General Synthetic Methods

All chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO), AK Scientific (Union City, CA), or Fischer Scientific (Hampton, NH), unless otherwise indicated, and used without further purification. All solvents were distilled before use, including THF (Na / benzophenone), DCM (CaH), and MeCN (4 Å MS). Anhydrous DMF was purchased from Sigma Aldrich and used without further purification.

Preparative HPLC methods were carried out on an Agilent 1100 (Santa Clara, CA) series HPLC equipped with a multi-wavelength UV-Vis detector using one of the following HPLC prep methods:

HPLC Preparatory Method A: [binary gradient: water +0.1% TFA (solvent A), acetonitrile +0.1% TFA (solvent B), 4.5 mL/min]: 0-30 min gradient 10% to 90% B; 30-31 min gradient 90% to 10% B; 31-35 min isocratic 10% B. Phenomenex Kinetex® 5 µm C18 100 Å LC column 250 x 10 mm.

HPLC Preparatory Method B: [binary gradient: water +0.1% TFA (solvent A), acetonitrile +0.1% TFA (solvent B), 4.5 mL/min]: 0-30 min gradient 0% to 60% B; 30-31 min gradient 60% to 0% B; 31-35 min isocratic 100% A. Phenomenex Kinetex® 5 µm C18 100 Å LC column 250 x 10 mm.

HPLC Preparatory Method C: [binary gradient: water +0.1% TFA (solvent A), acetonitrile +0.1% TFA (solvent B), 5.0 mL/min]: 0-30 min gradient 10% to 90% A; 30-35 min isocratic 90% A; 36-40 min isocratic 10% A. Waters XBridge® BEH Prep Amide 5 µm LC column 250 x 10 mm.

HPLC Preparatory Method D: [binary gradient: water (solvent A), acetonitrile, (solvent B) 5.0 mL/min]: 0-30 min gradient 10% to 90% A; 30-35 min isocratic 90% A; 36-40 min isocratic 10% A. Waters XBridge® BEH Prep Amide 5 µm LC column 250 x 10 mm.

UPLC-HRMS experiments to determine exact masses and purity levels of organic compounds were carried out on a Waters Acquity / Xevo-G2 UPLC-MS system at the Johns Hopkins Mass Spectrometry Facility. NMR spectra were recorded on either 400 MHz or 300 MHz Bruker (Billerica, MA) Advance NMR spectrometers. Chemical shifts are reported relative to the reference shift for the solvent used relative to TMS. Many ¹H-NMR resonances are broad owing to amide and carbamate configurational equilibria. ¹³C NMR spectra were recorded on a 400 MHz Bruker Advance operating at 101 MHz. Chemical shifts are reported relative to the reference chemical shift of the NMR solvent. In ¹³C experiments for which D₂O is the solvent, an internal standard of
acetone was added, and the spectrum adjusted to this reference (215.94 ppm, 30.89 ppm).

Reagent abbreviations used are as follows. PyBOP: benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DIPEA: N,N-Diisopropylethylamine, TFA: trifluoroacetic acid, DCC: N,N’-Dicyclohexylcarbodiimide. HOBt: hydroxybenzotriazole. Thin layer chromatography (TLC) was carried out on silica gel coated glass plates with the elution conditions indicated, CV refers to column volumes of mobile phase used in silica gel chromatography, and tR indicates retention time.
Synthesis of peptide CoA compounds.

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**N-Boc-t-butyl-D-pyroglutamate (6):** Protected D-pyroglutamate 6 was synthesized according to a known literature procedure\(^1\)^\(^2\). All spectral data were as reported.

**N-Boc-1-t-butyl-D-glutamate (7):** Diprotected D-pyroglutamate 7 was synthesized according to a known literature procedure\(^3\). All spectral data were as reported.

**D-Alanine benzyl ester (8):** D-Alanine benzyl ester HCl was synthesized and isolated according to the literature. Spectral data were as reported\(^4\).

**D-δ-[α-(t-Butyl-N-boc)-glutamyl]-D-Ala-OBn dipeptide (9):** Free acid 7 (875 mg, 2.88 mmol) was stirred in 20 mL anhydrous DCM under an inert atmosphere of argon. PyBOP (1.65 g, 3.17 mmol) was added, followed by DIPEA (0.775 mL, 4.33 mmol). D-Alanine benzyl ester S3 (567 mg, 3.17 mmol) was dissolved in 5 mL anhydrous DCM and DIPEA (0.775 mL, 4.33 mmol) was added. The D-alanine benzyl ester solution was added to the solution of S2 and PyBOP and allowed to stir under argon for 3 h. The reaction mixture was then poured over saturated aq. NH\(_4\)Cl (30 mL), the organic layer was separated, and the aqueous phase was back extracted 2×10 mL DCM. The pooled organics were washed with saturated NaHCO\(_3\) (30 mL) and dried over anhydrous MgSO\(_4\). Rotary evaporation followed by column chromatography on silica gel (gradient 90:10 – 50:50 hex:EtOAc) afforded pure dipeptide benzyl ester 9 (1.24 g, 2.67 mmol, 93%) as a white solid. \(^1\)H-NMR (400 MHz; CDCl\(_3\)): δ 7.39-7.31 (m, 5H), 6.50 (brs, 1H), 5.23-5.14 (m, 3H), 4.63 (quintet, \(J = 7.2\) Hz, 1H), 4.17 (brs, 1H), 2.33-2.25 (m, 2H), 2.21-2.11 (m, 1H), 1.93-1.80 (m, 1H), 1.50-1.39 (m, 21H). \(^13\)C-NMR (101 MHz, CDCl\(_3\)): δ 172.83, 171.39, 171.38, 155.74, 135.41, 128.54, 128.31, 128.03, 82.05, 79.72, 66.96, 53.49, 48.16, 32.31, 28.89, 28.29, 27.94, 18.10. UPLC-HRMS (ESI) calcd for C\(_{24}\)H\(_{37}\)N\(_2\)O\(_7\) \([M+H]^+\) 465.2595; found 465.2610 \([M+H]^+\).

**D-δ-[α-(t-Butyl-N-boc)-glutamyl]-D-alanyl-L-Dap-OBn tripeptide (10):** 9 (124 mg, 0.267 mmol) was added to 15 mL of anhydrous THF under an inert atmosphere of argon. 12 mg (10 w/w %) Pd/C was added and the resulting black suspension was sparged with hydrogen gas then stirred for 1 h at room temperature under balloon pressure of hydrogen until TLC indicated complete consumption of starting material. The mixture was then filtered through a 0.2 µM syringe filter and concentrated \(\text{in vacuo}\). The freshly hydrogenated free acid was used immediately without further purification. Freshly deprotected 9 was stirred in 40 mL of anhydrous DMF under an inert atmosphere of argon at 0 °C. To this solution was added 16 (86.5 mg, 0.294 mmol) as a 100 mg/mL solution in DMF. PyBOP (153 mg, 0.294 mmol) was subsequently added, along with DIPEA (52.6 µL, 0.294 mmol) in single portions. The resulting solution was stirred at 0 °C for 25 min. The reaction mixture was then diluted with 100 mL EtOAc and washed with 100 mL saturated aq. NH\(_4\)Cl. The aqueous phase was back-extracted.
and the combined organics were washed with saturated NaHCO$_3$ and dried over anhydrous MgSO$_4$. The solution was concentrated by rotary evaporation, and the resulting crude mixture was purified by column chromatography on silica gel (gradient 50-70% EtOAc in hexanes) yielding 115 mg of 10 as a clear oil (0.177 mmol, 66%), which was subsequently crystallized from EtOAc/hexane/cyclohexane. $^1$H-NMR (400 MHz, CDCl$_3$): δ 7.47-7.45 (m, 1H), 7.33 (s, 5H), 6.74-6.71 (m, 1H), 5.32-5.27 (m, 1H), 5.14 (brs, 3H), 4.59-4.58 (m, 1H), 4.49 (q, J = 7.6 Hz, 1H), 4.19-4.07 (m, 1H), 3.53 (brs, 2H), 2.28 (brs, 2H), 2.15-2.12 (m, 1H), 2.03-2.03 (m, 1H), 1.89-1.83 (m, 1H), 1.44-1.39 (m, 30H).

$^{13}$C-NMR (101 MHz, CDCl$_3$): δ 172.52, 171.44, 170.05, 169.91, 156.56, 155.82, 135.23, 128.59, 128.45, 128.34, 82.20, 79.88, 67.46, 60.38, 53.75, 53.48, 49.09, 41.89, 32.39, 28.31, 28.29, 27.98, 18.14, 14.18. UPLC-HRMS (ESI) calcd for C$_{32}$H$_{51}$N$_4$O$_{10}$+ 651.3600; found 651.3616 [M+H]$^+$. 

Protected Tripeptide CoA (11). Freshly deprotected 10 (71 mg, 0.109 mmol) was dissolved in 4 mL of anhydrous DMF. To this solution was added coenzyme A sodium salt hydrate (50 mg, 0.0651 mmol) followed by PyBOP (74 mg, 0.141 mmol) and DIPEA (57 µL, 0.327 mmol). The solution was stirred at room temperature for 1 h, then directly purified by HPLC prep method A ($t_R = 13.9$ min). Fractions containing the product were pooled, frozen, and lyophilized to dryness to yield 11 (16.2 mg, 0.012 mmol, 19%) as a white powder. $^1$H-NMR (400 MHz, D$_2$O): δ 8.67 (s, 1H), 8.45 (s, 1H), 6.23 (d, J = 5.4 Hz, 1H), 4.93-4.89 (m, 2H), 4.63-4.61 (m, 2H), 4.36-4.27 (m, 3H), 4.04 (s, 1H), 3.98-3.94 (m, 1H), 3.92-3.88 (m, 1H), 3.66-3.52 (m, 2H), 3.49-3.45 (m, 2H), 3.37-3.33 (m, 2H), 3.07-3.00 (m, 2H), 2.47-2.44 (m, 4H), 2.13-2.10 (m, 2H), 1.92-1.88 (m, 2H), 1.46-1.40 (m, 30H), 1.24 (s, 1H), 0.96 (s, 3H), 0.83 (s, 3H). UPLC-HRMS (ESI) calcd for C$_{46}$H$_{79}$N$_{11}$O$_{25}$P$_3$S$^+$ 1310.4177; found 1310.4193 [M+H]$^+$. 

D$\delta$-(Glutamyl)-D-alanyl-L-Dap-CoA (12). 11 (3.3 mg, 0.0025 mmol) was dissolved in 0.5 mL TFA, and the solution allowed to stand for 30 min at room temperature. The reaction was concentrated in vacuo, dissolved in water, then HPLC purified using HPLC prep method B ($t_R = 7.5$ min). Fractions containing the product were pooled, frozen, and lyophilized to dryness to yield 12 (1.1 mg, 0.001 mmol, 41%) as a white solid. $^1$H-NMR (400 MHz, D$_2$O-3mm): δ 8.59 (s, 1H), 8.35 (s, 1H), 6.14 (d, J = 5.7 Hz, 1H), 4.51 (brs, 1H), 4.27 (q, J = 7.3 Hz, 2H), 4.18-4.17 (m, 2H), 3.95 (s, 1H), 3.76 (dd, J = 10.0, 4.8 Hz, 3H), 3.65-3.62 (m, 1H), 3.55-3.48 (m, 3H), 3.41-3.35 (m, 3H), 3.32-3.20 (m, 4H), 2.97 (t, J = 3.2 Hz, 3H), 2.42-2.34 (m, 6H), 2.09-2.04 (m, 3H), 1.37 (d, J = 7.3 Hz, 4H), 0.85 (s, 3H), 0.74 (s, 3H). UPLC-HRMS (ESI) calcd for C$_{32}$H$_{55}$N$_{11}$O$_{25}$P$_3$S$^+$ 1054.2502 found 1054.2494 [M+H]$^+$. 

Protected Dipeptide CoA (13). To a solution of freshly deprotected 9 (42 mg, 0.0989 mmol) in anhydrous THF was added DCC (25 mg, 0.119 mmol). The solution was stirred for 30 min, then concentrated by rotary evaporation. The white residue was taken up in 3 mL of anhydrous DMF to which coenzyme A sodium salt hydrate (30 mg,
0.0391 mmol) and DIPEA (100 µL) were subsequently added. The reaction was stirred at room temperature for 1 h at which time the product was directly purified from the reaction mixture by HPLC prep method A (tr = 11.5 min) as the protected acyl CoA intermediate 13. Fractions containing the product were pooled, frozen on dry ice and lyophilized to dryness to yield 13 (13.5 mg, 0.012 mmol, 30%) as a white solid.

\[ ^1H-NMR (400 MHz, D_2O): \delta 8.54 (brs, 1H), 8.34 (brs, 1H), 6.11 (d, J = 5.9 Hz, 1H), 4.86-4.77 (m, 2H), 4.36 (q, J = 7.1 Hz, 1H), 4.24-4.17 (m, 2H), 3.93 (s, 1H), 3.88 (dt, J = 9.7, 5.0 Hz, 1H), 3.83-3.79 (m, 1H), 3.59-3.54 (m, 1H), 3.37 (t, J = 6.5 Hz, 2H), 3.24 (t, J = 6.2 Hz, 2H), 2.94-2.89 (m, 2H), 2.37-2.29 (m, 4H), 2.06-2.01 (m, 2H), 1.85-1.79 (m, 2H), 1.35 (s, 9H), 1.32 (s, 9H), 1.28 (d, J = 7.3 Hz, 3H), 0.86 (s, 3H), 0.73 (s, 3H). UPLC-HRMS (ESI) calcd for C_{38}H_{65}N_{9}O_{22}P_{3}S_{1} 1124.3172; found 1124.3138 [M+H]^+.

D-\delta-(Glutamyl)-D-alanyl-CoA (14): Protected dipeptide CoA 13 was dissolved in 3 mL of trifluoroacetic acid. The solution was allowed to stand at room temperature for 20 min, concentrated in vacuo, dissolved in 2 mL of water, and then purified by prep method B (tr = 8.0 min). The pooled fractions containing pure 14 were frozen on dry ice and lyophilized to dryness. \[ ^1H-NMR (400 MHz, CDCl_3): \delta 8.71 (d, J = 3.6 Hz, 1H), 8.47 (s, 1H), 6.34 (d, J = 5.4 Hz, 1H), 6.23 (s, 1H), 4.93-4.86 (m, 2H), 4.63 (brs, 1H), 4.49 (q, J = 7.4 Hz, 2H), 4.29 (brs, 2H), 4.06 (s, 1H), 4.02-3.97 (m, 1H), 3.90-3.87 (m, 1H), 3.68-3.61 (m, 1H), 3.48 (t, J = 6.6 Hz, 2H), 3.36 (d, J = 5.8 Hz, 2H), 3.04 (t, J = 5.6 Hz, 2H), 2.55 (q, J = 7.3 Hz, 2H), 2.4 (t, J = 6.6 Hz, 2H), 2.28-2.18 (m, 2H), 1.40 (d, J = 7.2 Hz, 3H), 0.97 (s, 3H), 0.85 (s, 3H). UPLC-HRMS (ESI) calcd for C_{29}H_{49}N_{9}O_{20}P_{3}S_{1} 968.2022; found 968.2001 [M+H]^+.

N-(\alpha-Fmoc)-N-(\beta-boc)-L-2,3-Dap (15): Fmoc-L-Dap(Boc)-OH (2 g, 4.69 mmol) was dissolved in 18 mL of anhydrous DMF and stirred at room temperature under an inert atmosphere of argon. Benzyl bromide (2.2 mL, 18.8 mmol) was added in a single portion, followed by NaHCO_3 (790 mg, 9.40 mmol). The solution was stirred overnight at room temperature, then diluted with 200 mL DCM and washed with 200 mL saturated NH_4Cl. The organic layer was washed with 200 mL saturated NaHCO_3 and dried over MgSO_4. The pooled organics were concentrated by rotary evaporation, and column chromatography on silica gel (90:10 to 50:50 hexanes:EtOAc) afforded pure 15 (2.4 g, 4.65 mmol, 98%) as a white solid. \[ ^1H-NMR (400 MHz, CDCl_3): \delta 7.77 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.2 Hz, 2H), 7.42-7.29 (m, 10H), 5.98-5.97 (m, 1H), 5.21-5.20 (m, 2H), 4.79-4.77 (m, 1H), 4.50-4.37 (m, 2H), 4.22 (t, J = 7.0 Hz, 1H), 3.58 (brs, 2H), 1.44 (s, 9H). \[ ^13C-NMR (101 MHz, CDCl_3): \delta 170.32, 156.45, 156.04, 143.73, 141.31, 141.28, 135.18, 128.65, 128.53, 128.38, 128.36, 127.72, 127.08, 125.16, 119.97, 80.08, 67.58, 67.17, 65.71, 55.20, 47.15, 42.21, 28.28. UPLC-HRMS (ESI) calcd for C_{30}H_{33}N_{3}O_{9}P_{3}S_{1} 517.2333; found 517.2338 [M+H]^+. 

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**N-(β-Boc)-L-2,3-Dap (16):** Triprotected diaminopropionate 15 (500 mg, 0.968 mmol) was stirred in 5 mL anhydrous DCM under an inert atmosphere of argon. Diethylamine (10 mL) was added and the reaction monitored by TLC (50:50 hexanes:EtOAc). After 3 h stirring at room temperature, the reaction was complete and the volatiles were removed by rotary evaporation. Column chromatography on silica gel (1 CV EtOAc + 1% TEA, 2 CV 95:5 DCM:MeOH + 1% TEA) afforded deprotected 15 (228 mg, 0.77 mmol, 80%) as a clear oil. $^1$H-NMR (400 MHz; acetone-d$_6$): δ 7.42-7.31 (m, 5H), 5.98-5.96 (m, 1H), 5.16 (q$_{AB}$, $J = 2.3$ Hz, 12.6 Hz, 1H), 4.39 (t, $J = 6.2$ Hz, 1H), 3.55 (dt, $J = 13.4$, 6.2 Hz, 1H), 3.34 (dt, $J = 13.3$, 6.2 Hz, 1H), 2.98 (brs, 1H), 1.97-1.83 (m, 1H), 1.39 (s, 9H). $^{13}$C-NMR (101 MHz, acetone-d$_6$): δ 172.03, 171.52, 156.76, 137.48, 129.42, 128.96, 128.85, 79.04, 66.93, 63.92, 43.99, 28.75. UPLC-HRMS (ESI) calcd for C$_{15}$H$_{23}$N$_4$O$_4^+ 295.1652$; found 295.1653 [M+H]$^+$. 

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Synthesis of pantetheine and SNAC thioester PCP₃ mimics.

Protected SNAC tripeptide (17). Freshly deprotected 11 (42 mg, 0.075 mmol) was stirred in 20 mL anhydrous DCM. To this solution was added N-acetylcysteamine (SNAC) (10.7 mg, 0.09 mmol) followed by PyBOP (46.8 mg, 0.09 mmol), and DIPEA (29.5 μL, 0.165 mmol). The reaction mixture was stirred for 4 h at room temperature before it was concentrated by rotary evaporation. The resulting oil was purified by
silica gel chromatography to yield the SNAC tripeptide thioester 17 (23 mg, 0.034 mmol, 46%). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.82 (d, $J = 7.1$ Hz, 1H), 6.89 (d, $J = 6.1$ Hz, 1H), 6.62-6.61 (m, 1H), 5.50-5.47 (m, 1H), 5.35-5.33 (m, 1H), 4.60-4.58 (m, 1H), 4.49 (t, $J = 6.2$ Hz, 1H), 4.14 (t, $J = 7.1$ Hz, 1H), 3.56-3.53 (m, 2H), 3.42 (q, $J = 6.3$ Hz, 1H), 3.33-3.29 (m, 1H), 3.05-2.97 (m, 2H), 2.37 (q, $J = 7.7$ Hz, 2H), 2.16-2.08 (m, 1H), 1.97 (s, 3H), 1.96-1.91 (m, 1H), 1.46 (s, 9H), 1.45-1.42 (m, 21H).

$^{13}$C-NMR (101 MHz, CDCl$_3$): $\delta$ 199.66, 173.41, 172.96, 171.81, 157.49, 156.19, 82.74, 80.43, 80.37, 61.09, 53.85, 49.64, 49.59, 42.81, 42.34, 38.65, 32.48, 29.15, 28.60, 28.26, 24.80, 23.31, 17.93. UPLC-HRMS (ESI) calcd for $\text{C}_{29}\text{H}_{52}\text{N}_{5}\text{O}_{10}\text{S}$ $^{+}$ 662.3429; found 662.3429 [M+H]$^+$. D-$\delta$-(Glutamyl)-D-alanyl-L-Dap-SNAC (18). Protected SNAC tripeptide 17 (22 mg, 0.033 mmol) was dissolved in 4 mL of a 3:1 TFA:DCM mixture and stirred for 90 min at room temperature. The solvent was then removed in vacuo, the crude oil taken up in 2 mL water, and purified directly by HPLC prep method B. Fractions containing the product were pooled, frozen, and lyophilized to dryness. $^1$H-NMR (400 MHz, D$_2$O-3mm): $\delta$ 4.75-4.71 (m, 1H), 4.30 (q, $J = 7.3$ Hz, 1H), 3.84 (t, $J = 6.4$ Hz, 1H), 3.51 (dd, $J = 13.7, 5.5$ Hz, 1H), 3.27 (td, $J = 13.7, 7.8$ Hz, 3H), 3.00 (t, $J = 6.3$ Hz, 2H), 2.43 (dq, $J = 16.5, 8.1$ Hz, 1H), 2.10 (qd, $J = 7.1, 1.9$ Hz, 1H), 1.88 (s, 3H), 1.36 (d, $J = 7.3$ Hz, 3H).

$^{13}$C-NMR (101 MHz, D$_2$O-3mm): $\delta$ 199.48, 175.80, 174.49, 174.30, 172.89, 56.83, 53.22, 49.83, 39.17, 38.16, 30.70, 28.18, 25.65, 21.81, 15.99. UPLC-HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{28}\text{N}_{5}\text{O}_{6}\text{S}$ $^{+}$ 406.1755; found 406.1756 [M+H]$^+$. Protected pantetheine tripeptide (20). Freshly hydrogenated 11 (62 mg, 0.095 mmol) was dissolved in 10 mL of anhydrous DCM and brought to 0 $^\circ$C. To this solution was added pantetheine acetal $^6$ (36.3 mg, 0.11 mmol) followed by PyBOP (59.5 mg, 0.11 mmol) and DIPEA (41 $\mu$L, 0.23 mmol). The solution was stirred at 0 $^\circ$C for 4 h before the solvent was removed by rotary evaporation. The crude oil was then chromatographed using silica to isolate pure pantetheine tripeptide 20 (75 mg, 0.087 mmol, 92%) as a clear oil. $^1$H-NMR (400 MHz, acetone-d$_6$): $\delta$ 8.08 (d, $J = 7.7$ Hz, 1H), 7.60 (d, $J = 6.7$ Hz, 1H), 7.51 (brs, 1H), 7.43-7.42 (m, 1H), 6.43-6.39 (m, 1H), 6.32-6.28 (m, 1H), 4.60 (t, $J = 5.7$ Hz, 1H), 4.47 (t, $J = 7.1$ Hz, 1H), 4.12 (s, 1H), 4.06-4.03 (m, 1H), 3.73 (d, $J = 11.6$ Hz, 1H), 3.56 (dd, $J = 13.5, 6.6$ Hz, 2H), 3.46-3.36 (m, 4H), 3.24 (d, $J = 11.6$ Hz, 1H), 2.99 (t, $J = 6.4$ Hz, 2H), 2.43 (td, $J = 6.7, 2.1$ Hz, 4H), 2.14-2.10 (m, 1H), 1.99-1.93 (m, 1H), 1.46-1.36 (m, 36H), 1.01 (s, 3H), 0.97 (s, 3H). $^{13}$C-NMR (101 MHz, acetone-d$_6$): $\delta$ 199.33, 173.21, 172.52, 172.03, 171.75, 169.88, 169.84, 157.19, 156.08, 99.05, 81.07, 79.16, 78.77, 77.29, 71.27, 60.84, 54.60, 54.50, 49.41, 42.12, 38.60, 35.90, 35.18, 33.03, 32.09, 28.18, 28.15, 27.71, 24.13, 21.96, 18.87, 18.61, 18.57, 17.42. UPLC-HRMS (ESI) calcd for $\text{C}_{39}\text{H}_{69}\text{N}_{5}\text{O}_{13}\text{S}^{+}$ 861.4638; found 861.4644 [M+H]$^+$.

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1 We acknowledge Dr. E. A. Hill for the synthesis of this compound.
D-δ-(Glutamyl)-D-alanyl-L-Dap-pantetheine (21). Protected pantetheine tripeptide 20 (53 mg, 0.062 mmol) was dissolved in 4 mL of a 3:1 TFA:DCM solution. The reaction mixture was stirred at room temperature for 1 h and the volatiles removed in vacuo. To the obtained oil was added add 2 mL water, and the crude mixture was directly purified by HPLC prep method B. Fractions containing the product were pooled, frozen, and lyophilized to dryness. ¹H-NMR (400 MHz, D₂O): δ 4.29 (q, J = 7.3 Hz, 1H), 3.95 (td, J = 6.5, 1.2 Hz, 1H), 3.51 (dd, J = 13.5, 5.6 Hz, 1H), 3.40 (q, J = 6.5 Hz, 3H), 3.28 (dd, J = 11.7, 6.6 Hz, 4H), 2.99 (t, J = 6.5 Hz, 2H), 2.46 (q, J = 7.8 Hz, 2H), 2.38 (t, J = 6.6 Hz, 2H), 2.16-2.10 (m, 2H), 1.35 (d, J = 7.3 Hz, 3H), 0.82 (s, 3H), 0.78 (s, 3H). ¹³C-NMR (101 MHz, D₂O): δ 199.70, 176.01, 175.37, 174.57, 174.29, 172.26, 76.07, 68.67, 57.15, 52.89, 50.12, 39.55, 38.93, 38.53, 35.73, 35.57, 30.98, 28.48, 25.81, 20.80, 19.43, 16.38. UPLC-HRMS (ESI) calcd for C₂₂H₄₁N₆O₉S⁺ 565.2650; found 565.2652 [M+H]⁺.
Synthesis of sulfonated product standards.

Benzylic (2R)-1-carboxybenzyl-pyroglutamate (22). 22 was prepared as previously described, all spectral data were as reported.

Benzylic (2R)-1-carboxybenzyl-glutamate (23). To a solution of 22 (2 g, 5.97 mmol) in 30 mL of THF at 0 °C was added 1.0 M aqueous LiOH (6.26 mL) dropwise. The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for an addition 2 h. The reaction was then extracted with 1 M NaOH (2 × 50 mL), and washed with 50 mL of DCM. The pH of the solution was then adjusted to 1 with 1 M HCl. The acidified aqueous suspension was then extracted with DCM (3 × 50 mL) and dried over Na2SO4 before filtration and concentration by rotary evaporation. The resulting crude free acid (727 mg, 1.95 mmol, 33%) was used without further purification. 1H-NMR (400
MHz, acetone- d₆): δ 10.75-10.70 (brs, 1H), 7.39-7.31 (m, 10H), 6.83 (d, J = 8.3 Hz, 1H), 5.17 (s, 2H), 5.07 (s, 2H), 4.36 (td, J = 8.8, 5.0 Hz, 1H), 2.49-2.44 (m, 2H), 2.23-2.14 (m, 1H), 2.01-1.95 (m, 1H). ¹³C-NMR (101 MHz, acetone-d₆): δ 173.36, 171.59, 156.14, 136.85, 135.90, 128.22, 128.13, 127.86, 127.76, 127.60, 127.55, 66.19, 65.88, 53.43, 29.32, 26.43.

UPLC-HRMS (ESI) calcd for C₂₀H₂₀NO₆: 370.1296; found 370.1283 [M-H].

D-Ś-(Glutamyl)-D-alanyl-0-t-bu (24). D-alanine t-butyl ester HCl (437 mg, 2.419 mmol) and DIPEA (0.65 mL, 3.628 mmol) were dissolved in 12 mL of anhydrous DCM. In another flask, free acid 23 was dissolved in 35 mL anhydrous DCM. To this solution was added PyBOP (1.257 g, 2.419 mmol) and DIPEA (0.65 mL, 3.628 mmol), and the resulting yellow solution was allowed to stir for 2 min. The D-alanine solution was then added by syringe to the activated acid solution dropwise and the reaction stirred for 2 days. The reaction mixture was then washed with saturated NH₄Cl and NaHCO₃, dried over MgSO₄, filtered, and concentrated. The resulting residue was purified by silica gel column chromatography (EtOAc:Hexanes 30-80% gradient EtOAc) to yield the product as clear oil, which subsequently crystallized (280 mg, 0.562 mmol, 28%). ¹H-NMR (400 MHz, CDCl₃): δ 7.34 (s, 10H), 6.19 (d, J = 6.8 Hz, 1H), 5.73 (d, J = 8.0 Hz, 1H), 5.20-5.13 (m, 2H), 5.10 (s, 2H), 4.42 (quintet, J = 7.1 Hz, 2H), 2.24 (brs, 3H), 2.04-1.94 (m, 1H), 1.45 (s, 9H), 1.33 (d, J = 7.1 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃): δ 172.23, 171.78, 171.06, 156.15, 136.22, 135.22, 128.63, 128.50, 128.35, 128.31, 128.14, 128.08, 82.02, 67.29, 67.04, 53.57, 48.69, 32.08, 28.14, 27.95, 18.59. UPLC-HRMS (ESI) calcd for C₂₇H₃₅N₂O₇+: 499.2439; found 499.2452 [M+H].

OBn-N-α-Cbz-β-lactam-sulfamate (25). 24 (96 mg, 0.200 mmol) was stirred in a 5 mL solution of a 1:1 TFA:DCM. Upon complete t-butyl ester deprotection as indicated by TLC, the volatiles were removed in vacuo. 27 (271 mg, 0.500 mmol) was stirred in 10 mL of anhydrous THF with a catalytic amount of Pd/C. The solution was sparged with H₂ and stirred under balloon pressure of H₂ until TLC indicated complete deprotection had occurred. Freshly deprotected 24 and 28 were then combined and the volatiles removed in vacuo. The atmosphere was exchanged with argon and the flask charged with anhydrous DMF (8 mL). DCC (49.4 mg, 0.24 mmol), followed by HOBt (36.7 mg, 0.24 mmol) were added to the solution. The reaction mixture was allowed to stir under argon for 5 h at room temperature. The product was purified as the tetrabutylammonium salt by HPLC preparatory method B. Fractions containing the product were pooled and lyophilized to yield 25 (21.4 mg, 0.036 mmol, 18%) as a white solid. ¹H-NMR (400 MHz, MeOD-3mm): δ 7.38-7.32 (m, 10H), 5.19 (s, 2H), 5.11 (d, J = 2.7 Hz, 2H), 5.00 (dd, J = 5.8, 3.1 Hz, 1H), 4.35 (q, J = 7.4 Hz, 1H), 4.26 (dd, J = 9.4, 4.8 Hz, 1H), 3.83 (t, J = 5.8 Hz, 1H), 3.51 (dd, J = 5.6, 3.2 Hz, 1H), 3.28-3.23 (m, 9H), 2.41-2.35 (m, 2H), 2.21-2.17 (m, 1H), 1.99-1.90 (m, 1H), 1.68 (dt, J = 16.3, 8.0 Hz, 9H), 1.43 (dq, J = 14.8, 7.4 Hz, 9H), 1.33 (d, J = 7.1 Hz, 3H), 1.04
(t, J = 7.4 Hz, 12H). $^{13}$C-NMR (101 MHz; CD$_3$CN): δ 173.74, 173.72, 173.10, 173.08, 172.98, 172.97, 172.87, 172.86, 157.25, 157.23, 138.09, 138.08, 137.08, 137.07, 129.47, 129.46, 129.41, 129.40, 129.11, 128.97, 128.95, 128.84, 128.83, 128.70, 128.69, 67.46, 67.46, 67.04, 67.05, 59.27, 59.24, 59.23, 59.21, 59.20, 55.10, 54.96, 50.10, 48.36, 32.46, 32.45, 32.44, 27.52, 27.51, 24.24, 20.27, 20.26, 18.26, 18.25, 13.75, 13.75.

Desmethoxysulfazecin (2). Spectral data are consistent with the literature. 25 (7 mg, 0.019 mmol) was stirred in 4 mL THF. To this solution was added 0.3 mL of a 0.5 M aqueous potassium phosphate buffer at pH=7.0. A catalytic amount of Pd/C was added and the solution sparged with H$_2$ for 3 h at room temperature before filtration through a 0.2 μm filter. The solution was diluted with water and directly purified by UPLC-MS to yield desmethoxysulfazecin 2 (1.5 mg, 0.4 mmol).

Hydrolyzed desmethoxysulfazecin (4). An analytical standard of 25 was subjected to hydrolysis in a 1% TFA:ACN solution for 1 hr. The solvent was then exchanged for a 10% KH$_2$PO$_4$ in THF solution (pH = 7). After addition of a catalytic amount of Pd/C, the solution was hydrogenated in a Parr shaker at 50 PSI for 2 hr, filtered, and directly purified by HPLC method D to yield an analytical sample of hydrolyzed compound 3.

3-(N-CBz)-β-lactam (26). Synthesized as reported in the literature, all spectral data were as reported.

3-(N-Cbz)-β-lactam-sulfamate (27). A protocol was adapted from the literature. 26 (425 mg, 1.93 mmol) was stirred in 7.5 mL anhydrous DCM. To this solution was added 2.9 mL (2.897 mmol) of a freshly prepared 1M DMF•SO$_3$ solution (459 mg DMF•SO$_3$ dissolved in 3 mL anhydrous DMF) by syringe. The solution was allowed to stir for 30 min at which time it was extracted with 30 mL of a 0.5 M solution of KH$_2$PO$_4$. The aqueous phase was washed with an equal volume of DCM and tetrabutylammonium hydrogen sulfate (TBAHS) (622 mg, 1.83 mmol) was added, effecting precipitation of the tetrabutylammonium salt. The solution was then extracted 3 x 30 mL of DCM, the organics were pooled and dried over MgSO$_4$ before concentration by rotary
evaporation. The resulting solid was taken up in hot ethyl acetate and 27 was crystallized (718 mg, 1.32 mmol, 69%) by addition of hexanes. 1H-NMR (400 MHz, CDCl₃): δ 7.30 (s, 5H), 5.69 (d, J = 8.6 Hz, 1H), 5.06 (s, 2H), 4.86-4.82 (m, 1H), 3.87 (t, J = 5.8 Hz, 1H), 3.48 (dd, J = 6.0, 2.9 Hz, 1H), 3.23-3.18 (m, 9H), 1.63-1.55 (m, 9H), 1.39 (sextet, J = 7.3 Hz, 9H), 0.96 (t, J = 7.3 Hz, 12H). 13C-NMR (101 MHz, CDCl₃): δ 163.42, 155.61, 136.11, 128.49, 128.14, 128.01, 67.03, 58.58, 56.19, 49.18, 23.89, 19.64, 13.66. UPLC-HRMS (ESI) calcd for C₁₁₁₁H₁₁N₂O₆: 299.0343; found 299.0342 [M-H].

Synthesis of unsulfonated product standards.

**t-Butylester-N-α-boc-β-lactam-Cbz (29).** 32 (168 mg, 0.525 mmol) was stirred in 5 mL of a 80:20 DCM:TFA solution until TLC indicated complete Boc-deprotection had occurred (visualized by ninhydrin stain). The volatiles were removed in vacuo, followed by addition of a solution of freshly deprotected 9 (163 mg, 0.437 mmol) in 10 mL DCM. To this solution was added PyBOP (88 mg, 0.525 mmol) followed by DIPEA (175 µL, 0.963 mmol). The solution was allowed to stir for 6 h at room temperature. The reaction mixture was then washed with saturated NH₄Cl and NaHCO₃, dried over MgSO₄, filtered, and concentrated by rotary evaporation. Silica gel column chromatography (rf = 0.5 100% EtOAc) afforded β-lactam 29 (176 mg, 0.305 mmol, 70%)
as a white crystalline solid. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.96 (d, $J = 6.7$ Hz, 1H), 7.68 (d, $J = 7.6$ Hz, 1H), 7.37-7.32 (m, 5H), 6.80-6.79 (m, 1H), 6.48 (d, $J = 6.0$ Hz, 1H), 5.34 (d, $J = 8.2$ Hz, 1H), 5.22 (d, $J = 0.1$ Hz, 2H), 5.07 (d, $J = 0.7$ Hz, 1H), 4.82-4.81 (m, 1H), 4.69 (dd, $J = 12.6$, 5.0 Hz, 1H), 4.54 (t, $J = 6.9$ Hz, 1H), 4.43 (t, $J = 6.2$ Hz, 1H), 4.14-4.06 (m, 1H), 3.87-3.71 (m, 2H), 2.31-2.25 (m, 2H), 1.99-1.97 (m, 1H), 1.88-1.86 (m, 1H), 1.42 (dd, $J = 14.6$, 6.5 Hz, 2H).

$^{13}$C-NMR (101 MHz, CDCl$_3$): $\delta$ 173.84, 172.99, 172.53, 171.84, 149.34, 135.11, 135.07, 128.84, 128.82, 128.79, 128.77, 128.68, 128.61, 128.55, 128.50, 77.68, 77.36, 77.04, 68.30, 56.70, 56.56, 53.92, 53.36, 48.93, 46.55, 46.52, 32.32, 32.30, 31.95, 28.53, 28.45, 28.18, 28.17, 17.95, 17.73. UPLC-HRMS (ESI) calcd for C$_{28}$H$_{41}$N$_4$O$_9$ $^+$ 577.2868; found 577.2878 [M+H]$^+$.  

**D-δ-(Glutamyl)-D-alanyl-β-lactam (1).** Protected β-lactam 29 (80 mg, 0.139 mmol) was stirred in 5 mL of a 60:40 DCM:TFA solution at room temperature for 4.5 h. The volatiles were then removed in vacuo overnight. To the resulting oil was added 5 mL MeOH and a catalytic amount of Pd/C. The solution was sparged with H$_2$ and stirred under balloon pressure of H$_2$ for 2 h. The reaction mixture was then 0.2 µm filtered and the product directly HPLC purified using HPLC preparatory method C ($t_R$= 7.0 min.). $^1$H-NMR (400 MHz, D$_2$O): $\delta$ 4.79-4.77 (m, 1H), 4.17 (d, $J = 7.2$ Hz, 1H), 3.86-3.84 (m, 1H), 3.55 (dd, $J = 6.1$, 5.3 Hz, 1H), 3.29 (dd, $J = 6.1$, 2.5 Hz, 1H), 2.43-2.40 (m, 2H), 2.10-2.08 (m, 2H), 1.31-1.27 (m, 3H).

$^{13}$C-NMR (101 MHz, D$_2$O): $\delta$ 175.51, 174.37, 174.22, 170.65, 56.26, 53.46, 52.78, 49.76, 43.43, 30.81, 25.55, 16.52. UPLC-HRMS (ESI) calcd for C$_{11}$H$_{19}$N$_4$O$_5$ $^+$ 287.1350; found 287.1356 [M+H]$^+$.  

**D-δ-(Glutamyl)-D-alanyl-L-Dap-OH (30).** 10 was stirred in 5 mL of anhydrous THF. A catalytic amount of Pd/C was added and the solution was sparged with H$_2$. The solution was then stirred under balloon pressure of H$_2$ until TLC indicated complete deprotection of substrate (80:20 EtOAc:Hex). The solution was then filtered through Celite and concentrated in vacuo. The resulting oil was then dissolved in 4 mL of a 3:1 TFA:DCM solution and stirred for 3 h at room temperature. The solution was then concentrated in vacuo and subsequently dissolved in water. The deprotected tripeptide was then purified by HPLC preparatory method B ($RT= 9.0$ min.). $^1$H-NMR (400 MHz, D$_2$O): $\delta$ 4.51 (dd, $J = 7.7$, 5.9 Hz, 1H), 4.28-4.22 (m, 1H), 3.87 (t, $J = 6.4$ Hz, 1H), 3.41 (dd, $J = 13.3$, 5.8 Hz, 1H), 3.22 (dd, $J = 13.3$, 7.7 Hz, 1H), 2.45-2.40 (m, 2H), 2.12-2.06 (m, 2H), 1.29 (d, $J = 7.3$ Hz, 1H). $^{13}$C-NMR (101 MHz, D$_2$O): $\delta$ 175.75, 174.37, 174.22, 170.65, 56.26, 53.46, 52.78, 49.76, 43.43, 30.81, 25.55, 16.52. UPLC-HRMS (ESI) calcd for C$_{11}$H$_{21}$N$_4$O$_6$ $^+$ 305.1456; found 305.1462 [M+H]$^+$.  

**3-(N-Boc)-β-lactam (31).** 31 was synthesized from L-serine as described in the literature$^9$. All spectral data were as reported.
3-(N-Boc)-1-(N-Cbz)-β-lactam (32). The β-lactam nitrogen of 31 was protected with a Cbz group as outlined in the literature\textsuperscript{10}. All spectral data were as reported.
NMR spectral data of synthetic compounds.
Supplementary References