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Host−Guest Interactions between Molecular Clips and Multistate Systems Based on Flavylium Salts

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Abstract: Flavylium salts contain the basic structure and show a pH-dependent sequence of reactions identical to natural anthocyanins, which are responsible for most of the red and blue colors of flowers and fruits. In this work we investigated the effect of the water-soluble molecular clips **C1** and **C2** substituted by hydrogen phosphate or sulfate groups on the stability and reactions of the flavylium salts **1**−**4** by the use of UV−vis absorption, fluorescence, and NMR spectroscopy as well as of the time-resolved pH jump and flash photolysis methods. Clip **C1** forms highly stable host−guest complexes with the flavylium salts **1** and **2** and the quinoidal base **3A** in methanol. The binding constants were determined by fluorometric titration to be log $K = 4.1$, 4.7, and 5.6, respectively. Large complexation-induced ^{1}H NMR shifts of guest signals, $\Delta\delta_{max}$, indicate that in the case of the flavylium salts **1** and **2** the pyrylium ring and in the case of the quinoidal base **3A** the *o*-hydroxyquinone ring are preferentially bound inside the clip cavity. Due to the poor solubility of these host−guest complexes in water, the association constants could be only determined in highly diluted aqueous solution by UV−vis titration experiments for the complex formation of clip **C1** with the flavylium salt **3A**H$^+$ at pH = 2 and the quinoidal base **3A** at pH = 5.3 to be log $K = 4.9$ for both complexes. Similar results were obtained for the formation of the complexes of the sulfate-substituted clip **C2** with flavylium salt **4A**H$^+$ and its quinoidal base **4A** which are slightly better soluble in water (log $K =$ 4.3 and 4.0, respectively). According to the kinetic analysis (performed by using the methods mentioned above) the thermally induced trans−cis chalcone isomerization (**4Ct** → **4Cc**) and the H_2O addition to flavylium cation **4A**H$^+$ followed by H$^+$ elimination leading to hemiketal **4B** are both retarded in the presence of clip **C2**, whereas the photochemically induced trans−cis isomerization (**4Ct** → **4Cc**) is not affected by clip **C2**. The results presented here are explained with dominating hydrophobic interactions between the molecular clips and the flavylium guest molecules. The other potential interactions (ion−ion, cation−π, π−π, and CH−π), which certainly determine the structures of these host−guest complexes to a large extent, seem to be of minor importance for their stability.

Introduction

Synthetic flavylium salts constitute a versatile family of compounds possessing the same basic structure and a network of chemical reactions identical to anthocyanins, the ubiquitous compounds responsible for most of the red and blue colors of flowers and fruits.[1−5]

The species involved in the general network of chemical reactions starting from a flavylium salt in moderately acidic aqueous solutions are shown in Figure 1.[3−5] The flavylium cation itself, **A**H$^+$, is the thermodynamically stable species in water at acidic pH values. When the pH is increased, two reaction pathways occur: an acid−base reaction forming the quinoidal base **A**, and a hydration reaction forming the hemiketal **B**. The rate of the hydration reaction is strongly pH-dependent and typically occurs in the second or subsecond time scale. The ring-opening of hemiketal ring in **B** leads to cis-chalcone **Cc**; this reaction occurs also in the subsecond time scale and is a tautomerization catalyzed by both acids and bases. Finally, the trans-chalcone, **Ct**, is formed from its cis isomer in a time scale that can range from several minutes to days. The quinoidal base **A** is a kinetic product that is drained through **A**H$^+$, **B**, and **Cc** to **Ct**, the thermodynamically stable species in the neutral pH range.

Among the reactions described in Figure 1, the hydration reaction is particularly relevant. The hydration reaction (and the coupled fast tautomerization) is important in the establishment of color in the vacuoles of plant cells, since it is the key step for color loss of these dyes at moderately acidic pH values. The pH inside the vacuoles of plant cells, where anthocyanins

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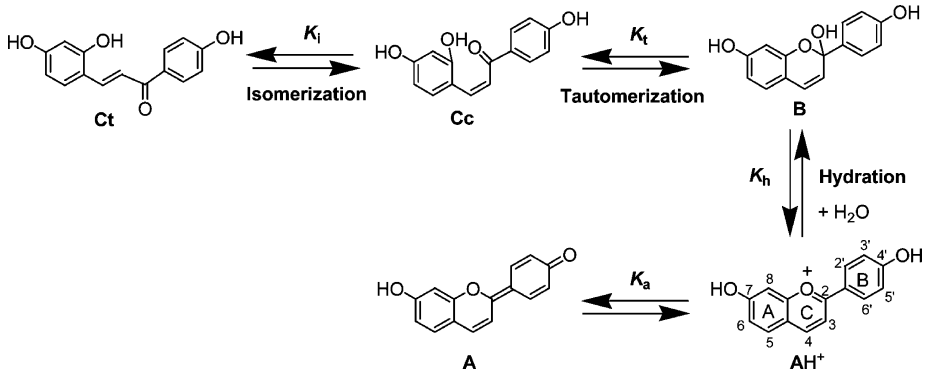

Figure 1. General network of chemical reactions of 7,4′-dihydroxyflavylium cation $\mathbf{AH}^+$ in acidic/neutral medium.

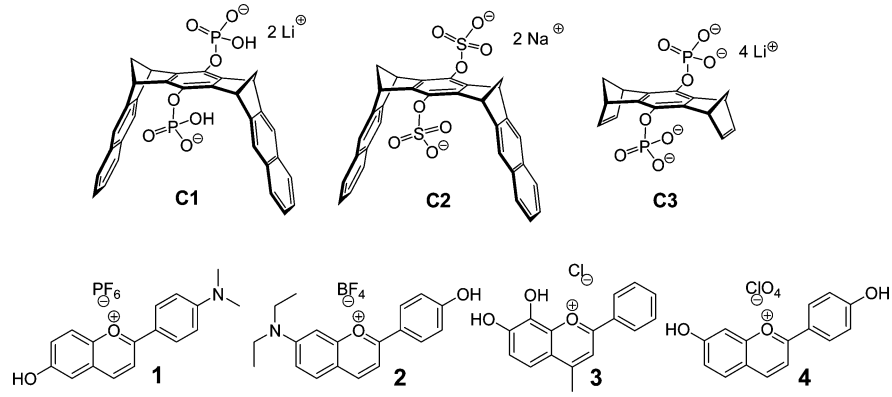

Figure 2. Structure of the molecular clips (**C1** and **C2**), model compound **C3** (phosphate-substituted bridge), and flavylium salts **1**−**4** used throughout this work.

are located in vivo, ranges roughly between 3 and 6.[6] At these pH values, anthocyanin solutions in vitro are essentially colorless, and nature had to develop strategies for stabilizing the color of anthocyanins inside the vacuoles. These strategies involve the association of flavylium cations and their quinoidal bases with other polyphenols (copigmentation) and complexation with metal ions, giving rise to beautifully organized supramolecular structures.[7] It is usually believed that copigmentation is driven by hydrophobic stacking and that hydration is reduced mainly by exclusion of water from the vicinity of the reactive center, but recent work[8] shows that hydration is actually very efficient in low-dielectric media suggesting the existence of other stabilization effects, such as charge-transfer interactions.[9] The study of flavylium salts upon host−guest complexation could in principle contribute to uncover the thermodynamic contributions to the copigmentation effect in nature.

During the past few years, Klärner and co-workers have introduced various molecular tweezers and clips designed for the inclusion of electron-poor guests such as aromatics with −M substituents, pyridinium cations, or even sulfonium cations.[10] These guests were mainly bound by $\pi-\pi$, CH$-\pi$, and $\pi-$cation interactions inside their electron-rich concave cavity. However, due to the lipophilic nature of these hosts, molecular recognition was restricted to organic solvents. On a later development, the molecular clips were decorated with phosphonate monoester anions, leading to water-soluble host molecules, flanked by two negatively charged functionalities for hydrogen bonding and/or additional ion pairing with cationic guests.[11] More recently, the hydrogen phosphate- and sulfate-functionalized clips **C1** and **C2** (see Figure 2) were synthesized presenting superior inclusion properties for electron-poor

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guests.[12] Flavylium salts, with their positive 1-benzopyrylium moieties, seem excellent candidates to be hosted by these molecular clips.

Figure 2 shows the structures of the flavylium salts **1**−**4**, the water-soluble molecular clips **C1** and **C2** substituted with hydrogen phosphate or sulfate groups in the central bridging unit, and the phosphate-substituted bridge **C3** to be used as guest and host molecules, respectively, in this study. Flavylium salts **1**[13] and **2**,[14] with amino substituents, were chosen on the basis of their stability until pH 4−5 where they start to be hydrated; they are in principle suitable to study the interaction of these flavylium salts with the hydrogen phosphate-substituted clip **C1** in a pH range where **C1** remains unprotonated, whereas the phosphate groups (OPO_3^{2-}) of bridge **C3** are certainly protonated to the corresponding hydrogen phosphate groups ($OP(O-H)O_2^-$) under these slightly acidic conditions. Flavylium salt **3**[15] is hardly hydrated in aqueous solutions due to the methyl group in position 4 (that reduces positive charge on position 2); compound **3** was chosen to allow NMR studies on both states $\mathbf{AH}^+$ and **A**, with no worries with the hydration reaction. Flavylium salt **4**,[16] a very well studied compound, is hydrated at pH 2−3, and the resulting chalcones are photochromic. It was chosen to test the effect of host−guest complex formation on the network of reactions shown in Figure 1. Particularly, we were interested in the following two questions: (1) Does the host molecule in the host−guest complex protect the flavylium core of the guest molecule from water attack? (2) Is the trans−cis isomerization of the chalcones affected upon complexation?

Experimental Section

Synthesis. Synthesis of water-soluble clip **C1** and **C2** and the bridge **C3** was recently described.[11,12] Flavylium salts **1**−**4** were available from previous studies.[13–16]

Mass Spectrometry. Electrospray ionization (ESI) mass spectra were recorded on a Bruker BioTOF II mass spectrometer.

UV−Vis Absorption and Emission Studies. All flavylium solutions were freshly prepared using acidified (HCl) water or methanol. The association constants were determined by adding increasing amounts of a stock solution of the clip to 2 mL of the flavylium. After each addition the absorption and emission spectra were taken. Absorption spectra were run on a Shimadzu UV-2501PC or a CARY 100Bio, and fluorescence spectra were run on a Jobin-Yvon Spex, Fluorolog FL3-22.

Table 1. ESI-MS Data Found for the Precipitates of the 1:1 Mixtures of Molecular Clip **C1** with the Flavylium Salts **1**−**3** from Water (Dissolved in Methanol)

ion	$(m/z)_{exptl}$	$(m/z)_{calcd}$
$[\mathbf{C1} - 2Li^+]^{2-}$	298.0347	298.0400
$[\mathbf{1} + \mathbf{C1} - PF_6^- - 2Li^+]^-$	862.1909	862.1965
$[\mathbf{1} - PF_6^-]^+$	619.0641	619.0699
$[\mathbf{C1} - 2Li^+]^{2-}$	298.0360	298.0400
$[\mathbf{2} + \mathbf{C1} - BF_4^- - 2Li^+]^-$	890.2230	890.2289
$[\mathbf{2} + \mathbf{C1} - BF_4^- - 2Li^+ - H^+]^{2-}$	444.6075	444.6108
$[\mathbf{2} - BF_4^-]^+$	294.1495	294.1498
$[\mathbf{C1} - 2Li^+]^{2-}$	298.0367	298.0400
$[\mathbf{C1} - 2Li^+ + H^+]^-$	597.0837	597.0874
$[\mathbf{3} + \mathbf{C1} - Cl^- - 2Li^+]^-$	849.1598	849.1660
$[\mathbf{3} - Cl^-]^+$	253.0848	253.0859

Fluorescence quantum yields were determined using rhodamine 6G in ethanol as standard for flavylium salts **1** and **2** ($\mathbf{AH}^+$ form) and **3** (**A** form); perylene in toluene was used as standard for **3** ($\mathbf{AH}^+$ form). Standard fluorescence quantum yields and refraction indexes were taken from literature.[17,18]

Time-resolved fluorescence decays with picosecond resolution were obtained by the single-photon time technique using laser excitation at 390 nm and recording the emission at 510 nm. The setup consisted of a Ti:Sapphire laser Tsunami (Spectra Physics) pumped with a solid-state laser Millennia Xs (Spectra Physics), delivering 70 fs pulses at a repetition rate of 80 MHz. The laser repetition rate was reduced to 4 MHz using a pulse-picker (APE), and the output was frequency doubled to 390 nm (∼1 nJ per pulse) and vertically polarized. The fluorescence passed through a polarizer set at the magic angle and was selected by a Jobin-Yvon HR320 monochromator with a grating of 100 lines/mm and detected by a Hamamatsu 2809U-01 microchannel plate photomultiplier. The experimental excitation pulse (fwhm = 35 ps) was measured using a scattering solution (Ludox AM30, Aldrich) in water. The decays were stored in a multichannel analyzer working with 1024 channels. The fluorescence emission was observed at 510 nm using a cutoff filter to effectively eliminate the scattered light from the sample. The experimental decay curves were fitted to simulated curves using a nonlinear least-squares reconvolution method.

^{1}H NMR, ^{13}C NMR, DEPT H,H-COSY, C,H-COSY, NOESY, HMQC, HMBC, ^{1}H NMR Titration Experiments. A Bruker DRX 500 was used. The undeuterated amount of the solvent was used as an internal standard. The ^{1}H and ^{13}C NMR signals were assigned by the 2D experiments mentioned above. In the titration experiments, the total guest concentration $[S]_0$ was kept constant, whereas the total host concentration $[R]_0$ was varied. This was achieved by dissolving a defined amount of receptor R in 0.6 mL of the solution containing the guest concentration $[S]_0$. The association constants K and the maximum complexation-induced ^{1}H NMR shifts, $\Delta\delta_{max}$, were determined from the dependence of the guest ^{1}H NMR shifts, $\Delta\delta$, on the host concentrations by nonlinear regression analysis using the computer program TableCurve 2D, version 5.01.

Kinetic Studies. pH jumps were carried out by mixing 100 μL of universal buffer at desired pH,[19] 400 μL of NaOH 0.01 M, and 500 μL of a stock solution of the flavylium **4** at pH 2.0 (1.4×10^{-5} M) or 200 μL of NaOH 0.01 M, 200 μL of a stock solution of the flavylium cation at pH 2.0 (3.4×10^{-5} M), and 500 μL of water. In the cases where the clip is present, 30 μL of clip **C2** 2.9×10^{-3} M or 28.5 μL of clip **C2** 3.1×10^{-3} M were previously mixed with the used amount of the flavylium ion. The absorption

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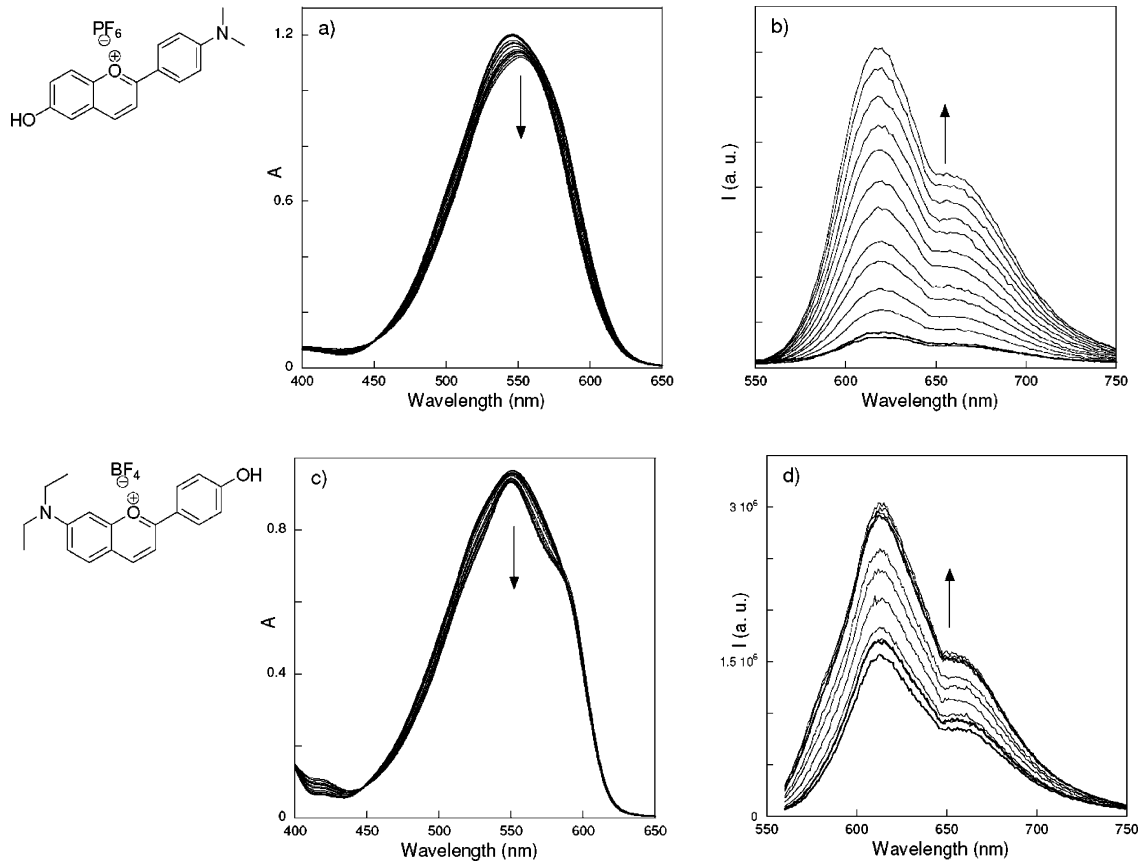


Figure 3. Spectral modifications observed upon addition of molecular clip, [**C1**] = 0−7 × 10^{-4} M, to methanolic solutions of 4′-dimethylamino-6-hydroxyflavylium hexafluorophosphate, [**1**] = 1.92 × 10^{-5} M, followed by absorption (a) and fluorescence emission (b) (2 nm slits, λ_{exc} = 545 nm) and to methanolic solutions of 7-diethylamino-4′-hydroxyflavylium hexafluorophosphate, [**2**] = 2.26 × 10^{-5} M, followed by absorption (c) and fluorescence emission (d) (2 nm slits, λ_{exc} = 550 nm).

Table 2. Photophysical Properties of **1**−**3**, **C1**, and Their Complexes in Methanol

	absorption	luminescence		
compd	λ_{max}/nm (ε/M^{-1} cm^{-1})	λ/nm	Φ_f[a]	τ/ns
C1	252 sh (33 300)	331		
	308 (2000)			
	322 (2300)			
1	547 (62 600)	618	0.0002	0.08
2	551 (42 700)	613	0.03	0.6
3	383 (25 583)	<650	$<10^{-5}$	
	462 (9223)			
3A	335 (11 300)	641	0.0004	
	545 (5300)			
1@C1	553 (58 300)	616	0.002	
2@C1	550 (41300)[b]	613	0.07	1.8
3A@C1	545 (5300)	653	0.0008	

[a] Estimated error of ± 20%. [b] When molecular clip **C1** is added to **2**, a shoulder at ca. 590 nm is evidenced.

spectrum was read immediately after the pH jump and followed along the time.

Flash photolysis experiments were performed as described elsewhere,[16b] using a CARY 5000 with fiber-optics adapter and a head to which the flash was adapted. A filter was used in the reference beam (neutral density, T = 0.01) in order to maximize the signal. The kinetics was followed at 450 nm, average time 0.1 s, and 5 nm slits.

Photochemical Reactions. The photoisomerization reaction of **4Ct** was studied by irradiating 3 mL of an aqueous solution of equilibrated [**4**] = 6.3 × 10^{-6} M at pH = 5.7 in presence and in absence of clip [**C2**] = 4.0 × 10^{-5} M, following the reaction by UV−vis spectroscopy. The irradiations were carried out using as light source the 450 W xenon lamp of a Fluorolog FL3-22, with 5 nm slits in both monochromators.

Results and Discussion

Host−Guest Formation with Molecular Clip C1. Studies in Methanol. When aqueous acidic solutions of clip **C1** and flavylium salts **1**−**4** are mixed together, a red shift in color and a precipitation of solid materials are observed. ^{1}H NMR spectra of the precipitates dissolved in [D_6]DMSO suggest the formation of 1:1 complexes between clip **C1** and the corresponding flavylium salt. The 1:1 complex stoichiometry was also confirmed by ESI-MS spectra, Table 1. In each case, the ion peaks corresponding to the free clip and the 1:1 complex with the flavylium cation (without the counterions) were observed in the negative spectral mode, whereas in the positive spectral mode only the ion peaks corresponding to the free flavylium cations were detected (see the Supporting Information).

The mixture of clip **C1** and the flavylium salts **1**−**3** was soluble in methanol. Thus, the host−guest interactions between these compounds could be studied in methanol by UV−vis absorption spectroscopy, steady-state and time-resolved spectrofluorimetry, and ^{1}H NMR dilution titrations.

UV−Vis and Fluorescence. The color of the solution of 4′-dimethylamino-6-hydroxyflavylium hexafluorophosphate, **1**, and 7-diethylamino-4′-hydroxyflavylium tetrafluoroborate, **2**, in methanol, is intensely purple and pink, respectively. In both cases the observed color corresponds to the presence of the flavylium cation, AH^+, which is stable in solution for days. The

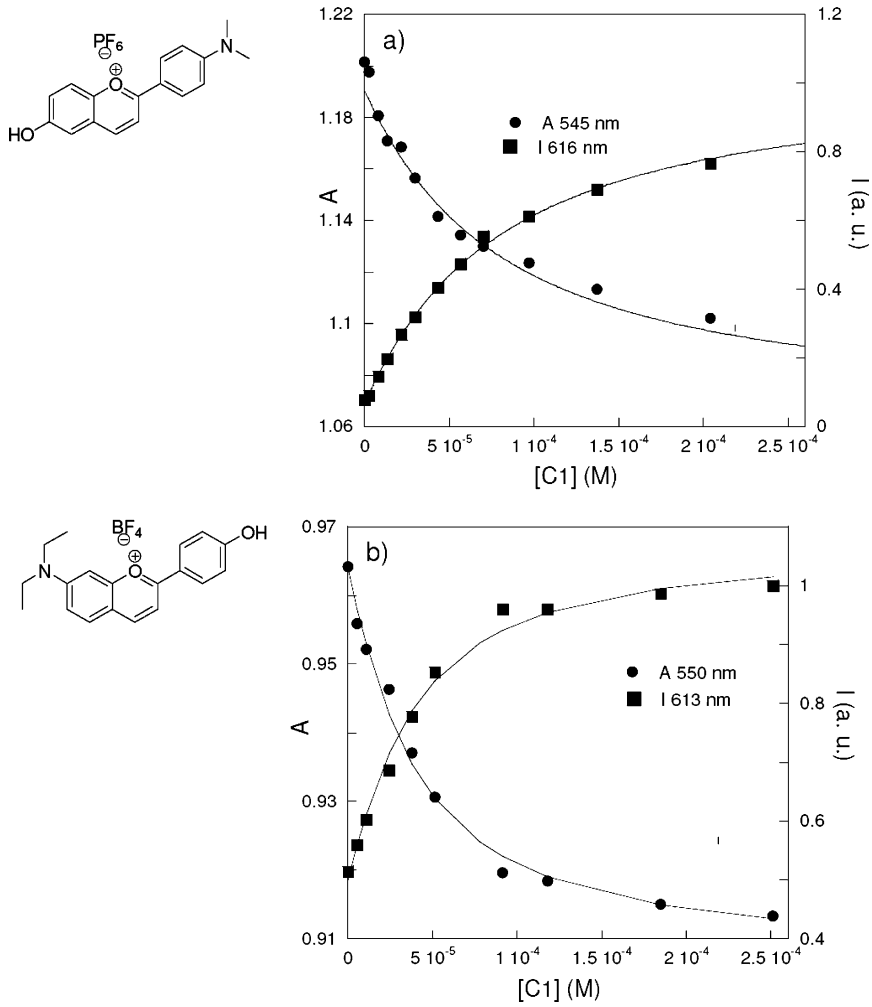


Figure 4. Fitting of the data in Figure 3 using eq 1 for the emission data and a similar equation for absorption data: (a) **1@C1**, log $K = 4.2 \pm 0.1$; (b) **2@C1**, log $K = 4.7 \pm 0.1$.

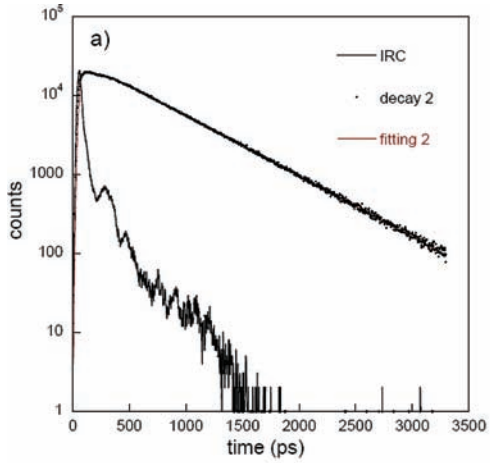
maximum in the UV−vis absorption spectrum of flavylium salt **1** and **2** in methanol is at 547 and 551 nm, respectively. These maxima are red-shifted relative to the values observed in acidic aqueous solution (540 nm for **1**[13]), as expected for positively charged heterocycles characterized by $\pi-\pi^*$ transitions with strong charge-transfer character, which present negative solvatochromism.[20]

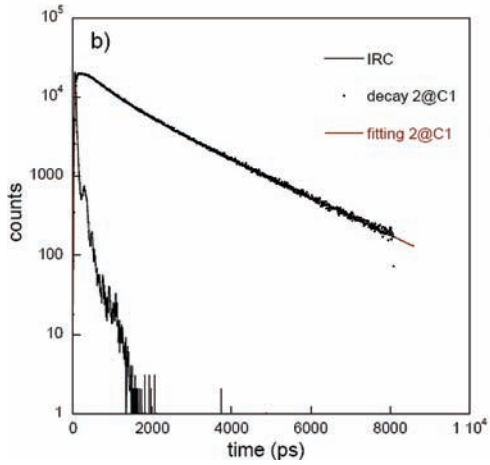
The addition of increasing amounts of molecular clip **C1** to methanolic solutions of **1** and **2** was followed by UV−vis absorption spectroscopy and spectrofluorimetry (Figure 3). Figure 3a shows that the addition of clip **C1** to flavylium salt **1** causes a red shift in the absorption maxima as well as a small decrease in the absorption intensity. In the case of the addition of **C1** to flavylium salt **2** the red shift is observed in the shoulder at ca. 585 nm and not at λ_{max}. The red shifts of the absorption maxima or shoulder indicate the positioning of the flavylium ions into a less polar medium than the original methanol and, hence, the interaction of the flavylium cations with the molecular clip. Further evidence for this interaction comes from the finding that the intensity of the emission band at 616 and 613 nm, respectively, for flavylium salts **1** and **2**, strongly increases upon addition of clip **C1**. This result suggests that each flavylium cation is bound inside the cavity of clip **C1**, since in the more rigid environment of the clip cavity the nonradiative transitions become less important, leading to an increase in the fluorescence quantum yield. Table 2 resumes the relevant photophysical data for **1**−**3**, **C1**, and their complexes in methanol.

The concentration-dependent spectral evolutions shown in Figure 3 were used to obtain the association constants, K, between molecular clip **C1** and flavylium salts **1** and **2**. A 1:1 stoichiometry was assumed, as suggested by ESI-MS data and confirmed by ^{1}H NMR (vide infra). The emission data was fitted using eq 1[21] where I_0 is the emission intensity of the flavylium in the absence of host **C1**, I_{lim} is the emission intensity of the complex of the flavylium salt with clip **C1**, S refers to the flavylium salt, R to the clip **C1**, and K is the association constant. The UV−vis data were fitted using a similar equation for the concentration dependence of the absorption. The simultaneous fitting of absorption and emission data for each host−guest complex are shown in Figure 4, allowing us to obtain log K values of 4.2 ± 0.1 and 4.7 ± 0.1 for **1@C1** and **2@C1**, respectively.

$$I = I_0 + (I_{lim} - I_0) \times \frac{([R]_0 + [S]_0 + 1/K) - \sqrt{([R]_0 + [S]_0 + 1/K)^2 - 4[R]_0[S]_0}}{2[S]_0} \quad (1)$$

For flavylium salt **2** in the absence and presence of clip **C1**, fluorescence lifetimes were measured, Figure 5. The decay of **2** in methanol is monoexponential with a lifetime of 0.6 ns. When clip





c)

	τ_1 (ns)	τ_2 (ns)	χ^2
2	0.6 (100 %)	-	1.28
2@C1 (84 %)	0.6 (24 %)	1.8 (76 %)	1.07

Figure 5. Fluorescence decays of flavylium **2** (a) and **2@C1** (84% complexation) (b) in methanol. Excitation at 390 nm, emission at 650 nm, scale 3.44 ps/channel for **2**, 8.4 ps/channel for **2@C1**. (c) Table with fitting parameters for flavylium salt **2** in the absence (lifetime 0.6 ns) and in presence of clip **C1** (lifetime 1.8 ns).

C1 is added, the decays become biexponential with lifetimes of 0.6 and 1.8 ns. This longer lifetime corroborates the emission data, where the intensity increases upon addition of clip **C1**, which was assigned to complexed **2**. The weighed amplitudes of these lifetimes are in reasonable agreement with the mole fractions of **2** and **2@C1** calculated on the basis of the association constant log $K = 4.7$: accordingly, a solution of clip **C1** (1.5×10^{-4} M) and flavylium salt **2** (1.8×10^{-5} M) contains 16% of free salt **2** and 84% of complex **2@C1**, a contribution of 76% of the longer lifetime is calculated from the amplitudes.

The quinoidal bases of flavylium salts **1** and **2** can be easily obtained by adding a drop of NaOH (1 M) to a previously prepared solution in methanol. However, these species are not stable. They undergo subsequent reactions to the more stable trans-chalcone species as final products, as could be easily detected by the color evolution of the solutions. For this reason, it was not possible to study the interaction between the molecular clip **C1** and these quinoidal bases.

Contrary to **1** and **2**, flavylium salt **3** does not hydrate, and in the presence of methanol **3** exists in its flavylium form $\mathbf{AH}^+$ and/or its quinoidal base **A**. When flavylium salt **3** is dissolved in methanol a color between yellow and purple may be observed indicating a mixture of both forms. This is certainly due to the presence of acid or base trace impurities in the solvent or in the flask. To overcome these problems, small aliquots of dilute HCl (0.1 M) or NaOH (0.1 M) were added until constant absorption spectra of **3** were observed, in order to obtain either the flavylium cation, $\mathbf{AH}^+$, or the quinoidal base, **A**. The interaction between the flavylium salt $\mathbf{3AH}^+$ (obtained by addition of small amounts of dilute HCl to the methanolic solution) and clip **C1** is very weak (see the Supporting Information) contrary to that with quinoidal base **3A**. The association constant for the formation of complex $\mathbf{3AH}^+\mathbf{@C1}$ in methanol was determined by ^{1}H NMR titration to be log $K = 2.0 \pm 0.1$ (vide infra). Figure 6 shows the spectral changes observed upon addition of **C1** to methanolic solutions of the quinoidal base **3A**. The absorption spectra show no changes on the flavylium absorption band, whereas a small increase is observed in the fluorescence intensity. These changes, although small, are reproducible and allowed us to determine the association constant for the formation of complex **3A@C1** to be log $K = 5.6 \pm 0.2$. This result of a highly stable host−guest complex **3A@C1** is in agreement with the findings of the ^{1}H NMR titration experiments which only gave a lower limit of the association constant of log $K \geq 4.2$ (vide infra).

^{1}H NMR. The host−guest complex formation between clip **C1** and flavylium salts **1**−**3** could be also detected by ^{1}H NMR. In the spectra of a methanol solution containing clip **C1** and one of the flavylium salts, either **1**, **2**, or **3**, profound upfield shifts of the

(20) Mataga, N.; Kubota, T. *Molecular Interactions and Electronic Spectra*; Marcel Dekker, Inc.: New York, 1970.
(21) Cudic, P.; Zinic, M.; Tomisic, V.; Simeon, V.; Vigneron, J.-P.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1995**, 1073–1075.

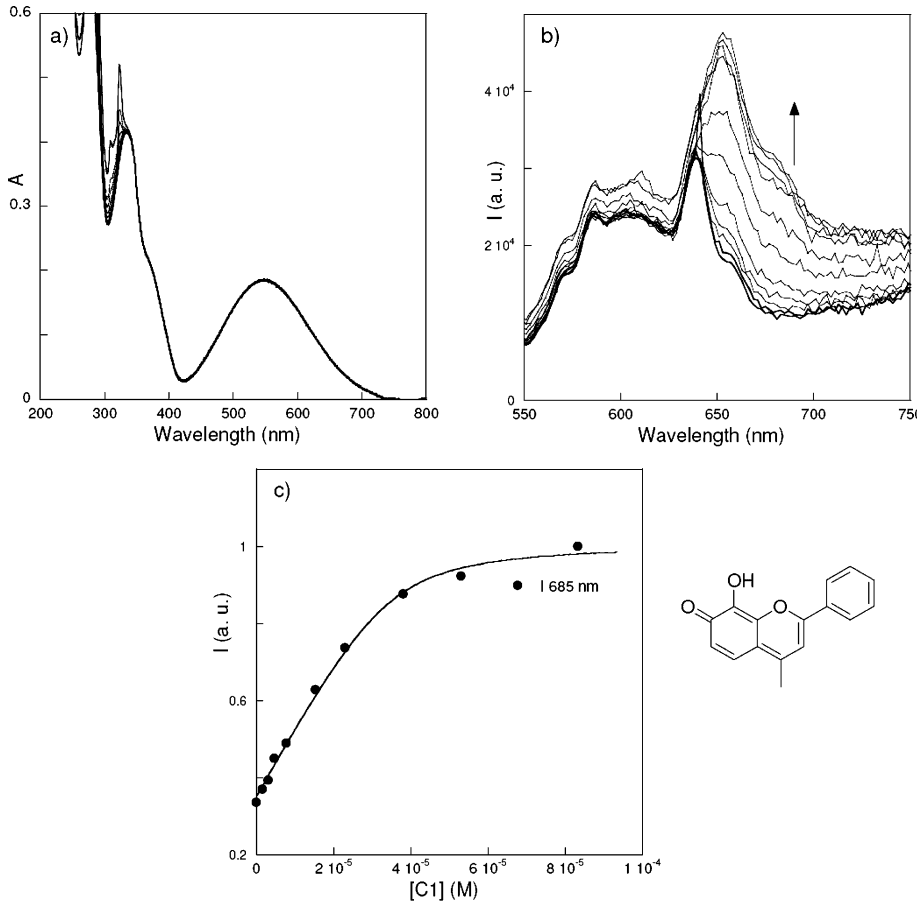


Figure 6. Spectral modifications observed upon addition of clip **C1**, [**C1**] = 0−8.3 × 10^{-5} M, to a methanolic solution of **3A**, [**3A**] = 3.46 × 10^{-5} M, followed by absorption (a) and fluorescence emission (b) (λ_{exc} = 520 nm). (c) Fitting of the data in panel b using eq 1 leads to log K = 5.6 ± 0.2.

signals assigned to the flavylium as well as to the clip arene protons are observed, as illustrated for clip **C1** and flavylium salt **2** in Figure 7 (see the Supporting Information for data of the other flavylium salts). The ^{1}H NMR signals of host **C1** and guests **1**−**3** could be assigned by the use of two-dimensional ^{1}H NMR experiments (see the Supporting Information). The observation of upfield shifts of the guest and host ^{1}H NMR signals indicate that both species are facing each other's aromatic regions, and hence, the flavylium guest is bound inside the clip cavity.

In the ^{1}H NMR spectra of the mixture between clip **C1** and flavylium salt **1**, **2**, or **3** the signals of the guest protons are broad (Figure 7). This finding indicates that the mutual host−guest complex formation and dissociation is already slow compared to the time which is required to observe sharp NMR signals resulting from an averaging between the signals of complexed and free guest. However, this mutual interconversion is still too fast to observe separate signals for complexed and free guest. We tried to determine the association constants K and the maximum complexation-induced shifts of the guest ^{1}H NMR signals, $\Delta\delta_{max}$, by means of ^{1}H NMR dilution titration experiments. In these experiments, the guest concentration of the flavylium salt was kept constant and the host concentration of clip **C1** was varied. In the range of concentrations which could be studied, the observed complexation-induced ^{1}H NMR shifts of the guest signals, $\Delta\delta_{obs}$, of the flavylium salts **1**, **2**, and quinoidal base **3A** did not vary significantly (Figure 8). This small concentration dependence of $\Delta\delta_{obs}$ already indicates that the complexes of clip **C1** with these guest molecules are very stable. For each guest signal, the dependence of the chemical shift, $\Delta\delta_{obs}$, on the clip concentration was fitted with eq 2 (similar to eq 1), as exemplified in Figure 8 for one kind of proton of each flavylium salt **1**, **2**, **3A**, or **3AH**$^+$ (see the Supporting Information for data of other flavylium protons). This fitting allowed us to determine the $\Delta\delta_{max}$ values very accurately (Figure 9) but not the association constants, K (Table 3). These experiments only provide lower limiting values for the association constants, log $K \geq 4.3$. In the case of **3** the formation of the stable complex with clip **C1** in methanolic solution was only observed for the deprotonated quinoidal **3A** form but not for salt **3AH**$^+$ which can be produced by the addition of dilute DCl to the methanol solution of **3A**. The association constant (determined by ^{1}H NMR titration) for the complex formation of **3AH**$^+$ with clip **C1** in an acidified methanolic solution is with log $K = 2.0 \pm 0.1$ surprisingly small, whereas the $\Delta\delta_{max}$ values (extrapolated from the clip-concentration dependence of the guest $\Delta\delta_{obs}$ values) are large comparable to those obtained for the complexes of **C1** with the other flavylium salts **1** and **2** (Figure 9).

$$\Delta\delta_{max} = \frac{\Delta\delta_{obs}[S]_0}{\frac{1}{2}\left([R]_0 + [S]_0 + \frac{1}{K_a}\right) - \sqrt{\frac{1}{4}\left([R]_0 + [S]_0 + \frac{1}{K_a}\right)^2 - [R]_0[S]_0}} \quad (2)$$

The large complexation-induced maximum upfield shifts, $\Delta\delta_{max}$, observed for the signals of the aromatic protons show

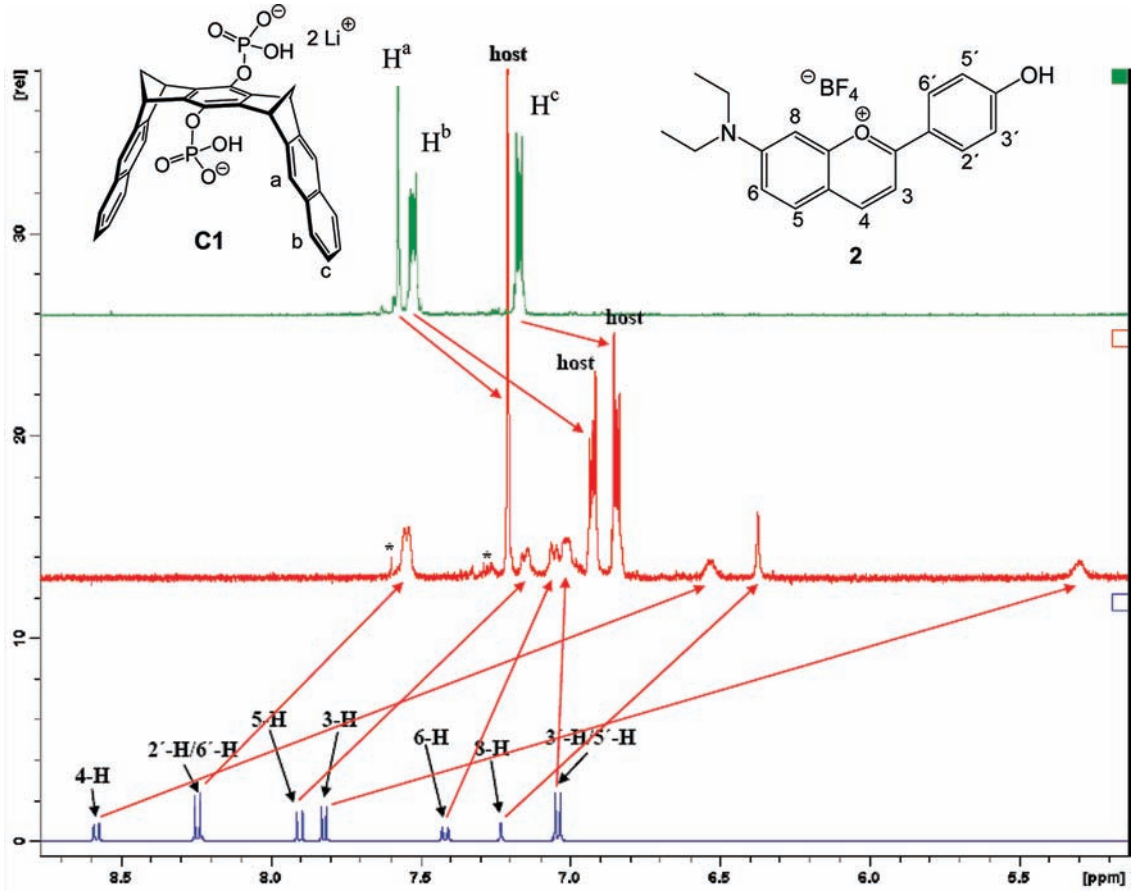


Figure 7. ^{1}H NMR spectra (500 MHz, CD_3OD, 25 °C) of clip **C1** (5.07 mM) (top), a mixture of **C1** (1.53 mM) and flavylium salt **2** (1.50 mM) (middle), and flavylium salt **2** (1.50 mM) (bottom) (aromatic range); * impurities.

that all flavylium cations are bound inside the clip cavity. For flavylium cations **1** and **2**, the signals of the protons 3-H and 4-H show the highest $\Delta\delta_{max}$ values indicating that the positively charged pyrylium ring is positioned inside the clip cavity presumably with the protons 3-H and 4-H pointing toward the central spacer-unit of clip **C1**. In the complex of **C1** with the flavylium salt **3A**H$^+$, the methyl group at C-4 evidently prevents this position of the pyrylium ring inside the clip cavity. In this case, the $\Delta\delta_{max}$ value of proton 3-H is, with 2.8 ppm, smaller than those of flavylium salt **1** ($\Delta\delta_{max}$ = 3.5 ppm) and **2** ($\Delta\delta_{max}$ = 3.1 ppm); on the other hand, the $\Delta\delta_{max}$ values of 5-H and 6-H (1.9 and 0.9 ppm, respectively) of **3A**H$^+$ are larger than those of **1** ($\Delta\delta_{max}$ (5-H) = 0.5 ppm) and **2** ($\Delta\delta_{max}$ (5-H, 6-H) = 0.9 and 0.4 ppm, respectively). These findings suggest that the methyl group at C-4 in **3A**H$^+$ points toward the tips of the clip naphthalene sidewalls and causes a shift of the guest position toward the inclusion of the terminal dihydroxy-substituted benzene ring inside the clip cavity. In the complex of clip **C1** with the quinoidal base **3A** the large value of $\Delta\delta_{max}$ for proton 6-H (1.5 vs 0.9 ppm in **3A**H$^+$) and the low value for 3-H (1.6 vs 2.8 ppm in **3A**H$^+$) suggest that the *o*-hydroxyquinone ring of **3A** is encapsulated in the clip cavity.

Attempts to model the host−guest complex structures of the flavylium salts by the use of force field methods (MMFF94, Monte Carlo conformer search implemented in SPARTAN)[23] were not very successful because these gas-phase calculations overestimate the ion−ion interaction between the differently charged flavylium salts and clip **C1** and the hydrogen bonds. Since in Macromodel the oxonium ion parameters are missing in the force fields (AMBER*, MMFF, MM2*, MM3*, OPLS), it was not possible to perform calculations including corrections for the solvation of the charged species with water or octanol which led to important information concerning the complex structures of this clip with pyridinium salts such as *N*-methylnicotinamide and nicotinamide adenine dinucleotide (NAD^+).[11] However, successful force field calculations could be run for the complex between the quinoidal form **3A** and clip **C1** (Figure 10). The Monte Carlo conformer search gave several structures in which the guest molecule is positioned inside the clip cavity. In the structure which is calculated to be only 1.1 kcal/mol higher in energy than the energy-minimum structure, the guest protons attached to the terminal *o*-hydroxyquinone ring point toward the central aromatic spacer-unit of clip **C1**. This finding suggests that these protons are particularly strongly influenced by the magnetic anisotropy of the clip arene units and is in good agreement with the large $\Delta\delta_{max}$ values determined for these protons by ^{1}H NMR titration experimentally. The large $\Delta\delta_{max}$ value observed for guest proton 3-H (attached to the pyrane ring) indicates, however, that several conformers (equilibrating rapidly with respect to the NMR time scale) are involved in the overall complex structure.

The host−guest complexes of hydrogen phosphate-substituted clip **C1** with the flavylium salts **1** and **2** as guest

(22) SPARTAN 04 version 1.0.0; Wave Function Inc.; Irvine, CA, 2004.
(23) (a) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467. (b) Macromodel, v. 7.1; Schrödinger: Portland, OR, 2001.

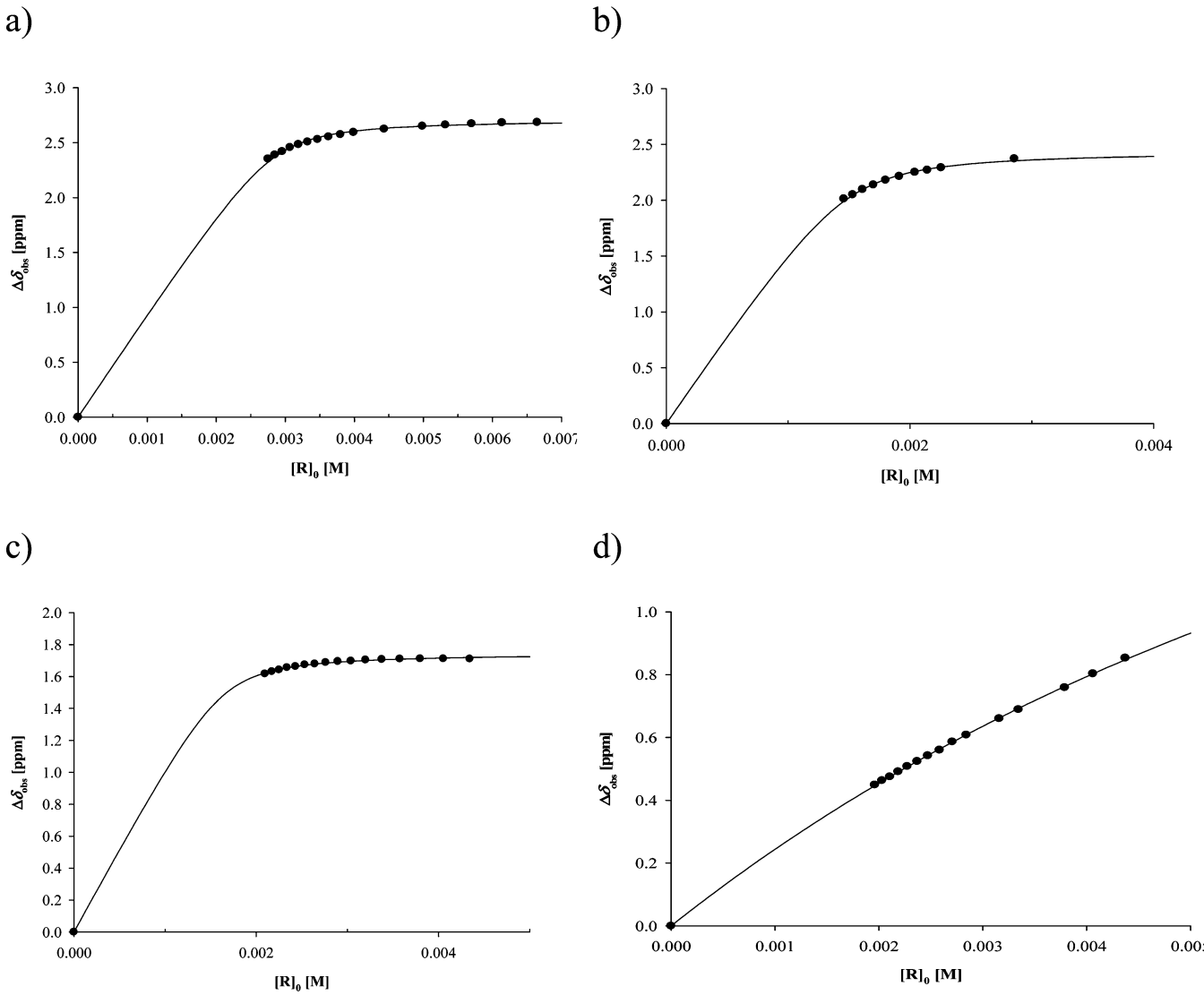


Figure 8. Dependence of $\Delta\delta_{obs}$ (flavylium salt) from the concentration of clip **C1** ($\equiv [R]_0$): (a) $\Delta\delta_{obs}$ (4-H) of **1**, (b) $\Delta\delta_{obs}$ (4-H) of **2**, (c) $\Delta\delta_{obs}$ (4-H) of **3A**, and (d) $\Delta\delta_{obs}$ (4-H) of **3A**H^+.

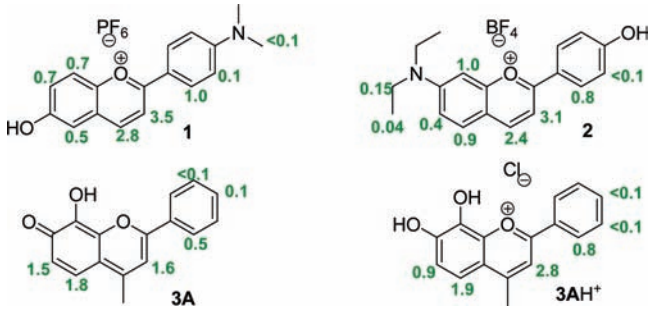


Figure 9. Complexation-induced maximum ^{1}H NMR shifts, $\Delta\delta_{max}$ ($= \delta_{complex} - \delta_0$) in ppm, determined by ^{1}H NMR titrations for the flavylium protons of **1**, **2**, and **3A**H^+ and the quinoidal base **3A** in the host−guest complexes with clip **C1**.

molecules are highly stable. ^{1}H NMR structural analysis (described above) suggests the positively charged guest pyrylium ring to be bound inside the clip cavity in both complexes. Thus, cation−π interactions between the pyrylium ring and the clip arene units seem to provide a significant contribution to the stability and structure of these complexes besides the Coulombic forces (between the negatively charged clip hydrogen phosphate groups and positively charged flavylium salt), dispersive, and solvophobic interactions. The result of the surprisingly high stability of the complex of **C1** with neutral quinoidal base **3A**, however, suggests that the arene−arene (CH−π) and the solvophobic interactions are major factors for the stability observed for this complex (Table 3). Quantum chemical calculation of the electrostatic potential surface of clip **C1** substituted by dihydrogen phosphate groups ($OPO(OH)_2$ instead of $OPO_2(OH)^-Li^+$) and quinoidal base **3A** indicate attractive electrostatic forces between the positively polarized hydrogen atoms 5- and 6-H

Table 3. Association Constants, log *K*, Determined for the Host−Guest Complex Formation of Clip **C1** with Flavylium Salts **1**, **2**, **3A**H^+, and the Quinoidal Base **3A** in Methanol, at 298 K Determined by UV−Vis Spectroscopic and/or Fluorometric Titration and ^{1}H NMR Titration in a Methanolic Solution Acidified by Addition of DCl

complex	**1@C1**	**2@C1**	**3AH$^+$@C1**	**3A@C1**
log *K*	4.1 ± 0.1[a]	4.7 ± 0.1[a]	2.0 ± 0.1[b]	5.6 ± 0.2[a]

[a] Determined by UV−vis spectroscopic and/or fluorometric titration. [b] Determined by ^{1}H NMR titration in a methanolic solution acidified by addition of DCl.

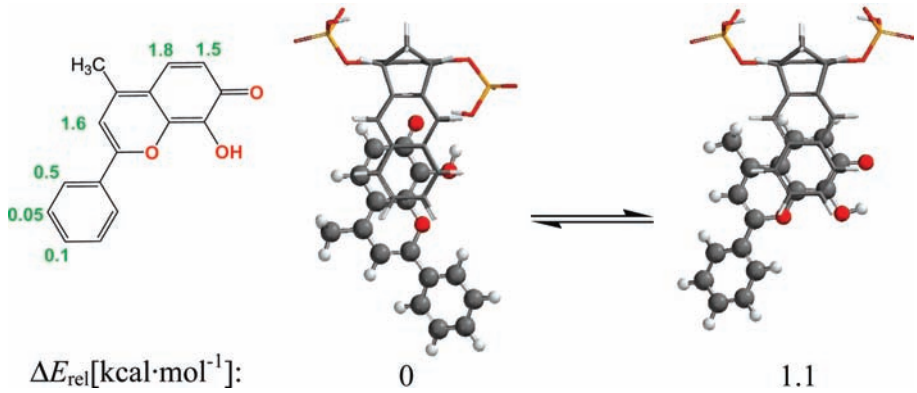


Figure 10. Comparison of the $\Delta\delta_{max}$ values (determined by ^{1}H NMR titration) with lowest-energy structures of the host−guest complex of the quinoidal base **3A** with clip **C1** calculated by a Monte Carlo conformer search (force field: AMBER*/H_2O, 5000 structures implemented in Macromodel 9.0) (ref 22).

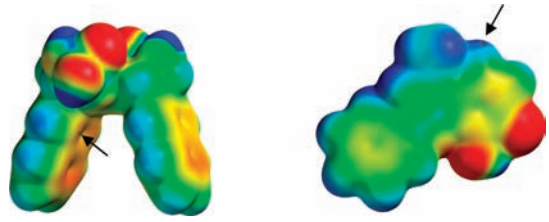


Figure 11. Electrostatic potential surface (EPS) calculated for clip **C1** substituted by dihydrogen phosphate groups ($OPO(OH)_2$ instead of $OPO_2(OH)^-Li^+$) (left) and quinoidal base **3A** (right) by the use of density functional B3LYP/6-31G**//AM1 (implemented in SPARTAN) (ref 24). The color code ranges from −25 kcal/mol (red) to +25 kcal/mol (blue). The molecular electrostatic potential (MEP) was calculated at the marked position to be −19 kcal/mol at the clip naphthalene sidewalls and +18 kcal/mol at hydrogen atom 5-H of **3A**.

attached to the *o*-hydroxyquinone ring of **3A** and the negatively polarized clip arene units (Figure 11).

The finding that clip **C1** only forms a very weak complex with the flavylium salt **3AH**$^+$ is surprising and not well understood. Speculatively, we assume that the addition of DCl to the mixture of clip **C1** and **3A** in methanol causes concomitant deuteration of the hydrogen phosphate groups of **C1** and the quinoidal base **3A**. Evidently, the noncharged neutral clip substituted by dihydrogen phosphate groups ($OPO(OH)_2$ or $OPO(OD)_2$) binds the flavylium salt **3AH**$^+$ (or **3AD**$^+$) only weakly. Since phosphoric acid is a weak acid compared to sulfuric acid, we examined the complex formation of flavylium salt **3AH**$^+$ with the sulfate-substituted clip **C2** in the presence of a small amount of DCl with the hope that under these conditions only the quinoidal base **3A** is deuterated and not the clip's sulfate groups. But also under these conditions only the formation of a weak complex was observed in methanol at 25 °C by ^{1}H NMR titration (log K = 2.2 ± 0.1). The large complexation-induced shifts of the guest signals ($\Delta\delta_{max}$ = 2.8 (3-H), 1.5 (5-H), and 0.4 (6-H)) again indicate that the guest pyrylium ring is bound inside the clip cavity. The small association constant suggests that the sulfate groups of clip **C2** were protonated (respectively, deuterated) concomitantly with the protonation/deuteration of the quinoidal base comparable to the hydrogen phosphate groups of clip **C1**.

Studies in Aqueous Solutions. The study of the host−guest interactions of flavylium salts **1** and **2** in aqueous solutions could not be performed because the complexes precipitate from water. However, water is by far the most interesting medium to study the effects of host−guest formation on the network of reactions of flavylium salts.

The compound 7,8-dihydroxyflavylium-4-methylflavylium chloride, **3AH**$^+$, does not suffer hydration in aqueous solution to give the hemiketal species. The only observed processes are the acid−base reactions to form the neutral quinoidal base **3A** with pK_{a1} = 4.0 (eq 3) and the anionic deprotonated species **3A**$^-$ with pK_{a2} = 6.8 (eq 4).[15]

$$\mathbf{AH}^+ + H_2O \rightleftarrows \mathbf{A} + H_3O^+ \quad (3)$$

$$\mathbf{A} + H_2O \rightleftarrows \mathbf{A}^- + H_3O^+ \quad (4)$$

Flavylium salt **3** could be associated with clip **C1** in aqueous media, under dilute conditions. The addition of clip **C1** to an acidic solution of the flavylium salt at pH = 2 gives rise to a red shift from 380 to 387 nm accompanied by a decrease of the absorption intensity. This red shift was also observed upon addition of **C1** to flavylium salts **1** and **2** in methanol and is an indicator for the flavylium salts entering into the clip cavity. Although the host−guest complex that is being formed (1:1) slowly precipitates, it was possible to determine the association constant for the complex formation between the flavylium salt **3AH**$^+$ and clip **C1** by UV−vis titration (log K = 4.9 ± 0.1), see Figure 12, parts a and b.

When clip **C1** is added to a solution of the quinoidal base of **3A** at pH = 5.3, a decrease in the absorption band centered at 512 nm is observed with no shift in λ_{max} (Figure 12c). The association constant was determined (log K = 4.9 ± 0.1, Figure 12d) for the host−guest complex formation between **3A** and the clip **C1** which was identical within the limits of the experimental error with that obtained for the formation of **3AH**$^+$**@C1**. The finding of same values of log K for the formation of **3AH**$^+$**@C1** and **3A@C1** suggests that the hydrophobic interactions are the major force in aqueous solution that determines the host−guest complex stability here. Evidently, the loss of the attractive electrostatic interactions (which are present in **3AH**$^+$**@C1**) has no apparent consequences in the binding constant of **3A@C1**. A confirmation of the importance of naphthalene sidewalls in clip **C1** for the binding

(24) The electrostatic potential surfaces (EPS) shown in Figure 11 were calculated by the use of computer program SPARTAN 04 version 1.0.0 (Wave Function Inc.) as described by (a) Kamieth, M.; Klärner, F.-G.; Diederich, F. *Angew. Chem., Int. Ed.* **1998**, *37*, 3303–3306. (b) Klärner, F.-G.; Panitzky, J.; Preda, D.; Scott, L. *J. Mol. Model.* **2000**, *6*, 318–327.

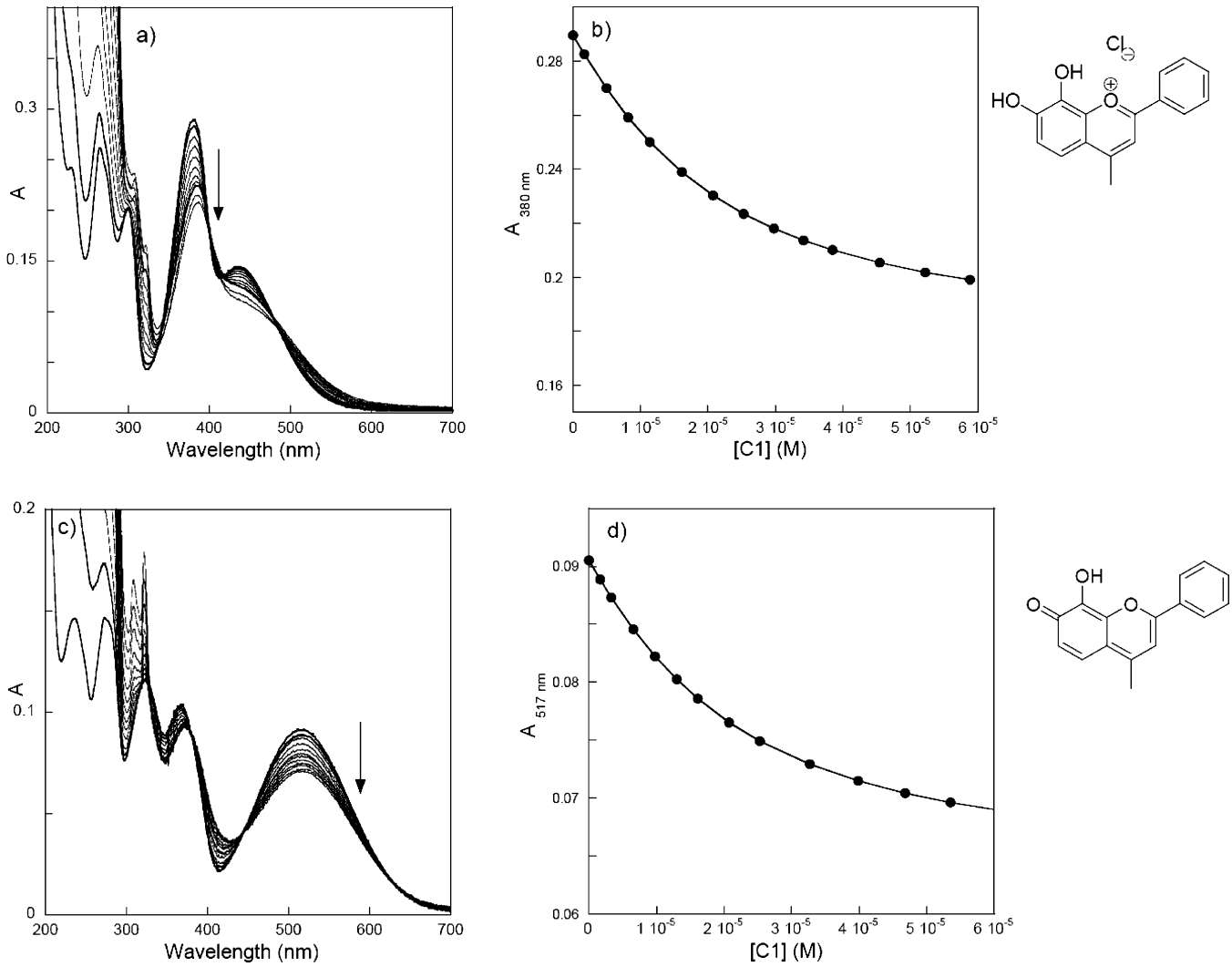


Figure 12. Dependence of the UV−vis spectra of compound **3** ([**3**] = 1.3 × 10^{-5} M) from the concentration of clip **C1** ([**C1**] = 0−6 × 10^{-5} M) in aqueous solution: (a and b) at pH = 2; (c and d) at pH = 5.3. The association constants were determined from theses concentration dependencies by UV−vis titration to give the same value of log $K = 4.9 \pm 0.1$ for the formation of both complexes **3A**H^+**@C1** and **3A@C1**. At both pH values, clip **C1** exists in its negatively charged hydrogen phosphate form, whereas **3** exist as **3A**H^+ at pH = 2 and as **3A** at pH = 5.3.

of the flavylium salt **3A**H^+ comes from a UV−vis titration experiment of **3A**H^+ ([**3A**H^+] = 4.2 × 10^{-5} M) with the phosphate-substituted bridge **C3** ([**C3**] = 0−1.2 × 10^{-3} M, lacking the naphthalene sidewalls) at pH = 2.0. The phosphate groups (OPO_3^{2-}) of C3 are certainly protonated to the hydrogen phosphate groups ($OP(OH)O_2^-$) under these acidic conditions. No change in the spectra of **3A**H^+ was observed upon the addition of **C3** indicating that here the electrostatic interaction between the positively and negatively charged systems is not sufficient to observe the host−guest complex formation.

The high association constants of **3A**H^+**@C1** and **3A@C1** allowed us to study the influence of the molecular clip on the acid−base behavior of flavylium salt **3** through a pH titration ([**C1**] = 5.9 × 10^{-5} M, [**3**] = 1.3 × 10^{-6} M; 81% association). Figure 13a shows the pH dependence of UV−vis spectral changes in the acidic pH range resulting from the evolution of complex **3A**H^+**@C1** to **3A@C1** (eq 5). A further increase in the pH value leads to the deprotonation of the quinoidal base **3A** to **3A**$^-$ in the complex with clip **C1** (Figure 13b). These data could be fitted to obtain $pK_{a1} = 4.8$ and $pK_{a2} = 7.0$ (Figure 13c). The increase in pK_{a1} of **3** from 4.0 to 4.8, when clip **C1** is present, can be explained by the "intramolecular" charge compensation in the complex between the positively charged flavylium salt inside the negatively charged host cavity. The slight increase in pK_{a2} from 6.8 to 7.0 in the presence of clip is explainable with a destabilization of negatively charged unprotonated quinoidal base in the presence of the negatively charged clip **C1** relative to the neutral quinoidal base. Furthermore, in this pH region, the deprotonation of the hydrogen phosphate to the phosphate groups of clip **C1** also occurs[11] which contributes to such a destabilization.

$$\mathbf{3A}H^+\mathbf{@C1} \rightleftarrows \mathbf{3A@C1} + H^+ \qquad (5)$$

Host−Guest Formation with Molecular Clip C2 in Aqueous Solutions. Molecular clip **C2**, containing two sulfate groups in the central spacer-unit, led to host−guest complexes with flavylium salts that are more soluble in water than the corresponding complexes with hydrogen phosphate-substituted clip **C1**. The solubility is, however, limited to the range of concentrations used in absorption and fluorescence measurements. In aqueous solution the compound 7,4′-dihydroxyflavylium perchlorate, **4**, undergoes a network of reactions that is well-characterized in both thermodynamic and kinetic terms.[16] It also exhibits photochromic behavior

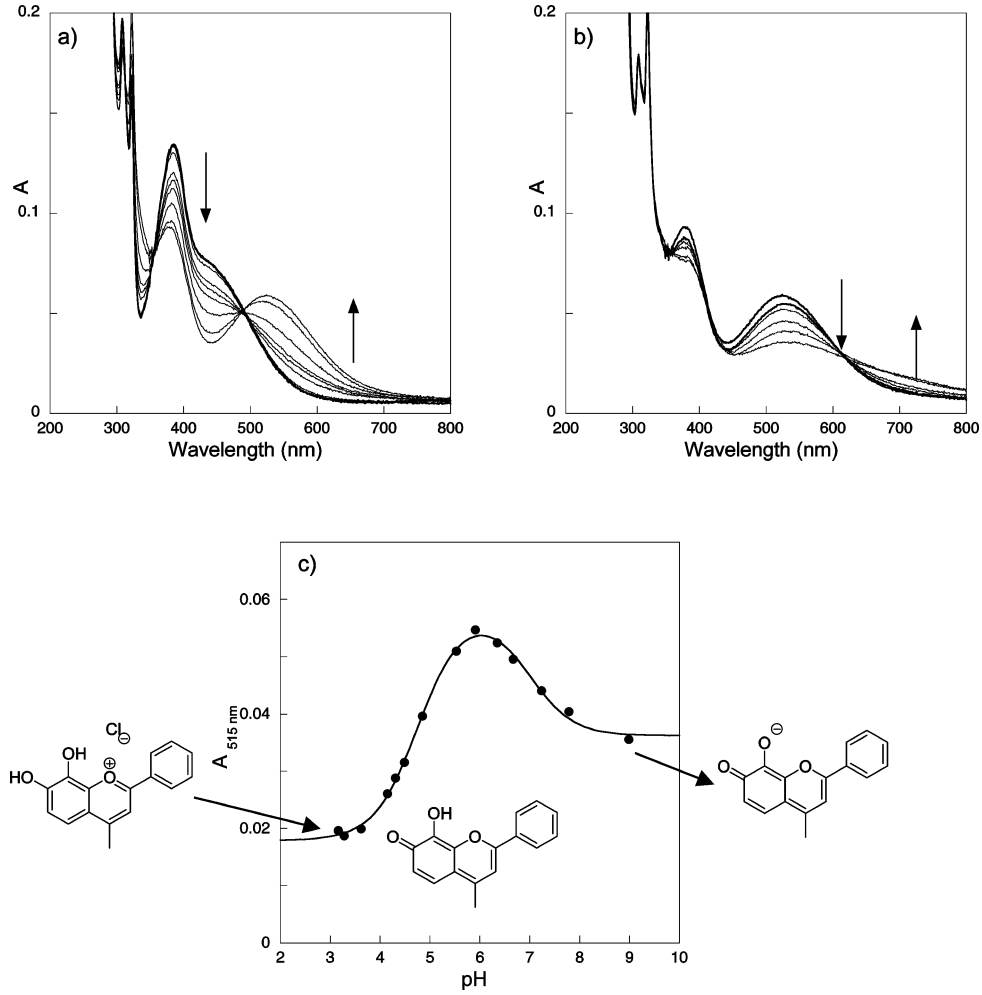


Figure 13. pH titration of the host−guest complex **3@C1** ([**C1**] = 5.9 × 10^{-5} M, [**3**] = 1.3 × 10^{-5} M) in aqueous solution. (a) Change in the UV−vis spectra of **3@C1** observed in the range from pH = 3.2 to pH = 5.9; (b) pH = 5.9 to pH = 9.0. (c) The pH dependence of the absorption intensity at 515 nm. The continuous curve presents the fit of these data points leading to the pK_a values of 4.8 and 7.0.

in water that has been exploited in liquid solutions as well as in organized media.[16] For these reasons this flavylium salt was chosen to study the influence of host−guest complex formation with molecular clips on the thermal and photochemical behavior of flavylium salts.

At acidic pH values, the flavylium cation **4AH**$^+$ is the thermodynamically stable species, Figure 1. By increasing the pH value, the quinoidal base **4A** is only formed as a transient in the equilibrium with cation **4AH**$^+$ (eq 3, pK_a = 4.1).[16c] The main reaction path goes from the cation **4AH**$^+$ via the hemiketal **4B** and the cis-chalcone **4Cc** to the trans-chalcone **4Ct** which is the thermodynamically stable species in slightly acidic and neutral pH regions. The addition of water to C-2 of the flavylium cation, leading to the hemiketal **B** and its subsequent ring-opening to the cis-chalcone **Cc**, proceeds in the subsecond time scale. Finally, **Ct** is formed through an isomerization of **Cc**, a process that ranges from minutes to hours depending on pH. Light can actuate the system at this point through the photoisomerization of **Ct** to form **Cc** (and consequently **AH**$^+$/**A**, depending on pH), a reaction that occurs in aqueous solutions as well as in ionic liquids, micelles, and hydrogel polymers.[16]

The thermodynamics of the network of reactions in Figure 1 is conveniently described considering a global reaction, with equilibrium constant K'_a, between the flavylium salt **AH**$^+$, and the sum of all other species of the network, **CB**, eqs 6 and 7.[5] The global constant K'_a can be expressed as a function of the equilibrium constants for each reaction in Figure 1, eq 8.[5]

$$\mathbf{AH}^+ + 2H_2O \rightleftarrows \mathbf{CB} + 2H_3O^+ \quad (6)$$

$$[\mathbf{CB}] = [\mathbf{A}] + [\mathbf{B}] + [\mathbf{Cc}] + [\mathbf{Ct}] \quad (7)$$

$$K'_a = K_a + K_h + K_hK_t + K_hK_tK_i \quad (8)$$

When sulfate clip **C2** is added to an aqueous solution of **4** at pH = 2, the UV−vis absorption band corresponding to the flavylium cation decreases and the maximum wavelength of absorption is shifted to higher wavelengths, from 457 to 465 nm (Figure 14a), similar to the observation for host−guest complex formation between clip **C1** and the flavylium salts **1** and **2** described above. Upon addition of clip **C2** the emission spectra show no wavelength change in the emission maximum but a quenching of the emission intensity. It is known that flavylium salt **4** exhibits excited-state proton transfer (ESPT) in protic solvents[25] so that the emission

(25) Pina, F.; Melo, M. J.; Santos, H.; Lima, J. C.; Abreu, I.; Ballardini, R.; Maestri, M. *New J. Chem.* **1998**, *22*, 1093–1098.

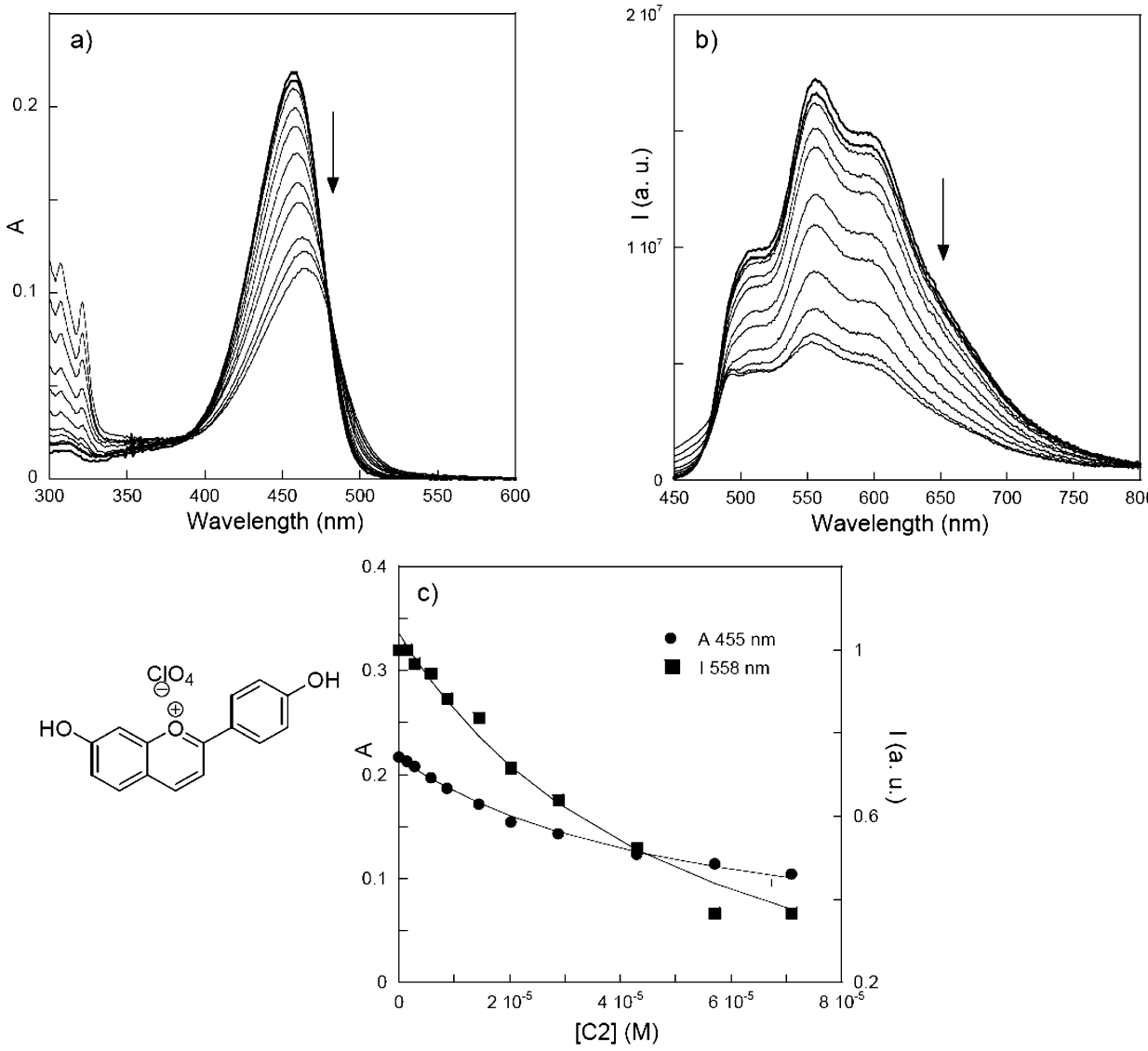


Figure 14. Spectral modifications observed upon addition of clip **C2**, [**C2**] = 0−7.2 × 10^{-5} M, to an aqueous solution of **4** at pH = 2, [**4**] = 6.9 × 10^{-6} M, in the presence of buffer (ref 19), followed by absorption (a) and fluorescence emission (b) (λ_{exc} = 420 nm). (c) The dependence of the absorption data on panel a at 455 nm and emission data on panel b at 558 nm from the clip concentration were fitted by the use of eq 1 to give log K = 4.3 ± 0.1 for **4A**H^+**@C2**.

spectra shown in Figure 14b correspond to the fluorescence of the quinoidal base **4A**. Taking into account the results observed above for **1@C1**, **2@C1**, and **3A@C1**, we expected that the inclusion of flavylium cation **4A**H^+ into the clip cavity would lead to an increase in the emission quantum yield. The observed quenching shown in Figure 14b might be correlated with a more efficient ESPT in the flavylium cation **4A**H^+ encapsulated by clip **C2**. Indeed, the presence of the two sulfate groups in the host could change the proton transfer from intermolecular to "intrasupramolecular", accelerating the process and thus decreasing the fluorescence quantum yield relative to that of the free flavylium ion. Assuming a 1:1 stoichiometry, the observed spectral changes lead to an association constant for the formation of **4@C2** of log K = 4.3 ± 0.1, Figure 14c.

The thermodynamics of the network of reactions shown in Figure 1 in the presence of clip **C2** was assessed by determining the value of pK'_a = 4.0 ± 0.2, Figure 15. The comparison of this value with that of pK'_a = 3.1 obtained for the free flavylium salt in water[16c] shows that the association of **4** with clip **C2** extends the pH stability region of the flavylium cation. The value of pK'_a is a measure of the effective color loss of solution of a flavylium salt, defining the pH range to which its color exists. In nature, anthocyanins often participate into supramolecular structures that also extend the color of the flavylium cations to the pH region of the vacuoles where they accumulate in plant cells.[7]

The fact that the pK'_a for **4@C2** is higher than for free **4** proves that the complexation of **C2** with **A**H^+ is stronger than with the other species comprised in **CB** (eq 7). Among these species, the quinoidal base **A** results from deprotonation of **A**H^+ and can be observed upon pH jumps to higher pH values from an acidic solution, where only **A**H^+ exists. This experimental approach allowed us to determine the pK_a = 4.5 ± 0.2 for **4@C2**, Figure 16.

The association constant for the formation of complex **4A@C2** could be determined from the values of log K(**4A**H^+**@C2**) = 4.3 ± 0.1, pK_a(**4@C2**) = 4.5 ± 0.2, and pK_a(**4**) = 3.1:[16c] log K(**4A@C2**) = 4.3 − 4.5 − (−4.2) = 4.0; this value is slightly lower than log K(**4A**H^+**@C2**) = 4.3 ±

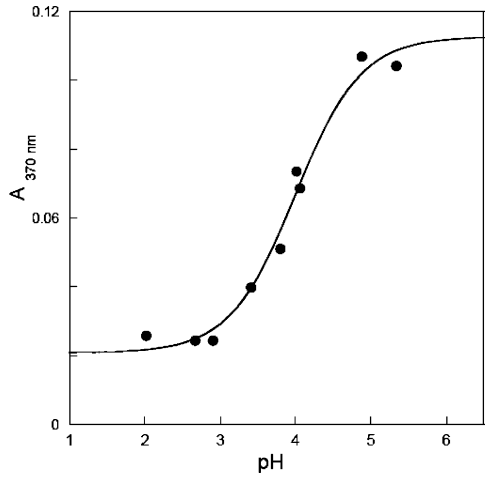


Figure 15. Variation of the absorbance at 370 nm (λ_{max} of **Ct**) of equilibrated aqueous solutions of flavylium salt **4** (7.2×10^{-6} M) and clip **C2** (8.7×10^{-5} M) as a function of pH. Fitting was obtained with $pK'_a = 4.0 \pm 0.2$; $T = 21 \pm 1$ °C.

0.1 as expected for the case that the electrostatic interaction between the charged host and guest system is important. But the relatively small difference between these two association constants indicates that other effects (such as the hydrophobic interactions) are responsible for the comparable stability of the host−guest complexes of sulfate-substituted clip **C2** with the charged flavylium salt **4AH**$^+$ and neutral quinoidal base **4A** in agreement with the results obtained for the host−guest complexes of the hydrogen phosphate-substituted clip **C1** with **3AH**$^+$ and **3A**. The association constants for **C2** with the hemiketal **B** and with the cis-chalcone **Cc** could not be determined because they only exist as transient species during the period when **AH**$^+$ (which is with the quinoidal base **A** in a fast acid−base equilibrium) reacts to the final equilibrium mixture at slightly acidic to neutral pH values. For free flavylium salt **4** in aqueous solution, the final equilibrium mixture in this pH range contains ca. 90% **4Ct** and 10% **4A**.[16a−e] Adding clip **C2** to such an equilibrated mixture of **4** at pH = 5.19 leads to a change in the UV−vis spectrum of **4** which is constant after few hours (see the Supporting Information). This spectral change indicates that the final equilibrium mixture in the presence of clip **C2** contains ca. 80% **4Ct** and 20% **4A**, showing that **C2** binds **4A** more strongly than it binds **4Ct**. A fast titration of an equilibrated mixture at pH = 5.7 was carried out in order to determine the association constant for complex formation between **C2** and **4Ct**. However, the changes in UV−vis absorption were too small to allow the determination of this association constant. Furthermore, emission spectroscopy could not be used to determine this constant since the incident light (used for the electronic excitation of **4Ct**) led to a trans−cis photoisomerization of **4Ct**, as verified by UV−vis spectroscopy.

^{1}H NMR titration experiments confirmed the assumed host−guest complex formation between clip **C2** and trans-chalcone **4Ct**. Since the solubility of **4Ct** is not sufficient in water for an NMR analysis, the association constant was determined in methanol to be log $K = 3.2 \pm 0.3$, see the Supporting Information. The small complexation-induced ^{1}H NMR shifts of the guest signals assigned to the aromatic protons ($\Delta\delta_{max} \leq 0.3$ ppm) and the protons attached to the C=C double bond ($\Delta\delta_{max} = 0.14$ (2-H) and -0.04 (3-H)) indicate that in this complex neither the aromatic rings nor the double bond of **4Ct** is included inside the clip cavity to a major extend. According to a Monte Carlo conformer search by the use of force field calculations (SPARTAN, MMFF94, gas phase) there are many low-energy conformers where either the aromatic rings or the C=C double bond of **4Ct** are positioned outside the clip cavity. The mutual exchange between these conformers certainly is a rapid process with respect to the NMR time scale so that only averaged NMR signals are observed. Therefore, the overall effect of the clip arene units on the shifts of the ^{1}H NMR guest signals may be small in this case.

The kinetics of the system consisting of flavylium salt **4** and clip **C2** was studied by carrying out pH jumps from solutions at pH = 2 to higher pH values, using UV−vis absorption spectroscopy to follow the course of reaction. At all pH values, the time dependence of the changes observed in the spectra of **4** in the presence and absence of clip **C2**, until the equilibrium between the different forms of **4** was reached, could be fitted as monoexponential processes. The observed rate constants, k_{obs}, as a function of pH are plotted in Figure 17.

To describe the observed kinetics, the simplified scheme 9 can be used. It was demonstrated before that, on the basis of this scheme, the kinetic process describing the evolution toward equilibrium upon a pH jump from an acidic solution containing only **AH**$^+$ to higher pH values can be accounted for by eq 10 (see the Supporting Information):[26]

$$\mathbf{4A} \overset{K_a}{\rightleftharpoons} \mathbf{4AH^+} \underset{k_{-h}[H^+]}{\overset{k_h}{\rightleftharpoons}} \mathbf{4B} \overset{K_t}{\rightleftharpoons} \mathbf{4Cc} \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} \mathbf{4Ct} \tag{9}$$

$$k_{obs} = \frac{\dfrac{[H^+]}{[H^+] + K_a} k_i K_t K_h + k_{-i}[H^+]}{[H^+] + \dfrac{k_i K_t}{k_{-h}}} \tag{10}$$

The fitting of eq 9 to the experimental data in the presence and absence of clip is represented in Figure 17. The value of K_a was determined independently (Figure 16) and used in the fitting where the following three parameters were adjusted (see eq 10): $k_i K_t K_h$, k_{-i} and $k_i K_t / k_{-h}$. Table 4 resumes the thermodynamic and kinetic data for both systems (k_h was obtained through $k_i K_t K_h / (k_i K_t / k_{-h})$).

The data for free **4** are in reasonable agreement within the limits of experimental error with previously published data.[16c] Most importantly, Figure 17 shows that the binding of **4** to clip **C2** causes a ca. 60% reduction of the overall rate constants determined for the conversion of **4AH**$^+$ into the species comprised in **CB**. When the pH of a solution containing the flavylium salt is increased, the first (kinetic) product is the quinoidal base **A** which then equilibrates to **Ct** passing through **AH**$^+$, **B**, and **Cc** as transient intermediates (Figure 1). Table 4 evidences that the rate constant for the hydration reaction, k_h, is reduced by a factor of 3 in the presence clip **C2**. This might happen because the complexed flavylium cation **4AH**$^+$ is more protected against the attack of a water molecule at C-2 than the free cation. The data in Table 4 also show that the trans−cis isomerization of **4Ct** to **4Cc** proceeds more slowly in the presence of clip **C2** indicating that also the chalcones interact with the clip.

To gain further insight into the effect of the molecular clip **C2** on the network of reactions of the various forms of

(26) Gomes, R.; Parola, A. J.; Laia, C. A. T.; Pina, F. *Photochem. Photobiol. Sci.* **2007**, 1003–1009.

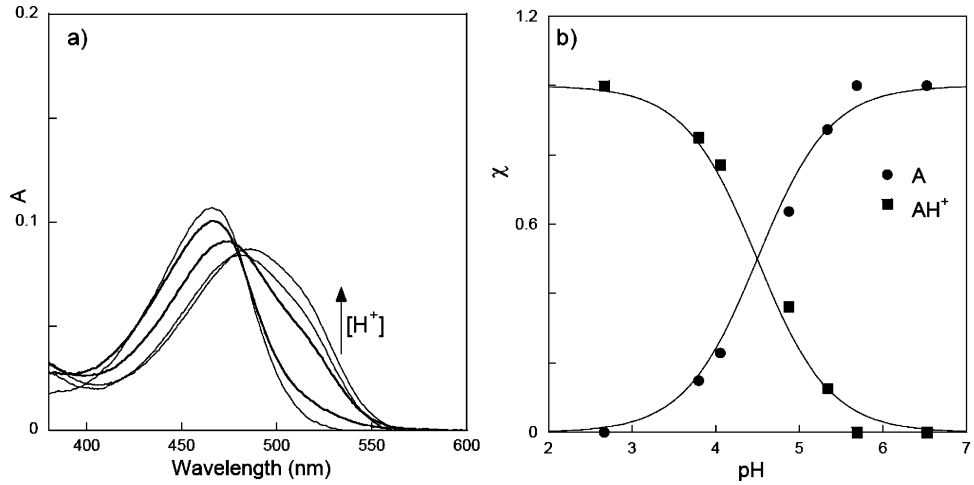


Figure 16. (a) Change in the UV−vis spectra of **4** in the presence of **C2** observed upon pH jumps from pH = 2.0 to higher pH values with final concentrations of [**4**] = 7.2 × 10^{-6} M and [**C2**] = 8.7 × 10^{-5} M; (b) simultaneous fitting of the mole fractions of $\mathbf{AH}^+$ and **A** calculated from the spectral data leads to pK_a = 4.5 ± 0.2 for **4@C2**, at 21 ± 1 °C, aqueous solution.

Table 4. Thermodynamic and Kinetic Constants for the Reactions of Free **4** and Complexed **4@C2** at 21 ± 1 °C

	4	**4@C2**
pK_a	4.1[a]	4.5 ± 0.2
pK'_a	3.1[a]	4.0 ± 0.2
$k_iK_tK_h$/s^{-1} M^{-1}	(2.1 ± 0.5) × 10^{-7}	(6.2 ± 0.5) × 10^{-8}
k_{-i}/s^{-1}	(1.0 ± 0.9) × 10^{-4}	(7.9 ± 0.9) × 10^{-5}
$(k_iK_T)/(k_{-h})$/M	(1.8 ± 0.7) × 10^{-5}	(1.6 ± 0.7) × 10^{-5}
k_h/s^{-1}	(1.2 ± 0.9) × 10^{-2}	(3.9 ± 0.9) × 10^{-3}

[a] Ref 16c.

4 we performed flash photolysis experiments in aqueous solutions of equilibrated **4** at various pH values in the presence and absence of clip **C2**. These rate constants display the usual linear dependency[16,26] with $[H^+]$ (eq 11, see Figure 18), but for higher proton concentrations the rate becomes constant, because the rate-determining step is no longer the pH-dependent dehydration rate, $k_{-h}[H^+]$ but the rate of formation of the hemiketal species, k_{-t}. The results indicate an effect of the clip on the various reactions of **4**. Since some of the parameters are missing, for example, the equilibrium constant K_t, this effect cannot be unambiguously assigned to the single reaction steps. With the assumption that K_t is not affected by the presence of the clip, the proton-mediated H_2O elimination of hemiketal **4B** is calculated from the data in Figure 18b to be faster in the complex with **C2** by a factor of 1.4 than in free **4B**. This suggests that the certainly positively polarized transition state of this reaction is stabilized by binding to the clip comparable to that of the flavylium cation $\mathbf{4AH}^+$.

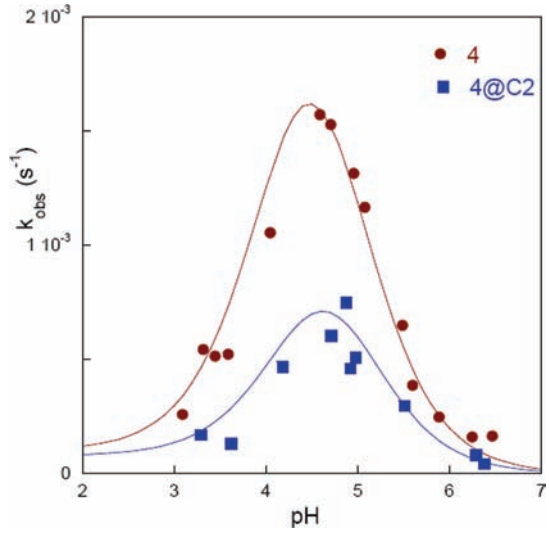


Figure 17. k_{obs} as a function of pH for **4** in aqueous solution (red) and in the presence of clip (blue, **4@C2**), using [**4**] = 7.2 × 10^{-6} M and [**C2**] = 8.7 × 10^{-5} M. Fitting was obtained with eq 10, for pK_a = 4.2 for **4** and pK_a = 4.5 for **4@C2**; T = 21 ± 1 °C.

$$k_{flash} = k_i\frac{K_t}{1 + K_t} + k_{-h}\frac{[H^+]}{1 + K_t} \quad (11)$$

The photoisomerization of the trans-chalcone **4Ct** was studied in the presence of clip **C2** to gain further information about the effect of the clip on the network of reactions. To an aqueous solution of equilibrated **4** at pH = 5.7, containing ca. 90% of **4Ct** and 10% of **4A**, clip **C2** was added and the solution irradiated at 390 nm (where clip **C2** no longer absorbs), Figure 19. A solution of equilibrated **4** in the absence of clip was also irradiated.

Figure 19a shows that irradiation of **4Ct** at pH = 5.7 in the presence of clip leads to the formation of **4A**, as expected at this pH (p$K_a(\mathbf{AH}^+)$ = 4.2). The presence of a clean isosbestic point in the spectra shows that no degradation accompanies the photoisomerization reaction and also that the reactions passing through **4Cc**, **4B**, $\mathbf{4AH}^+$ as intermediates to **4A** occur in the subsecond time scale. The solutions in the absence and in presence of clip have the same absorbance at λ_{irr} = 390 nm so that the slopes in Figure 19b are a direct measure of the trans−cis photoisomerization quantum yields, and these are the same for both experiments within the limits of the experimental error indicating that the clip **C2** has no significant effect on the photochemical trans−cis isomerization of **4Ct** to **4Cc**.

$$\phi = \phi_0\frac{[H^+]}{[H^+] + \dfrac{k_iK_t}{k_{-h}}} \quad (12)$$

The quantum yield is given by eq 12 (see the Supporting Information), where ϕ_0 is the intrinsic quantum yield and

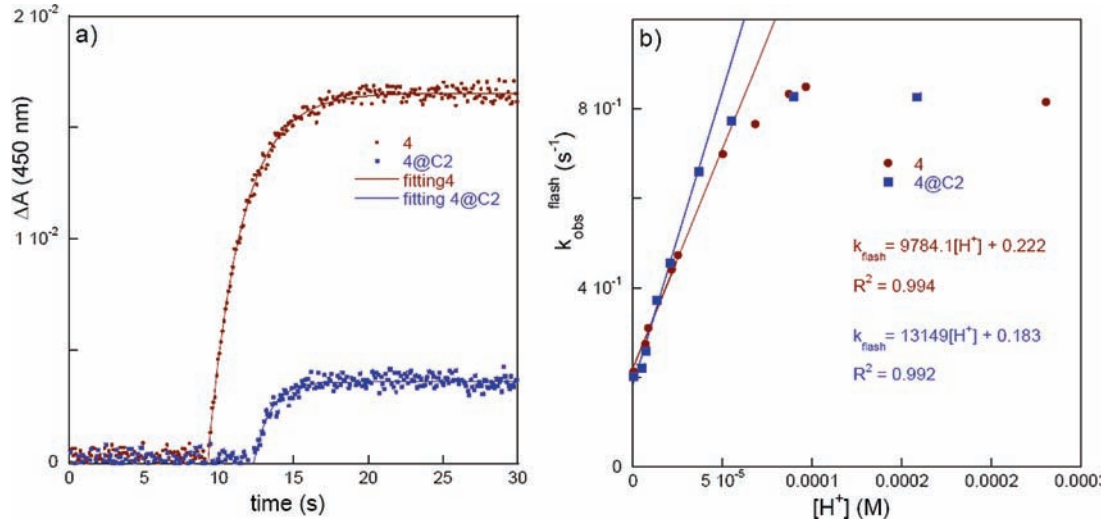


Figure 18. (a) Differential absorbance at 450 nm vs time in seconds in the absence (red, **4**, pH 4.66) and in presence of **C2** (blue, **4@C2**, pH 4.06), [**4**] = 7.2×10^{-6} M, [**C2**] = 8.7×10^{-5} M. Fitting was obtained using an exponential function with $k_{flash}^{obs} = 0.91$ s^{-1} in the presence of clip pH 4.06 and 0.45 s^{-1} without clip pH 4.66. (b) Rates of flash photolysis vs [H^+] for **4** (red) and **4@C2** (blue). Linear regressions and obtained equations and correlation coefficients are shown.

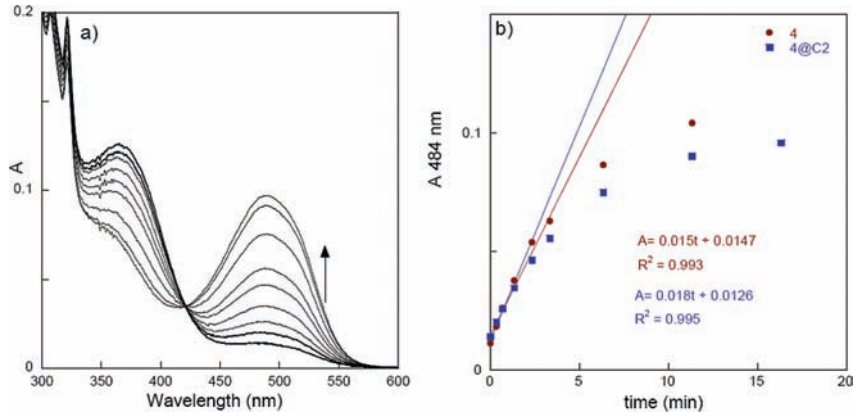


Figure 19. (a) Spectral modifications that occur upon irradiation at 390 nm of an aqueous solution of equilibrated [**4**] = 6.3×10^{-6} M at pH = 5.7 in presence of clip [**C2**] = 4.0×10^{-5} M. (b) Changes in absorbance at 484 nm in the absence (red circles, **4**) and in presence of **C2** (blue squares, **4@C2**) with time.

the factor dependent on [H^+] accounts for the efficiency of formation of **4A** from the **4Cc** produced under irradiation. Since Table 4 shows that the ratio $k_i K_t/k_{-h}$ is the same within the limits of experimental error independently of the presence of clip, the pH-dependent factor is also the same. The similarity of slopes in Figure 19b would then mean that ϕ_0 is not much affected by the addition of clip. This conclusion is, however, to be taken with caution since the measured quantum yields are actually a combination of the quantum yield of the **Ct** inside the clip and in bulk solution and there is large uncertainty in the association constant between **4Ct** and **C2**.

Conclusion

In this work we could demonstrate that UV−vis absorption and fluorescence spectroscopy on one hand and ^{1}H NMR spectroscopy on the other hand are complementary techniques for the investigation of host−guest complexes. Due to the different range of concentrations required for the measurements, the electronic spectroscopic methods are particularly suited for the determination of high binding constants, *K*, whereas NMR is better suited for the determination of lower *K* values. NMR provides, however, valuable data on the structures of host−guest complexes which cannot be gained by the other methods. The hydrogen phosphate-substituted molecular clip **C1** forms highly stable host−guest complexes with the flavylium salts **1** and **2** in methanol. The large binding constants (log K = 4.1 and 4.7 determined by fluorometric titration for the formation of **1@C1** and **2@C1**, respectively) and the large complexation-induced ^{1}H NMR shifts of the guest signals assigned to the protons 3- and 4-H of **1** or **2** allow the conclusion that in each complex the positively charged pyrylium ring of **1** or **2** is bound inside the clip cavity, and hence, cation−π interactions provide an important contribution to the host−guest complex formation besides other noncovalent bonds, for example, the Coulombic ion−ion and the solvophobic interactions. To explain the surprising result that the neutral quinoidal base **3A** forms an even more stable complex with clip **C1** in methanol (log K = 5.6), we assume that the solvophobic interactions contribute to the noncovalent host−guest binding substantially. In water the solvophobic effect (in this case the hydrophobic effect) should be even stronger than that in methanol. Indeed, clip **C1** forms stable 1:1 complexes with the flavylium salts **1**, **2**, and **3A**H^+ and the quinoidal base **3A**. But these complexes are insoluble

in water and precipitate during the mixing of aqueous solutions containing either the clip or one of the guest compounds. In highly diluted aqueous solution the association constants for the complex formation of clip **C1** with the flavylium salt **3A**H^+ at pH = 2 and the quinoidal base **3A** at pH = 5.3 could be determined by UV−vis titration experiments to be log $K = 4.9$ for the formation of both complexes. The finding that the complexes of the positively charged flavylium salt and the neutral quinoidal base are of the same stability indicates that here the hydrophobic interactions are dominating for the host−guest binding. The increase in the pK_a value of the acid−base reaction (**3A**$H^+ \rightleftharpoons$ **3A** + H^+) in the presence of clip **C1** from 4.0 to 4.8 shows that the proton dissociation from **3A**H^+ is more difficult inside the negatively charged clip cavity than in the free flavylium salt. As a consequence of this finding, the pH stability region of the flavylium cation is extended in the clip cavity to higher pH values.

Host−guest complexes of the sulfate-substituted clip **C2** with the flavylium salts **1**−**4** and their derivatives are slightly better soluble in water than the corresponding complexes of the hydrogen phosphate clip **C1**. In this work we focused on the effect of clip **C2** on the complex formation with the flavylium salt **4A**H^+ and its quinoidal base **4A** as well as on the kinetics and thermodynamics of the sequence of the following reactions, **4Ct** $\rightleftharpoons$ **4Cc** $\rightleftharpoons$ **4B** $\rightleftharpoons$ **4A**H^+ $\rightleftharpoons$ **4A**, that could be determined by a combination of UV−vis and fluorescence spectroscopy, pH jump, and flash photolysis methods. The finding that clip **C2** forms complexes with **4A**H^+ and **4A** of comparable stability confirms the results obtained for the complex formation of clip **C1** with **3A**H^+ and **3A** and provides further evidence for the importance of the hydrophobic interaction for the host−guest binding observed here. According to the partial analysis of the complex kinetics, the thermally induced trans−cis isomerization of **4Ct** to **4Cc** and the H_2O addition to **4A**H^+ followed by H^+ elimination leading to **4B** are retarded in the presence of clip **C2**. These findings allow the following conclusions: (1) the trans-chalcone **4Ct** is obviously also bound inside the clip cavity, and (2) the clip-induced deceleration of both reactions is presumably due to the steric constraints of the clip cavity in which the monomolecular sterically certainly demanding trans−cis isomerization of **4Ct** or the bimolecular H_2O addition to **4A**H^+ has to proceed. On the other hand, the photochemically induced trans−cis isomerization of the chalcones is accordingly not affected by encapsulation. Most recently, the conclusion from the kinetic analysis that trans-chalcone **4Ct** forms a host−guest complex with clip **C2** could be confirmed by ^{1}H NMR titration experiments independently. Since the solubility of **4Ct** is not sufficient in water for an NMR analysis, the association constant was determined in methanol to be log $K = 3.2 \pm 0.3$. The small complexation-induced ^{1}H NMR shifts of the guest signals ($\Delta\delta_{max} \leq 0.3$ ppm) indicate that in this complex there is no preference for one complex conformer having one of the aromatic rings and/or the C═C double bond of **4Ct** included inside the clip cavity.

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Supporting Information Available: Full experimental data, including ESI-MS, 2D NMR, ^{1}H NMR, and UV−vis spectra of titration experiments for host−guest formation, addition of clip **C2** to a pre-equilibrated solution of **4Ct**, and deduction of eqs 10, 11, and 12. This material is available free of charge via the Internet at http://pubs.acs.org.

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