Supporting Information

Dhar *et al.* 10.1073/pnas.0809154105

SI Methods

X-Ray Crystallographic Study

Light yellow crystals (0.3 × 0.2 × 0.12 mm) of **1** suitable for X-ray diffraction were grown by slow diffusion of diethyl ether into a saturated solution of **1** in CH_3CN at room temperature. A single crystal of **1** was mounted at room temperature within a microfiber loop immersed in Paratone-N oil and cooled to 110 K under a stream of cold nitrogen. Intensity data were collected on a Bruker APEX CCD diffractometer using the SMART software, with Mo-K_α radiation (λ = 0.71073 Å). The SAINT software was used for data integration. The structure of **1** was solved by the Patterson method and refined by the SHELXTL (1, 2) software. Empirical absorption corrections were applied with SADABS (3), part of the SHELXTL program package, and the structures were checked for higher symmetry by the PLATON program (4). All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed at calculated positions and assigned thermal parameters equal to either 1.5 (methyl hydrogen atoms) or 1.2 (non-methyl hydrogen atoms) times the thermal parameters of the atoms to which they were attached. A perspective view of the molecule was obtained with ORTEP (Fig. S4).

Cell Culture

Medium. Cells were cultured in RPMI medium 1640 (Invitrogen) with 10% FBS, 2 mM glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin. Cells were routinely passed by treatment with trypsin (0.05%)/EDTA.

Cell Lines. Androgen-sensitive human prostate adenocarcinoma LNCaP cells ($PSMA^+$) and androgen independent human prostate adenocarcinoma PC3 cells ($PSMA^-$) were cultured in RPMI for several passages. Cells were passed every 3 to 4 days and reseeded from frozen stocks after reaching passage number 20.

Endocytosis of Apt-Targeted Pt(IV)-Encapsulated PLGA-b-PEG NPs. To study the internalization of the Pt(IV)-encapsulated NPs, we coencapsulated a green fluorescein-based cholesterol, 22-NBD-cholesterol. LNCaP and PC3 cells were seeded on microscope coverslips (1.0 cm) at a confluence of 1×10^5 cells per coverslip and grown overnight in a humidified incubator with 5% CO_2 at 37 °C in RPMI medium 1640. The medium was changed and a suspension of **1** and NBD-cholesterol-encapsulated NPs containing aptamer on the surface was added to a final fluorophore concentration of 10 μM. The cells were incubated for 2.0 h at 37 °C. The medium was removed and the cells were fixed using a 2% paraformaldehyde solution for 1 h at room temperature. The cells were washed 3 times with PBS (pH 7.4). The cells were then permeabilized with 0.1% Triton-X 100 in PBS for 10 min followed by 5 washes using PBS. The cells were then blocked with blocking buffer (PBS, 0.1% goat serum, 0.075% glycin) for 30 min at room temperature. The cells were incubated for 1 h at 37 °C with the early endosomal marker, mouse monoclonal EEA-1, in a wet box according to the manufacturer-recommended procedure. After 2 washes with PBS, the cells were blocked with blocking buffer for 30 min at RT and then incubated with the secondary Cy5 goat anti-mouse antibody for 1 h at 37 °C. After 4 washes with PBS and 2 washes with water, cells were mounted on microscope slides using the mounting solution [20 mM Tris (pH 8.0), 0.5% *N*-propyl gallate, and 70% glycerol] for imaging. Images were collected at 500 msec for both FITC and rhodamine channels.

MTT Cell Proliferation Assay. Cytotoxicities of the compounds were determined by the MTT assay. LNCaP and PC3 were grown in 96-well tissue culture plates for overnight in RPMI medium. Cells were then treated with various concentrations of A10 aptamer targeted Pt-NP-Apt, non-targeted Pt-NP, **1**, or cisplatin at different concentrations. The plates were incubated for 72 h at 37 °C under 5% CO_2. The cells were then incubated for 5 h with 20 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/ml in PBS). The medium was removed, the cells were lysed by adding 100 μL of DMSO, and the absorbance of the purple formazan was recorded at 550 nm using a BioTek Synergy HT multidetection microplate plate reader. Each well was performed in triplicate in 3 independent experiments for each cell line.

Detection of Cisplatin 1,2-d(GpG) Intrastrand Adduct by Immunofluorescence. Detection of platinum 1,2-d(GpG) adduct was carried out by following a procedure recently reported by us using an antibody specific to this adduct (5). Briefly, LNCaP cells were seeded in a 6 well plate using RPMI medium and incubated overnight at 37 °C. A suspension of **1** and 22-NBD-cholesterol-encapsulated NPs with aptamer on the surface was added to a final concentration of 20 μM and incubated at 37 °C. After 12 h, cells were trypsinized, washed with PBS, the cells were resuspended in HAES-sterile-PBS at a density of 1×10^6 per mL and placed onto a precoated slide (ImmunoSelect, Squarix) and air-dried. Cell fixing was carried out at −20 °C in methanol for 45 min. Nuclear DNA was denatured by alkali (70 mM NaOH, 140 mM NaCl, 40% methanol vol/vol) treatment for 5 min at 0 °C, and cellular proteins were removed by proteolytic procedure involving 2 steps. The cells were first digested with pepsin at 37 °C for 10 min and then with proteinase K at 37 °C for 5 min. After blocking with milk (1% in PBS; 30 min; 25 °C) slides were incubated with anti-(Pt-DNA) Mabs (R-C18, 0.1 mg/ml in milk) for overnight at 4 °C. After washing with PBS, immunostaining was performed by incubation with FITC-labeled goat anti-(rat Ig) antibody at 37 °C for 1h. The nuclei of the cells were stained by using Hoechst (H33258) (250 μg/L) and mounted using the mounting solution for imaging.

1. Sheldrick GM (2000) SHELXTL00: Program for Refinement of Crystal Structures, Universit of Göttingen, Germany, 2000.
2. Sheldrick GM (2001) SHELXTL, Program Library for Structure Solution and Molecular Graphics, v.6.10, Bruker AXS, Madison, WI, 2001.
3. Sheldrick GM (2001) SADABS: Area-Detector Absorption Correction, University of Göttingen, Germany, 2001.
4. Spek AL (2000) PLATON, A Multipurpose Crystallographic Tool, Utrecht University, Uterecht, The Netharlands, 2000.
5. Dhar S, *et al.* (2008) Targeted single-wall carbon nanotube-mediated Pt(IV) prodrug delivery using folate as a homing device. *J Am Chem Soc* 130:11467–11476.

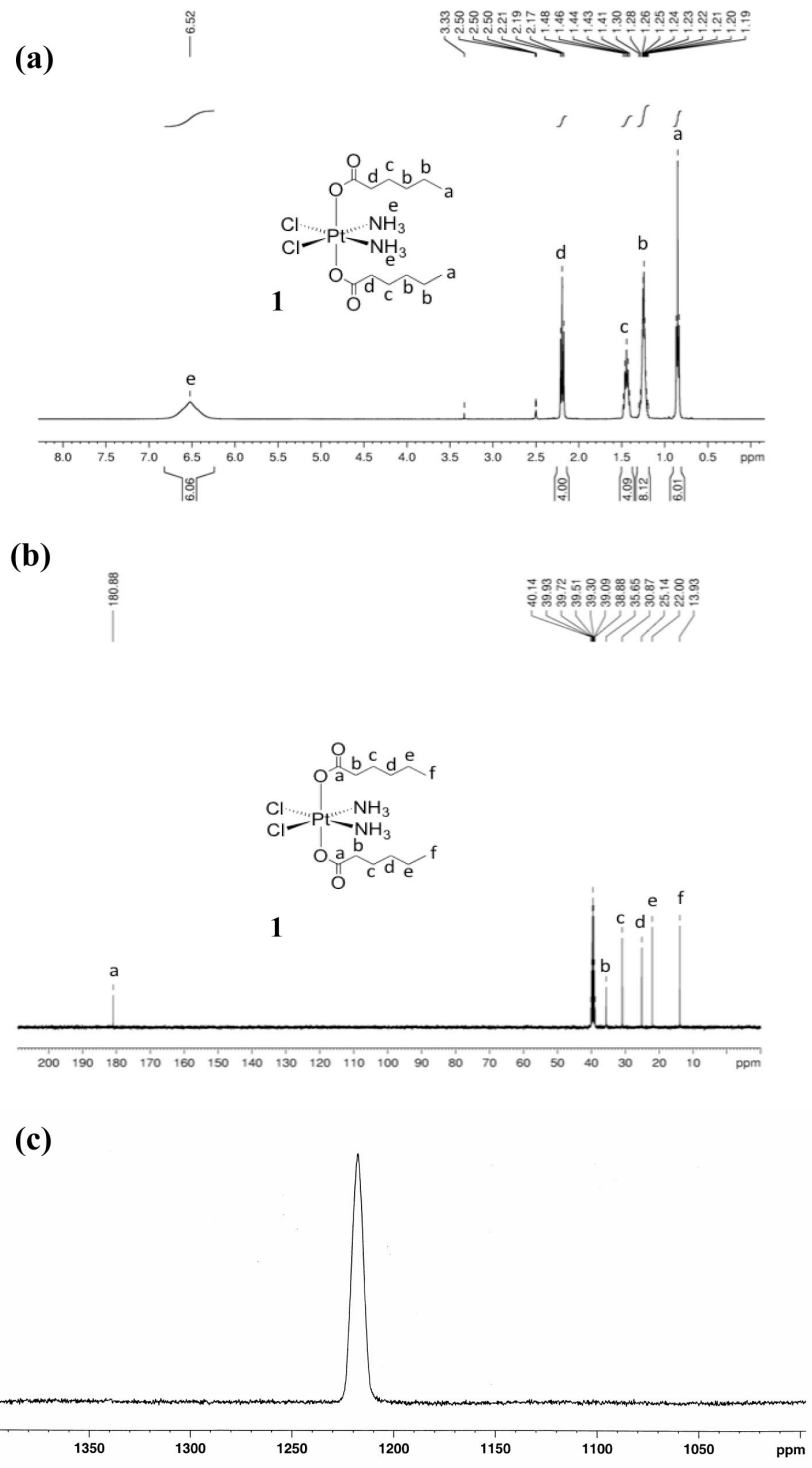

Fig. S1. (*a*) ^{1}H NMR spectrum of **1** in DMSO-d_6, (*b*) ^{13}C NMR spectrum of **1** in DMSO-d_6, (*c*) ^{195}Pt NMR spectrum of **1** in DMSO-d_6.

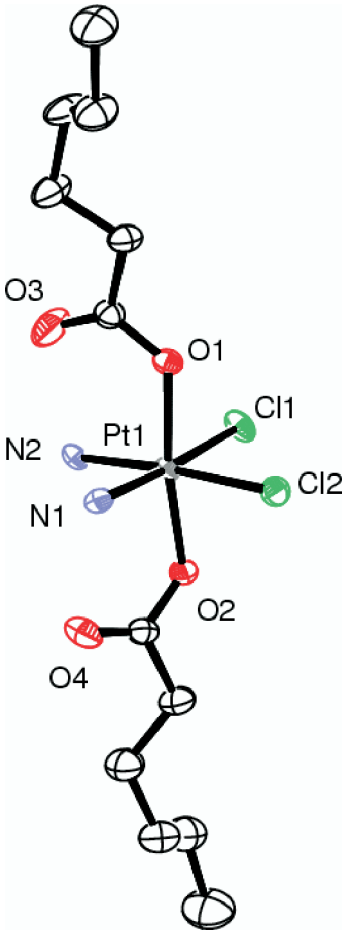

Fig. S2. ORTEP diagram showing 50% probability thermal ellipsoids and selected atom labels for **1**.

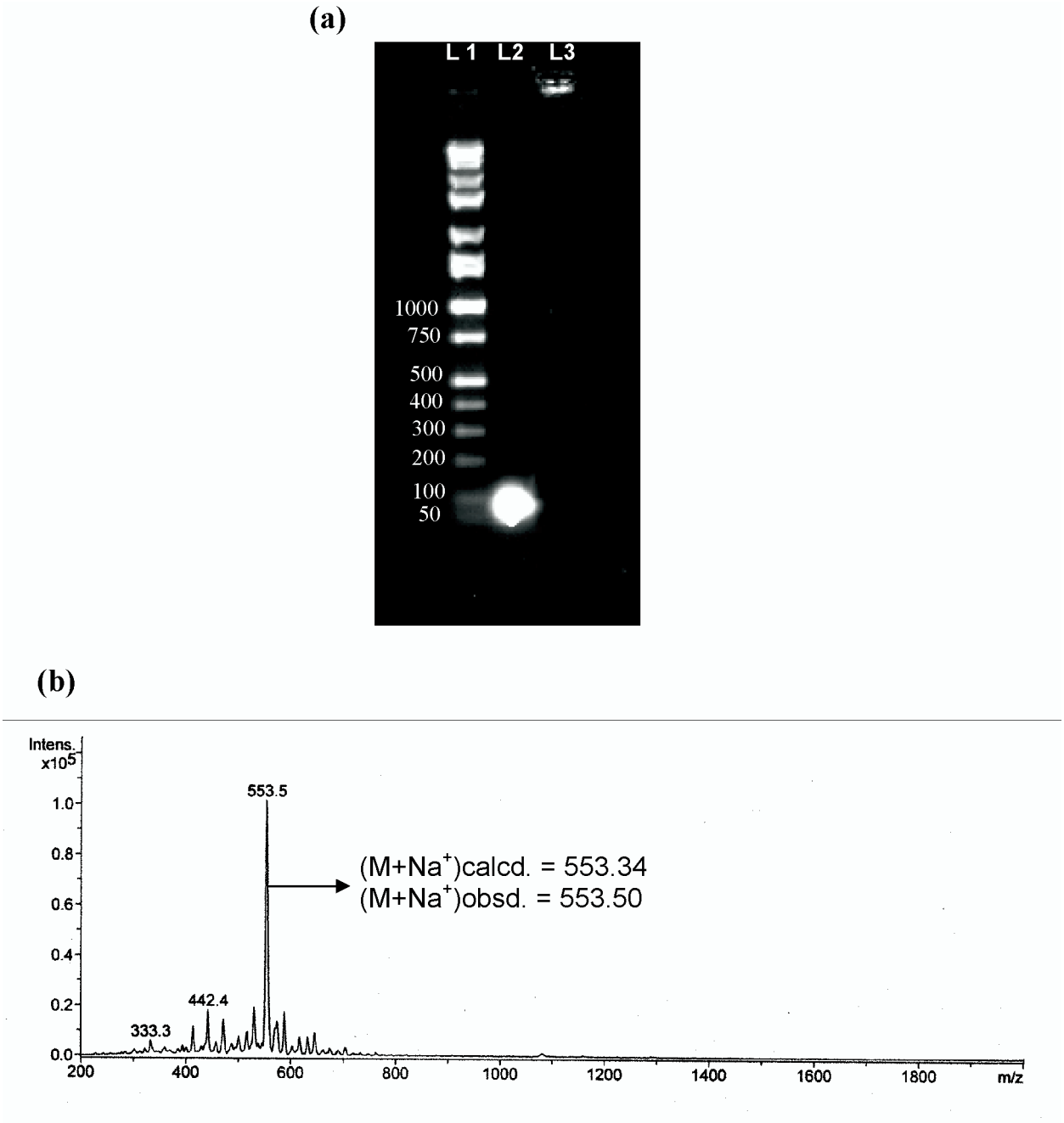

Fig. S3. (*a*) Analysis of aptamer conjugation to **1**-encapsulated PLGA-*b*-PEG nanoparticles by agarose gel electrophoresis. Lanes are as follows: lane 1: DNA ladder; lane 2: free 57-bp aptamer; lane 3: aptamer conjugated nanoparticles (Pt-NP-Apt). (*b*) Positive ion ESI-MS spectrum of the PBS dialysate against which **1**-encapsulated NPs were dialyzed showing the presence of compound **1**.

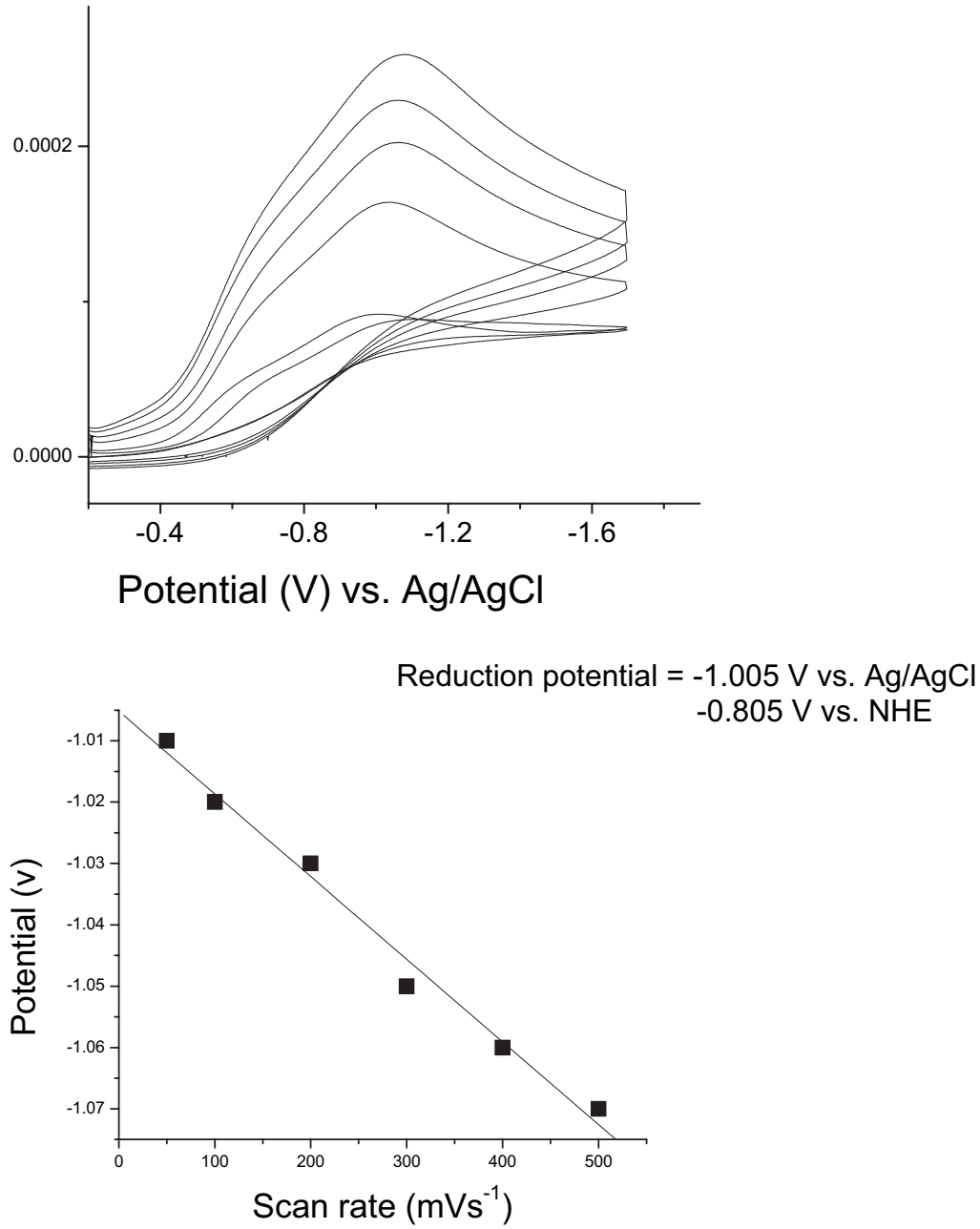

Fig. S4. Cyclic voltammograms of **1** in MeCN-0.1 M $TBAPF_6$ at various scan rates (*Upper*) and plot of reduction peak potential maxima of **1** in the voltammograms vs. scan rate (*Lower*).

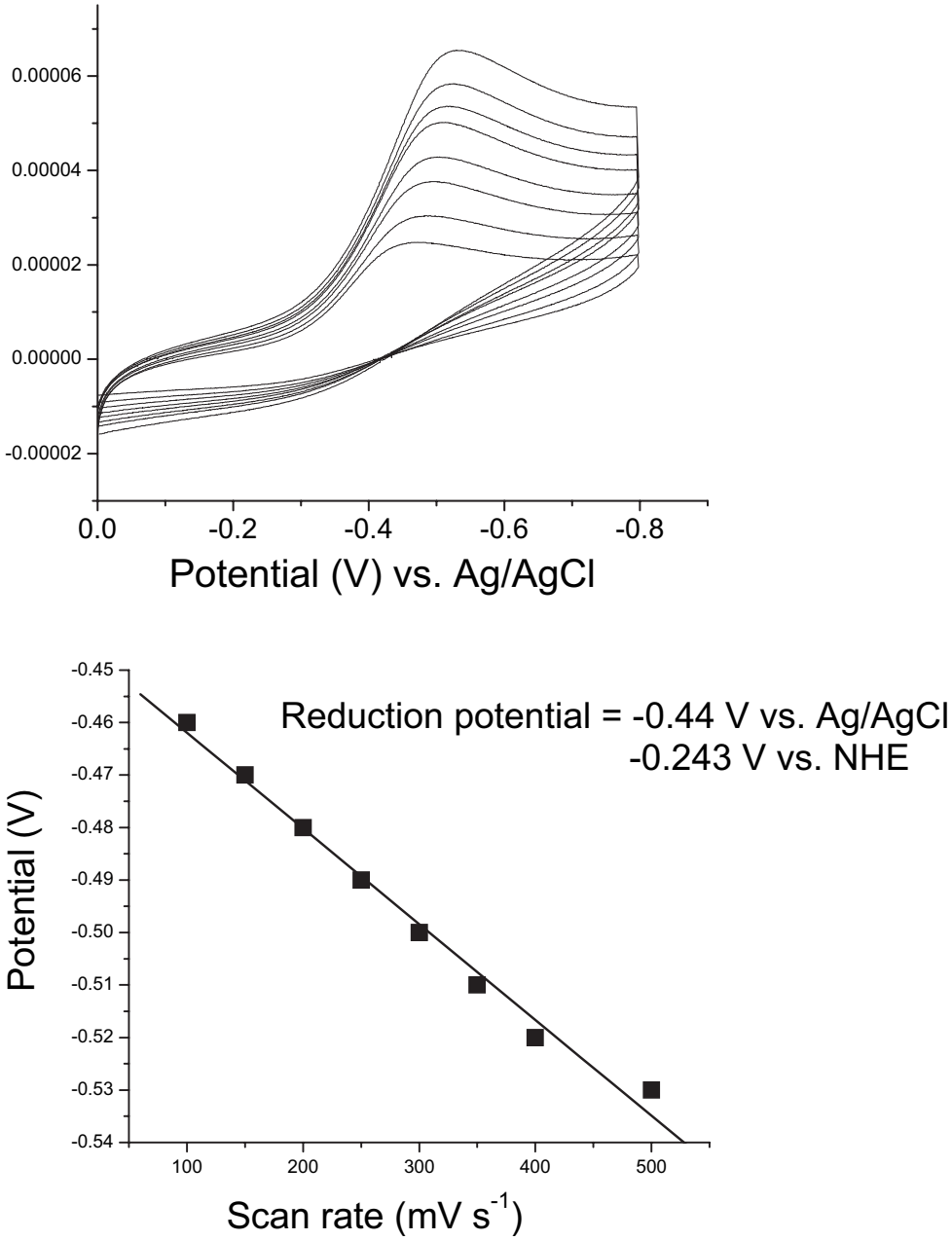

Fig. S5. Cyclic voltammograms of **1** in 1:4 DMF-phosphate buffer-0.1 M KCl of pH 7.4 with varied scan rates (*Upper*) and plot of reduction peak potential maxima of **1** at pH 7.4 from the voltammograms as a function of scan rate.

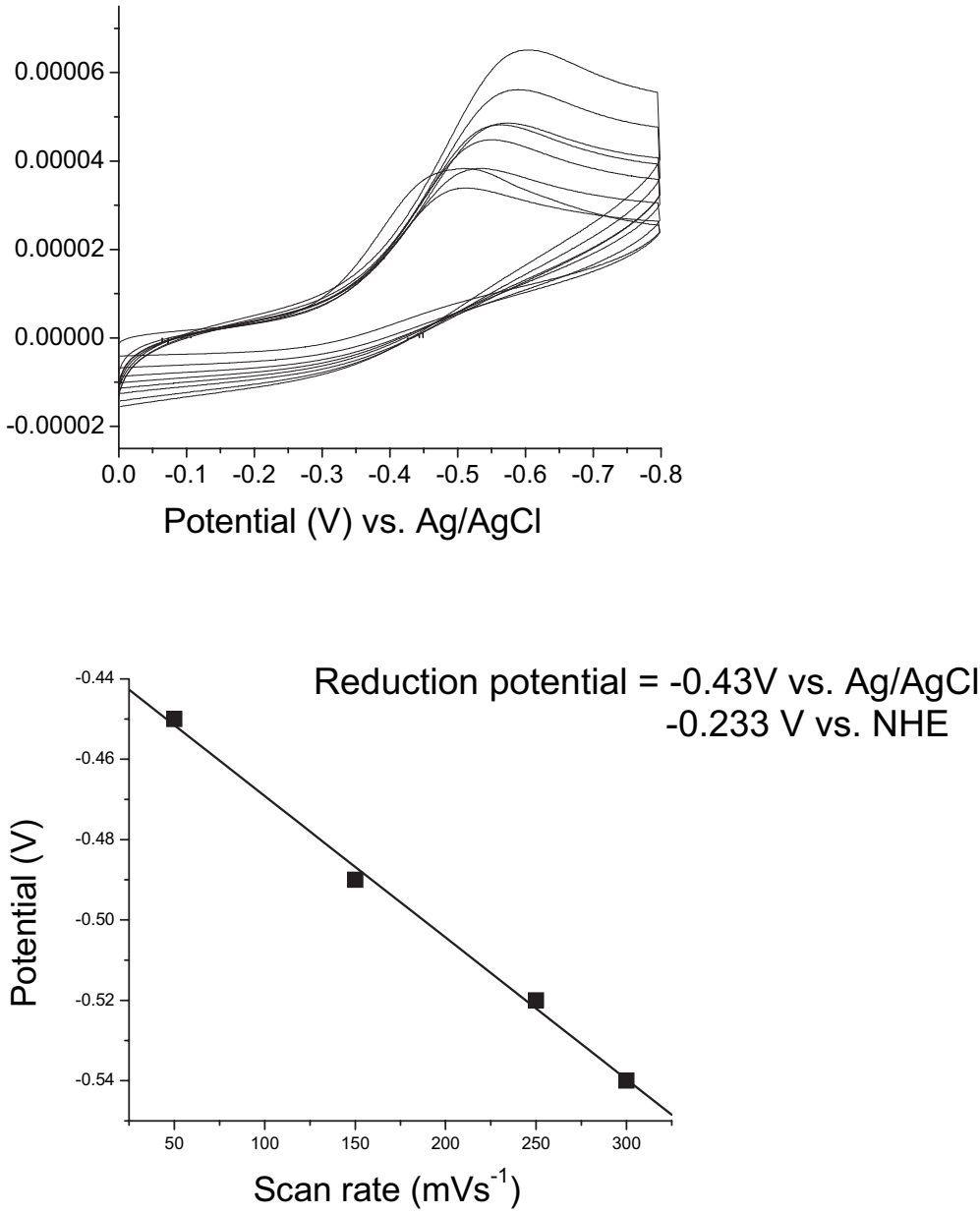

Fig. S6. Cyclic voltammograms of **1** in 1:4 DMF-phosphate buffer-0.1 M KCl of pH 6.0 with varied scan rates (*Upper*) and plot of reduction peak potential maxima of **1** at pH 6.0 from the voltammograms as a function of scan rate (*Lower*).

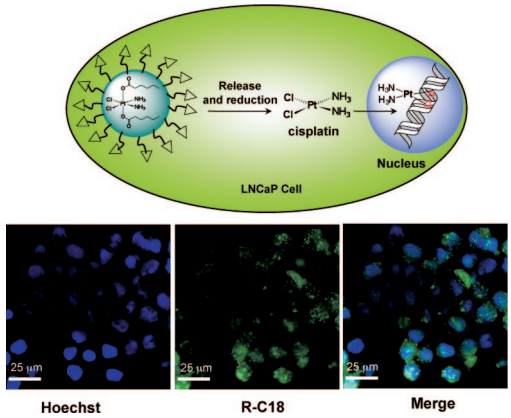

Fig. S7. Visualization of Pt-1,2-d(GpG) intrastrand cross-links in the nuclear DNA of LNCaP cells after a 12-h treatment of Pt-NP-Apt. Nuclei were stained with Hoechst (blue) and Pt-1,2-d(GpG) in DNA were identified using Mab R-C18 (green).

Table S1. Summary of crystal data, intensity collection parameters, refinement parameters, and geometry for 1

	1
Formula	$C_{12}H_{28}Cl_2N_2O_4Pt$
Molecular mass, g·mol^{-1}	530.35
Morphology	Yellow Block
Dimensions, mm	0.30 × 0.20 × 0.12
Crystal system	Monoclinic
Space group, Å	*C*2/c
a	30.105(3)
b	7.3980(6)
c	18.1809(15)
β	116.5050(10)
V, Å^3	3623.6(5)
Z	8
ρ_{calc}, g·cm^{-3}	1.944
μ, mm^{-1}	0.8054
F(000)	2064
θ range, deg.	2.27 to 28.31
Total/unique reflections	21,533/4,483
completeness to 2θ, %	99.6%
Data/restraints/parameters	4,483/0/190
GoOF	1.081
R_1*	0.0307
wR_2†	0.0725
Largest diff. peak and hole, *e* Å^{-3}	3.147 and −0.910

Bond or angle	Distance (Å) or angle (deg)
Pt(1)-O(1)	2.009(3)
Pt(1)-N(1)	2.014(4)
Pt(1)-O(2)	2.022(3)
Pt(1)-N(2)	2.047(4)
Pt(1)-Cl(1)	2.3020(10)
Pt(1)-Cl(2)	2.3067(11)
O(1)-Pt(1)-N(1)	87.76(14)
O(1)-Pt(1)-O(2)	172.23(13)
N(1)-Pt(1)-O(2)	97.48(14)
O(1)-Pt(1)-N(2)	97.21(14)
N(1)-Pt(1)-N(2)	89.43(15)
O(2)-Pt(1)-N(2)	88.61(15)
O(1)-Pt(1)-Cl(1)	89.00(10)
N(1)-Pt(1)-Cl(1)	176.31(11)
O(2)-Pt(1)-Cl(1)	85.92(9)
N(2)-Pt(1)-Cl(1)	89.23(11)
O(1)-Pt(1)-Cl(2)	86.49(10)
N(1)-Pt(1)-Cl(2)	89.02(11)
O(2)-Pt(1)-Cl(2)	87.86(11)
N(2)-Pt(1)-Cl(2)	175.93(10)
Cl(1)-Pt(1)-Cl(2)	92.54(4)

*$R_1 = \Sigma \, ||F_o| - |F_c|| / \Sigma \, |F_o|$
†$wR_2 = \{\Sigma[w(F_o^2 - F_C^2)^2] / \Sigma[w(F_o^2)^2]\}^{1/2}$