A Peptide/Oligourea/Azapeptide Hybrid That Adopts a Hairpin Turn

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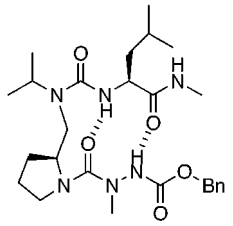
Introduction

The creation of compounds in which noncovalent forces stabilize secondary structure is an exciting new focus of peptidomimetic chemistry.[1] Seebach et al. and Gellman et al. have introduced β-peptides, oligomers of β-amino acids, that adopt intramolecularly hydrogen-bonded helices.[2,3] Gennari et al. have reported that sulfonylpeptides can adopt hydrogen-bonded turns.[4] A number of laboratories, including our own, have reported compounds that adopt linear strand conformations.[5] Zuckermann et al. have recently reported helical peptoids.[6]

These compounds are oligomers built from one type of monomer. A growing number of reports focus on structured peptidomimetic compounds built from two or more types of monomers. Schreiber et al. have reported "hybrids" of peptides and vinylogous peptides, one of which adopts a turn structure and another of which adopts a helical structure.[5d] Hamilton et al. have reported helical hybrids containing oligoanthranilamides.[5b,7] We have been hybridizing various oligomer types to create artificial β-sheets.[8] Gellman et al. have recently prepared β-peptide/depsipeptide hybrids that adopt sheet structures.[9] Lokey and Iverson have reported conceptually related polyaromatics that adopt unique π-stacked structures.[10]

In this paper, we report the synthesis and structural studies of peptidomimetic compound **1**, a hybrid that adopts an intramolecularly hydrogen-bonded hairpin turn. Components of **1** were chosen from the arsenal of building blocks introduced in the many recent reports of unnatural oligomers.[1,11] These monomeric building blocks have been used to create a variety of homooligomers including peptoids,[6,12] vinylogous polypeptides,[5d] β-peptides,[2,3] β-peptoids,[13] oligocarbamates,[14] oligoureas,[15−17] azatides,[18] ureapeptoids,[19,20] oligothioureas,[21] oligosulfonamides,[4,22,23] and oligoethoxyformacetals.[24] We anticipate that a continuing direction in the unnatural oligomer area will be to mix and match these building blocks to create structured hybrids, as we demonstrate with **1**.



1

Hairpin **1** is a hybrid of three oligomer types: a peptide, an oligourea,[15−17] and an azapeptide (Scheme

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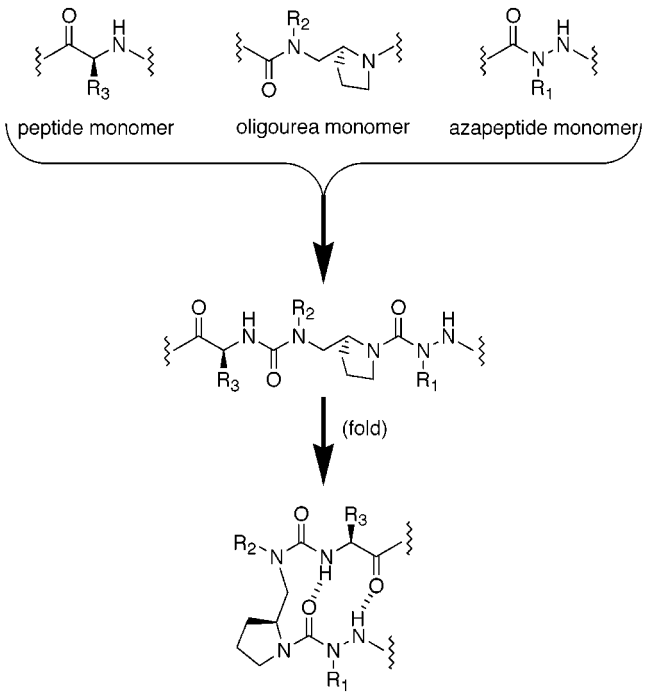
Scheme 1

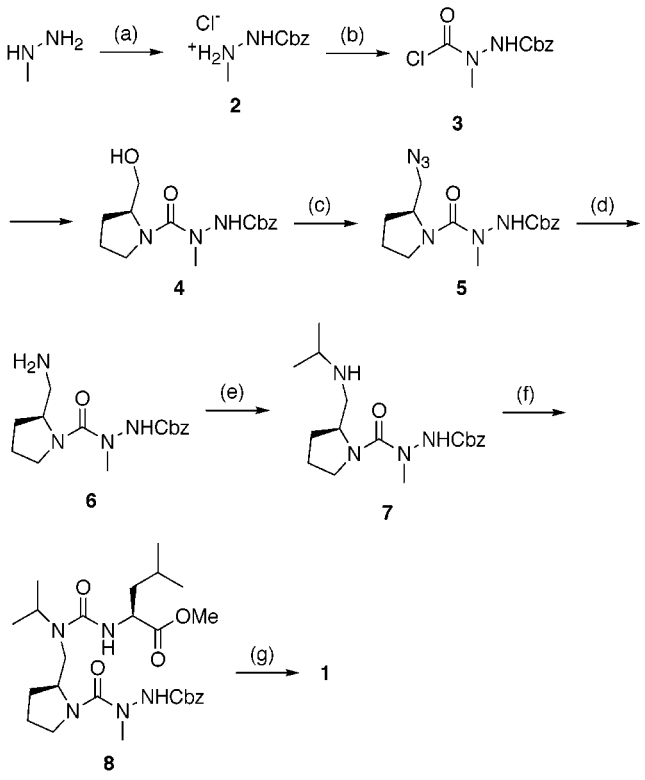


1).[18,25] The design of **1** relies on the propensity of ureas linked by two-carbon spacers to adopt hydrogen-bonded turn structures.[8,17,26] The expected turn conformation of **1** has side chains in relative positions similar to those in a peptide β-turn, an important element of protein structure and a popular target for mimicry.[27] Hairpin turns are of special interest[28,29] because they can serve as nucleators of antiparallel β-sheet structure; in addition, hairpins are often sites for molecular recognition of proteins.[30]

Results and Discussion

Synthesis of Hairpin 1. The synthesis of hairpin **1** is shown in Scheme 2. Methylhydrazine was Boc-protected on its substituted end, the remaining end was Cbz-protected, and the Boc group was removed with HCl

Scheme 2. Synthesis of Hairpin 1[a]



[a] Key: (a) (i) Boc_2O, MeOH, (ii) CbzCl, CH_2Cl_2/saturated aqueous NaOH, (iii) HCl, MeOH (68%); (b) (i) $COCl_2$, CH_2Cl_2/saturated aqueous $NaHCO_3$, (ii) L-prolinol, CH_2Cl_2/saturated aqueous $NaHCO_3$ (92%); (c) (i) MsCl, THF, 0 °C, (ii) NaN_3, DMF, 64 °C (60%); (d) PPh_3, H_2O, THF, Δ; (e) $(CH_3)_2CO$, $NaCNBH_3$, MeOH; (f) L-leucine methyl ester isocyanate, CH_2Cl_2 (76%, three steps); (g) $MeNH_2$, MeOH (93%).

in methanol to furnish the hydrochloride salt **2**.[31] Although this protection procedure involves three steps, the overall process is quick and convenient, requiring only a few hours. The Cbz-protected hydrazine **2** was converted to the corresponding carbamoyl chloride **3**,[17a,32] and the crude carbamoyl chloride was coupled with L-prolinol to generate alcohol **4**, which was sufficiently pure to be used without purification. Alcohol **4** was converted to the corresponding mesylate by reaction with methanesulfonyl chloride, and the mesylate was converted to azide **5** by reaction with sodium azide. Azide **5** was reduced under Staudinger conditions, the resulting primary amine **6** was reductively alkylated with acetone to introduce an isopropyl substituent, and the reductive alkylation product (**7**) was coupled with L-leucine methyl ester isocyanate to generate diurea **8**.[33] Diurea **8** was converted to hairpin **1** by aminolysis with methylamine.

Structural Studies of Hairpin 1. ^{1}H NMR chemical shift, variable-temperature (VT), and nuclear Overhauser effect (NOE) studies provide evidence that **1** adopts a hairpin conformation in $CDCl_3$ solution. In the chemical shift studies, compounds **9** and **10** were used as controls for the upper (leucine) and lower (methylhydrazine)

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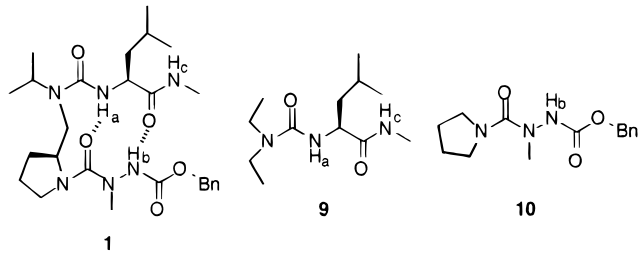


Figure 1. NH chemical shifts for hairpin **1** and controls **9** and **10** (1 mM solution in $CDCl_3$, 295 K).

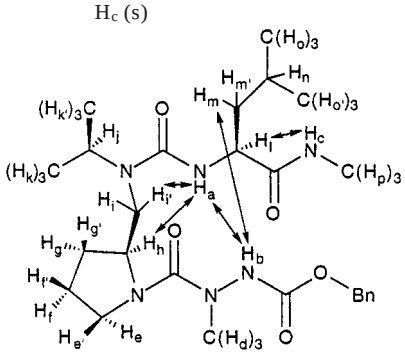
strands of **1**, respectively (Figure 1).[34] ^{1}H NMR spectra for **1**, **9**, and **10** were acquired at 1 mM in $CDCl_3$ solution, at which concentration these compounds are negligibly self-associated.[35] The spectrum for control **10** shows two conformers, with two different NH chemical shift values (6.59 and 6.35 ppm in 1 mM $CDCl_3$). These conformers presumably have extended (urea carbonyl trans to the hydrazine methyl) and folded (urea carbonyl cis to the hydrazine methyl) conformations.[25,36]

These ^{1}H NMR chemical shift studies indicate that the urea and hydrazine NH protons of **1** are intramolecularly hydrogen bonded in 1 mM $CDCl_3$ solution (Figure 1). The urea resonance of **1** (H_a) is shifted 1.7 ppm downfield from the corresponding resonance of control **9**. The hydrazine resonance of **1** (H_b) is shifted 2.1−2.3 ppm downfield from the corresponding resonance of control **10**. In contrast, the amide resonance of **1** (H_c) is shifted only 0.2 ppm downfield from the corresponding resonance of control **9**. These downfield shifts are consistent with a hairpin structure in which the urea and hydrazine NH protons are intramolecularly hydrogen bonded, and the amide NH proton is not hydrogen bonded.

^{1}H NMR VT studies offer additional insight on the hydrogen-bonding experienced by the NH protons of **1**. In 1 mM chloroform solution over a range of 294−330 K, the ^{1}H NMR chemical shifts of the urea resonance (H_a) and the amide resonance (H_c) show a small temperature dependence (−0.4 and −1.8 ppb/K, respectively). In a noncompetitive solvent, a small temperature dependence indicates that a proton is either completely hydrogen-bonded or completely non-hydrogen-bonded.[8b,37] The chemical shift of the hydrazine resonance (H_b) shows a large temperature dependence (−13.3 ppb/K). In a noncompetitive solvent, a large temperature dependence indicates that a proton participates in an equilibrium between hydrogen-bonded and non-hydrogen-bonded states.[8b,37] The VT and chemical shift data collectively indicate that the urea proton is completely hydrogen bonded, the amide proton is not hydrogen bonded, and the hydrazine proton is largely, but not completely, hydrogen bonded.

Table 1. NOESY Cross-Peaks Observed for Hairpin 1[a−c]

proton	NOESY cross-peaks[d]
H_a	H_b (w), H_h (m), $H_{i'}$ (m), H_l (w), $H_{m/m'/n}$ (m)
H_b	H_a (w), H_d (w), H_e (w), $H_{e'}$ (w), $H_{m/m'/n}$ (w)
H_c	H_j (w), H_l (s), $H_{m/m'/n}$ (w), H_p (s)
H_d	H_b (w)
H_e	H_b (w), $H_{f/f'}$ (m), H_h (w)
$H_{e'}$	H_b (w), $H_{f/f'}$ (m)
$H_f/H_{f'}$[e]	H_e (m), $H_{e'}$ (m), $H_{g'}$ (m)
H_g	H_h (m)
$H_{g'}$	$H_{f/f'}$ (m), H_i (m), H_j (w), H_k (m)
H_h	H_a (m), H_e (w), H_g (m), H_i (m), $H_{i'}$ (m)
H_i	$H_{g'}$ (m), H_h (m), H_k (m), $H_{k'}$ (m)
$H_{i'}$	H_a (m), H_h (m)
H_j	H_c (w), $H_{g'}$ (w), H_k (s), $H_{k'}$ (s)
H_k	$H_{g'}$ (m), H_i (m), H_j (s), $H_{k'}$ (s)
$H_{k'}$	H_i (m), H_j (s), H_k (s)
H_l	H_a (w), H_c (s), $H_{m/m'/n}$ (s), H_o (m), $H_{o'}$ (m)
$H_m/H_{m'}/H_n$[e]	H_a (m), H_c (w), H_b (w), H_l (s)
H_o	H_l (m), $H_{m/m'/n}$ (s)
$H_{o'}$	H_l (m), $H_{m/m'/n}$ (s)
H_p	H_c (s)



[a] 10 mM solution in $CDCl_3$, 295 K. [b] Protons of the benzyl group do not show any cross-peaks to protons of the rest of the molecule and are not listed in this table. [c] Geminal NOEs are not listed. [d] Cross-peaks were identified as strong (s), medium (m), or weak (w) on the basis of their relative intensities. [e] These protons have overlapping resonances. [f] For this structure, H_m was arbitrarily chosen from the $H_{m/m'/n}$ set of overlapping resonances; the NOESY cross-peak with H_b may be for any or all of these protons, without changing conclusions on interstrand proximity.

Two-dimensional proton magnetic resonance nuclear Overhauser effect (NOESY) studies provide compelling evidence for the hairpin turn conformation of **1** (Table 1).[35] Most significantly, both the urea proton (H_a) and the leucine β-/γ-protons (H_m, $H_{m'}$, and/or H_n) show NOE cross-peaks with the hydrazine proton (H_b).[38,39] These NOEs demonstrate the proximity of the leucine and hydrazine strands. H_a also shows NOE cross-peaks with backbone protons H_h and $H_{i'}$, but not with any protons of the isopropyl group. These NOEs provide evidence for the turn portion of the hairpin.

The leucine α-proton (H_l) shows a strong NOE cross-peak with the amide proton (H_c) and only a weak NOE cross-peak with the urea proton (H_a). This NOE pattern is consistent with an extended strand conformation for

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(35) ^{1}H NMR chemical shift studies, in which the chemical shifts of the amide and hydrazine NH's of **1** were measured at varying concentrations (1−100 mM range) in $CDCl_3$ solution, indicate that **1** self-associates with an estimated K_{dim} of 5 M^{-1}. Thus, **1** is ca. 1% self-associated in 1 mM solution and ca. 10% self-associated in 10 mM solution (NOE studies were performed at 10 mM).

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(39) The hydrazine NH_b resonance was broad, and all NOESY cross-peaks involving this resonance were weak. To corroborate these weak NOEs, we performed one-dimensional difference NOE experiments. These experiments were performed in triplicate to ensure that all enhancements were real and not subtraction artifacts. The same NOEs were seen as in the NOESY experiment. Upon irradiation of NH_b, the following enhancements were observed: urea NH_a (1%), leucine β-CH_m/β-$CH_{m'}$/γ-CH_n (0.8%), hydrazine $C(H_d)_3$ (1.7%), pyrrolidine CH_e (1.9%), and pyrrolidine $CH_{e'}$ (1.7%).

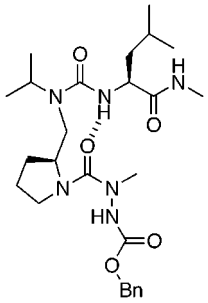

Figure 2. Possible minor conformation of **1**.

the leucine portion of **1**. Diastereotopic protons H_i and $H_{i'}$ exhibit different sets of NOEs: H_i shows NOE cross-peaks with the isopropyl methyl groups (H_k and $H_{k'}$) and with pyrrolidine ring proton H_g, while $H_{i'}$ shows an NOE cross-peak with the urea proton (H_a). This observation suggests that H_i and $H_{i'}$ are in completely different environments and is consistent with the proposed turn conformation, in which these protons are on different faces of a hydrogen-bonded ring.

Three NOEs are not consistent with a hairpin turn structure. The hydrazine proton (H_b) shows weak NOE cross-peaks with pyrrolidine ring protons H_e and $H_{e'}$. These NOEs suggest the conformer shown in Figure 2; this conformer must be minor, because the chemical shift studies indicate that the hydrazine NH is hydrogen-bonded.[40] The amide proton (H_c) shows an NOE cross-peak with isopropyl proton H_j. This NOE is very weak, but it furthers the point that nonhairpin conformations are present.

To gain further insight into the structure of the major conformer of **1**, we attempted to generate a model using MacroModel[41] and the AMBER*[42] force field. This force field has poor parameters for the diacylhydrazine functional group and did not allow the generation of a local minimum of hairpin **1** in which both hydrogen bonds were present. We attribute the failure to achieve a hydrogen-bonded conformation to the force field's strong torsional bias toward a planar geometry of the diacylhydrazine functional group. Crystallographic studies of related methylhydrazine−pyrrolidine ureas[32,36] and theoretical studies of diacylhydrazines[43] indicate that these groups should adopt nonplanar conformations. When we constrained the diacylhydrazine group to a twisted geometry, a hairpin was obtained in which both hydrogen bonds were present (Figure 3). This model is consistent with the NOEs attributed to the major conformer.

Conclusion

This work demonstrates the potential of a "mix and match" strategy toward the creation of structured peptidomimetic compounds. Three different types of oligomers were hybridized to create **1**. The chemical shift, VT, and NOESY experiments indicate that **1** adopts a tight simple turn (containing one intramolecular hydrogen bond), with a large population also adopting a hairpin turn (containing two intramolecular hydrogen bonds).

We are currently developing solid-phase syntheses for analogues of **1**. This structure is a promising scaffold for combinatorial chemistry, since it is made in a modular synthesis from readily varied building blocks. We will report structurally and biologically interesting analogues as they are discovered.

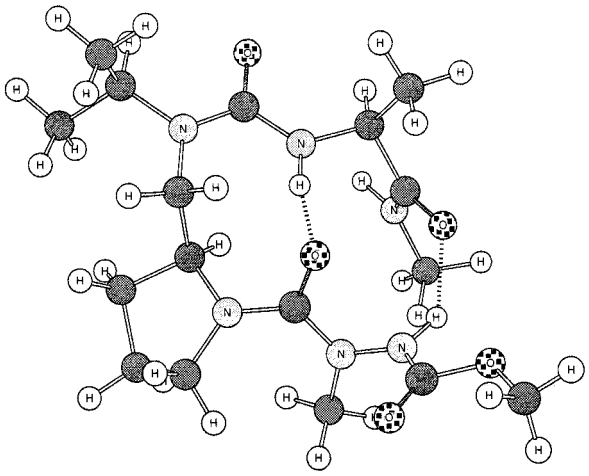

Figure 3. Model of hairpin **1** in a minimum energy conformation (local minimum). The benzyl group and leucine side chain are truncated for clarity. The model was generated using MacroModel V5.5 and the AMBER* force field; the diacylhydrazine group was constrained to a twisted (90°) geometry by constraining its C(O)−N−N−C(O), C(O)−N−N−H, CH_3−N−N−C(O), and CH_3−N−N−H torsions.

Experimental Section

General Methods. Commercially available reagents and solvents were used without further purification. Tetrahydrofuran was distilled from sodium and benzophenone under nitrogen. Phosgene was obtained from Fluka as a 20% (1.93 M) solution in toluene. High-resolution mass spectra (HRMS) were obtained by electron ionization (EI) at 70 eV, chemical ionization (CI) using isobutane or ammonia, or liquid secondary ion mass spectrometry (LSIMS) of samples in a *m*-nitrobenzyl alcohol matrix bombarded with Cs^+ ions at 25 kV (instrumental variation $\sigma = \pm 2$ mmu). Combustion analyses were performed by Desert Analytics, Tucson, AZ.

NOESY and NOE studies on hairpin **1** were performed using a 10 mM sample in $CDCl_3$ (solvent was predried over basic alumina) that was degassed by three freeze−pump−thaw cycles on a high-vacuum line (<0.001 mmHg) and sealed under vacuum. Peak assignments used in the interpretation of NOE data were based on COSY spectra.

Protected Hydrazine 2.[31] A solution of methyl hydrazine (1.4 mL, 26 mmol) and di-*tert*-butyl dicarbonate (4.32 g, 19.8 mmol) in 25 mL of methanol was stirred for 30 min and then concentrated by rotary evaporation to 2.99 g of a yellow liquid. Dichloromethane (20 mL) and 20 mL of a 1 M NaOH solution were added, and the mixture was rapidly stirred as benzyl chloroformate (2.8 mL, 20 mmol) was added in one portion. The mixture was rapidly stirred for 1 h, the layers were separated, and the organic layer was sequentially washed with 20 mL of a 1 M HCl solution, 20 mL of water, and 20 mL of a saturated aqueous NaCl solution, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to 5.12 g of a yellow oil. The oil was dissolved in 40 mL of methanol, and the solution was chilled in an ice bath for ca. 15 min. Acetyl chloride (10 mL) was added

(40) Most of the peaks in the ^{1}H NMR spectra of **1** are ill-resolved, suggesting that the rate of interconversion between conformations is intermediate or slow on the NMR time scale. This possibility appears very likely considering that the ^{1}H NMR spectra for control **10** show two conformers.

(41) Macromodel V5.5: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caulfield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

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(43) (a) Reynolds, C. H.; Hormann, R. E. *J. Am. Chem. Soc.* **1996**, *118*, 9395. (b) Graybill, T. L.; Ross, M. J.; Gauvin, B. R.; Gregory, J. S.; Harris, A. L.; Ator, M. A.; Rinker, J. M.; Dolle, R. E. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1375.

over 30 s, the ice bath was removed, and the solution was stirred for 20 min. The solution was then concentrated by rotary evaporation to a white solid, which was recrystallized from 15 mL of 2-propanol to afford 2.89 g (68%) of **2** as white flakes: mp 165−166 °C; IR (KBr) 3462, 3145, 3007, 2962, 2789, 2700, 2416, 1741, 1551 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 11.48 (br s, 2 H), 10.80 (s, 1 H), 7.44−7.37 (m, 5 H), 5.19 (s, 2 H), 2.73 (s, 3 H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 154.8, 135.6, 128.5, 128.3, 128.1, 67.2, 35.6; HRMS (EI) *m/e* for $C_9H_{12}N_2O_2$ $(M)^+$, calcd 180.0900, found 180.0896. Anal. Calcd for $C_9H_{13}ClN_2O_2$: C, 49.89; H, 6.05; Cl, 16.36; N, 12.93. Found: C, 49.80; H, 6.36; Cl, 16.04; N, 12.85.

Alcohol 4. A 1.93 M solution of phosgene in toluene (12 mL, 24 mmol) was added to a rapidly stirred, ice-cooled mixture of hydrazine **2** (1.02 g, 4.72 mmol), 25 mL of dichloromethane, and 25 mL of a saturated aqueous $NaHCO_3$ solution. The mixture was rapidly stirred at 0 °C for 20 min. The layers were separated, the aqueous layer was extracted with 25 mL of dichloromethane, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford 1.121 g (4.63 mmol) of crude carbamoyl chloride **3**, which was used immediately.

The oil was dissolved in 25 mL of dichloromethane and 25 mL of a saturated aqueous $NaHCO_3$ solution. L-Prolinol (0.483 g, 4.78 mmol) was added in 5 mL of dichloromethane, and the mixture was rapidly stirred for 24 h. The layers were separated, the aqueous layer was extracted with 25 mL of dichloromethane, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford 1.34 g (92%) of **4** as a colorless oil: IR (neat) 3431, 3244, 1722, 1632 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1 H), 7.34 (s, 5 H), 5.14 (s, 2 H), 4.10−4.00 (m, 1 H), 3.81 (br s, 1 H), 3.67 (br s, 1 H), 3.40−3.36 (m, 2 H), 3.22−3.18 (m, 1 H), 3.00 (s, 3 H), 1.95−1.82 (m, 1 H), 1.76−1.68 (m, 1 H), 1.64−1.62 (m, 2 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 161.5, 155.3, 135.5, 128.6, 128.5, 128.3, 67.8, 64.0, 61.0, 49.8, 39.1, 27.5, 25.2; HRMS (CI) *m/e* for $C_{15}H_{22}N_3O_4$ $(M + H)^+$, calcd 308.1611, found 308.1597. An analytical sample was further purified by column chromatography (2:1 EtOAc/hexanes). Anal. Calcd for $C_{15}H_{21}N_3O_4$: C, 58.62; H, 6.89; N, 13.67. Found: C, 58.36; H, 7.05; N, 13.37.

Azide 5. A solution of alcohol **4** (0.605 g, 1.97 mmol), 20 mL of tetrahydrofuran, and triethylamine (0.830 mL, 5.95 mmol) was chilled in an ice bath for ca. 15 min. Methanesulfonyl chloride (0.305 mL, 3.94 mmol) was added in one portion; a white precipitate immediately started to form. The suspension was stirred for 20 min, diluted with 60 mL of dichloromethane, and sequentially washed with two 50 mL portions of a saturated aqueous $NaHCO_3$ solution and 50 mL of a saturated aqueous NaCl solution. The dichloromethane layer was dried over $MgSO_4$, filtered into a round-bottomed flask, and concentrated by rotary evaporation to 0.783 g (2.0 mmol crude) of a colorless viscous oil. To the flask were added sodium azide (0.769 g, 11.8 mmol) and 20 mL of dimethylformamide, and the suspension was stirred, heating to 64 °C, for 16 h. The suspension was diluted with 50 mL of diethyl ether and then sequentially washed with 100 mL of water, three 50 mL portions of water, and 50 mL of a saturated aqueous NaCl solution. The ether layer was dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to 0.469 g of a pale yellow oil, which was purified by column chromatography (1.5:1 hexanes/EtOAc), affording 0.391 g (60%) of **5** as a colorless oil: IR (neat) 3248, 2102, 1736, 1632 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26 (s, 5 H), 6.82 (s, 1 H), 5.17 (s, 2 H), 4.16 (br s, 1 H), 3.56 (br s, 1 H), 3.43 (br s, 1 H), 3.36−3.29 (m, 1 H), 3.24 (appar d, J = 11.5 Hz, 1 H), 3.05 (s, 3 H), 2.04−1.98 (m, 1 H), 1.85−1.81 (m, 1 H), 1.73−1.81 (m, 2 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 160.7, 155.2, 135.6, 128.5, 128.4, 128.2, 67.6, 58.1, 52.4, 49.6, 38.9, 28.0, 25.3; HRMS (LSIMS) *m/e* for $C_{15}H_{21}N_6O_3$ $(M + H)^+$, calcd 333.1677, found 333.1686. Anal. Calcd for $C_{15}H_{20}N_6O_3$: C, 55.16; H, 6.94; N, 24.12. Found: C, 54.88; H, 6.92; N, 23.93.

Urea 8. A solution of azide **5** (0.405 g, 1.22 mmol), 10 mL of tetrahydrofuran, triphenylphosphine (0.638 g, 2.43 mmol), and water (0.045 mL, 2.5 mmol) was heated to reflux, stirred for 6 h, and then concentrated by rotary evaporation to a pale yellow liquid, which was dissolved in 25 mL of diethyl ether and 25 mL of a 0.1 N HCl solution. The aqueous layer was extracted with two 25 mL portions of diethyl ether, made basic with 5 mL of a 1 N NaOH solution, and then extracted with six 20 mL portions of dichloromethane. The combined dichloromethane layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford 0.324 g (87%) of amine **6** as a colorless oil, which was used without further purification: ^1H NMR (300 MHz, $CDCl_3$) δ 8.05 (br s, 1 H), 7.34 (s, 5 H), 5.14 (s, 2 H), 4.03 (m, 1 H), 3.38−3.36 (m, 1 H), 3.24−3.19 (m, 1 H), 3.01 (s, 3 H), 2.85−2.78 (m, 1 H), 2.60−2.56 (m, 1 H), 1.89−1.95 (m, 1 H), 1.77−1.46 (m, 5 H).

To a solution of amine **6** (0.324 g, 1.06 mmol) in 10 mL of methanol were added acetone (0.80 mL, 11 mmol) and sodium cyanoborohydride (0.065 g, 1.03 mmol). After 21 h, the colorless solution was concentrated by rotary evaporation to a colorless oil, which was partitioned between 20 mL of diethyl ether and 20 mL of a saturated aqueous NaCl solution. The aqueous layer was extracted with 20 mL of diethyl ether, and the combined ether layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford 0.364 g (98%) of amine **7** as a colorless oil, which was used without further purification: ^1H NMR (300 MHz, $CDCl_3$) δ 7.36 (appar s, 6 H), 5.16 (s, 2 H), 4.12 (br s, 1 H), 3.40 (br s, 1 H), 3.36−3.29 (m, 1 H), 3.03 (s, 3 H), 2.85 (br s, 2 H), 2.72 (appar d, J = 3.7 Hz, 2 H), 2.06−2.01 (m, 1 H), 1.83−1.80 (m, 1 H), 1.70−1.62 (m, 2 H), 1.08 (br s, 6 H).

A 1.93 M solution of phosgene in toluene (1.6 mL, 3.1 mmol) was added to a rapidly stirred, ice-cooled mixture of L-leucine methyl ester hydrochloride (0.271 g, 1.49 mmol), 10 mL of dichloromethane, and 10 mL of a saturated aqueous $NaHCO_3$ solution. The mixture was rapidly stirred for 20 min. The layers were separated, the aqueous layer was extracted with 10 mL of dichloromethane, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to a colorless oil (crude leucine methyl ester isocyanate). The oil was dissolved in 10 mL of dichloromethane, and the solution was added to amine **7** (0.364 g, 1.04 mmol). After 21 h, the colorless solution was concentrated by rotary evaporation, and the resultant oil was purified by column chromatography (2:1 EtOAc/hexanes) to afford 0.481 g (76% three steps) of **8** as a foamy white solid: IR (CH_2Cl_2) 3415, 3316, 1740, 1642 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.45 (br s, 1 H), 7.36 (s, 5 H), 6.96 (br s, 1 H), 5.19 (d, AB pattern, J = 11.5 Hz, 1 H), 5.14 (d, AB pattern, J = 11.8 Hz, 1 H), 4.42 (br s, 1 H), 4.37−4.35 (m, 1 H), 4.24 (br s, 1 H), 3.70 (s, 3 H), 3.45−3.33 (m, 2 H), 3.25 (br s, 1 H), 3.04 (s, 3 H), 2.70 (br s, 1 H), 2.20−2.13 (m, 1 H), 1.84−1.75 (m, 4 H), 1.58−1.44 (m, 2 H), 1.14 (appar d, J = 5.4 Hz, 3 H), 1.03 (br s, 3 H), 0.94 (appar d, J = 6.5 Hz, 3 H), 0.91 (m, 3 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 176.0, 161.7, 158.8, 155.1, 135.8, 128.5, 128.4, 128.2, 67.5, 58.7, 52.7, 51.9, 49.5, 46.6, 46.0, 40.3, 38.5, 31.1, 25.5, 24.8, 23.1, 21.5, 21.4, 20.2; HRMS (LSIMS) *m/e* for $C_{26}H_{42}N_5O_6$ $(M + H)^+$, calcd 520.3137, found 520.3134. Anal. Calcd for $C_{26}H_{41}N_5O_6$: C, 60.10; H, 7.95; N, 13.48. Found: C, 60.08; H, 7.94; N, 13.56.

Hairpin 1. Urea **8** (0.154 g, 0.297 mmol) was dissolved in 5 mL of a methylamine in methanol solution (10 M, 5 mmol). After 4 days, the colorless solution was concentrated by rotary evaporation to 0.151 g of a glassy solid, which was further purified by column chromatography (19:1 EtOAc/MeOH) to afford 0.143 g (93%) of **1** as a foamy white solid: IR (5 mM $CHCl_3$) 3448, 3303, 1735, 1660, 1645 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.86 (br s, 1 H), 7.36−7.30 (m, 5 H), 6.69 (s, 1 H), 6.38 (s, 1 H), 5.22 (d, AB pattern, J = 12.1 Hz, 1 H), 5.15 (d, AB pattern, J = 12.1 Hz, 1 H), 4.37−4.33 (m, 2 H), 4.20−4.10 (m, 1 H), 3.43 (br s, 1 H), 3.29−3.20 (m, 1 H), 3.23 (dd, J = 15.5 Hz, 6.8 Hz, 1 H), 3.07 (s, 3 H), 2.89 (appar d, J = 13.4 Hz, 1 H), 2.77 (d, J = 4.8 Hz, 3 H), 1.99−1.97 (m, 1 H), 1.84−1.79 (m, 2 H), 1.60−1.53 (m, 3 H), 1.53−1.46 (m, 1 H), 1.19 (d, J = 6.1 Hz, 3 H), 1.05 (d, J = 5.8 Hz, 3 H), 0.85 (d, J = 4.5 Hz, 3 H), 0.81 (d, J = 4.7 Hz, 3 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 175.2, 162.5, 159.2, 155.9, 135.9, 128.4, 128.2, 128.1, 67.2, 59.1, 53.2, 49.5, 47.1, 46.9, 40.7, 38.1, 29.6, 26.2, 25.4, 24.8, 22.9, 21.9, 21.1, 19.8; HRMS (LSIMS) *m/e* for $C_{26}H_{43}N_6O_5$ $(M + H)^+$, calcd 519.3298, found 519.3297. Anal. Calcd for $C_{26}H_{42}N_6O_5$: C, 60.21; H, 8.16; N, 16.20. Found: C, 60.03; H, 8.24; N, 16.12.

Control 10. A 1.93 M solution of phosgene in toluene (1.0 mL, 1.93 mmol) was added to a rapidly stirred mixture of hydrazine **2** (0.100 g, 0.461 mmol), 5 mL of dichloromethane, and 5 mL of a saturated aqueous $NaHCO_3$ solution. The mixture was vigorously stirred for 30 min. The layers were separated,

and the aqueous layer was further extracted with 5 mL of dichloromethane. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to a colorless liquid (carbamoyl chloride **3**). To the liquid was added 5 mL of dichloromethane, 5 mL of a saturated aqueous $NaHCO_3$ solution, and pyrrolidine (0.077 mL, 0.926 mmol). The mixture was vigorously stirred for 24 h, and then the layers were separated. The dichloromethane layer was sequentially washed with 5 mL of a 1 M HCl solution and 5 mL of a saturated aqueous NaCl solution, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford 0.106 g (83%) of **10** as a colorless oil, which slowly solidified to a white solid: mp 69−70 °C; IR (5 mM $CHCl_3$) 3427, 3377, 1741, 1645 cm^{-1}; ^1H NMR (500 MHz, 5 mM $CDCl_3$) δ 7.36 (5 H), 6.61 (br s, 0.6 H), 6.38 (br s, 0.4 H), 5.18 (br s, 2 H), 3.35 (br s, 4 H), 3.05 (s, 3 H), 1.78 (br s, 4 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 160.5, 155.4, 135.8, 128.5, 128.4, 128.2, 67.7, 67.5, 58.5, 48.0, 39.2, 25.4; HRMS (CI) *m/e* for $C_{14}H_{20}N_3O_3$ $(M + H)^+$, calcd 278.1506, found 278.1496. Anal. Calcd for $C_{14}H_{19}N_3O_3$: C, 60.63; H, 6.91; N, 15.15. Found: C, 60.91; H, 6.58; N, 15.11.

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Supporting Information Available: One-dimensional, COSY, and NOESY ^1H NMR spectra for hairpin **1** (7 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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