Supplementary Information.

Synthesis of a Selenocysteine-Containing Peptide by Chemical Ligation.

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General. Protected amino acids were purchased from either Advanced ChemTech or Chem Impex. Wang resin pre-loaded with the *C*-terminal Fmoc-protected amino acid was obtained from Advanced ChemTech and 4-sulfamylbutyryl AM (safety-catch) resin was obtained from Novabiochem. Water was purified using a Milli-Q Millipore system. THF was distilled from sodium/benzophenone, and CH_2Cl_2 was distilled from calcium hydride before use. Pyridine was distilled from calcium hydride onto 4 Å molecular sieves and stored under Ar. Some solvents were degassed immediately prior to use by bubbling Ar through the solvent for 30 min if indicated. Other solvents were freeze/thaw degassed as indicated. All other chemicals and solvents were obtained from Aldrich or Acros and were used without further purification. Peptide syntheses were carried out on a Rainin model PS3 peptide synthesizer. RP-HPLC was carried out on a Beckman System Gold with a Vydac C18 analytical or preparative column. Solution A was 0.1 % TFA in H_2O, and solution B was 80 % MeCN/20 % H_2O with 0.086 % TFA. A linear gradient of 10 % to 90 % B over 30 min. was used for all runs. ^1H and ^{13}C NMR data were obtained on either a Varian U400 or U500 spectrophotometer in $CDCl_3$. Mass spectrometry was performed by the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois using MALDI or FAB ionization techniques. CHN elemental analysis was performed by the Microanalysis Laboratory, School of Chemical Sciences, University of Illinois with a Leeman Labs Inc. Model DE 440 elemental analyzer. Melting points were obtained with a Thomas Hoover Capillary Melting Point Apparatus and optical rotations were obtained with a Perkin Elmer Polarimeter. IR data was recorded with a Perkin Elmer FT-IR system.

Methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-hydroxypropionate. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 1.45 (9H, s), 3.78 (3H, s), 3.93 (2H, m), 4.39 (1H, m), 5.44 (1H, br d, *J* = 7.6 Hz). These data agree with literature values.[1] ***Methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-(p-tolylsulfonyloxy)propionate.*** ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 1.44 (9H, s), 2.43 (3H, s), 3.68 (3H, s), 4.26 (1H, dd, *J* = 10.0, 3.0 Hz), 4.37 (1H, dd, *J* = 10.0, 3.0 Hz), 4.5 (1H, m), 5.34 (1H, d, *J* = 7.2 Hz), 7.34 (2H, d, *J* = 8.2 Hz), 7.75 (2H, d, *J* = 8.2 Hz). These data agree with literature values.[1] ***Methyl (2R)-2-2[(tert-butoxycarbonyl)amino]-3-bromopropionate*** **(3).** ^1H NMR ($CDCl_3$, 500 MHz) δ ppm 1.29 (9H, s), 3.54 (1H, dd, *J* = 10.8, 3.8 Hz), 3.63 (3H, s), 3.65 (1H, dd, *J* = 10.8, 3.8 Hz), 4.57 (1H, m), 5.3 (1H, br d, *J* = 7.5 Hz). These data agree with literature values.[1] ***Dimethyl bis(N-tert-butoxycarbonyl)-L-selenocystine*** **(4).** ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 1.38 (9H, s), 3.40 (2H, m), 3.78 (3H, s), 4.52 (1H, m), 5.39 (1H, br). These data agree with literature values.[1] ***L-Selenocystine*** **(5).** To an ice-cooled solution of **4** (3.33 g, 5.92 mmol) in CH_2Cl_2 (37 mL) was slowly added a mixture of CH_2Cl_2/TFA (4:1, v/v, 37 mL) and the mixture was stirred at rt for 3 h. The solvent was evaporated and the residue was placed under vacuum overnight. Then the residue was taken up in 6 N HCl, placed under N_2 and the temperature was brought to 75 °C for 12 h. Removal of the volatiles gave crude **5** as a yellow solid. The crude material was dissolved in a small amount of H_2O. To this mixture, 2.0 N $NH_3 \bullet H_2O$ was added to adjust the pH to 5. A precipitate was formed after 12 h at 4 °C.

Filtration followed by washing with cold H_2O, EtOH, and Et_2O gave a yellow powder (1.61 g). The pH of the combined mother liquors was adjusted to 1.0 with 1 N HCl and the solution was purified by cation exchange chromatography (Dowex 50 x 8). The compound **5** was eluted with 2 N $NH_3 \bullet H_2O$ (1.62 g, 82 %). mp 190 °C (decomp.). ^{1}H NMR (DCl-D_2O, 400 MHz) δ ppm 3.2 (1H, dd, *J* = 14.1, 7.6 Hz), 3.36 (1H, dd, *J*= 14.1, 4.7 Hz), 4.23 (1H, dd, *J* = 7.6, 4.7 Hz). This data matches the literature values.[1] ***(Se)-p-methoxybenzylselenocysteine* (6).** ^{1}H NMR (0.1 N DCl-D_2O, 400 MHz) δ ppm 2.25 (2H, m), 3.02 (3H, s), 3.14 (2H, s), 3.40 (1H, m), 6.20 (2H, m), 6.59 (2H, m). These data match literature values.[2]

***Fmoc-(Se)-p-methoxybenzylselenocysteine* (1)**. Method 1. To an ice-cooled suspension of 6 (0.31 g, 1.075 mmol) in a mixture of H_2O (3.68 mL) and TEA (0.15 mL, 1.08 mmol) was added Fmoc-OSu (0.35 g, 1.00 mmol) in MeCN (2 mL). The mixture was stirred at rt for 13 h. The solution was then acidified with 1.0 N HCl and extracted with EtOAc. The organic layer was washed with 1.0 N HCl, brine, and dried over Na_2SO_4. Removal of the solvent *in vacuo* gave crude 1, which was purified by flash chromatography to yield a white solid (0.31 g, 55 %). Method 2. Fmoc-PMB-Sec-OAll (4.61 g, 8.4 mmol) and $(Ph_3P)_4Pd$ (0.48 g, 0.42 mmol) were dissolved in THF (50 mL) and stirred under Ar. Morpholine (0.88 mL, 10.0 mmol) was added dropwise over 40 min. The solution was then stirred an additional 20 min, and then EtOAc was added (100 mL). The solution was washed with 2 M HCl (3 x 30 mL), dried over $MgSO_4$, filtered and the solvent was evaporated to yield a yellow solid. The crude product was flushed through three silica plugs, first with 30:1 CH_2Cl_2:MeOH, then 35:1 CH_2Cl_2:MeOH, and finally 40:1 CH_2Cl_2:MeOH containing 0.1 % AcOH, until the product **1** was recovered as a white solid. (R_f = 0.24, 9:1 CH_2Cl_2:MeOH, 4.23 g, 99 %). $[\alpha]_D^{21}$ = -31.6^o (c 5.7, DMF); ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.97 (2H, br s), 3.75 (3H, s), 3.76 (2H, br s), 4.23 (1H, t, *J* = 6.8 Hz), 4.42 (2H, d, *J* = 6.8 Hz), 4.69 (1H, m), 5.63 (1H, d, *J* = 7.2 Hz), 6.80 (2H, d, *J* = 8.3 Hz), 7.18 (2H, d, *J* = 8.1 Hz), 7.30 (2H, t, *J* = 7.4 Hz), 7.39 (2H, m), 7.60 (2H, t, *J* = 6.7 Hz), 7.76 (2H, d, *J* = 7.6 Hz), 8.84 (1H, br s). This data matches that found in the literature.[2]

***Fmoc-L-Serine-OAll*.** Fmoc-L-serine (6.99 g, 21.4 mmol) and $NaHCO_3$ (1.79 g, 21.4 mmol) were dissolved in H_2O (32 mL) under N_2. Allyl bromide (2.04 mL, 23.5 mmol) and aliquat 336 (8.66 g, 21.4 mmol) were dissolved in CH_2Cl_2 (32 mL) and added to the reaction mixture. The reaction was stirred vigorously under N_2 for 72 h. The reaction was extracted with CH_2Cl_2 (3 x 30 mL), and the combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated. The reaction was purified by flash chromatography (2:1 hexanes:EtOAc) to give the product as a white solid (R_f = 0.14, 15:1 CH_2Cl_2:MeOH, 6.00 g, 76 %). mp 82.5-84 ^{o}C; IR (CH_2Cl_2) 3425, 3064, 2952, 2892, 1725, 1513, 1198 cm^{-1}; $[\alpha]_D^{20}$ = +0.3^o (c 7.5, EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 2.33 (1H, br s), 3.94 (1H, br d, *J* = 10.3 Hz), 4.03 (1H, br d, *J* = 10.1 Hz), 4.23 (1H, t, *J* = 6.9 Hz), 4.43 (2H, m), 4.48 (1H, m), 4.69 (2H, d, *J* = 5.3 Hz), 5.26 (1H, dd, *J* = 0.8, 10.5 Hz), 5.35 (1H, dd, *J* = 0.8, 17.2 Hz), 5.79 (1H, d, *J* = 7.4 Hz), 5.91 (1H, ddt, *J* = 16.6, 11.0, 5.5 Hz), 7.32 (2H, br t, *J* = 7.4 Hz), 7.41 (2H, br t, *J* = 7.4 Hz), 7.61 (2H, m), 7.77 (2H, d, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm 47.08 (CH_3/CH), 56.05 (CH_3/CH), 63.26 (C/CH_2), 66.35 (C/CH_2), 67.19 (C/CH_2), 119.02 (C/CH_2), 119.99 (CH_3/CH), 125.07 (CH_3/CH), 127.06 (CH_3/CH), 127.74 (CH_3/CH), 131.29 (CH_3/CH), 141.31 (C/CH_2), 143.72 (C/CH_2), 156.23 (C/CH_2), 170.21 (C/CH_2); FAB-LRMS *m/z* 368 (M + 1); Anal. Calcd for $C_{21}H_{21}O_5N$: C, 75.44; H, 5.51; N, 2.84. Found: C, 73.64; H, 5.58; N, 2.69.

***Fmoc-(OTs)-Serine-OAll (7)*.** Fmoc-L-Serine-OAll (2.29 g, 6.23 mmol) and TsCl (5.94 g, 31.16 mmol) were dissolved in pyridine (11 mL). The flask was immediately cooled to 0 ^{o}C and stirred under Ar for 11 h. The mixture was taken up in Et_2O (100 mL) and the organic layer was

washed with H_2O (1 x 50 mL), 10 % $KHSO_4$ (4 x 50 mL), saturated $NaHCO_3$ (1 x 50 mL), brine (1 x 50 mL), and H_2O again (2 x 50 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated to give a light yellow solid. Purification was accomplished by flash chromatography to give **7** as a white solid (R_f = 0.26, 2:1 hexanes:EtOAc, 3.01 g, 94 %). mp 65-67 ^oC; IR (CH_2Cl_2) 3350, 1720, 1512, 1364, 1177 cm^{-1}; $[\alpha]_D^{20}$ = +0.03^o (c 7.5, EtOAc); ^1H NMR ($CDCl_3$, 500 MHz) δ ppm 2.36 (3H, s), 4.19 (1H, t, *J* = 7.5 Hz), 4.27 (2H, dd, *J* = 10.2, 7.2 Hz), 4.36 (2H, m), 4.47 (2H, dd, *J* = 10.2, 3.0 Hz), 4.61 (2H, m), 4.67 (1H, m), 5.27 (1H, dd, *J* = 10.4, 0.9 Hz), 5.32 (1H, dd, *J* = 17.2, 1.0), 5.67 (1H, d, *J* = 7.7 Hz), 5.85 (1H, ddt, *J* = 16.3, 11.0, 5.9 Hz), 7.27 (2H, *J* = 8.7 Hz), 7.33 (2H, dd, *J* = 12.9, 6.7 Hz), 7.42 (2H, td, *J* = 7.1, 2.2 Hz), 7.60 (2H, t, *J* = 8.4 Hz), 7.77 (4H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm 21.53 (CH_3/CH), 46.89 (CH_3/CH), 53.39 (CH_3/CH), 66.82 (C/CH_2), 67.48 (C/CH_2), 69.01 (C/CH_2), 119.36 (C/CH_2), 119.99 (CH_3/CH), 125.15 (CH_3/CH), 127.12 (CH_3/CH), 127.79 (CH_3/CH), 127.98 (CH_3/CH), 129.94 (CH_3/CH), 130.96 (CH_3/CH), 132.15 (C/CH_2), 141.25 (C/CH_2), 143.62 (C/CH_2), 145.24 (C/CH_2), 155.52 (C/CH_2), 167.90 (C/CH_2); FAB-LRMS *m/z* 522 (M^+); Anal. Calcd for $C_{28}H_{27}O_7NS$: C, 64.48; H, 5.22; N, 2.69. Found: C, 64.44; H, 5.23; N, 2.78.

Di-p-Methoxybenzyl diselenide ((PMBSe)$_2$). Selenium powder (5.00 g, 63.3 mmol) was suspended in THF (30 mL) and 1 M $LiB(Et)_3H$ in THF was added (63 mL, 63.0 mmol). The mixture was refluxed for 45 min under Ar. In a separate 3-necked flask, *p*-methoxybenzyl chloride (9.02 mL, 66.5 mmol) was dissolved in THF (30 mL) and cooled to –78 ^oC. The refluxing mixture was cooled to rt and transferred to the cold flask via cannula. The mixture was stirred at –78 ^oC for 45 min, warmed to 0 ^oC over 30 min., and to rt over another 30 min. The mixture was filtered through a silica plug (5:1 hexanes:EtOAc), purified by flash chromatography (11:1 hexanes:EtOAc), and finally recrystallized from EtOH to give the product as a yellow solid (R_f = 0.50, 4:1 hexanes:EtOAc, 10.86 g, 84 %). mp 67-68 ^oC; ^1H NMR ($CDCl_3$, 500 MHz) δ ppm 3.80 (6H, s), 3.84 (4H, s), 6.84 (4H, d, *J* = 8.4), 7.16 (4H, d, *J* = 8.4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm 32.42 (C/CH_2), 55.48 (CH_3/CH), 114.07 (CH_3/CH), 130.33 (CH_3/CH), 131.30 (C/CH_2), 159.00 (C/CH_2). This data matches that found in the literature.[3,4]

Fmoc-(Se)-p-methoxybenzylselenocysteine-OAll. ($PMBSe)_2$ (6.71 g, 16.7 mmol) was placed in a round bottom flask fitted with a reflux condenser. The yellow/orange solid was dissolved in THF (40 mL) and 50 % H_3PO_2 (60 mL) and refluxed under Ar for 1 h. The solution was cooled and extracted under Ar with Et_2O (2 x 30 mL) and the organic layers were transferred via syringe to a round bottom flask that was flushed with Ar containing degassed brine solution. The solution was shaken and then the organic layer was transferred via syringe to a Ar flushed round bottom flask containing $MgSO_4$. After drying, the solution was rapidly filtered and the solvent was evaporated. The resulting selenol was dissolved in degassed DMF (40 mL) in a round bottom flask under Ar. NaOH (0.46 g, 11.5 mmol) dissolved in degassed H_2O (10 mL) was added immediately followed by Fmoc-(OTs)-serine-OAll (**7**, 5.43 g, 10.4 mmol) dissolved in degassed acetone (40 mL). The mixture was stirred under Ar at 0 ^oC for 4 h. The reaction was then taken up in EtOAc (100 mL) and washed with satd. NH_4Cl (3 x 40 mL) and brine (3 x 40 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated. The product was purified by flash chromatography (6:1 hexanes:EtOAc) to give the product as a white solid (R_f = 0.25, 4:1 hexanes:EtOAc, 5.29 g, 92 %). mp 92-93 ^oC; IR (CH_2Cl_2) 3351, 2951, 1723, 1510, 1450, 1247, 1034 cm^{-1}; $[\alpha]_D^{20}$ = -10.0^o (c 7.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 2.94 (1H, B of ABX, J_{AB} = 13.0 Hz, J_{BX} = 5.5 Hz), 2.97 (1H, A of ABX, J_{AB} = 13.0 Hz, J_{AX} = 4.4 Hz), 3.77 (5H, s), 4.26 (1H, t, *J* = 6.8 Hz), 4.43 (2H, d, *J* = 6.8 Hz), 4.67 (2H, d, *J* = 5.6 Hz), 4.72 (1H, X of ABX, J_{BX} = 5.5 Hz, J_{AX} = 4.4 Hz), 5.29 (1H, dd, *J* = 10.4, 1.1 Hz),

5.36 (1H, dd, *J* = 17.2, 1.0 Hz), 5.63 (1H, d, *J* = 7.9 Hz), 5.93 (1H, ddt, *J* = 16.5, 11.1, 5.8 Hz), 6.83 (2H, d, *J* = 8.4 Hz), 7.21 (2H, d, *J* = 8.4 Hz), 7.32 (2H, td, *J* = 7.5, 1.2 Hz), 7.41 (2H, td, *J* = 7.2, 3.0 Hz), 7.63 (2H, t, *J* = 6.2 Hz), 7.78 (2H, dd, *J* = 7.8, 2.1 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm 25.82 (C/CH_2), 27.69 (C/CH_2), 47.28 (CH_3/CH), 54.07 (CH_3/CH), 55.40 (CH_3/CH), 66.52 (C/CH_2), 67.35 (C/CH_2), 114.19 (CH_3/CH), 119.31 (C/CH_2), 120.19 (CH_3/CH), 125.29 (CH_3/CH), 127.28 (CH_3/CH), 127.93 (CH_3/CH), 130.21 (CH_3/CH), 130.62 (C/CH_2), 131.52 (CH_3/CH), 141.49 (C/CH_2), 143.93 (C/CH_2), 155.89 (C/CH_2), 158.78 (C/CH_2), 170.81 (C/CH_2); FAB-LRMS *m/z* 552 (M+1); Anal. Calcd for $C_{29}H_{29}NO_5Se$: C, 63.27; H, 5.31; N, 2.54. Found: C, 63.32; H, 5.34; N, 2.75.

***Fmoc-L-Serine-ODpm*.** The procedure of Lapatsanis et al was modified.[5] Fmoc–L-Serine (15.29 g, 46.7 mmol) was ground into a fine powder, placed in a round bottom flask with diphenylmethyl hydrazone (13.76 g, 70.1 mmol), and the mixture was suspended in CH_2Cl_2 (115 mL). A 1% (w/v) solution of I_2 in CH_2Cl_2 (8.0 mL) was added and the mixture was cooled to –10 ^{o}C. Iodobenzenediacetate (22.56 g, 70.0 mmol) was added slowly over 1.5 h as the mixture was stirred under Ar. The reaction was allowed to warm to 0 ^{o}C over 45 min. The solvent was evaporated and the resulting yellow oil was dissolved in EtOAc (250 mL). The organic layer was washed with H_2O (1 x 100 mL), saturated aqueous $NaHCO_3$ (3 x 100 mL), and H_2O again (1 x 100 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated to give a yellow oil. The oil was dissolved in EtOAc and loaded onto a silica plug. Impurities were eluted with 5:1 hexanes:EtOAc, then the plug was washed with pure EtOAc to elute the product. The product was crystallized from EtOH to give a white solid (R_f = 0.60, 9:1 CH_2Cl_2:CH_3OH, 1st crop = 8.35 g, 2nd crop = 7.34 g 68 % overall). mp 103-105 ^{o}C; IR (CH_2Cl_2) 3419, 3065, 1716, 1520, 1450, 1197, 1080 cm^{-1}; $[\alpha]_D^{20}$ = -3.7^o (c 8.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.15 (1H, br t, *J* = 5.8 Hz), 3.96 (1H, B of ABX of doublets, *J* = 3.3 Hz, J_{AB} = 11.4 Hz, J_{BX} = 6.0 Hz), 4.08 (1H, A of ABX of doublets, *J* = 3.6 Hz, J_{AB} = 11.4 Hz, J_{AX} = 5.9 Hz), 4.21 (1H, X of ABX, J_{BX} = 5.5 Hz, J_{AX} = 5.8 Hz), 4.39 (1H, B of ABX, J_{AB} = 10.0 Hz, J_{BX} = 5.5 Hz), 4.43 (1H, A of ABX, J_{AB} = 10.0 Hz, J_{AX} = 5.8 Hz), 4.57 (1H, X of ABX of doublets, J_{BX} = 6.0 Hz, J_{AX} = 5.9 Hz), 5.78 (1H, br d, *J* = 7.4 Hz), 6.94 (1H, s), 7.26 – 7.38 (12H, br m), 7.40 (2H, t, *J* = 7.5 Hz), 7.59 (2H, d, *J* = 7.0 Hz), 7.77 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 47.02 (CH_3/CH), 56.22 (CH_3/CH), 63.31 (C/CH_2), 67.21 (C/CH_2), 78.57 (CH_3/CH), 119.95 (CH_3/CH), 125.05 (CH_3/CH), 126.91 (CH_3/CH), 127.07 (CH_3/CH), 127.70 (CH_3/CH), 128.18 (CH_3/CH), 128.59 (CH_3/CH), 137.27 (C/CH_2), 141.25 (C/CH_2), 143.68 (C/CH_2), 156.19 (C/CH_2), 169.62 (C/CH_2); FAB-HRMS *m/z* calcd for $C_{31}H_{27}O_5N$ $(M + 1)^+$ 494.1969, found 494.1967. This data matches that found in the literature.[5]

Fmoc-(OTs)-Serine-ODpm (8). Fmoc-L-Serine-ODpm (11.21 g, 22.7 mmol) and TsCl (8.71 g, 45.7 mmol) were placed in a round bottom flask and dissolved in pyridine (30 mL) and the same procedure for the preparation of **7** was followed. The crude reaction product was recrystallized twice from EtOH to give **8** as a white solid (R_f = 0.38, 2:1 hexanes:EtOAc, 10.79 g, 73 %). mp 128.5–130 ^{o}C; IR (CH_2Cl_2) 3380, 3065, 3034, 1726, 1513, 136, 1191, 1056 cm^{-1}; $[\alpha]_D^{20}$ = +4.5^o (c 6.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.33 (3H, s), 4.17 (1H, X of ABX, J_{BX} = 7.3 Hz, J_{AX} = 7.5 Hz), 4.26 (1H, B of ABX, J_{AB} = 10.4 Hz, J_{BX} = 7.3 Hz), 4.34 (1H, A of ABX, J_{AB} = 10.4 Hz, J_{AX} = 7.5 Hz), 4.43 (1H, B of ABX, J_{AB} = 10.2 Hz, J_{BX} = 3.0 Hz), 4.53 (1H, A of ABX, J_{AB} = 10.2 Hz, J_{AX} = 3.0 Hz), 4.71 (1H, X of ABX, J_{BX} = 3.0 Hz, J_{AX} = 3.0 Hz), 5.70 (1H, br d, *J* = 7.9 Hz), 6.90 (1H, s), 7.19 (2H, d, *J* = 8.1 Hz), 7.28 – 7.39 (12H, br m), 7.42 (2H, t, *J* = 7.4 Hz), 7.59 (2H, t, *J* = 6.6 Hz), 7.65 (2H, d, *J* = 8.3 Hz), 7.79 (2H, d, *J* = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 21.54 (CH_3/CH), 46.86 (CH_3/CH), 53.54 (CH_3/CH), 67.52 (C/CH_2),

68.85 (C/CH_2), 79.32 (CH_3/CH), 119.95 (CH_3/CH), 125.12 (CH_3/CH), 126.91 (CH_3/CH), 127.12 (CH_3/CH), 127.76 (CH_3/CH), 127.90 (CH_3/CH), 128.25 (CH_3/CH), 128.60 (CH_3/CH), 129.86 (CH_3/CH), 131.91 (C/CH_2), 138.89 (C/CH_2), 141.18 (C/CH_2), 143.56 (C/CH_2), 145.11 (C/CH_2), 155.46 (C/CH_2), 167.45 (C/CH_2); FAB-LRMS *m/z* 648 $(M + 1)^+$; Anal. Calcd for $C_{38}H_{33}NO_7S$: C, 70.50; H, 5.10; N, 2.20. Found: C, 70.39; H, 5.19; N, 2.12.

Fmoc-(Se)-Phenylselenocysteine-ODpm (9). PhSeH (1.88 mL, 17.7 mmol) was dissolved in degassed DMF (26 mL) at 0 ^oC. NaOH (0.54 g, 13.6 mmol) was dissolved in degassed H_2O (8 mL) and added to the mixture followed immediately by **8** (8.80 g, 13.6 mmol) dissolved in degassed acetone (26 mL). The reaction was stirred under Ar at 0 ^oC for 3.5 h. The product precipitated as the reaction progressed, and was filtered. The yellow solid obtained was recrystallized from EtOH to give **9** as a white solid (R_f = 0.26, 4:1 hexanes:EtOAc, 6.35 g, 75 %). mp 103-105 ^oC; IR (CH_2Cl_2) 3339, 3064, 1730, 1506, 1450, 1185 cm^{-1}; $[\alpha]_D^{20}$ = -25.2^o; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 3.37 (1H, B of ABX, J_{AB} = 13.0 Hz, J_{BX} = 5.3 Hz), 3.50 (1H, A of ABX, J_{AB} = 13.0 Hz, J_{AX} = 4.9 Hz), 4.16 (1H, X of ABX, J_{BX} = 7.5 Hz, J_{AX} = 8.1 Hz), 4.27 (1H, B of ABX, J_{AB} = 10.7 Hz, J_{BX} = 7.5 Hz), 4.31 (1H, A of ABX, J_{AB} = 10.7 Hz, J_{AX} = 8.1 Hz), 4.86 (1H, X of ABX, J_{BX} = 5.3 Hz, J_{AX} = 4.9 Hz), 5.58 (1H, br d, *J* = 8.0 Hz), 6.84 (1H, s), 7.16 – 7.23 (3H, br m), 7.27 – 7.37 (12H, br m), 7.41 (2H, t, *J* = 7.4 Hz), 7.51 (2H, m), 7.55 (2H, d, *J* = 7.4 Hz), 7.77 (2H, d, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm 30.06 (C/CH_2), 47.00 (CH_3/CH), 54.42 (CH_3/CH), 67.22 (C/CH_2), 78.66 (CH_3/CH), 119.96 (CH_3/CH), 125.16 (CH_3/CH), 127.07 (CH_3/CH), 127.12 (CH_3/CH), 127.59 (CH_3/CH), 127.71 (CH_3/CH), 128.18 (CH_3/CH), 128.58 (CH_3/CH), 128.99 (C/CH_2), 129.21 (CH_3/CH), 139.27 (C/CH_2), 141.24 (C/CH_2), 143.74 (C/CH_2), 155.46 (C/CH_2), 169.65 (C/CH_2); FAB-LRMS *m/z* 634 $(M+1)^+$; Anal. Calcd for $C_{37}H_{31}NO_4Se$: C, 70.25; H, 4.94; N, 2.21. Found: C, 70.03; H, 5.05; N, 2.13.

Fmoc-(Se)-Phenylselenocysteine (2). Thioanisole (2.38 mL, 20.1 mmol) was dissolved in TFA (45 mL) and added to Fmoc-Ph-Sec-ODpm (**9**, 6.36 g, 10.1 mmol). The mixture was stirred under N_2 for 40 min. The solvent was evaporated to give a dark oil that was taken up in $CHCl_3$ and evaporated repeatedly (5 x 50 mL). The yellow solid was recrystallized three times from hexanes/EtOAc to give **2** as a white solid (4.01 g). The combined mother liquors were concentrated and the product crystallized to give a second crop (0.63 g), (R_f = 0.30, 9:1 CH_2Cl_2:MeOH, 4.64 g, 98 %). mp 121-124 ^oC; IR ($CHCl_3$) 3425, 2950, 1720, 1509, 1210 cm^{-1}; $[\alpha]_D^{22}$ = -32.4^o (c 7.0, EtOAc); ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 3.36 (1H, B of ABX, J_{AB} = 13.2 Hz, J_{BX} = 5.4 Hz), 3.43 (1H, A of ABX, J_{AB} = 13.2 Hz, J_{AX} = 4.5 Hz), 4.18 (1H, X of ABX, J_{BX} = 6.8 Hz, J_{AX} = 7.3 Hz), 4.32 (1H, B of ABX, J_{AB} = 10.5 Hz, J_{BX} = 6.8 Hz), 4.35 (1H, A of ABX, J_{AB} = 10.5 Hz, J_{AX} = 7.3 Hz), 4.75 (1H, X of ABX, J_{BX} = 5.4 Hz, J_{AX} = 4.5 Hz), 5.58 (1H, br d, *J* = 7.7 Hz), 7.20 – 7.31 (5H, br m), 7.32 (2H, br t, *J* = 7.1 Hz), 7.41 (2H, br t, *J* = 7.7 Hz), 7.56 (2H, br s), 7.77 (2H, d, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm 30.0 (C/CH_2), 47.0 (CH_3/CH), 54.2 (CH_3/CH), 67.6 (C/CH_2), 120.3 (CH_3/CH), 125.4 (CH_3/CH), 127.4 (CH_3/CH), 128.0 (CH_3/CH), 128.1 (CH_3/CH), 128.8 (C/CH_2), 129.5 (CH_3/CH), 134.0 (CH_3/CH), 141.6 (C/CH_2), 144.0 (C/CH_2), 156.0 (C/CH_2), 175.0 (C/CH_2); FAB-HRMS *m/z* calcd for $C_{24}H_{22}NO_4Se$ M^+ 466.0722, found 466.0723.

Solid Phase Peptide Synthesis. Peptides were synthesized on a 0.1 mmol scale with a Rainin automated synthesizer using Fmoc chemistry. Wang resin or sulfamylbutyryl safety catch resin were swollen in DMF (3 x 6 mL, 10 min). Fmoc deprotection was then accomplished with 20 % v/v piperidine/DMF (4 x 6 mL, 6 min). A four-fold excess of the amino acids were activated with *O*-benzotriazole-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HBTU) and 0.4 M N-methylmorpholine and coupled to the resin. Each amino acid was coupled for 1 h or until

ninhydrin test of the resin indicated the coupling was complete. At the end of the synthesis, peptide **10** was deprotected to yield the free amine and peptide **13** was capped for 20 min with acetic anhydride (2 mL).

***Peptide cleavage*.** Wang resin with peptide **10** attached was washed with DMF (3 x 3 mL), EtOH (3 x 3 mL), and CH_2Cl_2 (3 x 3 mL), and dried in a vacuum dessicator for 3 h. The dry resin was swollen in a mixture consisting of H_2O (0.5 mL), anisole (0.5 mL), thioanisole (0.5 mL), and TFA (10 mL) and stirred for 2 h. The solvent was then filtered, and the resin was washed with TFA (3 x 3 mL), The TFA was evaporated, and the peptide was precipitated with cold Et_2O. The resulting white solid was isolated by filtration, dissolved in a 1:1 mixture of MeCN:H_2O, and lyophilized. The white solid was purified by preparative RP-HPLC to give **10** as a white solid (63 mg, 58 %). Peptide **13** was cleaved from the safety catch resin by alkylating with iodoacetonitrile and then cleaving with sodium phenylthiolate and benzyl mercaptan in CH_2Cl_2 according to the method of Ingenito et. al.[6] The product, **13**, was obtained as a white solid after purification by preparative RP-HPLC (39 mg, 61 %). FAB-MS for peptide **10** M+1 calc. 1066.2, found 1066.4; M+Na calc. 1088.2, found 1088.4; M+K calc. 1104.3, found 1104.4. MALDI-MS for peptide **13** M+Na calc. 1114.2, found 1113.4; M+K calc. 1130.3, found 1129.3; M+2Na calc. 1137.2, found 1136.4; M+Na+K calc. 1153.3, found 1152.3.

***Selenocysteine Deprotection*.** Method 1. Peptide, **10** (4.1 mg, 0.004 mmol), was dissolved in a mixture of 8:1:1 AcOH:H_2O:MeCN (5 mL). I_2 (15 mg, 0.058 mmol) was added to the mixture and it was stirred at ambient temperature for 1 h 15 min. The mixture was then immediately purified by preparative RP-HPLC and lyophilized to give peptide **11** as a white powder (2.2 mg, 61 %). MALDI-MS M+1 calc. 1889.1, found 1889.2; M+Na calc. 1911.0, found 1912.3; M+K calc. 1927.1, found 1926.0. A small amount of the free selenol monomer was also detected in the mass spectrum.

Method 2. Peptide **10** (12.6 mg, 0.012 mmol) was dissolved in a mixture of 8:1:1 AcOH:H_2O:MeCN (20 mL) containing I_2 (3.2 mg, 0.013 mmol). The solution was stirred at ambient temperature for 12 h, and then purified directly by preparative RP-HPLC and lyophilized. Peptide **11** was recovered as a white solid (1.3 mg, 12 %). Peptide **12** was recovered as a white powder (7.5 mg, 74 %) MALDI-MS M+1 calc. 855.9, found 856.2; M+Na calc. 877.9, found 878.2.

***Native Ligation Reactions*.** Method 1. Peptide **11** (2.2 mg, 0.001 mmol) and peptide **13** (1.4 mg, 0.001 mmol) were dissolved in 0.1 M sodium phosphate buffer (pH 7.5) with 6 M Gn•HCl that had been freeze/thaw degassed (0.5 mL). Thiophenol (4 %) was added and the solution was vigorously stirred under Ar for 20 h at ambient temperature. The mixture was directly purified by preparative RP-HPLC and lyophilized to give **14** as a white solid (1.3 mg, 56 %). Method 2. Peptide **12** (5.9 mg, 0.007 mmol) and **13** (6.9 mg, 0.006 mmol) were dissolved in 0.1 M sodium phosphate buffer (pH 7.5) with 6 M Gn•HCl that had been freeze/thaw degassed (1 mL). Thiophenol (4 %) was added and the mixture was stirred for 34 h under Ar at ambient temperature. The mixture was purified directly by RP-HPLC and lyophilized to give **14** as a white solid (6.9 mg, 60 %). MALDI-MS M+1 calc. 1822.9, found 1822.6; M+Na calc. 1844.9, found 1844.5; M+K calc. 1861.0, found 1860.5.

***Lanthionine Synthesis*.** Peptide **12** (6.9 mg, 0.008 mmol), peptide **13** (7.9 mg, 0.007mmol) and TCEP (20.8 mg, 0.07 mmol) were dissolved in 0.1 M sodium phosphate buffer (pH 7.5) with 6 M Gn•HCl that had been freeze/thaw degassed (1.5 mL). Thiophenol (4 %) was added and the mixture was stirred for 7 d under Ar at rt. The reaction was purified directly by preparative RP-

HPLC to give **15** as a white powder (3.1 mg, 25 %). MALDI-MS M+1 calc. 1743.9, found 1745.0; M+Na calc. 1765.9, found 1767.0; M+K calc. 1782.0, found 1783.2.

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