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Sugar thiacrown-ether appended calix[4]arene as a selective chemosensor for Fe^{2+} and Fe^{3+} ions

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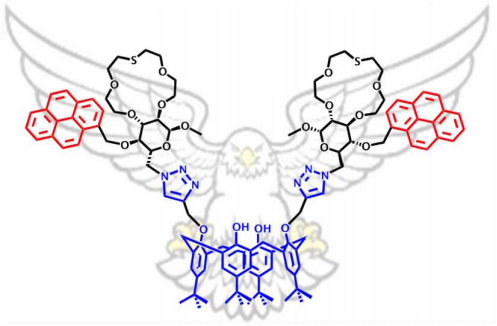
A fluorescent chemosensor derived from sugar-thiacrown-ether appended calix[4]arene, coupled with pyrene units as fluorophore was designed and synthesized. Upon the addition of Fe^{2+}, or Fe^{3+}, the fluorescence intensities are significantly quenched indicating high selectivity for these two metal ions over other transition metals investigated.



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A new fluorescent chemosensor derived from sugar-thiacrown-ether appended calix[4]arene, coupled with pyrene units was designed and synthesized. Upon the addition of Fe^{2+}, or Fe^{3+}, changes observed in both the fluorescence and absorption spectra. The fluorescence intensities are significantly quenched indicating high selectivity for these two metal ions over other transition metals investigated.

Introduction

Iron is an essential element in biological and chemical processes notably in cellular metabolism and enzymatic reactions in human body.[1-5] Both its deficiency and excess can provoke a variety of pathological conditions to a human body, where low levels of iron can lead to anemia, while its excess presence leads to hemochromatosis which causes damage to lipids, nucleic acids, and proteins.[6-9] Significant efforts have been devoted to the development of iron selective chemosensors. However, the design and synthesis of suitable sensors remain challenging.[10-13] Sensors derived from the metal ion-induced changes in fluorescence are appealing owing to the practical benefits in biochemistry, analytical and environmental chemistry.[14]

In recent years, calixarenes are being selected as one of the potential ionophores due to their intrinsic selectivity and recognition ability towards specific metal cations with appropriate modifications on either the upper or lower rims, which lead to a large variety of derivatives.[15-18] Numerous ion-binding properties of fluoroionophores involving combination of calix[4]arenes and pyrenes, have proved to be useful in ion sensing and recognition.[19-22] Pyrene is one of the preferred fluorophore due to its efficiency in excimer formation and emission and it can be attached either on the upper or lower rim of the calixarene.[23] Most of the sensors made up of two covalently linked pyrene moeties connected through a common binding unit, often show ratiometric fluorescence response due to the conformational changes before and after binding that directly affects the excimer-monomer emissions.[24]

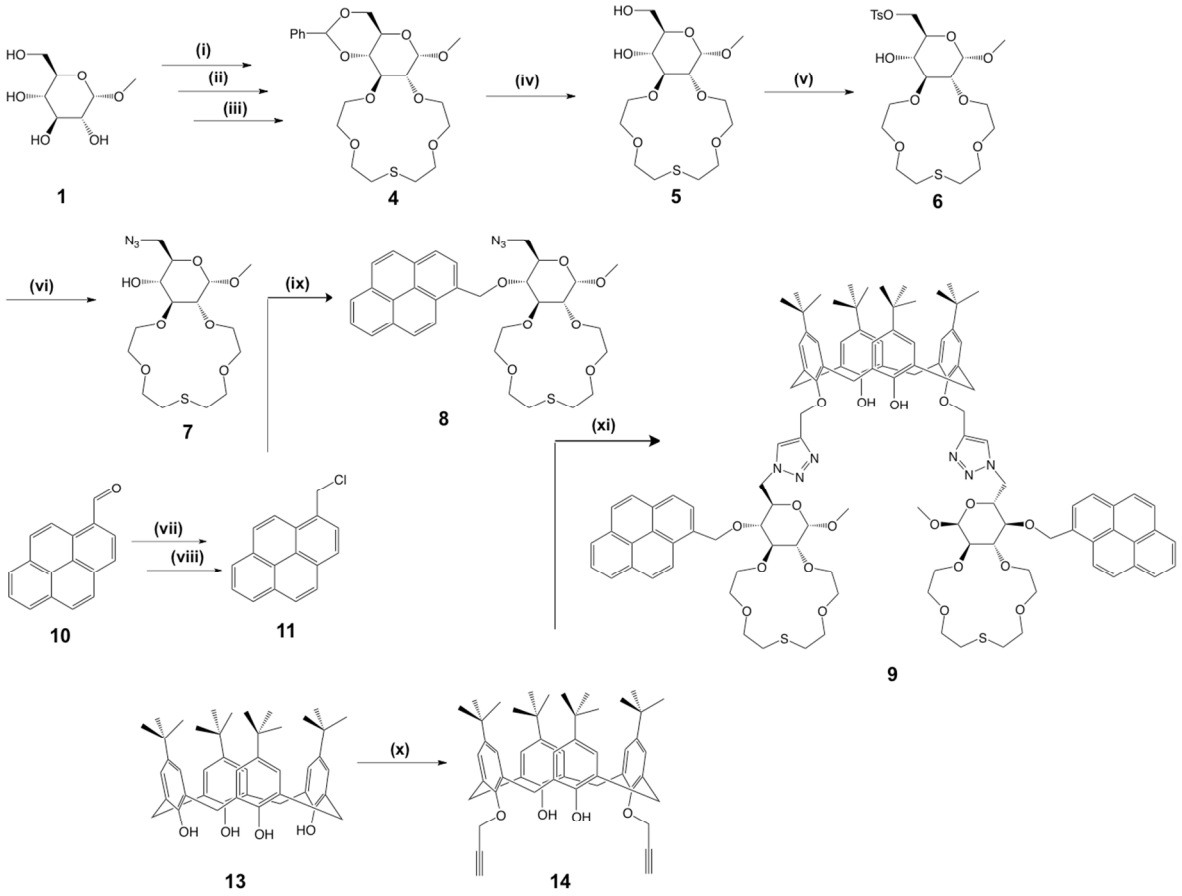
Recently, we have developed an interest in the synthesis of sugar incorporating crown ethers for various purposes.[25,26] In an ensuring present study, we design a fluorescent chemosensor using a sugar unit modified with thiacrown-ether developed in our previous work. This new design incorporates crown-ether moieties to calix[4]arene differently from those reported calix[4]crown chemosensors.[15,27] Two sugar thiacrown-ether units are appended to the lower rim of a tert-butylcalix[4]arene unit by 1,2,3-triazole linker as an ionophore along with a pyrene (a flourophore) attached directly to each sugar ring. Bioinspired by "an eagle hunting its prey", where the eagle's body is represented by a calix[4]arene, while the pyrene attached thiacrown-ether is its wings, the present design has notable advantage. For example, the triazole linker allows flexibilities to the crown ether units to move closer or further from each other to fit certain ionic species amongst many while the oxygen units of the crown ether form a rigid base, which is expected to enhance the binding affinity for metal ions. Related chemosensor system involving calix[4]arene linked by bistriazoles has been previously reported by Vicens and coworkers where two pyrenyl appended calix[4]arenes coupled with 1,2,3-triazole linkers displayed selective sensing towards Cd^{2+} and Zn^{2+}.[20] In a separate study reported by Chang and coworkers, a chromogenic calix[4]arene sensor bearing bistriazoles and azophenols showed selectivity towards Ca^{2+} and Pb^{2+} ions.[28]

In this study, we report an enhanced fluorescent quenching effect for Fe^{2+} and Fe^{3+} chemosensor for which the thiacrown-ether moieties act as the Fe^{2+} and Fe^{3+} chelator, while the pyrene groups experience changes in the optical signal during the event of metal ion recognition. It is noteworthy that many reported Fe^{3+} chemosensors response to significant fluorescence quenching *via* electron transfer or energy transfer by paramagnetic d^5 Fe^{3+} ion and commonly suffered from interference from either Al^{3+}, Cr^{3+} and Cu^{2+}.[29-31] Several iron metal chemosensors related to calix[4]arene integrating various chelators has been reported. For example, Liu et al. demonstrated that selective quenching of fluorescent intensity was achieved for calixarene possessing two 3-alkoxy-2-naphthoic acid moieties in the presence of Fe^{3+} while, Zhan et al. synthesized a pyridyl-appended calix[4]arene, which exhibits distinct colorimetric response to Fe^{3+}.[32,33]

Results and discussion

The chemosensor compound was prepared from an α-methyl glucopyranoside by a multi-step synthesis approach as summarized in Scheme 1. Due to the different reactivities of the sugar hydroxyl groups, the methyl glucopyranoside plays an important role in this synthetic outline. Selective protection of 4,6-hydroxyls can be achieved by benzylidination with

benzaldehyde dimethyl acetal,[34] while the thiacrown-ether is constructed at the hydroxyls in 2,3-positions *via* two steps. Compound **2** was subsequently treated with bis(2-chloroethyl) ether under basic condition (NaOH) in the presence of phase transfer catalyst, (PTC) to produce **3** and followed by cyclization with sodium sulphide nonahydrates in aqueous ethanol which affords compound **4**. The benzylidene group has been removed under acidic condition in mixture of chloroform:methanol (50:50) affording **5**. The treatment of **5** with *p*-toluene sulfonyl chloride in pyridine at 0□C furnished compound **6**. Selective tosylation takes place at the primary hydroxyl group on the 6-position since this position is more reactive than the secondary on the 4-position. Nucleophilic substitution of tosylate with azide group can be achieved in DMF at 80□C to give **7** in an almost quantitative yield. The (chloromethyl)pyrene, **12**, was prepared by two steps from the commercially available pyrenecarbaldehyde, **10**, by reduction with sodium borohydride in methanol followed by hydrochloric acid in the presence of THF:water. Williamson etherification[35] of **7** and **12** under phase transfer catalyst gave compound **8**. In a separate synthetic step, compound **14** can be prepared from calix[4]arene with propargyl bromide according to the standard procedure.[36] Following to the observation reported by Sharpless and Meldal, the Huisgen1,3-dipolar cycloaddition of alkynes and azides to give 1,2,3-triazoles can be catalyzed by Cu(I).[37] The final step in this sequence was performed by the coupling of compound **8** and **14** using the "click chemistry"[38] to give **9** in 68% using CuCl catalyst. Full synthetic procedures are described in Experimental Section.



Reagents and catalysts (i) $PhCH(OCH_3)_2$, $TsOH.H_2O$, DMF; (ii) $ClCH_2CH_2OCH_2CH_2Cl$, NaOH, toluene; (iii) $Na_2S.xH_2O$, EtOH, H_2O; (iv) $CHCl_3$/MeOH, $TsOH.H_2O$; (v) TsCl, pyridine, 0°C; (vi) NaN_3, DMF, 80°C; (vii) $LiAlH_4$, THF; (viii) HCl, THF/H_2O; (ix) NaOH 50%, toluene, *n*-butyl amomonium hydrogen sulfate; (x) propargyl bromide, K_2CO_3, acetone; (xi) CuCl, sodium ascorbate, EtOH, DCM

Scheme 1: Synthesis routes affording compound **9**.

In the absence of metal ion, the fluorescence spectrum of pristine **9** exhibits both monomer (378, 395 nm) and excimer (472 nm) emission bands when irradiated at 344 nm which is attributable to the interaction of two intramolecular pyrene units through a π–π interaction coupled by triazole group[20,39,40] in $MeCN/CHCl_3$, Figure 1. We investigated the metal ion binding abilities of **9** based on fluorescence and UV/Visible changes of their $MeCN/CHCl_3$ (1:1, v/v) solutions produced by addition of acetate and halide salts of the transition metal cations (Cr^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Sn^{2+}, Hg^{2+} and Pb^{2+}). The fluorescence spectra of **9** in the presence of different metal ions, given in Figure 1, shows that the fluorescence intensities become suppressed to a lower yet significant level, when the metal ions (100 equiv.) were added to **9**. Binding of metal ion through the coordination of O-atoms of the thiacrown-ether ring in **9** causes the quenching effect for both the excimer and monomer emission. Dramatic fluorescence intensity quenching for **9** due to the addition of Fe^{2+} and Fe^{3+} ions (in excess) suggests that **9** uniquely recognize the Fe^{2+} and Fe^{3+} ions in preference to other related heavy transition metal

ions. Both of the ions experience excited state electron transfer from **9** during interaction and consequently form the ground state non-fluorescent complexes. Substantial absorbance enhancement of **9** in the UV-visible absorption spectra (Figure 2) is observed in the presence of Fe^{2+} and Fe^{3+} along with an induced blue-shift and significant broadening which is caused by efficient positive polarization of the thiacrown-ether oxygen atoms during the metal ion interaction thus results in destablisation of the excited state in electron transition.[41] We also note that the quenching effect in fluorescence and enhanced absorbance in UV-visible are more significant notably for Fe^{3+} than for Fe^{2+}.

Competition experiments to evaluate the effect of competing metal ions which were carried out by adding Fe^{2+} and Fe^{3+} (100 equiv.) to **9** (10 μM) in the presence of other metal ions (100 equiv.) demonstrate that the Fe^{2+} and Fe^{3+}-induced fluorescent responses were not extensively interfered by the coexistent metal ions. Figure 3 illustrates the fluorescence intensity of **9**-Fe^{2+} and Fe^{3+}, which was marginally quenched upon adding other transition metals, whereas no significant variation was found upon adding Zn^{2+}. These observations indicate that **9** has high selectivity for Fe^{2+} and Fe^{3+} metals when distinguishing between numerous transition metal ions.

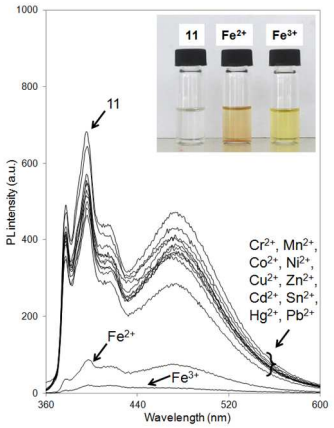


Figure 1. Fluorescence spectral change for **9** (1.0 x 10^{-5} M) recorded in MeCN: $CHCl_3$ (1:1, v/v) in presence of various cations. Inset: color change of **9** upon addition of Fe^{2+} and Fe^{3+}.

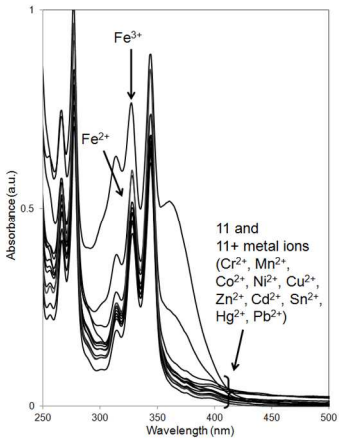


Figure 2. UV/Vis spectral change for **9** (1.0 x 10^{-5} M) recorded in MeCN: $CHCl_3$ (9:1, v/v) in presence of various cations.

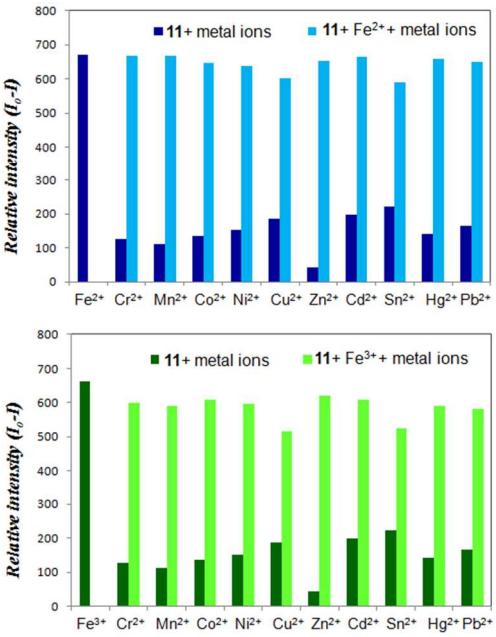


Figure 3. Fluorescence changes $(I - I_o)$ of (a) Fe^{2+} and (b) Fe^{3+} in **9** upon addition of 10 eq. of various metal ions in MeCN/$CHCl_3$ (1:1, v/v), excitation at 343 nm.

We investigated the binding properties of **9** with Fe^{2+} and Fe^{3+} by carrying out fluorescence and UV/Vis titration studies in MeCN/$CHCl_3$ (1:1, v/v) with the ligand concentration fixed at 10 μM in all titration experiments in order to further understand the sensing behaviour of **9** towards Fe^{2+} and Fe^{3+} at molecular level (Figure 4 & 5). Upon addition of increasing concentration of Fe^{2+} and Fe^{3+} (0–100 equiv.) to **9**, the fluorescence emission intensity decreased progressively on the excitation of **9** at 344 nm and reached the saturation level when 100 equiv. of Fe^{2+} and Fe^{3+} ions were added. A detailed representation of the fluorescent response of **9** versus gradual titration of Fe^{2+} and Fe^{3+} is illustrated in Figure 4. The metal complexation of **9** also causes absorbance or the wavelength changes in the UV/Vis spectra. A gradual increase in absorbance was observed with addition of Fe^{2+} and Fe^{3+} as shown in the titration profiles in Figure 5.

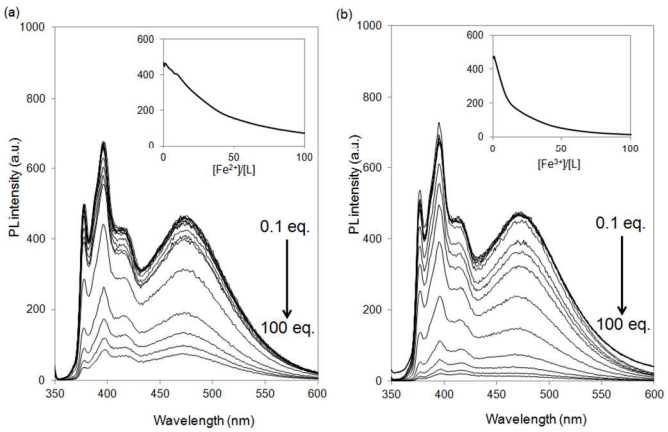


Figure 4. Titration profiles for fluorescence emission spectra of (a) Fe^{2+} and (b) Fe^{3+} complex in MeCN:$CHCl_3$. Excitation wavelength is 344 nm. Ligand concentration is 1.0 x 10^{-5} M.

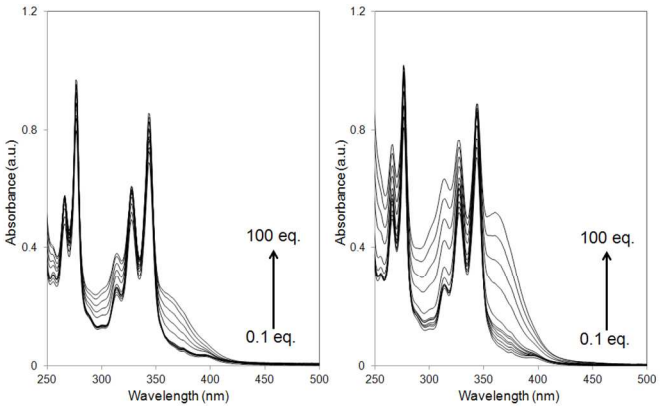


Figure 5. Titration profiles for UV/Vis absorbance spectra of (a) Fe^{2+} and (b) Fe^{3+} complex in MeCN:$CHCl_3$. Ligand concentration is 1.0 x 10^{-5} M.

Analysis of the Benesi–Hildebrand[42] plots reveals the 1:1 stoichiometry formation between **9** and Fe^{2+} or Fe^{3+} ions and the findings are in good agreement with the Job's plot, (Figure S1, ESI). The stoichiometry for **9** and Fe^{2+} or Fe^{3+} was found to be 1 : 1 based on the Job's method of continuous variation. The apparent binding constants K_a for **9**-metal complexes were estimated to be 900 M^{-1} for Fe^{2+} and 875 M^{-1} for Fe^{3+}, obtained from the slope and intercept in the plot of $1/\Delta F$ against $1/[Fe^{2+}]$ or $1/[Fe^{3+}]$ (Figure S2, ESI). The kinetics of the photophysical quenching process involving homogeneous medium with single component of decay is investigated using the Stern-Volmer relationship, which is represented by a simple equation:

$$\frac{Io}{I} = 1 + K_{sv}[Q] \quad (1)$$

where I_o and I are the fluorescence intensities in the absence and presence of a quencher, respectively, K_{sv} is the Stern-Volmer quenching constant and $[Q]$ is the concentration of the quencher. However, the plots of I_o/I versus Fe^{2+} or Fe^{3+} ion concentration deviate from linearity of the Stern-Volmer equation, showing a steep upward curvature in the plot, which suggests that the simple Stern-Volmer relationship did not sufficiently describe the data, (Figure S3, ESI). This can be related to the presence of transient component in the complicated quenching phenomena involving combination of both dynamic (diffusive encounters between the excited state fluorophore and the quencher) and static (ground state complex formation between the fluorophore and the quencher) quenching. We used a modified Stern–Volmer relationship, proposed in many reported systems[43,44] involving the mechanisms described above to obtain a calibration plot.

$$Log\left[\frac{Io}{I}\right] = K_{sv}[Q] + C \quad (2)$$

where I, I_o and $[Q]$ are the same parameters as mentioned earlier while C is the constant of equation. The equation depicts that I_o/I and concentration of the quencher have good linearity in the range of 1 to 100 μM (R^2 = 0.9967 for Fe^{2+}; R^2 = 0.9857 for Fe^{3+}). From slope of the plots, the Stern–Volmer constant (K_{sv}) is calculated to be for 947 M^{-1} Fe^{2+} while 1636 M^{-1} for Fe^{3+}.

In the event where combined dynamic and static quenching may be the cause of the reduction in fluorescence intensity, a relationship of the form of second order in quencher concentration is used to explain the quenching data:

$$\frac{Io}{I} = (1 + K_D[Q])\ (1 + K_s[Q]) \quad (3)$$

$$\frac{Io}{I} = 1 + (K_D + K_s)[Q] + K_D \cdot K_s[Q]^2 \quad (4)$$

$$\left(\frac{Io}{I} - 1\right) \cdot \frac{1}{[Q]} = (K_D + K_s) + K_D \cdot K_s[Q] \quad (5)$$

where K_D is the collisional quenching constant and K_S is the constant for static quenching or known as the association constant for complex formation between the quencher and the fluorophore. A plot of $(I_o/I - 1)/[Q]$ versus $[Q]$, (Figure S4, ESI) shows good linearity for both Fe^{2+} and Fe^{3+} complexes (R^2= 0.984 and R^2= 0.979 respectively). Considering the greater static quenching constant as opposed to the collisional quenching constant, the static quenching mechanism is predominantly operative in this system. It is reasonable that the quenching phenomenon is rationalized by the molecular conformational change caused by two outward-facing thiacrown-ether groups turning inward to coordinate with Fe^{2+} and Fe^{3+}, Figure 6. In such conformation, intramolecular parallel stacking of the two pyrene units is no longer possible. This destroys the excited state of the conjugated pyrene units and essentially quenches the monomer and excimer emissions of **9**.

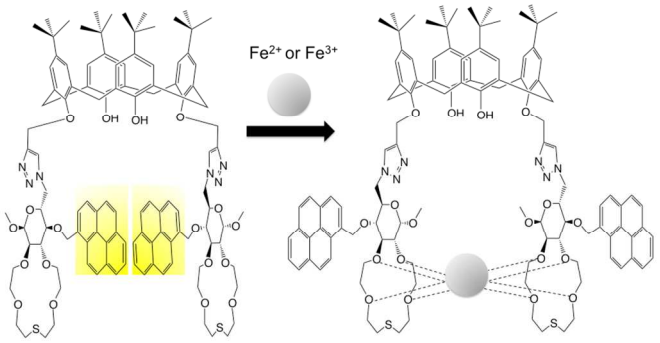


Figure 6. The proposed binding structure of **9**-Fe^{2+} and **9**-Fe^{3+}.

To confirm the postulation of the **9**-Fe^{2+} complex and **9**-Fe^{3+} binding structures, we optimized the geometries of the 1:1 ligand-to-metal ratio of these complexes (determined from the Job's plot measurement) using molecular mechanics energy optimization routine to determine the possible structure of the **9**-Fe^{2+} and **9**-Fe^{3+} complexes. The plausible binding mechanism with the lowest energy conformation of the complexes is schematically depicted in Figure 7. The energy-minimized geometry shows that both the pyrene moieties are oriented far from each other, and the metal ion is coordinated to the thiacrown-ether oxygen atoms (ball and stick represents thiacrown-ether, space-filling represents metal ion). The Fe^{2+}

and Fe^{3+} coordination with the thiacrown-ether resulted in the rigidification of **9** and inhibits the π–π stacking of the pyrene moieties subsequently prevents the formation of excimer.

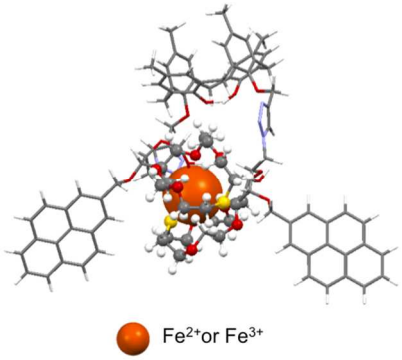


Figure 7: Energy-minimized geometry of **9** binding Fe^{2+} or Fe^{3+}.

Conclusions

In summary, we have synthesized a novel fluorescent chemosensor of a sugar thiacrown ether attached to the lower rims of tert-butylcalix[4]arene linked by 1,2,3-triazoles and coupled with pyrene units. The binding site geometry of the sugar thiacrown-ether calix[4]arene hybrid, in which the oxygens of thiacrown-ethers are arranged distally offers a suitable size and selectivity for metal ion recognition notably for Fe^{2+} and Fe^{3+}. Upon the addition of Fe^{2+} and Fe^{3+} the fluorescence emissions are quenched, implying higher selectivity for these two metal ions compared with other transition metals investigated. This study provides an alternative strategy to prepare organic sensing molecule, which has selectivity towards iron metals.

Experimental

General synthetic and characterization procedures

All reagents were obtained from commercial sources and used without further purification. Flash column chromatography was carried out on Silica Gel 60 (230-400 mesh, E. Merk). TLC was performed on pre-coated aluminum plates of Silica Gel 60 F254 (0.25 mm, E. Merck); detection was executed by immersing in a solution of (ethanol: water: H_2SO_4) (90:8:2) and subsequent heating by heat gun. Specific rotations were taken at ambient conditions and reported in 10^{-1} deg·cm^2·g^{-1}; the sample concentrations are in g·dL^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 600 MHz spectrometer. Chemical shifts are in ppm from Me_4Si, calibrated using the residual proton and carbon of the deuterated solvent. Proton peak assignments were performed with the aid of 2D NMR techniques (^{1}H-^{1}H COSY, HSQC/HMQC, and *J*-resolved); the hydrogen multiplicities of carbon peaks were determined using DEPT and PENDANT experiments. High-resolution mass spectra were recorded on an Agilent Technologies 6530 Accurate Q-TOF LC-MS system (MeOH–water eluents, 250 ºC hot nitrogen gas flow at 5 ml min^{-1} and electrospray ionization at 125 V) and Thermo Scientific Orbitrap Fusion with Orbitrap mass analyzer. Fluorescence and UV/Vis spectra were recorded with a Varians Cary Eclipse and Cary 60 spectrofluorophotometer. Stock solutions (1.0 mM) of the metal acetate and halide salts were prepared in MeCN/$CHCl_3$ (1:1, v/v).

Synthesis of Methyl 2,3-O-thia[15CR5]-glucopyranoside, 5

Compound **4** (4.6 g, 10 mmol) was dissolved in chloroform: methanol 1:1 (150 ml), *p*-toluenesulfonic acid (200 mg) was added. The mixture was stirred for 12 hours at room temperature. Distilled water (150 ml) was added and the organic phase washed with saturated sodium bicarbonate solution (2 x 50 ml). The organic phase was dried over magnesium sulfate, and the solvents were evaporated. The product was sufficient clean for the next step. [α]D -12.4° (c 1, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 4.86 (d, 1H; H-1, $3J_{1,2}$ = 3.8 Hz), 4.47 (dd, 1H; H-1, H-6eq, 3*J*6eq,6ax=12 , 3J5,6 =4.5 Hz), 4.40-4.03 (m, 5H; CH_2O, H-6eq), 3.87-3.46 (m, 13H; H-2, H-3, H-4, H-5, CH_2O), 3.43 (s, 3H; OCH_3), 2.95-2.68 (m, 4H; CH_2S); 13C NMR (150 MHz, $CDCl_3$) δ (ppm) 98.20 (C-1), 81.27/ 80.02(C-2/ C-3), 72.50/ 72.40/ 71.86/ 70.52/ 70.12 (5CH_2O), 70.00 (C-4), 69.91 (CH_2O), 69.35 (C-5), 63.43 (C-6), 55.26 (OCH_3), 31.66/ 31.54 (CH_2S); HRMS: [M+Na] calcd. for $C_{15}H_{28}O_8SNa$: 391.1403, 392.1436 (16%, 1^{13}C); found: 391.1403 (100%), 392.1426 (18%).

Synthesis of Methyl 6-O-toly-2,3-O-thia[15CR5]-glucopyranoside, 6

Compound **5** (3.7 g, 10 mmol) was dissolved in pyridine (30 ml) in two-necks round bottom flask and cool down to (5ºC) in ice bath. A solution of *p*-toluenesulfonyl chloride (2.47 g, 13 mmol) in pyridine (20 ml) was added dropwise over a period of 1 hour. The reaction was stirred for 2 hours, and then the temperature gradually rises to room temperature. The pyridine was evaporated and the residue was dissolved in chloroform and extracted with dilute solution of HCl (5%), then washed extensively with water. The chloroform layer was separated and dry with magnesium sulphate. The pure product was obtained after chromatography as yellow syrup, yield (4.1 g, 78 %); [α]D + 57.5° (c 1, CH_2Cl_2), ^{1}H NMR(600 MHz, $CDCl_3$) δ (ppm) 7.82 (d, 2H; Ph-CH, ^3J = 8 Hz), 7.36 (d, 2H; Ph-CH, ^3J = 8 Hz), 4.80 (d, 1H; H-1, $^3J_{1,2}$ = 3.8 Hz), 4.34-4.29 (m, 3H; CH_2O, H-6eq), 3.80-3.72 (m, 10H; CH_2O), 3.69 (dt, 1H; H-6ax, $^2J_{6ax,eq}$ =10, $^3J_{5,6}$ =3.1 Hz), 3.62-3.54 (m, 3H; H-4, CH_2O), 3.52 (dd □ t, 1H; H-3, $^3J_{3,4}$ = 9.5, $3J_{2,3}$ = 8.5), 3.34 (dd, 1H; H-2, $^3J_{2,3}$ = 8.5, $^3J_{1,2}$ = 3.4), 3.39 (s, 3H; OCH_3), 2.94-2.81 (m, 2H; CH_2S), 2.76-2.69 (m, 2H, CH_2S), 2.47 (s, 3H; CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 144.88/ 132.96 (2 C-Ph), 129.87(2 CH-Ph), 128.02 (2 CH-Ph), 97.40 (C-1), 80.87 (C-3), 79.90 (C-2), 72.47 (C-6), 72.42/ 71.88/ 70.47, 70.15/ 69.87 (CH_2O), 69.50 (C-4), 69.08 (C-5), 68.77 (CH_2O), 55.26 (OCH_3), 31.62/ 31.51 (CH_2S), 21.66 (Ph-CH_3). HRMS: [M+Na] calcd. for $C_{36}H_{68}O_{15}Na$: 545.1491, 546.1525 (24 %, 1 ^{13}C); found: 545.1492 (100 %), 546.1527 (22 %).

Synthesis of Methyl 4-O-pyrenomethylene-6-azido-2,3-O-thia[15CR5]-glucopyranoside, 8

Compound 6 (2.5 g, 4.8 mmol) was dissolved in DMF (30 ml), sodium azide (0.65 g, 10 mmol) was added the reaction was heated to 90 ºC overnight. After the TLC showed there was no starting material left, the DMF was evaporated and the mixture was dissolved in Toluene (50 ml) and washed extensively with water to remove the excess of sodium azide. The solution was cool down to 0 ºC, sodium hydroxide solution (20 ml, 50%), tetraammonium hydrogen sulphate (0.82 g, 2.4 mmol) were added and stirred for 30 minutes. 1(chloromethyl)pyrene (1.5 g, 6 mmol) was added and the mixture was stirred overnight. Distilled water (50 ml) was added, and the organic layer was separated, dried over magnesium sulphate and the toluene was evaporated. The flash chromatography was applied to get the desired product as yellow syrup, yield (2.1 g, 72%); [α]D + 39° (c 1, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 8.33 (d, 1H; Ph-CH) , ^3J = 8.0), 8.26-8.14 (m, 8H; Ph-CH), 5.66 (d, 1H; Pyr-CH, ^3J = 12 Hz), 5.36 (d, 1H; Pyr-CH, ^3J = 12 Hz), 4.88 (d, 1H; H-1, $^3J_{1,2}$ =3.8 Hz), 4.39 (dt, 1H; CH_2O, ^2J =9.5, ^3J =2.8 Hz), 3.88-3.74 (m, 11H; CH_2O, H-4), 3.72 (dd□t, 1H; H-33, $J_{3,4}$ =9.5, $^3J_{3,2}$ =8.5 Hz), 3.66-3.54 (m, 2H; H-5, CH_2O), 3.44 (s, 3H; OCH_3), 3.38 (dd, 1H; H-6eq, $^2J_{6eq,ax}$ = 12, 3J5,6 = 2 Hz), 3.23 (dd, 1H; H-6ax, $^2J_{6eq,ax}$ = 12, ^3J = 5.5 Hz), 3.01-2.87 (m, 2H; CH_2S), 2.78-2.73 (m, 2H, CH_2S); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 132.01/ 131.21/ 130.90/ 130.7/ 129.91/ 129.23/ (6 C-Ph), 128.20/ 127.98/ 127.41/ 127.13/ 126.87/ 126.40/ 126.06/ 125.49/ 125.05/ 124.83 (9 CH-Ph), 97.47 (C-1), 81.68 (C-4), 80.42 (C-3), 77.92 (C-2), 73.05 (CH_2-Pyr), 72.53/ 72.42/ 71.86/ 70.68/ 70.19/ 70.16 (6 CH_2O), 70.00 (C-5), 55.26 (OCH_3), 51.27 (C-6), 31.61/ 31.51 (CH_2S). HRMS: [M+Na] calcd.

for $C_{32}H_{37}N_3O_7SNa$: 630.2250, 631.2283 (35 %, 1 ^{13}C); found: 630.2249 (100 %), 631.2282 (32 %).

Synthesis of Bis-O-[methyl 4-pyrenemethylene-2,3-O-thia(15CR5)-6-triazolyl] methylene calix[4]rene, 9

Compound **8** (0.6 g, 1 mmol) was dissolved in dichloromethane (30 ml), then compound 14 (0.36 g, 0.5 mmol) was added and stirred for 15 minutes. Sodium ascorbate (30 mg, 0.1 mmol) and cuprous chloride (10mg, 0.05 mmol) was added and the reaction stirred at room temperature for 24 hours. The solution was washed extensively with water, dried and the solvent was evaporated. Size exclusion chromatography using sephadex LH20 was performed followed by purification on silica gel column to furnish the desired product. Yield (0.5 g, 53 %) pale yellow syrup; [α]D + 140.9° (c 1, CH_2Cl_2); ^1H NMR(600 MHz, $CDCl_3$) δ (ppm) 8.53-8.35 (dd, 2H; Ph-CH , ^3J = 8.0, ^3J = 8.0), 8.31-7.93 (m, 16H; Ph-CH), 7.5 (s, 1H; CH-triazole), 7.3 (s, 1H; CH-triazole), 7.07-6.73 (m, 8H, Ph-CH), 5.55 (d, 1H; Pyr-CH_2, ^2J = 12 Hz), 5.45 (d, 1H; Pyr-CH_2, ^2J = 12 Hz), 5.35 (d, 1H; Pyr-CH_2, ^2J = 12 Hz), 5.22 (1, 2H; Pyr-CH_2, ^2J = 12 Hz), 4.80 (d, 2H; calix-CH_2, ^2J = 12 Hz), 4.35 (d, 2H; calix-CH_2, ^2J = 12 Hz), 3.80-3.25 (m, 28H; CH_2O, H-2, H-3, H-4, H-5), 3.20 (s, 6H; OCH_3), 2.95-2.80 (m, 4H; CH_2S), 2.69-2.60 (m, 4H, CH_2S), 2.27-2.04 (m, 8H; $PhCH_2O$) 1.35-1.25 (s, 36H; C-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 150.53/ 150.40/ 149.61/ 149.58/ 149.52/ 147.56/ 147.39/ 147.34/ 144.46/ 141.88/ 141.79/ 141.70/ 132.77/ 132.60/ 132.45/ 132.35/ 132.30/ 131.49/ 131.46/ 131.27/ 131.25/ 131.16/ 130.82/ 130.71 (C-Ar), 126.04/ 126.00/ 125.94/ 125.86/ 125.80/ 125.72/ 125.66/ 125.56/ 125.53/ 125.36/ 125.31/ 125.20/ 125.17/ 125.11/ 125.06/ 124.96/ 124.79/ 124.74/ 124.69/ 123.31/ 123.20 (CH-Ar), 97.07 (C-1), 97.04 (C-1'), 81.71 (C-4), 81.39 (C-4'), 80.36 (C-3), 80.24 (C-3'), 78.85 (C), 77.75 (C-2), 77.64 (C-2'), 76.28 (C), 72.90/ 72.84/ 72.47/ 72.37/ 72.33/ 71.97/ 71.89/ 70.61/ 70.49/ 70.35/ 70.27/ 70.01/ 69.79 (CH_2O), 69.16 (C-5), 69.03 (C-5'), 63.81/ 63.16 (CH_2O), 55.26/ 55.22 (2 OCH_3), 50.80 (C-6), 50.44 (C-6'), 34.04/ 33.95/ 33.90/ 33.85 (4 $\underline{C}CH_3$), 32.01/ 31.93/ 31.81/ 31.71 (4 CH_2), 31.53/ 31.51/ 31.48/ 31.41 (4 CH_2S), 31.02/ 30.99/ 30.94 ($C\underline{C}H_3$). HRMS: calcd. for $C_{114}H_{13}4N_6O_{18}S_2$: 1938.9196 (81 %), 1939.9230 (100 %, 1 ^{13}C); found: 1938.7349 (100 %), 1939.7396 (98 %)

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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