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A simple and facile NMR method for the determination of hydrogen bonding by amide N–H protons in protein models and other compounds

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It is shown that measurements of the ^{1}H chemical shifts of amide N–H protons in chloroform and in DMSO solvents are sufficient to determine the extent of hydrogen bonding of the N–H protons in a variety of compounds containing the amide group. Two recent examples are presented from protein and structural chemistry.

Over the years, we have constructed a data base of properties, or 'descriptors' of compounds as solutes, using physicochemical properties of the solutes such as water–solvent partitions, solubilities, and gas- and liquid-chromatographic retention data. The general procedure has been extensively reviewed,[1–5] and is widely used, especially as the data base is available both commercially[6] and in the public domain.[7] The set of descriptors for solutes can then be used to correlate and estimate other physicochemical properties,[8,9] environmental properties[10] and biochemical properties,[11–16] and has been applied to general structural chemistry.[17] One of the descriptors thus determined is the solute hydrogen bond acidity, *A*.[1,18] This parameter is a quantitative indicator as to how well an N–H group (or an O–H group) can hydrogen bond to an external hydrogen bond base. *A*-Values for some N–H groups are given in Table 1.[6,7] If *A* is zero or nearly zero, then the N–H group must be taking part in a strong internal hydrogen bond and therefore the group has no ability to hydrogen bond to an external base because it is itself already hydrogen bonded. If *A* is above about 0.20, then the N–H group is free to form a hydrogen bond with an external base, and is itself not part of a hydrogen bond. The *A*-value of an N–H (and also an O–H and an S–H group) is thus an indicator of the hydrogen bond state of the N–H group.

We have also carried out studies on NMR shifts in chloroform and DMSO solvents,[19–21] and have shown[22] that there is a quantitative connection between the difference in chemical shifts, $\delta\Delta$, eqn (1), and values of *A* for N–H, O–H and S–H compounds, eqn (2).

$$\delta\Delta = \delta(\mathrm{DMSO}) - \delta(\mathrm{CDCl_3}) \quad (1)$$

$$A = 0.0065 + 0.133\ \delta\Delta \quad (2)$$

We subsequently applied eqn (2) to the assessment of hydrogen bonding in a number of different systems.[23–25] The usefulness of eqn (1) and (2) is that measurement of N–H NMR shifts in chloroform and DMSO is sufficient to determine *A* and thus to estimate the extent of hydrogen bonding. We give two examples of the use of this method in protein and structural chemistry.

Table 1 Values of the descriptor *A*, and estimates of internal hydrogen bond formation of N–H groups

Compound	*A*	Assessment
N-Methylacetamide	0.40	No hydrogen bond
N-Ethylacetamide	0.37	No hydrogen bond
Compound **2C**	0.27	No hydrogen bond
N-*tert*-Butylacrylamide	0.25	No hydrogen bond
Compound **2B**	0.21	No hydrogen bond
Compound **2A**	0.06	Strong hydrogen bond
2-Nitroacetanilide	0.00	Strong hydrogen bond
2-Nitro-*N*-methylaniline	0.00	Strong hydrogen bond

Ever since Mirsky and Pauling[26] suggested that hydrogen bonding between amide N–H and O=C groups was an essential feature of the structure of proteins, there has been continued interest in the assessment of hydrogen bonding involving amide N–H groups. Numerous hydrogen bond motifs have been identified in peptides.[27,28] Quite recently, Newberry and Raines[29] have made a notable contribution by identifying a particular hydrogen bond motif consisting of an amide N–H hydrogen bonded to an amide O=C group in a five-membered ring, known as 'C5' geometry, see Fig. 1. They used a combination of computational analysis and infrared spectroscopy to study hydrogen bonding in three model compounds (Fig. 2), *viz.*: two derivatives of diethylglycine: AcDegNHMe, (**2A**), and AcDegOMe, (**2B**), and 3-acetamido-3-methylpentane (**2C**). Newberry and Raines[29] showed that hydrogen bonding in the three model compounds increased

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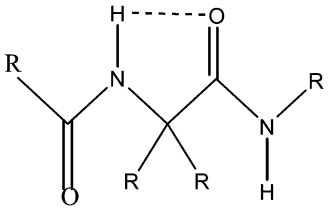

Fig. 1 The C5 configuration of N–H···O=C hydrogen bonding.

in the order **2C** < **2B** < **2A**. Since compound **2A** contains the C5 moiety, this is good evidence for the new hydrogen bond motif, C5.

Newberry and Raines[29] also measured the chemical shifts of the N–H proton in compounds **2A**, **2B** and **2C** in $CDCl_3$ and DMSO solvents, and gave the differences, $\delta\Delta$, eqn (1), for the three model compounds. They showed that there was a connection between values of $\delta\Delta$ for the three model compounds and the experimental infrared shifts of the N–H groups, but did not take this any further.

We can calculate *A* for the three compounds of Fig. 2 from the experimental NMR shifts,[29] using eqn (2), as shown in Table 1. Then just from the chemical shifts alone we can deduce that there is a strong hydrogen bond in compound **2A**, a weak hydrogen bond in compound **2B**, and no hydrogen bond in compound **2C**. This in itself is enough to deduce that the C5 motif contains a strong N–H hydrogen bond.

We suggest that the determination of ^{1}H NMR chemical shifts in chloroform and in DMSO of N–H protons in protein model compounds is a useful additional method for the assessment of hydrogen bonding through eqn (1) and (2). One caveat is that the N–H proton should not undergo exchange on the NMR time scale. However, Skinner *et al.*[30] have shown that N–H protons that are hydrogen bonded do not normally exchange on the NMR time scale.

Although assessment of N–H hydrogen bonding in protein model compounds is of exceptional interest, there are many other areas where our simple NMR method can be applied. Shalaeva *et al.*[31] have used a procedure based on the determination of water–solvent partition coefficients to investigate N–H hydrogen bonding in a series of compounds (Fig. 3). The experimental work is considerable, as it requires the determination of water–octanol and water–toluene partitions for a test molecule and a control molecule. That is four separate partition measurements are needed. Shalaeva *et al.*[31] also determined

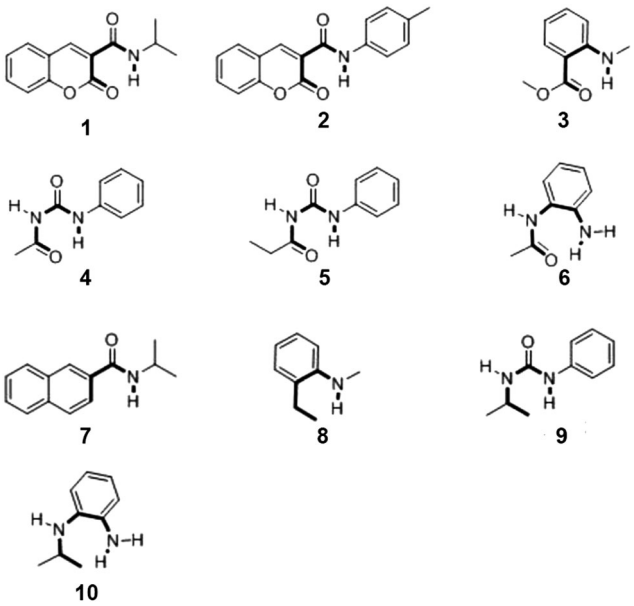

Fig. 3 Compounds **3-1** to **3-10** studied by Shalaeva *et al.*[31]

values of $\delta\Delta$ in eqn (2) for several compounds, but did not make any use of these measurements in assignment of hydrogen bonding. We used the $\delta\Delta$ values to deduce the strength of the N–H hydrogen bond in compounds **1–10**, as shown in Table 2. Compounds **3-1** to **3-5** possess strong internal hydrogen bonds, through traditional six-membered ring systems. Compound **3-6** has only a weak internal hydrogen bond, in a less-favored seven-membered ring system, and compounds **3-7** to **3-10** as expected from their structure have no internal hydrogen bond at all.

The NMR chemical shift procedure is therefore a much more convenient and definitive method than using partition coefficients, and we suggest that it could be a general method for the assessment of hydrogen bonding by N–H groups. However, we point out that use of the *A*-descriptor is an even more general method for the assessment of hydrogen bonding. For example, the *A*-descriptor for 2-nitro-*N*-methylaniline (Table 1) shows unambiguously that the N–H group takes part in a very strong internal hydrogen bond.

We show that technically quite simple measurements of ^{1}H chemical shifts of amide N–H protons in chloroform and in

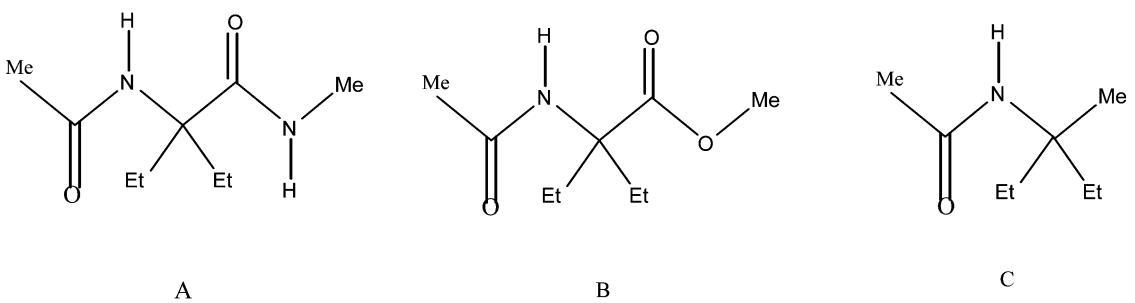

Fig. 2 Model compounds for the C5 configuration of N–H···O=C hydrogen bonding.

Table 2 NH chemical shifts, δ, in DMSO and chloroform,[31] their differences, $\delta\Delta$(DMSO − $CDCl_3$), and deduced A values for NH groups in compounds **3-1** to **3-10**[22,23]

Compound	DMSO	$CDCl_3$	$\delta\Delta$(NH)	A	IntraHB
3-1	8.52	8.69	−0.17	−0.02	Strong
3-2	10.60	10.78	−0.18	−0.02	Strong
3-3	7.53	7.64	−0.11	−0.01	Strong
3-4	10.51	10.37	0.14	0.03	Strong
3-5	10.56	10.44	0.12	0.02	Strong
3-6	4.83	3.90	0.93	0.13	Weak
3-7	8.38	6.06	2.32	0.32	None
3-8	5.03	3.68	1.35	0.19	None
3-9	8.25	6.01	2.24	0.30	None
3-10	4.45	3.30	1.15	0.16	None

DMSO solvents lead to the determination of the hydrogen bond acidity of the N–H group, A. These A-values lead directly to an quantitative assessment of the hydrogen bonding ability of the N–H group.

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