Design, Synthesis, and Conformational Analysis of a Novel Spiro-Bicyclic System as a Type II β-Turn Peptidomimetic

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Abstract: A novel highly constrained spiro-bicyclic system (**5**) has been developed to mimic the type II β-turn, a secondary structural feature found in many bioactive peptides. This system simultaneously restricts three (Φ_2, Ψ_2, and Φ_3) of the four torsion angles that characterize the type II β-turn. As a test of the design, the asymmetric synthesis and conformational analysis of derivative **6** starting from (*R*)-2-allylproline is reported. Temperature dependent NMR chemical shift studies in $CDCl_3$ suggest that the amide proton of **6** is involved in an intramolecular hydrogen bond. Also, NOE measurements place this hydrogen under the plane of the bicyclic ring system in proper proximity for this hydrogen bond to form with the acetyl carbonyl oxygen. Modeling studies of **6** produced eight minimum-energy conformations with torsion angles close to those of the classical type II β-turn. A comparison of the minimum-energy conformer of this molecule with the classical type II β-turn gave an RMS fit = 0.161 Å.

In the past two decades, a host of biologically active peptides have been discovered. Because of their therapeutic potential, structural analogues of many of these biologically active peptides have been synthesized for the purpose of studying the biological systems with which each of the peptides interact. In addition, the relationship between conformation and biological function of these peptides is a topic of great interest and importance. This has stimulated much work in the field of peptide structural and conformational analysis with such techniques as X-ray crystallography,[1] circular dichroism,[2] NMR spectroscopy,[3] and molecular modeling.[4]

Although the above methods provide information about the conformation of a peptide either in the solid state, in solution, or in the computed minimum-energy conformation, they do not in most instances tell us about the conformation that the peptide is in when it actually interacts with its receptor. In order to elucidate the bioactive conformation of a peptide, a more indirect method has been employed whereby conformationally constrained analogues of a peptide are synthesized. Efforts, thus, have been directed toward preparing compounds to mimic certain secondary structural features of peptides which may play important roles in receptor recognition and biological activity.[5-7]

One major structural feature observed in peptides is the reverse turn.[8] Turns are fundamental to peptide structure and occur in regions where the peptide reverses its direction by folding over on itself. There are a number of reverse-turn conformations found in peptides. One of the most common is the β-turn. The β-turn consists of four residues, which are designated as *i*, *i*+1, *i*+2, and *i*+3 (**1**, Figure 1). Several different types of β-turns are possible depending upon the Ψ and Φ torsion angles of the *i*+1 and *i*+2 residues.[8] In addition, these turns may or may not be stabilized by an intramolecular hydrogen bond involving the amide NH of the *i*+3 residue and the carbonyl oxygen of the *i* residue.

Several non-peptide systems have been designed to mimic the different types of β-turns.[6,9] The incorporation of some of these mimics into biologically active peptides has led to peptidomimetics with enhanced activity or metabolic stability.[10] The β-turn mimics that have been developed to date have in general restricted only one or two of the torsion angles to values close to those found in the type of β-turn being mimicked. In this paper, we report the synthesis and conformational features of a novel spiro-bicyclic type II β-turn mimic (**6**, Figure 1) in which three of the four torsion angles are simultaneously restricted to values characteristic of a type II β-turn.

Design Rationale

A type II β-turn is identified by its Φ_2, Ψ_2, Φ_3, and Ψ_3 torsion angles (**1**, Figure 1). In an ideal type II β-turn, these torsion angles possess values of −60, 120, 80, and 0°, respectively.[8] In the design of a type II β-turn mimic, the corresponding Φ_2, Ψ_2, Φ_3, and Ψ_3 torsion angles of the mimic should be restricted to values near those found in an ideal type II β-turn. In previous attempts to develop type II β-turn mimics, usually only one or two of the Φ_2, Ψ_2, Φ_3, and Ψ_3 torsion angles were restricted at one time. For example, Freidinger et al.,[9a,b] in one of the first attempts to mimic a β-turn, proposed the (*S*)-γ-lactam function. Subsequently, the (*R*)-γ-lactam function, as found in structure **2** (Figure 1), has been used by us[10d] and Freidinger and co-workers[9b,10c] as a type II β-turn mimic. The (*R*)-γ-lactam conformational constraint restricts the Ψ_2 torsional angle to about +142°.[11]

More recently, the spirolactam system illustrated in structure **3** (Figure 1) has been shown to be a good mimic of the type II β-turn.[9g,12] In this mimic, the Φ_2 and Ψ_2 angles are constrained

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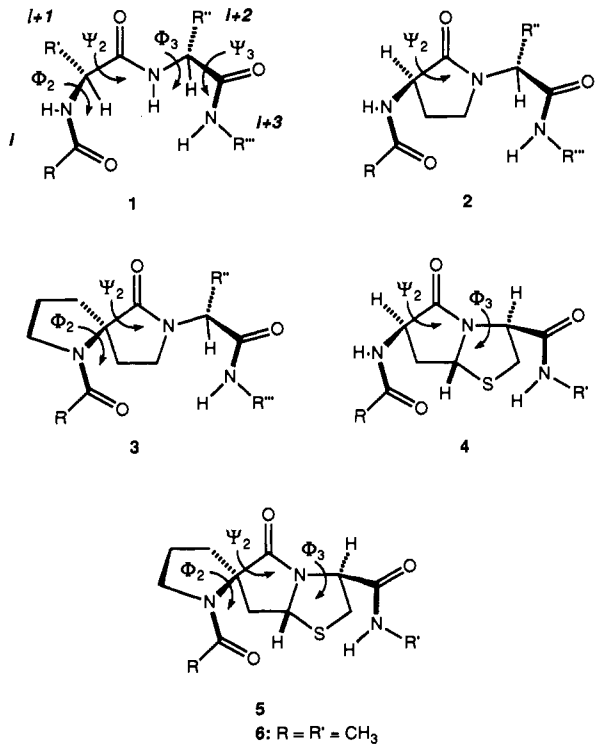


Figure 1. A comparison of the structural features of a type II β-turn and various β-turn mimics with those of the newly designed novel spiro-bicyclic type II β-turn mimic **5**.

to values of −75 ± 20 and +140 ± 10°, respectively. Modeling studies by Hinds et al.[12b] have shown that this spirolactam when incorporated into a peptide produces energy minima in which the distances and torsion angles are in good agreement with those predicted for the type II β-turn.

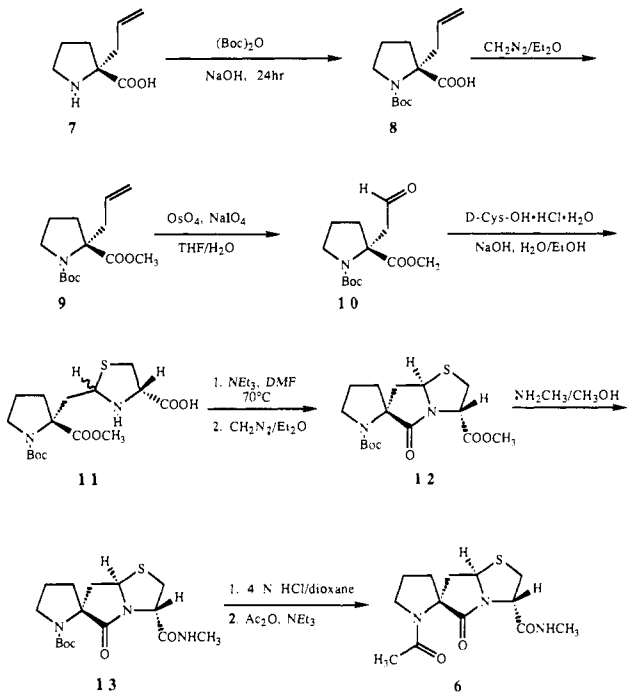
The 5,5-bicyclic lactam thiazolidine residue illustrated in structure **4** has been developed by us as a type II β-turn mimic. This bicyclic system restricts the Ψ_2 and Φ_3 angles that make up the turn to values around +130 and +80°, respectively. This bicyclic lactam thiazolidine constraint was adapted from an isomeric 6,5-bicyclic lactam thiazolidine system developed by Nagai et al.[9d] as a type II′ β-turn mimic.

On the basis of the conformational characteristics of the above constraints, we designed the novel spiro-bicyclic system illustrated in structure **5** (Figure 1), which incorporates the various constraints contained in compounds **2–4** into one molecule. This rigid system simultaneously restricts three (Φ_2, Ψ_2, Φ_3) of the four torsion angles to values similar to those that characterize a type II β-turn. In order to determine the validity of our design rationale, we synthesized the *N*-acetyl *N*-methylamide derivative **6** and examined its conformational properties.

Synthesis

The key starting point for the asymmetric synthesis of **6**, as outlined in Scheme I, was (*R*)-2-allylproline (**7**), which was prepared from (*S*)-proline using Seebach's α-alkylation with self-reproduction of chirality methodology.[13] This material was protected with the *tert*-butoxycarbonyl (Boc) group with NaOH and di-*tert*-butyl dicarbonate to give **8**. This reaction proceeded in somewhat lower yields (64%) than is normally observed for this type of amine protection. This is probably due to steric factors resulting from the presence of the new quaternary center which has been introduced α to the amino group. Increasing reaction times and/or increasing the amount of di-*tert*-butyl dicarbonate

Scheme I



did not have any appreciable effects on the yield. Compound **8** was converted to the methyl ester derivative **9** with excess CH_2N_2/Et_2O. Oxidative cleavage of the double bond of **9** with OsO_4 and $NaIO_4$ at room temperature afforded aldehyde **10** in good yields.

Condensation of aldehyde **10** with D-Cys-OH·HCl·H_2O yielded the thiazolidine derivative **11** as a mixture of diastereoisomers. A modification of the procedure developed by Baldwin and coworkers[14] was used to cyclize the diastereomeric thiazolidine derivative to the desired spiro-bicyclic system. In this modified method, intermediate **11** and NEt_3 were heated in dry DMF at 70 °C for 3 days. The spiro-bicyclic carboxylic acid product of this reaction was converted directly to the methyl ester **12** with excess CH_2N_2/Et_2O in order to facilitate the chromatographic purification. Only one of the two possible spiro-bicyclic diastereoisomers from this cyclization reaction was isolated. No trace of the other diastereoisomer could be found in the chromatographic fractions. However, an appreciable amount (20%) of the methyl ester of the uncyclized derivative **11** was recovered. This result differs from the work of Baldwin et al.[14] on γ-lactam analogues of penems. They observed that the cyclization of an analogous mixture of diastereomeric thiazolidines to a bicyclic lactam system gave a mixture of diastereoisomers, albeit with one diastereomer as the major product. Although it is not clear why only one diastereoisomer is being formed in the case of the spiro-bicyclic system, it is possible that only one of the two diastereoisomers of **11** is able to achieve the proper orbital approach of 109° between the amine lone pair electrons and the C=O bond to form the necessary tetrahedral intermediate.

The transformation of **12** into the *N*-methylamide compound **13** was carried out at room temperature with a saturated solution of methylamine in MeOH. This reaction proceeded quite rapidly and was complete in about 1 h. Subsequent deprotection of **13** with 4 N HCl/dioxane followed by acylation of the deprotected product with Ac_2O gave the desired spiro-bicyclic type II β-turn peptidomimetic **6**.

The stereochemistry at the bridgehead carbon of the spiro-bicyclic system was delineated through the use of 1D NOE measurements in $CDCl_3$. For the Boc-spiro-bicyclic-NHMe analogue **13** a prominent NOE was observed between the bridgehead hydrogen and the amide NH, a distance of about 2.7

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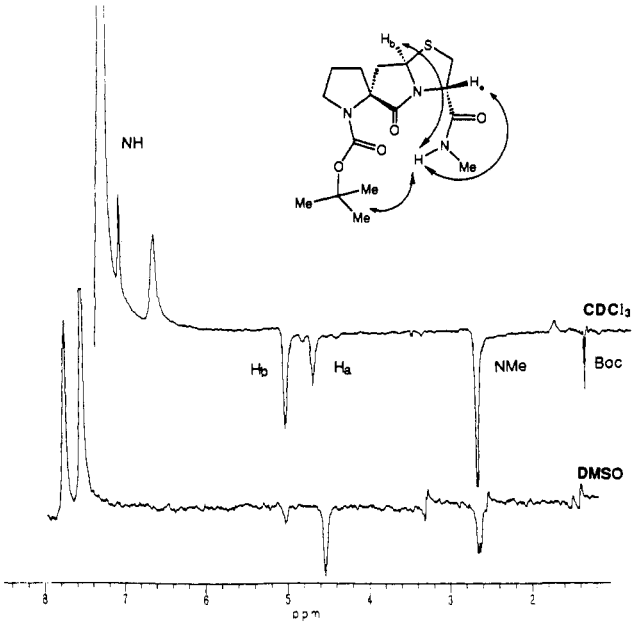

Figure 2. Difference NOE experiment on compound **13**. NOEs are those observed upon irradiation of the amide hydrogen in $CDCl_3$ (top) and DMSO (bottom).

Å (Figure 2). No NOE, however, was observed between the bridgehead hydrogen and the α-hydrogen of the thiazolidine ring. These results support a trans stereochemical relationship between the bridgehead hydrogen and the thiazolidine ring α-hydrogen. If these two hydrogens were in a cis relationship, the interatomic distance would be about 3 Å, well within the distance in which an NOE should be observed. Similar results were observed for the final *N*-acetyl compound **6** as is shown in Figure 3 and discussed below.

Conformational Analysis

NMR Studies. In an effort to determine the solution conformation of **6**, and in particular whether the acetyl and/or the lactam carbonyl oxygen(s) and the amide hydrogen are participating in an intramolecular hydrogen bond, several different types of NMR studies were conducted in $CDCl_3$ and DMSO. In one type of study, nuclear Overhauser effect (NOE) measurements were carried out on **6** (Figure 3) to determine if the amide hydrogen lies in an area of space where hydrogen bonding might be possible with the acetyl carbonyl oxygen. In $CDCl_3$, irradiation of the amide hydrogen of **6** resulted in strong reciprocal NOEs with the proton resonances at δ 4.7 (H_a) and 5.2 (H_b) ppm. Also, a small NOE was observed with the acetyl methyl group at δ 1.9 ppm. These NOE results in $CDCl_3$ are different from those observed for **6** in DMSO. Although, irradiation of the amide hydrogen produced a large NOE with H_a, as was observed in $CDCl_3$, the NOE observed between the amide NH and bridgehead hydrogen H_b was much smaller in DMSO than that seen in $CDCl_3$. In addition, in DMSO, irradiation of the amide hydrogen did not result in a significant NOE with the acetyl methyl group. Instead, a strong NOE appeared between the amide hydrogen and the SCH_2 of the thiazolidine ring system. This NOE was very small in $CDCl_3$.

These NOE results indicate that in $CDCl_3$ the amide hydrogen is oriented below the plane of the bicyclic ring system and, thus, is likely in proper proximity with relationship to the acetyl carbonyl oxygen and the lactam carbonyl oxygen to form a hydrogen bond. The results obtained in DMSO, on the other hand, suggest that the *N*-methylamide group has moved away from H_b and out from the underside of the bicyclic ring to a position in closer vicinity to the SCH_2 and H_a hydrogens. This effect is most likely due to the fact that in contrast to $CDCl_3$, which is a non-hydrogen-bonding solvent that promotes the formation of intramolecular hydrogen bonds, DMSO is a good hydrogen bond acceptor and thus is capable of disrupting any hydrogen bond that might be

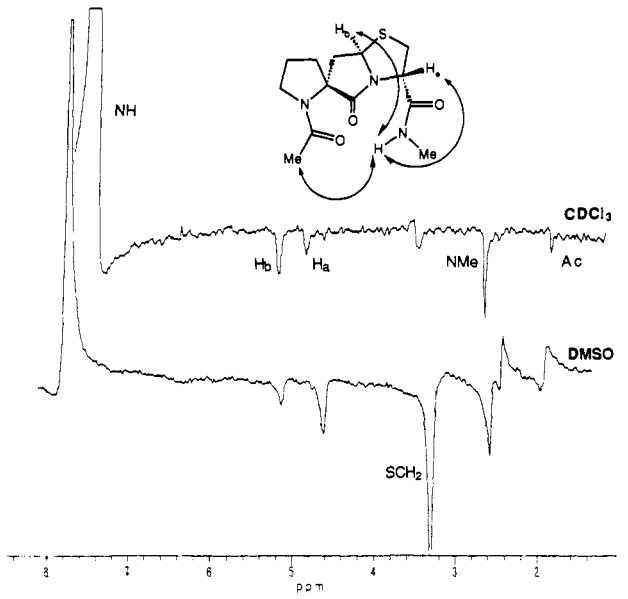

Figure 3. Difference NOE experiment on compound **6**. NOEs are those observed upon irradiation of the amide hydrogen in $CDCl_3$ (top) and DMSO (bottom).

forming between the acetyl carbonyl oxygen and the amide hydrogen.

Similar NOE experiments were also carried out on the synthetic intermediate **13** (Figure 2). Like the results obtained for **6** in $CDCl_3$, irradiation of the amide hydrogen resulted in strong reciprocal NOEs with the proton resonances at δ 4.8 (H_a) and 5.2 (H_b) ppm. In addition, an NOE was seen between the amide hydrogen and the *tert*-butyl group at δ 1.4 ppm. When the NOE experiments were carried out in DMSO, the NOE between the amide NH and the bridgehead hydrogen H_b diminished significantly, while the NOE between the amide NH and H_a remained quite large. Furthermore, the NOE observed in $CDCl_3$ between the amide hydrogen and the *tert*-butyl group was now absent. We attribute this loss of NOE in DMSO to the breakup of the carbamate C=O amide NH hydrogen bond. The loss of this hydrogen bond would also explain the observed difference in the ratios of trans to cis rotamers about the carbamate bond between these two solvents. This ratio was about 3:2 in DMSO whereas it was 10:1 in $CDCl_3$. If DMSO is disrupting the hydrogen bonding between the amide NH and the carbamate C=O, the *tert*-butoxycarbonyl group would be freer to interconvert between trans and cis rotamers.

Because hydrogen bonding cannot be shown conclusively via the above types of NOE experiments, temperature and solvent dependent chemical shift NMR studies on **6** and **13** were carried out. It has been well documented in the literature that changes in temperature have little effect on the chemical shifts of protons which are involved in an intramolecular hydrogen bond or which are otherwise shielded from the medium.[3,15] It is observed that exposed hydrogens, those which are accessible to the solvent, exhibit a larger temperature coefficient (>4 ppb/T) than do intramolecularly hydrogen-bonded hydrogens (<3 ppb/T).[3,15]

The temperature dependence of the amide hydrogen chemical shift was recorded in five-deg intervals over the range 295–325 K in both DMSO and $CDCl_3$. The temperature coefficients ($\Delta\delta/\Delta T$) which were calculated from these experiments are summarized in Table I. The $\Delta\delta/\Delta T$ of both compounds in DMSO were quite large, >5 ppb/K, suggesting that the amide hydrogen is not intramolecularly hydrogen bonded in this solvent. In $CDCl_3$, on the other hand, the coefficients were smaller. For compound **6**, the $\Delta\delta/\Delta T$ was 2.04 ppb/K, indicating that this hydrogen is indeed involved in an intramolecular hydrogen bond in this solvent. The coefficient for **13**, however, was 3.61 ppb/K. This number is in the region which falls between the two cutoff

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Table I. Temperature and Solvent Composition Dependence of the Amide Hydrogen Chemical Shifts of **6** and **13**

compound	NH $\Delta\delta/\Delta T$ (ppb/K) DMSO	NH $\Delta\delta/\Delta T$ (ppb/K) $CDCl_3$	NH $\Delta\delta$ (ppm) DMSO → 70% TFE/30% DMSO	
6	5.19	2.04	0.383	
13	6.42	3.61	0.291[a]	0.403[b]

[a] Trans rotamer about the carbamate bond. [b] Cis rotamer about the carbamate bond.

Table II. Torsion Angles and Energies of Eight Minimized Conformations of **6**

conformer	energy (kcal/mol)	dihedral angle (deg) Φ_2	Ψ_2	Φ_3	Ψ_3
A	8.25	-43.9	+111.0	+111.1	-28.4
B	6.80	-40.3	+108.1	+77.7	-16.7
C	7.38	-61.0	+111.7	+82.5	-6.4
D	12.70	-70.9	+107.9	+33.7	+32.5
E	13.17	-42.6	+104.1	+27.3	+37.2
F	13.35	-46.1	+115.5	+46.5	+24.8
G	13.45	-47.4	+110.1	+39.1	+29.8
H	13.56	-45.9	+125.8	+51.1	+22.9

points where it is difficult to draw concrete conclusions with respect to hydrogen bonding. This higher number may result from an averaging of H-bonded and non-H-bonded conformers due to the fact that the *tert*-butoxycarbonyl group can interconvert between cis and trans rotamers. Only the trans conformer is capable of hydrogen bonding to the amide hydrogen. In the cis rotamer, the amide hydrogen will be exposed to the medium and thus the temperature effects will be greater. These temperature dependence results support the findings of the above NOE experiments.

In addition to the temperature dependence of amide proton chemical shifts, their chemical shift dependence on the solvent composition is also a useful way to distinguish between exposed and intramolecularly hydrogen-bonded protons.[3,15] Protons which are involved in an intramolecular hydrogen bond display little dependence on changes in solvent composition.[3,15] Therefore, 2,2,2-trifluoroethanol (TFE) titration studies were performed on **6** and **13**. The dependence of the amide proton chemical shift on solvent composition when titrating from 100% DMSO to 70% TFE/30% DMSO was measured. The results are summarized in Table I. In all instances the amide hydrogen showed moderate dependence on solvent composition. Changes in chemical shifts ranged from 0.29–0.40 ppm, suggesting that this hydrogen is exposed to the medium and therefore it is sensitive to changes in the solvent. This result is not surprising, since the other studies suggested that DMSO disrupts the hydrogen bonding and twists the CONHMe side chain toward the medium, thereby exposing the amide proton.

Molecular Modeling Studies. Energy minimization and conformational analysis studies were performed on **6** using the Random Incremental Pulse Search (RIPS) method developed by Ferguson and Raber.[16,17] Eight different energy-minimized conformations were generated. Their energies and torsion angles are tabulated in Table II. All bond angles and distances are in agreement with those predicted for the hydrogen-bonded type II β-turn. For the lowest energy conformer, B, the calculated Φ_2, Ψ_2, Φ_3, and Ψ_3 torsion angles are approximately -40.3, +108.1, +77.7, and -16.7°, respectively. These angles are in good agreement with those of the classical type II β-turn. An overlay of the eight energy-minimized structures of **6** gives a picture (Figure 4) of the regions in space that are occupied by the acetyl and CONHMe groups. Only slight conformational variations are seen in the orientations of the acetyl group as well as in the puckering of the rigid spiro-bicyclic ring system. The CONHMe group, however, shows more variability in its range of motion. The amide backbone of all of these structures shares a region in space which closely resembles that occupied by the type II β-turn. A fit was carried out in which nine atoms of the amide backbone of B, the lowest energy conformer of **6**, were compared to their counterparts in an ideal type II β-turn. This comparison, which is shown in Figure 5, gave an RMS fit of 0.161 Å.

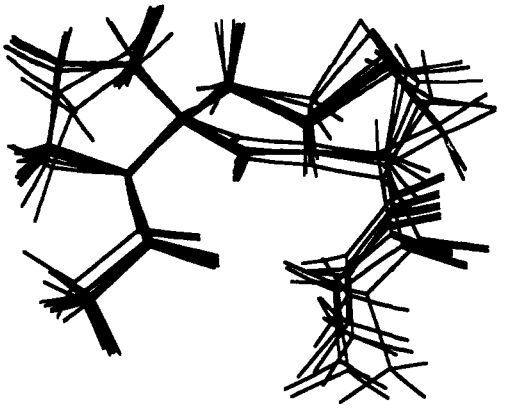

Figure 4. Overlay of the eight energy-minimized structures (A–H) of **6**.

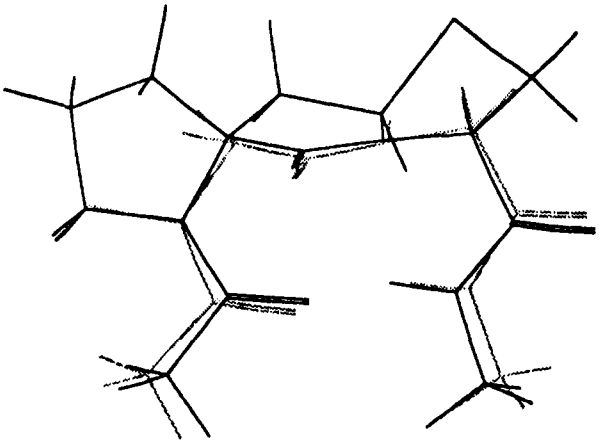

Figure 5. Fit of the minimum energy structure of **6**, structure B, with a model of a classical type II β-turn. The comparison was made by fitting nine atoms of **6** with the corresponding atoms in an ideal type II β-turn backbone. RMS fit = 0.161 Å.

The amide NH was also seen to form a bifurcated hydrogen bond with the carbonyl oxygens of both the acetyl and the lactam moieties. The NH···OC bond distances are about 2 Å, which is suitable for hydrogen bond formation. Also, the NH to CH-bridgehead distance is about 2.7 Å in the minimized structures. This distance is in accord with a large NOE that was observed between these two protons (Figures 2 and 3). These results are in very good agreement with the data obtained from the NMR experiments.

Conclusion

This study has shown the rational design and synthesis of a novel type II β-turn peptidomimetic compound (**6**). This compound restricts three of the four torsion angles that make up a type II β-turn. Modeling studies and NMR studies have shown that the conformation of this molecule is in close agreement with that of the hydrogen-bonded type II β-turn with respect to the torsion angles and the interatomic distances. NMR experiments have shown that the amide proton of **6** is intramolecularly hydrogen bonded in $CDCl_3$; however, DMSO was shown to disrupt this hydrogen bond. Nevertheless, we believe that this spiro-bicyclic system will restrict peptides into the type II β-turn conformation even if hydrogen bonding does not take place due to the highly

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(17) The structures were generated using the RIPS program (Ferguson & Raber, QCPE #588) combined with Allinger's molecular mechanics program MM2(87). For a copy of the RIPS–MM2 program, contact D. M. Ferguson at the Department of Medicinal Chemistry, University of Minnesota, 308 Harvard St. S.E., Minneapolis, MN 55455.

rigid nature of the spiro-bicyclic ring system. We also believe that this system can serve as a useful conformational constraint which, when incorporated into the structure of selected bioactive peptides, will yield new conformationally constrained peptide analogues for structure activity relationship studies.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus 6406-K and are uncorrected. Specific rotations were measured with a Rudolph Research Autopol III polarimeter at 589 nm (Na D line). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. ^{1}H NMR spectra were recorded on a General Electric GN-Ω-300-MHz spectrometer or a Varian 300-MHz Unity spectrometer. The chemical shifts are reported in parts per million (ppm) relative to TMS in $CDCl_3$ or DMSO-d_6. ^{13}C NMR spectroscopy was performed on the General Electric 300-MHz instrument at 75.5 MHz. When $CDCl_3$ was used as solvent, it worked as the internal standard at δ 77.0. When DMSO-d_6 was used, it worked as the internal standard at δ 39.5. FAB mass spectra were recorded on a Kratos MS25 spectrometer. Column chromatography was performed with silica gel, Merck, grade 60 (240–400 mesh, 60 Å) from Aldrich Chemical Company, Inc. Thin-layer chromatography (TLC) was carried out on Analtech 250-μm silica gel GHLF uniplates. Visualization was done with either UV, I_2, ninhydrin spray, $KMnO_4$ spray, or (2,6-dinitrophenyl)-hydrazine spray.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-2-allylproline (8). (*R*)-2-Allylproline (**7**, 4.0 g, 25.8 mmol) prepared as described by Seebach et al.[13] was dissolved in H_2O (60 mL) and cooled in an ice bath. To this solution was added 10% NaOH (11.7 mL) followed by a solution of di-*tert*-butyl dicarbonate (22.5 g, 103.1 mmol) in dioxane (60 mL). The reaction was allowed to stir at 0 °C for 5 h, after which time the ice bath was removed and stirring was then continued at room temperature overnight. The mixture was washed with Et_2O, and the aqueous layer was acidified with 2 N HCl to pH 4. This was then extracted with EtOAc (3 × 75 mL). The aqueous layer from this extraction can be stripped of solvent in vacuo to isolate unreacted starting material. The combined organic extracts were washed with saturated NaCl solution and dried ($MgSO_4$). Removal of solvent in vacuo gave a light yellow solid which crystallized from Et_2O as white needles to yield 4.2 g (64%) of **8**: mp 118–119 °C; $[\alpha]_D$ +72.5° (*c* 1.2, MeOH); TLC R_f (CH_2Cl_2/MeOH, 20:1) = 0.28, R_f (EtOAc/hexane, 3:1) = 0.51. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.44 (s, 9 H, Boc CH_3), 1.74–1.93 (m, 2 H, γ-CH_2), 1.96–2.00 and 2.33–2.39 (m, 1 H, β-CH_2), 2.11–2.15 (m, 1 H, β-CH_2), 2.54 and 2.62 (dd, J = 14.1 and 7.9 Hz, 1 H, CH_2CH=CH_2), 2.91 and 2.98 (dd, J = 14.3 and 7.1 Hz, 1 H, CH_2CH=CH_2), 3.26–3.39 (m, 1 H, δ-CH_2), 3.49–3.54 and 3.65–3.70 (m, 1 H, δ-CH_2), 5.08–5.16 (m, 2 H, CH=CH_2), 5.60–5.80 (m, 1 H, CH=CH_2), 11.61 (br s, 1 H, COOH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.48 and 22.66 (γ-C), 28.21 and 28.29 (Boc CH_3), 34.92 and 36.97 (β-C), 37.89 and 39.07 (CH_2CH=CH_2), 48.37 and 49.00 (δ-C), 66.79 and 68.68 (α-C), 80.51 and 81.04 (Boc C—O), 119.13 and 119.63 (CH=CH_2), 132.14 and 132.98 (CH=CH_2), 153.59 and 155.69 (Boc C=O), 177.23 and 180.60 (COOH); FAB MS *m/e* 256 $[MH]^+$. Anal. Calcd for $C_{13}H_{21}NO_4$: C, 61.15; H, 8.29; N, 5.49. Found: C, 61.29; H, 8.38; N, 5.49.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-2-allylproline Methyl Ester (9). (*R*)-Boc-Allylproline (**8**, 1.15 g, 4.5 mmol) was dissolved in MeOH (20 mL) and cooled in an ice bath. A solution of CH_2N_2 in Et_2O was added dropwise with stirring until the yellow color persisted. The solvent was removed in vacuo to give a light yellow oil which was chromatographed on a 3 × 35 cm silica gel flash column using EtOAc/hexane (1:3) as the eluting solvent. Methyl ester **9** was isolated as a colorless oil in a yield of 1.2 g (99%): $[\alpha]_D$ +72.4° (*c* 0.90, MeOH); TLC R_f (CH_2Cl_2/MeOH, 20:1) = 0.75, R_f (EtOAc/hexane, 1:3) = 0.47. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 and 1.44 (s, 9 H, Boc CH_3), 1.76–1.92 (m, 2 H, γ-CH_2), 2.00–2.14 (m, 2 H, β-CH_2), 2.59 (dd, J = 13.5 and 8.7 Hz, 1 H, CH_2CH=CH_2), 2.91 and 3.09 (dd, J = 13.9 and 6.8 Hz, 1 H, CH_2CH=CH_2), 3.31–3.41 (m, 1 H, δ-CH_2), 3.54–3.69 (m, 1 H, δ-CH_2), 3.71 (s, 3 H, OCH_3), 5.08–5.14 (m, 2 H, CH=CH_2), 5.70–5.77 (m, 1 H, CH=CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.36 and 22.85 (γ-C), 28.06 and 28.13 (Boc CH_3), 35.40 and 36.72 (β-C), 38.06 and 39.36 (CH_2CH=CH_2), 48.16 and 48.26 (δ-C), 51.86 (OCH_3), 66.69 and 67.24 (α-C), 79.19 and 79.74 (Boc C—O), 118.47 and 118.76 (CH=CH_2), 133.05 and 133.38 (CH=CH_2), 153.28 and 153.53 (Boc C=O), 174.60 and 174.81 (ester C=O); FAB MS *m/z* 270 $[MH]^+$. Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.26; H, 8.57; N, 5.07.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-2-(formylmethyl)proline Methyl Ester (10). The fully protected species **9** (390 mg, 1.45 mmol) was dissolved in THF/H_2O (4:1, 25 mL). This solution was stirred under N_2, and OsO_4 (20 mg) was added. The reaction turned dark brown. After 5 min, $NaIO_4$ (780 mg) was added in three batches over a 10-min period. The reaction turned light yellow, and stirring of the reaction mixture was continued for 5 h. The reaction was diluted with Et_2O (20 mL) and H_2O (10 mL). The aqueous layer was extracted with Et_2O (3 × 25 mL). The Et_2O layers were combined, washed with H_2O, and dried ($MgSO_4$). Removal of solvent in vacuo gave a tan oil which turned black on standing. This material was chromatographed on a 1 × 45 cm silica gel flash column with EtOAc/hexane (1:3) as the eluting solvent. Aldehyde **10** was isolated as a colorless oil in a yield of 360 mg (92%): $[\alpha]_D$ +35.4° (*c* 1.60, $CHCl_3$); TLC R_f (Et_2O) = 0.64; R_f (EtOAc/hexane, 1:3) = 0.25. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 and 1.39 (s, 9 H, Boc CH_3), 1.80–2.00 (m, 2 H, γ-CH_2), 2.13–2.27 (m, 2 H, β-CH_2), 2.71–3.08 (m, 2 H, CH_2CO), 3.41–3.77 (m, 2 H, δ-CH_2), 3.71 and 3.72 (s, 3 H, OCH_3), 9.77 and 9.78 (s, 1 H, CHO); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 23.25 and 23.81 (γ-C), 28.87 and 28.92 (Boc CH_3), 37.74 and 38.79 (β-C), 48.53 and 49.26 (δ-C), 49.79 (CH_2CO), 53.23 (OCH_3), 66.35 and 66.83 (α-C), 80.94 and 81.70 (Boc C—O), 153.68 and 153.80 (Boc C=O), 174.43 and 174.65 (ester C=O), 200.56 and 200.82 (CHO); FAB MS *m/z* 272 $[MH]^+$. Anal. Calcd for $C_{13}H_{21}NO_5$: C, 57.55; H, 7.80; N, 5.16. Found: C, 57.38; H, 7.91; N, 5.04.

2-[[2′-(*R*)-*N*-(*tert*-Butoxycarbonyl)-2′-(methoxycarbonyl)-pyrrolidinyl]methyl]thiazolidine-4-carboxylic Acid (11). D-Cys-OH·HCl·H_2O (123 mg, 0.70 mmol) was dissolved in H_2O (2 mL). NaOH (1 equiv) in H_2O (1 mL) was added followed by a solution of **10** (200 mg, 0.74 mmol) in EtOH (3 mL). The reaction was stirred overnight at room temperature, after which time it was diluted with H_2O (5 mL) and extracted with EtOAc (3 × 25 mL). The organic layers were combined and dried ($MgSO_4$). The solvent was removed in vacuo to give 300 mg (87%) of pure diastereomeric mixture **11** as a white solid: mp 88–90 °C; FAB MS *m/z* 375 $[MH]^+$. Anal. Calcd for $C_{16}H_{26}N_2O_6S$: C, 51.32; H, 7.00; N, 7.48; S, 8.56. Found: C, 51.24; H, 7.13; N, 7.38; S, 8.69.

Methyl [3′-(*S*)-[3′α,6′α(*R),7′aα]]-1-(*tert*-Butoxycarbonyl)tetrahydro-5′-oxospiro[pyrrolidine-2,6′-(5′*H*)-pyrrolo[2,1-*b*]thiazolidine]-3′-carboxylate (12).** The thiazolidine mixture (**11**, 0.94 g, 2.51 mmol), prepared as described above, was dissolved in dry DMF (100 mL) under N_2. NEt_3 (0.35 mL, 2.51 mmol) was added, and the solution was heated to 70 °C with stirring under N_2 for 3 days. The solution turned a golden yellow color. The solvent was removed in vacuo to give a dark yellow oil which was dissolved in Et_2O (25 mL). An excess of CH_2N_2/Et_2O solution was added until the evolution of gas ceased. Removal of solvent in vacuo gave a yellow oil which was dissolved in EtOAc (100 mL) and washed with 10% critic acid, saturated NaCl solution and dried ($MgSO_4$). The solvent was evaporated in vacuo to give a yellow oil which was chromatographed on a 2.5 × 45 cm silica gel flash column with Et_2O/hexane (1:1) as the eluting solvent. The product (**12**) was isolated as a colorless oil in a yield of 380 mg (43%): $[\alpha]_D$ +115.2° (*c* 1.83, $CHCl_3$); TLC R_f (Et_2O) = 0.55, R_f (EtOAc/hexane, 1:1) = 0.26. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond. ^{1}H NMR (300 MHz, $CDCl_3$, pro = pyrrolidine) δ 1.32 and 1.34 (s, Boc CH_3), 1.68–1.82 (m, 1 H, pro γ-CH_2), 1.85–1.95 (m, 1 H, pro γ-CH_2), 1.95–2.12 (m, 3 H, pro β-CH_2 and lactam β-CH_2), 2.69 and 2.82 (dd, J = 14.0 and 8.0 Hz, 1 H, lactam β-CH_2), 3.23–3.32 (m, 2 H, SCH_2), 3.34–3.47 (m, 2 H, pro δ-CH_2), 3.65 and 3.66 (s, 3 H, OCH_3), 4.98 (dd, J = 7.8 and 4.2 Hz, 1 H, thiazolidine α-CH), 5.07 and 5.21 (d, J = 7.5 Hz, 1 H, SCHN); ^{13}C NMR (75.5 MHz, $CDCl_3$, pro = pyrrolidine) δ 22.61 and 23.32 (pro γ-C), 27.97 and 28.23 (Boc CH_3), 34.04 and 34.51 (SCH_2), 35.93 (pro β-C), 39.63 and 40.72 (lactam β-C), 46.92 and 47.19 (pro δ-C), 52.36 and 52.59 (OCH_3), 58.57 (thiazolidine α-C), 62.51 and 62.88 (SCN), 66.48 and 66.81 (pro α-C), 79.73 and 80.68 (Boc C—O), 152.69 and 152.75 (Boc C=O), 169.91 and 170.00 (CON), 177.95 (CO_2Me); FAB MS *m/z* 357 $[MH]^+$. Anal. Calcd for $C_{16}H_{24}N_2O_5S$: C, 53.91; H, 6.79; N, 7.86; S, 9.00. Found: C, 54.12; H, 6.55; N, 7.57; S, 8.87.

[3′-(*S*)-[3′α,6′α(*R),7′aα]]-1-(*tert*-Butoxycarbonyl)tetrahydro-5′-oxospiro[pyrrolidine-2,6′-(5′*H*)-pyrrolo[2,1-*b*]thiazolidine]-3′-*N*-methylcarboxamide (13).** Spiro-bicyclic ester **12** (90 mg, 0.25 mmol) was dissolved in a saturated solution of methylamine in MeOH (10 mL). The solution was stirred for 30 min at room temperature, after which time the solvent and excess H_2NCH_3 were evaporated in vacuo to give a light yellow residue. This material was chromatographed on a 1.5 × 45 cm silica gel flash column using EtOAc/hexane (3:1) as the eluting solvent. The amide (**13**) was isolated as an oil in a yield of 75 mg (84%): $[\alpha]_D$ +158.1° (*c* 1.00, MeOH). ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond. ^{1}H NMR (300 MHz, $CDCl_3$, pro = pyrrolidine) δ 1.38 and 1.43 (s, 9 H, Boc CH_3), 1.79–1.93 (m, 2 H, pro γ-CH_2), 2.01–2.09 (m, 1 H, pro β-CH_2), 2.18 (dd, J = 14.1 and 4.2

Hz, 1 H, lactam β-CH_2), 2.35–2.46 (m, 1 H, pro β-CH_2), 2.69 (dd, J = 14.1 and 7.8 Hz, 1 H, lactam β-CH_2), 2.77 (d, J = 4.8 Hz, 3 H, NCH_3), 3.46 (dd, J = 8.0 and 5.6 Hz, 2 H, pro δ-CH_2), 3.56–3.58 (m, 2 H, SCH_2), 4.80–4.85 (m, 1 H, thiazolidine α-CH), 5.18 (dd, J = 7.4 and 5.0 Hz, 1 H, SCHN), 7.49 (br s, 1 H, CONH); ^{13}C NMR (75.5 MHz, $CDCl_3$, pro = pyrrolidine) δ 23.61 (pro γ-C), 26.23 (pro β-C), 28.36 (Boc CH_3), 36.52 (lactam β-C), 39.38 and 39.54 (SCH_2 and NCH_3), 47.79 (pro δ-C), 57.42 (thiazolidine α-C), 62.69 (SCHN), 69.52 (pro α-C), 80.64 (Boc C—O), 153.72 (Boc C=O), 169.47 (CONH), 172.54 (lactam C=O); FAB MS m/z 356 $[MH]^+$. Anal. Calcd for $C_{16}H_{25}N_3O_4S$: C, 54.06; H, 7.09; N, 11.82; S, 9.02. Found: C, 53.84; H, 7.05; N, 11.64; S, 8.88.

[3′-(*S*)-[3′α,6′α(*R),7′aα]]-1-Acetyltetrahydro-5′-oxospiro[pyrrolidine-2,6′-(5′*H*)-pyrrolo[2,1-*b*]thiazolidine-3′-*N*-methylcarboxamide (6).** *N*-Methylamide **13** (40 mg, 0.11 mmol) was deprotected in 4 N HCl/dioxane (5 mL) at room temperature for 1 h. The solvent was removed in vacuo; and the residue was taken up in CH_2Cl_2, whereupon the solvent was again removed in vacuo. The residue was dried in vacuo overnight. It was then dissolved in Ac_2O (10 mL) and cooled in an ice bath, whereupon NEt_3 (1 equiv) and DMAP (cat.) were added. The solution was stirred at room temperature overnight. The solvent was evaporated in vacuo, and the residue was chromatographed on a 1.5 × 45 cm silica gel flash column using CH_2Cl_2/MeOH as the eluting solvent. The product (**6**) was isolated as an oil: $[\alpha]_D$ +139.2° (*c* 0.90, MeOH); ^1H NMR (300 MHz, $CDCl_3$, pro = pyrrolidine) δ 1.90–2.07 (m, 2 H, pro γ-CH_2), 2.09 (s, 3 H, $COCH_3$), 2.16–2.22 (m, 2 H, β-CH_2), 2.31–2.45 (m, 1 H, lactam β-CH_2), 2.72 (dd, J = 14.1 and 8.1 Hz, 1 H, lactam β-CH_2), 2.82 (d, J = 5.1 Hz, 3 H, NCH_3), 3.56–3.65 (m, 4 H, pro δ-CH_2 and SCH_2), 4.85 (dd, J = 8.5 and 5.0 Hz, 1 H, thiazolidine α-CH), 5.15 (dd, J = 8.0 and 4.4 Hz, 1 H, SCHN), 7.24 (br s, 1 H, CONH); ^{13}C NMR (75.5 MHz, $CDCl_3$, pro = pyrrolidine) δ 23.60 (pro γ-C), 24.13 ($COCH_3$), 26.50 (pro β-C), 36.31 (lactam β-C), 38.34 (SCH_2), 39.11 (NCH_3), 48.87 (pro δ-C), 57.70 (thiazolidine α-C), 62.67 (SCHN), 69.50 (pro α-C), 169.57 and 169.72 (CONH and $COCH_3$), 173.04 (lactam C=O); FAB MS m/z 298 $[MH]^+$. Anal. Calcd for $C_{13}H_{19}N_3O_3S$: C, 52.50; H, 6.44; N, 14.13; S, 10.78. Found: C, 52.29; H, 6.55; N, 13.98; S, 10.56.

NMR Studies. Nuclear Overhauser effects (NOE's) were detected using the Varian 300-MHz instrument. Difference NOE measurements were measured as negative enhancements upon irradiation of the proton of interest. The experiment was repeated in triplicate for each compound at 21 °C with varying d2 delay times. The decoupler power was set to 18 dB and the 90-deg pulse width was measured for each compound and set appropriately. The FIDs were multiplied by a heavy line broadening (lb = 5) apodization function before subtraction.

Temperature studies were performed on the GE 300-MHz instrument at a sample concentration of 25 mM. The temperature was set to 295 K, and a spectrum was taken at this temperature and at 5-deg intervals up to 325 K. The chemical shifts of the amide hydrogen versus temperature were plotted, and the temperature coefficients were measured as ppb/K from the slope of the line.

TFE titration studies were performed on the same instrument at 25 °C. A spectrum was first taken in DMSO; then TFE was added in portions so as to increase the amount of TFE by 10% with each addition. After each addition, another spectrum was recorded. This was repeated to a final concentration of 70% TFE in DMSO. The change in chemical shifts (Δδ) of the amide hydrogen was then measured.

Molecular Modeling Studies. Energy minimization studies were performed as described by Ferguson and Raber[16,17] using the Random Incremental Pulse Search (RIPS) method. Eight energy-minimized structures were generated, and the figures were displayed using a Macintosh Quadra 700 computer with Alchemy III software, TRIPOS Associates, Inc. 1992.

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Design of Peptides That Bind in the Minor Groove of DNA at 5′-(A,T)G(A,T)C(A,T)-3′ Sequences by a Dimeric Side-by-Side Motif

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Abstract: The designed peptides pyridine-2-carboxamide–netropsin (2-PyN) and 1-methylimidazole-2-carboxamide–netropsin (2-ImN) are crescent-shaped synthetic analogs of the natural products netropsin (N) and distamycin A (D). Footprinting experiments indicate that the peptides 2-PyN and 2-ImN bind specifically the 5 base pair sequence 5′-TGTCA-3′. Affinity cleaving data suggest that the complexes, 2-ImN·5′-TGTCA-3′ and 2-PyN·5′-TGTCA-3′, are composed of two equivalent orientations which disfavor a 1:1 model. The footprinting and affinity cleaving data are in accord with a 2:1 complex where a novel side-by-side antiparallel dimer binds in the minor groove of double-helical DNA.

Netropsin and Distamycin A. Netropsin (N) and distamycin A (D) are natural products that bind in the minor groove of double-helical DNA at sites of 4 or 5 successive A,T base pairs (Figure 1).[1-4] X-ray[5] and NMR[6] studies of netropsin–DNA and distamycin–DNA complexes reveal how sequence specificity is accomplished. The crescent-shaped di- and tripeptides are bound in the middle of the minor groove of an A,T-rich sequence. The amide hydrogens of the *N*-methylpyrrolecarboxamides form bifurcated hydrogen bonds with the N3 of adenine and the O2 of

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