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The Limit of Intramolecular H-Bonding

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Supporting Information

ABSTRACT: Hydrogen bonds are ubiquitous interactions in molecular recognition. The energetics of such processes are governed by the competing influences of pre-organization and flexibility that are often hard to predict. Here we have measured the strength of intramolecular interactions between H-bond donor and acceptor sites separated by a variable linker. A striking distance-dependent threshold was observed in the intramolecular interaction energies. H-bonds were worth less than −1 kJ mol^{-1} when the interacting groups were separated by ≥6 rotating bonds, but ranged between −5 and −9 kJ mol^{-1} for ≤5 rotors. Thus, only very strong external H-bond acceptors were able to compete with the stronger internal H-bonds. In addition, a constant energetic penalty per rotor of ∼5−6 kJ mol^{-1} was observed in less strained situations where the molecule contained ≥4 rotatable bonds.

Hydrogen bonds are one of the most widely recognized molecular interactions[1] due to their role in determining the properties of water[2] and the activities of biomolecules.[3] H-bonds have been exploited in catalysis[4] and contribute to mechanical behavior in both macroscopic[5] and nanomechanical contexts.[6] Quantitative H-bonding parameters derived empirically,[7] semi-empirically,[8] or entirely from theory[9] are routinely employed in pharmaceutical and agrochemical design.[8b,10] It is also known that binding affinity in molecular recognition events is modulated by conformational flexibility.[11] For example, remarkable binding energies are observed in pre-organized arrays of interactions,[12] while the flexibilities of both proteins and ligands are important descriptors in quantitative structure−activity relationships.[13] Similarly, attaining an appropriate balance of conformational flexibility and pre-organization is also essential in the synthesis of complex supramolecular topologies.[14] The cost of restricting the rotation of a C_{sp^3}−C_{sp^3} bond at 298 K has been estimated between 1 and 7 kJ mol^{-1} based on the properties of alkanes,[15] ring-closing reactions,[16] and molecular recognition events occurring in both biomolecules[13c,17] and supramolecular complexes.[18] While broadly similar behavior is seen in many different contexts, there are numerous interesting examples where generalized principles of flexibility do not account for the observed behavior. For example, Whitesides found a trade-off between flexibility and the ideality of interaction geometry as the length of a tether between a protein and a ligand was varied.[19] Meanwhile, a series of investigations by Hunter has revealed a complicated dichotomy between flexibility and pre-organization in supramolecular complexes that can also be influenced by factors including the solvent and the strength and geometry of the interactions involved.[18c,d,20]

Here, we present an experimental investigation of the influence of conformational flexibility on H-bonding in a strictly intramolecular context using a series of synthetic compounds (Figure 1). The interactions between a H-bond acceptor and donor separated by a variable linker were measured using competitive binding experiments (Figure 2) and the energies compared to the number of rotatable bonds (Figure 3).

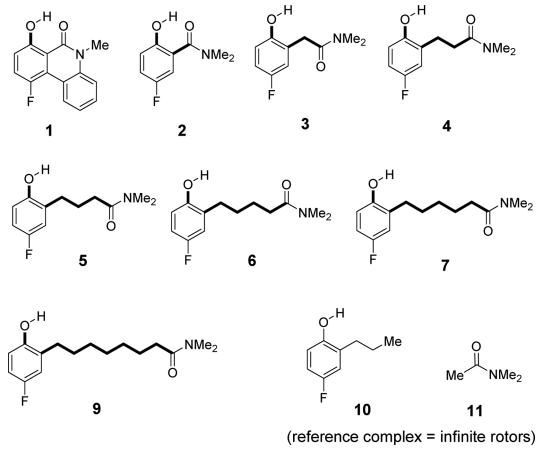

Figure 1. Compounds used to examine the influence of a variable linker on intramolecular H-bonding. Compound numbers **1**−**9** correspond to the number of rotatable bonds (indicated in bold).

The compounds selected for the present investigation each contain a phenolic hydroxyl and an amide carbonyl group that act as strong H-bond donors and acceptors, respectively (Figure 1).[7b] The compound numbers **1**−**9** correspond to the number of rotatable bonds separating the H-bond donor and acceptor in each case. Compounds **1**−**9** are in constant exchange between two major conformations in which the intramolecular H-bond is either formed (Figure 2B and Figures S1 in the Supporting Information (SI)) or broken (Figure 2C). Such a conformational exchange process can be deconvoluted into a series of bond rotations (Figure S2). Thus, if there is a large penalty to rotating the bonds such that a H-bond can be formed, then the internal H-bond will be weak and K_{intra} will be

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small. In contrast, if there is little energetic penalty associated with folding then the intramolecular H-bond will be strong and K_{intra} will be large.

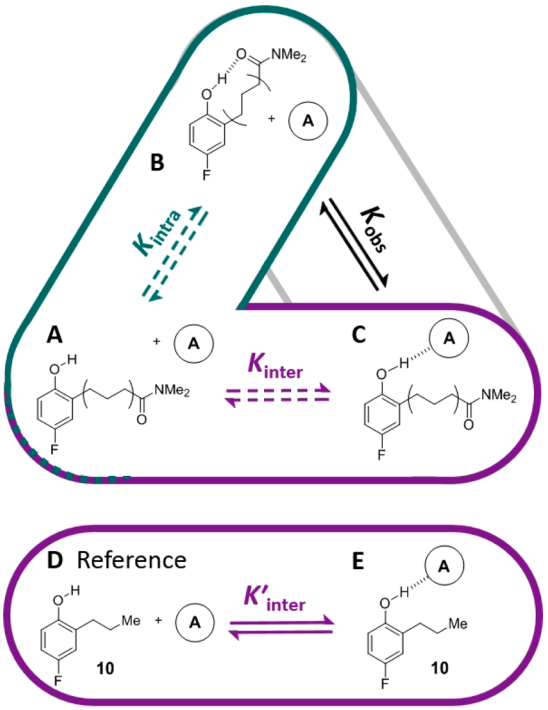

Figure 2. Competition of intramolecular folding (A) to (B) with intermolecular binding to an external acceptor (C). Experimentally non-observable equilibria are indicated with dashed arrows. (D) and (E) show reference complex used to estimate K_{inter}.

Intramolecular interactions can be measured in folding molecules where the folded/unfolded conformers are in slow exchange.[21] However, such an approach cannot be adopted to examine the compounds shown in Figure 1 due to their rapid conformational dynamics on the NMR time scale. Instead, a competition experiment was performed that allowed the energy of the intramolecular H-bond to be determined from the weakening effect that the internal H-bond had on a competing intermolecular binding event (K_{obs}, Figure 2, A and B versus C). Thus, in an equimolar solution of an acceptor **A** and any one of the compounds **1**–**9** (Figure 1), intramolecular folding (K_{intra}, green in Figure 2) is only in direct competition with intermolecular H-bonding to the external acceptor (K_{inter}, purple in Figure 2).[22] Since the observed equilibrium constant for a system that folds is given by

$$K_{obs} = K_{inter}/(1 + K_{intra}) \quad (1)$$

then K_{intra} can be determined if both K_{obs} and K_{inter} are known. K_{obs} can be determined from fitting changes in the NMR chemical shift of a signal on acceptor **A** during the dilution of a 1:1 solution of the acceptor **A** and any one of the compounds **1**–**10** (see SI). Although not directly observable, K_{inter} (Figure 2A–C) can be estimated to a high degree of certainty using a reference binding experiment where there is no competition from an intramolecular hydrogen bond (K'_{inter} in Figure 2D,E, cf. K_{inter} in Figure 2A,C). Compound **10** (Figure 1) was selected as an appropriate control due its steric and electrostatic similarity to compounds **1**–**9**, as confirmed by previous experiments[23] and DFT calculations (Table S1). Following the synthesis and purification of compounds **1**–**12** (see SI), NMR dilutions were performed on 1:1 mixtures of each combination of compounds **1**–**10** with acceptors **12** and **13** in $CDCl_3$ at 298 K. Figure 3A shows that no binding was detected between the weaker acceptor **12** (blue) and any of the donors **1**–**5** indicating that the internal H-bond in each of these compounds was substantially stronger than any potential intermolecular interactions.[24] In contrast, compounds **6**–**9** bound almost as strongly to acceptor **12** as the reference compound **10**, which lacked the ability to form any competitive internal H-bonding interactions (equivalent to infinite free rotors between the donor and acceptor). A similar structure–activity relationship was observed in the binding patterns to the stronger, phosphine oxide acceptor **13** (black); compounds **1**–**5** bound weakly to the external acceptor, while compounds **6**–**9** bound almost as strongly as the control compound **10** that lacked any internal competitive H-bond. Substituting in the values of K_{obs} and K'_{inter} into eq 1 yielded K_{intra} and thus ΔG_{intra} from $\Delta G_{intra} = -RT \ln K_{intra}$ in each of the compounds **1**–**9** (Figure 3B).

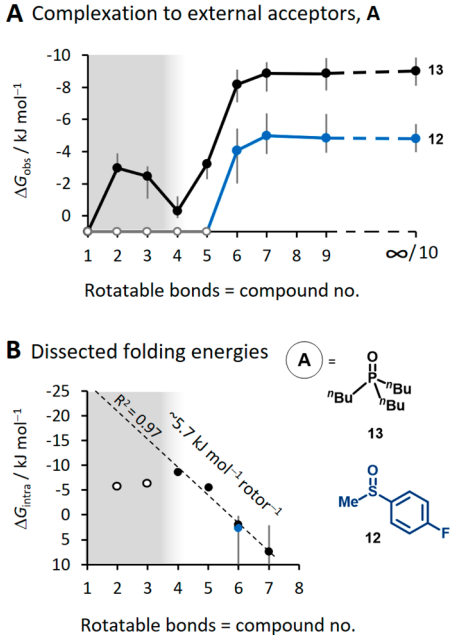

Figure 3. (A) Observed experimental binding free energies of compounds **1**–**10** with compounds **12** and **13** ($\Delta G_{obs} = -RT \ln K_{obs}$). Gray points indicate situations where no measurable binding was observed (i.e., $\Delta G_{obs} > +1$ kJ mol^{-1}). (B) Free energies of intramolecular folding in compounds **2** to **7** (ΔG_{intra}) dissected using eq 1. Hollow points indicate data not included in the straight line fit due to intramolecular strain. Only energies determined with reasonable certainty are shown. Data obtained in $CDCl_3$ at 298 K and are listed in Tables S3–S29.

Figure 3B reveals an interesting energetic pattern in the intramolecular folding energies. The trend for the compounds containing ≤4 rotors is likely attributed to enthalpic differences arising from non-ideality of the intramolecular H-bond geometry due to the strain associated with forming ring structures.[16c] In contrast, the five black and blue ΔG_{intra} values for compounds with ≥4 rotors form a steep linear correlation corresponding to an entropic cost of ~5–6 kJ mol^{-1} for restricting each C_{sp^3}–C_{sp^3} rotor at 298 K, which is

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commensurate with the values proposed by numerous seminal physical organic investigations.[16b,f,17a,c,d,18d,25,26]

In addition, the effective molarities (EM) of the intramolecular H-bonding interactions could be determined using

$$EM = K_{intra}/K_{inter} \quad (2)$$

where K_{inter} corresponded to the **10·11** intermolecular reference complex containing the same phenol donor and amide acceptor groups as folding compounds **1**–**9** (Figure 1).[27] The effective molarities of the internal H-bonds (Table S30) that could be accurately determined were all <3 M—below the ∼10−100 M upper limit proposed for non-covalent interactions[20a,25] and contrasting with the extremely high effective molarities reported for chemical reactions of up to 10^{14} M.[28]

In summary, our experimental investigation of intramolecular H-bond energies as a function of the number of rotatable bonds has revealed that the synergistic effects of both rotational entropy and conformational strain result a discrete limit for the occurrence of intramolecular H-bonding. Compounds with up to five rotatable bonds between the donor and acceptor contained strong hydrogen bonds worth at least −5 to −9 kJ mol^{-1}, while a ∼5−6 kJ mol^{-1} penalty per rotor (at 298 K) resulted in a sharp transition where internal H-bonding became negligible for more flexible compounds. In real terms, this means that only extremely strong external H-bond acceptors such as phosphine oxides are able to compete with the strong internal H-bonds in compounds **1**–**5**. Notably, this sharp transition in behavior occurs in a size regime similar to that of small-molecule pharmaceuticals and agrochemicals, and thus may be of some significance in the context of protein−ligand binding.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b09130.

Computational data, synthesis details, and characterization data, including Figures S1−S41 and Tables S1−S30 (PDF)

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Notes

The authors declare no competing financial interest.

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(24) The same binding pattern was observed with a weaker ketone acceptor, 1-(4-fluorophenyl)ethan-1-one, but binding was too weak to be accurately determined.

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(26) Although the errors for the most extreme energies are large due to small equilibrium constants and conservative error estimations (see SI), some confidence can be derived from the independent but coincident values of ΔG_{intra} determined using different acceptors for compound **6** (overlapping black and blue points).

(27) Control titrations were also performed with *N*,*N*-dimethylpentanamide, which gave binding constants similar to those observed with compound **10**, ruling out substantial steric influences (see SI).

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