.* **1986**, *51*, 2405−2408.

(92) Using 1000 ps of mixed Monte Carlo−stochastic dynamics, the GB/SA water model, and the AMBER/OPLS* force field, we obtained the following results: Ac-Ala-D-Pro-NMeAla-Ala-NMe ($|\beta| < 30$, 54%; $d <$ 7 Å, 76%; d(C═O···H−N) < 4 Å, 75%), Ac-Ala-D-Pro-Pip-Ala-NMe ($|\beta| < 30$, 62%; $d < 7$ Å, 84%; d(C═O···H−N < 4 Å, 74%). These results are in extremely good agreement with the data shown in Table 3. Similarly good agreement was found for the most populated backbone angles and rms deviations. Values given here are as listed in Table 4: Ac-Ala-D-Pro-NMeAla-Ala-NMe, 61° (73), −127° (20), −122° (34), 57° (37); Ac-Ala-D-Pro-Pip-Ala-NMe, 64° (13), −146° (18), −103° (14), 56° (38).