A Fluorescent Heteroditopic Hemicryptophane Cage for the Selective Recognition of Choline Phosphate

Dawei Zhang, Guohua Gao, Laure Guy, Vincent Robert, Alexandre Martinez, Jean-Pierre Dutasta

1. Materials and instrumentation
2. Synthesis
3. Fluorescence Job plot
4. Fluorescence spectroscopic titration
5. \(^1\)H NMR spectroscopic titration
6. Computational method
7. References
1. Materials and instrumentation

All solvents used were of commercial grade. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Avance spectrometer operating at 500.10 MHz and 125.76 MHz for $^1$H NMR and $^{13}$C NMR spectra, respectively. $^1$H NMR chemical shifts (δ) are reported in ppm and referenced to the protonated residual solvent signal. Fluorescence spectra were carried out with a Horiba-Jobin Yvon spectrofluorimeter. Mass spectra were recorded by the Centre de Spectrométrie de Masse, Institute of Chemistry, Lyon.

2. Synthesis

![Scheme S1. The synthesis of Zn(II)@1 complex.](image)

Hemicryptophane 1 was synthesized according to our previously reported procedure. Zn(II)@1 complex was prepared as follow: to a solution of 1 (90.3 mg, 0.082 mmol) in 6 mL CHCl$_3$, 20 µL triethylamine was added under argon followed by addition of the solution of Zn(ClO$_4$)$_2$(H$_2$O)$_6$ (30.5 mg, 0.082 mmol, 1.0 equivalent) in 6 mL CH$_3$OH. After stirring the reaction mixture at room temperature for 2 hours, a large amount of precipitate appeared. The precipitate was collected, washed thoroughly with Et$_2$O and dried under vacuum to give the
final product as a white solid (70.8 mg, yield 63%). The ligand 1 is soluble in most of the common solvents, for example CH$_2$Cl$_2$, CHCl$_3$, acetone and DMSO. However, the Zn(II)@1 complex is only soluble in DMSO, and moderate soluble in acetone.

**Ligand 1:**

$^1$H NMR (500.1 MHz, 298 K, CDCl$_3$) $\delta$ 7.33 (d, 3H, $J = 8.4$ Hz); 7.16 (d, 3H, $J = 8.3$ Hz); 7.13 (s, 3H); 7.07 (s, 3H); 7.00 (d, 3H, $J = 9.0$ Hz); 6.92 (s, 3H); 6.89 (s, 3H); 6.56 (d, 3H, $J = 8.6$ Hz); 4.84 (d, 3H, $J = 13.8$ Hz); 4.58-4.61 (m, 3H); 4.39-4.43 (m, 3H); 4.25 (t, 6H, $J = 4.90$ Hz); 3.69 (s, 9H); 3.65 (d, 3H, $J = 13.3$ Hz); 3.63 (d, 3H, $J = 13.7$ Hz); 3.53 (d, 3H, $J = 13.3$ Hz); 2.54-2.69 (m, 12H).

$^{13}$C NMR (125.7 MHz, 298 K, CDCl$_3$) $\delta$ 156.8, 148.7, 146.5, 133.6, 133.2, 131.9, 129.3, 128.9, 127.2, 126.9, 126.5, 119.4, 116.7, 113.7, 107.3, 67.6, 67.5, 56.0, 52.9, 47.7, 36.7.

ESI-MS m/z: found 1101.5350 [M+H]$^+$; calcd for C$_{69}$H$_{73}$N$_4$O$_9$: 1101.5372.

IR $\tilde{\nu}$ = 2931, 1606, 1508, 1263 cm$^{-1}$.

M.p. > 310 °C (decomp.).

**Zn(II)@1 complex:**

$^1$H NMR (500.1 MHz, 298 K, DMSO-$d_6$) $\delta$ 7.43-7.63 (broad, 12H); 7.20 (s, 3H); 7.05-7.11 (broad, 9H); 4.66 (d, 3H, $J = 13.3$ Hz); 4.21-4.43 (broad, 12H); 4.03 (broad, 3H); 3.93 (broad, 3H); 3.69 (s, 9H); 3.47 (d, 3H, $J = 13.4$ Hz); 2.96-3.18 (broad, 12H).

$^1$H NMR (500.1 MHz, 373 K, DMSO-$d_6$) $\delta$ 7.57 (bs, 9H); 7.32 (bs, 3H); 7.03-7.10 (m, 12H); 4.68 (d, 3H, $J = 13.5$ Hz); 4.28 (bs, 12H); 3.88 (bs, 6H); 3.70 (s, 9H); 3.50 (d, 3H, $J = 13.5$ Hz); 2.97 (bs, 12H).

$^{13}$C NMR (125.7 MHz, 298 K, DMSO-$d_6$) $\delta$ 156.6, 148.4, 146.5, 133.9, 133.0, 132.0, 129.4, 128.4, 127.4, 119.3, 116.4, 107.4, 66.9, 66.3, 57.2, 54.6, 51.0, 49.4, 35.4.

ESI-MS m/z: found 1199.4224 [M$^{2+}$ + Cl]$^+$; calcd for C$_{69}$H$_{73}$N$_4$O$_9$: 1199.4274.

IR $\tilde{\nu}$ = 3237, 2934, 1612, 1507, 1483, 1263, 1218, 1282, 1085 cm$^{-1}$.

M.p. > 350 °C (decomp.).
\(^1\)H NMR spectrum (DMSO-\(d_6\), 500.1 MHz, 298K) of the Zn(II)@ I complex.

\(^1\)H NMR spectrum (DMSO-\(d_6\), 500.1 MHz, 373K) of the Zn(II)@ I complex.
$^{13}$C NMR spectrum (DMSO-$d_6$, 125.7 MHz, 298K) of the Zn(II)@1 complex.

ESI-MS spectrum of the Zn(II)@1 complex.
3. Fluorescence Job plot

The continuous variation method was used for determining the binding stoichiometry.\[^2\] In this method, solutions of the host and guest at the same concentration (5 µM) were prepared in DMSO containing 2% H₂O. Then the two solutions were mixed in different proportions maintaining a total volume of 3 mL and a total concentration of 5 µM. After incubating the mixture for 30 s, the spectra of the solutions for different compositions were recorded.

![Fig. S1](image1.png)

**Fig. S1** Fluorescence Job plot of Zn(II)@I with choline phosphate 2 (a) and choline 3 (b).

4. Fluorescence spectroscopic titration

2 mL Zn(II)@I complex solution (5 µM) was taken into the cuvette, and then certain equivalents of a concentrated guest solution (0.5 mM or 5 mM) were added stepwise with a syringe. As a very small volume of guest solution was added, the final amount of the solution was almost unchanged (2 mL). The mixed solution was incubated for 30 s and then irradiated at 300 nm. The corresponding emission values at 350 nm during titration were then recorded.

![Fig. S2](image2.png)

**Fig. S2** Fluorescence titrations of 5 µM Zn(II)@I with choline 3 excited at 300 nm in DMSO containing 2% water. Inset: the intensity at 350 nm as a function of the added choline 3.
**Fig. S3** Fluorescence titrations of 5 µM Zn(II)@I with choline phosphate 2 (a) and choline 3 (b) excited at 300 nm in DMSO/H₂O (80/20, v/v). Inset: the intensity at 350 nm as a function of the guest.

**Fig. S4** Fluorescence titrations of 5 µM Zn(II)@I excited at 300 nm with guest 4 (a) and guest 5 (b) in DMSO containing 2% water. Insets: the intensity at 350 nm as a function of the guest.

**Fig. S5** Fluorescence titrations of 5 µM Zn(II)@I excited at 300 nm with taurine 6 in DMSO containing 2% water.

**Fig. S6** Fluorescence titrations of 5 µM ligand 1 excited at 300 nm with choline phosphate 2 in DMSO containing 2% water.
5. \(^1\)H NMR spectroscopic titration

0.5 mL Zn(II)@1 complex solution was taken into the NMR spectroscopy tube, and then certain equivalents of a concentrated guest solution were added stepwise with a syringe. As a very small volume of guest solution was added, the final amount of the solution was almost unchanged (0.5 mL). The mixed solution was incubated for 30 s and then the measurement of \(^1\)H NMR spectroscopy of the solution was performed.

Fig. S7 \(^1\)H NMR titrations of 1 mM Zn(II)@1 with choline phosphate 2 at 298 K in DMSO-\(d_6\)/D\(_2\)O (80/20, v/v). H atoms in blue are attributed to the four diastereotopic protons of the encaged 2.

Fig. S8 \(^1\)H NMR titrations of 1 mM Zn(II)@1 with choline phosphate 2 at 353 K and then return to 298 K in DMSO-\(d_6\)/D\(_2\)O (80/20, v/v).
Fig. S9 The up-field region of the 2D COSY NMR spectrum for the mixture of Zn(II)@1 and 5 equiv. of choline phosphate 2 in DMSO-d6/D2O (80/20, v/v).

Fig. S10 1H NMR titrations of 1 mM Zn(II)@1 with choline 3 at 298 K in DMSO-d6/D2O (80/20, v/v). H atoms in blue are attributed to the diastereotopic protons of methylene and N(CH3)3 of the encaged 3.

Fig. S11 1H NMR titrations of 1 mM Zn(II)@1 with choline 3 at 353 K in DMSO-d6/D2O (80/20, v/v).
Fig. S12 $^{31}$P NMR titrations of 1 mM choline phosphate 2 with Zn(II)@1 at 298 K in DMSO-d$_6$/D$_2$O (80/20, v/v).

6. Computational method

Ab initio evaluations were performed using the Gaussian 03 package17 within a restricted DFT framework.[3] In order to access geometrical information upon the host-guest species, full geometry optimizations were performed using DFT calculations. A combination of BP86 function and an all electron 6-31G* basis set including polarization functions has proven to be very satisfactory for similar issues.[4] We checked using the hybrid B3LYP function that our results do not suffer from the arbitrariness of the exchange correlation function.

7. Reference
