

Support information

Fluorescent Bio-nanocomposites Based on Chitosan Reinforced Hemicyanine Dye-Modified Montmorillonite

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Experimental

Unless stated otherwise, all reagents and solvents were purchased from commercial suppliers and were used without further purification. 2-methylbenzothiazole, Ethyl iodide, p/o-substituted benzaldehydes and solvents were obtained from Sigma Aldrich. The clay used in this study was a montmorillonite, purchased from "Southern clay". The cation-exchange capacity of this montmorillonite was 92 meq/100g. A high molecular weight (HMW) chitosan from crab shells (viscosity > 400 mPa.s, DDA = 75–85%) was purchased from Aldrich and used without further purification.

Fourier transform infrared spectra (FT-IR). The FT-IR spectra were recorded on an ABB Bomem FTLA 2000–102 FTIR instrument. (ATR: SPECAC GOLDEN GATE). The spectra were obtained with an accumulation of 16 scans and with a resolution of 4 cm⁻¹. The FT-IR of the used organo-modified montmorillonite was carried out to show the main characteristic peaks before and after use as reinforcement in the chitosan matrix. **X-Ray Diffraction (XRD).** The crystalline structure and the morphology of organo-modified montmorillonite were determined using a Bruker D8 Discover diffractometer equipped with CuK α radiation, and a GADDS detector. The angular range (2 θ) was scanned from 2 to 10° at a step size of 0.02°, the working voltage and current were 45 kV and 100 mA. **Thermogravimetric analysis (TGA).** The thermal decomposition of dyes and organoclay were evaluated by thermogravimetric analysis (TGA), using a Q500 instrument from TA Instruments. Samples were heated under air to 800 °C at a rate of 10 °C/min to yield the decomposition temperature, mass loss and maximum decomposition peak. **Surface hydrophobicity.** The water contact angle (CA) was measured using an optical video contact angle instrument (OCA 40, Dataphysics, Germany) at ambient temperature. The CA was determined 60 s after a water droplet of 5 μ l was placed on the surface of the film using a micro syringe. The automatic contact CA values were taken from five measurements at different positions of the same sample. **Fluorescence characterization.** Fluorescence spectrofluorometer (HORIBA Jobin-Yvon, NanoLog FL3- 2iHR) with a 450 W xenon lamp was used to obtain fluorescence spectra. The Fluorescence images were obtained using Leica microscope. ¹H and ¹³C NMR spectra were recorded in DMSO-d₆ solution with TMS as an internal reference using an Avance 300 (Bruker) instrument, chemical shifts (δ are given in ppm downfield from TMS. Multiplicities of ¹³C NMR resources were assigned by distortion less enhancement by polarization transfer (DEPT) experiments. Accurate mass measurement ESI-MS spectrum for compound was acquired in the positive ion mode using a SYNAPT G2 HDMS quadrupole orthogonal time-of-flight (QqToF)-MS/MS mass spectrometer (WATERS). **Evaluation of Film solubility in water.** The solubility of the film was determined as the percentage of soluble matter to initial dry matter of film sample. The initial dry matter of samples (3x3 cm) was determined by drying films at 105°C for 24 h. The films were then immersed in 50 mL distilled water and then placed in a shaker incubator at 25 °C and stirred for 24 hours. Then, the samples were filtered through Whatman No. 1 filter paper. The papers containing any insolubilized film were dried at 105 °C for 24 h. The film solubility (%) was calculated using the following equation:

$$\text{Solubility in water (\%)} = (\text{intial dry weight} - \text{final dry weight}) \times 100 / \text{Intial dry weight}$$

Synthesis of 3-ethyl-2-(p/o-substituted styryl)benzothiazol-3-ium iodide.

The synthesis of hemicyanine dyes have been performed in two steps. First, 3-ethyl-2-methyl-1,3-benzothiazol-3-ium iodide was prepared as follows: A solution of 2-methylbenzothiazole (2.86 ml, 22.45 mmol) and iodoethane (6.44 ml, 80.15 mmol) in DMF (5 ml). The mixture was heated under reflux to 90°C for 5 hours. After cooling, the desired salt collected by filtration under reduced pressure and washed several times with diethyl ether and ethanol. Drying under vacuum. The process was repeated 3 times to achieve a suitable yield as white crystal solid (3.69 g, 92%). The second step consists in melting 3-ethyl-2-methyl-1,3-benzothiazol-3-ium iodide (1 g, 5.61 mmol) and p/o-substituted benzaldehyde (16.83mmol). The mixture was stirred in an oil bath at 120-150°C for 5-7 hours. The progress of the reaction was monitored by TLC. After the reaction was complete, the mixture was slowly cooled which in most cases solidified or became a thick semi-solid mass. Further purification was recrystallization in methanol.

(E)-3-ethyl-2-(4-methoxystyryl)benzothiazol-3-ium iodide (HDa). Yield 94%; mp 250°C; IR (ATR): 2962, 2854, 1564, 1442, 1314, 762 cm⁻¹; ¹H NMR (300 MHz, DMSOd₆) δ 1.44(t, 3H, CH₃, ³J = 7.2 Hz), 3.85(s, 3H, OCH₃), 4.93(q, 2H, CH₂, ³J = 7.2 Hz), 7.86(d, 1H, =CH, J_{trans} = 15 Hz), 8.20(d, 1H, =CH, J_{trans} = 15 Hz), 7.08-8.41(m, 8H, CH_{Ar}); ¹³C NMR (75 MHz, DMSOd₆) δ 171.4, 162.7, 149.0, 140.6, 132.1, 129.2, 127.9, 127.7, 126.5, 124.1, 116.2, 114.5, 110.3, 55.4, 44.1, 40.1, 39.8, 39.5, 39.5, 39.2, 38.9, 38.7, 38.4, 13.9; HRMS-ESI(+): calculated for C₁₈H₁₈NOS⁺: 296.1104, found: 296.1104.

(E)-3-ethyl-2-(4-hydroxystyryl)benzothiazol-3-ium iodide (HDb).Yield 84%; mp 264 °C; IR (ATR): 3111, 2925, 2889, 1580, 1442, 1329, 1038, 746 cm⁻¹; ¹H NMR (300 MHz, DMSOd₆) δ 1.43(t, 3H, CH₃, ³J = 7.1 Hz), 4.90(q, 2H, CH₂, ³J = 7.1 Hz), 7.98(d, 1H, =CH, J_{trans} = 15.8 Hz), 8.24(d, 1H, =CH, J_{trans} = 15.8 Hz), 7.10-8.42(m, 8H, CH_{Ar}), 10.60(s, 1H, OH); ¹³C

NMR (75 MHz, DMSO_{d6}) δ 14.6, 44.7, 109.9, 116.7x2, 116.8, 124.8, 125.8, 128.3, 128.5, 129.8, 133.2x2, 141.3, 150.3, 162.6, 172.1; HRMS-ESI(+): calculated for C₁₇H₁₆NOS⁺: 282.0947, found: 282.0950.

(E)-3-ethyl-2-(4-chlorostyryl)benzothiazol-3-ium iodide (HDc). Yield 80%; mp 234°C; IR (ATR): 2974, 2898, 1586, 1498, 1323, 759 cm⁻¹; ¹H NMR (300 MHz, DMSO_{d6}) δ 1.46(t, 3H, CH₃, ³J = 7.2 Hz), 4.98(q, 2H, CH₂, ³J = 7.2 Hz), 8.05(d, 1H, =CH, J_{trans} = 15.8 Hz), 8.23(d, 1H, =CH, J_{trans} = 15.8 Hz), 7.59-8.48(m, 8H, CH_{Ar}); ¹³C NMR (75 MHz, DMSO_{d6}) δ 171.8, 147.9, 141.4, 137.4, 133.3, 132.0, 130.2, 129.7, 129.1, 129.0, 125.0, 117.3, 114.6, 45.32, 40.8, 40.5, 40.3, 40.0, 39.7, 39.4, 39.1, 14.8; HRMS-ESI(+): calculated for C₁₇H₁₅ClNS⁺: 300.0608, found: 300.0647.

(E)-3-ethyl-2-(2-methoxystyryl)benzothiazol-3-ium iodide (HDd). Yield 83%; mp 220°C; IR (ATR) :2939, 2828, 1563, 1432, 1337, 1205, 751 cm⁻¹; ¹H NMR (300 MHz, DMSO_{d6}) δ 1.45(t, 3H, CH₃, ³J = 7.2 Hz), 3.94(s, 3H, OCH₃), 4.92(q, 2H, CH₂, ³J = 7.2 Hz), 7.98(d, 1H, =CH, J_{trans} = 15.8 Hz), 8.24(d, 1H, =CH, J_{trans} = 15.8 Hz), 7.10-8.42(m, 8H, CH_{Ar}); ¹³C NMR (75 MHz, DMSO) δ 172.3, 159.1, 143.5, 141.5, 135.0, 130.3, 130.1, 128.9, 128.6, 124.9, 122.5, 121.5, 117.2, 113.7, 112.7, 56.7, 45.1, 40.8, 40.5, 40.3, 40.0, 39.7, 39.4, 39.1, 14.6; HRMS-ESI(+): calculated for C₁₈H₁₈NOS⁺: 296.1104, found: 296.1156.

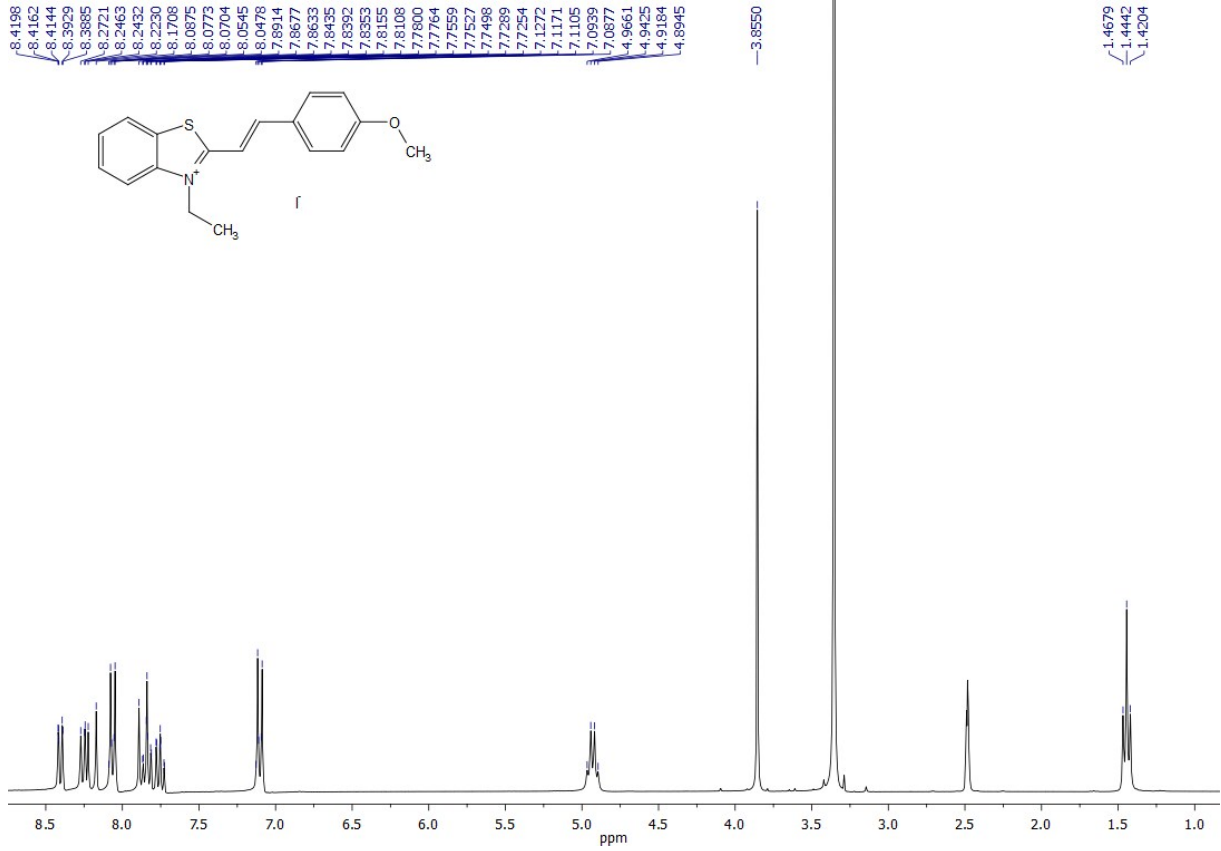
Montmorillonite-hemicyanine dye (MMT-HD) preparation.

In a two-neck round-bottom flask of 500 mL equipped with a condenser, 1.5 g of sodium-montmorillonite were dispersed in 300 ml of deionized water-ethanol (1:1) and vigorously stirred for 3h followed by sonication for at least 30 min. Sonication is necessary to homogenously disperse the MMT in the mixture. The suspension was heated at 80°C after which a solution of the corresponding dye (1.5 CEC) in ethanol (50ml) was added dropwise over 1hour. The stirring was continued for 24 hours at 80 °C. The organoclay (MMT-HD) was isolated by centrifugation, washed by a deionized water–ethanol (1:1) solution and then by ethanol (five times) to remove any excess dye and dried at room temperature. Next, the organoclay modified powder was ground in a mortar and sifted through 200-mesh sieve and then stored in a vacuum desiccator until use. The montmorillonite clay (MMT) modified with 3-ethyl-2-(p/o-substituted styryl)benzothiazol-3-ium iodide was named MMT-HDa-d.

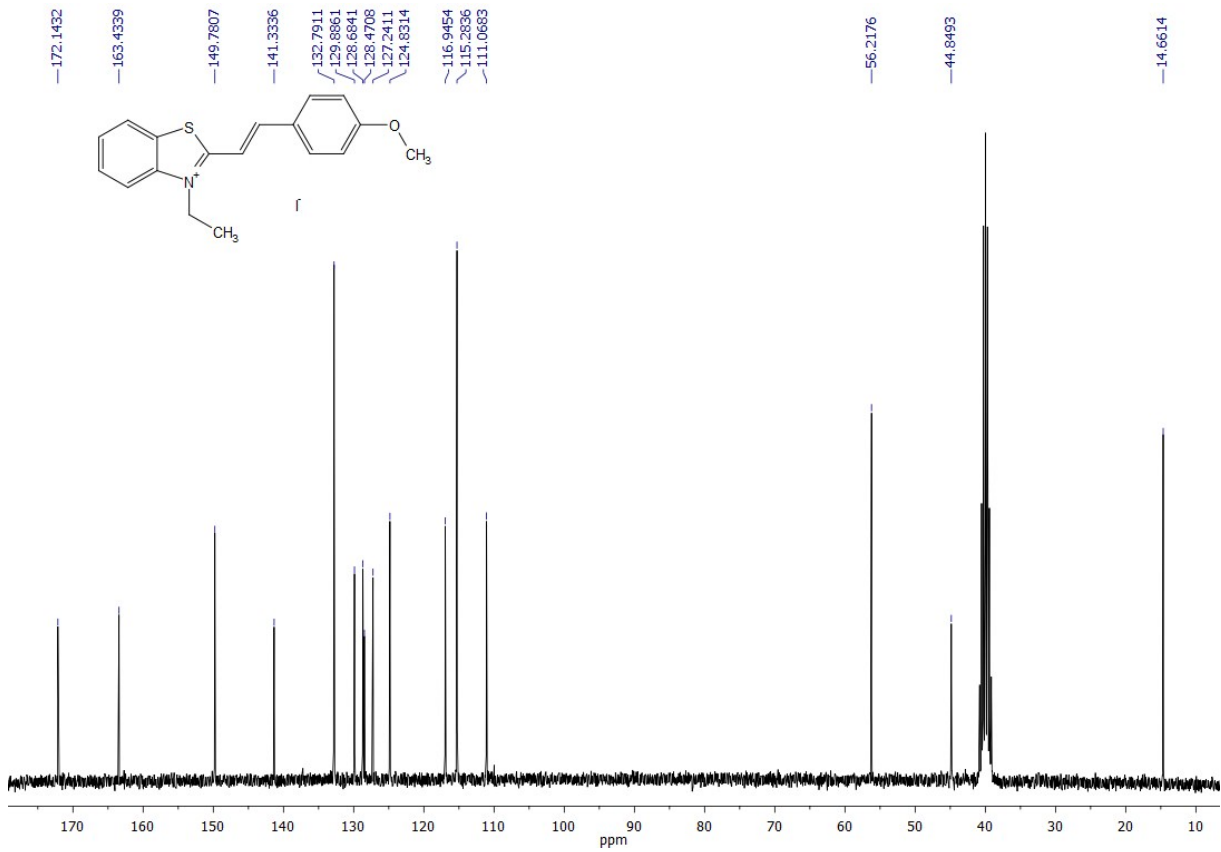
CS/MMT-hemicyanine dye bionanocomposites preparation.

Bionanocomposite samples in the form of films, whose thickness was about 70 μm, were produced using the solvent method with some modifications to obtain flexible and homogeneous films. The chitosan solution was prepared using 1% acetic acid solution. 0.5g chitosan was dissolved in 40 ml acetic acid solution (1%) and stirred at 50°C until fully dissolved. Afterward, the solutions were filtered with cheese cloth by vacuum aspiration to remove foam and any undissolved impurities. The pH of the polysaccharide solution was adjusted to ≈4.9 with acetic acid to avoid any structural alteration of the MMT-HD. The hemicyanine-modified montmorillonite at different weight percentage (1, 2, 5 and 10wt.%) were prepared by dispersing appropriate amounts of MMT-HDx into 10 ml of deionized water–ethanol (1:1) and vigorously stirred. After 1h, the suspension was treated with a high-performance dispersing instrument (T 25 digital ULTRA-TURRAX® - IKA) for 1 min and subsequently with an ultrasonic homogenizer for 4 min. The resulting dispersion of MMT-HD was added to the chitosan solution. The CS/MMT-HDx mixture was stirred continuously at 50 °C overnight and then sonicated for 30min to obtain a good dispersion of clay in chitosan. The solution was degassed to eliminate the air bulb. The applied method of homogenisation improved dispersion of the modified clay nano-particle within the polymer matrix. Then the solution was cast on a Petri dish and left to evaporate at room temperature. A film of pure chitosan was used as the reference sample. Hereafter, the bionanocomposite samples are designated as CS, CS/MMT-HDa-d.

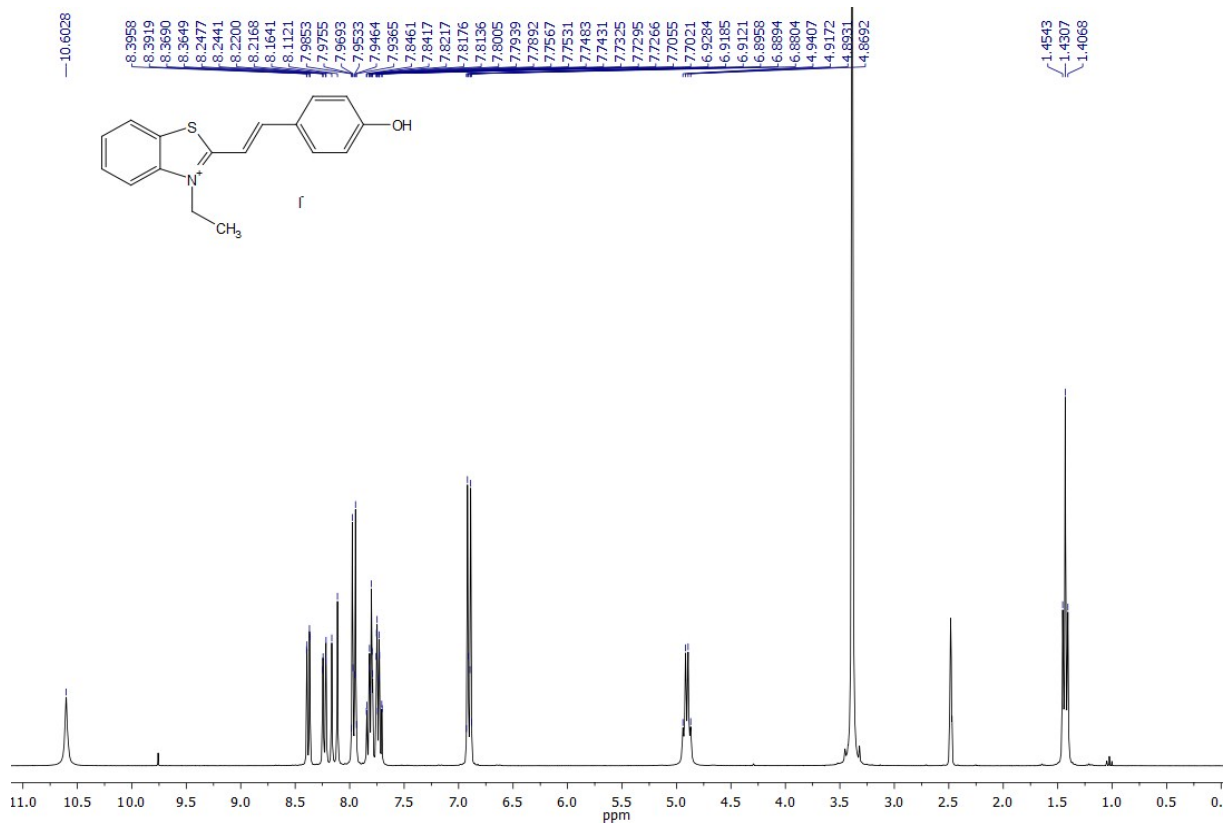
¹H NMR of HDa



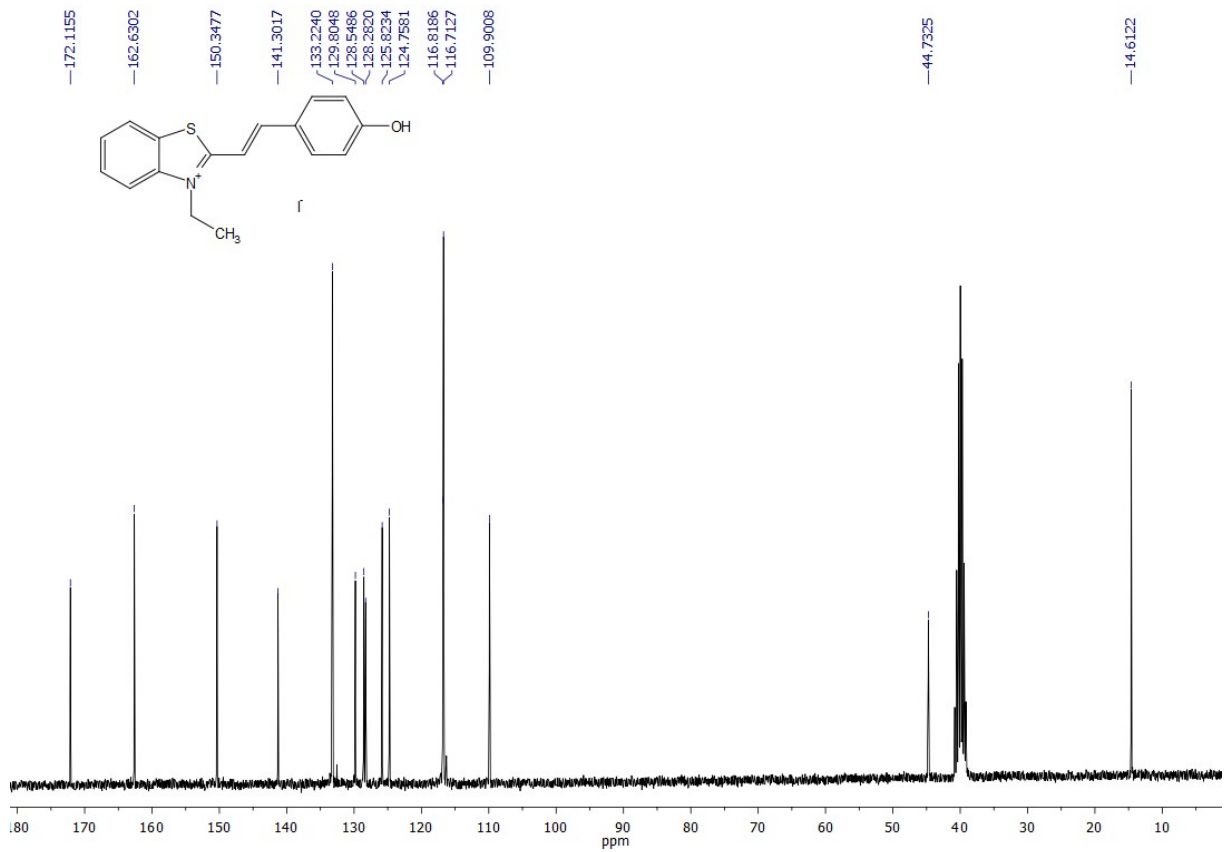
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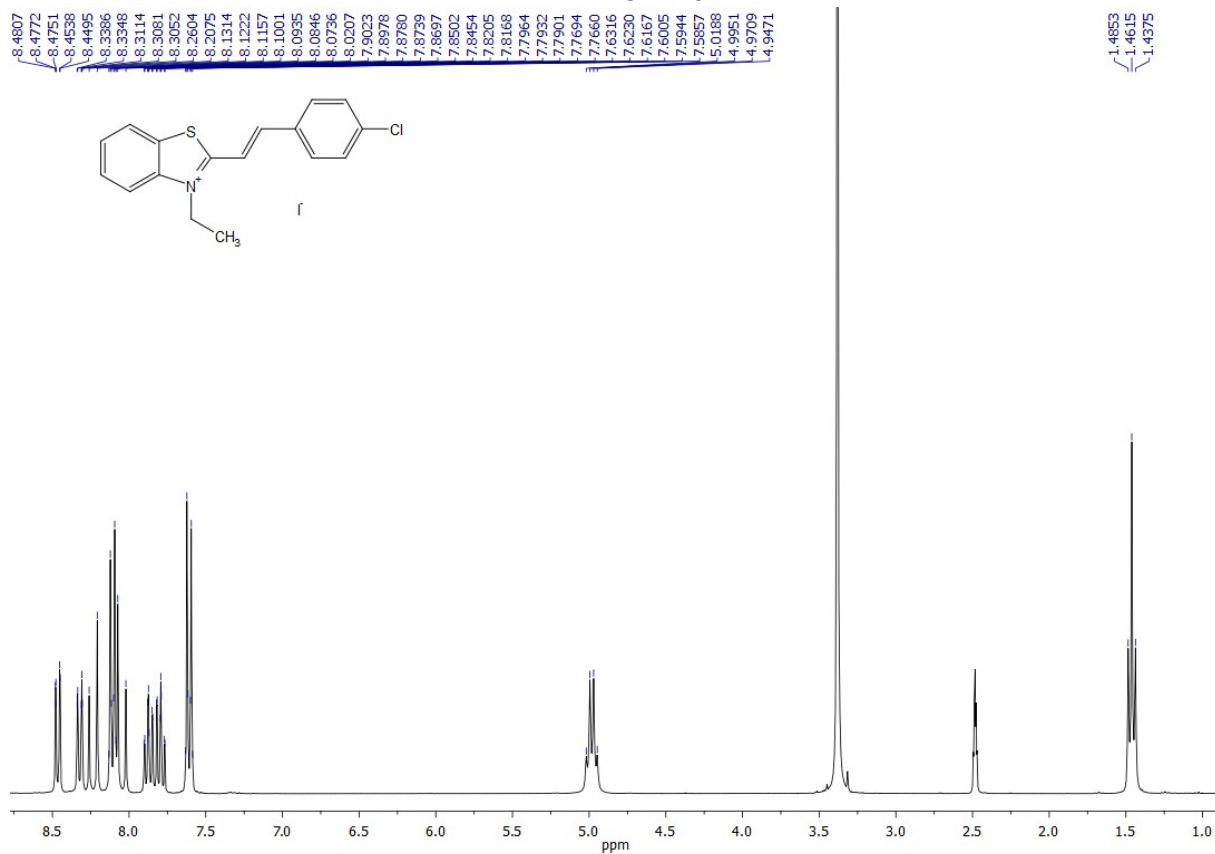
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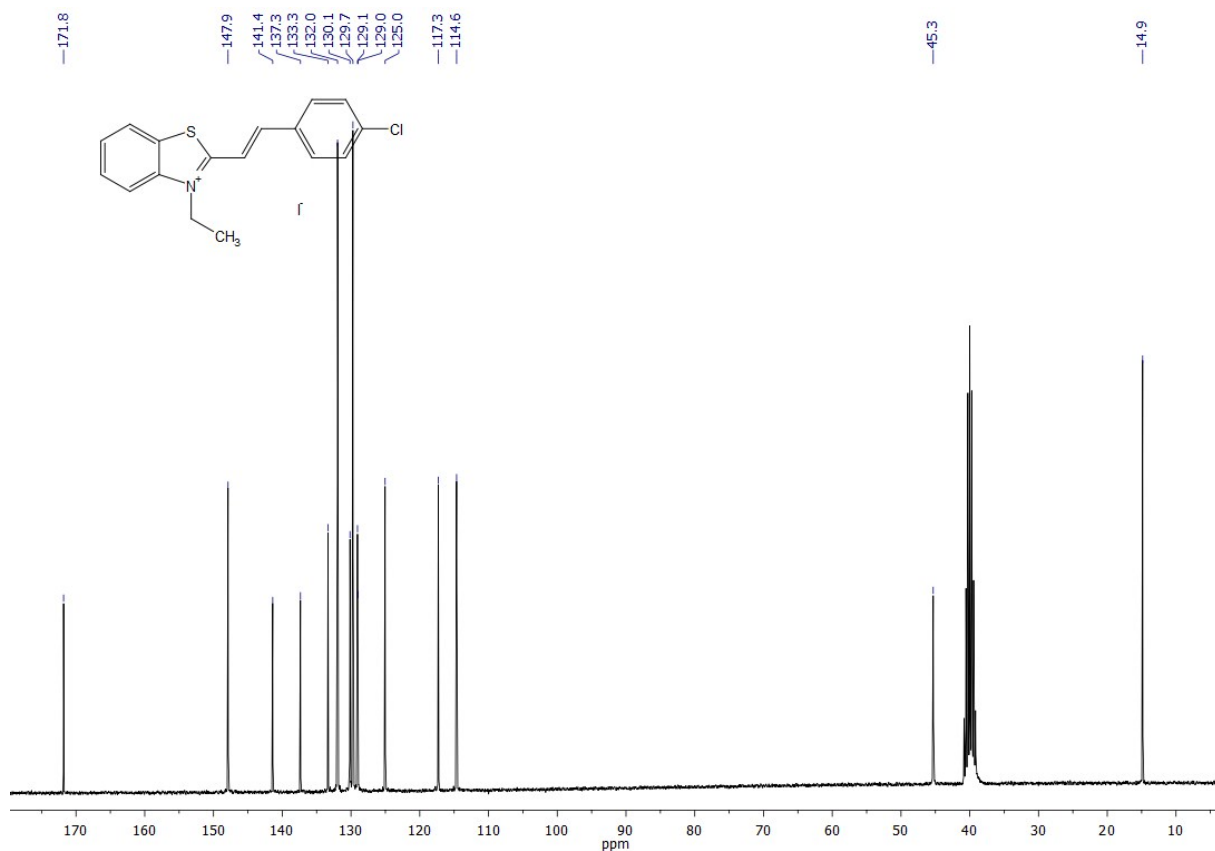
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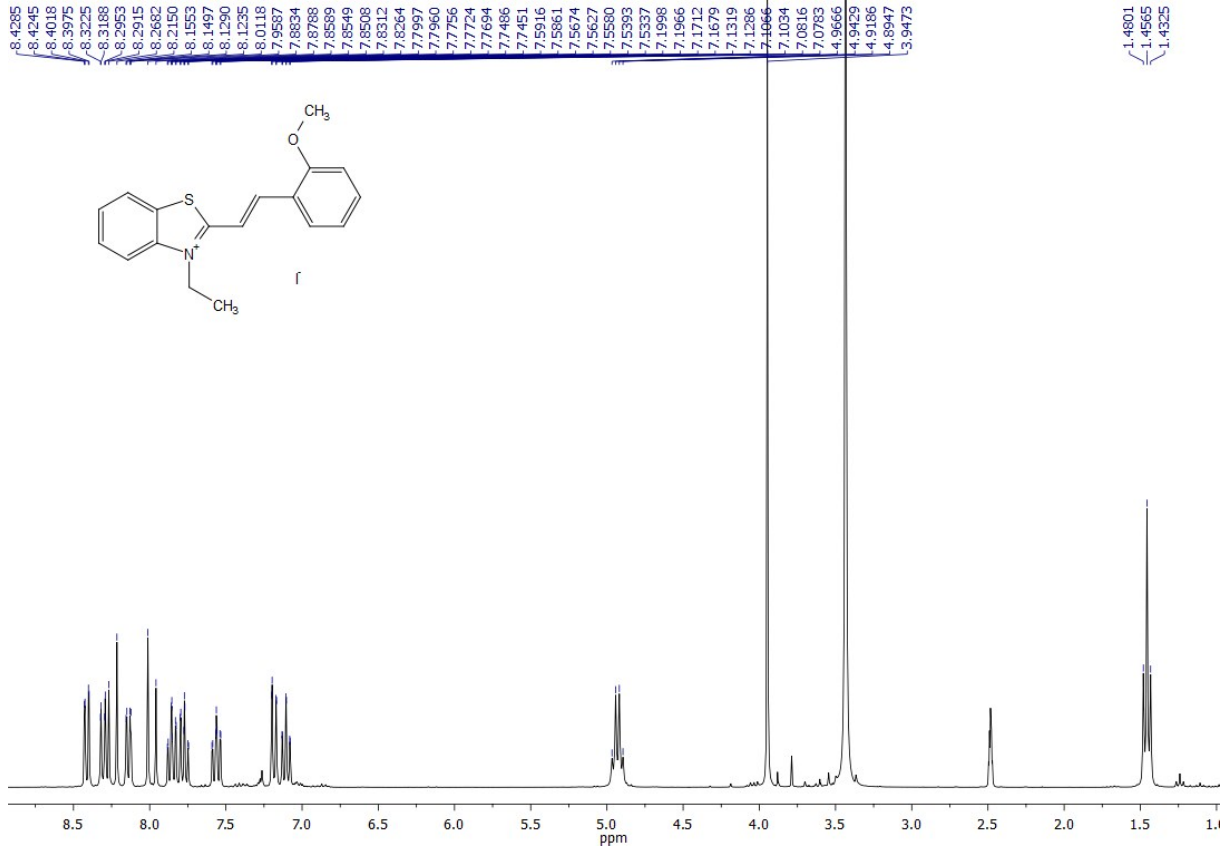
¹H NMR of HDc



¹³C NMR of HDc



¹H NMR of HDd



¹³C NMR of HDd

