Supporting Information for:

Nitrone Modified Gold Nanoparticles: Synthesis, Characterization and Their Potential as ^{18}F-Labeled PET Probes via I-SPANC

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General Materials and Methods

The following reagents were used as received. Triethylene glycol monomethylether (MeO-EG_3-OH), tetraethylene glycol (HO-EG_4-OH), 4-dimethylaminopyridine (DMAP), potassium thioacetate, deuterated acetonitrile (CD_3CN), deuterated chloroform ($CDCl_3$), phosphorous pentoxide (P_2O_5), tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium borohydride ($NaBH_4$), *p*-toluenesulfonyl chloride (TosCl), *N*-methylhydroxylamine hydrochloride ($CH_3NHOH \cdot HCl$), sodium azide (NaN_3), triphenylmethanethiol ($HSCPh_3$), triisopropylsilane (TIPS), *N*,*N*-Diisopropylethylamine (DIPEA), phthalimide potassium, sodium iodide (NaI), hydrazine monohydrate ($H_2N–NH_2 \cdot H_2O$), cesium fluoride (CsF) and O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Sigma-Aldrich. All common solvents, triethyleneamine (Et_3N), sodium sulfate anhydrous (Na_2SO_4) , dry methanol (CH_3OH), tert-butanol (*t*BuOH), hydrochloric acid (HCl), trifluoroacetic acid (TFA), sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$) and potassium carbonate (K_2CO_3) were purchased from Caledon. Deuterated water (D_2O) was purchased from Cambridge Isotope Laboratories. Ethanol (EtOH) was purchased from Commercial Alcohols. Glacial acetic acid (99.7%) was purchased from BDH. Dialysis membranes (MWCO 6000-8000) were purchased from Spectra/Por.

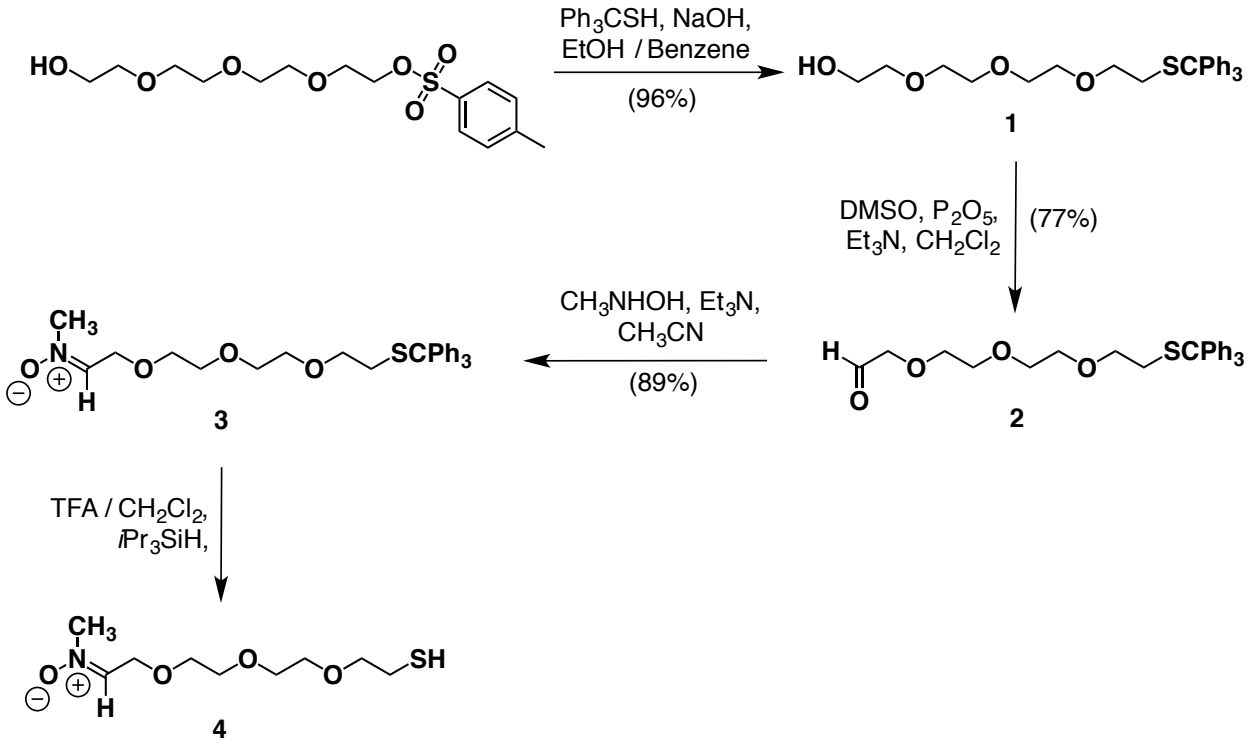
^{18}F-Fluoride was obtained from the Nordal Cyclotron & PET Radiochemistry Facility at the Lawson Health Research Institute, London, Canada. An automated synthesis unit (TracerLab, GE Healthcare, Schenectady, NY) was used to prepare and purify [^{18}F] SA-64. A V-10 evaporator (Biotage, Charlotte, NC) was used to remove the solvent after HPLC purification. PD-10 desalting column was purchased from GE Healthcare. HPLC analysis and purification was performed on a Waters HPLC system (Milford, MA) using a dual detector system (UV and

radiometric), with the mobile phase being CH_3CN (solvent A)/H_2O (solvent B) containing 0.1 % TFA. The HPLC columns were as follows: Sunfire RP-18 analytical column (Waters): 4.6 × 250 mm, 5 μm, flow rate: 1.5 mL/min; Sunfire RP-18 semipreparative column (Waters): 10 × 250 mm, 5 μm, flow rate: 4 mL/min. The UV detector was set to λ_{max} =230 nm. C57/B6 mice (male, 10-12 weeks age) were ordered from Charles River (Wilmington, MA) for PET imaging. All animal studies were performed following the Canadian Council on Animal Care guidelines and animal use protocol approved by animal facility of Western University. Imaging was performed using the Inveon preclinical PET system (Siemens Medical Solutions, Knoxville TN) on age-matched littermate mice using list mode scanning. Acquisition, histograms, and reconstructions were all performed using the Siemens Inveon acquisition and reconstruction software supplied with the scanner.

^{1}H, and ^{13}C and ^{19}F $\{^{1}H\}$ NMR spectra were recorded on either a Varian Inova 400 MHz, Varian Inova 600 MHz or a Varian Mercury 400 MHz spectrometer and were calibrated against the residual protonated solvents. Transmission electron microscopy (TEM) images were recorded from a TEM Philips CM10. The TEM grids (Formvar carbon film on 400 mesh copper grids) were purchased from Electron Microscopy Sciences and prepared by drop casting solution of nanoparticles directly onto the grid surface. Mass spectrometry measurements were carried out using a Micro mass LCT (electrospray time-of-flight) mass spectrometer. Thermogravimetric Analysis (TGA) measurements were recorded by loading the sample in 70 μL ceramic crucible and heating from 25-750 °C with a rate of 10 °C min^{-1}. The experiments were performed under a nitrogen flow of 70 ml min^{-1} in a Mettler Toledo TGA/SDTA 851 instrument. The XPS analyses were carried out with a Kratos Axis Ultra spectrometer using a monochromatic Al K(alpha) source (15mA, 14kV). The instrument work function was calibrated to give a binding energy

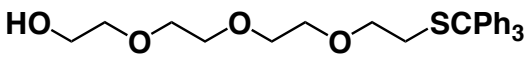
(BE) of 83.96 eV for the Au $4f_{7/2}$ line for metallic gold and the spectrometer dispersion was adjusted to give a BE of 932.62 eV for the Cu $2p_{3/2}$ line of metallic copper. Specimens were mounted on a double sided adhesive tape and the Kratos charge neutralizer system was used on all specimens. Survey scan analyses were carried out with an analysis area of 300 x 700 microns and pass energy of 160 eV. High resolution analyses were carried out with an analysis area of 300 x 700 microns and pass energy of 20 eV. Spectra have been charge corrected when needed to the main line of the carbon 1s spectrum set to 285.0 eV for aliphatic carbon. Spectra were analyzed using CasaXPS software (version 2.3.14). UV-Vis spectra were collected employing a Varian Cary 300 Bio spectrometer in CH_3CN. The FTIR spectra were carried out using KBr pellets on a Bruker Vector33 spectrometer.

Synthetic details



Scheme S1. Synthetic procedure for the preparation of nitrone-terminated thiol ligand (**compound 4**).

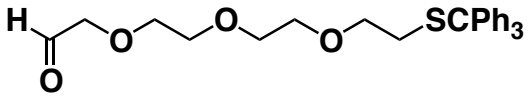
3.1 Synthesis of Compound 1



Ph_3CSH (0.99 g, 3.58 mmol) was dissolved in a solution of EtOH/benzene (1:1, 10 mL) and NaOH (0.18 g, 4.00 mmol) in 4 mL of H_2O was added. Then HO-EG_4-Tos (1.05 g, 3.00 mmol) was also dissolved in a solution of EtOH/benzene (1:1, 8 mL) and added to the Ph_3CSH mixture. The new reaction mixture was stirred overnight at room temperature. Once the reaction was completed (checked by TLC) all the mixture was poured into a saturated solution of $NaHCO_3$ and washed three times. The organic layer was separated and added into a saturated solution of NaCl and also washed for three times. Afterward the organic layer was separated, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel

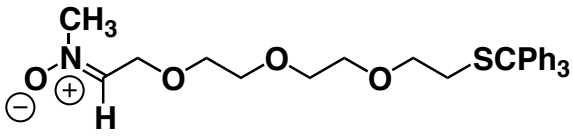
using EtOAc as the eluent to give **compound 1** in 96% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 2.44 (t, *J* = 6.8 Hz, 2H), 3.31 (t, *J* = 6.8 Hz, 2H), 3.46 (dd, *J* = 5.7, 3.7 Hz, 2H), 3.57-3.72 (m, 10H), 7.19-7.30 (m, 9H), 7.42 (m, 6H),; ^{13}C NMR (400 MHz $CDCl_3$); 31.6, 61.7, 66.6, 69.6, 70.1, 70.3, 70.4, 70.6, 72.5, 126.6, 127.8, 129.6, 144.8. HRMS (ESI) $[M]^+$ calcd for $C_{27}H_{32}O_4S$ 452.2023; found 452.2021.

3.2 Synthesis of Compound 2



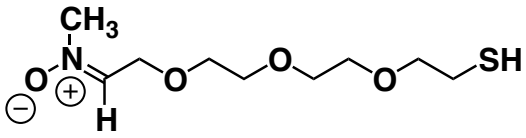
A flame-dried, two-necked, 250 mL round-bottomed flask was purged with Ar and then was loaded with **compound 1** (2.1 g, 4.6 mmol) dissolved in dry CH_2C1_2 (0.2 M in **compound 1**). The flask was immersed in an ice-water bath. DMSO (360.1 mg, 9.2 mmol) and P_2O_5 (1.3 g, 9.2) were added sequentially. The reaction mixture was allowed to stir and warm up to room temperature until disappearance of starting material by TLC (4 h). The flask was immersed again in an ice-water bath; then Et_3N (1.63 g, 16.1 mmol) was added dropwise over 10 minutes. Stirring was continued for 45 minutes. The reaction was quenched with 10% aqueous HCl and extracted with CH_2Cl_2. The organic extracts were washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4 filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using hexane/EtOAc (1:3 v/v) as the eluent providing aldehyde **compound 2** as a light yellow oil in 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 2.42 (t, *J* = 6.7 Hz, 2H), 3.29 (t, *J* = 7.0 Hz, 2H), 3.44 (m, 2H), 3.55-3.70 (m, 6H), 4.11 (m, 2H), 7.18-7.26 (m, 9H), 7.40 (m, 6H), 9.67 (s, 1H); ^{13}C NMR (400 MHz $CDCl_3$); 31.3, 66.3, 69.6, 69.8, 70.2, 70.4, 70.9, 126.3, 127.5, 129.3, 144.9, 200.5. HRMS (ESI) $[M]^+$ calcd for $C_{27}H_{30}N_2O_4S$ 450.1866; found 450.1864.

3.3 Synthesis of Compound 3



Aldehyde **compound 2** (1.1 g, 2.3 mmol), *N*-methylhydroxylamine hydrochloride (450 g, 4.70 mmol), and Et_3N (875 mg, 9.40 mmol) were mixed in dry CH_3CN (50 mL). After reaction completion (3h, checked by TLC) the solvent was evaporated and substituted with CH_2Cl_2. The organic was then washed with saturated aqueous NaCl three times, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using CH_2Cl_2/EtOAc/MeOH (10:3:1 v/v) as the eluent to give nitrone **compound 3** as a yellow oil in 89% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 2.44 (t, J = 7.0 Hz, 2H), 3.32 (t, J = 7.0 Hz, 2H), 3.48 (m, 2H), 3.58 (m, 2H), 3.62-3.67 (m, 6H), 4.44 (m, 2H), 6.89 (t, J = 4.5 Hz, 1H) 7.20-7.30 (m, 9H), 7.41 (m, 6H); ^{13}C NMR (400 MHz $CDCl_3$); 31.6, 52.0, 66.5, 66.6, 69.6, 70.1, 70.2, 70.5, 70.9, 126.6, 127.8, 129.5, 138.5, 144.7; HRMS (CI) $[M+H]^+$ calcd for $C_{28}H_{33}NO_4S$ 480.2209; found 480.2210.

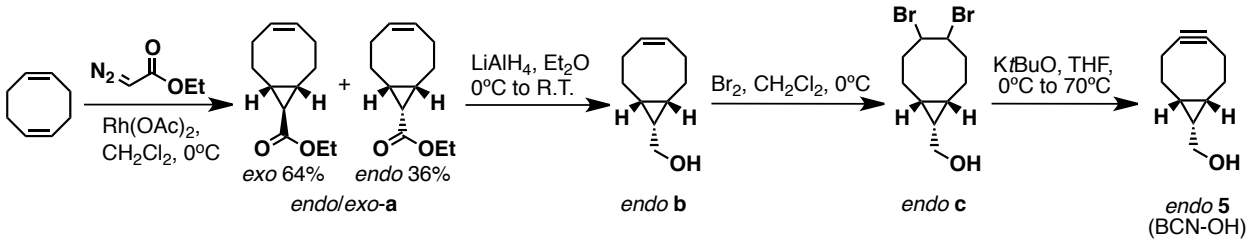
3.4 Synthesis of Compound 4



To a solution of nitrone **compound 3** (400 mg, 0.800 mmol) and TFA (1.90 g, 16.7 mmol) in CH_2Cl_2 (20 mL) was added TIPS (0.21 mL, 1.0 mmol) as a carbocation scavenger. The reaction mixture was stirred under Ar for 1 h. The solution was washed with 0.2 M $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography over silica gel using CH_3CN /EtOAc/MeOH (10:3:1 v/v) as the eluent to give thiol **compound 4** as a pale yellow oil. We were unsuccessful in purifying

compound 4 completely as a small percentage hydrolyzes back to the aldehyde over the course of purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 1.54 (t, *J* = 8.2 Hz, 1H), 2.56 (m, 2H), 3.54-3.63 (m, 14H), 4.40 (m, 2H), 6.86 (t, *J* = 4.5 Hz, 1H); ^{13}C NMR (400 MHz $CDCl_3$); 24.2, 52.1, 66.6, 70.2, 70.3, 70.5, 70.9, 72.8, 138.4. HRMS (CI) $[M+H]^+$ calcd for $C_9H_{20}NO_4S$ calculated: 238.1113, found: 238.1119.

3.5 Synthesis of Compound 5



Scheme S2. Synthetic procedure for the preparation of *endo* BCN-OH **5**.

All the steps in preparation of compound **5** were carried out according to previous reports as illustrated in Scheme S2.[1] In a typical synthesis, $Rh_2(OAc)_4$ (0.776 g, 1.76 mmol) was dissolved in dry CH_2Cl_2 (20 mL) under argon atmosphere. Cyclooctadiene (40 mL, 0.33 mmol) was then added to the solution. In a separate flask, ethyl diazoacetate (4.3 mL, 41 mmol) was also dissolved in dry CH_2Cl_2 (20 mL) and then slowly added to the cyclooctadiene solution. Evolution of nitrogen gas was observed right away. The dark green solution was stirred at room temperature for two days. After the solvent was evaporated, the excess cyclooctadiene was recovered by column chromatography on silica gel using hexanes as the eluent. After elution of cyclooctadiene, the *endo*/*exo*-**a** isomers were separated using hexanes/Et_2O 9:0.5 as the eluent. The two isomers were obtained as a colorless oil (6.979 g, 88% overall yield, 36% *endo* and 64% *exo*). R_f *exo* = 0.6, R_f *endo* = 0.4 in hexanes/Et_2O 9:0.5.

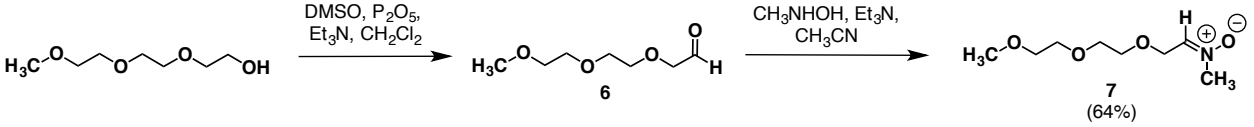
Only the *endo* isomer was used for the following steps. A suspension of $LiAlH_4$ (1.371 g, 36.11 mmol) in dry Et_2O (80 mL) was cooled to 0 ºC. Compound *endo*-**a**, from the previous step, (7.8 g, 40.4 mmol) was also dissolved in dry Et_2O (90 mL) and cooled to 0 ºC before dropwise addition to the reaction flask. The gray solution was stirred for 15 min at room temperature, then it was cooled again to 0 ºC and distilled water was added dropwise under normal atmosphere

until formation of a white precipitate was observed. The mixture was dried over Na_2SO_4, and the solids were filtered off and washed with Et_2O. The solvent was evaporated to obtain intermediate *endo* alcohol. No further purification was performed.

Compound *endo*-**b** from the previous step was dissolved in CH_2Cl_2 (250 mL) and cooled to 0 ºC. A solution of Br_2 (2.6 mL, 51 mmol) in CH_2Cl_2 was added dropwise at 0 ºC to the alkene solution until a light yellow color persisted. The reaction mixture was quenched with a 10% $Na_2S_2O_3$ solution (70 mL). After vigorous stirring for a few minutes, the solution turned colorless and was then extracted with CH_2Cl_2 (4 x 15 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated *in vacuo* to give *endo*-**c** as a thick yellow oil in 90% yield over the two last steps. No further purification was performed.

The *endo*-**c** from the previous step was dissolved in dry THF (80 mL) and cooled to 0 ºC. A 1 M solution of KO*t*Bu in THF (120 mL) was added dropwise into the reaction flask. After addition the solution turned orange. At this point the ice-bath was removed, the temperature was raised to 70 ºC, and the reaction mixture was refluxed for 2 h. After cooling to room temperature the reaction mixture was quenched with saturated NH_4Cl (200 mL) and the product was extracted with CH_2Cl_2. The collected organic fractions were dried with $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (3:1 Et_2O/hexanes) to give *endo*-**BCN 5** as a white solid in 62% yield. ^{1}H NMR ($CDCl_3$, 400 MHz); δ (ppm) 0.90-0.99 (m, 2H), 1.30- 1.39 (m, 2H), 1.56-1.66 (m, 2H), 2.20-2.35 (m, 6H), 3.74 (d, J = 8.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): 20.0, 21.4, 21.5, 29.0, 60.0, 98.9. HRMS (CI) $[M+H]^+$ calcd for $C_{10}H_{15}O$ 151.1123; found 151.1118.

3.6 Synthesis of Compound 7

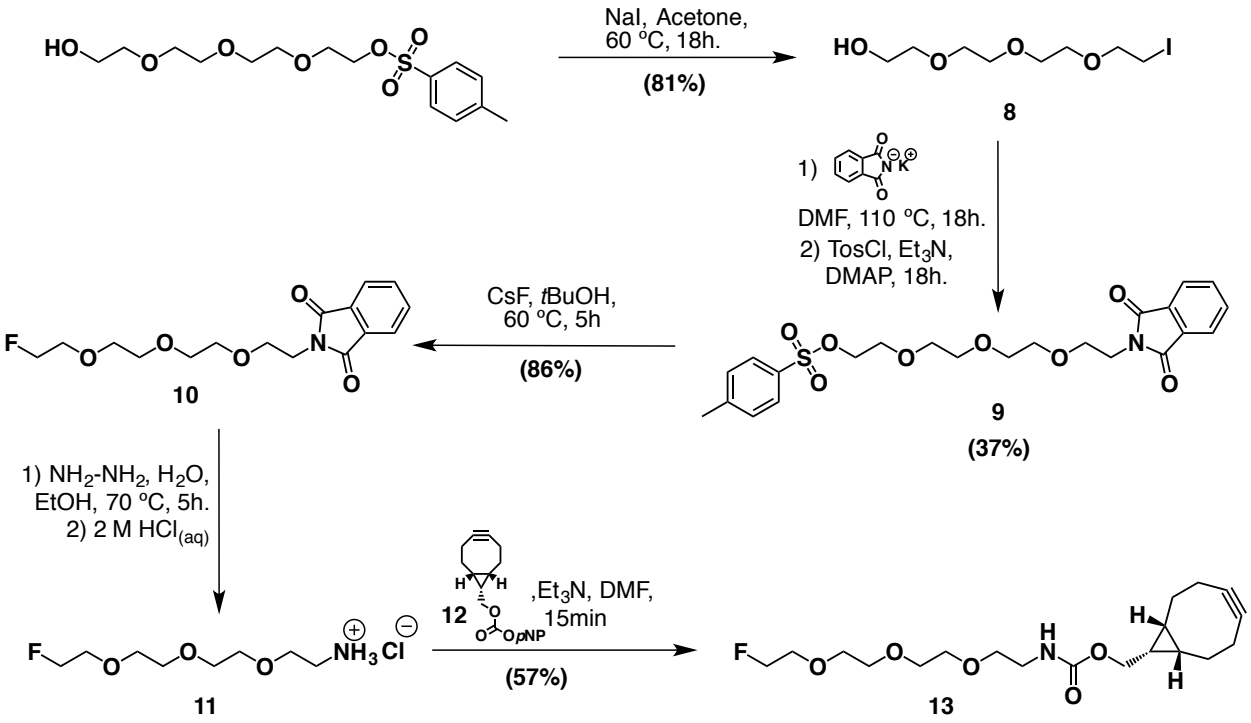


Scheme S3. Synthetic procedure for the preparation of model **compound 7**.

Compound **6** was prepared according to Scheme S3. A flame-dried, two-necked, 250 mL round-bottomed flask was purged with Ar and then was charged with MeO-EG_3-OH (2.00 g, 12.2 mmol) in dry CH_2C1_2 (0.2 M in starting alcohol). The flask was immersed in an ice-water bath. DMSO (955 mg, 24.4 mmol) and P_2O_5 (3.56 g, 24.4) were added sequentially. The reaction mixture was allowed to stir and warm up to room temperature until disappearance of starting material by TLC (~3 h). The flask was immersed in the ice-water bath again; then triethylamine (4.32 g, 42.7 mmol) was added dropwise over 10 minutes. Stirring was continued for 45 minutes. The reaction was quenched with 10% aqueous HCl and extracted with CH_2Cl_2. The organic extracts were washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4 filtered, and concentrated *in vacuo* to obtain **compound 6**.

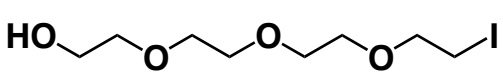
Subsequently, without further purification the crude aldehyde (720 mg, 4.40 mmol), $CH_3NHOH{\cdot}HCl$ (742 mg, 8.80 mmol), and Et_3N (1.8 g, 9.4 mmol) were mixed in 50mL of dry CH_3CN. After reaction completion (3h, checked by TLC) the solvent was evaporated and substituted with CH_2Cl_2. The organic was then washed with saturated aqueous NaCl three times, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using CH_3CN /EtOAc/MeOH (10:3:1 v/v) as the eluent to provide model nitrone **compound 7** as a yellow oil in 64% overall yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 3.93 (s, 3H), 3.57 (m, 2H), 3.65-3.70 (m, 10H), 4.46 (m, 2H), 6.95

(t, 1H, J = 4.5 Hz); ^{13}C NMR (400 MHz $CDCl_3$); 52.0, 58.9, 65.6, 70.2, 70.4, 70.8, 71.8, 138.6. HRMS (CI) $[M+H]^+$ calcd for $C_8H_{17}NO_4$ 192.1236; found 191.1236.



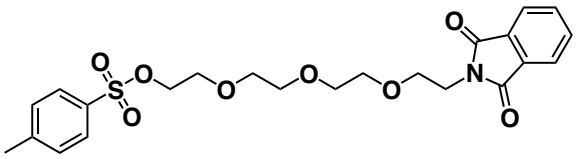
Scheme S4. Synthetic procedure for the preparation of compound **13**.

3.7 Synthesis of Compound 8



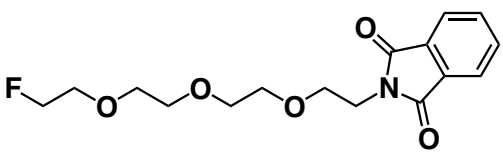
HO-EG$_4$-Tos (2.0 g, 5.7 mmol) and NaI (0.9 g, 6.0 mmol) were dissolved in acetone (50 mL). The reaction mixture was stirred and heated at 60 °C in a pre-heated oil bath overnight and monitored by TLC (acetone/CH_2Cl_2 1:1) using UV light and potassium permanganate stain. The crude product purified by flash column chromatography (acetone/CH_2Cl_2 1:1) to give a light yellow oil as the desired intermediate in 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 3.27 (t, J = 8 Hz, 2H), 3.62 (t, J = 4 Hz, 2H), 3.63–3.68 (m, 8H), 3.73–3.78 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$); 2.8, 61.8, 70.2, 70.3, 70.5, 70.7, 72.0, 72.5; HRMS (CI) $[M+H]^+$ calcd for $C_8H_{17}IO_4$ 305.0244; found 305.0243.

3.8 Synthesis of Compound 9



Compound 8 (1.24 g, 4.1 mmol) and potassium phthalimide (0.76 g, 4.1 mmol) were added into the reaction flask and dissolved in dry DMF (5 mL) under Ar atmosphere. While stirring, the reaction flask was immersed in a pre-heated oil bath at 110˚C and stirred overnight. The reaction progress was monitored by TLC (hexane/EtOAc/MeOH 7:7:1) using UV light. The reaction mixture was allowed to cool down and it was filtered and concentrated *in vacuo*. Without further purification crude product (1.3 g, 4.1 mmol), Et_3N (1.0 g, 10.1 mmol) and DMAP (0.1 g, 0.9 mmol) were dissolved in CH_2Cl_2 (30 mL). The reaction mixture was cooled to 0˚C then TosCl (0.75 g, 4.4 mmol) was slowly added while stirring vigorously. The reaction was allowed to cool to room temperature overnight and the progress was monitored by TLC (7:7:1 hexane/EtOAc/MeOH). The crude product was purified by flash column chromatography (7:7:1 hexane/EtOAc/MeOH) to give the final product as a clear and colorless oil in 37 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 2.45 (s, 3H), 3.50–3.53 (m, 4H), 3.55–3.58 (m, 2H), 3.61–3.66 (m, 4H), 3.73 (t, *J* = 4 Hz, 2H), 3.90 (t, *J* = 4 Hz, 2H), 4.14 (t, *J* = 4 Hz, 2H), 7.34 (dd, *J* = 8, 184 Hz, 2H), 7.72 (ddd, *J* = 4, 6, 52 Hz, 2H), 7.80 (dd, *J* = 8, 184 Hz, 2H), 7.85 (ddd, *J* = 4, 6, 52 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) 21.6, 37.3, 67.9, 68.7, 69.2, 70.1, 70.6, 70.7, 123.3, 128.0, 129.8, 132.1, 133.0, 144.7, 168.2. HRMS (ESI) $[M]^+$ calcd for $C_{23}H_{27}NO_8S$ 477.1457; found 477.1468.

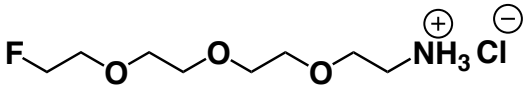
3.9 Synthesis of Compound 10



Compound 9 (480 mg, 1 mmol) and CsF (760 mg, 5 mmol) were dissolved in *t*BuOH (10 mL) and stirred at

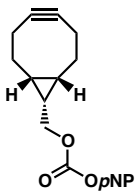
60 °C for 5 h. after removal of the solvent, the crude was purified using column chromatography (CH_2Cl_2/MeOH 19:1) to provide **compound 9** as a white solid in 86% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 3.62-3.76 (m, 12H), 3.91 (t, *J* = 5.8, 2H), 4.55 (dt, *J* = 4.0, 32.0, 2H), 7.72 (m, 2H), 7.86 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) 37.3, 67.9, 70.1, 70.3, 70.6, 70.7, 82.3, 84.0, 123.2, 132.1, 133.9, 144.7, 168.2. HRMS (CI) $[M+H]^+$ calcd for $C_{16}H_{20}FNO_5$ 325.1326; found 325.1335.

3.10 Synthesis of Compound 11



Compound 10 (470 mg, 1.45 mmol) was dissolved in 95% ethanol (15 mL). $H_2N–NH_2·H_2O$ (500 mg, 0.49 mL) was added to it and the reaction mixture was refluxed for 5 h. After TLC showed the reaction was complete, the reaction mixture was cooled to room temperature and the precipitated phthalyl hydrazide was filtered. The filtrate was then treated with 2M HCl and the solid residue was separated by filtration. The crude was used in preparation of **compound 12** without any further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 2.71 (t, *J* = 5.5, 2H), 3.40 (t, *J* = 5.5, 2H), 3.52-3.59 (m, 8H), 4.45 (m, 1H), 4.57 (m, 1H); HRMS (ESI) $[M]^+$ calcd for $C_8H_{18}FNO_3$ 195.12714; found 195.1275.

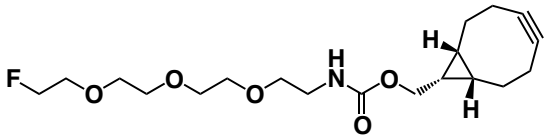
3.11 Synthesis of Compound 12



Compound 12 was prepared according to the previous reports[1] and was used in preparation of **compound 13**. To a solution of *endo*-**BCN 5** (500 mg, 3.33 mmol) in CH_2Cl_2 (25 mL) was added pyridine (229 µL, 8.31 mmol) and *p*-NO_2PhOC(O)Cl (838 mg, 416 mmol). After stirring for 15 min at room temperature the mixture was quenched with saturated NH_4Cl-solution (75 mL) and extracted with CH_2Cl_2 (3 x 15 mL). The organic

layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (Et_2O/Hexanes, 1:1) to afford **compound 12** as a white solid in 76% yield. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm); 1.02-1.12 (m, 2H), 1.47-1.56 (m, 1H), 1.57-1.67 (m, 1H), 2.22-2.40 (m, 6H), 4.41 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 9.6 Hz, 2H), 8.28 (d, J = 9.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz) 17.2, 20.5, 21.3, 29.0, 68.0, 98.7, 121.7, 125.3, 145.3, 152.5, 155.6.

3.12 Synthesis of Compound 13



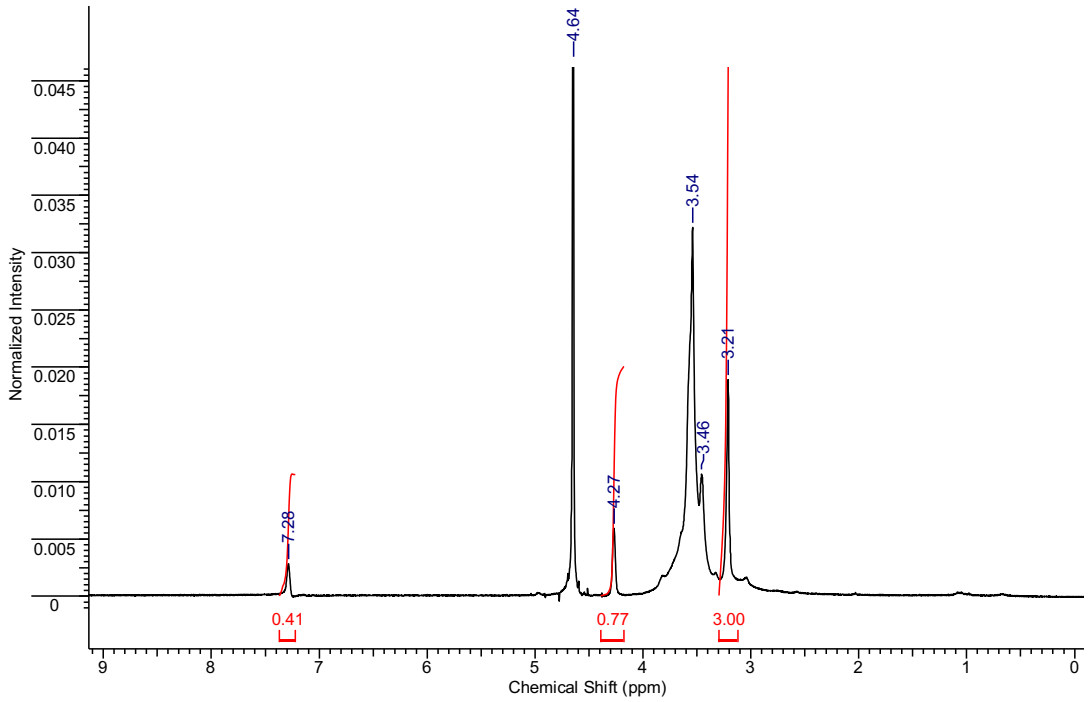
Compound 12 (81 mg, 0.26 mmol) was dissolved in dry DMF (0.5 mL) and was cooled on an ice-bath while purging with argon. In a separate flask, **compound 11** (200 mg, 1.02 mmol) was dissolved in dry DMF (1 mL) and Et_3N (0.2 mL, 1.02 mmol) was added. After purging this mixture with argon for 15 min, it was transferred to the first flask using a cannula and the reaction mixture was stirred at 0 °C for 20 min. After the TLC showed reaction was completed, the mixture was concentrated and then extracted using EtOAc. The combined organic fractions were washed with brine and dried over Na_2SO_4 and concentrated. The crude was purified using column chromatography using EtOAc as the solvent, and **compound 13** was obtained as a very light yellow oil in 57% yield. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm); 0.95 (m, 2H), 1.36 (m, 1H), 1.60 (m, 2H), 2.21-2.32 (m, 6H), 3.36 (m, 2H), 3.57 (t, J = 4.9 Hz, 2H), 3.62-3.73 (m, 8H), 3.74 (t, J = 4 Hz, 1H), 3.79 (t, J = 4 Hz, 1H), 4.14 (d, J = 8.2, 2H), 4.58 (dt, J = 32, 2.4, 2H), 5.28 (br.s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) 18.1, 20.4, 21.7, 29.3, 41.1, 42.1, 63.0, 70.4, 70.6, 70.8, 70.9, 70.9, 71.1, 82.6, 84.3, 99.1, 157.1. HRMS (ESI) $[M]^+$ calcd for $C_{19}H_{30}FNO_5$ 371.2109; found 371.2108.

3.13 Synthesis of Nitrone-AuNPs

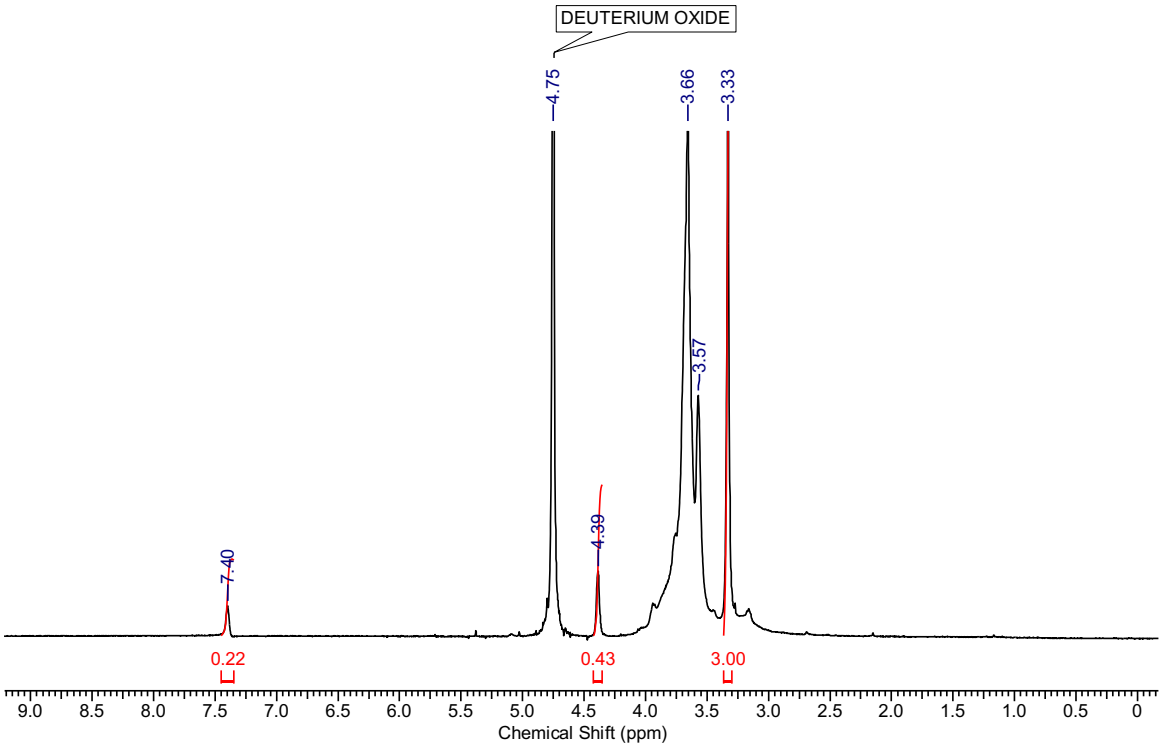
The MeO-EG_3-AuNPs were synthesized according to our previously established procedure.[53] $HAuCl_4 \cdot 3H_2O$ (1.4564 g, 3.7 mmol, 1.0 eq.) was dissolved in a mixture of dry MeOH (503 mL) and glacial acetic acid (83 mL). To this yellow solution was added MeO-EG_3-SH (2.0 g, 11 mmol, 3.0 eq.). The slightly darkened solution was stirred vigorously for 2 h and the solution color slightly faded. A solution of $NaBH_4$ (1.3997 g, 37 mmol, 10.0 eq.) in Milli-Q water (96 mL) was added dropwise to the reaction mixture under vigorous stirring. The mixture turned dark brown immediately. After overnight stirring at ambient temperature, the solution was concentrated and diluted with brine. The MeO-EG_3-AuNPs were extracted with toluene while adding NaCl to the aqueous phase after each extraction to maintain the saturation. The aqueous phase was eventually colorless. The combined organic phases were then concentrated *in vacuo*. Evaporation of the solvent left a thin film of nanoparticles, which was then rinsed with hexanes to remove the excess free thiol. The crude MeO-EG_3-AuNPs were dissolved in nanopure H_2O and further purified by overnight dialysis.

Next, to a solution of MeO-EG_3-AuNPs (180 mg) in CH_2Cl_2 (25 mL) was added a solution of nitrone-terminated thiol ligand **compound 4** (30 mg, 0.13 mmol) in CH_2Cl_2 (5 mL) while stirring vigorously. After 30 minutes of stirring, the solvent was evaporated to form a film of nanoparticles. This film was rinsed 5 times with haxanes/isopropanol (10:1) to remove most of the unbound thiols present. Next, **Nitrone-AuNPs** were dissolved in milli-Q water and further purified by overnight dialysis.

A)


4.64
3.54
3.21
3.46
4.27
7.28
0.41
0.77
3.00
Normalized Intensity
Chemical Shift (ppm)

B)


DEUTERIUM OXIDE
4.75
3.66
3.33
3.57
4.39
7.40
0.22
0.43
3.00
Chemical Shift (ppm)

C)

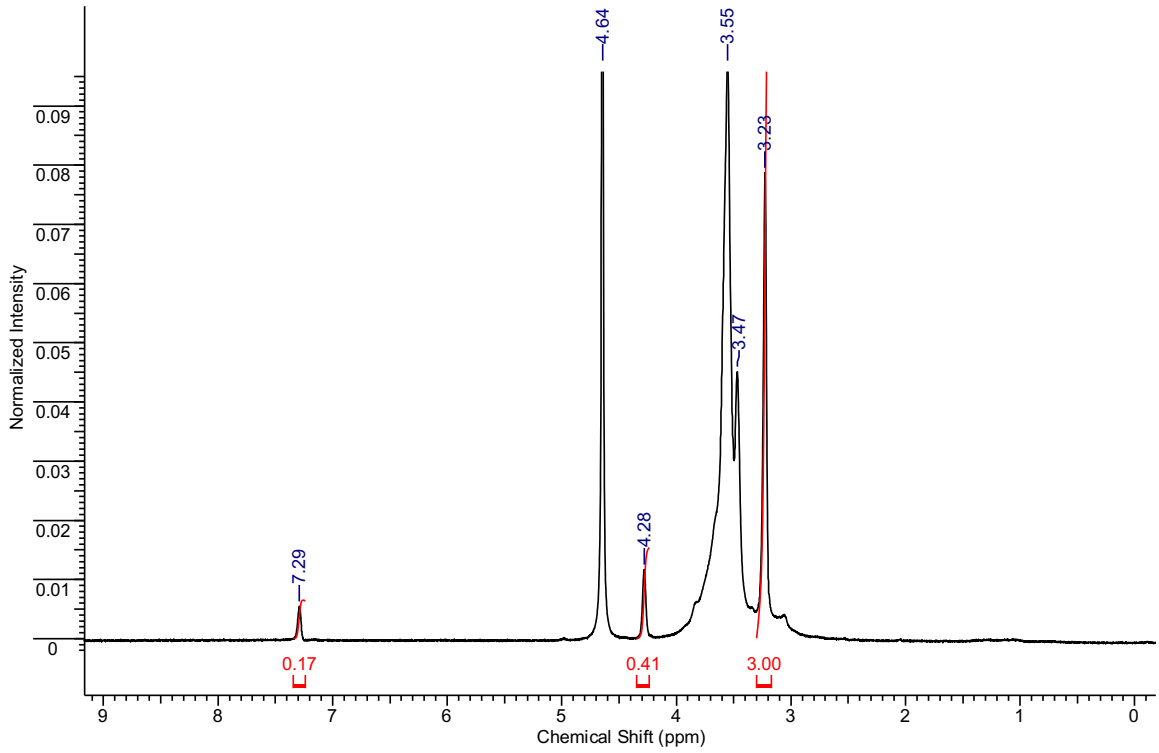


Figure S1. ^{1}H NMR spectra of three different samples of **Nitrone-AuNPs** with different amounts of nitrone ligand **4** recorded in D_2O and referenced against residual H_2O. Sample A has 29% of nitrone ligands, sample B has 18% of nitrone ligand, while sample C has 15% of nitrone ligands. Sample B is water-soluble and has the maximum amount of nitrone ligand. This sample was used for the rest of our investigations.

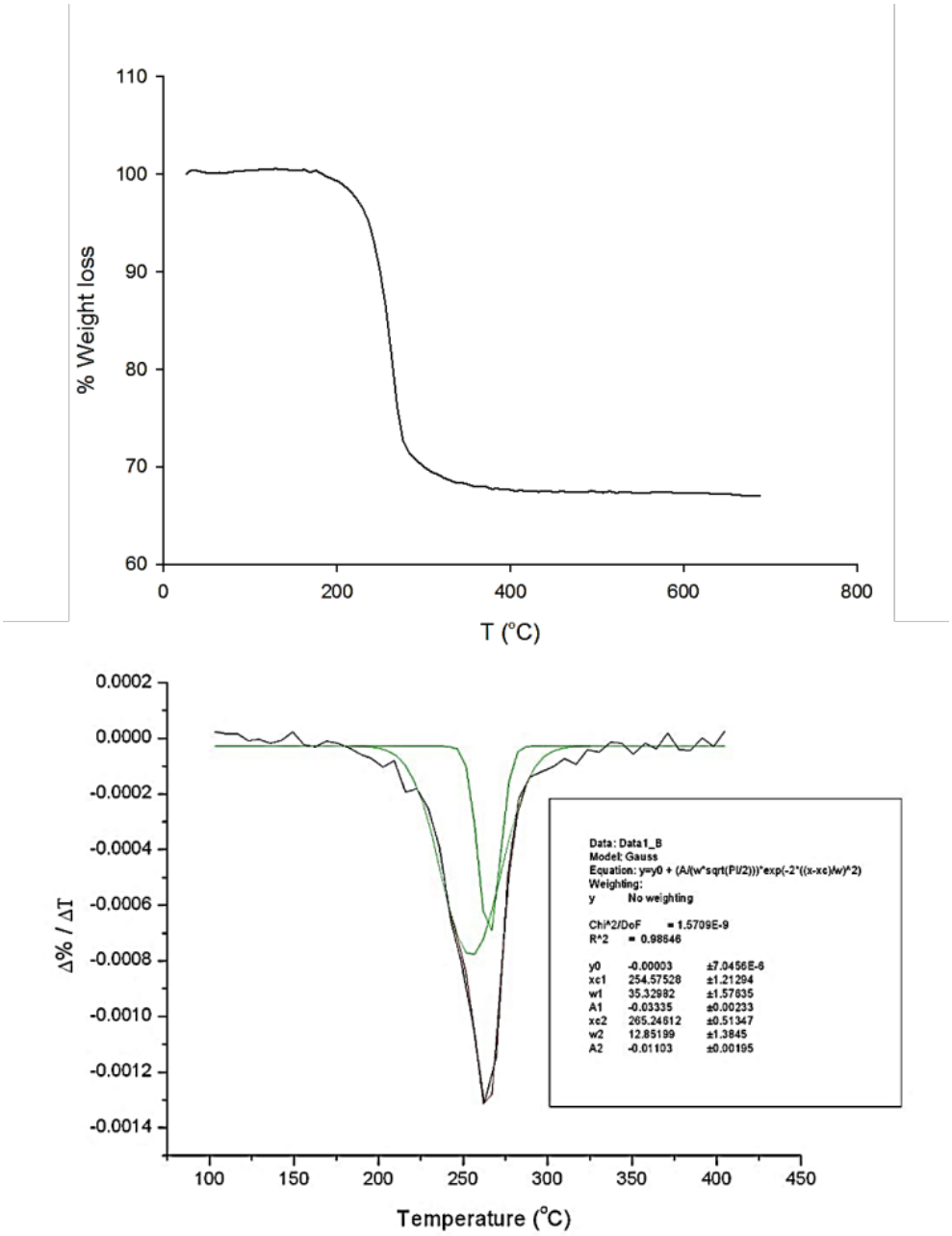


Figure S2. TGA (top) and deconvolution of the TGA first derivative curve (bottom) of **Nitrone-AuNPs**.

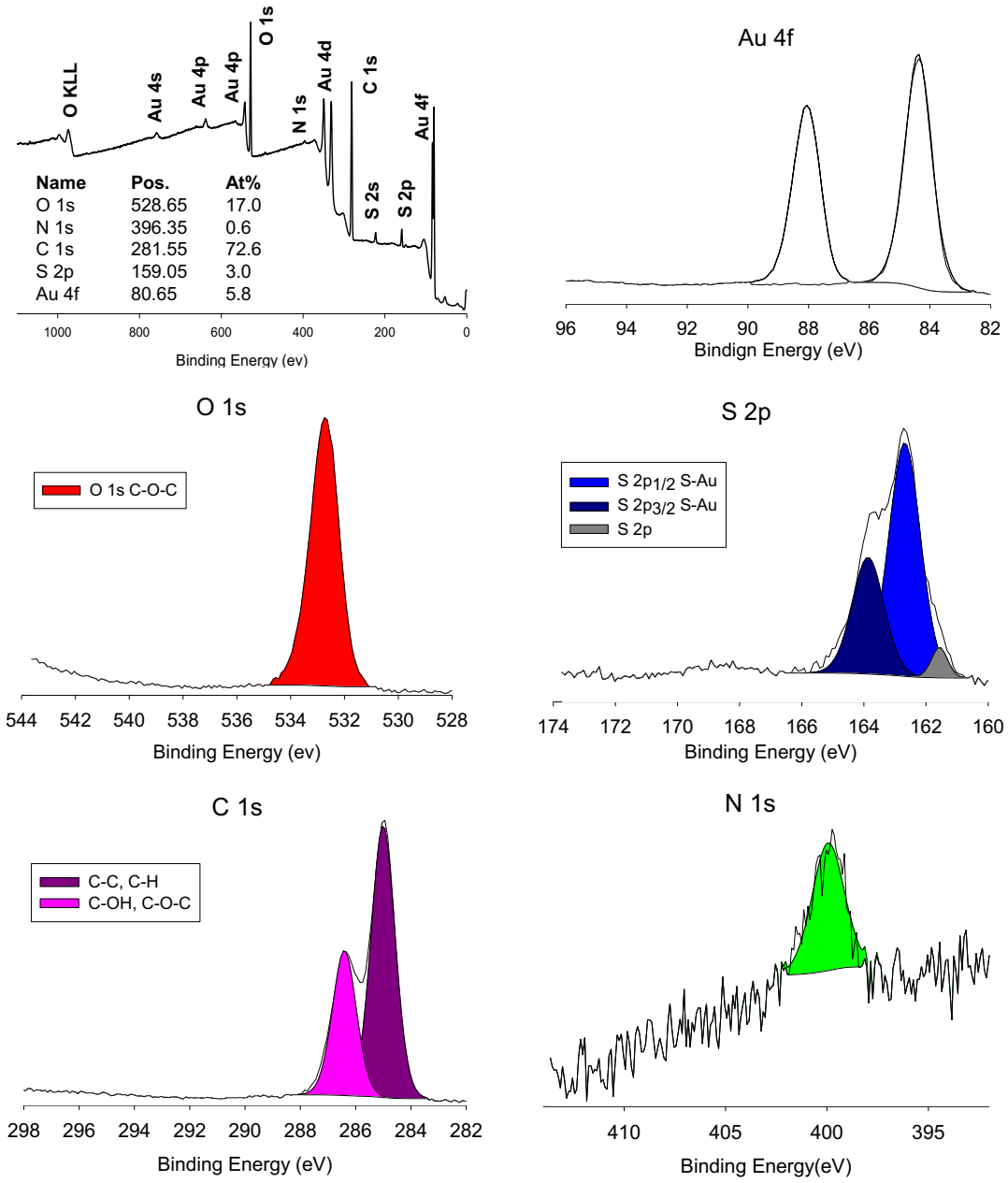
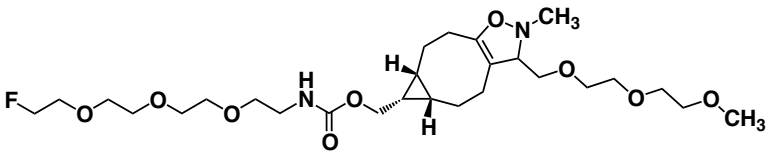


Figure S3. Survey scan and high resolution XPS spectra of **Nitrone-AuNPs**.

Proof of concept SPANC reactions using model nitrone 6 and Nitrone-AuNPs

3.14 Synthesis of cycloaddition product 14



In a typical experiment, nitrone **compound 7** (19.1 mg, 100 µmol) was mixed with endo-**BCN 13** (37.1 mg, 100 µmol) at room temperature in a mixture of CH_3CN/H_2O (3:1) (2 mL). After reaction went to completion, the solvent was removed. The crude product was purified by column chromatography using EtOAc as the eluent to afford the *endo*-cycloadduct **compound 14** as an inseparable mixture of isomers in 91% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 0.86-1.05 (m, 2H), 1.13–1.22 (m, 1H), 1.48-1.63 (m, 2H), 1.91-2.02 (m, 2H), 2.07-2.40 (m, 2H), 2.68 (s, 3H), 3.33-3.42 (m, 5H), 3.50-3.74 (m, 21H), 3.80 (t, *J* = 4, 1H), 4.15 (m, 2H), 4.58 (dt, J = 48, 4, 2H), 5.21 (br.s 1H); ^{13}C NMR (400 MHz, $CDCl_3$); 18.5, 18.8, 18.9, 19.8, 20.1, 20.6, 20.7, 21.8, 22.1, 25.4, 25.6, 26.0, 26.1, 46.6, 59.0, 60.0, 70.5, 70.6, 70.7, 71.9, 72.8, 101.9, 102.2, 146.6, 146.9; ^{19}F {^{1}H} NMR (400 MHz, $CDCl_3$) -223.36. HRMS (CI) $[M+H]^+$ calcd for $C_{27}H_{47}FN_2O_9$ 563.3345; found 563.3341.

3.15 Synthesis of [^{19}F]-AuNPs

In a typical experiment **Nitrone-AuNPs** (10 mg, 8.4 µmol of nitrone) were mixed with **compound 13** (3.1 mg, 8.4 µmol) in a mixture of CH_3CN/H_2O (3:1) (1 mL). Reaction was followed using ^{1}H NMR spectroscopy following the disappearance of nitrone's signature signals at 4.4 and 7.4 ppm. After reaction completion, the solvent was evaporated to form a film of nanoparticles. This film was then washed repeatedly 7 times with haxanes/isopropanol (10:1) to

remove any excess **BCN 13**. The resulting **[^{19}F]AuNPs** were then further purified by dialysis in Milli-Q water.

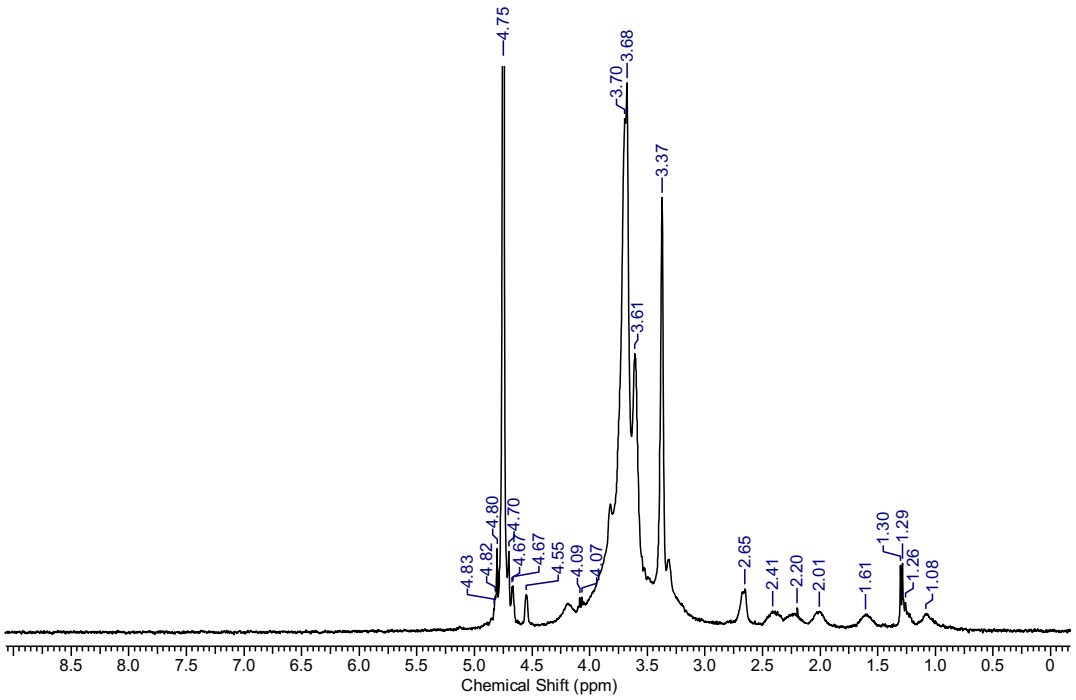


Figure S4. ^{1}H NMR spectrum of compound **[^{19}F]AuNPs** recorded in D_2O, and referenced against residual H_2O.

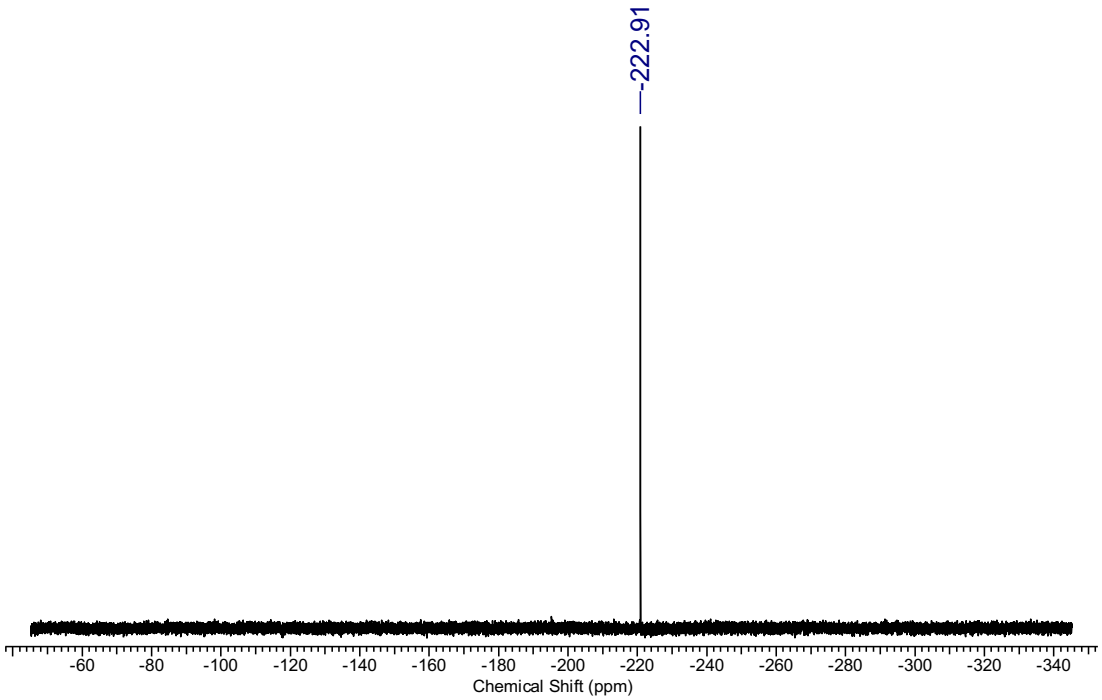


Figure S5. ^{19}F $\{^{1}H\}$ NMR spectrum of compound **[^{19}F]AuNPs** recorded in D_2O.

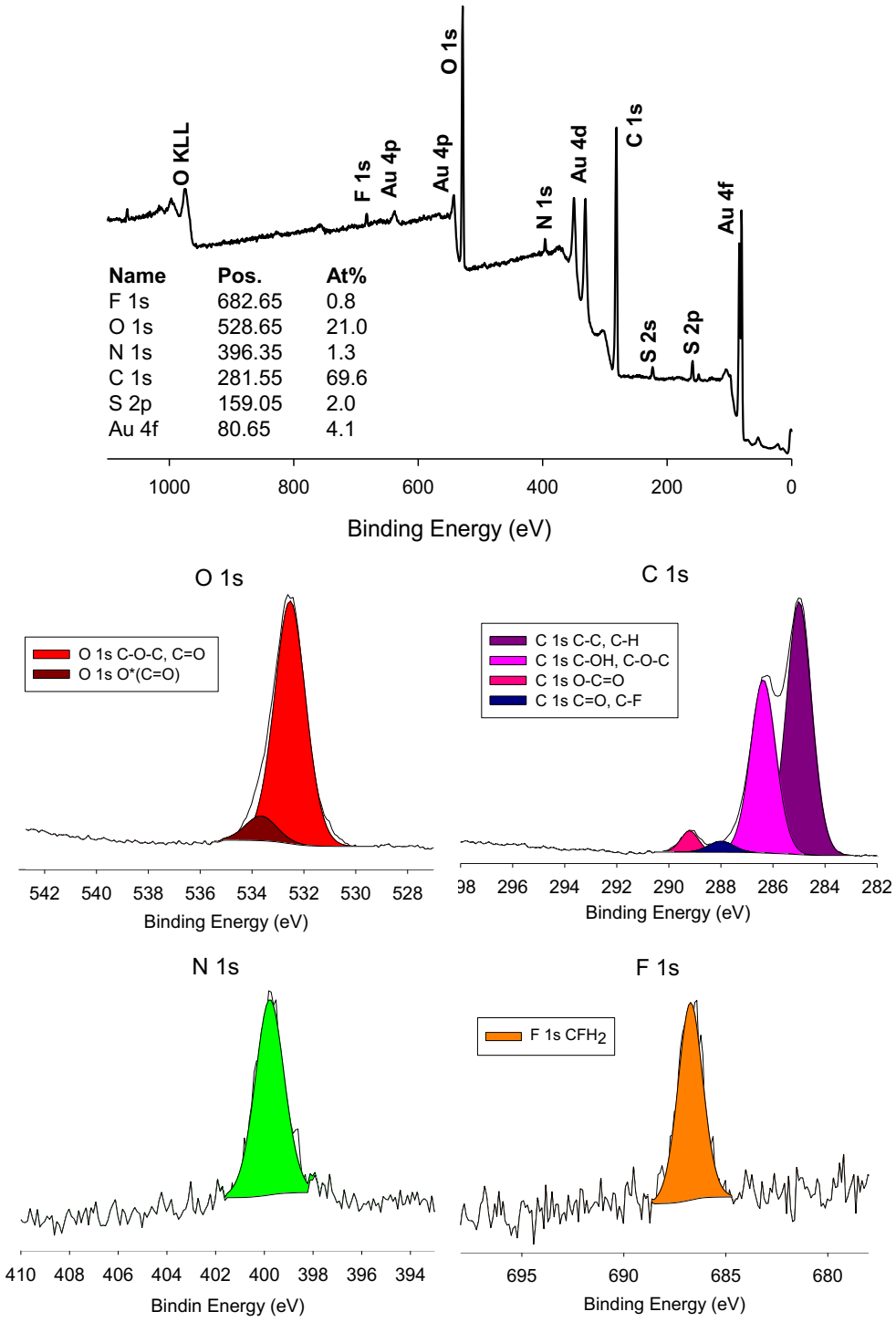


Figure S6. Survey scan and high resolution XPS spectra of **[^{19}F]AuNPs**.

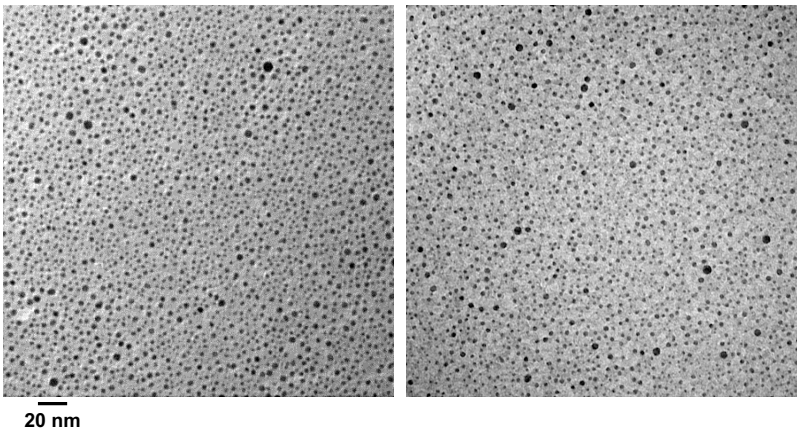
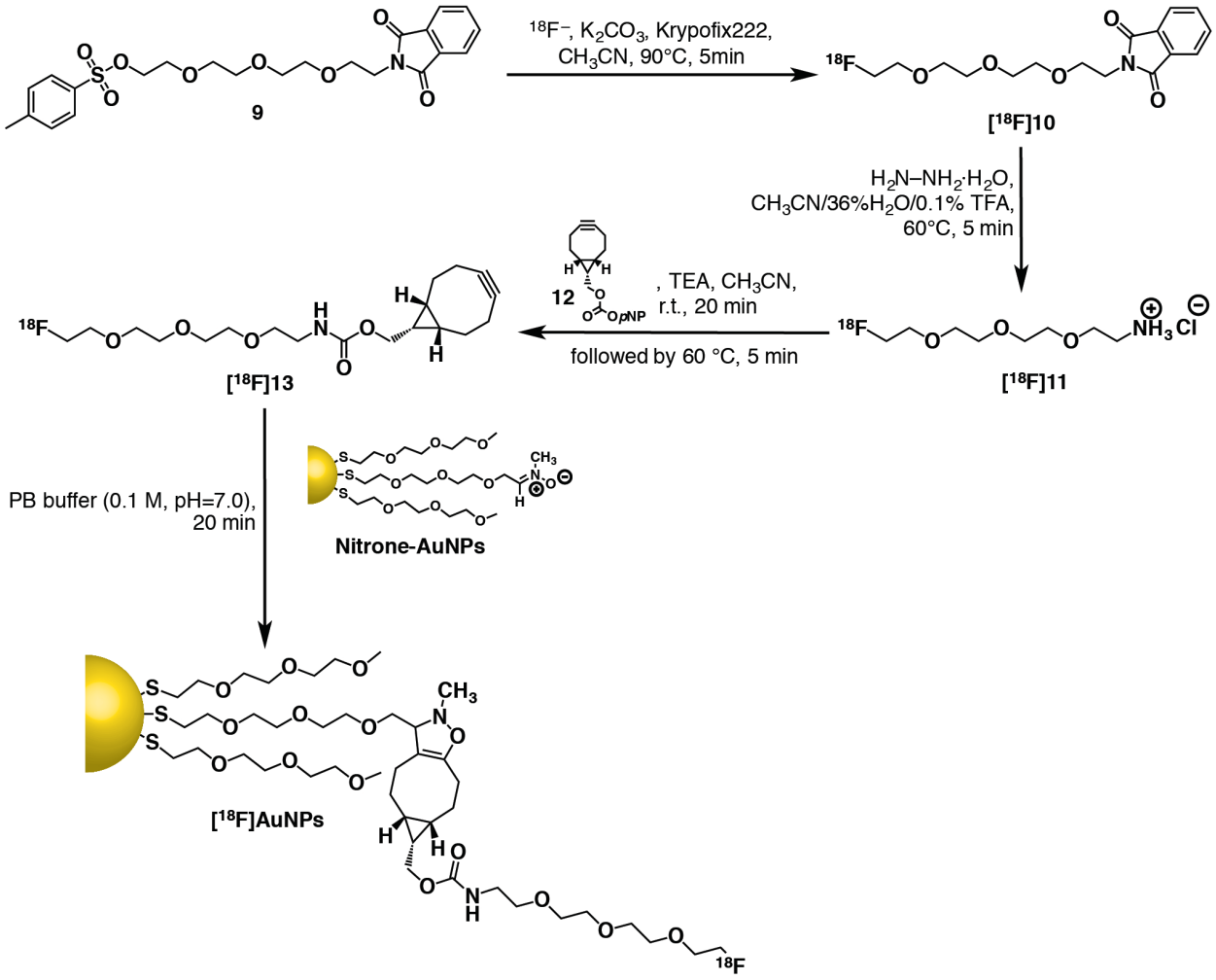


Figure S7. TEM images of **Nitrone-AuNPs** (left) and **[^{19}F]AuNPs** (right).

Radiochemistry

3.16 Synthesis of [^{18}F]AuNPs



Scheme S5. Reaction pathway towards the synthesis of **[^{18}F] 13** prosthetic group and their I-SPANC reaction to prepare **[^{18}F]AuNPs**.

[^{18}F]AuNPs were prepared following reaction Scheme S5. Compound **[^{18}F]10** was prepared on an automated synthesis unit with a built-in preparative HPLC system. Aqueous [^{18}F]fluoride (37 GBq) solution was acquired from the cyclotron and trapped on a Sep-Pak Accell plus light QMA cartridge (Waters). CH_3CN/H_2O (80:20 v/v) solution (1 mL) containing potassium carbonate (2

mg) and Kryptofix 2.2.2 (7 mg) was used to elute [^{18}F]fluoride into the reaction vial. The solvent was removed under vacuum in the presence of helium gas at 75 °C. The [^{18}F]fluoride was dried twice by adding 1 mL of anhydrous CH_3CN under a flow of helium at 75 °C. Anhydrous CH_3CN (500 μL) containing 10 mg of **compound [^{18}F]10** was added into the vial of dried [^{18}F]F^- under helium atmosphere. The reaction vial was sealed and heated at 90 °C for 5 min. The reaction mixture was cooled down to 40 °C, diluted with 3 mL of H_2O and purified by HPLC (eluent: CH_3CN /H_2O, 36% with 0.1% TFA) to provide **compound [^{18}F]10** (12.7 GBq, yield: 44% decay corr.)

Hydrazine monohydrate (500 μL) was added into the vial containing **compound [^{18}F]10**. The reaction mixture was heated at 60 °C for 5 min, diluted with 1 mL of water and trapped on a Sep-Pak C18 plus light cartridge (Waters). CH_3CN (500 μL) was used to elute **compound [^{18}F]11** (3.7 GBq) into a vial containing 3 mg of **compound 12** in 200 μL of CH_3CN, followed by the addition of 50 μL Et_3N. The reaction mixture was stirred at room temperature for 15 min and heated at 60 °C for 5 min, then diluted with 700 μL of water. **Compound [^{18}F]13** was purified on a semi-preparative C18 column (eluent: CH_3CN/H_2O, 50% containing 0.1% TFA, flow rate 4 ml/min) and dried on a V-10 evaporator at 50 °C to get 740 MBq of [^{18}F]13 (yield:49% decay corr.)

The **[^{18}F]AuNPs** were prepared *via* the I-SPANC reaction between **compound [^{18}F]13** and **Nitrone-AuNPs**. 200 μL of PB (phosphate buffer) (0.1 M, pH = 7.0) containing 500 μg of AuNP was added and reacted at room temperature for 20 min with the reaction mixture being passed through a PD-10 desalting column with PB buffer (0.1 M, pH = 7.0) to get **[^{18}F]AuNP** in

solution (26 MBq, yield 6% decay corr). **[^{18}F]AuNP** was used for the following *in vivo* and i*n vitro* biological evaluations.

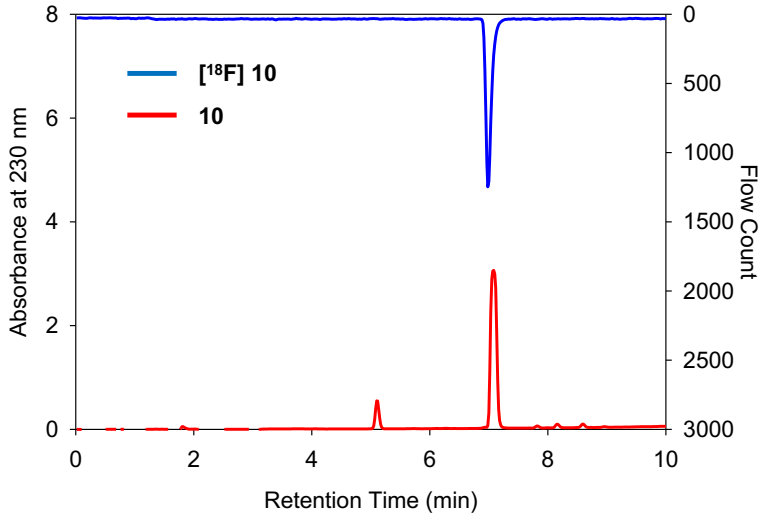


Figure S8. HPLC and flow count for **compound 10** and **compound [^{18}F]10**. HPLC condition: Sunfire RP-18 analytical column: 4.6 × 250 mm, 5 µm; mobile phase: CH_3CN (solvent A)/H_2O (solvent B) containing 0.1 % TFA; gradient: solvent A/B from 10/90 at 0 min to 90/10 at 10 min. flow rate: 1.5 mL/min. UV detector: λ_{max}=230 nm (red curve); radioactive detector (blue curve).

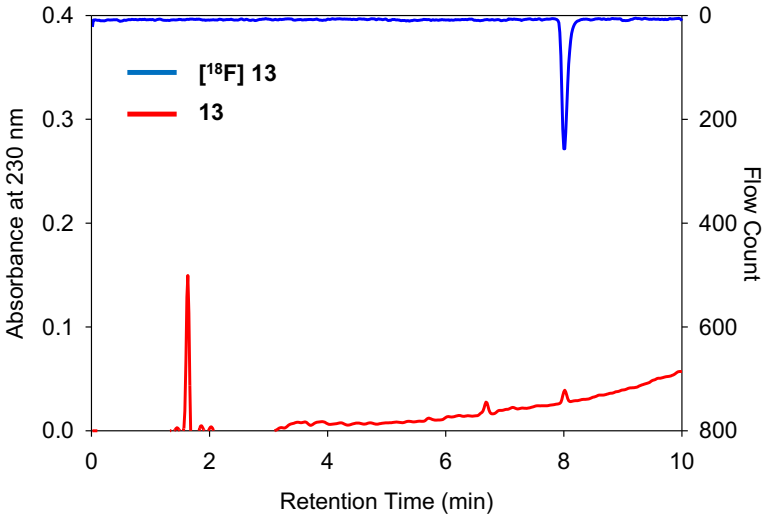


Figure S9. HPLC and flow count for **compound 13** and **compound [^{18}F]13**. HPLC condition: Sunfire RP-18 analytical column: 4.6 × 250 mm, 5 µm; mobile phase: CH_3CN (solvent A)/H_2O (solvent B)

containing 0.1 % TFA; gradient: solvent A/B from 10/90 at 0 min to 90/10 at 10 min. flow rate: 1.5 mL/min. UV detector: λ_{max}=230 nm (red curve); radioactive detector (blue curve).

3.17 PET imaging of [^{18}F]AuNP

C57/B6 mice were used for PET imaging of **[^{18}F]AuNP. [^{18}F]AuNP** with the dose of ≈ 10 MBq was injected into the mouse while under anesthesia and followed by whole-body scanning in dynamic mode for 60 min under 1.5% isoflurane/O_2 flow. The microPET image voxel intensities correspond to units of Bq/cc. Analysis was performed using AsiPro software (Concorde Microsystems, Knoxville, Tenn, USA) to view and analyze the microPET images. Measurements were done by measuring the signal from a 3D volume of interest (VOI) that was drawn over different organs including the bladder, brain, lungs, liver, kidneys to guide volume placement as well as inherent CA accumulation and resultant signal in 3D whole body PET images. Measurements taken from the fixed head of the animal and the brain which is clearly and easily delineated are relatively simple. In contrast, the choice of VOI for other organs within a live, breathing animal is less accurate (especially liver and lungs) without the use of gating and image registration. For this preliminary study, organ VOI segmentation was guided solely by an anatomical reference image and activity (signal visualized in PET image). This added error to the absolute activity measurements, and therefore, normalized data is displayed instead. The signal accumulation/washout of the radioisotope was observed in the axial, coronal and sagittal planes. The average ± standard deviation of the signal intensity was used for analysis. SUV data in vivo was drawn and calculated based on the VOI data.

Calculation of the nanoparticles raw formula

From the deconvolution of the TGA derivative (see Error! Reference source not found.**,** bottom) i t is possible to calculate the amount (in milligram) of the two ligands (MeO-EG_3-SH and Nitrone-EG_4-SH) per milligram of **Nitrone-AuNPs**. Having this information and using the following equations we can calculate the raw formula for **Nitrone-AuNPs**.

$$N_{Au} = \frac{\pi \rho d^3 N_A}{6 M_{Au}}$$

ρ = density of face centered cubic (fcc) gold lattice (19.3 g cm^{-1})

d = average diameter of nanoparticles in centimeters (from TEM images)

N_A = Avogadro constant

M_{Au} = mole atomic weight of gold (196.9665 g mol^{-1})

This is assuming that the AuNPs are spherical and that they are monodispersed.

The total number of ligands (N_L) can be calculated using the following formula:

$$N_L = \frac{N_{Au} M_{Au} M_{TGAOrg}}{(1 - M_{TGAOrg})(MW_1 n_1 + MW_2 n_2)}$$

MW_{TGAOrg} = organic percentage mass loss from TGA

MW_1 = molecular weight of ligand 1 (MeO-EG_3-S^-)

n_1 = molar percentage of ligand 1 (MeO-EG_3-S^-)

MW_2 = molecular weight of ligand 2 (Nitrone-EG_4-S^-)

n_2 = molar percentage of ligand 2 (Nitrone-EG_4-S^-)

References

(1) Gobbo, P., Romagnoli, T., Barbon, S. M., Price, J. T., Keir, J., Gilroy, J. B., and Workentin, M. S. (2015) Expanding the scope of strained-alkyne chemistry: a protection-deprotection strategy via the formation of a dicobalt-hexacarbonyl complex. *Chem Commun 51*, 6647-6650.