

Electronic Supplementary Information for

Convenient Detection of Metal-DNA, Metal-RNA, and Metal-Protein Adducts With a Click-Modified Pt(II) Complex

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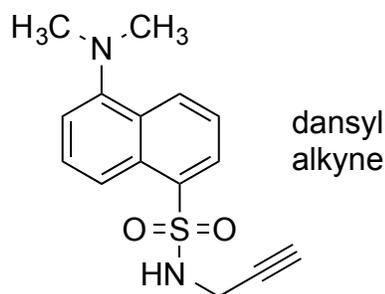
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Experimental Details

General Comments. Materials were purchased from commercial vendors and used without further purification. Anhydrous N,N'-dimethylformamide (DMF) was purchased from EMD Millipore (DriSolv). 1,3-Propanediamine was purchased from TCI America, KI and KCl was purchased from EMD, and AgNO₃ was purchased from Strem Chemicals, Inc. K₂PtCl₄ was purchased from Sigma Aldrich and deuterated solvents were purchased from Cambridge Isotope Laboratories. ¹H, ¹³C, and ¹⁹⁵Pt NMR spectra were recorded in d₇-DMF using a Varian Mercury 300 MHz (¹H: 300.09 MHz) or Bruker Advance III HD 600 MHz (¹H: 600.02 MHz, ¹³C: 150.87 MHz, ¹⁹⁵Pt: 128.99 MHz) NMR spectrometer with a Prodigy multinuclear broadband cryoprobe. Chemical shifts (δ) are expressed in ppm relative to the residual DMF (¹H: 8.03 ppm, ¹³C: 29.76) or the external reference K₂PtCl₄ (¹⁹⁵Pt: -1604 ppm). Gels were visualized with methylene blue stain and UV exposure (AlphaImager HP System). All DNA substrates were purchased directly from Integrated DNA Technologies, Inc., dissolved in nanopure ddH₂O to a final stock concentration of 1 mM, and used directly without further purification.

cis-[Pt(2-azido-1,3-propanediamine)Cl₂] (4). The azide-containing **4** was prepared according to the method of Urankar et al. (reference 8b in manuscript).

cis-[Pt(1,3-propanediamine)Cl₂] (5). The azide-free **5** was prepared following the method described by Dhara for cisplatin (S. C. Dhara, *Ind. J. Chem.*, 1970, **8**, 193-194). Briefly, to a clear-red solution of K₂PtCl₄ (0.54 g, 1.31 mmol) in H₂O (15 mL) was added KI (1.31 g, 7.87 mmol) in H₂O (5 mL) and the reaction was stirred in the dark for 20 min. 1,3-Propanediamine (0.01 g, 1.32 mmol) in H₂O (10 mL) was added dropwise over ca. 15 min. and stirred for another 2 h in the dark. The resulting yellow precipitate was then filtered and washed thoroughly with H₂O and dried in a desiccator. All of the yellow solid (0.61 g, 1.17 mmol) was suspended in H₂O (8 mL) to which AgNO₃ (0.38 g, 2.22 mmol) in H₂O (2 mL) was added at once. The mixture was stirred in the dark overnight (16.5 h). The opaque-tan mixture was filtered through a 0.2 μm syringe filter to reveal a clear-yellow filtrate. One drop of 1 M HCl was added to the filtrate, followed by 1.01 g KCl. The mixture was allowed to sit for 2 h in the dark at rt, after which a yellow solid was filtered from a clear-yellow filtrate. The yellow solid was rinsed with H₂O and dried in a desiccator to furnish the desired product (0.28 g, 0.83 mmol, 63%). ¹H NMR (300 MHz, d₇-DMF): δ 4.99 (Pt-NH₂-, s, 4H, ³J_{Pt-H} = ~60 Hz), 2.88-2.60 (Pt-NH₂-CH₂-, m, 4H, obscured by residual DMF solvent peak), 1.78 (CH₂-CH₂-, m, 1H); ¹³C NMR (151 MHz, d₇-DMF): δ 43.2 (-NH₂-CH₂-), 28.3 (-CH₂-CH₂-); ¹⁹⁵Pt NMR (129 MHz, d₇-DMF): δ -2256 (Pt). HRMS (ESI) m/z calculated for C₃H₁₁N₂Cl₂Pt, 339.9947 (M⁺+H); found, 339.9932.



5-(Dimethylamino)-N-(2-propynyl)-1-naphthalenesulfonamide (dansyl alkyne fluorophore). The dansyl alkyne was synthesized according to previously published methods (F. Bolletta, D. Fabbri, M. Lombardo, L. Prodi, C. Trombini and N. Zaccheroni, *Organometallics*, 1996, **15**, 2415-2417).

Platinum Activation (aquaation). Picazoplatin (**1**), *cis*-[Pt(2-azido-1,3-propanediamine)Cl₂] (**4**), or *cis*-[Pt(1,3-propanediamine)Cl₂] (**5**) (5 μmol) was added to a solution of AgNO₃ in ddH₂O (10 mM, 1 mL). The solution was incubated at 50 °C for 4 to 18 h with stirring, at which time AgI precipitated as a white solid and was separated by centrifugation (10,000 rpm for 10 min). The resulting supernatant was removed and used for DNA and RNA binding studies. Solutions were stored in the dark at 4 °C and used for up to one week.

Platinum–DNA Binding (HPLC analysis). The DNA duplex (280 μM each of 5'-T₅GGT₆-3' and 5'-A₆CCA₅-3', typically 28 nmol) or the DNA hairpin (280 μM of 5'-TATGGTATTTTTATAACCATA-3', typically 28 nmol) was folded by rapid heating to 90 °C and slow cooling to 4 °C in 10 mM Na₂PO₄ (pH 7.1), 0.1 M NaNO₃, and 10 mM Mg(NO₃)₂. An activated solution of **4** or **5** was added in equimolar proportions (for HPLC) or two-fold excess (for click reactions) and the solution was incubated at 37 °C for approx. 16 h. Pt-bound DNA was purified with Sephadex G-25 Medium size exclusion resin (GE Healthcare) on laboratory-prepared spin columns (BioRad) to remove unbound Pt.

Platinum–DNA Binding (PAGE analysis). The DNA duplex (280 μM each of 5'-T₅GGT₆-3' and 5'-A₆CCA₅-3', typically 28 nmol) was folded by rapid heating to 90 °C and slow cooling to 4°C in 10 mM Na₂HPO₄/NaH₂PO₄ buffer (pH 7.1), 0.1 M NaNO₃, and 10 mM Mg(NO₃)₂. An activated solution of **1** or **4** was added in twofold excess and the solution was incubated at 37 °C for 16 h. Pt-bound DNA was purified with Sephadex G-25 Medium size exclusion resin (GE Healthcare) on laboratory-prepared spin columns (BioRad) to remove unbound platinum.

Platinum–DNA Click Reactions. Sodium ascorbate (18 μL, 10 mM), CuI (18 μL, 10 mM), dansyl alkyne (10 μL, 2mM), and Et₃N (10 μL, 7.2 M) were added to a solution of water and acetonitrile (98:2 v/v, respectively) containing Pt-bound DNA (18 μL, 180 μM). The samples were incubated at 50 °C for 30 min to 4 h. The samples were then purified with

Sephadex G-25 Medium spin columns. The samples were dried *in vacuo* and then re-suspended in a 3:2 v/v mixture of ddH₂O and formamide, respectively, for dPAGE analysis. Alternatively, the dried samples were dissolved in ddH₂O for HPLC analysis.

HPLC Conditions. DNA was separated using reverse-phase HPLC (Akta purifier; Amersham Biosciences) on a C18 column (Hypersil GOLD, 5 mm 4.6/250 mm; Thermo Scientific). A mobile phase of 10 mM triethylammonium acetate (TEAA) and 80% acetonitrile/20% 10 mM TEAA were used. A multistep gradient was used that provided baseline separation of major peaks.

Molecular Modeling. DFT calculations were performed using a model from an NMR solution structure of cisplatin-bound DNA (pdb: 3LPV, reference 9b in manuscript) in Spartan '10 (Wavefunction Inc.) Version 1.1.0. The ammine ligands of cisplatin were replaced with 2-Azidopropane-1,3-diamine, and the bond angles and bond distances resulting from **4** bound to the two guanine residues were restricted (see below). The DNA atoms were held static while the equilibrium geometry of Pt was calculated using molecular mechanics (MMFF). The structures were then imported into PyMOL for visualization.

Bovine Serum Albumin Platination and Click Reaction Conditions. BSA (50 μM) was incubated with 0, 50, 100, or 250 μM **4** in 10 mM Na₂HPO₄/NaH₂PO₄ buffer (pH 7.0), 10 mM NaNO₃, and 100 μM Mg(NO₃)₂ at 37 °C for 16 h. Pt-bound BSA was purified with Sephadex G-25 Medium size exclusion resin (GE Healthcare) on laboratory-prepared spin columns (BioRad) to remove unbound Pt. To perform the click reaction, 100 μM dansyl-alkyne fluorophore, 100 μM CuSO₄, and 2 mM sodium ascorbate was added to platinated BSA and allowed to react at 37 °C for 1 h. Free fluorophore was removed using Sephadex G-25 spin-columns. Purified protein was then analyzed by 10% SDS-PAGE.

***S. cerevisiae* Treatment with **4**.** Liquid cultures of *S. cerevisiae* strain BY4741 (MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0), a gift from the Tom Stevens laboratory at the University of Oregon, were grown at 30 °C with shaking at 200 rpm in synthetic dextrose medium (0.67% yeast nitrogen base and 2% glucose supplemented with amino acids and nucleotide bases). Overnight cultures were diluted to OD₆₀₀ ~0.25 in synthetic dextrose medium supplemented with 0 or 250 μM 2-ADAP Pt. Cells were grown at 30 °C for 6 h with aeration, harvested by centrifugation at 4000 rpm at 4 °C, resuspended in fresh medium, and returned to shaker at 30 °C. At 0, 40, 80, and 120 min post treatment, aliquots were harvested by centrifugation at 4000 rpm, supernatant was discarded, and pellets were stored at -20 °C. Total RNA was extracted with the MasterPure Yeast RNA Purification Kit (Epicentre) according to a modified manufacturer's protocol. RNA concentration was calculated using absorbance at 260 nm. For the click reaction, 8-14 μg of total RNA (normalized to OD₆₀₀ of initial culture at timepoint collected) was incubated overnight at 37 °C in a 10 μL reaction mixture containing 20 U RiboGuard RNase Inhibitor and 0.25 mM Alexa Fluor 488 DIBO. Unbound fluorophore was removed using an RNeasy Mini Kit (Qiagen) according to a modified manufacturer's protocol.

Samples were diluted 2-fold in formamide and analyzed on an 8% (29:1) mono/bis polyacrylamide gel. Fluorescence signal intensities from Fig. 6, normalized to RNA (methylene blue stain) and culture OD, are shown below indicating significant retention of Pt-rRNA adducts after 2 h in 4-free media.

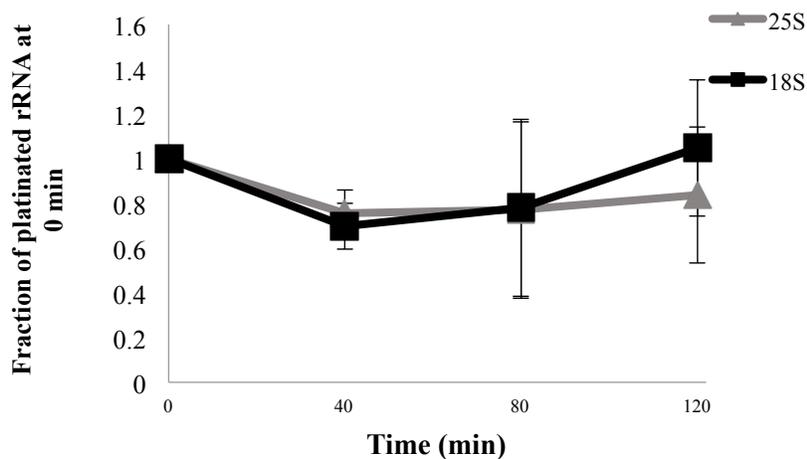


Figure S1. Normalized Pt-rRNA signal over time. Fluorescence signal from click reaction was normalized to total loaded RNA (methylene blue stain) and OD₆₀₀ of initial culture at timepoint collected.

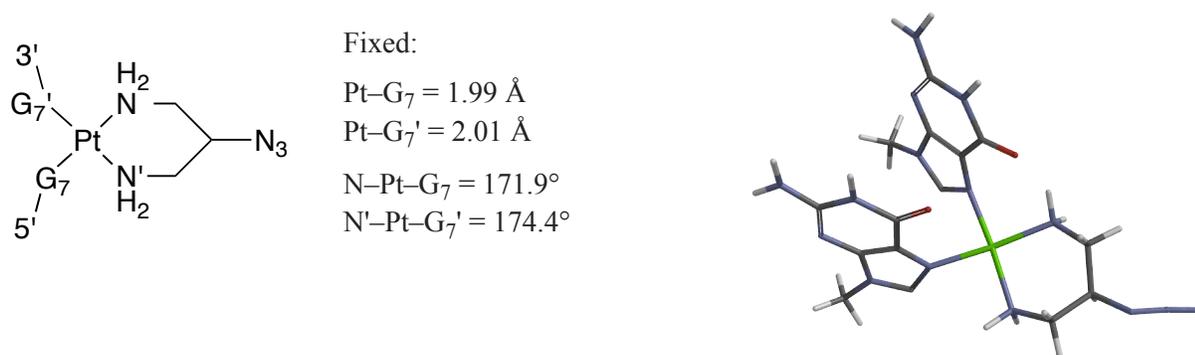
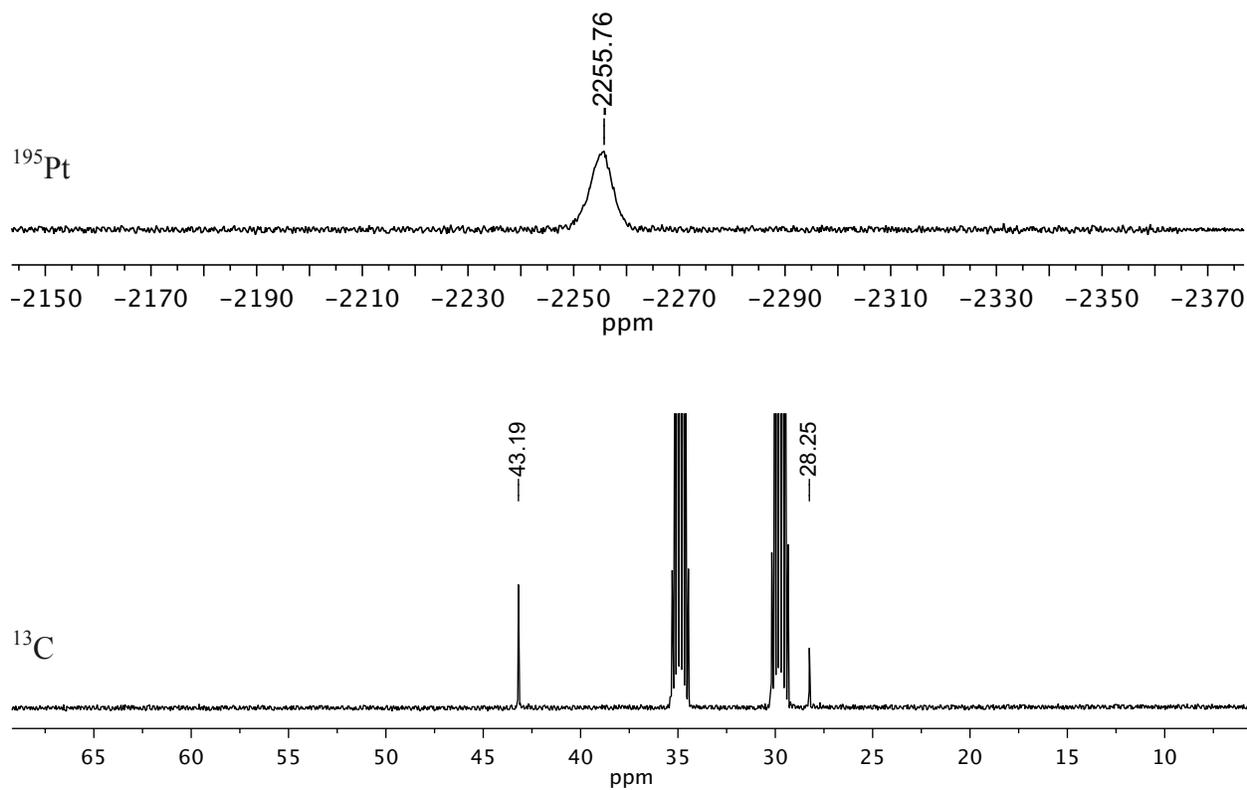
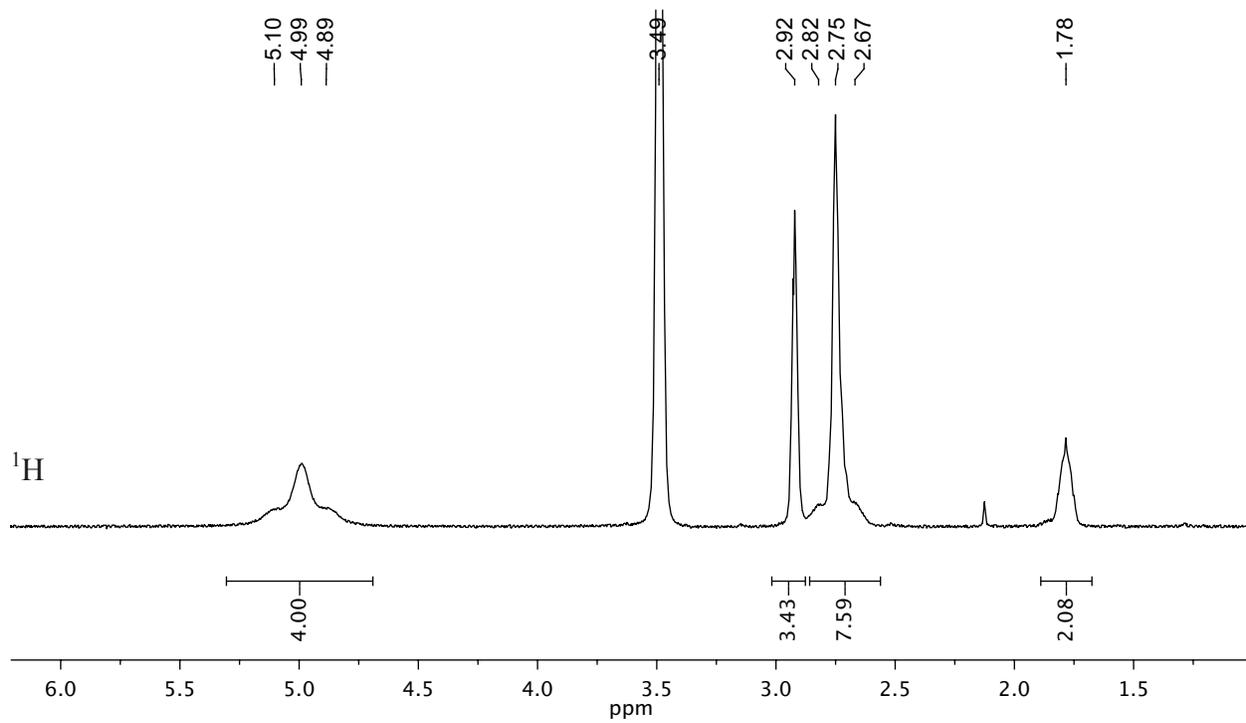


Figure S2. Results of DFT calculations of **4** bound to DNA. DFT-calculated (B3LYP 6-31G* with pseudopotentials) minimized ground-state geometry of **4** bound to the guanines of double-stranded DNA (3LPV)

Figure S3. NMR (¹⁹⁵Pt, ¹³C, ¹H) spectra of **5**:





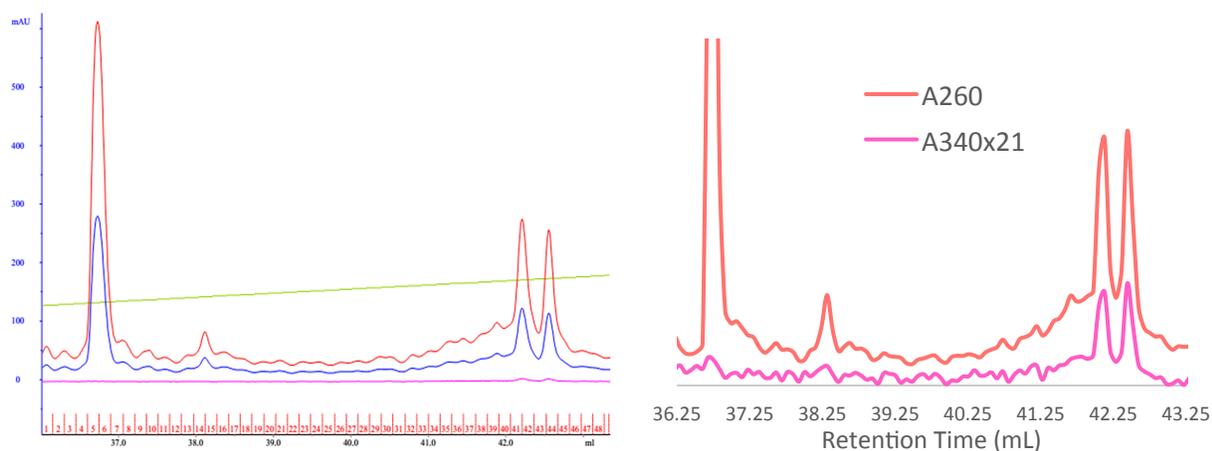
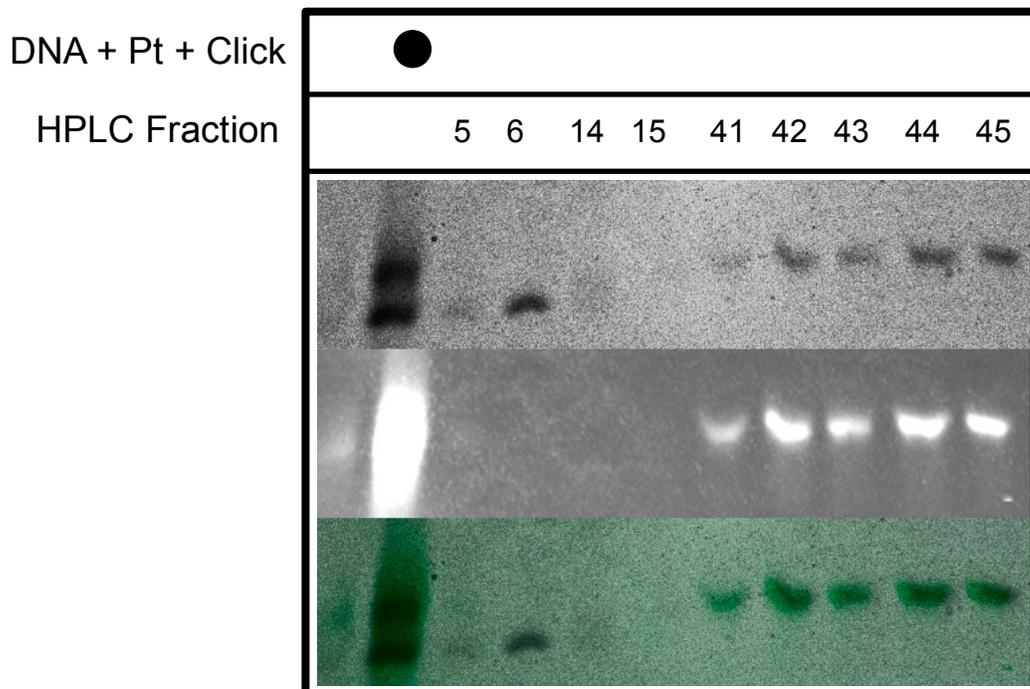
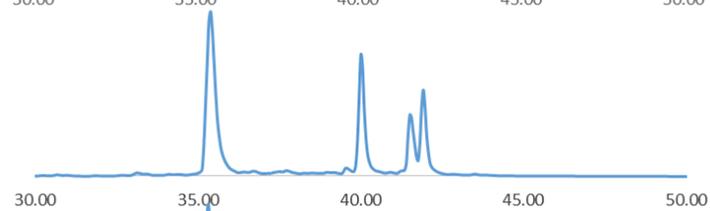
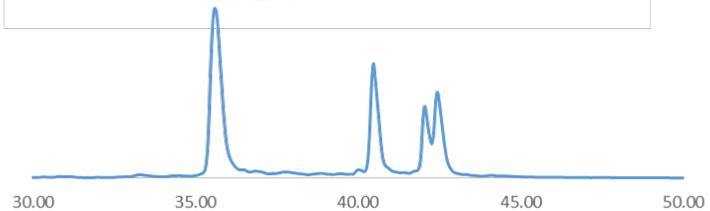
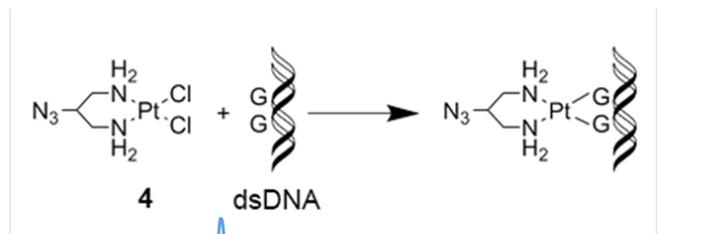
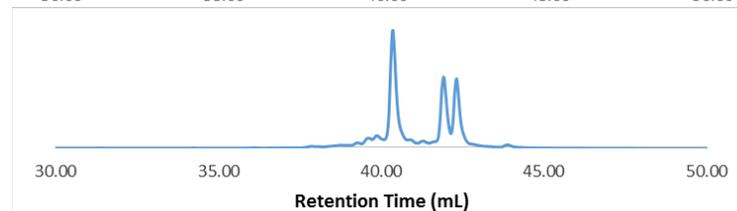
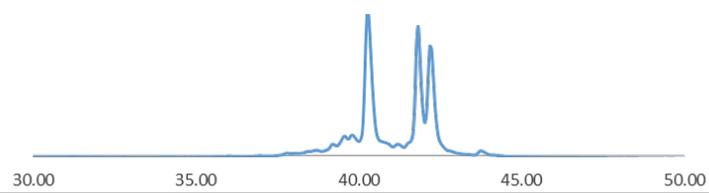
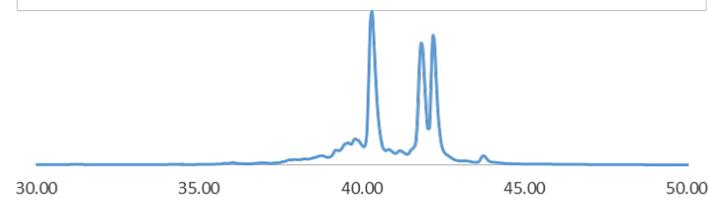
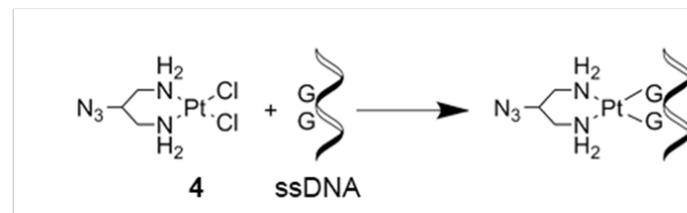


Figure S4. HPLC and dPAGE analysis of 4-bound hairpin DNA-Pt adduct following click reaction with the dansyl alkyne. HPLC peaks at 5, 6, 14, 15, and 41-45 mL were isolated, evaporated *in vacuo* and then re-suspended for dPAGE analysis. In the first lane of the gel (above) contains the crude reaction mixture, and in lanes 2-9 are the corresponding HPLC fractions. The HPLC chromatogram on the right reveals the absorbance values for the fluorophore, multiplied (scaled) by a factor of 21, correlating to the corresponding absorbance peaks at 260 nm (DNA). This scaling is based upon the approximate ratio between the extinction coefficient of the dansyl fluorophore and that of the 21-base hairpin.



ssDNA			Ratio Pt2/Pt1
DNA	Pt1	Pt2	
48	28	25	0.90
46	27	27	1.00
41	30	29	0.97
		AVG	0.96
		STD	0.05



dsDNA			Ratio Pt2/Pt1
DNA	Pt1	Pt2	
43	23	34	1.49
45	24	32	1.33
42	25	33	1.33
		AVG	1.38
		STD	0.09

Figure S5. HPLC chromatograms of the single-stranded construct (ssDNA) and the double-stranded construct (dsDNA) bound by **4**. Normalized areas of DNA parent peaks and first and second platinum-bound peaks are listed. The ratio of the Pt-DNA peaks are listed along with the average for each construct and their respective standard deviations.

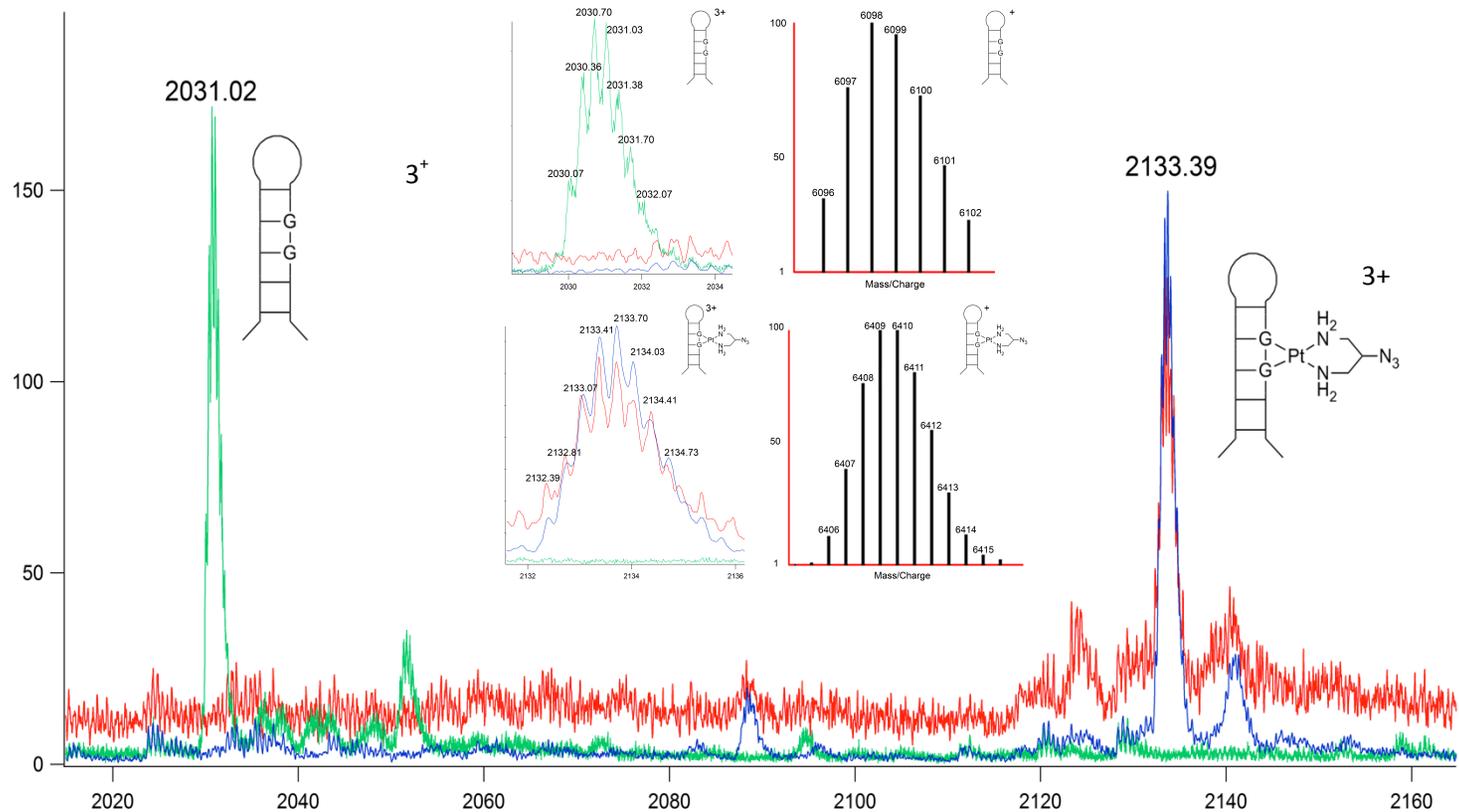


Figure S6. Results from ESI-MS experiment. Traces for the parent DNA strand (green) and the two isolated **4** bound to DNA (blue and red traces) are shown. Peaks in the spectrum are attributed to the 3⁺ DNA strand (2031 m/z) and the 3⁺ **4**-DNA strand (2133 m/z). The inset image shows the zoomed-in range of data as well as the predicted isomeric distributions.

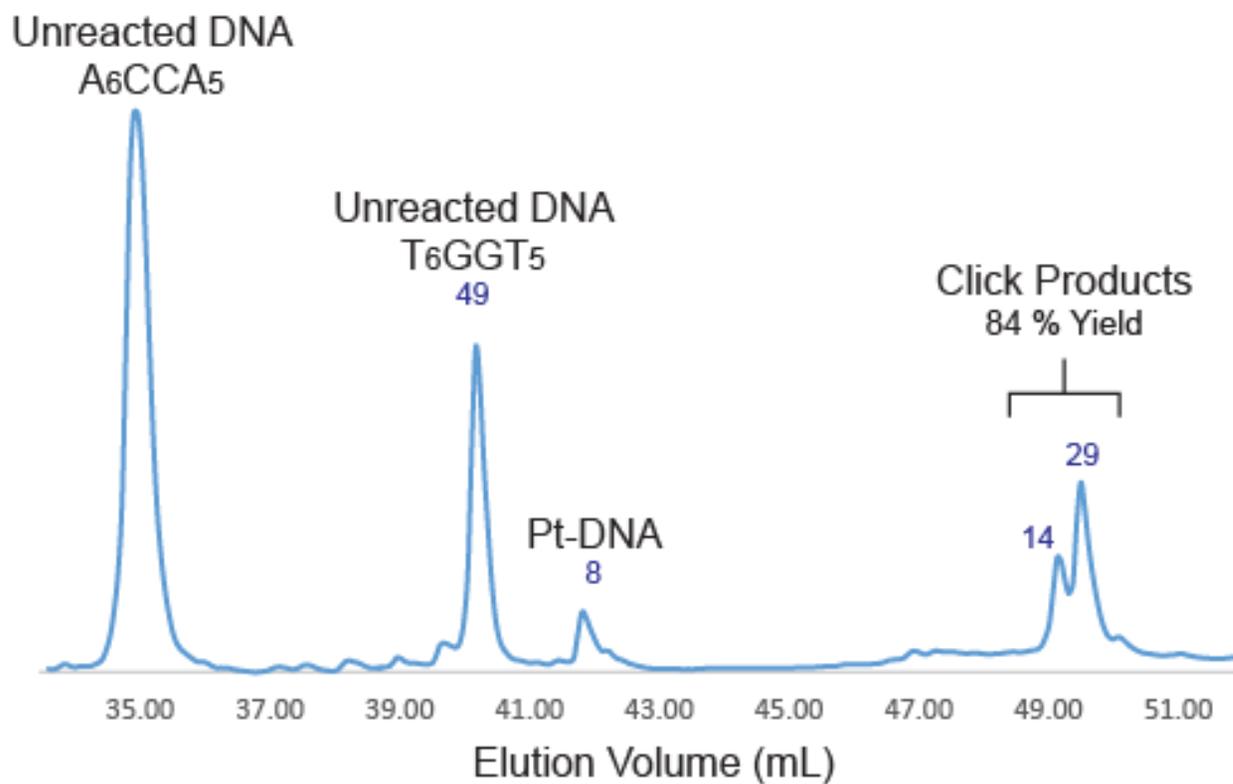


Figure S7. HPLC chromatogram for dsDNA bound by **4** and clicked to the dansyl alkyne fluorophore. Peaks near 35 and 40 mL elution volume correlate to unbound DNA, A₆CCA₅ and T₆GGT₅, respectively. The peak near 42 mL elution volume correlates to **4** bound DNA. Peaks near 49 mL elution volume correlate to the clicked products. The yield of the click reaction is approximately 84 %.