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Detection of weak hydrogen bonding to fluoro and nitro groups in solution using H/D exchange†

C. R. Shugrue, J. R. DeFrancisco, A. J. Metrano, B. D. Brink, R. S. Nomoto and B. R. Linton*

Hydrogen/deuterium (H/D) exchange can be a sensitive technique for measuring the strength of hydrogen bonding to neutral organic nitro and fluoro groups. The slower rates of reaction in comparison to suitable controls suggest that hydrogen bonding is present, albeit rather weak.

Introduction

There has been a history of uncertainty about the nature of solution–phase interactions with weak hydrogen bond acceptor groups, such as halides or as neutral nitro groups.[1] Hydrogen bonding has been observed in solution with anionic nitro compounds,[2] anionic fluoride,[3] and in metal-fluoride complexes,[4] but as these interactions become weaker, the situation becomes less clear. Failure to detect a hydrogen bond could suggest that either there is no interaction, or that the method of detection is not sensitive enough for an accurate determination. A third possibility is that these weak interactions could exist, but as is often the case, a stronger hydrogen bond acceptors is also present and predominates in any structural determination.

Hydrogen bonds to neutral nitro groups appear in a variety of examples both in solution[5] and in designed solid-state crystal structures.[6] On the other hand, hydrogen bonding interactions with C–F have been described as rare.[1] An analysis of crystal structure databases has suggested that organic fluorine hardly ever accepts hydrogen bonds,[7] while another presents evidence for their existence.[8] Of course this does not mean that these weak hydrogen bond acceptors cannot form hydrogen bonds, but rather that they are more transient interactions and difficult to discern with certainty. Earlier vibrational spectroscopy[9] and modern computational efforts[10] have supported the delicate interactions between alkyl halides and a variety of hydrogen bond donors. Some recent crystal structures document C–F···H–N hydrogen bonds and probe the changes between positional isomers.[11] Foldamers have been designed that rely on organic fluorine as a hydrogen bond acceptor.[12] Evidence for the strength of hydrogen bonds to organic fluorine in solution, however, remains elusive.

Solution-phase hydrogen/deuterium (H/D) exchange using ^{1}H-NMR provides an additional method for analyzing hydrogen bonding and can serve as a sensitive technique for investigating weak interactions. H/D exchange is a kinetic analysis of the rate at which a hydrogen atom is replaced by deuterium using a deuterated solvent.[13] This technique has proven to be quite illustrative in the field of protein folding, where amides making strong hydrogen bonds show significant protection from chemical exchange, while solvent-accessible amides exchange more rapidly. Our group and others have applied this technique to look at the intramolecular hydrogen bonding that is present in small-molecules in organic solvents.[14] This approach permits the analysis of the hydrogen bonding in comparison to similar controls that lack the ability to form hydrogen bonds.

Results and discussion

N-Phenylbenzamide **1** was used to position a single hydrogen bond donor within a rigid scaffold that might maximize hydrogen-bonding interactions. This scaffold has been used previously to show the application of H/D exchange to hydrogen bonding in small molecules.[15] Additional derivatives (**2–13**) positioned cyano, nitro and halide groups in a manner that may or may not be able to form intramolecular hydrogen bonds.[16] Placement of the nitro group on the aniline side, and the halides on the benzoyl side permits comparative analysis of six-membered ring hydrogen bonds, which have been shown to be more robust than similar five-membered rings on this scaffold.[15] To separate any inherent electronic effects from potential hydrogen bonding, the H/D exchange rates for both *ortho* and *para* derivatives for each functional group were determined by following the disappearance of the amide N–H in the ^{1}H-NMR. In an effort to ensure we were looking at intra-

Department of Chemistry, College of the Holy Cross, Worcester, MA, USA.
E-mail: blinton@holycross.edu; Tel: +1 508 793 3560

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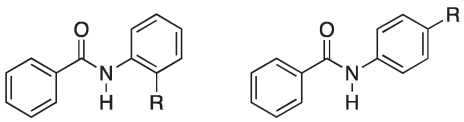
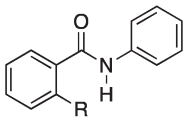
molecular hydrogen bonding instead of aggregation effects, amide chemical shifts were measured at a series of concentrations and all H/D exchange experiments are performed at concentrations below any observed signs of intermolecular assembly.

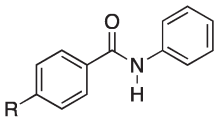


1: R = H
2: R = CN
4: R = NO_2

3: R = CN
5: R = NO_2



6: R = CN
8: R = F
10: R = Cl
12: R = I



7: R = CN
9: R = F
11: R = Cl
13: R = I

The hydrogen/deuterium exchange rates for **1–13** using 1% $CD_3OD/CDCl_3$ are shown in Table 1. The unsubstituted *N*-phenylbenzamide **1** showed a rate of H/D exchange that was slower than all *para*-substituted derivatives (**3**, **5**, **7**, **9**, **11**, **13**). The more strongly withdrawing groups (cyano **3**, **7** and nitro **5**) showed the greatest effects. While several mechanisms of H/D exchange are possible, the inherently electron-withdrawing ability of all of these groups would best stabilize any increase in electron density that results from the removal of the hydrogen. However, this does not rule out a concerted mechanism of exchange.[17] Molecules containing an *ortho*-functional group fell into two categories. The derivatives that contained either chloro (**10**), iodo (**12**), or cyano (**2** and **6**) groups all showed H/D exchange rates that were even faster than their *para*-complements (**11**, **13**, **3** and **7**). This would suggest that the electron-withdrawing effect is slightly greater in the *ortho* position than in the *para* position. By contrast, the slowest rates of H/D exchange were observed with the positioning of either a nitro or fluoro group in the *ortho* position. Both were even slower than the rate of unsubstituted *N*-phenylbenzamide **1**, despite the presence of a significant electron-withdrawing group. This selective protection from H/D exchange is consistent with the formation of an attractive interaction such as a hydrogen bond to nitro and fluoro, but not to cyano, chloro or iodo. A steric effect must be considered regarding the *ortho* substituent. However, positioning a methyl groups in either *ortho* positions results in rates that are almost identical to the parent *N*-phenylbenzamide (**1**),[15] suggesting that steric effects are not a major factor in the exchange reactions involving this scaffold.

The fluoro derivative **8** has the slowest rate of exchange, but this does not mean that it makes the strongest hydrogen bond. The exchange data for the cyano, chloro and iodo derivatives would suggest that in the absence of hydrogen bonding, *ortho* substitution leads to a slightly faster rate of H/D exchange than *para* substitution. It is also obvious that the effect of the *para* nitro is much greater than that of the *para* fluoro, and it seems likely that there is also an inherent electronic effect occurring with the *ortho* positioning that is overwhelmed by the presence of hydrogen bonding. One advantage of H/D exchange in the analysis of hydrogen bonds is the ability to correlate the rate of the reaction with hydrogen bond stability. This has been described for strongly hydrogen bonding methoxy groups,[15] and can also be applied here to the more weakly interacting halides and nitro. Englander has detailed that if the overall rate of H/D exchange is known, and the inherent rate of exchange of the functional group itself has also been determined, then the relative strength of the hydrogen bond can be calculated.[18] This complete treatment is not possible in this case, since there is obviously an inherent electronic effect from the electron-withdrawing group in the *ortho* position. If we use the *para*-derivatives as a crude control, we can obtain at best a rough estimate of hydrogen bond strength. This analysis would suggest the hydrogen bond to the nitro has a strength somewhere around 1.6 kcal mol^{-1}, and the hydrogen bond to the fluoro is around 0.4 kcal mol^{-1}.

In an effort to show that the observed effects are from hydrogen bonding and not the presence of lone-pair repulsion between the *ortho* functional groups and the amide oxygen, additional data can be considered. Support for the presence of these hydrogen bonds can be seen in the chemical shifts of individual amides in the ^{1}H-NMR. The shift of an N–H signal to a higher frequency is a typical indication of hydrogen bonding.[19] For the series of molecules presented in Table 1, most N–H chemical shifts are between 7.3 and 7.9 ppm (column 3). The notable exceptions are the nitro compounds (**4** and **5**) and the *ortho*-fluoro compound **8**. There is certainly a slight electronic effect of the *para*-nitro **5**, but it is difficult to explain the additional 3 ppm shift observed in **4** without the presence of hydrogen bonding. Similarly, the *ortho*-fluoro positioning (**8**) could be an extreme local effect, but is also consistent with the existence of a hydrogen bond.

Table 1 H/D exchange half-lives for derivatives **1–13**, measured in 1% $CD_3OD/CDCl_3$. Chemical shifts are listed for pure $CDCl_3$, and the change in chemical shifts after adding 1% CD_3OD

Compound	$t_{1/2}$ (min)	Chemical shift in $CDCl_3$ (ppm)	Change in ppm in 1% CD_3OD
1	83	7.79	0.15
2 (CN *ortho*)	3.2	7.90	0.26
3 (CN *para*)	9.8	7.78	0.30
4 (NO_2 *ortho*)	**130**	**11.37**	**0.00**
5 (NO_2 *para*)	8.9	8.29	0.18
6 (CN *ortho*)	3.4	7.90	0.17
7 (CN *para*)	18	7.78	0.30
8 (F *ortho*)	**158**	**8.45**	**0.03**
9 (F *para*)	70	7.72	0.20
10 (Cl *ortho*)	12	7.85	0.17
11 (Cl *para*)	28	7.73	0.26
12 (I *ortho*)	12	7.38	0.27
13 (I *para*)	55	7.84	0.11

A similar pattern is observed when comparing the change in chemical shift with the addition of CD_3OD (Table 1, column 4). Since methanol can readily form hydrogen bonds, one would expect any free amide NMR signal to experience a shift to a higher frequency upon the addition of methanol. Most derivatives show a shift between 0.10 and 0.30 ppm upon addition of 1% CD_3OD. Derivatives **4** and **8**, however, both show almost no effect with added methanol. This would be the case if they were already engaged in hydrogen bonding and did not compete as well for the added methanol. Again, this is most consistent with hydrogen bonding.

Changes in chemical shift at different concentrations can also be used as an indicator of hydrogen bonding ability. If a signal is sensitive to changes in concentration it can indicate increased intermolecular hydrogen bonding at increased concentration, while a similar derivative that shows little change with concentration may indicate that it is already engaged in intramolecular hydrogen bonding and therefore experiences less intermolecular interactions. The *ortho*-fluoro **8** and *ortho*-chloro **10** derivatives were prepared at a concentration of 500 mM and diluted to 0.25 mM (Fig. 1). Unfortunately *para*-derivatives were not of sufficient solubility for useful comparisons. Over this concentration range, the chloro derivative experienced a change in chemical shift of 0.40 ppm, while the fluoro showed a much smaller effect of 0.04 ppm. This insensitivity to changes in concentration is also consistent with the C–F bond functioning as an intramolecular hydrogen bond acceptor and the chloro failing to do so. A similar insensitivity to changes in concentration was observed with the *ortho*-nitro derivative **4**, which did not shift at all in the range of 250 mM to 25 mM.[20]

There is also a comparison to be made in the peakshape of the N–H signal in the ^{1}H-NMR for the fluoro derivatives. The region between 7–9 ppm is shown for each in Fig. 2. *Para*-fluoro **9** showed a characteristic broad N–H signal, but the amide signal in *ortho*-fluoro **8** was a broad doublet. This appears to be a result of long-range through-space coupling between the amide hydrogen and the NMR-active fluorine.

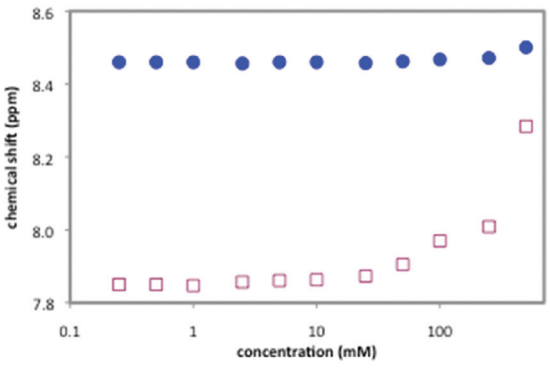


Fig. 1 Change in the N–H chemical shifts for *ortho*-fluoro 8 (●) and *ortho*-chloro **10** (□) with varying concentration.

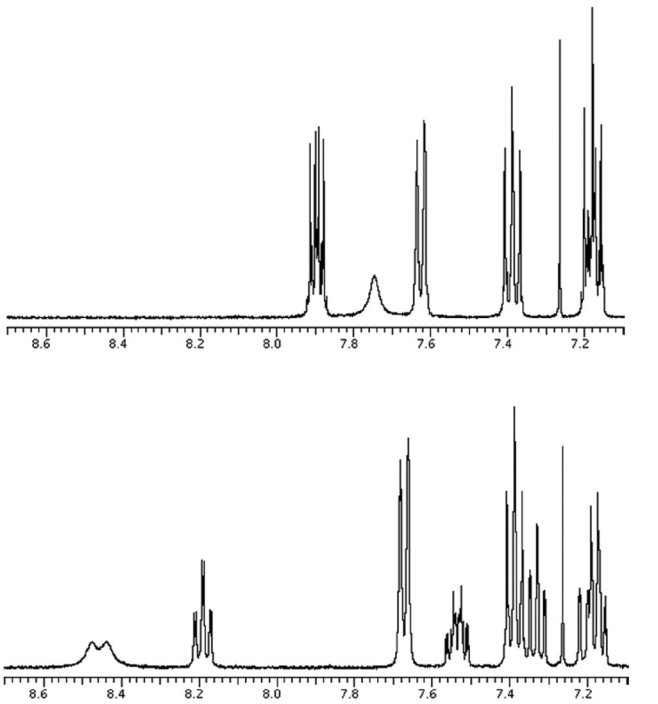


Fig. 2 NMR of *para*-fluoro **9** (top) and *ortho*-fluoro **8** (bottom) in $CDCl_3$ (400 MHz, 22 °C). Coupling can be observed in the NH signal at the bottom left.

A similar effect has been documented for intramolecular F···H–N interactions in other substrates.[21]

All of the evidence presented supports the ability of both nitro and fluoro groups to serve as a hydrogen bond acceptors in solution. Granted, the molecules involved have been chosen for the express purpose of maximizing both the structural rigidity and the ring-size for promotion of hydrogen bonding. There is also little doubt that the interactions to nitro and fluoro are still rather weak. They fail to show clear indications of hydrogen bonding using variable-temperature NMR or by comparing infrared spectra,[20] further illustrating the usefulness of H/D exchange as part of the toolbox to probe hydrogen bonds. The lack of similar interactions with cyano groups is consistent with geometric restrictions for the formation of a hydrogen bond.[22] It is unclear if the lack of interactions with chloro and iodo are due to the increased atom-size affecting hydrogen bond geometry, or due to differences in electron density. These results are consistent with crystal structures where hydrogen bonding to halide of nitro groups has been used for crystal engineering.[6,11] There are numerous examples in the literature where fluorine either does not form hydrogen bonds under a given set of conditions or engages in hydrogen bonding with a stronger hydrogen bond acceptor.[23] This does not mean that it cannot form hydrogen bonds, but rather it remains possible that competition with either solvent molecules or better hydrogen bond acceptors in the substrate itself makes hydrogen bonds to organic fluorine unlikely in many cases.

Conclusions

Because of the role that hydrogen bonds play in a variety of chemical processes, it is important to accurately assess their presence. Unfortunately, as hydrogen bonds get weaker, this task becomes more difficult. Hydrogen/deuterium exchange can provide an additional method to investigate hydrogen bond strength. In these rigid molecules in organic solvents, there is evidence for both neutral nitro groups and organic fluorine serving as hydrogen bond acceptors.

Experimental

All H/D exchange kinetics were performed in 1% CD_3OD/$CDCl_3$ and at 22 °C. An initial NMR spectrum was acquired with 990 μL of an analyte solution with a concentration of 5.0505 mM in $CDCl_3$. Immediately prior to use, the deuterochloroform was passed through a small plug of neutral alumina to remove any acidic impurities and then used immediately. To this was added 10 μL of CD_3OD marking time = 0, and resulting in a final substrate concentration of 5 mM, which was well below the concentrations where any evidence of aggregation has been observed. The added methanol created a 1% methanol : chloroform solution, with the final methanol concentration of 247 mM, which ensured pseudo-first-order kinetics. For a typical experiment, spectra were acquired starting at 3 minutes, then every 1 minute until 10 minutes, every 5 minutes through 100 minutes, at 120, 150 and 200 minutes, and then every 50 minutes thereafter until the disappearance of the proton signal into the baseline.

Acknowledgements

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