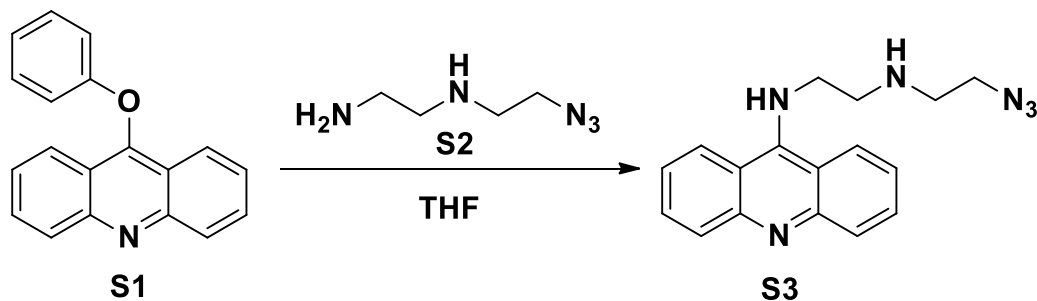


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## 1. Synthetic Procedures and Product Characterization

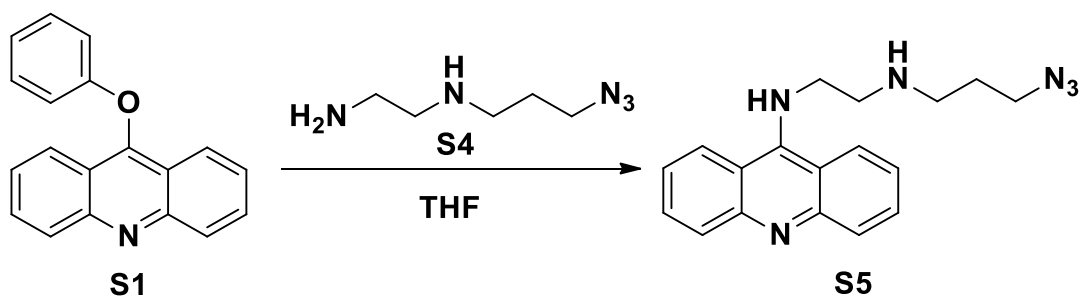
**Materials and Methods.** The synthetic precursors **S2**<sup>1</sup>, **S4**<sup>1</sup>, **S6**<sup>2</sup> and **S10**<sup>3</sup> were synthesized according to the methods described previously. All reagents were used as obtained from commercial sources without further purification unless indicated otherwise. <sup>1</sup>H NMR spectra of the target compounds and intermediates were recorded on a Bruker Advance 300 MHz instrument. Chemical shifts ( $\delta$ ) are given in parts per million (ppm) relative to internal standard tetramethylsilane (TMS). <sup>1</sup>H NMR data is reported in the conventional form including chemical shift ( $\delta$ , ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants (Hz), and signal integrations. <sup>13</sup>C{<sup>1</sup>H} NMR data are reported as chemical shift listings ( $\delta$ , 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<sub>2</sub> pressure of 50 psi and solvent evaporation was assisted by a flow of N<sub>2</sub> 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  $\mu$ m) analytical column at 25 °C. The purities of compound **S3** and compound **S5** 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 15 minutes. The purity of compound **2** 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 30 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.

### Scheme 1.1



A mixture of phenoxyacridine (**S1**) (2.71 g, 0.01 mol) and *N*<sup>1</sup>-(2-azidoethyl) ethane-1,2-diamine (**S2**) (1.42 g, 0.011 mol) in 25 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 conc. HCl, and the mixture was stirred at 4 °C for 3 hours. A yellow precipitate formed which was recovered by filtration, resuspended in 50 mL of 2 M ammonium hydroxide, and stirred at room temperature for 30 min. The aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> and the organic phase was collected, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated using rotary evaporation, affording 2.57 g of the free base (**S3**) as a yellow solid (Yield: 87%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ = 8.16 (d, *J* = 7.2 Hz, 2H), 8.06 (d, *J* = 8.4 Hz, 2H), 7.65 (t, *J* = 7.8 Hz, 2H), 7.35 (t, *J* = 8.1 Hz, 2H), 6.24 (br s, 1H), 3.82 (t, *J* = 5.6 Hz, 2H), 3.46 (t, *J* = 5.6 Hz, 2H), 2.93 (t, *J* = 6.4 Hz, 2H), 2.87 (t, *J* = 6.3 Hz, 2H), 1.39 (br s, 1H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 151.65, 149.51, 129.80, 122.97, 117.11, 51.57, 49.19, 49.09, 47.91. MS (ESI, positive-ion mode): calculated for C<sub>17</sub>H<sub>18</sub>N<sub>6</sub> ([M+H]<sup>+</sup>), 306.16; found: 307.2.

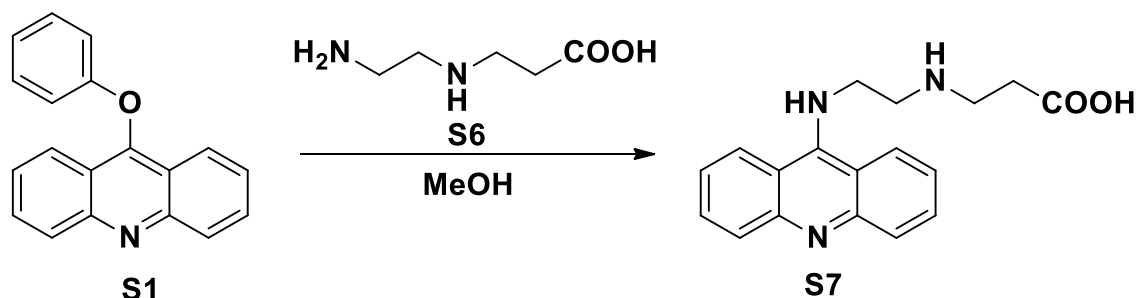
### Scheme 1.2



**S5** was prepared using the procedure described for **S3** starting from phenoxyacridine (**S1**) (2.71 g, 0.01 mol) and *N*<sup>1</sup>-(3-azidopropyl)ethane-1,2-diamine (**S4**). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.15 (d, *J* = 8.7 Hz, 2H), 8.06 (d, *J* = 8.9 Hz, 2H), 7.65 (t, *J* = 8.5 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 6.26 (brs, 1H), 3.81 (t, *J* = 5.4 Hz, 2H), 3.40 (t, *J* = 6.6, 2H), 2.90 (t, *J* = 5.7 Hz, 2H), 2.75 (t, *J* =

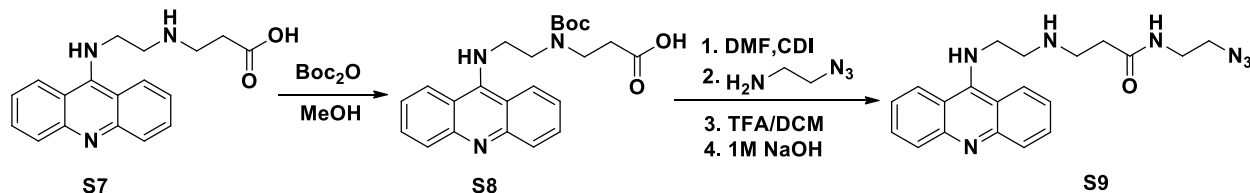
6.8, 2H), 1.80 (p,  $J = 6.7$ , 2H), 1.12 (brs, 1H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  151.70, 149.47, 129.82, 129.78, 123.00, 122.84, 116.87, 49.50, 49.43, 49.09, 46.28, 29.42. MS (ESI, positive-ion mode): calculated for  $\text{C}_{18}\text{H}_{20}\text{N}_6$  ( $[\text{M}+\text{H}]^+$ ), 320.17; found: 321.2.

### Scheme 1.3



A mixture of phenoxyacridine (**S1**) (2.71 g, 0.01 mol) and 3-((2-aminoethyl)amino) propanoic acid (**S6**) (1.45 g, 0.011 mol) in 20 mL of dry MeOH was refluxed for 3 h. The yellow solid that precipitated during the reaction was collected by filtration, washed with hot THF and ether, and dried in a vacuum, affording 2.6 g of the product **S7** as a yellow solid (Yield: 84 %).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  8.04 (d,  $J = 8.7$  Hz, 2H), 7.83 (dd,  $J = 8.4$ , 7.0 Hz, 2H), 7.58 - 7.34 (m, 4H), 4.33 (t,  $J = 6.1$  Hz, 2H), 3.60 (t,  $J = 5.8$  Hz, 1H), 3.33 (t,  $J = 6.3$  Hz, 2H), 3.33 (t,  $J = 6.3$  Hz, 2H).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  166.95, 157.31, 152.15, 130.13, 129.21, 125.84, 118.56, 115.13, 49.87, 48.80, 45.42, 27.92. MS (ESI, positive-ion mode): calculated for  $\text{C}_{18}\text{H}_{20}\text{N}_3\text{O}_2$  ( $[\text{M}+\text{H}]^+$ ), 310.37; found: 310.3.

### Scheme 1.4

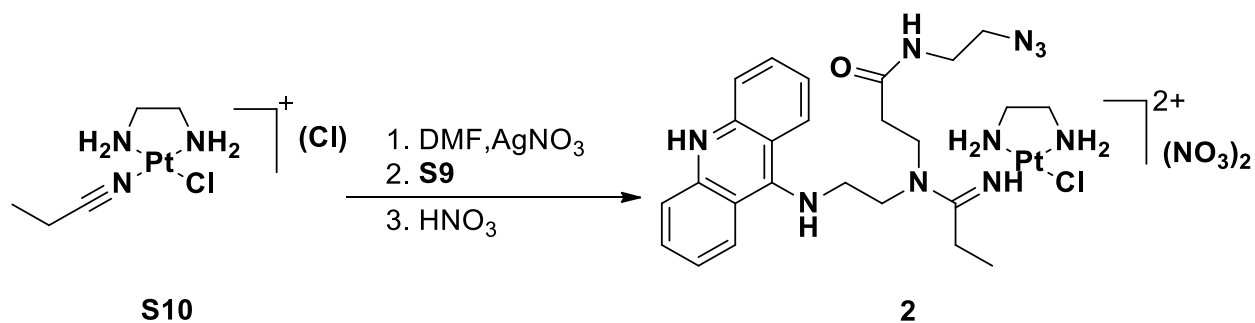


The Boc-protected acridine derivative (**S8**) was synthesized as follows. **S7** (1.43 g, 4.6 mmol) was suspended in 30 mL of anhydrous methanol, to which was added  $\text{Boc}_2\text{O}$  (1.3 g, 6 mmol) in 5 mL of anhydrous MeOH at 0-5 °C maintained with an ice bath. The mixture was then stirred at room temperature for 4 h. To remove excess  $\text{Boc}_2\text{O}$ , the solvent was removed by rotary evaporation and residue was suspended in 200 mL of anhydrous diethyl ether and stirred at room temperature for 1h. The solid was recovered by filtration and dried in a vacuum affording 1.79 g

(99%) of the product **S8** as a yellow solid, which was used in the next step without further purification.

Compound **S8** (1 g, 2.44 mmol) and 1,1'-carbonyldiimidazole (CDI, 533 mg, 3.28 mmol) were combined in 20 mL of anhydrous DMF. The mixture was heated to 40-50 °C and stirred for 6 h. Then the solution was cooled to 0-5 °C in an ice bath and 264 mg of 2-azidoethanamine, dissolved in 3 mL of anhydrous DMF, were added. The mixture was stirred at 0-5 °C for 4 h. DMF was removed by vacuum distillation at 35-40 °C, and the residue was redissolved in 40 mL of dichloromethane and washed with 1 M HCl (3 × 20 mL). The organic phase was collected, dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated to afford an orange oil. To remove the Boc group, the residue was dissolved in 6 mL of a 1:1 mixture of anhydrous dichloromethane and trifluoroacetic acid and stirred at room temperature for 3 h. The reaction was quenched by adding 10 mL of 1 M NaOH solution. The crude product was extracted from NaOH solution with DCM, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The product was purified by flash chromatography (Al<sub>2</sub>O<sub>3</sub>, DCM:MeOH, 30:1). Yield: 0.59 g (64 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.09 (d, *J* = 8.7 Hz, 2H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.59 (t, *J* = 7.6 Hz, 2H), 7.41 (s, 1H), 7.27 (t, *J* = 7.5 Hz, 2H), 4.95 (brs, 2H), 3.92 (t, *J* = 5.6 Hz, 2H), 3.44 (s, 4H), 2.91-3.14 (m, 4H), 2.53 (t, *J* = 6.0 Hz, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 152.09, 149.60, 129.65, 129.23, 123.60, 121.91, 115.75, 51.37, 51.32, 36.51, 29.15. MS (ESI, positive-ion mode): calculated for C<sub>20</sub>H<sub>24</sub>N<sub>7</sub>O ([M+H]<sup>+</sup>), 378.45; found: 378.3.

### Scheme 1.5



Platinum complex **S10** (381 mg, 1 mmol) was converted to its nitrate salt by reaction with AgNO<sub>3</sub> (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 **S9** (378 mg, 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, then the precipitate was recovered by membrane filtration and dried in a vacuum overnight. The solid was dissolved in anhydrous methanol containing one equivalent of 1 M HNO<sub>3</sub>, 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 432 mg of compound **2** as a yellow microcrystalline solid (Yield: 51%; analytical purity, HPLC: 98%). <sup>1</sup>H NMR (300 MHz, MeOH-*d*<sub>4</sub>) δ 8.45 - 8.39 (m, 2H), 7.89 (ddd, *J*=8.3, 6.9, 1.2 Hz, 2H), 7.78 - 7.70 (m, 2H), 7.52 (ddd, *J*=8.8, 6.9, 1.3 Hz, 1H), 5.37 - 5.15 (m, 4H), 4.27 (t, *J*=6.8 Hz, 2H), 3.84 (t, *J*=6.8 Hz, 2H), 3.59 (t, *J*=6.6 Hz, 2H), 3.30 - 3.22 (m, 4H), 2.94 (q, *J*=7.6, 2H), 2.58 - 2.26 (m, 7H), 1.18 (t, *J*=7.6, 3H). <sup>13</sup>C NMR (75 MHz, MeOD) δ 173.27, 171.13, 160.17, 141.38, 136.67, 125.45, 119.80, 114.17, 51.38, 49.86, 39.96, 29.19, 11.99.

## 2. NMR spectra

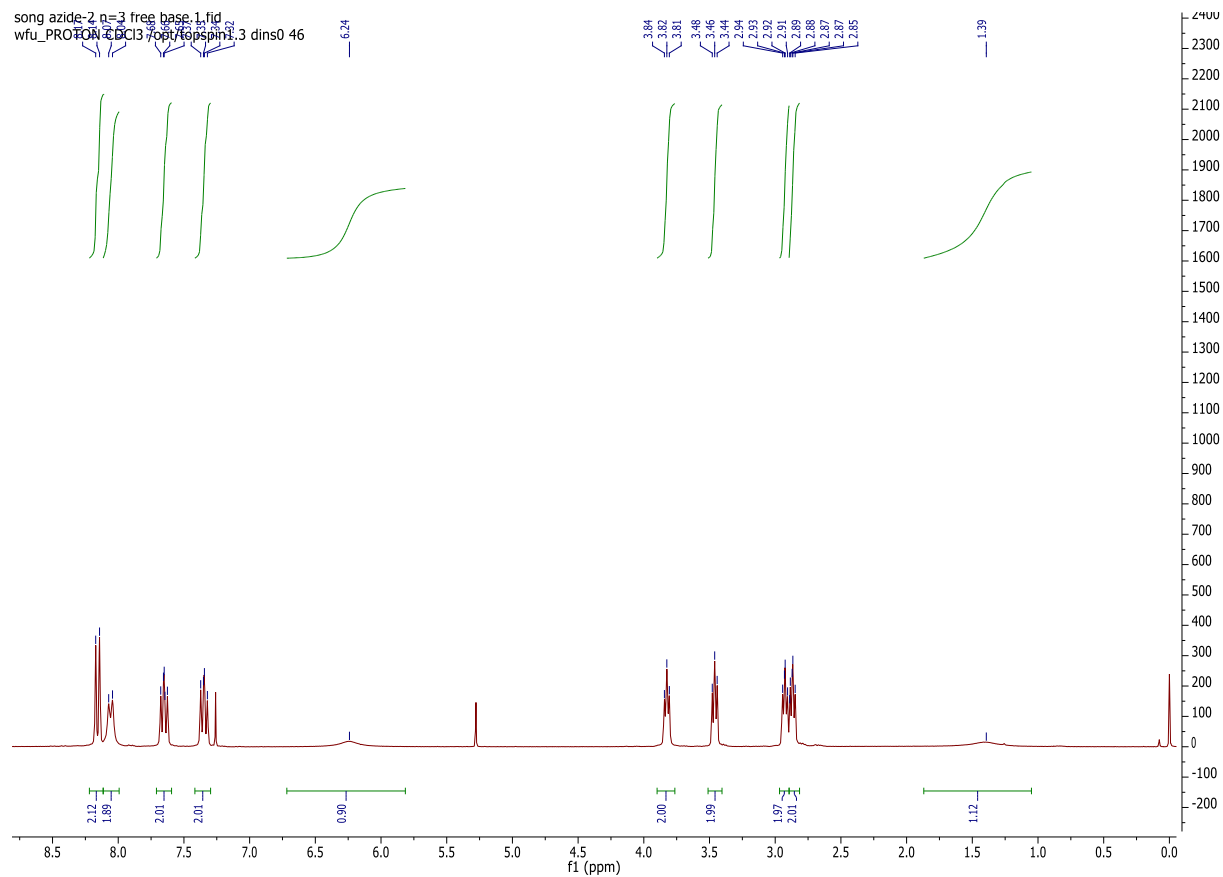
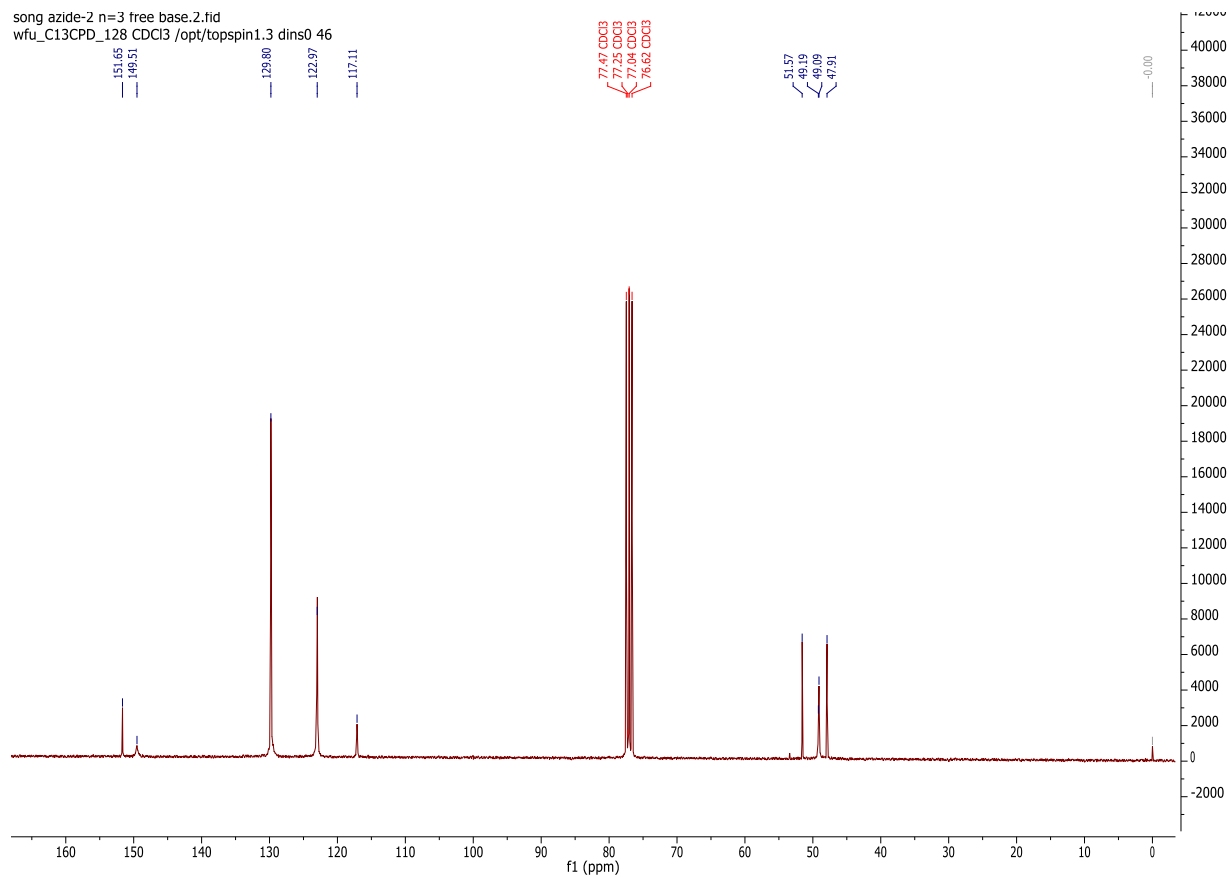
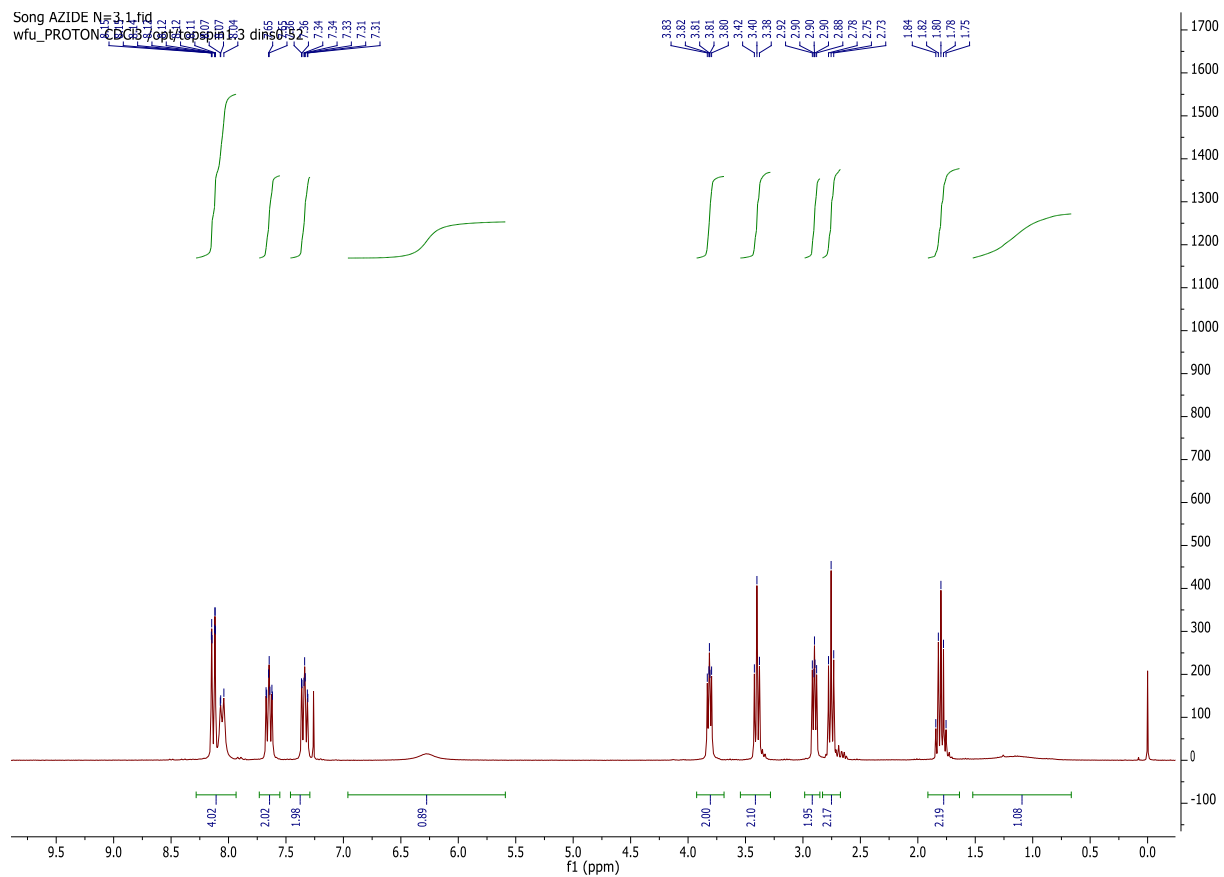


Figure S2.1.  $^1\text{H}$  NMR spectrum of compound **S3** in  $\text{CDCl}_3$ .

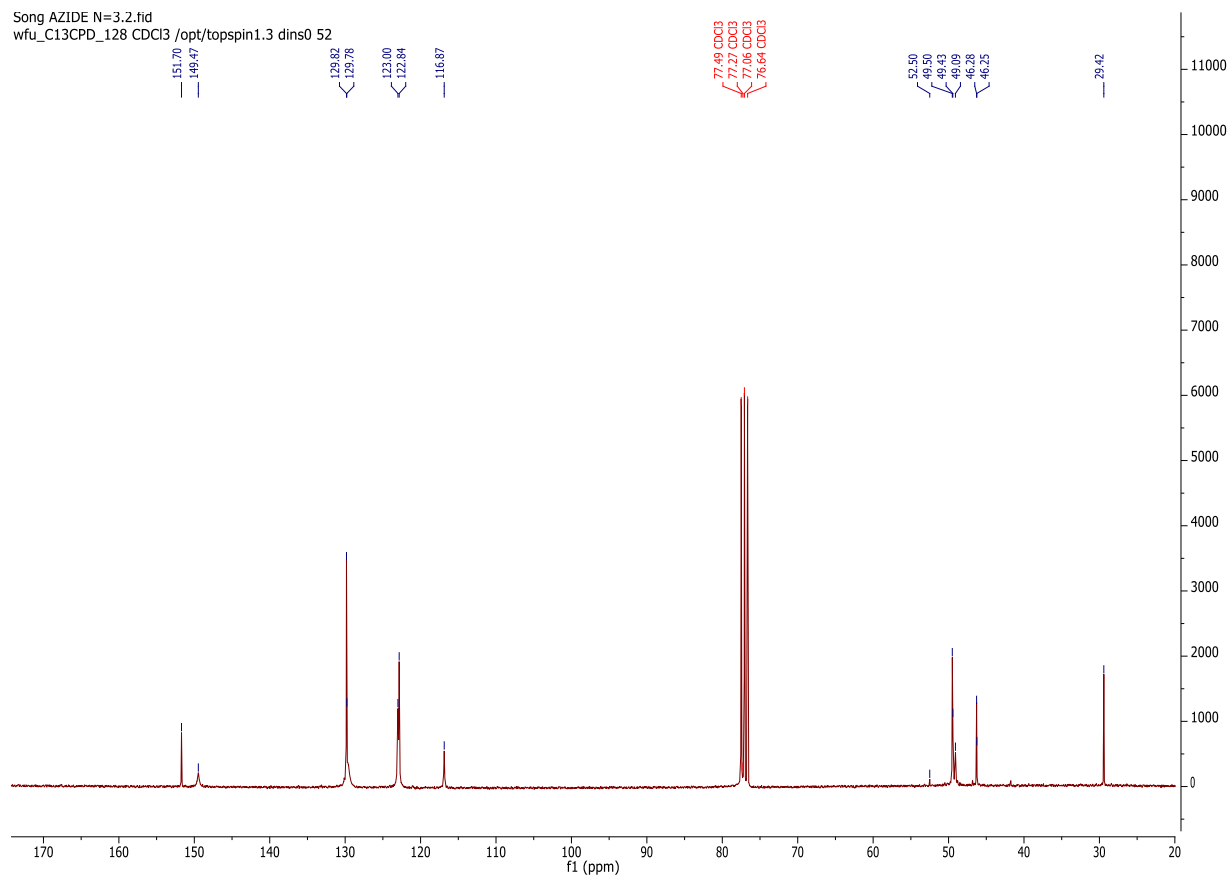


**Figure S2.2.**  $^{13}\text{C}$  NMR spectrum of compound **S3** in  $\text{CDCl}_3$ .

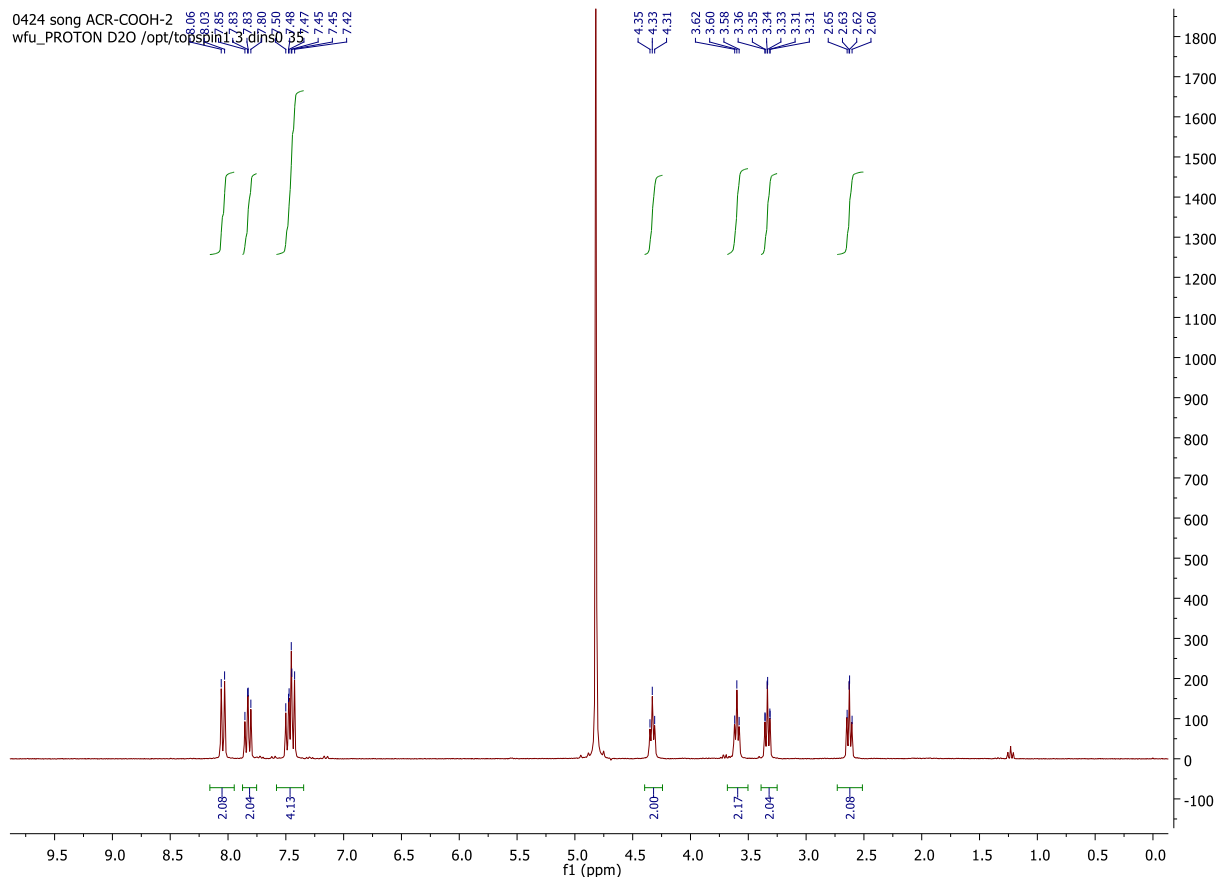




**Figure S2.3.**  $^1\text{H}$  NMR spectrum of compound **S5** in  $\text{CDCl}_3$ .



**Figure S2.4.** <sup>13</sup>C NMR spectrum of compound **S5** in CDCl<sub>3</sub>.



**Figure S2.5.**  $^1\text{H}$  NMR spectrum of compound **S7** in  $\text{D}_2\text{O}$ .

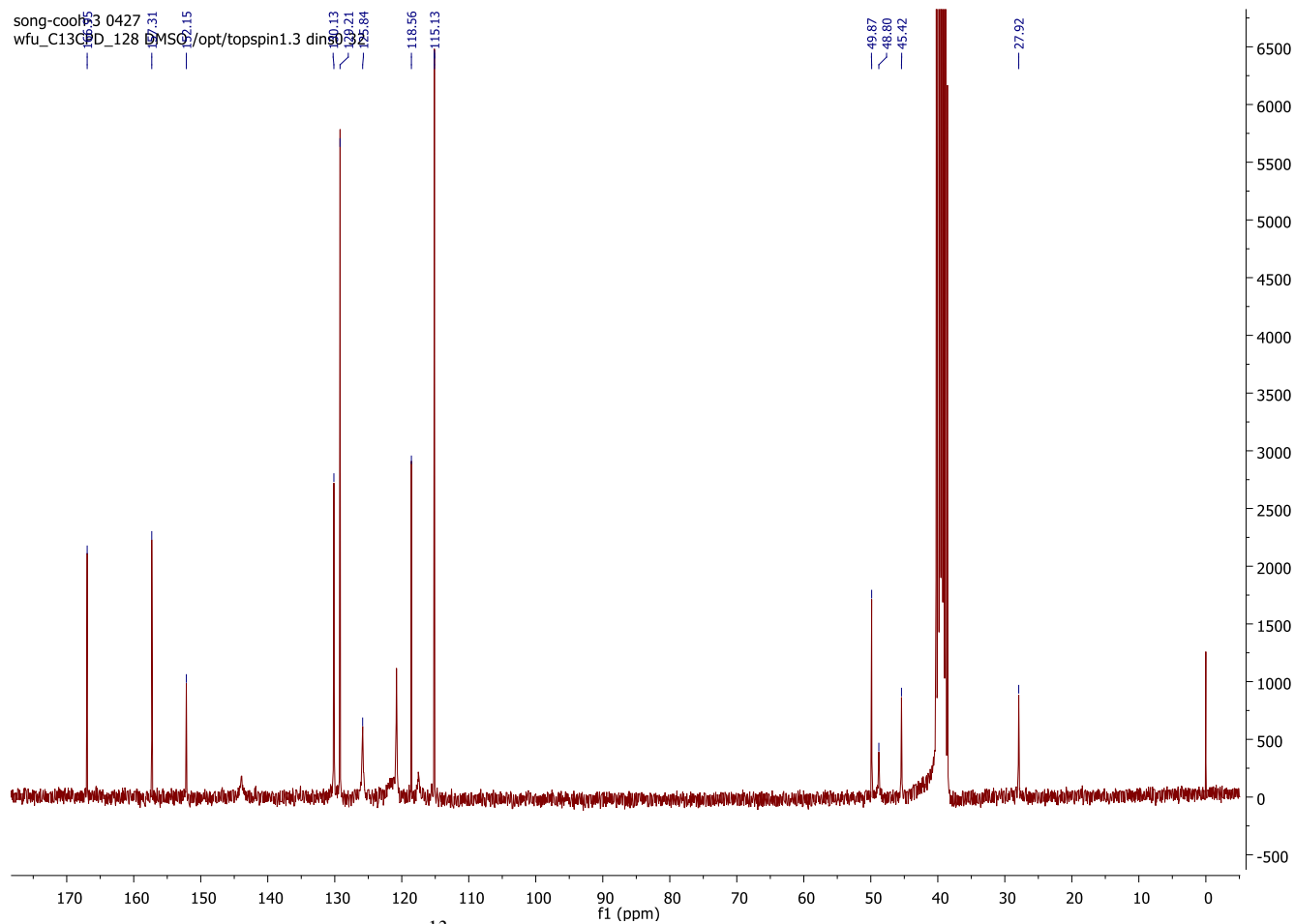


Figure S2.6.  $^{13}\text{C}$  NMR spectrum of compound S7 in  $\text{D}_2\text{O}$ .

0502 song Acr-Azide-2  
wfu\_PROTON CDCl3 /opt/topspin1.2

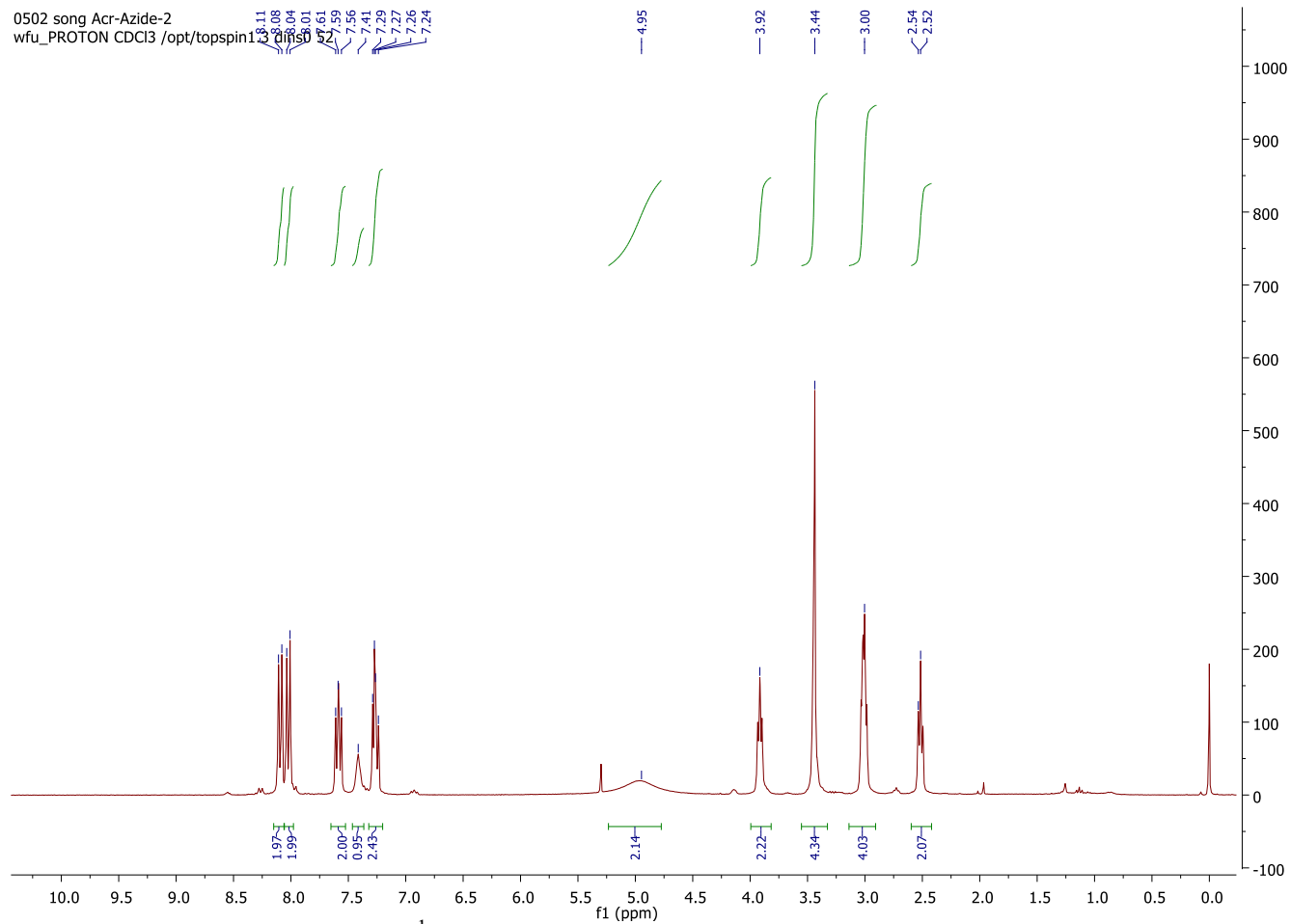
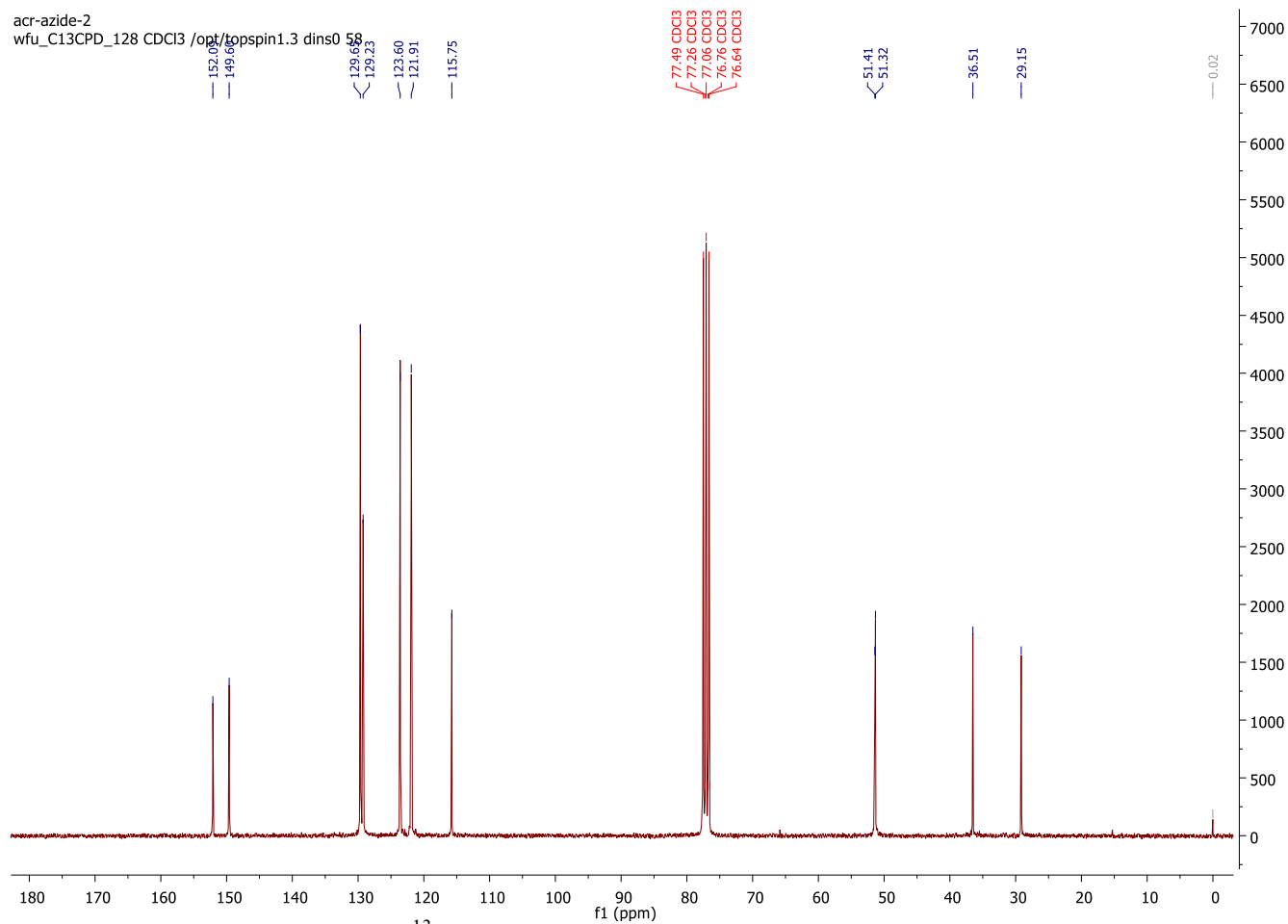
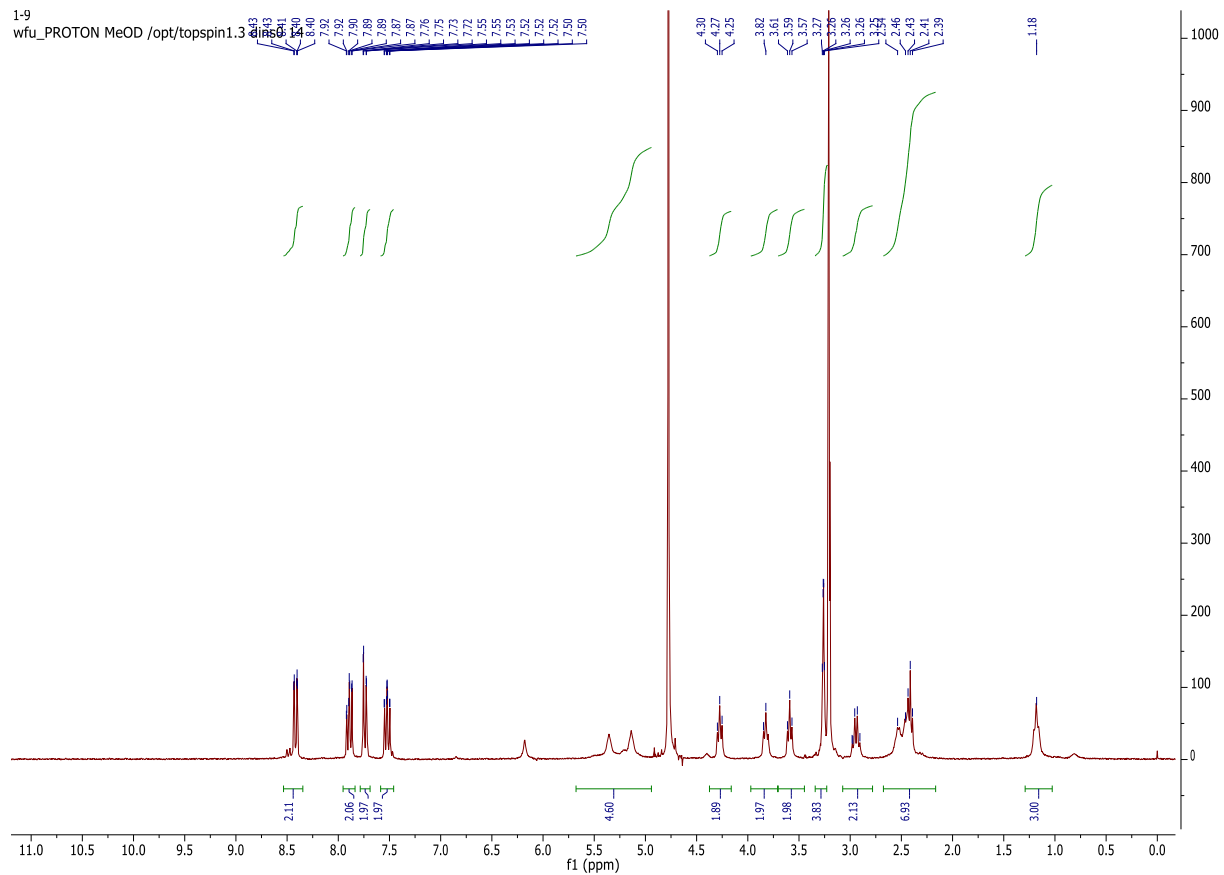


Figure S2.7.  $^1\text{H}$  NMR spectrum of compound S9 in  $\text{CDCl}_3$ .



**Figure S2.8.**  $^{13}\text{C}$  NMR spectrum of compound S9 in  $\text{CDCl}_3$ .



**Figure S2.9.**  $^1\text{H}$  NMR spectrum of compound **2** in MeOD.

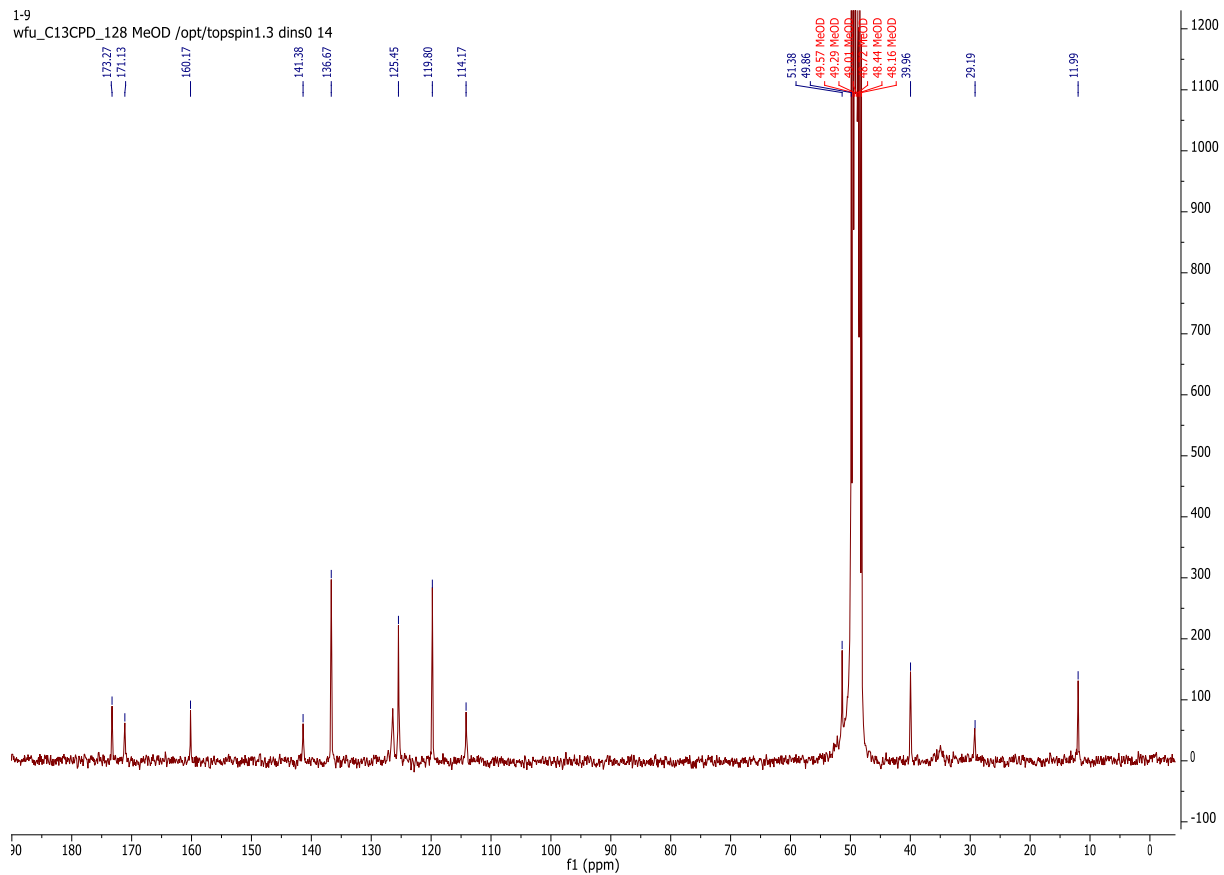
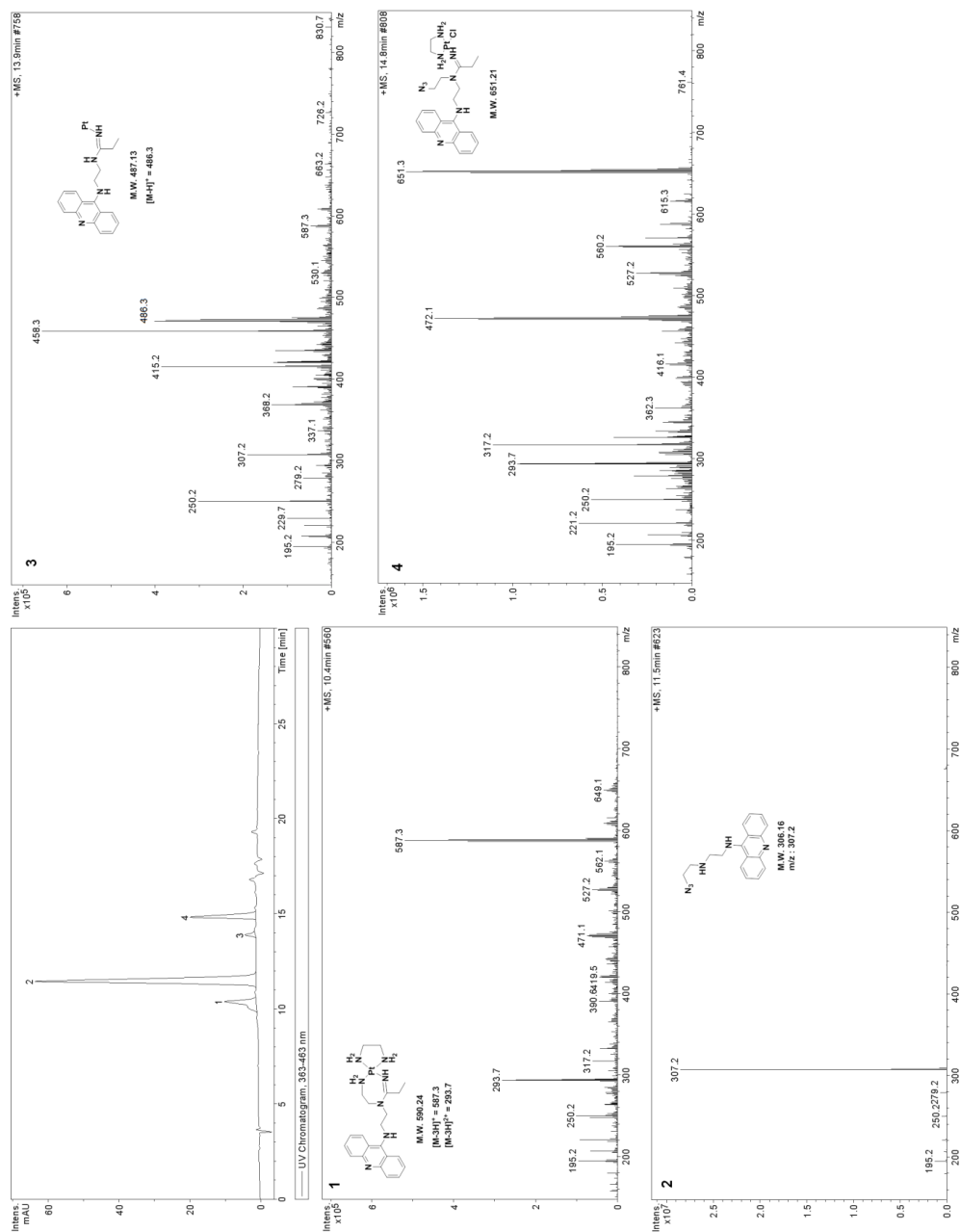
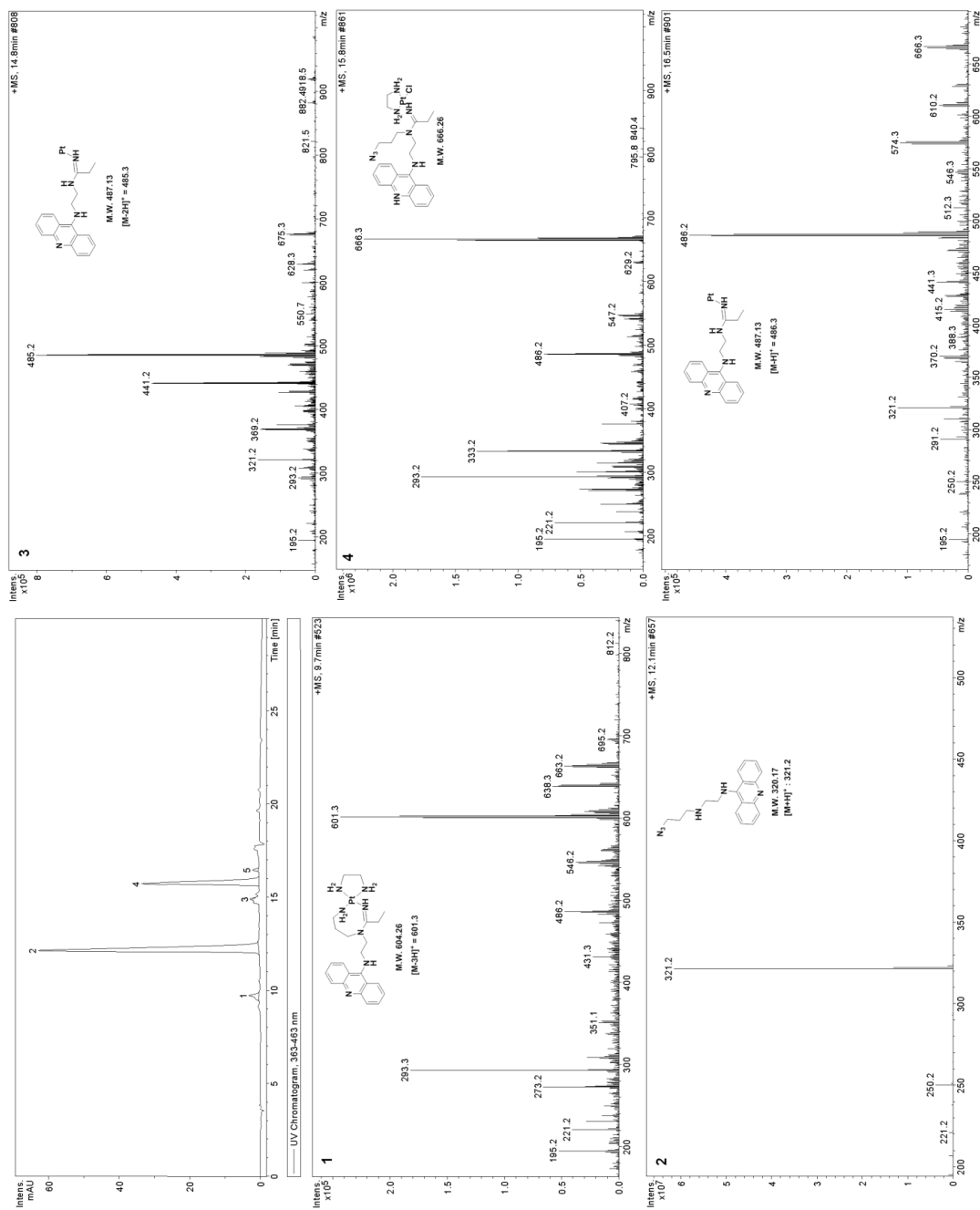


Figure S2.10.  $^{13}\text{C}$  NMR spectrum of compound **2** in MeOD.



### 3. LC-MS analysis of reaction mixtures containing unstable azide-modified platinum-acridines





**Figure S3.2.** LC-ESMS analysis of the reaction mixture between **S5** and **S10** suggesting decomposition of the target compound (peak 4) via loss of  $N_2$  and N-chelate formation (peak 1) along with unidentified platinum-containing products (peaks 3 and 5).

#### 4. In-gel Alexa Fluor 488 fluorescence detection

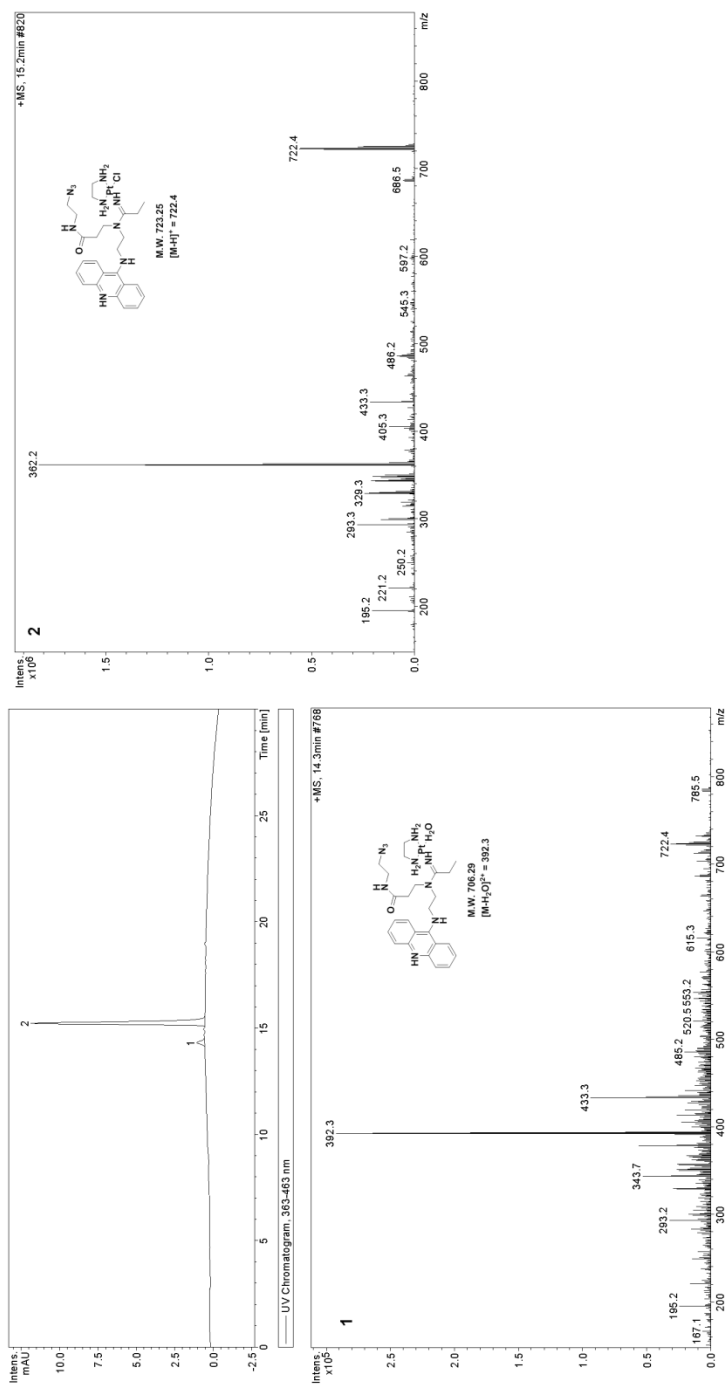
Plasmid DNA (pUC19, 2686 bp) was generated by transforming a chemically competent *E. coli* (NovaBlue, Novagen) with pUC19 and extracted from cells using Qiagen's MegaPrep Plasmid kit (Qiagen, Valencia, CA). The plasmid was linearized with restriction endonuclease BamH I (New England BioLabs, Ipswich, MA) at 37 °C overnight. A small aliquot was removed and analyzed on an agarose gel to confirm complete conversion to the linear form. After heat-inactivation of enzyme at 65 °C, the DNA was purified with the QIAquick PCR Purification kit (Qiagen, Valencia, CA). DNA concentrations and purity were determined spectrophotometrically at 260 nm with  $\epsilon = 6500 \text{ M}^{-1} \cdot \text{cm}^{-1}$  (nucleotides, n.t.). The linearized pUC19 was incubated with compound **2** at drug-to-nucleotide ratios ( $r_b$ ) of 0.2, 0.1, and 0.05 in 5  $\mu\text{L}$  of 10 mM PBS at 37 °C for 24 hours. To the samples were then added 45  $\mu\text{L}$  of click reaction buffer ( $\text{CuSO}_4$ , TBTA (tris-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine), Alexa Fluor 488-alkyne (Invitrogen, Carlsbad, CA) and sodium ascorbate in 10 mM PBS, pH 7.4) to produce the final concentrations listed in Table S.1. (TBTA is used as a chelating agent to stabilize Cu(I) and minimize oxidative degradation of DNA observed in the presence of redox-active copper.<sup>4</sup>) These mixtures were incubated for 3 hours at 37 °C in the dark. To remove the low-molecular-weight compounds in the click reaction mixture, the clicked plasmid DNA were purified by using the MinElute PCR purification columns (Qiagen, Valencia, CA). To further support that the fluorescent dye was ligated with to platinum complexes covalently bound to the DNA, 3 mM NaCN was incubated with the purified Alexa Fluor 488-modified plasmid at 37 °C for 12 hours to remove the platinum complex from DNA, followed by purification on MinElute PCR purification column. The samples and control plasmid were analyzed by agarose gel (1 %) electrophoresis and visualized through in-gel fluorescence using a Typhoon Trio Variable Mode imager (GE Healthcare, Piscataway, NJ). Images were collected with a 488 nm excitation laser and a 520 bp40 emission filter at 600 V PMT. The same gels were further stained with ethidium bromide and documented using the Kodak Electrophoresis Documentation and Analysis System 290 (Rochester, NY).

**Table S1.** Composition of CuAAC Reactions Analyzed by Gel Electrophoresis.

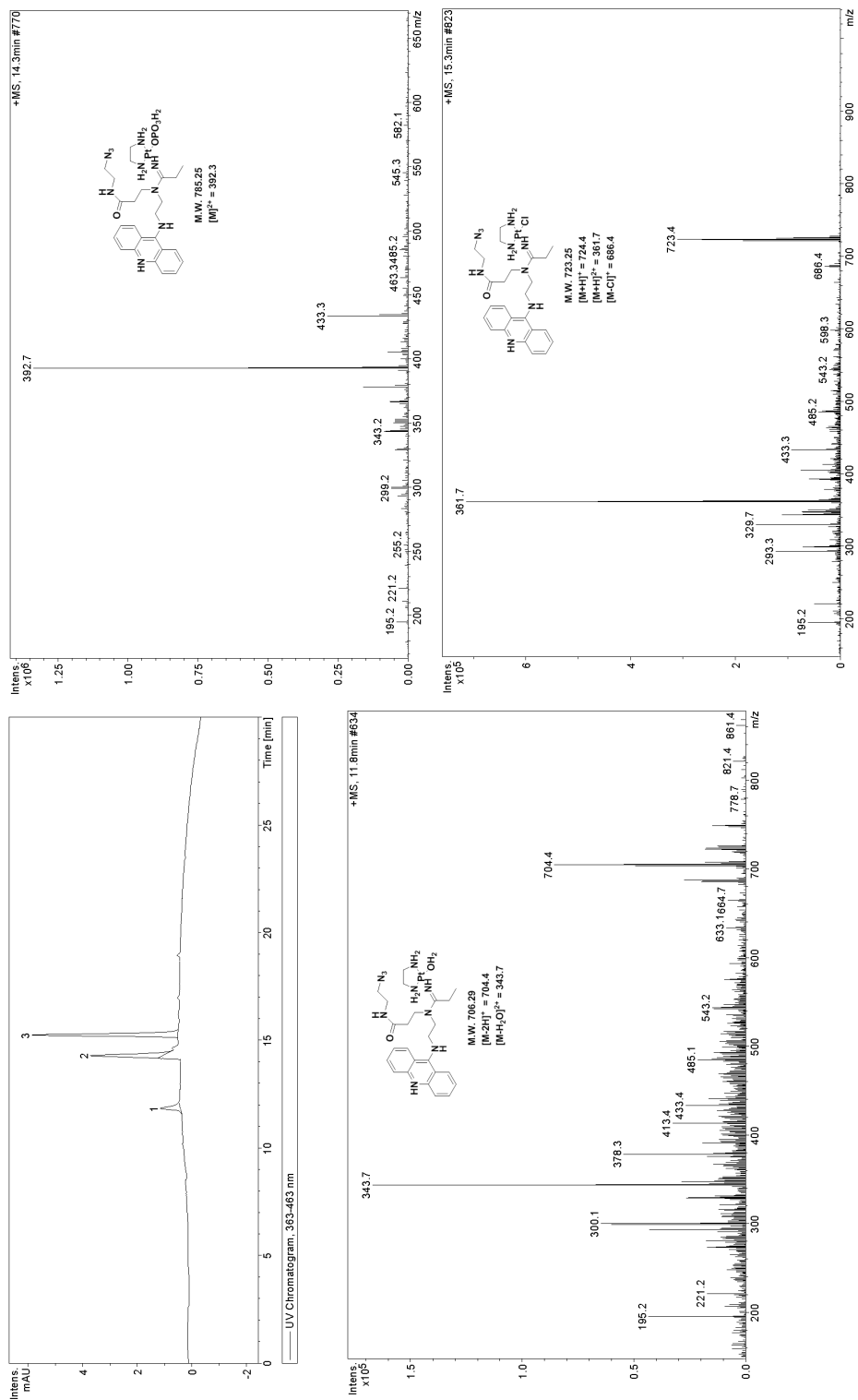
Lane	$r_b$	pUC19 (nucleotides, $\mu\text{M}$ )	<b>2</b> ( $\mu\text{M}$ )	CuSO <sub>4</sub> ( $\mu\text{M}$ )	TBTA ( $\mu\text{M}$ )	Sodium Ascorbate ( $\mu\text{M}$ )	Alexa Fluor 488 ( $\mu\text{M}$ )	NaCN (mM)
1	0	1.82	0	0	0	0	0	0
2	0	1.82	0	0.182	0.182	9.1	0.91	0
3	0.05	1.82	0.091	0.091	0.091	4.55	0.455	0
4	0.1	1.82	0.182	0.182	0.182	9.1	0.91	0
5	0.2	1.82	0.364	0.364	0.364	18.2	1.82	0
6	0.1	1.82	0.182	0.182	0.182	9.1	0.91	3

## 5. Stability of Compound 2 in buffers

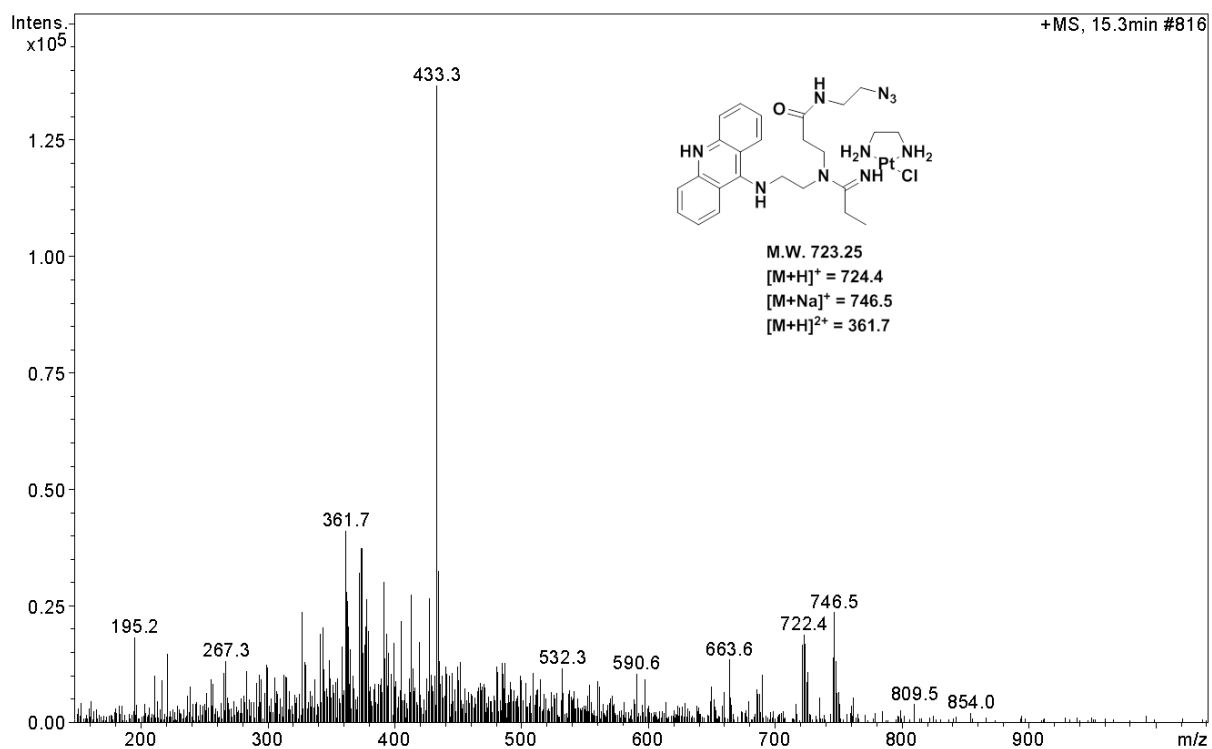
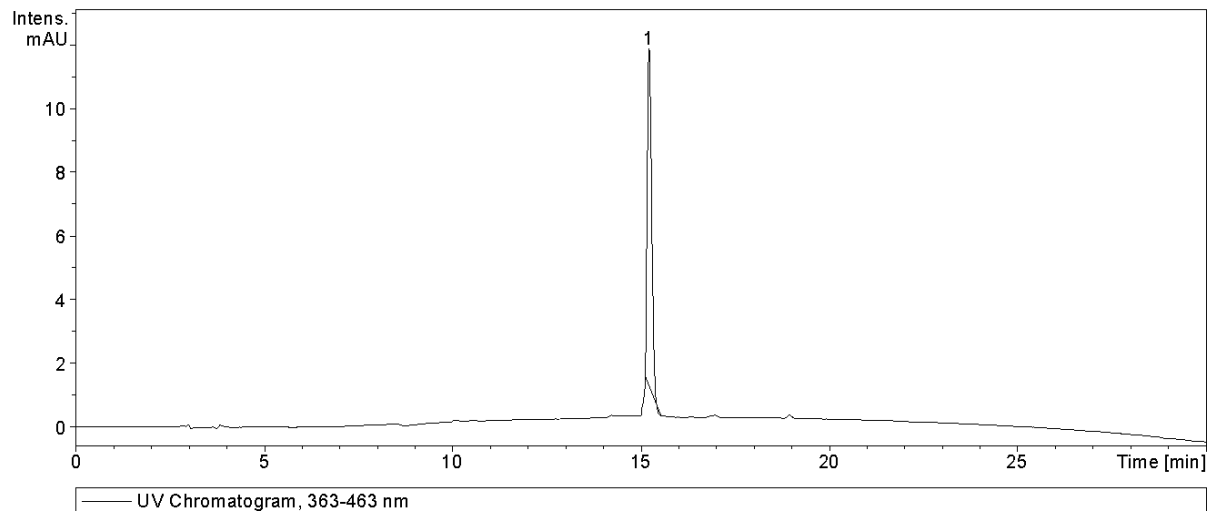
10  $\mu$ M solutions of compound 2 were prepared in the following buffers: 10 mM phosphate-buffered saline (PBS), pH 7.4; 10 mM phosphate (PB), pH 7.4; acetate, pH 5.0. Each sample was heated at 37 °C in the dark for 24 h and then analyzed by LC-ESMS. The chromatographic separations were performed with a 4.6 mm x 150 mm reverse-phase Agilent ZORBAX SB-C18 (5  $\mu$ m) 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 30 minutes and a flow rate of 0.5 mL/min.



**Figure S5.1.** LC-ESMS analysis of the mixture of compound **2** in 10 mM PBS (pH 7.4) after incubation for 24 h at 37 °C, showing minor aquation of the chloro leaving group. The azide group and amide linkage are stable under these conditions.



**Figure S6.2.** LC-ESMS analysis of the mixture of compound 2 in 10 mM PB (pH 7.4) after incubation for 24 h at 37 °C, showing aquation and substitution of chloride by phosphate.



**Figure S6.3.** LC-ESMS analysis of the mixture of compound **2** in 10 mM acetate buffer (pH 5.0) incubating for 24 h at 37 °C.



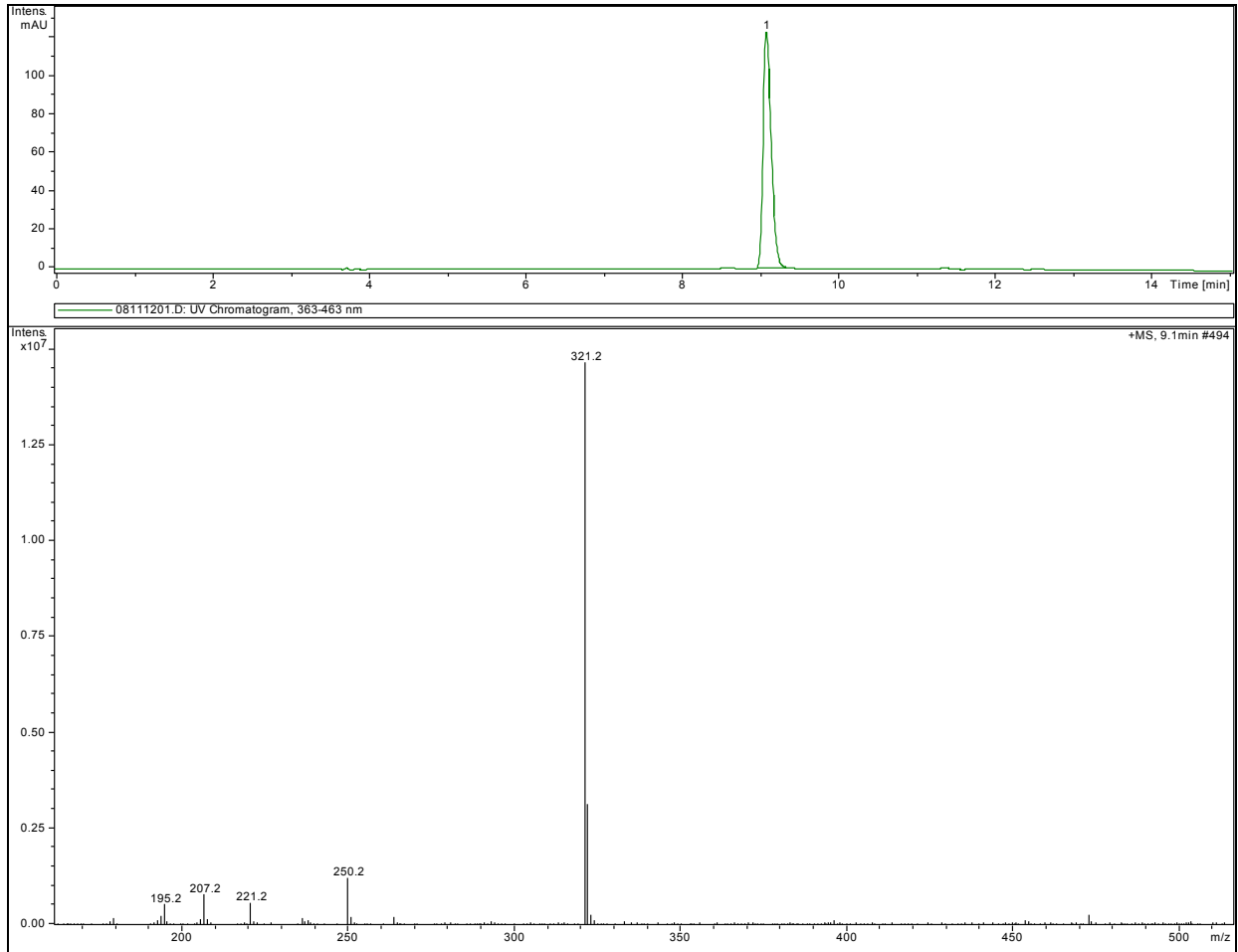
## 6. LC-MS analysis of purified compounds

### Compound Chromatogram Report - MS

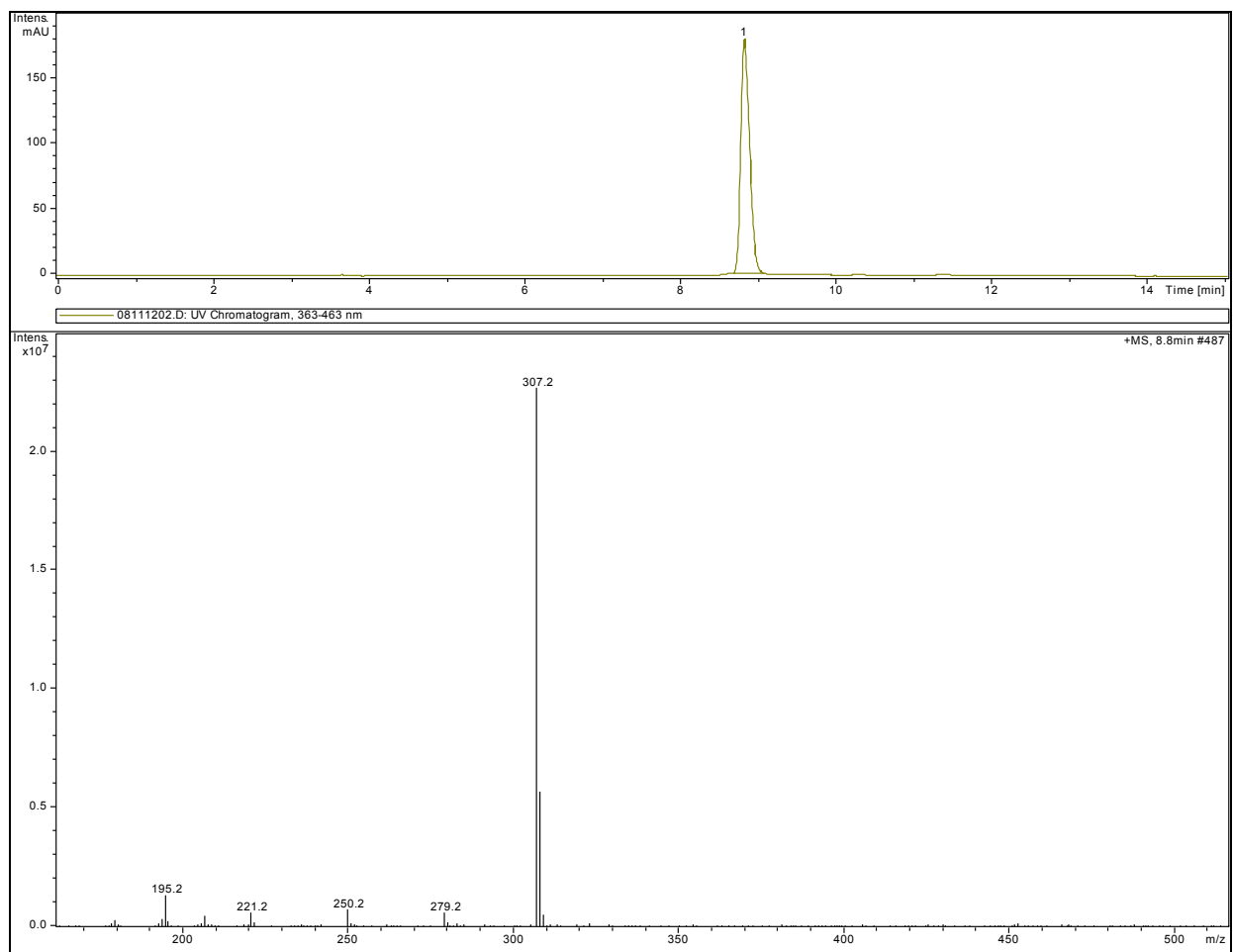
**Analysis Name:** 02091207.D      **Instrument:** LC-MSD-Trap-SL      **Print Date:** 06/30/2012 11:55:45 PM  
**Method:** PTAMID~1.M      **Operator:** Administrator      **Acq. Date:** 2/9/2012 1:57:33 PM  
**Sample Name:** pt-azide  
**Analysis Info:** n=2

#### 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



**Figure S6.1.** LC-ESMS analysis of compound S3.



**Figure S6.2.** LC-ESMS analysis of compound S5.

# Compound Chromatogram Report - MS

**Analysis Name:** 02091207.D    **Instrument:** LC-MSD-Trap-SL    **Print Date:** 01/20/2013 02:27:51 PM  
**Method:** PTAMID~1.M    **Operator:** Administrator    **Acq. Date:** 2/9/2012 1:57:33 PM  
**Sample Name:** pt-azide  
**Analysis Info:** n=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	13.0	12.9 - 13.2	1	4	0.7
2	15.1	14.9 - 15.6	43	546	98.0
3	17.0	16.8 - 17.2	1	7	1.3

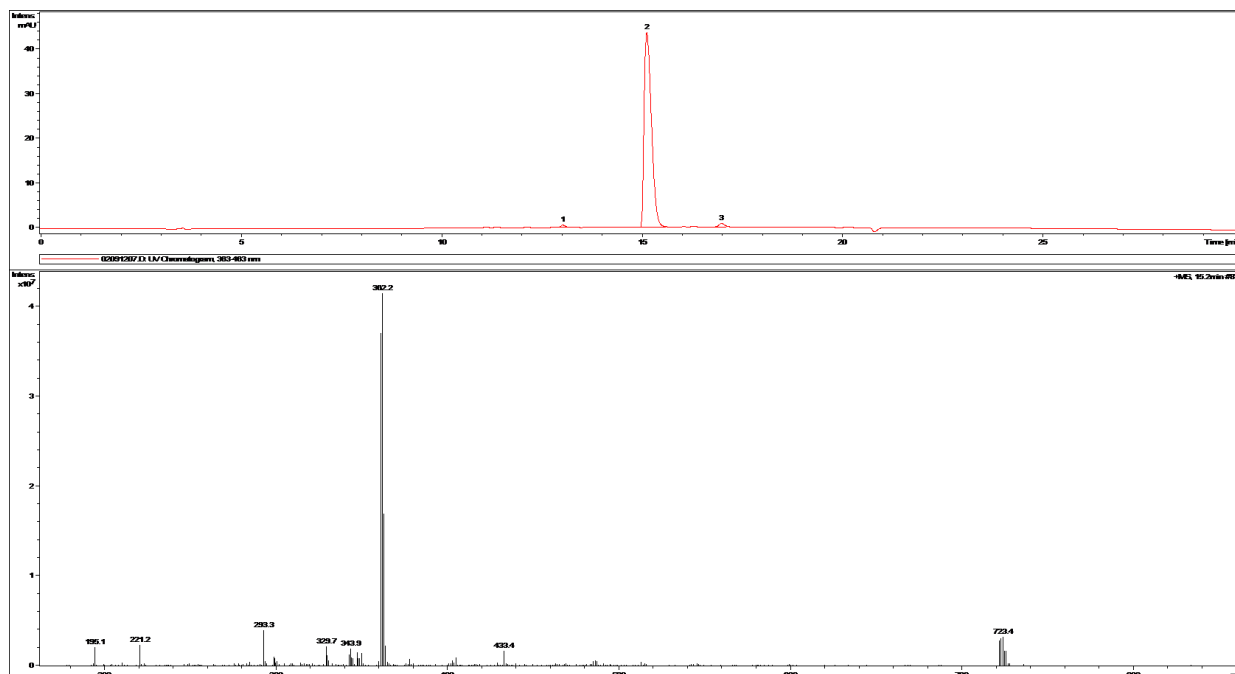
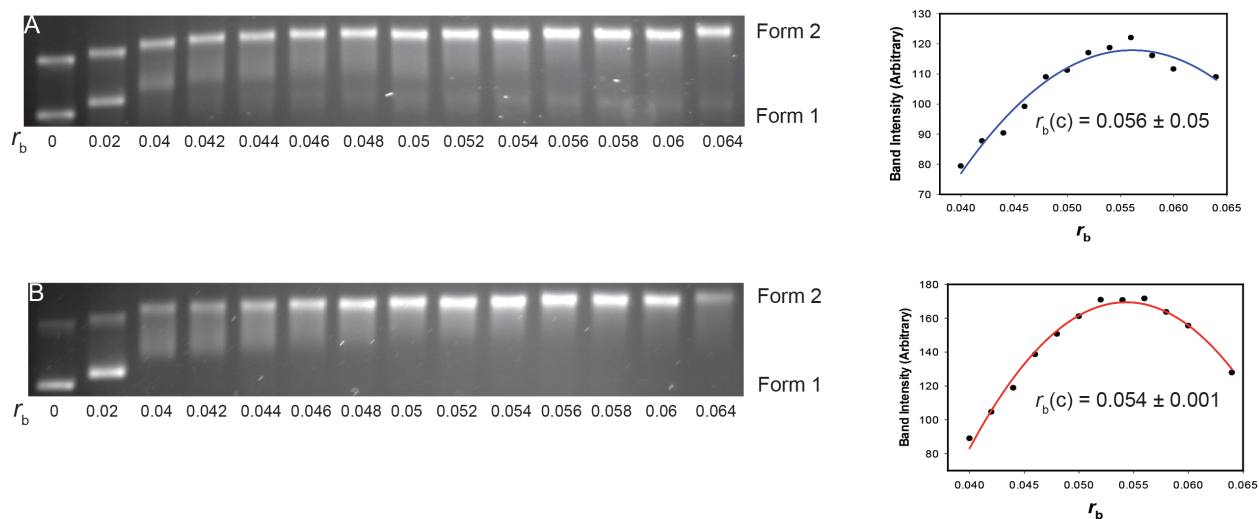


Figure S6.3. LC-ESMS analysis of compound 2 (analytical purity: 98%).

## 7. Plasmid Unwinding Experiments.

Unwinding experiments were performed according to a previously published procedure.<sup>5</sup>



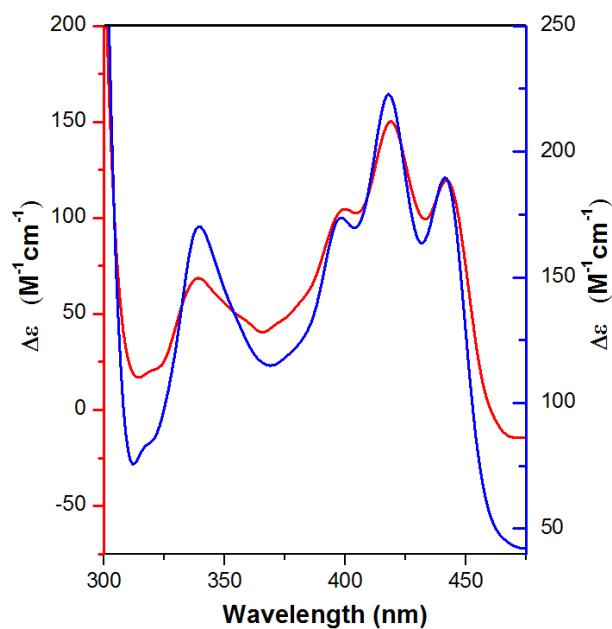
**Figure S7.1.** Gel electrophoresis of platinum–acridine-modified, negatively supercoiled plasmid DNA. pUC19 modified with compound **2** (A) with compound **1** (B). Each gel is shown with the corresponding plot of relaxed DNA band intensities vs.  $r_b$ . The unwinding angles ( $\phi$ ) calculated from  $\phi = 18\sigma/r_b(c)$ , where  $\sigma$  is the superhelical density of the plasmid ( $-0.050$ ) and  $r_b(c)$  is the maximum of the quadratic polynomial curve fits, for compounds **1** and **2** are  $17 \pm 1^\circ$  and  $16 \pm 1^\circ$ , respectively.

## 8. CD experiments and molecular modeling

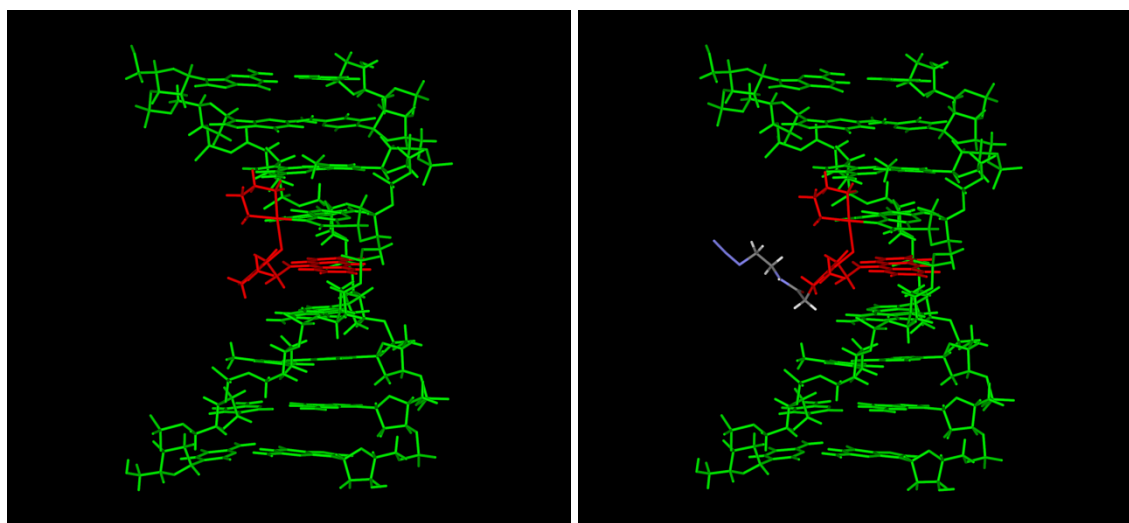
Solutions of calf thymus DNA (lyophilized powder, Sigma, St. Louis, MO) were prepared in 10 mM phosphate buffer (pH 7.4). The DNA samples were annealed by slow cooling from 90 °C to room temperature, and the final concentration was determined spectrophotometrically at 260 nm with  $\epsilon = 6500 \text{ M}^{-1} \cdot \text{cm}^{-1}$  (nucleotides, n.t.).

To modify the DNA with platinum agent, calf thymus DNA (1 mM, n.t.) was incubated in phosphate buffer (10 mM, pH 7.4) overnight at 37 °C with **1** and **2** at a platinum-to-nucleotide ratio ( $r_b$ ) of 0.05. To perform the click reaction, calf thymus DNA (1 mM n.t.) was incubated with platinum complex **2** with  $r_b$  0.05 at 37 °C overnight. The sample was then incubated with click reaction buffer at 37 °C for 2 hour with the following final concentrations:  $\text{CuSO}_4$  (50  $\mu\text{M}$ ), TBTA (50  $\mu\text{M}$ ), Alexa Fluor 488 Alkyne (50  $\mu\text{M}$ ) and sodium ascorbate (500  $\mu\text{M}$ ).

CD experiments were performed on an AVIV Model 215 spectrophotometer equipped with a thermoelectrically-controlled cell-holder. All the experiments were performed in quartz cells with 0.5 cm path length. Spectra were recorded in the range 200-600 nm at 25 °C in 1-nm increments with an averaging time of 1 s. CD profiles were base-line adjusted by subtracting buffer background.



**Figure S8.1.** Circular dichroism (CD) spectra (ICD region) of calf thymus DNA modified with platinum–acridines **1** (blue trace) and **2** (red trace). CD spectra were recorded for  $\sim 1$  mM (n.t) DNA in 10 mM phosphate buffer (25 °C, pH 7.4) at a drug-to-nucleotide ratio ( $r_b$ ) of 0.05.



**Figure S8.2.** Molecular models (AMBER) of the monofunctional–intercalative adducts at guanine–N7 in the DNA major groove formed by compound **1** (left) and compound **2** (right) based on the NMR solution structure of the hybrid adduct (PDB 1XRW). The platinum–acridine and the newly installed azide linker are shown in red and element-specific colors, respectively.

## 9. Cell culture maintenance

The human non-small cell lung cancer cell line, NCI-H460, was obtained from the American Type Culture Collection (Rockville, MD, USA) and was cultured in RPMI-1640 media (HyClone) containing 4.5 g·L<sup>-1</sup> glucose, 1.5 g·L<sup>-1</sup> sodium bicarbonate, 10 mM HEPES, and 110 mg·L<sup>-1</sup> sodium pyruvate supplemented with 10% fetal bovine serum (FBS), 10% penstrep (P&S), and 10% L-glutamine. Cells were incubated at a constant temperature at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub> and were subcultured every 2 to 3 days in order to maintain cells in logarithmic growth.

## 10. Cell treatment and Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) ligation

NCI-H460 cells were seeded into poly-D-lysine coated glass bottom Petri dishes (MatTeck Corporation, Ashland, MD, USA) with 10<sup>5</sup> cells·mL<sup>-1</sup> suspended in 2 mL of medium per dish. Cells were incubated overnight and then treated with compound **2** (5 μM) (or media for controls) for 3 h. After rinsing with chilled PBS (3×), cells were fixed by treatment with 3.7% formaldehyde solution (in PBS, pH 7.4) for 15 min at room temperature and subsequently washed with a 3% solution of bovine serum albumin (BSA; Sigma) in PBS (pH 7.4) twice for 10 min. For colocalization experiments using mitochondrial stain, cells were treated with 100 nM MitoTracker Deep Red TM (Invitrogen) in 2 mL pre-warmed phenol red-free media at 37 °C for 30 min prior to fixing them using the abovementioned procedure. Cells were then permeabilized by treatment with 0.5% Triton X-100 (in PBS, pH 7.4) at room temperature for 20 min. Permeabilization buffer was quenched with 3% BSA in PBS (2 × 10 min) and the cells were incubated with 250 μL of click reaction mixture (1 mM CuSO<sub>4</sub>; 0.5 μM Alexa Fluor 488-alkyne (Invitrogen); 10 mM sodium ascorbate; 50 mM Tris-HCl, pH 7.3) at room temperature for 30 min. For the no-copper control, the copper solution in the click buffer was replaced with water. Before co-staining with organelle-specific dyes, cells were subjected to extensive washes with gentle agitation: 1) 3% BSA in PBS (5 min); 2) 0.5% Triton X-100 in PBS (2 × 10 min); 3) PBS

for (3 × 10 min). Nuclei were stained by 5 µg·mL<sup>-1</sup> Hoechst 33342 (Sigma) in PBS for 5 min. Three final PBS washes were performed immediately prior to image capture.

## 11. Confocal microscopy and fluorescence intensity analysis

Images were collected using a Zeiss LSM 710 confocal microscope (Carl Zeiss MicroImaging, Thornwood, NY) using either a 40x (PLAN APO, 0.95 NA) or a 63x (PLAN APO, 1.2 NA) objective lens. All images were acquired in multi-track configuration mode to minimize excitation cross talk and emission bleed-through. We utilized a 405 nm laser line (for Hoechst 33342) with an emission range of 424–466 nm, a 488 nm laser line (for Alexa Fluor 488) with an emission range of 489–553 nm, and a 633 nm laser line (for MitoTracker Deep Red TM) with an emission range of 645–740 nm. For comparative fluorescence intensity analysis, great care was taken to equalize excitation power, pinhole settings, PMT gain, and offset values across and within imaging sessions for each respective channel. For all images, the pinhole value was kept at or below 1.2 airy units, and images were acquired with 8x line averaging at 1024 x 1024 pixels. Images were collected at 12-bit sampling to provide a wide dynamic intensity range for analysis (0–4096). Zen software was used for image acquisition.

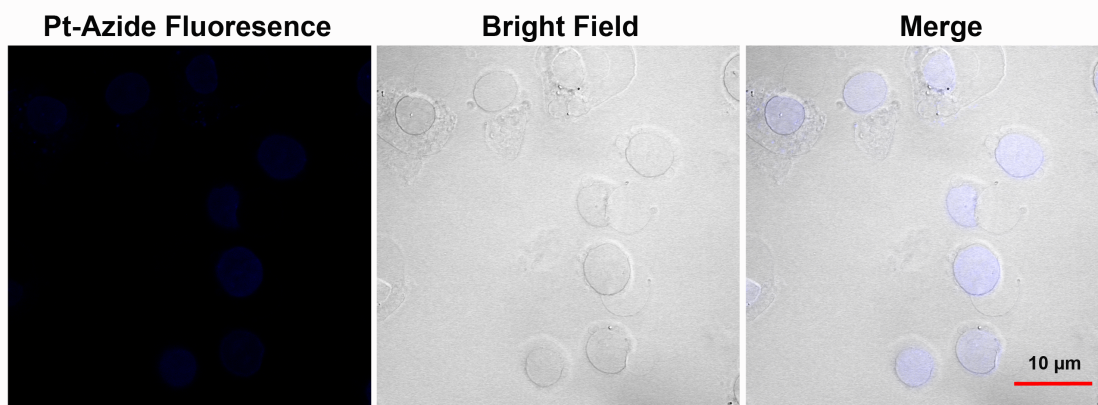
The intensity of platinum–azide-related Alexa Fluor 488 fluorescence in nuclei and nucleoli was compared quantitatively using Zen software. The fluorescence signals from the nuclei of labeled cells were measured by drawing a region of interest (ROI) around each nucleus, with the Hoechst 33342 image used to verify nucleus location. The fluorescence intensity of each object,  $F_{obj}$ , was calculated using the following formula:

$$F_{obj} = I_{ROI} - I_{bkgd},$$

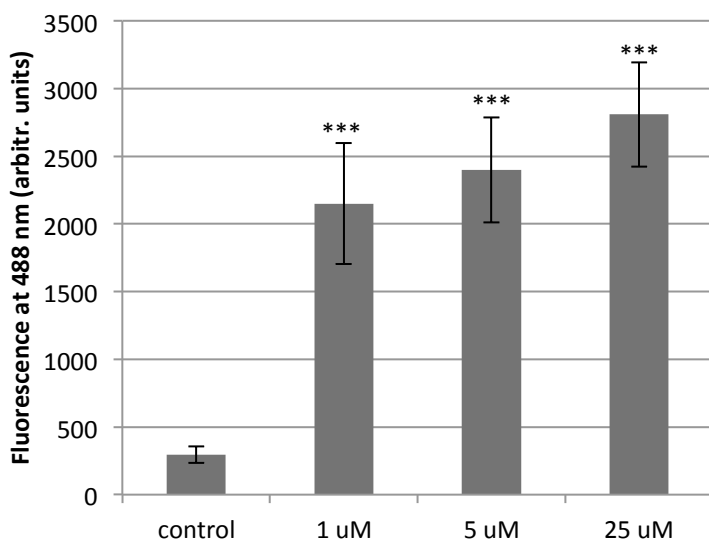
where  $I_{ROI}$  and  $I_{bkgd}$  are the averaged signal intensities from all the pixels in the ROI and background, respectively.<sup>6,7</sup> Background was determined by drawing an ROI of the same size in an empty region of the image. The fluorescence intensity in the nucleoli was determined in the same manner. A total of 40–50 cells in 8 different fields were analyzed in this manner for each treatment (cell counts were analyzed by one-way ANOVA, indicating there was no statistical difference between fields;  $P = 0.54$ ). The relative enhancement of Pt-Alexa Fluor 488



fluorescence in the nucleoli was determined from the ratio of intensities in nucleoli and in the nuclei for each group. Statistical analysis was done using one-way ANOVA (GraphPad Prism).  $P < 0.05$  was considered significant.

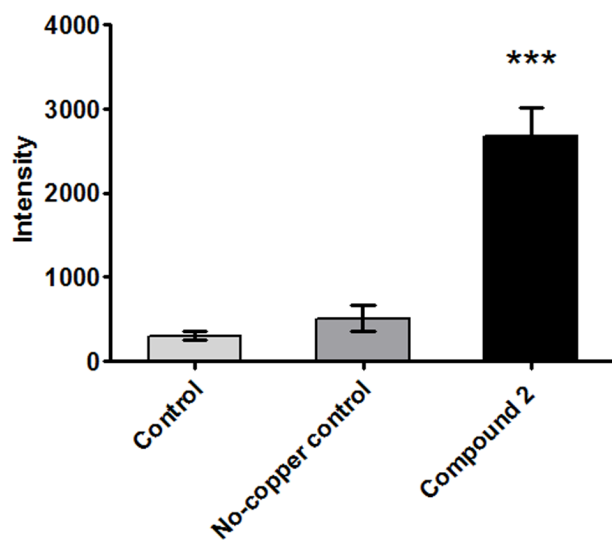


**Figure S11.1.** Acridine fluorescence in NCI-H460 cells (excitation wavelength: 405 nm, emission wavelength range: 414-470 nm) after treatment with 5 µM compound **2** (“Pt-Azide”) for 3 h. Close inspection of the merged-channel panel shows that weak blue fluorescence of the acridine chromophore is localized to the nuclei of the cells. No blue fluorescence is observed outside the nuclei, suggesting that other cellular organelles are not targeted by the hybrid agents.

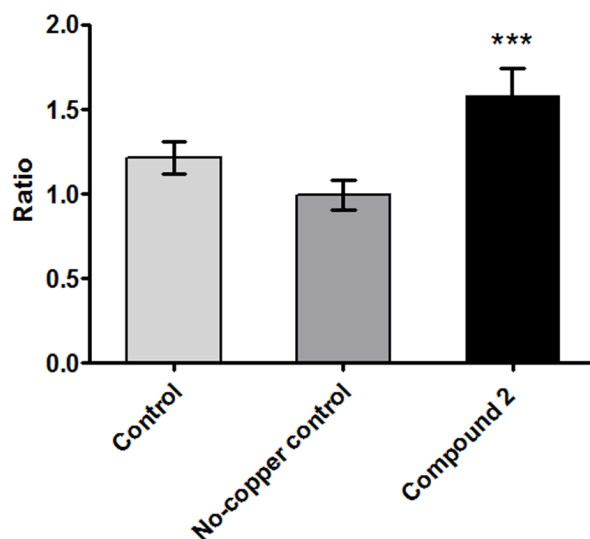


**Figure S11.2.** Total background-corrected fluorescent intensities in nuclei of cells treated with compound **2** (1, 5, 25 µM) and in untreated cells. Plotted data are the mean fluorescence

intensities ( $\pm$  S.D.) determined for  $n > 50$  cells. The data was analyzed with one-way ANOVA ( $***P < 0.001$ ;  $P < 0.05$  was considered significant).



(a)



(b)

**Figure S11.3.** (a) Background-corrected fluorescent intensities in nuclei of cells treated with compound **2** ( $5 \mu\text{M}$ ) and in untreated cells. (b) Nucleolus-to-nucleus fluorescence intensity ratios ( $F_{\text{nucleolus}}/F_{\text{nucleus}}$ ) in cells treated with compound **2** ( $5 \mu\text{M}$ ) and in untreated cells. Plotted data are the mean fluorescence intensities ( $\pm$  S.D.) determined for  $n = 40\text{--}50$  cells. The data was analyzed with one-way ANOVA ( $***P < 0.001$ ;  $P < 0.05$  was considered significant).

## 12. References

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