Supplemental Information for

Targeted and armed oncolytic adenovirus via chemoselective modification

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Supplementary Methods.

All chemical reagents were obtained from commercial sources and used without further purification unless otherwise noted. NMR spectra were recorded on a Varian 300 MHz NMR spectrometer or Varian 400 MHz NMR spectrometer. Mass spectra for the small molecules were obtained using an Agilent 1100 LC/MSD VL instrument. Thin Layer Chromatography (TLC) was performed on Merck DC-alufolien with Kieselgel 60F-254 and column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM). The α-FLAG M2 conjugate was obtained from Sigma (St. Louis, MO). Centri Sep spin columns were obtained from Princeton Separations (Adelphia, NJ). Mouse breast cancer cell line, 4T1 was obtained from ATCC (Manassas, VA).RP-HPLC was performed using a L201243 Shimadzu on a C12 Jupiter column (250 x 10mm; Phenomenex). UV-Visible absorbance was recorded on a Beckmann Coulter DU 730. Electrophoresis gels were scanned on a Typhoon 9400 fluorescent gel scanner.

Synthesis of Homopropargylglycine (HPG). Homopropargylglycine was synthesized as reported by Dong *et.al.*[1]

Synthesis of SB-T-1214-Linker-PEG$_3$-Azide (1-11). Shown in supplemental scheme 1.

3*H*-Benzo[b]thiophen-2-one (1-1). A 5.0 g (28 mmol) aliquot of thianaphthene-2-boronic acid was dissolved in ethanol and 9.2 mL of hydrogen peroxide was added to the mixture drop wise. The solution was stirred for 24 hours at room temperature. The reaction was monitored by TLC (Rf = 0.73; developed on 3:1 hexanes: ethyl acetate). After 24 h, the mixture was diluted with water and the organic layer was extracted with three allotments of $CHCl_3$. The organic layer was combined and dried over $MgSO_4$. The solvent was removed and purification of the crude product was done using column chromatography on silica gel using increasing amounts of ethyl acetate in hexanes to yield **1-1** (3.2 g, 86%), a light-brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 2 H), 7.17 (d, 1 H), 7.21 (dd, 2 H), 7.24 (d, 1 H). All data are consistent with literature reported values.[2,3]

2-Sulfhydrylphenylacetic acid (1-2). A 2.0 g (13 mmol) aliquot of **1-1** was dissolved in 65 mL THF and warmed to 60 °C. A solution of 3.4 g (80 mmol) LiOH dissolved in 65 mL H_2O was degassed and added dropwise to the warmed solution containing **1-1**, producing a cloudy brown solution. The reaction was stirred overnight at 60 °C and monitored via TLC. After 24 hours, the mixture was removed from heat and cooled to room temperature. At room temperature, the mixture was diluted with H_2O and diethyl ether. The pH of the mixture was adjusted to pH 2 using 1 M HCl. The organic layer was extracted, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification was done using column chromatography on silica gel using increasing amounts of ethyl acetate in hexanes to yield **1-2** with impurity to be purified. All data are consistent with literature reported values.[2,3]

1,2-di(pyridine-2-yl)disulfane (1-3). A 12.5 g (113 mmol) aliquot of pyridine-2(1H)-thione was dissolved in 225 mL CH_2Cl_2. Subsequently, 53 g KMnO4 was added slowly

over a period of 20 minutes and the solution was stirred vigorously producing a black solution. The reaction was monitored via TLC (Rf = 0.66; developed on 1:1 hexanes: ethyl acetate). After 3.5 hours, the solution was filtered over celite and concentrated *in vacuo* to yield **1-3** (11.7 g, 95%), as a light-yellow solid without further purification: m.p. 55-56 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (dd, J = 7 Hz, 2 H), 7.61 (d, J = 7 Hz, 2 H), 7.62 (dd, J = 7 Hz, 2 H) 8.46 (d, J = 7 Hz, 2 H). In a second trial 7.35 g (66 mmol) pyridine-2(1H)-thione was used to obtain 3-III (7.5 g, 100% yield). All data are consistent with literature reported values.[4,5]

4-Sulfhydrylpentanoic acid (1-5). A 5.25 g (52.4 mmol) aliquot of γ-valerolactone was refluxed with 21.2 g of HBr to 70 ^{o}C. After reflux was established, 20.0 g of thiourea was added to the mixture and solution was further refluxed for 24 hours. After 24 hours of reflux, the clear solution was diluted with ice-water and washed with three allotments CH_2Cl_2 and either. The aqueous layer was then treated with 8 N NaOH to adjust the pH to 10. The mixture was refluxed for 24 hours. After 24 h, the reaction was allowed to cool to room temperature. The pH was adjusted to 1 with 1 N HCl. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine and H_2O. The organic layer was dried under $MgSO_4$ and concentrated *in vacuo* to yield **1-5** (3.01 g, 40%), as a yellow oil with strong stench: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.5 (bs, 1 H), 2.904-2.950 (m, 1H), 2.461-2.501 (m, 2 H), 1.900-1.951 (m, 1H), 1.696-1.792 (m, 1 H), 1.417 (d, *J* = 3.5, 1 H), 1.323 (d, *J* = 3.5 Hz, 3 H). All data are consistent with literature reported values.[6]

4-(Pyridin-2-yldisulfanyl)pentanoic acid (1-6). A 0.67 g (5.00 mmol) aliquot of **1-3** was dissolved in 25 mL absolute ethanol. To the solution was added 6.61 g (30.0 mmol) of **1-5** dissolved in 150 mL. The reaction was allowed to stir at room temperature and monitored via TLC (Rf = 0.296; developed on 1:1 hexanes: ethyl acetate). After 16 h, the reaction solution was evaporated. The resulting yellow oil was dissolved in CH_2Cl_2 at purified via silica gel column chromatography to obtain unreacted **1-3** and desired **1-6** (hexanes: ethyl acetate 3:1), and to obtain dimmers and reaction side products (hexanes: ethyl acetate 1:1). 1 g of **1-6** was obtained (82.4 %) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.311 (d, J = 3.3 Hz, 3 H), 1.875-2.003 (m, 2 H), 2.525 (t, J = 3.9 Hz, 2 H), 2.981-3.026 (m, 1H), 7.060-7.101 (m, 1 H), 7.604-7.661 (dt, 1 H), 7.721-7.748 (m, 1 H), 8.456 (m, 1 H). [M^{1+}] 244.0.

Triisopropylsilyl 4-(pyridin-2-yldisulfanyl)pentanoate (1-7). A 1.00 g (4.1 mmol) aliquot of **1-6** and 0.83 g of TEA was dissolved in 20 mL of CH_2Cl_2 and cooled to 0 °C under inert conditions. To the mixture, 1.05 mL of TIPSCl was added dropwise. The mixture was stirred at room temperature and monitored *via* TLC (Rf = 0.93; developed on 3:1 hexanes: ethyl acetate). The reaction was quenched with NH_4Cl and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification was done *via* column chromatography on silica gel with increasing amounts of eluent (hexanes: ethyl acetate) to yield 1.49 g of light yellow oil, **1-7** (91 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.421 – 8.396 (dq, 1 H), 7.721 – 7.688 (dt, 1 H), 7.621 – 7.563 (m, 1H), 7.619 (t, *J* = 7.6, 1 H), 7.058 (t, *J* = 5.2 Hz, 1 H), 3.011 – 2.960 (m, 1 H), 2.253 – 2.468 (m, 2 H), 2.004 – 1.866 (m, 2 H), 1.300 (m, 3 H), 1.007 (m, 21 H).

2-(2-5-Oxo-5-(triisopropylsilyloxy)pentan-2-yldisulfanylphenyl)acetic acid (1-8). A 100 mg (0.251 mmol) aliquot of **1-7** was dissolved in 0.63 mL of THF and cooled to -10 °C under inert conditions. To this mixture, 40 mg of **1-2** previously dissolved in 0.63 mL of THF was added dropwise. The mixture was stirred at -10 °C for 30 minutes and was then warmed to room temperature for 60 minutes. The reaction was monitored *via* TLC (Rf = 0.77; developed on 3:1 hexanes: ethyl acetate). The solvent was be evaporated and the residual was purified *via* column chromatography on silica gel with increasing amounts of eluent (hexanes: ethyl acetate) to yield unreacted **4-V**, **1-8**, and pyridine-2(*1H*)-thione. Dark brown oil was obtained with a weight of 81 mg of **1-8** (71%). In a second trail, 280 mg (0.501 mmol) of **1-7** was used to obtain 279 mg of **1-8** (88 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.803 – 7.784 (d, *J* = 7.6 Hz, 1 H), 7.297 – 7.267 (m, 1H), 7.211 – 7.178 (m, 1 H), 3.896 (s, 2 H), 2.918 – 2.884 (m, 1 H), 2.435 – 2.388 (m, 2 H), 1.954 – 1.917 (m, 1 H), 1.840 – 1.805 (m, 1 H), 1.310 – 1.235 (m, 3 H), 1.060 (m, 21 H).

SB-T- 1214-Linker-CO_2TIPS (1-9). To 80 mg (0.2 mmol) **1-8** dissolved in 1.6 mL dry CH_2Cl_2 at 0 °C was added 0.03 mL (1.2 eq) of DIC, dropwise. The temperature was maintained and the solution was stirred for 10 min during which the solution became cloudy. After 10 min, to the solution was added a mixture of 150 mg (0.2 mmol) SB-T-1214 and 0.52 mg (0.3 eq) DMAP dissolved in dry CH_2Cl_2, dropwise. The temperature was maintained and the mixture was stirred for 5 hour. After 5 h, the solvent was evaporated and the resulting crude was purified via column chromatography on silica gel to give **1-9** (134 mg 60% yield), as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.988 (m, 2 H), 1.046 (m, 21 H), 1.079 (m, 2 H), 1.147 (m, 3 H), 1.255 (m, 8 H), 1.358 (s, 6 H), 1.663 (s, 2 H), 1.717 (s, 2 H), 1.775 (m, 3 H), 1.903 (m, 2 H), 2.041 (s, 3 H), 2.380 (m, 4 H), 2.540 (m, 1 H), 3.784 (s, 1 H), 4.125 (s, 2 H), 4.183 (m, 1 H), 4.313 (m, 1 H), 4.407 (m, 1 H), 4.981 (m, 2 H), 5.142 (m, 1 H), 5.678 (d, *J* = 7 Hz, 1 H), 6.221 (m, 1 H), 6.295 (d, *J* = 7 Hz, 1 H), 7.298 (m, 1 H), 7.587 – 7.616 (t, *J* = 5, 1 H), 7.784 – 7.822 (t, *J* = 11 Hz, 1 H), 8.094 – 8.110 (d, *J* = 6.4 Hz, 1 H).

SB-T- 1214-Linker-CO_2H (1-10). A 50 mg (0.04 mmol) aliquot of **1-9** was dissolved in 2.5 mL of a 1:1 mixture of acetonitrile: pyridine and cooled to 0 °C under inert conditions. To the mixture, 0.60 mL of HF/pyridine was added dropwise. The reaction was stirred at room temperature for 24 hours and monitored *via* TLC (developed on 1:1 hexanes: ethyl acetate). Upon completion, the reaction was quenched with aqueous 10% citric acid and extracted with ethyl acetate. The organic layer was collected, was washed with $CuSO_4$, then with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done *via* column chromatography on silica gel with increasing amounts of eluent (hexanes: ethyl acetate) to yield **1-10** (40 mg, 91%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.953-1.003 (m, 1 H), 1.144 (s, 3 H), 1.251 (m, 9 H), 1.659 (s, 3 H), 1.708-1.726 (d, *J* = 7.2 Hz, 3 H), 1.854 (m, 1 H), 1.903 (s, 3 H), 1.936-2.133 (m, 2 H), 2.352 (m, 4 H), 2.490-2.566 (m, 1 H), 2.640-2.677 (t, J_1 = 7.2 Hz, J_2 = 7.6 Hz, 1 H), 2.998 (m, 1 H), 3.790-3.808 (d, *J* = 4 Hz), 4.167-4.189 (d, *J* = 8.8 Hz), 4.291-4.312 (d, *J* = 8.4 Hz), 4.400-4.444 (q, J_1 = 6.8 Hz, J_2 = 4 Hz, J_3 = 6.8 Hz, 1 H), 4.785-4.806 (m, 1 H), 4.926-4.976 (m, 2 H), 5.085-5.106 (m, 1 H), 5.660-5.677 (d, *J* = 6.8 Hz, 1 H), 6.165-6.210 (m,

1 H), 6.279 (s, 1 H), 7.276-7.341 (m, 1 H), 7.454-7.493 (t, J_1 = 7.6 Hz, J_2 = 8 Hz, 2 H), 7.581-7.618 (t, J_1 = 7.6 Hz, J_2 = 7.2 Hz, 1 H), 7.783-7.805 (m, 1 H), 8.101-8.122 (m, 2 H);; $C_{58}H_{74}NO_{18}S_2$ (M = 1136.4), TOF MS ES+: m/z 1136.4.

SB-T-1214-Linker-PEG$_3$-Azide (1-11). To a solution of 15 mg (0.01 mmol) **1-10** and 1 mg DMAP dissolved in CH_2Cl_2 (0.01 M) was added 2 mg EDC dissolved in CH_2Cl_2 (0.5 mL). The mixture was stirred at room temperature and monitored via thin-layer chromatography. After 16 h, the reaction solvent was evaporated, and the resulting crude was dissolved in 5 mL ethyl acetate. The organic layer was washed with saturated aqueous NH_4Cl, then twice with water (5 mL) and once with brine (5 mL). The organic layer was collected, and concentrated *in vacuo*. The residual was purified via flash column chromatography on silica gel (hexanes: ethyl = 2:1) to give **1-11** (13 mg, 72% yield), as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.821-0.848 (m, 1 H), 0.939-0.968 (m, 2 H), 1.093-1.123 (m, 12 H), 1.223 (s, 5 H), 1.318 (s, 12 H), 1.725 (s, 8 H), 1.868 (d, J = 4.4 Hz, 6 H), 2.337 (s, 5 H), 2.600-2.633 (m, 2 H), 2.697 (bs, 1 H), 3.035 (m, 1 H), 3.772-3.825 (m, 2 H), 4.028 (bs, 1 H), 4.142 (d, J = 8.4 Hz, 1 H), 4.267 (d, J = 8.4 Hz, 1 H), 4.417 (m, 1 H), 4.793 (m, 1 H), 4.887-4.955 (m, 3 H), 5.150 (bs, 1 H), 5.637 (d, J = 6.8 Hz, 1 H), 6.142 (m, 1 H), 6.273 (s, 1 H), 7.029-7.059 (t, $J_1 = J_2$ = 6.8 Hz, 1 H), 7.420-7.458 (t, $J_1 = J_2$ = 7.6 Hz, 2 H), 7.550-7.619 (m, 2 H), 7.677 (d, J = 8 Hz, 1 H), 8.068 (d, J = 7.6 Hz, 2 H), 8.400 (bs, 1 H); TOF MS ES+: m/z 1304.5 [M^{28-}].

Synthesis of Phosphine-PEG-folate. Folic acid (50 mg, 0.114 mmol) was activated by *N,N'*-dicyclohexylcarbodiimide (82.4 mg, 0.4 mmol) and *N*-hydroxysuccinimide (46 mg, 0.4 mmol) in dry dimethylsulphoxide at RT for 12 hours and filtered with a syringe filter (pore size 0.2 µm). *O*-(*N*-trityl-3-aminopropyl)-*O*'-(3-aminpropyl)-diethyleneglycol (MW 462) was added to the solution at a molar ratio of 5:1 (folate-NHS: PEG) and stirred vigorously overnight. The resulting solution was acidified with trifluoroaceticacid (final concentration 1%) to remove C- protecting trityl group. The reaction was then neutralized with triethyl amine and concentrated in *vacuo*. A solution of the phosphine-PFP ester (10 mg, 0.02 mmol) and diisopropylethylamine (5 µL, 0.02 mmol) was made in anhydrous DMSO and added to the Folate-PEG (8.0 mg, 0.012mmol) under an atmosphere of argon. The mixture was rocked for 3 hours. The Phosphine-PEG-folate was precipitated by addition of diethyl ether. Crude product was purified on a Jupiter C-18 column with a gradient of 5% to 25% acetonitrile containing 0.1% TFA. Product eluted at 30 minutes. ^{1}H NMR (300 Mz, D_2O): δ 1.07 (2H, t), 1.10 (4H, t), 1.89 (4H, m), 1.92 (12H, m), 2.71 (1H. s), 3.40 (2H, m), 3.50-3.51 (2H, m), 3.92 (3H,s), 6.7 (2H, d), 7.5 (2H, d), 7.26 (4H, m), 7.78 (6H, m), 8.04 (1H, s), 8.47 (1H, d), 8.56 (1H, d), 9.0 (1H, s) ppm. ESIMS calculated for $C_{50}H_{56}O_{11}N_9P$ (MH^+) 990.0, found 992.02.

Synthesis of phosphine FLAG. Synthesized as described by Bertozzi *et. al.* [7] Briefly, all peptides were synthesized by standard Fmoc solid phase peptide synthesis protocol. In this case activated esters were formed using *N,N'*-diisopropylcarbodiimide and 1-Hydroxybenzotriazole. Alkyne functionalized peptides were produced by on-bead *N*-terminal functionalization with propynoic acid. Phosphine FLAG was obtained by on-bead *N*-terminal derivitization with 2-(Diphenylphosphino)terephthalic acid 1-methyl 4-

pentafluorophenyl diester (Sigma-Aldrich 679011). During all de-blocking steps 0.1 M HOBt was added to the 20% piperidine solution to alleviate aspartamide formation.

Cell culture. Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, Penicillin/Streptomycin and 0.5% Trypsin-EDTA were purchased from GIBCO (Grand Island, NY). RPMI 1640 minus Folic acid was obtained from Sigma (St. Louis, MO). Fetal calf serum (FCS) and Bovine Calf Serum (BCS) was from HyClone (Logan, UT). 293 cells were maintained in DMEM supplemented with 10% BCS, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin. ID8 cells were maintained in RPMI 1640 medium containing 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. 4T1 cells were maintained in RPMI 1640 medium containing 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. For 4T1 cells grown without Folate, RPMI 1640 minus Folic acid was supplemented with 10% FCS, 0.3 mg/mL L-glutamine, 2 mg/mL sodium bicarbonate, 100 U/mL penicillin, 100 μg/mL streptomycin. All cells were maintained in 100 x 20 mm Tissue Culture Dishes obtained from BD biosciences (Franklin Lakes, NJ) at 37°C and 5% CO_2.

Metabolic labeling of adenovirus type 5 with N-azidoacetygalactosamine.[8] HEK 293 cells were infected with wild type adenovirus particles with an MOI of 5pfu/cell. The complete media was supplemented with 50 μM peracetyl-N-azidoacetygalactosamine and the infected cells incubated at 37°C. The plates were harvested 42-46 hours post infection and virus particles purified over a gradient of 1.4 g/mL and 1.25 g/mL CsCl centrifuged at 32,000 rpm for 1 hour at 15°C. The virus band at the junction of the two CsCl bands was collected and further purified by an 18 hour centrifugation at 35,000 rpm over 1.33 g/mL of CsCl.

Metabolic labeling of adenovirus type 5 with Homopropargyglycine (HPG). HEK 293 cells were infected with wild type adenovirus particles with an MOI of 5 pfu/ cell. 18 hours post infection complete media was removed and the cells washed with TD buffer (25 mM Tris, 125 mM NaCl, 5 mM KCl and 1 mM Na_2HPO_4 at pH 7.5) at 37 °C for 20 minutes. HPG supplemented DMEM (-Met) media was then added to the infected cells and allowed to grow until 24 hours post infection. The labeling media was then removed and the cells were supplemented with complete media. The plates were harvested 46-48 hours post infection and virus particles purified over a gradient of 1.4 g/mL and 1.25 g/mL CsCl centrifuged using an SW41 rotor (Beckman) at 32,000 rpm for 1 hour at 15 °C. The virus band at the junction of the two CsCl bands was collected and further purified using an SW60 rotor (Beckman) by an 18 hour centrifugation at 35,000 rpm over 1.33 g/mL CsCl.

Metabolic labeling of adenovirus type 5 with Homopropargyglycine (HPG) and N-azidoacetylglucosamine. . HEK 293 cells were infected with wild type adenovirus particles with an MOI of 5pfu/cell. The complete media was supplemented with 50 μM peracetyl-N-azidoacetygalactosamine and the infected cells incubated at 37°C. 18 hours post infection growth media was removed and the cells washed with TD buffer (25 mM Tris, 125 mM NaCl, 5 mM KCl and 1 mM Na_2HPO_4 at pH 7.5) at 37 °C for 20 minutes. 4 mM HPG and 50 μM $Ac_4GalNAz$ supplemented DMEM (-Met) media was then added

to the infected cells and allowed to grow until 24 hours post infection. The labeling media was then removed and the cells were supplemented with complete media containing 50 μM Ac_4GalNAz. The plates were harvested 46-48 hours post infection and virus particles purified over a gradient of 1.4 g/mL and 1.25 g/mL CsCl centrifuged using an SW41 rotor (Beckman) at 32,000 rpm for 1 hour at 15 °C. The virus band at the junction of the two CsCl bands was collected and further purified using an SW60 rotor (Beckman) by an 18 hour centrifugation at 35,000 rpm over 1.33 g/mL CsCl.

Reaction of HPG and GalNAz labeled virus with phosphine-FLAG and azido-TAMRA. 50 μL of 1 x 10^{12} metabolically labeled viral particles/mL in a 100 mM tris buffer at pH 8.0 was treated with phosphine–FLAG at a final concentration of 300 μM in room temperature for 3 hours. After which time, to the solution bathophenanthroline disulphonic acid disodium salt (3 mM) and azido-TAMRA (500 μM), 5-tetramethylrhodamine (GBiosciences, Maryland Heights, MO) was added and kept in a argon-filled glove bag for 6 hours to deoxygenate. Next copper bromide at a final concentration of 1 mM was added to the mixture and the reaction allowed to proceed for 12 hours [9] inside the glove bag. The samples were then removed from the glove bag and quenched by addition of 10 mM EDTA. The particles were purified using Centri-Sep spin columns and quantified with QuantIT Picogreen Dye labeling[10]. The samples were then analyzed by western blotting technique to determine incorporation of FLAG on the viral proteins, and by fluorescent gel scanning to determine incorporation of TAMRA.

Western Blotting. To all samples coupled with phosphine-FLAG, loading dye was added and boiled at 95°C for 10 minutes. The samples were run on a 10% polyacryamide electrophoresis gel and transferred onto nitrocellulose at 40V over 2 hours in a western transfer buffer (25 mM tris, 192 mM glycine, 0.5% SDS and 10% methanol). Blots were blocked by 5% milk in PBST and treated with anti-FLAG M2 HRP conjugate at a ratio of 1:12000 in 5% milk in PBST. Blots were washed with milk and PBST and developed by chemiluminescence (Millipore Immobilon Western kit).

Fluorescent Gel Scanning Assay. Samples chemically treated with azido-TAMRA dye using Cu catalyzed "Click" chemistry as described above were purified using Centri-Sep spin columns and quantified with QuantIT Picogreen Dye labeling[10]. 1 x 10^{12} viral particles/ mL were run on a 10% polyacrylamide electrophoresis gel using the azido-TAMRA dye as standard. Standard dye was loaded 10 minutes before the end of the run. Gels were scanned using a typhoon gel scanner in the fluorescence mode with excitation filter at 532 nm and emission filter at 580 ± 15 nm. The scans were subsequently analyzed with Image Quant TL 1D gel analyzer software. All gels were run at 4°C for 60 minutes and scanned within 10minutes of the end of run.

Plaque Assay. HEK 293 cells cultivated in 6 mL culture plates were infected with 600 μL of HPG + GalNAz, HPG, GalNAz and non-labeled virus particles at concentrations of 10^3 particles/ml. The plates were overlaid with complete media containing 2.8% bactoagar and 10% BCS. After 3 days the cells were again overlaid with same agar solution containing 2% BCS. After the 6^{th} day when plaques became visible the cells

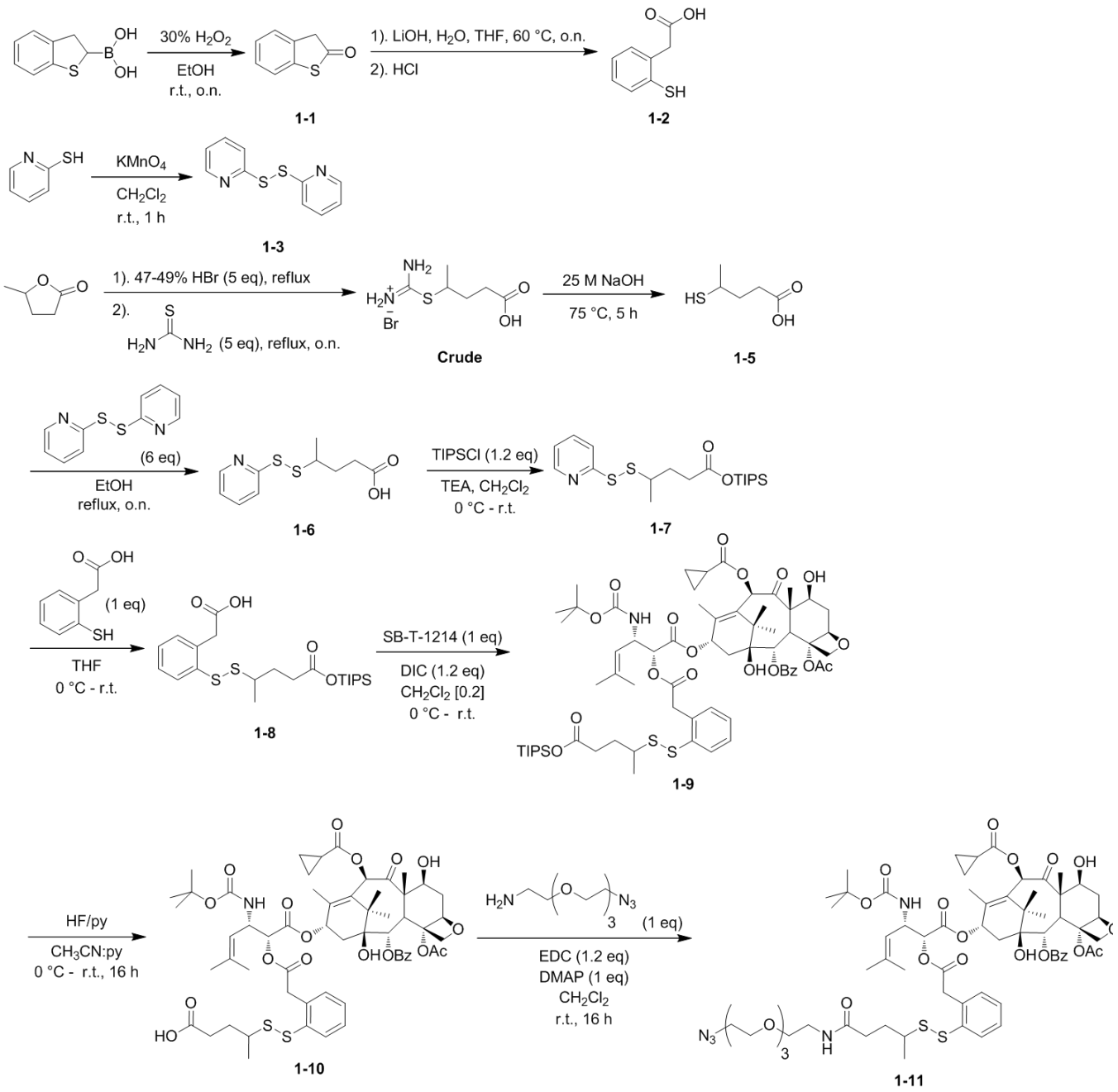
were overlaid with agar solution containing 0.1% neutral red. The plaques were counted within a day after the final overlay.

Reaction of HPG and GalNAz labeled AdTRAIL with phosphine-Folate and SBT-1214-PEG-azide. AdTRAIL was obtained from Prof. Andre Lieber, University of Washington, Seattle. The virions were metabolically labeled with HPG and GalNAz as described above. 50 μL of 1 x 10^{12} metabolically labeled viral particles/mL in a 100 mM tris buffer at pH 8.0 was treated with phosphine–Folate at a final concentration of 300 μM in room temperature for 3 hours. After which time, bathophenanthroline disulphonic acid disodium salt (3 mM) and SBT-1214-PEG-azide (500 μM), was added and the resultant mixture kept in an argon-filled glove bag for 6 hours to deoxygenate. Next copper bromide at a final concentration of 1 mM was added to the mixture and the reaction allowed to proceed for 12 hours [9] inside the glove bag. The samples were then removed from the glove bag and quenched by addition of 10 mM EDTA. The particles were purified using Centri-Sep spin columns and quantified with QuantIT Picogreen Dye labeling[10]. Subsequently, MTT and Targeting assays were performed using these modified virions.

MTT assay. Adenovirus bearing TRAIL were metabolically labeled with HPG and GalNAz as described. Chemical labeling of these viruses with SBT-1214-PEG-azide was carried via the copper catalyzed "click" reaction in deoxygenating conditions (as for azido-TAMRA). The viruses were purified over centriSep spin columns and quantified using QuantiT Picogreen assay. Human ovarian cancer cells, ID8 were seeded in 96 well plates at concentrations of 1 x 10^5 cells/mL. 24 hours after seeding, these cells were infected with different MOI of SBT-1214 labeled AdTRAIL. Control experiments were run by infecting cells with metabolically unlabeled AdTRAIL (these were chemically treated exactly as the azide, alkyne enabled virions). Cells were also treated with increasing concentrations of SBT-1214-PEG-azide – to account for the increasing MOI of infection. The cells were incubated at 37°C incubator for 5 days. After this they were treated with MTT reagent obtained from Cell Proliferation KitI (Roche, Eugene,OR). After a 4 hour incubation at 37°C the cells were again treated with solubilization buffer and left overnight. Absorbance was measured at 580 nm using a PerkinElmer Victor X plate reader.

Targeting Assay. Luciferase transgene bearing Ad5 were metabolically labeled with HPG and GalNAz as described. The viruses were chemically labeled with folate bearing phosphine-probe (phosphine-PEG-folate) and SBT-1214-PEG-azide using Staudinger ligation and Cu catalyzed "click" chemistry in a deoxygenated glove under same conditions (as for FLAG/ TAMRA labeling) and the reaction quenched with 10 mM EDTA. Viruses were purified on Centri Sep spin columns and quantified using QuantIT Picogreen assay (Molecular Probes, Eugene, OR) and stored in a 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$ buffer in PBS containing 10% glycerol. Mouse breast cancer cell line 4T1 was cultivated in minus folate media for 2 weeks after which they were seeded in 96 well plates at a density of 1 x 10^5 cells/well and cultivated for a day in minus folate media containing 2% FCS. After 24 hours the cells were infected with labeled virus at an MOI of 50. 24 hours post infection the cells were treated with 100 μL of reconstituted

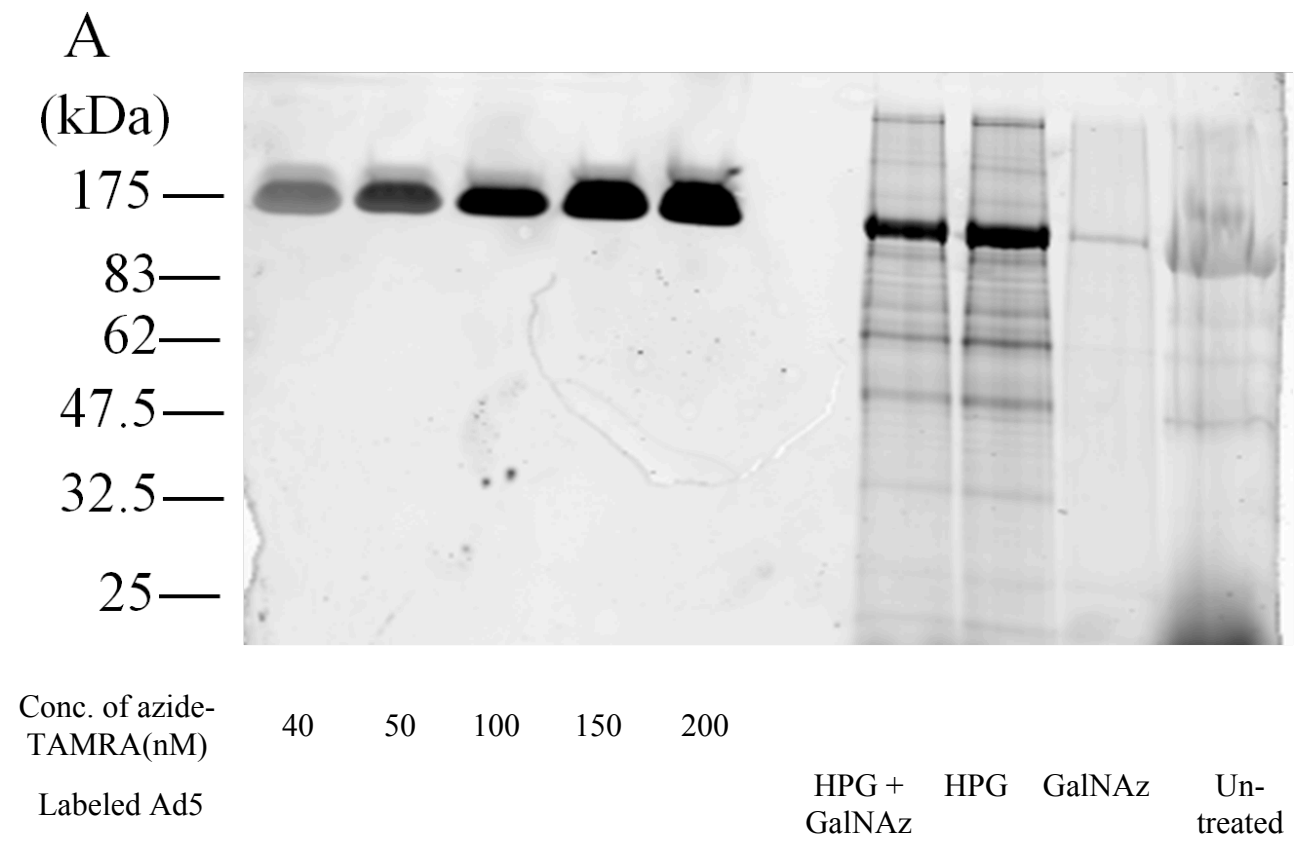
luciferase substrate from Bright Glo™ Luciferase assay Kit (Promega, Madison,WI) ; luciferase expression was evaluated using a PerkinElmer Victor X luminescence plate reader (emission 528 ± 10 nm).

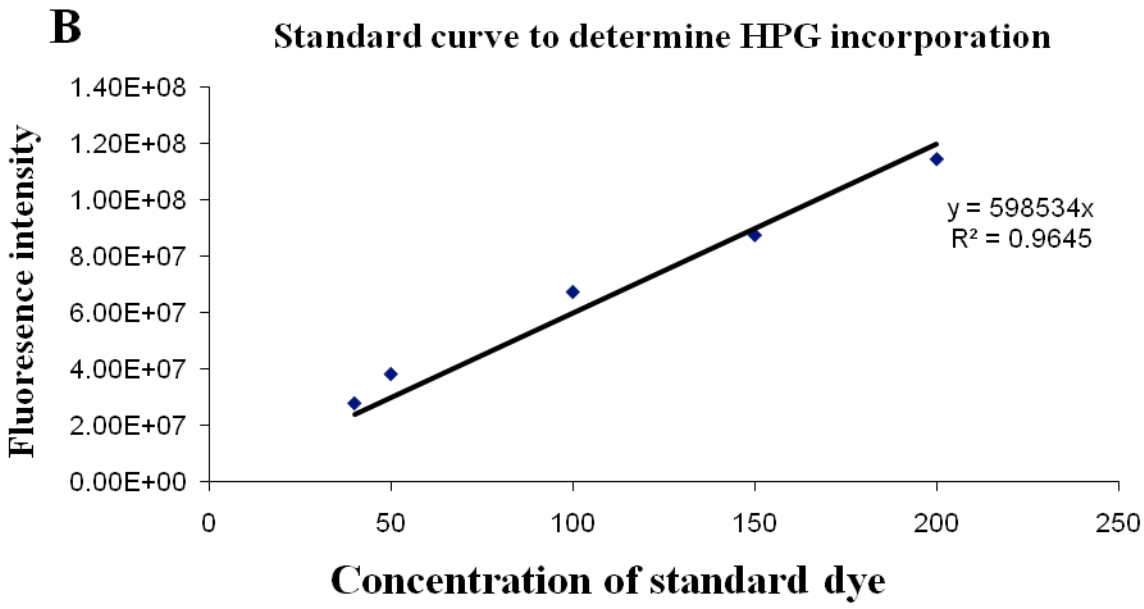


Supplemental Scheme 1. Synthesis of SBT-1214-PEG-azide.

Supplemental Figures

S1





Supplementary Figure S1. Determination of labeling of HPG enabled virus with azide-fluorophore by Cu catalyzed "click" chemistry. A) Fluorescent gel scanned image of HPG + GalNAz, HPG, GalNAz and unlabeled virus treated with azide-TAMRA . The "click" reaction was run overnight in a deoxygenated glove bag. Well 1-5: Increasing concentrations of standard dye (TAMRA) for determining the concentration of label. The gel was run at 200V for 1 hour at 4ºC and scanned within 10 minutes of the end of run. B) Standard curve drawn between fluorescence intensity and concentration of dye loaded on gel. Slope of graph was used to determine concentration of labeled protein.

Supplemental Table 1

Estimated number of solvent exposed methionine residues on adenoviral capsid proteins based on X-ray and Cryo-EM structural information (PDB ID 1P30, 1X9P, 1X9T AND 1QHV) and the observed number of HPG residues incorporated in each of the discussed proteins.

Adenoviral structural proteins*	**Estimated total no of solvent exposed Met per VP**	**Average number of chemically labeled dyes per VP with 4 mM HPG**
Hexon	5760	132.3 ± 6.6
Penton	300	36.6 ± 3.8
Fiber	108	23.1 ± 1.6

*only these 3 protein X-ray crystal structures were used to estimate the average number of exposed methionine residues per VP

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