Supporting Information for:

Non-invasive Imaging of Cell Death Using a Hsp90 Ligand

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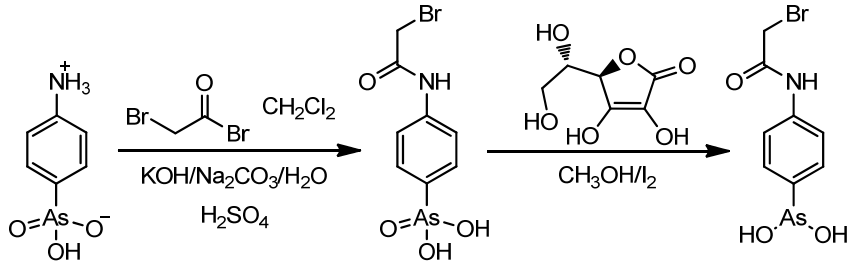
Experimental Procedures

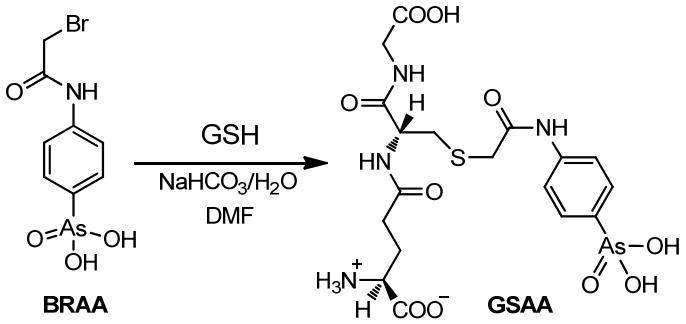
The following chemicals were purchased and used without further purification: *p*-aminobenzoic acid, bromoacetyl bromide (Acros Organics), *p*-arsanilic acid, dimethylformamide, glutathione (Sigma-Aldrich), L-ascorbic acid, potassium hydroxide, sodium bicarbonate, sodium carbonate (Mallinckrodt), acetonitrile, dichloromethane, 37% hydrochloric acid, iodine, methanol, 98% sulfuric acid (Fisher).

Preparation of GSAO and GSAA

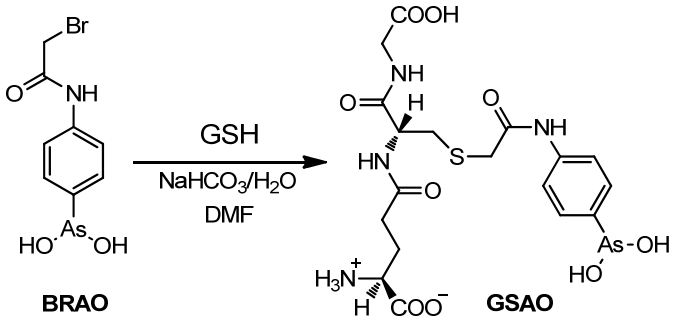


Synthesis of 4-(N-(bromoacetyl)amino)phenylarsonic acid (BRAA)
Potassium hydroxide (5.1g, 91.8mmol) was dissolved in 125mL deionized water, and while being stirred *p*-arsanilic acid (10.0g, 46.3mmol) and sodium carbonate (14.5g, 137.1mmol) were added slowly to the solution. The warm solution was cooled in ice-water bath for some time and bromoacetyl bromide (6mL, 13.9g, 68.9mmol) in 25mL dichloromethane was dripped quickly into the cooled solution. The reaction mixture was stirred for 15 mins and the ice-water bath removed. The cloudy reaction mixture was transferred to a separatory funnel and the organic layer separated by draining off. The aqueous solution was carefully acidified with 98% sulfuric acid to pH 1 with precipitation of product. It was filtered by suction, dried in air and then under vacuum overnight to give BRAA (11.2g, 33.0mmol, 71.4%).
^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm): 4.08 (s, 2H), 5.78 (br s, 2H), 7.71 (d, 2H), 7.79 (d, 2H), 10.74 (s, 1H).
^{13}C NMR (75.4 MHz, DMSO-d_6) δ(ppm): 30.3, 119.3, 127.8, 131.0, 142.4, 165.2.

Synthesis of 4-(N-(bromoacetyl)amino)phenylarsonous acid (BRAO)
BRAA (10.0g, 29.6mmol) and L-ascorbic acid (16.1g, 91.4mmol) were stirred in 100mL de-aerated methanol under argon atmosphere. Iodine (2.5g, 9.7mmol) was added and continued to stir reaction mixture under argon at room temperature. After about 30 mins, the reaction mixture turned clear. Stirring was continued for 2 hours, and the solution was filtered by suction to remove some insoluble material. The filtrate was evaporated under reduced pressure to about 50mL. While stirring filtrate, 50mL of 1N HCl was added to precipitate the product and the stirring continued for another 20 mins. The solid was filtered by suction, rinsed with 30mL methanol:water: (1:2), and dried under vacuum overnight to give BRAO (10.2g, quantitative).
^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm): 3.85 (s, 2H), 4.06 (s, 2H), 7.66 (s, 4H), 10.45 (s, 1H).
^{13}C NMR (75.4 MHz, DMSO-d_6) δ(ppm): 30.2, 118.5, 118.7, 130.3, 140.4, 166.7.



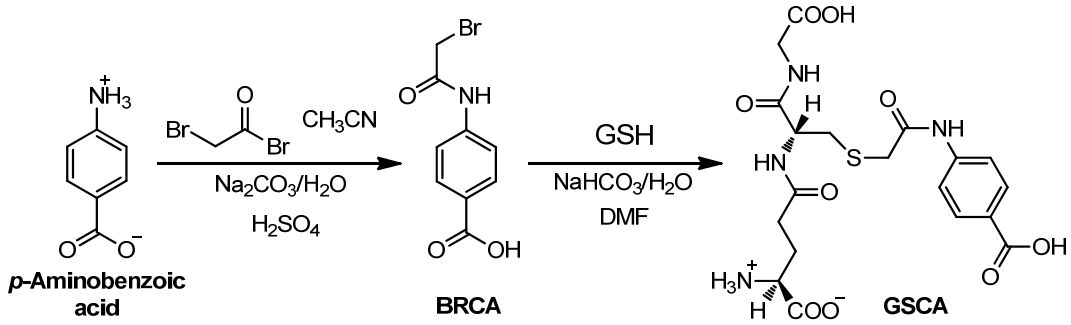
Synthesis of 4-(N-(S-glutathionylacetyl)amino)phenylarsonic acid (GSAA)
BRAA (1.0g, 3.0mmol) was dissolved in 10mL dimethylformamide. A solution of glutathione (GSH, 1.1g, 3.6mmol) in 10mL of 1M sodium bicarbonate was added to the BRAA in dimethylformamide, and the reaction mixture stirred at room temperature overnight. The reaction mixture was acidified with 20mL of 1N HCl and evaporated to dryness. The solid residue was dissolved in HCl(aq) to pH1 (15mL). The crude product solution was purified by C_{18} flash chromatography (70g column) with water-acetonitrile gradient as eluant (40mL/min flow rate monitoring at 257nm) to yield product (1.1g, 2.0mmol, 67.2%) upon lyophilization.
^{1}H NMR (300 MHz, D_2O) δ(ppm): 1.93 (q, 2H), 2.32 (t, 2H), 2.80 (m, 1H), 2.98 (m, 1H), 3.30 (m, 2H), 3.66 (t, 1H), 3.78 (s, 2H), 4.44 (m, 1H), 7.48 (d, 2H), 7.56 (d, 2H).
^{13}C NMR (75.4 MHz, D_2O) δ(ppm): 25.8, 31.1, 33.6, 36.3, 41.3, 52.8, 53.4, 121.0, 125.4, 131.1, 142.1, 170.7, 172.3, 173.0, 173.2, 174.4.



Synthesis of 4-(N-(S-glutathionylacetyl)amino)phenylarsonous acid (GSAO)

BRAO (1.0g, 3.1mmol) was dissolved in 10mL dimethylformamide by warming to 50°C under argon atmosphere. After the solution cooled to room temperature, it was cooled in an ice-water bath. A solution of glutathione (GSH, 1.0g, 3.3mmol) in 15mL of 0.5M sodium bicarbonate (prepared with de-aerated deionized water) was added to the BRAO in dimethylformamide, the ice-water bath was removed and the reaction mixture stirred at room temperature overnight. The reaction mixture was acidified to pH2 with 37% HCl and evaporated to dryness. The solid residue was dissolved in HCl(aq) to pH1 (12mL). The crude product solution was purified by C_{18} flash chromatography (25g column) in three portions with water-acetonitrile gradient as eluant (40mL/min flow rate monitoring at 257nm) to yield product (0.92g, 1.7mmol, 53.3%) upon lyophilization.
^{1}H NMR (300 MHz, D_2O) δ(ppm): 1.92 (q, 2H), 2.33 (t, 2H), 2.83 (m, 1H), 3.02 (m, 1H), 3.33 (m, 2H), 3.60 (t, 1H), 3.79 (s, 2H), 4.45 (m, 1H), 7.41 (d, 2H), 7.56 (d, 2H).
^{13}C NMR (75.4 MHz, D_2O) δ(ppm): 26.2, 31.4, 33.8, 36.4, 41.7, 53.2, 53.9, 121.6, 130.2, 139.0, 144.3, 171.1, 172.5, 173.4, 173.5, 174.7.

Preparation of GSCA



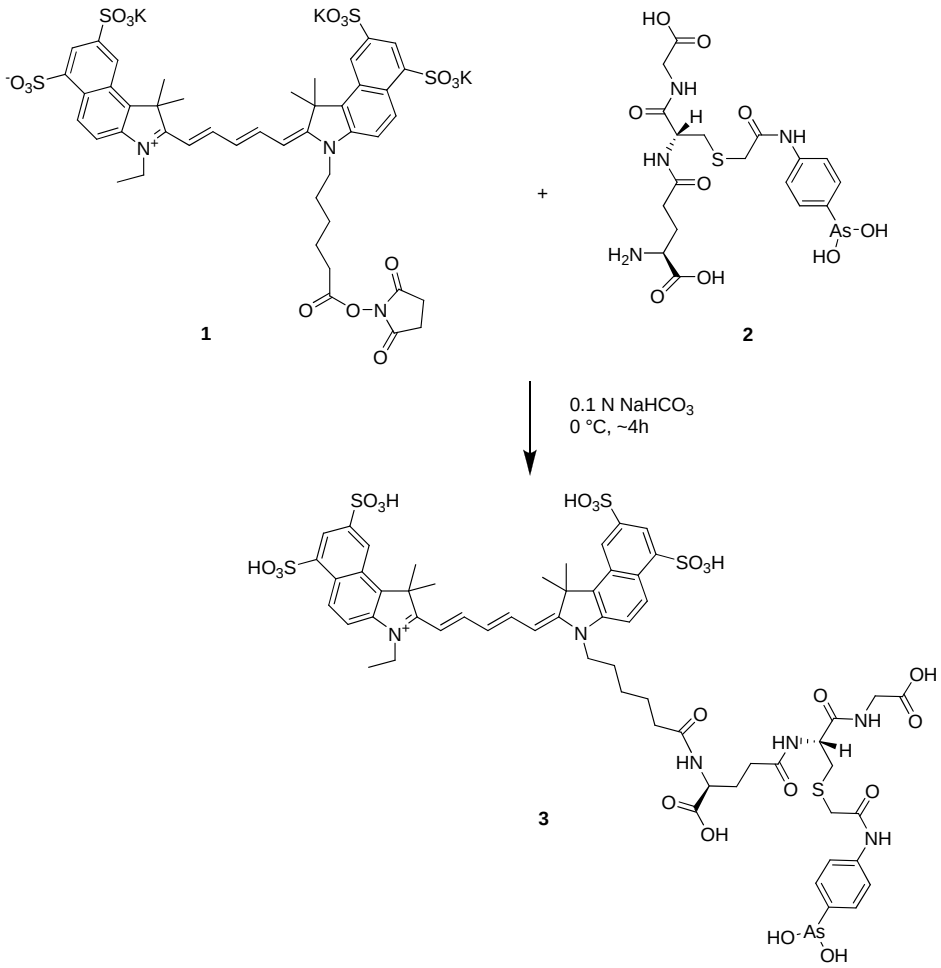
Synthesis of 4-(N-(bromoacetyl)amino)benzoic acid (BRCA)
Sodium carbonate (70.1g, 661.7mmol) was dissolved in 300mL deionized water, and while being stirred *p*-aminobenzoic acid (30.5g, 222.3mmol) was added slowly to the solution. Additional 400mL water was added and the resulting solution was cooled in ice-water bath until 3°C. Bromoacetyl bromide (27mL, 62.6g, 309.9mmol) was dripped quickly into the cooled solution and rinsed with 10mL acetonitrile. The reaction mixture was stirred for 10 mins and the ice-water bath was removed. After another 10 mins of stirring, 98% sulfuric acid was carefully added until the suspension was pH 1. The white product was filtered by suction and air dried overnight. The solid was resuspended in 250mL water, acidified again with 98% sulfuric acid to pH 1, filtered and dried in air then under vacuum overnight to give BRCA (39.6g, 153.6mmol, 69.1%).
^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm): 4.05 (s, 2H), 6.59 (d, 1H), 7.68 (d, 2H), 7.88 (d, 2H), 10.64 (s, 1H).
^{13}C NMR (75.4 MHz, DMSO-d_6) δ(ppm): 30.3, 118.4, 130.3, 130.9, 142.4, 165.1, 166.6.

Synthesis of 4-(N-(S-glutathionylacetyl)amino)benzoic acid (GSCA)
BRCA (1.2g, 4.5mmol) was dissolved in 10mL dimethylformamide. A solution of glutathione (GSH, 1.5g, 4.93mmol) in 25mL of 0.5M sodium bicarbonate was added to the BRCA in dimethylformamide, and the reaction mixture stirred at room temperature overnight. The reaction mixture was evaporated and the residue dissolved in 50mL water. Resulting solution was

acidified with 37% HCl to pH 0.7 and filtered by suction to remove some impurities. The filtrate was adjusted to pH2.8 with 50% (w/w) sodium hydroxide solution. After standing overnight, the product was filtered by suction and dried in air then under vacuum overnight to give GSCA (1.2g, 2.56mmol, 56.7%).
^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm): 1.92 (m, 2H), 2.35 (m, 2H), 2.79 (m, 1H), 3.05 (m, 1H), 3.42 (s, 2H), 3.42 (m, 1H), 3.71 (d, 2H), 4.52 (m, 1H), 7.72 (d, 2H), 7.86 (d, 2H), 8.55 (m, 2H), 10.66 (s, 1H).
^{13}C NMR (75.4 MHz, DMSO-d_6) δ(ppm): 26.8, 31.6, 34.0, 36.0, 41.2, 52.4, 53.0, 118.3, 125.2, 130.1, 142.9, 166.7, 168.2, 170.2, 170.3, 170.7, 171.7.

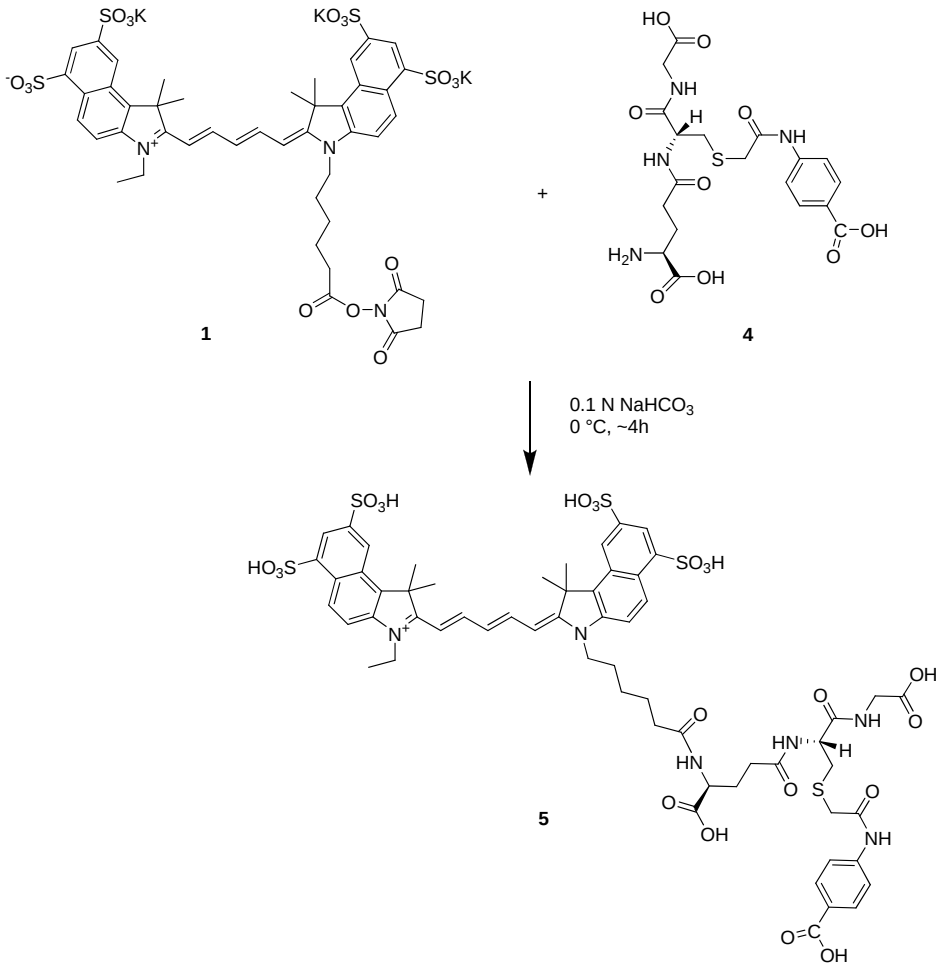
Preparation of conjugates with fluorescein, Oregon Green or biotin.
A solution of fluorescein-5-EX succinimidyl ester (Molecular Probes, Invitrogen) or Oregon Green 488-X succimidyl ester (Molecular Probes, Invitrogen) (10 mg per mL in DMSO) was added to GSAO, GSAA or GSCA (10 mg per mL in 0.1 M bicarbonate buffer, pH 9) and incubated for 60 min in the dark. The molar ratio of fluorophore to pendant is ~5:1. The conjugate was separated from unreacted fluorophore by passing the reaction through at least two Sephadex G10 spin columns equilibrated with 0.1 M bicarbonate buffer, pH 8.3 (Sigma, St Louis, MO). The unreacted dye binds to the Sephadex matrix while the conjugate elutes in the void volume. Purity of the conjugates was confirmed by HPLC (1200 Series; Agilent Technologies) on a Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm; Agilent Technologies) using a mobile phase of acetonitrile-water (25:75 vol/vol), flow rate of 0.5 mL per min and detection by absorbance at 256 nm. Purity was also assessed by thin layer chromatography using a 3:1:1 butanol/acetic acid/water mobile phase. Biotin-tagged GSAO was prepared as described [1].

Preparation of GSAO-Cy5.5
Cy5.5 mono NHS ester was purchased from GE Amersham (PA15604). The 0.1 N Sodium Bicarbonate (pH ~8.3) used as solvent in the reactions was degassed by bubbling argon through the solution for about one hour prior to starting reactions. RP-HPLC analyses were carried out on Agilent 1200 series system equipped with a UV detector (column: XTerra RP18 5 µm 100 Å 250 × 4.6 mm; λ_{max}: 280 nm; flow rate: 1 mL/min; gradient: 5-50% B/20min; mobile phase A: 0.1% TFA in H_2O; mobile phase B: 0.1% TFA in CH_3CN). Preparative RP-HPLC was carried out using a Waters Dual Pump system equipped with a Liquid Handler and a PDA detector [column: Waters XBrdige™ Prep C18 OBD™ 10 µm 30 × 250 mm; λ_{max}: PDA (200–800 nm); flow rate: 30 mL/min; gradient: 5/10 to 20/50% B/19 min; mobile phase A: 0.1% TFA in H_2O; mobile phase B: 0.1% TFA in CH_3CN]. RP-LC/ESI-MS analyses (negative-ion mode) were carried out on a Thermo Scientific LTQ system equipped with a Surveyor LC and a Surveyor PDA Plus detector using an XTerra C18 5 µm (250 mm or 150 mm × 3.0 mm) with a flow rate range of 200-400 µL using a gradient condition (5 to 50%B/20 min; mobile phase A: 0.1% TFA in H_2O; mobile phase B: 0.1% TFA in CH_3CN. HRMS (ESI) data was obtained on a Thermo Scientific LTQ-Orbitrap Discovery mass spectrometer equipped with an IonMax electro spray ionization source in FTMS (Fourier Transform) mode with resolution ≤30K.



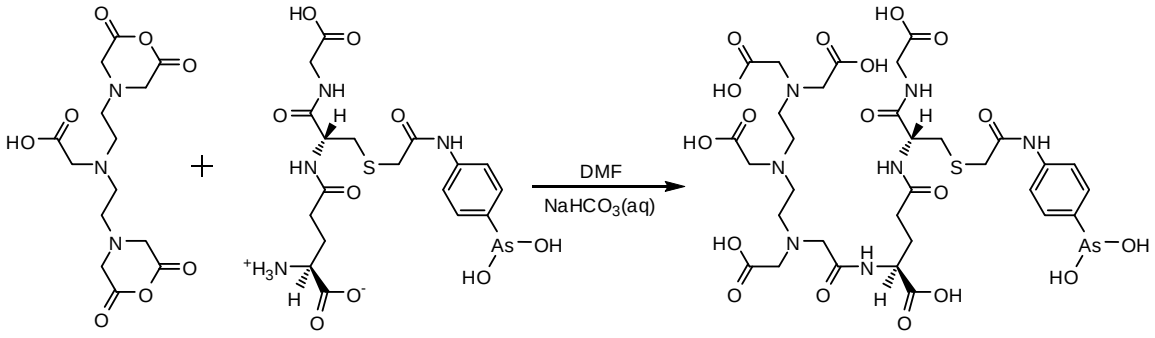
2-((1*E*,3*E*,5*E*)-5-(3-(6-((*S*)-4-((*R*)-3-(2-(4-arsonophenylamino)-2-oxoethylthio)-1-(carboxymethylamino)-1-oxopropan-2-ylamino)-1-carboxy-4-oxobutylamino)-6-oxohexyl)-1,1-dimethyl-6,8-disulfo-1*H*-benzo[e]indol-2(3*H*)-ylidene)penta-1,3-dienyl)-3-ethyl-1,1-dimethyl-6,8-disulfo-1*H*-benzo[e]indolium (3). GSAO (**2**, 19.7 mg, 35.92 µmol) was dissolved in degassed 0.1 N aq sodium bicarbonate (6.5 mL) under argon and the solution was cooled in an ice-bath. Then Cy5.5 (**1**, 20 mg, 17.7 µmol) was added as a solid in two portions of 10 mg each with an interval of 1.5 h. The reaction mixture was stirred for a total of 4 h at ice-bath temperature under argon. The mixture was then freeze dried on a lyophilizer to obtain crude solid product which was purified and isolated by preparative HPLC. Fractions containing the desired pure product were combined, organic solvent (CH_3CN) was evaporated on rotovap using $t \leq 32$ °C and the remaining aqueous solution was freeze dried on the lyophilizer to give pure product **3** (13.6 mg, 53%) as a dark blue fluffy solid: RP-HPLC (280 nm) area% purity 96.7% (t_R = 12.89 min, 5–50%B/20min); RP-LC/MS (ESI) *m/z* 1427.0 $(M^+–2H–H_2O)^-$, 1449.0 $(M^+–2H–H_2O+Na)^-$, 713.2 $(M^+–2H–H_2O)^{2–}$, (t_R = 11.57 min, 3.0 × 150 mm, 5–50%B/20min).

Preparation of GSCA-Cy5.5

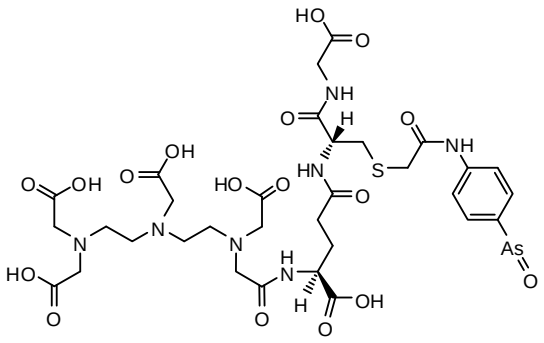


2-((1E,3E,5E)-5-(3-(6-((S)-1-carboxy-4-((R)-1-(carboxymethylamino)-3-(2-(4-carboxyphenylamino)-2-oxoethylthio)-1-oxopropan-2-ylamino)-4-oxobutylamino)-6-oxohexyl)-1,1-dimethyl-6,8-disulfo-1H-benzo[e]indol-2(3H)-ylidene)penta-1,3-dienyl)-3-ethyl-1,1-dimethyl-6,8-disulfo-1H-benzo[e]indolium (5). GSCA (**4**, 17.6 mg, 36.3 μmol) was dissolved in degassed 0.1 N aq sodium bicarbonate (6.5 mL) under argon and the solution was cooled in an ice-bath. Then Cy5.5 (**1**, 20 mg, 17.7 μmol) was added as a solid in two portions of 10 mg each with an interval of 1.5 h. The reaction mixture was stirred for a total of 4 h at ice-bath temperature under argon. The mixture was then freeze dried on a lyophilizer to obtain crude solid product which was purified and isolated by preparative HPLC. Fractions containing the desired pure product were combined, organic solvent (CH_3CN) was evaporated on rotovap using $t \leq 32$ °C and the remaining aqueous solution was freeze dried on the lyophilizer to give pure product pure product **5** (17.6 mg, 71%) as a dark blue fluffy solid: RP-HPLC (280 nm) area% purity 100% (t_R = 13.04 min, 5–50%B/20min); RP-LC/MS (ESI) *m/z* 1381.1 $(M^+–2H)^–$, 690.2 $(M^+–2H)^{2–}$, (t_R = 3.6 min, 3.0 × 150 mm, 5–50%B/20min).

Preparation of GSAO-DTPA



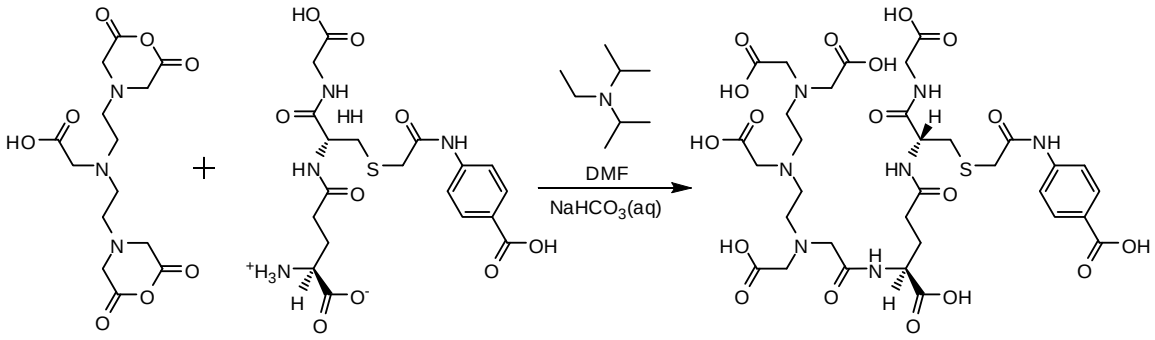
DTPA-bis(anhydride) (DTPAA, 4.0g, 11.4mmol) and sodium bicarbonate (2.6g, 30.5mmol) in 30mL dimethylformamide were stirred under argon at room temperature. 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsonous acid (GSAO, 0.20g, 0.37mmol) was dissolved in 2mL of deaerated deionized water and the solution added dropwise into the reaction mixture which was stirred at room temperature overnight. The reaction mixture was acidified with conc HCl (6mL) and then evaporated. The crude product was dissolved with 1N HCl(aq) and purified by C_{18} flash chromatography (70g column) in two portions with water-acetonitrile gradient as eluant (40mL/min flow rate monitoring at 257nm) to yield product (99.6mg, 29.5%) upon lyophilization.
^{1}H NMR (300 MHz, D_2O) δ(ppm): 1.89 (m, 1H), 2.08 (m, 1H), 2.32 (t, 2H), 2.88 (m, 1H), 3.05 (m, 1H), 3.13 (t, 2H), 3.17 (t, 2H), 3.26 (t, 2H), 3.35 (t, 2H), 3.38 (s, 2H), 3.62 (s, 2H), 3.74 (s, 2H), 3.82 (s, 4H), 3.87 (s, 2H), 3.89 (s, 2H), 4.29 (m, 1H), 4.54 (m, 1H), 7.50 (t, 2H), 7.59 (d, 2H).
^{13}C NMR (75.4 MHz, D_2O) δ(ppm): 26.3, 31.6, 33.8, 36.3, 41.2, 50.3, 50.8, 51.8, 51.9, 52.3, 52.8, 54.3, 56.2, 56.3, 121.1, 130.2, 139.2, 144.1, 168.4, 170.6, 170.7, 171.4, 172.3, 172.4, 172.9, 174.5, 174.8.



Chemical Formula: $C_{32}H_{44}AsN_7O_{17}S$
Exact Mass: 905.1730
Molecular Weight: 905.7151

HRMS (negative ion mode) for $C_{32}H_{44}N_7O_{18}SAs$: calcd 904.1658, found 904.1651.

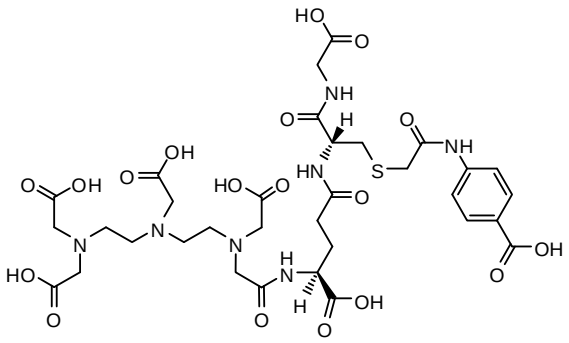
<u>Preparation of GSCA-DTPA</u>



DTPA-bis(anhydride) (DTPAA, 3.1g, 8.6mmol) and diisopropylethylamine (10mL, 7.4g, 57.4mmol) in 40mL dimethylformamide were stirred under argon at 60°C until clear, then allowed to cool to room temperature. 4-(*N*-(*S*-glutathionylacetyl)amino)benzoic acid (GSCA, 0.21g, 0.43mmol) was dissolved in 3mL of 0.5M sodium bicarbonate solution and then added dropwise into the reaction mixture which was stirred at room temperature overnight. The reaction mixture was evaporated and the residue dissolved in HCl(aq) to pH1 (16mL). The crude product solution was purified by C_{18} flash chromatography (25g column) in two portions with water-acetonitrile gradient as eluant (40mL/min flow rate monitoring at 254nm) to yield product (222.1mg, 60.5%) upon lyophilization.

^{1}H NMR (300 MHz, D_2O) δ(ppm): 1.79 (m, 1H), 1.96 (m, 1H), 2.24 (t, 2H), 2.82 (m, 1H), 3.00 (m, 1H), 3.06 (t, 2H), 3.11 (t, 2H), 3.22 (t, 2H), 3.33 (t, 2H), 3.34 (s, 2H), 3.54 (s, 2H), 3.69 (s, 2H), 3.78 (s, 4H), 3.82 (s, 2H), 3.84 (s, 2H), 4.18 (m, 1H), 4.45 (m, 1H), 7.42 (t, 2H), 7.81 (d, 2H).

^{13}C NMR (75.4 MHz, D_2O) δ(ppm): 26.5, 31.8, 34.1, 36.7, 41.5, 50.4, 51.0, 52.2, 52.3, 52.5, 53.1, 54.6, 56.6, 56.7, 120.5, 126.0, 131.1, 141.9, 168.3, 169.9, 170.6, 171.1, 171.4, 172.5, 172.6, 173.0, 174.6, 175.0.



Chemical Formula: $C_{33}H_{45}N_7O_{18}S$
Exact Mass: 859.2542
Molecular Weight: 859.8115

HRMS (negative ion mode) for $C_{33}H_{45}N_7O_{18}S$: calcd 858.2469, found 858.2448.

<u>Incorporation of ^{111}In</u>

A stock solution of GSCA-DTPA or GSAO-DTPA is prepared in double-distilled water at 1 mg per mL and stored at 4°C. 9.2 µg of DTPA conjugate in 0.50 mM sodium acetate trihydrate, 0.25

mM ascorbic acid buffer is incubated with 1 mCi of ^{111}In at room temperature for 10-20 minutes. Aliquots are stored frozen.

Concentrations of the organoarsenicals were measured by titrating with dimercaptopropanol and calculating the remaining free thiols with 5,5'-dithiobis(2-nitrobenzoic acid) (Sigma) [2]. The conjugates were sterile filtered and stored at 4°C in the dark until use. There was no significant loss in the active concentration of arsenical stock solutions for at least one week when stored under these conditions

Flow cytometry

Jurkat A3 cell were cultured in RPMI medium, and HT1080 and Lewis lung carcinoma cells (ATCC) in DMEM medium, supplemented with 10% foetal bovine serum, 2 mM L-glutamine, and 1 U.mL^{-1} penicillin/streptomycin. Cell culture plasticware was from Techno Plastic Products (Trasadingen). All other cell culture reagents were from Gibco.

Jurkat A3 cells were seeded at a density of 5 x 10^5 cells per well and incubated without or with 0.1 μg per mL anti-human Fas (CD95) antibody (Medical and Biological Laboratories), 4 μM staurosporine (Sigma) or 20 μM doxorubicin (Pfizer) for discrete times up to 24 h. On some occasions, cells were first pre-treated with 20 μM carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (Z-VAD-FMK, Promega) for 1 min. Human fibrosarcoma HT1080 cells were treated for 20 h with 1 μg per mL camptothecin (Calbiochem), then detached with phosphate-buffered saline containing 2.5 mM EDTA and combined with cells that had detached during incubation. Cells were washed twice with ice cold phosphate-buffered saline, re-suspended in ice cold 10 mM Hepes, pH 7.4 buffer containing 0.14 M NaCl and 2.5 mM $CaCl_2$ and incubated at room temperature with 1 μM of the GSAO-fluorophore conjugates for discreet times with shaking. Cells were washed twice with ice cold phosphate-buffered saline and then incubated with 5 μL per 100μL of annexin V APC (BD Pharmingen) and either 1 μg per mL propidium iodide (Molecular Probes, Invitrogen) or 1 μM Sytox Blue Dead Cell Stain (Molecular Probes, Invitrogen) for 15 min in the dark. Flow cytometry was performed using a FACS Canto II Flow Cytometer (Becton Dickinson) and data analysed using FlowJo software version 8.7.

Confocal microscopy

HT1080 cells were treated for 20 h with 1 μg per mL^{-1} camptothecin and incubated for 15 min in 10 mM Hepes, pH 7.4 buffer containing 0.14 M NaCl and 2.5 mM $CaCl_2$, 10 μM GSAO-F and 1:20 (v/v) Alexa594-conjugated annexin V (Molecular Probes, Invitrogen). The cells were washed twice and transferred onto a microscope slide. Transverse cell sections were captured using an Olympus BX60 microscope and an Optiscan F900e confocal unit and software (Optiscan).

Identification of GSAO-biotin binding proteins in apoptotic cells

1×10^8 Jurkat A3 cells were seeded at a density of 5×10^5 cells per mL and treated with 4 μM staurosporine for 24 h. Cells were washed twice, suspended in 10 mM Hepes, pH 7.4 buffer containing 0.14 M NaCl and 2.5 mM $CaCl_2$ and incubated without or with 50 μM GSAO-biotin for 15 min. Washed cells were re-suspended in 50 mM Tris, pH 8 buffer containing 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and a cocktail of

protease inhibitors (Roche) and incubated at 4 °C for 20 min. The cells were sonicated on ice and the lysate clarified by centrifugation at 5250 g for 20 min at 4 °C. Approximately 3.5 mg of total protein was incubated with 0.5 mg of streptavidin-agarose beads (Sapphire Bioscience) at 4 °C for 2 h on a rotating wheel. The beads were washed 5 times and the bound proteins eluted at 4 °C overnight into 50 µl of 50 mM Tris, pH 8 buffer containing 150 mM NaCl, protease inhibitor cocktail (Roche) and 2 mM dimercaptopropanol. The eluted proteins were resolved on a 4-12% gradient NUPAGE Bis-Tris gel (Invitrogen) and stained with Sypro Ruby Protein Gel Stain (Molecular Probes, Invitrogen).

Identification of proteins eluted from the beads was performed essentially as previously described [3]. Briefly, the proteins were separated on a Coomassie stained gel and the bands of interest were excised. These samples were then reduced with dithiothreitol, cysteines alkylated with iodoacetamide and digested with modified trypsin (Promega) (1:100 enzyme:protein) for 3 h at 37°C. Following digestion, tryptic peptides were separated by nano-liquid chromatographic system (Dionex) over a C18 column using a 0.5% gradient for 60 min at a flow rate of 250 nL per min. Eluting peptides were directly analysed using a Micromass Q-ToF 2 (Waters Corporation) mass spectrometer equipped with a nanospray source. The mass spectrometer was operated in data dependent acquisition mode to generate MS/MS data for protein identification. Mass spectral data were searched using Inspect (v20090202) [4] against the Uniprot/Swiss-Prot database (release 15.4). Search parameters were: Precursor tolerance 100 ppm and product ion tolerances ± 0.4 Da, variable modification of Met-O and Cys-carboxyamidomethyl, full tryptic cleavage and up to 1 missed cleavage. The significance threshold was set at $p < 0.05$.

5 µg of reduced Hsp90 (Prospec) was incubated without or with the thiol alkylators, 20 mM iodoacetamide (IAM), 10 mM N-ethylmaleimide (NEM) or 10 mM methyl methanethiolsulfonate (MMTS) for 30 min, labelled with 200 µM GSAO-biotin for 1 h, resolved on a 4-12% gradient NUPAGE Bis-Tris gel, transferred to PVDF membrane and blotted with streptavidin-horseradish peroxidase (1:2000 dilution; Dako Cytomation). The blot was developed using ECL chemiluminescence (NEN). To control for non-specific blotting with streptavidin-peroxidase, GSAO-biotin was omitted in one experiment.

Labeling of apoptotic tumor cells with GSAO-Cy5.5

0.5×10^6 colorectal carcinoma (CT26.WT) cells were implanted subcutaneously in the shoulder area of Balb/c mice [5]. On day 9 post tumor cell implantation mice were treated with a single tail vein injection of 10 mg/kg doxorubicin [6]. The following day the mice received a tail vein injection of either 5 mg/kg GSCA-Cy5.5 or GSAO-Cy5.5 and 1 h later the tumors were excised. Tumors were embedded in paraffin, 4 micron sections prepared and stained with cleaved caspase-3 (Asp175) rabbit primary monoclonal antibody (5A1E, 1:200 dilution, Cell Signalling), secondary Cy2 conjugated AffiniPure donkey anti-rabbit IgG (1:200 dilution, Jackson ImmunoResearch) and DAPI/AntiFade Reagent-Prolong Gold (Invitrogen). Images were collected with an Olympus FV1000 Laser Scanning Microscope at excitation wavelengths of 405 nm (DAPI), 488 nm (Cy2) and 633 nm (Cy5.5).

Imaging of tumor cell death in mice using a GSAO-radioisotope conjugate

2×10^5 Jurkat A3 cells were untreated or treated with 500 nM staurosporine without or with 50 µM of the caspase inhibitor, Z-VAD-FMK, for 5 h. 5 µCi of control GSCA-^{111}In or GSAO-^{111}In

was incubated with the cells for 15 min, unbound compound was removed by washing 3 times, and bound radioactivity measured using a Perkin Elmer Wizard 1470 Automatic Gamma Counter.

0.5×10^6 Lewis lung carcinoma cells were implanted subcutaneously in the shoulder area of C57BL/6 mice [7]. Mice bearing ~0.5 g Lewis lung carcinoma tumors were injected with 350-750 μCi of GSCA-^{111}In or GSAO-^{111}In in the tail vein and 5 h later the blood, kidneys, livers, lungs, spleens and tumors were harvested and % injected dose per gram of tissue weight measured.

Tabulation of the results shown in Fig. 6b. Biodistribution of control GSCA-^{111}In or GSAO-^{111}In in C57BL/6 mice bearing Lewis lung carcinoma tumors.

compound	% injected dose per gram of tissue, mean ± SD (n = 4-68)					
	blood	kidney	liver	lung	spleen	tumor
GSCA-^{111}In	0.011 ± 0.002	1.089 ± 0.333	0.053 ± 0.015	0.032 ± 0.010	0.047 ± 0.011	0.090 ± 0.007
GSAO-^{111}In	0.309 ± 0.186	19.62 ± 8.604	1.959 ± 0.556	0.791 ± 0.991	1.007 ± 0.370	2.242 ± 1.262

C57BL/6 mice bearing Lewis lung carcinoma tumors or Balb/c mice bearing CT26 colorectal carcinoma tumors in the shoulder area were injected with 350-750 μCi of GSCA-^{111}In or annexin V-^{99m}Tc (HYNIC-annexin V, Theseus Imaging Corp.) in the tail vein and dual images in separate energy windows collected 5 h later using a NanoSPECT/CT camera (Bioscan). Acquisition time was 28 min. Image intensities were calibrated to account for the difference in decay between indium and technetium.

Statistical analyses

Results are presented as means ± SD and were calculated using GraphPad Prism (GraphPad, San Diego, CA).

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