^{15}N NMR Spectroscopy

28†—Solvent Effects on the ^{15}N—^{13}C Coupling Constants of Amides, Imides, Ureas and Polypeptides

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The ^{15}N—^{13}C coupling constants of ^{15}N-enriched acetamide, *N*-acetylglycine anilide, phthalimide, α,α′-bisphthalimido-*p*-xylene, *N*-methyl-*N*′-phenylurea, poly-L-alanine, poly-L-leucine and poly-L-valine were measured in various solvents. It was found that the ^{15}N—^{13}C coupling constants depend largely on the nature of the solvent. Increasing acidity leads to higher one-bond and lower two-bond coupling constants. A change of concentration does not have any influence on the ^{15}N—^{13}C couplings while a large increase in temperature leads to a slightly lower ^{1}J(NC). It is concluded that solvent effects may limit the use of ^{15}N—^{13}C coupling constants for the elucidation of solvent-induced conformational changes.

INTRODUCTION

^{1}H—^{1}H coupling constants, widely used for conformational analyses of low molecular weight compounds, are in most cases worthless in the field of polypeptides and proteins, because the natural line width resulting from intra- and intermolecular dipole–dipole interactions prevents accurate measurements of these couplings. Even in the case of small peptides, the determination of the angles φ (rotation around NH—C-α) and ψ (rotation around C-α—CO) by means of ^{1}H—^{1}H coupling constants are difficult or impossible because of proton exchange. Measurements of ^{13}C—^{13}C coupling constants require ^{13}C enrichment and both ^{13}C—^{13}C and ^{1}H—^{13}C couplings are worthless for the determination of φ. Thus, it is not surprising that ^{15}N—^{1}H and ^{15}N—^{13}C coupling constants have found more and more interest as analytical tools for conformational analyses. However, ^{1}J(NH), although quite large (*c.* 87–97 for amide groups), is insensitive to conformational changes. ^{2}J(NH) and ^{3}J(NH) are, on the other hand, so small (0–4 Hz) that they cannot be determined in the case of polypeptides or proteins because of the natural line width in the ^{15}N and ^{1}H NMR spectra. Because one-bond and two-bond ^{15}N—^{13}C coupling constants are larger (*c.* 5–25 Hz) than ^{2}J(NH) and ^{3}J(NH), several authors have expressed the optimistic expectation that measurements of ^{15}N—^{13}C coupling constants will be useful for conformational analyses of peptides.[1–3] Since it is known that the ^{15}N NMR chemical shift is sensitive to solvent effects, where the solvation shell strongly influences the electron density distribution in the amide groups, it was our intention to investigate whether ^{15}N—^{13}C coupling constants of amides and related compounds are, likewise, sensitive to solvent effects.

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EXPERIMENTAL

Materials

99% ^{15}N-enriched acetamide, phthalimide, aniline, alanine, leucine and valine were purchased from Stohler Isotope Chemicals (Waltham, Massachusetts, USA). Dimethyl sulphoxide, trifluoroacetic acid, methanesulphonic acid and fluorosulphonic acid were purchased from Fluka (Buchs, Switzerland) and were used after simple distillation.

N-Acetylglycine ^{15}N-anilide

50 mmol aniline containing *c.* 9% ^{15}N were diluted with 10 ml dry dioxane and added dropwise to a solution of 50 mmol *N*-acetylglycine-*N*-carboxy-anhydride in 30 ml dry dioxane. In the course of the exothermic reaction, which was accompanied by CO_2 evolution, most of the product crystallized from the reaction mixture. The reaction mixture was concentrated *in vacuo*, the residue was treated with 100 ml diethyl ether and the product isolated by filtration (yield: 93%). The structure and purity were evaluated by C-, H- and N-elemental analyses.

α,α′-Bis-^{15}N-phthalimido-*p*-xylene

1.72 g (10 mmols) potassium ^{15}N-phthalimide and 1.32 g (5 mmol) α,α′-dibromo-*p*-xylene were heated in 25 ml dry dimethyl formamide at 140 °C for 2 h. The cooled reaction mixture was diluted with 100 ml tetrahydrofuran and filtered from potassium bromide. The filtrate was concentrated *in vacuo*, the residue treated with 50 ml ice cold diethyl ether and the crystallized product isolated by filtration (yield: 77%).
Analyses: Calc.; for $C_{24}H_{16}N_2O_4$; C, 72.72; H, 6.73; N, 7.07;
Found; C, 72.49; H, 6.85; N, 7.13.

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Measurements

The ^{15}N—^{13}C coupling constants were determined by means of 22.63 MHz ^{13}C NMR spectra using a Bruker WH-90 PFT spectrometer. 200 mg product dissolved in 1.5 ml solvent were measured in 10 mm diameter sample tubes with a coaxial 4 mm capillary containing TMS and dioxane-d_6 (1:1 by volume) for lock purpose and shift referencing. A pulse width of 4 μs (*c.* 30°) and 8 K data points for a spectral width of 5000 Hz (or 4000 Hz for acetamide) were used for acquisition. The Fourier transform was carried out with 16 K data points and 0.8 Hz exponential line broadening; 3000–50 000 transients were accumulated depending on the concentration of ^{15}N. At 80–130 °C a pulse interval of 2.0 s was used. The ^{15}N—^{1}H coupling constants were measured with the same PFT spectrometer by means of ^{15}N NMR spectroscopy. The above-mentioned solutions were diluted with 5 ml solvent and measured in 20 mm diameter sample tubes with a coaxial 5 mm tube containing a 30% (by weight) solution of $^{15}NH_4{}^{15}NO_3$ in pure D_2O. The $NO_3^{\ominus}$ ion served for shift referencing; its shift relative to the $NH_4^{\oplus}$ ion was 356.5 ppm. For acquisition, gated decoupling with the following parameters was applied: pulse width 30 μs (*c.* 30°); 1 K data points for a spectral width of 500 Hz zero filled to 2 K before Fourier transform; delay time 6.0 s; exponential line broadening 1.0 Hz; 100–1000 transients depending on the concentration of ^{15}N.

For the measurements shown in Fig. 1, 100% H_2SO_4 was prepared from 96% H_2SO_4 and SO_3. The 100% H_2SO_4 was then mixed with ice to obtain well-defined concentrations of diluted H_2SO_4. The relationship between Hammett acidity (H_0) and the weight concentration of H_2SO_4 was taken from Ref. 4. 1 M solutions of acetamide in diluted or 100% H_2SO_4 were measured at 30–32 °C.

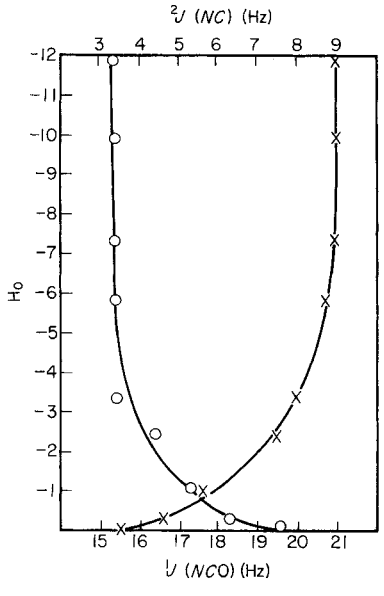
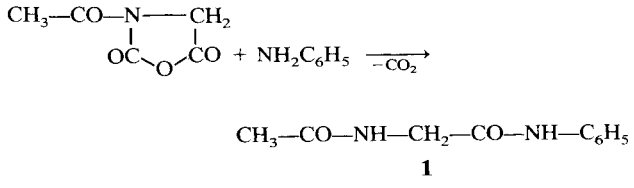


Figure 1. Dependence of ^{1}J(NCO) (×) and ^{2}J(NC) (○) of acetamide on the Hammett acidity (H_0) of H_2O/H_2SO_4 mixtures at 31–32 °C.

RESULTS AND DISCUSSION

Amides

In addition to 99% ^{15}N-enriched acetamide, 9% enriched *N*-acetylglycine anilide (**1**) was measured. This anilide was synthesized according to a new method from *N*-acetylglycine-*N*-carboxyanhydride[5] and 9% ^{15}N-enriched aniline. This one-step procedure has the advantage that the expensive ^{15}N-enriched aniline is almost quantitatively converted to the end product **1**.



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Acetamide (1 M solutions) was measured in pyridine, dimethyl sulphoxide (DMSO), water, trifluoroacetic acid (TFA), 100% H_2SO_4 and fluorosulphonic acid (FSA) to study the influence of solvation on the ^{15}N—^{13}C coupling constants. The data of Table 1 clearly demonstrate that both ^{1}J(NC) and ^{2}J(NC) depend strongly on the acidity of the solution, whereas other properties of the solvents do not have a significant influence. This conclusion is underlined by measurements carried out in water/H_2SO_4 mixtures.

Figure 1 displays a plot of ^{1}J(NC) and ^{2}J(NC) versus the Hammett acidity.[4] The resulting curves strongly resemble one half of a titration curve. According to these 'titration curves' acetamide is almost completely protonated in 80% (by weight) H_2SO_4: $^{1}J(NCO) = 21.0$ Hz at $H_0 = -7.24$. The lower ^{1}J(NC) value (18.5 Hz) found in pure TFA (Table 1) indicates that acetamide is not completely protonated in this solvent, in agreement with its lower acidity. The fact that the highest ^{1}J(NC) and the lowest ^{2}J(NC) values were found in the strongest acid, namely FSA, fits well into this picture. However, it must be emphasized that not only protonation but also H bonds directed to the carbonyl oxygen of acetamide affect the ^{15}N—^{13}C coupling constants, as shown by a comparison of DMSO (or pyridine) and water solutions (Table 1). This observation is noteworthy, because it allows the prediction that any conformational change of a peptide which affects the strength or direction of H bonds will influence the ^{15}N—^{13}C coupling constants.

It is known from ^{1}H—^{1}H, ^{1}H—^{13}C, ^{13}C—^{13}C, ^{13}C—^{19}F and ^{15}N—^{1}H coupling constants that solvent effects are weak or absent. Thus, the unusually strong solvent effects on ^{1}J(NC) and ^{2}J(NC) of acetamide deserve an explanation. The one-bond ^{15}N—^{13}C coupling constants reported for DMSO solutions of acetamide (14.2 Hz)[3] and acetanilide (13.1 Hz)[6] are usually considered to confirm Binsch's rule, which connects ^{1}J(NC) with the s character of the hybridization of both the ^{15}N and ^{13}C nuclei.[7] Since protonation of the carbonyl oxygen[8] does not markedly

Table 1. $^{15}N—^{13}C$ and $^{15}N—^{1}H$ coupling constants (Hz) of amides and polypeptides

	Solvent	Concentration (molarity)	Temperature (°C)	Acyl residue $^1J(NCO)$	Acyl residue $^2J(NC)$	N-residue $^1J(NC)$	N-residue $^1J(NH)$
	Pyridine	1.0	29–30	14.1	8.8	—	—
	Pyridine	1.0	99–100	13.6	9.3	—	—
	DMSO	1.0	29–30	14.1	8.8	—	88.0
	DMSO	1.0	129–130	14.1	8.8	—	—
Acetamide	DMSO	0.1	29–30	14.1	8.8	—	88.0
	H_2O	1.0	29–30	15.5	7.3	—	—
	TFA	1.0	29–30	18.5	3.9	—	—
	H_2SO_4 (100%)	1.0	32–34	21.0	3.4	—	—[a]
	FSA	1.0	32–34	21.5	2.9	—	—[a]
N-Acetyl-glycine anilide	DMSO	1.0	29–30	14.6	9.5	15.0	89.9
	DMSO	1.0	129–130	14.0	—	14.2	
	TFA	1.0	29–30	—[b]	—[b]	14.1	—[b]
Polyala-nine	TFA	1.0	29–30	16.5	—[b]	—	
	FSA	1.0	32–33	20.0	—[b]	—	—[a]
Poly-leucine	TFA+10% MSA	1.0	29–30	18.1	—[b]	—	—[a]
	FSA	1.0	32–33	19.5	—[b]	—	—[a]
Polyva-line	TFA	1.0	29–30	17.0	—[b]	—	—[a]
	TFA+10% MSA	1.0	29–30	18.0	—[b]	—	—[a]
	FSA	1.0	32–33	19.5	—[b]	—	—[a]

[a] Not measurable because of fast proton exchange.
[b] The doublet of the ^{15}N isotopomer is obscured by the signal of the ^{14}N isotopomer.

change the sp^2 hybridization of nitrogen or carbon, it is clear in the case of amide groups that, first, the validity of Binsch's rule is limited to neutral, aprotic solvents and, second, other factors than hybridization must have, likewise, a strong influence on $^1J(NCO)$. Most one-bond coupling constants, e.g. $^1H—^{13}C$, $^{13}C—^{13}C$, $^{13}C—^{19}F$ and $^{15}N—^1H$ are known to increase with increasing electronegativity of the substituents and with an increasing number of electronegative substituents.[9] Since the protonation of an amide group diminishes the electron density at both carbon and nitrogen nuclei, protonation can be considered as the introduction of an extremely electronegative substituent. However, an interpretation of the observed solvent effects exclusively based on charge density considerations is not satisfactory when the $^{15}N—^{13}CO$ coupling constants of imides are taken into account (see below).

In this connection still another aspect deserves discussion, namely that $^1J(NC)$ and $^2J(NC)$ alter in opposite directions with increasing acidity of the solution. Since the scalar coupling interaction of two nuclei is transmitted by the σ (and π) electrons between the two nuclei, it is theoretically obvious and experimentally proved that the signs of one-bond and two-bond couplings, as well as the substituent effects on these coupling constants, have an opposite sense. Because it has been demonstrated that the $^{15}N—^{13}CO$ coupling constants of acetamide[3] and formamide[10] have a negative sign, one might conclude from the solvent effects that $^2J(NC)$ of acetamide has a positive sign. However, this conclusion would contrast sharply with the negative sign determined from selective coupling experiments by Marco and Llinas.[3] In our opinion, the currently available data do not allow the establishment of simple relationships between solvent effects and the sign of the coupling constant in question.

Polypeptides

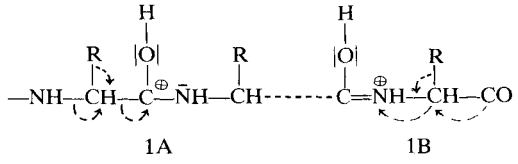
Poly-L-alanine, poly-L-leucine and poly-L-valine containing 45–50% ^{15}N were prepared from the corresponding α-amino acid-*N*-carboxyanhydrides[11] by means of a benzylamine initiated polymerization in dioxane. Whereas poly-L-alanine and poly-L-valine are soluble in TFA, poly-L-leucine is only soluble in sulphonic acids or mixtures of sulphonic acids with TFA. Because of the line width of the ^{14}N isotopomers (8–16 Hz) the $^{15}N—^{13}C$-α and $^{15}N—^{13}C$-β coupling constants, which are expected to be only of the order of 3–9 Hz, were not measurable. The line widths are mainly due to the short transverse relaxation time (T_2) of the carbons in question. Crude longitudinal relaxation time (T_1) measurements of poly-L-alanine in TFA have shown that T_1 ($\geq T_2$) of the α-carbon is shorter than 0.05 s.

In contrast to the $^{15}N—^{13}C$-α and $^{15}N—^{13}C$-β couplings the $^{15}N—^{13}CO$ coupling constants were easily measurable, on the one hand because they are larger (*c.* 15–20 Hz) than the former *J* values and, on the other hand, because the line widths of the CO signals are smaller (4–5 Hz) than those of C-α or C-β. The $^{15}N—^{13}CO$ coupling constants of all three polypeptides agree on the one hand, with those of acetamide in that they increase with increasing acidity of the solution (Table 1). On the other hand, it is conspicuous that $^1J(NCO)$ values of the polypeptides are lower than those of acetamide **1** when compared in the same solvent. This is, in our opinion, a consequence of the lower degree of protonation, because polypeptides are less basic than acetamide **1**. There are three reasons for this lower basicity. First, amino acids are stronger acids than acetic acid, which means that the carboxyl groups and their derivatives (esters, amides) are less basic. Second, protonated polypeptides behave as

polyelectrolytes, which means their basicity decreases with increasing degree of protonation. Finally, it must be considered that the solvation of the protonated amide groups in polypeptides is sterically more hindered than in the case of acetamide and **1**. In a previous paper we have shown that the protonation of polypeptides and polyamides is well monitored by their ^{15}N NMR chemical shift.[12] From this investigation[12] we can conclude that the degree of protonation of polypeptides is below 5% (if not zero) in TFA and in the range of 80–95% in FSA. For acetamide the following ^{15}N NMR chemical shifts were found (relative to external $NO_3^{\ominus}$ in D_2O): $\delta = -266.3$ ppm in DMSO, $\delta = -263.0$ ppm in H_2O, $\delta = -255.8$ ppm in TFA and $\delta = -247.8$ ppm in FSA. Hence, we assume that acetamide is protonated to 50–70% in TFA and almost 100% protonated in FSA, in good agreement with the order of the ^{15}N—^{13}C coupling constants found in these solvents (Table 1).

In this connection the conformational aspects of the ^{15}N—^{13}C couplings must also be discussed. As demonstrated in a previous paper[13] by means of ^{13}C NMR spectra, poly-L-alanine can adopt the helix conformation in TFA. The extent of helix formation depends on the optical purity and on the degree of polymerization. The polyalanine used in this work is *c.* 70% helical so that $^1J(NCO)$ mainly reflects the helix form. The data in Table 1 show that $^1J(NC)$ of polyalanine is slightly lower (*c.* 0.6 Hz) than that of polyvaline, which possesses exclusively the random coil conformation. The difference of 0.6 Hz equals our digital resolution, and thus does not necessarily mean that a helical polypeptide has a lower ^{15}N—^{13}CO coupling constant than its random coil form. However, even if we assume that a helix–coil transition causes an increase of $^1J(NCO)$ of *c.* 0.6 Hz, studies of helix–coil transitions by means of $^1J(NCO)$ measurements are, in practice, worthless for the following reasons. The digital resolution must be better than 0.1 Hz and the expensive ^{15}N enrichment should be better than 50%. Thus, the measurements of ^{13}C and ^{15}N NMR chemical shifts, which are good or moderate monitors of helical–coil transitions,[13–18] are more practical. Furthermore, a change of $^1J(NCO)$ in the course of a solvent induced helix–coil transition is the result of at least two influences: first the net conformational effect and, second, the direct solvent effect. Hence, a reliable method must first be found which allows one to extract the net conformational effect from the global effect on $^1J(NCO)$.

Finally, we have also measured a simple α-amino acid, namely alanine, in the betaine form (in D_2O, pH 7), as the cation (in 5% D_2SO_4) and as the cation with a protonated carboxyl group (in 97% D_2SO_4). These measurements were carried out for the following reason. The protonation of an individual amide group in an oligo- or polypeptide can, in principle, influence the ^{15}N—^{13}C-α and ^{15}N—^{13}C-β couplings of the neighbouring amino acid units in two ways (Scheme 1A and 1B). Measurements of *N*-substituted amides, imides and ureas (see below) can serve as models for the protonation effect of scheme 1B, whereas the measurement of an amino acid with a protonated carboxyl group can serve as a model for the interaction outlined in scheme 1A. Our measurements of alanine with a digital resolution of 0.49 Hz/point did not allow us to detect any ^{15}N—^{13}C two-bond coupling, and $^1J(NCH)$ (5.85 Hz) was identical for all three solutions. Thus, one can conclude that solvent effects on the ^{15}N—^{13}C couplings of oligo- and polypeptides are almost exclusively the result of an interaction according to scheme 1B.

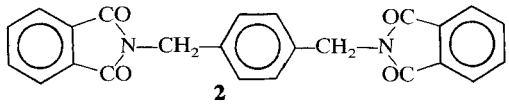


1A 1B

Scheme 1. Influence of protonation of a peptide group on the electron density of neighbouring σ bonds.

Imides

In addition to 99% ^{15}N-enriched phthalimide, α,α'-bisphthalimido-*p*-xylene (**2**) was measured to obtain ^{15}N—^{13}C coupling constants of a saturated *N*-substituent. For comparison with the data of acetamide, phthalimide was measured in pyridine, DMSO, TFA, 100% H_2SO_4 and FSA. Again, it was observed that $^1J(NCO)$ increases while $^2J(NC)$ decreases with increasing acidity of the solvent (Table 2). An analogous result is obtained upon comparison of $^1J(NCO)$ of **2** in TFA and FSA. However, it is noteworthy that the two one-bond coupling constants ^{15}N—^{13}CO and ^{15}N—$^{13}CH_2$ behave differently, in that the former increases while the latter decreases with increasing acidity of the solution. It is highly probable that $^1J(NCO)$ of imides has a negative sign like that of amides,[3,10] and that $^1J(NCH_2)$ of imide **2** has also a negative sign, by analogy with primary amines.[10] This conclusion can be drawn because all one-bond ^{15}N—^{13}C coupling constants dominated by the Fermi contact term (J^{Fc}) have a negative sign[10,19] and, on the basis of Schulman's work,[10,19] there would seem to be no reason why $^1J(NCO)$ and $^1J(NCH_2)$ should not be dominated by J^{Fc}. If this conclusion is true, our measurements on **2** again demonstrate that the sense of the solvent effects on the ^{15}N—^{13}C coupling constants is not connected with their sign.



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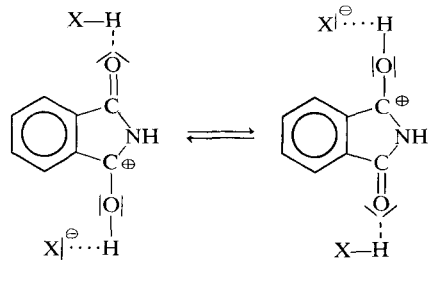
The most interesting aspect of the data in Table 2 concerns the ^{15}N—^{13}CO couplings, because they are lower than those of acetamide and anilide **1** when compared in DMSO and because the increase on protonation is far less. The stronger delocalization of the nitrogen lone pair slightly increases the *s* character of the *N*-hybridization in imides. Thus, because of Binsch's rule, and because of the electronegative second acyl group, a slightly higher ^{15}N—^{13}CO coupling constant is expected for imides than for amides. The failure of this conclusion indicates that $^1J(NCO)$ of amides and imides must be strongly influenced by another factor,[20] and this is, in our opinion, the so called 'π density' of the N—C double bond. It is well known from vicinal and other long-range ^1H—^1H couplings in

Table 2. $^{15}N—^{13}C$ and $^{15}N—^{1}H$ coupling constants (Hz) of imides

Compound	Solvent	Concentration (molarity)	Temperature (°C)	Acyl residue $^{1}J(NCO)$	Acyl residue $^{2}J(NC)$	Amine residue $^{1}J(NC)$	Amine residue $^{2}J(NC)$	$^{1}J(NH)$
	Pyridine	1.0	29–30	12.8	—[a]	—	—	—
	DMSO	1.0	29–30	13.4	7.4	—	—	93.7
	TFA	1.0	29–30	14.0	6.1	—	—	97.7
	TFA	0.1	29–30	14.0	6.1	—	—	97.7
Phthalimide	H_2SO_4 (100%)	1.0	33–34	14.6	5.3	—	—	—
	FSA	1.0	32–33	14.6	—[a]	—	—	—
	DMSO	0.1	29–30	13.4	7.3	—	—	93.7
	DMSO	0.3	29–30	13.4	7.3	—	—	93.7
	DMSO	1.5	29–30	13.4	7.3	—	—	93.7
	DMSO	1.5	79–80	12.8	7.3	—	—	93.7
	DMSO	1.5	129–130	12.8	7.3	—	—	93.7
α,α′-Bisphthal-imido-*p*-xylene	TFA	0.5	29–30	13.4	7.3	9.8	2.0	—
	FSA	0.5	32–33	14.6	—[a]	6.1	2.0	—

[a] The doublet of the quaternary carbons was partially obscured by a more intense CH signal.

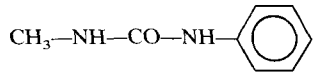
olefines, acetylenes and aromatic systems that the scalar interaction is strongly, or even exclusively, transmitted by the π electrons. If this is, likewise, true for amide groups, the spectroscopic behaviour of amides, imides, ureas and the observed solvent effects on $^{1}J(NCO)$ can be explained. Protonation of the carbonyl oxygen of amide groups leads to a nearly perfect $^{15}N—^{13}C$ double bond and the enhanced π density between the two atoms causes the increase in $^{1}J(NCO)$. The lone pair of an imide nitrogen is delocalized by two acyl groups and, thus, the π density of one individual N—CO bond can never reach the level of a normal amide group. Hence, $^{1}J(NCO)$ of an imide must be lower than that of an amide, even in an aprotic solvent such as DMSO. Furthermore, H bonds and protonation attack both carbonyls of an imide (Scheme 2), so that the increased delocalization of the lone pair by one of the two acyl groups cannot reach the extent of a protonated amide group. Hence, the spectroscopic effect of acidic solvents must be substantially lower in the case of imides, in as much as they are less basic than amides. Finally, it should be mentioned that a comparison of various aromatic or olefinic compounds has shown that vicinal $^{1}H—^{1}H$ coupling constants increase with decreasing bond length of the π bonds.[21] It is highly probable that protonation of amides, imides and ureas leads to a shortening of the N—C distance and this effect is more pronounced in the case of amides. This effect may also contribute to the observed solvent effects and to the differences between $^{15}N—^{13}CO$ coupling constants of amides and imides.



Scheme 2. Solvation of phthalimide in acidic solution.

Ureas and temperature effects

We have also extended our investigation to ureas because urea groups occur in biologically active compounds, such as citrulline, uracile or barbituric acid, and because intermolecular interactions of urea groups play an important role in the properties of polyurethanes and polyureas. *N*-Methyl-*N*′-phenylurea (**3**) was prepared from 99% ^{15}N-enriched aniline and methylisocyanate. The measurements in pyridine, DMSO, D_2O/acetone, formic acid (FA), TFA and FSA show, on the one hand, a distinct enhancement of the $^{15}N—^{13}CO$ coupling with increasing solvent acidity (Table 3). The $^{15}N—^{13}C$ coupling constant of the aniline residue, on the other hand, decreases slightly in acidic solution by analogy with the $^{15}N—^{13}CH_2$ coupling of imide **2** (Table 2), because the electron density of the σ bond is lowered (see Scheme 1B).



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We have shown in a previous paper[11] that the $^{15}N—^{13}CO$ couplings of various carbamic acid derivatives are substantially higher than those of amides for still unknown reasons. Hence, it is interesting to see that the solvent effects on $^{1}J(NCO)$ of urea **3** strongly resemble those on acetamide. However, the maximum difference found on comparison of pyridine and FSA solutions is *c.* 1.5 Hz smaller for urea **3** ($\Delta\nu = 6.0$ Hz) than for acetamide ($\Delta\nu = 7.4$ Hz). The weaker solvent effects on **3** can also be explained by the 'π-density' hypothesis. In the case of ureas, protonation causes the delocalization of two nitrogen lone pairs, and the positive charge is distributed on two N—H groups. Hence the increase of the 'π density' of one individual NH—CO bond, as well as the shortening of this bond, must be lower than in the case of an amide. Finally, it should be mentioned that we have observed two- and three-bond $^{15}N—^{13}CH$ couplings in the aniline ring. As expected, these coupling constants are small and also show solvent effects, but we cannot detect a simple relationship with solvent acidity.

Table 3. $^{15}N—^{13}C$ coupling constants (Hz) of *N*-methyl-*N'*-phenylurea[a]

Solvent	Concentration (molarity)	Temperature (°C)	$^1J(NCO)$	$^1J(NC)$	$^2J(NCH)$	$^3J(NCH)$
Pyridine	1	29–30	20.1	15.9	2.0	*c.* 1.6
DMSO	1	29–30	20.1	15.9	1.2	1.8
D_2O/Acetone[b]	1	29–30	—	15.9	<2.0	2.5
FA	1	29–30	22.5	15.3	<2.0	<2.0
TFA	1	29–30	23.6	14.6	2.4	2.4
FSA	1	29–30	26.1	14.6	—	2.0
DMSO	0.16	29–30	20.1	15.9	1.2	1.8
DMSO	0.4	29–30	20.1	15.9	1.2	1.8
DMSO	1.6	29–30	20.1	15.9	1.2	1.8
DMSO	1.6	79–80	19.5	15.9	1.2	—
DMSO	1.6	129–130	18.9	15.3	1.2	1.8

[a] Only the aromatic nitrogen was ^{15}N enriched and measured.
[b] 1:3 by volume.

In addition to the effect of the solvent acidity we have investigated the potential influence of concentration and temperature on the $^{15}N—^{13}C$ coupling constants. An effect by these two parameters is not unlikely because, depending on the solvent and concentration, ureas, amides and imides can undergo association via H bonds, and our measurements in non-acidic solvents demonstrate that the formation of H bonds affects $^1J(NCO)$. Unfortunately, a variation of the concentration is strongly limited by the solubility of the products under investigation and by the low signal-to-noise ratio. In the accessible range of concentrations constant coupling constants were found (Tables 1–3). However, when the temperature was raised a decrease of $^1J(NCO)$ was observed, except for acetamide/DMSO. In the case of acetamide/pyridine, anilide **1**/DMSO and phthalimide/DMSO the temperature effect is within the margin of error resulting from our digital resolution (0.5 Hz). However, in the case of statistical scattering of the data, one would expect that values higher than those obtained at 30 °C should also be found. Yet, only lower $^1J(NCO)$ values were measured at higher temperatures and, in the case of urea **3**/DMSO the temperature effect clearly exceeds the margin of error (Table 3). Since acetamide, *N*-acetylglycine anilide, phthalimide and urea **3** behave analogously, and because phthalimide possesses a rigid ring structure, conformational changes cannot play a major role in the temperature effect. Hence, it can be concluded that a decrease in association, together with a weakening of the H bonds, is responsible for the decrease of $^1J(NCO)$. Measurements of a compound with a tertiary nitrogen at various temperatures should show whether the above assumption is correct. Unfortunately, the imide **2** which cannot associate via H bonds, was no longer available when we detected the temperature effect. Hence, the identification of the origin of the temperature effect must at the moment remain open.

CONCLUSIONS

Our measurements of the $^{15}N—^{13}C$ coupling constants of amides, imides, polypeptides and ureas in various solvents have shown that all coupling constants depend on the solvent acidity. A large change in temperature has only a weak influence on the $^{15}N—^{13}C$ coupling constants. Because not only protonation but also the formation and strength of H bonds affect these couplings, all conformational changes which cause a rearrangement of the solvation shell or an alteration of association equilibria must have a complex influence on the $^{15}N—^{13}C$ coupling constants. Hence, it will be difficult to analyse solvent induced conformational changes by means of $^{15}N—^{13}C$ coupling constants. Furthermore, it must be emphasized that any quantitative relationship between hybridization and $^{15}N—^{13}C$ coupling constants is limited to measurements in aprotic solvents, and should be standardized to a well-defined association equilibrium.

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