Supplementary Information

Convergent Synthesis of Peptide Conjugates using Dehydroalanines for Chemoselective Ligations.

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General. Protected amino acids were purchased from either Advanced ChemTech or Chem-Impex. Wang resins pre-loaded with the *C*-terminal Fmoc-protected amino acid were obtained from Advanced ChemTech. MeOH was purified via treatment with Mg and I_2 followed by distillation, THF was distilled from sodium-benzophenone, and CH_2Cl_2 was distilled from CaH_2 before use. Dimethylallyl-, geranyl- and farnesylthioacetates were synthesized via literature procedures.[1] The preparation of *N*-(9-fluorenylmethoxycarbonyl)-L-β-phenylselenocysteine has been published previously.[2] 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 (125 solvent module and 166 detector) with a Vydac C18 analytical or preparative column, monitoring at 220 nm. Solution A was 100% MeCN containing 0.1% TFA, solution B was 0.1% TFA in water. ^{1}H and ^{13}C NMR data were obtained on either a Varian U400 or U500 spectrophotometer in $CDCl_3$. Optical rotation was recorded on a JASCO DIP-360 Digital Polarimeter. Melting points were measured on a Fisher melting apparatus without correction. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum BX spectrophotometer. 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.

Synthesis of Di-& Tripeptides. Di- & tripeptides were prepared via standard solution phase Fmoc chemistry.[3] They were purified by silica gel flash chromatography.

Fmoc-Sec(Ph)-Gly-OtBu R_f = 0.29 (CH_2Cl_2 : MeOH = 50:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.48 (s, 3 H), 3.27-3.47 (m, 2 H), 3.76-3.93 (m, 2 H), 4.19-4.23 (t, 1 H, J = 6.97 Hz), 4.37-4.41 (m, 2 H), 4.52 (m, 1 H), 5.84 (d, 1 H, J = 7.88 Hz), 6.81 (br s, 1 H, N–H), 7.26–7.79 (m, 13 H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 28.29, 30.54, 133.31, 141.53, 143.93, 156.18, 168.69, 170.30; M.p. 58-60 °C; $[\alpha]_D^{27}$ = -21.61 ($CHCl_3$, 2.96 w/v %); IR ($CHCl_3$) 3308, 3066, 2978, 1735, 1663, 1529, 1478, 1450, 13678, 1249, 1226 and 1156 cm^{-1}. FAB-HRMS *m/z* calc'd for $C_{30}H_{33}N_2O_5{}^{80}Se$ $(M+H)^+$ 581.1555, found 581.1556.

Ac-Gly-Sec(Ph)-Gly-OtBu R_f = 0.23 (CH_2Cl_2 : MeOH = 20:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.46 (s, 9 H), 2.03 (s, 3 H), 3.24-3.32 (m, 2 H), 3.78 (A of ABX, 1 H, J_{AB} = 18.00 Hz, J_{AX} = 5.33 Hz), 3.87 (B of ABX, 1 H, J_{AB} = 18.00 Hz, J_{BX} = 5.08 Hz), 3.89 (d, 2 H, J = 4.84 Hz), 4.86 (m, 1 H), 6.87 (t, 1 H, J = 5.1 Hz), 7.24-7.26 (m, 3 H), 7.32 (t, 1 H, J = 5.09 Hz), 7.44 (d, 1 H, J = 8.11 Hz), 7.51-7.53 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 23.1, 28.3, 29.9, 42.4, 43.4, 53.4, 82.5, 127.6, 129.5, 129.6, 133.2, 168.7, 169.3, 170.3, 171.2; M.p. 155-156 °C; $[\alpha]_D^{27}$ = -19.86 ($CHCl_3$, 2.61 w/v %); IR ($CHCl_3$) 3293, 3073, 2979, 2933, 1743, 1650, 1534, 1368, 1225 and 1156 cm^{-1}. FAB-HRMS *m/z* cacld for $C_{19}H_{28}N_3O_5{}^{80}Se$ $(M+H)^+$ 458.1194, found 458.1194.

Preparation of Ac-Gly-Dha-Gly-OtBu (4). Ac-Gly-Sec(Ph)-Gly-OtBu (58 mg, 0.10 mmol) was dissolved in 2 mL of 1:1 CH_3CN/H_2O. A solution of 30 % H_2O_2 in water (24 μL) was

added to the reaction, and the mixture was stirred at rt until the reaction was completed as determined by TLC (30 min). The reaction mixture was then diluted with EtOAc and washed with water, saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The resulting yellow oil was purified by silica gel flash chromatography with 30:1 of CH_2Cl_2 : MeOH (R_f = 0.10) to give 34 mg (88 %) of the product **4**. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.48 (s, 9 H), 2.08 (s, 3H), 4.00 (d, 2 H, *J* = 5.28 Hz), 4.04 (d, 2 H, *J* = 4.31 Hz), 5.39 (s, 1 H), 6.45 (d, 1 H, *J* = 1.42 Hz), 6.48 (t, 1 H, *J* = 4.87 Hz), 6.89 (t, 1 H, *J* = 4.37 Hz), 8.49 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 23.16, 28.24, 42.65, 44.12, 83.18, 103.18, 133.75, 163.85, 168.10, 169.01, 170.94; M.p. 61-63 °C; IR ($CHCl_3$) 3307, 2976, 2928, 1740, 1655, 1630, 1509, 1368, 1227 and 1156 cm^{-1}; FAB-HRMS *m/z* calc'd for $C_{13}H_{22}N_3O_5$ $(M+H)^+$ 300.1559, found 300.1559.

Preparation of FarSTIPS. Farnesyl bromide (95 mg, 0.33 mmol) was added to a solution of sodium triisopropylsilanethiolate[4] (71 mg, 0.33 mmol) in 1 mL of THF at -78 °C. The reaction was then warmed to rt and stirred for 5 h. Pentane was added to the mixture, and the solution was washed with water. The pentane phase was dried over $MgSO_4$, and the product was purified by flash column chromatography eluting with hexane (80 mg, 62 %, R_f = 0.37). ^{1}H NMR (500 MHz) δ ppm 1.14 (s, 9 H), 1.15 (s, 9 H), 1.25-1.31 (m, 5 H), 1.68 (s, 3 H), 1.70 (s, 3 H), 1.96-2.15 (m, 8 H), 3.20 (d, 2 H, *J* = 7.61 Hz), 5.12 (q, 2 H, *J* = 6.4 Hz), 5.34 (t, 1 H, *J* = 7.7 Hz). ^{13}C NMR (125 MHz) δ ppm 12.9, 16.2, 17.9, 18.8, 23.6, 25.9, 26.6, 26.9, 39.8, 39.9, 123.0, 124.2, 124.6, 131.5, 135.4, 137.95; IR ($CHCl_3$): 2967, 2918, 2855, 1694, 1445, 1382, 1352, 1231, 1133, and 1107 cm^{-1}; FAB-HRMS *m/z* calc'd for $C_{24}H_{45}SiS$ (M-H) 393.3011, found 393.3009.

GerGerSTIPS was synthesized similarly to the preparation of FarSTIPS from geranylgeranyl bromide. R_f = 0.31 in hexane. ^{1}H NMR (400 MHz) δ ppm 1.12 (d, 18 H, *J* = 4.14 Hz), 1.22-1.30 (m, 3 H), 1.59 (s, 9 H), 1.66 (s, 3 H), 1.67 (s, 3 H), 1.96-2.10 (m, 12 H), 3.17 (d, 2 H, *J* = 7.78 Hz), 5.07-5.12 (m, 3 H), 5.31 (t, 1 H, *J* = 7.2 Hz). ^{13}C NMR (100 MHz) δ ppm 12.94, 16.25, 16.26, 17.95, 18.86, 23.62, 25.96, 26.61, 26.89, 27.01, 39.81, 39.94, 39.97, 123.00, 124.19, 124.50, 124.65, 131.54, 135.19, 135.49, 137.91; IR ($CHCl_3$): 2956, 2942, 2925, 2866, 1664, 1462, 1382 and 882 cm^{-1}; FAB-HRMS *m/z* calc'd for $C_{29}H_{53}SiS$ (M-H) 461.3637, found 461.3637.

Solid Phase Peptide Synthesis. Peptides were synthesized on an automated peptide synthesizer using standard Fmoc protocols.[5] 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 the Kaiser test.[6] For every coupling, four equivalents of each amino acid and HBTU with respect to the resin loading capacity were used. Following each coupling, the resin was rinsed with DMF (3 x 6 mL, 30 s). *N*-Acetylamino acids were used in the last couplings to give *N*-terminal acetamides.

Peptide Cleavage[5] Peptides were cleaved from the solid phase after isolation of the resin by filtration, washing with ethanol (1 mL) and CH_2Cl_2 (2 x 1 mL), and drying in vacuo in a desiccator for 1 h. In general peptides were cleaved with water (0.5 mL), anisole (0.5 mL), thioanisole (0.5 mL), and TFA (10 mL). 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 Et_2O (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 an analytical column and 8 mL/min on a preperative column. AcGLPU(Ph)VIA (**1**) was obtained in 40 % yield after purification. MALDI-LRMS for (M + Na) calc'd. 860.8 ; found 860.4. AcISVU(Ph)RSTS (**4**) was synthesized in 34 % yield. MALDI-LRMS for (M + H) calc'd. 1018.9 ; found 1018.9

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. AcGLPDhaVIA (**2**) was obtained from AcGLPU(Ph)VIA (**1**) in 82 % yield. MALDI-LRMS *m/z* for (M + Na) calc'd. 701.8; found 702.2. AcISVU(Ph)RSTS (**4**) afforded AcISVDhaRSTS (**5**) in 82 % yield. MALDI-LRMS *m/z* for (M + H) calc'd. 860.9; found 860.8.

Preparation of AcGLPC(Far)VIA (3) from FarSAc. Farnesyl thioacetate (FarSAc) (28 mg, 0.10 mmol) was dissolved in 1 mL of dry MeOH under Ar. A solution of NaOMe in MeOH (0.10 M, 1 mL) was added, followed by a solution of AcGLPDhaVIA (**2**) (10 mg, 0.015 mmol) in 0.5 mL of dry MeOH. The reaction was stirred at rt and was complete in 140 min as shown by HPLC. The reaction was quenched with 2 N HCl, and extracted with EtOAc. The organic layer was dried and the solvent was removed by rotary evaporation. The solid residue was purified by HPLC to give 8.5 mg (63 %) of AcGLPC(Far)VIA (**3**). MALDI-LRMS *m/z* calc'd for (M+Na) 941.2, found 941.0.

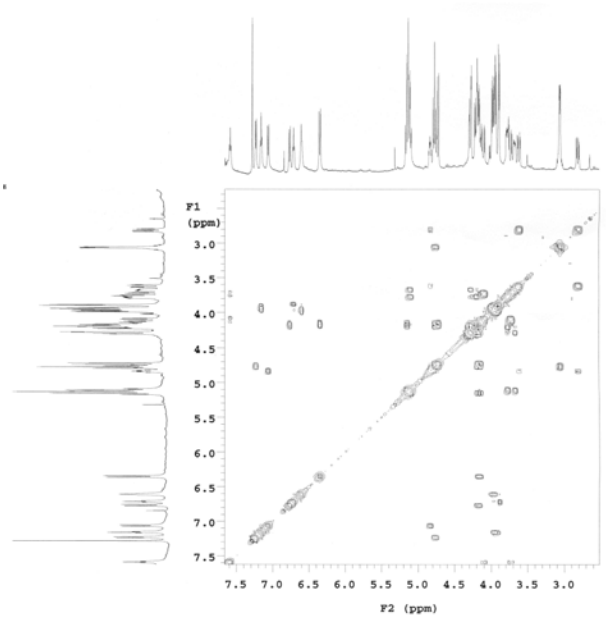
Ac-GLPC(Dimethylallyl)VIA-OH was obtained similarly in 69 % yield, FAB-HRMS *m/z* calc'd for $C_{37}H_{63}N_7O_9NaS$ (M + Na) 804.4306, found 804.4306; **Ac-GLPC(Ger)VIA-OH** was obtained in 72% yield, FAB-HRMS *m/z* calc'd for $C_{42}H_{72}N_7O_9NaS$ (M + Na) 850.5112, found 850.5112.

Preparation of AcGLPC(Far)VIA from FarSTIPS. FarSTIPS (8.7 mg, 0.022 mmol), CsF (13.4 mg, 0.088 mmol), AcGLPDhaVIA (3.7 mg, 0.0054 mmol) and DMF were separately purged with Ar for 30 min. DMF (0.5 mL) was added to FarSTIPS and the peptide. Both solutions were transferred into the flask containing CsF. The reaction was stirred, and the reaction was complete in 120 min as shown by HPLC. HPLC purification afforded 3.4 mg of AcGLPC(Far)VIA (65 % yield).

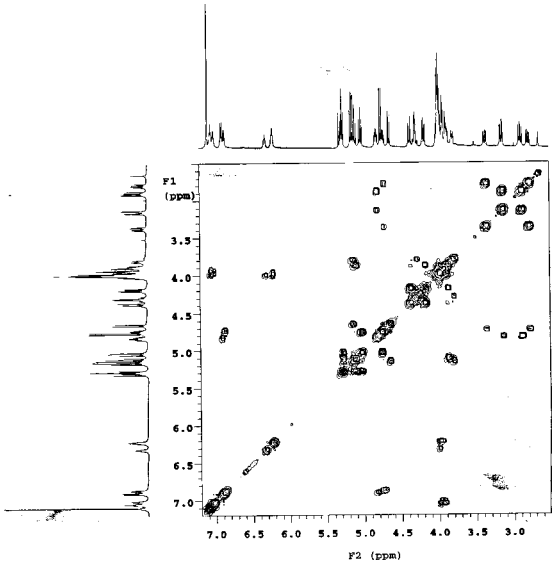
Ac-GLPC(GerGer)VIA-OH was obtained similarly in 76 % yield. FAB-HRMS *m/z* calc'd for $C_{52}H_{87}N_7O_9NaS$ $(M + Na)^+$ 1008.6184, found 1008.6182.

Preparation of Ac-GLPC(Ac)VIA-OH. Potassium thioacetate (AcSK) (3 mg, 0.026 mmol) and AcGLPDhaVIA (3 mg, 0.0044 mmol) were suspended in 1 mL of MeOH. The mixture was stirred at rt for 4 d affording the Michael adduct in 67 % yield after HPLC purification. FAB-HRMS *m/z* calc'd for $C_{34}H_{57}N_7O_{10}NaS$ (M + Na) 778.3785, found 778.3785.

Preparation of glycopeptides. NaOMe in MeOH (0.13 M, 0.45 mmol) was transferred to the thioglycoside (0.058 mmol) under Ar. A solution of the dehydropeptide (0.017 mmol) in 0.5 mL of MeOH was then cannulated into the reaction mixture. The reaction was stirred at rt and followed by HPLC or TLC. Purification by HPLC gave peptide **6** in 75 % yield. FAB-LRMS *m/z* calc'd for $C_{49}H_{82}N_{12}O_{22}NaS$ $(M + Na)^+$ 1245.2, found 1245.2. Similar procedures provided the products in entries 1 & 2, Table 2. After purification by flash chromatography the peptides in entries 1 & 2 were both obtained in 62 % yields. Entry 1: R_f = 0.20 (CH_2Cl_2 : MeOH = 10:1); FAB-HRMS *m/z* calc'd for $C_{27}H_{42}N_4O_{13}NaS$ $(M + Na)^+$ 685.2367, found 685.2368. Entry 2: R_f = 0.34 (CH_2Cl_2 : MeOH = 20:1); FAB-HRMS *m/z* calc'd for $C_{27}H_{41}N_3O_{14}NaS$ $(M + Na)^+$ 686.2207, found 686.2204.



COSY of the product in entry 1, Table 2



COSY of the product in entry 2, Table 2

Preparation of Ac-Gly-Cys(β-glucosyl)-Gly-OtBu. The sodium salt of 1-thio-β-glucosylpyranose (24 mg, 0.11 mmol) and Ac-Gly-Dha-Gly-OtBu (**7**) (6.6 mg, 0.022 mmol) were dissolved in 0.5 mL of water and 0.2 mL of CH_3CN under Ar. The mixture was stirred at rt for 4 h and purified by HPLC to give 8.0 mg of the glycopeptide product (74 %) of entry 3, Table 2. FAB-HRMS *m/z* calc'd for $C_{19}H_{33}N_3O_{10}NaS$ $(M + Na)^+$ 518.1784, found 518.1785.

Preparation of Ac-Gly-Ala-Cys(β-glucosyl)-Ala-Ser-Thr-Ser-OH (10) on solid phase. Ac-Gly-Ala-Sec-Ala-Ser(OtBu)-Thr(OtBu)-Ser(OtBu)-Wang resin **9** (0.58 g) was obtained by solid phase synthesis following the general procedures described above. To ascertain the identity of the peptide on the resin, a few beads (10 mg) were treated with 3 mL of TFA/CH_2Cl_2 (95/5) and 3 drops of triisopropylsilane (TIS) for 1 h. Removal of TFA by rotary evaporation afforded an

oily residue, which changed into a white solid upon the addition of Et_2O. More ether (3×1 mL) was used to wash the peptide. MALDI-LRMS *m/z* calc'd for $C_{29}H_{43}N_7O_{12}SeNa$ $(M + Na)^+$ 784.2, found 783.9. A clean HPLC trace further confirmed that the vast majority of the peptide on the resin corresponded to the desired product.

The resin loaded with Ac-Gly-Ala-Sec-Ala-Ser(tBu)-Thr(tBu)-Ser(tBu) (0.25 g) was swollen in DMF (7 mL) for 0.5 h, and H_2O_2 (30 % aq., 71 μL) was added. To achieve proper mixing N_2 was periodically purged through the reaction mixture over a period of 2 h. The resin was washed with DMF, MeOH and CH_2Cl_2, and dried under vacuum. A few beads (3 mg) were cleaved in 3 mL of TFA/CH_2Cl_2 (95/5) with 3 drops of TIS for 1 h. Removal of TFA on a rotary evaporator afforded an oily residue, which changed into white solid upon the addition of Et_2O. Additional ether (3×1 mL) was used to wash the peptide, which was analyzed by MALDI-LRMS: *m/z* calc'd for $C_{23}H_{37}N_7O_{12}Na$ $(M + Na)^+$ 626.2, found 626.1. An analytical HPLC trace of the crude cleavage mixture indicated that the dehydropeptide constituted the main product. The sodium salt of 1-thio-β-glucosylpyranose (50 mg, 0.23 mmol, 10 equiv. based on resin loading) was passed through a cation exchange column that had been equilibrated to the H^+-form, and the eluent was collected into 0.1 M aqueous NEt_3. The solution was lyophilized to give a white foam of the triethylammonium salt. The foam was dissolved in 1 mL of DMF, and the solution was transferred to 40 mg of Wang resin loaded with Ac-Gly-Ala-ΔAla-Ala-Ser(tBu)-Thr(tBu)-Ser(tBu). The mixture was stirred for 2 d under argon. The resin was filtered and washed with DMF, MeOH, and CH_2Cl_2. After drying the resin the peptide was cleaved off with 10 mL of 95/3/2 TFA/CH_2Cl_2/TIS. Removal of TFA by rotary evaporation afforded an oily residue, which changed into white solid upon the addition of ether. Additional ether (3×3 mL) was used to wash the peptide. The peptide was purified by RP-HPLC to provide 8 mg (overall 45 %) of the desired peptide. MALDI-LRMS *m/z* calc'd for $C_{29}H_{49}N_7O_{17}SNa$ $(M + Na)^+$ 822.2, found 821.9.

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