Synthesis of a water-soluble pillar[9] arene and its pH-responsive binding to paraquat

Zhengtao Li,^a Jie Yang,^a Guocan Yu,^a Jiuming He,^b Zeper Abliz^b and Feihe Huang^{*a}

^a State Key Laboratory of Chemical Engineering, Department of Chemistry, Zhejiang University, Hangzhou 310027, P. R. China; Fax and Tel: +86-571-8795-3189; Email address: fhuang@zju.edu.cn.

^b Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, P. R. China

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1. Materials and methods

All reagents were commercially available and used as supplied without further purification. Solvents were either employed as purchased or dried according to procedures described in the literature. Compounds **DEP9**^{S1} and **G**^{S2} were synthesized according to published literature procedures. NMR spectra were recorded with a Bruker Avance DMX 400 spectrophotometer or a Bruker Avance DMX 500 spectrophotometer with the deuterated solvent as the lock and the residual solvent or TMS as the internal reference. Low-resolution electrospray ionization mass spectra (LRESI-MS) were obtained on a Bruker Esquire 3000 Plus spectrometer (Bruker-Franzen Analytik GmbH Bremen, Germany) equipped with an ESI interface and an ion trap analyzer. High-resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Bruker 7-Tesla FT-ICR mass spectrometer equipped with an electrospray source (Billerica, MA, USA). The melting points were collected on a SHPSIC WRS-2 automatic melting point apparatus. UV-vis spectroscopy was performed on a Shimadzu UV-2550 instrument at room temperature. The fluorescence experiments were conducted on a RF-5301 spectrofluorophotometer (Shimadzu Corporation, Japan).

2. Synthesis of compound WP9





2.1. Synthesis of compound 2



To a solution of **DEP9** (400 mg, 0.250 mmol) in dried chloroform (25 mL) was added boron tribromide (3.38 g, 13.5 mmol). The mixture was stirred at room temperature for 24 h. Then the mixture was added into water. The resulting precipitated product **1** was collected by filtration, washed with water and dried completely under vacuum (0.260 g, 96%). To a solution of **1** (0.260 g, 0.240 mmol) in DMF (30 mL) was added methyl chloroacetate (2.34 g, 21.6 mmol) and K₂CO₃ (1.20 g, 8.64 mmol). The mixture was heated in a flask at 100 °C under nitrogen gas protection for 2 d. Then the reaction mixture was cooled to room temperature and filtered. The filter cake was washed with chloroform (2 × 30 mL). Then the filtrate was concentrated under vacuum, and then the residue was purified by column chromatography on silica gel with dichloromethane/ethyl acetate (6:1 ν/ν) as the eluent to get product **2** as a white solid (110 mg, 19%), mp: 120.2–121.5 °C. The ¹H NMR spectrum of **2** is shown in Figure S1. ¹H NMR (400 MHz, chloroform-*d*, 293 K) δ (ppm): 6.89 (s, 18H), 4.51 (s, 36H), 3.87 (s, 18H), 3.64 (s, 54H). The ¹³C NMR spectrum of **2** is shown in Figure S2. ¹³C NMR (100 MHz, chloroform-*d*, 293 K) δ (ppm): 169.77, 149.75, 128.12, 114.88, 65.75, 51.77 and 30.05. LRESIMS is shown in Figure S3: *m/z* 2413.7 [M + H₂O + H]⁺. HRESIMS: *m/z* calcd for [M + H₂O + H]⁺ C₁₁₇H₁₂₉O₅₅⁺, 2413.72973; found 2413.73111; error 1 ppm.



Figure S1. ¹H NMR spectrum (400 MHz, chloroform-*d*, 293K) of **2**.



Figure S2. ¹³C NMR spectrum (100 MHz, chloroform-*d*, 293K) of **2**.



Figure S3. Electrospray ionization mass spectrum of **2**. Assignment of the main peak: m/z 2413.7 [M + H₂O + H]⁺.

2.2. Synthesis of compound 3



A solution of **2** (110 mg, 0.0460 mmol) in anhydrous ethyl alcohol (20 mL) was treated with 40% aqueous sodium hydroxide (20 mL) at 67 °C for 12 h. Then the reaction mixture was evaporated under vacuum, diluted with water (15 mL) and acidified with aqueous HCl solution. The resulting precipitate was filtered, washed with water and dried to afford product **3** (86.0 mg, 88%) as a white powder, mp: 215.3–216.2 °C. The ¹H NMR spectrum of **3** is shown in Figure S4. ¹H NMR (400 MHz, DMSO-*d*₆, 293 K) δ (ppm): 12.99 (s, 18H), 6.86 (s, 18H), 4.49 (s, 36H), 3.71 (s, 18H). The ¹³C NMR spectrum of **3** is shown in Figure S5. ¹³C NMR (100 MHz, DMSO-*d*₆, 293 K) δ (ppm): 170.63, 149.25, 126.86, 114.34 and 65.07. LRESIMS is shown in Figure S6: *m*/*z* 1070.2 [M – 2H]^{2–}. HRESIMS: *m*/*z* calcd for [M – 2H]^{2–} C₉₉H₈₈O₅₄^{2–}, 1070.20699; found 1070.20689; error –0.1 ppm.



Figure S4. ¹H NMR spectrum (400 MHz, DMSO-*d*₆, 293K) of **3**.



Figure S5. ¹³C NMR spectrum (100 MHz, DMSO-*d*₆, 293K) of **3**.



Figure S6. Electrospray ionization mass spectrum of **3**. Assignment of the main peak: $m/z \ 1070.2 \ [M - 2H]^{2-}$.

2.3. Synthesis of compound WP9



Compound **3** (86.0 mg, 0.0400 mmol) and ammonium hydroxide solution (25–28 %, 10 mL) were stirred at room temperature for 1 h. Water was then removed by rotary evaporation to gain **WP9** as a colorless powder (100 mg, 100%), mp: 175.2–176.4 °C. The ¹H NMR spectrum of **WP9** is shown in Figure S7. ¹H NMR (400 MHz, D₂O, 293 K) δ (ppm): 6.66 (s, 18H), 4.24 (s, 36H), 3.92 (s, 18H). The ¹³C NMR spectrum of **WP9** is shown in Figure S8. ¹³C NMR (100 MHz, D₂O, 293 K) δ (ppm): 176.77, 150.25, 128.43, 115.79, 68.27 and 29.73. LRESIMS is shown in Figure S9: *m/z* 1070.3 [M – 2NH₄ – 16NH₃]^{2–} C₉₉H₈₈O₅₄^{2–}, 1070.20699; found 1070.21054; error 4 ppm.



Figure S7. ¹H NMR spectrum (400 MHz, D₂O, 293K) of **WP9**.



Figure S8. ¹³C NMR spectrum (100 MHz, D₂O, 293K) of **WP9**.



Figure S9. Electrospray ionization mass spectrum of WP9. Assignment of the main peak: $m/z \ 1070.3 \ [M - 2NH_4 - 16NH_3]^{2-}$.

3. Comparison of the ¹H NMR spectra of WP5, WP6, and WP9



Figure S10. Partial ¹H NMR spectra (400 MHz, D₂O, 293 K): (a) 1.00 mM **WP5**; (b) 1.00 mM **WP6**; (c) 1.00 mM **WP9**.

4. Fluorescence titration experiments of WP9 and G in H_2O

To determine the stoichiometry and association constant for the complexation between **WP9** and **G**, fluorescence titration experiments were done with solutions which had a constant concentration of **WP9** $(1.00 \times 10^{-5} \text{ M})$ and varying concentrations of **G**. By a non-linear curve-fitting method, the association constant (K_a) of **WP9** \supset **G** was determined. By a mole ratio plot, 1:1 stoichiometry was obtained for the complexation between **WP9** and **G**.

The non-linear curve-fittings were based on the equation:

 $\Delta F = (\Delta F_{\infty}/[H]_0) (0.5[G]_0 + 0.5([H]_0 + 1/K_a) - (0.5([G]_0^2 + (2[G]_0 (1/K_a - [H]_0)) + (1/K_a + [H]_0)^2)^{0.5}))$

Where ΔF is the fluorescence intensity changes at 326 nm at $[G]_{0,} \Delta F_{\infty}$ is the fluorescence intensity changes at 326 nm when **WP9** is completely complexed, $[G]_{0}$ is the initial concentration of **G**, and $[H]_{0}$ is the fixed initial concentration of **WP9**.



Figure S11. Fluorescence spectra of **WP9** at a concentration of 1.00×10^{-5} M in aqueous solution at room temperature upon different concentrations of **G**: 0, 0.249, 0.498, 0.744, 0.990, 1.23, 1.48, 1.72, 1.96, 2.44, 3.38, and 6.10×10^{-5} M in aqueous solution.



Figure S12. Mole ratio plot for WP9 and G, showing a 1:1 complexation stoichiometry.



Figure S13. The fluorescence intensity changes of **WP9** upon addition of **G**. The red solid line was obtained from the non-linear curve-fitting method based on the above equation.

5. Partial ¹H NMR spectra of $WP9 \supset G$



Figure S14. Partial ¹H NMR spectra (400 MHz, D₂O, 293 K): (a) **G** (1.00 mM); (b) **G** (1.00 mM) and **WP9** (1.00 mM); (c) **WP9** (1.00 mM).

6. UV-vis spectroscopy investigations of the complexation between WP9 and G in H_2O



Figure S15. UV-vis spectra of (a) 5.00×10^{-4} M G, (b) 5.00×10^{-4} M WP9 and (c) 5.00×10^{-4} M WP9 with equimolar G in water at room temperature.

7. Photographs of pH-controlled experiments



Figure S16. Photographs of aqueous solutions: (a) 1.00 mM G and WP9; (b) after adding 2.0 μ L aqueous DCl solution (20 %) to a; (c) after adding 1.0 μ L aqueous NaOD solution (30 %) to b.

8. Electrospray ionization mass spectra of WP9 and G in H_2O



Figure S17. Positive electrospray ionization mass spectrum of **WP9** and **G** in H₂O. Mass fragment at m/z 1164.4, corresponding to $[WP9 \supset G - 2I - 18NH_3]^{2+}$, confirmed the 1:1 complexation stoichiometry between **WP9** and **G**.

References:

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