Design and Enantioselective Synthesis of a Peptidomimetic of the Turn in the Helix−Turn−Helix DNA-Binding Protein Motif

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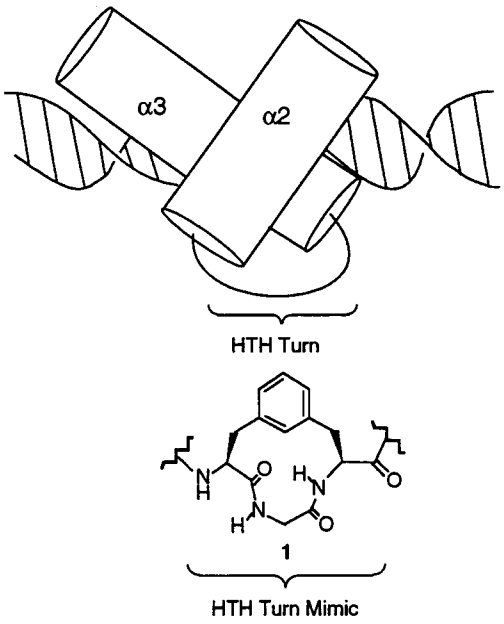
A peptidomimetic of the turn in the helix−turn−helix (HTH) motif of DNA-binding proteins was designed and synthesized. Conformational constraint was achieved by an unusual linking of two amino acids with a side chain carbon−carbon bond. A phenyl ring provides the potential for new hydrophobic contacts with the hydrophobic core of the HTH motif. In the mimic, the peptide backbone and the central residue were retained in native form within a 12-membered cyclic tripeptide. The target compound **1b** was synthesized by two sequential Horner−Wittig couplings followed by enantioselective hydrogenation with Rh(MeDuPHOS) in eight steps and 35% overall yield. The stereochemical outcome of the key hydrogenation was determined by aromatic ring oxidation with $RuO_2/NaIO_4$ to give 2 equiv of Boc-Asp-OMe.

The helix−turn−helix (HTH) structural motif is found in DNA-binding proteins. From prokaryotic repressors of transcription to eukaryotic homeodomain transcription activators, the motif is well-suited to bind DNA. Of the two helixes, one binds DNA directly in the major groove and is stabilized by a second backing helix at right angles to the first (Figure 1). Between these two helixes is a hydrophobic core that serves to stabilize the tertiary interactions between the two helixes. The helixes are connected via a tight turn that resembles the seven-membered hydrogen-bonded ring of the classical γ-turn. The turn of the HTH motif is distinct from the more common β-turn. The HTH-turn is tighter, containing only three amino acid residues, while β-turns have four residues.[1] The HTH turn lacks the hydrogen bond of the γ-turn, and consequently the backbone Φ and Ψ angles differ from the classical γ-turn angles.

To study the molecular recognition process between proteins and DNA, we wish to synthesize peptidomimetics corresponding to the small DNA-binding HTH motif. Since short peptides are usually not conformationally stable in the absence of the constraints from the whole protein, we designed a small peptidomimetic turn, **1**, to stabilize the HTH motif in small peptides (Figure 1). The mimic was designed to stabilize the tertiary structure of the HTH motif found in DNA-binding proteins such as the bacteriophage 434 Cro protein[2,3] or eukaryotic homeodomain proteins, such as Oct-1, that are key developmental transcription factors.[4,5]

Two principles were used to design the turn peptidomimetic: (1) conformational constraint via side chain covalent bonds and (2) introduction of additional hydrophobic contacts in the core of the HTH motif.[6] A wide variety of covalent bonds, from disulfides and amides to carbon−carbon bonds, could constrain the conformation, but the new bonds must not interfere with the existing sterics or electronics of the motif. Mullen and Bartlett designed a template for the outside of the turn region of the HTH motif.[7] Our template is designed to be tucked into the hydrophobic core of the HTH motif, inside the turn, so that the nativelike backbone is retained. We chose to make a hydrocarbon-bridged turn mimic because the carbon−carbon bond would be stable and would suit the steric constraints in the core of the tight turn. Although the mimic is rigidified relative to the natural turn motif, the 12-membered ring is somewhat flexible. This flexibility is desirable when the conformation of the natural motif cannot be matched



2a Native Cro Peptide: Ac-TQTELATKAGVKQQSIQLIEAGV-NH_2

2b Cro Peptidomimetic: Ac-TQTELATK--1--KQQSIQLIEAGV-NH_2

Figure 1. Illustration of the HTH motif bound to DNA. Beneath is the HTH turn mimic **1a** used in molecular modeling design work. Sequence of the Cro HTH native peptide **2a** and peptide with HTH turn mimic **2b** used in modeling.

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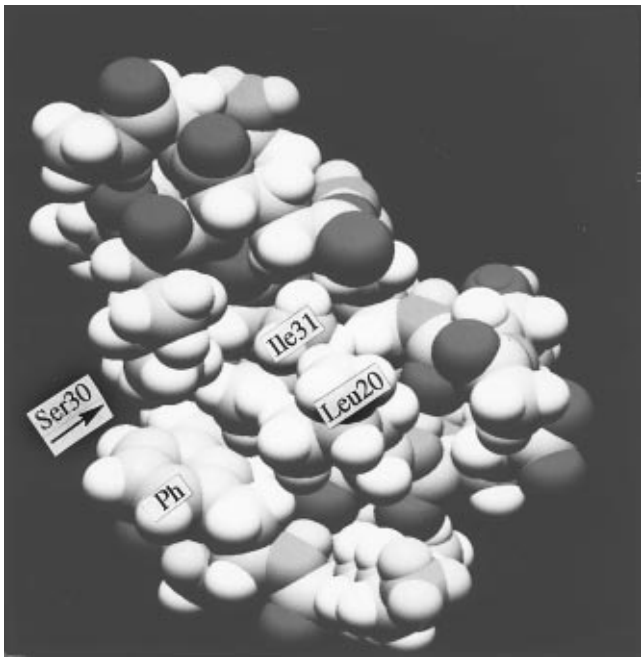

Figure 2. CPK model of the HTH turn mimic minimized in the context of the X-ray structure of Cro peptide,[3] **2b**. The phenyl ring and three of the helix side chains involved in hydrophobic interactions are labeled. Ala21 is buried.

exactly or is bounded by the uncertainty of the X-ray or NMR structure that the mimic was modeled upon.

Hydrophobic contacts between side chains within the two helixes and the turn mimic are expected to stabilize the hydrophobic core of this peptide fragment taken from the Cro protein. The phenyl ring thus satisfies the second criterion of introducing hydrophobic contacts, as shown in Figure 2. Hydrophobic contacts between peptide side chains and β-turn mimics have been used successfully to stabilize β-sheet peptides[6,8−10] and a β-sheet/α-helix tertiary motif.[11] The central amino acid residue of the HTH turn mimic can be readily varied in casette fashion to accommodate the various hydrophilic side chains found in different members of the HTH structural family. The molecule was designed in an iterative process of molecular modeling and synthetic considerations.

The synthetic considerations in the design of mimic **1** required construction of a unique α,ω-diaminodicarboxylate with β-carbons from two α-amino acid backbones attached meta- to a single phenyl side chain. Because of this requirement, each of the four functional groups had to be orthogonally protected. Two stereocenters corresponding to L-amino acid configurations had to be incorporated to retain the designed hydrophobic interactions. Finally, a medium-sized ring had to be closed either at a carbon−carbon bond or at an amide bond. We chose the amide-bond closure for synthetic simplicity.

Stereoselective amino acid synthesis has been reviewed.[12] The turn mimic is an example of the less common side-chain bridged α,ω-diaminodicarboxylates,

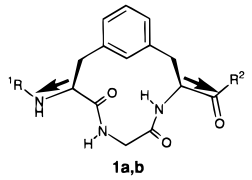

Figure 3. Turn mimic used in molecular modeling showing vectors superimposed on Cro peptide, **1a**: $R^1 = CH_3CO$, $R^2 =$ NHMe. Synthetic target **1b**: $R^1 =$ tBuOCO, $R^2 =$ OH.

of which there are many examples,[13−17] but few that are carbon−carbon linked.[18,19] Synthesis of unnatural amino acids was greatly advanced by the development of stereoselective catalysts for the hydrogenation of didehydroamino acids.[20−22] A C_2-symmetric chiral catalyst, rhodium 2(*S*),5(*S*)-dimethyl-1,2-bisphospholanobenzene (MeDUPHOS), cleanly reduces didehydroamino acids to the corresponding L-amino acid in high yield and high enantioselectivity.[23−25] Synthesis of the precursor didehydroamino acids was facilitated by the development of an α-amino acid Horner−Wittig reagent.[26] The Wittig reaction and Horner−Wadsworth−Emmons modifications were reviewed in 1989.[27] We have taken advantage of the powerful enantioselective hydrogenation of didehydroamino acids to synthesize turn mimic **1b**. Simultaneous enantioselective hydrogenation of two aminoacrylate centers in a single molecule is a key feature of our synthesis.[28]

Results and Discussion

Design of the Mimic. A model of the HTH turn mimic **1a** (Figure 3), was constructed and the global minimum conformation was explored using Macromodel v 3.5. First, the X-ray crystal structure of the Cro protein bound to OR1 DNA[3] was edited to remove the DNA and most of the protein, leaving a 23-residue HTH peptide, Cro16−38 (Figure 1). Only helical residues were included at the C- and N-termini. The C-terminus was modified to a carboxamide and the N-terminus was acetylated for all modeling. The turn mimic **1a** was built by removing all but the β-carbons from the side chains

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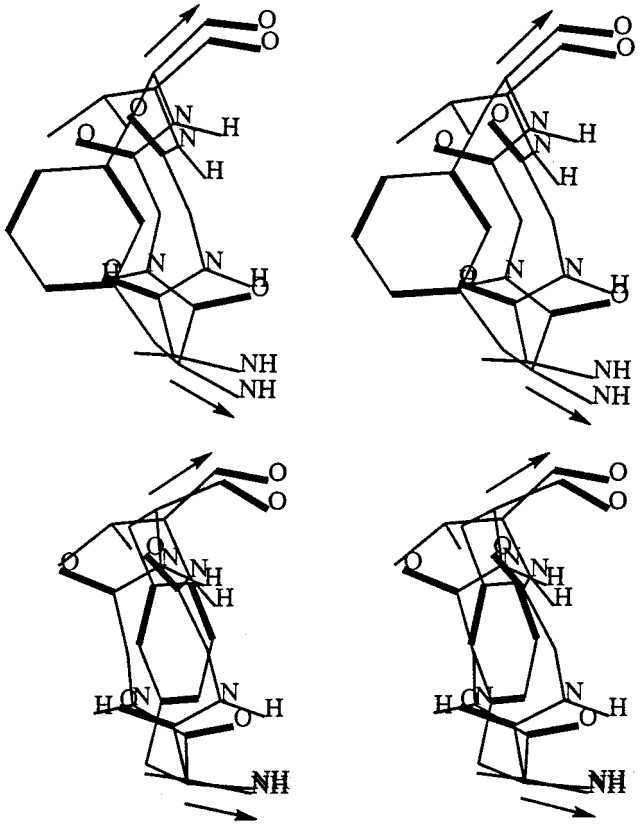
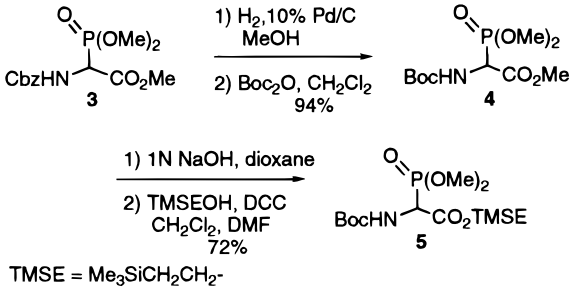


Figure 4. Stereodrawing of the superposition of the ΦAla24 and ΨVal26 backbone vectors of the turn residues of **1a**, shown with arrows, with the corresponding vectors of the two low-energy conformers in **2b**, rmsd 0.38 Å with the lowest energy conformer (top) and 0.16 Å with the second lowest (bottom).

of Ala21 and Val23 and connecting a phenyl group to the two β-carbons. To search for the global minimum, 1000 starting structures of **1a** were generated by the Macromodel Monte Carlo dihedral angle conformational search option and minimized using the Amber force field with water solvation. Of the 129 unique conformations found, 65 minimized with good convergence. The lowest energy conformation, found 15 times with good convergence, was 6 kJ/mol lower than the next conformer, found 17 times.

The two lowest energy conformers were incorporated into the backbone of the HTH Cro peptide **2a** and minimized using MacroModel Amber and water solvation. Superposition of all α-carbons of the resulting HTH peptidomimetics **2b** with Cro HTH peptide **2a** (lowest energy root mean squared deviation (rmsd) of 0.80 Å; second lowest, 0.71 Å) suggests minimal alteration of the three-dimensional structure of the HTH motif (data not shown). The ΦAla24 and ΨVal26 backbone vectors[29−31] set the direction for the two helixes with respect to the ring. Figure 4 shows the superposition of these vectors in the turn of **1a** with the corresponding vectors of the two low-energy conformers in **2b**, rmsd 0.38 Å with the lowest energy conformer, and 0.16 Å with the second lowest. The two conformers have different orientations of the phenyl ring with respect to the backbone, reflecting

Scheme 1. Synthesis of Orthogonally Protected Amino Acid Synthon



the range of conformational flexibility designed into the turn mimic (Figure 4).

In the lowest energy conformer of the turn mimic, no hydrogen bond was formed between the C═O and NH that normally would form in a γ-turn. Since this hydrogen bond is also absent in the native turn, this implies that the C═O and NH of the mimic will be available for hydrogen bonding to the helixes as in the native protein. In the X-ray structure, the extra-turn hydrogen bond to the α2 helix is implied by a distance of 2.01 Å between the helix Ala21 C═O and the turn Val26 NH. The analogous hydrogen bond is also found in the HTH peptidomimic **2b**. In the second lowest energy conformer of **1a** the γ-turn hydrogen bond is present. The existence of this structure in a nearby minimum indicates that the HTH turn mimic may make a satisfactory γ-turn mimic as well.

Five hydrophobic contacts between the turn mimic and helix side chains were found in the minimized peptidomimetic **2b** (Figure 2), specifically, (1) Ph1-proS-βH to Leu20-proS-δCH_3, 2.1 Å; (2) Ph2H to Ala21-αH, 1.4 Å; (3) Ph2H to Ile31-δCH_3, 2.2 Å; (4) Ph3-proR-βH to Ile31-δCH_3, 2.3 Å; and (5) Ph3-proR-βH to Ser30-βH, 1.8 Å. Two hydrophobic contacts were found in the turn of the native Cro HTH peptide **2a**: (1) Val26-γH to Ile31-δCH_3, 2.3 Å, and (2) Ala24-βCH_3 to Leu20-proS-δCH_3, 3.1 Å. The design of the turn mimic thus incorporates potential hydrophobic stabilization of the HTH motif using the aryl side chain conformational constraint.

Synthesis

α,ω-Diaminodiacrylate. Despite the apparent simplicity, the synthesis of the aryl turn mimic requires orthogonal protection on four functional groups, two amines and two carboxylic acids. The amino acid synthon, **3**, was first reported in a series of protected amino phosphoryl acetate esters.[26] Trimethylsilylethyl 2-Boc-2-(dimethoxyphosphoryl)acetate (**5**) fits our criteria of ease of synthesis and orthogonal protection. The use of **5**, without its synthesis, has been reported.[32] We now report the synthesis and characterization of this key starting material (Scheme 1).

Preparation of the aminoacrylate **6** was achieved in 94% yield by reaction of a 10-fold excess of the commercially available benzene-1,3-dialdehyde with phosphonate **3** (Scheme 2). Reaction of phosphonate **5** with monoaldehyde **6** produced orthogonally protected **7** in

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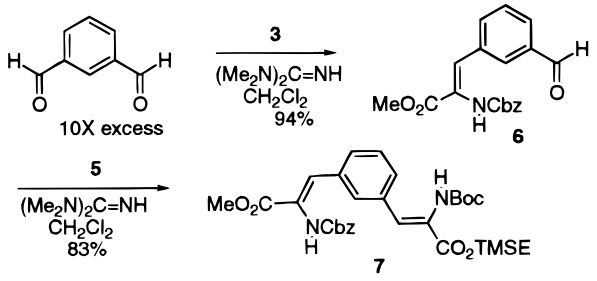
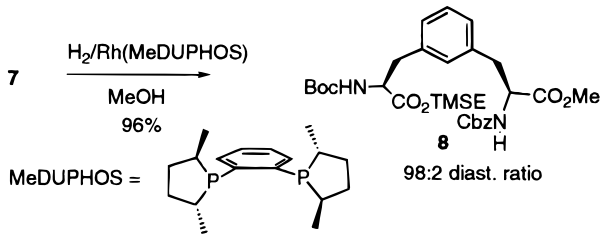
Scheme 2. Synthesis of Bis-Alkene



Scheme 3. Asymmetric Hydrogenation of 7

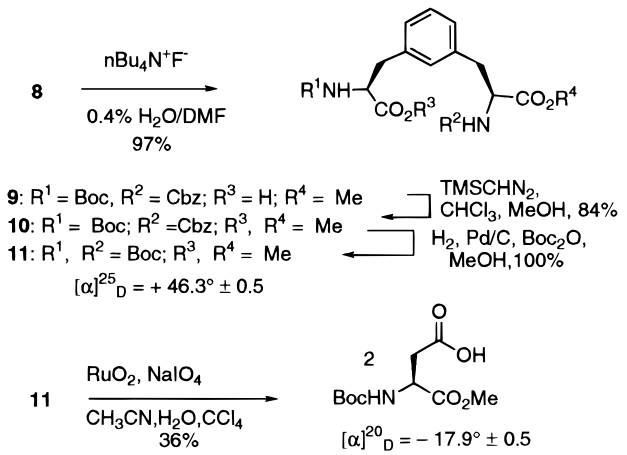


83% yield.[33] Under conditions with tetramethylguanidine as base, we observed predominantly one diastereomer in the ^{1}H NMR (see the Supporting Information), presumably *Z* based on the literature precedence.[28] The one-step synthesis of a meta-substituted phenyl α,ω-diaminodiacrylate has been reported, though it is not orthogonally protected.[26] Hydrogenation to the α,ω-diamino dicarboxylate was not reported.

Enantioselective Hydrogenation. Rh(MeDUPHOS)[34] gives excellent enantioselectivity and high yields for both *Z* and *E* olefins regardless of the amino acid protecting groups.[23] The double enantioselective hydrogenation of **7** produced α,ω-diamino dicarboxylate **8** in excellent yield (Scheme 3). Hydrogenation using Wilkinson's catalyst gave a racemic mixture of diastereomers **8**. The aryl singlets were well-resolved in the ^{1}H NMR spectra of partially purified diastereomeric mixtures of **8**. Integration[35] of the aryl singlet of the hydrogenated product gave a 98:2 diastereomeric ratio (see the Supporting Information). Since the catalyst is known to produce L-amino acid stereochemistry,[23] this indicates that small amounts of *S,R*- or *R,S*-isomer were formed in the hydrogenation, with the major product being the *S,S*-diastereomer, mimicking the natural L,L-stereochemistry of the turn.

Proof of the *S,S*-stereochemistry was obtained by chemical degradation of the reduced intermediate **8**

Scheme 4. Proof of Stereochemistry by Chemical Degradation to Boc-Asp-OMe



(Scheme 4). Preparation of a symmetrical derivative followed by oxidative cleavage of the aromatic ring produced Boc-Asp-OMe of known configuration. Boc-protected amines are stable to RuO_4 oxidative conditions.[36] We avoided the presence of Cbz because it is not stable to the reaction conditions.[37] Thus we chose to produce the Boc and methyl ester protected symmetric derivative **11** for oxidative cleavage.

Removal of the trimethylsilylethyl (TMSE) ester with fluoride gave acid **9**, also the next step in the synthesis (Scheme 4). Under rigorously dry conditions in the desilylation, we observed epimerization. However, deliberate addition of water to the reaction resulted in clean removal of the TMSE ester without epimerization (see the Supporting Information). Esterification with (trimethylsilyl)diazomethane gave the bis-methyl ester **10**. The Cbz amino protecting group was switched to Boc by hydrogenolysis in the presence of di-*tert*-butyl dicarbonate (Boc_2O) to afford **11**. The optical rotation of this intermediate, $[\alpha]_D = +46.3° \pm 0.5°$, demonstrated that the major product was one of the C_2 symmetric stereoisomers (*R,R*- or *S,S*-) and not the meso isomer. Oxidation of **11** with RuO_2 and an excess of $NaIO_4$ for 12 h produced Boc-Asp-OMe with $[\alpha]_D = -17.9° \pm 0.5°$ (lit. −17.8°[38] and −19°[39]). We have thus synthesized the unusual α,ω-diamino dicarboxylate **8** with the desired *S,S*-stereochemistry.

Cyclization. Coupling of the tosylate salt of benzyl glycinate with acid **9** proceeded smoothly to give the protected acyclic precursor **12** (Scheme 5). Simultaneous hydrogenolysis of the Cbz and Bn protecting groups followed by cyclization with diphenylphosphoryl azide (DPPA) in dilute DMF solution afforded cyclic tripeptide methyl ester **13** in high yield.[40] In anticipation of future incorporation of amino acids besides Gly into the mimic, DPPA was chosen for cyclization because of the low

(33) Efficient synthesis of the cyclization precursor **12** (Scheme 5) could conceivably have been achieved by reaction of a dipeptide phosphonate with the monoaldehyde **6**, followed by hydrogenation. The Horner−Wittig reaction of dipeptide synthons having the phosphonate in the C-terminal position such as methyl Boc-(S)-Ile-2-(dimethoxyphosphoryl)acetate with aryl aldehydes has been reported.[25] However, in our sterically congested target, the reaction of benzyl 2-(Cbz)amino-2-(dimethoxyphosphoryl)acetylglycinate with **6** gave a very poor yield in the second Horner−Wittig reaction. Inverting the order by reacting the dipeptide phosphonate in the first step and **3** in the second step did not improve the yield. We concluded that stepwise synthesis of the tripeptide backbone was necessary and suitable for cassette synthesis with amino acids other than Gly.

(34) The activity of the catalyst from Strem varied from batch to batch and the turnover ratio did not approach that reported in the literature. In our experience, rigorously oxygen-free conditions are required to attain total conversion to product. In this initial work, Rh(MeDUPHOS) was used, but Rh(EtDUPHOS) gives slightly better enantioselectivity.

(35) The recycle delay was increased to 10 s and no line-broadening was used in processing to minimize integration errors due to the long relaxation times for aromatic protons. The spectra were acquired at 500 MHz for enhanced resolution.

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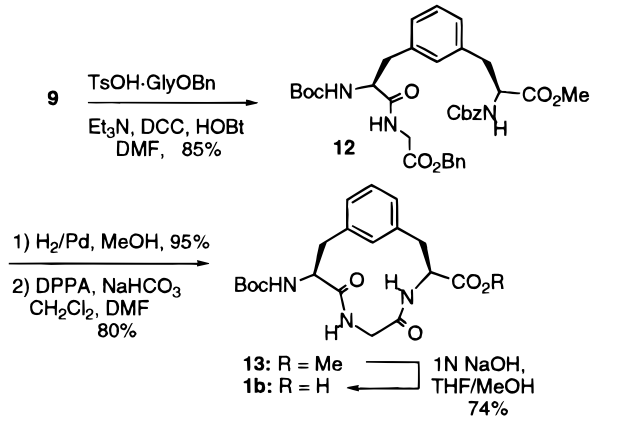
(40) NMR samples at normal concentrations in $CDCl_3$ were found to form gels upon standing for a few hours. The target ester **13** was soluble only in DMF, DMSO, or mixtures of THF/MeOH, $CHCl_3$/DMSO. Boc-protected intermediates **8** and **13** were found to decompose upon standing in $CDCl_3$ due to the presence of DCl.

Scheme 5. Tripeptide Formation, Cyclization, and Ester Hydrolysis of Turn Mimic



propensity of acyl azides to racemize.[41] This cyclization has also been performed with Ala in the central position in 80% yield (J.M.T and F.A.E, unpublished results).

The methyl ester was hydrolyzed with 1 equiv of 1 N NaOH in THF/MeOH to give 74% yield of the free acid product **1b**. This brief, high-yielding synthesis has allowed the preparation of the quantities of **1b** necessary for peptide synthesis (825 mg). Peptidomimetic **2b** has been synthesized on solid phase using compound **1b** (J.M.T, Hart, S., and F.A.E., unpublished results).

Conclusion

We have synthesized a mimic of either the HTH-turn or γ-turn that is designed to stabilize the HTH motif by both conformational constraint and hydrophobic interactions. The mimic was synthesized in eight steps from phosphonates **3** and **5**, using two sequential Horner–Wittig reactions followed by simultaneous enantioselective hydrogenation of both aminoacrylates to give the nativelike stereochemistry. Macrocyclization of the tripeptide proceeded in 80% yield. Turn mimic **1b** was synthesized in eight steps and 35% overall yield.

Experimental Section

All chemicals were reagent grade and commercially available. Rh(MeDUPHOS) was obtained from Strem Chemicals Inc.[34] Unless otherwise indicated, all reactions were carried out under a N_2 atmosphere, in flame-dried flasks. THF and Et_2O were distilled from dark blue solutions with K and benzophenone. Benzene, toluene, CH_2Cl_2, CH_3CN, Et_3N, and diisopropylethylamine (DIEA) were distilled from CaH_2 under a N_2 atmosphere. DMF and MeOH were dried with 3 Å molecular sieves. Chromatography was on 32–63 μm silica gel with reagent grade solvents. TLC was performed using aluminum-backed silica gel plates. Melting points are uncorrected. Proton (300 MHz) and carbon-13 (75 MHz) NMR spectral data were recorded on a General Electric spectrometer using deuterated solvents at approximately 22 °C.

2-(Trimethylsilyl)ethyl 2-[(*tert*-Butoxycarbonyl)amino]-2-(dimethoxyphosphoryl)acetate (5). Methyl phosphorylglycinate **4** (876 mg, 2.95 mmol) was dissolved in dioxane (3 mL) and cooled to 0 °C. NaOH (1 N, 2.95 mL) was added dropwise over 15 min and the reaction was stirred until the starting material disappeared (~1 h) (TLC: R_f = 0.5 1:1 pet. ether/EtOAc, ninhydrin stain). The solution was acidified to ~pH 1 with 20% HCl and extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with water (5 mL) and brine (10 mL) and dried over $MgSO_4$. Concentration followed by recrystallization (EtOAc/pet. ether) yielded 616 mg (74%) of a white solid. mp 155–156 °C. ^{1}H NMR ($CDCl_3$): δ 9.21 (br s, 2H), 5.57 (d, 1H, J = 9 Hz), 4.94 (dd, 1H, J = 9.0, 22.6 Hz), 3.88 (d, 3H, J = 11.1 Hz), 3.84 (d, 3H, 11.6 Hz), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 168.05, 155.45, 81.41, 55.17 (d, J_{COP} = 7.6 Hz), 55.07 (d, J_{COP} = 7.6 Hz), 52.12 (d, J_{CP} = 150.4 Hz) 26.69. The acid (2.25 g, 7.94 mmol) and (trimethylsilyl)-ethanol (1.42 mL, 9.93 mmol) were dissolved in CH_2Cl_2 (30 mL) and cooled to 0 °C. DCC (1.80 g, 8.74 mmol) was added and the reaction was stirred for 8 h. The urea byproduct was removed by filtration and the solution was concentrated. The residue was taken up in EtOAc (60 mL), washed with citric acid (2 × 10 mL), $NaHCO_3$ (2 × 10 mL), water (10 mL), and brine (20 mL), and dried over $MgSO_4$. Concentration followed by chromatography (6:4 pet. ether/EtOAc) gave 3.01 g (98%) of the TMSE ester **5** as a colorless oil that slowly solidified at −20 °C. ^{1}H NMR ($CDCl_3$): δ 5.31 (br d, 1H, J = 8.4 Hz), 4.81 (dd, 1H, J = 9.4, 22.1 Hz), 4.28 (m, 2H), 3.82 (d, 3H, J = 3.1 Hz), 3.78 (d, 3H, J = 3.1 Hz), 1.43 (s, 9H), 1.05 (m, 2H), 0.03 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 166.98, 148.44, 89.49, 64.94, 53.88 (d, J_{COP} = 5.5 Hz), 51.96 (d, J_{CP} = 147.1 Hz), 28.18, 17.37, −1.63.

Methyl (*Z*)-2-[[(Benzyloxy)carbonyl]amino]-3-(3-formylphenyl)-2-propenoate (6). Phosphorylglycinate **3** (4.00 g, 12.1 mmol) was dissolved in 150 mL of CH_2Cl_2, and tetramethylguanidine (1.67 mL, 13.3 mmol) was added. The reaction was stirred for 30 min, then isophthalaldehyde (16.20 g, 120.8 mmol) was added. The reaction was judged to be complete after 10 min by monitoring the disappearance of **3** by TLC (R_f = 0.3, 1:1 pet. ether/EtOAc, UV, veratraldehyde stain). The solution was diluted with 50 mL of CH_2Cl_2, washed with 10% citric acid (2 × 30 mL), $NaHCO_3$ (30 mL), water (30 mL), and brine (80 mL), and then dried over $MgSO_4$. Concentration followed by chromatography (90:10 pet. ether/EtOAc eluted excess isophthalaldehyde; 85:15 pet. ether/EtOAc eluted product) gave 3.87 g (94%) of **6** as a thick colorless oil which solidified after several days at 4 °C. mp = 77–83 °C. ^{1}H NMR ($CDCl_3$): δ 9.89 (s, 1H), 7.94 (s, 1H), 7.80 (d, 1H, J = 7.7 Hz), 7.72 (d, 1H, J = 7.7 Hz), 7.47 (t, 1H, J = 7.7 Hz), 7.37 (s, 1H), 7.32 (br s, 5H), 6.67 (s, 1H), 5.08 (s, 2H), 3.84 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 191.71, 165.34, 153.28, 136.47, 135.69, 135.01, 134.92, 134.56, 130.88, 129.73, 129.13, 128.50, 128.34, 128.27, 125.12, 67.60, 52.85. Anal. Calcd for $C_{19}H_{17}NO_5$: C, 67.25 H, 5.05; N, 4.13. Found: C, 67.05; H, 5.18; N, 4.04.

Methyl (*Z*)-2-[[(Benzyloxy)carbonyl]amino]-3-[3-[(*Z*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-(trimethylsilyl)-ethoxy]-3-oxo-1-propenyl]phenyl]-2-propenoate (7). Phosphorylglycinate **5** (2.80 g, 7.30 mmol) was dissolved in 10 mL of CH_2Cl_2, tetramethylguanidine (1.25 mL, 9.96 mmol) was added, and the mixture was stirred for 30 min at rt. Aldehyde **6** (2.40 g, 7.08 mmol), dissolved in 10 mL of CH_2Cl_2, was then added and the reaction was stirred for 24 h. The solution was diluted to 50 mL with CH_2Cl_2, washed with 10% citric acid (2 × 10 mL), $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL), and then dried over $MgSO_4$. Concentration followed by chromatography (9:1 pet. ether/EtOAc) gave 3.50 g (83%) of **7** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.63 (s, 1H), 7.47 (d, 2 H, J = 6 Hz), 7.33–7.26 (m, 7H), 7.18 (s, 1H), 6.52 (br s, 1H), 6.27 (br s, 1H), 5.1 (s, 2H), 4.34 (m, 2H), 3.79 (s, 3H), 1.37 (s, 9H), 1.1 (m, 2H), 0.08 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 165.55, 165.42, 153.78, 152.51, 135.91, 134.81, 133.87, 131.02, 130.91, 130.29, 129.73, 128.64, 128.45, 128.18, 128.14, 126.87, 125.51, 124.84, 80.97, 67.48, 64.10, 52.58, 28.03, 17.42, −1.52. Anal. Calcd for $C_{31}H_{40}N_2O_8Si$: C, 62.39; H, 6.76; N, 4.69. Found: C, 62.14; H, 6.96; N, 4.60.

Methyl (*S*)-2-[[(Benzyloxy)carbonyl]amino]-3-[3-[(*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-(trimethylsilyl)-ethoxy]-3-oxopropyl]phenyl]propanoate (8). The diacrylate **7** (1.50 g, 2.51 mmol) was dissolved in 8 mL of MeOH and degassed for 30 min with N_2. Rh(MeDUPHOS) catalyst was added (16 mg) and the reaction was subjected to 45–50 psi of H_2 on a Parr shaker for 12 h. The MeOH was evaporated. The residue was dissolved in $CHCl_3$ and passed through a layer of silica (7:3 pet. ether/EtOAc) to give 1.45 g (96%) of a colorless oil. Diastereomeric ratios were determined

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to be 98:2 by ^{1}H NMR analysis (see the Supporting Information). ^{1}H NMR ($CDCl_3$): δ 7.33 (m, 5H), 7.19 (t, 1H, J = 7.5 Hz), 7.00 (d, 1H, J = 11.4 Hz), 6.98 (d, 1H, J = 11.4 Hz), 6.88 (s, 1H), 5.27 (br d, 1H, J = 7.9 Hz), 5.10 (s, 2H), 4.98 (br d, 1H, J = 7.91 Hz), 4.63 (m, 1H), 4.50 (m, 1H), 4.17 (m, 2H), 3.71 (s, 3H), 3.07−2.96 (m, 4H), 1.41 (s, 9H), 0.95 (m, 2H), 0.03 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 172.36 (two peaks), 156.12, 155.58, 137.06, 136.80, 136.41, 130.99, 129.23, 129.01, 128.66, 128.61, 128.38, 127.45, 80.33, 67.46, 64.23, 55.32, 55.03, 52.82, 38.67 (two peaks), 28.79, 17.89, −1.03. Anal. Calcd for $C_{31}H_{44}N_2O_8Si$: C, 61.98; H, 7.38; N, 4.66. Found: C, 61.88; H, 7.38; N, 4.69.

3-[3-[(*S*)-2-[[(Benzyloxy)carbonyl]amino]-3-methoxy-3-oxopropyl]phenyl]-(*S*)-2-[(*tert*-butoxycarbonyl)amino]-propanoic Acid (9). TMSE ester **8** (1.00 g, 1.66 mmol) was dissolved in 15 mL of DMF and cooled to 0 °C. Water (8 drops) was added to the solution. $Bu_4N^+F^-$ (1.10 g, 3.49 mmol) was added and the reaction was stirred for 12 h, during which time gas was evolved. The reaction was concentrated and the residue was dissolved in 40 mL of EtOAc. HCl (0.5 N, 20 mL) was added to the organic layer and the layers were separated. The aqueous layer was extracted with EtOAc (3 × 10 mL), and the combined organic extracts were dried over $MgSO_4$. Concentration gave 802 mg (97%) of crude product which was used without purification for all subsequent transformations. ^{1}H NMR ($CDCl_3$) δ 8.7−9.1 (br s, 1H), 6.8−7.4 (m, 9H), 6.06 (d, 0.5 H, J = 8.4 Hz), 5.46 (d, 0.5 H, J = 7.9 Hz), 5.0−5.3 (m, 3H), 4.5−4.7 (m, 2H), 3.70 (m, 3H), 2.8−3.2 (m, 4H), 1.4 (m, 9H). ^{13}C NMR ($CDCl_3$) δ 174.74, 174.33, 172.51, 170.72, 157.40, 156.41, 155.76, 155.45, 136.91, 136.51, 136.04, 135.37, 131.65, 130.73, 129.21, 129.07, 128.84, 128.75, 128.61, 128.55, 128.06, 80.53, 80.29, 68.60, 68.43, 67.75, 55.62, 55.23, 54.77, 52.95, 38.88, 38.58, 38.49, 28.88, 26.09. Compound **9** appears to exist in two conformations in chloroform as indicated by the doubling of some resonances in the ^{1}H and ^{13}C spectra. (See the Supporting Information.)

Methyl (*S*)-2-[[(Benzyloxy)carbonyl]amino]-3-[3-[(*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-phenyl]propanoate (10). Crude acid **9** (119 mg, 0.238 mmol) was dissolved in 4.0 mL of $CHCl_3$:MeOH (3:1) and stirred. (Trimethylsilyl)diazomethane (2 M in hexanes) was added dropwise until evolution of N_2 ceased and the solution remained yellow. Concentration followed by chromatography (3:1 pet. ether/EtOAc) yielded 101 mg (84%) of product as a colorless glass. $[\alpha]^{25}_D$ = +57.6° ± 0.8° (c = 1.25 $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.33 (s, 5H), 7.19 (t, 1H, J = 7.5 Hz), 6.98 (m, 2H), 6.86 (s, 1H), 5.29 (d, 1H, J = 7.5 Hz), 5.09 (s, 2H), 5.00 (d, 1H, J = 7.5 Hz), 4.64 (m, 1H), 4.54 (m, 1H), 3.71 (s, 3H), 3.67 (s, 3H), 3.04 (m, 4H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.74, 172.38, 156.15, 155.57, 136.88, 136.77, 136.51, 130.87, 129.29, 129.01, 128.64, 128.45, 80.46, 67.48, 55.32, 54.90, 52.83, 52.70, 38.67, 28.78. Anal. Calcd for $C_{27}H_{34}N_2O_8$: C, 63.02; H, 6.66; N, 5.44. Found: C, 60.42; H, 6.49; N, 5.13.

Methyl (*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[3-[(*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-phenyl]propanoate (11). The ester **10** (94 mg, 0.18 mmol) was dissolved in MeOH (5 mL). Boc_2O (81 mg, 0.37 mmol) and 5% Pd/C (20 mg) were added, followed by H_2 addition via balloon while the reaction stirred for 10 h. In reactions where the reprotection of the amine was slow, another equivalent of Boc_2O was added along with a catalytic amount of DMAP (5%). When judged to be complete by TLC (disappearance of free amine, R_f = 0.1, 7:3 pet. ether/EtOAc, ninhydrin stain) the reaction was filtered, the solvent was evaporated, and the residue was chromatographed (excess Boc_2O eluted with 9:1 pet. ether/EtOAc; product eluted with 7:3 pet. ether/EtOAc) to give 88 mg (100%) of a colorless glass. $[\alpha]^{25}_D$ = +46.3° ± 0.5° (c = 2.2 $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.19 (t, 1H, J = 7.3 Hz), 6.98 (d, 2H, J = 7.9 Hz), 6.86 (s, 1H), 5.01 (d, 2H, J = 7.3 Hz), 4.53 (m, 2H), 3.68 (s, 6H), 3.02 (m, 4H), 1.39 (s, 18H). ^{13}C NMR: ($CDCl_3$) δ 172.15, 154.97, 136.19, 130.37, 128.66, 127.83, 79.85, 54.36, 52.10, 38.11, 28.19. Anal. Calcd for $C_{24}H_{36}N_2O_8$: C, 59.98; H, 7.55; N, 5.83. Found: C, 59.83; H, 7.52; N, 5.75.

α-Methyl *N*-(*tert*-Butoxycarbonyl)aspartate. The amino ester **11** (131 mg, 0.273 mmol) was dissolved in a mixture of CH_3CN (8 mL), CCl_4 (8 mL), and water (14 mL). RuO_2 (12 mg, 0.090 mmol) was added and the mixture was stirred vigorously. $NaIO_4$ (2.00 g, 9.35 mmol) was added in three portions over the course of 4 h, each time causing a gas emission and solution color change from black/gray to yellow. Additional $NaIO_4$ was added in three ∼300 mg portions until all starting material had disappeared by TLC (R_f = 0.4, 7:3 pet. ether/EtOAc, ninhydrin stain). The mixture was diluted with 10 mL of CH_3CN and 10 mL of $CHCl_3$ and filtered. ^{i}PrOH was added to reduce the RuO_4, during which time the solution turned from yellow to black, and the mixture was filtered and concentrated. The residue was taken up in 10 mL of saturated $NaHCO_3$ and extracted with CH_2Cl_2 (2 × 4 mL). The water layer was acidified to ∼pH 1 with 2 N HCl and extracted with CH_2Cl_2 (5 × 4 mL). The organic extracts were washed with water (5 mL) and brine (10 mL) and dried ($MgSO_4$). Concentration of the solution left 48 mg (36%) of a colorless oil that slowly solidified at 4 °C. mp 81−87 °C, lit. mp 84−88 °C,[38] 89 °C.[39] $[\alpha]^{20}_D$ = −17.9° ± 0.5 (c = 1.45 MeOH); lit. $[\alpha]^{22}_D$ = −17.8° (c = 1 MeOH),[38] $[\alpha]^{23}_D$ = −19.0° (c = 1 MeOH).[39] ^{1}H NMR ($CDCl_3$) δ 8.81 (br s, 1H), 5.54 (d, 1H, J = 8 Hz), 4.58 (m, 1H), 3.75 (s, 3H), 3.04 (dd, 1H, 3.8, J = 17.2 Hz), 2.85 (dd, 1H, 4.3, J = 17.2 Hz), 1.44 (s, 9H). ^{13}C NMR: ($CDCl_3$) δ 175.85, 171.50, 155.50, 80.38, 52.75, 49.71, 36.62, 28.23.

Methyl (*S*)-2-[[(Benzyloxy)carbonyl]amino]-3-[3-[3-[[2-(benzyloxy)-2-oxoethyl]amino]-(*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl]phenyl]propanoate (12). Crude acid **9** (515 mg, 1.03 mmol) was dissolved in DMF (12 mL) along with HOBT (192 mg, 1.42 mmol), triethylamine (190 μL, 1.37 mmol), and glycine benzyl ester hydrotosylate (462 mg, 1.25 mmol). The reaction was cooled to 0 °C and DCC (249 mg, 1.20 mmol) was added. The reaction was allowed to warm to rt and was stirred for 24 h. The solution was diluted with 50 mL of EtOAc and cooled to 0 °C to precipitate salts. The solution was filtered through a bed of Celite and then extracted with 10% citric acid (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL), water (5 × 5 mL), and brine (25 mL). Concentration of the solution followed by column chromatography (65:35 pet. ether/EtOAc) gave the 567 mg (85%) of the coupled product **12** as a colorless glass. ^{1}H NMR ($CDCl_3$): δ 7.26−7.32 (m, 10H), 7.16 (t, 1H, J = 7.5 Hz), 7.06 (d, 1H, J = 7.9 Hz), 7.04 (s, 1H), 6.95 (d, 1H, J = 7.5 Hz), 6.46 (br s, 1H), 5.54 (d, 1H, J = 8.4 Hz), 5.21 (br s, 1H), 5.12 (s, 2H), 5.08 (br m, 1H), 5.01 (s, 2H), 4.63 (m, 1H), 3.90 (br d, 2H, J = 4.9 Hz), 3.73 (s, 3H), 2.93−3.17 (m, 4H), 1.40 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 172.39, 172.02, 169.95, 156.13, 155.76, 137.46, 136.81, 136.65, 135.64, 130.76, 129.16, 129.03, 128.92, 128.81, 128.61, 128.49, 128.32, 80.52, 67.52, 67.28, 56.16, 55.48, 52.81, 41.67, 39.00, 38.68, 28.70. Anal. Calcd for $C_{35}H_{41}N_3O_9$: C, 64.90; H, 6.38; N, 6.49. Found: C, 65.01; H, 6.39; N, 6.35.

Methyl (*S*)-9-[(*tert*-Butoxycarbonyl)amino]-5,8-dioxo-(*S*)-4,7-diazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3-carboxylate (13). The fully protected dipeptide **12** (1.61 g, 2.49 mmol) was dissolved in MeOH (40 mL) and 5% Pd/C (300 mg) was added. H_2 was added via balloon and the reaction was stirred vigorously for 12 h. TLC analysis showed the disappearance of starting material (R_f = 0.5, 50:50 pet. ether/EtOAc, ninhydrin stain). The catalyst was removed by filtration and the solution was concentrated. Redissolving the semisolid in MeOH followed by addition of EtOAc and evaporation gave 1.00 g (95%) of a hygroscopic granular white solid. Proton NMR showed the absence of benzylic groups. ^{1}H NMR ($CDCl_3$) (peaks broad due to zwitterion): δ 7.9 (br s, 3H), 6.8−7.2 (m, 5H), 5.4 (br s, 1H), 4.4 (br s, 1H), 4.0 (br s, 1H), 3.62 (s, 3H), 3.58 (br s, 2H), 2.7−3.2 (m, 4H), 1.23 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 174.21, 172.32, 155.94, 138.90, 137.26, 131.05, 128.77, 128.45, 127.90, 78.89, 56.33, 55.69, 52.52, 42.51, 25.38, 28.95. Anal. Calcd for $C_{20}H_{29}N_3O_7\ H_2O$: C, 54.41; H, 7.08; N, 9.52. Found: C, 54.52; H, 7.15; N, 8.94. The amino acid (1.00 g, 2.36 mmol) was dissolved in DMF (1.25 L) and cooled to 0 °C. DPPA (709 μL, 2.83 mmol) was added, followed by DIEA (1.03 mL, 5.90 mmol). The reaction was stirred under N_2 for 7 d at 4 °C. The solvent was removed in vacuo, dissolved in minimal $CHCl_3$, and chromatographed (120:2:1 $CHCl_3$/MeOH/AcOH), to give 800 mg (80%) of a white solid. mp 245 °C (dec). ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 7.95 (t, 1H, J = 6.4 Hz), 7.12

(d, 1H, J = 8.8 Hz), 7.06 (t, 1H, J = 7.5 Hz), 6.90 (d, 1H, J = 7.5 Hz), 6.82 (d, 1H, J = 7.5 Hz), 6.60 (s, 1H), 5.98 (d, 1H, J = 7.9 Hz), 4.52 (m, 1H), 4.11 (m, 1H), 3.72 (dd, 1H, J = 6.8, 14.1 Hz), 3.61 (s, 3H), 3.40 (dd, 1H, J = 6.2 , 14.1 Hz), 3.01 (dd, 1H, J = 3.1, 13.8 Hz), 2.86 (d, 2H, J = 6.7 Hz), 2.60 (dd, 1H, J = 7.5 Hz, 12.7 Hz), 2.31 (dd, 1H, J = 5.7, 13.2 Hz), 1.30 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 172.07 (two peaks), 169.27, 155.45, 136.98, 136.40, 133.26, 129.31, 128.69, 127.83, 79.41, 56.57, 53.01, 52.49, 44.00, 39.06, 37.05, 29.11. Anal. Calcd for $C_{20}H_{27}N_3O_6$: C, 59.25; H, 6.71; N, 10.36. Found: C, 59.15; H, 6.76; N, 10.29.

(*S*)-9-[(*tert*-Butoxycarbonyl)amino]-5,8-dioxo-(*S*)-4,7-diazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3-carboxylic Acid (1b). Cyclic peptide ester **13** (190 mg, 0.45 mmol) was dissolved in warm THF/MeOH (40 mL) and cooled to rt. NaOH (1 N, 0.5 mL) was slowly added with stirring. After 1 h, the completed reaction (TLC showed the disappearance of **13**; 120:2:1 $CHCl_3$/MeOH/AcOH, ninhydrin stain) was concentrated and the residue was dissolved in water. Dropwise addition of 2 N HCl induced precipitation of the product which was collected on a filter and dried under vacuum in a desiccator to give a white solid (140 mg, 74%). mp >250 °C. ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 8.30 (br s, 1H), 7.98 (t, 1H, J = 6.3 Hz), 7.23 (d, 1H, J = 9.4 Hz), 7.14 (t, 1H, J = 7.0 Hz), 6.96 (d, 1H, J = 7.0 Hz), 6.66 (s, 1H), 6.37 (d, 1H, J = 7.8 Hz), 4.39 (m, 1H), 4.05 (m, 1H), 3.85 (dd, 1H, J = 8.8, 14.0 Hz), 3.27 (1H, obscured by H_2O peak), 3.02−2.78 (m, 3H), 2.67 (dd, 1H, 8.2 Hz, J = 12.9 Hz), 1.34 (s, 9H); ^{13}C NMR (DMSO-d_6; CD_3-OD): δ 173.27, 173.05, 169.97, 156.14, 137.34, 136.43, 133.51, 129.55, 128.80, 128.18, 80.24, 56.76, 52.70, 49.45, 44.16, 37.58, 28.73. Anal. Calcd for $C_{19}H_{25}N_3O_6 \cdot H_2O$: C, 55.74; H, 6.65; N, 10.26. Found: C, 55.16; H, 6.59; N, 9.90.

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Supporting Information Available: Experimental procedures for compound **3** and its precursors, the ^{1}H and ^{13}C NMR spectra of compounds **5**,**7**,**8**,**9**,**10**,**13,** and **1b**, and the HPLC and ^{1}H NMR of enriched diastereomeric mixtures of **8** (17 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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