Supporting Information

Synthesis of pillar[5]arene functionalized graphene as a fluorescent probe for paraquat in living cells and mice

Xiaowei Mao,† Ting Liu,† Jiahai Bi, Li Luo, Demei Tian, and Haibing Li*

Key Laboratory of Pesticide and Chemical Biology (CCNU), Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, (P.R. China)
E-mail: lhbing@mail.ccnu.edu.cn
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**Chemical reagent:** graphite powder (99%, Sinopharm Chemical Reagent co., Ltd), 1-ethyl-(3-dimethyl amino propyl) carbodiimide hydrochloride (EDC·HCL,99%, Fluka), N-hydroxysuccinimide (NHS, 99%) were purchased from Shanghai covalent chemical science and technology limited Company, paraquat and analogs(99%, Sinopharm Chemical Reagent co., Ltd) and these commonly used solvent, secondary distilled water used for experiment were all prepared on the lab own.

**Laboratory apparatus:** 1H NMR spectra was recorded on a Bruker Advance DMX-400 spectrometer. Transmission electron microscopy (TEM) investigations were carried out on a JEM-1200EX instrument. The fluorescence experiments were conducted on a RF-5301 spectrofluorophotometer (Shimadzu Corporation, Japan). Thermal gravimetric analysis (TGA) was recorded on PerkinElmer Pyris 1 instrument.
The synthesis of 4-methoxy-propargyloxy benzene 1

A mixture of methoxy phenol 7.44 g (60 mmol), 120 mL anhydrous acetonitrile, 11.04 g (80 mmol) K₂CO₃ was stirred to reflux for 30 min, then adding 3-bromine propiolic 4.7 mL (60 mmol), reacting for 10 h under 90 ℃, then cooling down to room temperature and removing acetonitrile, adding in saturated salt water, using the chloroform extraction 3 times and combined organic phase, washing with 10% NaOH solution 3 times and then water is used for washing to neutral, drying with Na₂SO₄, after filtration, spin dry, gaining the brown solid product 8.4 g the yield was 87%. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 6.94-6.83 (m, 4H), 4.65 (s, 2H), 3.77 (s, 3H), 2.51 (t, \( J = 6.0 \) Hz, 1H). Anal.caled for C₁₀H₁₀O₂: C, 74.06%; H, 6.21%; found: C, 74.00%; H, 6.28%. The anytical data of pillar[5]arene 2 is same as the literature.¹²
Figure S1 $^1$H NMR spectrum of the 4-methoxy-propargyloxy benzene 1
The synthesis of alkynyl pillar[5]arene 2

A mixture of 4-methoxy-propargyloxy benzene 650 mg (4 mmol), paraformaldehyde 240 mg (8 mmol), boron trifluoride diethyl etherate 0.75 mL (6 mmol), 1,2-dichloroethane 80 mL was stirred in N₂ at room temperature for 3 h. Then adding the methanol to stop the reaction, and taking evaporation of the solvent, dichloromethane dissolve the residues, vacuum suction filtration. H₂O and 5% Na₂CO₃ are used to wash the filtrate 3 times, respectively. Then dry for column chromatography with the petroleum ether and ethyl acetate (PE:EA) ratio 5:1 to gain the product 469 mg, as the yield is 67.4%. ¹H NMR (400 MHz, CDCl₃) δ 7.02 – 6.80 (m, 10H), 4.66 – 4.43 (m, 10H), 4.02 (s, 10H), 3.96 (s, 6H), 3.94 (s, 9H), 2.56 – 2.31 (m, 5H). MALDI-TOF-MS Calcd. for m/z = 870.34, found: m/z = 893.416 [M+Na]⁺. Anal. calcd. for C₅₅H₅₀O₁₀: C, 75.84%; H, 5.79%; found: C, 75.80%; H, 5.75%. The analytical data of pillar[5]arene 2 is same as the literature.¹²
Figure S2  $^1$H NMR spectrum of the alkynyl pillar[5]arene 2
Figure S3  Mass spectrum of the alkynyl pillar[5]arene 2
The synthesis of ester-pillar[5]arene 3

A mixture of 10.4 mg (0.5 mmol) alkynyl pillar[5]arene, azide ethyl acetate 3.5mmol, CuSO$_4$·5H$_2$O 0.8g (3 mmol), NaVc 2.72g (15mmol) and 10 mL DMF was stirred at 80°C for 8h. After the reaction, cooled and crushed ice, add 5g with methylene chloride extraction 3 times, combination of organic phase, organic phase 3 times washed with water, drying, concentrating column. With EA:PE ratio 4:1 80mg white solid ester-pillar[5]arene 2 is gained. The yield is 10.6%.$^1$H NMR (400 MHz, DMSO-d$_6$) δ 8.49-8.14 (m, 5H), 7.17-6.61 (m, 10H), 5.43 (s, 5H), 5.34 (s, 5H), 5.00 (m, 5H), 4.85 (m, 5H), 4.15 (s, 5H), 4.11-3.98 (m, 5H), 3.83-3.46 (m, 25H), 1.38-0.91 (m, 15H). Anal.calcd for: C$_{77}$H$_{85}$N$_{15}$O$_{20}$: C, 59.40%; H, 5.65%; N, 13.85% found: C, 59.30%; H, 5.75%; N, 13.72%. The anytical data of pillar[5]arene 2 is same as the literature.$^{12}$
Fig S4  $^1$H NMR spectrum of the ester-pillar[5]arene 3
The synthesis of the graphene oxide

In all the methods of the preparation of graphene, graphene can be more simply, fast and abundantly prepared in aqueous solution though the chemical oxidation process. Therefore, the graphene oxide was prepared according to the report of Hummer, and the specific procedures are as follows:

pre-oxidation: a certain amount 325 mesh (size ≤45 nm) natural flake graphite was placed to be standby in the drying oven at 80 °C for 24 h. H₂SO₄ (30 mL), K₂S₂O₈ (10 g), P₂O₅ (10 g) were mixed in three-necked flask to be heated to 80 °C, and then 20 g the above dried natural graphite was added in. When the reactants changed to be blue-black, stopped heating, cooled down to room temperature and placed for 6 h. The mixture was washed with water, filtered until the filtrate was neutral. The product was dried at room temperature.

Oxidation procedure of Hummer: the above step air-dried product (2 g) and H₂SO₄ (92 mL) were mixed in three-necked flask, then added in KMnO₄ (12 g) at 0 °C slowly in water bath. The temperature was controlled to be no higher than 20 °C. After stirring for 15 min, NaNO₃ (2 g) was added in the reacting solution. The mixture was stirring for 2 h at room temperature, finally 200 mL distilled water was add in. This reaction was stopped by adding in 560 mL distilled water. When 10 mL H₂O₂ was added in, the color of the mixture turned from tan to bright yellow about 15 min. Then 1:10 HCl solution was used to wash and filter the above reactant to remove part of the metal ions. The graphene oxide washed with HCl was dialyzed in distilled water once every 2 h, until the filtrate tested with BaCl₂ and AgNO₃ solution resulting to be no SO₄²⁻ or Cl⁻. The dialytic product was placed in the centrifuge centrifuging with the speed of 4000 r.p.m for 40 min to remove a small amount of not oxidized graphite particles and gaining sticky and brown colloid, graphite oxide. Finally, a certain amount of graphite oxide was dispered in distilled water and stirred for 3 h. Then the dispersion system was placed in a 120 W ultrasonic machine for 3 h to flake the graphite flake oxide layer and get brown dispersion. The precipitate was removed
by centrifuging with the speed of 4000 r.p.m. for 40 min, which is the peeling graphite oxide, ultimately to gain the graphene oxide dispersion. There are large amounts of oxygen containing functional groups on the surface of the layer structure of graphene oxide in the dispersion, which make the lamella achieve good dispersion by electrostatic repulsion with each other. The surface of the graphene prepared on this method exists lots of oxygen-containing groups, which will be easy to be functionalized.
The synthesis of hydrazino-pillar[5]arene HP

A mixture of 0.117g (0.075mmol) ester-pillar[5] 2 and 0.19 mL (3 mmol) 85% hydrazine hydrate, 10 mL ethanol, 10mL toluene was stirred at room temperature for 6h. Then remove the solvent, adding chloroform to get the white solid precipitation, as the yield is 42.4%. $^1$H NMR (600 MHz, DMSO-D6) $\delta$9.54 (d, $J$ = 41.3 Hz, 5H), 8.30-8.09 (m, 5H), 7.09-6.72 (m, 15H), 5.04 (d, $J$ = 18.6Hz, 15H), 4.87 (d, $J$ = 32.4Hz, 5H), 4.58 (d, $J$ = 25.2 Hz, 5H), 4.39 (s, 10H), 3.64 (m, 15H). MALDI-TOF-MS Calcd for m/z = 1445.59, found: m/z =1446.64 [MH]$^+$. Anal.caled for C$_{65}$H$_{75}$N$_{25}$O$_{15}$: C, 53.97%; H, 5.23%; N,24.21% found: C, 53.87%; H, 5.21%; N, 24.32%.
Fig S5  $^1$H NMR spectrum of the hydrazino-pillar[5]arene HP
**Fig S6.** FT-IR spectra of the graphene (curve red), and HP-G (curve black).
Fig S7. Fluorescence spectra of safranine T ($10^{-4}$ M), and safranine T in the presence of different concentrations of HP-G (0–14 mg/mL). Successive fluorescence quenching was observed as the concentration of HP-G increased. B) The decrease in relative fluorescence intensity at the same HP-G/safranine T concentration, fitted according to the competitive binding model performed in a nonlinear manner according to spectrofluorometric titrations.
Fig S8. A) Fluorescence spectra of the HG/safranine T complexes via different concentrations of paraquat (0~86 μg/mL). Successive fluorescence recovery was observed as the concentration of paraquat increased. B) The increase in relative fluorescence intensity at the same HP-G/safranine T concentration, fitted according to the competitive binding model performed in a nonlinear manner according to spectrofluorometric titrations. It can be seen that the paraquat has stronger binding affinity with HP than that of safranine T.
Figure S9. Relative cell viability of Hela treated with HP-G. HeLa cells were incubated with different concentrations of HP-G for 24 h in fresh medium. With various concentrations of HP-G, the activity of cells is still in a high state, thus meaning the well biocompatibility of modified G.
Figure S10. Hela cells, incubated with HP-G/safranine T and then 1, or incubated with HP-G/safranine T and then 2, or incubated with HP-G/safranine T and then 3, or incubated with HP-G/safranine T and then 4, or incubated with HP-G/safranine T and then 5 was shown by confocal fluorescence microscopy images (bottom) and wide-field fluorescence images (top). However, after adding the pesticides, the fluorescence didn’t show any recover while the paraquat displays good fluorescence recovery.