Supporting Information

for

Novel Arylhydrazone-conjugated Gold Nanoparticles with DNA-Cleaving Ability: the First DNA-Nicking Nanomaterial

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General Procedure. All reactions were carried out in oven-dried glassware (120 °C) under an atmosphere of nitrogen unless as indicated otherwise. Acetone, ethanol, ethyl acetate, hexane, and tetrahydrofuran were purchased Mallinckrodt Chemical Co. Acetone was dried with 4Å molecular sieves and distilled. Ethanol was dried and distilled from magnesium turnings under an atmosphere of

nitrogen. Ethyl acetate (EtOAc) and hexane were dried and distilled from CaH_2. Tetrahydrofuran was dried by distillation from sodium and benzophenone under an atmosphere of nitrogen. 4-Hydroxybenzaldehyde, phenylhydrazine, tetraethylene glycol, and thioacetic acid were purchased from Aldrich Chemical Co. Potassium carbonate and sodium hydroxide were purchased from Riedel-de Haën Chemical Co. Supercoiled circular *ϕX174* RFI DNA was purchased from New England Biolabs.

Analytical thin layer chromatography (TLC) was performed on precoated plates (silica gel 60 F-254), purchased from Merck Inc. Gas chromatographic analyses were performed on a SHIMADZU GC-2014 instrument, which was equipped with a 25-m crosslinked methyl silicone gum capillary column (0.32 mm i.d.). Nitrogen gas was used as a carrier gas and the flow rate was kept constant at 14.0 mL/min. The retention time t_R was measured under the following conditions: injector temperature 260 °C, isothermal column temperature 280 °C. Gas chromatography and low resolution mass spectral analyses were performed on a Agilent Technology 6890N Network GC System equipped with a Agilent 5973 Network Mass Selective Detector and HP-1 capillary column. Purification by gravity column chromatography was carried out by use of Merck Reagents Silica Gel 60 (particle size 0.063–0.200 mm, 70–230 mesh ASTM). Purity of all compounds was >99.5%, as checked by GC.

Ultraviolet (UV) spectra were measured on Hitachi U3300 UV/VIS spectrophotometer. Infrared (IR) spectra were measured on Jasco FT-IR-5300 Fourier transform infrared spectrometer. The wave numbers reported are referenced to the polystyrene 1601 cm^{-1} absorption. Absorption intensities are recorded by the following abbreviations: s, strong; m, medium; w, weak. The fluorescent intensity was measured on Hitach F-4500 Florescence Spectrophotometer. Proton NMR spectra were obtained on a Varian Mercury-400 (400 MHz) spectrometer by use of chloroform-*d* as the solvent and tetramethylsilane as an internal standard. Carbon-13 NMR spectra were obtain on a Varian Mercury-400 (100 MHz) spectrometer by use of chloroform-*d* as the solvent. Carbon-13 chemical shifts are referenced to the center of the $CDCl_3$ triplet (δ 77.0 ppm). Multiplicities are recorded by the following

abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (hertz). Hight-resolution mass spectra were obtain by means of a JEOL JMS-HX110 mass spectrometer.

Photolytic experiments with UV light were carried out at room temperature by use of an FB-UVM-80 UV hand lamp with 16-watt and 312-nm wavelength from Fisher Scientific Co. The NIH 1.60 image program, provided by Dr. Wayne of National Institute of Health, U.S.A., was used for the quantitative analysis of DNA cleavage.

11-Bromo-3,6,9-trioxaundecyl Thioacetate (3). To a solution containing tetraetylene glycol dibromide[1] (**2**, 1.05 g, 3.28 mmol 1.0 equiv) and thioacetic acid (249.1 mg, 3.275 mmol, 1.0 equiv) in THF (20 mL) was added NaOH (144.3 mg, 3.593 mmol, 1.1 equiv). After the solution was stirred at room temperature for 6.0 h, it was quenched with water (10 mL). The THF was removed under reduced pressure and the resultant mixture was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine, dried over $MgSO_{4(s)}$, and concentrated under reduced pressure to give a pale yellow oil. Purification of the oil by flash chromatography packed with silica gel (10% EtOAc in hexane as eluent) gave **3** (801.2 mg, 2.549 mmol) as a pale yellow oil in 77% yield: ^{1}H NMR ($CDCl_3$, 400 MHz,) δ.2.29 (s, 3 H, CH_3), 3.06 (t, *J* = 3.2 Hz, 2 H, CH_2), 3.44 (t, *J* = 6.2 Hz, 2 H, CH_2), 3.55–3.65 (m, 10 H, 5 × CH_2), 3.78 (t, *J* = 6.4 Hz, 2 H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.76, 30.26, 30.51, 69.71, 70.25, 70.47, 70.51, 70.58, 71.15, 195.49; IR KBr 2867 (m), 1692 (s), 1424 (m), 1353 (m), 1113 (s), 1039 (m) cm^{-1}; MS (EI) *m/e* (relative intensity) 316 (M^+ + 2, 1), 314 (M^+, 1), 239 (3), 195 (11), 151 (15), 107 (57), 87 (46), 73 (12), 61 (21), 43 (100); HRMS calcd for $C_{10}H_{19}BrO_4S$ 314.0187, found 314.0182.

11-(4-Formylphenoxy)-3,6,9-trioxaundecyl Thioacetate (4). A solution containing **3** (389.2 mg, 1.267 mmol, 1.0 equiv), 4-hydroxybenzaldehyde (150.1 mg, 1.229 mmol, 1.0 equiv), and potassium carbonate (210.1 mg, 1.522 mmol, 1.2 equiv) in acetone (25 mL) was heated to reflux for 16 h. The reaction was quenched with water (20 mL) and acetone was removed under reduced pressure. After the aqueous phase was extracted with EtOAc (3 × 20 mL), the combined organic layer was washed with

brine, dried over $MgSO_{4(s)}$, and concentrated under reduced pressure to give a pale yellow oil. Purification of the oil by flash chromatography packed with silica gel (20% EtOAc in hexane as eluent) gave the desired **4** (357.9 mg, 1.034 mmol) in 81% yield as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3 H, CH_3), 3.03 (t, *J* = 6.6 Hz, 2 H, CH_2), 3.57–3.78 (m, 10 H, 5 × CH_2), 3.84 (t, *J* = 4.8 Hz, 2 H, CH_2), 4.17 (t, *J* = 4.8 Hz, 2 H, CH_2), 6.97 (d, *J* = 8.8 Hz, 2 H, 2 × ArH), 7.78 (d, *J* = 8.8 Hz, 2 H, 2 × ArH), 9.83 (s, 1 H, CHO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.59, 30.39, 67.58, 69.29, 69.58, 70.11, 70.36, 70.47, 70.70, 114.72, 129.84, 131.81, 163.69, 190.74, 195.41; IR KBr 2870 (m), 2735 (m), 1690 (s), 1601 (s), 1578 (m), 1508 (m), 1455 (w), 1426 (w), 1258 (m), 1161 (m), 1110 (m), 835 (m), 621 (m) cm^{-1}; MS (EI) *m/e* (relative intensity) 356 (M^+, 2), 311 (5), 281 (32), 254 (27), 237 (20), 211 (27), 193 (12), 149 (65), 121 (44), 103 (63), 77 (32), 43 (100); HRMS (FAB) calcd for $C_{17}H_{24}O_6S$ 346.0511, found 346.0514.

11-(4-Formylphenoxy)-3,6,9-trioxaundecyl Thiol (5). A solution containing **4** (438 mg, 1.22 mmol, 1.0 equiv) and NaOH (48.8 mg, 1.22 mmol, 1.0 equiv) in methanol (20 mL) was stirred at room temperature for 5.0 h. The reaction was quenched with water (10 mL) and methanol was removed under reduced pressure. After the aqueous phase was extracted with EtOAc (3 × 20 mL), the combined organic layer was washed with brine, dried over $MgSO_{4(s)}$, and concentrated under reduced pressure to give a pale yellow oil. Purification of the oil by flash chromatography packed with silica gel (30% EtOAc in hexane as eluent) gave the desired **5** (298.1 mg, 0.949 mmol) as a yellow oil in 77% yield: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.56 (t, *J* = 8.2 Hz, 1 H, SH), 2.72 (dt, *J* = 7.2 Hz, 7.2 Hz, 2 H, CH_2), 3.51–3.78 (m, 10 H, 5 × CH_2), 3.85 (t, *J* = 4.8 Hz, 2 H, CH_2), 4.13 (t, *J* = 4.8 Hz, 2 H, CH_2), 6.99 (d, *J* = 8.8 Hz, 2 H, 2 × ArH), 7.81 (d, *J* = 8.8 Hz, 2 H, 2 × ArH), 9.85 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.05, 67.58, 69.26, 69.99, 70.36, 70.45, 70.69, 72.65, 114.71, 129.83, 131.75, 163.65, 190.63; IR KBr 2869 (m), 2731 (w), 1686 (s), 1601 (m), 1574 (m), 1508 (m), 1454 (w), 1255 (m), 1109 (w), 835 (m), 652 (w) cm^{-1}; MS (FAB) *m/e* (relative intensity) 314 (M^+, 2), 255 (28), 211 (53), 149 (59), 121 (52), 105 (16), 77 (38), 61 (100); HRMS (FAB) calcd for $C_{15}H_{22}O_5S$ 314.1187, found 314.1183.

11-(4-Benzaldehyde)phenylhydrazone-3,6,9-trioxaundecyl Thiol (6). To a solution of compound **5** (209.2 mg, 0.665 mmol, 1.0 equiv) in water (4.0 mL) and ethanol (16 mL) was added phenylhydrazine (71.4 mg, 0.661 mmol, 1.0 equiv) and then three drops of acetic acid. After the solution was stirred for 6.0 h, it was quenched with water (10 mL). The solution was extracted with ethyl acetate (3 × 25 mL) and the combined organic layer was washed with saturated brine, dried over $MgSO_{4(s)}$, filtered, and concentrated under reduced pressure to give **6** (220 mg, 0.544 mmol) in 81% yield as a brown oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.57 (t, *J* = 8.2 Hz, 1 H, SH), 2.67 (dt, *J* = 7.2 Hz, 7.2 Hz, 2 H, CH_2), 3.61–3.73 (m, 10 H, 5 × CH_2), 3.85 (t, *J* = 4.8 Hz, 2 H, CH_2), 4.14 (t, *J* = 4.8 Hz, 2 H, CH_2), 6.82 (m, 1 H, ArH), 6.88 (d, *J* = 8.8 Hz, 2 H, 2 × ArH), 7.06 (d, *J* = 8.8 Hz, 2 H, 2 × ArH), 7.24 (d, *J* = 8.8 Hz, 2 H, 2 × ArH), 7.51 (s, 1 H, NH), 7.55 (d, *J* = 8.8 Hz, 2 H, 2 × ArH), 7.63 (s, 1 H, CH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.5, 67.7, 69.9, 70.5, 70.8, 70.9, 71.1, 73.1, 112.9, 115.0, 119.9, 127.72, 128.6, 129.56, 129.46, 137.56, 145.22, 159.39; IR KBr 2922 (m), 2869 (m), 1660 (w), 1602 (s), 1507 (s), 1508 (m), 1448 (w), 1248 (s), 1131 (s), 1102 (m), 831 (w), 752 (w) cm^{-1}; UV (EtOH): λ_{max} 243 (ε 22,344), 302 (ε 30,679), 345 (ε 47,771) nm; MS (FAB) *m*/*e* (relative intensity) 404 (M^+, 19), 344 (4), 297 (4), 223 (2), 212 (15), 211 (9), 93 (23), 92 (33), 77 (22), 67 (100); HRMS (FAB) calcd for $C_{21}H_{28}N_2O_4S$ 404.1769, found 404.1765.

Self-Assembly of Thiolated PEG-Arylhydrazone 6 on Gold Nanoparticles. Thiolated PEG-arylhydrazone **6** (2.0 mL, 10 mM) in ethanol was added to freshly prepared citrate-reduced gold nanoparticles (10 mL).[2,3] After being kept at 25 °C for 12 h, the solution was diluted with water (90 mL) and then centrifuged at 8000 rpm for 30 min. The supernatant was removed to give crude particles of **7.** Then water (90 mL) was added to the crude **7**, to which the centrifugation step was repeated for another three times. The remaining solution was then re-suspended back to the original volume (10 mL) by addition of water.

Figure 3

The average number of the hydrazone moieties bound on each gold nanoparticle was calculated on the basis of the UV adsorption at 345 nm and the molar extinction coefficient of hydrazone **6** (ε = 47,771) by the Nie's displacement method.[4] Accordingly, mercaptoethanol (9.79 g, 125 mmol) was used to react with **7** (1.01 mM, 10.0 mL) to give a solution containing **6,** which absorbed UV light with A = 2771 in a 1.0-cm cell. The number of hydrazone moiety attached on each gold nanoparticle was calculated as 58.

General Procedure for DNA Cleavage by Use of Gold Nanoparticles 7 and Thiolated PEG-Arylhydrazone 6. The reaction mixtures (10 μL) containing a DNA cleaver (20 pM–2.0 nM for **7** or 10–2000 μM for **6**) supercoiled circular *ϕX174* RFI DNA stock solution (form I, 50 μM/base pair), phosphate buffer (0.10 M, pH 7.4), and 10% ethanol in a Pyrex vial was preincubated at 37 °C for 30 min. The concentrations of conjugate nanoparticles **7** and thiolated PEG-arylhydrazone **6** were determined by UV spectrometry. The reaction mixture was then irradiated with UV light (312 nm, 1.43 mW/cm^2) under aerobic conditions at room temperature for 2.0 h. After addition of gel-loading buffer (2.5 μL containing 0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol), the reaction mixture was loaded on a 1.0% agarose gel with ethidium bromide staining. The electrophoresis tank was attached to a power supply at a constant current (~100 mA). The gel was visualized by 312-nm UV transilluminator and photographed by an FB-PBC-34 camera. Quantitation of DNA-cleaving activities was performed by integration of the optical density as a function of the band area by use of a Hewlett–Packard scanner and NIH 1.60 image program. The results are shown in Figure 1 for **7** and Figure 3 for **6**.

Figure 4

LITERATURE CITED

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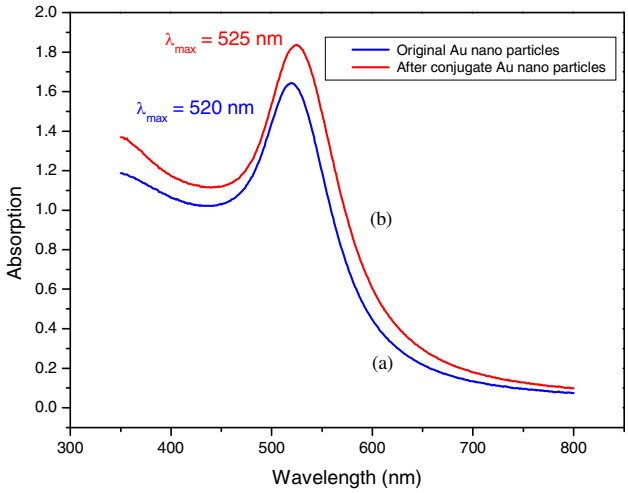

Figure 3. UV–Vis absorption spectra of solutions containing (a) Au-NPs, and (b) Au-NPs–PEG–arylhydrazone hybrid (**7**).

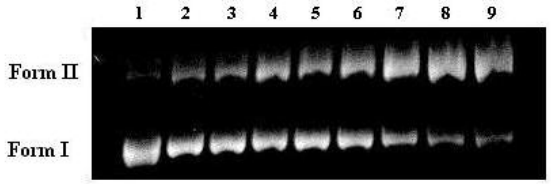

Figure 4. Dose measurement of HS–TEG–phenylhydrazone (**6**) for its DNA cleaving ability in sodium phosphate buffer (pH = 7.4) upon irradiation with UV light (312 nm, 16W) at various concentration and room temperature for 2.0 h: Lane 1, DNA alone; Lane 2–8: 10, 20, 50, 100, 200, 500, 1000, 2000 μM,

respectively. The resultant products were subjected to electrophoresis on 1% agarose gel followed by ethidium bromide staining.