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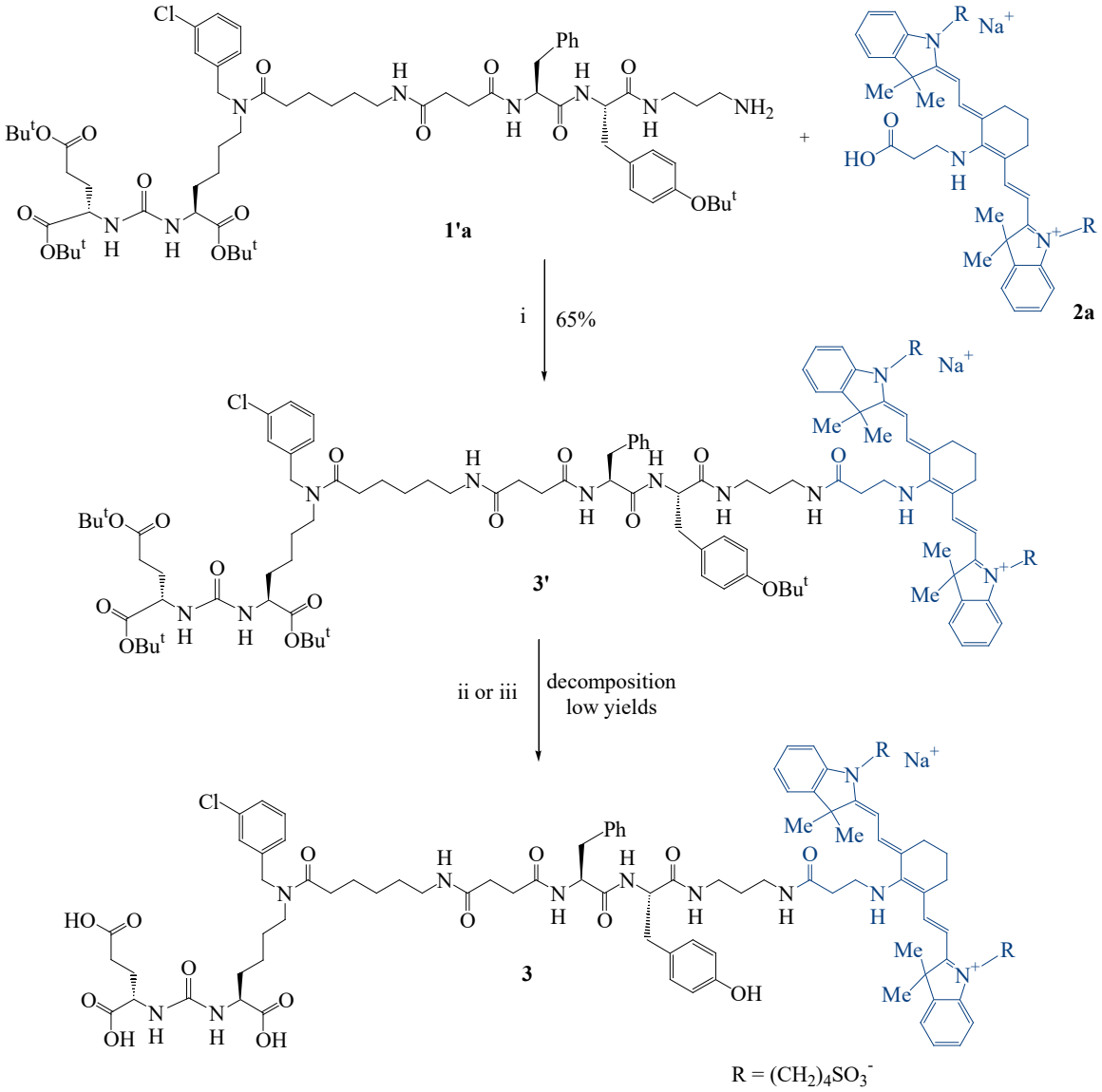
Fluorescent conjugates of PSMA-targeting ligand with cyanine dyes: synthetic approaches and photochemical properties

Anastasiia A. Uspenskaia, Irina A. Doroshenko, Kseniia A. Popovicheva, Nazar V. Shmychkov, Irina I. Levina, Mikhail K. Beklemishev, Vadim N. Tashlitsky, Tatiana A. Podrugina, Aleksei E. Machulkin and Elena K. Beloglazkina

^{1}H and ^{13}C NMR spectra were registered on Bruker Avance 400 spectrometer (400 MHz for ^{1}H and 101 MHz for ^{13}C) in $CDCl_3$ or DMSO-d_6. Preparative column chromatography was performed on INTERCHIM puriFlash 430. For analysis of samples we used Shimadzu Prominence LC-20 system with Phenomenex Luna 3µm C18 100A (150 x 4.6 mm) column in column oven at 40ºC and fraction collector coupled to single quadrupole mass-spectrometer Shimadzu LCMS-2020 with dual DUIS-ESI-APCI ionization source. Mobile phases: A - 0.1% formic acid in water, B - 10 mM ammonium formate in water, D - acetonitrile. LC parameters for analyses were: gradient flow of 1 ml/min (0-0.5 min - 5% D, 0.5 -10.5 min - 5% to 100% D, 10.5-12 min - 100% D, 12-14.5 min - 100% to 5% D) with optional UV detection for some compounds. MS parameters: drying gas 15.0 L/min, nebulizing gas 1.5 L/min, desolvation line temperature 250ºC, heat block temperature 400ºC, interface voltage -3.5 kV, corona needle voltage -3.5 kV. Positive (mass range 250-2000 Da, in some cases 155-2000 Da) and negative ions (mass range 100-2000 Da) were registered simultaneously. High resolution mass spectra were registered on Orbitrap Elite mass spectrometer (Thermo Scientific) with ESI ionization source. Compounds with concentration of 0.1-10 µg/ml (in 1% formic acid in acetonitrile) were directly infused into the ion source with syringe pump (5 µl/min). We didn't use auxiliary and sheath gases, spray voltage was +-3.5 kV, capillary temperature was set to 275ºC. MS spectra were registered by Orbitrap analyzer with 480000 resolution (1 microscan, AGC target value of 1×106, max inject time 1000 ms, averaged on 10 spectra, MS range 100-2000 Da, in some cases 200-4000 Da). We used DMSO and di-iso-octyl phthalate as internal calibration signals (m/z 157.03515 and 413.26623) in positive mode and dodecylsulfate (m/z 265.14790) in negative mode.

The fluorescence spectra were obtained on Fluorat-02 Panorama (Lumex, Russia). The UV–vis spectra were recorded using an SF-102 spectrophotometer (Interfotofizika, Moscow, Russia) in 1.0 cm × 2.0 cm quartz cells (an optical path length of 2 cm) or U-5100 spectrophotometer (Hitachi, Japan) in 1.0 cm × 1.0 cm quartz cells. The NIR quantum yields (761–817 nm) were measured with respect to zinc (II) phthalocyanine at the excitation of 660–700 nm;

Synthesis of starting compounds and fluorophores obtained from them was carried out according to the previously described methodology.



Scheme S1 *Reagents and conditions:* i, HOBt, HBTU, DIPEA, DMF;
ii, $CF_3CO_2H/CH_2Cl_2/H_2O/Pr^i_3SiH$ (46.25:46.25:5:2.5), iii, 25% CF_3CO_2H/ CH_2Cl_2, 0 °C.

The removal of *tert*-butyl protection groups in the urea and phenolic parts of **3′** was carried out in a solution of $CF_3CO_2H/CH_2Cl_2/H_2O/Pr^i_3SiH$ (46.25:46.25:5:2.5) using standard techniques (Scheme S1, procedure ii, Uspenskaya *et al*., *Med. Chem. Res*., 2023, **32**, 32; doi:10.1007/s00044-022-03002-w). At this stage, we encountered a rapid degradation of the fluorescent dye, which was confirmed by absorption spectra. Consequently, the concentration of CF_3CO_2H was reduced to 25% and the reaction temperature was lowered to 0 °C (procedure iii, Chen *et al*., *Chem. Eur J*., 2015, **21**, 11696; doi:10.1002/chem.201502226). However, under these conditions the degradation also occurred (Figure S1). Further decrease in the concentration of CF_3CO_2H in the system is inappropriate, as it can only lead to partial removal of *tert*-butyl protecting groups from the vector fragment. Decreasing the concentration of CF_3CO_2H while increasing the exposure time is also not suitable in this case, since prolonged stay of the vector moiety under acidic conditions leads to urea cyclization and formation of by-products that are not selective to the marker protein (N. L. Benoiton, *Chemistry of Peptide Synthesis*, CRC Press, Boca Raton, 2006; doi.org/10.1201/9781420027693), (Konnert *et al*., *Chem Rev*., 2017, **117**, 13757; doi:10.1021/acs.chemrev.7b00067). Thus, this synthetic Scheme S1 turned out to be inefficient.

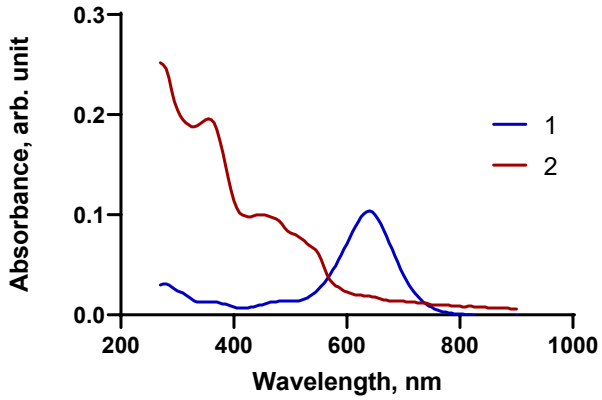
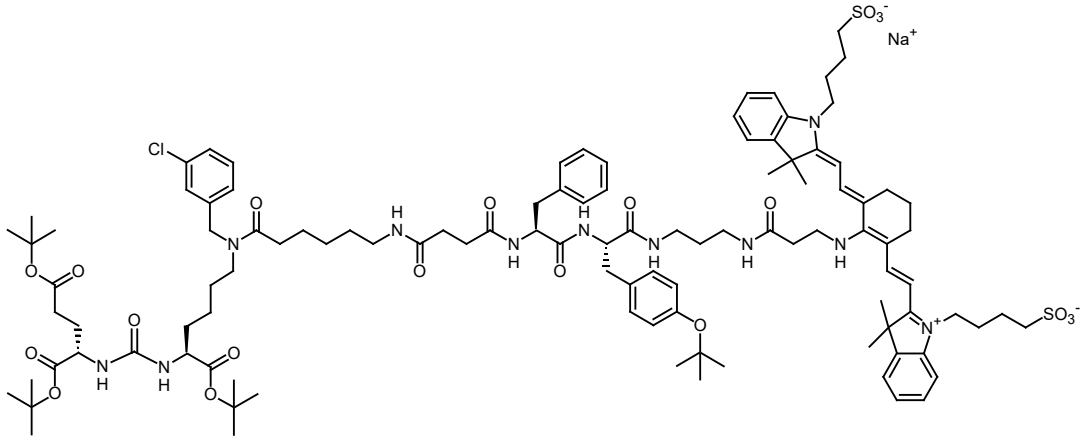


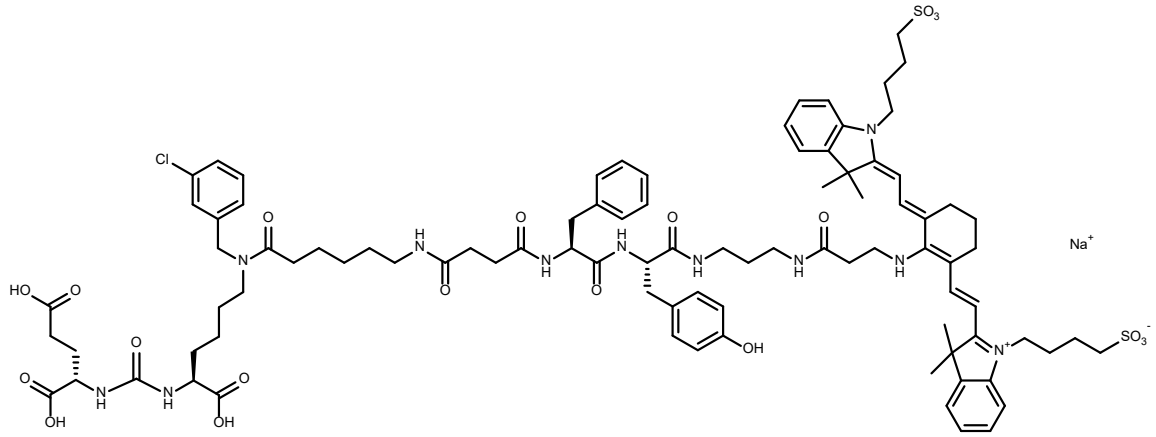
Figure S1 Absorption spectra of the reaction mixture (see Scheme S1, steps ii or iii) before (line *1*) and after (line *2*) addition of a solution containing CF_3CO_2H.

Compound 3′ (*all-O-tert*-butyl derivative of **3**)



Ligand **1′a** (26 mg, 0.02 mMol), fluorophore **2a** (16.765 mg, 0.021 mMol), HOBt (2.69 mg, 0.02 mMol), HBTU (11.326 mg, 0.03 mMol), DIPEA (13.87 μL, 0.08 mMol) were dissolved in DMF (5 mL) and left to stir overnight. The reaction mixture was then extracted in dichloromethane/water system. The organic extract was then evaporated under reduced pressure. The resulting mixture was chromatographed in ethyl acetate/hexane system. A blue-colored amorphous solid **3′** was obtained in 65% yield (54 mg). ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 8.28 (s, 1 H), 8.16 (s, 1 H), 7.93 (s, 1 H), 7.57 - 7.63 (m, 1 H), 7.52 (d, J=5.07 Hz, 1 H), 7.27 - 7.37 (m, 3 H), 7.18 - 7.25 (m, 4 H), 7.09 - 7.17 (m, 7 H), 6.81 - 6.89 (m, 2 H), 6.68 - 6.79 (m, 1 H), 6.27 - 6.40 (m, 2 H), 4.53 (d, J=6.72 Hz, 1 H), 4.46 (s, 1 H), 4.23 - 4.37 (m, 2 H), 3.97 - 4.05 (m, 1 H), 3.89 - 3.97 (m, 1 H), 3.74 - 3.87 (m, 1 H), 3.56 - 3.67 (m, 1 H), 3.11 - 3.21 (m, 3 H), 2.95 - 3.06 (m, 6 H), 2.93 (s, 1 H), 2.79 - 2.91 (m, 4 H), 2.32 (br. s., 5 H), 2.20 (dd, J=17.45, 8.59 Hz, 6 H), 1.58 - 1.70 (m, 7 H), 1.41 - 1.53 (m, 11 H), 1.35 (br. s., 27 H), 1.22 - 1.25 (m, 7 H), 1.18 - 1.22 (m, 12 H). ESI-HRMS for $C_{107}H_{148}ClN_{11}O_{20}S_2$: m/z calculate $[M-2H]^{2-}$: 1004.0055, found: 1004.0057.

Compound 3



Attempted synthesis from **3′**

Procedure ii (see Scheme S1). Conjugate **3′** (1 eq.) was dissolved in a solution of trifluoroacetic acid, DCM, distilled water and triisopropylsilane (46.25 : 46.25 : 5 : 2.5), and this was stirred for 3 hours at room temperature. The solvent was removed under reduced pressure. The obtained substance was re-evaporated 3 times with DCM. Diethyl ether was added to the resulting oily precipitate yielding a white amorphous product. The diethyl ether was decanted and the procedure was repeated 2 more times. The product was purified by reversed-phase column chromatography using 0.1% trifluoroacetic acid in water : acetonitrile as eluent (Puriflash C18-HP, 15μ, 20g, eluent: acetonitrile-0.1% trifluoroacetic acid in water system: from 10% acetonitrile to 100 % in 20 minutes). The degradation of the substance was indicated by absorption spectra.

Procedure iii (see Scheme S1) Compound **3′** (25.5 mg, 0.013 mMol) was dissolved in DCM (3 mL) and cooled to 0˚C for 30 minutes. The system was purged with argon. Then, a cold solution of 25% TFA/DCM (4 mL) was slowly added to the reaction mixture. The maximum processing time was 6 hours. The degradation of the substance was indicated by absorption spectra.

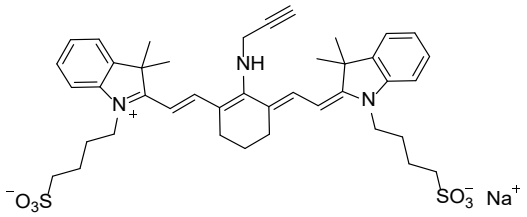
Preparative synthesis form **2a**.

Procedure 1. To a solution of carbocyanine **2a** (31 mg, 0.038 mMol) in DMF (2 mL) under argon atmosphere was added NHS (4 mg, 0.038 mMol), and the reaction mixture was cooled to 0-5˚C. Then DCC (8 mg, 0.038 mMol) was added, and this was stirred overnight in the refrigerator. Ligand **1a** (39 mg, 0.038 mMol) dissolved in DMF (1 mL) and triethylamine (5 μL, 0.038 mMol) were then added under argon, and the reaction was left overnight. Evaporation of the solvent on a rotary evaporator, precipitation from diethyl ether (3 mL) gave the substance which was then separated by reversed phase column chromatography in H_2O/MeCN system (15 min 5% to 100% MeCN, 5 min 100% MeCN). An amorphous blue colored solid 5(Route I) was obtained in 22 % yield (15 mg).

Procedure 2. To a solution of carbocyanine **2a** (24 mg, 0.029 mMol) in DMF (2 mL) under argon atmosphere was added HOBt (4.04 mg, 0.029 mMol), and the reaction mixture was cooled to 0-5˚C. Then DCC (6.17 mg, 0.029 mMol) was added, and this was stirred overnight in the refrigerator. Ligand **1a** (26 mg, 0.029 mMol) dissolved in DMF (1 mL) and triethylamine (4 μL, 0.029 mMol) were then added under argon. The mixture was stirred overnight, evaporated under reduced pressure, the product was precipitated from diethyl ether (3 mL). The obtained substance was separated by reversed phase column chromatography in H_2O/MeCN system (15 min 5% to 100% MeCN, 5 min 100% MeCN). Blue solid 5(Route II) was obtained in 19% yield (10 mg).

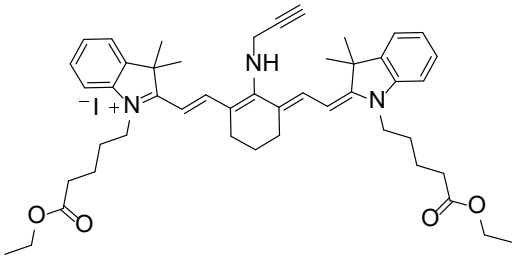
^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.35 (br. s., 3H), 9.17 (s, 1H), 8.45-8.43 (s, 1H), 8.35-8.25 (m, 1H), 8.11-8.07 (m, 1H,), 7.66-7.55 (m, 1H), 7.34-7.28 (m, 6H), 7.23-7.20 (m, 8H), 7.43 – 7.08 (m, 11H), 7.01 (s, 3H), 6.65 (d, 2H, J = 8.2 Hz), 6.31 (d, 2H, J = 16.6 Hz), 4.54 (s, 1H), 4.46 (s, 1H), 4.26 (s, 1H), 4.07 (s, 2H), 3.92 (s, 1H), 3.17 – 3.13 (m, 3H), 3.03-2.92 (m, 20H), 2.67-2.64 (m, 3H), 2.34-2.30 (m, 9H), 2.23-2.16 (m, 10H), 1.71-1.61 (m, 10H), 1.49-1.41 (m, 16H), 1.24-1.18 (m, 12H). ESI-HRMS for $C_{91}H_{117}$ Cl $N_{11}O_{20}S_2Na$: m/z calculate $[M-2H]^{2-}$: 901.8679, found: 901.8689.

Compound 5a.



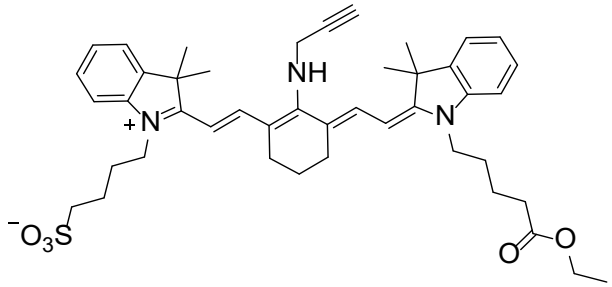
A mixture of tricarbocyanine **4a** (0.3 g, 0.41 mmol) with propargylamine (0.04 mL, 0.58 mmol) in absolute DMF (5 mL) was stirred at 65 °C for 1.5 hours with the addition of Et_3N (0.08 mL, 0.58 mmol). The reaction mixture was precipitated with methyl *tert*-butyl ether and the precipitate was filtered off. The target dye was purified by preparative chromatography on silica gel in the eluent mixture CH_2Cl_2 : CH_3OH:= 2.7:1. The yield was 0.08 g (26.7%) λ_{abs} = 654 nm (in DMF). λ_{fl} = 777 nm (in DMF). ε = 1.56•10^5 $dm^3*mol^{-1}*cm^{-1}$. ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 1.63 (s, 12 H), 1.67 - 1.80 (m, 14 H), 2.45 - 2.49 (m, 4 H), 3.60 (br. s., 1 H), 3.99 (t, 4 H, $^3J_{HH}$ = 6.46), 4.35 (d, 2 H, $^3J_{HH}$ = 3.91), 5.86 (d, 2 H, $^3J_{HH}$ = 12.91), 7.08 (t, 2 H, $^3J_{HH}$ = 7.43), 7.21 (d, 2 H, $^3J_{HH}$ = 8.22), 7.30 (t, 2 H, $^3J_{HH}$ = 7.63), 7.44 (d, 2 H, $^3J_{HH}$ = 7.04), 7.75 (d, 2 H, $^3J_{HH}$ = 12.91), 8.31 (br. s., 1 H). ^{13}C NMR (126 MHz, DMSO-d6, δ ppm): 21.25, 22.57, 24.95, 25.48, 28.00, 28.84, 42.59, 47.43, 50.88, 76.79, 81.08, 95.47, 109.51, 120.54, 121.95, 122.74, 128.14, 138.66, 140.06, 142.81, 167.47, 167.69. ESI-HRMS for $C_{41}H_{50}N_3O_6S_2^-$: m/z calculate $[M-H]^-$: 744.3147, found: 744.3149.

Compound 5b



A mixture of 0.30 g (0.42 mmol) of tricarbocyanine **4b** with 0.03 g (0.59 mmol) propargylamine and 0.06 g (0.59 mmol) triethylamine was dissolved in 5 mL absolute DMF and stirred at room temperature for 24 hours. The reaction mixture was precipitated with diethyl ether and the precipitate was filtered off. The target dye was purified by preparative chromatography on silica gel in the eluent mixture CH_2Cl_2:EtOH:= 3.5:1. The yield was 0.13 g (36.1%) λ_{abs} = 666 nm (in DMF). λ_{fl} = 776 nm (in DMF). ε = 1.87•10^5 $dm^3*mol^{-1}*cm^{-1}$. ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 1.14 (t, 6 H, $^3J_{HH}$ = 7.09), 1.45 - 1.59 (m, 4 H), 1.64 (12 H), 1.65 - 1.80 (m, 6 H), 2.37 (t, 4 H, $^3J_{HH}$ = 6.76), 2.50 - 2.54 (m, 4 H), 3.61 (br. s., 1 H), 4.02 (q, 8 H, $^3J_{HH}$ = 7.07), 4.35 (br. s., 2 H), 5.85 (d, 2 H, $^3J_{HH}$ = 12.99), 7.10 (t, 2 H, $^3J_{HH}$ = 7.40), 7.20 (d, 2 H, $^3J_{HH}$ = 7.95), 7.31 (t, 2 H, $^3J_{HH}$ = 7.49), 7.46 (d, 2 H, $^3J_{HH}$ = 7.21), 7.75 (d, 2 H, $^3J_{HH}$ = 12.99), 8.29 (br. s., 1 H). ^{13}C NMR (126 MHz, CHLOROFORM-*d,* δ ppm): 14.21, 21.26, 22.43, 25.62, 26.02, 28.49, 28.59, 29.64, 33.72, 47.55, 48.15, 60.43, 74.28, 80.64, 94.40, 108.16, 109.87, 121.56, 122.00, 122.50, 122.99, 128.01, 128.51, 129.69, 137.47, 140.09, 143.07, 166.62, 172.91. ESI-HRMS for $C_{47}H_{60}N_3O_4$: m/z calculate $[M]^+$: 730.4578, found: 730.4589.

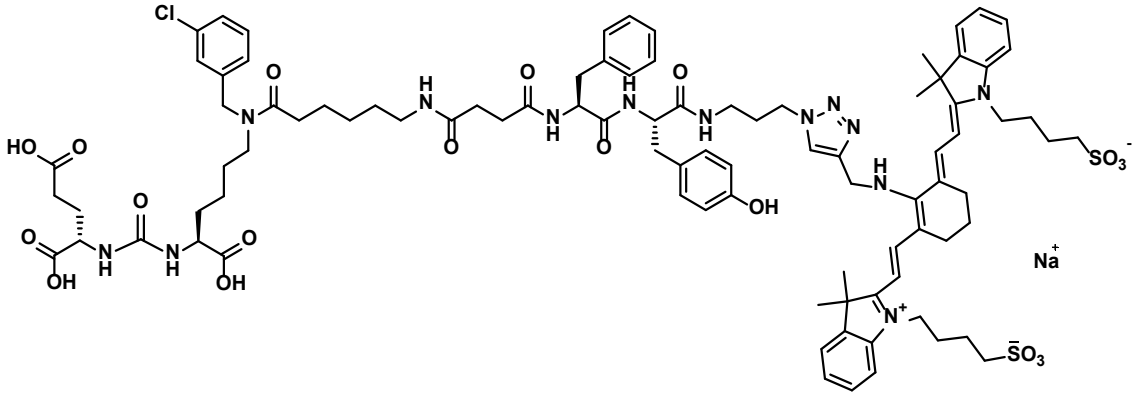
Compound 5c



A mixture of 0.07 g (0.10 mmol) of tricarbocyanine **4c** with 0.01 mL (0.15 mmol) propargylamine in 4 mL absolute DMF was stirred at 65 °C for 5 h with the addition of 0.02 mL (0.148 mmol) triethylamine. The reaction mixture was precipitated with methyl tert-butyl ether and the precipitate was filtered off. The target dye was purified on preparative chromatograph on silica gel in the eluent mixture CH_2Cl_2:EtOH:= 3:1. The yield was 0.043 g (56.6%) λ_{abs} = 654 nm (in DMF). λ_{fl} = 777 nm (in DMF). ε = 2.01•10^5 ^{1}H NMR (400 MHz, DMSO-d6,

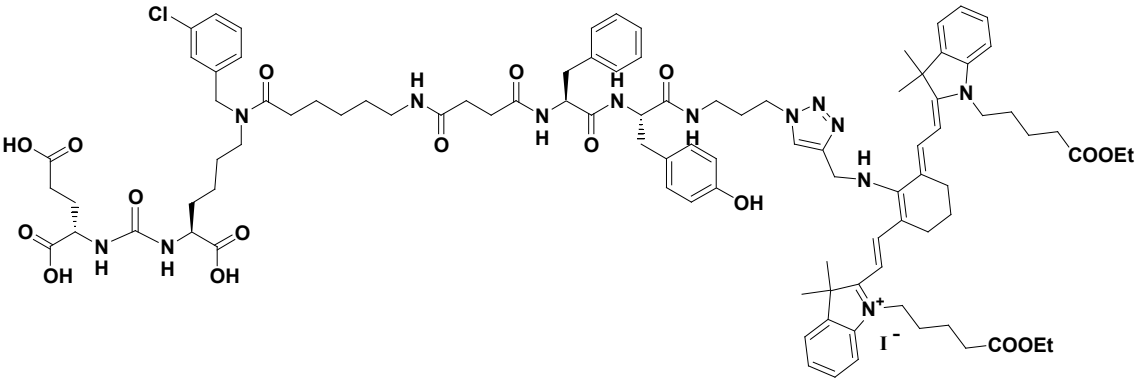
δ, ppm): 1.14 (t, 3 H, $^3J_{HH}$ = 7.12), 1.64 (s, 12 H), 1.66 - 1.80 (m, 10 H), 2.30 - 2.41 (m, 4 H), 2.43 - 2.49 (m, 4 H, CH_2), 3.62 (br. s., 1 H), 3.97 - 4.06 (m, 6 H), 4.35 (d, 2 H, $^3J_{HH}$ = 2.87), 5.87 (t, 2 H, $^3J_{HH}$ = 12.96), 7.09 (q, 2 H, $^3J_{HH}$ = 7.19), 7.18 (d, 1 H, $^3J_{HH}$ = 7.95), 7.21 - 7.26 (m, 1 H), 7.31 (td, 2 H, $^3J_{HH}$ = 7.34, 4.22), 7.45 (d, 2 H, $^3J_{HH}$ = 7.89), 7.75 (t, 2 H, $^3J_{HH}$ = 12.96), 8.26 (br. s., 1 H). ^{13}C NMR (126 MHz, CHLOROFORM-*d,* δ, ppm):14.22, 21.26, 22.45, 22.66, 26.04, 28.58, 29.33, 29.63, 29.67, 31.90, 33.72, 42.95, 47.57, 48.33, 58.42, 60.50, 74.31, 80.67, 108.23, 121.86, 122.05, 122.65, 123.05, 128.09, 128.45, 128.54, 129.72, 132.05, 132.13, 137.91, 140.04, 143.03, 166.82, 172.96. ESI-HRMS for $C_{44}H_{55}N_3O_5S$: m/z calculate $[M+H]^+$: 738.3946, found: 738.3946.

Compound 6a



The fluorescent label **5a** (1.15 eq., 27 mg, 0.035 mmol) and the ligand **1b** (1 eq., 32 mg, 0.03 mmol) were dissolved in DMF/H_2O solution (3:1). The reaction was carried out in an argon atmosphere. Then $CuSO_4$*$5H_2O$ (0.4 eq., 3,5 mg, 0.014 mmol) and sodium ascorbate (1.2 eq., 8.5 mg, 0.042 mmol) were added. The mixture was stirred for 24 hours. After that EDTA (0.8 eq., 8.9 mg, 0.028 mmol) was added to the system and stirred for 3 hours. The reaction mixture was filtered off the EDTA and the solvent was removed under reduced pressure. The target compound was isolated by reverse phase column chromatography (Puriflash C18-HP, 15μ, 20g, eluent: acetonitrile / 0.1% trifluoroacetic acid solution in water: 10% acetonitrile to 100% in 15 min, 100% acetonitrile in 5 min). The bright blue solid **6a** was obtained in 44% yield (24 mg). λ_{abs} = 650 nm (in DMF). λ_{fl} = 775 nm (in DMF). ε = 6.54•10^4 dm^3*mol^{-1}*cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 12.1 (br. s., 3H), 9.19 (s, 1 H), 8.27 - 8.36 (m, 1 H), 8.21 (s, 1 H), 8.11 (d, J=8.80 Hz, 1 H), 7.94 (s, 2 H), 7.69 (s, 2 H), 7.62 (d, J=16.38 Hz, 1 H), 7.42 (d, J=7.09 Hz, 2 H), 7.38 (d, J=9.48 Hz, 4 H), 7.24 - 7.35 (m, 5 H), 7.18 - 7.24 (m, 2 H), 7.15 (t, J=8.25 Hz, 5 H), 6.96 - 7.06 (m, 4 H), 6.64 (d, J=8.56 Hz, 1 H), 6.54 (s, 1 H), 6.24 - 6.36 (m, 1 H), 5.89 - 5.92 (m, 2 H), 5.84 (d, J=2.87 Hz, 2 H), 5.73 - 5.80 (m, 2 H), 4.54 (s, 1 H), 4.46 (s, 1 H), 4.24 - 4.38 (m, 2 H), 4.02 - 4.12 (m, 1 H), 3.87 - 3.92 (m, 5 H), 3.24 – 3.22 (m, 2 H), 2.63 - 2.68 (m, 2 H), 2.31 (s, 3 H), 2.13 - 2.28 (m, 3 H), 1.82 - 1.99 (m, 2 H), 1.59 - 1.80 (m, 15 H), 1.55 (s, 12 H), 1.34-1.42 (3 H), 1.23 (d, J=6.60 Hz, 4 H). ESI-HRMS for $C_{91}H_{116}ClN_{13}O_{19}S_2$: m/z calculate $[M-2H]^{2-}$: 895.8747, found: 895.8748; for $C_{91}H_{116}ClN_{13}O_{19}S_2K$: m/z calculate $[M-2H]^{2-}$: 915.3544, found: 915.3502.

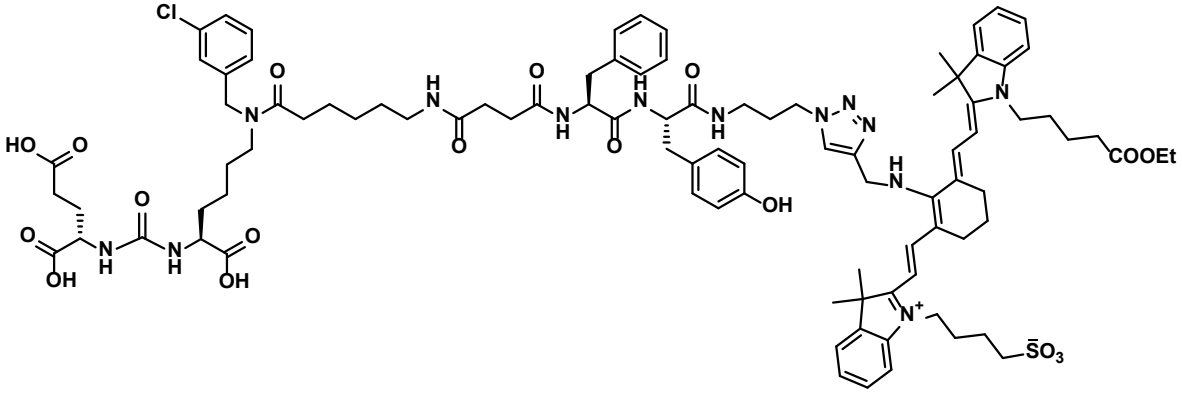
Compound 6b



Fluorescent label **5b** (1.025 eq., 19 mg, 0.026 mmol) and the ligand **1b** (1 eq., 26 mg, 0.025 mmol) were dissolved in DMF/H_2O solution (3:1). The reaction was carried out in an argon atmosphere. Then $CuSO_4$*$5H_2O$ (0.8 eq., 6 mg, 0.02 mmol) and sodium ascorbate (2.4 eq., 12 mg, 0.06 mmol) were added. The mixture was stirred for 24 hours. After that EDTA (1.6 eq., 14 mg, 0.04 mmol) was added to the system and stirred for 3

hours. The reaction mixture was filtered off the EDTA and the solvent was removed under reduced pressure. The target compound was isolated by reverse phase column chromatography (Puriflash C18-HP, 15μ, 20g, eluent: acetonitrile / 0.1% trifluoroacetic acid solution in water: 10% acetonitrile to 100% in 15 min, 100% acetonitrile in 5 min). The bright blue solid **6b** was obtained in 30% yield (13 mg). λ_{abs} = 634 nm (in DMF). λ_{fl} = 761 nm (in DMF). ε = 2.31•10^3 ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 12.12 (br.s, 3H), 8.24 (s, 1 H), 8.05 (s, 1 H), 7.62 (d, *J*=12.65 Hz, 2 H), 7.38 (d, *J*=6.85 Hz, 3 H), 7.26 (td, *J*=14.92, 7.76 Hz, 6 H), 7.13 - 7.16 (m, 4 H), 7.10 (d, *J*=8.50 Hz, 8 H), 6.96 - 7.04 (m, 7 H), 6.65 (d, *J*=8.50 Hz, 4 H), 6.37 – 6.39 (m, 1 H), 6.26 -6.28 (m, 1 H), 4.86 - 4.88 (m, 2 H), 4.44 (s, 2 H), 4.36 (d, *J*=7.82 Hz, 2 H), 4.24 – 4.30 (m, 3 H), 3.89 - 3.96 (m, 1 H), 3.11 – 3.17 (m, 8 H), 3.03 -3.05 (m, 9H), 2.92 - 2.99 (m, 5 H), 2.64 – 2.67 (m, 3 H), 2.32 – 2.34 (t, *J*=6.60 Hz, 7 H), 2.26 – 2.28 (m, 6 H), 2.16 (d, *J*=7.46 Hz, 6 H), 1.89 - 2.01 (m, 5 H), 1.70 - 1.77 (m, 4 H), 1.55 - 1.68 (m, 12 H), 1.26 - 1.28 (d, 7 H), 1.10 -1.17 (m, 4 H). ESI-MS for $C_{97}H_{125}ClN_{13}O_{17}$ m/z calculate [M-H]$^-$: 1778.89, found: 1778.75; m/z calculate [M-2H]$^{2-}$: 888.94, found: 888.90.

Compound 6c



The fluorescent label **5c** (1.05 eq., 30 mg, 0.041 mmol) and the ligand **1b** (1 eq., 40 mg, 0.039 mmol) were dissolved in DMF/H_2O solution (3:1). The reaction was carried out in an argon atmosphere. Then $CuSO_4$*$5H_2O$ (0.8 eq., 7,5 mg, 0.02 mmol) and sodium ascorbate (2.4 eq., 18 mg, 0.06 mmol) were added. The mixture was stirred for 24 hours. After that EDTA (1.6 eq., 16 mg, 0.04 mmol) was added to the system and stirred for 3 hours. The reaction mixture was filtered off the EDTA and the solvent was removed under reduced pressure. The target compound was isolated by reverse phase column chromatography (Puriflash C18-HP, 15μ, 20g, eluent: acetonitrile / 0.1% trifluoroacetic acid solution in water: 10% acetonitrile to 100% in 15 min, 100% acetonitrile in 5 min). The bright blue solid **6c** was obtained in 25% yield (17 mg). λ_{abs} = 640 nm (in DMF). λ_{fl} = 761 nm (in DMF). ε = 4.89•10^4 ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 12.82 (br. s, 3 H), 9.17 (s, 1 H), 8.61 (s, 1 H), 8.35 (t, J=11.46 Hz, 1 H),8.14 (t, J=12.26 Hz, 1 H), 7.93 (d, J=14.06 Hz, 2 H), 7.56 - 7.74 (m, 3 H), 7.38 (d, J=7.34 Hz, 4 H), 7.19 - 7.34 (m, 5 H), 7.08 - 7.18 (m, 8 H), 7.01 (d, J=4.52 Hz, 5 H), 6.57 - 6.69 (m, 2 H), 6.32 (t, J=8.59 Hz, 2 H), 5.79 (t, J=12.59 Hz, 2 H), 4.87 (s., 1 H), 4.52 (s., 1 H), 4.45 (s., 1 H), 4.35 (d, J=5.81 Hz, 2 H), 4.28 (d, J=14.43 Hz, 3 H), 4.07 (s, 1 H), 3.94 (d, J=0.67 Hz, 4 H), 3.07 - 3.21 (m, 7 H), 2.98 (d, J=15.41 Hz, 6 H), 2.90 (m., 6 H), 2.61 - 2.66 (m, 3 H), 2.28 - 2.43 (m, 9 H), 2.09 - 2.27 (m, 7 H), 1.82 - 2.01 (m, 4 H), 1.58 - 1.82 (m, 13 H), 1.48 (d, J=14.73 Hz, 4 H), 1.16 - 1.37 (m, 8 H). ESI-MS for $C_{94}H_{120}ClN_{13}O_{18}S_2$: m/z calculate [M-2H]$^{2-}$: 892.41, found: 892.50; [M-2H]$^{2-}$: 1785.82, found: 1785.56.

NMR spectra

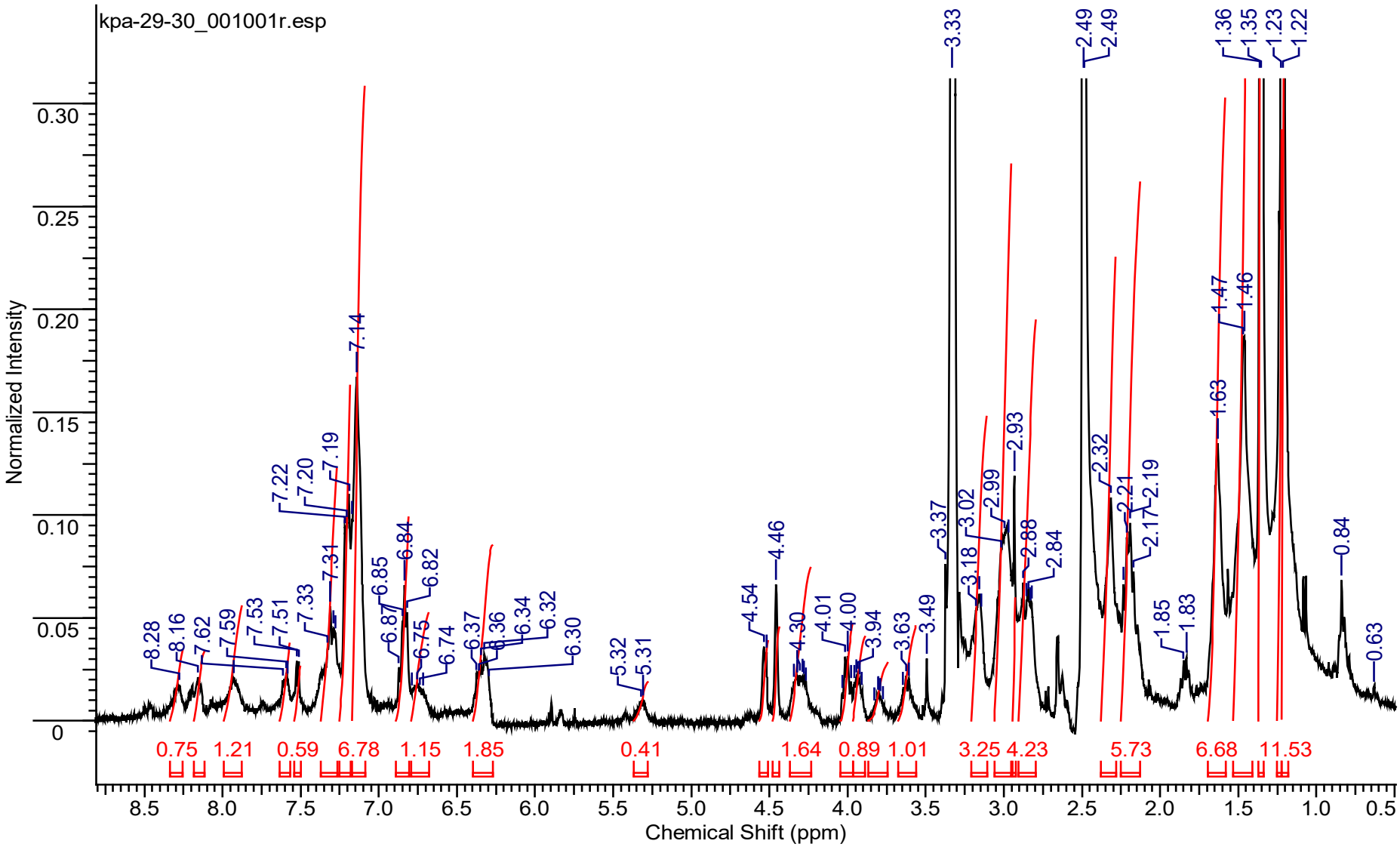


Figure S2. [1]H-NMR of compound **3'**

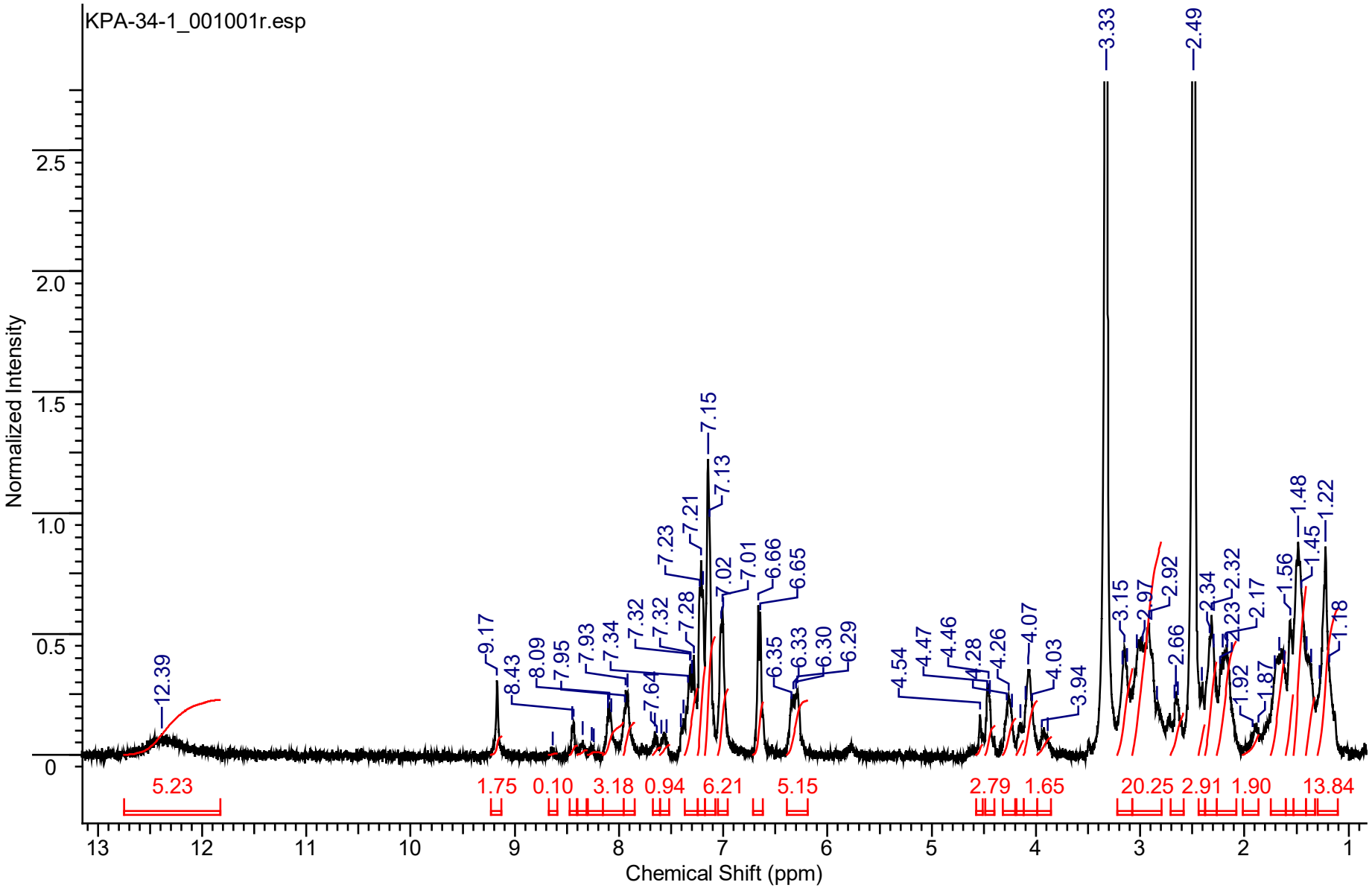


Figure S3. ^{1}H-NMR of compound **3**

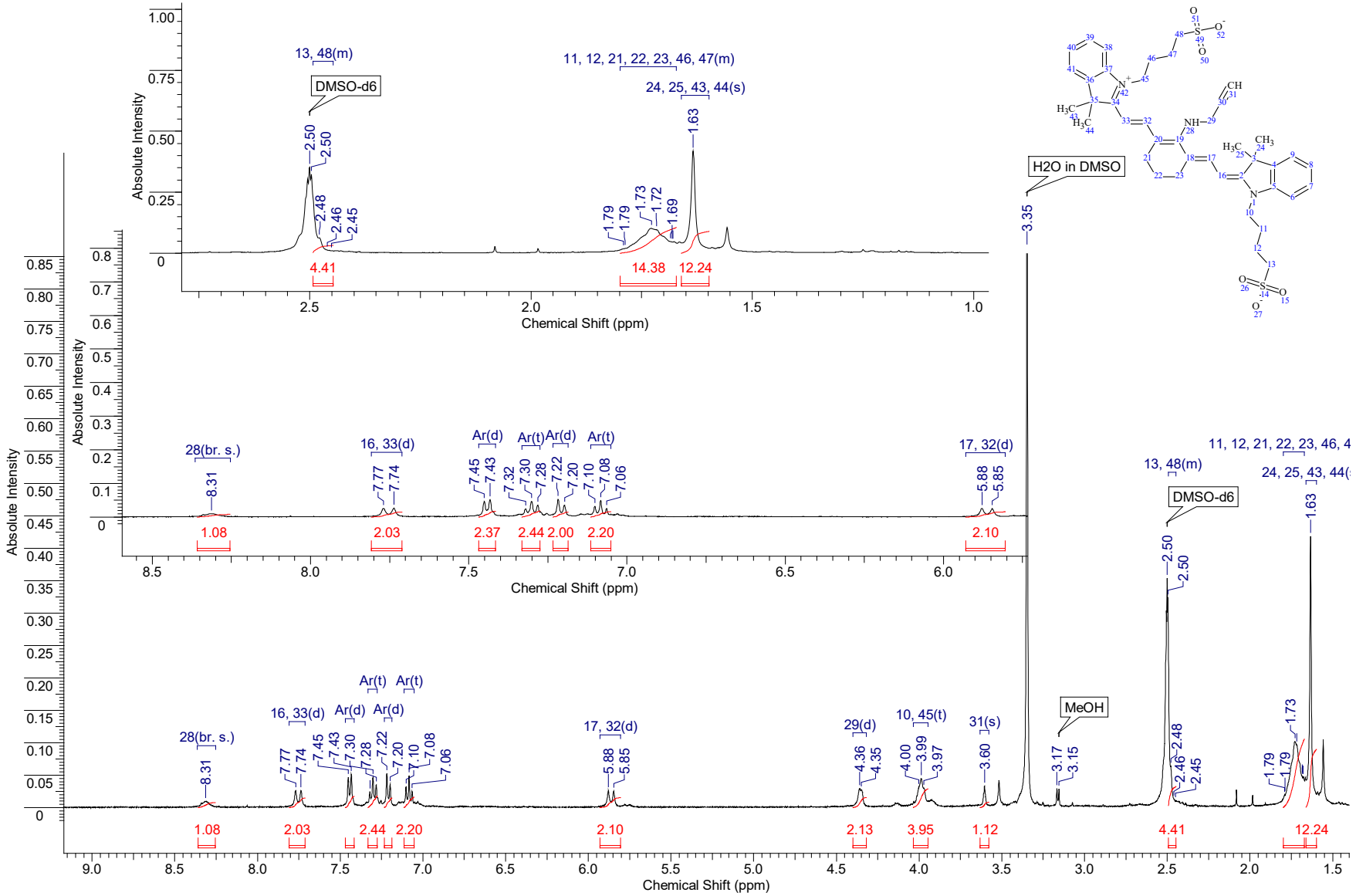


Figure S4. [1]H-NMR of compound **5a**

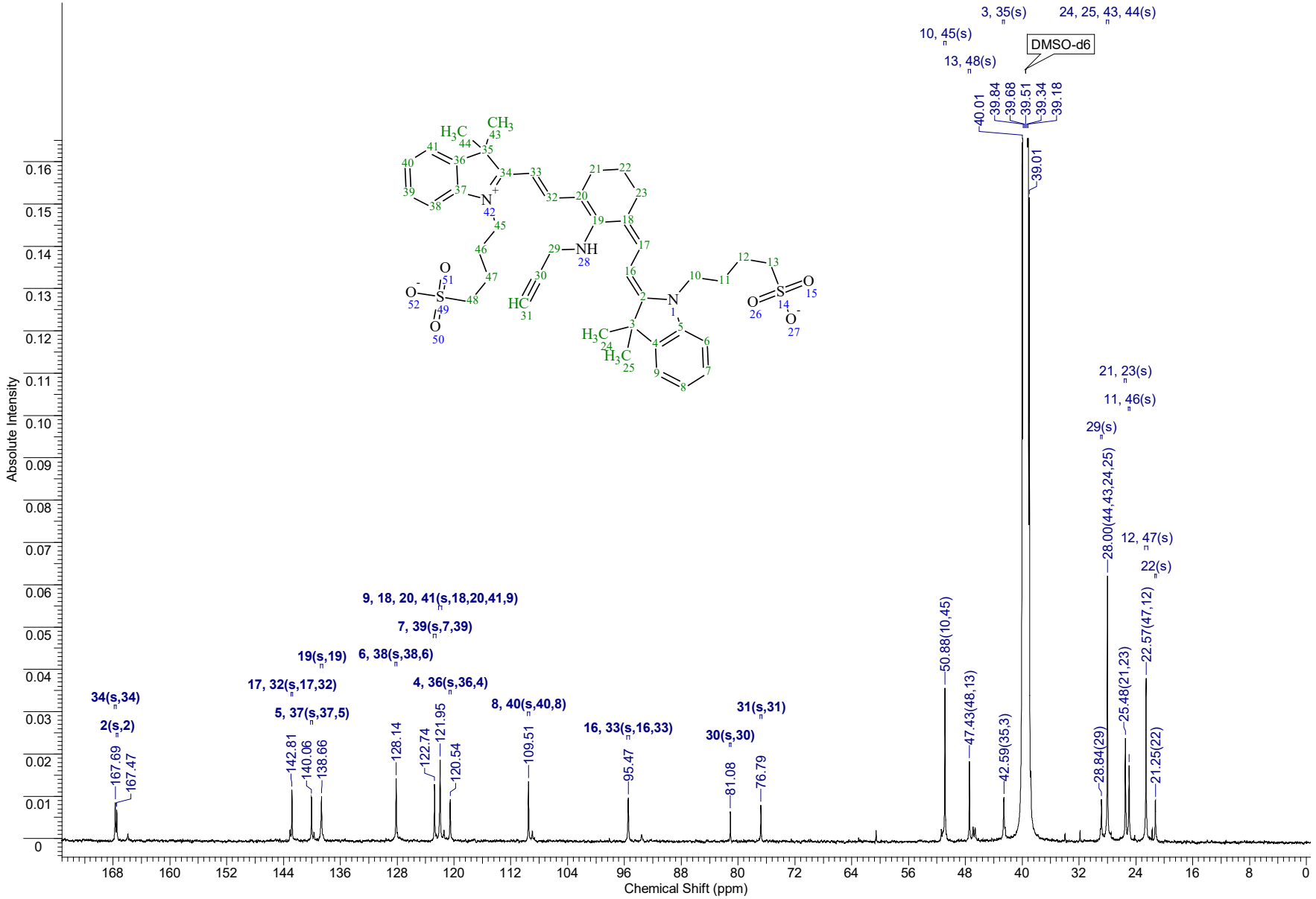


Figure S5. ^{13}C NMR of compound **5a**

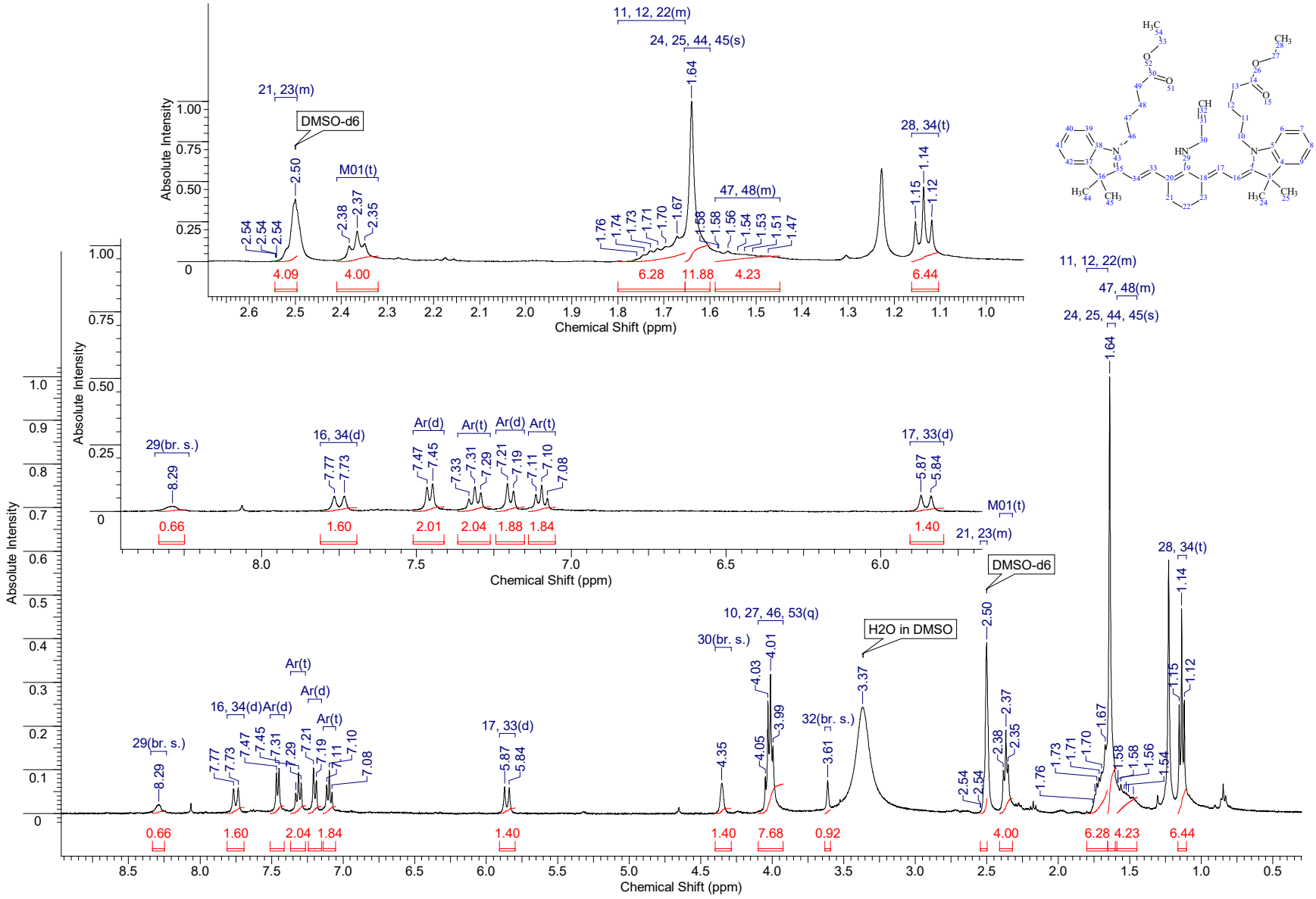


Figure S6. [1]H-NMR of compound **5b**

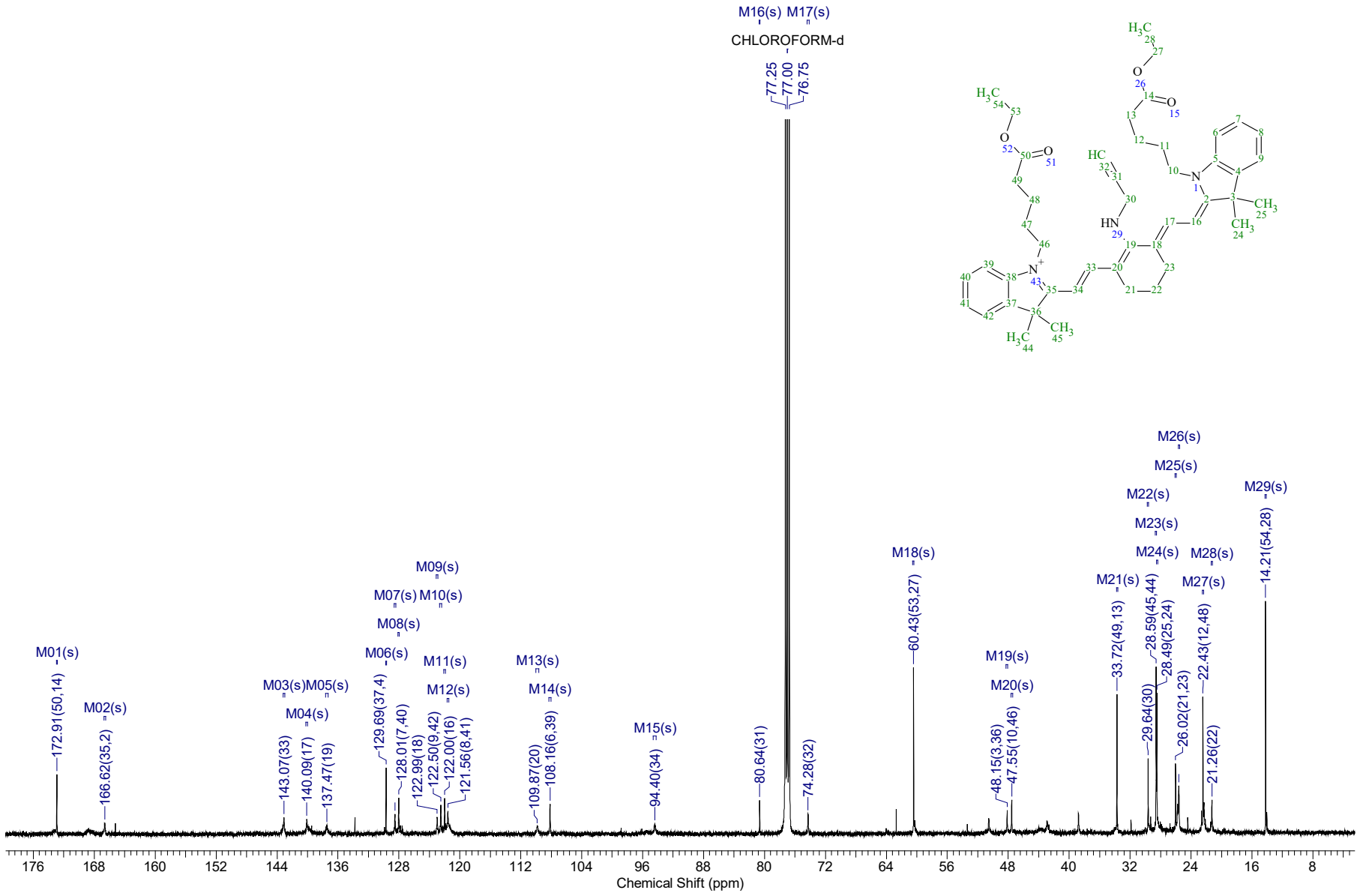


Figure S7. ^{13}C NMR of compound **5b**

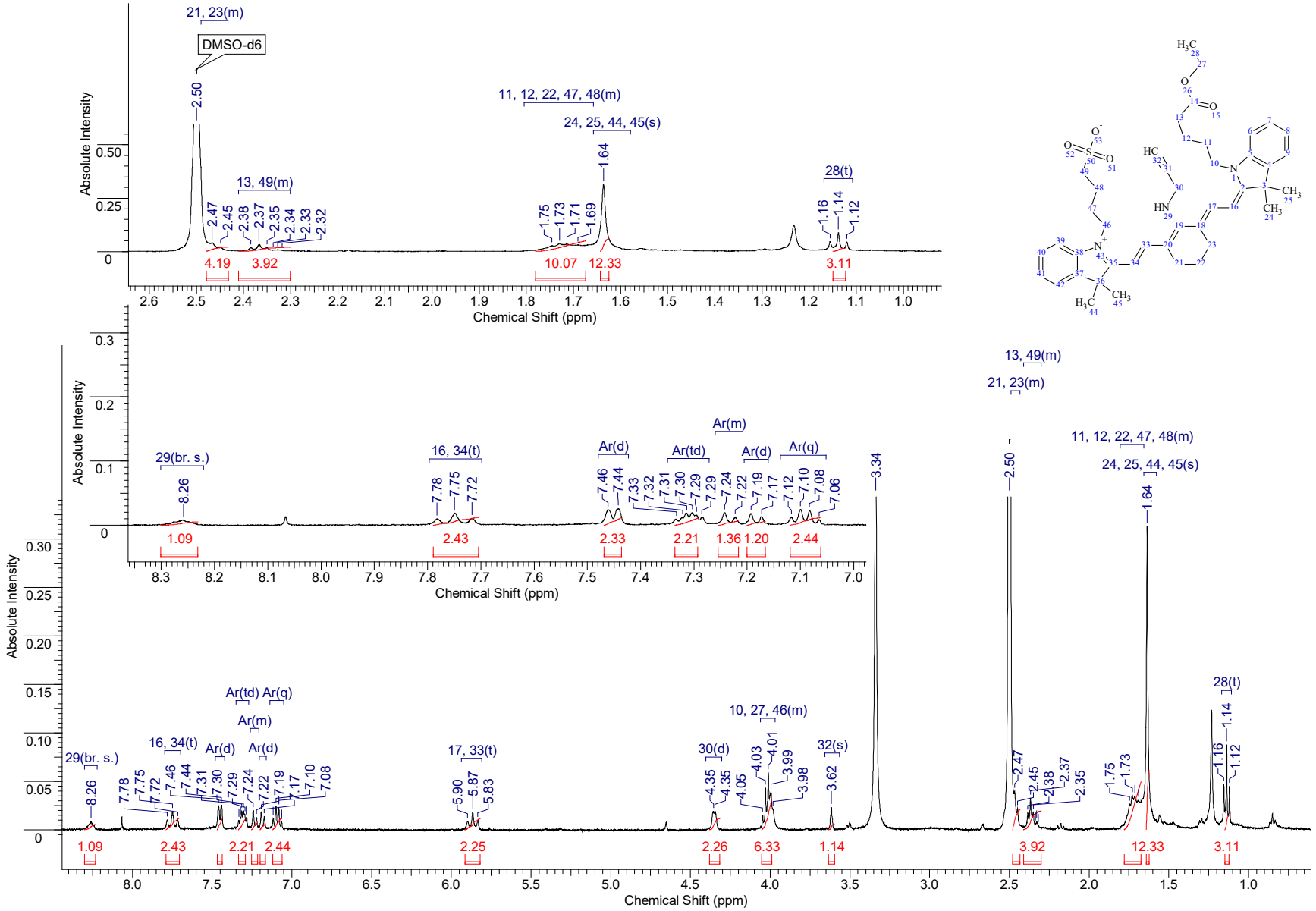


Figure S8. ^{1}H NMR of compound **5c**

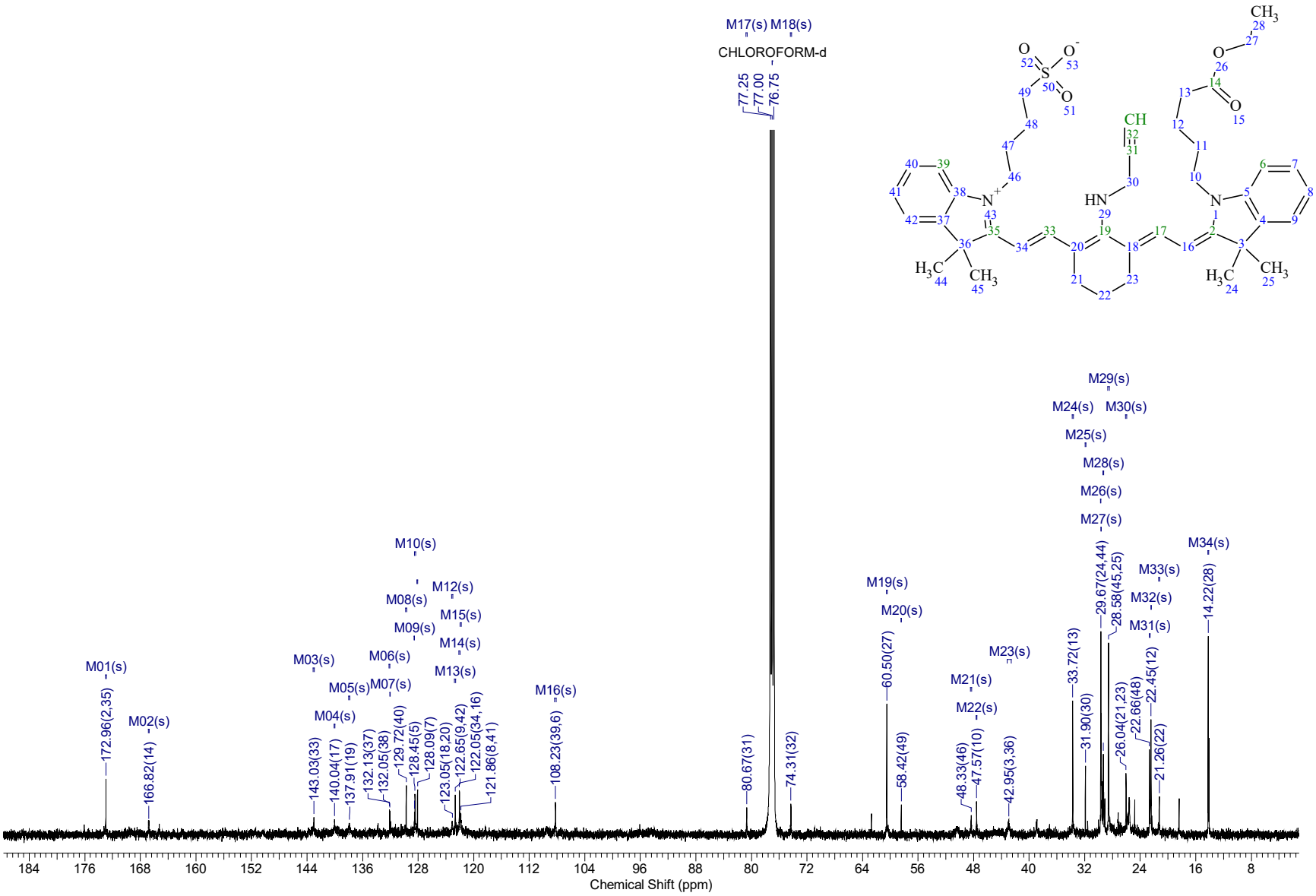


Figure S9. ^{13}C NMR of compound **5c**

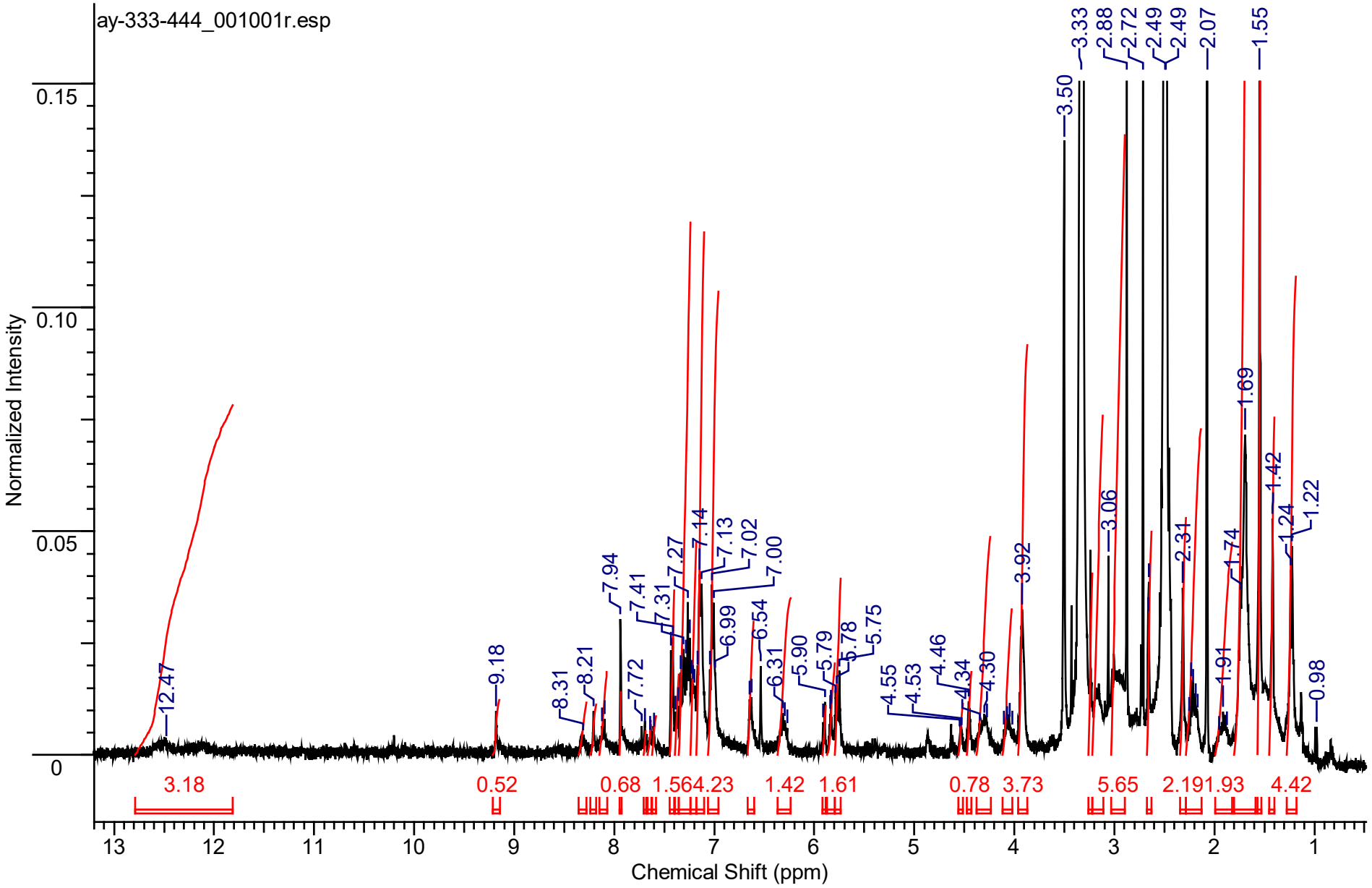


Figure S10. ^{1}H NMR of compound **6a**

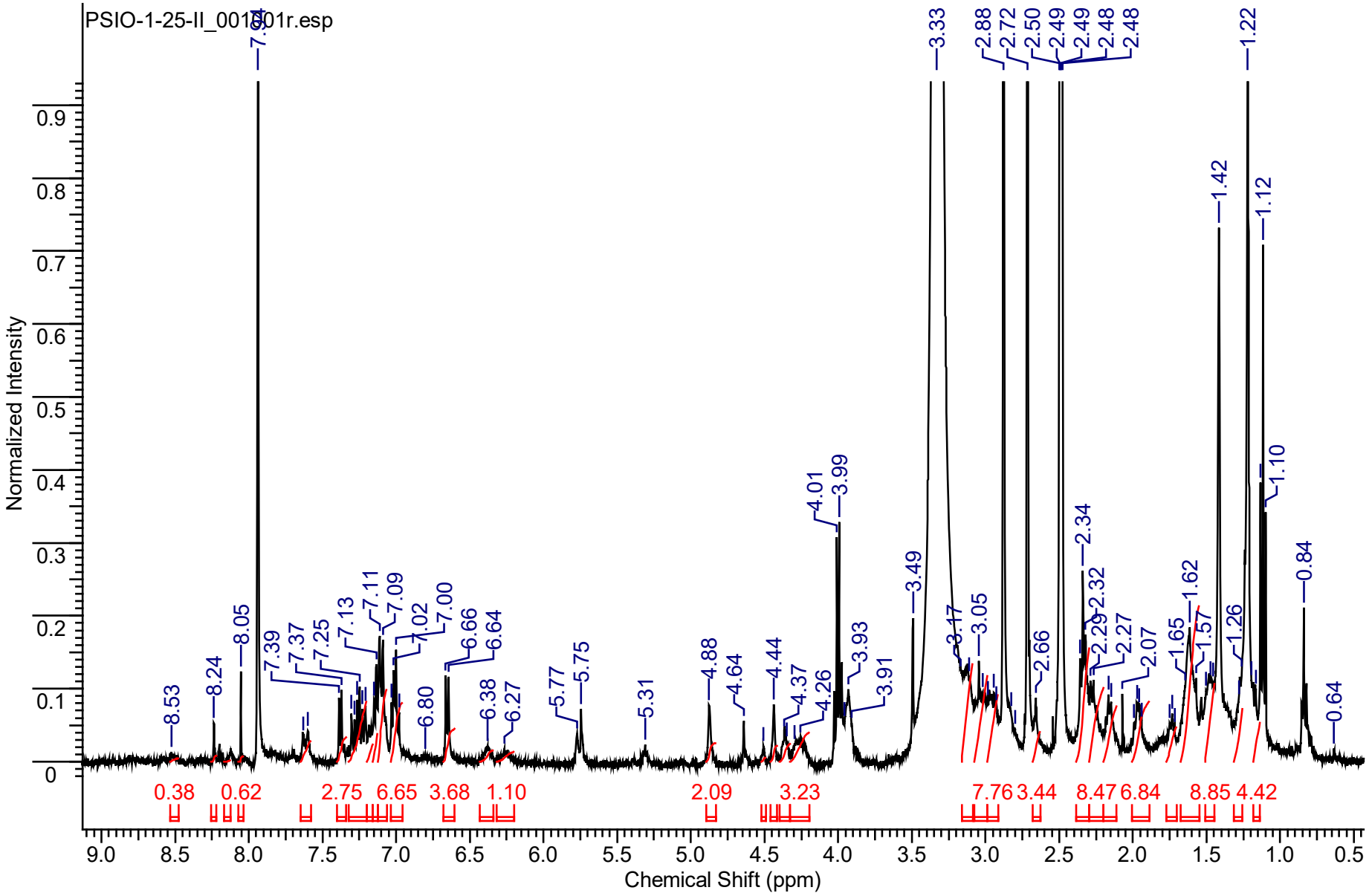


Figure S11. ^{1}H NMR of compound **6b**

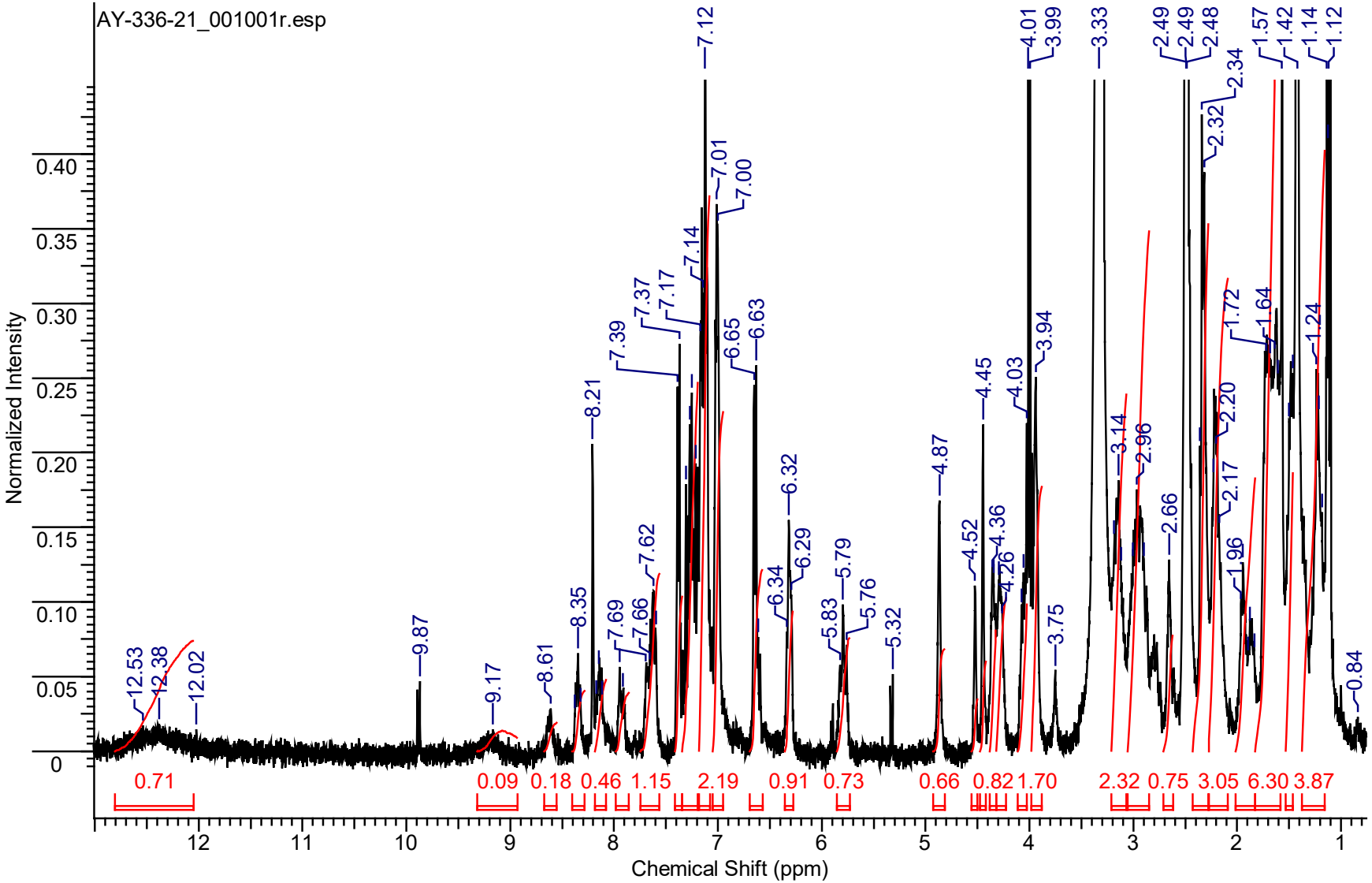


Figure S12. ^{1}H NMR of compound **6c**

Mass-spectra

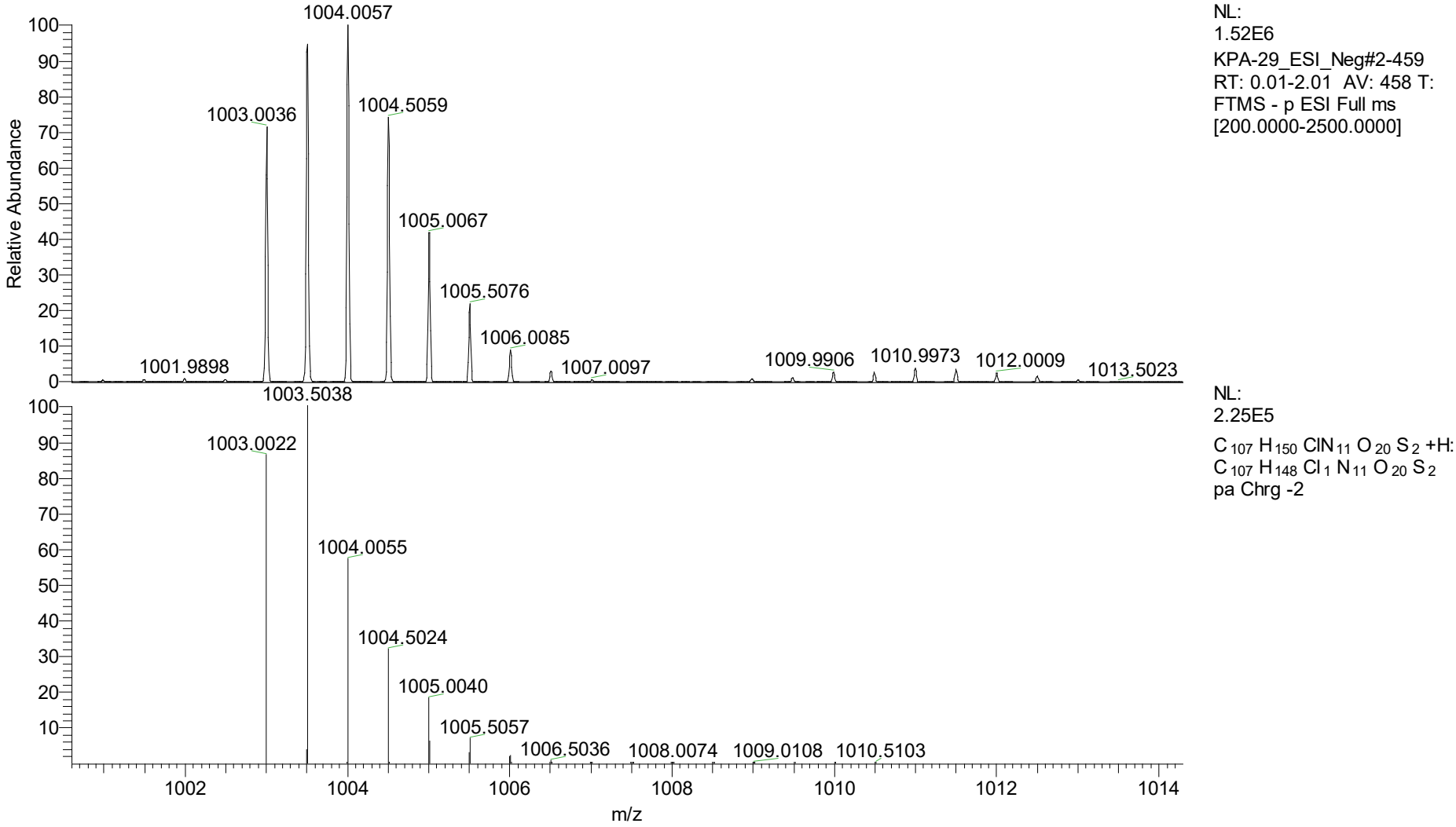


Figure S13. ESI-HRMS of compound **3'**

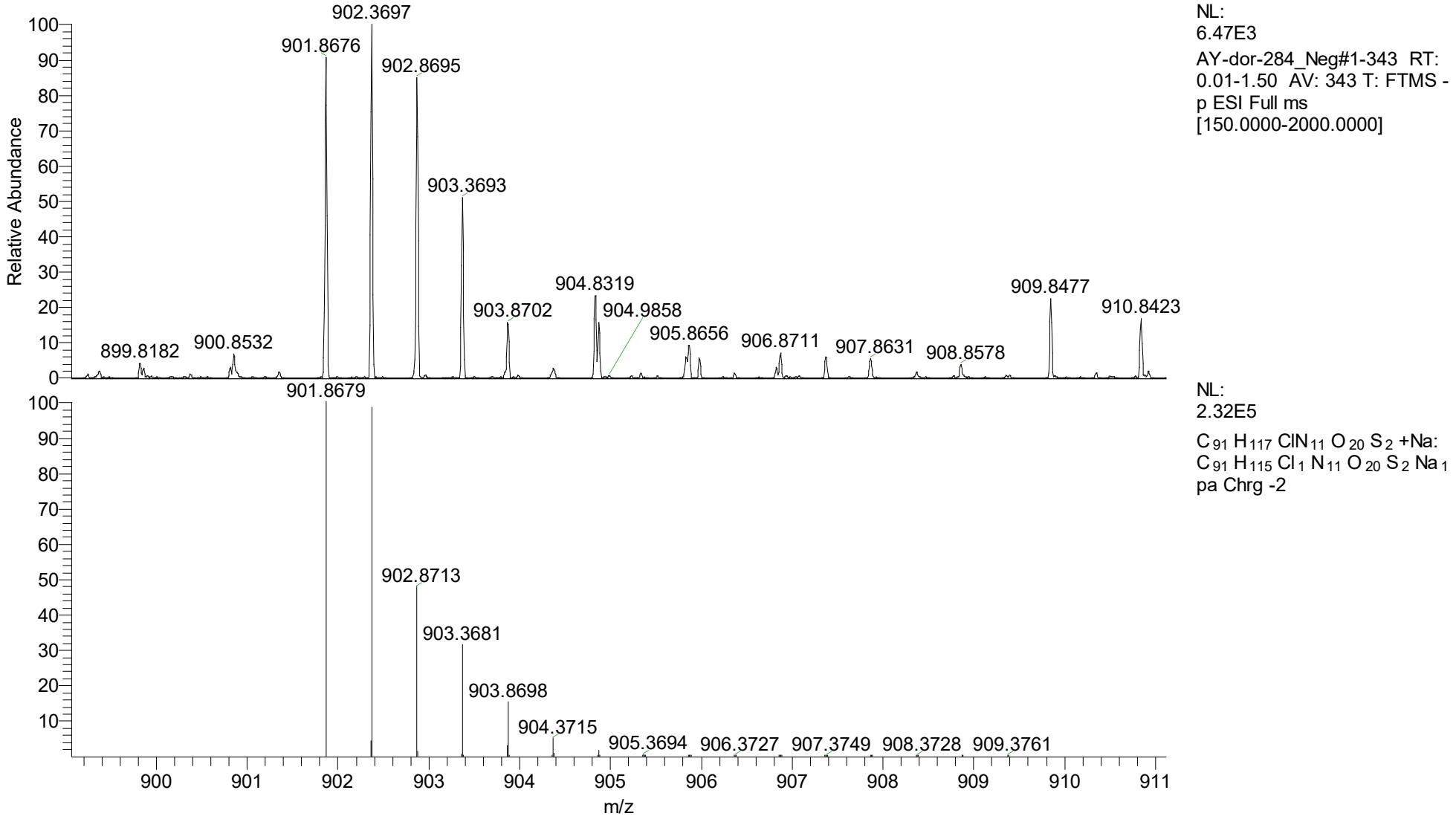


Figure S14. ESI-HRMS of compound **3**

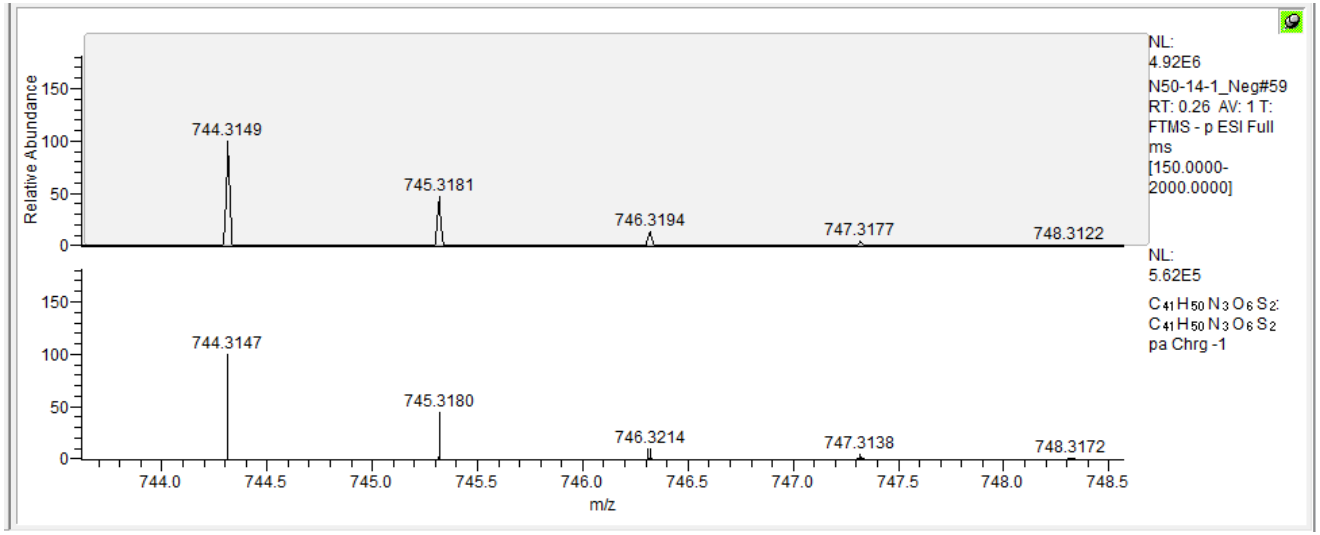


Figure S15. ESI-HRMS of compound **5a**

Peak Mass	Display Formula	Delta [ppm]	Theo. mass	Pattern Cov. [%]
730.4589	$C_{47}H_{60}O_4N_3$	1.48	730.4578	99.77

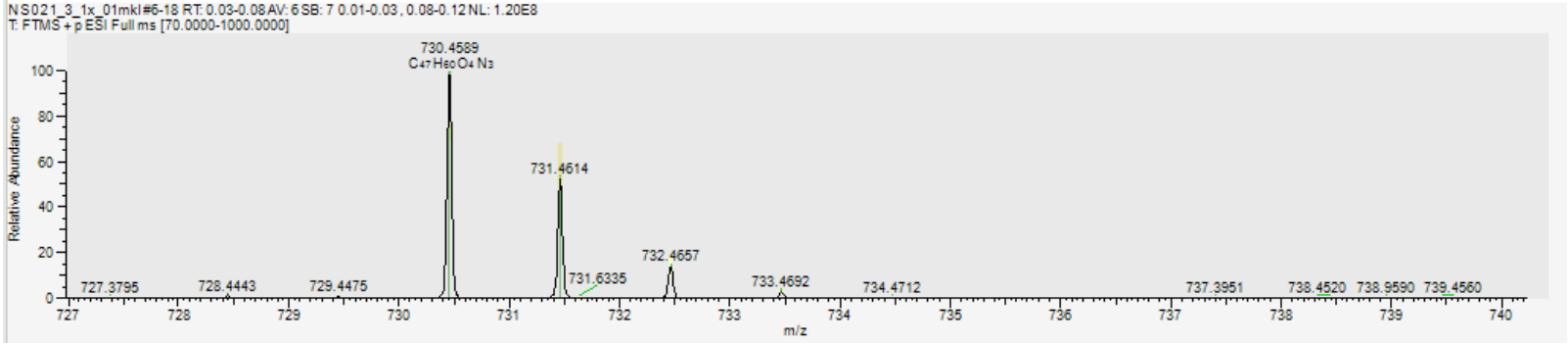


Figure S16. ESI-HRMS of compound **5b**

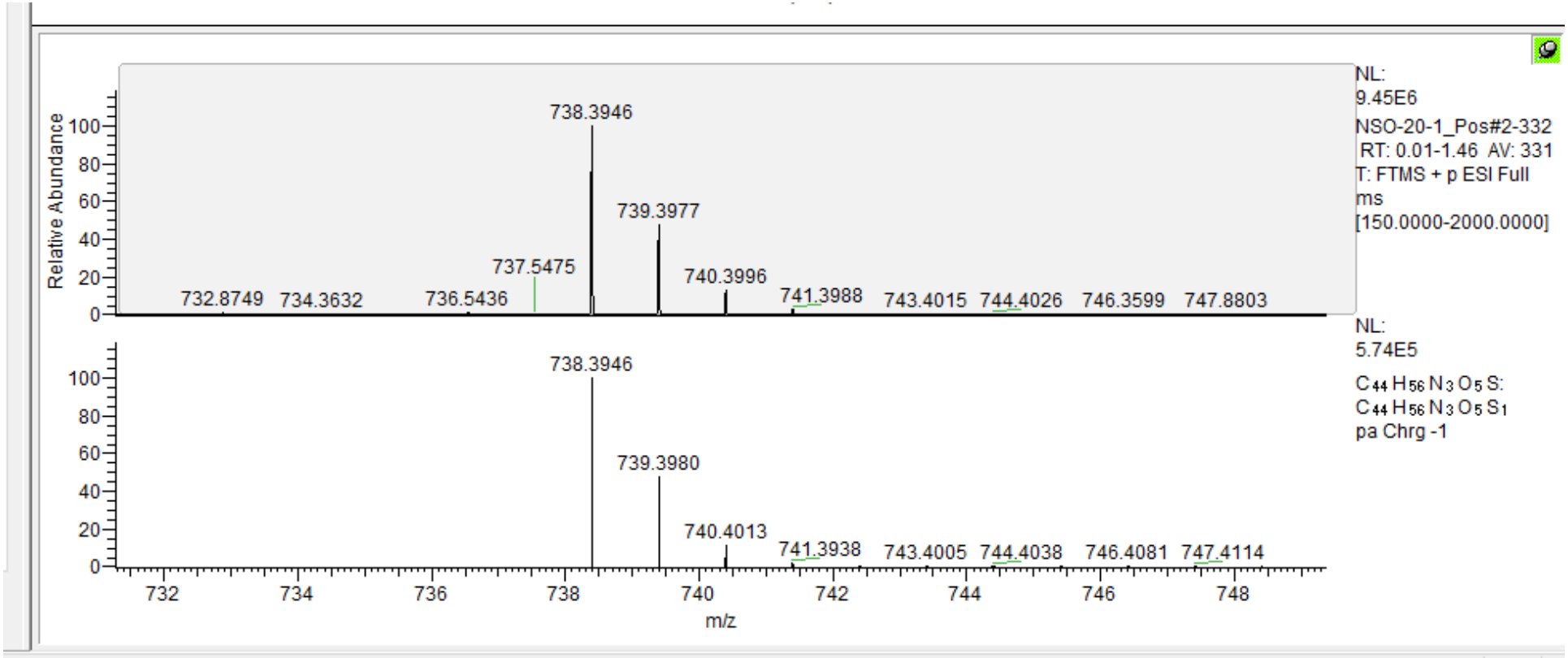


Figure S17. ESI-HRMS of compound **5c**

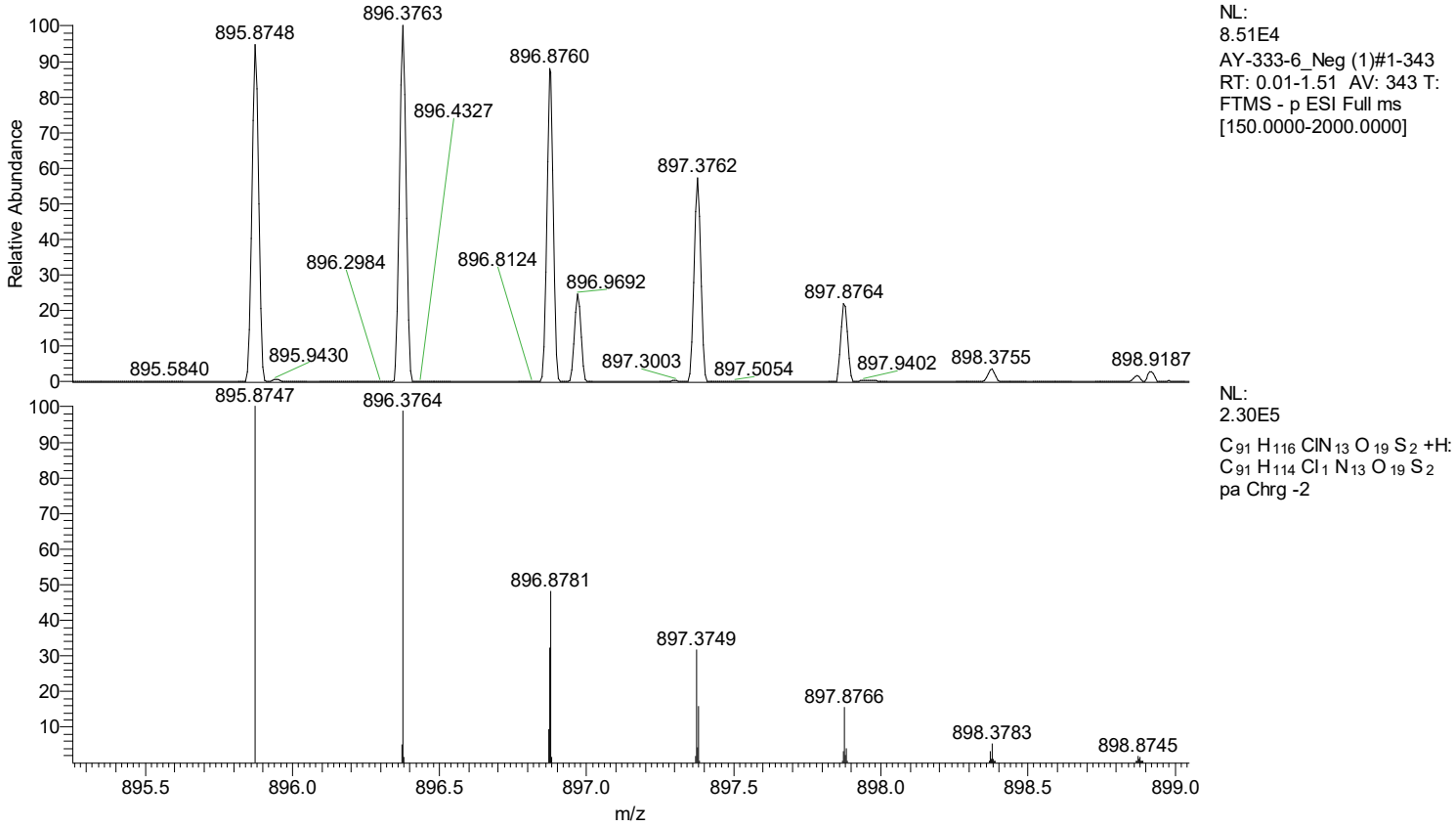


Figure S18. ESI-HRMS of compound **6a** ($C_{91}H_{116}ClN_{13}O_{19}S_2$)

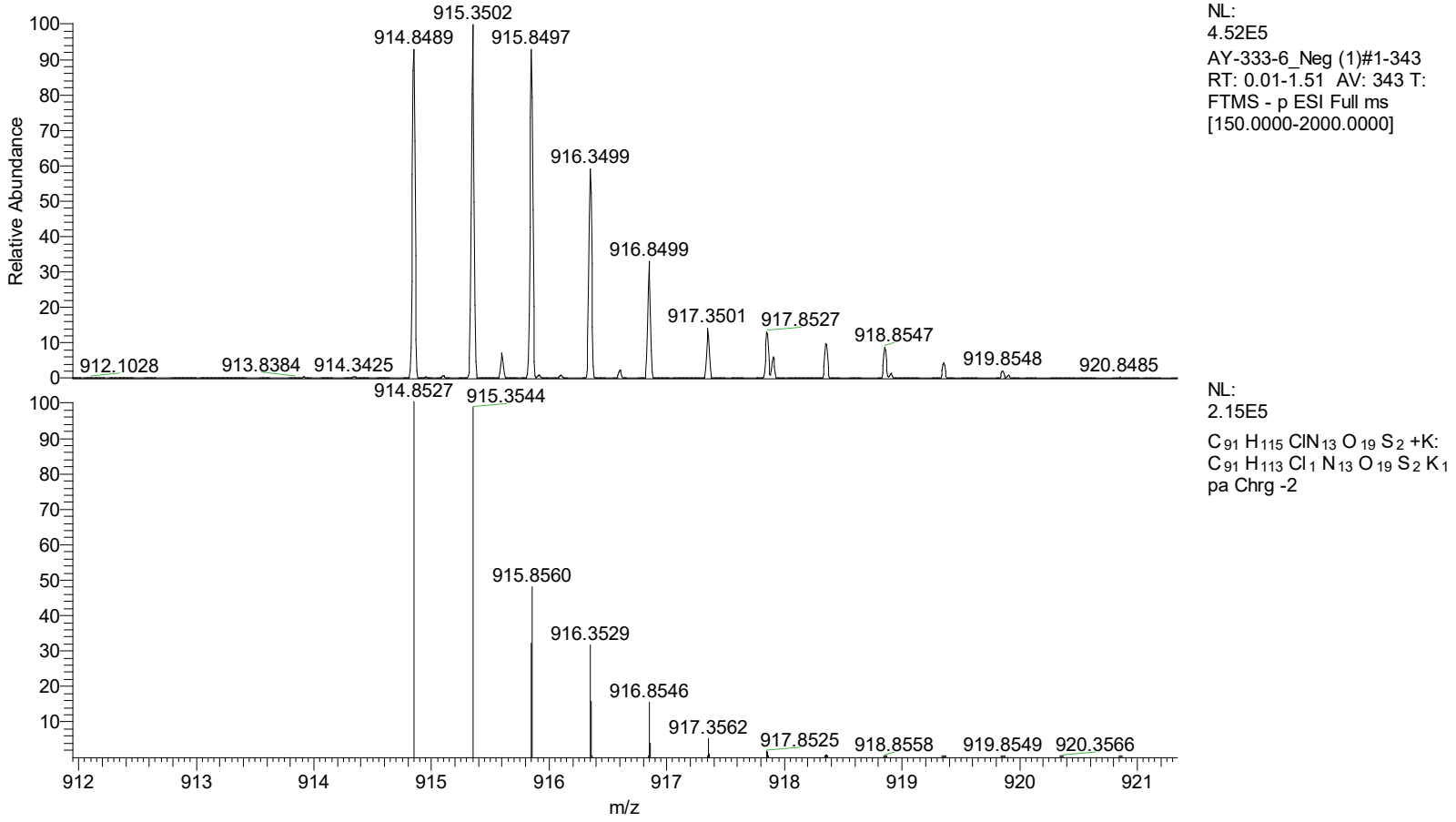


Figure S19. ESI-HRMS of compound **6a** ($C_{91}H_{116}ClN_{13}O_{19}S_2K$)

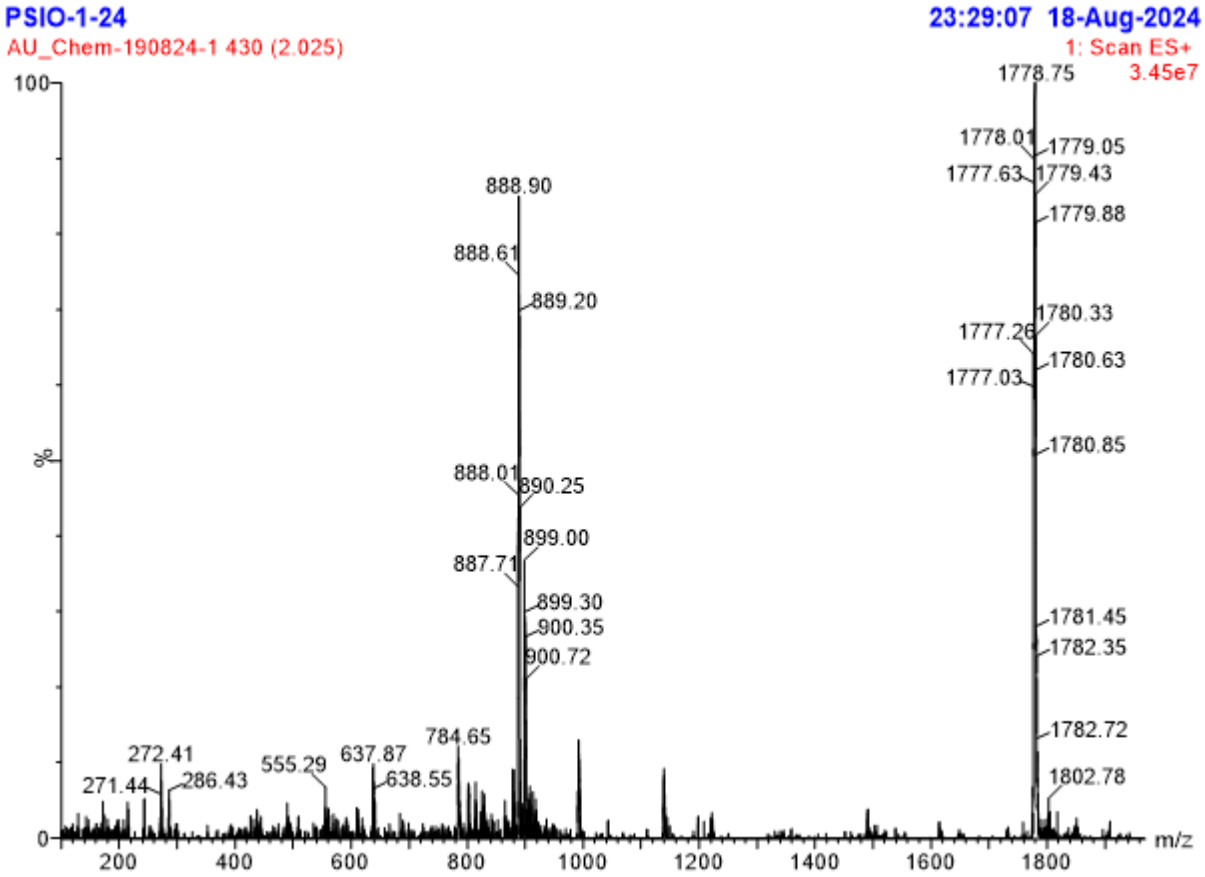


Figure S20. ESI-MS of compound **6b**

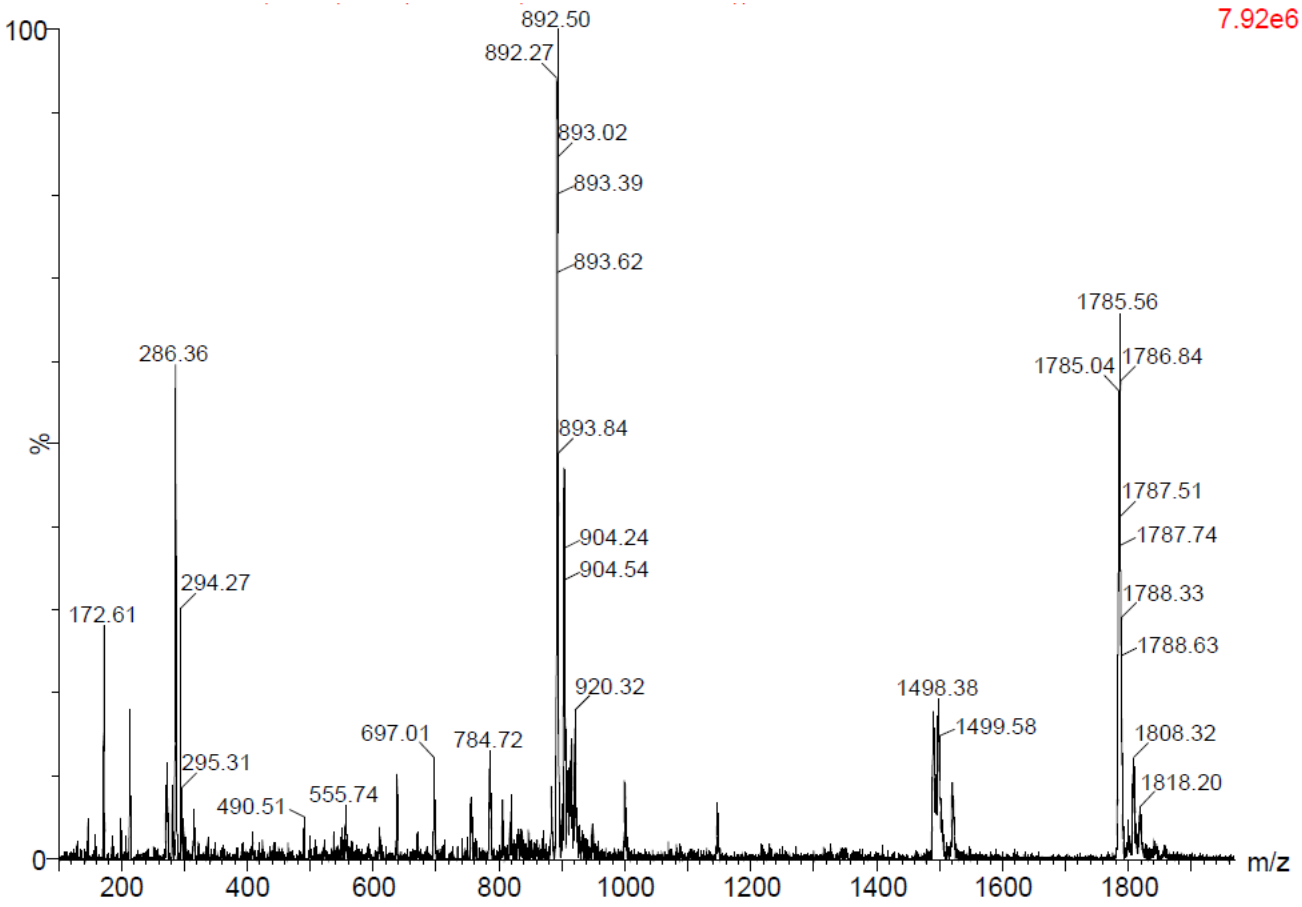


Figure S21. ESI-MS of compound **6c**