

Synthesis of modified conformationally fixed tricyanocyanine dyes for conjugation with therapeutic agents

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1. Experimental part

¹H and ¹³C NMR spectra were recorded on Bruker Avance 400 (400 and 100 MHz, respectively), Agilent 400-MR spectrometers (400 and 100 MHz, respectively), Bruker Avance 500 (500 and 125 MHz) and Bruker Avance 600 (600 and 150 MHz). Residual signals of solvents were used as a reference (¹H: CDCl₃, δ 7.26, DMSO-d₆, δ 2.49, CD₃OD, δ 3.31. ¹³C: CDCl₃, δ 77.1, CD₃OD, δ 49.0).

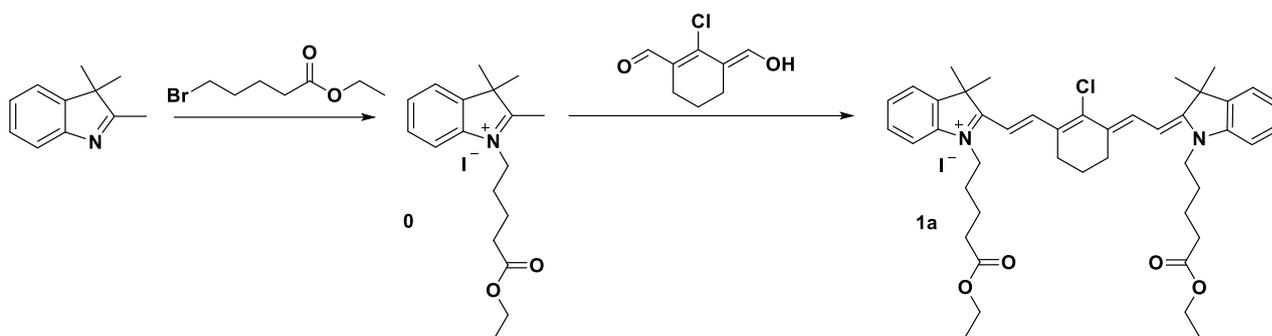
IR spectra were recorded on a Nicolet IR 200 Fouriertransform spectrometer (Thermo Scientific) using an internal reflectance accessory with a ZnSe attenuated total reflection (ATR) element with an incidence angle of 45°. The resolution was 4 cm⁻¹, the number of scans was 20.

High resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD 1100 SL instrument with atmospheric pressure electrospray ionization (AP-ESI) in the positive ion detection mode (ion trap mass analyzer). Registration conditions: the nebulizer gas temperature (nitrogen) 300 °C at a rate of 12 dm³ min⁻¹, the source potential 5000 V, the capillary outlet potential 150 V, solvent acetonitrile.

Reaction progress and purity of chromatographically separated compounds were monitored by thin-layer chromatography (TLC) on Silica gel 60 F254 plates (Merck).

Chromatographic separation was carried out on a column with MN Kieselgel 60 silica gel, 0.04—0.063 mm (230-400 mesh) ASTM and Interchim puriflash 60 si hp 50 μm particle size.

Compounds **1c-d** were obtained as described [S1,S2].



Scheme S1.

1-(5-Ethoxy-5-oxopentyl)-2,3,3-trimethyl-3H-indol-1-ium iodide (0)

2,3,3-Trimethyl-3H-indole (0.5 g, 3 mmol) was dissolved in acetonitrile (5 ml). Ethyl 5-bromopentanoate (0.71 ml, 4.5 mmol) and potassium iodide (1.02 g, 6.15 mmol) were added. The mixture was heated (72 °C) with stirring for 24 hours. After cooling to room temperature, excess diethyl ether was added. The resulting precipitate was filtered off, washed with CH₂Cl₂, and the filtrate was evaporated. The yield was 0.863 g (69%).

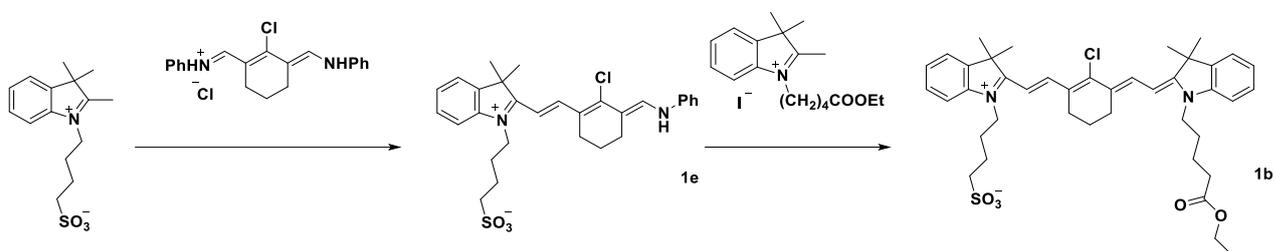
¹H NMR (400 MHz, Chloroform-d, δ, ppm, J/Hz): 1.18 (t, 3H, ³J_{HH} = 7.15, OCH₂CH₃), 1.63 (s, 6H, C(CH₃)₂), 1.72 - 1.82 (m, 2H, CH₂), 1.97 - 2.08 (m, 2H, CH₂), 2.39 (t, 2H, ³J_{HH} = 6.88, CH₂COOEt), 3.12 (s, 3H, =C(CH₃)), 4.04 (q, 2H, ³J_{HH} = 7.13, OCH₂CH₃) 4.71 (t, 2H, ³J_{HH} = 7.67, CH₂N⁺), 7.53 - 7.58 (m, 3H, Ar), 7.72 - 7.77 (m, 1H, Ar). ¹³C NMR (100 MHz, Chloroform-d δ, ppm, J/Hz): 14.05 (s, =C(CH₃)), 17.03 (s, COOCH₂CH₃), 21.71 (s, C(CH₃)₂), 23.08 (s, C(CH₃)₂, CH₂), 27.05 (s, CH₂), 32.90 (s, CH₂), 49.58 (s, CH₂N⁺), 54.58 (s, C(CH₃)₂), 60.41 (s, COOCH₂CH₃), 115.41, 123.24, 129.44, 130.02, 140.90, 141.44 (all s, Ar), 172.64 (s, COOEt), 195.86 (s, =C(CH₃)). IR, ν/cm⁻¹: 1726.94 (C=O), 1590.99 - 1623.77 (Ar). HRMS-ESI: found m/z 288.1939 [M⁺] C₁₈H₂₆NO₂⁺. calculated M=288.1958.

2-((E)-2-((E)-2-Chloro-3-(2-((E)-1-(5-ethoxy-5-oxopentyl)-3,3-dimethylindolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-1-(5-ethoxy-5-oxopentyl)-3,3-dimethyl-3H-indol-1-ium iodide (1a)

A mixture of 1-(5-ethoxy-5-oxopentyl)-2,3,3-trimethyl-3H-indol-1-ium iodide from the previous step (1 g, 2.4 mmol), (E)-2-chloro-3-(hydroxymethylidene)cyclohex-1-ene-1-carbaldehyde (0.208 g, 1.2 mmol) and sodium acetate (0.256 g, 2.12 mmol) ethanol (7 ml) was stirred at 70 °C for 2.5 hours. After cooling to room temperature, an excess of diethyl ether was added, the precipitate was filtered off, washed on the filter with diethyl ether and dried. The dye was purified by column chromatography on silica gel in a mixture of eluents CH₂Cl₂:EtOH 50:1. The yield was 0.7 g (71%). λ_{max} = 780 nm. ε = 2.4•10⁵ dm³*mol⁻¹*cm⁻¹.

¹H NMR (400 MHz, CD₃OD, δ, ppm, J/Hz): 1.21 (t, 6H, ³J_{HH} = 7.12, 2 OCH₂CH₃), 1.74 (s, 12H, 2C(CH₃)₂), 1.75 - 1.81 (m, 4H, 2CH₂), 1.83 - 1.91 (m, 4H, 2CH₂) 1.95 - 1.99 (m, 2H, CH₂), 2.43

(t, 4H, $^3J_{\text{HH}} = 7.00$, $2\text{CH}_2\text{COOEt}$), 2.76 (t, 4H, $^3J_{\text{HH}} = 6.11$, 2CH_2), 4.10 (q, 4H, $^3J_{\text{HH}} = 7.13$, OCH_2CH_3) 4.22 (t, 4H, $^3J_{\text{HH}} = 7.21$, CH_2N^+), 6.32 (d, 2H, $^3J_{\text{HH}} = 14.18$, $=\text{CH}$), 7.27 - 7.33 (m, 2H, Ar), 7.33 - 7.38 (m, 2H, Ar), 7.40 - 7.47 (m, 2H, Ar), 7.54 (d, 2H, $^3J_{\text{HH}} = 7.46$, Ar), 8.45 (d, 2H, $^3J_{\text{HH}} = 14.18$, $=\text{CH}$). ^{13}C NMR (100 MHz, CD_3OD δ , ppm, J/Hz): 14.11 (s, $2\text{COOCH}_2\text{CH}_3$), 20.57 (s, CH_2), 22.16 (s, 2CH_2), 26.64 (s, 4CH_2), 28.05 (s, $2\text{C}(\text{CH}_3)_2$), 33.47 (s, 2CH_2), 44.65 (s, $2\text{C}(\text{CH}_3)_2$), 49.23 (s, $2\text{CH}_2\text{N}^+$), 60.35 (s, $2\text{COOCH}_2\text{CH}_3$), 93.32 (s, 2CH), 101.30 (s, Ar), 110.85 (s, $\text{C}=\text{C}(\text{Cl})-\text{C}$), 116.55 (s, Ar), 122.16 (s, Ar), 125.25 (s, Ar), 127.46 (s, 2CH), 128.74 (s, Ar), 140.87 (s, Ar), 142.01 (s, Ar), 144.27 (s, Ar), 150.44 (s, CCl), 172.96 (s, $2\text{COOCH}_2\text{CH}_3$), 176.57 (s, $2\text{C}=\text{N}$). IR, ν/cm^{-1} : 714.01 (C-Cl), 1368,25 (N sec.), 1550.01 - 1512.40 (Ar), 1728.87 (C=O). HRMS-ESI: found m/z 711.3929 $[\text{M}^+]$ $\text{C}_{44}\text{H}_{56}\text{ClN}_2\text{O}_4^+$. calculated $M=711,3923$.



Scheme S2.

4-(2-((*E*)-2-((*E*)-2-Chloro-3-(phenylaminomethylidene)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-3*H*-indol-1-ium-1-yl)butane-1-sulfonate (**1e**)

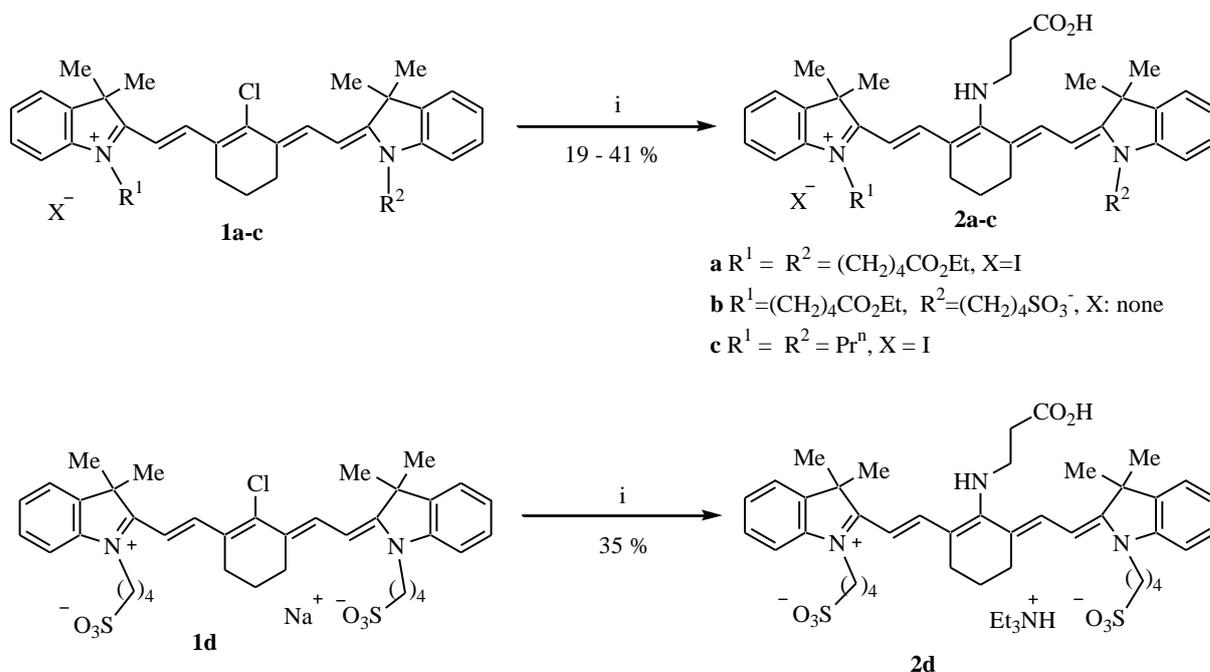
A mixture of 4-(2,3,3-trimethyl-3*H*-indol-1-ium-1-yl)butane-1-sulfonate (1 g, 3.38 mmol), *N*-((1*E*,3*E*,5*E*)-5-(phenylimino)penta-1,3-dien-1-yl)aniline hydrochloride (2.19 g, 6.77 mmol) and sodium acetate (0.56 g, 6.77 mmol) in acetonitrile (15 ml) was stirred at room temperature for 17 hours. After the completion of the reaction, excess diethyl ether was added, the precipitate was filtered off, washed on the filter with diethyl ether and dried. The product was isolated by column chromatography on silica gel in a mixture of eluents $\text{CH}_2\text{Cl}_2:\text{MeOH}$ 17:1. The yield was 0.427 g (24%). $M.p.$ = 184°C. λ_{max} = 645 nm.

^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ , ppm, J/Hz): 1.69 (s, 6H, $\text{C}(\text{CH}_3)_2$), 1.72-1.80 (m, 2H, CH_2), 1.82-1.91 (m, 4H, 2CH_2), 2.65-2.71 (m, 2H, CH_2), 2.71-2.77 (m, 2H, CH_2), 3.46 (br.s., 2H, CH_2SO_3), 4.33-4.42 (m, 2H, $+\text{NCH}_2$), 6.59 (d, 1H, $^3J_{\text{HH}} = 14.98$, $=\text{CH}$), 7.12-7.20 (m, 1H, Ar), 7.36-7.45 (m, 5H, Ar), 7.50 (t, 1H, $^3J_{\text{HH}} = 7.61$, Ar), 7.64-7.75 (m, 2H, Ar), 8.20 (s, 1H, CHNH), 8.41 (d, 1H, $^3J_{\text{HH}} = 14.61$, $=\text{CH}$), 10.23 (br.s., 1H, NH). IR, ν/cm^{-1} : 731 (C-Cl), 1241 (SO_3), 1347 (N sec.).

4-(2-((*E*)-2-((*E*)-2-Chloro-3-(2-((*E*)-1-(5-ethoxy-5-oxopentyl)-3,3-dimethylindolin-2-ylidene)-ethylidene)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-3*H*-indol-1-ium-1-yl)butane-1-sulfonate (1b)

A mixture of compound **3** (0.417 g, 0.79 mmol), compound **1** (0.329 g, 0.79 mmol) and AcONa (0.130 g, 1.58 mmol) in EtOH (20 ml) was stirred at 70 °C for 6 hours. After cooling to room temperature, excess diethyl ether was added. The resulting precipitate was filtered off, purified by column chromatography on Interchim puriflash silica gel in a mixture of eluents CH₂Cl₂:EtOH 7:1. The yield was 0.261 g (46%). $\lambda_{\text{max}} = 785 \text{ nm}$. $\epsilon = 1.6 \cdot 10^5 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

¹H NMR (600 MHz, Chloroform-*d*, δ , ppm, J/Hz): 1.20 (t, 3H, ³*J*_{HH} = 7.11, OCH₂CH₃), 1.67 (s, 6H, C(CH₃)₂), 1.69 (s, 6H, C(CH₃)₂), 1.73 - 1.79 (m, 2H, CH₂), 1.80 - 1.88 (m, 2H, CH₂), 1.91 - 1.99 (m, 2H, CH₂), 2.02 - 2.11 (m, 4H, 2CH₂), 2.37 (t, 2H, ³*J*_{HH} = 6.92, CH₂), 2.62 (t, 2H, ³*J*_{HH} = 5.36, CH₂), 2.76 (t, 2H, ³*J*_{HH} = 5.32, CH₂), 2.98 (t, 2H, ³*J*_{HH} = 5.55, CH₂), 3.99 (t, 2H, ³*J*_{HH} = 7.20, CH₂N), 4.09 (q, 2H, ³*J*_{HH} = 7.06, OCH₂CH₃), 4.22 - 4.28 (m, 2H, CH₂N), 5.97 (d, 1H, ³*J*_{HH} = 13.66, CH), 6.51 (d, 1H, ³*J*_{HH} = 14.30, CH), 7.02 (d, 1H, ³*J*_{HH} = 7.89, Ar), 7.15 (t, 1H, ³*J*_{HH} = 7.43, Ar), 7.24 - 7.26 (m, 1H, Ar), 7.28 - 7.35 (m, 3H, Ar), 7.36 (d, 1H, ³*J*_{HH} = 7.34, Ar), 7.40 (t, 1H, ³*J*_{HH} = 7.61, Ar), 8.21 (d, 1H, ³*J*_{HH} = 13.66, CH), 8.41 (d, 1H, ³*J*_{HH} = 14.40, CH). ¹³C NMR (150 MHz, Chloroform-*d*, δ , ppm, J/Hz): 14.15 (s, COOCH₂CH₃), 20.67 (s, CH₂), 22.21 (s, CH₂), 22.87 (s, CH₂), 26.08 (s, CH₂), 26.36 (s, CH₂), 26.39 (s, CH₂), 26.47 (s, CH₂), 27.96 (s, C(CH₃)₂), 28.18 (s, C(CH₃)₂), 33.50 (s, CH₂), 43.72 (s, C(CH₃)₂), 45.18 (s, C(CH₃)₂), 48.64 (s, CH₂SO₃), 49.79 (s, CH₂N⁺), 49.91 (s, CH₂N⁺), 60.48 (s, COOCH₂CH₃), 98.99 (s, CH), 103.66 (s, CH), 111.85 (s, Ar), 122.10 (s, C=C(Cl)-C), 122.13 (s, C=C(Cl)-C), 124.29 (s, Ar), 126.15 (s, Ar), 126.98 (s, Ar), 128.56 (s, CH), 128.57 (s, CH), 129.09 (s, Ar), 140.45 (s, Ar), 141.43 (s, Ar), 141.71 (s, Ar), 142.11 (s, Ar), 142.41 (s, Ar), 146.77 (s, Ar), 150.49 (s, CCl), 169.92 (s, COOCH₂CH₃), 172.84 (s, C=N), 174.41 (s, C=N). IR, ν/cm^{-1} : 1727.42 (C=O), 1549.04 - 1511.44 (Ar), 1247.24 (SO₃⁻). HRMS-ESI: found *m/z* 719.3276 [M+H]⁺ C₄₁H₅₁ClN₂O₅S. calculated M=718.3207.



Scheme S3

Compounds **2a-d** were obtained by the general method. A mixture of 1 equivalent of dye **1a-d** (1 equiv.) and β -alanine (6.8 equiv.) was dissolved in anhydrous DMF (6 ml) under argon and heated at 85 °C for 3-22 hours. The product was precipitated with diethyl ether, and the precipitate was filtered off. The target dye was purified by column chromatography on Interchim puriflash silica gel in a mixture of eluents.

2-((E)-2-((E)-2-((2-Carboxyethyl)amino)-3-(2-((E)-1-(5-ethoxy-5-oxopentyl)-3,3-dimethylindolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-1-(5-ethoxy-5-oxopentyl)-3,3-dimethyl-3H-indol-1-ium iodide (2a) was prepared in 0.069 g (19%) yield from compound **1a** (0.300 g, 0.4 mmol), heated for 12 hours and purified in a mixture of eluents 30:1 $CH_2Cl_2:EtOH$. $\lambda_{max} = 625$ nm. $\epsilon = 4.2 \cdot 10^4$ $dm^3 \cdot mol^{-1} \cdot cm^{-1}$.

1H NMR (400 MHz, Chloroform-d, δ , ppm, J/Hz): 1.21 - 1.25 (m, 6H, $2OCH_2CH_3$), 1.66 (s, 12H, 4 CH_3), 1.72 - 1.81 (m, 8H, 4 CH_2), 1.81 - 1.87 (m, 2H, CH_2), 2.38 (t, 4H, $^3J_{HH} = 5.96$, $2CH_2COOEt$), 2.46 - 2.54 (m, 4H, $CH_2CH_2CH_2$), 2.64 - 2.71 (m, 2H, CH_2COOH), 3.74 - 3.85 (m, 4H, $2CH_2N$), 3.89 - 3.99 (m, 2H, CH_2NH), 4.13 (q, 4H, $^3J_{HH} = 6.91$, $2OCH_2CH_3$), 5.56 (d, 2H, $^3J_{HH} = 12.47$, 2CH), 6.82 (d, 2H, $^3J_{HH} = 7.52$, Ar), 7.04 (t, 2H, $^3J_{HH} = 7.37$, Ar), 7.20 - 7.26 (m, 4H, Ar), 7.67 (d, 2H, $^3J_{HH} = 12.35$, 2CH). ^{13}C NMR (100 MHz, Chloroform-d δ , ppm, J/Hz): 14.19 (s, $2COOCH_2CH_3$), 21.38 (s, CH_2), 22.45 (s, $2CH_2$), 25.32 (s, $2CH_2$), 25.94 (s, $2CH_2$), 28.81 (s, $2C(CH_3)_2$), 33.72 (s, $2CH_2$), 36.22 (s, $2C(CH_3)_2$), 42.72 (s, CH_2COOH), 47.52 (s, $2CH_2N^+$), 47.82 (s, CH_2NH), 60.48 (s, $2COOCH_2CH_3$), 93.32 (s, 2CH), 107.92 (s, Ar), 119.34 (s, $C=C(NH)-C$), 122.17 (s, Ar), 122.47 (s, Ar), 127.97 (s, Ar), 137.02 (s, 2CH), 140.11 (s, Ar),

142.94 (s, Ar), 166.69 (s, COOH), 168.80 (s, CNH), 172.96 (s, 2COOCH₂CH₃), 176.57 (s, 2C=N). IR, ν/cm^{-1} : 3363.73 (OH), 1564.95 - 1532.65 (Ar), 1732.25 (C=O),. HRMS-ESI: found m/z 764.4627 [M]⁺ C₄₇H₆₂N₃O₆. calculated M=764.4633.

4-(2-((E)-2-((E)-2-((2-Carboxyethyl)amino)-3-(2-((E)-1-(5-ethoxy-5-oxopentyl)-3,3-dimethylindolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-3H-indol-1-ium-1-yl)butane-1-sulfonate (2b) was prepared in 0.102 g (41%) yield from compound **1b** (0.232 g, 0.3 mmol), heated for 22 hours and purified in a mixture of eluents 10:1 CH₂Cl₂:EtOH. $\lambda_{\text{max}} = 630 \text{ nm}$. $\epsilon = 3.5 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

¹H NMR (400 MHz, CD₃OD, δ , ppm, J/Hz): 1.18 (t, 3H, ³J_{HH} = 7.12, OCH₂CH₃), 1.63 (s, 12H, 2C(CH₃)₂), 1.67 - 1.86 (m, 6H, 3CH₂), 1.86 - 1.95 (m, 4H, 2CH₂), 2.38 (t, 2H, ³J_{HH} = 7.06, CH₂COOEt), 2.48 - 2.58 (m, 4H, 2CH₂), 2.68 (t, 2H, ³J_{HH} = 6.02, CH₂COOH), 2.85 (t, 2H, ³J_{HH} = 6.57, CH₂), 3.86 - 4.02 (m, 6 H, 2CH₂N, CH₂NH), 4.07 (q, 2H, ³J_{HH} = 7.06, COOCH₂CH₃), 5.74 - 5.96 (m, 2H, CH), 6.98 - 7.13 (m, 4H, Ar), 7.23 - 7.37 (m, 4H, Ar), 7.64-7.85 (m, 2H, Ar). ¹³C NMR (150 MHz, CD₃OD δ , ppm, J/Hz): 14.72 (s, COOCH₂CH₃), 18.52 (s, CH₂), 23.03 (s, CH₂), 23.52 (s, CH₂), 23.82 (s, CH₂), 26.14 (s, CH₂), 26.94 (s, CH₂), 27.21 (s, CH₂), 29.30 (s, 2C(CH₃)₂), 34.66 (s, CH₂), 43.81 (s, 2C(CH₃)₂), 44.02 (s, CH₂COOH), 47.96 (s, CH₂SO₃⁻), 52.18 (s, 2CH₂N⁺), 58.46 (s, CH₂NH), 61.66 (s, COOCH₂CH₃), 96.12 (s, CH), 96.33 (s, CH), 110.24 (s, Ar), 110.47 (s, Ar), 121.82 (s, C=C(NH)-C), 121.89 (s, C=C(NH)-C), 122.03 (s, Ar), 123.03 (s, Ar), 123.17 (s, CH), 123.22 (s, CH), 124.14 (s, Ar), 124.23 (s, Ar), 129.53 (s, Ar), 129.59 (s, Ar), 140.19 (s, Ar), 140.63 (s, Ar), 141.60 (s, Ar), 144.56 (s, Ar), 169.42 (s, COOH), 169.74 (s, CNH), 170.66 (s, COOCH₂CH₃) 175.04 (s, 2C=N). IR, ν/cm^{-1} : 3374.82 (OH), 1724.53 (C=O), 1563.50 - 1519.63 (Ar), 1288.70 (SO₃⁻). HRMS-ESI: found m/z 772.3980 [M+H]⁺ C₄₄H₅₇N₃O₇S. calculated M=771.3917.

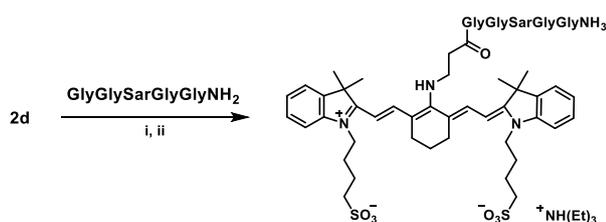
2-((E)-2-((E)-2-((2-Carboxyethyl)amino)-3-(2-((E)-3,3-dimethyl-1-propylindolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-propyl-3H-indol-1-ium iodide (2c) was prepared in 0.044 g (21%) yield from compound **1c** (0.200 g, 0.3 mmol), heated for 2.5 hours and purified in a mixture of eluents 10:1 CH₂Cl₂: MeOH. $\lambda_{\text{max}} = 630 \text{ nm}$. $\epsilon = 7.1 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

¹H NMR (500 MHz, CD₃OD, δ , ppm, J/Hz): 1.03 (t, 6H, ³J_{HH} = 7.39, 2CH₂CH₃), 1.66 (s, 12H, C(CH₃)₂), 1.76 - 1.87 (m, 6H, 2CH₂CH₃, CH₂CH₂CH₂), 2.53 (t, 4H, ³J_{HH} = 6.32, CH₂CH₂CH₂), 2.66 (t, 2H, ³J_{HH} = 5.75, CH₂COOH), 3.88 - 3.98 (m, 6H, CH₂N), 5.85 (d, 2H, ³J_{HH} = 13.01, CH), 7.03 - 7.13 (m, 4H, Ar), 7.26 - 7.34 (m, 2H, Ar), 7.37 (d, 2H, ³J_{HH} = 7.45, Ar), 7.77 (d, 2H, ³J_{HH} = 13.14, CH). ¹³C NMR (125 MHz, CD₃OD δ , ppm, J/Hz): 11.91 (s, 2CH₂CH₃), 21.31 (s, 2CH₂), 23.03 (s, CH₂), 26.12 (s, CH₂CH₂CH₂), 29.33 (s, 2C(CH₃)₂), 30.88 (s, 2C(CH₃)₂), 37.13 (s, CH₂COOH), 45.70 (s, 2CH₂N⁺), 48.18 (s, CH₂NH), 96.10 (s, 2CH), 110.39 (s, Ar), 121.60 (s,

$\underline{\text{C}}=\text{C}(\text{NH})-\underline{\text{C}}$, 123.20 (s, Ar), 124.18 (s, Ar), 129.51 (s, Ar), 140.43 (s, 2CH), 141.64 (s, Ar), 144.74 (s, Ar), 169.82 (s, 2C=N, $\underline{\text{C}}\text{NH}$), 170.45 (s, COOH). IR, ν/cm^{-1} : 3375.30 (OH), 1726.46 (C=O), 1563.02 - 1520.60 (Ar). HRMS-ESI: found m/z 592.3892 $[\text{M}]^+$ $\text{C}_{39}\text{H}_{50}\text{N}_3\text{O}_2$. calculated $M=592.3898$.

Triethylammonium 4-((*E*)-2-((*E*)-2-(2-((2-carboxyethyl)amino)-3-((*E*)-2-(3,3-dimethyl-1-(4-sulfonatobutyl)-3*H*-indol-1-ium-2-yl)vinyl)cyclohex-2-en-1-ylidene)ethylidene)-3,3-dimethylindolin-1-yl)butane-1-sulfonate (2d) was prepared in 0.132 g (35%) yield from compound **1d** (0.349 g, 0.4 mmol), heated for 11 hours and purified in a mixture of eluents 10:1:0.5 MeCN:MeOH:Et₃N. $\lambda_{\text{max}} = 645$ nm. $\epsilon = 7.3 \cdot 10^4$ $\text{dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

¹H NMR (500 MHz, CD₃OD, δ , ppm, J/Hz): 1.31 (t, 9H, ³ $J_{\text{HH}} = 7.34$, 3CH₃ (Et₃NH⁺)), 1.66 (s, 12H, 2C(CH₃)₂), 1.81 - 1.89 (m, 2H, CH₂), 1.89 - 1.97 (m, 8H, 4CH₂), 2.56 (t, 4H, ³ $J_{\text{HH}} = 6.24$, $\underline{\text{C}}\text{H}_2\text{CH}_2\text{CH}_2$), 2.70 (t, 2H, ³ $J_{\text{HH}} = 5.58$, $\underline{\text{C}}\text{H}_2\text{COOH}$), 2.87 (t, 4H, ³ $J_{\text{HH}} = 7.26$, CH₂SO₃), 3.20 (q, ³ $J_{\text{HH}} = 7.34$, 6H, 3CH₂ (Et₃NH⁺)), 3.93 - 4.03 (m, 6H, 2CH₂N, CH₂NH), 5.87 (d, 2H, ³ $J_{\text{HH}} = 12.91$, 2CH), 7.04 - 7.13 (m, 4H, Ar), 7.30 (t, 2H, ³ $J_{\text{HH}} = 7.85$, Ar), 7.35 (d, 2H, ³ $J_{\text{HH}} = 7.34$, Ar), 7.77 (d, 2H, ³ $J_{\text{HH}} = 13.06$, 2CH). ¹³C NMR (125 MHz, CD₃OD δ , ppm, J/Hz): 7.32 (s, 3CH₃ (Et₃NH⁺)), 20.96 (s, CH₂), 21.82 (s, 2CH₂), 24.10 (s, 2CH₂), 24.86 (s, 2CH₂), 27.29 (s, 2C($\underline{\text{C}}\text{H}_3$)₂), 28.81 (s, 2C(CH₃)₂), 35.07 (s, $\underline{\text{C}}\text{H}_2\text{COOH}$), 41.95 (s, 2CH₂N⁺), 45.94 (s, 3CH₂ (Et₃NH⁺)), 46.14 (s, CH₂NH), 50.19 (s, 2CH₂SO₃), 94.08 (s, 2CH), 108.32 (s, Ar), 119.86 (s, $\underline{\text{C}}=\text{C}(\text{NH})-\underline{\text{C}}$), 121.12 (s, Ar), 122.06 (s, Ar), 127.50 (s, Ar), 138.21 (s, 2CH), 139.57 (s, Ar), 142.55 (s, Ar), 167.39 (s, COOH), 168.52 (s, $\underline{\text{C}}\text{NH}$), 174.64 (s, 2C=N). IR, ν/cm^{-1} : 3406.64 (OH), 1711.03 (C=O), 1563.02 - 1496.97 (C=C), 1294.48 (SO₃⁻). HRMS-ESI: found m/z 778.3205 $[\text{M}]^-$ $\text{C}_{41}\text{H}_{52}\text{N}_3\text{O}_8\text{S}_2$. calculated $M=778.3201$.



3

Scheme S4.

Cy- β -Ala-Gly-GLy-Sar-Gly-Gly-NH₂ (3)

Compound **2d** (0.053 g, 0.06 mmol) was dissolved in DMF (2 ml) under argon atmosphere. *N*-Hydroxysuccinimide (0.007 g, 0.06 mmol) was added, cooled to 0-5 °C and then DCC (0.012 g, 0.06 mmol) was added. The mixture was stirred for 30 min, and then left in the refrigerator for 15 hours. The abovementioned peptide (0.027 g, 0.06 mmol) and Et₃N (0.008 ml, 0.06 mmol) were added, and the mixture was stirred at room temperature for 5 days. After completion of the

reaction, an excess of diethyl ether was added. The precipitate was filtered off and purified by column chromatography on Interchim puriflash silica gel in a mixture of eluents 20:1:0.5 CH₂Cl₂:CH₃OH:Et₃N. The yield was: 0.024 g (34%). $\lambda_{\text{max}} = 635 \text{ nm}$.

¹H NMR (500 MHz, CD₃OD, δ , ppm, J/Hz): 1.31 (t, 9H, ³J_{HH} = 7.32, 3CH₃ (Et₃NH⁺)), 1.64 (s, 12H, 2C(CH₃)₂), 1.82 - 1.86 (m, 2H, CH₂), 1.89 - 1.97 (m, 8H, 4CH₂), 2.56 (t, 4H, ³J_{HH} = 6.18, CH₂CH₂CH₂), 2.72 (t, 2H, ³J_{HH} = 5.80, CH₂CONH) 2.87 (t, 4H, ³J_{HH} = 6.94, 2CH₂SO₃⁻), 2.95 (s, 1H, NH), 3.12 (s, 3H, NCH₃), 3.21 (q, 6H, ³J_{HH} = 7.32, 3CH₂ (Et₃NH⁺)), 3.84 (s, 1H, NH), 3.85 (s, 2H, NHCH₂), 3.87 (s, 2H, NHCH₂), 3.91 (s, 1H, NH), 3.97 - 4.05 (m, 8H, 2CH₂N, 2NHCH₂), 4.06 (s, 1H, NH), 4.08 (s, 2H, NHCH₂), 4.15 (s, 1H, NH), 4.18 (s, 2H, NCH₂), 4.60 (s, 2H, CONH₂), 5.86 (d, 2H, ³J_{HH} = 11.60, 2CH), 7.05 - 7.13 (m, 4H, Ar), 7.30 (t, 2H, ³J_{HH} = 7.40, Ar), 7.36 (d, 2H, ³J_{HH} = 7.32, Ar), 7.78 (d, 2H, ³J_{HH} = 12.66, 2CH). ¹³C NMR (125 MHz, CD₃OD δ , ppm, J/Hz): 9.37 (s, 3CH₃ (Et₃NH⁺)), 22.99 (s, CH₂), 23.87 (s, 2CH₂), 26.28 (s, 2CH₂), 26.99 (s, 2CH₂), 29.31 (s, 2C(CH₃)₂), 30.89 (s, 2C(CH₃)₂), 33.21 (s, NCH₃), 36.70 (s, CH₂CONH), 42.03 (s, NHCH₂), 42.23 (s, NHCH₂), 43.31 (s, NHCH₂), 43.62 (s, NHCH₂), 43.94 (s, NHCH₂), 48.10 (s, 3CH₂ (Et₃NH⁺)), 52.23 (s, 2CH₂N⁺), 53.00 (s, CH₂N), 53.77 (s, 2CH₂SO₄), 96.21 (s, 2CH), 110.42 (s, Ar), 121.81 (s, C=C(NH)-C), 123.21 (s, Ar), 124.12 (s, Ar), 129.56 (s, Ar), 140.21 (s, 2CH), 141.63 (c, Ar), 144.66 (s, Ar), 169.45 (s, CONH), 170.34 (s, CONH), 171.48 (s, CONH), 171.74 (s, CONH), 172.10 (s, CONH), 172.16 (s, CNH), 174.43 (s, 2C=N), 174.55 (s, C=N). HRMS-ESI: found m/z 1076.4575 [M]⁻ C₅₂H₇₀N₉O₁₂S₂. calculated M=1076,4591.

2. NMR spectra of compounds

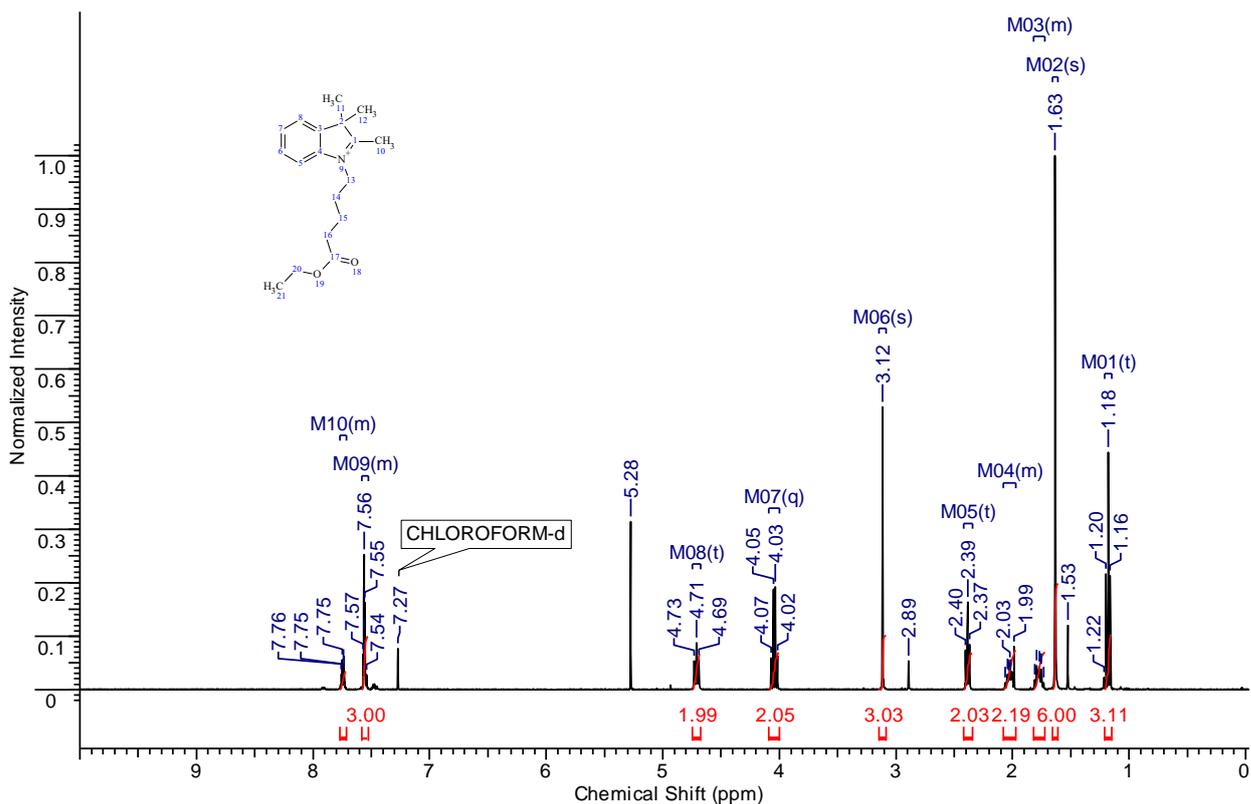


Figure S1 ^1H NMR of 1-(5-ethoxy-5-oxopentyl)-2,3,3-trimethyl-3*H*-indol-1-ium iodide.

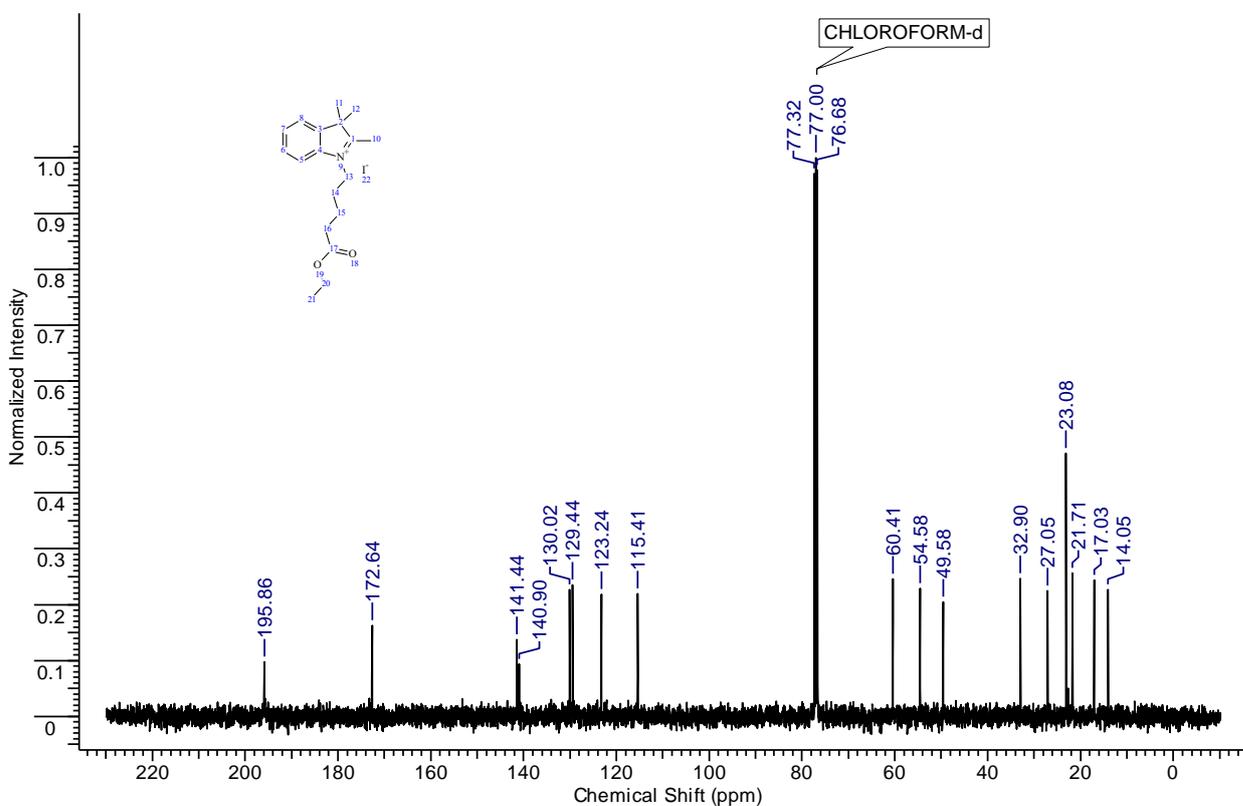


Figure S2 ^{13}C NMR of 1-(5-ethoxy-5-oxopentyl)-2,3,3-trimethyl-3*H*-indol-1-ium iodide.

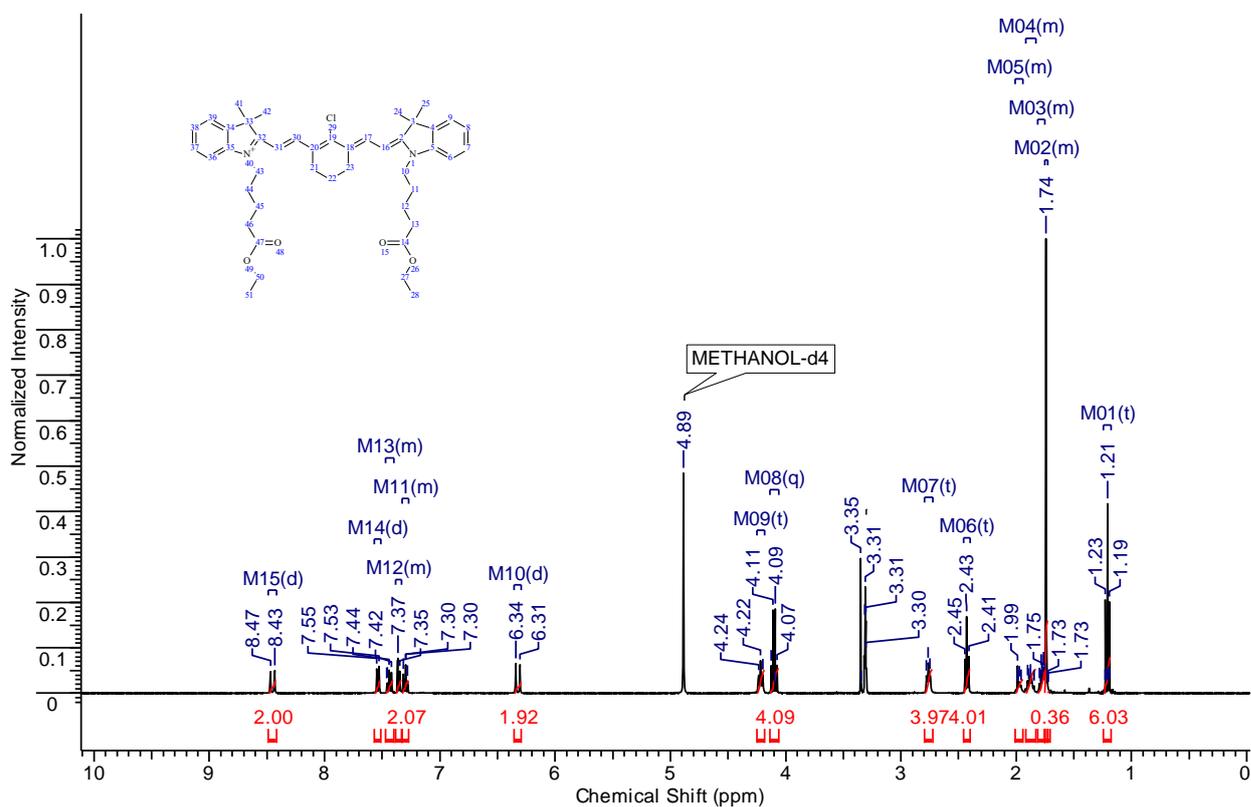


Figure S3 ^1H NMR spectrum of compound **1a**.

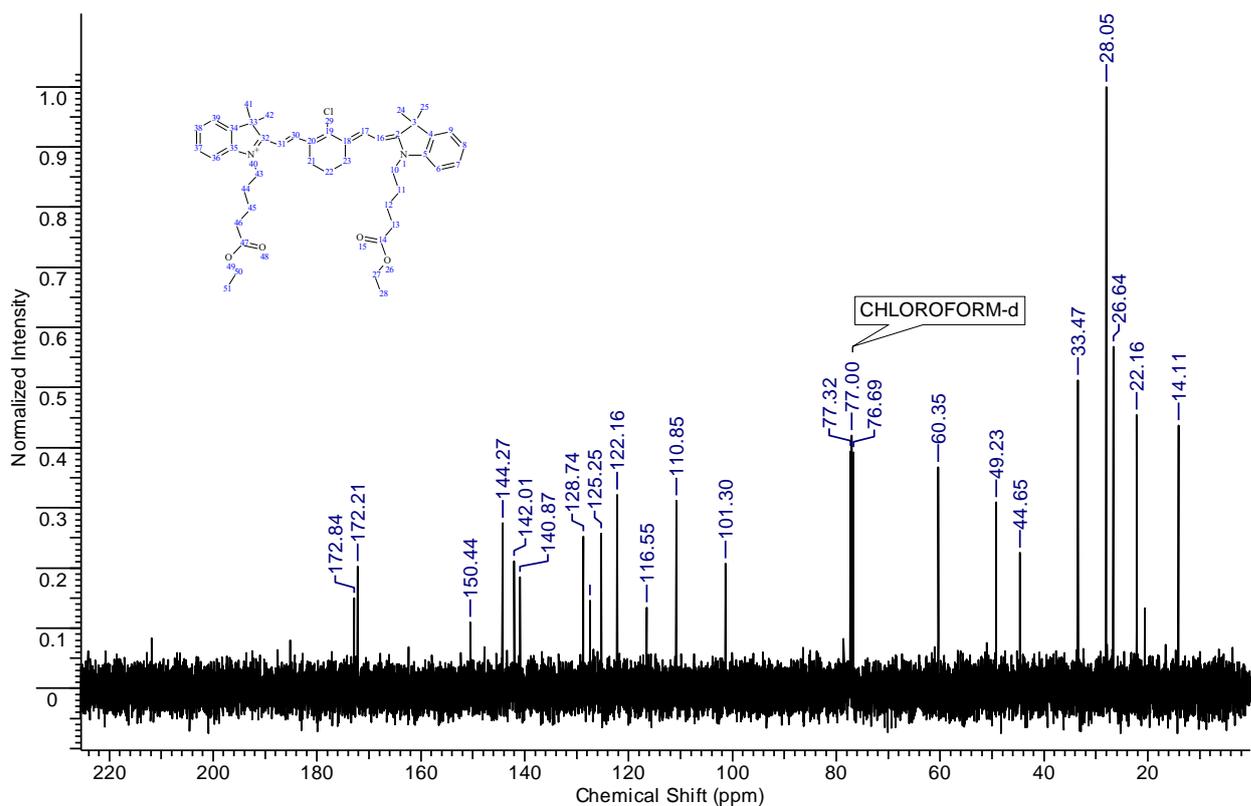


Figure S4 ^{13}C NMR spectrum of compound **1a**.

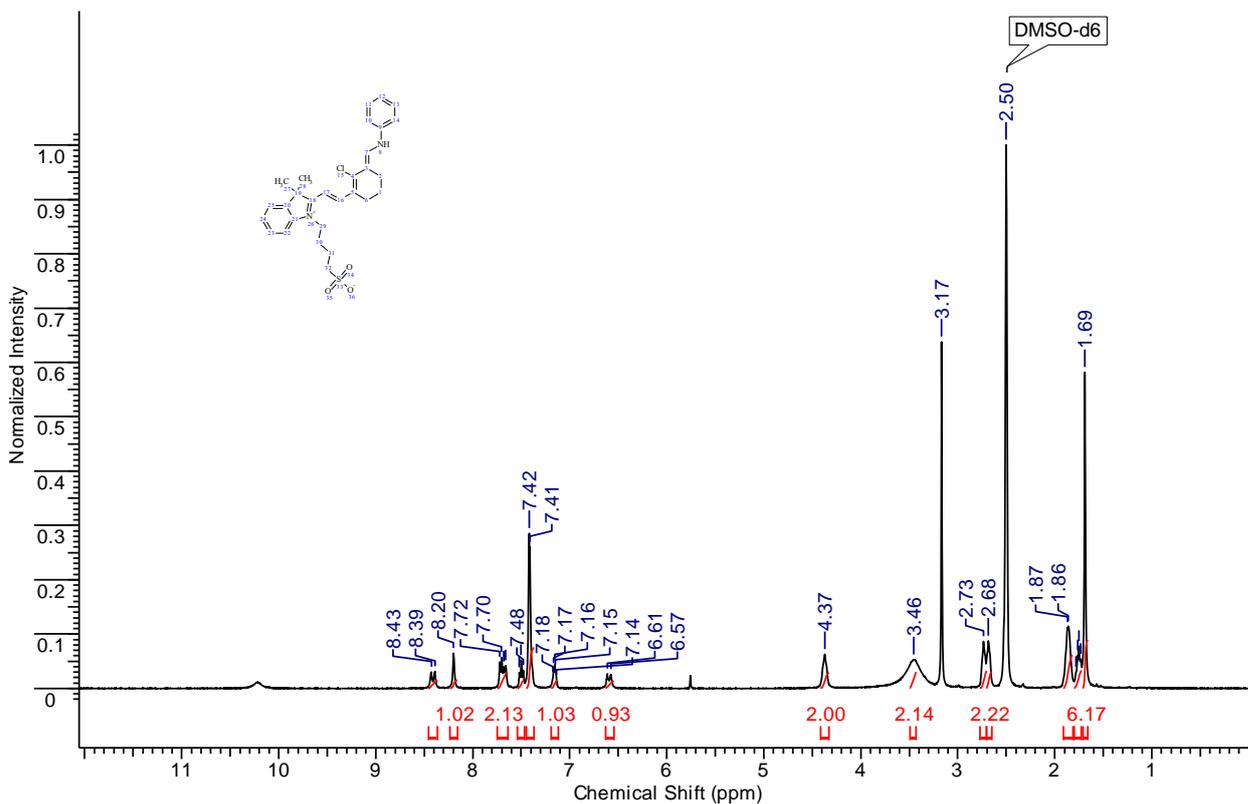


Figure S5 ^1H NMR spectrum of compound **1e**.

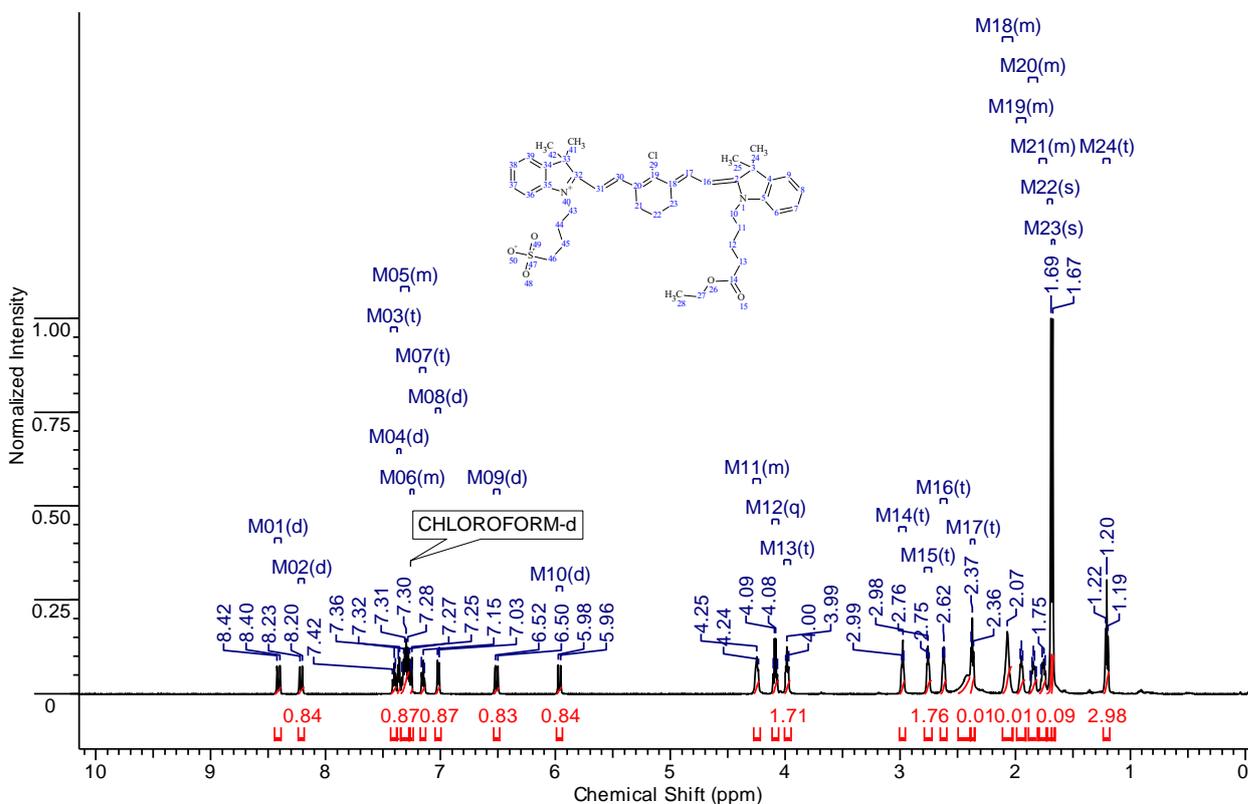


Figure S6 ^1H NMR spectrum of compound **1b**.

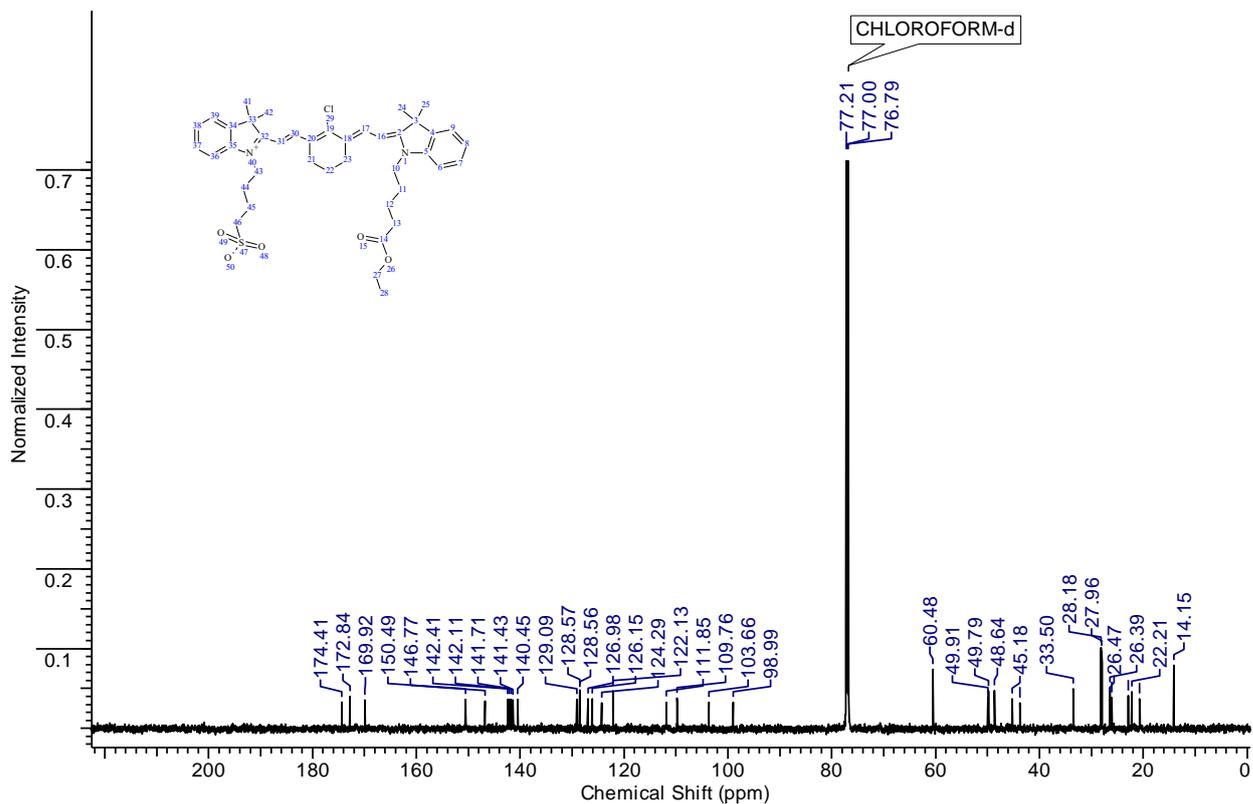


Figure S7 ^{13}C NMR spectrum of compound **1b**.

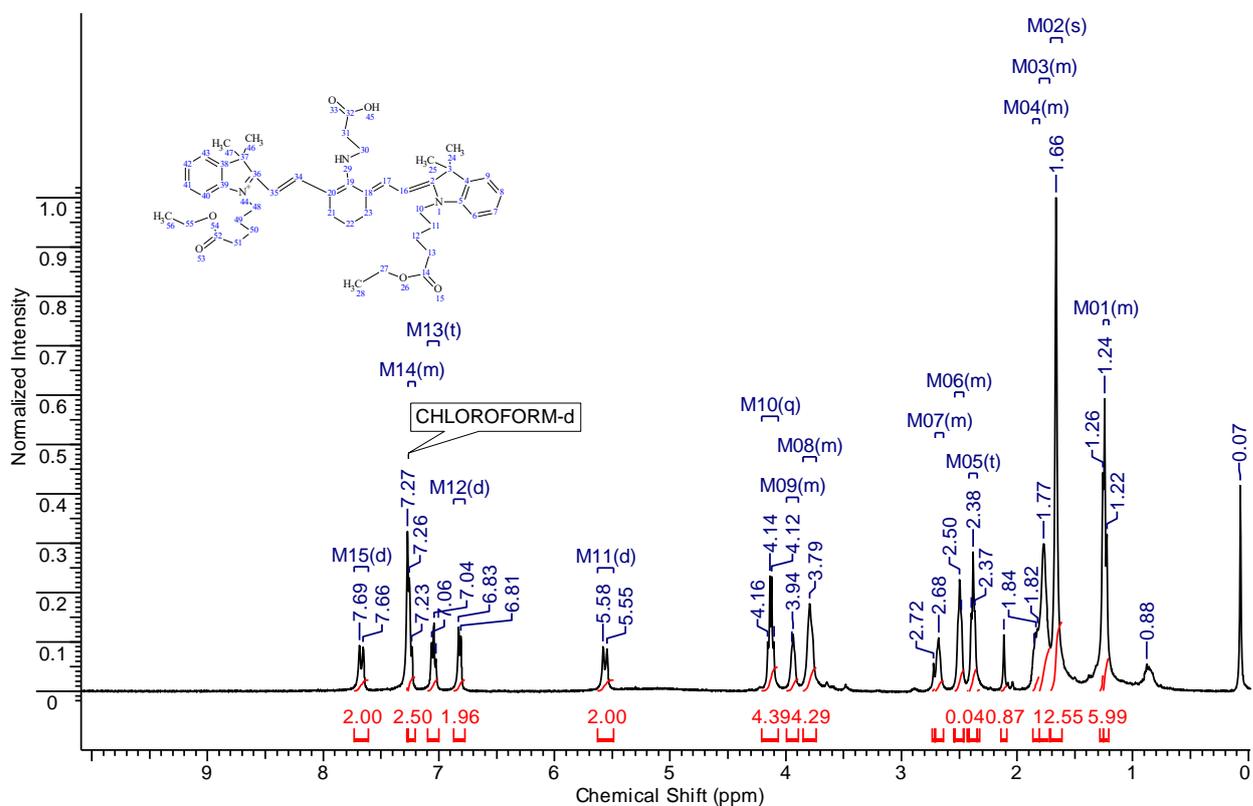


Figure S8 ^1H NMR spectrum of compound **2a**.

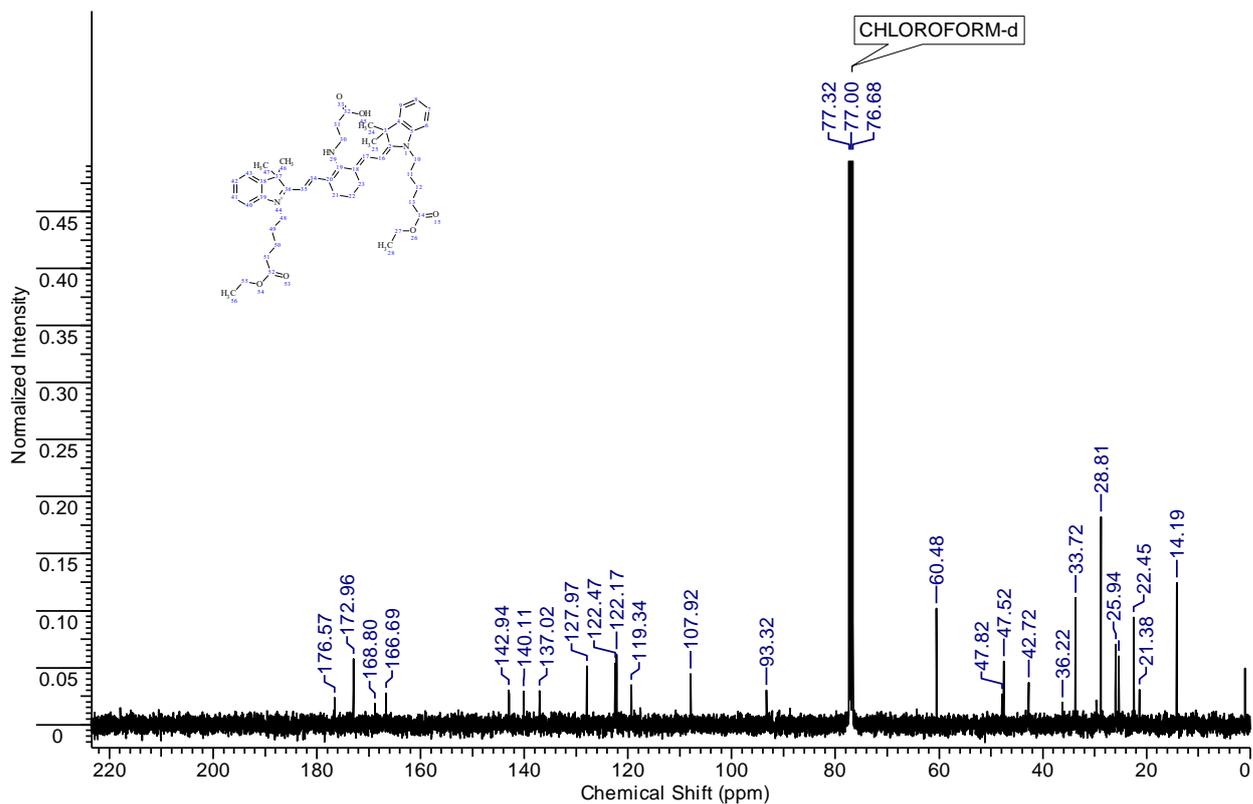


Figure S9 ^{13}C NMR spectrum of compound **2a**.

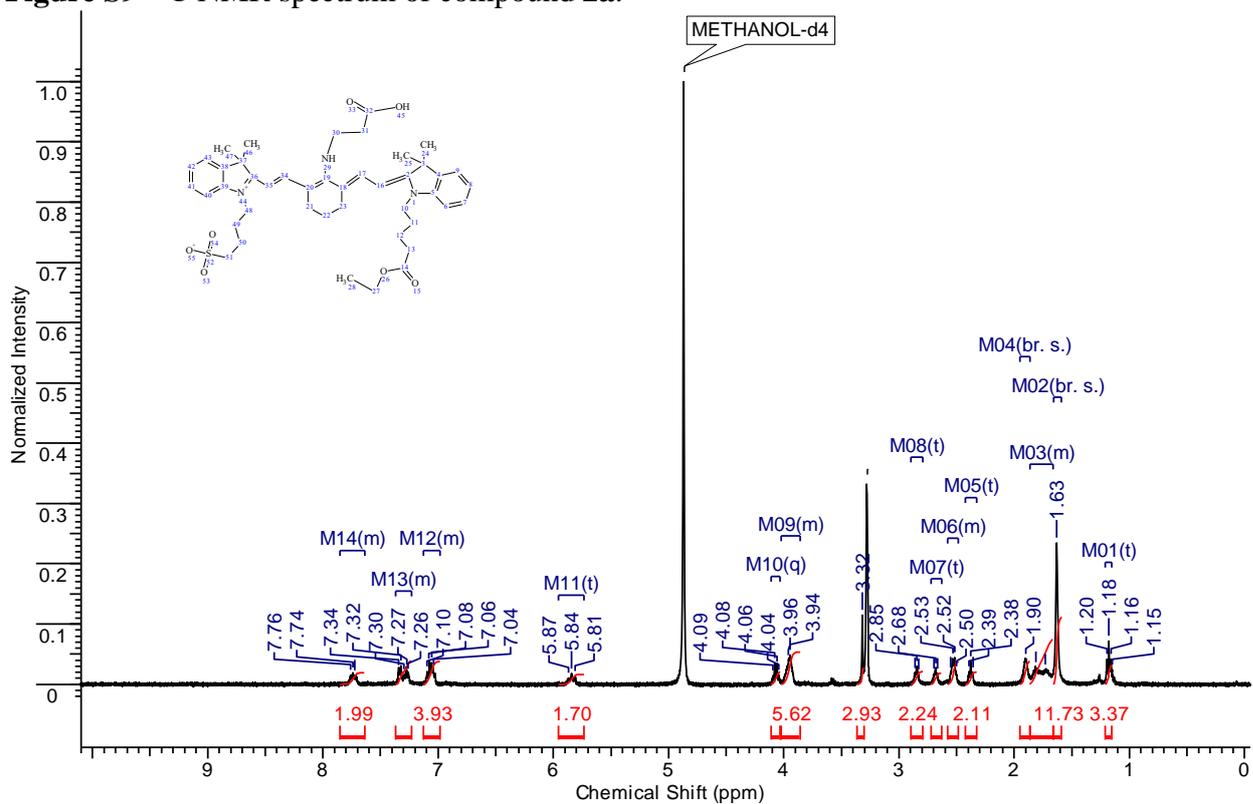


Figure S10 ^1H NMR spectrum of compound **2b**.

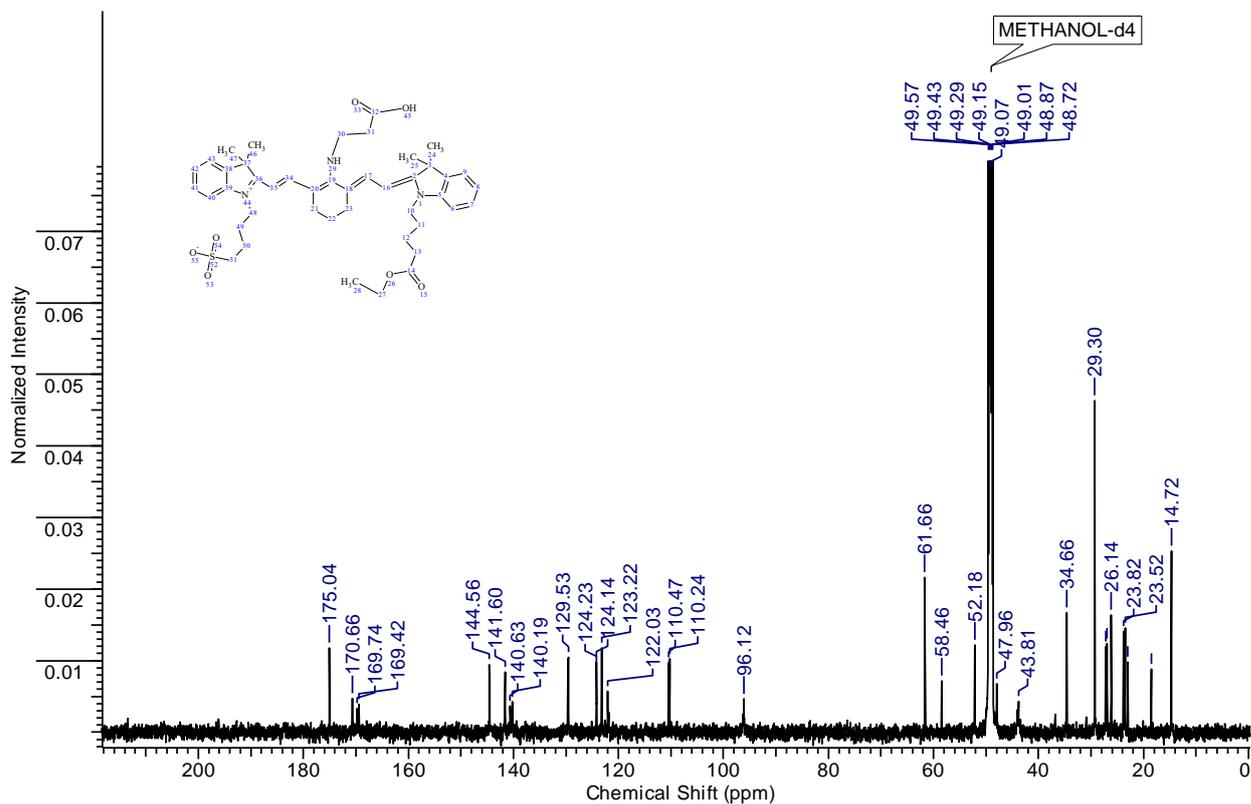


Figure S11 ^{13}C NMR spectrum of compound **2b**.

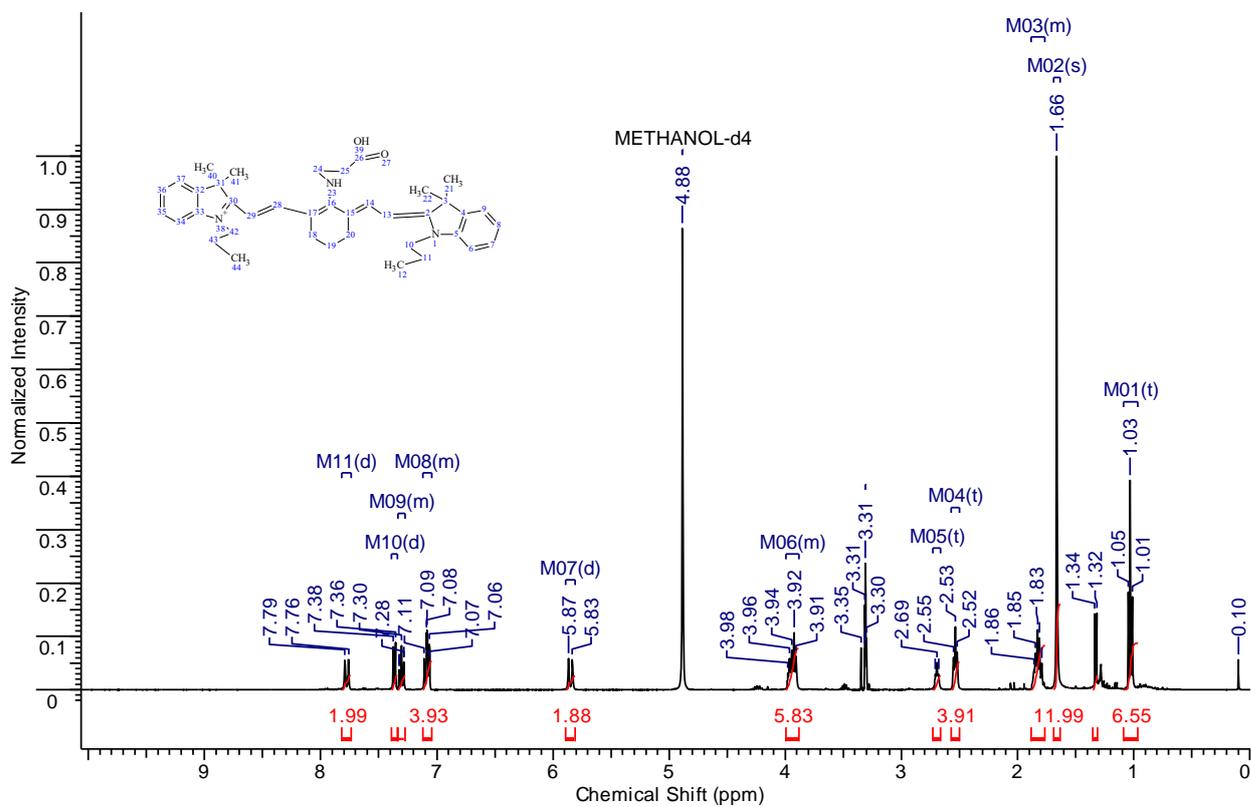


Figure S12 ^1H NMR spectrum of compound **2c**.

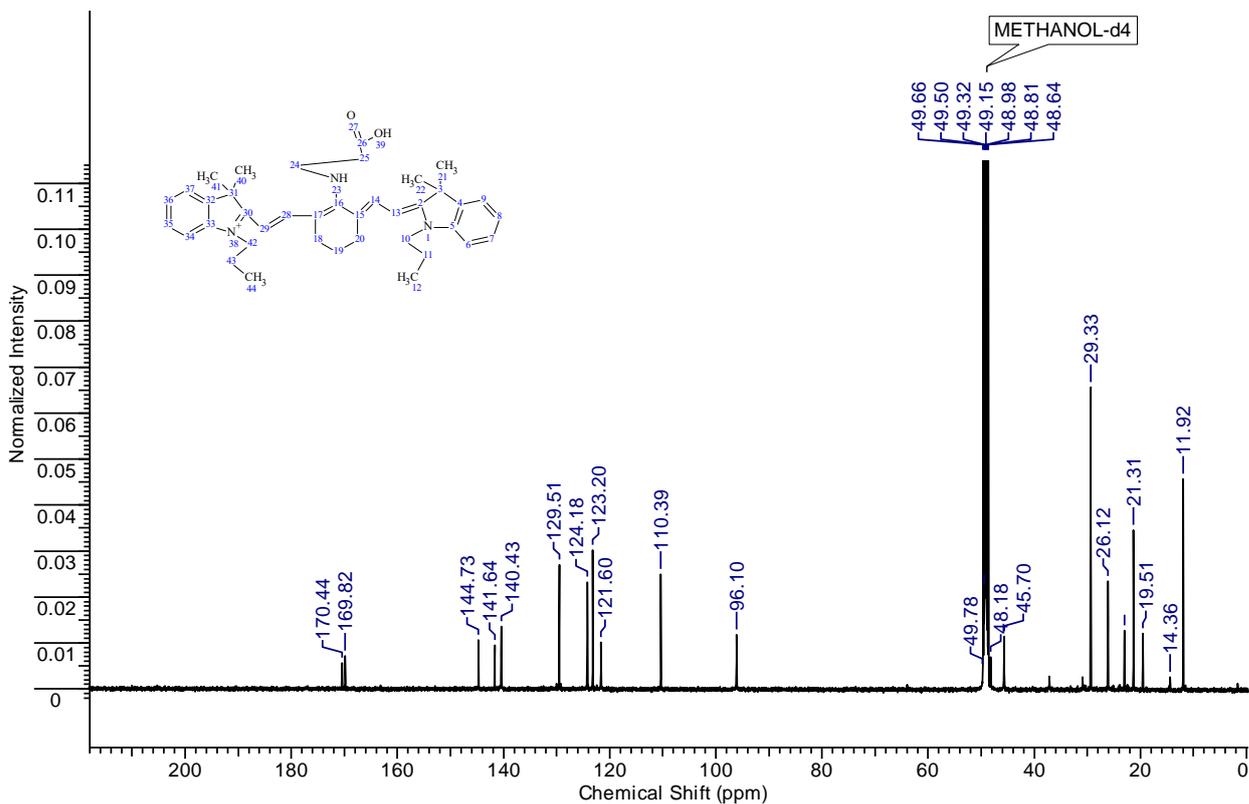


Figure S13 ^{13}C NMR spectrum of compound **2c**.

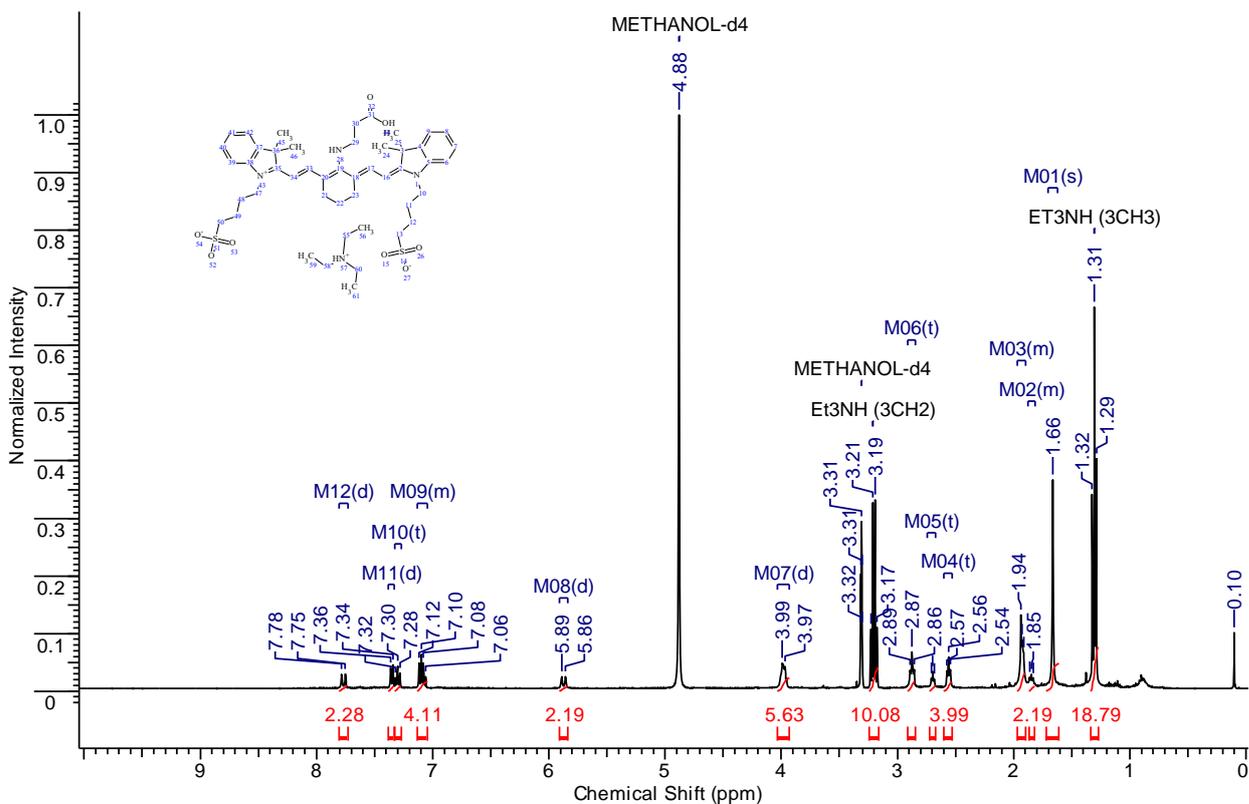


Figure S14 ^1H NMR spectrum of compound **2d**.

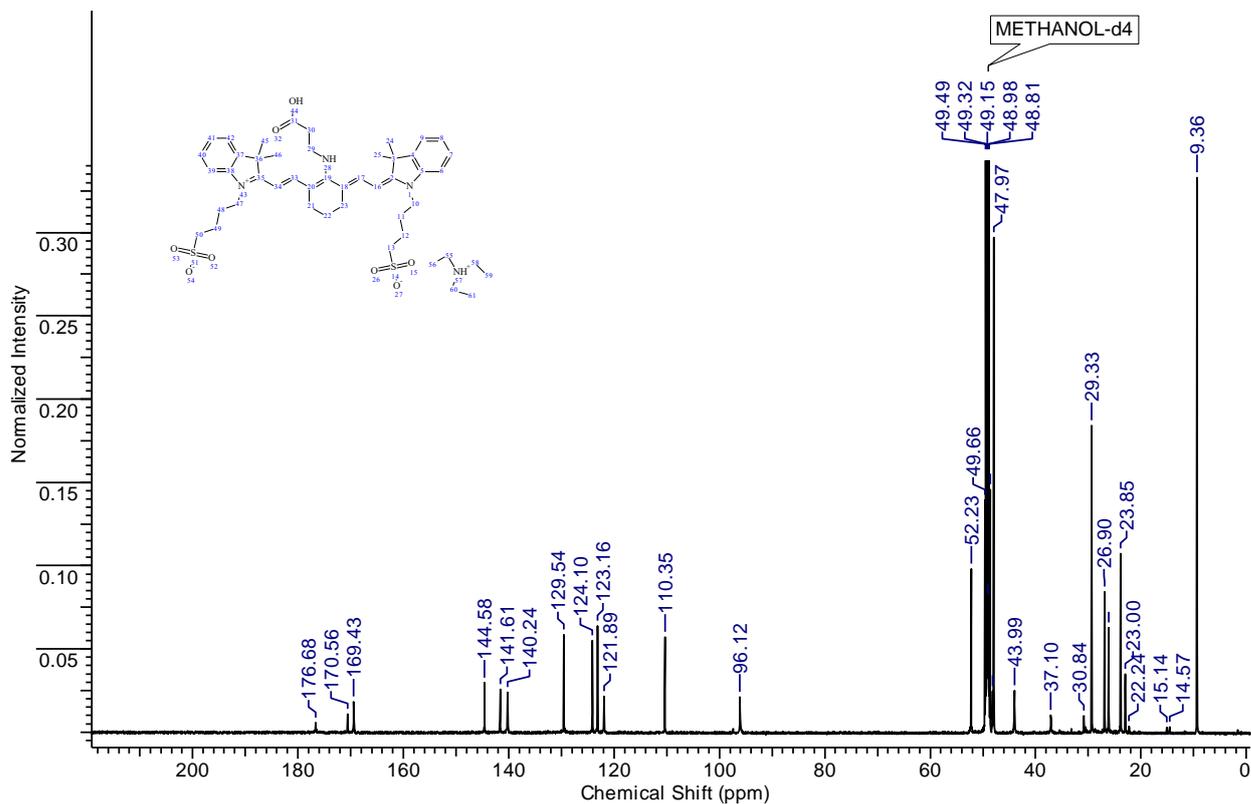


Figure S15 ^{13}C NMR spectrum of compound **2d**.

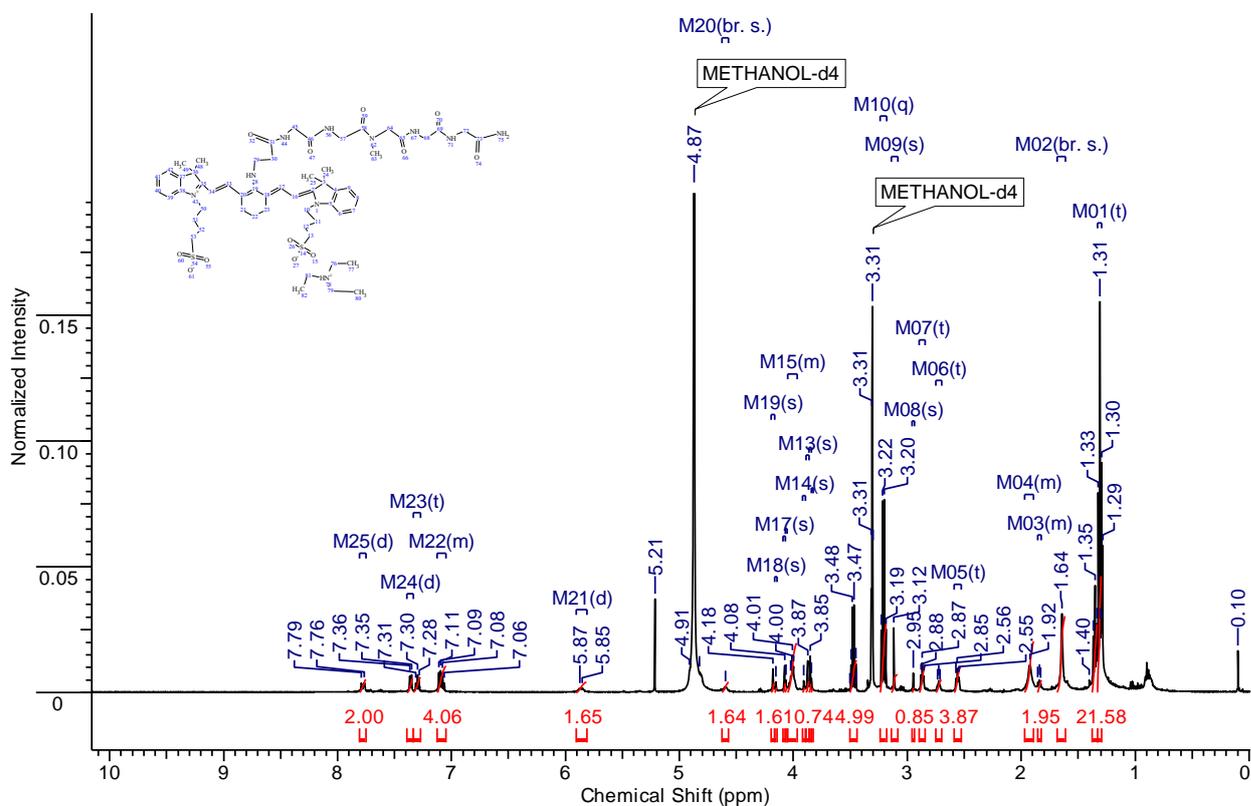


Figure S16 ^1H NMR spectrum of compound **3**.

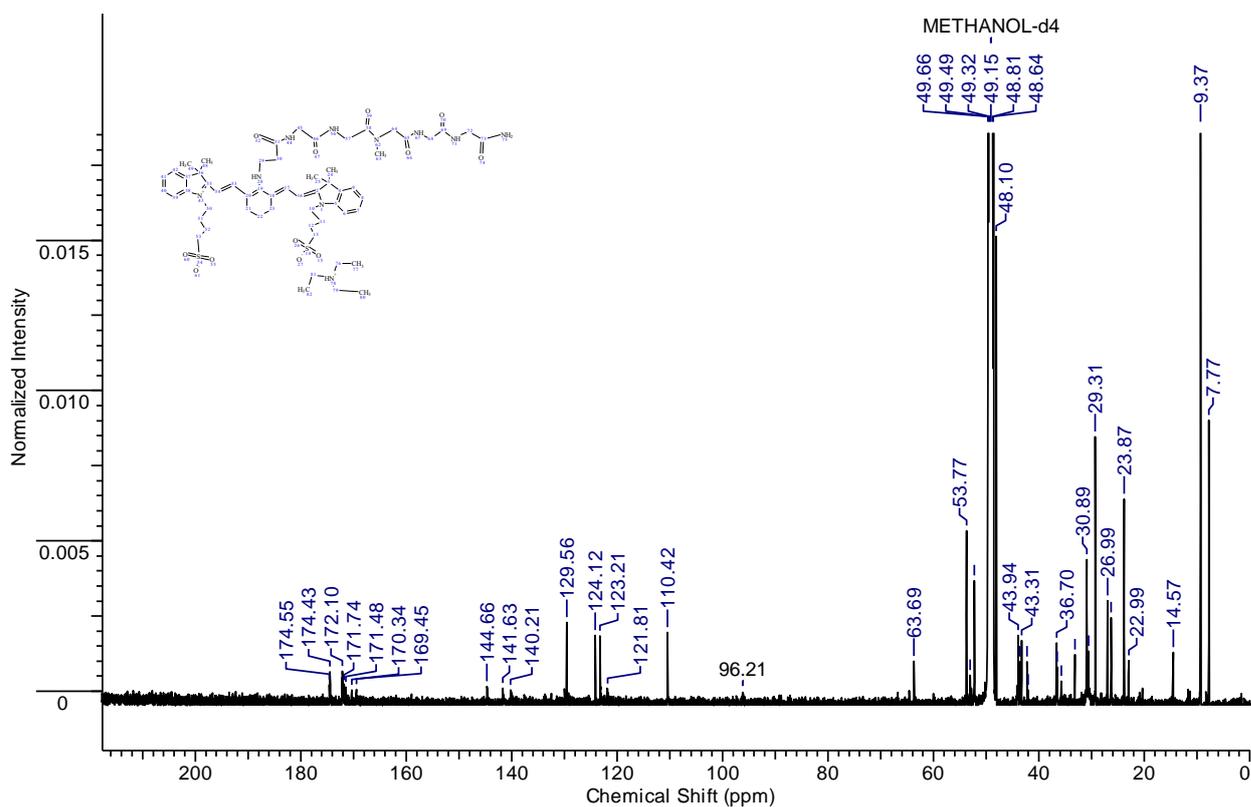


Figure S17 ^{13}C NMR spectrum of compound **3**.

2. Fluorescence intensity of compounds **2a-d**, **3**.

Fluorescence measurements of dyes with HCT116 cells in DMED medium with glucose 4.5 g dm^{-3} with glutamine were carried out on a Molecular Devices SpectraMax i3 plate photometer. $10 \mu\text{l}$ of a compound with a concentration of **2a** - $1.2 \times 10^{-4} \text{ mol dm}^{-3}$, **2b** - $1.3 \times 10^{-4} \text{ mol dm}^{-3}$, **2c** - $1.4 \times 10^{-4} \text{ mol dm}^{-3}$, **2d** - $1.3 \times 10^{-4} \text{ mol dm}^{-3}$ and **3** - $0.8 \times 10^{-4} \text{ mol dm}^{-3}$ in DMSO was dropped into $100 \mu\text{l}$ of medium with a cell line and further recorded the value of the fluorescence of the substrates.

Table S1 Fluorescence intensity of compounds **2a-d**, **3** in HCT116 cells.

	λ_{fl} , nm	Fluorescence of cells before washing	Fluorescence of cells after washing	Fluorescence of the medium
2a	750	566758	68320	1010286
2b	750	1626891	78301	1418616
2c	760	1288686	103224	1010286
2d	750	29286	5411	725050
3	730	38103	5421	718167

Table S2. Cytotoxicity of compounds **2a-d**.

Compound	IC ₅₀ , μm HCT116	IC ₅₀ , μm fibroblasts
2a	2.3	4.6
2b	>50	>50
2c	2.7	3.4
2d	>50	>50

Mean IC₅₀ values are calculated based on 3 measurements (MTT assay after 72 h cell exposure to compounds; standard deviations <10%).

3. Cytotoxicity tests.

Cell survival after 72 h exposure to **2a-d** was determined in a colorimetric MTT-test as described [S3].

References

- [S1] N.-Y. Kang, S.-J. Park, X. W. E. Ang, A. Samanta, W. H. P. Driessen, V. Ntziachristos, K. O. Vasquez, J. D. Peterson, S.-W. Yun and Y.-T. Chang, *Chem. Commun.*, 2014, **50**, 6589.
- [S2] O. A. Okoh, R. H. Bisby, C. L. Lawrence, C. E. Rolph and R. B. Smith, *J. Sulfur Chem.*, 2014, **35**, 42.
- [S3] A. S. Tikhomirov, V. A. Litvinova, D. V. Andreeva, L. G. Dezhenkova, Y. L. Volodina, D. N. Kaluzhny, V. B. Tsvetkov, I. D. Treshalin, D. Schols, A. A. Shtil and A. E. Shchekotikhin, *Eur. J. Med. Chem.*, 2020, **199**, 112294.