Supporting Information

A Selective Turn-On Fluorescent Sensor for Sulfur Mustard Simulants

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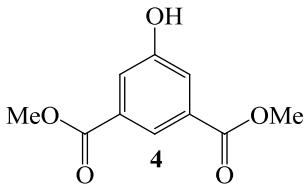
General

The ^{1}H NMR, ^{13}C NMR and HRMS were recorded from the University of Texas at Austin instrumental facility. Fluorescence measurements were carried our using a Photon Technology International Quanta Master spectrofluorimeter with an 814 photomultiplier detection system using a 75W xenon short arc lamp. All chemicals and reagents were bought from Aldrich or Fluka and used without further purification. Deionized water was degassed and used for analysis.

Caution: *CEES is a blistering agent and potential vesicant so it should be handled inside the fuming hood by wearing hand gloves.*

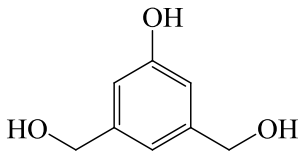
Synthesis of 5-hydroxyisophthalic acid dimethylester (4)



4

This compound was prepared by known method and the spetral data were matched with known one.[1]

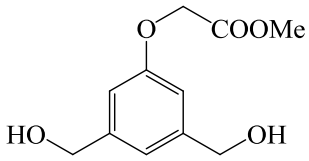
Synthesis of 5-hydroxybenzene-1,3-dimethanol (5)



5

This compound was prepared by known method and the spetral data were matched with known one.[2]

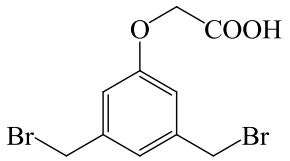
Synthesis of 2-[3,5-bis(hydroxymethyl)phenoxy]acetate 6



6

Compound **4** was prepared by known method and the spetral data were matched with known one.[2]

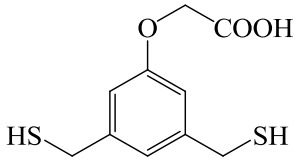
Synthesis of compound 2-[3,5-bis(bromomethyl)phenoxy]acetic acid 7



7

HBr 30% in acetic acid (3 ml) was added to a solution of compound **4** (0.5g) in acetic acid (2 mL). The reaction mixture was stirred at room temperature for 48 h. A while precipiate formed which was further diluted with water (50 mL). The precipitate was filtered and washed thoughly with water in order to remove any trace of acetic acid. The white precipitate was dried and charaterized. mp 147-149 ^{o}C; ^{1}HNMR (400 MHz, DMSO-*d*6) δ 13.05 (s, br, 1H), 7.10 (s, 1H,), 6.93 (s, 2H,), 4.66 (s, 2H), 4.63 (s, 4H), .22 (d, 1H, *J*=9.0 Hz), 7.57 (dd, 1H, *J*=8.4, 7.5 Hz); ^{13}CNMR (400 MHz, DMSO-*d*6) δ 170.3, 158.2, 140.2, 123.2, 115.6, 64.8, 34.2;HRMS m/z=336.8900 (M-H^{+}), calculated 336.8903.

Synthesis of 2-(3,5-bis(mercaptomethyl)phenoxy)acetic acid 1



1

2-[3,5-bis(bromomethyl)phenoxy]acetic acid **5** (0.48 g) was combined with thiourea (0.228 g) in ethanol (30 mL). The reaction mixture was stirred for 20 h and the solvent was removed at reduced pressure, leaving the bis(thiouronium) salt as a white solid. A solution of sodium hydroxide (0.5 g) in degassed water (30 mL) was added and the solution was refluxed for 4 h. The reaction mixture was cooled to 0 ^{o}C and acidified to pH 2 with 6N hydrochloric acid.The precipitate formed was filtered and washed thoughly with water in order to remove any trace of acid. The white precipitate of **6**was dried and charaterized.mp 112-114 ^{o}C. ^{1}H NMR (400 MHz, DMSO-*d*6)δ12.94 (s,br, 1H), 6.88 (s, 1H,), 6.74 (s, 2H,), 4.63 (s, 2H), 3.65 (d, 4H, *J*=8.4 Hz), 2.85 (t, 2H, *J*=8.4 Hz);^{13}CNMR (400 MHz, DMSO-*d*6) δ 170.5, 158.2, 143.4, 121.1, 113.0, 64.8, 28.0. HRMS m/z= 243.0155. (M-H^{+}), calculated 243.0155.

Fluorescence titration studies:

Fluorescence titrations of 6,7-dihydroxy-4-methyl coumarin in water with $Cd(NO_3)_2.4H_2O$

A stock solution of 6,7-dihydroxy-4-methyl coumarin(1.0 mg, 5.2 mM) was prepared by dissolving 6,7-dihydroxy-4-methyl coumarin (1.0 mg) in1 mL of buffer solution (bicarbonate-hydroxidebuffer, pH 9.5). pH of this solution was brought down to 9.1. This stock solution was then used to prepare a 51.5 μM solution of indicator using pure water (pH 9). A separate stock solution of $Cd(NO_3)_2.4H_2O$ (1.6 mg, 5.2 mM) was also prepared in pure water. This second solution wasthen used to prepare a 0.41mM solution of $Cd(NO_3)_2.4H_2O$. A 2 mL aliquot of the 6,7-dihydroxy-4-methyl coumarin solution was transferred to the fluorescence cuvette and the initial fluorescence was measured. The pH of indicator and $Cd(NO_3)_2.4H_2O$ solution was maintained at 9 by the addition of 0.1M NaOH solution. The titration was performed by adding successive 100μL aliquots of the titrant solution to the cuvette and recording the spectrum after each aliquot addition. A Cd^{2+} ion-indicator complex solution in which 90% saturated solution of indicator with $Cd(NO_3)_2.4H_2O$ was used for further titration study. This solution was prepared by mixing $Cd(NO_3)_2.4H_2O$ solution (64.4 μM) and indicator (26 μM) solution. pH at 9 was maintained by the addition of 0.1M NaOH solution

Fluorescence titrations of 1 with 8

Dithiol (0.5mg, 2.04m M) was dissolved in 1 mL of buffer solution (bicarbonate-hydroxide buffer, pH 9.5). pH of this solution was brought down to 9.1.This solution was titrated with aCd^{2+} ion-indicator complex solution (90% saturated solution of indicator with Cd^{2+} ion as prepared above). A 2 mL aliquot of the Cd^{2+} ion-indicator complex solution was transferred to the fluorescence cuvette and the initial fluorescence was measured. The titration was performed by adding successive 5 μL aliquots of the dithiol solution (1.02 mM) to the cuvette and recording the spectrum after each aliquot addition.

Fluorescence titrations of podand with Cd^{2+} ion-indicator complex, 8

Dithiol (1.0 mg, 4.09 mM) was dissolved in 1 mL of buffer solution (bicarbonate-hydroxide buffer, pH 9.5). pH of this solution was brought down to 9. This solution was reacted with CEES (1.12mg, 9.0 mM).The pH of this solution goes down which was maintained at 9 by the addition of 0.1M NaOH solution. This solution was titrated with aCd^{2+} ion-indicator complex solution (90% saturated solution of indicator with Cd^{2+} ion as prepared above). A 2 mL aliquot of the Cd^{2+} ion-indicator complex solution was transferred to the fluorescence cuvette and the initial fluorescence was measured. The titration was performed by adding successive 100 μL aliquots of the podand solution (CEES at 4.5 mM) to the cuvette and recording the spectrum after each aliquot addition.

Procedure for the capping of 1 by 4-phenyl-3-butyn-2-one, 10

Dithiol (1.0 mg, 4.09 mM) was dissolved in 1 mL of buffer solution (bicarbonate-hydroxide buffer, pH 9.5). pH of this solution was brought down to 9. This solution was reacted with 4-phenyl-3-butyn-2-one (8.6 mM).The reaction takes place in less than a min at 80 ^{o}C to adduct **11**. The pH of this solution remains at 9.

Fluorescence titrations of 2 with complex 8 (using capping agent)

Dithiol (1.0 mg, 4.09 mM) was dissolved in 1 mL of buffer solution (bicarbonate-hydroxide buffer, pH 9.5). pH of this solution was brought down to 9. This solution was reacted with CEES (1.12 mg, 9.0 mM) The reaction takes place in less than a min at 80 ^{o}C to give podand **2**. The pH of this solution goes down which was maintained at 9 by the addition of 0.1M NaOH solution. This solution was then reacted with 4-phenyl-3-butyn-2-one (4.3 mM) at 80 ^{o}C for a min. The pH of this solution remains at 9. This solution was titrated with a Cd^{2+} ion-indicator complex solution (90% saturated solution of indicator with metal, as prepared above). A 2 mL aliquot of the Cd^{2+} ion-indicator complex solution was transferred to the fluorescence cuvette and the initial fluorescence was measured. The titration was performed by adding successive 100 μL aliquots of the podand solution (CEES at 4.5 mM) to the cuvette and recording the spectrum after each aliquot addition.

Procedure for the detection of CEES on the surfaces by fluorescence titration

CEES was placed on the surfaces and then it was absorbed by filter paper, and this paper was extracted with DCM. The solvent was evaporated by nitrogen blow down, and the residue was reacted with aqueous solution of dithiol (1.0 mg, 4.09 mM) in 1 mL of buffer solution (bicarbonate-hydroxidebuffer, pH 9.5) at 80 ^{o}C for a min to give a solution of podand **2**. The pH of this solution goes down which was maintained at 9 by the addition of 0.1M NaOH solution. This solution was then reacted with 4-phenyl-3-butyn-2-one (8.6 mM) for at 80 ^{o}C for min. This solution was titrated with a Cd^{2+} ion-indicator complex solution (90% saturated solution of indicator with metal, as prepared above). A 2 mL aliquot of the Cd^{2+} ion-indicator complex solution was transferred to the fluorescence cuvette and the initial fluorescence was measured. The titration was performed by adding the entire solution of the podand to the cuvette and recording the spectrum after addition.

Procedure for prepared of CEES spiked soil sample and its detection in by fluorescence titration

2.0 g of soil was mixed with CEES in diethyl ether (2 mL) and this was allowed to stand for 20 min. The solvent from soil sample was evaporated by nitrogen blow down. CEES spiked soil sample was reacted with aqueous solution of dithiol (1.0 mg, 4.09 mM) in 1 mL of buffer solution (bicarbonate-hydroxide buffer, pH 9.5) at 80 ^{o}C for a min to give a solution of podand **11**.The suspension solution was

centrifuged and aqueous solution was carefully decanted. The pH of this solution goes down which was maintained at 9 by the addition of 0.1M NaOH solution. This solution was then reacted with 4-phenyl-3-butyn-2-one (8.6 mM) for at 80 ^{o}C for a min. This solution was titrated with a Cd^{2+} ion-indicator complex solution (90% saturated solution of indicator with Cd^{2+} as prepared above). A 2 mL aliquot of the Cd^{2+} ion-indicator complex solution was transferred to the fluorescence cuvette and the initial fluorescence was measured. The titration was performed by adding the entire solution of the podand to the cuvette and recording the spectrum after addition.

Detection of mustard simulant (without capping). In an attempt to investigate the interaction between **2** and **8** without capping of the solution of **2**. It was intended to react the **1** with CEES in similar manner as mentioned above to give **2**. In a fluorescence titration of **2** into the solution of **8**, we observed the enhancement of fluorescence intensity at 460 nm (Figure S1). The change in fluorescence was almost identical to that of previous case with capping process. This confirms that with the 2.2 equivalents of CEES (4.5 mM) with respect to **1**, the reaction goes to completion. Further, there are no remaining **1** to displace the indicator from the **8** complex. However, the capping is an important step in a detection system if mustard is present in lesser equivalent than required to react with **1**.

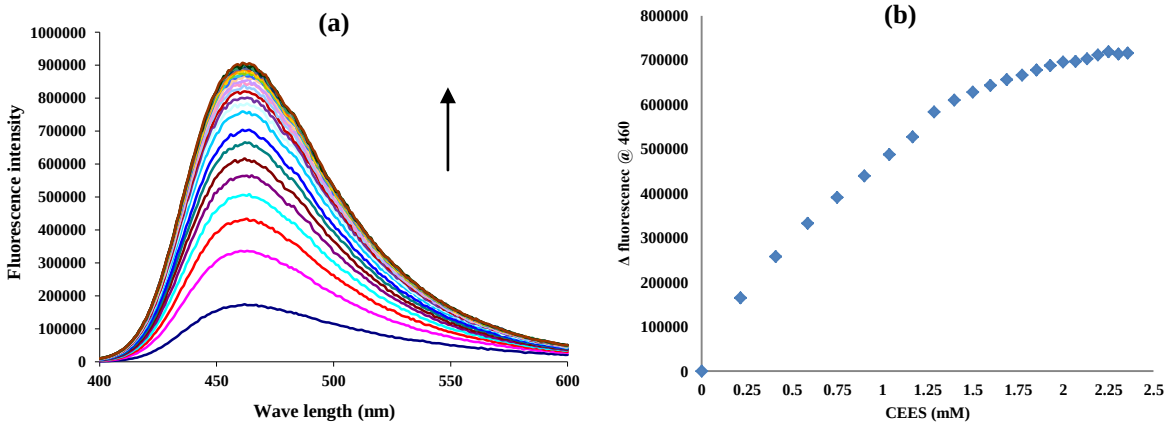


Figure S1. (a) Fluorescence titration of a solution of Cd^{2+} ion-indicator complex solution (containing $Cd(NO_3)_2$ at 64.4 µM and indicator at 26 µM) with podand **2** (containing CEES at 4.5 mM). All experiments were performed at 25 ^{o}C in aqueous solution, pH 9, using bicarbonate–hydroxide.(b) Isotherm showing increase in fluorescence intensity of **8** solution with added podand solution.

Figure S2. Mass spectrum of capped-dithiol, **11**

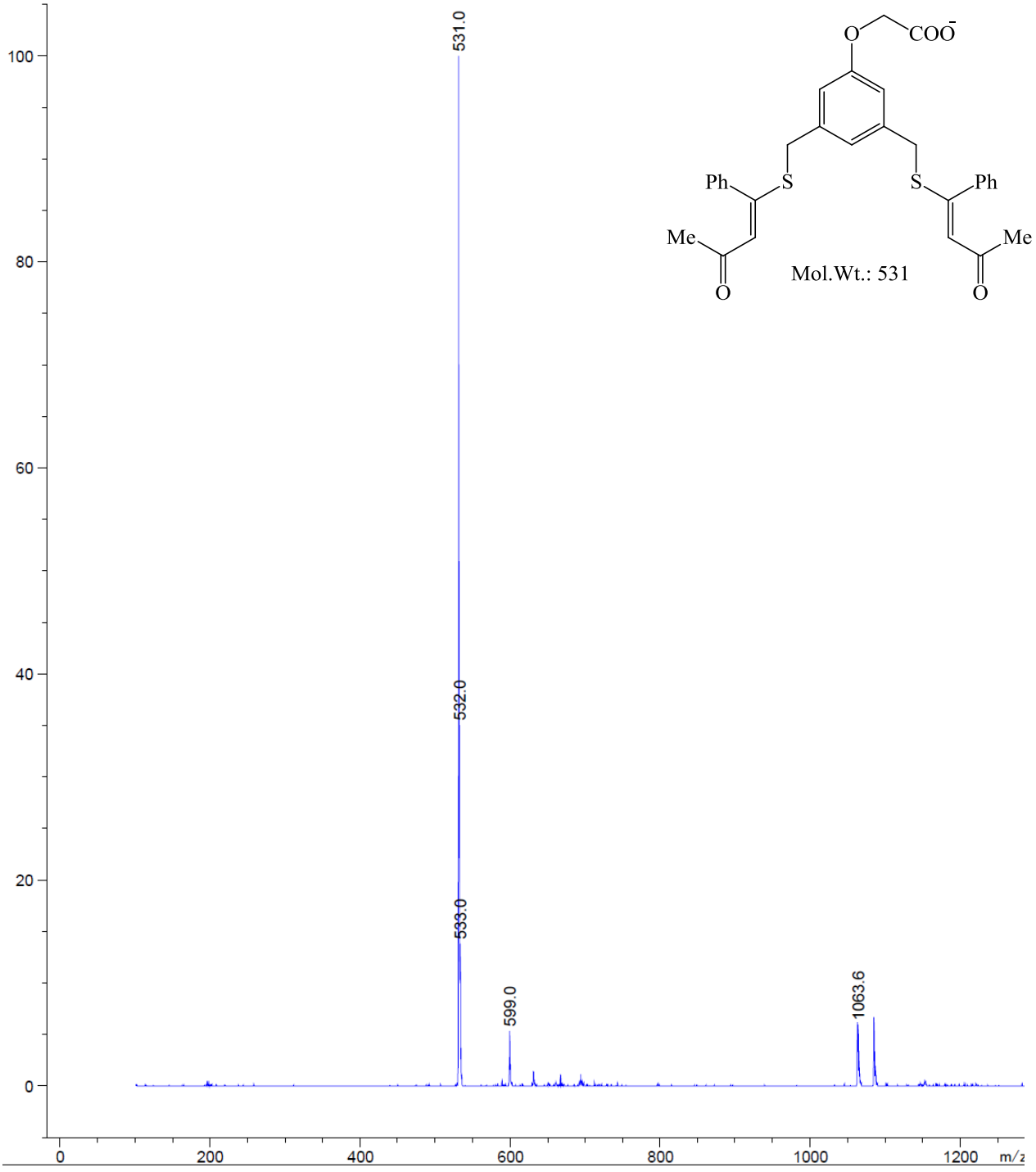
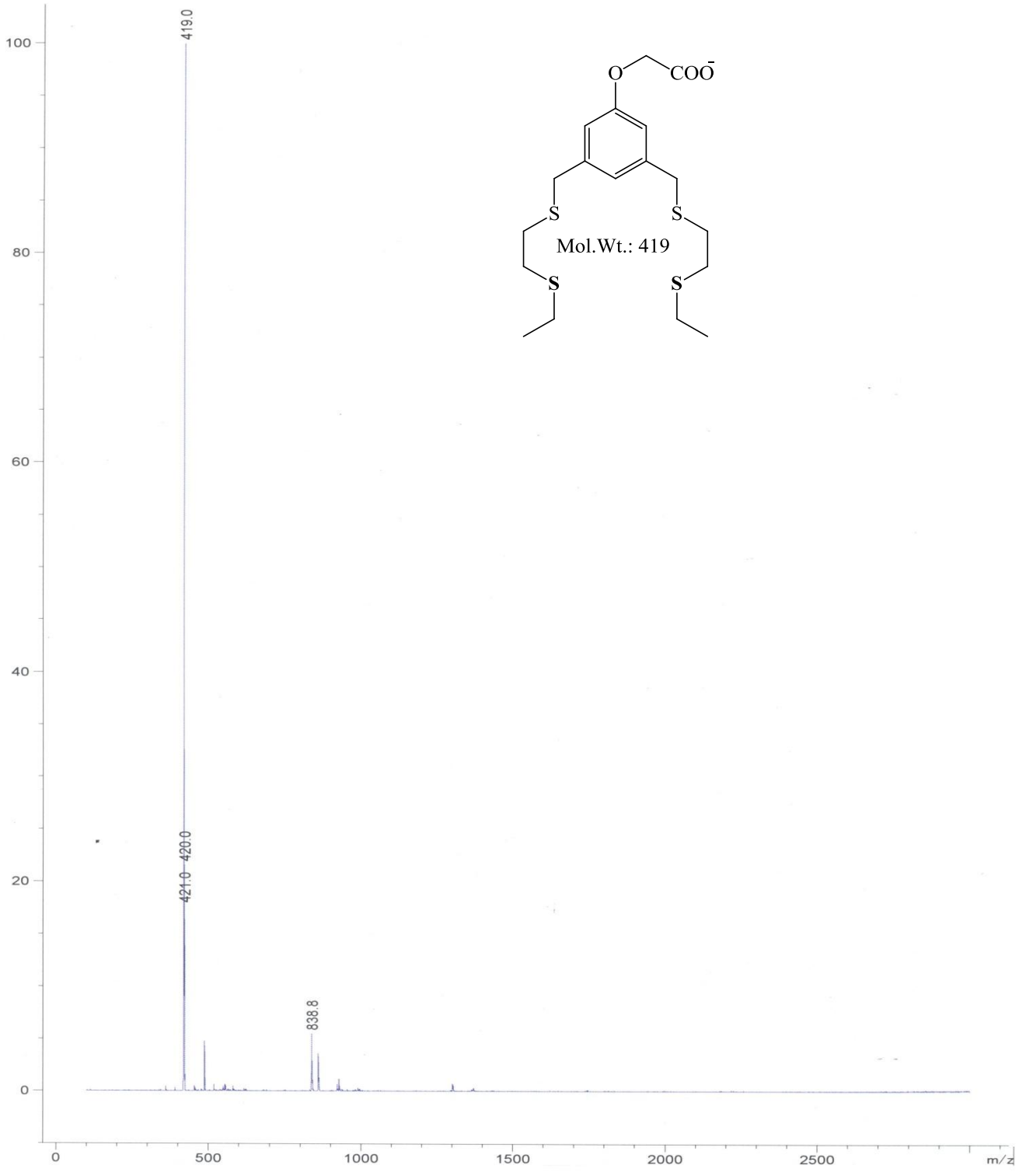


Figure S3 Mass spectrum of podand, **2**



Fluorescence titration studies

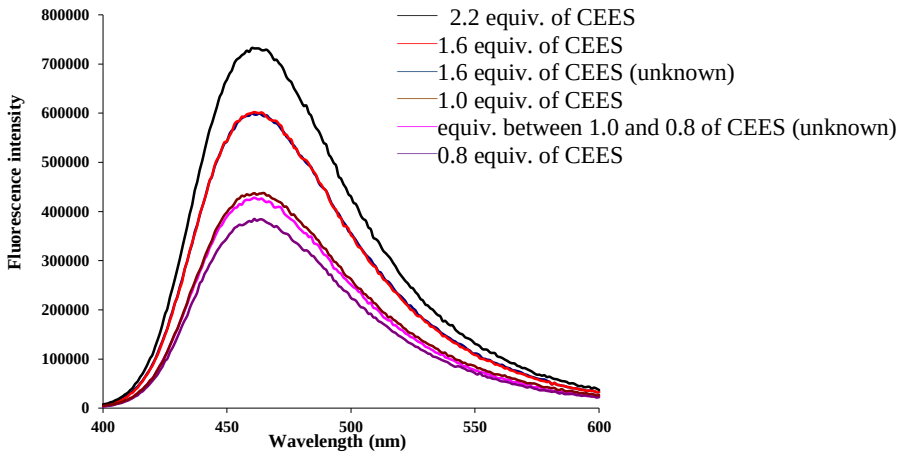


Figure S4. Fluorescence spectra of two unknown samples of CEES.

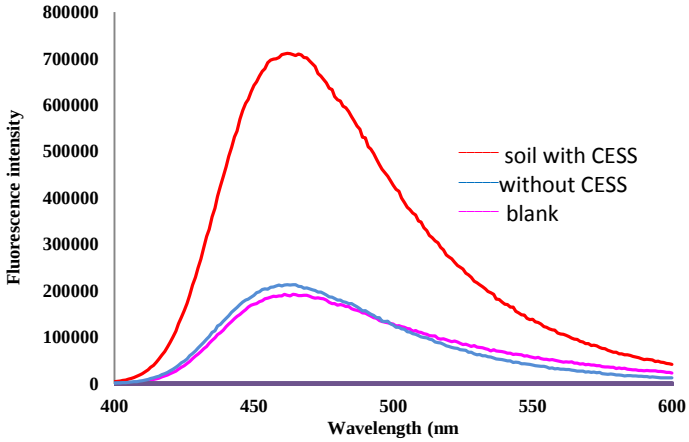


Figure S5. Fluorescence spectra of a Cd^{2+} ion-indicator complex solution with soil treated sample with CEES and without CEES.

References

(1) Jain, A. K.; Reddy, V. V.; Paul, A.; Muniyappa, K.; Bhattacharya, S. *Biochemistry*, **2009**, *48*, 10693.

(2) Li, W.; Fishkin, N. E.; Zhao, R. Y.; Miller, M. L.s; Chari, Ravi, V. J. *PCT Int. Appl*., 2010091150