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New fluorescent probes based on supramolecular diastereomers for the detection of 2-nitrophenol

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ABSTRACT

A couple of new fluorescent probes, based on diethoxycarbonyl glycoluril, were synthesized and characterized by ^{1}H NMR, X-ray crystal structure. The probes were found to be relatively highly fluorescence with quantum yields of 0.20 and 0.18, respectively, at room temperature in THF solution. Both of the proposed probes were tested using different phenolic compounds, showing very high selectivity and sensitivity for 2-nitrophenol by forming a 1:1 complex in THF-MeOH (9:1, v/v). Additionally, the sensors could be used for the qualitative analysis of 2-nitrophenol in real water samples.

1. Introduction

Phenols of natural and anthropogenic origin are dangerous contaminants in medical, food and environmental [1]. Although some of them are used as detergents or disinfectants even added to medicines, the phenols could cause damage to the urinogenital organs, liver and kidneys [2,3]. Nitroaromatic compounds are considered by the United States Environmental Protection Agency as main pollutants, because they are toxic to human health [4]. Approximately 165 phenolic compounds are known to have a toxic effect on the environment, among them, nitrophenols are considered as major toxic pollutants [5]. Hence, it is necessary to develop accurate and rapid detection methods for monitoring phenols in environmental and biological samples.

The analysis of phenolic compounds is difficult due to their high polarity. Although many analytical techniques have been applied to phenols detection, such as colorimetry [6–8], chromatography [9–11], most of these methods are time-consuming and require a tedious sample pretreatment. In contrast to such methods, the fluorescence spectroscopy has been considered as a promising method for monitoring of phenol because of the advantages such as good selectivity and low cost. Moreover, synthetic organic fluorescence probes show high stability and flexibility due to the versatility of the organic synthesis [12].

Glycoluril as a covalent building block for supramolecular chemistry has became popular for the remarkable recognition properties of the cucurbit [n] uril family of molecular containers [13,14] and their application in areas as diverse as molecular machines [15], drug delivery [16], and fluorescent sensors [17–19]. Glycoluril moiety has been introduced into the framework of hosts to produce host molecules, which are capable of binding bromide ion or iron ion by the cooperative action of multiple hydrogen bonds [20,21]. Nolte and co-workers reported diphenylglycoluril-based molecular clips were excellent receptors for neutral aromatic guests, particularly phenols and dihydroxybenzenes [19]. In our exploration of diethoxycarbonyl glycoluril derivatives, we have found that the glycoluril derived systems are essential for selective phenol binding [22]. Furthermore, the modification of the size and electron density of the sidewall group for the clips allows facile synthesis of various derivatives and influences the guest binding, which is an important feature for the development of fluorescence probes. Herein, we report the synthesis, and photophysical properties of two new fluorescent molecular clips **1** and **2**. Supramolecular diastereomers **1** and **2** showed high selective and sensitive response to 2-nitrophenol at room temperature in THF-MeOH (9:1, v/v).

2. Experimental

2.1. Reagents

All starting materials and catalysts were obtained commercially and used without further purification. Most of solvents were distilled under N_2 over appropriate drying reagents (sodium or

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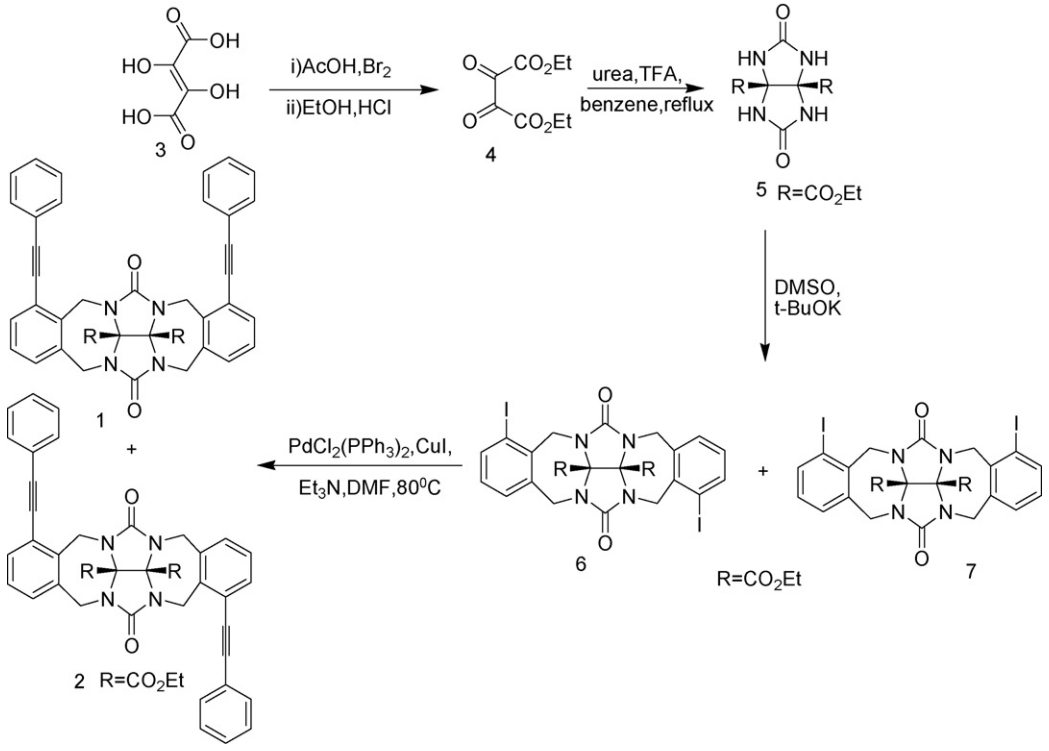


Scheme 1. Synthesis of molecule Clip **1** and **2**.

calcium hydride). Column chromatography: silica gel 200–300 mesh.

2.2. Apparatus

Absorption spectra were determined on UV-2501 PC spectrophotometer. Fluorescence spectra measurements were performed on a FluoroMax-P spectrofluorimeter equipped with a 150 W xenon discharge lamp, 1 cm quartz cells at room temperature (about 298 K). Typical scanning parameters were integration time of 0.1 s per point, intervals of 1 nm, and excitation/emission slits set at 4 nm. NMR spectra were measured on a Varian Mercury 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C relative to tetramethylsilane as internal standard. MS spectra were obtained on a Finnigan Trace MS spectrometer. IR spectra were recorded on a PerkinElmer PE-983 infrared spectrometer as KBr pellets with absorption reported in cm^{-1}. The X-ray crystal structure determinations of **1** and **2** were obtained on a Bruker SMART APEX CCD system.

2.3. Synthesis of molecular clip *1* and *2*

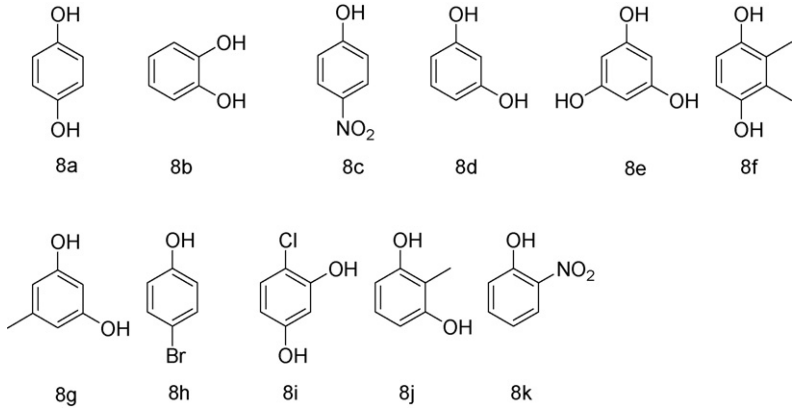
The compound **4**, **5** and phenylacetylene were synthesized, according to our previous work [23,24]. Preparation of compounds **1** and **2** from compound **4** and **5** is shown in Scheme 1. Phenylacetylene (101 mg, 1 mmol) was added to a solution of $PdCl_2$ $(PPh_3)_2$ (18 mg, 0.025 mmol), CuI (9.5 mg, 0.05 mmol) and the mixture of **4** and **5** (186 mg, 0.25 mmol) in freshly distilled Et_3N (10 mL) and DMF (10 mL) under Ar. The mixture was heated at 80 °C for 12 h, the solvent was evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc/Hexane, 1:4) to give pure compound **1** as a white solid (39.2 mg, 0.0533 mmol, 43%) and compound **2** as a white solid (44.1 mg, 0.0588 mmol, 47%).(**1**) M.p.: >250 °C. IR (ν_{max}, KBr, cm^{-1}): 3053, 2987, 2921, 1755(s), 1724(s), 1459(s), 1421, 1261, 1150, 757, 692; ^{1}H NMR ($CDCl_3$), δ (ppm): 7.62–7.60(m, 4H, Ar–H), 7.43–7.41(m, 4H, Ar–H), 7.31–7.16(m, 8H, Ar–H), 5.64(d, 2H, J = 16.0 Hz, CH_2), 4.75(d, 2H, J = 15.6 Hz, CH_2), 4.47(d, 2H, J = 16.0 Hz, CH_2), 4.40(d, 2H, J = 15.6 Hz, CH_2), 4.23(q, 4H, J = 7.2 Hz, $COOCH_2CH_3$), 1.31(t, 6H, J = 7.2 Hz, $COOCH_2CH_3$);^{13}C NMR ($CDCl_3$), δ (ppm): 166.0, 156.3, 155.5, 137.9, 136.5, 132.1, 131.7, 129.9, 128.33, 128.27, 127.8, 123.7, 123.1, 94.2, 87.1, 80.0, 63.4, 45.7, 42.7, 14.0. ESI mass spectrometry: m/z 691 (100% $[M+H]^+$); M^+ calculated 690.(**2**) M.p.: 254–255 °C. IR (ν_{max}, KBr, cm^{-1}): 3035, 2985, 2925, 1757(s), 1718(s), 1460(s), 1422, 1252, 757, 691; ^{1}H NMR ($CDCl_3$), δ (ppm): 7.63–7.59(m, 4H, Ar–H), 7.41–7.14(m, 12H, Ar–H), 5.67(d, 2H, J = 16.0 Hz, CH_2), 4.80(d, 2H, J = 16.0 Hz, CH_2), 4.43(d, 4H, J = 16.0 Hz, CH_2), 4.27(q, 4H, J = 7.2 Hz, $COOCH_2CH_3$), 1.33(t, 6H, J = 7.2 Hz, $COOCH_2CH_3$); ^{13}C NMR ($CDCl_3$), δ(ppm): 166.0, 155.8, 137.8, 136.6, 132.0, 131.7, 130.1, 128.4, 128.3, 127.8, 123.5, 123.0, 94.1, 87.0, 80.0, 63.4, 45.7, 42.6, 14.0. ESI mass spectrometry: m/z 690 (100% M^+); M^+ calculated 690.

2.4. X-ray diffraction analysis of compounds *1* and *2*

The crystals of **1** and **2** that were suitable for X-ray crystal structure analysis were grown by slow evaporation of solutions of the compounds in CH_2Cl_2–CH_3CN (20:1, v/v) mixture. The details of the crystal data have been deposited with Cambridge Crystallographic Data Centre as Supplementary Publication No. CCDC 757311 and 757312.

2.5. Binding titration

The stock solutions of **1** and **2** (1.0×10^{-5} M) were prepared by dissolving **1** and **2** respectively in THF-MeOH (9:1, v/v). The hydroxybenzene derivatives (Scheme 2) stock solutions were prepared in CH_3OH with a concentration of 3.0×10^{-3} M for fluorescence spectral analysis. Each time a 3 mL solution of **1** was filled in a quartz cell of 1 cm optical path length, and we increased concentrations of hydroxybenzene derivatives by stepwise addition of different equivalents using a micro-syringe. An excitation wavelength of 300 nm and room temperature were employed in all experiments.



Scheme 2. Guests **8** used in this study.

2.6. UV–vis spectrophotometric titrations

To a solution of 1.0×10^{-5} M of **1** in THF-MeOH, a solution of 2-nitrophenol in MeOH was added. Spectra in the range of 450–200 nm were recorded at 8 different concentrations of the 2-nitrophenol in a range of 0–14 equiv. The absorbance of the 2-nitrophenol was monitored in 1.0×10^{-4} M THF/MeOH (9:1, v/v) at room temperature.

3. Results and discussion

3.1. Synthesis and structural characteristics of *1* and *2*

The synthesis of diethoxycarbonyl glycoluril and some of their intermediates have already been described [19,20,25]. We synthesized compounds **1** and **2** by Pd-catalyzed coupling reactions of phenylacetylene with **6** and **7**. Spectroscopic identification of the cis and trans diastereomers is based on a combination of ^{1}H and ^{13}C NMR spectroscopy and symmetry arguments [20]. The trans diastereomer **2** is *C*2-symmetric, while **1** is Cs-symmetric form. Because of these symmetry differences, for **1** we observe a pair of doublets for the carbonyl groups of glycoluril ring (156.3 and 155.5 ppm), whereas for **2** we observe a singlet (155.8 ppm) for the chemically equivalent ureidyl C=O carbon atoms in ^{13}C NMR spectroscopy.

X-ray crystallography of our compounds, as shown in Figs. 1 and 2, has corroborated symmetry arguments described above. The C=O···O=C distances of the ureidyl C=O groups in the crystal of compound **1** (Fig. 1) is 5.65 Å, the *o*-xylylene walls define a tapered cavity, the walls at an angle of 41.11°, with the centers of the benzene rings 6.53 Å apart. The dimensions of this tapered cavity for **2** (Fig. 2) are somewhat smaller than those observed for **1**. In compound **2**, the distance between the ureidyl C=O oxygen atoms is 5.56 Å, the dihedral angle between the two phenyl rings of the sidewalls is 38.11° and the distance between the centroids of *o*-xylylene is 6.49 Å. These geometrical features of **1** and **2** were ideal to engage in π–π interactions and H-bonds with flat aromatic guest molecules [26].

3.2. Spectral characteristics

As shown in Fig. 3, compound **1** exhibited a strong absorption band centered at 300 nm in neutral THF solution, whereas the compound **2** showed a similar characteristic absorption peak. The high-energy peak around 300 nm was expected to $\pi \rightarrow \pi^{*}$ electronic transitions.

The fluorescence emission spectrum of chemosensor **1** consists of two peaks centered at 406 nm and 432 nm. It is found a distinct difference of fluorescence spectra of the compound **1** and compound **2** in the FL intensity. Although the fluorescence profile of compound **2** has peaks at the same wavelengths as that observed from compound **1**, which owns the same luminescent fragments, the **2** has higher fluorescence intensity.

The fluorescent quantum yield of diastereomers was measured by comparing with quinine sulphate as the standard compound in sulphuric acid according to the following equation [27,28]:

$$\Phi_u = \frac{\Phi_s \times A_s \times F_u \times n^2}{A_u \times F_s \times n_0^2}$$

where Φ_u and Φ_s are quantum yield for the sample and reference, F_u and F_s are the integrated area under the corrected fluorescence spectra for the sample and reference, A_u and A_s are the absorbance for the sample and reference, n and n_0 are the refractive indexes of the solvents used for samples and reference. The quantum yield for compound **1** (Φ = 0.20) was a little higher than that observed for compound **2** (Φ = 0.18).

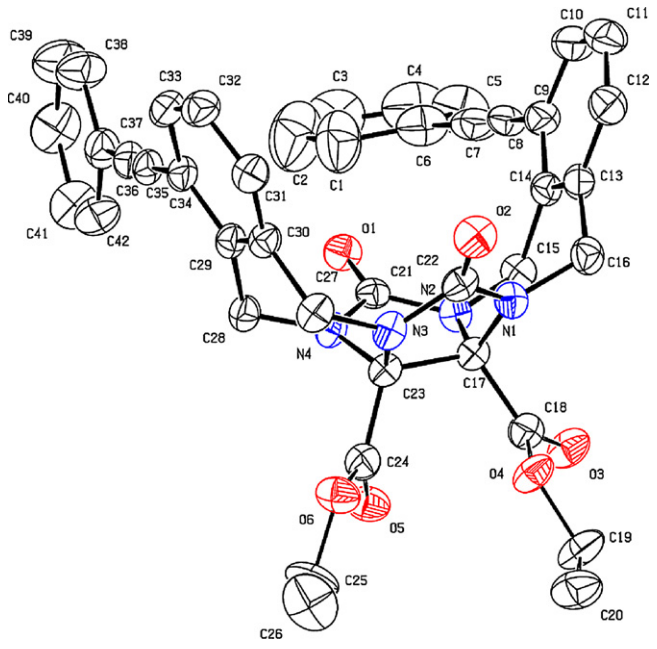


Fig. 1. ORTEP figure of **1**. Hydrogen atoms and solvent molecules omitted for clarity.

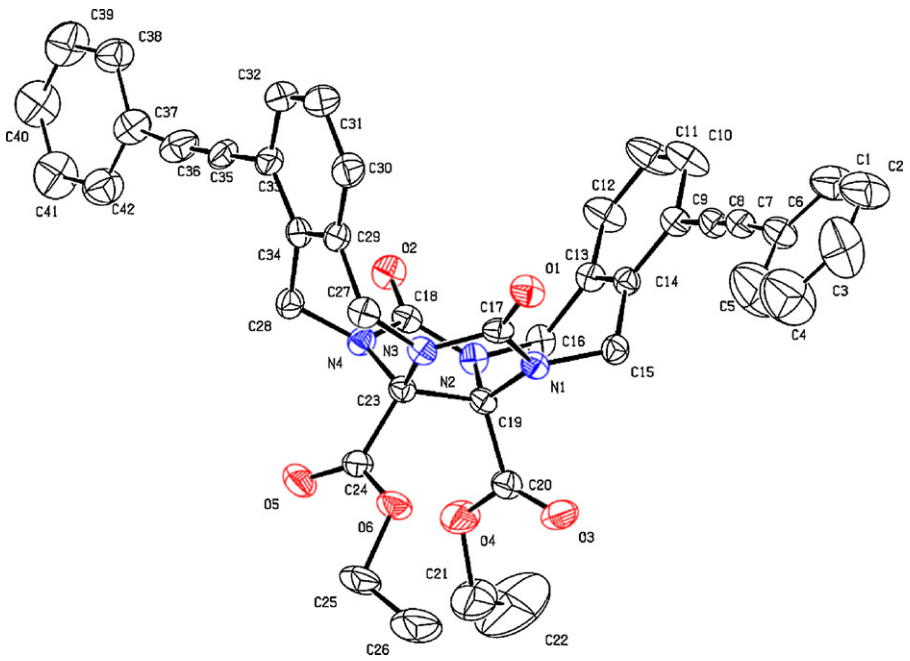


Fig. 2. ORTEP figure of **2**. Hydrogen atoms and solvent molecules omitted for clarity.

3.3. Selectivity

The titration of molecular clips with phenols including hydroquinone, pyrocatechol, 4-nitrophenol, resorcinol, benzene-1, 3, 5-triol, 2, 3-dimethylbenzene-1, 4-diol, 5-methylbenzene-1, 3-diol, 4-bromophenol, 4-chlorobenzene-1, 3-diol, 2-methylbenzene-1, 3-diol, 2-nitrophenol was conducted to examine the selectivity. As summarized in Fig. 4 and Fig. S1, the fluorescence of **1** and **2** around 430 nm was not influenced by **8a**–**j**, which exists even at as high a concentration as 16 equiv. Under such conditions, 2-nitrophenol (**8k**) greatly quenched the emissions (Fig. 4, Fig. S1). It is of particular interest that **1** and **2** did not sense 4-nitrophenol, though 4-nitrophenol would compete with 2-nitrophenol. The relatively strong quenching by 2-nitrophenol comparing to other phenols seems to be related to more electron deficiency.

3.4. Analytical figures of merit

In order to estimate the specific concentration for selective 2-nitrophenol, the fluorescence spectra of **1** and **2** in the presence of different concentrations of 2-nitrophenol were measured. A characteristic fluorescence emission maximum centered at about 430 nm was recorded and the fluorescence intensity of the **1** and **2** were significantly quenched with increase of the 2-nitrophenol concentration (Fig. 5, Fig. S2).

To know the stoichiometry between the quencher (**8k**) and acceptor (**1** or **2**) molecule in THF-MeOH (9:1) solution, Job's plot (insets of Fig. 5, Fig. S2) has been drawn. It shows the maxima at 0.5 molar fraction for 1:1 stoichiometry between the two interacting species [29].

Stern–Volmer plots are a useful method of presenting data on emission quenching. The nature of the quenching process in quencher and acceptor was probed by Stern–Volmer analysis [30–32]. Based on the fluorescence titration of **1** and **2** in THF-MeOH with 2-nitrophenol, their Stern–Volmer constants (K_{sv}) are determined to be $(5.75 \pm 0.19) \times 10^4\ M^{-1}$ and $(2.34 \pm 0.006) \times 10^4\ M^{-1}$, respectively. A practically usable range for quantitative determination of **1** covers a range from 2.4×10^{-6} M to 3.2×10^{-4} M

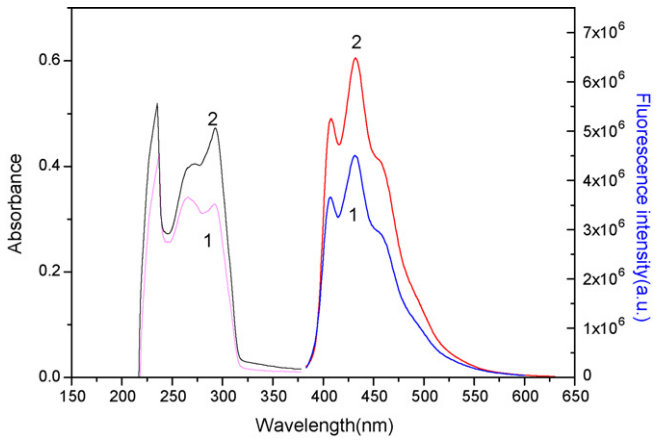


Fig. 3. UV–vis absorption and emission spectra of **1** and **2** at an excitation wavelength of 300 nm.

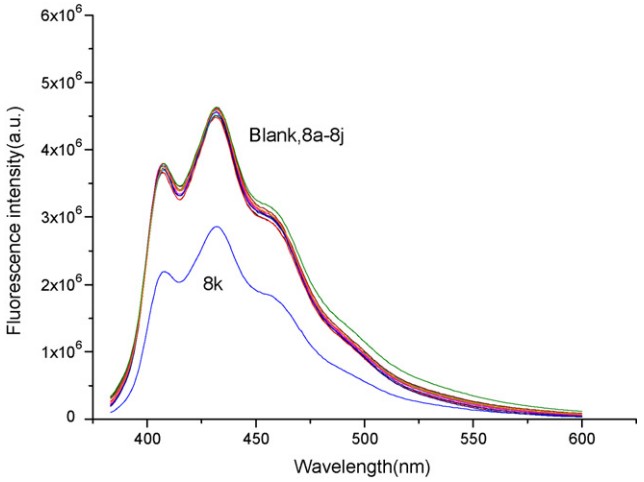


Fig. 4. Fluorescence emission changes of molecule **1** (1.0×10^{-5} M) in THF/MeOH (9:1, v/v) in the presence of 16 equiv. various phenols (excitation at 300 nm).

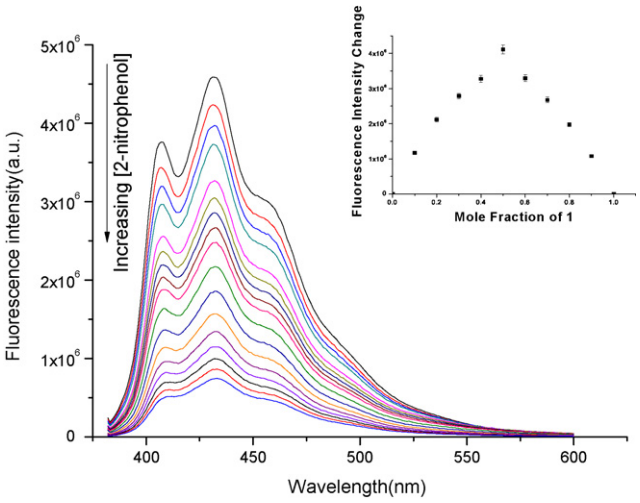


Fig. 5. Fluorescence emission spectra (excitation at 300 nm) of **1** (1.0×10^{-5} M) in THF/MeOH (9:1, v/v) in the presence of 0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 18.0, 22.0, 26.0, 30.0, 34.0, 38.0, 42.0, 46.0, and 50.0 equiv of 2-nitrophenol predissolved in MeOH. Inset: Job plot for mixtures of **1** and **8k** ([**1**] + [**8k**] = 2.0×10^{-5} M).

(correlation coefficient $R^2 = 0.9905$). The fluorescence decrease of **2** is linear with 2-nitrophenol concentration within the concentration range from 2.9×10^{-6} M to 4.0×10^{-4} M (correlation coefficient $R^2 = 0.9919$). The detection limits of **1** and **2**, based on the definition by IUPAC [33], were 4.7×10^{-7} M and 5.7×10^{-7} M from ten blank solutions. The detection limits of this work are lower than fluorescence sensors reported earlier by Yu (Table 1).

3.5. Quenching mechanism of 2-nitrophenol

In contrast to the typical Stern-Volmer quenching behavior driven by a collision between quencher and luminescent molecules, the fluorescence quenching of the hosts and guests is attributed to the complex formation between electron deficient guest 2-nitrophenol molecules (**8k**) and molecular clips **1** and **2** as shown in Fig. 6. As the Job's plot indicated, the stoichiometric ratio of guests to hosts is proved to be 1:1. Some published relative studies also indicate the quenching mechanism by ^{1}H NMR spectra [22].

Table 1
Summary of reported determination used for the 2-nitrophenol.

Determination	Detection limits	Ref.
HPLC	0.54 μg L^{-1}	[34]
HPLC/MS	55 ng L^{-1}	[11]
Fluorescence	8×10^{-5} mol L^{-1}	[35]
Fluorescence	2×10^{-6} mol L^{-1}	[36]
This paper	4.7×10^{-7} mol L^{-1} [a]	
	5.7×10^{-7} mol L^{-1} [b]	

[a] Detection limit for the senor **1**.
[b] Detection limit for the senor **2**.

From the view of signaling, the phenol signaling mechanism for the present systems is considered as the PET process [37,38]. When the 2-nitrophenol is added into solution, they can be embedded into the cavities of fluorescence probe **1** or **2** and the formation of the **1**·8k and **2**·8k complexes result in electron transfer from the excited state of the phenylethynyl-conjugated sidewalls of **1** and **2** to the aromatic ring of electron-poor 2-nitrophenol, then fluorescence is quenched. This conjecture has been supported by a UV–vis spectral study: the absorption spectrum change of compound **1** induced by the addition of phenol is shown in Fig. 7. The addition of 2-nitrophenol caused the wavelength at 230 nm blue shifted for the decrease of electron density of **1**. The concomitant high increase in the absorption intensity is relative to the formation of host-guest complexes which benefit from Ar–OH···O=C H-bonds, π–π stacking interactions, and a cavity effect [18]. The UV–vis spectral study indicates that the direct interaction between 2-nitrophenol and host **1** is weak.

3.6. Interferences study

To examine the selectivity of the 2-nitrophenol sensors, responses to potential interferents such as 4-nitrophenol and other phenols were recorded for their possible cooccurrence with 2-nitrophenol in the real world under the conditions selected above [39,40]. The results show, as expected, that the 4-nitrophenol and diphenols do not interfere with the 2-nitrophenol signals with deviations below 5%(Table 2, S1). As can be seen, the compound **1** and **2** can be used even under complex conditions involving an increase of other possibly coexisting substances without remarkable interference for the determination of 2-nitrophenol.

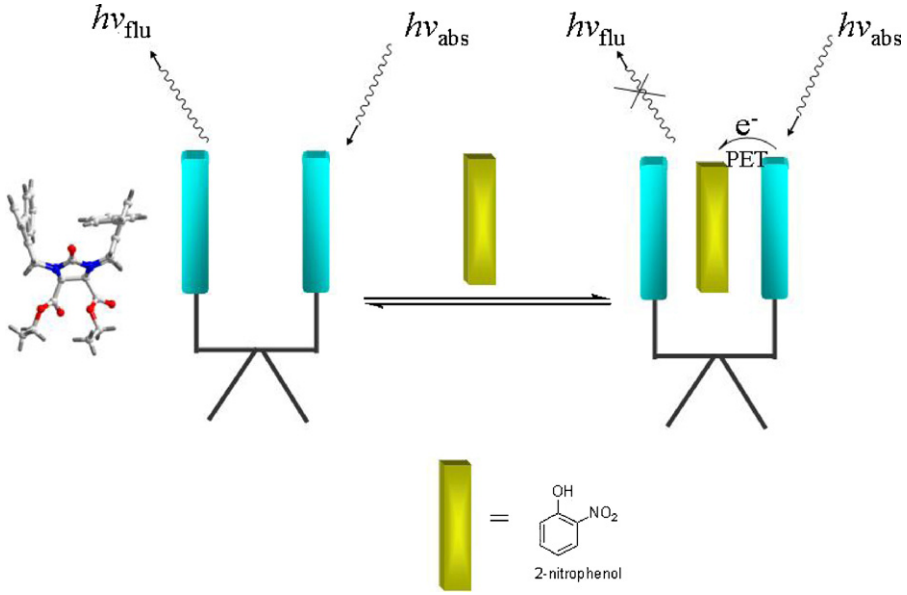


Fig. 6. Schematic illustration of a possible formation mechanism of **1**·**8k** complex.

Table 2
Results of the analysis of prepared samples by sensor 1(n = 5).

Sample	4-Nitrophenol added (10^{-5} mol L^{-1})	2-Nitrophenol added (10^{-5} mol L^{-1})	Fluorescence change value $\Delta F = (F_2 - F_1)$[c]	Deviations (%) $(\Delta F/F_1) \times 100$
Sample 1[a]	1	1	−0.04	−0.19
	1	3.5	−0.16	−0.76
	5	1	−0.61	−2.90
	5	3.5	−0.46	−2.20
Sample 2[b]	1	1	−0.03	−0.14
	1	3.5	−0.57	−2.72
	50	1	−0.59	−2.80
	50	3.5	−0.52	−2.48

[a] Synthetic samples containing other phenols ($\times 10^{-5}$ mol L^{-1}):resorcinol 1; pyrocatechol 1; hydroquinone 1.
[b] Synthetic samples containing other phenols($\times 10^{-4}$ mol L^{-1}):resorcinol 1; pyrocatechol 1; hydroquinone 1.
[c] F_1 and F_2 are the fluorescence intensities of the sensor **1** contacted with 2-nitrophenol solution without and with the interferents, respectively.

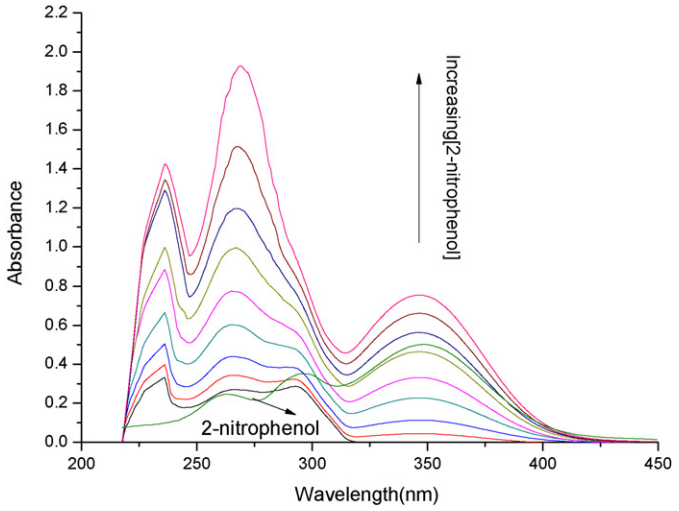


Fig. 7. Changes in UV–vis spectra for the receptor **1** (1.0×10^{-5} M) in THF/MeOH (9:1, v/v) solution in the presence of 0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 14.0 equiv of 2-nitrophenol predissolved in MeOH and UV–vis spectra of the 2-nitrophenol (1.0×10^{-4} M) in THF/MeOH (9:1, v/v).

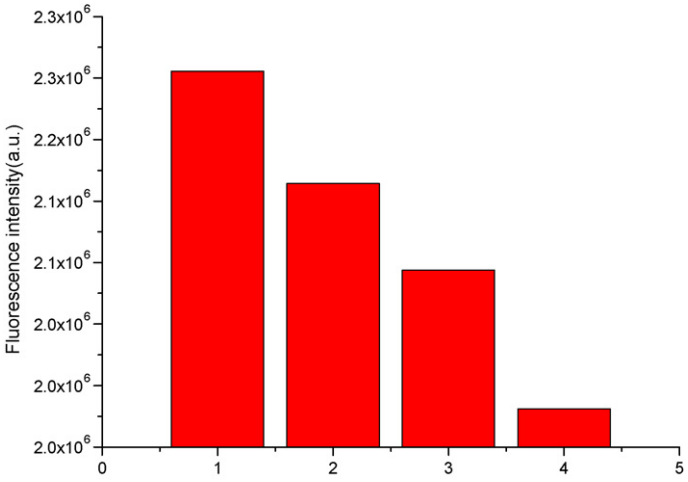


Fig. 8. The proof of qualitative analysis for 2-nitrophenol in THF-MeOH-Lake water. Fluorescence intensity changes of **1** (1.0×10^{-5} M) upon addition of 2-nitrophenol. (1) **1** in THF-MeOH-Lake water; (2) **1** + 1.0 equiv of 2-nitrophenol in THF-MeOH-Lake water; (3) **1** + 3.0 equiv of 2-nitrophenol in THF-MeOH-Lake water; (4) **1** + 8.0 equiv of 2-nitrophenol in THF-MeOH-Lake water.

3.7. Application of the method

The proposed probes were evaluated for the determination of 2-nitrophenol in lake water and synthetic samples. The lake water samples were simply filtered and no 2-nitrophenol signal was observed for them by reported method [41]. For a general test, 5.00 mL of a sample solution and 95.00 mL THF-MeOH (9:1) solution was transferred to a 250 mL conical flask. Then 1 mL of 1.00×10^{-3} M compound **1** or **2** stock solution was added and sonicated for 5 min. After that, 3.0 mL of this solution was pipetted into a 1 cm cell and the fluorescence measurement was carried out by excitation/emission at 300 nm. The sensors were applied to lake water samples spiked with 2-nitrophenol at a certain concentration. The results were shown in Fig. 8 and Fig. S3. It was shown that the signals from the 2-nitrophenol added water solutions (2, 3 and 4) were weaker than that from no additive one (1). The fluorescent quench phenomenon in water solutions also depends on 2-nitrophenol, which is similar to that observed in the THF-MeOH. Thus, the sensors **1** and **2** could be useful for the qualitative analysis of 2-nitrophenol in real water samples.

4. Conclusion

In conclusion, compound **1** and **2** were designed and synthesized with a view to developing new fluorescent sensors for phenols by making use of the non-covalence binding of ureidyl C=O with phenol to enhance the communication between the host and the guest components. The crystal structure of hosts **1** and **2** revealed that the similar cavities do not influence the guest binding selectivity. Fluorescent spectral results clearly indicate that diastereomers **1** and **2** can be used as fluorescent probes for 2-nitrophenol with good selectivity and sensitivity in the THF-MeOH. The insolubility of synthesized sensors **1** and **2** in water is a clear limitation for quantitative analysis of environmental water samples. However, modification of the sidewall structure in the diethoxycarbonyl glycoluril derivatives, by adding different hydrophilic groups, is already progressing in our laboratory. Further studies include the design of new analogues of **1** and **2** with good solubility in water, which will enable the practical application of these types of phenol sensors to be implemented.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.03.018.

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