Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Information

Generation of Oligonucleotide Conjugates *via* One-pot Diselenide-Selenoester Ligation Deselenization / Alkylation

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Experimental Procedures

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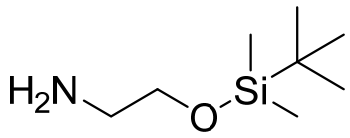
Reagents and general methods for the preparation and characterization of small molecules

N,N′-Diisopropylamino cyanoethyl phosphonamidic chloride (Cl-POCEN(*i*Pr)$_2$) was purchased from ChemGenes Corporation (Wilmington, MA). 5′-*O*-Dimethoxytrityl-2′-deoxyribonucleoside-3′-*O*-(β-cyanoethyl-*N,N*′-diisopropyl) phosphoramidites and protected 2′-deoxyribonucleoside controlled pore glass supports (500 Å) were purchased from Glen Research (Sterling, VA). All other chemicals and solvents were purchased from Sigma Aldrich (Milwaukee, WI) or EMD Chemicals Inc. (Gibbstown, NJ). Flash column chromatography was performed using silica gel 60 (230–400 mesh) purchased from Canadian Life Science (Pointe-Claire, QC). Thin layer chromatography (TLC) was carried out with pre-coated TLC plates (Merck, Kieselgel 60 F_{254}, 0.25 mm), purchased from EMD Chemicals Inc. (Gibbstown, NJ), using UV light for visualization. NMR spectra were recorded on a Varian 500 MHz NMR spectrometer at room temperature. ^{1}H NMR spectra were recorded at a frequency of 500.0 MHz and chemical shifts were reported in parts per million (ppm). ^{13}C NMR spectra (^{1}H decoupled) were recorded at a frequency of 125.7 MHz and chemical shifts were reported in ppm. The NMR spectra were calibrated using the proton or carbon signals of residual, nondeuterated solvent peaks: δ_H 7.26 and δ_C 77.0 for $CDCl_3$ and δ_H 2.05 and δ_C 29.84 for $(CD_3)_2CO$. ^{31}P NMR spectra (^{1}H decoupled) were recorded at a frequency of 202.3 MHz and chemical shifts were reported in ppm with H_3PO_4 used as an external standard.

^{77}Se NMR spectra (^{1}H decoupled) were recorded at a frequency of 95.3 MHz and chemical shifts were reported in ppm with dimethyl selenide used as an external standard. High resolution mass spectrometry was performed using a 7T-LTQ FT ICR mass spectrometer (Thermo Scientific) at the Concordia University Centre for Structural and Functional Genomics. The mass spectrometer was operated in full scan, positive ion detection mode.

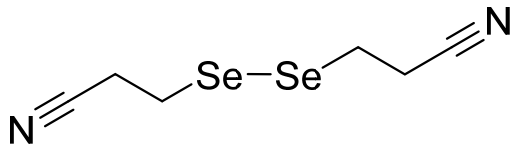
Chemical synthesis of small molecules

2-((*tert*-butyldimethylsilyl)oxy)ethan-1-amine; i.e. TBS protected ethanolamine (synthesis adapted from previous report[1]):



To a stirring solution of ethanolamine (0.999 g; 16.4 mmol) and imidazole (1.35 g; 19.8 mmol) in anhydrous DCM (20 mL) at 0 °C was added TBS-Cl (2.724 g; 18.1 mmol) directly as a solid. The flask was then raised from the ice bath and left to come up to RT overnight (22 hours). The reaction mixture was then washed with saturated $NaHCO_3$ (30 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by flash column chromatography using DCM/MeOH/NH_4OH (90:9:1; v/v/v) to afford 1.67 g (58 %) of TBS protected ethanolamine as a pale yellow liquid. $\mathbf{R_f}$ (SiO_2 TLC): 0.40 (90:9:1 DCM/MeOH/NH_4OH, v/v/v). **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 3.57 (t, J = 5.3 Hz, 2H), 2.72 (t, J = 5.3 Hz, 2H), 1.23 (br s, 2H), 0.85 (s, 9H), 0.01 (s, 6H).

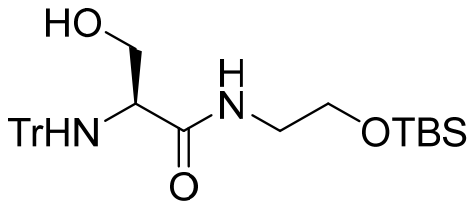
Di-2-cyanoethyl diselenide (synthesis adapted from previous report[2]):



To a flask containing selenium powder (5.02 g; 63.6 mmol) and $NaBH_4$ (2.02 g; 53.4 mmol) on ice and under an argon atmosphere was added a solution of 4:1 (v/v) dioxane-ethanol (100 mL) dropwise (exit needle recommended as reaction is vigorous, forming dark brown-red suspension). After stirring the reaction mixture for 1 hour on ice, 3-bromopropionitrile (8 mL; 96.4 mmol; 1.5 equiv.) was added dropwise. The reaction mixture was again stirred on ice for 1 hour before diluting the contents with H_2O (300 mL). The suspension was extracted with EtOAc (4×150 mL) and the combined organic layers washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by flash column chromatography using a gradient of $CHCl_3$/hexanes (50 %, 60 %, 70 %, 80 %, 90 % and 100 % $CHCl_3$; 300 mL each) to afford 4.15 g (49 %) of di-2-cyanoethyl diselenide as a light orange oil

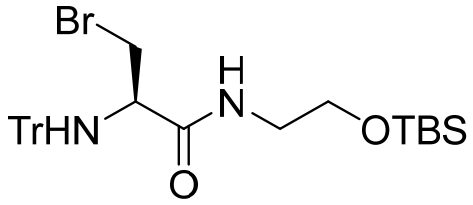
(only place product on high-vacuum for short time to remove residual solvent). **^{1}H NMR** (500 MHz, $CDCl_3$, ppm) δ 3.08 (t, *J* = 7.1 Hz, 4H), 2.87 (t, *J* = 7.1 Hz, 4H). **^{77}Se NMR** (95.3 MHz, $CDCl_3$, ppm): δ 310.49.

N-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3-hydroxy-2-(tritylamino)propanamide (Amide **2**):



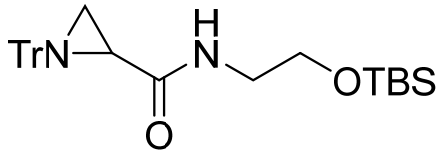
To a flask containing *N*-trityl-L-serine methyl ester **1** (0.600 g; 1.66 mmol) and LiOH (0.045 g; 1.88 mmol) was added 1:1 (v/v) H_2O/MeOH (20 mL). The suspension was stirred and refluxed for 4 hours (solution clear after heating), before concentrating the reaction mixture *in vacuo*. The resulting residue was co-evaporated with toluene (3×20 mL) and the white solid dried *in vacuo* overnight. In the same flask, the carboxylate salt intermediate was dissolved in anhydrous DMF (5 mL) before adding the TBS protected ethanolamine (0.434 g; 2.48 mmol) and PyBOP (1.042 g; 2 mmol). The atmosphere of the flask was exchanged with argon and the reaction stirred at RT for 20 hours. The reaction mixture was diluted with EtOAc (100 mL) then washed with H_2O (50 mL), 1:1 (v/v) H_2O/brine (2×50 mL) and brine (2×50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by flash column chromatography using EtOAc/hexanes (1:1; v/v) to afford 0.559 g (67 % over 2 steps) of amide **2** as a white foam. **R_f** (SiO_2 TLC): 0.26 (1:1 EtOAc/hexanes, v/v). **$\lambda_{max\ (MeCN)}$**: 228 nm. **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 7.70 (s, 1H, amide NH), 7.46 – 7.44 (m, 6H, Ar), 7.29 – 7.26 (m, 6H, Ar), 7.21 (t, *J* = 7.3 Hz, 3H, Ar), 3.71 – 3.64 (m, 2H), 3.52 – 3.48 (m, 1H), 3.34 – 3.27 (m, 3H), 2.88 (d, *J* = 6.8 Hz, 1H), 2.62 – 2.58 (m, 1H), 2.02 (t, *J* = 6.0 Hz, 1H), 0.91 (s, 9H), 0.08 (app d, *J* = 9.0 Hz, 6H).**^{13}C NMR** (125.7 MHz, $CDCl_3$, ppm): δ 173.76, 145.76, 128.65, 128.05, 126.79, 71.58, 63.51, 62.03, 59.01, 41.54, 25.92, 18.28, -5.29, -5.33. **HRMS** (ESI-MS) *m/z* calculated for $C_{30}H_{40}N_2O_3SiNa^+$: 527.2700; found 527.2698 $[M + Na]^+$.

3-bromo-*N*-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-2-(tritylamino)propanamide (Alkyl bromide **3**):



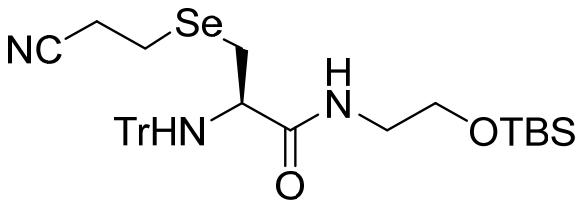
To a flame-dried flask containing a solution of amide **2** (0.319 g; 0.63 mmol) and carbon tetrabromide (0.280 g; 0.84 mmol) in anhydrous DCM (6 mL) at 0 °C and under argon atmosphere was added triphenylphosphine (0.177 g; 0.67 mmol). The reaction mixture was stirred at 0 °C for 30 minutes before lifting the flask from the ice bath and letting it come up to RT over 1.5 hours. The reaction mixture was then concentrated *in vacuo* and the crude material was purified (without workup) by flash column chromatography using hexanes/EtOAc (4:1; v/v; minimal DCM used to transfer crude material to column) to afford 0.256 g (71 %) of alkyl bromide **3** as a white foam. $\mathbf{R_f}$ (SiO_2 TLC): 0.87 (1:1 EtOAc/hexanes, v/v). $\lambda_{max\ (MeCN)}$: 228 nm. **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 8.03 (s, 1H, amide NH), 7.48 (d, *J* = 7.5 Hz, 6H, Ar), 7.29 (t, *J* = 7.6 Hz, 6H, Ar), 7.23 (t, *J* = 7.3 Hz, 3H, Ar), 3.82 – 3.75 (m, 2H), 3.71 (d, *J* = 9.9 Hz, 1H), 3.52 – 3.47 (m, 3H), 2.71 (d, *J* = 8.7 Hz, 1H), 1.82 (dd, *J* = 9.9, 4.2 Hz, 1H), 0.94 (s, 9H), 0.13 (app d, *J* = 9.5 Hz, 6H). **^{13}C NMR** (125.7 MHz, $CDCl_3$, ppm): δ 171.58, 145.65, 128.46, 128.25, 126.97, 71.81, 62.27, 57.45, 41.86, 37.91, 25.97, 18.29, -5.24, -5.26. **HRMS** (ESI-MS) *m/z* calculated for $C_{30}H_{39}BrN_2O_2SiNa^+$: 589.1856; found 589.1854 $[M + Na]^+$.

N-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-1-tritylaziridine-2-carboxamide (aziridine - **undesired byproduct**):



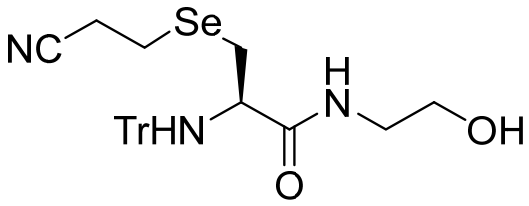
To a solution of triphenylphosphine (0.039 g; 0.15 mmol) and imidazole (0.022 g; 0.32 mmol) in anhydrous DCM (2 mL) on ice was added iodine (0.039 g; 0.15 mmol). The reaction mixture was left stirring at 0 °C for 30 minutes before adding a solution of amide **2** (0.051 g; 0.10 mmol) in anhydrous DCM (1 mL) dropwise over 2 minutes. The reaction mixture was stirred at 0 °C for 1 hour and left to come up to RT over another hour. The reaction was quenched with 10 % sodium thiosulfate (10 mL) and extracted with DCM (25 mL). The organic layer was washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by flash column chromatography using EtOAc/hexanes (1:1; v/v) to afford 0.021 g of aziridine as a white foam. $\mathbf{R_f}$ (SiO_2 TLC): 0.76 (1:1 EtOAc/hexanes, v/v). **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 7.43 – 7.41 (m, 6H, Ar), 7.29 – 7.21 (m, 10H, Ar and amide NH), 3.80 – 3.73 (m, 2H), 3.53 – 3.42 (m, 2H), 2.02 (dd, *J* = 6.6, 2.7 Hz, 1H), 1.97 (dd, *J* = 2.8, 1.0 Hz, 1H), 1.46 (dd, *J* = 6.6, 0.9 Hz, 1H), 0.92 (s, 9H), 0.11 (app d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (125.7 MHz, $CDCl_3$, ppm): δ 170.80, 143.34, 129.31, 127.69, 127.06, 74.55, 61.96, 41.07, 34.04, 29.83, 25.87, 18.18, -5.30, -5.39. **HRMS** (ESI-MS) *m/z* calculated for $C_{30}H_{38}N_2O_2SiNa^+$: 509.2595; found 509.2593 $[M + Na]^+$.

N-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3-((2-cyanoethyl)selanyl)-2-(tritylamino)propanamide (Protected selenide **4**):



To a flask containing $NaBH_4$ (0.033 g; 0.87 mmol) under argon atmosphere was added EtOH (2 mL). In a separate flask, di-2-cyanoethyl diselenide (0.225 g; 0.85 mmol) was dissolved in EtOH (2 mL) and stirred at 0 °C under an argon atmosphere. The $NaBH_4$ suspension was then added dropwise to the flask of diselenide. After 10 minutes, the solution became clear (indicating all diselenide had reduced to sodium selenolate) and a solution of alkyl bromide **3** (0.235 g; 0.41 mmol) in EtOH (6 mL) was injected into the flask. The reaction mixture was stirred for 30 minutes at 0 °C before lifting the flask from the ice bath and letting it come up to RT over 1 hour and 20 minutes. The reaction mixture was then quenched with H_2O (25 mL) and extracted with EtOAc (2 ×40 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by flash column chromatography using DCM/hexanes (9:1; v/v) to afford 0.191 g (74 %) of protected selenide **4** as a white foam. $\mathbf{R_f}$ (SiO_2 TLC): 0.45 (9:1 DCM/EtOAc, v/v). $\boldsymbol{\lambda_{max\ (MeCN)}}$: 228 nm. **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 8.12 (s, 1H, amide NH), 7.47 – 7.46 (m, 6H, Ar), 7.29 – 7.25 (m, 6H, Ar), 7.23 – 7.20 (m, 3H, Ar), 3.82 – 3.72 (m, 2H), 3.55 – 3.45 (m, 2H), 3.40 – 3.29 (m, 1H), 3.04 (dd, *J* = 13.0, 2.4 Hz, 1H), 2.90 (d, *J* = 8.7 Hz, 1H), 2.66 – 2.34 (m, 4H), 1.16 (dd, *J* = 13.0, 5.8 Hz, 1H), 0.93 (s, 9H), 0.12 (app d, *J* = 10.4 Hz, 6H). **^{13}C NMR** (125.7 MHz, $CDCl_3$, ppm): δ 173.13, 145.80, 128.43, 128.24, 126.89, 118.56, 71.67, 62.01, 55.85, 41.83, 28.24, 25.95, 19.15, 18.28, 17.54, -5.23, -5.27. **^{77}Se NMR** (95.3 MHz, $CDCl_3$, ppm): δ 115.81. **HRMS** (ESI-MS) *m/z* calculated for $C_{33}H_{43}N_3O_2SeSiNa^+$: 644.2182; found 644.2181 $[M + Na]^+$.

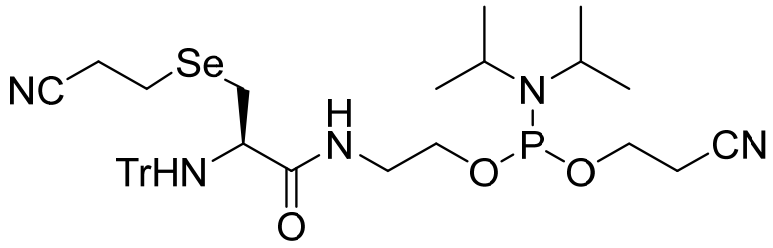
3-((2-cyanoethyl)selanyl)-*N*-(2-hydroxyethyl)-2-(tritylamino)propanamide (Alcohol **5**):



To a stirring solution of protected selenide **4** (0.190 g; 0.31 mmol) in anhydrous THF (3 mL) was added triethylamine trihydrofluoride (150 µL; 0.92 mmol) and triethylamine (85 µL; 0.61 mmol). The reaction mixture was stirred at RT for 3 hours. The solvent was evaporated *in vacuo* and the crude material purified (without workup) by flash column chromatography using 100 % EtOAc to afford 0.136 g (88 %) of alcohol **5** as a white foam. $\mathbf{R_f}$ (SiO_2 TLC): 0.63 (100 % EtOAc). $\boldsymbol{\lambda_{max\ (MeCN)}}$: 228 nm. **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 7.80 (t, *J* = 5.9 Hz, 1H, amide NH), 7.46 – 7.44 (m, 6H, Ar), 7.31 – 7.28 (m, 6H, Ar), 7.25 – 7.21 (m, 3H, Ar), 3.73 (t, *J* = 4.8 Hz, 2H), 3.52

(ddd, J = 8.6, 6.4, 2.7 Hz, 1H), 3.46 – 3.40 (m, 1H), 3.37 – 3.31 (m, 1H), 3.06 (dd, J = 12.9, 2.7 Hz, 1H), 2.99 (d, J = 8.2 Hz, 1H), 2.67 – 2.59 (m, 2H), 2.57 – 2.46 (m, 3H), 1.55 (dd, J = 12.9, 6.4 Hz, 1H). **^{13}C NMR** (125.7 MHz, $CDCl_3$, ppm): δ 174.38, 145.66, 128.50, 128.26, 126.99, 118.72, 71.68, 62.11, 56.21, 42.47, 28.57, 19.24, 17.83. **^{77}Se NMR** (95.3 MHz, $CDCl_3$, ppm): δ 119.87. **HRMS** (ESI-MS) *m/z* calculated for $C_{27}H_{29}N_3O_2SeNa^+$: 530.1317; found 530.1316.

2-cyanoethyl (2-(3-((2-cyanoethyl)selanyl)-2-(tritylamino)propanamido)ethyl) diisopropylphosphoramidite
(Selenide phosphoramidite **6**):



To a stirring solution of alcohol **5** (0.101 g, 0.20 mmol) in anhydrous THF (2 mL) was added DIPEA (138 µL, 0.79 mmol), followed by the dropwise addition of Cl-P(OCE)N(*i*Pr)$_2$ (132 µL, 0.59 mmol). After 20 minutes, the reaction mixture was taken up in EtOAc (40 mL) and washed with 3 % (aq., w/v) $NaHCO_3$ (40 mL) and brine (40 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by short flash column chromatography using EtOAc/hexanes (4:1, v/v) (with 2 % NEt_3, v/v) to afford 0.119 g (85 %) of selenide phosphoramidite **6** as a white semi-solid. **R_f** (SiO_2 TLC): 0.85 (4:1 EtOAc/hexanes, v/v). **$\lambda_{max\ (MeCN)}$**: 228 nm. **^{1}H NMR** (500 MHz, acetone-d_6, ppm): δ 7.82 – 7.77 (m, 1H, amide NH), 7.57 – 7.55 (m, 6H, Ar), 7.34 – 7.30 (m, 6H, Ar), 7.25 – 7.22 (m, 3H, Ar), 3.94 – 3.80 (m, 2H), 3.77 – 3.65 (m, 4H), 3.49 – 3.39 (m, 2H), 3.31 – 3.21 (m, 1H), 3.15 (dd, J = 8.7, 3.0 Hz, 1H), 2.98 (dt, J = 12.6, 3.0 Hz, 1H), 2.80 – 2.56 (m, 6H), 1.78 – 1.72 (m, 1H), 1.22 – 1.18 (m, 12H). **^{13}C NMR** (125.7 MHz, acetone-d_6, ppm): δ 173.45, 147.24, 129.58, 129.57, 128.89, 128.88, 127.51, 127.49, 119.86, 119.05, 119.00, 72.38, 63.00, 62.97, 62.88, 62.85, 59.71, 59.68, 59.57, 59.53, 57.06, 43.80, 43.70, 41.36, 41.34, 41.31, 41.28, 25.06, 25.00, 24.96, 24.90, 20.85, 20.81, 20.79, 20.76, 19.56, 18.81, 18.79. **^{31}P NMR** (202.3 MHz, acetone-d_6, ppm): δ 147.76, 147.61. **^{77}Se NMR** (95.3 MHz, acetone-d_6, ppm): δ 127.62, 127.20. **HRMS** (ESI-MS) *m/z* calculated for $C_{36}H_{46}N_5O_3PSeNa^+$: 730.2396; found 730.2405 $[M + Na]^+$.

Selenoester peptide synthesis and purification

General Solid-Phase Peptide Synthesis (SPPS):

General amino acid coupling: A solution of protected amino acid (4 equiv.), PyBOP (4 equiv.) and *N*-methylmorpholine (8 equiv.) in DMF (final amino acid concentration: 0.1 M) was added to

the resin. After 1 h, the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL).

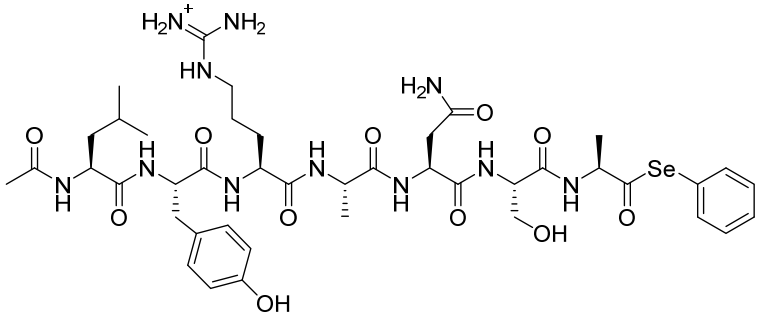
Deprotection: The resin was treated with 20 % piperidine/DMF (2 × 3 mL, 3 minutes) and washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL).

Capping: Acetic anhydride/pyridine (1:9, v/v) was added to the resin (3 mL). After 3 min, the resin was washed with DMF (5 × 3mL), DCM (5 × 3mL) and DMF (5 × 3 mL).

HPLC purification:

All purifications of selenoester peptides were performed on a Waters Sunfire 5 µm, 19 x 150 mm preparative column (C-18) operating at a flow rate of 7 mL/min. Buffer A: H_2O + 0.1 % TFA, buffer B: acetonitrile + 0.1 % TFA.

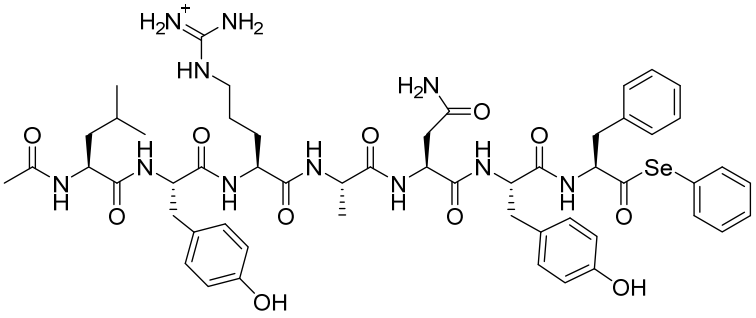
Ac-LYRANSA-SePh (**8**):



2-chloro-trityl chloride resin (81 mg, 1.22 mmol/g loading) was swollen in dry DCM for 30 min then washed with DCM (5 × 3 mL). A solution of Fmoc-SerAla-OAll dipeptide (2 equiv. relative to resin functionalization) and *i*Pr_2NEt (4 equiv. relative to resin functionalization) in DCM (final amino acid concentration: 0.2 M) was added and the resin was shaken at RT for 16 h. The Fmoc-SerAla-OAll was prepared as outlined by Hanna *et al.*[3] The resin was washed with DMF (5 × 3 mL) and DCM (5 × 3 mL). The resin was treated with a solution of DCM/MeOH/*i*Pr_2NEt (17:2:1 v/v/v, 3 mL) for 1 h and washed with DMF (5 × 3 mL), DCM (5 × 3mL), and DMF (5 × 3 mL). The peptide was then elongated *via* standard Fmoc-strategy SPPS as outlined in the general SPPS section. To the resin (25 µmol_aliquot) was added a solution of $Pd(PPh_3)_4$ (25 mg, 22 µmol) and $PhSiH_3$ (123 µL, 1 mmol) in dry DCM (2 mL) and the resin was shaken for 1 h, then the resin was washed with DCM (10 × 5 mL) and DMF (5 × 5 mL). A solution of diphenyldiselenide (DPDS) (234 mg, 0.75 mmol, 30 equiv.) and tri-*n*-butylphosphine (184 µL, 0.75 mmol, 30 equiv.) in DMF (3 mL) was added to the resin. The resin was left at 0 °C in an ice bath for 3 h. Afterwards, the resin was washed with DMF (5 × 5 mL) and DCM (5 × 5 mL). For resin cleavage, a mixture of TFA : triisopropylsilane : H_2O (5 mL, 90:5:5 v/v/v) was added. After 2 h, the resin was washed with TFA (2 × 2 mL). The combined solutions were concentrated under a stream of nitrogen. The residue was dissolved in H_2O containing 30 % ACN + 0.1 % TFA, purified by preparative HPLC

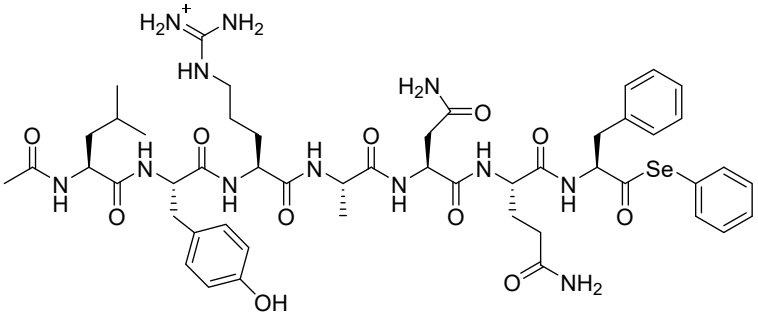
(0 to 60 % B over 30 min, 0.1 % TFA) and lyophilized to afford the desired peptide selenoester (15.8 mg, 65 %). See **Figure S17** for characterization.

Ac-LYRANYF-SePh (**15**):



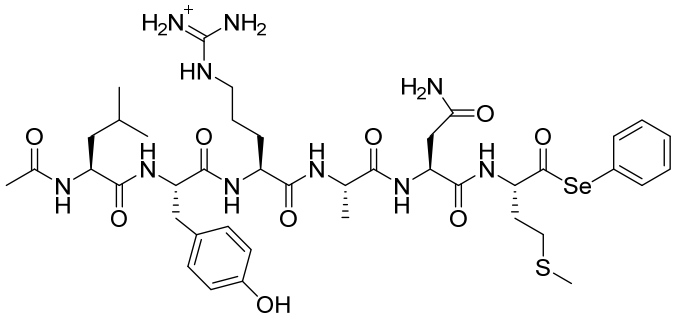
Wang resin was washed with DCM (5 × 3 mL). Fmoc-TyrPhe-OAll dipeptide (1 equiv.) and triphenylphosphine (1.1 equiv.) were dissolved in DCM and the solution was added to the resin. The Fmoc-TyrPhe-OAll was prepared as outlined by Hanna *et al.*[3] A solution of diisopropyl azodicarboxylate (1.1 eq.) was diluted 5 fold in DCM and added dropwise to the resin at 0 °C. The resin was shaken at RT for 16 h, and then washed with DCM (5 × 3 mL), DMF (5 × 3 mL), and DCM (5 × 3 mL). The resin was capped with acetic anhydride/pyridine (1:9, v/v) (2 × 3 min) and washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL). The peptide was then elongated *via* standard Fmoc-strategy SPPS as outlined in the general SPPS section. To the resin (25 μmol aliquot) was added a solution of $Pd(PPh_3)_4$ (25 mg, 22 μmol) and $PhSiH_3$ (123 μL, 1 mmol) in dry CH_2Cl_2 (2 mL) and the resin was shaken for 1 h, then the resin was washed with DCM (10 × 5 mL) and DMF (5 × 5 mL). A solution of diphenyldiselenide (DPDS) (234 mg, 0.75 mmol, 30 equiv.) and tri-*n*-butylphosphine (184 μL, 0.75 mmol, 30 equiv.) in DMF (3 mL) was added to the resin. The resin was left at 0 °C in an ice bath for 3 h. Afterwards, the resin was washed with DMF (5 × 5 mL) and DCM (5 × 5 mL). For resin cleavage, a mixture of TFA: triisopropylsilane: H_2O (5 mL, 90:5:5 v/v/v) was added. After 2 h, the resin was washed with TFA (2 × 2 mL). The combined solutions were concentrated under a stream of nitrogen. The residue was dissolved in water containing 30 % ACN + 0.1 % TFA, purified by preparative HPLC (0 to 60 % B over 30 min, 0.1 % TFA) and lyophilized to afford the desired peptide selenoester (20.3 mg, 72 % yield). See **Figure S18** for characterization.

Ac-LYRANQF-SePh (**16**):



Rink amide resin was initially washed with DCM (5 × 3 mL) and DMF (5 × 3 mL), followed by Fmoc deprotection with 20 % piperidine/DMF (2 × 5 min). The resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL). PyBOP (4 equiv.) and *N*-methylmorpholine (8 equiv.) were added to a solution of Fmoc-GluPhe-OAll dipeptide (4 equiv.) in DMF. The Fmoc-GluPhe-OAll was prepared as outlined by Hanna *et al.*[3] After 5 min of pre-activation, the mixture was added to the resin. After 2 h, the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL), capped with acetic anhydride/pyridine (1:9, v/v) (2 × 3 min) and washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL). The peptide was then elongated *via* standard Fmoc-strategy SPPS as outlined in the general SPPS section. To the resin (25 µmol aliquot) was added a solution of $Pd(PPh_3)_4$ (25 mg, 22 µmol) and $PhSiH_3$ (123 µL, 1 mmol) in dry DCM (2 mL) and the resin was shaken for 1 h, then the resin was washed with DCM (10 x 5 mL) and DMF (5 x 5 mL). A solution of diphenyldiselenide (DPDS) (234 mg, 0.75 mmol, 30 equiv.) and tri-*n*-butylphosphine (184 µL, 0.75 mmol, 30 equiv.) in DMF (3 mL) was added to the resin. The resin was left at 0 °C in an ice bath for 3 h. Afterwards, the resin was washed with DMF (5 × 5 mL) and DCM (5 x 5 mL). For resin cleavage, a mixture of TFA: triisopropylsilane : H_2O (5 mL, 90:5:5 v/v/v) was added. After 2 h, the resin was washed with TFA (2 × 2 mL). The combined solutions were concentrated under a stream of nitrogen. The residue was dissolved in H_2O containing 30 % ACN + 0.1 % TFA, purified by preparative HPLC (0 to 60 % B over 30 min, 0.1 % TFA) and lyophilized to afford the desired peptide selenoester (17.1 mg, 63 %). See **Figure S19** for characterization.

Ac-LYRANM-SePh (**17**):



2-CTC resin (81 mg, 1.22 mmol/g loading) was swollen in dry DCM for 30 min then washed with DCM (5 × 3 mL). A solution of Fmoc-Met-OH (0.5 equiv. relative to resin functionalization) and *i*Pr$_2$NEt (2.0 equiv. relative to resin functionalization) in DCM (final amino acid concentration: 0.2 M) was added and the resin was shaken at RT for 16 h. The resin was washed with DMF (5 × 3 mL) and DCM (5 × 3 mL). The resin was capped with a solution of DCM/MeOH/*i*Pr$_2$NEt (17:2:1 v/v/v, 3 mL) for 1 h and washed with DMF (5 × 3 mL), DCM (5 × 3 mL), and DMF (5 × 3 mL). The peptide was then elongated *via* standard Fmoc-strategy SPPS as outlined in the general SPPS section. To cleave the protected peptide from the resin, the resin (25 µmol scale) was treated with hexafluoroispropanol (HFIP, 30 % in DCM, 3 mL) for 1 h. The resin was filtered and the filtrate was isolated. The resin was again treated with HFIP (30 % in DCM, 3 mL) for 1 hour, filtered, and the combined filtrates were concentrated under a stream of nitrogen. The remaining residue was

treated with a solution of diphenyldiselenide (DPDS) (234 mg, 0.75 mmol, 30 equiv.) and tri-*n*-butylphosphine (184 µL, 0.75 mmol, 30 equiv.) in DMF (3 mL). The reaction was left at 0 °C in an ice bath for 3 h, and then the solvent was evaporated under a stream of nitrogen. A mixture of TFA: triisopropylsilane : H_2O (5 mL, 90:5:5 v/v/v) was added at 0 °C to remove the side chain protecting groups and the reaction was left for 2 h. On completion, the reaction mixture was concentrated under a stream of nitrogen. The residue was dissolved in H_2O containing 30 % ACN + 0.1 % TFA, purified by preparative HPLC (0 to 50 % B over 30 min, 0.1 % TFA) and lyophilized to afford the desired peptide selenoester (20.8 mg, 45 % yield). See **Figure S20** for characterization.

Selenoester peptide characterization

Analytical HPLC was performed on a Waters System 2695 separations module with a 2996 photodiode array detector and an Alliance series column heater at 40 °C. Separations were carried out using a Waters Sunfire C-18 column (5 µm, 2.1 × 150 mm) at a flow rate of 0.2 mL/min. Buffer A: H_2O + 0.1 % TFA, buffer B: acetonitrile + 0.1 % TFA.

UPLC-MS analysis was performed using Shimadzu LC-30AD Liquid Chromatography pump modules and a DGU-20A5R degassing unit connected to a SPD-M30A Diode Array Detector. A CTO-20A Column Oven and Waters C-18 BEH 1.7 µm, 2.1 mm x 50 mm column were used at a 0.60 mL/min flow rate. A CBM-20A Communications Bus Module connected these S4 systems with a Nexera X2 SIL-30AC Autosampler and a Shimadzu 2020 Mass Spectrometer operating in positive mode. Samples were analyzed with a mobile phase of Milli-Q water containing 0.1 vol.% formic acid and HPLC grade acetonitrile containing 0.1 vol.% formic acid, operating with a linear gradient. Analysis was performed using Shimadzu LabSolutions LCMS software.

Oligonucleotide solid-phase synthesis

All oligonucleotide sequences were assembled with an Applied Biosystems Model 3400 synthesizer on a 2 µmol scale using standard β-cyanoethyl phosphoramidite cycles supplied by the manufacturer with slight modifications to coupling times as described below. The oligonucleotides were prepared using commercially available 3'-*O*-2'-deoxynucleoside phosphoramidites, containing standard protecting groups (N6-benzoyl-2'-deoxyadenosine, N4-benzoyl-2'-deoxycytidine and N2-isobutyryl-2'-deoxyguanosine), which were dissolved in anhydrous acetonitrile at a concentration of 0.1 M. Selenide phosphoramidite **6** was prepared in anhydrous acetonitrile/tetrahydrofuran 85:15 (v/v) at a concentration of 0.15 M. Oligonucleotide sequence assembly was carried out as previously described.[4,5] The capping step of the assembly was performed using acetic anhydride/pyridine/tetrahydrofuran 1:1:8 (v/v/v; solution A) and 1-methyl-imidazole/ tetrahydrofuran 16:84 (w/v; solution B). The coupling time for selenide phosphoramidite **6** was extended to 900 seconds (compared to 120 seconds for the commercially available phosphoramidites). The selenide oligonucleotides were synthesized without final acid treatment (DMTr-on) but the control oligonucleotide (dTCCCGTTTCCA) had their 5'-terminal trityl groups removed on the synthesizer by acid treatment.

Oligonucleotide deprotection and purification

The solid-phase column was removed from the synthesizer and half of the CPG-bound oligonucleotide was transferred to a 2 mL Teflon lined screw cap vial. Protected selenide oligonucleotide **7** and the control oligonucleotide (dTCCCGTTTCCA) were deprotected and cleaved from the solid support by treatment with 1 mL NH_4OH/EtOH (3:1; v/v) for 25 hours at 37 ºC (the lower temperature was chosen to avoid the thermal loss of the trityl group and the longer time chosen to ensure complete guanine deprotection, the rate-limiting step of the reaction), whereas protected selenide oligonucleotide **14** was treated with 1.5 mL NH_4OH/EtOH (3:1; v/v) for 25 hours at 37 ºC (to minimize thymine acrylonitrile adduct formation). After the incubation period, the supernatant was transferred to a separate screw cap vial and the CPG washed twice with 200 µL aqueous acetonitrile (50 %; v/v). The crude oligonucleotides were lyophilized in a speed-vac concentrator.

All oligonucleotides were purified by ion exchange (IEX) HPLC using a Dionex DNAPAC PA-100 column (0.4 cm x 25 cm) purchased from Dionex Corp (Sunnyvale, CA). The column was eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl). The column was monitored at 260 nm for analytical runs (0.1-1.0 OD_{260} injected) and 260 nm and 280 nm for preparative runs (20-30 OD_{260} injected). The purified control oligonucleotide was desalted on a C-18 SEP PAK cartridge (Waters): 2×10 mL 18 MΩ H_2O. Tritylated diselenide oligonucleotide **7** was loaded onto the C-18 SEP PAK cartridge for detritylation and desalting: 3× 5 mL washes of 2 % TFA/H_2O (v/v), with a flow rate of 2.5 mL/min, followed by 2×10 mL 18 MΩ H_2O washes. Tritylated diselenide oligonucleotide **14** was loaded onto a C-18 SEP PAK cartridge for detritylation and desalting: alternating washes of 5 mL 2 % TFA/H_2O (v/v) and 5 mL 18 MΩ H_2O, where each was performed a total of 3 times. The oligonucleotides were eluted from the cartridge by washing with 4 mL 1:1:2 H_2O/ACN/MeOH (v/v/v). Diselenide oligonucleotide **14** was eluted with the same mixture, but with an additional 0.5 % NH_4OH (to prevent depurination). The samples were lyophilized in a speed-vac concentrator, dissolved in 18 MΩ H_2O and stored long-term at -20 °C.

Oligonucleotide characterization by LC-MS

LC-MS analyses of oligonucleotides were obtained at the Concordia University Centre for Biological Applications of Mass Spectrometry (CBAMS) using an Agilent 1100 LC system coupled to a Thermo LTQ Orbitrap Velos mass spectrometer equipped with a heated electrospray ion source in negative mode. A Spursil C18-EP column (50×2.1 mm and 3 µm particle diameter, Dikma Technologies) was used and oligonucleotides were eluted using a 20 minute gradient at an initial flow rate of 250 µL/min with mobile phase A (10 mM ammonium acetate and 1 mM ammonium fluoride water solution) and B (acetonitrile). The gradient started at 2 % B and held for 3 min, linear gradients were achieved to 50 % B at 8 min, to 90 % B at 10 min, then followed

by isocratic with 90 % B for 2 min. The column was reconditioned from 13 min with 2 % B at a flow rate of 400 µL/min for 5 min and at 250 µL/min for extra 2 min. Dried samples were reconstituted in 50 µL of mobile phase A and the injection volume was 10 µL. The divert valve was set at 0 min to the waste, and at 4.0 min to the detector. MS spectra (*m/z* 300-2000) were acquired in the Orbitrap at a resolution of 60,000. The uncharged monoisotopic mass of oligonucleotides were calculated using Thermo FreeStyle™ software (v1.7 SP2).

UV thermal denaturation studies of i-motif DNA

Molar extinction coefficients for the unmodified and diselenide oligonucleotides were calculated using the nearest-neighbor approximations (M^{-1} cm^{-1}) of the mononucleotides and dinucleotides. The 5'-functionality was assumed to minimally absorb at 260 nm and not included in the calculation. The calculated molar extinction coefficient of the monomer strand was doubled for the diselenide oligonucleotides. The control oligonucleotide, diselenide oligonucleotide **7** and POC **13** (1.0 A_{260} units each) were lyophilized to dryness in a speed-vac concentrator. The resulting pellets were dissolved in 1 mL buffer (pH 5.5) containing 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) and 100 mM KCl, to give a final i-motif concentration of 5.3 µM. Prior to the thermal run, samples were degassed in a speed-vac concentrator for 2 minutes. Samples were held at 95 °C for 5 minutes to ensure complete unfolding. Annealing profiles were acquired at 295 nm at a rate of cooling of 0.5 °C/min, from 95 to 15 °C, using a Varian CARY Model 3E spectrophotometer equipped with a 6 sample thermostated cell block and a temperature controller. The data were analyzed according to the published procedure from Puglisi and Tinoco[6] and transferred to Microsoft Excel™ software.

Circular dichroism (CD) spectroscopy of i-motif DNA

Circular dichroism spectra were obtained on a Jasco J-815 spectropolarimeter equipped with a Julaba F25 circulating bath. Samples were allowed to equilibrate for 5 minutes at 15 °C in 1 mL buffer (pH 5.5) containing 10 mM MES and 100 mM KCl, to a final i-motif concentration of 5.3 µM. Each spectrum was an average of 5 scans, collected at a rate of 20 nm/min, with a bandwidth of 1 nm and sampling wavelength of 0.2 nm using fused quartz cells (Starna 29-Q-10). The spectra were recorded from 320 to 200 nm at 15 °C. The molar ellipticity [θ] was calculated from the equation $[\theta] = \varepsilon/Cl$, where ε is the relative ellipticity (mdeg), C is the molar concentration of the i-motif DNA (M), and l is the path length of the cell (cm). The data were processed using software supplied by the manufacturer (JASCO, Inc.) and transferred to Microsoft Excel™ software.

General procedure for reductive diselenide-selenoester ligation (rDSL)-deselenization to prepare peptide-oligonucleotide conjugates (POCs)

Prior to using any buffer, they were sparged with argon for 10 minutes to adequately remove any dissolved O_2 (**this applies to all DSL procedures**). The ligation buffer containing tris(2-carboxyethyl)phosphine (TCEP) and diphenyl diselenide (DPDS) was sonicated for 30 minutes before use to ensure optimal dissolution of phenylselenolate.

A solution of diselenide oligonucleotide (50 nmol; 1 equiv.; 500 μM) and selenoester peptide (200 nmol; 2 equiv. with respect to monomer oligonucleotide; 2 mM) in ligation buffer (0.1 M MES, pH 5.5, 100 μL) containing 30 mM TCEP and 20 mM DPDS was prepared and incubated at 37 °C. Aliquots (1 μL taken after 5 minutes, 1 hour, 2 hours and 3 hours) were quenched with 1 μL hydrazine monohydrate (0.0206 mmol) at RT for 7 minutes before adding 50 μL 2 % TFA/H_2O (0.261 M; 0.0131 mmol) and diluting up to 500 μL with 18 MΩ H_2O (448 μL). This solution was then extracted with diethyl ether (3 × 500 μL) and the residual ether evaporated over a stream of argon before IEX HPLC injection. IEX HPLC details: the column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

Once ligation was complete after 3 hours (confirmed by IEX HPLC), the reaction mixture was extracted with diethyl ether (3 × 500 μL) and the residual ether evaporated over a stream of argon. To the reaction mixture was then added 100 μL of a solution of 0.1 M MES (pH 6.7) containing 50 mM TCEP (5000 nmol; 50 equiv. with respect to monomer oligonucleotide) and 50 mM DTT (5000 nmol; 50 equiv. with respect to monomer oligonucleotide). This solution was sparged with argon for 10 minutes, before incubating at 37 °C under an argon atmosphere. Aliquots (2 μL taken after 1 hour, 2 hours and 3 hours; no post-treatment) were diluted up to 500 μL with 18 MΩ H_2O (498 μL) for IEX HPLC injection. Once deselenization was complete after 3 hours (confirmed by IEX HPLC), the resulting POC was purified by IEX HPLC (or reverse-phase and IEX HPLC in the case of POC **13**) and subsequently desalted on a C-18 SEP PAK cartridge (Waters): 2×10 mL 18 MΩ H_2O washes. **Should deselenization be sluggish, extracting the reaction mixture once more with diethyl ether (to remove any remaining DPDS) and adding a second portion of equal volume of deselenization buffer will aid in the reaction progress.

Reverse-phase (RP) HPLC of the crude POC sample allowed for yield determination, comparing the areas of the POC and deselenized/reduced oligonucleotide starting material. RP HPLC details: the C-18 column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-60 % buffer B over 30 minutes (buffer A: 50 mM sodium phosphate, pH 5.8, 2 % acetonitrile; buffer B: 50 mM sodium phosphate, pH 5.8, 50 % acetonitrile).

Additive-free DSL-alkylation for the preparation of POC micelles

A solution of diselenide oligonucleotide **7** (50 nmol; 1 equiv.; 500 μM) and selenoester peptide **8** (200 nmol; 2 equiv. with respect to monomer oligonucleotide; 2 mM) in ligation buffer (0.1 M MES, pH 5.5, 100 μL) was incubated at 37 °C for 4 hours. Once the ligation was complete (confirmed by IEX HPLC), the reaction mixture was extracted with diethyl ether (3 × 500 μL) and the residual ether evaporated over a stream of argon. To the reaction mixture was then added 100 μL of a solution of 0.1 M MES (pH 6.7) containing 150 mM DTT (15000 nmol; 150 equiv. with respect to monomer oligonucleotide) followed by 200 μL of a 0.25 M solution of bromodecane (50000 nmol; 500 equiv. with respect to monomer oligonucleotide) in DMF (final monomer

oligonucleotide concentration: 250 μM). The resulting mixture was vortexed, centrifuged and incubated at 37 °C for 23 hours. Aliquots (5 μL taken after 5 minutes, 1 hour, 3 hours, and 23 hours; no post-treatment) were analyzed by IEX HPLC. After 23 hours of reaction time, the sample was lyophilized in a speed-vac concentrator on high heat.

Alkylation of diselenide oligonucleotides

To a lyophilized sample of diselenide oligonucleotide **7** (50 nmol) was added 50 μL of 0.1 M MES buffer (pH 6.7) containing 300 mM DTT (15000 nmol; 150 equiv. with respect to monomer oligonucleotide). To this solution was added 50 μL of a 1.0 M solution of bromodecane (50000 nmol; 500 equiv. with respect to monomer oligonucleotide) in DMF (final monomer oligonucleotide concentration: 1 mM). The reaction mixture was vortexed, centrifuged and incubated at 37 °C for 22 hours. Aliquots (2 μL taken after 5 minutes, 1 hour, 3 hours, 5 hours, 7 hours and 22 hours; no post-treatment) were analyzed by IEX HPLC. After 48 hours of reaction time, the sample was lyophilized in a speed-vac concentrator on high heat.

To a lyophilized sample of diselenide oligonucleotide **7** (5 nmol) was added 25 μL of 0.1 M MES buffer (pH 6.7) containing 300 mM DTT (7500 nmol; 750 equiv. with respect to monomer oligonucleotide). To this solution was added 25 μL of a 0.20 M solution of bromopentane (5000 nmol; 500 equiv. with respect to monomer oligonucleotide) in DMF (final monomer oligonucleotide concentration: 200 μM). The reaction mixture was vortexed, centrifuged and incubated at 37 °C for 3 hours. Aliquots (5 μL taken after 1 hour and 3 hours; no post-treatment) were analyzed by IEX HPLC and LC-MS.

To a lyophilized sample of diselenide oligonucleotide **14** (10 nmol) was added 50 μL of 0.1 M MES buffer (pH 6.7) containing 300 mM DTT (15000 nmol; 750 equiv. with respect to monomer oligonucleotide). To this solution was added 50 μL of a 0.10 M solution of 4-bromomethyl-6,7-dimethoxycoumarin (5000 nmol; 250 equiv. with respect to monomer oligonucleotide) in DMF (final monomer oligonucleotide concentration: 200 μM). The reaction mixture was vortexed, centrifuged and incubated at 37 °C. Once alkylation was complete after 1 hour (confirmed by IEX HPLC), the sample was quenched by the addition of 18 MΩ H_2O (500 μL), lyophilized in a speed-vac concentrator on high heat, resuspended in 18 MΩ H_2O (200 μL), centrifuged and the supernatant collected for purification by IEX HPLC. The pure dimethoxycoumarin labelled oligonucleotide was analyzed by LC-MS, UV-Vis (Varian CARY Model 3E spectrophotometer) and fluorescence (Varian CARY Eclipse spectrophotometer; excitation and emission slits: 10 nm) spectroscopy.

Dynamic light scattering (DLS) and transmission electron microscopy (TEM) of micelles

DLS measurements were acquired at the Concordia University Centre for NanoScience Research on a Malvern Zetasizer Nano ZSP at 25 °C. Following alkylation of diselenide oligonucleotide **7** and POC **13** with bromodecane, a 100 μM micelle solution was prepared by adding 1 mL 0.1 M

MES buffer (pH 6.7) to the lyophilized sample (containing 100 nmol monomer oligonucleotide from 50 nmol diselenide). The sample was vortexed, centrifuged and left to incubate for 3 hours at RT before DLS measurements. The control oligonucleotide (dTCCCGTTTCCA) was prepared in the same way, without prior alkylation.

A 5 μL drop of sample (100 μM in 0.1 M MES buffer, pH 6.7) was drop cast onto a 200-mesh Cu TEM grid with carbon support film (Agar Scientific, Stansted, UK). After 10 minutes, the excess solution was wicked off with filter paper. The grid was immediately stained with a 5 μL drop of 2 % aqueous uranyl acetate. After 1 minute, the excess solution was wicked off with filter paper and the grid was allowed to dry under ambient conditions. The TEM grid was imaged at the McGill University Facility for Electron Microscopy Research (FEMR) by an FEI Tecnai G^2 Spirit 120 kV TEM (FEI Co., Hillsboro, OR) equipped with a Gatan Ultrascan 4000 CCD Camera Model 895 (Gatan Inc., Warrendale, PA). The proprietary Digital Micrograph 16-bit images (DM3) were converted to unsigned 8-bit TIFF images.

Dealkylation of oligonucleotide micelles

Half of the micelle sample (50 nmol), generated from the alkylation of diselenide oligonucleotide **7** with bromodecane**,** was lyophilized in a speed-vac concentrator. To this pellet was added 20 μL of 18 MΩ H_2O. The sample was vortexed, centrifuged and incubated at RT for 1 hour to provide adequate time for the micelles to self-assemble. To this solution was added 80 μL of a solution of 100 mM H_2O_2 (8000 nmol; 160 equiv.; 80 mM) in 18 MΩ H_2O (final monomer oligonucleotide concentration: 500 μM). The reaction mixture was vortexed, centrifuged and incubated at 37 °C for 10 minutes. An aliquot (5 μL taken after 10 minutes; no post-treatment) was lyophilized in a speed-vac concentrator and analyzed by LC-MS.

Figure S1: Model of the NMR solution structure of the 5'-d**T**CCCGTTTCCA-3' dimer (PDB: 1C11).[7] A) Top-down view. B) Side view. The 5'-thymidine of each individual strand is labelled for clarity.

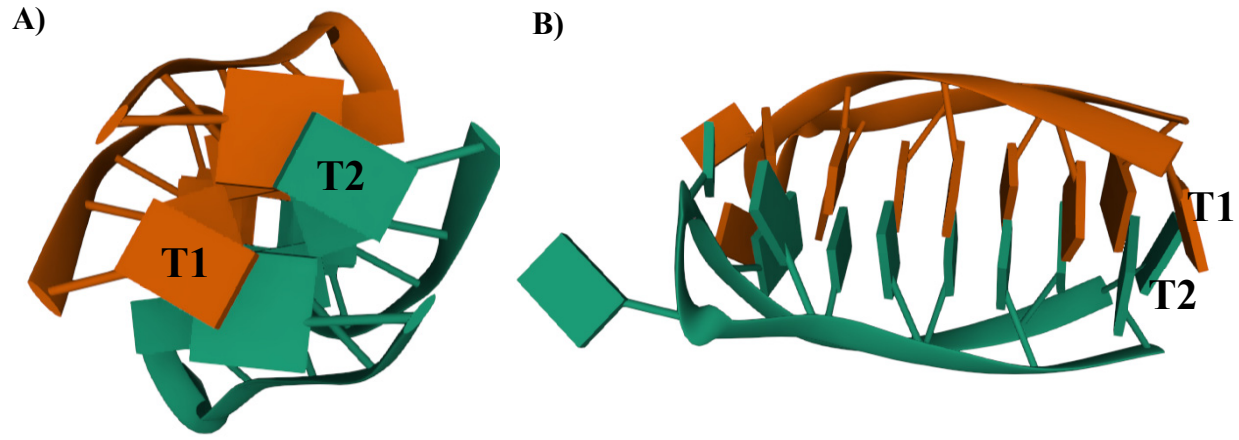


Figure S2: IEX HPLC traces of crude A) dTCCCGTTTCCA oligonucleotide and B) tritylated diselenide oligonucleotide **7**. The shoulder peak corresponding to oligonucleotide with a thymine acrylonitrile adduct is identified with an arrow. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

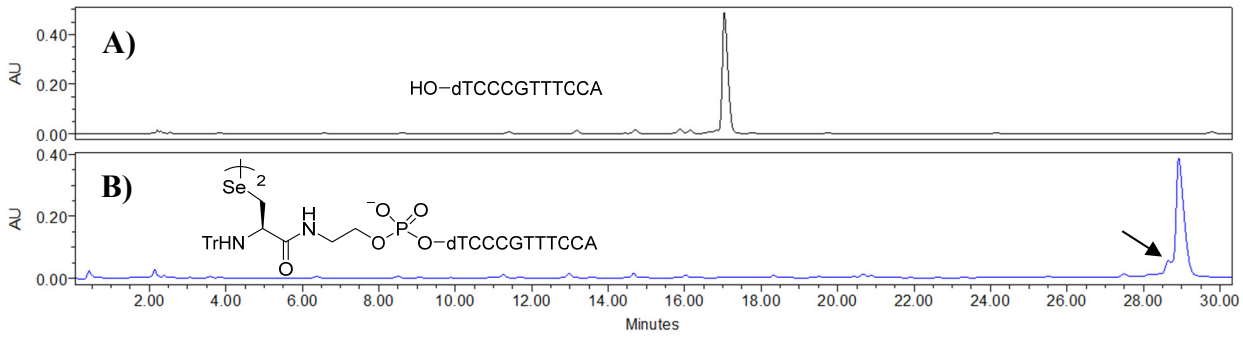


Figure S3: ESI-MS spectrum of crude tritylated diselenide oligonucleotide **7** (expected mass: 7513.30). Also observed, the thymine acrylonitrile adduct oligonucleotide (expected mass: 7566.30)

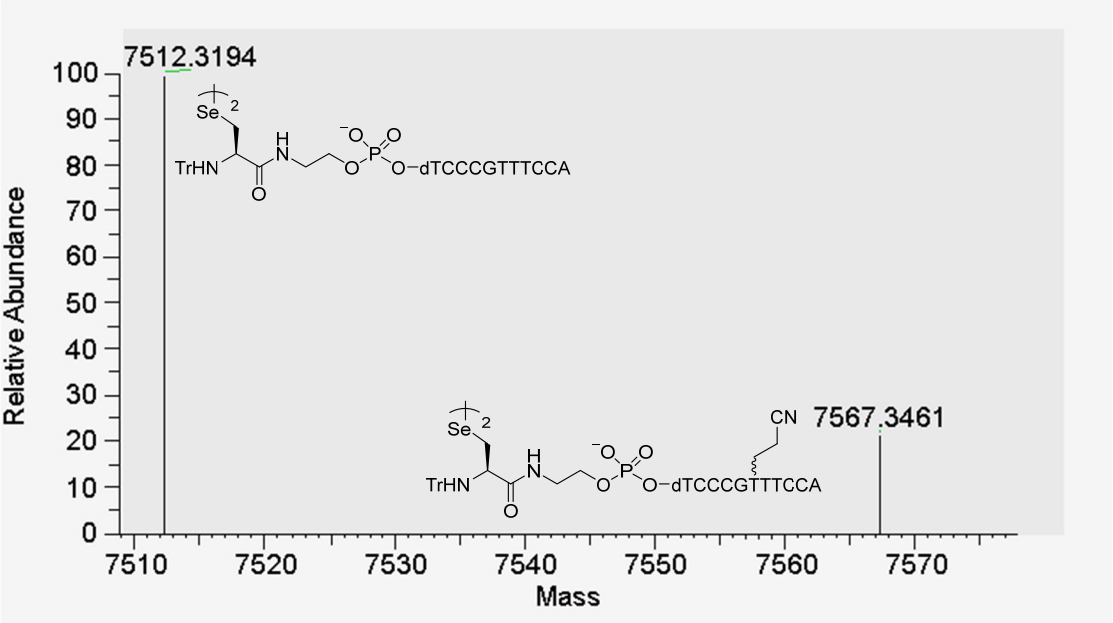


Figure S4: IEX HPLC trace of pure diselenide oligonucleotide **7** after only one 2 % TFA/H_2O wash. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

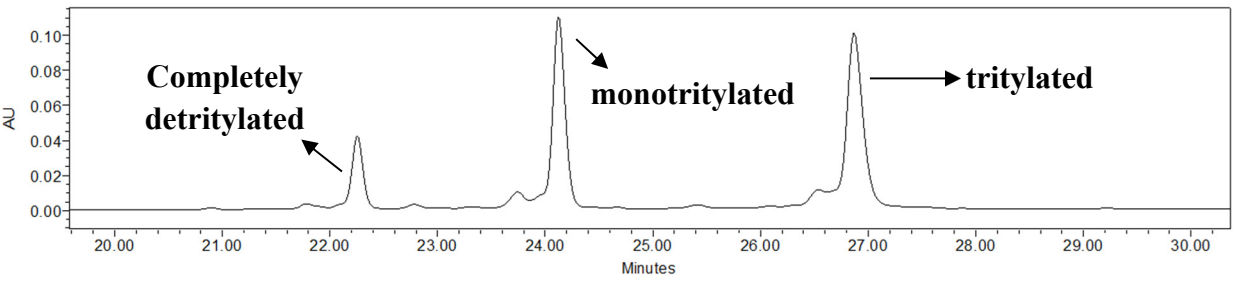


Figure S5: IEX HPLC trace of pure detritylated and desalted diselenide oligonucleotide **7**. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

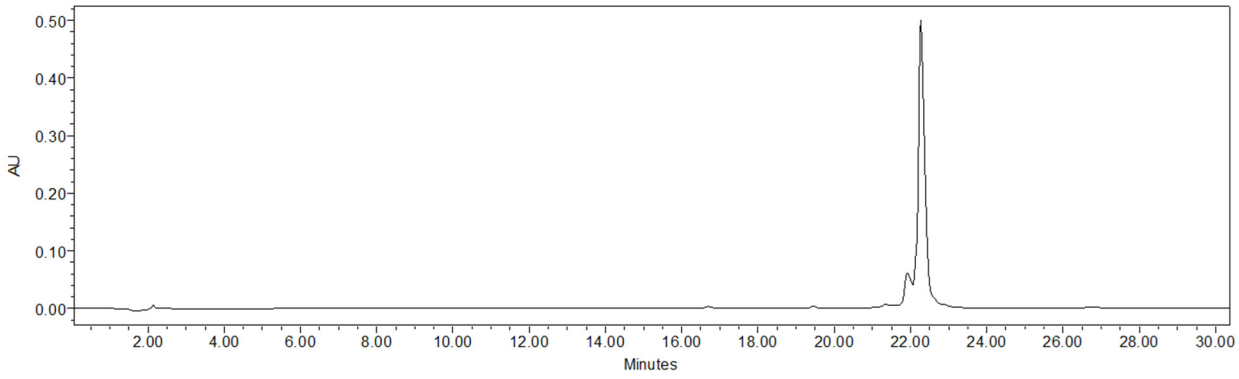


Figure S6: ESI-MS spectrum of pure diselenide oligonucleotide **7** (expected mass: 7029.05). Also observed, depurinated oligonucleotide (loss of 3'-adenine; expected mass: 6912.01). AP = apurinic site. $[M - 5H]^{5-}$ for diselenide oligonucleotide **7** shown in inset.

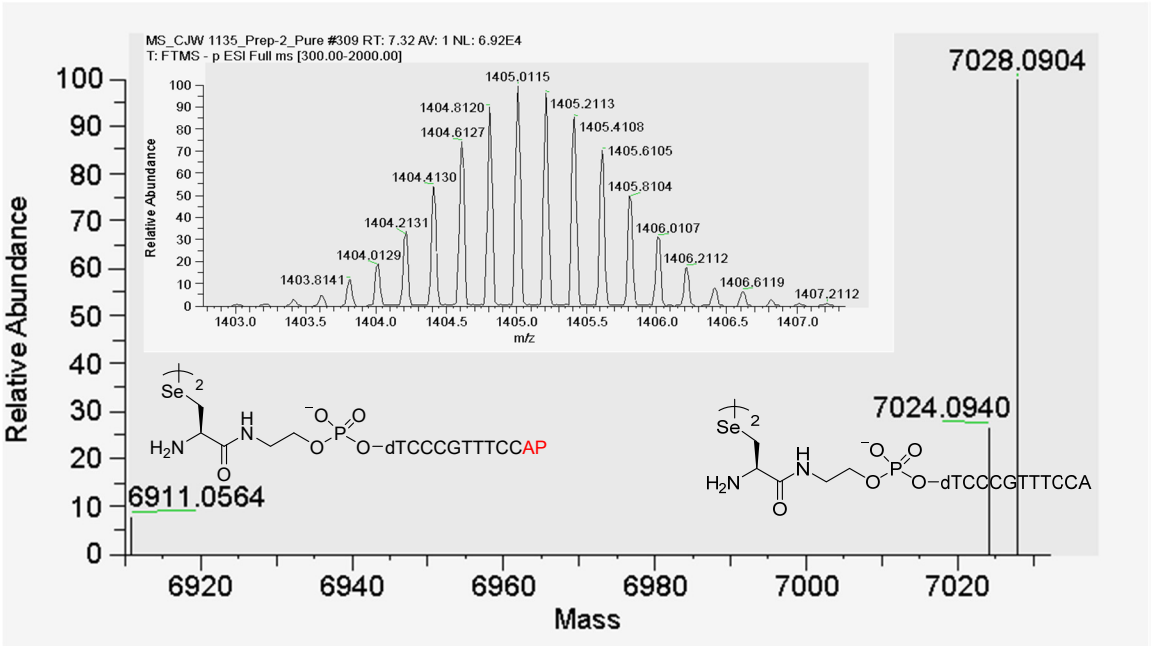


Figure S7: IEX HPLC traces of crude A) tritylated diselenide oligonucleotide **7** and B) tritylated diselenide oligonucleotide **14**. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

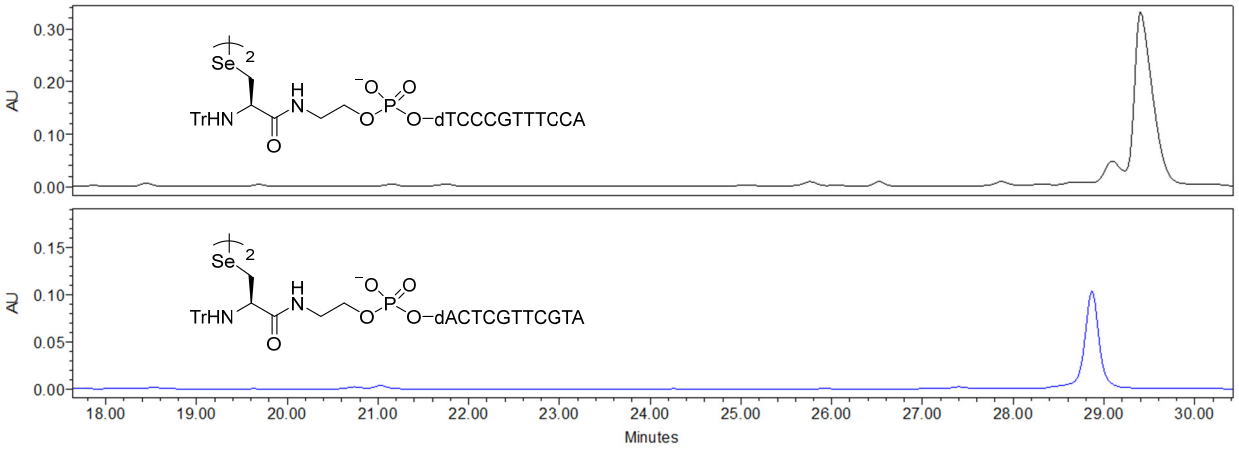


Figure S8: IEX HPLC trace of pure detritylated and desalted diselenide oligonucleotide **14**. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

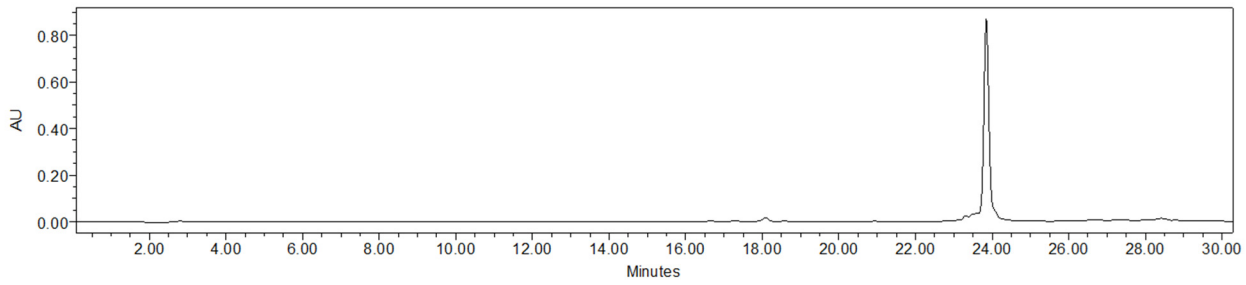


Figure S9: ESI-MS spectrum of pure diselenide oligonucleotide **14** (expected mass: 7157.08). [M - 5H]$^{5-}$ for diselenide oligonucleotide **14** shown in inset.

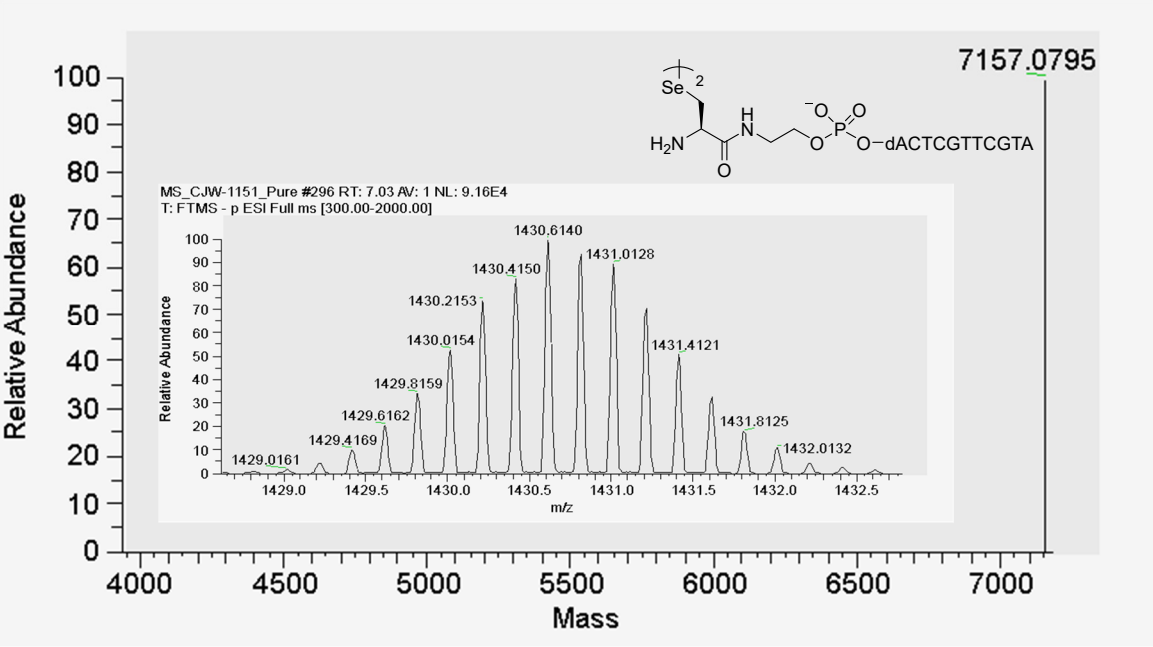


Figure S10: ESI-MS spectrum of 1 hour aliquot of rDSL between diselenide oligonucleotide **7** and selenoester peptide **8**. Intermediates **9-12** are identified.

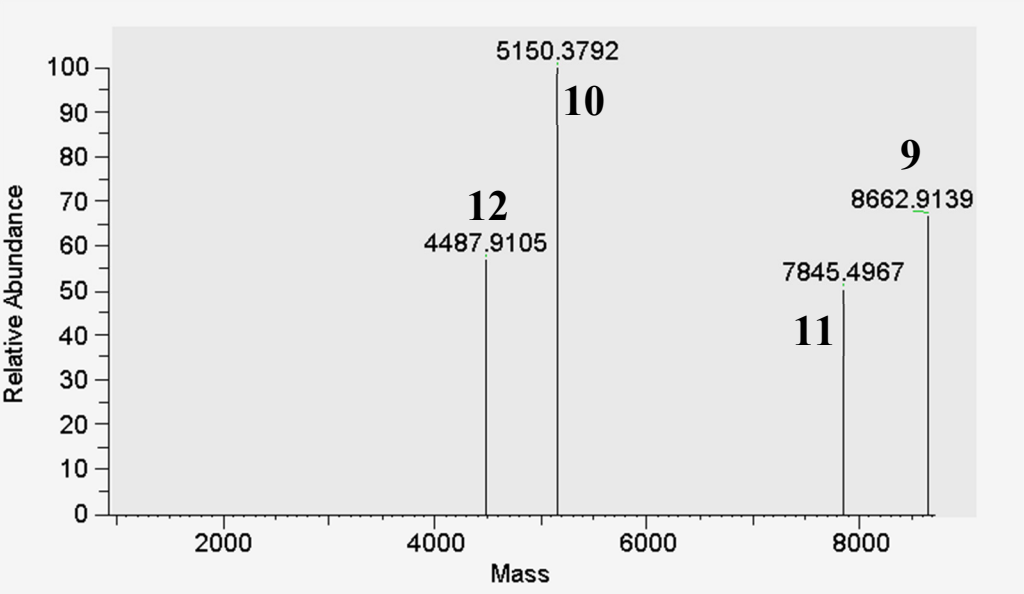


Figure S11: ESI-MS spectrum of 3 hour aliquot of rDSL between diselenide oligonucleotide **7** and selenoester peptide **8**. Intermediates **9**, **11** and **12** are identified.

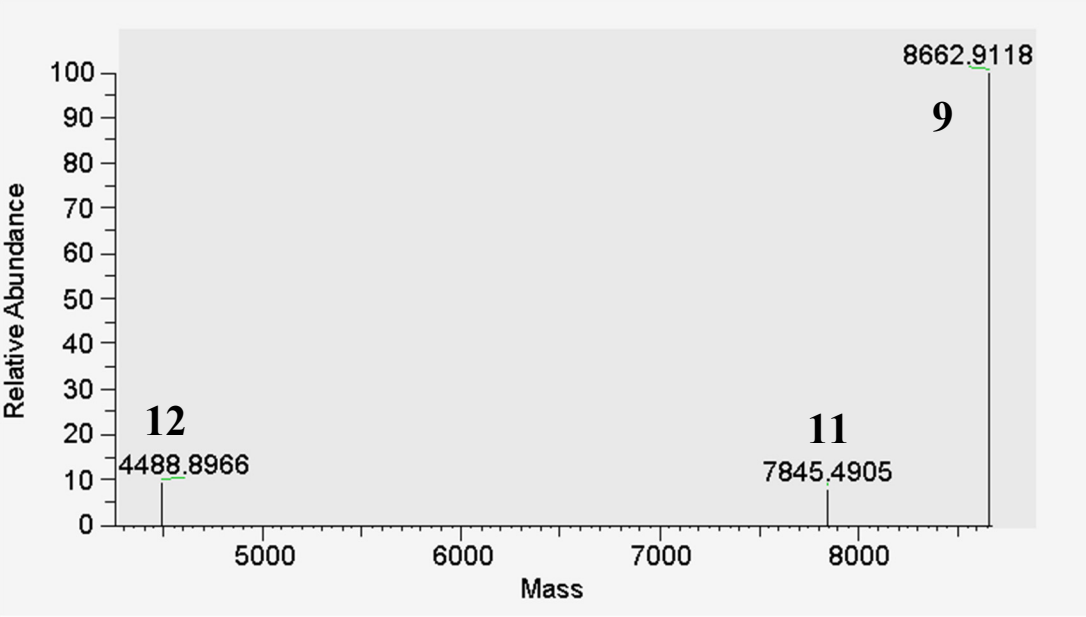


Figure S12: ESI-MS spectrum of 3 hour aliquot of deselenization following rDSL between diselenide oligonucleotide **7** and selenoester peptide **8** (expected mass for POC **13**: 4253.02). $[M - 3H]^{3-}$ for POC shown in inset.

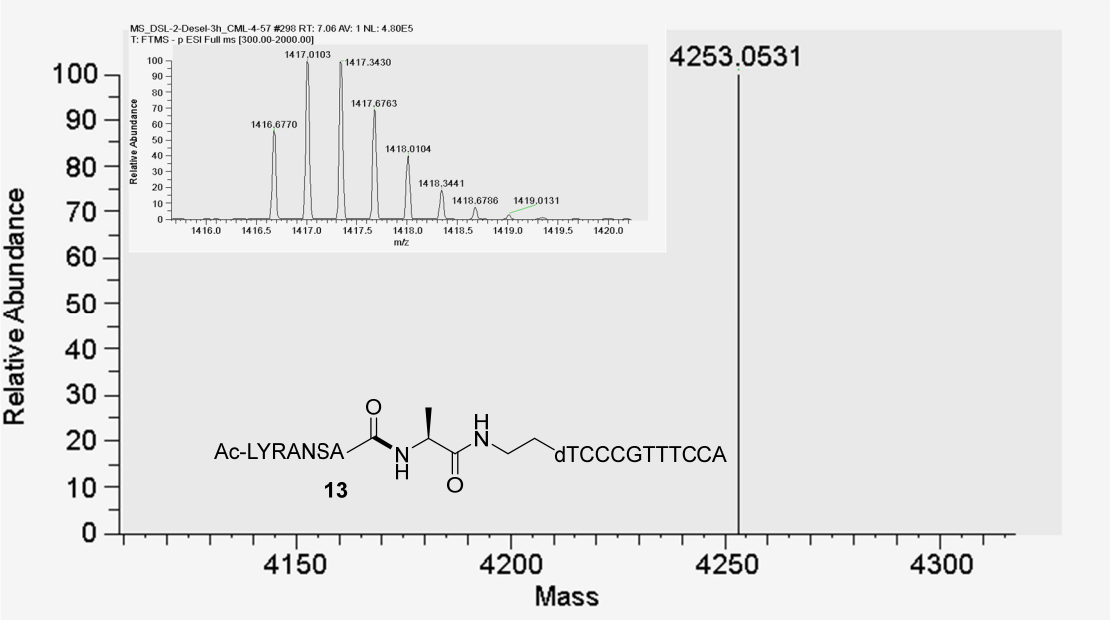


Figure S13: RP HPLC of crude POC **13** after 3 hour rDSL and 3 hour deselenization. The C-18 column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-60 % buffer B over 30 minutes (buffer A: 50 mM sodium phosphate, pH 5.8, 2 % acetonitrile; buffer B: 50 mM sodium phosphate, pH 5.8, 50 % acetonitrile).

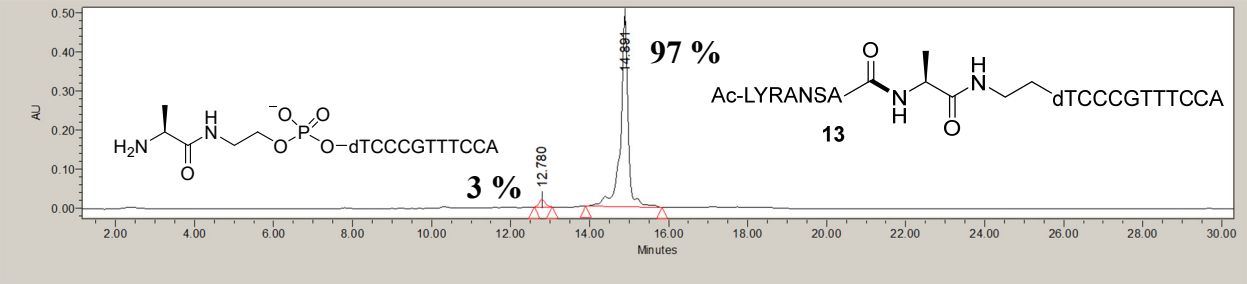


Figure S14: IEX HPLC traces of A) crude POC **13** B) RP-purified POC **13** and C) RP and IEX purified POC **13**. The deselenized starting material is identified with an arrow. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

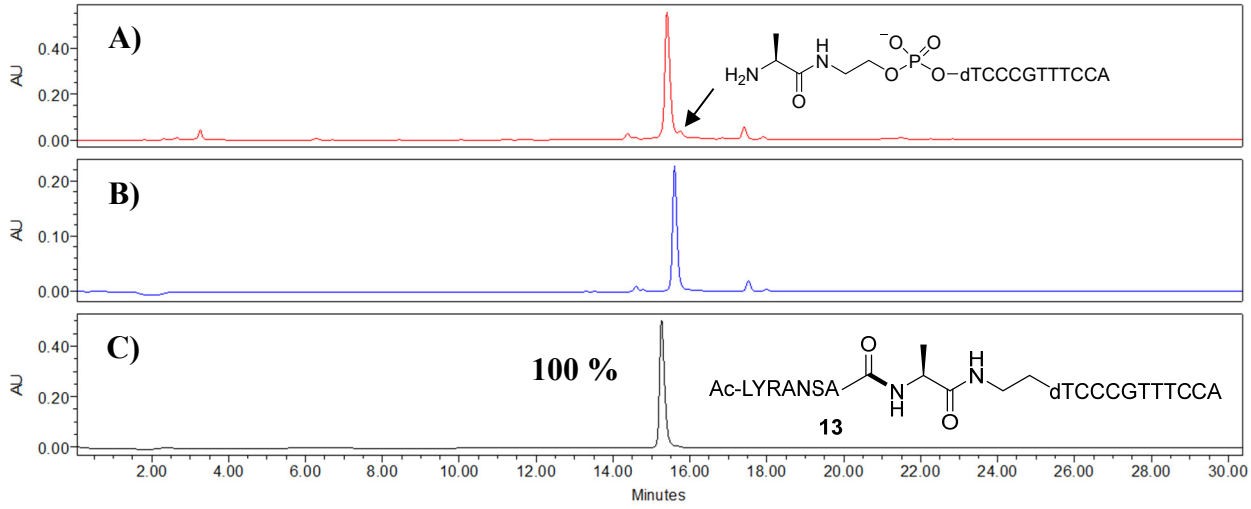


Figure S15: UV-thermal denaturation curves of i-motif oligonucleotides. The melting temperatures (T_m) of each i-motif are shown in the inset. Solutions containing a final i-motif concentration of 5.3 µM were prepared in a pH 5.5 buffer consisting of 10 mM MES and 100 mM KCl. Data was acquired at 0.5 °C/min from 95 °C to 15 °C, monitoring UV absorption at 295 nm. Native = 5'-dTCCCGTTTCCA-3' oligonucleotide.

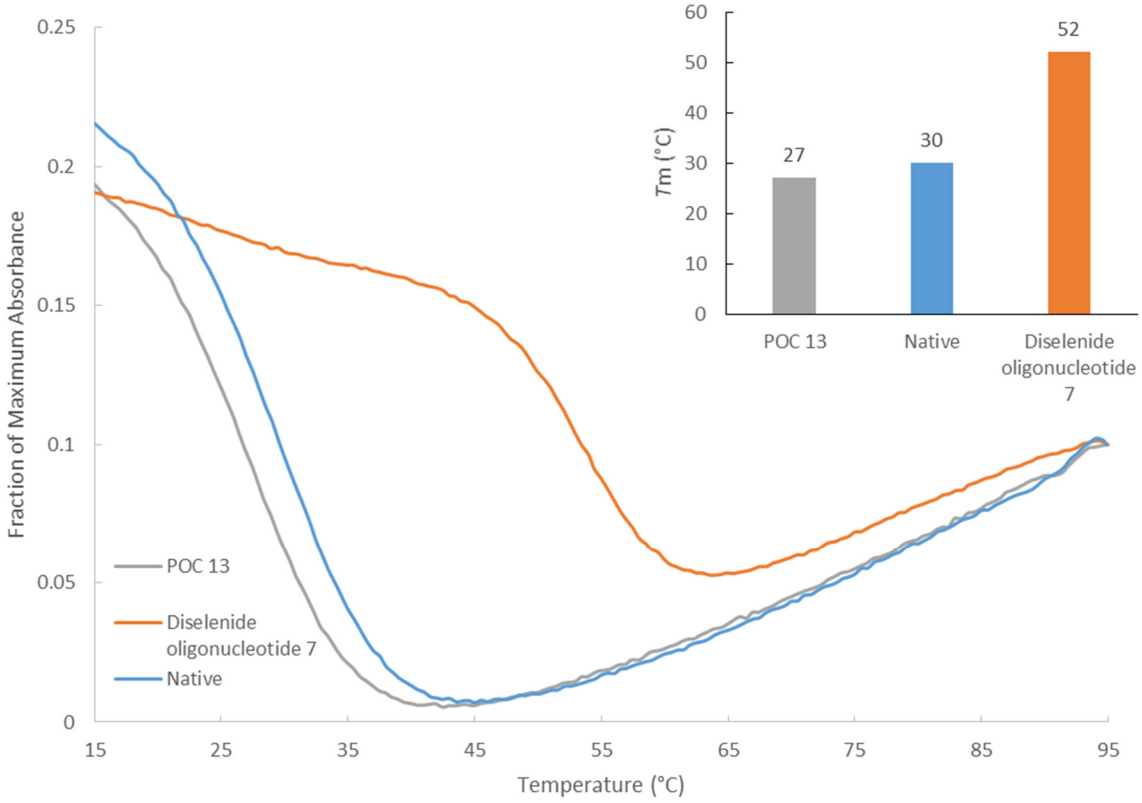


Figure S16: Far-UV circular dichroism spectra of i-motif oligonucleotides. Solutions containing a final i-motif concentration of 5.3 µM were prepared in a pH 5.5 buffer consisting of 10 mM MES and 100 mM KCl. Native = 5'-dTCCCGTTTCCA-3' oligonucleotide.

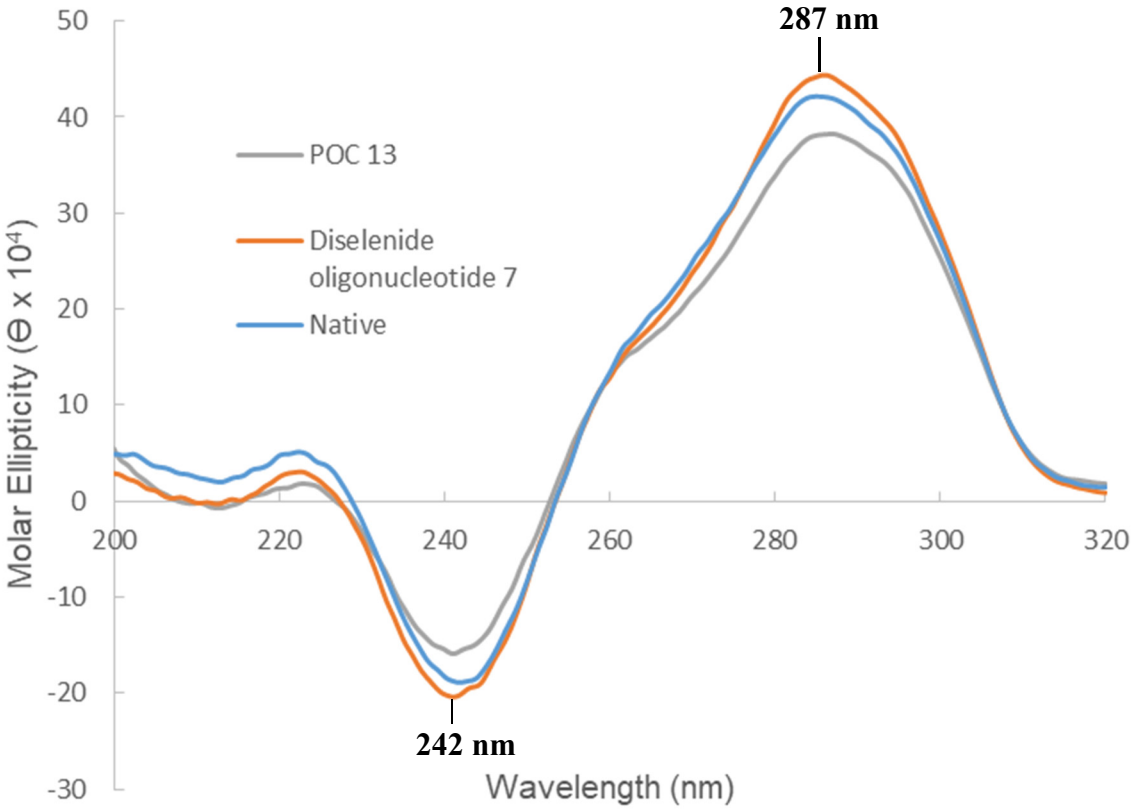


Figure S17: A) Analytical HPLC trace of pure Ac-LYRANSA-SePh (**8**) and B) ESI-MS spectrum of pure **8**; R_t 4.44 min (0-60 % B over 5 min, λ = 214 nm); Calc. Mass $[M + H]^+$: 976.4, $[M + 2H]^{2+}$: 488.7; Found; $[M + H]^+$: 976.3, $[M + 2H]^{2+}$: 488.8. *$[M(-SePh) + 2H]^{2+}$: 410.5 (artefact ion).

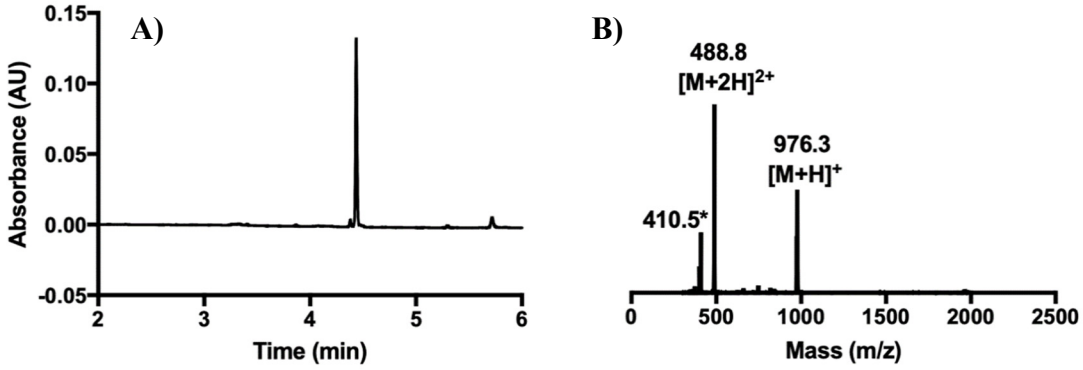


Figure S18: A) Analytical HPLC trace of pure Ac-LYRANYF-SePh (**15**) and B) ESI-MS spectrum of pure **15**; R_t 5.18 min (0-60 % B over 5 min, λ = 214 nm); Calc. Mass $[M + H]^+$: 1128.4, $[M + 2H]^{2+}$ 564.7; Found; $[M + H]^+$: 1128.2, $[M + 2H]^{2+}$: 564.6.

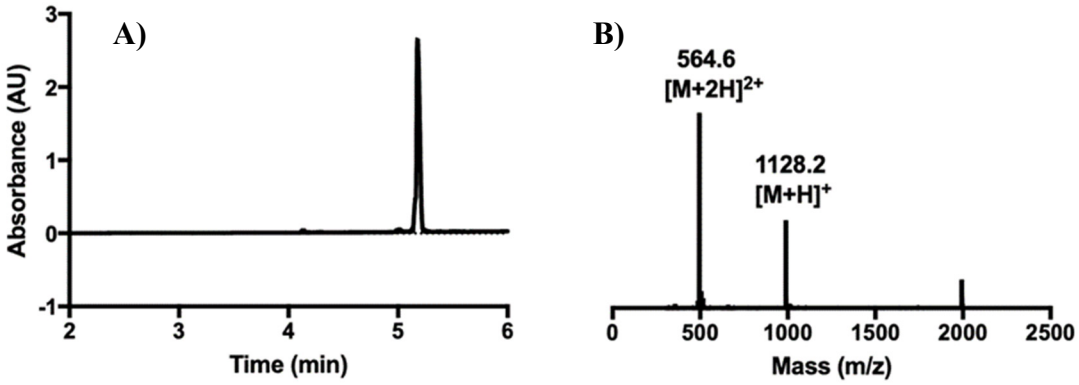


Figure S19: A) Analytical HPLC trace of pure Ac-LYRANQF-SePh (**16**) and B) ESI-MS spectrum of pure **16**; Rt 4.84 min (0-60 % B over 5 min, λ = 214 nm); Calc. Mass $[M + H]^+$: 1093.4, $[M + 2H]^{2+}$: 547.2; Found; $[M + H]^+$: 1093.1, $[M + 2H]^{2+}$: 547.0. *$[M(-SePh) + 2H]^{2+}$: 469.3 (artefact ion).

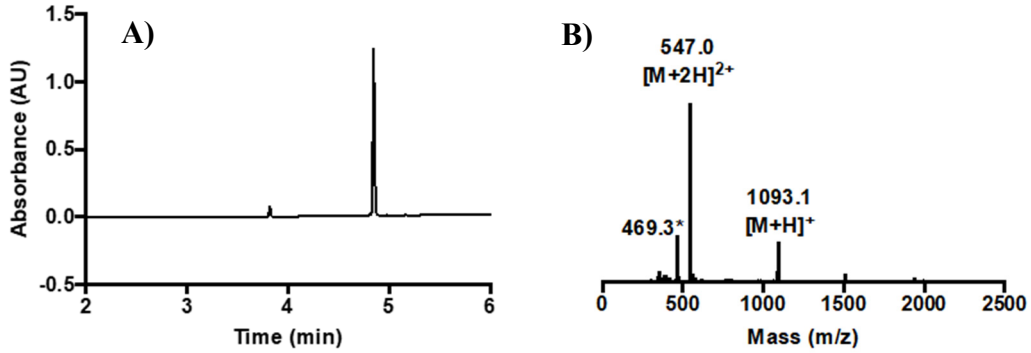


Figure S20: A) Analytical HPLC trace of pure Ac-LYRANM-SePh (**17**) and B) ESI-MS spectrum of pure **17**; Rt 5.08 min (0-60 % B over 5 min, λ = 214 nm); Calc. Mass $[M + H]^+$: 949.3, $[M + 2H]^{2+}$: 475.2; Found; $[M + H]^+$: 949.5, $[M + 2H]^{2+}$: 475.3.

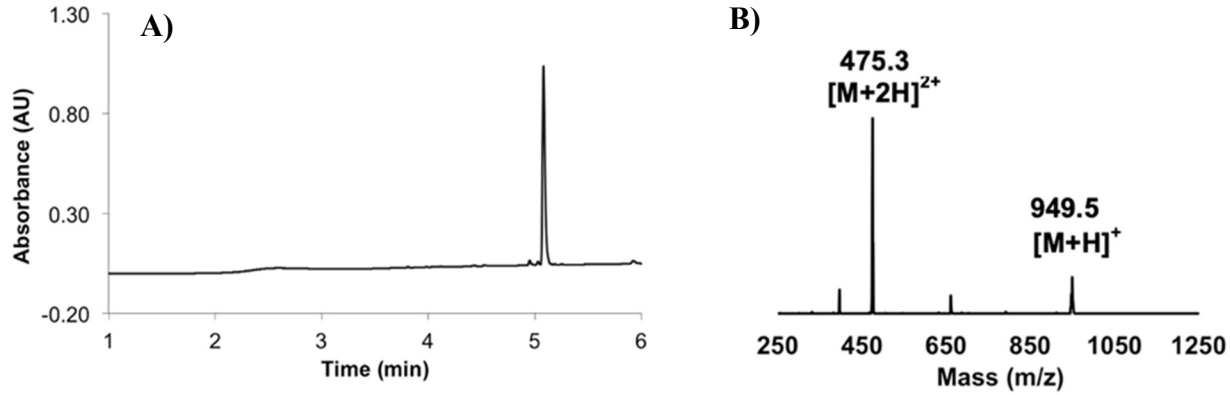


Figure S21: IEX HPLC traces of A) pure diselenide oligonucleotide **14** B) 3 hour aliquot of rDSL between diselenide oligonucleotide **14** and selenoester peptide **8** (Ac-LYRANSA-SePh) and C) 3 hour aliquot of deselenization following rDSL. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

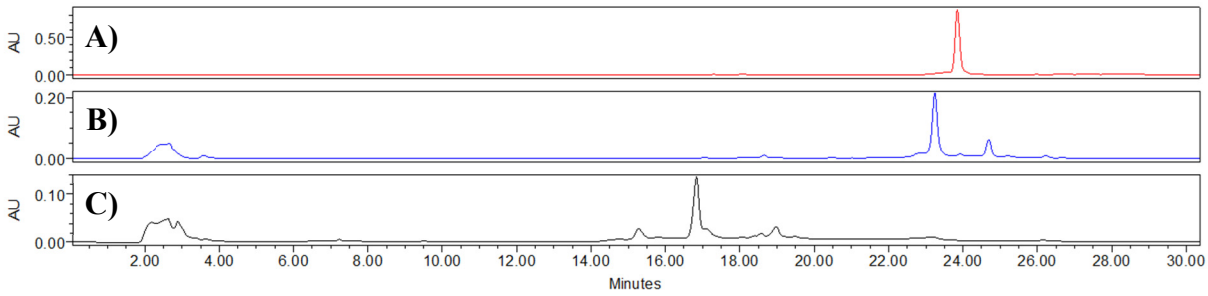


Figure S22: IEX HPLC traces of A) pure diselenide oligonucleotide **14** B) 3 hour aliquot of rDSL between diselenide oligonucleotide **14** and selenoester peptide **15** (Ac-LYRANYF-SePh) and C) 3 hour aliquot of deselenization following rDSL. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

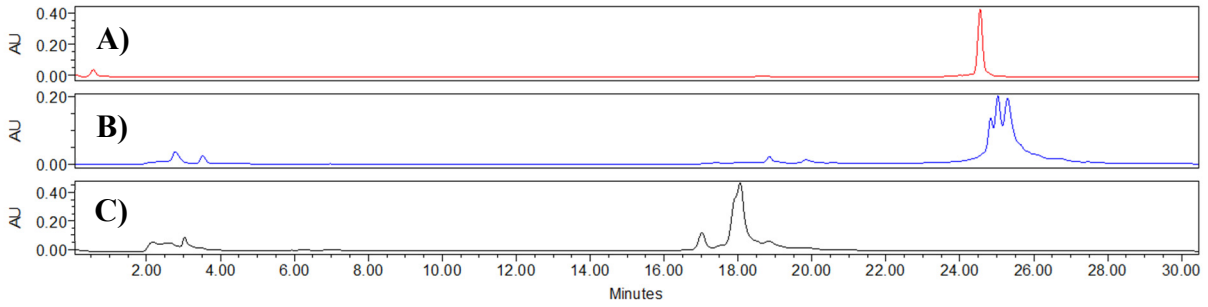


Figure S23: IEX HPLC traces of A) pure diselenide oligonucleotide **14** B) 3 hour aliquot of rDSL between diselenide oligonucleotide **14** and selenoester peptide **16** (Ac-LYRANQF-SePh) and C) 3 hour aliquot of deselenization following rDSL. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

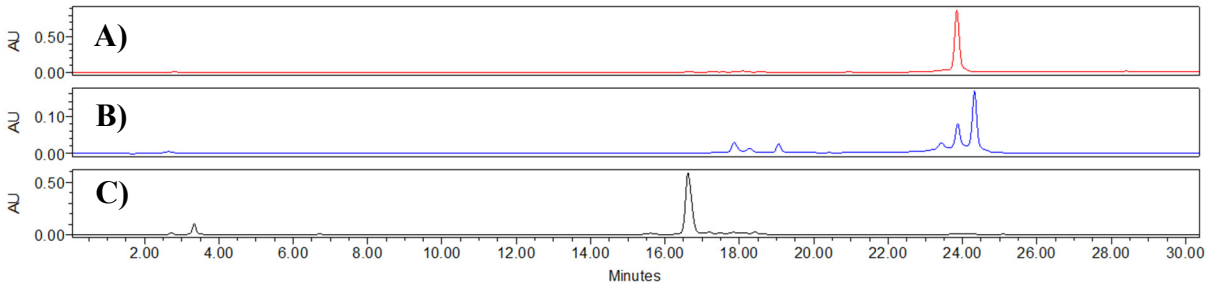


Figure S24: IEX HPLC traces of A) pure diselenide oligonucleotide **14** B) 3 hour aliquot of rDSL between diselenide oligonucleotide **14** and selenoester peptide **17** (Ac-LYRANM-SePh) and C) 3 hour aliquot of deselenization following rDSL. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

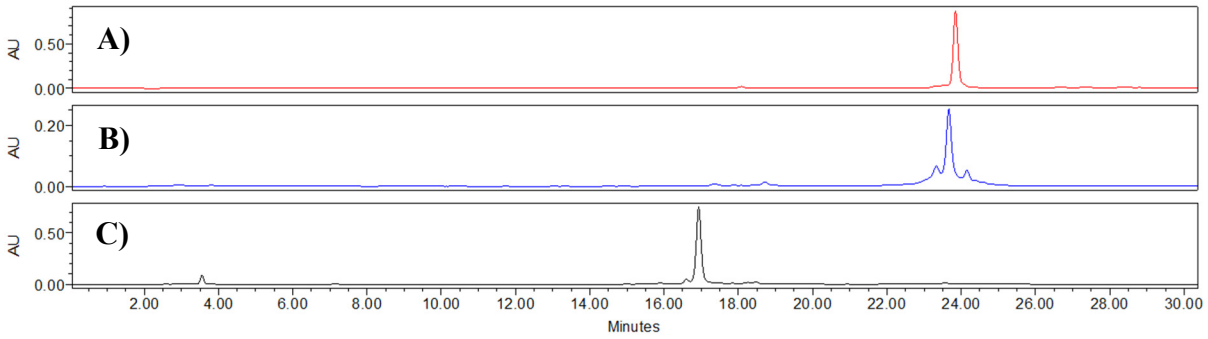


Figure S25: IEX HPLC trace of IEX purified LYRANSA POC. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

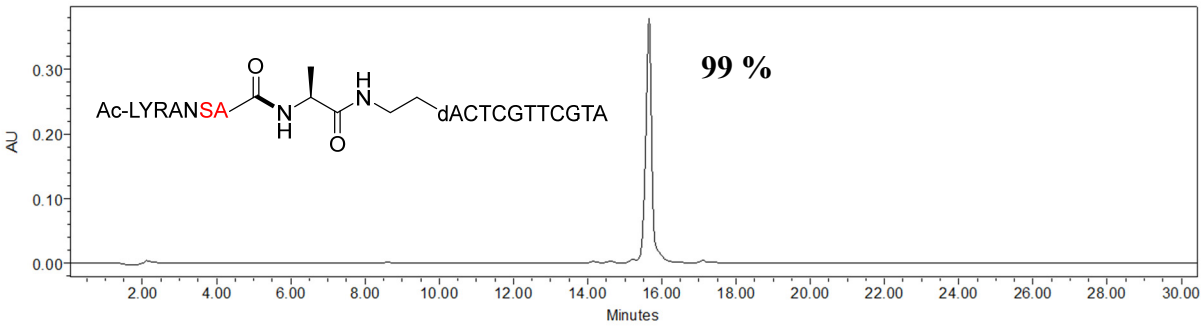


Figure S26: IEX HPLC trace of IEX purified LYRANYF POC. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

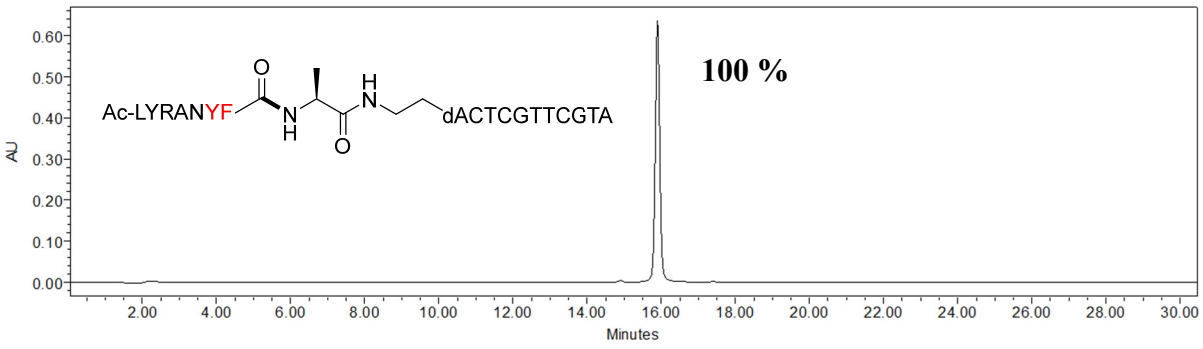


Figure S27: IEX HPLC trace of IEX purified LYRANQF POC. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

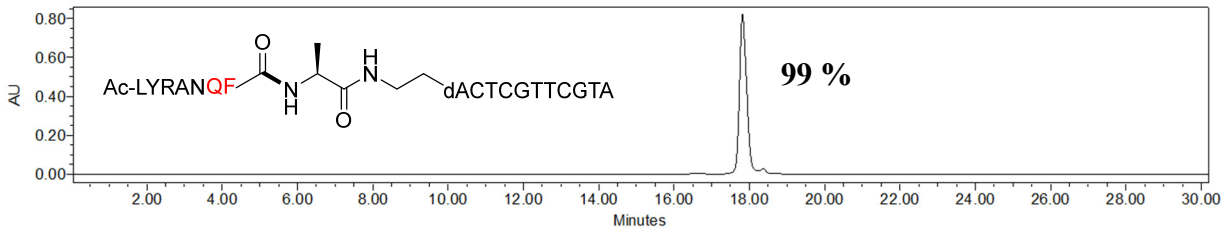


Figure S28: IEX HPLC trace of IEX purified LYRANM POC. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

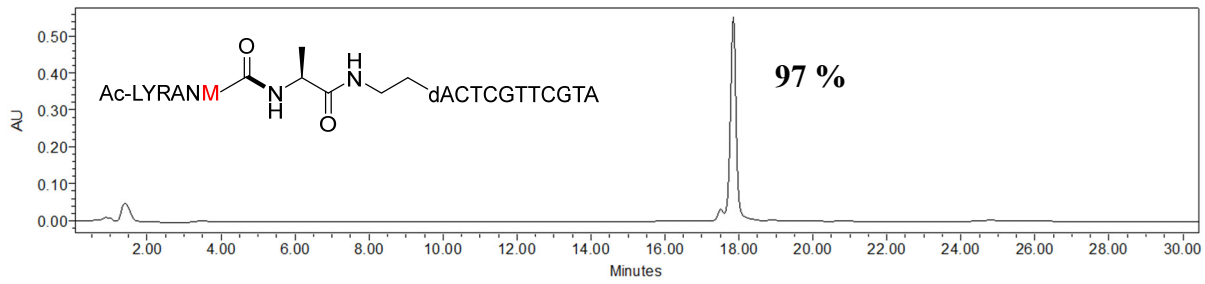


Figure S29: ESI-MS spectrum of pure LYRANSA POC (expected mass: 4317.04). [M - 3H]$^{3-}$ for POC shown in inset.

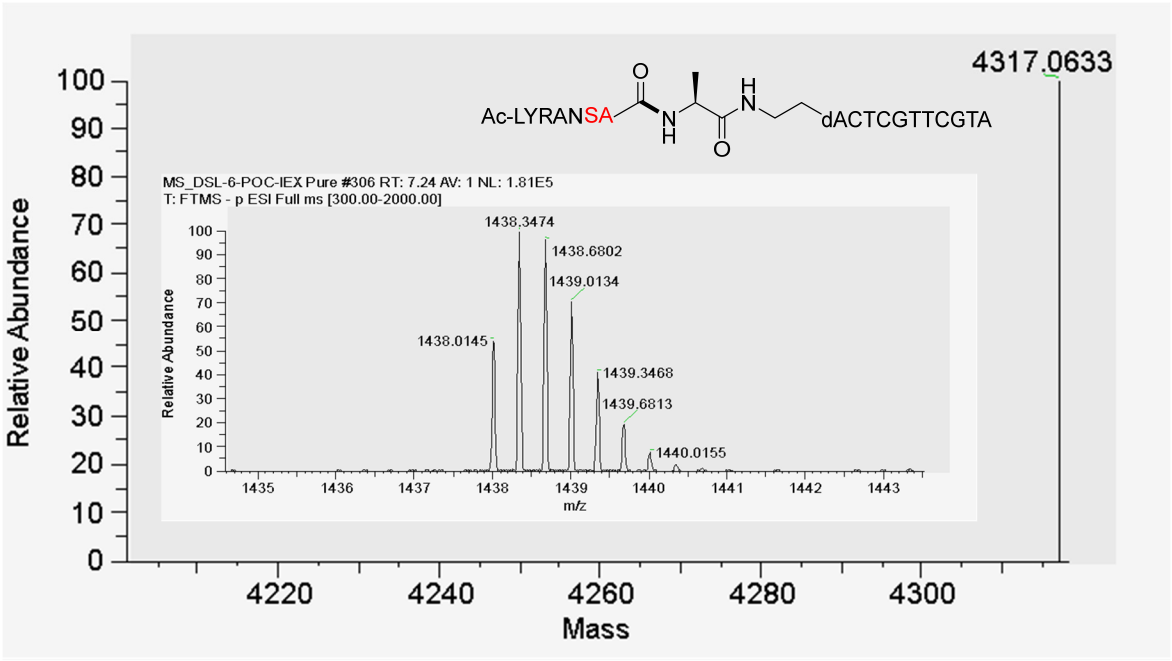


Figure S30: ESI-MS spectrum of pure LYRANYF POC (expected mass: 4469.10). [M - 3H]$^{3-}$ for POC shown in inset.

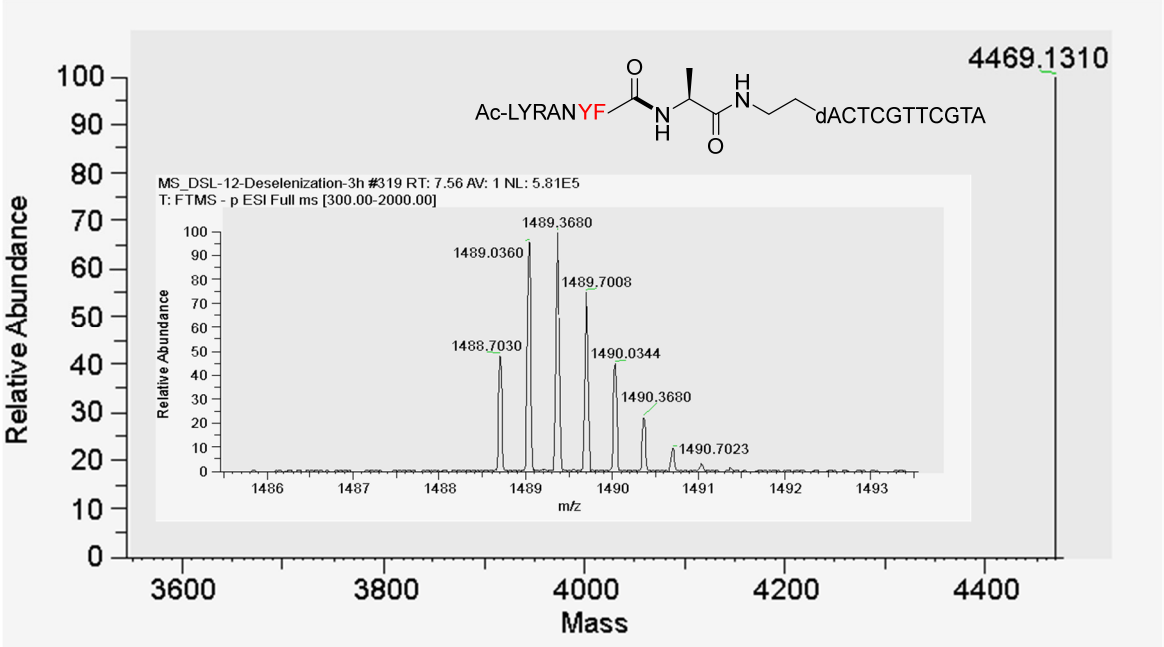


Figure S31: ESI-MS spectrum of pure LYRANQF POC (expected mass: 4434.10). [M - 3H]$^{3-}$ for POC shown in inset.

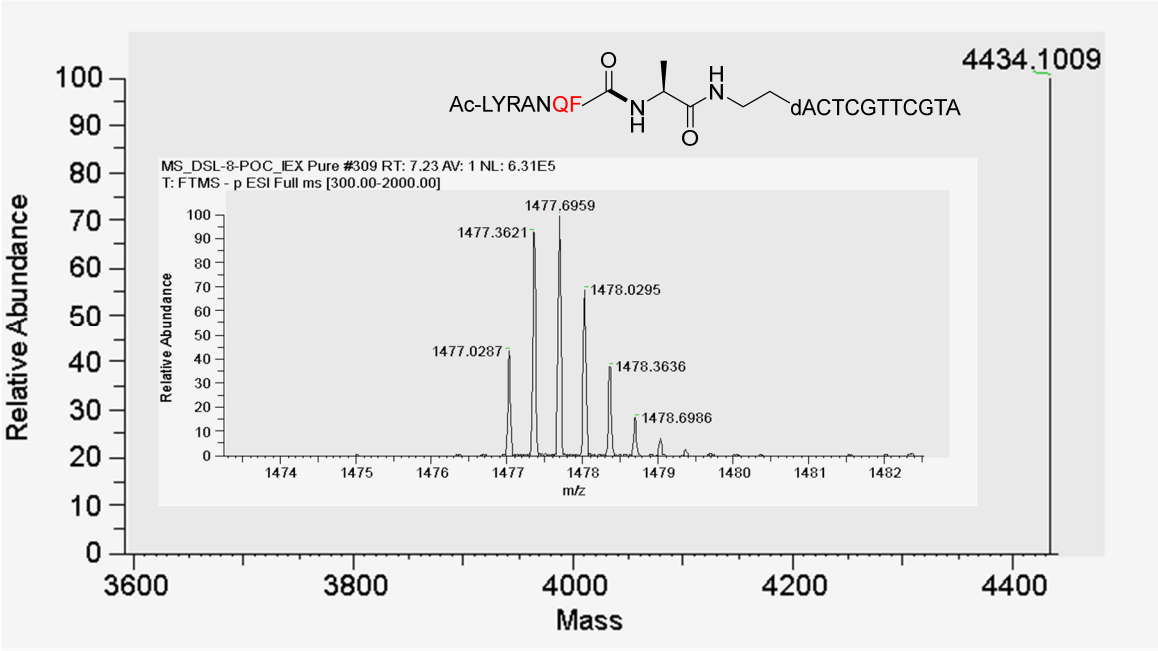


Figure S32: ESI-MS spectrum of pure LYRANM POC (expected mass: 4290.01). Also observed, β-hydroxylated POC (expected mass: 4306.01). [M - 3H]$^{3-}$ for POC shown in inset.

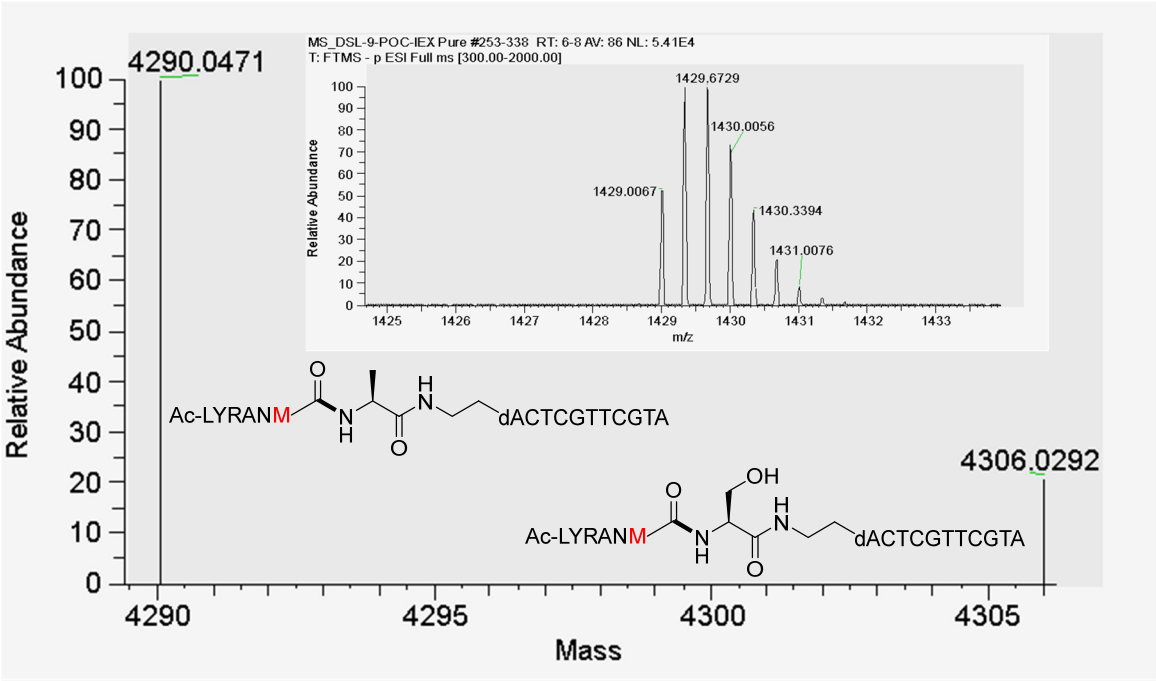


Figure S33: IEX HPLC traces tracking alkylation of POC **13** intermediates with bromodecane following additive-free DSL. Traces depict reaction progress after A) 0 minutes B) 5 minutes C) 1 hour D) 3 hours and E) 23 hours. The absence of any single-stranded oligonucleotides is highlighted by the red box. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

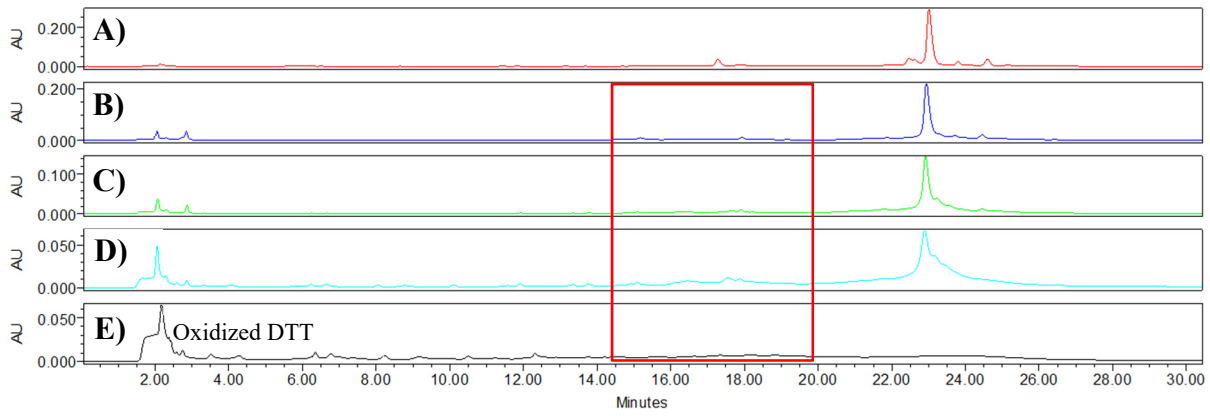


Figure S34: DLS measurements of alkylated POC **13**. Solution contained micelles at a concentration of 100 µM prepared in a buffer of 0.1 M MES, pH 6.7. Average particle size: 84 nm. Average PDI: 0.42.

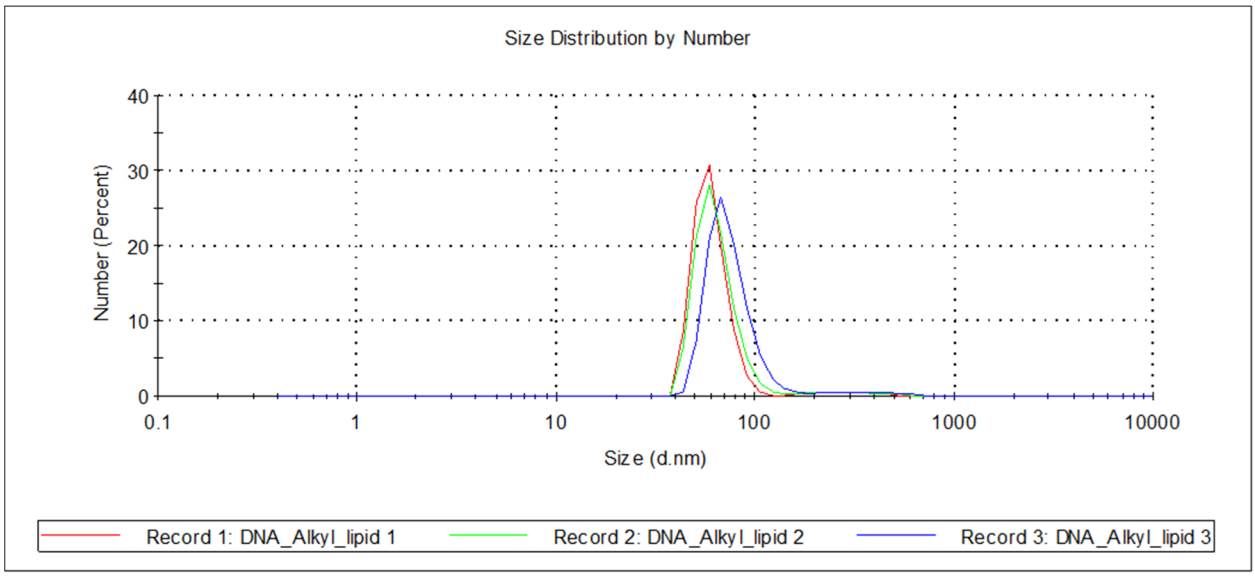


Figure S35: IEX HPLC traces tracking alkylation of diselenide oligonucleotide **7** with bromodecane. Traces depict reaction progress after A) 5 minutes B) 1 hour C) 3 hours D) 5 hours E) 7 hours and F) 22 hours. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

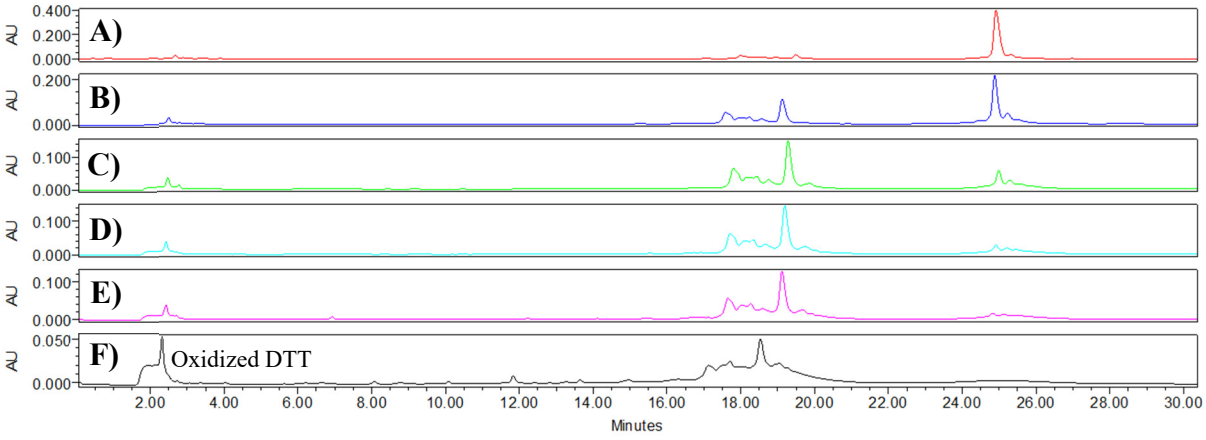


Figure S36: DLS measurements of alkylated diselenide oligonucleotide **7**. Solution contained micelles at a concentration of 100 µM prepared in a buffer of 0.1 M MES, pH 6.7. Average particle size: 77 nm. Average PDI: 0.52.

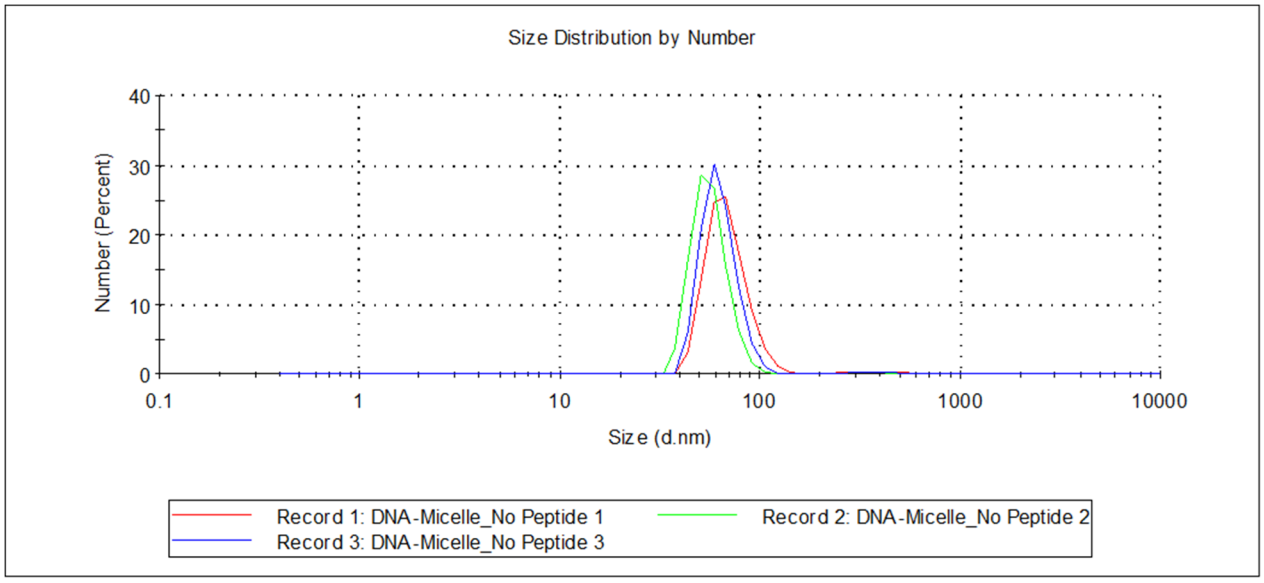


Figure S37: DLS measurements of unmodified oligonucleotide dTCCCGTTTCCA. Solution contained oligonucleotide at a concentration of 100 μM prepared in a buffer of 0.1 M MES, pH 6.7. Average particle size: 1.5 nm. Average PDI: 0.66.

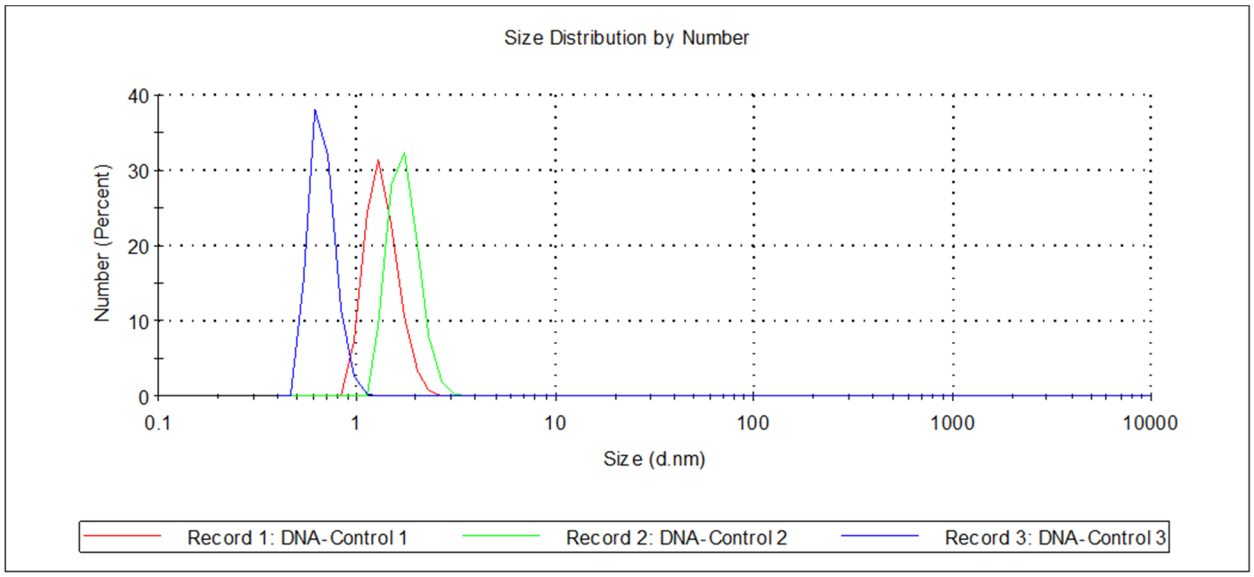


Figure S38: Negative-stain TEM images of POC **13** micelles.

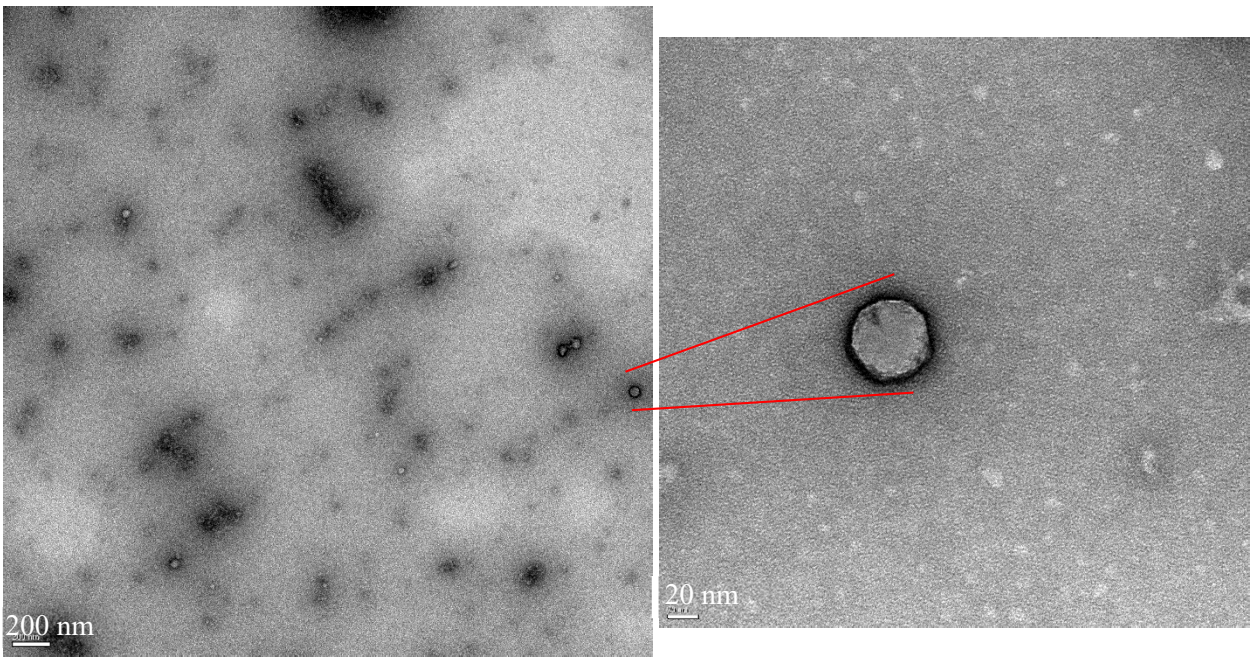


Figure S39: Negative-stain TEM image of unmodified oligonucleotide dTCCCGTTTCCA (control).

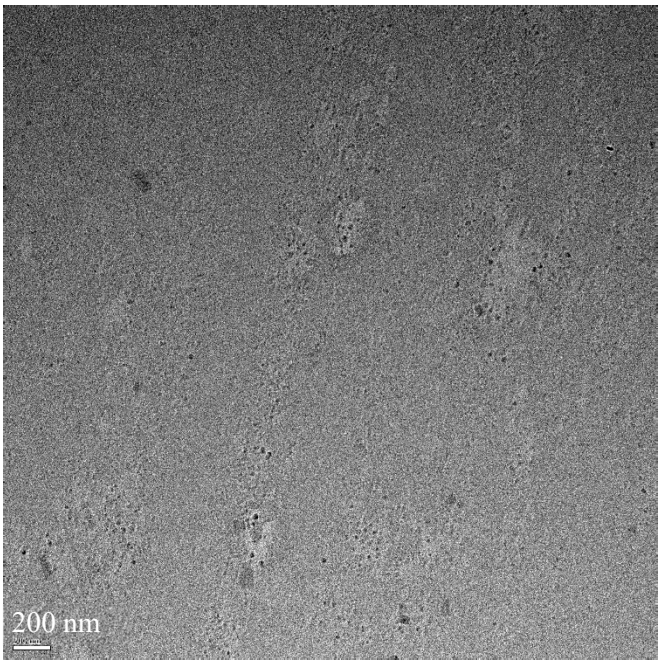


Figure S40: IEX HPLC traces tracking alkylation of diselenide oligonucleotide **7** with bromopentane. Traces depict reaction progress after A) 0 minutes (i.e. pure diselenide oligonucleotide **7**) B) 1 hour and C) 3 hours. The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

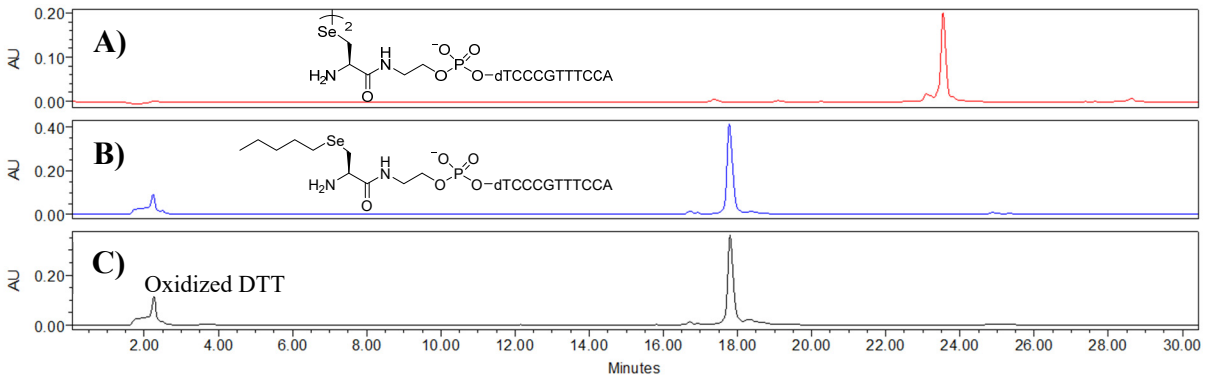


Figure S41: ESI-MS spectrum of alkylated diselenide oligonucleotide **7** (expected mass: 3585.61).

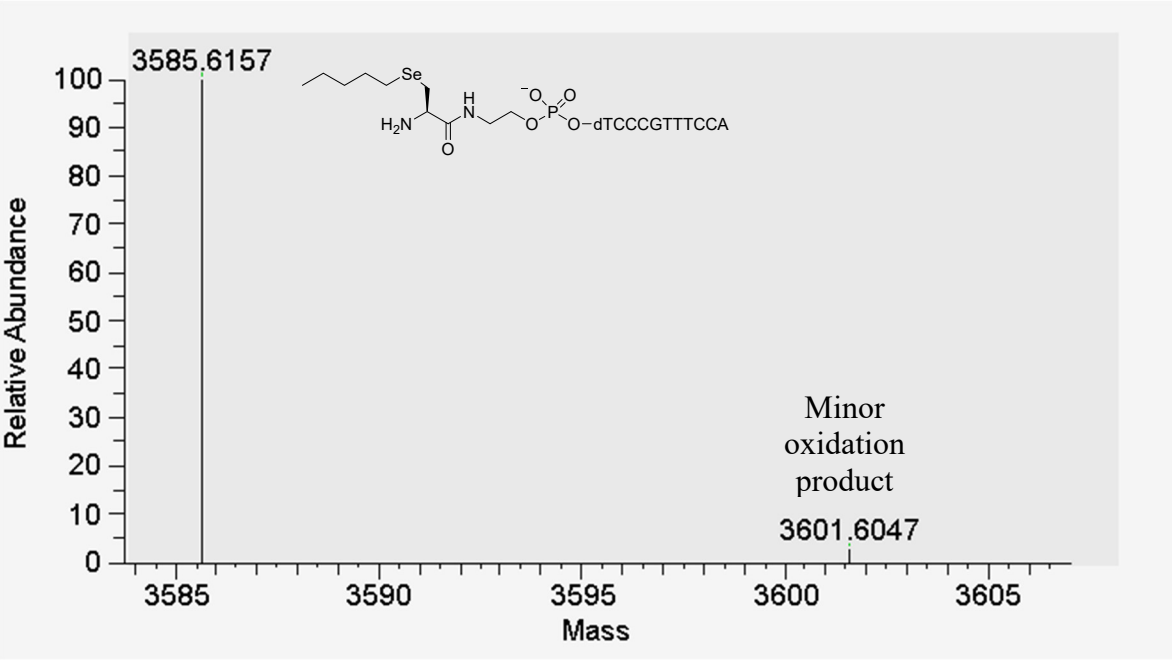


Figure S42: ESI-MS spectrum of alkylated diselenide oligonucleotide **7** ($[M - 3H]^{3-}$). Demonstrating the characteristic isotopic distribution of Se-containing compounds.

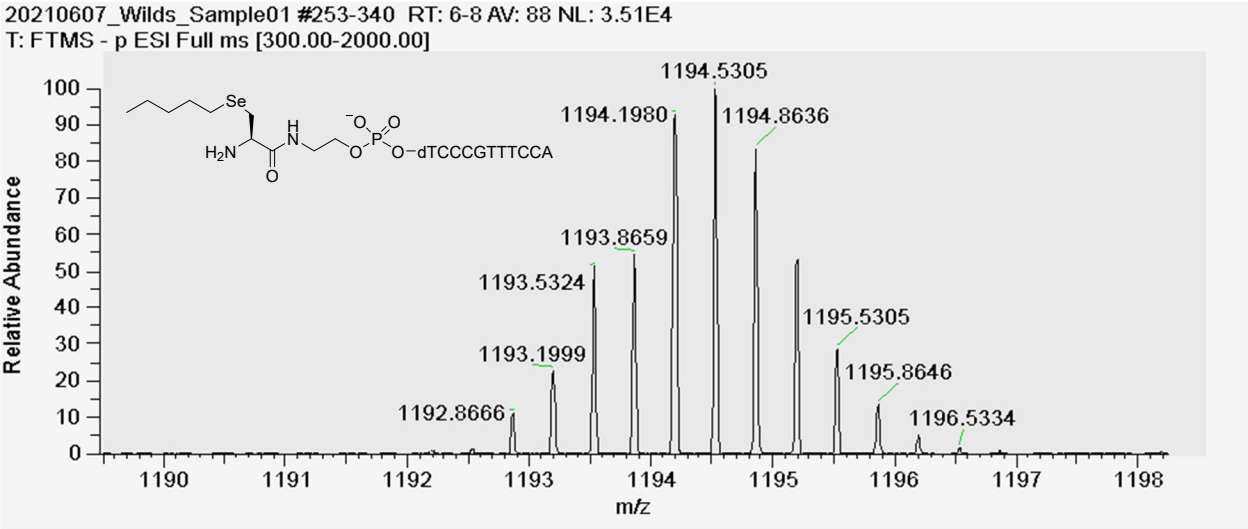


Figure S43: IEX HPLC traces following 1 hour alkylation of diselenide oligonucleotide **14** with 4-bromomethyl-6,7-dimethoxycoumarin (Br-DMC). The column was monitored at A) 260 nm and B) 350 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

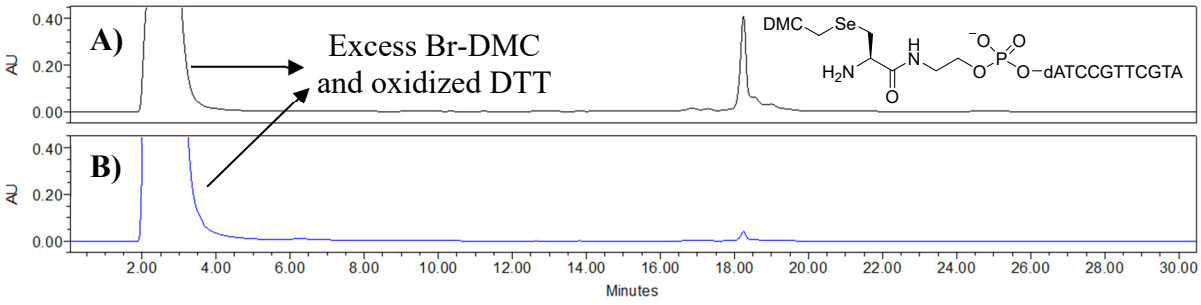


Figure S44: ESI-MS spectrum of dimethoxycoumarin labelled diselenide oligonucleotide **14** (expected mass: 3797.61).

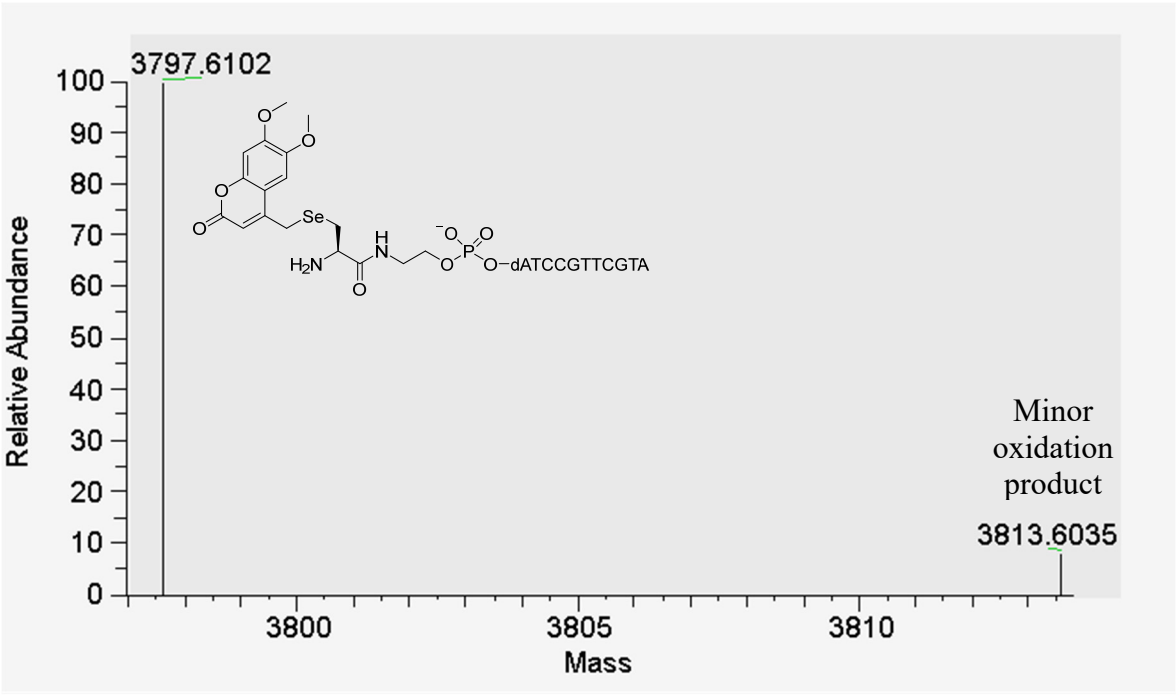


Figure S45: IEX HPLC trace of pure and desalted dimethoxycoumarin (DMC) labelled diselenide oligonucleotide **14**. The column was monitored at A) 260 nm and B) 350 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

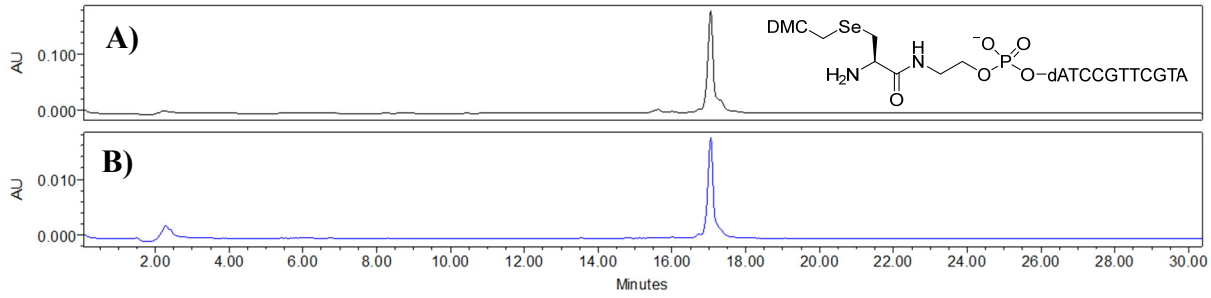


Figure S46: A) UV-Vis and B) emission spectra of dimethoxycoumarin labelled diselenide oligonucleotide **14**. For emission spectrum, sample excited at 350 nm. Control DNA = diselenide oligonucleotide **14**.

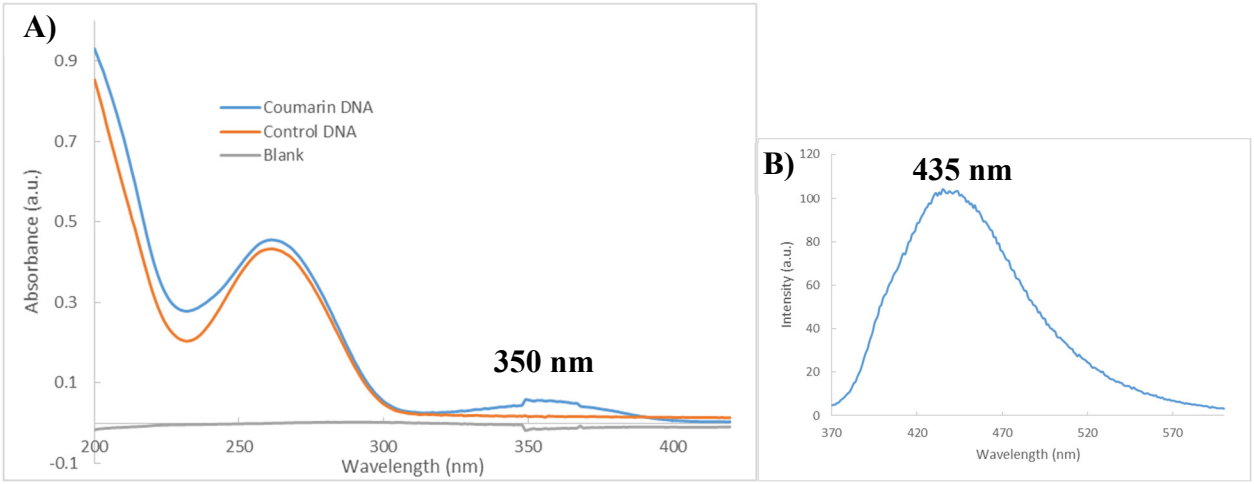


Figure S47: ESI-MS spectrum of dicarbonyl oligonucleotide **18** (expected mass: 3434.58).

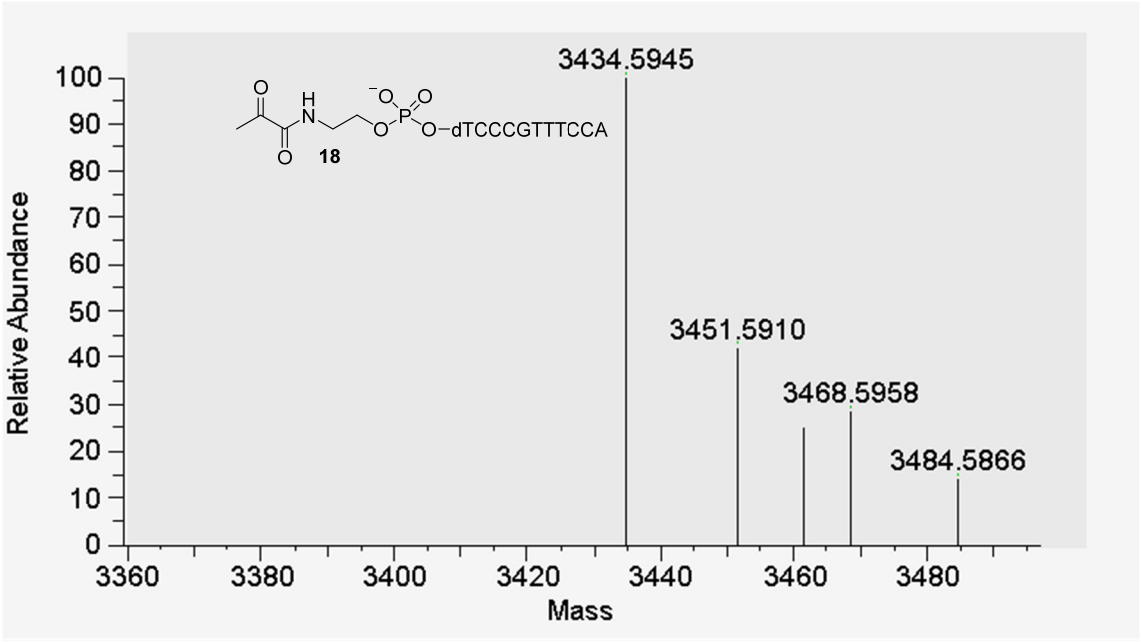


Figure S48: ESI-MS spectrum of dicarbonyl oligonucleotide **18** ($[M - 3H]^{3-}$). Demonstrating the loss of the characteristic isotopic distribution of Se-containing compounds.

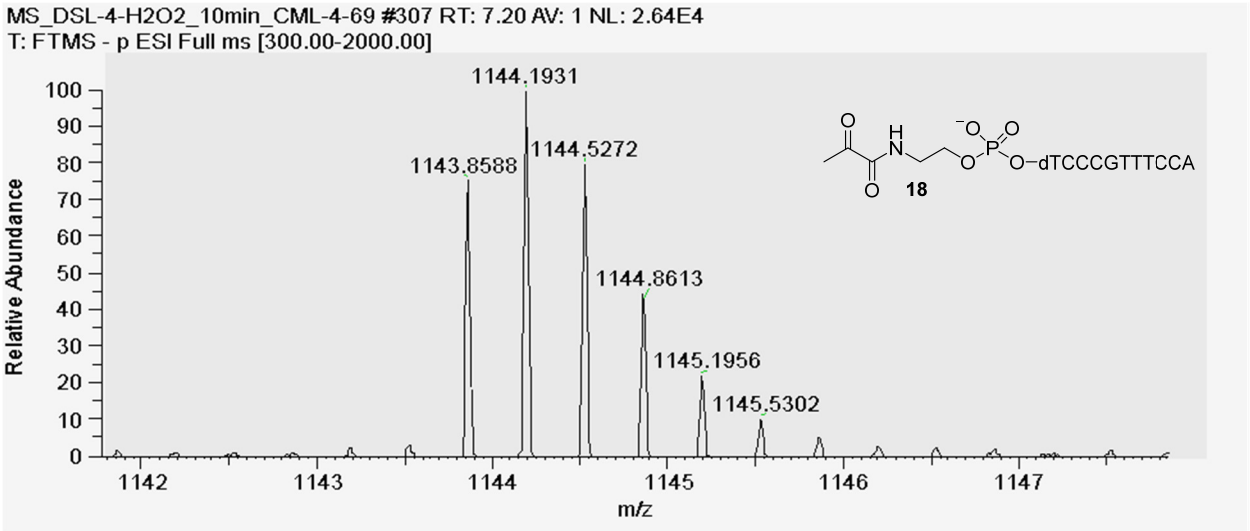
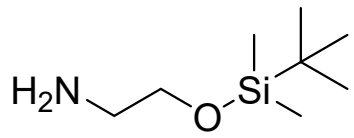


Figure S49: 500 MHz ^{1}H NMR spectrum of TBS protected ethanolamine (in $CDCl_3$)



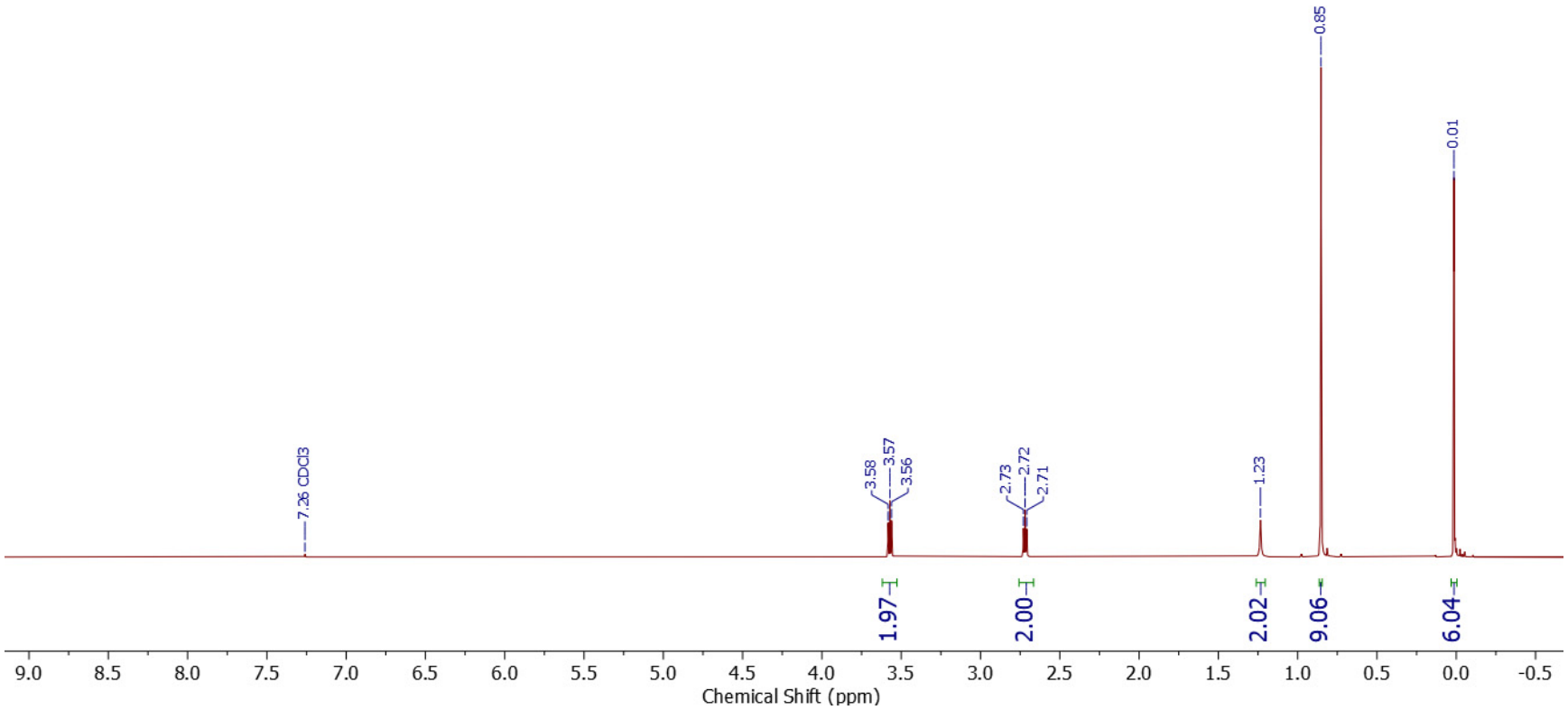


Figure S50: 500 MHz ^{1}H NMR spectrum of di-2-cyanoethyl diselenide (in $CDCl_3$)

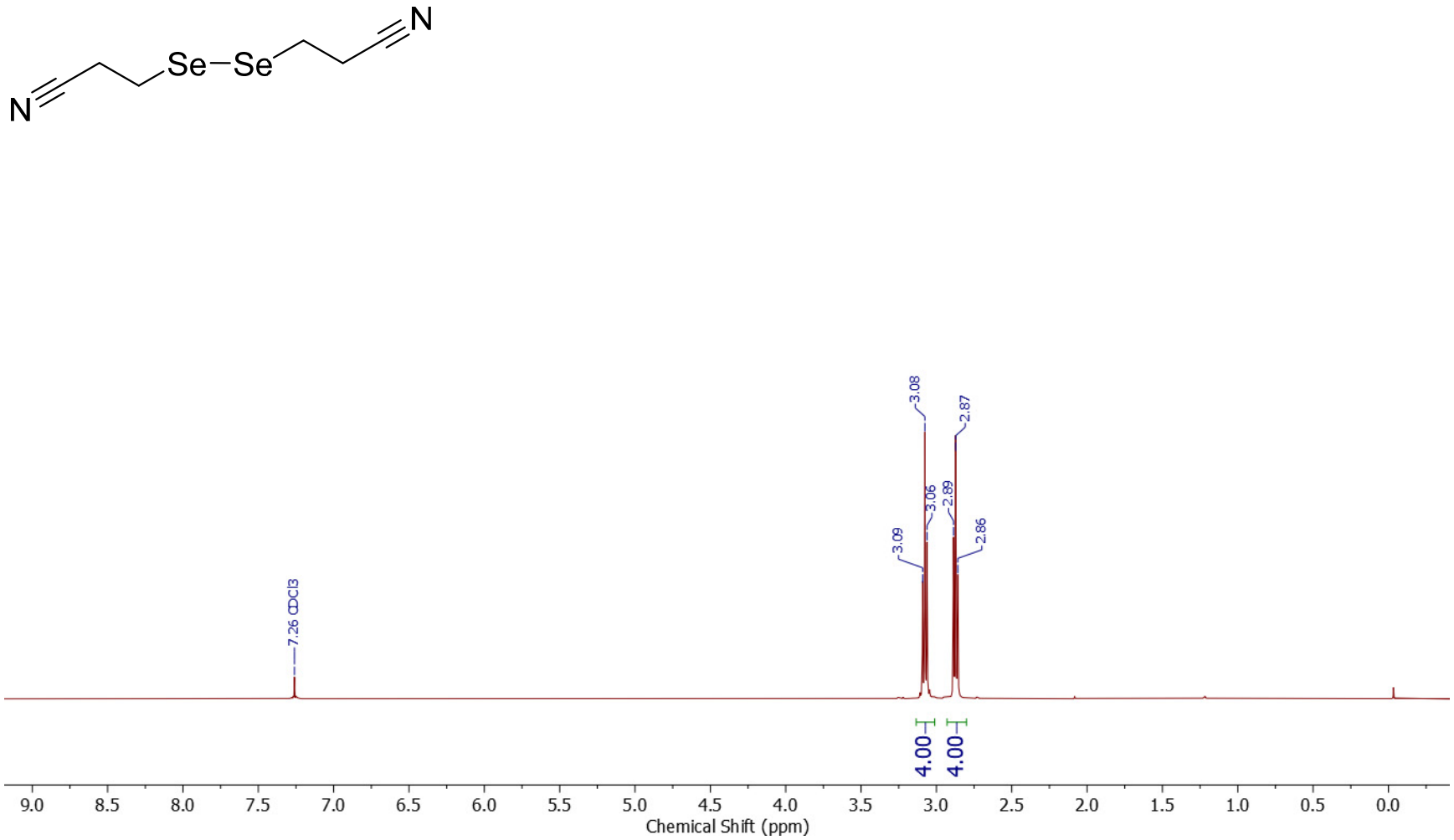


Figure S51: 95.3 MHz ^{77}Se NMR spectrum of di-2-cyanoethyl diselenide (in $CDCl_3$)

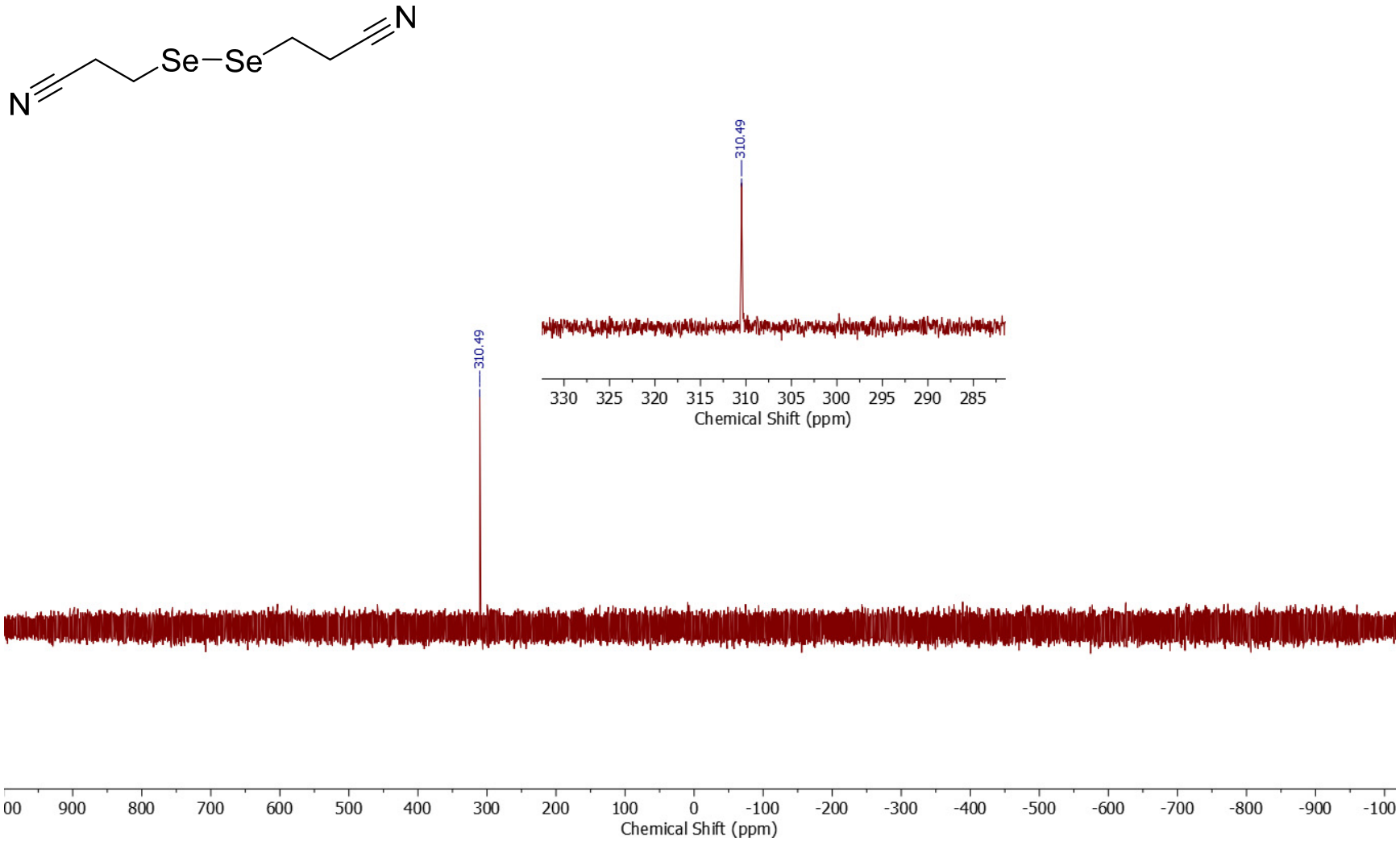


Figure S52: 500 MHz ^{1}H NMR spectrum of amide **2** (in $CDCl_3$)

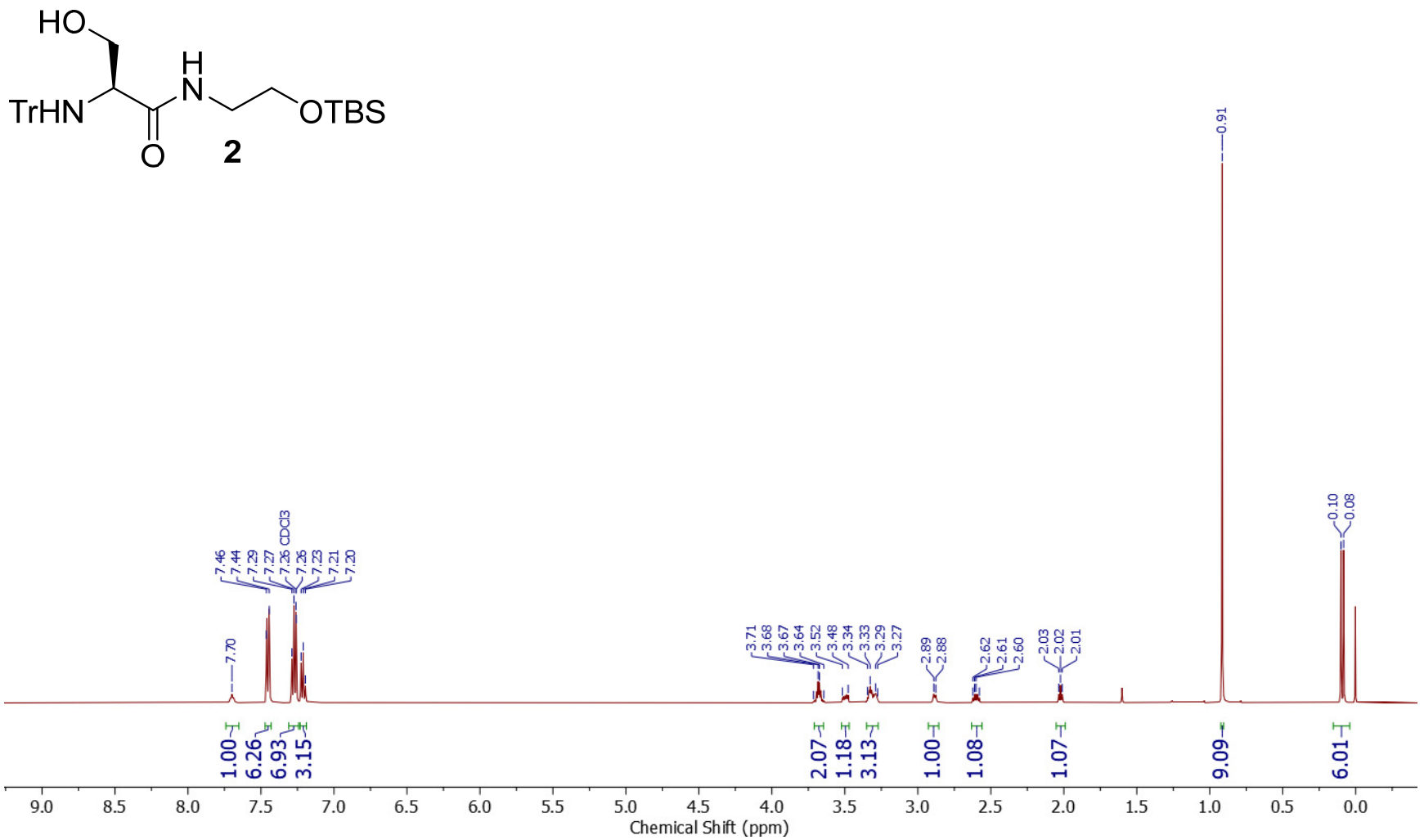


Figure S53: 125.7 MHz ^{13}C NMR spectrum of amide **2** (in $CDCl_3$)

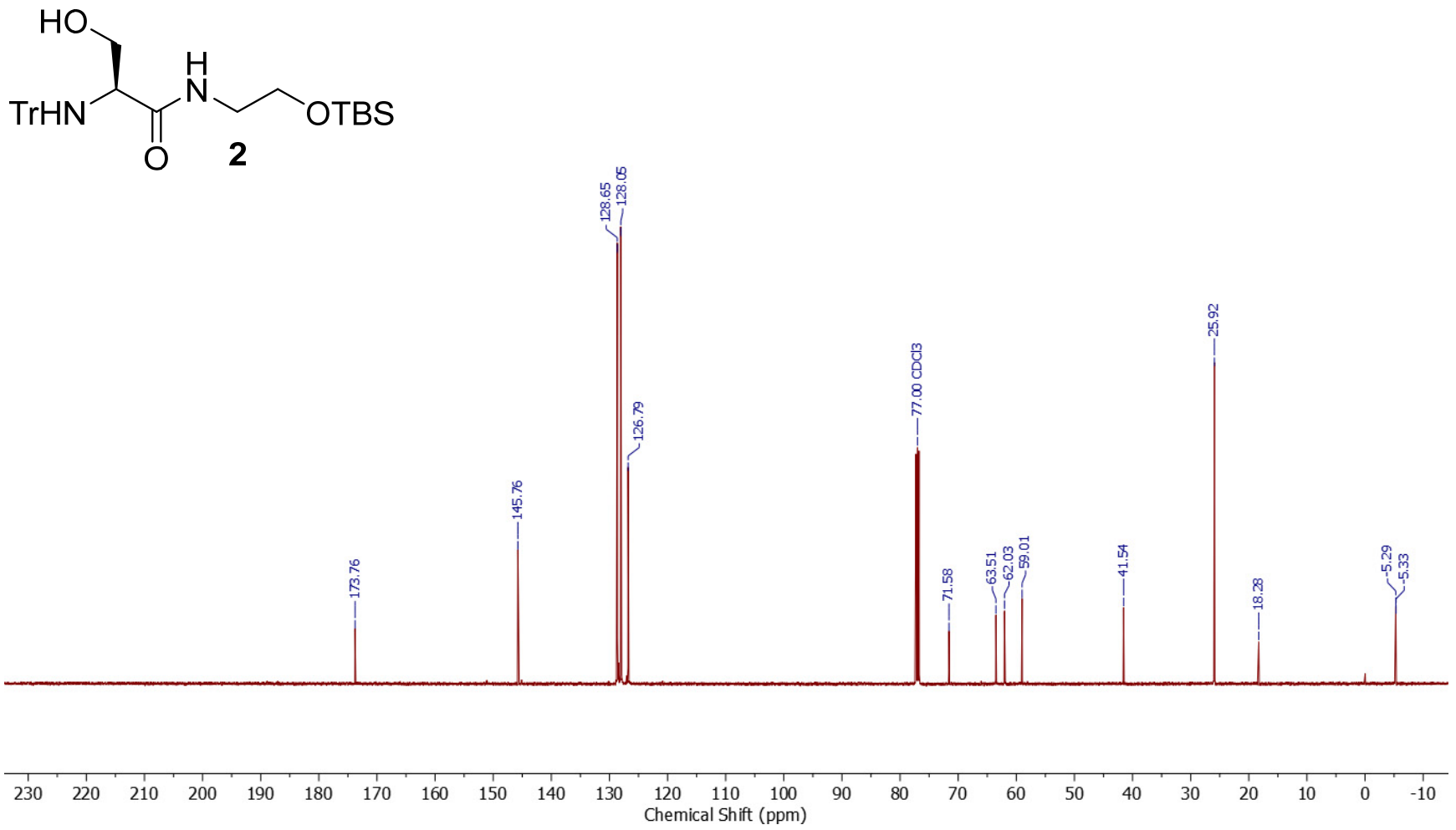
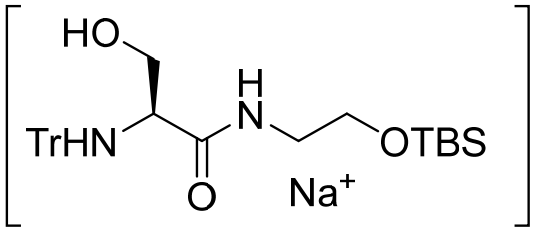


Figure S54: HR ESI-MS spectrum of amide **2** (expected mass: 527.2700)



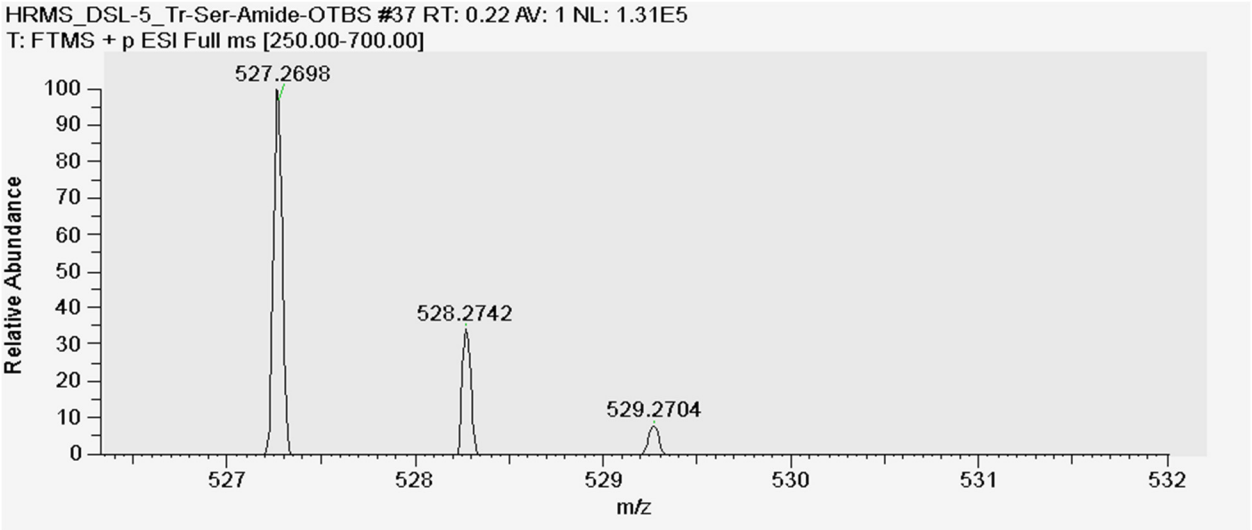


Figure S55: 500 MHz ^{1}H NMR spectrum of alkyl bromide **3** (in $CDCl_3$)

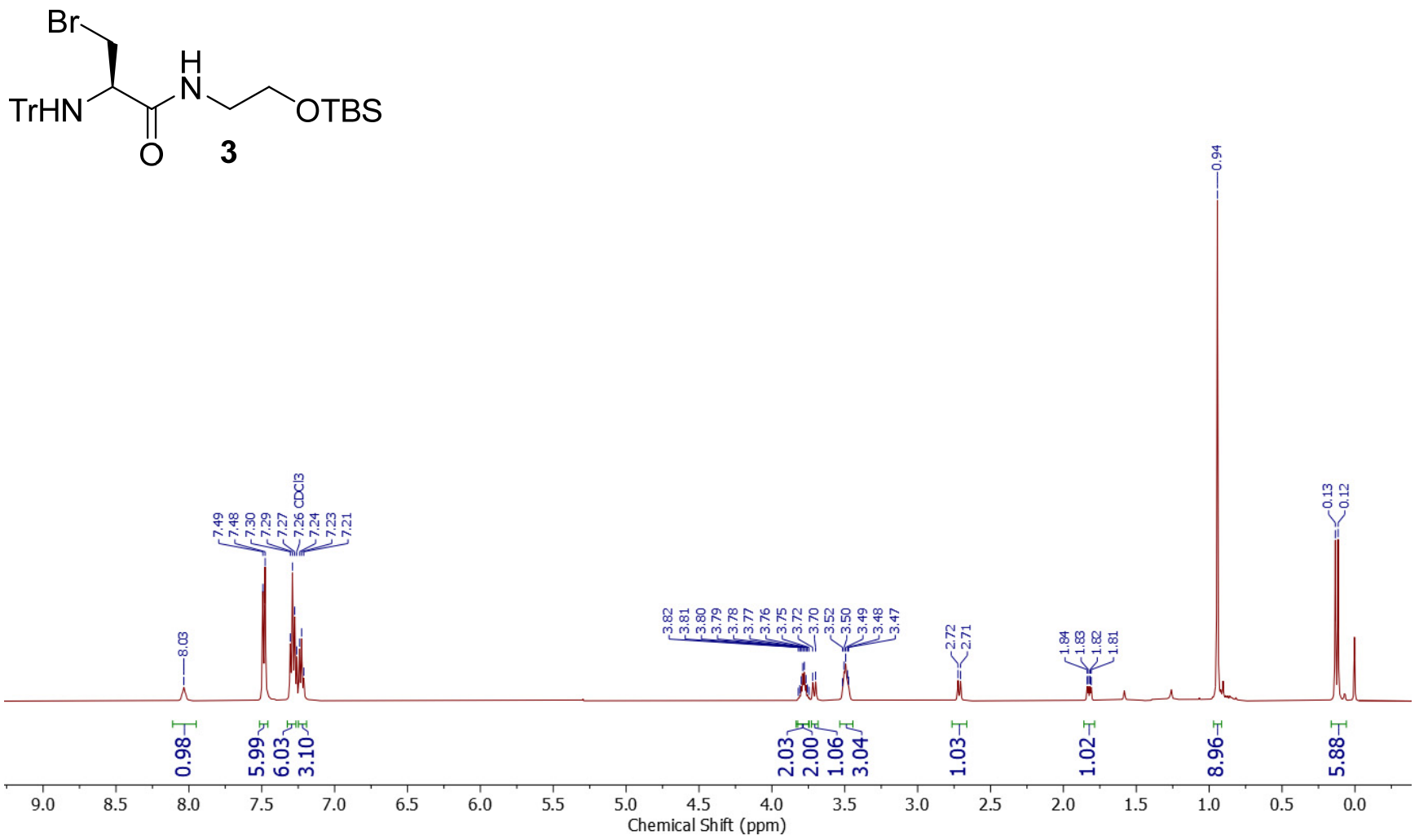


Figure S56: 125.7 MHz ^{13}C NMR spectrum of alkyl bromide **3** (in $CDCl_3$)

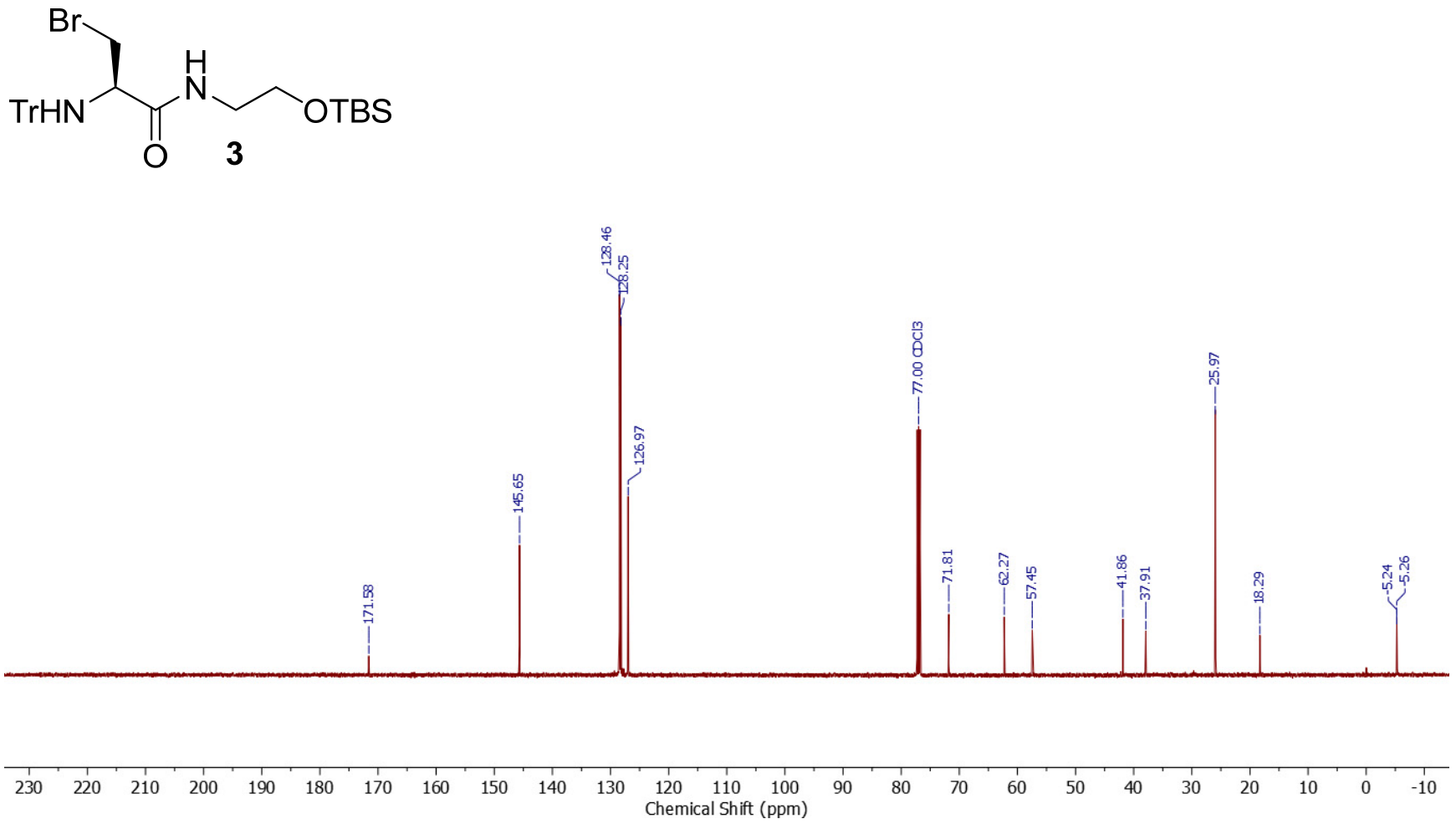
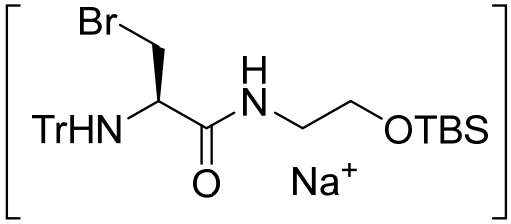


Figure S57: HR ESI-MS spectrum of alkyl bromide **3** (expected mass: 589.1856)



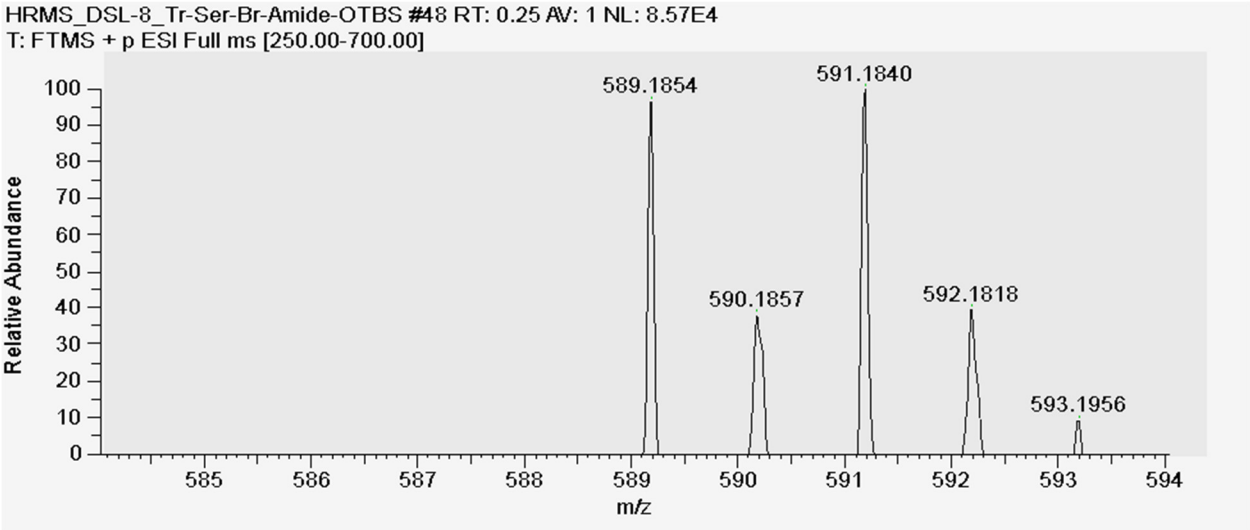


Figure S58: 500 MHz ^{1}H NMR spectrum of aziridine (in $CDCl_3$)

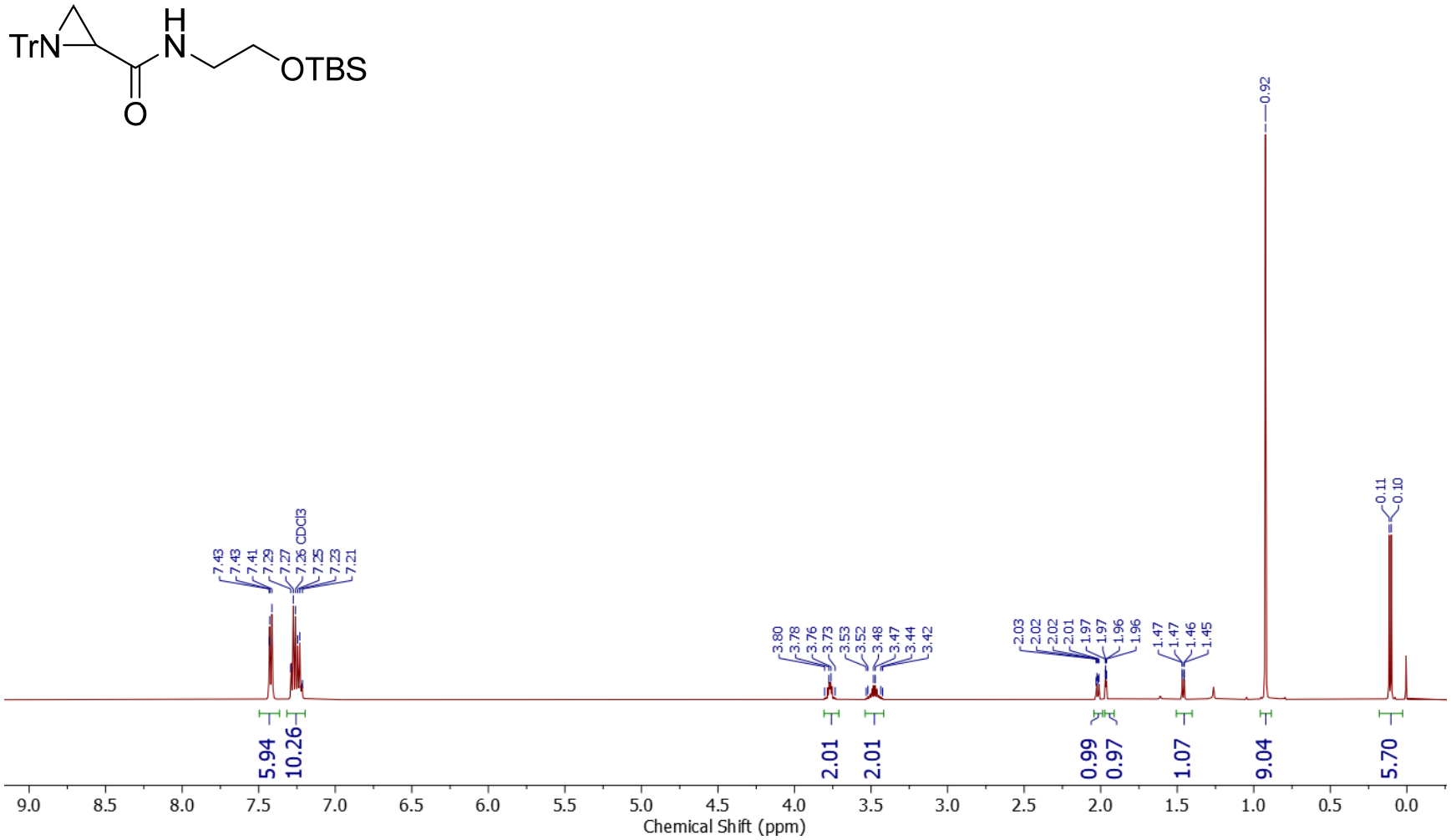


Figure S59: 125.7 MHz ^{13}C NMR spectrum of aziridine (in $CDCl_3$)

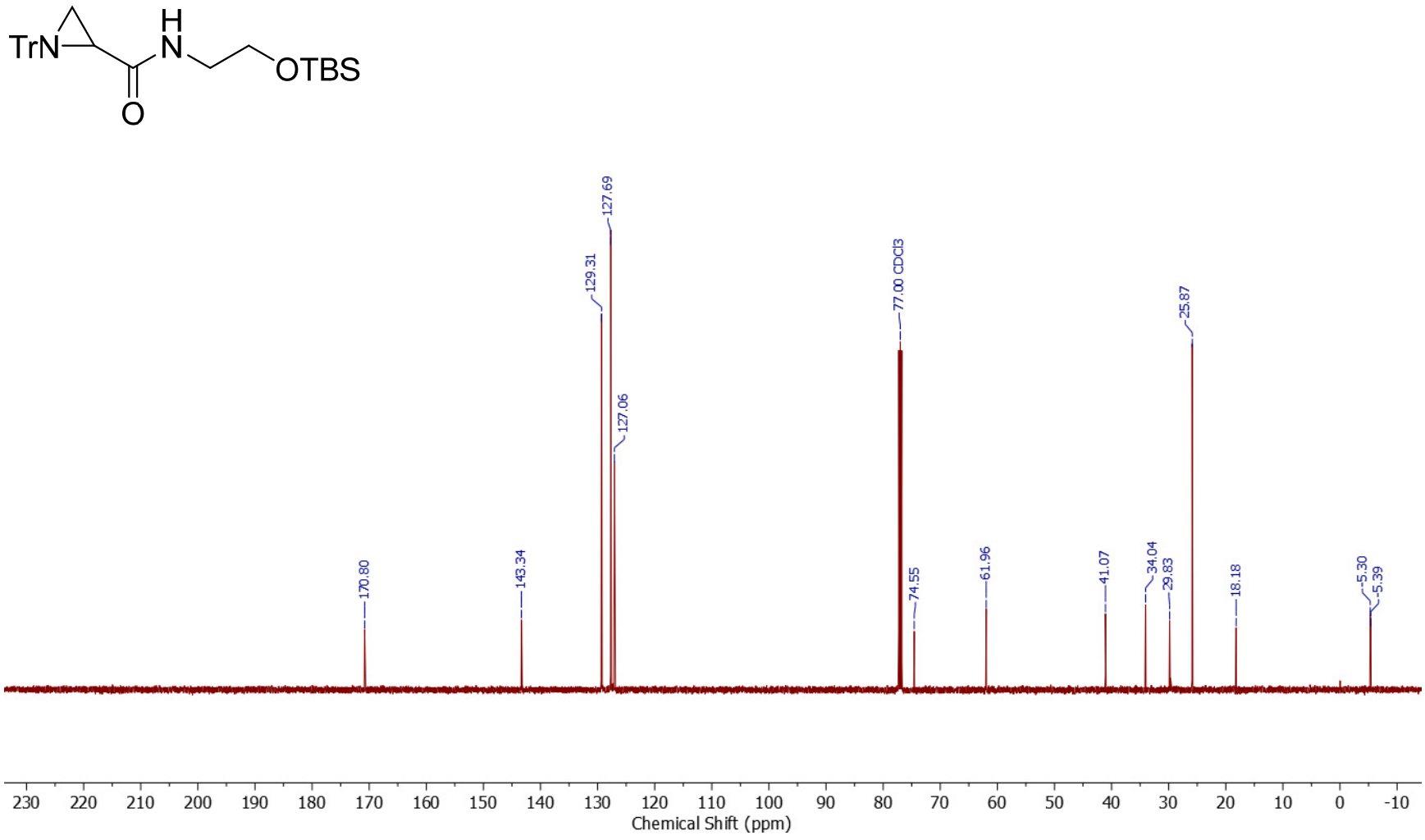
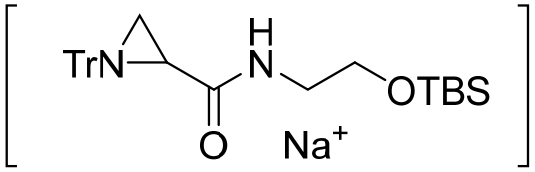


Figure S60: HR ESI-MS spectrum of aziridine (expected mass: 509.2595)



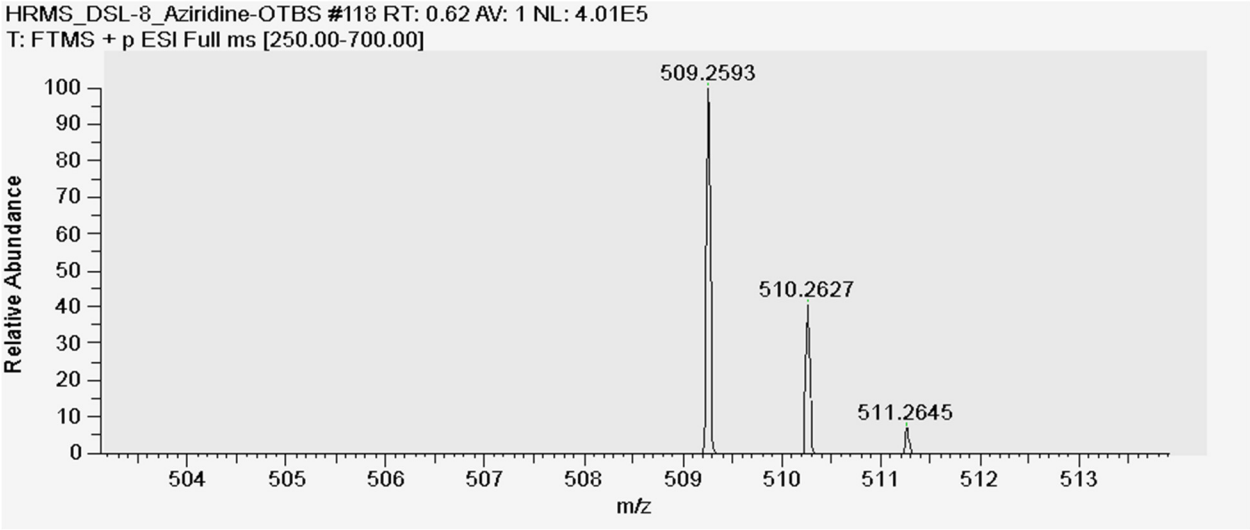


Figure S61: 500 MHz ^{1}H NMR spectrum of protected selenide **4** (in $CDCl_3$)

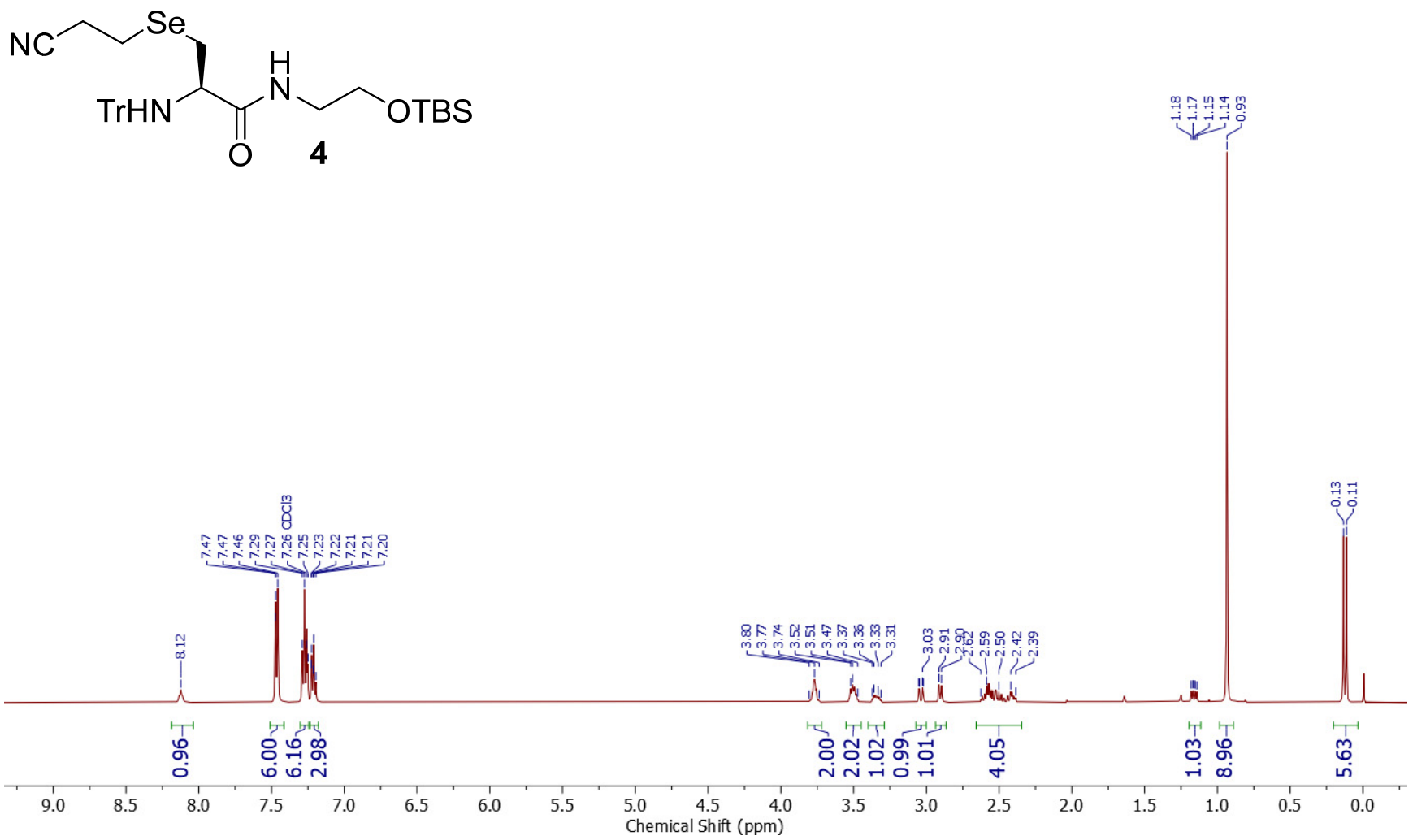


Figure S62: 125.7 MHz ^{13}C NMR spectrum of protected selenide **4** (in $CDCl_3$)

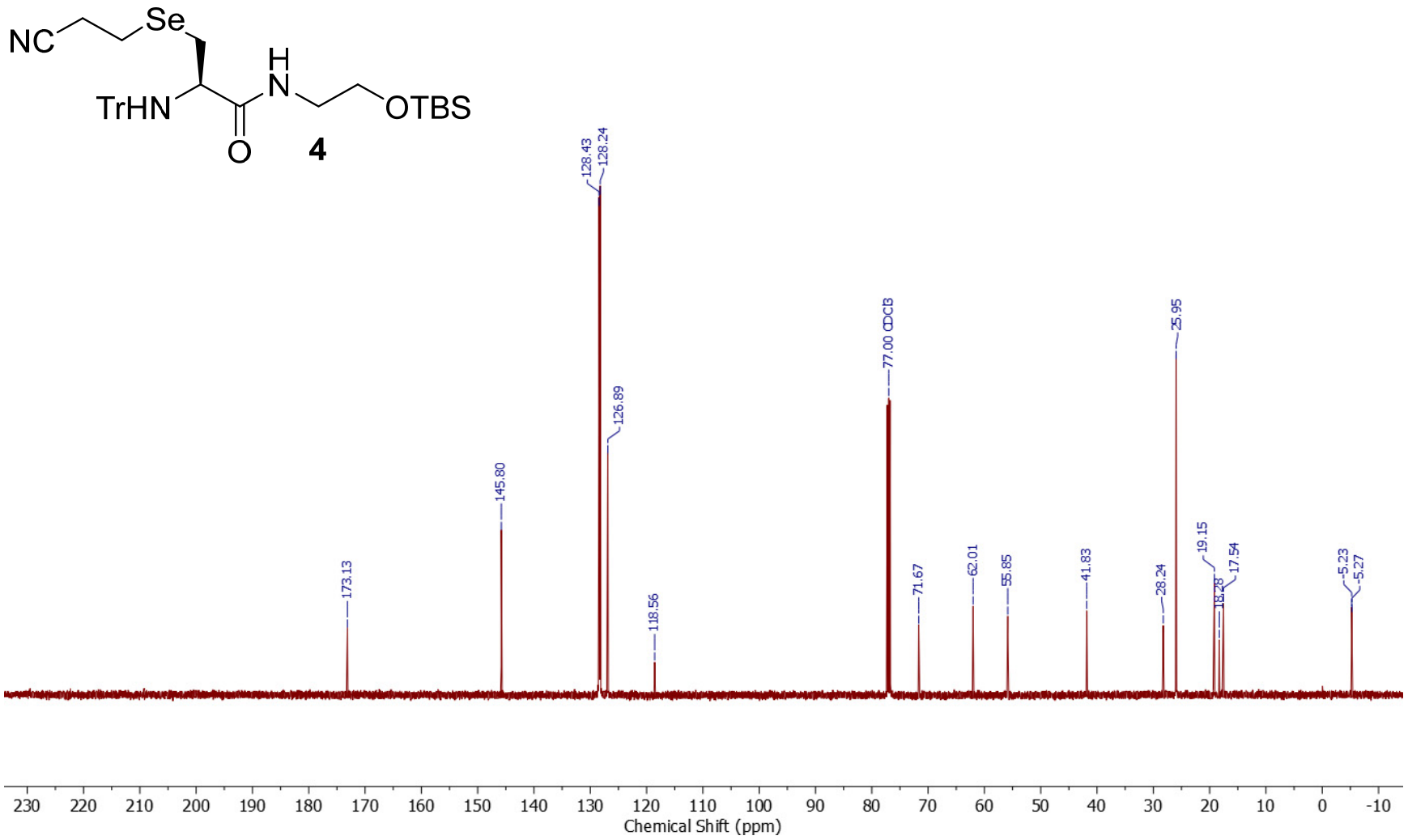


Figure S63: 95.3 MHz ^{77}Se NMR spectrum of protected selenide **4** (in $CDCl_3$)

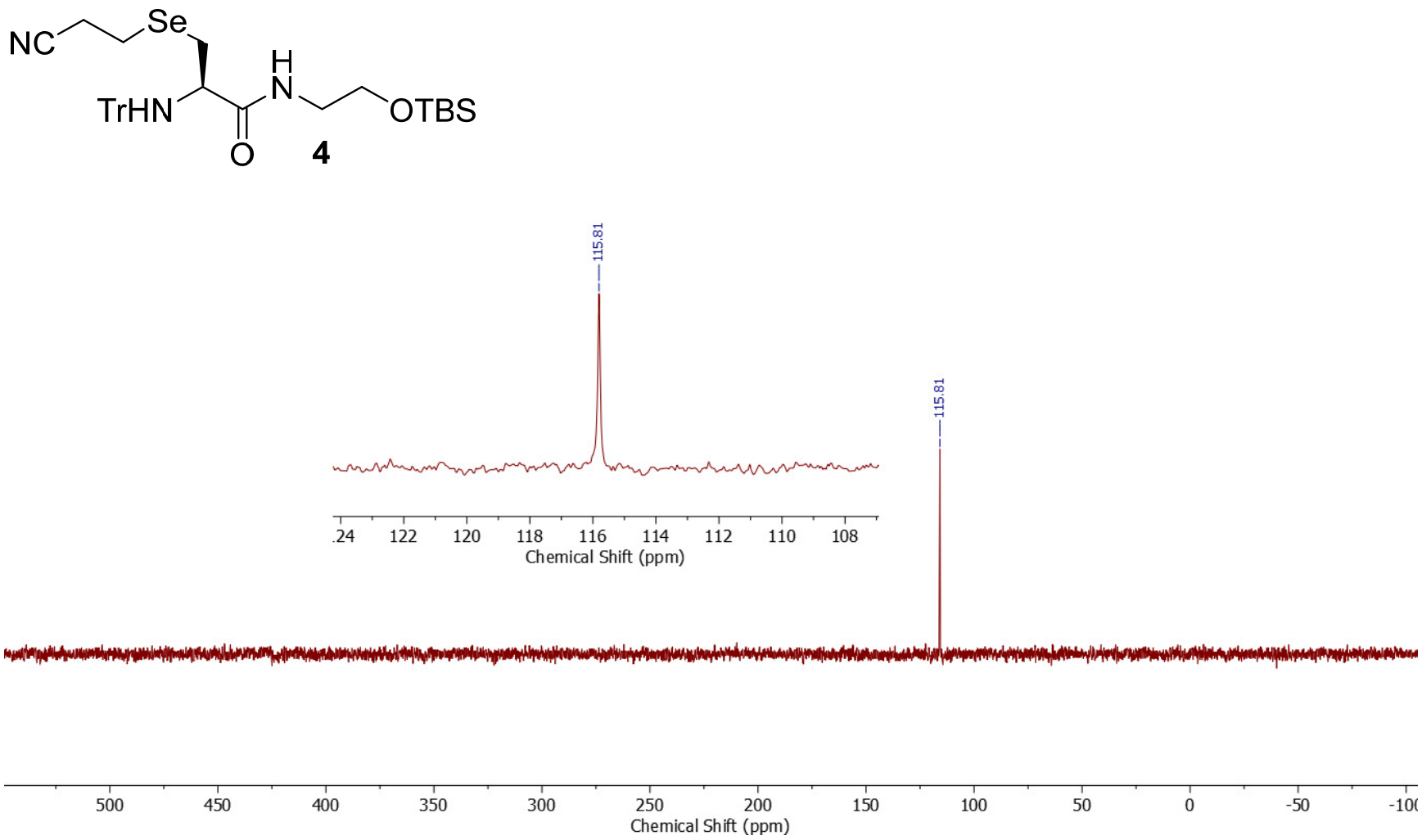
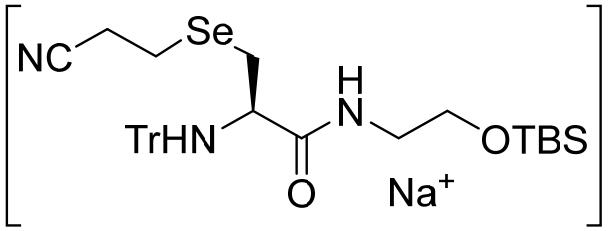


Figure S64: HR ESI-MS spectrum of protected selenide **4** (expected mass: 644.2182)



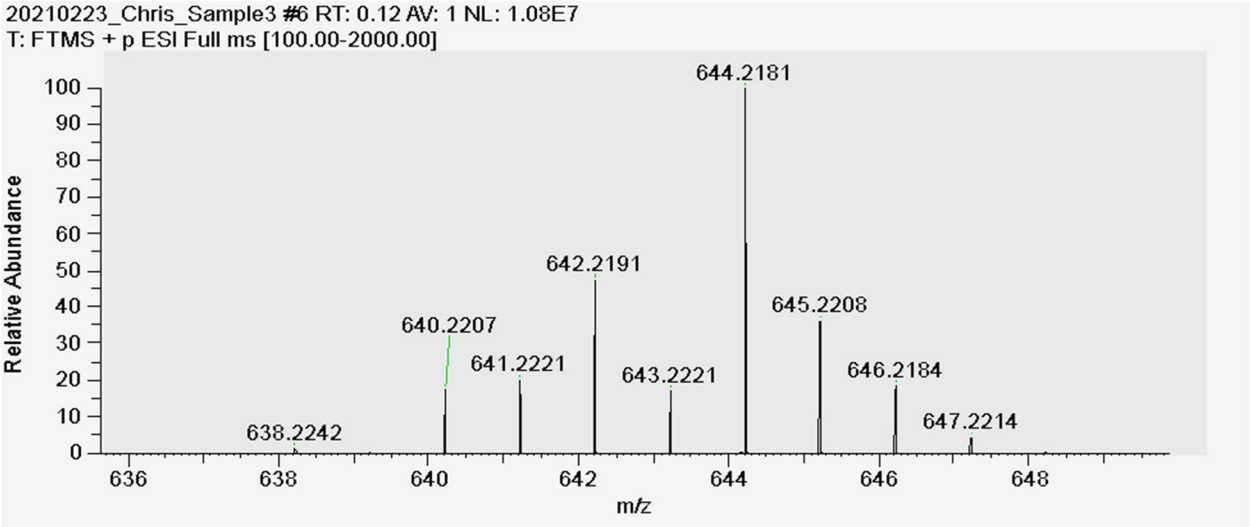


Figure S65: 500 MHz ^{1}H NMR spectrum of alcohol **5** (in $CDCl_3$)

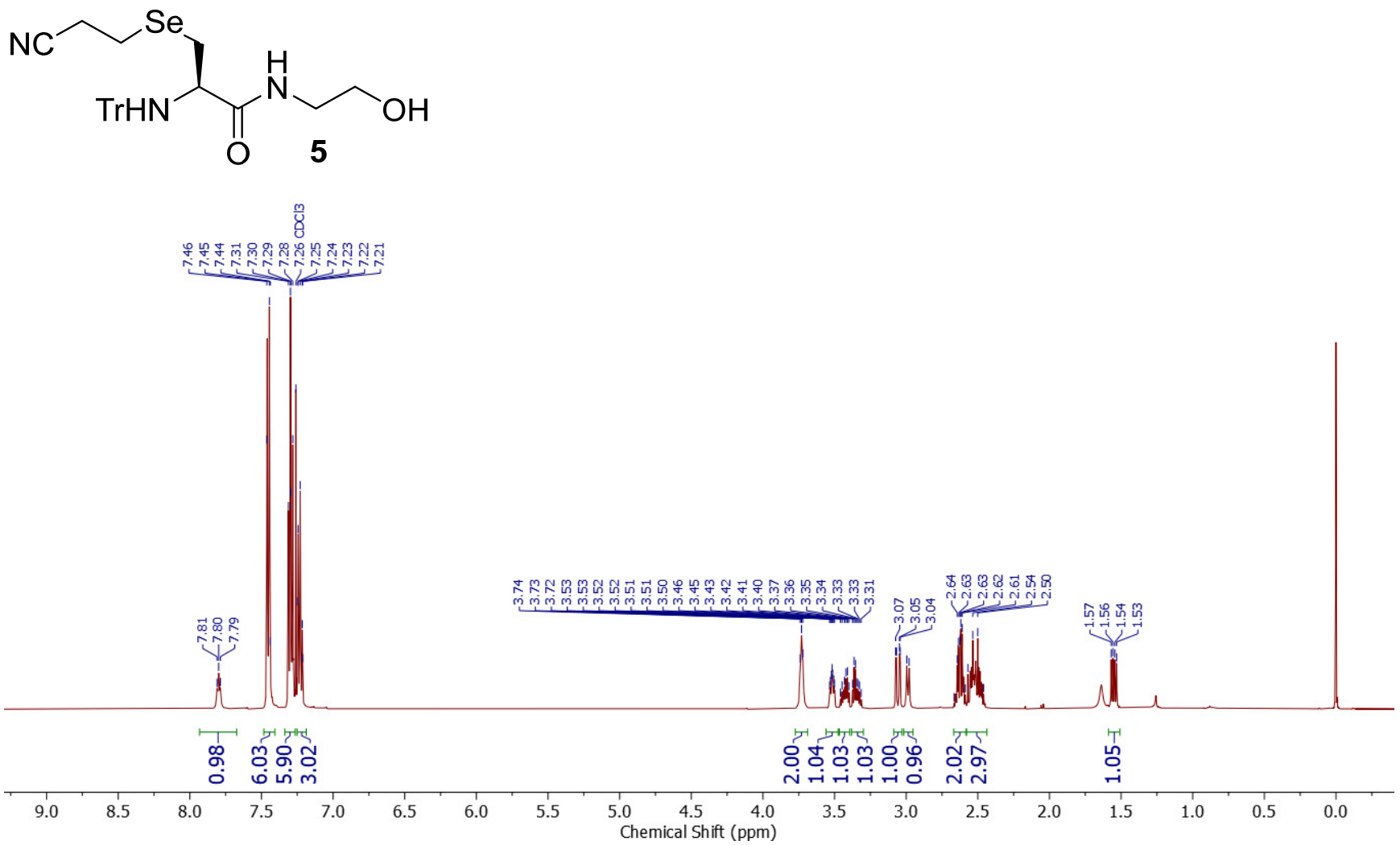


Figure S66: 125.7 MHz ^{13}C NMR spectrum of alcohol **5** (in $CDCl_3$)

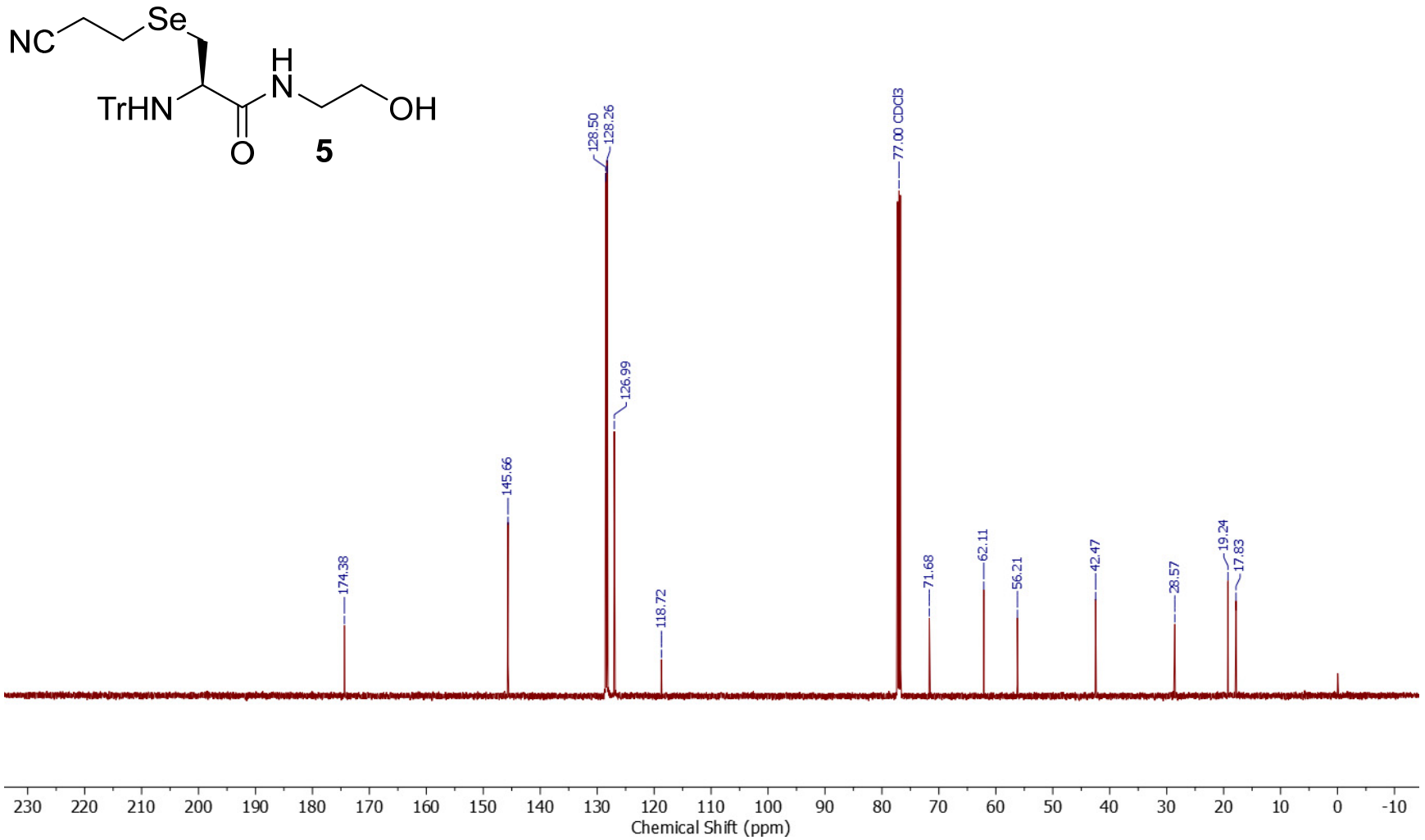
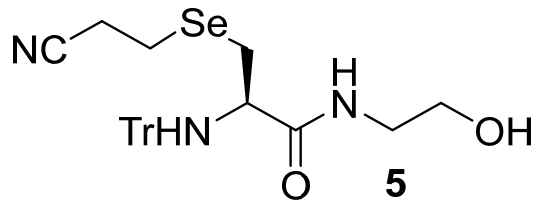


Figure S67: 95.3 MHz ^{77}Se NMR spectrum of alcohol **5** (in $CDCl_3$)



5

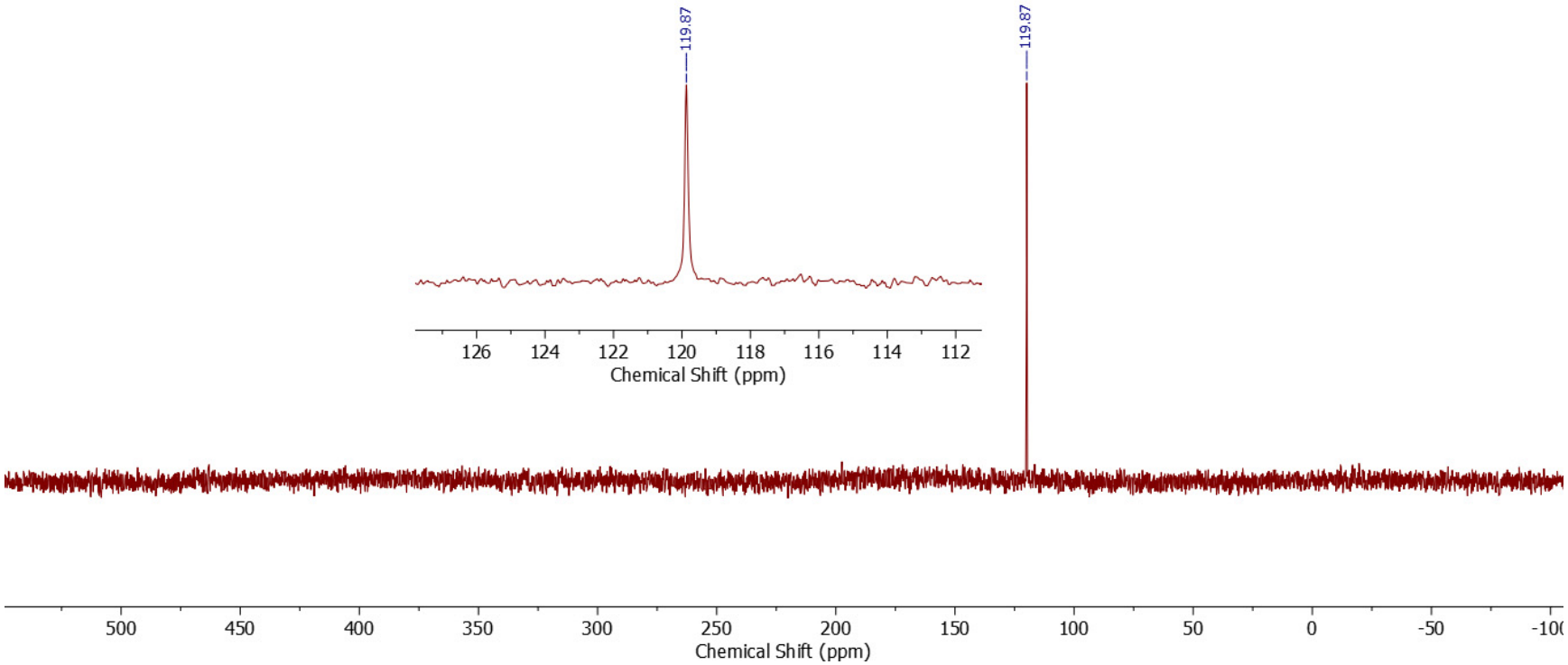
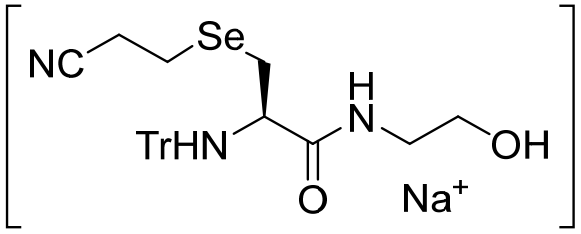


Figure S68: HR ESI-MS spectrum of alcohol **5** (expected mass: 530.1317)



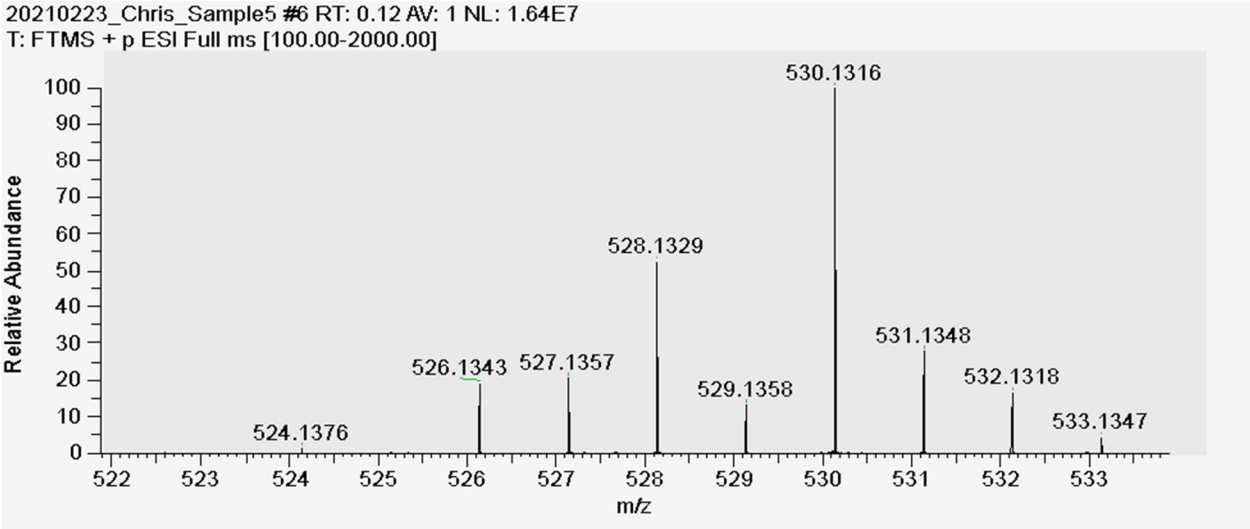


Figure S69: 500 MHz ^{1}H NMR spectrum of selenide phosphoramidite **6** (in acetone-d_6)

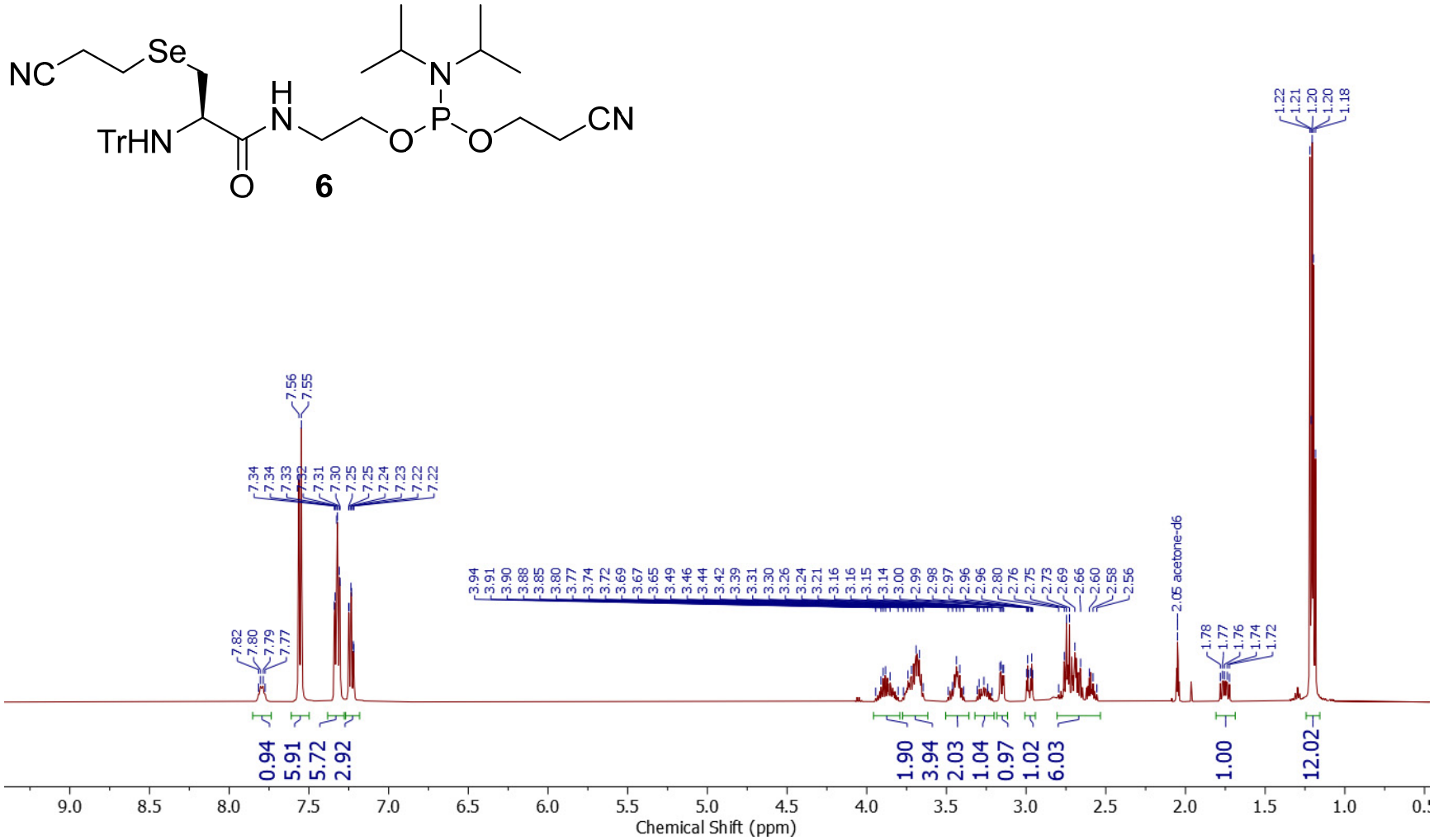


Figure S70: 125.7 MHz ^{13}C NMR spectrum of selenide phosphoramidite **6** (in acetone-d$_6$)

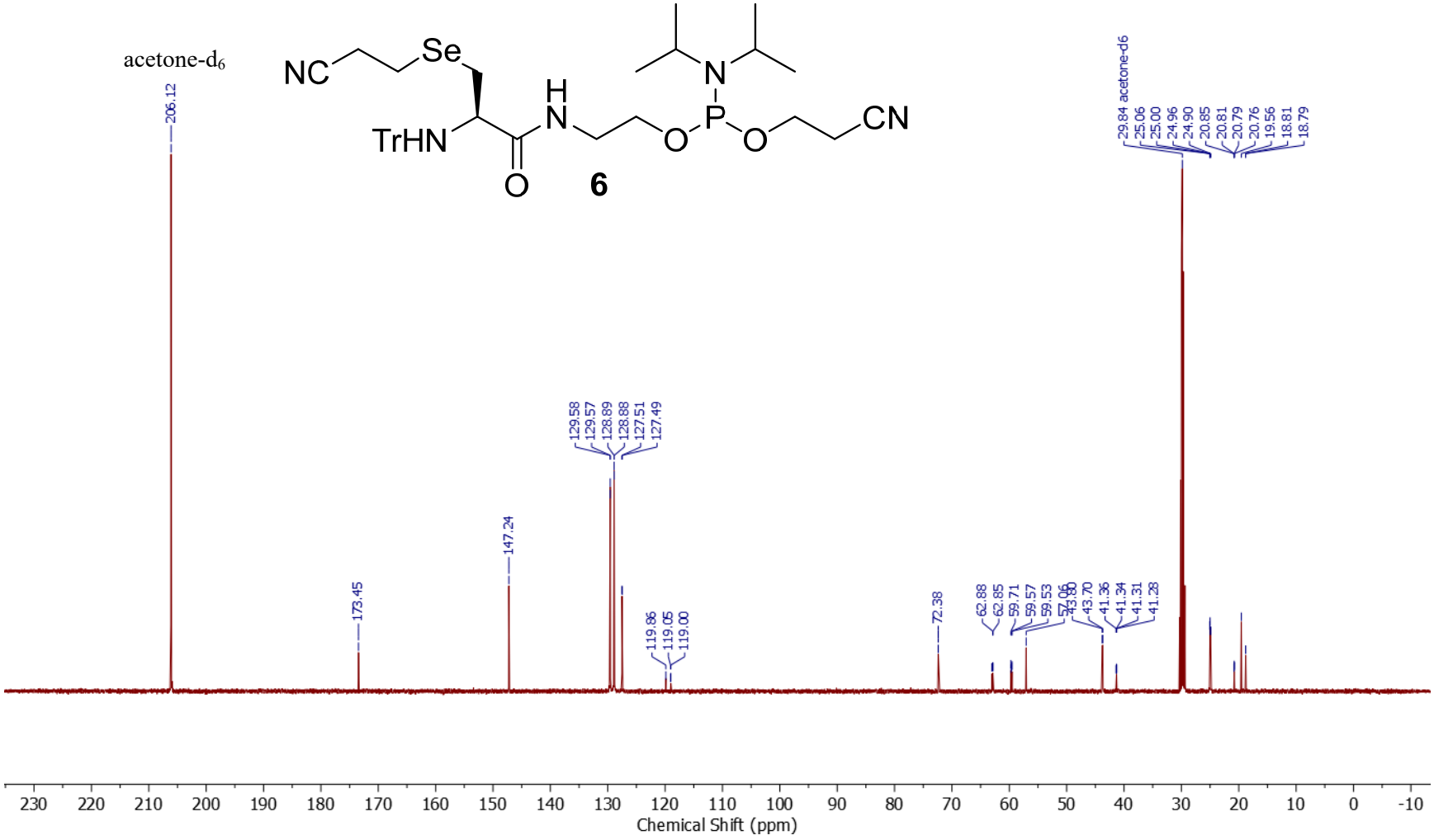


Figure S71: 202.3 MHz ^{31}P NMR spectrum of selenide phosphoramidite **6** (in acetone-d_6)

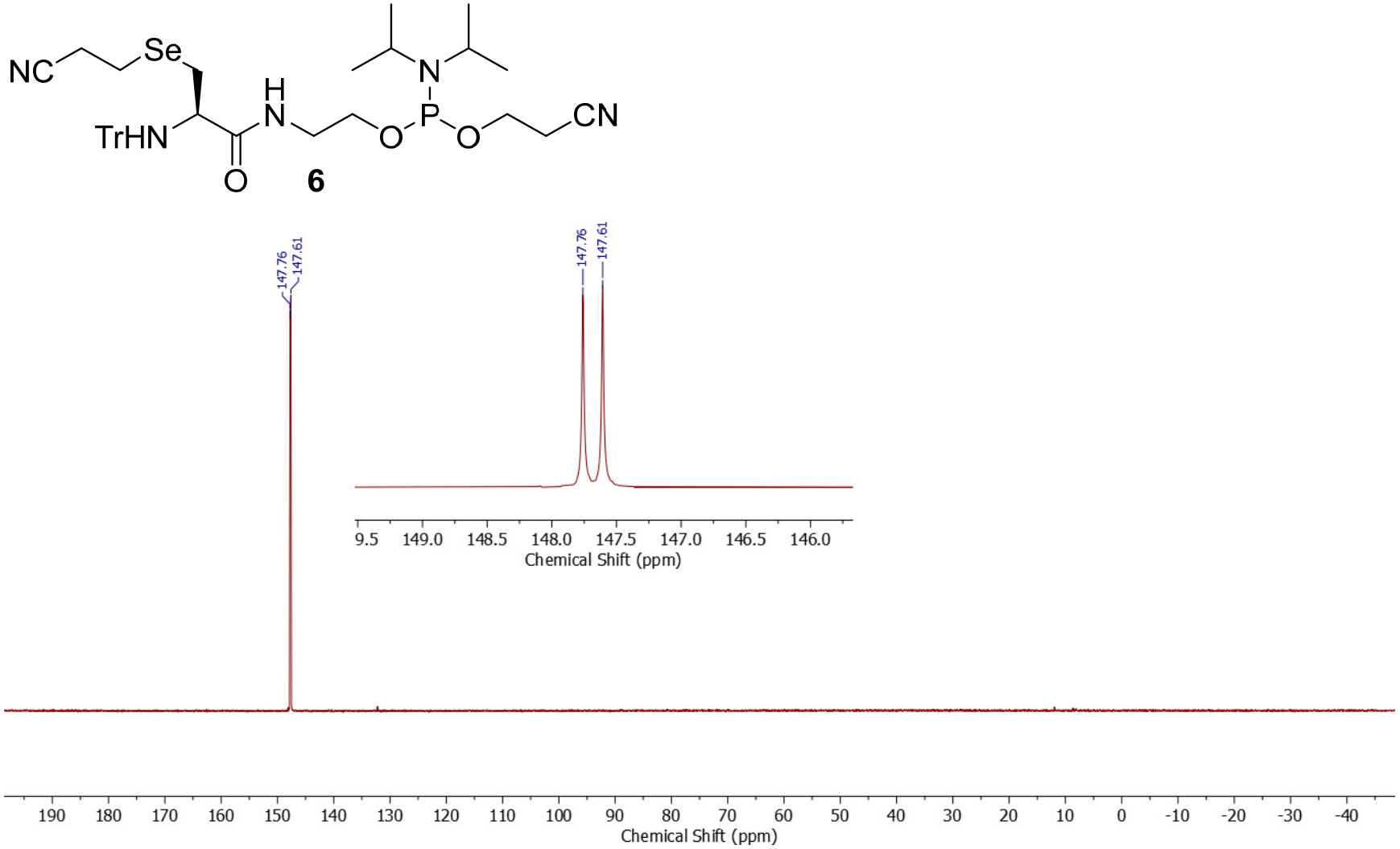


Figure S72: 95.3 MHz ^{77}Se NMR spectrum of selenide phosphoramidite **6** (in acetone-d_6)

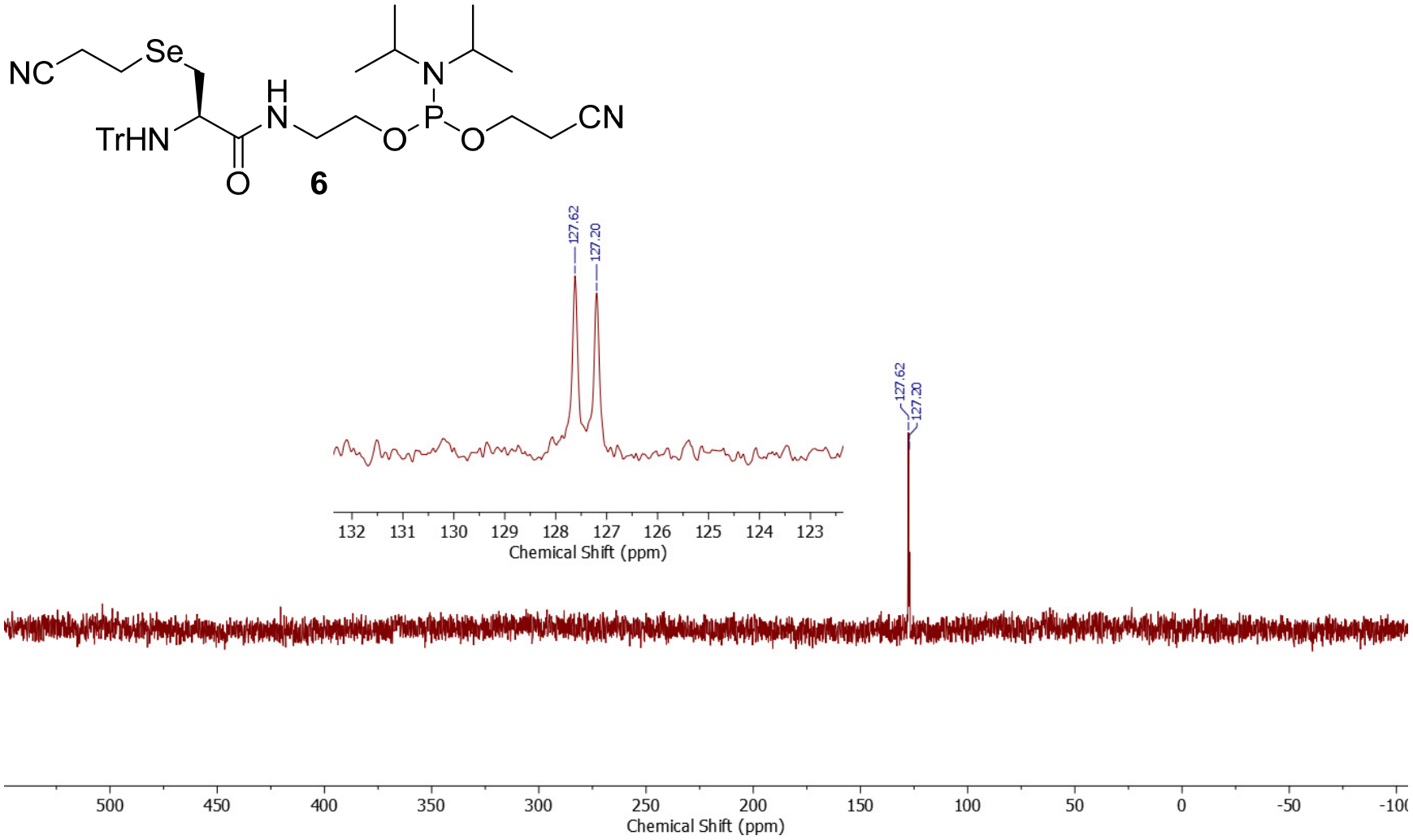
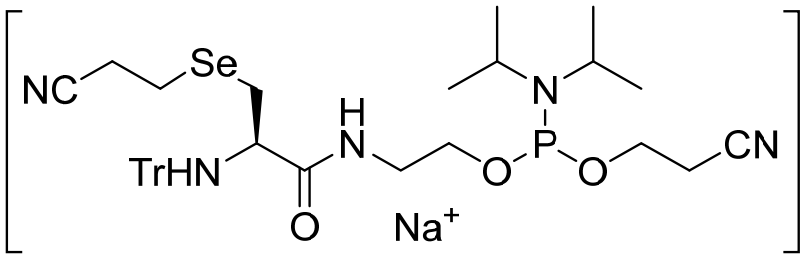
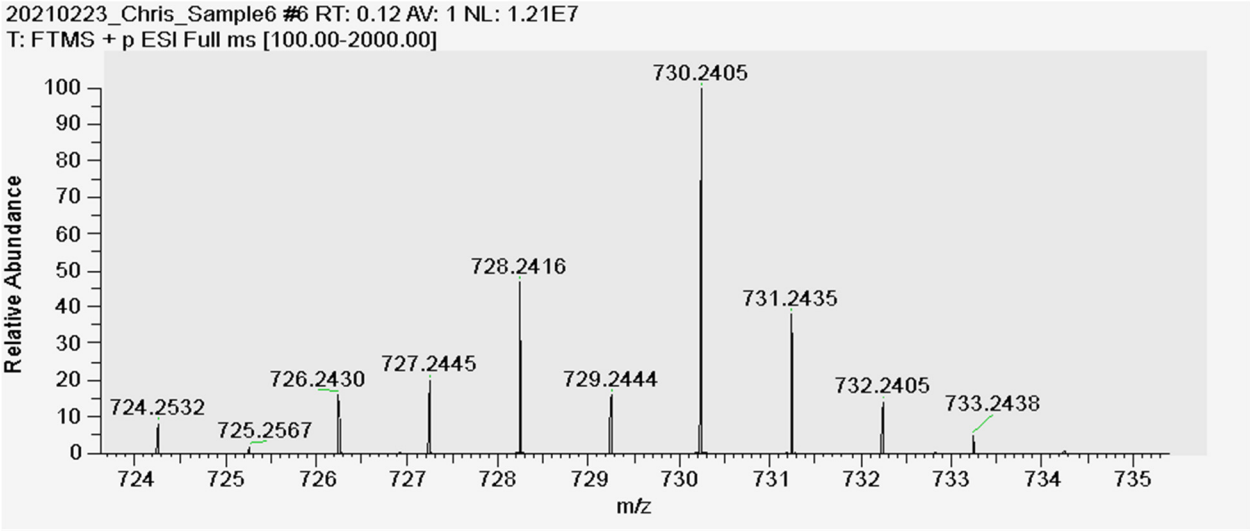


Figure S73: HR ESI-MS spectrum of selenide phosphoramidite **6** (expected mass: 730.2396)





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