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Photochemical properties of new bis-carbocyanine dye as a promising agent for *in vivo* imaging

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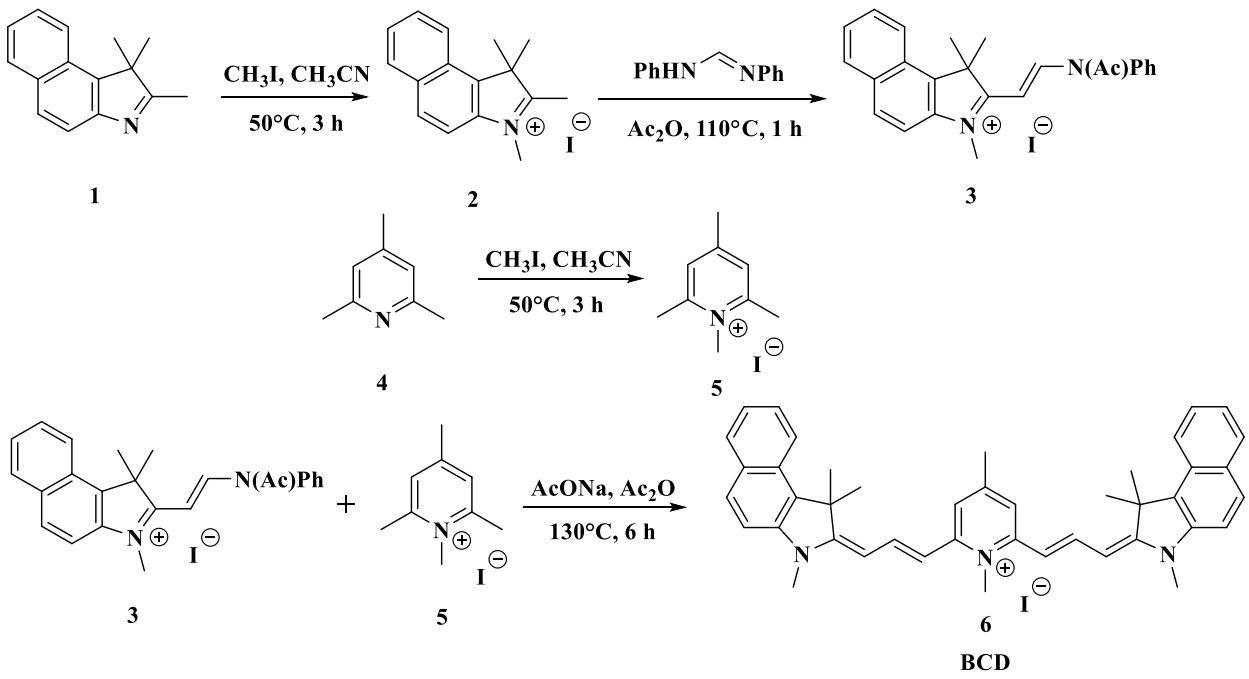
Materials and methods:

All of the chemicals used in the current study were purchased from commercial vendors and used as received without further purification, unless otherwise noted. Human serum albumin (HSA) (Sigma, US) was used. Experiments in water phase (Millipore, 18 MOhm×cm**)** were carried out in phosphate buffered saline (PBS) at pH 7.4 at room temperature. ^{1}H and ^{13}C NMR spectra were recorded on Avance-400 (Bruker, Germany, 400.0 MHz) in CD_3OD, $CDCl_3$, DMSO-d6, D_2O. High-resolution mass spectra were recorded on the Bruker microTOF II setup by the method of electrospray ionization (ESI). Measurements were performed on positive (capillar voltage – 4500 V) or negative (capillar voltage-3200 V) ions. The scanning range of the mass is within *m/z* = 50-3000 Da. For external or internal calibration Electrospray Calibrant Solution (Fluka) was used. Syringe injection of the substance solution in acetonitrile, methanol or water, the flow rate 3 μl/min, nebulizer gas was nitrogen (4 l/min) and the interface temperature was 180°C.

Procedure of BCD Synthesis



Scheme 1. Synthesis of BCD**.**

Compounds **2**, **3**, **5** and **8** were synthesized by modified methods described in the literature[1,2].

Preparation of 1,1,2,3-tetramethyl-1H-benzo[e]indol-3-ium iodide (**2**).

The reaction mixture of 1,1,2-trimethyl-1H-benzo[e]indole (**1**) (2 g, 9.57 mmol) and methyl iodide (1.8 ml, 28.77 mmol) in absolute acetonitrile (10 ml) was stirred at 50°C for 3h. The reaction was cooled to room temperature. Diethyl ether (70 ml) was added. The precipitate was filtered, washed with diethyl ether and dried *in vacuo* to give **2** as white solid. Yield: 92%. mp 230°C. ^{1}H NMR (DMSO-d6; δ, ppm; J, Hz) : 1.75 (s, 6 H, C(CH_3)$_2$), 2.87 (s, 3 H, =C-CH_3), 4.09 (s, 3 H, +NCH_3), 7.72 (t, 1 H, $^3J_{HH}$ = 7.8, arom), 7.78 (t, 1 H, $^3J_{HH}$ = 7.4, arom), 8.11 (d, 1 H, $^3J_{HH}$ = 8.9, arom), 8.21 (d, 1 H, $^3J_{HH}$ = 8.1, arom), 8.29 (d, 1 H, $^3J_{HH}$ = 8.9, arom), 8.37 (d, 1 H, $^3J_{HH}$ = 8.2, arom).

Preparation of (E)-1,1,3-trimethyl-2-(2-(N-phenylacetamido)vinyl)-1H-benzo[e]indol-3-ium iodide (**3**).

1,1,2,3-tetramethyl-1H-benzo[e]indol-3-ium iodide (**2**) (0.7 g, 2 mmol) and N,N′-diphenylformamidine (0.6 g, 3 mmol) were suspended in acetic anhydride (2 ml). The suspension was stirred at 110°C for 1h. The reaction mixture was cooled to room temperature.

Diethyl ether (60 ml) was added. The obtained precipitate was filtered, washed with diethyl ether and dried *in vacuo* to give **3** as brown powder. Yield: 75%. mp 180-185°C. ^{1}H NMR (DMSO-d6; δ, ppm; J, Hz): 1.92 (s, 6 H, $C(CH_3)_2$), 2.11 (s, 3 H, $C(O)CH_3$), 3.73 (s, 3 H, $+NCH_3$), 5.38 (d, 1 H, $^{3}J_{HH}$ = 14.3, =CH), 7.59 (d, 2 H, $^{3}J_{HH}$ = 6.9, arom), 7.65-7.77 (m, 5 H, arom), 7.96 (d, 1 H, $^{3}J_{HH}$ = 8.9, arom), 8.17 (d, 1 H, $^{3}J_{HH}$ = 8.1, arom), 8.22 (d, 1 H, $^{3}J_{HH}$ = 8.9, arom), 8.40 (d, 1 H, $^{3}J_{HH}$ = 8.4, arom), 9.15 (d, 1 H, $^{3}J_{HH}$ = 14.3, =CH). ^{13}C NMR (DMSO-d6; δ, ppm): 23.2 (1 C), 26.1 (2 C), 33.6, 52.7, 94.9 (all 1 C), 112.6 (2 C), 126.4, 126.5 (all 1 C), 128.1 (2 C), 129.7 (1 C), 130.1, 130.5 (all 2 C), 132.5 (1 C), 135.9 (2 C), 136.1, 139.2 (all 1 C), 147.8 (1 C), 170.2 (1 C), 182.5 (1 C). HRMS calculated for $C_{25}H_{25}N_2O$ [M] m / z = 369.1961, found m / z = 369.1951.

Preparation of 1,2,4,6-tetramethylpyridin-1-ium iodide (**5**).

The reaction mixture of 2,4,6-trimethylpyridine (**4**) (2 ml, 15 mmol) and methyl iodide (2.85 ml, 45 mmol) in absolute acetonitrile (7 ml) was stirred at 50 ° C for 3h. The reaction was cooled to room temperature. Diethyl ether (50 ml) was added. The obtained precipitate was filtered, washed with diethyl ether and dried *in vacuo* to give **5** as pale yellow-white solid. Yield: 88%. mp 123-125°C. ^{1}H NMR ($CDCl_3$-d; δ, ppm): 2.51 (s, 3 H, CH_3), 2.86 (s, 6 H, CH_3), 4.19 (s, 3 H, $+NCH_3$), 7.55 (s, 2 H, arom).

1,4-dimethyl-2,6-bis-((1E,3E)-3-(1,1,3-trimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)prop-1-ene-1-yl)-pyridin-1-ium iodide (BCD) (**6, BCD**).

A solution of 1,2,4,6-tetramethylpyridin-1-ium iodide (**5**) (0.09 g, 0.32 mmol), (E)-1,1,3-trimethyl-2-(2-(N-phenylacetamido)vinyl)-1H-benzo[e]indole-3-ium iodide (**3**) (0.48 g, 0.97 mmol) and sodium acetate (0.11 g, 1.34 mmol) in acetic anhydride (2 ml) was stirred at 130° C for 6 h. The reaction mixture was cooled to room temperature. Diethyl ether (50 ml) was added. The resulting precipitate was filtered, washed twice with diethyl ether and dried *in vacuo* to give dark powder. The pure product was isolated by column chromatography (Al_2O_3, eluent CH_2Cl_2:MeOH = 50:1) to give **6** as blue powder. Yield: 17%.

^{1}H NMR ($CDCl_3$; δ, ppm; J, Hz): 2.07 (s, 12 H, $C(CH_3)_2$), 2.91 (s, 3 H, CH_3), 3.90 (br. s., 6 H, NCH_3), 4.27 (s, 3 H, $+NCH_3$), 6.01 (d, 2 H, $^{3}J_{HH}$ = 14.8 Hz, =CH), 7.36 (d, 2 H, $^{3}J_{HH}$ = 8.8 Hz, arom), 7.49 - 7.52 (m, 2 H, arom), 7.54 (s, 2 H, arom), 7.65 (t, $^{3}J_{HH}$ = 7.7 Hz, 2 H, arom), 7.96 (t, $^{3}J_{HH}$ = 7.6Hz, 6 H, arom, =CH), 8.01 (d, $^{3}J_{HH}$ = 14.5 Hz, 2 H, =CH), 8.13 (d, $^{3}J_{HH}$ =8.5 Hz, 2 H,

arom). ^{13}C NMR ($CDCl_3$; δ, ppm): 22.9 (1 C), 27.6, 29.6 (all 2 C), 35.2 (2 C), 41.7 (1 C), 51.3 (2 C), 100.9, 110.7 (all 2 C), 122.0, 125.3, 126.8, 127.7, 127.8, 128.3, 129.1, 132.1, 134.1, 138.5, 145.0 (all 2 C), 146.1 (2 C), 154.7 (1 C), 157.3 (2 C), 175.8 (2 C). HRMS calculated for $C_{43}H_{44}N_3$ $[M]^+$ m/z = 602.3530, found m/z = 602.3511.

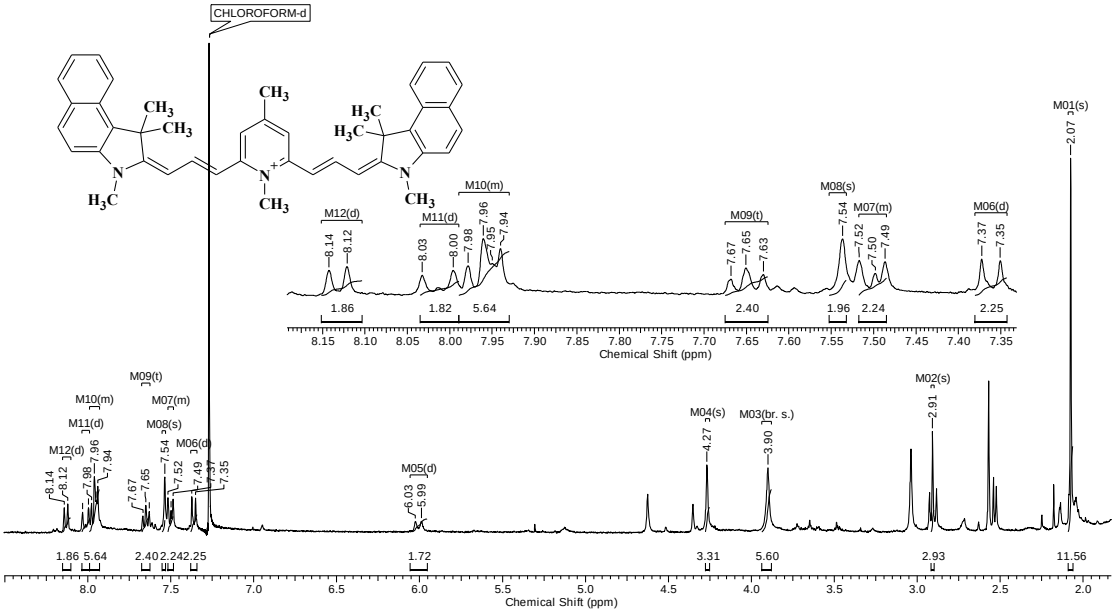


Figure S1 ^{1}H NMR spectrum of BCD in $CDCl_3$.

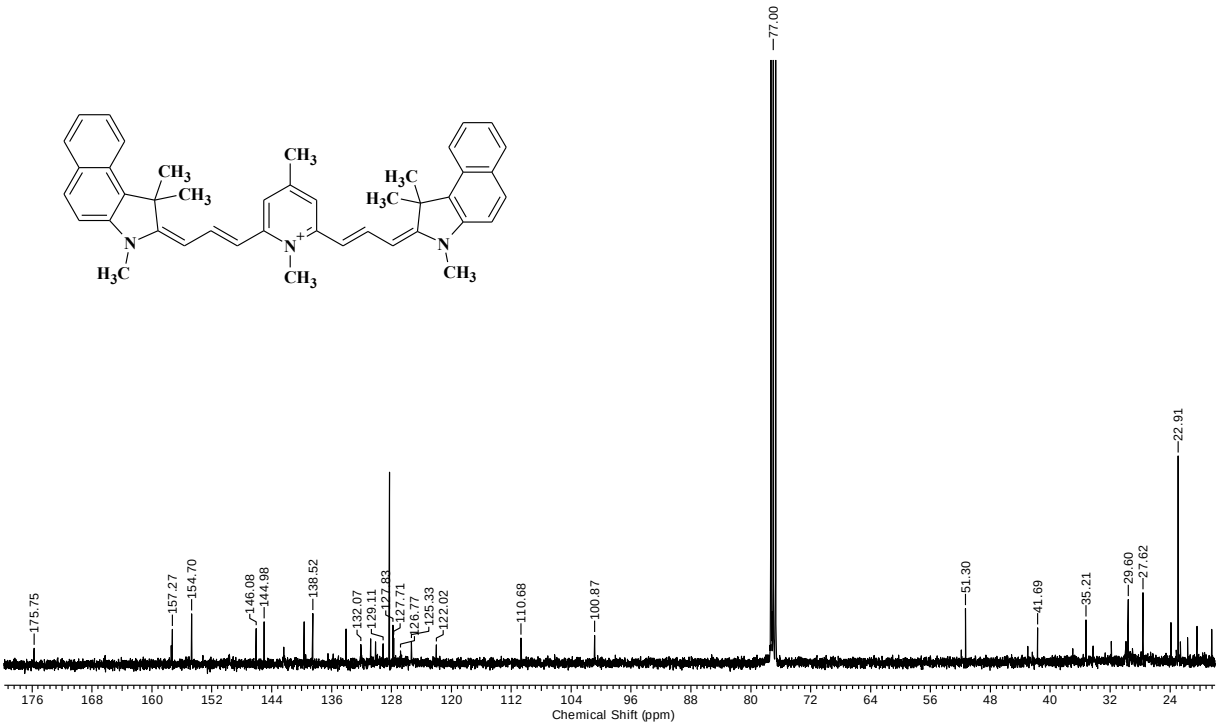


Figure S2 ^{13}C NMR spectrum of BCD in $CDCl_3$.

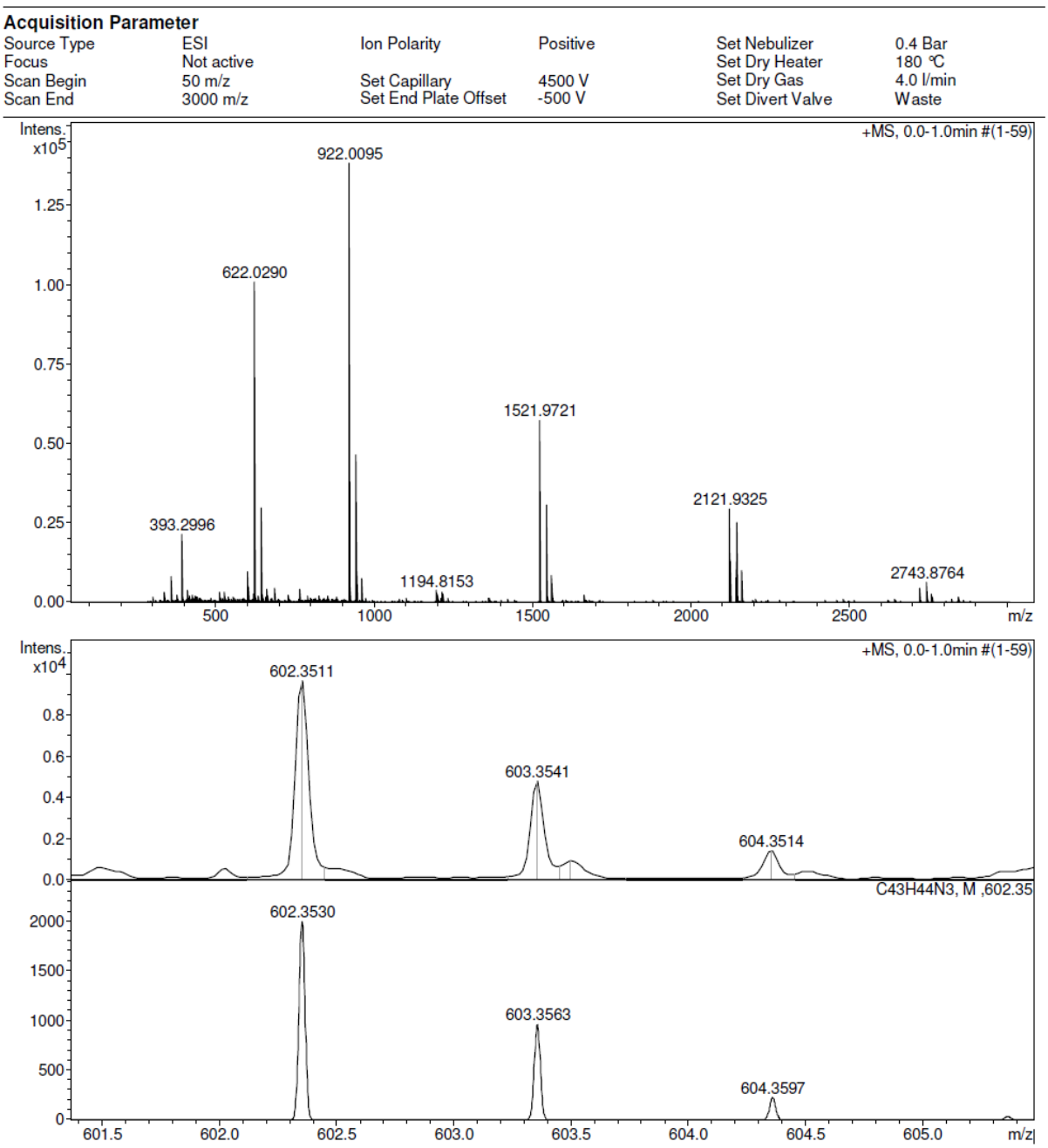


Figure S3 BCD HRMS spectra.

References

1 M. Gerowska, L. Hall, J. Richardson, M. Shelbourne and T. Brown, *Tetrahedron*, 2012, **68**, 857.

2 F. Yang, X.-L. Xu, Y. Gong, W.-W. Qiu, Z.-R. Sun, J.-W. Zhou, A. Pierre and J. Tang, *Tetrahedron*, 2007, **63**, 9188.