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Supporting Information

An Enolizable Barbiturate with Adjustable Hydrogen-Bonding Structure for UV/vis Detection of Nucleic Acid Bases and Related Compounds

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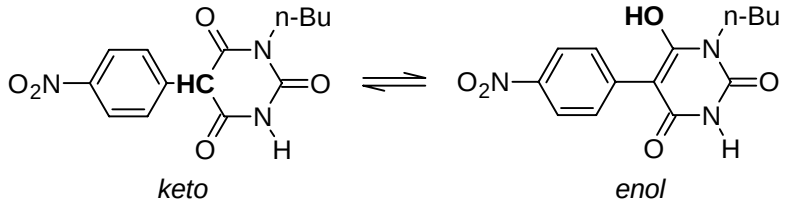
Table of contents

Materials. Unless otherwise noted, all materials were received from commercial suppliers and used without further purification. All reaction and deuterated solvents were dried over appropriate drying agents and freshly distilled prior to use. Synthesis of the chromophor 1-*n*-butyl-5-(4-nitrophenyl)-barbituric acid **1** is described in the literature.[S1] 2,6-Diaminopyridine (**DAP**; Acros Organics, 98%) was purified by recrystallization from hot chloroform after filtration with charcoal. The receptors 2,6-diacetamidopyridine **DAC**[S2], 2,6-bis(trifluoroacetamido)pyridine **TFA**[S3], 9-ethyladenine **EtAd**[S4], 1-butylcytosine **BuCy**[S5] and 1-butylthymine **BuTy**[S6] were synthesized by literature-known procedures. 9-Ethylguanine was received from Sigma Aldrich (≥ 98 %).

Instrumentation. The UV/Vis absorption spectra of freshly prepared solutions were obtained by means of an MCS 400 diode-array spectrometer (Carl Zeiss Jena). Multiple regression analysis and the linear curve-fitting were performed with Origin 5.0 statistical program. The ^{1}H NMR titration experiments were obtained on a Varian Inova-400 spectrometer. Chemical shifts were reported as δ-values in parts per million (ppm) relative to $Si(CH_3)_4$ as relative reference (δ = 0 ppm) and to the solvent as internal reference. Minimization of the sum of squared deviations between the observed experimental data points and those calculated with the proposed model was performed employing the nonlinear least-squares fitting program Origin 5.0 which is based on a modified Levenberg-Marquardt algorithm. A detailed explanation for UV/Vis- and ^{1}H-NMR spectroscopic analyses is given in the literature.[S7]

***Keto-enol*-tautomerism and self-aggregation of 1.**



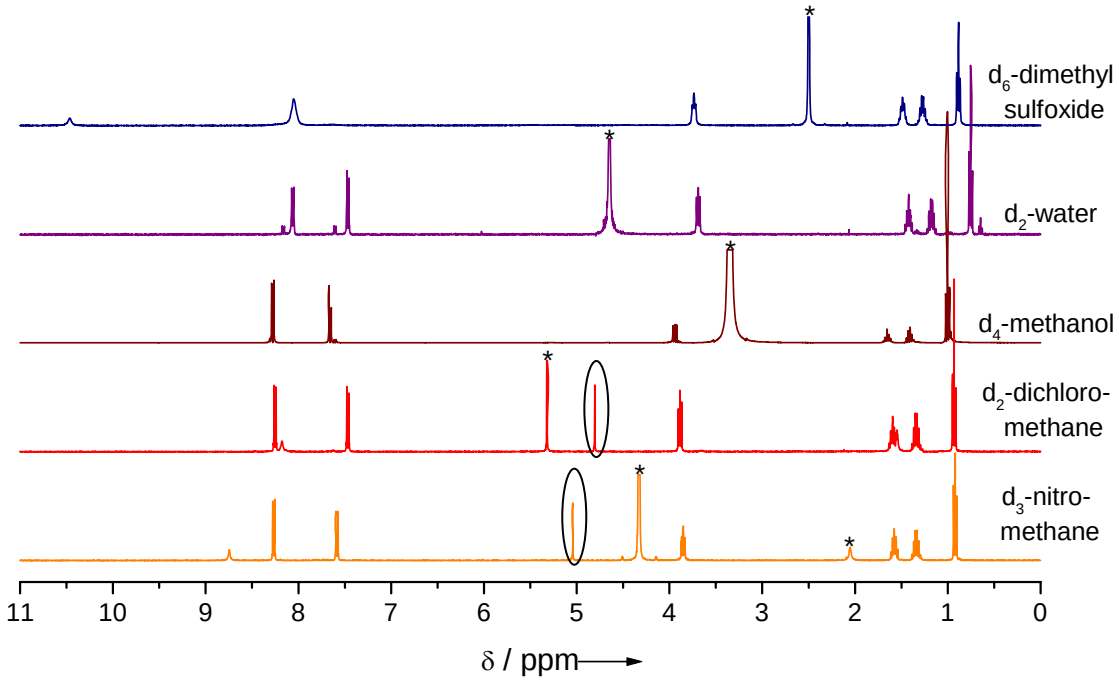


Figure S1. ^{1}H NMR spectra of **1** (8.19 mmol/l) in different deuterated solvents (marked with asterisk). The presence of a methine proton (black framed) supports the *keto*-structure for **1** in nitromethane and dichloromethane. In water a protonation-deprotonation equilibrium is observed.

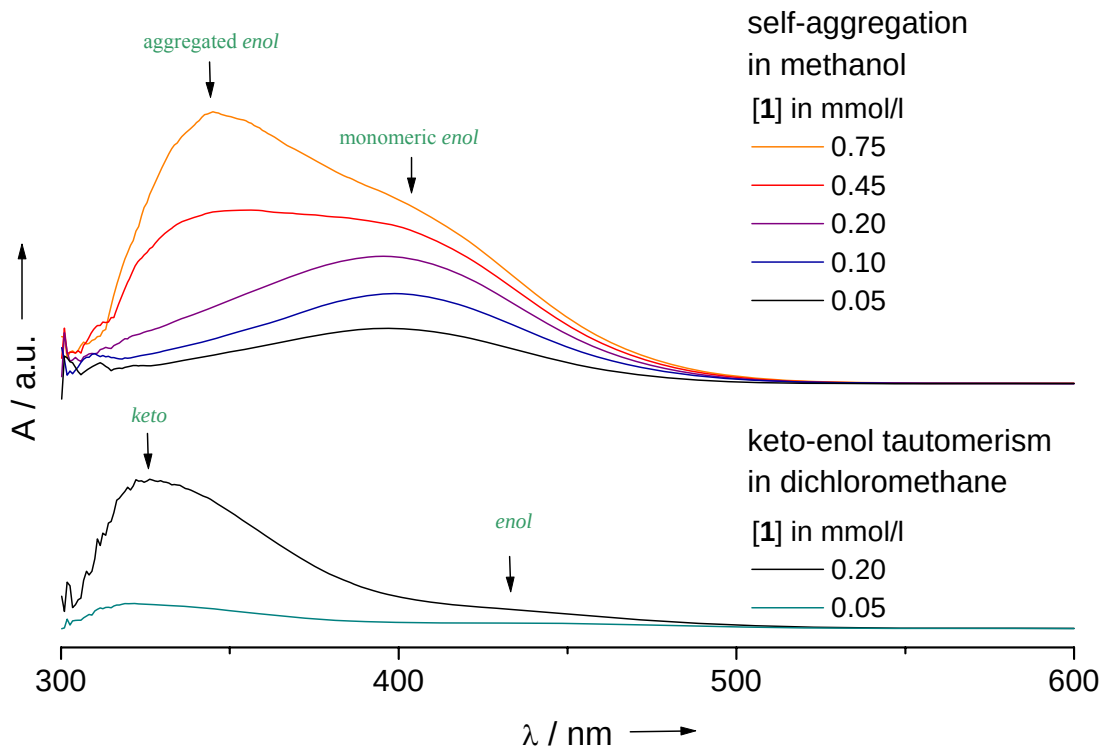


Figure S2. Concentration-dependent UV/Vis titration of **1** in methanol and dichloromethane. Self-aggregation as well as *keto-enol* tautomerism of **1** is dependent on the polarity of the solvent. In methanol ($E_T(30)$ = 55.4)[S8d] the stacking of the *enol* form is observed. In the weaker polar solvent dichloromethane ($E_T(30)$ = 40.7)[S8d] the non-solvatochromic *keto* form is preferred.

Solvatochromism of 1.

Table S1. UV/Vis absorption maxima ($\tilde{\nu}_{max}$) of the barbiturate **1** in various solvents, the empirical Kamlet-Taft parameters α, β, π* and the extend of the solvatochromic absorption shift.[S8, S9]

solvent	**α**	**β**	**π***	$\tilde{\nu}_{max}$ **[10^{-3} cm^{-1}]**
1,1,1,3,3,3-hexafluoro-2-propanol (HFP)	1.96	0	0.65	27.13[a]
2,2,2-trifluoroethanol (TFE)	1.49	0	0.73	**26.54**[e]
water	1.17	0.47	1.09	25.90[a]
1,2-ethanediol	0.90	0.52	0.92	24.74
methanol	0.98	0.66	0.60	25.14
ethanol	0.54	0.75	0.86	24.74
2-propanol	0.76	0.84	0.48	24.29
1-butanol	0.84	0.84	0.47	24.74
anisole	0	0.22	0.73	24.28[a]
m-cresol	1.13	0.34	0.68	–[b]
acetone	0.08	0.48	0.71	21.87
ethyl acetate	0	0.45	0.55	23.43
γ-butyrolactone	0	0.49	0.87	21.78
acetonitrile	0.19	0.40	0.75	22.44
benzene	0	0.10	0.59	24.28
toluene	0	0.11	0.54	24.35
chloroform	0.20	0.10	0.58	25.08
dichloromethane	0.13	0.10	0.82	22.81
tetrachloromethane	0	0.05	0.28	–[c]
1,1,2,2-tetrachloroethane	0	0	0.95	22.61
diethyl ether	0	0.47	0.27	–[b]
tetrahydrofuran	0	0.55	0.58	23.05
triethylamine	0	0.71	0.14	–[b]
pyridine	0	0.64	0.87	21.33
N,*N*,*N*',*N*'-tetramethyl urea (TMU)	0	0.80	0.83	**21.30**[d]
formamide	0.71	0.48	0.96	23.67
N-methylformamide	0.62	0.80	0.90	23.26
N,*N*-dimethylformamide	0	0.69	0.88	21.52
dimethyl sulfoxide	0	0.76	1.00	21.50
$[C_6\text{-mim}]^+ BF_4^-$	0.44	0.60	0.96	22.55
$[C_6\text{-mim}]^+ Br^-$	0.35	0.88	1.06	21.88
$[C_6\text{-mim}]^+ Cl^-$	0.30	0.97	1.06	21.58
Δλ [nm]				93
$\Delta\tilde{\nu}$ [cm^{-1}]				5232

[a]excluded for correlation, [b]compound present in not-solvatochromic higher aggregation or *keto*-form, [c]compound insoluble, [d]highest bathochromic shift, [e]highest hypsochromic shift

Table S2. The solvent-independent correlation coefficients of the solvatochromic compounds **1** using the Kamlet–Taft linear solvation energy relationship (excluding HFP, H_2O, anisole).[S8]

$\tilde{\nu}_{max,0}$	a	b	s	r	SD	F	n
25.9	+2.41	–1.89	–3.26	0.954	0.473	<0.0001	25
23.5	+2.67	–2.41	———	0.864	0.775	<0.0001	25
27.2	———	–1.39	–4.24	0.668	1.144	0.0015	25
25.5	+2.23	———	–3.97	0.883	0.722	<0.0001	25
22.3	+2.50	———	———	0.722	1.041	<0.0001	25
24.3	———	–2.014	———	0.399	1.378	0.0482	25
26.8	———	———	–4.72	0.612	1.189	0.0012	25

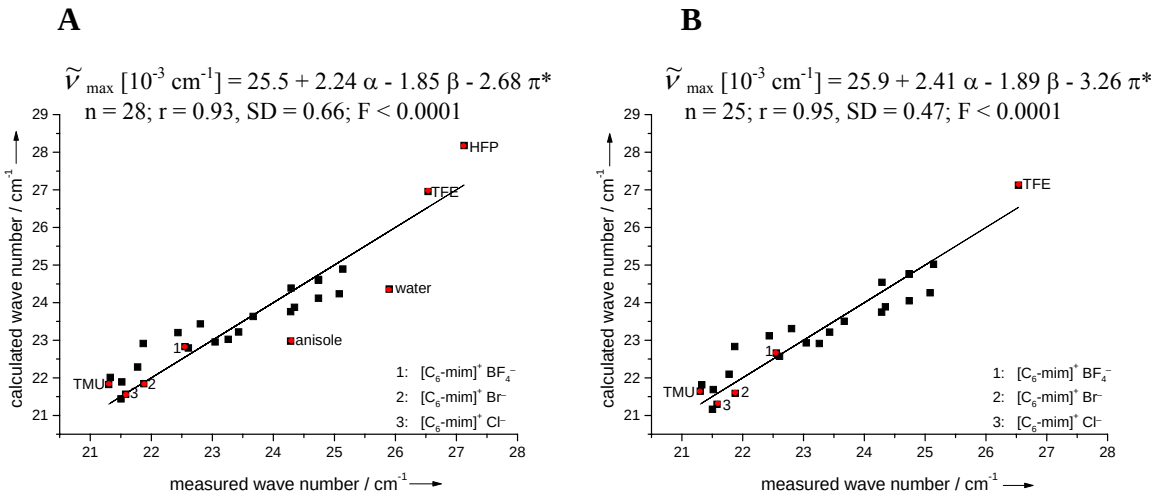


Figure S3. Comparison of the results of the Kamlet-Taft analysis of **1** in all solvent used (**A**) and without 1,1,1,3,3,3-hexafluoro-2-propanol (HFP), water and anisole (**B**).

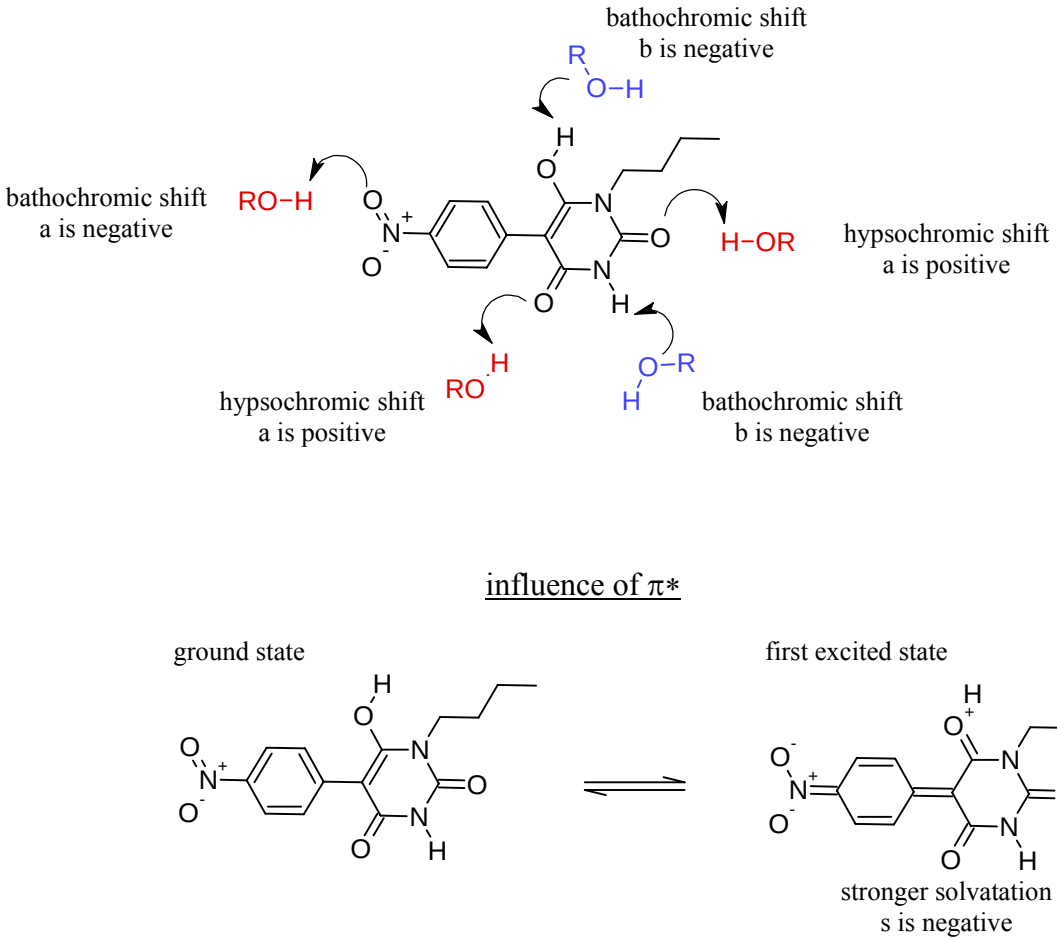


Figure S4. Possible solute-solvent interactions. The multiple correlation analyses of the $\tilde{\nu}_{max}$ data with the used Kamlet-Taft parameters show a significant influence of the hydrogen bonding acidity α and basicity β of the solvent on $\tilde{\nu}_{max}$.

^{1}H NMR titration with the ionic liquid $[C_6\text{-mim}]^+$ Cl^-. The stock solutions of **1** (5.45 mmol/l) and the ionic liquid 1-Hexyl-3-methylimidazolium chloride **$[C_6\text{-mim}]^+$ Cl^-** (107 mmol/l) were prepared in dried deutero-dichloromethane under Ar-atmosphere. The **$[C_6\text{-mim}]^+$ Cl^-**-solution was added in 10 μl steps to **1**.

Determination of the pK_A values of 1. The UV/Vis spectra of **1** were recorded in aqueous solution (0.195 mmol/l) from 180 to 1000 nm. The *p*H was varied by addition of 1 M HCl or 1 M NaOH and was measured with the VARIO *p*H-meter (WTW, Weilheim). Three different maxima of the longest wavelength band λ_{max} were found as function of *p*H value: at 378 nm (*p*H < 1.8), 385 nm (*p*H 2.40–8.78) and at 412 nm (*p*H > 12.93). In every spectrum the absorbances at 378 nm (A1), 385 nm (A2) and 412 nm (A3) were determined (figure S5). The pK_A-values were estimated as the intercept of the plotting log(A2/A1) or log (A3/A2) as a function of *p*H according to the Henderson-Hasselbalch equation.[S7a]

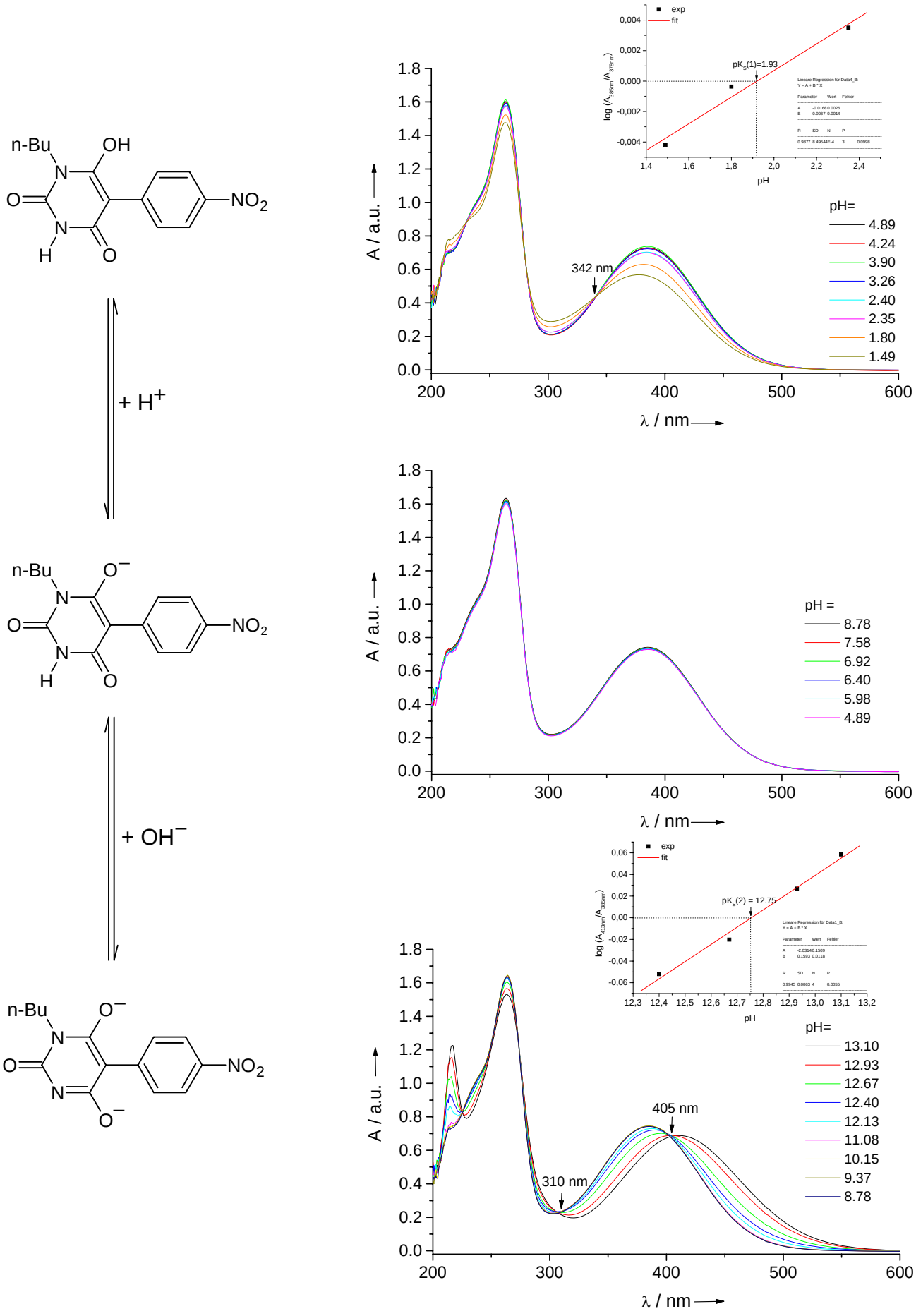


Figure S5. Set of UV/Vis absorption spectra of **1** under the variation of *p*H value.

Binding studies of 1+DAP. The binding study between the model base **DAP** and the host **1** was investigated by UV/Vis and ^{1}H NMR spectroscopy.

UV/Vis titration. The UV/Vis titration was performed using dichloromethane (freshly distilled from CaH_2). A typical binding experiment involved the titration of **1** (25 ml, 0.131 mmol/l) where ten aliquots of 10 µl and five aliquots of 20 µl of the **DAP** stock solution (32.989 mmol/l) were added. The increase in UV/Vis absorption intensity at 411 nm was monitored as a function of guest concentration. The 1:1 stoichiometry of the adduct **1+DAP** was confirmed with the method of continuous variations (Job's method, figure S6).[S7a]

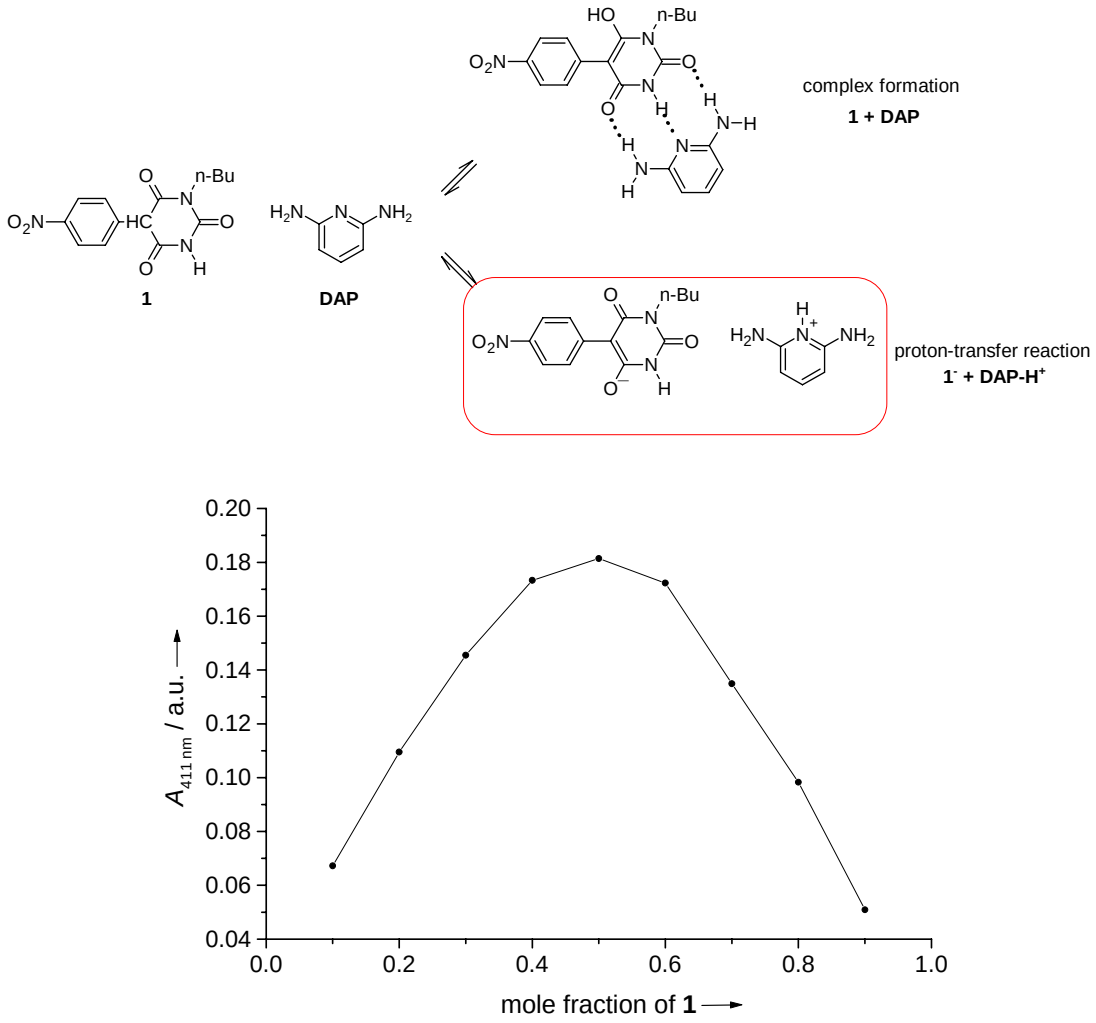


Figure S6. Job's plot between **1** and **DAP** (every stock solution: 0.131 mmol/l) observed by UV/Vis spectroscopy in dichloromethane. The simple acid-base reaction should be favourable.

^{1}H NMR spectroscopy. ^{1}H NMR binding studies were carried out in dried d_6-dimethyl sulfoxide (residue water concentration < 0.1%). In a typical experiment, the ^{1}H NMR spectrum of the host solution (**1**, 2.2 mmol/l, 1.0 ml of solvent) was recorded and then small portions of **DAP** were added to the NMR tube under argon atmosphere. The concentration of **DAP** was calculated by the comparison of the integrals. After the 1:1 stoichiometry was reached, the chemical shifts of the NH proton of **1** were monitored as a function of **DAP** concentration for the determination of the association constant K_A (figure S7).[S7a]

K_A for the complex formation of the enolate anion $\mathbf{1}^-$ with **DAP** was then obtained as a constant using the simple equilibration equation (1).

$$\mathbf{1} + \mathbf{DAP} \longrightarrow \mathbf{1}^- + \mathbf{DAP\text{-}H}^+$$

$$\mathbf{1}^- + \mathbf{DAP} \underset{}{\overset{K_A}{\rightleftharpoons}} \mathbf{1}^- + \mathbf{DAP} \qquad \text{(equ. 1)}$$

If we assume that the proton transfer reaction is complete and only a 1:1 complex is formed, then the association constant K_A is given by

$$K_A = \frac{x}{(\mathbf{1}_0 - x)(\mathbf{DAP_0} - x)} \qquad \text{(equ. 2)}$$

where x is the concentration of the formed complex and $\mathbf{1}_0$ and $\mathbf{DAP}_0$ are the initial concentrations in mol/l. The observed chemical shift of the enolate NH proton δ_{obs} is given by

$$\delta_{obs} = \frac{\mathbf{1}_0 - x}{\mathbf{1}_0}\delta_{\mathbf{1}^-} + \frac{x}{\mathbf{1}_0}\delta_{\mathbf{1}_c^-} \qquad \text{(equ. 3)}$$

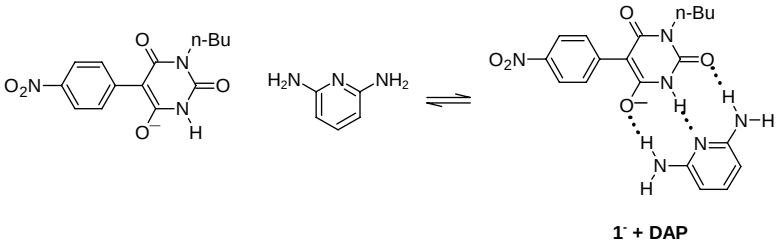
where $\delta_{\mathbf{1}^-}$ is the chemical shift of the NH proton of the free enolate anion $\mathbf{1}^-$ and $\delta_{\mathbf{1}_c^-}$ the chemical shift of the NH proton of the complexed enolate anion $\mathbf{1}^-_\mathbf{c}$.

The titration curve (equ. 4) represents the relationship between the observed chemical shift of the enolate NH proton δ_{obs} and the total concentration of DAP $\mathbf{DAP_0}$ with the three unknown parameters $\delta_{\mathbf{1}^-}$, $\delta_{\mathbf{1}_c^-}$ and K_A:

$$\delta_{obs} = \delta_{\mathbf{1}^-} + \frac{\delta_{\mathbf{1}_c^-} - \delta_{\mathbf{1}^-}}{2 \cdot \mathbf{1_0}} \cdot \left[\left(\mathbf{DAP_0} + \mathbf{1_0} + K_A^{-1}\right) - \sqrt{\left(\mathbf{DAP_0} - \mathbf{1_0}\right)^2 + \left(2 \cdot \mathbf{1_0} \cdot K_A^{-1}\right) + \left(2 \cdot \mathbf{DAP_0} \cdot K_A^{-1}\right) + K_A^{-2}} \right]$$

(equ. 4)

Minimization of the sum of squared deviations between the observed experimental data points and those calculated with the proposed model was performed employing the nonlinear least-squares fitting program ORIGIN which is based on a modified Levenberg-Marquardt algorithm.



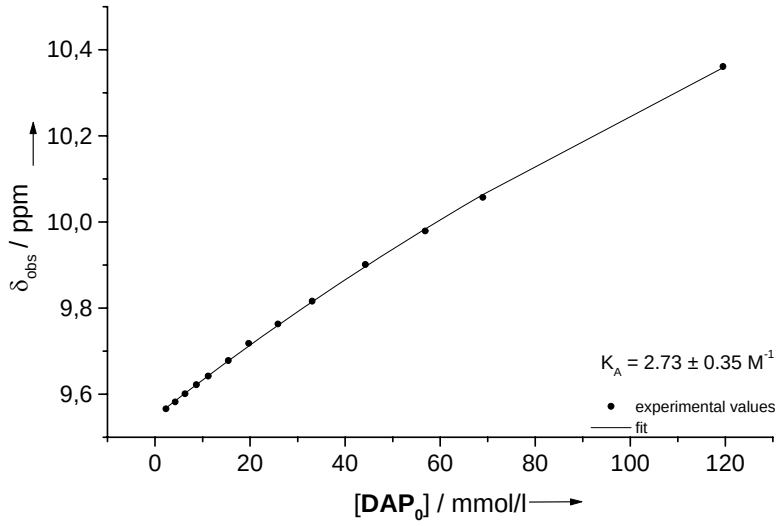


Figure S7. Observed chemical shift of the enolate NH proton of **1** δ_{obs} ([$\mathbf{1_0}$] = 2.16 mmol/l) as function of the total concentration of $\mathbf{DAP_0}$ in dried d_6-DMSO.

Self-association study of the salt 1+PS. *UV/Vis titration.* The binding study between **1** and the proton sponge® **PS** was performed according to the UV/Vis binding study of **1** and **DAP**. To the stock solution of **1** (25 ml, 0.131 mmol/l) were added ten aliquots of 10 µl and five aliquots of 20 µl of the **PS** stock solution (32.664 mmol/l) in dichloromethane (freshly distilled from CaH_2). The increase in UV/Vis absorption intensity at 451 nm was monitored as a function of guest concentration [$\mathbf{PS_0}$]. Saturation is reached with the 1 : 1 stochiometry which shows that self-aggregation of the enolate anion is neglible in this concentration range. The 1:1 stoichiometry of the adduct **1+PS** was confirmed with the method of continuous variations (Job's method, figure S8).[S7a]

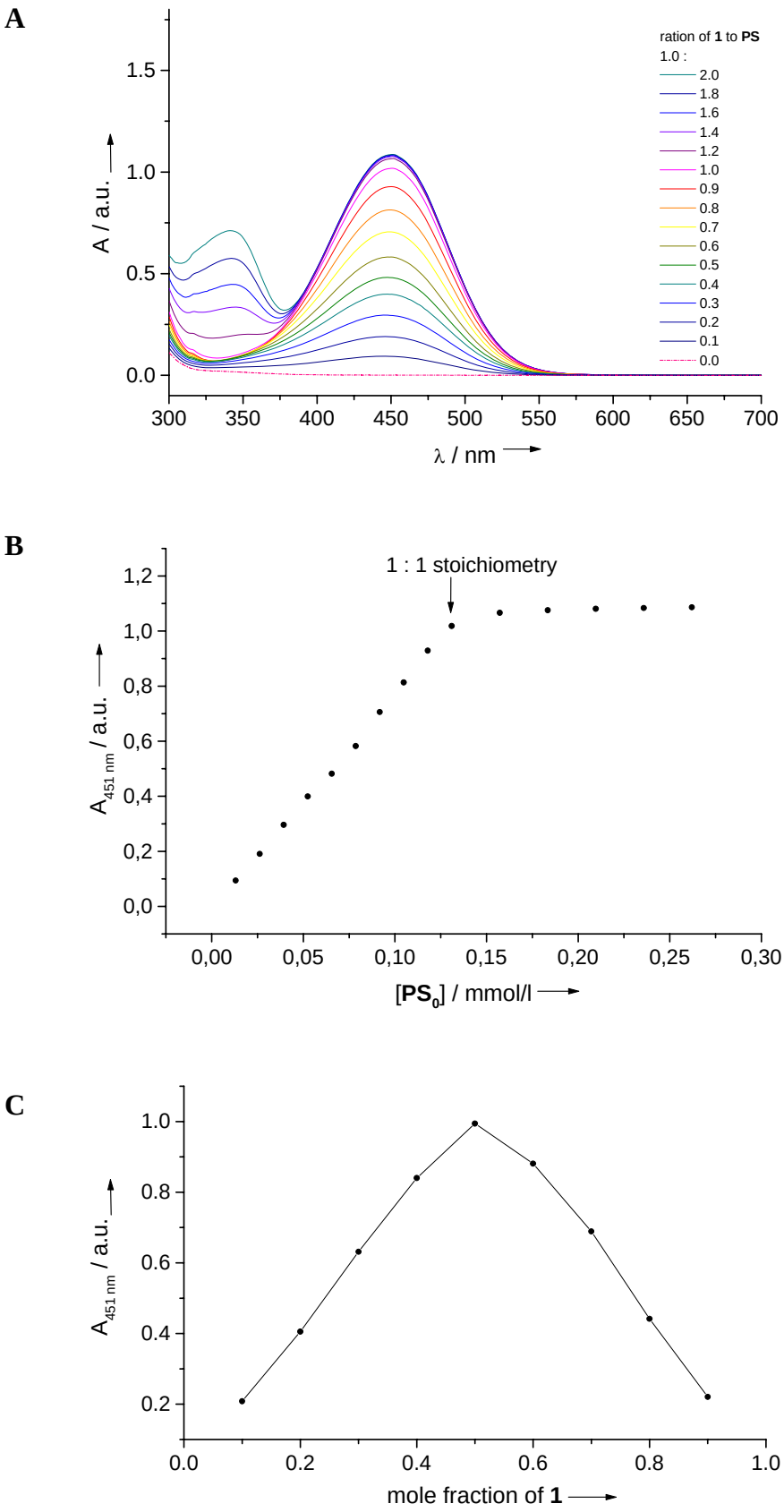


Figure S8. UV/Vis absorption spectra (**A**) of **1** (0.131 mmol/l, dotted line) with **PS** (0.013-0.262 mmol/l), the corresponding binding curve (**B**) and Job's plot (**C**, every stock solution: 0.131 mmol/l) in dichloromethane.

Concentration-dependent ^1H NMR spectroscopy. According to the literature[S9], the salt **1**+**PS**, which is a 1 : 1 mixture of the enolate anion of **1** and the proton sponge®,[S1] was concentrated stepwise in the concentration range 1 – 33 mmol/l. Each time a ^1H NMR spectrum was recorded. The measurements were carried out under an argon atmosphere in d_6-DMSO that had been dried over 4 Å molecular sieves (residue water concentration < 0.1%). The concentration-dependent shifts of the NH proton of the enolate anion were fitted with the non-linear least-squares program ORIGIN similar to the procedure described (figure S9).

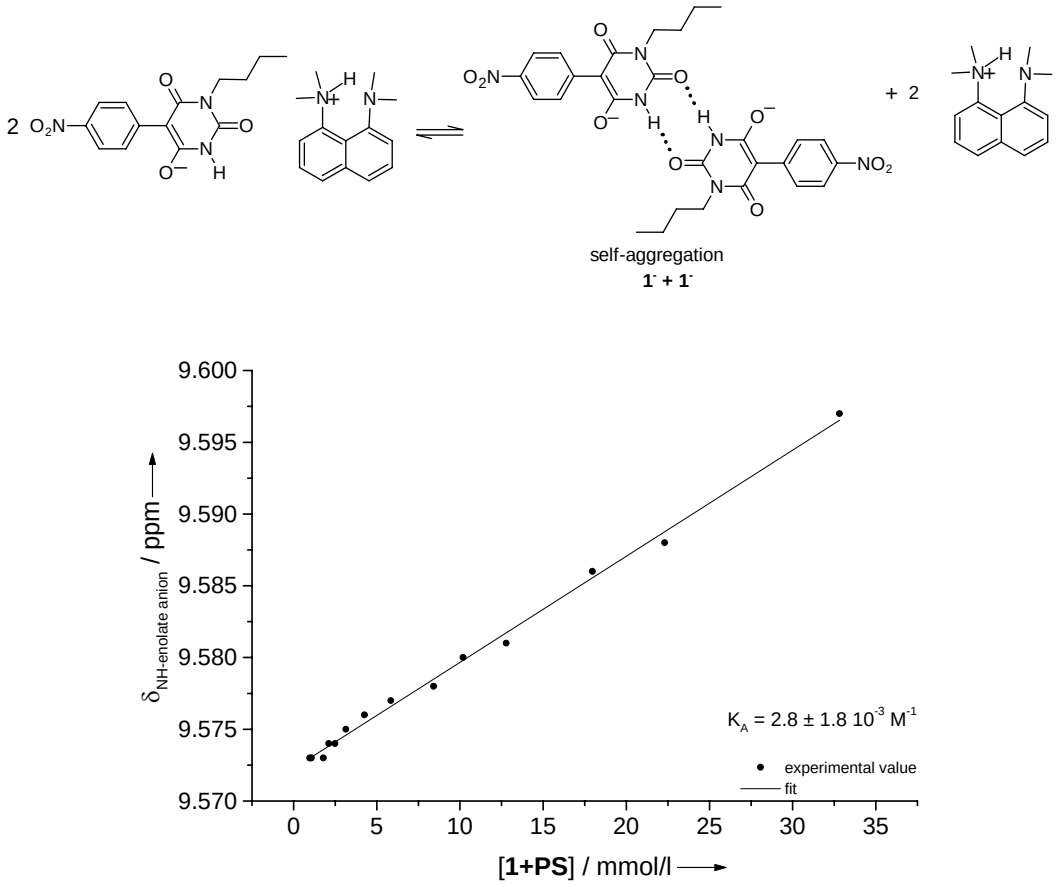


Figure S9. Concentration-dependent ^1H NMR measurements of the equimolar mixtures of **1** and the proton sponge® **PS** in dried d_6-DMSO.

UV/Vis-binding studies with six artificial receptors. The *UV/Vis titration* was performed using dichloromethane and methanol at a ratio of 1.00 to 0.04 (both freshly distilled from appropriate drying agents). A typical binding experiment involved the titration of **1** (1.0 ml, 1.96 10^{-7} mol/l) where aliquots of 0.04 – 4.00 ml of the receptor stock solution (24.56 10^{-3} mol/l) were added. Due to the poor solubility, the **BuCy**-stock solution was 8.791 10^{-3} mol/l (added aliquots 0.11 – 3.91 ml). The stock solution of **EtGu** was only 0.692 10^{-3} mol/l, the added aliquots (0.70 – 2.80 ml) correspond to a molar ratio of **EtGu** from 1 to 4. The solutions were filled up to 5.0 ml. The increase in absorption intensity was monitored as a function of guest concentration. The quantitative determination of the association constant K_A is based on the absorbance variation of the host (H, **1**) in the presence of the guest (G, receptor) with certain concentration.[S7] The UV/Vis-control experiments were performed in dichloromethane using the same titration procedure. Figure S10 shows the UV/Vis titration of **1** with the receptor **DAC** and with **EtAd** in both solvent systems.

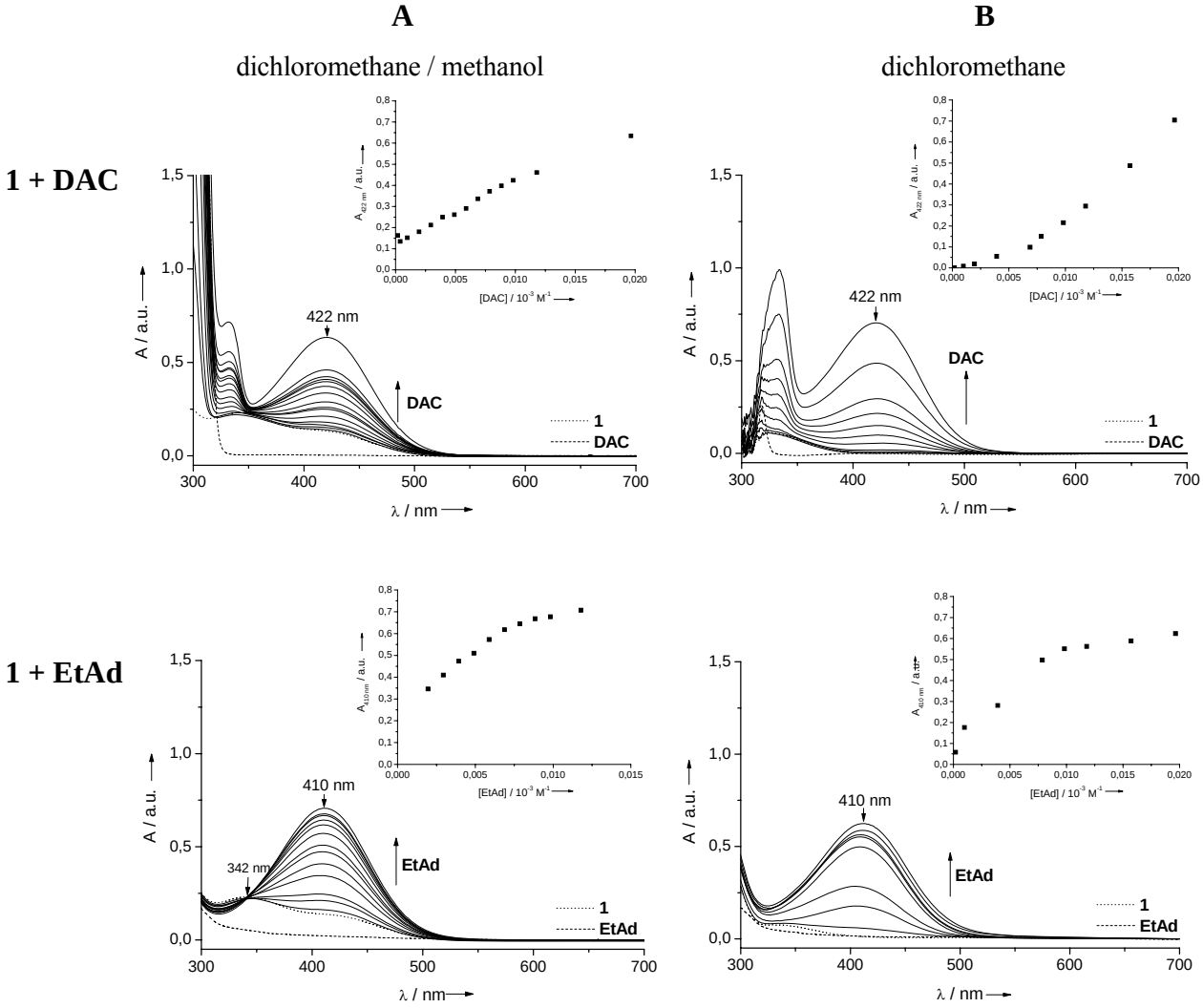


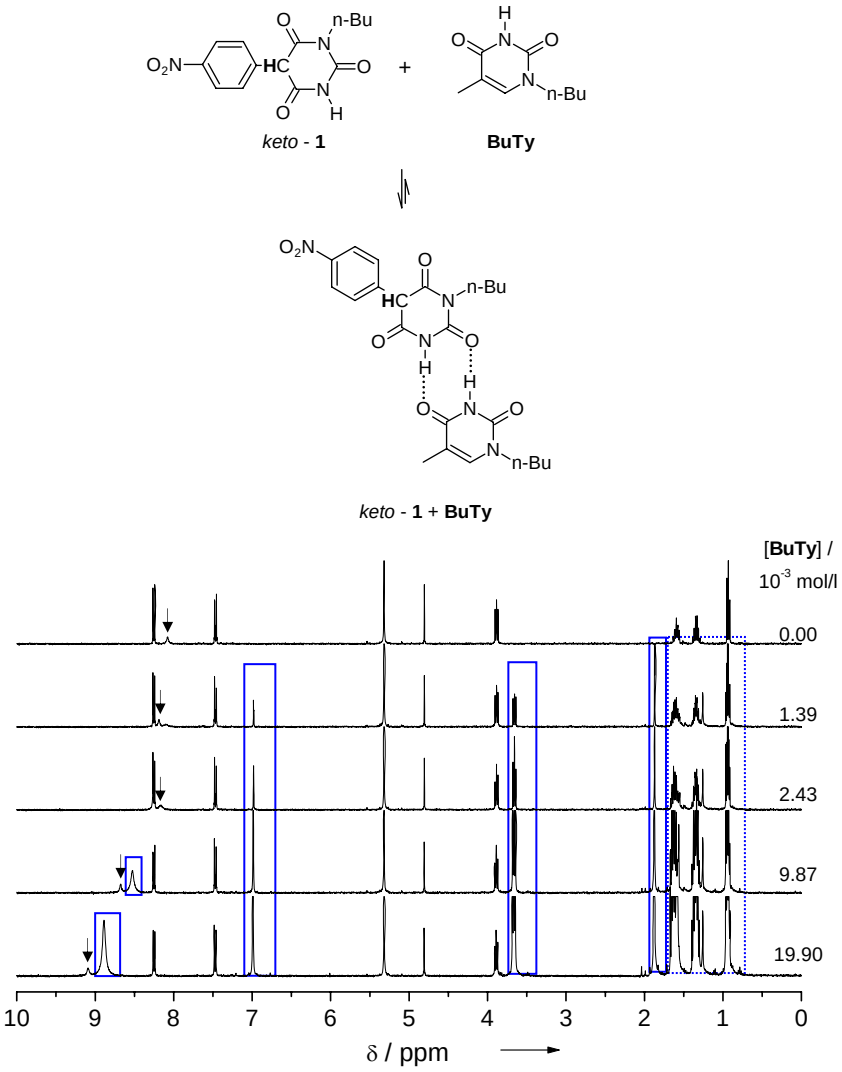
Figure S10. UV/Vis absorption spectra of **1** (1.96 10^{-7} mol/l) with the receptors **DAC** (1.96 10^{-7} - 1.96 10^{-5} mol/l) and **EtAd** (1.96 10^{-7} - 1.18 10^{-5} mol/l) in dichloromethane/methanol (ratio 1.00:0.04, **A**) and in dichloromethane (**B**).

^{1}H NMR binding studies with five artificial receptors. ^{1}H NMR titrations were carried out in dried d_2 dichloromethane (residue water concentration < 0.1%). In a typical experiment, the ^{1}H NMR spectrum of the host solution (**1**, 2.17 10^{-3} mol/l, 1.0 ml of solvent) was recorded and then small portions of the receptor were added to the NMR tube under argon atmosphere. The concentration of the receptor was calculated by the comparison of the integrals. The chemical shift of the NH proton of **1** was monitored as a function of the receptor concentration for the determination of the association constant K_A.[S7a]

In the following sections adequate ^{1}H NMR experiments are shown. The ^{1}H NMR resonances of the receptor are blue highlighted. The overlapping of the alkyl chains of the chromophor and the receptor are marked with blue dotted lines. NH-protons are marked with arrows.

^{1}H NMR Titration with 1-*n*-butylthymine BuTy.

[**BuTy**] 10^{-3} mol/l	$\delta_{NH(Keto)}$ ppm	$\delta_{CH\text{-}5(Keto)}$ ppm
0.00	8.080	4.806
0.20	8.095	4.806
0.28	8.103	4.806
0.48	8.117	4.806
0.62	8.130	4.806
0.85	8.150	4.806
1.14	8.171	4.806
1.39	8.189	4.806
1.66	8.204	4.806
2.00	-	4.806
2.43	-	4.806
3.02	8.309	4.806
3.72	8.335	4.807
5.05	8.429	4.807
6.48	8.506	4.807
7.84	8.586	4.807
9.87	8.674	4.808
11.48	8.748	4.808
13.11	8.825	4.808
15.64	8.916	4.809
17.13	8.974	4.809
19.90	9.090	4.809
23.23	9.205	4.810
31.02	9.357	4.811
37.59	9.473	4.812
44.87	9.632	4.814
73.35	9.853	4.817

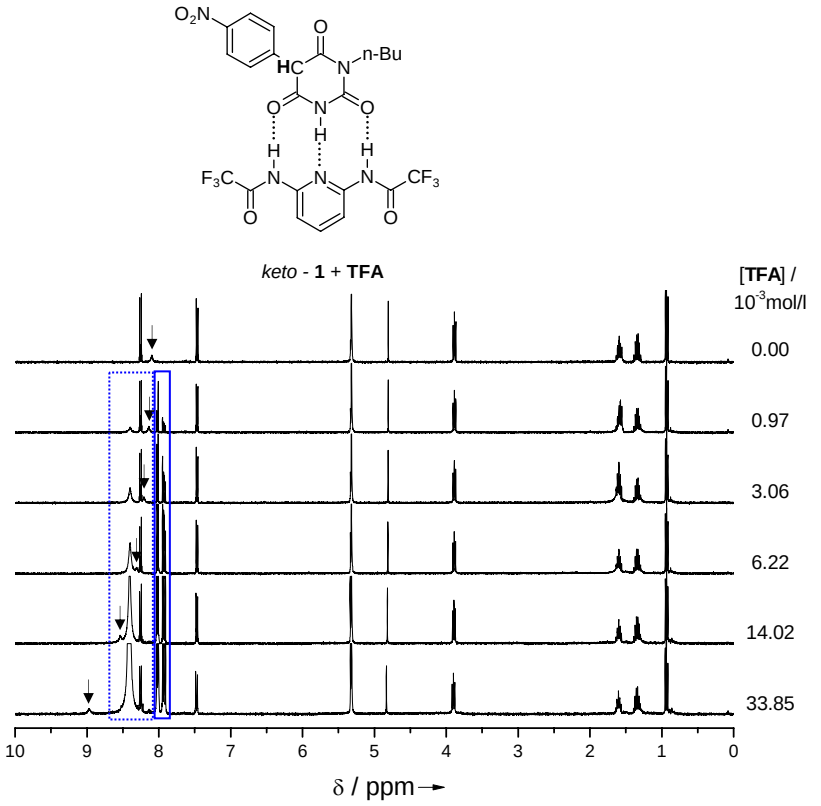


[**1**] = 2.1717 10^{-3} mol/l

^{1}H NMR Titration with 2,6-bis(trifluoroacetamido)pyridine TFA.

[**TFA**] 10^{-3} mol/l	$\delta_{NH(Keto)}$ ppm	$\delta_{CH\text{-}5(Keto)}$ ppm
0.00	8.109	4.806
0.05	8.106	4.806
0.68	8.130	4.807
0.97	8.138	4.807
1.68	8.156	4.808
2.63	8.191	4.809
3.06	8.206	4.809
4.83	-	4.811
6.22	8.308	4.812
8.30	-	4.814
11.13	-	4.816
14.02	8.538	4.819
17.83	8.629	4.822
23.42	8.768	4.827
27.33	8.851	4.830
33.85	8.905	4.833

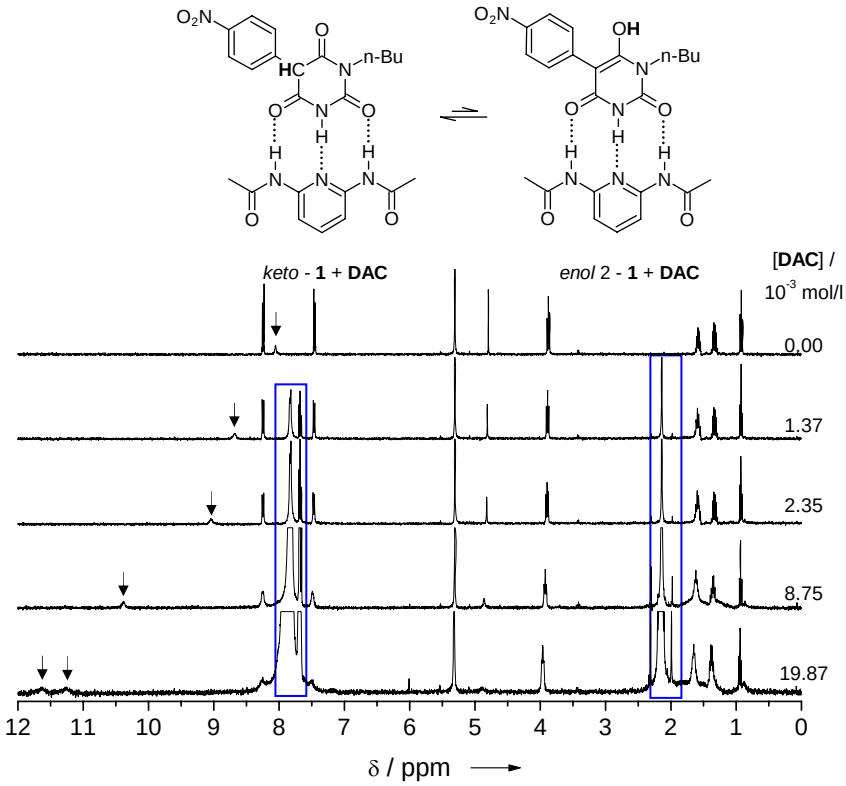
[**1**] = 2.1610 10^{-3} mol/l



^{1}H NMR Titration with 2,6-diacetamidopyridine DAC.

[**DAC**] 10^{-3} mol/l	$\delta_{NH(Keto)}$ ppm	$\delta_{CH\text{-}5(Keto)}$ ppm
0.00	8.069	4.806
0.13	8.131	4.807
0.30	8.204	4.810
0.59	8.362	4.814
0.83	8.458	4.817
1.37	8.692	4.822
2.35	9.060	4.832
3.27	9.335	4.840
4.14	9.586	4.847
5.13	9.778	4.852
5.77	9.942	4.858
7.60	10.261	4.867
8.75	10.392	4.872
10.64	10.616	-
16.52	11.061	-
19.87	11.225	-

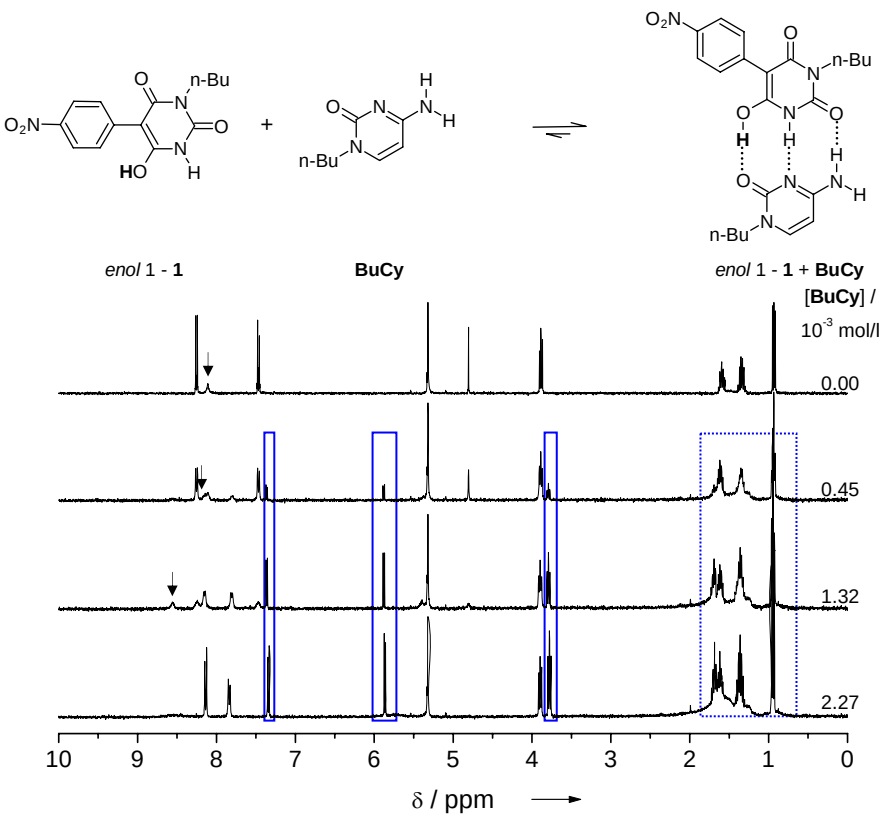
[**1**] = 2.1619 10^{-3} mol/l



^{1}H NMR Titration with 1-*n*-butylcytosine BuCy.

[**BuCy**] 10^{-3} mol/l	$\delta_{NH(Keto)}$ ppm	$\delta_{CH\text{-}5(Keto)}$ ppm
0.00	8.108	4.805
0.11	8.107	4.805
0.45	8.110 / 8.554*	4.805
0.97	8.554*	4.803
1.32	8.560*	-
1.71	8.561*	-
2.27	-	-
4.52	-	-
4.84	-	-
5.64	-	-
6.50	9.499*	-

*$\delta_{NH(Enol)}$ / ppm; [**1**] = 2.1610 10^{-3} mol/l

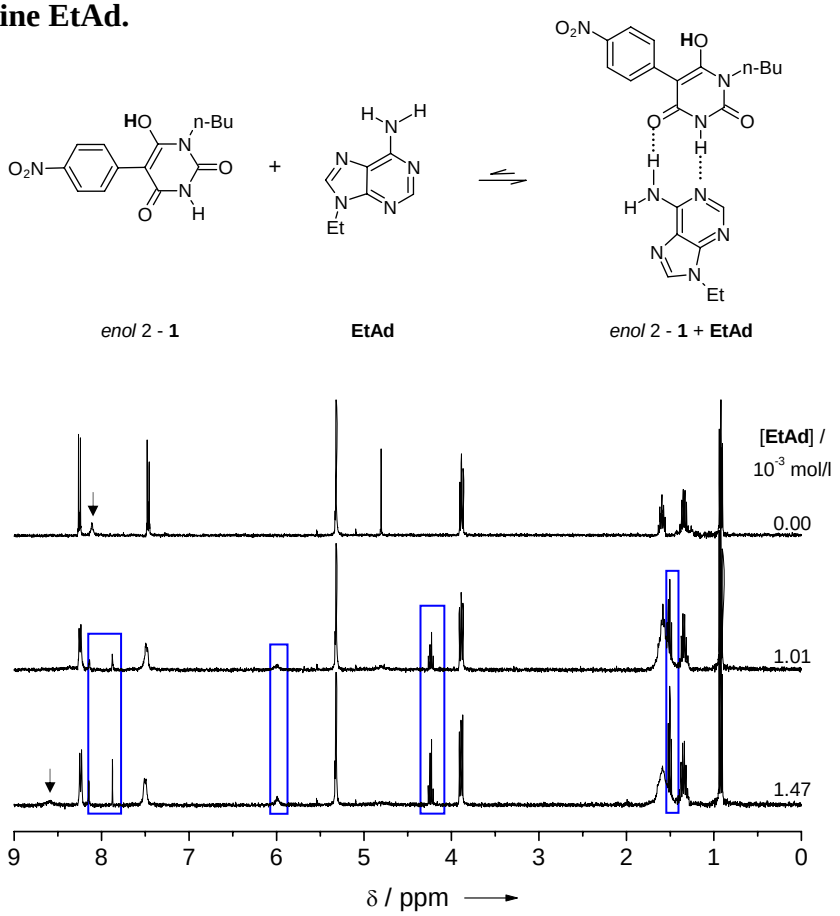


^{1}H NMR Titration with 9-ethyladenine EtAd.

[**EtAd**] 10^{-3} mol/l	$\delta_{NH(Keto)}$ ppm	$\delta_{CH\text{-}5(Keto)}$ ppm
0.00	8.113	4.805
1.01	-	-
1.47	8.586	-
1.76*	-	-
2.46*	-	-
3.08*	-	-
2.34*	-	-

*complex precipitated;

[**1**] = 2.1610 10^{-3} mol/l



References

S1 I. Bolz, C. Moon, V. Enkelmann, G. Brunklaus, S. Spange, *J. Org. Chem*. **2008**, *73*, 4783 – 4793.

S2 K. Kurita, R. L. Williams, *J. Heterocyclic Chem.* **1975**, *12*, 789 – 791.

S3 E. Beinlinger, A. Niemz, V. M. Rotello, *J. Am. Chem. Soc.* **1995**, *117*, 5379 – 5380.

S4 J. S. Nowick, J. S. Chen, G. Noronha, *J. Am. Chem. Soc.* **1993**, *115*, 7636 – 7644.

S5 D. L. Helfer II, R. S. Hosmane, N. J. Leonard, *J. Org. Chem.* **1981**, *46*, 4803 – 4804.

S6 A. D. Hamilton, D. van Engen, *J. Am. Chem. Soc.* **1987**, *109*, 5035 – 5036.

S7 a) K. A. Connors, *Binding Constants*, J. Wiley & Sons, New York, **1987**; b) I. Bolz, C. May, S. Spange, *New J. Chem.* **2007**, *31*, 1568 – 1571.

S8 a) M. J. Kamlet, J.-L. M. Abboud, M. H. Abraham, R. W. Taft, *J. Org. Chem.* **1983**, *48*, 2877–2887; b) Y. Marcus, *Chem. Soc. Rev.* **1993**, *22*, 409–416; b) A. Oehlke, K. Hofmann, S. Spange, *New J. Chem.* **2006**, *30*, 533–536; c) R. Lungwitz, M. Friedrich, W. Linert, S. Spange, *New J. Chem.* **2008**, DOI:10.1039/b805932b; d) C. Reichardt, *Solvents and Solvent Effects in Organic Chemistry*, VCH, Weinheim, **2003**.

S9 K. S. Schmidt, R. K. O. Sigel, D. V. Filippov, G. A. van der Marel, B. Lippert, J. Reedijk, *New J. Chem.* **2000**, *24*, 195–197.