Supporting Information

Inhibition of DD-Peptidases by a Specific Trifluoroketone: Crystal Structure of a Complex with the *Actinomadura* R39 DD-Peptidase

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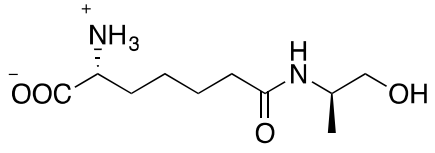
Synthesis of Inhibitors



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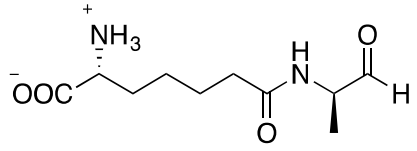

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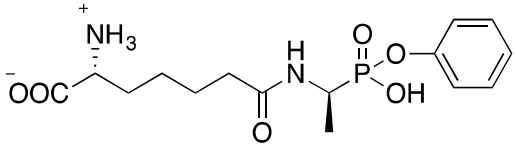

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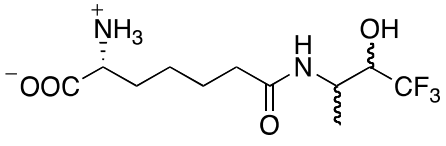

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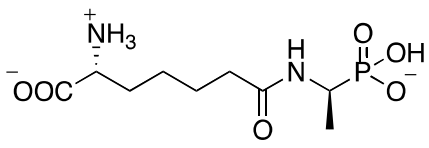

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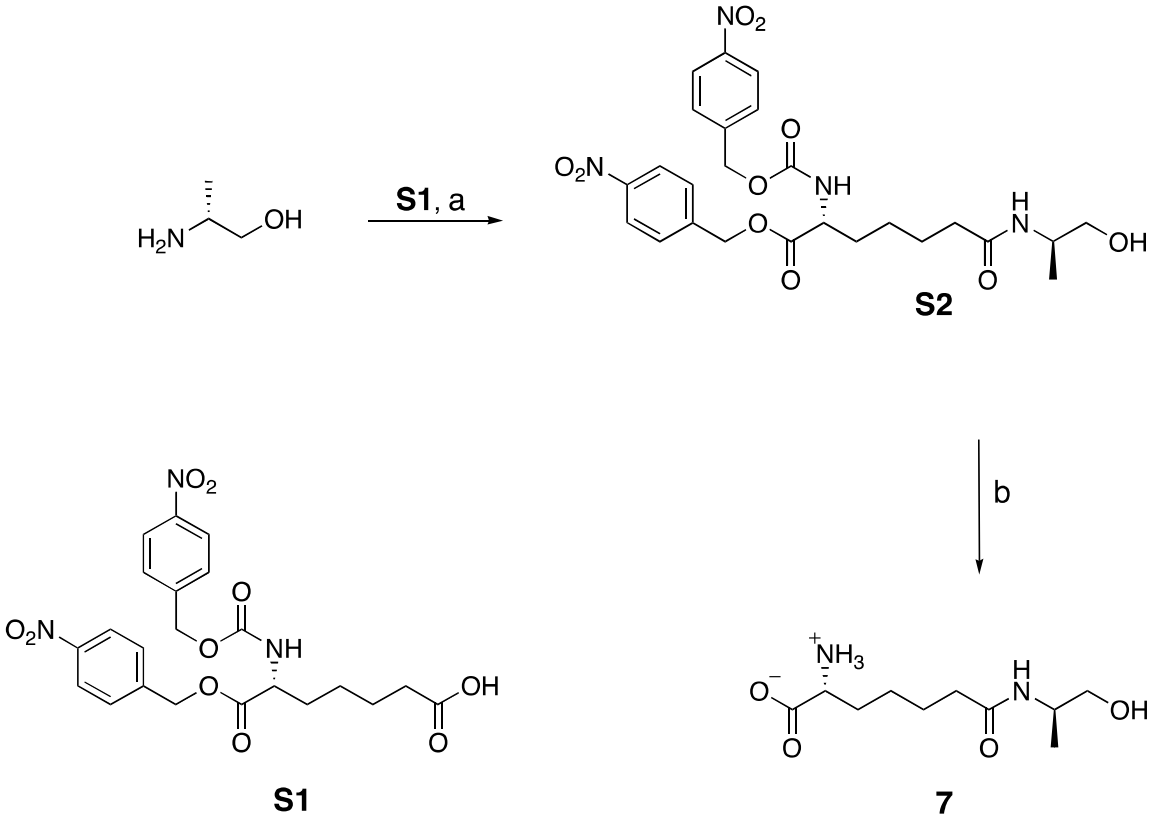
*D-α-Aminopimelyl-D-2-aminopropanol **7** (Scheme S1)*

*p-Nitrobenzyl [N-(p-nitrobenzyloxycarbonyl)]-D-α-aminopimelyl-D-(2-amino) propanol **S2***. To a solution of p-nitrobenzyl-[N-(p-nitrobenzyloxycarbonyl)]-D-α-aminopimelic acid **S1** [prepared as described in ref. 28, main text) (215 mg, 0.44 mmol, 1 eq.) in DCM (4.4 mL) were added sequentially: water (4.4 mL), D-2-aminopropanol (Acros Organics) (33 mg, 0.44 mmol, 1 eq,) and 1-hydroxybenzotriazole (HOBt), (Aldrich) (60 mg, 0.44 mmol, 1 eq.). The mixture was cooled in an ice bath to 0 – 5 °C with stirring. To the resulting mixture, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (93 mg, 0.484 mmol, 1.1 eq.) was added and the subsequent reaction mixture was left stirring for 48 hours at 0 – 5 °C. Aqueous hydrochloric acid (2 M, 1 mL) was added to quench the reaction and the layers were separated. The organic phase was further washed with aqueous hydrochloric acid (0.5 M, 2 mL), brine (2 mL), aqueous sodium bicarbonate (1 M, 2 × 2 mL) and brine (2 × 2 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was removed from the filtrate by evaporation. The crude product was recrystallized from benzene, providing the title compound **S2** as a colorless solid, in 43% yield. ^{1}H NMR (300 MHz, d_6 DMSO), δ: 1.00 (d, 3H, J = 7.5 Hz, CH_3), 1.2 – 1.8 (m, 6H, CH_2 αβγ), 2.02 (t, 2H, J = 7.5 Hz, CH_2 δ), 3.1 -3.4 (m, 2H, CH_2, CH_2OH), 3.75 (m, 1H, CH α), 4.12 (m, 1H, $CH$$CH_3$), 4.64 (t, 1H, J = 6.5 Hz, OH), 5.20 (AB q, 2H), 5.29 (s, 2H), 7.56 (d, 1H, J = 10 Hz, NH), 7.58 (d, 4H, J = 8Hz, Ar), 8.00 (d, 1H, J = 10 Hz, NH), 8.20 (m, 5H, Ar, NH).

Scheme S1.



[a] Reagents and conditions: (a) HOBt, EDC, Et_3N, CH_2Cl_2, H_2O, 0 °C, 48 h, 43%; (b) H_2, 40 psi, 10% Pd on carbon, MeOH, 8 h, 45%.

D-α-Aminopimelyl-D-2-aminopropanol 7. Compound **S2,** dissolved in MeOH, was hydrogenated in the presence of 10% Pd/C for 8 hours under 40 psi of H_2. The product **7** was purified by Sephadex G10 size exclusion chromatography (eluent water) and reverse phase HPLC (0.5% MeOH, 0.03% NH_4HCO_3 in water) and isolated in 45% yield. ^{1}H NMR (300 MHz, D_2O), δ: 0.98 (d, 3H, J = 7.5 Hz, CH_3), 1.2-1.8 (m, 6H, CH_2 αβγ), 2.25 (t, 2H, J = 7.5 Hz, CH_2δ), 3.39 (m, 2H, J = 7.5 Hz, CH_2OH), 3.60 (t, J = 7.5 Hz, 1H, CHα), 4.32 (m, 1H, $CHCH_3$).

D-α-Aminopimelyl-D-2-amino-propanal ***11*** *(Scheme S2)*

N-Cbz-D-Alanyl(N-methoxymethyl)amide ***S3*****.** N-Cbz-D-alanine (ChemImpex) (1 g, 4.5 mmol, 1 eq.) was dissolved in DMF (20 mL) and then HBTU (1.865 g, 4.95 mmol, 1.1 eq.), HOBt (0.75 g, 4.95 mmol, 1.1 eq.), DIEA (2.018 g, 11.7 mmol, 2.6 eq.) and N-methoxymethylamine **36** (1.1 g, 11.25 mmol, 2.5 eq.) were added sequentially to the solution. After being stirred for 2 hours at room temperature the reaction mixture was diluted with ethyl acetate (200 mL) and the separated organic layer washed with 5% $NaHCO_3$ (50 mL), 10 % citric acid, and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent in vacuo provided a colorless solid in 87% yield. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 1.34 (d, 3H, J = 7.2 Hz, $CH$$CH_3$), 3.21 (s, 3H, NCH_3), 3.78 (s, 3H, OCH_3), 5.67 (t, 1H, J = 7.0 Hz, CH), 5.09 (AB q, J = 5.4, 20 Hz, CH_2O), 5.48(d, J=5 Hz, NH), 7.34 (s, 5H, Ar).

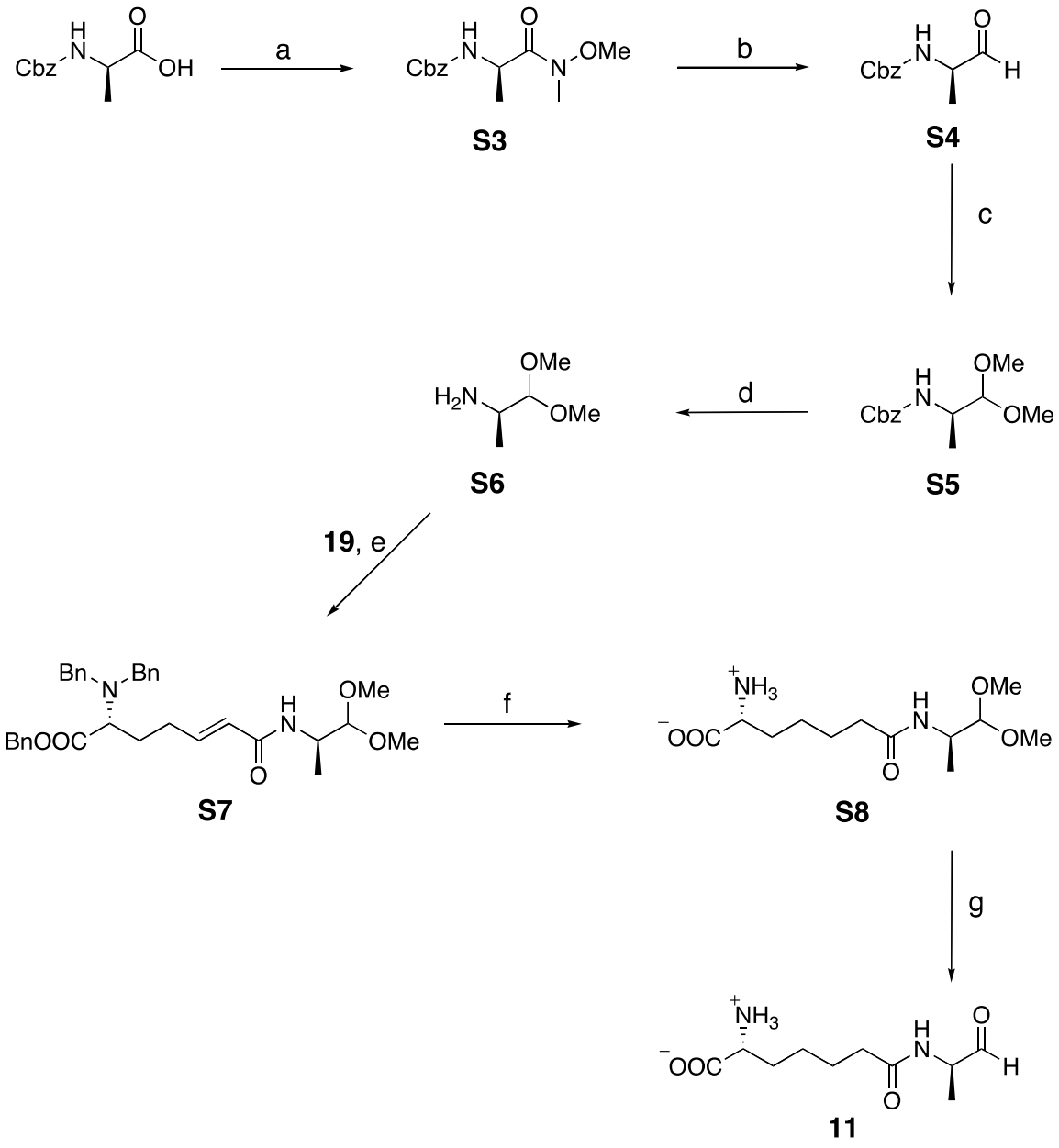
N-Cbz-D-2-aminopropanal ***S4***. Lithium aluminum hydride (1M in THF, 3 eq.) was added dropwise at 0 °C to a solution of **S3** (1.05 g, 3.92 mmol, 1 eq.) in THF (25 mL) under nitrogen atmosphere. The ice bath was removed, and the reaction was allowed to proceed for 30 min to room temperature. The reaction mixture was diluted with diethyl ether (100 mL) and quenched with 0.5 N HCl (20 mL). The aqueous phase was extracted with diethyl ether and the combined organic layers were washed with 1.0 N HCl, dried over sodium sulfate and concentrated in vacuo to provide the title compound as a colorless oil in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 1.37 (d, 3H, J = 7.2 Hz, CH_3), 4.32 (t, 1H, J =7 .0 Hz, CH), 5.08 (AB q, J = 5.4, 20 Hz, CH_2O), 5.4 (s, 1H, NH), 7.34 (s, 5H, Ar), 9.48 (s, 1H, COH).

N-Cbz-D-2-Amino-3,3-dimethoxypropane ***S5***. A solution of **S4** (0.6 g, 2.9 mmol, 1eq.) and p-toluenesulfonic acid (70 mg, 3.5 mmol, 1.2 eq.) in MeOH (70 mL) was refluxed for 6 hours under a Dean-Stark trap. Following concentration in vacuo, the crude product mixture was taken up in ethyl acetate (40 mL), washed with water and brine to yield 89% of **S5**, which was used below without further purification. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 1.12 (d, 3H, J = 7.2 Hz, CH_3), 3.40 (s, 3H, OCH_3), 3.46 (s, 3H, OCH_3), 3.89 (t, 1H, J = 7.0 Hz, CH), 5.08 (AB q, J = 5.4, 20 Hz, CH_2O), 7.34 (s, 5H, Ar).

D-2-Amino-3,3-dimethoxypropane ***S6***. As in a typical procedure, **S5** was dissolved in MeOH (10 mL) and hydrogenated in the presence of 10% Pd on carbon for 12 hours at 40 psi. The product, **S6**, was obtained in 85% yield and used without purification. ^{1}H NMR (300 MHz, D_2O) δ: 1.00 (d, 3H, J = 7.2 Hz, CH_3), 3.00 (t, 1H, J = 7.2 Hz, CH), 3.34 (s, 3H, OCH_3), 3.36 (s, 3H,OCH_3), 4.23 (d, 1H, J = 7.5 Hz, $CH(OMe)_2$).

D-1-[6-(N,N-Dibenzylamino)-6-(benzyloxycarbonyl)-trans-hex-2-enoylamino]-D-2-amino-3,3-dimethoxypropane ***S7***. To a stirred solution of D-1-[6-(N,N-dibenzylamino)-6-(benzyloxycarbonyl)-trans-hex-2-enoic acid, **19**, (133 mg, 0.3 mmol, 1 eq.) and *D-2-amino-3,3-dimethoxypropane* **S6** (35 mg, 0.3 mmol, 1 eq.) in DCM (5 mL), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (58 mg, 0.3 mmol, 1 eq.) and 1-hydroxybenzotriazole (405 mg, 0.3 mmol, 1 eq.) were added. The reaction mixture was stirred for 24 hours at 0 – 5 °C. Aqueous citric acid (0.1 M, 1 mL) was added to the reaction and the layers were separated. The organic phase was further washed with aqueous citric acid (0.5 M, 2 mL), brine (2 mL), aqueous sodium bicarbonate (5%, 2 × 2 mL) and brine (2 × 2 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and the filtrate concentrated to dryness by evaporation under reduced pressure. The product **S7** was

Scheme S2.



[a] Reagents and conditions: (a) MeNHOMe, HBTU, HOBt, DIEA, DMF, 25 °C, 2 h, 87% (b) $LiAlH_4$, THF, 0 °C, 30 min, 80% (c) TsOH (cat.), MeOH, reflux, 6 h, 89 % (d) H_2, 40 psi, Pd on carbon, 10% w/w, MeOH, 12 h, 85% (e) **19**, HOBt, EDC, CH_2Cl_2, 79% (f) H_2, Pd/C, MeOH,12h, 85% (g) 1N HCl, D_2O, 2 h, 100%.

obtained as a colorless gum in 79% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.12 (d, 3H, *J* = 7.2 Hz, CH_3), 1.75 – 1.98 (m, 2H), 2.06 – 2.20 (m, 1H), 2.24 – 2.36 (m, 1H), 3.32 (t, 1H, *J* = 7.5 Hz, CH), 3.40 (s, 3H, OCH_3), 3.46 (s, 3H, OCH_3), 3.53, 3.87 (AB q, *J* = 13.5 Hz, 4H), 3.95 (m, 1H), 5.19, 5.25 (AB q, *J* = 10.8 Hz, 2H), 5.75 (d, J = 16 Hz, 1H), 6.70 (quint, J = 7.2 Hz, 1H), 7.2 – 7.4 (m, 15H).

D-α-Aminopimelyl-D-2-amino-3,3-dimethoxypropane ***S8***. The product from above, **S7**, (40 mg, 0.15 mmol) was hydrogenated overnight at 40 psi in MeOH (10 mL) with 10% Pd on carbon. The solution was then filtered and the filtrate was evaporated to dryness to give a colorless solid. The crude product was dissolved in water and the solution washed three times with ethyl acetate. The final aqueous solution was freeze-dried to give a colorless solid. The product was further purified on a C_{18} reverse phase HPLC column (0.5% MeOH, 0.03% NH_4HCO_3 in water) in 85 % yield. ^{1}H NMR (300 MHz, D_2O), δ: 0.94 (d, 3H, *J* = 6.6 Hz), 1.23 (m, 2H), 1.46 (m, 2H), 1.70 (m, 2H), 2.09 (t, 2H, *J* = 7.5 Hz), 3.27 (s, 3H), 3.29 (s, 3H), 3.55 (m, 1H), 3.89 (m, 1H), 4.18 (d, 1H, *J* = 5.1 Hz).

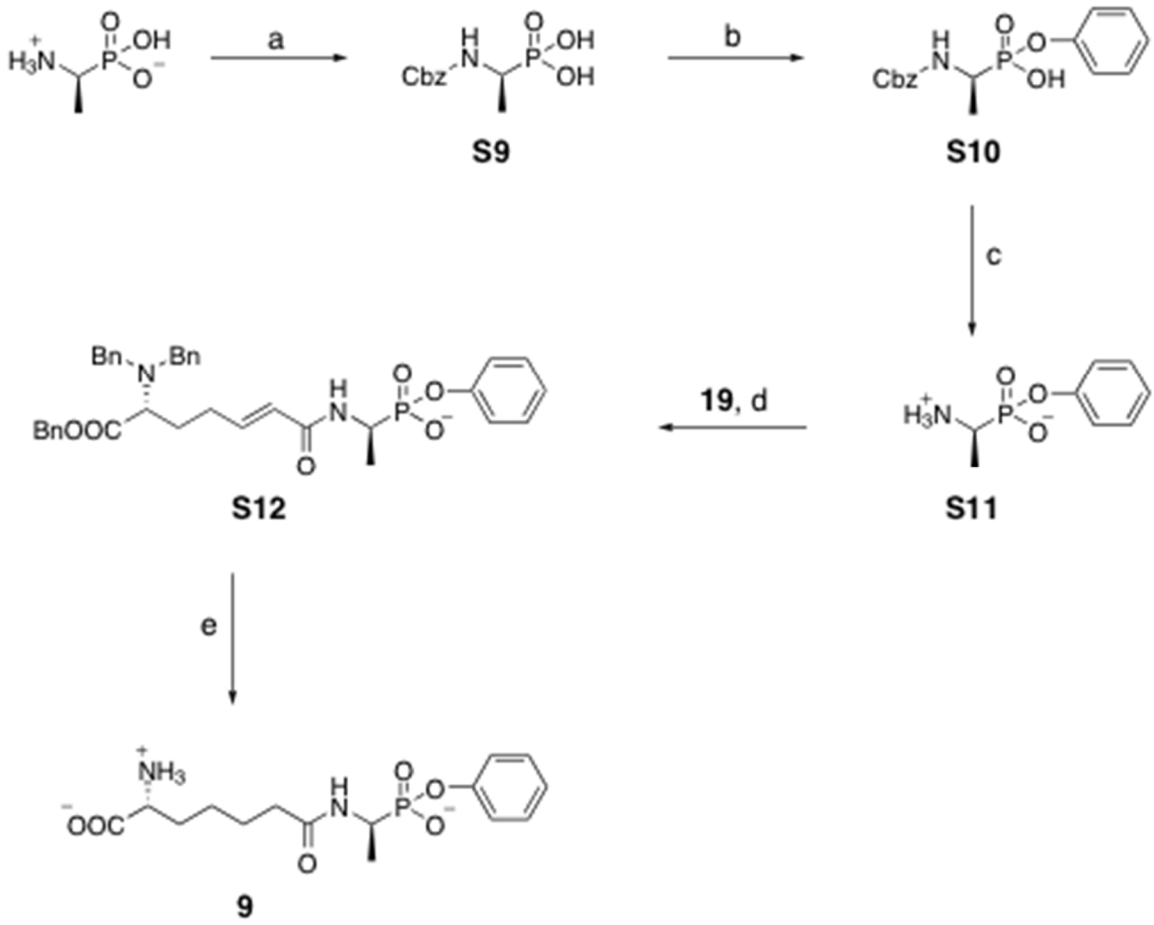
D-α-Aminopimelyl-D-2-amino-propanal ***11***. The dimethyl acetal **S8** was hydrolyzed to afford the aldehyde **11**. Thus, **S8** (1.5 mg) was dissolved in 0.1 N hydrochloric acid (0.5 mL) and the solution stirred for 1 hour. The resulting solution was freeze-dried, producing the title compound in quantitative yield. ^{1}H NMR (300 MHz, D_2O), δ: 0.94 (d, 3H, *J* = 6.6 Hz), 1.23 (m, 2H), 1.46 (m, 2H), 1.70 (m, 2H), 2.09 (t, 2H, *J* = 7.5 Hz), 3.55 (m, 1H), 3.89 (m, 1H). HRMS (ESI) $[M+H]^+$ found 231.1349 (calc. 231.1345).

Phenyl D-α-aminopimelyl-D-1-aminoethylphosphonate **9** *(Scheme S3)*

D-1-N-Cbz-aminoethylphosphonic acid ***S9***. A mixture of D-1-aminoethyl phosphonic acid (Aldrich) (0.5 g, 4 mmol, 1 eq.), sodium bicarbonate (1.1 g, 13.2 mmol, 3.3. eq.),

potassium carbonate (0.77 g, 5.6 mmol, 1.4 eq.) and benzyl chloroformate (0.9 g, 5.6 mmol, 1.4 eq.) was stirred at room temperature for 24 hours. Water was added to bring

Scheme S3.



[a] Reagents and conditions: (a) $PhCH_2COCl$, $NaHCO_3$, K_2CO_3, 25 °C, 24 h, 57% (b) $(PhO)_3P$, Py, 120 °C, 8 h, 66% (c) H_2, 40 psi, 10% Pd on carbon, 24 h, 87% (d) **19**, HOBt, EDC, Et_3N, H_2O, DMF, 0 °C, 24 h, 84% (e) H_2, 40 psi, 10% Pd on carbon, 24 h, 87%.

the total volume to 50 ml and the pH was adjusted to 2 with concentrated HCl. An additional 5 mL of concentrated HCl was added and the mixture was evaporated to dryness. The residue was extracted twice with hot 1/1 v/v MeOH/CH_3CN. The hot

solution was filtered and the solvent evaporated from the filtrate. The residue was purified on a Dowex 50W cation-exchange column, affording the product as a yellowish solid in 57% yield. ^{1}H NMR (300 MHz, D_2O), δ: 1.15 (AX q, 3H, *J* = 8 Hz), 3.69 (m, 1H), 4.90 (AB q, 2H, *J* = 6, 12 Hz), 7.27 (s, 5H).

Phenyl D-1-N-Cbz-aminoethylphosphonate ***S10***. Triphenyl phosphite (1.8 g, 5.81 mmol, 3 eq.) was added to a solution of **S9** (0.5 g, 1.9 mmol, 1 eq.) in pyridine (26 mL). The reaction mixture was then heated under reflux in an oil bath at 120 °C for 8 hours, after which the pyridine was removed by evaporation with application of a vacuum pump. The residue was dissolved in minimal amount of water and the solution acidified with concentrated HCl and cooled in an ice bath. The precipitated product **S10** was collected by filtration as colorless solid in 66% yield. ^{1}H NMR (300 Hz, D_2O), δ: 1.18 (AX q, 3H, *J* = 8 Hz), 3.77 (m, 1H), 4.90 (AB q, 2H, *J* = 6, 12 Hz), 7.0 (d, 2H, *J* = 10 Hz), 7.10 (t, 1H, *J* = 10 Hz), 7.20 (t, 2H, *J* = 10 Hz), 7.3 (s, 5H).

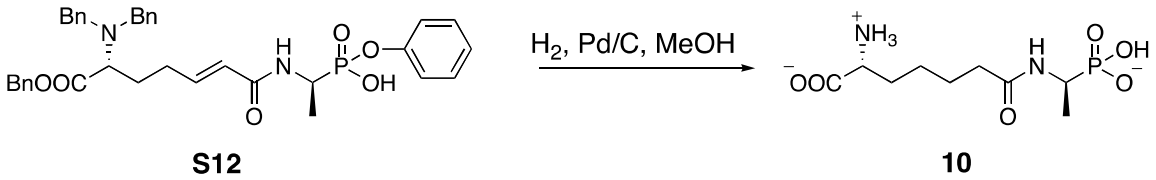
Phenyl D-1-aminoethylphosphonate ***S11***. A solution of **S10** (117 mg, 0.38 mmol) in MeOH (5 mL) was hydrogenated in the presence of 10% Pd on carbon (13 mg) at 40 psi for 24 hours. The solvent was removed in vacuo yielding the product **S11** as colorless solid in 87% yield. ^{1}H NMR (300 MHz, D_2O), δ: 1.42 (AX q, 3H, J = 10 Hz), 3.48 (m, 1H), 7.06 (d, 2H, *J* = 7.5 Hz), 7.10 (t, 1H, *J* = 7.5 Hz), 7.29 (t, 2H, *J* = 7.5 Hz).

Phenyl D-1-[6-(N,N-Dibenzylamino)-6-(benzyloxycarbonyl)-trans-hex-2-enoylamino]-D-1-aminoethylphosphonate ***S12***. To a solution of D-1-[6-(N,N-dibenzylamino)-6-(benzyloxycarbonyl)-trans-hex-2-enoic acid, **19**, (133 mg, 0.3 mmol, 1 eq.) in DMF (2 mL) , **S11** (60 mg, 0.3 mmol, 1 eq.), dissolved in water (1 mL), was added at 0 – 5 °C. To this mixture, triethylamine (100 μL, 0.6 mmol, 2 eq.) was added, followed by

1-hydroxybenzotriazole (60 mg, 0.3 mmol, 1 eq.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (116 mg, 0.6 mmol, 2 eq.) at 0 – 5 °C and the mixture stirred for 24 hours. Solvent was removed by evaporation under reduced pressure and the residue was dissolved in ethyl acetate (10 mL) and the resulting solution filtered. The filtrate was washed with 0.1 N hydrochloric acid (3 × 1 mL) and brine and concentrated in vacuo. The product **S12** was thus obtained as a yellow oil in 84 % yield. ^{1}H NMR (300 MHz, d_6 DMSO, d_6), δ: 1.20 (AX q, 3H, *J* = 7.5 Hz), 1.8– 2.1 (m, 3H), 2.2 – 2.36 (m, 1H), 3.26 (m, 1H), 3.57, 3.7 (AB q, 4H, *J* = 15 Hz), 4.20 (m, 1H), 5.20 (AB q, 2H, *J* = 15, 22.5 Hz), 6.00 (d, 1H, *J* = 15 Hz), 6.52 (m, 1H), 7.1-7.8 (d, 20H), 7.9 (d, 1H, *J* = 9 Hz).

*Sodium phenyl D-α-aminopimelyl-D-1-aminoethylphosphonate **9***. Prior to deprotection, the starting material **S12** was converted into its sodium salt: **S12** was dissolved in water with a stochiometric amount of sodium bicarbonate and the solution freeze-dried. The salt (100 mg, 0.15 mmol) was dissolved in methanol (5 mL) and hydrogenated in the presence of 10% Pd on carbon (10 mg) at 40 psi for 24 hours. The solvent was removed in vacuo and the crude product was purified on a Sephadex G10 size-exclusion chromatography column (eluent water) and then on a C_{18} reverse phase HPLC column (0.5% MeOH, 0.03% NH_4HCO_3 in water). The required product **9** was isolated in 40% yield. ^{1}H NMR (300 Hz, D_2O), δ: 1.2 (AX q, 3H, J = 10 Hz), 1.2-1.8 (m, 6H), 2.2 (t, 2H, J = 7Hz), 3.66 (m, 1H), 3.94 (m, 1H), 7.06 (d, 2H, *J* = 7.5 Hz), 7.10 (t, 1H, *J* = 7.5 Hz), 7.29 (t, 2H, *J* = 7.5 Hz). ^{31}P NMR (300 MHz, D_2O) ppm: 15.74.

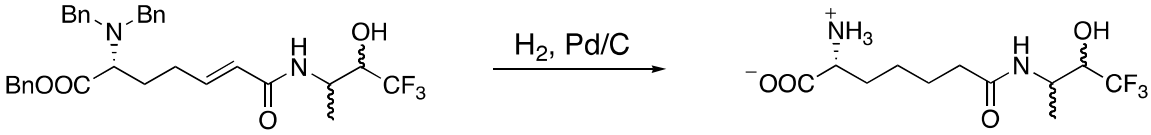
Scheme S4.



S12 **10**

*Sodium D-α-aminopimelyl-D-1-aminoethylphosphonate **10***. This compound was prepared in a similar fashion to **9** starting, however, from **S12** in the acidic form. It was purified on a Sephadex G10 size-exclusion chromatography column (eluent water) and then on a C_{18} reverse phase HPLC column (0.5% MeOH, 0.03% NH_4HCO_3 in water). The product, **10**, was isolated in 40% yield. ^{1}H NMR (300 MHz, D_2O), ppm: 1.1 (AX q, 3H, J = 10 Hz), 1.2-1.8 (m, 6H), 2.2 (t, 2H, J = 7Hz), 3.60 (m, 1H), 3.94 (m, 1H). ^{31}P NMR (300 MHz, D_2O) ppm: 16.87.

Scheme S5.



22 **8**

*D-α-Aminopimelyl-(1,1,1-trifluoro-3-amino)-2-butanol **8** (Scheme S5)*. This compound was prepared starting from D-1-[6-(N,N-dibenzylamino)-6-(benzyloxycarbonyl)-trans-hex-2-enoyl-3-amino-1,1,1-trifluoro-2-butanol **22** (main text) by catalytic hydrogenation in a similar fashion as in the preparation of **11**. The crude product was purified by passage twice through a Sephadex G10 size-exclusion chromatography column (eluent water). The product was isolated as a colorless solid in 30% yield. ^{1}H NMR (400 MHz, D_2O), α: 1.05 (d,3H, *J* = 7.2 Hz), 1.23 (m, 2H), 1.48 (m, 2H), 1.70 (m, 2H), 2.10 (t, 2H, *J*

= 7.2 Hz), 3.57 (t, 1H, *J* = 6 Hz), 3.93 (m, 1H), 4.05 (m, 1H). ^{19}F NMR (300 MHz, D_2O) ppm -75.17, -75.20, -75.20, -75.22 (CF_3, mixture of 4 diastereoisomers).