Fluorescent water-soluble molecular clips. Self-association and formation of adducts in aqueous and methanol solutions†‡

Barbara Branchi,[a] Paola Ceroni,*[a] Vincenzo Balzani,[a] Marçal Casas Cartagena,[b] Frank-Gerrit Klärner,*[b] Thomas Schrader[b] and Fritz Vögtle*[c]

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We have synthesized the water-soluble molecular clip **AC** containing two anthracene sidewalls connected by semirigid aliphatic units to a benzene bridge which carries two sodium sulfate substituents. For comparison purposes, the analogous clip **NC** with naphthalene instead of anthracene sidewalls has been synthesized. In methanol solution both clips exist in their monomeric form, while in aqueous solution the anthracene clips **AC** self-assemble to give a highly stable dimer with $\log K_{dim} = 5.1$ (for the naphthalene clip **NC**, $\log K_{dim} = 2.5$). The ^{1}H NMR spectra of the dimers have evidenced the interaction of two intertwined clip molecules in a perpendicular arrangement. The non-associated clip **AC** exhibits a very strong blue fluorescence typical of the anthracene chromophoric unit. The $(\mathbf{AC})_2$ dimer has a broader absorption spectrum and a much weaker and red shifted emission band. Addition of acetylcholine leads to the disruption of the dimer $(\mathbf{AC})_2$ and the revival of the very strong fluorescence typical of the monomeric clip **AC**. Both clips interact with nicotinamide adenine dinucleotide (NAD^+) in buffered aqueous solution at pH = 7.2. A ^{1}H NMR analysis shows that the naphthalene clip **NC** forms a 1 : 1 complex with NAD^+ including the active site of the cofactor inside the clip cavity. In methanol, formation of a stable 1 : 1 adduct between **AC** and a fluorescent first-generation dendrimer containing four dansyl groups appended to a 1,4-diaminobutane core is driven by acid addition. In such adduct, a very efficient energy transfer takes place from the excited anthracene units of the clip to the dansyl chromophores of the dendrimer. The adduct can be reversibly disassembled by addition of base or of competitive guests.

Introduction

Molecular clips[1,2] are preorganized synthetic receptors in which solubility, photophysical, and redox properties together with selectivity towards a specific substrate can be tuned by changing the nature of the sidewalls and/or of the substituents appended to the bridging unit. They can mimic natural receptors in both binding and carrying biological relevant substrates. In recent years, clips and tweezers of various structures containing chiral,[3] luminescent,[4] or redox-active groups[5] have been studied. Most of them, however, are not soluble in water, thereby preventing their use as hosts for biologically relevant molecules.

The synthesis of molecular clips and tweezers soluble in water thanks to bis-phosphonate monoester anions appended to the bridging unit has been recently reported.[6] These synthetic receptors have an electron-rich inner cavity in which they can host flat electron-poor aromatic guests, for example *N*-alkylpyridinium salts. In particular, good complexation ability towards *N*-alkyl or *N*-arylnicotinamide salts, some nucleotides, and nicotinamide adenine dinucleotide (NAD^+, the important cofactor of many redox enzymes) was demonstrated.[6]

We report on a fluorescent water-soluble molecular clip **AC** (Scheme 1) which contains two anthracene sidewalls and two sulfate substituents in the benzene bridging unit. This clip exhibits a very strong blue fluorescence typical of the anthracene chromophoric unit. By monitoring the absorption, emission, and ^{1}H NMR spectra we have found that clip **AC** undergoes dimerization in aqueous solution but not in methanol. We present the results of a detailed investigation on (i) the dimerization process in water, (ii) the disruption of the dimer and the formation of an adduct by addition of acetylcholine (**1**, Scheme 1) or NAD^+ (**2**) in aqueous solution, (iii) the reversible adduct formation in methanol solution, driven by acid/base stimuli, of **AC** with a luminescent dendrimer of first generation (**3**) containing two alkylamine units in its interior and four dansyl (5-dimethylamino-1-naphthalenesulfonamido) groups at the periphery, and (iv) the very

[a] *Dipartimento di Chimica "G. Ciamician", Università di Bologna, via Selmi 2, I-40126 Bologna, Italy. E-mail: paola.ceroni@unibo.it; Fax: +39 051 2099456; Tel: +39 051 2099535*
[b] *Institut für Organische Chemie der Universität Duisburg-Essen, Campus Essen, Universitätsstrasse 5, 45117 Essen, Germany. E-mail: frank.klaerner@uni-duisburg-essen.de*
[c] *Kekulé-Institut für Organische Chemie und Biochemie der Universität Bonn, Gerhard-Domagk Strasse 1, D-53121 Bonn, Germany. E-mail: voegtle@uni-bonn.de; Fax: +49 228 735662; Tel: +49 228 733495*

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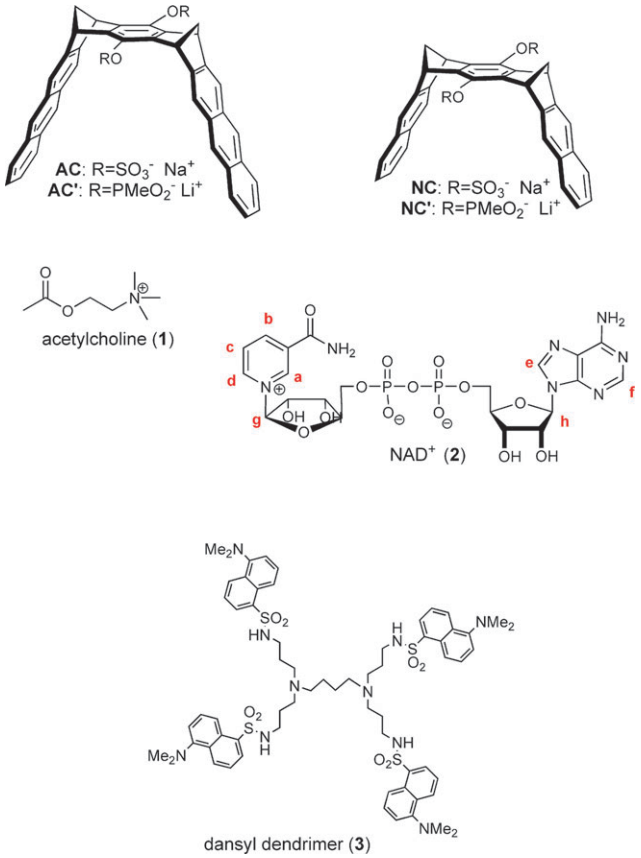
‡ Dedicated to Prof. Jean-Pierre Sauvage on the occasion of his 65th birthday.



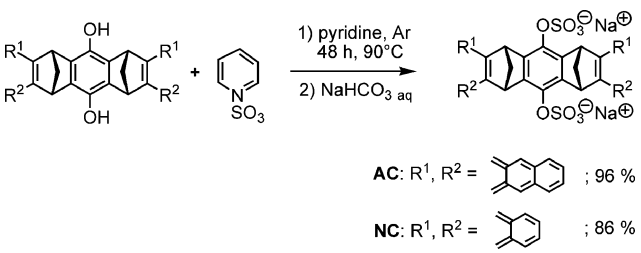
Scheme 1

efficient (>90%) energy transfer taking place from the excited anthracene units of the clip to the dansyl chromophores in the adduct. For comparison purposes, we have synthesized and studied the corresponding water-soluble clip **NC** (Scheme 1) containing two naphthalene sidewalls and two sulfate substituents in the benzene bridging unit and we make references to the results obtained[6a,b] with two analogous clips **AC**′ and **NC**′ which carry two methanephosphonate substituents in the benzene bridging unit.

Results and discussion

Synthesis and characterization of clips AC and NC

The molecular clips **AC** and **NC** were prepared in a one-pot reaction starting from the corresponding hydroquinone clips containing either anthracene[7] or naphthalene[8] sidewalls (Scheme 2). The reaction of the hydroquinone clip with an excess of the sulfur trioxide pyridinium complex in anhydrous pyridine at 90 °C and subsequent work-up with a saturated aqueous solution of $NaHCO_3$ led to clip **AC** or **NC** in a very good yield of 96 and 86%, respectively. From the naphthalene clip **NC** the lithium sulfate derivative was also prepared which has properties similar to those of the sodium salt. The structures of all new compounds were assigned by their spectral data listed in the Experimental section.



Scheme 2

The ^{1}H NMR spectra of both clips are surprisingly different in CD_3OD and D_2O and provide experimental evidence for a self-association of both compounds in aqueous solution (Fig. 1). By changing the solvent from CD_3OD to D_2O the ^{1}H NMR signal of **AC** (assigned to the anthracene protons H_b) experience a dramatic up-field shift of $\Delta\delta = 2.1$ ppm (from $\delta = 8.1$ to $\delta = 6.0$). The ^{1}H NMR signals assigned to the other anthracene protons $H_{a,c,d}$ of **AC** also experience up-field shifts by the solvent change, but less pronounced than that of H_b. A similar solvent dependence was observed for the ^{1}H NMR signals of the naphthalene protons of clip **NC**. Also in this case the ^{1}H NMR signal (assigned to the naphthalene protons H_b) shows the most significant up-field shift of $\Delta\delta = 0.5$ ppm (from $\delta = 7.5$ to $\delta = 7.0$) which is, however, smaller than that observed for the signal of the corresponding anthracene protons H_b of clip **AC**. Concentration dependent

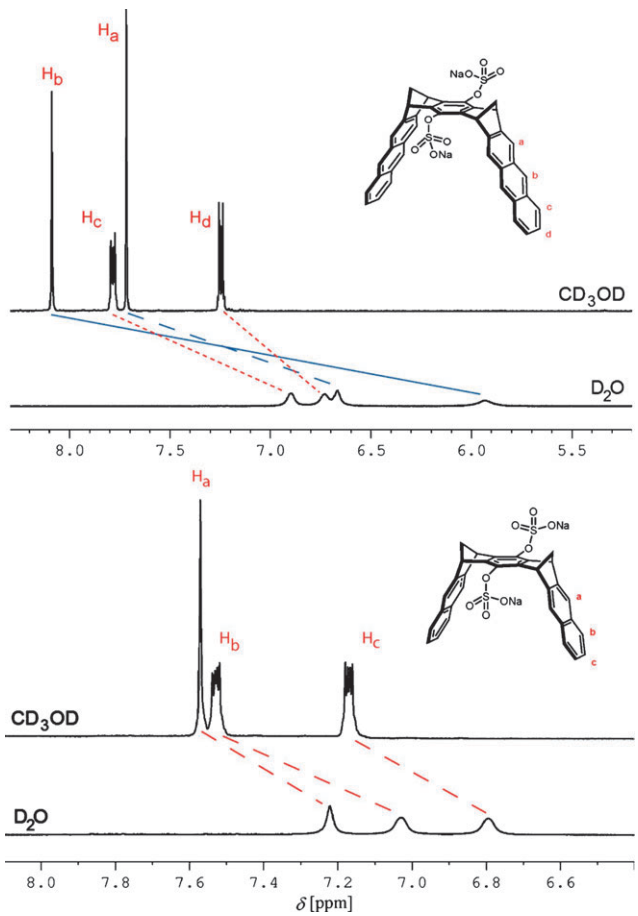


Fig. 1 Solvent effect on the ^{1}H NMR spectra (500 MHz) of the clips **AC** and **NC**.

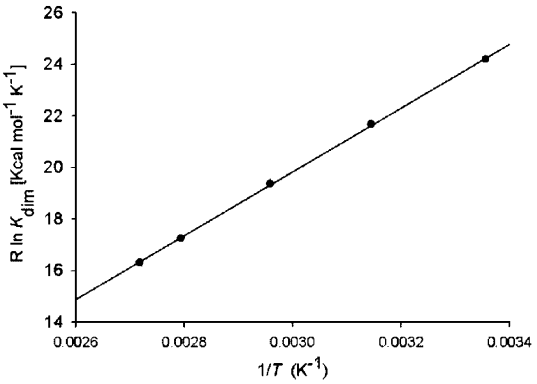


Fig. 2 Dimerization-induced ^{1}H NMR shifts, $\Delta\delta_{max}$, and thermodynamic parameters of the dimerization of **AC** at 298 K in aqueous solution derived from the temperature dependence of the dimerization constants, K_{dim}, determined by ^{1}H NMR dilution titrations at various temperatures between 298 and 368 K.

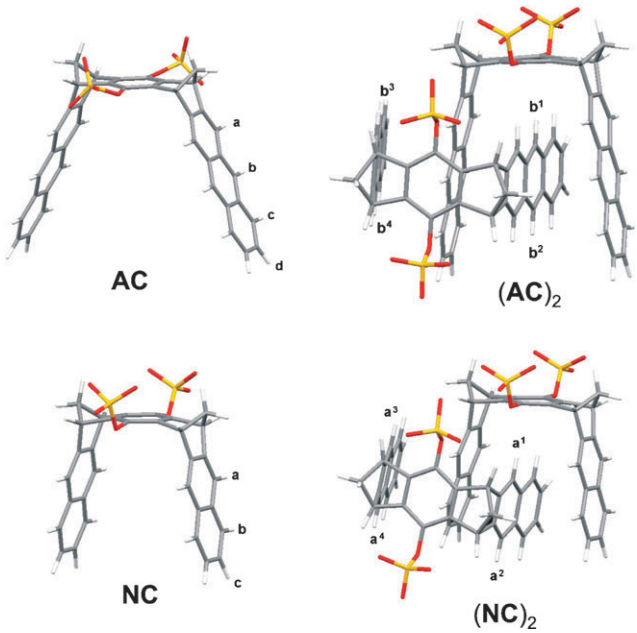


Fig. 3 Structures of the monomers and dimers of molecular clips **AC** and **NC** calculated by conformational search (MacroModel 9.0, Monte Carlo simulation, AMBER*/H_2O, 5000 structures).

NMR spectra indicate that both molecular clips form self-assembled dimers in aqueous solution.

The dimerization constants, K_{dim}, and the corresponding shifts of the ^{1}H NMR signals of the clip protons ($\Delta\delta_{max} = \delta_{(clip)} - \delta_{(clip)2}$) were determined by dilution titration experiments of both clips in aqueous solution. In the case of the anthracene clip **AC** the dilution titration experiments were carried out at various temperatures between 298 and 368 K. The thermodynamic parameters—the enthalpy of association, ΔH, and entropy of association, ΔS (Fig. 2), could be determined from the temperature dependence of the K_{dim} values. The $\Delta\delta_{max}$ values (also listed in Fig. 2) do not show any significant temperature dependence. They are of similar size as those found for anthracene clip substituted by methanephosphonate groups in the benzene bridging unit (**AC**′ in Scheme 1: $\Delta\delta_{max}$ = 0.8 (H_a), 2.3 (H_b)).[6a] In the case of the naphthalene clip **NC** the ^{1}H NMR titration experiment was only performed at 298 K giving a dimerization constant $K_{dim} = 310 \pm 30$ M^{-1} and the dimerization-induced ^{1}H NMR shifts of $\Delta\delta_{max}$ = 0.6 (H_a), 0.8 (H_b), and 0.6 (H_c) for the formation of the self-assembled dimer (**NC**)$_2$. The details of all NMR titration experiments are described in the ESI.‡

Geometry optimization of monomeric **AC**, **NC** and dimeric (**AC**)$_2$, (**NC**)$_2$ by a Monte Carlo conformer search (MacroModel 9.0, AMBER*/H_2O)[9] led to the low-energy structures shown in Fig. 3. In particular, the large $\Delta\delta_{max}$ value observed for the ^{1}H NMR signal of the anthracene protons H_b is well explained with the structure calculated for the dimer (**AC**)$_2$. In this structure all four H_b protons are no longer chemically equivalent: one (H_{b1}) of the two protons (which are positioned inside the cavity of the second clip molecule) points toward and the other one (H_{b2}) away from the central benzene spacer-unit of the second clip molecule. The other two protons (H_{b3} and H_{b4}) are located outside the cavity with different orientation relative to the central spacer unit of the second clip molecule. Therefore, in the ^{1}H NMR spectrum of (**AC**)$_2$ four signals are expected for the H_b protons. The (H_{b1}) proton (pointing toward the central arene spacer-unit of the second clip molecule) is expected to experience the largest shielding. In the spectrum of **AC** in D_2O (Fig. 1) only one signal is observed for these H_b protons, but its specific broadening indicates that a fast exchange between at least two signals takes place leading to an averaged signal. According to the broadening of all ^{1}H NMR signals of **AC** in D_2O, this exchange evidently results from an association and dissociation [2 **AC** $\rightleftharpoons$ (**AC**)$_2$] which proceeds at a rate comparable to the NMR "time-scale". These assumptions are strongly supported by quantum chemical ^{1}H NMR shift calculations for the protons of the monomeric and dimeric structure of clip **AC**′. Indeed, very different chemical shifts are calculated for each type of the protons H_{a1}–H_{a4}, H_{b1}–H_{b4}, or H_{c1}–H_{c4} but the mean values calculated for each type agree well with the experimental data obtained for H_a, H_b or H_c. Similar conclusions can be drawn for the formation of the self-assembled dimer of the naphthalene clip **NC**. The $\Delta\delta_{max}$ values were found to be smaller for the protons H_a, H_b and H_c of (**NC**)$_2$ than those of the corresponding protons of the anthracene clip (**AC**)$_2$. This finding may be explained with a slightly different structure of dimer (**NC**)$_2$ as is shown in Fig. 3.

The profound solvent dependence found for the dimerization of the clips **AC** and **NC** allows the conclusion that this event is largely the result of a hydrophobic effect. The self-assembly of the anthracene clip **AC** was found to be strongly enthalpy driven ($\Delta H < 0$). The enthalpic driving force is partially compensated by an unfavorable entropy loss ($T\Delta S < 0$). Similar results of negative enthalpies and entropies of association were also observed for the dimerization of the **AC**′ clip and the naphthalene tweezer[6a] as well as several host–guest complexations in chemical and biological systems in aqueous solution.[10] All these findings are, however, in sharp contrast to a classical hydrophobic effect (large favorable positive association entropy, small favorable enthalpy, and negative ΔC_p term) but they agree well with the so-called nonclassical hydrophobic effect.[11] Evidently, attractive

aromatic π–π and CH–π interactions are responsible for the formation of the self-assembled dimers $(\mathbf{AC})_2$ and $(\mathbf{NC})_2$. The finding, that the dimer $(\mathbf{AC})_2$ is very much more stable than $(\mathbf{NC})_2$, indicates that the enlargement of the π-faces of the sidewalls from the naphthalene in **NC** to anthracene in **AC** has a strong effect on the stability of the dimer and, hence, on the hydrophobic interactions. The results obtained for the anthracene clip **AC** by the use of NMR spectroscopy are in very good agreement with those derived from its photophysical properties.

Photophysical and photochemical properties of clip AC

Aqueous solution. The absorption spectrum of clip **AC** in dilute aqueous solution shows the typical absorption bands due to ππ* transition of the anthracene chromophore[12] between 315 and 380 nm (Fig. 4(a)), but broadening of these bands upon increasing **AC** concentration was evidenced (Fig. 4(a) inset), together with a non-linear increase in the absorbance. Clip **AC** exhibits the typical anthracene structured fluorescence band between 360 and 440 nm, with a much lower emission quantum yield (Table 1), which is accompanied by an unstructured band with maximum at 505 nm. The ratio between the intensities of the two emission bands changes with the clip concentration, as reported in the inset of Fig. 4(b). The decay of emission intensity registered at either 400 or 505 nm as a function of time can be fitted by a single exponential corresponding to lifetimes of 4 and 18 ns,

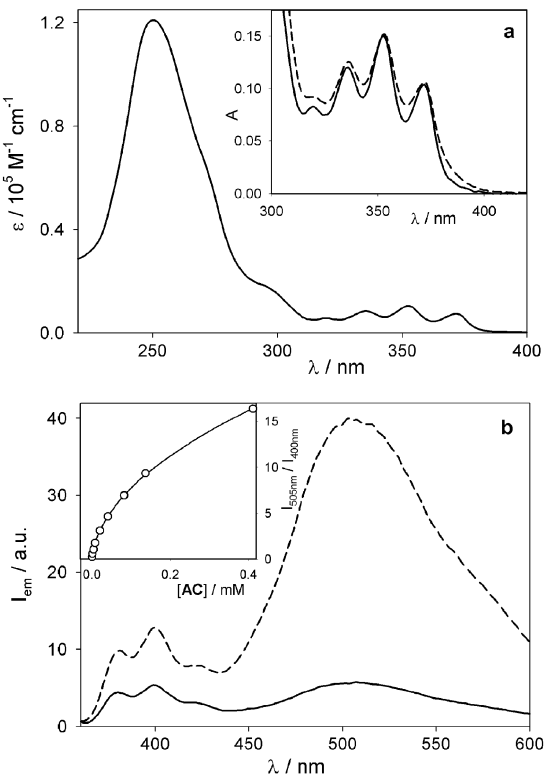


Fig. 4 Absorption (a) and emission (b) spectra of 4×10^{-6} M (solid line) and 2×10^{-5} M (dashed line) aqueous solutions of clip **AC** at 298 K. The absorption spectra reported in the inset of panel (a) are normalized. The inset of panel (b) shows emission intensity ratio at 505 and 400 nm as a function of clip concentration. $\lambda_{ex} = 320$ nm.

respectively (Table 1). The excitation spectra performed upon setting the emission wavelength at 400 or 505 nm are slightly different in the 315–380 nm region: the latter is broader than the former, demonstrating the presence of two emitting species. All these experimental results are consistent with the presence, even in dilute solutions (4×10^{-6} M), not only of the monomeric clip ($\lambda_{em} = 400$ nm), but also of a dimer ($\lambda_{em} = 505$ nm), in which the two clips are associated in a structure that allows electronic interaction between the anthracene units. This hypothesis is consistent with the ^{1}H NMR spectra and quantum chemical ^{1}H NMR shift calculations (see above). The dimeric structure is responsible for the broader absorption spectrum and the emission band at 505 nm corresponding to the longer excited state lifetime. Global fitting of absorption spectra by Specfit software[13] leads to $\log K_{dim} = 4.9$. Fitting of the intensity ratio (I_{505nm}/I_{400nm}) reported in the inset of Fig. 4(b) (see Experimental section for details) yields $\log K_{dim} = 5.1$. These values agree well with that obtained by ^{1}H NMR ($\log K_{dim} = 5.3$).

Anthracene and, in particular, molecules containing two anthracene chromophores in a pre-organized structure are known to photoreact upon irradiation in deaerated solutions, giving rise to a covalently bound species.[14] In our case, no evidence of photoreaction was observed upon irradiation at $\lambda > 300$ nm of a 1.3×10^{-4} M solution of clip **AC** in deaerated aqueous solution. The lack of photoreaction is consistent with a dimer structure in which there is no parallel alignment between anthracene chromophores.

Methanol solution. The absorption and emission spectra of clip **AC** in CH_3OH (Fig. 5) do not depend on concentration. A comparison with anthracene in the same solvent shows that for the clip: (i) the molar absorption coefficient is lower than expected considering that two chromophores are present in each molecule; (ii) the absorption bands are much broader, particularly the one centered at 255 nm; (iii) new absorption bands at 270 and 295 nm are present; (iv) the low energy absorption bands and the emission band are slightly blue-shifted; (v) the emission quantum yield is lower; (vi) the excited state lifetime is shorter (Table 1). These results are consistent with a small perturbation of the anthracene chromophore caused by the 1,2-methyl substituents and a contribution of the sulfate-substituted benzene in the bridge to the absorption bands at 270 and 295 nm. However, there is no evidence of a through space interaction of the two anthracene chromophores, as expected because of the relatively long distance (*ca.* 1 nm). The lack of dimer/excimer emission shows that dimerization of **AC** in methanol solution can be ruled out.

Adduct formation

Adduct between clip AC and acetylcholine. Upon titration of a 4×10^{-6} M aqueous solution of **AC** with acetylcholine chloride **1** (Scheme 1), very small perturbations of the absorption spectrum are observed in agreement with the fact that **1** does not absorb at $\lambda > 250$ nm. Dramatic changes, however, are evidenced in the emission spectra, where the disappearance of the dimer emission band is accompanied by the increase of the monomer emission (Fig. 6). The normalized emission intensity changes at 400 (solid circles in Fig. 4) and 505 nm

Table 1 Photophysical properties in aqueous and methanol solution at 298 K

	Absorption			Emission		
	Solvent	λ_{max}[a]/nm	$\varepsilon/10^4$ M^{-1} cm^{-1}	λ_{max}[b]/nm	Φ_{em}	τ/ns
AC	H_2O	372	—[c]	375	—[c]	4
$(\mathbf{AC})_2$	H_2O	372	—[c]	505	—[c]	18
AC	CH_3OH	372	0.8	375	0.18	4
Anthracene	CH_3OH	375	0.7	377	0.21	5
3	CH_3OH	335	1.7	518	0.26	12
Dansyl	CH_3OH	337	0.4	518	0.26	12

[a] Lowest energy band. [b] Highest energy band. [c] These values cannot be accurately estimated because in the investigated concentration range (6×10^{-7}–3×10^{-5} M), both the monomer and the dimer are present.

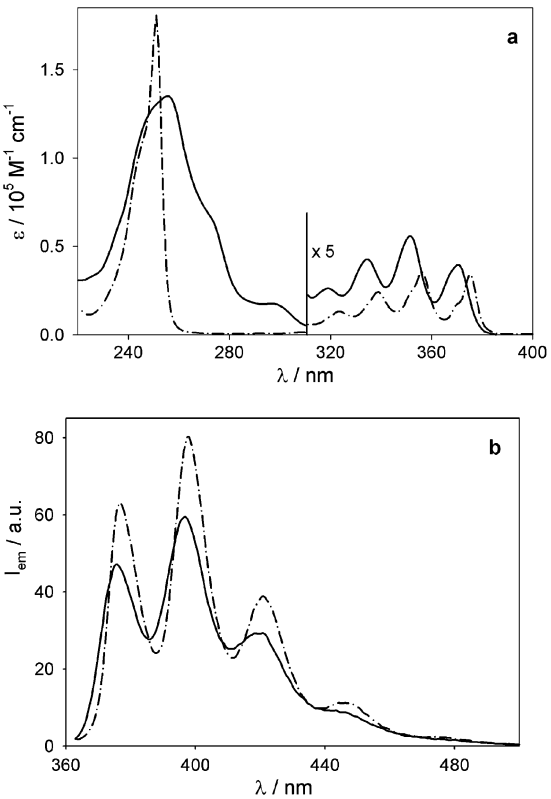


Fig. 5 Absorption (a) and emission (b) spectra of a 4×10^{-6} M (solid line) solution of clip **AC** and a 6.5×10^{-5} M (dashed–dotted line) solution of anthracene in methanol at 298 K; λ_{ex} = 320 nm.

(empty squares in Fig. 6) are mirror-like and reach a plateau at *ca.* 200 equivalents of **1** per clip. These results demonstrate that an excess of acetylcholine is able to disrupt the very stable dimer of clip **AC** in aqueous solution ($\log K_{dim}$ = 5). The association of **AC** with **1** is likely driven by electrostatic interaction between the sulfate groups and the ammonium ion. Global fitting of the emission spectra, taking into account the competition between dimerization of **AC** and adduct formation of **AC** with **1**, leads to $\log K_a$ = 3.9 for the association constant.

Host–guest complex formation of clips AC and NC with NAD^+. In buffered aqueous solution clip **NC** forms a 1 : 1 complex with NAD^+ (compound **2** in Scheme 1). The large complexation-induced shifts of the ^1H NMR signals assigned to the nicotinamide ring protons of NAD^+ and the similar

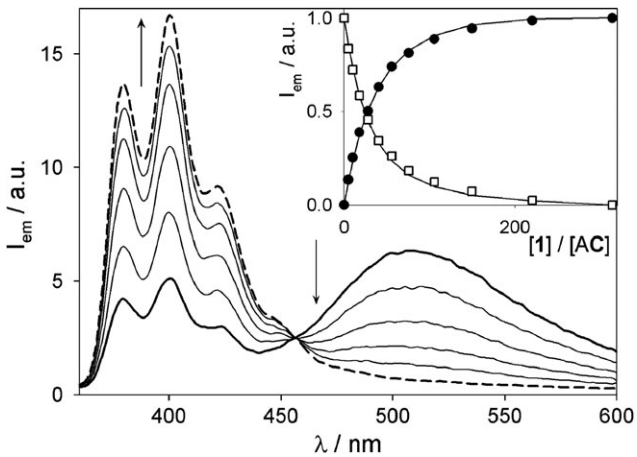


Fig. 6 Emission spectra of a 4×10^{-6} M aqueous solution of **AC** upon titration with a 1.3×10^{-2} M solution of **1**. λ_{exc} = 295 nm. Inset shows the normalized emission intensity changes at 400 nm (solid circles) and 505 nm (empty squares) with the corresponding fitting lines.

results obtained with the methanephosphonate naphthalene clip **NC′** (Table 2) allowed the conclusion that the active site (the nicotinamide ring) of NAD^+ is included inside the clip cavity.

The association constant of **NC** with NAD^+ is significantly lower than that of **NC′** (K_a = 1200 *vs.* 4000 M^{-1}). Indeed, a competitive dimerization process has been evidenced only in the case of the sulfate substituted clip **NC** (K_{dim} = 310 M^{-1}).[15] Therefore, the apparent association constant estimated for **NC** is related to the overall process, *i.e.* dissociation of the clip dimer and binding of NAD^+. This endothermic dissociation of the dimer has to be overcompensated by the binding energy of the host–guest complex.

In the case of the highly stable dimer of sulfate-substituted anthracene clip **AC** this process, however, is much more difficult. In the ^1H NMR spectrum of a mixture containing **AC** and NAD^+ in a molar ratio of (1 : 13) the signals assigned to NAD^+ are only slightly shifted and the signals of the clip are too broad for an unambiguous analysis, as previously observed for the methanephosphonate anthracene clip **AC′**. At higher temperature (323 K) the signals are sharper but not much different to those measured for the separate compounds.[16]

Evidence of association between **AC** and NAD^+ have been obtained by fluorimetric titration in diluted solution

Table 2 The maximum complexation-induced chemical ^{1}H NMR shifts, $\Delta\delta_{max}$ [ppm], apparent binding constants, K_a [M^{-1}], and Gibbs binding enthalpies, ΔG [kcal mol^{-1}], of the complexes between NAD^+ and the naphthalene clips **NC** and **NC′**[6b,c] in aqueous buffer (pH = 7.2) at 298 K

	δ_{max}							
	H_a	H_b	H_c	H_d	H_e	H_f	K_a	ΔG
NC	1.2	2.1	2.9	1.6	0.2	0.4	1200 ± 30	−4.3
NC′	0.8	2.8	3.2	1.5	1.6	0.5	4000 ± 330	−4.9

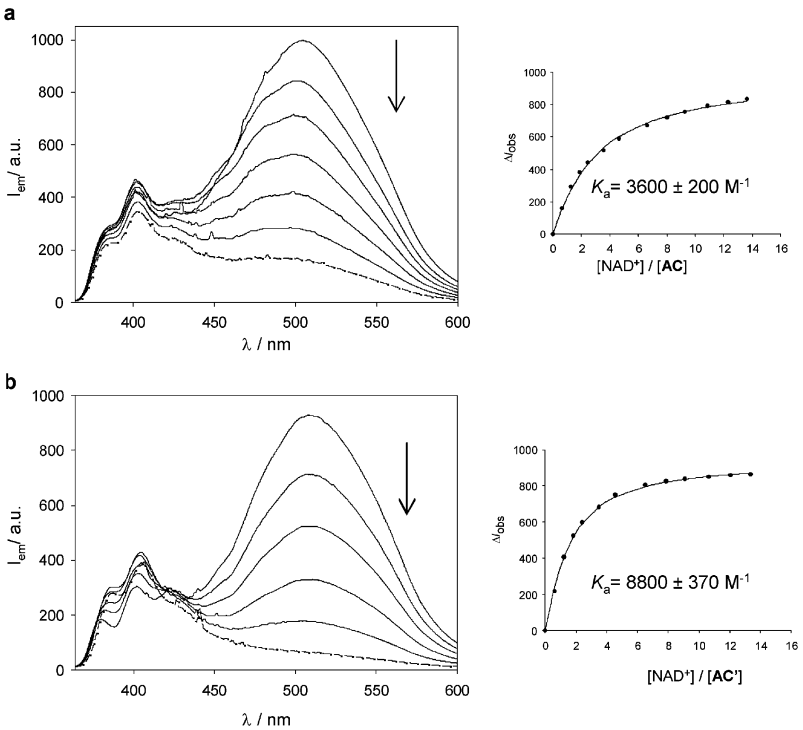


Fig. 7 Emission spectra of an aqueous buffered (pH = 7.2) solution of **AC** (a) and **AC′** (b) upon titration with NAD^+ at 298 K. Apparent association constants are determined from the emission intensity changes at 505 nm upon addition of NAD^+.

(micromolar range): the emission band at 505 nm, assigned to the dimer $(\mathbf{AC})_2$, decreases in intensity upon addition of NAD^+ (Fig. 7). Apparent association constants K_a related to the overall reaction (dissociation of the clip dimer and binding of NAD^+) were determined showing that NAD^+ is bound by **AC** and **AC′** quite substantially. It is worth noting that upon binding of NAD^+, no revival of the typical anthracene fluorescence band at 400 nm has been observed, at variance with the previous example with acetylcholine **1**. These results suggest that a quenching of the anthracene fluorescent excited state by photoinduced electron transfer is thermodynamically allowed for NAD^+, but not for acetylcholine.

Adduct formation between clip AC and first-generation dendrimer 3 in methanol solution. Compound **3** is a first-generation dendrimer constituted by four dansyl chromophores appended to a 1,4-diaminobutane core (Scheme 1). It exhibits intense absorption bands in the near UV spectral region (λ_{max} = 252 and 335 nm) and a strong fluorescence band in the visible region (λ_{max} = 517 nm; Φ = 0.26, τ = 12 ns). These photophysical properties are characteristic of the dansyl chromophore. Indeed, a comparison with the 5-dimethylamino-1-naphthalenesulfonamido model compound (hereafter called dansyl) shows: (i) no significant change in the position and shape of the absorption and emission bands, (ii) a molar absorption coefficient four times higher in the case of **3**, and (iii) no significant difference in the emission properties (energy, emission quantum yield and excited-state lifetime, see Table 1). These results point out that there is no ground- or excited-state interaction between the four dansyl moieties in **3**, as previously observed for CH_3CN–CH_2Cl_2 5 : 1 (v/v) solution.[17]

Upon addition of four equivalents of $HClO_4$ to a 5×10^{-6} M solution of **3**, a small red-shift of the lowest-energy absorption and emission band is observed (Fig. 8) and a concomitant slight decrease of the emission quantum yield and excited-state lifetime (τ = 11 ns). These results are consistent with protonation of the aliphatic amine units, which are more basic than the aromatic dansyl amine units.[17] Protonation of the aliphatic amines slightly perturbs the photophysical properties of the external dansyl chromophores since the fluorescent excited state of dansyl has a charge-transfer (CT) character.[18] Further addition of acid leads to protonation of the dansyl units, as

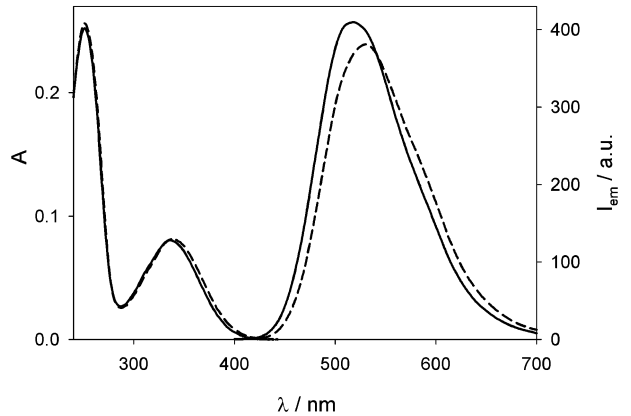


Fig. 8 Absorption and emission spectra of a 5×10^{-6} M solution of dendrimer **3** in CH_3OH before (solid line) and after (dashed line) addition of four equivalents of $HClO_4$; $\lambda_{exc} = 332$ nm.

evidenced by the decrease of both the absorption and emission bands (not shown in Fig. 8). These spectral changes are completely reversible upon addition of base.

Addition of four equivalents of $HClO_4$ to a 7×10^{-6} M solution of clip **AC** does not cause any change in the absorption and emission spectra, showing that the clip is stable under these experimental conditions.

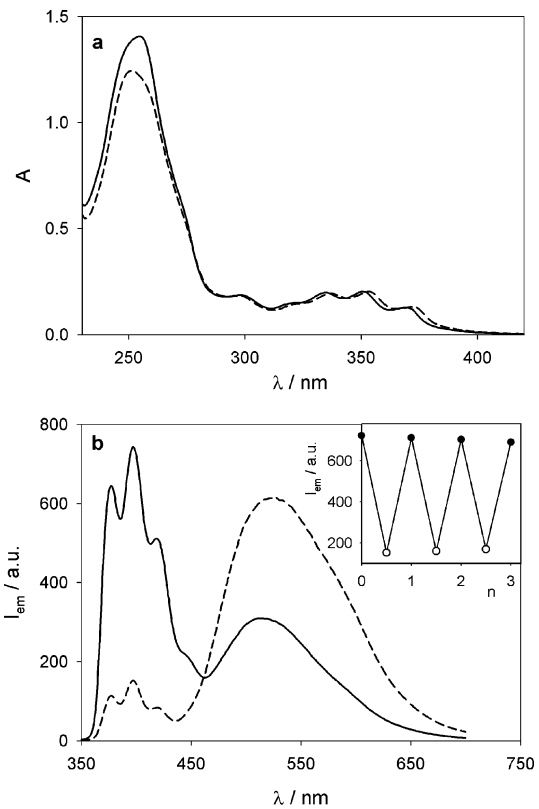


Fig. 9 Absorption (a) and emission (b) spectra of clip **AC** and dendrimer **3** ($c = 7 \times 10^{-6}$ M) in CH_3OH solution before (solid line) and after (dashed line) addition of four equivalents of $HClO_4$. Inset shows the emission intensity changes at 520 nm upon addition of $HClO_4$ and NaOH: n is referred to the number of cycles. $\lambda_{exc} = 295$ nm.

An equimolar methanol solution of **3** and **AC** (7×10^{-6} M) shows absorption and emission spectra (solid line in Fig. 9) characteristic of the two components without any evidence of ground- or excited-state interactions. Upon addition of four equivalents of $HClO_4$, which causes protonation of the internal amine units of **3**, the absorption spectrum (dashed line in Fig. 9(a)) is consistent with the sum of the two components, while profound changes are evidenced in the emission spectrum (Fig. 9(b)). In particular, upon excitation at 295 nm a strong quenching of the clip emission at 400 nm is accompanied by an increase of the dansyl emission at 520 nm. Such a quenching-sensitization process cannot be due to a dynamic energy transfer mechanism because of the short lifetime of the emitting excited-state of anthracene (Table 1) and the low concentration of dendrimer. Therefore, the occurrence of energy transfer requires formation of an adduct between the clip and dendrimer **3**. Indeed, the resemblance of the absorption and excitation spectrum of the mixture performed with $\lambda_{em} = 520$ nm confirms that an efficient energy transfer process takes place. The emission intensity decay at 400 nm for an equimolar solution of **AC** and **3** (in the presence of acid) can be fitted by a single exponential function with $\tau = 4$ ns, assigned to the very small fraction of clip that is not involved in the adduct.[19] The emission intensity decay at 520 nm corresponds to $\tau = 12$ ns, typical of the dansyl chromophore. The main driving force for adduct formation is likely the electrostatic interaction between the protonated amine units and the sulfate anions of the clip, which can either point outwards or inwards the clip cavity. The former conformation is most likely because steric hindrance prevents inclusion of the dendrimer in the clip. Adduct formation is completely reversible upon addition of base, as shown by the inset of Fig. 9(b).

In order to establish the stoichiometry of the adduct, a titration of a 5×10^{-6} M solution of **3** containing four equivalents of $HClO_4$ with a 5.7×10^{-4} M solution of **AC** was monitored by the changes in the emission intensity upon excitation at 295 nm.[20] The clip emission intensity (solid circle in Fig. 10) is very low up to addition of 1 equivalent of clip and then increases linearly, while the dansyl emission intensity

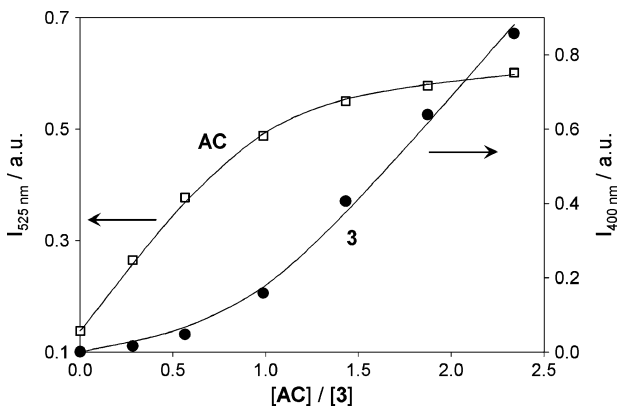


Fig. 10 Emission intensity changes at 400 (solid circles) and 525 nm (empty squares) of a 5×10^{-6} M solution of **3** in CH_3OH containing 2×10^{-5} M $HClO_4$ upon titration with **AC**; $\lambda_{exc} = 295$ nm. The lines represent the fitting obtained by Specfit.

(empty squares in Fig. 10) shows a linear increase up to addition of *ca.* 1 equivalent of clip and then reaches a plateau. These results indicate a 1 : 1 stoichiometry for the adduct and confirm that the energy transfer process from anthracene to dansyl chromophores is very efficient (>90%). Global fitting of the emission spectra leads to $\log K_a = 6.4$ for the 1 : 1 adduct between the protonated dendrimer **3** and clip **AC**.

Disassembly of the adduct can also be obtained by addition of diprotonated *N*,*N*,*N*′,*N*′-tetramethyl 1,4-diaminobutane which competes with approximately the same affinity of compound **3** in binding to the clip.[21] Tetrabutylammonium perchlorate or protonated tributylamine are much less efficient in this competition, underlying the importance of the cooperative effect brought about by the presence of two protonated amine functions in the adduct formation.

Conclusions

Fluorescent water-soluble molecular clips are interesting because (i) fluorescence offers a handle to study their self-association and complexation ability, and (ii) solubility in water allows the investigation of their interaction with biologically relevant guest molecules. We have prepared and characterized molecular clip **AC** which contains fluorescent anthracene sidewalls and sulfate substituents in the bridging benzene unit which favour solubilization in polar solvents. For comparison purposes, we also synthesized the sulfate-substituted molecular clip **NC** based on naphthalene sidewalls. The anthracene clip **AC** dissolves in water as a dimeric species, as evidenced by NMR and emission spectra, whereas no self-association takes place in methanol. The clip shows a strong blue fluorescence in its monomeric form and a much lower green–orange emission when it is self-associated. Both the NMR spectra (including simulation) and the photophysical and photochemical properties show that in the dimer there is no parallel alignment between anthracene chromophores. In contrast to the anthracene clip **AC**, the naphthalene clip **NC** forms a self-assembled dimer only of modest stability.

In water, **AC** gives rise to adducts with suitable guest, such as acetylcholine, resulting in the dissociation of the dimer $(\mathbf{AC})_2$, as evidenced by the revival of the strong blue fluorescence. This result could be exploited in biological imaging by fluorescence technique since this system shows an OFF–ON behavior in the absence and presence of acetylcholine. In highly dilute buffered aqueous solution the dissociation of the clip dimer $(\mathbf{AC})_2$ can be also achieved by host–guest complex formation with NAD^+. The stability of the resulting NAD^+ complex is comparable to that of the naphthalene clip **NC** which forms only a weak dimer.

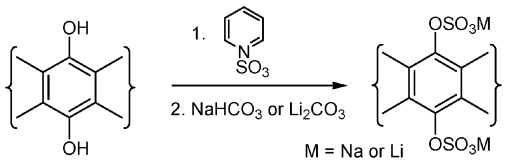
In methanol solution, clip **AC** forms a stable and reversible 1 : 1 adduct with a first-generation dendrimer containing four dansyl groups appended to a 1,4-diaminobutane core (**3**). The adduct is formed upon protonation of the inner amine groups of **3**, and it can be fully reverted by addition of base or a competitive guest, such as protonated tetramethyl 1,4-diaminobutane. In the adduct, a very efficient energy transfer from the excited anthracene units of the clip to the four dansyl groups of **3** has been evidenced.

Experimental

Acetylcholine chloride and *N*,*N*,*N*′,*N*′-tetramethyl 1,4-diaminobutane were high purity reagents.

Dansyl and dendrimer **3** were synthesized according to previously published procedure.[17]

Preparation of sulfate-substituted molecular clips AC and NC



General procedure of the sulfonation of hydroquinone derivatives: A mixture of the hydroquinone derivative (1 eq.) and sulfur trioxide pyridinium complex (4 eq.) are dissolved in dry pyridine (31 mL/mmol) and stirred under argon at 90 °C. After 24 h additional sulfur trioxide pyridinium complex (3 eq.) is added and after 24 h of additional reaction time the mixture is cooled to room temperature and quenched with an aqueous saturated solution of either $NaHCO_3$ or Li_2CO_3. The excess of inorganic salt is filtered off over a filtration plate (pore 4). The solution is washed three times with diethyl ether and the aqueous phase is concentrated *in vacuo*. The resulting dark solid is redissolved in ethanol and filtered again. The ethanolic solution is diluted with isopropanol and the organic solvents are removed in the rotatory evaporator under reduced pressure. A light brown solid is obtained. The yield of the reactions is between 85 and 99%.

Clip NC. The reaction of the naphthalene clip substituted by hydroxyl groups in the central benzene spacer-unit (290 mg, 0.66 mmol or 271 mg, 0.62 mmol) with the sulfur trioxide pyridinium complex and $NaHCO_3$ or Li_2CO_3 gave 364.3 mg (0.57 mmol) of the sodium sulfate substituted clip **NC**, yield: 86% and 346 mg (0.57 mmol) of the lithium sulfate substituted naphthalene clip, yield: 91%, respectively.

NC(OSO_3Na). Molecular formula: $C_{32}H_{20}Na_2O_8S_2$ (642.61 g mol^{-1}); mp >300 °C. HR-MS (ESI-TOF, MeOH, negative ionization), m/z = 298.0295 $[M - 2{\cdot}Na]^{2-}$ (calc. for $C_{32}H_{20}O_8S_2^{2-}$ = 596.0600/2 = 298.0300), 619.0487 $[M - Na]^-$ (calc. for $C_{32}H_{20}NaO_8S_2$ = 619.0497). ^{1}H NMR (500 MHz, CD_3OD, Fig. 11) δ[ppm]: 2.36 (d, 2H, ^2J(H-19a,H–19i) = 7.9 Hz, H-19a + H-20a), 2.65 (d, 2H, H-19i + H-20i), 4.80 (s, 4H, H-6 + H-8 + H-15 + H-17), 7.17 (dd, 4H, ^3J(H-2,H-1) = 6.3 Hz, ^4J(H-2,H-4) = 3.3 Hz, H-2 + H-3 + H-12 + H-11), 7.53 (dd, 4H, H-1 + H-4 + H-10 + H-13), 7.57 (s, 4H, H-5 + H-9 + H-14 + H-18); ^{13}C NMR (126 MHz, CD_3OD) δ[ppm]: 49.66 (C-6 + C-8 + C-15 + C-17), 65.74 (C-19 + C-20), 120.97 (C-5 + C-9 + C-14 + C-18), 125.79 (C-2 + C-3 + C-11 + C-12), 128.57 (C-1 + C-4 + C-10 + C-13), 133.55 (C-4a + C-9a + C-13a + C-18a), 139.64 (C-7 + C-16), 144.02 (C-6a + C-7a + C-15a + C-16a), 148.59 (C-5a + C-8a + C-14a + C-17a). ^{1}H NMR (500 MHz, D_2O) δ[ppm]: 2.37 (d, 2H, ^2J(H-19a,H-19i) = 8.1 Hz, H-19a + H-20a), 2.63 (d, 2H, H-19i + H-20i), 4.70 (s, 4H, H-6 + H-8 + H-15 + H-17), 6.78 (br, 4H, H-2 +

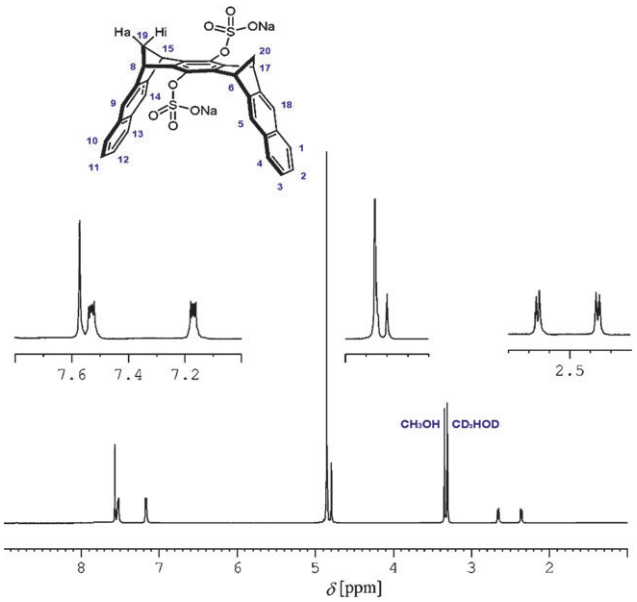


Fig. 11 ^{1}H NMR spectrum (500 MHz) of naphthalene clip **NC** in CD_3OD.

H-3 + H-12 + H-11), 7.01 (br, 4H, H-1 + H-4 + H-10 + H-13), 7.20 (s, 4H, H-5 + H-9 + H-14 + H-18). ^{13}C NMR (126 MHz, D_2O) δ[ppm]: 50.28 (C-6 + C-8 + C-15 + C-17), 66.11 (C-19 + C-20), 121.20 (C-5 + C-9 + C-14 + C-18), 126.31 (C-2 + C-3 + C-11 + C-12), 128.56 (C-1 + C-4 + C-10 + C-13), 132.67 (C-4a + C-9a + C-13a + C-18a), 139.26 (C-7 + C-16), 144.45 (C-6a + C-7a + C-15a + C-16a), 147.48 (C-5a + C-8a + C-14a + C-17a). IR (Diffuse reflection) $\tilde{\nu}$ [cm^{-1}]: 2870 (st, Csp^3–H), 1232 (st S═O), 1622 (st C═C), 1044 (st S═O), 945, 835, 743. UV/vis (H_2O): λ_{max} [nm] (log ε) = 219 (4.87), 274 (4.17), 308 (3.37), 322 (3.34).

NC(OSO$_3$Li). Molecular formula: $C_{32}H_{20}Li_2O_8S_2$ (610.50 g mol^{-1}); mp >300 °C; HR-MS (ESI-TOF, MeOH, negative ionization), m/z = 298.0295 $[M - 2{\cdot}Li]^{2-}$ (calc. for $C_{32}H_{20}O_8S_2^{2-}$ = 596.0600/2 = 298.0300), 603.0743 $[M - Li]^-$ (calc. for $C_{32}H_{20}LiO_8S_2$ = 603.0760). ^{1}H NMR (500 MHz, CD_3OD, Fig. S1) δ[ppm]: 2.36 (dt, 2H, ^2J(H-19a,H-19i) = 7.6 Hz, ^3J(H-19a,H-8) = 1.6 Hz, H-19a + H-20a), 2.66 (dt, 2H, ^3J(H-19i,H-8) = 1.6 Hz, H-19i + H-20i), 4.80 (t, 4H, H-6 + H-8 + H-15 + H-17), 7.18 (dd, 4H, ^3J(H-2,H-1) = 5.9 Hz, ^4J(H-2,H-4) = 3.0 Hz, H-2 + H-3 + H-12 + H-11), 7.54 (dd, 4H, H-1 + H-4 + H-10 + H-13), 7.58 (s, 4H, H-5 + H-9 + H-14 + H-18). ^{13}C NMR (126 MHz, CD_3OD) δ[ppm]: 49.68 (C-6 + C-8 + C-15 + C-17), 65.75 (C-19 + C-20), 120.95 (C-5 + C-9 + C-14 + C-18), 125.76 (C-2 + C-3 + C-11 + C-12), 128.60 (C-1 + C-4 + C-10 + C-13), 133.58 (C-4a + C-9a + C-13a + C-18a), 139.63 (C-7 + C-16), 144.00 (C-6a + C-7a + C-15a + C-16a), 148.60 (C-5a + C-8a + C-14a + C-17a). ^{1}H NMR (500 MHz, D_2O) δ[ppm]: 2.33 (d, 2H, ^2J(H-19a,H-19i) = 8.0 Hz, H-19a + H-20a), 2.63 (d, 2H, H-19i + H-20i), 4.68 (s, 4H, H-6 + H-8 + H-15 + H-17), 6.28 (br, 4H, H-2 + H-3 + H-12 + H-11), 6.59 (br, 4H, H-1 + H-4 + H-10 + H-13), 6.97 (s, 4H, H-5 + H-9 + H-14 + H-18). ^{13}C NMR (126 MHz, D_2O) δ[ppm]: 48.18 (C-6 + C-8 + C-15 + C-17), 64.78 (C-19 + C-20), 119.79 (C-5 + C-9 + C-14 + C-18), 124.59 (C-2 + C-3 + C-11 + C-12), 126.88 (C-1 + C-4 + C-10 + C-13), 131.14 (C-4a + C-9a + C-13a + C-18a), 137.92 (C-7 + C-16), 143.18 (C-6a + C-7a + C-15a + C-16a), 145.76 (C-5a + C-8a + C-14a + C-17a). IR (Diffuse reflection) $\tilde{\nu}$ [cm^{-1}]: 2970 (st, Csp^3–H), 1627 (st C═C), 1235 (st S═O), 1050 (st S═O), 946, 890, 837, 745, 697. UV/vis (H_2O): λ_{max} [nm] (log ε) = 219 (4.97), 274 (4.25), 308 (3.31), 322 (3.32).

Clip AC. The reaction of the anthracene clip substituted by hydroxyl groups in the central benzene spacer-unit (173.7 mg, 0.32 mmol) with the sulfur trioxide pyridinium complex and $NaHCO_3$ gave 220.0 mg (0.30 mmol) of the sodium sulfate-substituted anthracene clip **AC**, yield: 92%. Molecular formula: $C_{40}H_{24}Na_2O_8S_2$ (742.07 g mol^{-1}); mp >300 °C. HR-MS (ESI-TOF, MeOH, negative ionization), m/z = 348.0484 $[M - 2{\cdot}Na]^{2-}$ (calc. for $C_{40}H_{24}O_8S_2^{2-}$ = 696.0918/2 = 348.0459), 719.0870 $[M - Na]^-$ (calc. for $C_{40}H_{24}NaO_8S_2$ = 719.0816). ^{1}H NMR (500 MHz, CD_3OD, Fig. 12) δ[ppm]: 2.70 (dt, 2H, ^2J(H-23a,H-23i) = 7.8 Hz, ^3J(H-23a,H-9) = 1.5 Hz, H-23a + H-24a), 2.68 (dt, 2H, ^3J(H-23i,H-9) = 1.3 Hz, H-23i + H-24i), 4.85 (s, 4H, H-7 + H-9 + H-18 + H-20), 7.25 (dd, 4H, ^3J(H-2,H-1) = 6.5 Hz, ^4J(H-2,H-4) = 3.2 Hz, H-2 + H-3 + H-13 + H-14), 7.72 (s, 4H, H-6 + H-10 + H-17 + H-21), 7.78 (dd, 4H, H-1 + H-4 + H-12 + H-15), 8.09 (s, 4H, H-5 + H-11 + H-16 + H-22). ^{13}C NMR (126 MHz, CD_3OD) δ[ppm]: 64.33 (C-23 + C-24), 64.80 (C-7 + C-9 + C-18 + C-20), 120.58 (C-6 + C-10 + C-17 + C-21), 125.52 (C-2 + C-3 + C-13 + C-14), 126.60 (C-5 + C-11 + C-16 + C-22), 128.85 (C-1 + C-4 + C-12 + C-15), 132.54 (C-5a + C-10a + C-16a + C-21a), 132.82 (C-4a + C-11a + C-15a + C-22a), 139.69 (C-8 + C-19), 143.62 (C-7a + C-8a + C-18a + C-19a), 147.72 (C-6a + C-9a + C-17a + C-20a). ^{1}H NMR (500 MHz,

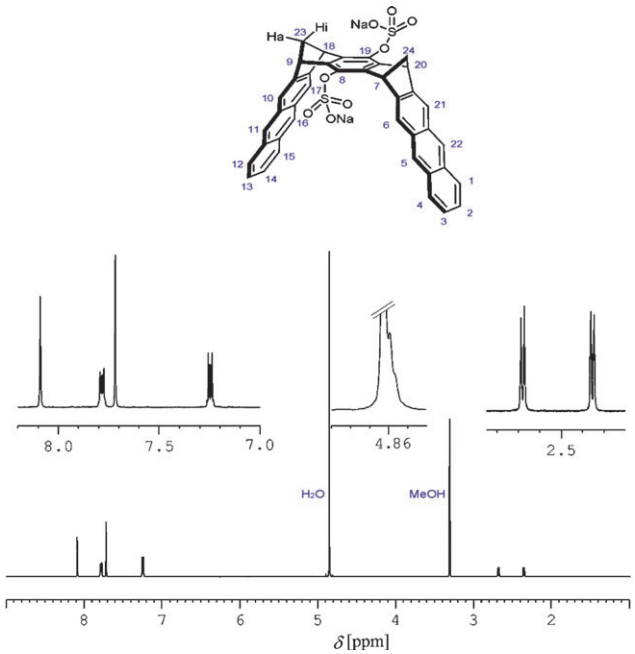


Fig. 12 ^{1}H NMR spectrum (500 MHz) of naphthalene clip **AC** in CD_3OD.

D_2O) δ[ppm]: 2.44 (br, 2H, H-23i + H-24i), 2.75 (br, 2H, H-23♀ + H-24♀), 4.76 (s, 4H, H-7 + H-9 + H-18 + H-20), 5.95 (br, 4H, H-5 + H-11 + H-16 + H-22), 6.65 (br, 8H, H-1 + H-4 + H-6 + H-10 + H-12 + H-15 + H-17 + H-21), 6.79 (br, 4H, H-2 + H-3 + H-13 + H-14). ^{13}C NMR (126 MHz, D_2O) δ[ppm]: 48.57 (C-7 + C-9 + C-18 + C-20), 62.54 (C-23 + C-24), , 120.03 (C-6 + C-10 + C-17 + C-21), 124.28 (C-2 + C-3 + C-13 + C-14), 124.73 (C-5 + C-11 + C-16 + C-22), 127.63 (C-1 + C-4 + C-12 + C-15), 129.88 (C-5a + C-10a + C-16a + C-21a), 130.47 (C-4a + C-11a + C-15a + C-22a), 138.65 (C-8 + C-19), 143.72 (C-7a + C-8a + C-18a + C-19a), 144.58 (C-6a + C-9a + C-17a + C-20a). IR (Diffuse reflection) $\tilde{\nu}$ [cm^{-1}]: 2944 (st, Csp^3–H), 1626 (st C═C), 1231 (st S═O), 1041 (st S═O), 946, 902, 839, 768, 739. UV/Vis (H_2O): λ_{max} [nm] ($\log\varepsilon$) = 252 (4.93), 337 (3.76), 354 (3.82), 371 (3.68).

Determination of K_{dim} and K_a by ^{1}H NMR titration method: see ref. 6*a*.

Photophysics. The experiments were carried out in aqueous or methanol solution at 298 K. UV-Vis absorption spectra were recorded with a Perkin Elmer λ40 spectrophotometer. Fluorescence spectra were obtained with a Perkin Elmer LS-50 spectrofluorimeter, equipped with a Hamamatsu R928 phototube, on air-equilibrated solutions. Fluorescence quantum yields were measured following the method of Demas and Crosby[22] (standard used: anthracene in deaerated ethanol solution).[23] Global fitting of absorption or emission spectra has been performed by Specfit software.[13] Fitting of the emission intensity ratio of the two bands observed for clip **AC** in water has been performed according to the following equation:

$$I_D/I_M = (A_D\Phi_D)/(A_M\Phi_M) = k[D]/[M]$$

where the subscripts $_D$ and $_M$ refer to the monomer and dimer, respectively; I is the emission intensity estimated at 400 and 505 nm for the monomer and dimer, respectively; A is the absorbance at the excitation wavelength; Φ is the emission quantum yield and k is a constant.

Fluorescence lifetime measurements were performed by an Edinburgh FLS920 spectrofluorometer equipped with a TCC900 card for data acquisition in time-correlated single-photon counting experiments (0.5 ns time resolution) with a D_2 lamp and a LDH-P-C-405 pulsed diode laser. The estimated experimental errors are: 2 nm on the band maximum, 5% on the molar absorption coefficient, fluorescence lifetime, and $\log K$ values, 10% on the fluorescence quantum yield.

Acknowledgements

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