Supporting Information

Chemoselective Methionine Bioconjugation: Site-Selective Fluorine-18 Labeling of Proteins and Peptides

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Materials and Instrumentation

All reagents were of ACS grade or better. Oxaziridine **10** was prepared as previously described by Lin *et al.*[13] and iodonium salt **14** was prepared as previously described by Taylor *et al.*[17] Non-radioactive semi-preparative HPLC purifications were performed using a Varian Chromatography system. Non-radioactive analytical HPLC analyses were performed on a Shimadzu model SCL-10A system with an SPD-10AV UV-vis detector or an Agilent 1200 Infinity UPLC system. Radioactive semi-preparative HPLC purifications were performed using a TRACERlab FX2N or a custom built remote controlled synthesis unit. Radioanalytical HPLC analyses were performed on an Agilent model 1100, with a variable wavelength UV detector equipped with a posi-ram radio-HPLC (Lab Logic) radioactive detector in line with its flow path. LC-MS analyses were performed on an Agilent 1100 series HPLC and a Finnigan LXQ ion trap mass spectrometer (Desolvation Gas: Nitrogen; Capillary Temp: 270 °C; Positive Electrospray).

LC/HRMS. Bovine serum albumin (BSA) conjugate was analyzed on a Waters Synapt G2 HDMS (Waters Manchester, U.K.) operated under sensitivity mode with ESI voltage at 3.5 kV; cone voltage at 35 V, and source temperature at 120 °C. Prior to MS analysis, 1 µg of sample was injected into an ACQUITY UPLC system (Waters Manchester, U.K.). Proteins were eluted from a Waters BEH C4 column (2.1 x 150 mm, 300 Å, 1.7 µm), heated at 60 °C, using a 20 min LC gradient. The gradient setting was 10% to 38% solvent B in 10 min at 0.2 mL/min flow rate. Solvent A was 0.1% formic acid in water and solvent B was 0.1% formic acid in acetonitrile. Peptides and small molecules were analyzed on a QEPlus mass spectrometer (ThermoFisher Scientific, Waltham, MA) connected to Aquity UPLC (Waters, Milford, MA). MS was operated under positive mode with ESI voltage at 3.5 kV; sheath gas flow rate of 45, and capillary temperature 320 °C. Sample of 1 µg was injected and eluted on a Waters BEH C18 column (2.1 x

50 mm, 300 Å, 1.7 µm), heated at 60 °C, using a 6 min LC gradient. The gradient setting was 2% to 100% solvent B in 5 min at 0.6 mL/min flow rate. Solvent A was 0.1% formic acid in water and solvent B was 0.1% formic acid in acetonitrile.

BSA concentrations were measured using a NanoDrop 2000 UV-Vis spectophotometer. Reactions were monitored by HPLC and LC-MS with comparisons made to authentic unlabeled materials when available.

NMR. Small molecule proton and carbon NMR spectra were recorded on a Bruker 400 MHz Avance II NMR spectrometer with a 5mm $^{3}H/^{1}H/^{13}C$ QNP probe at 27°C. One and two-dimensional NMR spectra for structure elucidation of methionine modified peptide isomers **21a** and **b** were collected on a Bruker Avance III HD 700 MHz NMR spectrometer equipped with a 5-mm TCI triple resonance cryoprobe (Bruker, Billerica, MA) at 27 °C. All ^{1}H and ^{13}C chemical shifts are reported on the δ scale (ppm) downfield from tetramethylsilane.

Synthesis of Methyl (2S)-2-acetamido-4-S-methyl-N-(prop-2-yn-1-ylcarbamoyl) sulfinimidoyl)butanoate (11). To a solution of methyl acetyl-L-methioninate (**9**, 10 mg, 0.049 mmol) in Water (0.25 mL) placed in a reaction vial was added a solution of 3-phenyl-N-(prop-2-yn-1-yl)-1,2-oxaziridine-2-carboxamide (**10**, 10.84 mg, 0.054 mmol) dissolved in Methanol (0.25 mL). The resulting solution was stirred at ambient temp for 30 min. The progress of the reaction was monitored by LC/MS (mobile phase A = 0.1% formic acid, B = acetonitrile, linear gradient 5% B to 95% B over 7 min, Luna column, 1.8µ, 2.1 x 50 mm) which showed the desired product (R_T = 0.79 min, m/z = 332 ([M+H]+), 247, 158) with trace amounts of ester **9** remaining (R_T = 0.32 min, m/z = 208), and a non-ionizing slowly eluting component (R_T = 5.74 min) which is presumably benzaldehyde. Analysis of this crude product by UPLC (A = 0.05% TFA, B = 0.05% TFA in acetonitrile, 0.2% B over 2 min then 0.2% B to 30% B over 9 min, flow rate = 0.45

mL/min, ZORBAX Eclipse Plus C18, 1.8 μ, 2.1 x 50 mm) showed the presence of **11** as two closely eluting isomers (R_T = 3.58 min and 3.69 min). Purification of **11** as an inseparable isomer mixture was achieved by flash filtration through a small plug of silica gel (15:85 methanol:methylene chloride [v:v]) affording pure isomer mixture **11** (8.8 mg, 0.029 mmol, 60%) as a white solid: LC-MS m/z = 302, 247; HRMS (ESI) [M+H]+ calcd for $C_{12}H_{19}N_3O_4S$: 302.1169, found: 302.1161; ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.58 (dd, 0.5 H, *J* = 4.0, 5.2 Hz, NC-H, isomer), 4.56 (dd, 0.5 H, *J* = 4.0, 5.8 Hz, NC-H), 3.91 (m, 2 H), 3.76 (s, 1.5 H, OCH_3), 3.75 (s, 1.5 H, OCH_3), 2.95-3.10 (m, 2 H) 2.68 (s, 1.5 H, acetyl CH_3), 2.68 (s, 1.5 H, acetyl CH_3), 2.51 (brt, 1 H, J = 2.5, alkyne CH), 2.24-2.36 (m, 1 H), 2.04-2.15 (m, 1 H), 2.02 (s, 1.5 H, S-CH_3), 2.02 (s, 1.5 H, S-CH_3); ^{13}C NMR (100 MHz, MeOD) δ 172.1, 171.3, 166.9 (urea), 80.9 (alkyne), 70.0 (alkyne), 53.4, 51.6, 51.5, 51.4, 51.1, 44.5, 44.3, 30.3, 30.5, 25.0, 24.9, 21.0, 20.9.

Synthesis of Methyl(2S)-2-acetamido-4-(N-(((1-(2-(2-(2-(2-((2-fluoropyridin-3-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)carbamoyl)-S-methylsulfinimidoyl)butanoate (13). To a solution of **11** (4 mg, 0.013 mmol) in methanol (0.5 mL) charged into a 2 mL vial was added **12**[1] (8.34 mg, 0.027 mmol) dissolved in 200 μL of methanol. A solution of copper(II) sulfate (3 mg, 0.019 mmol) and sodium ascorbate (4.5 mg) in 0.5 mL of water was prepared and added to the vial. Stirring at ambient temp was continued for 30 min during which time the solution changed from light yellow to a clear, light blue color. The progress of the reaction was monitored by LC/MS (Mobile phase A = 0.1% formic acid, B = acetonitrile, gradient 5% B to 95% B over 7 min, Luna column 1.8 μ, 2.1 x 50 mm) which showed formation of a major slow eluting product (4.82 min, m/z = 616) consistent with **13**. After dilution with 0.5 mL of acetonitrile the crude was subjected to semi-preparative HPLC (Column = Luna 10 μm C18 prep (2), 100 Å , 10 x 250 mm, mobile phase: A = water (0.1% Formic acid), B = acetonitrile, λ = 232 nm, gradient: 5% B to 95% B over 20 min, flow rate = 5 mL/min) which afforded purified **13** (5 mg, 0.008 mmol, 62%) as a clear oil: HRMS (ESI) $[M+H]^+$ calcd for $C_{25}H_{38}FN_7O_8S$: 616.2559, found 616.2544; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.89 (s, 1 H, triazole C-H), 7.71 (dt, 1 H, *J* = 7.9, 1.5 Hz), 7.60 (ddd, 1H, J = 10.3, 7.9, 1.5 Hz), 7.24 (ddd, 1 H, *J* = 7.9, 4.9, 0.6), 4.56 (m, 1 H), 4.55 (brt, 2 H, *J* = 5 Hz), 4.26 (m, 2 H), 3.89 (m, 4 H), 3.85-3.92 (m, 4 H), 3.75 (s, 3 H, O-CH_3), 3.68-3.72 (m, 2 H), 6.63 (m, 2 H), 3.61 (brs, 4 H), 2.95-3.10 (m, 2 H), 2.67 (s, 3 H, acetyl CH_3), 2.24-2.36 (m, 1 H), 2.04-2.15 (m, 1 H), 2.02 (brs, 3 H S-CH_3); ^{13}C NMR (100 MHz, MeOD) δ 172.0, 171.4, 167.0, 154.8, 152.4, 142.5, 142.2, 136.6, 136.5, 123.7, 123.7, 123.2, 123.2, 122.1,

122.1, 70.44, 70.2, 70.1, 70.1, 69.1, 69.0, 68.7, 51.6, 51.5, 51.1, 50.0, 30.7, 30.5, 25.0, 25.0, 21.0, 21.0.

Synthesis of ((S)-3-acetamido-4-methoxy-4-oxobutyl)(2-(4-(1-(2-(2-(2-(2-((2-fluoropyridin-3-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)butoxy)-2-oxoethyl)(methyl)sulfonium BF_4 salt (19). To a solution of sulfonium salt **18** (5 mg, 0.012 mmol) in water (2 mL) was added azide **12** (8 mg, 0.025 mmol). A stock solution of $CuSO_4$ and sodium ascorbate was prepared by combining 20 mg of copper sulfate and 30 mg of sodium ascorbate in 5 mL of water and allowed to stir for 5 min. A 250 μL aliquot of this stock solution was added to the alkyne/azide mixture and the reaction stirred at room temp for 30 min. Reaction monitoring by LC-MS by LC/MS (mobile phase A = 0.1% formic acid, B = acetonitrile, linear gradient 5% B to 95% B over 7 min, Luna column, 1.8 μ, 2.1 x 50 mm) showed none of the sulfonium salt **18** remaining. Purification by semi-preparative HPLC (Mobile phase A = 0.1% formic acid, B = acetonitrile, linear gradient 5-95 over 25 min, flow = 5 mL/min, column = Luna C18(2), product R_T = 14 min) afforded 6.2 mg (0.008 mmol, 69%) of CuAAC product **19**: HRMS (ESI) $[M+H]^+$ calcd for $C_{29}H_{45}FN_5O_9S$: 658.2917, found: 658.2892; ^1H NMR (400 MHz, acetonitrile-d_3) δ 8.33 (brs, 1 H, NH), 7.73 (m, 1 H), 7.60 (s, 1H), 7.50 (brt, 1 H, *J* = 8 Hz), 7.22 (m, 1 H), 4.46 (m, 2 H), 4.21 (m, 4 H, OCH_2), 3.83 (m, 4 H), 3.60 (s, 3 H, OCH_3, 3.50-3.70 (m, 15 H), 3.40 (brt, 1 H, J = 5.2 Hz, HC-N), 2.71 (m, 4 H), 1.72 (m, 4 H).

Synthesis of methionine-modified peptide mixture 21. Peptide **20** (45 mg, 0.026 mmol) was dissolved into 40 mL of phosphate buffered saline. A solution of oxaziridine **10** (5.8 mg, 0.029 mmol) in 500 μL of DMF was added to the peptide solution over 2 min and the resulting mixture was stirred at ambient temp for an additional 30 min. Reaction monitoring by LC-MS analysis (Positive ion, Mobile phase A = 0.05% TFA in water, B = 0.05% TFA in acetonitrile, gradient 2%

B to 98% B over 1 min, flow = 0.8 mL/min, Column = BEH C18 1.7µ, 2.1 x 50 mm) showed quantitative conversion of peptide **20** to the desired conjugated product **21** (m/z = 909.5 $[M+2H]^{2+}$). Isolation by semi-preparative HPLC semi-preparative HPLC (Column = Luna 10 µm C18 prep (2), 100 Å , 10 x 250 mm, mobile phase: A = water (0.1% Formic acid), B = acetonitrile, λ = 232 nm, gradient: 5% B to 95% B over 20 min, flow rate = 5 mL/min, product R_t = 6.7 min) afforded 35.6 mg (74%) of the pure peptide mixture **21** as a white solid after lyophilization. Analysis by UPLC (Mobile phase A = 0.05% TFA in water, B = 0.05% TFA in acetonitrile, gradient: 10% B held for 2 min then 10% B to 20% B over 9 min, flow = 0.45 mL/min, Column = BEH C18 1.7 µ, 2.1 x 50 mm, λ = 232 nm) showed a 1:1 mixture of isomers **21a** and **21b** with R_T = 7.0 and 7.4 min respectively. An identical batch of crude peptides **21** was prepared as above and the isomers separated by semi-preparative HPLC (Column = Xselect RP Prep C18 OBD Column, 5µm, 19 X 150 mm, mobile phase: A = 100 mM aqueous NH_4OAc (pH: 4.7), B = acetonitrile, λ = 232 nm, isocratic gradient: 12% B over 20 min, flow rate = 20 mL/min, product R_T: **21a** = 17.2 min, **21b** = 21.1 min) which afforded 14.5 mg (31%) of isomer **21a**: HRMS (ESI) $[M+2H]^{2+}$ calcd for $C_{77}H_{121}N_{23}O_{26}S$: 908.9359, found 908.9338; and 15.1 mg (33%) of **21b**: HRMS (ESI) $[M+2H]^{2+}$ calcd for $C_{77}H_{121}N_{23}O_{26}S$: 908.9359, found 908.9338. A series of NMR analyses (^1H, ^{13}C, ^{19}F, TOCSY, NOESY, ^1H-^{13}C multiplicity-edited HSQC, ^1H-^{13}C HMBC, ^1H-^{15}N HMBC, see S19) of both **21a** and **21b** confirmed the sulfimide structures of the peptide conjugates.

Synthesis of the unlabeled standard of peptide isomer mixture 22: CuAAC of peptide mixture 21 with unlabeled azide 12. The peptide mixture **21** (3 mg, 0.0017 mmol) was dissolved into 2 mL of water. To the stirred solution was added 5 mg (0.016 mmol) of azide **12**. Finally, a solution of copper (II) sulfate (5 mg, 0.010 mmol) and sodium ascorbate (4 mg, 0.020 mmol) in

0.5 mL of water was prepared and added to the vial. The resulting solution was stirred for 15 min at ambient temp and the reaction monitored by LC/MS (Positive ion, Mobile phase A = 95:5 water:acetonitrile 10 mM NH_4OAc B = 5:95 water:acetonitrile 10 mM NH_4OAc; flow rate = 0.8 mL/min, gradient 5% B to 95% B over 1 min, Column = BEH C18 1.7μ, 2.1 x 50) which showed clean formation of a new product (R_T = 0.52 min, m/z = 413$[M+4H]^{4+}$, 711.5 $[M+3H]^{3+}$, 1066 $[M+2H]^{2+}$) consistent with unlabeled peptide isomers **22** which were inseparable under these conditions. The crude material was diluted with approximately 100 μL of acetonitrile and subjected to semi-preparative HPLC (Column = Luna 10 μm C18 prep (2), 100 Å, 10 x 250 mm, mobile phase: A = water (0.1% Formic acid), B = acetonitrile, λ = 232 nm, gradient: 5% B to 95% B over 20 min, flow rate = 5 mL/min, product R_T = 7.0 min) affording 2.6 mg (74%) of purified unlabeled peptide isomer mixture **22**: HRMS (ESI) $[M+3H]^{3+}$ calcd for $C_{90}H_{140}FN_{27}O_{30}S$: 711.0061, found 711.0040. UPLC and MS analysis of unlabeled peptide **22** (Mobile phase A = 0.05% TFA in water, B = 0.05% TFA in acetonitrile, gradient: 15% B held for 2 min then 15% B to 30% B over 9 min, flow = 0.45 mL/min, Column = ACQUITY UPLC BEH C18 1.7 μ, 2.1 x 50 mm, λ = 220 nm) showed a 1:1 mixture of isomers (R_T = 6.29 and 6.43 min, m/z = 1066 $[M+2H]^{2+}$,711 $[M+3H]^{3+}$).

Synthesis of methionine conjugated peptide 23. To a 10 mL vial that contained Fmoc-Met-OH (92.8 mg, 0.25 mmol) and HCTU (103.2 mg, 0.25 mmol) in DMF (4 mL), was added a 2M solution of DIEA in NMP (0.25 mL, 0.5 mmol). The resulting solution was then added into another 20 mL vial that contained Fmoc deprotected peptide A20FMDV2 on rink resin[2] (0.5 g, 005 mmol, resin substitution = 0.1 mmol/g). After stirring at ambient temp for 16 h, the resin was filtered, washed with DMF (6 mL) and dichloromethane (6 mL). The resin was then suspended in 20% solution of piperidine in DMF (2 mL) and stirred at ambient temp for 20 min. It was then filtered,

washed twice with DMF (2 x 6 mL) and once with dichloromethane (6 mL), and then suspended in 4 mL of a mixture of phenol:thioanisole:dithiothreitol:H_2O:TIS:TFA (750 mg:0.5 mL:20 mg:0.5 mL:0.2 mL:10 mL). The reaction mixture was stirred at ambient temp for 2.5 h and the resin removed by filtration. After concentrating the filtrate to 2 mL, ice cold ether was added giving a white precipitate which was filtered, washed with cold ether (2 x 6 mL) and vacuum dried for 3 h. LC-MS analysis (mobile phase A = 0.1% formic acid, B = acetonitrile, linear gradient 5% B to 95% B over 7 min, Luna column, 1.8 μ, 2.1 x 50 mm) showed the desired crude product (m/z: $[M+2H]^{2+}$ = 1147.8, $[M+3H]^{3+}$ = 765.6, $[M+4H]^{4+}$ = 574.4). Purification by semi-preparative HPLC (Mobile phase A = 0.1% TFA in water, B = 0.1% TFA in acetonitrile, isocratic gradient 5% B over 13 min, λ = 220 nm, product R_T = 9.7 min) gave pure methionine extended peptide as a white solid, (41.5 mg, 36 % yield) after lyophilization.

To a solution of the methionine extended peptide (10 mg, 4.36 μmol) in PBS (1.25 mL) charged into a 5 mL vial was added a solution of oxaziridine **10** (0.969 mg, 4.79 μmol) in DMF (0.2 mL) via syringe. The resulting solution was stirred at ambient temp for 20 min. Progress of the conjugation was monitored by LC/MS (Mobile phase A = 0.1% formic acid, B = acetonitrile, gradient 5% B to 95% B over 7 min, Luna column 1.8μ, 2.1 x 50) which showed formation of methionine-modified peptide (R_t = 4.8 min, m/z = 1196 ($[M+2H]^{2+}$, m/z = 797 ($[M+3H]^{3+}$, and m/z = 598 ($[M+4H]^{4+}$). This material was diluted with 100 μL of acetonitrile and subjected to semi-preparative HPLC (Column = Luna 10 μm C18 prep (2), 100 Å , 10 x 250 mm, mobile phase: A = water (0.1% Formic acid), B = acetonitrile, λ = 232 nm, gradient: 5% B to 95% B over 20 min, flow rate = 5 mL/min) which afforded purified **23** (8.9 mg, 0.0037 mmol, 86%) as a white solid after lyophilization: HRMS (ESI) $[M+3H]^{3+}$ calcd for $C_{102}H_{176}N_{34}O_{30}S$: 797.4409, found 797.4476.

Synthesis of peptide 24 standard: CuAAC of peptide 23 with azide 12. Peptide **23** (1 mg) was weighed into a 2 mL vial and then dissolved into 0.5 mL of water. To the solution was added 1 mg of azide **12** (1 mg, 3.18 μmol) dissolved into 100 μL of DMSO. Finally, a solution of copper(II) sulfate (3 mg, 0.006 mmol) and sodium ascorbate (2 mg, 0.010 mmol) in 0.5 mL of water was prepared and added to the vial. The resulting solution was stirred for 0.5 h at ambient temp and the reaction monitored by LC/MS (Mobile phase A = 0.1% formic acid, B = acetonitrile, gradient 5% B to 95% B over 7 min, Luna column 1.8μ, 2.1 x 50) which showed formation of a new product (R_T = 5.02 min, m/z = 677, 902, 1352) consistent with unlabeled peptide **24**. Also observed in this analysis was a side product (RT = 4.89 min, m/z = 578, 771, 1156) which appears to result from loss of the prosthetic group. The crude material was diluted with approximately 100 μL of acetonitrile and subjected to semi-preparative HPLC (Column = Luna 10 μm C18 prep (2), 100 Å , 10 x 250 mm, mobile phase: A = water (0.1% Formic acid), B = acetonitrile, λ = 232 nm, gradient: 5% B to 95% B over 20 min, flow rate = 5 mL/min, product R_t = 9.5-10.5 min) affording 0.59 mg (52%) of purified unlabeled peptide **24**: HRMS (ESI) $[M+4H]^{4+}$ calcd for $C_{115}H_{195}FN_{38}O_{34}S$: 676.6154, found 676.6201.

Figure S1. ^{1}H NMR (400 MHz, d_4-methanol) of **11**

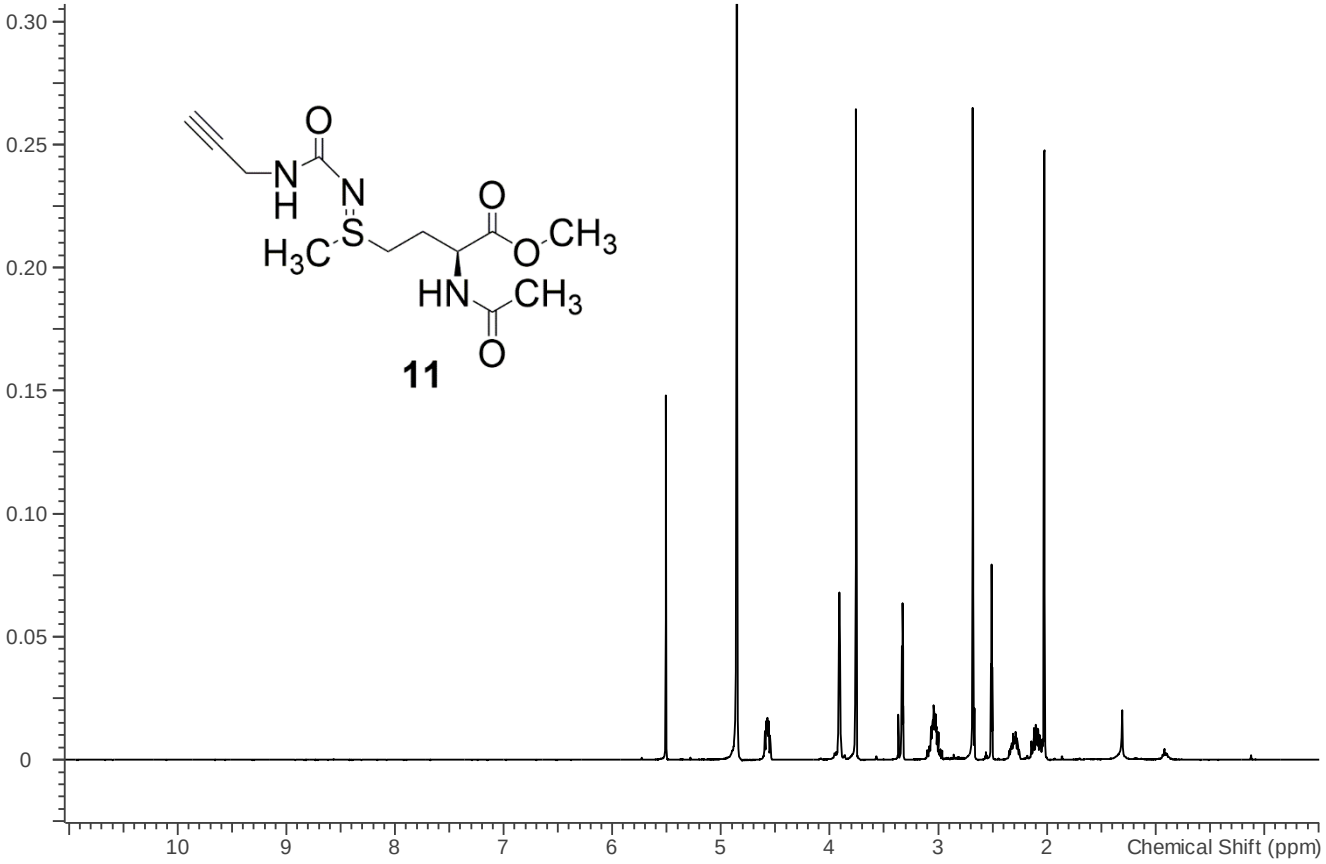


Figure S2. ^{13}C NMR (100 MHz, d_4-methanol) of compound **11**

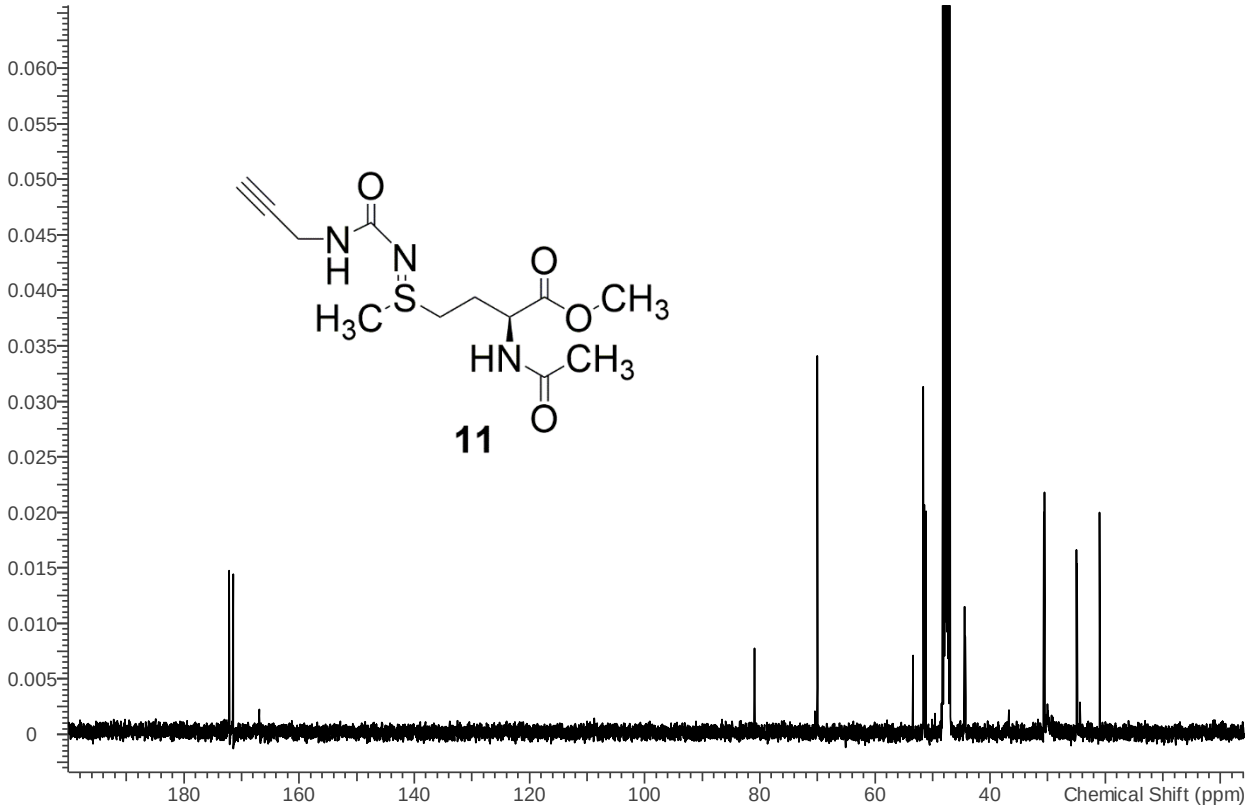


Figure S3. ^{1}H NMR (400 MHz, d_4-methanol) of **13.**

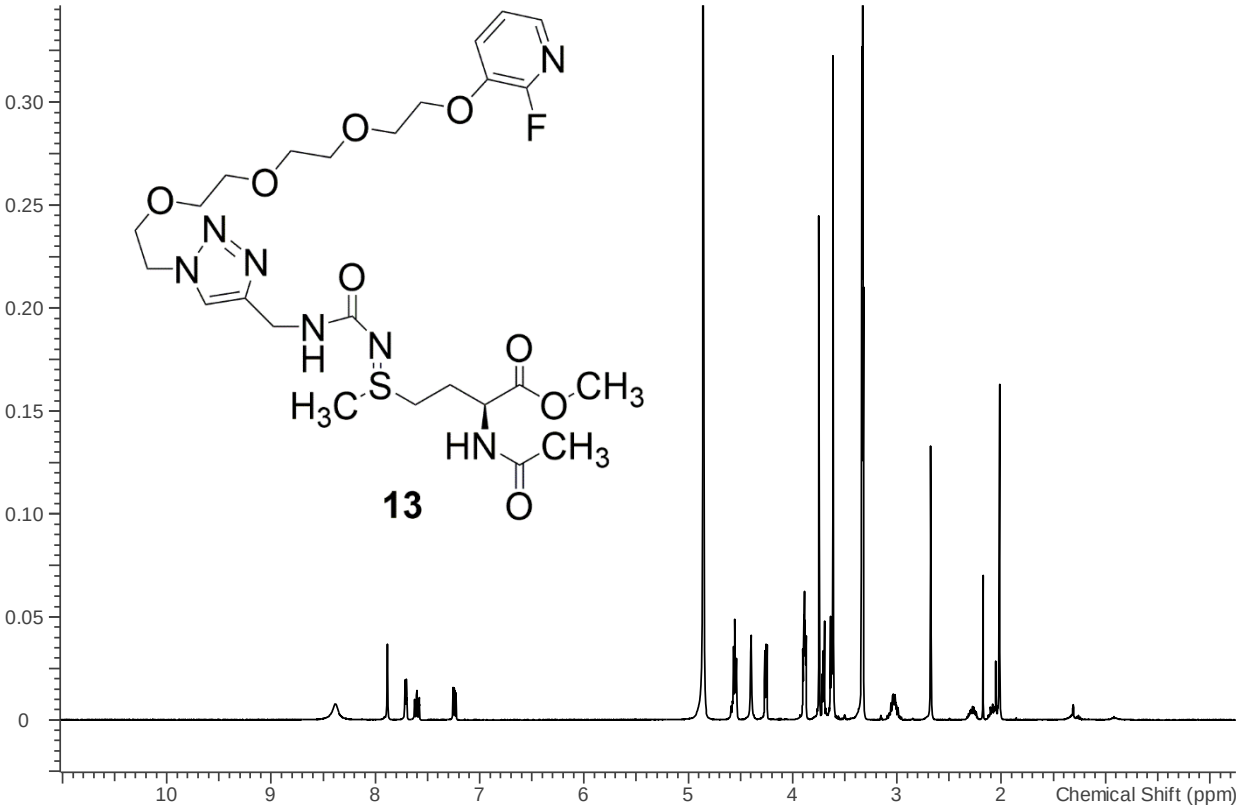


Figure S4. ^{13}C NMR (100 MHz, d_4-methanol) of **13**

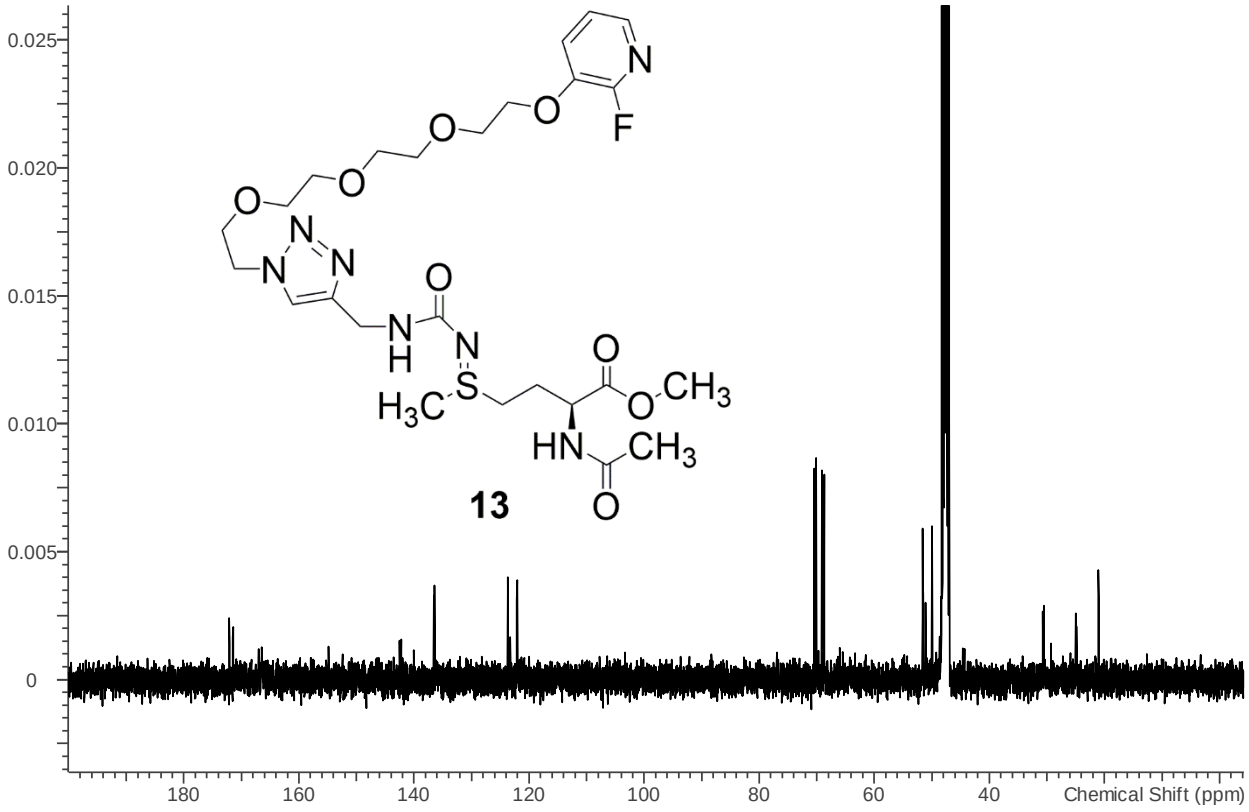


Figure S5. ^{1}H NMR (400 MHz, d_3-acetonitrile) of **18**

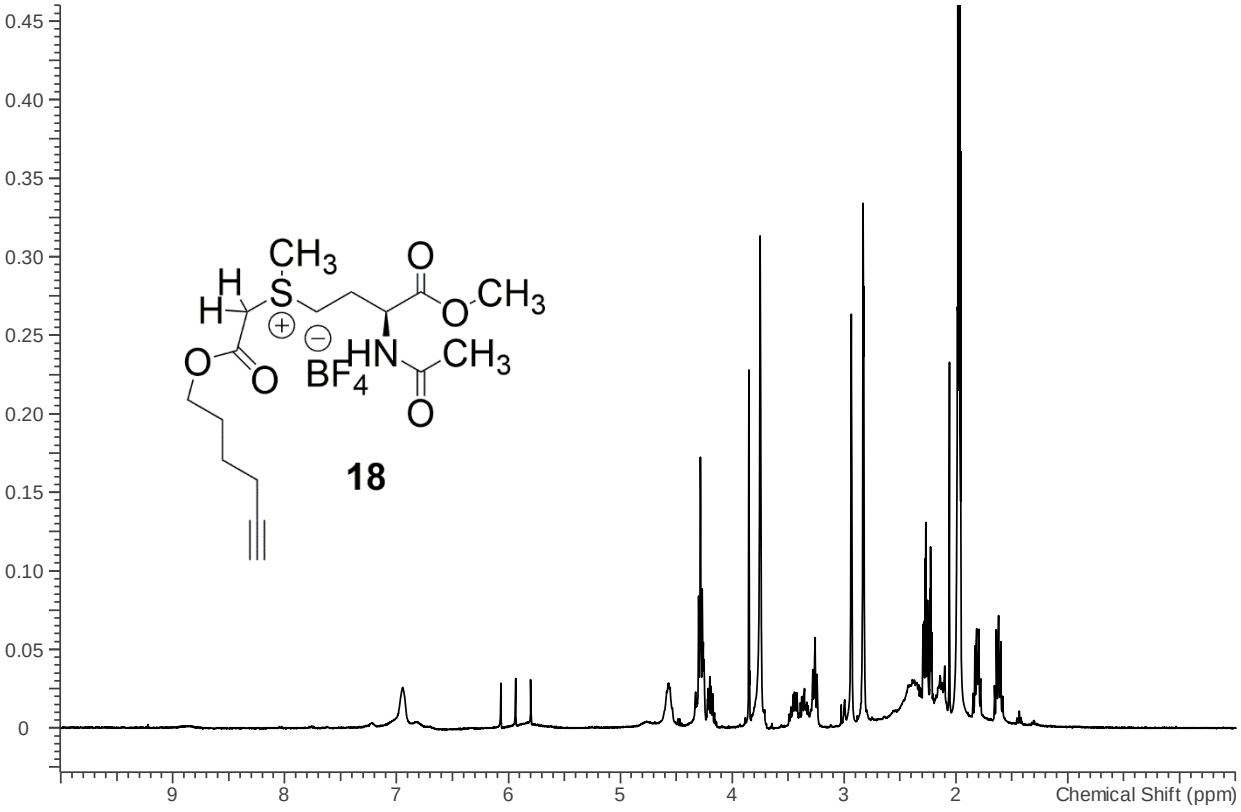


Figure S6. ^{13}C NMR (100 MHz, d_3-acetonitrile) of **18**

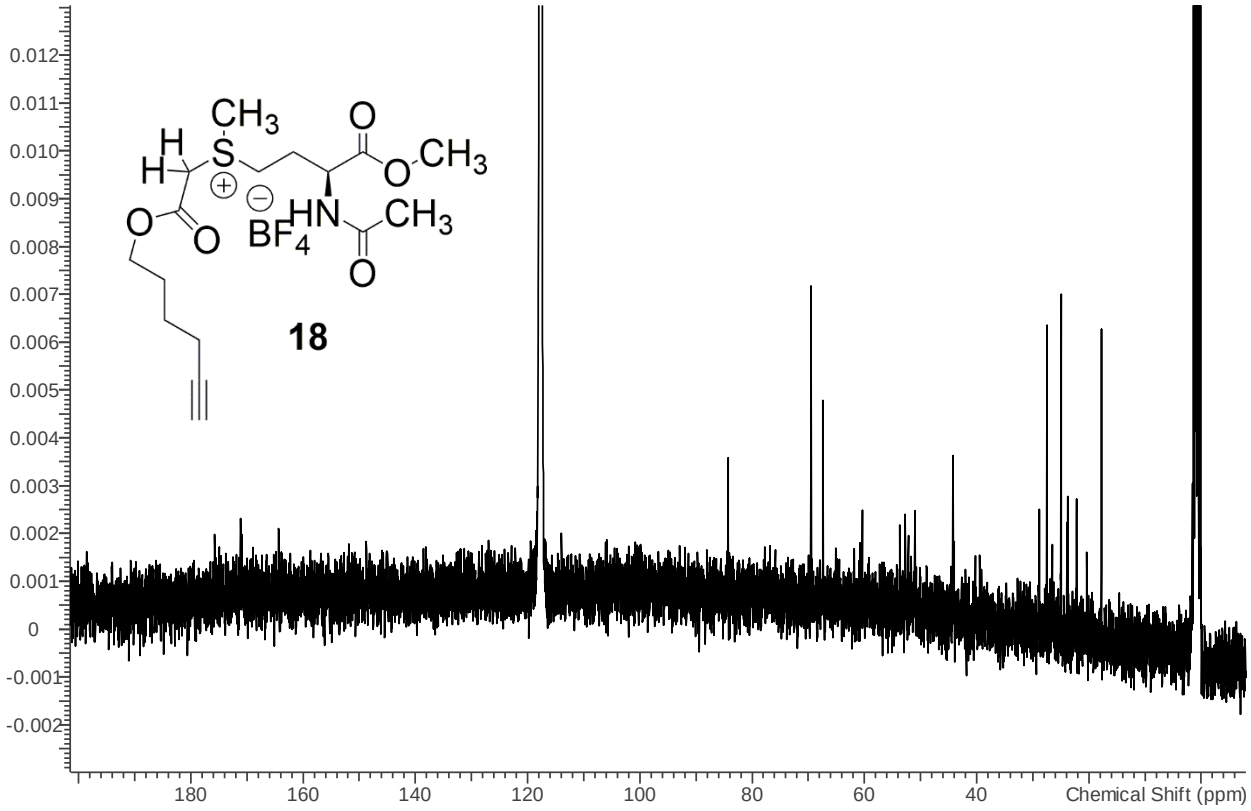
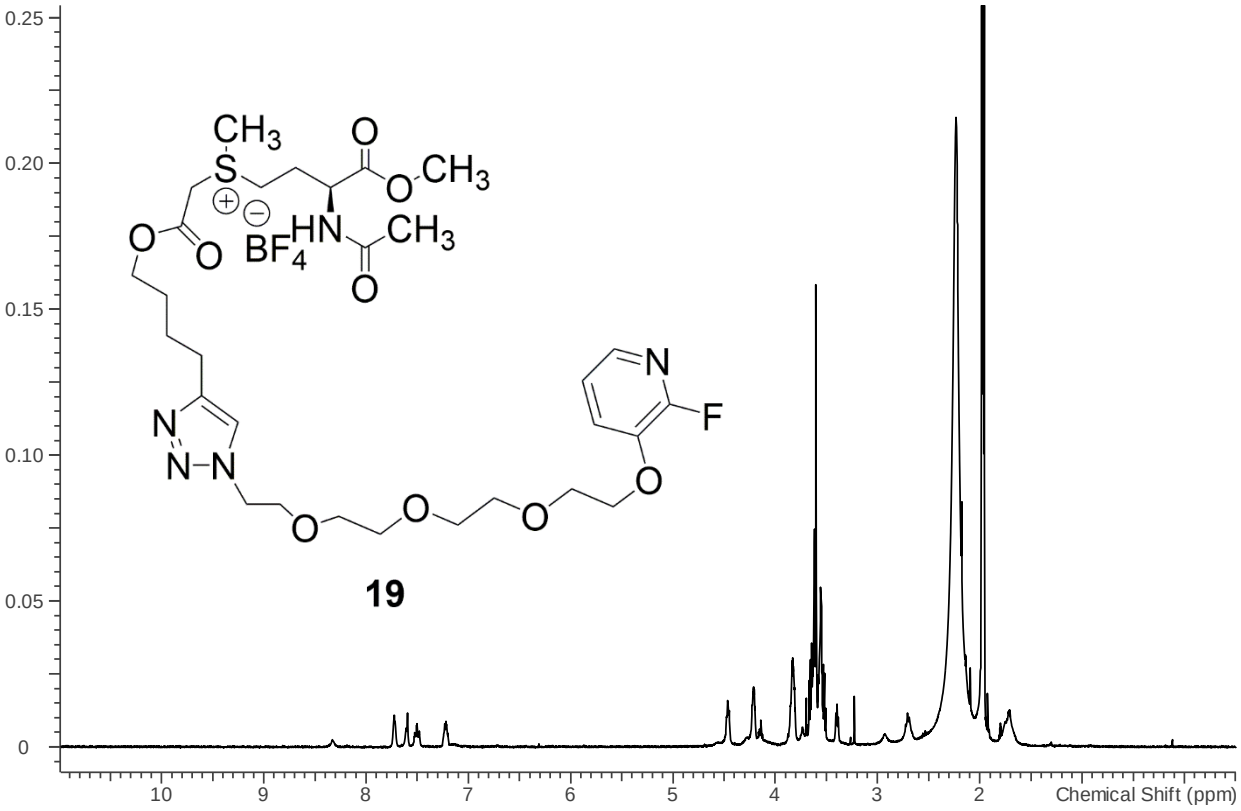


Figure S7. ^{1}H NMR (400 MHz, d_3-acetonitrile) of **19**



NMR structural characterization of methionine modified peptides, isomers 21a and 21b.

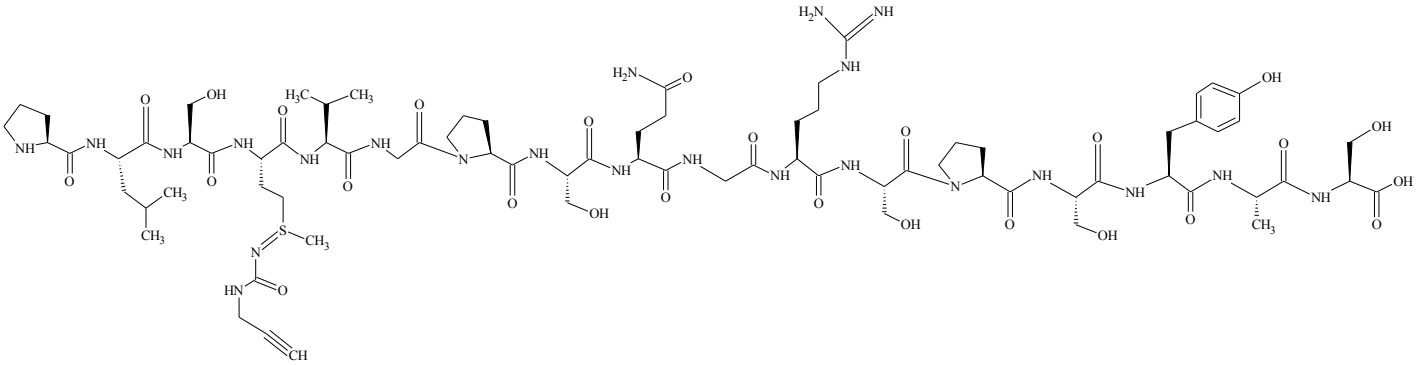
Each isomer was dissolved in approximately 0.65 mL dimethylsulfoxide-d6 (0.75 mL ampoule containing 100%, 99.96 atom % D, with 0.03 % (v/v) TMS, Sigma-Aldrich). Data was collected on a Bruker Avance III HD 700 MHz NMR spectrometer equipped with a 5-mm TCI triple resonance cryoprobe (Bruker, Billerica, MA) at 27 °C. All the ^{1}H and ^{13}C chemical shifts are reported on the δ scale (ppm) downfield from tetramethylsilane. Proton, carbon and nitrogen chemical shift data were elucidated from ^{1}H, proton decoupled ^{13}C, Total Correlation Spectroscopy (TOCSY), Nuclear Overhauser Enhancement Spectroscopy (NOESY), multiplicity-edited heteronuclear single quantum coherence (1H-13C edited HSQC) and heteronuclear multiple-bond correlation (^{1}H-^{13}C HMBC and ^{1}H-^{15}N HMBC) experiments. ACD/NMR prediction software (ACD/LABS Release 2015 Pack 2) and ACD NMR Spectrus Workbook (ACD/NMR Workbook 2019.1.1) from Advanced Chemistry Development Inc. (Toronto, ON, Canada) were used.

Isomers 21a and 21b:

The structures of isomers **21a** and **21b** were assigned from 1D and 2D NMR data and the chemical shifts are captured in tables 1 and 2. Numerous peaks in the proton and carbon spectra appear broad. Data suggested the presence of conformers. The chemoselective modification of methionine to sulfimide in both isomers was verified by a long-range ^{1}H-^{15}N HMBC correlation between methionine proton 4-6 and urea nitrogen 4-7 (Figure S8). A ^{1}H-^{1}H NOESY correlation was observed between 4-6 and urea proton 4-10. Additionally, carbon 4-6 exhibited a 16-ppm downfield shift as compared to underivatized starting molecule **20**. The alkyne was identified by its carbon peaks at 71.8 and 83.2 ppm. In the ^{1}H-^{13}C HSQC-DEPT spectrum, a one-bond correlation is observed between the terminal alkyne proton (H13) and its attached carbon (C13). There is also a weak two-bond correlation between the terminal alkyne proton (H13) and the other

alkyne carbon (C12). TOCSY correlations were observed between protons 4-10, 4-11 and 4-13. Long-range ^{1}H-^{13}C HMBC correlations were observed between protons 4-11 and carbons 4-12, 4-13 from the alkyne and 4-8 from the urea.

Structure:



ACD Numbering:

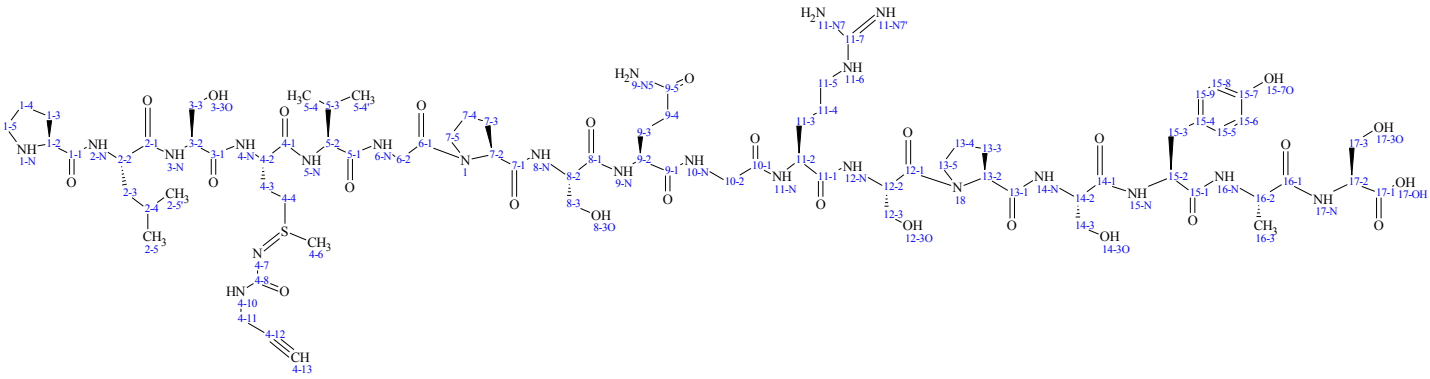
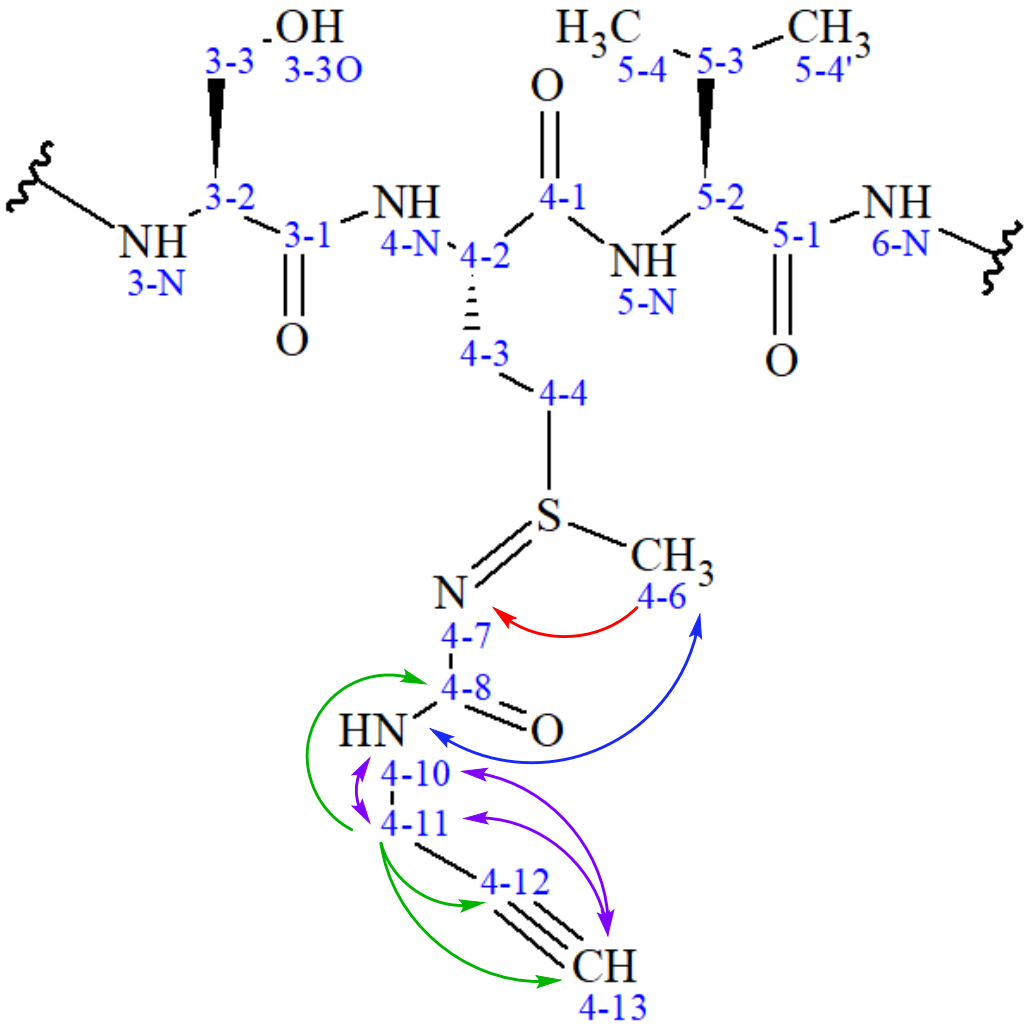


Figure S8. Key 2D NMR correlations confirming the chemoselective modification of methionine to sulfimide for isomers 21a and 21b.



1H-1H TOCSY, 1H-1H NOESY, 1H-13C HMBC, 1H-15N HMBC

Figure S9. 1D proton spectrum of isomer 21a in DMSO-d6 at 27°C.

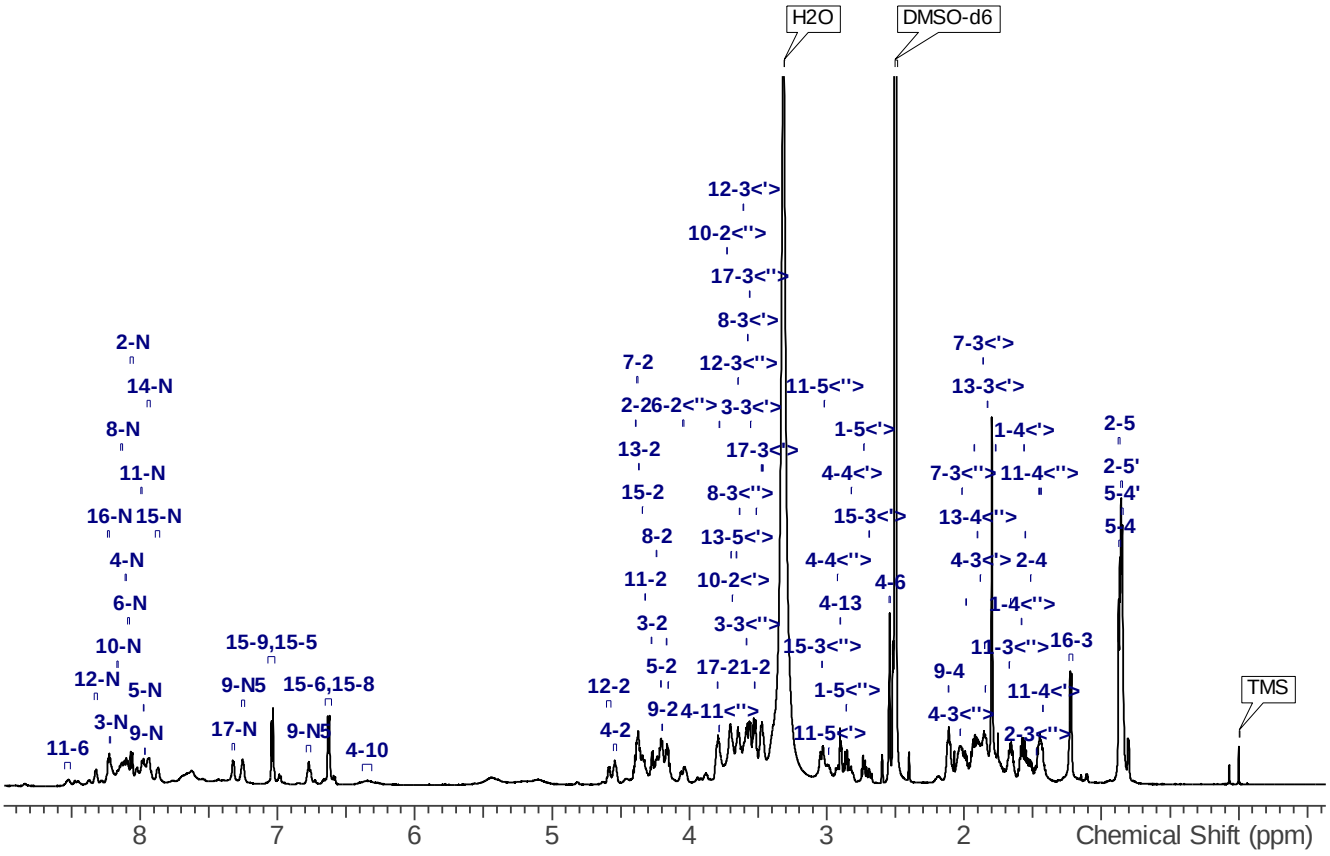


Figure S10: 1D carbon spectrum of isomer 21a in DMSO-d6 at 27°C.

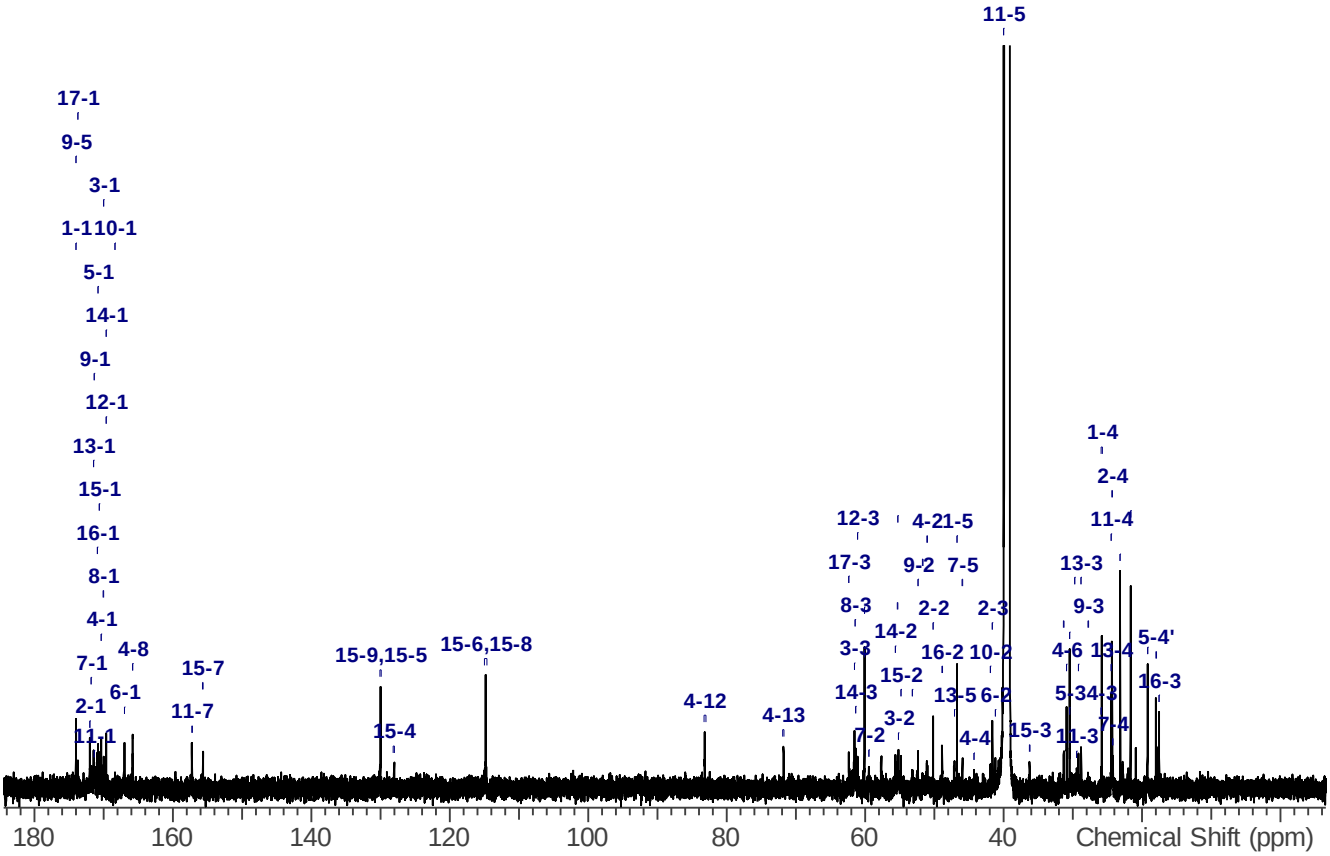


Table S1: Chemical Shifts of Isomer 21a in DMSO-d6 at 27°C.

Atom#	XHn	H Shift	C Shift	N Shift
1-5	CH2	2.73	46.7	
1-5	CH2	2.86	46.7	
1-4	CH2	1.58	25.8	
1-4	CH2	1.56	25.8	
1-3	CH2	1.94	30.5	
1-3	CH2	1.66	30.5	
1-2	CH	3.53	60.1	
1-1	C		174.1	
1-N	NH			42.0
2-5	CH3	0.87	23.1	
2-5'	CH3	0.85	21.7	
2-4	CH	1.52	24.3	
2-3	CH2	1.47	41.6	
2-3	CH2	1.44	41.6	
2-2	CH	4.39	50.2	
2-1	C		172.0	
2-N	NH	8.06		115.7

3-3	CH2	3.55	61.5	
3-3	CH2	3.58	61.5	
3-2	CH	4.28	55.2	
3-1	C		170.0	
3-N	NH	8.22		
4-13	CH	2.90	71.8	
4-12	C		83.2	
4-11	CH2	3.70	29.8	
4-11	CH2	3.79	29.8	
4-10	NH	6.35		
4-8	C		165.9	
4-7	N			110.0
4-6	CH3	2.54	30.9	
4-4	CH2	2.92	44.2	
4-4	CH2	2.82	44.2	
4-3	CH2	1.88	25.9	
4-3	CH2	2.03	25.9	
4-2	CH	4.55	51.0	
4-1	C		170.4	
4-N	NH	8.11		
5-4	CH3	0.87	19.2	
5-4'	CH3	0.85	18.0	
5-3	CH	1.99	30.4	
5-2	CH	4.21	57.7	
5-1	C		170.8	
5-N	NH	7.97		
6-2	CH2	3.78	41.2	
6-2	CH2	4.05	41.2	
6-1	C		167.0	
6-N	NH	8.09		
7-5	CH2	3.47	45.9	
7-5	CH2	3.54	45.9	
7-4	CH2	1.85	24.2	
7-4	CH2	1.90	24.2	
7-3	CH2	2.01	29.2	
7-3	CH2	1.86	29.2	
7-2	CH	4.38	59.5	
7-1	C		171.9	
8-3	CH2	3.57	61.4	
8-3	CH2	3.63	61.4	
8-2	CH	4.24	55.2	
8-1	C		170.1	
8-N	NH	8.14		
9-5	C		174.0	
9-4	CH2	2.11	31.3	
9-3	CH2	1.77	27.8	
9-3	CH2	1.93	27.8	
9-2	CH	4.20	52.3	
9-1	C		171.4	
9-N	NH	7.97		
9-N5	NH2	6.77		
9-N5	NH2	7.25		
10-2	CH2	3.73	41.9	

10-2	CH2	3.69	41.9	
10-1	C		168.4	
10-N	NH	8.16		
11-7	C		157.3	
11-6	NH	8.52		
11-5	CH2	2.99	40.0	
11-5	CH2	3.02	40.0	
11-4	CH2	1.45	24.4	
11-4	CH2	1.43	24.4	
11-3	CH2	1.55	29.4	
11-3	CH2	1.67	29.4	
11-2	CH	4.32	51.7	
11-1	C		171.4	
11-N	NH	7.99		
12-3	CH2	3.61	61.1	
12-3	CH2	3.65	61.1	
12-2	CH	4.58	53.1	
12-1	C		169.7	
12-N	NH	8.32		
13-5	CH2	3.66	47.1	
13-5	CH2	3.70	47.1	
13-4	CH2	1.90	24.4	
13-4	CH2	1.84	24.4	
13-3	CH2	1.83	28.8	
13-3	CH2	2.04	28.8	
13-2	CH	4.37	60.1	
13-1	C		171.5	
14-3	CH2	3.51	61.3	
14-3	CH2	3.47	61.3	
14-2	CH	4.17	55.6	
14-1	C		169.7	
14-N	NH	7.94		
15-7	C		155.7	
15-6, 15-8	CH	6.63	114.8	
15-5, 15-9	CH	7.04	130.0	
15-4	C		128.1	
15-3	CH2	2.69	36.2	
15-3	CH2	3.03	36.2	
15-2	CH	4.34	54.8	
15-1	C		170.6	
15-N	NH	7.87		
16-3	CH3	1.22	17.5	
16-2	CH	4.15	48.9	
16-1	C		171.0	
16-N	NH	8.23		120.0
17-3	CH2	3.56	62.3	
17-3	CH2	3.47	62.3	
17-2	CH	3.79	55.3	
17-1	C		173.8	
17-N	NH	7.32		

Figure S11. 1D proton spectrum of isomer 21b in DMSO-d6 at 27°C.

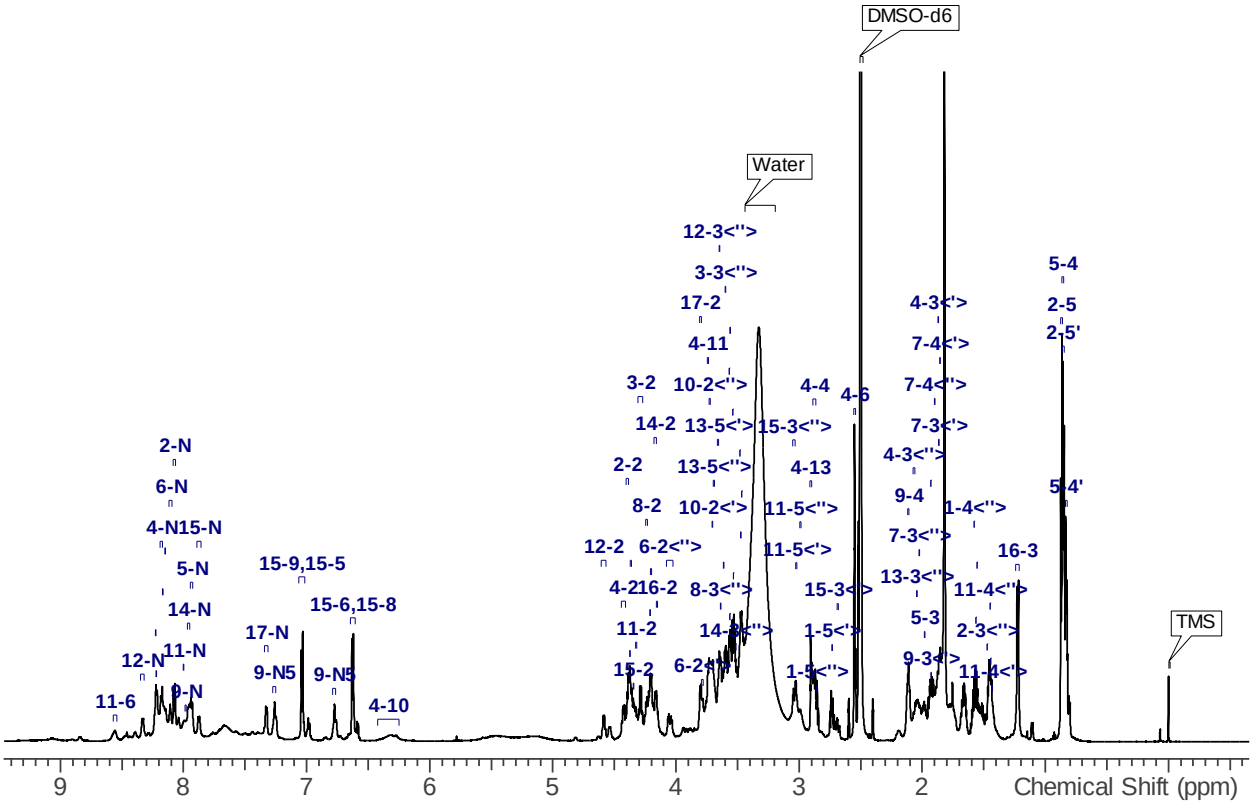


Figure S12: 1D carbon spectrum of isomer 21b in DMSO-d6 at 27°C.

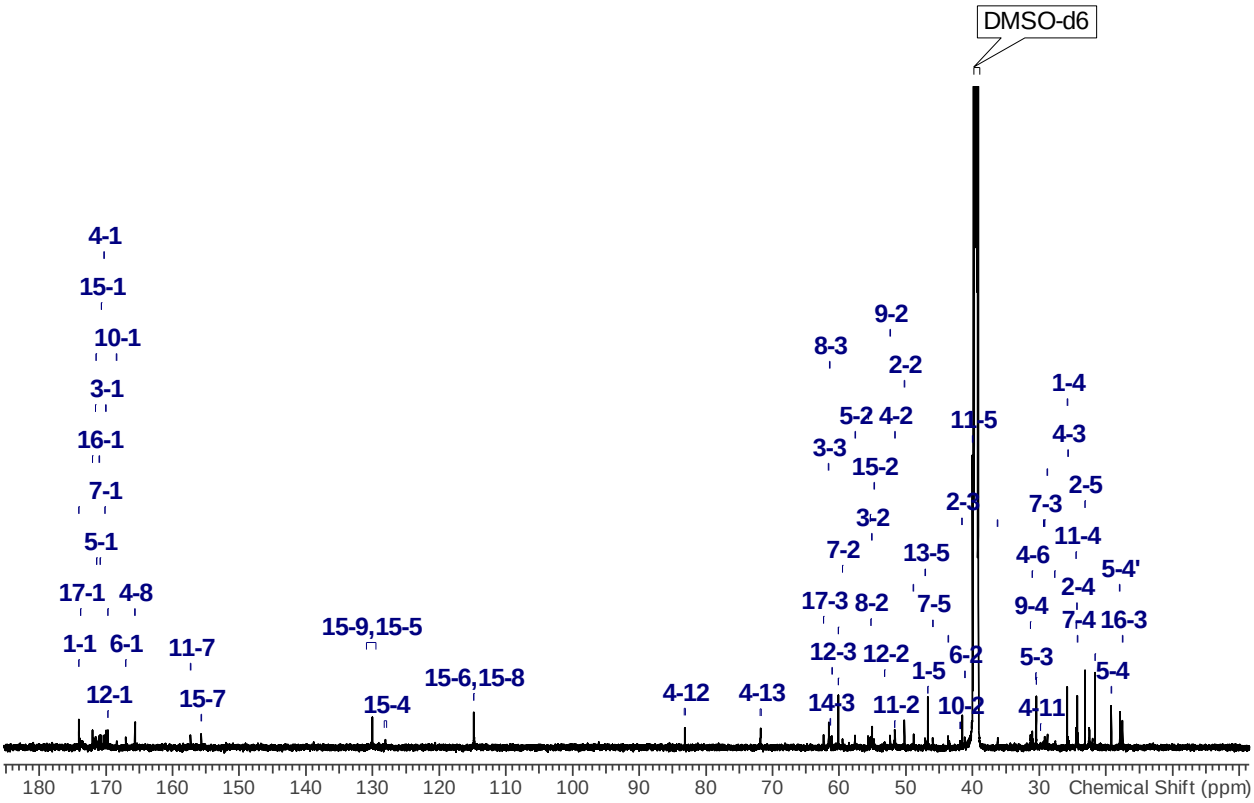


Table S2: Chemical Shifts of Isomer 21b in DMSO-d6 at 27°C.

Atom#	XHn	H Shift	C Shift	N Shift
1-5	CH2	2.86	46.7	
1-5	CH2	2.73	46.7	
1-4	CH2	1.58	25.8	
1-4	CH2	1.56	25.8	
1-3	CH2	1.93	30.5	
1-3	CH2	1.67	30.5	
1-2	CH	3.53	60.1	
1-1	C		174.1	
1-N	NH2			42.0
2-5	CH3	0.87	23.1	
2-5'	CH3	0.85	21.6	
2-4	CH	1.52	24.3	
2-3	CH2	1.44	41.6	
2-3	CH2	1.47	41.6	
2-2	CH	4.39	50.2	
2-1	C		172.0	
2-N	NH	8.08		115.6
3-3	CH2	3.56	61.6	
3-3	CH2	3.60	61.6	

3-2	CH	4.29	55.1	
3-1	C		170.0	
3-N	NH	8.22		
4-13	CH	2.91	71.8	
4-12	C		83.2	
4-11	CH2	3.74	29.8	
4-10	NH	6.31		
4-8	C		165.6	
4-7	N			109.2
4-6	CH3	2.55	31.1	
4-4	CH2	2.88	43.7	
4-3	CH2	2.06	25.7	
4-3	CH2	1.87	25.7	
4-2	CH	4.42	51.6	
4-1	C		170.3	
4-N	NH	8.18		
5-4	CH3	0.86	19.2	
5-4'	CH3	0.83	17.9	
5-3	CH	1.98	30.5	
5-2	CH	4.21	57.6	
5-1	C		170.8	
5-N	NH	7.93		
6-2	CH2	3.79	41.2	
6-2	CH2	4.05	41.2	
6-1	C		167.0	
6-N	NH	8.10		
7-5	CH2	3.47	46.0	
7-5	CH2	3.54	46.0	
7-4	CH2	1.90	24.2	
7-4	CH2	1.86	24.2	
7-3	CH2	2.03	29.2	
7-3	CH2	1.86	29.2	
7-2	CH	4.37	59.5	
7-1	C		170.2	
8-3	CH2	3.64	61.4	
8-3	CH2	3.57	61.4	
8-2	CH	4.24	55.3	
8-N	NH	8.15		
9-5	C		174.0	
9-4	CH2	2.11	31.3	
9-3	CH2	1.93	27.6	
9-3	CH2	1.77	27.6	
9-2	CH	4.20	52.4	
9-1	C		171.4	
9-N	NH	7.98		
9-N5	NH2	7.26		
9-N5	NH2	6.77		
10-2	CH2	3.70	41.9	
10-2	CH2	3.72	41.9	
10-1	C		168.4	
10-N	NH	8.17		
11-7	C		157.3	
11-6	NH	8.56		

11-5	CH2	3.03	40.0	
11-5	CH2	2.99	40.0	
11-4	CH2	1.45	24.4	
11-4	CH2	1.43	24.4	
11-3	CH2	1.55	29.4	
11-3	CH2	1.67	29.4	
11-2	CH	4.32	51.7	
11-1	C		171.4	
11-N	NH	8.00		
12-3	CH2	3.61	61.1	
12-3	CH2	3.65	61.1	
12-2	CH	4.59	53.2	
12-1	C		169.7	
12-N	NH	8.33		
13-5	CH2	3.69	47.1	
13-5	CH2	3.66	47.1	
13-4	CH2	1.91	24.4	
13-4	CH2	1.85	24.4	
13-3	CH2	1.83	28.8	
13-3	CH2	2.04	28.8	
13-2	CH	4.37	60.1	
13-1	C		171.5	
14-3	CH2	3.47	61.3	
14-3	CH2	3.51	61.3	
14-2	CH	4.17	55.7	
14-1	C		169.7	
14-N	NH	7.96		111.7
15-8, 15-6	CH	6.63	114.8	
15-7	C		155.7	
15-5, 15-9	CH	7.04	130.0	
15-4	C		128.1	
15-3	CH2	3.04	36.2	
15-3	CH2	2.69	36.2	
15-2	CH	4.35	54.8	
15-1	C		170.7	
15-N	NH	7.87		
16-3	CH3	1.22	17.5	
16-2	CH	4.16	48.9	
16-1	C		171.0	
16-N	NH	8.23		119.9
17-3	CH2	3.48	62.4	
17-3	CH2	3.56	62.4	
17-2	CH	3.80	55.3	
17-1	C		173.8	
17-N	NH	7.33		

Radiochemistry General Methods

Fluorine-18 fluoride was produced using a General Electric (GE) PETTrace cyclotron via the $^{18}O(p,n)^{18}F$ nuclear reaction. Synthesis and purification of ^{18}F-labeled PEGylated azide [^{18}F]**12** was completed using a GE TRACERlab FX2N remote controlled synthesis unit.[1] Purification of ^{18}F-labeled BSA was completed on a custom-built remote controlled system. The radiochemical conversion and purity was determined using an HPLC (Agilent) system and a Posi-Ram (Lab Logic) radio-HPLC detector. Protein concentration was measure using a NanoDrop 2000.

Analytical HPLC method A: Zorbax SB C18 (Agilent) 5 micron column (4.6 x 250 mm) with a gradient program using a mobile phase that went from 10% acetonitrile in aqueous 0.1% trifluoroacetic acid (TFA) at a flow rate of 1 mL/min to 90% acetonitrile in aqueous 0.1% TFA at a flow rate of 1 mL/min over 30 minutes. UV detector was set at 205 nm and ^{18}F-peak associated with the product was structurally confirmed by co-injection of non-radioactive reference standard. ^{18}F-peak associated with the product co-eluted with non-radioactive reference standards between 0.1-0.3 minutes using this method as shown in Figure S13 and Figure S14.

Analytical HPLC method B: Zorbax SB C18 (Agilent) 5 micron column (4.6 x 250 mm) with a gradient program using a mobile phase that went from 10% acetonitrile in aqueous 0.1% trifluoroacetic acid (TFA) at a flow rate of 1 mL/min to 45% acetonitrile in aqueous 0.1% TFA at a flow rate of 1 mL/min over 25 minutes. UV detector was set at 220 nm and ^{18}F-peak associated with the product was structurally confirmed by co-injection of non-radioactive reference standard. ^{18}F-peak associated with the product co-eluted with non-radioactive reference standards between 0.1-0.3 minutes using this method as shown in Figure S15.

Analytical HPLC method C: Zorbax SB C18 (Agilent) 5 micron column (4.6 x 250 mm) with an isocratic HPLC program using a 39% acetonitrile in an aqueous 10 mM ammonium acetate

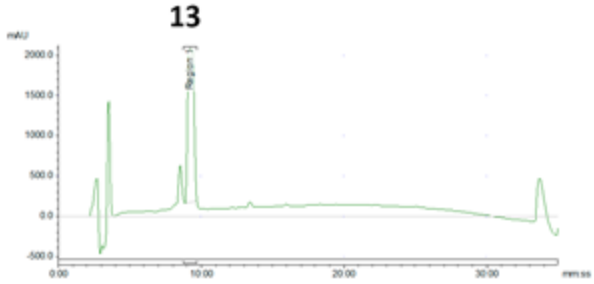
buffer at a flow rate of 1 mL/min over 30 minutes. UV detector was set at 220 nm and ^{18}F-peak associated with the product was structurally confirmed by co-injection of non-radioactive reference standard. The^{18}F-peak associated with the product co-eluted with non-radioactive reference standards between 0.1-0.3 minutes using this method as shown in Figure S16.

Analytical HPLC method D: Superdex 200 Increase 10/300 GL (GE Lifesciences) (10 x 300 mm) with an isocratic HPLC program using 1X PBS mobile phase at a flow rate of 0.75 mL/min over 45 minutes. UV detector was set at 280 nm and ^{18}F-peak associated with the product was structurally confirmed by co-injection of non-radioactive reference standard and molecular weight standard (Gel Filtration Standard, Bio-Rad, 1511901). ^{18}F-peak associated with the product co-eluted with non-radioactive reference standards between 0.1 minutes using this method as shown in Figure S17 and 18. This method was also used for radiochemical purity and stability studies as shown in Figure S19.

Figure S13. Reverse phase HPLC analysis of ^{18}F**[13]**

UV (205 nM) Dectector Response

13

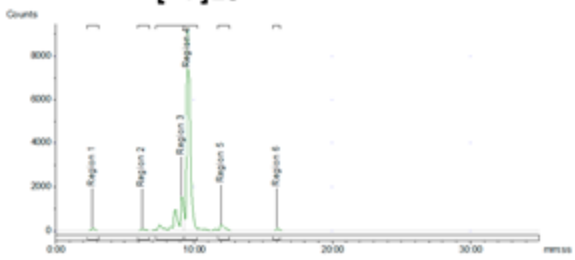


Regions UV (205 nM)

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU·s)	%ROI (%)
13	**8:46**	**9:43**	**9:14**	**52294.7**	**100.00**

Gamma Detector Response

[^{18}F]13



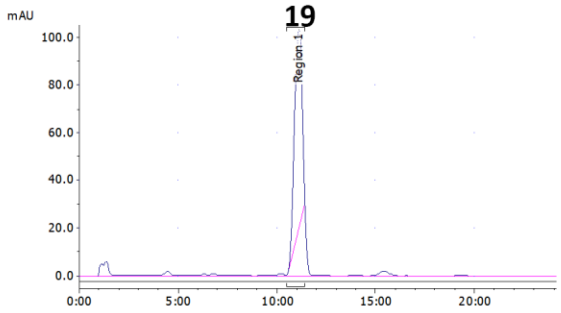
Regions ^{18}F

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	Radiochemical conversion (%)
Region 1	2:17	3:06	2:39	2149	1.23
Region 2	5:57	6:45	6:14	1953	1.12
Region 3	7:14	9:18	9:06	40113	22.99
[^{18}F]13	**9:18**	**10:16**	**9:31**	**122835**	**70.42**
Region 5	11:44	12:34	11:59	5890	3.38
Region 6	15:43	16:15	16:00	1504	0.86
6 Peaks				174444	100.00

Figure S14. Reverse phase HPLC analysis of **[^{18}F]19**

UV (205 nM) Dectector Response

19

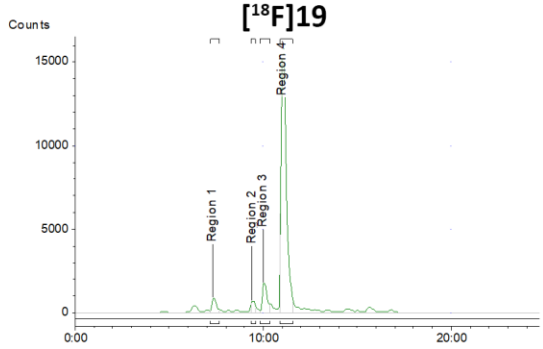


Regions UV (205 nM)

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU·s)	%ROI (%)
19	**10:34**	**11:26**	**10:55**	**2274.2**	**100.00**
1 Peak				2274.2	100.00

Gamma Detector Response

[^{18}F]19

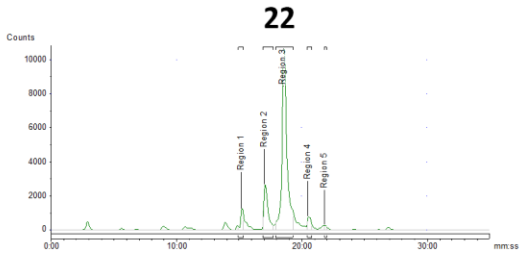


Regions ^{18}F

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	Radiochemical conversion (%)
Region 1	7:12	7:40	7:19	12134	4.17
Region 2	9:22	9:35	9:25	7269	2.50
Region 3	9:49	10:23	10:01	26596	9.14
[^{18}F]19	**10:54**	**11:33**	**11:00**	**244863**	**84.19**

Figure S15. Reverse phase HPLC analysis of **[^{18}F]22**

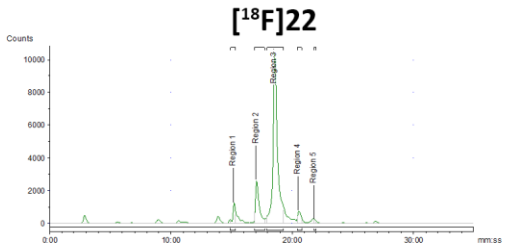
UV (220 nM) Dectector Response



Regions UV (220 nM)

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU·s)	%ROI (%)
Region 1 (precursor)	16:28	17:27	16:52	1278.2	72.97
22	**17:53**	**18:46**	**18:22**	**473.5**	**27.03**
2 Peaks				1751.7	100.00

Gamma Detector Response

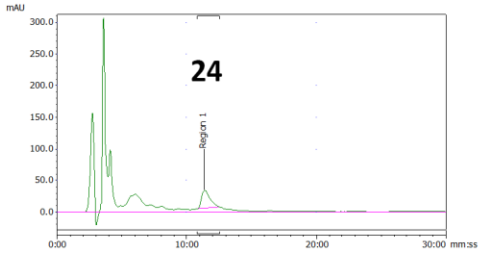


Regions ^{18}F

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	%Radiochemical conversion
Region 1	14:56	15:21	15:11	15296	4.69
Region 2	16:55	17:44	17:02	49048	15.05
[^{18}F]22	**17:56**	**19:19**	**18:32**	**248271**	**76.18**
Region 4	20:26	20:47	20:30	11401	3.50
Region 5	21:48	21:58	21:48	1870	0.57
5 Peaks				325886	100.00

Figure S16. Reverse phase HPLC analysis of **[^{18}F]24**

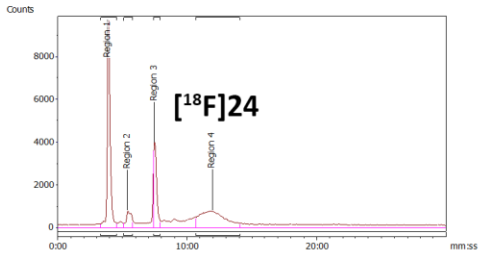
UV (220 nM) Dectector Response



Regions UV (220 nM)

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU·s)	%ROI (%)	%Total (%)
24	**10:51**	**12:30**	**11:21**	**1176.5**	**100.00**	**8.16**
1 Peak				1176.5	100.00	8.16

Gamma Detector Response



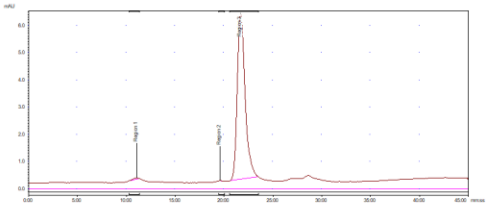
Regions ^{18}F

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	Radiochemical conversion (%)
Region 1	3:18	4:32	3:53	122834	41.49
Region 2	5:07	5:48	5:24	19319	6.53
Region 3	7:24	7:55	7:27	51309	17.33
[^{18}F]24	**10:39**	**14:05**	**11:57**	**102610**	**34.66**
4 Peaks				296072	100.00

Figure S17. Size exclusion chromatography analysis of **^{18}F-labeled BSA**

UV (280 nM) Dectector Response

BSA

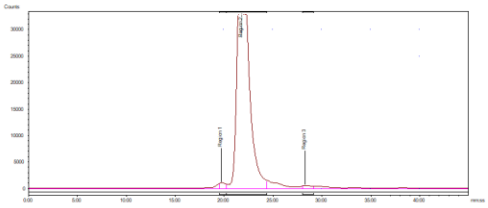


Regions UV (280 nM)

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU·s)	%ROI (%)
Region 1	10:18	11:19	11:06	2.0	0.60
Region 2	19:29	20:04	19:36	0.1	0.03
BSA	**20:34**	**23:30**	**21:39**	**334.8**	**99.37**
3 Peaks				336.9	100.00

Gamma Detector Response

^{18}F-labeled BSA



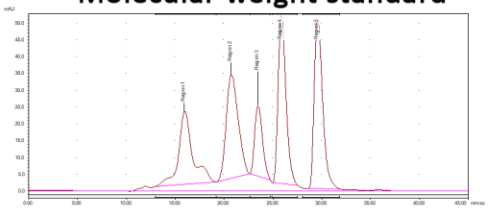
Regions ^{18}F

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	Radiochemical purity (%)
Region 1	19:30	20:12	19:44	40849	1.23
^{18}F-labeled BSA	**20:12**	**24:21**	**21:49**	**3259573**	**97.79**
Region 3	28:01	29:08	28:16	32808	0.98
3 Peaks				3333230	100.00

Figure S18. Size exclusion chromatography analysis of ^{18}F-labeled BSA with molecular weight standard

UV (280 nM) Dectector Response

Molecular weight standard

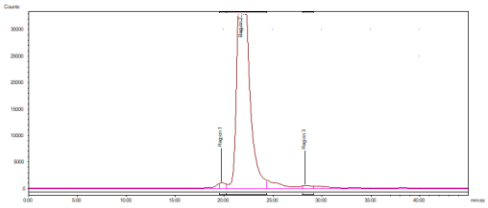


Regions UV (280 nM)

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU·s)	%ROI (%)
670 kDa	12:58	19:13	15:56	2160.3	18.69
158 kDa	19:13	22:38	20:43	2570.0	22.23
44 kDa	22:38	24:46	23:26	1143.0	9.89
17 kDa	24:53	27:32	25:50	2732.8	23.64
1.4 kDa	28:02	31:51	29:33	2953.3	25.55
5 Peaks				11559.4	100.00

Gamma Detector Response

^{18}F-labeled BSA

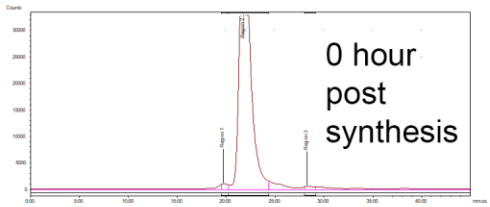


Regions ^{18}F

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	Radiochemical purity (%)
Region 1	19:30	20:12	19:44	40849	1.23
^{18}F-labeled BSA	**20:12**	**24:21**	**21:49**	**3259573**	**97.79**
Region 3	28:01	29:08	28:16	32808	0.98
3 Peaks				3333230	100.00

Figure S19. Size exclusion chromatography analysis of 6-hour stability of **^{18}F-labeled BSA**

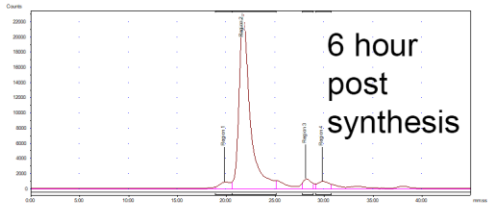
A) Gamma Detector Response



Regions ^{18}F

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	Radio-chemical purity (%)
Region 1	19:30	20:12	19:44	40849	1.23
^{18}F-labeled BSA	**20:12**	**24:21**	**21:49**	**3259573**	**97.79**
Region 3	28:01	29:08	28:16	32808	0.98
3 Peaks				3333230	100.00

B) Gamma Detector Response



Regions ^{18}F

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	Radio-chemical purity (%)
Region 1	18:50	20:34	19:48	70284	3.57
^{18}F-labeled BSA	**20:34**	**25:06**	**21:38**	**1755688**	**89.15**
Region 3	27:44	28:52	28:07	68498	3.48
Region 4	29:07	30:44	29:48	74831	3.80
4 Peaks				1969301	100.00

A) Radiochemical purity of **^{18}F-labeled BSA** at the end of synthesis B) Radiochemical purity of **^{18}F-labeled BSA** at 6-hours post synthesis

References

1. Donnelly D.J., Smith R.A., Morin P., Lipovsek D., Gokemeijer J., Cohen D., Lafont V., Tran T., Cole E.L., Wright M., Kim J., Pena A., Kukral D., Dischino D.D., Chow P., Gan J., Adelakun O., Wang X.-T., Cao K., Leung D., Bonacorsi S.J. Jr., Hayes W. (2018) Synthesis and Biologic Evaluation of a Novel ^{18}F-Labeled Adnectin as a PET Radioligand for Imaging PD-L1 Expression. J Nucl Med 59, 529–535.
2. Hausner S.H., DiCara D., Marik J., Marshall J.F., Sutcliffe J.L. (2007) Use of a Peptide Derived from Foot-and-Mouth Disease Virus for the Noninvasive Imaging of Human Cancer: Generation and Evaluation of 4-[^{18}F]Fluorobenzoyl A20FMDV2 for In vivo

Imaging of Integrin $\alpha_v\beta_6$ Expression with Positron Emission Tomography. *Cancer Res 67*, 7833-7840.