Reversible pH-responsive supramolecular aggregates from viologen based amphiphiles – A molecular design perspective

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Electronic Supplementary Information (ESI)

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1. Materials and Methods

1.1 Reagents

Benzyl bromide, Benzyl chloride, Carbon tetrabromide, para-toluic acid, Lithium Aluminium Hydride, N-bromosuccinimide and Phosphorous tribromide were purchased from Spectrochem, India. NBS was used after recrystallisation from hot water. 4,4'-bipyridine and Methyl gallate were procured from Alfa Aesar. Potassium carbonate, Sodium sulphate, Sodium borohydride, Sodium bicarbonate, and Triphenylphosphine were obtained from Merck

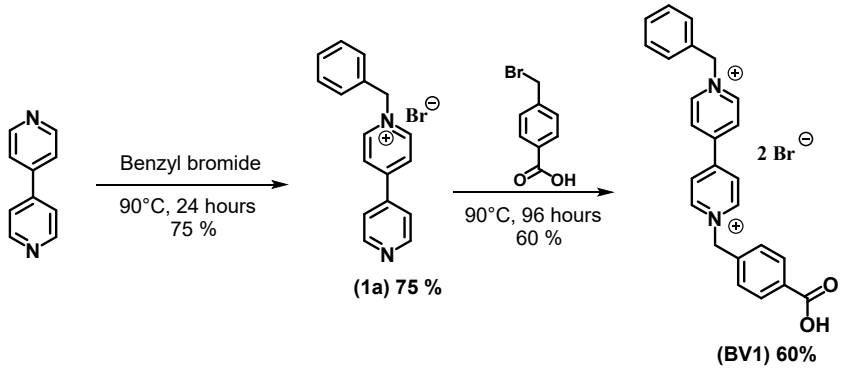
India and used as such. HPLC grade organic solvents (Acetone, Acetonitrile, Chloroform, Dichloromethane and Ethyl acetate) were used as received. Freshly distilled THF over Sodium and benzophenone under N_2 gas was used for reactions. Dimethyl formamide was distilled and stored over molecular sieves under N_2 atmosphere. Ultrapure water (Merck Millipore) having a resistivity of 18 $M\Omega cm^{-1}$ (25°C) was used for preparing aqueous solutions of molecules under investigation.

1.2 Instrumentation

^1H NMR spectra were recorded on Bruker avance 400 and 500 MHz Bruker Avance spectrometers. Chemical shift values were reported in parts per million (ppm) relative to the tetramethylsilane (δ 0.00 ppm), $CDCl_3$ (δ 7.26 ppm), D_2O (δ 4.75 ppm) and dmso-d_6 (δ 2.50 ppm). ^{13}C NMR spectra were recorded on 100 and 125 MHz spectrometers with complete decoupling. Chemical shift values were reported in ppm relative to TMS (δ 0.0 ppm), $CDCl_3$ (δ 77.0 ppm) or dmso-d_6 (δ 39.52 ppm). Coupling constants were reported in Hertz. Molecular mass of the synthesized compounds was measured using Agilent 6200 series TOF and 6500 series Q-TOF LC/MS system. UV-Visible absorbance spectra of solutions were recorded on a Shimadzu UV-3100 UV-Vis-NIR spectrophotometer at 25°C. Emission spectra were recorded using a JASCO FP-6300 spectrofluorometer. Helma cuvettes (path length = 1 cm) received from Sigma Aldrich were used to record absorption and emission spectra. SEM images were recorded using EVO 18 SEM, Carl ZEISS. Powder XRD patterns were recorded on a Bruker D8 Advance X-ray diffractometer using CuK_a radiation (I = 1.54178 Å). Particle size analysis of the CT aggregates was carried out using a Nano-ZS90 zetasizer nanoseries instrument procured from Malvern. Thermogravimetric analysis (TGA) of the dried samples was conducted under a nitrogen atmosphere at a heating rate of 10°C per min using a TGA Q500 V20.10 Build 36 instrument.

2. Synthesis of Benzyl Viologen Derivatives:

2.1 Synthesis of BV1

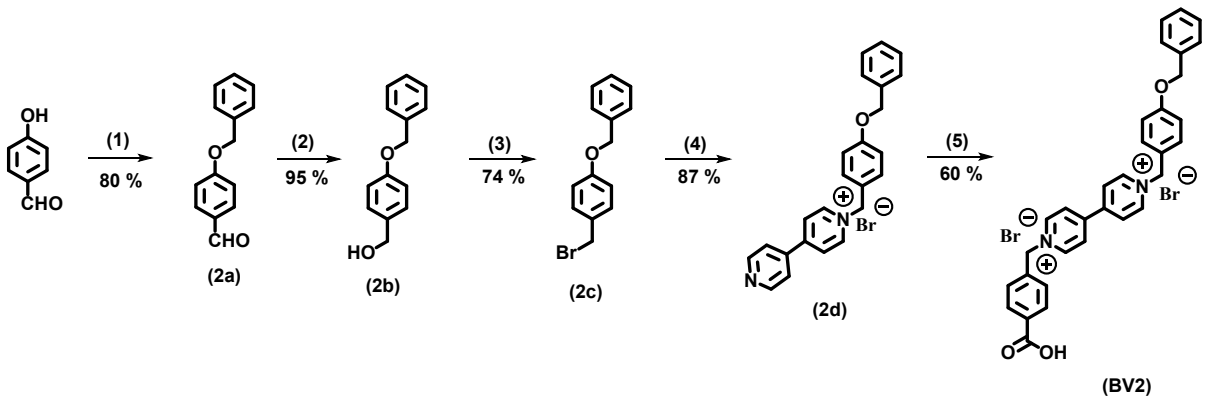


Scheme S1. Synthetic scheme of BV1

1a: 4,4-bipyridine (1.5 g, 0.0096 moles) and benzyl bromide (0.3798 mL, 0.0032 moles) were dissolved in 40 mL of acetonitrile and the mixture was refluxed under nitrogen atmosphere for 24 hours. After cooling the reaction mixture to room temperature, the solvent was evaporated to obtain the crude solid material which was triturated with hot hexane to obtain 1a 0.7848 grams (75% yield).

BV1: 1a (0.7 g, 0.00229 moles) and 4-(bromomethyl)benzoic acid (0.735 g, 0.0034 moles) in 20 mL of acetonitrile were refluxed for 96 hours with stirring under nitrogen atmosphere. After cooling the reaction mixture, obtained precipitate was filtered and washed with hot acetonitrile (5 mL x 5) to obtain the yellow solid in 0.7458 grams (60% yield) ^{1}H NMR (dmso-d_6): 13.17 (s, 1H), 9.54-9.52 (d, 4H), 8.78-8.75 (t, 4H), 8.02-8.0 (d, 2H), 7.70-7.68 (d, 2H), 7.62-7.61 (d, 2H), 7.49-7.48 (d, 3H), 6.04 (s, 2H), 5.96 (s, 2H); ^{13}C NMR: 166.7, 149.3, 149.1, 145.9, 145.7, 138.6, 134.1, 131.6, 130.0, 129.5, 129.3, 129.0, 128.9, 127.3, 127.2, 63.3, 62.7 (Figure S1, S2). **HRMS**: calculated for $C_{25}H_{22}Br_2N_2O_2$ [M] 544.0007, $C_{25}H_{22}Br_2N_2O_2Na$ $[M+Na]^+$ 566.9905, found 566.9880.

2.2 Synthesis of BV2



Scheme S2. Synthetic scheme of BV2. (1) benzyl bromide, K_2CO_3, CH_3CN, reflux - 24 hours; (2) $NaBH_4$, CH_3OH, 0°C – room temperature, 6 hours; (3) PBr_3, CH_2Cl_2, 0°C; (4) 4,4'-bipyridine, CH_3CN, reflux, 24 hours; (5) 4-bromomethyl benzoic acid, CH_3CN, reflux, 96 hours.

2a: A mixture of 4-hydroxybenzaldehyde (1 g, 0.008 moles) and potassium carbonate (3.39 g, 0.0246 moles) in a double-necked round-bottomed flask was dried under vacuum for 30 minutes and purged with nitrogen. To the above mixture, 1.46 mL of benzyl bromide and 15 mL of dry DMF were subsequently added dropwise using a syringe under an N_2 atmosphere and refluxed with stirring for 24 hours. The reaction

mixture was cooled after the reflux and the contents were poured onto crushed ice. The precipitate obtained was filtered, washed with water, and partitioned between dichloromethane and water. The organic fraction collected was dried over dry sodium sulphate followed by solvent evaporation under reduced pressure to obtain the crude product, which was chromatographed over silica gel and eluted with 5% ethyl acetate in hexane to yield 2a as a white solid in 80 % yield (1.38 gram).

2b: To a mixture of 2a (1 gram, 0.0047 moles) and $NaBH_4$ (0.1739 gram, 0.0047 moles), 20 mL of dry methanol was added and the reaction mixture was stirred over 2 hours under an N_2 atmosphere. After 2 hours, methanol was evaporated under reduced pressure and the crude solid was dissolved in diethyl ether and washed with water, 1M HCl, and brine solution. After removal of the solvent under reduced pressure, the residue was chromatographed over silica gel and eluted with 1:3 ethyl acetate-hexane solution to obtain 2b as a white solid in 95 % yield (0.95 grams).

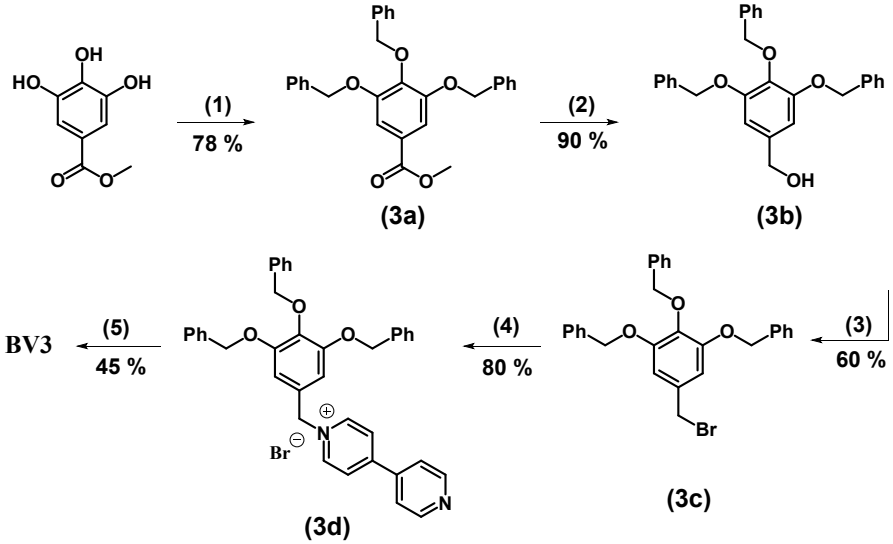
2c: 2c was synthesized using 2b (0.9 g, 0.0042 mole) and Phosphorous tribromide (0.40 mL, 0.0042 mole) as reported in literature[284] with a yield of 74% (0.8578 gram). Product 2C obtained was used immediately towards the synthesis of 2d without further purification.

2d: A double-necked RB flask was charged with 2c (0.8 gram, 0.0028 moles) and 4,4'-bipyridine (1.311 gram, 0.0084 moles), dried under vacuum, and purged with nitrogen. The mixture was dissolved in 40 mL of dry acetonitrile and refluxed with stirring for over 24 hours under an N_2 atmosphere. After 24 hours, the solvent was evaporated and the residue was triturated with hot hexane (10 mL x 7) to obtain 2d in 87% yield (1.052 gram) as a light-yellow solid.

BV2: A mixture of 2d (0.7 gram, 0.00162 mole) and 4-(bromomethyl)benzoic acid (0.52 gram, 0.00243 mole) was dissolved in 20 mL of acetonitrile under N_2 atmosphere and resultant mixture was refluxed under stirring over 96 hours and cooled to room temperature. After 96 hours, product obtained was filtered and the residue was washed with hot acetonitrile to yield BV2 in 60 % yield (0.627 gram) as a yellow solid. **^{1}H NMR** (dmso-d_6): δ 13.18 (s, 1H), 9.54-9.50 (t, 4H), 8.78 – 8.74 (q, 4H), 8.02-8.0 (d, 2H), 7.71-7.69 (d, 2H), 7.62-7.56 (d, 2H), 7.44-7.31 (m, 5H), 7.11-7.09 (d, 2H), 6.05 (s, 2H), 5.88 (s, 2H), 5.13 (s, 2H). **^{13}C NMR**: 166.6, 159.1, 149.3, 148.9, 145.8, 145.4, 138.5, 136.7, 131.6, 130.8, 129.9, 129.0, 128.4, 127.8, 127.6, 127.2, 127.1, 126.2,

115.4, 69.2, 62.9, 62.6. (Figure S4, S5). **HRMS**: calculated for $C_{32}H_{28}Br_2N_2O_3$ [M] 648.0170, $C_{32}H_{28}Br_2N_2O_3Na$ $[M+Na]^+$ 671.0068, found 671.0284.

2.3 Synthesis of BV3



Scheme S3. Synthetic scheme of BV3 - (1) benzyl chloride, K_2CO_3, DMF, reflux for 24 hours; (2) $LiAlH_4$, Anhydrous THF, 0°C – room temperature, 6 hours; (3) CBr_4, PPh_3, THF; (4) 4,4'-bipyridine, CH_3CN, reflux, 24 hours; (5) 4-bromomethyl benzoic acid, CH_3CN, reflux, 96 hours.

3a: A mixture of methyl 3,4,5-trihydroxybenzoate (2 grams, 0.0108 mole) and potassium carbonate (9.0 grams, 0.0652 mole) was dried under vacuum for 30 minutes and purged with Nitrogen. To the mixture, 5.62 mL (0.04891 moles) of benzyl chloride and 40 mL of dry DMF were subsequently added dropwise by syringe and refluxed with stirring over 24 hours under an N_2 atmosphere. The reaction mixture was cooled to room temperature after reflux and poured into 500 grams of crushed ice. The precipitate obtained was filtered and dissolved in dichloromethane and washed with saturated sodium bicarbonate, water and brine. After evaporating the solvent, the crude solid was purified by column chromatography (7.5% ethyl acetate in hexane) to obtain the white powder as 3a in 60 % yield (2.96 grams).

3b: 3a (2 grams, 0.0044 moles) in a double-necked round-bottomed flask was dried under vacuum, dissolved in 15 mL of dry THF and the mixture was cooled to 0°C in an ice bath for 10 minutes under an N_2 atmosphere. Then, $LiAlH_4$ (167 mg, 0.0044 moles) was added portion-wise to the solution and stirred for 6 hours under an N_2 atmosphere. The reaction mixture was quenched with dropwise addition of 0.1 mL of

water, followed by 0.1 mL of 1M HCl, and filtered through Whatman filter paper. The solid residue was washed with THF and the filtrate was evaporated to obtain the crude 3b, which was further purified by column chromatography to obtain pure 3b as a white solid in 90 % yield (1.68 grams).

3c: 3c was synthesized as reported in the literature with 3b (1.5 g, 3.51 mmol), carbon tetrabromide (1.167 g, 3.51 mmol), triphenylphosphine (1.224 g, 4.66mmol) in dry THF.[285] The product was obtained as a spongy white solid with a yield of 60 % (1.02 grams).

3d: A mixture of 3c (0.8 grams, 1.6 mmol) and 4,4'-bipyridine (0.75 grams, 4.9 mmol) was dissolved in dry acetonitrile and refluxed with stirring for 24 hours under the N_2 atmosphere. After cooling the reaction mixture to room temperature, the solvent was evaporated and triturated with hot hexane. The remaining crude was subjected to silica gel column chromatography to obtain 3d as a greenish-yellow foam in 70 % yield (0.73 grams).

BV3: A procedure similar to BV2 synthesis was adopted where the reaction was carried out with 3d (0.6 gram, 0.93 mmol) and 4-(bromomethyl)benzoic acid (0.298 grams, 1.39 mmoles) in 20 mL of dry acetonitrile resulted in a product yield of 45 % (0.359 grams). ^{1}H NMR: 13.16 (broad s, 1H), 9.608-9.564 (dd, 4H), 8.83-8.77 (dd, 4H) 8.03-8.0 (d, 2H), 7.73-7.71 (d, 2H) 7.47-7.25 (m, 17H), 6.08 (s, 2H), 5.85 (s, 2H), 5.16 (s, 4H), 4.92 (s, 2H). ^{13}C NMR: 166.7, 152.5, 149.198, 148.8, 145.9, 145.5, 138.5, 138.0, 137.4, 136.6, 131.6, 130.0, 129.2, 129.0, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.2, 127.0, 108.6, 74.2, 70.3, 63.5, 62.7 (Figure S5, S6). **HRMS**: calculated for $C_{46}H_{40}Br_2N_2O_5$ [M] 858.1304, $C_{46}H_{40}Br_2N_2O_5Na$ $[M+Na]^+$ 881.1203, found 881.1209.

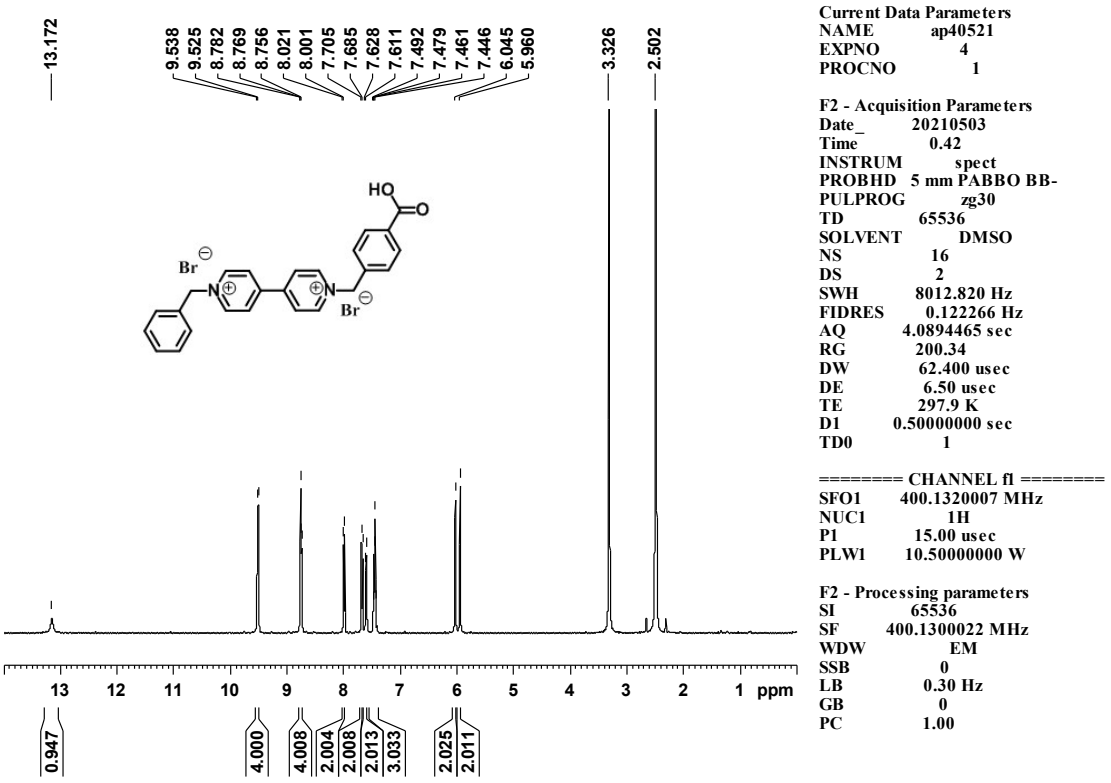


Figure S1 ^{1}H NMR Spectra of BV1 (DMSO-d_6) at 25°C.

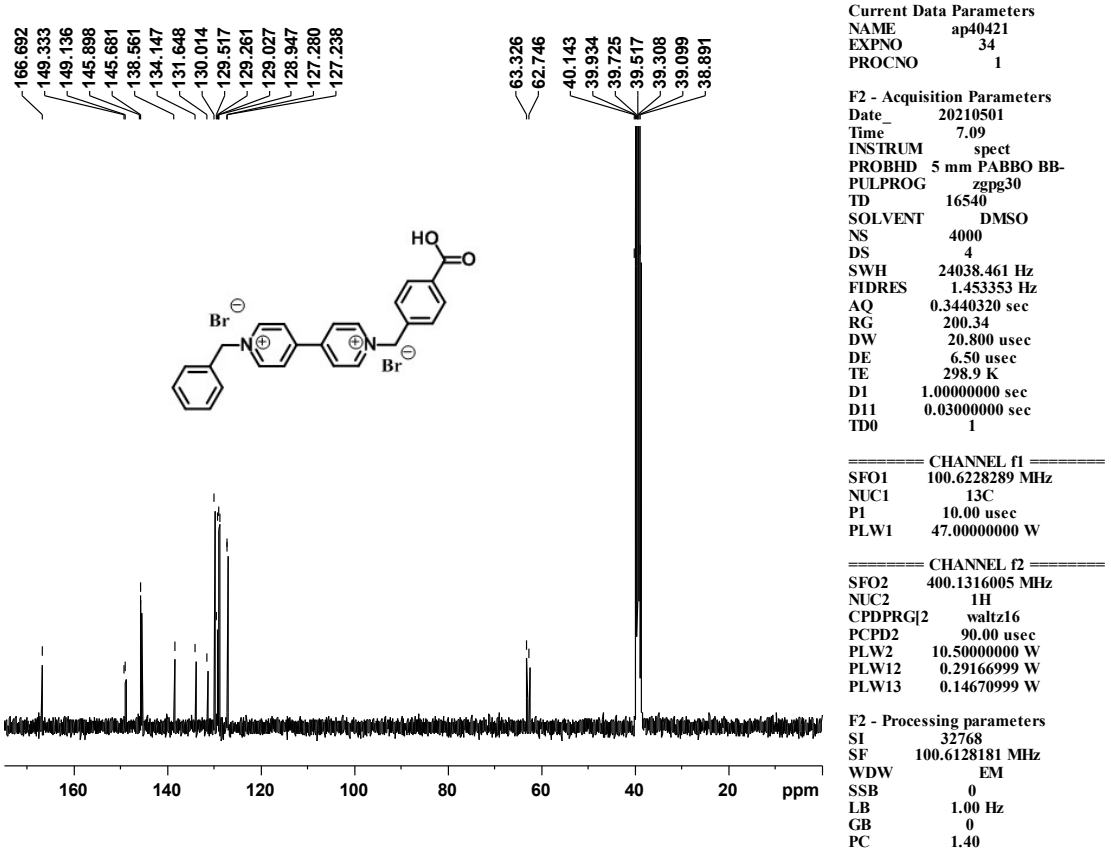
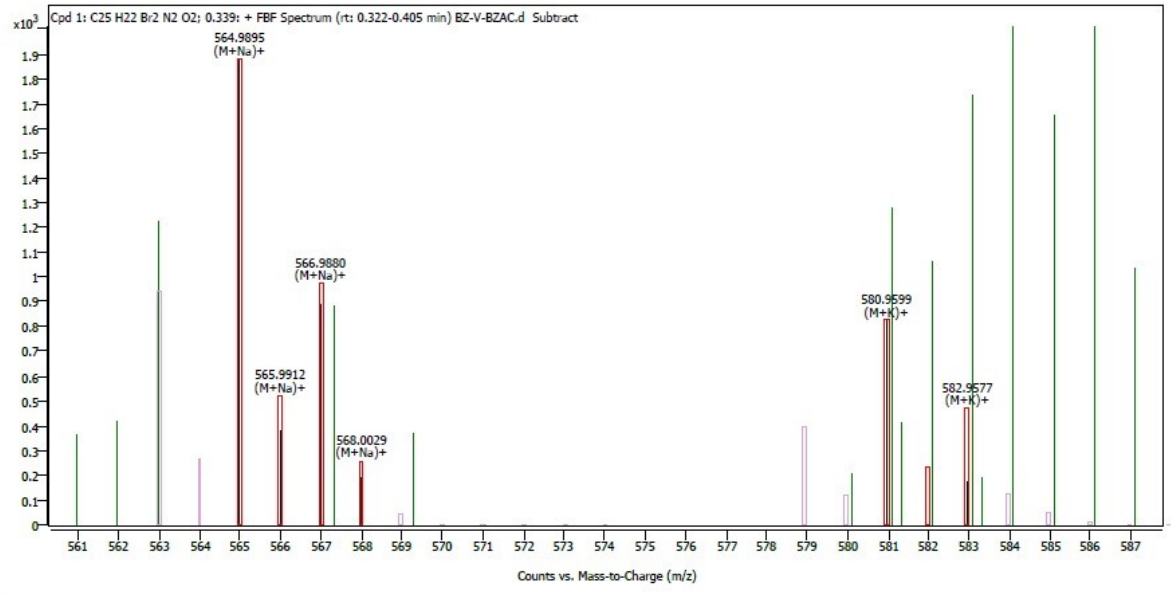


Figure S2 ^{13}C NMR Spectra of BV1 (DMSO-d_6) at 25°C.



Compound ID Table

Cpd	Formula	Mass (Tgt)	Calc. Mass	Mass	Species	Diff(Tgt,ppm)	mDa
1	C25 H22 Br2 N2 O2	540.0048	540.0017	564.9895 580.9599	(M+Na)+ (M+K)+	-5.72	-3.09

Figure S3: HRMS of BV1

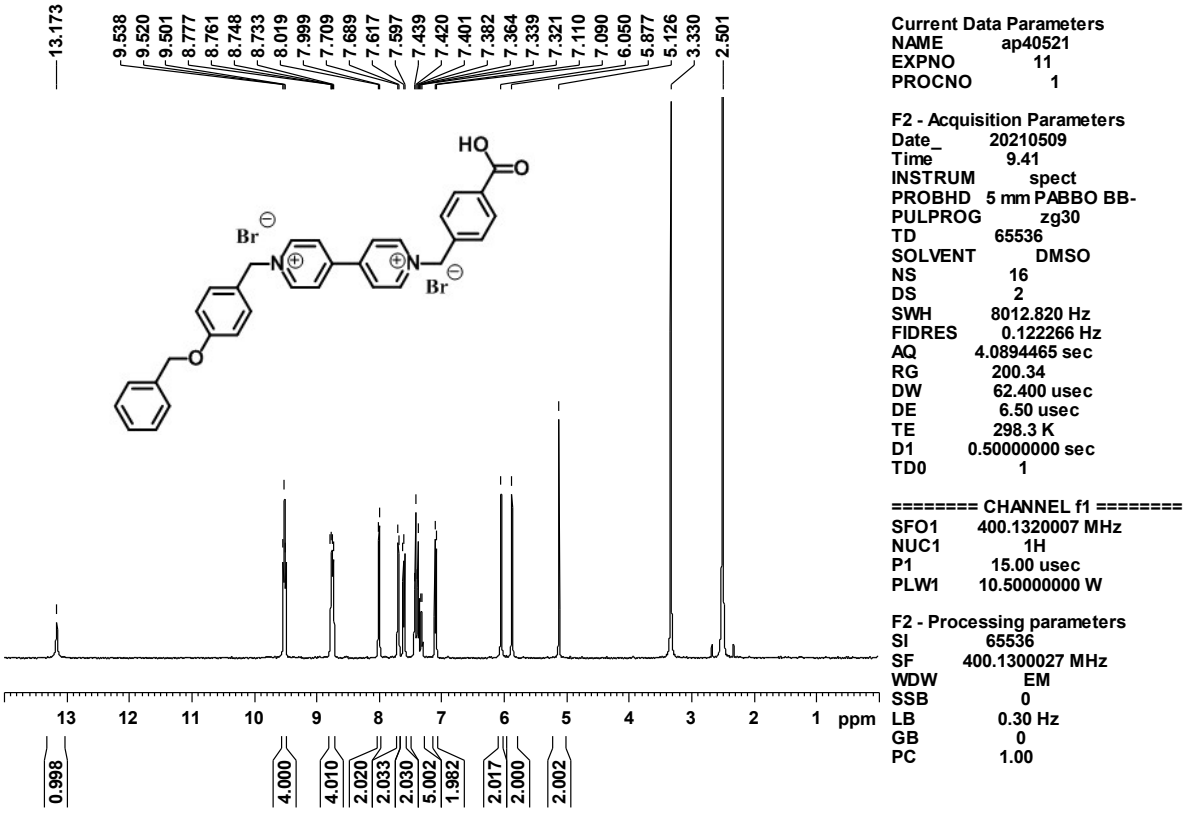


Figure S4 ^{1}H NMR Spectra of BV2 (DMSO-d_6)

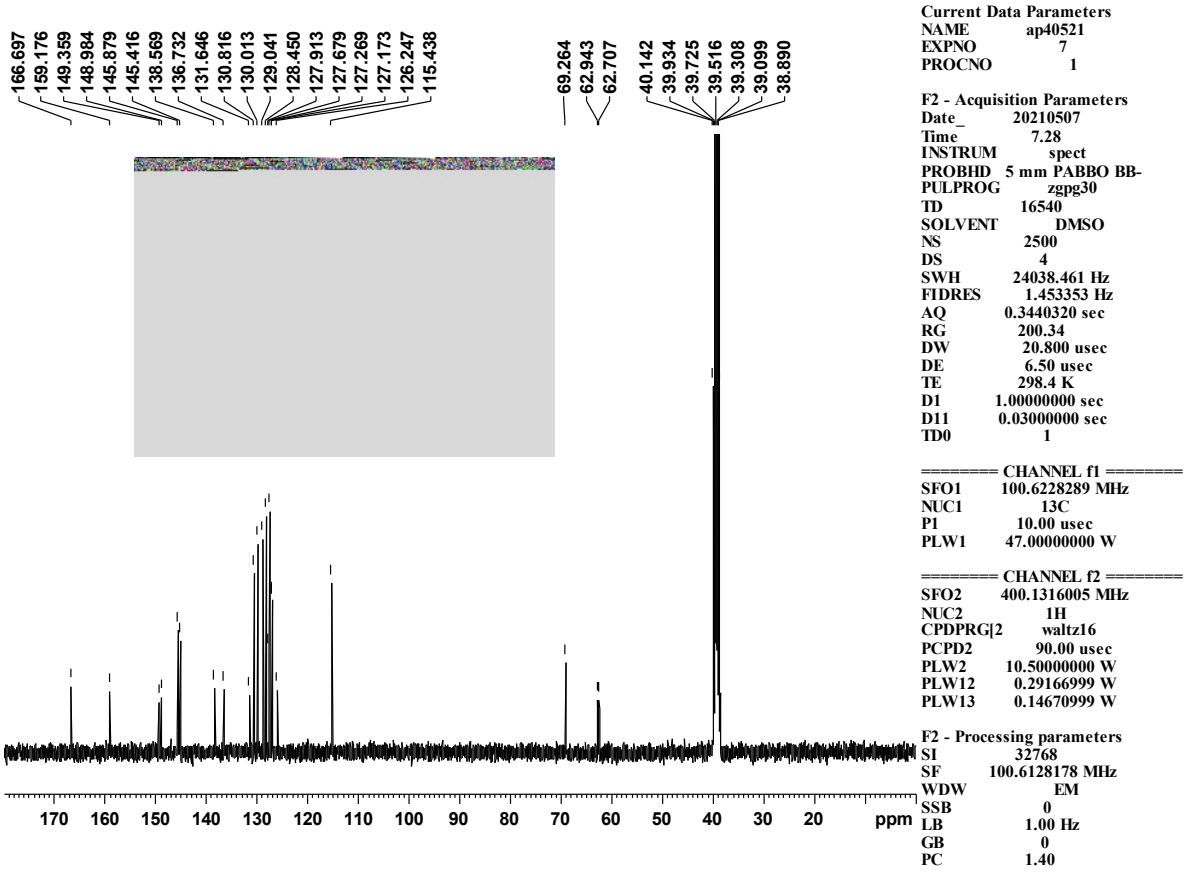
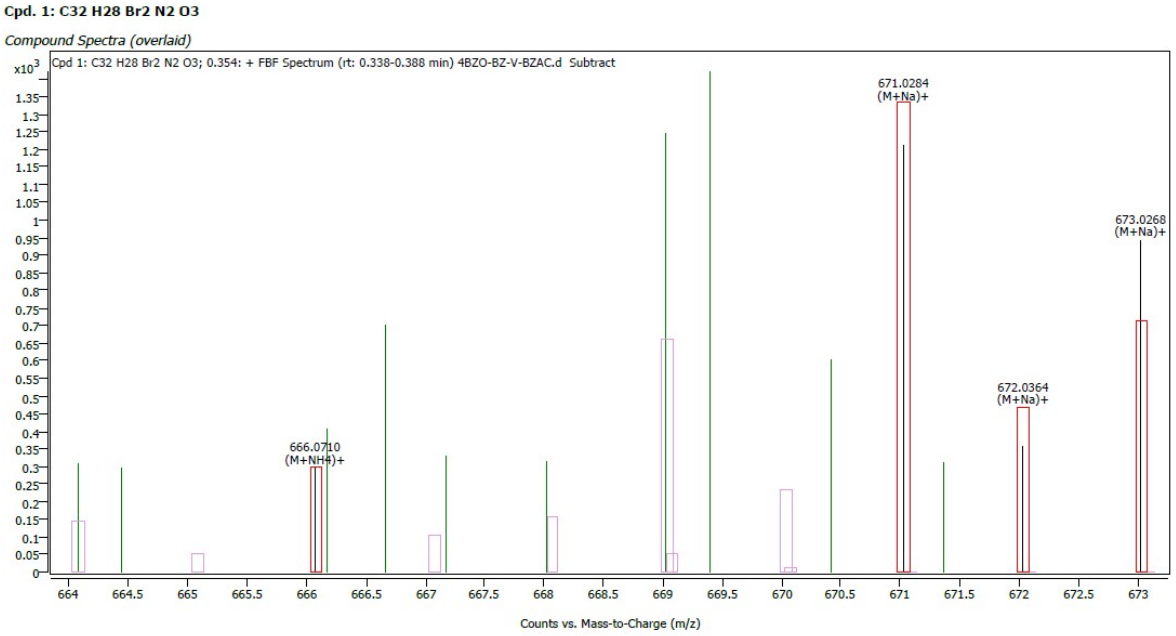


Figure S5: ^{13}C NMR Spectra of BV2 (DMSO-d_6) at 25°C.



Compound ID Table

Cpd	Formula	Mass (Tgt)	Calc. Mass	Mass	Species	Diff(Tgt.ppm)	mDa
1	C32 H28 Br2 N2 O3	646.0467	646.0412	666.0710 671.0284	(M+NH4)+ (M+Na)+	-8.39	-5.42

Figure S6: HRMS of BV2

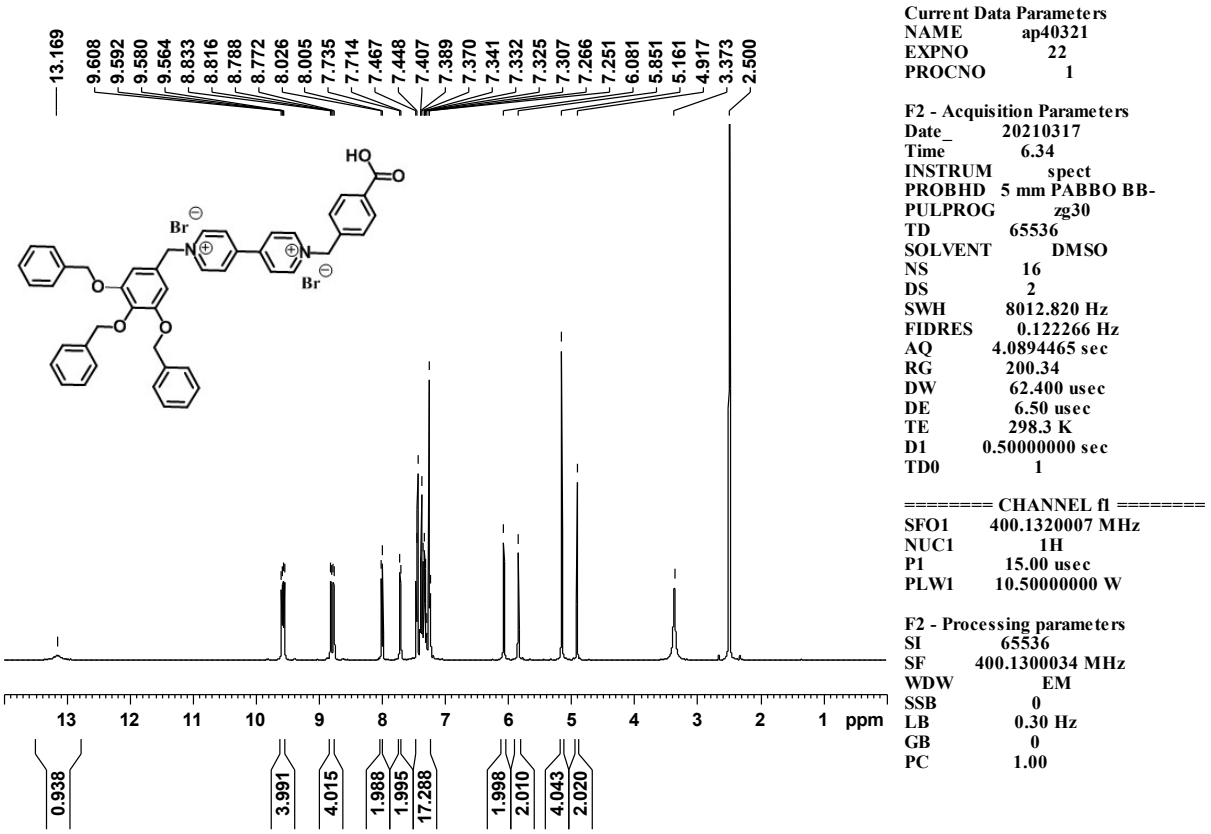


Figure S7 ^{1}H NMR Spectra of BV3 (DMSO-d_6) at 25°C.

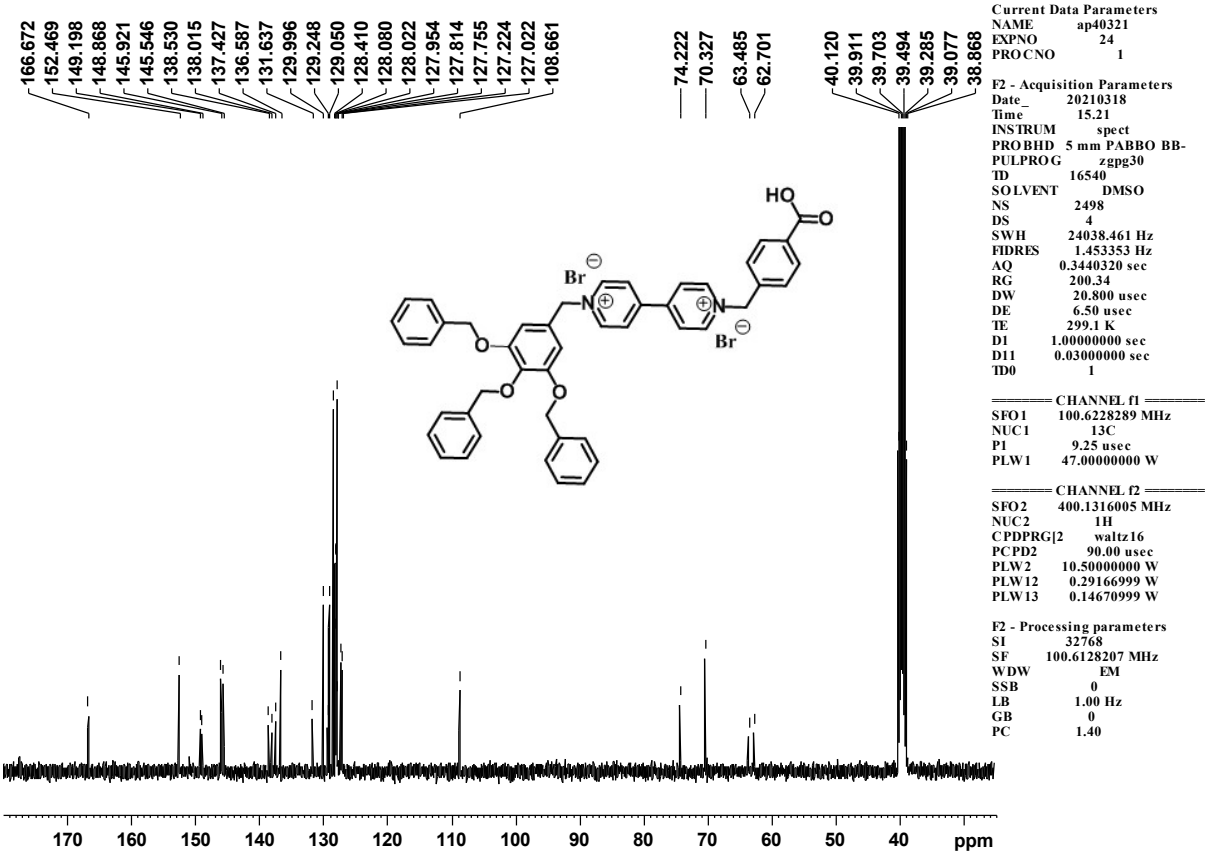
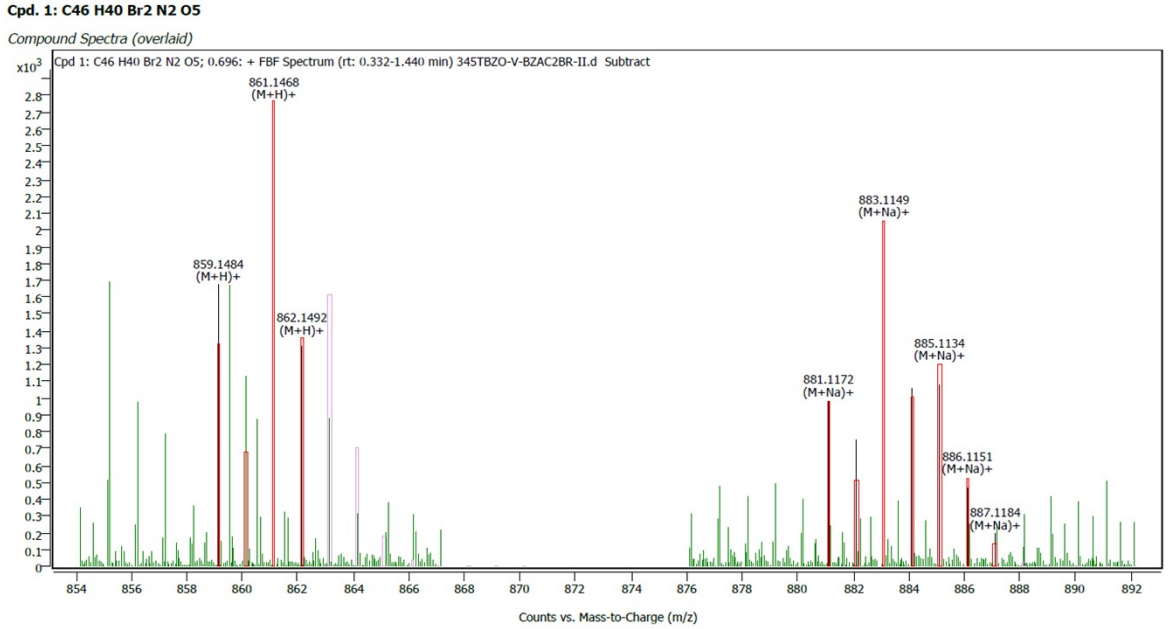


Figure S8 ^{13}C NMR Spectra of BV3 (DMSO-d_6) at 25°C.



Compound ID Table

Cpd	Formula	Mass (Tgt)	Calc. Mass	Mass	Species	Diff(Tgt.ppm)	mDa
1	C46 H40 Br2 N2 O5	858.1304	858.1334	859.1484 881.1172	(M+H)+ (M+Na)+	3.54	3.04

Figure S9: HRMS of BV3

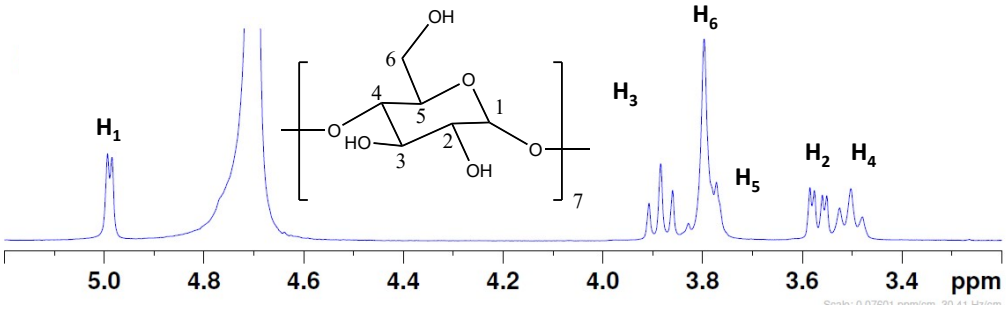


Figure S10: ^{1}H NMR Spectrum of β-CD (D_2O) at 25°C

Protons	δ (ppm)		
	BV1	BV1 ⊂ 1 eq. β-CD	BV1 ⊂ 2 eq. β-CD
H_a (singlet)	7.45	7.48	7.49
H_b (doublet)	7.52	7.61	7.65
H_c (doublet)	8.02	8.04	8.06
H_d (quartet)	8.45	8.60, 8.54	8.65, 8.58
H_e (quartet)	9.09	9.20, 9.08	9.22, 9.10
H_x (singlet)	5.85	5.87	5.90
H_y (singlet)	5.94	6.02	6.05

Table 1. Change in ^{1}H NMR Chemical Shifts of BV1 as a result of interaction with β-CD

Protons	δ (ppm)		
	BV2	BV2 ⊂ β-CD	BV2 ⊂ 2 eq. β-CD
H_a (singlet)	5.08	5.12	5.13
H_b (singlet)	5.75	5.8	5.81
H_c (singlet)	5.92	5.99	6.03
H_d (doublet)	7.06-7.03	6.99-6.96	6.95-6.94
H_e (multiplet)	7.35-7.27	7.31 (s)	7.33-7.28 (t)
H_f (triplet)	7.41-7.37	7.44-7.42 (d)	7.45-7.43 (d)
H_g (doublet)	7.52-7.49	7.59-7.57	7.63-7.61
H_h (doublet)	8.01-7.99	8.03-8.01	8.05-8.02
H_i (dd)	8.45-8.43; 8.41-8.39	8.55-8.53 (d) 8.51-8.49 (d)	8.61-8.59 (d) 8.55-8.53 (d)
Hj (dd)	9.09-9.07; 9.03-9.01	9.16-9.14 (d) 9.06-9.04 (d)	9.11-9.09 (d) 9.07-9.05 (d)

Table 2 Change in ^{1}H NMR Chemical Shifts of BV2 as a result of interaction with β-CD

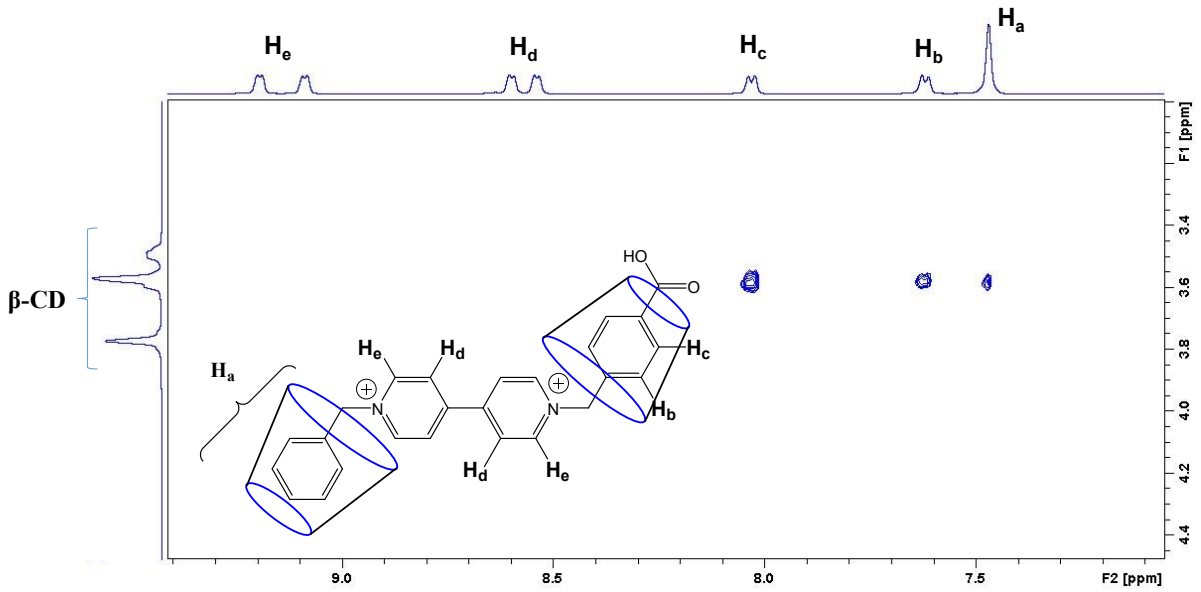


Fig. S11: Enlarged ^{1}H-^{1}H ROESY NMR spectra of of BV1 ⊂ 1 eq. β-CD (D_2O, 25°C)

Benesi-Hildebrand Equation:

1:1 binding constant (K_b) for the host-guest complexes of BV1 – β-CD and BV2 – β-CD, were calculated using the following equation.

$$\frac{1}{\Delta\delta_{obs}} = \frac{1}{K_b \Delta\delta_c [\beta\text{-CD}]} + \frac{1}{\Delta\delta_c}$$

K_b – binding constant

$\Delta\delta_{obs}$ – observed chemical shift changes of proton in BV molecule in the absence and presence of β-CD.

$\Delta\delta_c$ - chemical shift change between the free and complexed guest

[β-CD] – concentration of β-CD

$1/\Delta\delta_{obs}$ was plotted against 1/[β-CD] and the binding constant was obtained by dividing intercept by slope

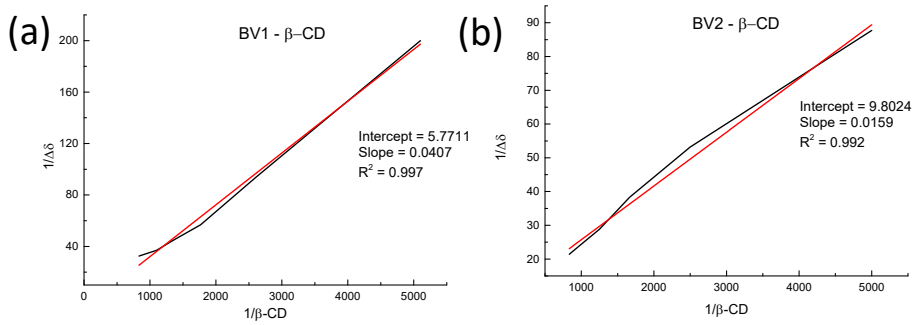


Fig. S12: Benesi Hildebrand Plot of (a) BV1 - β-CD, and (b) BV2 - β-CD (black line – experimental, red line – linear fit)**.** The x-axis scale in both figures is 1 / [β-CD].

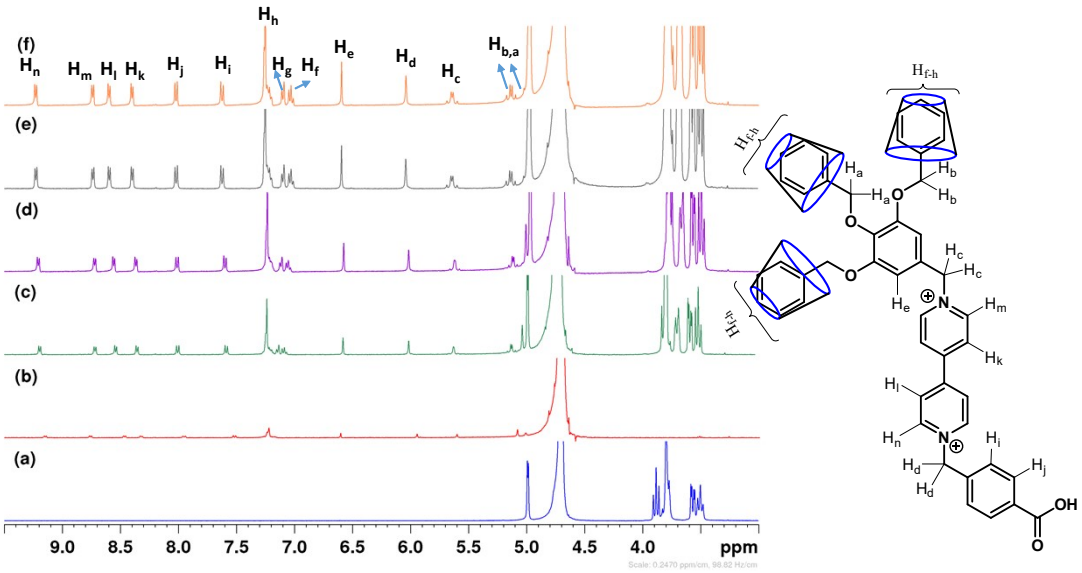


Figure S13 Stacked ^{1}H NMR spectra of (a) β-CD, (b) BV3, (c) BV3 ⊂ 1 eq. β-CD, (d) BV3 ⊂ 2 eq. β-CD, (e) BV3 ⊂ 3 eq. β-CD, and (f) BV3 ⊂ 3.3 eq. β-CD in D_2O at 25°C.

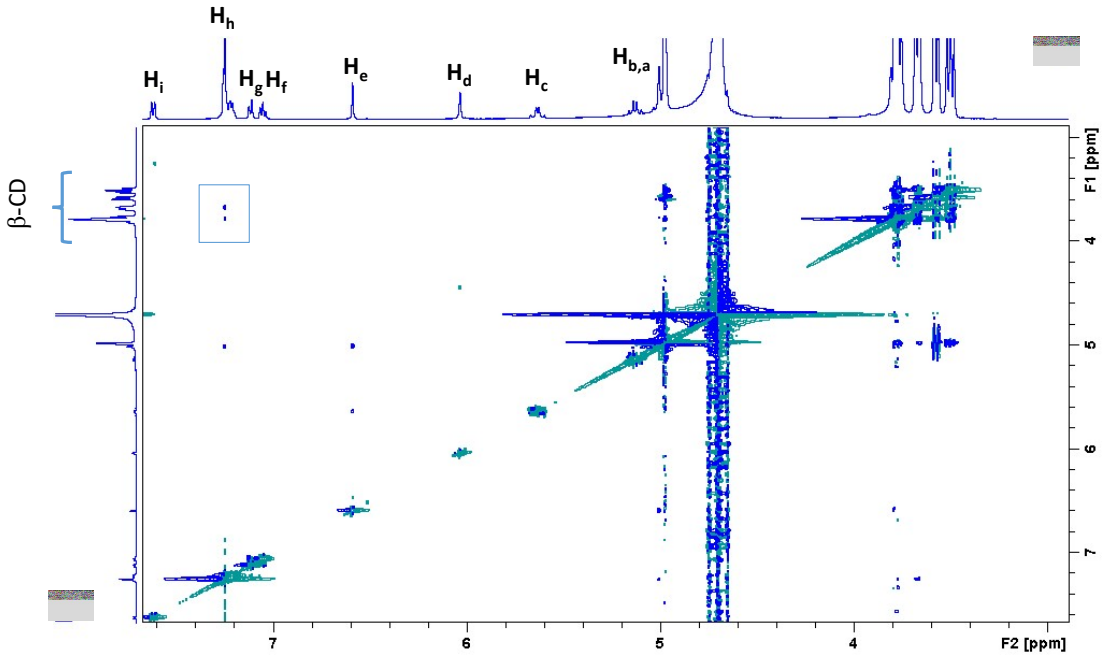


Figure S14 ^{1}H-^{1}H ROESY NMR spectra of BV3 ⊂ 3 eq. β-CD in D_2O at 25°C.

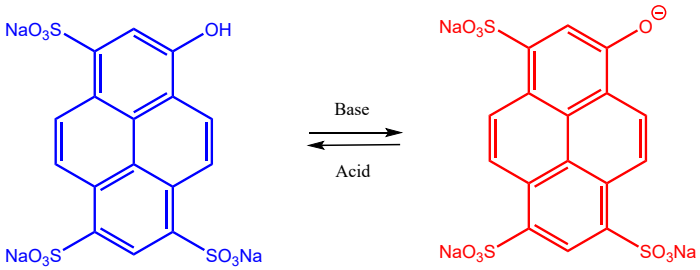


Figure S15: Molecular structure of pyranine and its deprotonated form

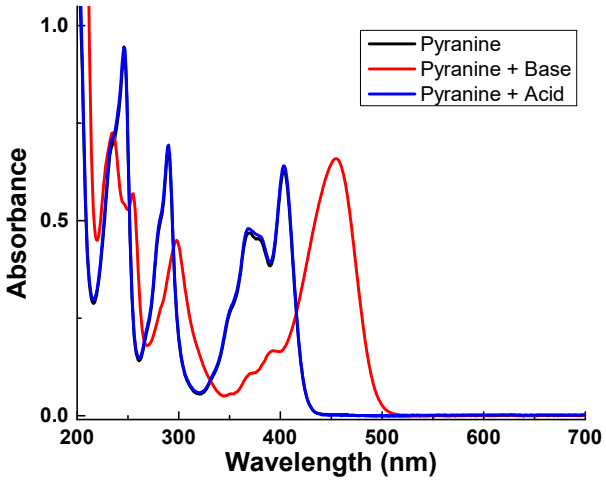


Figure S16: UV-visible spectra of pyranine at different pH in water at 25°C.

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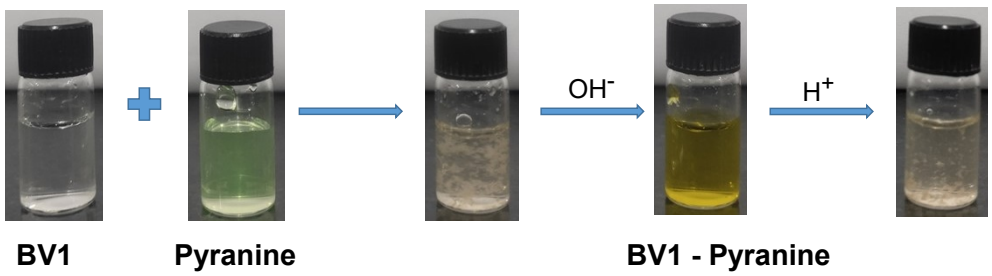


Figure S17: Photograph of solution phase interaction between BV1 and pyranine

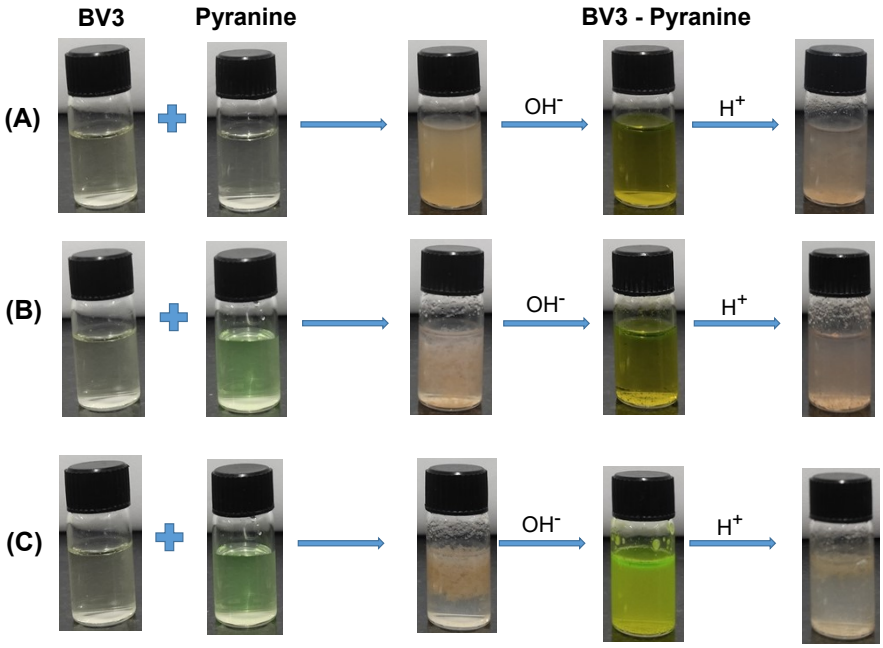


Figure S18: Photograph of solution phase interaction between BV3 and pyranine in (a) CH_3OH (b) 1:1 CH_3OH – H_2O and (c) 1:3 CH_3OH – H_2O solvent mixture.

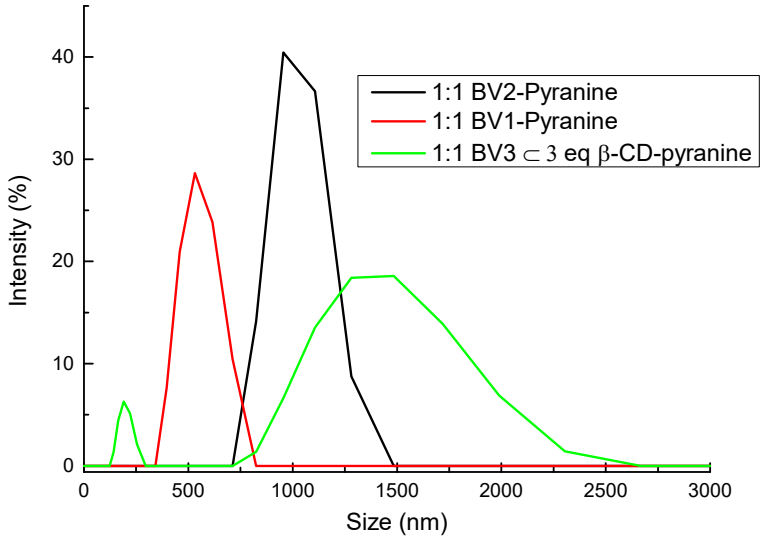


Figure S19: Particle size analysis of BV-pyranine aggregates at 25°C.

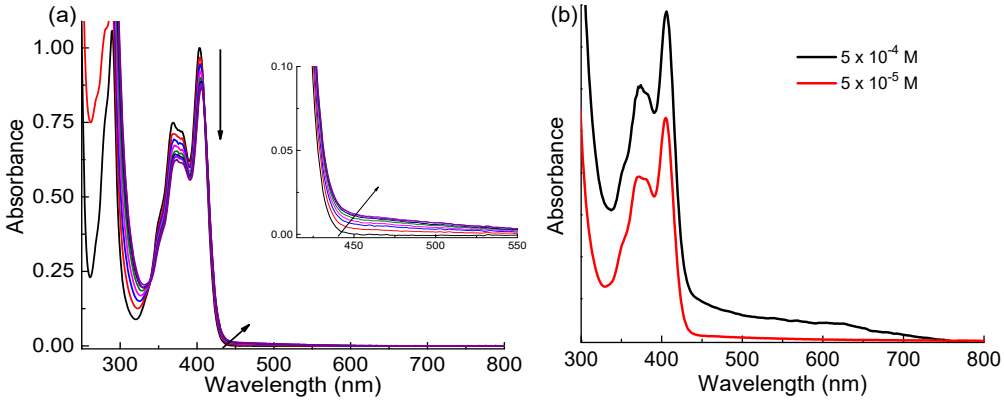


Figure S20: (a) UV-Vis spectral titration of pyranine vs BV1 ([pyranine] = 10^{-4} M); (inset) - enlarged portion of spectra to highlight Mie scattering. (b) Effect of concentration on aggregation revealed by the increase of Mie Scattering in the UV-visible spectral titration of pyranine vs BV1 (at 25°C).

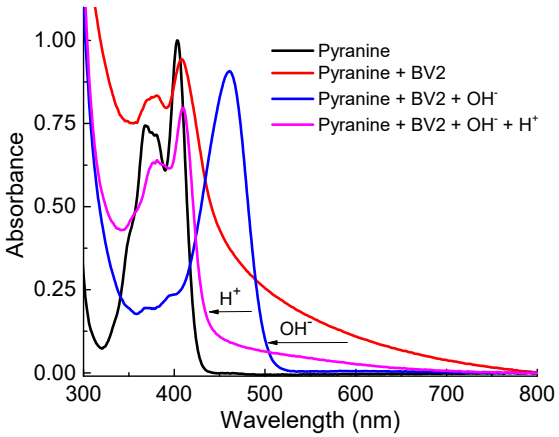


Figure S21: UV-visible spectra of pH switchable aggregation of BV2-Pyranine in water at 25°C.

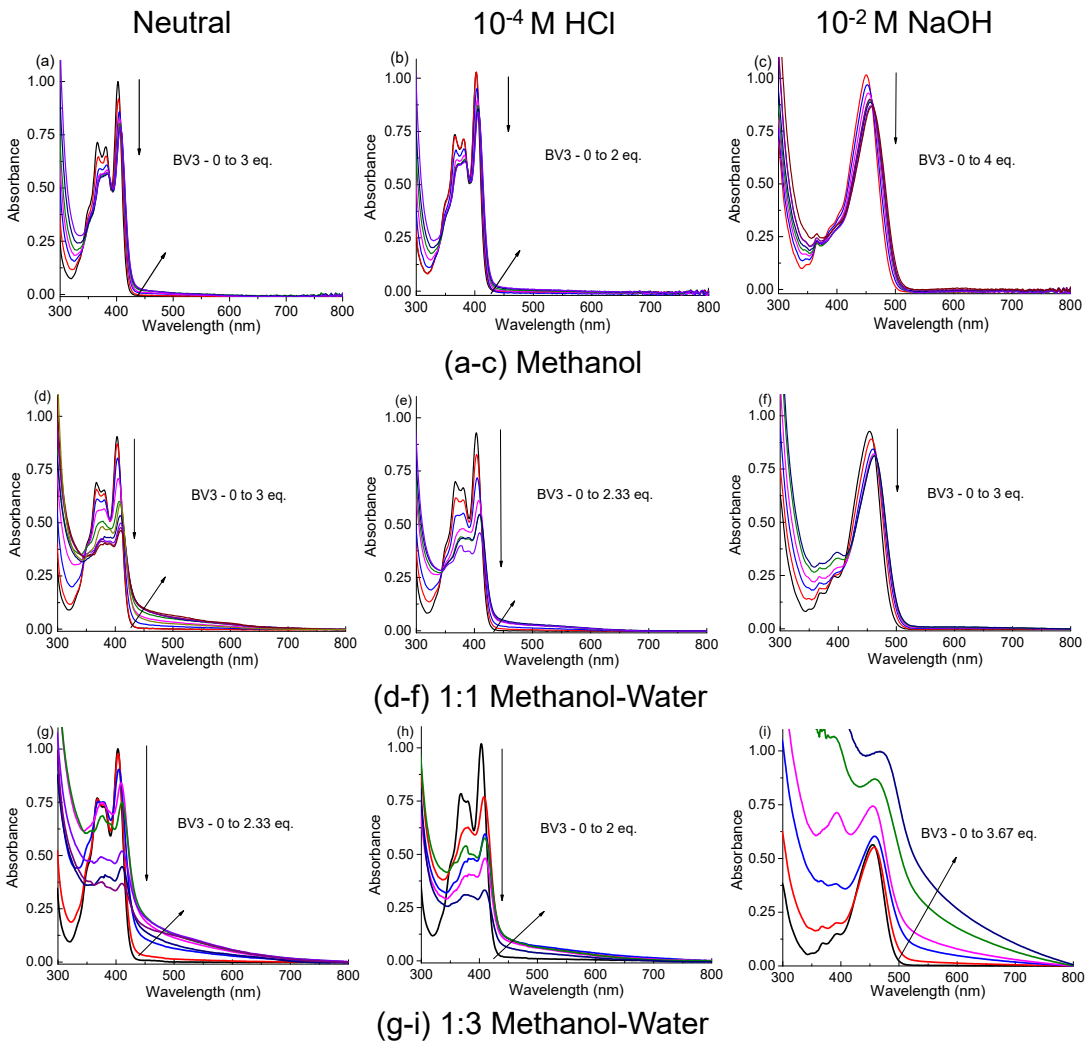


Figure S22: UV-Visible Spectra of interaction between BV3 and pyranine in methanol-water solvent mixtures at 25°C.

CT aggregates	Critical aggregation concentration	
	Water	Acidic pH
Pyranine-BV1	100 μM - 84 μM	100 μM - 50 μM
Pyranine-BV2	50 μM – 50 μM	50 μM – 16.7 μM
Pyranine-BV2 ⊂ 1 eq. β-CD	50 μM – 50 μM	50 μM – 16.7 μM
Pyranine-BV2 ⊂ 2 eq. β-CD	50 μM – 50 μM	50 μM – 16.7 μM
Pyranine-BV3 ⊂3 eq. β-CD	10 μM – 5 μM	10 μM – 3 μM

Table 3: Critical aggregation concentration of BV-pyranine (measured by UV-visible spectroscopy by monitoring change in absorption of pyranine at 480 nm upon addition of viologens).

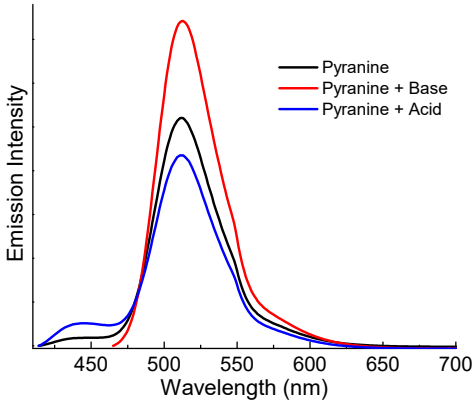


Figure S23: Emission Spectra of Pyranine at different pH in water at 25°C.

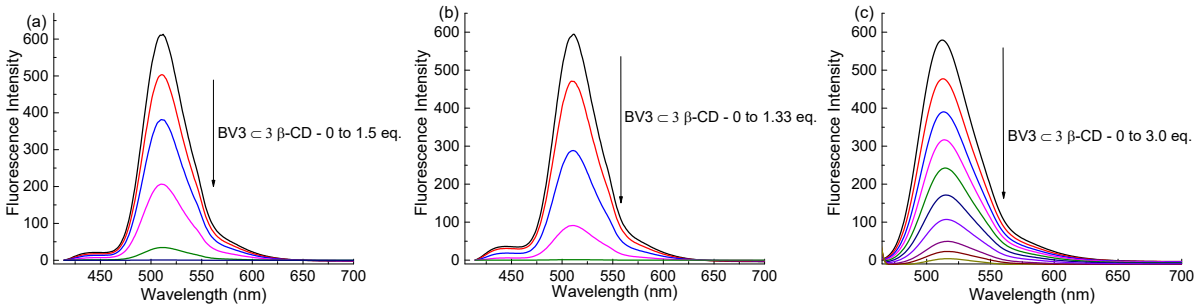


Figure S24: Fluorescence titration of pyranine vs BV3 ⊂ 3 eq. β-CD in (a) water, (b) pH = 4, and (c) pH = 12 at 25°C.

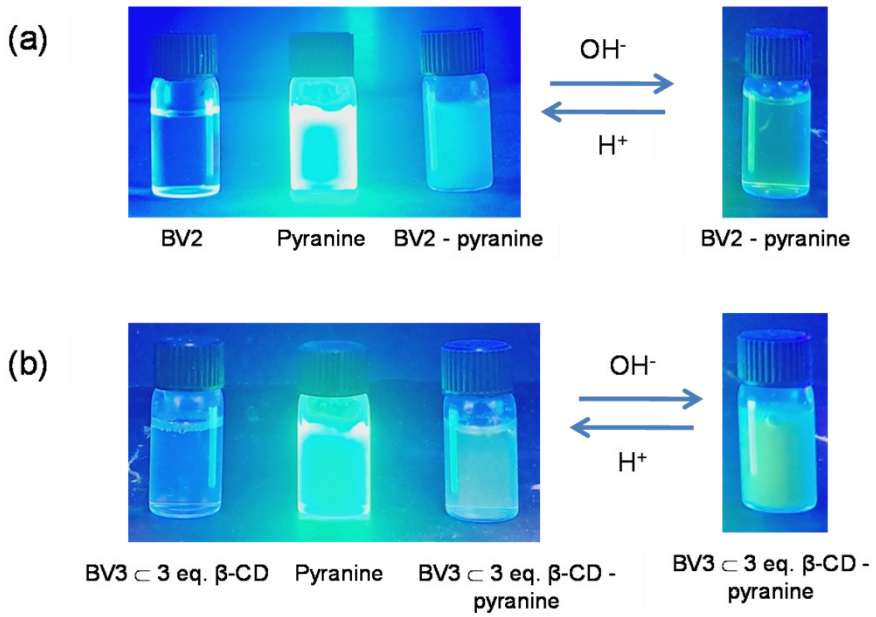


Figure S25. Quenching of pyranine's emission by BV2 and BV3 ⊂ 3 eq. β-CD.

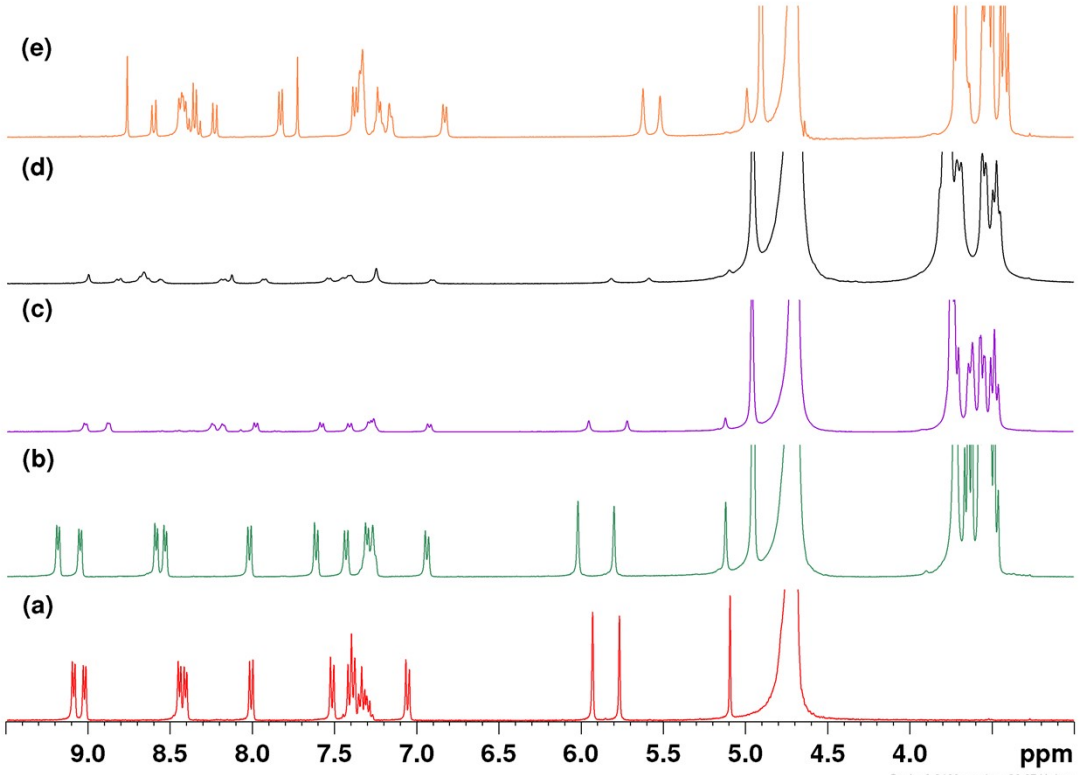


Figure S26. Stacked ^{1}H NMR spectra of (a) BV2, (b) BV2 ⊂ 2 eq. β-CD, (c) BV2 ⊂ 2 eq. β-CD + pyranine (0.5 eq.), (d) BV2 ⊂ 2 eq. β-CD + pyranine (1 eq.), (e) BV2 ⊂ 2 eq. β-CD + pyranine (1 eq.) + K_2CO_3 in D_2O at 25°C.

Preparation of BV2-Pyranine CT aggregates

15 mL of 10^{-2} M aqueous solution of BV2 (3 eq.) and 10 mL of 10^{-2} M aqueous solution of pyranine (2 eq.) were mixed under stirring in a round bottomed flask over a duration of 5 minutes and the aggregates formed were instantly filtered through a Whatman filter paper, washed with deionized water till the filtrate answered negative towards halides and pyranine. Aggregates obtained were dried under vacuum and subjected to Powder XRD and thermal studies. BV2-pyranine aggregates were also prepared under acidic conditions (pH = 4).

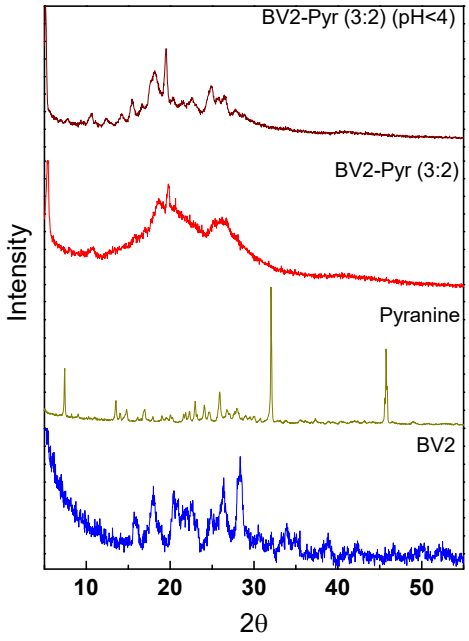


Figure S27. PXRD pattern of BV2- pyranine aggregates.

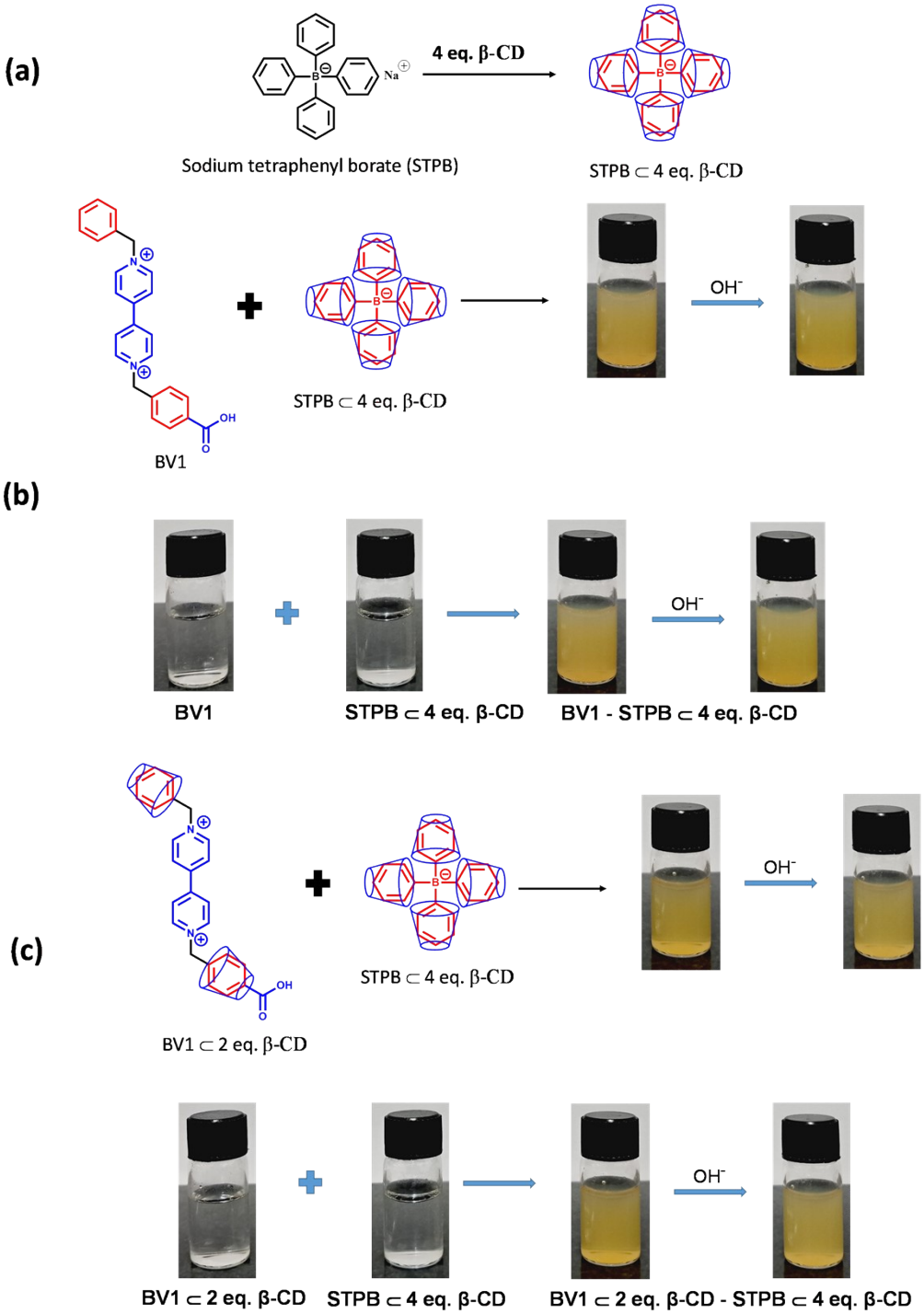


Figure S28. (a) Structure of STPB and STPB ⊂ 4 eq. β-CD; Digital photograph of solution state interaction between (b) BV1 and STPB ⊂ 4 β-CD, and (c) BV1 ⊂ 2 eq. β-CD and STPB ⊂ 4 eq. β-CD. ([BV1] = [BV1 ⊂ 2 eq. β-CD] = [STPB] = 5 x 10^{-4} M)

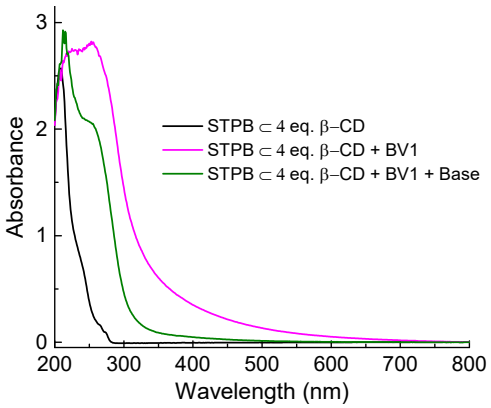
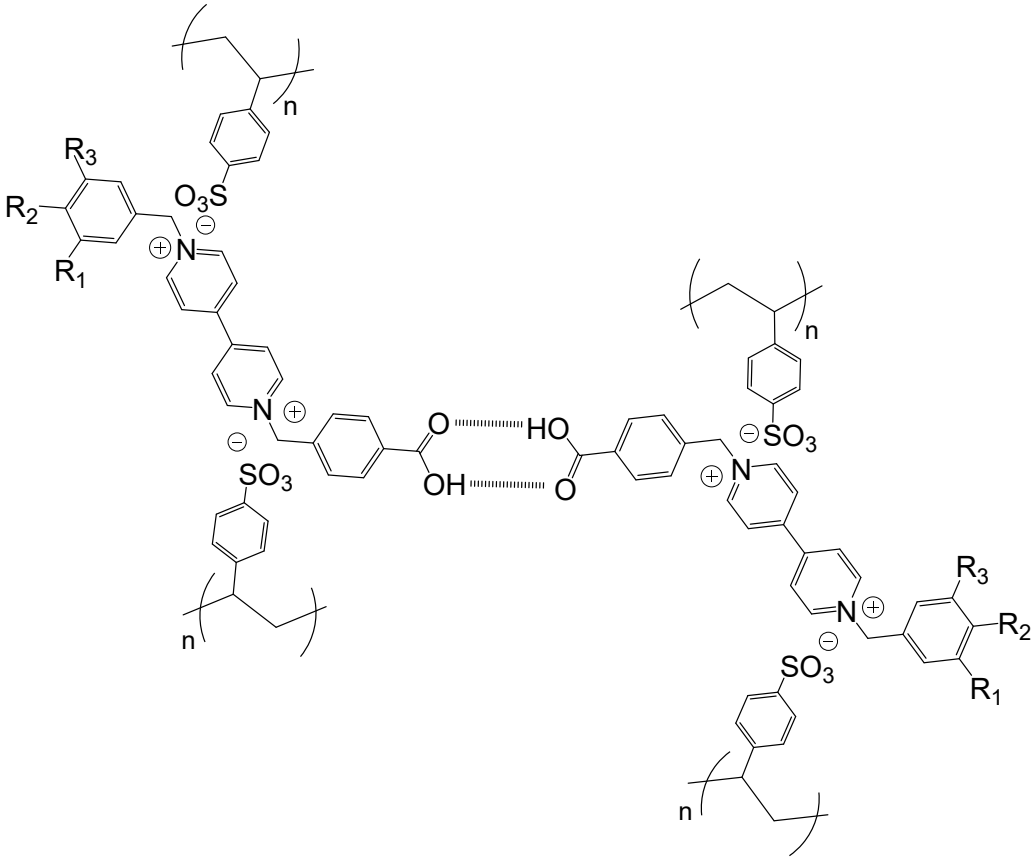


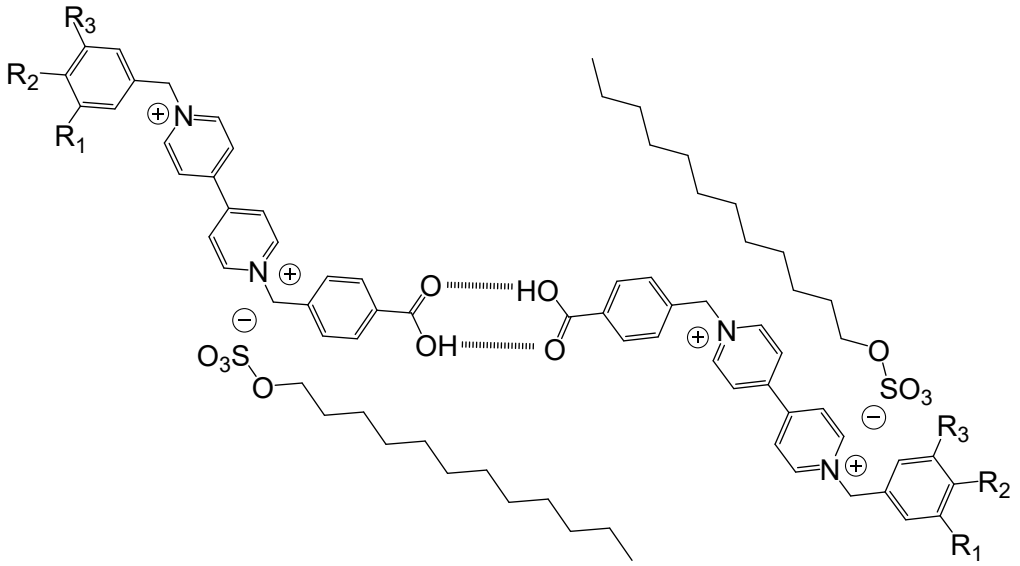
Figure S29. UV-visible spectra of the interaction between STPB ⊂ 4 eq. β-CD - BV1. [BV1] = [STPB ⊂ 4 eq. β-CD] = 50 μM with respect to STPB at 25°C.

CT aggregates	Critical aggregation concentration
BV1- PSS	1 mM – 0.5 mM
BV2- PSS	1 mM – 0.3 mM
BV3 ⊂3 eq. β-CD- PSS	0.1 mM – 0.1 mM
BV1 - SDS	100 μM – 75 μM
BV2 - SDS	50 μM – 25 μM
BV2 ⊂ 1 eq. β-CD-SDS	50 μM – 25 μM
BV2 ⊂ 2 eq. β-CD-SDS	50 μM – 35 μM
BV2 ⊂ 2.5 eq. β-CD-SDS	50 μM – 35 μM
BV3 ⊂ 3 eq. β-CD-SDS	10 μM – 10 μM

Table 4: Critical aggregation concentration of BV-PSS and BV-SDS (measured by UV-visible spectroscopy by monitoring change in absorption of viologen at 300 nm by adding aqueous solution of polystyrenesulfonate (PSS) and sodium dodecyl sulphate (SDS)).



Scheme S4. Plausible mechanism for the pH responsive aggregation of BV viologens - Polystyrene sulphonate. ($R_1 = R_2 = R_3 = H$ for BV1, $R_1 = R_3 = H$ and $R_2 = C_6H_5CH_2O$- for BV2 and $R_1 = R_2 = R_3 = C_6H_5CH_2O$- for BV3).



Scheme S5. Plausible mechanism for the pH responsive aggregation of BV viologens – Sodium dodecyl sulphate. ($R_1 = R_2 = R_3 = H$ for BV1, $R_1 = R_3 = H$ and $R_2 = C_6H_5CH_2O$- for BV2 and $R_1 = R_2 = R_3 = C_6H_5CH_2O$- for BV3).