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Intramolecular Hydrogen Bond Strength and pK_a Determination of *N,N′*-Disubstituted Imidazole-4,5-dicarboxamides

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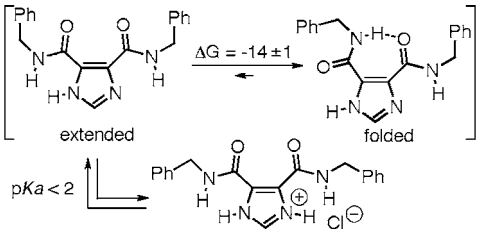
ABSTRACT



***N,N′*-Disubstituted imidazole-4,5-dicarboxamides (I45DCs) form an intramolecular hydrogen bond worth an estimated 14 ± 1 kcal/mol, as measured with a model structure in DMSO-d_6 at 3 mM, thereby predisposing the molecular conformation to a folded rather than extended form. The I45DCs also show evidence of aggregation in both $CDCl_3$ (>1 mM) and DMSO-d_6 (>10 mM) solutions. These compounds are uncharacteristically weak bases in comparison with imidazoles bearing similar electron-withdrawing groups.**

The intramolecular hydrogen bond, intermolecular aggregation, and pK_a's for select *N,N′*-disubstituted imidazole-4,5-dicarboxamides (I45DCs) were studied, as the solution behavior of the I45DCs is important in understanding the biological activity of this class of compounds against target macromolecules such as HIV-1 protease[2] and the protein−protein interaction of CD81 with the hepatitis C glycoprotein E2.[3] Earlier work has shown that the I45DCs form a robust seven-membered intramolecular hydrogen bond in the solid state, along with a relatively small number of different intermolecular aggregation motifs.[4] The solution conformation of I45DCs and other closely related analogues such as ring alkylated I45DCs has been previously reported.[5−7] The torsional potential of the substituent amide bond of a ring alkylated I45DC was estimated to be worth 20 kcal/mol, a value that reflects the strength of this intramolecular hydrogen bond.[6] Despite this interest in the intramolecular hydrogen-bonded conformation, no empirical determination of the hydrogen bond strength has ever been reported. That is the subject of this paper, along with illustration of the impact this hydrogen bond has on the pK_a values for the I45DCs in comparison with other substituted imidazoles.

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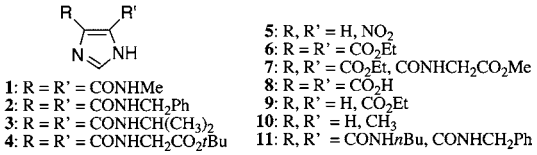

Figure 1. Compounds used in this study.

Figure 1 shows the compounds included in this study, including four symmetrically disubstituted I45DCs (**1**−**4**). The synthesis and characterization of **1**−**3** and **7** has been previously reported,[4] and **4** was prepared in an analogous fashion.[8] Likewise, a previously reported dissymmetrically disubstituted analogue (**11**) is included in this study.[9] Diester **6** is known[10] and was prepared for this study by refluxing 5,10-dioxo-5*H*,10*H*-diimidazo{1,5-*a*:1′,5′-*d*}pyrazine-1,6-diethyl diester in absolute ethanol. The remaining compounds (**5**, **8**, **9**, and **10**) were purchased commercially and used without added purification.

It is a reasonable assumption that aggregation patterns observed in the solid state might be found in the aggregation pattern(s) of a sufficiently concentrated solution. Thus, it was necessary to identify a concentration and solvent where a model I45DC existed exclusively (or largely) as a monomer before undertaking experiments to determine the strength of the intramolecular hydrogen bond in solution. Three of the intermolecular hydrogen-bonding motifs identified in I45DC crystals are shown in Figure 2.[4] The NH···O hydrogen-

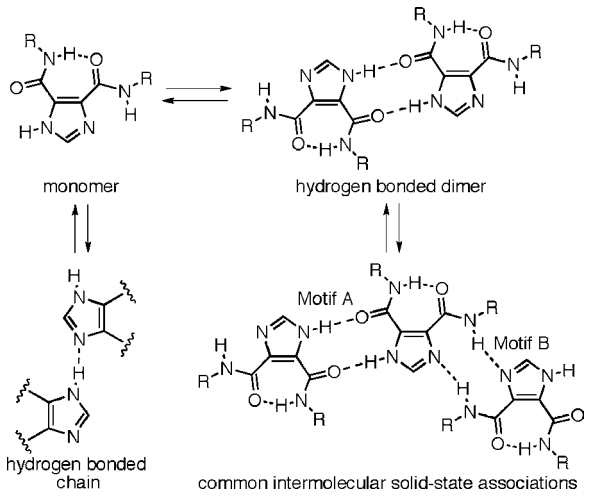

Figure 2. Postulated solution interactions for I45DCs that lead to solution hydrogen-bonded dimers or larger aggregates and ultimately to the observed crystalline interactions such as Motifs A and B reported in ref 4.

bonded dimer (Motif A) observed in the solid-state structure of **1** is 8.7 ± 1.4 kcal/mol more favorable than the dimer associated by NH···N hydrogen bonds (Motif B), according to Spartan calculations with the PM3 force field.[11] In contrast, **2** forms a hydrogen-bonded NH···N chain along with CH···O interactions (C2−H···O═C) in the solid state.[4] We chose to examine the solution aggregation for **2** expecting that the NH···N and C2−H···O interactions in the solid-state structure were likely weaker than either Motif A or Motif B in **1**.

The concentration dependence of the ring NH for **2** in both $CDCl_3$ and DMSO-d_6 was determined, and the results are shown in Figure 3. Although **2** aggregates in both solvents,

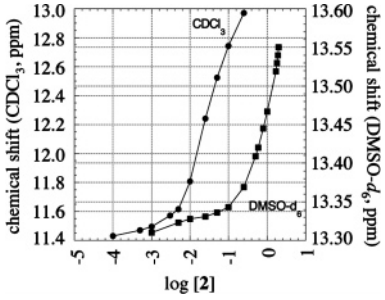

Figure 3. Concentration-dependent chemical shift for the imidazole NH in **2** as measured in $CDCl_3$ and DMSO-d_6.

it is significantly more associated in $CDCl_3$ between 1 and 10 mM as compared with DMSO-d_6. We decided to study **2** in DMSO-d_6 at 3 mM for the purpose of determining the strength of the intramolecular hydrogen bond. We note that the aggregation characteristics of imidazole itself are similar to those of **2** in $CDCl_3$ and consistent with the dominant association of **2** in solution by intermolecular NH···N hydrogen bonding.[12] It is also noteworthy that the aggregation appears to be a continuum between monomer, dimer, and higher aggregates, thereby making it difficult to determine meaningful association constants.

Additional evidence that **2** is largely monomeric even at 10 mM in DMSO-d_6 was obtained by measuring the chemical shift values of the amide and ring NHs for **11** in $CDCl_3$, DMSO-d_6, and mixtures (v/v) of these two solvents. These results are shown in Figure 4. In neat $CDCl_3$, two hydrogen-bonded conformations are observed as evidenced by two broad but distinct ring NHs (ca. 11.5 ppm), two intramolecularly hydrogen-bonded amide NHs (ca. 11.1 and 11.7 ppm), and two non-hydrogen-bonded amide NHs (<8 ppm). After addition of 10% DMSO-d_6, the ring NHs coalesce and shift downfield to 12.7 ppm. Addition of more DMSO-d_6 results in a continued shift downfield of the ring NH to a final value of 13.4 ppm in DMSO-d_6. A downfield shift of the non-hydrogen-bonded amide NH is also observed as the

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(12) See Supporting Information.

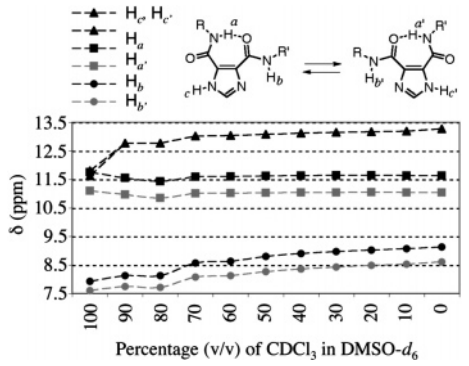

Figure 4. Chemical shift values for the amide and ring NHs in **11** (10 mM) as observed in $CDCl_3$, DMSO-d_6, or mixtures (v/v) of these two solvents. Two intramolecular hydrogen bonded conformations are observed in approximately a 1:1 ratio.

concentration of DMSO-d_6 is increased, while leaving the intramolecularly hydrogen-bonded amide NH largely unaffected. This result illustrates that I45DCs are intramolecularly hydrogen-bonded in both $CDCl_3$ and DMSO-d_6 and also shows that the major change in chemical shifts between the two solvents occurs with addition of a relatively small volume of DMSO-d_6 in $CDCl_3$. This chemical shift change is hypothesized to result from the disruption of hydrogen-bonded chains and larger aggregates present in the neat $CDCl_3$ solution by the competing hydrogen bond acceptor, DMSO-d_6.

Dynamic NMR spectroscopy (DNMR) was employed on **2** at 3 mM in DMSO-d_6 in order to determine the strength of the intramolecular hydrogen bond. The temperature of the instrument was calibrated by using the chemical shift values of 0.5% ethylene glycol in CD_3OD.[13] Kinetic analysis of the line broadening for the intramolecularly hydrogen-bonded amide NH from 20 to 64 °C (coalescence temperature) was done as described by Stewart and Siddall[14] to give a free energy of activation value of 14 ± 1 kcal/mol for this interaction.[12] We think the conditions of the DNMR experiment for **2** (3 mM in DMSO-d_6) are suitable for estimating the intramolecular hydrogen bond strength, although it is noteworthy that the hydrogen bond strength is seemingly linked to the aggregation. This association is evident in variable-temperature NMR experiments with a related I45DC at 30 mM, a concentration where aggregation of the I45DC is beginning to occur even in DMSO-d_6, which do not show such significant line broadening even up to 90 °C.[12] Thus, we are unable to formally exclude aggregation or even the possibility of other participating interactions in solution, meaning that our measured activation energy represents a lower limit for this interaction.[15] On the other hand, disruption of the hydrogen bond is likely coupled to other unfavorable consequences, such as loss of coplanarity between the imidazole and amide bond(s). Thus, the measured activation energy is best described as a lower limit for exchange of the intramolecular hydrogen-bonded conformation of **2** at 3 mM in DMSO-d_6.

The measured strength of this intramolecular hydrogen bond in DMSO-d_6 was expected from published work[5−7] and our experience with this chemical class. However, it was of interest to determine whether the intramolecular hydrogen bond and folded conformation was stable in water and, more specifically, whether the conformation was stable under similar conditions to those used to measure the bioactivity of I45DC analogues against HIV-1 protease (100 mM NaOAc, pH 4.7).[2] It was expected that inhibition of HIV-1 protease at the active site by this chemical class would require an extended conformation and not the folded conformation readily observed in $CDCl_3$ or DMSO-d_6.[2]

The pH-dependent ^{1}H NMR spectra from 7 to 11.5 ppm for **3** at 10 mM in H_2O/DMSO-d_6 (4:1, v/v) is shown in Figure 5. Both an intramolecular hydrogen-bonded amide

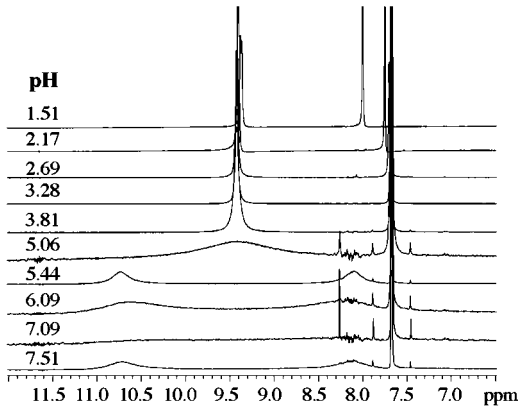

Figure 5. Amide NH region for **3** (10 mM) as determined in H_2O/DMSO-d_6 (4:1, v/v) at different pH values. The amide resonances are distinct above pH 5.44 and coalesce at and below pH 5.06.

NH (ca. 10.8 ppm) and non-hydrogen-bonded amide NH (ca 8.1 ppm) are evident at pH 7.51. The spectrum is similar at pH 5.44, although significantly broadened signals are observed at pH values of 6.09 and 7.09 for the same sample. The cause of the broadened amide NH signals at pH 6.09 and 7.09 is not clear. At pH 5.06 a single and broad NH is observed at 9.4 ppm. This single NH is consistent with disruption of the intramolecular hydrogen bond and folded conformation, thereby making the two substituents chemically equivalent through tautomerization and rapid exchange of the ring NH on the NMR time scale. This exchange is amplified by going to even lower pH, with sharp NH signals observed at and below pH 3.81.

The coalescence of the amide NH signals at pH 5.06 is evidence that the intramolecular hydrogen bond is in rapidly equilibrium under these conditions and that an extended conformation would be sampled more frequently in an acetate buffer at pH 4.7 than at pH 7. We expect that protonation of the I45DC ring at lower pH values causes the intramolecular

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hydrogen bond to be either less favorable or even unfavorable altogether. In either case, there still may be an enthalpic penalty for the initial disruption of the intramolecular hydrogen bond as the pH is lowered, and this penalty could affect the basicity of the imidazole ring. To estimate the degree of ring protonation and thereby measure the pK_a of the imidazole ring, we followed the chemical shift of the C2-H as a function of pD.[16] This is a technique often employed for proteins in order to determine the environment and possible role of imidazole rings in histidine side chains.[17,18]

The chemical shift value of the C2−H for several imidazole derivatives as a function of pD in D_2O is shown in Figure 6. Strong electron-withdrawing groups such as the

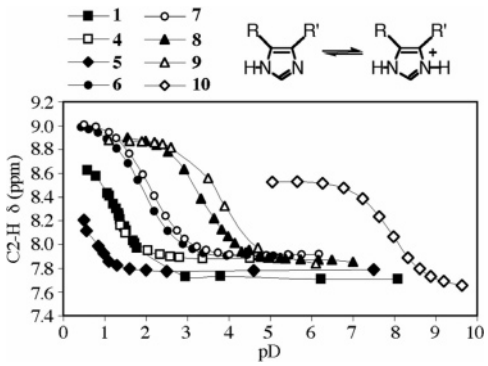

Figure 6. Observed chemical shift of the ring C2-H for different imidazole derivatives as a function of pD. Samples were prepared at 5 mM in D_2O with 2 mM TSP as the reference.

nitro group in **5** result in an imidazolium ring that is a significantly stronger acid (pK_a < 1) than unsubstituted imidazolium (pK_a ≈ 6.9). In contrast, the electron-donating methyl group in **10** leads to a weaker imidazolium (pK_a ≈ 7.8).

Amides and ester groups have comparable electron-withdrawing ability as evidenced by their similar Hammett σ values.[19] A single ester substituent in **9** leads to an imidazolium with a pK_a ≈ 3.8, while the two esters in **6** lead to an imidazolium with a pK_a ≈ 1.8. The amide-ester analogue **7** has a very similar imidazolium pK_a ≈ 2.0. It is expected that a hydrogen bond in **7** would be present at more neutral pD values in D_2O, which suggests that this intramolecular hydrogen bond does not perturb the measured imidazolium pK_a. The imidazolium pK_a in I45DCs **1** and **4** is approximately a half log unit less (pK_a ≈ 1.5) than either diester **6** or amide-ester **7**. We think this is because of the repulsion between the imidazolium NH and the non-hydrogen-bonded amide NH at lower pH values. This repulsion could lead to this observed stronger imidazolium acidity in comparison to the diester or amide-ester. This is also consistent with diacid **8**, which has a weaker imidazolium ring (pK_a ≈ 3.5), since the zwitterion at this pD helps stabilizes the monocarboxylate expectedly involved in an intramolecular OH···O hydrogen bond.

The repulsive interaction between the amide and ring NHs for **1** and **4** in an acidic environment is analogous to results previously reported for the effect of ring methylation on the hydrogen bonding in amide-ester analogues.[5] As shown in Figure 7, it was observed that methylation on the donor side

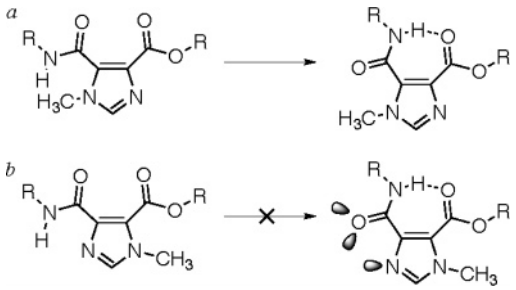

Figure 7. Ring methylation as a control of intramolecular hydrogen bonding and overall amide-ester conformation. The figure is based on results from ref 5.

(amide) allowed for intramolecular hydrogen bonding to occur, whereas ring methylation on the acceptor side (ester) prevented intramolecular hydrogen bonding. This was hypothesized to result from steric interactions between the ring methyl and ester substituent seen in Figure 7b. However, we think this difference results from the steric interaction that drives the methyl and amide NH away from one another (thereby strengthening the intramolecular hydrogen bond in Figure 7a), as well as the electron pair repulsion that precludes the formation of the intramolecular hydrogen bond in Figure 7b.

Both the pK_a determinations and the earlier ring methylation experiments of amide-ester analogues show how a relatively small structural change can result in a large influence on the overall molecular conformation. This information will be invaluable in designing I45DC and analogues that have predictable conformational[20] and aggregation behavior for either biological applications or material properties.

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Supporting Information Available: Experimental methods and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) These experiments were done in D_2O. The difference between pH and pD is small relative to the highlighted differences.

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