Supporting Information for:

Design of a Platinum–Acridine–Endoxifen Conjugate Targeted at Hormone-Dependent Breast Cancer

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1. Synthetic Procedures and Product Characterization

Materials and Methods. All reagents were used as obtained from commercial sources without further purification unless indicated otherwise. ^{1}H NMR spectra of the target compounds and intermediates were recorded on Bruker Advance DRX-500 and 300 MHz instruments. Proton-decoupled ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument operating at 125.8 MHz. 2-D ^{1}H-^{13}C gradient-selected Heteronuclear Multiple Bond Coherence (gsHMBC) experiments and temperature-dependent spectra were acquired on a Bruker DRX-500 instrument equipped with a TBI probe and a variable-temperature unit. 2-D HMBC spectra were collected with 2048 pts in t_2 (sw = 6510 Hz) , 256 pts in t_1 (sw = 27670 Hz), 128 scans per t_1 increment, and a recycle delay (d_1) of 1.5 s. Chemical shifts (δ) are given in parts per million (ppm) relative to internal standard tetramethylsilane (TMS). ^{1}H NMR data is reported in the conventional form including chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants (Hz), and signal integrations). ^{13}C{^{1}H} NMR data are reported as chemical shift listings (δ, ppm). The NMR spectra were processed and analyzed using the MestReNova software package. HPLC-grade solvents were used for all HPLC and mass spectrometry experiments. LC-ESMS analysis was performed on an Agilent 1100LC/MSD ion trap mass spectrometer equipped with an atmospheric pressure electrospray ionization source. Eluent nebulization was achieved with a N_2 pressure of 50 psi and solvent evaporation was assisted by a flow of N_2 drying gas (350 °C). Positive-ion mass spectra were recorded with a capillary voltage of 2800 V and a mass-to-charge scan range of 150 to 2200 *m/z*. To establish the purity of target compounds, samples were diluted in methanol containing 0.1 % formic acid and separated using a 4.6 mm x 150 mm reverse-phase Agilent ZORBAX SB-C18 (5 μm) analytical column at 25 °C. The purities of compound **9,** compound **10**, compound **9'** and compound **10'** were assessed using the following solvent system: solvent A, optima water, and solvent B, methanol/0.1% formic acid, with a flow rate of 0.5 mL/min and a gradient of 95% A to 5% A over 45 minutes. The purity of compound **5** was assessed using the following solvent system: solvent A, optima water, and solvent B, methanol/0.1% formic acid, with a flow rate of 0.5 mL/min and a gradient of 95% A to 5% A over 25 minutes. HPLC traces were recorded with a monitoring wavelength range of 363-463 nm. Peak integration was done using the LC/MSD Trap Control 4.0 data analysis software.

Acronyms used for reagents:

DMF: Dimethylformamide

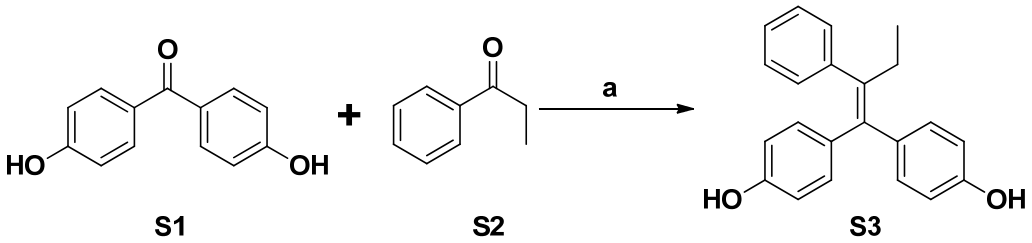
DCM: Dichloromethane

MeOH: Methanol

TEA: Triethylamine

TFA: Trifluoroacetic acid

Scheme 1. Synthesis of S3.

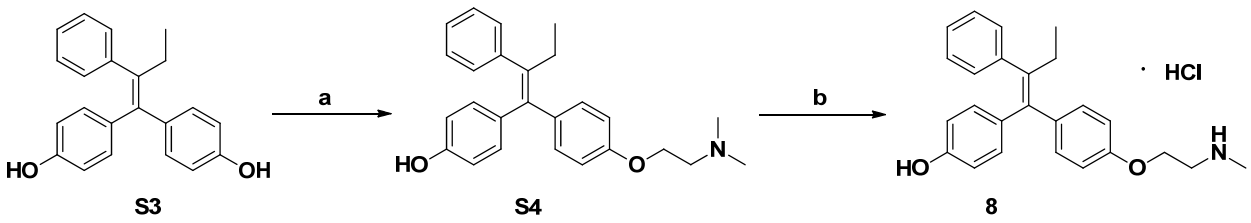


Reagents and conditions: (a) Zn dust, $TiCl_4$, THF, reflux, 82%.

1, 1-Bis(4-hydroxyphenyl)-2-phenylbut-1-ene (**S3**). This compound was synthesized according to the method reported previously[1] with modifications. To a stirred suspension of zinc powder (8 g, 0.124 mol) in 80 mL of anhydrous THF, $TiCl_4$ (6 mL, 0.056 mol) was added dropwise under argon at -20 °C. When the addition was complete, the mixture was warmed to room temperature and refluxed for 3 h. The reaction was cooled in an ice bath and a solution of 4,4'-hydroxybenzophenone (2 g, 0.0092 mol) and propiophenone (4.0 g, 0.0296 mol) in 10 mL of anhydrous THF was added. The reaction mixture was refluxed in the dark for 12 h. The reaction was quenched with 100 mL of 10% aqueous K_2CO_3 and extracted with ethyl acetate. The organic layers were collected, washed with saturated NaCl, dried over $MgSO_4$, and concentrated by rotary evaporation, affording a pink oil. To purify the crude product, the oil was dissolved in 10 mL of ethanol and precipitated by adding 40 mL of water. The white precipitate was recovered by filtration and recrystallized from 35 mL of hot DCM to afford **S3** (2.4 g, 82%) as a white solid. ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 9.35 (s, 1H), 9.10 (s, 1H), 7.21 – 7.04 (m, 5H), 6.97 (d,

J = 8.4 Hz, 2H), 6.74 (d, *J* = 8.5 Hz, 2H), 6.60 (d, *J* = 8.5 Hz, 2H), 6.39 (d, *J* = 8.5 Hz, 2H), 2.38 (q, *J* = 7.4 Hz, 2H), 0.84 (t, *J* = 7.3 Hz, 3H).

Scheme 2. Synthesis of 8.



S3 S4 8

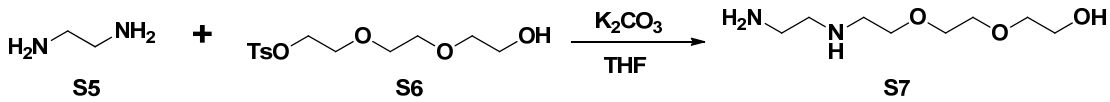
Reagents and conditions: (a) $Me_2NCH_2CH_2Cl$, Cs_2CO_3, DMF, 120 °C, 67%. (b) (1)ACE-Cl, DCM, reflux; (2)MeOH, 6 M HCl, reflux, 81%.

(*E,Z*)-1-[4-(2-Dimethylaminoethoxy)phenyl]-1-(4-hydrox-yphenyl)-2-phenylbut-1-ene (S4). This compound was synthesized according to the method[1] reported previously with slight modifications. A solution of 1,1-bis(4-hydroxyphenyl)-2-phenylbut-1-ene (1 g, 3.2 mmol) in DMF (15 mL) was mixed with Cs_2CO_3 (3.1 g, 9.5 mmol) and refluxed for 30 min. To this mixture was added 2-(dimethylamino)ethylchloride hydrochloride (0.56 g, 3.8 mmol) in three portions over 1 h. The mixture was heated at 120 °C for another 6 h. After cooling to room temperature, the mixture was poured into 20 mL of saturated NH_4Cl and extracted with ethyl acetate (3 × 20 mL). The organic phases were combined, washed with saturated NaCl (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated to give brown oil. The crude material was purified by recrystallization from 10 mL of hot ethanol to afford 0.82 g white crystals (**S4**) with a 1:1 Z/E ratio (yield: 67%). ^{1}H NMR ($(CD_3)_2SO$) δ 9.38 (s, 0.5H), 9.13 (s, 0.5H), 7.23 - 7.04 (m, 5H), 6.98 (d, *J* = 8.4 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.71 (d, *J* = 8.7 Hz, 1H), 6.63 - 6.53 (m, 2H), 6.40 (d, *J* = 8.6 Hz, 1H), 4.05 (t, *J* = 5.8 Hz, 1H), 3.88 (t, *J* = 5.8 Hz, 1H), 2.62 (t, *J* = 5.8 Hz, 1H), 2.56 - 2.49 (m, 1H), 2.44 - 2.33 (m, 2H), 2.22 (s, 3H), 2.15 (s, 3H), 0.84 (t, *J* = 7.3 Hz, 3H).

(*E,Z*)-Endoxifen Hydrochloride (**8·HCl**). This compound was synthesized according to the method[2] reported previously with modifications. 1-Chloroethyl chloroformate (ACE-Cl, 668 μL, 61.9 mmol) was added dropwise to a solution of **S4** and 1,8-bis(dimethylamino)naphthalene

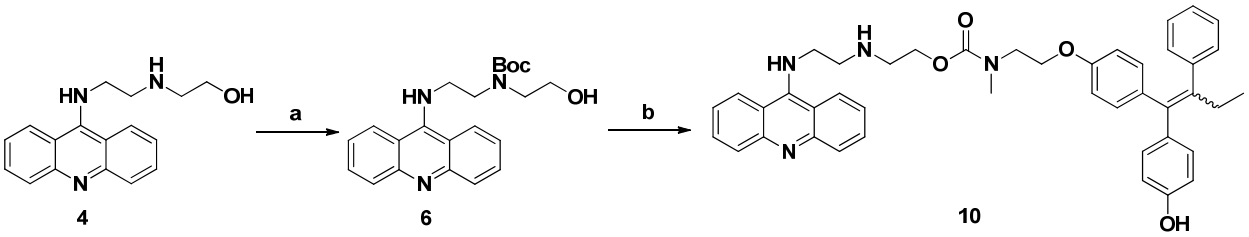
(proton sponge, 249 mg, 11.6 mmol) in 9 mL of DCM at 0 ºC. The resulting solution was stirred for 30 min. at 0 ºC and then refluxed for 12 h. The reaction mixture was cooled to room temperature and removal of the solvent under reduced pressure gave a brown crude oil. This residue was dissolved in a mixture of MeOH (20 mL) and 6 M HCl (5 mL) and refluxed for 3 h and then cooled to room temperature. The MeOH was evaporated off and the residue was dissolved in 50 mL of ethyl acetate, washed with saturated NaCl and water, dried over anhydrous Na_2SO_4, and concentrated to give 0.260 g of **8** as white solid, (yield: 81%), as 1:1 mixture of Z/E Endoxifen isomers, which was used in the next step without further purification. ^{1}H NMR ($(CD_3)_2SO$) δ 9.30 (br s, 2H), 7.25 - 7.06 (m, 5H), 7.00 (d, *J* = 2.7 Hz, 1H), 6.96 (d, *J* = 2.7 Hz, 1H), 6.79 - 6.72 (m, 2H), 6.65 (d, *J* = 8.5 Hz, 1H), 6.60 (d, *J* = 8.5 Hz, 1H), 6.42 (d, *J* = 8.5 Hz, 1H), 4.26 (t, *J* = 4.6 Hz, 1H), 4.08 (d, *J* = 5.7 Hz, 1H), 3.19 (d, *J* = 5.7 Hz, 1H), 2.60 (s, 1.5H), 2.54 (s, 1.5H), 2.40 (m, 2H), 0.85 (t, *J* = 7.3 Hz, 3H).

Scheme 3. Synthesis of S7.



The mixture of ethylene diamine (**S5**, 3.05g, 0.05 mol) and anhydrous potassium carbonate (1.15 g, 0.0083 mol) in 30 ml anhydrous THF was heated under reflux, to which was added monotosylated triethylene glycol (**S6**, 1.71g, 0.0056 mol) in 15 ml anhydrous THF dropwisely over 1 h, then the reaction was refluxed for another 16 hours. When the reaction was completed, the white solids were filtered off after the mixture was cooled to room temperature. To remove THF and unreacted ethylene diamine, the filtrate was then concentrated under reduced pressure followed by dried under vacume at 45 ºC overnight to give a pale yellow crude oil (1.34 g, yield 87%), which can be used in the next step without any purification. ^{1}H NMR ($CDCl_3$) δ 3.85 - 3.51 (m, 10H), 2.92 - 2.63 (m, 10H), 2.31 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 72.86, 70.46, 70.29, 70.12, 61.30, 52.17, 48.94, 41.46.

Scheme 4. Synthesis of 8.



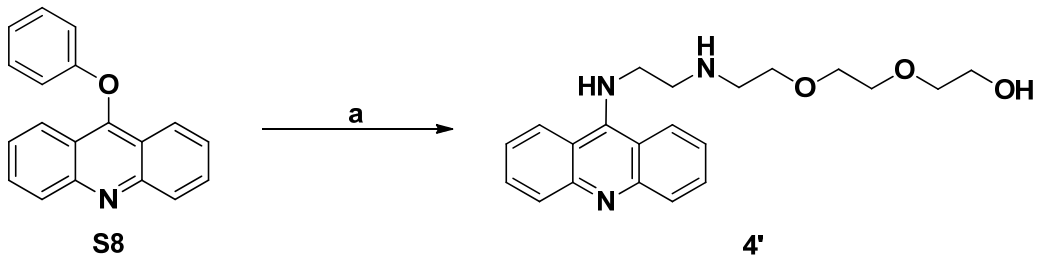
Reagents and conditions: (a) Boc_2O/DCM. (b) 1. 4-nitrophenyl chloroformate, TEA, DCM, room temperature; 2. **8**, DCM, room temperature; 3. TFA, DCM, room temperature; 4. 1 M NaOH, DCM, room temperature, 56%.

The Boc protected acridine derivative **6** was synthesized as follows. 2-((2-(Acridin-9-ylamino) ethyl)amino)ethanol[3] (**4**) (2.82 g, 0.01 mol) was suspended in 30 mL of anhydrous dichloromethane, to which was added Boc_2O (2.18 g, 0.01 mol) in 5 mL of DCM at 0-5 ºC, maintained with an ice bath. The mixture was then stirred at room temperature for 4 h. The solvent was removed by rotary evaporation and the residue was dissolved in 10 mL of DCM and precipitated with 200 mL of anhydrous diethyl ether. The solid was recovered by filtration and dried in a vacuum, affording **6** as yellow solid 3.57 g (yield: 93%), which was used in the next step without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 7.8 Hz, 2H), 7.45 (t, J = 7.8 Hz, 2H), 7.12 (t, J = 7.8 Hz, 2H), 4.15 - 3.95 (m, 2H), 3.78 - 3.54 (m, 4H), 3.42 - 3.24 (m, 2H), 1.43 - 1.10 (m, 9H).

Compound **6** (1 g, 3.55 mmol), TEA (1.79 g, 17.7 mmol) and 4-nitrophenyl chloroformate (0.99 g, 4.6 mmol) were dissolved in 20 mL of anhydrous dichloromethane. The mixture was stirred at room temperature for 16 h. To the resulting solution of the 4-nitrophenyl-activated form of the acridine (**7**) was added **8** (1.74 g, 4.23 mmol) in 5 mL of anhydrous DCM and the reaction was stirred for another 8 h. The solvent was removed in a high vacuum and the residue was redissolved in 40 mL of DCM and washed with 1 M HCl (3 × 20 mL). The organic phase was collected, dried over anhydrous Na_2SO_4, and concentrated to afford orange oil. To remove the Boc group, the orange oil was dissolved in 6 mL of a 1:1 mixture of anhydrous DCM and TFA and stirred at room temperature for 3 h. The reaction was quenched by adding 10 mL of 1 M NaOH solution. The free base was extracted from NaOH solution with DCM, dried over anhydrous Na_2SO_4, and concentrated. The product was purified by flash chromatography (Al_2O_3,

DCM:MeOH, 30:1) to give 1.35 g of **9** as an orange oil with a 1:1 Z/E ratio (yield: 56%). ^{1}H NMR (500 MHz, $CDCl_3$, 60 ºC) δ 8.13 - 8.05 (m, 4H), 7.51 (s, 2H), 7.27 - 6.41 (m, 16H), 4.34 - 4.29 (t, *J* = 5.4 Hz, 1H), 4.28 (t, *J* = 5.3 Hz, 1H), 4.17 - 3.94 (m, 4H), 3.69 (t, *J* = 5.4 Hz, 1H), 3.58 (t, *J* = 5.3 Hz, 1H), 3.11 - 2.94 (m, 7H), 2.55 - 2.40 (m, 2H), 1.00 – 0.84 (m, 3H). MS (ESI, positive mode): for $C_{43}H_{44}N_4O_4$ ($[M+H]^+$), 681.3; found: 681.5.

Scheme 5. Synthesis of 4'.

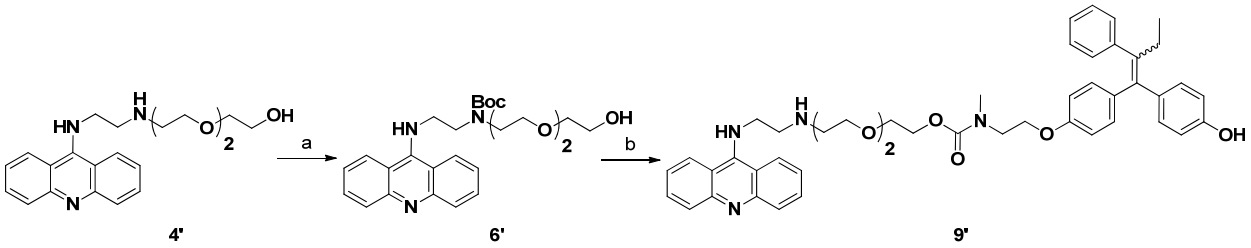


S8 **4'**

Reagents and conditions: (a) **S7**, THF, reflux.

A mixture of phenoxyacridine (**S8**, 2.71 g, 0.01 mol) and 2-(2-(2-((2-aminoethyl) amino) ethoxy) ethoxy) ethanol (**S7**, 2.31 g, 0.011 mol) in 30 mL of anhydrous THF was refluxed for 16 h. The solvent was evaporated off and the residue was dissolved in 30 mL of acetone. To this solution were added 5 mL of concentrated HCl and the mixture was stirred at 4 ºC for 3 hours. A yellow precipitate formed which was recovered by filtration, resuspended in 50 mL of 1 M sodinum hydroxide, and stirred at room temperature for 30 min. The aqueous phase was extracted with CH_2Cl_2 and the organic phase was collected, dried over Na_2SO_4, and concentrated using rotary evaporation, affording 3.18 g of the free base as an orange oil (Yield: 86%). ^{1}H NMR ($CDCl_3$) δ 8.17 (d, *J* = 8.8 Hz, 1H), 8.05 (d, *J* = 8.7 Hz, 1H), 7.69 – 7.59 (m, 1H), 3.84 (t, *J* = 5.5 Hz, 1H), 3.74 (d, *J* = 4.7 Hz, 1H), 2.93 (t, *J* = 5.5 Hz, 1H), 2.84 (t, *J* = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.98, 149.25, 141.24, 133.06, 129.86, 129.11, 126.91, 123.21, 122.70, 121.31, 120.94, 116.86, 116.77, 72.58, 70.50, 70.34, 70.23, 61.51, 49.32, 49.02, 48.45. MS (ESI, positive mode): for $C_{26}H_{35}N_3O_5$ ($[M+H]^+$), 370.26; found: 370.5.

Scheme 6. Synthesis of 9'.

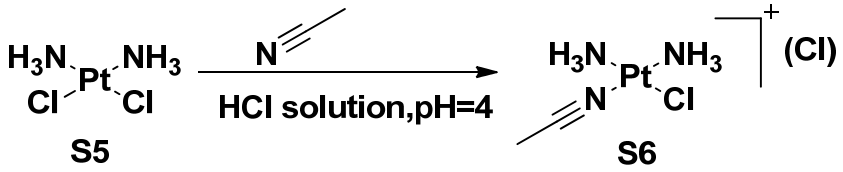


Reagents and conditions: (a) Boc_2O/DCM. (b) 1. 4-nitrophenyl chloroformate, TEA, DCM, room temperature; 2. **8**, DCM, room temperature; 3. TFA, DCM, room temperature; 4. 1 M NaOH, DCM, room temperature, 37%.

Compound **6'** was prepared according to the procedure described for **6**, by using **4'** as the precursor with the yield of 95%. ^{1}H NMR ($CDCl_3$) δ 8.31 - 7.86 (m, 4H), 7.62 - 7.45 (m, 2H), 7.20 (s, 2H), 4.12 - 3.06 (m, 16H), 1.40 - 1.08 (m, 9H). MS (ESI, positive mode): for $C_{26}H_{35}N_3O_5$ ($[M+H]^+$), 470.26; found: 470.5.

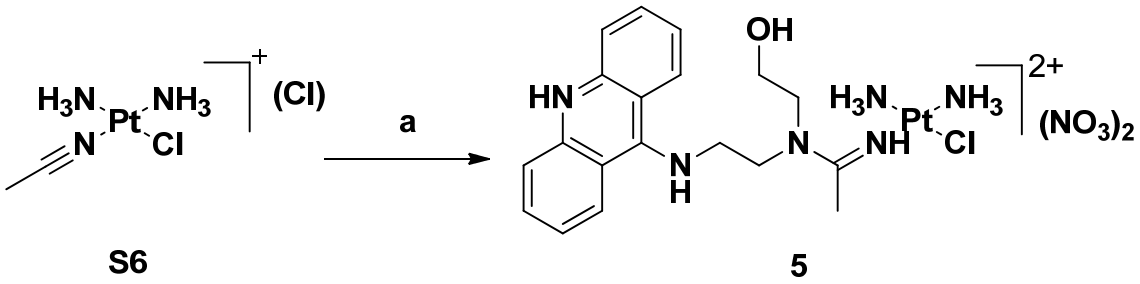
Compound **9'** was prepared according to the procedure described for **9**, by using **6'** as the precursor with the yield of 37%. ^{1}H NMR ($CDCl_3$, 60 ºC) δ 8.38 - 8.01 (m, 3H), 7.77 - 7.24 (m, 3H), 7.22 - 6.98 (m, 6H), 6.86 - 6.25 (m, 6H), 4.33 - 3.39 (m, 16H), 3.07 - 2.79 (m, 6H), 2.62 - 2.37 (m, 2H), 1.79 - 1.66 (m, 2H), 1.31 (s, 1H), 0.95 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.33, 152.38, 148.61, 141.17, 137.80, 132.09, 132.06, 130.67, 130.67, 130.30, 129.73, 127.86, 125.90, 122.83, 122.83, 116.48, 115.36, 115.28, 114.62, 114.56, 113.96, 113.79, 113.23, 113.04, 70.62, 70.36, 69.58, 48.91, 48.70, 48.58, 48.21, 47.91, 35.98, 28.99, 26.40, 13.65, 1.03. MS (ESI, positive mode): for $C_{47}H_{52}N_4O_6$ ($[M+H]^+$), 769.39; found: 769.8.

Scheme 7. Synthesis of S6.



The platinum precursor complex **S6** was prepared according to a previously described method.[4] Briefly, *cis*-diamminedichloridoplatinum(II) (cisplatin)[5] (**S5**) (0.5 g, 1.67 mmol) was heated under reflux in 25 mL of dilute HCl (pH 4) with propionitrile (6.85 mL , 98.5 mmol) until the yellow suspension turned into a colorless solution (~2 h). Solvent was removed by rotary evaporation, and the pale-yellow residue was redissolved in 10 mL of dry MeOH. A small amount of an insoluble yellow residue was removed by membrane filtration and the colorless filtrate was added directly into 250 mL of vigorously stirred dry diethyl ether, affording 342 mg of **S6** (yield: 63%) as an off-white microcrystalline precipitate. ^{1}H NMR (D_2O) δ 2.53 (3 H, m, Pt satellites), 4.35, 4.48 (4 H, HD exchange, 2 br s).

Scheme 8. Synthesis of 4.

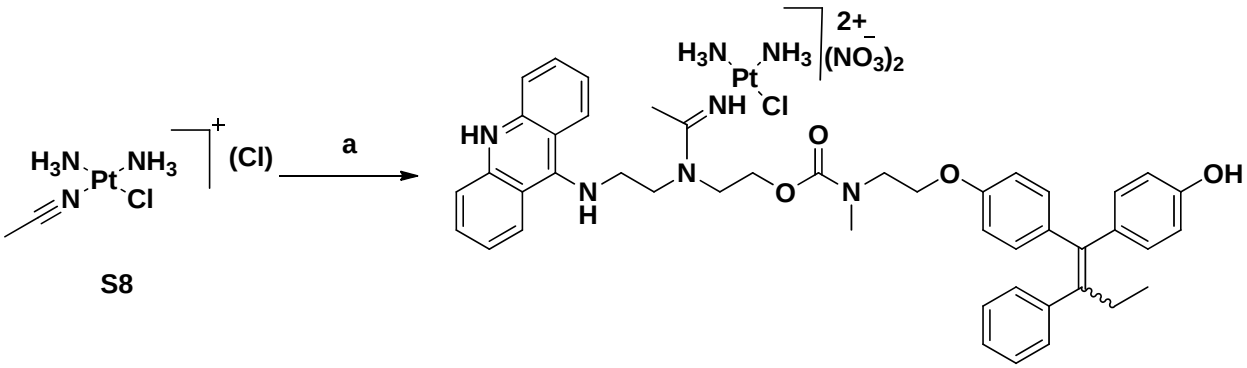


Reagents and conditions: (a) 1. DMF, $AgNO_3$; 2. **4**, DMF, -10 °C; 3. 1M HNO_3, MeOH, 87%.

Platinum complex **S6** (341 mg, 1 mmol) was converted to its nitrate salt by reaction with $AgNO_3$ (162 mg, 0.95 mmol) in 7 mL of anhydrous DMF. AgCl was removed by syringe filtration, and the filtrate was cooled to -10 °C. Acridine precursor **4** (282 mg, 0.1 mmol) was added to the solution, and the suspension was stirred at -10 °C for 24 h. After treatment with activated carbon, the reaction mixture was added into 300 mL of diethyl ether. The yellow slurry was stirred for 30 min, and the precipitate was recovered by membrane filtration and dried in a vacuum overnight. The solid was dissolved in anhydrous MeOH and treated with 1 equivalent of 1 M HNO_3, stirred at room temperature for 30 minutes and precipitated by 300 mL anhydrous diethyl ether. The product was further purified by recrystallization from hot ethanol to give 618 mg of hybrid **5** a microcrystalline orange material (yield: 87%). ^{1}H NMR (MeOH-d4) δ 8.40 (d, J = 8.8 Hz, 2H), 7.87 (t, J = 6.8, 2H), 7.72 (dd, J = 8.7, 1.2 Hz, 1H), 7.49 (d, J = 8.4 Hz, 2H), 4.31 (t, J = 7.0 Hz,

2H), 4.10 (brs, 2H), 3.91 (t, J = 6.8 Hz, 1H), 3.75 (s, 2H), 3.65 (t, J = 4.9 Hz, 2H), 3.49 (t, J = 4.9 Hz, 2H), 2.58 (s, 3H). ^{13}C NMR (MeOD) δ 167.49, 160.02, 141.40, 136.55, 126.49, 125.30, 119.77, 114.14, 60.73, 49.86, 48.16, 47.39, 23.02. MS (ESI, positive mode): for $C_{19}H_{28}ClN_6OPt$ ($[M]^+$), 587.00; found: 585.2.

Scheme 9. Synthesis of 10.

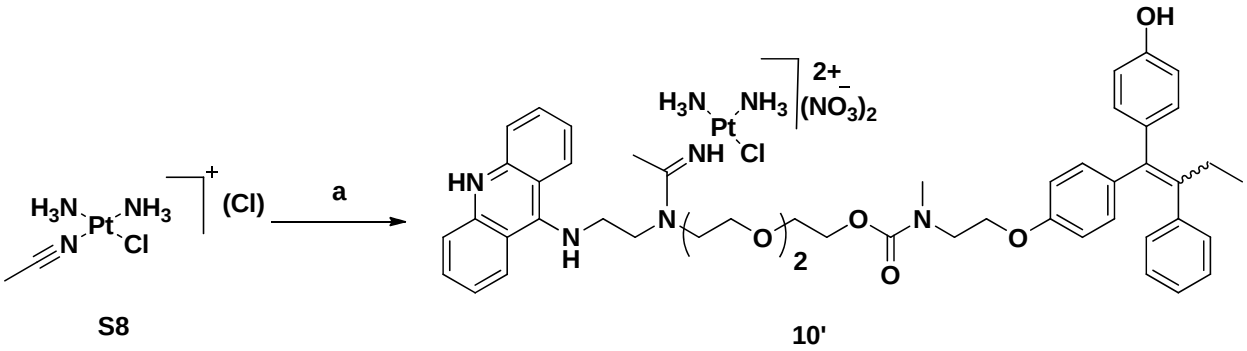


Reagents and conditions: (a) 1. DMF, $AgNO_3$; 2. **9**, DMF, 4 °C; 3. 1 M HNO_3, MeOH, r.t., 47%.

Platinum complex **S8** (75 mg, 0.22 mmol) was converted to its nitrate salt by reaction with $AgNO_3$ (35.6 mg, 0.21 mmol) in 7 mL of anhydrous DMF. AgCl was removed by syringe filtration, and the filtrate was cooled to -10 °C. Acridine precursor **9** (150 mg, 0.22 mmol) was added to the solution, and the mixture was stirred at 4 °C for 5 days. After treatment with activated carbon, the reaction mixture was added into 300 mL of diethyl ether. The yellow slurry was stirred for ~30 min, and the precipitate was recovered by membrane filtration, washed by 10 mL of anhydrous DCM and dried in a vacuum overnight. The solid was dissolved in anhydrous MeOH containing 1 equivalent of 1 M HNO_3, stirred at room temperature for 30 minutes and precipitated with 300 mL of anhydrous diethyl ether. The product was further purified by recrystallization from hot ethanol to give 116 mg of **10** (yield 47 %). ^{1}H NMR (500 MHz, DMF-*d*7) δ 14.28 (s, 0.5H), 10.61 (s, 0.5H), 9.64 (s, 0.5H), 9.40 (s, 0.5H), 8.81 (s, 2H), 7.10 (s, 2H), 7.28 - 6.00 (m, 15H), 4.74 (s, 3H), 4.50 (s, 3H), 4.59 - 3.87 (m, 10H), 3.65 (t, J = 5.6 Hz, 1H), 3.55 (t, J = 6.3 Hz, 1H), 3.02 (s, 2H), 2.96 (s, 3H), 2.78 (s, 3H), 2.54 - 2.37 (m, 2H), 0.97 - 0.82 (m, 3H). ^{13}C NMR (126 MHz, DMF-*d*7) δ 165.54, 161.88, 158.26, 157.48, 156.61, 156.01,

155.67, 142.56, 140.32, 138.34, 136.31, 135.06, 131.60, 130.41, 129.75, 127.93, 125.71, 123.69, 118.93, 115.13, 113.87,113.36, 65.84, 62.39, 48.38, 21.99, 12.96. MS (ESI, positive mode): for $C_{45}H_{54}ClN_7O_4$ Pt([M - 2H]$^+$), 986.37; found: 985.6.

Scheme 10. Synthesis of 10'.



Reagents and conditions: (a) 1. DMF, $AgNO_3$; 2. **9'**, DMF, 4 °C; 3. 1 M HNO_3, MeOH, r.t., 84%.

Compound **9'** was prepared according to the procedure described for **10**, by using **9'** as the precursor with the yield of 84%. ^{1}H NMR (500 MHz, DMF-*d7*) δ 9.65 (s, 0.5H), 9.39 (s, 0.5H), 8.64 (s, 2H), 8.06 - 7.92 (m, 3H), 7.78 - 6.35 (m, 16H), 4.67 - 3.87 (m, 14H), 3.87 - 3.35 (m, 22H), 3.01- 2.98 (m, 1H), 2.59 - 2.36 (m, 2H), 1.79 (m, 6H), 0.90 (s, 3H). ^{13}C NMR (126 MHz, DMF-*d7*) δ 165.75, 161.87, 156.85, 156.00, 142.79, 140.58, 140.30, 138.43, 136.29, 134.49, 131.81, 130.48, 129.74, 127.92, 126.02, 115.11, 114.37, 114.15, 113.33, 97.85, 70.12, 69.22, 67.32, 65.53, 64.56, 34.29, 34.12, 29.17, 29.00, 25.36, 21.97, 13.15. MS (ESI, positive mode): for $C_{49}H_{61}ClN_7O_6Pt$ ([M+H]$^+$), 1074.40; found: 1074.8.

2. Stability Study of Compound 10. 10 μM solutions of compound **10** were prepared in the following buffers: 10 mM phosphate-buffered saline (PBS), pH 7.4; 10 mM phosphate (PB), pH 7.4; acetate, pH 5.0. Each sample was heated at 37 °C in the dark for 48 h and then analyzed by LC-ESMS. The chromatographic separations were performed with a 4.6 mm x 150 mm reverse-phase Agilent ZORBAX SB-C18 (5 μm) analytical column with the column temperature maintained at 25 °C and a binary mobile phase system consisting of: solvent A, optima water, and solvent B, methanol/0.1% formic acid, a gradient of 95% A to 5% A over 20 minutes and a flow rate of 0.5 mL/min.

3. NMR spectra

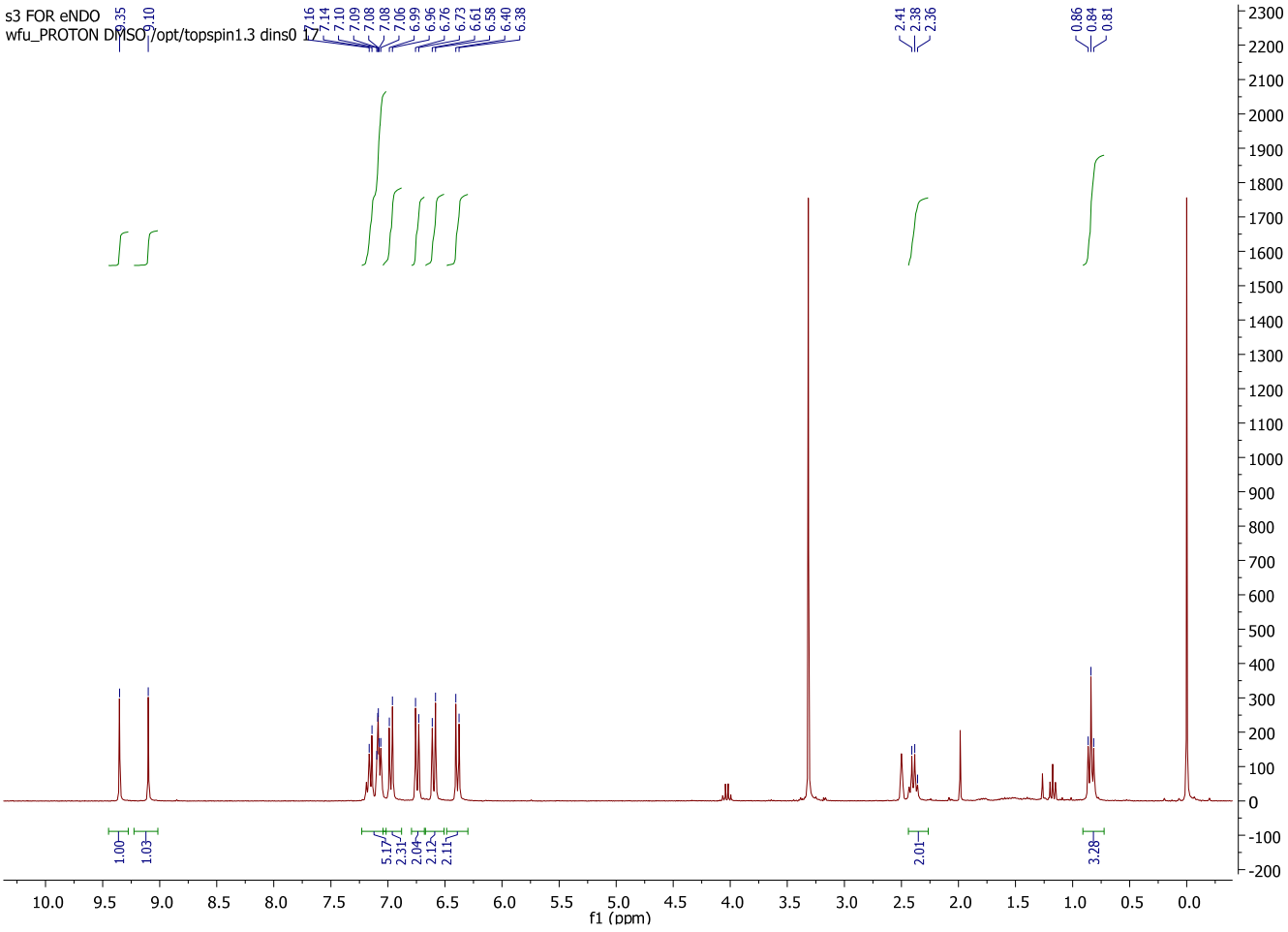


Figure S3.1. ^{1}H NMR spectrum of compound S3 in $(CD_3)_2SO$.

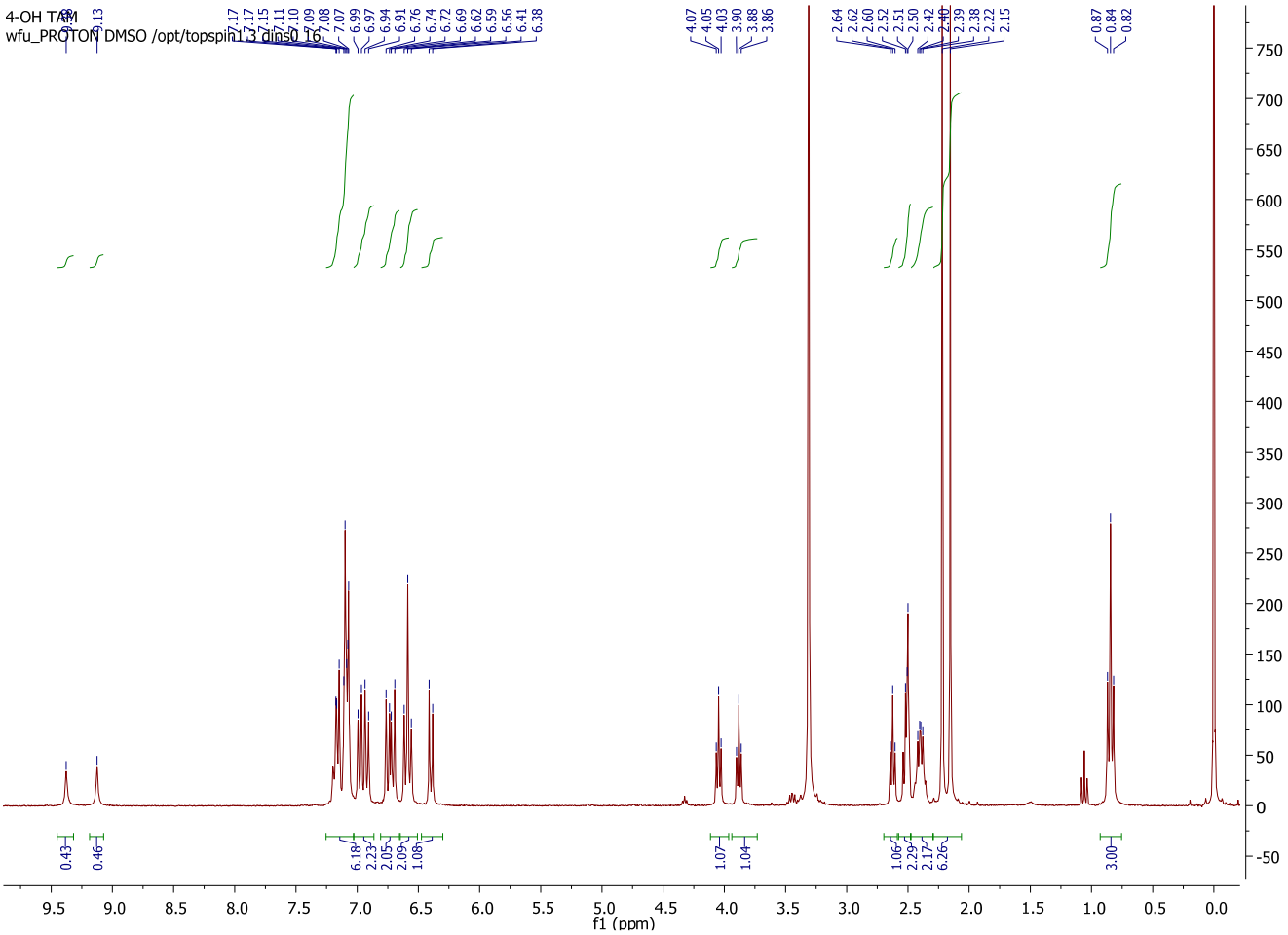


Figure S3.2. ^{1}H NMR spectrum of compound S4 in $(CD_3)_2SO$.

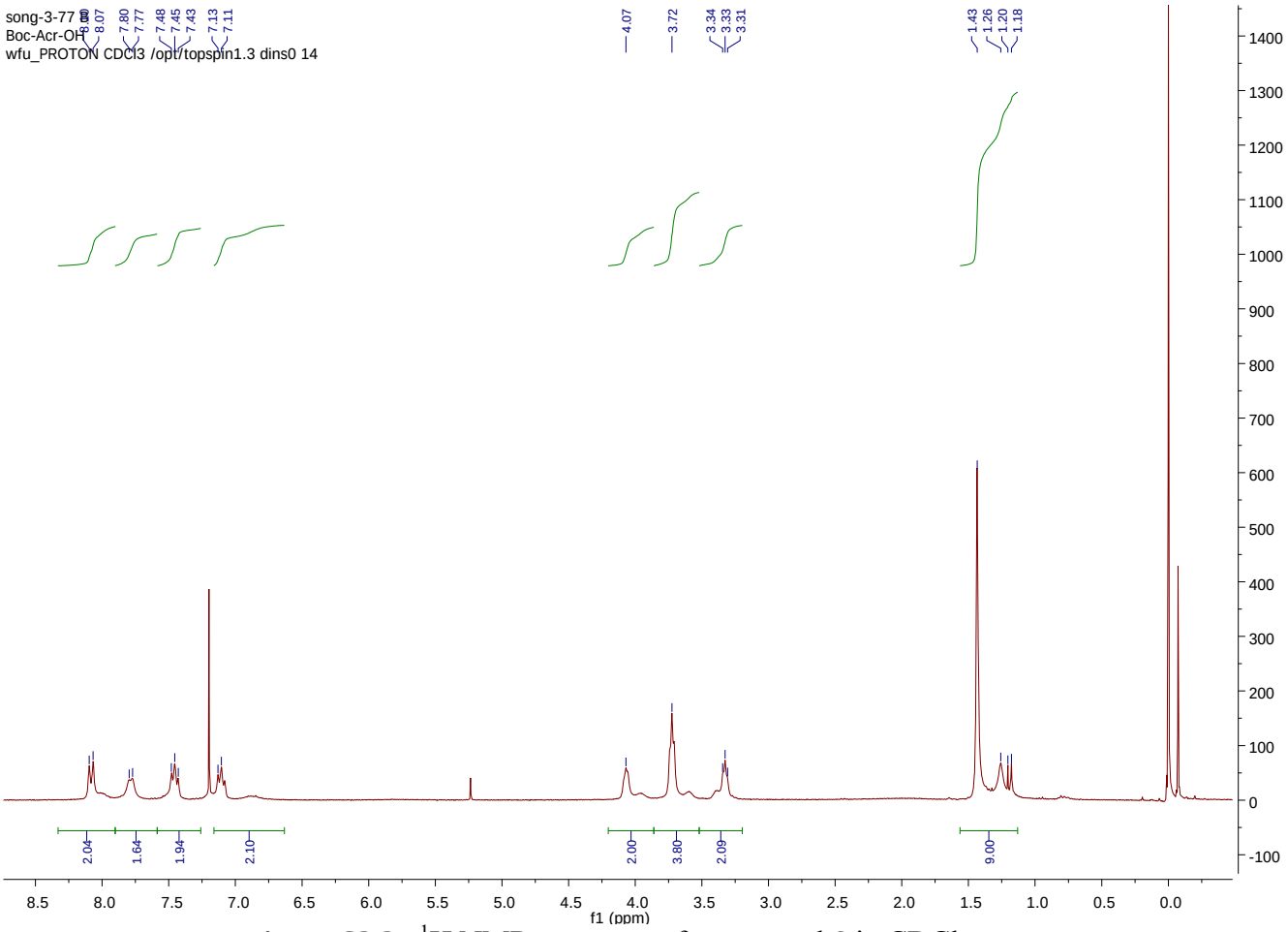


Figure S3.3. ^{1}H NMR spectrum of compound **6** in $CDCl_3$.

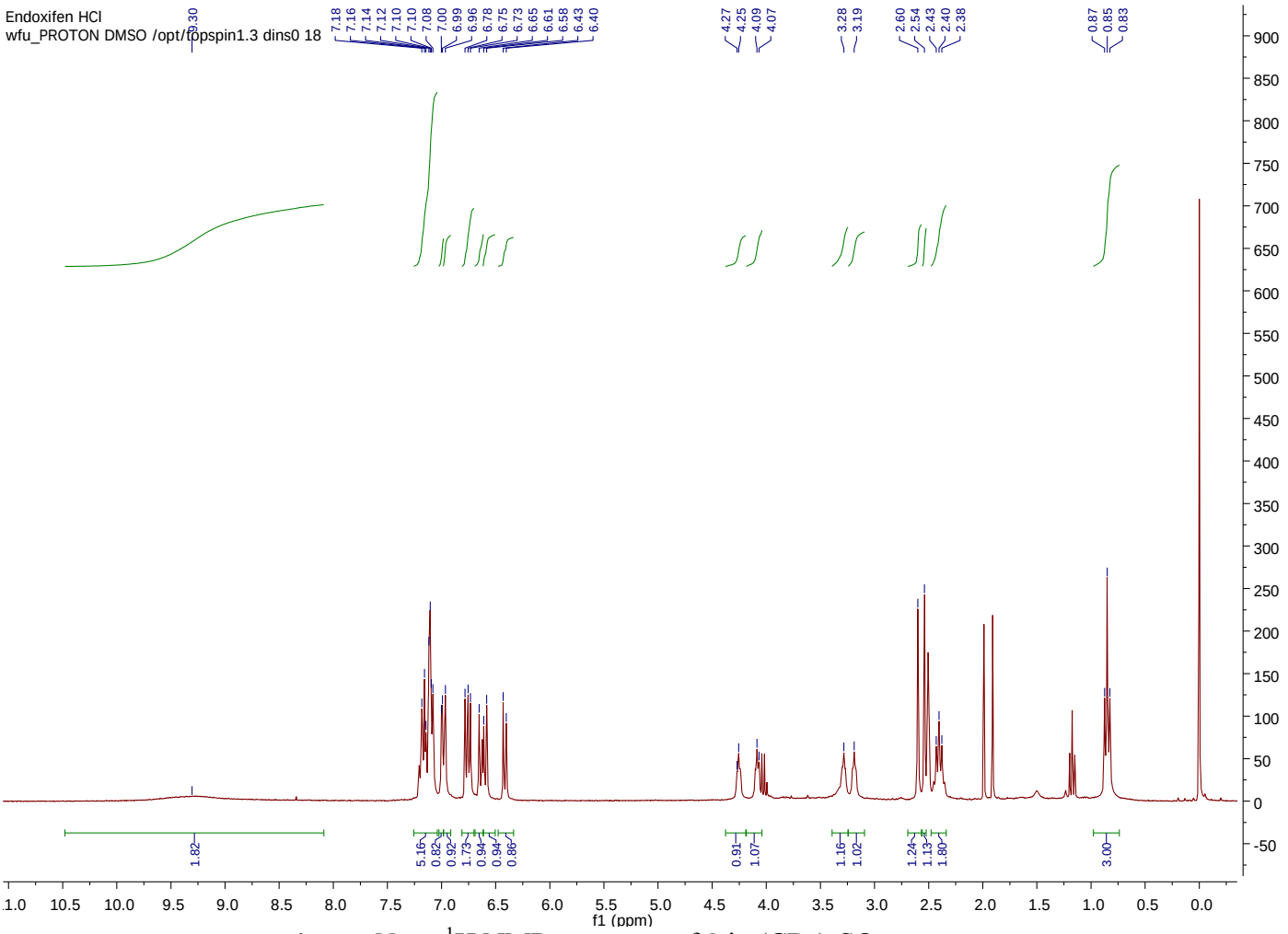


Figure S3.4. ^{1}H NMR spectrum of **8** in $(CD_3)_2SO$.

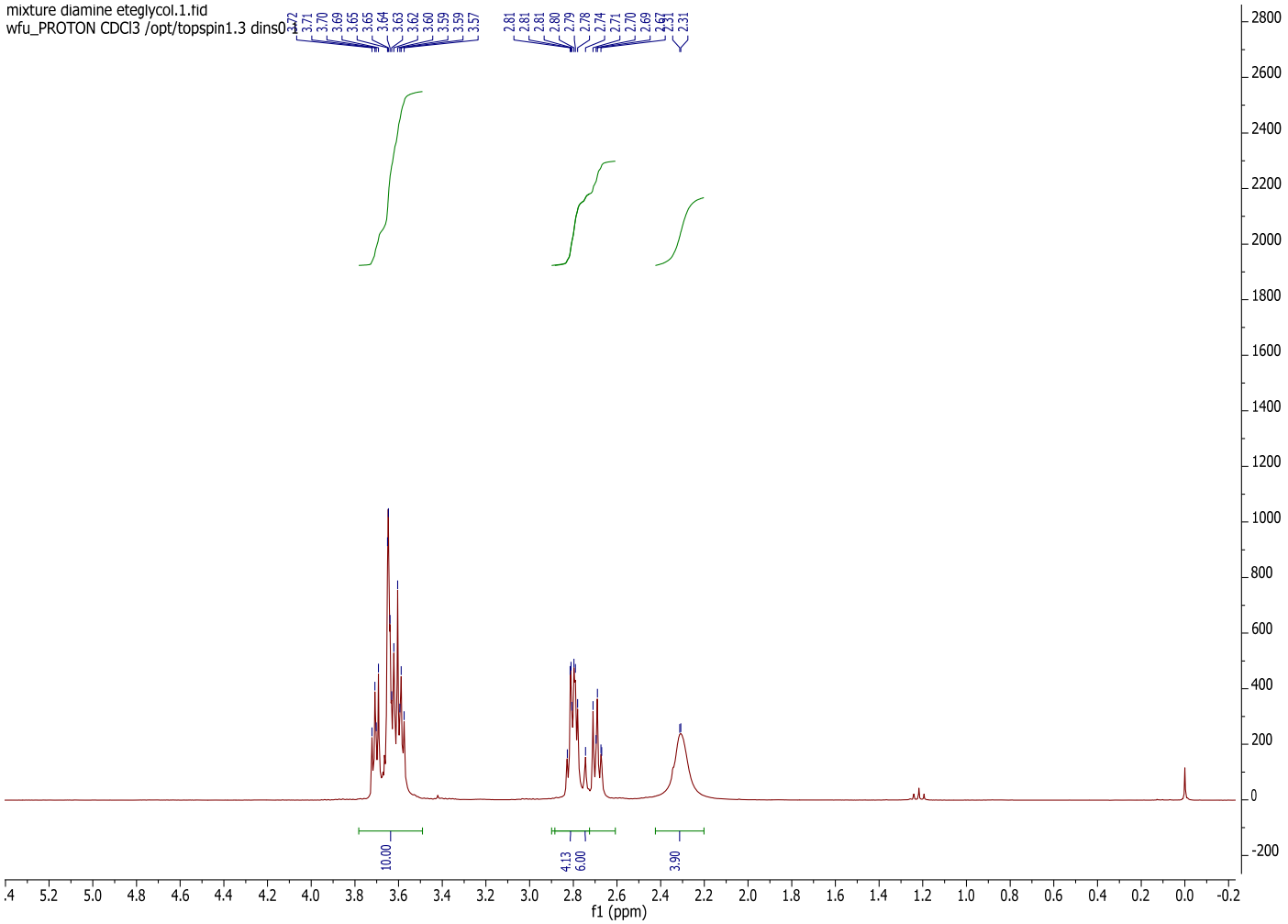


Figure S3.5. ^{1}H NMR spectrum of S7 in $CDCl_3$.

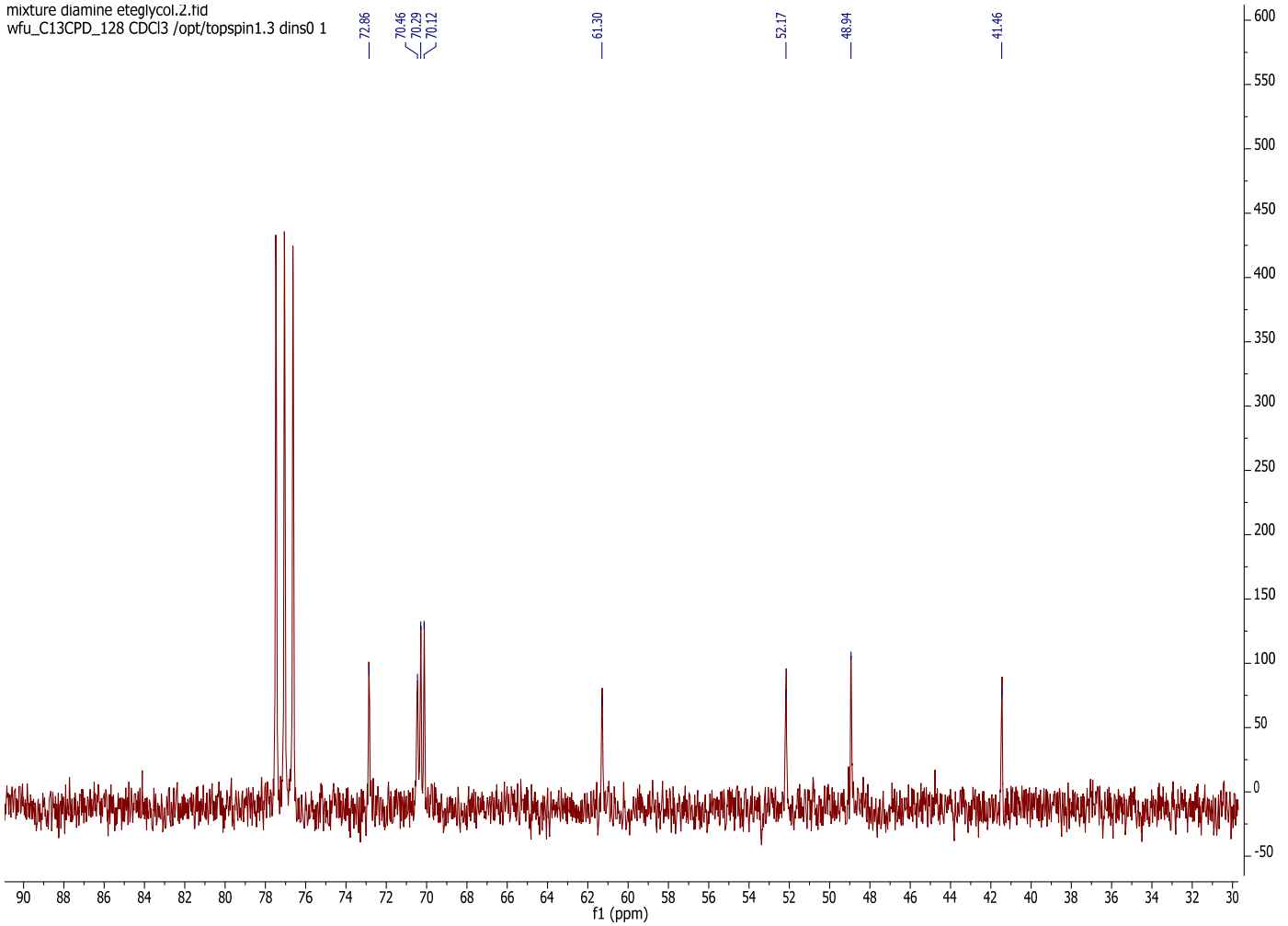


Figure S3.6. ^{13}C NMR spectrum of S7 in $CDCl_3$.

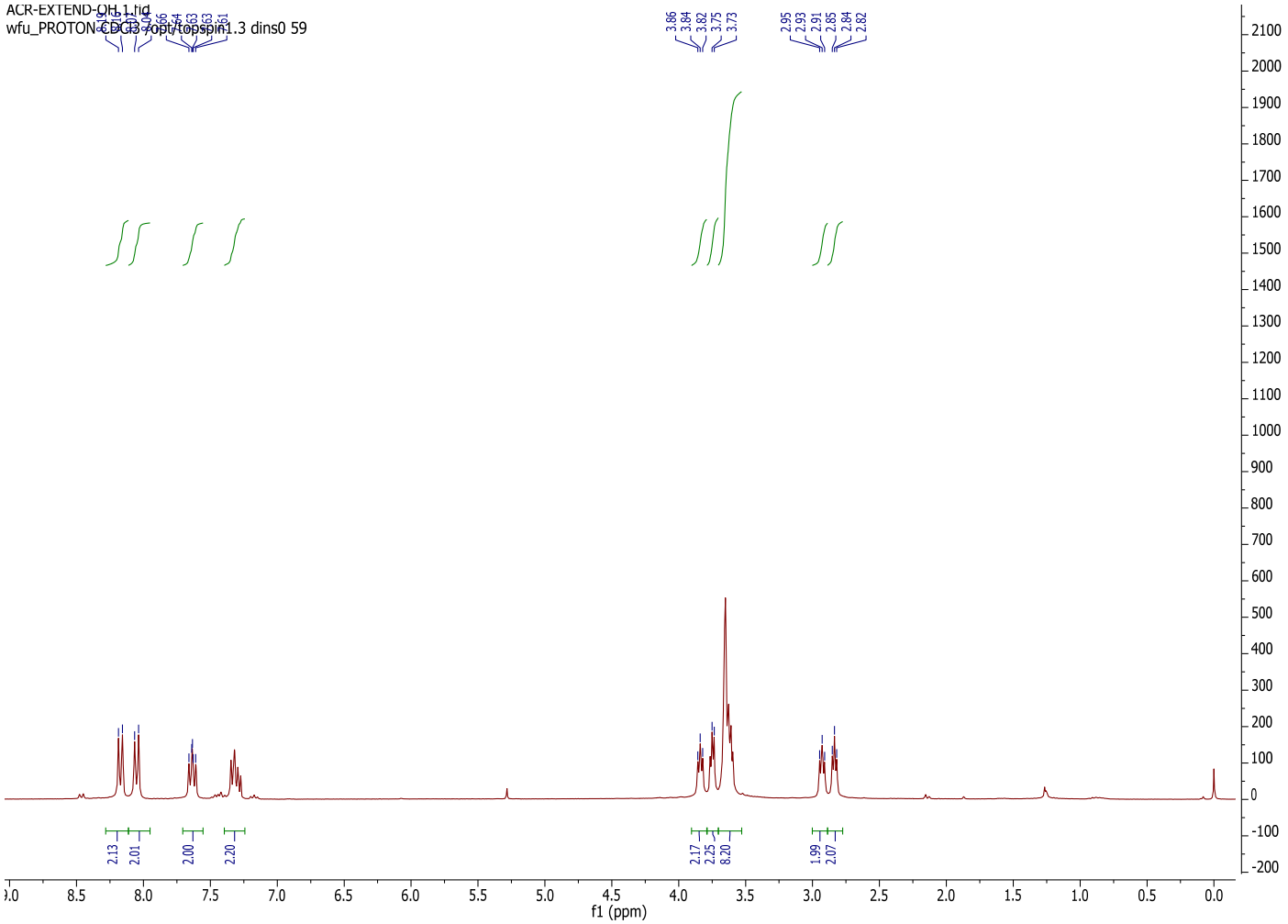


Figure S3.7. ^{1}H NMR spectrum of **4'** in $CDCl_3$.

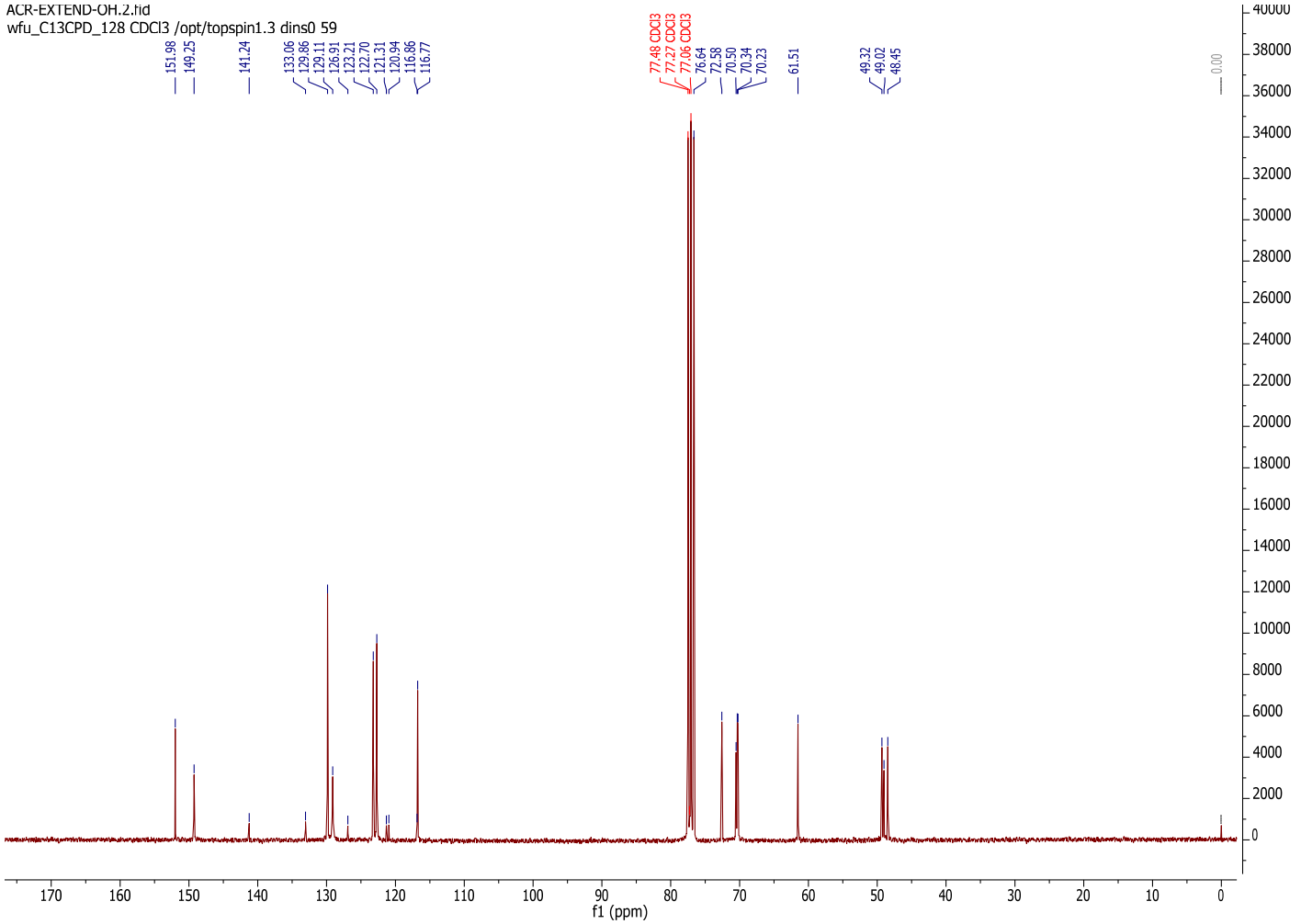


Figure S3.8. ^{13}C NMR spectrum of **4'** in $CDCl_3$.

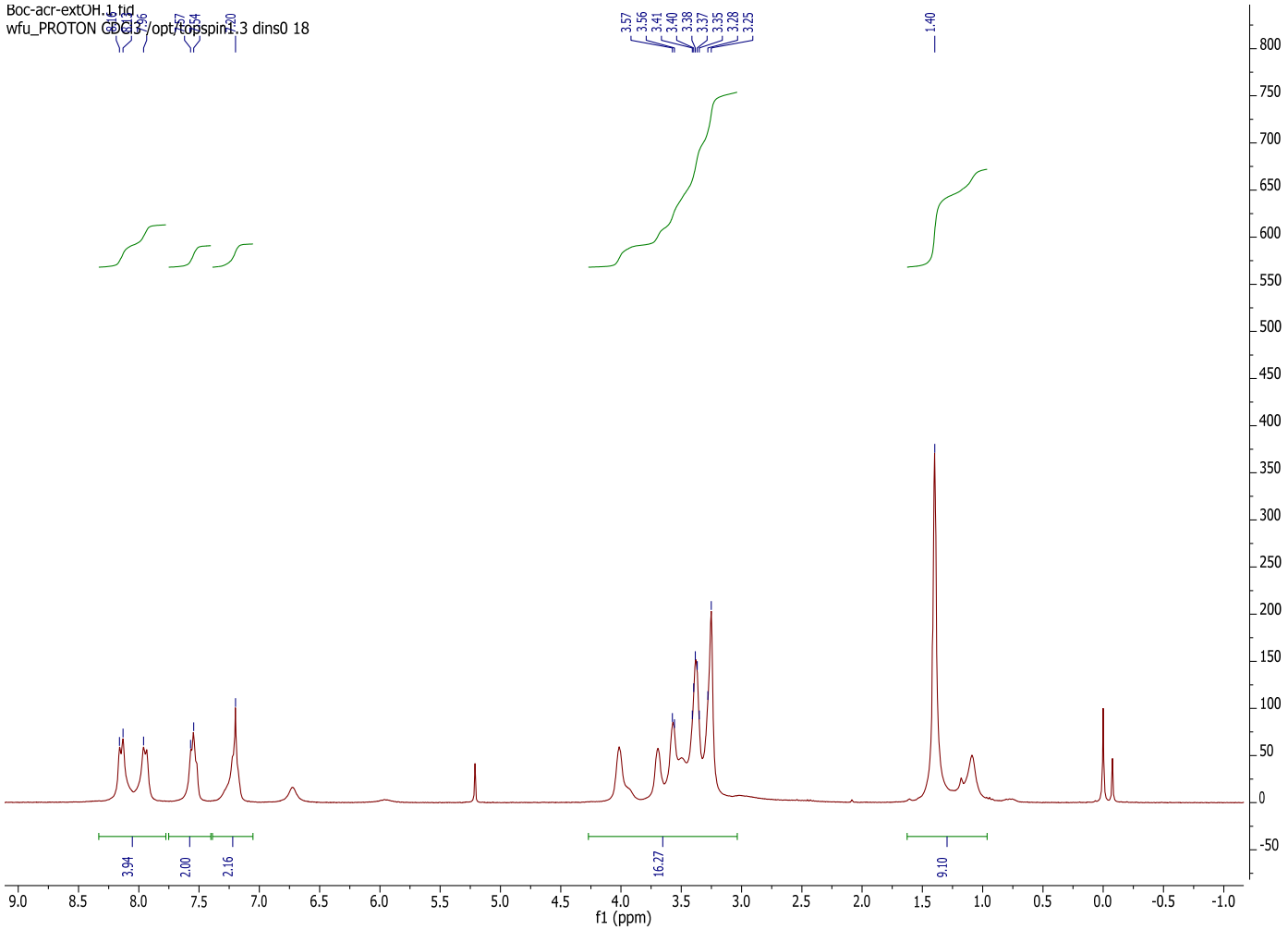


Figure S3.9. ^{1}H NMR spectrum of **6'** in $CDCl_3$.

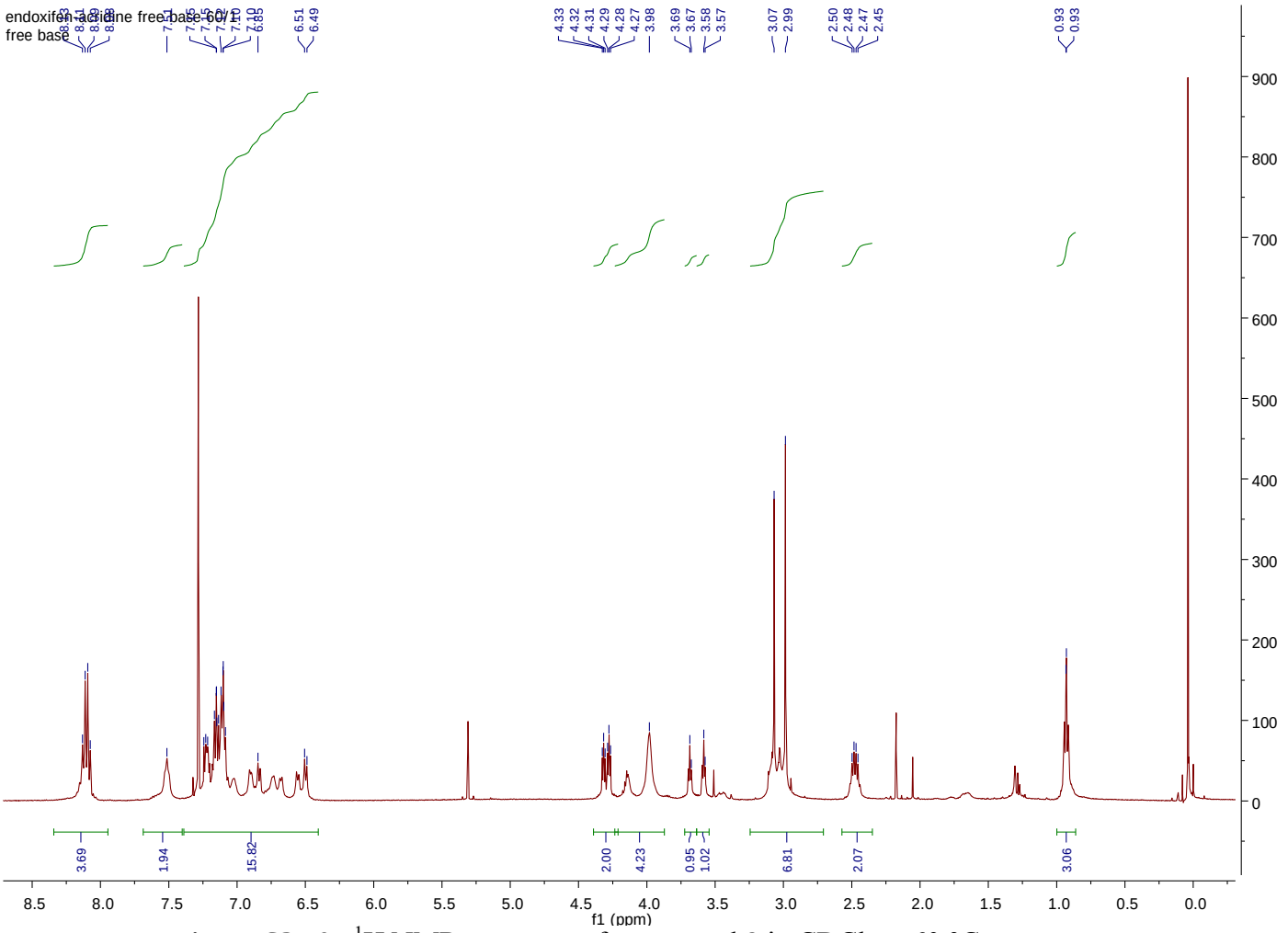


Figure S3.10. ^{1}H NMR spectrum of compound **9** in $CDCl_3$ at 60 °C.

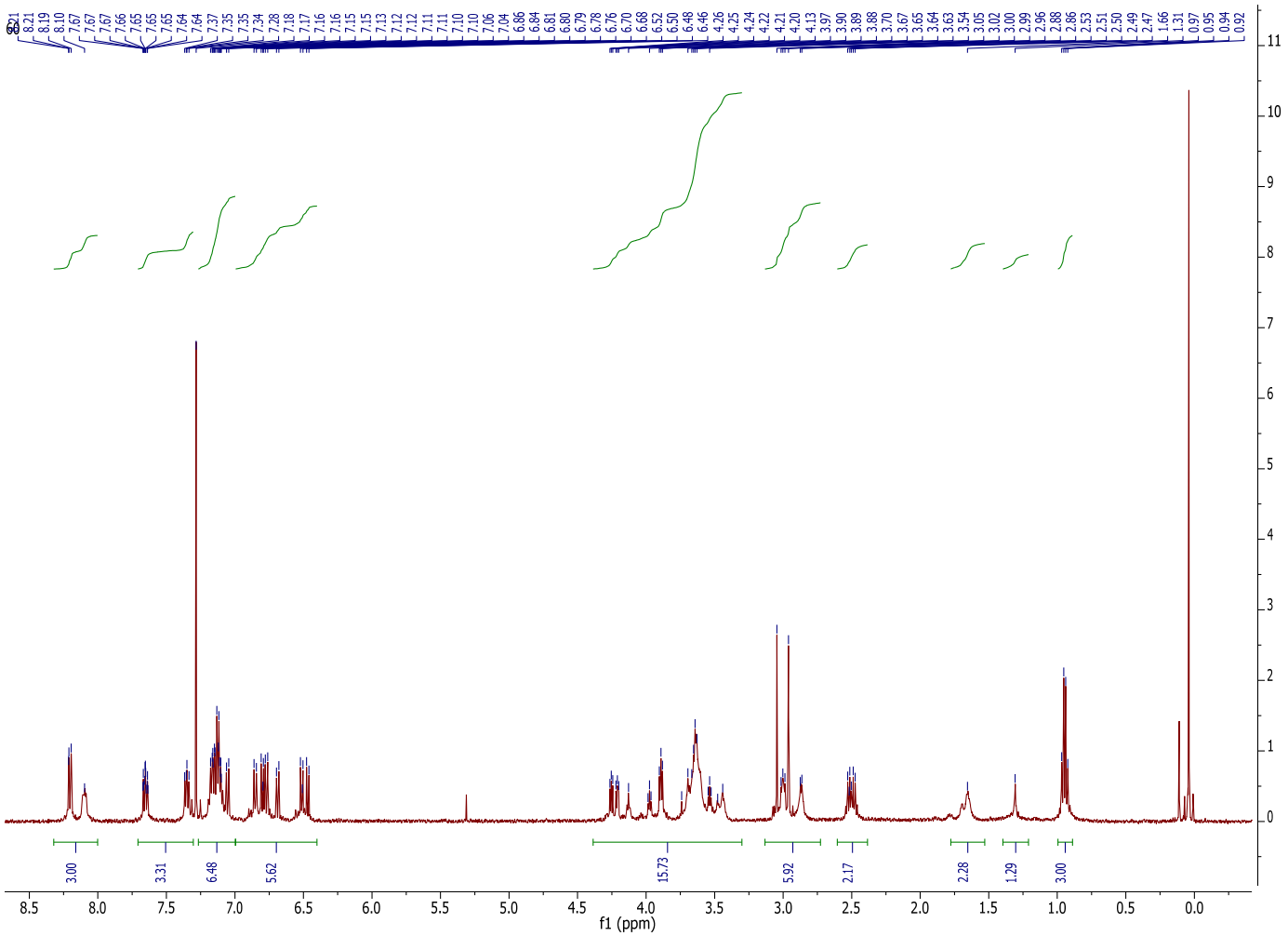


Figure S3.11. ^{1}H NMR spectrum of compound **9'** in $CDCl_3$ at 60 °C.

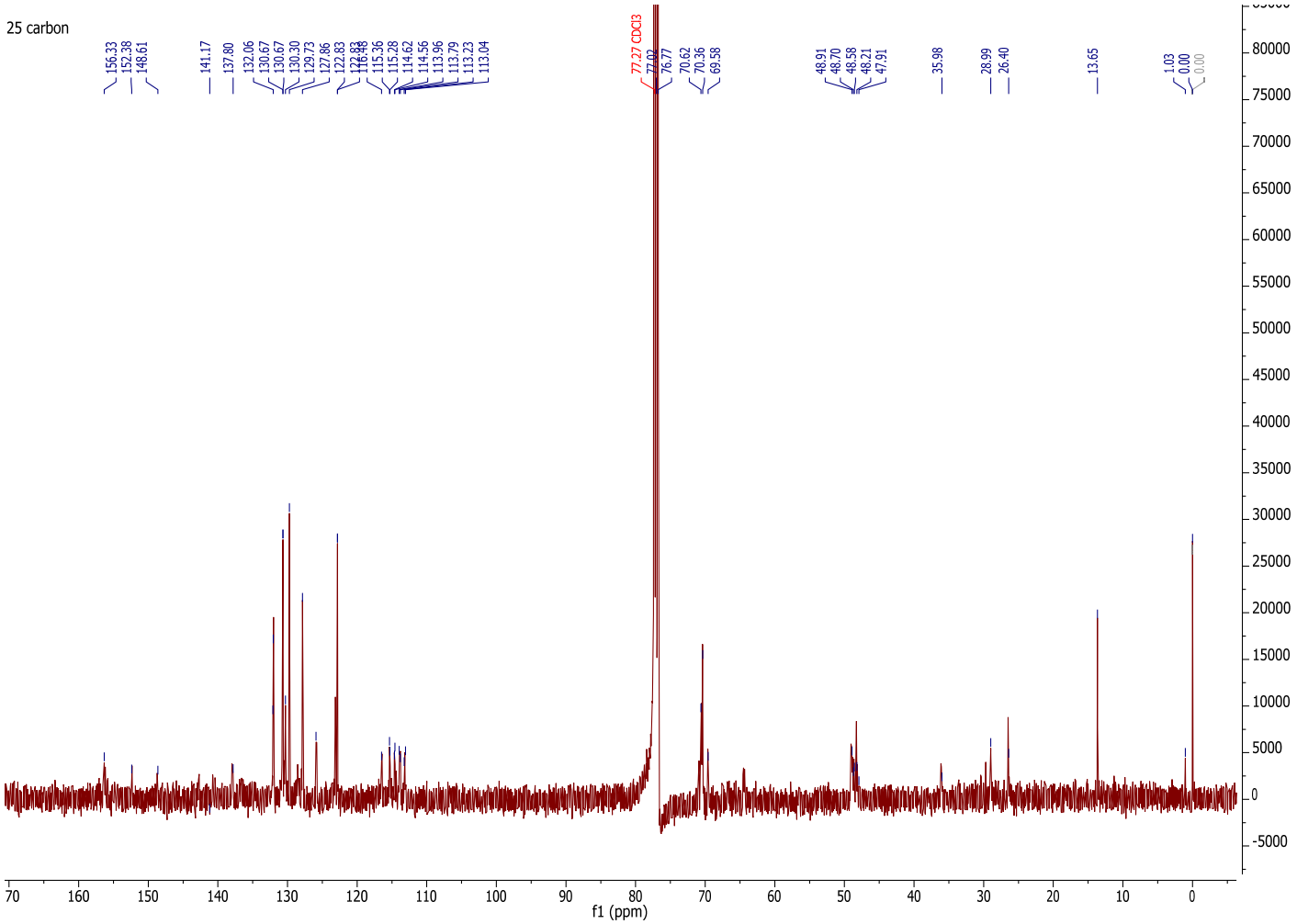


Figure S3.12. ^{13}C NMR spectrum of compound **9'** in $CDCl_3$ at 25 °C.

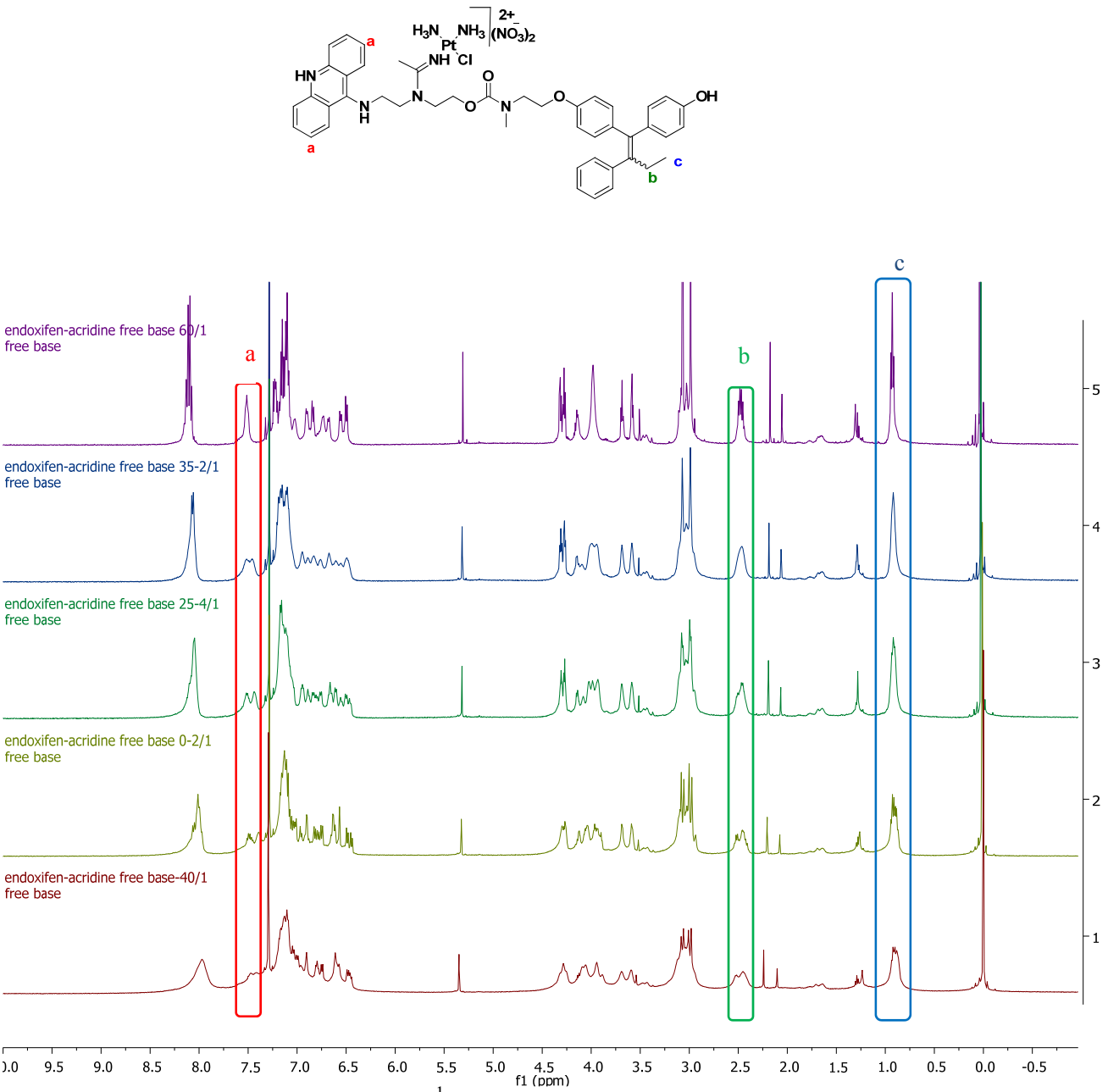


Figure S3.13. Variable Temperature ^{1}H NMR spectrum of compound **9** in $CDCl_3$ at -40 °C (red), 0 °C (yellow), 25 °C (green), 35 °C (blue), 60 °C (purple), confirming interconversion of the E- and Z-isomeric forms. Coalescence of endoxifen based peaks due to rapid interconversion of E- and Z- isomers is highlighted by red, green and blue box.

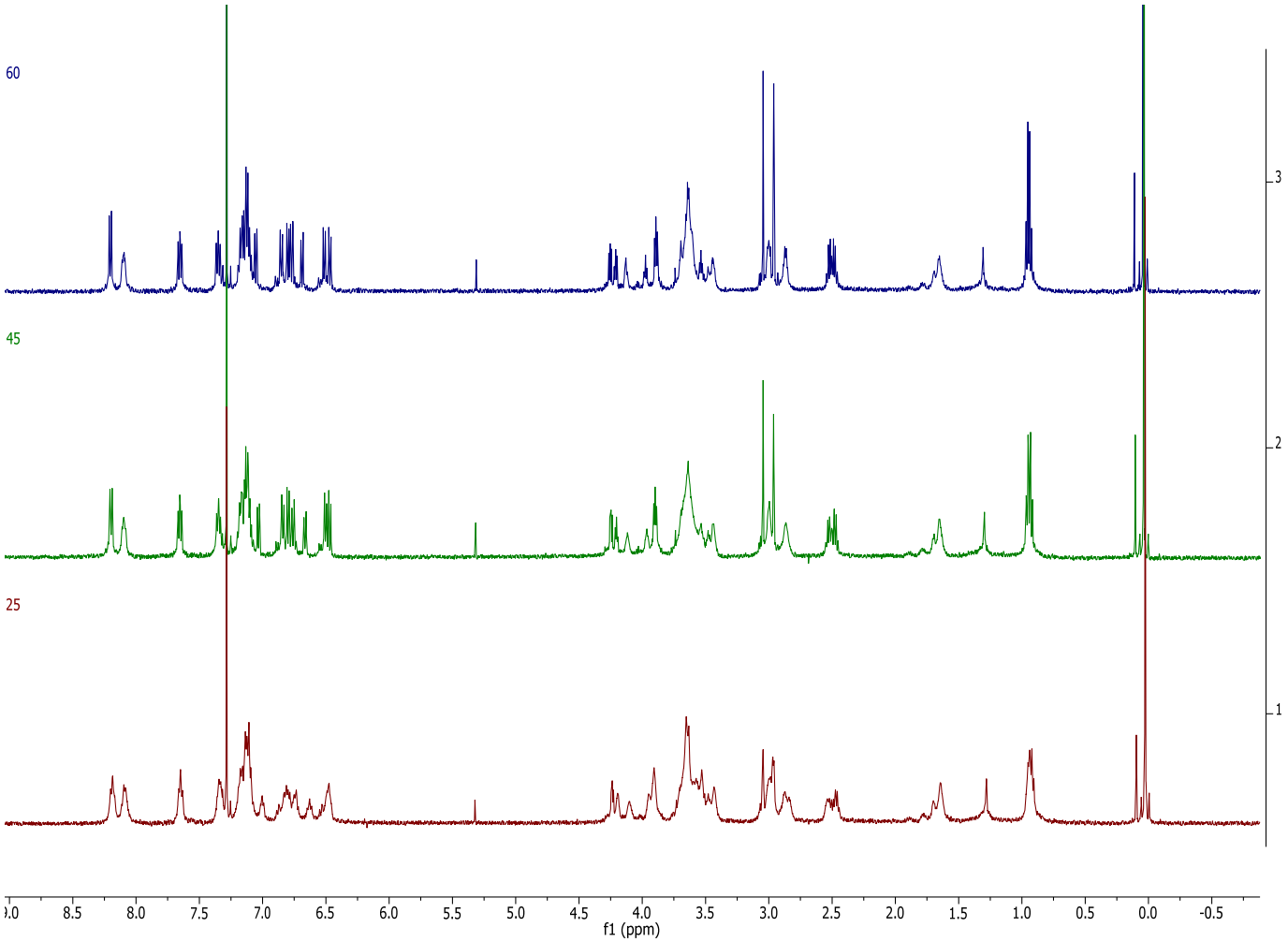


Figure S3.14. Variable Temperature ^{1}H NMR spectrum of compound **9'** in $CDCl_3$ at 25 °C (red), 45 °C (green), 60 °C (blue).

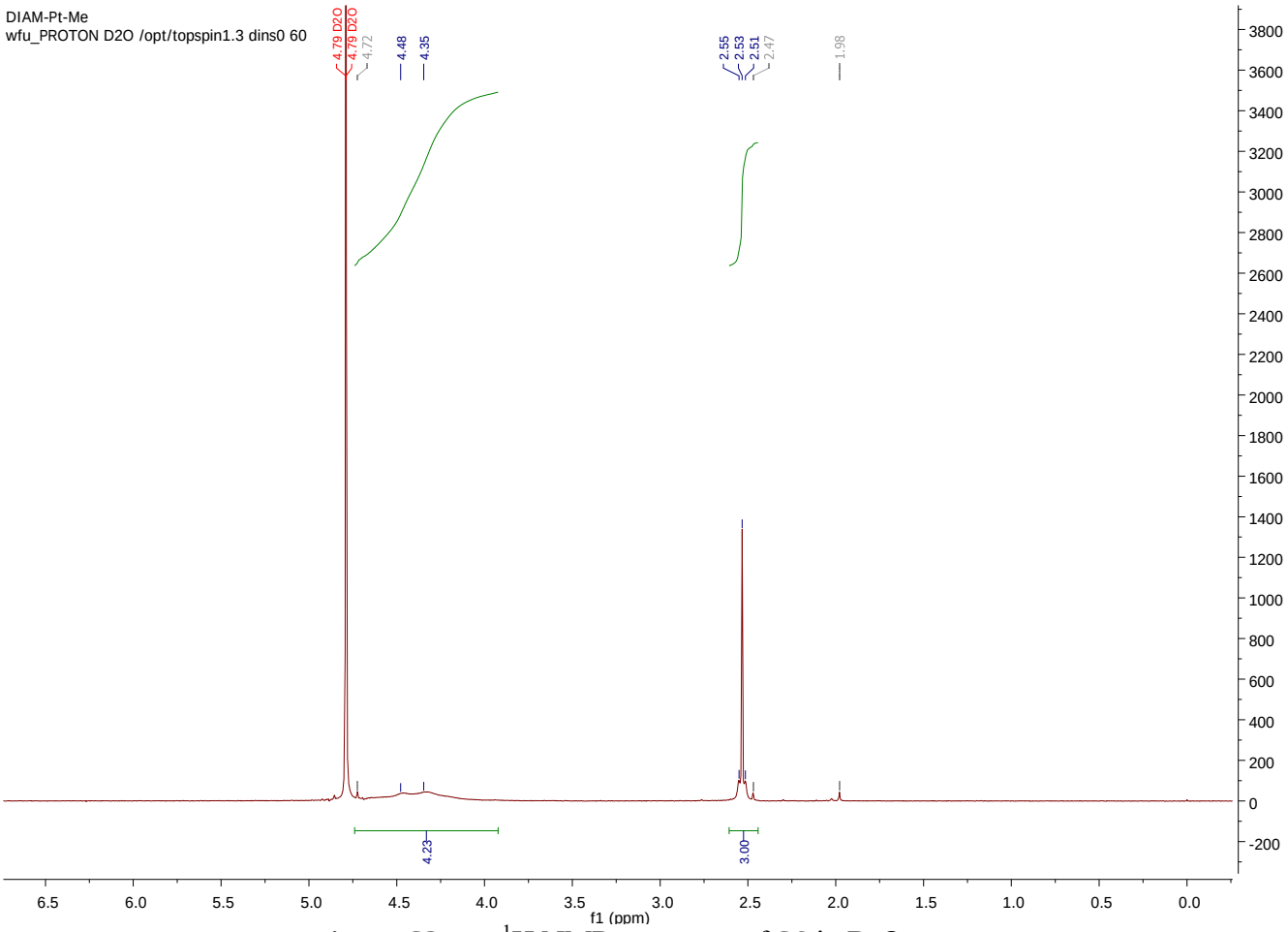


Figure S3.15. ^{1}H NMR spectrum of **S6** in D_2O.

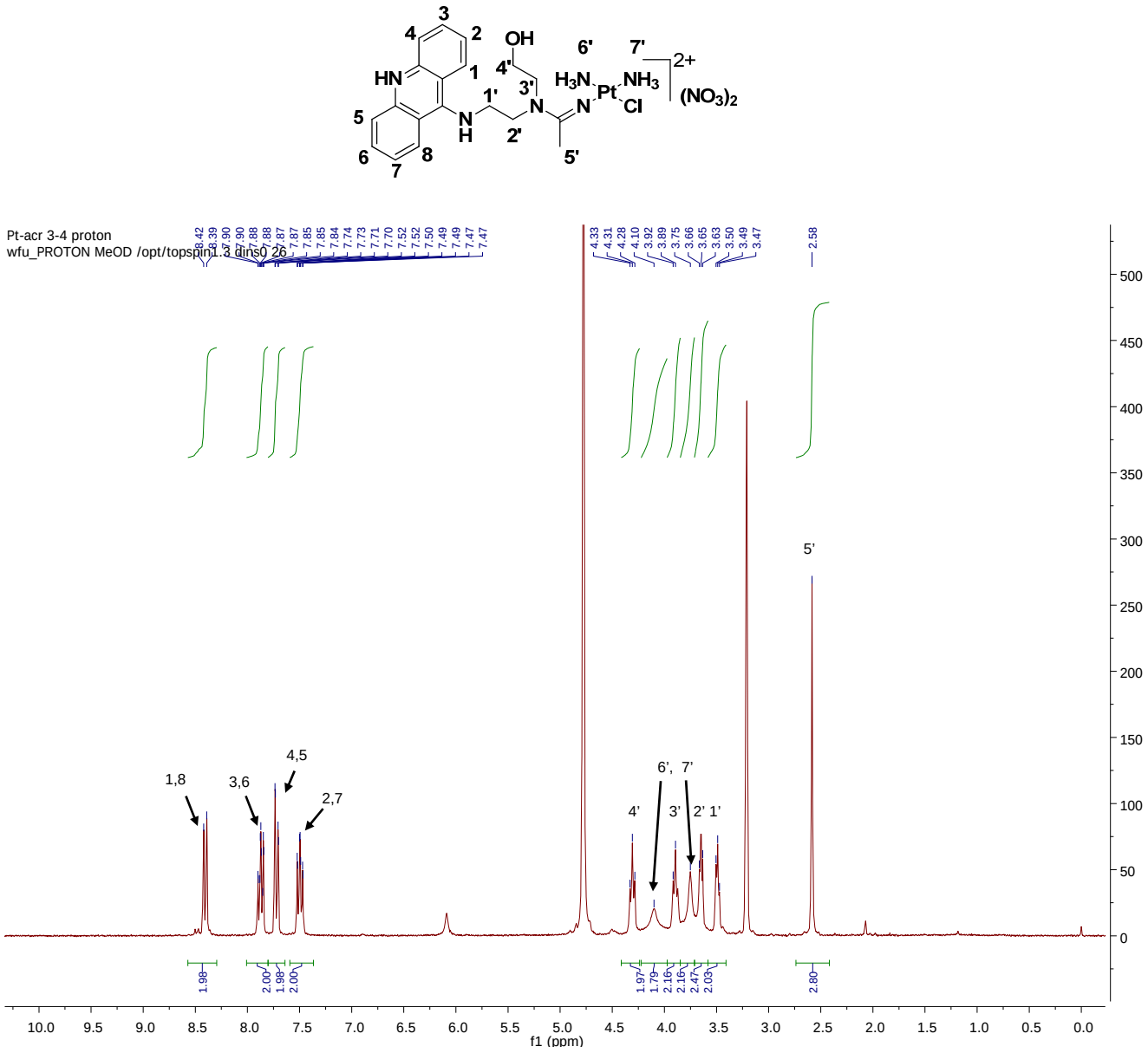


Figure S3.16. ^{1}H NMR spectrum of compound **5** in MeOH-d4.

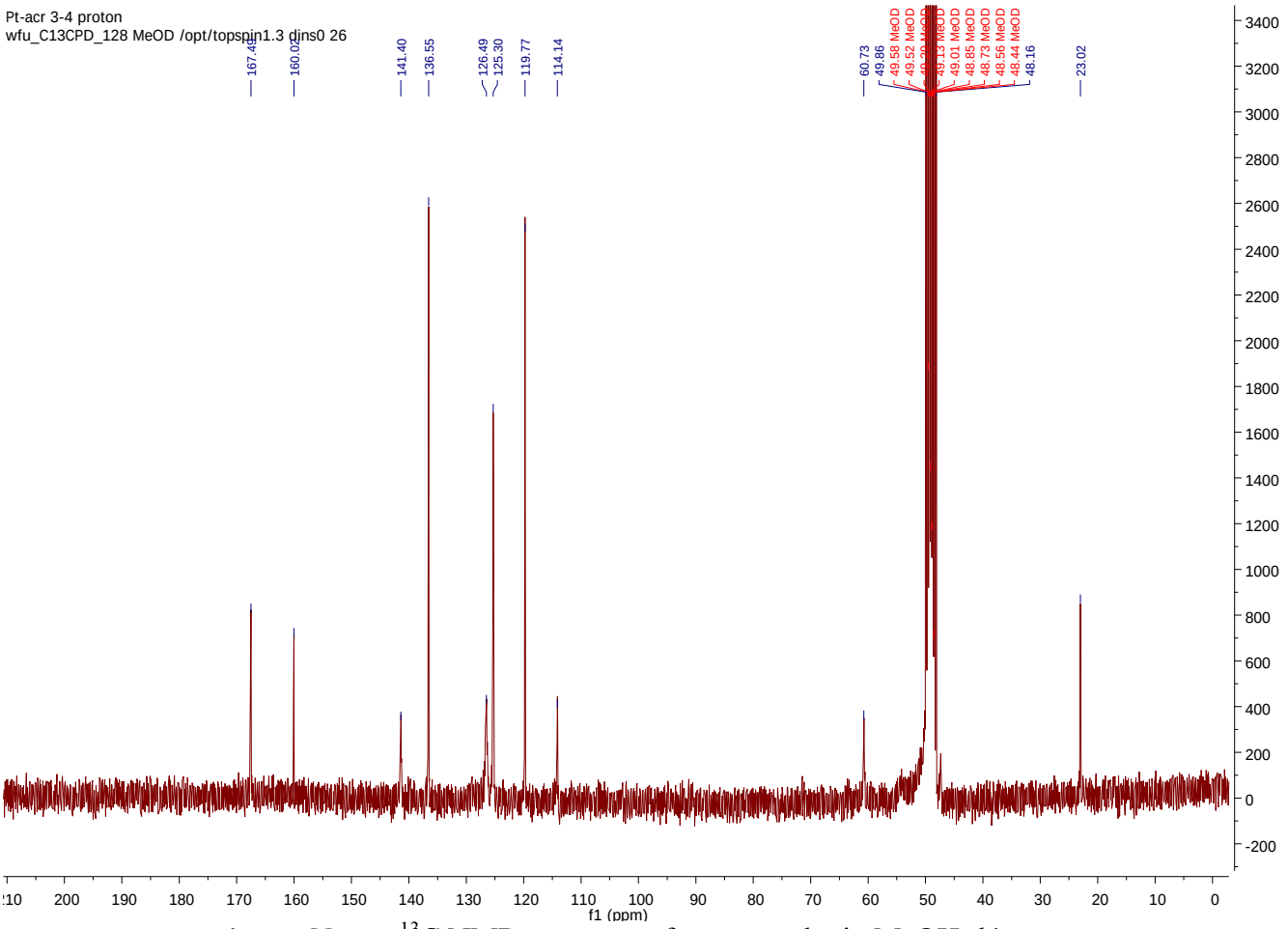


Figure S3.17. ^{13}C NMR spectrum of compound **5** in MeOH-d4.

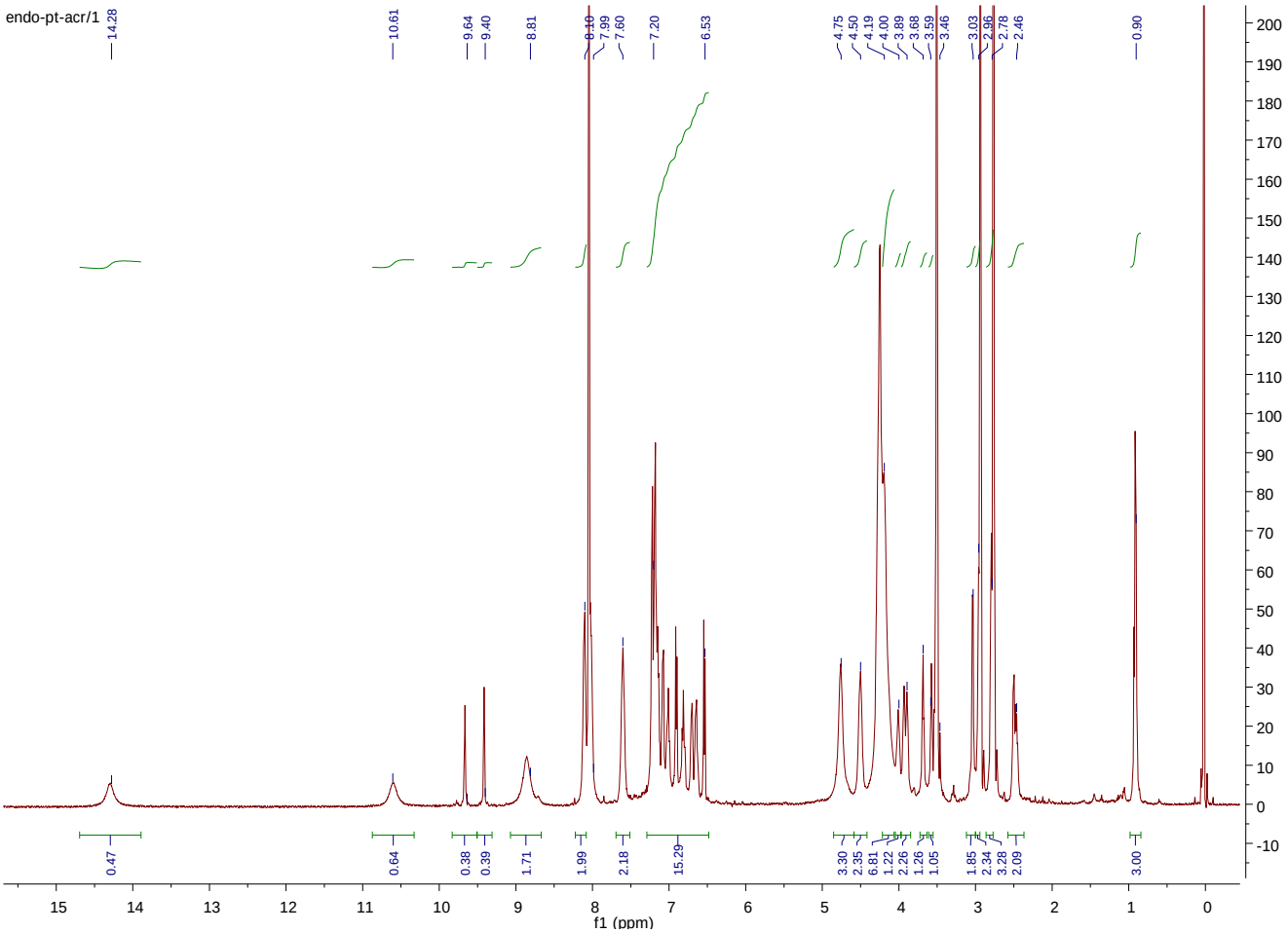


Figure S3.18. ^{1}H NMR spectrum of compound **10** in DMF-*d*7.

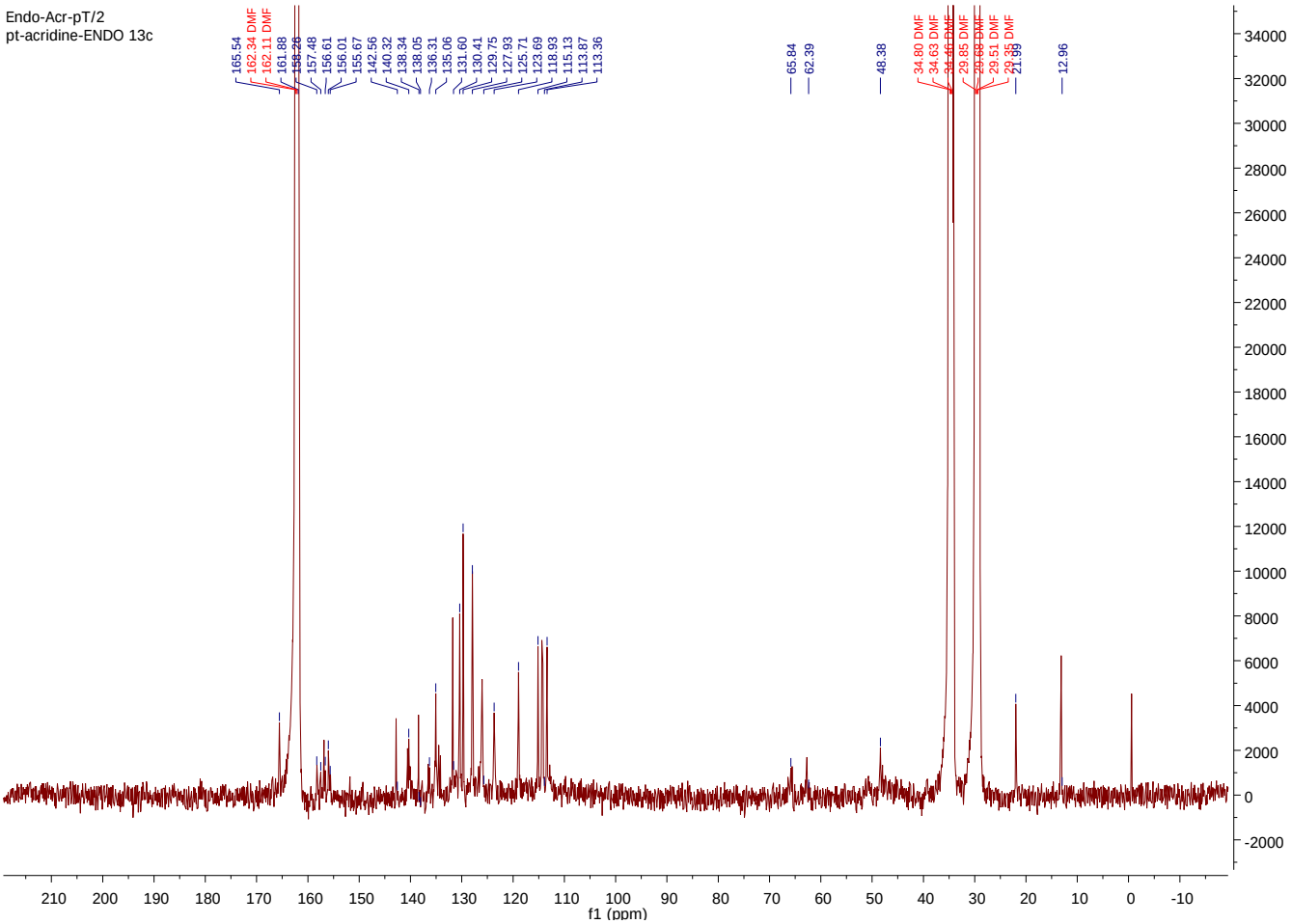


Figure S3.19. ^{13}C NMR spectrum of compound **10** in DMF-*d*7.

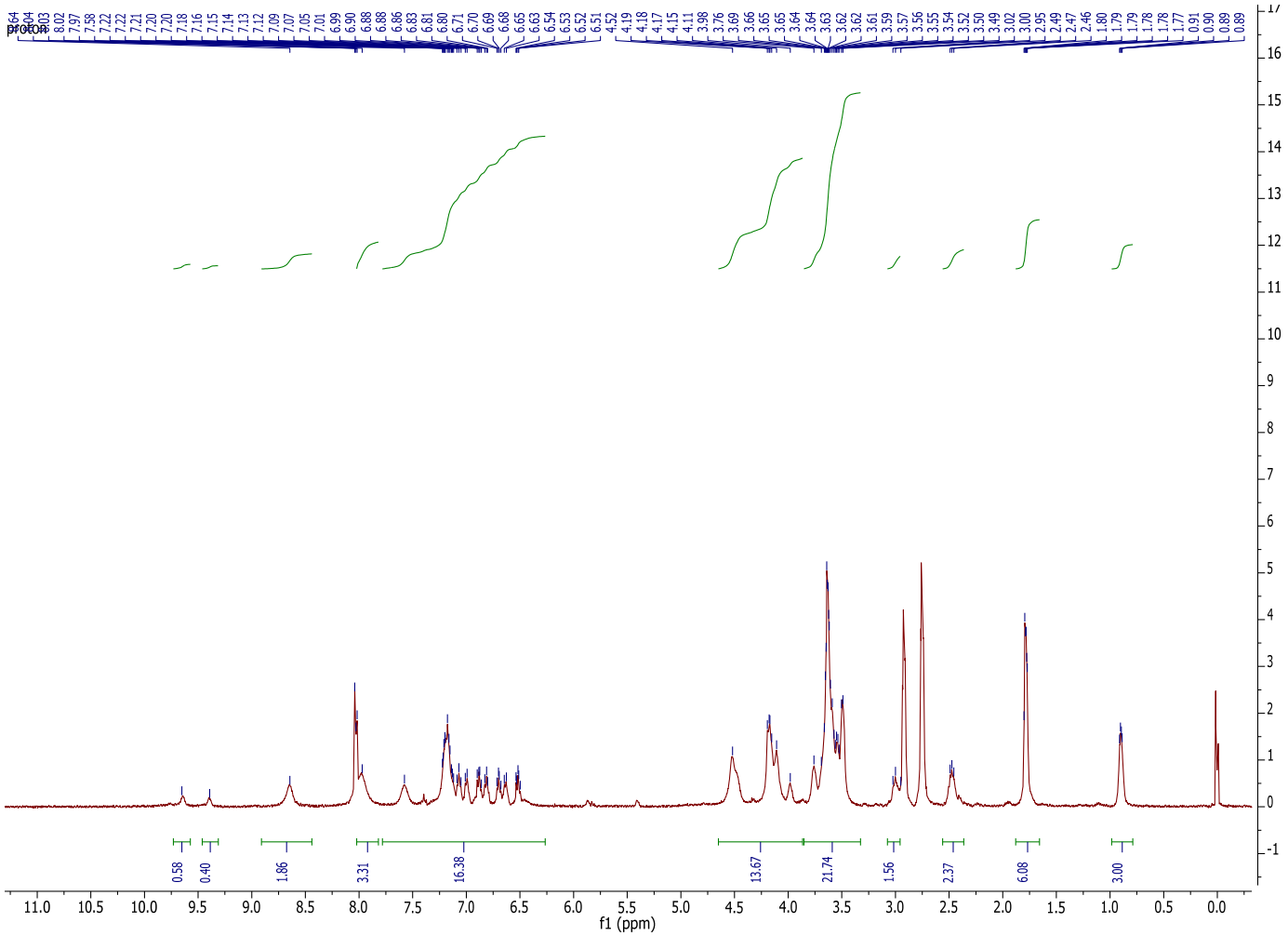


Figure S3.20. ^{1}H NMR spectrum of compound **10'** in DMF-*d*7.

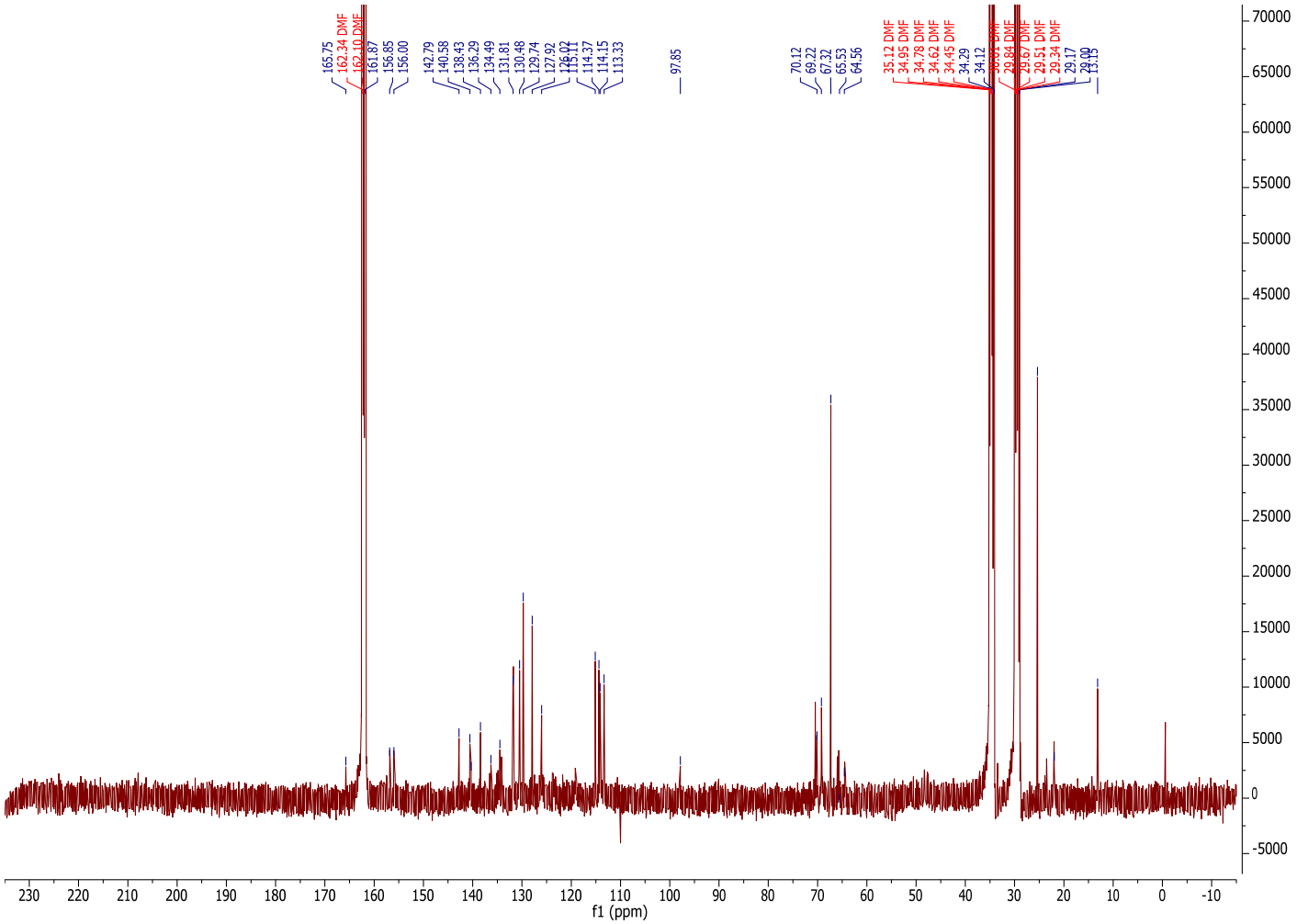


Figure S3.21. ^{13}C NMR spectrum of compound **10'** in DMF-*d*7.

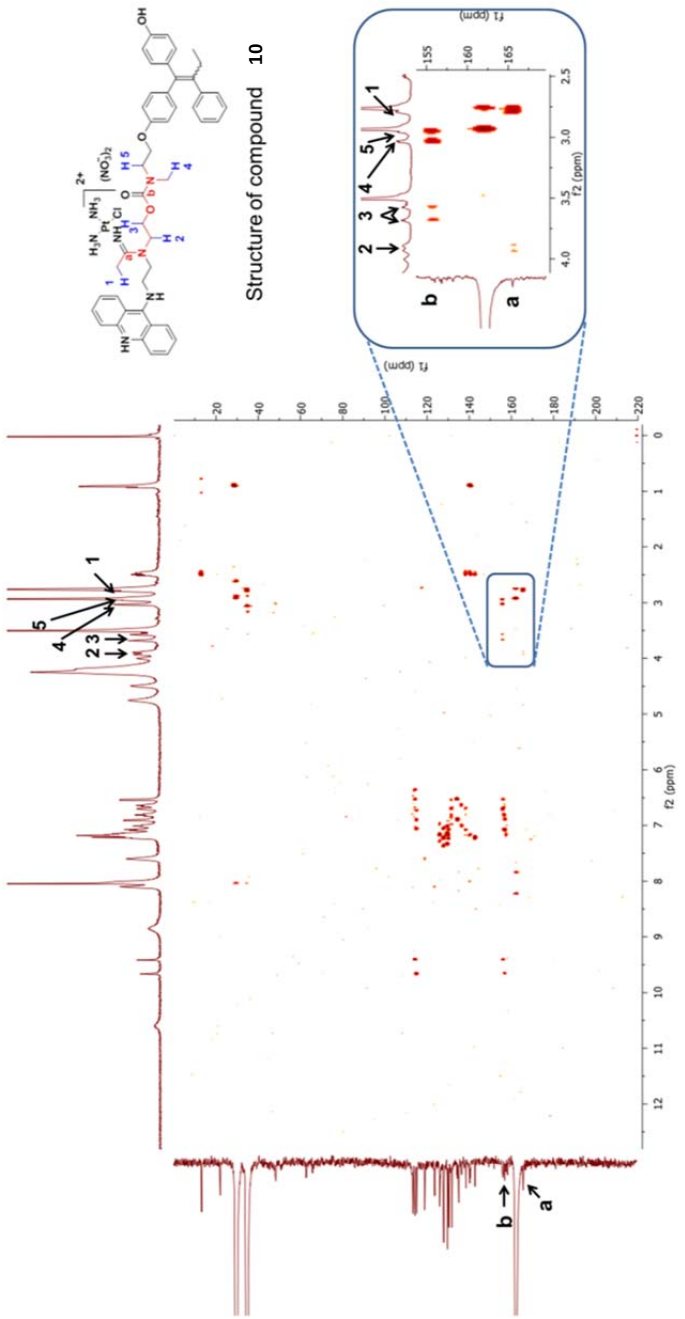


Figure S3.22. 2-D ^{1}H–^{13}C HMBC spectra for compound **10** in DMF-d_7 giving connectivities in the carbamate linker. The two sets of cross-peaks observed for several of the proton and carbon nuclei is due to the presence of E- and Z-isomers, which were assigned in the expanded view of the spectrum. No attempts were made to assign the two sets of signals to the specific isomeric forms.

4. LC-MS analysis

Compound Chromatogram Report - MS

Analysis Name: 05261205.D **Instrument:** LC-MSD-Trap-SL **Print Date:** 06/30/2012 11:46:43 PM
Method: PTAMID~1.M **Operator:** Administrator **Acq. Date:** 5/28/2012 2:58:04 PM
Sample Name: diam-Pt-OH
Analysis Info: N=2,2+

Acquisition Parameter:

Mass Range Mode	Std/Normal	Trap Drive	52.5	Scan Begin	150 m/z
Ion Polarity	Positive	Octopole RF Amplitude	200.0 Vpp	Scan End	2200 m/z
Ion Source Type	ESI	Capillary Exit	135.7 Volt	Averages	5 Spectra
Dry Temp (Set)	350 °C	Skimmer	40.0 Volt	Max. Accu Time	200000 µs
Nebulizer (Set)	50.00 psi	Oct 1 DC	12.00 Volt	ICC Target	30000
Dry Gas (Set)	11.00 l/min	Oct 2 DC	1.73 Volt	Charge Control	on

Compound List:

#	RT [min]	Range [min]	Height	Area	Area Frac %
1	10.0	9.8 - 10.2	19	141	100.0

Chromatograms:

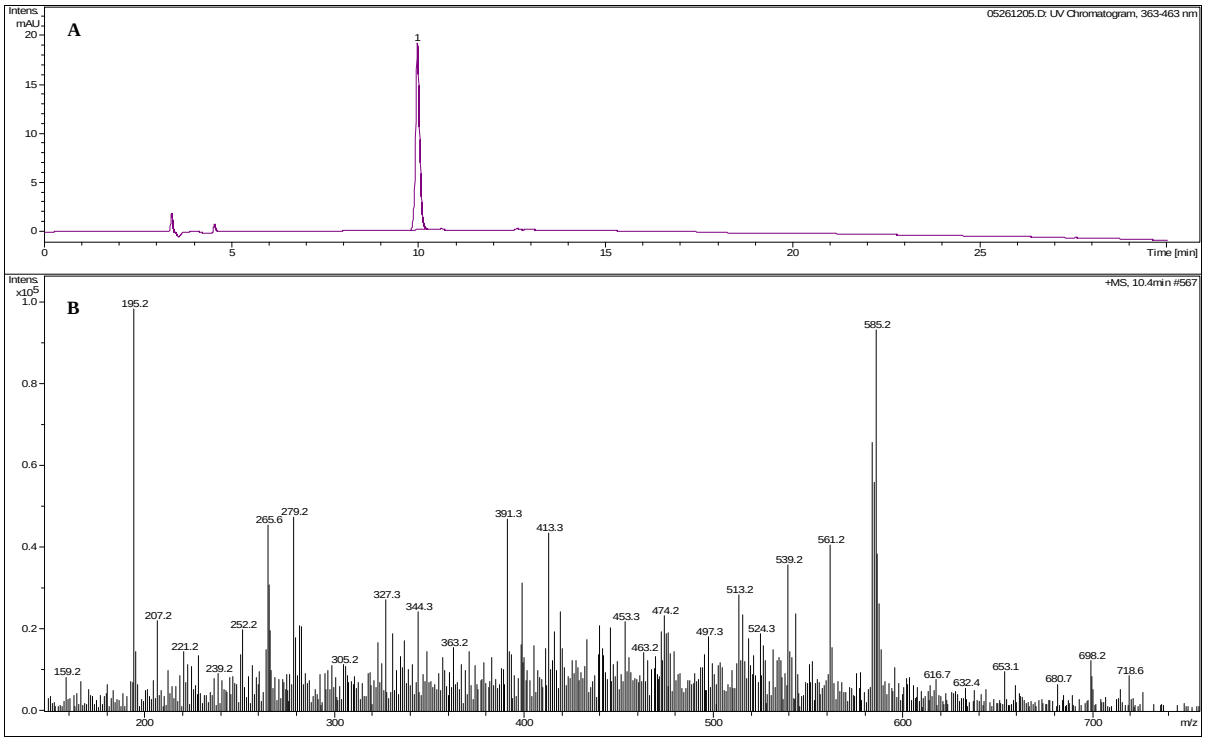


Figure S4.1. LC-ESMS analysis of compound **5**. (A) Reverse-phase HPLC trace of compound **5**. (B) ESMS spectrum of compound **5** (HPLC fraction with retention time 10.0 min) recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M]^+$ (m/z 585.2).

Compound Chromatogram Report - MS

Analysis Name: 04271211.D **Instrument:** LC-MSD-Trap-SL **Print Date:** 07/11/2012 03:48:11 PM
Method: SONG-E~1.M **Operator:** Administrator **Acq. Date:** 4/27/2012 7:37:21 PM
Sample Name: endo-acr
Analysis Info: free base

Acquisition Parameter:

Mass Range Mode	Std/Normal	Trap Drive	52.5	Scan Begin	150 m/z
Ion Polarity	Positive	Octopole RF Amplitude	200.0 Vpp	Scan End	2200 m/z
Ion Source Type	ESI	Capillary Exit	135.7 Volt	Averages	5 Spectra
Dry Temp (Set)	350 °C	Skimmer	40.0 Volt	Max. Accu Time	200000 µs
Nebulizer (Set)	50.00 psi	Oct 1 DC	12.00 Volt	ICC Target	30000
Dry Gas (Set)	11.00 l/min	Oct 2 DC	1.73 Volt	Charge Control	on

Compound List:

#	RT [min]	Range [min]	Height	Area	Area Frac %
1	34.0	33.7 - 34.6	3	52	100.0

Chromatograms:

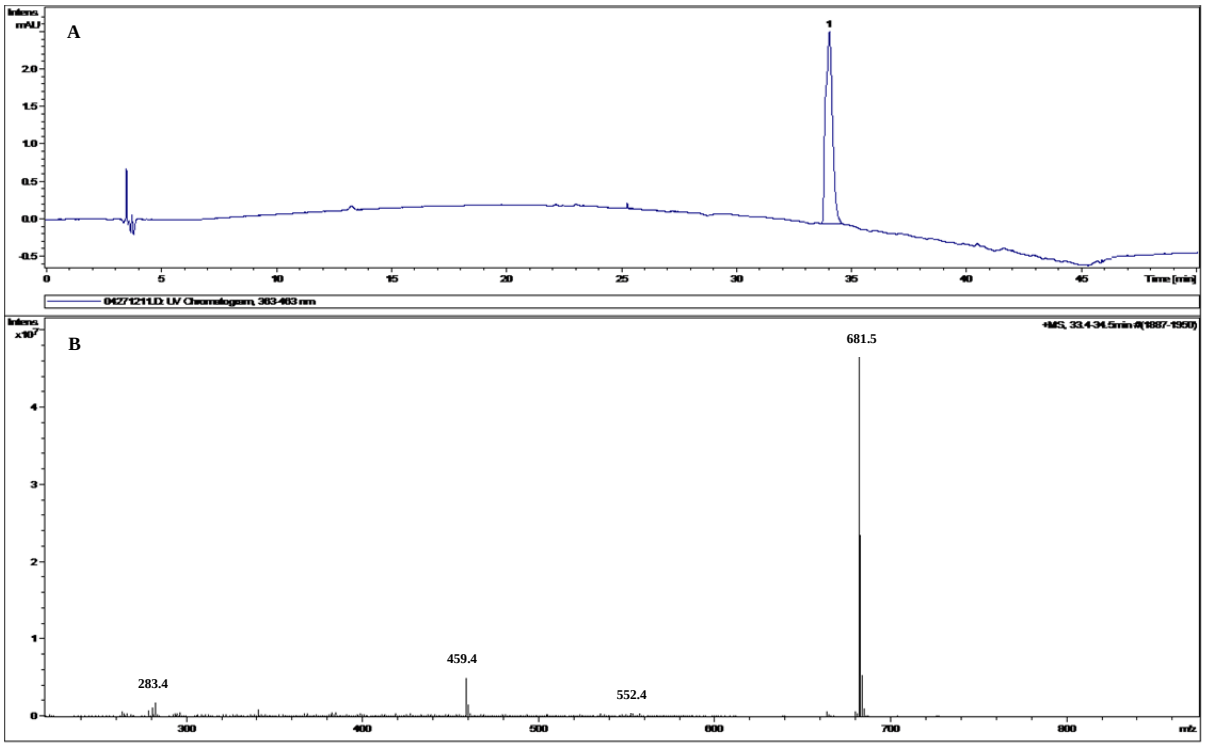


Figure S4.2. LC-ESMS analysis of compound **9**. (A) Reverse-phase HPLC trace of compound **9**. (B) ESMS spectrum of compound **9** (HPLC fraction with retention time 34.0 min) recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M]^+$ (m/z 681.5).

Compound Chromatogram Report - MS

Analysis Name: 04051203.D **Instrument:** LC-MSD-Trap-SL **Print Date:** 07/11/2012 03:49:13 PM
Method: SONG-E~1.M **Operator:** Administrator **Acq. Date:** 4/5/2012 12:20:13 PM
Sample Name: endo-acr-pt
Analysis Info: 2+

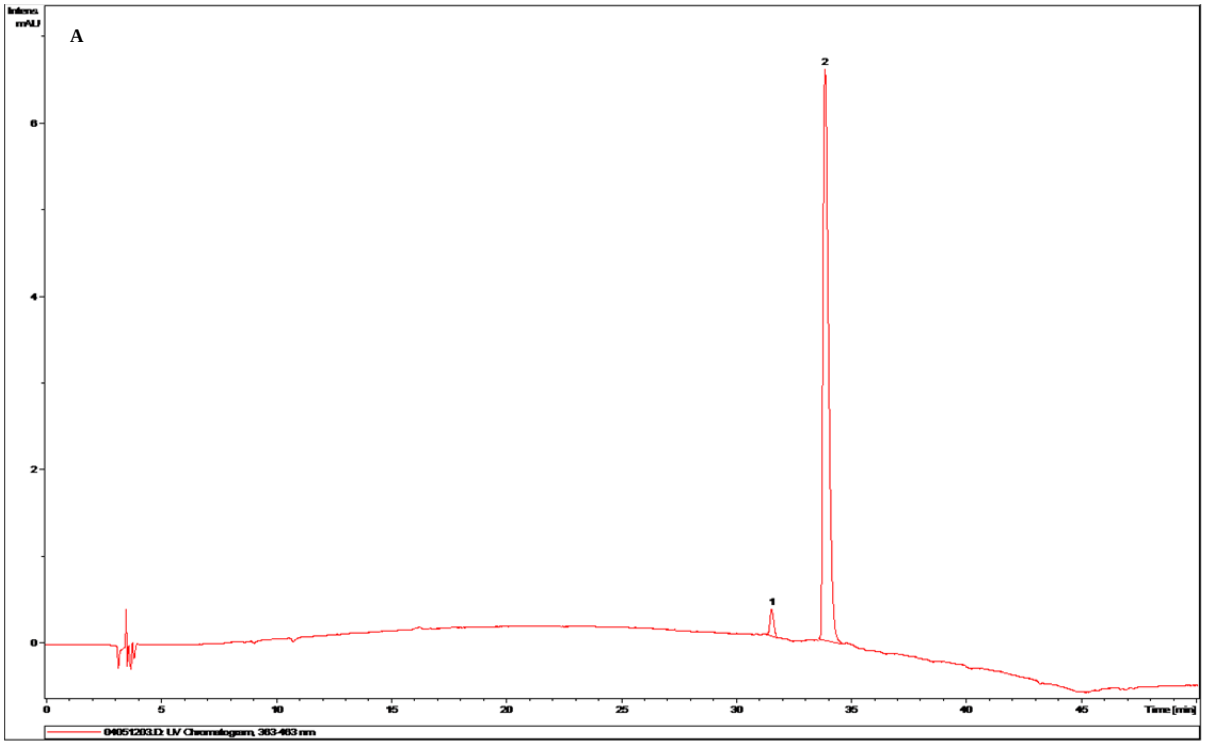
Acquisition Parameter:

Mass Range Mode	Std/Normal	Trap Drive	52.5	Scan Begin	150 m/z
Ion Polarity	Positive	Octopole RF Amplitude	200.0 Vpp	Scan End	2200 m/z
Ion Source Type	ESI	Capillary Exit	135.7 Volt	Averages	5 Spectra
Dry Temp (Set)	350 °C	Skimmer	40.0 Volt	Max. Accu Time	200000 µs
Nebulizer (Set)	50.00 psi	Oct 1 DC	12.00 Volt	ICC Target	30000
Dry Gas (Set)	11.00 l/min	Oct 2 DC	1.73 Volt	Charge Control	on

Compound List:

#	RT [min]	Range [min]	Height	Area	Area Frac %
1	31.5	31.3 - 31.7	0	3	3.0
2	33.8	33.6 - 34.6	7	110	97.0

Chromatograms:



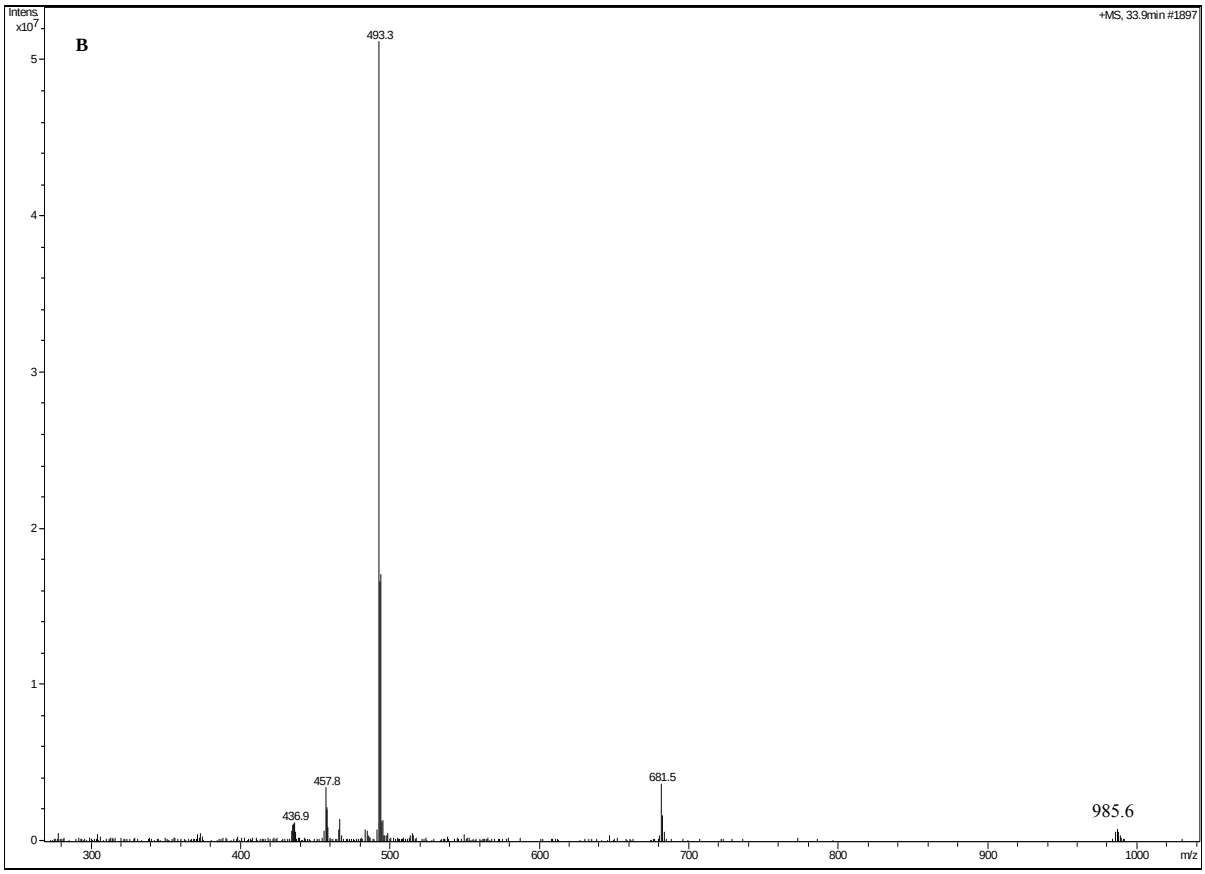


Figure S4.3. LC-ESMS analysis of compound **10**. (A) Reverse-phase HPLC trace of compound **10**. (B) ESMS spectrum of compound **10** (HPLC fraction with retention time 33.8 min) recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M-2H]^{+}$ (m/z 985.6), $[M-2NH_3\text{-Pt-Cl-MeCN}]^{+}$ (m/z 681.5), $[M-H]^{2+}$ (m/z 493.3, z = 2), $[M-2NH_3\text{-Cl}]^{2+}$ (m/z 457.8, z = 2), $[M-2NH_3\text{-Cl-Et-OH}]^{2+}$ (m/z 436.9, z =2).

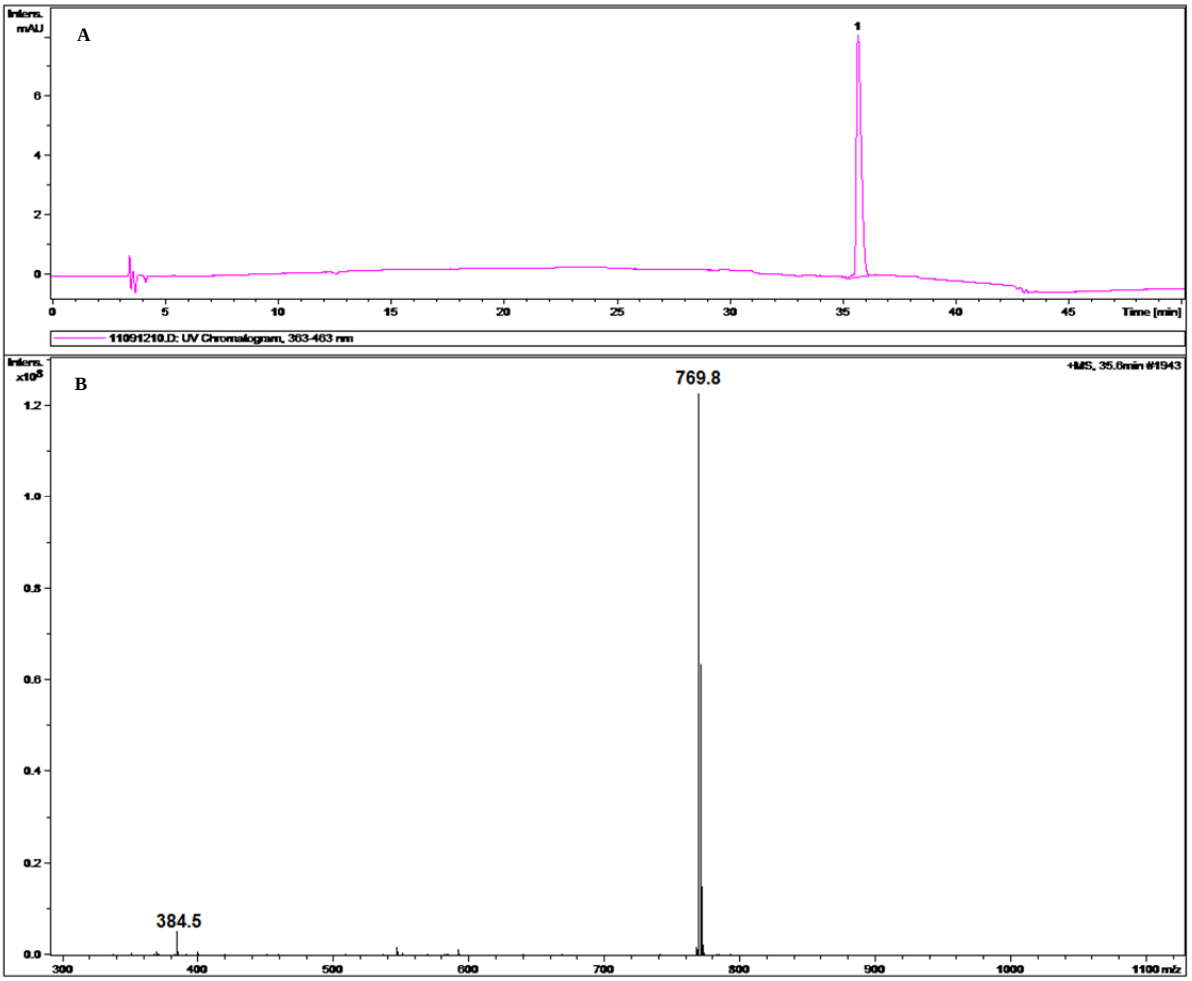


Figure S4.4. LC-ESMS analysis of compound **9'**. (A) Reverse-phase HPLC trace of compound **9'**. (B) ESMS spectrum of compound **9'** (HPLC fraction with retention time 35.6 min) recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M+H]^+$ (m/z 769.8), $[M+H]^{2+}$ (m/z 384.5, z=2).

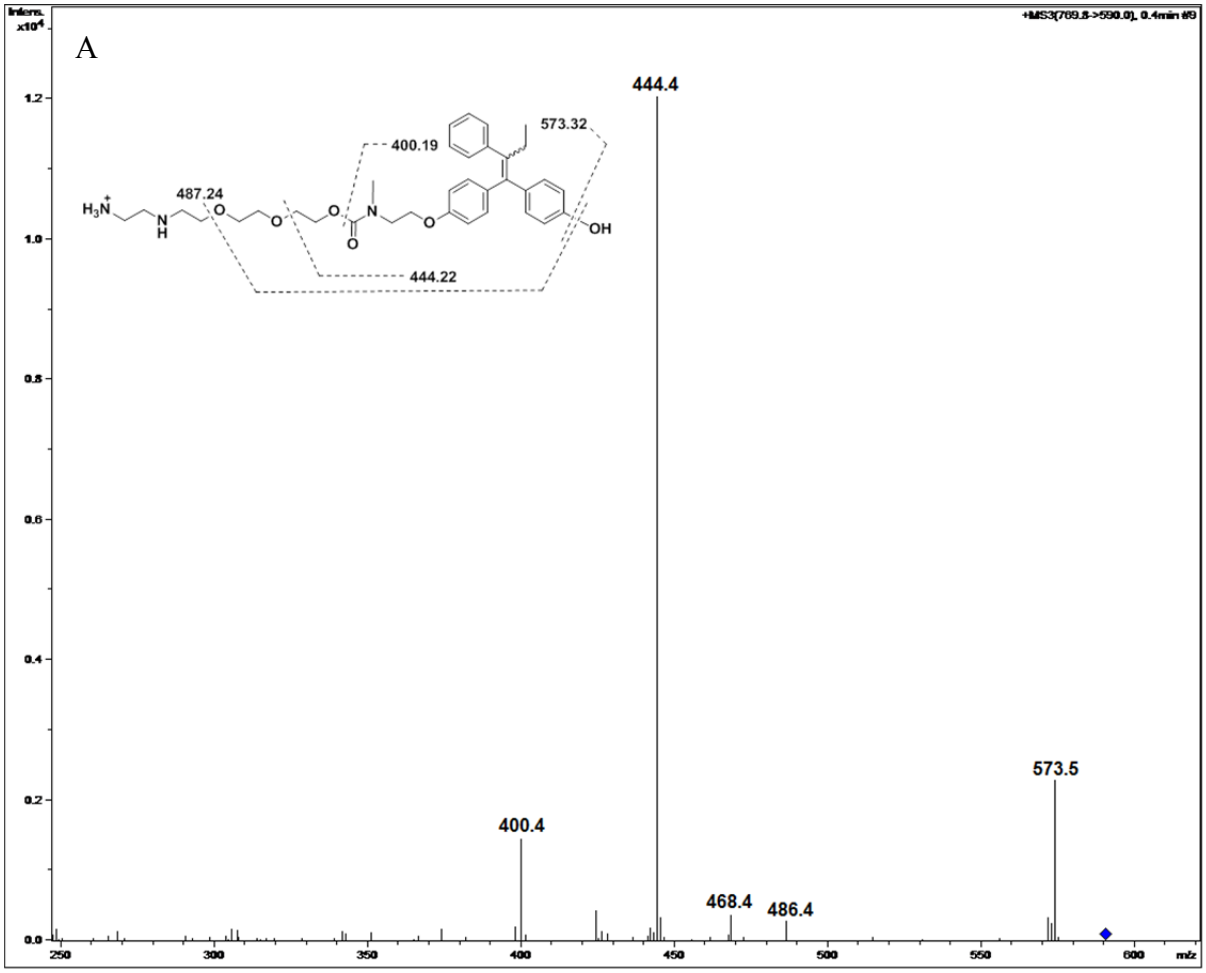
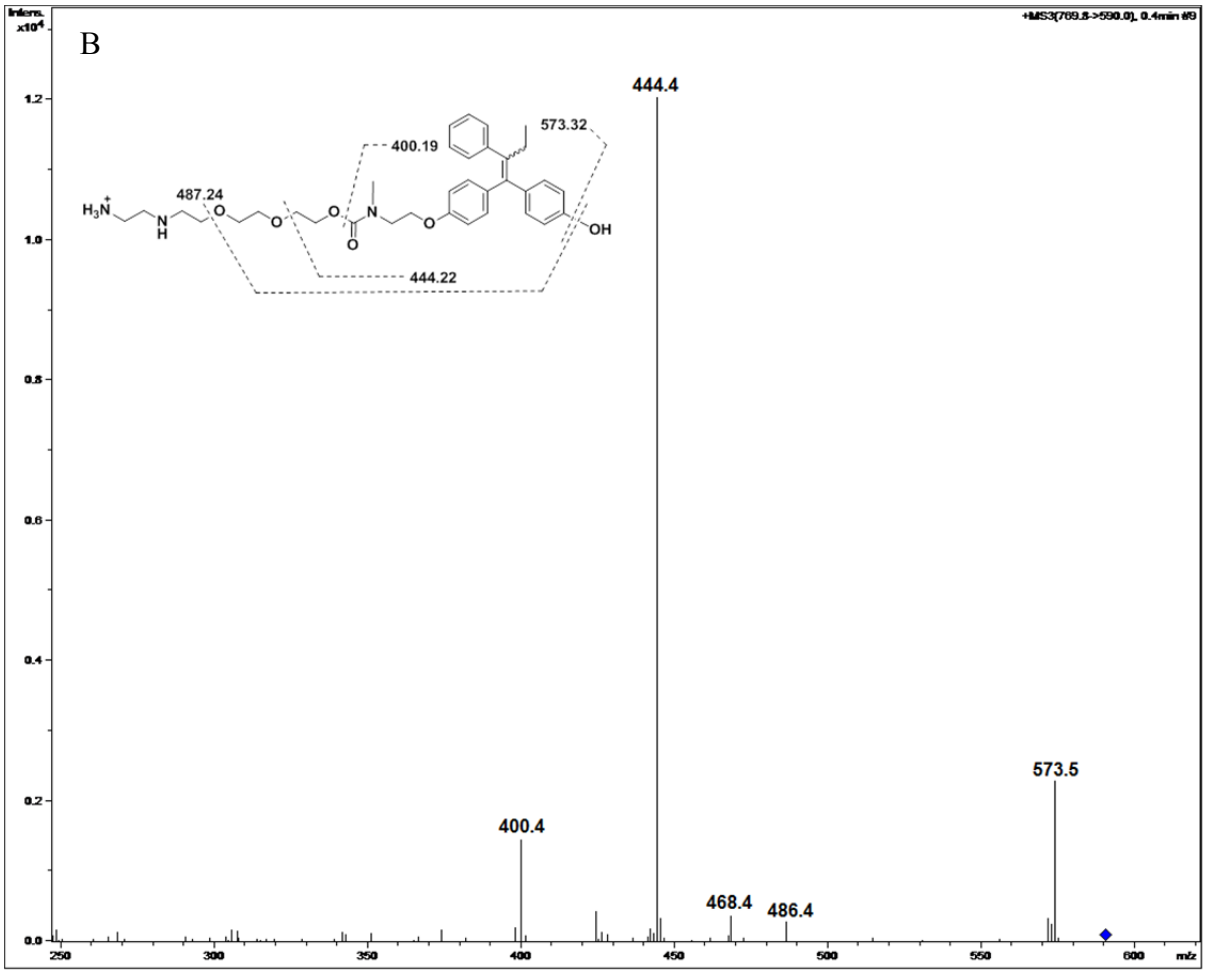


Figure S4.5. Product-ion mass spectra of 9' (A) $[M+H]^+$ (m/z 769.8, MS^2) and (B) [M-acridine]$^+$ (m/z 590.5, MS^3). MS/MS parameters: (A, B) ESI+, capillary voltage: 3.5 kV, cone voltage: 40 V, collision energy: 52.5 eV;

Figure S2.5 continued.



Acquisition Parameter:

Mass Range Mode	Std/Normal	Trap Drive	52.5	Scan Begin	150 m/z
Ion Polarity	Positive	Octopole RF Amplitude	200.0 Vpp	Scan End	2200 m/z
Ion Source Type	ESI	Capillary Exit	135.7 Volt	Averages	5 Spectra
Dry Temp (Set)	350 °C	Skimmer	40.0 Volt	Max. Accu Time	200000 µs
Nebulizer (Set)	50.00 psi	Oct 1 DC	12.00 Volt	ICC Target	-1
Dry Gas (Set)	11.00 l/min	Oct 2 DC	1.73 Volt	Charge Control	on

Compound List:

#	RT [min]	Range [min]	Height	Area	Area Frac %
1	18.4	18.2 - 18.7	2	25	3.0
2	35.2	35.0 - 35.8	4	64	7.5
3	36.4	36.2 - 37.0	31	712	83.5
4	37.2	37.0 - 37.3	2	24	2.8
5	37.5	37.3 - 37.8	3	27	3.2

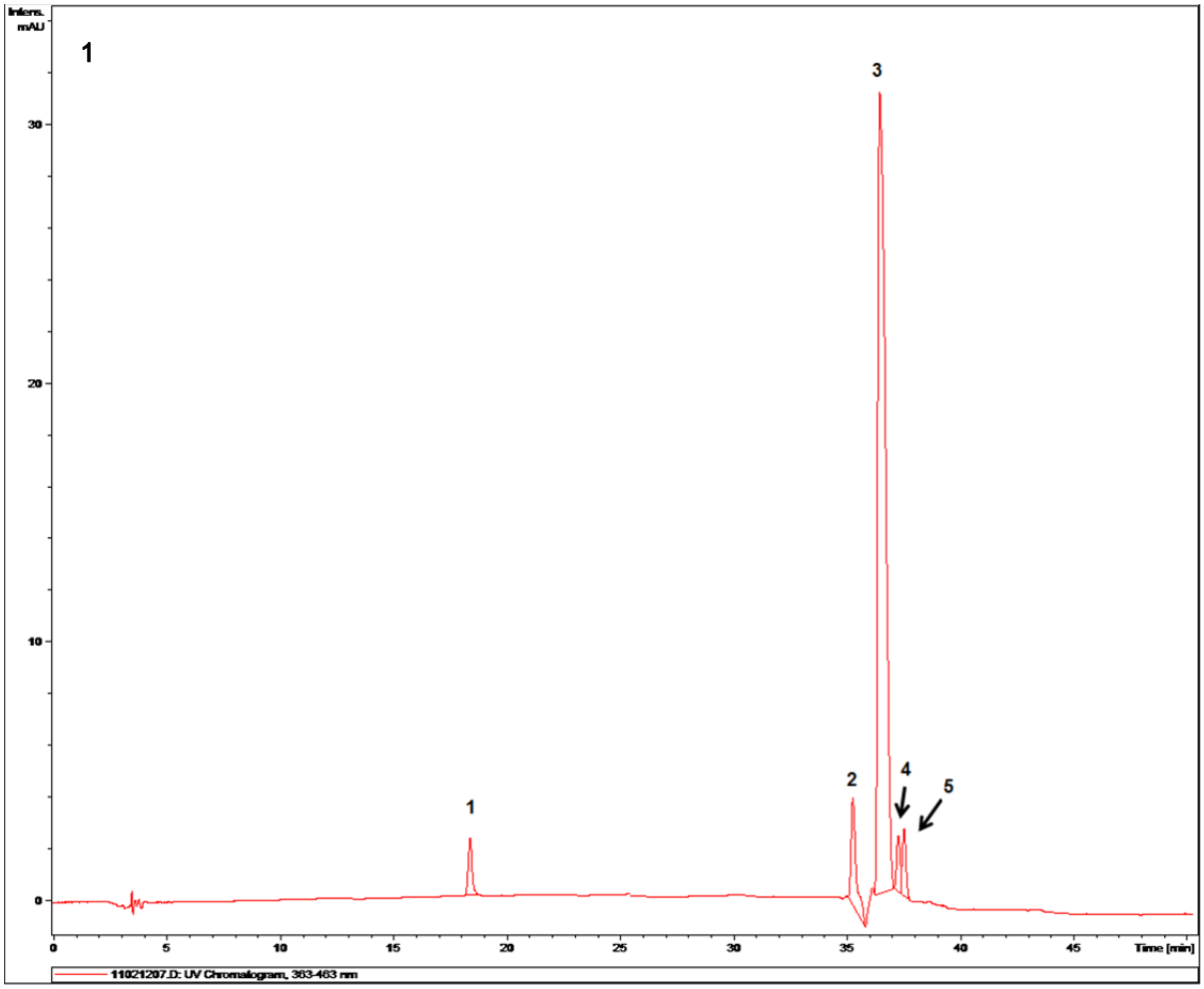


Figure S4.6. LC-ESMS analysis of compound **10'**. (1) Reverse-phase HPLC trace of compound **10'**. Peaks 2–5 show identical masses and can be assigned to rotational isomers as a

result of hindered rotation within the amidine and (E,Z)-endoxifen moieties. Peak 1 is an unidentified impurity. Combined % purity for peaks 2–5 (rotamers): 97%. (2) ESMS spectrum of one species of compound **10'** (HPLC fraction with retention time 35.2 min) recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M+H]^+$ (m/z 1074.8), $[M+H]^{2+}$ (m/z 537.5, z = 2), $[M-2NH_3-Cl]^{2+}$ (m/z 502.4, z = 2). (3) ESMS spectrum of one species of compound **10'** (HPLC fraction with retention time 36.4 min) recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M+H]^+$ (m/z 1074.8), $[M]^{2+}$ (m/z 537.5, z = 2), $[M-2NH_3-Cl]^{2+}$ (m/z 502.4, z = 2). (4) ESMS spectrum of one species of compound **10'** (HPLC fraction with retention time 37.2 min) recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M]^+$ (m/z 1073.8), $[M+H]^{2+}$ (m/z 537.8, z = 2), $[M-2NH_3-Cl]^{2+}$ (m/z 502.4, z = 2). (5) ESMS spectrum of one species of compound **10'** (HPLC fraction with retention time 37.2 min) recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M]^+$ (m/z 1073.8), $[M]^{2+}$ (m/z 537.4, z = 2), $[M-2NH_3-Cl]^{2+}$ (m/z 502.4, z = 2)

Figure S2.6 continued.

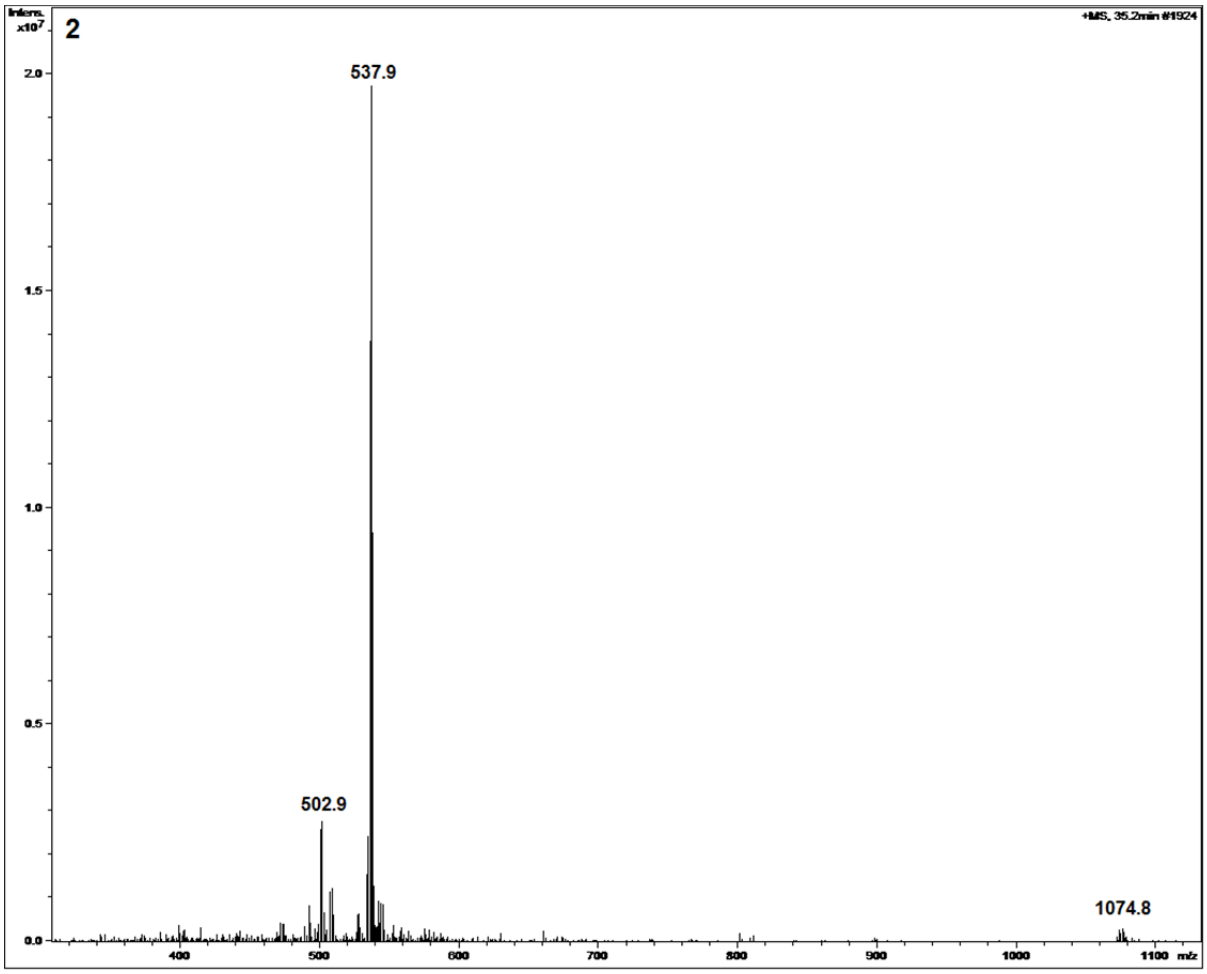


Figure S4.6 continued.

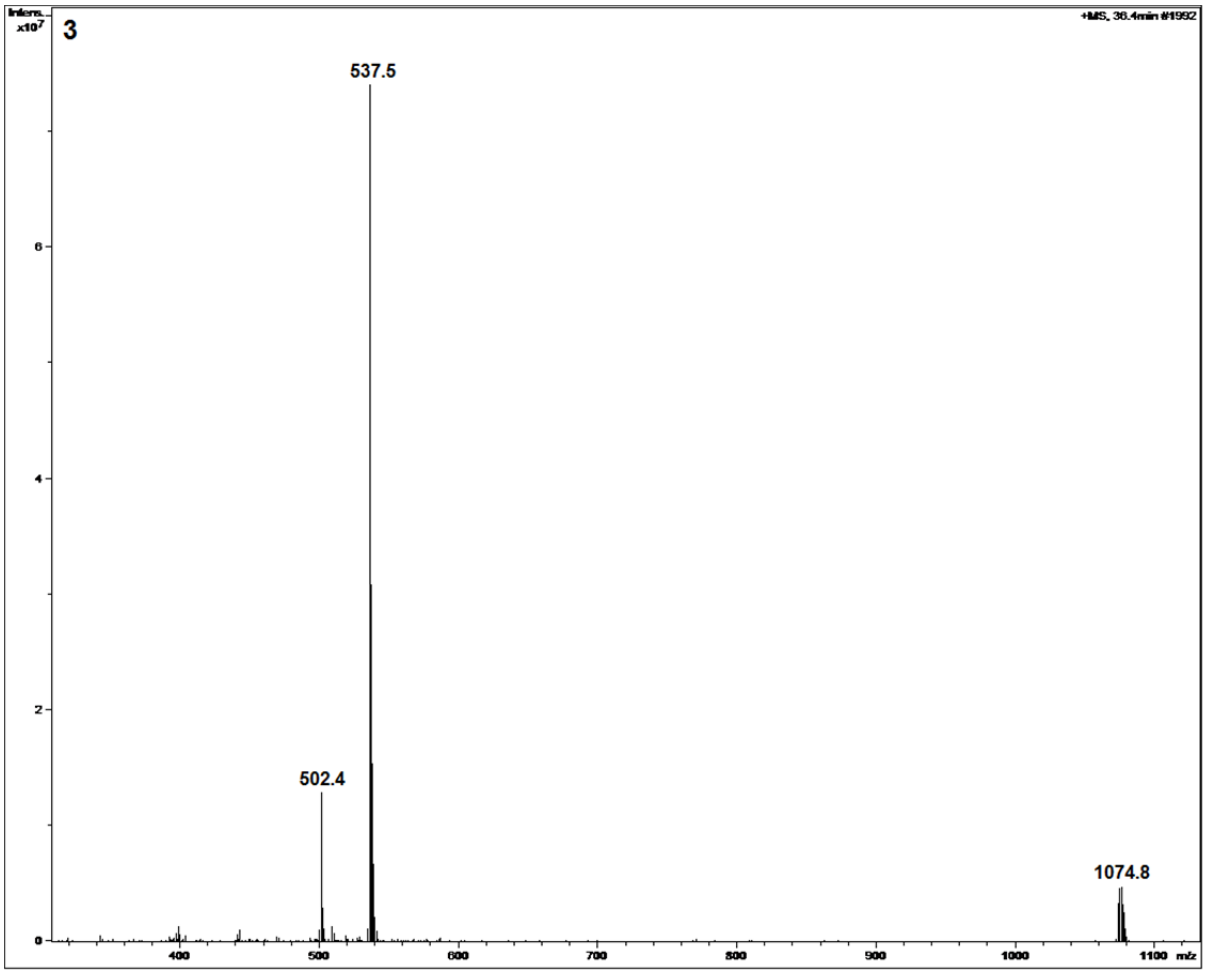


Figure S4.6 continued.

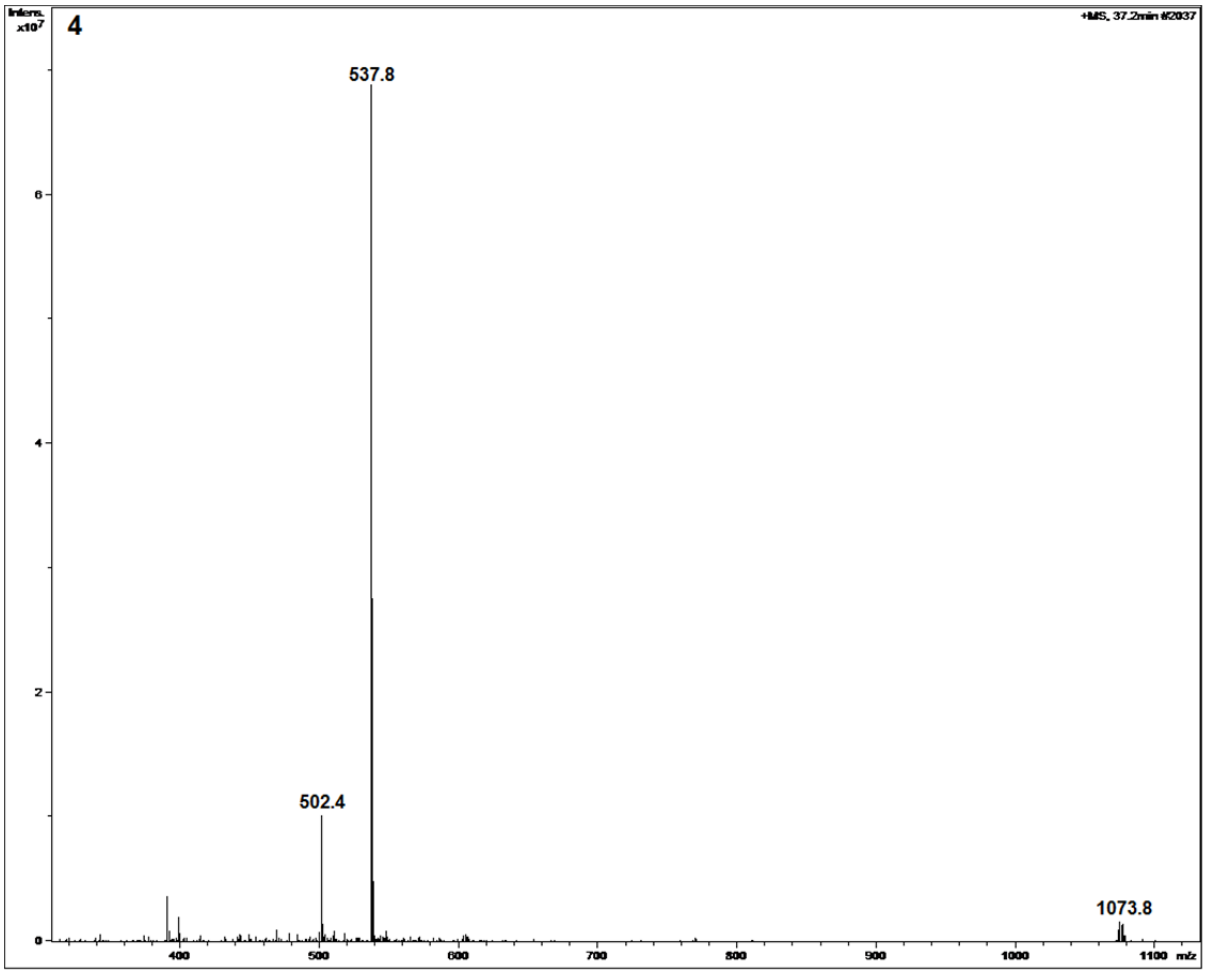
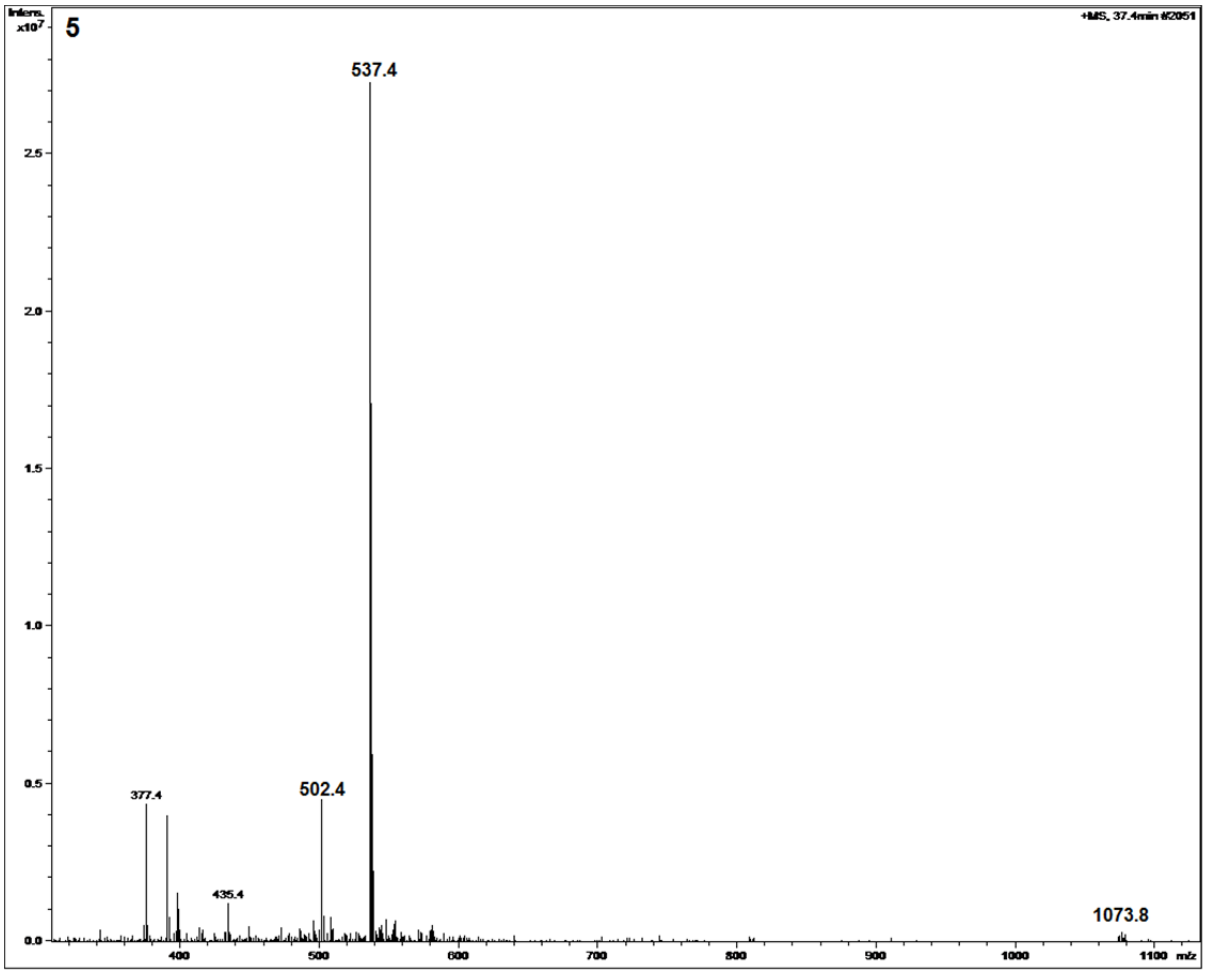
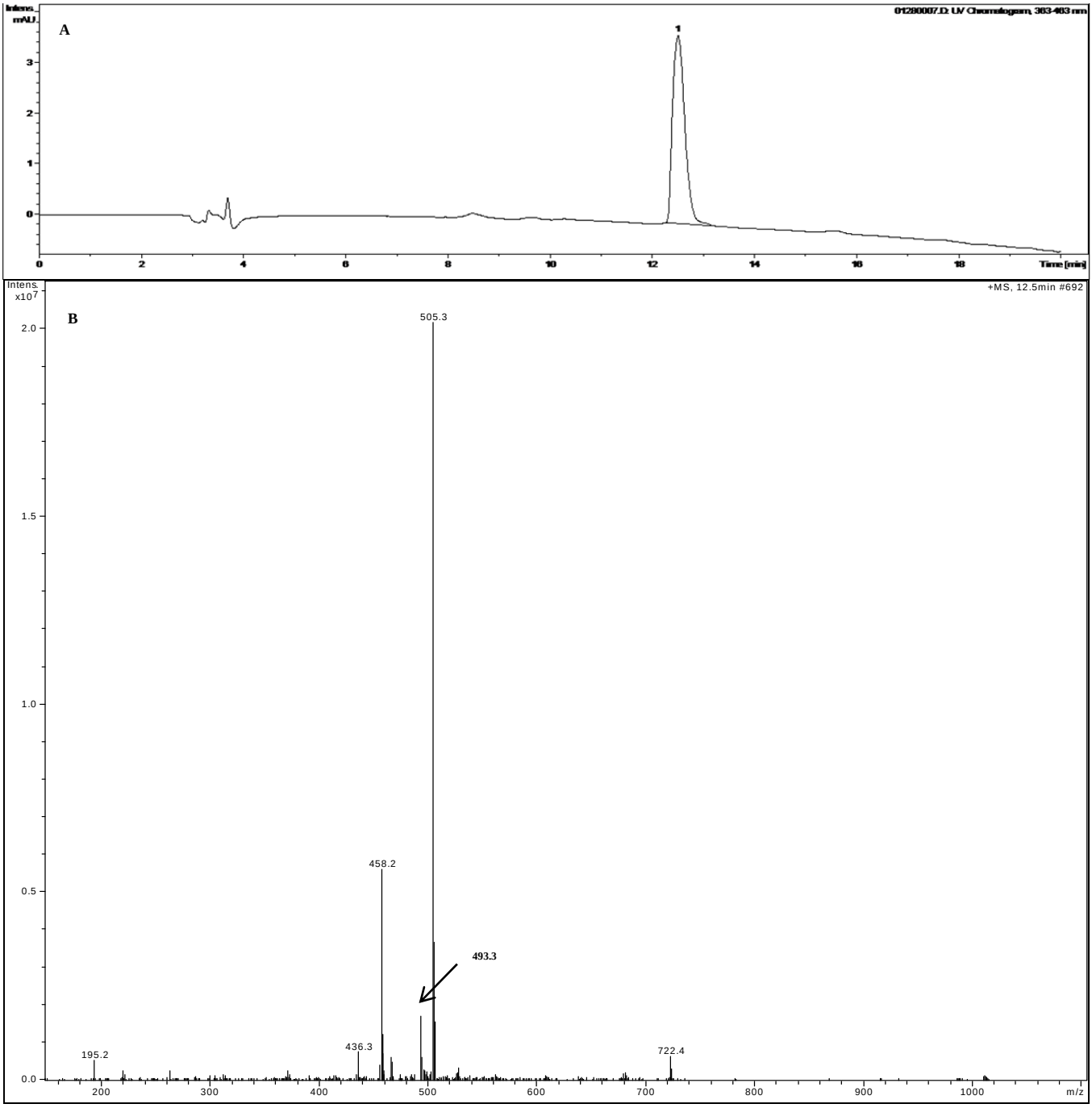


Figure S4.6 continued.



5.1. Stability of compound 10 in acetate buffer (pH = 5.0)



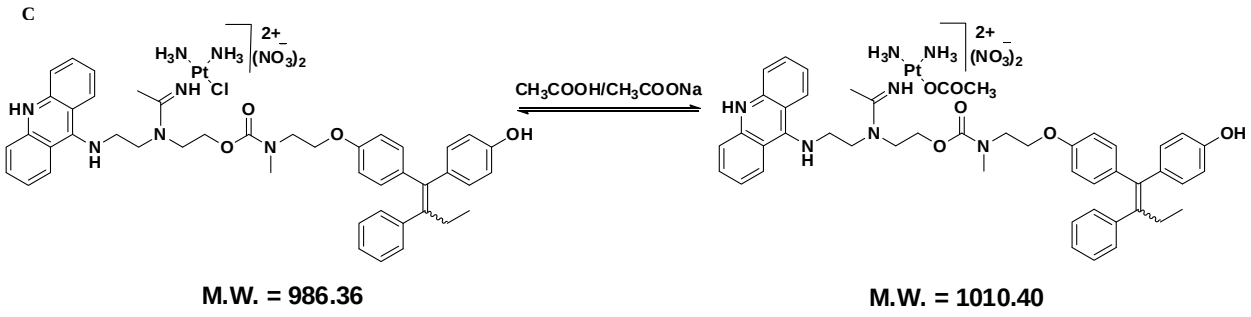
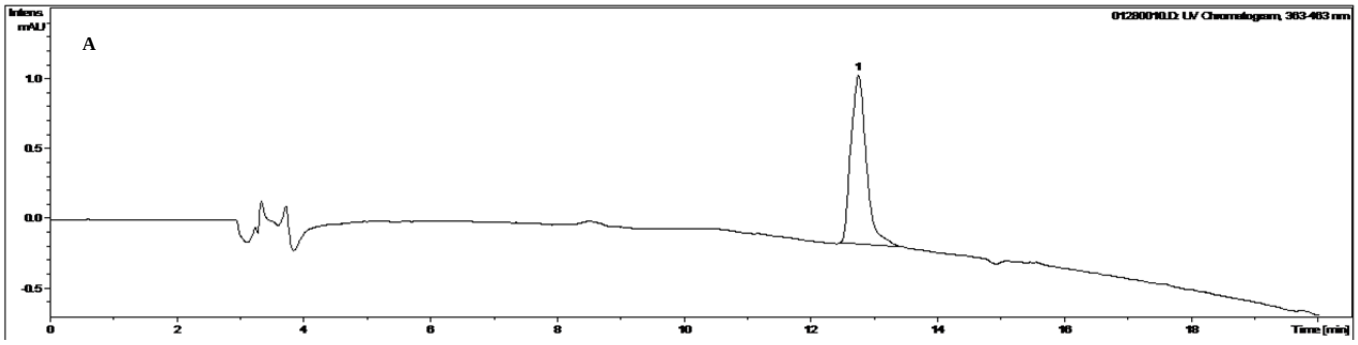
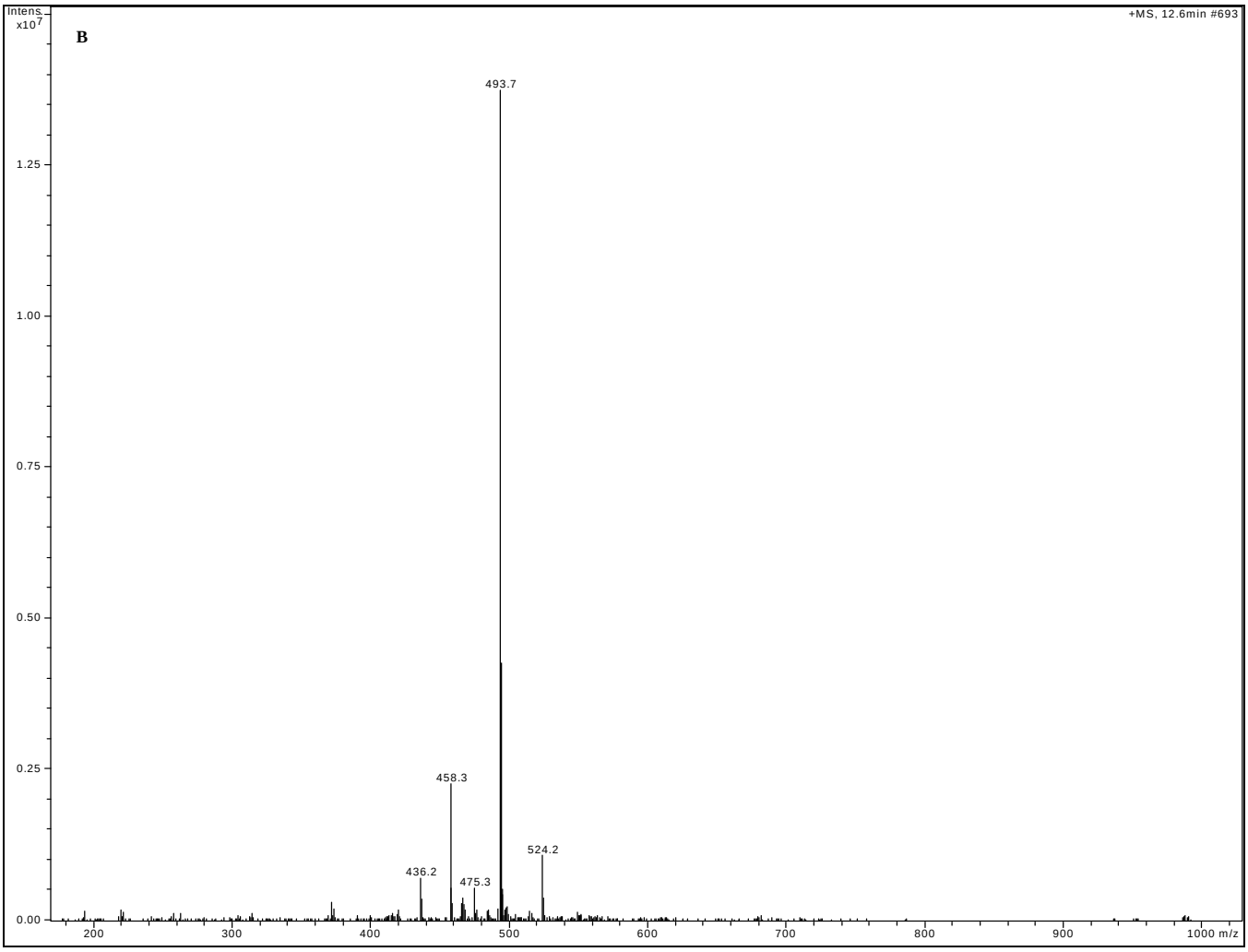


Figure S5.1. LC-ESMS analysis of the mixture of compound **10** in 10 mM acetate buffer (pH = 5.0) incubating for 48 h at 37 °C. ESMS spectrum was recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M\text{-}2NH_3\text{-}Pt\text{-}Cl]^+$ (m/z 722.4), $[M\text{-}Cl+MeCOO]^{2+}$ (m/z 505.3, z =2), $[M\text{-}H]^{2+}$ (m/z 493.3, z = 2), $[M\text{-}2NH_3\text{-}Cl+H]^{2+}$ (m/z 458.2, z = 2), $[M\text{-}2NH_3\text{-}Cl\text{-}Et\text{-}OH]^{2+}$ (m/z 436.9, z =2). (C) Compound **10** is chemically stable in the incubation condition. Only the displacement of chloride ligand with buffer was observed.

5.2 Stability of compound 10 in phosphate-buffered saline (pH = 7.4)





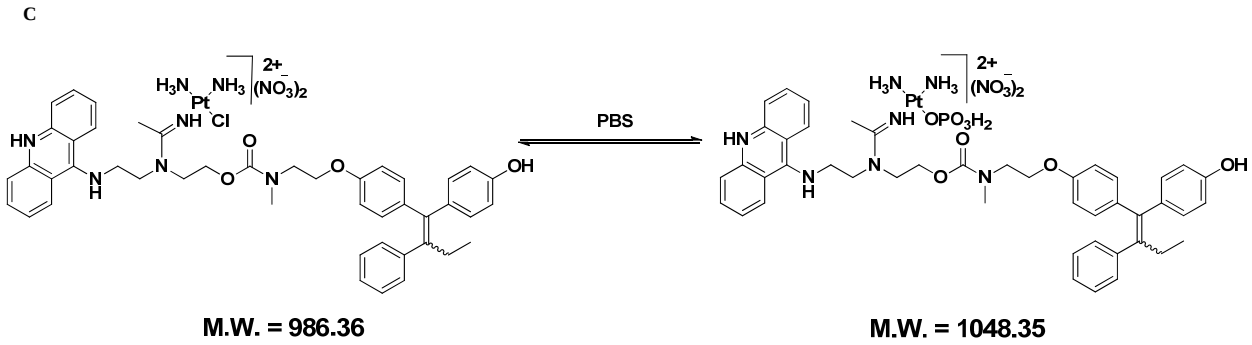
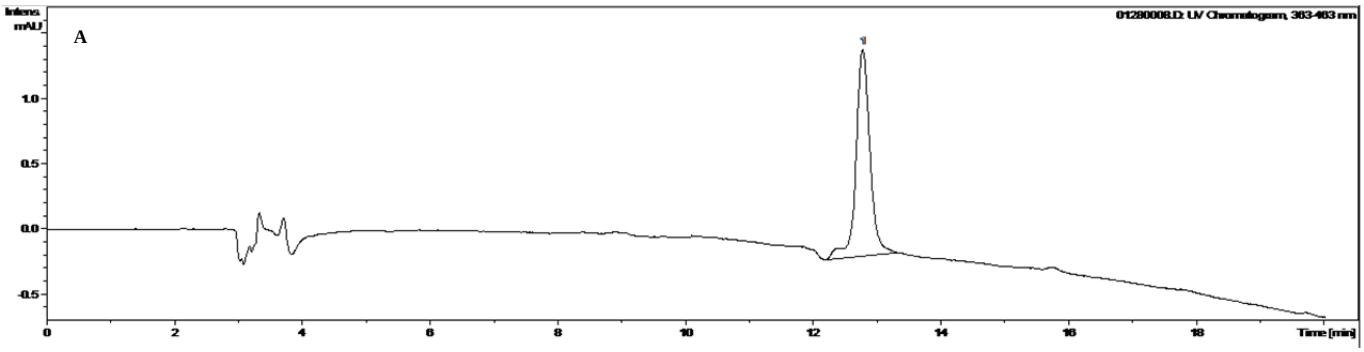


Figure S5.2. LC-ESMS analysis of the mixture of compound **10** in 10 mM phosphate buffer saline (pH = 7.4) incubating for 48 h at **37** °C. ESMS spectrum was recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M\text{-}Cl+H_2PO_4]^{2+}$ (m/z 524.3, z =2), $[M\text{-}H]^{2+}$ (m/z 493.3, z = 2), $[M\text{-}2NH_3\text{-}Cl]^{2+}$ (m/z 458.3, z = 2), $[M\text{-}2NH_3\text{-}Cl\text{-}Et\text{-}OH]^{2+}$ (m/z 436.2, z =2).

(C) Compound **10** is chemically stable in the incubation condition. The displacement of chloride ligand with buffer was observed, while dramatically inhibited by the presence of chloride ions in buffer.

5.3 Stability of compound 10 in phosphate buffer (pH = 7.4)



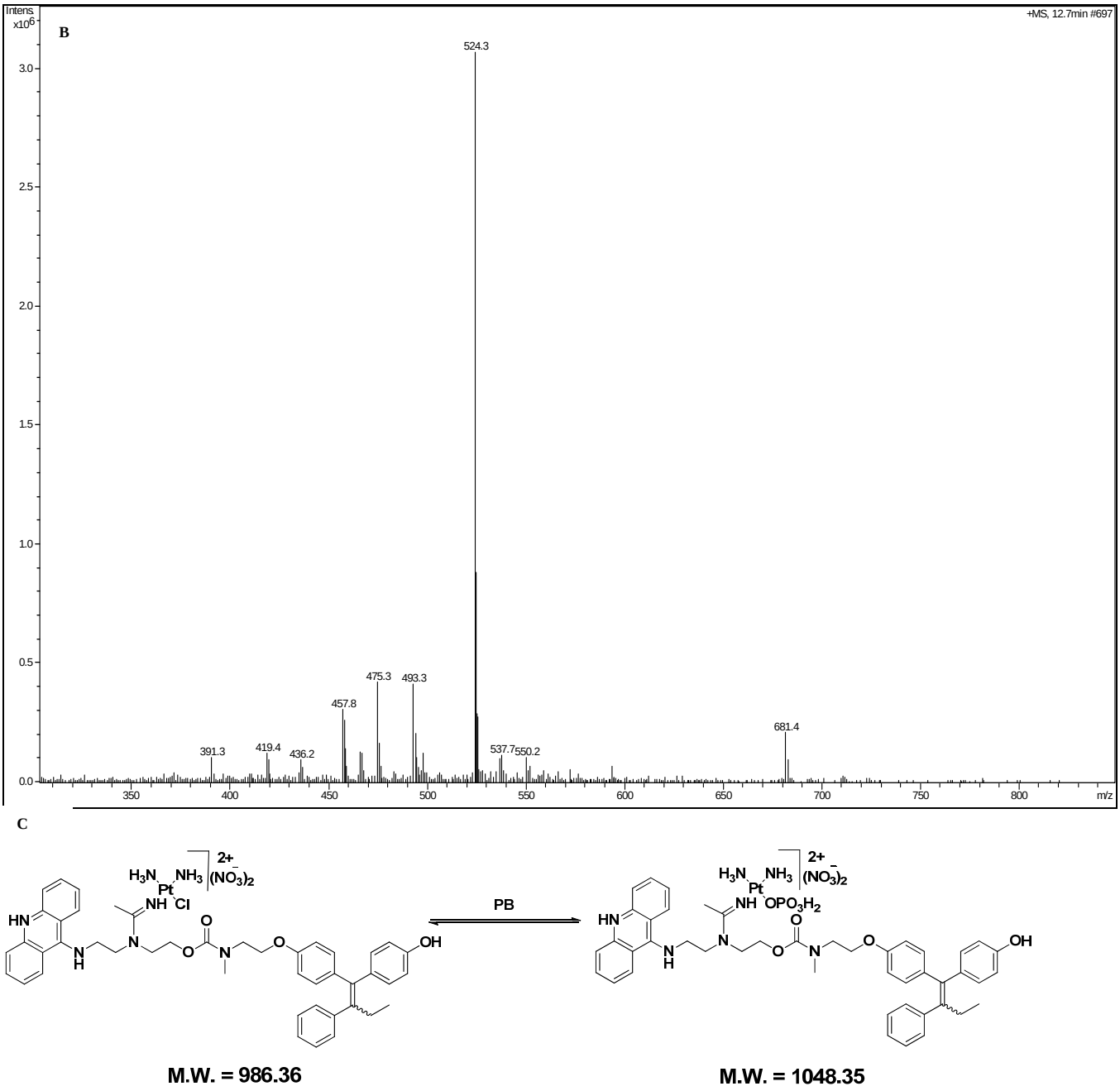


Figure S5.3. LC-ESMS analysis of the mixture of compound **10** in 10 mM phosphate buffer (pH = 7.4) incubating for 48 h at **37** °C. ESMS spectrum was recorded in positive-ion mode. Characteristic molecular and fragment ions : $[M\text{-}2NH_3\text{-}Pt\text{-}Cl\text{-}MeCN]^+$ (m/z 681.4), $[M\text{-}Cl+H_2PO_4]^{2+}$ (m/z 524.3, z =2), $[M\text{-}H]^{2+}$ (m/z 493.3, z = 2), $[M\text{-}2NH_3\text{-}Cl]^{2+}$ (m/z 457.8, z = 2), $[M\text{-}2NH_3\text{-}Cl\text{-}Et\text{-}OH]^{2+}$ (m/z 436.2, z =2). (C) Compound **10** is chemically stable in the incubation condition. Only the displacement of chloride ligand with buffer was observed.

6. Cytotoxicity studies and cell viability assays

Sample preparation and cell culture

Stock solutions of compound **5**, compound **10** and compound **10'** were prepared in DMF and concentrations were determined spectrophotometrically by $\varepsilon_{413} = 10000\ M^{-1}\ cm^{-1}$. Cisplatin, endoxifen and estradiol were purchased from Sigma. Stock solution of cisplatin was prepared in phosphate-buffered saline (PBS) and the concentration was determined by $\varepsilon_{300} = 132\ M^{-1}\ cm^{-1}$.[1] Stock solutions of endoxifen and tamoxifen were prepared in DMSO.

The human breast adenocarcinoma cells MCF-7 (ER-positive), and MDA-MB-231 (ER-negative) were obtained from American Type Culture Collection (Rockville, MD, USA). MCF-7 cells were maintained in phenol red DMEM/F-12 media (Gibco) containing 2.4 g/L sodium bicarbonate and 2.5 mM L-glutamine supplemented with 10% fetal bovine serum (FBS), 10% penstrep (P&S), and 10 μg/mL insulin. MDA-MB-231 cells were cultured in high glucose DMEM media (Gibco) containing 4.5 g/L D-glucose, 110 mg/L sodium pyruvate supplemented with 10% fetal bovine serum (FBS), 10% penstrep (P&S), and 10% L-glutamine. All cultures were grown at a constant temperature at 37 °C in a humidified atmosphere containing 5% CO_2, and cells were subcultured once a week in order to maintain cells in logarithmic growth.

Cytotoxicity studies

The cytotoxicity studies of selected compounds were carried out by the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA) as described previously.[2] Briefly, cells suspensions were harvested and seeded into 96-well microplates at a

certain density (MCF-7, 10000 cells/well; MDA-MB-231, 12000 cells/well). After overnight incubation, cells cells were treated with varying concentrations (starting concentrations were 80 or 100 μM, and DMF or DMSO amount was less than 0.5%) of test compound for 72 h at 37 ºC in an atmosphere of 5% CO_2. After incubation periods of 72 h, 20 μL of MTS/PMS solution was added to each well and incubated at 37 °C for 4 h. The absorbance of tetrazolium dye was measured at 490 nm using an enzyme-linked immunesorbent assay (ELISA) reader. The reported IC_{50} data were calculated from non-linear curve fits using a sigmoidal dose-response equation in GraphPad Prism (GraphPad Software, La Jolla, CA) and are averages of two individual experiments performed in triplicate.

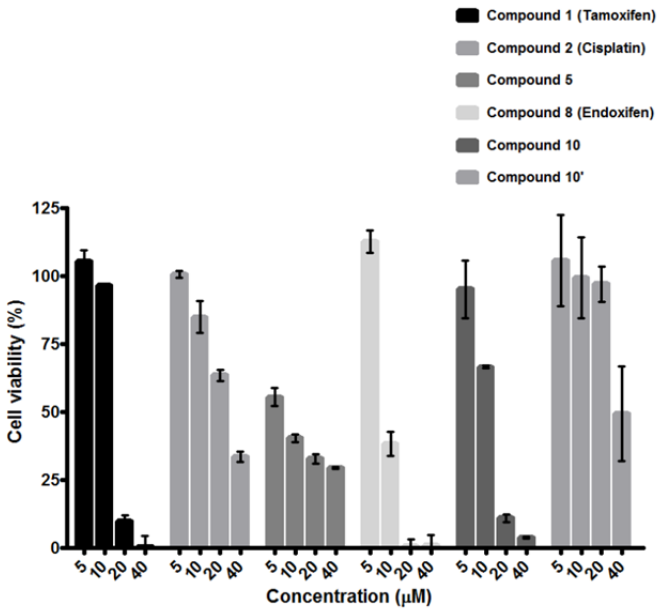


Fig. S6.1. Differential response of MCF-7 cancer cells to compounds tested (48-h incubations in cells in phenyl red-free media).

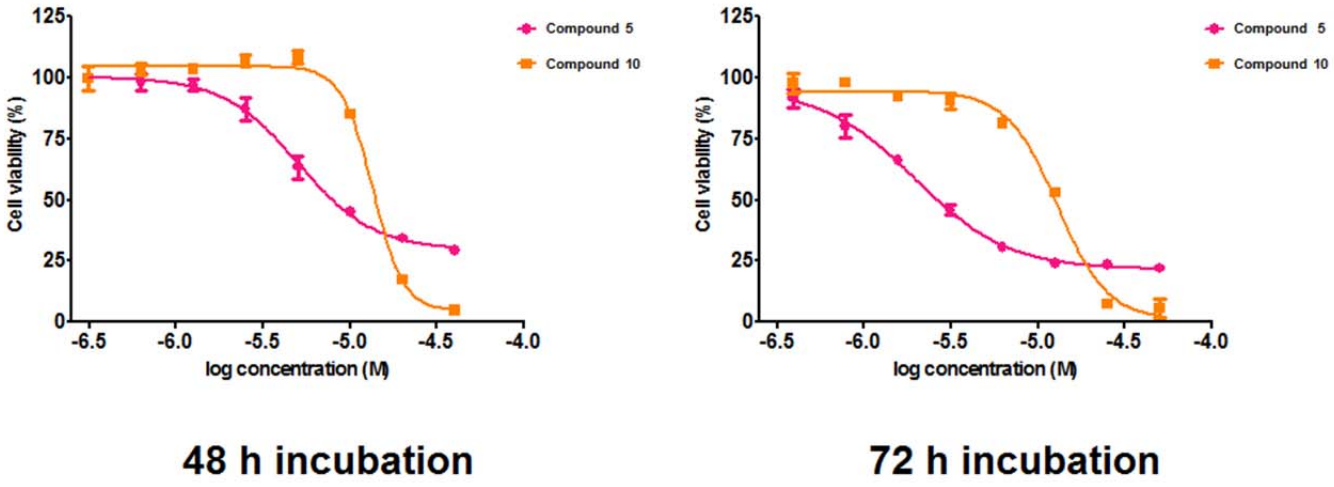


Fig. S6.2. Dose–response curves for 48-h and 72-h incubations of MCF-7 cells with compounds **5** and **10**. Note the inability of compound **5** to kill the entire population of cells even at the highest concentrations applied. This suggests that a subpopulation of cells exists that exhibits a high degree of resistance to the cytotoxic agent **5** but not to conjugate **10.** In this experiment, MCF-7 cells were cultured for 4 days in phenol red-free DMEM/F-12 media supplemented with 5% dextran-coated charcoal-stripped FBS (DCC-FBS) to remove steroids. Inhibitory concentrations, therefore, vary from those reported in Table 1 (main text).

7. References

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