Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information

Heparin Triggered Dose Dependent Multi-Color Emission Switching in Water: A Convenient Protocol for Heparinase I Estimation in Real-Life Biological Fluids

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Materials and Instrumentations

All reagents and starting materials were purchased from the best known commercial sources and were used without further purification. FTIR spectra were recorded on a Perkin-Elmer FT-IR Spectrum BX system. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Advance DRX 400 spectrometer operating at 400 and 100 MHz respectively. Chemical shifts were reported in ppm downfield with respect to tetramethylsilane (the internal standard). Mass spectra were recorded on Micromass Q-TOF Micro TM spectrometer.

Sampling Procedure of Sensing

For sensing of the polysaccharides, 10 μL (for UV-vis) or 2.5 μL (for fluorescence) of 1 mM DMSO solution of all compounds were added in pure water to make the final volume of 1 mL (with concentration 1×10^{-5} M for UV-vis and 0.25×10^{-5} M for fluorescence studies). Similar procedure were also followed for the sensing in buffered media of different pH (HCO_2Na/HCl buffer for pH 2-4.5, NaOAC/HCl buffer for pH 4.5-6.5, Tris/HCl for pH 7-9 and $Na_2B_4O_7{\cdot}10H_2O$/NaOH for pH 9-10). The final concentration of DMSO in the final solution did not exceed 1% in any case.

Optical Spectroscopy

The UV–vis and fluorescence spectroscopy were recorded on a Shimadzu model 2100 spectrometer and Cary Eclipse spectrofluorimeter respectively. The slit-width for the fluorescence spectrometer was kept at 5 nm (excitation) and 5 nm (emission) and the excitation wavelength was set at 400 nm for the probe compounds.

Atomic Force Microscopy

A diluted aqueous solution of 1 ($1x10^{-5}$ M) with and without heparin (1 mg/mL) were drop-coated on clean glass substrates. After the formation of a stable film, images were collected using an Agilent Technologies 5500 AFM instrument and were processed using 'Picoview 1.6' software. Imaging was taken in non-contact mode in presence of air using silicon cantilevers of resonance frequencies of 290 kHz and a nominal tip radius of curvature of 10-15 nm.

^{1}H NMR Titration Studies

^{1}H NMR titration of compound 1 was performed upon dissolving 1 (1.2 mM) in D_2O. To that heparin was added and the spectra were recorded using identical parameters.

Fluorescence Decay Experiment

Fluorescence decay constants were measured by using a time-correlated single photon counting fluorimeter (Horiba Jobin Yvon). The system was excited with 400 nm nano LED of Horiba - Jobin Yvon with pulse duration of 1.2 ns (slit width of 2/2). The emission wavelength was fixed at both 470 and 600 nm. Average fluorescence decay time (τ_{av}) for the exponential iterative fitting were calculated from the decay times (τ_i) and the relative amplitudes (a_i) using the following relation

$$\tau_{av} = (a_1\tau_1^2 + a_2\tau_2^2 + a_3\tau_3^2)/(a_1\tau_1 + a_2\tau_2 + a_3\tau_3)$$

Where a_1, a_2 and a_3 are the relative amplitudes and τ_1, τ_2, and τ_3 are the lifetime values, respectively. For data fitting, a DAS6 analysis software version 6.2 was used.

Sensing in biological fluid: serum medium

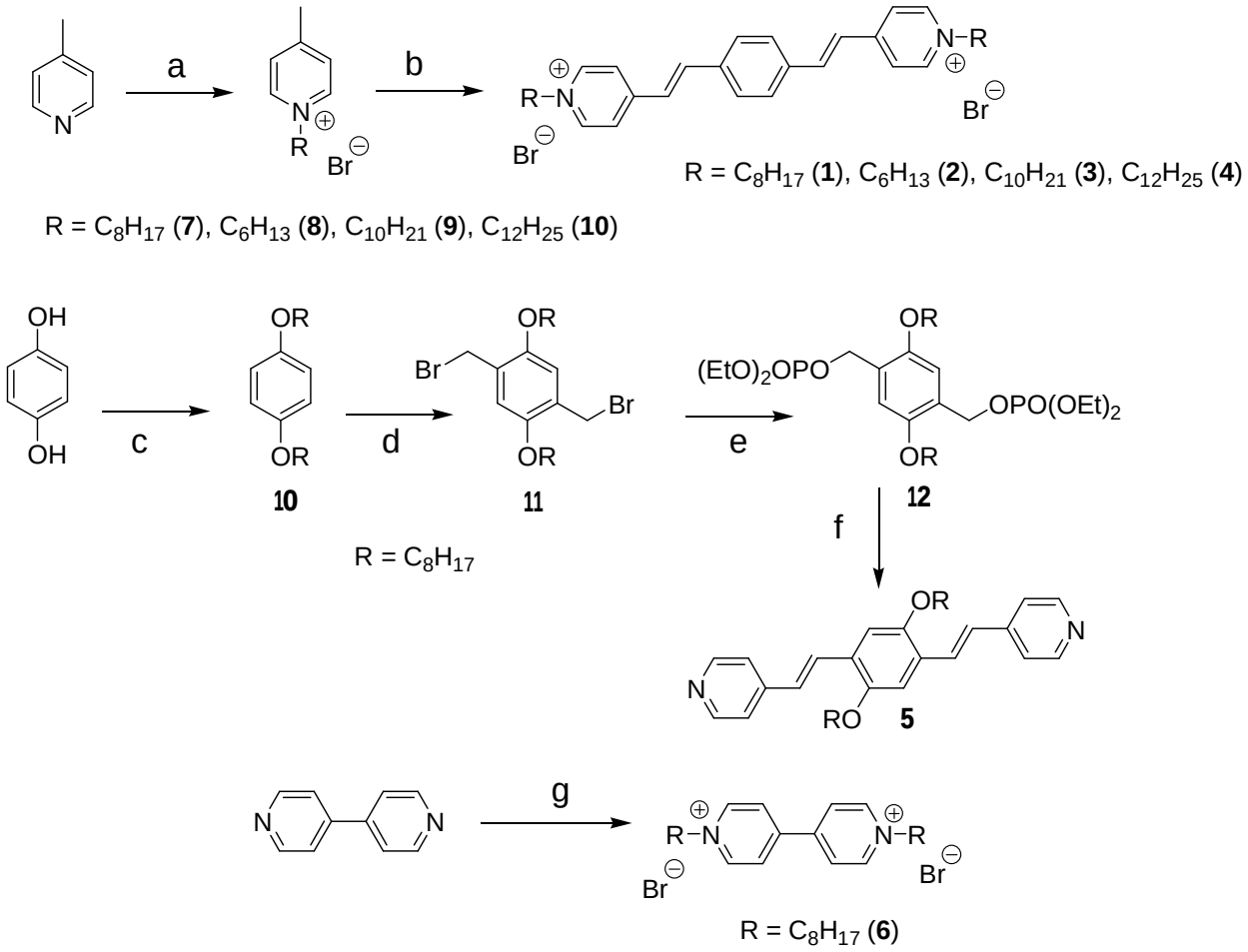
For analysis, 50 µL of serum was added to 950 µL of buffered solution (pH 7.4) containing 2.5 µM of compound. For heparin interaction studies, the solution was then spiked with different concentration of heparin. On the other hand, during enzymatic hydrolysis 1 + Heparin ([1] = 2.5 µM, Heparin = 1.0 µg/mL) conjugate was utilized as the substrate. The spectra were measured after 2 minute incubation after each addition.

Development of Low-cost color strips for sensing

For preparing dye-coated filter paper, rectangular pieces (1.0 × 1.5 cm) of Whatman-40 filter paper were cut and attached on a black chart paper using glue. Then the MeOH solution compound **1** (0.02 mM) was drop casted onto the filter paper. Special care was taken so that the solution can spread evenly to form uniform film. The solution was completely absorbed in filter paper within 15 minute and then the strips were kept overnight for air dry. Finally the coated paper strips were visualized under a 365 nm UV lamp to confirm the uniform blue emission. When the strips were dipped into the aqueous solution of heparin, a gradual change in color from blue to yellow was observed.

Synthesis of compounds used in present study

The synthesis of compounds **1-4** (along with their respective precursors **7, 8, 9, 10**)[1], **5** (along with precursors **10, 11, 12**)[2] and **6**[3] were executed following the literature reported procedure.



Sceheme 1. (a) R-Br, EtOH, reflux, 12 h; (b) Terephthalaldehyde, K_2CO_3 or NaOH, EtOH/H_2O, 0ºC, 2 h. (c) Octyl bromide, K_2CO_3, CH_3CN, reflux, 48 h; (d) Paraformaldehyde, HBr, AcOH, 80 ℃, 2 h; (e) $P(OEt)_3$, 140-150 ℃, 4 h; (f) K^tBuO, pyridine carboxaldehyde, dry THF, 10 min. (g) Octyl bromide, DMF, reflux, 8 h.

References

1. S. Bhattacharya, S. K. Samanta, *Chem. Eur. J.* 2012, **18**, 16632 – 16641.

2. S. Bhattacharjee, S. Datta, S. Bhattacharya, *Chem. Eur. J.* 2013, **19**, 16672 – 16681.

3. D. Mandler, Y. Degani, I. Willner, *J. Phys. Chem.* 1984, **88**, 4366-4370.

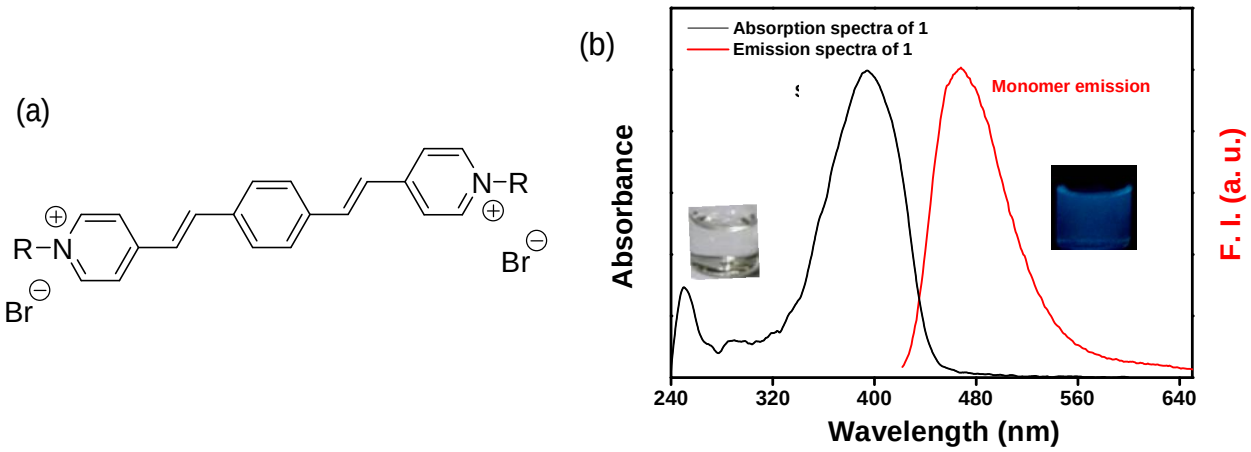


Figure S1. (a) Molecular structure of compound **1** used for the present study. (b) Optical spectra (Absorption and emission spectra) of **1** in water.

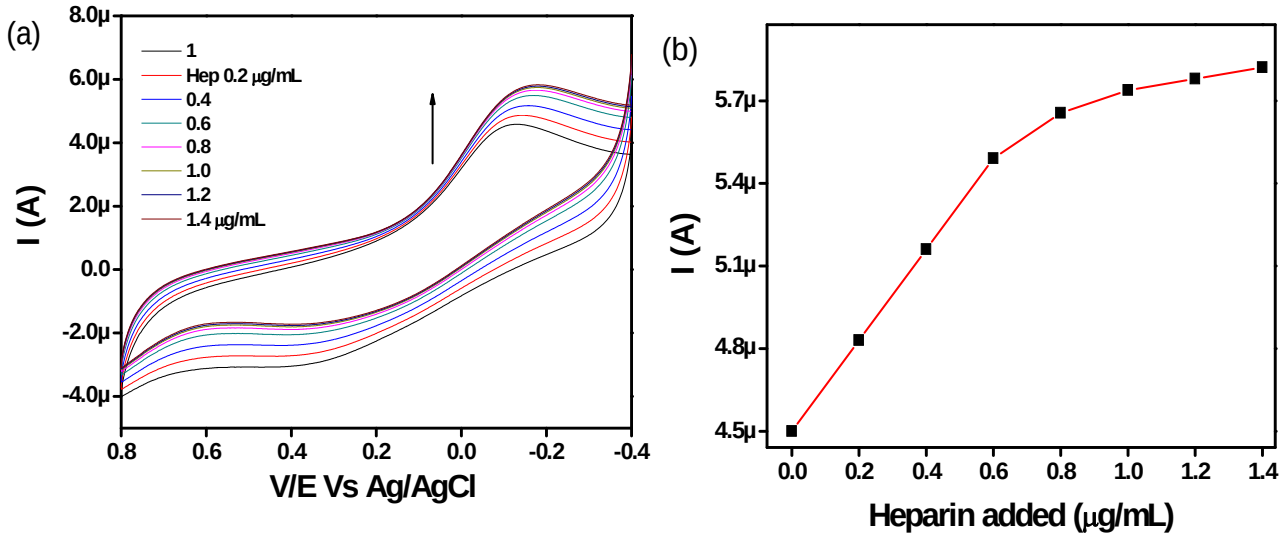


Figure S2. (a) Change in current value of **1** (scan rate: 200 mV/s, KCl: 10 mM) upon addition of heparin in water. (b) Change in current value (monitored at -0.16 V) upon addition of heparin in water.

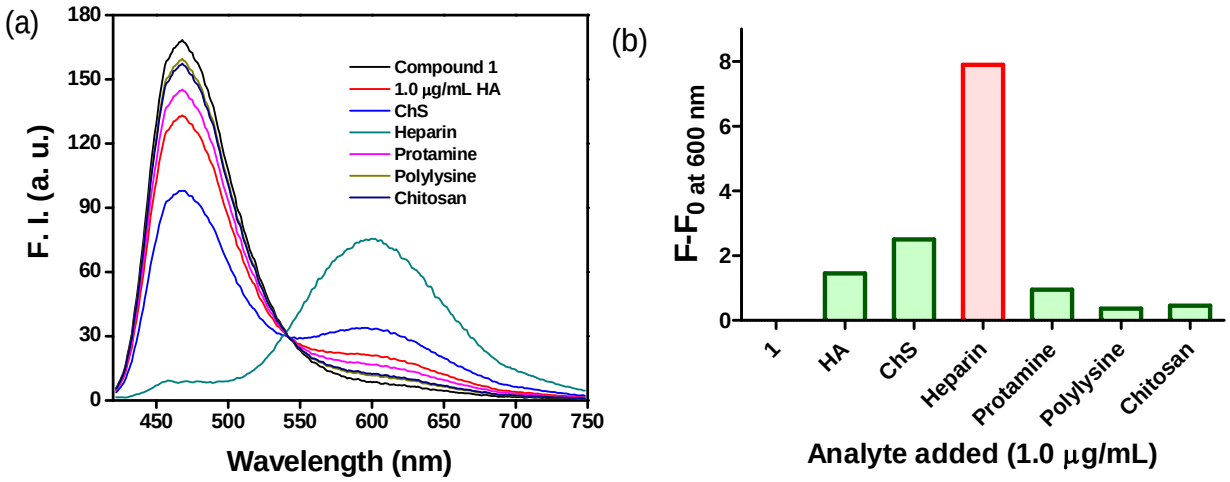


Figure S3. (a) Emission spectra of **1** (2.5 μM, λ_{ex} = 400 nm) in presence of different polysaccharides in water (pH 7.4). (b) Change in emission intensity of **1** (2.5 μM, λ_{ex} = 400 nm) at 600 nm upon addition of different analytes in water (pH 7.4).

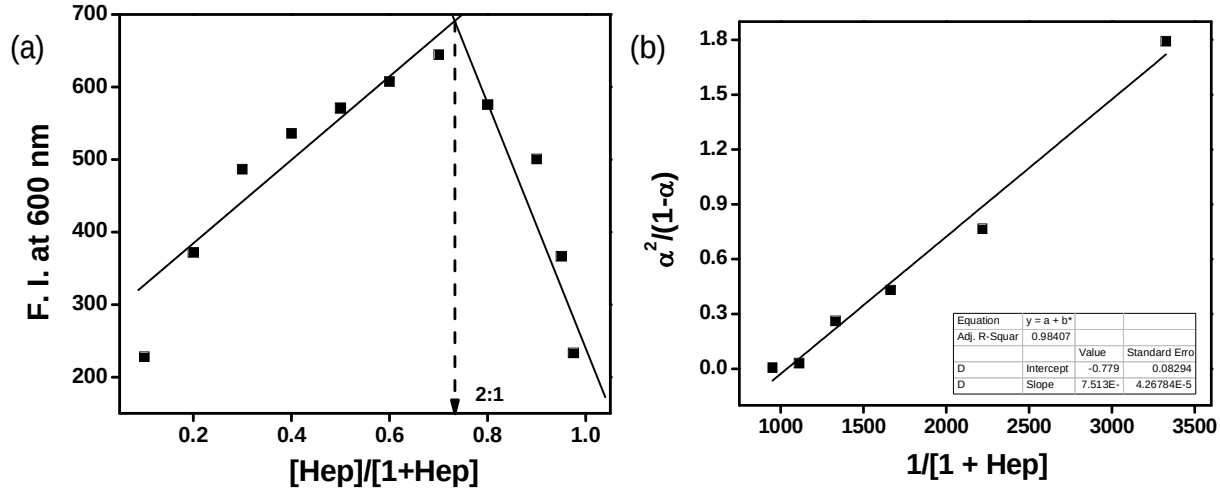


Figure S4. (a) Determination of stoichiometry of **1** and heparin interaction from Job's plot analysis. (b) Estimation of affinity constant of **1** towards heparin in water considering Benesi-Hildebrand method for 1:2 interaction.

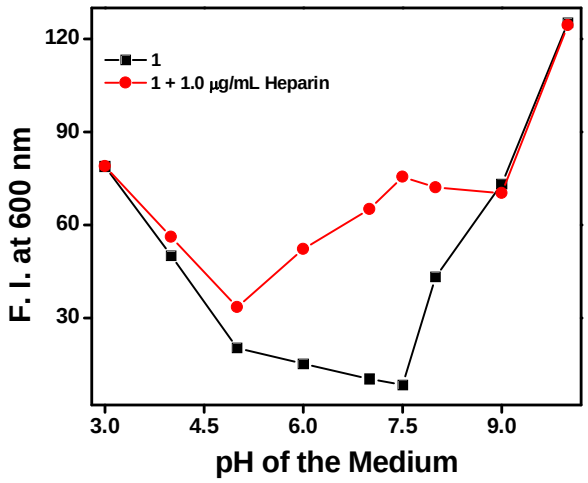


Figure S5. Effect of pH on the interaction of **1** (2.5 μM, λ_{ex} = 400 nm) towards heparin in water (pH 3 to 9.5).

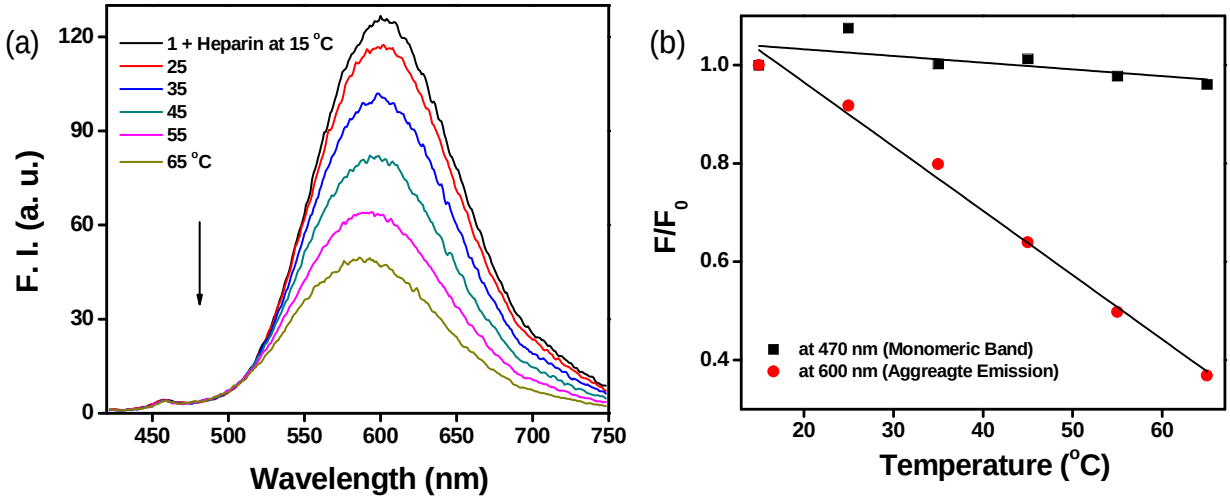


Figure S6. (a) Effect of temperature on the response of **1** towards heparin in water (pH 7.4). (b) Change in emission intensity (at 470 and 600 nm) of **1**+Heparin conjugate at different temperatures in water.

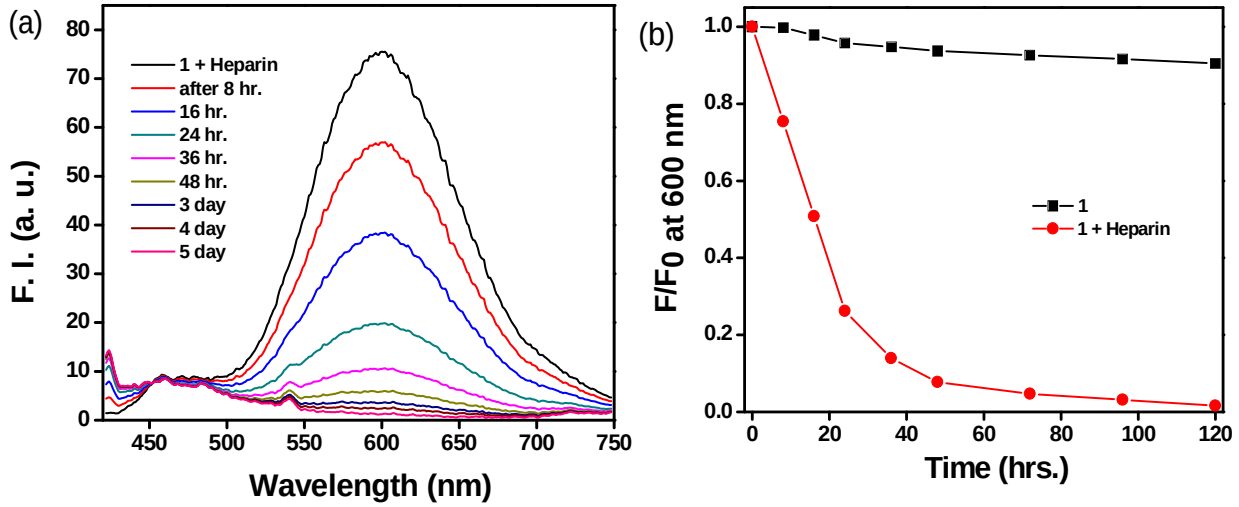


Figure S7. (a) The stability of **1**+heparin conjugate with time in water (pH 7.4). (b) Compare the stability of **1** and **1**+Heparin with time (monitored at 600 nm) in water (pH 7.4).

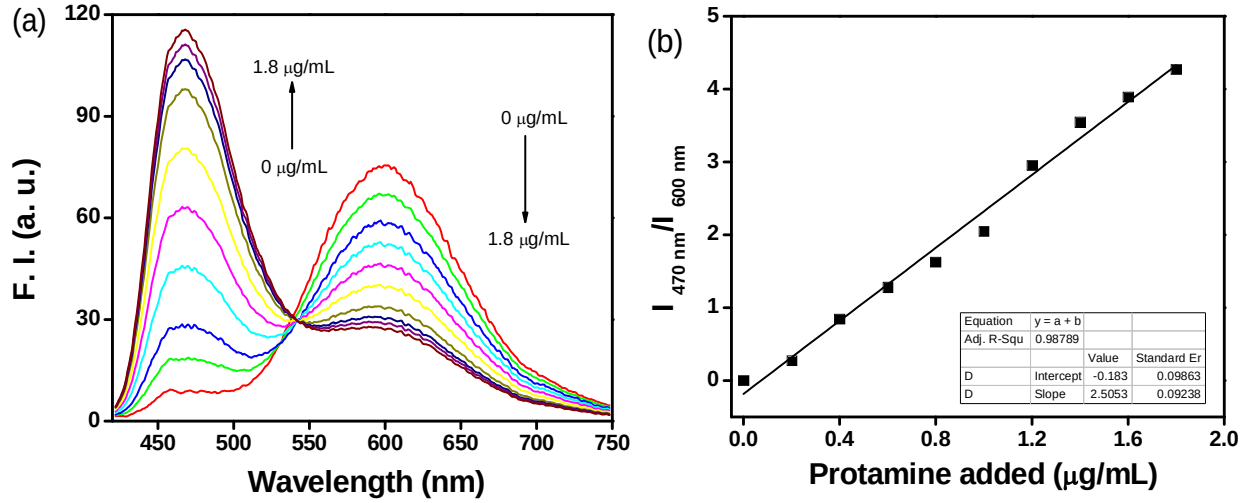


Figure S8. (a) Reversibility checking of heparin interaction (1.0 μg/mL) with compound **1** (2 μM) by protamine in water (pH 7.4). (b) Ratiometric interaction of protamine towards **1**+heparin conjugate in water (pH 7.4).

Table S1. Heparin induced increase in fluorescence decay time of **1** (2 mM) monitored at 470 and **1**+heparin at 600 nm in water (pH 7.4).

System	ι1	A1	ι2	A2	<ι>	χ^2
1	-	-	0.13 ns	100 %	0.13 ns	1.10
1 + Hep	16.2 ns	14 %	6.7 ns	86 %	9.4 ns	1.15

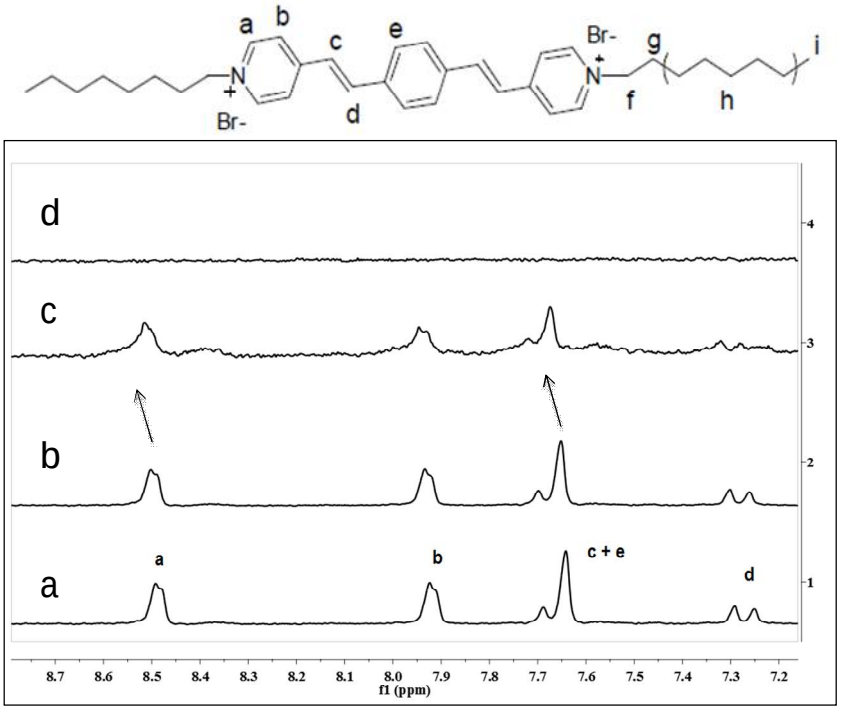


Figure S9. (a) Partial ^{1}H-NMR spectra of **1** (1.5 mM) upon addition of Heparin (a: 0 mM, b: 0.5 mM, c: 1 mM, d: 1.5 mM) in D_2O. The relevant protons have been mentioned in the structure.

Table S2. Compare the interaction parameters of compound with Heparin in water (pH 7.4).

Probes	Binding Constant (log K)	K_{sv} (10^4 M^{-1})	Detection limit (ng/L)
2	10.70	1.332	7.82
1	11.23	8.793	6.54
3	11.85	13.848	1.91
4	12.50	19.213	1.74

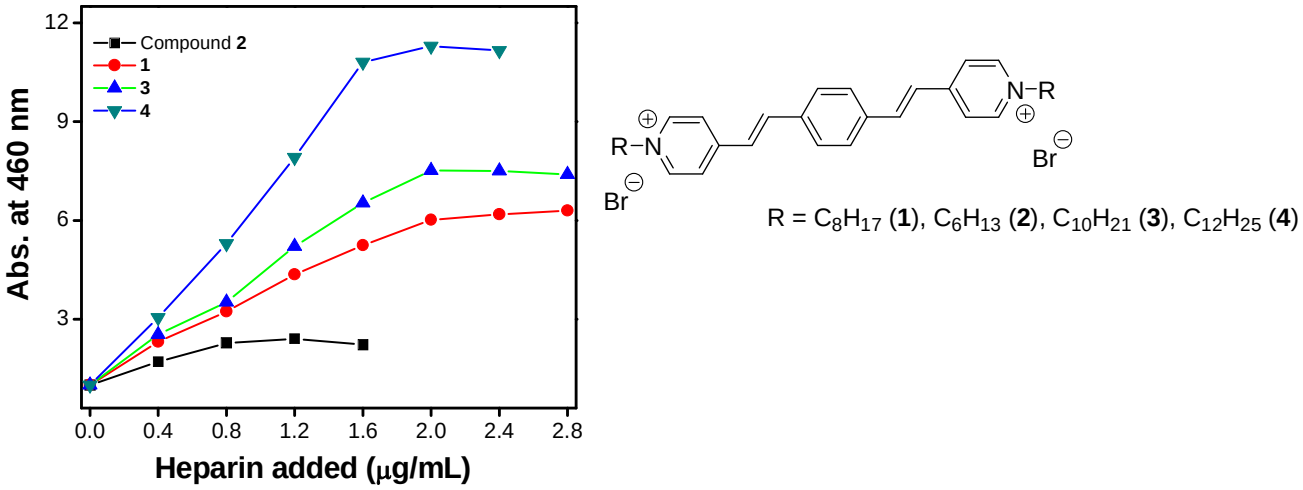


Figure S10. Comparison the extent of interaction of compounds **1-4** with heparin in water (pH 7.4).

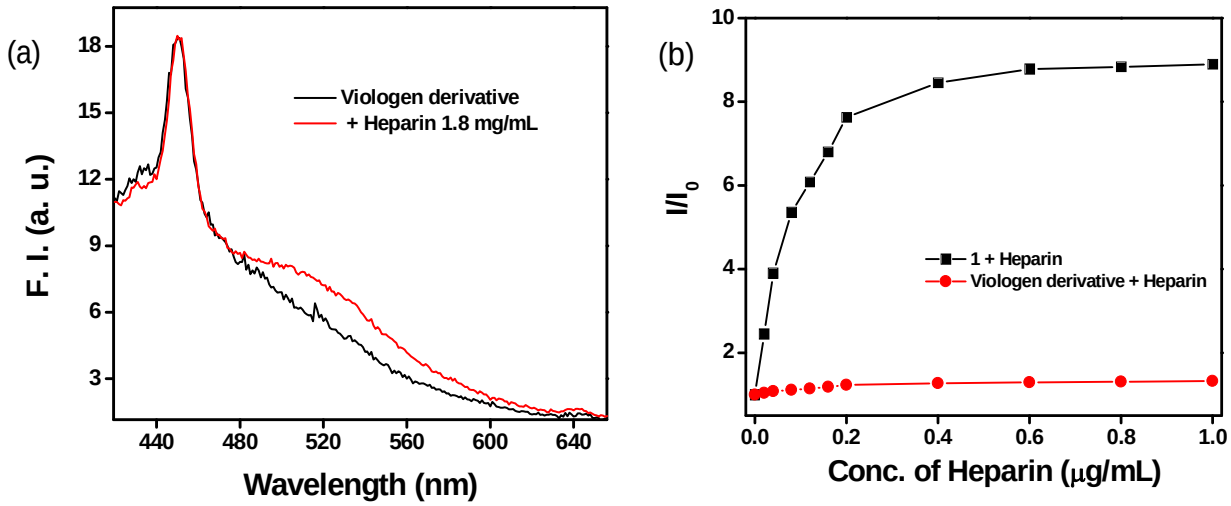


Figure S11. (a) Interaction of compound **6** (2.5 μM, λ_{ex} = 400 nm) with heparin in water (pH 7.4). (b) Compare the change in emission intensity (at 600 nm) of **1** and viologen derivative **6** upon addition of heparin in water (pH 7.4).

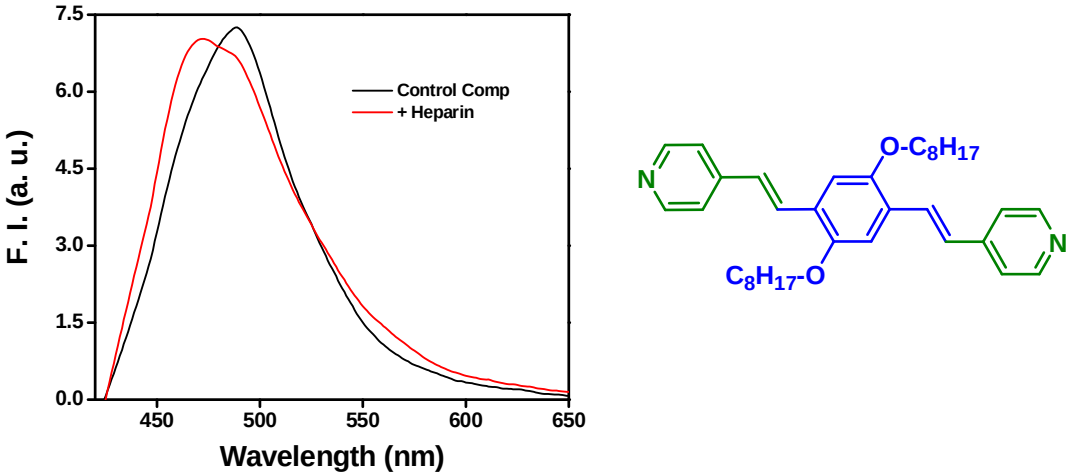


Figure S12. Interaction of control compound **5** (2.5 μM, λ_{ex} = 400 nm) with heparin in water (pH 7.4).

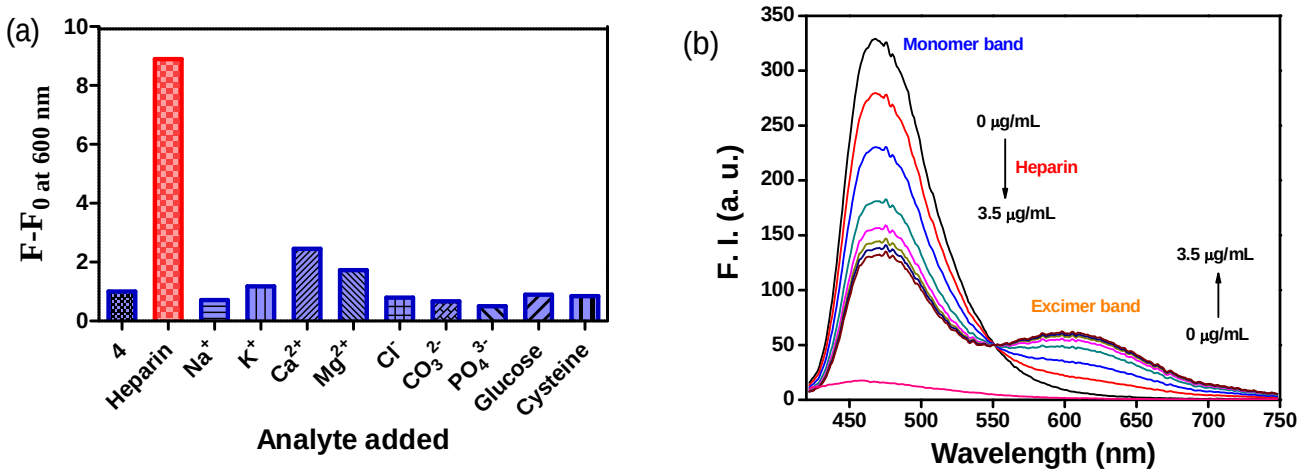
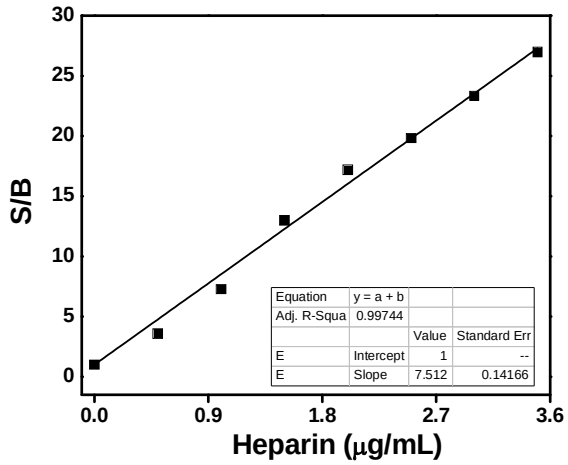


Figure S13. (a) Interaction of different common ionic analytes with **1** (2.5 μM) in 5% FBS containing water solution (pH 7.4). (b) Titration of **1** (2.5 μM) with heparin in 5% FBS containing water solution (pH 7.4).

For calculation in serum sample, signal to noise (S/B) ratio has been considered to eliminate the background signal.



$$S/B = \frac{(I_{600nm}/I_{465nm})_{\text{with Heparin}}}{(I_{600nm}/I_{465nm})_{\text{No Heparin}}}$$

Figure S14. Change in S/B of sensor **1** (2.5 μM) as a function of the heparin concentration in 5% FBS containing water solution (pH 7.4).

Heparinase I or Heparin lyase

Heparinase belongs to lyase group of enzymes, specifically known for cleaving C-O bond of highly sulfated polysaccharide chains containing 1-4 linkages between hexosamines and O-sulfated iduronic acid residues. The conventional heparinase I activity assay is to measured the absorbance (at 235 nm) of the formed unsaturated uronic acid during enzymatic cleavage. But as different components of biological matrices also generally absorb in this range, this procedure often suffers from significant interference from background.

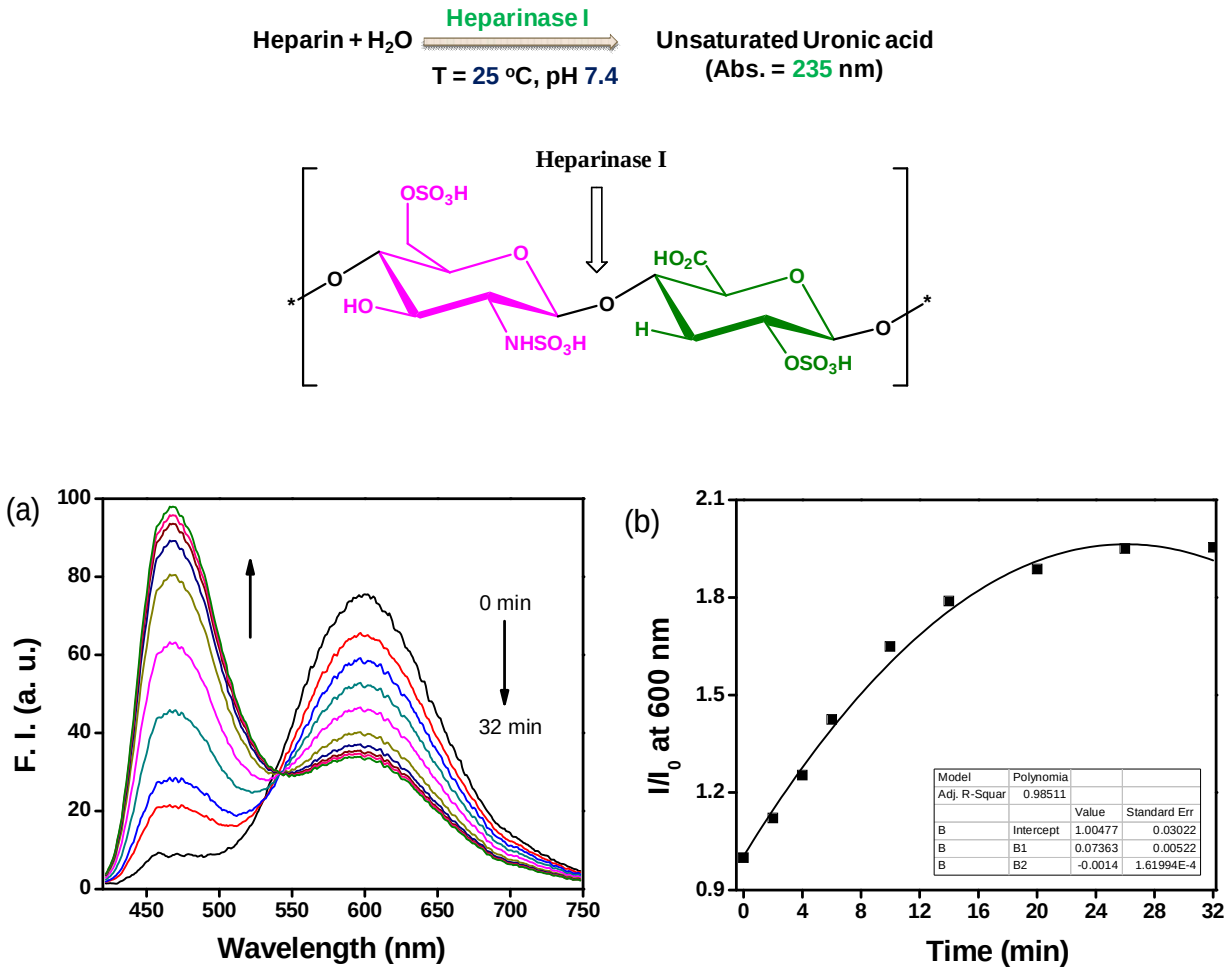


Figure S15. (a) Change in emission intensity of (**1**+heparin 1.0 μg/mL) upon addition of heparinase I (0.2 μg/mL) with time in water (pH 7.4). (b) Change in emission intensity (at 600 nm) of **1**+heparin conjugate upon addition of heparinase I in water (pH 7.4).

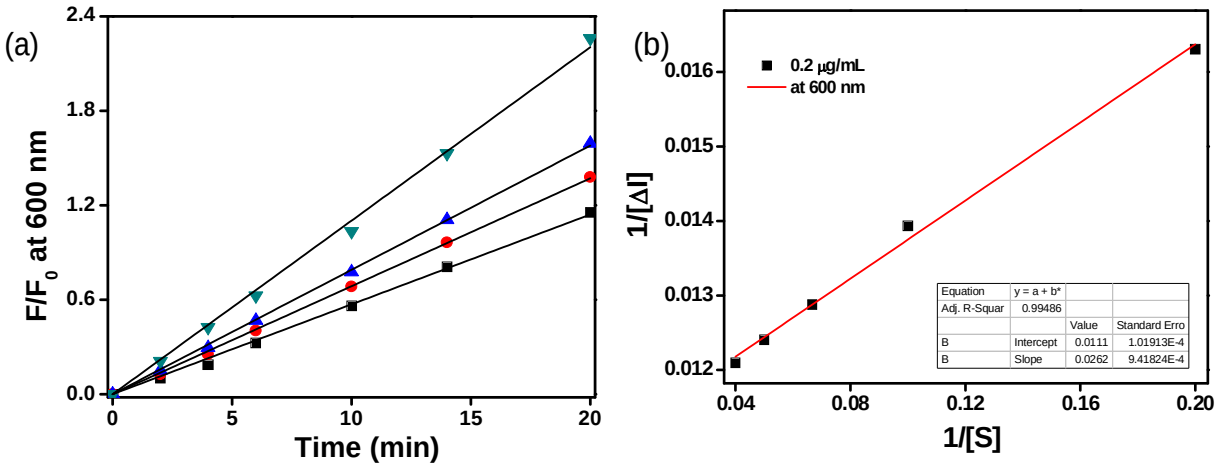


Figure S16. (a) Change in emission intensity of (**1**+heparin 1.0 μg/mL) upon addition of different amount of heparinase I (0.05-0.2 μg/mL) in water (pH 7.4). (b) Lineweaver–Burk plot for determination of Michalis-Menten constant value for heparinase I in water (pH 7.4) at 25 ^{o}C

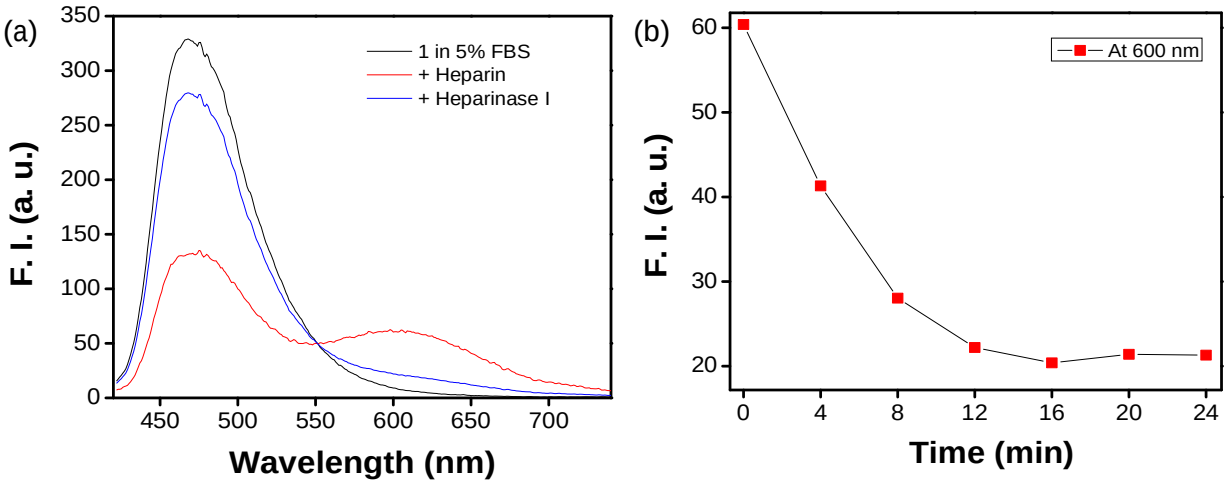


Figure S17. (a) Change in emission spectra of **1** + heparin conjugate ([1] = 2.5 μM, Heparin = 3.5 μg/mL) upon addition of heparinase I (0.2 μg/mL) in 5% serum medium (pH 7.4). (b) Change in emission intensity (at 600 nm) of **1** + heparin conjugate upon addition of heparinase I (0.2 μg/mL) with time in 5% serum medium (pH 7.4).