

Electronic Supplementary Information

**Self-assembly of a Benzothiazolone Conjugate to Panchromatic
Fluorescent Fibres and its Application in Cellular Imaging**

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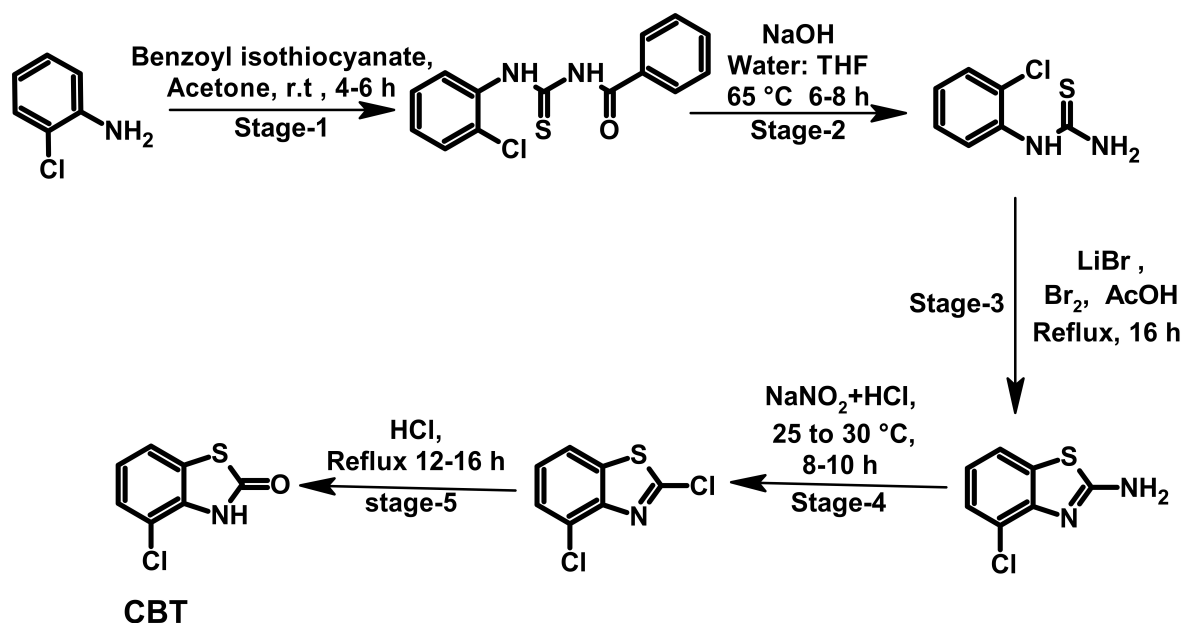
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Material and methods:

All the starting materials for the synthesis of **CBT** were obtained from the commercial suppliers and used as received. 2-chloroaniline and benzoyl isothiocyanate were purchased from Spectrochem, India. Acetone, THF, sodium sulphate was purchased from Sisco Research Laboratories (SRL), India. Moisture-sensitive reactions were performed under an atmosphere of dry nitrogen. All the solvents used for the reactions were distilled prior to use. The R_f was recorded in Analytical TLC Silica Gel 60F₂₅₄ purchased from Merck (Germany). ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on an Avance III 400 NMR spectrometer. Proton chemical shifts are reported in parts per million. HPLC was done using the Waters E2695 machine. HPLC was performed using 0.1% OPA (Orthophosphoric acid in water Mobile phase: 40% ACN:60% (ABC) 20 minutes run time and water: ACN as mobile phase symmetry C-18 column (250 mmX4.6 u): 5 mm diameter Flow rate: 0.8 ml/min Isocratic. UV-visible spectra were recorded on a Shimadzu UV-Visible spectrophotometer 1900 with 10 mm quartz cell at 25 °C. While, the fluorescence spectra were recorded on Jasco spectrofluorometer FP 8300 using 5 mm quartz cell at 25 °C.

Synthesis of CBT:

Synthesis of CBT has been carried out via the previously reported methodology.^{1,2}



Scheme S1: Synthesis scheme of CBT

Synthesis of CBT Stage-1: In a three-necked round bottom flask-fitted with a dropping funnel filled with 120 mL of dry acetone. 10.0 g (7.8 mmol) of 2-chloroaniline was placed followed by dropwise addition of the dry acetone under the nitrogen atmosphere during the constant stirring of the reaction mixture. Next, 14.0 g (8.6 mmol) of benzoyl isothiocyanate was added and the reaction mixture was then allowed to stir for another 4 to 6 hours at room temperature. The progress of the reaction was monitored by analytical TLC by using the ethyl acetate-hexane (3:7) solvent mixture as a mobile phase. After the completion of the reaction, the reaction mixture was then poured carefully with the stirring into 500 mL of cold water and the resulting yellow precipitate of N-((2-chlorophenyl)carbamothioyl)benzamide is separated by suction filtration followed by the washing of precipitate with water (3x200 mL).

The solid wet cake was further washed with the cooled water under reduced pressure, subsequently the wet cake transfers to a 1 litre round bottom flask and dried under reduced pressure which yielded the desired product (22.0 g, 7.5 mmol, Yields 97%) as solid, off-white material. R_f 0.53. ^1H NMR (400 MHz, $\text{DMSO-}d_6$, 25°C , TMS) $\delta(\text{ppm}) = 12.88$ (s, 1H), 11.95 (s, 1H), 8.06-7.95 (m, 3H), 7.70-7.66 (t, $J=8.00$ Hz, 1H), 7.61-7.54 (m, 3H), 7.45-7.29 (m, 2H).

Synthesis of CBT Stage-2: To the 100 mL of THF solvent a 20 gm CBT Stage-1 in two neck round bottom flask was added, 100 mL of an aqueous solution of NaOH (4.0 eq.) was added dropwise during the course of 20 minutes. The reaction mixture was then allowed to heat at 60°C for 4 to 8 hours. The progress of the reaction was monitored by analytical TLC by using ethyl acetate: hexane (8:2) solvent mixture. After the completion of the reaction, the mixture was concentrated under reduced pressure and the residue was re-dissolved in ethyl acetate followed by extractions and washing with brine. To the combined organic layer, anhydrous sodium sulphate was added. The organic layer was then filtered and the solvent was evaporated under reduced pressure to give CBT Stage-2 (12.5 g, Yields 88%). The compound was then characterized by ^1H -NMR and used in the next step without any further purification. ^1H NMR (400 MHz, $\text{DMSO-}d_6$, 25°C , TMS) $\delta(\text{ppm})= 9.43$ (s, 1H), 7.66-7.64 (dd, $J=4.0$ Hz, $J=10.0$ Hz, 1H), 7.50-7.48 (dd, $J=2.4$ Hz, $J=8.8$ Hz, 1H), 7.34-7.29 (dt, $J=2.0$ Hz, $J=8.00$ Hz, 1H), 7.25-7.21 (dt, $J=4.0$ Hz, $J=8.00$, 1H).

Synthesis of CBT Stage-3: CBT Stage-2 (10.0 g, 53 mM, 1.0 eq.) was dissolved in acetic acid and lithium bromide (4.7 g, 111 mM, 2.1 eq.) was added at room temperature. The reaction mixture was then stirred for 45 minutes at room temperature. Further, bromine (Br_2) (6.85 g, 42 mM, 0.8 eq.) was added at room temperature and the reaction mixture was refluxed at 60°C for 10-12 hours. TLC observation in 50% ethyl acetate: hexane showed the starting material was consumed and the product was formed. The reaction mixture was

allowed to cool and distilled under the reduced pressure and remove the acetic acid, the solid residue was observed, added the ice-cooled water and stirred well. Followed by filtered the reaction mass and washed with cooled water, and dried till the removal excess of water. Isolate the wet cake and dry it at 60 °C in the oven. The crude product was isolated (8.3 g, 44 mM, Yields 83%), the crude product has been used in the next step without any further purification. ¹H-NMR (400 MHz, DMSO-d₆ 25 °C, TMS: 7.89 (s, 2H), 7.63 (dd, J=8.1 Hz, 1.3 Hz, 1H), 7.29 (dd, J=8.1 Hz, 1.3 Hz, 1H), 7.00 (t, J=8.1, 1H).

Synthesis of CBT-Stage-4: CBT stage-3 (8.0 g, 43 mM, 1.0 eq.) was dissolved in 36% concentrated HCl, stirred for 30 minutes at room temperature till the solution become clean, the reaction mixture was then allowed to cool at 15 to 20 °C and subsequently 40% aqueous solution of NaNO₂ was added by using the addition funnel and maintaining the temperature between 20 to 30 °C. This reaction mixture was stirred for 10 to 12 hours by maintaining the temperature in the range of 20 to 30 °C. The reaction was monitored by TLC in 50% ethyl acetate: hexane which revealed the starting material has been consumed and the product formation was observed. The reaction mixture was allowed to cool at 15 °C followed by the addition of 18% aqueous solution of urea such that exothermic reaction and foam formation is controlled. The reaction mixture was then checked by using a starch paper, it did not show any blue colour which indicating excess nitrous acid (HNO₂) is eliminated. Again, the reaction mixture was cooled at 0 to 5 °C and stirred for 1 hour and the reaction mixture is filtered by maintaining the temperature and washed with cooled water which yielded the orange colour wet cake. Further, the wet cake was dried under reduced pressure which yielded orange colour crude compound, which is used in the next step without further purification (7.8 g, 42 mM, Yields 88.2% ¹H-NMR (400 MHz, DMSO-d₆, 25°C, TMS) δ(ppm) = 8.11 (d, J = 8.0 Hz, 1H), 7.68 (d, J= 8.0 Hz, 1H), 7.52 (t, 1H). ¹³C-NMR, 400 MHz, DMSO-d₆, 25 °C) 154.98, 147.67, 137.82, 127.90, 122.09.

Synthesis of CBT-Stage-5: CBT Stage-4 crude (7.0 g, 37 mM, 1.0 eq.) was dissolved in 36% concentrated HCl and heat it at temperature 110 to 115 °C for 10 to 12 hours on an oil bath with a water condenser. The reaction is monitored and it shows the formation of the small particles after hydrolysis. As the reaction proceeds, there is loss of HCl by evaporation, so we need to add excess 36% HCl to maintain the volume. The reaction is monitored by using a TLC system 50% ethyl acetate: hexane which revealed that the starting material was consumed. Subsequently, the heating was stopped and the reaction mixture is cooled at room temperature. After that, the reaction mixture is again cooled at 0 to 5 °C and filtered further wet solid precipitates. Take this into 250 mL single neck round bottom flasks and then dry the wet precipitate using rotavapor under reduced pressure which gives 6.5 gm crude material. This was further purified by the crystallization process in acetone. The crude material was dissolved in acetone and refluxed for 30 to 45 minutes and filtered at 60 °C, the solid wet material obtained was further dried using rota vapour under the reduced pressure to yield 5 gm final product of **CBT**, the purity of **CBT** has been confirmed by the HPLC purification method. Rf 0.38, ¹H NMR (400 MHz, DMSO-d₆, 25°C, TMS) δ(ppm) = 12.20 (s, 1H), 7.57 (dd, J = 8.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.16-7.12 (t, 3H), ¹³C-NMR, 400 MHz, DMSO-d₆, 25 °C) 177.27, 134.16, 126.96, 125.02, 124.09, 115.92, LC-Mass for was calculated 185.19 and found [M+H] +=186.19.

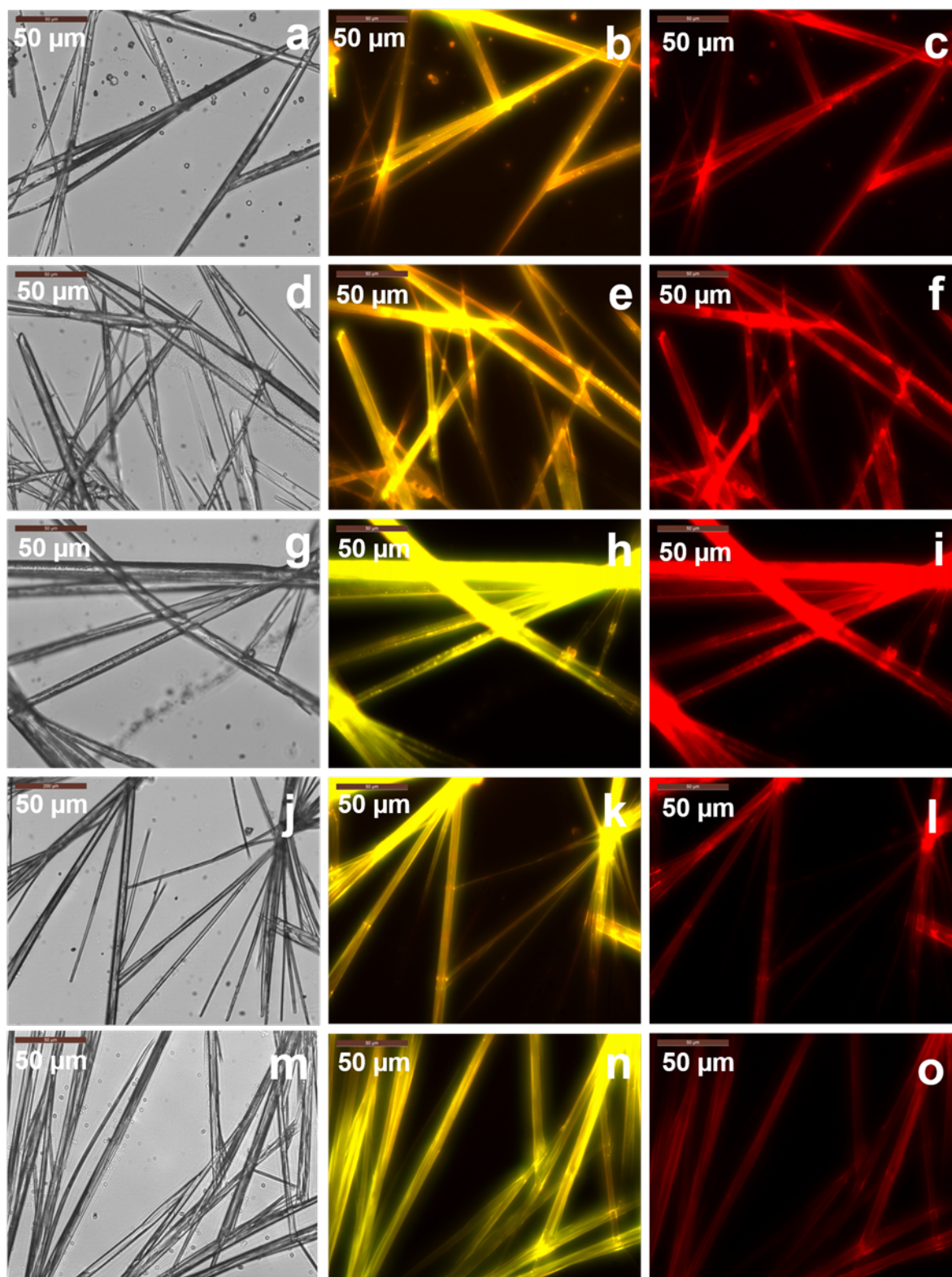


Fig. S1: Microscopy images of self-assembled structure formed by CBT in DMSO, (a) at 1 mM under bright field; (b) at 1 mM under green filter; (c) at 1 mM under red filter; (d) at 3 mM under bright field; (e) at 3 mM under green filter; (f) at 3 mM under red filter; (g) at 5 mM under bright field; (h) at 5 mM under green filter; (i) at 5 mM under red filter; (j) at 7

mM under bright field; (k) at 7 mM under green filter; (l) at 7 mM under red filter; (m) at 9 mM under bright field; (n) at 9 mM under green filter; (o) at 9 mM under red filter.

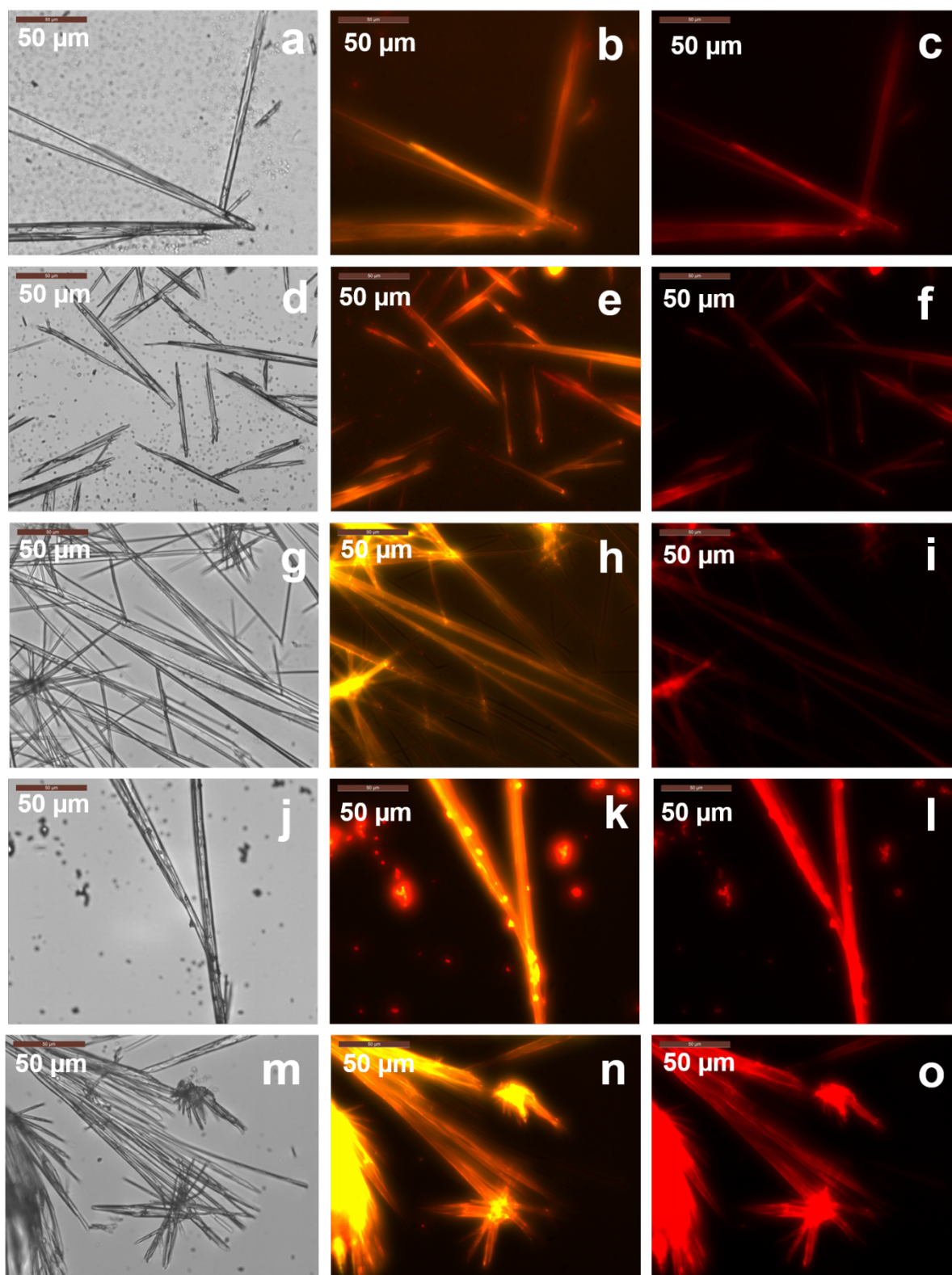


Fig. S2: Microscopy images of self-assembled structure formed by CBT in methanol, (a) at 1 mM under bright field; (b) at 1 mM under green filter; (c) at 1 mM under red filter; (d) at

3 mM under bright field; (e) at 3 mM under green filter; (f) at 3 mM under red filter; (g) at 5 mM under bright field; (h) at 5 mM under green filter; (i) at 5 mM under red filter; (j) at 7 mM under bright field; (k) at 7 mM under green filter; (l) at 7 mM under red filter; (m) at 9 mM under bright field; (n) at 9 mM under green filter; (o) at 9 mM under red filter.

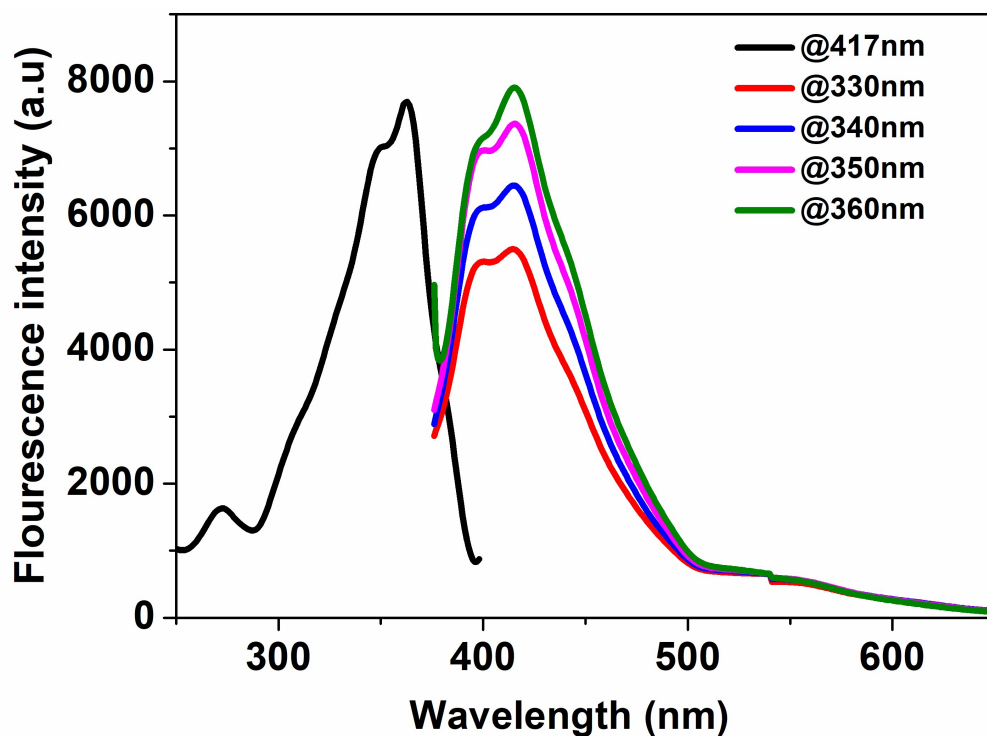


Fig. S3: Emission spectra of CBT under different excitation wavelengths (λ_{ex}): (red) 330 nm, (blue) 340 nm, (pink) 350 nm, (green) 360 nm, since at 360 nm emission spectra is the mirror image of excitation spectra real excitation of CBT is at 360 nm.

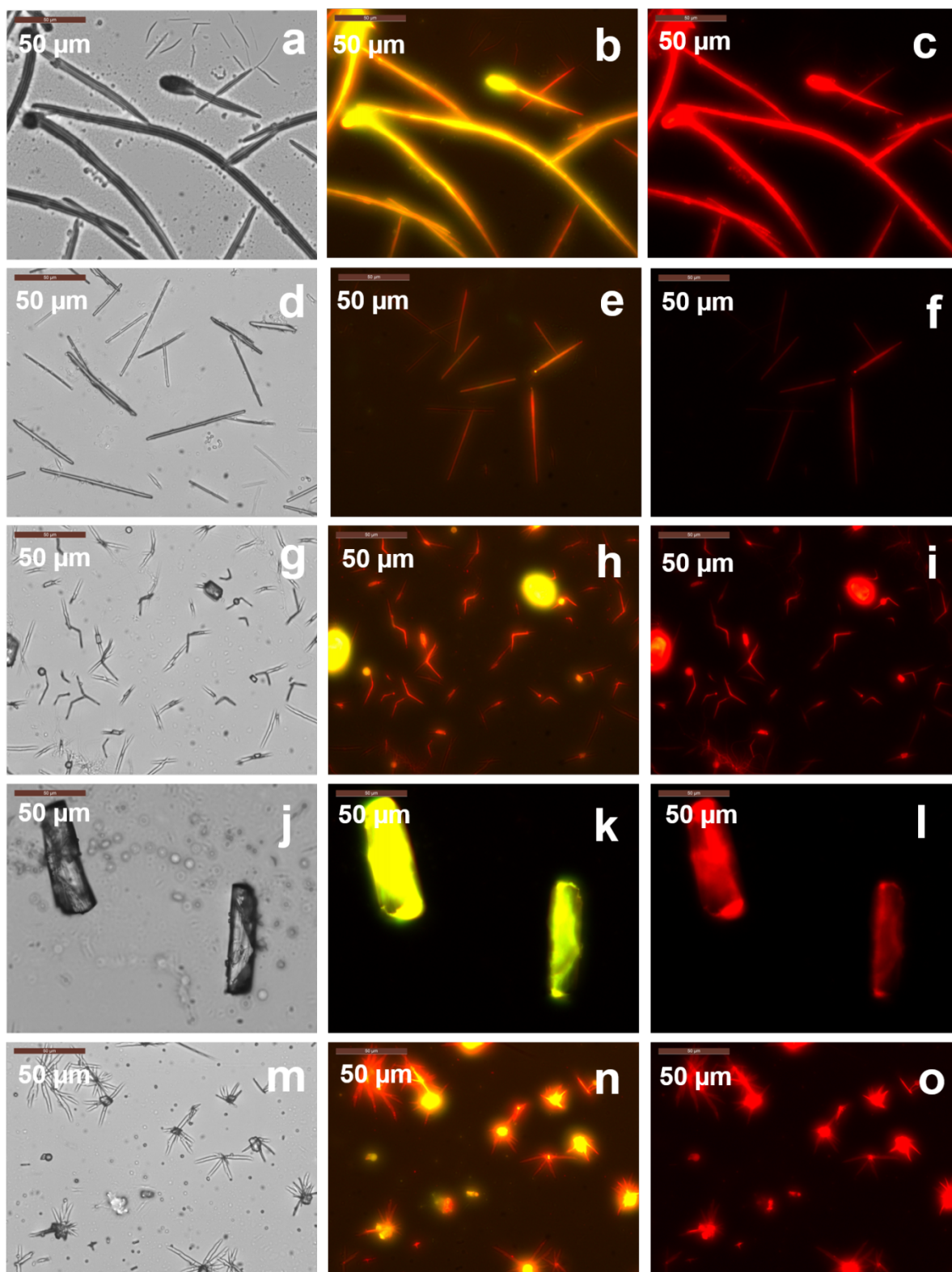


Fig. S4: Microscopy images of the self-assembled structure formed by CBT at increasing % of THF in water, (a) in 10% THF: water under bright field; (b) in 10% THF: water under green filter; (c) in 10% THF: water under red filter; (d) in 30% THF: water under bright field; (e) in 30% THF: water under green filter; (f) in 30% THF: water under red filter; (g) in 50%

THF: water under bright field (h) in 50% THF: water under green filter; (i) in 50% THF: water under red filter; (j) in 70% THF: water under bright field; (k) in 70% THF: water under green filter; (l) in 70% THF: water under red filter; (m) in 90% THF: water under bright field; (n) in 90% THF: water under green filter; (o) in 90% THF: water under red filter.

ATR-FTIR studies of CBT: Further, to understand the role of other non-covalent interaction forces such as hydrogen bonding, electrostatic interactions, and hydrophobic interactions in the self-assembled structures formed by **CBT** we resorted to ATR-FTIR studies. Hence, we recorded the ATR-FTIR spectra of **CBT** at 1, 3, 5, 7, and 9 mg/mL in DMSO. The study revealed that ATR-FTIR spectra of **CBT** at 1 mg/mL depicts -OH stretching vibration at 3458.01 cm^{-1} , while the C-H stretching vibrations were observed at 2995.96 cm^{-1} .

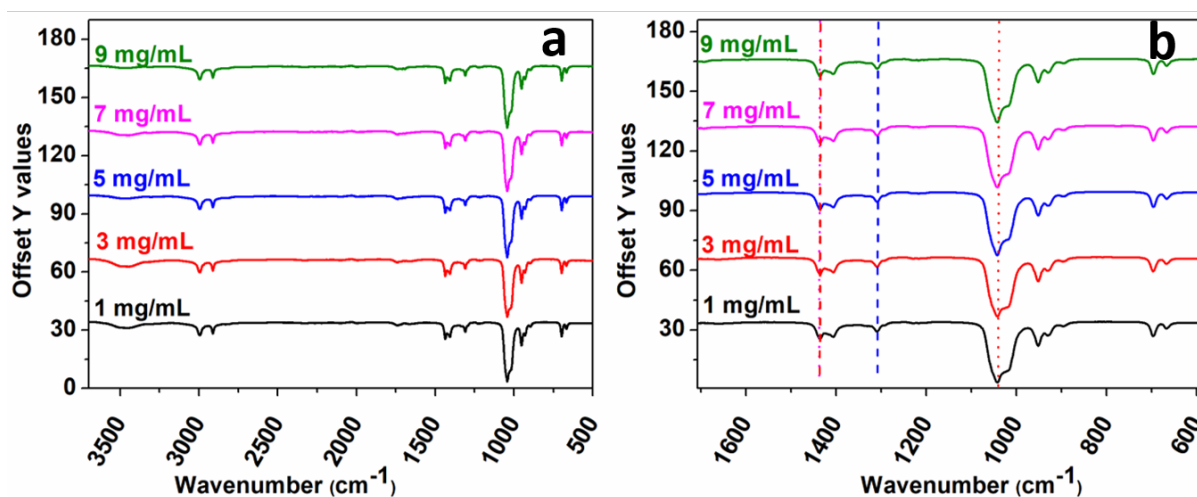


Fig. S5: ATR-FTIR spectra of **CBT** at 1, 3, 5, 7, and 9 mg/mL in DMSO (a) Full ATR-FTIR spectra of **CBT**, (b) expansion of ATR-FTIR spectra at $1600\text{-}600\text{ cm}^{-1}$.

The peak at 1738.50 cm^{-1} was attributed to $-\text{CO}$ stretching vibration, while the $-\text{NH}$ deformation peaks were observed at 1436.30 cm^{-1} and the $-\text{C-O}$ peak at 1042.61 cm^{-1} . However, when we recorded the ATR-FTIR spectra at 3, 5, 7, and 9 mg/mL in DMSO we could not observe any promising shifting (Fig. 5a, 5b). Since the ATR-FTIR spectra could not be recorded in liquid mode since the solvent dominated peaks, we need to do it by lyophilizing the solutions which we observed in self-assembly. Hence probably, because of this reason, prominent peak shift attributing to hydrogen bonding could not be seen.

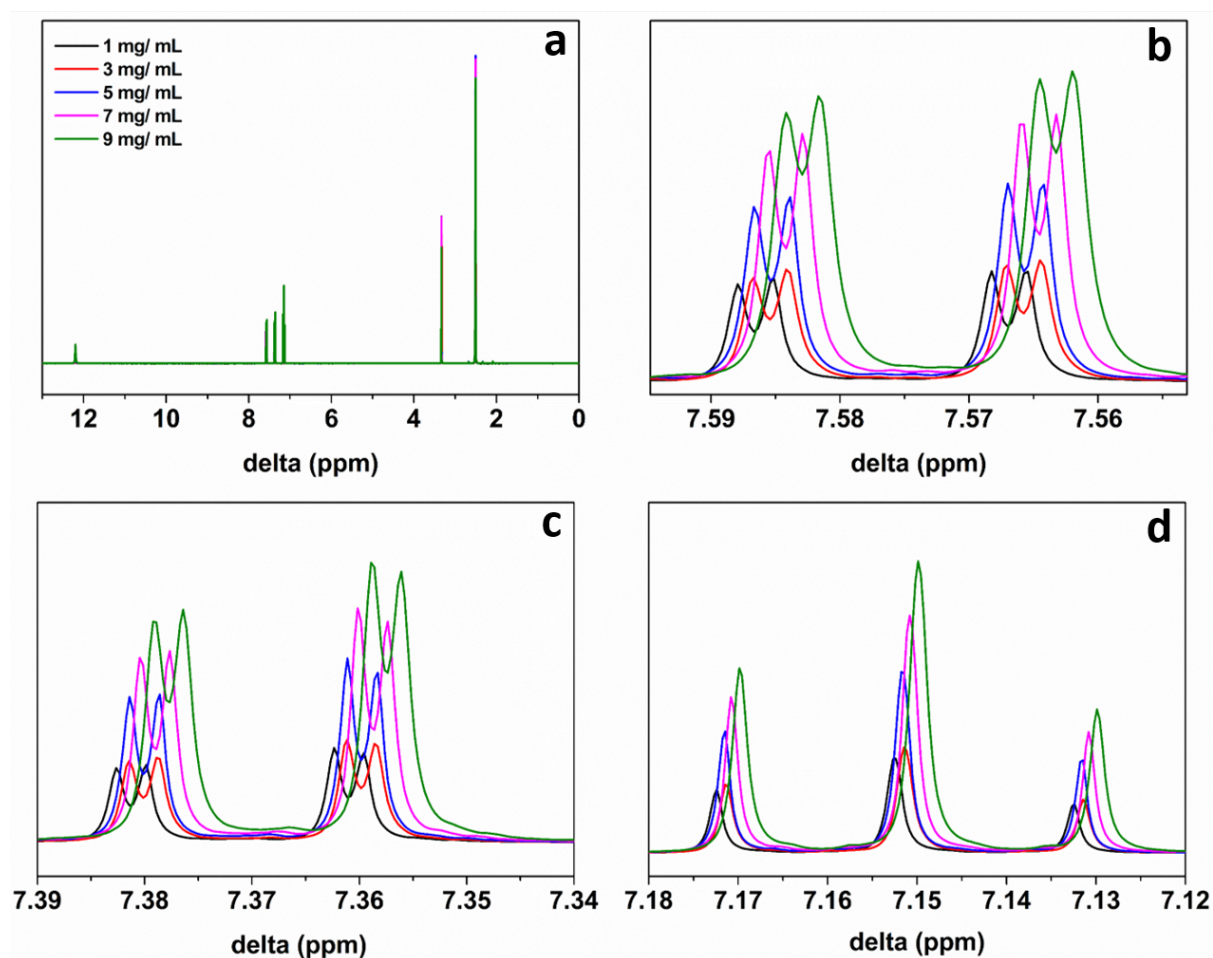


Fig. S6: $^1\text{H-NMR}$ spectra of CBT (a) Full spectra; (b) expansion spectra 7.58-7.55 ppm; (c) expansion 7.39-7.34 ppm; (d) expansion 7.18-7.12 ppm.

Supplementary Figures of characterization of CBT (NMR and HPLC):

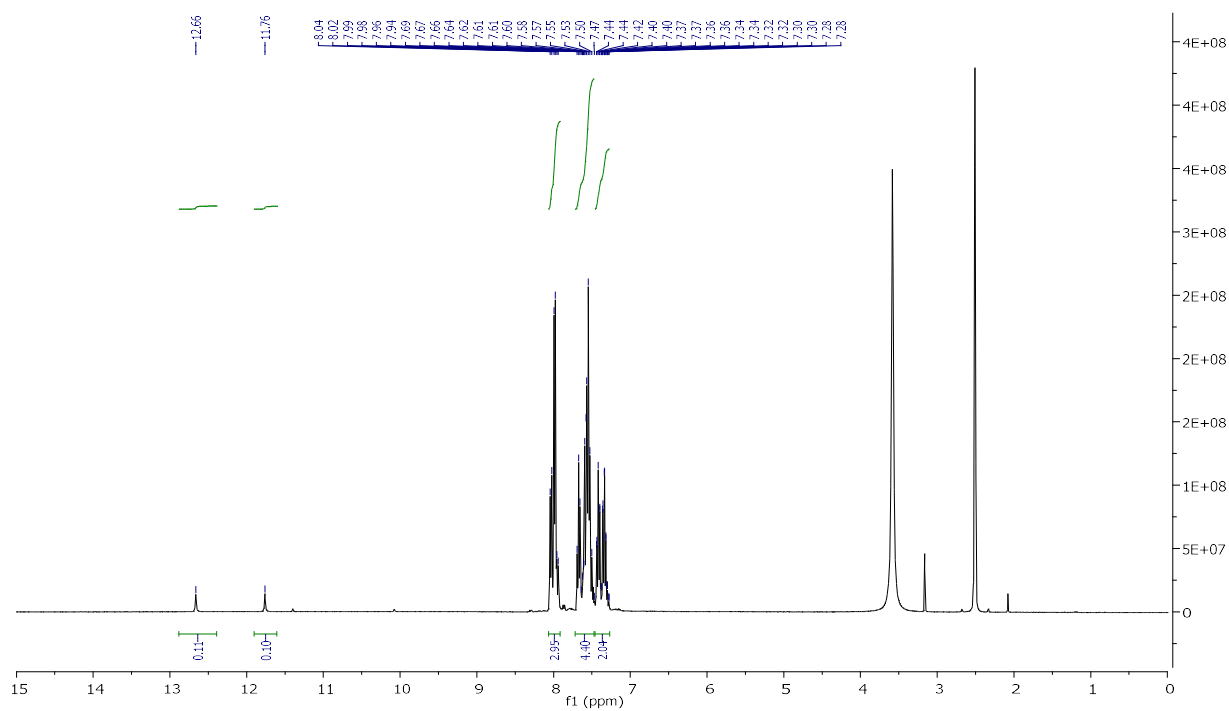


Fig. S7: ¹H-NMR spectra of CBT stage-1

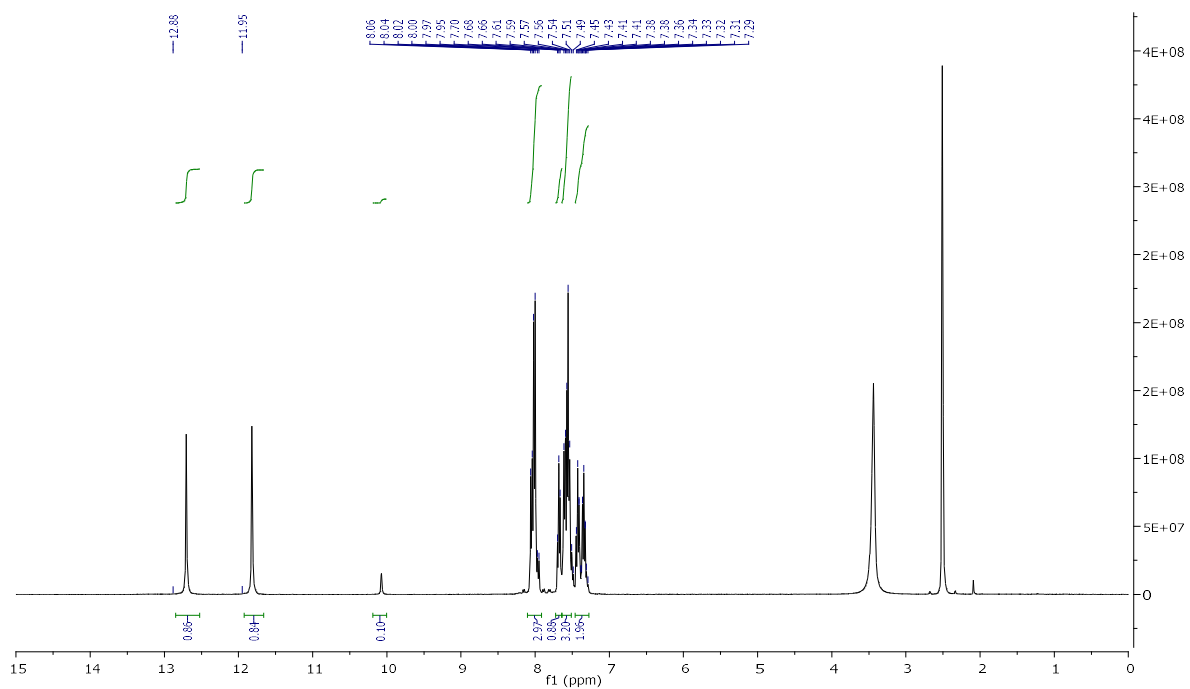


Fig. S8: D₂O exchange of CBT stage-1

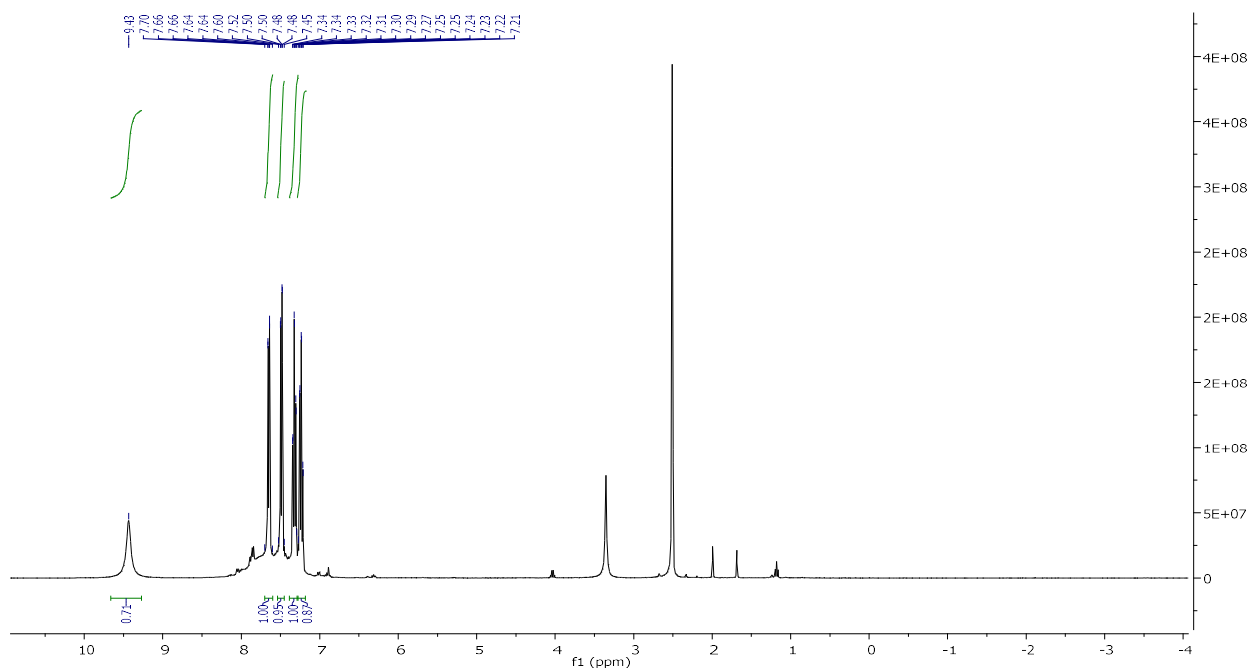


Fig. S9: $^1\text{H-NMR}$ spectra of CBT stage-2

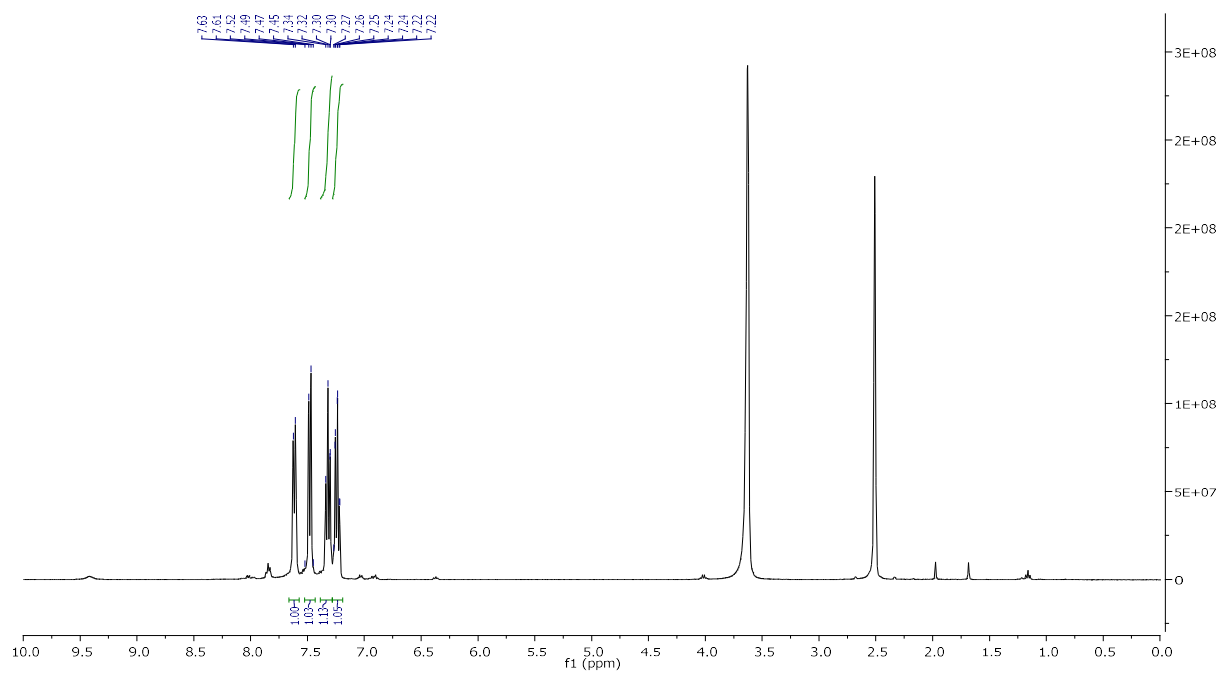


Fig. S10: D_2O exchange of CBT-stage-2

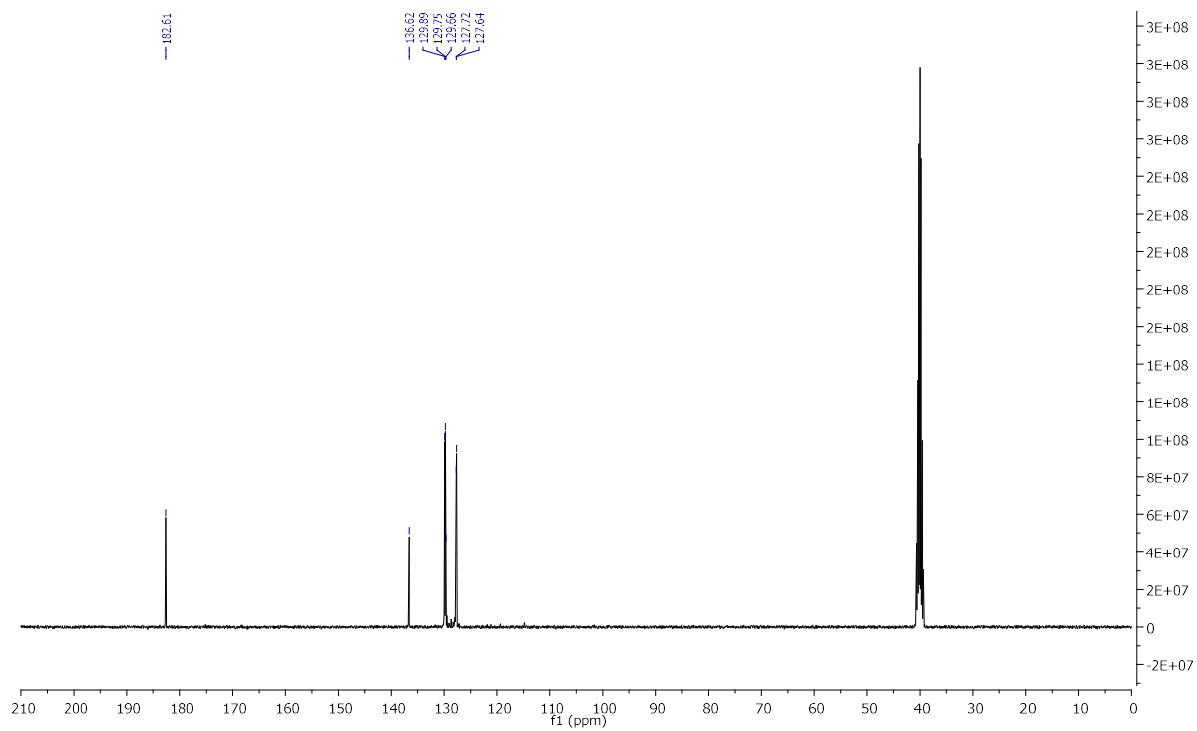


Fig. S11: ^{13}C -NMR spectra of CBT stage-2

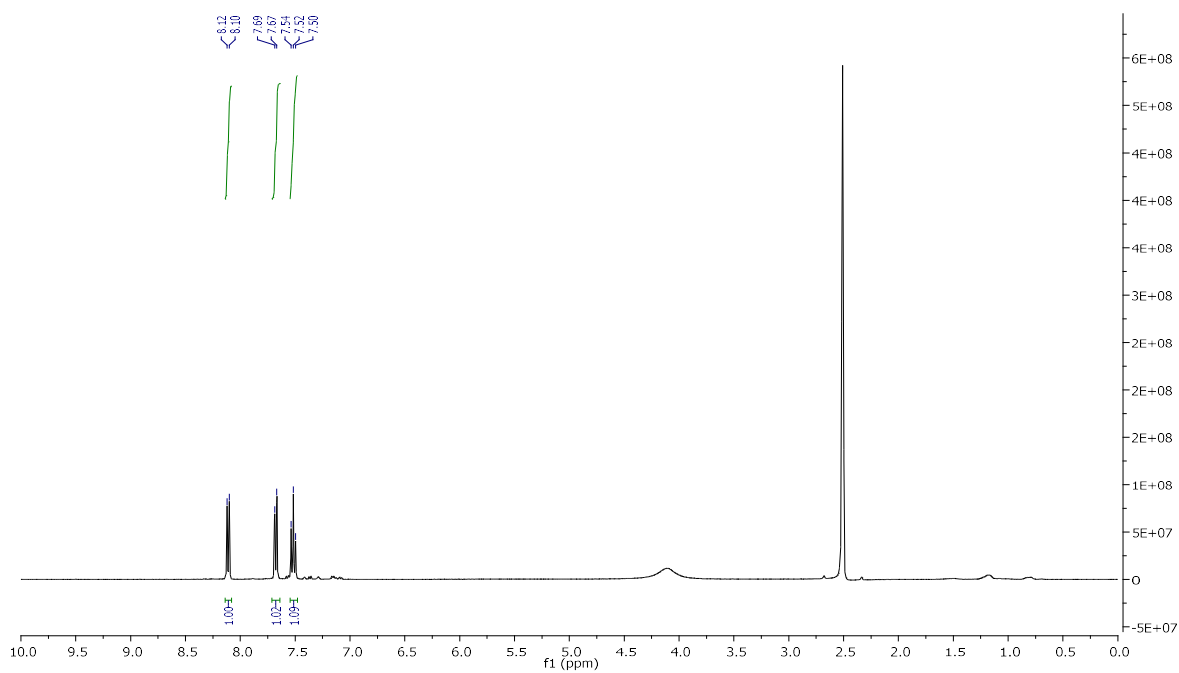


Fig. S12: ^1H -NMR spectra of CBT stage-4

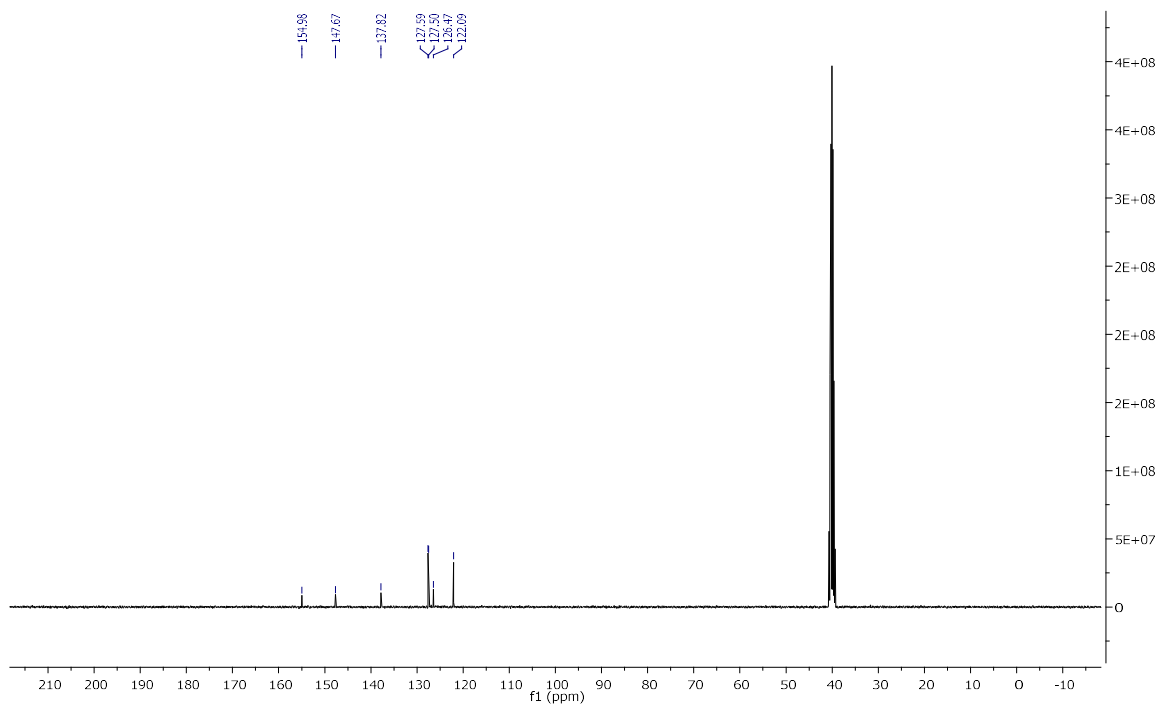


Fig. S13: ^{13}C NMR spectra of CBT Stage-4

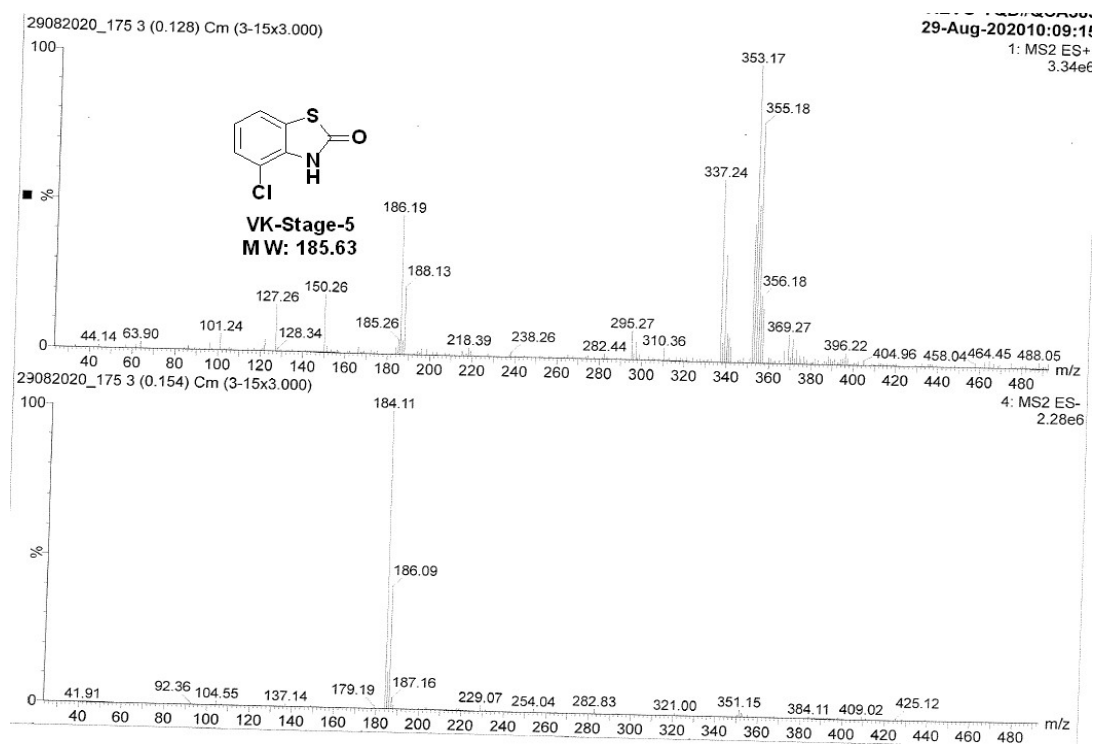
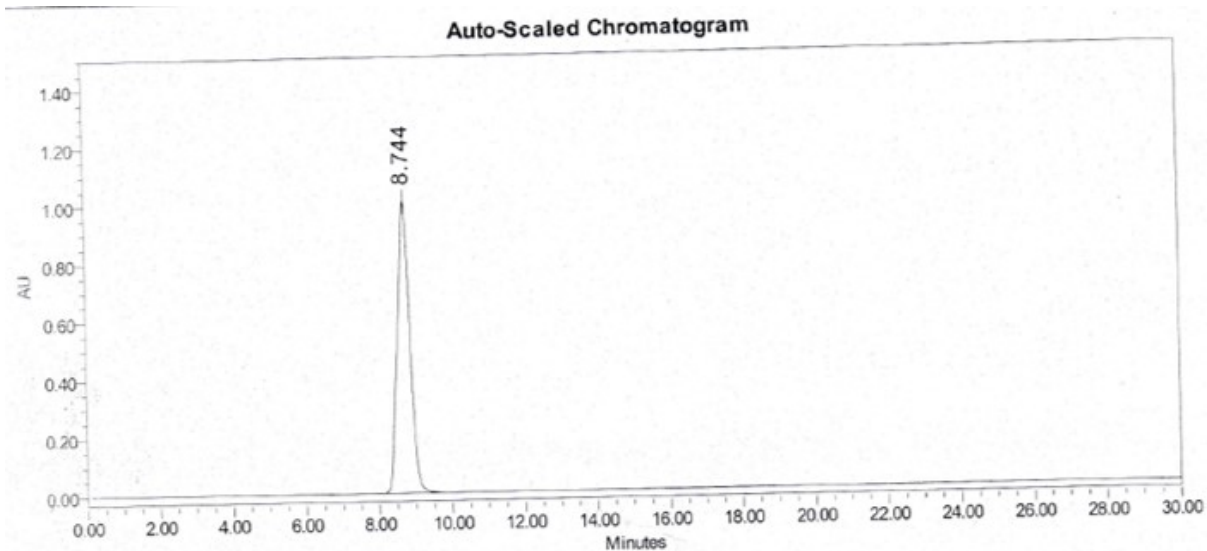


Fig. S14: Mass spectra of CBT in positive and negative mode



Peak Results

Name	RT	Area ($\mu\text{V}\cdot\text{sec}$)	% Area	RT Ratio
1	8.74	23735149	100.00	

Figure S15: HPLC Chromatogram of CBT

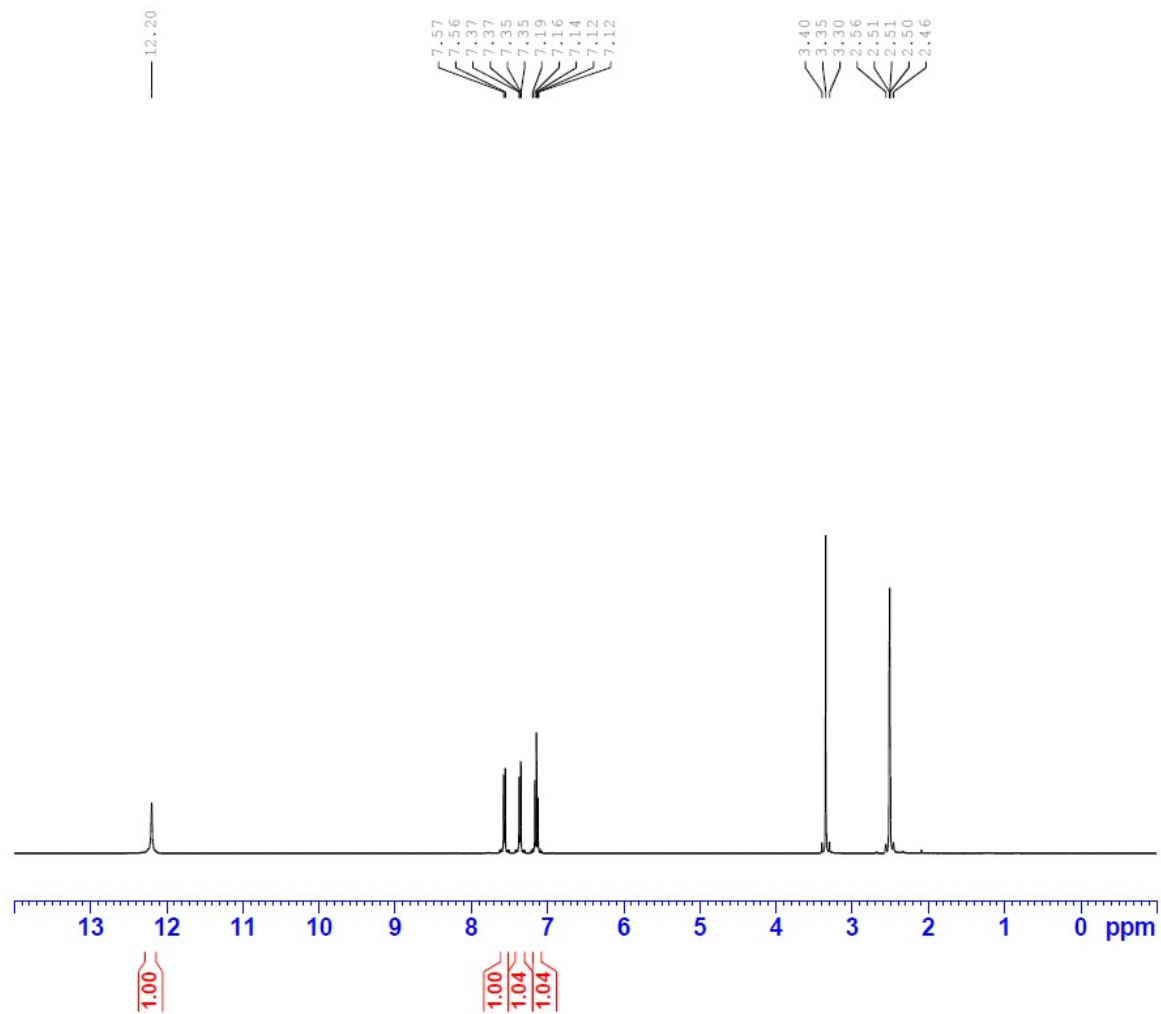


Fig. S16: $^1\text{H-NMR}$ spectra of **CBT**

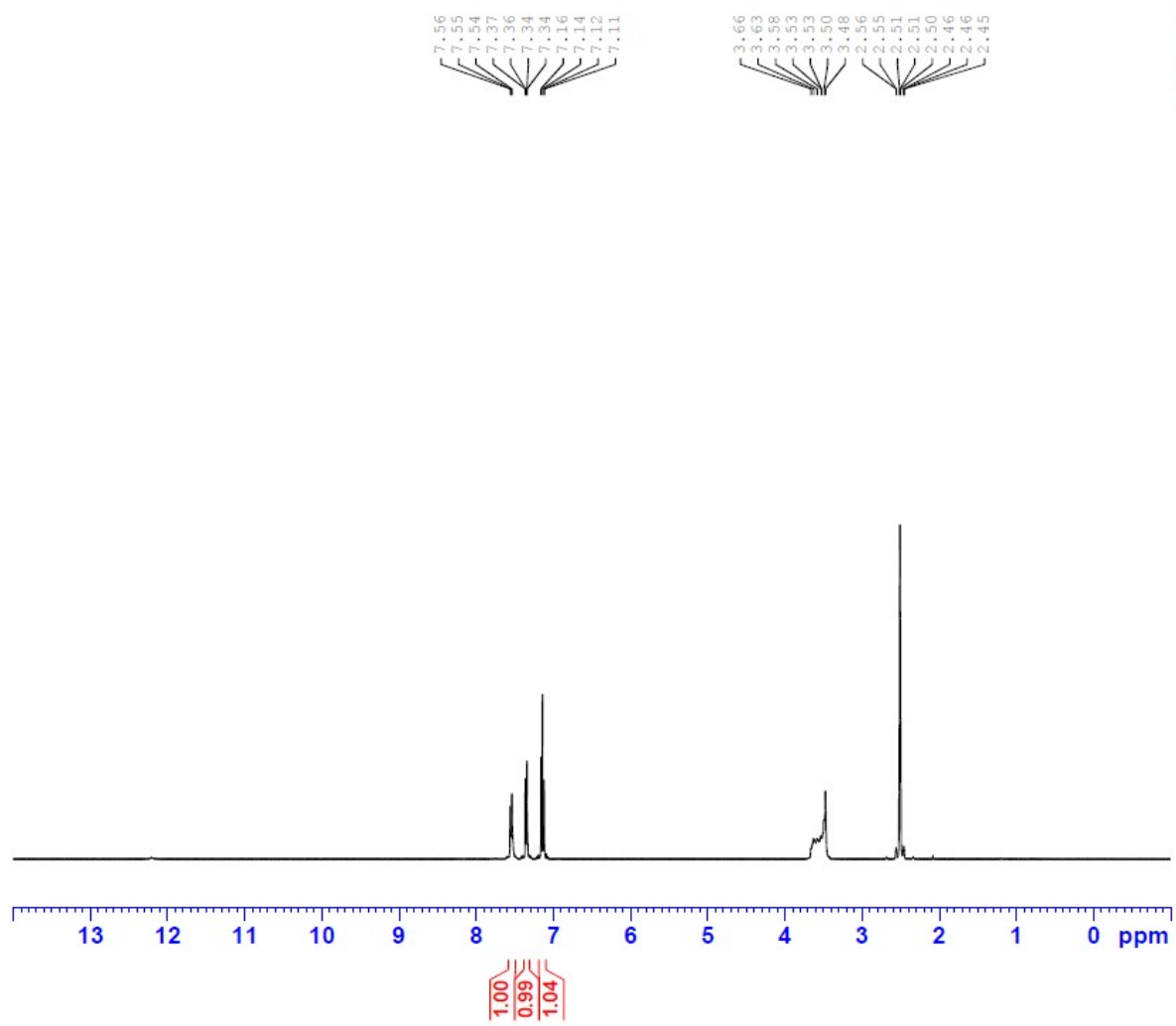


Fig. S17: D₂O exchange of CBT

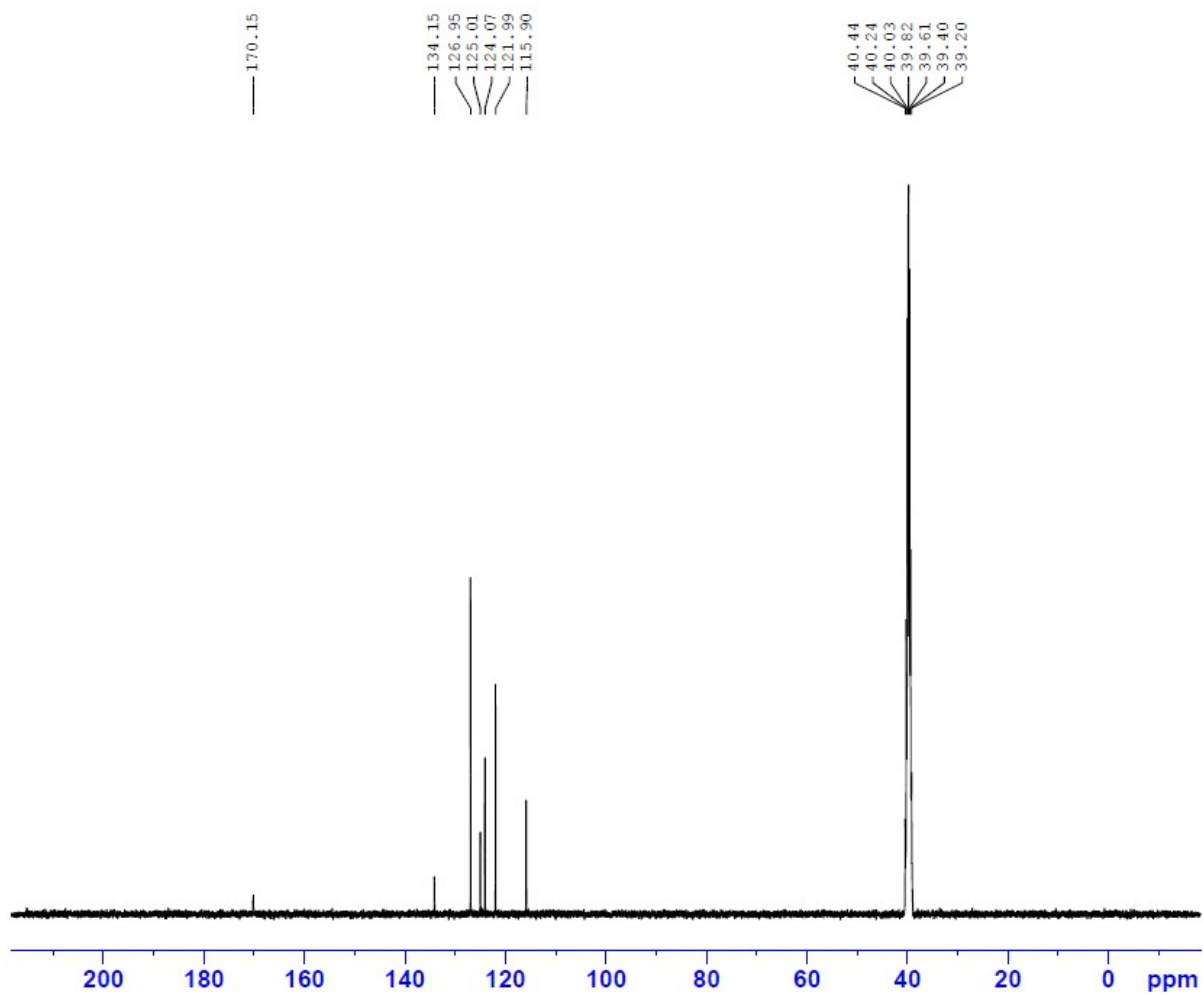


Fig. S18: ^{13}C NMR spectra of CBT

Reference:

1. M. Kakuno, S. Izawa, T. Takemoto, and Y. Tanabe, *Heterocycles.*, 2018, **96**, 1925-1939. 10.3987/COM-18-13984.
2. N. Gour, V. Kshtriya, S. Gupta, B. Koshti, R. Singh, D. Patel, and K. B. Joshi, *ACS Appl. Bio Mater.*, 2019, **2**, 4442-4455. <https://doi.org/10.1021/acsabm.9b00627>.