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

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Abstract: The use of hydrogen-bonding patterns in the same way as is known from DNA building blocks is a challenge for the construction of novel types of suitable chromophoric probes. This feature has been utilised for the construction of a novel type of UV/Vis probe for detection of supramolecular AAD or DAD sequences (A = hydrogen bond acceptor, D = hydrogen bond donor). Here we report on the structure of the enolisable chromophore 1-*n*-butyl-5-(4-nitrophenyl)barbituric acid (**1**), which has an adjustable hydrogen-bonding pattern. The position of the keto–enol equilibrium of this dye is strongly influenced both by the solvent polarity and by the chemical environment. Furthermore, the recognition properties of the barbiturate were examined by the use of seven artificial receptors: the pyridine bases 2,6-diaminopyridine (**DAP**), 2,6-diacetamidopyridine (**DAC**) and 2,6-bis(trifluoroacetamido)pyridine (**TFA**), as well as the nucleic acid bases 9-ethyladenine (**EtAd**), 9-ethylguanine (**EtGu**), 1-*n*-butylcytosine (**BuCy**) and 1-*n*-butylthymine (**BuTy**). It was found that **1** can interact with these bases either through acid–base interaction or by hydrogen-bonding complexation. The balance between the interactions is dependent both on the basicity strength and on the presence of a suitable recognition sequence in the base. The induced formation of the enol form of **1** thus causes a significant UV/Vis shift as function of the nature of the base.

Keywords: dyes/pigments · molecular recognition · solvent effects · supramolecular chemistry · tautomerism

Introduction

For the sequence-specific detection of molecular species such as Watson–Crick base pairs, enzyme–substrate complexes or antibody–antigen complexes, synthetic models are still not able to copy nature.[1] For analytical processes, therefore, adjustable model compounds through which we can achieve an improved understanding of noncovalent interactions are still being sought. In order, for example, to differentiate between multiple hydrogen bonds, an elegant and simple way would be to prepare UV/Vis probe molecules in which an incorporated push–pull system would be altered by the electronic effect of complex formation, so that an altered chromophoric system would be induced.[2–4] For analytical applications an enhancement of the UV/Vis signal is desirable, as in the case of the so-called off–on fluorophores.[5]

We therefore set ourselves the task of developing an adjustable probe molecule, complexation of which with nucleic acid bases or similar compounds would produce a differentiated UV/Vis signal, depending on the hydrogen-bonding sequence (Scheme 1).

In the creation of new sensor molecules it should be noted that supramolecular complex formation based on desolvation effects is often entropy-controlled. An important prerequisite is therefore that, on complexation with the receptor, the probe molecule should make a contribution to the binding energy ($\Delta_R H$) larger than that obtainable through desolvation of both the receptor and the probe molecule.[6] Furthermore, it is known that the stability of a hydrogen-bonded complex can be raised and also lowered significantly by the polarisation of hydrogen-bonding atoms.[7]

Certain merocyanine dyes contain the barbituric acid moiety as an electron-withdrawing substituent showing a DAD molecular recognition sequence (D: hydrogen bond donor, A: hydrogen bond acceptor) related to those of the nucleic acid bases. On complex formation with melamine derivatives or Hamilton receptors, which have an ADA sequence, only small UV/Vis shifts have been found,[8–10] and

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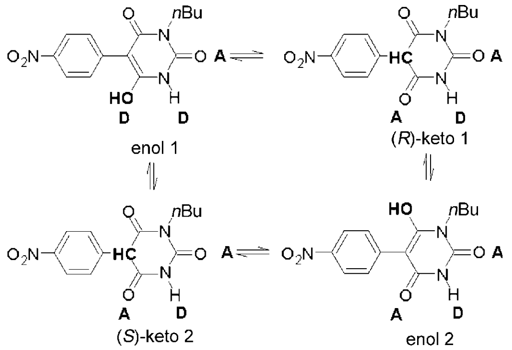


Scheme 1. Hydrogen-bonding sequences (D: H-donor, A: H-acceptor) of the keto and enol tautomers of 1-*n*-butyl-5-(4-nitrophenyl)barbituric acid (**1**).

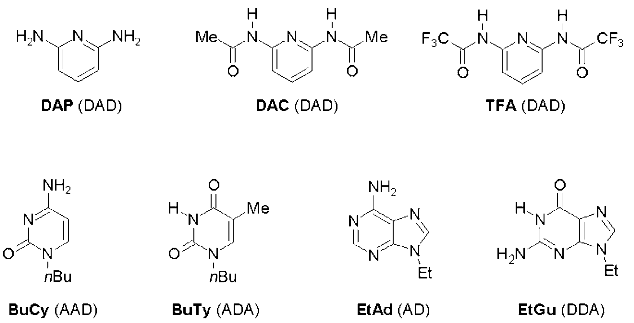
nothing has been reported on the detection of nucleic acid bases.

We considered that on complex formation with bases a push–pull substituted π system would be induced in the probe molecule. The targeted adjustable properties require the presence of a (−M)-activated enol.[11] These conditions are fulfilled by the chromophore 1-*n*-butyl-5-(4-nitrophenyl)barbituric acid (**1**), which must show a total acid or base strength significantly different from that of the molecule to be detected or the solvent. As a result of the single *N*-substituent, there is only one adjustable hydrogen-bonding sequence in **1**, while the *n*-butyl group also serves as the solubility-enhancing substituent. Whether a positively (acidic) or negatively (basic) hydrogen-bonding sequence is present has been investigated by use of LSE (linear solvation energy) relationships with the aid of the well-known simplified Kamlet–Taft equation [Eq. (1)], in which the coefficients of the individual interaction contributions are determined by multiple correlation analysis.[12,13]

$$\tilde{\nu}_{\max} = \tilde{\nu}_{\max,0} + a\alpha + b\beta + s\pi^* \qquad (1)$$

The overall polarity of a solvent is described by the effects of the hydrogen-bonding acidity (α), the hydrogen-bonding basicity (β) and the dipolarity/polarisability (π*) on the solute.

Besides investigations of the solvatochromism and acidity of **1** in pure organic solvents and room-temperature ionic liquids, (RTILs), we also present others relating to the interaction of **1** with seven structurally different receptors. Pyridine derivatives—2,6-diaminopyridine (**DAP**), 2,6-diacetamidopyridine (**DAC**) and 2,6-bis(trifluoroacetamido)pyridine (**TFA**, Scheme 2)—allow a systematic investigation of the effects of the acidity of the complex-forming partner on the electronic structure of the chromophoric π system of **1** in solution, because of their different amide substitution patterns. Furthermore, complexation with four synthetic nucleic acid bases capable of entering into Watson–Crick base pairing—9-ethyladenine (**EtAd**), 9-ethylguanine (**EtGu**), 1-*n*-butylcytosine (**BuCy**) and 1-*n*-butylthymine (**BuTy**)—have also been studied.

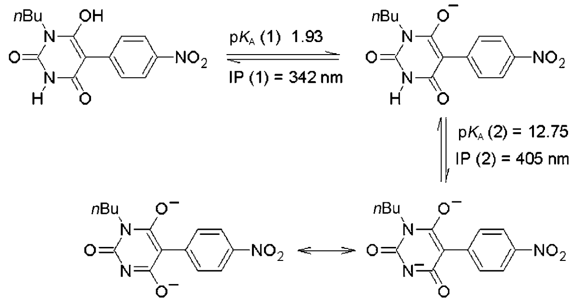


Scheme 2. Receptors employed and their hydrogen-bonding sequences (D: hydrogen bond donor, A: hydrogen bond acceptor).

The prototypic tautomerism of the dye allows, in principle, the molecules to adapt to DDA and ADA bonding sequences (cf. Scheme 1). It should thus be possible to determine whether only one tautomeric form is stabilised, depending on the structure and the polarity of the receptor. Furthermore, **1** can also act as a Brønsted acid, if the base strength of the complex-forming partner is appropriate.

Results and Discussion

Acidochromic and solvatochromic properties: The pK_A value of **1** was determined by a pH-dependent UV/Vis titration in water by application of the Henderson–Hasselbalch method.[14] Two pK_A values of 1.93 and 12.75 were determined. From the molecular structure of **1**, two proton-transfer reactions could be formulated (Scheme 3). The (+)-M effect of the enol oxygen of **1** is strengthened by deprotonation and the mesomeric stabilisation of the enolate anion by the nitrophenyl substituent, resulting in a lower pK_A value for the barbiturate **1** than for barbituric acid (pK_A 4.02 in H_2O) or 5-phenylbarbituric acid (pK_A 2.54 in H_2O).[15a] The acidity of the NH proton is caused by the delocalisation of the charge over the barbituric acid function, which is



Scheme 3. Protonation–deprotonation equilibrium of **1**, its isosbestic points (IP) and the measured pK_A values.

strengthened by the electron-withdrawing NO_2 substituent. In the literature, comparable dianionic species and their stabilisation have been discussed in a large number of investigations, with the pK_A values found lying between 12.5 (barbituric acid) and 13.5 (5-*tert*-butylbarbituric acid).[15b–f]

The ^1H NMR and UV/Vis spectroscopic structure determination of the barbiturate **1** confirm its existence in its keto form in nonpolar solvents. In protic solvents such as methanol, on the other hand, the enol form is predominant and can aggregate, depending on the concentration. Neither these higher associated forms nor the keto form show any significant solvatochromism. In strongly hydrogen-bonding acceptor (HBA) and electron pair donor (EPD) solvents such as DMSO or DMF, the UV/Vis maxima of the monomeric enol form are observed exclusively. Through HBA solvation the enol oxygen experiences negative polarisation, through which the electron-donating properties of the barbituric acid unit are strengthened. This solvent effect has been described in terms of the basicity [β value in Eq. (1)], so that for the position of the keto–enol equilibrium to favour the enol, higher β and π^* values of the solvent are important. This effect of the polarity of the surrounding solvent is exemplified in the following ^1H NMR experiment with deuterodichloromethane (CD_2Cl_2, low β, high π^* value) and the ionic liquid 1-hexyl-3-methylimidazolium chloride ($[C_6\text{-mim}]^+Cl^-$).[16] The RTIL used has a high β value, while the α and π^* parameters are similar to those of CD_2Cl_2. Figure 1 shows the ^1H NMR spectrum of the chromophore **1** in CD_2Cl_2, which only shows signals of the keto form. Through addition of $[C_6\text{-mim}]^+Cl^-$ the basicity of the solvent surrounding **1** is significantly raised, and as a result the enol form is increasingly stabilised. With a two-molar excess of $[C_6\text{-mim}]^+Cl^-$, based on **1** at 22 ± 1 °C, only the ^1H NMR resonances of the enol form are detectable.

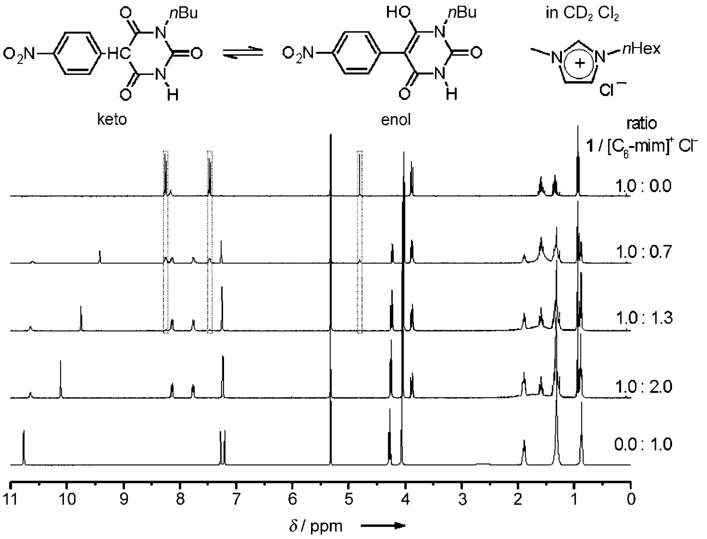


Figure 1. Shift of the keto–enol equilibrium (simplified), based on a ^1H NMR titration of **1** (5.45×10^{-3} mol L^{-1}) in CD_2Cl_2 with increasing concentration of $[C_6\text{-mim}]^+Cl^-$, with the protons of the keto form marked.

The question now arises as to whether and how the (+M)-barbituric acid unit has an effect on the chromophoric π electron system through interaction with the surroundings of the molecule and of how far dipole–dipole and/or hydrogen-bonding and acid–base interactions play a role.

In order to separate the individual solvation effects the chromophore **1** was examined in 29 organic solvents, with the UV/Vis absorption maxima in 22 solvents being correlated with the Kamlet–Taft parameters corresponding to Equation (2), below. For the reasons discussed above, only the UV/Vis maxima of the solvatochromic, monomeric enol form, measured at high dilution, are included in the correlation analysis.

Figure 2 shows selected UV/Vis spectra of **1** in six organic solvents and three 1-hexyl-3-methylimidazolium salts ($[C_6\text{-mim}]^+$). Table 1 shows the $\tilde{\nu}_{max}$ values and polarity parameters of the characteristic solvents listed in Figure 2. For the RTILs used the β value is fixed by the anion used (Cl^-, Br^- and BF_4^-), while the α und π^* values in these three solvents are practically of the same order of magnitude. The polarity parameters of the RTILs determined by an improved method[16] are incorporated into the correlation equation. The data for the complete correlation analysis can be found in the Supporting Information.

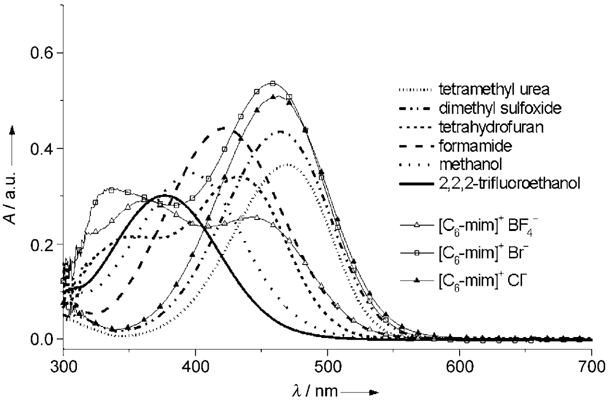


Figure 2. UV/Vis spectra of **1** in six solvents of different polarities and in three room-temperature ionic liquids.

The solvatochromic range ($\Delta\tilde{\nu}$) on moving from 2,2,2-trifluoroethanol (TFE, high α value) to *N,N,N′,N′*-tetramethylurea (TMU, high β value) is 5232 cm^{-1}, which indicates a strongly positive solvatochromic effect. However, the classification of this class of compound by the established terms of positive or negative solvatochromism, based only on the dipolarity of the solvents, makes less sense here. In comparison with the small magnitude of the conjugated systems, the solvatochromic range determined is, however, enormous and indicates a pronounced push–pull system.[13b] This is a good conceptually targeted result for the expected measurable interaction with complementary bases.

Equation (2) shows the result of the multiple correlation analysis.

Table 1. UV/Vis absorption maxima ($\tilde{\nu}_{max}$) of **1** in representative solvents and ionic liquids, the empirical polarity parameters α, β, and π^*, and the calculated solvatochromic range.[12a,b,16]

Solvent	α	β	π^*	$\tilde{\nu}_{max}$
tetrahydrofuran	0.00	0.55	0.58	23.0
DMSO[a]	0.00	0.69	0.88	21.5
TMU[b]	0.00	0.76	1.00	21.3[d]
dichloromethane	0.13	0.10	0.82	22.8
formamide	0.71	0.48	0.97	23.7
methanol	0.98	0.66	0.60	25.1
TFE[c]	1.49	0.00	0.73	26.5[e]
$[C_6\text{-mim}]^+BF_4^-$	0.44	0.60	0.96	22.6
$[C_6\text{-mim}]^+Br^-$	0.35	0.88	1.06	21.9
$[C_6\text{-mim}]^+Cl^-$	0.30	0.97	1.06	21.6
$\Delta\lambda$ [nm]	93			
$\Delta\tilde{\nu}$ [cm^{-1}]	5232			

[a] Dimethyl sulfoxide. [b] Tetramethylurea. [c] 2,2,2-Trifluoroethanol. [d] Largest bathochromic shift. [e] Largest hypsochromic shift.

$$\begin{aligned}&\tilde{\nu}_{max}\,[10^{-3}\,\text{cm}^{-1}] = 25.9{+}2.41\,\alpha{-}1.89\,\beta{-}3.26\,\pi^*\\&n = 25;\ r = 0.95;\ \text{SD} = 0.47;\ F\ <\ 0.0001\end{aligned}\tag{2}$$

The complex solvatochromism of the enol form is determined by the acidity (α), the basicity (β) and the dipolarity and polarisation effects (π^*) of the solvent.

The strongly negative coefficient s for the π^* value shows that the excited state of the enol form, which has a higher dipole moment than the ground state, experiences stronger solvation than the ground state. The strong influence of the α term on the hypsochromic shift represents the interaction of a protic solvent, which can interact with the free electron pairs of the oxygen atoms of the barbiturate both as an HBD and as an EPA. The effect of the β term on the bathochromic shift shows that the HBA solvation plays an important role in the negative polarisation of the enol oxygen. In particular, the bathochromic shift, measured in the chloride of $[C_6\text{-mim}]^+$, as a comparison with the tetrafluoroborate, underlines the dominance of this interaction. This is particularly important with regard to the conception of the hoped-for result, as the complexation with complementary bases should result in analogous effects.

Acid–base behaviour: 2,6-Diaminopyridine (**DAP**) represents the ideal model compound for more precise study of the balance between salt formation and complexation. In supramolecular chemistry, **DAP** derivatives have proved to be useful receptors for the nucleic acid bases and structurally related compounds.[17] **DAP** possesses a hydrogen-bonding sequence complementary both to the keto form and to the enol2 structure of **1** (cf. Scheme 1). Furthermore, proton-transfer reactions of Brønsted acids in the presence of **DAP** have been intensively investigated.[18] The pK_A value of **DAP** has been reported to be 6.00 in water and 14.77 in acetonitrile.[19]

In a UV/Vis experiment, **1** was titrated in equimolar steps with **DAP** in CH_2Cl_2 (Figure 3). With increasing **DAP** concentration, an increase in the absorbance and a smaller bathochromic shift of 4 nm was observed. The 1:1 stoichiometry could be determined by UV/Vis spectroscopy by Job's method,[14] which indicates the formation of the salt (**1**$^-$+**DAP-H**$^+$). The absence of an isosbestic point and a further intensification of the UV/Vis absorption band after 1:1 stoichiometry has been reached shows the overlap of the acid–base reaction with complex formation through hydrogen bonding.[20]

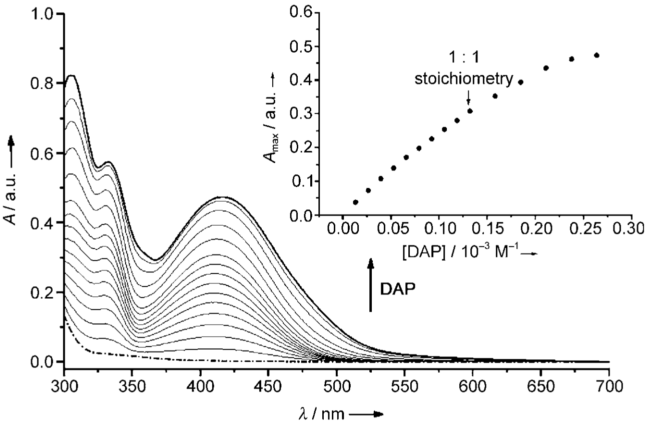
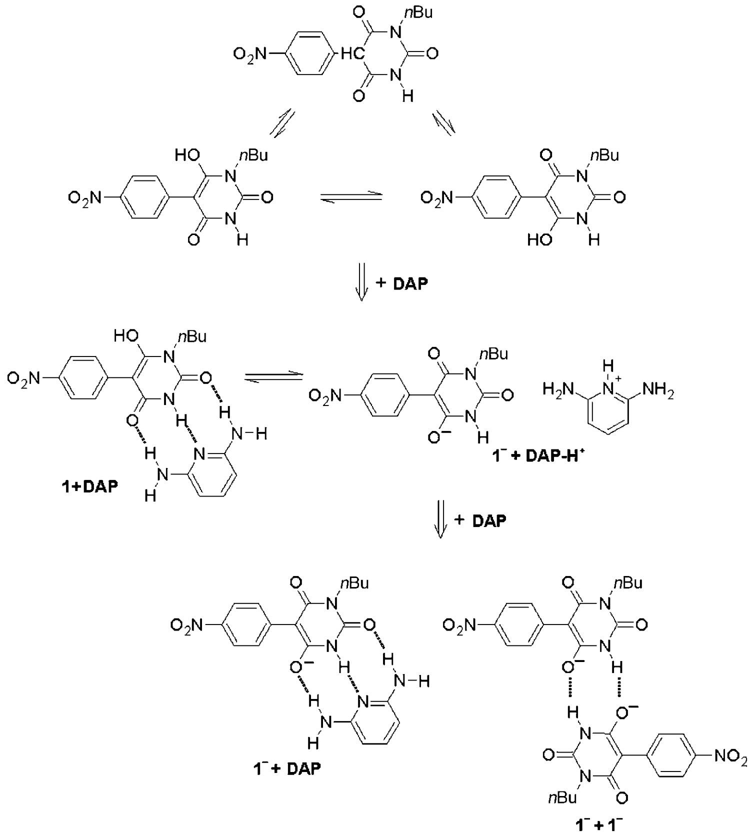


Figure 3. UV/Vis absorption spectra of **1** (0.13×10^{-3} mol L^{-1}, dotted line) in the presence of 2,6-diaminopyridine (**DAP**, 0.01–0.26×10^{-3} mol L^{-1}) in dichloromethane, together with a plot of the absorbance (at 411 nm) against the concentration of **DAP**.

The competing equilibrium reactions are shown in Scheme 4. In particular, interactions of the enolate anion of **1** with itself (self-aggregation) or with **DAP** (complexation) make the interpretation of the structure more difficult.

A further indication of the consecutive proton-transfer reaction and complexation was derived from control experiments with **DAC** and **TFA**. Because of the amide substitution, both receptors are less basic than **DAP**, so acid–base reactions do not play a role. Under analogous conditions, there is therefore no change in the UV/Vis spectrum of **1** on addition of **DAC** or **TFA** (see Supporting Information). A further study of the aggregate of **1** with **DAP** by ^{1}H NMR spectroscopy was not possible in CD_2Cl_2, as a CD_2Cl_2-insoluble product precipitated in the required concentration range, even with the smallest traces of **DAP**. Further investigations were therefore carried out in deutero-dimethylsulfoxide ([D$_6$]DMSO), an aprotic solvent that also favours proton-transfer reactions.[6b,17d,21] With increasing **DAP** concentration, signal broadening of the NH$^+$ proton and a downfield shift of the NH proton of the enolate anion **1**$^-$ were observed, which indicates the presence of a hydrogen-bonding complex (**1**$^-$+**DAP**). Self-aggregation as shown in Scheme 4 can, however, be ruled out.[22] The nonlinear regression curve for 1:1 complexation (**1**$^-$+**DAP**) gave an association constant of $K_A = 2.7\pm0.4\,\text{M}^{-1}$ (Figure 4). Unfortunately, there are very few descriptions of complex formation with DMSO in the literature. Thus, for example, for the complexation of different ruthenium(II) bipyridine barbiturates with a 2,6-diaminopyridine derivative, values of 10–



Scheme 4. Possible acid–base and complex formation interactions of **1** and **DAP**.

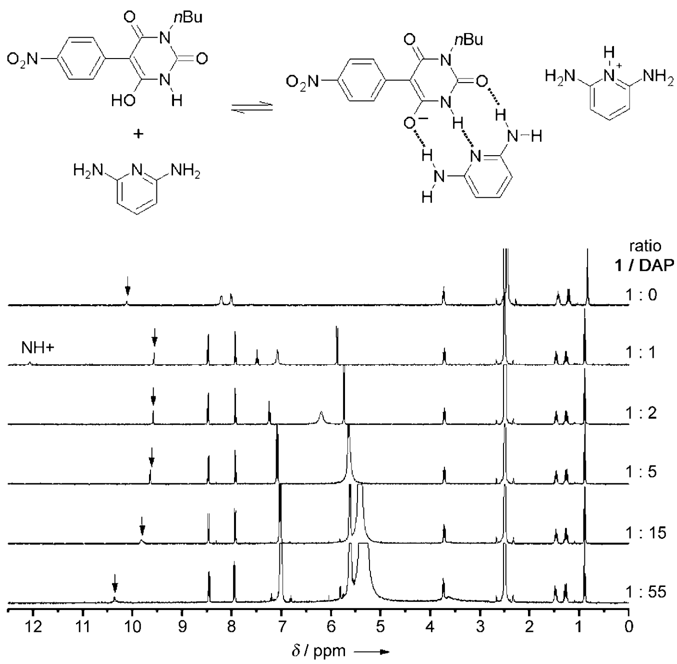


Figure 4. ^{1}H NMR titration of **1** with **DAP** in [D$_6$]DMSO. The formation of the diaminopyridinium cation confirms the formation of the salt **1$^-$+DAP-H$^+$**, while the downfield shift of the marked NH proton of the enolate anion with increased **DAP** concentration indicates the formation of the complex **1$^-$+DAP**.

$170\,\text{M}^{-1}$ have been reported. In this case, however, there are aggregates stabilised by six hydrogen bonds.[23] For the formation of the cytidine-guanosine three-hydrogen-bond complex in DMSO, a value of $K_A = 3.7 \pm 0.6\,\text{M}^{-1}$ has been reported.[24] The value of $K_A = 2.7 \pm 0.4\,\text{M}^{-1}$ determined for the binding pair (**1$^-$+DAP**) thus indicates that strong complexation involving three hydrogen bonds has occurred.

Supramolecular complex formation: In the following section the shifts in the keto–enol equilibrium of **1** induced by complex formation with the receptors **DAC**, **TFA**, **EtGu**, **BuTy**, **EtAd** and **BuCy** are described. The keto and enol2 forms feature a complementary sequence involving three hydrogen bonds to **DAC** and **TFA**, while **BuCy** has a structure complementary to the enol1 (see Figure 7, below) form. On the basis of their geometries, the receptors **BuTy**, **EtAd** and **EtGu** each have a DA sequence complementary to all tautomeric forms of **1**.

The ^{1}H NMR titrations of **1** with the receptors shown in Figures 5 and 6 (below) were performed in CD_2Cl_2, in which pure **1** exists in the keto form.[25] However, it was not possible to investigate the interactions with **EtGu** by NMR spectroscopy because of the insolubility of the receptor.

From Figure 5 it can be seen that the NH proton of the keto form is shifted downfield with increasing concentration of **BuTy** or **TFA**, which indicates participation in hydrogen bonding. The nonlinear regression curve[14] gave an association constant (K_A) of $36 \pm 4\,\text{M}^{-1}$ for complex formation of keto-**1** with **BuTy**, which confirms the presence of a doubly H-bonded complex. The triply hydrogen-bonded complex keto-**1+TFA** has an unexpectedly low K_A of $14 \pm 4\,\text{M}^{-1}$, due to an unfavourable electronic effect of the trifluoroacetamido function.[17c] The titration with **DAC** shows that the position of the keto–enol equilibrium is moved more to the side of the enol form with increasing concentration of the receptor, as is demonstrated by the broadening of the aromatic proton signal as well as that of the methine protons (Figure 5). At higher concentrations, as well as in the solid state, only the resonances of the complexed enol form of **1+DAC** were found.[11c] Figure 6 shows the shift of the keto–enol equilibrium of **1** induced by **BuCy**; at a stoichiometry of 1:1, compound **1** exists completely in the enol form. During the titration of **1** with **EtAd** with up to 0.3 equivalents of the receptor significant broadening of the methine proton and aromatic resonances is observed. At higher **EtAd** concentrations an insoluble adduct is precipitated from CD_2Cl_2; according to quantitative elemental analysis it has a 1:1 stoichiometry.

The desired conceptual application of the chromophore **1** as a hydrogen-bond-sensitive UV/Vis sensor is addressed in more detail in the following UV/Vis titrations (Figure 7). Here the concentration of **1** was kept constant, and the effect of the presence of different numbers of equivalents (1–100) of the corresponding receptor was observed. Because of the poor solubilities of **BuCy**, **EtAd** and **EtGu**, a solvent mixture of dichloromethane and methanol (MeOH) in the ratio of 1.00:0.04 was used throughout.[26] The position

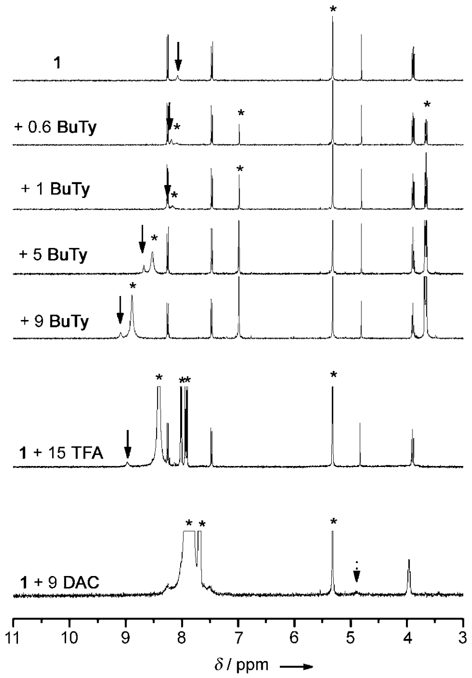


Figure 5. ^{1}H NMR spectra of **1** (2.2×10^{-3} mol L^{-1}) alone and in the presence of **BuTy**, **TFA** and **DAC** in CD_2Cl_2. Arrows mark the downfield shift of the NH proton and the broadening of the methine proton of **1**; receptor and solvent signals are marked with a small star.

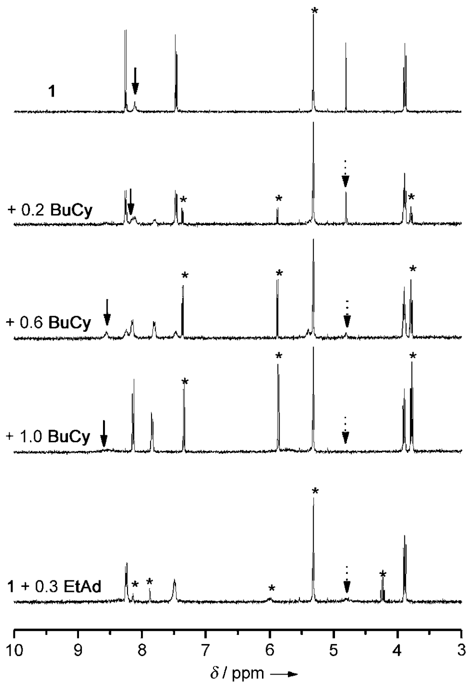


Figure 6. Comparison of the ^{1}H NMR spectra of **1** (2.2×10^{-3} mol L^{-1}) with different stoichiometric amounts of **BuCy** and **EtAd** in CD_2Cl_2. The receptor and solvent signals are marked (with small stars), as are the downfield shifts of the NH signals and the broadening of the methine proton signals of **1** (with arrows).

of the UV/Vis shift of **1** is not influenced by complex formation with MeOH, as was shown by UV/Vis control experiments in pure CH_2Cl_2 (see Supporting Information). A small threefold excess of **EtGu** leads to no important change in the UV/Vis spectrum, so no conclusions about the ease of complex formation with this receptor can be drawn. With up to a 100-fold excesses of **BuTy** or **TFA**, again no increase in the absorbance of the solvatochromic enol form was observed, which supports the complexation of the keto form of **1** with **BuTy** or **TFA**. As is already known from the ^{1}H NMR spectroscopic analyses, complex formation with **DAC** leads to a rapid equilibration of both complementary forms with the receptor. The linear, **DAC**-concentration-dependent increase in the intensity of the UV/Vis absorption at 422 nm, shown in the titration curve of **1+DAC**, and the absence of an isosbestic point (IP) are evidence for the presence of three mutually influencing equilibria: the keto–enol tautomerism and the complex formation of the keto and the enol2 forms with **DAC** (Scheme 5). The favoured complex formation of the enol2 form with **DAC** has also been found in the solid state.[11c]

On the addition of the receptors **EtAd** or **BuCy** to **1**, even at low receptor concentrations, significant increases in the intensity of the UV/Vis absorption of the chromophore are observed. For each UV/Vis titration an IP was observed (IP = 342 nm with **EtAd**; IP = 383 nm with **BuCy**), starting from a tenfold excess of **EtAd**, and a fivefold excess of **BuCy**. This supports the stabilisation of the enol form and the presence of a 1:1 equilibrium with the receptor. Evaluation of the UV/Vis data as described by Scatchard[11b,14] gave association constants for **1+EtAd** as $K_A = 147\pm7\,\text{M}^{-1}$ and for **1+BuCy** as $K_A = 1172\pm36\,\text{M}^{-1}$ (Table 2).

In comparison with known literature complexes,[27,28] we conclude that **1+EtAd** forms a doubly hydrogen-bonded complex, whereas for **1+BuCy** we postulate a triply hydrogen-bonded complex (Scheme 5).

Conclusion

In this work we have presented the enolisable barbiturate 1-*n*-butyl-5-(4-nitrophenyl)barbituric acid (**1**) as a new UV/Vis probe. The prototropic tautomerisation, and thus the electron donor strength of the barbituric acid substituent, is determined through the complex polarity of the solvent environment. In the example of the interaction with 2,6-diaminopyridine (**DAP**), consecutive acid–base reactions and complex formation were demonstrated, whereas with the more acidic 2,6-diacetamidopyridine (**DAC**) a rapid equilibration between the complexed keto and enol2 forms took place. Addition of the most acidic 2,6-bis(trifluoracetamido)pyridine (**TFA**) to **1** produced no substantial change in keto–enol tautomerism and only weak stabilisation of the complexed keto form. During the systematic study of the supramolecular interactions of **1** with synthetic nucleic acid bases, it was found that with 1-*n*-butylthymine (**BuTy**) only complexation with the keto form was observed, while with the receptors **BuCy** and **EtAd** strong preferences for the complementary enol form from the tautomeric equilibrium

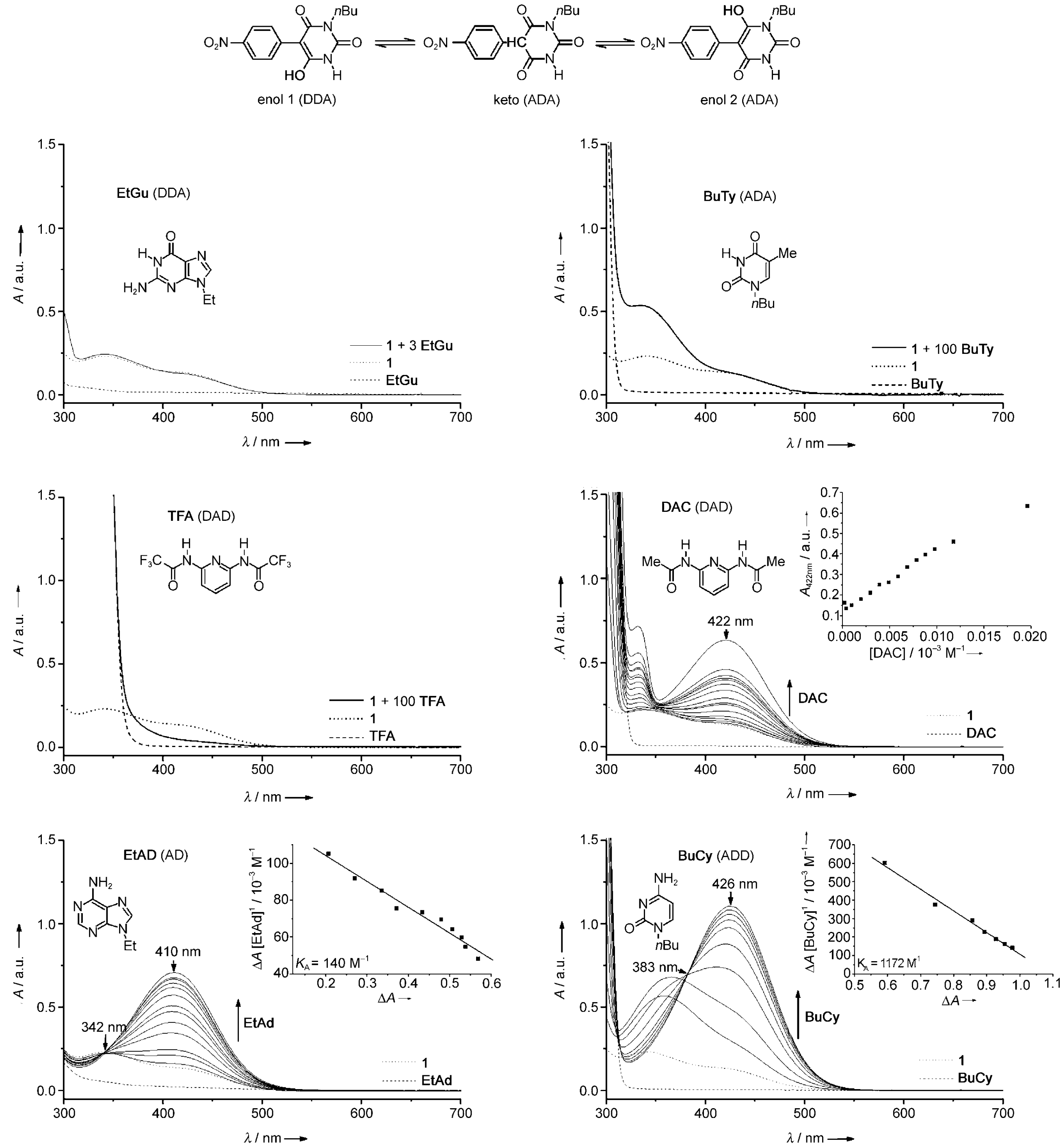
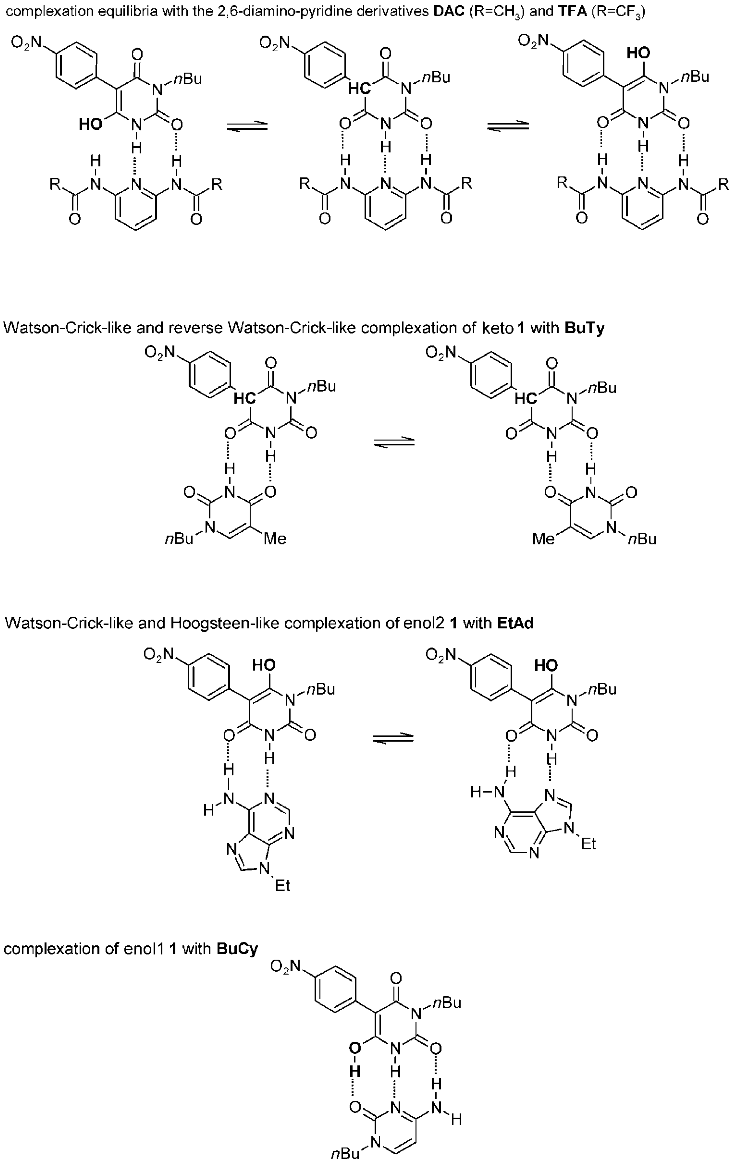


Figure 7. Comparison of the results from the UV/Vis titrations of **1** (1.96×10^{-7} mol L^{-1}) with the artificial receptors **EtGu** (5.88×10^{-7} mol L^{-1}), **BuTy** (1.96×10^{-5} mol L^{-1}), **TFA** (1.96×10^{-5} mol L^{-1}), **DAC** (1.96×10^{-7}–1.96×10^{-5} mol L^{-1}), **EtAd** (1.96×10^{-7}–1.18×10^{-5} mol L^{-1}) and **BuCy** (1.96×10^{-7}–0.69×10^{-5} mol L^{-1}), measured in dichloromethane/methanol (1.00:0.04).

were found. Because of the nature of supramolecular interactions, each complexation example represents a differential change in the electron-donating effect of the enolisable barbituric acid unit, and therefore a gradual change in the push–pull system. These structural changes can be measured by means of the shift in the UV/Vis absorption bands. We have therefore found the first simple system capable in principle of discriminating between the different hydrogen-bonding patterns in nucleic acid bases and related compounds, based on a differentiated UV/Vis signal. This has opened up potential for developing a new class of UV/Vis probe molecules.



Scheme 5. Possible complex formation equilibria of the enolisable barbiturate **1** with the 2,6-diaminopyridine derivatives 2,6-diacetamidopyridine (**DAC**, R=CH_3) and 2,6-bis(trifluoroacetamido)pyridine (**TFA**, R=CF_3), as well as with the artificial nucleic acid bases 1-*n*-butylthymine (**BuTy**), 9-ethyladenine (**EtAd**) and 1-*n*-butylcytosine (**BuCy**).

Table 2. Complexation properties of the enolisable chromophore **1** with the synthetic receptors.

Base	λ_{max} [nm] [a]	Type of H-bonding in **1**		Number of H-bonds	K_A [M^{-1}][d]
DAC	422	DAD	equil[c]	3	–
TFA	–[b]	DAD	keto	3	14±4
EtGu	–[b]	AD	–[b]	–	–
BuCy	426	DDA	enol1	3	1172±36
EtAd	410	DA	enol2	2	147±7
BuTy	–[b]	AD	keto	2	36±4[e]

[a] Measured in CH_2Cl_2/MeOH 1:0.04. [b] Cannot be determined. [c] Keto–enol equilibrium. [d] Determined by UV/Vis titration (Scatchard plot) in CH_2Cl_2/MeOH 1.0:0.04. [e] Determined by ^{1}H NMR titration in CD_2Cl_2.

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