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Enhanced Intramolecular Amide-Amide Hydrogen Bonding Through Cooperativity

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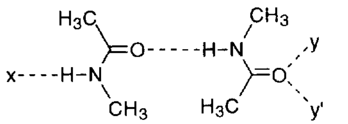
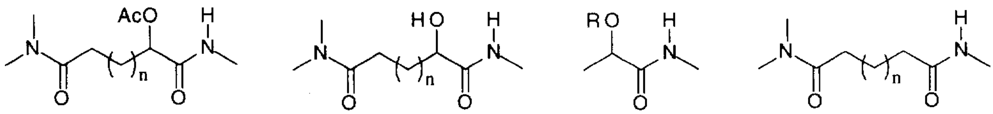
Summary: Model peptides **1-6** are prepared and studied by IR and variable temperature ^{1}H NMR spectroscopies. The α-hydroxy diamides **3** and **4** form intramolecular amide-amide hydrogen bond through an eight-membered and a nine-membered ring, respectively. When compared to Gellman's simple diamides of same chain length, the enhanced hydrogen bond strength is considered as an indication of a cooperative effect.

Recently, Karplus reported a theoretical study of cooperative effect on solvent influence on the stability of the peptide hydrogen bond.[1] It was found that a variety of solvents (water, ethanol, ethylene glycol, and trifluoroethanol) strengthen amide-amide hydrogen bonding. This cooperative stabilizing effect ranges from 0.8 (y = water) to 4.5 kcal/mol (x = y' = formamide, y = water). This study is important



because there is considerable uncertainty with regard to the contribution of hydrogen bonding to the stability of proteins.[2-6] Although the existence of a cooperative effect in peptide hydrogen bonding has been discussed in earlier work,[7] both theoretical and experimental elucidation of this effect have been limited.[8,9]

We wish to report a model system, in which the cooperative effect is demonstrated through an intramolecular hydrogen bonding network. The spectroscopy (IR and ^{1}H NMR) studies of model peptides **1-6** [10] are carried out and compared to the results reported by Gellman on simple diamides **7** and **8**.[12]



1 (n = 1), **2** (n = 2) **3** (n = 1), **4** (n = 2) **5** (R = Ac) **6** (R = H) **7** (n = 1), **8** (n = 2)

Intramolecular amide-amide hydrogen bonding can be formed through either an eight-membered (e.g. **1** and **3**) or a nine-membered ring (**2** and **4**). Diamides **3** and **4** have a free α-hydroxy group, which can form a five-membered ring through hydrogen bond to the amide carbonyl group. Monoamides **5** and **6** are prepared to serve as control compounds since they cannot form intramolecular amide-amide hydrogen bond.

A series of solutions with different concentrations of the model amides in CH_2Cl_2 were prepared

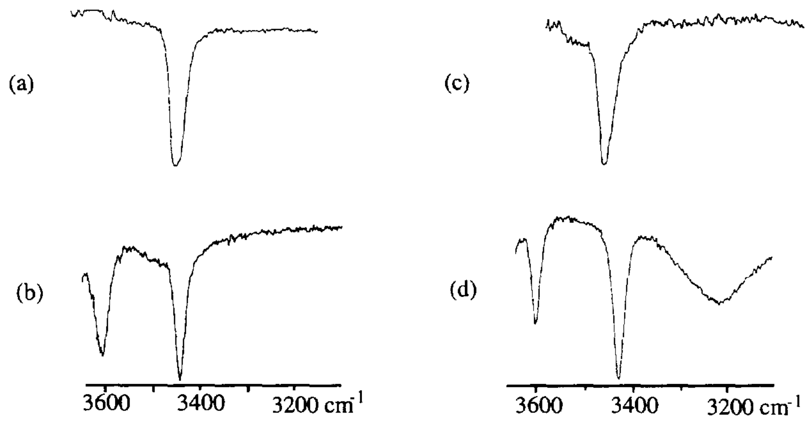
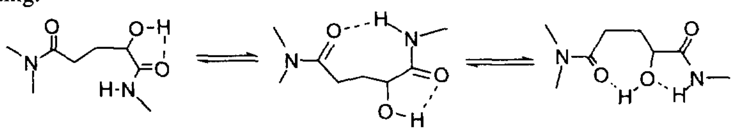


Figure 1. NH stretch region of infrared spectra recorded in CH_2Cl_2 at 5 mmol concentration: (a) monoamide **5**, (b) α-hydroxymonoamide **6**, (c) diamide **1**, and (d) α-hydroxydiamide **3**.

and the IR spectra were recorded on a Perkin-Elmer 1650 instrument. At greater than 100 mmol concentrations both a sharp peak at ~3450 cm^{-1} and a broad peak at ~3300 cm^{-1} are observed for all six compounds. However, at lower concentrations (< 100 mmol) diamides **1** and **2** and monoamides **5** and **6** show only a single NH absorption at ~3450 cm^{-1} while the α-hydroxydiamide **3** and **4** display not only a sharp peak but also a broad peak, Figure 1.

The sharp absorption at ~3450 cm^{-1} is assigned to the free NH stretching frequency and the broad peak at ~3200 cm^{-1} the hydrogen-bonded NH.[12] Both **3** and **4** show two NH stretching bands, a sharp one at 3426 cm^{-1} and a broad band at ~3201 cm^{-1} for **3** and ~3281 cm^{-1} for **4**. The current results indicate that at dilute concentration the α-hydroxy diamides **3** and **4** form intramolecular amide-amide hydrogen bonding (Table) while no such a bond is present for compounds **1** and **2.** The diamides **1** and **2** have their hydroxy function blocked by an acetyl group. Hence they lack the structural feature for cooperative hydrogen bonding.



I **II** **III**

An equilibrium can be envisioned among the three conformations (**I-III**) for diamide **3**. The free NH observed for **3** may come from structure **I**. The broad absorption at 3201 cm^{-1} is expected to originate from conformation **II** where the secondary amide function is hydrogen bonded to both the tertiary amide carbonyl and the α-hydroxy group. This assignment is supported by the stretching frequencies of the secondary amide carbonyl group. The diamides **1** and **2** have a stretching frequency of 1681 cm^{-1} for the secondary amide carbonyl group while the corresponding frequency for diamides **3** and **4** is 1670 cm^{-1}.

Both diamides 3 and 4 show a 11 cm^{-1} reduction in the frequencies of the secondary amide carbonyl group, which is consistent only with structure **II**.

Table IR frequencies (cm^{-1}) observed for compounds **1-4** in one mmol CH_2Cl_2 solution.

Compound	Free N-H	N-H···O=C	CO(NHMe)	CO(NMe_2)
1	3455	---	1681	1642
2	3448	---	1681	1640
3	3426	3201 br	1670	1617
4	3426	3281 br	1670	1634
Gellman's diamide[12]	3456	3310 br		

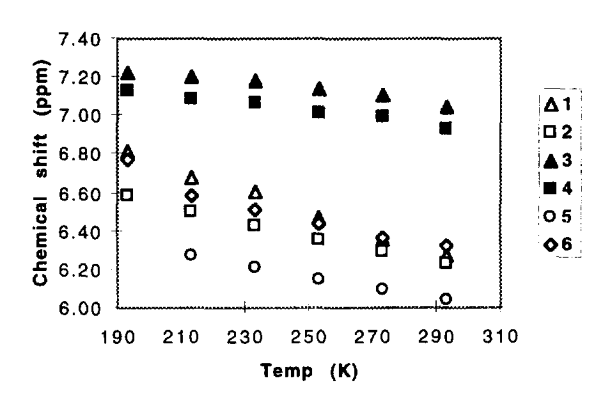


Figure 2. The NH chemical shifts as a function of temperature for diamides **1-6**.

Figure 2 shows the graph with the NH proton chemical shifts plotted as a function of temperature for diamides **1-6**. It is evident that the NH chemical shifts of compound **3** and **4** are further downfield than that of diamides **1** and **2** and monoamides **5** and **6** at all temperatures. Furthermore, the NH chemical shifts of compound **3** and **4** are also more downfield than Gellman's diamide 7.[12] Since a downfield chemical shift is considered to be an indication of a greater proportion of the hydrogen bonded form, these observations indicate an enhanced intramolecular hydrogen bonding between the NH and the N,N-dimethylamide carbonyl group in diamide **3** and **4**. It is worth noting that Gellman's simple diamide **7** shows very little folding[12] while the corresponding α-hydroxy amide **3** exhibits significant intramolecular hydrogen bonding.

In summary, diamides **3** and **4**, which have a free α-OH group, form intramolecular amide-amide hydrogen bonding. Diamides **1** and **2**, which have an α-acetyl group, showed no sign of intramolecular hydrogen bonding. The intramolecular amide-amide hydrogen bond formed in diamides **3** and **4** are judged to be stronger than Gellman's diamide **8** on the basis of the observed NH and CO stretching frequencies and the NH chemical shifts.[13]

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(10) The model diamides **1-4** are prepared starting from γ-butyrolactone and δ-valerolactone in four steps. The key reaction is a three-component, one-pot Passerini reaction involving a ω-formyl amide, methyl isocyanide, and acetic acid.[11]

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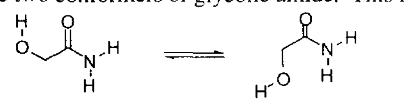
(13) A reviewer has suggested that the enhanced intramolecular amide-amide hydrogen bonding of the diamides **3** and **4** are due to the conformational restriction from the five-membered ring between the α-hydroxy and the amide carbonyl group. Our *ab initio* (MP2/6-31*) calculations show a difference of only 0.7 kcal/mol for the two conformers of glycolic amide. This means that both forms are



0.0 0.7 kcal/mol

available to diamide **3** and **4**. Therefore, we believe that it is hydrogen bonding cooperativity which enhances the intramolecular amide-amide H-bond. Furthermore, it is not clear how can one clearly separate hydrogen bonding cooperativity from conformational restriction. For example, in the initiation process of an α-helical peptide, the establishment of the first hydrogen bond certainly restricts the conformation to the advantage of the second H-bond formation. However, this process is normally considered as hydrogen bonding cooperativity, rather than conformational restriction.

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