**L,D-Transpeptidase Assay:** Purified *M. tuberculosis* L,D-Transpeptidase (5 μg each) was incubated with a peptide substrate (23-26) (5 mM), 14C-D-Ala (180 μM) in a 10 μL reaction containing sodium cacodylate buffer (10 mM, pH 6.0) and Triton X-100 (0.1 % v/v) at 37 °C for 1 h. The reaction was terminated by boiling at 100 °C for 3 min. An aliquot (1.0 μL) of this reaction was analyzed on a cellulose TLC plate using methanol:0.12 M DMAP in H₂O, pH 6.0 (2:1, v/v) solvent system, and visualized by phosphor-imaging.

Reagents and solvents were purchased from Sigma Aldrich and Fisher Scientific. Protected amino acids were purchased from Bachem and used without further purification. THF was distilled from sodium metal and benzophenone and CH₂Cl₂ was distilled from CaH₂. Solvent mixtures are reported as a volume ratio. LCMS was performed on an Agilent 1100 system equipped with G1946D SL Mass Selective Detector (positive mode electrospray ionization), G1315A DAD detector, and Phenomenex Luna (2) 2 × 50mm 3μm 100Å C₁₈ column. For analysis of the final compounds (23-26), the following method was used: flow rate 0.3 mL/min of 90% H₂O (containing 0.1% formic acid) and 10% MeCN (containing 0.1% formic acid). The DAD was monitored at 270 nm and 310 nm. ESI monitored masses in positive ionization mode from 60 to 2000 Da. NMR spectra were obtained on a Varian Mercury 300 MHz spectrometer.
(R)-benzyl (1-amino-1-oxopent-4-en-2-yl)carbamate (7). A solution of \(3^2\) (1.42 g, 5.70 mmol) in THF (25 mL) was cooled to 0 °C, treated dropwise with Et\(_3\)N (0.83 mL, 5.98 mmol), ethyl chloroformate (0.57 mL, 5.98 mmol) and then warmed to 25 °C for 2 h. The solution was cooled to 0 °C, treated with NH\(_4\)OH (28%, 1.0 mL), stirred for 0.5 h and then warmed to 25 °C for 2 h. The mixture was diluted with ethyl acetate (300 mL), washed with H\(_2\)O (100 mL), 1 M aqueous HCl (100 mL), saturated aqueous NaHCO\(_3\) (100 mL), saturated aqueous NaCl (100 mL), dried (Na\(_2\)SO\(_4\)) and concentrated in vacuo. The residue was dissolved in a minimal amount of Et\(_2\)O and precipitated by the slow addition of hexanes to provide 7 (1.08 g, 76%) as a white solid: mp. 103-104 °C (Et\(_2\)O-hexanes); [\(\alpha\)]\(_D\) = -1 (c = 1.0, CH\(_2\)Cl\(_2\)); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.35 (5H, comp), 6.11 (1H, br s), 5.84-5.70 (1H, m), 5.67 (1H, br s), 5.40-5.30 (1H, m), 5.18 (1H, d, \(J = 5.4\) Hz), 5.14 (1H, s), 5.12 (2H, s), 4.32-4.20 (1H, m), 2.54 (2H, t, \(J = 5.7\) Hz); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 166.6, 164.3, 132.8, 129.9, 128.8, 128.5, 128.3, 119.7, 67.5, 50.8, 36.9; HRMS m/z 249.1246 (M + H\(^+\), C\(_{13}\)H\(_{16}\)N\(_2\)O\(_3\) requires 249.1239).

(R)-2-acetamidopent-4-enamide (8). A solution of \(3^3\) (0.500 g, 3.18 mmol) in THF (10 mL) was cooled to 0 °C, treated dropwise with Et\(_3\)N (0.460 mL, 3.34 mmol), ethyl chloroformate (0.320 mL, 3.34 mmol), and then warmed to 25 °C for 2 h. The solution was cooled to 0 °C, treated with NH\(_4\)OH (28%, 1.0 mL), stirred for 30 minutes and then warmed to 25 °C. The mixture was diluted with ethyl acetate (300 mL), washed with H\(_2\)O (100 mL), 1 M aqueous HCl (100 mL), saturated aqueous NaHCO\(_3\) (100 mL), saturated aqueous NaCl (100 mL), dried (Na\(_2\)SO\(_4\)) and concentrated in vacuo. The residue was purified by flash chromatography (SiO\(_2\), 5% MeOH-CH\(_2\)Cl\(_2\)) to afford 8 (0.313 g, 63%) as a white solid: mp. 169-170 °C (Et\(_2\)O-hexanes); [\(\alpha\)]\(_D\) = -0.2 (c = 1.0, CH\(_3\)OH); \(^1\)H NMR (300 MHz, CD\(_3\)OD) \(\delta\) 5.86-5.72 (1H, m), 5.17-5.06 (2H, comp), 4.39 (1H, dd, \(J = 8.1, 5.1\) Hz), 2.60-2.51 (1H, m), 2.43-2.33 (1H, m); \(^{13}\)C NMR (75 MHz, CD\(_3\)OD) \(\delta\) 176.5, 173.4, 134.9, 118.6, 54.3, 37.6, 22.6; HRMS m/z 157.0974 (M + H\(^+\), C\(_7\)H\(_{12}\)N\(_2\)O\(_2\) requires 157.0977).

(R)-benzyl 2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(trityloxy)butanamido)propanoate (S1). A solution of 9 (1.00 g, 1.71 mmol) in DMF (10.0 mL) was cooled to 0 °C and treated sequentially with EDCI (0.361 g, 1.88
mmol), HOBt (0.255 g, 1.88 mmol) and D-Ala-OBn p-TsOH salt (10, 0.602 g, 2.11 mmol). The solution was warmed to 25 °C and stirred 14 h. The mixture was poured over saturated aqueous NaCl (100 mL) and extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with 1 M aqueous HCl (100 mL), saturated aqueous NaHCO3 (100 mL), saturated aqueous NaCl (2 × 100 mL), dried (Na2SO4) and concentrated in vacuo. The residue was purified by flash chromatography (SiO2, 25% ethyl acetate-hexanes) to afford S1 (0.630 g, 50%) as a white solid: mp. 129-130 °C (Et2O-hexanes); [α]D = −12 (c = 1.0, CH2Cl2); 1H NMR (300 MHz, CDCl3) δ 7.76 (2H, d, J = 7.5 Hz), 7.53 (2H, dd, J = 7.2, 2.7 Hz), 7.42-7.37 (8H, comp), 6.80-6.72 (1H, m), 6.00-5.90 (1H, m), 5.14 (2H, dd, J = 20.1, 12.3 Hz), 4.52 (1H, quintet, J = 7.2 Hz), 4.42-4.31 (2H, comp), 4.22 (1H, t, J = 5.1 Hz), 3.74-3.66 (2H, m), 2.10-1.94 (1H, m), 1.92-1.80 (2H, comp), 1.42 (3H, d, J = 6.9 Hz); HRMS m/z 503.2180 (M + H+, C29H30N2O6 requires 503.2182).

(R)-benzyl 2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-hydroxybutanamido)propanoate (11). A solution of S1 (0.630 g, 0.846 mmol) in CH2Cl2 (10 mL) was cooled to 0 °C and treated with TFA (0.5 mL). The solution was warmed to 25 °C and stirred for 1 h. The mixture was concentrated in vacuo and purified by flash chromatography (SiO2, 25-33% ethyl acetate-hexanes) to afford 11 (0.220 g, 52%) as a white solid: mp. 59-60 °C (Et2O-hexanes); [α]D = −8 (c = 0.37, CH2Cl2); 1H NMR (300 MHz, CDCl3) δ 7.77 (2H, d, J = 6.9 Hz), 7.59 (2H, d, J = 6.9 Hz), 7.41 (2H, t, J = 7.8 Hz), 7.35-7.22 (7H, m), 6.72 (1H, d, J = 6.6 Hz), 5.88 (1H, d, J = 9.0 Hz), 5.17 (2H, d, J = 5.1 Hz), 4.63 (1H, t, J = 7.5 Hz), 4.52-4.32 (3H, comp), 4.22 (1H, t, J = 7.2 Hz), 3.74-3.66 (2H, m), 2.10-1.94 (1H, m), 1.92-1.80 (2H, comp), 1.42 (3H, d, J = 6.9 Hz); HRMS m/z 503.2180 (M + H+, C29H30N2O6 requires 503.2182).

(R)-benzyl 2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)but-3-enamido)propanoate (12). A solution of 11 (0.220 g, 0.438 mmol) in THF (5.0 mL) was cooled to 0 °C and treated dropwise with Bu3P (0.220 mL, 0.875 mmol), and 2-nitrophenylselenocyanate (0.199 g, 0.875 mmol). The solution was warmed to 25 °C and stirred for 1 h. The solution was then cooled to 0 °C, treated with H2O2 30% solution (4.0 mL) and stirred for 14 h. The mixture was poured over saturated aqueous NaCl (100 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with saturated aqueous NaCl (100 mL), dried (Na2SO4) and concentrated in vacuo. The
residue was purified by flash chromatography (SiO2, 25-50% ethyl acetate-hexanes) to afford 12 (0.0579 g, 27%) as a white solid that was identical in all respects to the compound reported in the experimental section.

(2R,6S,E)-benzyl 6-(((9H-fluoren-9-ylmethoxy)carbonyl)amino)-7-(((R)-1-(benzyloxy)-1-oxopropan-2-yl)amino)-2-(((benzyloxy)carbonyl)amino)-7-oxohept-4-enoate (15). A solution of 12 (0.250 g, 0.516 mmol) in CH2Cl2 (5.0 mL) was treated with 6 (0.350 g, 1.03 mmol) and Grubbs Second Generation Catalyst (0.087 g, 0.103 mmol), then Ar (g) was bubbled through the solution. The vial was capped and heated to 70 °C for 48 h. The solution was cooled to 25 °C, treated with DMSO (0.5 mL), and stirred for 8 h. The solution was diluted with CH2Cl2 (100 mL), washed with water (2 x 20 mL), dried (Na2SO4), concentrated in vacuo, and purified by flash chromatography (SiO2, 50% Et2O-hexanes) to provide 15 (0.156 g, 38%) as a clear oil: [α]D = +14 (c = 1.0, CH2Cl2); 1H NMR (300 MHz, CDCl3) δ 7.75 (2H, d, J = 7.2 Hz), 7.58 (2H, d, J = 7.5 Hz), 7.41-7.21 (19H, comp), 6.70-6.60 (1H, m), 5.78-5.79 (1H, m), 5.68-5.62 (1H, m), 5.54-5.38 (1H, m), 5.16-5.11 (4H, comp), 5.06 (2H, d, J = 2.4 Hz), 4.56 (1H, t, J = 6.6 Hz), 4.50-4.40 (1H, m), 4.40-4.32 (2H, comp), 4.23-4.18 (1H, m), 2.62-2.52 (1H, m), 2.52-2.40 (1H, m), 1.40 (3H, d, J = 6.9 Hz); 13C NMR (300 MHz, CDCl3) δ 172.5, 171.3, 169.1, 156.1, 155.9, 144.1, 144.0, 143.8, 141.5, 135.2, 130.6, 130.3, 128.9, 125.84, 128.78, 128.76, 128.7, 128.5, 128.38, 128.36, 127.9, 127.3, 125.3, 120.2, 67.7, 67.4, 67.2, 57.1, 54.1, 48.9, 47.4, 35.9, 29.9, 17.9; HRMS m/z 796.3209 (M + H+, C47H45N3O9 requires 796.3194).

A solution of 15 (0.043 g, 0.054 mmol) in ethyl acetate-MeOH-acetic acid (2:1:1, 4.0 mL) was treated with 10% Pd/C (0.010 g), and stirred under a hydrogen atmosphere (1 atm) at 25 °C for 2 h. The mixture was filtered through Celite, concentrated in vacuo, and dried azeotropically with toluene. The residue was dissolved in EtOH:H2O (1:1, 4.0 mL), treated with aqueous 1 M HCl (0.10 mL), filtered, and concentrated to dryness in vacuo. The residue was washed with 10% Et2O-hexanes to afford 19 (0.0186 g, 67%) as a beige film: [α]D = +3 (c = 1.0, CH3OH); 1H NMR (300 MHz, CD3OD) δ 7.80 (2H, d, J = 7.5 Hz), 7.73-7.76 (2H, comp), 7.40 (2H, t, J = 6.9 Hz), 7.32 (2H, t, J = 7.2 Hz), 4.42-4.32
(2R,6S)-2,6-diamino-7-(((R)-1-carboxyethyl)amino)-7-oxoheptanoic acid trifluoroacetic acid salt (23). Compound 19 (4.7 mg, 9.0 µmol) was suspended in DMF (0.01 M, 171 µL) treated with piperidine (9.0 µL), and stirred for 0.5 h. The mixture was concentrated under a stream of N₂ (g). The crude solid was dissolved in H₂O (800 µL), loaded onto a Waters Sep-Pak® (C₁₈, 500 mg cartridge) preconditioned with a mixture of MeCN (5 mL) and 0.1% aqueous TFA (15 mL). The loaded sample was washed with 0.1% aqueous TFA (5 mL), and the product was eluted with 60% aqueous MeCN. The eluted fractions were combined, concentrated in vacuo to remove MeCN, and lyophilized to dryness to provide 23 as a white solid (3.1 mg, 8.3 µmol, 84%): LCMS: retention time 1.41 min; (ESI⁺) m/z 262 (M + H⁺).

(R)-benzyl 2-((2S,6R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-7-amino-6-(((benzyloxy)carbonyl)amino)-7-oxoheptanamido)propanoate (17). A solution of 12 (0.375 grams, 0.773 mmol) in ClCH₂CH₂Cl (5.0 mL) was treated with 7 (0.384 g, 1.55 mmol), Grubbs Second Generation Catalyst (0.131 g, 0.155 mmol), and then Ar (g) was bubbled through the solution. The vial was capped and heated to 70 °C for 48 h. The solution was cooled to 25 °C, treated with DMSO (0.5 mL) and stirred for 8 h. The solution was diluted with CH₂Cl₂ (100 mL), washed with water (2 x 20 mL), dried (Na₂SO₄), concentrated in vacuo and purified by flash chromatography (SiO₂, 5% MeOH-CH₂Cl₂). The combined fractions were concentrated, suspended in Et₂O and precipitated with the addition of hexanes to afford 17 (0.0841 g, 15%) as a white solid collected by filtration, and additional material obtained from concentration of the mother liquor (0.211 g, 39%): mp. 173-174 °C (Et₂O-hexanes); [α]D = +17 (c = 0.5, CH₃OH); ¹H NMR (300 MHz, CD₃OD) δ 7.78 (2H, d, J = 7.5 Hz), 7.63 (2H, br s), 7.38 (4H, t, J = 7.5 Hz), 7.34-7.22 (10H, comp), 5.82-5.70 (1H, m), 5.60 (1H, dd, J = 10.2, 5.7 Hz), 5.13 (2H, s), 5.05 (2H, s), 4.69-4.65 (1H, m), 4.39 (1H, q, J = 7.5 Hz), 4.35 (2H, d, J = 7.2 Hz), 4.23-4.13 (2H, comp), 2.58-2.45 (1H, m), 2.44-2.31 (1H, m), 1.36 (3H, d, J = 7.2 Hz).
Hz): $^{13}$C NMR (75 MHz, CD$_3$OD) $\delta$ 176.7, 173.9, 172.8, 171.4, 170.2, 145.44, 145.36, 142.8, 138.1, 130.8, 130.3, 129.7, 129.6, 129.4, 129.3, 129.2, 129.1, 128.9, 128.4, 126.37, 126.35, 121.1, 68.2, 68.1, 68.0, 64.1, 58.2, 55.9, 36.4, 24.0, 17.5; HRMS m/z 705.2917 (M + H$^+$, C$_{40}$H$_{40}$N$_4$O$_8$ requires 705.2924).

(R)-2-((2S,6R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6,7-diamino-7-oxoheptanamido)propanoic acid hydrochloride (21). A solution of 17 (0.0300 g, 0.0426 mmol) in ethyl acetate-MeOH-acetic acid (1:1:1, 3.0 mL) was treated with 10% Pd/C (0.005 g) and stirred under a hydrogen atmosphere (1 atm) at 25 °C for 2 h. The mixture was filtered through Celite and concentrated in vacuo. The residue was dissolved in EtOH:H$_2$O (1:1, 4.0 mL), treated with aqueous 1 M HCl (0.10 mL), filtered, and concentrated to dryness. The residue was suspended in a minimal amount of Et$_2$O, and the white solid collected by filtration to afford 21 (0.0204 g, 92%), as a white solid: mp. 247-248 °C (dec., Et$_2$O); $[\alpha]_D = +4$ (c = 0.5, CH$_3$OH); $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 7.80 (2H, d, $J = 7.5$ Hz), 7.69-7.63 (2H, m), 7.40 (2H, t, $J = 7.2$ Hz), 7.32 (2H, t, $J = 7.5$ Hz), 4.48-4.41 (1H, m), 4.37 (2H, d, $J = 7.2$ Hz), 4.24 (1H, t, $J = 6.6$ Hz), 4.15 (1H, t, $J = 5.7$ Hz), 3.85 (1H, t, $J = 6.6$ Hz), 1.92-1.79 (4H, comp), 1.56-1.45 (2H, comp), 1.39 (3H, d, $J = 7.2$ Hz); $^{13}$C NMR (75 MHz, CD$_3$OD) $\delta$ 175.9, 174.3, 172.3, 158.6, 145.4, 142.8, 129.0, 128.4, 126.3, 121.1, 68.2, 56.1, 54.2, 49.6, 33.0, 32.3, 22.4, 17.9; HRMS m/z 481.2090 ([M - H$^-$], C$_{25}$H$_{30}$N$_4$O$_6$ requires 481.2087).

(R)-2-((2S,6R)-2,6,7-triamino-7-oxoheptanamido)propanoic acid ditrifluoroacetic acid salt (25). A solution of 21 (5.1 mg, 9.8 $\mu$mol) in DMF (0.05 M, 186 $\mu$L) was treated with piperidine (10 $\mu$L), stirred for 0.5 h, then concentrated under a stream of N$_2$ (g). The crude solid was dissolved in H$_2$O (800 $\mu$L), loaded onto a Waters Sep-Pak® (C$_{18}$, 500 mg cartridge) preconditioned with a mixture of MeCN (5 mL) and 0.1% aqueous TFA (15 mL). The loaded sample was washed with 0.1% aqueous TFA (5 mL), and the product was eluted with 60% aqueous MeCN. The eluted fractions were combined, concentrated in vacuo to remove MeCN, and lyophilized to dryness to provide 25 as a white solid (2.5 mg, 6.7 mmol, 69%); LCMS: retention time 1.33 min; (ESI) m/z 261 (M + H$^+$), 283 (M + Na$^+$).
(R)-2-((2S,6R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-acetamido-7-amino-7-oxoheptanamido)propanoic acid (22). A solution of 17 (0.034 g, 0.0482 mmol) in ethyl acetate-MeOH-acetic acid (2:1:1, 4.0 mL) was treated with 10% Pd/C (10 mg), and stirred under a hydrogen atmosphere (1 atm) at 25 °C for 2 h. The mixture was filtered through Celite, concentrated in vacuo, and dried azeotropically with toluene. The crude sample of 21 was suspended in H$_2$O (1.0 mL), treated with NaHCO$_3$ (0.0202 g, 0.241 mmol) and Ac$_2$O (5.0 µL, 0.053 mmol) and stirred for 3 h at 25 °C. The crude mixture was acidified with aqueous 1 M HCl until the pH < 2, extracted into ethyl acetate (3 × 20 mL), dried (Na$_2$SO$_4$), concentrated in vacuo, and purified by flash chromatography (SiO$_2$, 15% MeOH-ethyl acetate with 1% acetic acid) to provide 22 (0.0186 g, 67%) as a beige film: [α]$_D$ = -1 (c = 0.5, CH$_3$OH); $^1$H NMR (300 MHz, CD$_3$OD) δ 7.79 (2H, d, J = 7.5 Hz), 7.70-7.62 (2H, comp), 7.39 (2H, t, J = 7.8 Hz), 7.31 (2H, t, J = 6.9 Hz), 4.38 (2H, t, J = 6.6 Hz), 4.31-4.23 (3H, comp), 4.13-4.11 (1H, m), 1.98 (3H, s), 1.84-1.76 (2H, comp), 1.68-1.58 (2H, comp), 1.38 (3H, d, J = 6.9 Hz), 1.29 (2H, br s); $^{13}$C NMR (75 MHz, CD$_3$OD) δ 178.7, 177.2, 174.4, 173.5, 154.9, 145.4, 142.8, 129.0, 128.4, 126.4, 121.1, 68.1, 54.5, 51.7, 50.8, 33.6, 33.1, 32.9, 23.3, 22.7, 18.1; HRMS m/z 525.2339 (M + H$,^+$, C$_{27}$H$_{32}$N$_4$O$_7$ requires 525.2349).

(R)-2-((2S,6R)-2,6,7-triamino-7-oxoheptanamido)propanoic acid trifluoroacetic acid salt (26). Compound 22 (1.9 mg, 3.6 µmol) was suspended in DMF (0.05 M, 69 µL) was treated with piperidine (3.6 µL), stirred for 0.5 h, then concentrated under a stream of N$_2$ (g). The crude solid was dissolved in H$_2$O (800 µL), loaded onto a Waters Sep-Pak® (C18, 500 mg cartridge) preconditioned with a mixture of MeCN (5 mL) and 0.1% aqueous TFA (15 mL). The loaded sample was washed with 0.1% aqueous TFA (5 mL), and the product was eluted with 60% aqueous MeCN. The eluted fractions were combined, concentrated in vacuo to remove MeCN, and lyophilized to dryness to provide 26 as a white solid (1.1 mg, 2.7 µmol, 75%); LCMS: retention time 1.48 min; (ESI$^+$) m/z 303 (M + H$,^+$), 325 (M + Na$,^+$).
References


2, 300 MHz, DMSO-\textit{d}_6
5, 300 MHz, CDCl₃
6, 300 MHz, CDCl$_3$
NHCbz

\[ \text{NH}_{2} \]

7, 300 MHz, CDCl\textsubscript{3}
8. 300 MHz, CD$_3$OD
S1, 300 MHz, CDCl₃
11, 300 MHz, CDCl₃
13, 300 MHz, CDCl₃
14, 300 MHz, CDCl₃
300 MHz, CDCl₃
12, 300 MHz, CDCl₃
15, 300 MHz, CDCl₃
19, 300 MHz, CD$_3$OD
16, 300 MHz, CDCl₃
20, 300 MHz, CD$_3$OD
17, 300 MHz, CD$_3$OD
$\text{NH}_2\text{HCl}$, 300 MHz, CD$_3$OD

21, 300 MHz, CD$_3$OD
22, 300 MHz, CD$_2$OD