Electrochemical Cyclization of Dipeptides toward Novel Bicyclic, Reverse-Turn Peptidomimetics. 1. Synthesis and Conformational Analysis of 7,5-Bicyclic Systems

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Abstract: Novel, highly constrained, 7,5-bicyclic dipeptides (1-aza-6-oxa-2-oxobicyclo[5.3.0]decane ring skeletons, **3Sa** and **7Sa**) have been synthesized on a 40 mmol scale in ~50% yield by a one-step electrochemical cyclization from the dipeptides Boc-L-homoserine-L-proline-OMe (Boc-Hse-Pro-OMe) and Boc-Hse-D-Pro-OMe. The reaction involved a selective anodic amide oxidation which was highly diastereoselective, generating a new chiral center having an *S* configuration from both precursors. In terms of conformation, the bicyclic system restricts two ($\psi 2$ and $\phi 3$) of the four torsion angles that characterize a reverse turn. Conformational analysis of these molecules and analogs having an *R* configuration at the ring fusion revealed some families of minimum energy conformations with torsion angles close to those of classical β-turns, a secondary structural feature found in many bioactive peptides. This new ring skeleton was stable to trifluoroacetic acid, dilute base, and anhydrous hydrofluoric acid, making it compatible with standard solid phase peptide synthesis methodologies.

Introduction

Reverse turns are common motifs in protein structure and have often been implicated as recognition elements.[1,2] The β-turn (or β-bend) consists of four consecutive residues where the chain changes direction by almost 180°.[1] Examples of turns as recognition motifs can be found in crystal structures of antibody−peptide complexes.[2−4] These complexes are entirely consistent with the receptor recognition of turn motifs deduced from structure−activity studies of the peptide hormones, angiotensin II and bradykinin.[5]

The prevalence of this motif in recognition has led to the development of numerous cyclic and bicyclic dipeptide analogs[6−8] intended to stabilize the peptide chain in a reverse turn. Examples of modifications which enhance reverse-turn propensity are the dipeptide lactam;[9] the bicyclic dipeptide, BTD;[10] spiro-bicyclic systems based on proline;[11−14] substitution by α,α-dialkyl amino acids; and substitution by dehydroamino acids.[21−24] In other turn mimetics, hydrogen-bonding groups stabilizing the turn are replaced by covalent bonds.[15−20] Benzodiazepines have also been used as turn mimetics.[21−23]

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(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) Rini, J. M.; Schulze-Gahmen, U.; Wilson, I. A. *Science* **1992**, *255*, 959−965.
(4) Garcia, K. C.; Ronco, P. M.; Veroust, P. J.; Brunger, A. T.; Amzel, L. M. *Science* **1992**, *257*, 502−507.
(5) 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.
(6) Ball, J. B.; Alewood, P. F. *J. Mol. Recognit.* **1990**, *3*, 55−64.
(7) Holzemann, G. *Kontakte (Darmstadt)* **1991**, 3−12.
(8) Holzemann, G. *Kontakte (Darmstadt)* **1991**, 55−63.
(9) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104−109.
(10) Nagai, U.; Sato, K.; Nakamura, R.; Kato, R. *Tetrahedron Lett.* **1993**, *49*, 3577−3592.
(11) Hinds, M. G.; Richards, N. G. J.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1447−1449.
(12) Ward, P.; Ewan, G. B.; Jordan, C. C.; Ireland, S. J.; Hagan, R. M.; Brown, J. R. *J. Med. Chem.* **1990**, *33*, 1848−1851.
(13) 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.
(14) Genin, M. J.; Johnson, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8778−8783.
(15) Kahn, M.; Nakanishi, H.; Chrusciel, R. A.; Fitzpatrick, D.; Johnson, M. E. *J. Med. Chem.* **1991**, *34*, 3395−3399.
(16) 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.
(17) 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.
(18) Gardner, B.; Nakanishi, H.; Kahn, M. *Tetrahedron* **1993**, *49*, 3433−3448.
(19) 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.
(20) Callahan, J. F.; Newlander, K. A.; Burgess, J. L.; Eggleston, D. S.; Nichols, A.; Wong, A.; Huffman, W. F. *Tetrahedron* **1993**, *49*, 3479−3488.
(21) Ripka, W. C.; DeLucca, G. V.; Bach, A. C., II; Pottorf, R. S.; Blaney, J. M. *Tetrahedron* **1993**, *49*, 3593−3608.
(22) Ripka, W. C.; Lucca, G. V. D.; Bach, A. C., II; Pottorf, R. S.; Blaney, J. M. *Tetrahedron* **1993**, *49*, 3609−3628.
(23) 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.
(24) Shono, T.; Matsumura, Y.; Inoue, K. *J. Org. Chem.* **1983**, *48*, 1388−1389.

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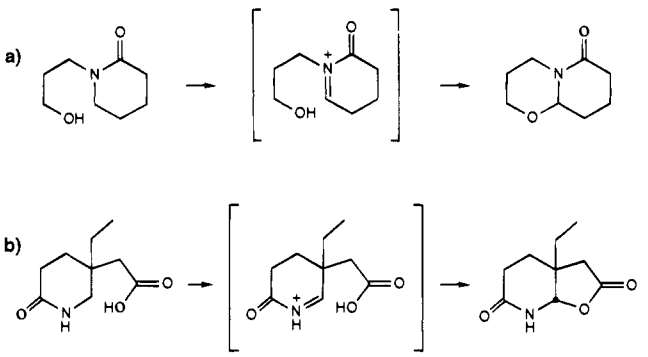
Scheme 1. Examples of Electrochemical Annulation by Anodic Amide Oxidation[a]



[a] (a) Taken from ref 69; (b) taken from ref 50.

One approach to the rapid construction of reverse-turn mimetics involves organic electrochemistry of dipeptides. In general, electrochemical oxidations have received considerable interest because they allow selective oxidation of a variety of functional groups under neutral conditions.[24-28] Applications of electrochemical oxidation to the functionalization of amino acids[24-28] and, in one case, dipeptides[29,30] have been reported. Among electrochemical oxidations, the oxidation of amides is one of the most developed synthetic methods.[31-36] Indeed, the potential power of this reaction lies in the oxidative alternative it provides to the more common methods of *N*-acyliminium formation[37] and the possibility it affords for developing a general annulation procedure for amides.[34,38-43] The one-pot electrochemical generation of an *N*-acyliminium cation followed by trapping of this active intermediate by an intramolecular nucleophilic center (Scheme 1) was reported some years ago.[44,45]

These findings combined with our interest in reverse-turn mimetics prompted us to survey the applicability of this method to the synthesis of the 7,5-bicyclic compounds **3** and **7** (Figure

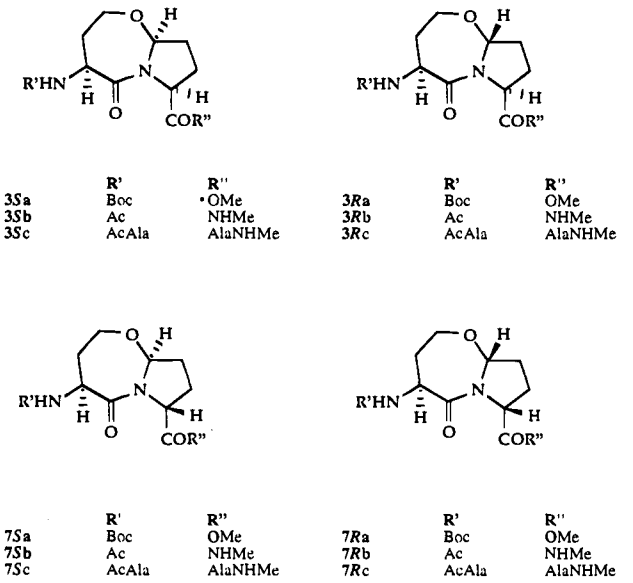
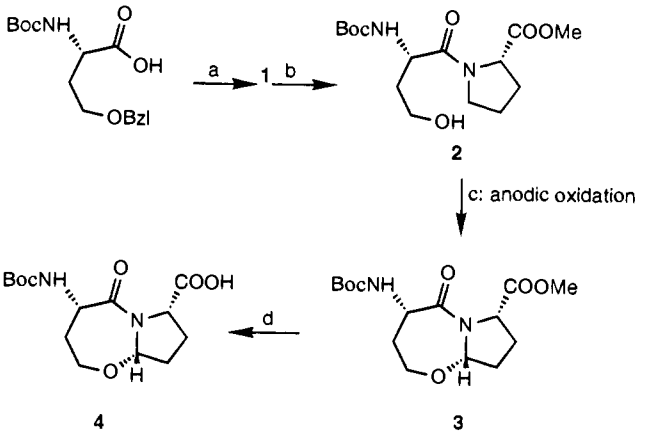


Figure 1. Bicyclic dipeptide derivatives either prepared or used in modeling to assess their turn propensity.

Scheme 2. Synthetic Scheme for the Preparation of Compound **3Sa**[a]



[a] The same protocol, substituting H-D-Pro-OMe for H-L-Pro-OMe, results in the production of compound **7Sa**. (a) H-L-Pro-OMe, TBTU, diisopropylethylamine, overnight, room temperature, 81% yield. (b) H_2, 45 psi, Pd/C, 48 h, room temperature, 77% yield. (c) Platinum electrodes, 1 M Bu_4NBF_4 in acetonitrile/isopropyl alcohol 95:5; constant current 138 mA (density 6.9 mA/cm^2), 3.8 F; 48% yield. (d) 1 N NaOH, MeOH, 1 h at room temperature, followed by addition of 1 M $KHSO_4$; 84% yield.

1) directly from protected proline dipeptides. A similar bicyclo-[5.3.0]decane derivative analogous to BTD[10] was prepared from L-4-thiaproline by Wyvratt et al.[46] in their studies of ACE inhibitors. To be successful, the synthetic transformation would require the previously undemonstrated selective oxidation of the proline tertiary amide in the presence of the urethane-protected amide (Scheme 2). If this were possible, then the starting materials for the synthesis of **3** and **7** would consist of the readily accessible dipeptides Boc-L-homoserine-L-proline-OMe (Boc-Hse-Pro-OMe) and Boc-Hse-D-Pro-OMe. From a chemical point of view, this synthesis would represent a unique and highly selective application of organic electrochemistry to the construction of peptide mimetics.[34]

(25) Thaning, M.; Wistrand, L.-G. *Helv. Chim. Acta* **1986**, *69*, 1711.
(26) Ginzel, K.-D.; Brungs, P.; Steckhan, E. *Tetrahedron* **1989**, *45*, 1691-1701.
(27) Barrett, A. G. M.; Pilipauskas, D. *J. Org. Chem.* **1991**, *56*, 2787-2800.
(28) Moeller, K. D.; Rutledge, L. D. *J. Org. Chem.* **1992**, *57*, 6360-6363.
(29) Papadopoulos, A.; Heyer, J.; Ginzel, K.-D.; Steckhan, E. *Chem. Ber.* **1989**, *122*, 2159.
(30) Papadopoulos, A.; Lewall, B.; Steckhan, E.; Ginzel, K.-D.; Knoch, F.; Nieger, M. *Tetrahedron* **1991**, *47*, 563-572.
(31) Ross, S. D.; Finkelstein, M.; Peterson, C. *J. Am. Chem. Soc.* **1964**, *86*, 4139-4143.
(32) Ross, S. D.; Finkelstein, M.; Peterson, R. C. *J. Org. Chem.* **1966**, *31*, 128-133.
(33) Ross, S. D.; Finkelstein, M.; Peterson, R. C. *J. Am. Chem. Soc.* **1966**, *88*, 4657-4660.
(34) Shono, T. *Tetrahedron* **1984**, *40*, 811-850.
(35) Shono, T.; Matsumura, Y.; Uchida, K.; Tsubata, K.; Makino, A. *J. Org. Chem.* **1984**, *49*, 300-304.
(36) Shono, T.; Matsumura, Y.; Tsubata, K. In *Organic Synthesis*; Saucy, G., Ed.; John Wiley & Sons: New York, 1984; Vol. 63, p 206.
(37) Speckamp, W. N.; Hiemstra, H. *Tetrahedron* **1985**, *41*, 4367-4416.
(38) Okita, M.; Wakamatsu, T.; Mori, M.; Ban, Y. *Heterocycles* **1980**, *14*, 1089-1092.
(39) Irie, K.; Ban, Y. *Heterocycles* **1981**, *15*, 201-206.
(40) Irie, K.; Ban, Y. *Heterocycles* **1982**, *18*, 255-258.
(41) Okita, M.; Wakamatsu, T.; Ban, Y. *Heterocycles* **1983**, *20*, 401-404.
(42) Shono, T.; Matsumura, Y.; Uchida, K.; Tagami, K. *Chem. Lett.* **1987**, 919-922.
(43) Mori, M.; Kagechika, K.; Tohjima, K.; Shibasaki, M. *Tetrahedron Lett.* **1988**, *29*, 1409-1412.
(44) Okita, M.; Wakamatsu, T.; Ban, Y. Unpublished work, 1979.
(45) Irie, K.; Okita, M.; Wakamatsu, T.; Ban, Y. *Nouv. J. Chim.* **1980**, *4*, 275-277.
(46) Wyvratt, M. J.; Tischler, N. H.; Ikelar, T. J.; Springer, J. P.; Tristram, E. W.; Patchett, A. A. In *Peptides*, Proceedings of the 8th American Peptide Symposium; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; pp 551-554.

In this report, the synthesis of the *N*-Boc-protected bicyclic dipeptidic analogs **3Sa** and **7Sa** using an electrochemical approach is presented. It was hoped that this constrained bicyclic system, when introduced in the sequence of a biologically active peptide, would prove useful in enforcing particular backbone conformations and thereby assist in determining the bioactive conformation.[47-49] Computational analyses designed to elucidate the conformational preferences of dipeptide and tetrapeptide analogs of these bicyclic systems are reported. In addition to highlighting the synthetic potential of the electrochemical approach, this study suggests that different bicyclic systems increase the propensity for certain reverse-turn types once introduced into peptides.

Results and Discussion

Synthesis. The synthesis of the bicyclic compound **3Sa** proceeded as shown in Scheme 2. Standard conditions were used to couple *tert*-butoxycarbonyl-*O*-benzyl-L-homoserine to L-proline methyl ester, and the benzyl ether was removed by hydrogenolysis. The resulting compound **2** (40 mmol, $c = 0.5$ M) was then electrolyzed in a 95:5 mixture of acetonitrile/isopropyl alcohol using 1 M tetrabutylammonium tetrafluoroborate as supporting electrolyte in an undivided cell, with two 20 cm^2 platinum foil electrodes and a constant current of 138 mA (current density = 6.9 mA/cm^2). The reaction was monitored by TLC and HPLC and found to be complete following the passage of 3.8 F/mol through the solution. These conditions resulted in 6.3 g (48% yield) of compound **3Sa** after column chromatography. The structure of the product was established by 2D NMR experiments (see below). Although no major side products were detected, a number of unidentified minor products were observed. In creating one new chiral center, the cyclization reaction proved to be highly diastereoselective. The isomer having an *S*-configuration at the ring fusion dominated the product by a ratio of 15:1 or greater based on ^{13}C and ^{1}H NMR (see below). In the last step of the synthesis (Scheme 2), the hydrolysis of **3** by 1.1 equiv of NaOH in methanol during 1 h at room temperature produced compound **4** (84% yield) after acidification. NMR analysis revealed that no racemization occurred during this step.

The conditions used for the electrochemical oxidation were similar to those described by Irie et al.[45] (Scheme 1b). We substituted isopropyl alcohol for water in order to minimize hydroxylation of the *N*-acyliminium ion generated, necessitating an increase in the concentration of electrolyte because of the lower conductivity of the solution. In modifying parameters in order to optimize the purity and yield of the product obtained, we found that a platinum anode worked better than a carbon anode. The use of constant potential conditions instead of constant current conditions slowed the reaction rate and resulted in the formation of more byproducts. The most important parameter proved to be current density. The yield of the reaction increased from 21% to 48% on a 4 mmol scale just by changing the current density from 19.5 mA/cm^2 to 6.9 mA/cm^2. Keeping this in mind, we scaled up the reaction 10-fold (40 mmol) using the same current density (6.9 mA/cm^2). The size of the electrodes and the current intensity were increased 10-fold in order to run the reaction in the same time. As found in the smaller scale, the reaction went to completion after 3.8 F/mol was passed through the solution. Once again, a 48% yield of product was obtained.

The electrochemical oxidation in this sequence was intriguing for two reasons. First, the results of this oxidation serve to refine our picture of what substituents are compatible with the anodic oxidation of proline derivatives. Earlier studies had established that alkoxy substituents on the carbon α to the amide carbonyl interfered with the oxidation reaction. In these cases, a high yield of the recovered starting material was obtained (K. D. Moeller and S. L. Rothfus, unpublished results). Although speculative, this result was attributed to the electron-withdrawing nature of the alkoxy substituent. Carbon substituents on the carbon α to the amide did not adversely affect the oxidation.[50,51] It was not obvious how a substrate with a nitrogen substituent would fare in the electrolysis reaction since its electronegativity falls between that of oxygen and carbon. With the success of the current oxidations, we now know that the dividing line between a successful oxidation and no reaction for a substituent on the carbon α to the amide lies between a secondary amide and an oxygen. Information concerning dividing lines of this type are essential for planning future syntheses. Second, the above oxidation reactions serve to highlight the utility of electrochemistry for accomplishing both chemo- and regioselective oxidations. Note that only one of three possible *N*-acyliminium ions was presumably generated, that of the tertiary amide of proline with removal of a proton from position 5. This selectivity allows use of the protected dipeptide as starting material and greatly simplifies the overall synthesis. The only previously reported electrochemical oxidation of dipeptides (Boc-Val-Gly-OMe and Boc-Pro-Gly-OMe[29,30]) utilized an indirect oxidation with chloride as a redox catalyst to replace the α-proton of a C-terminal glycine dipeptide by a methoxy group.

The electrochemical oxidation–cyclization sequence has been successfully extended to the cyclization of Boc-Hse-D-Pro-OMe to produce the bicyclic system **7** (Figure 1). Under the same conditions described above for the L-Pro isomer, the 7,5-bicyclic compound **7Sa** was produced in a 52% yield. It is noteworthy that electrochemical cyclization of Boc-Hse-D-Pro-OMe exhibits the same high stereoselectivity for the *S*-diastereoisomer at the ring fusion as was observed for Boc-Hse-L-Pro-OMe. The high stereoselectivity obtained is not surprising in the context of reports on intramolecular cyclization of *N*-acyliminium intermediates[37,52,53] and experience with similar carbocyclic systems.[54] In this case, it is clear that the cyclization is not influenced by the chirality of the α-carbon of the proline precursors and, therefore, must be dictated by the chirality of the homoserine residue.

Studies on the stability of the bicyclic compound **3Sa** to acids such as trifluoroacetic acid (TFA) and anhydrous hydrofluoric acid (HF) were performed in order to ascertain if this new bicyclic system were suitable for solid phase peptide synthesis. Except for the removal of the *tert*-butoxycarbonyl (Boc) group, this molecule proved stable to pure TFA for 2 h at room temperature and to HF for 1 h at 0 °C. This conclusion was confirmed by resynthesis of **3Sa** from the deprotection product through reintroduction of the Boc group and comparison of the NMR and HPLC of the resulting product with an original sample of bicyclic **3Sa**. It must be mentioned, however, that, in order to avoid formation of side products, no scavenger can be used

(47) Hruby, V. J. *Life Sci.* **1982**, *31*, 189–199.
(48) Fauchere, J. L. In *QSAR in Drug Design and Toxicology*; Hadzi, D., Jerman-Blazic, B., Ed.; Elsevier Science Publishers B. V.: Amsterdam, 1987; p 221.
(49) Marshall, G. R. *Tetrahedron* **1993**, *49*, 3547–3558.
(50) Moeller, K. D.; Rothfus, S. L.; Wong, P. L. *Tetrahedron* **1991**, *47*, 583–592.
(51) Wong, P. L.; Moeller, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 11434–11445.
(52) Zaugg, H. E. *Synthesis* **1984**, *85*, 181–212.
(53) Speckamp, W. N. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 345–354.
(54) Shono, T. *Top. Curr. Chem.* **1988**, *148*, 131–151.

under HF deprotection conditions, probably due to an equilibrium between the bicyclic compound and the *N*-acyliminium isomer with trapping of the latter by the scavenger. The relative stability of the 7,5-bicyclic system toward acids such as TFA and HF facilitates the synthesis of peptide analogs by a standard solid phase approach. Compounds **3Sa** and **7Sa** have been incorporated after saponification of the methyl ester into two gonadotropin hormone-releasing hormone (GnRH, LHRH) analogs by solid phase synthesis and treatment with pure HF (Slomczynska et al., in preparation). Stability of the cyclic ether to acids is potentially problematic, as the acyliminium ion is probably in equilibrium under these conditions. The facility with which the compounds can be prepared and incorporated, however, argues for their use in structure–activity exploration with preparation of the more stable carbocyclic lactam analog[51,55,56] if an interesting lead is discovered.

In an effort to synthesize the related 6,5 bicyclo[4.3.0]nonane ring skeleton, the dipeptide starting material was prepared with serine, threonine, or β-phenylserine replacing homoserine. In these instances, the same conditions described for Boc-homoserine-L-proline methyl ester gave an unexpected cleavage of the β-hydroxy side chain as the aldehyde and addition of methanol to produce Boc-α-methoxy-glycine-L-proline methyl ester in a yield of 70–75% (Slomczynska and Marshall, unpublished results). No oxidation of the proline ring was observed. A reaction of this type has been observed (K. D. Moeller and S. L. Rothfus, unpublished results) in the oxidation of the analogous 2-(hydroxymethyl)pyrrolidine derivative. This elimination was not observed in the reactions of the homoserine dipeptides used in this study. Although α-methoxylation of protected amino acids and dipeptides using indirect electrochemical oxidation with chloride as the catalyst has been reported,[24,26,30] the absence of chloride ion in our studies suggests that a different mechanism is operative. During preparation of this article, we became aware of an alternate chemical route to the 6,5-bicyclic analog, Boc-Ser-(ec)-Pro-OH, using the unusual amino acid, (*S*)-but-3-enylglycine.[57]

Conformational Analyses. Previous conformational analyses of β-turn mimetics have generally been done *in vacuo*. Solvation energies of molecules containing polar functionality may significantly alter *in vacuo* free energies. Furthermore, solvation effects may produce new conformers that are not minima on the *in vacuo* potential energy surface. In order to explore the relative stability of different low-energy conformations of the bicyclic turn mimetics in water, the solvent of biological interest, inclusion of a solvation model was necessary. The AMBER/OPLS force field[58] developed for simulations in liquids and the solution model GB/SA[59] in MACROMODEL[60] were used in a Monte Carlo conformational search strategy to identify the solvated low-energy minima of the bicyclic systems **3** and **7** (Figure 1). The search protocol was similar to those reported in the literature.[61]

In this study, both the dipeptide (Ac-Hse-(ec)-Pro-NHMe) and tetrapeptide (Ac-Ala-Hse-(ec)-Pro-Ala-NHMe) forms of **3** and **7** were analyzed. In addition, all combinations of the three chiral centers of compounds **3** and **7** were considered, since at the outset it was not clear that the cyclization would be as stereoselective as it eventually proved to be. For each molecule the set of low-energy conformers was evaluated to identify those which were turn-like. β-Turns in proteins are identified as any tetrapeptide sequence occurring in a nonhelical region in which the $\alpha C_1-\alpha C_4$ distance is less than or equal to 7.0 Å.[1] Conformers which satisfied this requirement are designated as turns in the discussion that follows. The definition of a specific class of β-turn is based on the ϕ and ψ backbone torsion angles (±20°) of residues 2 and 3.[62,63] Assignment of a low-energy conformation to a particular turn type was made where possible on the basis of these canonical ϕ and ψ torsion angles.

Table 1. Characteristics of Low-Energy Conformers Found for Dipeptide (**3Sb**) and Tetrapeptide (**3Sc**) Analogs of L-Hse-(*S*-ec)-L-Pro[a]

conformer	E_{total}[b]	E_u[b]	E_v[b]	μ (D)	$\alpha C_1-\alpha C_4$ distance
3Sb					
1	−365.9	−306.7	59.2	3.3	8.3
2	−360.9	−305.5	−55.4	4.3	7.7
3	−357.3	−294.7	−62.6	5.7	7.6
4	−356.9	−297.2	−59.7	3.4	8.0
5	−351.4	−278.0	−59.3	3.7	7.3
6	−351.2	−277.8	−73.4	5.9	8.3
7	−341.6	−256.6	−85.0	14.8	7.8
3Sc					
2	−654.3	−569.3	−85.0	9.0	6.2
3	−654.2	−542.2	−112.0	11.8	6.9
4	−653.0	−561.6	−91.4	12.8	8.1
5	−652.6	585.6	−97.9	7.9	6.1

[a] No equivalent turn types were identified. [b] All energies are reported in kilojoules/mole. E_v and E_u are the energy of the conformation *in vacuo* and the solute–solvent interaction energy, respectively.

The results from the conformational search of Ac-L-Hse-(*S*-ec)-L-Pro-NHMe (**3Sb**), in which the bridgehead carbon has an *S* configuration, are shown in Table 1. In this case, none of the conformations found had an $\alpha C_1-\alpha C_4$ distance of less than 7.0 Å. Therefore, no β-turn classes were identified. The most stable conformation appeared to be the most extended in nature on the basis of the long $\alpha C_1-\alpha C_4$ distances. When this bicycle was modeled in the tetrapeptide form **3Sc**, a total of 63 conformations were found (Table 1). Only 10 of these conformations (including conformers 2, 3, and 5) had the desired $\alpha C_1-\alpha C_4$ distance of less than 7.0 Å. None of the conformations could be classified into standard β-turn classes on the basis of their $\phi 2$, $\psi 2$, $\phi 3$, and $\psi 3$ torsion angles. The most stable conformation (conformer 1) could again be considered as extended in nature. Interestingly, the incorporation of the extra Ala residues appears to help stabilize reverse-turn conformations, as the protected bicycle (**3Sb**) itself does not appear to adopt any β-turn conformations.

In the case of Ac-L-Hse-(*R*-ec)-L-Pro-NHMe (**3Rb**), a total of 27 conformations were found, of which 21 were β-turn mimetics (Table 2). With the exception of conformer 3, the first six conformations all had $\alpha C_1-\alpha C_4$ distances greater than 7.0 Å. Of the 87 conformations found for compound **3Rc** (the tetrapeptide analog), 72 had an $\alpha C_1-\alpha C_4$ distance of less than 7.0 Å. In fact, the first 29 conformations (within 14 kJ/mol) could be classified as β-turn mimetics. In contrast to the findings for the Hse-(*S*-ec)-Pro compounds (Table 1), several

(55) Moeller, K. D.; Wong, P. L. *BioMed. Chem. Lett.* **1992**, *2*, 739–742.
(56) Moeller, K. D.; Hanau, C. E.; D'Avignon, D. A. *Tetrahedron Lett.* **1994**, *35*, 835–838.
(57) Baldwin, J. E.; Hulme, C.; Schofield, C. J.; Edwards, A. J. *J. Chem. Soc., Chem. Commun.* **1993**, 935–936.
(58) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657–1666.
(59) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.
(60) 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.
(61) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386.
(62) Lewis, P. N.; Momany, F. A.; Scheraga, H. A. *Biochim. Biophys. Acta* **1973**, *303*, 211–229.
(63) Chou, P. Y.; Fasman, G. D. *J. Mol. Biol.* **1977**, *115*, 135–175.

Table 2. Characteristics of Low-Energy Conformers Found for Dipeptide (**3Rb**) and Tetrapeptide (**3Rc**) Analogs of L-Hse-(*R*-ec)-L-Pro[a]

conformer	turn type	E_{total}	E_u	E_v	μ (D)	$\alpha C_1-\alpha C_4$ distance
3Rb						
		−342.5	−268.2	−74.3	3.6	7.4
3		−340.9	−278.2	−62.7	2.5	6.7
10	I or III	−332.2	−236.9	−95.3	14.6	5.0
13	II′	−330.4	−261.0	−69.4	2.5	5.4
14	II′	−330.0	−268.9	−61.1	1.9	4.9
15	II′	−328.8	−268.5	−60.3	1.1	5.5
24	I or III	−322.9	−212.6	−110.3	8.9	4.3
25	V′	−322.5	−263.3	−59.2	2.5	5.3
3Rc						
1		−646.6	−551.4	−95.2	2.5	6.0
2		−646.4	−538.5	−107.9	2.2	5.9
3		−645.5	533.0	−112.5	2.2	5.8
36	I or III	−631.2	−522.9	−108.3	8.8	5.5
41	I′	−630.6	−537.9	−92.7	7.6	5.0
72	II′	−628.3	−542.1	−86.2	0.1	5.1
77	I	−628.1	−535.3	−92.8	10.1	4.9
83	II′	−627.2	−610.7	−16.5	0.9	5.1

[a] Assessment of turn type is based on comparison of torsion angles $\phi 2$, $\psi 2$, and $\psi 3$ with those observed in canonical turn types.[62,63] Where no entry is given, no equivalent turn type was identified. (See footnote *b* in Table 1 for description of energies.)

Table 3. Characteristics of Low-energy Conformers Found for Dipeptide (**7Sb**) and Tetrapeptide (**7Sc**) Analogs of L-Hse-(S-ec)-D-Pro[a]

conformer	E_{total}	E_u	E_v	μ (D)	$\alpha C_1-\alpha C_4$ distance
7Sb					
1	−359.5	−308.9	−50.6	2.8	7.3
2	−357.0	−289.5	−67.5	3.2	7.7
3	−348.3	−280.3	−68.0	9.2	7.5
4	−333.7	−271.1	−259.4	5.2	6.9
7Sc					
1	−653.5	−551.0	−102.5	13.2	7.4
2	−653.3	−571.7	−81.6	1.9	6.2
3	−648.7	−545.0	−103.7	13.2	6.9
7	−645.3	−543.0	−102.3	6.4	6.9
10	−644.4	−554.8	−89.6	2.1	6.1

[a] See fottnotes to Table 1.

standard β-turn classes could be identified among the low-energy conformers obtained (Table 2).

For Ac-L-Hse-(*S*-ec)-D-Pro-NHMe (**7Sb**), a total of four conformations were found, of which one (conformer 4) had an $\alpha C_1-\alpha C_4$ distance of less than 7.0 Å (Table 3). When modeled in a tetrapeptide analog (**7Sc**), a total of 157 conformations were found, of which 68 had the desired $\alpha C_1-\alpha C_4$ distance of less than 7.0 Å (Table 3). None of the conformations found for these two peptides could be classified into standard β-turn classes on the basis of their ϕ and ψ torsion angles. The most favorable reverse-turn conformation was conformation 2 of the tetrapeptide **7Sc**, which is only 0.2 kJ/mol above the lowest energy conformation found. Although only a single conformer appears to be turn-like, the small number of total conformations accessible to this compound means that a relatively high percentage of them are reverse-turn-like.

In the case of Ac-L-Hse-(*R*-ec)-D-Pro-NHMe (**7Rb**), a total of 11 conformations were found. None of these conformations had an $\alpha C_1-\alpha C_4$ distance of less than 7.0 Å (Table 4). When modeled as a tetrapeptide (compound **7Rc**), eight of the 187 low-energy conformations were found to be reverse turns. None of these conformations could be classified into β-turns on the basis of their ϕ and ψ torsion angles.

Table 4. Characteristics of Low-Energy Conformers Found for Dipeptide (**7Rb**) and Tetrapeptide (**7Rc**) Analogs of L-Hse-(*R*-ec)-D-Pro[a]

conformer	E_{total}	E_u	E_v	μ (D)	$\alpha C_1-\alpha C_4$ distance
7Rb					
1	−347.7	−267.7	−80.0	9.8	9.1
2	−342.5	−272.6	−69.9	6.0	9.3
3	−342.4	−276.7	−65.7	4.3	7.9
4	−340.5	−269.6	−70.9	10.6	7.8
5	−338.6	−243.5	−95.1	9.0	9.0
7Rc					
1	−637.5	−533.7	−103.8	19.9	9.0
7	−632.9	−550.7	−82.2	3.6	6.2
25	−628.0	−515.1	−112.9	12.3	6.9
31	−627.6	−525.1	−102.5	5.9	6.7
34	−627.9	−515.0	−112.0	4.2	6.9

[a] See footnotes to Table 1.

Table 5. Characteristics of Low-Energy Conformers Found for Ac-Ala-D-Hse-(*S*-ec)-D-Pro-Ala-NHMe[a]

conformer	turn type	E_{total}	E_u	E_v	μ (D)	$\alpha C_1-\alpha C_4$ distance
1		−651.2	−539.8	−111.4	5.3	5.9
2	I′	−647.3	−531.0	−116.3	2.5	5.2
25	I′	−635.9	−546.3	−89.6	4.1	5.1
32	I′	−634.0	−511.3	−122.7	7.4	5.0

[a] See footnote to Table 2.

Table 6. Characteristics of Low-Energy Conformers Found for Ac-Ala-D-Hse-(*R*-ec)-D-Pro-Ala-NHMe[a]

conformer	E_{total}	E_u	E_v	μ (D)	$\alpha C_1-\alpha C_4$ distance
1	−655.5	−548.2	−107.3	11.5	8.0
2	−653.7	−569.4	−84.3	11.2	6.3
9	−650.1	−567.9	−82.2	5.1	6.4
10	−647.1	−535.3	−111.8	17.3	6.9
33	−643.8	−538.5	−105.3	3.2	6.2

[a] See footnotes to Table 1.

Conformational data for Ac-D-Hse-(*S*-ec)-D-Pro-NHMe, Ac-D-Hse-(*R*-ec)-D-Pro-NHMe, Ac-D-Hse-(*S*-ec)-L-Pro-NHMe, and Ac-D-Hse-(*R*-ec)-L-Pro-NHMe were not calculated, but can be deduced from their enantiomers (**3Rb**, **3Sb1**, **7Rb**, and **7Sb**, respectively). When these chiral β-turn mimetics are incorporated into tetrapeptides with two new Ala chiral centers, however, the equivalence is destroyed. Consequently, the tetrapeptide analogs of these enantiomers were modeled independently. For Ac-Ala-D-Hse-(*S*-ec)-D-Pro-Ala-NHMe, a total of 50 conformations were found, of which 44 had $\alpha C_1-\alpha C_4$ distances of less than 7.0 Å. Several of these reverse-turn conformations could be classified into standard type-I′ β-turn classes on the basis of their ϕ and ψ torsion angles (Table 5). The conformational search data for the tetrapeptide analog Ac-Ala-D-Hse-(*R*-ec)-D-Pro-Ala-NHMe indicates that 16 conformations of the observed 77 had $\alpha C_1-\alpha C_4$ distances of less than 7.0 Å (Table 6). For the tetrapeptide analog Ac-Ala-D-Hse-(*S*-ec)-L-Pro-Ala-NHMe, a total of 33 conformations were found, of which 19 had the desired $\alpha C_1-\alpha C_4$ of less than 7.0 Å (Table 7). For the same compound with the *R* ring fusion (Ac-Ala-D-Hse-(*R*-ec)-L-Pro-Ala-NHMe), a total of 146 conformations were found, with 23 having the desired $\alpha C_1-\alpha C_4$ distance of less than 7.0 Å (Table 8).

In order to gauge how well the various constrained bicyclic systems stabilize reverse-turn conformations, we also carried out conformational searches of several linear peptides as

Table 7. Characteristics of Low-Energy Conformers Found for Ac-Ala-D-Hse-(*S*-ec)-L-Pro-Ala-NHMe[a]

conformer	E_{total}	E_u	E_v	μ (D)	$\alpha C_1-\alpha C_4$ distance
1	−658.6	−571.9	−86.7	4.7	6.1
2	−650.1	−550.7	−99.4	10.8	6.9
3	−650.0	−556.5	−93.5	1.0	5.9
4	−646.5	−546.0	−100.6	2.4	5.9

[a] See footnotes to Table 1.

Table 8. Characteristics of Low-Energy Conformers Found for Ac-Ala-D-Hse-(*R*-ec)-L-Pro-Ala-NHMe[a]

conformer	E_{total}	E_u	E_v	μ (D)	$\alpha C_1-\alpha C_4$ distance
1	−637.5	547.0	−90.5	4.2	9.0
8	−634.4	−547.9	−86.5	4.1	6.1
9	−633.8	−552.1	−81.7	2.8	6.0
14	−633.1	−539.8	−93.3	11.0	6.1
22	−630.4	−525.6	−104.8	12.7	6.8

[a] See footnotes to Table 1.

Table 9. Summary of Conformational Search Data Using Aqueous Solvation Model[a]

peptide modeled	no. of low-energy conformations	reverse turns: no. and %[b]	no. of β-turn types and classes[c]
Hse-Pro (linear)	46	0 (0)	0
Ala-Ala (linear)	42	20 (48)	1 (I′)
Hse-("R-ec)-Pro (**3Rb**)	27	21 (78)	7 (I, II′, III, V′)
Hse-(*S*-ec)-Pro (**3Sb**)	7	0 (0)	0
Ala-Hse-(*R*-ec)-Pro-Ala (**3Rc**)	87	72 (83)	5 (I, II′, III)
Ala-Hse-(S-ec)-Pro-Ala (**3Sc**)	63	10 (16)	0
Hse-D-Pro (linear)	107	91 (85)	8 (I′)
Ala-D-Ala (linear)	39	19 (49)	3 (I′, I or III, II′)
Hse-(*R*-ec)-D-Pro (**7Rb**)	11	0 (0)	0
Hse-(*S*-ec)-D-Pro (**7Sb**)	4	1 (25)	0
Ala-Hse-(*R*-ec)-D-Pro-Ala (**7Rc**)	189	8 (4)	0
Ala-Hse-(*S*-ec)-D-Pro-Ala (**7Sc**)	157	68 (43)	0
Ala-D-Hse-(*S*-ec)-D-Pro-Ala	50	44 (88)	3
Ala-D-Hse-(*R*-ec)-D-Pro-Ala	77	16 (21)	0
Ala-D-Hse-(*S*-ec)-Pro-Ala	33	19 (56)	0
Ala-D-Hse-(*R*-ec)-Pro-Ala	50	44 (88)	0

[a] All peptides were capped as *N*-acetyl and *C*-terminal methylamides. [b] Defined as conformations having an $\alpha C_1-\alpha C_4$ distance less than or equal to 7.0 Å.[1,62,63] Percentages given in parentheses. [c] Assessment of turn type was based on comparison of torsion angles $\phi 2$, $\psi 2$, $\phi 3$, and $\psi 3$ with those observed in canonical turn types.[62,63]

controls. These included Ac-Hse-Pro-NHMe, Ac-Ala-Ala-NHMe, Ac-Hse-D-Pro-NHMe, and Ac-Ala-D-Ala-NHMe. The conformational search data for these linear peptides and all of the constrained analogs mentioned above are summarized in Table 9. In comparing results from the linear peptides with their bicyclic counterparts, several important points are apparent. For the L,L series of compounds, one can see that the presence of a Pro at the $i+2$ position in the linear peptides destabilizes reverse-turn conformations (0% for Ac-Hse-Pro-NHMe) with respect to the alanine dipeptide (48% reverse-turn conformations for Ac-Ala-Ala-NHMe). The cyclization of L-Hse to L-Pro with the formation of an *R* stereocenter results in a molecule whose conformation is significantly restricted to reverse-turn regions (78% for compound **3Rb** versus 0% for Ac-Hse-Pro-NHMe). In contrast, cyclization of L-Hse to L-Pro with formation of an *S* stereocenter at the bridgehead does little to stabilize reverse-turn conformations, resulting in an $\alpha C_1-\alpha C_4$ distance range of 7.3−8.3 Å for the seven lowest energy conformers of compound **3Sb** (Table 1).

For the linear L,D peptide series, one can see that the presence of D-Pro results in a higher percentage (85%) of reverse-turn conformations in comparison to the alanine dipeptide (49%). However, the addition of cyclic constraints (either *S* or *R* ring fusion, **7Sb** and **7Rb**, respectively) results in a significant reduction of reverse-turn conformations in relation to the linear peptides, which may reflect a preference for a more extended geometry with the bicyclic constraint. It must be remembered that these minima reflect the enthalpic surface. In order for a true quantitative comparison to be made, one must evaluate the contribution of each minimum according to its free energy.

When modeled as tetrapeptides, the D,D-bicyclic compounds show turn stabilization which is quantitatively and qualitatively similar to that of tetrapeptides derived from the enantiomeric L,L series. One of the ring fusion epimers (Ac-Ala-D-Hse-(*S*-ec)-D-Pro-Ala-NHMe, 88%) is markedly greater in turn proclivity than the other (Ac-Ala-D-Hse-(*R*-ec)-D-Pro-Ala-NHMe, 21%). The D,D and L,L tetrapeptides which most strongly favor turns are related by inversion about the three chiral centers of the bicycle. In contrast, when the D,L-bicyclic compounds were modeled in tetrapeptides, both ring fusion epimers showed a significant turn stability which is greater than that seen in the L,D series (56% vs 4% for one stereopair and 88% vs 43% for the other (given as D,L vs L,D)). These data illustrate the influence of the chiral environment in selection of low-energy conformations.

Several important points can be concluded from the results of the conformational analyses. Reverse-turn propensity occurs with only some of these bicyclic dipeptides (Hse-(*R*-ec)-Pro, D-Hse-(*S*-ec)-D-Pro, and D-Hse-(*R*-ec)-Pro), and the quantitative preference (sometimes lower than the linear peptide controls, Table 9) depends on the model system studied. The conformations found for the acetyl- and NHMe-protected mimetics contain only a small percentage of reverse-turn conformations. In contrast, the tetrapeptide conformers contain a significantly higher percentage of turn conformations. Therefore, one would expect that, upon insertion of this bicycle into peptides, the sequence of the peptides would provide different "environments" that may help stabilize, or destabilize, particular turn conformations. Certainly, due to the conformational restriction of the bicyclic constraint, irrespective of sequence, the peptide chain will not be fully extended and will be forced (by the constraint) to change direction. However, the tightness of the turn and the mimicry of a particular turn type will depend on the sequence of the peptide as well as the bicyclic mimetic. The second important point is that the lowest energy conformations found are primarily extended-like in nature. It is not surprising to find extended-like conformations in water, simply because of the screening effect of the solvent on charge interactions and the resulting destabilization of hydrogen bonding. This obviously differs from *in vacuo* simulations where no such screening exists. At least qualitatively, these conclusions agree with the conclusions of Tobias et al.[64] Their data showed that type-I reverse turns in short model peptides are intrinsically unstable in water.

NMR Structural Studies. Two-dimensional NMR studies were used to establish the absolute configuration of the electrochemically-formed ring fusion in compounds **3Sa** and **7Sa**. Degeneracy of the Hse and Pro β-protons of **3Sa** made it impossible to exploit the known chirality at the α-carbons by measuring the relative intensity of NOE connectivities ($\alpha'-\beta'$, $\alpha-\beta''$, $\beta'-\gamma'$, $\beta'-\gamma''$, etc.) around the two rings. As an alternative, interproton distances within the ensemble of low-

(64) Tobias, D. J.; Sneddon, S. F.; Brooks, C. L., III. *J. Mol. Biol.* **1990**, *216*, 783−796.

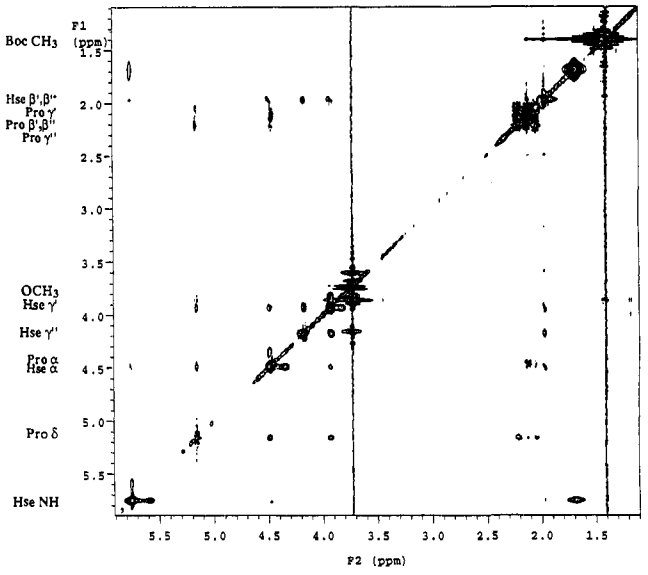


Figure 2. NOESY spectrum (600 MHz, 25 °C, τ_m = 300 ms, $CDCl_3$) of compound **3Sa** demonstrating that the ring fusion has an *S* absolute configuration. Resonant frequencies are labeled along F_1. All NOE cross peaks were of opposite sign from the diagonal, indicating that the molecule is in the extreme narrowing limit. The absence of NOE connectivities from Hse γ′ (4.20 ppm) to Hse α and Pro δ and the presence of NOE connectivities from Hse γ″ (3.96 ppm) to both Hse α and Pro δ can only be explained if the absolute configuration at Pro δ is *S*.

energy conformers found for the *R*- and *S*-isomers of compound **3** (Tables 1 and 2) were examined. For the *R*-conformations, the Hse Hα−Pro Hδ distance ranged from 3.9 to 4.9 Å, while in the *S*-conformations, this distance was much smaller, ranging from 2.3 to 2.8 Å. The Hse Hα−Pro Hδ distance calculated from the observed NOE cross-peak volume was 2.2 ±0.4 Å, which is consistent with an *S*-configuration at the ring fusion rather than *R*. Further support for this assignment lies in NOE connectivities to the Hse γ protons. The NOESY spectrum of Figure 2 shows no connectivities for Hse Hγ′ (4.20 ppm) to either Hse Hα (4.52 ppm) or Pro Hδ (5.20 ppm). In contrast, Hse Hγ″ at 3.96 ppm exhibits NOE connectivities to *both* Hse Hα and Pro Hδ. The longest distance observable in the NOESY spectrum was calculated to be 3.5 Å. Examination of the entire ensemble of *R*-isomer conformations (Table 2) revealed that neither of the Hse γ protons could simultaneously be within 3.5 Å of the Hse α and Pro δ hydrogens. In contrast, several of the *S*-isomer conformations in Table 1 satisfied this restraint.

For the D-Pro bicyclic analog (compound **7Sa**), the NOESY spectrum revealed the same pattern with respect to the Hse Hγ′ and Hγ″ protons. Only one of these protons exhibited NOE connectivities to protons other than the Hse Hβ protons, and, as in the case of compound **3**, these were NOEs to Hse Hα and Pro δ. Consequently, an *S*-configuration was assigned to the ring fusion Pro δC of compound **7**.

The turn-inducing potential of compound **3** was evaluated by ^1H NMR analysis of a peptide into which it had been incorporated. The shortened LHRH analog, Ac-D-Nal[1]-D-Cpa[2]-D-Pal[3]-Hse[4]-(*S*-ec)-Pro[5]-Pro[6]-Ala[7]-NH_2 gave a single set of signals in 50% DMSO/H_2O at 5 °C, with no evidence of *cis*/*trans*-isomerization at Pro[6]. NOESY analysis (data not shown) revealed only intraresidue and sequential connectivities; no medium- or long-range NOEs were present. Only the backbone coupling constants ($J_{NH-\alpha H}$) for Ala[7] and Pal[3] could be measured, as the rest were obscured by overlap. The observed values of ~7 Hz were consistent with conformational averaging. Signal dispersion was poor in both the amide and α regions. Overall, the results suggested that the peptide is disordered in 50% DMSO/H_2O.

Conclusions

The synthetic approach outlined in this study, with its key electrochemical cyclization, leads to *N*-Boc-protected bicyclic dipeptides in four steps with a 25% overall yield starting from commercially available amino acids. These compounds are suitable for solid phase peptide synthesis, which facilitates synthesis of analogs of biologically active peptides with this template incorporated into their primary sequence. The use of chiral precursors for the cyclization and the generation of only one new chiral center at the ring fusion with high stereoselectivity highlight the potential of this approach for the generation of peptidomimetics with a required geometry. In addition, introduction of appropriate side chain functionality, into either the proline or homoserine component, does not present a major synthetic challenge as routes to such derivatives are available in the literature. The relatively rigid bicyclic ring system would appear to provide an appropriate scaffold for orienting side chains. The exploration of such modifications and their impact on the electrochemical cyclization are current topics for research (Slomczynska, Chalmers, and Marshall, unpublished).

Although the bicyclic dipeptides prepared in this study, Hse-(*S*-ec)-Pro and Hse-(*S*-ec)-D-Pro, do not appear to stabilize formation of β-turn conformations but force a more extended conformation, they should still prove useful to constrain the backbone conformation of peptides as a probe of molecular recognition. In addition, epimerization of the chiral bridgehead would yield Hse-(*R*-ec)-Pro, whose geometry is more conducive to β-turn stabilization. In the case of the 6,5-bicyclic (1-aza-5-oxabicyclo[4.3.0]nonane) analog (Boc-Ser-(*S*-ec)-Pro-OH in comparable nomenclature),[57] epimerization of the analogous chiral center was accomplished by prolonged treatment with trifluoroacetic acid. Our studies to date suggest a number of alternative bicyclic structures as turn-inducing elements of peptides. Comparison of the reverse-turn propensities of these compounds with other peptidomimetics described in the literature is the subject of ongoing research.

Experimental Section

Synthesis. Infrared spectra (IR) were obtained using a Perkin-Elmer 1710 FT-IR spectrometer. Chemical ionization mass spectral data were obtained on a 500 VG Biotek-Trio 3A mass spectrometer (Parke-Davis Research Laboratories) using 1% ammonia in methane. Carbon, hydrogen, and nitrogen analyses were obtained from Galbraith Laboratories Inc., Knoxville, TN. Melting points were obtained on a Thomas Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured in a 1-dm cell (1 mL) on a Perkin-Elmer polarimeter (Model 241) at 589 nm (Na D line).

For thin-layer chromatography (TLC) 250-nm silica gel GF precoated uniplates (Analtech) were used with the solvent system indicated. The chromatograms were developed with chlorine followed by starch/KI spray. For flash chromatography, columns packed with silica gel 60 (Merck) were used (5.5 × 15 cm). Analytical high-performance chromatography (HPLC) was performed on a Spectra-Physics instrument with an SP8800 ternary pump, using a Vydac C_{18} column (0.46 × 25 cm, particle size 5 μm) at a flow rate of 1.0 mL/min, UV detection at 220 nm, and solvents (A) 0.05% trifluoroacetic acid in H_2O and (B) 0.038% trifluoroacetic acid in 90:10 acetonitrile/H_2O. The gradient used was 10−90% B in 25 min.

Electrolyses were conducted using a Model 630 coulometer, a Model 410 potentiostatic controller, and a Model 420A power supply purchased from the Electrosynthesis Company, Inc. Tetrabutylammonium tetrafluoroborate was purchased from Aldrich and stored in a vacuum desiccator (ca. 0.5 mmHg). *N*-Boc-*O*-benzyl-L-homoserine was purchased from Bachem California; L-proline methyl ester, D-proline

methyl ester, and diisopropylethylamine were purchased from Advanced Chemtech; and TBTU was obtained from Richelieu. All solvents were purchased from Baxter and used without purification.

Boc-Hse(Bzl)-Pro-OMe (1). L-Proline methyl ester hydrochloride (13.26 g, 80 mmol), *N-tert*-butoxycarbonyl-*O*-benzyl-L-homoserine (24.72 g, 80 mmol), and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (25.68 g, 80 mmol) were dissolved in 400 mL of dichloromethane. The solution was stirred and cooled in an ice water bath while *N,N*-diisopropylethylamine (36.2 g = 48.8 mL, 280 mmol) was added. Stirring was continued overnight at room temperature. The solvent was evaporated *in vacuo*. The residue was redissolved in ethyl acetate (500 mL), and the organic phase was washed with 1 M $KHSO_4$ (3 × 150 mL), saturated $NaHCO_3$ (3 × 150 mL), and brine (3 × 150 mL). The solution was dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. The resulting crude peptide was purified using a silica gel column (5.5 × 20 cm) with ethyl acetate (50% in hexane) as eluent ($R_f = 0.30$). The pure compound **1** (27.3 g, 81%) was a clear oil: $[\alpha]^{25}_D$ −52.9° ($c = 1$, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 5H), 5.62 (d, 1H, $J = 8.2$ Hz), 4.68 (m, 1H), 4.54 (m, 1H), 4.53 (AB system, 2H, $J = 11.84$ Hz), 3.74 (m, 2H), 3.71 (s, 3H), 3.60 (t, 2H, $J = 6.2$ Hz), 2.19 (m, 1H), 1.98 (m, 5H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.43, 171.14, 155.46, 138.26, 128.26, 127.59, 127.44, 79.46, 73.06, 66.28, 58.60, 52.13, 49.44, 46.77, 33.05, 29.96, 28.27, 24.77; IR (neat/NaCl) 3313, 2977, 1747, 1710, 1652, 1498, 1440, 1366, 1326, 1250, 1169, 1101 cm^{-1}; MS (PCI) *m/z* 421 (MH^+), calcd for $C_{22}H_{32}N_2O_6$ 420.51. Anal. Calcd for $C_{22}H_{32}N_2O_6$: C, 62.84; H, 7.67; N, 6.66. Found: C, 61.74; H, 7.54; N, 6.52 (results of two determinations, subsequent products analyzed within limits).

Boc-Hse-Pro-OMe (2). A solution of Boc-L-homoseryl-L-prolyl methyl ester (**1**) (27.1 g, 64.4 mmol) in 50% acetic acid in methanol (50 mL) was prepared in a 500-mL hydrogenation flask fitted with a mechanical shaker and a gas inlet−outlet tube connected to a hydrogen tank. The reaction mixture was degassed by bubbling nitrogen through the solution for 5 min, and 10% palladium-on-charcoal (2.7 g = 0.27 g of metal) was added. The system was exposed to vacuum for 5 min and then to a pressure of 45 psi of hydrogen. After 48 h at room temperature, the solution was filtered through Celite, and the filtrate was evaporated. The crude oil was purified on a silica gel column with ethyl acetate as eluent ($R_f = 0.33$) and then recrystallized from a mixture of ethyl acetate/hexane to afford 16.5 g (77% yield) of white crystals: $[\alpha]^{25}_D$ −95.6° ($c = 1$, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.62 (d, 1H, $J = 8.2$ Hz), 4.62 (m, 1H), 4.56 (m, 1H), 3.73 (s, 3H), 3.72 (m, 4H), 2.92 (s, 1H), 2.24 (m, 1H), 2.05 (m, 4H), 1.59 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.79, 170.60, 156.71, 79.14, 58.17, 57.40, 51.61, 48.52, 46.32, 34.93, 28.39, 27.63, 24.27; IR (neat/NaCl) 3424, 2977, 1746, 1707, 1644, 1508, 1438, 1393, 1367, 1250, 1171, 1052 cm^{-1}; MS (PCI) *m/z* 331 (MH^+), calcd for $C_{15}H_{26}N_2O_6$ 330.38. Anal. Calcd for $C_{15}H_{26}N_2O_6$: C, 54.53; H, 7.93; N, 8.48. Found: C, 54.82; H, 7.70; N, 8.42.

(3*S*,7*S*,10*S*)-3-((*tert*-Butyloxycarbonyl)amino)-10-(methoxycarbonyl)-1-aza-6-oxa-2-oxobicyclo[5.3.0]decane, Boc-Hse-(ec)-Pro-OMe (3). A two-hole rubber stopper was fitted with a needle as a nitrogen inlet and two platinum foil electrodes (20 cm^2 each). The stopper was placed on the top of a vial charged with 4 mL of isopropyl alcohol, 76 mL of acetonitrile, 26.24 g (80 mmol) of tetrabutylammonium tetrafluoroborate, and 13.20 g (40 mmol) of Boc-Hse-Pro-OMe (**2**). The reaction mixture was degassed by sonication and then electrolyzed with a constant current of 138 mA (current density 6.9 mA/cm^2). After 3.8 F/mol was passed (29 h), no more starting material could be detected by HPLC ($t_R = 10.5$ min for **2**, 12.32 min for **3**). The reaction mixture was concentrated *in vacuo* and immediately chromatographed on silica gel using 30% ethyl acetate/hexane and then 50% ethyl acetate/hexane as eluent to afford 6.30 g (48%) of foamy solid **3**: TLC $R_f = 0.27$ in 50% ethyl acetate/hexane; HPLC purity 99%, $t_R = 12.32$ min (C_{18} column); $[\alpha]^{25}_D$ −143.6° ($c = 1$, methanol). TOCSY and ^{1}H{^{13}C} HMQC experiments enabled assignment of proton and carbon NMR resonances: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.76 (Hse NH, d, 1H, $J =$ 6.0 Hz), 5.16 (Pro Hδ, dd, 1H, $J_{\gamma\delta} = 6.6$, 3.0 Hz), 4.48 (Hse Hα, m, 1H), 4.46 (Pro Hα, dd, 1H, $J_{\alpha\beta} = 6.6$, 7.8 Hz), 4.20 (Hse γ'', dt, 1H, $J_{\gamma\gamma} = 12.0$ Hz, $J_{\gamma''\beta} = 3.0$, 3.6 Hz), 3.93 (Hse γ', m, 1H), 3.73 (OMe, s, 3H), 2.25 (Pro Hγ'', m, 1H), 2.16 (Pro Hβ'/Hβ'', m, 2H), 2.08 (Pro Hγ', m, 1H), 1.97 (Hse Hβ'/Hβ'', m, 2H), 1.45 (Boc CH_3, s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.57, 171.14 (Hse, Pro C=O), 155.14 (Boc C=O), 89.26 (Pro δ), 79.63 (Boc quarternary C), 70.80 (Hse β), 59.39 (Pro α), 53.93 (Hse α), 52.35 (OMe), 33.33 (Hse β), 32.67 (Pro γ), 28.25 (Boc CH_3), 26.55 (Pro β); IR (neat/NaCl) 3406, 3360, 2977, 1750, 1714, 1665, 1499, 1440, 1393, 1367, 1326, 1271, 1250, 1200, 1172, 1089, 1059 cm^{-1}; MS (PCI) *m/z* 329 (MH^+), calcd for $C_{15}H_{24}N_2O_6$ 328.37. Anal. Calcd for $C_{15}H_{24}N_2O_6$: C, 54.87; H, 7.37; N, 8.53. Found: C, 54.97; H, 7.80; N, 8.24.

(3*S*,7*S*,10*S*)-3-((*tert*-Butyloxycarbonyl)amino-10-carboxy-1-aza-6-oxa-2-oxobicyclo[5.3.0]decane, Boc-Hse-(ec)-Pro-OH (4). A solution of compound **3** (0.492 g, 1.5 mmol) in methanol (1 mL) was surrounded by an ice-cooled water bath, and 1 N NaOH (1.65 mL) was added with stirring. The mixture was stirred at room temperature for 1 h. The methanol was removed *in vacuo*, and the aqueous phase was acidified with 1 M $KHSO_4$ (10 mL) and extracted with ethyl acetate (40 mL). The organic phase was washed with brine (2 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. The crude peptide was purified on a silica gel column with 95:5:1 CH_2Cl_2/MeOH/AcOH as eluent ($R_f = 0.30$). The pure compound **4** (0.398 g, 84%) was a glassy white solid: HPLC purity 98%, $t_R = 10.46$ min (C_{18} column); $[\alpha]^{25}_D$ −86.2° ($c = 1$, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 5.87 (d, 1H, $J = 4.1$ Hz), 5.24 (dd, 1H, $J = 5.7$ Hz, $J = 3.0$ Hz), 4.59 (dd, 1H, $J = 6.0$ Hz, $J = 13.8$ Hz), 4.50 (dd, 1H, $J = 14.1$ Hz, $J = 5.7$ Hz), 4.18 (dt, 1H, $J = 12.5$ Hz, $J = 3.1$ Hz), 3.98 (m, 1H), 2.25 (m, 3H), 2.10 (m, 1H), 1.98 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.00, 171.60, 155.34, 89.39, 79.96, 70.63, 59.41, 53.77, 33.13, 32.60, 28.24, 26.32; IR (neat/NaCl) 3424, 2980, 1743, 1710, 1662, 1500, 1443, 1368, 1367, 1250, 1166, 1090, 1060 cm^{-1}; MS (PCI) *m/z* 315 (MH^+), calcd for $C_{14}H_{22}N_2O_6$ 314.34. Anal. Calcd for $C_{14}H_{22}N_2O_6$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.61; H, 7.29; N, 8.64.

Boc-Hse(Bzl)-D-Pro-OMe (5). Boc-Hse(Bzl)-D-Pro-OMe was synthesized from Boc-Hse(Bzl)-OH and HCl·D-Pro-OMe as described for **1**, yield 87%. The pure compound was a clear oil: $[\alpha]^{25}_D$ 16.3° ($c =$ 1, MeOH); IR (neat/neat) 3315, 3000, 1746, 1746, 1709, 1646, 1498, 1453, 1393, 1367, 1249, 1169 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), 5.45 (d, 1H), 4.69 (m, 1H), 4.48 (AB system, $J = 11.7$ Hz), 4.38 (m, H), 3.80 (m, 2H), 3.71 (s, 3H), 3.59 (m, 2H), 1.78−2.32 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (172.58), 172.3, (171.82), 170.69, (155.48), 155.19, (138.28), 138.12, 128.20, (128.15), 127.60, 127.46 (127.33), (79.45), 79.38, 73.08, (72.93), 66.37, (66.26), (59.09), 58.84, (52.27), 52.10, 49.50, (49.23), 46.69, (46.683), 33.30, (32.42), (31.14) 28.91, 28.20, 24.56, (22.33) (resonances in parentheses are due to an impurity (<5%) which could be traced to contamination by the L-Pro isomer); MS (PCI) *m/z* 421 (MH^+), calcd for $C_{22}H_{32}N_2O_6$ 420.51.

Boc-Hse-D-Pro-OMe (6). Boc-Hse(Bzl)-D-Pro-OMe (1.26 g, 3 mmol) was hydrogenated as described for **2**, yielding 0.83 g (84%) of **6** as a glassy powder: $[\alpha]^{25}_D$ 28.1 ($c = 1$, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.71 (d, $J = 8.1$ Hz), 4.64 (m, 1H), 4.42 (m, 1H), 3.73 (s, 3H), 3.72−3.87 (m, 2H), 3.55−3.72 (m, 2H), 2.23 (m, 1H), 1.85−2.18 (m, 4H), 1.50−1.65 (m, 1H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (172.47), 172.16, (171.48), 156.30, (155.89), 79.87, (79.71), (59.12), 58.75, (57.90), 57.54, (52.47), 52.03, (48.80), 48.62, 46.67, (46.37), 35.79, (35.16), (30.94), 28.84, 27.86, 24.36, (22.10) (resonances in parentheses are due to an impurity (<5%) which could be traced to contamination by the L-Pro isomer); IR (neat/NaCl) 3400, 3000, 1745, 1703, 1640, 1510, 1440, 1367, 1170, 1056 cm^{-1}; MS (PCI) *m/z* 331 (MH^+), calcd for $C_{15}H_{26}N_2O_6$ 330.38.

(3*S*,7*S*,10*R*)-3-((*tert*-Butyloxycarbonyl)amino)-10-(methoxycarbonyl)-1-aza-6-oxa-2-oxobicyclo[5.3.0]decane, Boc-Hse-(ec)-D-Pro-Ome (7). Boc-Hse-D-Pro-OMe **6** (0.33 g, 1 mmol) was electrolyzed under the same conditions as described for **3**. After flash column chromatography (1:1 ethyl acetate/hexane) the pure compound was obtained (0.17 g, 52% yield) as a foamy solid: TLC $R_f = 0.28$; HPLC purity 98%, $t_R = 13.58$ min (C_{18} column); $[\alpha]^{25}_D$ 77.8° ($c = 1$, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.88 (Hse NH, d, 1H, $J_{NH-\alpha H} = 5.4$ Hz), 5.24 (Pro Hδ, dd, 1H, $J_{\gamma\delta} = 1.2$, 6.0 Hz), 4.62 (Pro Hα, dd, 1H, $J_{\alpha\beta} = 1.2$, 9.0 Hz), 4.59 (Hse Hα, qd, 1H, $J_{\alpha\beta} = 2.4$, 12.0 Hz), 4.11

(Hse Hγ'', m, 1H), 3.97 (Hse Hγ', td, 1H), 3.72 (OCH_3, s, 3H), 2.38 (Pro Hβ'', m, 1H), 2.22 (Pro Hγ'', m, 1H), 2.02 (Pro Hγ', m, 1H), 2.03 (Hse Hβ'', m, 1H), 2.00 (Pro Hβ', m, 1H), 1.82 (Hse Hβ', m, 1H), 1.43 (Boc CH_3, s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.75, 171.18 (Hse, Pro C=O), 154.87 (Boc C=O), 89.08 (Pro δ), 79.51 (Boc quarternary C), 70.50 (Hse γ), 59.20 (Pro α), 53.75 (Hse α), 52.29 (OMe), 33.68 (Hse β), 32.06 (Pro γ), 28.16 (Boc CH_3), 26.17 (Pro β); IR (neat/NaCl) 3406, 3000, 1747, 1713, 1615, 1493, 1439, 1393, 1361, 1167, 1090, 1058 cm^{-1}; MS (PCI) *m/z* 329 (MH^+), calcd for $C_{15}H_{24}N_2O_6$ 328.37.

(3*S*,7*S*,10*R*)-3-((*tert*-Butyloxycarbonyl)amino)-10-carboxy-1-aza-6-oxa-2-oxobicyclo[5.3.0]decane, Boc-Hse-(ec)-D-Pro-OH (8). Boc-Hse-(ec)-D-Pro-OMe (0.492 g, 1.5 mmol) was hydrolyzed under conditions as described for **4**. The pure compound (0.381 g, 81%) was obtained after crystallization from ethyl acetate: mp 191−192 °C; HPLC purity 99%, t_R = 11.74 min (C_{18} column); $[\alpha]^{25}_D$ 20.9° (*c* = 1, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.85 (d, 1 H, *J* = 5.5 Hz), 5.21 (d, 1H, *J* = 5.1 Hz), 4.67 (d, 1H, *J* = 8.8 Hz), 4.60 (m, 1H) 4.16, 4.18 (dt, 1H, *J* = 12.8 Hz, *J* = 3.24 Hz, *J* = 3.6 Hz), 3.98 (td, 1H, *J* = 11.9 Hz, *J* = 12.3 Hz, *J* = 1.5 Hz), 2.28−2.42 (m, 1H); 2.28−2.12 (m, 2H); 2.0−2.1 (m, 2H), 1.76−1.93 (m, 1H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.99, 172.49, 155.08, 89.35, 79.97, 70.60, 59.53, 53.87, 33.66, 32.27, 28.29, 25.95; IR (neat/NaCl) 3426, 2975, 1745, 1725, 1655, 1508, 1438, 1368, 1248, 1167, 1092 cm^{-1}; MS (PCI) *m/z* 315 (MH^+), calcd for $C_{14}H_{22}N_2O_6$ 314.34.

Conformational Analyses. An arbitary conformation of a given dipeptide mimetic (Ac-Hse-(ec)-Pro-NHMe) was built using MACROMODEL.[60] The Monte Carlo searches of torsional space were done with BATCHMIN [M5] (version 3.5X). For the bicyclic systems, two ring closure bonds were defined. An iterative conformational search was then initiated. BATCHMIN first selected a random subset of the rotatable torsions (each subset comprises 1 to $n - 1$ torsion angles, where n is the total number of rotatable torsions[64]) and a random rotational increment between 0° and 360°. After application of the random torsional rotations, the structure was tested for ring closure and high-energy nonbonded contacts. If the structure had no pair of atoms separated by less than one-fourth of the sum of their van der Waals radii, it was energy-mimimized for 500 iterations using the AMBER/OPLS force field[58] and the GB/SA solvation model.[59] The resulting conformer was kept only if its energy was within 25 kJ/mol of the instantaneous global minimum and did not duplicate any previously stored structures. The next search cycle began by using the usage-directed search. In this search, each conformer within 25 kJ/mol of the global minimum was used an equal number of times as the starting geometry. Such a protocol[61] has been found to be the most efficient in a conformational search.

The resulting conformations were then minimized using the OPLS force field and the GB/SA solvation model to a gradient of less than 0.0001 using full-matrix Newton−Raphson minimization, and duplicate conformations were discarded. The question of saddle point structures was answered umambiguously by examining the second derivative matrix for negative eigenvalues. Thus it was possible to distinguish true minima from poorly converged or saddle point structures. In the resulting conformations, the $\alpha C_1-\alpha C_4$ distance and the torsion angles $\phi 2$, $\psi 2$, $\phi 3$, and $\psi 3$ were determined in order to identify those consistent with ideal β-turn types.

The conformation search protocol for the tetrapeptide mimics (Ac-Ala-Hse-(ec)-Pro-Ala-NHMe) used the same approach except for the following: The search was allowed to run for 5000 iterations and is, therefore, not considered converged. Conformations were collected if they were within 50 kJ/mol of the lowest energy conformation found. The conformations found were then minimized to a gradient of 0.01 using conjugate gradients, and only those within 25 kJ/mol of the lowest energy conformation found were kept. Duplicate conformations were also discarded. These conformations were then again minimized using the full-matrix Newton−Raphson and tested for saddle point structures as described above.

NMR Analyses. One-dimensional spectra were recorded using either a Varian Gemini 300 or Varian Unity 600 spectrometer. Chemical shifts are reported as parts per million (ppm) downfield from tetramethylsilane. All two-dimensional experiments were done on a Varian Unity 600 spectrometer. HMQC experiments were recorded with 1984 points in F_2 (sweep width 4941 Hz) and 128 increments in F_1 (sweep width 12 070 Hz), zero-filled to 4K × 2K, and processed with a shifted sine−bell filter. TOCSY analyses were recorded with a sweep width of 4941 Hz, 2048 points in F_2, and 256 × 2 points in F_1. Mix times of 110 and 25 ms were used; the data sets were zero-filled to 4K × 2K and processed with a Gaussian filter. NOESY analyses of compounds **3** and **7** were done at 25 °C in $CDCl_3$ with a mix time of 300 ms, sweep width 5500 Hz, 3000 points in F_2, and 300 × 2 increments in F_1. The data set was zero-filled to 4K × 2K and processed with a 90° shifted sine−bell filter. Both molecules were in the extreme narrowing limit, with the cross peaks and diagonal peaks having opposite signs. Integrated cross−peak intensities were converted to interproton distances using an isolated spin-pair approximation and the NOE cross peak for the fixed Hse $\gamma'-\gamma''$ distance as a reference. A 20% error range in the calculated distances was assumed.

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Supplementary Material Available: ^{1}H NMR one-dimensional spectra for compounds **3** and **7**, TOCSY and ^{1}H{^{13}C} HMQC spectra for compound **3**, and NOESY spectrum for compound **7** (600 MHz) (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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