Electronic Supplementary Material (ESI) for ChemComm.

Visualization of third-level information in latent fingerprints by a new fluorogenic L-tyrosine analogue

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EXPERIMENTAL SECTION

1. Materials and Methods

Reagent grade chemicals and solvents were used for synthetic procedures and were used as received from vendors unless stated otherwise. Analytical grade Dry DMF and CH_2Cl_2 were purchased from Finar Chemicals and were used for solid phase peptide synthesis. Methanol, DMSO, acetonitrile used for photophysical investigations and analytical/preparative HPLC were of HPLC standard. The crude products were purified using column chromatography with silica gel 100-200 mesh. Fmoc-Phe-OH and rink amide resin were procured from NovaBioChem. L-Tyrosine, Fmoc-OSu were purchased from SRL Pvt. Limited. Silica gel 60 F254 coated aluminium sheets were purchased from Merck, Darmstadt to perform TLC. MilliQ water was used for spectroscopic investigations.

2. Instrumentations

- NMR spectra were recorded on JEOL-JNM spectrometers operating at 500, 400 MHz for ^1H; 125, 100 MHz for ^{13}C, respectively using deuterated solvents ($CDCl_3$ and DMSO-d_6) and were reported as ppm (δ). TMS and deuterated solvents were used as internal references for ^1H and ^{13}C NMR experiments. The splitting patterns for respective peaks were abbreviated as s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet.
- The absorption spectra were recorded on Varian CARY 100 Bio UV-Vis spectrophotometer from Agilent Technologies, Inc. using quartz cells of 10 mm path length. The fluorescence investigations were performed on Varian Cary Eclipse fluorescence spectrophotometer using excitation laser at 350 nm for **FHBY** (slit width, excitation = 10 nm, emission = 5 nm) and **BHBY** (slit width, excitation/emission = 10 nm), unless stated otherwise.
- Waters, Q-Tof Premier Micromass HAB 213 mass spectrometer using capillary voltage of 2.6-3.2 kV was used for high resolution mass spectroscopy.
- Dynamic Light Scattering (DLS): DLS measurements were performed at 25.0 ± 0.1°C using a light-scattering apparatus (Delsa TM Nano from Beckman Coulter India). The solutions were filtered with a Millipore membrane filter (Acrodisc syringe filter, 0.45 μm Supor membrane) before measurements.
- SEM measurements were performed on a FEI QUANTA 200 microscope operating at an acceleration voltage of 10 KV with tungsten filament as electron source.
- AFM measurements were obtained using Asylum Research, Oxford Instruments, MFP-3D Origin microscope in tapping mode. Silicon nitride cantilever, purchased form Nanosensors,

was used having following specifications: Force constant = 21 N/m, Thickness = 7.0 ± 1 µm, Length = 225 ± 10 µM, Width = 38 ± 7.5 µm, Resonance frequency = 170 kHz.

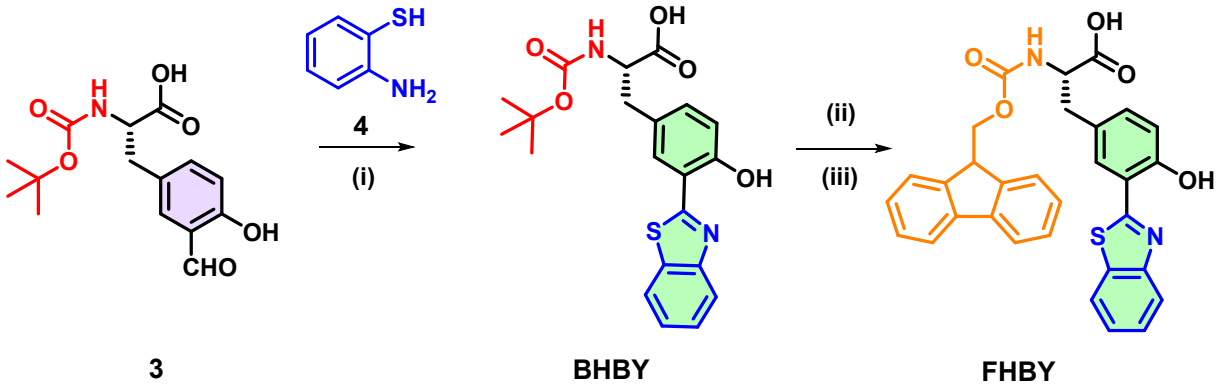
3. Preparation of Samples:

(i) UV-visible and fluorescence titrations:

The stock solutions of **FHBY**, **BHBY** and conjugate **5** were prepared and diluted to desired concentrations to perform the photophysical measurements using UV-Visible and Fluorescence spectroscopy. All data was saved as comma delimited (.csv) files followed by further graphed using Origin™ 2015 software.

(ii) Sample preparation for SEM and AFM imaging:

Stock solutions of **FHBY** and **BHBY** were used for preparation of diluted samples (100 µM) dissolved in CH_3OH and CH_3OH:H_2O (1:99). 5 µL of these samples were deposited on glass/silicon wafer surface using drop casting method and were allowed to evaporate at 25°C. These samples were further gold coated for imaging using SEM technique.



Scheme 1 Schematic diagram showing synthetic protocol for synthesis of conjugates **BHBY** and **FHBY**.

4. Synthetic Procedures

*(i) Synthesis of **BHBY:***

Compound **3** (600 mg, 1.94 mmol), synthesized using a reported procedure,[1] was transferred to a 50 mL round bottomed flask containing ethanol (12 mL), followed by addition of 2-aminothiophenol (**4**, 243 mg, 1.94 mmol), 37% aq. HCl (0.53 mL, 5.82 mmol) and 30% aq. H_2O_2 (1.2 mL, 11.64 mmol). The resultant reaction mixture was allowed to stir at room temperature. The progress of reaction was followed with thin layer chromatography (TLC) and showed complete transformation of reactants to product in 30 mins. The precipitated solid was filtered to get crude **BHBY** and was purified using column chromatography using 5% CH_3OH:CH_2Cl_2 (containing 1%

acetic acid) as eluent to get pure **BHBY** as off-white powder. Yield = 660 mg (82%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (d, 1H, *J* = 8.0 Hz), 7.89 (d, 1H, *J* = 8.0 Hz), 7.49 (t, 1H, *J* = 7.2 Hz), 7.47 (s, 1H), 7.40 (t, 1H, *J* = 7.6 Hz), 7.18 (dd, 1H, *J* = 8.4 Hz), 7.00 (d, 1H, *J* = 8.0 Hz), 5.05 (d, 1H, *J* = 7.2 Hz), 4.62 (d, 1H, *J* = 6.0 Hz), 3.19-2.89 (m, 2H), 1.43 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.11, 169.09, 157.06, 155.48, 151.87, 133.85, 132.66, 129.09, 126.80, 125.68, 122.30, 121.61, 118.16, 54.40, 36.96, 28.39 ppm; HRMS-ESI calculated for $C_{21}H_{22}N_2O_5S$, *m/z* = 414.1249; experimental = 413.1166 $[M-H]^-$; UV-Vis = 340 nm ; fluorescence λ_{em} = 475 nm (H_2O:CH_3CN = 99:1).

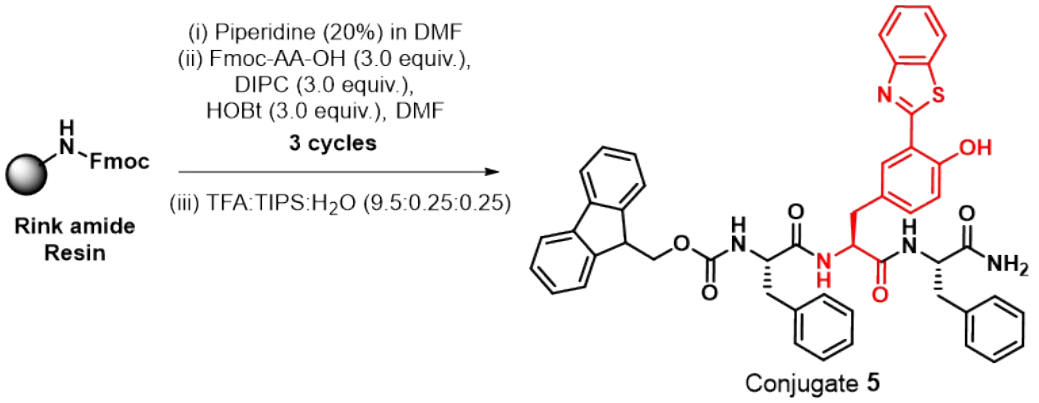
*(ii) Synthesis of **FHBY:***

Conjugate **BHBY** (200 mg, 0.483 mmol) was dissolved in solution of 4M HCl in Dioxan (10 mL) under N_2 atmosphere along with stirring at room temperature. The progress of reaction was monitored using TLC that showed that the reaction completed in 30 mins. The reaction mixture was concentrated under vacuum using rotary evaporator followed by precipitation with diethyl ether. The resulting solid was dried under vacuum and was dissolved in a solution containing 10% aq. Na_2CO_3 (10 mL, pH = 10.0) and Dioxan (5mL) in an ice bath. To this solution, Fmoc-OSu (195 mg, 0.5796 mmol) dissolved in dioxan (5 mL) was added dropwise. After the completion of Fmoc-OSu addition, the reaction was transferred to room temperature and was allowed to run overnight. After TLC showed completion of reaction, dioxan was evaporated from solution under vacuum. The resulting aqueous solution was acidified using citric acid and was extracted using ethyl acetate. The ethyl acetate layer was concentrated under vacuum and resultant solid was purified using column chromatography to give pure **FHBY** as white solid. Yield = 205 mg (79%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.42 (s, 1H), 8.08 (d, 2H, J = 8.0 Hz), 8.00 (d, 1H, J = 8.0 Hz), 7.79 (d, 3H, J = 7.2 Hz), 7.57 (d, 1H, J = 7.6 Hz), 7.54 (d, 1H, J = 7.6 Hz), 7.49 (t, 1H, J = 8.0 Hz), 7.40 (t, 1H, J = 7.6 Hz), 7.30 (t, 3H, J = 7.2 Hz), 7.20-7.14 (m, 2H), 6.96 (d, 1H, J = 8.4 Hz), 4.15-4.11 (m, 4H), 3.09-3.05 (m, 1H), 2.89-2.82 (m, 1H) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.84, 165.55, 156.54, 155.43, 151.95, 144.24, 144.17, 141.13, 134.91, 133.77, 129.71, 129.48, 128.08, 127.50, 126.96, 125.68, 125.54, 122.50, 120.58, 118.58, 117.34, 66.15, 56.28, 47.04, 36.12 ppm; HRMS-ESI calculated for $C_{31}H_{24}N_2O_5S$, *m/z* = 536.1406; experimental = 535.1317 $[M-H]^-$ and 1071.2724 $[2M-H]^-$; UV-Vis = 340 nm; fluorescence λ_{em} = 525 nm (H_2O:CH_3OH = 99:1).

*(iii) Synthesis of Conjugate **5**:*

Rink amide resin having substitution 0.54 mmol/g (370 mg, 0.2 mmol) was transferred to the reaction vessel. Resin was washed with DMF (3 x 3 mL) and swollen for 30 minutes in DCM (3 mL). Fmoc deprotection of resin was achieved using phased treatment 20% piperidine in DMF. For this, the resin was then treated with 20% piperidine in DMF (3 mL) and stirred for 5 minute and filtered. The resin was again

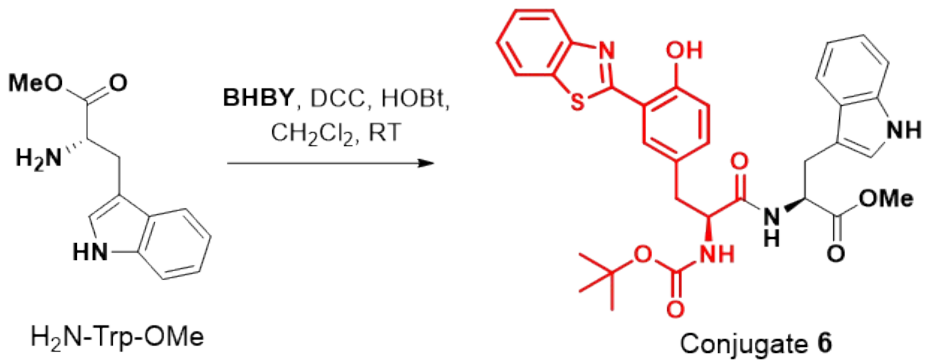
treated with 20% piperidine in DMF (5 mL) and stirred for 25 minutes under nitrogen and filtered followed by washing with DMF (3x3 mL). To this resin, a solution of Fmoc–Phe-OH (232 mg, 0.6 mmol) and HOBt (81 mg, 0.6 mmol) dissolved in 4 mL of DMF was added and reaction mixture was stirred by purging nitrogen gas followed by addition of DIC (94 µL, 0.6 mmol) and the reaction mixture was stirred by purging nitrogen gas for 3 h under nitrogen. Sample of resin was tested for monitoring the coupling by Kaiser's test. If the test is found positive, stirring was continued till Kaiser's test is negative. After completion of reaction, the resin was then washed with DMF (3x3 mL). The resin after proper washing was treated with 20% piperidine in DMF (3 mL) and stirred for 5 minute and filtered. The resin was again treated with 20% piperidine in DMF (5 mL) and stirred for 20 minutes under nitrogen and filtered. The resin was washed with DMF (3x3 mL). To this resin a solution of **FHBY** (215 mg, 0.4 mmol) and HOBt (54 mg, 0.4 mmol) dissolved in 4 mL of DMF was added and reaction mixture was stirred by purging nitrogen gas followed by addition of DIC (64 µL, 0.4 mmol) and the reaction mixture was stirred by purging nitrogen gas for 4 h under nitrogen. Sample of resin was tested for monitoring the coupling by Kaiser's test. After completion of reaction, the resin was then washed with DMF (3x3 mL) followed by treatment with 20% piperidine in DMF (3 mL) for 5 minute and filtration. The resin was again treated with 20% piperidine in DMF (5 mL) and stirred for 20 minutes under nitrogen and filtered. The resin was washed with DMF (3x3 mL). To this resin, a solution of Fmoc–Phe-OH (232 mg, 0.6 mmol) and HOBt (81 mg, 0.6 mmol) dissolved in 4 mL of DMF was added and reaction mixture was stirred by purging nitrogen gas followed by addition of DIC (94 µL, 0.6 mmol) and the reaction mixture was stirred by purging nitrogen gas for 3 h under nitrogen. After completion of this cycle, the resin was then washed with DMF (3x3 mL) and DCM (3x3 mL). The resin obtained was then dried under vacuum. The above resin was then treated with 10 mL mixture of TFA:TIPS:water (95:2.5:2.5 %) and the reaction mixture was stirred for 2 h. The resin was removed by filtration and the filtrate was reduced to half by evaporation under reduced pressure below 45 °C. Peptide was precipitated with diethyl ether. Precipitate obtained was dried under vacuum to give crude peptide **5** which was purified by subsequent washes with DCM and methanol (Scheme 2). Yield = 120 mg, 72%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.40 (s, 1H), 8.16 (d, 1H, J = 8.0 Hz), 8.08–8.04 (m, 2H), 8.02 (d, 1H, J = 2.0 Hz), 7.86 (d, 1H, J = 8.0 Hz), 7.81 (d, 2H, J = 7.5 Hz), 7.51–7.45 (m, 4H), 7.39–7.32 (m, 4H), 7.25-7.17 (m, 8H), 7.13-7.08 (m, 6H), 6.90 (d, 1H, J = 8.5 Hz), 4.60–4.42 (m, 2H), 4.21–4.13 (m, 1H), 4.09–3.97 (m, 3H), 3.04–2.97 (m, 2H), 2.85–2.64 (m, 4H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.13, 171.87, 171.22, 156.10, 155.48, 151.95, 144.26, 144.16, 141.14, 138.57, 138.25, 134.74, 134.20, 129.72, 129.62, 129.33, 128.60, 128.46, 128.10, 127.56, 126.90, 126.77, 126.65, 125.79, 125.72, 125.52, 122.53, 122.44, 120.56, 118.36, 117.18, 79.70, 66.13, 56.68, 54.32, 47.04, 46.31, 38.20, 37.37; HRMS-ESI calculated for $C_{56}H_{71}N_{19}O_7S$, *m/z* = 829.2934; experimental = 830.2993 $(M + H)^+$; fluorescence λ_{em} = 390 nm (DMSO), 525 nm (H_2O:DMSO = 99.9:0.1).



Scheme 2 Schematic diagram showing synthesis of conjugate **5**.

*(iv) Synthesis of Conjugate **6**:*

To a solution of **BHBY** (200 mg, 0.4825 mmol) in DMF, N-hydroxybenzotriazole (HOBt, 65 mg, 0.4825 mmol) and dicyclohexylcarbodiimide (120 mg, 0.5790 mmol) were added and stirred at 0°C for one hour. After completion of one hour, the reaction mixture was allowed to warm to room temperature, followed by subsequent addition of L-Tryptophan methyl ester hydrochloride salt (148 mg, 0.5750 mmol) and triethylamine (0.34 mL, 2.4125 mmol). The progress of the reaction was monitored using TLC. After completion of the reaction, the reaction mixture was passed through celite bed to remove dicyclohexylurea. Filtrate was concentrated under vacuum on rotary evaporator. The residue was acidified using 1N HCl solution and was extracted using ethyl acetate. The organic layer was subsequently washed with 10% $NaHCO_3$ and brine solution. The resultant organic layer was evaporated, column chromatographed (using ethyl acetate:hexane = 2:3 as eluent) and gave conjugate **6**. Yield = 87%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.45 (s, 1H), 8.13 (s, 1H), 7.98 (d, 1H, J = 8.0 Hz), 7.88 (d, 1H, J = 7.6 Hz), 7.49 (t, 1H, J = 7.6 Hz), 7.43-7.36 (m, 3H), 7.25 (d, 1H, J = 7.6 Hz), 7.18 (d, 1H, J = 8.4 Hz), 7.06-7.01 (m, 2H), 6.96 (d, 1H, J = 8.4 Hz), 6.81 (s, 1H), 6.31 (bs, 1H), 5.07 (bs, 1H), 4.89-4.84 (m, 4H), 4.33 (bs, 1H), 3.61 (s, 3H), 3.29-2.95 (m, 4H), 1.39 (s, 9H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.78, 170.76, 169.23, 156.90, 155.32, 151.82, 136.06, 133.90, 132.75, 129.19, 127.69, 127.47, 126.82, 125.69, 122.81, 122.24, 121.66, 119.70, 118.35, 118.16, 116.71, 111.44, 109.60, 80.27, 55.79, 53.07, 52.50, 37.78, 28.34, 27.56 ppm; HRMS-ESI calculated for $C_{33}H_{34}N_4O_6S$, *m/z* = 614.2199; experimental = 615.2272 $(M + H)^+$, 637.2092 $(M + Na)^+$; fluorescence λ_{em} = 390 nm (DMSO), 525 nm (H_2O:DMSO = 99.9:0.1).



Scheme S1. Schematic diagram showing synthesis of conjugate **6**.

5. Procedure for Latent Fingerprinting:

Colleagues at the laboratory served as volunteers during this experiment and donated LFPs. The volunteers were asked to wash their hands with soap before donating LFPs. The fingertips were wiped across the forehead and LFPs were transferred to designated surface by gently pressing fingertips on it. These LFPs were covered with 1 mL of 25 µM aqueous solutions (containing 0.1 %, *v/v* DMSO) of **FHBY** followed by incubation at room temperature for one minute. After completion of the incubation time, the LFPs were washed with copious amount water to wash off **FBHY** solution. The LFP containing substrates were dried under room temperature conditions and were photographed under 365 nm light illumination using Samsung Galaxy Note 20 Ultra. The obtained photographs were further processed using Image J software. The sections of these images were analyzed for contrast of fluorescence intensity between adjacent ridges and furrows of LFPs and were graphed as variation of grey value with distance (pixels) using Origin 2015 software. The processed photographs were carefully observed to delineate the characteristic details up to third level of information of the developed LFPs.

6. Procedure for stability studies of LFPs:

The freshly developed LFPs were photographed under 365 nm UV irradiation. These developed LFPs were stored at room temperature and under ambient light conditions available in chemistry research laboratory for a period of three weeks. The LFPs substrate was again photographed after completion of three weeks. Same sections of the photographs of fresh LFPs and three weeks aged LFPs were analyzed for contrast of fluorescence intensity between adjacent ridges and furrows of LFPs and were graphed as variation of grey value with distance (pixels) using Origin 2015 software. The fluorescence intensity contrast traces produced from the photographs of fresh LFP and 3 weeks aged LFP showed the overlapping of traces suggesting high stability of developed LFPs under ambient conditions.

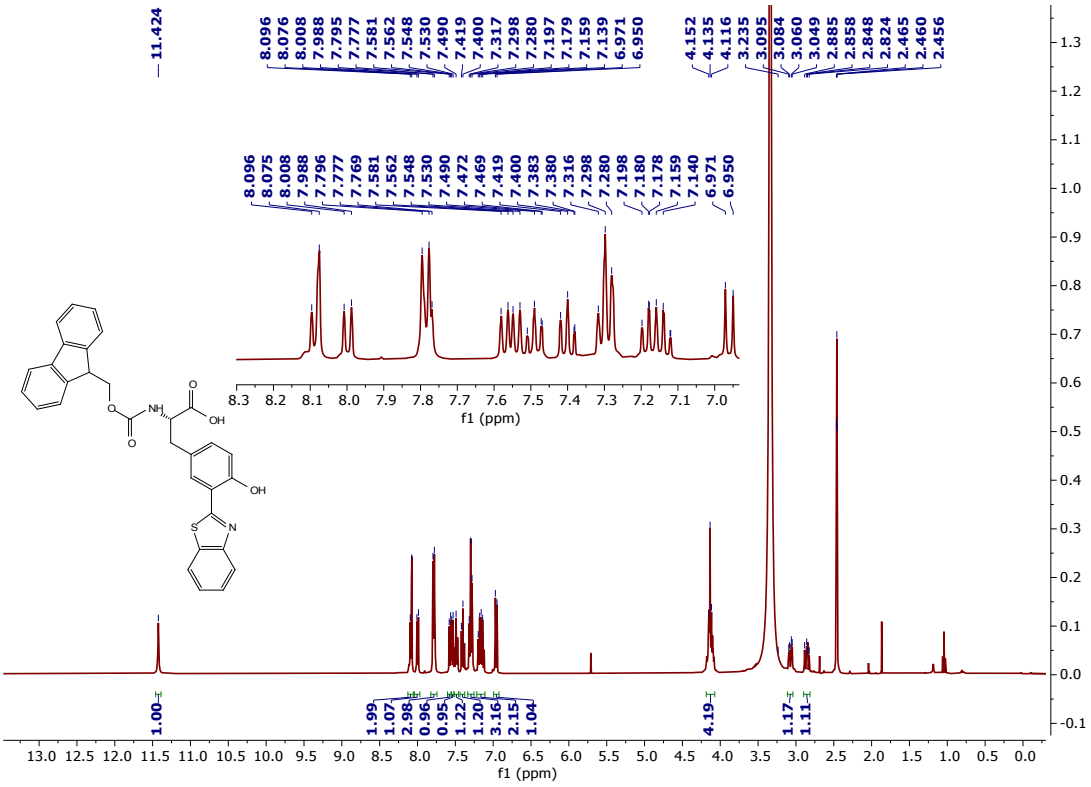


Figure S1a. [1]H NMR spectrum of **FHBY**.

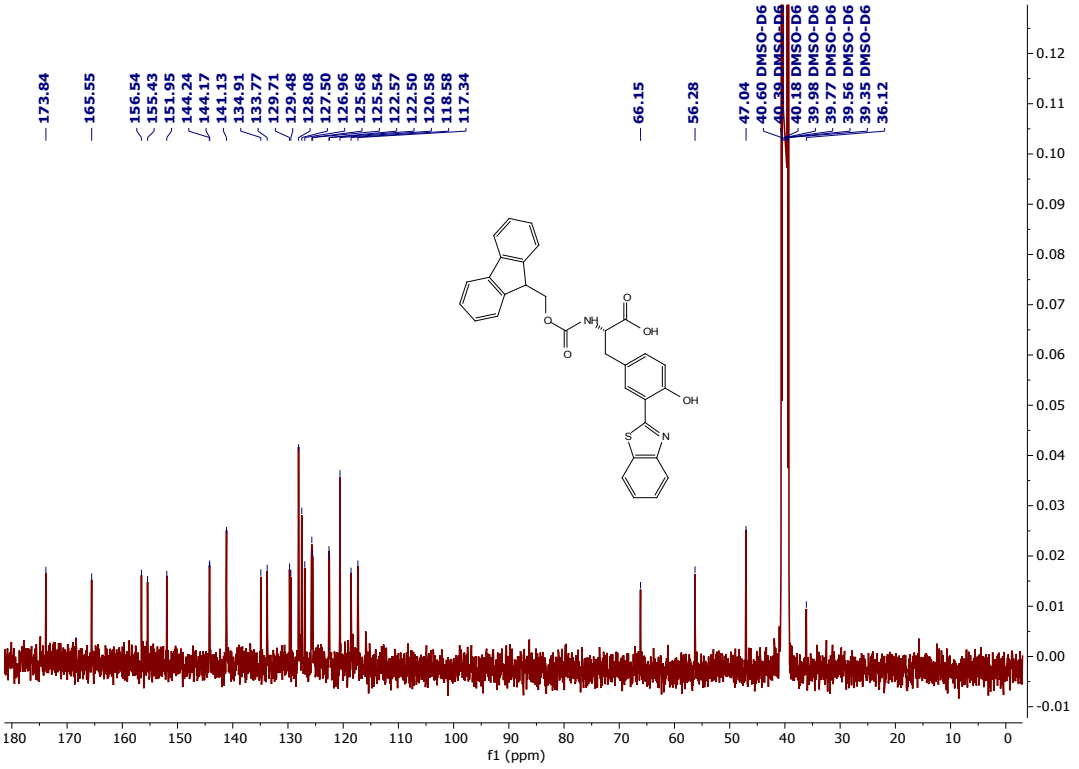


Figure S1b. [13]C NMR spectrum of **FHBY**.

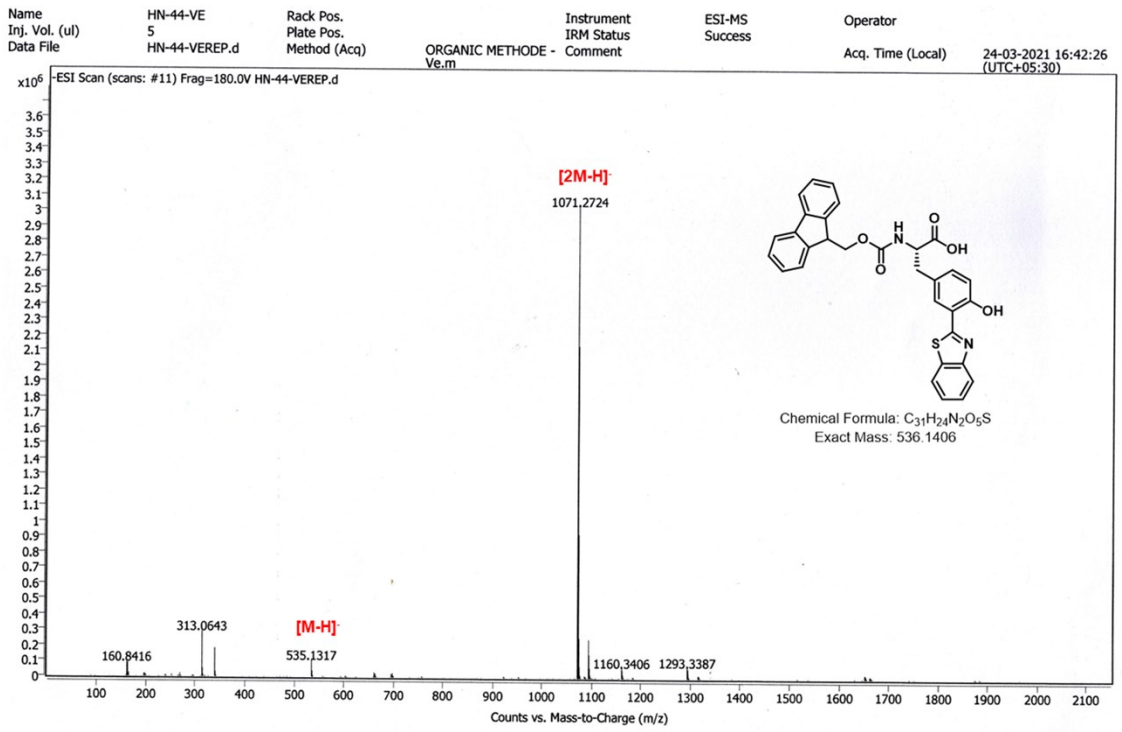


Figure S1c. Mass spectrum of **FHBY**.

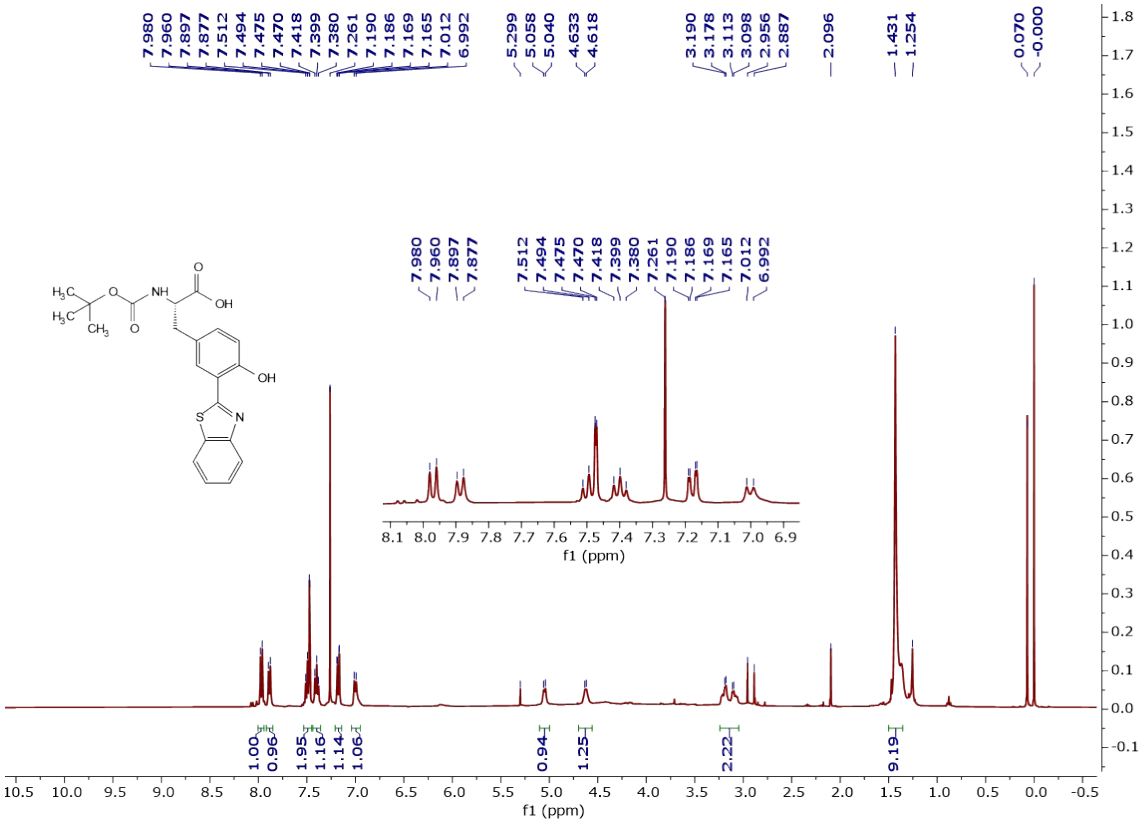


Figure S2a. [1]H NMR spectrum of **BHBY**.

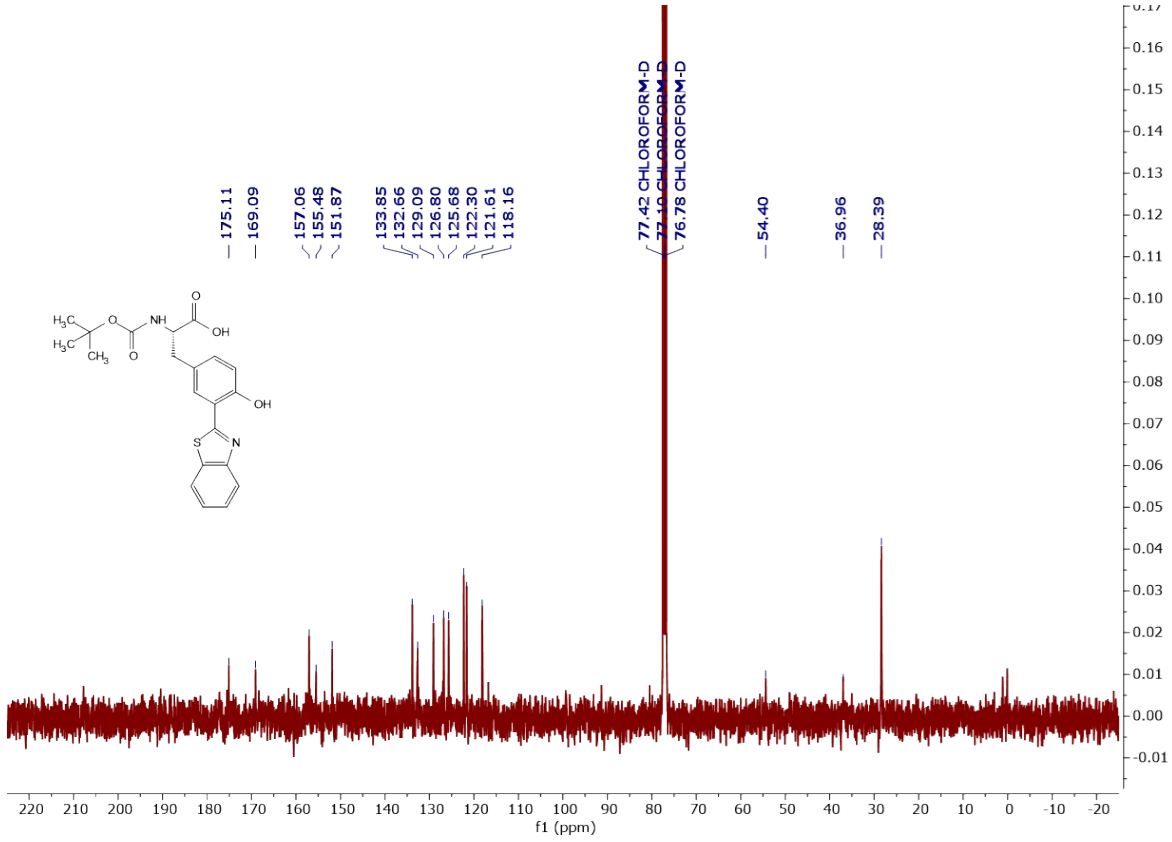


Figure S2b. ^{13}C NMR spectrum of **BHBY**.

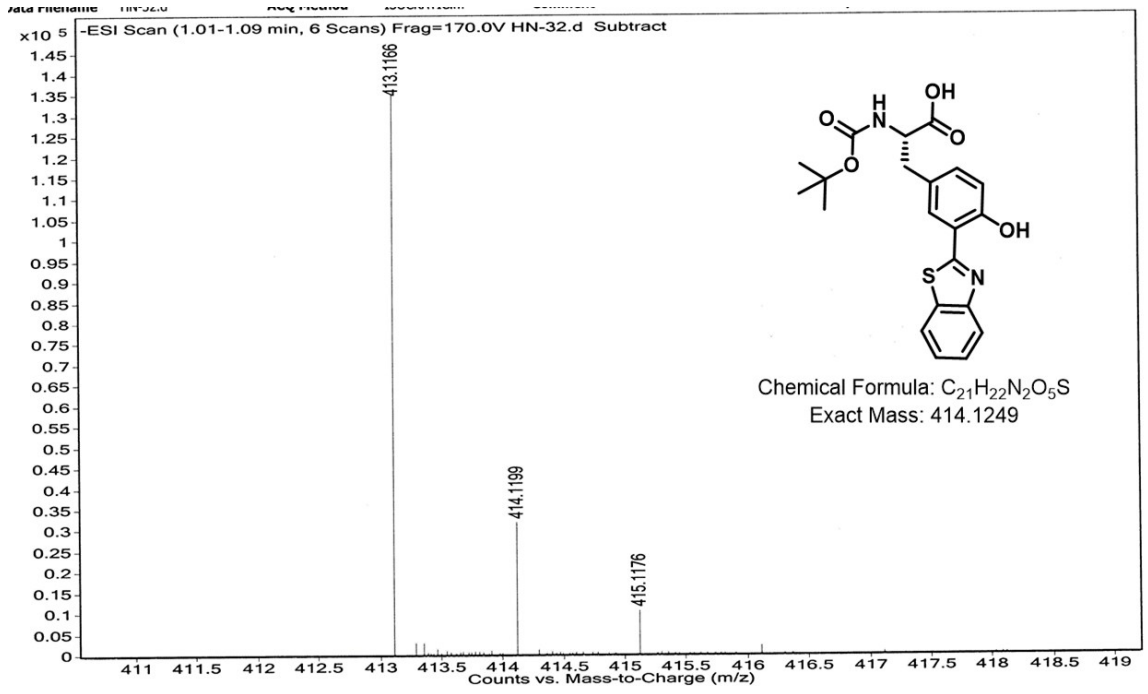


Figure S2c. Mass spectrum of **BHBY**.

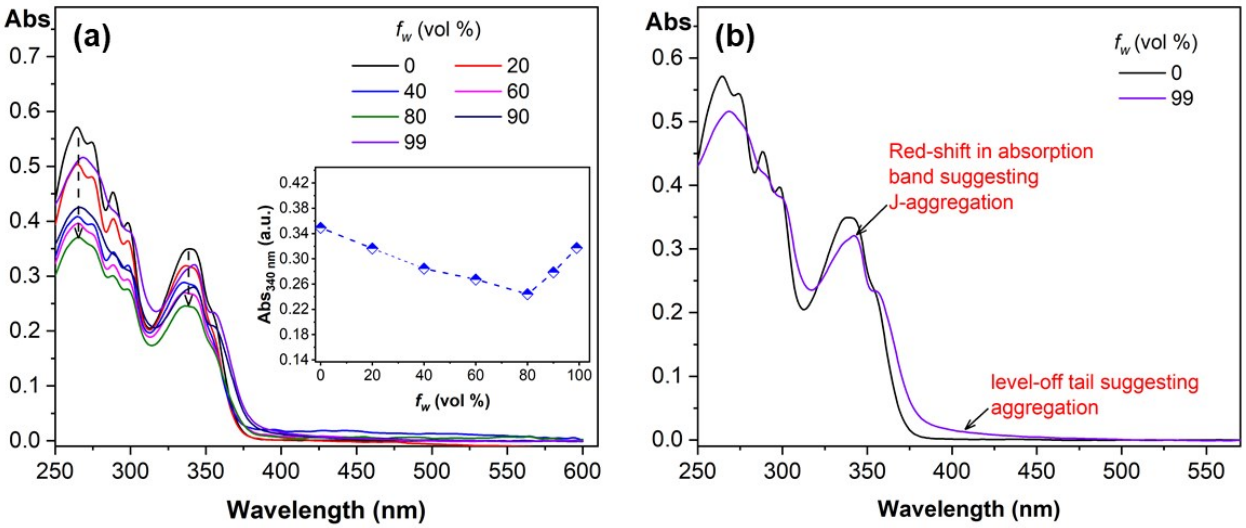


Figure S3 (a) UV-Vis spectrum and titration profiles (inset) of **FHBY** (2.5 x 10^{-5} M) upon gradual increase in volume fraction of H_2O in CH_3OH; (b) UV-Vis traces of **FHBY** in CH_3OH (black) and in H_2O:CH_3OH = 99:1, *v/v* (violet) suggesting formation of J-aggregates.

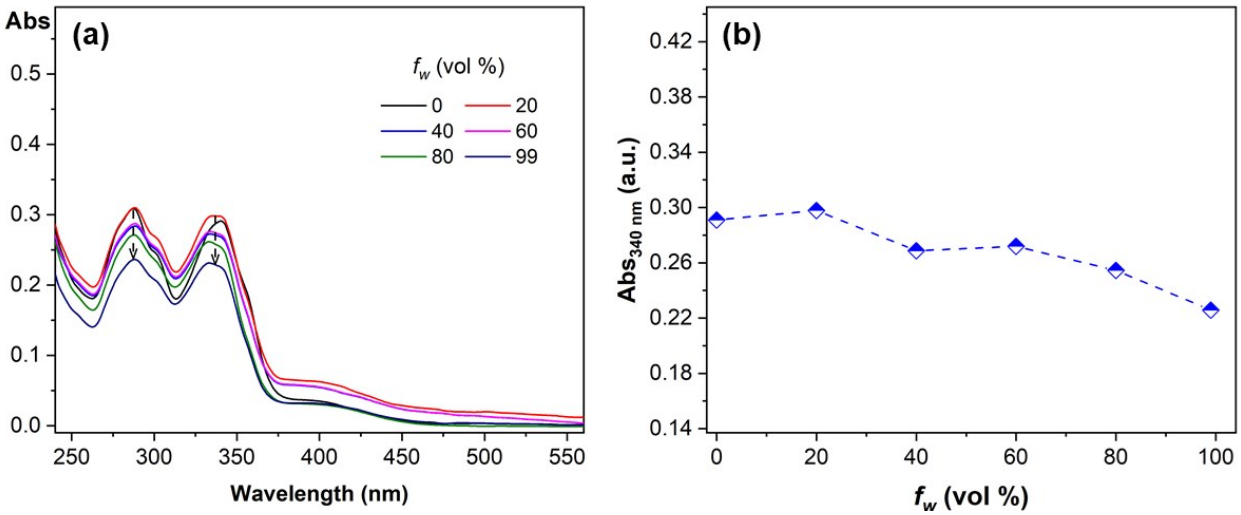


Figure S4 (a) UV-Vis spectrum and (b) UV-Vis titration profiles of **BHBY** (2.5 x 10^{-5} M) upon gradual increase in volume fraction of H_2O in CH_3OH.

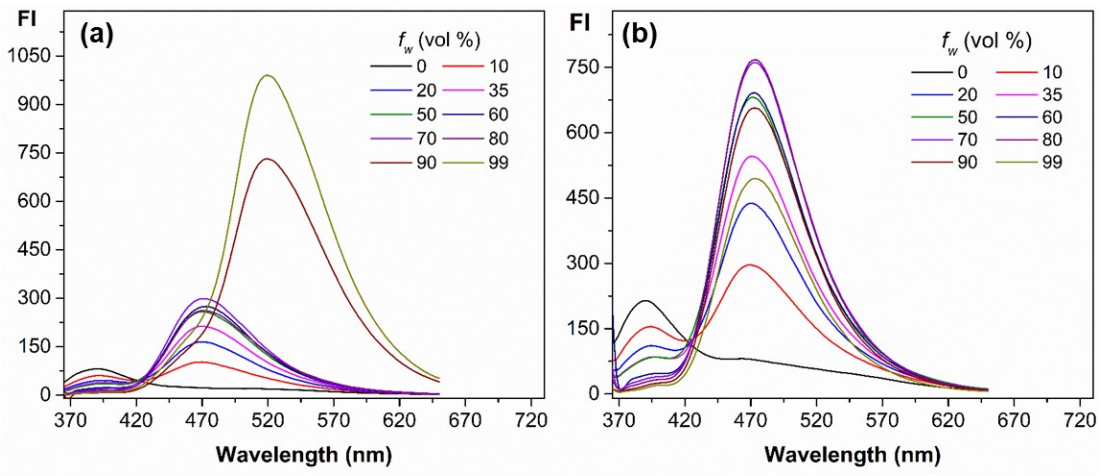


Figure S5. Fluorescence spectrum of 1 x 10^{-5} M solutions of (a) **FHBY** and (b) **BHBY** showing variations in fluorescence intensity at different wavelengths upon gradual increase in volume percentage of water (f_w) in CH_3OH.

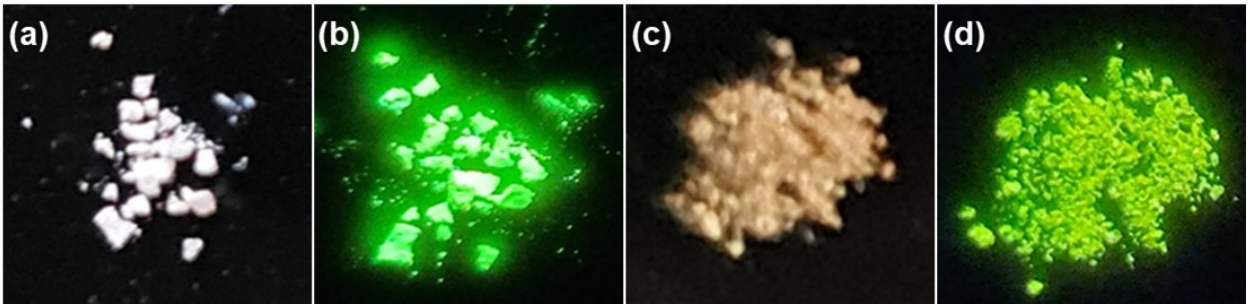


Figure S6. Photographs of **FHBY** (a,b) and **BHBY** (c,d) under daylight (a,c) and 365 nm UV illumination (b,d) showing solid-state fluorescence.

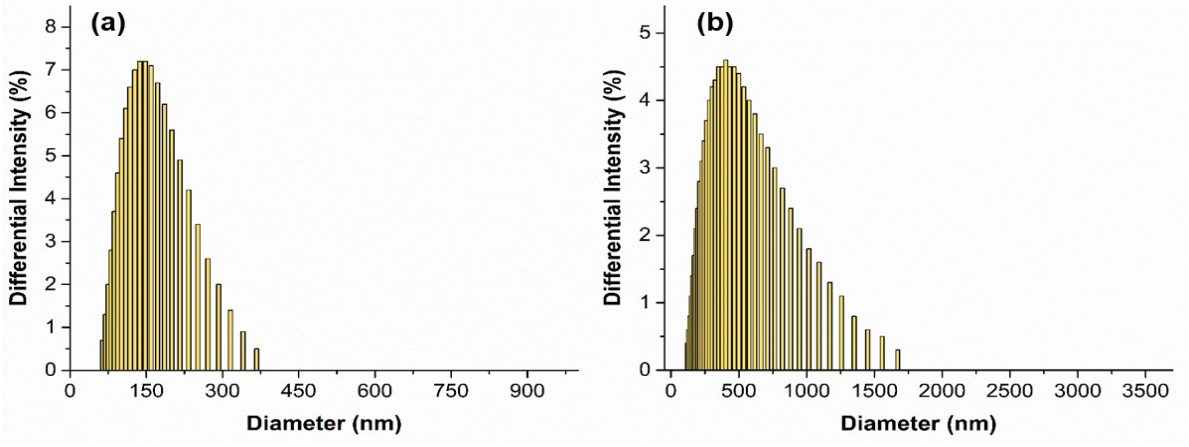


Figure S7. DLS bar graphs of (a) **BHBY** and (b) **FHBY** in mixed binary solvent system (H_2O:CH_3OH = 99:1).

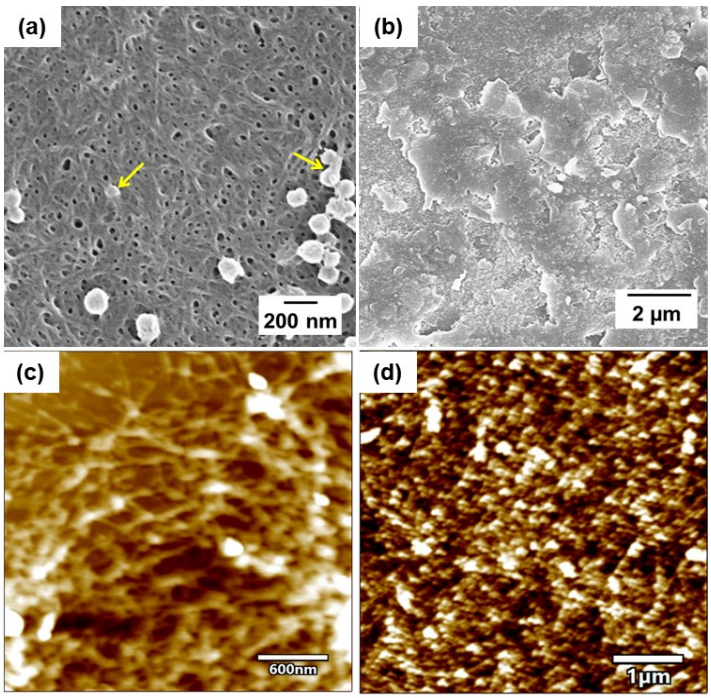


Figure S8. (a,b) SEM and (c,d) AFM micrographs of **FHBY** showing self-assembled nanofibers in CH_3OH (a,c) and flake shaped morphology in H_2O:CH_3OH = 99:1 (b,d).

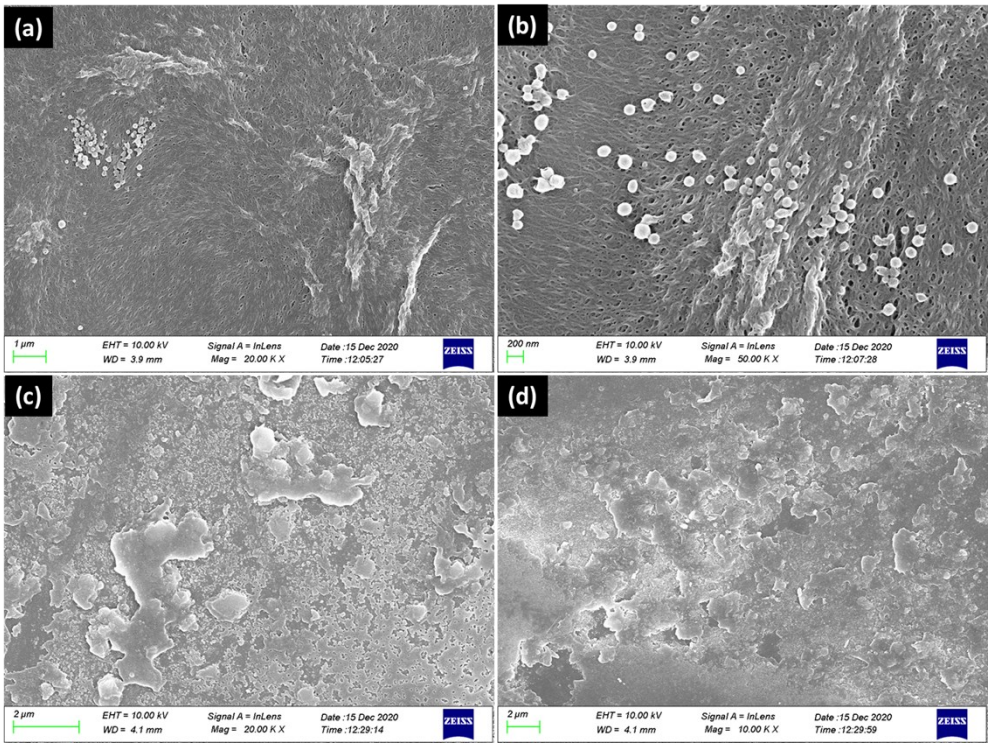


Figure S9. SEM micrographs of drop casted samples of **FHBY** (1 x 10^{-4} M) dispersed in (a,b) CH_3OH and (c,d) CH_3OH:H_2O (1:99) showing nanofiber and flake shaped morphologies, respectively.

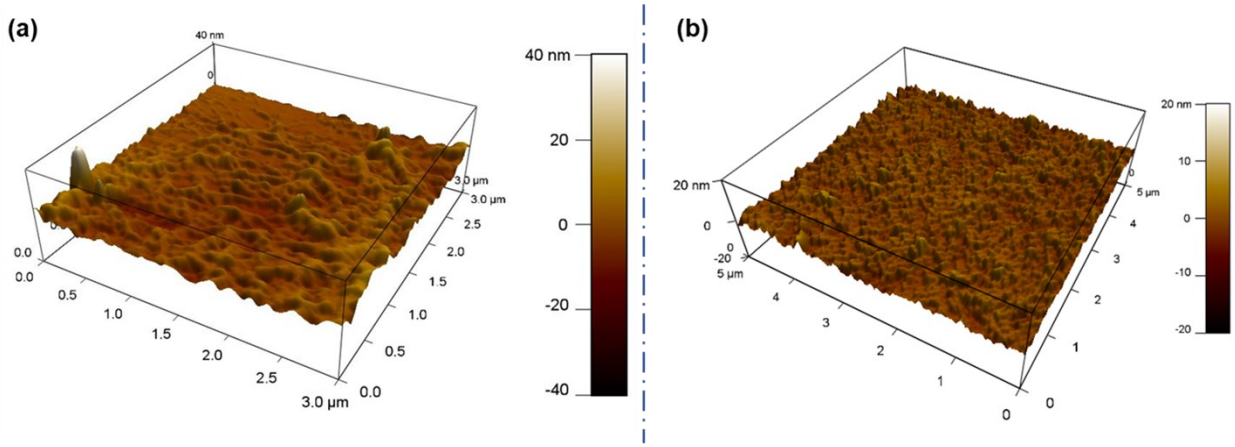


Figure S10. AFM micrographs showing 3D cross sectional view of drop casted samples of **FHBY** (1 x 10^{-4} M) dispersed in (a) CH_3OH and (b) CH_3OH:H_2O (1:99).

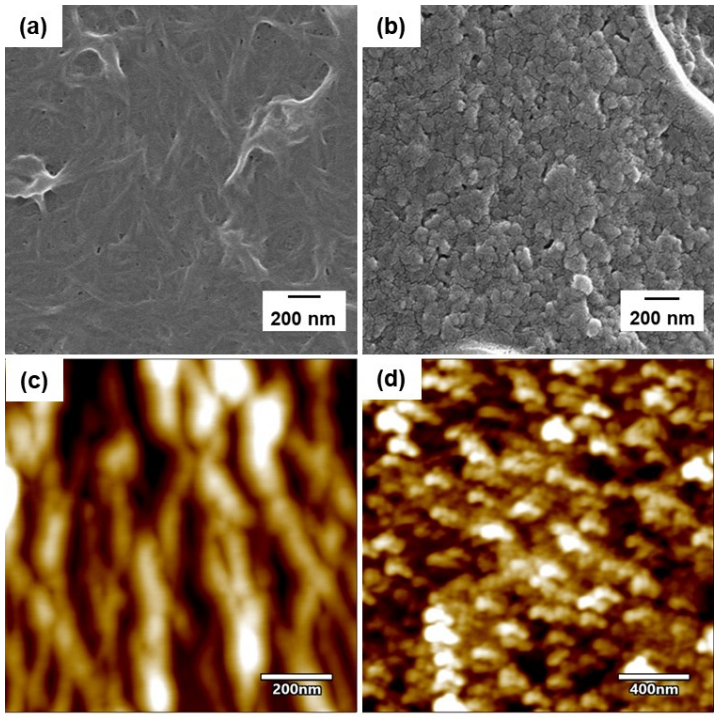


Figure S11. (a,b) SEM and (c,d) AFM micrographs of **BHBY** (1 x 10^{-4} M) showing self-assembled nanofibers in CH_3OH (a,c) and nanospherical morphology in H_2O:CH_3OH = 99:1 (b,d).

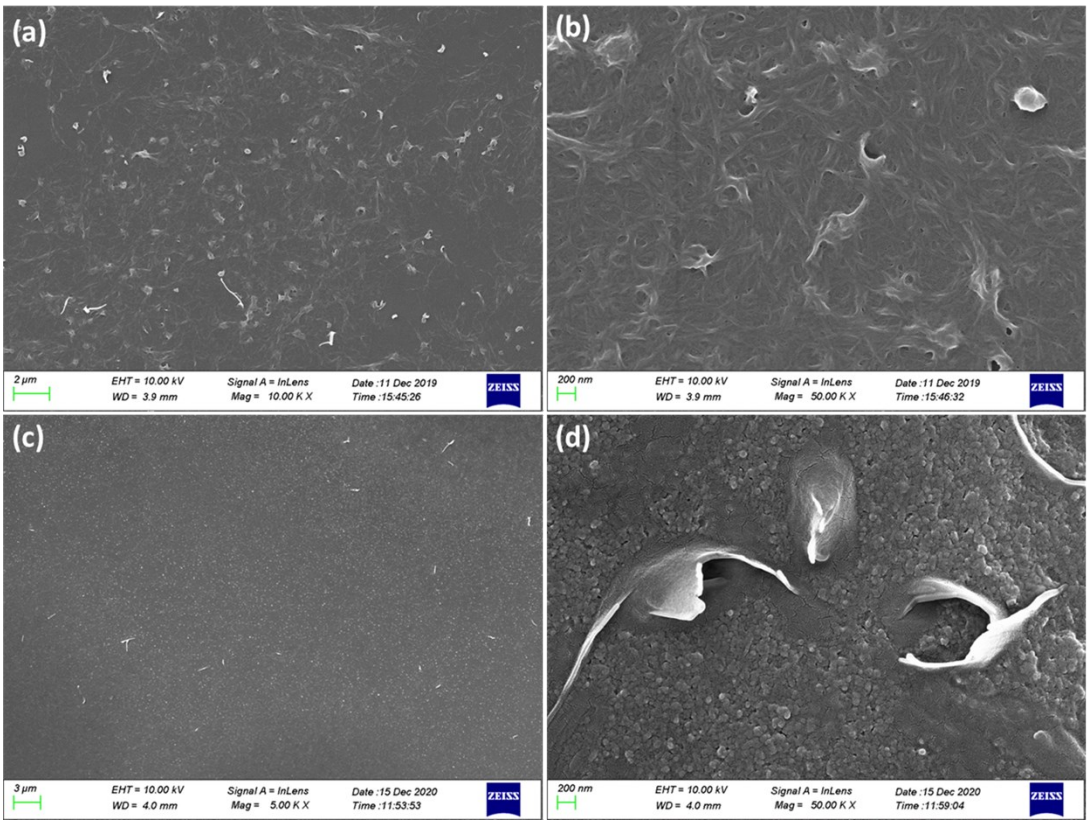


Figure S12. SEM micrographs of drop casted samples of **BHBY** (1 x 10^{-4} M) dispersed in (a,b) CH_3OH and (c,d) CH_3OH:H_2O (1:99) showing nanofiber and nanospherical morphologies, respectively.

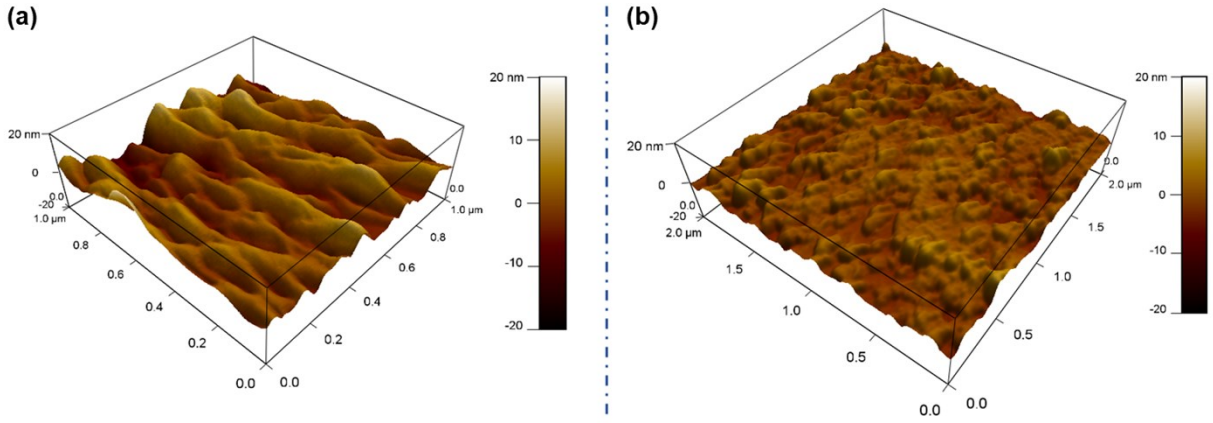


Figure S13. AFM micrographs showing 3D cross sectional view of drop casted samples of **BHBY** (1 x 10^{-4} M) dispersed in (a) CH_3OH and (b) CH_3OH:H_2O (1:99).

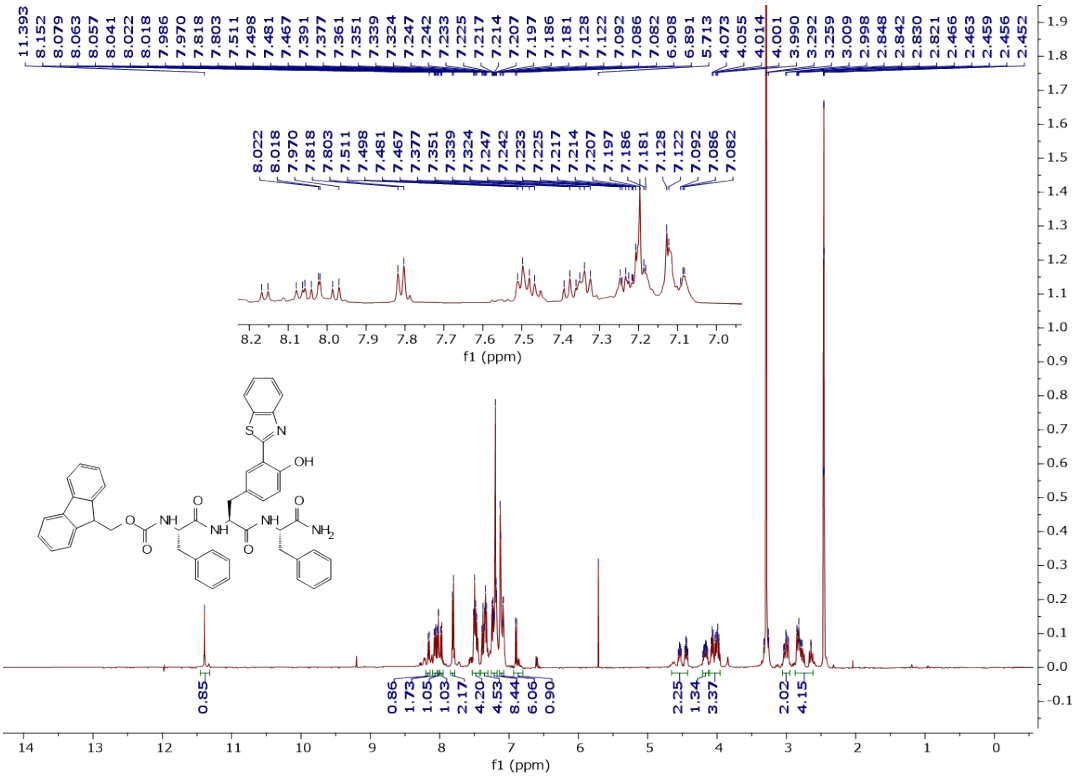


Figure S14a. [1]H NMR spectrum of Conjugate **5**.

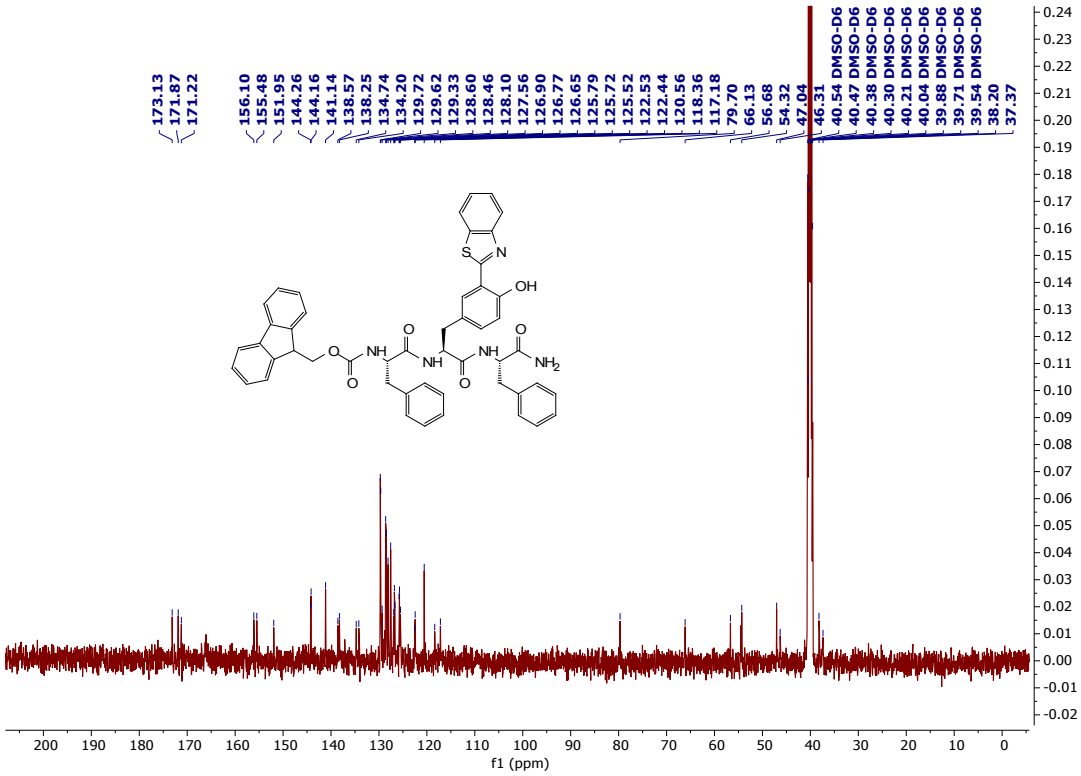


Figure S14b. [13]C NMR spectrum of Conjugate **5**.

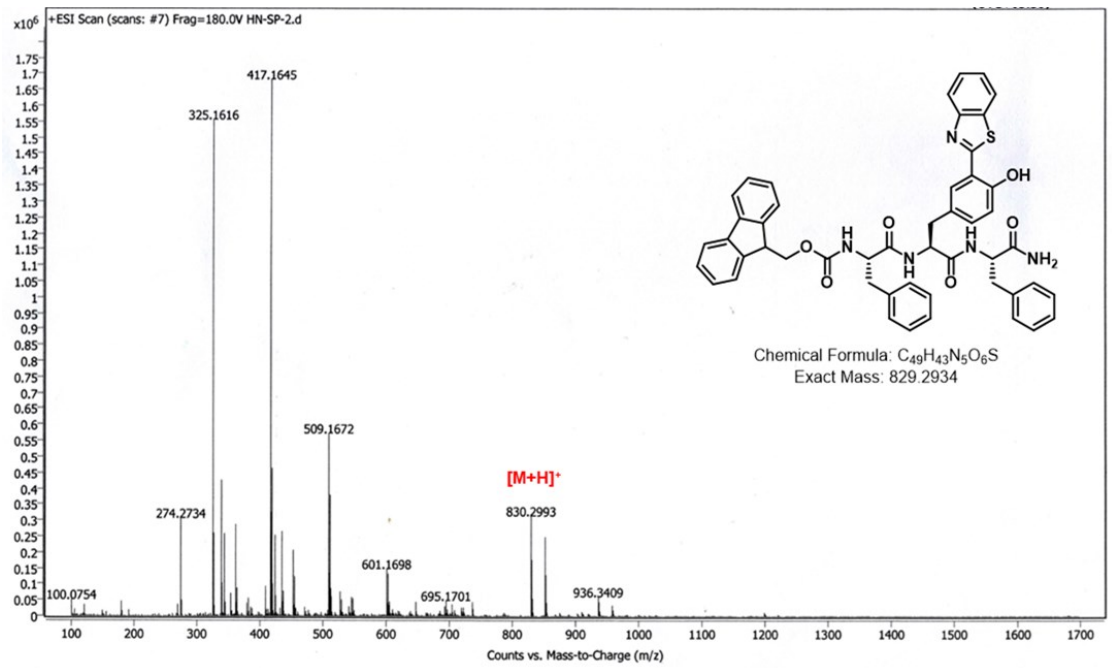


Figure S14c. Mass spectrum of Conjugate **5**.

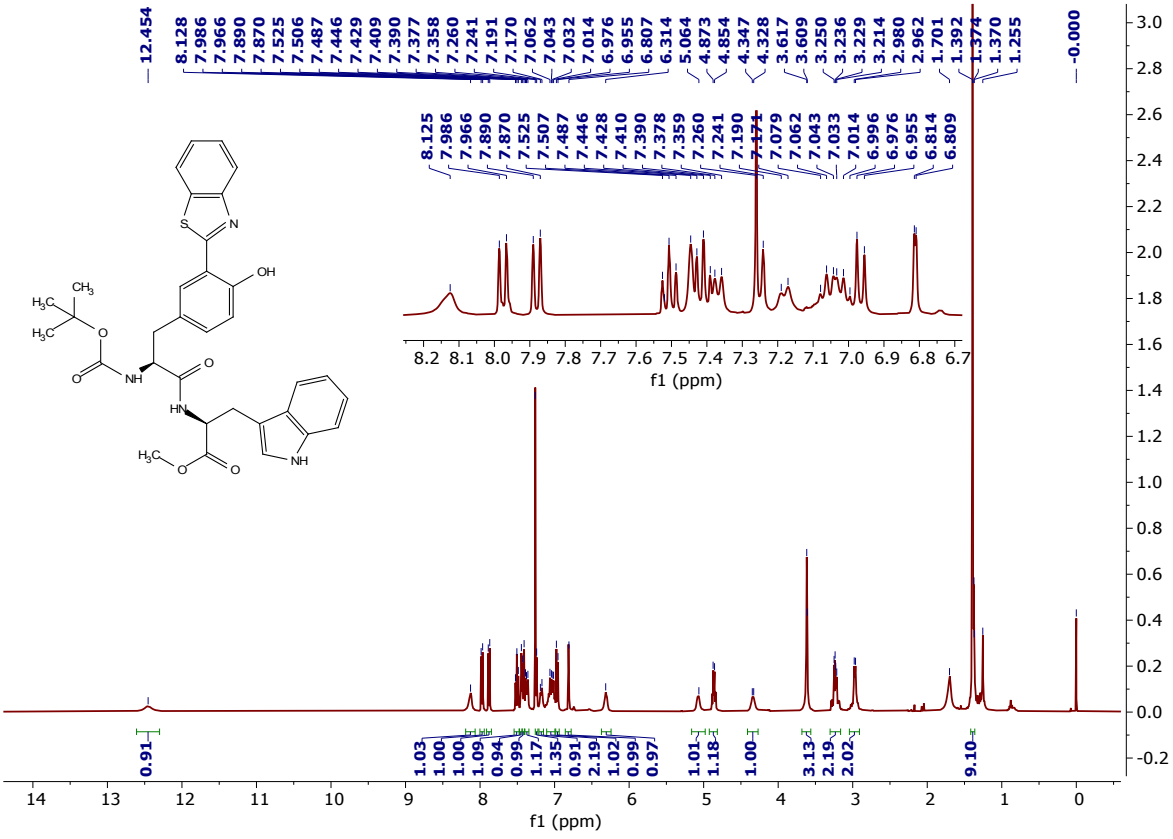


Figure S15a. ^{1}H NMR spectrum of Conjugate **6**.

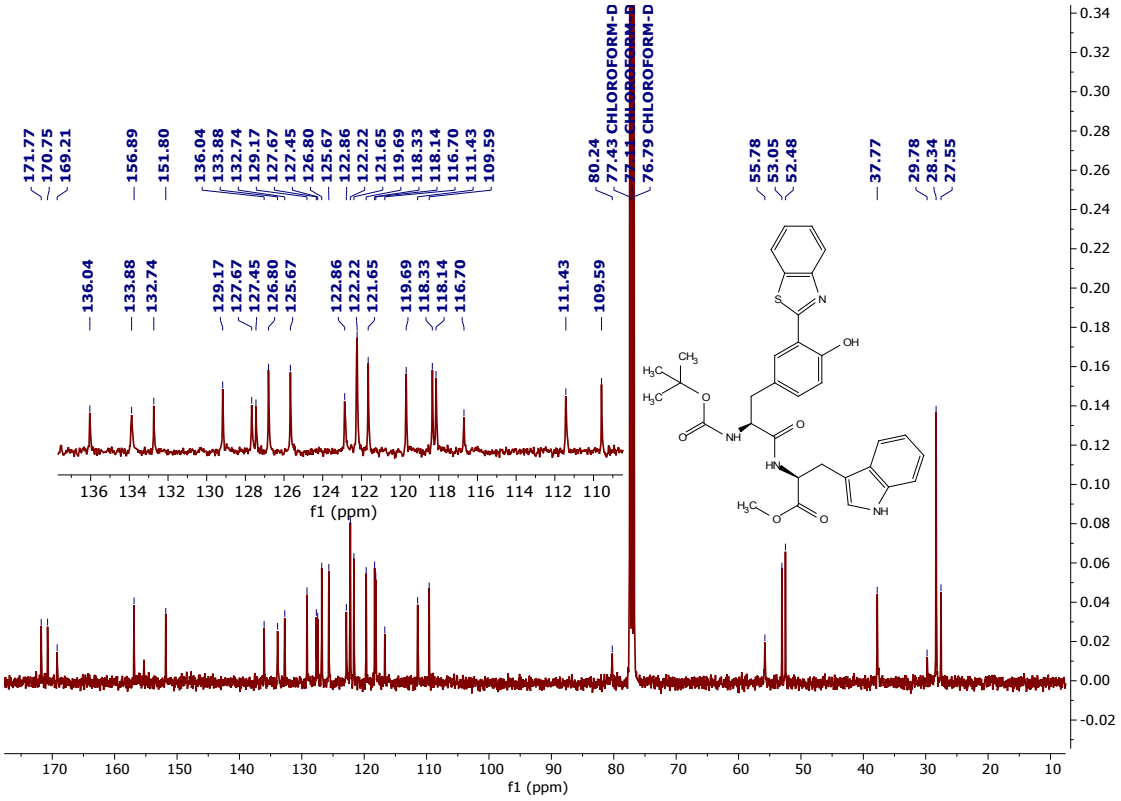


Figure S15b. ^{13}C NMR spectrum of Conjugate **6**.

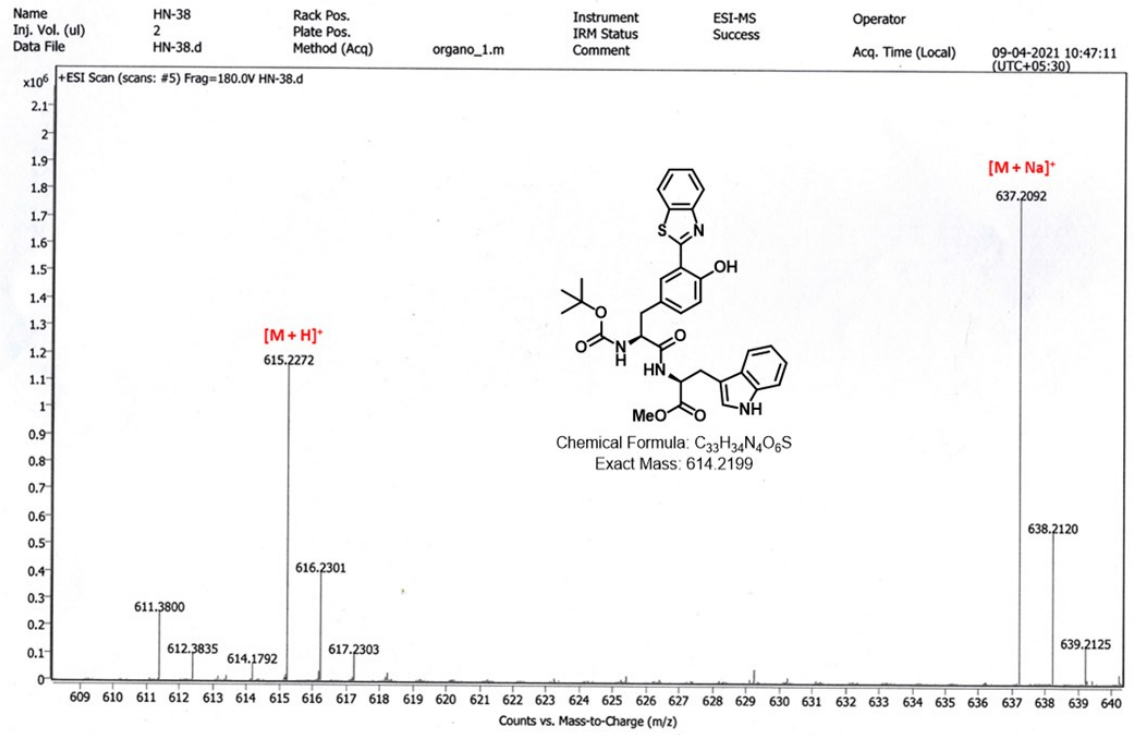


Figure S15b. ^{13}C NMR spectrum of Conjugate **6**.

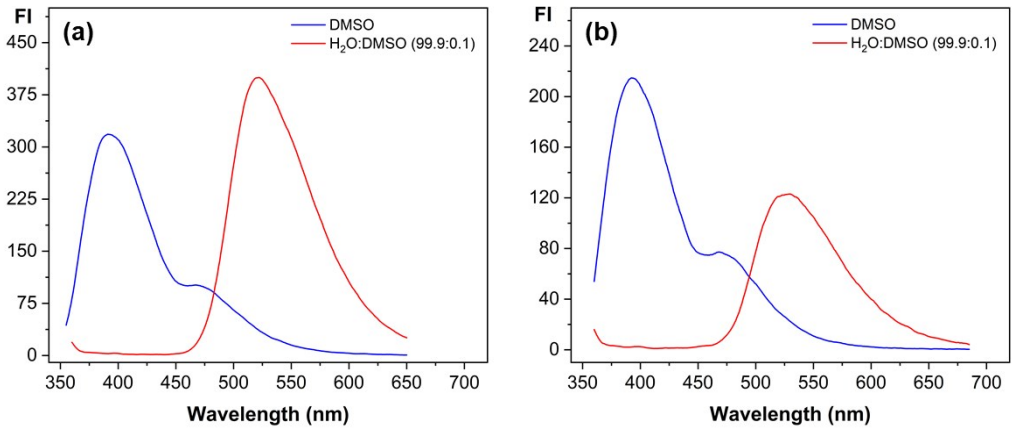


Figure S16. Fluorescence traces of 1 x 10^{-5} M solution of (a) conjugate **5**, and (b) conjugate **6** in DMSO (blue) and H_2O:DMSO (99.9:0.1, red), λ_{ex} = 350 nm, slit width Ex/Em = 5/5 nm.

Table S1. Comparison with literature reports of LFP visualization agents based on aggregation induced emission.

Citation	Concentration used	Solvent mixture	Water content	Fluorescence maximum	Level of Information
Present Manuscript	25 µM	H_2O/DMSO	99.9%	525 nm	Third
J. Am. Chem. Soc. 2020, 142, 16, 7497–7505	30 µM	H_2O	100%	658 nm	Third
New J. Chem., 2021, DOI: 10.1039/D1NJ00678A	50 µM	CH_3CN/H_2O	50%	541 nm	Third
New J. Chem., 2018, **42**, 12900	1 mM	CH_3CN/H_2O	90%	564 nm	Second
Sensors and Actuators B, 2018, **258**, 184	0.3 mM	THF/H_2O	60%, 70%	440 nm	Second
Sensors and Actuators B, 2017, **244**, 777	0.25 mM	CH_3CN/H_2O	60%, 70%	470 nm	Second
J. Mater. Chem. C, 2016, **4**, 11180	0.25 mM	CH_3CN/H_2O	50%	526 nm	Second
			40%	518 nm	Second
RSC Adv., 2015, **5**, 87306	0.25 mM	CH_3CN/H_2O	70%, 80%	650 nm	Second
Analyst, 2014, **139**, 2332	0.25 mM	C_2H_5OH/H_2O	30, 40%	450 nm	Second
			50%, 70%	470 nm	Second
Chem. Commun., 2012, **48**, 4109	0.25 mM	CH_3CN/H_2O	40%, 50%	440 nm	Second

References:

1 A. Banerjee, T. D. Panosian, K. Mukherjee, R. Ravindra, S. Gal, D. L. Sackett and S. Bane, *ACS Chem. Biol.*, 2010, **5**, 777–785.