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# Sensor for Nitrophenol Based on a Fluorescent Molecular Clip

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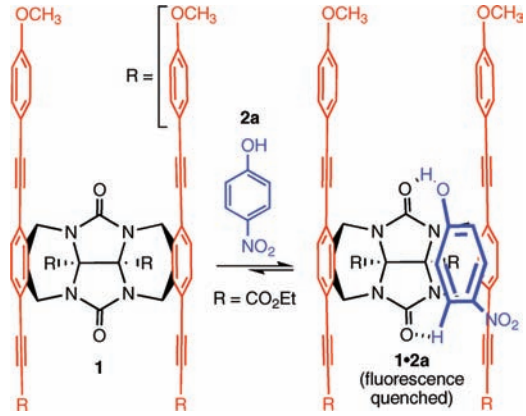
## ABSTRACT




**We report the synthesis, X-ray crystal structure, and photophysics of a fluorescent molecular clip (1). Binding studies of 1 toward phenols 2a−j were carried out using fluorescence, $^1$H NMR, and IR spectroscopy, which revealed a high affinity and selectivity for 4-nitrophenol (2a) due to formation of the 1·2a complex driven by H-bonding and $\pi-\pi$ stacking interactions.**

Contemporary supramolecular systems aim to meld form with function. Accordingly, supramolecular design principles are being employed for the development of molecular devices and machines,[1] drug delivery systems,[2] artificial ion channels,[3] supramolecular catalysts,[4] and optical sensing systems.[5]

A popular route to the development of optical (colorimetric or fluorescence) sensors relies on indicator displacement assays which have been used to sense a variety of molecular and ionic guests.[6] Alternatively, fluorophores may be incorportated into the covalent structure of the host and thereby participate directly in the sensing process.[7] In all of these application areas, host systems that exhibit high affinity and highly selective binding processes are essential.

Glycoluril—as the essential component of Nolte's molecular clips,[8] Rebek's capsules,[9] and the cucurbit[*n*]uril family of macrocycles[10]—has become a popular building block for the preparation of new host systems.[11] Of particular relevance

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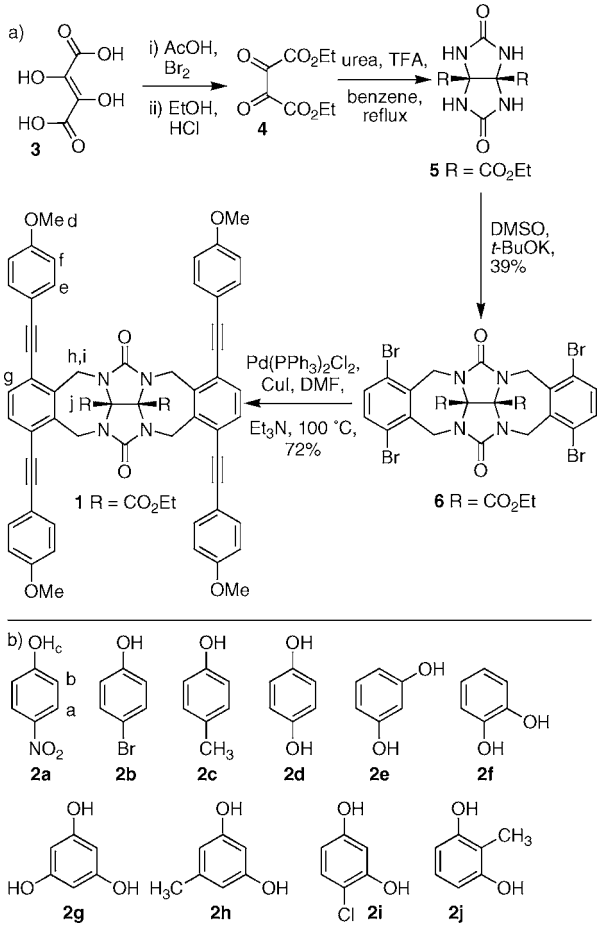
to the work reported here are the reports by Nolte on the use of U-shaped glycoluril-based molecular clips as receptors for 1,3-dihydroxybenzene derivatives whose complexes benefit from Ar−OH···O═C H-bonds, $\pi-\pi$ stacking interactions, and a cavity effect.[12] In ongoing efforts, we aim to combine the outstanding recognition properties of glycoluril derived systems with the high sensitivity and low detection limits of fluorescence spectroscopy.[13] In this paper, we describe the design, synthesis, and highly selective recognition properties of fluorescent molecular clip **1** toward 4-nitrophenol **2a**. Compound **2a**—which is of interest because of its mutagenic properties and its use in active ester coupling reactions—has previously been complexed by cyclodextrin, calixarene, and cucurbit[*n*]uril molecular containers.[14,15]

Scheme 1 shows the synthesis of fluorescent molecular clip **1**. In brief, dihydroxyfumaric acid **3** was oxidized with

**Scheme 1.** (a) Synthesis of Molecular Clip **1** and (b) Guests **2** Used in This Study




bromine in acetic acid to the corresponding diketone which was esterified to give **4**. Transformation of **4** into diethoxylcarbonyl glycoluril **5** proceeded smoothly according to the literature procedure.[16] Alkylation of **5** with 2,3-bis(bromomethyl)-1,4-dibromobenzene under basic conditions (DMSO, *t*-BuOK) gave **6** in 39% yield. Installation of the 4-methoxyphenylethynyl arms under Sonogashira cross-coupling conditions[17] gave molecular clip **1** in 72% yield. By virtue of the extended $\pi$-system of the *o*-xylylene sidewalls and the attached phenylethynyl arms, clip **1** is highly fluorescent.

We were fortunate to obtain X-ray quality crystals of molecular clip **1** which allowed us to confirm the structural assignment and elucidate the three-dimensional structure of **1** by single-crystal X-ray diffraction (Figure 1). The crystal

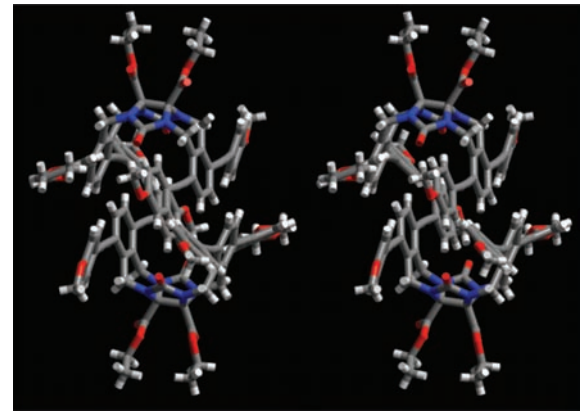


**Figure 1.** Cross-eyed stereoview of the structure of **1** in the crystal. Color code: C, gray; H, white; N, blue; O, red.

structure of molecular clip **1** clearly reveals the presence of a deep cavity, which is defined by two extended aromatic sidewalls. To achieve efficient packing in the crystal, molecular clip **1** undergoes dimerization[18] by the reciprocal insertion of the aromatic sidewall of one clip into the cleft of the opposing clip. The distance between the ureidyl C═O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oxygen atoms in **1** amounts to 5.6 Å; this spacing is appropriate for H-bonding to 1,3-dihydroxybenzenes.[10] The dihedral angle between the two phenyl rings of the sidewalls is 26° and the distance between the centroids of *o*-xylylene is 6.1 Å. These geometrical features enable **1** to engage in $\pi-\pi$ interactions and H-bonds with suitable guests.[12]

Molecular clip **1** displayed strong fluorescence emission in THF as shown in Figure 2. The excitation and emission

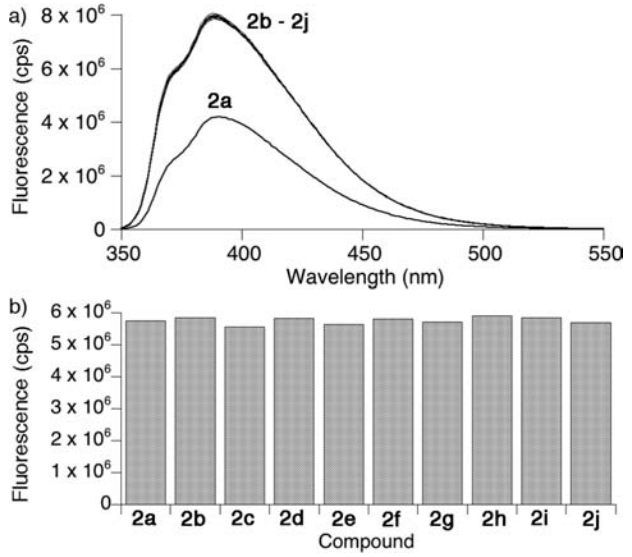


**Figure 2.** (a) Fluorescence emission changes of **1** (10 $\mu$M) in THF−MeOH (9:1, v/v) in the presence of **2a**−**j** (400 $\mu$M). (b) Fluorescence intensity at 389 nm of a solution of **1** (10 $\mu$M), **2a** (160 $\mu$M), and **2b**−**j** (160 $\mu$M). $\lambda_{ex} = 339$ nm.

wavelengths were 339 and 388 nm, respectively. The quantum yield of **1** ($\Phi = 0.65$) was determined by comparing the ratio of the fluorescence emission intensity at 389 nm to UV−vis absorbance at the excitation wavelength used relative to that of quinine ($\Phi = 0.546$) as reference.[19]

Once the synthesis, structural characterization, and preliminary photophysical investigations of **1** were complete, we decided to investigate the use of **1** as a fluorescence sensor for phenols **2**. Figure 2 shows the changes in fluorescence intensity of molecular clip **1** upon addition of 40 equiv of **2a**−**j**. Quite interestingly, significant fluorescence quenching is observed for 4-nitrophenol **2a** but not for any of the other phenols **2b**−**j**. To gauge whether the fluorescence quenching observed for **2a** was due to a higher binding constant for **1**·**2a** or whether the electron-poor aromatic ring of **2a** was simply more efficient at quenching the fluorescence

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of the electron rich sidewalls of **1** relative to **2b**−**j**, we performed competition experiments. Figure 2b shows the fluorescence intensity of solutions containing **1** (10 $\mu$M), **2a** (160 $\mu$M), and individually **2b**−**j** (160 $\mu$M). Clearly, the presence of **2b**−**j** does not significantly effect the overall fluorescence intensity which establishes that molecular clip **1** binds more tightly to **2a** than to phenols **2b**−**j**.[20]

Given the interesting ability of **2a** to quench the fluorescence of **1**, we decided to investigate this process in more detail. We first performed a titration where the emission spectrum of **1** (10 $\mu$M) was monitored as the concentration of **2a** was increased (Figure 3). The inset to Figure 3 shows

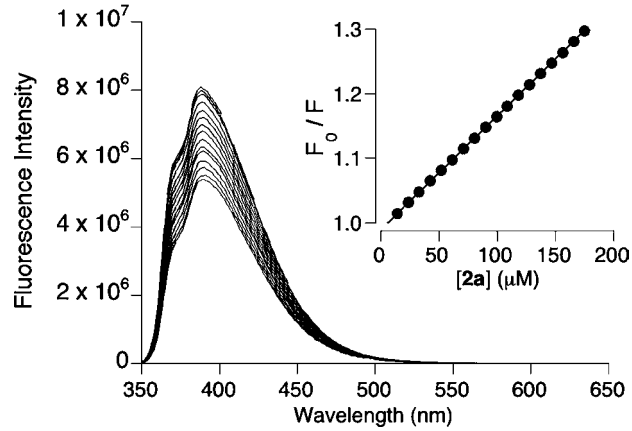


**Figure 3.** Fluorescence spectra (excitation at 339 nm) of **2** (10 $\mu$M) in THF−MeOH (9:1, v/v) in the presence of 0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, 22.0, 24.0, 26.0, 28.0, 30.0, 35.0, and 40.0 equiv of 4-nitrophenol predissolved in MeOH. Inset: Stern−Volmer plot of the emission data.

the linearity of the Stern−Volmer plot which confirms the formation of a single type of complex between **1** and **2a**. The stoichiometry of the complex between **1** and **2a** is 1:1 based on the fluorescence Job plot shown in Figure 4.[21] The

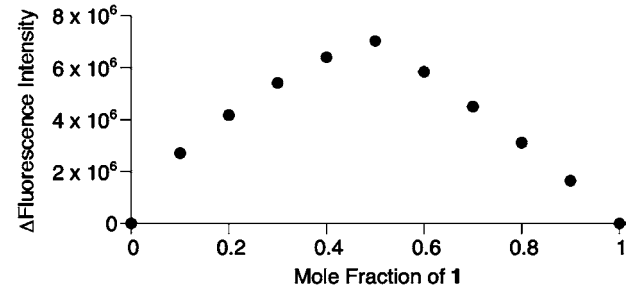


**Figure 4.** Job plot for mixtures of **1** and **2a** ([**1**] + [**2a**] = 20 $\mu$M).

association constant for the formation of **1**·**2a** ($K_a = 1.09 \times 10^4 \pm 1.82 \times 10^2$ M$^{-1}$) in THF−MeOH (9:1) was determined from a Benesi−Hildebrand plot.[22]

To further elucidate the geometry of the **1**·**2a** complex we performed $^1$H NMR and IR studies. Figure 5 shows the $^1$H

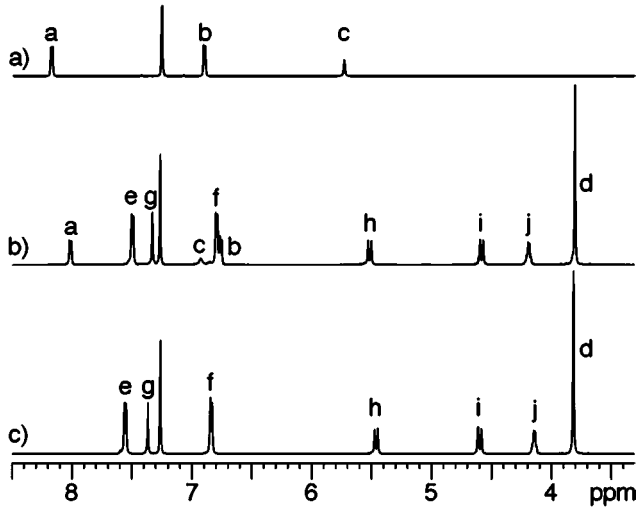


**Figure 5.** $^1$H NMR spectra (400 MHz, $CDCl_3$, 298 K) recorded for (a) **2a**, (b) **1·2a**, and (c) **1**.

NMR spectra recorded for **1**, **2a**, and the **1•2a** complex. We find that protons on the aromatic ring of **2a** undergo upfield shifting ($H_a$, −0.164 ppm; $H_b$, −0.154 ppm) whereas the OH group ($H_c$, +0.553 ppm) undergoes significant downfield shifting. The upfield shifts indicate that **2a** binds in the cavity of **1** by $\pi-\pi$ interactions whereas the downfield shift indicates that the OH group of **2a** is involved in H-bonding interactions with the ureidyl C═O group of **1**. Infrared spectroscopy was also used to investigate the H-bonding between **1** and **2a** (Supporting Information). Molecular clip **1** displays two $\nu_{C=O}$ stretching vibrations at 1750 and 1726 $cm^{-1}$ due to the presence of ureidyl and $CO_2Et$ functional groups.[12] The carbonyl stretching vibration band of the host is influenced by hydrogen bonding. IR spectra of molecular clip **1** mixed with 4-nitrophenol shows that $\nu_{C=O}$ splits into three bands (1755, 1723, and 1690 $cm^{-1}$). This splitting indicates that the carbonyl groups of host **1** in the complexes are involved in hydrogen bonding. Overall, the binding of **2a** in the cavity of molecular clip **1** described here is due to three factors, namely H-bonding between the phenolic OH and the urea carbonyl group, $\pi-\pi$ stacking interactions between the electron-poor aromatic guest and the electron-rich sidewalls of the host, and a cavity effect.[12,23] The MMFF-minimized geometry of the **1·2a** complex is shown in Figure 6.

(20) The fact that the fluorescence intensity of a solution of **1·2a** (10 mM, $K_a = 1.09 \times 10^4$ $M^{-1}$) and **2a** (150 $\mu$M) does not change significantly (<10%) upon addition of **2b**−**j** (160 $\mu$M) allows us to determine an upper limit on the binding constants for the **1·2b**−**1·2j** complexes ($K_a \leq 1.09 \times 10^3$ $M^{-1}$).

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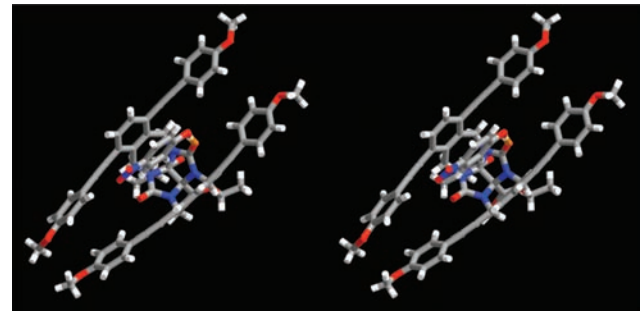

**Figure 6.** Cross-eyed stereoview of the MMFF-minimized model of **1·2a**. Color code: C, gray; H, white; N, blue; O, red.

As described above, molecular clip **1** binds strongly and selectively to electron deficient guest **2a** driven by a combination of H-bonding and $\pi-\pi$ stacking interactions. In addition to the strong and selective binding observed between **1** and **2a**, it is critical that **1** undergo a significant modulation in fluorescence upon binding. In this case, we believe that the formation of the **1·2a** complex results in electron or energy transfer from the excited state of the 4-methoxyphenylethynyl-conjugated sidewalls of **1** to the aromatic ring of electron-poor **2a**. For the other phenols examined, **2b**−**j**, a combination of weaker binding and lower fluorescence modulation upon binding result in the high selectivity observed for **2a** in this fluorescence assay.

In conclusion, we have synthesized a molecular clip **1** with extended ethynylated *o*-xylylene sidewalls which exhibits strong fluoresence emission. By virtue of the aromatic sidewalls and the glycoluril ureidyl C═O groups, **1** exhibits high binding affinity and selectivity toward electron-poor aromatic phenol **2a** driven by a combination of H-bonding and $\pi-\pi$ interactions as revealed by the detailed fluorescence, $^1$H NMR, and IR studies. The high efficiency of the sensing mechanism is due to the interplay of the H-bonds to the ureidyl C═O groups of the glycoluril which are stronger for more acidic electron-poor phenols and the fluorescence quenching which is also enhanced by electron-poor aromatic guests. Overall, the work highlights the advantages of a sensor design wherein the determinants of binding affinity and fluorescence modulation reinforce one another.

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**Supporting Information Available:** Synthesis of molecular clip **1** and details of the fluorescence and IR experiments. Crystallographic information file for **1** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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