Antiandrogen Gold Nanoparticles Dual-Target and Overcome Treatment Resistance in Hormone-Insensitive Prostate Cancer Cells

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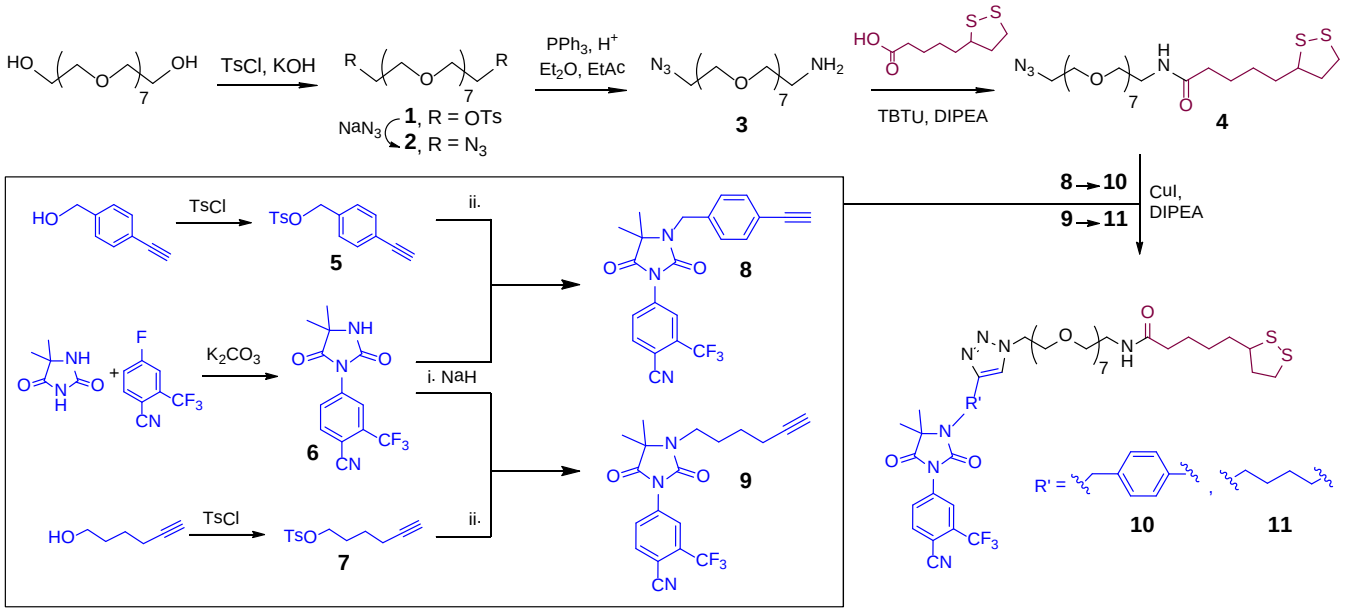
SUPPORTING INFORMATION

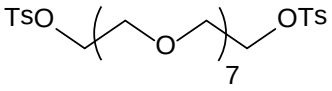
Experimental Methods

Scheme S1| Synthesis of α-Bicalutamide and β-Bicalutamide antiandrogen ligands.



Octaethylene glycol was purchased from Polypure. All other chemicals were purchased from Sigma–Aldrich. Anhydrous solvents and other reagents were purchased and used without further purification. Analtech silica gel plates (60 F_{254}) were used for analytical TLC, and Analtech preparative TLC plates (UV 254, 2000 μm) were used for purification. UV light was used to examine the spots. Silica gel (200–400 Mesh) was used in column chromatography. NMR spectra were recorded on a Varian-Gemini 400 magnetic resonance spectrometer, unless otherwise specified. ^{1}H NMR spectra were recorded in parts per million (ppm) relative to the peak of

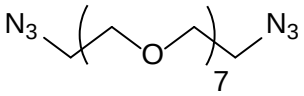
$CDCl_3$, (7.26 ppm), Acetone-d_6 (2.05 ppm) or DMSO-d_6 (2.50 ppm). ^{13}C spectra were recorded relative to the central peak of the $CDCl_3$ triplet (77.0 ppm), Acetone-d_6 septet (29.84 ppm) or the DMSO-d_6 septet (39.7 ppm), and were recorded with complete heterodecoupling. Multiplicities are described using the abbreviation s, singlet; d, doublet, t, triplet; q, quartet; m, multiplet. High-resolution mass spectra (HRMS) were recorded at the Georgia Institute of Technology mass spectrometry facility in Atlanta. Common abbreviations include: TBTU (O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate), EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide), DMF (*N,N*'-dimethylformamide), DCM (dichloromethane), TLC (thin layer chromatography), THF (tetrahydrofuran), PEG (polyethylene glycol), DIPEA (*N,N*'-Diisopropylethylamine), DMSO (Dimethyl sulfoxide).



1

Synthesis of Ditosylated-octaethylene glycol (1) ***3,6,9,12,15,18,21-heptaoxatricosane-1,23-diyl bis(4-methylbenzenesulfonate)***

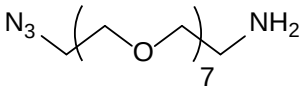
This procedure was modified from previous work.[1] Octaethylene glycol (7.09 g, 19.14 mmol) and tosylchloride (10.8 g, 56.70 mmol) was dissolved in 20 mL THF at zero degrees while stirring. Potassium hydroxide (7.40 g, 132.2 mmol) dissolved in 14 mL H_2O/THF (1:1 mixture) was slowly added over one hour. The reaction was then allowed to warm to room temperature overnight, followed by dilution with 300 mL diethyl ether/ethyl acetate mixture (2:1). Organic layer was washed twice with 200 mL aqueous sodium bicarbonate and water, dried over sodium sulfate and concentrated under reduced pressure to yield 1 (12.44 g, 95.7%) as a clear oil. ^1H NMR (400 MHz, $CDCl_3$) δ = 7.78 (d, *J*=8.4, 4H), 7.33 (d, *J*=7.9, 4H), 4.17 – 4.10 (m, 4H), 3.69 – 3.64 (m, 4H), 3.64 – 3.52 (m, 24H), 2.43 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 144.76, 132.98, 129.80, 127.94, 70.71, 70.58, 70.54, 70.48, 69.23, 68.64, 21.62.



2

Synthesis of Diazido-octaethylene glycol (2) ***1,23-diazido-3,6,9,12,15,18,21-heptaoxatricosane***

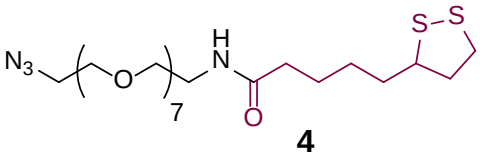
Ditosylated-octaethylene glycol 1 (6.41 g, 9.46 mmol) and sodium azide (4.30 g, 66.10 mmol) was dissolved in DMF (100 mL) and stirred at 110 °C under inert atmosphere of argon overnight. DMF was evaporated off, and the residue was dissolved in ethyl acetate and filtered to remove excess salt. The organic layer was washed with brine three times, the aqueous layer was extracted with ethyl acetate/methanol 10:1 three times, and the organic layers were combined, dried over sodium sulfate and then concentrated under reduced pressure to obtain product 2 as clear yellow oil (3.51 g, 88% yield). ^1H NMR (400 MHz, $CDCl_3$) δ = 3.67 – 3.61 (m, 28H), 3.36 (t, *J*=5.1, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 69.49, 69.44, 68.92, 49.55.



3

Synthesis of Azido-amine-octaethylene glycol (3) ***23-Azido-3,6,9,12,15,18,21-heptaoxatricosan-1-amine***

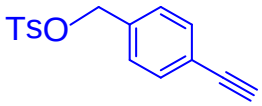
To a solution of diazido-octaethylene glycol 2 (470 mg, 1.12 mmol) in 50 mL ether/ethyl acetate (1:1) was added 40 mL of 5% HCl in water at 0 °C, followed by slow portion-wise addition of PPh_3 (286 mg, 1.09 mmol). The reaction was then allowed to warm to room temperature and stirred for an additional three hours, after which the organic layer was removed, and the aqueous layer was washed twice with DCM. The aqueous layer was then basified with sodium hydroxide to a pH of 12, and then extracted with DCM/methanol (10:1) three times, dried over sodium sulfate and concentrated under reduced pressure to furnish compound 3 (290 mg, 67.4%) as a clear yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ = 3.90-3.57 (m, 24H), 3.50 (t, *J*=5.1, 2H), 3.38 (t, *J*=4.8, 2H), 1.47 (broad s, 2H).



4

Synthesis of Azido-octaethylene glycol-lipoic acid conjugate (4) ***N-(23-azido-3,6,9,12,15,18,21-heptaoxatricosyl)-5-(1,2-dithiolan-3-yl)pentanamide***

Azido-amine-octaethylene glycol 3 (100 mg, 0.252 mmol), lipoic acid (52.0 mg, 0.252 mmol) and EDC (48.0 mg, 0.252 mmol) were dissolved in 3 mL anhydrous DMF at room temperature, followed by addition of DMAP (1.4 mg, 0.010 mmol). Reaction stirred for 4.5 hours, then was concentrated under reduced pressure. Crude product was purified on preparative silica TLC using DCM/methanol 12:1 to obtain compound 4 as a clear yellow liquid (100 mg, 68.1%). ^1H NMR (400 MHz, $CDCl_3$) δ = 6.30 (s, 1H), 3.68 – 3.48 (m, 26H), 3.42 – 3.37 (m, 2H), 3.37 – 3.29 (m, 2H), 3.19 – 3.01 (m, 2H), 2.41 (dt, *J*=19.2, 6.5, 1H), 2.15 (t, *J*=7.5, 2H), 1.86 (dq, *J*=13.8, 6.9, 1H), 1.72 – 1.53 (m, 4H), 1.49 – 1.34 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 172.66, 70.54, 70.50, 70.47, 70.42, 70.41, 70.39, 70.37, 70.36, 70.07, 69.88, 69.79, 56.29, 50.54, 40.09, 39.03, 38.32, 36.15, 34.53, 28.78, 25.24.

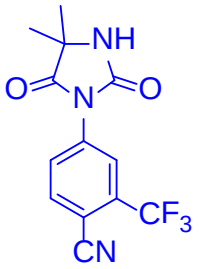


5

Synthesis of 4-Ethynylbenzyl tosylate (5) ***4-ethynylbenzyl 4-methylbenzenesulfonate***

4-Ethynylbenzyl alcohol (2.59 g, 19.59 mmol) was dissolved in 200 mL THF. Potassium hydroxide (11.0 g, 195.9 mmol) and tosylchloride (11.2 g, 58.8 mmol) were added while stirring, and reacted for 12 hours at ambient temperature. Solids were then

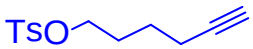
filtered off, and solution was concentrated *in vacuo*. Column chromatography (eluent 10:1 hexanes/ethyl acetate), followed by recrystallization in hexane/ethyl acetate gave 5 as an off white solid (2.74 g, 49%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.37 (3H, s), 3.11 (1H, s), 5.00 (2H, s), 7.16 (2H, d, J = 8.0), 7.28 (2H, d, J = 8.0), 7.37 (2H, d, J = 8.0), 7.74 (2H, d, J = 8.0).



6

Synthesis of cyano-nilutamide (6) ***4-(4,4-dimethyl-2,5-dioxoimidazolidin-1-yl)-2-(trifluoromethyl)benzonitrile***

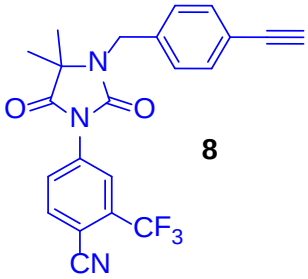
4-Fluoro-2-(trifluoromethyl)benzonitrile (4.02 g, 21.3 mmol) was added to Hydantoin (13.6 g, 106.3 mmol) and Potassium Carbonate (4.40 g, 31.9 mmol) in 60 mL DMF and stirred at 45 °C under argon for 48 hours. Reaction mixture was then diluted in ethyl acetate and washed three times with water. Organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. Column chromatography (eluent 30:1 DCM/Methanol) gave 1 as a white solid (4.62 g, 74%). ^{1}H NMR (400 MHz, $(CD_3)_2$-CO) δ 1.54 (6H, s), 7.80 (1H, s), 8.13 (1H, dd, J = 1.8 Hz, J = 8.4 Hz), 8.20 (1H, d, J = 8.4 Hz), 8.26 (1H, d, J = 1.8 Hz)



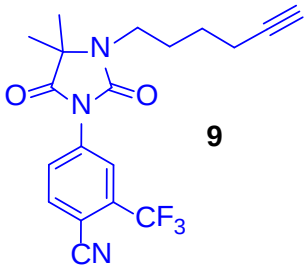
7

Synthesis of 5-Hexynyl tosylate (7) ***hex-5-yn-1-yl 4-methylbenzenesulfonate***

5-Hexynyl alcohol (3.00 g, 30.6 mmol), triethylamine (4.64 g, 45.8 mmol) and tosylchloride (8.74 g, 45.8 mmol) were dissolved in 100 mL DCM, followed by addition of catalytic 4-dimethylaminopyridine. Reaction stirred for 48 hours at ambient temperature, then solution was washed with 200 mL H_2O, 150 mL saturated aqueous NH_4Cl, and lastly 150 mL brine. Organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. Column chromatography (eluent 12:1 hexanes/ethyl acetate) gave 7 as a clear liquid (6.95 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 – 1.60 (2H, m), 1.61 – 1.81 (2H, m), 1.89 (1H, s), 2.10 (2H, t, J = 5.5 Hz), 2.39 (3H, s), 4.00 (2H, t, J = 6.1 Hz), 7.30 (2H, d, J = 7.8 Hz), 7.73 (2H, d, J = 7.9 Hz) ppm.



8



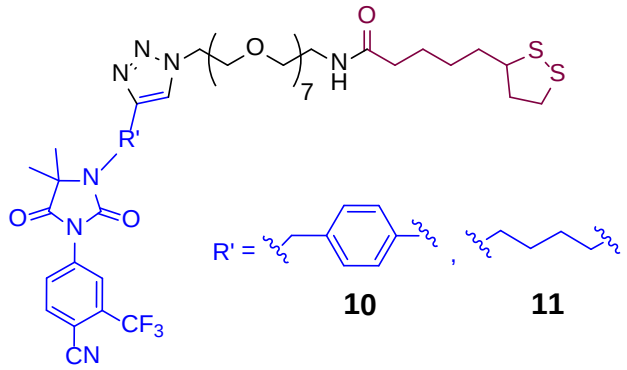
9

Synthesis of Aryl-cyano-nilutamide-alkyne (8) ***4-[3-[(4-ethynylphenyl)methyl]-4,4-dimethyl-2,5-dioxo-1-imidazolidinyl]-2-(trifluoromethyl)-benzonitrile***

Compound 6 (565.2 mg, 1.90 mmol) was dissolved in 7 mL DMF under argon, followed by addition of NaH (60% in mineral oil, 129.3 mg, 3.23 mmol) and stirring for 2 hours at ambient temperature. Then 5 (1,089 mg, 3.80 mmol) was added and reaction was stirred for 11 hours at 53 °C. Mixture was then dissolved in 100 mL 3:1 ethyl acetate/hexanes and washed 3 times with 150 mL brine. Organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. Column chromatography (eluent 3:1 hexanes/ethyl acetate) gave 8 as a white solid (537.9 mg, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (6H, s), 3.09 (1H, s), 4.57 (2H, s), 7.30 (2H, d, J = 8.4 Hz), 7.41 (2H, d, J = 8.3 Hz), 7.86 (1H, d, J = 8.4 Hz), 8.00 (1H, dd, J = 1.9, 8.4 Hz), 8.14 (1H, d, J = 7.3 Hz) ppm.

Synthesis of Alkyl-cyano-nilutamide alkyne (9) ***4-[3-(4-ethynylbutyl)-4,4-dimethyl-2,5-dioxo-1-imidazolidinyl]-2-(trifluoromethyl)benzonitrile***

Reaction of 6 (1.00 g, 3.364 mmol) with NaH and then 7 (1.273 g, 5.046 mmol) as described for the synthesis of 8, followed by column chromatography (eluent 3:1 hexanes/ethyl acetate) gave 9 as a white solid (1.192 g, 94%). ^{1}H NMR (400 MHz, cdcl3) δ 1.50 (5H, s), 1.52 – 1.63 (2H, m), 1.67 – 1.85 (2H, m), 1.88 – 2.02 (1H, m), 2.05 – 2.33 (2H, m), 3.18 – 3.46 (2H, m), 7.87 (1H, d, J = 8.4 Hz), 7.97 (1H, dd, J = 1.8, 8.4 Hz), 8.11 (1H, d, J = 1.5 Hz) ppm.



Synthesis of Aryl-cyano-nilutamide-triazole-PEG-lipoic acid conjugate (β-Bic, 10) ***N-(23-(4-(4-((3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-2,4-dioxoimidazolidin-1-yl)methyl)phenyl)-1H-1,2,3-triazol-1-yl)-3,6,9,12,15,18,21-heptaoxatricosyl)-5-(1,2-dithiolan-3-yl)pentanamide***

Aryl-cyano-nilutamide alkyne 8 (128.9 mg, 0.3415 mmol) and azido-octaethylene glycol-lipoic acid 4 (199 mg, 0.3415 mmol) were dissolved in anhydrous DMSO under inert atmosphere. DIPEA (0.12 mL, 0.6830 mmol) and CuI (32.5 mg, 0.1708 mmol) were then added, and stirred overnight. Reaction was then diluted with 25 mL of NH_4OH/saturated NH_4Cl (1:4) and 25 mL DCM and stirred vigorously for 5 minutes, followed by washing organic layer twice with NH_4OH/saturated NH_4Cl (1:4), dried over sodium sulfate and concentration under reduced pressure. Purification on silica gel with a gradient mobile phase of DCM/methanol from 130:1 to 20:1 afforded compound 10 as a viscid semisolid (257.6 mg, 78.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.11 (s, 1H), 8.01 – 7.93 (m, 2H), 7.85 (d, *J*=8.4, 1H), 7.74 (d, *J*=7.7, 2H), 7.34 (d, *J*=7.9, 2H), 6.43 (s, 1H), 4.56 (s, 2H), 4.49 (t, *J*=4.8, 2H), 3.81 (t, *J*=4.8, 2H), 3.61 – 3.41 (m, 26H), 3.36 – 3.29 (m, 2H), 3.10 – 2.94 (m, 2H), 2.42 – 2.26 (m, 1H), 2.09 (t, *J*=7.5, 2H), 1.78 (dq, *J*=13.9, 6.9, 1H), 1.67 – 1.45 (m, 5H), 1.42 – 1.27 (m, 8H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 174.53, 172.88, 153.17, 146.79, 136.57, 136.50, 135.28, 130.65, 128.46, 128.04, 126.02, 123.31, 122.97, 122.92, 121.24, 114.99, 107.97, 70.40, 70.37, 70.06, 69.76, 69.35, 62.16, 56.36, 50.29, 43.38, 40.13, 39.08, 38.38, 36.14, 34.55, 28.82, 25.32, 23.58; HRMS (MALDI) calculated for $C_{46}H_{63}F_3N_7O_{10}S_2^+$ [*M*+H$^+$] 994.4030, observed 994.4019.

Synthesis of Alkyl-cyano-nilutamide-triazole-PEG-lipoic acid conjugate (α-Bic, 11) ***N-(23-(4-(4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-2,4-dioxoimidazolidin-1-yl)butyl)-1H-1,2,3-triazol-1-yl)-3,6,9,12,15,18,21-heptaoxatricosyl)-5-(1,2-dithiolan-3-yl)pentanamide***

Reaction of alkyl-cyano-nilutamide alkyne 9 (140 mg, 0.3415 mmol) and azido-octaethylene glycol-lipoic acid 4 (199 mg, 0.3415 mmol) as described for compound 10 afforded compound 11 as a viscid semisolid (270.4 mg, 80.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.05 (d, *J*=1.6, 1H), 7.92 (dd, *J*=8.5, 1.9, 1H), 7.82 (d, *J*=8.5, 1H), 7.42 (s, 1H), 6.41 (s, 1H), 4.40 (t, *J*=5.0, 2H), 3.75 (t, *J*=5.1, 2H), 3.59 – 3.41 (m, 28H), 3.37 – 3.21 (m, 5H), 3.13 – 2.93 (m, 2H), 2.83 (s, 1H), 2.66 (t, *J*=6.6, 2H), 2.34 (td, *J*=12.4, 6.5, 1H), 2.08 (t, *J*=7.5, 2H), 1.79 (td, *J*=13.8, 6.9, 1H), 1.73 – 1.63 (m, 4H), 1.62 – 1.48 (m, 4H), 1.41 (s, 6H), 1.38 – 1.26 (m, 2H); ^{13}C NMR (400 MHz, $CDCL_3$) δ = 174.57, 172.81, 152.61, 147.00, 136.49, 135.22, 127.93, 122.88, 121.99, 115.00, 107.84, 70.40, 70.38, 70.33, 70.06, 69.77, 69.44, 61.80, 56.35, 50.00, 40.13, 39.98, 39.05, 38.38, 36.14, 34.56, 28.82, 28.77, 26.77, 25.31, 24.95, 23.36; HRMS (MALDI) calculated for $C_{43}H_{65}F_3N_7O_{10}S_2^+$ [*M*+H$^+$] 960.4156, observed 960.4186.

Nanoparticle Synthesis and Characterization

Gold nanoparticles were synthesized using the methods of Turkevich[2] and Frens.[3] Briefly, 10 mL of 16.6 mM trisodium citrate was rapidly added to 190 mL of 0.638 mM aqueous chloroauric acid solution under reflux with stir. The solution was allowed to react for 20 min and the crude nanoparticle product was centrifuged (4185 × g) for 20 min. Particle sizing was performed using transmission electron microscopy (TEM, JEOL 100CX II) and image analysis software (ImageJ). Octanol/water partition coefficient (log *P*) was determined using the shake-flask method and experimentally determined particle molar extinction cross sections reported by Liu et al. for a 26 ± 6 nm diameter gold nanoparticle[4]. Hydrodynamic diameter was measured using a NanoZS Zetasizer particle analyzer (Malvern, 633 nm). Optical extinction was characterized by UV-Vis absorption spectroscopy (Ocean Optics, HR4000CG-UV-NIR). Particles used in these studies were 29 ± 4 nm in diameter (λ_{max}~532 nm).

Conjugation of the Nanoparticles

Thiol-PEGylated antiandrogen ligands were solubilized in DMSO and added to aqueous solutions of purified gold nanoparticles at varying molar excesses and allowed to react overnight under sonication, in dark, at 30 °C (Figure S1). The conjugates were purified by centrifugation (30 min, 4185 × g) and stored at 4 °C prior to use. Surface adsorbate coverages were determined by UV absorption assay (α-Bic, 280 nm; β-Bic, 262 nm) using experimentally determined cross sections for the particle[4] and ligand. α-Bic and β-Bic nanoconjugates used in these studies were functionalized with 95% PEG-thiol (5 kDa, Lysan Bio) and 5% antiangrogen ligand (2.25 ± 0.02 × 10^3 α-Bic particle^{-1}; 1.56 ± 0.08 × 10^3 β-Bic particle^{-1}) and were conjugated at a 1.0378 × 10^4 and 1.5567 × 10^4 molar excess of thiolated ligands, respectively. Control particles were fully PEGylated.

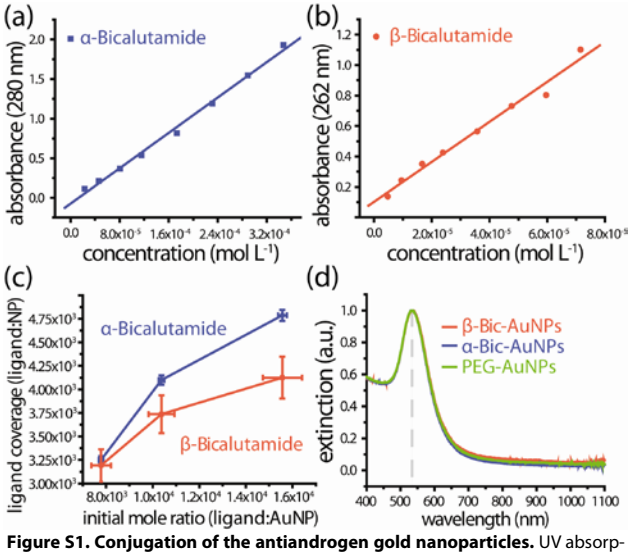


Figure S1. Conjugation of the antiandrogen gold nanoparticles. UV absorption calibration curves for **(a)** α-Bicalutamide and **(b)** β-bicalutamide antiandrogen ligands in water. **c,** Antiandrogen ligand coverages on 29 ± 4 nm diameter gold nanoparticles as a function of ligand excess present during conjugation. **d,** Normalized optical extinction spectra of the purified antiandrogen and PEGylated gold nanoparticles in water. Error bars represent SD.

Docking Studies

Docking was performed using Autodock Vina,[5] and rendered using PyMOL 1.6. The apo, human, wild-type AR homology model[6] was used as a target macromolecule, and has the dynamic, hinge-like helix-12 in the open position. This is vital as crystal structures of the AR are in the closed, agonist form, although helix-12 has shown highly varied positioning in antagonist-bound crystal structures for other steroid receptors such as the estrogen receptor (ER). The ligands were prepared using Autodock Tools 1.5.4 by assigning Gasteiger charges, reducing the linker length, merging non-polar hydrogens and setting torsional bonds. Docking runs were performed with a 20Å cubic grid surrounding the binding pocket, with solutions found using an exhaustiveness of 8.

Cell Culture and In Vitro Analysis

DU-145 human prostate carcinoma cells (ATCC) were subcultured in Dulbecco's modified eagle's medium (DMEM) supplanted with 10% v/v fetal bovine serum (FBS), 20 I.U./mL penicillin, 100 ug/mL streptomycin, and 250 ng/mL amphotericin B at 37 °C in a 5% CO_2 humidified atmosphere. Cell viability was determined from mitochondrial dehydrogenase activity by tetrazolium assay (XTT, Sigma). All experiments were performed on cells passaged 12 h prior. Unless otherwise noted, nanoparticle concentration indicates particle molarity.

Radioligand Binding

Radioligand binding (Ricerca Biosciences) was performed using rat androgen receptor and [^{3}H]mibolerone (PanVera) in triphosphate buffer (pH 7.4). 78 ng of AR was incubated with 1.5 nM [^{3}H]mibolerone for 4 h at 4 °C, then incubated with a hydroxyapatite slurry over 15 minutes and filtered. The filters are washed 3 times and counted to determine [^{3}H]mibolerone specifically bound.

GPRC6A Expression and Stimulation

PC-3, 22Rv1, and LNCaP prostate carcinoma cells (ATCC) and non-malignant RWPE-1 prostate cells (ATCC) were subcultured in RPMI 1640 supplemented with 10% v/v fetal bovine serum (Gibco). Cells (10^3 well^{-1}) were cultured in triplicate in 96-well flat-bottomed microculture dishes in the presence and absence of various compounds for 72 hr. *Gprc6a* expression levels were analyzed by total RNA levels isolated using a quantitative real-time polymerase chain reaction RT-PCR protocol (Perkin-Elmer), as described previously.[7] Briefly, PCR reactions contained 100 ng of template (cDNA or RNA), 300 nM each of forward and reverse primer, and 1× iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) in 50 µL. Samples were amplified for 40 cycles in an iCycler iQ Real-Time PCR Detection System (Bio-Rad) with an initial melt at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. PCR product accumulation was monitored at multiple points during each cycle by measuring the increase in fluorescence caused by the binding of SybrGreen I to dsDNA. The threshold cycle (C_t) of tested-gene product from the indicated genotype was normalized to the C_t for cyclophilin A. The primers for human *Gprc6a* consisted of hGPRC6A.F130: cataattggaggtttgtttgc and hGPRC6A.R346: cactgtgacttctgtacaagtgtc. Dissociation analysis was used to confirm the presence of a single transcript and lack of primer-dimer amplification in all PCR reactions.

Cyclic adenosine monophosphate (cAMP) accumulation in response to GPRC6A stimulation was determined via spectrophotometric enzyme-linked immunosorbent assay (EIA kit, Cayman Chemical) of GPRC6A$^-$/AR$^-$ HEK-293 (ATCC) and transfected[8] GPRC6A$^+$/AR$^-$ HEK-293.mGPRC6A cell extracts according to the manufacturer's instructions. HEK-293 (ATCC) and HEK-293.mGPRC6A cells (10^5 well^{-1}) were subcultured in triplicate in DMEM containing 10% v/v fetal calf serum and 1% v/v penicillin/streptomycin (Gibco). Quiescent cells were treated overnight with stimulators as indicated, then 100 nM forskolin for 30 minutes at 37 °C. Treatment was stopped and the cells were lysed by replacing media with 0.5 ml 0.1 N HCl. cAMP levels were measured following the manufactory's protocol.

Imaging

Nanoconjugate localization was determined by optical dark-field scattering microscopy. Sterile glass coverslips (18 mm dia) were incubated with 0.04 mg/mL rat tail collagen/DPBS for 6 h at 37 °C in a 5% CO_2 humidified atmosphere and rinsed in DPBS. Cells were passaged onto the coverslips and after 12 h, growth solutions were replaced with fresh media containing 0.2 nM gold nanoparticle conjugates. After incubation (24 h), cell monolayers were rinsed in DPBS and fixed in 4 % paraformaldehyde/DPBS at 4 °C for 15 min. The fixed coverslips were coated with glycerol, mounted, and sealed onto glass slides. Optical dark-field scattering microscopy was performed using an inverted objective Olympus IX70 microscope fitted with a dark-field condenser (U-DCW), 100x/1.35 oil Iris objective (UPLANAPO), (white light) tungsten lamp, and a Nikon D200 digital SLR camera. Please note that dark-field scattering optics are distinct from confocal optics and that fluorescence images were obtained on a separate instrument.

Fluorescence microscopy was performed on a Zeiss NLO META confocal microscope. Antiandrogen nanoparticles were labeled with a carboxyfluorescein-terminal PEG-SH (5 kDa). 5-(and-6)-carboxyfluorescein succinimidyl ester (Molecular Probes) was re-

acted with amine-terminal PEG-SH (5 kDa, Lysan Bio) in pH 7.4 DPBS buffer for 24 h in dark, with sonication. Fluorescently-labeled PEG-SH was dialyzed twice (5 Da MWCO, Spectra/Por) at a 10^3 volume excess for 24 h with three solvent exchanges. Carboxyfluorescein-terminal PEG-SH was incubated with the antiandrogen nanoparticles for 12 h with sonication at RT and purified by centrifugation (6000 rpm, 15 min). Cell cultures were incubated with 5.0 μM Alexa Fluor 647-dextran (10 kDa) to label endo/lysosomal compartments and 0.33 nM of the fluorescently-labeled nanoconjugates. After 12 h, DAPI was added to 300 nM and allowed to incubate for 15 min. The cell monolayers were then twice rinsed with DPBS and fixed in 4 % paraformaldehyde/DPBS at 4 °C for 30 min. The microscopy samples were again twice rinsed with DPBS and incubated with 1 mg/mL $NaBH_4$ at 4 °C to minimize fixative-induced fluorescence. After 5 min, the borohydride solution was replaced with fresh solution and allowed to incubate for another 5 min at 4 °C. The samples were then rinsed three times with DPBS and imaged.

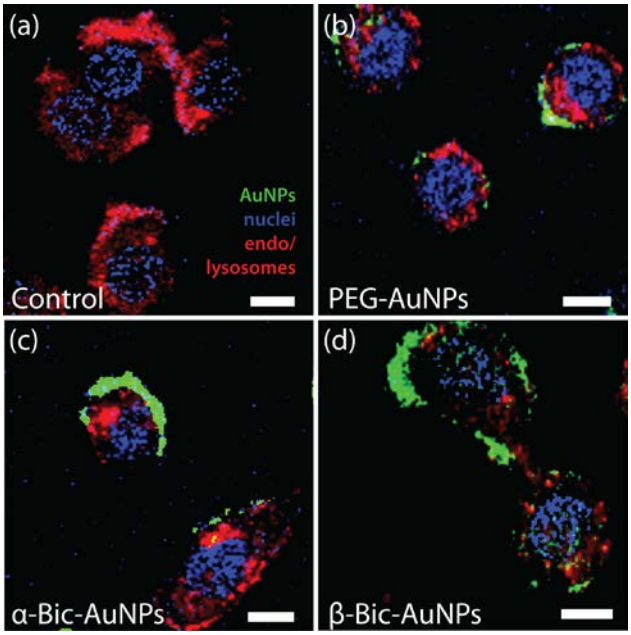


Figure S2. Nonspecific cell surface binding of antiandrogen gold nanoparticles with an androgen receptor null cancer cell line. Confocal fluorescence microscopy of androgen receptor negative human squamous cell carcinoma (SCC) cells illustrating non-specific membrane binding antiandrogen gold nanoparticle conjugates. HSC-3 cells were incubated with antiandrogen- or control-gold nanoparticles (green) and a dextran endo/lysosomal marker (red) for 12 h. Nuclei were stained with DAPI (blue). Scale bar represents 10 μm.

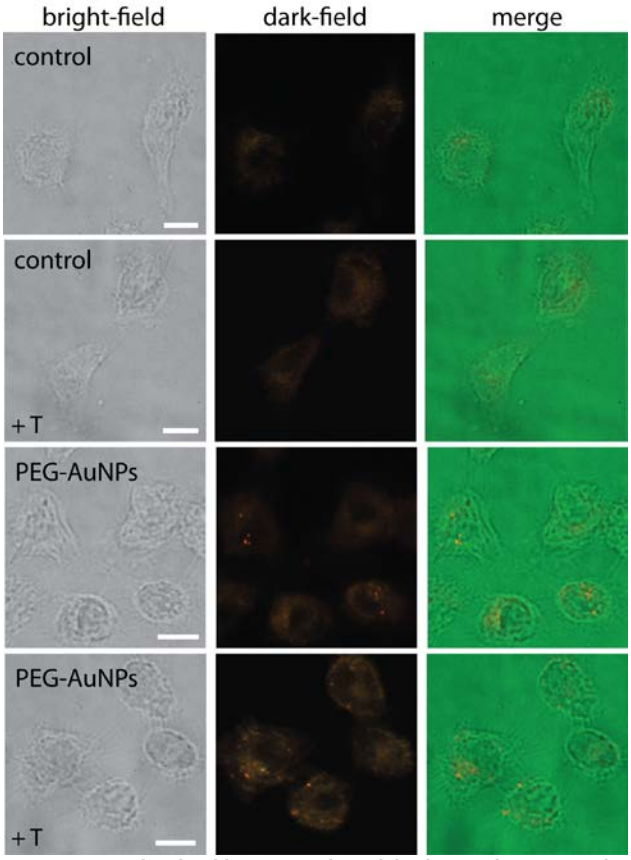


Figure S3. PEGylated gold nanoparticles exhibit low, androgen stimulation-independent accumulation in antiandrogen treatment-resistant prostate cancer cells expressing membrane-androgen receptor (mAR) and G protein-coupled receptor GPRC6A. Optical dark-field scattering microscopy of DU-145 prostate cancer cells showing baseline accumulation levels of PEGylated control gold nanoparticles both in the presence and absence of androgen-stimulated mAR-upregulation by testosterone (T, 10^{-6} M). Scale bars represent 10 μm.

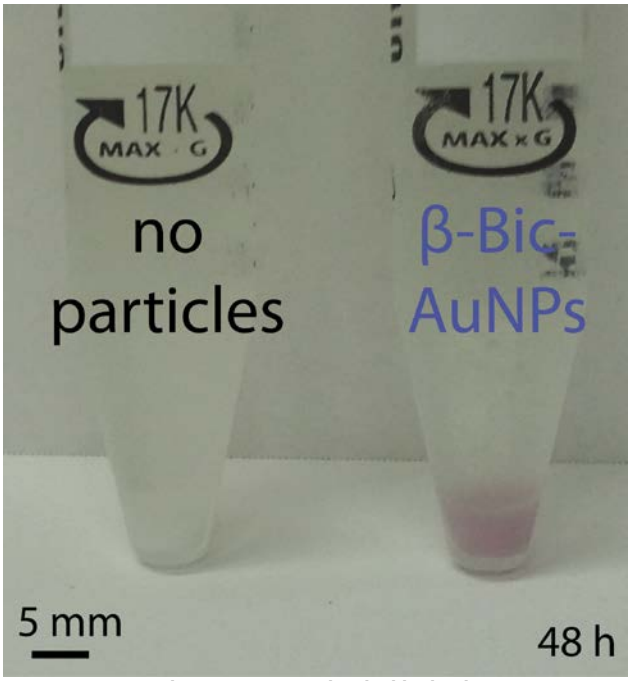


Figure S4. Antiandrogen nanoparticles highly localize in treatment-resistant prostate cancer cells. β-Bicalutamide gold nanoparticles were incubated with DU-145 prostate cancer cells at 0.34 nM for 48 h. Cell monolayers were washed with buffer and viable, adherent cells were trypsinized, and centrifuged at 1500 rpm for 7 min. Cell pellets incubated with β-Bic gold nanoparticles display the characteristic ruby color observed from solutions of spherical gold colloid.

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