Supporting Information

Tan et al. 10.1073/pnas.1100195108

SI Materials and Methods.

All commercial materials (Aldrich, Fluka, EMD Biosciences, Chem-Impex International) were used without further purification. All solvents were reagent grade or HPLC grade (Fisher). Anhydrous THF, diethyl ether, CH_2Cl_2, toluene, and benzene were obtained from a dry solvent system (passed through column of alumina) and used without further drying. All reactions were performed under an atmosphere of prepurified dry Ar(g). NMR spectra (^1H and ^{13}C) were recorded on a Bruker Advance II 600 MHz or Bruker Advance DRX-500 MHz, referenced to tetramethylsilane or residual solvent. Low-resolution mass spectral analyses were performed with a JOEL JMS-DX-303-HF mass spectrometer or Waters Micromass ZQ mass spectrometer. Analytical TLC was performed on E. Merck silica gel 60 F254 plates, and flash column chromatography was performed on E. Merck silica gel 60 (40–63 mm). Yields refer to chromatographically pure compounds.

Liquid chromatography and mass spectrometry (LC-MS) and HPLC: All separations involved a mobile phase of 0.05% TFA (vol/vol) in water (solvent A)/0.04% TFA in acetonitrile (solvent B). LC-MS analyses were performed using a Waters 2695 Separations Module and a Waters 996 Photodiode Array Detector equipped with Varian Microsorb 300-5, C4 250×2.0 mm columns at a flow rate of 0.2 mL/ min. Preparative separations were performed using a Ranin HPLC solvent delivery system equipped with a Rainin UV-1 detector and Varian Dynamax using Varian Microsorb 300-5, C4 250×21.4 mm columns at a flow rate of 16.0 mL/ min.

Solid Phase Peptide Synthesis.

Automated peptide synthesis was performed on an Applied Biosystems Pioneer continuous flow peptide synthesizer. Peptides were synthesized under standard automated Fmoc protocols. The deblock solution was a mixture of 100/5/5 of dimethylformamide/piperidine/1,8-Diazabicyclo[5.4.0]undec-7-ene. The peptide cleavage solution was a mixture of 95/2.5/2.5 of TFA/triisopropyl silane (TIS)/H_2O. The following Fmoc amino acids from EMD Biosciences were employed: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Met-OH, Fmoc-Phe-OH, Fmoc-Leu-Ser(psiMe,Mepro)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Val-OH.

Preparation and Characterization of Compounds 7–19

Fmoc-Glu(OtBu)-OFm (10). In a 50-mL round-bottom flask, Fmoc-Glu(OtBu)-OH **9** (4.0 g, 9.4 mmol, 1.0 equiv), Fm-OH (1.5 g, 7.8 mmol, 0.83 equiv), and DMAP (115 mg, 0.94 mmol, 0.10 equiv) were dissolved in CH_2Cl_2 (24 mL). The solution was cooled to 0 °C. N,N′-Dicyclohexylcarbodiimide (DCC) (2.1 g, 10.3 mmol, 1.1 equiv) was added at 0 °C. The mixture was stirred at room temperature (RT) for 20 min. The reaction mixture was filtered under reduced pressure, and the residue was washed with 20 mL of cold CH_2Cl_2. The filtrate was concentrated in vacuo. The crude product was purified by silica flash chromatography (4:1 hexanes/EtOAc) to yield **10** (4.6 g, 99%) as a white solid.

TLC: R_f 0.26 (4:1 hexanes/EtOAc). **^{1}H-NMR** ($CDCl_3$, 500 MHz): δ 7.78 (m, 4H), 7.63 (m, 4H), 7.42 (m, 4H), 7.36 (m, 4H), 5.52 (d, 1H, $J = 8.0$), 4.55–4.41 (m, 5H), 4.27 (m, 2H), 2.25 (m, 2H), 2.15 (m, 1H), 1.93 (m, 1H), 1.49 (s, 9H). **^{13}C-NMR** ($CDCl_3$, 600 MHz) δ 172.03, 171.97, 155.98, 143.92, 143.74, 143.50, 143.32, 141.37, 141.34, 141.32, 127.95, 127.93, 127.74, 127.24, 127.11, 125.15, 125.13, 124.98, 120.12, 120.10, 120.01, 120.00, 80.89, 67.22, 67.13, 53.63, 47.18, 46.80, 31.33, 28.12, 27.38. **Electrospray ionization (ESI)-MS** m/z calculated for $C_{38}H_{37}NNaO_6$ $[M + Na]^+$: 626.25, found 626.3.

Fmoc-Glu(OCH$_2$CH = CHCH$_2$CH$_2$CO$_2$tBu)-OFm (11). In a 100-mL round-bottom flask, compound **10** (4.6 g, 7.8 mmol, 1.0 equiv) was dissolved in 1:1 (vol/vol) TFA/CH_2Cl_2 (44 mL). After stirring at the ambient temperature for 30 min, the solvent was removed by nitrogen stream. The residue was coevaporated with toluene (2×20 mL) to give 4.1 g of crude product. The deprotected product was used in the next step without further purification.

In a 100-mL round-bottom flask, Bu_4NBr (322 mg, 1.0 mmol, 1.0 equiv) was dissolved in saturated $NaHCO_3$ (16 mL). The crude material from the previous step (547 mg, ∼1.0 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (16 mL) and THF (8 mL) and was added dropwise to the aqueous solution of Bu_4NBr and $NaHCO_3$ in 30 min. The mixture was stirred at room temperature for 7.5 h. CH_2Cl_2 (150 mL) was added and the mixture was washed with H_2O (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. Purification by silica flash chromatography (3:1 hexanes/EtOAc) yielded **11** (442 mg, 60%) as a white solid.

TLC: R_f 0.24 (3:1 hexanes/EtOAc). **^{1}H-NMR** ($CDCl_3$, 500 MHz): δ 7.78 (m, 4H), 7.62 (m, 4H), 7.42 (m, 4H), 7.35 (m, 4H), 5.79 (m, 1H), 5.64 (m, 1H), 5.47 (m, 1H), 4.54 (m, 4H), 4.43 (m, 3H), 4.26 (m, 2H), 2.35 (m, 6H), 2.16 (m, 1H), 1.94 (m, 1H), 1.47 (m, 9H). **^{13}C-NMR** ($CDCl_3$, 600 MHz) δ 172.37, 172.14, 171.79, 155.91, 143.88, 143.70, 143.44, 143.25, 141.38, 141.34, 141.32, 134.50, 127.97, 127.95, 127.76, 127.26, 127.25, 127.11, 125.11, 124.94, 124.67, 120.14, 120.12, 120.02, 120.01, 80.40, 67.23, 67.15, 65.28, 53.43, 47.17, 46.79, 34.68, 30.03, 28.13, 27.68, 27.41. **ESI-MS** m/z calculated for $C_{44}H_{45}NNaO_8$ $[M + Na]^+$: 738.30, found 738.4.

Fmoc-Glu(OCH$_2$CH = CHCH$_2$CH$_2$CO$_2$H)-OFm (12). In a 10-mL round-bottom flask, compound **11** (440 mg, 0.62 mmol, 1.0 equiv) was mixed with 1:1 (vol/vol) TFA/CH_2Cl_2 (12 mL) and stirred for 35 min. The reaction mixture was then blown down under nitrogen to an oily residue. After the coevaporation with toluene (2×20 mL), the reaction mixture was purified by silica-gel chromatography (3:1 hexanes/EtOAc + 10% AcOH) to provide **12** (350 mg, 86%) as a white foam.

TLC: R_f 0.33 (3:1 hexanes/EtOAc + 10% AcOH). **^{1}H-NMR** ($CDCl_3$, 500 MHz): δ 7.68 (m, 4H), 7.50 (m, 4H), 7.34 (m, 4H), 7.25 (m, 4H), 5.69 (m, 1H), 5.57 (m, 1H), 5.40 (m, 1H), 4.47 (m, 4H), 4.36 (m, 3H), 4.16 (m, 2H), 2.37–2.16 (m, 6H), 2.07 (m, 1H), 1.83 (m, 1H). **^{13}C-NMR** ($CDCl_3$, 600 MHz) δ 177.32, 172.31, 171.90, 156.03, 143.85, 143.68, 143.44, 143.24, 141.38, 141.34, 133.80, 129.06, 128.25, 127.96, 127.95, 127.76, 127.25, 127.12, 125.24, 125.11, 124.96, 120.13, 120.11, 120.03, 67.27, 67.21, 65.07, 53.39, 47.14, 46.78, 33.07, 29.99, 27.42, 27.19. **ESI-MS** m/z calculated for $C_{40}H_{37}NNaO_8$ $[M + Na]^+$: 682.24, found 682.5.

Fmoc-Glu[OCH$_2$CH = CHCH$_2$CH$_2$C(= O)-Arg(Pbf)-OtBu]-OFm (13). In a 10-mL round-bottom flask, compound **12** (330 mg, 0.50 mmol, 1.0 equiv), H-Arg(Pbf)-OtBu (290 mg, 0.60 mmol, 1.2 equiv), and DMAP (6.0 mg, 0.050 mmol, 0.1 equiv) were dissolved in CH_2Cl_2 (1.3 mL) and cooled to 0 °C. DCC (114 mg, 0.55 mmol, 1.1 equiv) was then added in one portion and the reaction mixture

was stirred at room temperature for 30 min. The precipitate was filtered off and the solvent was removed. The residue was purified by silica-gel chromatography (hexanes/EtOAc, 1:2 and 1:5) to give **13** (416 mg, 74%) as a white solid.

TLC: R_f 0.31 (1:5 hexanes/EtOAc). **^{1}H-NMR** (CD_3Cl_3, 500 MHz): δ 7.77 (m, 4H), 7.60 (m, 4H), 7.41 (m, 4H), 7.35 (m, 4H), 6.47 (m, 1H), 6.13 (m, 3H), 5.70 (m, 1H), 5.61 (m, 2H), 4.53 (m, 4H), 4.44 (m, 4H), 4.22 (m, 2H), 3.35 (m, 1H), 3.20 (m, 1H), 2.95 (s, 2H), 2.61 (s, 3H), 2.55 (s, 3H), 2.34 (m, 6H), 2.14 (m, 1H), 2.10 (s, 3H), 1.92 (m, 1H), 1.80 (m, 1H), 1.56 (m, 3H), 1.46 (m, 16H). **^{13}C-NMR** ($CDCl_3$, 600 MHz) δ 172.49, 171.84, 171.35, 158.66, 156.09, 143.84, 143.66, 143.43, 143.24, 141.37, 141.32, 141.31, 138.37, 133.67, 133.20, 132.33, 127.96, 127.77, 127.27, 127.24, 127.13, 125.14, 125.11, 124.95, 124.55, 120.12, 120.11, 120.02, 117.41, 86.32, 82.69, 67.24, 67.19, 65.00, 53.37, 47.12, 46.78, 43.25, 40.69, 35.44, 30.80, 30.00, 28.60, 27.98, 27.36, 25.02, 19.28, 17.90, 12.48. **ESI-MS** *m/z* calculated for $C_{63}H_{73}N_5NaO_{12}S$ $[M + Na]^+$: 1,146.49, found 1,146.7.

Fmoc-Glu[OCH$_2$CH = CHCH$_2$CH$_2$C(= O)-Arg(Pbf)-OtBu]-OH (7). In a 25-mL round-bottom flask, compound **13** (400 mg, 0.36 mmol, 1.0 equiv) was dissolved in 1:4 (vol/vol) piperidine/CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 20 min. The solvent was removed and the residue was coevaporated with toluene (2 × 10 mL). The crude compound was used directly in the next step without further purification.

In a 25-mL round-bottom flask, the residue from the previous step was dissolved in a 1:1 mixture of THF and H_2O (12 mL). To the resulting solution were added $NaHCO_3$ (90 mg, 1.08 mmol, 3.0 equiv) and Fmoc-OSu (243 mg, 0.72 mmol, 2.0 equiv). The reaction mixture was stirred at ambient temperature for 2.5 h. Subsequently, 50 mL of saturated NH_4Cl solution was added and the solution was extracted with CH_2Cl_2 (4 × 25 ml). The organic layers were combined, dried over Na_2SO_4, and concentrated. The residue was purified by silica-gel chromatography (hexanes/EtOAc, 1:1, 1:2, and 1:6 + 10% AcOH) to give **7** (100 mg, 29%) as a white solid.

TLC: R_f 0.38 (1:6 hexanes/EtOAc + 10% AcOH). **^{1}H-NMR** (CD_3OD, 500 MHz): δ 7.80 (d, 2H, $J = 7.5$), 7.69 (t, 2H, $J = 7.7$), 7.40 (t, 2H, $J = 7.4$), 7.32 (t, 2H, $J = 7.4$), 5.78 (m, 1H), 5.59 (m, 1H), 4.50 (m, 2H), 4.37 (m, 2H), 4.24 (t, 2H, $J = 6.9$), 4.17 (m, 1H), 3.19 (m, 2H), 2.99 (s, 2H), 2.59 (s, 3H), 2.52 (s, 3H), 2.43 (m, 2H), 2.30 (m, 4H), 2.20 (m, 1H), 2.10 (s, 3H), 1.92 (m, 1H), 1.79 (m, 1H), 1.62 (m, 1H), 1.54 (m, 2H), 1.47 (m, 16H). **^{13}C-NMR**($CDCl_3$, 600 MHz) δ 176.20, 173.20, 171.59, 158.77, 156.42, 156.10, 143.83, 143.68, 141.28, 137.89, 134.49, 132.42, 129.05, 128.24, 127.12, 125.32, 125.15, 120.00, 86.41, 82.65, 67.20, 65.79, 53.46, 47.10, 43.23, 40.54, 35.23, 30.20, 30.05, 29.94, 28.60, 27.97, 27.95, 27.54, 27.37, 25.08, 19.30, 17.91, 12.50. **ESI-MS** *m/z* calculated for $C_{49}H_{63}N_5NaO_{12}S$ $[M + Na]^+$: 968.41, found 968.5.

Fmoc-Lys(Boc)-OFm (15). In a 50-mL round-bottom flask, Fmoc-Lys (Boc)-OH **14** (5.0 g, 10.6 mmol, 1.0 equiv), Fm-OH (1.7 g, 8.9 mmol, 0.83 equiv), and DMAP (130 mg, 1.1 mmol, 0.10 equiv) were dissolved in CH_2Cl_2 (24 mL). The solution was cooled to 0 °C. DCC (2.1 g, 10.3 mmol, 1.1 equiv) was added at 0 °C. The mixture was stirred at RT for 20 min. The reaction mixture was filtered. The filtrate was concentrated and chromatographed on silica gel (3:1 hexanes/EtOAc) to afford **15** (1.6 g, 28%) as a white solid.

TLC: R_f 0.19 (3:1 hexanes/EtOAc). **^{1}H-NMR** ($CDCl_3$, 500 MHz): δ 7.69 (m, 4H), 7.51 (m, 4H), 7.34 (m, 4H), 7.23 (m, 4H), 5.27 (d, 1H, $J = 6.7$), 4.51–4.43 (m, 3H), 4.33–4.27 (m, 3H), 4.16–4.06 (m, 2H), 3.29 (m, 2H), 1.63 (m, 1H), 1.49 (m, 1H), 1.35 (s, 9H), 1.20–1.03 (m, 4H). **^{13}C-NMR** ($CDCl_3$, 600 MHz) δ 172.35, 156.06, 155.95, 143.91, 143.77, 143.53, 143.32, 141.43, 141.37, 141.33, 127.94, 127.73, 127.22, 127.09, 125.12, 124.88, 120.11, 120.08, 120.01, 119.99, 79.95, 67.06, 66.82, 53.79, 47.19, 46.87, 40.01, 29.66, 28.45, 25.64, 22.20. **ESI-MS** *m/z* calculated for $C_{40}H_{42}N_2NaO_6$ $[M + Na]^+$: 669.30, found 669.5.

Fmoc-Lys-OFm (16). In a 100-mL round-bottom flask, compound **15** (1.6 g, 2.8 mmol, 1.0 equiv) was dissolved in 4 M HCl in dioxane (38 mL) and was stirred for 1 h. The solvent was evaporated off by nitrogen gas. The residue was purified by column chromatography on silica gel (1:3 EtOAc/MeOH) to give **16** (700 mg, 50%) as white solid.

TLC: R_f 0.18 (1:3 EtOAc/MeOH). **^{1}H-NMR** (MD_3OD, 500 MHz): δ 7.60 (m, 4H), 7.47 (m, 4H), 7.20 (m, 4H), 7.12 (m, 4H), 4.18 (m, 4H), 4.03 (m, 2H), 3.85 (m, 1H), 2.71 (m, 2H), 1.64 (m, 1H), 1.51 (m, 2H), 1.37 (m, 1H), 1.25 (m, 2H). **^{13}C-NMR** (MD_3OD, 600 MHz) δ 173.75, 158.67, 145.29, 145.17, 144.98, 142.76, 142.64, 129.90, 128.92, 128.83, 128.28, 128.26, 128.20, 128.17, 126.25, 126.21, 126.07, 126.05, 121.01, 120.97, 120.70, 67.99, 67.54, 55.26, 40.49, 31.77, 28.03, 23.75. **ESI-MS** *m/z* calculated for $C_{35}H_{35}N_2O_4$ $[M + H]^+$: 547.26, found 547.5.

***(E)-tert*-butyl 6-((2,5-dioxopyrrolidin-1-yloxy)carbonyloxy)hex-4-enoate (17).** In a 100-mL round-bottom flask, (*E*)-*tert*-butyl 6-bromohex-4-enoate (1.97 g, 7.9 mmol, 1.0 equiv) and Bu_4NOAc (4.79 g, 15.9 mmol, 2.0 equiv) were stirred with acetone (40 mL) for 30 min at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The solvent was evaporated and the residue was pumped overnight. The crude product, (*E*)-*tert*-butyl 6-acetoxyhex-4-enoate, was used in the next step without further purification.

In a 500-mL round-bottom flask, the residue from the previous step was dissolved in 200 of MeOH. To the resulting solution was added K_2CO_3 (2.19 g, 15.9 mmol, 2.0 equiv). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was concentrated and the residue was purified by silica-gel chromatography (hexanes/EtOAc, 5:1) to give (*E*)-*tert*-butyl 6-hydroxyhex-4-enoate (1.21 g, 82%) as an oily substance.

TLC: R_f 0.50 (1:1 hexanes/EtOAc). **^{1}H-NMR** (CD_3OD, 500 MHz): d 5.42 (m, 2H), 3.83 (d, 2H, $J = 3.5$), 2.07 (m, 4H), 1.67 (s, 1H), 1.19 (s, 9H). **^{13}C-NMR** ($CDCl_3$, 600 MHz) δ 172.57, 130.20, 130.14, 80.33, 62.95, 34.94, 27.99, 27.57. **ESI-MS** *m/z* calculated for $C_{10}H_{18}O_3M^+$: 186.13, found 186.2.

In a 100-mL round-bottom flask, (*E*)-*tert*-butyl 6-hydroxyhex-4-enoate (3.80 g, 20.4 mmol, 1.0 equiv) was dissolved in DMSO (50 mL). N,N′-Disuccinimidyl carbonate (DSC, 5.2 g, 20.4 mmol, 1.0 eq) was then added and the mixture was heated at 60 °C for 45 min. After that, another portion of DSC (2.6 g, 10.2 mmol, 0.5 eq) was added and the reaction was continued for another 5 h at 60 °C. The reaction was stopped by the addition of $NaHCO_3$ solution (100 mL). The mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic solution was washed with H_2O (5 × 30 mL) and dried (Na_2SO_4). The solvent was evaporated and the crude product was purified by silica-gel chromatography (hexanes/EtOAc, 3:1 and 2:1) to give **17** (3.99 g, 60%) as a slight yellow oil.

TLC: R_f 0.50 (1:1 hexanes/EtOAc).**^{1}H-NMR** (CD_3OD, 500 MHz): d 5.72 (m, 1H), 5.47 (m, 1H), 4.55 (d, 2H, $J = 6.7$), 2.64 (s, 4H), 2.20–2.13 (m, 4H), 1.26 (s, 9H). **^{13}C-NMR** ($CDCl_3$, 500 MHz) δ 171.96, 168.59, 151.42, 137.37, 122.52, 80.53, 71.56, 34.41, 28.10, 27.61, 25.46. **ESI-MS** *m/z* calculated for $C_{15}H_{21}NNaO_7$ $[M + Na]^+$: 350.12, found 350.3.

Fmoc-Lys[C(= O)OCH$_2$CH = CHCH$_2$CH$_2$C(= O)-OtBu]-OFm (18). In a 100-mL round-bottom flask, compound **16** (2.5 g, 4.6 mmol, 1.5 equiv) and $NaHCO_3$ (0.79 g, 6.1 mmol, 2.0 equiv) were mixed with H_2O (15 mL). The solution of compound **17** (1.0 g, 3.1 mmol, 1.0 equiv) in CH_3CN was added to the flask in one portion. The mixture was sonicated and stirred at room temperature for 1 h.

The organic solvent was evaporated off and the remaining solution was diluted by CH_2Cl_2 (100 mL). The organic phase was washed with water (50 mL) and brine (50 mL) and was dried over sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 2:1 and 1:1) to give **18** (0.91 g, 40%) as a white solid.

TLC: R_f 0.20 (2:1 hexane/EtOAc). **^{1}H-NMR** ($CDCl_3$, 500 MHz): δ 7.69 (m, 4H), 7.51 (m, 4H), 7.31 (m, 4H), 7.23 (m, 4H), 5.63 (m, 1H), 5.51 (m, 1H), 5.29 (d, 1H, $J = 7.6$), 4.61 (m, 1H), 4.52–4.31 (m, 5H), 4.29–4.25 (m, 2H), 4.14 (m, 2H), 3.04 (m, 2H), 2.25–2.20 (m, 4H), 1.63–1.59 (m, 1H), 1.51–1.44 (m, 1H), 1.36 (s, 9H), 1.37–1.10 (m, 4H). **^{13}C-NMR** ($CDCl_3$, 600 MHz) δ 172.33, 172.01, 156.46, 155.98, 143.91, 143.77, 143.52, 143.31, 141.43, 141.36, 141.33, 133.61, 127.94, 127.89, 127.74, 127.22, 127.10, 125.55, 125.12, 124.87, 120.11, 120.08, 120.01, 120.00, 80.52, 80.35, 67.04, 66.81, 65.30, 53.73, 47.19, 46.87, 40.44, 34.76, 31.98, 29.47, 28.13, 27.68, 25.42. **ESI-MS** m/z calculated for $C_{46}H_{50}N_2NaO_8$ $[M + Na]^+$: 781.35, found 781.4.

Fmoc-Lys[C(= O)OCH$_2$CH = CHCH$_2$CH$_2$C(= O)-Arg(Pbf)-OtBu]-OFm (19). In a 50-mL round-bottom flask, compound **18** (0.90 g, 1.2 mmol, 1.0 equiv) was dissolved in 1:1 mixture of TFA (5 mL) and CH_2Cl_2 (5 mL). The reaction solution was stirred at room temperature for 40 min. After that, the organic solvent was blown off in a stream of nitrogen. The residue was coevaporated with toluene (2 × 10 mL) and was used in the next step without further purification.

In a 25-mL round-bottom flask, the compound from the previous step (0.85 g, 1.2 mmol, 1.0 equiv), H-Arg(Pbf)-OtBu (0.71 g, 1.5 mmol, 1.2 equiv) and DMAP (15 mg, 0.12 mmol, 0.10 equiv) were mixed with CH_2Cl_2 (3 mL) and cooled to 0 °C. After the addition of DCC, the reaction solution was warmed to and stirred at room temperature for 10 min. The precipitate was filtered off and washed with hexane. The organic solution was concentrated under reduced pressure and purified by column chromatography on silica gel (hexane/EtOAc, 1:4 and 1:10) to give **19** (0.70 g, 50%) as a white solid.

TLC: R_f 0.25 (1:10 hexane/EtOAc). **^{1}H-NMR** ($CDCl_3$, 500 MHz): δ 7.66 (m, 4H), 7.51 (m, 4H), 7.31 (m, 4H), 7.20 (m, 4H), 6.35 (d, 1H, $J = 7.8$), 6.15 (s, 3H), 5.63–5.44 (m, 3H), 4.48–4.25 (m, 8H), 4.13 (d, 2H, $J = 6.1$), 3.23–2.98 (m, 4H), 2.83 (s, 2H), 2.50 (s, 3H), 2.43 (s, 3H), 2.30–2.17 (m, 4H), 1.99 (s, 3H), 1.73–1.58 (m, 2H), 1.51–1.42 (m, 4H), 1.35 (s, 15H), 1.36–1.09 (m, 4H). **^{13}C-NMR** ($CDCl_3$, 600 MHz) δ 172.41, 171.42, 171.18, 158.63, 156.90, 156.77, 156.26, 156.18, 143.90, 143.77, 143.56, 143.34, 141.40, 141.34, 141.29, 138.36, 133.24, 133.12, 132.31, 127.91, 127.73, 127.21, 127.10, 125.99, 125.17, 124.95, 124.92, 124.54, 120.08, 120.05, 119.99, 117.40, 86.32, 82.64, 67.10, 66.87, 65.13, 60.41, 53.85, 51.66, 49.12, 47.15, 46.84, 43.25, 40.65, 40.32, 35.19, 33.93, 31.72, 30.67, 29.34, 28.59, 27.98, 27.73, 25.63, 25.06, 24.97, 22.09, 21.07, 19.29, 17.92, 14.22, 12.49. **ESI-MS** m/z calculated for $C_{65}H_{78}N_6NaO_{12}S$ $[M + Na]^+$: 1189.53, found 1189.7.

Fmoc-Lys[C(= O)OCH$_2$CH = CHCH$_2$CH$_2$C(= O)-Arg(Pbf)-OtBu]-OFm (8). In a 25-mL round-bottom flask, compound **19** (0.70 g, 0.60 mmol, 1.0 equiv) was dissolved in a 1:4 mixture of piperidine (1.8 mL) and CH_2Cl_2 (7.2 mL) and stirred at room temperature for 10 min. The reaction solution was concentrated under reduced pressure and the residue was coevaporated with toluene (2 × 10 mL).

To a mixture of the residue from the previous step (~0.60 mmol, 1.0 equiv), $NaHCO_3$ (0.14 g, 1.0 mmol, 1.7 equiv) and H_2O (10 mL) was added the solution of Fmoc-OSu (0.55 g, 1.6 mmol, 2.7 equiv) in THF (10 mL). The reaction mixture was stirred at room temperature for 2 h and was quenched with saturated NH_4Cl (50 mL). The aqueous phase was extracted with CH_2Cl_2 (6 × 20 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated. The crude product was purified by silica-gel chromatography (hexane/EtOAc, 1:2 and 1:4+ 10% AcOH) to give **8** (0.55 g, 94%) as a yellow solid.

TLC: R_f 0.43 (1:4 hexanes/EtOAc + 10% AcOH). **^{1}H-NMR** (CD_3OD, 500 MHz): δ 7.68 (d, 2H, $J = 7.6$), 7.57 (t, 2H, $J = 7.8$), 7.28 (t, 3H, $J = 7.4$), 7.21 (m, 2H), 5.50 (m, 1H), 5.39 (m, 1H), 4.31 (d, 2H, $J = 5.6$), 4.23 (d, 2H, $J = 7.1$), 4.11 (m, 2H), 4.02 (m, 1H), 3.05 (m, 2H), 2.99 (m, 2H), 2.87 (s, 2H), 2.47 (s, 3H), 2.40 (s, 3H), 2.19 (m, 4H), 1.97 (s, 3H), 1.81–1.47 (m, 4H), 1.41 (m, 4H), 1.38–1.28 (m, 17H). **^{13}C-NMR** (CD_3OD, 600 MHz) δ 175.52, 173.01, 172.77, 159.88, 158.93, 158.56, 158.13, 145.43, 145.24, 142.61, 139.39, 134.45, 134.03, 133.52, 128.81, 128.22, 128.20, 127.25, 126.34, 126.33, 126.05, 120.95, 118.47, 87.68, 82.80, 67.93, 66.05, 61.56, 56.06, 54.24, 44.00, 41.53, 36.10, 32.84, 30.52, 29.93, 29.39, 28.76, 28.31, 26.32, 24.14, 20.99, 20.90, 19.64, 18.45, 14.51, 12.58. **ESI-MS** m/z calculated for $C_{51}H_{68}N_6NaO_{12}S$ $[M + Na]^+$: 1,011.45, found 1,011.5.

Preparation and Characterization of hEPO(43–77) Variants Upon completion of automated synthesis on a 0.05-mmol scale, the peptide resin was washed into a peptide cleavage vessel with CH_2Cl_2. The resin cleavage was effected by treatment with TFA/H_2O/TIS (95:2.5:2.5) for 45 min to yield the unprotected peptides. TFA was removed by N_2. The oily residue was triturated with diethyl ether and centrifuged to give a white pellet. After the ether was decanted, the solid was dissolved in MeCN/H_2O/AcOH (47.5:47.5:5) for HPLC purification.

Each purified synthetic peptide variant was dissolved in MeCN/H_2O/AcOH (47.5:47.5:5) to make a 0.12-mM solution. At time zero, 10 μL of the peptide solution were injected into the LC-MS system operated as described above. The analyte was eluted using a linear gradient method: 0 min/30% solvent B and 30 min/95% solvent B. After standing in air for 3 d, each analyte was characterized again using the same conditions.