Comparison of an HXH Three-Center Hydrogen Bond with Alternative Two-Center Hydrogen Bonds in a Model System
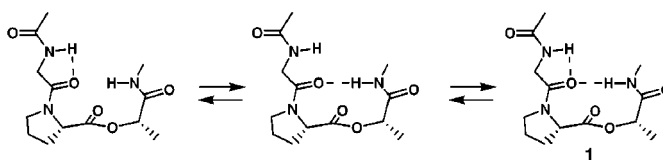
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ABSTRACT

Little information is available on the stability of three-center hydrogen bonds relative to two-center hydrogen bonds. In an XHX three-center hydrogen bond, two donors interact with one acceptor; here, we compare an HXH interaction with the component two-center hydrogen bonds (H–X and X–H) in a small depsipeptide, 1. The results show that there is negative cooperativity between the two components of the HXH interaction.

Three-center ("bifurcated") hydrogen bonds are commonly observed within and between polar molecules, particularly in the solid state.[1] There is very little information on the relative energetics of two- and three-center hydrogen bonds, but several groups have speculated on this subject.[1−3] Two types of three-center interaction can be distinguished, one involving two donors and one acceptor, which we designate "HXH", and the other involving one donor and two acceptors, which we designate "XHY" (Scheme 1). We have

Scheme 1
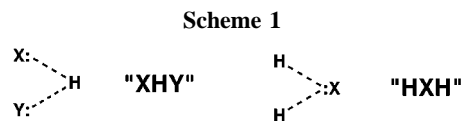
recently reported a direct thermodynamic comparison of an XHY three-center interaction with the alternative two-center hydrogen bonds (X−H or H−Y).[4] There is no enthalpic advantage to forming the XHY three-center interaction once one of the two-center hydrogen bonds has formed in the depsipeptide model system we used. Here we employ a similar approach to compare an HXH three-center interaction with the alternative two-center hydrogen bonds.

The HXH three-center interaction was examined by using **1** and reference molecules **2**−**5**. Depsipeptide **1** has two

(1) (a) Baur, W. H. *Acta Crystallogr.* **1965**, *19*, 909. (b) Taylor, R.; Kennard, O.; Versichel, W. *J. Am. Chem. Soc.* **1982**, *104*, 244. (c) Jeffrey, G. A.; Mitra, J. *J. Am. Chem. Soc.* **1982**, *104*, 5546. (d) Reutzel, S. M.; Etter, M. C. *J. Phys. Org. Chem.* **1992**, *5*, 44. (f) Baker E. N.; Hubbard, R. E. *Prog. Biophys. Mol. Biol.* **1984**, *44*, 97. (g) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *J. Mol. Biol.* **1985**, *103*, 553. (h) Jain, S.; Zon, G.; Sundaralingam, M. *Biochemistry* **1989**, *28*, 2360. (i) Nelson, H. C. M.; Finch, J. T.; Luisi, B. F.; Klug, A. *Nature* **1987**, *330*, 221. (j) Coll, M.; Frederick, C. A.; Wang, A. H.-J.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8385. (k) Aggarwal, A. K.; Rodgers, D. W.; Drottar, M.; Ptashne, M.; Harrison, S. C. *Science* **1988**, *242*, 899. (l) Sundaralingam, M.; Sekharudu, Y. C. *Science* **1989**, *244*, 1333. (m) Preissner, R.; Egner, U.; Saenger, W. *FEBS Lett.* **1991**, *288*, 192. (n) Askew, A.; Ballester, P.; Buhr, C.; Jeong, K. S.; Jones, S.; Parris, K.; Williams, K.; Rebek, J. *J. Am. Chem. Soc.* **1989**, *111*, 1082. (o) Allain, F. H.-T.; Varani, G. *J. Mol. Biol.* **1995**, *250*, 333. (p) Conn, M. M.; Rebek, J. *Chem. Rev.* **1997**, *97*, 1647. (q) Aurora, R.; Rose, G. D. *Protein Sci.* **1998**, *7*, 21.

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(3) For opposing views on the significance of bifurcated hydrogen bonding in protein hydration, see: (a) Savage, H. J.; Elliot, C. J.; Freeman, C. M.; Finney, J. L. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 2609. (b) McDonald, I. K.; Thornton, J. M. *J. Mol. Biol.* **1994**, *238*, 777.

(4) Yang, J.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 9090.
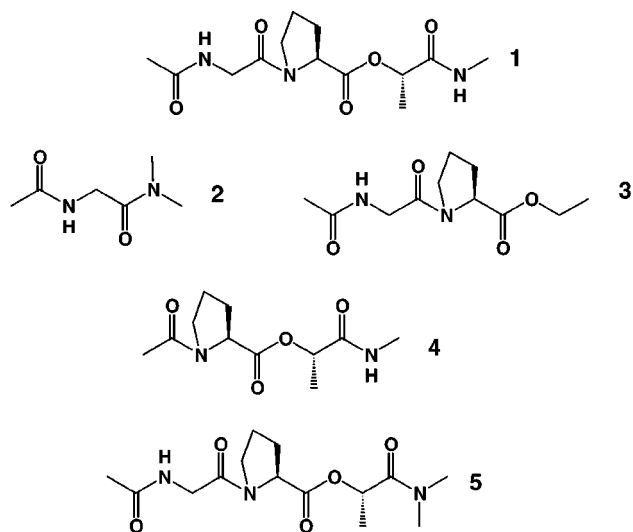
hydrogen-bond donor sites (N−H) and four acceptor sites (C=O); therefore, several intramolecular hydrogen bonds are possible. All hydrogen-bonding patterns other than **1a** (5-membered ring hydrogen bond), **1b** (10-membered ring hydrogen bond), and **1c** (HXH three-center interaction) could be ruled out as significant options for **1**.[5] The N−H stretch region IR spectrum of **1** (Figure 1) shows three maxima:
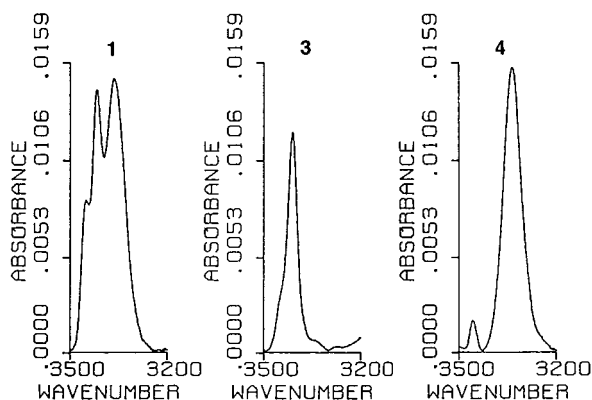

Figure 1. N−H stretch region FT-IR data for 1 mM samples of compounds **1**, **3**, and **4** in CH_2Cl_2 at room temperature, after subtraction of the spectrum of pure CH_2Cl_2 (nominal resolution 2 cm^{-1}). Data acquired on a Nicolet 740 spectrometer. From left to right: **1**, maxima at 3454, 3420, and 3360 cm^{-1}; **3**, maximum at 3411 and shoulder at ∼3447 cm^{-1} (ref 4); **4**, maxima at 3456 and 3334 cm^{-1}.

(i) The small band at 3454 cm^{-1} can be assigned to non-hydrogen-bonded N−H, based on extensive precedent.[6] (ii) The band at 3420 cm^{-1} can be assigned to the N-terminal N−H involved a two-center hydrogen bond in a 5-membered ring ("C_5 interaction"),[7] based on the behavior of reference compounds **2** (major band at 3406 cm^{-1} (ref 8)) and **3** (major band at 3411 cm^{-1}). This 5-membered ring hydrogen bond could stand alone (**1a**) or be part of a three-center interaction (**1c**). (iii) The band at 3360 cm^{-1} can be assigned to the

C-terminal N−H involved in a two-center hydrogen bond in a 10-membered ring, on the basis of the behavior of **4** (major band at 3334 cm^{-1} (refs 4 and 9)). This 10-membered ring hydrogen bond could stand alone (**1b**) or be part of a three-center interaction (**1c**). (The fact that the latter two bands occur at higher energy than seen in reference compounds **2**−**4** will be discussed below.) Since only very small populations of non-hydrogen-bonded N−H are observed for reference compounds **2**−**4**, we conclude that the completely non-hydrogen-bonded state of **1** is not significantly populated (<2%) under these conditions.

^{15}N labeling[10] confirmed the assignments of the two larger N−H stretch bands observed for **1**. Labeling at the N-terminal nitrogen caused the 3420 cm^{-1} band to shift to 3411 cm^{-1}, and labeling at the C-terminal nitrogen caused the 3360 cm^{-1} band to shift to 3352 cm^{-1}. Neither labeled version of **1** displayed a clear shift in the small band at 3454 cm^{-1}, which prevented definitive assignment of this band to either of the NH groups.

We used IR data to estimate the population of non-hydrogen-bonded N−H in **1** and reference compounds **3** and **4**. As previously described,[6,11] such quantification involves

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(6) There are six potential two-center hydrogen bonds within **1**, arising from the combination of each of two NH groups with each of three carbonyls. The N-terminal NH of **1** could be involved in 11-, 8-, or 5-membered ring hydrogen bonds. The IR data for reference compounds **2** (ref 8) and **3** (Figure 1) shows that the C_5 interaction can occur, and IR data for control compound **5** (maximum at 3411 cm^{-1}, shoulder at ∼3447 cm^{-1} (not shown)) reveal that the 11-membered ring hydrogen bond does not occur. The C-terminal NH of **1** could be involved in 13-, 10-, or 7-membered ring hydrogen bonds. As discussed in the text, precedent rules out the 7-membered ring hydrogen bond, the behavior of **4** (Figure 1) shows that the conformation containing the 10-membered ring hydrogen bond is favorable, and the behavior of **3** suggests that the 8-membered ring hydrogen bond is not populated. The 13-membered ring hydrogen bond between the acetyl C=O and the methylamide NH was assessed with Monte Carlo−stochastic dynamics simulations (AMBER*/MacroModel with GB/SA $CHCl_3$), which suggested that this hydrogen-bonded conformation would not be significantly populated. This prediction is likely to be reliable, since calculations of this type often over-estimate the tendency for intramolecular hydrogen bond formation (Gellman, S. H.; Dado, G. P. *Tetrahedron Lett.* **1991**, *32*, 7377). Monte Carlo−stochastic dynamics method: Still, W. C.; Guarnieri, F. *J. Comput. Chem.* **1994**, *15*, 1302. AMBER force field: Weiner, S.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230. AMBER* modification: McDonald, D. Q.; Still, W. C. *Tetrahedron Lett.* **1992**, *33*, 7747. MacroModel: Mohamdi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440. GB/SA solvation: Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.

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(8) (a) Avignon, M.; Huong, P. V.; Lascombe, J.; Marraud, M.; Néel, J. *Biopolymers* **1969**, *8*, 69. (b) Burgess, A. W.; Scheraga, H. A. *Biopolymers* **1973**, *12*, 2177. (c) Ashish; Kishore, R. *Tetrahedron Lett.* **1997**, *38*, 2767.

(9) (a) Cung, M. T.; Marraud, M.; Néel, J. In *Proceedings of the 5th Jerusalem Symposium on Quantum Chemisty and Biochemistry*; Bergman, E., Pullman, B., Eds.; Israel Academy of Sciences and Humanities: Jerusalem, 1973; pp 69ff. (b) Reference 6b.

(10) 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.] This calculation predicts a localized ^{15}N−H stretch to be ∼12 cm^{-1} lower in energy than a ^{14}N−H stretch.

determination of an integrated extinction coefficient for the non-hydrogen-bonded N−H stretch band by using a simple secondary amide (*N*-methylcyclohexaneacetamide in this case) in dilute solution. For the spectra of **1**, **3**, and **4**, we employed a deconvolution/curve fitting procedure to "isolate" the non-hydrogen-bonded N−H band. In each case, the amount of non-hydrogen-bonded N−H stretch was estimated by applying the integrated N−H stretch extinction coefficient to the mathematically isolated band. For 1 mM solutions, this procedure suggested that there is ~0.1 mM non-hydrogen-bonded N−H for **3** (5-membered ring hydrogen-bond reference compound), ~0.1 mM non-hydrogen-bonded N−H for **4** (10-membered ring hydrogen bond reference compound), and ~0.6 mM non-hydrogen-bonded N−H for **1**. Two important qualitative conclusions may be drawn from these approximate values. First, folding pattern **1c** must be populated to some extent, because when the total N−H concentration is 2 mM there is < 1 mM non-hydrogen-bonded N−H. Second, there is negative cooperativity between the 5- and 10-membered ring hydrogen-bonding interactions, because the total non-hydrogen-bonded N−H concentration for **1** is significantly greater than the non-hydrogen-bonded N−H concentration for **3** and **4** combined (~0.6 mM vs ~0.2 mM). The IR data indicate that the extent of formation of one or both of the two-center interactions in **1** (i.e., the 5-membered ring interaction in **1a** and/or **1c** and the 10-membered ring interaction in **1b** and/or **1c**) is diminished relative to the extent of formation of the corresponding two-center interactions in reference compounds **3** and **4**. The IR data also suggest that each of the two-center hydrogen bonds in **1** may be weakened relative to the corresponding two-center hydrogen bonds in the reference compounds since, as mentioned above, both of the bands assigned to hydrogen-bonded N−H in **1** occur at significantly higher wavenumber ($9-26$ cm^{-1}) than the corresponding bands of the reference compounds.[12] Thus, the 5-membered ring two-center interaction that is part of the three-center interaction in **1c** may be weaker than an isolated 5-membered ring two-center interaction, as in **3**, and the 10-membered ring two-center interaction in **1c** may be weaker than an isolated 10-membered ring two-center action, as in **4**.

The thermodynamic relationship among folding patterns containing two- and three-centered hydrogen bonds was examined by estimating the population of non-hydrogen-bonded N−H at various temperatures (using a temperature-dependent extinction coefficient for the N−H stretch band).[6,11] As the temperature drops, the non-hydrogen-bonded N−H stretch band observed for **1** becomes less intense relative to the two hydrogen-bonded N−H stretch bands. This observation suggests that the population of the three-center hydrogen-bonded state grows at lower temperatures, i.e., that **1c** is enthalpically more favorable than **1a** or **1b**. A van't Hoff

analysis was carried out on the basis of the two-state model in eq 1. The total concentration of non-hydrogen-bonded
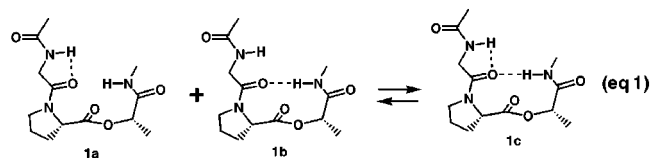
N−H at each temperature was assumed to represent the combined concentrations of **1a** and **1b**, and the remainder of the amide concentration (1 mM total) was attributed to **1c**. This analysis suggests that **1c** is ~1 kcal/mol more enthalpically favorable but ~3 eu less entropically favorable than the combination of **1a** and **1b**.[13] In contrast, previous study of the *N*-methyl amide of acetyl-alanyl-glycolic acid in methylene chloride indicated an enthalpic favorability of ~2 kcal/mol and an entropic unfavorability of ~4 eu for closure of a hydrogen-bonded ring related to that in **1b**.[11] It should be noted that these thermodynamic values reflect differences among conformational states (in the present case, [**1a** + **1b**] vs **1c**); therefore, the enthalpy values do not correspond directly to the strengths of the hydrogen bonds involved.

These results reveal both similarities and differences between HXH and XHY three-center hydrogen-bonding interactions in our depsipeptide model systems. The previous study[4] showed a negative cooperativity between the two-center components of an XHY three-center interaction, which parallels the negative cooperativity documented here between the two-center components of an HXH three-center interactions. Thus, both donors and acceptors appear to become less avid hydrogen-bonding partners once they are engaged in a hydrogen bond, a trend that is qualitatively consistent with the secondary interaction hypothesis of Jorgensen.[14] The difference between XHY and HXH three-center interactions in our model systems is that addition of a second acceptor (Y) to X−H has no enthalpic benefit,[4] while addition of a second donor to X−H is modestly favorable enthalpically. The approach we have taken to evaluating three-center hydrogen-bonding interactions is applicable to other sets of donor and acceptor groups and to other geometric arrangements; additional model studies should reveal whether our findings represent general trends.

(11) Gallo, E. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 9774.

(12) The extent to which a hydrogen-bonded N−H stretch band is shifted from its non-hydrogen-bonded position should be directly related to the strength of the hydrogen bond: Pimentel, G. C.; McClellan, A. L. *The Hydrogen Bond*; Freeman: San Francisco, 1960. However, positions observed for amide N−H stretch bands may be influenced by Fermi resonance: Miyazawa, T. *J. Mol. Spectroscopy* **1960**, *4*, 168.

(13) The IR-derived thermodynamic parameters derived for **1** are reproducible to within 10% in parallel independent determinations. Systematic error in these values, however, is difficult to evaluate. We have previously shown that IR-derived and NMR-derived enthalpy values agree well in related systems, but that agreement can be poorer for entropy values (refs 6a and 6b).

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