Electronic Supplementary Information

Discovery of heparin chemosensors through diversity oriented fluorescence library approach

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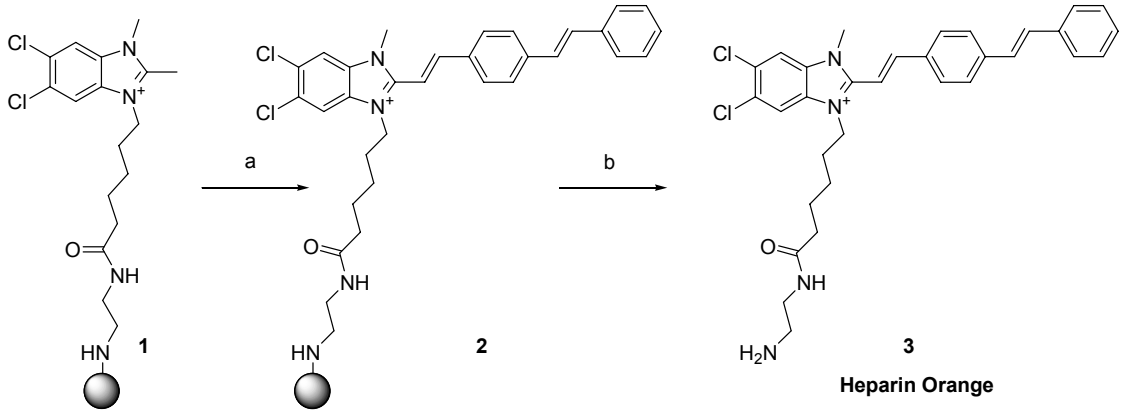
General Information

All chemicals and solvents were purchased from Sigma-Aldrich or Acros and used without further purification. 2-Chlorotrityl alcohol resin (1.37 mmol g^{-1}) were purchased from BeadTech Inc., Korea. All compounds were tested with LC-MSD (ChemStation 1100, Agilent Technologies.) equipped with a Phenomenex Luna 3μC18 column (20 × 4.0 mm). High accuracy mass spectrometry was obtained from Agilent 1200 series HPLC and 6210 TOF MS system. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker Avance 400 NMR spectrometer and were recorded at 400 and 100 MHz respectively. Chemical shifts are reported relative to tetramethylsilane. Heparin from porcine intestinal mucosa (180 U mL^{-1}) and low molecular weight heparin from porcine intestinal mucosa (mw 4000-6000 Da) were purchased from Sigma-Aldrich with sodium as the counter cation. Pooled normal human plasma with citrate was purchased from Innovative.

Synthesis of G26 (Heparin Orange) [1]



To resin **1** (10 mg, 1 eq) was added trans-stilbenecarboxaldehyde (10 eq) in 1-methyl-2-pyrrolidinone (300 μL) solution and pyrrolidine (2 μL). The reaction was shaken in dark and under a positive pressure ofnitrogen for 24 hrs. The resin was filtered and washed with DMF (5 times), alternatively dichloromethane and methanol (5 times), dichloromethane (5 times)

and dried in vacuum.

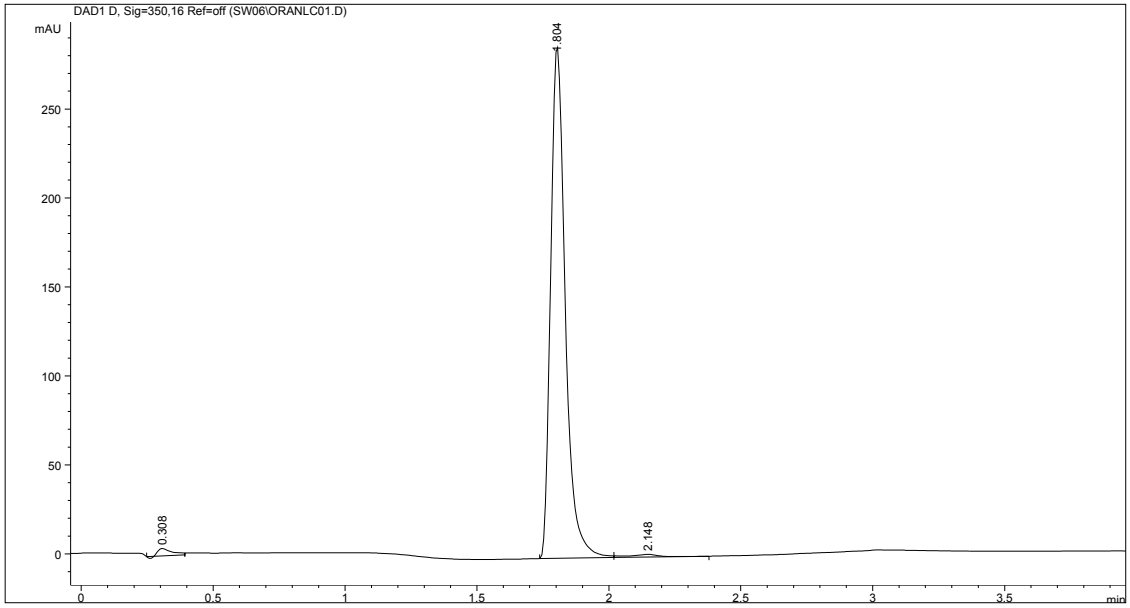
Resin **2** (10 mg) was suspended in 5% trifluoroacetic acid/dichloromethane cleavage cocktail solution (0.5 mL) and shook for 15 min. The resin was filtered off and washed with dichloromethane (1 mL) and methanol (1mL). The solutions were collected and evaporated to dryness to obtain the benzimidazolium dye **3**.

^{1}H-NMR (DMSO): 8.624(s, 1H), 8.515(s, 1H), 8.030(t, 1H), 7.720(d, 1H, J=13Hz), 7.592(m, 2H), 7.581(d, 1H, J=8Hz), 7.380(m, 2H), 7.299(m, 4H), 7.155(d, 1H, J=8), 6.811(d, 1H, J=13Hz), 4.292(m, 2H), 3.633(s, 3H), 3.242(m, 2H), 2.828(m, 2H), 2.041(t, 2H), 1.694(m, 2H), 1.463(m, 2H), 1.273(m, 2H).

^{13}C-NMR (DMSO): 172.634, 158.316, 158.010, 157.704, 157.397, 150.272, 145.782, 139.186, 136.528, 132.647, 131.424, 130.490, 130.459, 129.674, 129.520, 128.911, 128.685, 128.077, 127.232, 127.196, 126.638, 118.711, 115.726, 115.581, 115.293, 107.827, 46.055, 38.514, 36.288, 34.913, 32.814, 28.131, 25.436, 24.351.

Figure S1.

LC-MS spectrum of **G26(Heparin Orange).** ESI-MS (m/z) calcd (found): 561.2(560.8) for $[M]^+$.



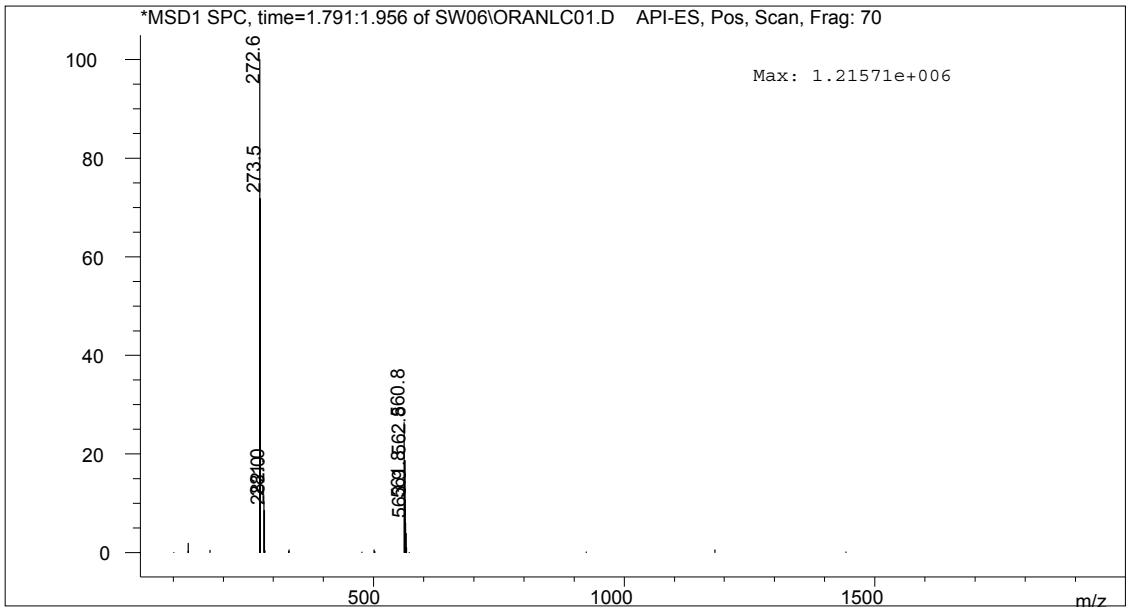


Figure S2. High Accuracy ESI-TOF Mass Spectrometry of **G26 (Heparin Orange)** in MeOH**.** Calcd (Found): 561.2182(561.2178) for $[M]^+$

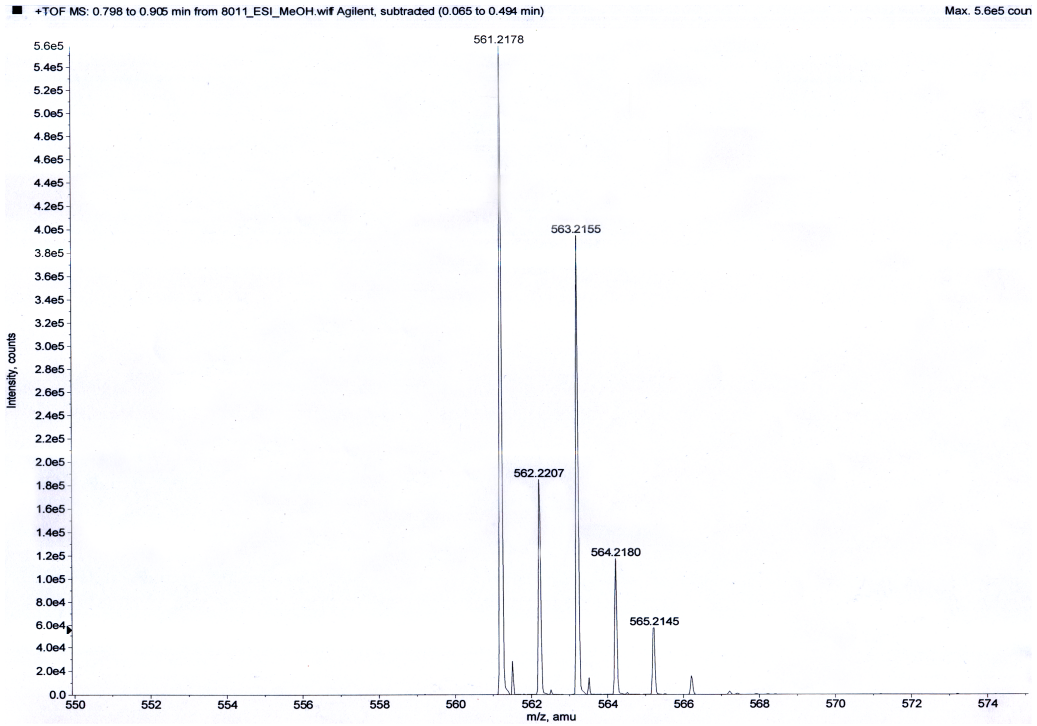
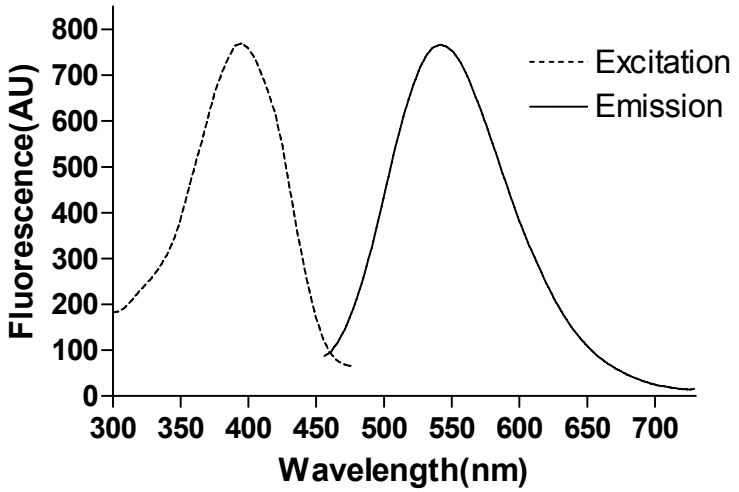
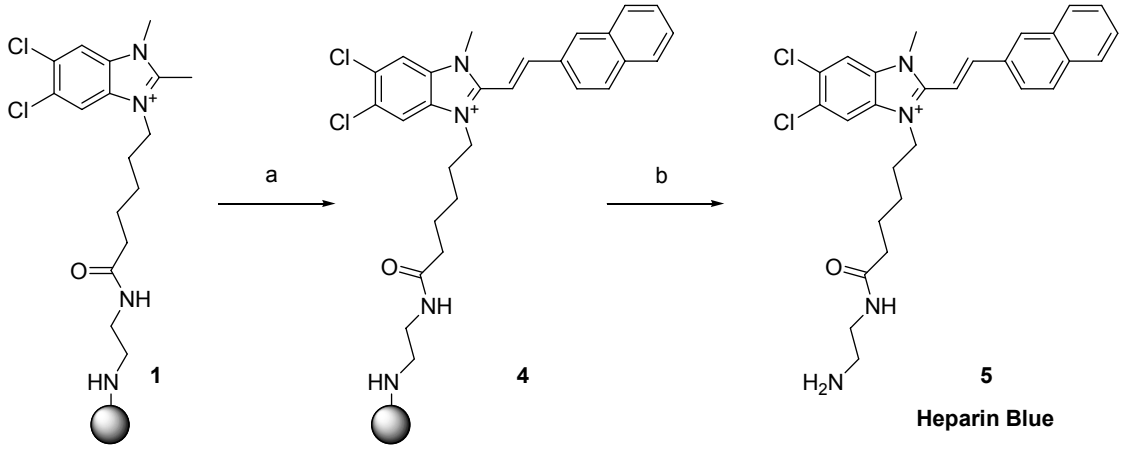


Figure S3. Excitation and emission, spectra of **G26 (Heparin Orange)** in MeOH**.**



Fluorescence excitation (emission: 540 nm) and emission (excitation: 390 nm) spectra of 20 μM G26 in methanol (100 μL) in a Grainer 96 well black polypropylene plate.

Synthesis of G45 (Heparin Blue) [1]



To resin **1** (10 mg, 1 eq) was added 2-naphthaldehyde (10 eq) in 1-methyl-2-pyrrolidinone (300 µL) solution and pyrrolidine (2 µL). The reaction was shaken in dark and under a positive pressure ofnitrogen for 24 hrs. The resin was filtered and washed with DMF (5 times), alternatively dichloromethane and methanol (5 times), dichloromethane (5 times) and dried in vacuum.

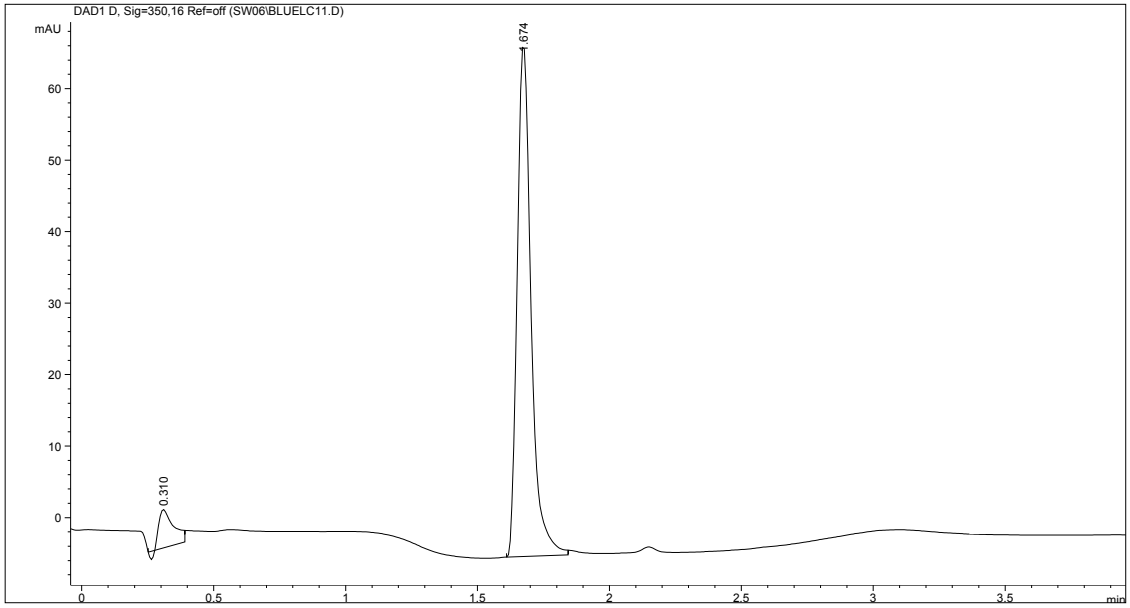
Resin **4** (10 mg) was suspended in 5% trifluoroacetic acid/dichloromethane cleavage cocktail solution (0.5 mL) and shook for 15 min. The resin was filtered off and washed with dichloromethane (1 mL) and methanol (1mL). The solutions were collected and evaporated to dryness to obtain the benzimidazolium dye **5**.

^{1}H-NMR (DMSO): 8.618(s, 1H), 8.499(s, 1H), 8.010(t, 1H), 7.924(m, 3H), 7.905(d, 1H, J=13Hz), 7.829(d, 1H), 7.575(m, 2H) 7.064(m, 1H), 6.910(d, 1H, J=13Hz), 4.290(t, 2H), 3.618(s, 3H), 3.246(m, 2H), 2.830(m, 2H), 2.010(t, 2H), 1.684(m, 2H), 1.407(m, 2H), 1.254(m, 2H).

^{13}C-NMR (DMSO): 172.621, 157.804, 150.179, 146.198, 133.251, 132.621, 131.414, 131.151, 130.510, 129.753, 129.691, 129.531, 129.090, 128.525, 127.794, 127.585, 127.008123.945, 115.544, 115.291, 108.503, 48.475, 46.072, 38.428, 36.246, 34.849, 32.846, 28.064, 25.392, 24.289.

Figure S4.

LC-MS spectrum of **G45(Heparin Blue).** ESI-MS (m/z) calcd (found): 509.2(508.9) for $[M]^+$.



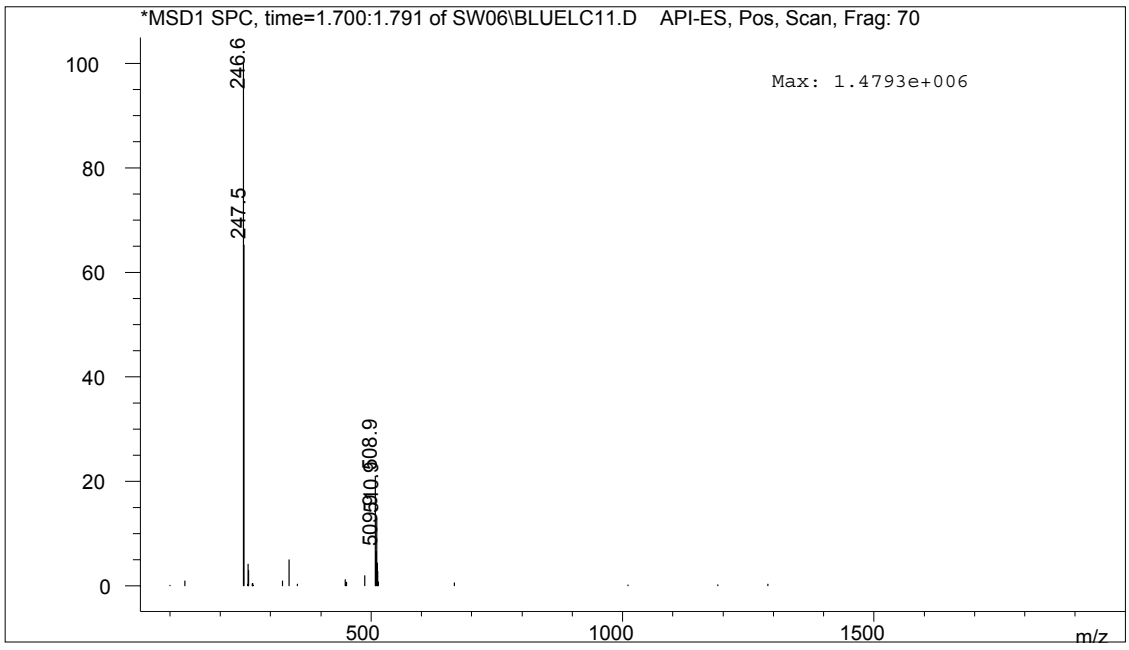


Figure S5. High accuracy ESI-TOF Mass Spectrometry **G45(Heparin Blue)** in MeOH**.** Calcd (Found): 509.1838(509.1862) for $[M]^+$

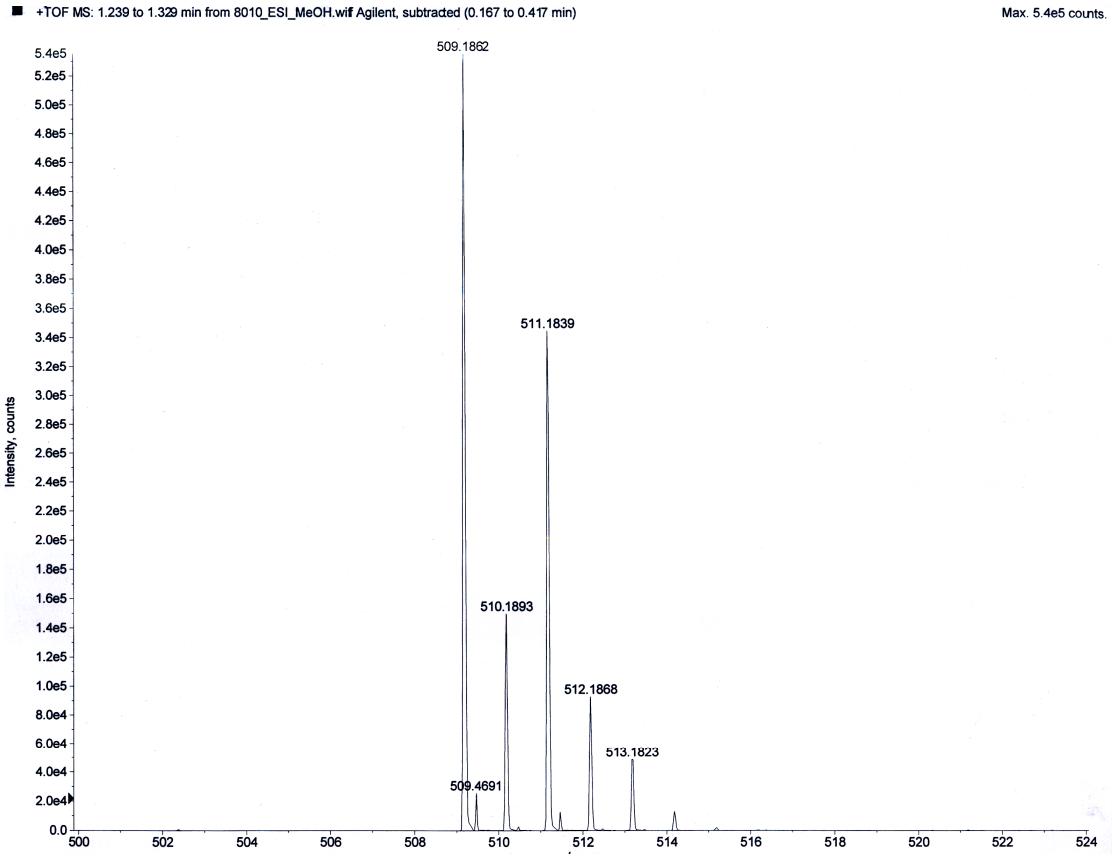
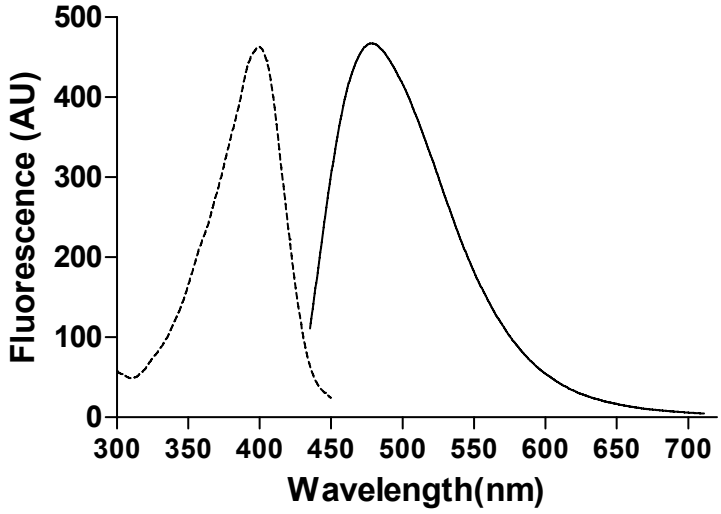


Figure S6. Excitation, emission, and absorbance spectra of **G45(Heparin Blue)** in MeOH**.**



Fluorescence excitation (emission: 477 nm) and emission (excitation: 410 nm) spectra of 1 mM G45 in methanol (100 μL) in a Grainer 96 well black polypropylene plate.

UFH and LMWH concentration calculation

Due to the complexity of heparin, in this research we determined heparin molecular weight as the common repeating disaccharide = 644.2 g mol^{-1}. [2] Since UFH is 180 U mg^{-1}, 1 μM UFH is corresponding to 0.12 U mL^{-1}.

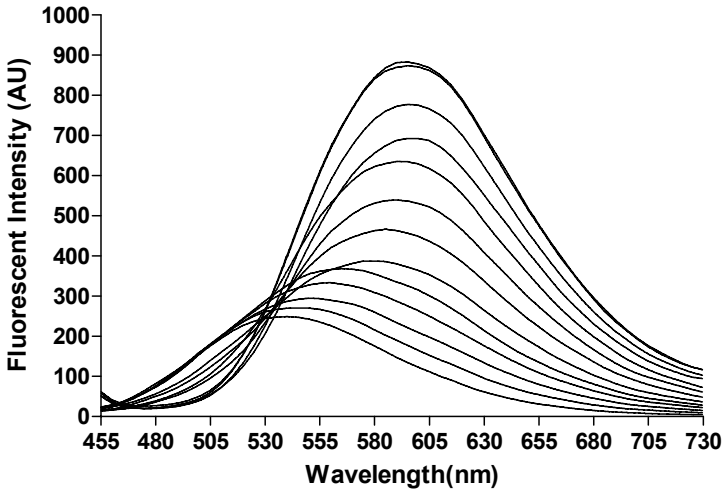
Primary screening procedure

1. Benzimidazolium compounds were transferred to Grainer 96 well black polypropylene plates (final concentration as 10 μM) and tested with 2 μM, 5 μM, 10 μM and 20 μM heparin and blank control in 10 mM HEPES buffer (pH = 7.4) with 1% DMSO. Fluorescent spectra were recorded on a Gemini XS fluorescent plate reader with excitation at 350 nm (cutoff: 420 nm), 400 nm (cutoff: 435 nm), 450 nm (cutoff: 495 nm).

2. Benzimidazolium compounds were transferred to Grainer 96 well black polypropylene plates (final concentration as 10 μM) and tested with 10 μM heparin and 0.5mg mL^{-1} protamine, and blank control in 10 mM HEPES buffer (pH = 7.4) with 1% DMSO. Fluorescent spectra were recorded on a Gemini XS fluorescent plate reader with excitation at 350 nm (cutoff: 420 nm), 400 nm (cutoff: 435 nm), 450 nm (cutoff: 495 nm).

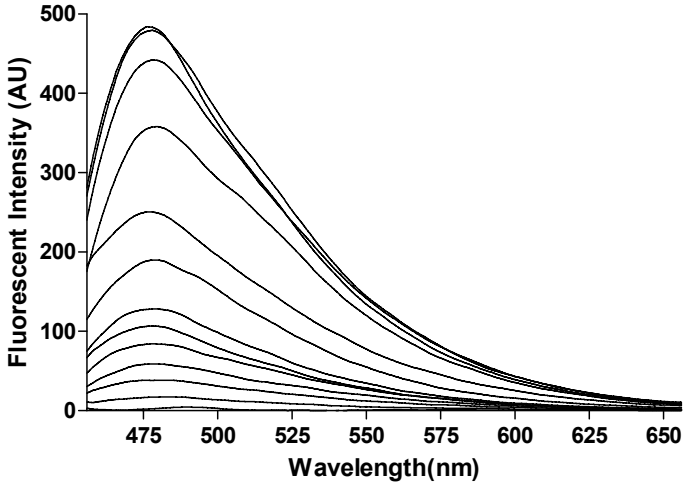
3. Benzimidazolium hit compounds were transferred to Grainer 96 well black polypropylene plates (final concentration as 10 μM) and tested with 2 μM, 5 μM, 10 μM and 20 μM heparin in 20% Human plasma/HEPES buffer. Fluorescent spectra were recorded on a Gemini XS fluorescent plate reader with corresponding excitation wavelength.

Figure S7. Fluorescence emission spectra of **Heparin Orange** with different concentrations of LMWH.



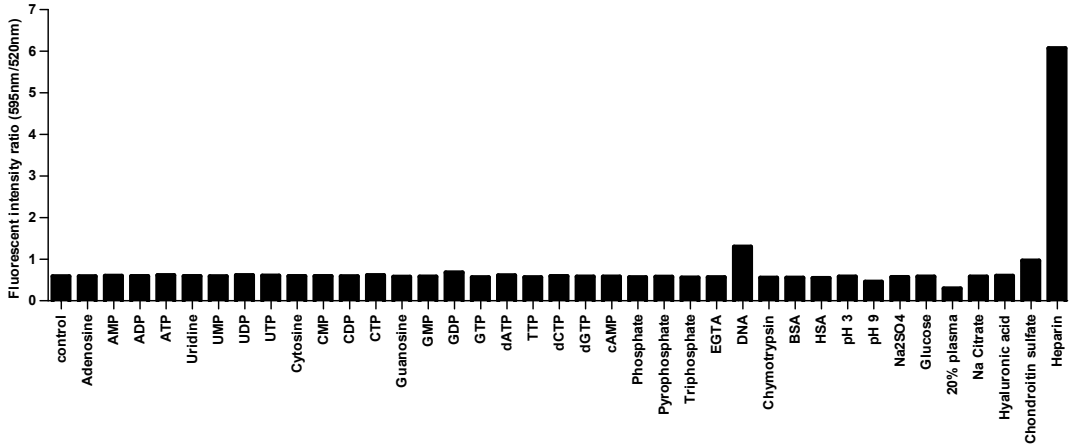
Fluorescence emission spectra (excitation: 380 nm, cutoff: 420 nm) of Heparin Orange (10 μM) with 0, 0.12, 0.2, 0.3, 0.4, 0.6, 0.8, 1, 1.2, 2, 4, 5, 10 μM of LMWH in 10 mM HEPES buffer (pH = 7.4) with 1% DMSO. The quantum yield (Φ) of Heparin Orange with and without heparin is 0.061 and 0.023, respectively.[3]

Figure S8. Fluorescence emission spectra of **Heparin Blue** with different concentrations of LMWH.



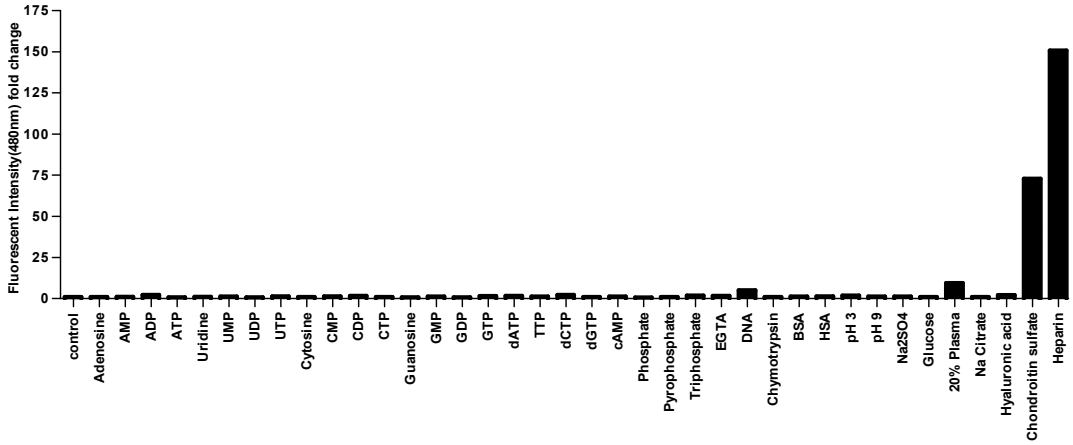
Fluorescence emission spectra (excitation: 420 nm, cutoff: 455 nm) of Heparin Blue (10 μM) with 0, 0.2, 0.4, 0.1, 0.6, 0.8, 1, 1.2, 1.5, 2, 3, 4, 5, 10 μM LMWH in 10 mM HEPES buffer (pH = 7.4) with 1 % DMSO. The quantum yield (Φ) of Heparin Blue with and without heparin is 0.31 and 0.048, respectively. [3]

Figure S9. Heparin Orange selectivity test.



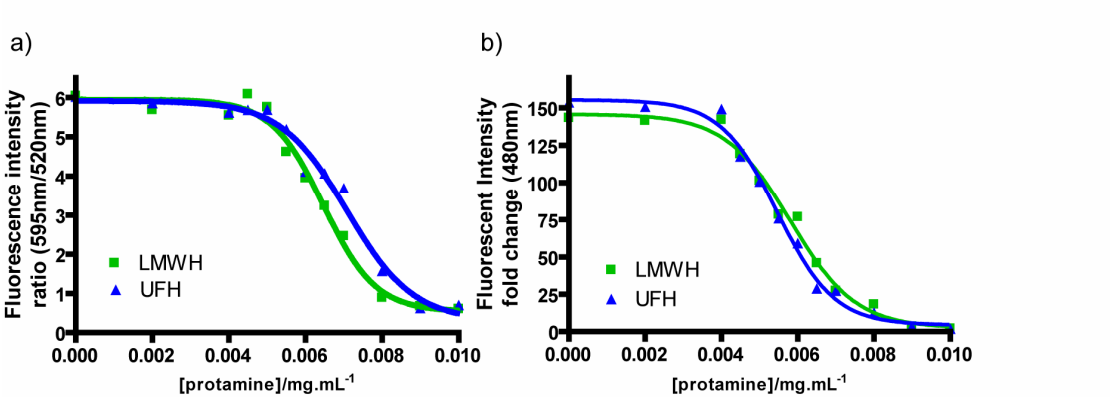
Heparin Orange (10 μM) fluorescent emission ratio of 595 nm / 520 nm upon addition of analytes. All nucleosides and nucleotides, phosphate, pyrophosphate, triphosphate, EGTA, Na_2SO_4, glucose, Na Citrate were at 50 μM in 10 mM HEPES buffer (pH = 7.4). DNA was at 0.025 mg mL^{-1} (~50 μM). Chymotrypsin, bovine serum albumin(BSA) and human serum albumin (HSA) were at 0.5 mg mL^{-1}. Heparin, hyaluronic acid and chondroitin sulfate were at 6.5 μg mL^{-1} (~10 μM). pH =3 and pH=9 solution were prepared by HCl and NaOH respectively. 20% plasma solution were prepared by dilution of pooled normal human plasma in 10mM HEPES buffer (pH = 7.4). Control is 10mM HEPES buffer (pH = 7.4). Due to the auto-fluorescence of blood plasma sample at short wavelength, the background ratio of fluorescent emission of 595nm/520nm was lower compared to control condition.

Figure S10. Heparin Blue selectivity test.



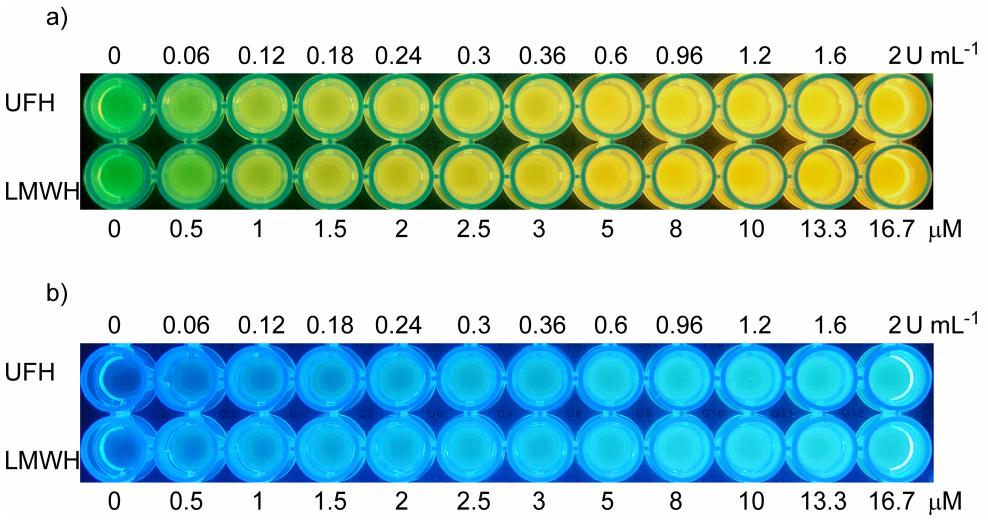
Heparin Blue (10uM) fluorescent emission intensity fold change at 480nm upon addition of analytes. All nucleosides and nucleotides, phosphate, pyrophosphate, triphosphate, EGTA, Na_2SO_4, glucose, Na Citrate were at 50 µM in 10 mM HEPES buffer (pH = 7.4). DNA was at 0.025 mg mL^{-1} (~50 µM). Chymotrypsin, bovine serum albumin(BSA) and human serum albumin (HSA) were at 0.5 mg mL^{-1}. Heparin, hyaluronic acid and chondroitin sulfate were at 6.5 µg mL^{-1} (~10 µM). pH =3 and pH=9 solution were prepared by HCl and NaOH respectively. 20% plasma solution were prepared by dilution of pooled normal human plasma in 10mM HEPES buffer (pH = 7.4). Control is 10mM HEPES buffer (pH = 7.4). Due to the auto-fluorescence of blood plasma sample at short wavelength, the background of fluorescence emission at 480nm was higher compared to control condition. These tests demonstrated that our dye is selective for sulfated carbohydrate polymer.

Figure S11. Protamine titrations of Heparin Orange and Heparin Blue.



Briefly, 1 µL of 0.2, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.8, 0.9, and 1 mg mL^{-1} of protamine in 10 mM HEPES buffer (pH = 7.4) were added to 100 µL of equilibrated 10 µM Heparin Orange(a) / Heparin Blue(b) and 10 µM UFH(blue) / LMWH(green) in a Grainer 96 well black polypropylene plate and the fluorescence emission were recorded on a Gemini XS fluorescent plate reader.

Figure S12. Picture of Heparin Orange and Heparin Blue with different concentrations of UFH and LMWH in human plasma.



Picture of Heparin Orange (20 μM) (a) and Heparin Blue (20 μM) (b) upon addition of UFH and LMWH at indicated concentrations in 20% pooled human plasma in 10 mM HEPES buffer (pH = 7.4) with 1% DMSO in a 96 well plate under 365 nm UV lamp light. Corresponding U mL^{-1} is the calculated corresponding concentrations of UFH based on 180 U mg^{-1}. Concentrations indicate the final concentrations in the assay solutions, and the corresponding concentrations in original plasma should be timed by 5.

References

1. Wang, S.; Chang, Y. T. *J. Am. Chem. Soc.* **2006**, *128*, 10380-10381.
2. Wright, A. T.; Zhong, Z. L.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2005**, *44*, 5679-5682., supporting information.
3. Fluorescence quantum yields of Heparin Orange and Heparin Blue were determined by reference to quinine sulfate in 0.1 M H_2SO_4 (Φ = 0.54) and fluorescein in 0.1 M NaOH (Φ = 0.79) respectively and following Fery-Forgues, S.; Lavabre, D. *J. Chem. Educ.* **1999**, *76*, 1260-1264.