Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

A BODIPY-*O*-glycoside based near-infrared fluorescent sensor for serum albumin

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1. Materials and General Methods.

Chemical reactions are carried under argon atmosphere using anhydrous solvent in round bottom flask fitted with rubber septa or screw-cap schlenk tube with magnetic stirring unless stated otherwise. Reagents are obtained from commercial supplier and were used without further purification except triethyl amine (TEA). This reagent was purified by distillation, kept over KOH for overnight and then dried by distillation with calcium hydride and stored over 3Å molecular sieves (activated) in septum fitted reagent bottle before use. Solvents like methanol, ethanol and iso-propanol procured from commercial source are further purified by distillation. Tetrahydrofuran (THF) was dried by distillation under argon over sodium-benzophenone. Reactions were monitored by Thin-layer chromatography which was performed in commercially available 0.25 mm fluorescent silica plate (F-254) and visualized using a UV-lamp (254/365 nm wavelength) or developed in iodine chamber or in alkaline $KMnO_4$ solution after heating. Normal phase flash chromatography was performed for separation using silica gel (40-63 μm) and eluted with distilled solvents.

The ^1H and ^{13}C spectra were recorded at room temperature with a 200 MHz, 300 MHz or 600 MHz spectrometer, using perdeuterated solvents as the internal standards. The ^1H and ^{13}C chemical shifts are given in ppm relative to the residual protiated solvent or the solvent, respectively. The following abbreviations are used to express multiplicity of ^1H NMR signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplate. Proton coupling constants (*J* values) are expressed in Hertz (Hz). Yields represent to chromatographically and spectroscopically (^1H NMR) homogeneous materials. High Resolution Mass Spectrometric (HRMS) analysis was done using 6540 UHD Accurate- Mass Agilent Q-TOF LC/MS instrument. CHN analysis was done in Thermo finnigan make FLASH EA 1112 series CHNS (O) Analyzer.

2. Synthesis of BODIPY dyes.

2,6-Diethyl-4,4-difluoro-3,5-di(4′-hydroxystyryl)-1,7-dimethyl-8-phenyl-4-bora-3a,4a-diaza-*s*-indecene (2): A mixture of **1** (0.6 g, 1.579 mM), *p*-hydroxybenzaldehyde (0.386 g, 3.158 mM), glacial acetic acid (0.9 ml) and piperidine (6.0 ml) in toluene (60 ml) was refluxed with simultaneous azeotropic removal of water (formed during the reaction). After the consumption of **1**, H_2O (100 ml) was added into the reaction mixture, which was extracted with $CHCl_3$ (3 × 20 ml). The organic layer was dried and concentrated in vacuo to give a residue, which on column chromatography (silica gel, hexane-EtOAc) furnished **2** (0.4 g, 43%) as a dark green powder. mp: >250 °C; ^1H NMR (600 MHz, $(CD_3)_2SO$): δ 1.09 (t, *J* = 7.2 Hz, 6H),

1.29 (s, 6H), 2.57 (q, J = 7.2 Hz, 4H), 6.86 (d, J = 7.8 Hz, 4H), 7.22 (d, J = 16.8 Hz, 2H), 7.39–7.45 (m, 4H), 7.47 (d, J = 8.4 Hz, 4H), 7.56–7.58 (m, 3H); The ^{13}C NMR spectrum could not be recorded due to poor solubility; EI-MS (*m/z*): 587.5 $[M-1]^+$; Anal. Calcd. For $C_{37}H_{35}BF_2N_2O_2$: C, 75.51; H, 5.99; N, 4.76%; Found: C, 75.14; H, 5.83; N, 4.87%.

2,6-Diethyl-4,4-difluoro-1,7-dimethyl-3,5-di(4′-[[2″,3″,4″,6″-tetra-*O*-acetyl-α-D-glucopyra-nosyl]oxy]styryl)-8-phenyl-4-bora-3a,4a-diaza-*s*-indecene (3): A mixture of Bu_4NBr (0.132 g, 0.408 mM) in 1:1 H_2O–$CHCl_3$ (10 ml) was stirred at 40 °C for 10 min. Solutions of 2- bromoglucose tetraacetate (0.168 g, 0.408 mM) and **2** (0.120 g, 0.204 mM) in $CHCl_3$ (10 ml) and of K_2CO_3 (0.168 g) in H_2O (1.0 ml) were simultaneously added into the reaction mixture, which was heated to 60 °C for 6 h with vigorous stirring. It was brought to room temperature, washed with aqueous 5% NaOH (3 × 20 ml), H_2O (2 × 20 ml) and brine (1 × 20 ml), and dried. Column chromatography of the residue (silica gel, hexane/EtOAc) gave **3** (0.071 g, 28%) as a dark green powder. mp: 218 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.14 (t, J = 7.6 Hz, 6H), 1.34 (s, 6H), 2.04–2.09 (m, 24H), 2.67 (q, J = 7.4 Hz, 4H), 3.88–4.01 (m, 2H), 4.14–4.46 (m, 4H), 5.12–5.45 (m, 8H), 7.09 (d, J = 8.6 Hz, 4H), 7.28 (d, J = 17.0 Hz, 2H), 7.33–7.38 (m, 2H), 7.51–7.63 (m, 7H), 7.76 (d, J = 17.0 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 11.4, 14.0, 18.3, 20.5, 20.7, 22.6, 29.4, 29.6, 31.8, 33.8, 61.8, 68.2, 71.1, 72.0, 72.6, 98.8, 114.0, 117.1, 119.3, 128.6, 129.1, 132.7, 133.0, 133.7, 134.7, 135.8, 138.6, 139.1, 150.2, 157.0, 169.3, 169.4, 170.2, 170.6; HRMS (ESI-TOF) *m/z*$[M + K]^+$ Calcd. For $C_{65}H_{71}BF_2N_2O_{20}$ 1287.4298; Found 1287.4273.

2,6-Diethyl-4,4-difluoro-1,7-dimethyl-3,5-di(4′-[[α-D-glucopyranosyl]oxy]styryl)-8-phenyl-4-bora-3a,4a-diaza-*s*-indecene (4): 3 (0.175 g, 0.140 mM), was added to methanolic solution (10 ml) of NaOMe (0.7 mM) and stirred for 1 h at room temperature. Solvent removal in vacuo followed by column chromatography (silica gel, $CHCl_3$/MeOH) of the residues produced **4** (0.058 g. 45%) as a dark green powder. mp: >250 °C; ^{1}H NMR (400 MHz, d_8-THF): δ 1.16 (t, J = 8.0 Hz, 6H), 1.36 (s, 6H), 2.65 (q, J = 8.0 Hz, 4H), 3.76–3.92 (m, 4H), 4.49 (s, 2H), 4.59 (s, 2H), 4.78 (s, 2H), 4.93–4.95 (m, 4H), 7.12 (d, J = 12.0 Hz, 4H), 7.29 (d, J = 16.0 Hz, 2H), 7.38–7.40 (m, 2H), 7.53–7.56 (m, 7H), 7.74 (d, J = 16.0 Hz, 2H); ^{11}B NMR (128 MHz, d_8-THF): δ 1.19 (t, J = 34.6 Hz); ^{19}F NMR (376 MHz, d_8-THF): δ -139.09 (q, J = 33.8 Hz); The ^{13}C NMR could not be recorded due to poor solubility; HRMS (ESI-TOF) *m/z*$[M + Na]^+$ Calcd. for $C_{49}H_{55}BF_2N_2O_{12}$ 935.3714; Found 935.3683.

3. Photophysical studies.

Ground state absorption measurements were carried out using a Shimadzu (UV-2700) UV-Vis spectrophotometer and steady-state (SS) fluorescence studies were performed in Horiba fluoromax-4 spectrofluorimeter. In the SS measurements the dye samples were excited at 620 nm in ordered to get almost full emission spectrum.

The following equation was used to determine the relative fluorescence quantum yield:

$$\phi(X) = (A_S/A_X)(F_X/F_S)(n_X/n_S)^2\phi(S)$$

Where A is the absorbance at the excitation wavelength (in the range 0.01-0.1 A.U.), F is the area under the corrected emission curve, n is the refractive index of the solvents (at 25°C) used in measurements, and the subscripts S and X represent standard and unknown, respectively.

Excited-state lifetime measurements were performed using a Time Correlated Single Photon Counting (TCSPC) set up, obtained from Edinburgh, U.K. In TCSPC measurements, A 638 nm pulsed diode laser (EPL-640, pulse width ~100 ps and repetition rate 10 MHz) was used for exciting the samples and the decay traces for the excited-state were recorded at the emission maximum of the dye-protein complexes (~665 nm) using a photomultiplier tube (PMT) based detection module. Aqueous suspension of ludox was used to measure the instrument response function (IRF) for the present TCSPC setup. The full-width at half-maximum of a typical IRF was measured to be ~100 ps. The excited-state lifetime measurements were performed at magic angle (54.7^o) configuration.

4. Aggregation studies of Dye 2.

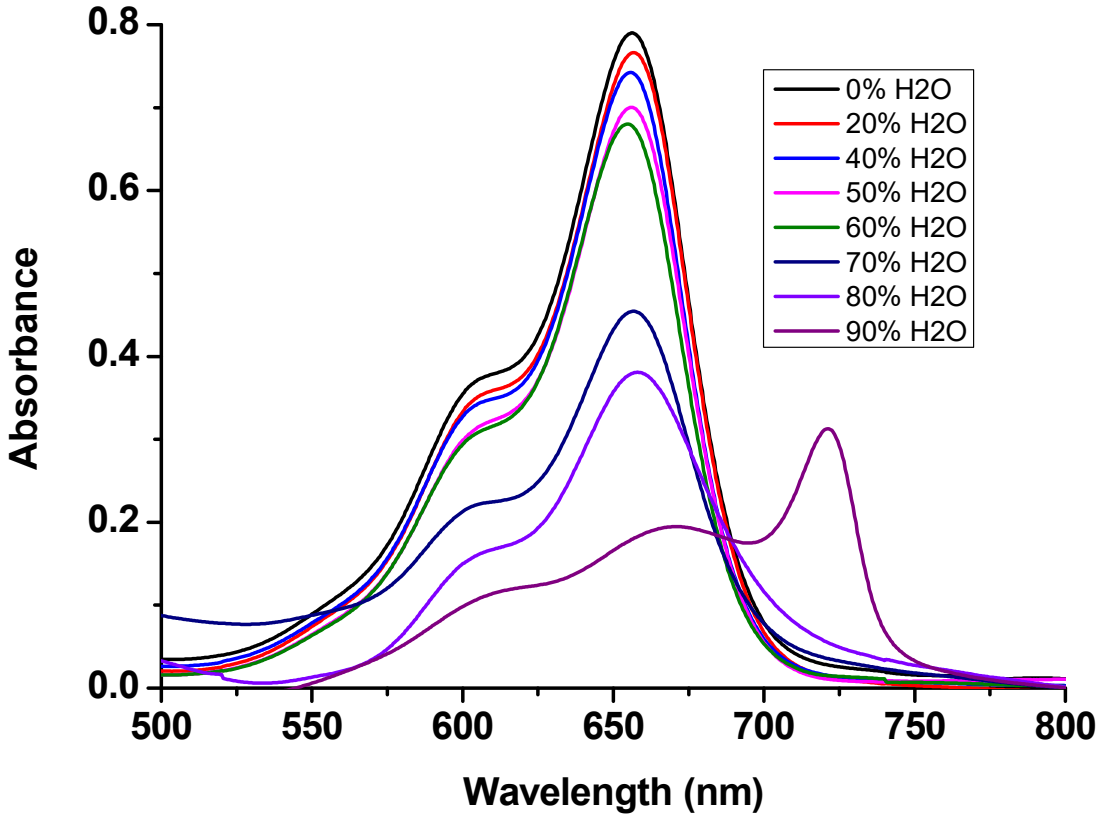


Figure S1. UV–vis spectra of the solutions of **2** (3.0 μM) in THF-water mixtures.

5. Aggregation studies of BDP-Glu (4).

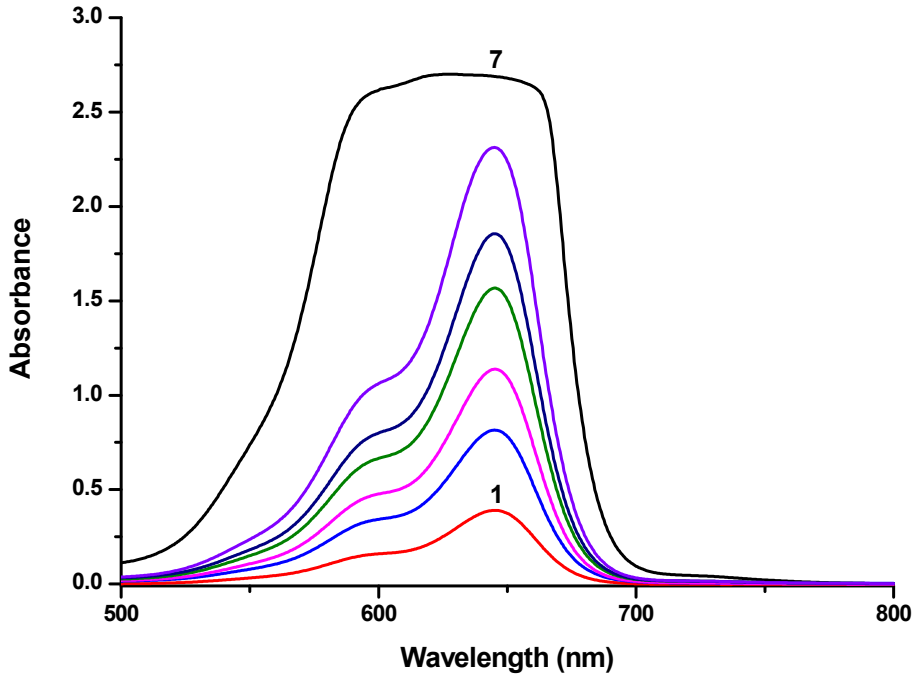


Figure S2. UV–vis spectra of dye **4** in ethanol with increasing concentrations of **4** from (1) 4.2 μM to (7) 84.8 μM.

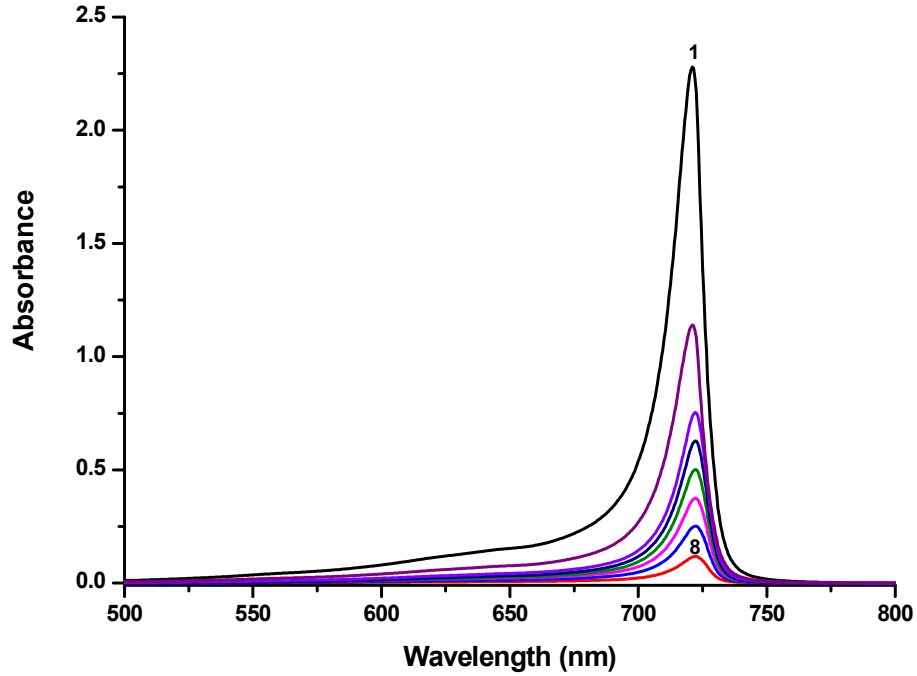


Figure S3. UV–vis spectra of dye **4** in water/ethanol (80/20, v/v) solutions with decreasing concentrations of **4** from (1) 16.96 μM to (8) 0.85 μM.

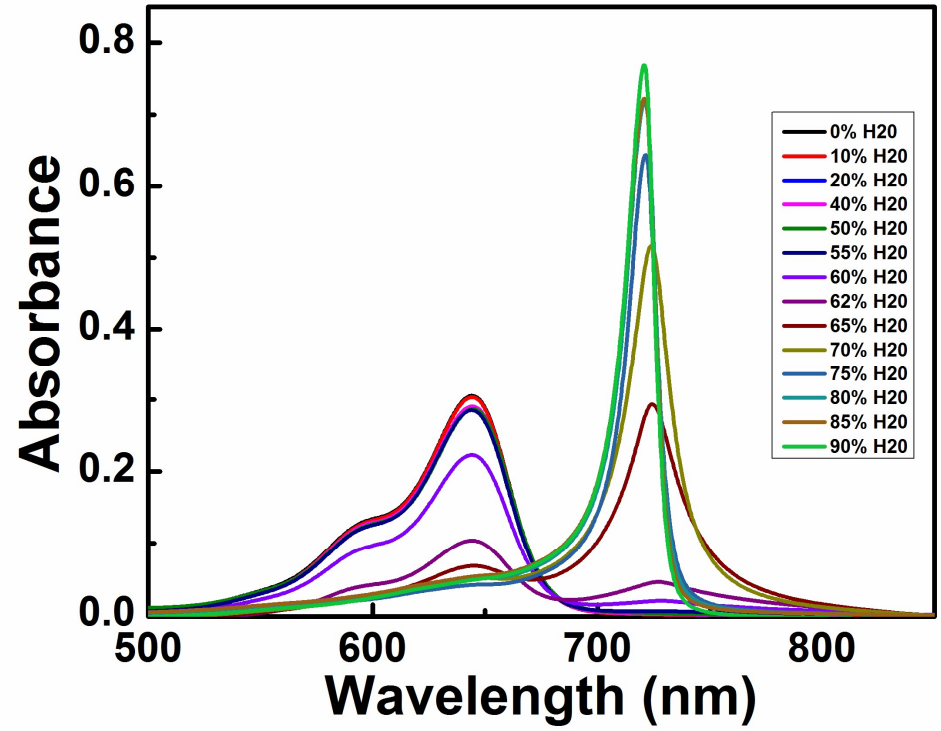


Figure S4. UV–vis spectra of the solutions of **4** (3.0 μM) in EtOH-water mixtures.

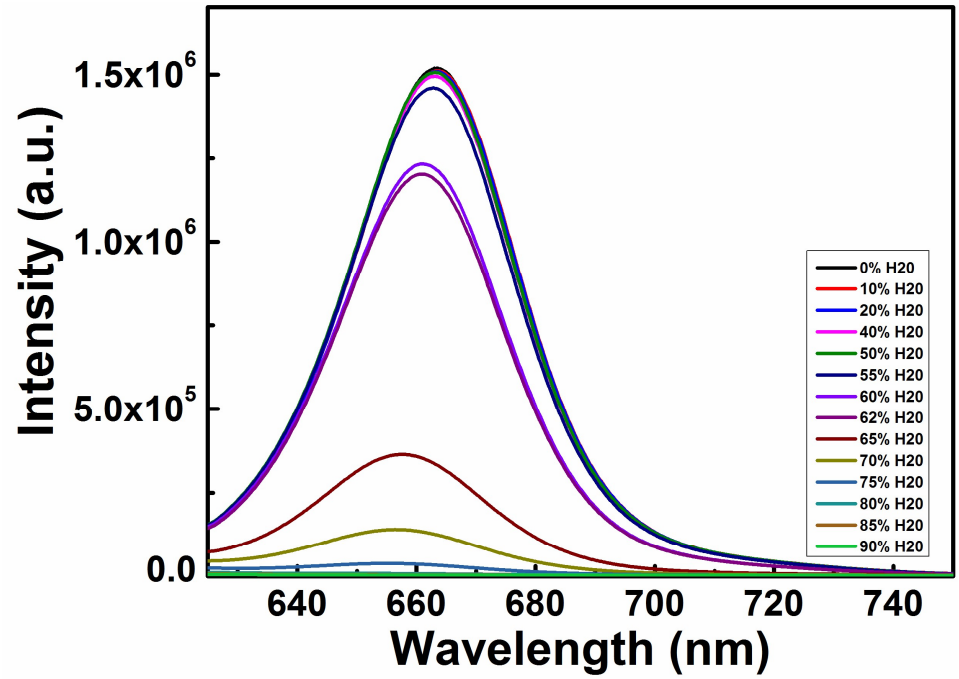
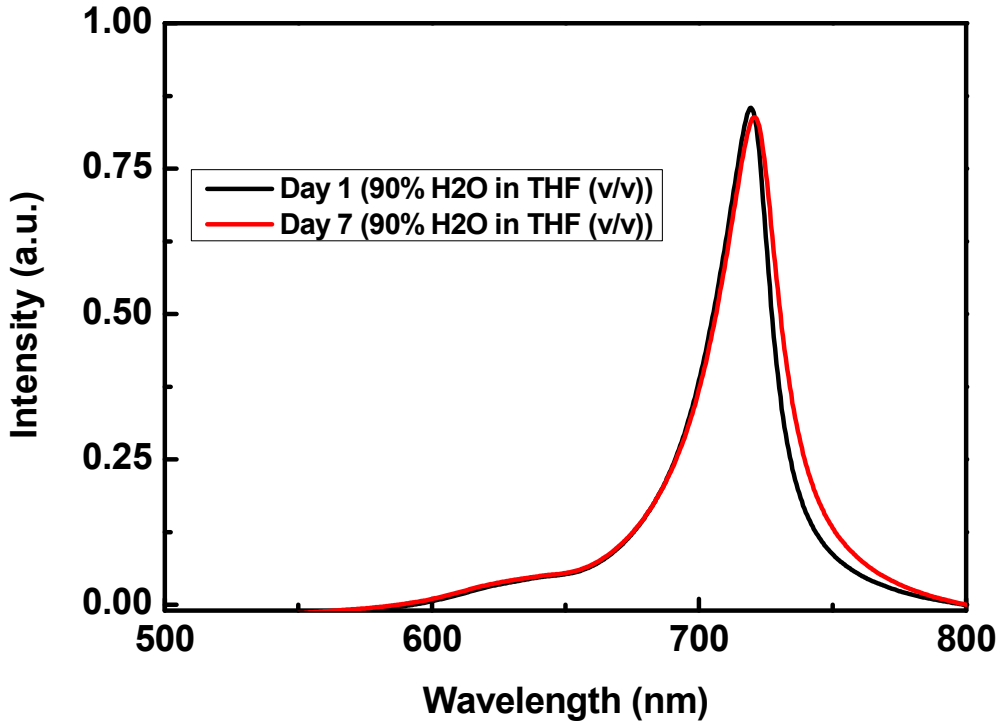
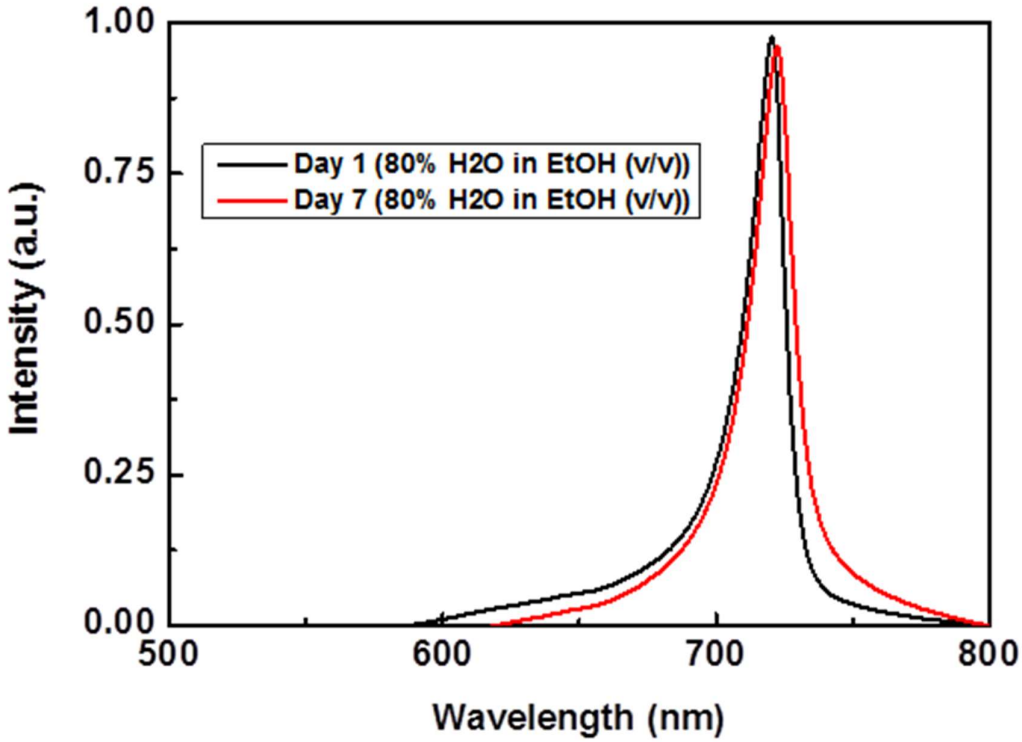


Figure S5. Fluorescence spectra of the solutions of **4** (3.0 μM) in EtOH-water mixtures.



Figures S6. Time dependent absorption spectra of the aggregated solution of dye **4** in 90% H_2O/THF (v/v).



Figures S7. Time dependent absorption spectra of the aggregated solution of dye **4** in 80% H_2O/EtOH (v/v)).

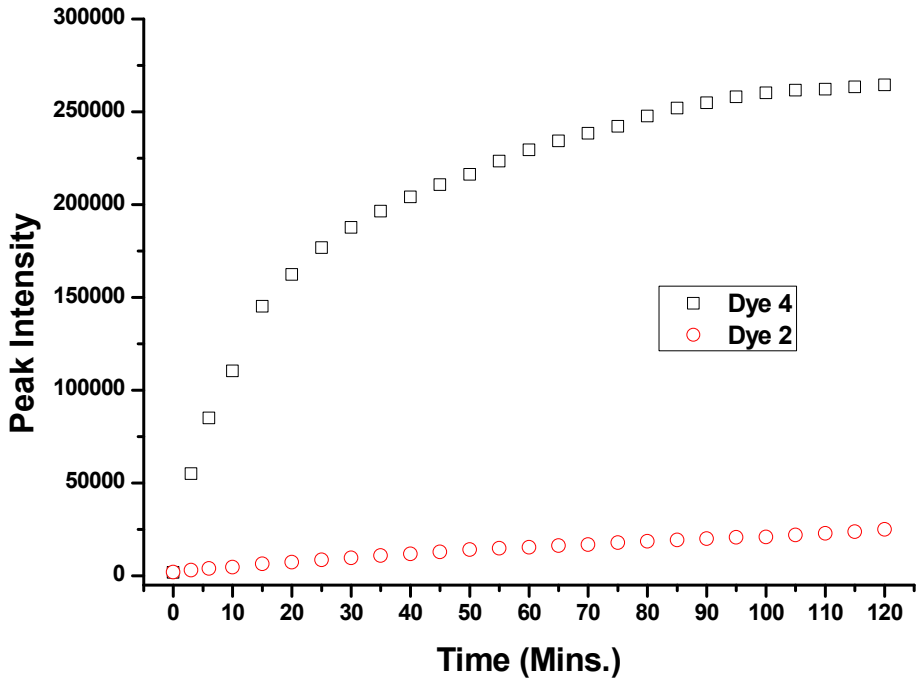


Figure S8. Fluorescence changes of dyes **2** and **4** (0.5 μM) with time upon the addition of BSA (413 μM) in buffer solutions.

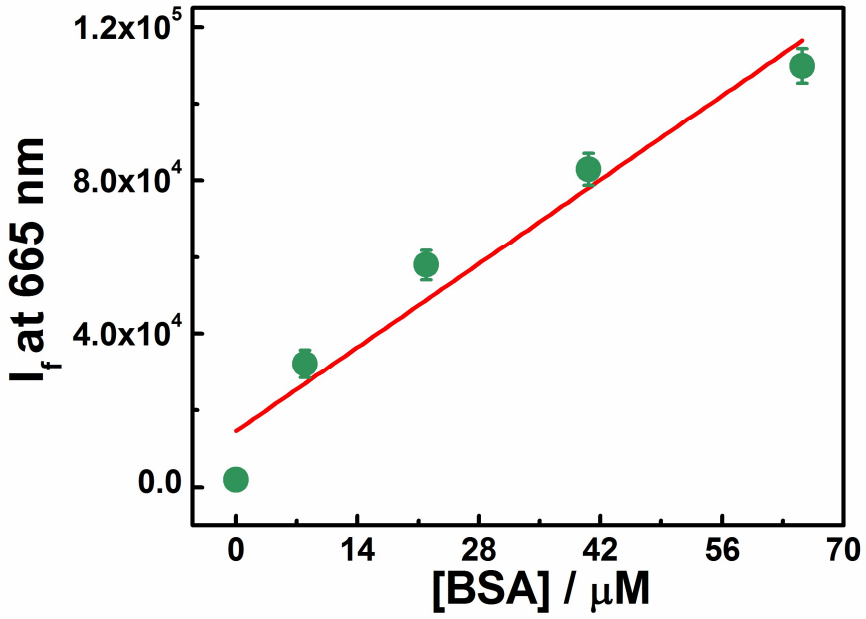


Figure S9. LOD determination plot for BSA using the linear changes in the emission intensity of the dye **4** as a function of BSA concentration from 0 to 65.2 μM. (I_{665} = 1.52 x 10^3 [BSA/μM] + 14576.4, R^2 = 0.937), LOD = 157.7 nM] [λ_{ex}= 620 nm].

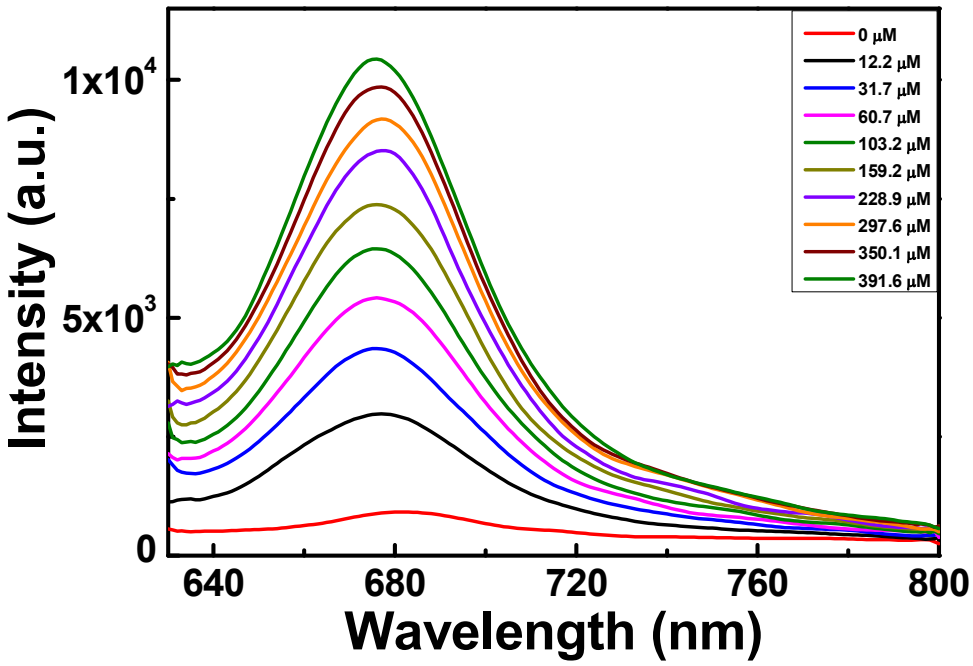


Figure S10. Steady-state emission spectra of dye **2** (0.5 μM) [λ_{ex} = 620 nm] in 10 mM PBS, pH 7.4 with step wise increase of BSA concentration.

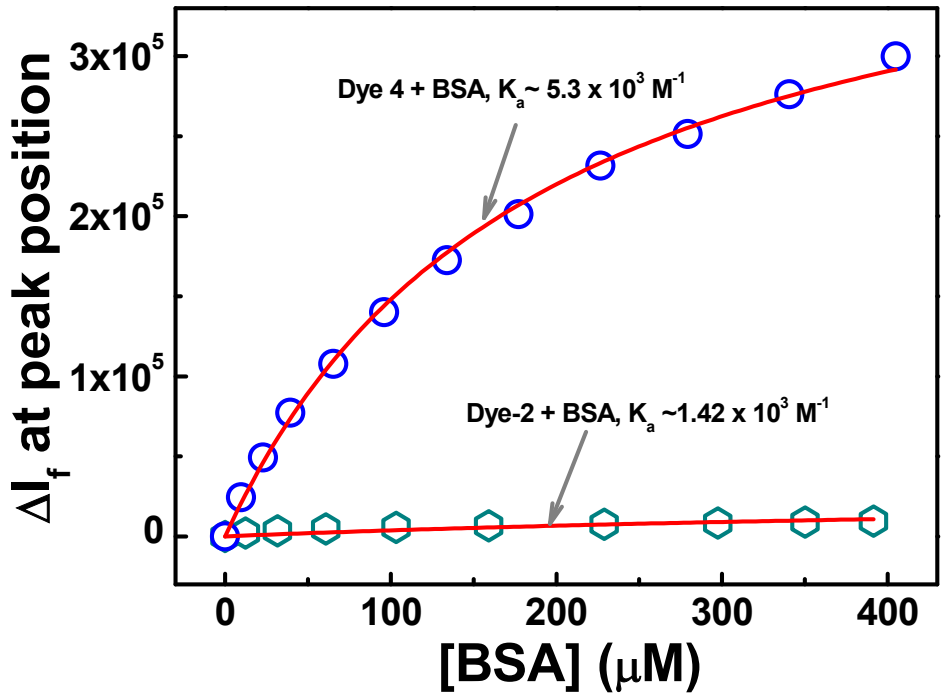
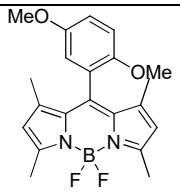
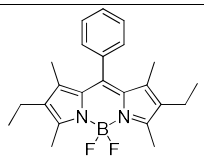
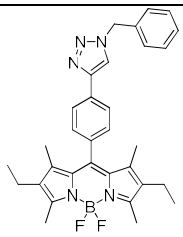
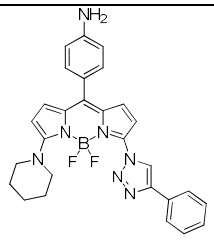
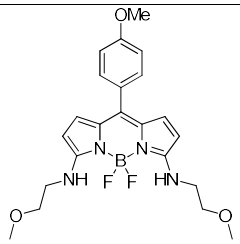
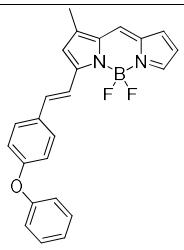
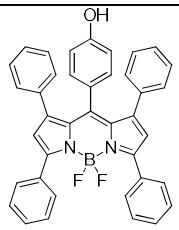
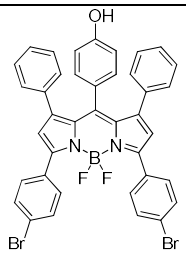
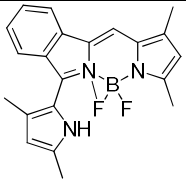
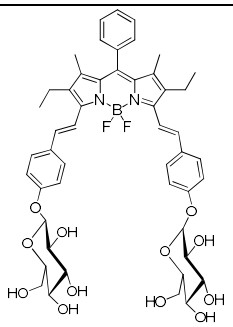


Figure S11. Binding isotherms of dyes **2** and **4** with BSA protein using 1:1 binding model.

Table T1: Comparison of performance of different reported BODIPY dyes for albumin sensing.

Sl. No.	BODIPY Dye	Emission maxima	Fluorescence enhancement	Reference
1		517 nm	Not reported	Ref[1]
2		538 nm	10 folds	Ref[2]
3		538 nm	40 folds	Ref[3]
4		575 nm	220 folds	Ref[4]
5		580 nm	33 folds	Ref[5]
6		580 nm	212 folds	Ref[6]

7		598 nm	30 folds	Ref[7]
8		605 nm	20 folds	Ref[7]
9		622 nm	Not reported	Ref[8]
10		**662 nm**	**150 folds**	**This work**

6. NMR spectra of dyes 2-4

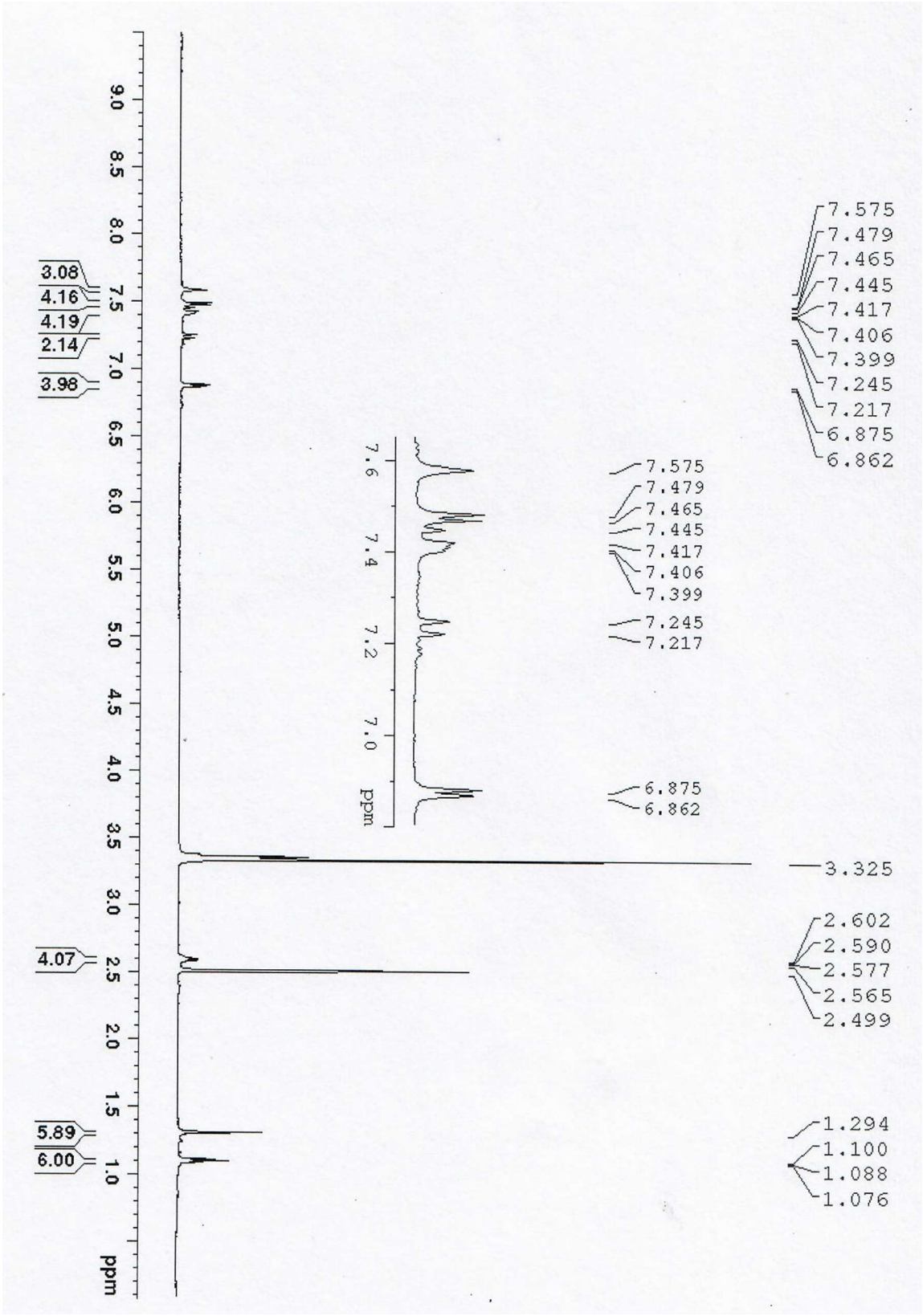


Figure S9. ^{1}H NMR spectrum of dye **2**.

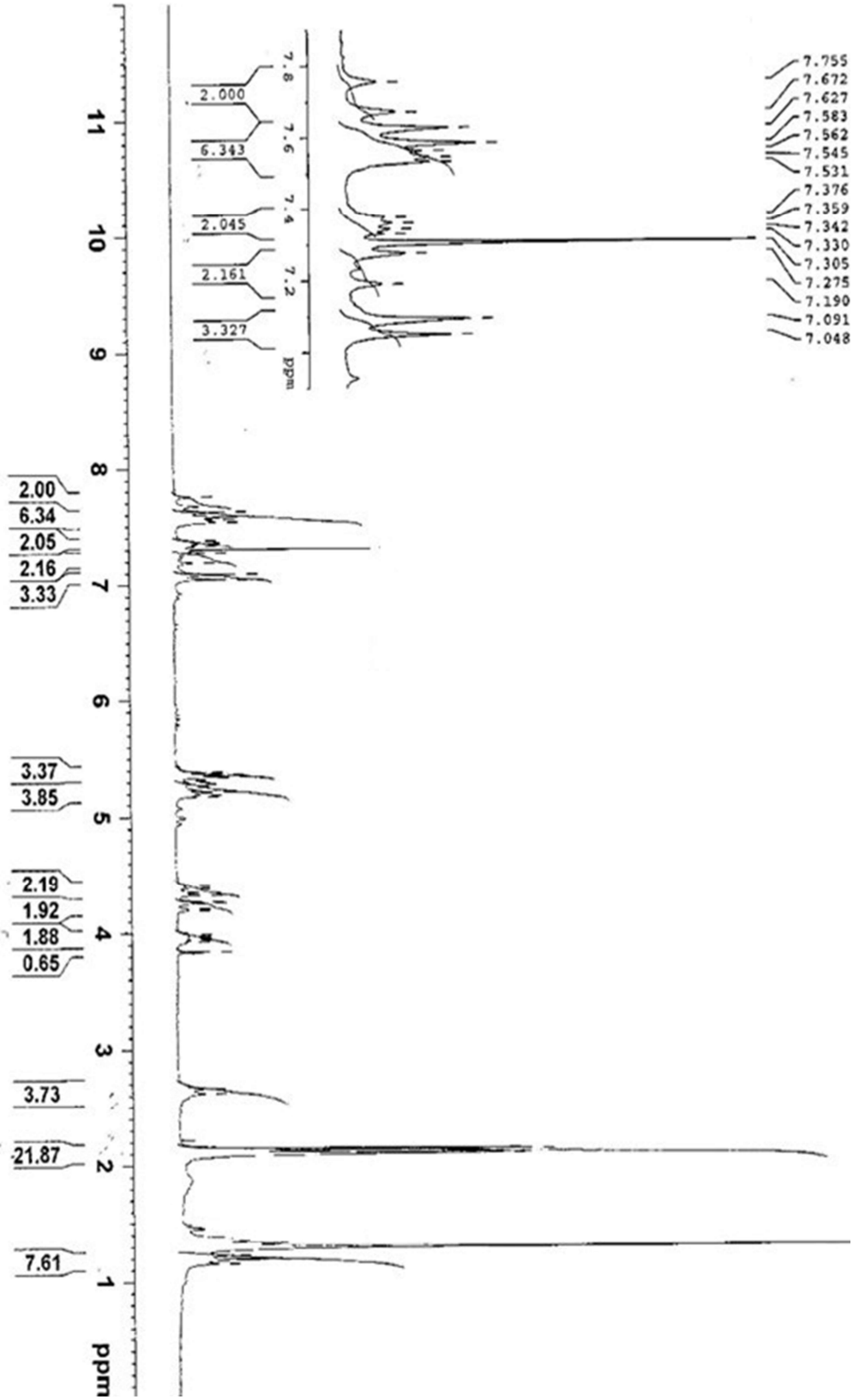


Figure S10. ^{1}H NMR spectrum of dye **3**.

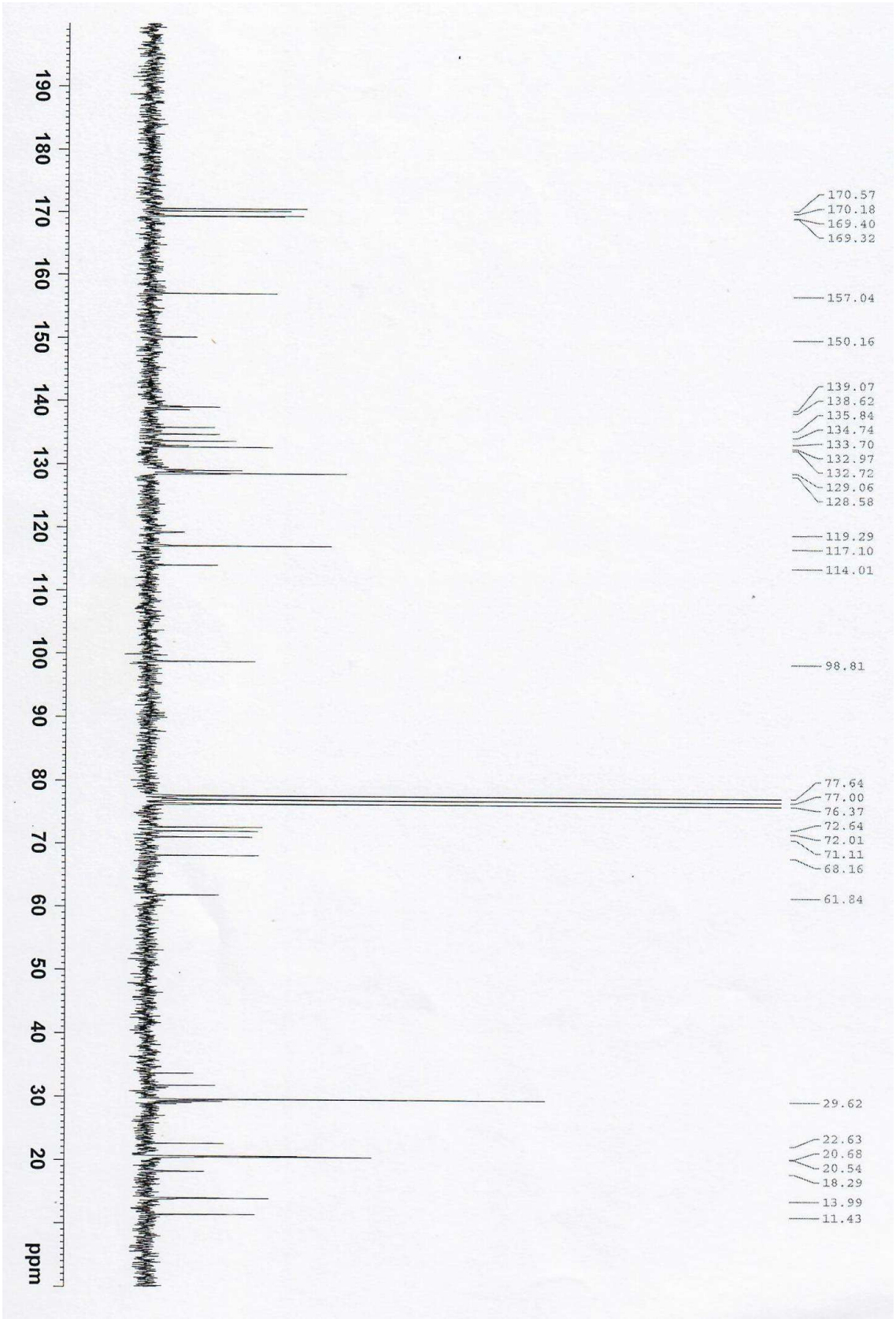


Figure S11. ^{13}C NMR spectrum of dye **3**.

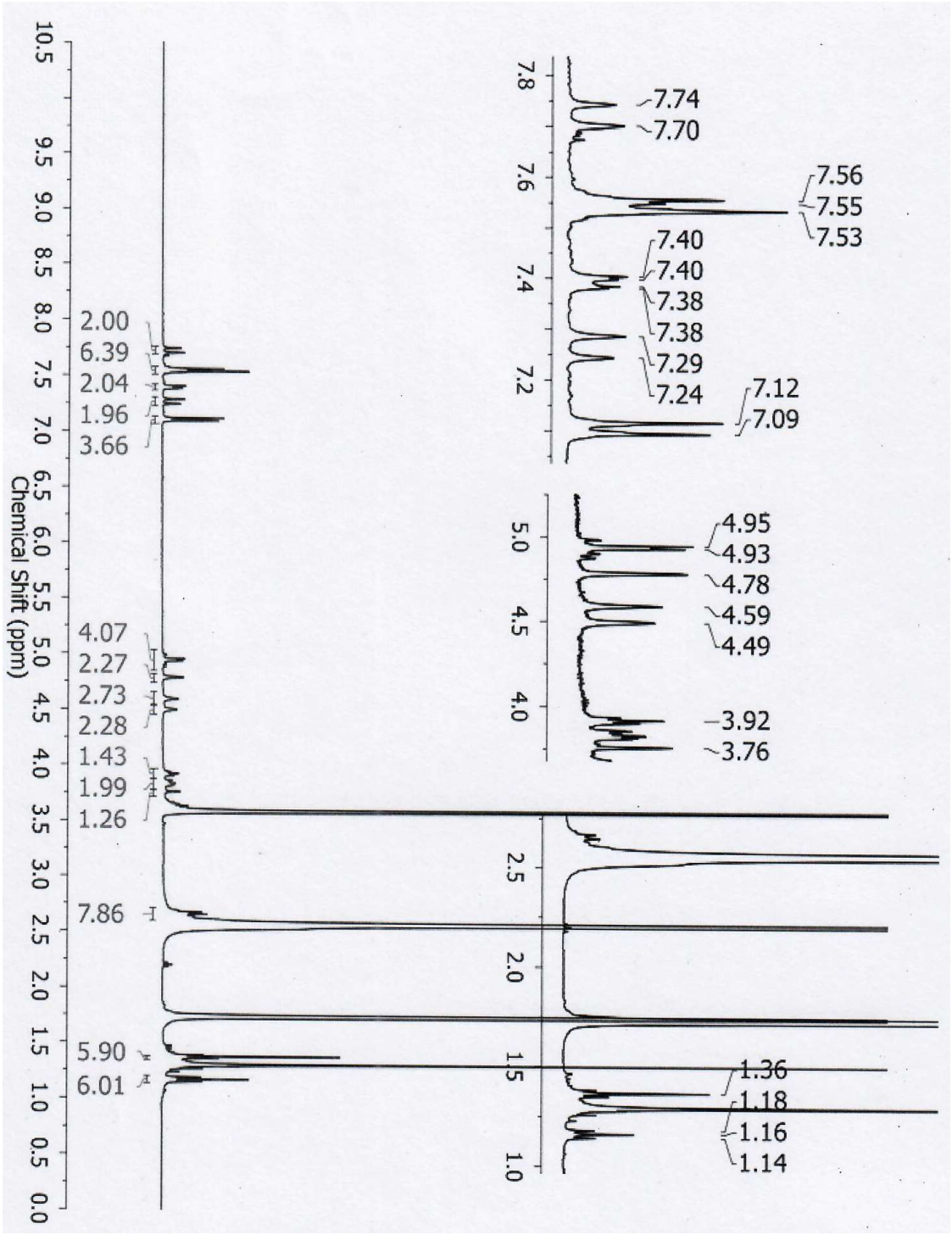


Figure S12. ^{1}H NMR spectrum of dye **4**.

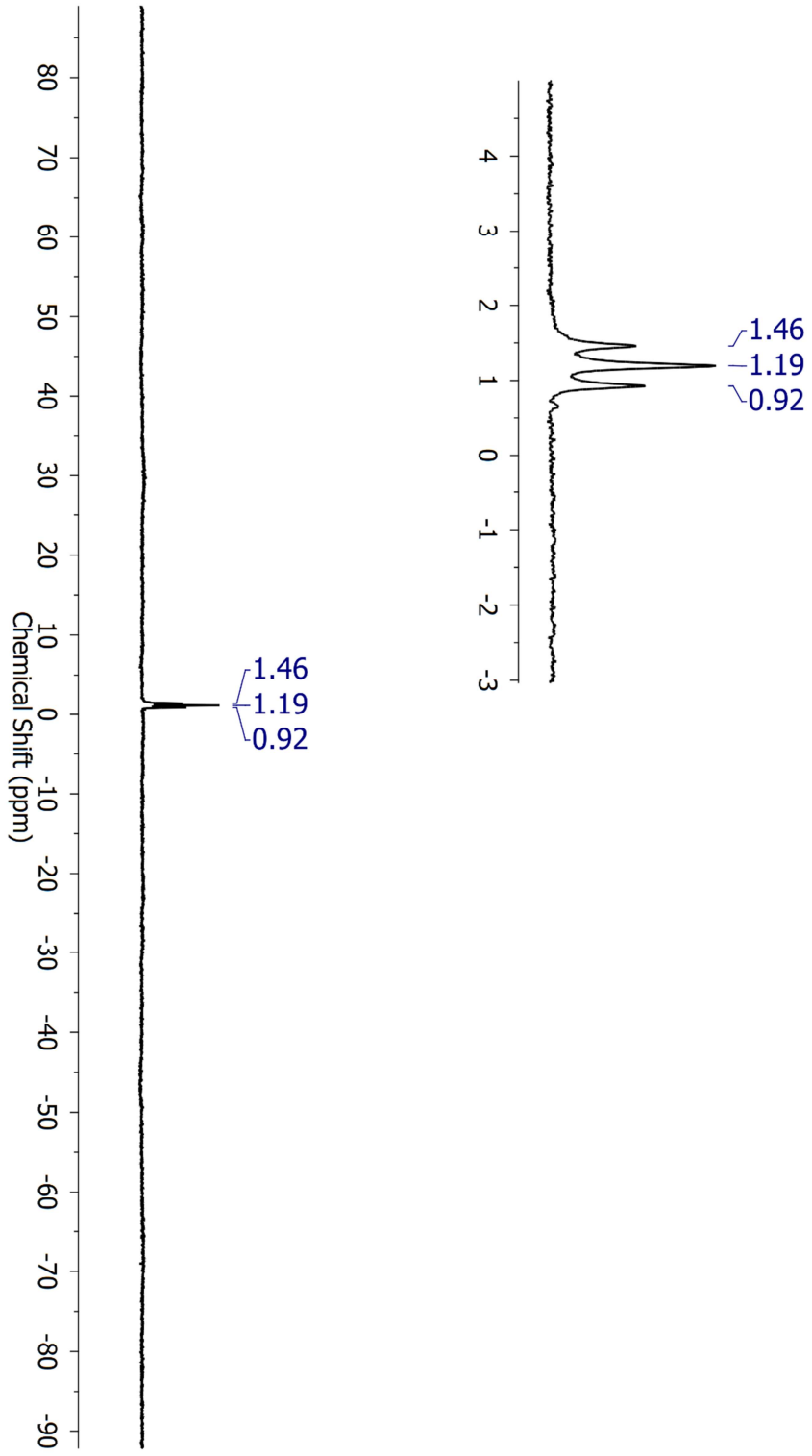


Figure S13. ^{11}B NMR spectrum of dye **4**.

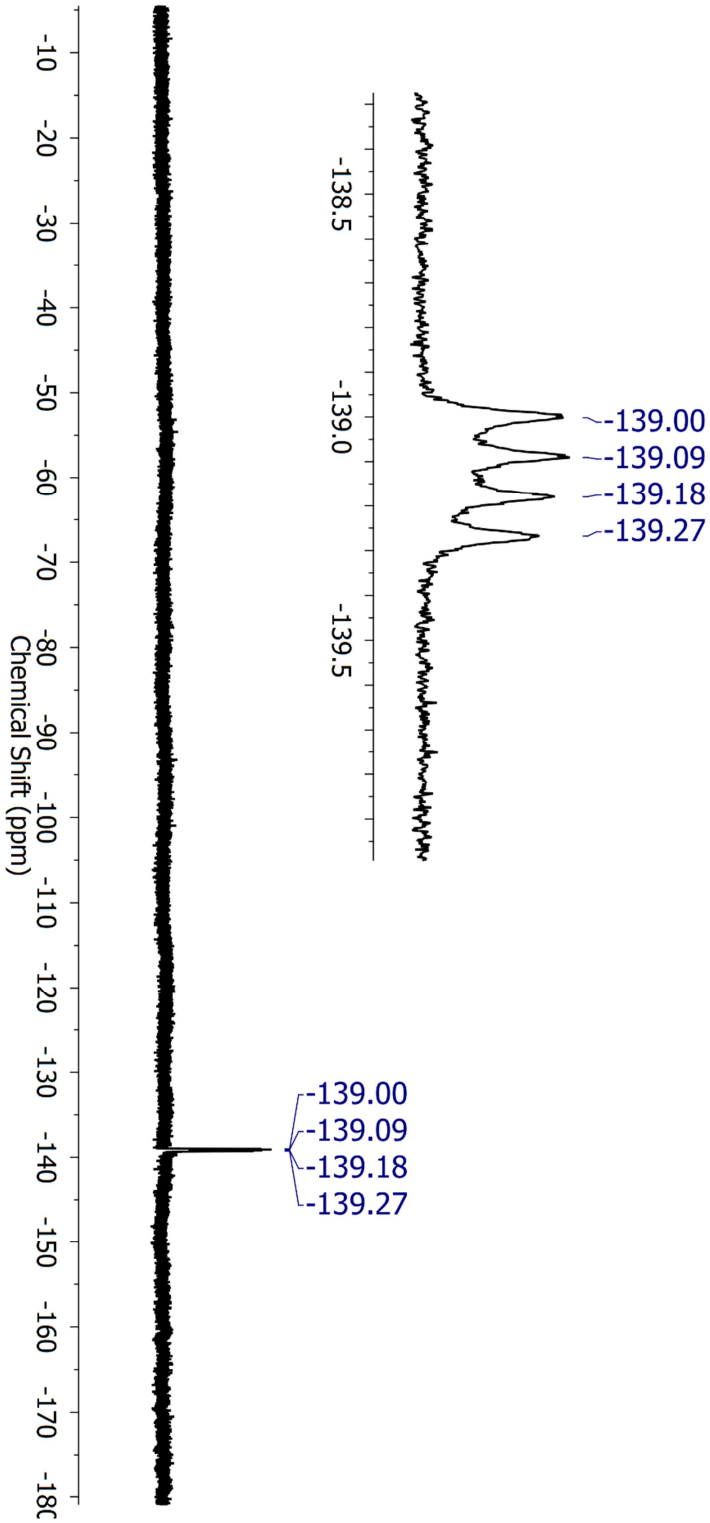


Figure S14. ^{19}F NMR spectrum of dye **4**.

7. References

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