Supporting Information

A Survey of Reagents for Selenocysteine Conjugation and the Stability of Resulting Selenocysteine Adducts

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1. General Information:

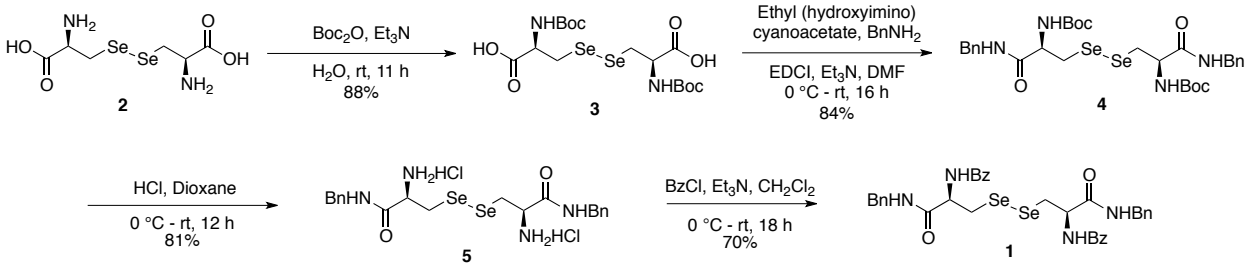
Dimethylformamide and dichloromethane were purified by passing through a solvent column of activated alumina (A-1). Unless otherwise indicated, all starting materials were purchased from different commercial sources and were used without further purification. Triethylamine was distilled before use. Reactions were conducted under an atmosphere of argon using flamed-dried glassware. Standard techniques for handling air-sensitive compounds were employed for all the operations. Removal of solvents was accomplished on a rotary evaporator at reduced pressure.

2. Physical Properties and Spectroscopic Measurements:

^{1}H NMR spectra were recorded on a Bruker spectrometer at 400 MHz. ^{13}C NMR spectra were recorded on a Bruker spectrometer at 100 MHz. The proton signal for non-deuterated solvent (δ 7.26 for $CHCl_3$) was used as an internal reference for ^{1}H NMR spectra. For ^{13}C NMR spectra, chemical shifts are reported relative to the δ 77.0 resonance of $CHCl_3$. Infrared (IR) spectra were recorded on neat samples using a Perkin-Elmer Spectrum One FT-IR Spectrometer equipped with a Universal ATR Sampling Accessory. High-resolution mass spectra were recorded at the University of Illinois Mass Spectrometry Laboratory. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25-mm thickness of silica gel. TLC plates were visualized with UV light and/or by staining with potassium permanganate (1.5g $KMnO_4$, 10 g K_2CO_3, 1.25 10% NaOH in 200 mL H_2O). Column chromatography was generally performed using a Biotage Isolera™ purification system with Biotage pre-packed columns. LC-MS was performed on an Agilent Infinity LC with an Agilent Technologies 500 MS.

3. Experimental Section

3.1. Selenocystine Synthesis



(2*R*,2'*R*)-3,3'-diselanediylbis(2-((*tert*-butoxycarbonyl)amino)propanoic acid) (3): To a suspension of L-selenocysteine (500 mg, 1.50 mmol, 1 equiv) in water (7.5 mL) was added Et_3N (0.63 mL, 4.49 mmol, 3 equiv) and

the resulting in a brown solution was placed in an ice bath. Di-*tert*-butyl-dicarbonate (980 mg, 4.49 mmol, 3 equiv) was added in small portions with vigorous stirring (over 5 minutes). The suspension was stirred at rt for 11 h, diluted with EtOAc (20 mL), washed with 1N HCl (20 mL), extracted with EtOAc (2 x 20 mL), washed with brine (20 mL), dried ($MgSO_4$ and Na_2SO_4), filtered, and concentrated under reduced pressure to give title compound (700 mg, 88%) as a yellow foam that was used in the next step without further purification. ^{1}H NMR (400 MHz, Methanol-d_4): δ 4.40 (dd, *J* = 8.8, 4.7 Hz, 2H), 3.45 (dd, *J* = 12.6, 4.9 Hz, 2H), 3.20 (dd, *J* = 12.7, 9.0 Hz, 2H), 1.45 (s, 18H). ^{13}C NMR (101 MHz, MeOD) δ 174.38, 157.82, 80.74, 55.65, 32.51, 28.77; IR (neat) 3380, 2981, 1683, 1512, 1286, 1243, 1162, 1050 cm^{-1}; HRMS (ESI) calcd for $C_{16}H_{28}N_2O_8Se_2Na$ $(M+Na)^+$ 559.0074, found 559.0068

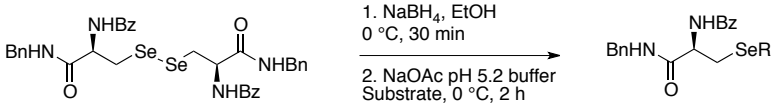
Di-*tert*-butyl ((2*R*,2'*R*)-diselanediylbis(1-(benzylamino)-1-oxopropane-3,2-diyl))dicarbamate (4): To (2*R*,2'*R*)-3,3'-diselanediylbis(2-((*tert*-butoxycarbonyl)amino)propanoic acid) (**3,** 980 mg, 1.83 mmol, 1 equiv) in anhydrous DMF (9.2 mL) was added benzyl amine (0.44 mL, 4.04 mmol, 2.2 equiv), ethyl (hydroxyimino)cyanoacetate (782 mg, 5.50 mmol, 3 equiv), and Et_3N (0.77 mL, 5.5 mmol, 3 equiv). The reaction mixture was stirred in an ice bath for 30 min before EDCI (1.06 g, 5.5 mmol, 3 equiv) was added. The reaction mixture was stirred at rt overnight (16 h) and quenched with half-saturated NH_4Cl (60 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL), and the combined organics were dried ($MgSO_4$ and Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography using a Biotage™ purification system with increasing concentration of EtOAc in hexanes to give title compound (1.10 g, 84% yield) as a yellow solid. Rf: 0.19/7:3/H:E ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (t, *J* = 6 Hz, 2H), 7.32-7.22 (m, 11H), 5.57 (d, *J* = 9.7 Hz, 2H), 4.90 (td, *J* = 4.6, 10.1 Hz, 2H), 4.60 (dd, *J* = 6.6, 15.0, 2H), 4.32 (dd, *J* = 5.4, 15.0, 2H), 3.28-3.17 (m, 4H), 1.23 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.70, 155.72, 138.16, 128.58, 127.70, 127.37, 79.99, 55.22, 43.44, 37.74, 28.17; IR (neat) 3252, 3029, 2909, 1685, 1549, 1505, 1469, 1456, 1256, 1121, 753, 699 cm^{-1}; HRMS (ESI) calcd for $C_{30}H_{43}N_4O_6Se_2$ $(M+H)^+$ 715.1513, found 715.1513

(2*R*,2'*R*)-3,3'-diselanediylbis(2-amino-*N*-benzylpropanamide) dihydrochloride (5): To di-*N*-boc-selenocystine-*bis*-benzyl amide (1.10 g, 1.54 mmol, 1 equiv) was added dioxane (2 mL) and the suspension was placed in an ice bath. 4 M HCl in dioxane (3.9 mL, 15.4 mmol, 10 equiv) was added dropwise and the resulting brown solution was stirred at rt overnight (12 h). The reaction mixture was concentrated under reduced pressure to give title compound

(731 mg, 81%) as a beige solid that was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.48 (t, J = 6 Hz, 2H), 8.66 (s, 6H), 7.36-7.29 (m, 8H), 7.29-7.22 (m, 2H), 4.40-4.29 (m, 4H), 4.19 (t, J = 6.4 Hz, 2H), 3.57 (dd, J = 6, 12.8 Hz, 2H), 3.45 (dd, J = 7.0, 12.9, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 167.01, 138.35, 128.24, 127.35, 126.91, 52.58, 42.37, 29.46; IR (neat) 3252, 2919, 1687, 1550, 1471, 1304, 1257, 1058, 751, 699 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{27}N_4O_2Se_2$ $(M+H)^+$ 515.0464, found 515.0464

***N*,*N*'-((2*R*,2'*R*)-diselanediyl*bis*(1-(benzylamino)-1-oxopropane-3,2-diyl)) dibenzamide (1):** To Selenocystine-*bis*-benzyl amide dihydrochloride (717 mg, 1.23 mmol, 1 equiv) suspended in CH_2Cl_2 (4.7 mL) was added DMF (1 mL) forming a brown solution. Et_3N (0.79 mL, 5.64 mmol, 4.6 equiv) was then added and the solution was placed in an ice bath. Benzoyl chloride (0.34 mL, 2.94 mmol, 2.4 equiv) dissolved in CH_2Cl_2 (0.52 mL) was added dropwise and the resulting brown suspension was stirred at rt overnight. After 14 h, the reaction mixture had formed a thick suspension and was not stirring. DMF (*ca* 2 mL) was added resulting in a brown suspension. The suspension was stirred for a further 2.5 h, diluted with CH_2Cl_2 (30 mL), washed successively with HCl (30 mL, 10 %), water, $NaHCO_3$, and brine, and purified by flash chromatography using a Biotage™ purification system with increasing concentration of EtOAc in CH_2Cl_2 (up to 50 %) to give title compound (883 mg, 70%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90 (t, J = 5.4 Hz, 2H), 7.50-7.30 (m, 24H), 5.76 (td, J = 5.0, 9.6 Hz, 2H), 4.58 (dd, J = 5.7, 14.7 Hz, 2H), 4.47 (dd, J = 5.0, 14.7 Hz, 2H), 3.49-3.34 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.59, 167.43, 137.64, 133.28, 131.89, 128.72, 128.54, 128.24, 127.52, 127.14, 54.56, 44.03, 36.69; IR (neat) 3269, 1668, 1634, 1578, 1533, 1490, 1454, 1288, 1241, 665 cm^{-1}; HRMS (ESI) calcd for $C_{34}H_{35}N_4O_4Se_2$ $(M+H)^+$ 723.0989, found 723.0986

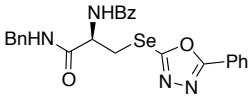
3.2. Selenocysteine Conjugation



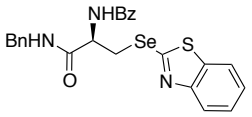
General Procedure for Conjugation Conditions

Bis-(BzHN-Sec-benzamide) (**1,** 67.8 mg, 0.094 mmol, 1.0 equiv) was suspended in EtOH (2.6 mL, degassed by bubbling argon through for about 20 minutes). The suspension was placed in an ice bath before $NaBH_4$ (10.7 mg, 0.282 mmol, 3.0 equiv) was added in one portion. The reaction mixture bubbled and formed a yellow solution and

then a white precipitate. The reaction was stirred in the ice bath for 30 min. NaOAc buffer (pH 5.2, 2.6 mL, degassed by bubbling argon through for about 20 min) was added to the reaction followed by substrate (2.5 equiv). The resulting suspension was stirred in the ice bath for 2 h, partitioned between half-saturated brine (25 mL) and EtOAc (30 mL), extracted with EtOAc (2 x 30 mL), washed with brine, dried ($MgSO_4$ and Na_2SO_4), and filtered. The crude product was purified by flash chromatography with increasing concentration of EtOAc in hexanes (up to 60%).

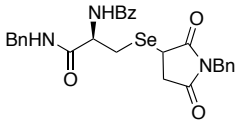


(*R*)-*N*-(1-(benzylamino)-1-oxo-3-((5-phenyl-1,3,4-oxadiazol-2-yl)selanyl)propan-2-yl)benzamide (Sec-ODA, 11): *Bis*-(BzHN-Sec-benzamide (**1,** 62.2 mg, 0.093 mmol, 0.83 equiv) was suspended in MeOH (3.1 mL, degassed by bubbling argon through, 1 min/mL) in a 10 mL 2-neck round bottom flask. The suspension was placed in an ice bath before $NaBH_4$ (10.5 mg, 0.279 mmol, 2.5 equiv) was added in one portion. The reaction was stirred for 3 min after which NaOAc buffer (pH 5.2, 3.1 mL, degassed by bubbling argon through, 1 min/mL) was added followed by phenyl methyl sulfone[1] (**6,** 25 mg, 0.111 mmol, 1 equiv). The reaction mixture was stirred for an overall 4 h, quenched by the addition of water, extracted with EtOAc, dried, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography with a Biotage™ purification system with increasing concentration of EtOAc in hexanes to give 26.9 mg of Sec-ODA as a brown solid (44%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 6.5 Hz, 1H), 8.04-7.99 (m, 2H), 7.95-7.90 (m, 2H), 7.58-7.42 (m, 8H), 7.41-7.20 (m, 7H), 5.23 (td, *J* = 6.5, 3.6 Hz, 1H), 4.49 (d, *J* = 5.9 Hz, 2H), 3.88 (dd, *J* = 13.5, 6.6 Hz, 1H), 3.76 (dd, *J* = 13.6, 3.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.08, 168.40, 167.34, 158.69, 137.65, 132.76, 132.17, 131.94, 129.09, 128.66, 127.50, 127.47, 127.44, 126.79, 123.27, 54.34, 43.65, 29.74; IR (neat) 3311, 1623, 1524, 1467, 1167, 663 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{23}N_4O_3Se$ $(M+H)^+$ 507.0935, found 507.0929

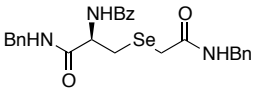


(*R*)-*N*-(3-(benzo[*d*]thiazol-2-ylselanyl)-1-(benzylamino)-1-oxopropan-2-yl)benzamide (Sec-BTA, 12): Substrate 2-(methylsulfonyl)benzo[*d*]thiazole[1] (**8,** 50.2 mg, 0.235 mmol, 2.5 equiv) was used to give 79.4 mg of product (85%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 (d, *J* = 5.6 Hz, 1H), 7.85-7.80 (m, 3H), 7.71-7.64 (m, 1H), 7.53 (bt, *J* = 5.2 Hz, 1H), 7.50-7.44 (m, 1H), 7.41 (ddd, *J* = 8.2, 7.2, 1.4 Hz, 1H), 7.38-7.35 (m, 1H), 7.34-7.27 (m, 6H), 7.25-7.21 (m, 1H), 5.17 (td, *J* = 6.3, 3.4 Hz, 1H), 4.50 (d, *J* = 5.7 Hz, 2H), 3.99 (dd, *J* = 13.7, 6.8 Hz, 1H), 3.74 (dd, *J* = 13.7, 3.4 Hz, 1H); ^{13}C NMR

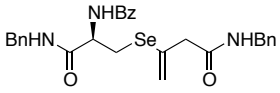
(101 MHz, $CDCl_3$) δ 170.47, 168.97, 161.36, 153.02, 137.89, 136.82, 133.23, 131.97, 128.67, 128.54, 127.52, 127.38, 126.24, 124.90, 121.31, 121.27, 55.13, 43.60, 29.95; IR (neat) 3287, 1634, 1523, 1454, 1423, 967, 753, 664 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{22}N_3O_2Se$ $(M+H)^+$ 496.0598, found 496.0598.



***N*-((2*R*)-3-((1-benzyl-2,5-dioxopyrrolidin-3-yl)selanyl)-1-(benzylamino)-1-oxopropan-2-yl)benzamide (Sec-MAL, 13):** Substrate *N*-benzyl maleimide (108.08 mg, 0.376 mmol, 4 equiv) was used to give 32.9 mg of product (32%) as a brown solid and as a mixture of diastereomers: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.90-7.83 (m, 2H), 7.57-7.50 (m, 1H), 7.50-7.42 (m, 2H), 7.42-7.21 (m, 11H), 5.08-4.95 (m, 1H), 4.74-4.38 (m, 4.5H), 4.25 (dd, *J* = 9.3, 3.9 Hz, 0.6H), 3.86-3.66 (m, 1H), 3.32-3.21 (m, 1H), 3.15-3.04 (m, 1H), 2.58 (ddd, *J* = 20.7, 19.1, 3.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.88, 178.81, 174.42, 174.24, 170.27, 170.13, 167.82, 167.46, 137.68, 137.60, 135.22, 135.19, 134.19, 133.34, 133.19, 132.06, 132.03, 128.72, 128.66, 128.63, 128.57, 128.38, 128.12, 127.72, 127.63, 127.51, 127.48, 127.40, 127.23, 53.73, 52.93, 43.73, 42.82, 41.43, 36.50, 35.94, 30.71, 30.04, 29.71, 28.09, 27.39; IR (neat) 3298, 2927, 1774, 1699, 1635, 1531, 1396, 1340, 1167, 908, 727, 664 cm^{-1}; HRMS (ESI) calcd for $C_{28}H_{28}N_3O_4Se$ $(M+H)^+$ 550.1245, found 550.1241

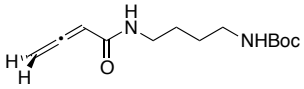


(*R*)-*N*-(1-(benzylamino)-3-((2-(benzylamino)-2-oxoethyl)selanyl)-1-oxopropan-2-yl)benzamide (Sec-IAM, 14): Substrate *N*-benzyl-2-iodoacetamide[2] (**9**, 64.7 mg, 0.253 mmol, 2.5 equiv) was used to give 48 mg of product (50%) was a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84-7.74 (m, 2H), 7.57-7.47 (m, 1H), 7.48-7.39 (m, 2H), 7.36-7.17 (m, 12H), 4.77 (dd, *J* = 7.6, 5.1 Hz, 1H), 4.49-4.36 (m, 4H), 3.40-3.25 (m, 2H), 3.19 (dd, *J* = 13.5, 4.9 Hz, 1H), 2.90 (dd, *J* = 13.5, 7.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$:CH_3OD/15:1) δ 171.16, 170.51, 167.82, 137.77, 137.47, 133.13, 131.93, 128.49, 128.48, 128.45, 127.64, 127.50, 127.32, 127.16, 53.36, 43.84, 43.59, 26.73, 25.47; IR (neat) 3298, 1657, 1633, 1522, 1453, 694, 663 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{28}N_3O_3Se$ $(M+H)^+$ 510.1296, found 510.1294

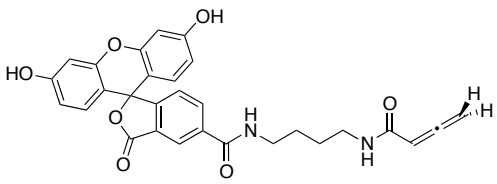


(*R*)-*N*-(1-(benzylamino)-3-((4-(benzylamino)-4-oxobut-1-en-2-yl)selanyl)-1-oxopropan-2-yl)benzamide (Sec-ALL, 15): Substrate *N*-benzylbuta-2,3-dienamide[3] (**10,** 65.2 mg, 0.376 mmol, 4 equiv) was used to give 83.6 mg of product (83%) as a brown solid:

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (d, J = 8.0 Hz, 1H), 8.63 (t, J = 6.0 Hz, 1H), 8.44 (t, J = 6.0 Hz, 1H), 7.95-7.89 (m, 2H), 7.58-7.51 (m, 1H), 7.51-7.44 (m, 2H), 7.34-7.17 (m, 11H), 5.62 (s, 1H), 5.31 (s, 1H), 4.73 (ddd, J = 9.7, 7.8, 4.9 Hz, 1H), 4.31 (dd, J = 6.0, 3.2 Hz, 2H), 4.25 (d, J = 6.0 Hz, 2H), 3.26 (dd, J = 12.2, 5.0 Hz, 1H), 3.23 (s, 2H), 3.14 (dd, J = 12.3, 9.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.33, 168.51, 166.43, 139.27, 139.24, 134.71, 133.92, 131.41, 128.22, 128.20, 128.17, 127.61, 127.20, 127.03, 126.73, 126.69, 116.73, 53.48, 45.81, 42.22, 27.14; IR (neat) 3298, 2974, 1640, 1534, 1086, 1045, 879, 697 cm^{-1}; HRMS (ESI) calcd for $C_{28}H_{30}N_3O_3Se$ $(M+H)^+$ 536.1452, found 536.1451



***tert*-Butyl (4-(buta-2,3-dienamido)butyl)carbamate (21):** 3-butynoic acid (100 mg, 1.13 mmol, 1.0 equiv) and 2-chloro-1-methyl pyridinium iodide (Mukaiyama's reagent, 446 mg, 1.70 mmol, 1.5 equiv) were suspended in CH_2Cl_2 (4.1 mL) and the resulting yellow suspension was stirred at rt for 1 h. A solution of boc-1,4-diaminobutane (0.27 mL, 1.36 mmol, 1.2 equiv) and Et_3N (0.24 mL, 1.70 mmol, 1.5 equiv) in CH_2Cl_2 (2.0 mL) was added dropwise (10 minutes) after which the resulting yellow solution was stirred at rt for another 30 min. Additional Et_3N (0.24 mL, 1.70 mmol, 1.5 equiv) was added and the resulting red solution was stirred at room temperature for 15 h, concentrated under reduced pressure, diluted with EtOAc, and washed with water. The aqueous layer was extracted once with EtOAc, the combined organics were washed with brine, dried (Na_2SO_4 and $MgSO_4$), and concentrated under reduced pressure. The crude product was purified by flash chromatography with increasing concentration of EtOAc in hexanes (up to 100%) to give product (127 mg, 44%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95 (s, 1H), 5.62 (t, J = 6.6 Hz, 1H), 5.21 (d, J = 6.7 Hz, 2H), 4.59 (s, 1H), 3.36-3.25 (m, 2H), 3.18-3.07 (m, 2H), 1.60-1.47 (m, 5H), 1.43 (s, 10H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 211.75, 164.56, 156.08, 90.84, 80.41, 79.13, 40.10, 39.35, 28.40, 27.52, 26.81; IR (neat) 3346, 3299, 2950, 2878, 1973, 1949, 1681, 1627, 1519, 1277, 1168 cm^{-1}; HRMS (ESI) calcd for $C_{13}H_{22}N_2O_3Na$ $(M+Na)^+$ 277.1528, found 277.1524



***N*-(4-(buta-2,3-dienamido)butyl)-3',6'-dihydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthene]-5-carboxamide (Fl-ALL, 19).** *tert*-Butyl (4-(buta-2,3-dienamido)butyl)carbamate (**21,** 24.6

mg, 0.097 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (0.29 mL) and placed in an ice bath before CF_3CO_2H (0.19 mL) was added dropwise. The reaction mixture was stirred in the ice bath for 1 h, concentrated under reduced pressure to give *N*-(4-aminobutyl)buta-2,3-dienamide trifluoroacetate salt as a yellow gel. To 5-carboxyfluorescein (18.2 mg, 48 μmol, 1 equiv), EDCI•HCl (18.5 mg, 97 μmol, 2 equiv), and Oxyma (14.1 mg, 97 μmol, 2 equiv) was added DMF (0.5 mL) and Et_3N (20.2 μL) followed by *N*-(4-aminobutyl)buta-2,3-dienamide trifluoroacetate (13.0 mg, 48 μmol, 1 equiv) in DMF (0.5 mL). The reaction mixture was stirred for 3.5 h, purified by preparative HPLC and lyophilized to give product (5.3 mg, 21%) as a white solid: Retention Time: 6.406 min, LRMS (ESI) calcd for $C_{29}H_{25}N_2O_7$ $[M+H]^+$ 513.17, found 513.5; 90 % purity by LC-MS at 254 nm; HRMS (ESI) calcd for $C_{29}H_{25}N_2O_7$ $(M+H)^+$ 513.1662, found 513.1651

3.3. Figures and Schemes

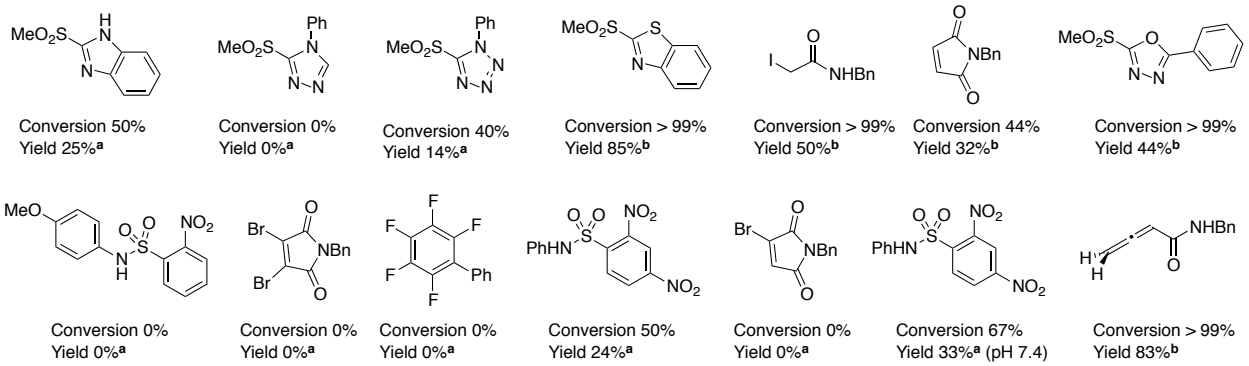
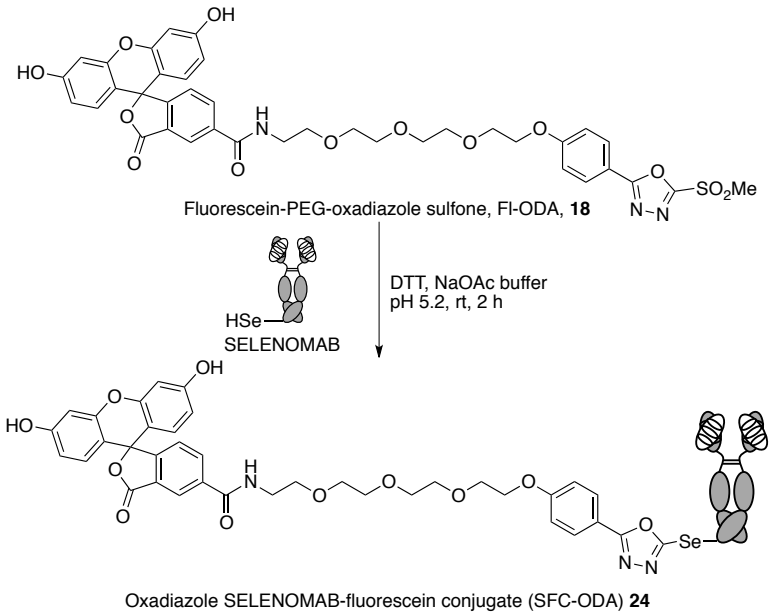


Fig. S1 All the electrophilic reagents surveyed for selenocysteine conjugation. Conversion determined by ^{1}H NMR of crude product. [a]Yield determined by LC-MS. [b]Isolated yield.



Scheme S1 Conjugation of SELENOMAB to fluorescein oxadiazole probe to give SELENOMAB-fluorescein conjugate **24** (SFC-ODA)

3.4. Antibody Conjugation and Analysis

SELENOMAB Conjugation Reactions

The cloning, expression, and purification of trastuzumab scFv-Fc-Sec and scFv-Fc-stop antibody were performed as previously described.[4] Conjugation reactions were carried out using 4 μM (~ 0.5 mg/mL) tratuzumab scFv-Fc-Sec or scFv-Fc-stop and 40 μM fluorescein-attached ALL **19** (Fl-ALL) or sulfone linker **18** (Fl-ODA) in 100 mM sodium acetate (pH 5.2) buffer in the presence of 0.1 mM dithiothreitol (DTT). The reactions were allowed to proceed for indicated time points at room temperature. Unconjugated compounds were removed by using NAP-5 columns (GE healthcare) and buffer-exchanged with PBS. The labeling was analyzed by SDS-PAGE.

SDS-PAGE

2 μg conjugates were incubated at 70 ºC for 5 min in NuPAGE LDS Sample Buffer (Life Technologies) supplemented with or without 50 mM DTT and then loaded on a NuPAGE Novex Bis-Tris 4-12% gradient gel (Life Technologies). Following SDS-PAGE and prior to staining with SimplyBlue SafeStain (Life Technologies), a picture of the gel was taken under blue light illumination (Life Technologies) to record the fluorescence.

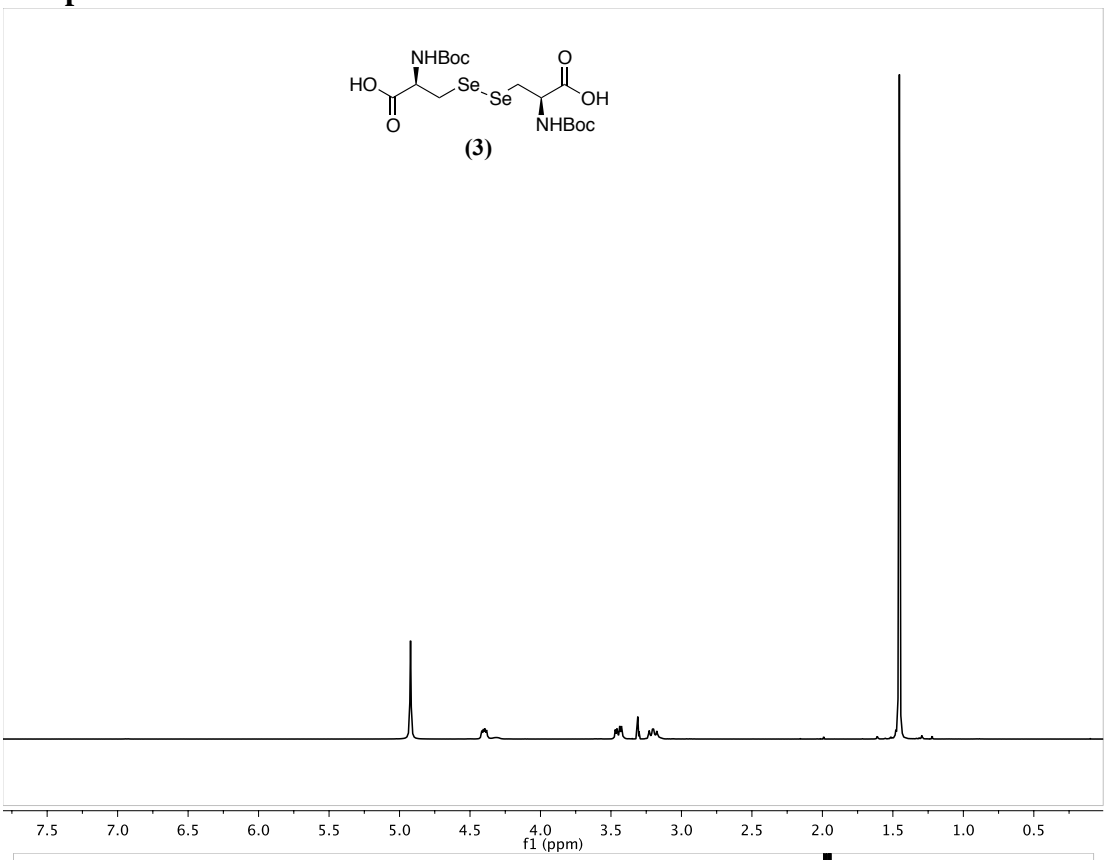
Analysis of HER2 Binding by Flow Cytometry

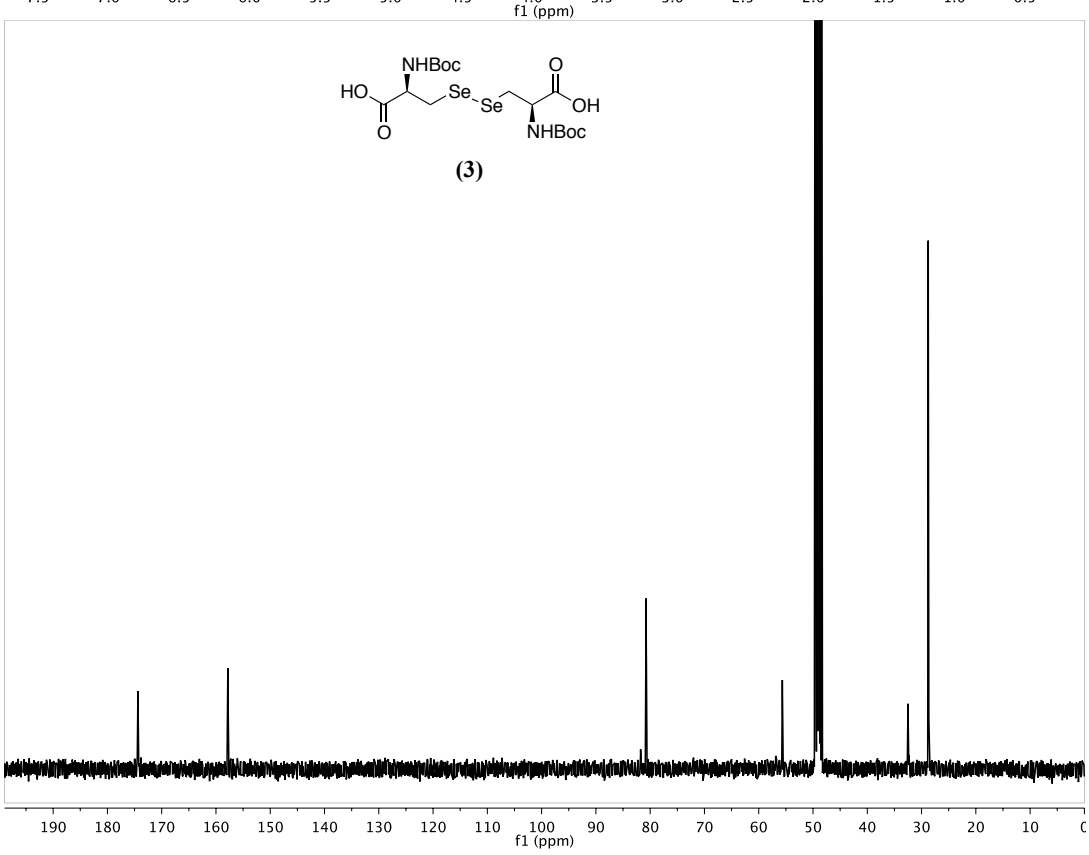
Human breast cancer cell lines SK-BR-3 and MDA-MB-468 were maintained at 37 °C in a humidified 5% CO_2 atmosphere in DMEM completed with 10% FBS and 1% Pen Strep. 2 x 10^5 cells were distributed in each well of a V-shaped 96-well plate. The cells were incubated with 10 μg SELENOMAB-fluorescein conjugates for 30 min on ice. Samples were washed three times with 200 μL FACS buffer and transferred to FACS tubes. Fluorescence was measured on an LSR II Flow Cytometer (Becton-Dickinson), and data were analyzed using FlowJo software (Tree Star, Inc.).

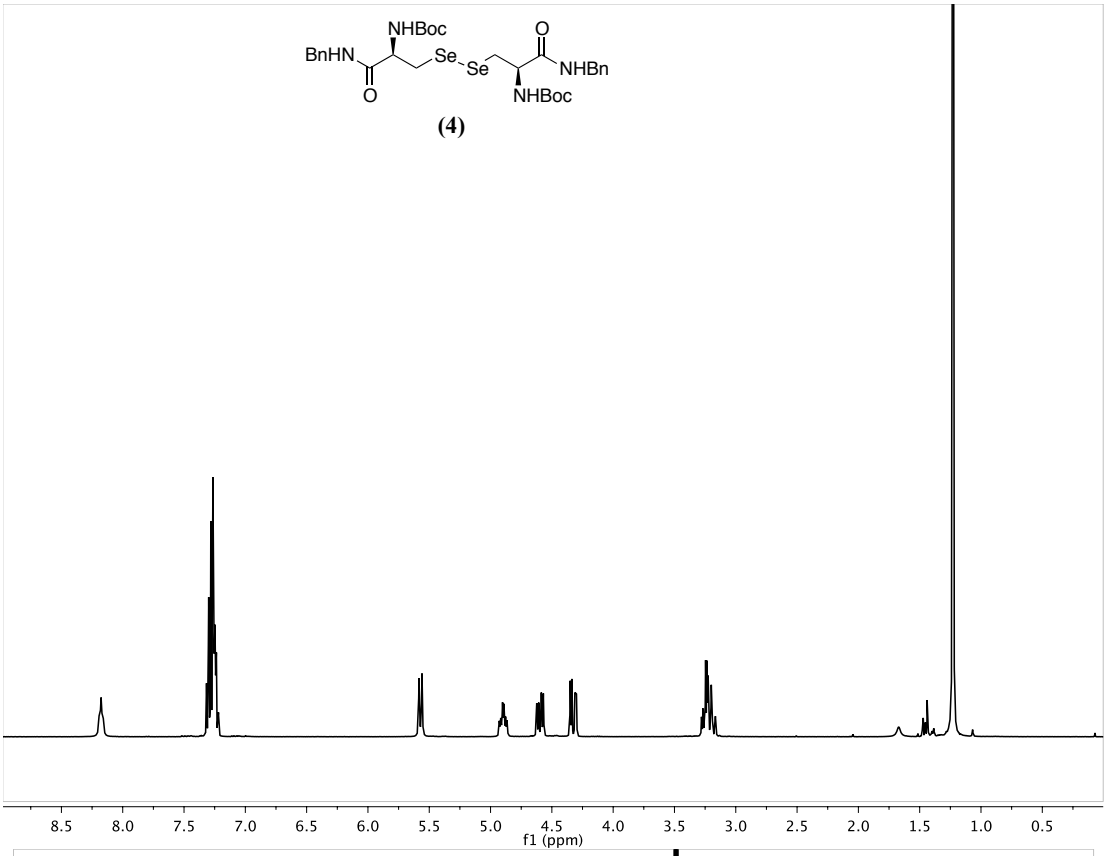
Human plasma stability

Conjugate plasma stability was carried out as previously described.[5] In brief, SELENOMAB-fluorescein conjugates at a concentration of 1 mg/mL were diluted 1:1 (v/v) into human plasma and incubated at 37 °C for the duration of the study. Aliquots were removed at various time points (0, 4, 8, 12, 24, 48, and 72 h) and stored at -80 °C, and then SDS-PAGE was performed as above.

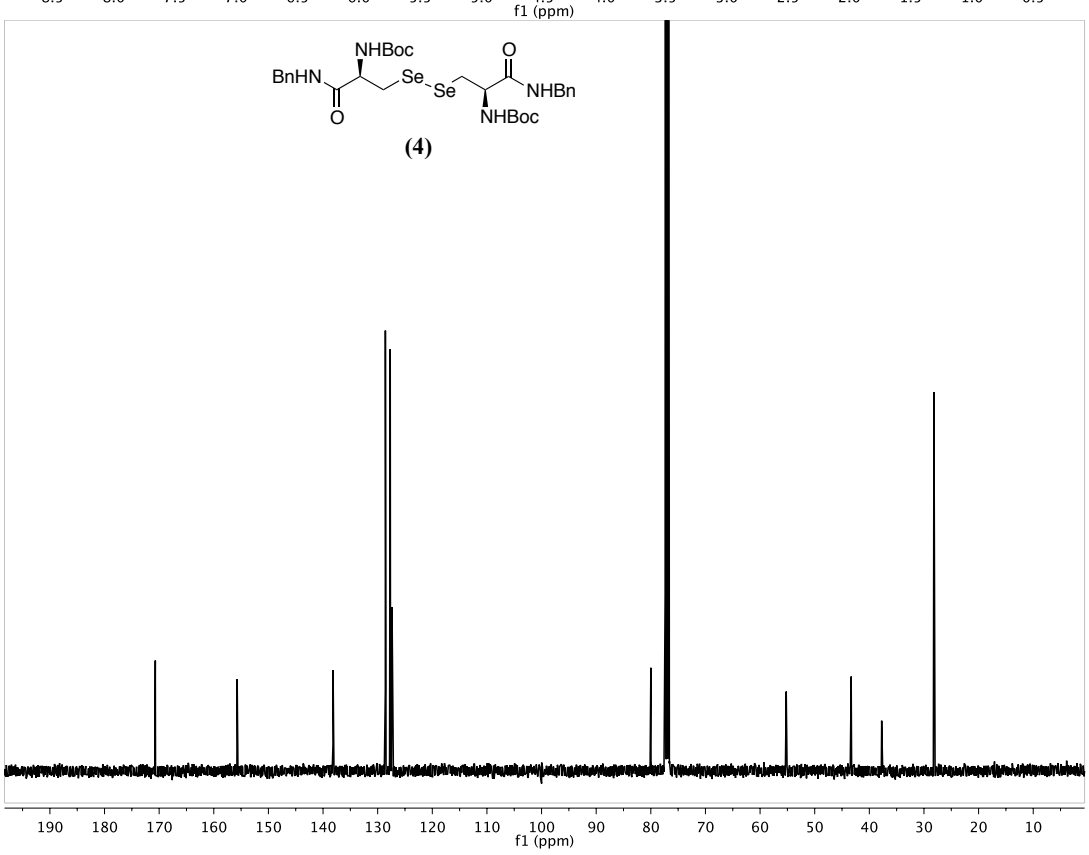
4. NMR Spectra






NHBoc
BnHN
Se
Se
O
NHBn
O
NHBoc
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

(4)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BnHN
Se
Se
O
NHBn
O
NHBoc
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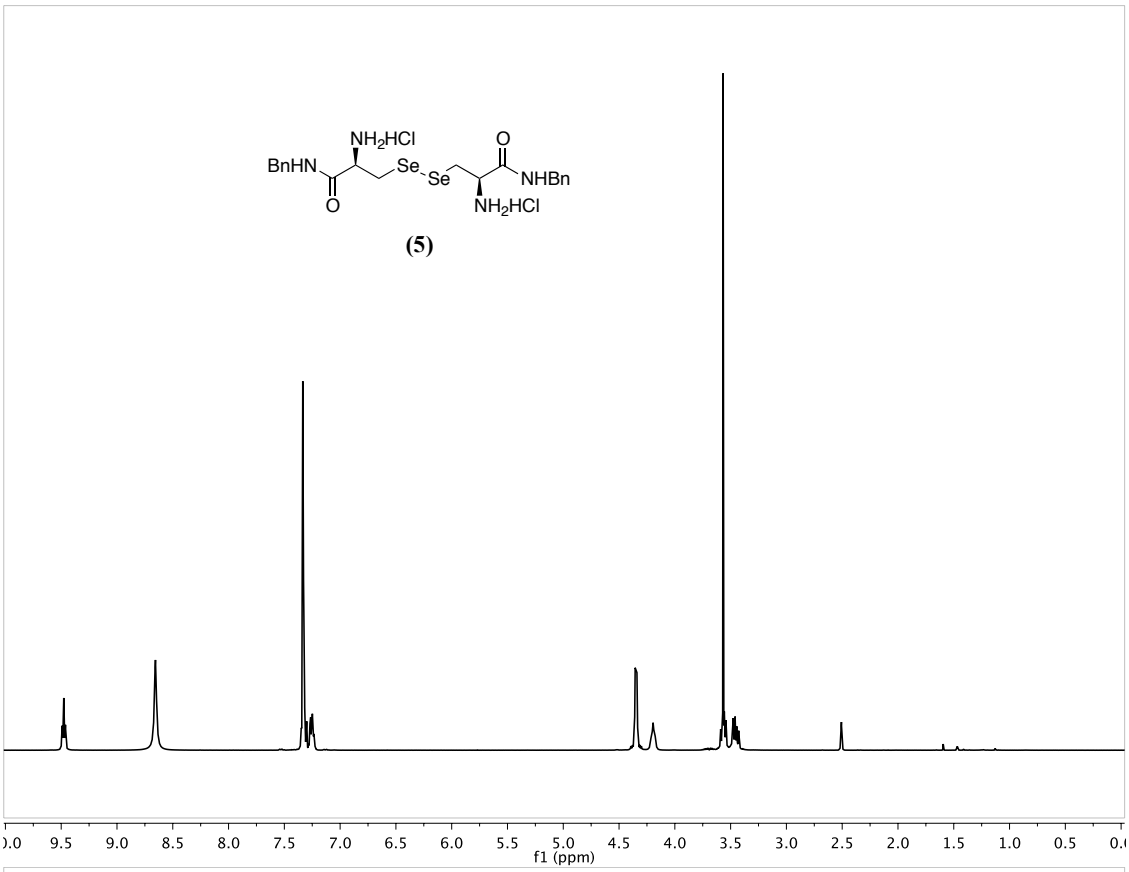
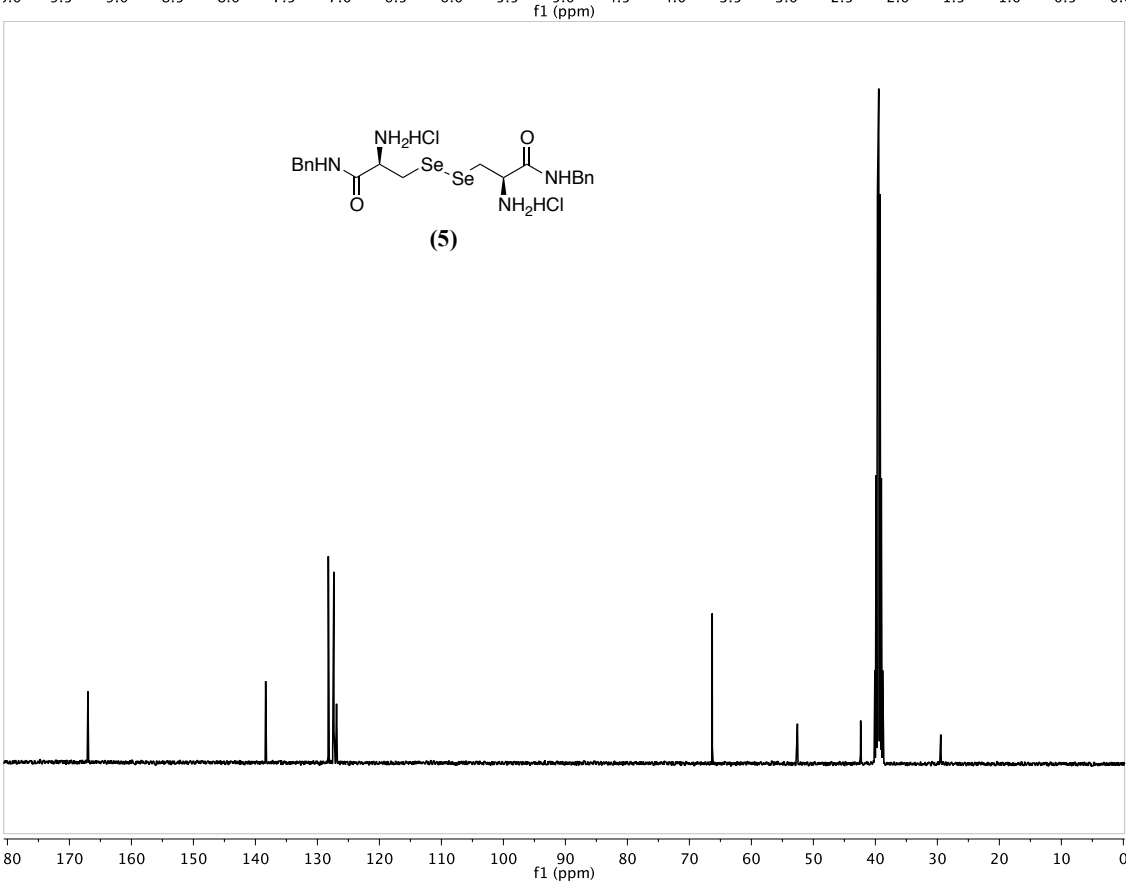
f1 (ppm)

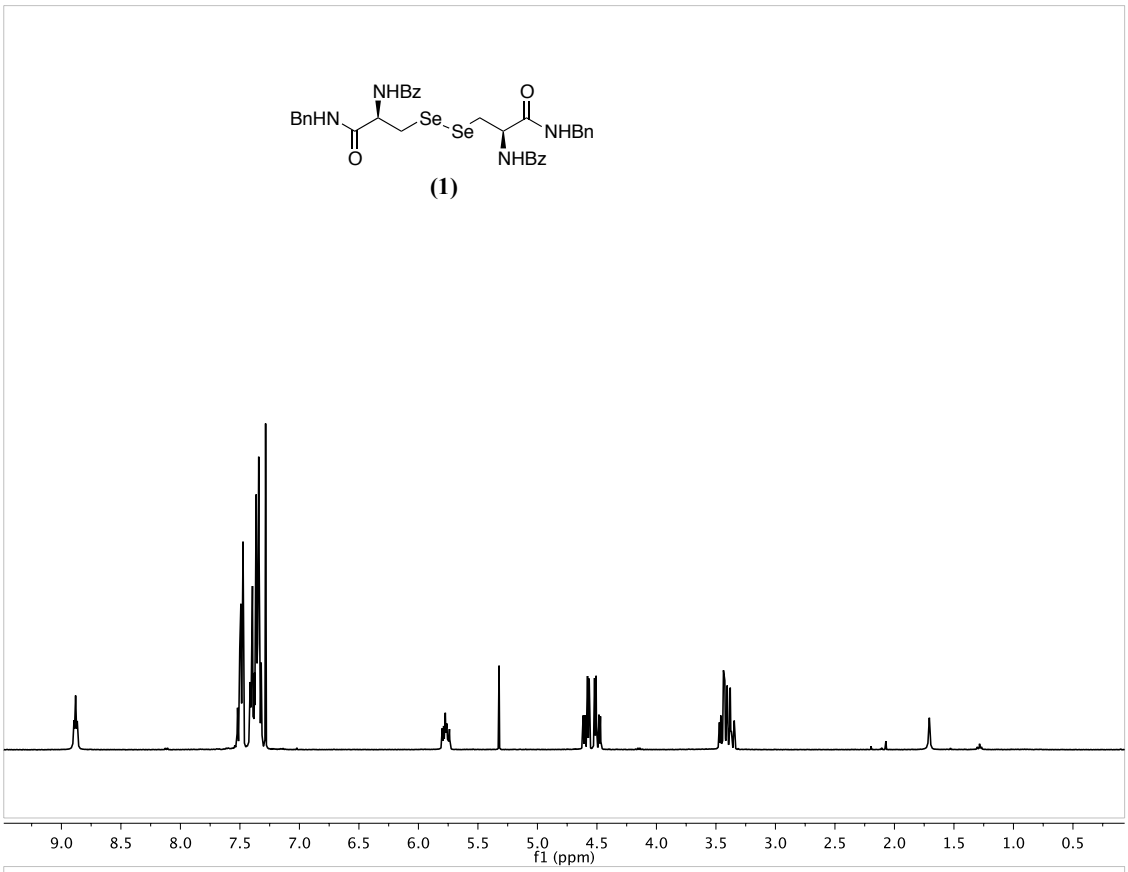
(4)

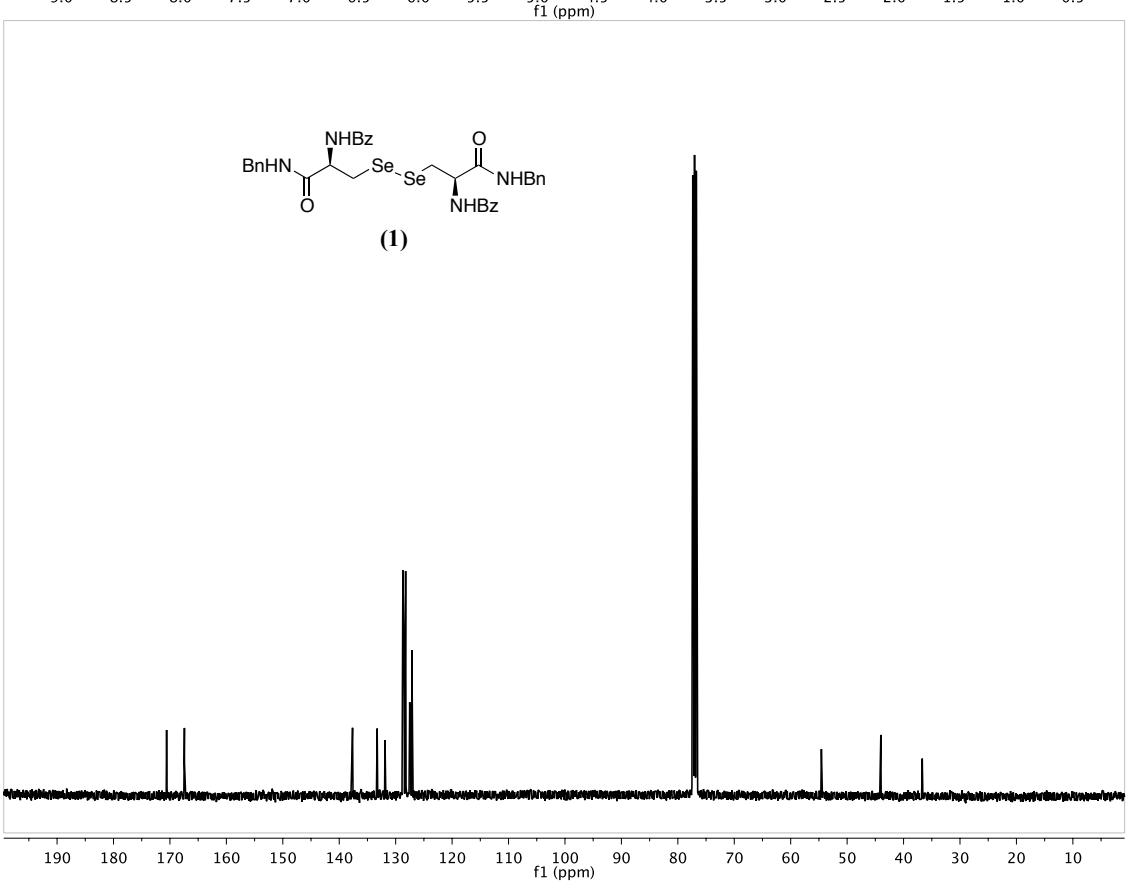

NH2HCl
BnHN
Se
Se
O
NHBn
NH2HCl
O
f1 (ppm)

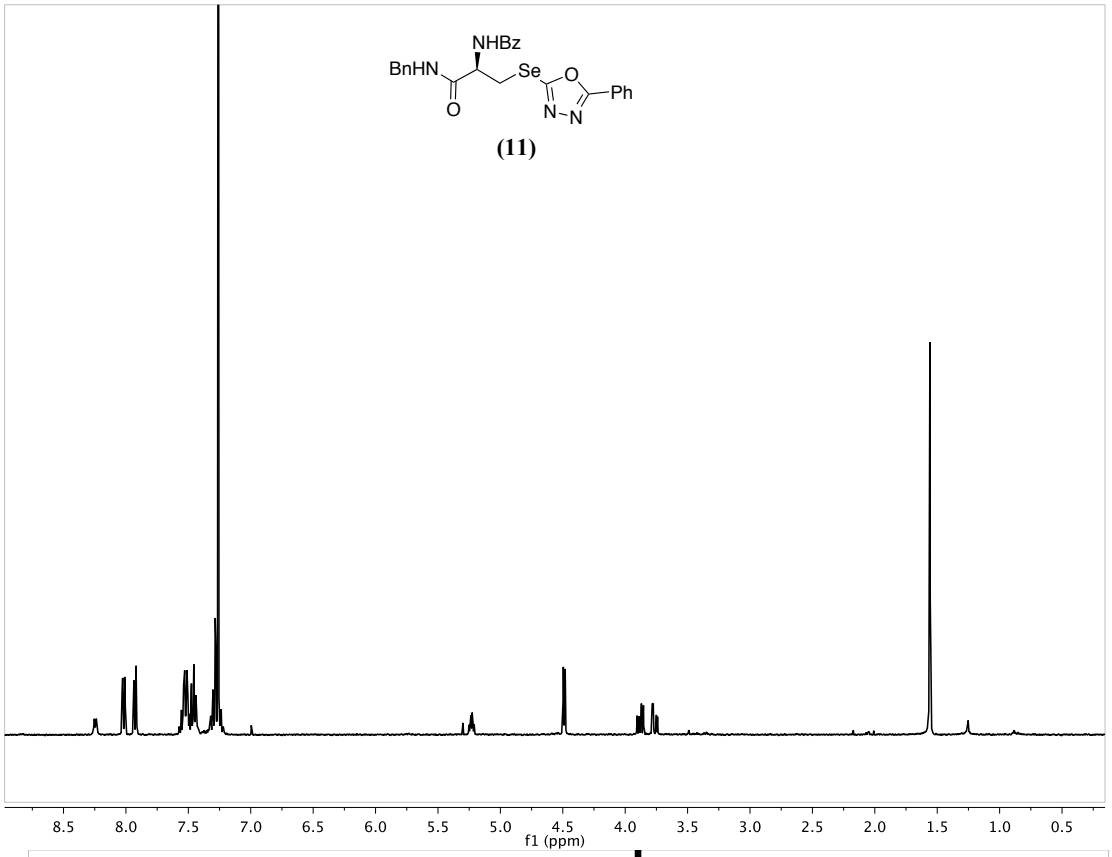
(5)


NH2HCl
BnHN
Se
Se
O
NHBn
NH2HCl
f1 (ppm)

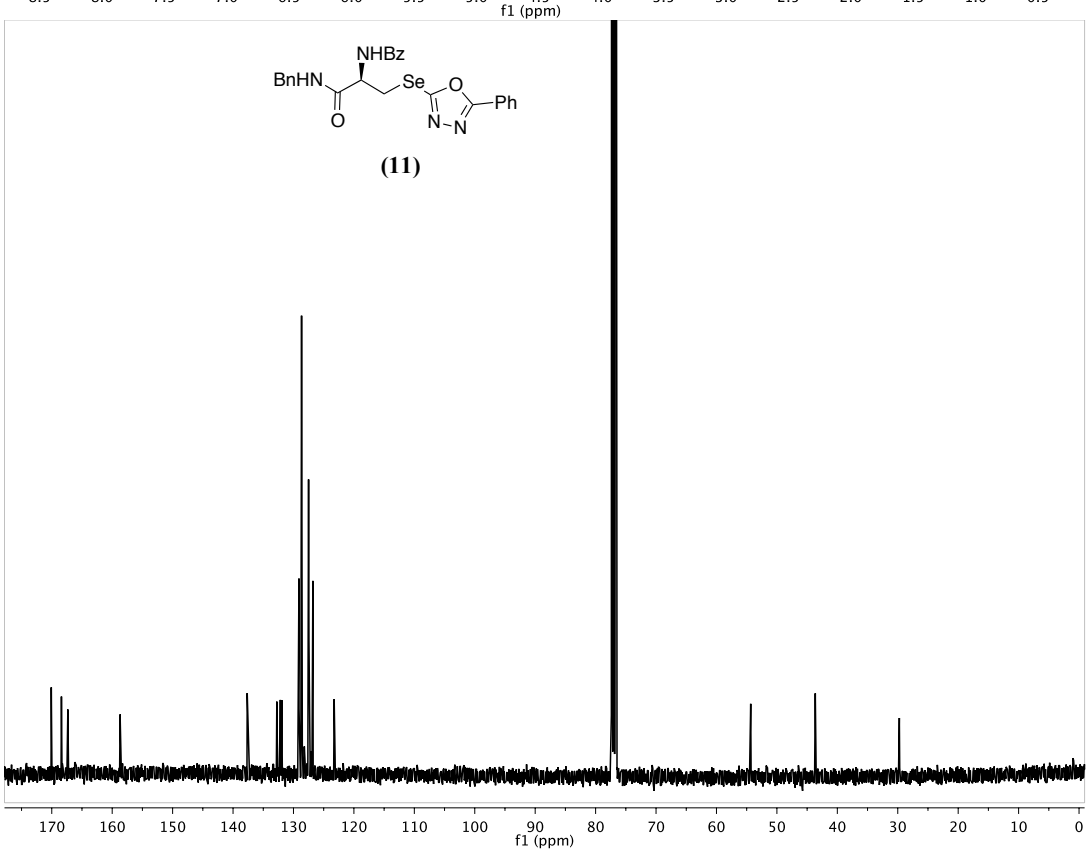
(5)


NHBz
O
BnHN
Se
Se
NHBz
O
NHBn
NHBz
(1)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

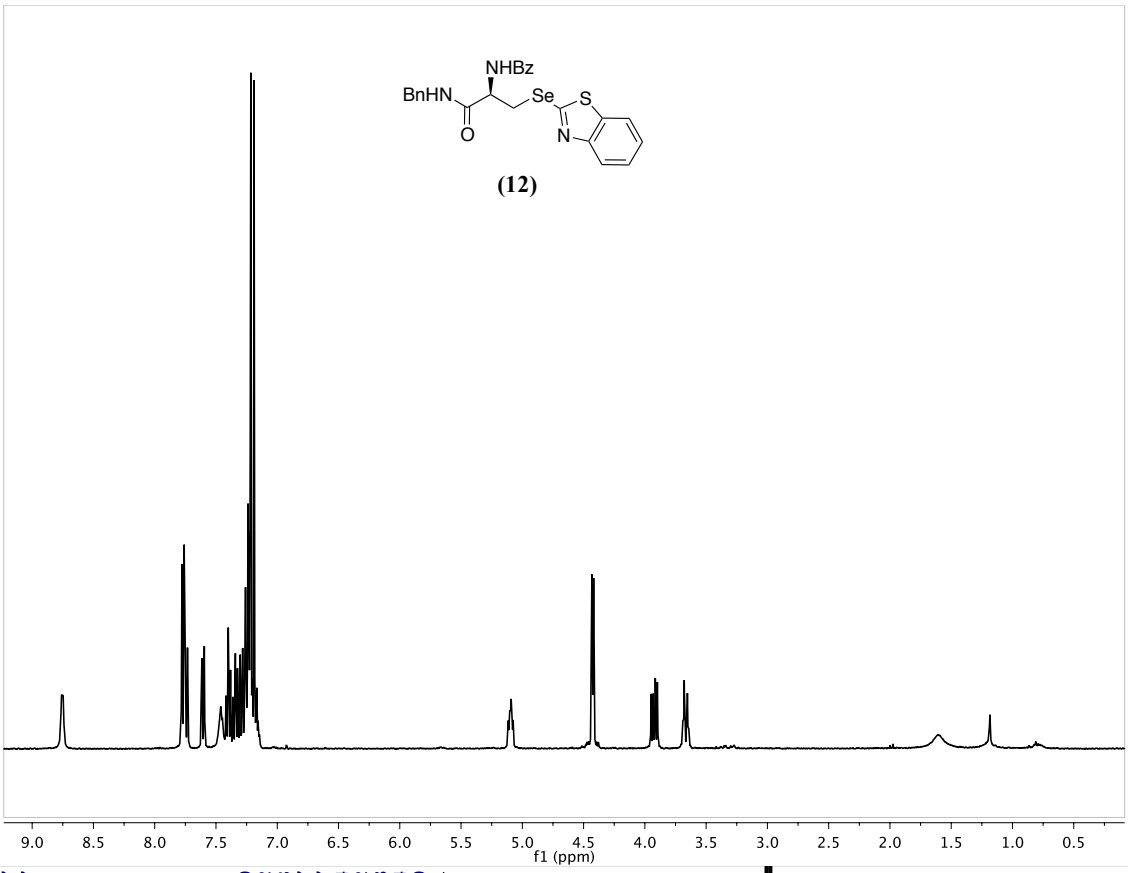

NHBz
O
BnHN
Se
Se
NHBz
O
NHBn
NHBz
(1)
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


NHBz
BnHN
Se
O
Ph
N
N
O
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

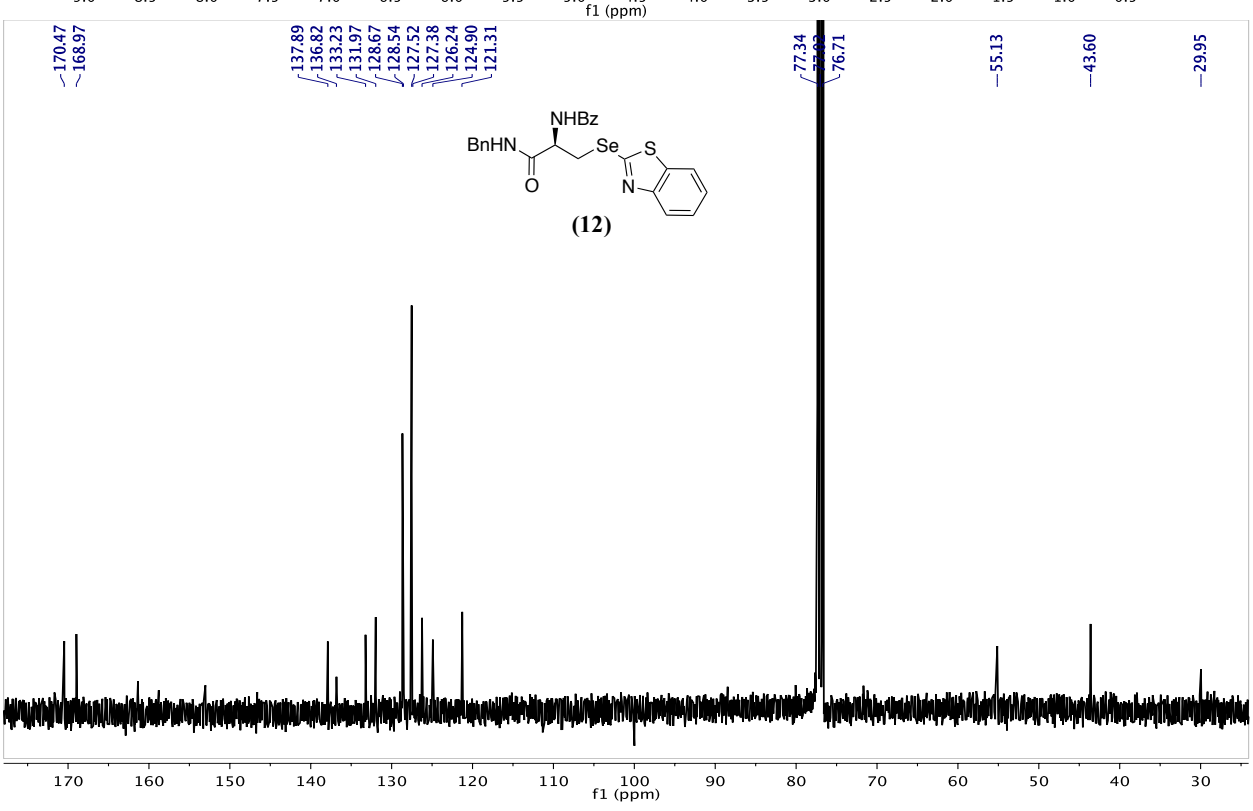
(11)


NHBz
BnHN
Se
O
Ph
N
N
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

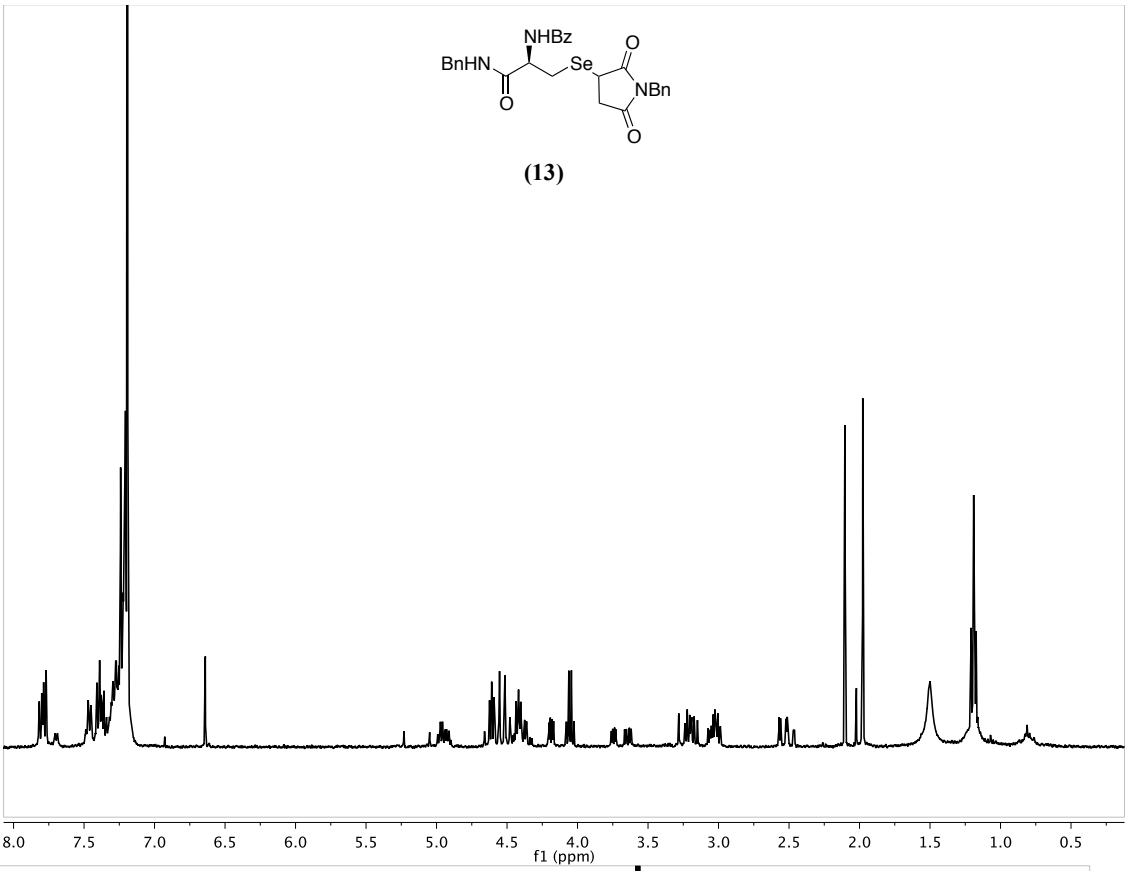
(11)


NHBz
BnHN
Se
S
N
O
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

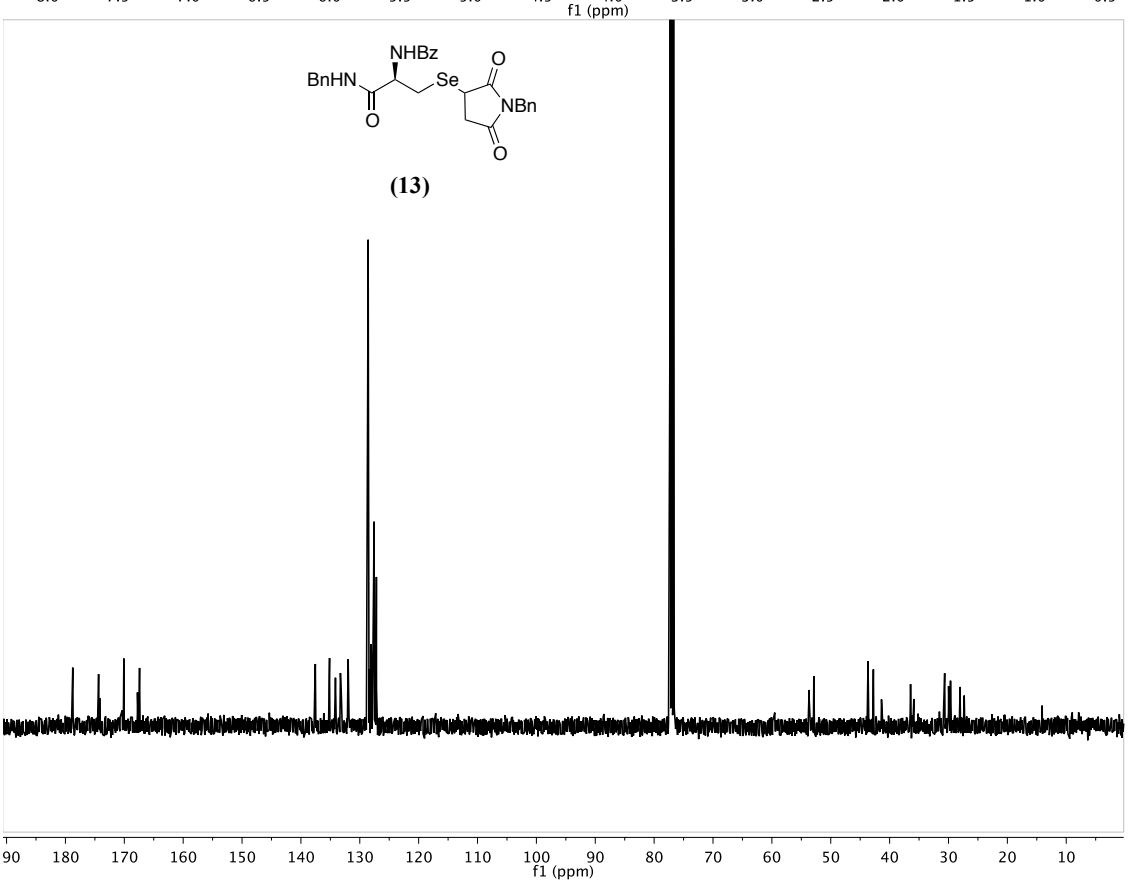
(12)


170.47
168.97
137.89
136.82
133.23
131.97
128.67
128.54
127.52
127.38
126.24
124.90
121.31
77.34
76.71
55.13
43.60
29.95
NHBz
BnHN
Se
S
N
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)

(12)


NHBz
BnHN
Se
O
NBn
O
O
O
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

(13)


NHBz
BnHN
Se
O
NBn
O
O
O
90
180
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160
150
140
130
120
110
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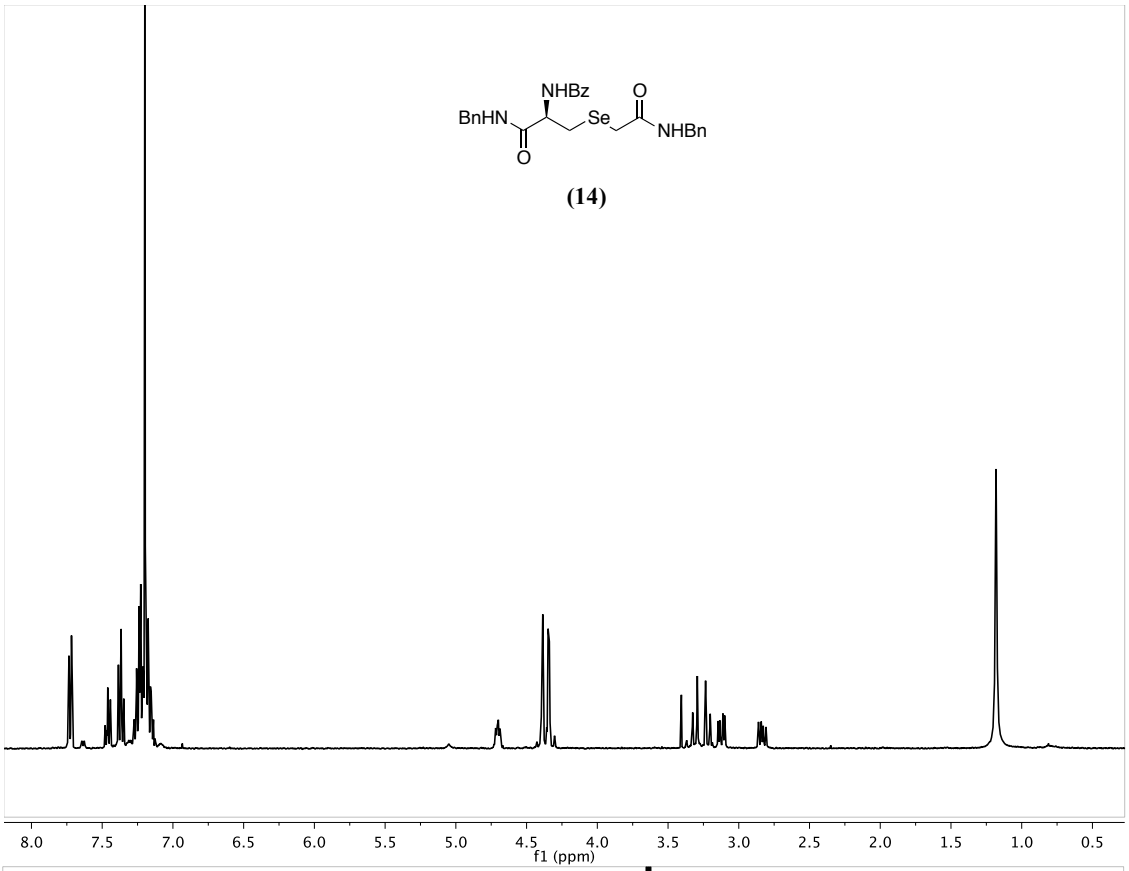
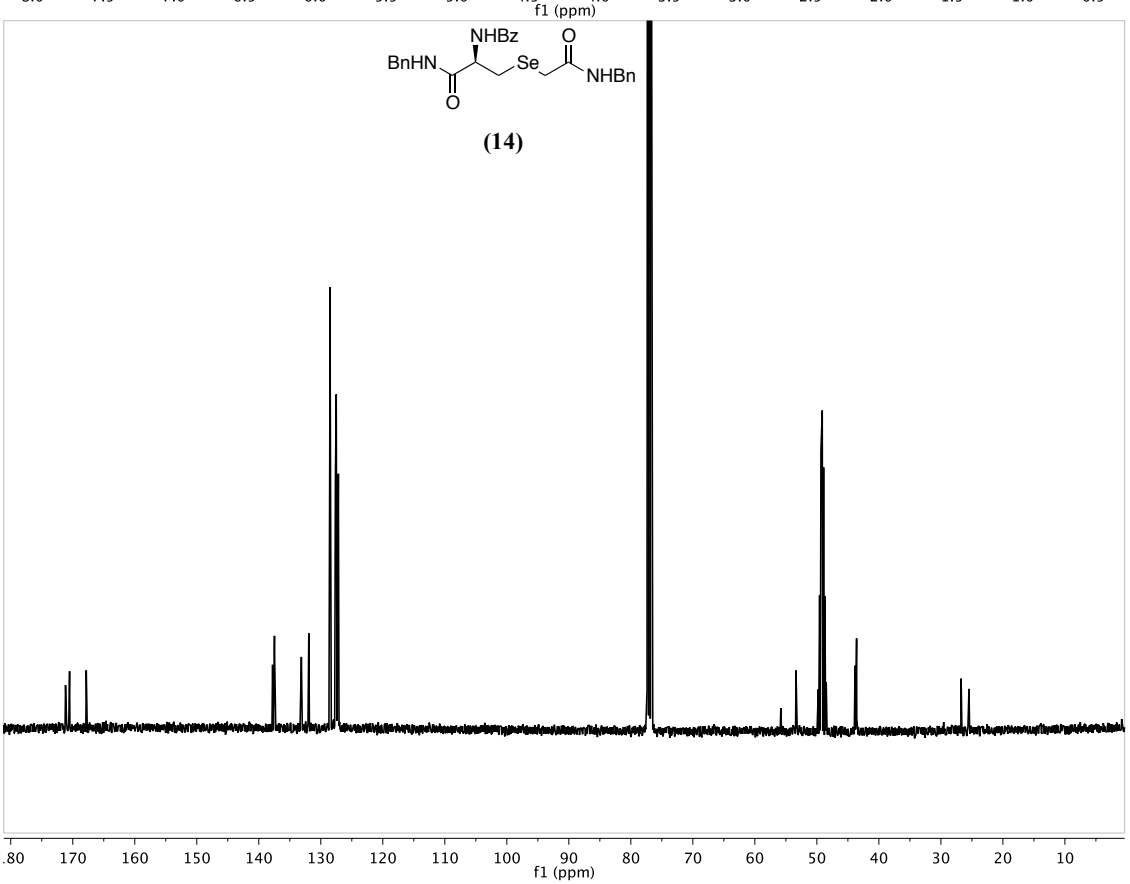
60
50
40
30
20
10
f1 (ppm)

(13)


NHBz
O
BnHN
Se
NHBn
O
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

(14)


NHBz
O
BnHN
Se
NHBn
O
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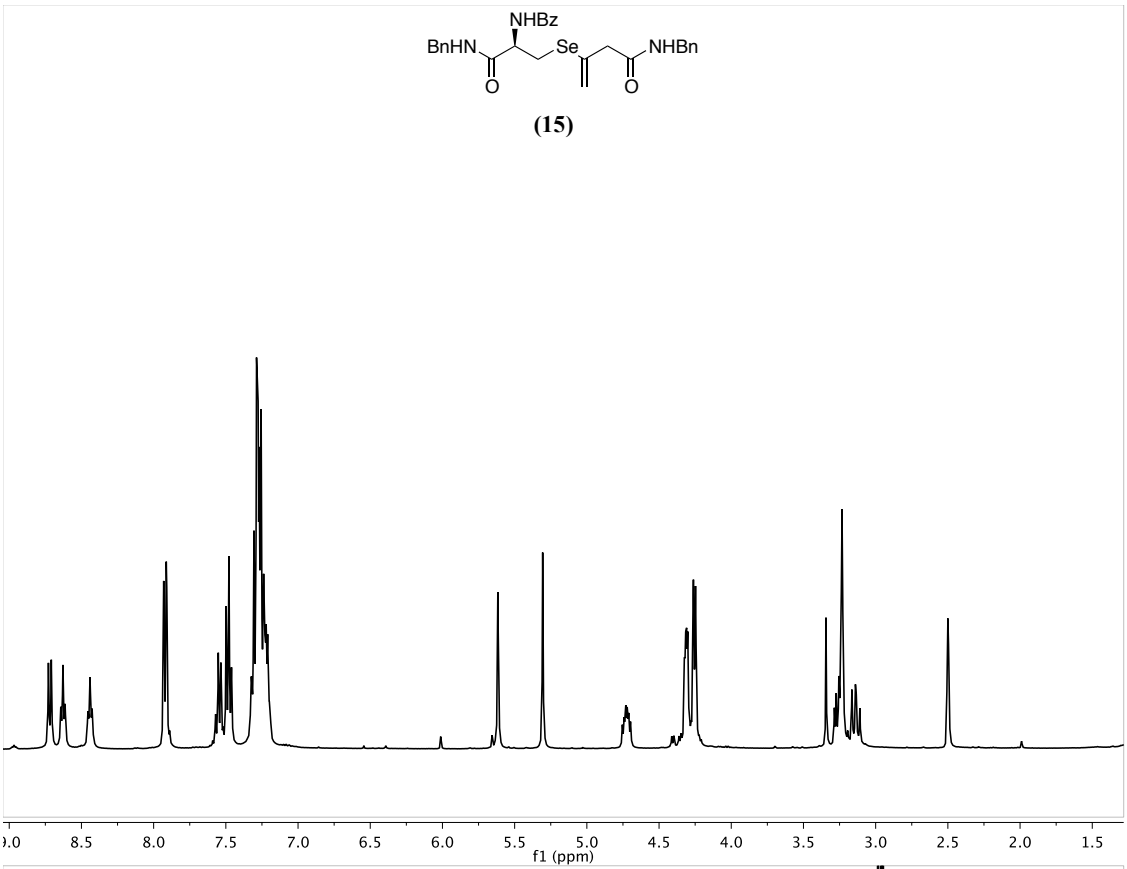
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

(14)



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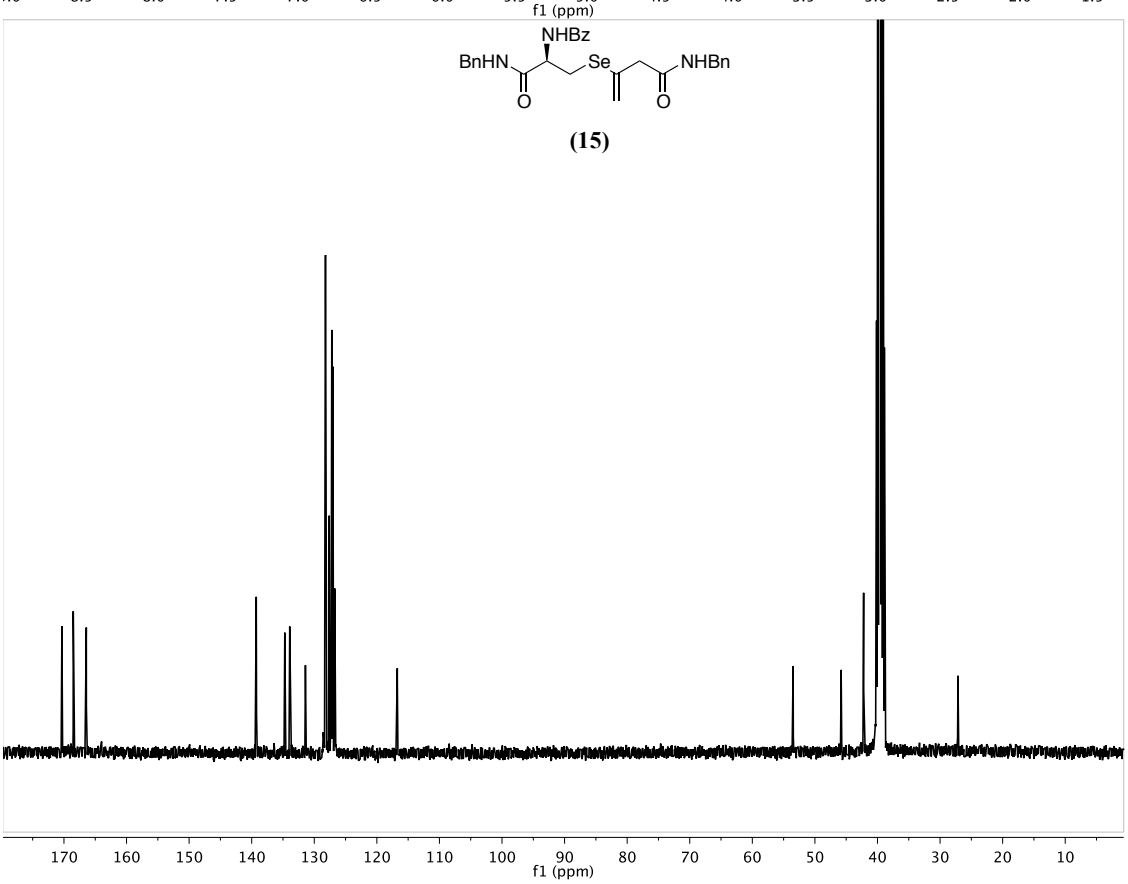

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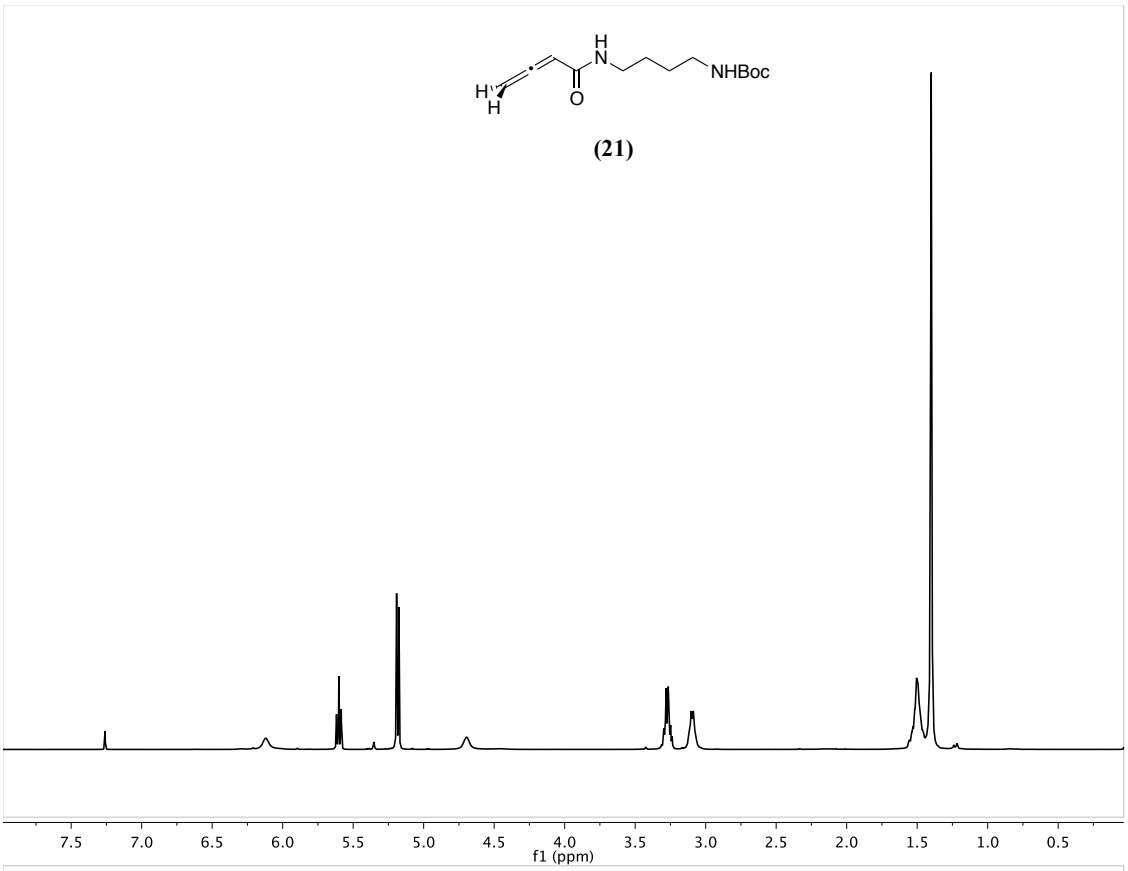
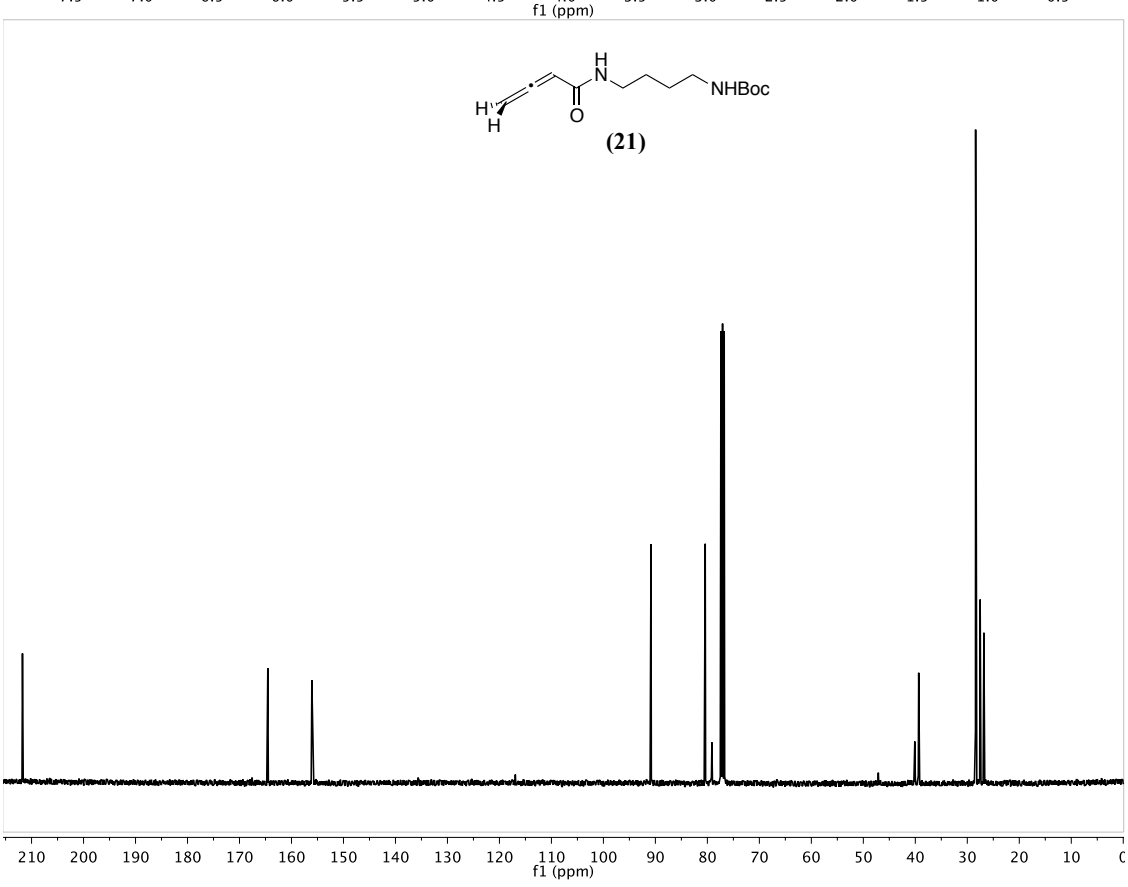

H
N
NHBoc
H
H
O
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

(21)


H
N
NHBoc
H
H
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

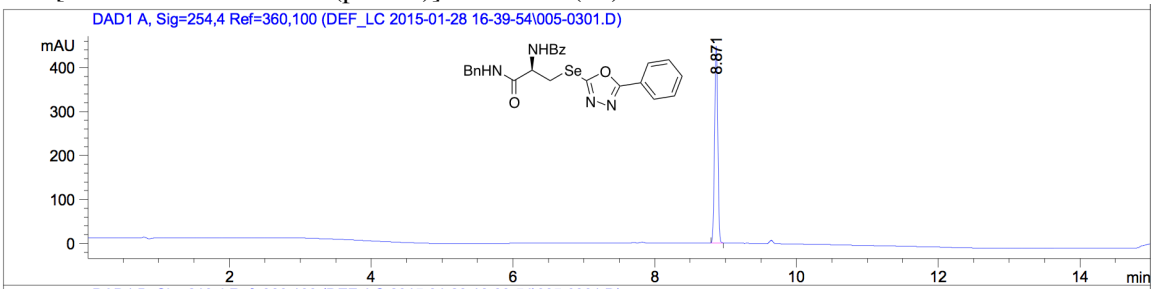
(21)

5. LC-MS Traces for Selenocysteine Adducts Stability Studies

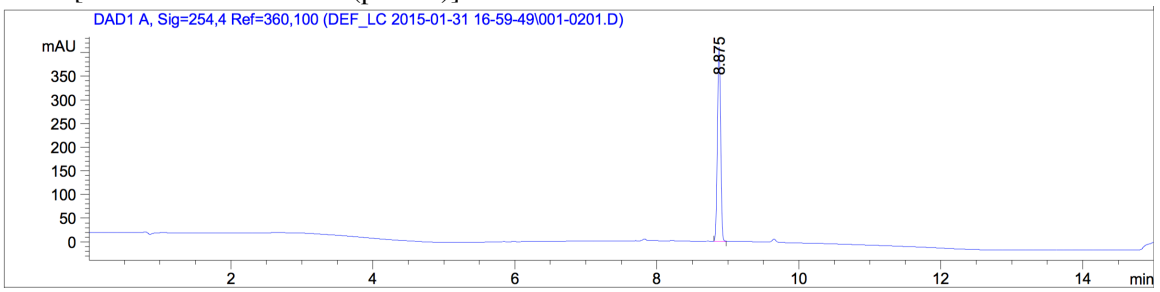
5.1. Stability experiments at pH 5.2 NaOAc buffer

The solution of the substrate (0.0025 mmol) in THF (0.75 mL) and 100 mM pH 5.2 NaOAc buffer (0.25 mL) was stirred for 3 days at room temperature. Remaining amounts of substrate were analyzed by HPLC at 0 and 72 h.

0 h [THF/100 mM NaOAc (pH 5.2)] Sec-ODA (**11**)

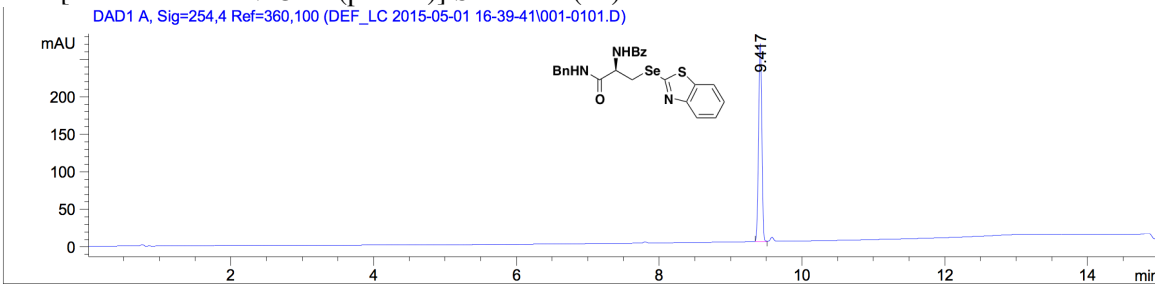


72 h [THF/100 mM NaOAc (pH 5.2)]

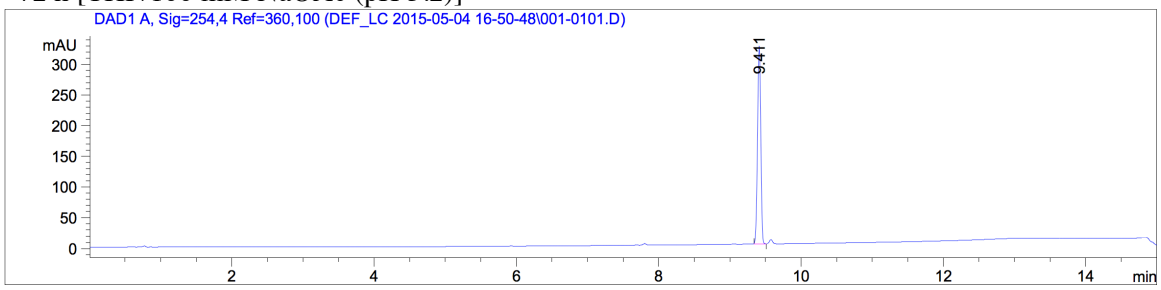


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.875	BB	0.0513	1316.81531	411.38223	100.0000

0 h [THF/100 mM NaOAc (pH 5.2)] Sec-BTA (**12**)



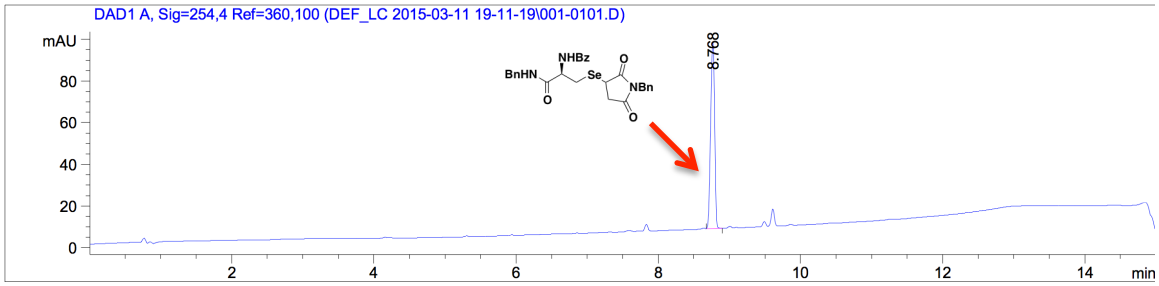
72 h [THF/100 mM NaOAc (pH 5.2)]



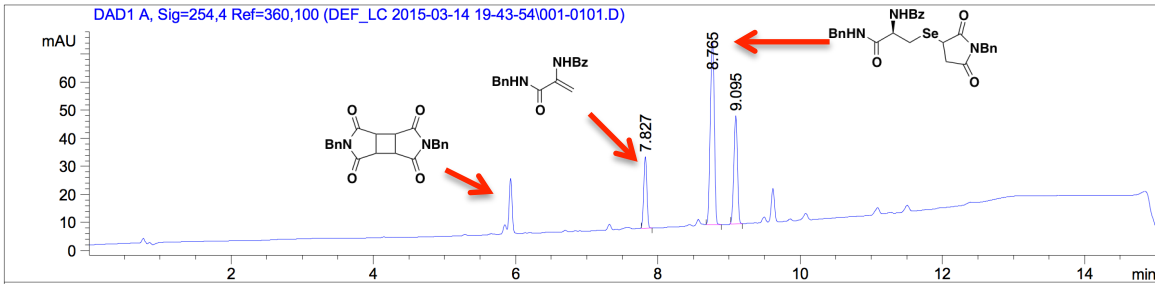
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.411	BB	0.0511	1026.44470	322.95502	100.0000

0 h [THF/100 mM NaOAc (pH 5.2)] Sec-MAL (**13**)



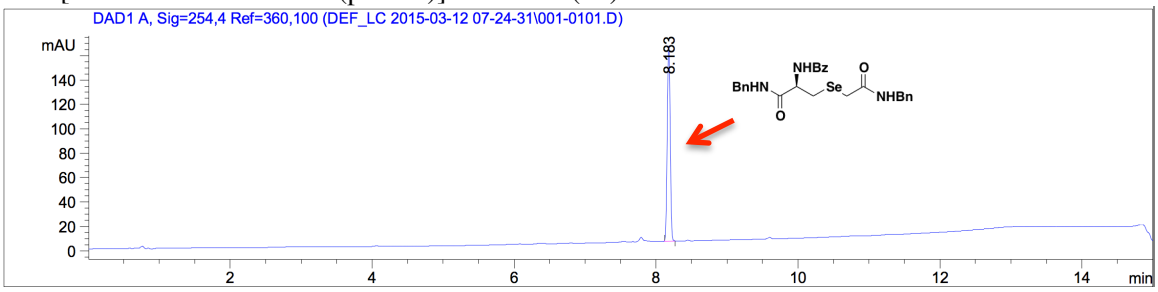
72 h [THF/100 mM NaOAc (pH 5.2)]



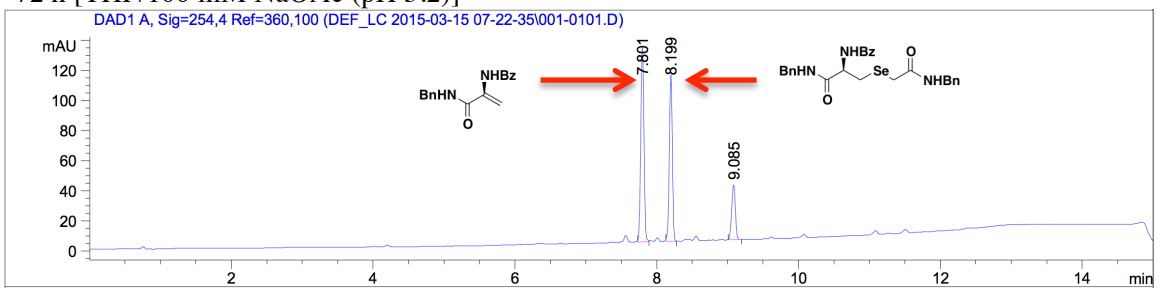
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.827	BB	0.0480	79.02779	25.60394	16.5923
2	8.765	BB	0.0643	259.63531	65.40703	54.5118
3	9.095	BB	0.0555	137.62874	38.66005	28.8959

0 h [THF/100 mM NaOAc (pH 5.2)] Sec-IAM (**14**)



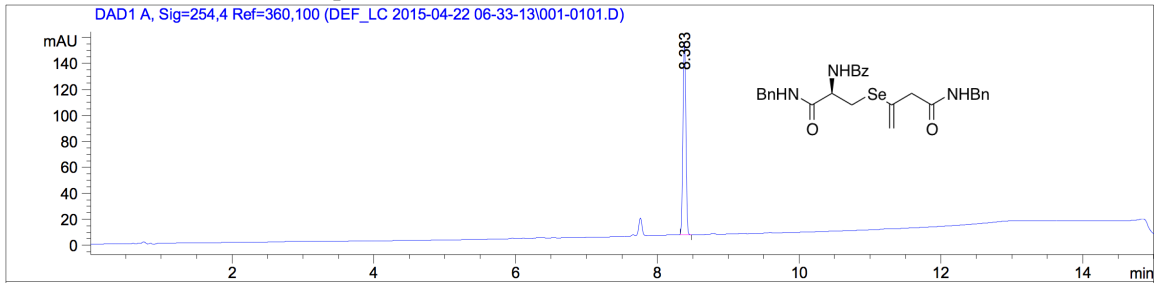
72 h [THF/100 mM NaOAc (pH 5.2)]



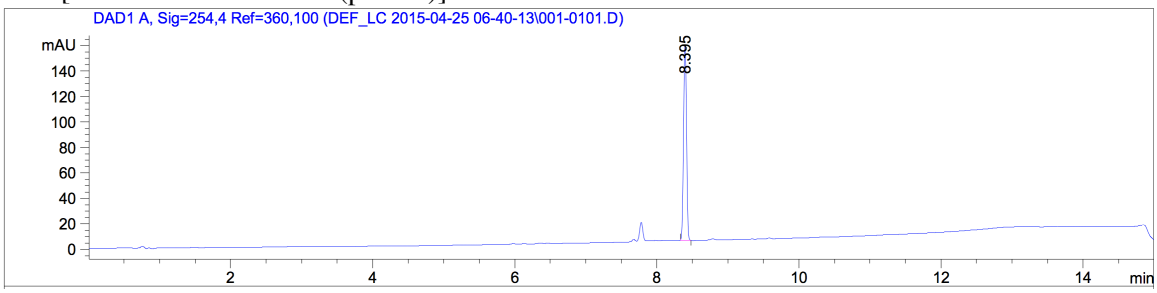
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.801	BB	0.0509	409.35870	129.48523	46.8871
2	8.199	BB	0.0470	331.40213	110.49889	37.9581
3	9.085	BB	0.0581	132.31171	36.67674	15.1547

0 h [THF/100 mM NaOAc (pH 5.2)] Sec-ALL (**15**)



72 h [THF/100 mM NaOAc (pH 5.2)]



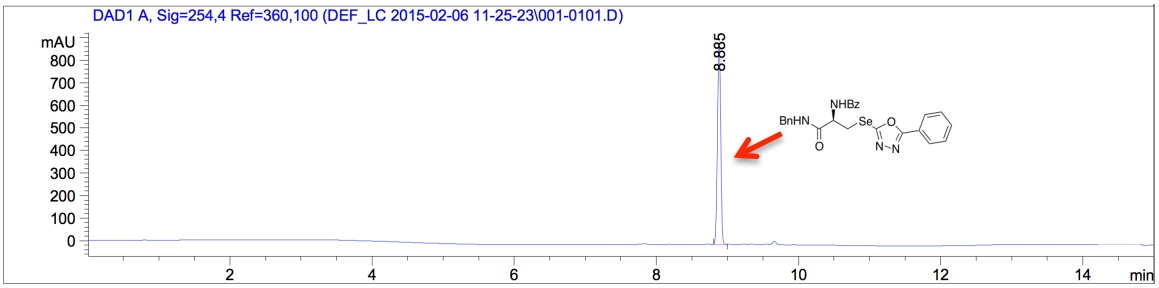
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.395	BB	0.0462	453.97052	154.90054	100.0000
Totals :				453.97052	154.90054	

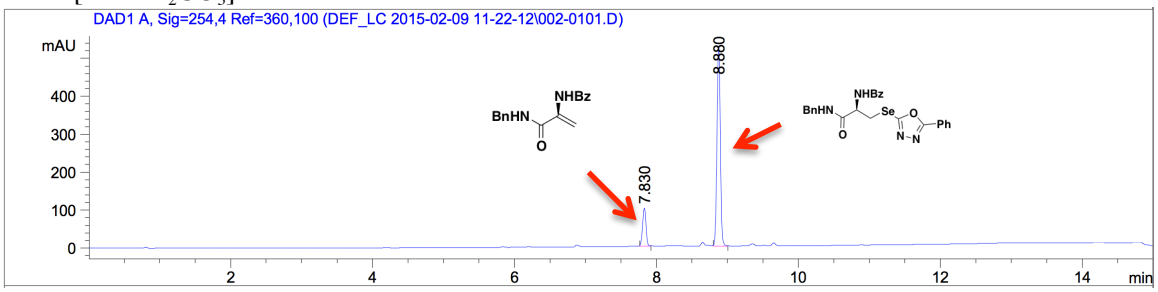
5.2. Stability experiments under basic conditions

The solution of the substrate (0.0025 mmol) in THF (0.75 mL), K_2CO_3 (0.01 mmol) and water (0.25 mL) was stirred for 3 days at room temperature. Remaining amounts of substrate were analyzed by HPLC at 0 and 72 h.

0 h [THF/K_2CO_3] Sec-ODA (**11**)

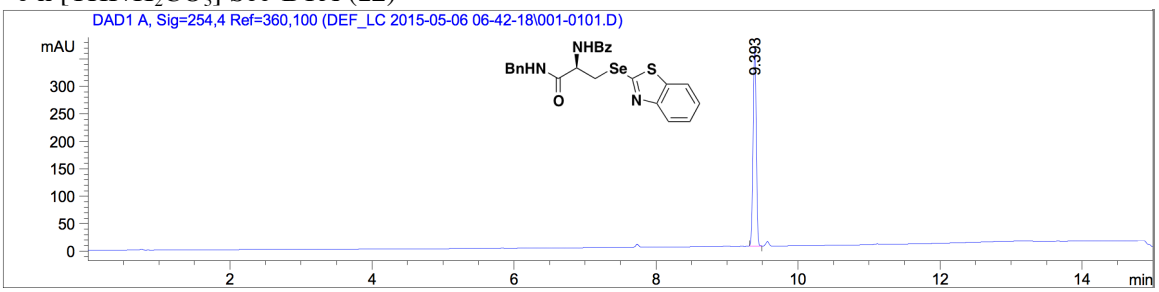


72 h [THF/K_2CO_3]

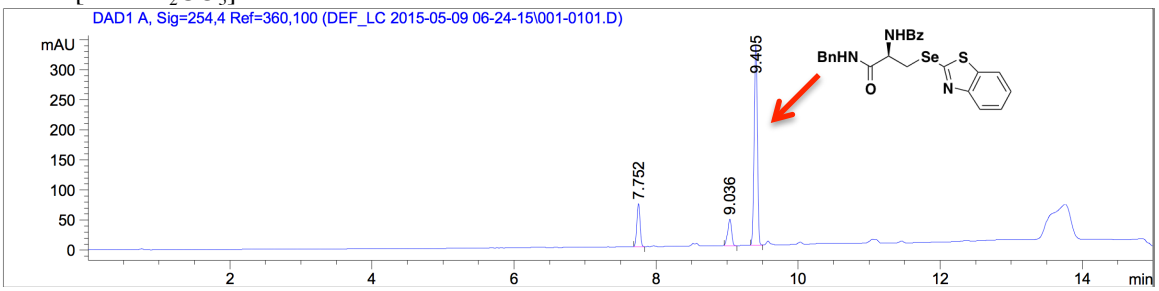


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.830	BB	0.0531	336.20941	100.44408	16.1632
2	8.880	BB	0.0504	1743.88831	530.40320	83.8368

0 h [THF/K_2CO_3] Sec-BTA (**12**)



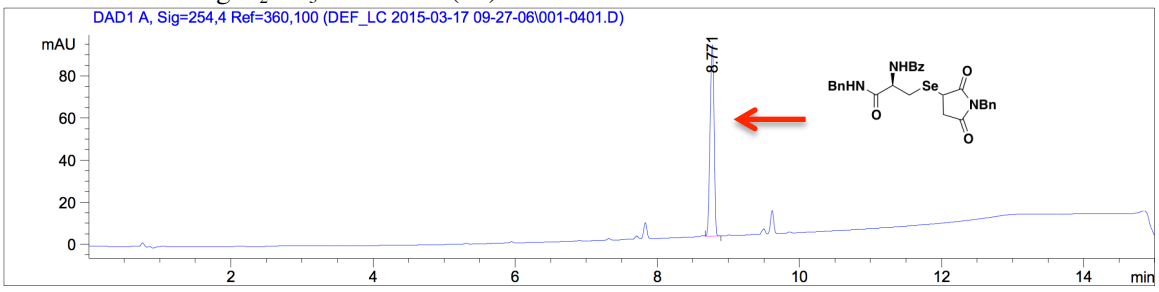
72 h [THF/K_2CO_3]



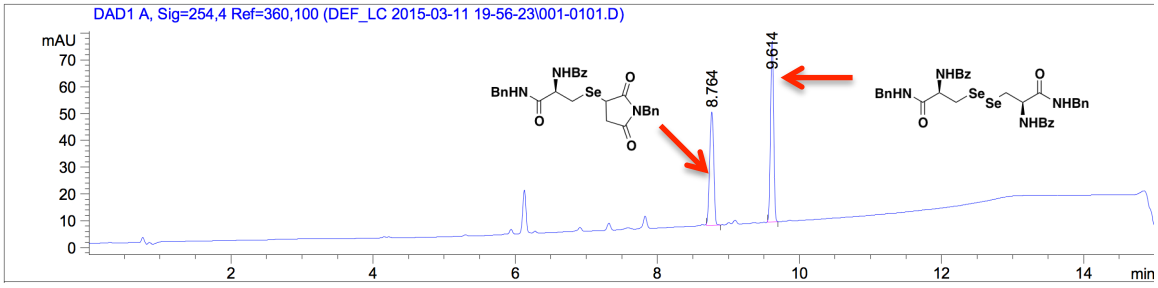
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.752	BB	0.0489	226.18492	71.56116	15.6437
2	9.036	BB	0.0572	163.73650	44.21599	11.3245
3	9.405	BB	0.0510	1055.93469	332.84763	73.0318

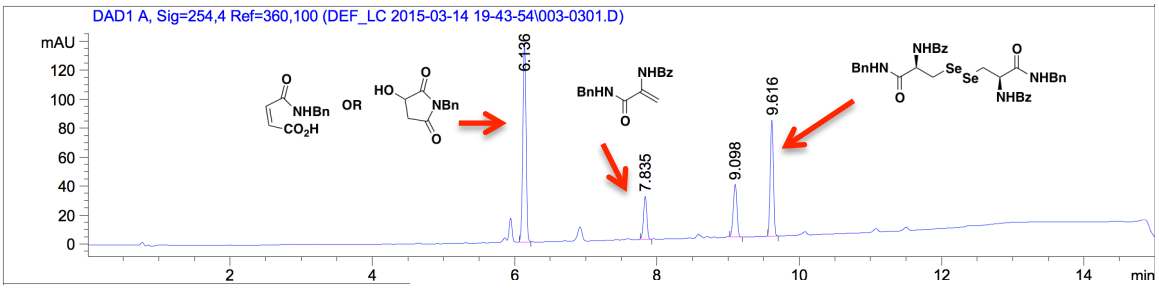
Just before adding K_2CO_3 Sec-MAL (**13**)



0 h [THF/K_2CO_3]



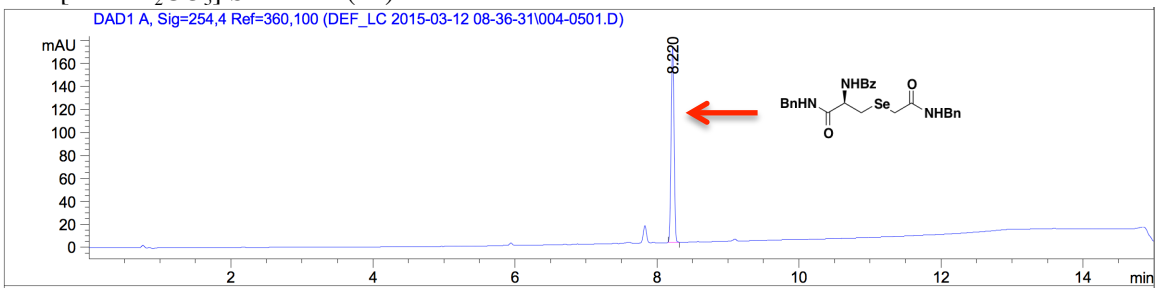
72 h [THF/K_2CO_3]



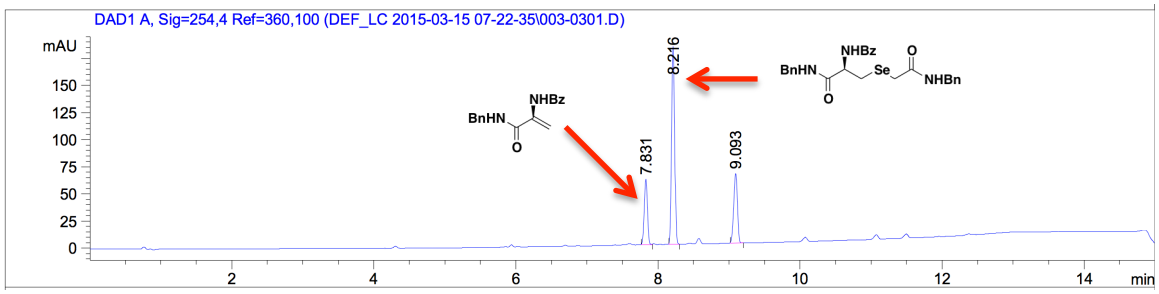
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.136	BB	0.0490	438.51343	138.19540	47.7976
2	7.835	BB	0.0496	96.40651	29.90200	10.5082
3	9.098	BB	0.0565	133.43469	36.60106	14.5443
4	9.616	BB	0.0481	249.08412	80.43068	27.1499

0 h [THF/K_2CO_3] Sec-IAM (**14**)



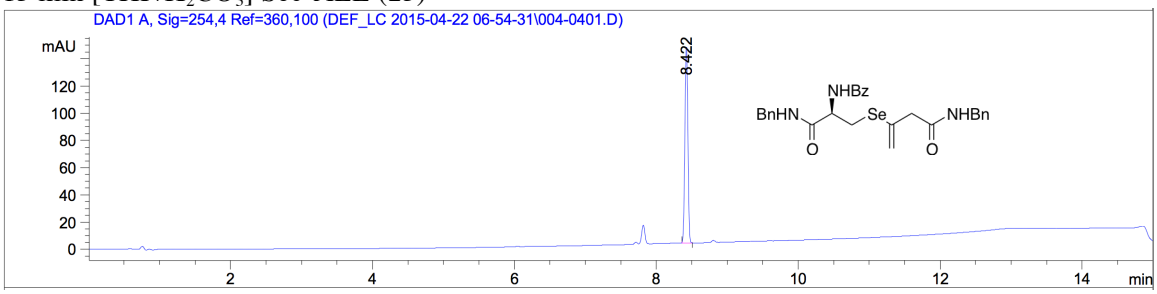
72 h [THF/K_2CO_3]



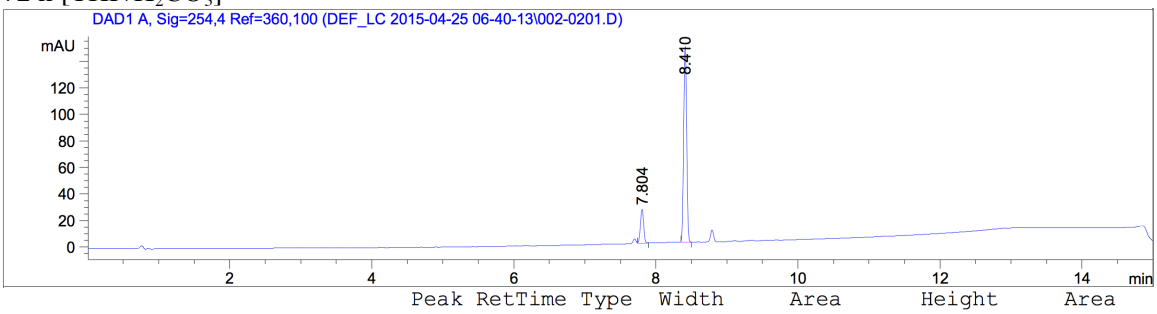
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.831	BB	0.0506	188.73688	60.13903	19.8100
2	8.216	BB	0.0459	529.01312	182.25276	55.5256
3	9.093	BB	0.0584	234.98654	64.58604	24.6644

15 min [THF/K_2CO_3] Sec-ALL (**15**)



72 h [THF/K_2CO_3]

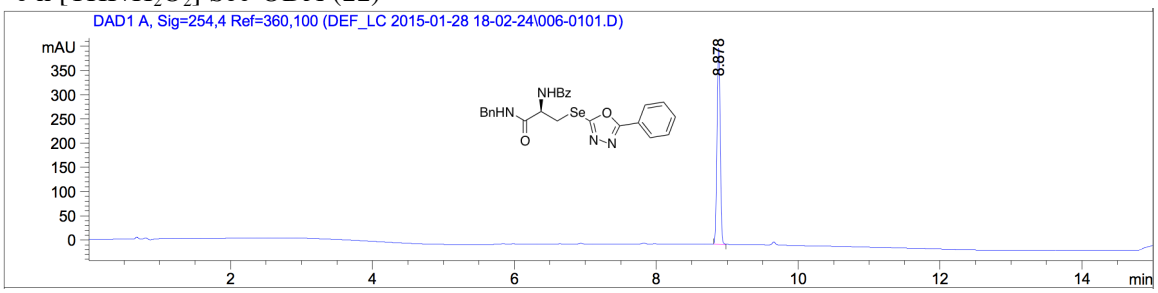


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.804	VB	0.0513	81.92669	25.65652	15.6854
2	8.410	BB	0.0467	440.38470	148.10350	84.3146

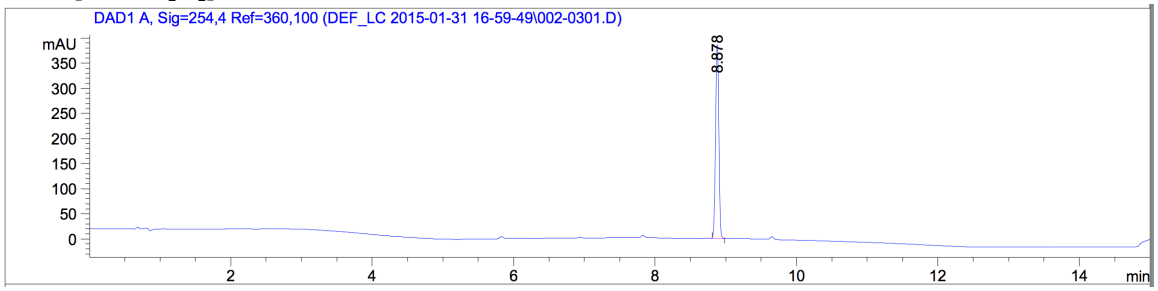
5.3. Stability experiments under oxidizing conditions

The solution of the substrate (0.0025 mmol) in THF (0.75 mL), H_2O_2 (5 mM) and pH 7 buffer (0.25 mL) was stirred for 3 days at room temperature. Remaining amounts of substrate were analyzed by HPLC at 0 and 72 h

0 h [THF/H_2O_2] Sec-ODA (**11**)

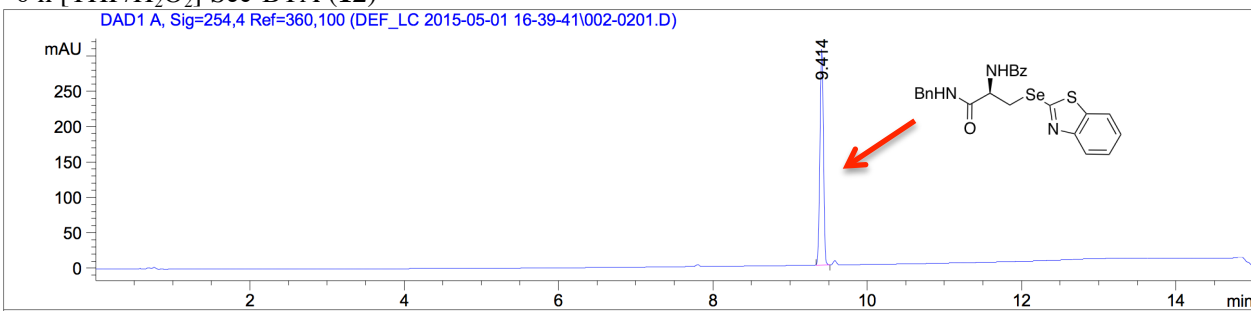


72 h [THF/H_2O_2]

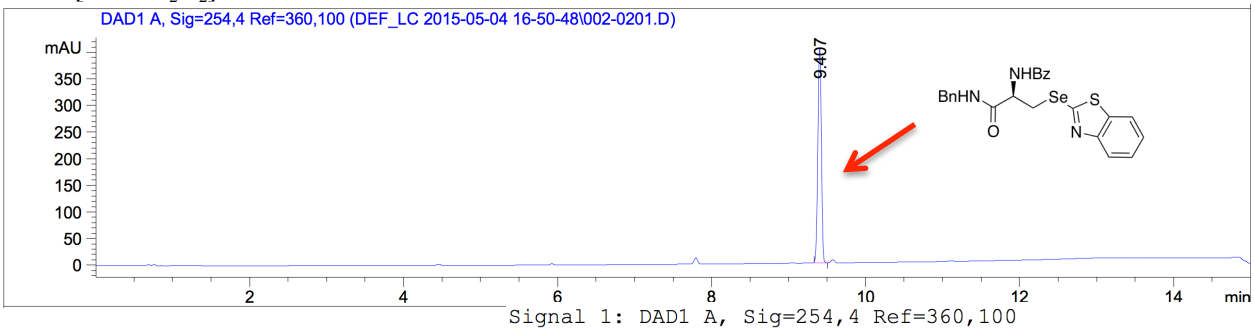


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.878	BB	0.0495	1249.99243	389.19852	100.0000

0 h [THF/H_2O_2] Sec-BTA (**12**)



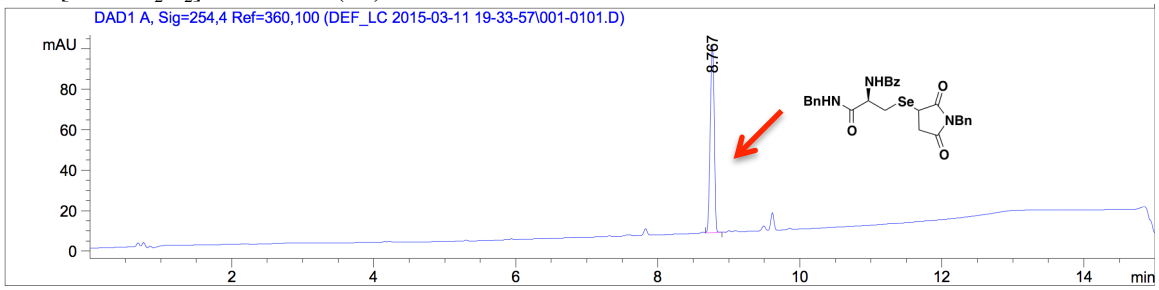
72 h [THF/H_2O_2]



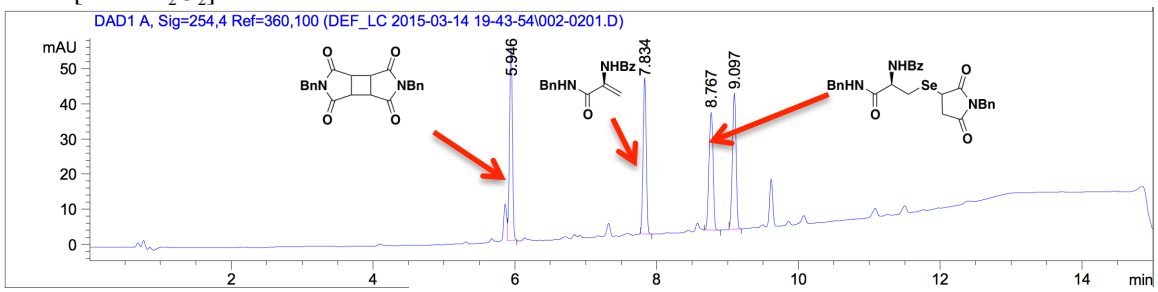
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.407	BB	0.0492	1296.25757	407.00394	100.0000

0 h [THF/H_2O_2] Sec-MAL (**13**)



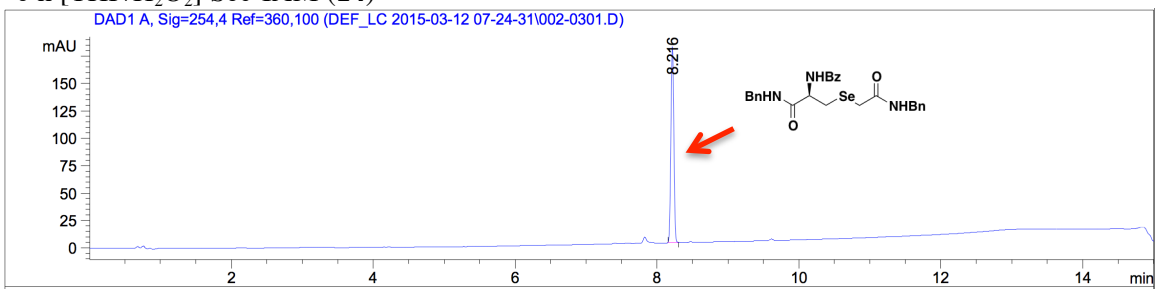
72 h [THF/H_2O_2]



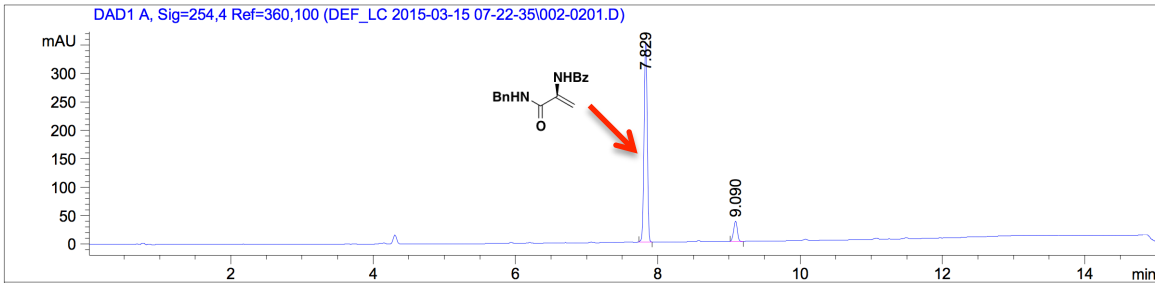
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.946	VB	0.0458	160.32913	55.33151	27.9014
2	7.834	BB	0.0506	139.63368	44.50903	24.2999
3	8.767	BB	0.0668	134.37415	33.50397	23.3846
4	9.097	BB	0.0563	140.29071	38.68728	24.4142

0 h [THF/H_2O_2] Sec-IAM (**14**)



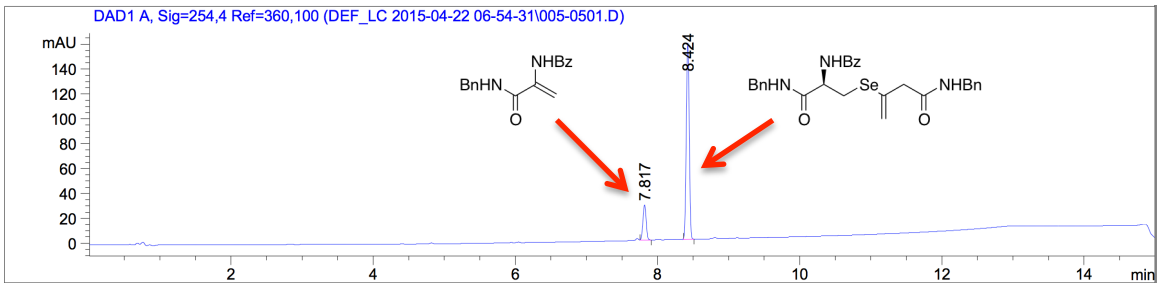
72 h [THF/H_2O_2]



Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.829	BB	0.0489	1119.75000	354.50128	89.6153
2	9.090	BB	0.0583	129.75697	35.75947	10.3847

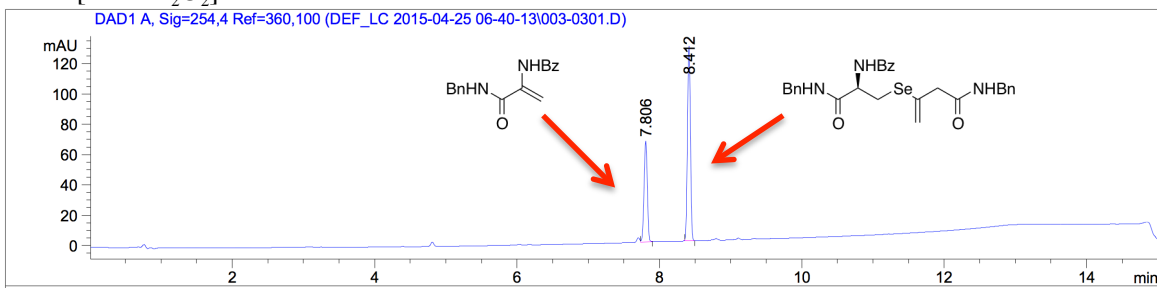
15 min [THF/H_2O_2] Sec-ALL (**15**)



Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.763	MM	0.0523	43.41139	13.83810	9.0446
2	8.383	BB	0.0482	436.55859	148.74916	90.9554

72 h [THF/H_2O_2]

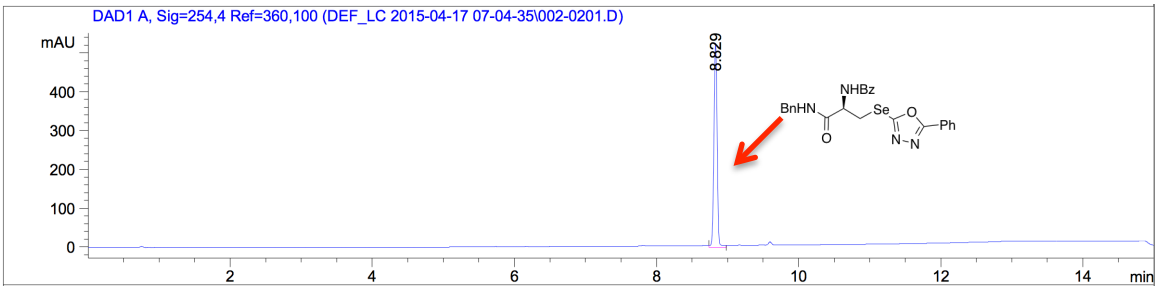


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.806	VB	0.0513	212.91008	66.63770	36.0686
2	8.412	BB	0.0462	377.38208	128.73642	63.9314

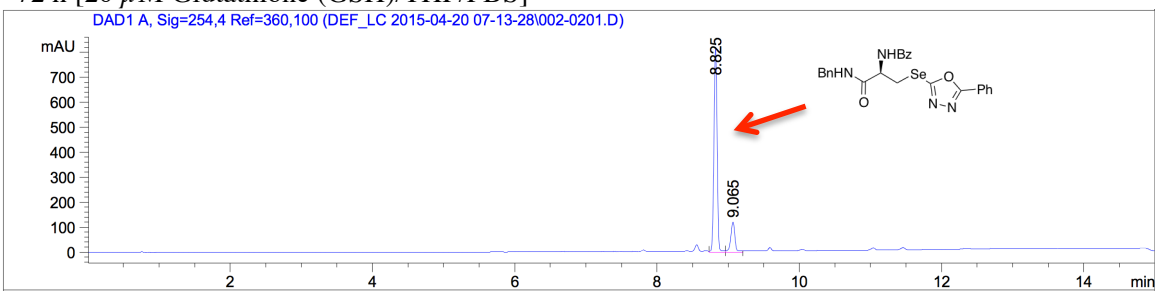
5.4. Stability study in the presence of glutathione

The solution of the substrate (2.5 μmol) in THF (0.75 mL) was added glutathione (GSH, 20 nmol, 20 μM final concentration) in PBS buffer pH 7.4 (0.25 mL) and the mixture was stirred at 37 °C for 3 days. Remaining amounts of substrate were analyzed by HPLC at 0 and 72 h.

0 h [20 μM Glutathione (GSH)/THF/PBS] Sec-ODA (**11**)



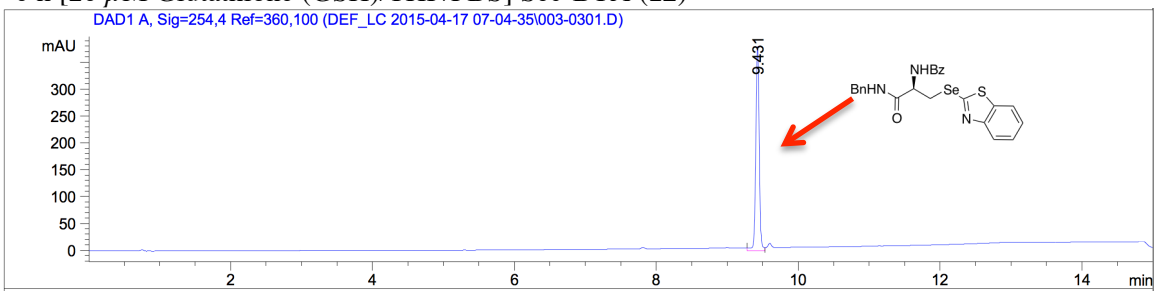
72 h [20 μM Glutathione (GSH)/THF/PBS]



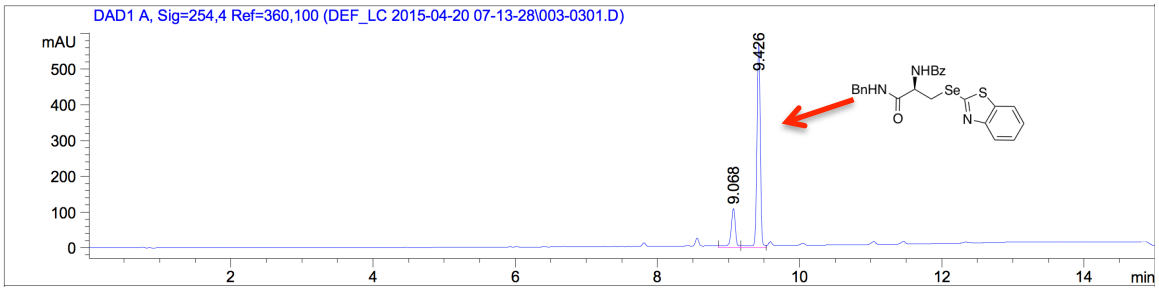
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.825	VV	0.0506	2580.00488	822.29919	83.3087
2	9.065	VV	0.0633	516.91522	122.26472	16.6913

0 h [20 μM Glutathione (GSH)/THF/PBS] Sec-BTA (**12**)



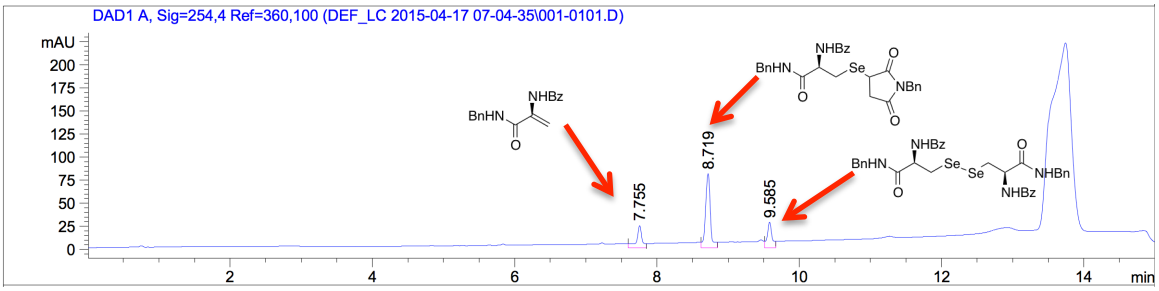
72 h [20 μM Glutathione (GSH)/THF/PBS]



Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.068	VV	0.0680	490.57880	110.04535	20.2209
2	9.426	VV	0.0533	1935.52014	574.12799	79.7791

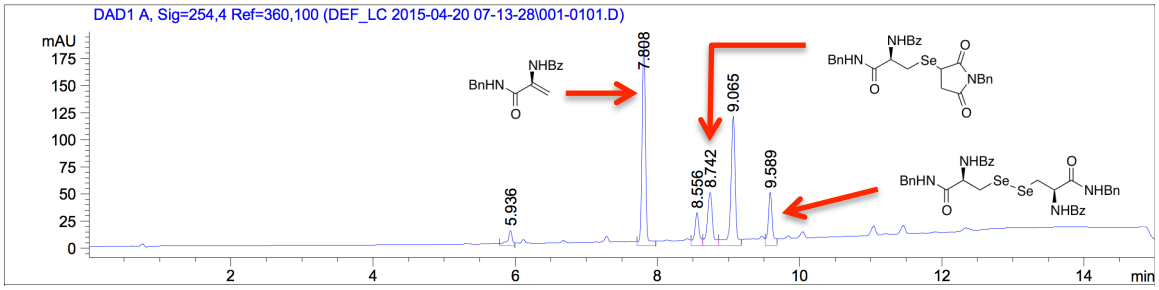
0 h [20 μM Glutathione (GSH)/THF/PBS] Sec-MAL (**13**)



Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.755	VV	0.0756	131.47903	24.11124	20.3689
2	8.719	VV	0.0755	383.08081	80.59831	59.3474
3	9.585	VV	0.0687	130.92946	27.90836	20.2838

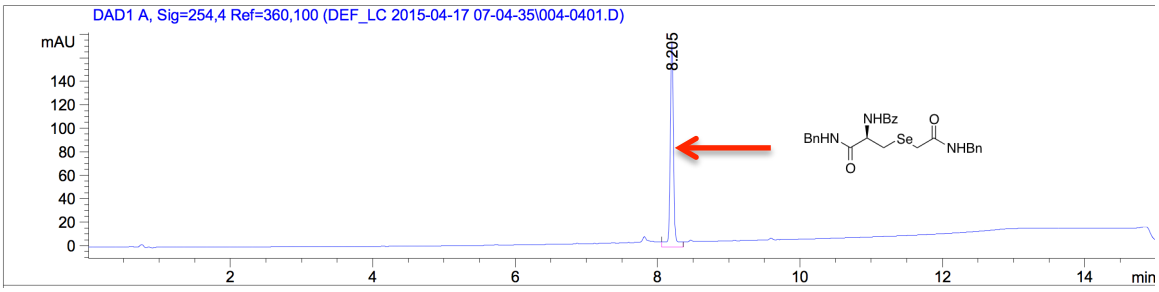
72 h [20 μM Glutathione (GSH)/THF/PBS]



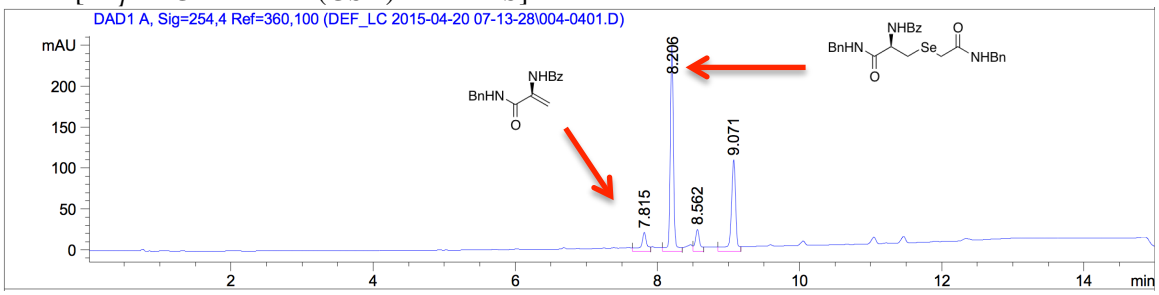
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.936	VV	0.0684	68.47449	14.16934	3.7185
2	7.808	VV	0.0523	655.33435	189.61926	35.5874
3	8.556	VV	0.0661	136.61131	30.58173	7.4186
4	8.742	VV	0.0806	256.21854	49.35325	13.9137
5	9.065	VV	0.0655	527.39581	119.42727	28.6398
6	9.589	VV	0.0588	197.44440	49.27272	10.7221

0 h [20 μM Glutathione (GSH)/THF/PBS] Sec-IAM (**14**)



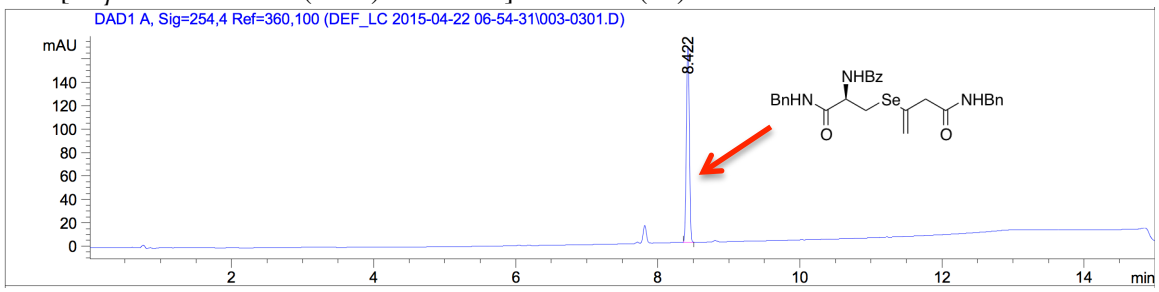
72 h [20 μM Glutathione (GSH)/THF/PBS]



Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.815	VV	0.0765	126.16600	22.81664	8.2566
2	8.206	VV	0.0509	791.66071	250.46336	51.8082
3	8.562	VV	0.0643	114.67382	26.59954	7.5045
4	9.071	VV	0.0678	495.55920	111.65112	32.4306

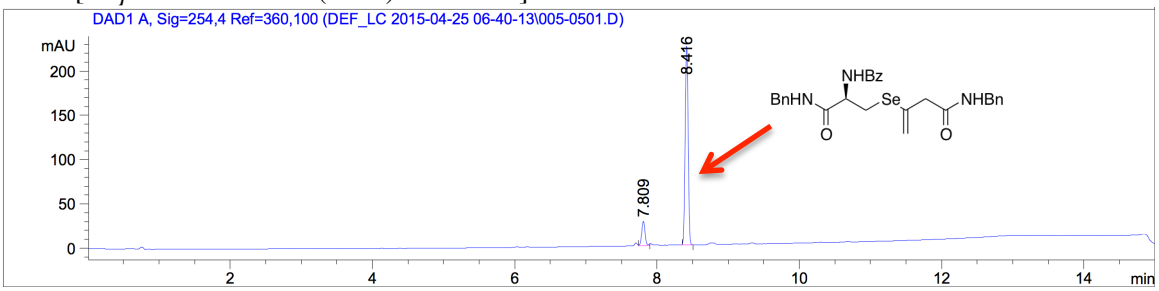
0 h [20 μM Glutathione (GSH)/THF/PBS] Sec-ALL (**15**)



Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.817	MM	0.0542	51.20485	15.74344	9.3682
2	8.422	BB	0.0461	495.37906	169.32568	90.6318

72 h [20 μM Glutathione (GSH)/THF/PBS]

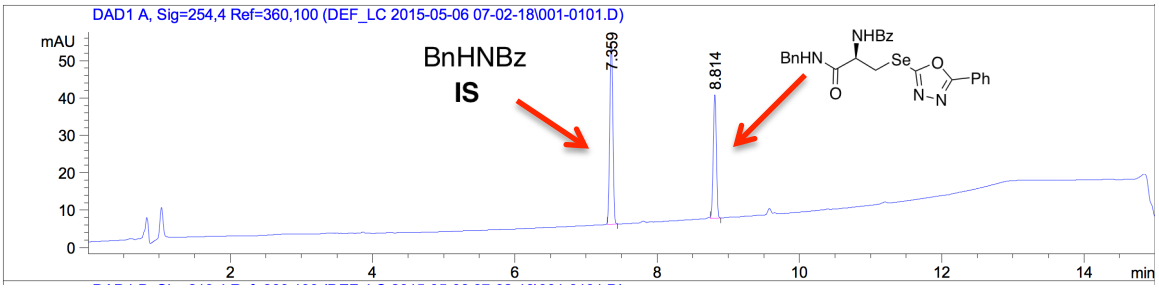


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.809	VB	0.0502	89.59914	27.37656	11.8890
2	8.416	BB	0.0462	664.03326	226.37442	88.1110

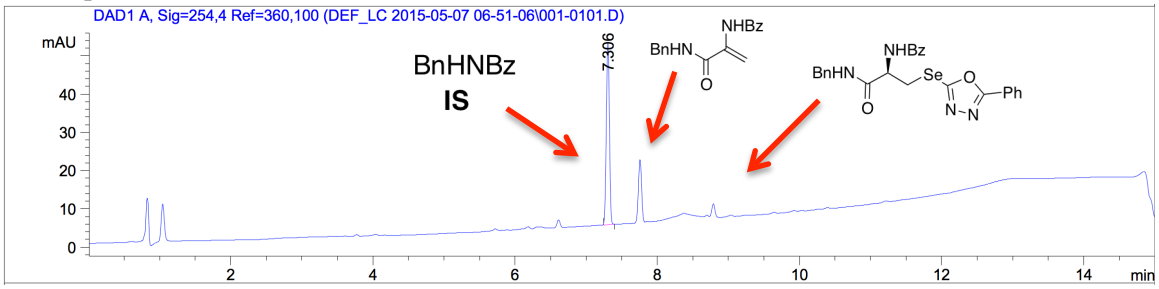
5.5. Stability study in human blood plasma

A solution of substrate (50 μL, 4 mM in DMSO) was added to human plasma (950 μL) at 0 °C. The mixture was incubated for 72 h at 37 °C. Samples for HPLC analysis (100 μL) were collected from the mixture at each time point. To the collected samples were added MeCN (600 μL) containing N-benzylbenzamide (internal standard, **IS**) and centrifuged for 5 minutes at 5000 rpm. The supernatant (600 μL) was analyzed by HPLC (254 nm).

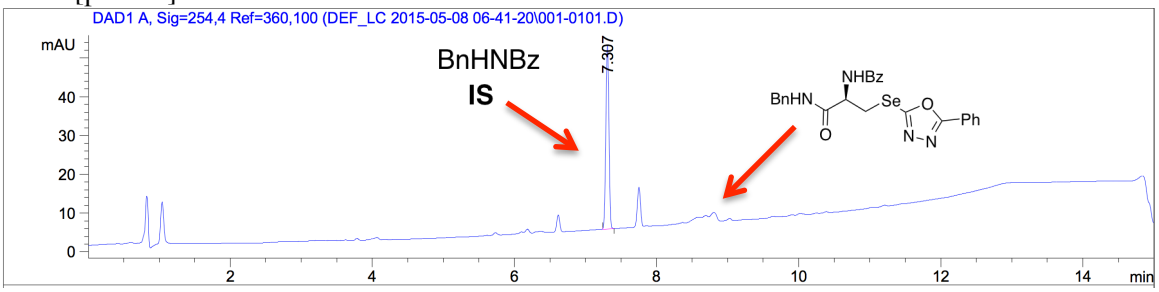
0 h [plasma] Phenyloxadiazole-Selenocysteine Adduct (Sec-ODA **11**)



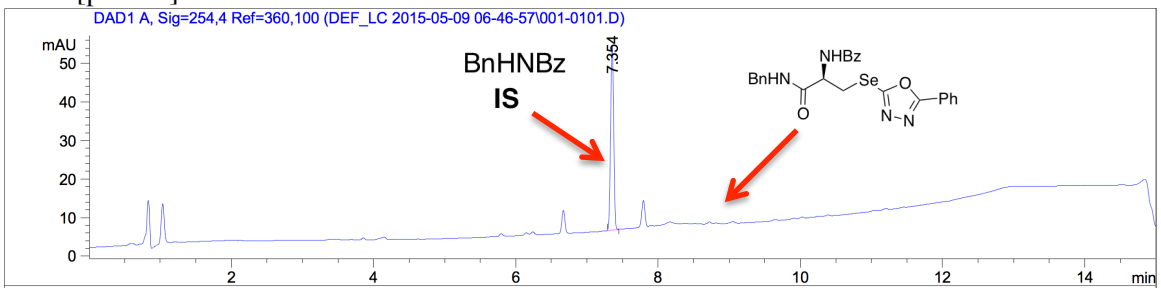
24 h [plasma]



48 h [plasma]

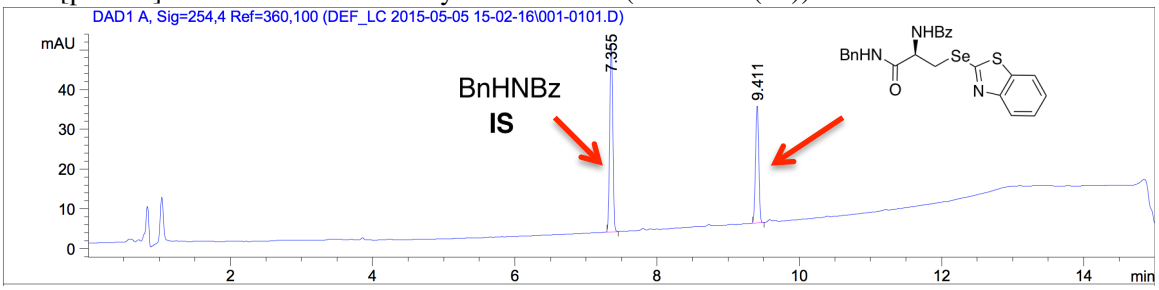


72 h [plasma]

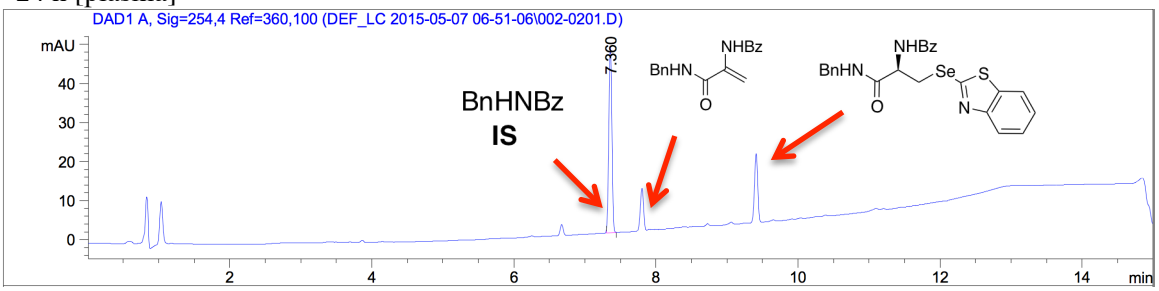


Time/h	IS area	Sec-ODA (**11**) area	% Remaining
0	150.21799	101.38508	100
24	145.25957	10.37195	10.6
48	144.67004	8.44145	8.65
72	146.65027	0	0.00

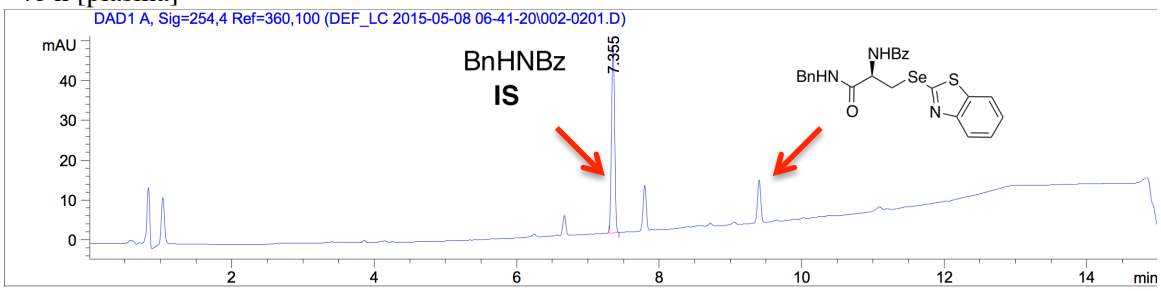
0 h [plasma] Benzothiazole-Selenocysteine Adduct (Sec-BTA (**12**))



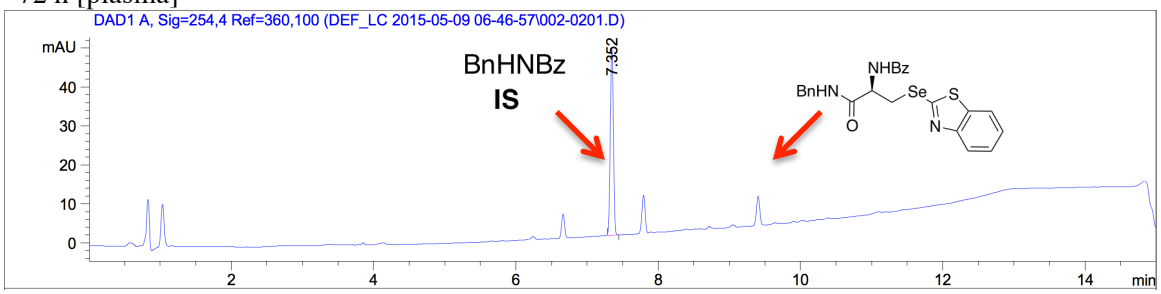
24 h [plasma]



48 h [plasma]

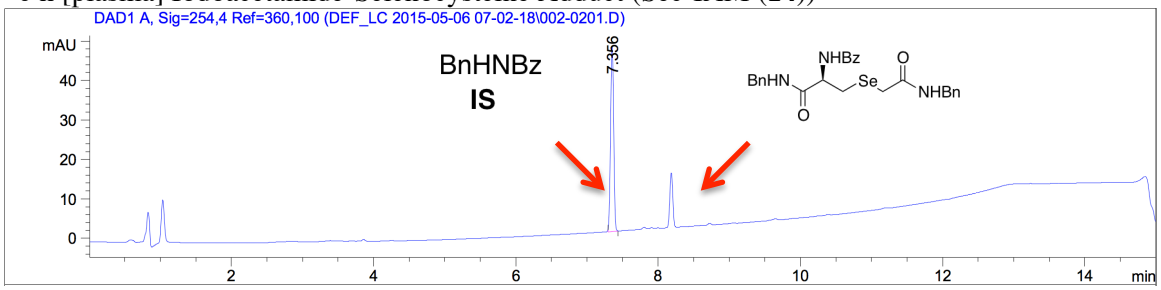


72 h [plasma]

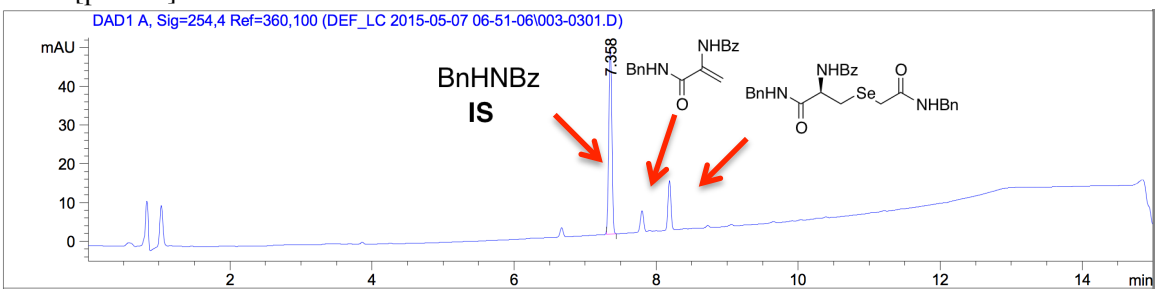


Time/h	IS area	Sec-BTA (**12**) area	% Remaining
0	144.55676	93.96006	100
24	146.74489	56.03693	58.7
48	145.50803	34.67171	36.7
72	147.53522	24.39896	25.4

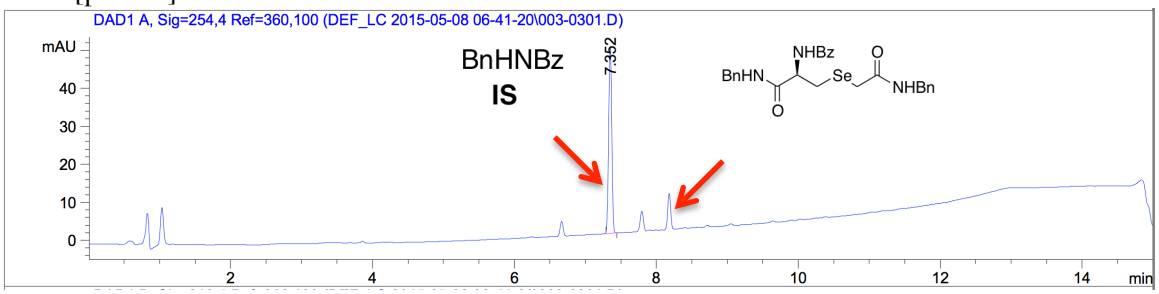
0 h [plasma] Iodoacetamide-Selenocysteine Adduct (Sec-IAM (**14**))



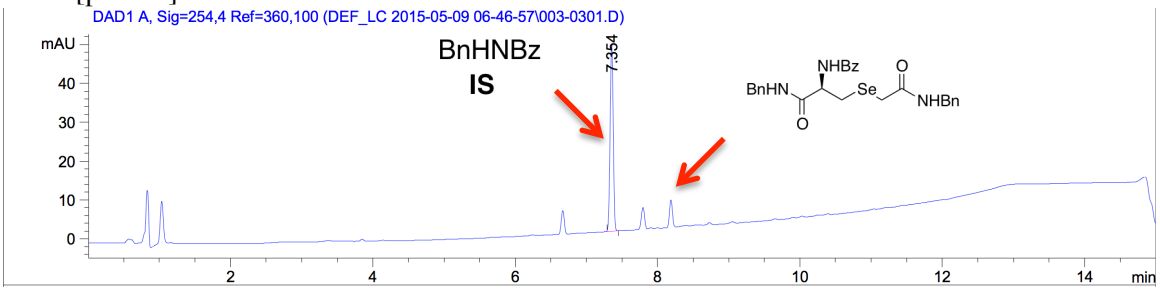
24 h [plasma]



48 h [plasma]

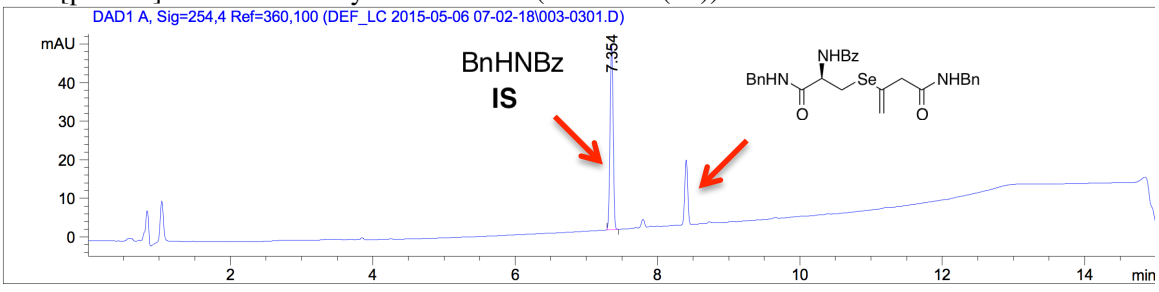


72 h [plasma]

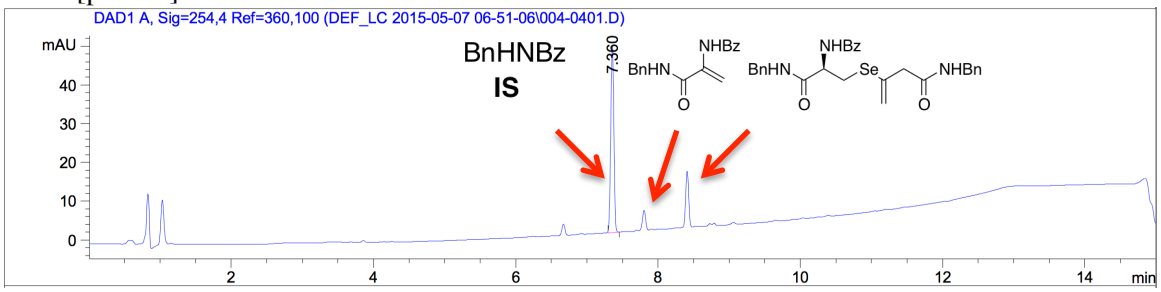


Time/h	IS area	Sec-IAM (**14**) area	% Remaining
0	145.50101	40.32212	100
24	148.23933	37.86126	92.2
48	150.33929	28.08746	67.4
72	147.77037	20.66275	50.5

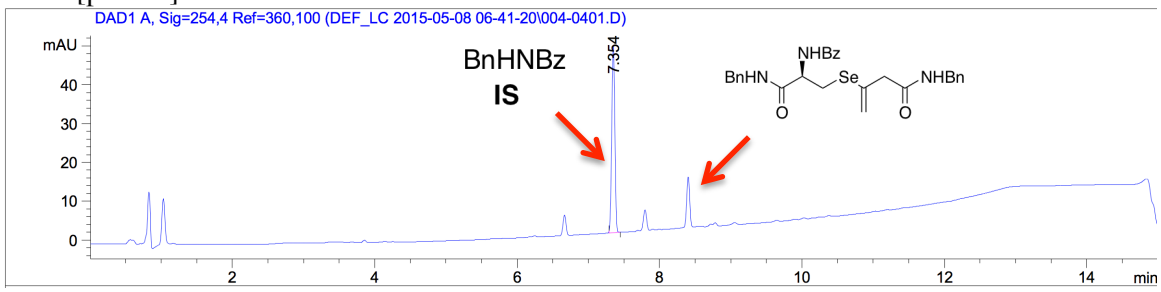
0 h [plasma] Allene-Selenocysteine Adduct (Sec-ALL (**15**))



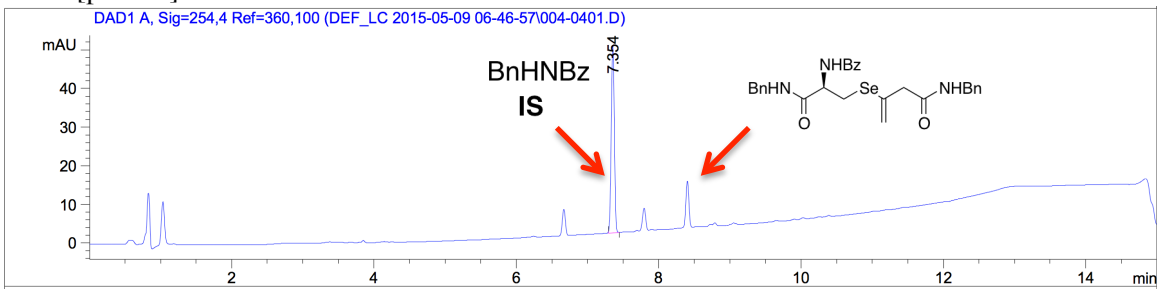
24 h [plasma]



48 h [plasma]



72 h [plasma]



Time/h	IS area	Sec-ALL (**15**) area	% Remaining
0	147.14783	49.82321	100
24	148.26094	43.2604	86.2
48	148.00545	39.35146	78.5
72	148.81026	36.97701	73.4

6. References

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