Electronic Supplementary Information (ESI)

Rapid One-Pot Iterative Diselenide-Selenoester Ligation using a Novel Coumarin-Based Photolabile Protecting Group

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Table of Contents

Methods and Materials

^{1}H Nuclear Magnetic Resonance (NMR) spectra were recorded at 300 K using a Bruker Avance DPX500, DPX400 or DPX300 at a frequency of 500, 400 and 300 MHz, respectively. Chemical shifts are reported in parts per million (ppm) and referenced to solvent residual signals: $CHCl_3$ (δ = 7.26 ppm) or MeOH (δ = 3.46 ppm). ^{1}H NMR data is reported as chemical shift (δ), relative integral, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, t = triplet, dt = doublet of triplets, q = quartet, m = multiplet), coupling constant, *J* in hertz (Hz) and with proton assignments where possible. ^{1}H NMR assignments for **1** and **4** were made in conjunction with Correlation Spectroscopy (COSY), selective Nuclear Overhauser Effect Spectroscopy (NOESY), Heteronuclear Single-Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Coherence (HMBC) experiments. All 2D NMR experiments were carried out using a Bruker Avance DPX500 or DPX400 spectrometer. ^{13}C NMR shifts are reported in ppm and are referenced to solvent resonance: $CHCl_3$ (δ = 77.2 ppm) or MeOH (δ = 49.8 ppm).

Thin layer chromatography was performed using Merck Kieselgel 60 F254 pre-coated aluminium sheets, with compounds visualized by UV light at λ = 254 nm or λ = 365 nm or *via* staining with phosphomolybdic acid (PMA).

Low-Resolution Electrospray Ionization Mass Spectrometry (LRMS) was performed on a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode. The total ion count was integrated over the entire UV peak. High-Resolution Electrospray Ionization Mass Spectrometry (HRMS) was performed on a Bruker–Daltonics Apex Ultra 7.0T Fourier transform mass spectrometer (FTICR). MALDI-TOF mass spectra were measured on a Bruker autoflex speed MALDI-TOF instrument using a matrix of sinapinic acid in water/acetonitrile (7:3 v/v) containing 0.1 vol. % TFA in linear mode. Infrared (IR) absorption spectra were recorded on a Bruker ALPHA Spectrometer with Attenuated Total Reflection (ATR) capability, using OPUS 6.5 software. Optical rotations of enantioenriched compounds were recorded on a Perkin-Elmer 341 polarimeter at 589 nm (sodium D line) with a cell path length of 1 dm, and the concentrations were reported in g/100 mL.

A Waters Acquity system was used for analytical HPLC using an XBridge^{TM} BEH C18, 300 Å 5 μm 4.6 mm x 250 mm column for purified peptides: **9, 17, 20, 21, 22** as well as monitoring of ligation reactions to generate **9** and **17;** XBridge^{TM} BEH C18, 130 Å 5 μm 4.6 mm x 150

mm column for purified peptides: **7, 8, 12, 13, 14, S3;** and Symmetry^{TM} C4, 300 Å 5 μm 2.1 mm x 150 mm column for purified protein **19** as well as monitoring of ligation reactions to generate **19**. The flow rate was 1.0 mL min^{-1} and operated using a mobile phase composed water with 0.1 vol.% TFA (solvent A) and acetonitrile with 0.1 vol.% TFA (solvent B). The analysis of the chromatograms was conducted using Empower 3 Pro software (2010).

UPLC-MS was performed on a Shimadzu LC-MS 2020 system equipped with a Nexera X2 LC-30AD pump and a Nexera X2 SPD-M30A diode array detector coupled to a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode. Peptides were analyzed using an Acquity UPLC BEH 1.7 μm (C18) 2.1 x 50 mm column at a flow rate of 0.6 mL min^{-1} using a mobile phase of water with 0.1 vol. % TFA and acetonitrile with 0.1 vol. % TFA.

Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable Wavelength Detector operating at 210 and 280 nm. All peptide fragments were purified using a Waters Sunfire 5 μm (C18; 19 x 150 mm) preparative column operating at a flow rate of 14 mL min^{-1} using a mobile phase of solvent A and solvent B with a linear gradient as specified. Peptide ligation-deselenization products were purified on a Waters Sunfire 5 μm C18 semi-preparative column operating at a flow rate of 4 mL min^{-1} using a mobile phase of solvent A and solvent B with a linear gradient as specified.

A Rayonet RPR-100 photoreactor (Rayonet) was used at λ = 254 nm, (35 W) and a PennOC Photoreactor M1 (PennOC) was used at λ = 450 nm or λ = 365 nm (36 W).

Reaction yields are calculated based on the amount of isolated product relative to the theoretical reaction yield. Reported yields are not adjusted to account for the removal of aliquots for reaction monitoring (e.g. UPLC-MS, measuring pH).

Materials: Reagents were used as received unless otherwise noted. Resins, coupling reagents, and amino acids were all obtained from GL Biochem, Mimotopes, or Novabiochem. *N*,*N*-dimethylformamide (DMF) was obtained as peptide synthesis grade from Merck or Labscan. All Gdn•HCl used to prepare buffers was dried *in vacuo*.

Handling of light-sensitive compounds: Compounds that contained or were precursors to the DEAMC group were deemed light sensitive. When these species were undergoing reactions, work-up, purification or being stored, they were always covered in aluminium foil (except during ligations).

Resins

Loading 2-chlorotrityl chloride resin: 2-chlorotrityl chloride resin (1.22 mmol/g loading) was swollen in DCM for 30 min. A solution of Fmoc-AA-OH (3 eq. relative to maximum loading) and DIPEA (15 eq.) in DCM (0.3 M of Fmoc-AA-OH) was added to the resin and agitated for 16 h at rt. The loading solution was drained and the resin was rinsed with DMF (5 x 3 mL), DCM (5 x 3 mL), and DMF (5 x 3 mL). The resin was treated with a capping solution of DCM/MeOH/DIPEA (17:2:1 v/v/v, 4 mL) for 1 h then washed with DMF (5 x 3 mL), DCM (5 x 3 mL), and DMF (5 x 3 mL).

Side chain loading 2-chlorotrityl chloride resin: 2-Chlorotrityl chloride resin (1.22 mmol/g loading) was swollen in DCM for 30 min. A solution of Fmoc-Ser(OH)-OAllyl (3 eq. relative to maximum loading) and DIPEA (15 eq.) in DCM (0.3 M of Fmoc-Ser(OH)-OAllyl) was added and the resin was shaken at rt for 96 h. The loading solution was drained and the resin rinsed with DMF (5 x 3 mL), DCM (5 x 3 mL), and DMF (5 x 3 mL). The resin was treated with a solution of DCM/MeOH/DIPEA (17:2:1 v/v/v, 4 mL) for 1 h and the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL), and DMF (5 × 3 mL).

Rink Amide resin loading: Rink amide resin (0.6 mmol/g) was swollen in DCM for 30 min then washed with DMF (5 x 3 mL). The Fmoc group was removed using 20 vol.% piperidine in DMF (2 x 5 mL, 3 min) followed by washing with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL). DIC (4 eq.) and Oxyma (4.4 eq.) were added to Fmoc-AA-OH (4 eq.) in DMF (0.125 M relative to Fmoc-AA-OH) and the mixture was added to the resin at room temperature with agitation for 2 h. The resin was washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL) then capped with Ac_2O (0.3 M) and DIPEA (0.3 M) in DMF (5 mL) for 5 min. The resin was then washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL).

Quantification of resin loading: A measured quantity of approximately 10 mg of the loaded resin was treated with a solution of 2 vol. % 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF (2 mL) and agitated for 30 min. The solution was removed from resin and diluted to 10 mL with acetonitrile. A 1 mL aliquot of this resultant solution was taken up and diluted to 12.5 mL with acetonitrile. The absorbance of the DBU-fulvene adduct (λ = 304 nm, ε = 9254 M^{-1} cm^{-1}) was measured to estimate the resin loading.

Fmoc-SPPS

General method using Symphony Automated Synthesizer: Automated Fmoc-SPPS was performed on a Gyros Protein Technologies Symphony automated synthesizer on a 100 µmol scale. Solutions of Fmoc-AA-OH (0.3 M) and Oxyma (0.33 M) in DMF, and DIC (0.3 M) in DMF were prepared for coupling. General synthetic protocols performed were:

Fmoc deprotection: The resin-bound peptide was shaken in a solution of piperidine in DMF (20 vol. %, 2 mL, 2 x 3 min). The deprotection solution was then drained and the resin was washed with DMF (4 x 4 mL).

Coupling: The resin-bound peptide was shaken in a solution of Fmoc-amino acid (8 eq., 0.1 M), Oxyma (8.8 eq., 0.11 M) and DIC (8 eq., 0.1 M) in DMF for 45 min at rt. The coupling solution was drained, and the resin was washed with DMF (6 mL, 4 x 30 s).

Capping: The resin-bound peptide was shaken in a 0.3 M solution of Ac_2O and DIPEA in DMF (2 mL, 3 min). The capping solution was then drained, and the resin was washed with DMF (4 mL, 5 x 30 s).

General method using SYRO I Automated Synthesizer: Heated automated Fmoc-SPPS was performed on a Biotage SYRO I automated synthesizer on a 50 µmol scale. Solutions of Fmoc-AA-OH (0.5 M) and Oxyma (0.55 M) in DMF, and DIC (0.5 M) in DMF were prepared for coupling. General synthetic protocols performed were:

Fmoc deprotection: The resin-bound peptide was shaken in a solution of 40 vol. % piperidine in DMF (800 µL, 4 min). The deprotection solution was then drained and the resin was treated again with a solution of 20 vol. % piperidine in DMF (800 µL, 4 min). The deprotection solution was then drained and the resin was washed with DMF (4 x 1 mL).

Coupling: The resin-bound peptide was shaken in a solution of Fmoc-AA-OH (4 eq., 0.17 M), Oxyma (4.4 eq., 0.18 M) and DIC (4 eq., 0.17 M) in DMF for 30 min at 50 °C or 1 h at rt. The coupling solution was drained, and the resin was washed with DMF (4 x 800 µL).

Capping: The resin-bound peptide was shaken in a solution of 5 vol. % Ac_2O and 10 vol. % DIPEA in DMF (800 µL, 6 min). The capping solution was then drained, and the resin was washed with DMF (4 x 800 µL).

Coupling of Specialised Amino Acids

The resin-bound peptide was shaken in a solution of the specialised amino acid (1.1 eq., 0.1 M), Oxyma (1.1 eq., 0.11 M) and DIC (1.1 eq., 0.1 M) in DMF at rt for times as specified in the experimental. The coupling solution was drained and the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL), and DMF (5 × 3 mL).

Cleavage of peptides from resin

Cleavage from resin with side-chain protecting groups intact: The fully extended resin-bound peptide was treated with 1,1,1,3,3,3-hexafluoro*iso*propanol (HFIP)/DCM (30% v/v, 5 mL for 50 µmol scale) and agitated at rt for 1 h. The resin was filtered and the treatment was repeated. The resin was finally washed with DCM (5 x 3 mL) and the combined filtrates were concentrated *in vacuo*.

Cleavage from resin along with deprotection of side-chain protecting groups: A mixture of trifluoroacetic acid (TFA), tri*iso*propylsilane (TIS) and water (18:1:1 v/v/v, 5 mL) was added to the resin-bound peptide (50 µmol) and agitated at rt for 2 h. The resin was then filtered and washed with TFA (2 x 3 mL) and the combined filtrates were concentrated under nitrogen flow. Diethyl ether (40 mL) was added and the suspension cooled to 0 °C for 10 min. The precipitate was pelleted by centrifugation at 6800 rcf for 10 min at 0 °C and the supernatant decanted.

General procedure for selenoesterification

Solution phase method: The cleavage of fully extended peptide from resin was performed using HFIP as described in the general procedure above. The crude side chain protected peptide was dissolved in dry DMF (20 mM) and cooled to 0 °C. The resulting solution was treated with diphenyldiselenide (DPDS, 30 eq.) followed by dropwise addition of tri-*n*-butylphosphine (Bu_3P, 30 eq.). The mixture was stirred for 3 h at 0 °C under an argon atmosphere before being warmed to rt and concentrated under nitrogen flow.

Side-chain anchored method: Fully extended resin-bound peptide was swollen in dry DCM (4 mL) for 30 min. For a 25 µmol scale, a solution of $Pd(PPh_3)_4$ (25 mg, 22 µmol, 0.9 eq.) and phenylsilane (123 µL, 1 mmol, 40 eq.) in dry DCM (2 mL) was added to the resin and agitated for 1 h at rt. The procedure was repeated once more and the resin was washed with DCM (5 x 3 mL), DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL). A solution of DPDS (50 eq.)

and Bu_3P (50 eq.) in DMF (4 mL) was added to the resin and agitated for 5 h at rt. The resin was then washed with DMF (5 x 3 mL) and dried with DCM (5 x 3 mL) before being subjected to acidolytic cleavage.[1]

Synthesis of Boc-Sec(DEAMC)-OH (1)

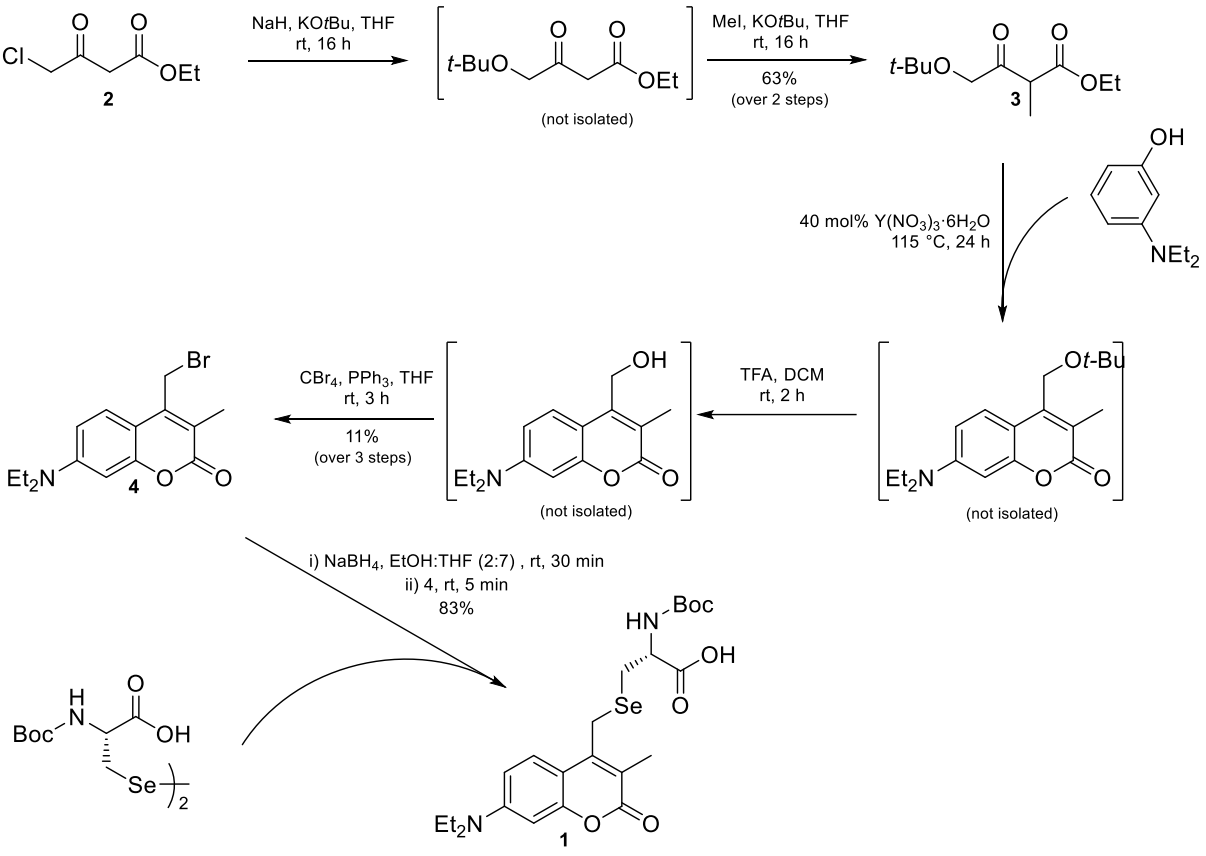
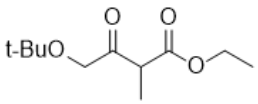


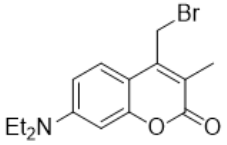
Figure S1. Synthesis of Boc-Sec(DEAMC)-OH building block **1**.

Ethyl 4-(*tert*-butoxy)-2-methyl-3-oxobutanoate (3)



Ethyl 4-chloroacetoacetate **2** (16.4 mL, 120.6 mmol, 1 eq.) was added dropwise (20 mL/h) to a stirred suspension of 60% NaH (7.4 g, 182 mmol, 1.5 eq.) and KO*t*Bu (19.6 g, 157 mmol, 1.3 eq.) in dry THF (160 mL). The resultant orange suspension was stirred at rt for 16 h, after which time TLC analysis (8:1 v/v hexanes:EtOAc) demonstrated complete consumption of the starting material. The reaction mixture was cooled to 0 °C and quenched with 1 M HCl (added dropwise with vigorous stirring) before concentration *in vacuo*. The crude product was dissolved in EtOAc (600 mL) and washed with saturated $NaHCO_3$ solution (2 x 200 mL) and brine (200 mL), then dried over Na_2SO_4 and concentrated *in vacuo* to afford a yellow liquid. The crude product was resuspended in dry THF (600 mL), and KO*t*Bu (16.2 g, 145 mmol, 1.2 eq.) was added portionwise at 0 °C. The thick yellow solution was stirred vigorously and iodomethane (9.0 mL, 144.7 mmol, 1.2 eq.) in dry THF (120 mL) was added dropwise over 1 h. The reaction was warmed to rt and stirred for 16 h. The reaction was then cooled to 0 °C, acidified with 1 M HCl, concentrated *in vacuo* and diluted with brine (200 mL). The aqueous phase was extracted with EtOAc (3 x 150 mL), and the combined organic extracts were dried over Na_2SO_4 then concentrated *in vacuo*. The resulting yellow liquid was purified by column chromatography (20:1 v/v hexanes:EtOAc) to yield **3** as a colourless liquid (15.5 g, 75.8 mmol, 63%). The characterization of **3** is consistent with published work.[2] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.14 (q, *J* = 7.2 Hz, 2H, OC$\underline{H_2}$$CH_3$), 4.06 – 3.99 (m, 2H, OCH_2CO), 3.74 (q, *J* = 7.2 Hz, 1H, C$\underline{H}$$CH_3$), 1.31 (d, *J* = 7.2 Hz, 3H, CHC$\underline{H_3}$), 1.24 (t, *J* = 7.2 Hz, 3H, $OCH_2C\underline{H_3}$), 1.18 (s, 9H, $C(CH_3)_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 206.2, 171.0, 74.5, 67.7, 61.4, 49.4, 27.5, 22.1, 14.4, 12.5.

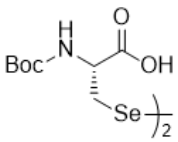
4-(bromomethyl)-7-(diethylamino)-3-methyl-2H-chromen-2-one (4)



To a mixture of diethylaminophenol (2.3 g, 13.9 mmol, 1 eq.) and $Y(NO_3)_3 \cdot 6H_2O$ (2.0 g, 5.24 mmol, 0.4 eq.) was added ethyl 4-(*tert*-butoxy)-2-methyl-3-oxobutanoate **3** (4.0 g, 18.5 mmol, 1.3 eq.). The reaction mixture was then heated to 115 °C using a distillation apparatus to

remove ethanol as the reaction proceeded. After 24 h the reaction mixture was cooled to rt and solvated in MeOH. The resulting solution was dry loaded onto celite and eluted with DCM. The crude reaction mixture was then again dry loaded onto celite and enriched using silica column chromatography (DCM with 0.1 vol.% Et_3N, then 2 vol. % MeOH in DCM with 0.1 vol. % Et_3N) to remove unreacted diethylaminophenol that proved difficult to handle in subsequent steps. This afforded DEAMC-O*t*Bu as a crimson oil that was used directly in the next step. To the crude intermediate (2.2 g, 6.93 mmol, 1 eq.), DCM (100 mL) and TFA (100 mL) was added at 0 °C. This reaction mixture was stirred for 2 h at rt before concentration under nitrogen and subsequent co-evaporation with toluene *in vacuo* which was used without further purification. Triphenylphosphine (2.75 g, 10.4 mmol, 1.5 eq.) and CBr_4 (3.50 g, 10.4 mmol, 1.5 eq.) was added to a solution of the crude in dry THF (100 mL). The reaction was stirred for 3 h at rt. Upon completion, the reaction mixture was dry loaded onto celite and purified using silica column chromatography (hexanes, then 20 vol. % EtOAc in hexanes) affording the desired bromide **4** as an orange oil (0.48 g, 1.5 mmol, 11% over 3 steps). **IR:** (ν cm^{-1}) = 2971, 2929, 1700, 1619, 1597. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 9.0 Hz, 1H, ArH), 6.62 (dd, *J* = 9.0, 2.6 Hz, 1H, ArH'), 6.49 (d, *J* = 2.6 Hz, 1H, ArH''), 4.47 – 4.45 (m, 2H, $BrCH_2Ar$), 3.40 (q, *J* = 7.1 Hz, 4H, 2 x $CH_3C\underline{H_2}N$), 2.18 (s, 3H, $ArCH_3$), 1.19 (t, *J* = 7.1 Hz, 6H, 2 x $C\underline{H_3}CH_2N$). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 163.2, 155.2, 150.3, 144.9, 125.0, 117.5, 109.1, 107.0, 98.1, 45.0, 24.1, 12.8, 12.7. **LRMS** (ESI^+) m/z: 324.0 ($[M+H]^+$, 100%); **HRMS** (ESI^+) m/z: calculated for $C_{15}H_{19}Br_1N_1O_2$ [$(M+H)^+$, 100%]: 324.0594, found 324.0591.

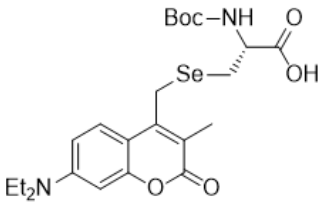
Boc-L-selenocystine (Boc-Sec-OH)$_2$



To L-selenocystine (1.00 g, 2.98 mmol, 1.0 eq.) in H_2O (15 mL) was added Et_3N (1.25 mL, 9.95 mmol, 3.0 eq.). The reaction mixture was cooled to 0 °C followed by the portion-wise addition of Boc_2O (1.95 g, 9.95 mmol, 3.0 eq.) over 5 min with vigorous stirring. The reaction was warmed to rt and after 16 h was diluted with 0.5 M HCl on ice. The product was extracted with EtOAc (3 x 50 mL) and the combined organic extracts dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash silica column chromatography (5 vol. % MeOH in DCM with 0.1 vol. % AcOH) afforded (Boc-Sec-OH)$_2$ as a yellow solid (1.20 g, 2.25 mmol, 75%). $[\alpha]_D^{25}$ = -96 ° (c. 0.17, MeOH). **^{1}H NMR** (300 MHz, MeOD) δ 4.53 (dd, *J* = 8.9, 4.8 Hz,

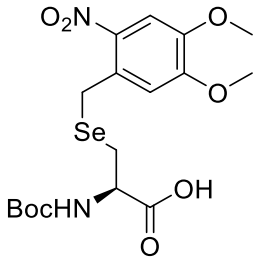
1H, NHC*H*COOH), 3.57 (dd, *J* = 12.6, 4.8 Hz, 1H, C*H*$_A$H$_B$Se), 3.33 (dd, *J* = 12.6, 8.8 Hz, 1H, CH$_A$*H*$_B$Se), 1.57 (s, 9H, C(CH$_3$)$_3$).

Boc-Sec(DEAMC)-OH (1)



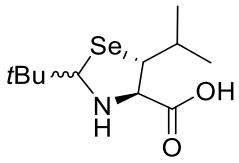
(Boc-Sec-OH)$_2$ (0.48 g, 0.89 mmol, 0.6 eq.) was dissolved in a degassed mixture of EtOH (3.5 mL) and dry THF (12 mL) and the resulting solution was treated with $NaBH_4$ (100 mg, 2.7 mmol, 1.8 eq.) at 0 °C. The reaction was then allowed to procced for 30 min at rt. This mixture was transferred in one portion to bromide **4** (0.48 g, 1.5 mmol, 1 eq.) and stirred for 5 min. The reaction mixture was quenched with deionised water (50 mL), extracted with DCM (50 mL), followed by acidification with 1 M HCl. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by silica column chromatography (DCM with 0.1 vol. % AcOH, then 4 vol. % MeOH in DCM with 0.1 vol. % AcOH) afforded Boc-Sec(DEAMC)-OH **1** as a yellow solid (0.63 g, 1.2 mmol, 83%). $[\alpha]_D^{25}$ = -12 ° (c. 0.21, MeOH). **IR:** (ν cm^{-1}) = 3340, 2973, 2930, 1690, 1614, 1592. **^{1}H NMR** (500 MHz, $CDCl_3$:MeOD (19:1)) δ 7.42 (d, *J* = 9.3 Hz, 1H, ArH), 6.58 (dd, *J* = 9.3, 2.5 Hz, 1H, ArH'), 6.45 (d, *J* = 2.5 Hz, 1H, ArH''), 5.52 (d, *J* = 8.0 Hz, 1H, HN), 4.68 – 4.60 (m, 1H, αH), 3.96 – 3.89 (m, 2H, SeCH_2Ar), 3.37 (q, *J* = 7.1 Hz, 4H, 2 x CH_3C*H*$_2$N), 3.24 (dd, *J* = 13.7, 4.8 Hz, 1H, β_aH), 3.12 (dd, *J* = 13.0, 5.3 Hz, 1H, β_bH), 2.13 (s, 3H, ArCH_3), 1.43 (s, 9H, C(CH_3)$_3$), 1.17 (t, *J* = 7.0 Hz, 6H, 2 x C*H*$_3$CH$_2$N). **^{13}C NMR** (126 MHz, $CDCl_3$:MeOD (19:1)) δ 173.4, 163.7, 155.7, 155.0, 150.2, 147.6, 125.6, 115.9, 109.1, 108.0, 97.9, 80.7, 53.7, 45.0, 28.6, 28.2, 20.6, 13.1, 12.8. **LRMS** (ESI$^+$) m/z: 513.1 ([M+H]$^+$, 100%); **HRMS** (ESI$^+$) m/z: calculated for $C_{23}H_{33}N_2O_6Se_1$ [(M+H)$^+$, 100%]: 513.1499, found 513.1494.

Boc-Sec(oNv)-OH (S1)



Boc-Sec(oNv)-OH **S1** was synthesized using a previously published synthetic protocol.[3] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.67 (s, 1H, ArH), 6.82 (s, 1H, ArH), 5.38 (s, 1H, NH), 4.60 (s, 1H, CH), 4.20 (d, *J* = 11.8 Hz, 1H, BnCH_AH$_B$Se), 4.11 (d, *J* = 11.9 Hz, 1H, BnCH$_A$$H_B$Se), 3.99 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.12 – 2.96 (m, 2H, $SeCH_2$), 1.45 (s, 9H, $C(CH_3)_3$).

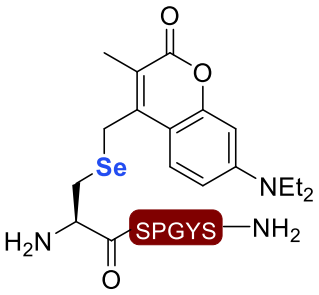
Sez(*t*Bu)-Leu-OH (S2)



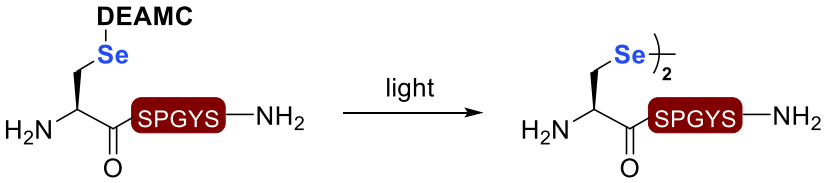
Sez(*t*Bu)-Leu-OH **S2** was synthesized using a previously published synthetic protocol to afford two diastereoisomers.[4] **^{1}H NMR** (500 MHz, MeOD) δ 5.05 (s, 1H, NHC*H*Se, minor), 4.78 (s, 1H, NHC*H*Se, major), 4.26 (d, *J* = 2.9 Hz, 1H, αH, minor), 3.88 (dd, *J* = 8.7, 2.8 Hz, 1H, βH, minor), 3.76 (dd, *J* = 9.2, 4.3 Hz, 1H, βH, major), 3.55 (d, *J* = 9.1 Hz, 1H, αH, major), 2.14 – 2.00 (m, 1H, C*H*$(CH_3)_2$, major), 1.85 – 1.72 (m, 1H, C*H*$(CH_3)_2$, minor), 1.12 (s, 9H, $C(CH_3)_3$, major), 1.11 (s, 9H, $C(CH_3)_3$, minor), 1.09 (d, *J* = 6.6 Hz, 3H, CH(C*H*$_3)_2$, minor), 1.09 (d, *J* = 6.6 Hz, 3H, CH(C*H*$_3)_2$, major), 1.06 (d, *J* = 6.6 Hz, 3H, CH(C*H*$_3)_2$, minor), 1.03 (d, *J* = 6.7 Hz, 3H, CH(C*H*$_3)_2$, major). **^{13}C NMR** (126 MHz, MeOD) δ 175.6 (major), 175.3 (minor), 78.9 (major), 78.4 (minor), 71.2 (major), 71.0 (minor), 64.6 (major), 60.8 (minor), 36.5 (minor), 36.3 (major), 35.1 (minor), 32.7 (major), 28.7 (major), 26.2 (major), 23.4 (minor), 22.8 (minor), 19.5 (major).

Synthesis of Peptide fragments

Synthesis of model peptide U(DEAMC)SPGYS (7) and photocleavage to USPGYS (8)



Fmoc-Ser(*t*Bu)-OH was loaded onto Rink-amide resin in accordance with the general methods. The loaded resin (50 µmol) was subjected to manual Fmoc SPPS as outlined in the general methods (see Symphony automated synthesizer section). Boc-Sec(DEAMC)-OH **1** was coupled for 3 h at rt using conditions described in the specialised amino acid coupling section in the general methods. The model peptide was then cleaved using a solution of TFA:TIS:H_2O as described in the general methods. Purification *via* preparative HPLC (0 to 40% B over 60 min, 0.1% TFA) followed by lyophilization afforded peptide **7** as a yellow solid (31 mg, 69% yield based on resin loading).



Photocleavage: The model peptide **7** (2.7 mg, 3.0 µmol, 5.0 mM) was dissolved in ligation buffer comprising of 6 M Gdn•HCl and 0.1 M Na_2HPO_4 at pH 6.0 (600 µL). The solution was separated into 2 equal aliquots. One aliquot was irradiated with 450 nm light (PennOC, 36 W) for a duration of 10 min and was analysed *via* UPLC-MS and analytical HPLC and converted cleanly to peptide **8** over this time. The other aliquot was left to stand in a clear Eppendorf tube (exposed to ambient light) for a duration of 24 hrs. Analysis *via* UPLC-MS showed no degradation.

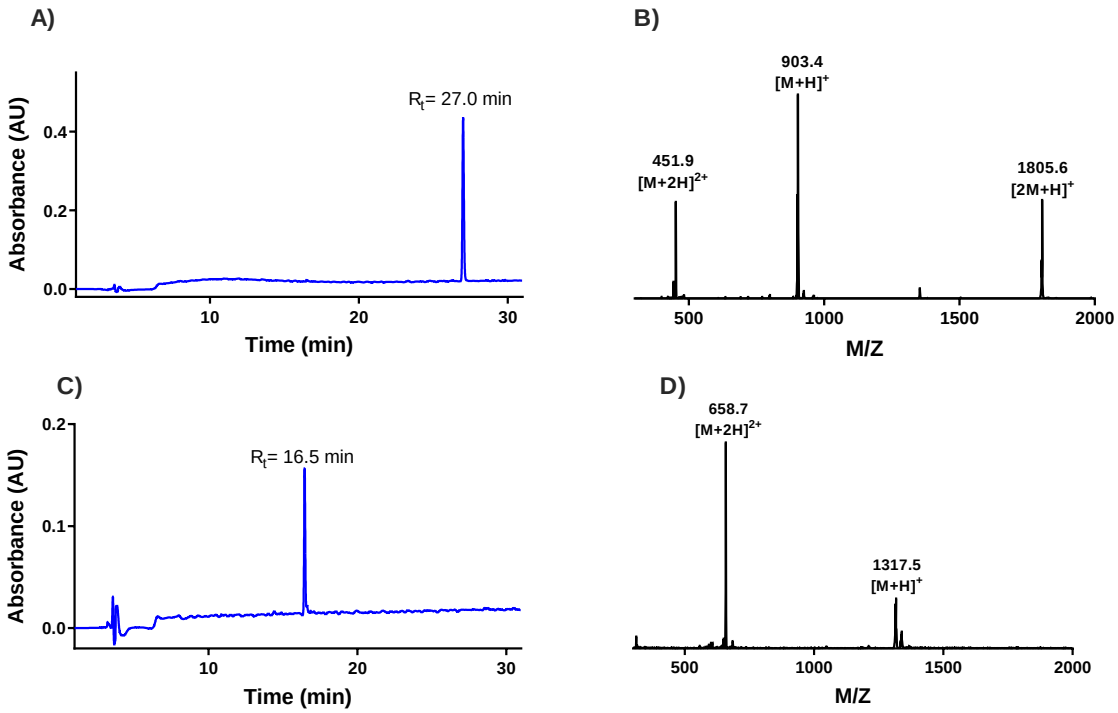
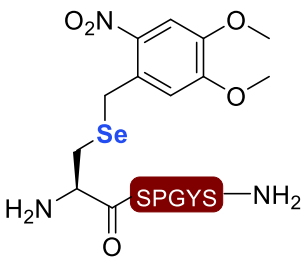


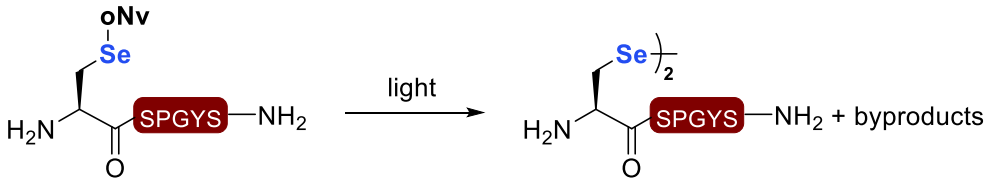
Figure S2. (A) Analytical HPLC, R_t 27.0 min (0-30% B over 30 min, λ = 214 nm), **(B)** ESI-MS of pure DEAMC protected peptide **7**; Calculated Mass: 1804.79 $[2M+H]^+$, 902.89 $[M+H]^+$, 451.95 $[M+2H]^{2+}$; Mass found (ESI^+): 1805.6 $[2M+H]^+$, 903.4 $[M+H]^+$, 451.9 $[M+2H]^{2+}$, **(C)** Crude Analytical HPLC, R_t 16.5 min (0-30% B over 30 min, λ = 214 nm), **(D)** ESI-MS of crude DEAMC deprotected peptide **8**; Calculated Mass: 1316.15 $[M+H]^+$, 658.58 $[M+2H]^{2+}$; Mass found (ESI^+): 1317.5 $[M+H]^+$, 658.7 $[M+2H]^{2+}$.

Synthesis of model peptide U(oNv)SPGYS (S3) and photocleavage to USPGYS (8)



Fmoc-Ser(*t*Bu)-OH was loaded onto Rink-amide resin in accordance with the general methods. The loaded resin (25 µmol) was subjected to manual Fmoc SPPS as outlined in the general methods (see Symphony automated synthesizer section). Boc-Sec(oNv)-OH **S1** was coupled for 3 h at rt using conditions described in the specialised amino acid coupling section in the general methods. The model peptide was then cleaved using a solution of TFA:TIS:H_2O as

described in the general methods. Purification *via* preparative HPLC (0 to 40% B over 60 min, 0.1% TFA) followed by lyophilization afforded peptide **S3** as a yellow solid (12.0 mg, 56% yield based on resin loading).



Photocleavage: Model peptide **S3** (1.0 mg, 1.2 µmol, 5.0 mM) was dissolved in ligation buffer comprising of 6 M Gdn•HCl, 0.1 M Na_2HPO_4 at pH 6.0 (234 µL). The solution was irradiated with 365 nm light (PennOC, 36 W) for a duration of 30 min. Analysis *via* UPLC-MS and analytical HPLC showed conversion to peptide **8** together with a series of byproducts.

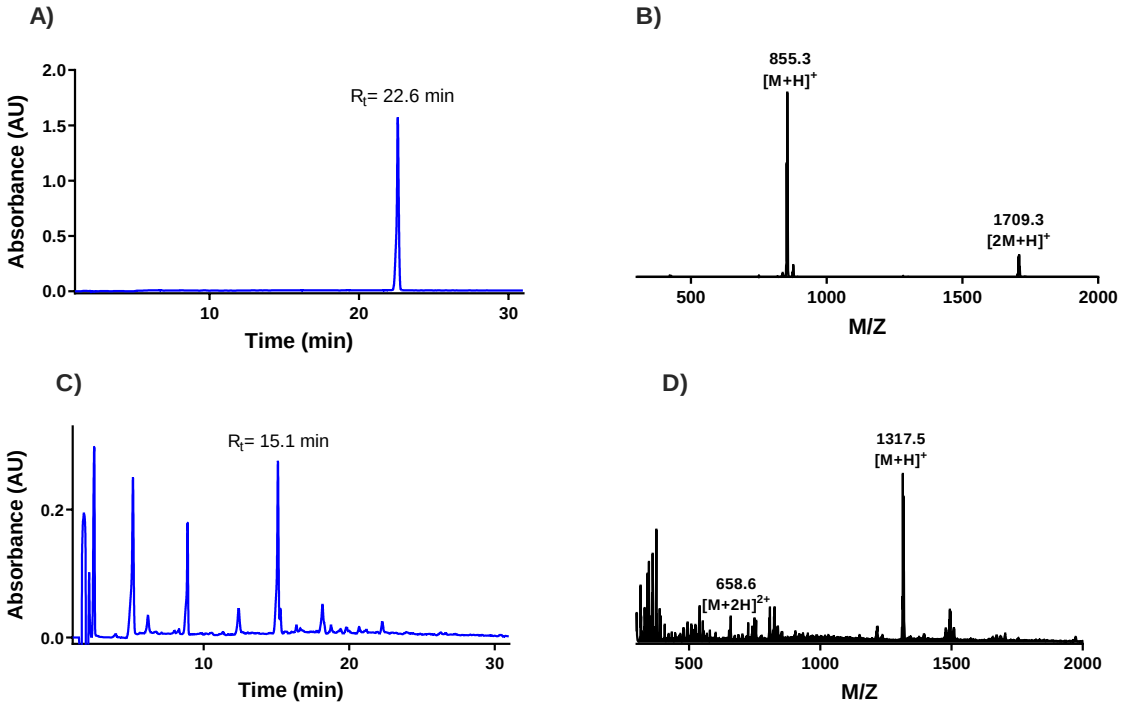


Figure S3. (A) Analytical HPLC, R_t 22.6 min (0-30% B over 30 min, λ = 214 nm), **(B)** ESI-MS of pure oNv protected peptide **S3**; Calculated Mass: 1708.53 $[2M+H]^+$, 854.77 $[M+H]^+$; Mass found (ESI^+): 1709.3 $[2M+H]^+$, 855.3 $[M+H]^+$, **(C)** Crude Analytical HPLC, R_t 15.1 min (0-30% B over 30 min, λ = 214 nm), **(D)** ESI-MS from 1-31 min for the crude photodeprotection reaction; Calculated Mass: 1316.15 $[M+H]^+$, 658.58 $[M+2H]^{2+}$; Mass found (ESI^+): 1317.5 $[M+H]^+$, 658.6 $[M+2H]^{2+}$.

Synthesis of MUC1 N-terminal selenoester (14)

Fmoc-Ser(*t*Bu)-OH was loaded onto 2-CTC resin in accordance with the general methods. The loaded resin (100 µmol) was subjected to Fmoc SPPS using a Gyros Protein Technologies Symphony automated synthesizer as outlined in the general methods (where the light blue coloured residues represent double couplings). The side-chain protected peptide was cleaved from resin using 30 vol. % HFIP in DCM, and subjected to solution phase selenoesterification followed by acidolytic cleavage as described in general methods. Purification *via* preparative reverse-phase HPLC (0 to 60% B over 60 min, 0.1 vol. % TFA) followed by lyophilization afforded peptide **14** as a white solid (25 mg, 12% yield based on the initial resin loading).

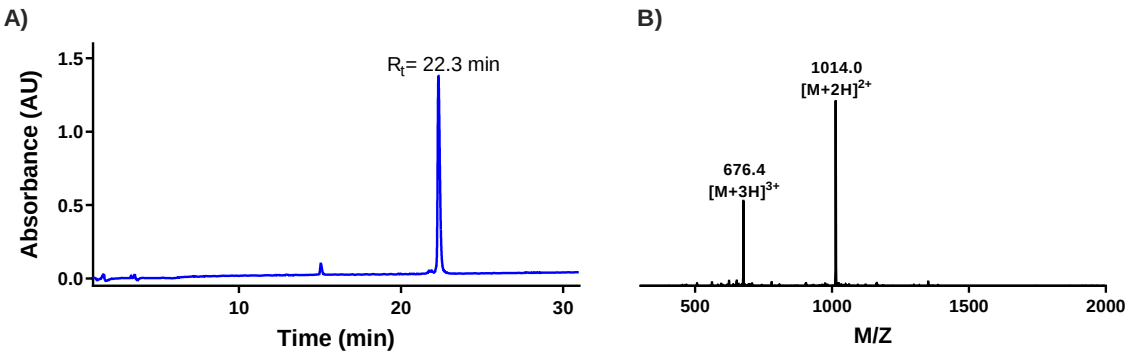


Figure S4. (A) Analytical HPLC, R_t 22.3 min (0-40% B over 30 min, λ = 214 nm), **(B)** ESI-MS of pure selenoester fragment **14**; Calculated Mass: 1014.1 $[M+2H]^{2+}$, 676.4 $[M+3H]^{3+}$; Mass found (ESI^+): 1014.0 $[M+2H]^{2+}$, 676.4 $[M+3H]^{3+}$.

Synthesis of MUC1 bifunctional peptide selenoester (12)

Fmoc-Ser(OH)-OAllyl was loaded onto 2-CTC resin through the side chain alcohol in accordance with the general methods for side-chain anchoring. The loaded resin (25 µmol) was subjected to Fmoc SPPS on a Gyros Protein Technologies Symphony automated synthesizer

as outlined in the general methods (where the light blue coloured residues represent double couplings). The peptide was subjected to allyl deprotection and selenoesterification on resin at room temperature as outlined in the general methods section. Finally, Boc-Sec(DEAMC)-OH **1** was coupled at the N-terminus for 3 h at rt using the specialised amino acid coupling conditions described in the general methods. The peptide selenoester was cleaved from resin as outlined in the general methods and purified by preparative reverse-phase HPLC (0 to 60% B over 60 min, λ = 214 nm) and lyophilized to afford the desired bifunctional peptide **12** as a yellow solid (5 mg, 9% yield).

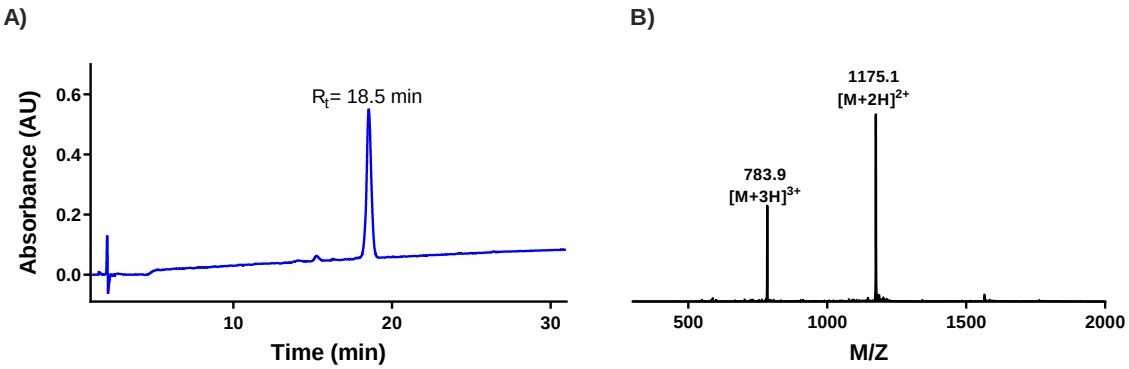


Figure S5. (A) Analytical HPLC, R_t 18.5 min (0-60% B over 30 min, λ = 214 nm), **(B)** ESI-MS of pure bifunctional peptide fragment **12**; Calculated Mass: 1175.2 $[M+2H]^{2+}$, 783.8 $[M+3H]^{3+}$; Mass found (ESI^+): 1175.1 $[M+2H]^{2+}$, 783.9 $[M+3H]^{3+}$.

Synthesis of MUC1 C-terminal diselenide dimer (13)

Fmoc-Ser(*t*Bu)-OH was loaded onto 2-CTC resin in accordance with the general methods. The loaded resin (100 µmol) was subjected to Fmoc SPPS on a Gyros Protein Technologies Symphony automated synthesizer as outlined in the general methods (where the light blue coloured residues represent double couplings). Finally, $(Boc\text{-}Sec\text{-}OH)_2$ was coupled for 16 h at rt in accordance to specialised amino acid coupling conditions in the general methods. Acidolytic cleavage and purification *via* preparative HPLC (0 to 60% B over 60 min, 0.1% TFA) followed by lyophilization afforded peptide diselenide **13** as a white solid (35 mg, 18% yield based on resin loading).

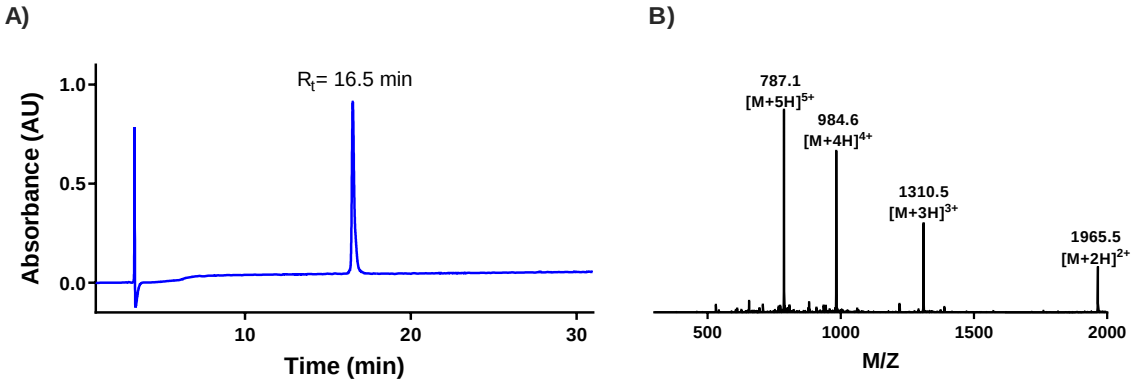


Figure S6. (A) Analytical HPLC, R_t 16.5 min (0-40% B over 30 min, λ = 214 nm), **(B)** ESI-MS of pure peptide diselenide fragment **13**; Calculated Mass: 1966.0 $[M+2H]^{2+}$, 1311.0 $[M+3H]^{3+}$, 986.0 $[M+4H]^{4+}$, 787.0 $[M+5H]^{5+}$; Mass found (ESI^+): 1965.5 $[M+2H]^{2+}$, 1310.5 $[M+3H]^{3+}$, 984.6 $[M+4H]^{4+}$, 787.1 $[M+5H]^{5+}$.

Synthesis of ApoCIII N-terminal peptide selenoester (22)

Fmoc-Thr(*t*Bu)-OH was loaded onto 2-CTC resin in accordance with the general methods. The loaded resin (50 µmol) was subjected to Fmoc SPPS on a Biotage SYRO I automated synthesizer (50 °C couplings as outlined in the general methods) to yield the extended resin-bound peptide (where the light blue coloured residues represent double couplings). The peptide was cleaved from resin using 30 vol. % HFIP in DCM, selenoesterified and subjected to acidolytic cleavage. Reverse-phase HPLC purification (0 to 60% B over 65 min, 0.1 vol. % TFA) followed by lyophilization afforded peptide selenoester **22** as a white solid (12 mg, 9% yield based on the initial resin loading).

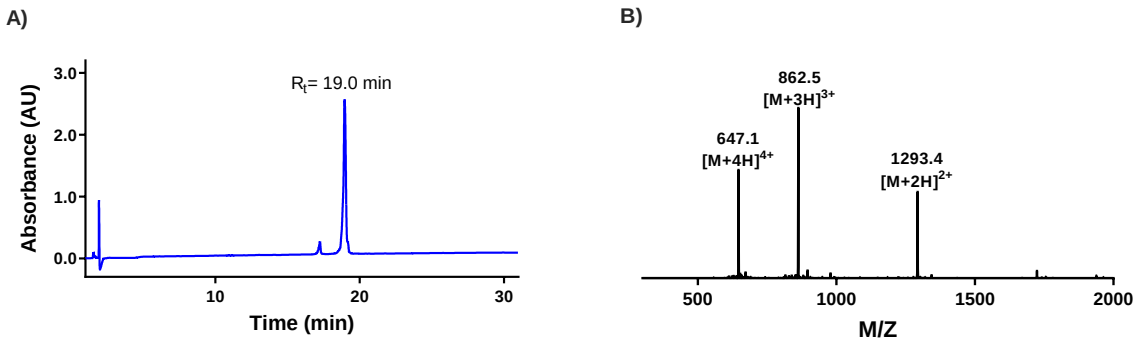


Figure S7. (A) Analytical HPLC, R_t 19.0 min (0-70% B over 30 min, λ = 214 nm), **(B)** ESI-MS of pure selenoester fragment **22**; Calculated Mass: 1293.4 $[M+2H]^{2+}$, 862.6 $[M+3H]^{3+}$, 647.2 $[M+4H]^{4+}$; Mass found (ESI^+): 1293.4 $[M+2H]^{2+}$, 862.5 $[M+3H]^{3+}$, 647.1 $[M+4H]^{4+}$.

Synthesis of ApoCIII bifunctional fragment (21)

Fmoc-Ser(OH)-OAllyl was loaded onto 2-CTC resin through the side chain alcohol in accordance with the general methods for side-chain anchoring. The loaded resin (25 µmol) was subjected to Fmoc SPPS on a Biotage SYRO I automated synthesizer (rt couplings as outlined in the general methods; the light blue coloured residues represent double couplings and yellow residues represents Asp-(DMB)Gly dipeptide). The fully extended peptide was subjected to allyl deprotection and selenoesterification at room temperature on resin as outlined in the general methods section. Finally, Boc-Sec(DEAMC)-OH **1** was coupled at rt for 3 h using the specialised amino acid coupling conditions described in the general methods and cleaved from resin. The crude peptide was purified by preparative reverse-phase HPLC (0 to 60% B over 65 min, 0.1 vol. % TFA) and lyophilized to afford the desired bifunctional peptide **21** as a yellow solid (5 mg, 6% yield based on the initial resin loading).

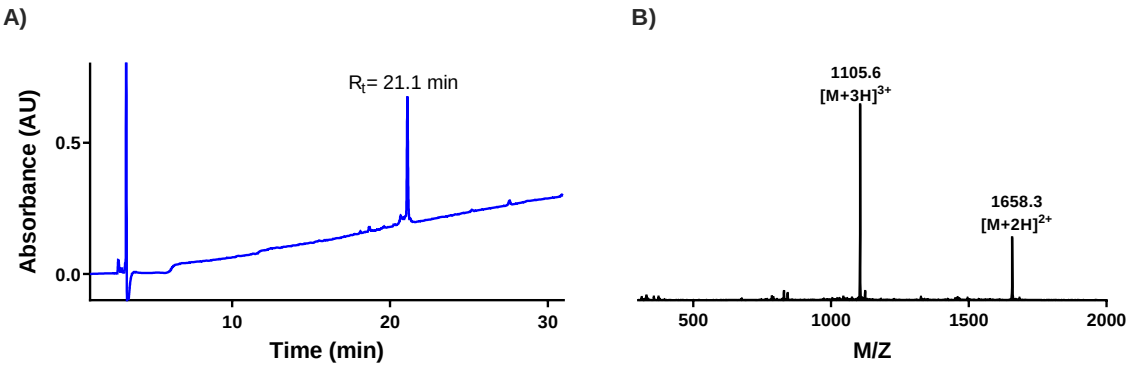


Figure S8. (A) Analytical HPLC, R_t 21.1 min (0-70% B over 30 min, λ = 214 nm), **(B)** ESI-MS of pure bifunctional peptide fragment **21**; Calculated Mass: 1658.2 $[M+2H]^{2+}$, 1105.8 $[M+3H]^{3+}$; Mass found (ESI^+): 1658.3 $[M+2H]^{2+}$, 1105.6 $[M+3H]^{3+}$.

Synthesis of ApoCIII C-terminal diselenide dimer (20)

Wang Resin pre-loaded with Fmoc-Ala-OH (20 µmol) was subjected to Fmoc SPPS on Biotage SYRO I automated synthesizer (as outlined in the general methods; light blue residues represent double couplings and yellow residues represent pseudoproline dipeptides). Finally, Sez(*t*Bu)-Leu-OH **S2** was coupled at rt for 16 h using the specialised amino acid coupling conditions described in the general methods. Cleavage of the peptide from resin along with side-chain deprotection was carried out using TFA:TIS:H_2O (90:5:5; v/v/v) as described in the general methods. The crude peptide (at 5.0 mM concentrations) was dissolved in ligation buffer comprising of 6 M Gdn•HCl, 0.1 M Na_2HPO_4 at pH 4.0 and 200 mM $MeONH_2$•HCl. The reaction mixture was incubated at 37 °C for 8 hours at which stage UPLC-MS indicated complete deprotection. Purification *via* preparative HPLC (0 to 60% B over 65 min, 0.1 vol. % TFA) followed by lyophilization afforded peptide **20** as a white solid (7 mg, 10% yield based on the initial resin loading).

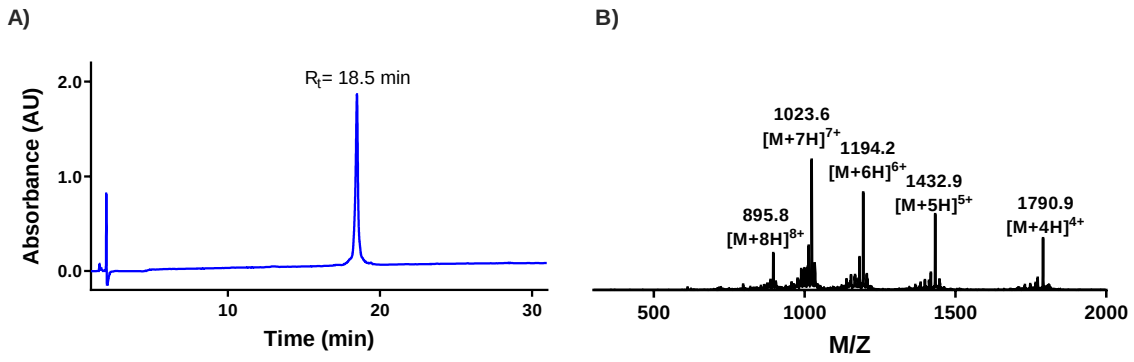


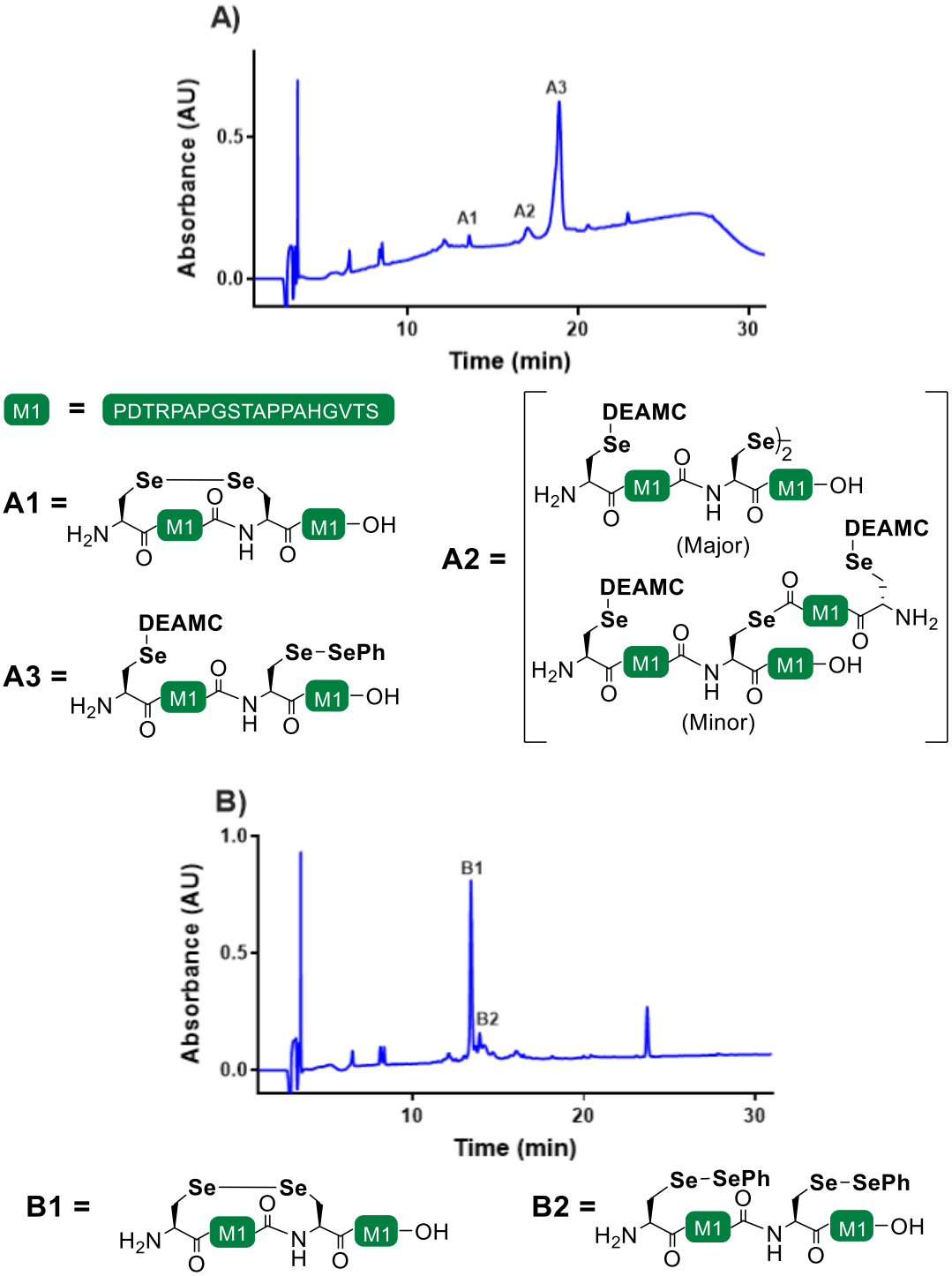
Figure S9. (A) Analytical HPLC, R_t 18.5 min (0-70% B over 30 min, λ = 214 nm), **(B)** ESI-MS of pure peptide diselenide fragment **20**; Calculated Mass: 1790.9 $[M+4H]^{4+}$, 1433.0 $[M+5H]^{5+}$, 1194.30 $[M+6H]^{6+}$, 1023.8 $[M+7H]^{7+}$, 896.0 $[M+8H]^{8+}$; Mass found (ESI^+): 1790.9 $[M+4H]^{4+}$, 1432.9 $[M+5H]^{5+}$, 1194.2 $[M+6H]^{6+}$, 1023.6 $[M+7H]^{7+}$, 895.8 $[M+8H]^{8+}$.

Ligations

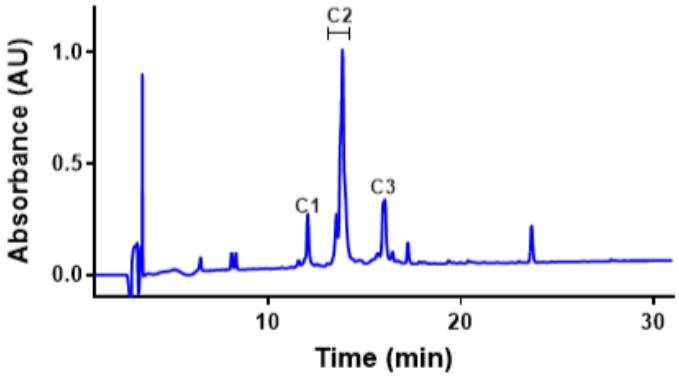
Synthesis of MUC1 60mer (9)

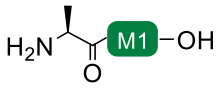
VNTR (1-20) diselenide dimer **13** (0.72 mg, 5.0 mM, 1.0 eq.) and VNTR (1-20) bifunctional fragment **12** (0.95 mg, 5.5 mM, 1.1 eq.) were dissolved in ligation buffer comprised of 6 M Gdn•HCl, 0.1 M Na_2HPO_4 at pH 7.5 (75 µL). The pH was adjusted to 6.0 using 0.1 M NaOH and the solution was incubated at rt. After completion of the ligation (15 min, as confirmed by UPLC-MS), the pH of the solution was increased to 8.0 using 5.0 M NaOH and left to stand for 5 min to hydrolyse unreacted and unproductive selenoesters. The pH was readjusted to 6.0 using 5.0 M HCl and the ligation mixture was irradiated with 450 nm light for 10 min (PennOC, 36 W) affording complete deprotection (as confirmed by UPLC-MS analysis). The resulting diselenide was reacted with VNTR (1-20) selenoester **14** (1.50 mg, 10 mM, 2.0 eq.) at rt for 15 min. The DPDS generated during ligations was then extracted with diethyl ether (3 x 0.5 mL) and the reaction mixture was treated with an equal volume of a degassed solution of 400 mM TCEP and 80 mM GSH in ligation buffer at pH 7. Thorough degassing followed by irradiation with 254 nm light for 5 min (Rayonet, 35 W) afforded the target MUC1 60mer. Upon reverse-

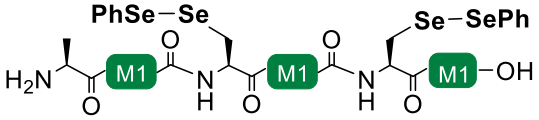
phase HPLC purification (0 to 40% B over 65 min, 0.1 vol. % TFA), the MUC1 60mer **9** was isolated as a white solid (1.3 mg, 60% yield over 4 steps).



C)



C1 = 

C3 = 

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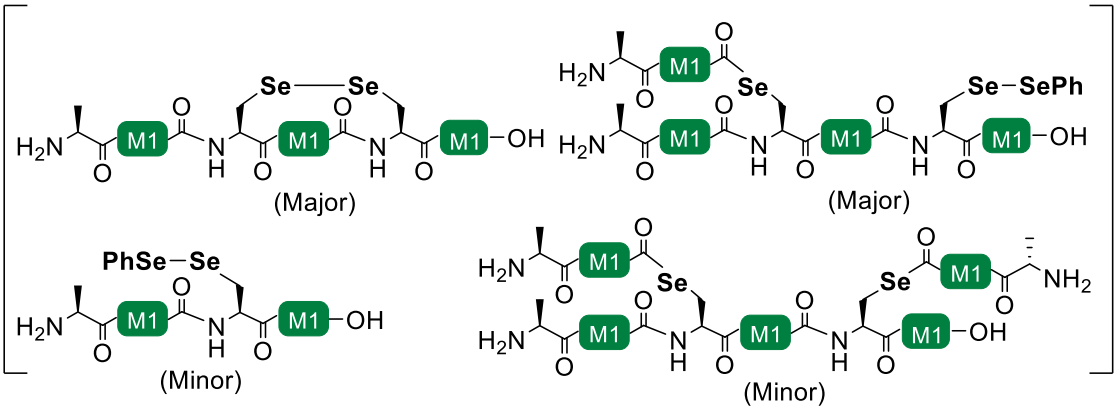
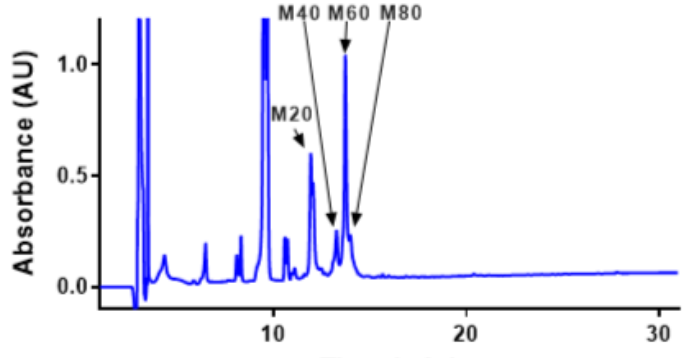
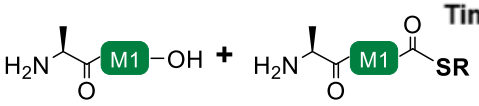
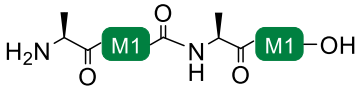
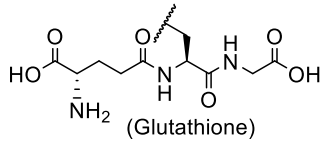


D)



M20 = 

M40 = 

R = 
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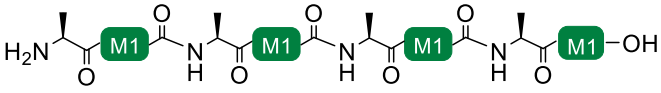
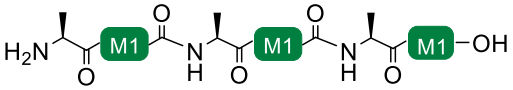
M80 = 

M60 = 
(desired final product **9**)

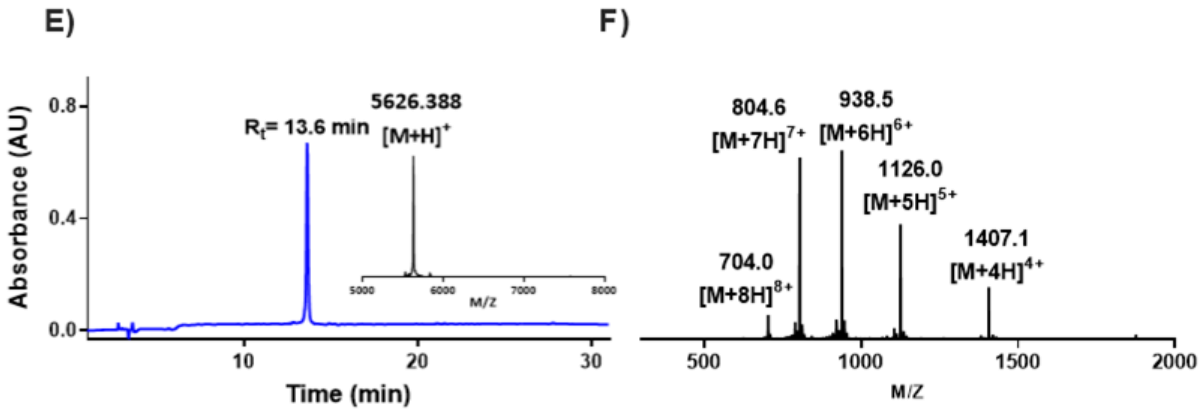
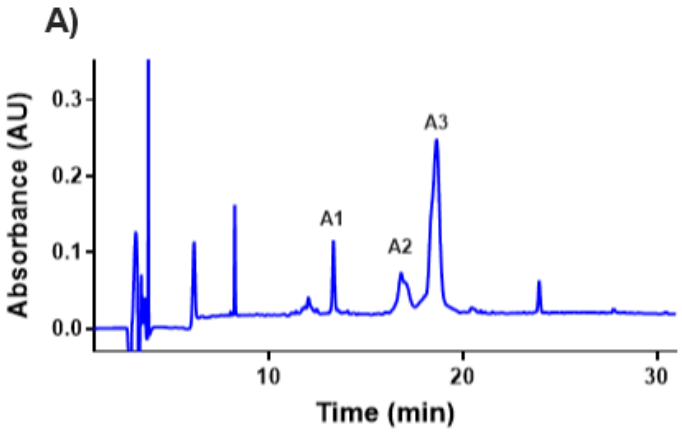


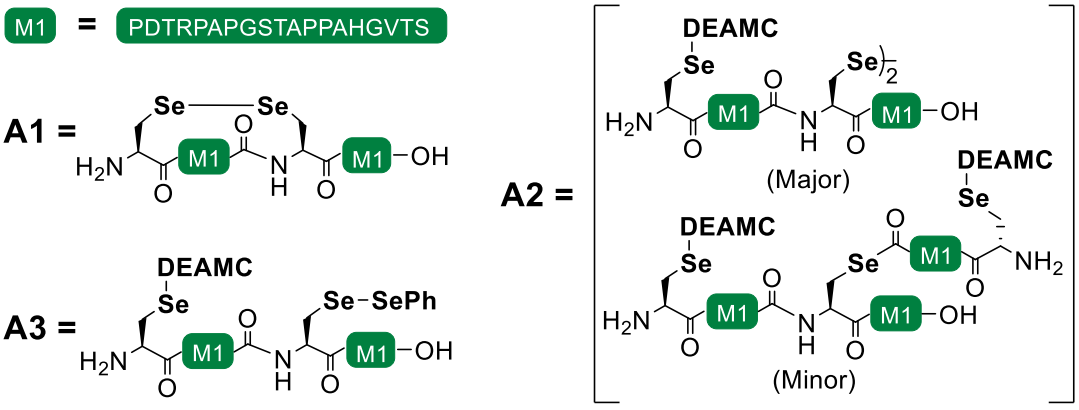
Figure S10: Analytical HPLC traces (0-60% B over 30 min, λ = 214 nm) of **(A)** Crude trace after 1st DSL between diselenide dimer **13** and bifunctional MUC1 fragment **12**, ligation products generated in the reaction are depicted underneath the chromatogram; **(B)** Crude trace after photodeprotection of DEAMC with 450 nm light, products from the crude deprotection reaction are shown underneath the chromatogram; **(C)** Crude trace after 2nd DSL with MUC1 selenoester **14**, ligation products generated in the reaction are depicted underneath the chromatogram; **(D)** Crude trace after photodeselenization with 254 nm light in presence of TCEP and GSH, products from the crude deselenization reaction are shown underneath the chromatogram. Analytical HPLC trace R_t 13.6 min (0-60% B over 30 min, λ = 214 nm) with MALDI **(E)** and ESI-MS **(F)** of purified MUC1 60mer **9**.

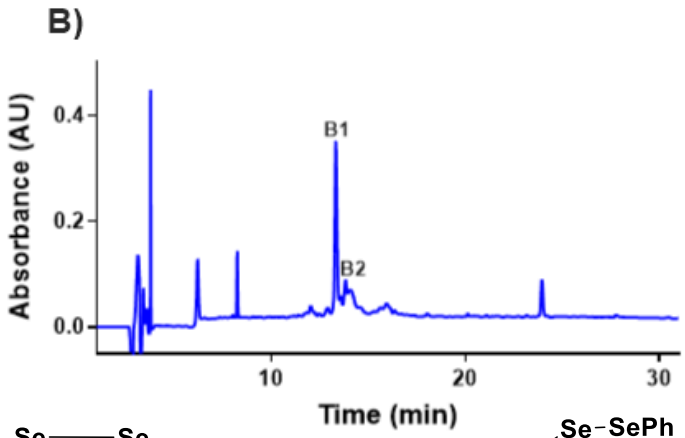
Synthesis of MUC1 80mer (17)

VNTR (1-20) diselenide dimer **13** (0.60 mg, 5.0 mM, 1.0 eq.) and VNTR (1-20) bifunctional fragment **12** (0.79 mg, 5.5 mM, 1.1 eq.) were dissolved in 60 µL of ligation buffer (6 M Gdn•HCl, 0.1 M Na_2HPO_4, pH 7.5). The pH was adjusted to 6.0 using 0.1 M NaOH and the reaction mixture was incubated at rt. After completion of the ligation (15 min, as confirmed by UPLC-MS analysis) the pH of the solution was increased to pH 8.0 using 5.0 M NaOH and left to stand for 5 min to hydrolyse unproductive selenoesters. The pH was readjusted to 6.0 using 5.0 M HCl and the ligation mixture was irradiated with 450 nm light for 10 min (PennOC, 36 W) affording complete deprotection (as confirmed by UPLC-MS analysis). The resulting diselenide was ligated with VNTR (1-20) bifunctional fragment **12** (1.15 mg, 8.0 mM, 1.6 eq.)

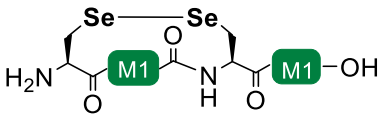
at pH 6.0 and the abovementioned steps (hydrolysis of unproductive selenoesters, photodeprotection and ligation with VNTR (1-20) selenoester **14** (1.50 mg, 12.0 mM, 2.4 eq.)) were repeated to afford MUC1 80mer as a mixture of ligation products (see Figure S11E). DPDS was then extracted with diethyl ether (3 x 0.5 mL) and the reaction mixture was treated with an equal volume of a degassed solution of 400 mM TCEP and 80 mM GSH in ligation buffer at pH 7.0. Thorough degassing followed by irradiation with 254 nm light for 5 min (Rayonet, 35 W) afforded the target MUC1 80mer **17** as a white solid (0.8 mg, 35% yield over 6 steps) upon reverse-phase HPLC purification (0 to 40% B over 65 min, 0.1 vol. % TFA).



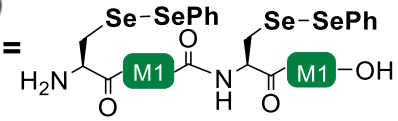


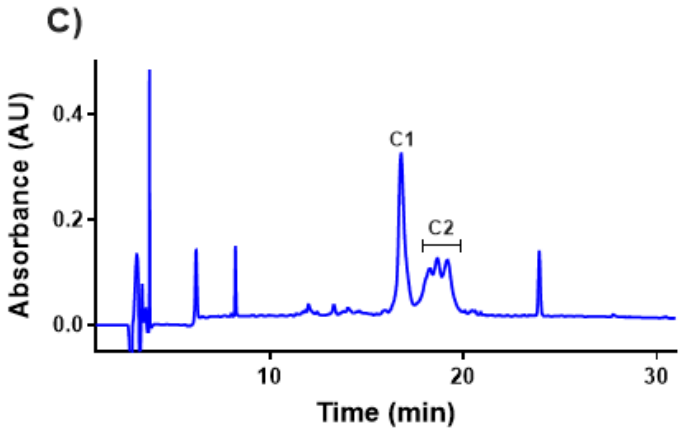

B)
Absorbance (AU)
0.4
0.2
0.0
B1
B2
10
20
30
Time (min)

B1 =

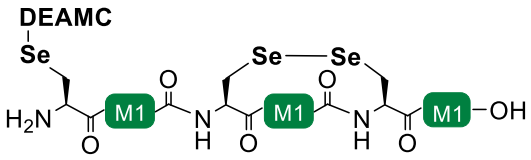

Se
Se
O
H2N
M1
N
H
M1
OH
O
O

B2 =

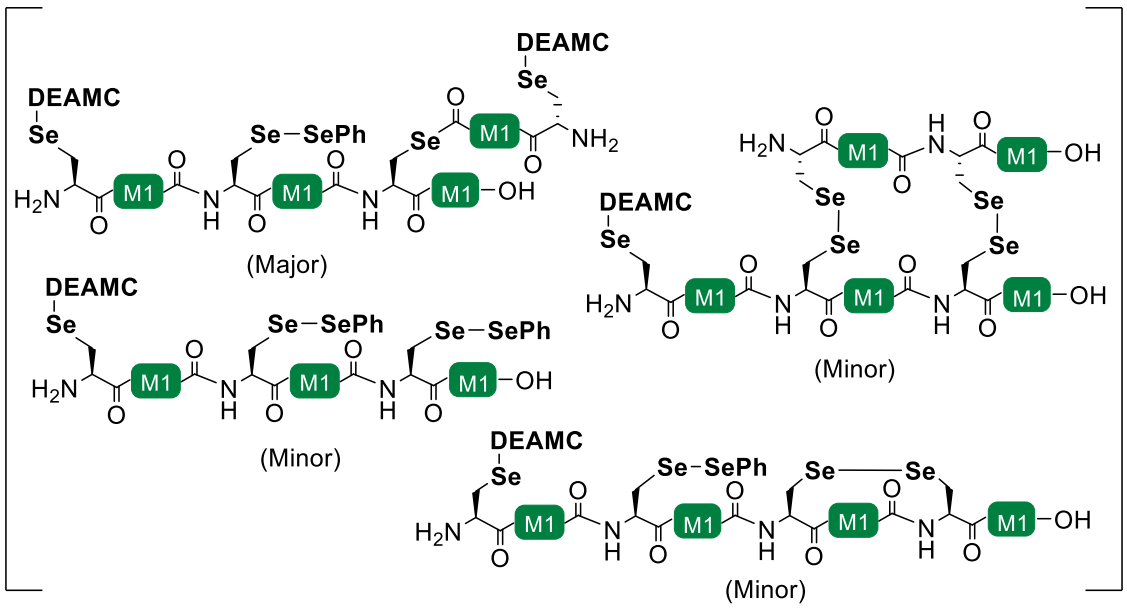

Se–SePh
Se–SePh
O
H2N
M1
N
H
M1
OH
O
O

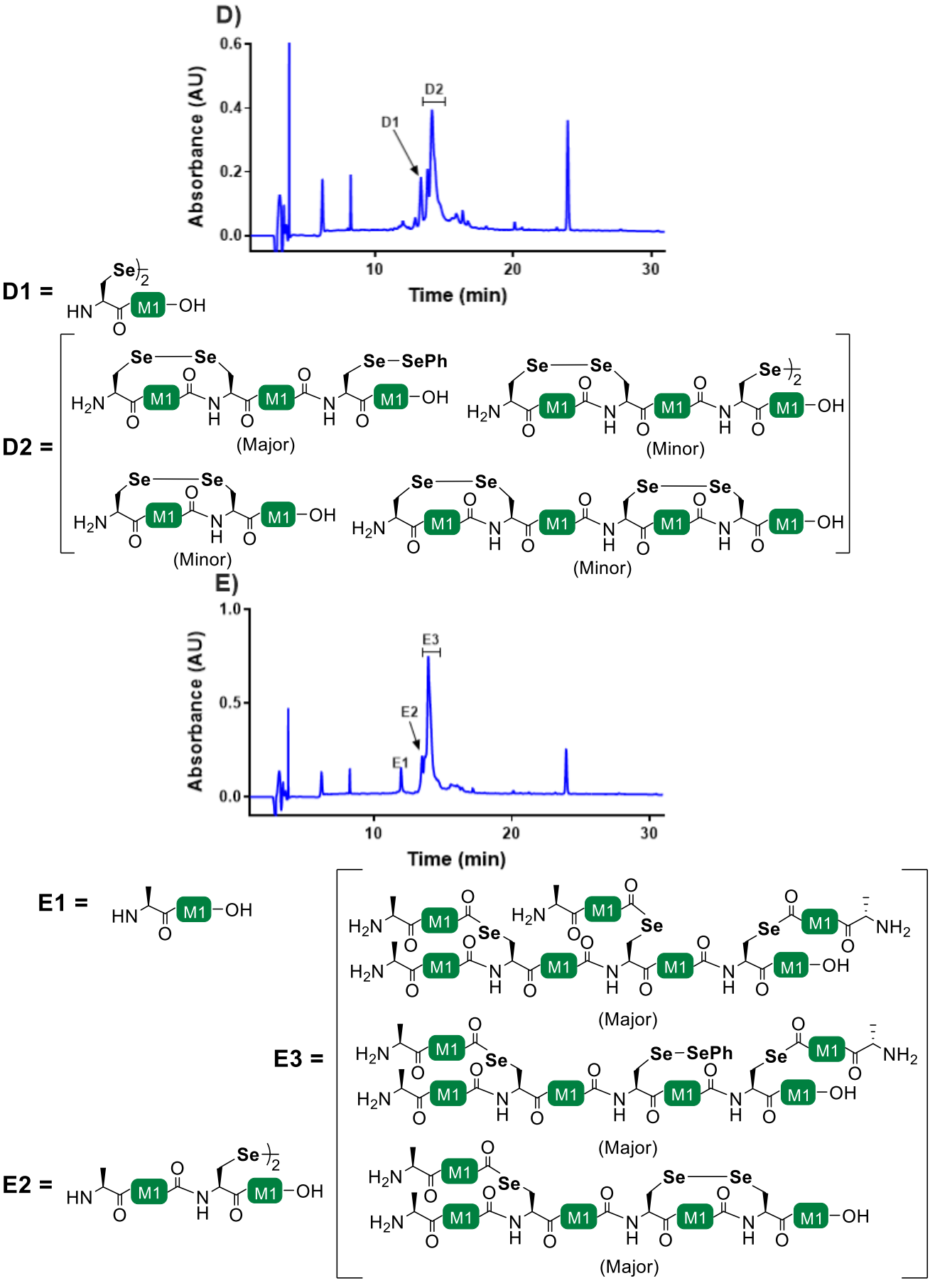

C)
Absorbance (AU)
0.4
0.2
0.0
C1
C2
10
20
30
Time (min)

C1 =


DEAMC
Se
Se
Se
O
H2N
M1
N
H
M1
N
H
M1
OH
O
O
O

C2 =


DEAMC
Se
DEAMC
Se
Se–SePh
Se
M1
NH2
H2N
M1
N
H
M1
N
H
M1
OH
(Major)
H2N
M1
N
H
M1
OH
Se
Se
Se
Se
DEAMC
Se
M1
N
H
M1
N
H
M1
OH
(Minor)
DEAMC
Se
Se–SePh
Se–SePh
H2N
M1
N
H
M1
N
H
M1
OH
(Minor)
DEAMC
Se
Se–SePh
Se
Se
H2N
M1
N
H
M1
N
H
M1
N
H
M1
OH
(Minor)


D)
Absorbance (AU)
Time (min)
D1
D2
D1 =
D2 =
(Major)
(Minor)
(Minor)
(Minor)
E)
Absorbance (AU)
Time (min)
E1
E2
E3
E1 =
E2 =
E3 =
(Major)
(Major)
(Major)

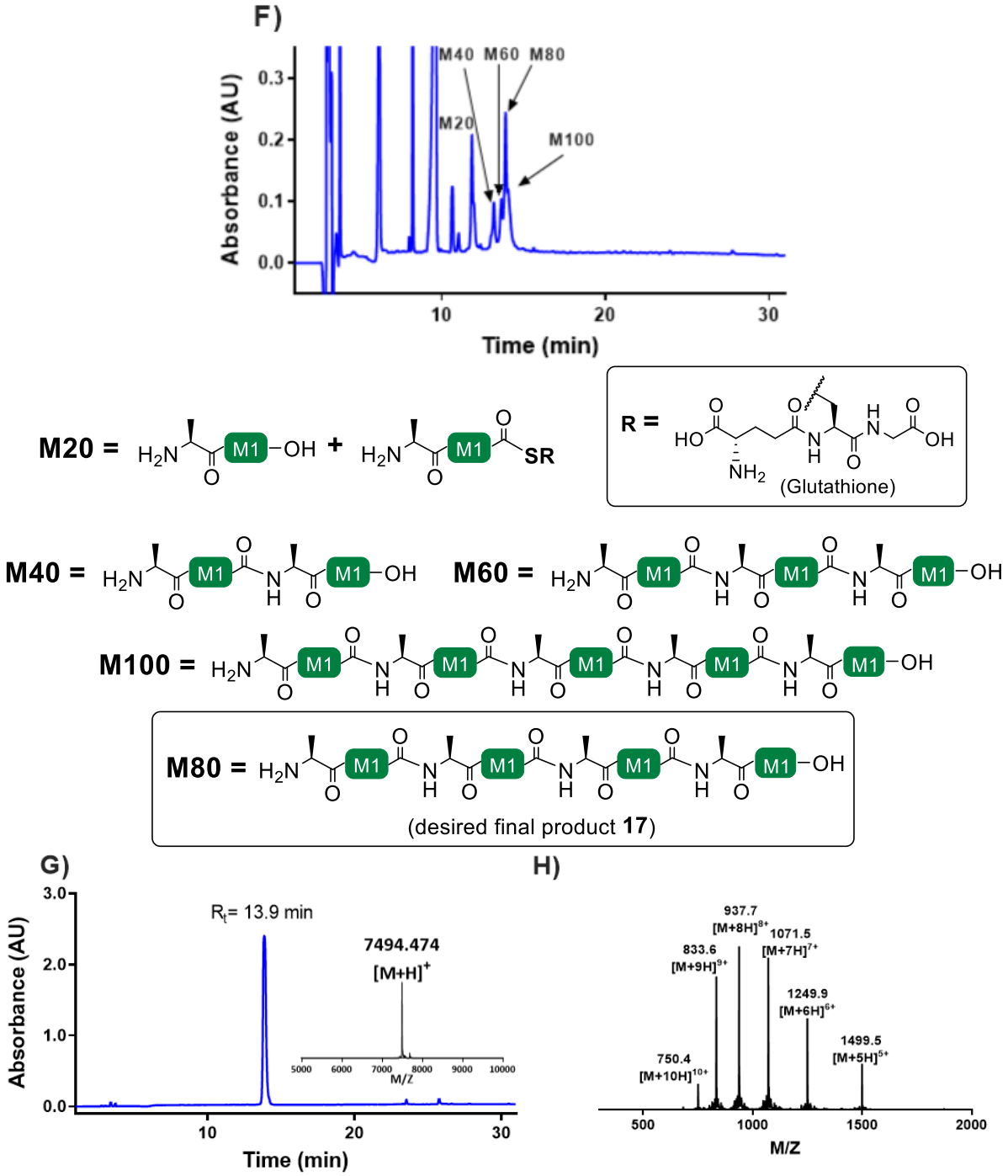
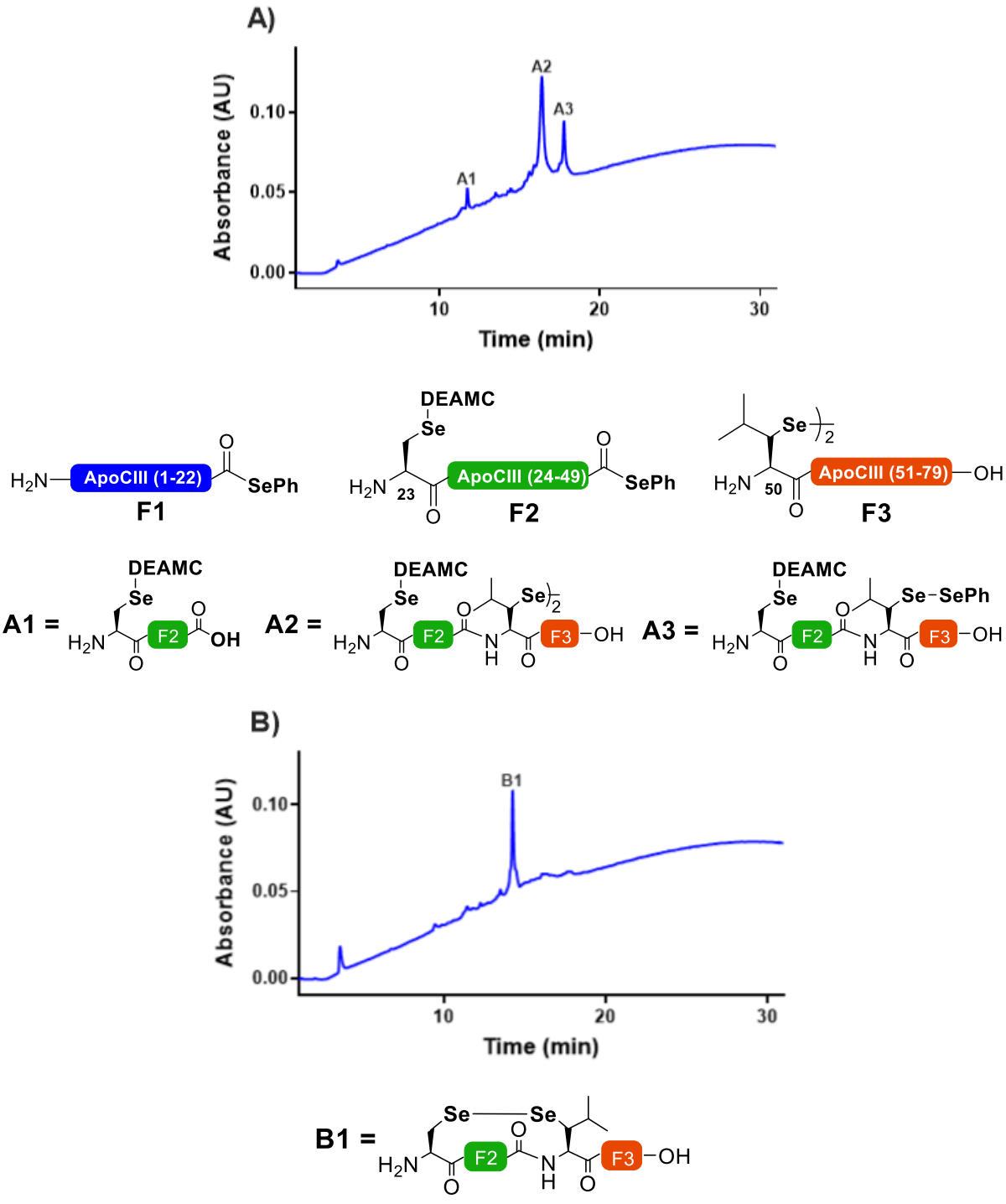


Figure S11: Analytical HPLC traces (0-60% B over 30 min, λ = 214 nm) of **(A)** Crude trace after 1st DSL between diselenide dimer **13** and bifunctional MUC1 fragment **12**, ligation products generated in the reaction are depicted underneath the chromatogram; **(B)** Crude trace after photodeprotection of DEAMC with 450 nm light, products from the crude deprotection reaction are shown underneath the chromatogram; **(C)** Crude trace after 2nd DSL with additional bifunctional MUC1 fragment **12**, ligation products generated in the reaction are depicted underneath the chromatogram; **(D)** Crude trace after second photodeprotection of DEAMC with 450 nm light, products from the crude deprotection reaction are shown

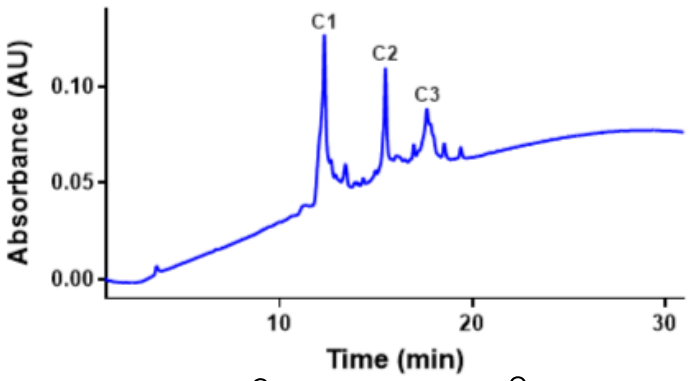
underneath the chromatogram; **(E)** Crude trace after 3rd DSL with MUC1 selenoester **11**, ligation products generated in the reaction are depicted underneath the chromatogram; **(F)** Crude trace after photodeselenization with 254 nm light in the presence of TCEP and GSH, products from the crude deselenization reaction are shown underneath the chromatogram. Analytical HPLC trace R_t 13.9 min (0-60% B over 30 min, λ = 214 nm) with MALDI **(G)** and ESI-MS **(H)** of purified MUC1 80mer **17**.

Synthesis of ApoCIII (19)

ApoCIII (50-79) diselenide **20** (0.70 mg, 5.0 mM, 1.0 eq.) and ApoCIII (23-49) bifunctional fragment **21** (1.04 mg, 8.0 mM, 1.6 eq.) were dissolved in 40 µL of degassed ligation buffer (6 M Gdn•HCl, 0.1 M Na_2HPO_4, pH 7.5) and the pH was adjusted to 6.2 using 0.1 M NaOH and ligation was allowed to proceed at 37 °C. After completion of the ligation (30 min, as confirmed by UPLC-MS analysis) the pH of the solution was raised to pH 8.0 with 5.0 M NaOH and left to stand for 10 min. The pH was readjusted to 6.2 and the reaction mixture was irradiated with 450 nm light for 10 min (PennOC, 36 W) affording complete deprotection (as confirmed by UPLC-MS analysis). The resulting mixture of diselenides was reacted with ApoCIII (1-22) selenoester **22** (1.21 mg, 0.48 mM, 2.4 eq.) in the presence of additives, TCEP (5.6 mg, 20 mM) and DPDS (9.15 mg, 30 mM) at pH 6.2 (950 µL, 200 µM final concentration with respect to ApoCIII (50-79) monomer of **20**). The reaction mixture was incubated at 37 °C and after completion of the ligation (2 h, as confirmed by UPLC-MS), DPDS was extracted with diethyl ether (6 x 1 mL). The reaction mixture was treated with an equal volume of a degassed solution of 400 mM TCEP and 80 mM GSH in ligation buffer at pH 7.0. Thorough degassing followed by irradiation with 254 nm light for 5 min (Rayonet, 35 W) afforded ApoCIII (1-79) **19** as a white solid (1.0 mg, 60% yield over 4 steps) upon reverse-phase HPLC purification (0 to 60% B over 65 min, 0.1 vol. % TFA at 50 °C). NB: HPLC was conducted at 50 °C to avoid aggregation of ApoCIII (1-79) **19** during purification (Figure S12. D).


A)
Absorbance (AU)
0.00
0.05
0.10
10
20
30
Time (min)
A1
A2
A3
H2N
ApoCIII (1-22)
SePh
F1
DEAMC
Se
23
ApoCIII (24-49)
F2
50
ApoCIII (51-79)
OH
F3
A1 =
A2 =
A3 =
OH
Se-SePh
B)
B1
B1 =

C)

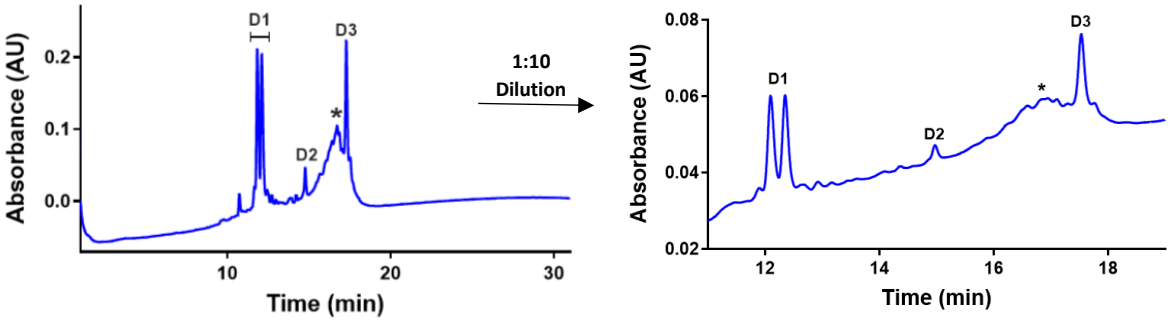


C1 = H_2N–F1–OH (Major) + H_2N–F1–SePh (Minor)

C2 = H_2N–F1–Se–Se–F1–F2–F3–OH

C3 = H_2N–F1–Se–Se–F1–F2–F3–OH

D)



D1 = H_2N–F1–OH + H_2N–F1–SR

R = HO, NH_2, O, H, N, OH (Glutathione)

D2 = H_2N–F1–F2–OH

D3 = H_2N–F1–F2–F3–OH (desired final product **19**)

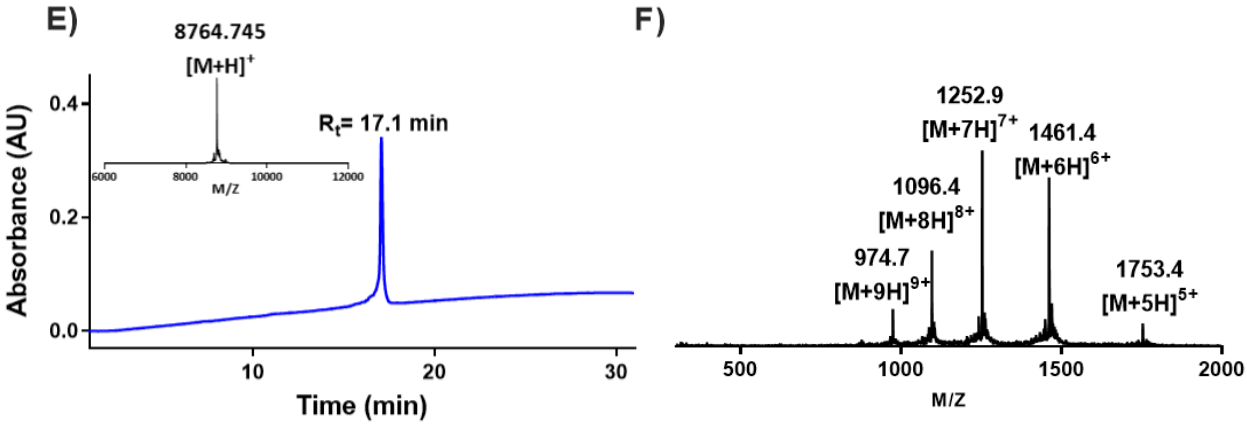


Figure S12 Analytical HPLC traces (0-70% B over 30 min, λ = 214 nm) of **(A)** Crude trace after 1st DSL between diselenide dimer **20** and bifunctional ApoCIII fragment **21**, ligation products generated in the reaction are depicted underneath the chromatogram; **(B)** Crude trace after photodeprotection of DEAMC with 450 nm light, products from the crude deprotection reaction are shown underneath the chromatogram; **(C)** Crude trace after rDSL with ApoCIII selenoester **22**, ligation products generated in the reaction are depicted underneath the chromatogram; **(D) (left)** Crude trace after photodeselenisation with 254 nm light in the presence of TCEP and GSH, *Aggregated ApoCIII (1-79) **19**. **(D) (right)** Crude trace after photodeselenisation with 254 nm light in the presence of TCEP and GSH after 10-fold dilution leading to a decrease in ApoCIII aggregation. Analytical HPLC trace R_t 17.1 min (1-70% B over 30 min, λ = 214 nm) with MALDI **(E)** and ESI-MS **(F)** and of purified ApoCIII (1-79) **19**.

Circular Dichroism (CD)

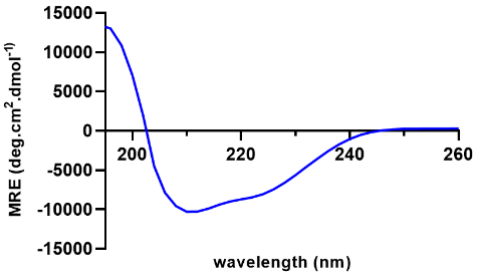


Figure S13. CD spectrum of ApoCIII **19** in SDS micelles. The sample contained 8 μM ApoCIII protein **19**, 10 mM Tris, 100 mM NaCl, SDS 1.8 mM (pH 7.4).

CD spectroscopy was performed as previously reported for recombinant ApoCIII.[5] A sample of synthetic ApoCIII was dissolved in SDS buffer (10 mM Tris, 100 mM NaCl, SDS 1.8 mM, pH 7.4) and left to stand for 30 min at rt. The protein concentration was then determined to be 8 µM using a NanoDrop Spectrophotometer ND-1000. Protein solutions were transferred to a quartz cuvette (1 mm path length), and far UV CD spectra were recorded at 22 °C with a scanning speed of 20 nm/min on a Jasco J-815 CD Spectrometer (Jasco, Easton, MD) equipped with a temperature controller. A 1 s time constant and a 2 nm band width were used during data acquisition over a wavelength range of 190–260 nm. For the protein solution, three spectra were recorded, averaged, and referenced by subtracting the average of the three spectra obtained from SDS buffer alone. The data was smoothed with 6 neighbouring points on each side using Graphpad Prism 8.

Nuclear Magnetic Resonance (NMR)

See following pages for NMR spectra of DEAMC-protected Sec building block **1** and synthetic precursors.

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8	Author	
9	Solvent	CDCl3
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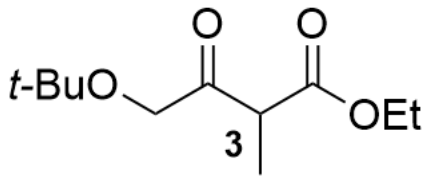

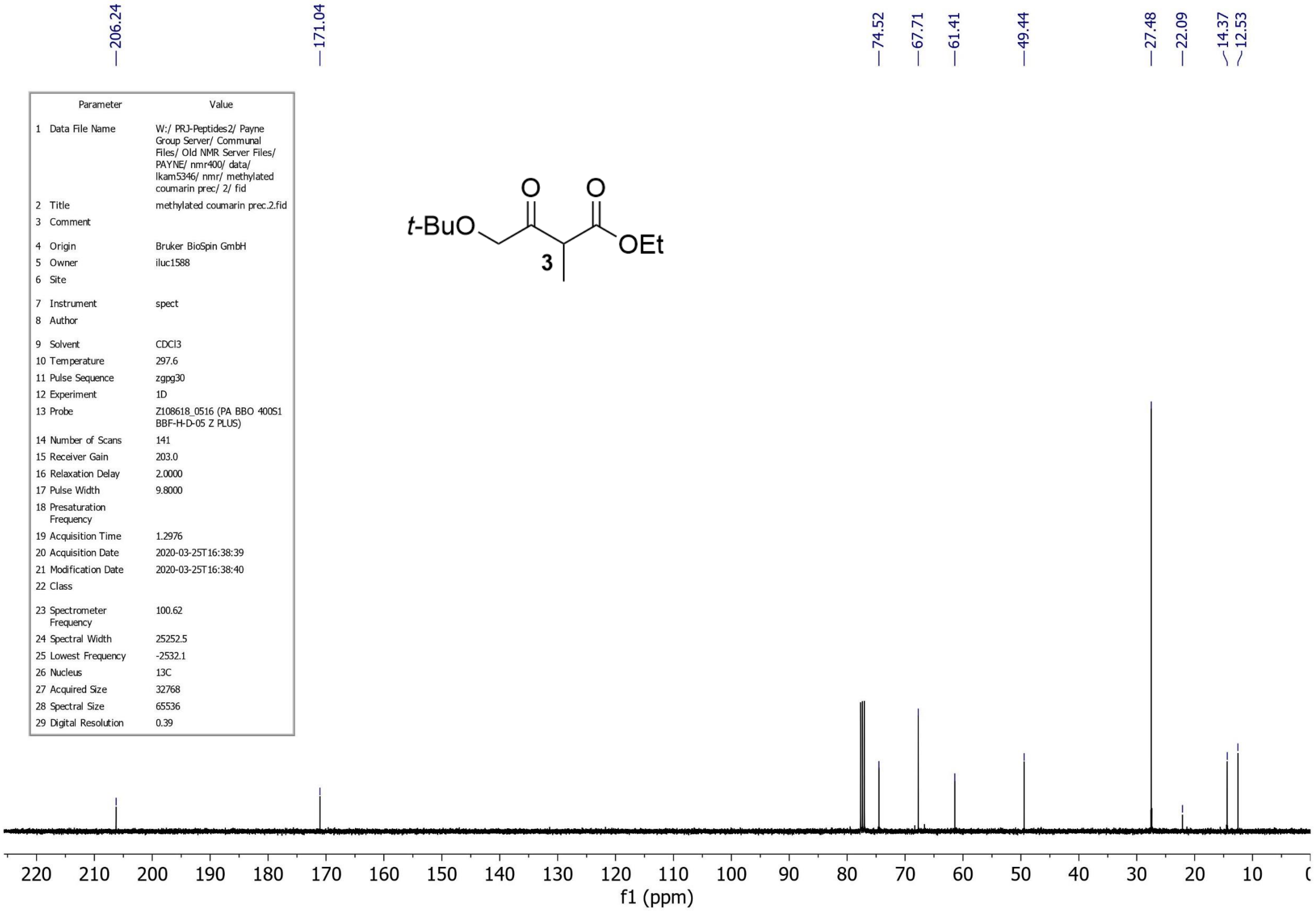

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8	Author	
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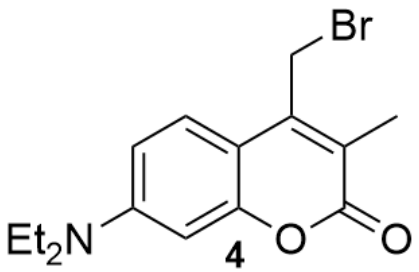

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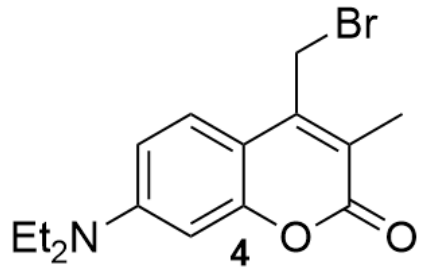

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8	Author	
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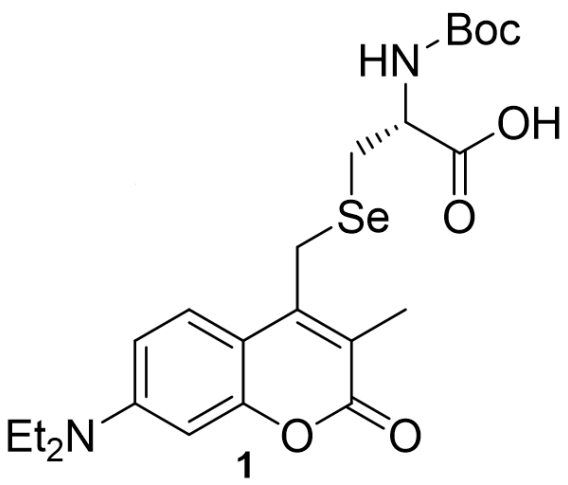

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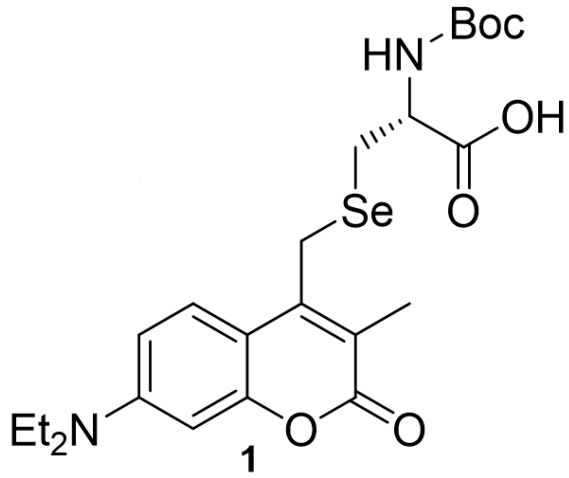

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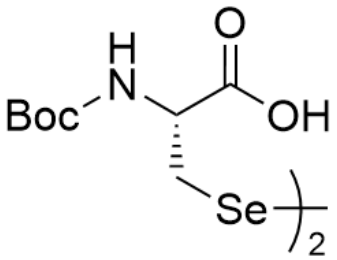
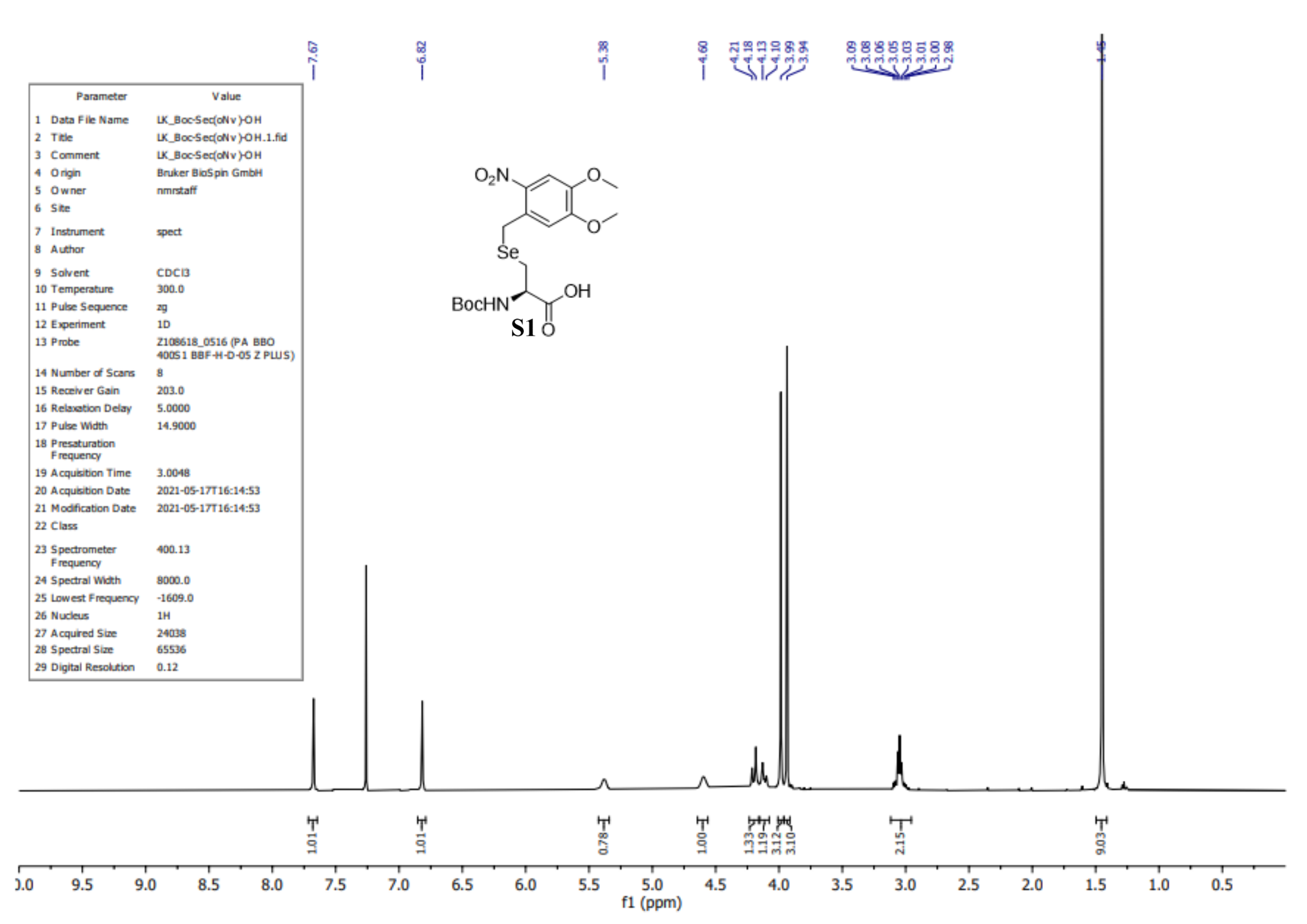
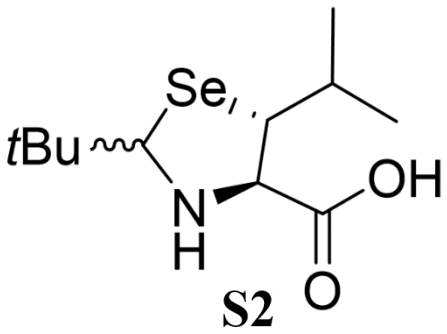
3	Comment	
4	Origin	Bruker BioSpin GmbH
5	Owner	lkam5346
6	Site	
7	Instrument	spect
8	Author	
9	Solvent	MeOD
10	Temperature	301.2
11	Pulse Sequence	zg
12	Experiment	1D
13	Probe	5 mm QNP 1H/ 13C/ 31P/ 19F Z-GRD Z8352/ 0119
14	Number of Scans	8
15	Receiver Gain	181.0
16	Relaxation Delay	2.0000
17	Pulse Width	13.7500
18	Presaturation Frequency	
19	Acquisition Time	2.7329
20	Acquisition Date	2020-02-11T14:03:46
21	Modification Date	2020-02-11T14:03:55
22	Class	
23	Spectrometer Frequency	300.13
24	Spectral Width	5995.2
25	Lowest Frequency	-1165.5
26	Nucleus	1H
27	Acquired Size	16384
28	Spectral Size	65536
29	Digital Resolution	0.09



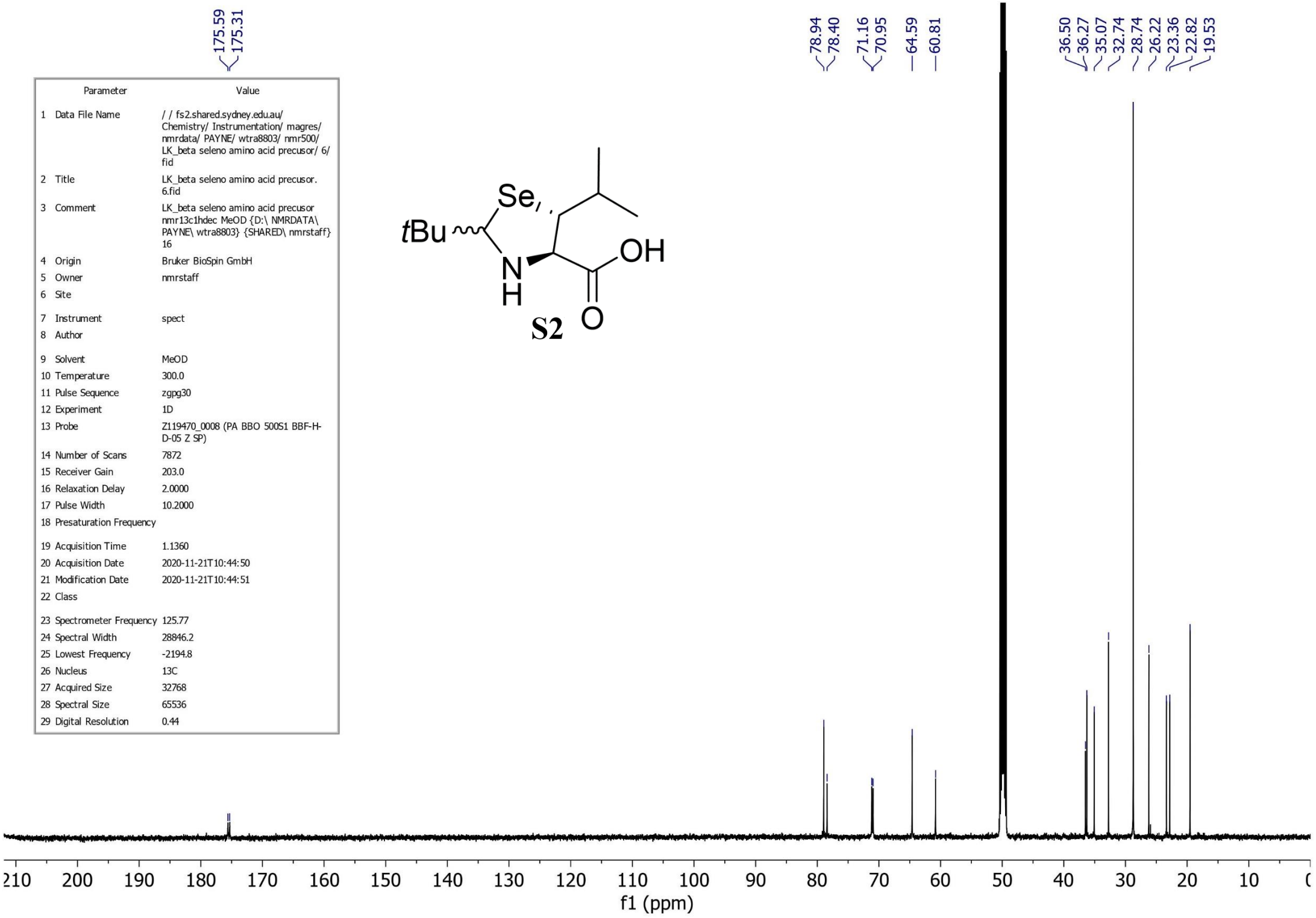


	Parameter	Value
1	Data File Name	LK_Boc-Sec(oNv)-OH
2	Title	LK_Boc-Sec(oNv)-OH.1.fid
3	Comment	LK_Boc-Sec(oNv)-OH
4	Origin	Bruker BioSpin GmbH
5	Owner	nmrstaff
6	Site	
7	Instrument	spect
8	Author	
9	Solvent	CDCl3
10	Temperature	300.0
11	Pulse Sequence	zg
12	Experiment	1D
13	Probe	Z108618_0516 (PA BBO 400S1 BBF-H-D-05 Z PLUS)
14	Number of Scans	8
15	Receiver Gain	203.0
16	Relaxation Delay	5.0000
17	Pulse Width	14.9000
18	Presaturation Frequency	
19	Acquisition Time	3.0048
20	Acquisition Date	2021-05-17T16:14:53
21	Modification Date	2021-05-17T16:14:53
22	Class	
23	Spectrometer Frequency	400.13
24	Spectral Width	8000.0
25	Lowest Frequency	-1609.0
26	Nucleus	1H
27	Acquired Size	24038
28	Spectral Size	65536
29	Digital Resolution	0.12

	Parameter	Value
1	Data File Name	/ / fs2.shared.sydney.edu.au/ Chemistry/ Instrumentation/ magres/ nmrdata/ PAYNE/ wtra8803/ nmr500/ LK_beta seleno amino acid precusor/ 1/ fid
2	Title	LK_beta seleno amino acid precusor.1.fid
3	Comment	LK_beta seleno amino acid precusor
4	Origin	Bruker BioSpin GmbH
5	Owner	nmrstaff
6	Site	
7	Instrument	spect
8	Author	
9	Solvent	MeOD
10	Temperature	300.0
11	Pulse Sequence	zg
12	Experiment	1D
13	Probe	Z119470_0008 (PA BBO 500S1 BBF-H-D-05 Z SP)
14	Number of Scans	8
15	Receiver Gain	45.2
16	Relaxation Delay	2.0000
17	Pulse Width	14.0000
18	Presaturation Frequency	
19	Acquisition Time	3.2768
20	Acquisition Date	2020-11-20T15:20:35
21	Modification Date	2020-11-20T15:20:36
22	Class	
23	Spectrometer Frequency	500.13
24	Spectral Width	10000.0
25	Lowest Frequency	-1994.3
26	Nucleus	1H
27	Acquired Size	32768
28	Spectral Size	65536
29	Digital Resolution	0.15



S2


175.59
175.31
78.94
78.40
71.16
70.95
64.59
60.81
36.50
36.27
35.07
32.74
28.74
26.22
23.36
22.82
19.53
Parameter
Value
1 Data File Name
/ / fs2.shared.sydney.edu.au/ Chemistry/ Instrumentation/ magres/ nmrdata/ PAYNE/ wtra8803/ nmr500/ LK_beta seleno amino acid precusor/ 6/ fid
2 Title
LK_beta seleno amino acid precusor. 6.fid
3 Comment
LK_beta seleno amino acid precusor nmr13c1hdec MeOD {D:\ NMRDATA\ PAYNE\ wtra8803} {SHARED\ nmrstaff} 16
4 Origin
Bruker BioSpin GmbH
5 Owner
nmrstaff
6 Site
7 Instrument
spect
8 Author
9 Solvent
MeOD
10 Temperature
300.0
11 Pulse Sequence
zgpg30
12 Experiment
1D
13 Probe
Z119470_0008 (PA BBO 500S1 BBF-H-D-05 Z SP)
14 Number of Scans
7872
15 Receiver Gain
203.0
16 Relaxation Delay
2.0000
17 Pulse Width
10.2000
18 Presaturation Frequency
19 Acquisition Time
1.1360
20 Acquisition Date
2020-11-21T10:44:50
21 Modification Date
2020-11-21T10:44:51
22 Class
23 Spectrometer Frequency
125.77
24 Spectral Width
28846.2
25 Lowest Frequency
-2194.8
26 Nucleus
13C
27 Acquired Size
32768
28 Spectral Size
65536
29 Digital Resolution
0.44
tBu
Se
N
H
OH
O
S2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

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