Supporting information

Rhodamine-alginate conjugate as self indicating gel bead for efficient detection and scavenging of Hg^{2+} and Cr^{3+} in aqueous media

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Contents

1. Experimental:

Materials and Methods

Sodium alginate used was extracted from brown seaweed (*Sargassum tenerrimum*) which was collected from the Gujarat coast of India.[1] The M/G ratio of sodium alginate used in this study was 0.61.[2,3] 1-ethyl-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) has been purchased from Sigma-Aldrich chemicals.

The non-modified and modified sodium alginate was characterized by ^{1}H NMR and ^{13}C NMR spectra of alginate, rhodamine and rhodamine-alginate conjugate were recorded on a Bruker Avance-II 500 (Ultra shield) Spectrometer, Switzerland. Samples were dissolved in D_2O (40 mg/ml) and the spectra were recorded with 15000 accumulations, pulse duration 5.9 µs, acquisition time 1.2059 s and relaxation delay 6 µs using d_6-DMSO (ca. δ 39.5) as internal standard.

The Uv-vis absorption spectra were recorded on a Varian CARY 500 UV-VIS-NIR spectrophotometer. The fluorescence spectra were recorded at room temperature on EDINBURGH INSTRUMENTS Xe 900.

Preparation of receptor 1 conjugate

The receptor 1 was prepared by adding 121 mg (0.25 mmol) of amino ethyl rhodamine[4] to a solution of alginate (25 mL of solution, 1.00 mmol of sodium alginate monomer, 200 mg) in distilled water. The reaction mixture was stirred at room temperature for 10 min to facilitate a homogeneous dispersion of the rhodamine reagent in the reaction solution. Then 15 mg (0.13 mmol) of NHS and 47.25 mg (0.25 mmol) of EDC were added (ratios of reagents were calculated for a theoretical 25% molar modification of the number of carboxylic groups of alginate). After 12 h of stirring at room temperature the resulting mixture was dialyzed against 1 mM aq. HEPES buffer solution using a 1200 molecular weight cut-off dialysis tube (Dialysis tubing, D-7884, Sigma Chemical Co., USA) (3 cycles) and finally the dialyzate was precipited in isopropyl alcohol (1:3 v/v) to obtained the modified alginate conjugate.

^{1}H NMR signals of Na-alginate (500 MHz, D_2O): δ 5.65 [1H, C*H-1* of M], 5.88 [1H, C*H-1* of G], 3.96 [1H, C*H-2* of M], 4.06 [1H, C*H-2* of G], 3.58 [1H, C*H-3* of M], 4.12 [1H, C*H-3* of G],

3.68 [1H, C*H-4* of M], 4.43 [1H, C*H-4* of G], 3.83 [1H, C*H-5* of M], 4.56 [1H, C*H-5* of G].[3] [M = mannuronic acid; G = guluronic acid].

^{1}H NMR signals of amino ethyl rhodamine (500 MHz, d_6-DMSO): δ 7.77–7.73 (1H, Ar-*H*, m), 7.49-7.45 (2H, Ar-*H*, m), 7.0-6.98 (1H, Ar-*H*, m), 6.34-6.32 (6H, Ar-*H*), 2.99-2.92 (8H, CH_2) 2.49-2.47 (2H, CH_2), 2.21-2.17 (2H, CH_2) and 1.1-1.03 (12H, CH_3).

^{1}H NMR signals of receptor 1 (500 MHz, D_2O): δ 6.94-7.88 (10H, Ar-*H* of Rhd), 3.46-4.94 (1H, C*H-1* to C*H-5* of M or G), 2.85 (2H, t from Rhodamine fragment), 2.75 (8H, m from Rhodamine fragment), 2.58 (2H, t from Rhodamine fragment) and 1.03-1.10 (12H, t from Rhodamine fragment).

^{13}C NMR signals of Na-Alg (125 MHz, D_2O, d_6 –DMSO as internal standard; δ ppm): 102.3 [C-1 of MM], 101.45 [C-1 of GG], 71.50 [C-2 of MM], 66.26 [C-2 of GG], 72.90 [C-3 of MM], 70.53 [C-3 of GG], 79.35 [C-4 of MM], 81.55 [C-4 of GG], 77.31 [C-5 of MM], 68.53 [C-5 of GG], 177.4 [C-6 of MM], 176.68 [C-6 of GG]. These values were in good agreement with those reported in our previous work.[3]

^{13}C NMR signals of amino ethyl rhodamine (125 MHz, d_6 –DMSO δ ppm): 167.53, 154.00, 152.97, 148.76, 133.02, 130.78, 128.70, 124.00, 122.67, 108.51, 105.47, 97.62, 64.36, 44.08, 43.56, 42.09 and 12.83.

^{13}C NMR signals of receptor 1 (125 MHz, D_2O, d_6 –DMSO as internal standard; δ ppm): 175.24, 171.11, 156.44, 152.80, 131.27, 102.00, 101.53, 100.77, 94.05, 80.59, 78.58, 75.91, 72.01, 70.56, 67.81, 65.36, 64.46, 52.71, 45.28, 43.51, 24.43 and 10.53.

Preparation of receptor 1 beads with Ca^{2+} ion

Alginate-rhodamine beads were prepared according to method described in the literature.[5] **Receptor 1** was dissolved in distilled water at a concentration of 1% (w/w). The polymer solution was added drop wise fashion at room temperature to a stirred $CaCl_2$ solution (2%) using a syringe with a 0.8 mm diameter needle. After allowing 10 min of contact time, nearly round shaped, colour less beads were filtered off and dried in air, kept in vacuum desiccator for complete drying.

2. Determination of degree of substitution (DS) in the receptor 1:

To determine DS three methods were employed and the results were compared and the nitrogen content was considered to be ~10%.

(i) Total nitrogen estimation to determine DS

Total nitrogen was estimated by Kjeldahl method on a KEL PLUS- KES 201 Digestion unit attached to a KEL PLUS-CLASSIC DX Distillation unit (M/s PELICAN equipments, Chennai, India). Total nitrogen contents in alginate, amino ethyl rhodamine- and **receptor 1** were 0.02 ± 0.01%, 11.5 ± 0.11%, 1.08 ± 0.10% respectively and which suggested formation of amide derivative (Scheme 1) and the DS was 11% , which was calculated on the basis of the nitrogen content of the product using the following equation.

Degree of Substitution (DS; %) = [Observed N% / Calculated N%] x 100;

while the calculated N% for the sample was found to be 9.2%.

(ii) Assay of alginic acid carboxyl groups to determine DS

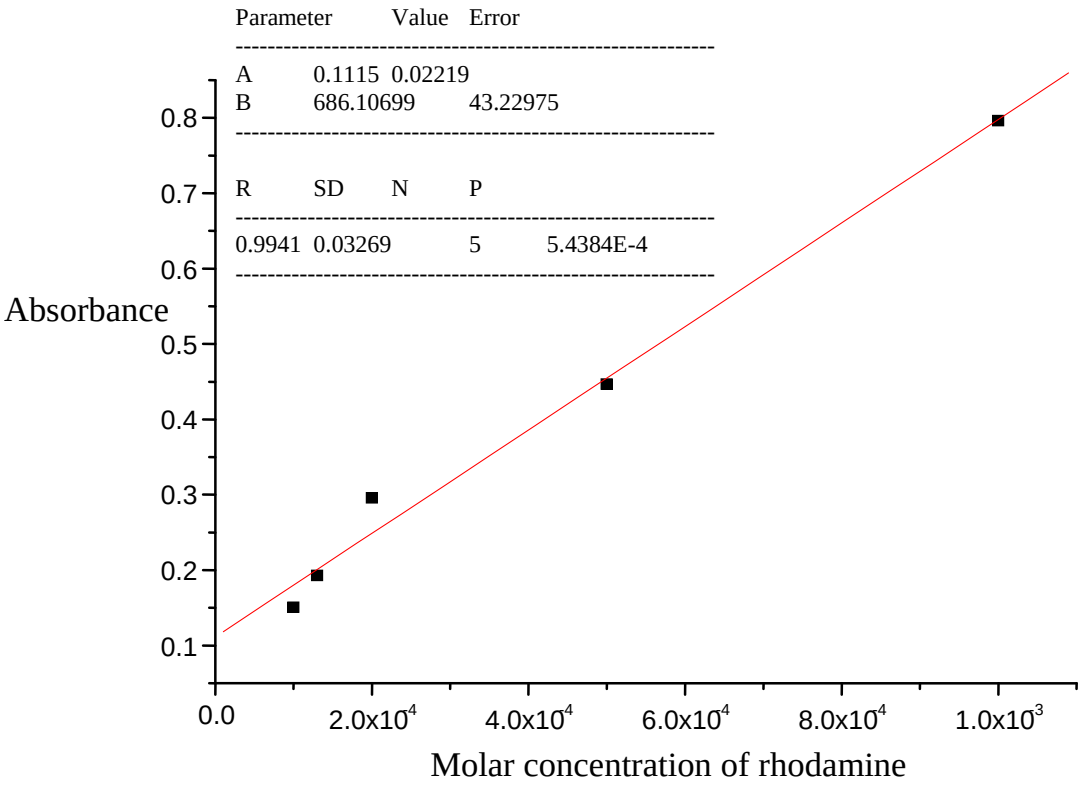
The degree of substitutions (DS) of **receptor 1** was determined to be 0.1 (10%) using the assay of alginic acid method as reported in the literature.[3,6]

(iii) Quantitative assay of rhodamine chromophores by Uv-vis spectroscopy to determine DS:

The extent of modification of Na-Alginate by amino ethyl rhodamine was evaluated by UV absorbance measurements at 560 nm, according to method reported in the literature.[7] **Receptor 1** was dissolved in acetate buffer pH 4.0 solution to produce 0.01 % (w/v) solution and the abosorbance was measured on a Varian CARY 500 UV-VIS-NIR spectrophotometer at 560 nm. The degree of modification was calculated from the calibration curve obtained by measuring the absorbances of different concentrations of **receptor 1** in 0.01 % (w/v) alginate solution (Figure 1). A solution of alginate at a concentration of 0.01 % (w/v) was used as a blank.

A $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition occurs in the amino ethyl rhodamine ring present in the **receptor 1**, allowing quantification of the amount of rhodamine by UV-vis absorption spectroscopy at 560 nm. Several samples within a concentration range (1.0×10^{-4} M to 1.0×10^{-3} M) of rhodamine dissolved in alginate solution (0.01 w/v %) was measured by UV absorption at 560 nm. The data collected from Uv absorbance measurements had a very good linear fit for the studied rhodamine concentrations ($R^2 = 0.99$). The average value of the degree of substitution (DS) obtained about

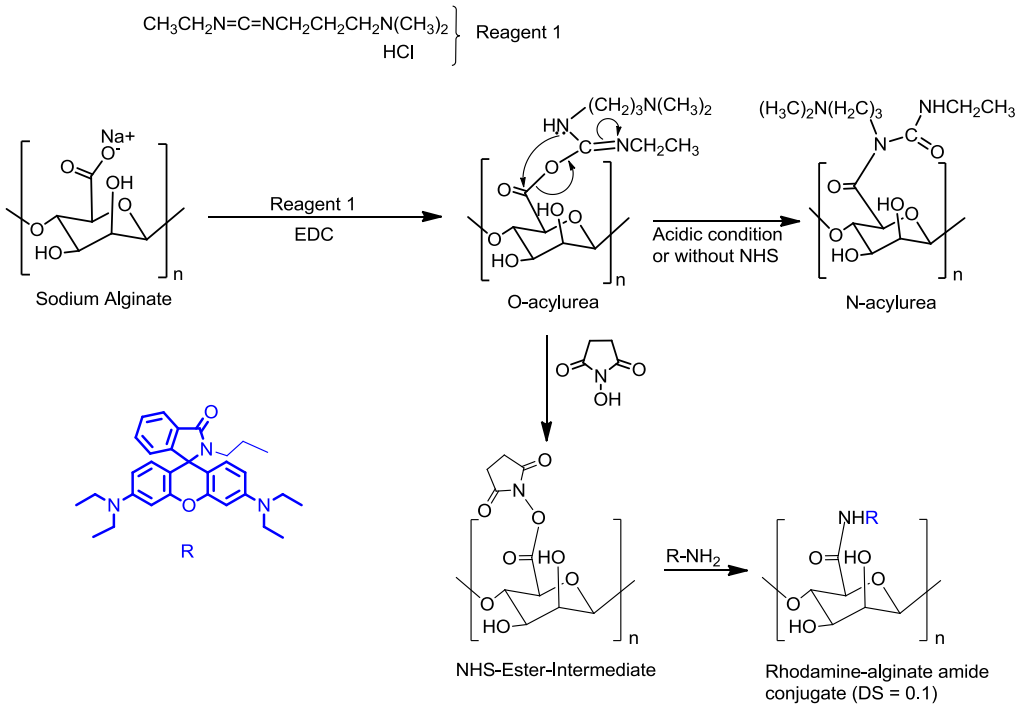
0.08 ± 0.02 of the **receptor 1**. Further, we calculated DS on the bases of %N (DS= 0.11 ± 0.03) and by alginic acid assay which indicated that *ca*. 10% substitution of rhodamine was occured.



SI Figure 1: Standard curve of amino ethyl rhodamine for determination of the degree of substitution at pH 4.0 in acetate buffer medium.

3. Proposed reaction Mechanism for amide bond formation:

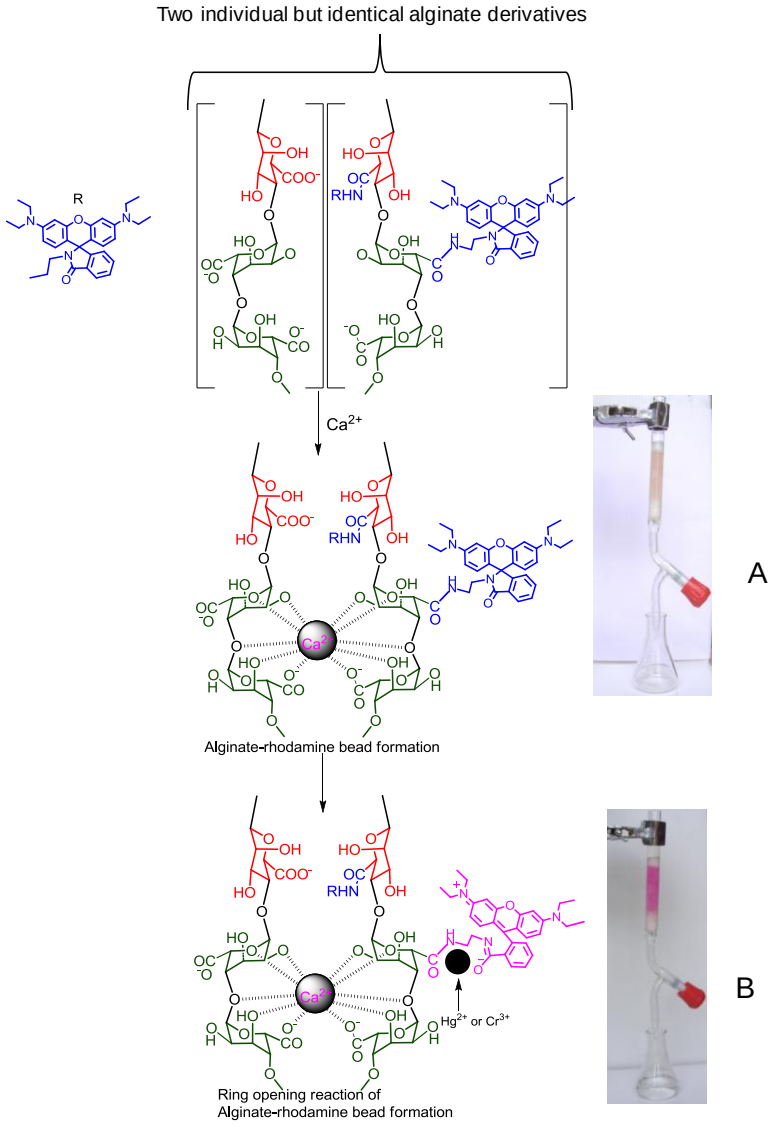
The amide bond formation between carboxylic acid functions and amines was catalysed by carbodiimide throughout activation of carboxylate functions (Scheme 1). Carbodiimide (EDC) reactes with carboxyl groups to form an unstable, intermediate *o*-acylurea which, in the absence of nucleophiles, rearranges to a stable N-acylurea by way of a cyclic electronic displacement.[8,9] This intermediate was unstable in aqueous solution and undergoes fast hydrolysis. For the procedure to be successful, the active form of alginate carboxyls would have to be more stable than the *o*-acylurea derivative (Scheme 1). NHS was further used as an activating reagent. Activated acid functions (NHS-ester intermediate) react with amines to form amide bonds.[10,11] NHS then reacted to form a less labile activated acid EDC was released subsequently like water soluble derivatives of urea.[12-13]



SI Scheme 1: Proposed reaction mechanism for the formation of Rhodamine-alginate conjugate (**receptor 1**).

For the synthesis of the **receptor 1**, 0.25 molar equivalent of amino ethyl rhodamine was used as compared to alginate polymer to ensure that a major proportion of **G**-residues remained available for binding to the Ca^{2+} ions forming **GB 1** (scheme 2). The structure of the ionotropic **receptor 1** gels has been described by the so-called ‘‘egg-box model’’, in which each divalent cation is

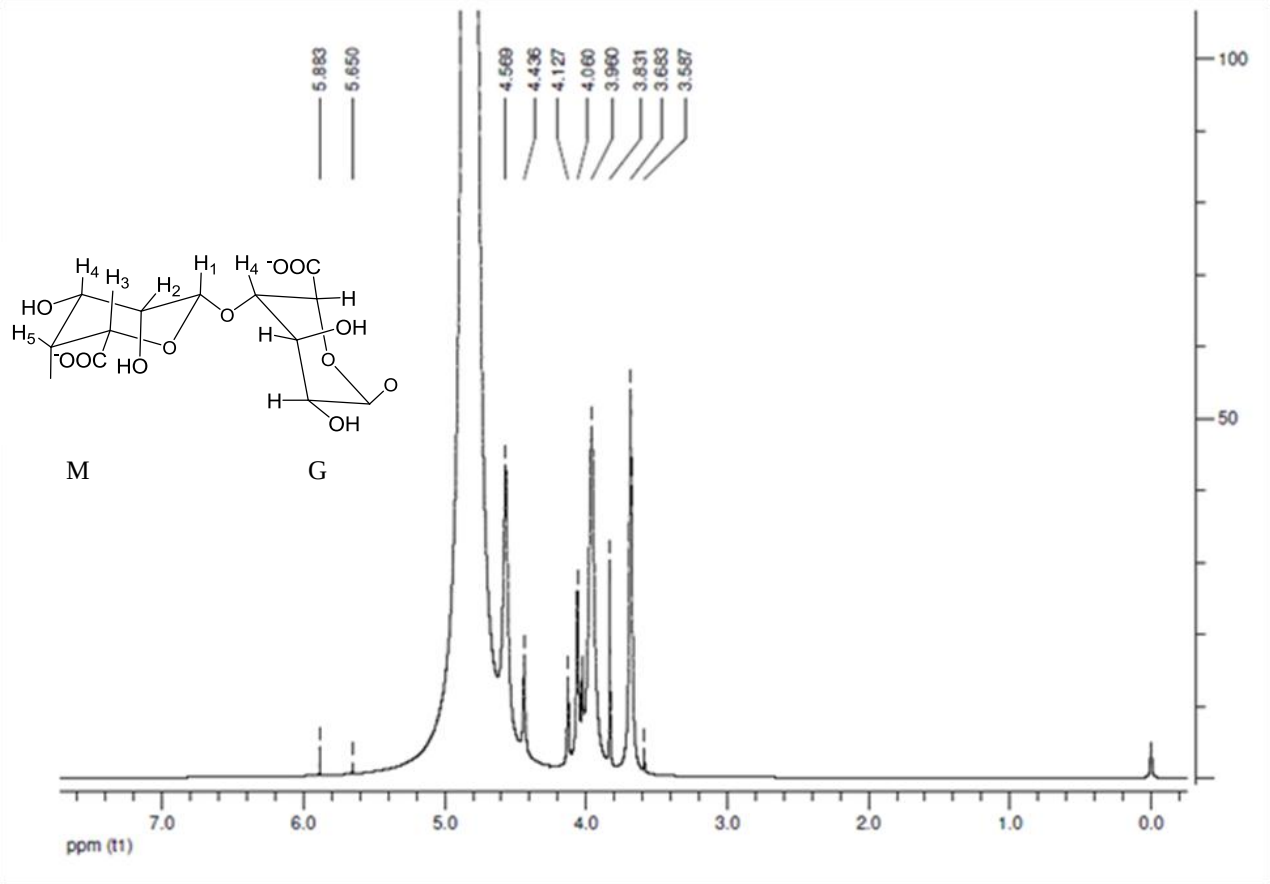
cooordinated to the carboxyl and hydroxyl groups of four guluronate monomers from the two adjacent chains of the polymer (scheme 2).[14,15]



SI Scheme 2: Proposed mechanistic representation of **GB 1** formation and spirolactam ring opening upon binding to Hg^{2+} or Cr^{3+}. Inset: (A) Column packed with **GB 1** as stationary phase; (B) Change in colour of the column with **GB 1** as stationary phase after binding to M^{n+} (Hg^{2+} or Cr^{3+}) solution as mobile phase.

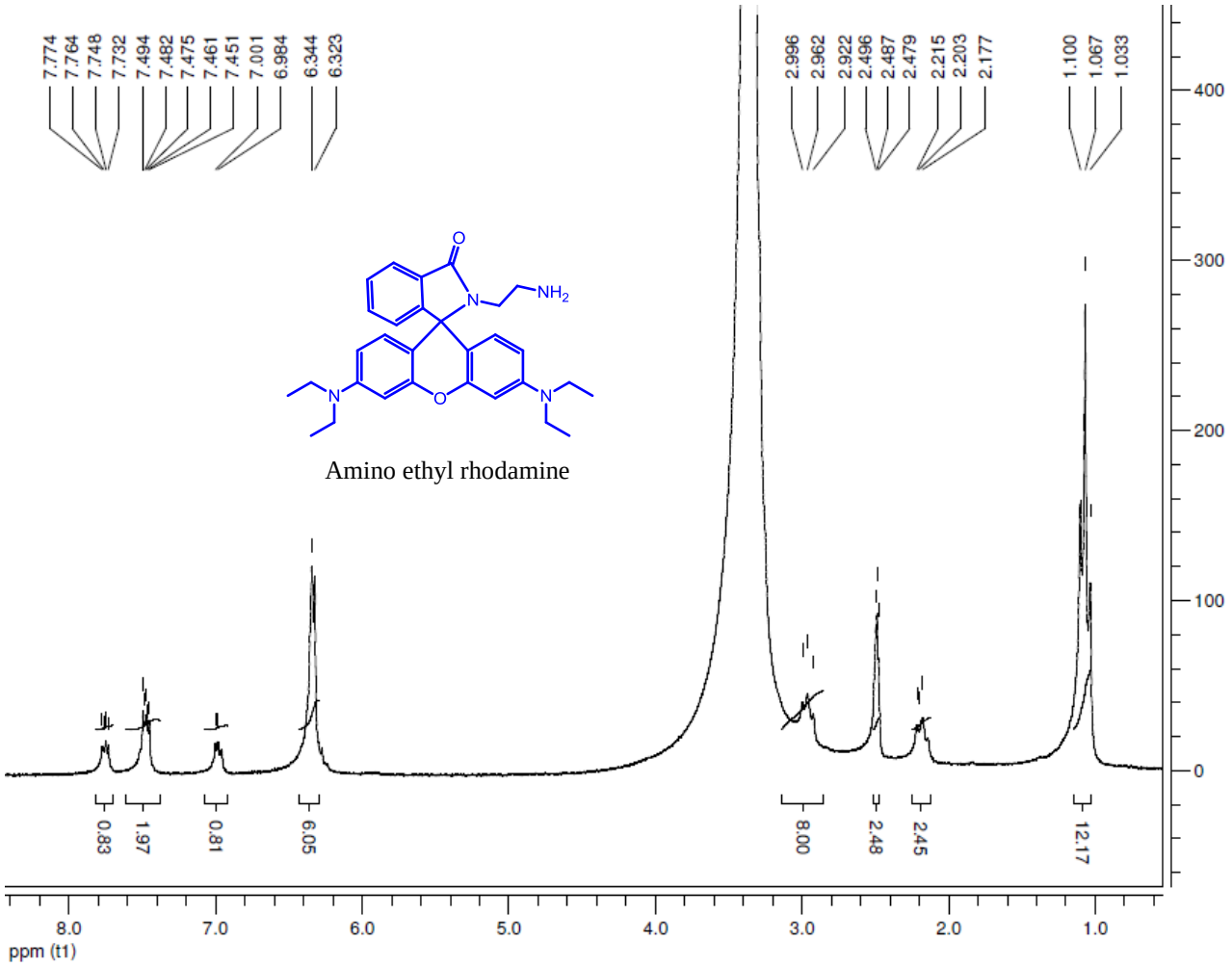
This rhodamine- alginate bead on exposure to the solution containing Hg^{2+} or Cr^{3+} changes its color from colorless to pink due to opening of rhodamine spirolactam ring on coordination to the metal ion (Scheme 2).

4. ^{1}H NMR of Na-alginate in D_2O:



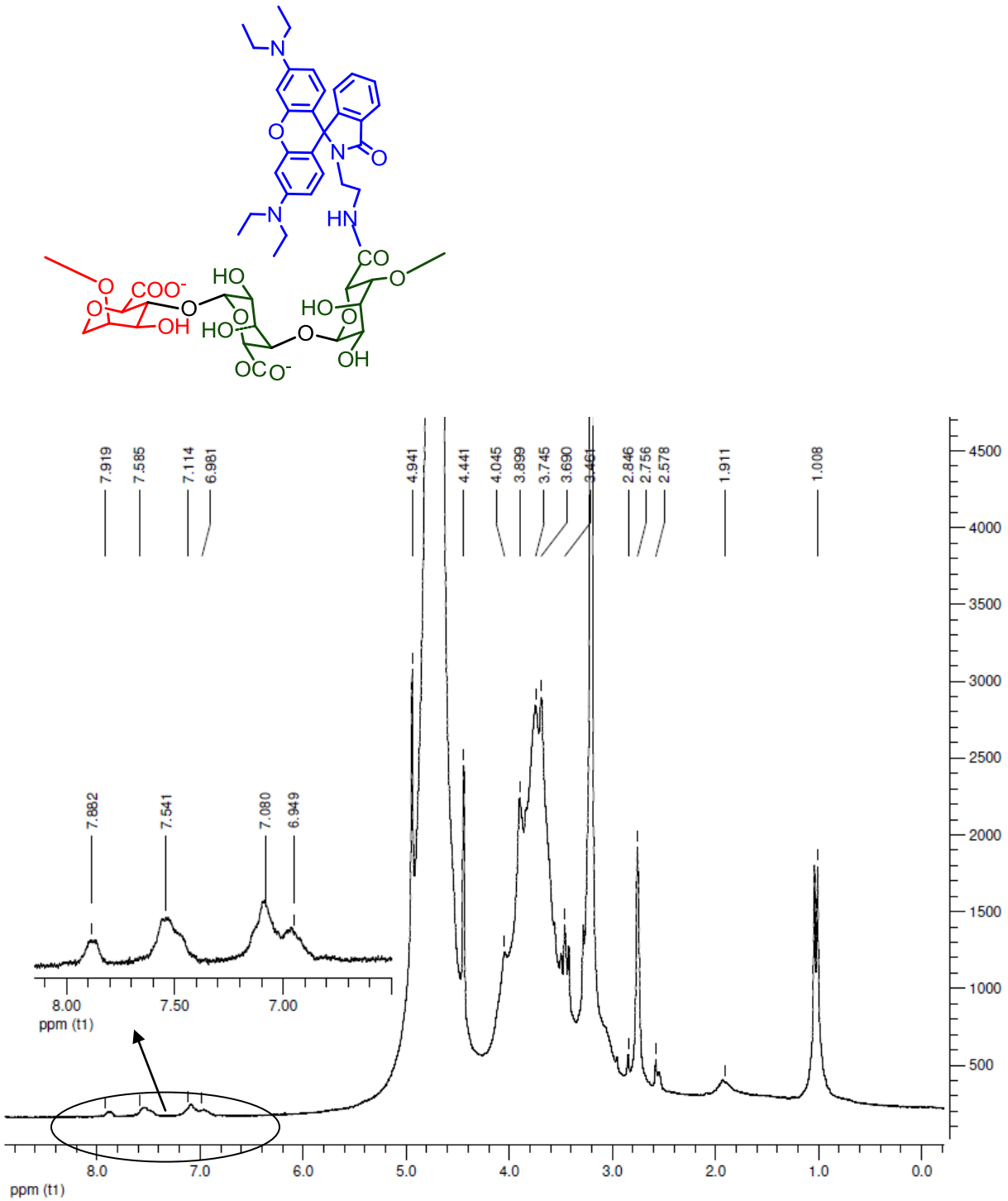
SI Figure 2: ^{1}H NMR of Na-alginate in D_2O.

5. ^{1}H NMR of amino ethyl rhodamine in d_6-DMSO:



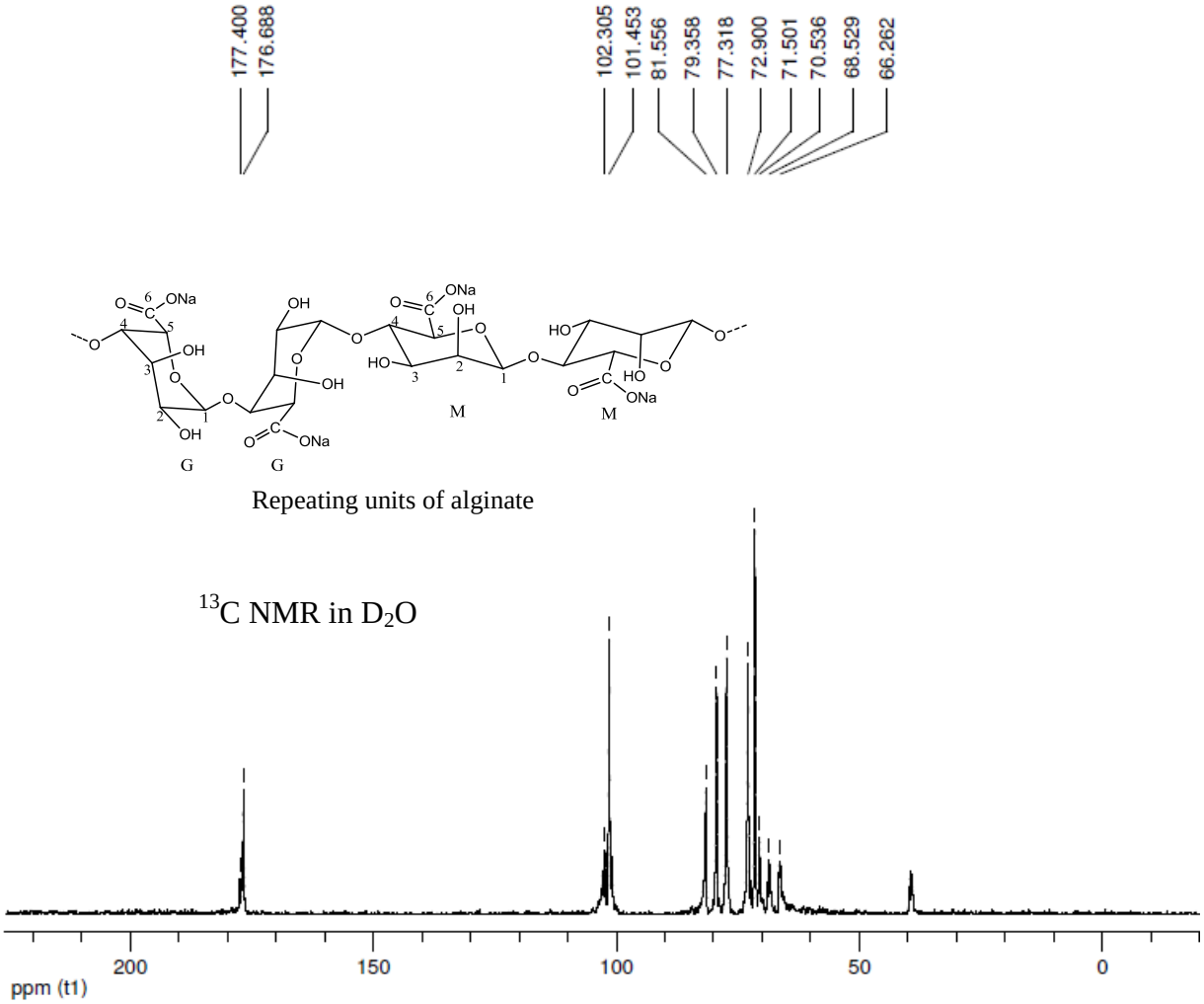
SI Figure 3: ^{1}H NMR of amino ethyl rhodamine in d_6-DMSO.

6. ^{1}H NMR of receptor 1 in D_2O:



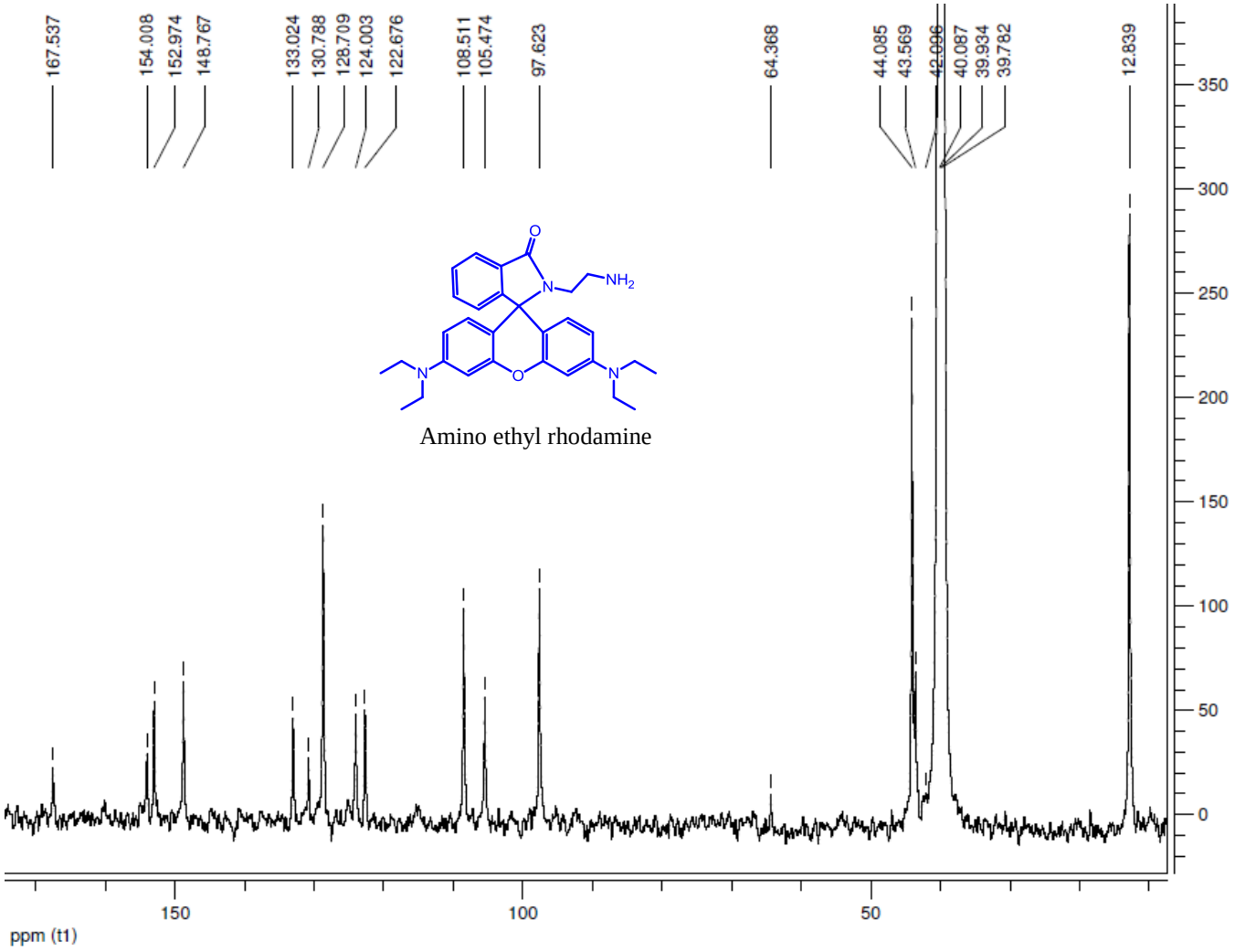
SI Figure 4: ^{1}H NMR of receptor 1 in D_2O.

7. ^{13}C NMR of Na-alginate in D_2O:



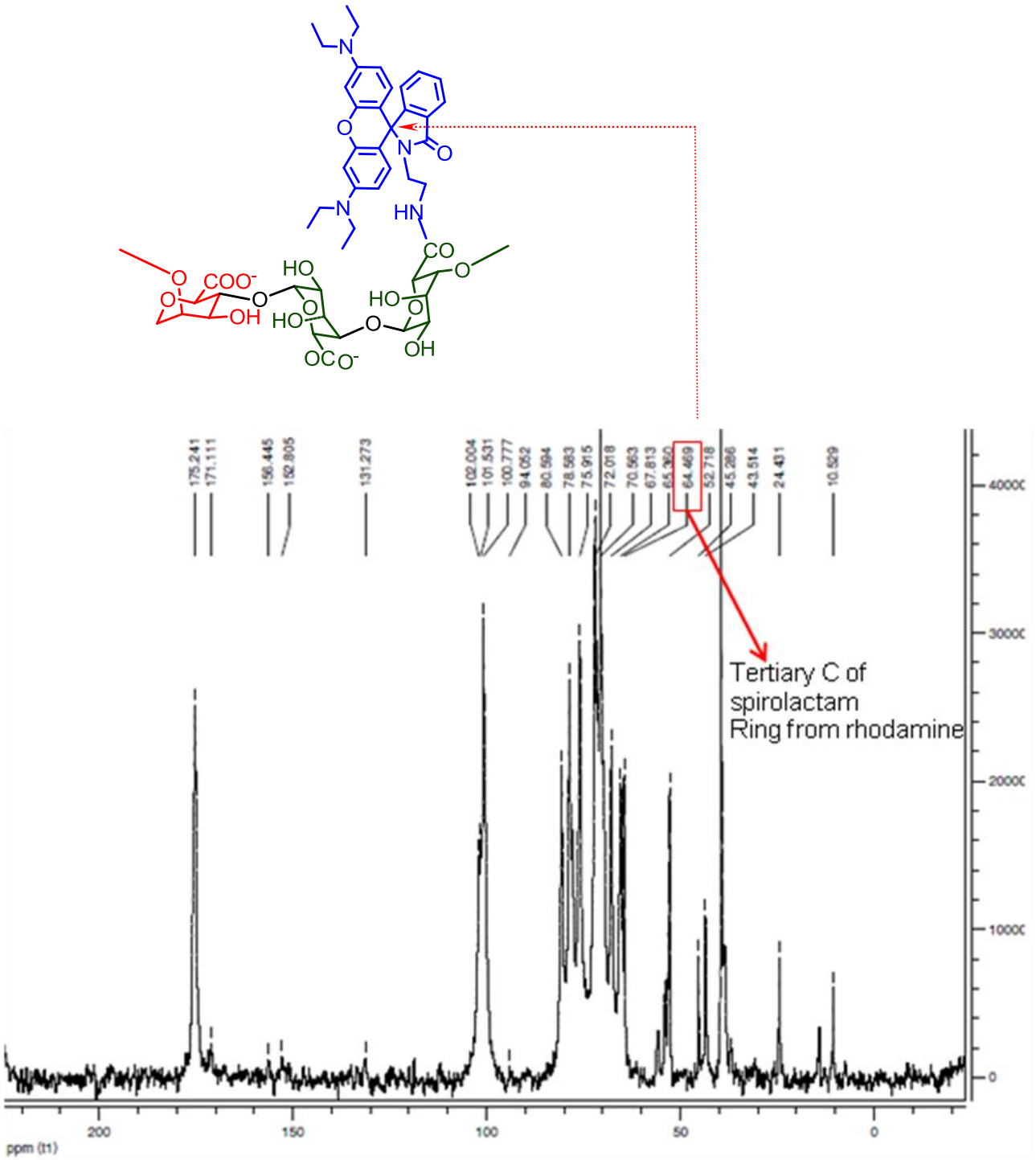
SI Figure 5: ^{13}C NMR of Na-alginate in D_2O.

8. ^{13}C NMR of amino ethyl rhodamine in d_6-DMSO:



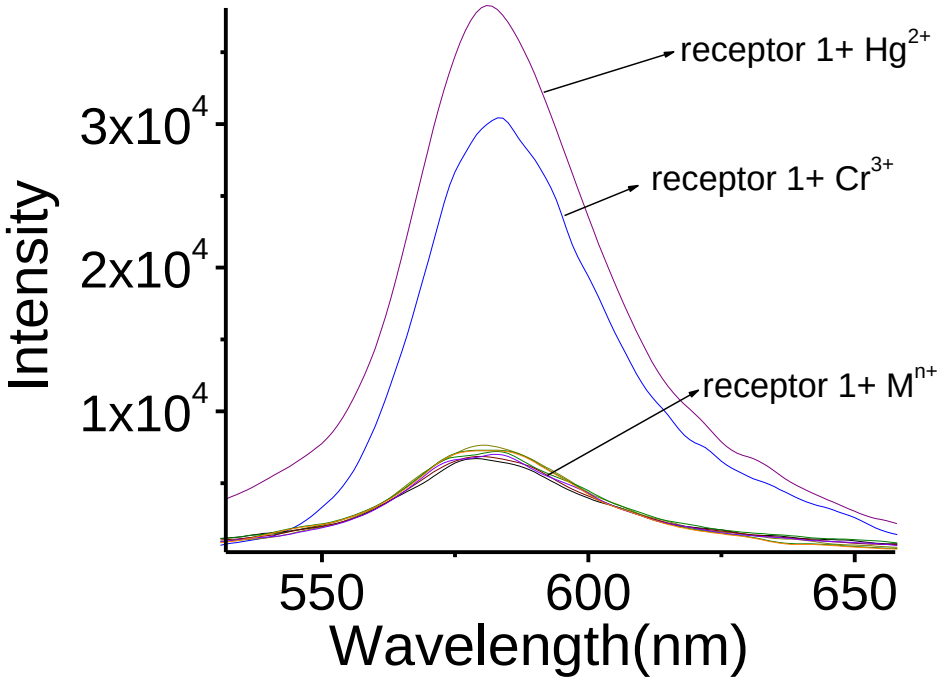
SI Figure 6: ^{13}C NMR of amino ethyl rhodamine in d_6-DMSO.

9. ^{13}C NMR of receptor 1 in D_2O:



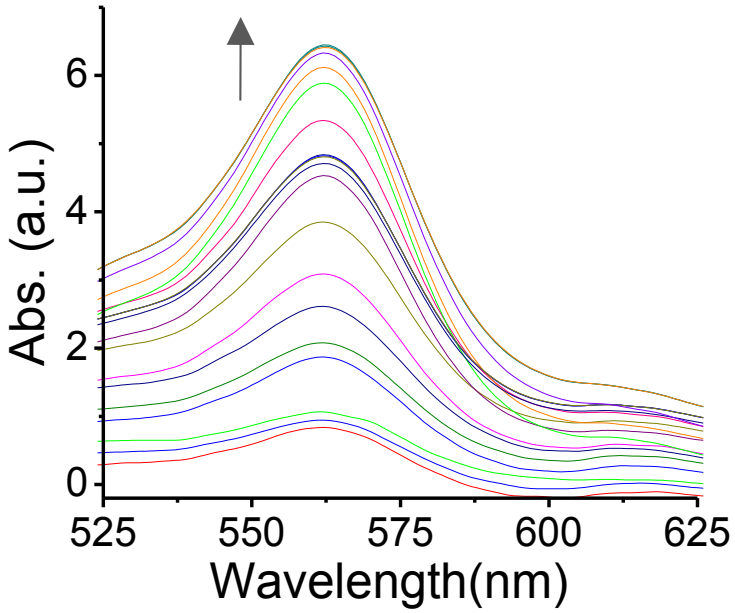
SI Figure 7: ^{13}C NMR of **receptor 1** in D_2O.

10. Change in emission spectra of receptor 1 with various cation in aqueous HEPES buffer medium:



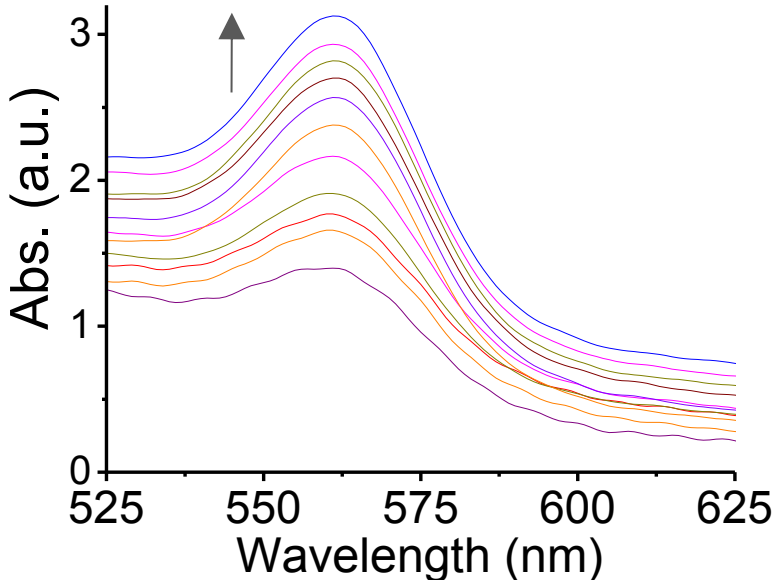
SI Figure 8: Change in emission spectra of **receptor 1** (2.5 mg/ml) with various cation 2.5 x 10^{-4} M in aqueous HEPES buffer medium (pH 7.1). M^{n+}: Li^{+}, Na^{+}, K^{+}, Cs^{+}, Mg^{2+}, Ba^{2+}, Ca^{2+}, Sr^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} (5.0 x 10^{-4} M) (λ_{ext}= 500 nm).

11. Spectrophotometric titration of receptor 1 with varying [Cr^{3+}] in aq. HEPES buffer medium:



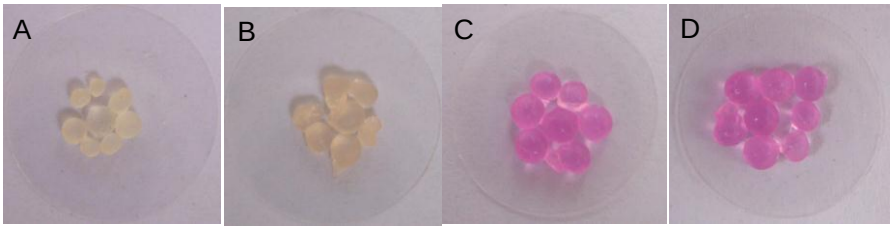
SI Figure 9: Spectrophotometric titration of **receptor 1** (2.5 mg/ml) with varying [Cr^{3+}] (0-2.2 x 10^{-4} M) in HEPES buffer medium (pH 7.1).

12. Spectrophotometric titration of receptor 1 with varying [Hg^{2+}] in aqueous HEPES buffer medium:



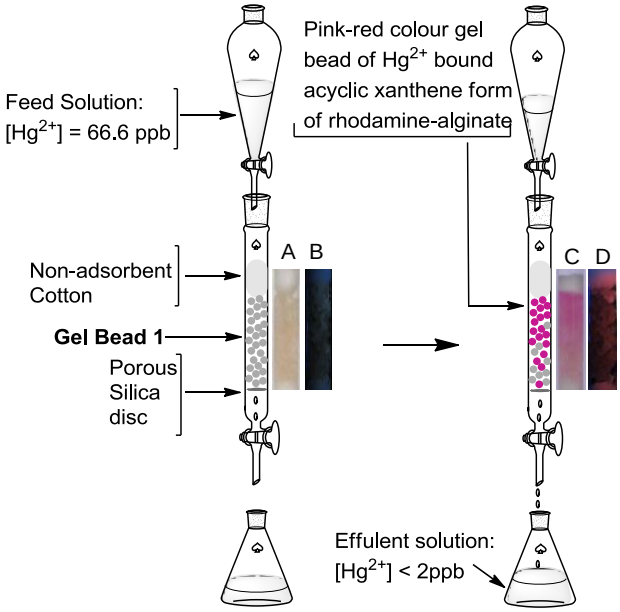
SI Figure 10: Spectrophotometric titration of **receptor 1** with varying [Hg^{2+}] (0-2.3 x 10^{-4} M) in HEPES buffer medium (pH 7.1).

13. Photograph of the Ca^{2+}-alginate bead, GB 1 and GB 1 pretreated with Cr^{3+} (10 μM) or Hg^{2+} (10 μM) aqueous solution:



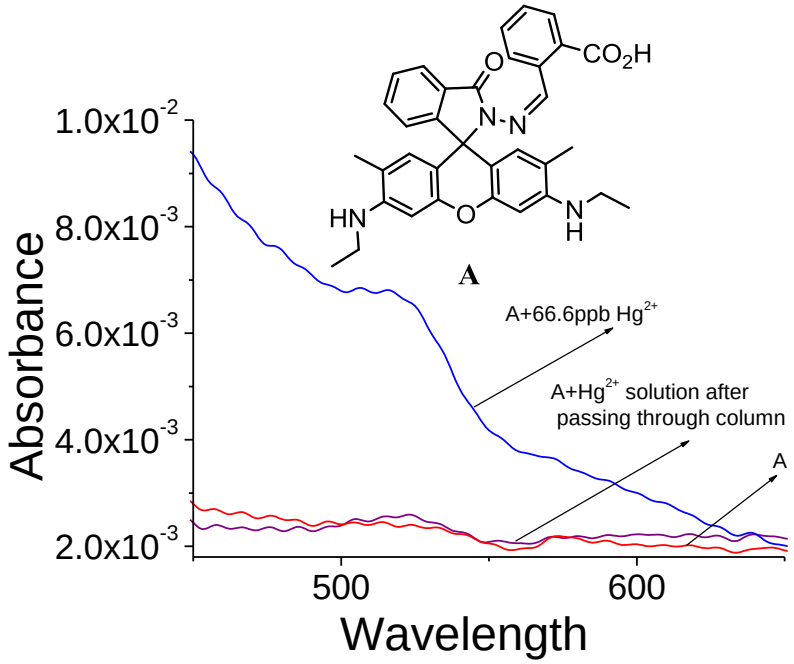
SI Figure 11: Optical photograph of (A) Ca^{2+}-alginate bead, (B) **GB 1**, (C) **GB 1** pretreated with aqueous solution of Cr^{3+} (10 μM) or (D) Hg^{2+} (10 μM). Generation of the pink-red coloration suggests the formation of acyclic xanthene form upon binding with Hg^{2+} or Cr^{3+} to the cyclic lactam form of the rhodamine fragment of the **GB 1**.

14. Removal of trace amount of Hg^{2+} from feed water using GB 1 as self indicating stationary phase of the column:



SI Figure 12: Removal of Hg^{2+} contaminated water by traditional column chromatography method using **GB 1** as stationary phase (200mg **receptor 1** was used for **GB 1** preparation). Insets: (A) Photograph of the quartz column (internal diameter of 10 cm) packed with **GB 1** in presence of normal light, (B) in dark and exposed to UV-radiation (~365 nm), (C) Photograph of the quartz column after passing 3 ml of Hg^{2+} feed solution having effective [Hg^{2+}] of 66.6 ppb in presence of normal light and (d) Photograph of the quartz column after passing 3 ml of Hg^{2+} feed solution having effective [Hg^{2+}] of 66.6 ppb in dark, while exposed to UV-radiation (~365 nm).

15. Determination of [Hg^{2+}] from the eluted solution after passing through the column of GB 1 using receptor A, according to the literature procedure:



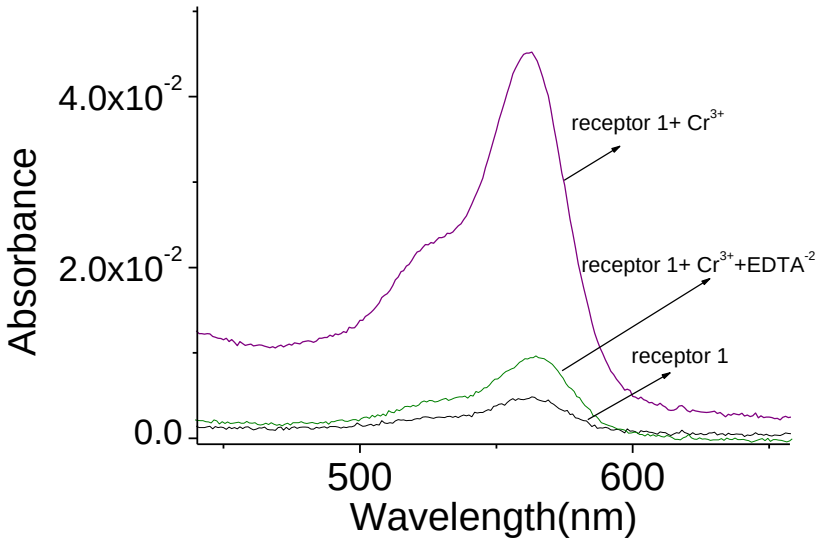
SI Figure 13: Electronic spectra of receptor **A**[16] (3.5×10^{-3} M) with the Hg^{2+} (66.6 ppb) solution before and after eluting through the **GB 1** column showing concentration reduced to detection limit of **A**.

For detailed literature procedure, please see the article of M. Suresh, A. Shrivastav, S. Mishra, E. Suresh and A. Das, *Org.Lett.*, 2008, **10**, 3013 (Reference 16).

Reversibility and reusability of reagent GB1.

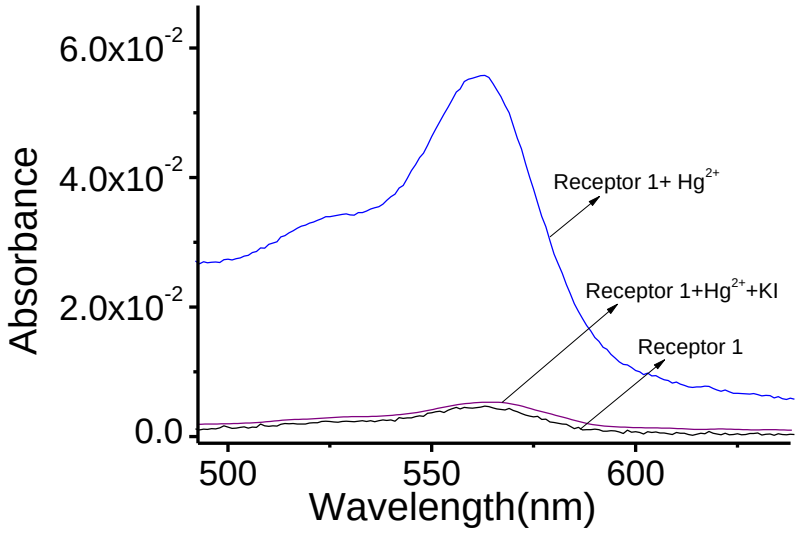
After adsorption of Hg^{2+} GB-1 is regenerated by treating it with 1% aqueous KI to remove Hg^{2+} followed by water wash. After 3 such cycles the beads started disintegrating presumably due to gradual exchange of Ca^{+2} in alginate beads with K^{+} ions (data not given).

16. Reversibility of the binding process of receptor 1 with Cr^{3+}:



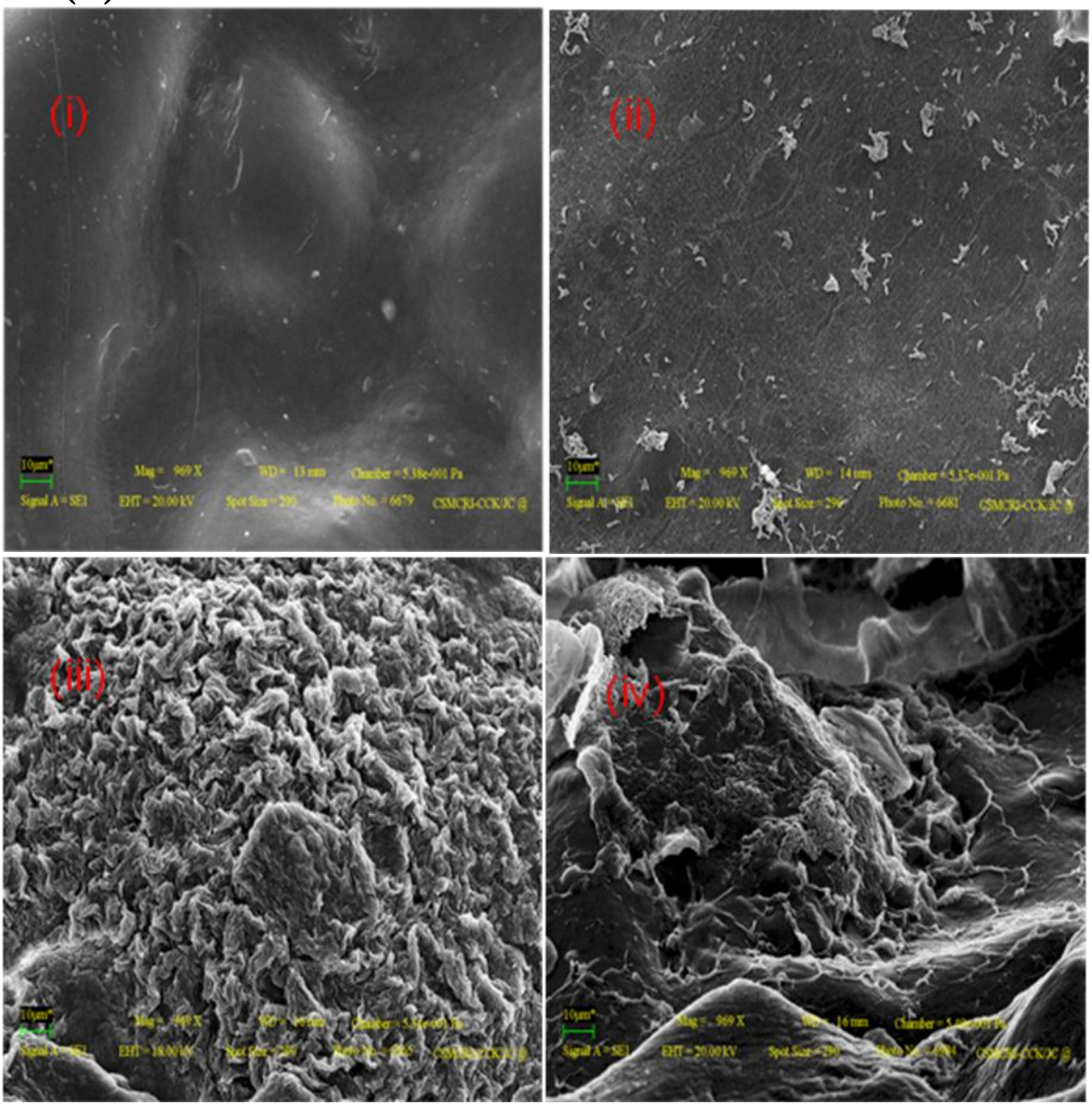
SI Figure 14: Absorption spectral changes that confirms the reversible binding of **receptor 1** (1 mg/ml) with Cr^{3+} (3.69 x 10^{-4} M) with Na_2EDTA (4.0 x 10^{-3} M).

17. Reversibility of the binding process of receptor 1 with Hg^{2+}:



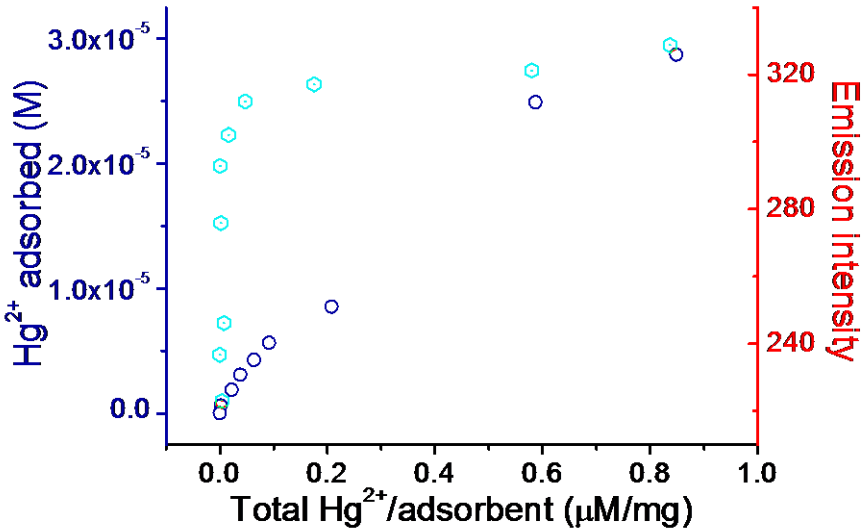
SI Figure 14: Absorption spectral changes that confirms the reversible binding of **receptor 1** (1 mg/ml) with Hg^{2+} (4.13 x 10^{-4} M) with KI (4.56 x 10^{-3} M).

18. SEM images of (i) Ca^{2+}-alginate bead, (ii) GB 1, (iii) GB 1 bound to Hg^{2+} and (iv) Cr^{3+}:



SI Figure 15: SEM images of (i) Ca^{2+}-alginate bead, (ii) **GB 1**and (iii) **GB 1** bound to Hg^{2+} and (iv) Cr^{3+}. Images were recorded using Scanning Electron Microscope (SEM) LEO 1430 VP, the material at an accelerating voltage of 20 kV.

19. Hg^{2+} adsorption isotherm at pH 7.1 and 25ºC [+]:



SI Figure 16: Hg^{2+} adsorption isotherm at pH 7.1 and 25ºC. Hg^{2+} adsorbed was evaluated from each $[Hg^{2+}]_{Total}$ added – $[Hg^{2+}]_{Unadsorbed}$ present in the respective supernatant aqueous solution after treated with 50 mg of the receptor **1**. Amount of receptor **1** kept unchanged while treating aqueous solution having different $[Hg^{2+}]_{Total}$ added. Hg^{2+} solution was allowed to equilibrate with receptor **1** for 15 minutes to ensure an efficient and complete adsorption of Hg^{2+}. Saturation was achieved with 0.1 μM of $[Hg^{2+}]_{Total}$ per 1.0 mg equivalent of Receptor **1** present in **GB1.** $[Hg^{2+}]_{Unadsorbed}$ was determined from the calibration curve obtained with receptor A (8.57×10^{-6}M) at different $[Hg^{2+}]$.

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