Supplementary Material. Facile Chemoselective Synthesis of Dehydroalanine-Containing Peptides. Nicole M. Okeley, Yantao Zhu, and Wilfred A. van der Donk

General. Protected amino acids were purchased from either Advanced ChemTech or Chem-Impex. Resins pre-loaded with the *C*-terminal Fmoc-protected amino acid were obtained from Advanced ChemTech. THF was distilled from sodium/benzophenone, and CH_2Cl_2 was distilled from calcium hydride before use. All other chemicals were obtained from Aldrich or Acros and, unless indicated, were used without further purification. Water was purified using a Milli-Q Millipore system. Peptide syntheses were performed on a Rainin model PS3 peptide synthesizer. RP-HPLC was performed on either a Rainin system (Dynamax model SD-200 pump and model UV-1 detector) or a Beckman System Gold (Model 125 solvent module and model 166 detector) with a Vydac C18 analytical or preparative column, monitoring at 220 nm. Solution A consisted of 80 % MeCN/20 % H_2O containing 0.086 % TFA, solution B was 0.1 % TFA in water. ^1H and ^{13}C NMR data were obtained on either a Varian U400 or U500 spectrophotometer in $CDCl_3$. Mass spectrometry of peptides was performed by the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois by matrix assisted laser desorption ionization for larger peptides (MALDI), or fast atom bombardment (FAB) ionization for smaller peptides.

N-(tert-Butoxycarbonyl)-L-serine β-Lactone. The procedure by Pansare *et al.* was followed.[1] The melting point and ^1H NMR data matched the reported values.

N-(tert-Butoxycarbonyl)-L-(Se)-phenylselenocysteine.[2a,b] Dry ethanol (distilled from Mg and I_2, 34 mL) was placed in a 100 mL round bottom flask equipped with a stir bar. Diphenyl diselenide (0.94 g, 3.0 mmol) was added, and the mixture was stirred under nitrogen until the suspension became homogeneous. $NaHB(OMe)_3$ (0.767 g, 6.00 mmol) was added, and the reaction was stirred for 50 min. BocSer-β-lactone (0.802 g, 4.28 mmol) was added in one portion to the pale peach solution, and the reaction mixture was stirred for 4 h at rt. Saturated aqueous $NaHCO_3$ (50 mL) was added, and the reaction was diluted with diethyl ether (50 mL). The aqueous layer was acidified to pH ~ 2 with 5 M aqueous HCl, and was extracted with EtOAc (3 x 50 mL). The combined EtOAc layers were washed with brine (50 mL) and dried over $MgSO_4$. Filtration and concentration gave a yellow oil. Addition of hexane induced the formation of white needles (1.33 g, three crops, 90 %). R_f = 0.43, 9:1 CH_2Cl_2: MeOH; mp 89-92

°C; ^{1}H NMR, 400 MHz: δ ppm 1.42 (9H, s), 3.30-3.36 (2H, m), 4.62 (1H, m), 5.28 (1H, br d, *J* = 7.1 Hz), 7.25-7.57 (5H, m). These values agree with reported literature values.[2c]

N-(9-Fluorenylmethoxycarbonyl)-L-β-phenylselenocysteine. Recrystallized BocSec(Ph) (2.96 g, 7.80 mmol) was placed in a 100 mL round bottom flask equipped with a stir bar. The flask was purged with N_2 and CH_2Cl_2 (15 mL) was added. Once the solids had dissolved, TFA (15 mL) was added, and the reaction was stirred at rt for 1 h. The solvent was removed by rotary evaporation. To remove remaining TFA, $CHCl_3$ was added and removed by rotary evaporation to provide a solid product. Water was added to the resulting residue (15 mL), followed by Et_3N (1.0 mL) to adjust the pH to about 9. An additional 1 mL of water was added, after which a solution of Fmoc-succinimide (2.96 g, 7.80 mmol) in acetonitrile (15 mL) was added. The pH of the reaction was checked every five min. More triethylamine was added, if necessary, to keep the pH of the solution ~9. At this point the solids had dissolved and the reaction was stirred at rt for 30 min.

The reaction was acidified with 1 M HCl to pH ~ 2 and was extracted with EtOAc (2 x 70 mL). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator. The resulting oil was basified with saturated $NaHCO_3$ and water (pH ~ 9). The resulting solution was extracted with diethyl ether (2 x 60 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (4 x 25 mL). The combined aqueous layers were acidified to pH ~2 with 1 M HCl and extracted with EtOAc (4 x 50 mL). The combined EtOAc layers were washed with brine (50 mL) and dried over $MgSO_4$. Filtration and concentration gave an off-white solid that was sufficiently pure for use in SPPS (3.30 g, 91 %). For characterization purposes, the solids were recrystallized from hexane:EtOAc to give white crystals (2.50 g, 69 %). mp 121-124 °C; IR ($CHCl_3$) 3425, 2950, 1720, 1509, 1210 cm^{-1}; ^{1}H NMR, 500 MHz: δ ppm 3.36-3.47 (2H, m), 4.16 (1H, t, *J* = 7.0 Hz), 4.29 (2H, quin, *J* = 7.5 Hz), 4.72 (1H, m), 5.70 (1H, br d, *J* = 7.5 Hz), 7.21 (4H, m), 7.30 (2H, m), 7.40 (2H, m), 7.56 (3H, m), 7.75 (2H, m); ^{13}C NMR, 125 MHz: δ ppm 30.0, 47.0, 54.2, 67.6, 120.3, 125.4, 127.4, 128.0, 128.1, 128.8, 129.5, 134.0, 141.6, 144.0, 156.0, 175.0; FAB-HRMS *m/z* calcd for $C_{24}H_{22}NO_4{}^{78}Se$ $(M+1)^+$ 466.0722, found 466.0723.

Synthesis of Dipeptides. Dipeptides were prepared via standard solution phase Boc and Fmoc chemistry,[3] and were purified by silica gel flash chromatography.

General Procedure for Dipeptide Oxidation. The dipeptide was dissolved in the requisite solvent or solvent system (see Table 1). Aqueous $NaIO_4$ was added dropwise to the reaction, and the mixture was stirred at rt until the reaction was finished as determined by the disappearance of starting material on TLC (45 min to 12 h). The reaction mixture was then diluted with CH_2Cl_2 and washed with water, saturated $NaHCO_3$, water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The resulting yellow oil was purified by silica gel flash chromatography.

FmocCys(SEt)Sec(Ph)ODPM ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28 (t, 3H, *J* = 7.0 Hz), 2.66 (br q, 2H, *J* = 7.0 Hz), 2.80 (dd, 1H, *J* = 13.6 Hz, 4.9 Hz), 2.94 (dd, 1H, *J* = 13.6 Hz, 4.9 Hz), 3.32 (dd, 1H, *J* = 13.3 Hz, 4.9 Hz), 3.47 (dd, 1H, J_{AB} = 13.6 Hz, 4.9 Hz), 4.21-4.27 (m, 1H), 4.31-4.38 (m, 1H), 4.39-4.52 (m, 2H), 5.03-5.08 (m, 1H), 5.15 (br d, 1H, *J* = 7.0 Hz, N–H), 6.84 (s, 1H), 6.94 (br d, 1H, *J* = 7.0 Hz, N–H), 7.27–7.45 (m, 19H, aromatic), 7.60 (br s, 2H), 7.78 (d, 2H, *J* = 7.6 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.46, 29.55, 32.50, 40.32, 41.65, 47.32, 57.76, 67.50, 79.01, 120.25, 125.30, 127.33, 127.41, 127.89, 128.01, 128.03, 128.47, 128.48, 128.81, 128.85, 129.59, 133.70, 139.42, 141.58, 143.94, 161.68, 169.22, 169.69; FAB-HRMS *m/z* calcd for $C_{42}H_{41}N_2O_5S_2{}^{80}Se$ $(M+1)^+$ 797.1623, found 797.1622.

FmocCys(SEt)DhaODPM (70 % yield) ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31 (t, 3H, *J* = 7.2 Hz), 2.70 (q, 2H, *J* = 7.5 Hz), 3.10 (m, 2H), 4.23 (br t, 1H, *J* = 7.2 Hz), 4.42 (br d, 2H, *J* = 7.2 Hz), 4.60 (br s, 1H), 5.62 (br s, 1H), 6.15 (s, 1H), 6.69 (s, 1H), 6.96 (s, 1H), 7.27-7.41 (m, 14H), 7.58 (br d, 2H, *J* = 7.1 Hz), 7.76 (d, 2H, *J* = 7.1 Hz), 8.60 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.43, 32.64, 40.02, 47.25, 57.76, 67.80, 79.19, 110.43, 120.23, 125.40, 127.23, 127.26, 127.37, 127.40, 128.01, 128.51, 128.55, 128.88, 128.90, 131.07, 139.52, 139.56, 141.54, 143.83, 143.91, 163.01, 164.23, 169.11; FAB-HRMS *m/z* calcd for $C_{36}H_{35}NO_5S_2$ $(M+1)^+$ 639.1984, found 639.1987.

[Boc-Sec(Ph)Cys-OMe]$_2$ ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45 (9H, s), 3.06-3.16 (m, 2H), 3.27 (m, 2H), 3.74 (s, 3H), 4.48 (br, 1H), 4.73-4.78 (dt, 1H, *J* = 7.4, 5.4), 5.60 (d, 1H, *J*=7.81), 7.22-7.27 (m, 3H), 7.30-7.35 (br s, 1H), 7.53-7.55 (m, 2H); ^{13}C NMR (126MHz, $CDCl_3$) δ 28.5, 30.0, 40.8, 52.2, 53.0, 54.5, 80.6, 127.63, 129.4, 129.7, 133.4, 155.8, 170.5, 171.1; FAB-HRMS *m/z* cacld for $C_{36}H_{51}N_4O_{10}S_2{}^{80}Se_2$ $(M+1)^+$ 923.1377, found 923.1377.

[Boc-DhaCys-OMe]$_2$ (75 % yield) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H), 3.23 (d, 2H, *J* = 5.2 Hz), 3.78 (s, 3H), 4.89 (dt, 1H, *J* = 7.0 Hz, 5.0 Hz), 5.21 (t, 1H, *J* = 1.7 Hz), 6.07 (s, 1H),

6.99 (d, 1H, *J* = 7.29 Hz), 7.21 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.4, 40.6, 52.2, 53.2, 80.9, 99.2, 134.5, 153.0, 164.1, 170.6; FAB-HRMS *m/z* calc for $C_{24}H_{39}N_4O_{10}S_2$ $(M+1)^+$ 607.2107, found 607.2115.

Boc-Sec(Ph)Cys(Trt)-OMe ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41 (s, 9H), 2.62 (d, 2H, *J* = 4.03), 3.15-3.30 (m, 2H), 3.70 (s, 3H), 4.33 (br s, 1H),.4.39-4.44 (m, 1H), 5.20 (br s, 1H), 6.63 (d, 1H, *J* = 8.21), 7.19-7.53 (m, 20H); ^{13}C NMR (126MHz, $CDCl_3$) δ 28.5, 30.2, 33.8, 51.5, 52.8, 53.2, 54.5, 67.1, 127.2, 127.7, 128.3, 129.5, 129.7, 133.3, 144.5, 155.4, 170.3, 170.6; FAB-HRMS *m/z* cacld for $C_{37}H_{41}N_2O_5S^{80}Se$ $(M+1)^+$ 705.1901, found 705.1904.

Boc-DhaCys(Trt)-OMe (83 % yield) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.64 (dd, 1H, *J* = 18.0, 4.5 Hz), 2.72 (dd, 1H, J_{AX} = 18.0, 5.68 Hz), 3.73 (s, 3H), 4.56-4.60 (m, 1H), 5.09 (t, 1H, *J* = 1.7 Hz), 6.05 (s, 1H), 6.60 (d, 1H, *J* = 7.46 Hz), 7.17 (br s, 1H), 7.20-7.39 (m, 15H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.5, 33.8, 51.8, 53.1, 67.3, 80.8, 98.9, 127.2, 128.3, 129.7, 134.5, 144.4, 152.9, 163.8, 170.7. FAB-LRMS (neg. ion) for $(M\text{-}Trt)^-$ *m/z* 303.1.

Fmoc-TrpSec(Ph)-ODPM. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.05 (dd, 1H, *J* = 14.3 Hz, 7.8 Hz), 3.22 (m, 2H), 3.30 (dd, 1H, *J* = 13.1 Hz, 4.8 Hz), 4.16-4.23 (m, 1H), 4.30 (br s, 1H), 4.35-4.50 (m, 2H), 4.95-5.00 (m, 1H), 5.22 (br d, 1H, *J* = 6.7 Hz), 6.75-7.80 (m, 28H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.74, 29.59, 47.32, 53.25, 55.34, 67.05, 78.76, 111.41, 119.03, 120.04, 120.18, 120.21, 122.56, 122.66, 123.54, 125.27, 125.32, 125.33, 125.34, 127.21, 127.23, 127.29, 127.34, 127.35, 127.40, 127.79, 127.92, 127.95, 128.41, 128.47, 128.80, 128.85, 129.37, 133.54, 136.39, 136.42, 139.42, 139.64, 141.53, 144.01, 169.17, 169.25, 170.92; FAB-HRMS *m/z* calcd for $C_{48}H_{42}N_3O_5{}^{80}Se$ $(M+1)^+$ 820.2289, found 820.2290.

Fmoc-TrpDha-ODPM (64 % yield) ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.21 (dd, 1H, *J* = 13.8 Hz, 6.9 Hz), 3.39 (br d, 1H, *J* = 13.8 Hz), 4.18 (t, 1H, 6.9 Hz), 4.38 (quin, 2H, *J* = 8.0 Hz), 4.58 (br s, 1H), 5.43 (br s, 1H), 6.07 (d, 1H, *J* = 1.2 Hz), 6.68 (s, 1H), 6.83 (s, 1H), 6.96 (s, 1H), 7.11 (t, 1H, *J* = 7.4 Hz), 7.18 (t, 1H, *J* = 7.4 Hz), 7.25-7.42 (m, 13H), 7.51(t, 2H, 9.3 Hz), 7.64 (s, 1H), 7.75 (d, 2H, *J* = 7.5 Hz), 7.87 (s, 1H), 8.03 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 31.10, 47.30, 56.58, 64.37, 67.49, 78.95, 109.96, 111.54, 118.83, 120.19, 120.21, 120.25, 122.72, 123.30, 125.37, 127.25, 127.27, 127.34, 127.95, 128.51, 128.88, 130.96, 136.46, 139.54, 139.67, 141.52, 141.54, 143.90, 144.01, 160.94, 162.89, 164.03, 169.22, 170.46; FAB-HRMS *m/z* calcd for $C_{42}H_{36}N_3O_5$ $(M+1)^+$ 662.2657, found 662.2655.

Peptide Synthesis. Peptides were synthesized in an automated peptide synthesizer using standard Fmoc protocols.[4] Resins (generally 0.6 mmol/g loading capacity) were swollen in DMF (3 x 6 mL, 10 min). Fmoc groups were removed with 20 % v/v piperidine (2 x 6 mL, 7 min). After deprotection, the resins were rinsed with DMF (6 x 6 mL, 30 s). Amino acids were activated by reaction with *O*-benzotriazole-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HBTU) and 0.4 M *N*-methylmorpholine (3 mL, 30 s). The activated solutions were transferred into the reaction vessel. The amino acid cartridges were rinsed with DMF (3 mL, 30 s) and this solution was also transferred to the reaction vessel. Coupling of the amino acids in general proceeded for 30 min or until complete as determined by Kaiser test.[5] For every coupling, four equivalents of each amino acid and HBTU with respect to the resin loading capacity were used. Following each coupling, the resins were rinsed with DMF (3 x 6 mL, 30 s). After the final coupling for each peptide, the *N*-terminal Fmoc group was left on the *N*-terminus, cleaved and replaced with an acetate group using acetic anhydride, or simply deprotected to yield the free amino terminus.

Peptide Cleavage[4] The resin was isolated by filtration, washed with ethanol (1 mL) and CH_2Cl_2 (2 x 1 mL), and dried in vacuo in a desiccator for 1 h. In general the peptides were cleaved with water (0.5 mL), anisole (0.5 mL), thioanisole (0.5 mL), and TFA (10 mL). When cleavage of the Trt-group from Cys was desired, triisopropylsilane was added to the cocktail. The cleavage reactions were stirred at rt for approximately 2 h. The solution was filtered through a coarse frit into a 250 mL round bottom flask and the vial was rinsed with TFA (4 x 1 mL). TFA was removed by rotary evaporation at 30 °C, and diethyl ether (10 mL) was added to precipitate the peptide. The resulting white residue was recovered by filtration. The peptides were analyzed and purified by RP-HPLC on Vydac C-18 columns (0.46 cm x 25 cm analytical, 2.2 cm x 25 cm preparative). They were eluted in 0.1% TFA in acetonitrile/water with linear gradients optimized for each peptide with a flow rate of 1 mL/min on the analytical column and 8 mL/min on the preparative column.

Peptide Oxidation. The purified peptides were dissolved in acetonitrile/water. An aqueous solution of $NaIO_4$ was added, and the reaction was stirred at rt until complete as determined by RP-HPLC. The reaction mixtures were purified by preparative RP-HPLC.

Reduction of Disulfides and Cyclization Reactions to Form Lanthionines. The dehydropeptides were dissolved in acetonitrile/20 mM aqueous NH_4Oac, pH 4.4. A solution of tris(carboxyethyl)phosphine, prepared using the procedure of Whitesides *et al*,[6] in 20 mM

NH_4Oac, pH 4.4, was added and the reactions were stirred at rt until complete as determined by RP-HPLC. Following the disulfide cleavage, the pH of the reaction was adjusted to ~ 8 by addition of NEt_3 and the cyclization was complete within 10 min at rt. The lanthionines were purified by RP-HPLC as described above for the peptides.

Characterization of peptides by MALDI-MS:

All masses are M+1, calculations are for M+1 (Unless otherwise noted)**.** Yields are given in parentheses.

FmocGLPU(Ph)VIA M+Na calc. 1040.1 ; found 1040.4 (44 %)

FmocGLPDhaVIA M+Na calc. 883.0 ; found 882.4 (72 %)

FmocISVU(Ph)RSTS calc. 1198.2 ; found 1198.3 (27 %)

FmocISVDhaRSTS calc. 1041.2 ; found 1041.6 (67 %)

AcGLPU(Ph)VIA M+Na calc. 860.8 ; found 860.4 (40 %)

AcGLPDhaVIA M+Na calc. 701.8 ; found 702.2 (82 %)

AcISVU(Ph)RSTS calc. 1018.9 ; found 1018.9 (34 %)

AcISVDhaRSTS calc. 860.9 ; found 860.8 (67 %)

AcGGS(S*t*Bu)PU(Ph)VIA M+Na calc. 995.8 ; found 995.6 (33 %)

AcGGS(S*t*Bu)PDhaVIA calc. 815.8 ; found 815.6 (84 %)

LU(Ph)PGC(Trt)VG M+Na calc. 1036.0 ; found 1036.1 (15 %)

LDhaPGC(Trt)VG M+Na calc. 878.4 ; found 878.4 (80 %)

Cyclic(LDhaPGCVG) calc. 614.4 ; found 614.6 (56 %)

$(LU(Ph)ANCKI)_2$ calc. 1773.7 ; found 1772.90 (30 %)

$(LDhaANCKI)_2$ calc. 1457.8 ; found 1457.5 (33 %)

Cyclic(LDhaANCKI) calc. 730.4 ; found 730.7 (75%)

RIAU(Ph)IALC(S*t*Bu)K calc. 1202.6 ; found 1201.9 (47 %)

RIADhaIALC(S*t*Bu)K calc. 1044.6 ; found 1044.5 (72 %)

References Suppl. Material

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