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Secondary Effects in Flexible Hydrogen Bonding Networks

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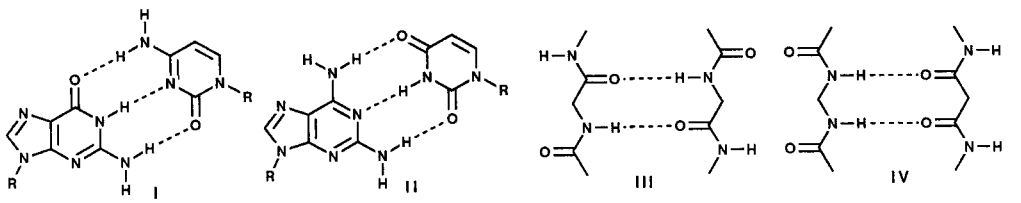
Abstract: Extension of Jorgensen's secondary interaction hypothesis to conformationally flexible systems has been examined. The results indicate that secondary interactions between covalently adjacent hydrogen bonding groups are as important as secondary interactions between hydrogen bonding groups brought together by the primary interactions. © 1997 Elsevier Science Ltd.

Physical organic chemists have traditionally devoted their attention to covalent bonding, particularly the manner in which covalent bonds are made and broken.[1] Increasing interest in biological chemistry, however, has evoked increasing interest in noncovalent attractions, since networks of noncovalent interactions often underlie structure and function in biomolecules. Dissecting noncovalent networks is challenging, because the energetic increments are often small, and because the chemist's eye is not yet trained to evaluate, or even recognize, all of the pertinent interactions.

In 1990, Jorgensen and Pranata pointed out a quandry in the literature on triply hydrogen bonded complexes between heterocycles: stability does not correlate with the number of hydrogen bonds.[2] For example, it has been known since the 1960s that hydrogen bonded pair **I**, composed of guanine and cytosine derivatives, is much more tightly associated in chloroform than is pair **II**, composed of uracil and 2-aminoadenine derivatives.[3] Jorgensen and Pranata rationalized this difference in affinities by focusing not on the "primary" hydrogen bonding interactions (dotted lines in **I** and **II**), but rather on the "secondary" interactions between hydrogen bond donors and acceptors.[2] These secondary interactions involve hydrogen bond donors and acceptors held near one another in the molecular complex, but not hydrogen bonded to one another. Since protons attached to nitrogen should be electron deficient (δ+) and the nitrogens and oxygens should be electron rich (δ-), one expects all four of the secondary interactions indicated in **II** to be electrostatically unfavorable, while two of the secondary interactions in **I** should be favorable and two unfavorable.

Subsequent papers from Jorgensen and co-workers, and from others, have shown that the secondary interaction hypothesis constitutes a powerful intellectual tool for evaluating relative strengths of hydrogen bonded heterocycle complexes. Rebek et al.,[4] and Jorgensen and Severance,[5] have reported that relative strengths of doubly hydrogen bonded complexes can be rationalized via the secondary interaction hypothesis, particularly if one takes account of "overhanging" groups, including polarized C-H units.

Zimmerman and co-workers have further extended the secondary interaction hypothesis by examining triply hydrogen bonded complexes in which all three donors occur on one heterocycle and all three acceptors occur on the other.[6] Jorgensen and Pranata predicted that such DDD-AAA complexes should be extremely stable, since all secondary interactions are favorable.[2] Zimmerman et al. found that this type of triply hydrogen bonded complex is indeed more favorable than related complexes with either the DDA-AAD or DAD-ADA pattern (e.g., **I** or **II**).[6]



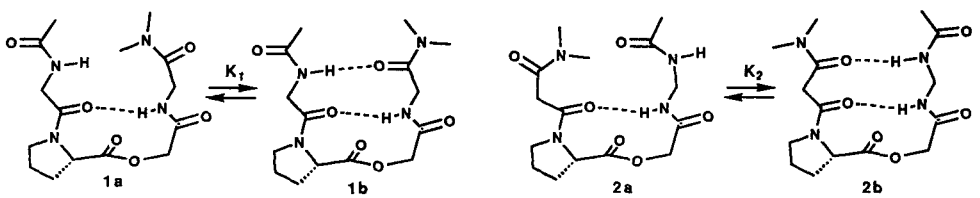
In originally formulating the secondary interaction hypothesis, Jorgensen and Pranata extended the concept to peptides and peptide-like molecules.[2a] Specifically, their calculations predicted that the doubly hydrogen bonded pairing of glycine derivatives, **III**, would be less favorable than the unsymmetrical doubly hydrogen bonded pairing of diaminomethane- and malonic acid-derived diamides, **IV**, when the individual molecules were constrained to remain planar. This prediction is intriguing in the context of drug design, since malonic acid and diaminomethane units are commonly found in "retro-inverso" peptidomimetics.[7] Complex **III** represents a hydrogen bonding pattern commonly found in antiparallel β-sheets of proteins; therefore, the considerably greater affinity predicted for **IV** is interesting in the context of designing unnatural polymers with well-defined folding patterns.[8]

We decided to compare hydrogen bonding patterns **III** and **IV** experimentally, in order to address the question left open by the calculations of Jorgensen and Pranata: does the large predicted difference in complex stabilities persist if the individual units are not constrained to be planar?[9] This question arises because the real molecules are conformationally mobile, and the unfavorable alignments of the C=O groups in the malonamide component of **IV** and the N-H groups in the diaminomethane component of **IV** would be expected to cause these molecules to prefer non-planar conformations. (These dipolar repulsions can be viewed as intramolecular analogues of the "secondary interactions" discussed above.) Thus, there should be an energetic cost associated with adoption of the planar conformations required for complex **IV**, and this cost will diminish the stability of the complex. No such conformational reorganization is required for formation of complexes between the rigid heterocycles in **I** and **II**.

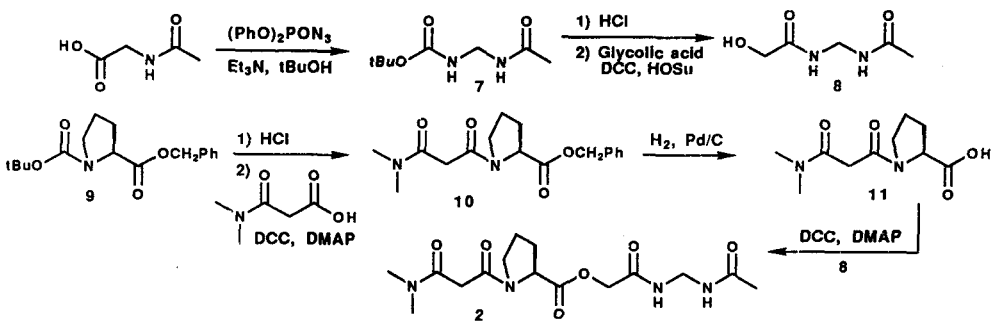
Results and Discussion

Experimental design. The doubly hydrogen bonded patterns illustrated in **III** and **IV** were compared in an intramolecular rather than intermolecular context, because of the potential difficulty of distinguishing self-association from heterogeneous complex formation in mixtures of the malonic acid- and diaminomethane-derived components of **IV**. In order to evaluate the relative stabilities of hydrogen bonding patterns **III** and **IV** by examining folding equilibria, we required a covalent link between the two

diamide fragments that would enforce one of the two hydrogen bonds, and allow but not enforce the other. The prolyl-glycolyl depsipeptide unit appears to meet this requirement, since Marraud et al. have shown that the β-turn-like 10-membered ring hydrogen bond available to this fragment is almost completely populated in organic solvents of low polarity.[10] (We have previously used this observation to develop a model system for minimal β-hairpin formation.[11]) We prepared **1** and **2**, with the expectation that in dilute methylene chloride solution the behavior of these molecules would conform largely to the two-state equilibria shown below. (Compound **2** was synthesized as outlined in Scheme 1.) Concentration-dependent ^{1}H NMR studies (0.05 to 50 mM) showed that these molecules do not aggregate at or below 1 mM in methylene chloride.[9]



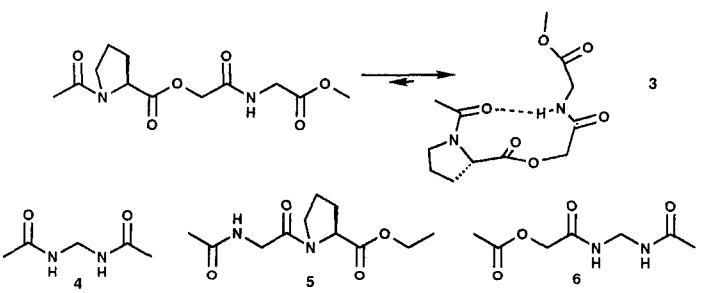
Scheme 1



Qualitative analysis of intramolecular hydrogen bonding via IR spectroscopy.[12,13] IR data from the N-H stretch region provided insight on the hydrogen bonding patterns in **1**, **2** and reference compounds **3-6**. Each sample was 1 mM, to ensure that no aggregation occured. Compound **3** can experience only one intramolecular amide-amide hydrogen bond, the 10-membered ring interaction that we expect to characterize all major folding patterns of **1** and **2** in methylene chloride solution. The data for **3** indicate that this molecule is indeed largely folded to form the 10-membered ring hydrogen bond, since the major band occurs at 3318 cm^{-1}.[9] Depsipeptide **3** also displays a small band at 3430 cm^{-1}, which is a bit low for an N-H completely free of hydrogen bonding in CH_2Cl_2 (expected in the range 3440-3460 cm^{-1}). The band at 3430 cm^{-1} presumably results from the N-H engaged in a weak interaction with the C-terminal ester carbonyl, a so-called "C_5 interaction."[14] Whether or not the C_5 interaction constitutes a true hydrogen bond is unclear, because the geometry is extremely poor (N-H--O angle around 100°), but small

shifts in N-H bands of the type seen for **3** (relative to non-hydrogen bonded N-H) suggest that there is a modest N-H--O=C attraction within the five-membered ring.

Diamide **4**[15] displays a single band, at 3441 cm^{-1}, indicating that there is no six-membered ring hydrogen bonding.[9] This result suggests that there should be no six-membered ring hydrogen bonding within the diaminomethane unit of **2**. Glycyl-proline dipeptide **5** represents the N-terminal half of **1**, and shows a major band at 3410 cm^{-1}, along with a small shoulder at 3448 cm^{-1} (Figure 1). The minor 3448 cm^{-1} absorbance is assigned to non-hydrogen bonded N-H stretch, and the major 3410 cm^{-1} is assigned to N-H engaged in a C_5 interaction. This assignment is supported by the observation that N-acetyl-glycine-dimethylamide displays a major band at 3406 cm^{-1} under identical conditions.[12d] The 3410 cm^{-1} band of **5** is lower than the band attributed to the C_5 interaction in **3** because the acceptor in **3** is an ester carbonyl, while the C_5 acceptor in **5** is an amide carbonyl, and amides are intrinsically superior to esters as hydrogen bond acceptors.



For both **1** and **2**, the major N-H stretch band occurs in the range 3330-3340 cm^{-1} (Figure 1), which can be assigned to N-H involved in a typical amide-amide hydrogen bond.[12,13] Compound **1** displays an additional shoulder at 3404 cm^{-1}, which may be assigned to N-H engaged in a C_5 interaction. Compound **2** displays an additional band at 3441 cm^{-1}, which may be assigned to non-hydrogen bonded N-H (this band appears at the same position as the lone band for reference compound **4**). Our two-state folding hypothesis for **1** predicts that the high energy shoulder should arise from the N-terminal N-H (folding pattern **1a**), and our two-state folding hypothesis for **2** predicts that the high energy band should arise from the outer N-H (folding pattern **2a**). These predictions were tested by preparing versions of **1** and **2** site-specifically labelled with ^{15}N. For a localized N-H unit, the standard calculation predicts that the ^{15}N-H stretch band will appear ca. 12 cm^{-1} lower that the ^{14}N-H stretch band.[16] When the N-terminal glycine residue of **1** is ^{15}N-labelled, the minor N-H stretch band shifts to 3399 cm^{-1} (from 3404 cm^{-1} in unlabelled **1**). This minor band appears at 3406 cm^{-1} in **1** that has the C-terminal glycine labelled (the nominal resolution of these spectra is ± 2 cm^{-1}.) These data indicate that the minor band arises from N-terminal N-H engaged in a C_5 interaction, but not in a "normal" amide-amide hydrogen bond, which supports our two-state conformational hypothesis for **1**. For **2**, only the "outer" N-H was ^{15}N labelled, and this modification caused the non-hydrogen bonded N-H stretch band to shift from 3441 to 3434 cm^{-1}. This result supports our two-state conformational hypothesis for **2**.

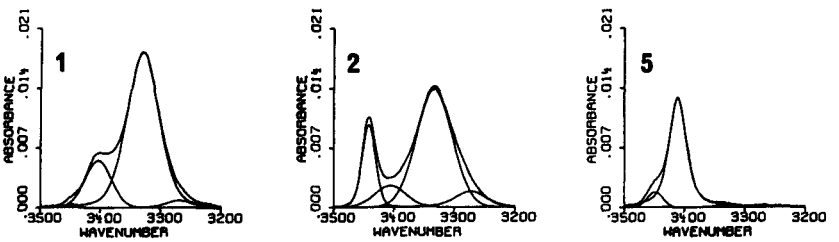


Figure 1. N-H stretch FT-IR data for 1 mM samples in CH_2Cl_2 at rm temp, after subtraction of the spectrum of pure CH_2Cl_2 (nominal resolution = 2 cm^{-1}): **1**, maxima at 3404 (shoulder) and 3328 cm^{-1}; **2**, maxima at 3441 and 3332 cm^{-1}; **5**, maxima at 3448 (shoulder) and 3410 cm^{-1}. In each case, bands implied by curve fitting analysis (ref. 12d) are also shown.

Quantitative analysis of infrared data. It should be possible to estimate equilibrium constant K_1 by determining the amount of amide N-H engaged in a C_5 interaction rather than in a strong intramolecular hydrogen bond in a dilute solution of **1**. This amount provides the concentration of folding pattern **1a**, and if the total concentration of **1** is known, then that portion of the total not accounted for as **1a** can be attributed to **1b**. Similarly, determination of the amount of non-hydrogen bonded N-H in a dilute solution of **2** should provide K_2. We have previously shown that two-state hydrogen bonding equilibria experienced by di- and triamides can be analyzed by determining the concentration of non-hydrogen bonded N-H, and that the results agree reasonably well with those obtained from independent NMR analysis.[12a,d]

IR-based quantification of hydrogen bonding equilibria depends upon the availability of reliable extinction coefficients for individual N-H stretch bands. Such extinction coefficients are often accessible for non-hydrogen bonded N-H, because it is easy to examine simple monoamides at sufficient dilution to preclude hydrogen bonding. Quantification based on *hydrogen bonded* N-H bands, however, is generally not possible, because it is difficult to identify fully hydrogen bonded model compounds. Further, the extinction coefficient of a hydrogen bonded N-H band is expected to depend upon the details of hydrogen bond geometry, while the extinction coefficient of a non-hydrogen bonded N-H band should be less sensitive to structural details.

Reference compound **4** should provide the integrated extinction coefficient required for determining the concentration of non-hydrogen bonded N-H in dilute solutions of **2**. The ideal reference compound for **1** would have an N-H group completely engaged in the C_5 interaction. We have not found such a compound, and we therefore employed **5** in order to estimate the required extinction coefficient. Visual inspection of the N-H region spectrum of **5** in Figure 1 reveals a minor shoulder at 3448 cm^{-1}, as discussed above. Mathematical decomposition of this spectrum (Figure 1) suggests that presence of a third, extremely weak band at 3330 cm^{-1}. We used the mathematically isolated band 3411 cm^{-1} to estimate the integrated extinction coefficient to be used for analysis of **1**. This approach provides a value that is slightly too high, and will lead us to overestimate the amount of N-H corresponding to the 3404 cm^{-1} band in the spectrum of **1**. (As will be seen below, this turns out to be a conservative error.)

Mathematical decomposition of the N-H stretch region IR spectrum for **1** (Figure 1) shows, in addition to the major band at 3330 cm^{-1} and the minor band at 3404 cm^{-1}, very small bands at 3447 cm^{-1} (presumably non-hydrogen bonded N-H) and 3270 cm^{-1} (possibly some type of hydrogen bonded N-H). Use of the integrated extinction coefficient derived from **5** suggests that 60±8% of the N-terminal N-H is engaged in a C_5 interaction (folding pattern **1a**) rather than in the 14-membered ring hydrogen bond (folding pattern **1b**). (The uncertainty is the standard deviation derived from multiple measurements.) If we assume that only folding patterns **1a** and **1b** are populated, then we conclude that K_1 is 0.67±0.13.

Mathematical decomposition of the N-H stretch region IR spectrum of **2** (Figure 1) indicates, in addition to the major bands at 3442 and 3337 cm^{-1}, the presence of minor but significant bands at 3411 and 3332 cm^{-1}. The origins of these minor bands are unclear. The latter could arise from some sort of amide-amide hydrogen bonded N-H. The 3411 cm^{-1} band is in the region attributable to C_5 interations, but none is possible in this molecule. The 3411 cm^{-1} band could also arise from N-H--O=C hydrogen bonding involving the ester carbonyl;[12e] however, the N-H stretch region IR spectrum of compound **6** is identical to that of **4**, which indicates that no N-H--O=C(ester) hydrogen bonding occurs in **6**. In any case, the presence of these minor bands in the N-H region IR spectrum of **2** does not undermine our previous conclusion that there are only two major folding patterns in CH_2Cl_2. Use of the integrated extinction coefficient derived from **4** suggests that 70±5% of the outer N-H is not hydrogen bonded (folding pattern **2a**) in dilute solutions of **2**. If we assume that only folding patterns **2a** and **2b** are populated, then we conclude that K_2 is 0.44±0.05. Given the uncertainties associated with our estimations of K_1 and K_2, it is most conservative to conclude that the doubly hydrogen bonded folding patterns (**1b** and **2b**) have similar stabilities.

Conclusions. The secondary interaction hypothesis predicts that hydrogen bonding pattern **III** is less stable than **IV**, if the constituent diamides are forced to remain planar.[2a] We have compared these two hydrogen bonding patterns experimentally in an intramolecular setting (K_1 vs. K_2), and the most conservative interpretation of our results is that the two hydrogen bonding patterns are similar in stability. (K_1 may actually be slightly larger than K_2.) The similarity of K_1 and K_2 can be rationalized by noting that adoption of the requisite planar conformations by diamides derived from malonic acid or diaminomethane (i.e., the components of **IX**, or the subunits of **2**) causes dipolar repulsion between the parallel adjacent C=O or N-H groups. These flexible diamide units are therefore expected to prefer non-planar conformations.[18] The energetic cost of the internal dipolar repulsions in the planar conformations of the malonic acid- and diaminomethane-derived units is presumably analogous to the cost of "secondary" dipolar repulsions between the C=O and N-H groups on separate glycine fragments in complex **III** and folding pattern **1b**.

Experimental Section

General. All reagents used in the synthesis of **1-11** are commercially available. Anhydrous solutions of HCl (4 N) in dioxane were purchased from Pierce. Anhydrous CH_2Cl_2 and *t*-BuOH were obtained by distillation from CaH_2. All other solvents used were reagant grade except for hexane, which

was purified by distillation. Anyhydrous reaction conditions were maintained under a slightly positive nitrogen atmosphere in oven dried glassware. All silica gel chromatography was performed using 230-400 mesh silica purchased from EM Science. Routine ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AM-300 spectrometer at 300.132 Mhz and 75.033 Mhz frequencies, respectively. All NMR spectra were reference to TMS or to the isotopic impurity peak of $CDCl_3$. Routine infrared spectra were obtained on a Nicolet 680 FT-infrared spectrometer. High resolution electron impact ionization mass spectroscopy was performed using a Kratos MS-25 spectrometer.

IR Studies Infrared spectra were obtained at 2 cm^{-1} resolution and 24 °C using a 1 mm CaF_2 solution cell and Nicolet 740 FT-infrared spectrometer. IR spectra were 1 mM solutions in anhydrous CH_2Cl_2. Compounds were dried *in vacuo* at elevated temperatures and in the presence of P_2O_5. CH_2Cl_2 was distilled from CaH_2 and stored over 4 Å molecular sieves. Sample preparation was performed in a nitrogen atmosphere. See refs. 8a, 12a, 12d and 12e for further details.

Compound 2 was prepared as outlined in Scheme 1. The synthesis is described below in detail, and the various steps are representative of the methods used to prepare compounds **1** and **3-6**.

A solution of acetylglycine (0.60 g, 5.13 mmol) in anhydrous *tert*-butanol was treated with diphenylphosphorylazide (1.79 g, 6.50 mmol) and triethylamine (13.0 mmol). The reaction was refluxed 24 hr under nitrogen.[19] The *t*-butanol was removed *in vacuo* and the resulting residue was purified by silica gel chromatography eluting with ethyl acetate. Purification yielded **7** as a white solid (0.36 g, 41%). **M.P.** (recryst. from ethyl acetate and hexane) 82-84 °C; **^{1}H-NMR** ($CDCl_3$/TMS, ppm): 1.44 (s, 9H, CH_3), 1.99 (s, 3H, CH_3C=O), 4.49 (t, J = 6.22 Hz, 2H, NCH_2N), 5.66 (broad, 1H, $NHCO_2$-), 6.73 (broad, 1H, NHC=O); **^{13}C-NMR** ($CDCl_3$, ppm): 22.9 ($\underline{C}H_3$), 28.2 (3 x CH_3), 45.9 ($N\underline{C}H_2N$), 79.9 ($\underline{C}(CH_3)_3$), 156.1 ($N\underline{C}(O)O$-), 171.1 ($\underline{C}$=O); **IR** (neat, cm^{-1}): 3356, 1693, 1660, 1525; **MS** (EI, m/z): 87.0577 (Calc. for $C_8H_{16}N_2O_5$ - $C_5H_9O_2$ 87.0558).

An anhydrous solution of **7** (0.35 g, 1.86 mmol) and HCl (12 mmol) in dioxane (3 ml) was stirred under nitrogen for four hr at 20 °C. The solution was concentrated to a white solid under a stream of nitrogen and placed *in vacuo* for 1 hr. The solid was dissolved in DMF (7 ml) and this solution was treated with glycolic acid (0.17 g, 2.20 mmol), O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) (0.83 g, 2.20 mmol) and triethylamine (0.40 g, 4.0 mmol). The solution was stirred overnight. The DMF was removed *in vacuo*, and the resulting residue was purified by silica gel chromatography eluting with methanol (10%, v/v) in chloroform. Purification yielded **8** as a white solid (0.18 g, 64%). **M.P.** (after recrystallization from ethyl acetate and hexane): 120-122 °C; **^{1}H-NMR** ($CDCl_3$/TMS, ppm): 1.99 (s, 3H, CH_3C=O), 4.13 (s, 2H, NCH_2N), 4.67 (t, J = 6.12 Hz, 2H, OCH_2), 6.57 (broad, 1H, NHC=O), 6.57 (broad, 1H, NHC=O); **^{13}C-NMR** ($CDCl_3$, ppm): 21.3 ($\underline{C}H_3$), 43.1 ($N\underline{C}H_2N$), 60.8 ($O\underline{C}H_2$), 172.2, 173.7 ($\underline{C}$=O); **IR** (neat, cm^{-1}): 3285, 1675, 1653, 1506; **MS** (EI, m/z): 146.0691 (Calc. for $C_5H_{16}N_2O_3$ 146.0691).

A solution of N-(*tert*-butoxycarbonyl)-proline (1.00 g, 4.65 mmol) in anhydrous CH_2Cl_2 (23 ml) was treated with dicyclohexylcarbodiimide (1.15 g, 5.60 mmol), N,N-dimethylaminopyridine (68 mg, 0.56 mmol) and benzyl alcohol (1.08 g, 10 mmol) and the mixture was stirred for 4 hr. The reaction mixture

was cooled to -30 °C, suction-filtered, and the filtrate was concentrated. The resulting residue was purified by silica gel chromatography eluting with ethyl acetate (20%, v/v) in hexane yielding **9** as a colorless oil (1.28 g, 90 %) **^{1}H-NMR** ($CDCl_3$/TMS, ppm): 1.35, 1.46 (2 x s, 9H, CH_3), 1.81-2.23 (m, 4H, β- and γ- CH_2), 3.40-3.60 1 (m, 2H, δ-CH_2), 4.27 (dd, J = 3.8 Hz, J= 8.6 Hz, 0.70H, α-CH), 4.38 (dd, J = 3.3 Hz, J = 8.4 Hz, 0.30H, α-CH), 5.08, 5.33 (AB quartet, J_{ab} = 12.44 Hz, 0.60H, OCH_2), 5.13, 5.19 (AB quartet, J_{ab} = 12.44 Hz, 1.4H, OCH_2), 7.29-7.35 (m, 5H, phenyl-CH); **^{13}C-NMR** ($CDCl_3$, ppm): 23.4 (γ- $\underline{C}H_2$), 28.0 ($\underline{C}H_3$), 30.7 (β-$\underline{C}H_2$), 46.1 (δ-$\underline{C}H_2$), 59.0 (α-$\underline{C}H$), 66.4 (O$\underline{C}H_2$), 79.7 ($\underline{C}(CH_3)_3$), 127.8, 127.9, 128.1, 128.3, 128.4, 135.4 (phenyl-$\underline{C}H$), 153.6 (N-$\underline{C}$(O)-O), 172.8 ($\underline{C}$=O); **IR** (neat, cm^{-1}): 1751, 1699, 1396, 1164; **MS** (EI, m/z): 305.1612 (Calc. for $C_{17}H_{23}NO_4$ 300.1627).

An anhydrous solution of **9** (1.00 g, 3.27 mmol) and HCl (12 mmol) in dioxane (3 ml) was stirred under nitrogen for four hr at 20 °C. The solution was concentrated to a white solid under a stream of nitrogen and placed *in vacuo* for 1 hour. N,N-dimethylamidomalonic acid[12a] (0.46 g, 3.50 mmol), dicyclohexylcarbodiimide (0.74 g, 3.60 mmol), N,N-dimethylaminopyridine (44 mg, 0.36 mmol), triethylamine (0.56 g, 5.50 mmol) and anhydrous CH_2Cl_2 (21 ml) were added and the mixture was stirred for 4 hr. The reaction mixture was cooled to -30 °C, suction-filtered, and the filtrate was concentrated. The resulting residue was purified by silica gel chromatography eluting with ethyl acetate (30%, v/v) in hexane yielding **10** as a colorless oil (0.71, 70%). **^{1}H-NMR** ($CDCl_3$/TMS, ppm): 1.80-2.35 (m, 4H, β- and γ- CH_2), 2.91, 2.94, 2.97, 3.04 (4 x s, 6H, NCH_3), 3.48, 3.57 (AB quartet, J_{ab} = 14.58 Hz, 2H, C(O)-CH_2), 3.65-3.75 (m, 2H, δ-CH_2), 4.50-4.75 (m, 1H, α-CH), 5.12 (AB quartet, J_{ab} = 12.28 Hz, 2H, OCH_2), 7.29-7.35 (m, 5H, phenyl-CH); **^{13}C-NMR** ($CDCl_3$, ppm): 24.6 (γ- $\underline{C}H_2$), 29.1 (β-$\underline{C}H_2$), 42.0 (C(O)-$\underline{C}H_2$), 47.5 (δ-$\underline{C}H_2$), 58.8 (α-$\underline{C}H$), 66.6 (O$\underline{C}H_2$), 127.8, 128.0, 128.3, 128.5, 128.6, 135.5 (phenyl-$\underline{C}H$), 165.6, 166.3, 171.7 ($\underline{C}$=O); **IR** (neat, cm^{-1}): 1743, 1652, 1420, 1173; **MS** (EI, m/z): 318.1591 (Calc. for $C_{17}H_{22}N_2O_4$ 300.1580).

A solution of **10** (1.0 g, 3.14 mmol) in methanol (60 ml) was mixed with 5% Pd/C (25 mg). A high pressure glass bottle containing this mixture was purged four times each with nitrogen gas (40 psi) followed by H_2 (40 psi). The reaction mixture was shaken for 24 hr under a H_2 (25 psi). The activated carbon was removed by filtration through celite, and the resulting filtrate was concentrated to a colorless oil. The oil was dried *in vacuo* for 4 hours yielding **11** as a white solid (0.67 g, 95%). **M.P.** (recryst. from ethyl acetate and hexane): 96-98 °C; **^{1}H-NMR** ($CDCl_3$/TMS, ppm): 1.90-2.30 (m, 4H, β- and γ- CH_2), 2.98, 2.99, 3.07, 3.10 (4 x s, 6H, NCH_3), 3.55, 3.57 (2 x s, 2H, C(O)-CH_2), 3.50-3.70 (m, 2H, δ-CH_2), 4.57 (m, 1H, α-CH), 8.10 (broad, 1H, COOH); **^{13}C-NMR** ($CDCl_3$, ppm): 24.4 (γ- $\underline{C}H_2$), 28.3 (β-$\underline{C}H_2$), 35.4, 37.6 (N$\underline{C}H_3$), 41.2 (C(O)-$\underline{C}H_2$), 47.9 (δ-$\underline{C}H_2$), 59.4 (α-$\underline{C}H$), 166.6, 168.5, 171.5 ($\underline{C}$=O); **IR** (neat, cm^{-1}): 1741, 1648, 1428, 1190; **MS** (EI, m/z): 228.1099 (Calc. for $C_{10}H_{16}N_2O_4$ 228.1110).

A solution of **11** (0.11 g, 0.48 mmol) in anhydrous CH_2Cl_2 (5 ml) was treated with dicyclohexylcarbodiimide (0.11 g, 0.53 mmol), N,N-dimethylaminopyridine (20 mg, 0.16 mmol) and **8** (0.110 g, 0.69 mmol), and the mixture was stirred for 4 hr. The reaction mixture was cooled to -30 °C, suction-filtered, and the filtrate was concentrated. The resulting residue was purified by silica gel chromatography eluting with methanol (10%, v/v) in chloroform yielding **2** as a white, crystalline solid

(0.11 g, 66%). **M.P.** (recryst. from ethyl acetate and hexane) 49-51 °C; **^{1}H-NMR** ($CDCl_3$/TMS, ppm): 1.96 (s, 3H, CH_3), 2.00-2.40 (m, 4H, β- and γ- CH_2), 3.01, 3.10 (2 x s, 6H, NCH_3), 3.63, 3.50 (AB quartet, J_{ab} = 14.97 Hz, 2H, C(O)-CH_2), 4.50 (m, 2H, δ-CH2), 4.68 (m, 2H, NCH_2N), 4.75, 4.58 (AB quartet, J_{ab} = 15.74 Hz, 2H, C(O)-CH_2), 6.72, 8.32 (broad, 1H, NH); **^{13}C-NMR** ($CDCl_3$, ppm): 23.0 (C(O)$\underline{C}H_3$), 25.0 (γ- $\underline{C}H_2$), 29.2 (β-$\underline{C}H_2$), 35.5, 35.7 (N$\underline{C}H_3$), 41.3 (C(O)-$\underline{C}H_2$), 44.3 (N$\underline{C}H_2$N), 47.7 (δ-$\underline{C}H_2$), 59.3 (α-$\underline{C}$H), 62.8 (O$\underline{C}H_2$), 166.3, 166.6, 168.5, 169.8, 170.9 ($\underline{C}$=O); **IR** (neat, cm^{-1}): 3495, 3290, 1750, 1645, 1434, 1173; **MS** (EI, m/z): 356.1682 (Calc. for $C_{15}H_{24}N_4O_6$ 356.1696).

Compound 1. M.P. (recryst. from ethyl acetate and hexane): 142-144 °C; **^{1}H-NMR** ($CDCl_3$/TMS, ppm): 2.09 (s, 3H, CH_3), 1.90-2.45 (m, 4H, β- and γ- CH_2), 3.00, 3.03 (2 x s, 6H, NCH_3), 3.61 (m, 2H, d-CH_2), 4.36, 3.87, 4.34, 3.86 (ABX, J_{ab} = 17.66 Hz, J_{ax} = 6.07 Hz, J_{bx} = 2.76 Hz, 2H, NCH_2), 4.48, 3.98, 4.46, 3.97 (ABX, J_{ab} = 17.27 Hz , J_{ax} = 6.62 Hz, J_{bx} = 2.98 Hz, 2H, NCH_2), 6.20, 3.94 (AB quartet, Jab = 15.74 Hz, 2H, CH_2), 4.56 (dd, J = 8.06 Hz, 4.61 Hz, 1H, α-CH), 7.50 (broad, 1H, NH), 7.68 (broad, 1H, NH); **^{13}C-NMR** ($CDCl_3$, ppm): 22.6 ($\underline{C}H_3$C=O), 24.9 (γ- $\underline{C}H_2$), 28.8 (β-$\underline{C}H_2$), 35.5, 36.1 (N$\underline{C}H_3$), 40.6, 42.0 (N$\underline{C}H_2$), 46.1 (δ-$\underline{C}H_2$), 59.3 (α-$\underline{C}$H), 62.7 (O$\underline{C}H_2$), 166.8, 167.9, 168.3, 170.4, 170.4 ($\underline{C}$=O); **IR** (neat, cm^{-1}): 3491, 3317, 2943, 1751, 1654, 1551, 1441, 1171; **MS** (EI, m/z): 356.1668 (Calc. for $C_{15}H_{24}N_4O_6$ 356.1666).

Compound 3. ^{1}H-NMR ($CDCl_3$/TMS, ppm): 1.90-2.30 (m, 4H, β- and γ- CH_2), 2.11 (s, 3H, CH_3), 3.55-3.78 (m, 2H, δ-CH_2), 3.73 (s, 3H, OCH_3), 4.05 (m, 2H, NCH_2), 4.45 (m, 1H, α-CH), 4.57, 5.01 (AB quartet, J_{ab} = 15.55 Hz, 2H, OCH_2), 7.81 (broad, 1H, NH); **^{13}C-NMR** ($CDCl_3$, ppm): 22.0 ($\underline{C}H_3$), 24.9 (γ- $\underline{C}H_2$), 29.2 (β-$\underline{C}H_2$), 40.5 (N$\underline{C}H_2$), 47.9 (δ-$\underline{C}H_2$), 51.9 (OCH_3), 58.8 (α-$\underline{C}$H), 62.5 (O$\underline{C}$H2), 167.6, 169.7, 170.3 171.3 (C=O); **IR** (neat, cm^{-1}): 3304, 1751, 1693, 1628, 1171; **MS** (EI, m/z): 286.1170 (Calc. for $C_{12}H_{18}N_2O_6$ 286.1164).

Compound 5. M.P. (recryst. from ethyl acetate and hexane): 47-49 °C; **^{1}H-NMR** ($CDCl_3$/TMS, ppm): 1.28 (t, J = 7.00 Hz, 3H, CH_3), 1.95-2.35 (m, 4H, β- and γ- CH_2), 2.17 (s, 3H, CH_3), 3.50-3.70 (m, 2H, δ-CH_2), 4.12 (m, 2H, NCH_2), 4.36 (d, J = 7.00 Hz, 2H), 4.53 (m, 1H, α-CH), 6.57 (broad, 1H, NH); **^{13}C-NMR** ($CDCl_3$, ppm): 13.9 ($\underline{C}H_3$), 22.6 ($\underline{C}H_3$), 24.3 (γ- $\underline{C}H_2$), 28.8 (β-$\underline{C}H_2$), 41.8 (N$\underline{C}H_2$), 45.8 (δ-$\underline{C}H_2$), 58.8 (α-$\underline{C}$H), 61.7 (O$\underline{C}H_2$), 79.4 ($\underline{C}(CH_3)_3$), 166.9, 170.0, 171.5 ($\underline{C}$=O); **IR** (neat, cm^{-1}): 3323, 2982, 1744, 1660, 1441, 1190; **MS** (EI, m/z): 242.1270 (Calc. for $C_{11}H_{18}N_2O_4$ 242.1267).

Compound 6. M.P. (recryst. from ethyl acetate and hexane): 154-156 °C; **^{1}H-NMR** ($CDCl_3$/TMS, ppm): 2.00 (s, 3H, CH_3C=O), 2.17 (s, 3H, CH_3CO_2), 4.56 (s, 2H, OCH_2), 4.65 (t, J = 6.22 Hz, 2H, NCH_2N), 6.99, 7.42 (broad, 1H, NHC=O); **^{13}C-NMR** ($CDCl_3$, ppm): 20.4, 22.7 ($\underline{C}H_3$), 44.0 (N$\underline{C}H_2$N), 62.5 (O$\underline{C}H_2$), 168.2, 169.3, 171.1 ($\underline{C}$=O); **IR** (neat, cm^{-1}): 3298, 1751, 1648, 1551; **MS** (EI, m/z): 188.0796 (Calc. for $C_7H_{12}N_2O_4$ 188.0797).

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References and Notes

1. See, for example: Doering, W. vE.; Roth, W. R. *Tetrahedron* **1962**, *18*, 67.
2. (a) Jorgensen, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008. (b) See also: Pranata, J.; Wierschke, S. G.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810.
3. (a) Kyogoku, Y.; Lord, R. C.; Rich, A. *Proc. Natl. Acad. Sci. USA* **1967**, *57*, 250. (b) Kyogoku, Y.; Lord, R. C.; Rich, A. *Biochim. Biophys. Acta* **1969**, *179*, 10.
4. Jeong, K. S.; Tjivikua, T.; Muehldorf, A.; Deslongchamps, G.; Famulok, M.; Rebek, J. *J. Am. Chem. Soc.* **1991**, *113*, 201.
5. Jorgensen, W. L; Severance, D. L. *J. Am. Chem. Soc.* **1991**, *113*, 209.
6. (a) Murray, T. J.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010. (b) Murray, T. J.; Zimmerman, S. C.; Kolotuchin, S. V. *Tetrahedron* **1995**, *51*, 635. (c) See also: Sartorius, J.; Schneider, H.-J. *Chem. Eur. J.* **1996**, *2*, 1446.
7. Goodman, M.; Chorev, M. *Acc. Chem. Res.* **1993**, *26*, 266, and references therein.
8. (a) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054. (b) Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 446. (c) Gennari, C.; Salom, B.; Potenza, D.; Williams, A. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2067.
9. A preliminary account of this work has appeared: Gardner, R. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 10411.
10. Boussard, G.; Marraud, M.; Neel, J.; Maigret, B.; Aubry, A. *Biopolymers* **1977**, *16*, 1033.
11. (a) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4105. (b) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975.
12. For previous studies from our laboratory involving qualitative and quantitative interpretation of N-H stretch region IR data, see: (a) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164. (b) Liang, G.-B.; Rito, C. J.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 4440. (c) Liang, G.-B.; Desper, J. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 925. (d) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4228. (e) Gallo, E. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 9774. (f) Ref. 8a.
13. For other studies involving qualitative and quantitative interpretation of N-H stretch region IR data, see: (a) Nowick, J. S.; Abdi, M.; Bellamo, K. A.; Love, J. A.; Martinez, E. J.; Noronha, G.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 89, and references therein. (b) Tsang, K. Y.; Kiaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988, and references therein. (c) Winningham, M. J.; Sogah, D. Y. *J. Am. Chem. Soc.* **1994**, *116*, 11173. (d) Gung, B. W.; Zhu, Z. *J. Org. Chem.* **1996**, *61*, 6482. (e) Yang, D.; Ng, F.-F.; Li, Z.-J.; Wu, Y.-D.; Chan, K.; Wang, D.-P. *J. Am. Chem. Soc.* **1996**, *118*, 9794.
14. (a) Avignon, M.; Huong, P. V.; Lascombe, J.; Marraud, M.; Neel, J. *Biopolymers* **1969**, *8*, 69. (b) Burgess, A. W.; Scheraga, H. A. *Biopolymers* **1973**, *12*, 2177.
15. Dado, G. P., Ph.D. Thesis, University of Wisconsin - Madison, 1993.
16. For a localized A-B stretch, the band position can be estimated from the equation: $\nu = (2\pi c)^{-1}[k(M_A + M_B)/M_A M_B]^{1/2}$ where c is the speed of light, k is the force constant of the A-B bond, M_A is the mass of atom A and M_B is the mass of atom B. (Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 5th Ed.; John Wiley & Sons: New York, 1991, p. 93).
17. For interpretation of $\Delta\delta NH/\Delta T$ data obtained in nonpolar solvents, see: (a) Ref. 12a. (b) Stevens, E. S.; Sugawara, N.; Bonara, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048.
18. (a) Stern, P. S.; Chorev, M.; Goodman, M.; Hagler, A. T. *Biopolymers* **1983**, *22*, 1901. (b) Tereshko, V.; Navarro, E.; Puiggali, J.; Subirana, J. A. *Macromolecules* **1993**, *26*, 7024.
19. Ninomiya, K.; Shiori, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.

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