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Derivatization of a rigid *meso*-substituted heptamethine cyanine dye

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All chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA) or Acros Organics (Pittsburgh, PA, USA). All chemicals and solvents were of American Chemical Society grade or HPLC purity and were used as received. The ^{1}H NMR spectra were recorded with a Bruker AMX-400 spectrometer (400 MHz) (Bruker, USA) in D_2O and DMSO-d_6 solutions. Chemical shifts (δ) are given in ppm. Coupling constants (*J*) are given in Hz. Multiplicities of the signals: s, singlet; d, doublet; t, triplet; m, multiplet. The mass spectroscopic analysis was performed with a 4800 Plus MALDI-TOF mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA). Mass spectra were recorded in the linear mode for positive ions. Fluorescence spectra were recorded using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Santa Clara, CA). UV spectra were measured using a Jasco V-550 spectrophotometer (JASCO International Co., Tokyo, Japan). The pH values were determined using a Thermo Orion 330 pH-meter (Thermo Scientific, Waltham, MA). Thin layer chromatography (TLC) was used as a quality control check of dyes and dye-labeled deoxyuridine triphosphate to ensure chemical purity ~95%. TLC was performed using silica gel 60 RP-18 F254S plates from Merck (Darmstadt, Germany) with mobile phase: MeCN/0.1 M TEAHC 30/70 v/v.

The quantum yield of fluorophores was measured at 25°C using a reference compound with a known quantum yield [V. E. Shershov *et al*, *Dyes Pigm.*, 2013, **97**, 353; F. Song *et al*, *J. Photochem. Photobiol., A*, 2004, **168**, 53]. Photo- and thermal stability were determined according to our previously published procedure [V. E. Shershov *et al*, *Mendeleev Commun.*, 2017, **27**, 360; M. A. Spitsyn *et al*, *Dyes Pigm.*, 2017, **147**, 199].

1-Ethyl-2,3,3-trimethylindoleninium-5-sulfonate 1 was synthesized according to the literature [V. E. Shershov *et al*, *Dyes Pigm.*, 2013, **97**, 353].[8] Fluorescently labeled 5-(3-aminoallyl)-2′-deoxyuridine 5′-triphosphate, PCR and Biochip Hybridization Analysis were performed according to our previously published procedure [V. E. Shershov *et al*, *Mendeleev Commun.*, 2017, **27**, 360; M. A. Spitsyn *et al*, *Dyes Pigm.*, 2017, **147**, 199].

***N*-[5-Anilino-3-chloro-2,4-(propane-1,3-diyl)-2,4-pentadien-1-ylidene]anilinium chloride 2** [A. Levitz *et al*, *Molecules*, 2018, **23**, 226]. Phosphorus oxychloride (0.1 mol, 11 ml) was added dropwise to a DMF (0.17 mol, 13 ml) at 0°C for 30 min under inert conditions. Then cyclohexanone (5.5 ml, 0.053 mol) was added to the obtained solution keeping the temperature below 45ºC. After addition was complete, the resulting mixture was heated at 90°C for 3 h. The solution was allowed to cool to room temperature and then aniline (98.57 mmol, 9 ml) in ethanol (9 ml) was added, and this was stirred at room temperature for 30 min. The resulting mixture was poured into a cooled (5°C) 1 N HCl and allowed to stand for 12 h at 5°C. The crude solid was collected and washed with cold water and dried to constant mass. The deep purple solid obtained (12.25 g, 64%) was used without further purification. MS (MALDI-TOF) *m/z*: calcd. for $C_{20}H_{20}ClN_2^+$ 323.84; found 324.4 $[M]^+$. ^{1}H NMR (DMSO-d_6) δ: 2.51 (m, 2H, $CH_2CH_2CH_2$), 2.77 (m, 4H, $CH_2CH_2CH_2$), 7.26 (t, 2H, Ar(4,4')*H*, *J* = 7.4 Hz), 7.45 (t, 4H, Ar(3,3')*H*, *J* = 7.4 Hz), 7.63 (t, 4H, Ar(2,2')*H*, *J* = 7.6 Hz), 8.52 (s, 2H, NC*H*). Anal. Calcd. for $C_{20}H_{20}Cl_2N_2$ (%): C, 66.86; H, 5.61; N, 7.80. Found: C, 67.64; H, 5.49; N, 7.89.

2-[(*E*)-2-((*E*)-2-Chloro-3-[(*E*)-2-{(3,3-dimethyl-5-sulfonato-1-ethyl)indolin-2-ylidene)ethylidene}]cyclohex-1-enyl)vinyl]-3,3-dimethyl-1-ethyl-3*H*-indoleninium-5-sulfonate, soduim salt (**3**)**.** A solution of indolenine **1** (3.2 mmol, 855 mg) and *N*-[5-anilino-3-chloro-2,4-(propane-1,3-diyl)-2,4-pentadien-1-ylidene]anilinium chloride **2** (1.6 mmol, 575 mg) in a mixture of ethanol (12 ml) and acetic anhydride (8 ml) in presence of potassium acetate (6.4 mmol, 0.64 g) was heated to 60°C for 7 h. The solvent was evaporated, and the cyanine dye was purified using reversed-phase (C18) chromatography. The cyanine dye was dissolved in 0.1 M triethylammonium acetate buffer (TEAA) and loaded onto the column. Elution of the dye was carried out using a linear gradient from 0.1 M TEAA to 50% MeCN in 0.1 M TEAA. Pure dye obtained as a triethylammonium salt was converted into its sodium salt in the following fashion: the solution of dye in Milli-Q water was loaded onto the C18-RP column, washed with 0.1 M NaCl and Milli-Q water, and eluted with an acetonitrile-Milli-Q water mixture. The yield of dye was 480 mg (43%). MS (MALDI-TOF) *m/z*: calcd. for $C_{34}H_{38}ClN_2O_6S_2^-$ 670.26; found 671.7 $[M]^+$. R_f (CH_3CN/0.1 M TEAA = 1:1) = 0.52. ^{1}H NMR (DMSO-d_6) δ: 1.27 (t, 6H, CH_2CH_3, *J* = 7.0 Hz), 1.64 (s, 12H, C(3,3)H_3, C(3',3')H_3), 1.82 (m, 2H, $CH_2CH_2CH_2$), 2.68 (m, 4H, $CH_2CH_2CH_2$), 4.22 (m, 4H, CH_2CH_3), 6.32 (d, 2H, α,α'-CH, *J* = 14.0 Hz), 7.36 (d, 2H, Ar(7,7')*H*, *J* = 8.0 Hz), 7.66 (dd, 2H, Ar(6,6')*H*, *J* = 8.0 Hz), 7.77 (d, 2H, Ar(4,4')*H*), 8.25 (d, 2H, β,β'-CH, *J* = 14.0 Hz). ^{13}C NMR (DMSO-d_6): 172.53, 148.53, 146.25, 143.48, 142.08, 141.09, 126.98, 126.79, 120.42, 110.89, 102.10, 49.49, 27.81, 26.37, 20.84, 12.65. Anal. Calcd. for $C_{34}H_{38}ClN_2NaO_6S_2$ (%): C, 58.91; H, 5.52; N, 4.04. Found: C, 58.24; H, 5.61; N, 4.11.

5-Aminosulfonyl-1-ethyl-3,3-dimethyl-2-[(*E*)-2-((*E*)-2-chloro-3-[(*E*)-2-{(3,3-dimethyl-5-aminosulfonyl-1-ethyl)indoline-2-ylidene)ethylidene}]cyclohex-1-enyl)vinyl]-3*H*-indolenin-1-ium chloride (**5**). A mixture of *meso*-chloro cyanine dye **3** (0.29 mmol, 200 mg) and phosphorus oxychloride (4 ml, 36 mmol) was heated to 80°C for 2 h to provide conversion into bis-sulfonyl chloride **4**. The solvent was evaporated, and the acetonitrile (8 ml) was added. The

solution obtained was cooled to -18°C, and concentrated ammonium hydroxide (10 ml) was added dropwise. The reaction mixture is warmed up to ambient temperature within 30 min and stirred for next 1 h. The solvent was evaporated, and the residue was dissolved in methanol (30 ml) and filtered to remove water-soluble inorganic salts. After that, the solvent was evaporated, and dye **5** was used directly in the next reaction step without further purification. R_f (MeCN/0.1 M TEAA = 1:1) = 0.09.

Disodium salt of 4-hydroxybenzenesulfonic acid. A solution of sodium hydroxide (6 mmol, 0.24 g) in water (4 ml) was added to a suspension of a monosodium salt of 4-hydroxybenzenesulfonic acid dihydrate (6.6 mmol, 1.5 g) in water (4 ml). The solvent was evaporated, and the residue was triturated with acetone (50 ml). The white crystals formed were filtered and dried in a vacuum desiccator over P_2O_5 to give 1.34 g (93%) of the phenolate.

5-Aminosulfonyl-1-ethyl-3,3-dimethyl-2-[(*E*)-2-((*E*)-2-(4-sulfonatophenyloxy)-3-[(*E*)-2-{(3,3-dimethyl-5-aminosulfonyl-1-ethyl)indoline-2-ylidene)ethylidene}]cyclohex-1-enyl)vinyl]-3*H*-indolenin-1-ium 6. A mixture of bis-sulfonamide **5** (0.29 mmol) and disodium salt of 4-hydroxybenzenesulfonic acid (1.43 mmol, 312 mg) in anhydrous DMSO (10 ml) was heated at 70°C with stirring under inert atmosphere for 9 h. The solution was diluted with 30% acetonitrile in 0.1 M TEAA, and the cyanine dye was purified using reversed-phase (C18) chromatography. Elution of the dye was carried out using a 45% acetonitrile in 0.1 M TEAA. The dye solutions was diluted with 0.1 M TEAA, loaded onto the C18-RP column, washed with 0.1 M NaCl and Milli-Q water, and eluted with an 60% MeOH in Milli-Q water. The yield of *meso*-sulfophenoxy dye **6** was 33 mg (14%). MS (MALDI-TOF) *m/z*: calcd. for $C_{40}H_{46}N_4O_8S_3$ 807.01; found 808.5 $[M]^+$. R_f (MeCN/0.1 M TEAA = 1:1) = 0.4. ^{1}H NMR (DMSO-d_6) δ: 1.22 (t, 6H, CH_2CH_3, *J* = 7.0 Hz), 1.29 (s, 12H, C(3,3)H_3, C(3',3')H_3), 1.91 (m, 2H, $CH_2CH_2CH_2$), 2.71 (m, 4H, $CH_2CH_2CH_2$), 4.17 (m, 4H, CH_2CH_3), 6.25 (d, 2H, α,α'-CH, *J* = 14.0 Hz), 7.07 (d, 2H, Ar(2",6")*H*, *J* = 9.0 Hz), 7.27 (s, 4H, SO_2NH_2), 7.50 (d, 2H, Ar(7,7')*H*, *J* = 8.5 Hz), 7.59 (d, 2H, Ar(3",5")*H*, *J* = 9.0 Hz), 7.85 (m, 6H, Ar(6,6')*H*, Ar(4,4')*H*, β,β'-CH). ^{13}C NMR (DMSO-d_6): 172.22, 163.99, 159.77, 144.65, 143.81, 141.92, 141.81, 140.71, 128.43, 127.35, 123.55, 120.41, 114.10, 111.55, 101.58, 49.08, 27.56, 24.28, 21.06, 12.48. Anal. Calcd. for $C_{40}H_{46}N_4O_8S_3$ (%): C, 59.53; H, 5.75; N, 6.94. Found: C, 59.41; H, 5.86; N, 6.87.

5-Aminosulfonyl-1-ethyl-3,3-dimethyl-2-[(*E*)-2-((*E*)-2-(4-sulfonatophenyloxy)-3-[(*E*)-2-{(3,3-dimethyl-5-acetylaminosulfonyl-1-ethyl)indoline-2-ylidene)ethylidene}]cyclohex-1-enyl)vinyl]-3*H*-indolenin-1-ium (**7**)**.** The *meso*-sulfophenoxy dye **6** (41 μmol, 33 mg) was reacted with acetic anhydride (0.3 ml) in DMF (1.5 ml) in the presence of *N*,*N*-diisopropylethylamine (10 μl) at 50°C. After 30 min, the mixture was diluted with 25% acetonitrile in 0.1 M TEAA, and the cyanine dye was purified using reversed-phase (C18) chromatography. Elution of the dye was carried out using a 40% acetonitrile in 0.1 M TEAA. The dye solutions was diluted with 0.1 M TEAA, loaded onto the C18-RP column, washed with 0.1 M NaCl and Milli-Q water, and eluted with an 45% MeOH in Milli-Q water. The yield of *N*-acetylaminosulfonyl **8** was 14 mg (40%). MS (MALDI-TOF) *m/z*: calcd. for $C_{42}H_{48}N_4O_9S_3$

849.05; found 850.4 $[M]^+$. R_f (MeCN/0.1 M TEAA = 1:1) = 0.55. ^{1}H NMR (DMSO-d_6) δ: 1.26 (m, 6H, CH_2CH_3, *J* = 7.0 Hz), 1.34 (d, 12H, C(3,3)H_3, C(3',3')H_3), 1.85 (s, 3H, $COCH_3$), 1.95 (m, 2H, $CH_2CH_2CH_2$), 2.75 (m, 4H, $CH_2CH_2CH_2$), 4.20 (m, 4H, CH_2CH_3), 6.27 (dd, 2H, α,α'-CH, *J* = 14.5 Hz), 7.11 (d, 2H, Ar(2",6")*H*, *J* = 9.0 Hz), 7.32 (s, 2H, SO_2NH_2), 7.49 (d, 1H, Ar(7)*H*, *J* = 8.5 Hz), 7.56 (d, 2H, Ar(7')*H*, *J* = 8.5 Hz), 7.63 (d, 2H, Ar(3",5")*H*, *J* = 9.0 Hz), 7.88 (m, 6H, Ar(6,6')*H*, Ar(4,4')*H*, β,β'-CH). ^{13}C NMR (DMSO-d_6): 171.31, 163.90, 159.73, 145.73, 144.56, 143.81, 142.56, 142.00, 141.41, 141.11, 141.00, 129.50, 128.43, 127.35, 123.90, 123.63, 121.85, 120.44, 114.11, 111.84, 111.01, 102.18, 101.34, 49.29, 48.82, 27.58, 27.48, 24.28, 21.05, 12.55, 12.43. Anal. Calcd. for $C_{42}H_{48}N_4O_9S_3$ (%): C, 59.41; H, 5.70; N, 6.60. Found: C, 59.53; H, 5.78; N, 6.68.

5-(7-Carboxy-1-oxoheptylaminosulfonyl)-1-ethyl-3,3-dimethyl-2-[(*E*)-2-((*E*)-2-(4-sulfonato-phenyloxy)-3-[(*E*)-2-{(3,3-dimethyl-5-acetylaminosulfonyl-1-ethyl)indoline-2-ylidene)ethylidene}]cyclohex-1-enyl)vinyl]-3*H*-indolenin-1-ium 8. A mixture containing *N*-acetylaminosulfonyl **7** (16.5 μmol, 14 mg), octane-1,8-dioic acid (165 μmol, 29 mg), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (165 μmol, 32 mg), 4-dimethylaminopyridine (DMAP, 165 μmol, 20 mg), *N*,*N*-diisopropylethylamine (10 μl) and DMF (1.5 ml) was stirred at room temperature for 2 h in inert atmosphere. The mixture was diluted with 0.1 M TEAA (5 ml), and loaded onto the C18-RP column. Elution of the dye was carried out using a 20% acetonitrile in 0.1 M TEAA. The dye solutions was diluted with 0.1 M TEAA, loaded onto the C18-RP column, washed with 0.1 M NaCl and Milli-Q water, and eluted with an 7.5% acetonitrile in Milli-Q water. The yield of dye **8** was 7 mg (42%). MS (MALDI-TOF) *m/z*: calcd. for $C_{50}H_{60}N_4O_{12}S_3$ 1005.23; found 1006.6 $[M]^+$. R_f (MeCN/0.1 M TEAA = 1:1) = 0.58. ^{1}H NMR (DMSO-d_6) δ: 1.26 (t, 6H, CH_2CH_3, *J* = 7.0 Hz), 1.12, 1.41 (both m, 10H, $COCH_2CH_2CH_2CH_2CH_2CH_2COOH$), 1.32 (s, 12H, C(3,3)$H_3$, C(3',3')$H_3$), 1.71 (s, 3H, $COCH_3$), 1.97 (m, 2H, $CH_2CH_2CH_2$), 2.13 (t, 2H, $COCH_2CH_2CH_2CH_2CH_2CH_2COOH$, *J* = 7.0 Hz), 2.73 (m, 4H, $CH_2CH_2CH_2$), 4.18 (m, 4H, CH_2CH_3), 6.26 (d, 2H, α,α'-CH, *J* = 14.5 Hz), 7.11 (d, 2H, Ar(2",6")*H*, *J* = 9.0 Hz), 7.41 (d, 2H, Ar(7,7')*H*, *J* = 8.0 Hz), 7.63 (d, 2H, Ar(3",5")*H*, *J* = 9.0 Hz), 7.80 (m, 6H, Ar(6,6')*H*, Ar(4,4')*H*, β,β'-CH). ^{13}C NMR (DMSO-d_6): 176.14, 174.99, 173.59, 171.95, 163.31, 159.77, 144.15, 143.70, 141.02, 128.88, 128.50, 123.42, 121.63, 121.47, 114.25, 110.73, 101.62, 49.08, 38.42, 34.19, 28.89, 28.70, 27.65, 26.20, 25.61, 24.94, 24.40, 21.18, 12.61. Anal. Calcd. for $C_{50}H_{60}N_4O_{12}S_3$ (%): C, 59.74; H, 6.02; N, 5.57. Found: C, 59.61; H, 6.14; N, 5.63.

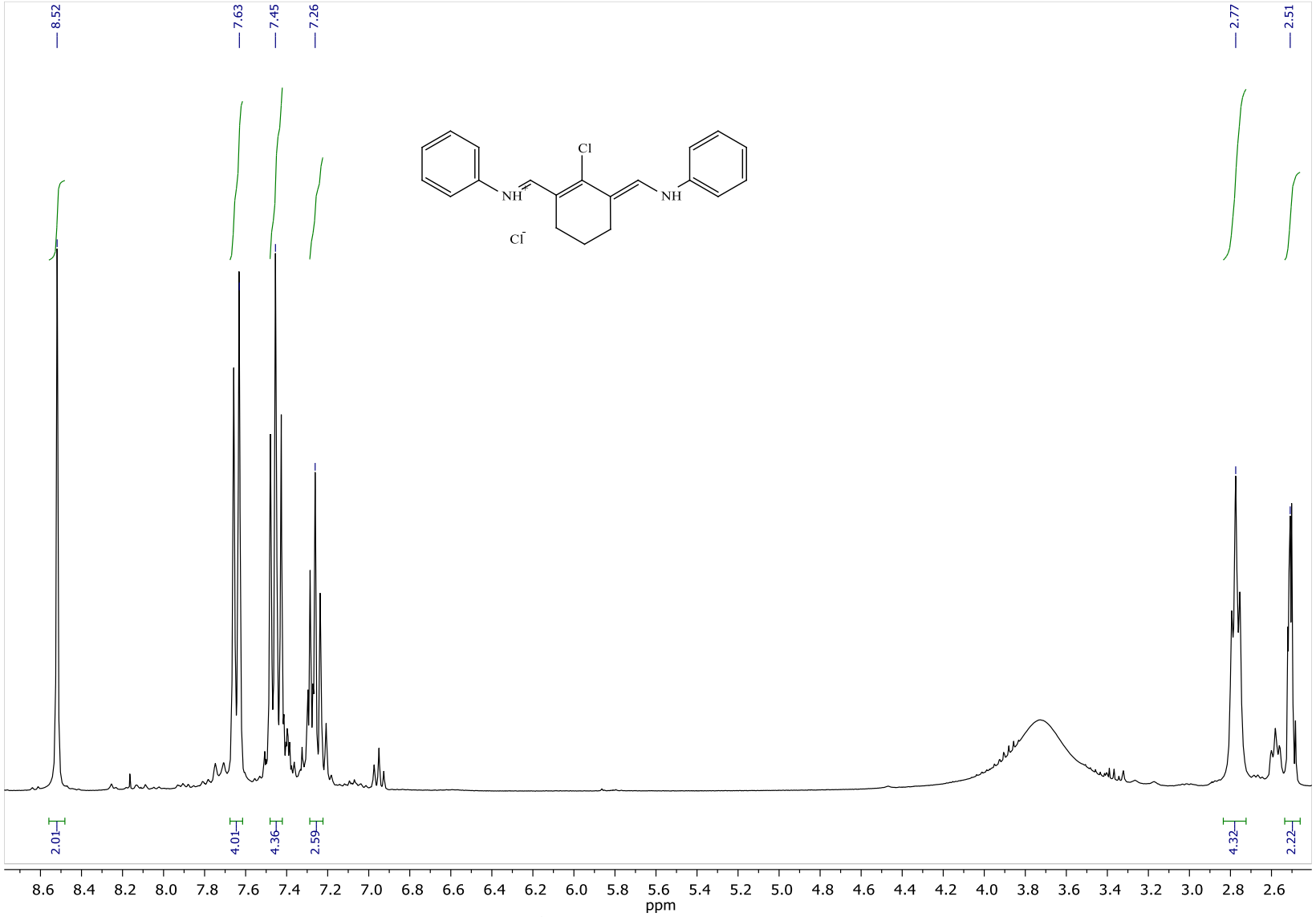

Figure S1. ^{1}H NMR spectrum of compound **2**.

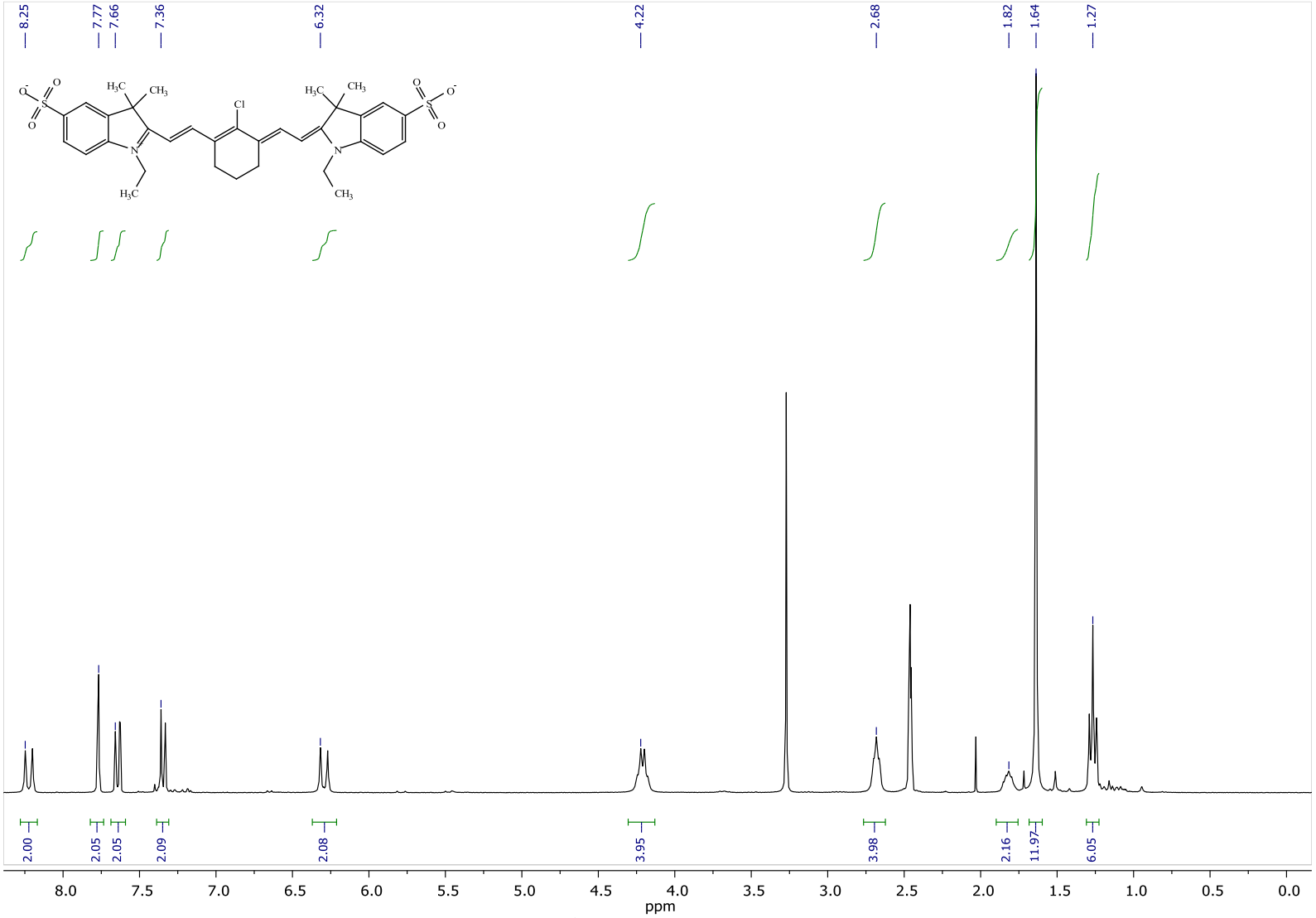

Figure S2. ^{1}H NMR spectrum of salt **3**.

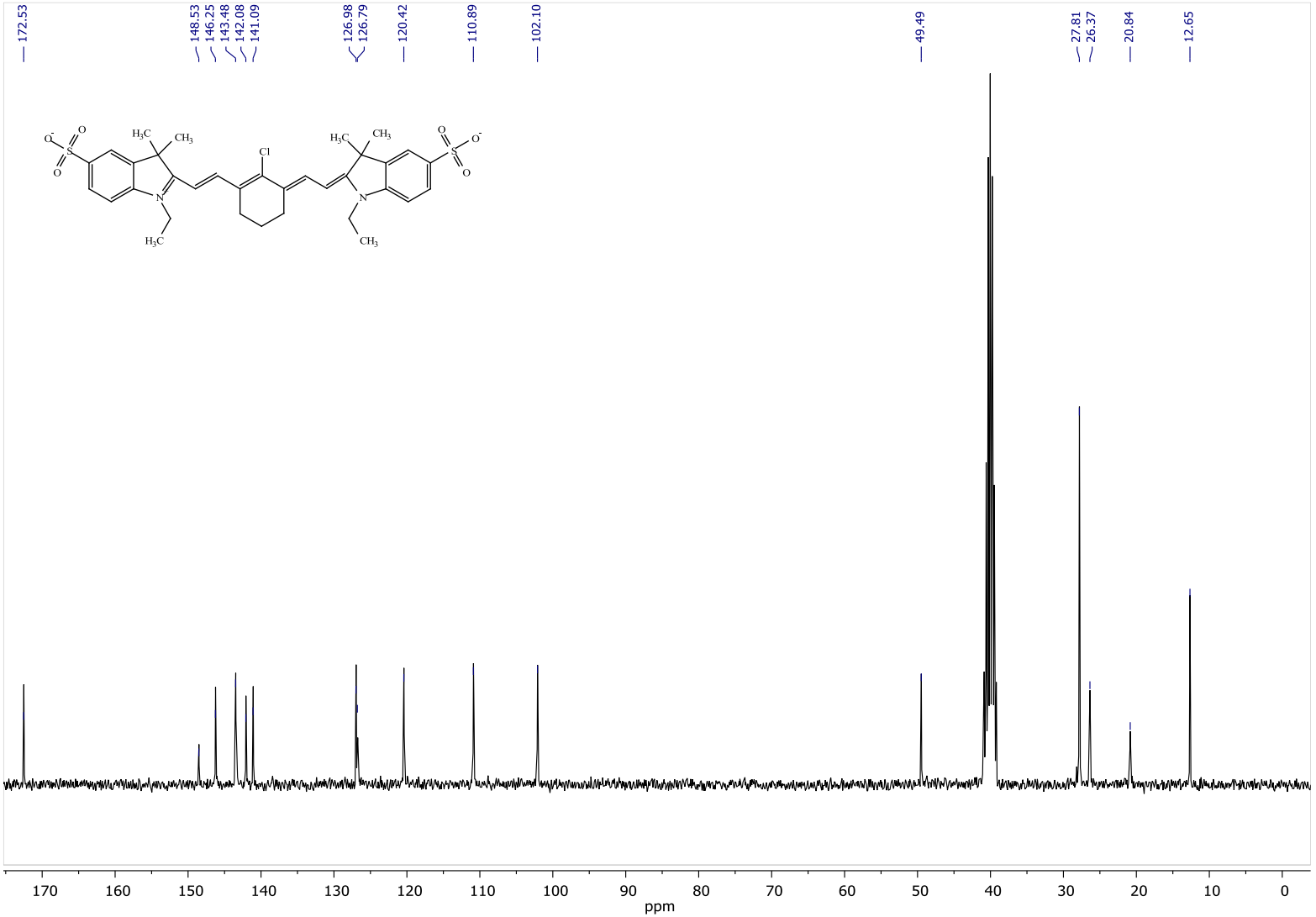

Figure S3. ^{13}C NMR spectrum of salt **3**.

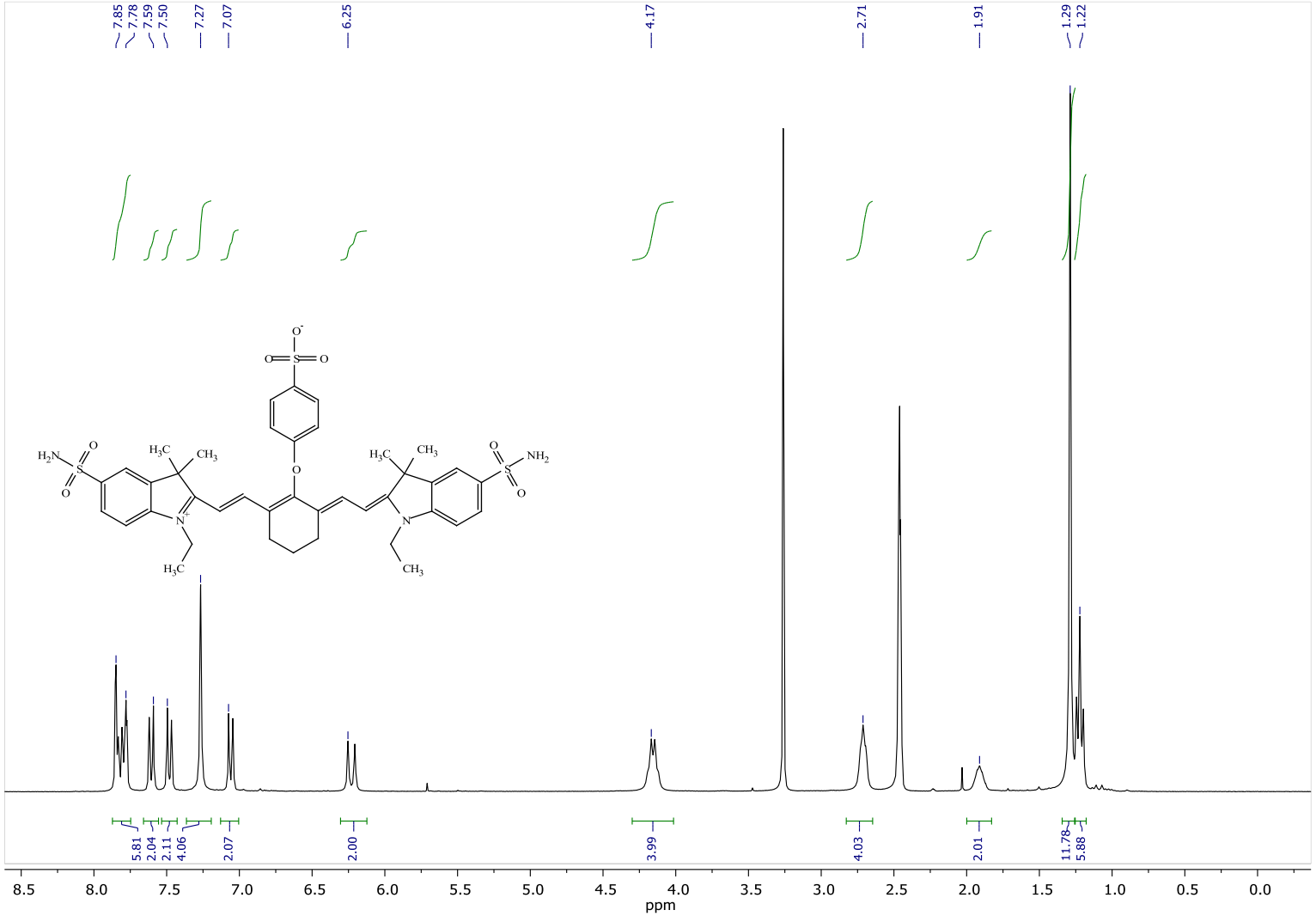

Figure S4. ^{1}H NMR spectrum of compound **6**.

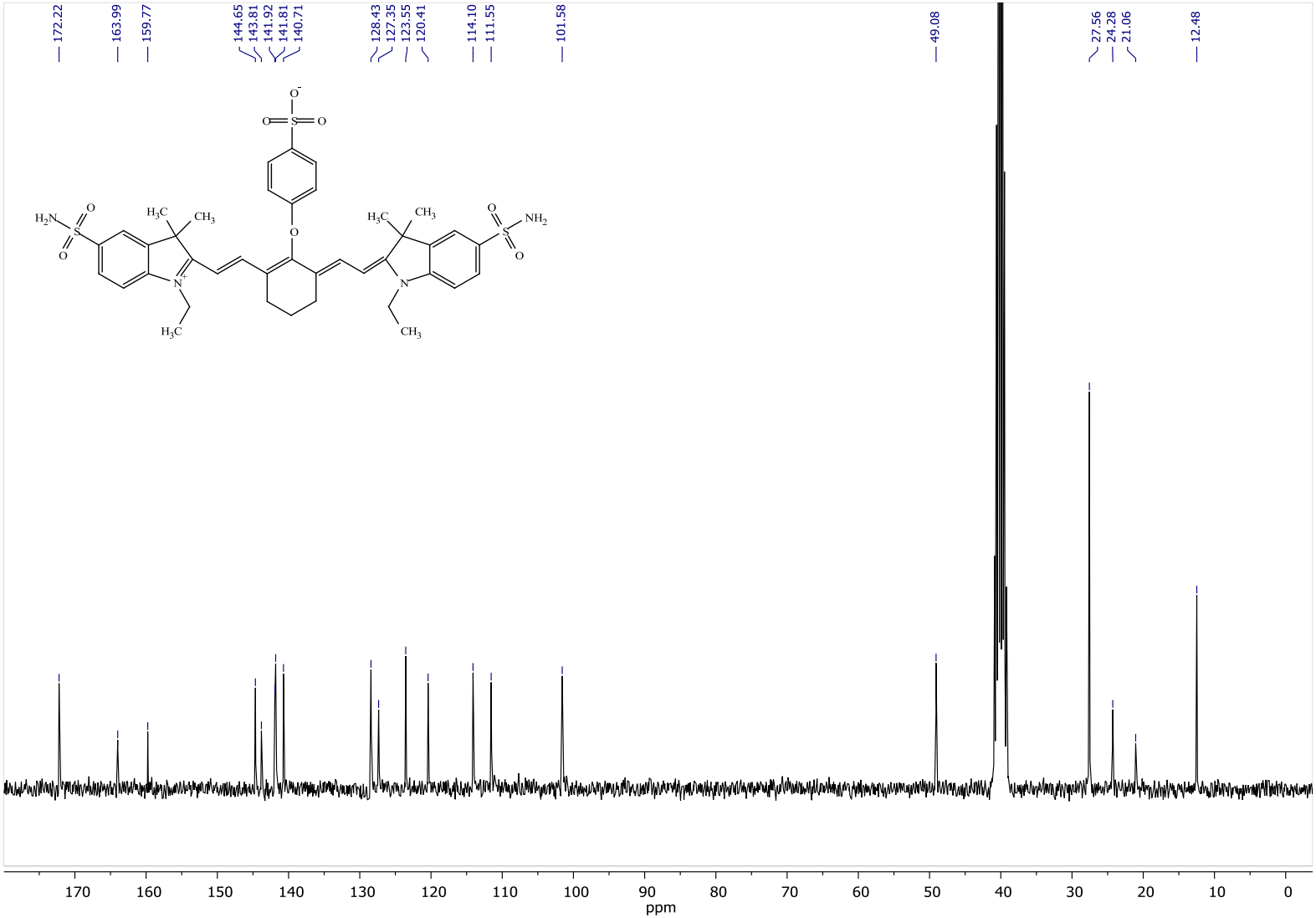

Figure S5. ^{13}C NMR spectrum of compound **6**.

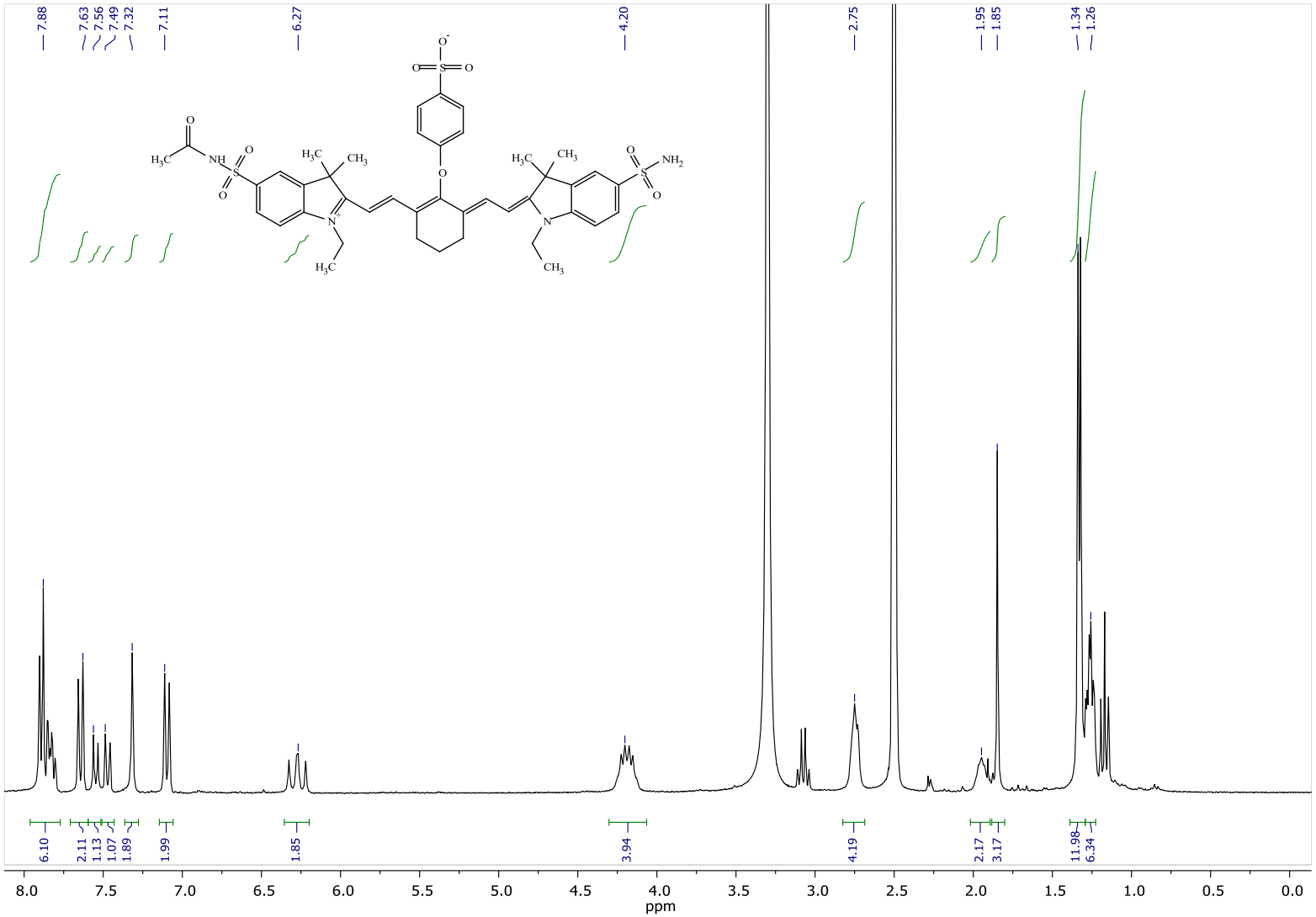

Figure S6. ^{1}H NMR spectrum of compound **7.**

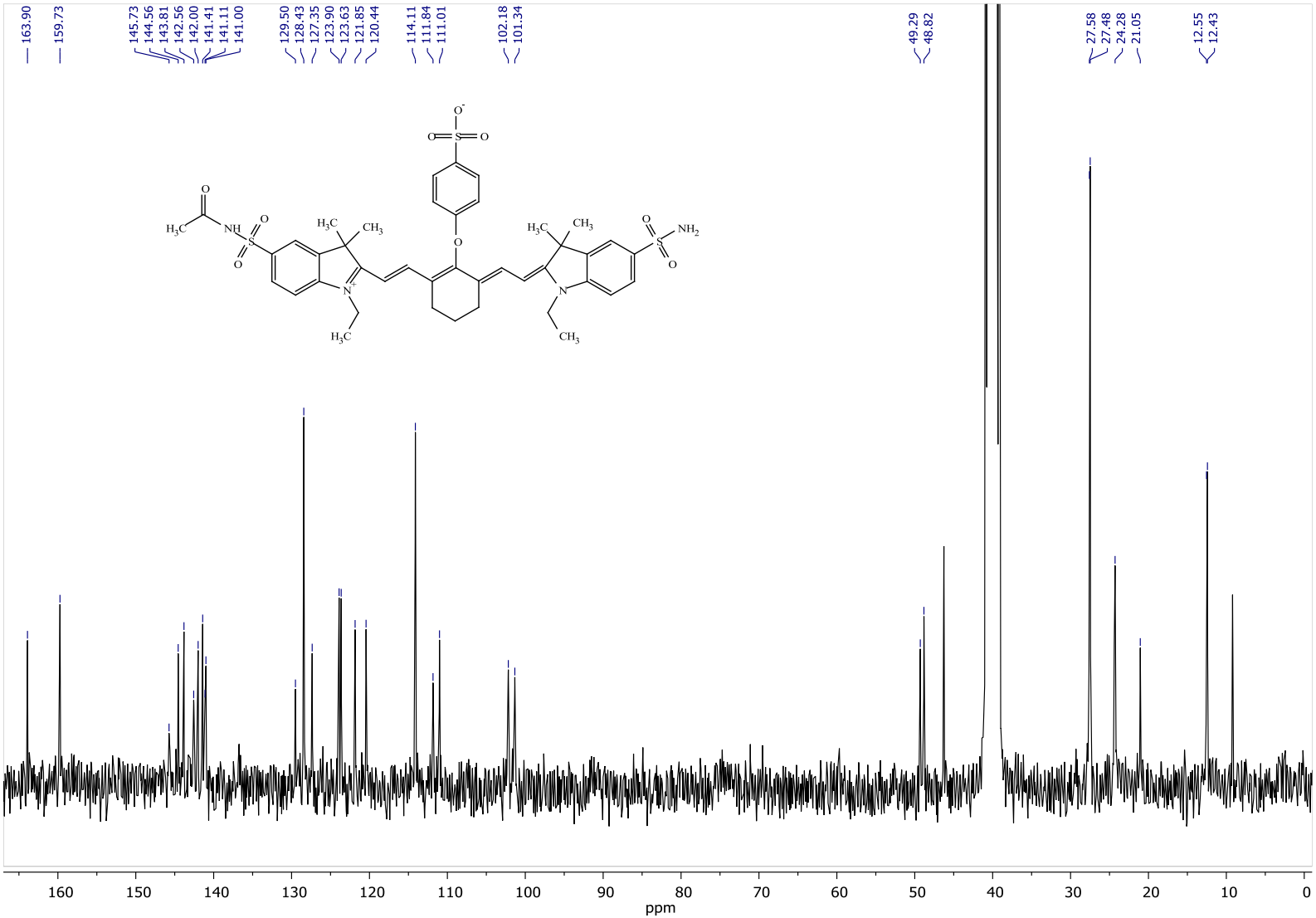

Figure S7. ^{13}C NMR spectrum of compound **7.**

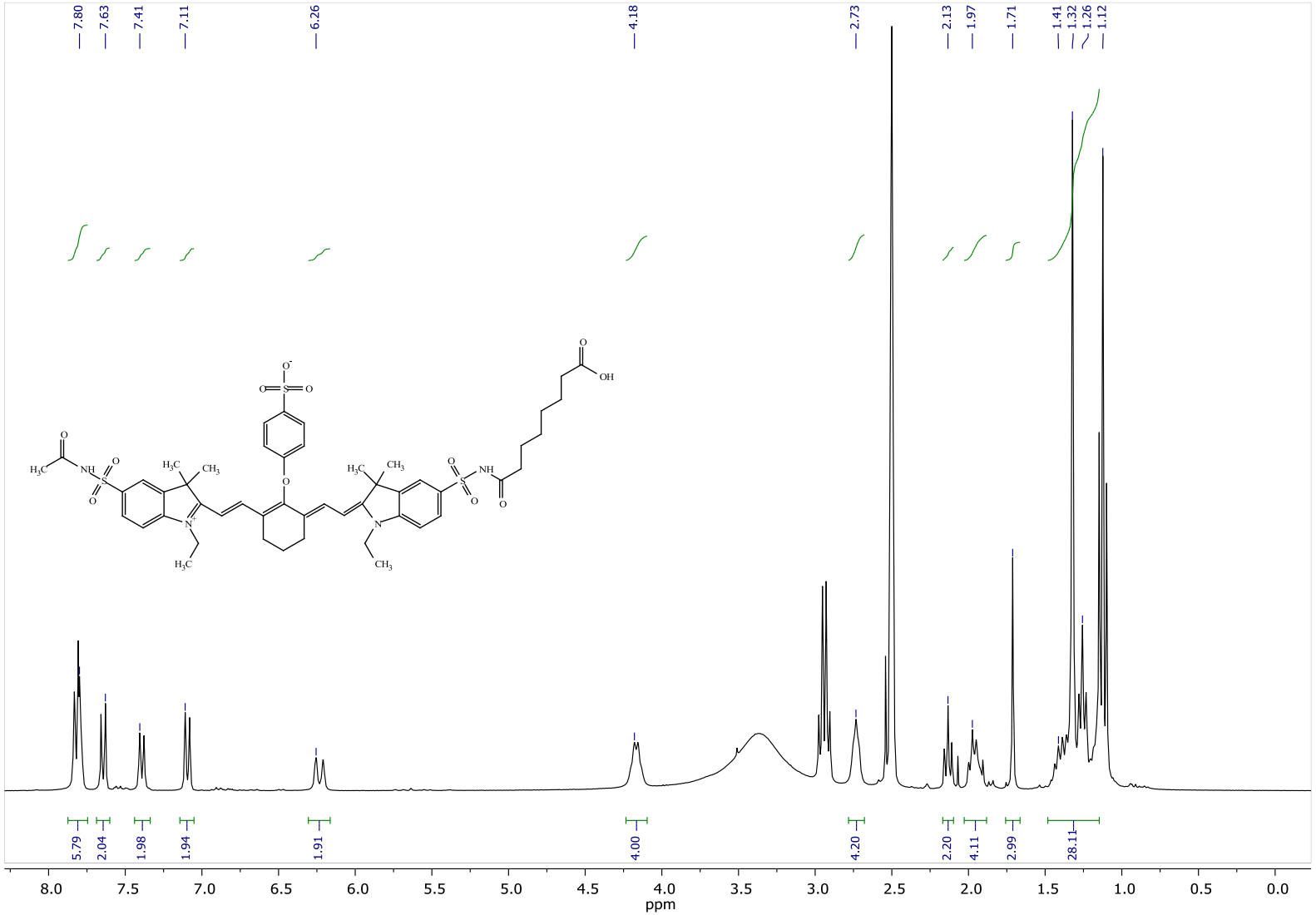

Figure S8. ^{1}H NMR spectrum of compound **8**.

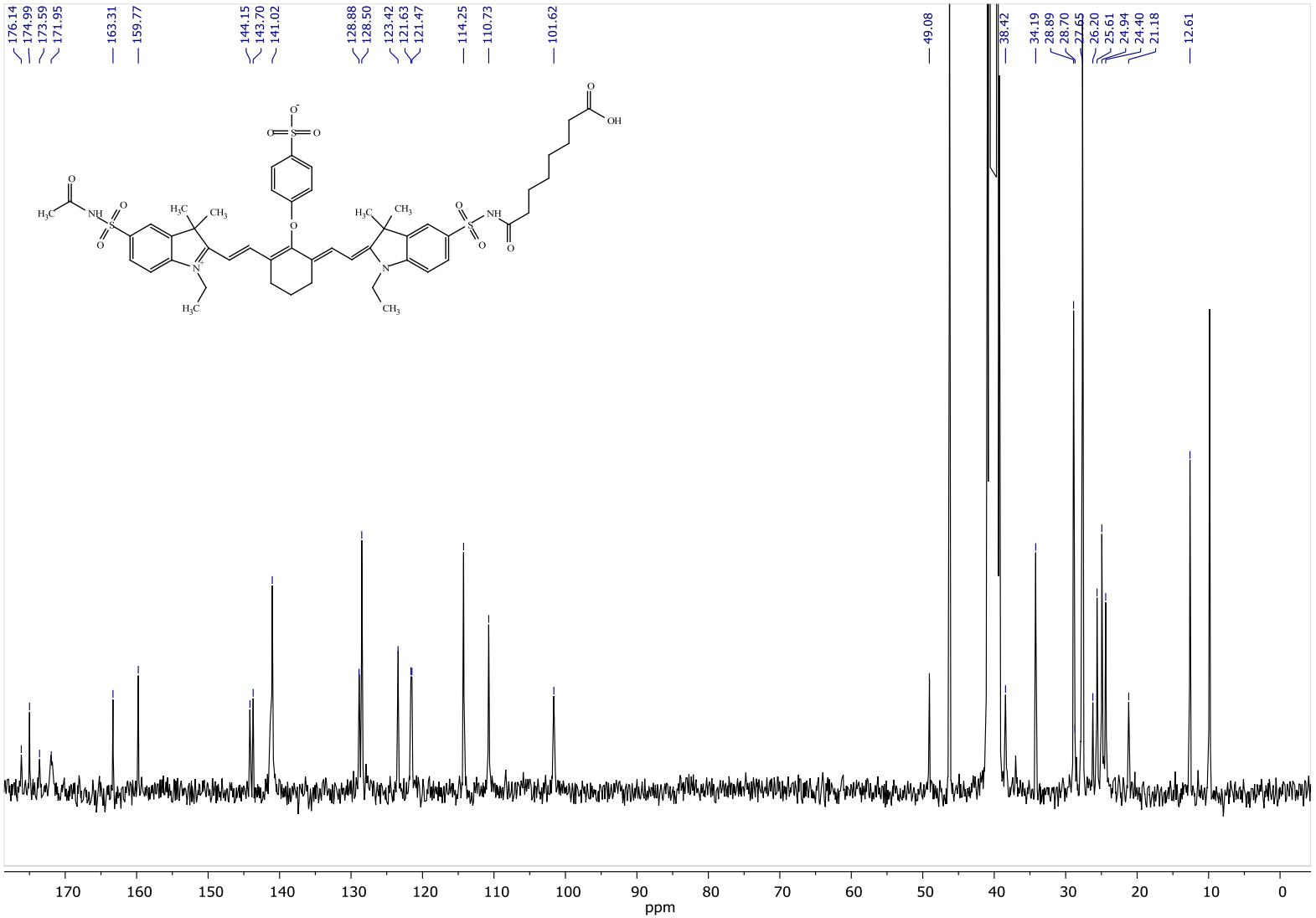

Figure S9. ^{13}C NMR spectrum of compound **8**.