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NMR and DFT investigations of the substituent and solvent effect on amino—imino tautomerism in acridin-9-amines substituted at the exocyclic nitrogen atom†

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ABSTRACT: The ^{1}H and ^{13}C NMR spectra of 9-(methoxyamino)acridine (**1**) and 9-hydrazinoacridine (**2**) show that these compounds exist principally in the imino tautomeric form in $CDCl_3$, acetone-d_6, CD_3CN, DMSO-d_6 and Py-d_5, all solvents with different polarities and abilities to participate in specific interactions. The spectra of the other two compounds investigated—*N*-(2-chloroethyl)acridin-9-amine (**3**) and *N*-(5-methylpyridin-2-yl)acridin-9-amine (**4**)—indicate that they coexist in the amino and imino forms. The amino tautomer of compound **3** predominates in $CDCl_3$, CD_3CN and Py-d_5 and that of compound **4** in $CDCl_3$ and Py-d_5. On the other hand, the amino and imino forms of compound **3** coexist in acetone-d_6 and probably DMSO-d_6, whereas those of compound **4** coexist in acetone-d_6 and DMSO-d_6. The positions of the signals in the NMR spectra compare qualitatively with those predicted computationally at the GIAO/DFT level of theory. The equilibrium constants predicted by the DFT(PCM) method are in agreement with the results of NMR spectral analysis. In general, both the data predicted at the DFT level of theory and x-ray structural data show that the imino tautomers display a 'butterfly'-type geometry, whereas the amino forms are characterized by an almost flat acridine moiety. Electron-attracting substituents at the exocyclic N atom improve the stability of the imino form, and electron-withdrawing substituents do likewise for the amino form. The importance of tautomeric phenomena in the context of the ability of acridin-9-amines to participate in specific interactions is outlined in brief, as are the possible applications of these compounds as probes of environmental properties.

KEYWORDS: acridin-9-amines; prototropic tautomerism; substituent and solvent effect; NMR and DFT investigations

INTRODUCTION

Acridin-9-amine is one of the simplest members of the family of heterocyclic nitrogen organic bases containing two centres, located at the endocyclic and exocyclic (amine) nitrogen atoms, that are susceptible to hydrogen bond formation and electron donor–acceptor interactions.[1–3] The compound itself and its derivatives interact selectively with macromolecules in living matter[4–14] and are thus convenient models or probes for investigating various features of natural systems.[15–20] There are good reasons for believing that the tautomeric forms of acridin-9-amines exhibit different capabilities of interacting with other molecules,[21–24] and consequently display different biological activities. Hence, the ability of these compounds to exist in the amino or imino form, or to coexist in various tautomeric forms, has been investigated by ourselves[21–27] and others.[28–32] We have used the results of theoretical predictions to demonstrate that amino–imino equilibria are largely affected by the features of the substituent at the exocyclic nitrogen atom.[24] The present paper covers NMR investigations and density functional theory (DFT) calculations of four selected acridin-9-amines (Scheme 1), and focuses on the influence of the substituent at the exocyclic nitrogen atom on the stability of tautomers in solvents with different polarities and abilities to undergo specific interactions. A second aim was to work out the main structural and physicochemical features of these compounds and to indicate possible applications.

EXPERIMENTAL

Compounds

N-Methoxyacridin-9-amine (**1**), *N*-2-chloroethylacridin-9-amine (**3**) and *N*-(5-methylpyridin-2-yl)acridin-9-amine (**4**) were synthesized by heating 9-phenoxyacridine at

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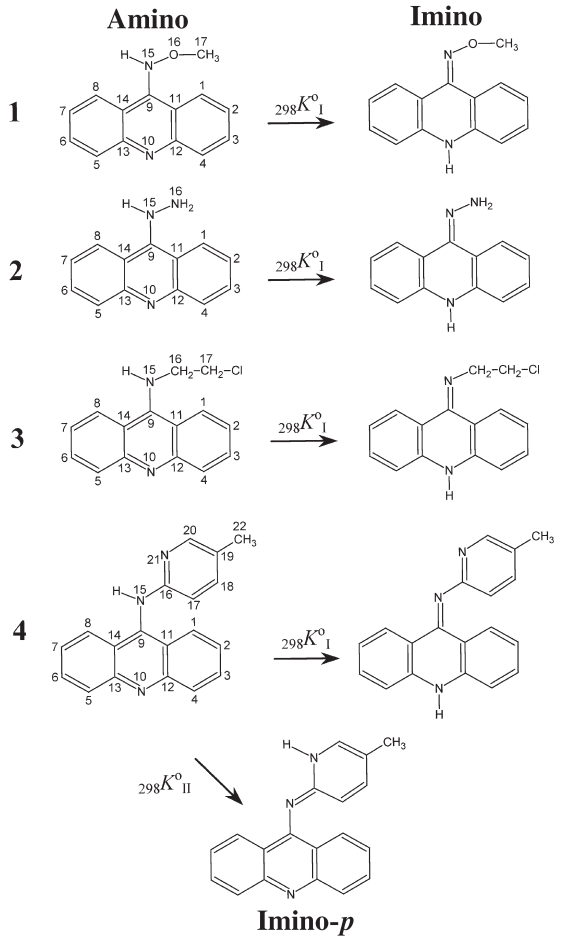

Scheme 1. Compounds and equilibria ($_{298}K^{o}$, equilibrium constant) investigated with the numbering of atoms indicated. **1**, 9-(methoxyamino)acridine; **2**, 9-hydrazinoacridine; **3**, *N*-(2-chloroethyl)acridin-9-amine, **4**, *N*-(5-methylpyridin-2-yl)acridin-9-amine

80–120 °C for 1.5 h with methoxyamine hydrochloride, 2-chloroethylamine hydrochloride and 5-methyl-2-amino pyridine, respectively, dissolved in phenol.[33] 9-Phenoxyacridine was synthesized according to the procedure described in Ref. 1. The crude products of compounds **1** and **3** were shaken with aqueous 1 M sodium hydroxide to liberate free bases. Compound **1** was then extracted from the reactant mixture with chloroform, purified by column chromatography (silica gel 60 from Merck, hexane–ethyl acetate = 2:1 v/v) and crystallized from isooctane to yield light-yellow crystals (m.p. 118–120 °C). Compound **3** was extracted with dichloromethane, purified on a silica gel 60 column (toluene–diethylamine = 10:1 v/v) and crystallized from cyclohexane to give yellow crystals of the monohydrate (m.p. 75–77 °C). The crude product of the preparation of compound **4** was dissolved in methanol, reprecipitated with ether, purified by column chromatography (silica gel 60, toluene–diethylamine = 10:1 v/v) and crystallized from ethanol to yield orange crystals (m.p. 120–122 °C). 9-Hydrazinoacridine (**2**) was obtained from 9-chloroacridine, which was gradually added to a stirred and refluxed solution of hydrazine monohydrate in ethanol.[34] After completion of the reaction, water was added (75 °C) and the suspension quickly filtered and refrigerated to give orange needles of the crude product. Recrystallization from benzene yielded pale-yellow needles (m.p. 170–172 °C, lit.171 °C[34]).

The purity of the final products was verified by thin-layer chromatography (TLC) and their chemical composition established by elemental analysis with a Carlo-Erba (model EAGER 200) instrument (% found/theoretical): C = 74.96/74.98, H = 5.62/5.39, N = 12.23/12.49 for compound **1**; C = 74.47/74.60, H = 5.26/5.30, N = 19.91/20.10 for compound **2**; C = 65.56/65.45, H = 5.32/5.45, N = 10.22/10.18 for compound **3** (monohydrate); and C = 80.20/79.98, H = 5.31/5.30, N = 14.73/14.73 for compound **4**. The identification of compound **3** (monohydrate) was confirmed by an x-ray method.[35]

Investigations by NMR

The ^{1}H and ^{13}C NMR spectra of saturated solutions of compounds **1**–**4** in the following organic solvents—chloroform-d_1 ($CDCl_3$), acetone-d_6 (Acn-d_6), acetonitrile-d_6 (CD_3CN), dimethyl sulfoxide-d_6 (DMSO-d_6) and pyridine-d_5 (Py-d_5) (purchased from Deutero GmbH, Kastellaun)—were measured at room temperature on a Varian Mercury 400 spectrometer operating at a proton frequency of 400.5 MHz and a carbon frequency of 100.7 MHz. The chemical shifts were referenced to tetramethylsilane (TMS). The homonuclear ^{1}H–^{1}H two-dimensional correlated diagrams were obtained using the COSY pulse sequence. One-bond heteronuclear (^{1}H–^{13}C) correlation spectra were recorded using the gHSQC technique and long-range heteronuclear (^{1}H–^{13}C) correlation spectra by the gHMBC technique.

Calculations

Unconstrained geometry optimizations of isolated molecules (Scheme 1) were carried out at the DFT level[36] using gradient techniques[37] and the 6-31G** basis set.[38,39] The calculations were carried out with the B3LYP functional, in which Becke's non-local exchange[40,41] and the Lee–Yang–Parr correlation functionals[42] were applied. After completion of each optimization, the Hessian (second derivatives of the energy as a function of the nuclear coordinates) was calculated and checked for positive definiteness to assess whether the structures were true minima.[36,39] The harmonic vibrational frequencies were then derived from the numerical values of these second derivatives and used to obtain the Gibbs free energy contributions at 298.15 K and standard pressure with the aid of a built-in computational program of statistical thermodynamics routines.[43]

The solvent effect was included in the single-point DFT calculations utilizing the polarized continuum model (PCM) (UAHF radii were used to obtain the molecular cavity).[44,45] The ^{1}H and ^{13}C magnetic shielding tensors (χ) for DFT-optimized structures of compounds **1**–**4**, and TMS as reference, were obtained following the gauge-including atomic orbital (GIAO) approach[46–48] at the DFT level. The differences between the isotropic magnetic shielding tensors of the nuclei in TMS and the compounds studied ($\chi_{TMS} - \chi_{\mathbf{1–4}}$) were considered to be relevant chemical shifts.[46] All calculations were done on computers of the Tri-city Academic Network Computer Centre in Gdańsk (TASK) using the Gaussian 03 program package.[49]

Dipole moments and atomic partial charges originating from Mulliken population analysis[50] were extracted directly from the data files following the geometry optimisations. Values of the Mulliken mean charge of the substituent at N15 and $\ln_{298}K^{o}$ (equilibrium constant) were obtained in the manner outlined in Ref. 24. Structural data (angles) in Table 1 were obtained using a program written by one of us.

RESULTS AND DISCUSSION

Structure and properties of acridin-9-amines

All the compounds investigated can coexist in the amino and imino tautomeric forms; additionally, compound **4** can exist in the imino-*p* form. Scheme 1 shows the canonical structure of these forms and gives the equilibrium constants of tautomerization. Figure 1 shows DFT-optimized structures of all the possible tautomers and Table 1 provides selected structural information. All the amino tautomers and the imino-*p* tautomer of compound **4** are nearly planar within the acridine moiety, a situation reflected by the respective dihedral angles listed in column C of Table 1. The valence angle reflecting the arrangement of the exocyclic N atom, the C9 and the endocyclic N atom (N10 . . . C9—N15 angle in Table 1) is only slightly smaller than the straight angle in the amino tautomers and the imino-*p* tautomer of compound **4**. This indicates that the exocyclic N atom, together with the substituent attached to it, is only slightly deflected from the plane defined by the acridine moiety (Table 1, column A). The structures of the imino tautomers of compounds **2**, **3** and **4** exhibit a 'butterfly'-type geometry that is reflected by the dihedral angles (Table 1, column C). The exocyclic N atom in these tautomers is deflected quite considerably from the N10 . . . C9 line (the N10 . . . C9—N15 angle in Table 1) and the mean plane of the acridine nucleus (angle given in column A of Table 1). The exception is compound **1**: in this case, hitherto unpublished x-ray data suggest a typical 'butterfly'-type structure, whereas the DFT method predicts a completely flat structure in which all the atoms of the acridine

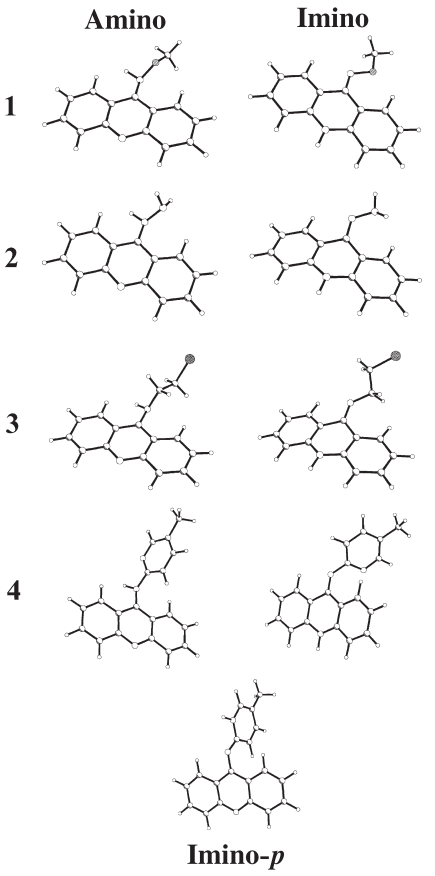

Figure 1. The DFT-optimized structures of the tautomers of acridin-9-amines (Scheme 1)

moiety, the exocyclic N atom and the O and C atoms of the methoxy group lie in one plane of symmetry (Table 1). This atypical structure is most probably stabilized by a C1—H1 . . . O16 interaction (the H1 . . . O16 distance of 2.05 Å is considered significant for this type of interaction).[51,52] The substituent at the exocyclic N atom is twisted relative to the acridine nucleus at an average angle of 57° in all the amino tautomers and at 17° in the imino tautomers of compounds **2**, **3** and **4** (Table 1, column B). The data predicted at the DFT level of theory and x-ray structural data compare quite well in most cases (Table 1), which implies that the geometry of the acridine nucleus is preserved when the molecules in the gaseous phase pass to the crystalline phase.

The amino tautomers of compounds **1** and **2** are more polar than the imino ones, but this tendency is reversed in the case of compounds **3** and **4**, i.e. the imino (also the imino-*p* in the case of compound **4**) tautomers are more polar than the amino ones (Table 2). Dipole moments generally increase with increasing polarity of the medium; the change is more distinct on passing from the gaseous to the liquid phase.

The substituent at the exocyclic N atom is clearly electron-attracting in compound **1**, slightly electron-donating in compound **2**, and quite strongly electron-withdrawing in

Table 1. Structural features of tautomers of acridin-9-amines

Compound (Scheme 1)	Tautomer	Source of structure	Angle[a]			
			N10 . . . C9—N15	A	B	C
1	Amino	DFT	176	3.4	51.5	2.2
	Imino	DFT	171	1.0	1.0	0.9
		X-ray[b]	150	9.2	9.2	9.1
			148	6.8	7.2	9.4
2	Amino	DFT	176	3.4	47.1	2.3
	Imino	DFT	162	18.7	18.9	17.6
3	Amino	DFT	179	0.3	65.8	3.7
		X-ray[c]	179	0.8	27.0	2.5
	Imino	DFT	165	14.6	16.3	13.3
4	Amino	DFT	178	1.2	63.8	3.3
	Imino	DFT	171	6.5	16.5	8.4
	Imino-*p*	DFT	177	1.5	70.0	1.8

[a] A, between the mean plane of the acridine nucleus and C9—N15; B, between the mean plane of the acridine nucleus and the plane delineated by C9, N15 and atom 16; C, between the mean planes delineated by C1, C2, C3, C4, C9, N10, C11, C12 (right-hand half of the acridine nucleus) and C5, C6, C7, C8, C9, N10, C13, C14 (left-hand half of the acridine nucleus).
[b] Unpublished results.
[c] Ref. 35.

Table 2. Physicochemical features of tautomers of acridin-9-amines (Scheme 1)[a]

Computational level	Dipole moments[b] (D)		Mulliken mean charge of substituent at N15[c]	$\ln_{298}K_I^{od}$
	Amino	Imino		
Compound **1**				
DFT	3.36	1.93	−0.106	20.4
DFT(PCM—$CHCl_3$)	4.29	3.15	−0.105	19.2
DFT(PCM—Acn)	4.69	3.70	−0.105	19.5
DFT(PCM—CH_3CN)	4.81	3.84	−0.106	19.3
DFT(PCM—DMSO)	4.79	3.85	−0.105	19.5
Compound **2**				
DFT	4.11	3.04	0.0698	12.7
DFT(PCM—$CHCl_3$)	6.23	3.65	0.0906	12.6
DFT(PCM—Acn)	6.86	4.10	0.0978	12.3
DFT(PCM—CH_3CN)	7.01	4.21	0.0990	12.2
DFT(PCM—DMSO)	7.02	4.23	0.0993	12.3
Compound **3**				
DFT	1.96	5.99	0.142	1.59
DFT(PCM—$CHCl_3$)	2.95	6.74	0.141	2.65
DFT(PCM—Acn)	3.39	7.34	0.141	2.78
DFT(PCM—CH_3CN)	3.52	7.46	0.141	2.66
DFT(PCM—DMSO)	3.52	7.47	0.141	2.97
Compound **4**				
DFT	1.95	3.54 (7.20)	0.121	−4.30 (−13.3)
DFT(PCM—$CHCl_3$)	2.67	4.39 (9.09)	0.114	−2.29 (−8.22)
DFT(PCM—Acn)	2.93	4.93 (9.74)	0.111	−0.613 (−6.38)
DFT(PCM—CH_3CN)	3.00	5.07 (9.85)	0.111	−0.482 (−6.44)
DFT(PCM—DMSO)	3.01	5.07 (9.87)	0.111	−0.216 (−6.06)

[a] Values of the Mulliken mean charge of the substituent at N15 and $\ln_{298}K^o$ were obtained following Ref. 24.
[b] The imino-*p* form, values are in parentheses.
[c] For the amino and imino forms.
[d] The $\ln_{298}K_{II}^o$ values are in parentheses.

compounds **3** and **4** (Table 2). As we showed earlier,[24] this parameter is related to the logarithm of the equilibrium constant for amino–imino tautomerization and hence to the stability of these tautomeric forms.

The NMR spectra of acridin-9-amines

The double bond between the exocyclic N atom and the acridine C9 atom in imino tautomers makes this fragment of the molecule rigid (Scheme 1). On the other

hand, rotation around single bonds can occur within the substituent at the exocyclic N atom. The same applies to this substituent in the amino tautomers. In the latter case, however, the single bond between the exocyclic N atom and the acridine C9 atom permits rotation of the whole fragment around this bond. The consequence of these structural features and possible conformational changes is that only half of the ^{1}H and ^{13}C signals for the imino forms are recorded for the amino tautomers; this applies to all the atoms of the acridine moiety except C9.[22] Therefore, the number of signals in the NMR spectra indicates unequivocally which form we are dealing with in the liquid phase. As far as the substituents at the exocyclic N atom in both amino and imino tautomers are concerned, one can expect averaged ^{1}H and ^{13}C signals because rotation around single bonds may occur. If the molecule exists predominantly in the amino or imino tautomeric form, ^{1}H signals of H1, H4, H5 and H8 occur as more or less resolved doublets, whereas the same signals of H2, H3, H6 and H7 occur as triplets. This is the result of conjugation with neighbouring H atoms. When tautomerization takes place, some or all of the signals are unresolved because the acridine moiety changes its structure from the planar (amino form) to the 'butterfly' type (imino form). The pattern of the ^{1}H NMR spectrum thus provides information on the presence or absence of tautomerization in solution.

As shown in Fig. 1, all the theoretically predicted structures of both amino and imino forms correspond to the lowest energy conformations. In consequence, each H and C atom is characterized by unique, theoretically predicted ^{1}H and ^{13}C signals (Tables 3 and 4). This implies that experimental and theoretical NMR characteristics are difficult to compare directly. Nonetheless, knowledge of theoretical NMR data makes it easier to assign the signals in experimental spectra.

One-dimensional ^{1}H and ^{13}C spectra appear to be insufficient for assigning signals to particular atoms,[53] even if we refer to the literature data on the NMR spectroscopy of acridin-9-amines.[22,54–58] We thus employed HSQC, HMBC and decoupling techniques[59] in order to complete the assignment; this is demonstrated in Tables 3 and 4. Compound **1** exists as an imino tautomer in all solvents: the unique ^{1}H and ^{13}C signals of atoms belonging to the acridine moiety testify to this. The single ^{1}H signal of CH_3 weighs in favour of free rotation around the O16—C17 bond. The ^{1}H signal of the H atom attached to the endocyclic N atom is quite sharp and occurs at relatively high ppm values. Like compound **1**, compound **2** in all solvents occurs in the imino form that results from the unique ^{1}H and ^{13}C signals of the atoms in the acridine moiety. The single ^{1}H signal of NH_2 indicates that this group very probably rotates freely around the N15—N16 bond. The ^{1}H signal of the H atom at the endocyclic N atom generally occurs at lower ppm values than in compound **1**. Occurring as an amino tautomer in the crystalline phase,[35] compound **3** also exists in this form when dissolved in $CDCl_3$ (at both 25 °C and −60 °C). If the compound is dissolved in acetone-d_6, the amino form is prevalent at 25 °C, but with decreasing temperature the amino and imino forms coexist without the rapid tautomerization demonstrated by the NMR spectra in Fig. 2. The NMR spectra of compound **3** in CD_3CN are typical of the amino form, although the ^{1}H signal of the H atom at the exocyclic N atom is not seen. The ^{1}H and ^{13}C signals for compound **3** in DMSO-d_6 and pyridine-d_6 are broad and poorly resolved, which indicates that rapid tautomerization is taking place. The ^{1}H signals of the migrating H atom are very broad and were tentatively assigned to the imino (DMSO-d_6) or amino (pyridine-d_6) forms.

Compound **4** is more susceptible to tautomerization than compound **3**, as the equilibrium constants in Table 2 show. Also, the pattern of the NMR spectra of compound **4** is more complicated, particularly when two tautomers coexist. This means that we were unable to assign all the ^{1}H and ^{13}C signals. Tables 3 and 4 show what was actually done. Fortunately, the ^{1}H signal relevant to the CH_3 group in the 5-methylpyridin-2-yl substituent is unique to the amino and imino forms, and was thus used to distinguish coexisting tautomeric forms. The ^{1}H and ^{13}C signals in the NMR spectra of compound **4** in $CDCl_3$ are broad, indicating rapid tautomerization with the amino form predominant. If compound **4** is dissolved in acetone-d_6, the amino form prevails at 25 °C, whereas with a drop in temperature more of the imino form occurs (Fig. 3 and Table 3). Tautomerization is slow at low temperatures, so the NMR spectra are superimposed on those due to the amino and imino forms (Fig. 3). In DMSO-d_6 two forms, not tautomerizing rapidly, can be noted: we see two separate signals, which are due to the migrating H atom and CH_3 group in the 5-methylpyridin-2-yl substituent. Compound **4** dissolved in pyridine-d_6 undergoes tautomerization because the ^{1}H and ^{13}C signals are broad and only one signal is seen for the CH_3 attached to the 5-methylpyridin-2-yl substituent.

Tautomerization of acridin-9-amines

The equilibrium constants of tautomerization for compounds **1** and **2** are so high that there can be no doubt that both compounds should occur mainly as imino tautomers (Table 2). Indeed, the NMR and preliminary x-ray data for compound **1** confirm this (Table 1). The substituent in compound **1** is electron-attracting, whereas in compound **2** it is almost equally strongly electron-withdrawing. With both these substituents, then, the imino tautomers are much more stable than the amino tautomers; this concurs with our recent findings.[24] Introducing them into molecules yields stable imino tautomers of acridin-9-amines (the imino form of compound **1** is further

Table 3. Theoretically predicted and experimental ^{1}H NMR chemical shifts (ppm) for the compounds investigated

				Hydrogen atom number[a]																	
										16			17						22		
Compound	Method	Solvent (temp. °C)	Form	1/8	2/7	3/6	4/5	10	15	a		b	a	b	c	18	20	21	a	b	c
1	Theory		Amino	7.95/8.53	7.54/7.53	7.77/7.80	8.26/8.21		7.84				3.77	4.34	3.85						
			Imino	8.51/9.34	7.00/6.88	7.21/7.26	6.47/6.48	5.54					3.87	4.01	4.01						
	Exp.	$CDCl_3$ (25)	Imino	8.09/8.85	6.99/6.99	7.31/7.31	6.87/6.87	6.86						4.08							
		$CDCl_3$ (−60)	Imino	8.04/8.81	7.04/7.04	7.35/7.38	6.93/6.95	7.10						4.09							
		Acn-d_6 (25)	Imino	8.06/8.87	6.97/6.93	7.32/7.36	7.06/7.11	9.29						4.00							
		Acn-d_6 (−50)	Imino	8.04/8.86	6.99/6.95	7.35/7.39	7.07/7.12	9.68						3.98							
		Acn-d_6 (−94)	Imino	8.01/8.85	7.01/6.97	7.38/7.42	7.07/7.12	9.96						3.96							
		CD_3CN (25)	Imino	8.00/8.82	6.97/6.93	7.34/7.37	7.01/7.05	8.35						3.99							
		DMSO-d_6 (25)	Imino	7.94/8.75	6.96/6.93	7.35/7.40	7.08/7.12	12.2						3.96							
		Py-d_5 (25)	Imino	8.48/9.25	7.10/7.05	7.35/7.38	7.28/7.35	11.1						4.16							
2	Theory		Amino	7.80/9.53	7.44/7.47	7.70/7.75	8.19/8.14		5.97	3.53		3.63									
			Imino	8.17/8.25	7.01/6.95	7.13/7.20	6.44/6.54	5.28		4.99		5.05									
	Exp.	$CDCl_3$ (25)	Imino	7.92/8.29	7.03/7.03	7.26/7.33	6.84/6.97	6.72			5.66										
		$CDCl_3$ (−60)	Imino	7.87/8.25	7.06/7.06	7.29/7.36	6.90/7.02	7.10			5.70										
		CD_3CN (25)	Imino	7.81/8.27	6.97/6.97	7.23/7.31	6.93/7.05	7.99			5.79										
		DMSO-d_6 (25)	Imino	7.75/8.23	6.87/6.90	7.19/7.29	6.94/7.06	9.68			6.27										
		Py-d_5 (25)	Imino	8.44/8.72	7.07/7.01	7.23/7.30	7.11/7.01	10.5			6.73										
3	Theory		Amino	7.93/8.21	7.58/7.59	7.80/7.82	8.29/8.28		3.84	3.48		3.69	4.07		3.57						
			Imino	8.55/7.95	7.13/7.10	7.36/7.40	6.65/6.76	5.85		3.98		4.16	3.93		3.44						
	Exp.	$CDCl_3$ (25)	Amino	8.14	7.42	7.69	8.07		5.4		4.07			3.72							
		$CDCl_3$ (−60)	Amino	8.20	7.50	7.77	8.12		5.51		4.11			3.68							
		Acn-d_6 (25)		8.26	7.26	7.57	7.67				4.22			3.91							
		Acn-d_6 (−50)						10.0	4.12		4.23			3.94							
		Acn-d_6 (−94)						10.36	4.39		4.23			3.97							
		CD_3CN (25)	Amino	8.18	7.31	7.60	7.73				4.11			3.81							
		DMSO-d_6 (25)		8.18	7.20	7.55	7.55	10.35			4.16			3.92							
		Py-d_5 (25)		8.47	7.24	7.54	7.75		5.3		4.34			4.01							
4	Theory		Amino	8.25/8.33	7.64/7.54	7.88/7.84	8.36/8.35		6.73					6.40		7.18	8.21		1.08	2.21	2.67
			Imino	8.90/7.56	7.21/6.74	7.44/7.31	6.74/6.73	6.08						6.93		7.56	8.14		1.78	2.28	2.32
			Imino-*p*	7.97/8.38	7.36/7.40	7.70/7.74	8.19/8.16							5.72		6.63	6.80	7.38	1.49	1.99	2.01
	Exp.	$CDCl_3$ (25)		8.18	7.47	7.78	8.25							6.14		7.18	8.00			2.19	
		Acn-d_6 (25)																		2.25	
		Acn-d_6 (−50)	Amino																	2.19	
			Imino																	2.32	
		Acn-d_6 (−94)	Amino																	2.17	
			Imino																	2.31	
		DMSO-d_6 (25)	Amino						9.56											2.15	
			Imino					11.0												2.28	
		Py-d_5 (25)		8.43	7.28	7.63	8.09							6.77		7.36	8.32			2.14	

[a] Identical to the carbon atom number to which it is attached (Scheme 1).

Table 4. Theoretically predicted and experimental ^{13}C NMR chemical shifts (ppm) for the compounds investigated at 25 °C

				Carbon atom number												
Compound	Method	Solvent	Form	1/8	2/7	3/6	4/5	9	11/14	12/13	16	17	18	19	20	22
1	Theory		Amino	122.0/114.5	119.4/118.7	123.0/123.9	127.8/126.7	142.4	112.0/115.3	144.0/145.1		60.4				
			Imino	121.6/129.5	115.7/114.3	123.6/124.9	108.4/108.4	138.8	117.9/115.2	131.4/133.5		59.9				
	Exp.	$CDCl_3$	Imino	125.1/132.2	121.6/120.0	129.9/131.0	114.6/115.1	143.8	118.7/118.7	137.6/139.8		62.8				
		Acn-d_6	Imino	125.5/132.7	121.6/120.0	130.6/131.8	115.9/116.3	143.9	119.0/115.8	139.1/141.4		62.7				
		CD_3CN	Imino	124.6/131.9	121.1/119.4	130.1/131.2	115.1/115.4	143.4	118.1/114.9	138.2/140.5		62.1				
		DMSO-d_6	Imino	124.1/131.3	120.5/118.8	129.9/131.1	115.1/115.5	142.9	117.0/114.1	137.8/140.1		62.2				
		Py-d_5	Imino	125.8/133.1	121.6/121.6	130.7/131.9	116.1/116.5	144.8	119.9/119.9	139.7/142.0		63.1				
2	Theory		Amino	114.2/123.8	118.5/117.5	122.7/123.8	127.8/126.2	143.6	112.8/114.5	143.9/114.5						
			Imino	121.3/123.4	116.3/113.4	122.4/123.7	107.7/108.9	133.8	120.9/114.7	132.0/136.0						
	Exp.	$CDCl_3$	Imino	124.7/128.3	119.7/122.0	129.0/130.2	114.3/115.7	140.1	122.3/115.5	138.1/141.4						
		CD_3CN	Imino	125.2/129.0	122.0/120.1	129.6/130.9	116.5/116.5		115.3/116.5	138.8/142.8						
		DMSO-d_6	Imino	123.8/127.7	120.3/118.5	128.1/129.5	114.3/115.3	136.2	122.0/115.6	138.4/141.5						
		Py-d_5	Imino	125.5/129.0	119.4/117.0	129.1/130.4	115.2/116.3	138.7	121.5/116.3	140.1/143.3						
3	Theory		Amino	115.3/119.1	119.9/119.6	123.6/124.0	128.0/127.7	144.6	116.5/116.0	144.5/145.2	54.0	47.7				
			Imino	123.8/126.1	116.4/114.1	124.8/116.4	108.3/109.7	148.4	121.7/116.1	132.2/114.1	57.7	51.4				
	Exp.	$CDCl_3$	Amino	124.1	130.3	130.3	129.3	150.7	118.6	149.0	52.2	45.6				
		Acn-d_6		126.2	125	130.9	130.9	152.9	122.7	146	54.4	46.2				
		CD_3CN	Amino	125.7	123.4	131.2	126.2	152.8	118.4	147.6	53.7	46.5				
		DMSO-d_6		125.8	120.9	130.2	125.8	152.3	118	144	53.3	45.9				
		Py-d_5	Amino	127.2	122.1	131.1	127.2	153.7			55.0	47.0				
4	Theory		Amino	117.7/121.4	118.5/119.9	123.9/120.9	127.4/127.5	136.6	118.5/118.4	145.1/145.2	150.5	101.1	132.1	120.5	143.9	18.6
			Imino	125.0/127.3	116.2/113.9	125.5/116.2	108.7/109.9	148.3	120.9/114.7	132.8/134.7	158.2	111.2	131.8	121.0	144.6	19.0
			Imino-*p*	120.3/121.5	117.9/117.9	123.1/123.6	127.1/126.0	149.4	115.4/118.0	145.2/145.3	143.1	111.9	134.4	109.3	126.3	18.2
	Exp.	$CDCl_3$		124.1	125.8	130.5	130.3			142.5	150.2	108.0	139.1	122.6	148.3	17.7
		DMSO-d_6	Amino	130.1	140.0	131.6	125.6		120.1	152.0	155.6	109.6	123.9	148.7	138.6	17.0
			Imino	129.3	124.8	140.0	130.1	147.1	121.9	127.2	162.9	113.2	124.6	149.3	138.8	17.4
		Py-d_5			125.6	131.3						111.4	139.3	127.0	149.5	18.0

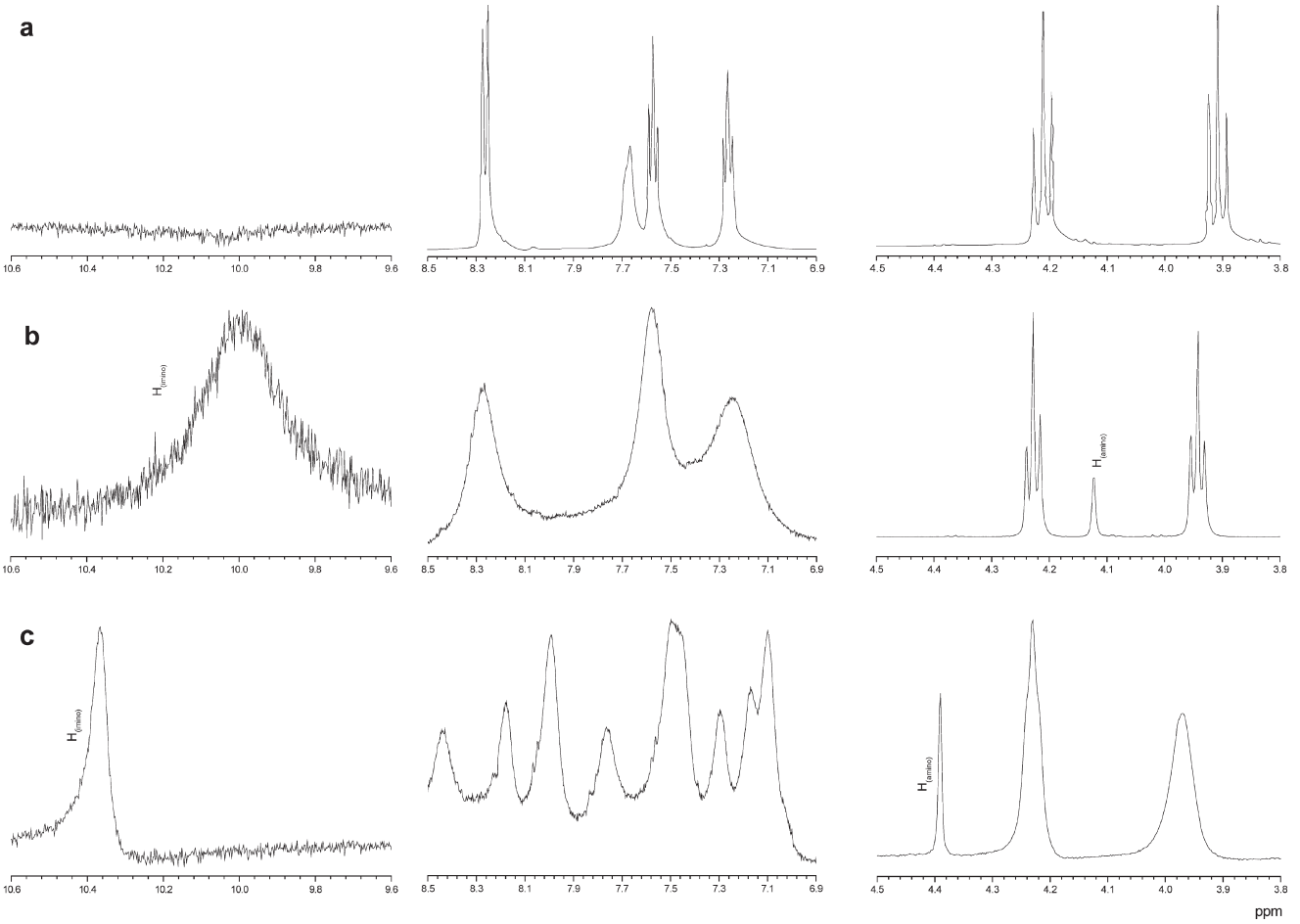

Figure 2. The ^{1}H NMR spectra of compound **3** in Acn-d_6: (a) at 25 °C; (b) at −50 °C; (c) at −94 °C

stabilized owing to the possible occurrence of a C—H . . . O interaction). The electron-withdrawing nature of the substituents is similar in compounds **3** and **4** and more distinct than that in compound **2**, which may be why the former compounds can coexist in two tautomeric forms (the imino-*p* tautomer of compound **4** is not as stable as the corresponding imino tautomer). The NMR investigations confirm this. The tautomeric equilibria in compounds **3** and **4** are strongly affected by the properties of the medium, because the tautomeric forms exhibit various polarities (Table 2) and electrostatic potential distributions around the molecules.[24] We were unable to find any simple relation between the abilities of compounds **3** and **4** to tautomerize and any well-defined property of the medium. However, if one could be found, these compounds could serve as probes for monitoring the properties of liquids.

It is perhaps worth mentioning that tautomeric equilibria in various acridin-9-amines have been monitored by NMR spectroscopy and our results correspond well with those reported elsewhere by others.[57,60–62] What arises from all of these investigations is that tautomeric phenomena are strongly influenced by the medium's properties. Unfortunately, we were unable to find any relationship combining these two facts.

CONCLUDING REMARKS

Of the four acridin-9-amines investigated, two—one with an OCH_3 (**1**) and one with an NH_2 (**2**) substituent at the exocyclic N atom—occur mainly (in solution and in the solid phase) as imino tautomers whereas the other two—one with CH_2CH_2Cl (**3**) and one with 5-methylpyridin-2-yl (**4**) as substituents—exhibit an ability to tautomerize that is strongly influenced by the properties of the medium. These findings go hand in hand with the results of our recent studies.[24]

It has long been a topic of discussion whether stable imino tautomers of acridin-9-amines substituted at the exocyclic N atom can exist. The results presented here prove that they do exist (compounds **1** and **2**), they can be obtained in crystalline form and they can serve as convenient models of such compounds.

Compounds **3** and **4** are examples of acridin-9-amines whose amino and imino tautomers coexist in liquid phases and whose tautomeric equilibria are strongly affected by the medium's properties. This finding indicates that these compounds may be of interest as molecular probes for monitoring the properties of liquids. Compound **4** also appears interesting as a probe in studies of DNA behaviour.[13,14]

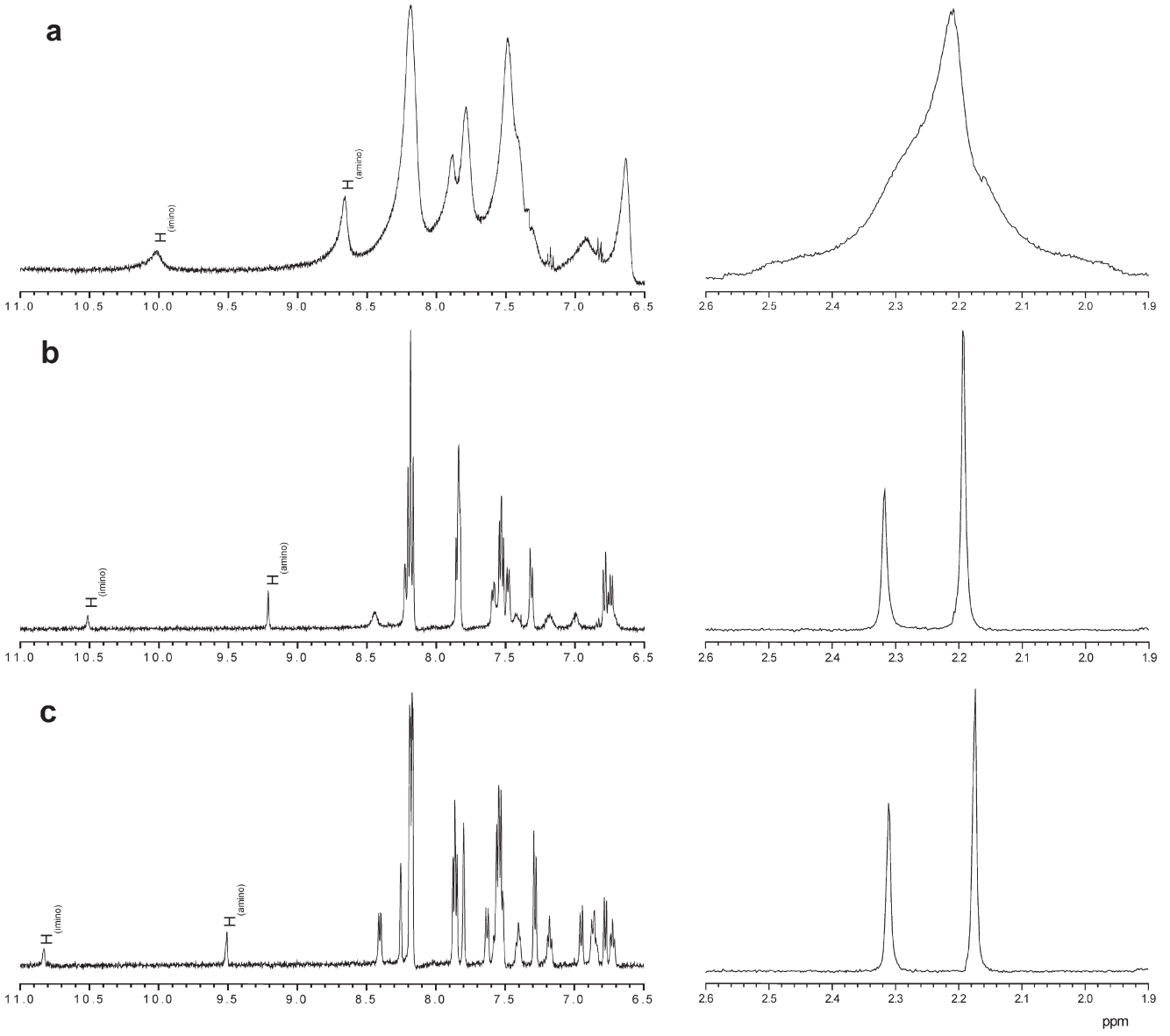

Figure 3. The ^{1}H NMR spectra of compound **4** in Acn-d_6: (a) at 25 °C; (b) at −50 °C; (c) at −90 °C

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