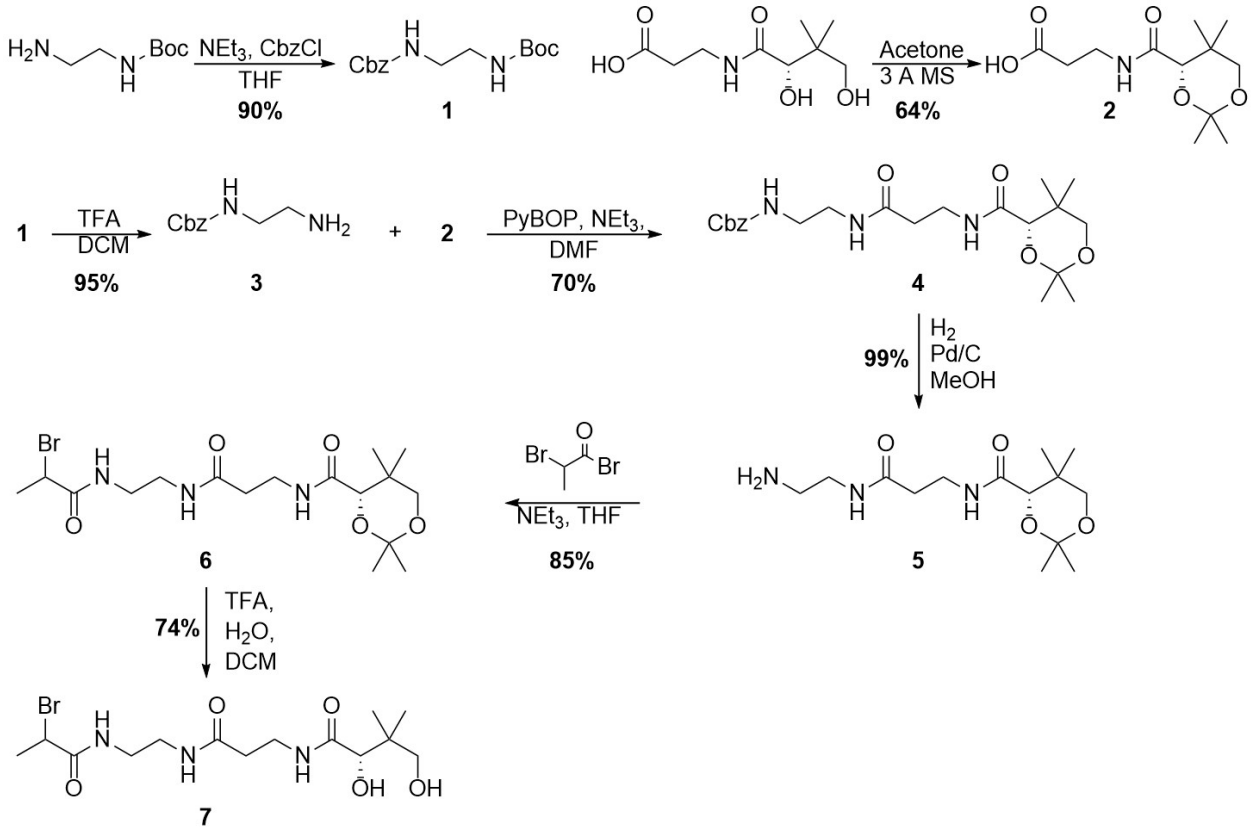
Supplementary Note 1 | Synthetic procedure for the synthesis of α-bromopropionyl-aminopantetheine (7)



Supplementary Scheme 1: ACP to SAT-KS-MAT crosslinker synthesis scheme

All reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated.

Benzyl *tert*-butyl ethane-1,2-diyldicarbamate (1): In a 500 mL round-bottomed flask, *N*-Boc-ethylenediamine (14 mL, 88.5 mmol) was stirred in anhyd. DCM (200 mL) under argon. Triethylamine (16 mL, 113.9 mmol) was added, followed by slow addition of Cbz-Cl (15 mL, 105 mmol). DMAP (1.1 g, 8.9 mmol) was added, and the solution was stirred at room temperature overnight. The reaction was quenched by the addition of a saturated aqueous solution of NH_4Cl (100 mL). The aqueous layer was washed with EtOAc (3×50 mL). The combined organic extracts were dried over anhyd. Na_2SO_4. Crystallization proceeded spontaneously upon concentration on a rotary evaporator and the resulting solid was filtered on a glass frit to give *N*-Boc,*N*'-Cbz-ethylenediamine **1** (23.1 g, 79.7 mmol, 90%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.35-7.33 p.p.m. (m, 5H), 5.09 (s, 2H), 3.29-3.24 (m, 4H), 1.42

(s, 9H). **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$): δ 136.41, 128.44, 128.04, 128.01, 79.46, 66.65, 45.71, 41.38, 40.52, 28.29, 8.51. **UPLC-HRMS** (ESI) calc'd for $C_{15}H_{22}N_2O_4Na^+$ 317.1472; found 317.1471 $[M+Na]^+$.

(*S*)-3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanoic acid (2): *D*-pantothenic acid hemicalcium salt (11 g, 46.2 mmol) and *p*-toluene sulfonic acid hydrate (17 g, 100 mmol) were added to acetone (200 mL) in a flame-dried, round-bottomed flask under argon. Molecular sieves (4 Å, 200 g) were added, and the reaction mixture was stirred vigorously enough to break up the sieves. The reaction was run overnight at room temperature. The sieves were filtered off through a bed of Celite. The solution was concentrated by rotary evaporation and the resulting syrup was redissolved in EtOAc (100 mL). The organic solution was washed with brine (2×50 mL) and dried with anhyd. Na_2SO_4. The solution was concentrated partially *in vacuo*, and hexanes were added. The ketal-protected pantothenic acid **2** (7.64 g, 29.4, 64%) precipitated as a white solid upon concentration to dryness. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.06 p.p.m. (t, J = 5.6 Hz, 1H), 4.10 (s, 1H), 3.67 (d, J = 11.6 Hz, A of AB_q, 1H), 3.61-3.56 (m, 1H), 3.50-3.44 (m, 1H), 3.27 (d, J = 11.6 Hz, B of AB_q, 1H), 2.59 (t, J = 6.0 Hz, 2H), 1.39 (s, 3H), 1.37 (s, 3H), 0.96 (s, 3H), 0.91 (s, 3H). **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$): δ 176.32, 170.22, 99.04, 77.00, 71.35, 34.08, 33.77, 32.92, 29.34, 21.96, 18.78, 18.66. **UPLC-HRMS** (ESI) calc'd for $C_{12}H_{21}NO_5Na^+$ 282.1312; found 282.1312 $[M+Na]^+$.

Benzyl (2-aminoethyl)carbamate (3): To a 250 mL round-bottomed flask containing DCM:TFA (30 mL:15 mL) was added **1** (10 g, 34 mmol). The solution was stirred for 2 h at room temperature. The reaction mixture was diluted with toluene, and concentrated by rotary evaporation three times to give Cbz-ethylenediamine (6.27 g, 32.3 mmol, 95%) as a yellow oil, which was taken directly to the next reaction. The observed spectral data matched those data reported previously[1].

Benzyl (*S*)-(2-(3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido) ethyl) carbamate (4): In a flame-dried, round-bottomed flask, **2** (8 g, 30.6 mmol), **3** (6.6 g, 34 mmol) and triethylamine (9.48 mL, 68 mmol) were dissolved in DCM (300 mL). The solution was cooled to 0 ℃ in an ice bath. PyBOP (19.45 g, 37.4 mmol) was added in one portion. The reaction mixture was stirred for 3 h, and allowed to warm to room temperature. The reaction was quenched by the addition of a saturated aqueous solution of NH_4Cl (150 mL). The aqueous layer was extracted with EtOAc (2×100 mL) and the combined organic extracts were dried over anhyd. Na_2SO_4, followed by concentration by rotary evaporation. A white solid precipitated from the solution and was filtered off. The remaining oil was fractionated by flash silica chromatography (100% EtOAc) to afford pure, diprotected Cbz-aminopantetheine **4** (9.32 g, 21.4 mmol, 70%) as a colorless solid, which was recrystallized from EtOAc/Hexanes. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.25 p.p.m. (m, 5H), 7.05-7.02 (m, 2H), 5.92 (s, 1H), 5.00 (s, 2H), 3.98 (s, 1H), 3.57 (d, J = 11.7 Hz, A of AB_q, 1H), 3.42 (septet, J = 6.6 Hz 2H), 3.30-3.21 (m, 4H), 3.17 (d, J = 11.7 Hz, B of AB_q, 1H), 2.33 (t, J = 6.4 Hz, 2H), 1.38 (s, 3H), 1.32 (s, 3H), 0.92 (s, 3H), 0.88 (s, 3H). **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$): δ 171.69, 170.39, 157.04, 136.37, 128.49, 128.14, 128.10, 99.09, 77.09, 71.32, 66.81, 40.81, 40.13, 36.12, 34.79, 32.90, 29.40, 22.05, 18.81, 18.64. **UPLC-HRMS** (ESI) calc'd for $C_{22}H_{34}N_3O_6^+$ 436.2442; found 436.2443 $[M+H]^+$.

(*S*)-*N*-(3-((2-aminoethyl)amino)-3-oxopropyl)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamide (5): Compound **4** (7 g, 18.4 mmol) was added to MeOH (10 mL) in a 100 mL Parr bomb flask. The reaction mixture and flask were purged with argon. 10% Pd/C (700 mg) was added. The reaction vessel was placed under a H_2 atmosphere (50 bar), and shaken for 3 h. The reaction mixture was filtered over Celite to remove the catalyst and the Celite washed with MeOH (3×10 mL). The filtrate was concentrated by rotary evaporation to afford ketal-protected aminopantetheine **5** (5.5 g, 18.3 mmol, 99%) as a pale yellow oil, which was taken directly to

the next step. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.07 p.p.m. (t, *J* = 8.0 Hz, 1H), 6.88 (s, 1H), 4.04 (s, 1H), 3.65 (d, *J* = 15.6 Hz, A of AB_q, 1H), 3.52-3.46 (m, 3H), 3.42 (s, 1H), 3.38-3.37 (m, 2H), 3.24 (d, *J* = 15.6 Hz, B of AB_q, 1H), 2.86 (t, *J* = 7.5 Hz, 2H), 2.42 (t, *J* = 8.7 Hz, 2H), 1.44 (s, 3H), 1.40 (s, 3H), 0.98 (s, 3H), 0.93 (s, 3H). **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$): δ 171.51, 170.05, 98.90, 76.96, 71.19, 41.54, 41.02, 35.81, 34.86, 32.75 29.26, 21.95, 18.70, 18.51. **UPLC-HRMS** (ESI) calc'd for $C_{14}H_{28}N_3O_4^+$ 302.2074; found 302.2079 $[M+H]^+$.

(4*S*)-*N*-(3-((2-(2-bromopropanamido)ethyl)amino)-3-oxopropyl)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamide (6): In a round-bottomed flask under argon, **5** (1.77 g, 5.87 mmol) was added to DCM (20 mL). To this solution was added triethylamine (1.82 mL, 12.9 mmol). The temperature of the solution was lowered to 0 ℃ in an ice bath. Bromopropionyl bromide (0.645 mL, 6.16 mmol) was added dropwise. The initially milky solution was stirred at 0 ℃ for 1 h and became clear. The reaction was quenched by the addition of a saturated aqueous solution of NH_4Cl (30 mL), and the organic layer was washed with saturated aqueous Na_2CO_3 (15 mL) and brine (15 mL). The organic layer was concentrated by rotary evaporation. The resulting off-white solid was purified by flash silica chromatography (1:9 MeOH:EtOAc) to give 2-bromopropionyl aminopantetheine **6** (2.13 g, 4.87 mmol, 83%) as a colorless solid. The isolated mixture of diastereomers gave the following analytical data: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.11 p.p.m. (s, 1H), 7.02 (t, *J* = 5.6 Hz, 1H), 6.57 (br s, 1H), 4.39/4.38 (2×q, *J* = 7.0 Hz, 1H), 4.09/4.07 (2×s, 1H), 3.68 (d, *J* = 12.0 Hz, A of AB_q, 1H), 3.59-3.52 (m, 2H), 3.43-3.38 (m, 4H), 3.28 (d, *J* = 12.0 Hz, B of AB_q, 1H), 2.46 (t, *J* = 6.2 Hz, 2H), 1.85/1.84 (2×d, *J* = 7.0 Hz, 3H), 1.46 (s, 3H), 1.42 (s, 3H), 1.02 (s, 3H), 0.97/0.96 (2×s, 3H). **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$): δ 172.10/172.07, 170.42, 170.10/170.07, 98.90/98.90, 76.95, 71.12, 43.81/43.80, 40.12/40.05, 38.93, 35.81/35.75, 34.85, 32.74/32.73, 29.26, 22.31/22.82, 21.96/21.95, 18.72, 18.53. **UPLC-HRMS** (ESI) calc'd for $C_{17}H_{31}BrN_3O_5^+$ 436.1442; found 436.1433 $[M+H]^+$.

(2*S*)-*N*-(3-((2-(2-bromopropanamido)ethyl)amino)-3-oxopropyl)-2,4-dihydroxy-3,3-dimethylbutanamide (7): To DCM (2 mL) stirring in a 25 mL round-bottomed flask, **6** (25 mg, 0.06 mmol) was added. 4 mL of deionized water, and 0.1 mL of TFA were added to the reaction mixture. The solution was allowed to stir at room temperature for 60 min. The reaction mixture was diluted with 1:1 H_2O:toluene and concentrated by rotary evaporation three times to remove residual TFA. The resulting pale yellow oil was purified by reverse-phase (C_{18}) flash silica chromatography (gradient: 5-95% ACN in H_2O) to afford 2-bromopropionyl aminopantetheine **7** (15 mg, 1.2 mmol, 63%) as a white solid after lyophilization. **^{1}H NMR** (400 MHz, MeOD): δ 4.47 p.p.m. (q, J = 6.8 Hz, 1H), 3.91 (s, 1H), 3.50-3.47 (m, 2H), 3.48 (d, J = 10.8 Hz, A of AB_q, 1H), 3.40 (d, J = 10.8 Hz, B of AB_q, 1H), 3.36-3.32 (m, 4H), 2.43 (t, J = 6.8 Hz, 2H), 1.77 (d, J = 6.8 Hz, 3H), 0.93 (s, 6H). **^{13}C{^{1}H} NMR** (101 MHz, MeOD): δ 176.00, 174.17, 172.76, 77.30, 70.28, 43.97, 40.32, 40.30, 39.70, 36.58, 36.38, 22.41, 21.35, 20.88. **UPLC-HRMS** (ESI) calc'd for $C_{14}H_{27}BrN_3O_5^+$ 396.1129; found 396.1120 $[M+H]^+$.

References

1 Barker, P. L., Gendler, P. L. & Rapoport, H. Acylation of dibasic compounds containing amino amidine and aminoguanidine functions. *The Journal of Organic Chemistry* **46**, 2455-2465, doi:10.1021/jo00325a006 (1981).