Articles

Electrochemical Cyclization of Dipeptides To Form Novel Bicyclic, Reverse-Turn Peptidomimetics. 2. Synthesis and Conformational Analysis of 6,5-Bicyclic Systems

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Novel, highly constrained, 6,5-bicyclic dipeptides (1-aza-5-oxa-2-oxobicyclo[4.3.0]nonane ring skeletons, **2**) have been synthesized by a one-step electrochemical cyclization from the dipeptides Boc-(*S*)-serine-(*S*)-proline-OMe (Boc-(*S*)-Ser-(*S*)-Pro-OMe, **3**) and Boc-(*R,S*)-α-methylserine-(*S*)-proline-OMe (Boc-(*R,S*)-α-MeS-(*S*)-Pro-OMe, **12**) in yields of 10−25% and 41%, respectively. The one-pot reaction uses selective anodic amide oxidation to generate an *N*-acyliminium cation which is trapped by an intramolecular hydroxyl group. The cyclization of Boc-(*S*)-Ser-(*S*)-Pro-OMe (**3**) to the 6,5-bicyclic skeleton **4** was highly diastereoselective, generating a new chiral center with an *S* configuration. This bicyclic compound was sufficiently stable to trifluoroacetic acid and anhydrous hydrofluoric acid for use in standard solid phase peptide synthesis methodologies. Oxidation of Boc-(*R,S*)-MeS-(*S*)-Pro-OMe (**12**) gave different results for each diastereoisomer. Cyclization only occurred for the *S,S*-diastereoisomer with very low stereoselectivity (6:4 ratio of diastereomers) at the newly-formed ring fusion. In terms of conformation, the 6,5-bicyclic system restricts two (ψ_2 and ϕ_3) of the four torsion angles that characterize a reverse turn. Conformational analyses of tetrapeptides containing the 6,5-bicyclic system were performed using Monte Carlo conformational searches and molecular dynamics simulations. All of the eight possible diastereomers arising from the three stereogenic centers (Ser Cα, Pro Cα, and newly formed bridgehead) were considered. These studies revealed that the 3*S*,7*S*,10*S* and 3*R*,7*R*,10*R* configurations are effective turn inducers although the torsion angles of the backbone do not exactly mimic those of classical β-turns. Other diastereomers were found to stabilize the peptide backbone in an extended conformation.

Introduction

Reverse-turn mimetics are generally cyclic or bicyclic compounds which, by their covalent geometry, force a peptide chain to fold back upon itself. Many of these molecules utilize small- and medium-sized lactam rings to conformationally constrain the peptide backbone.[1−15] We have recently been investigating the use of anodic amide oxidations for the construction of constrained building blocks of this type[1−5] and have shown that 7,5-bicyclic systems (**1**, Figure 1) can be prepared with high stereoselectivity by electrochemical oxidation of dipeptide derivatives such as Boc-homoserine-proline methyl ester.[1,2] These findings prompted us to survey the applicability of this method to the synthesis of 6,5-bicyclic compounds (**2**, Figure 1) directly from the corresponding serine-containing dipeptides. This procedure is particularly useful because complex peptidomimetics can be constructed in a small number of steps from readily available protected dipeptides. Although a synthesis of the 6,5-bicyclic system **2** has recently been reported by

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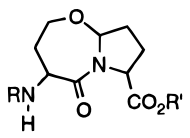

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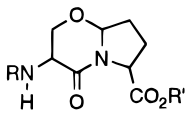

2

Figure 1. 7,5-Bicyclic **1** and 6,5-bicyclic dipeptides **2** produced by anodic oxidation of Boc-Hse-Pro-OMe and Boc-Ser-Pro-OMe, respectively.

Baldwin *et al.*,[16] the use of strong oxidants (OsO_4 and $NaIO_4$) and the need for chiral preparation of the unusual precursor, (*S*)-but-3-enylglycine, make this a less generally applicable procedure. Furthermore, the electrochemical oxidation used here provides an alternative to the more common methods of acyliminium formation[17] and affords the possibility to develop a general annulation procedure for amides.[17−33] In order to elucidate the consequences of incorporating the 6,5-bicyclic system into peptides, we have performed conformational analyses of tetrapeptides containing these systems and assessed their turn-inducing potential.

Results and Discussion

Cyclization of Boc-Ser-Pro-OMe. The most direct route to the 6,5-bicyclo[4.3.0]nonane system (Scheme 1) is analogous to our previously reported synthesis of the 7,5-bicyclic system **1**. The two-electron oxidation of the protected dipeptide Boc-Ser-Pro-OMe (**3**) generates an acyliminium ion which is trapped by the hydroxyl group of serine to give the bicyclic compound Boc-Ser-(ec)-Pro-OMe (**4**). Our initial efforts employed two sets of conditions: (a) the dipeptide (0.5 M) in methanol containing tetrabutylammonium tosylate (0.03 M) as supporting electrolyte, a charcoal anode, a platinum wire cathode, and a constant current of 53 mA; or (b) the dipeptide (0.5 M) in a mixture of acetonitrile with methanol or isopropyl alcohol (95:5) with tetrabutylammonium tetrafluoroborate as the supporting electrolyte (1 M), platinum foil electrodes, and a constant current of 13.8 mA/cm^2 in an undivided cell. Under both sets of conditions, we found an unexpected cleavage of the hydroxymethyl side chain which yielded a diastereomeric mixture of Boc-α-alkoxyglycine-proline-methyl esters **5** in 70−75% yield. Under these conditions, we found no evidence of the desired compound **4** or other oxidation products of proline. Similar results were obtained using dipeptides containing threonine and *β*-phenylserine as starting materials. In the oxidation of the *β*-phenylserine-containing dipeptide, benzaldehyde was also identified as a product of side chain cleavage. It is noteworthy that a similar cleavage of a hydroxymethyl side chain has been found (Moeller, K. D.; Rothfus, S. L., unpublished results) in the electrochemical oxidation of an acyl 2-(hydroxymethyl)-pyrrolidine derivative which also has a hydroxy group *β* to an acylamine. This side reaction was not observed during oxidation of Boc-Hse-Pro-OMe which we have reported previously.[2] Although conditions for indirect electrochemical α-methoxylation of protected amino acids and dipeptides using chloride as the oxidative catalyst have been reported,[18,25] the absence of chloride ion under our experimental conditions suggests a different mechanism in the conversion of Ser to α-methoxyglycine.

To eliminate cleavage of the hydroxymethyl side chain of serine, we explored several alternative routes (Scheme 2): (A) electrochemically functionalizing proline to generate the *δ*-methoxy compound **6** (R-Pro(5-OMe)-OMe) before coupling to Ser to form the dipeptide **7** (R-Ser-Pro(5-OMe)-OMe) which would lead to **4** by acid-catalyzed cyclization; (B) protecting the hydroxyl group of serine and oxidizing the protected dipeptide **8** (R-Ser-(R′)-Pro-OMe) in methanol to generate the *δ*-methoxy compound **9** (R-Ser(R′)-Pro(5-OMe)-OMe), followed by deprotection of the serine hydroxyl group and cyclization using an acid catalyst; and (C) direct electrochemical cyclization of R-Ser-Pro-OMe (**3**) in a non-nucleophilic solvent.

The first approach seemed straightforward in that protected 5-methoxy or hydroxy compounds **6** can be readily prepared in good yield by electrochemical oxidation of the appropriately N-protected proline methyl ester[18] or by nonelectrochemical oxidation.[34] The 5-cyano derivatives can be readily prepared by treating the 5-hydroxy or methoxy compound with trimethylsilyl cyanide.[35] Deprotection of the amide nitrogen in compound **6** and coupling of the 5-methoxyproline using standard techniques would in theory provide a dipeptide **7** appropriate for cyclization. Unfortunately, attempts to deprotect any of the compounds **6** (R = Boc) using even low concentrations of TFA, or by hydrogenation (R = Cbz), resulted in complete decomposition of the starting material and no identifiable products.

The second approach also involved an electrochemical oxidation followed by a conventional synthetic transformation and consequently required a set of protecting groups compatible with both steps. Among the amino protecting groups commonly used in peptide chemistry, only Boc and Cbz are compatible with electrochemical anodic oxidation. Possible side chain hydroxyl protecting groups were selected based on an orthogonal protection scheme which would allow selective deprotection of the hydroxyl group. Acetyl ester (Ac) and tetrahydropyranyl (THP), benzyl (Bzl), and *tert*-butyl (*t*-Bu) ethers were evaluated as hydroxyl protecting groups in this approach. Electrochemical anodic oxidation was carried out under

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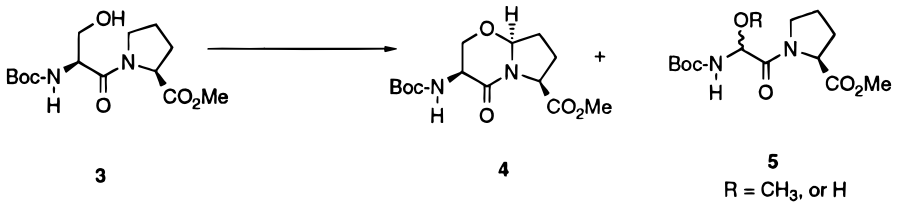
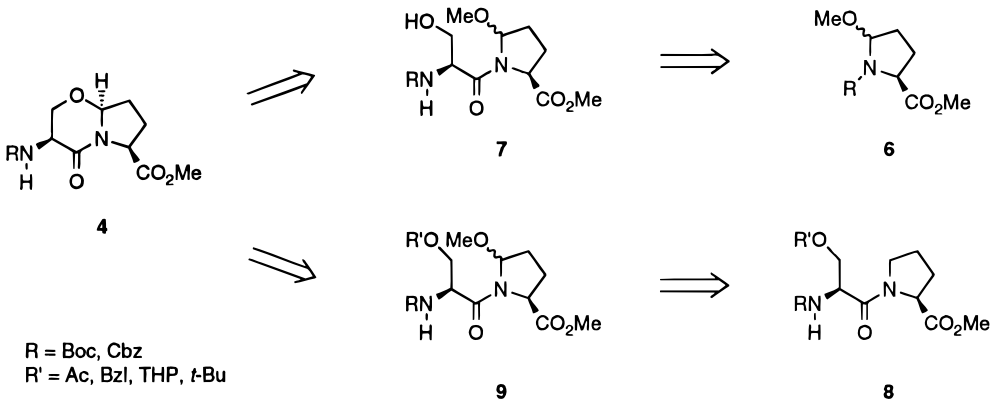
Scheme 1



Scheme 2



conditions that had been found to be optimal for the synthesis of Boc-Pro(5-OMe)-OMe (**6**) from Boc-Pro-OMe, methanol with tetrabutylammonium tosylate as the supporting electrolyte ($c = 0.03$ M) using a charcoal anode and platinum wire cathode at a constant current of 53 mA. Among the hydroxyl protecting groups evaluated, only the *tert*-butyl ether was found to be stable under these conditions. In the case of Boc-Ser(Ac)-Pro-OMe, Boc-Ser(Bzl)-Pro-OMe and Boc-Ser(THP)-Pro-OMe, cleavage of the hydroxyl protecting group and undesirable serine side chain cleavage was observed. Although the *tert*-butyl ether was stable under electrochemical oxidation conditions, anodic oxidation of the proline δ-carbon in the dipeptides CBz-Ser(*t*-Bu)-Pro-OMe and Boc-Ser-(*t*-Bu)-Pro-OMe met with failure, possibly due to steric effects of the bulky *tert*-butyl substituent. These difficulties led us to abandon the second strategy for generating the 6,5-bicyclic compounds.

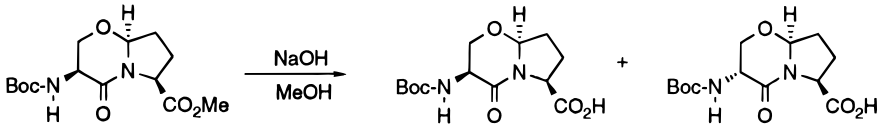
Our third approach was based on the assumption that the serine side chain cleavage under electrochemical oxidation is associated with the presence of solvents having a hydroxyl group such as methanol, isopropyl alcohol, or water. This assumption appears to be valid, as we successfully prepared the bicyclic compound **4** from Boc-Ser-Pro-OMe in dry acetonitrile. To minimize the side chain cleavage reaction caused by the presence of water in the supporting electrolyte, commercially available tetrabutylammonium tetrafluoroborate was dried over P_2O_5 under high vacuum. Platinum foil electrodes were found to be better than the charcoal anode and platinum wire cathode. A higher yield of **4** (25%, 1 mmol scale) was obtained when the applied current density was in the range 8−14 mA/cm^2. We found that optimal conditions for direct cyclization of Boc-Ser-Pro-OMe in dry acetonitrile ($c = 0.25$ M) involved tetrabutylammonium tetrafluoroborate as supporting electrolyte (1 M), two platinum foil electrodes, and a constant current density of 14 mA/cm^2.

Several aspects of this reaction deserve further comment. The use of dry acetonitrile was important for the cyclization. Unfortunately, it was impossible to eliminate water from the system to the extent that the undesired side chain cleavage was stopped completely. Therefore, cyclization of Boc-Ser-Pro-OMe (**3**) to the 6,5-bicycle **4** was always accompanied by some formation of Boc-α-hydroxyglycylproline methyl ester **5** as a byproduct. Although no other major side-products were detected, a number of unidentified minor products were observed. The pure 6,5-bicyclic compound **4** was isolated by two-step flash chromatography using hexane/ethyl acetate (gradient from 8:2 to 1:1) as eluent. Notably, scaling up the synthesis resulted in a decreased yield, giving 10−15% of the desired product when the oxidation was performed on a 10 mmol scale.

The cyclization reaction was diastereoselective with the major isomer having an *S*-configuration at the bridgehead. The bridge stereochemistry was assigned using a combination of NOE data and molecular modeling (see Experimental Section) and is similar to that observed in the oxidation of Boc-Hse-Pro-OMe and Boc-Hse-(*R*)-Pro-Me.[2] These results support our assumption that the bridgehead stereochemistry is controlled by the stereochemistry of the N-terminal amino acid.

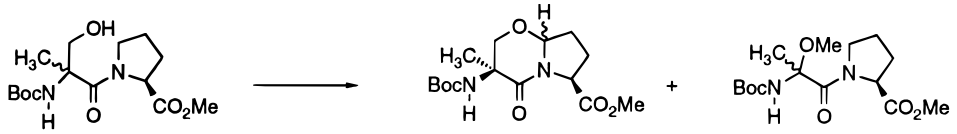
Stability Studies. As a prelude to incorporating the 6,5-bicyclic system into a peptide, hydrolysis of the methyl ester was attempted (Scheme 3). Treatment of **4** with 1.1 equiv of NaOH in methanol for 1 h at room temperature produced a mixture of two diastereoisomers of Boc-Ser-(ec)-Pro-OH after acidification. On the basis of the report of Baldwin and co-workers,[16] epimerization at the α-carbon of serine under basic conditions was anticipated. The two diastereoisomers, **10** (*S*,*S*,*S*) and **11** (*R*,*S*,*S*), could be easily separated by flash chromatography on silica gel using chloroform/methanol/acetic acid (95:5:1). This approach could, therefore, be used to generate both 6,5-bicyclic building blocks Boc-(*S*)-Ser-(*S*-ec)-(*S*)-Pro-OH (**10**) and Boc-(*R*)-Ser-(*S*-ec)-(*S*)-Pro-OH (**11**) for solid phase peptide synthesis. The overall yield of each diastereoisomer was 25−30%. To assign the configuration at the α-carbon of serine for each diastereoisomer, isomer **10** (*S*,*S*,*S*) was prepared independently by the electrochemical oxidation of Boc-Ser-Pro-OBzl and hydrogenolysis of the benyzl ester. Removal of the benzyl ester by hydrogenation did not cause the epimerization

Scheme 3



4 10 11

Scheme 4



12 13 14

of the serine α-carbon and it was, therefore, possible to obtain the reference compound corresponding to the *S*,*S*,*S*-diastereoisomer. This approach provided optically pure product; however, its practical application to the synthesis of compound **10** is limited due to a low yield in the anodic oxidation step of the benzyl ester and difficulties with isolation of the pure product.

Studies of the stability of compound **4** to trifluoroacetic acid (TFA) and anhydrous hydrofluoric acid (HF) were performed to ascertain the suitability of this bicyclic system for solid phase peptide synthesis. The ring system of **4** proved stable to pure TFA for 2 h at room temperature and to HF for 1 h at 0 °C, with removal of the Boc group seen as the only effect. The resynthesis of **4** from the deprotection product by reintroduction of the Boc group confirmed this conclusion. The NMR spectra, optical rotation and HPLC of the resulting product were identical to the original sample. Notably, the use of scavengers such as anisole and dimethyl sulfide during HF deprotection results in the formation of side products. These are probably due to ring opening and regeneration of the *N*-acyliminium ion under the acidic conditions, with subsequent intermolecular trapping of the cation by scavenger. The relative stability of the 6,5-bicyclic system toward TFA and HF allows their facile incorporation into peptides by a standard solid phase approach. Compounds **10** and **11** have been used in the synthesis of two gonadotropin releasing hormone analogs which incorporate the 6,5-bicyclic system by solid phase synthesis.[36]

Cyclization of Dipeptides Containing α-Methylserine. In an attempt to circumvent the side reaction which occurs upon oxidation of the Boc-Ser-Pro-OMe, we investigated oxidation of the compound in which the serine α-hydrogen was replaced by a methyl group (Scheme 4). The epimeric mixture, Boc-(*R*,*S*)-α-MeS-Pro-OMe (**12**), when electrolyzed in methanol or in a mixture of acetonitrile with methanol or isopropyl alcohol (95:5), was found to generate some cyclic product **13** but also underwent cleavage of the hydroxymethyl side chain in a fashion similar to that seen with Boc-Ser-Pro-OMe (Scheme 1) to produce the alkoxy compound **14**. Interestingly, the reaction pattern was found to be different for each epimer. For one diastereoisomer of **12** (Boc-(*R*)-α-MeS-Pro-OMe), cleavage of the side chain was observed under electrochemical oxidation, yielding the α-methoxyalanine derivative **14** in 35% yield based on the starting diastereoisomeric dipeptide **12** without any oxidation of proline. The desired mixture of 6,5-bicyclic compounds **13** was formed only from the Boc-(*S*)-α-MeS-Pro-OMe diastereomer in a yield of 41% based on the starting diastereoisomeric dipeptide **12**. The absolute configuration of α-methylserine in the bicyclic compound was determined by HPLC analysis of the peptide hydrolysate using Marfey's method[37] and comparison with authentic standards. Stereoselectivity at the newly-formed ring fusion was very low, with two diastereoisomers produced in a 6:4 ratio. It was possible to separate the two diastereoisomers using reverse phase HPLC; however, the compounds were found to be very labile toward acids (e.g. dilute TFA or lyophilization from 0.1% TFA in aqueous solution). Our results indicate that incorporating the 6,5-bicyclic systems **13** into peptides would require very mild conditions not compatible with the usual solid-phase methodologies.

Molecular Modeling Studies. Because there are three stereogenic centers present in the 6,5-bicyclic system **2**, there are eight different compounds having this general structure. All of these structures are potentially useful as peptidomimetics, but the configuration at each of the chiral centers can be expected to dictate the conformation that results when the compounds are incorporated into peptides. Of the eight distinct compounds, only those which have the carboxyl and amino groups on the same face of the ring system are of potential use as reverse turn mimetics. This arrangement is necessary to force a change in the direction of the peptide backbone. Compounds having the carboxyl and amino groups on opposite faces of the ring system are, however, also of interest because of their potential as rigid linear peptidomimetics. An analog of the turn mimetic, BTD, which has carboxyl and amino groups on opposite faces of a bicyclic ring system has found utility as a "spacer group" in peptidic fibrinogen antagonists.[38] Although we have not synthesized all of the eight possible diastereomers, they are available in principle by our synthetic approach or that of Baldwin et al.[16] and, consequently, all were considered in our conformational analysis of the 6,5-bicyclic system.

Molecular modeling studies were performed using blocked tetrapeptides of the type Ac-Ala-Ser-(ec)-Pro-Ala-NMe to mimic the incorporation of the peptidomimetic

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Table 1. Conformational Parameters for All of the Possible Stereoisomers of the 6,5-Bicyclic System 2, Alanine Tetrapeptide, and Selected Classes of Ideal β-Turns[a]

compound	no. of starting conformers	center of most populated 20° range and RMS_{tors} over all conformers, deg					% $d_{C\alpha1-C\alpha4}$, <7 Å	% d_{O-H}, <4 Å
		ϕ_2	ψ_2	ϕ_3	ψ_3	β		
type I β-turn		−60	−30	−90	0	34.5°	4.8 Å	1.9 Å
type I′ β-turn		60	30	90	0	−52.3°	5.3 Å	2.0 Å
type II β-turn		−60	120	80	0	4.8°	5.0 Å	1.9 Å
type II′ β-turn		60	−120	−80	0	1.8°	5.2 Å	2.0 Å
Ala_4	11	−162 (42)	140 (51)	−163 (48)	138 (59)	169 (81)	5	1
RRR	10	172 (21)	104 (16)	74 (18)	−127 (116)	−43 (32)	76	0
RRS	10	172 (31)	108 (14)	−96 (20)	125 (53)	−124 (24)	0	0
RSR	21	179 (72)	131 (16)	98 (19)	−114 (62)	−11 (22)	46	3
RSS	11	179 (49)	134 (22)	−76 (20)	121 (81)	−102 (36)	0	0
SRR	19	−179 (44)	−133 (23)	76 (17)	−122 (67)	94 (39)	0	0
SRS	29	−178 (74)	−127 (17)	−99 (21)	123 (81)	5 (26)	53	3
SSR	11	−174 (21)	−106 (16)	103 (19)	−122 (53)	131 (25)	0	0
SSS	10	−169 (32)	−111 (16)	−66 (21)	−19 (89)	−7 (36)	85	29

[a] Data was derived from approximately 1000 ps of molecular dynamics on the tetrapeptides Ac-Ala-Ala-Ala-Ala-NMe and Ac-Ala-Ser-(ec)-Pro-Ala-NMe starting from several conformations (no. of starting conformers) found to be 15 kJ above the global minimum found in a Monte Carlo search (see Computational Methods). Stereochemistries are given, in order, for Ser Cα, the ring fusion carbon, and Pro Cα. Angle values are the center of the most highly populated 20° range. The average RMS_{tors} for all conformations against this value is given in brackets. The virtual torsion β is defined by $C\alpha_1$, $C\alpha_2$, $C\alpha_3$, and N_4; % $d_{C\alpha1-C\alpha4}$ is the percentage of all conformers for which the distance between Cα1 and Cα4 <7 Å; and % d_{O-H} is the percentage of all conformers sampled from the simulation in which the distance between the carbonyl oxygen of residue 1 and the amide hydrogen of residue 4 was less than 4 Å.

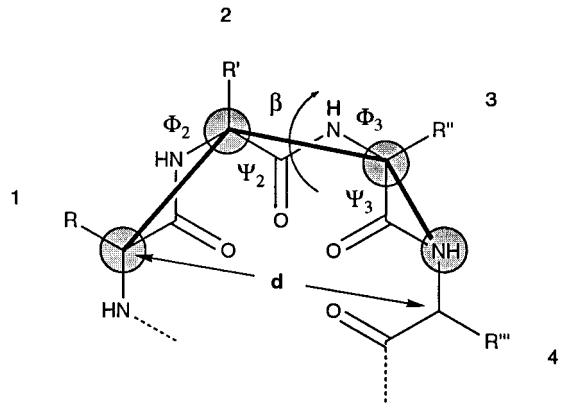

Figure 2. Parameters used to characterize β-turn propensity of 6,5-bicyclic ring systems. The virtual torsion β is defined by relative positions of highlighted atoms $C\alpha_1$, $C\alpha_2$, $C\alpha_3$, and N_4; d is the distance between Cα1 and Cα4; and Φ_2, Ψ_2, Φ_3, and Ψ_3 are the torsion angles of the bonds between the amide nitrogen and the α carbon of residue 2, between the α carbon and the carbonyl carbon of residue 2, between the amide nitrogen and α carbon of residue 3, and between the α carbon and the carbonyl carbon of residue 3, respectively.

into a peptide. Each model tetrapeptide was subjected to an extensive Monte Carlo conformational search to identify minimum energy conformations. Because energy minima by themselves only represent the lowest point of each energy well and give no indication of its size, each low-energy conformer falling within 15 kJ/mol of the global minimum was subjected to approximately 1000 ps of molecular dynamics. We have previously used this technique to model a wide range of reverse-turn mimetics.[39]

The results of the molecular dynamics calculations are summarized in Table 1. For purposes of comparison, the results obtained for the unconstrained tetrapeptide Ac-Ala-Ala-Ala-NMe are shown, along with torsional values for selected ideal β-turns. Several parameters (Figure 2) were used to characterize the conformational preferences of each compound: the center of the most highly populated 20° range for each of the peptide backbone angles ϕ_2, ψ_2, ϕ_3, and ψ_3 and the average RMS_{tors} for all conformers sampled from the simulation against this angle (given in parentheses), the virtual torsion angle β (defined by Cα1, Cα2, Cα3, N4) similar to that defined by Ball et al.;[40,41] the percentage of all conformers for which the distance between Cα1 and Cα4 was less than 7 Å (one definition of a β-turn); and the percentage of all conformers sampled from the simulation in which the distance between the carbonyl oxygen of residue 1 and the amide hydrogen of residue 4 was less than 4 Å (the classic definition of a β-turn).

The percentage of all conformers with a distance between Cα1 and Cα4 of less than 7 Å shows clearly that the *RRR*-, *RSR*-, *SRS*-, and *SSS*-diastereomers are able to force the peptide backbone to reverse direction with the *RRR* and *SSS* forms being the most effective. The RMS_{tors} for the backbone angles (Figure 2) show, as expected, that the bicyclic ring system tightly constrains ψ_2 and ϕ_3. Somewhat larger deviations occur for ϕ_2 and ψ_3 although in many cases these noncovalently constrained angles show less movement than the corresponding angles in tetraalanine. Comparison with the ideal turn angles shows that for those enantiomers which induce reverse turns, the angles ψ_2 and ϕ_3 are compatible with some classical turn values (II and II′). However, in all cases the angles ϕ_2 and ψ_3 are not compatible with classical values. The virtual torsion angle β shows that the remaining diastereomers hold residues 1 and 4 apart and induce approximately 90° kinks in the backbone. The parameter $d_{C\alpha1-C\alpha4}$ clearly shows that the *R*,*R*,*R*- and the *S*,*S*,*S*-isomers are the most effective turn restraints with the *S*,*S*,*S* compound slightly more efficient than its enantiomer. The values found for d_{O-H} shows that in all cases except the *S*,*S*,*S*-diastereomer, there is no hydrogen bond between residues 1 and 4. This interaction may be responsible for the slightly better turn induction by the *S*,*S*,*S*-compound than its enantiomer. The absence of a 1−4 hydrogen bond can be expected to result in different electrostatic and conformational properties for the 6,5-bicyclic system compared to a conventional β-turn. For this reason, we suggest that although several of the

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6,5-systems are effective reverse-turn constraints they differ significantly from classical peptide β-turns.

Experimental Section

General Synthetic Methods. Optical rotations were measured in a 1-dm cell (1 mL) at 589 nm (Na D line). For thin-layer chromatography (TLC), 250-nm silica gel GF precoated plates were used with the solvent system indicated. The chromatograms were developed with chlorine followed by starch/KI spray. For flash chromatography, columns (5.5 × 15 cm) packed with silica gel 60 were used. Analytical high-performance chromatography (HPLC) was performed using a 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 to 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 Co., Inc. Tetrabutylammonium tetrafluoroborate was purchased from Aldrich and stored in a vacuum desiccator (ca. 0.5 mmHg). N- and C-protected amino acids were purchased from Advanced Chemtech. (*R*,*S*)-β-Phenylserine and (*R*,*S*)-α-methylserine were purchased from Sigma. TBTU was obtained from Richelieu. All solvents were purchased from Baxter and used without purification. Boc-Ser-Pro-OMe, Boc-Ser(Bzl)-Pro-OMe, Boc-Ser(*t*-Bu)-Pro-OMe, Boc-Ser-Pro-OBzl, Cbz-Ser(*t*-Bu)-Pro-OMe, Boc-Thr-Pro-OMe, Boc-(*R*,*S*)-β-PhS-Pro-OMe, and Boc-(*R*,*S*)-MeS-Pro-OMe were prepared according to standard procedure using TBTU as a coupling reagent.[42] Boc-Ser-(THP)-Pro-OMe was prepared from Boc-Ser-Pro-OMe and dihydropyran in the presence of a catalytic amount of TsOH according to a procedure described by Bernady *et al.*[43] Boc-Ser(Ac)-Pro-OMe was prepared by acetylation of Boc-Ser-Pro-OMe with acetic anhydride.[44] Both ^{1}H and ^{13}C spectra were consistent with the assigned structures.

(3*S*,7*S*,10*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-10-carbomethoxy-1-aza-5-oxa-2-oxobicyclo[4.3.0]decane, Boc-Ser-(ec)-Pro-OMe (4). A two-hole rubber stopper was fitted with a needle as a nitrogen inlet and two platinum foil electrodes (5 cm^2 each). The stopper was placed into a vial charged with 40 mL of acetonitrile, 13.2 g (40 mmol) tetrabutylammonium tetrafluoroborate, and 3.16 g (10 mmol) of Boc-Ser-Pro-OMe (**3**). The reaction mixture was degassed by sonication and then electrolyzed with a constant current of 70 mA (current density 14 mA/cm^2). After 4.5 F/mol of charge was passed, the reaction was stopped and the mixture was concentrated *in vacuo*. Chromatography on silica gel using 20% ethyl acetate/hexane and then 50% ethyl acetate/hexane as eluent afforded a mixture of the 6,5-bicyclic product **4** and Boc-α-hydroxyglycyl-proline methyl ester (**5**). After a second purification using the same conditions the pure Boc-Ser-(ec)-Pro-OMe was isolated in 15% yield (0.471 g, 1.5 mmol). HPLC purity 99%, t_R = 11.3 min (C_{18} column); $[\alpha]^{25}_D$ −83.75 (c = 0.5, methanol). MS (FAB) m/z 315 (MH^+).

(3*S*,7*S*,10*S*)- and (3*R*,7*S*,10*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-10-carboxyl-1-aza-6-oxa-2-oxobicyclo[4.3.0]decane, Boc-Ser-(ec)-Pro-OH (10 and 11). A solution of **4** (0.492 g, 1.5 mmol) in methanol (1 mL) was cooled in an ice/water bath, and 1 N NaOH (1.65 mL) was added with stirring. The mixture was stirred at room temperature for 1 h. 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 mixture consisted of two diastereoisomers: **10** (3*S*,7*S*,10*S*) and **11** (3*R*,7*S*,10*S*) which were separated by flash chromatography on a silica gel column using 95/5/1 CH_2Cl_2/MeOH/AcOH as eluent.

Boc-(*S*)-Ser-(*S*-ec)-(*S*)-Pro-OH (**10**) was obtained in 25% yield (0.110 g); R_f = 0.51 (chloroform/MeOH/AcOH = 95:5:1); HPLC purity 97%, t_R = 10.2 min (C_{18} column); $[\alpha]^{25}_D$ −62.6° (c = 0.5, methanol). MS (ES) m/z 301 (MH^+), 245 ([MH − *t*Bu]$^+$).

Boc-(*R*)-Ser-(*S*-ec)-(*S*)-Pro-OH (**11**) was obtained in 30% yield (0.147 g); R_f = 0.38 (chloroform/MeOH/AcOH = 95:5:1); HPLC purity 95%, t_R = 10.2 min (C_{18} column); $[\alpha]^{25}_D$ −45.7° (c = 0.5, methanol). MS (ES) m/z 301 (MH^+), 245 ([MH − *t*Bu]$^+$).

(3*S*,7*R*,10*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-3-methyl-10-carbomethoxy-1-aza-5-oxa-2-oxobicyclo[4.3.0]-decane, Boc-*S*-MeS-(ec)-*S*-Pro-OMe (13). A two-hole rubber stopper was fitted with a needle as a nitrogen inlet and two platinum foil electrodes (5 cm^2 each). The stopper was placed into a vial charged with 7.6 mL of acetonitrile, 0.4 mL of methanol, 2.64 g (8 mmol) of tetrabutylammonium tetrafluoroborate, and 0.660 g (2 mmol) of Boc-(*R*,*S*)-MeS-(*S*)-Pro-OMe (**12**). The reaction mixture was degassed by sonication and then electrolyzed with a constant current of 70 mA (current density 14 mA/cm^2). After 4.5 F/mol of charge was passed, the reaction was stopped and the mixture was concentrated *in vacuo*. Chromatography on silica gel using 20% ethyl acetate/hexane and then 50% ethyl acetate/hexane as eluent afforded 0.270 g (41% based on starting Boc-(*R*,*S*)-MeS-(*S*)-Pro-OMe (**12**)) of the bicyclic product, Boc-MeS-(ec)-Pro-OMe (**13**). HPLC analysis of the hydrolysate of **13** produced using Marfey's method revealed an *S* configuration at the α-carbon of the resulting α-methylserine derivative by comparison with an authentic standard. Two diastereomers were formed at the new ring fusion in a 6:4 ratio (analytical HPLC, t_R = 11.6 min and 12.2 min, respectively). MS (PCI, CH_4) m/z 297 ([M − OCH_3]$^+$), 329 (MH^+), 357 ([M + C_2H_5]$^+$), 369 ([M + C_3H_5]$^+$).

Computational Methods

Molecular modeling studies were performed using Macromodel[45] version 4.5 using the Macromodel implementation of the Amber all-atom force field[46] and the implicit water GB/SA solvation model of Still *et al.*[47] Conformational searches were performed using the systematic Monte Carlo method of Goodman and Still.[48] Amide bonds were required to be *trans*. For each search, 5000 starting structures were generated and minimized using the truncated Newton−Raphson method[49] implemented in Macromodel. Duplicate conformations and those with an energy greater than 50 kJ/mol above the global minimum were discarded. Conformations were tested as described previously[2] to confirm that they were true minima. Conformers less than 15 kJ above the global minimum were used as starting conformations for molecular dynamics simulations (range of number of conformers = 10−29, Table 1). The 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.[50] At least 25 and up to 100 ps of dynamics were run for each starting

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conformation to generate approximately 1000 conformations for analysis in each case.

NMR Analyses

One dimensional spectra were recorded using either a Varian Gemini 300 or Varian Unity 600 spectrometer. Chemical shifts are reported as part per million downfield from tetramethylsilane. NOESY analysis of compound **4** was done on a Varian Unity 600 spectrometer at 25 °C in $CDCl_3$ with a mix time of 300 ms, sweep width 5500 Hz, 2048 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. The molecule was in the extreme narrowing limit, with cross peaks and diagonal peaks having opposite sign. Crosspeak integrals were measured using Varian software. Absolute stereochemistry at the ring fusion was established in a fashion analogous to that reported previously[2] for the 7,5-bicyclic system prepared from Boc-Hse-Ser-OMe. Observed NOE peak intensities were compared to those predicted from low-energy conformers of Boc-Ser-(*S*-ec)-Pro-NMe and Boc-Ser-(*R*-ec)-Pro-NMe and to interproton distances observed in the course of an unrestrained molecular dynamics simulation of both molecules. In both cases, the data were best explained by the *S* ring fusion isomer. In particular, the predicted Ser Hα-Pro Hδ distance for the *R* isomer was always greater than 4 Å, which exceeds the estimated maximum observable interproton distance of 3.5 Å (based on observed NOE intensities for fixed interproton distances in **4**). The NOEs observed, their relative intensities, and stereospecific assignments[51] were as follows: Ser HN-Ser Hα; Ser HN-Ser Hβ2; Ser Hα-Ser Hβ1 > Ser Hα-Ser Hβ2; Ser Hα-Pro Hδ; Ser Hβ1-Pro Hδ; Pro Hα-Pro Hδ; Pro Hδ-Pro Hγ2 > Pro Hδ-Pro Hγ1; Pro Hδ-Pro Hβ2 > Pro Hδ-Pro Hβ1.

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Supporting Information Available: One-dimensional ^1H and NOESY spectra of Boc-Ser-(*S*-ec)-Pro-OMe (**4**) in $CDCl_3$ (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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