



A highly selective fluorescent chemosensor for Ag⁺ based on calix[4]arene with lower-rim proximal triazolylpyrenes

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ABSTRACT

Ligand **3**, a calix[4]arene with lower-rim proximal triazolylpyrenes, is a ratiometric fluorescent chemosensor for Ag⁺ with higher sensitivity compared to ligand **5**, a calix[4]arene with lower-rim distal triazolylpyrenes, and the conventional fluoroionophore with non-conjugated triazole and pyrene. In a polar protic solvent, the binding ratios for **3**:Ag⁺ was determined to be 1:1 and the association constant was found to be $7.0 \times 10^3 \text{ M}^{-1}$. The detection of Ag⁺ by ligand **3** even works in aqueous methanol solution.

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1. Introduction

Silver ion has been used in killing harmful bacteria [1] and its complexes have been widely used in electric industry, photographic and imaging industry, and pharmacy. However, silver ion is also known to have negative impact on environment and human beings, for example, silver oxide may destroy the environmental benign bacteria by inhibiting their growth and disturbing their reproductive ability, and silver ions inactivate sulfhydryl enzymes and bind with various metabolites [2]. Additionally, there are also many reports on silver bioaccumulation and toxicity [3]. It should be noted that Ag⁺ has moderate coordination ability, making it quite difficult to be discriminated from other chemically similar heavy metal ions. Therefore, the design and synthesis of a selective and sensitive fluorescent chemosensor for Ag⁺, preferably a ratiometric one, is still highly desirable to date [4].

The 1,2,3-triazole unit, from the “click chemistry” [5] of an azide and an alkyne, has been frequently used in ionophores [6,7] especially those combining pyrenes as fluoroionophores [8,9]. Pyrene is one of the most popular fluorophores in the design of a fluorescent chemosensor, because of its high fluorescence quantum yield and its monomer to excimer emission is very sensitive to microenvironmental change [10]. Our group was one of

the first to use click chemistry on calix[4]arene to construct sensors for metal ions [6c,7]. In previous calixarene fluoroionophores, the triazole and pyrene (or other fluorophore) units are usually linked by a methylene-bridge and worked in polar nonprotic solvents because of the poor solubility of calixarenes in polar protic solvents, furthermore, polar protic solvents usually compete with the ligand making it inefficient in ion sensing [9]. Two research groups [9a,b] independently reported the synthesis of calix[4]arene with lower-rim distal 1,2,3-triazole-CH₂-pyrene units showing similar sensitivities toward Pb²⁺, Hg²⁺, and Cu²⁺ in CH₃CN with quenched fluorescence. Their system showed a decreased excimer but enhanced monomer emissions toward Zn²⁺. Recently, Yamato and co-workers also reported the incorporation of the triazole-CH₂-pyrene fluoroionophores into the lower-rim of homooxacalix[3]arenes [9c] and thiacalix[4]arenes [9d]. Homooxacalix[3]arenes, with lower-rim distal triazole-CH₂-pyrenes, led to quenched fluorescence in the presence of Cu²⁺ and Hg²⁺ in CH₃CN/H₂O/DMSO (1000:50:1, v/v) cosolvent; however, it showed a decreased excimer but increased monomer emission in the presence of Pb²⁺ [9c]. Interestingly, the thiacalix[4]arenes, appended with lower-rim distal triazole-CH₂-pyrenes, showed a quenched excimer but an enhanced monomer emission toward Ag⁺ in CH₃CN/CH₂Cl₂ (1000:1, v/v) cosolvent [9d].

Herein, we report the first synthesis of calix[4]arenes with conjugated triazolylpyrenes to study their metal ion selectivity in polar protic solvent and/or aqueous methanol solutions. In addition, ligands **3** and **5**, with proximal and distal triazolylpyrenes on the lower rim of calix[4]arenes, respectively, are synthesized to study the

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influence of the orientation of the fluoroionophores on metal ions selectivity. UV–vis, fluorescent emission and excitation spectra, ^1H NMR, and mass spectrometry are used to study the possible binding modes of ligands **3** and **5** toward metal ions. Molecular modeling (DMol3) [11] is also carried out to rationalize the observed spectral changes.

2. Experimental

2.1. General

All reported yields were isolated yields. Flash column chromatography was performed using silica gel (70–230 mesh). ^1H NMR spectra were recorded in a 300 MHz NMR spectrometer, using the CHCl_3 solvent peak as an internal standard. ^{13}C NMR spectra were recorded at 75.4 MHz. The following abbreviations are used: singlet (s); doublet (d); triplet (t); quartet (q); and multiplet (m). All the ^1H and ^{13}C NMR spectra are listed in Supporting Information. High resolution mass spectra were measured at FAB mode or Electron Impact by JMS-700 HRMS. Melting points were measured with a Yanaco MP500D apparatus and were uncorrected. UV–vis spectra were measured with an HP-8453 spectrophotometer and solvents were of HPLC grades. Fluorescence spectra were measured with an AMINCO-Bowman Series 2 or FluoroMax-3 luminescence spectrometer and solvents were of HPLC grades.

2.2. Synthesis of compound **2**

2.2.1.

5,11,17,23-tetra-*t*-butyl-25,26-bis(*O*-propargyl)calix[4]arene (**2**)

A solution of 5,11,17,23-tetra-*t*-butylcalix[4]arene **1** (0.5 g, 0.77 mmol), propargyl bromide (0.20 g, 1.70 mmol) in DMF (20 mL) and sodium hydride (0.27 g, 1.92 mmol) was stirred at 70 °C for 2 h. The reaction mixture was extracted twice with CH_2Cl_2 and the organic solution was dried over MgSO_4 and then evaporated to give the sticky crude products. Purification by column chromatography on silica gel eluting with ethyl acetate/hexane (v/v, 2:3) gave pale yellow solid compound **2** (0.31 g, 56%); $R_f = 0.9$; CH_2Cl_2 /hexane (v/v, 1:1); mp 120–130 °C; ^1H NMR (CDCl_3 , 300 MHz) δ 8.33 (s, 2H, OH), 7.04–7.00 (m, 8H, ph-H), 4.99–4.81 (m, 4H, OCH_2), 4.59 (d, $J = 12.8$ Hz, 1H, Ph- CH_2 -Ph), 4.52 (d, $J = 13.1$ Hz, 2H, Ph- CH_2 -Ph), 4.37 (d, $J = 13.4$ Hz, 1H, Ph- CH_2 -Ph), 3.40 (d, $J = 13.2$ Hz, 2H, Ph- CH_2 -Ph), 3.38 (d, $J = 13.5$ Hz, 2H, Ph- CH_2 -Ph), 2.67 (t, $J = 2.4$ Hz, 2H, $\text{OCH}_2\text{CC-H}$), 1.24 (s, 18H, *t*-Bu), 1.17 (s, 18H, *t*-Bu); ^{13}C NMR (CDCl_3 , 75.4 MHz) δ 150.8 (Cq), 148.8 (Cq), 147.3 (Cq), 142.7 (Cq), 133.8 (Cq), 133.6 (Cq), 128.6 (Cq), 128.5 (Cq), 126.0 (CH), 125.9 (CH), 125.3 (CH), 125.1 (CH), 79.5 (Cq), 76.0 (CH), 62.4 (CH_2), 53.2 (CH_2), 34.1 (Cq), 33.8 (Cq), 32.6 (CH_2), 32.3 (CH_2), 31.5 (CH_3), 31.2 (CH_3); EI-MS m/z 724 (M^+); HRMS m/z calcd. for $\text{C}_{50}\text{H}_{60}\text{O}_4$ 724.4492, found 724.4498.

2.3. Synthesis of compounds **3**, **5**, and **7**

2.3.1. General procedures for the synthesis of **3**, **5**, and **7**

A solution of 1-azidopyrene (0.25 g, 1.03 mmol) and CuI about (1 mg, 0.005 mmol) was added to **2** (0.37 g, 0.52 mmol), **4** (0.37 g, 0.52 mmol), and **6** (0.19 g, 1.03 mmol) in THF/ H_2O (v/v, 2:1, 30 mL), respectively, and the heterogeneous mixture were stirred at 50 °C for 1 day. The mixture was extracted twice with CH_2Cl_2 and all the organic layers were combined, dried over MgSO_4 , and then evaporated to give the solid crude products. Column chromatography on silica gel eluting with ethyl acetate and hexane gave white solid compounds **3**, **5**, and **7** in 74%, 72%, and 79% yield, respectively.

2.3.2. 5,11,17,23-tetra-*t*-butyl-25,26-bis[(*O*-methyl)-2H-triazole-1-pyrene]calix[4]arene (**3**)

The solid was eluted with ethyl acetate/hexane (v/v, 1:1) and gave **3** (0.45 g, 72%) as a white solid, mp 201–202 °C; $R_f = 0.3$, ethyl acetate/hexane (v/v, 1:3); ^1H NMR (CDCl_3 , 300 MHz) δ 9.17 (s, 2H, OH), 8.39 (s, 2H, CCHN), 8.02–7.52 (m, 18H, pyrene-H), 7.14 (d, $J = 2.4$ Hz, 2H, Ph-H), 7.09 (d, $J = 2.3$ Hz, 2H, Ph-H), 7.04 (d, $J = 2.3$ Hz, 2H, Ph-H), 7.00 (d, $J = 2.3$ Hz, 2H, Ph-H), 5.74 (d, $J = 11.5$ Hz, 2H, OCH_2), 5.16 (d, $J = 11.5$ Hz, 2H, OCH_2), 4.85 (d, $J = 12.7$ Hz, 1H, Ph- CH_2 -Ph), 4.54 (d, $J = 12.9$ Hz, 2H, Ph- CH_2 -Ph), 4.29 (d, $J = 13.5$ Hz, 1H, Ph- CH_2 -Ph), 3.61 (d, $J = 12.7$ Hz, 1H, Ph- CH_2 -Ph), 3.45 (d, $J = 12.9$ Hz, 2H, Ph- CH_2 -Ph), 3.38 (d, $J = 13.5$ Hz, 1H, Ph- CH_2 -Ph), 1.23 (s, 18H, *t*-Bu), 1.19 (s, 18H, *t*-Bu); ^{13}C NMR (CDCl_3 , 75.4 MHz) δ 151.4 (Cq), 149.1 (Cq), 147.3 (Cq), 144.1 (Cq), 142.7 (Cq), 134.0 (Cq), 133.2 (Cq), 131.3 (Cq), 130.6 (Cq), 130.0 (Cq), 129.6 (CH), 129.2 (Cq), 128.9 (CH), 128.3 (CH), 127.9 (Cq), 126.9 (CH), 126.4 (CH), 126.3 (CH), 126.3 (CH), 126.2 (CH), 125.9 (CH), 125.6 (CH), 125.5 (CH), 125.4 (CH), 124.7 (CH), 124.2 (Cq), 124.1 (CH), 123.4 (Cq), 122.5 (CH), 120.5 (CH), 69.1 (CH_2), 34.2 (Cq), 33.9 (Cq), 32.8 (CH_2), 31.5 (CH_3), 31.4 (CH_2), 31.3 (CH_3), 29.7 (CH_2); MS (FAB, m/z) 1211 [M^+]; HRMS m/z calcd. for $\text{C}_{82}\text{H}_{78}\text{N}_6\text{O}_4$ 1210.6085, found 1210.6100.

2.3.3. 5,11,17,23-tetra-*t*-butyl-25,27-bis[(*O*-methyl)-2H-triazole-1-pyrene]calix[4]arene (**5**)

The solid was eluted with ethyl acetate/hexane (v/v, 1:1) and gave **5** (0.47 g, 74%) as a white solid, mp 196–197 °C; $R_f = 0.3$, ethyl acetate/hexane (v/v, 1:3); ^1H NMR (CDCl_3 , 300 MHz) δ 8.08 (s, 2H, CCHN), 8.05–7.62 (m, 18H, pyrene-H), 7.30 (s, 2H, OH), 7.11 (s, 4H, Ph-H), 6.87 (s, 4H, Ph-H), 5.32 (s, 4H, OCH_2), 4.40 (d, $J = 13.1$ Hz, 4H, Ph- CH_2 -Ph), 3.40 (d, $J = 13.1$ Hz, 4H, Ph- CH_2 -Ph), 1.31 (s, 18H, *t*-Bu), 0.99 (s, 18H, *t*-Bu); ^{13}C NMR (CDCl_3 , 75.4 MHz) δ 150.5 (Cq), 149.7 (Cq), 147.4 (Cq), 144.1 (Cq), 141.8 (Cq), 132.6 (Cq), 131.7 (Cq), 130.7 (Cq), 130.3 (Cq), 129.8 (Cq), 129.3 (CH), 128.4 (CH), 127.8 (Cq), 126.6 (CH), 126.4 (CH), 126.1 (CH), 125.9 (CH), 125.7 (CH), 125.3 (CH), 125.2 (CH), 124.5 (CH), 123.7 (Cq), 122.7 (CH), 120.8 (CH), 69.6 (CH_2), 34.0 (Cq), 33.8 (Cq), 31.9 (CH_2), 31.7 (CH_3), 31.0 (CH_3); MS (FAB, m/z) 1211 (M^+ , 0.45), 1234 ([$\text{M}+\text{Na}^+$], 0.27); HRMS m/z calcd. for $\text{C}_{82}\text{H}_{78}\text{N}_6\text{O}_4$ 1210.6085, found 1210.6089.

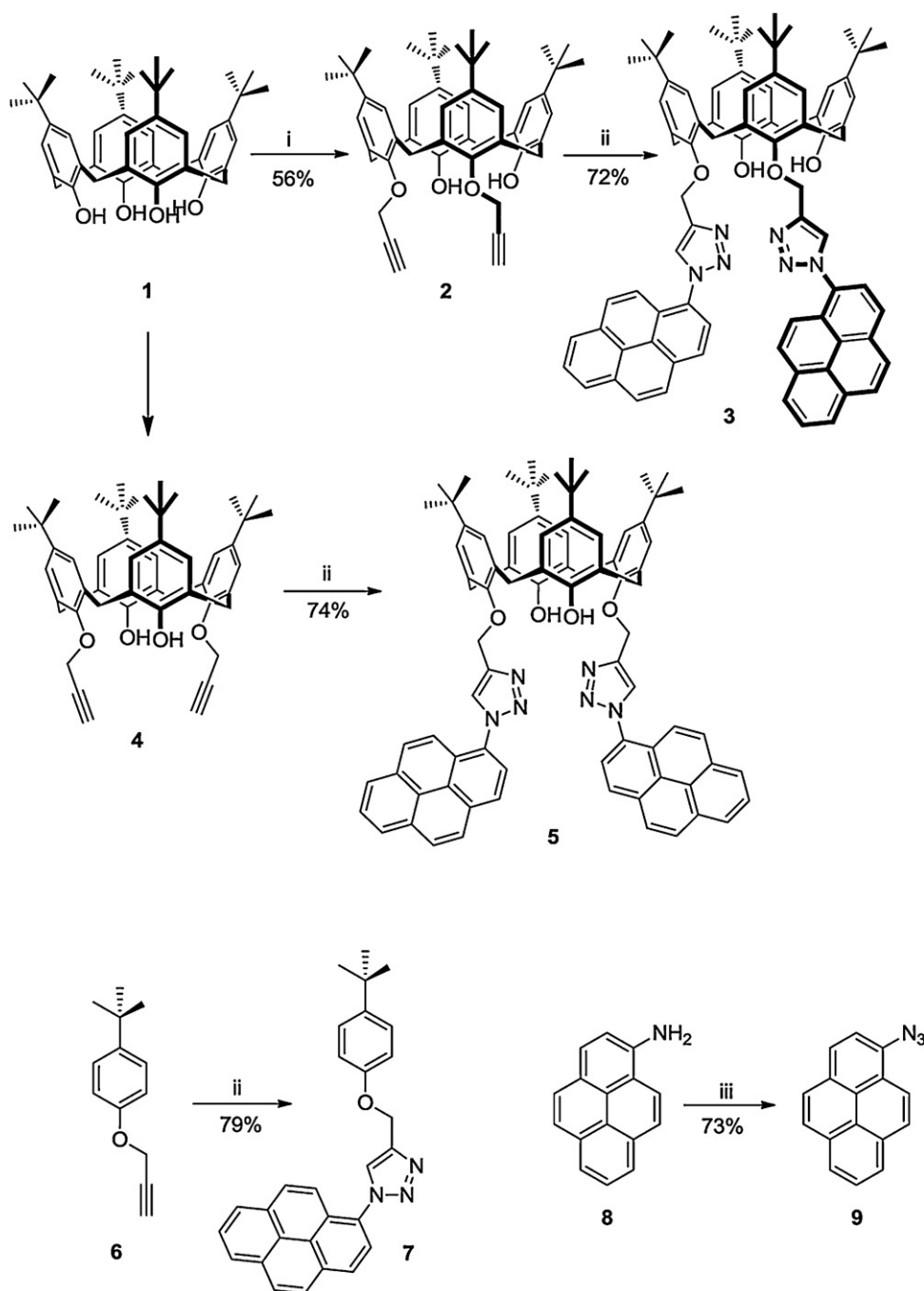
2.3.4. *t*-Butylphenyl-(*O*-methyl)-2H-triazole-1-pyrene (**7**)

The solid was eluted with ethyl acetate/hexane (v/v, 1:1) and gave **7** (0.35 g, 79%) as a white solid, mp 170–171 °C; $R_f = 0.6$, ethyl acetate/hexane (v/v, 1:3), ^1H NMR (CDCl_3 , 300 MHz) δ 8.32–8.06 (m, 9H, pyrene-H), 7.88 (d, $J = 9.3$ Hz, 1H, CCHN), 7.38–7.35 (m, 2H, Ph-H), 7.05–7.02 (m, 2H, Ph-H), 5.43 (s, 2H, OCH_2), 1.32 (s, 9H, *t*-Bu); ^{13}C NMR (CDCl_3 , 75.4 MHz) δ 156.0 (Cq), 144.7 (Cq), 144.1 (Cq), 132.3 (Cq), 131.1 (Cq), 130.6 (Cq), 130.3 (Cq), 129.8 (CH), 129.0 (CH), 127.0 (CH), 126.8 (CH), 126.4 (CH), 126.4 (CH), 126.2 (Cq), 126.1 (CH), 125.8 (CH), 125.0 (Cq), 124.7 (CH), 124.1 (Cq), 123.4 (CH), 121.1 (CH), 114.3 (CH), 62.3 (CH_2), 34.1 (Cq), 31.5 (CH_3); MS (EI, m/z) 431 [M^+]; HRMS m/z calcd. for $\text{C}_{29}\text{H}_{25}\text{N}_3\text{O}$ 431.1998, found 431.1999.

3. Results and discussion

3.1. The synthesis and differentiation of compounds **3**, **5**, and **7**

The synthetic pathways for ligands **3**, **5**, and a control compound **7** are shown in Scheme 1, in which the main fluoroionophores are composed of proximal (**3**) and distal (**5**) triazolyipyrenes at the lower rim of calix[4]arenes. It should be noted that the 1,2,3-triazolyipyrene not only functioning as a coordination site but also acting as a signaling unit. 1-Azidopyrene **9** [12a] was obtained from the diazotization of 1-aminopyrene **8**, followed by its click reaction with compounds **2**, **4** [12b], and **6** [12c] to afford triazolyipyrene



Scheme 1. Synthesis of fluorogenic calix[4]arenes **3**, **5**, and control compound **7**. Reagents and conditions: (i) propargyl bromide, sodium hydride, DMF, 70 °C, 2 h; (ii) 1-azidopyrene, CuI, THF/H₂O, 50 °C, 1 d; (iii) NaNO₂/concd HCl, acetone, sodium azide/H₂O, rt, 1 day.

ligands **3**, **5**, and **7** in 74%, 72%, and 79% yield, respectively. The structures of all ligands synthesized in this work were fully characterized by NMR (¹H and ¹³C), MS, and HRMS (see Section 2).

The structures of ligands **3** and **5** can be readily distinguished by their different symmetry in NMR spectra. The ¹H NMR spectra of the proximal ligand **3** exhibited six doublet signals around 3–5 ppm for the methylene-bridge protons because of its C_s symmetry; in contrast, the methylene-bridge protons of ligand **5** appeared as two doublet signals because of its C_{2v} symmetry (cf. Figs. S3 and S5, Supporting information). Furthermore, ligand **3** exhibited three methylene-bridge carbons (29.7, 31.4, and 32.8 ppm) but the more symmetric ligand **5** displayed only one methylene-bridge carbon at 31.9 ppm (see Section 2). All the methylene-bridge carbons of

ligands **3** and **5** exhibited signals around 31 ppm supporting that they were in cone conformations [13]. Moreover, the lower-rim O-methylene protons of ligand **3** exhibited two doublet signals around 5–6 ppm in the ¹H NMR spectra implying that the two O-methylene protons are nonequivalent (Fig. S3, Supporting information).

3.2. Spectral characteristics of compounds **3**, **5**, and **7**

The UV–vis and fluorescent emission spectra of ligands **3**, **5**, and the control compound **7** in MeOH/CHCl₃ (v/v, 98:2) polar protic cosolvent are shown in Fig. 1, where the concentration of all three compounds was fixed at 10 μM.

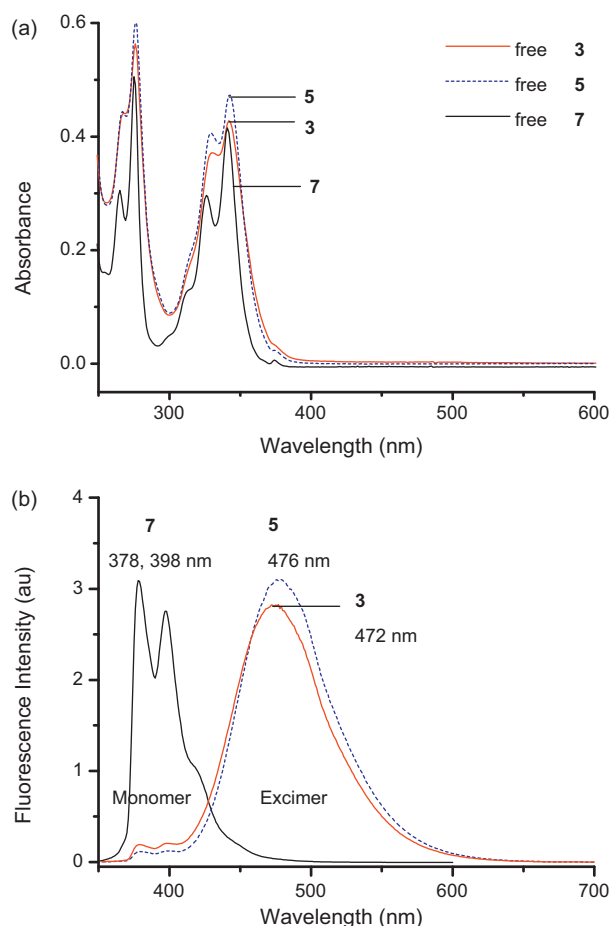


Fig. 1. (a) UV-vis and (b) fluorescence spectra (excitation wavelength = 342 nm) of ligands (10 μ M) **3**, **5**, and **7** in a polar protic MeOH/CHCl₃ (v/v, 98:2) cosolvent at 25 °C.

The two absorption bands, around 276 nm and 342 nm in the UV-vis spectra of **3**, **5**, and **7**, are from triazolylpyrenes (Fig. 1a) [14]. The fluorescence spectra of **3**, **5**, and **7** were taken when irradiating at the 342 nm of the triazolylpyrene band (Fig. 1b). Compound **7**, bearing only a single triazolylpyrene fluoroionophore, displayed very strong monomer emission bands around 378 and 398 nm without any emission from the excimer (Fig. 1b) indicating that intermolecular π - π stacking of the conjugated triazolylpyrene fluoroionophores did not exist under the low concentration (10 μ M) conditions. In contrast, both ligands **3** and **5**, displayed very weak monomer emissions (379 and 398 nm) but strong excimer emissions (around 476 nm, Fig. 1b), implying that strong intramolecular π - π interactions of the two neighboring triazolylpyrene units exist even in the very dilute condition.

3.3. Spectral characteristics of compounds **3**, **5**, and **7** for cations

The binding properties of ligands **3**, **5**, and **7** (all at 10 μ M) toward metal ions were assessed by UV-vis and fluorescence spectroscopy by the addition of 10 equiv of metal perchlorates (Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Ag⁺, Cu²⁺, Ni²⁺, Cd²⁺, Hg²⁺, Zn²⁺, Mn²⁺, Pb²⁺, and Cr³⁺) in MeOH/CHCl₃ (v/v, 98:2) cosolvent and the results are depicted in Figs. 2 and S9 (Supporting information).

Upon the addition of 15 different metal perchlorates, the UV-vis spectra of **3**, **5**, and **7** did not show any bathochromic or hypsochromic shift (Fig. S9, Supporting information); therefore throughout the work, the fluorescence spectra were taken using the excitation wavelength at 342 nm. In contrast, the fluorescence

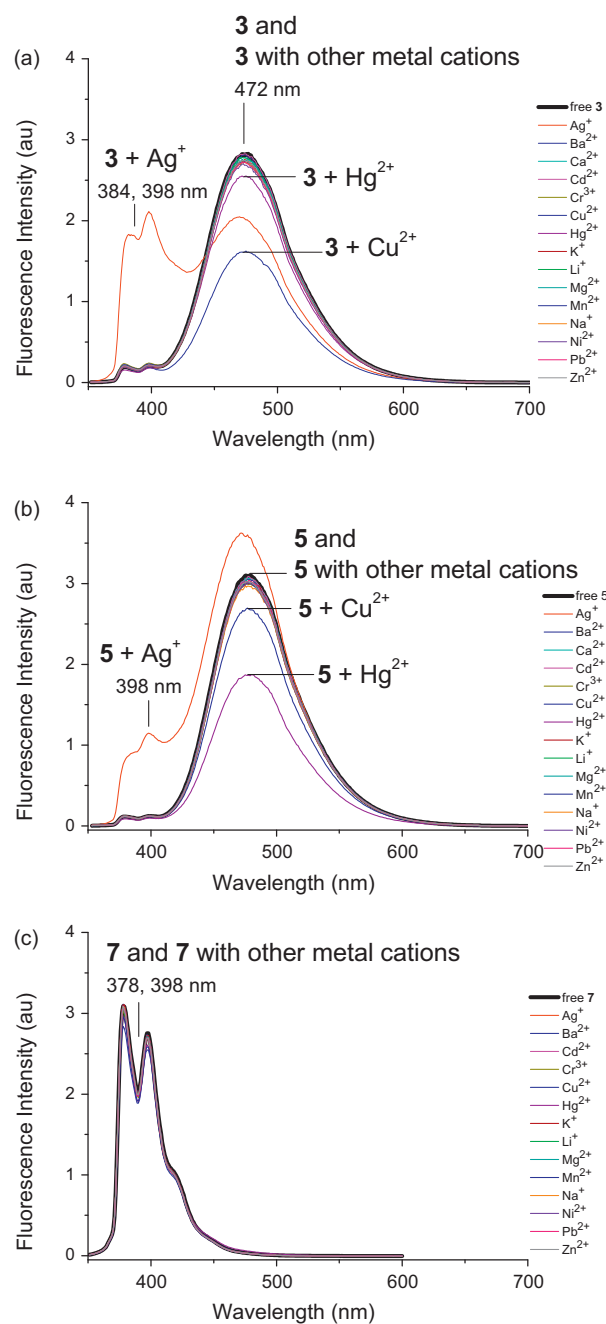


Fig. 2. Fluorescence spectra of ligands (a) **3**, (b) **5**, and (c) **7** (each 10 μ M) in the absence and presence of 10 equiv of 15 metal perchlorates (Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Ag⁺, Cu²⁺, Ni²⁺, Cd²⁺, Hg²⁺, Zn²⁺, Mn²⁺, Pb²⁺, and Cr³⁺) in polar protic MeOH/CHCl₃ (v/v, 98:2) cosolvent at 25 °C (excitation wavelength = 342 nm).

spectra of ligands **3** and **5** were significantly changed when Ag⁺, Cu²⁺, or Hg²⁺ was added (Fig. 2a and b); whereas, the control compound **7**, bearing only a single triazolylpyrene, did not show any obvious change upon the addition of 15 metal ions (Fig. 2c). The results indicate that the two triazolylpyrene fluoroionophores on the lower-rim of calix[4]arenes are necessary for the selective sensing of Ag⁺, Cu²⁺, and Hg²⁺.

Heavy atoms are well known to quench the fluorescence of nearby fluorophores via enhanced spin-orbital coupling [15a], and/or photoinduced electron transfer (PET) [15b]. As anticipated, Cu²⁺ and Hg²⁺ quenched the fluorescence of ligands **3** and **5** (Fig. 2a and b) through heavy atom effect [9b] and/or the reversed PET [7a,9a,d,16]. In the latter case, when Cu²⁺ or Hg²⁺ was bounded

to the nitrogen atoms of the triazole units, the pyrene units probably behaved as PET donors and the triazole groups behaved as PET acceptors. Interestingly, ligands **3** and **5** displayed very different fluorescent responses toward Ag^+ . Upon the addition of 10 equiv of Ag^+ , the excimer emission of ligand **3** decreased with the concomitant enhancement on its monomer emission (Fig. 2a). In contrast, the monomer and excimer emissions of ligand **5** were both enhanced by Ag^+ (Fig. 2b). The very different fluorescent responses of ligands **3** and **5** toward Ag^+ implying that the *orientation* of the lower-rim triazolylpyrenes of calix[4]arene plays an important role in their sensing toward Ag^+ .

3.4. Binding properties of compounds **3**, **5**, for Ag^+

The Job plot [17] experiments of ligands **3** and **5** with Ag^+ (Fig. S10, Supporting information) showed that the excimer emissions of **3** and **5** reached a minimum at 0.4 of the molar fractions of ligands to Ag^+ , indicating that both 1:1 and 1:2 complexation modes coexisted for both **3** and **5**. To clarify the binding ratio, the precipitates from the respective ligands (10 μM) with 40 equiv of Ag^+ in MeOH/ CHCl_3 (v/v, 98:2) cosolvent were obtained and measured by the electrospray mass spectrometry. The mass spectra showed a peak at $m/z=1317.5$ for complex **3**· Ag^+ and a peak at $m/z=1317.6$ for complex **5**· Ag^+ (Figs. S11 and S12, Supporting information), corresponding to the masses of [**3**+ Ag^+] and [**5**+ Ag^+], respectively. No signals from the 1:2 complexes of **3**·(Ag^+)₂ and **5**·(Ag^+)₂ were observed despite our repeated efforts.

In the titration of **3** with Ag^+ in MeOH/ CHCl_3 (v/v, 98:2) cosolvent (Fig. 3a), the fluorescence intensity of the monomer emission bands (at 379 and 398 nm) gradually increased with the concomitant decrease of the excimer emission (λ_{max} 476 nm) as the concentration of Ag^+ increased, and an isoemissive point was clearly identified at 443 nm. The spectral feature of the fluorescence titration of ligand **3** with Ag^+ was consistent with a 1:1 binding ratio; furthermore, the system features a highly selective *ratiometric* fluorescent chemosensor for Ag^+ . For comparison, both the monomer and excimer emissions of ligand **5** were gradually enhanced as the concentration of Ag^+ increased (Fig. 3b) and there was a small blue shift of its excimer emission maximum (λ_{max} from 476 to 474 nm). Since the absorption spectra of **5** showed very little intensity change upon the addition of Ag^+ (Fig. S13b, Supporting information), the small hypsochromic shift of the excimer emission was attributed to a slightly different orientation of the lower-rim distal triazolylpyrene units in the excited state when **5** complexed with Ag^+ . The association constants of complexes **3**· Ag^+ and **5**· Ag^+ , calculated by the nonlinear least-square curve-fitting method [18], were found to be $7.11 \pm 0.80 \times 10^3 \text{ M}^{-1}$ and $1.83 \pm 0.33 \times 10^4 \text{ M}^{-1}$, respectively (Fig. S14, Supporting information).

Upon the addition of 60 equiv of Ag^+ , the intensity of the monomer emission of **3** at 398 nm increased by 14-fold while that for the excimer emission at 476 nm decreased by 0.5-fold (inset of Fig. 3a). On the other hand, the intensity of monomer emission at 398 nm increased by 8-fold but that for excimer emission at 476 nm increased by only 0.2-fold, when 40 equiv of Ag^+ was added to ligand **5** (inset of Fig. 3b). For comparison, the reported fluorescence enhancement factors for calixarenes with *non-conjugated* triazole- CH_2 -pyrene upon the addition of metal ions were around 0.5–9-fold [9]. Our results demonstrated that *conjugated* triazolylpyrene is a more sensitive fluoroionophore than conventional triazole- CH_2 -pyrene in metal ion sensing; furthermore, the relative orientation of the two triazolylpyrenes on the lower-rim of calix[4]arene plays an important role in their unique fluorescent sensing toward Ag^+ .

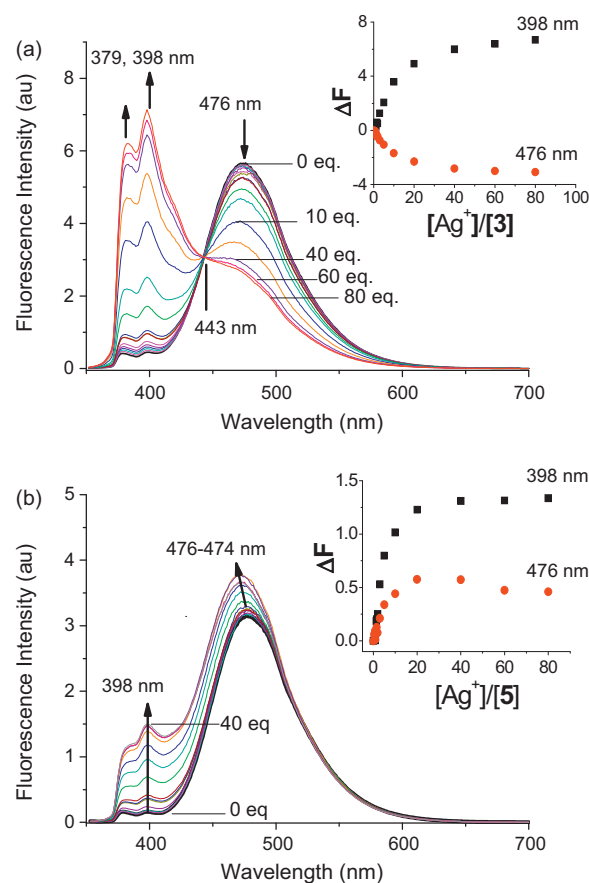


Fig. 3. Fluorescence titration spectra upon the addition of various amount of AgClO_4 to ligands (a) **3** and (b) **5** (10 μM) in a polar protic MeOH/ CHCl_3 (v/v, 98:2) cosolvent (excitation wavelength = 342 nm). The inset shows the plot of $\Delta F/F_0$ vs. $[\text{Ag}^+]/[\text{ligand}]$ based on the fluorescence titration of ligands (a) **3** and (b) **5** with various amount of AgClO_4 .

3.5. Excitation spectra of compounds **3** and **5** for Ag^+

In principle, there are two types of excimer for pyrene compounds: *dynamic* and *static* [10a]. The former results from a pyrene dimer formed in the excited state, whereas the latter arises from a pyrene dimer exists in the ground state. One of the powerful methods to distinguish a *dynamic* excimer from a *static* one is by comparing their excitation spectra. The excitation spectra of ligands **3** and **5** and complexes **3**· Ag^+ and **5**· Ag^+ were measured in MeOH/ CHCl_3 (v/v, 98:2) cosolvent, and we found that those monitored at the monomer ($\sim 398 \text{ nm}$) and excimer emissions ($\sim 475 \pm 1 \text{ nm}$) were not superimposable (Fig. S15, Supporting information). All the excitation spectra monitored at the excimer emission were red-shifted by at least 1–5 nm compared to those monitored at the monomer emission, indicating the *static* nature of the excimer emissions [10a], irrespective of the relative orientations of the two triazolylpyrene fluoroionophores on ligands **3** or **5** and their metal complexes.

3.6. ^1H NMR characterized the recognition behavior for Ag^+

In order to gain insight into the structures of the two complexes, **3**· Ag^+ and **5**· Ag^+ , ^1H NMR titration experiments on ligands **3** and **5** with various amount of Ag^+ were carried out in $\text{CD}_3\text{OD}/\text{CDCl}_3$ (v/v, 3:2) cosolvent (Figs. 4 and 5) and in $\text{CD}_3\text{OH}/\text{CDCl}_3$ (v/v, 3:2) cosolvent to observe the chemical shift changes of the hydroxy groups on calix[4]arene of complexes **3**· Ag^+ and **5**· Ag^+

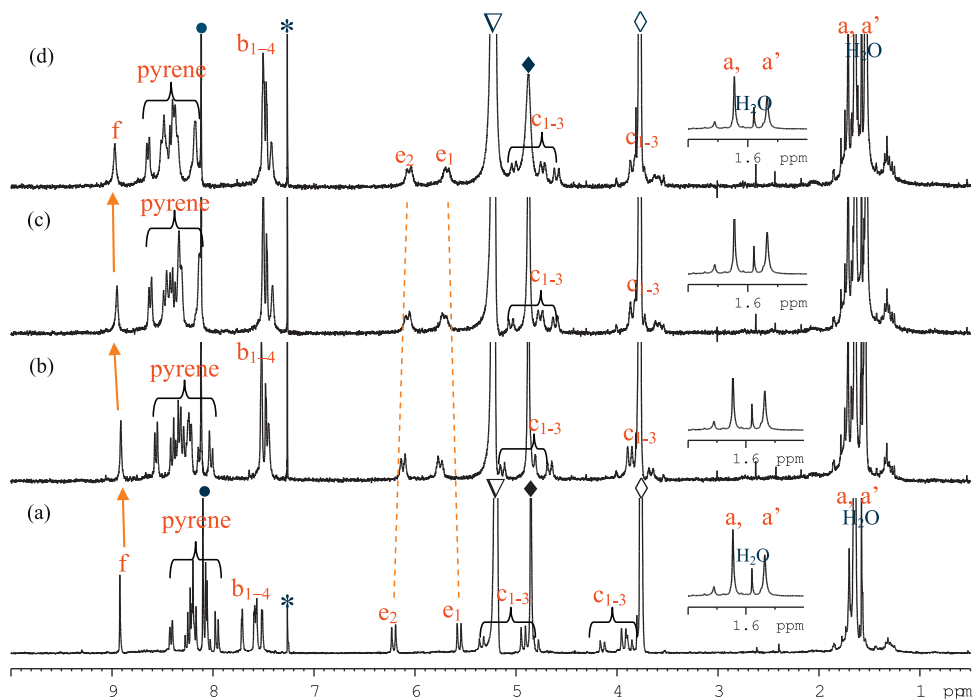


Fig. 4. ^1H NMR spectra of **3** (2.5 mM) in $\text{CD}_3\text{OD}/\text{CDCl}_3$ (v/v, 3:2) cosolvent and CDCl_3 as an external standard in the presence of different amount of AgClO_4 : (a) 0 mM, (b) 1.25 mM (0.5 equiv), (c) 2 mM (0.8 equiv), and (d) 2.5 mM (1 equiv). \diamond : CHD_2OD , \blacklozenge : CD_3OH , ∇ : H_2O , \bullet : internal CHCl_3 , and $*$: external CHCl_3 .

(Figs. S16, and S17, Supporting information). To avoid the interference of AgClO_4 on the chemical shifts of d-solvents, all ^1H NMR titration experiments were carried out using CDCl_3 as an external standard. Significant difference in spectral changes were observed when ligands **3** and **5** were each titrated with Ag^+ (cf. Figs. 4 and 5), in particular, the chemical shifts of OCH_2 -triazole protons (H_{e1} and H_{e2}), triazolyl protons (H_f), and pyrene protons.

3.6.1. ^1H NMR titration of compound **3** for Ag^+

The protons of OCH_2 (H_{e1} and H_{e2}) and pyrene groups showed more significant chemical shift changes in the 1:1 complex of **3**· Ag^+ (Fig. 4d) compared to those of **5**· Ag^+ (Fig. 5d). The chemical shift difference ($\Delta\delta$) between the two sets of the lower-rim OCH_2 protons (H_{e1} and H_{e2}) reduced from 0.65 ppm (Fig. 4a) to 0.38 ppm and most of the pyrene protons around 8–8.5 ppm were downfield shifted by 0.2 ppm (Fig. 4d). However, the triazolyl protons H_f were barely

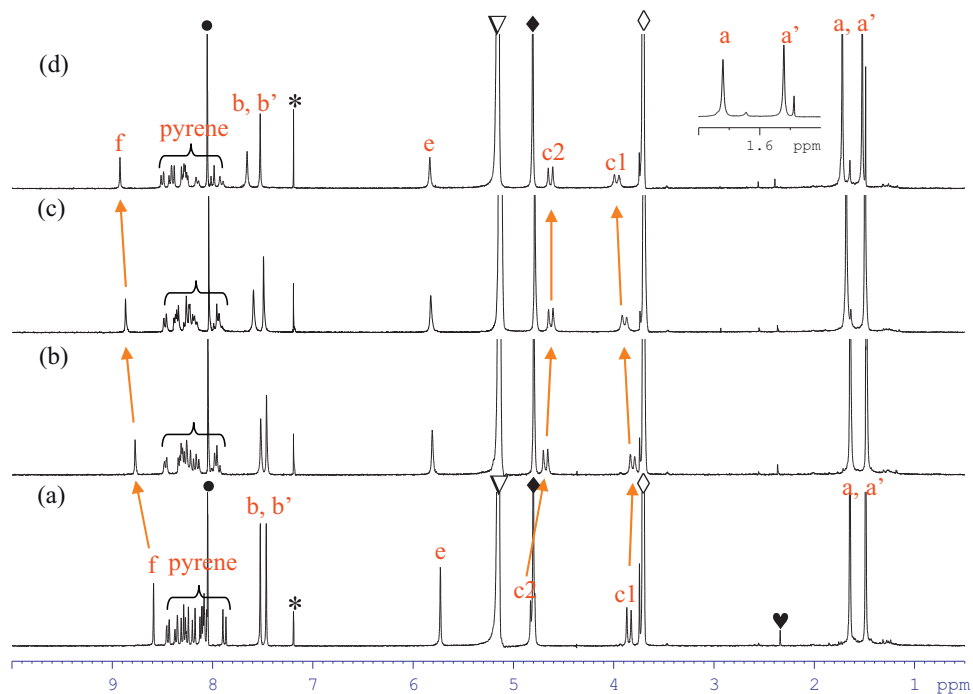
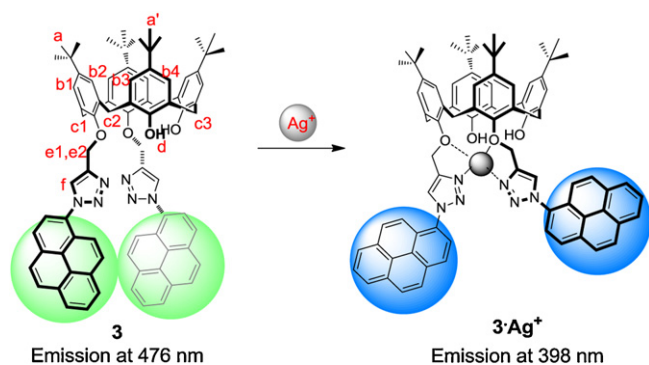


Fig. 5. ^1H NMR spectra of **5** (2.5 mM) in a $\text{CD}_3\text{OD}/\text{CDCl}_3$ (v/v, 3:2) cosolvent and CDCl_3 as an external standard in the presence of different amount of AgClO_4 : (a) 0 mM, (b) 1.25 mM, (c) 2 mM, and (d) 2.5 mM (1 equiv). \diamond : CHD_2OD , \blacklozenge : CD_3OH , ∇ : H_2O , \bullet : internal CHCl_3 , $*$: external CHCl_3 . \heartsuit denoted the sweeping noise from the instrument.



Scheme 2. A possible binding mode of 3-Ag^+ in MeOH/CHCl₃ (v/v, 98/2) cosolvent.

shifted (Fig. 4d) possibly due to the magnetic anisotropy effect from the moving pyrene units. Although the four sets of the phenyl protons on the calix[4]arene H_{b1–4} became overlapped in the presence of 1 equiv of Ag⁺ (Fig. 4d), the structure of the complex 3-Ag^+ was assumed to be still symmetrical because the splitting patterns of the protons on triazole units (H_f), OCH₂ (H_{e1}, H_{e2}), and the *t*-butyl groups (H_{a,a'}) on the upper rim of calix[4]arene were unchanged. Based on all ¹H NMR spectral features of **3** with Ag⁺, we propose that the proximal pyrene units moved apart from each other in the complex 3-Ag^+ (Scheme 2) [9d] causing substantial magnetic anisotropic shifts of the pyrene protons and the severe decrease of the excimer emission (vide supra).

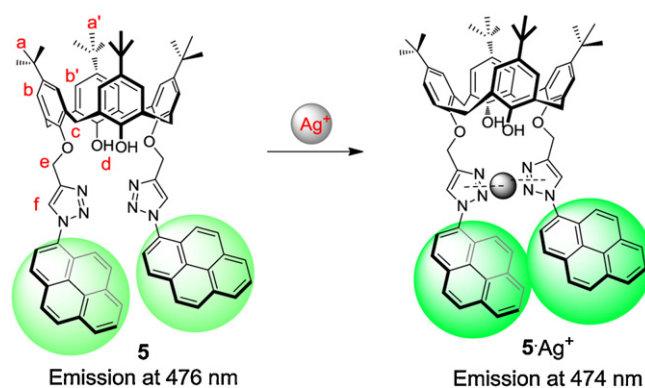
3.6.2. ¹H NMR titration of compound **5** for Ag⁺

In the titration of ligand **5** with Ag⁺ in CD₃OD/CDCl₃ (v/v, 3:2) cosolvent (Fig. 5d), the triazolyl proton H_f was downfield shifted by 0.34 ppm at 1 equiv of Ag⁺, which was the most affected proton among all the protons of ligand **5**. The chemical shifts of methylene protons of the OCH₂ (H_e) and the pyrene protons were little affected. Furthermore, the hydroxyl proton of phenol, H_d, was found to be buried in the protons of the pyrenes in the range of 8.52–7.94 ppm and the chemical shifts did not seem to have obvious change when **5** (2.5 mM) with 1 equiv of Ag⁺ was taken in CD₃OH/CDCl₃ (v/v, 3:2) cosolvent (Fig. S17, Supporting information). These spectral features implied that Ag⁺ was bound to the distal triazole units of ligand **5** with the two distal pyrene units remained in similar orientations as that in a free ligand **5**. The chemical shift difference ($\Delta\delta$) between the two sets of methylene-bridge protons H_{c1} and H_{c2} of ligand **5** decreased from 0.97 ppm (Fig. 5a) to 0.66 ppm in the 1:1 complex of 5-Ag^+ (Fig. 5d), indicating that the calix[4]arene in complex 5-Ag^+ was in a flattened cone conformation [19]. As the concentration of Ag⁺ increased (Figs. 5 and S17), signals of the two methyl protons (H_a and H_{a'}) of the upper-rim *t*-butyl and the O–CH₂ protons (H_e) remained to be singlets indicating that the structure of complex 5-Ag^+ was symmetrical.

Based on the spectral features of 5-Ag^+ , we propose that the complex 5-Ag^+ is in a symmetrical flattened cone conformation and the two distal pyrene units keep its face-to-face conformation as that in a free ligand **5** (Scheme 3). The enhancement of both the monomer and excimer emissions of ligand **5** in the presence of Ag⁺ (Fig. 3b) was attributed to the CHEF (chelation-enhanced fluorescence) effect [20] and the π – π stacking of the distal pyrene units. That is, when Ag⁺ was bonded to **5**, it helped to lock the two lower-rim triazolylpyrene units (Scheme 3), hence, enhanced the overall fluorescence intensity of the triazolylpyrenes (vide supra).

3.7. DMol3 characterized the recognition behavior for Ag⁺

In order to rationalize the quite different fluorescent responses of the two structurally related ligands on Ag⁺, we calculated the



Scheme 3. A possible binding mode of 5-Ag^+ in MeOH/CHCl₃ (v/v, 98:2) cosolvent.

optimized geometries of ligands **3** and **5** and their complexes 3-Ag^+ and 5-Ag^+ by the molecular modeling DMol3 [11] method and simulated them in MeOH environment. We used a B3LYP function with the double numeric plus d-functions (DND) basis set [21]. The size of the DND basis set is equivalent to the Gaussian 6-31+G** [21a]. The optimized geometries of ligands **3** and **5** and complexes 3-Ag^+ and 5-Ag^+ are displayed in Fig. 6, and related data of those calculation by DMol3 are summarized in Tables S5–S16 (Supporting information).

Based on the results from the DMol3 calculations, the distance between the two triazole units is about 3.7–3.9 Å in ligand **3** (Fig. 6a) and is about 7.2–7.4 Å in ligand **5** (Fig. 6b). In the complexation of ligands **3** and **5** with Ag⁺, there are two possible binding sites for each of the two triazole units: one is via the upper nitrogen atoms (N3) and the other is via the lower nitrogen atoms (N2) of the triazole units. The two possible situations for complexes 3-Ag^+ and 5-Ag^+ were optimized and are shown in Fig. S18 (Supporting information). When Ag⁺ was bound to N3 atoms of triazole units, the two pyrene units of complex would move apart from each other (Figs. S18a and c, Supporting information). The conformational change of complex 3-Ag^+ compared to free ligand **3** explains why the excimer emission decreased while the monomer emission increased which is consistent with the observed fluorescent changes and the proposed binding mode of complex (cf. Figs. 3a and 6c and Scheme 2). On the other hand, the complex of 5-Ag^+ revealed that Ag⁺ preferred to be bound to N2 atoms of the triazole units and the two pyrene units remained their face-to-face conformations as that in free ligand **5** (Fig. 6b and d). Such conformation would show stronger excimer than monomer emission and is in accord with the fluorescence results described above (cf. Figs. 3b and 6d and Scheme 3).

3.8. Properties of complex 3-Ag^+

3.8.1. Reversibility of complex 3-Ag^+

The complexation of ligand **3** with Ag⁺ in a MeOH/CHCl₃ (v/v, 98:2) cosolvent was set free by the addition of H₂O followed by extraction with dichloromethane (Fig. S19, Supporting information).

3.8.2. Competitive recognition of Ag⁺ with other metal cations

The fluorescence of ligand **3** (10 μM) in the presence of 10 equiv of Ag⁺ and in the presence of 14 other competing metal ions (10 equiv, Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cu²⁺, Ni²⁺, Cd²⁺, Hg²⁺, Zn²⁺, Mn²⁺, Pb²⁺, and Cr³⁺) in MeOH/CHCl₃ (v/v, 98:2) cosolvent were studied and the results are shown in Fig. 7. As can be seen, the enhanced monomer emission (398 nm) of complex 3-Ag^+ remained the same upon the addition of 13 competitive metal ions except Cu²⁺, in which a 20% decrease in the fluorescence enhancement

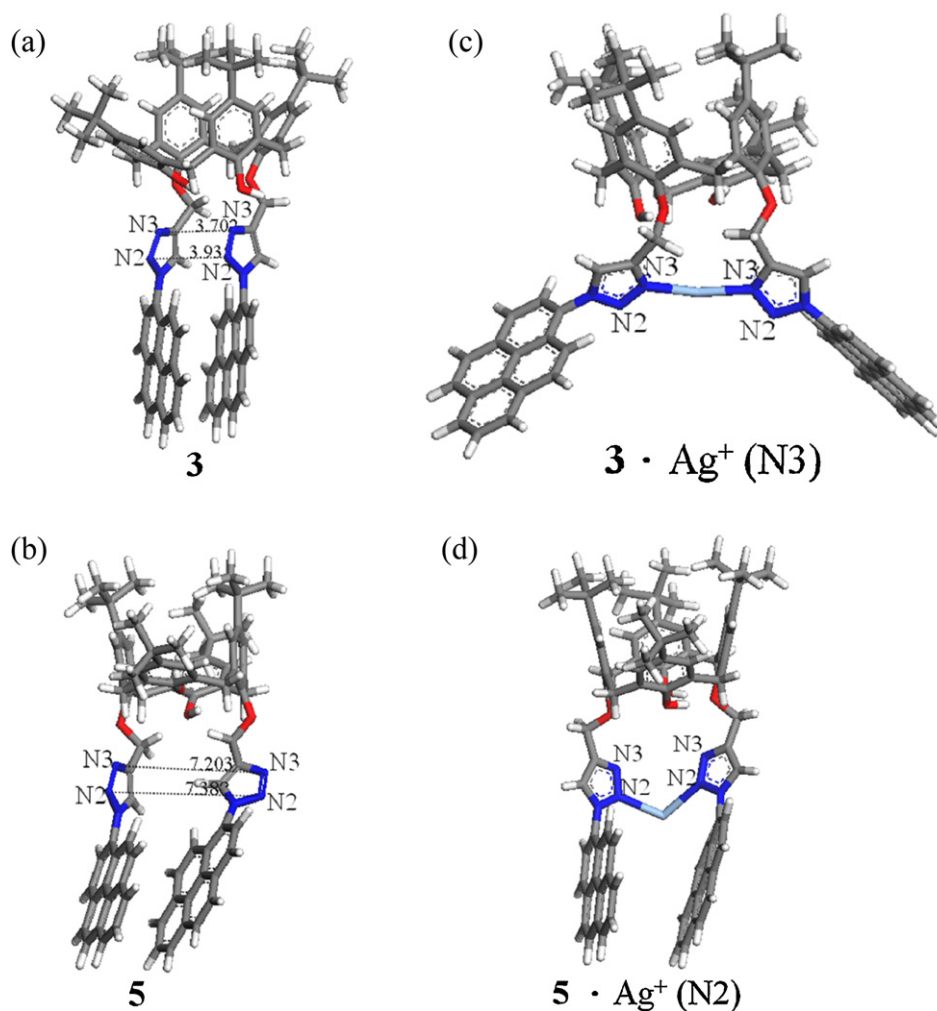


Fig. 6. Optimized geometries for ligands (a) **3**; (b) **5**, and complexes (c) **3**·Ag⁺ (N3); (d) **5**·Ag⁺ (N2) in MeOH by DMol3 calculation. Where “N3” means the binding site happened at the upper nitrogen atom (N3) of triazole units on ligands, and “N2” means the binding site happened at the lower nitrogen atom (N2) of triazole units on ligands.

was observed (Fig. 7h). Accordingly, ligand **3** can be claimed as a highly selective, ratiometric fluorescent chemosensor for Ag⁺ in the presence of most other competing metal ions in a polar protic solvent.

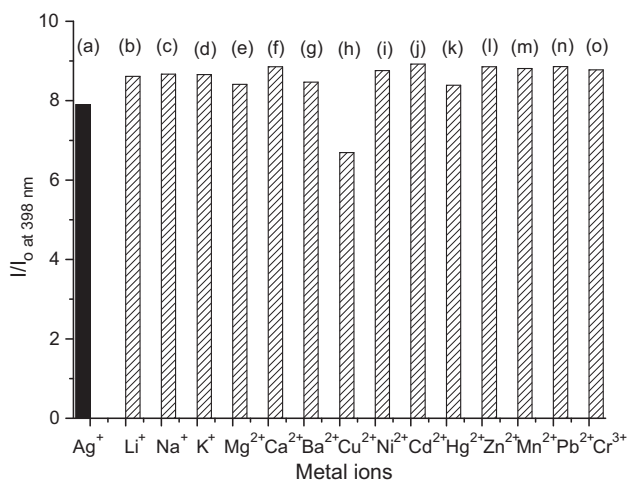


Fig. 7. Fluorescence intensity ratio of ligand **3** (10 μM) in the presence of 10 equiv (a) Ag⁺ and (b–o) Ag⁺ plus 14 other metal perchlorates (Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cu²⁺, Ni²⁺, Cd²⁺, Hg²⁺, Zn²⁺, Mn²⁺, Pb²⁺, and Cr³⁺) versus that of a free ligand **3** in MeOH/CHCl₃ (v/v, 98:2) cosolvent at 25 °C (excitation wavelength = 342 nm).

3.8.3. Recognition behavior of complex **3**·Ag⁺ in aqueous methanol solution

Furthermore, we also found that the unique ratiometric fluorescent sensing of Ag⁺ by ligand **3** still worked in 10% aqueous methanol solution, although the enhancement factor of the monomer emission of **3** dropped from 9.2 in MeOH/CHCl₃ (v/v,

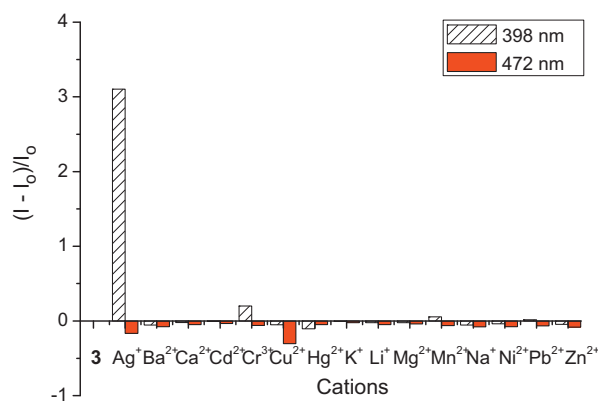


Fig. 8. Fluorescence intensity changes of ligand **3** (10 μM), upon the addition of 15 metal perchlorates (10 equiv, Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Ag⁺, Cu²⁺, Ni²⁺, Cd²⁺, Hg²⁺, Zn²⁺, Mn²⁺, Pb²⁺, and Cr³⁺) in aqueous H₂O/MeOH/CHCl₃ (v/v, 10:88:2) cosolvent at 25 °C (excitation wavelength = 342 nm).

98:2) cosolvent (Fig. 2a) to that of 3.1 in 10% aqueous methanol solution (Fig. 8) when 10 equiv of Ag^+ was added.

4. Conclusion

A conjugated triazolylpyrene fluoroionophore was synthesized and introduced into the lower-rim of a calix[4]arene scaffold with *proximal* disubstitution in ligand **3** and *distal* disubstitution in ligand **5**. Both ligands **3** and **5** possessed a high selectivity toward Ag^+ relative to most other metal ions in $\text{MeOH}/\text{CHCl}_3$ (v/v, 98:2) polar protic cosolvent where the conjugated triazolylpyrene fluoroionophore is proved to be not only an ionophore but also a more sensitive signaling unit than the conventional triazolyl- CH_2 -pyrene fluoroionophore. In addition, the orientation of the fluoroionophore was proved to influence the fluorescent response in the event of metal ion binding where the proximal orientation of the lower-rim fluoroionophores of calix[4]arene, ligand **3**, showed a ratiometric fluorescent response for Ag^+ ; whereas, the lower-rim distal orientation of fluoroionophores of calix[4]arene, ligand **5**, did not show ratiometric response to Ag^+ . Finally, the highly selective and ratiometric detection of Ag^+ by **3** is not interfered by most other metal ions in polar protic solvent $\text{MeOH}/\text{CHCl}_3$ (v/v, 98:2) and it works even in 10% aqueous methanol solution. In addition, the optimized geometries of complexes **3**- Ag^+ and **5**- Ag^+ were calculated by DMol3 which provided rational explanations for the observed fluorescence and ^1H NMR spectral changes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2012.06.014>.

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