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Synthesis of Coumarin-Pillar[5]arene as a Selective Fluorescent Probe for Methyl-Parathion

Fan Zhang, Xianliang Cao, Demei Tian,* and Haibing Li*

Key Laboratory of Pesticide and Chemical Biology of the Ministry of Education, *College of Chemistry*, *Central China Normal University*, *Wuhan, Hubei 430079*, *China*

A novel coumarin-pillar[5]arene derivative (**P5C10**) was prepared. Fluorescence spectroscopy indicates that **P5C10** shows good selectivity towards methyl-parathion. A combination of NMR spectra, MALDI-TOF spectra and computational calculations reveals the formation of a host-guest complex driven by π-π stacking interactions.

Keywords fluorescent probe, pillar[5]arene, methyl-parathion, host-guest

Introduction

Methyl-parathion is a highly toxic pesticide and widely used as an insecticide and acaricide on agricultural crops.[1] Due to improper use of pesticides, it has caused serious problems in the environment and food safety, which has posed a severe threat to human health.[2] Therefore, the development of efficient detection of methyl-parathion is in urgent demand. To meet this requirement, a large number of methyl-parathion detection methods have been developed, such as chromatography,[3-5] acetylcholinesterase biosensor,[6] fluorescence polarization immunoassay (FPIA)[7] and so forth. Among these methods of detecting methyl-parathion, the fluorescent chemosensor detection is the most widely applied due to its high sensitivity and efficiency. To our knowledge, design and synthesis of high efficient and sensitive fluorescent sensors for the selective recognition of methyl-parathion remains a challenging task, and it is also the focus of our current study.

Pillararenes as the new generation of macrocyclic host molecules in supramolecular chemistry have drawn considerable attention during the past few years.[8-10] With good host-guest properties, pillararene can further self-assemble and be applied in the artificial transmembrane proton channels, fluorescent sensors, or other functional materials.[11-14] Huang *et. al*.[15] have reported the pillar[5]arene can recognize paraquat derivatives by the cation-π-electron interactions. For the highly symmetrical and rigid structure, and being easier to functionalize with different substituents on all of the benzene rings,[16-20] we can introduce the special fluorophore to afford high selective binding to guests.

Herein, in this paper we design and synthesize a novel coumarin-pillar[5]arene (**P5C10**) as a selective fluorescent probe for methyl-parathion (**3d**). By introducing the coumarin moieties with high fluorescence quantum yield to the pillar[5]arene via alkyl chain,[21] we can get the fluorescent pillar[5]arene derivative (**P5C10**), which shows good selectivity and sensitivity to methyl-parathion on the fluorescence spectra. The prepared **P5C10** was characterized by ^{1}H NMR, MS, and elemental analysis. The fluorescence of **P5C10** was strongly quenched by methyl-parathion, which indicated that receptor **P5C10** can serve as a sensor for methyl-parathion.

Experimental

Apparatus and reagents

^{1}H NMR spectra were recorded on Varian Mercury Plus600 instrument at ambient temperature with TMS as the internal standard. Chemical shifts are expressed in parts per million and *J* values are given in hertz. MALD I-TOF-MS were recorded on matrix assisted laser desorption ionization/time of flight MS. Melting point was recorded on METTLER TOLEDO FP62 instrument. The absorption spectra were recorded on UV-2501. All fluorescence measurements were carried out on a Cary Eclipse FL10 08M018 instrument. Computational calculations were recorded at HF/6-31G levels using Gaussian 03. The guests are pesticide of methomyl, carbofuran, isoprocarb, methyl-parathion, tsumacide, iprodione, dichlorvos, profenofos, malathion, purchased from Germany Dr. Ehrenstorfer Company. All chemicals were A.R. grade and purified by standard procedures.

Synthesis of P5C10

As shown in Scheme 1, the **P5C10** was synthesized in two steps. Compounds **1** and **2** were prepared ac-

* E-mail: hbing@mail.ccnu.edu.cn
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cording to the literature reported.[22] To a solution of monomer **1** (1.9 g, 5 mmol) in 1,2-dichloroethane (35 mL) was added paraformaldehyde (0.45 g, 15 mmol) under nitrogen atmosphere. Then boron trifluoride diethyl etherate (BF_3•$(OC_2H_5)_2$, 0.65 mL, 5 mmol) was added to the solution and the mixture was stirred at room temperature for 30 min. Methanol (20 mL) was poured into the reaction mixture and the solution was concentrated and dissolved in dichloromethane (60 mL). After the solvent was removed, the obtained solid was purified by column chromatography on silica gel with petroleum ether/dichloromethane (V∶V=1∶1) as the eluent to get a white powder **2**, 1.3 g, yield 66.1%. ^{1}H NMR ($CDCl_3$, 600 MHz, TMS) δ: 6.87 (s, 10H), 3.96 (s, 20H), 3.75 (s, 10H), 3.51 (s, 20H), 2.13−2.10 (m, 20H), 2.00−1.95 (m, 20H); MALDI-TOF-MS calcd for *m*/*z*: 1960.6, found: *m*/*z* 1961.6 $[M+H]^+$. Anal. calcd for $C_{75}H_{100}Br_{10}O_{10}$: C 45.92, H 5.10, Br 40.82; found C 45.70, H 5.33, Br 40.65.

Compound **2** (0.2 g, 0.1 mmol) and anhydrous potassium carbonate (0.22 g, 1.6 mmol) were stirred in acetone for 30 min, then coumarins (0.27 g, 1.5mmol) was added. The mixture was refluxed for 12 h, then cool to room temperature, the product was collected by vacuum filtration, with 30 mL dichloromethane dissolved, thoroughly washed with saturated salt water (50 mL) and pure water. After removing the aqueous layer, the organic layer was collected, and dried over anhydrous Na_2SO_4, then purified by column chromatography with methylene chloride/methanol (V∶V=100∶2) as eluent, giving **P5C10** (1.5 g, yield 51.7%). m.p. 129.6−131.4 ℃; ^{1}H NMR ($CDCl_3$, 600 MHz, TMS) δ: 7.43 (d, J=8.9 Hz, 10H), 6.90 (s, 10H), 6.79 (d, J=8.7 Hz, 10H), 6.65 (s, 10H), 6.06 (s, 10H), 4.08 (s, 10H), 4.02 (s, 20H), 3.91 (s, 10H), 3.79 (s, 10H), 2.34 (s, 30H), 2.03 (d, J=24.6 Hz, 40H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.95, 161.36, 155.08, 152.41, 149.75, 128.43, 125.54, 114.93, 113.64, 112.87, 111.80, 101.24, 77.27, 76.95, 76.63, 68.19, 29.45, 26.34, 26.03, 25.35, 18.46. MALDI-TOF-MS calcd for *m*/*z*: 2914.1, found *m*/*z*: 2937.0, $[M+Na]^+$. Anal. calcd for $C_{175}H_{170}O_{40}$: C 72.16, H 5.84; found C 72.38, H 5.70.

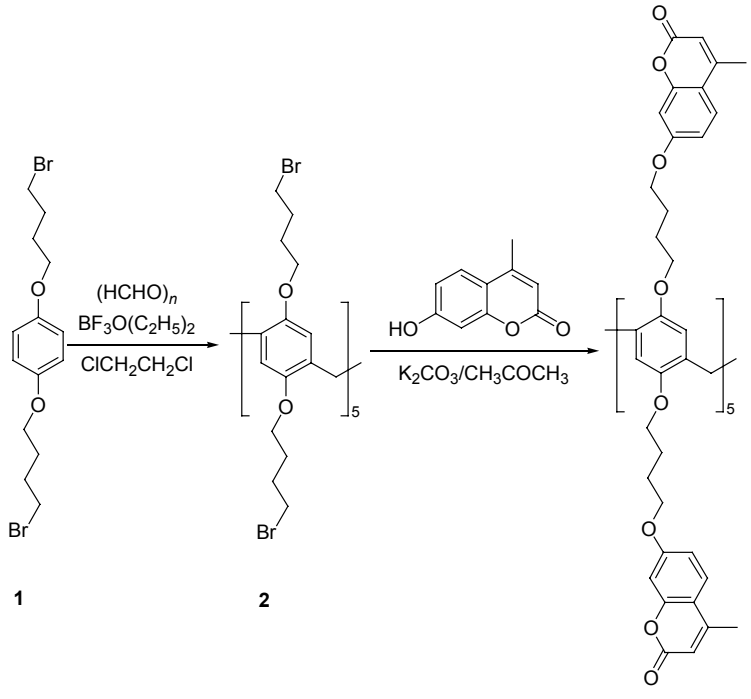
General procedure of fluorescent detection

P5C10 was dissolved in DMSO as the stock solution with a concentration of 5.0×10^{-6} mol/L for UV-vis spectroscopy and fluorescent spectroscopy analysis. And the pesticides (**3a**−**3i**) were dissolved in acetonitrile to prepare stock solutions with a concentration of 8.0×10^{-5} mol/L. The fluorescent spectra were recorded on Cary Eclipse FL1008 M018.

Results and Discussion

The molecular recognition behavior of the **P5C10** was studied toward pesticides **3a**−**3i** by fluorescence spectroscopy. Figure 1 shows the fluorescence response of P5C10 to 16 equiv. of pesticides (**3a**−**3i**), including methomyl, carbofuran, isoprocarb, methylparathion, tsumacide, iprodione, dichlorvos, profenofos and malathion. With the 310 nm excitation wavelength, there is a significant fluorescence quench of **P5C10** upon addition of **3d**, but other organic pesticides have little effects on the fluorescence (Figure 1b). To better elucidate the selective recognition of pesticide compounds, column chart showed the variation of the fluorescence quenching intensity at 391 nm (Figure 1c). When **3d** was added to the system, the intensity of fluorescence quenching

Scheme 1 Synthetic route to **P5C10**



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was 85% of the original. The results indicated that **P5C10** could be employed to distinguish **3d** from the other pesticide compounds by its fluorescence "on-off".

The fluorescence spectra of **P5C10** (5×10^{-6} mol/L) at increasing concentrations of **3d** are depicted in Figure 2. It was found that while no shift in the fluorescence maximum was observed, the fluorescence intensity of **P5C10** decreased with increasing concentrations of **3d**.

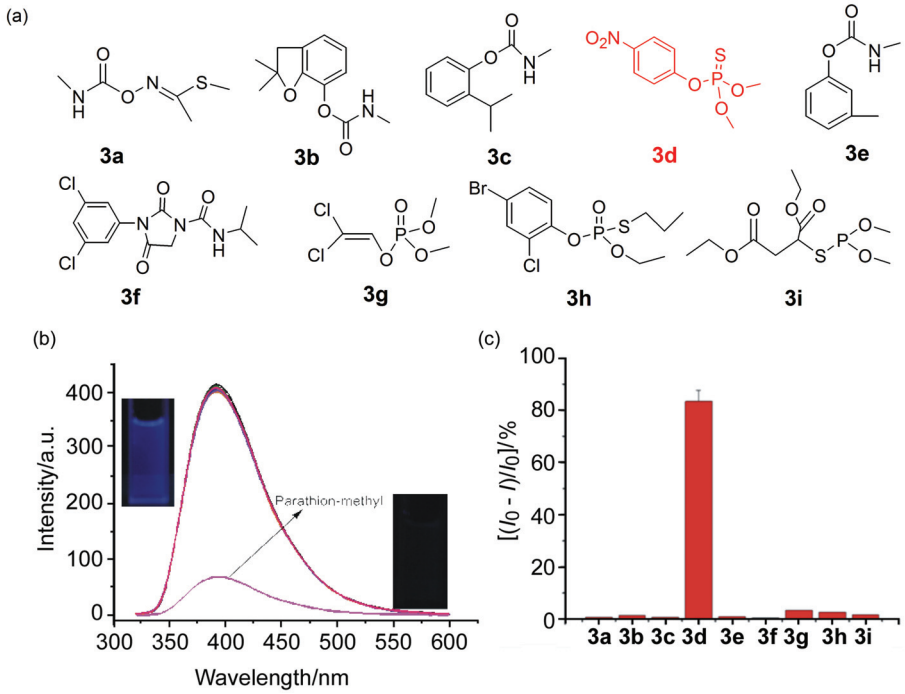

Figure 1 (a) The structures of the guests. (b) Fluorescence intensity changes for **P5C10** (5×10^{-6} mol/L) in CH_3CN/DMSO ($V/V=1/1$) upon addition of **3a**—**3i** (8×10^{-5} mol/L). (c) $(I_0–I)/I_0$ ($\lambda_{em}=391$ nm). I_0 is the fluorescent emission intensity of the host, and I is the fluorescent intensity after adding **3a**—**3i**. It shows that **P5C10** appeared an obvious selectivity of **3d**.

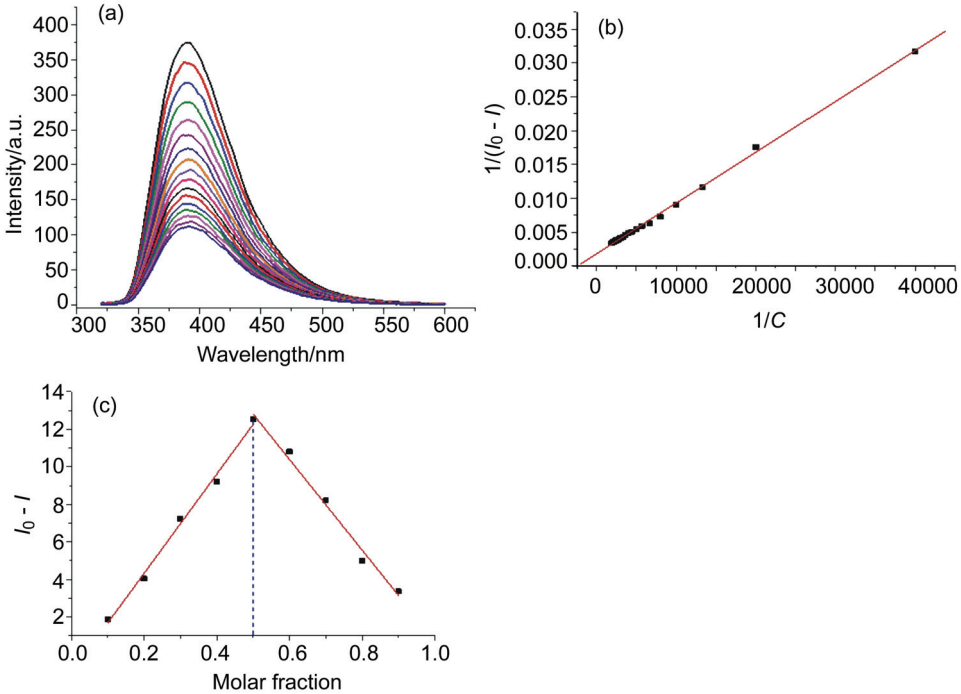

Figure 2 (a) Fluorescence spectra titration of **P5C10** (5×10^{-6} mol/L) with various equivalents of **3d** (0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52 equiv., $\lambda_{ex}=310$ nm). (b) Benesi-Hildebrand analysis of the fluorescence changes for the complexation between **P5C10** and **3d**. (c) Job's plots of **P5C10** toward **3d** at an invariant total concentration of 1×10^{-5} mol/L ($\lambda_{em}=391$ nm, slit=5). It illustrates the host-guest fluorescence quenching occurs in 1∶1 complexation.

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Simultaneously, the association constant of **P5C10** for **3d** was evaluated using the Benesi-Hildebrand equation and was found to be 2.38×10^4 L/mol, which indicated an important feature of **P5C10** is the high binding affinity toward **3d** over other pesticides. In the Job plot, a maximum fluorescence change was observed when the molar fraction of **P5C10** versus **3d** was 0.5, indicating 1∶1 complexation (Figure 2). Meanwhile, the computational calculations revealed the formation of a host-guest complex driven by π-π stacking interactions which formed a 1∶1 inclusion complex.

To seek more detailed information on the binding properties of **P5C10** with **3d**, the ^{1}H NMR spectra of mixtures of **P5C10** and **3d** were investigated as depicted in Figure 3. Because of the reciprocity, the signals from the aromatic ring protons of **3d** shifted upfield (H_a, δ 0.02; H_b, δ 0.01) and the signals of the protons on the benzene ring skeleton of pillar[5]arene (**P5C10**) were downfield shifted (ArH, δ 0.01). Possible mechanism of host-guest interaction was proposed in Figure 4, and the computational calculations were carried out at the HF/6-31G (d) levels using Gaussian 03.[23] It shows that due to π-π stacking interactions between **3d** and **P5C10**, the electron-poor compound of methyl-parathion penetrated into the rich-electron cavity of **P5C10**, which causes the fluorescence quenching for the electron transfer.

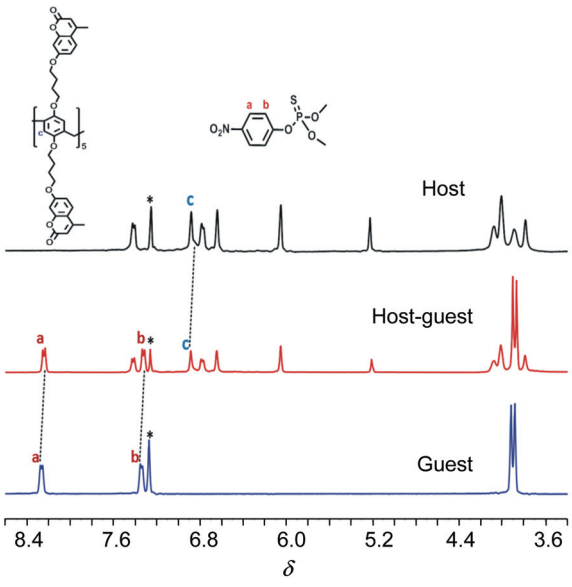

Figure 3 ^{1}H NMR spectra ($CDCl_3$, 600 MHz, 298 K) of (a) **P5C10** (10 mmol/L), (b) **P5C10** and **3d** (10 mmol/L each), and (c) **3d** (10 mmol/L).

Conclusions

In conclusion, a new fluorescent **P5C10** has been synthesized by Williamson reaction. It exhibits high selectivity toward methyl-parathion (**3d**) in the fluorescence studies. Job's plots showed a 1∶1 stoichiometry complexation between **P5C10** and **3d**. ^{1}H NMR and computational calculation shows that the electron-poor aromatic guest was included in the π-rich cavity of the host pillar[5]arene within a 1∶1 complex mode, which induces the fluorescence quenching. The **P5C10** shows excellent selectivity and sensitivity to methyl-parathion, which can be developed as pesticide-detecting sensor in our life.

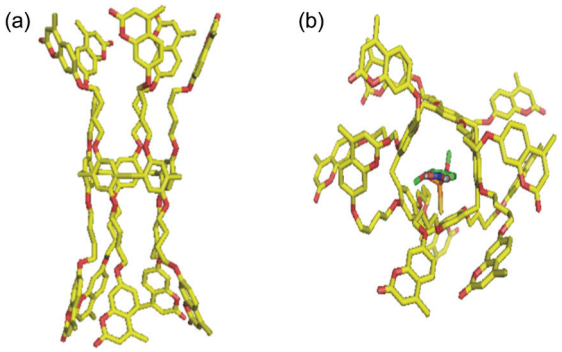

Figure 4 The possible complexation mode of host-guest.

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Supporting Information

Available experimental details, MALDI-TOF-MS, NMR spectra of all of the components, fluorescence spectra and other data mentioned in above paragraphs.

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