Supporting Information

For

Preparation of Water Soluble Maleimide-Functionalized 3 nm Gold Nanoparticles: A New Bioconjugation Template

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Content:

ligand contains 2 α to sulfur protons,. The mole ratio of the maleimide-PEG-thiolated:PEGylated determined from the integration is 1:2.

Figure S14. Transmission electron microscopy (TEM) images of a) **6**-AuNP and b) **7**-AuNP. Scale bar 20 nm.

Figure S15. ^{1}H NMR spectrum of the Diels-Alder adducts of **7**-AuNP and furan. The white and blue arrows are attributed to the endo- and exo- products, respectively. Solvent = D_2O.

Figure 16S. XPS spectra of **7**-AuNP. The peak at 400.28 eV and 162.35/163.61 eV are corresponding to the BE of N1s and S2p3/S2p1, respectively. Quantitative analysis showed the mole ratio of N:S=3:7.

Figure 17S. Fluorescent spectra for rhodamine-AuNP before and after treatment with I_2/KI. Excitation at 505 nm and emission at 530 nm.

Experimental Section

General Materials and Methods

Potassium tetrabromoaurate (III), hydrogen tetrachloroaurate(III), tetraoctylammonium bromide, tetrabutylammonium borohydride, triethylene glycol, carbone tetrabromide, triphenylphosphine, furan, maleimide, potassium thioacetate, thiourea, Rhodamine 123, and cysteine were all purchased from Aldrich and were used as received. Potassium carbonate (Caledon), chloroform-d_6 (Cambridge Isotope Laboratories), D_2O (Cambridge Isotope Laboratories) were also used as received. ^{1}H NMR and ^{13}C NMR spectra were obtained on Varian 300 (300MHz) and Inova 500 (500MHz) spectrometers in deuterated chloroform or deuterated water and are reported in parts per million (ppm), with the residual protonated solvent resonance used as a reference. HR-MS analyses were recorded at the Department of Chemistry,

McGill University. Infrared spectra were obtained on a Perkin Elmer ATR-FTIR spectrometer and data are reported in wavenumbers (cm^{-1}). TGA analysis was performed on a TA-TGA 500 instrument. Transmission electron microscopy images were collected from a Philips CM200 TEM. X-ray photoelectron spectra were collected using a VG ESCALAB 220i-XL spectrometer employing a monochromatic X-ray source and a hemispherical electrostatic analyzer.

1,2-bis(2-bromoethoxy)ethane (2). Triethylene glycol (2.0 g, 13.1 mmol) and CBr_4 (8.7 g, 26.2 mmol) were dissolved in 20 mL of dry THF in ice bath. To the solution was added triphenylphosphine (7.0 g, 26.2 mmol) in 20 mL of dry THF. The temperature was allowed to rise to room temperature overnight. Solvent was removed and the crude product was redissolved in 50 mL of dichloromethane (DCM). A white precipitate was formed after adding 50 mL of hexane. The white precipitate was filtered and the organic layer was concentrated to yield an orange residue. 1,2-bis(2-bromoethoxy)ethane was purified by flash column chromatography with an eluent of EtOAc:Hexane = 1:2 to give 3.1 g (yield 91 %) of a clear colorless oil. ^{1}H NMR ($CDCl_3$, 300MHz) δ(ppm): 3.80 (triplet, J=6.5 Hz, 4H), 3.66 (broad, 4H), 3.36 (triplet, J=6.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 60MHz); δ(ppm): 71.12 (2C), 70.39(2C), 30.29 (2C). ATR-IR (cm^{-1}): 2935, 2871, 2247, 1710, 1422, 1357, 1277, 1220, 1110, 1039, 911, 729, 665. MS (ESI): Exact mass ($C_6H_{12}Br_2O_2$) $[M+Na]^{+1}$, calc: 296.9101 found: 296.9091

2-(2-(2-(2-bromoethoxy)ethoxy)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (4). Maleimide (1.0 g, 10.3 mmol) and furan (1.05 g, 15.5 mmol) were dissolved in 15 mL of diethyl ether in a sealed tube, and then heated at 90 - 100 °C for 12 hours. The 3, 6-Endoxo-Δ^4-tetrahydrophthalimide (**3**) precipitated as a white solid after cooling the mixture to room temperature. The product was then filtered, washed with 3 x 10 mL of cold diethyl ether to remove the unreacted maleimide and gave rise to a white, crystalline **3** with a yield of 62%. The

^{1}H-NMR spectrum indicates that the product is exclusively the *exo* isomer. ^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 8.10 (broad singlet, 1H), 6.52 (doublet, J = 0.8 Hz, 2H), 5.31 (doublet, J = 0.8 Hz, 2H), 2.89 (singlet, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm): 175.9, 136.6, 80.9, 48.7. ATR-IR (cm^{-1}): 3185, 3089, 3029, 2995, 1705, 1356, 1258, 1189, 856, 822. MS (ESI): Exact mass ($C_8H_7O_3N$) calc. 165.0426; found: 165.0422.

Compound **2** (3.00 g, 11.6 mmol) and **3** (0.96 g, 5.8 mmol) were dissolved in 25 mL dimethylformamide (DMF). K_2CO_3 (0.80 g, 5.8 mmol) was added and the mixture was heated to 50 ºC overnight. The mixture was then dissolved in 200 mL of dichloromethane and washed with 3 X 50 mL water. The organic layer was dried with magnesium sulfate ($MgSO_4$), filtered and rotary evaporated to dryness. The crude product was then purified by flash liquid chromatography with an eluent starting with 1:2 hexane:ethyl acetate to remove excess of compound **2** and followed with 95:5 ethyl acetate: methanol to isolate 1.30 g compounds **4** (clear colorless oil, yield 63%). ^{1}H NMR ($CDCl_3$, 300MHz) δ(ppm): 6.49 (singlet, 2H), 5.24 (singlet, 2H), 3.76 (triplet, J=6.3 Hz, 2H), 3.67-3.55 (two triplet at 3.67, 3.61 merged with a broad singlet at 3.55, 8H), 3.44 (triplet, J=6.3 Hz, 2H), 2.84 (singlet, 2H). ^{13}C NMR ($CDCl_3$, 60MHz) δ(ppm): 176.05, 136.47, 80.82, 71.09, 70.40, 70.01, 67.14, 47.40, 38.12, 30.38. ATR-IR (cm^{-1}): 2935, 2870, 1772, 1696, 1428, 1398, 1336, 1281, 1193, 1130, 1022, 916, 853, 722, 650. MS (ESI): Exact mass ($C_{14}H_{18}BrNO_5$) $[M+Na]^{+1}$, calc: 382.0266 found: 382.0257

2-(2-(2-(2-(prop-1-en-2-ylthio)ethoxy)ethoxy)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (5). Compounds **4** (0.50 g, 1.4 mmol) and potassium thioacetate (0.17g, 1.6 mmol) were mixed in 20 mL of acetone and heated at 50 ºC overnight. Precipitate was removed by filtration and the solvent was removed. The residue was dissolved in 50 mL dichloromethane and washed with water and brine. The organic layer was dried with $MgSO_4$, filtered and rotary evaporated to dryness to generate compound **5** as a pale yellow oil in

quantitative yield. The crude product is pure by ^{1}H NMR and used without further purification. ^{1}H NMR ($CDCl_3$, 300MHz) δ(ppm): 6.49 (singlet, 2H),by 5.24 (singlet, 2H), 3.66-3.54 (three triplet at 3.66, 3.60, and 3.54 merged with a broad singlet at 3.55, 10H), 3.05 (triplet, J=6.4 Hz, 2H), 2.84 (singlet, 2H), 2.31 (singlet, 3H). ^{13}C NMR ($CDCl_3$, 60MHz) δ(ppm): 195.47, 176.03, 136.47, 80.81, 70.21, 69.88, 69.64, 67.08, 47.39, 38.07, 30.51, 28.77. ATR-IR (cm^{-1}): 2935, 2867, 1772, 1692, 1427, 1398, 1352, 1335, 1285, 1271, 1192, 1130, 1096, 1021, 956, 916, 877, 853, 813, 722, 649, 625. MS (ESI): Exact mass ($C_{16}H_{21}NO_6S$) $[M+Na]^{+1}$, calc: 378.0987 found: 378.0980

2-(2-(2-(2-mercaptoethoxy)ethoxy)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (6). Thiolate **5** (0.40 g, 1.1 mmol) was dissolved in 10 mL of argon-purged methanol. To this solution, 1.1 mL of argon purged NaOH (1 M in EtOH) was added and the mixture was stirred at room temperature for 50 min. Longer reaction times result in the generation of disulfide. The solvent was removed and the residue was dissolved in 30 mL of dichloromethane, followed by washing with water and brine. The organic layer was dried with $MgSO_4$, filtered, and rotary evaporated to dryness to generate compound **6** as a pale yellow oil with quantitative yield. ^{1}H NMR of the crude product shows trace amount of disulfide (<5%). This thiol sample **6** is used without further purification. ^{1}H NMR ($CDCl_3$, 300MHz) δ(ppm): 6.50 (singlet, 2H), 5.25 (singlet, 2H), 3.68-3.58 (three triplet at 3.68, 3.63, and 3.57 merged with a broad singlet at 3.57, 10H), 3.05 (triplet, J=6.4 Hz, 2H), 2.85 (singlet, 2H), 2.68 (quartet, J=8.10/6.4 Hz, 2H), 1.58 (triplet, J=8.10 Hz, 1H). ^{13}C NMR ($CDCl_3$, 60MHz) δ(ppm): 176.07, 135.50, 80.85, 72.79, 70.15, 69.95, 67.14, 47.44, 38.13, 24.26. ATR-IR (cm^{-1}): 3453, 3080, 2935, 2866, 1771, 1694, 1597, 1397, 1335, 1272, 1192, 1132, 1095, 1020, 914, 877, 853, 820, 720, 649, 594. MS (ESI): Exact mass ($C_{14}H_{19}NO_5S$) $[M]^{+1}$, calc: 313.0984 found: 313.0948

2-(2-(2-bromoethoxy)ethoxy)ethanol. To a mixture of 2,2'-(ethane-1,2-diylbis(oxy))diethanol (4.25 g, 28.3 mmol) in toluene (50 mL) was added concentrated HBr (5.1 mL of 48% aqueous solution, 45.3 mmol). The mixture was refluxed for 72 hours. The mixture was then cooled to room temperature. After phase separation, the aqueous layer was removed. The organic layer was diluted with 100 mL of dichloromethane; then washed with 1M NaOH and brine. Drying (with $MgSO_4$) and concentration of the organic layer gave a pale yellow oil. The crude product was then purified by flash liquid chromatography with eluent starting with 1:1 hexane: ethyl acetate, followed by 90:10 ethyl acetate: methanol to yield 3.60 g of 2-(2-(2-bromoethoxy)ethoxy)ethanol (clear colorless oil, yield 60%). ^{1}H NMR ($CDCl_3$, 300MHz) δ(ppm): 3.78 (triplet, J=6.2 Hz, 2H), 3.70 (triplet, J=4.7 Hz , 2H), 3.65 (broad, 4H), 3.57 (triplet, J=4.7 Hz , 2H), 3.45 (quartet, 2H), 2.68 (broad, 1H). ^{13}C NMR ($CDCl_3$, 60MHz); δ(ppm): 72.04, 71.01, 70.36, 70.16, 61.56, 30.16. ATR-IR (cm^{-1}): 3409, 2868, 1453, 1421, 1351, 1277, 1186, 1111, 1064, 92, 887, 818, 731, 665, 566. MS (ESI): Exact mass ($C_6H_{13}BrO_3$) $[M+Na]^{+1}$, calc: 234.9945 found: 234.9935

2-(2-(2-mercaptoethoxy)ethoxy)ethanol. 2-(2-(2-bromoethoxy)ethoxy)ethanol (2.00 g, 9.4 mmol) and thiourea (1.07 g, 14.1 mmol) were refluxed in 40 mL of water under argon for 18 hours. The mixture was allowed to cool to room temperature. 4 g of NaOH in 10 mL of water was purged with argon and added to the reactants, continue reflux for 3 hours. The mixture was cooled to room temperature again and dumped into ice/water under rapid stirring. The pH of the mixture was adjusted to 4 with concentrated HCl. The mixture was then extracted with 3 X 50 mL of DCM. The combined organic layer was dried with MgSO4, filtered and rotary evaporated to dryness to provide 1.30 g of 2-(2-(2-mercaptoethoxy)ethoxy)ethanol as a clear colorless liquid (>95% yield). ^{1}H NMR ($CDCl_3$, 300MHz) δ(ppm): 3.66 (triplet, J=4.3 Hz, 2H), 3.59 (two merged triplets, 4H), 3.54 (two merged triplets, 4H), 2.82 (broad, 1H), 2.65 (quartet, J=8.2/6.4 Hz, 2H), 1.55 (triplet, J=8.2 Hz , 1H). ^{13}C NMR ($CDCl_3$, 60MHz); δ(ppm): 72.54, 72.20, 69.98,

69.89, 61.37, 23.87. ATR-IR (cm^{-1}): 3407, 2865, 2550, 1701, 1656, 1453, 1350, 1293, 1244, 1105, 1064, 919, 887, 820, 729, 667. MS (ESI): Exact mass ($C_6H_{14}O_3S$) $[M+Na]^{+1}$, calc: 189.0561 found: 189.0553

General procedure to prepare PEGylated AuNP. Briefly, potassium tetrabromoaurate (III) (0.30 g, 0.54 mmol) or hydrogen tetrachloroaurate trihydrate (0.54 mmol) was dissolved in 20 mL of Milli-Q water resulting in a bright yellow solution, then extracted by mixing with 200 mL of a tetraoctylammonium bromide (TOAB, 0.36 g, 0.65 mmol) toluene solution. The contents were stirred vigorously for 20 minutes at room temperature to facilitate the phase transfer of the Au(III) into the toluene layer, which resulted in the organic layer turning to a dark orange color and the aqueous layer becoming clear colorless. After phase transfer, the aqueous layer was removed. The organic layer was dried with $MgSO_4$ and filtered to remove excess of water. The solution was cooled to 0°C in an ice bath; PEG-thiol (for example, 3 stoichiometric of TEG-thiol, 0.27 g, 1.64 mmol) in 10 mL of dichloromethane was added via a volumetric pipette and allowed to stir for ten minutes. The orange solution faded with time. At a thiol:gold molar ratio > 2:1, the solution turned clear and colorless. Different sizes of AuNP can be synthesized by varying the gold/thiol ratio. A fresh solution of tetrabutylammonium borohydride (1.40 g, 5.45 mmol) in 10 mL dichloromethane was then added to the rapidly stirring toluene solution over 5 seconds. The solution turned dark black instantly. The PEGylated nanoparticles start to precipitate from toluene after 2 hours. After stirring the mixture overnight (~12 hours), 30 mL of Milli-Q water was added under moderate stirring to extract the PEGylated AuNP. The organic layer was decanted and the aqueous layer was concentrated to form a film in the round bottom flask. This film was washed repeatedly with toluene, acetonitrile and iso-propanol, resulting in pure NP as judged by ^1H NMR spectroscopy, which showed no signs of free ligands. The PEGylated AuNP can also be purified by dialysis. Namely, the black aqueous layer was

transferred into the dialysis tube (1000 Da) and dialysis in 3L of Milli-Q water for 3 days, the dialysis water was changed every 10 hours. The pure AuNP were collected from the solution by rotary evaporation or lyophilisation. The resulting AuNP sample was dark brown in color. Unlike the previous reported place-exchanged PEGylated nanoparticles from citrate-nanoparticles or CTAB-nanoparticles, these PEGylated AuNPs are relatively small (3.0 ± 0.5 nm), exhibit excellent stability, and can be repeatedly dried and redissloved in water.

General procedure to preparation furan protected maleimide AuNP (6-AuNP). The preparation of **6**-AuNP was followed by a place-exchange reaction of furan-masked maleimide-PEG-thiol ligand (**6**) with the PEGylated AuNPs. A typical procedure involved mixing the previous prepared PEGylated NPs and thiol **6** in water for a period of time. Controlling of the thiol ratio and reaction time is crucial because high loading of the furan-protected maleimide thiol always irreversibly induces the formation of a precipitate. The optimized condition involves using a 1:1 mole ratio of thiol **6**:PEGylate ligand for one hour. The mixed ligand **6**-AuNP sample was then purified by washing with ethyl acetate, acetonitrile, and iso-propanol. ^{1}H-NMR spectroscopy was used to characterize and check the purity of the **6**-AuNP product.

Preparation of maleimide-tethered AuNP (7-AuNP). 100 mg of **6**-AuNP was dissolved in 15 mL of Milli-Q water, 30 mL of toluene was added to the solution under argon. The mixture was stirred and refluxed at 95 ºC for 2 hours and the resulting **7**-AuNP was purified by washing with iso-propanol and acetonitrile. The purity of the NP sample was checked by ^{1}H NMR and no sharp peaks, usually related to free ligands, were present. The organic shell of the resulting **7**-AuNP contains 1:2 mole ratio of maleimide-PEG-thiolate:PEGylated ligand. In a separate experiment, 5 mg of **6**-AuNP was dissolved in 1.0 mL of D_2O in NMR tube, variable temperature ^{1}H NMR spectra were collected thereafter to monitor the retro-Dials-Alder reaction.

General procedure of the Michael addition reaction. 7-AuNP was dissolved in water or PBS buffer (0.1M, PH7.0) and 5 mole ratio of corresponding rhodamine 123 or cysteine was added under Ar. The resulting NP sample was purified after reacting for 1 h. The rhodamine-NP sample was purified by washing with an ethyl acetate:ethanol (95:5) mixture solvent mixture until no fluorescence was detected in the wash-out solvent. Cysteine-NP were purified by dialysis as described before.

Determination of the maleimide-AuNP composition.

Analysis of the data derived from TEM, NMR, and TGA experiments leads to an estimation of the composition of the AuNPs. Previous research has indicated that the gold core has a truncated octahedron shape.[1] To simplify the calculation, we assume here that the maleimide-AuNP has a spherical shape. The average diameter of the maleimide-AuNP core can be derived from the planar sphere projection (from TEM as 3.2 nm). We also assumed that the gold core has the same density as bulk gold; the average number of gold atoms per AuNP is thus *ca*. 1000, using[2]

$N = \frac{\pi \rho d^3}{6 M_{Au}} N_A$, where

N = number of atoms per nanoparticle

ρ = density of face centered cubic (fcc) gold = 19.3g/cm^3

d = average diameter of nanoparticles

M_{Au} = mole atomic weight of gold, 196.9665 g/mol

N_A = Avogadro constant

Thus the total number of ligands per gold nanoparticle can be calculated from the weight percentage of the organic portion of MPN. For our case, the mixed ligand maleimide-AuNP is

capped with two different ligands, and the average protecting ligands number is determined to be 90, using

$$N_L = \omega \frac{NM_{Au}/(1-\omega)}{M_{L1}\varphi + M_{L2}(1-\varphi)}$$, where

N_L = total number of ligands per nanoperticle

ω = percentage of mass loss due to the protecting ligands

M_{L1} = molecule weight of the maleimide thiolate ligands ($C_{10}H_{14}O_4NS$ = 244.0643 g/mol)

M_{L2} = molecule weight of the TEGylated ($C_6H_{13}O_3S$ = 165.0585 g/mol)

φ = the mole percentage of the maleimide ligand

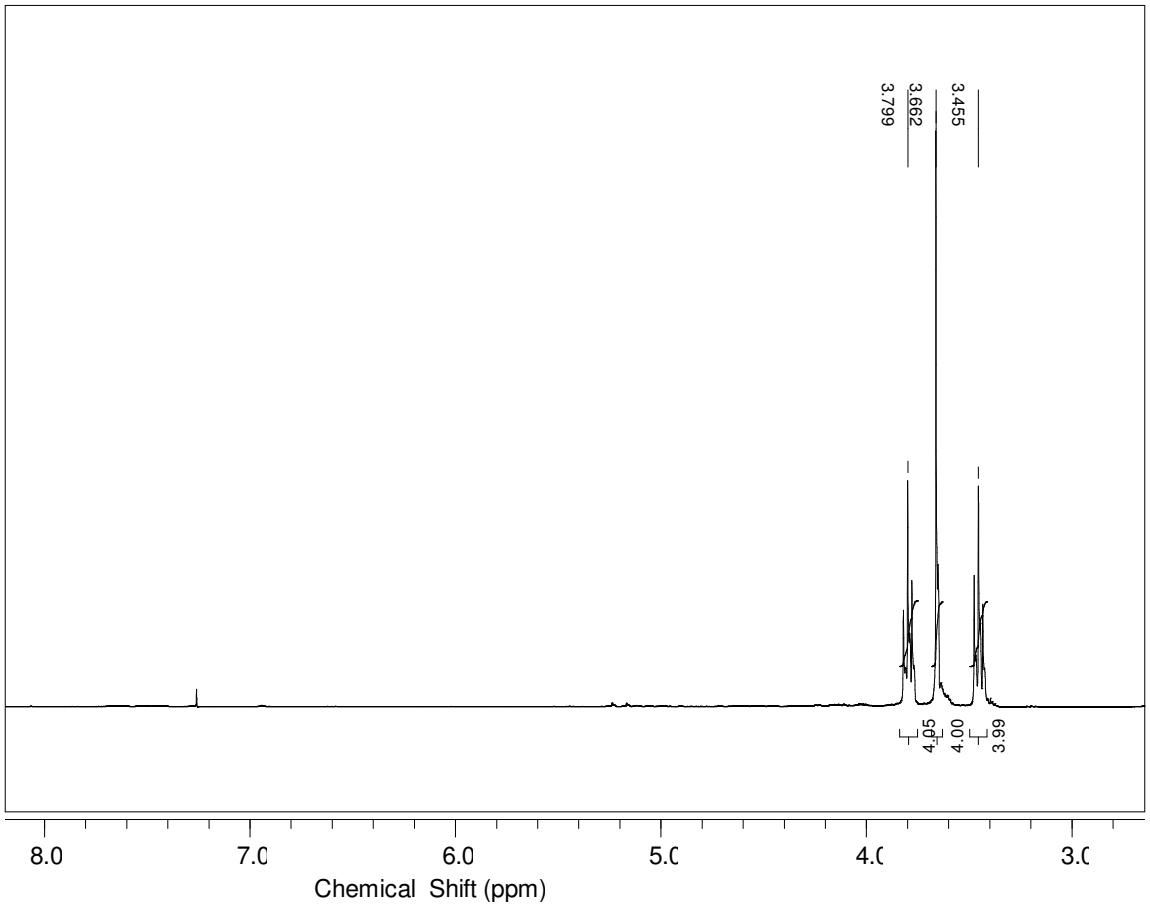


Figure S1. ^{1}H NMR spectrum of 1,2-bis(2-bromoethoxy)ethane (**2**) in $CDCl_3$

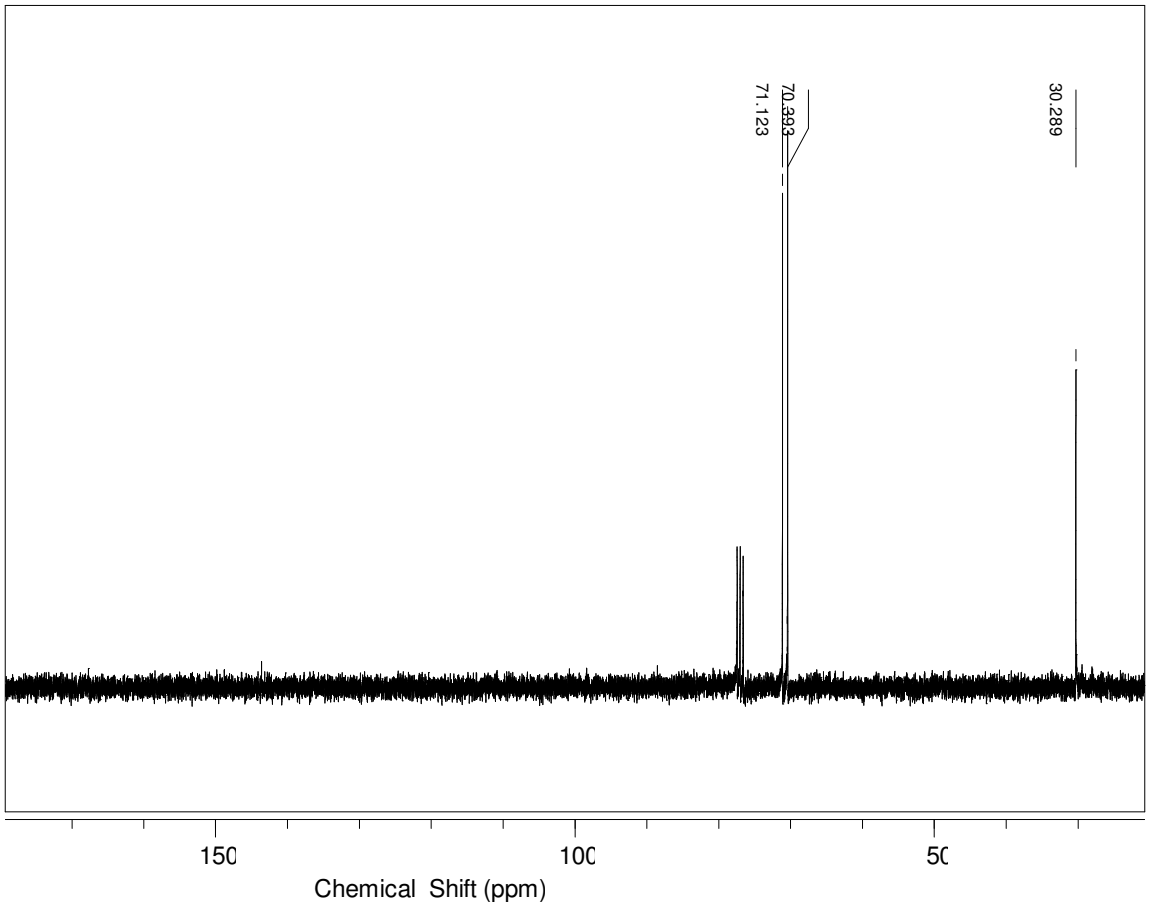


Figure S2. ^{13}C NMR spectrum of 1,2-bis(2-bromoethoxy)ethane (**2**) in $CDCl_3$

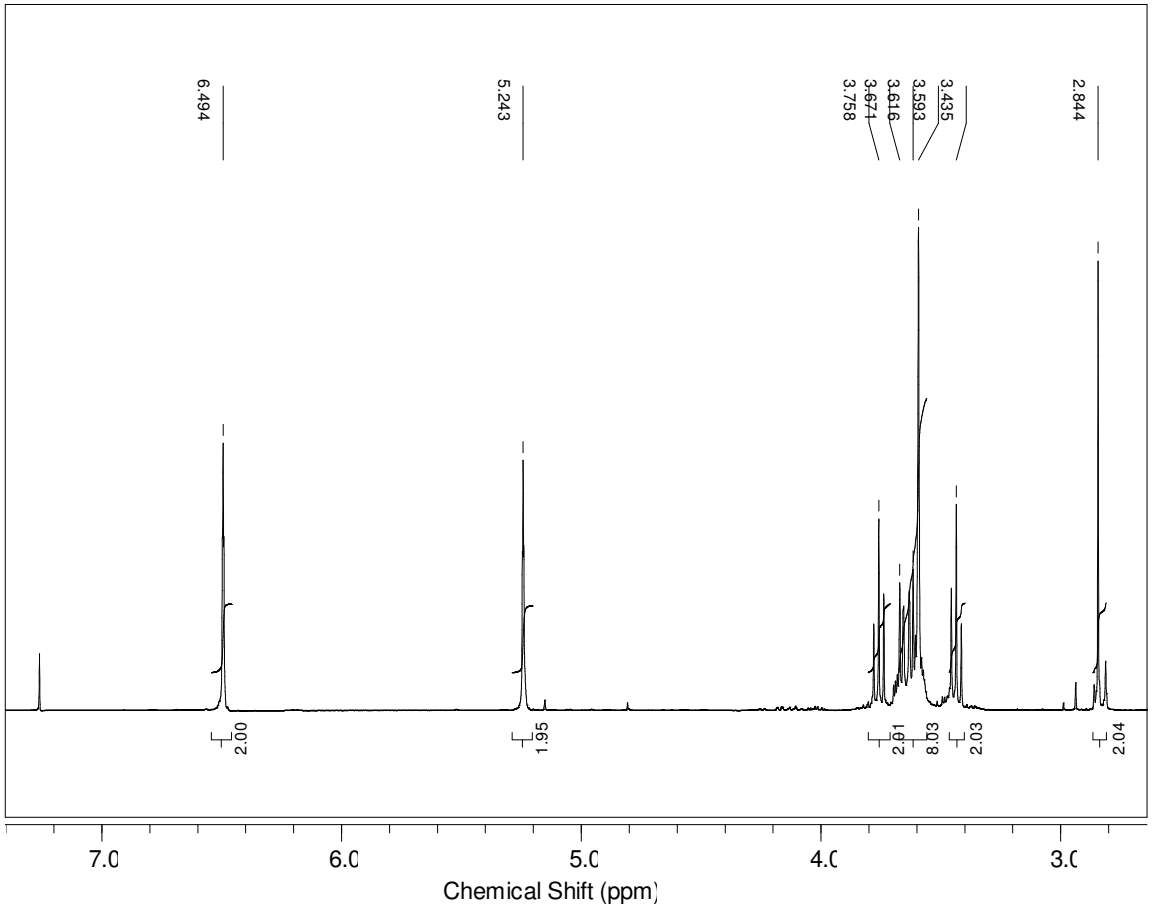


Figure S3. ^{1}H NMR spectrum of 2-(2-(2-(2-bromoethoxy)ethoxy)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**4**) in $CDCl_3$

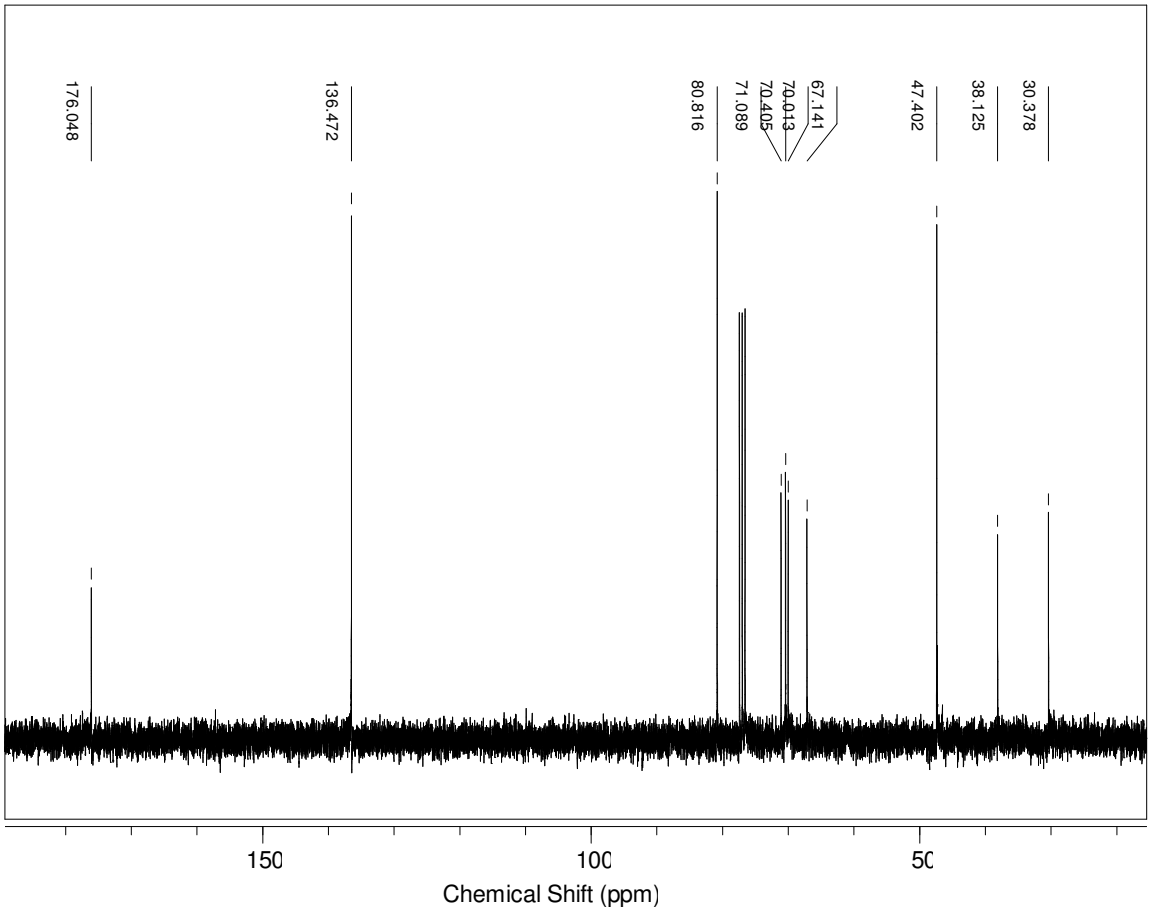


Figure S4. ^{13}C NMR spectrum of 2-(2-(2-(2-bromoethoxy)ethoxy)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**4**) in $CDCl_3$

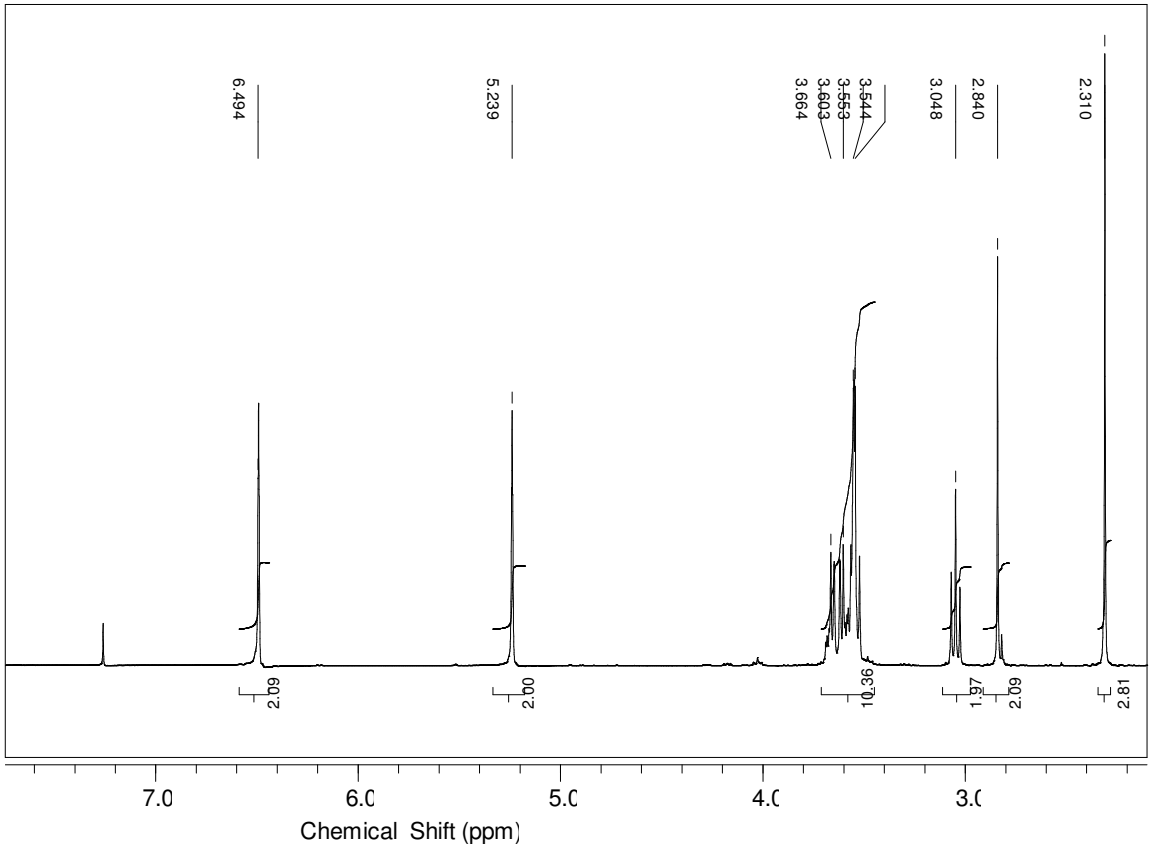


Figure S5. ^{1}H NMR spectrum of 2-(2-(2-(2-(prop-1-en-2-ylthio)ethoxy)ethoxy)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**5**) in $CDCl_3$

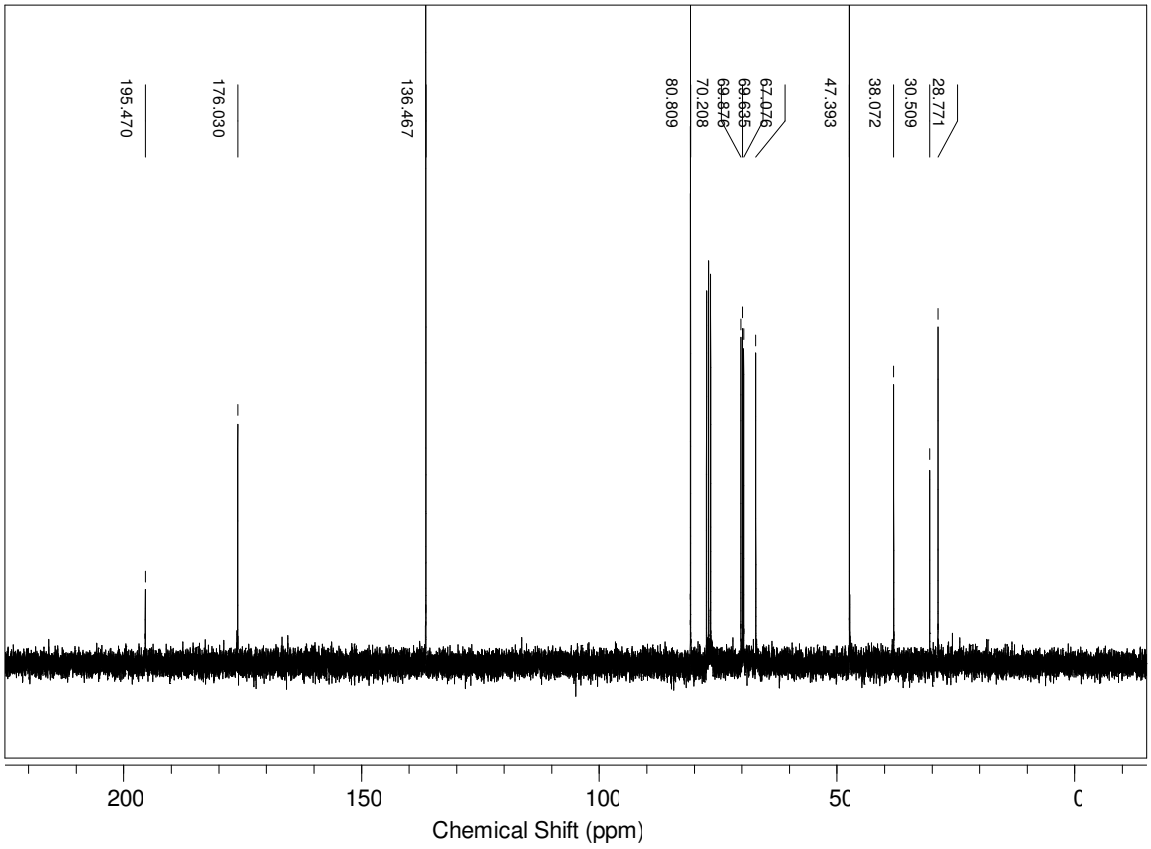


Figure S6. ^{13}C NMR spectrum of 2-(2-(2-(2-(prop-1-en-2-ylthio)ethoxy)ethoxy)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**5**) in $CDCl_3$

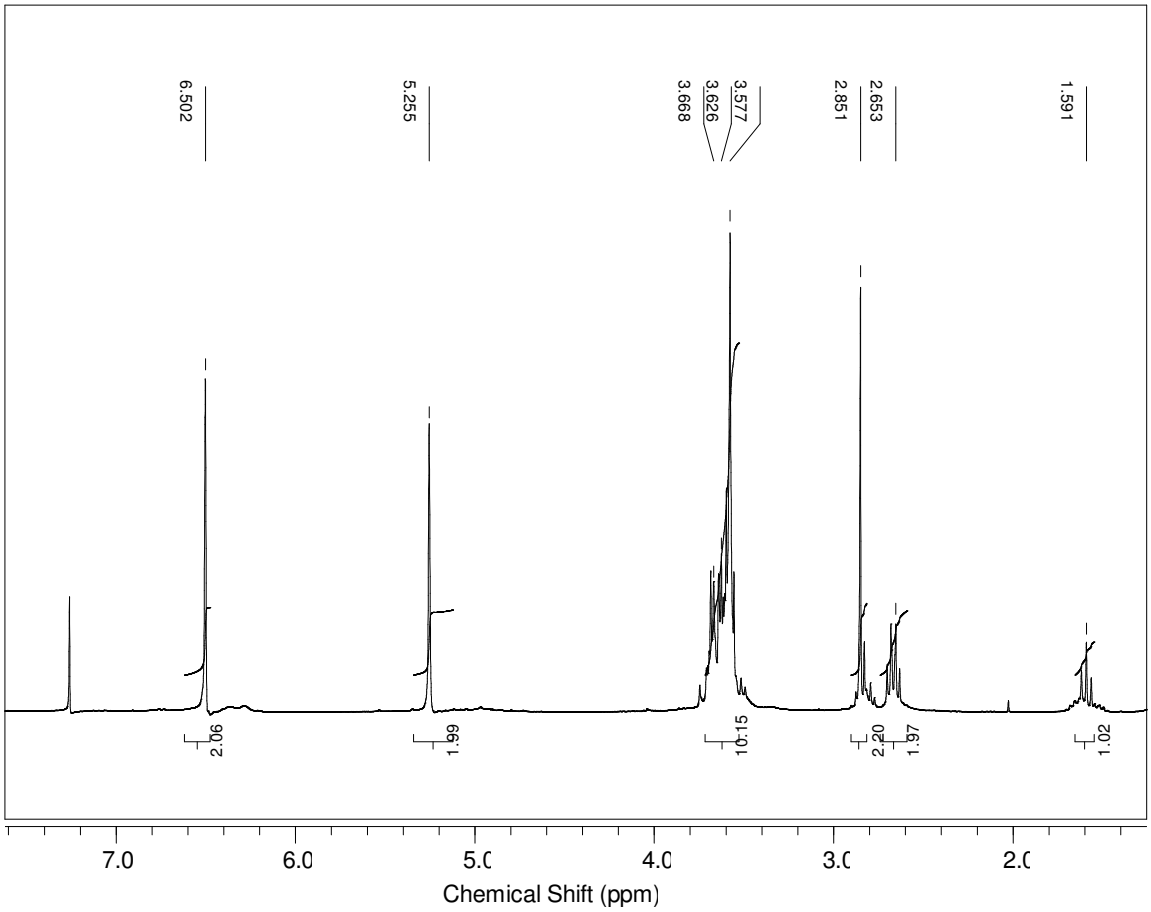


Figure S7. ^{1}H NMR spectrum of 2-(2-(2-(2-mercaptoethoxy)ethoxy)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**6**) in $CDCl_3$

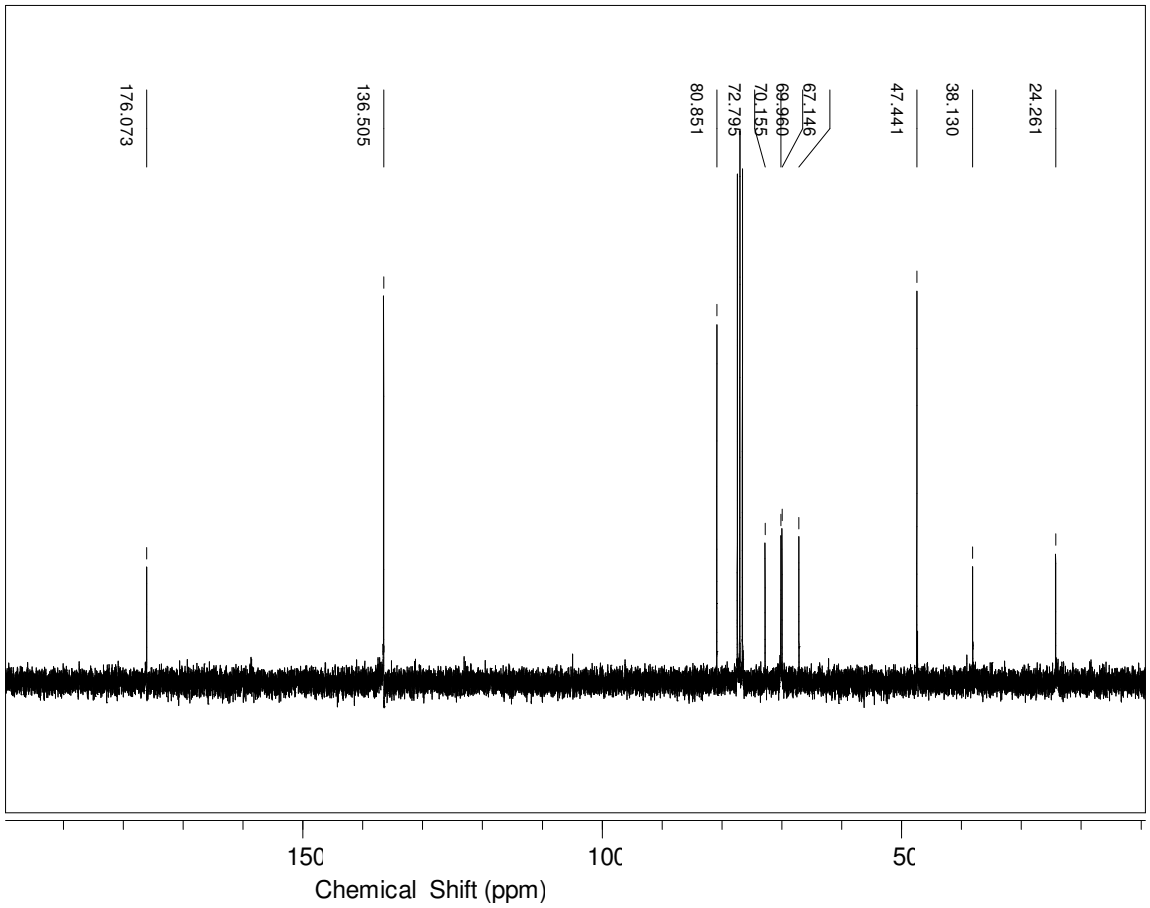


Figure S8. ^{13}C NMR spectrum of 2-(2-(2-(2-mercaptoethoxy)ethoxy)ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (**6**) in $CDCl_3$

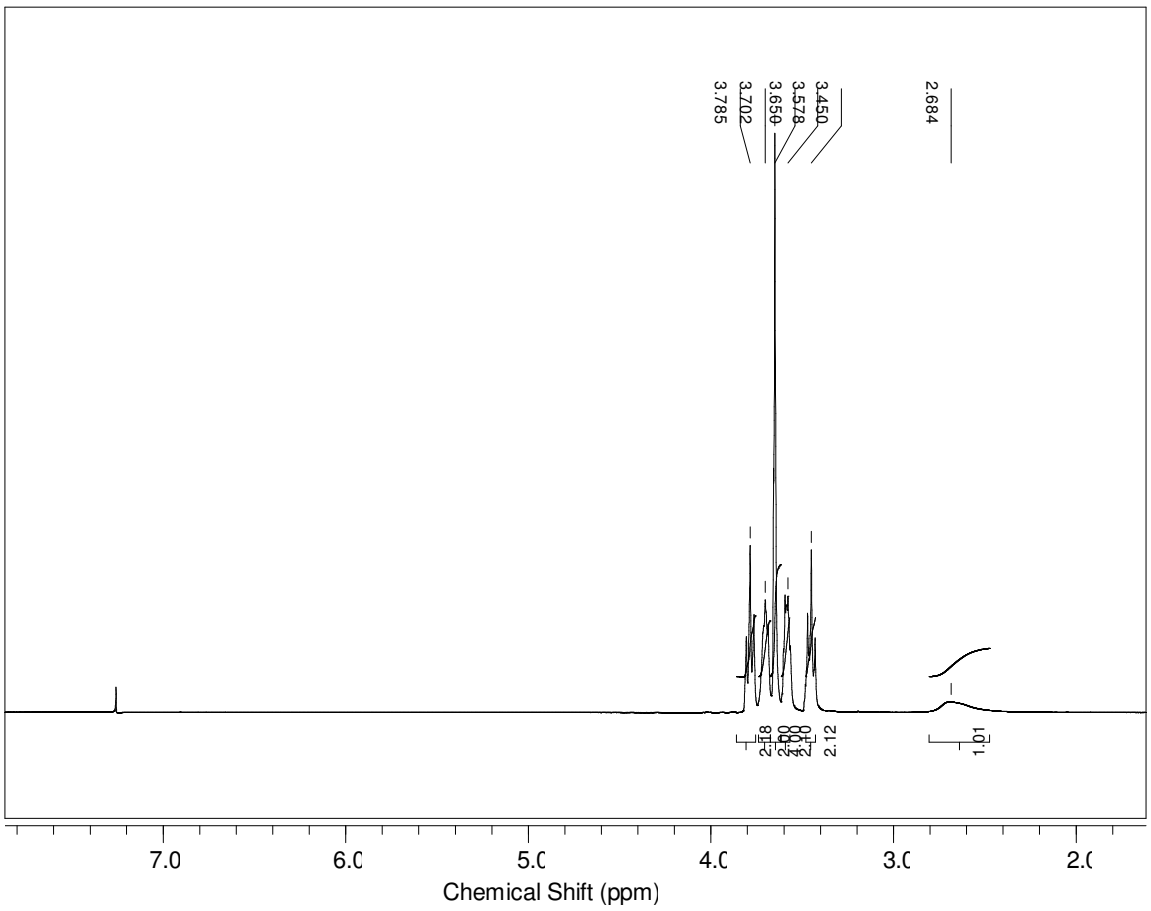


Figure S9. ^{1}H NMR spectrum of 2-(2-(2-bromoethoxy)ethoxy)ethanol in $CDCl_3$

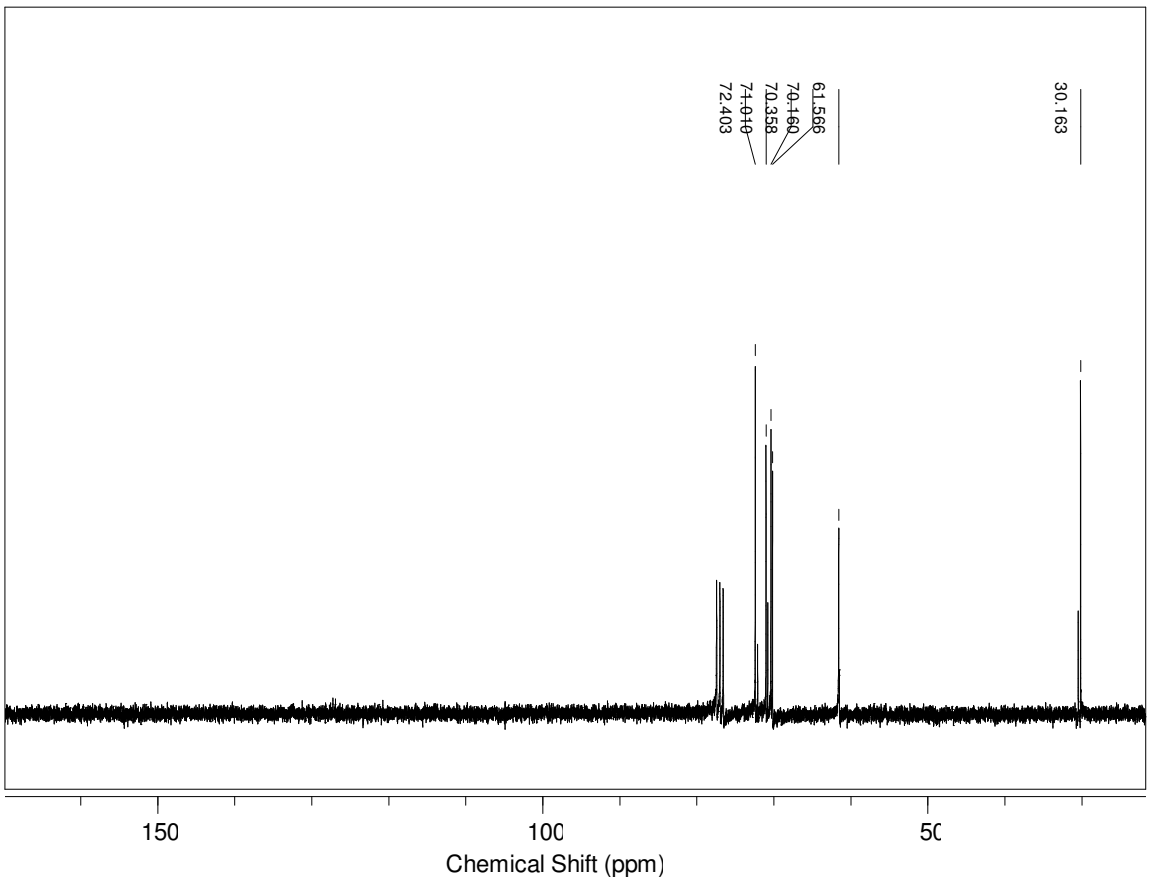


Figure S10. ^{13}C NMR spectrum of 2-(2-(2-bromoethoxy)ethoxy)ethanol in $CDCl_3$

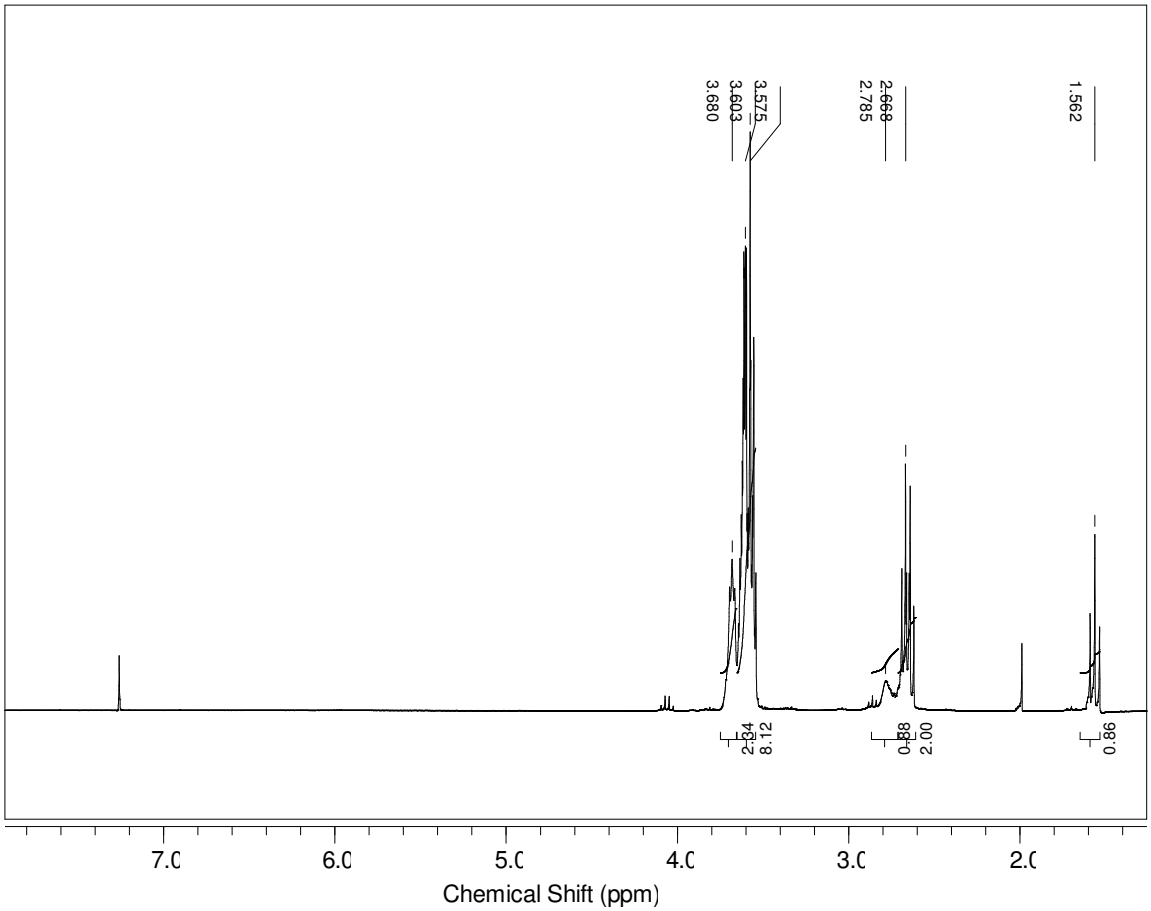


Figure S11. ^{1}H NMR spectrum of 2-(2-(2-mercaptoethoxy)ethoxy)ethanol in $CDCl_3$

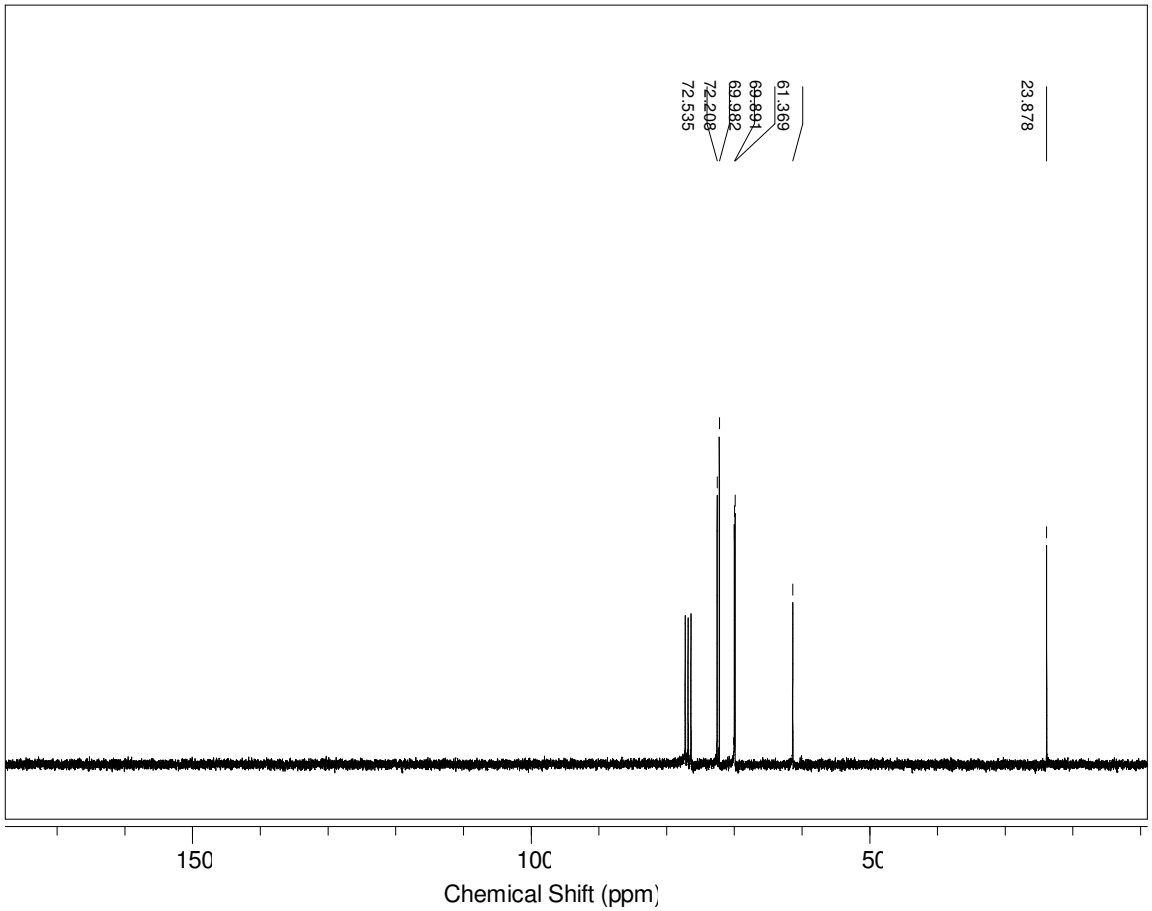


Figure S12. ^{13}C NMR spectrum of 2-(2-(2-mercaptoethoxy)ethoxy)ethanol in $CDCl_3$

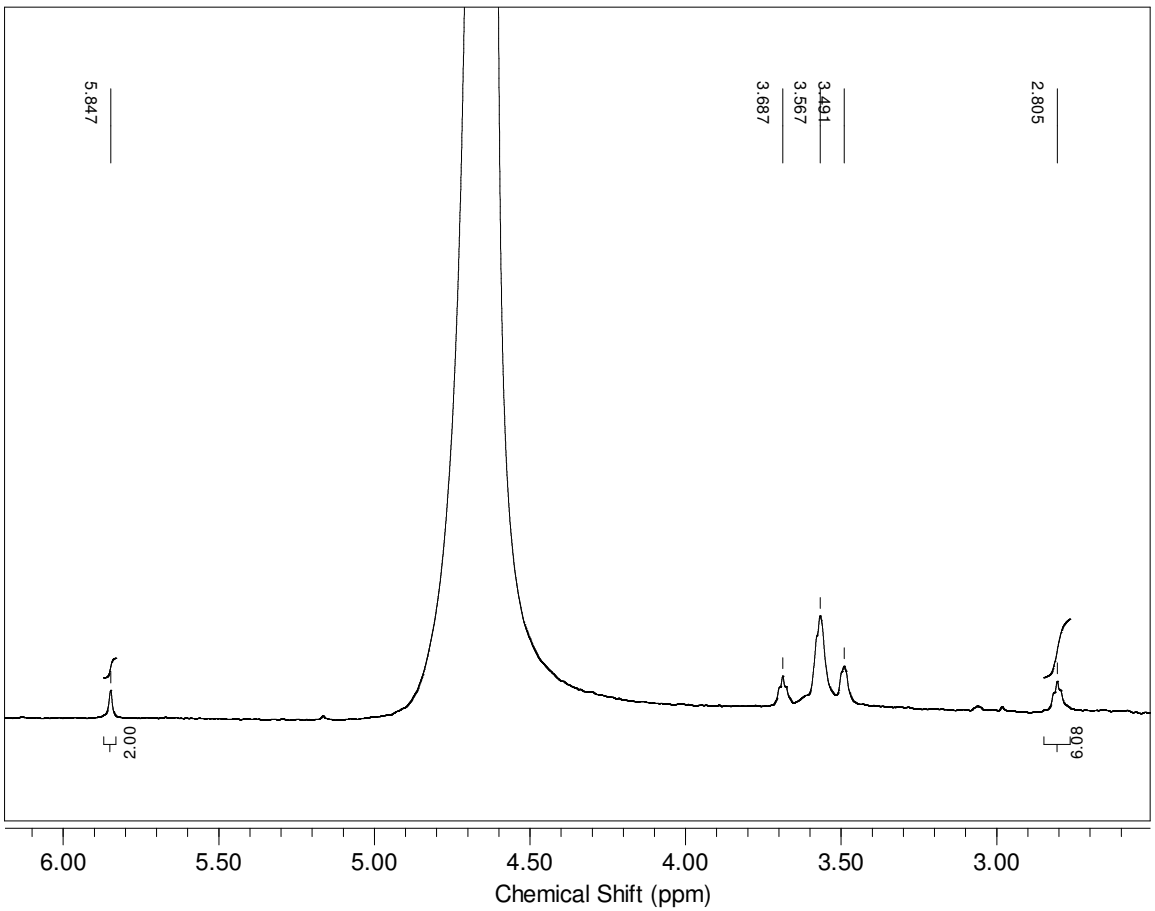


Figure. S13. ^{1}H NMR spectrum of the 7-AuNP. Peaks at 5.82 ppm and 2.80 ppm correspond to the alkene protons of maleimide and protons α to the sulfur, respectively. Solvent = D_2O. As each maleimide-PEG ligand contains 2 alkene protons plus 2 α to sulfur protons and each PEG ligand contains 2 α to sulfur protons,. The mole ratio of the maleimide-PEG-thiolated:PEGylated determined from the integration is 1:2.

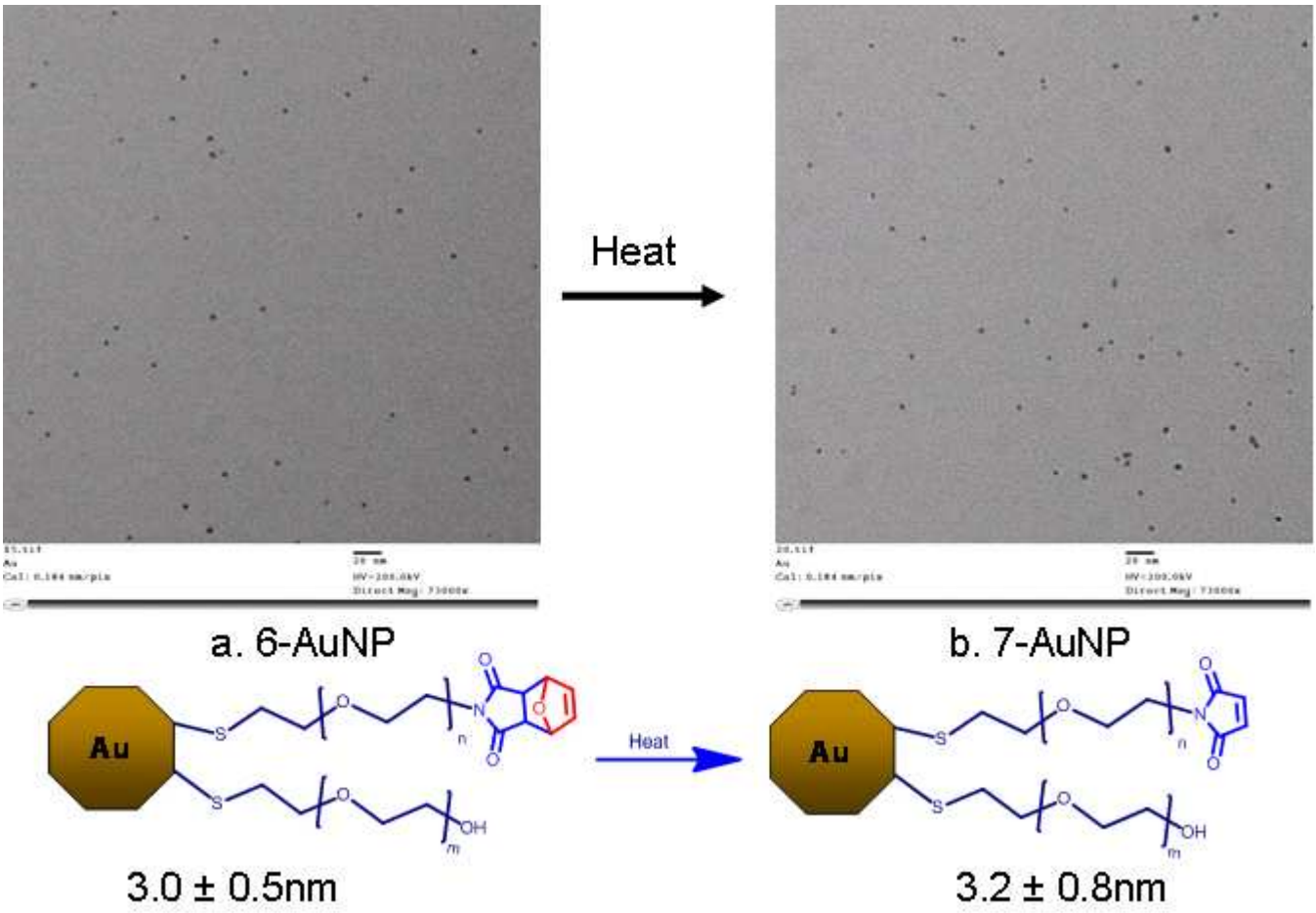


Figure S14. Transmission electron microscopy (TEM) images of a) **6**-AuNP and b) **7**-AuNP. Scale bar 20 nm.

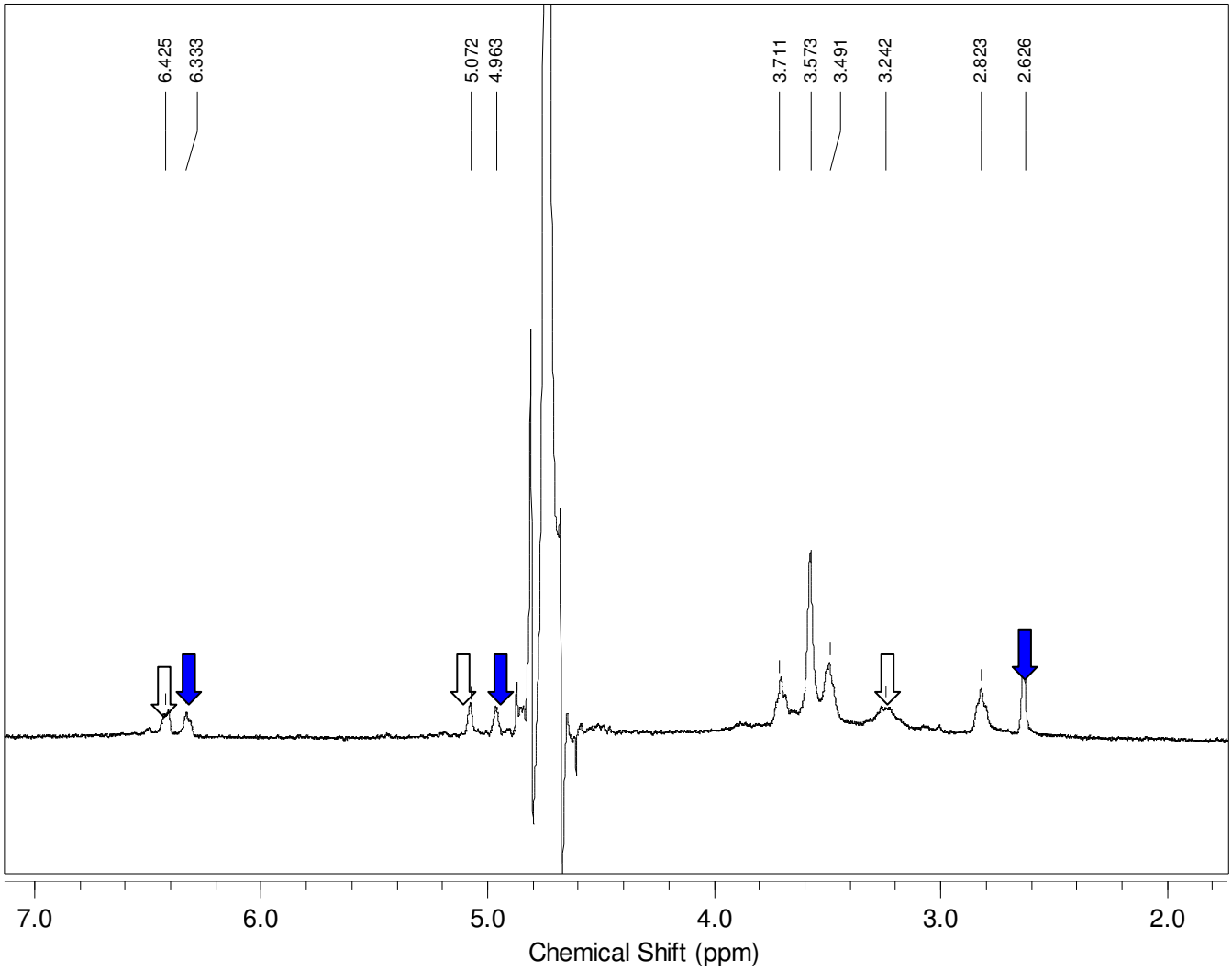
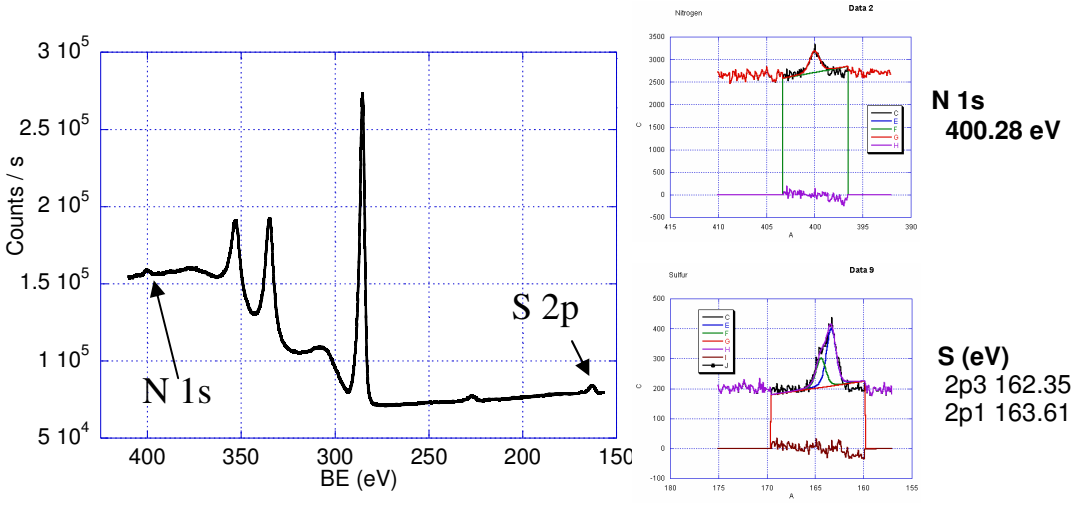


Figure S15. ^{1}H NMR spectrum of the Diels-Alder adducts of **7**-AuNP and furan. The white and blue arrows are attributed to the endo- and exo- products, respectively. Solvent = D_2O.



Name	Start BE	Peak BE	End BE	FWHM eV	Area (P) C	Area (N)	At. %	F	Q	SF	TXFN	Backgnd
C 1s Scan	292.18	286.23	281.28	2.06	26202.18	0	0	PF	0	1	1	Shir.
C 1s Scan	292.18	288.71	281.28	2.12	7221.01	0	0	PF	0	1	1	Shir.
C 1s Scan	292.18	285.01	281.28	1.6	45439.42	0	0	PF	0	1	1	Shir.
Au4f7 Sca	92.58	83.62	79.78	1.11	59955.97	0	0	PF	0	9.58	1	Shir.
Au4f5 Sca	92.58	87.29	79.78	1.12	47444.88	0	0	PF	0	7.54	1	Shir.
N1s Scan	405.97	400.3	395.48	3.1	1634.37	13.69	31.45	PF	1	1.8	1	Shir.
O1s Scan	539.88	533.61	527.78	2.27	20331.03	0	0	PF	0	2.93	1	Lin.
O1s Scan	539.88	532.26	527.78	1.49	19452.98	0	0	PF	0	2.93	1	Lin.
S 2p3 Scar	169.07	162.35	157.89	2.34	1234.42	14.89	34.2	PF	1	1.11	1	Shir.
S 2p1 Scar	169.07	163.61	157.89	2.35	632.9	14.96	34.35	PF	1	0.567	1	Shir.

Figure 16S. XPS spectra of **7**-AuNP. The peak at 400.28 eV and 162.35/163.61 eV are corresponding to the BE of N1s and S2p3/S2p1, respectively. Quantitative analysis showed the mole ratio of N:S=3:7.

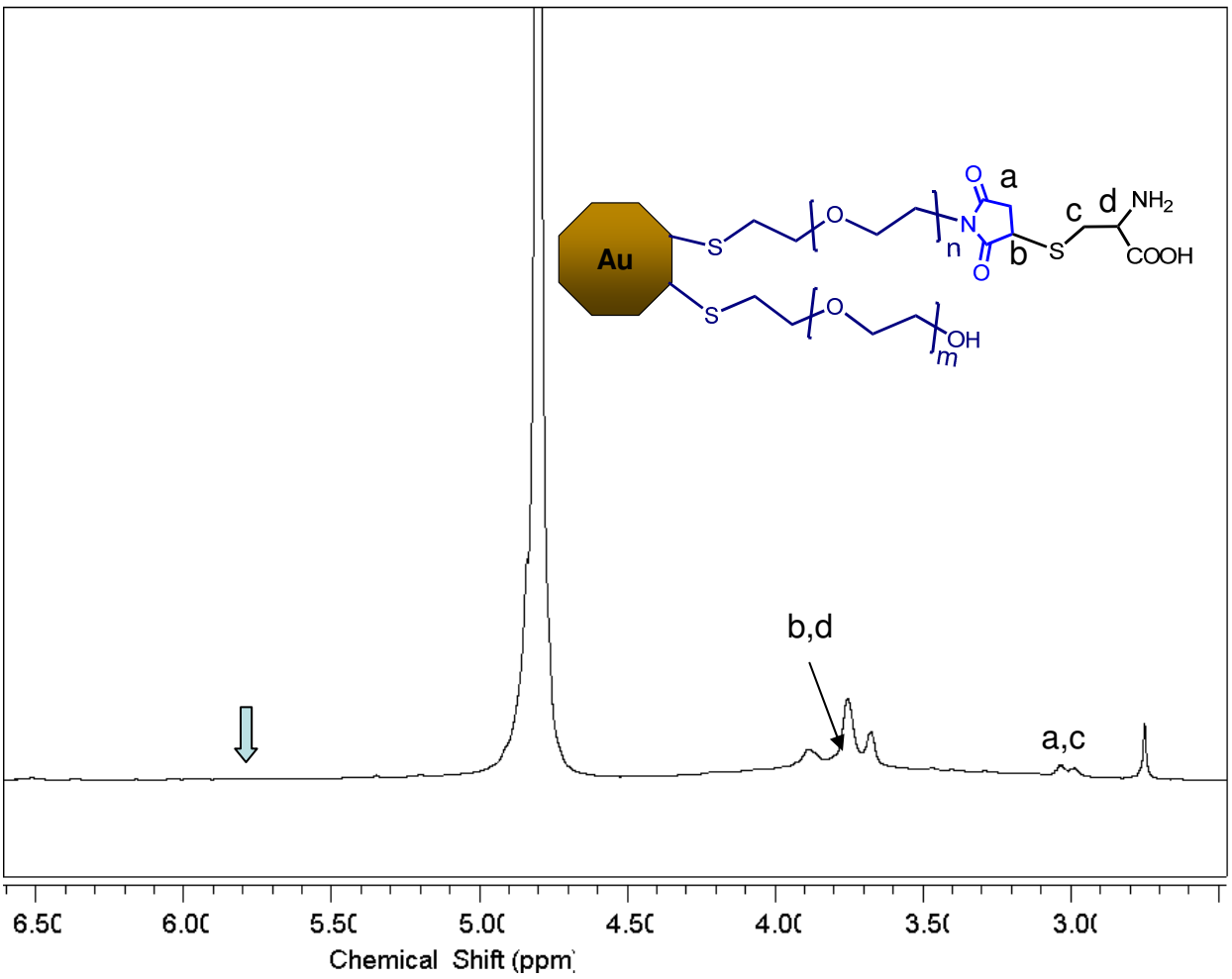


Figure 17S. ^{1}H NMR spectrum of the Michael addition reaction adducts of **7**-AuNP and L-cysteine. Protons b and d are merged with the PEGylated ligand protons. The disappearing of maleimide peak indicates the completion of the reaction. Solvent = D_2O.

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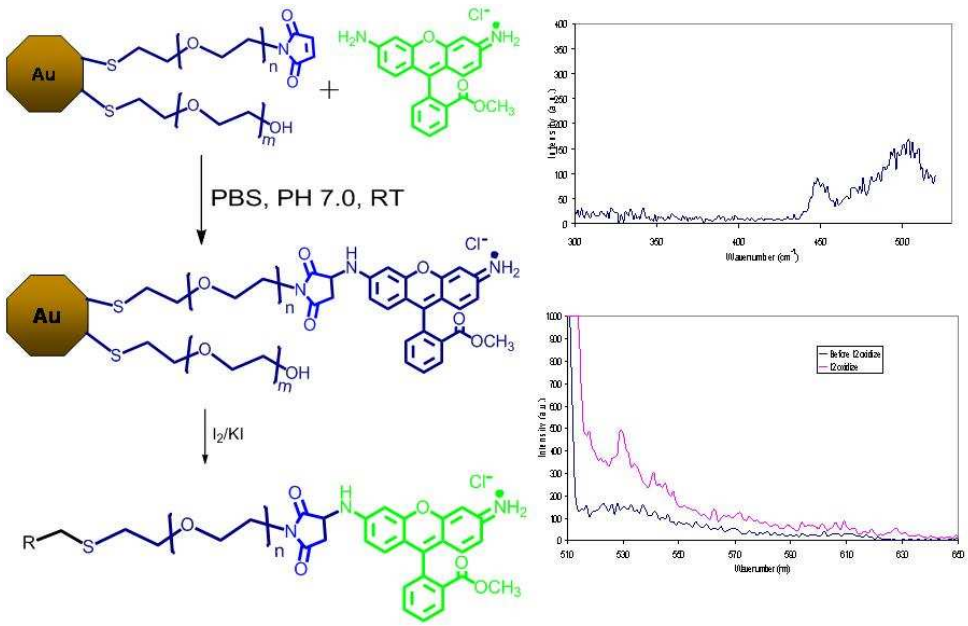

Figure 18S. Fluorescent spectra for rhodamine-AuNP before and after treatment with I_2/KI. Excitation at 505 nm and emission at 530 nm.

[1] (a) Whetten, R. L.; Khoury, J. T.; Alvarez, M. M.; Murthy, S.; Vezmar, I.; Wang, Z.; Stephens, P. W. *Adv. Mater.*, **1996**, *8*, 428-433. (b) Cleveland, C, L.; Landman, U.; Schaaff, T. G. ; Shafigullin, M. N.; Stephens, P. W.; Whetten, R. L. *Phys. Rev. Lett.* **1997**, *79*, 1873-1876. (c) Whetten, R. L.; Shafigullin, M. N.; Khoury, J. T.; Schaaff, T. G.; Vezmar, I.; Alvarez, M. M.; Wilkinson, A. *Acc. Chem. Res*., **1999**, *32*, 397-406

[2] Liu, X., Atwater, M., Wang, J., and Huo, Q. *Colloids Surf. B*, **2007**, *58*, 3-7