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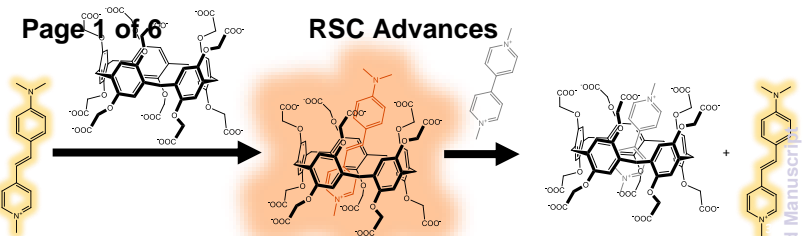


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VIS



UV

ARTICLE

Optical spectroscopic studies on the complexation of stilbazolium dyes with a water soluble pillar[5]arene

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The host-guest interactions of a water soluble carboxylato-pillar[5]arene (**WPA5**) and three stilbazolium dyes (containing 9-anthryl, 1-pyrenyl and 4-dimethylaminophenyl groups) were studied by UV-Vis and fluorescence spectroscopy with the detailed description of the spectral changes. Different spectroscopic response was observed in each case, with the most significant result of 28-fold fluorescence enhancement and intense color change in case of 4-dimethylaminostyryl-*N*-methylpyridinium iodide (**DAST**, **G3**). In addition, a FID (fluorescence indicator displacement) system comprised of **WPA5** and **G3** was shown to detect paraquat by turn-off fluorescence in aqueous solution.

Introduction

Since the foundation of supramolecular chemistry¹ the four generations of host molecules (cyclodextrins, crown ethers, calixarenes and cucurbiturils) have been the main targets in host-guest chemical studies. However, the recent discovery of pillararenes (**PAs**)² have attracted great attention as this novel type of host molecules possessing advantageous chemical (easy synthesis and modifications), structural (different cavity size, symmetry) and binding characteristics. Over the past three years numerous review papers³ discussing mainly the synthesis, conformations, chemical modifications and properties of pillararenes have been published. For years **PA5** was the only representative of the family with acceptable synthetic availability.⁴ Lately a similarly simple access to **PA6** was reported⁵ along with the identification and separation of some higher (up to 15) membered **PAs**.⁶ Although the parent **PAs** are efficiently soluble in organic solvents to study their binding properties by spectroscopic methods, a break-through was achieved by water soluble pillararenes (**WPAs**)^{7,8} making studies possible in aqueous systems.

A lot of pillararene-based host-guest interactions have been explored, especially with **PA5,6** and **WPA5,6**. The guest molecules consist of mono- and diamines,⁹ ammonium, imidazolium, pyridinium salts,¹⁰ *N*-methylacridinium iodide¹¹ and basic amino acids.¹² Among guest molecules, bipyridinium salts (paraquat and homologues) have been the most extensively studied and the complexations were primarily monitored by NMR, as generally neither **PAs** nor the guests

were sensitively detectable with optical spectroscopic methods. A pillar[5]arene host with pyrene fluorescent marker,⁹ a quaternary ammonium attached to tetraphenylethene¹³ and an *N*-methylacridinium fluorescent guest are to be mentioned as exceptions.¹¹ Beside the rare utilization of fluorescence detection in the complexation studies of **PAs**, the striking lack of stilbazolium dyes (mainly 4-styryl-*N*-methylpyridinium) among the numerous pyridinium guest molecules tested is surprising, albeit they are of great scientific and technologic interest as nonlinear optical materials,¹⁴ organelle-targeted fluorescent probes,¹⁵ norepinephrine transporter ligands,¹⁶ etc. In this paper the fluorescence spectroscopic investigations of host-guest interaction between **WPA5** and stilbazolium dyes **G1-G3** (see Fig. 1) in aqueous solution are presented. In addition, a fluorescence indicator displacement (FID) system comprised of **WPA5** and **DAST** (4-dimethylaminostyryl-*N*-methylpyridinium iodide, **G3**) is shown to sensitively detect paraquat herbicide.

Results and Discussion

Stilbazolium dyes (**G1-G3**) were prepared by the piperidine-catalyzed condensation of the respective aldehydes with 1,4-dimethylpyridinium iodide according to previously reported methods.¹⁷ **WPA5** was synthesized as described recently.¹⁸ The complexation studies were performed primarily by UV-Vis and fluorescence spectroscopy in aqueous media.

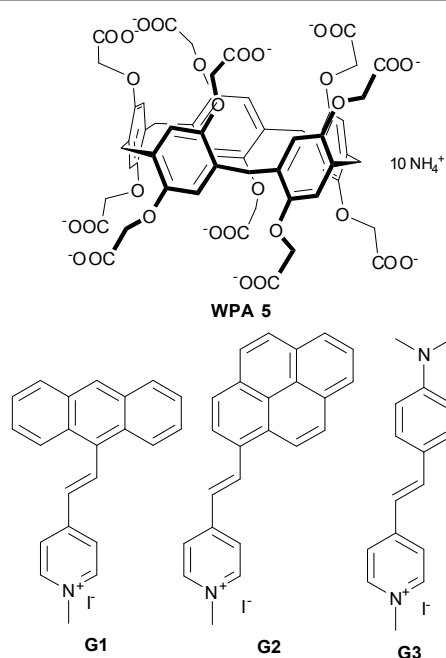


Fig. 1 Structures of WPA5 and guests G1-G3

Complexation studies

Upon addition of WPA5 to the aqueous solution of G1, an increase in fluorescence intensity (Fig. S1) was observed accompanied by a bathochromic shift of 18 nm. The maximum emission ($F/F^0 = 6.5$ at 616 nm) was reached at 5 equivalent of WPA5, as it can be seen in Fig. S1. The measurements were found, however, poorly reproducible presumably due to the E/Z-isomerism of G1 upon irradiation with the UV light.¹⁹ In this way, therefore, the complexation could not be evaluated quantitatively. Interestingly, an opposite emission behavior was experienced with G2, where ethanol-water 1:1 mixture was used for solubility reasons. Upon addition of WPA5 resulted in a slight decrease of fluorescence (Fig. S1) which may be attributed to different emission mechanisms originated from the planarity of G2 vs. the non-planar structure of G1.¹⁹ A Job's plot of the fluorescence intensities at 595 nm (see Fig. S2) indicated the formation of an 1:1 complex, for which an association constant of $K_1 = (2.64 \pm 0.2) \cdot 10^5 \text{ M}^{-1}$ was obtained by a least-square fitting of the fluorescence spectra (for details of calculations see section 4 in ESI). As G1 and G2 contain bulky aromatic fluorophore groups, we supposed the complex formation was simply driven by multiple electrostatic interactions between the anionic host and the cationic pyridinium moiety. NMR-studies carried out with G1 in D₂O – DMSO-*d*₆ = 1:1 mixture (for G2 we could not find a proper solvent mixture) revealed the proton signals in the complex were mostly unchanged with the exception of the signals of the pyridinium moiety (Fig. S5). We observed large upfield shifts (approx. 1 ppm) and broadening of the signals of the N⁺Me and Py⁺-2,6-H and a slight upfield shift (0.3 ppm) assigned to the Py⁺-3,5-H protons. The results clearly show that the bulky 9-

anthryl group prevent the complete inclusion of G1 and this assumption can be valid for the pyrene-substituted G2 as well.

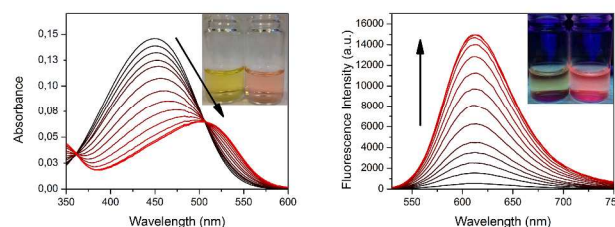


Fig. 2 Absorption and fluorescence spectra of G3 (5.0 μM) upon addition of WPA5 (0 to 1.46 equiv.) in water (excitation at 506 nm); inset: photographs showing colorimetric and fluorescent changes in aqueous solution (10.0 μM G3 (left vial), 10.0 μM G3 and 30.0 μM WPA5 (right vial)) Note: aqueous solution of WPA5 is colorless and therefore omitted

When WPA5 was added to the aqueous solution of G3, intense optical responses were observed. Both the colorimetric (from yellow to orange) and fluorescent changes were visible to the naked eye (Fig. 2). In contrast, a significant fluorescence enhancement ($F/F^0 = 28$ at 613 nm accompanied by a 20-fold increase in the fluorescence quantum yield) occurred even at low WPA5 concentrations (see Table 1 for spectroscopic details). The association constant for 1:1 complex (see Fig. S3 for Job's plot) calculated from the absorption spectra was $K_1 = (1.3 \pm 0.1) \cdot 10^6 \text{ M}^{-1}$. We speculated that the complex formation was reached by the penetration of G3 into the cavity of the host molecule driven by electrostatic attraction, hydrophobic interaction, π - π stacking and C-H \cdots π interactions. The strong binding affinity in this system is attributed to the accumulation of these non-covalent forces. The inclusion phenomena were confirmed by ¹H-NMR studies (Fig. 3). Because of the insufficient solubility of G3 in water, D₂O – DMSO-*d*₆ = 1:1 mixture was used as NMR solvent. Almost all signals deviated in the complex (1 equiv. G3). The most significant difference was observed in the case of the aromatic protons including the aminophenyl moiety as well: all the protons shifted upfield and/or broadened/disappeared from the spectrum which is attributed to the strong shielding by the electron-rich aromatic system surrounding the electron-deficient G3. Only the signals of the protons on the dimethylamino group did not show detectable changes. These changes were accompanied by the slight downfield shifts of the aromatic protons on the host WPA5 as well as the splitting of the O-CH₂ protons into two sets of peaks indicating the restriction of swinging of the constituent units observed previously with paraquat.⁷ To characterize the effect of the DMSO co-solvent on the complex formation, the association constant K_1 was also determined in water-DMSO 1:1 mixture from the absorption spectra. Its value, $(1.5 \pm 0.1) \cdot 10^5 \text{ M}^{-1}$, was lower than in neat water, showing the importance of the hydrophobic nature of the dye in the complex formation.

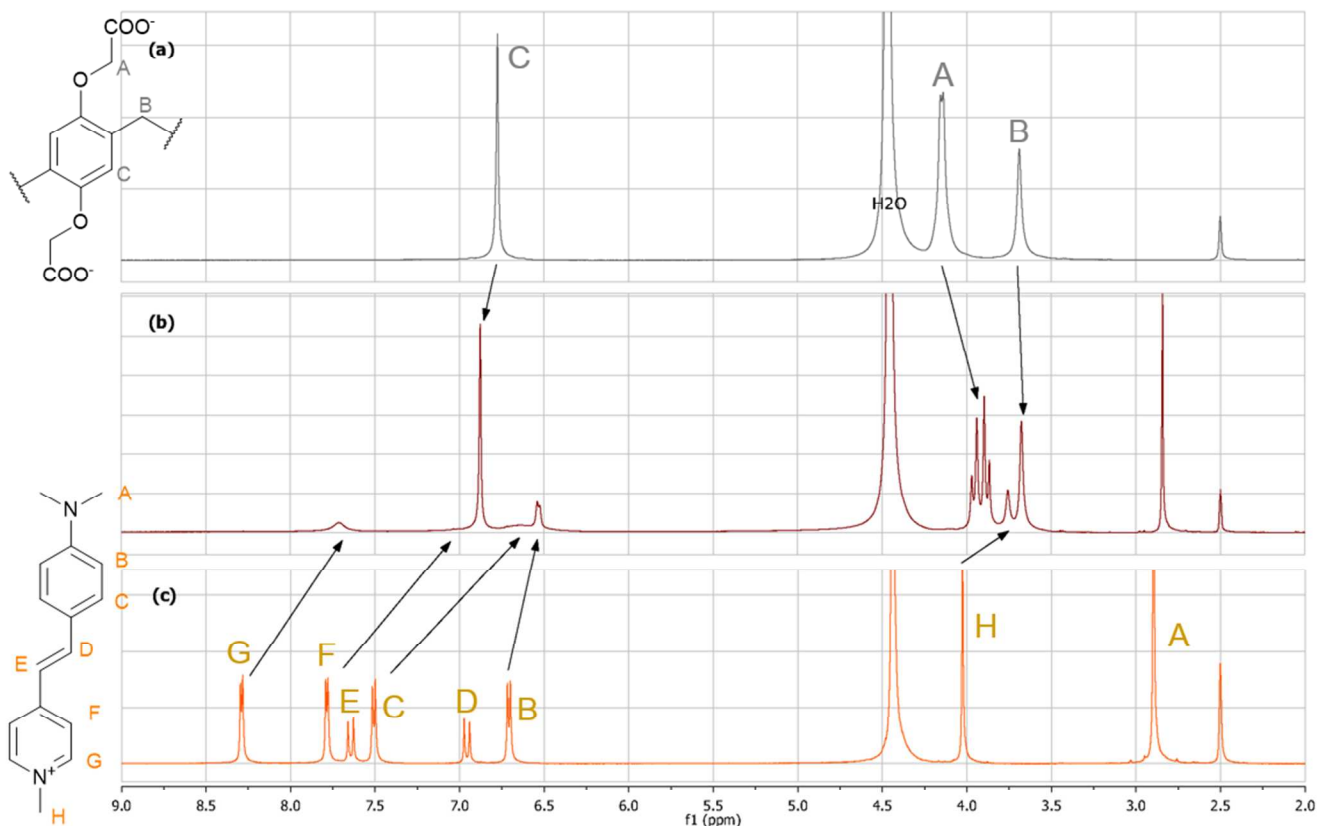


Fig. 3 Partial $^1\text{H-NMR}$ spectra (500 MHz, $\text{D}_2\text{O} - \text{DMSO-d}_6$ 1:1, 298 K) of (a) WPA5; (b) G3 and WPA5 (1 equiv.), (c) G3

Table 1 Wavelengths of the absorption and fluorescence bands (λ_{abs} , λ_{em}), absorption coefficients (ϵ), fluorescence quantum yields (Φ) and fluorescence decay time constants (τ) of **G3** and its **WPA5-G3** complex in aqueous solution.

	λ_{abs} (nm)	ϵ ($\text{M}^{-1}\text{cm}^{-1}$)	λ_{em} (nm)	Φ	τ_{F} (ps) with rel. weights
G3	449	29200	615	0.002	12 (100%) ^a
WPA5-G3	515 ^b	14300 ^b	607	0.041	310 (48%) ^c 640 (52%)

^a Stimulated emission decay time from ref.²⁰, $\lambda_{\text{pump}} = 400$ nm, $\lambda_{\text{probe}} = 620$ nm.

^b Calculated from the spectra of samples with constant **G3** and varying **WPA5** concentrations

^c $\lambda_{\text{exc}} = 470$ nm, $\lambda_{\text{em}} = 610$ nm.

Fluorescence analysis of G3 and WPA5-G3 complex

The optical spectral data of **G3** and its **WPA5-G3** complex in aqueous solution are collected in Table 1. The positions of the absorption and fluorescence bands of **G3** are sensitive primarily to the polarity and the hydrogen bond acceptor ability of the local environment, quantified by the π^* and β Kamlet-Taft solvatochromic parameters, respectively.²¹

The absorption maximum of the dye shifts to lower wavelengths with increasing solvent polarity (negative solvatochromism), in accordance with the results of theoretical calculations.²² These showed that **G3** is a charge transfer dye with a strongly polar S_0 state, in which the positive charge is concentrated on the pyridinium moiety, and a less polar S_1 state, where the charge is more evenly distributed on the pyridinium and aniline segments. Hydrogen bond acceptor, i.e. electron pair donor solvents stabilize the **G3** solute by interacting with the positive charge of the ground as well as the excited state solute. The net effect is a bathochromic shift of the absorption band with increasing β parameter. The absorption band of **G3** complexed by **WPA5** - 515 nm vs. 448 nm measured in water – falls at a higher wavelength than in the solution spectra measured in most of solvents (the exception is the CH_2Cl_2 solution of the dye with $\lambda_{\text{abs}} = 517$ nm),²³ demonstrating that the less polar excited state solute is stabilized strongly in the cavity of the **WPA5** host.

Unlike the absorption band, the fluorescence band of **G3** shows a bathochromic shift upon complexation, but this is also in accordance with the lower polarity of the environment, as the

fluorescence band of the dye shows a positive solvatochromism.

The spectacular enhancement of the fluorescence intensity upon complexation is a consequence of hindering the deactivation via non-emissive twisted intramolecular charge-transfer (TICT) states. The results of time-resolved fluorescence studies in low temperature ethanol solutions suggested that the planar excited-state of **G3** formed via vertical excitation from the coplanar ground state molecule, easily relaxes to energetically close non-planar structures.²⁴ Theoretical calculations showed that the non-planar species are formed presumably via the rotations of the aniline or pyridinium units around the single C-C bonds of the vinylene group, as the torsion of the dimethylamino group and the twisting around the C=C double bond are energetically less favored.²⁵ The correlation of the fluorescence lifetimes (τ_F) of **G3** measured in water-glycerol mixtures with viscosity of the solvent,²⁶ and the correlation of the τ_F values in polymer matrices with the elastic modulus of the medium²⁷ are also consistent with the decisive role of a non-emissive TICT state of the dye in the deactivation process.

The picosecond decay kinetics of **G3** in room temperature solutions can be mono-, bi- and triexponential, depending on the polarity and viscosity of the solvent, which can be interpreted in terms of an energy-level scheme with three sub-levels within the S_1 state: a weakly polar planar 'locally excited' (LE), a strongly polar, also planar intramolecular (ICT) state, and the above TICT state.²⁸ In strongly polar, low viscosity solvents, like water, the decay is fast and monoexponential, as the emission derives only from the LE state, the ICT state easily deactivates via dipole-dipole solute-solvent interactions. The TICT state may be emissive only in highly polar and viscous solvents, like glycerol, where it may contribute to the emission on the red edge of the fluorescence band. By a different model, describing the deactivation of the dye on the potential energy surface (PES),²⁹ the TICT excited state of **G3** can be considered a 'sink' region on the PES, and the relaxation of the molecule to the TICT state, following the excitation, is a coupled process involving contributions from the solvational, vibrational and torsional relaxations.

A similarly strong enhancement of fluorescence intensity was observed with the cucurbit[6]uril complex of **G3**,³⁰ whereas its β -cyclodextrin³¹ and cucurbit[7]uril³² complexes showed a somewhat smaller effect.

The fluorescence lifetime of **G3**, τ_F , in aqueous solution is shorter than ~ 20 ps,³³ the temporal resolution of our TCSPC system. The decay of the **WPA5-G3** complex was found much slower, and was best fitted by a biexponential function with two close time constants. The decay curves measured at different wavelengths on the rising and falling edges of the fluorescence band were found similar, their time constants and relative amplitudes were not sensitive to the wavelengths. This is illustrated in Fig. S6, showing the time-resolved emission spectra constructed from the decay curves, which did not shift on the picoseconds time scale. This differs from the decay kinetics of the dye in various solvents, which change markedly

with the wavelength, following the different phases of structural and solvational relaxation.^{33, 28, 29}

Fluorescence indicator displacement (FID)

DMV (dimethylviologen or 4,4'-dimethylbipyridinium salts like paraquat) are known to bind strongly to **WPA5** via inclusion in the cavity,⁷ therefore by designing a threaded macrocycle-dye supramolecular system with modified fluorescence compared to the free dye, the competitor paraquat can be detected or measured by the displacement of the dye. The fluorescence intensity of the complex can be higher or lower than the free dye, hence the analyte exhibits turn-off or turn-on fluorescence response, respectively.³⁴

To our knowledge, only a few examples of pillararene-based receptors are used in FID methods, thus we speculated that our most sensitive system could be used for the detection of **DMV** analyte. The addition of **DMV** (diiodide salt) to the **WPA5-G3** system (Fig. 4) caused the expected fluorescence quenching (turn-off sensing) due to the dethreading of the dye from the complex causing the regeneration of the almost non-fluorescent free state of **G3** in water. The displacement, caused by the stronger binding of **DMV** can be easily observed by the color changes and the reduced emission under a handheld UV lamp.

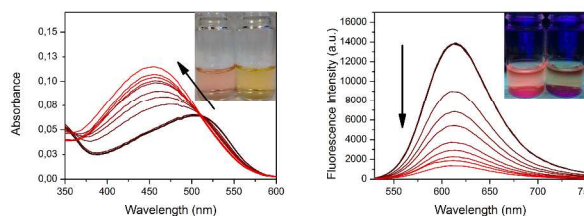


Fig. 4 Absorption and fluorescence spectra of **G3** (5.0 μM), **WPA5** (15.0 μM) upon addition of **DMV** (0 μM to 27.4 μM) in water (excitation at 506 nm); inset: photographs showing colorimetric and fluorescent changes in aqueous solution (10.0 μM **G3** and 30.0 μM **WPA5** (left vial), 10.0 μM **G3**, 30.0 μM **WPA5** and 30.0 μM **DMV** (left vial))

Fluorescence titration experiments proved our system to be very sensitive: a 60% reduction in fluorescence intensity can be observed by adding 15 μM of **DMV** to a solution of **WPA5-G3** (5.0 μM dye and 15 μM host concentration) which is a significantly better result than the previously reported.¹¹ From the absorption spectra in Fig. 4, $K_2 = (2.57 \pm 0.4) \cdot 10^6 \text{ M}^{-1}$ was obtained for the equilibrium constant of the binding of **DMV** by the pillararene host by a least square fitting calculation (see ESI for details). To characterize the sensitivity of this FID method, the fluorescence intensities of **WPA5-G3-paraquat** systems with fixed **WPA5** and **G3** initial concentrations and varying added paraquat concentrations were calculated from K_1 and K_2 . As can be seen in Fig S7, the addition of paraquat in 0.2 μM concentration to a solution containing $[\text{G3}]_0 = 1 \text{ } \mu\text{M}$ dye and $[\text{WPA5}]_0 = 3 \text{ } \mu\text{M}$ pillararene, results in a $\sim 5\%$ change of fluorescence intensity, that can be easily detected.

Conclusions

In summary, we have examined the interaction between different pyridinium-based stilbazolium salts and **WPA5** as new host-guest systems where the interactions are detectable by optical spectroscopic methods. The most significant effect was observed in the case of **G3** with remarkable turn-on fluorescence and color change thus **WPA5-G3** system could be used in various supramolecular systems in the future. This was demonstrated by using it as a FID-based probe to detect paraquat in a highly sensitive manner. In addition, detailed description on the mechanism of fluorescence enhancement is provided.

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Notes and references

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