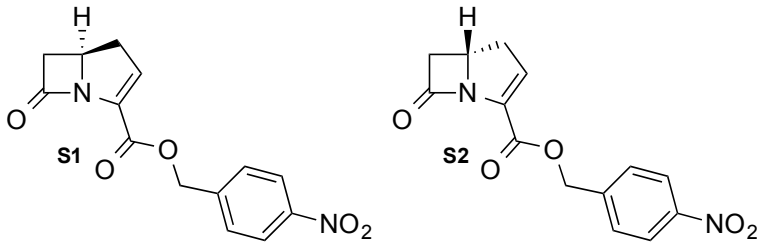
Supporting Information:

General methods and abbreviations

hydroxocobalamin (HOCbl)
4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES)
isopropyl β-D-1-thiogalactopyranoside (IPTG)
2-mercaptoethanol (BME)
methylcobalamin (MeCbl)
methyl viologen (MV)
nicotinamide adenine dinucleotide phosphate hydrate (NADPH)
phenylmethysulfonyl fluoride (PMSF)
other reagents were purchased from commercial sources unless otherwise noted

Restriction enzymes, DNA modifying enzymes, and PCR reagents were obtained from New England Biolabs (Ipswich, MA). Plasmid kits were from Thermo Scientific (Waltham, MA). *E. coli* DH5α (Invitrogen) was usually used for DNA cloning and ThnK expression was done in *E. coli* Rosetta 2(DE3) (Novagen). The plasmid pDB1282 was utilized for Fe/S cluster expression (1). SAM and *S*-adenosyl-L-[*methyl*-d_3]methionine (d_3-SAM) were synthesized as described previously (2). Recombinant flavodoxin (Flv) and flavodoxin reductase (Flx) were over-produced in *E. coli* as previously described (3). Plasmid DNA was sequenced by the Synthesis and Sequencing Facility of the Johns Hopkins University. A Bruker Avance (Billerica, MA) 300 or 400 MHz spectrometer was utilized for all ^{1}H and ^{13}C NMR spectra, which are reported in parts per million (δ) referenced against a TMS standard or residual solvent peak. The JHU Chemistry Department Mass Spectrometry Facility determined exact masses by fast atom bombardment (FAB) or electrospray ionization (ESI).

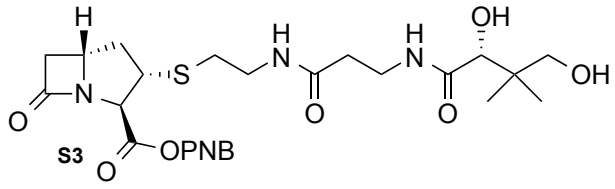
Synthesis of Substrates.
Compounds 10-12, 15:



The *p*-nitrobenzyl (5*R*)-carbapenem-3-carboxylate (**S1**) and the *p*-nitrobenzyl (5*S*)-carbapenem-3-carboxylate (**S2**) were prepared as previously described (4, 5).

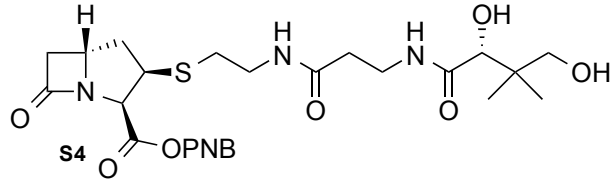
General procedure for pantetheine 1,4-addition:

***p*-Nitrobenzyl (2*R*,3*R*,5*R*)-2-pantetheinyl-carbapenam-3-carboxylate (S4).** To pantetheine acetonide (563.6 mg, 1.77 mmol, as described elsewhere (6)) was added tetrahydrofuran (1.7 mL) and 1 M HCl (1.7 mL) and the solution was stirred for about 30 min or until complete by TLC (10% ethanol/chloroform). Then 1 M NaOH (1.7 mL) was added followed by triethylamine (149.4 µL). This solution was added to a separate flask containing *p*-nitrobenzyl (5*S*)-carbapenem-3-carboxylate **S2** (4, 5) (340 mg, 1.18 mmol) (or *p*-nitrobenzyl (5*R*)-carbapenem-3-carboxylate **S1**) and acetonitrile (4.8 mL). The reaction mixture was stirred at room temperature for 40 min, then diluted with ethyl acetate (75 mL) and washed with saturated ammonium chloride (8 mL) in brine (20 mL). The aqueous layer was extracted with ethyl acetate (25 mL). The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield a yellow oil. The oil was purified by silica gel chromatography using 5% ethanol/chloroform to provide a mixture of the two isomers (398 mg, 55%) oil; TLC (ethanol:chloroform, 1:9 v/v) major isomer R_f = 0.33, minor isomer R_f = 0.29. Repeated chromatography enriched each isomer for characterization.

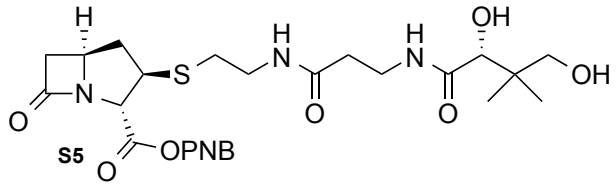


***p*-Nitrobenzyl (2*S*,3*R*,5*R*)-2-pantetheinyl-carbapenam-3-carboxylate (S3).** major isomer $[\alpha]^{24.0\ °C}$ = -60.6° (c = 1.020, $CHCl_3$); IR 3408, 1769, 1667, 1644, 1530, and, 1348 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.22 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.40 (bt, *J* = 5.8 Hz, 1H), 6.61 (bt, *J* = 5.4 Hz, 1H), 5.29 (s, 2H), 4.41 (d, *J* = 5.8

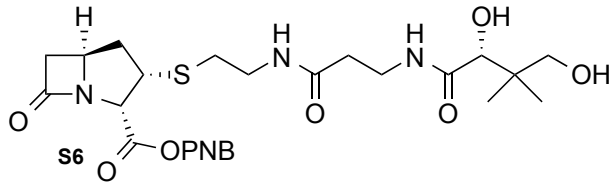
Hz, 1H), 3.97 (s, 1H), 3.86-3.93 (m, 1H), 3.78-3.85 (m,1H), 3.54 (q, *J* = 5.5 Hz, 1H), 3.46 (s, 2H), 3.36-4.44 (m, 2H), 3.35 (dd, *J* = 15.9, 5.1 Hz, 1H), 2.86 (dd, *J* = 16.0, 1.6 Hz, 1H), 2.74-2.82 (m, 1H), 2.58-2.70 (m, 2H), 2.44 (t, *J* = 5.5 Hz, 1H), 1.64-1.74 (m, 1H), 0.98 (s, 3H), 0.89 (s, 1H) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 175.8, 173.9, 171.7, 169.4, 147.6, 142.2, 128.3, 123.7, 77.2, 70.5, 65.7, 60.3, 52.5, 51.7, 44.1, 39.1, 38.5, 38.3, 35.6, 35.1, 31.7, 21.1, 20.4 ppm; **HRMS** *m/z*: $[M+H]^+$ calcd. for $C_{25}H_{34}N_4O_9S$ 567.2125; found 567.2126.



***p*-Nitrobenzyl (2*R*,3*R*,5*R*)-2-pantetheinyl-carbapenam-3-carboxylate (S4).** minor isomer $[\alpha]^{22.5\,°C}$ = -23.0° (c = 0.90, $CHCl_3$); IR 3396, 1769, 1660, 1650, 1608, 1522, and, 1347 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.32 (bt, *J* = 5.0 Hz, 1H), 6.33 (bt, *J* = 5.8 Hz, 1H), 5.29 (s, 2H), 4.80 (d, *J* = 7.1 Hz, 1H), 4.08-4.14 (m, 1H), 4.00 (s, 1H), 3.60-3.68 (q, *J* = 8.0 Hz, 1H), 3.58 (t, *J* = 5.9 Hz, 2H), 3.49 (s, 2H), 3.33-3.45 (m, 3H), 2.81 (dd, *J* = 16.5, 2.8 Hz, 1H), 2.60-2.80 (m, 2H), 2.41 (t, *J* = 5.1 Hz, 2H), 2.23-2.32 (m, 1H), 2.12-2.22 (m, 1H), 1.02 (s, 3H), 0.91 (s, 3H) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 176.5, 174.0, 171.7, 168.0, 147.7, 142.2, 128.7, 124.7, 77.2, 70.5, 65.4, 60.3, 52.1, 48.8, 44.2, 42.5, 39.2, 35.6, 35.2, 32.3, 24.1, 21.0, 20.4 ppm; **HRMS** *m/z*: $[M+H]^+$ calcd. for $C_{25}H_{34}N_4O_9S$ 567.21248; found 567.212229.



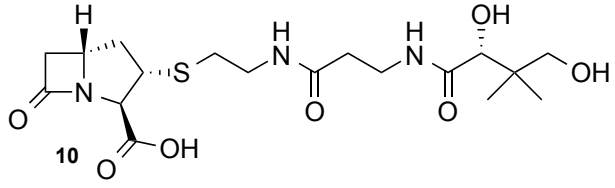
***p*-Nitrobenzyl (2*R*,3*S*,5*S*)-2-pantetheinyl-carbapenam-3-carboxylate (S5).** major isomer $[\alpha]^{24.8\,°C}$ = +29.3° (c = 1.55, CH_2Cl_2); IR 3408, 1769, 1667, 1644, 1609, 1530, and, 1348 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.22 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.40 (bt, *J* = 5.8 Hz, 1H), 6.61 (bt, *J* = 5.4 Hz, 1H), 5.29 (s, 2H), 4.41 (d, *J* = 5.8 Hz, 1H), 3.97 (s, 1H), 3.86-3.93 (m, 1H), 3.78-3.85 (m, 1H), 3.54 (q, *J* = 5.5 Hz, 1H), 3.46 (s, 2H), 3.36-4.44 (m, 2H), 3.35 (dd, *J* = 15.9, 5.1 Hz, 1H), 2.86 (dd, *J* = 16.0, 1.6 Hz, 1H), 2.74-2.82 (m, 1H), 2.58-2.70 (m, 2H), 2.44 (t, *J* = 5.5 Hz, 1H), 1.64-1.74 (m, 1H), 0.98 (s, 3H), 0.89 (s, 1H) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 175.9, 173.7, 171.7, 169.5, 147.8, 142.2, 128.4, 123.9, 77.2, 70.7, 65.9, 65.6, 52.6, 51.8, 44.2, 39.3, 38.6, 38.3, 35.7, 35.2, 31.8, 21.3, 20.4 ppm; **HRMS** *m/z*: $[M+H]^+$ calcd. for $C_{25}H_{34}N_4O_9S$ 567.2125; found 567.2124.



***p*-Nitrobenzyl (2*S*,3*S*,5*S*)-2-pantetheinyl-carbapenam-3-carboxylate (S6).** minor isomer $[\alpha]^{22.5\ °C}$ = +44.3° (c = 0.970, $CHCl_3$); IR 3373, 1749, 1650, 1523, and, 1347 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.25 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.32 (bt, *J* = 5.0 Hz, 1H), 6.33 (bt, *J* = 5.8 Hz, 1H), 5.29 (s, 2H), 4.80 (d, *J* = 7.1 Hz, 1H), 4.08-4.14 (m, 1H), 4.00 (s, 1H), 3.60-3.68 (q, *J* = 8.0 Hz, 1H), 3.58 (t, *J* = 5.9 Hz, 2H), 3.49 (s, 2H), 3.33-3.45 (m, 3H), 2.81 (dd, *J* = 16.5, 2.8 Hz, 1H), 2.60-2.80 (m, 2H), 2.41 (t, *J* = 5.1 Hz, 2H), 2.23-2.32 (m, 1H), 2.12-2.22 (m, 1H), 1.02 (s, 3H), 0.91 (s, 3H) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 176.5, 174.0, 171.7, 168.0, 147.7, 142.2, 128.7, 124.7, 77.2, 70.5, 65.4, 60.3, 52.1, 48.8, 44.2, 42.5, 39.2, 35.6, 35.2, 32.3, 24.1, 21.0, 20.4 ppm; **HRMS** *m/z*: $[M+H]^+$ calcd. for $C_{25}H_{34}N_4O_9S$ 567.2125; found 567.2124.

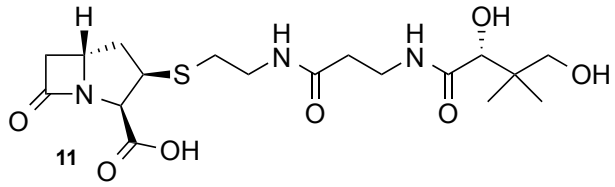
General procedure for deprotection of *p*-nitrobenzyl esters:

The *p*-nitrobenzyl ester (310 mg, 0.76 mmol) was dissolved in tetrahydrofuran (10 mL) and potassium bicarbonate (76.09 mg, 0.76 mmol) in water (5 mL) was added followed by 10% Pd on carbon (36 mg). Hydrogen (40 psi) was added to the reaction mixture in a pressure tube and shaken on a Parr apparatus for 2 h. The catalyst was removed by filtration on Celite. The filtrate was washed twice with ethyl acetate (15 mL). The aqueous layer was filtered through 0.2 µm nylon filter and lyophilized to give an amorphous solid. The solid was dissolved in a minimal amount of water and applied to a bed of Diaion® HP-20 resin and eluted with distilled water. After any excess salt was removed, 10% ethanol/water was used to elute the product. The presence of salt was detected using silver nitrate solution and the presence of desired compound was detected using potassium permanganate solution. Fractions that contained product and were salt free were pooled and lyophilized.



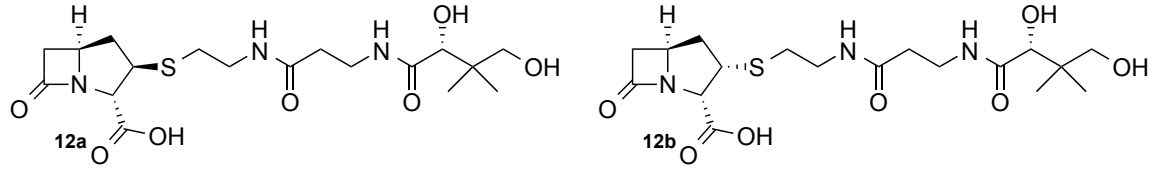
Potassium (2*S*,3*R*,5*R*)-2-pantetheinyl-carbapenam-3-carboxylate (10). major isomer $[\alpha]^{22.4\ °C}$ = -36.1° (c = 1.050, H_2O); **^{1}H NMR** (400 MHz, D_2O): δ 4.17 (d, *J* = 5.3 Hz, 1H), 3.97 (s, 1H), 3.94-4.00 (m, 1H), 3.78 (dd, *J* = 12.6, 6.6 Hz, 1H), 3.20-3.60 (m, 7H), 2.90 (dd, *J* = 16.6, 2.3 Hz), 2.74-2.95 (m, 3H), 2.60 (td, *J* = 13.6, 6.8 Hz, 1H), 2.50 (t, *J* = 6.4 Hz, 2H), 1.70 (td, *J* = 13.7, 6.8 Hz, 1H), 0.92 (s, 3H), 0.88 (s, 3H) ppm; **^{13}C NMR** (100 MHz, D_2O): δ 181.2, 177.4, 175.7, 174.5, 76.4, 69.4, 69.0, 53.1, 53.1, 43.6,

39.2, 39.2, 37.6, 36.1, 35.8, 31.5, 21.2, 19.8 ppm; **HRMS** *m/z*: [M-H]$^-$ calcd. for $C_{18}H_{29}N_3O_7S$ 430.1653; found 430.1665.



Potassium (2*R*,3*R*,5*R*)-2-pantetheinyl-carbapenam-3-carboxylate (11). minor isomer $[\alpha]^{23.1\ °C}$ = **-**59.0° (c = 0.7300, H_2O); **^{1}H NMR** (400 MHz, D_2O): δ 4.52 (d, *J* = 6.6 Hz, 1H), 4.05-4.14 (m, 1H), 3.99 (s, 1H), 3.90-3.97 (m, 1H), 3.35-3.55 (m, 6H), 3.31 (dd, *J* = 16.9, 4.5 Hz), 2.75-2.93 (m, 3H), 2.51 (t, *J* = 6.2 Hz, 2H), 2.30-2.37 (m, 1H), 2.07 (td, *J* = 13.4, 6.6 Hz, 1H), 0.92 (s, 3H), 0.88 (s, 3H) ppm; **^{13}C NMR** (100 MHz, D_2O): δ 181.7, 176.0, 175.7, 174.4, 76.3, 69.0, 67.5, 53.1, 51.7, 41.6, 39.2, 38.5, 37.7, 36.1, 35.9, 32.2, 21.2, 19.8 ppm; **HRMS** *m/z*: [M+H]$^+$ calcd. for $C_{18}H_{29}N_3O_7S$ 432.1805; found 432.1797.

The diastereomers **10** and **11** were separated from each other and purified using an Agilent model 1100 HPLC equipped with a multi-wavelength ultraviolet–visible detector in conjunction with a reverse-phase Phenomenex Luna 10µ C18(2) 100 Å preparatory column (250 x 21.20 mm ID). The mobile phase was 25% methanol and 75% buffer (10 mM potassium phosphate, pH 6.65) at a flow rate of 5 ml per min. Compound **10** eluted at 29 min and compound **11** eluted at 22 min.

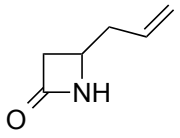


Potassium (2*R*,3*S*,5*S*)-2-pantetheinyl-carbapenam-3-carboxylate (12a). major isomer **^{1}H NMR** (400 MHz, D_2O): δ 4.17 (d, *J* = 5.0 Hz, 1H), 3.97 (s, 1H), 3.94-4.00 (m, 1H), 3.78 (dd, *J* = 12.8, 6.0 Hz, 1H), 3.20-3.60 (m, 7H), 2.90 (dd, *J* = 16.6, 2.4 Hz), 2.74-2.95 (m, 3H), 2.60 (td, *J* = 13.4, 6.8 Hz, 1H), 2.50 (t, *J* = 6.2 Hz, 2H), 1.70 (td, *J* = 13.7, 6.9 Hz, 1H), 0.92 (s, 3H), 0.88 (s, 3H) ppm; **^{13}C NMR** (100 MHz, D_2O): δ 181.2, 177.4, 175.7, 174.5, 76.3, 69.4, 69.0, 53.1, 53.1, 43.6, 39.2, 39.2, 37.6, 36.1, 35.9, 31.5, 21.2, 19.8 ppm; **HRMS** *m/z*: [M+H]$^+$ calcd. for $C_{18}H_{29}N_3O_7S$ 432.1799; found 432.1808.

Potassium (2*S*,3*S*,5*S*)-2-pantetheinyl-carbapenam-3-carboxylate (12b). minor isomer **^{1}H NMR** (400 MHz, D_2O): δ 4.52 (d, *J* = 6.6 Hz, 1H), 4.05-4.14 (m, 1H), 3.98 (s, 1H), 3.90-3.97 (m, 1H), 3.35-3.55 (m, 6H), 3.31 (dd, *J* = 17.0, 4.8 Hz), 2.75-2.93 (m, 3H), 2.51 (t, *J* = 6.2 Hz, 2H), 2.33 (td, *J* = 13.1, 5.0 Hz, 1H), 2.06 (td, *J* = 13.6, 6.8 Hz,

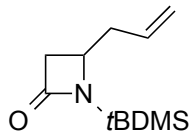
1H), 0.91 (s, 3H), 0.87 (s, 3H) ppm; **^{13}C NMR** (100 MHz, D_2O): δ 181.8, 176.1, 175.7, 174.5, 76.3, 69.0, 67.5, 53.1, 51.7, 41.5, 39.2, 38.5, 37.6, 36.1, 36.0, 32.2, 21.2, 19.7 ppm; **HRMS** *m/z*: $[M+H]^+$ calcd. for $C_{18}H_{29}N_3O_7S$ 432.1799; found 432.1780.

C2 and C3 stereochemical assignments were established by Nuclear Overhauser Effect (n.O.e.) NMR experiments and confirmed previous stereochemical analyses (7).



S7

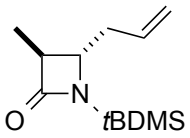
(±)-4-Allylazetidin-2-one (S7). The procedure was carried out as described previously (8). To 1,4-pentadiene (6.6 g, 96.9 mmol), chlorosulfonyl isocyanate (7.79 ml, 12.7 g) was added at 0 °C, then stirred at room temperature for 3 days in a pressure bottle. The reaction mixture was diluted with dichloromethane (110 mL) and was then add slowly to a solution of sodium sulfite (14.5 g) and dibasic potassium phosphate (8.51 g) in water (52.5 ml) at 0 °C. Then aqueous sodium hydroxide (1 M) was added to maintain pH = 7.0 - 8.0. After the addition, the reaction mixture was stirred at room temperature for 2 h, then extracted with ethyl acetate (3 × 100 ml). The organic layers were combined, dried with anhydrous sodium sulfate, filtered, and concentrated to give **S7** as a light yellow oil, which was used crude in the next reaction (5.57g, 52%). TLC (ethyl acetate:hexanes, 3:7 v/v): R_f = 0.67; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.67 (s, 1H), 5.64-5.78 (m, 1H), 5.00-5.10 (m, 2H), 3.58-3.66 (m, 1H), 2.97 (dd, *J* = 14.9, 5.1 Hz, 1H), 2.53 (dd, *J* = 14.9, 1.1 Hz, 1H), 2.24-2.36 (m, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 168.1, 133.1, 117.8, 46.9, 42.7, 39.2 ppm.



S8

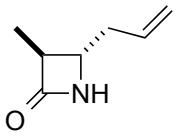
(±)-4-Allyl-1-dimethyl-*t*-butylsilyl-azetidin-2-one (S8). The procedure was carried out as described previously (9). Triethylamine (16.15 ml, 115.8 mmol) and *N,N*-dimethylaminopyridine (1.1g, 9.26 mmol) were added to **S7** (5.15g, 46.3 mmol) in tetrahydrofuran (173 ml) at 0 °C. *t*-Butyldimethylsilyl-methanesulfonate (12.8 ml, 55.56 mmol) was then added to the reaction mixture slowly. The solution was stirred at 0 °C for 20 min before it was allowed to warm to room temperature for 1

h. After concentration *in vacuo*, the mixture was dissolved in ethyl acetate (200 ml) and washed with saturated ammonium chloride (80 ml) and brine (100 ml), dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude oil was purified by silica gel chromatography (diethyl ether:hexanes, 1:9) to yield **S8** as a light yellow oil (8.71 g, 83%); TLC (ethyl acetate:hexanes, 3:7 v/v): R_f = 0.67; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.65-5.80 (m,1H), 5.50-5.15 (m, 2H), 3.54-3.63 (m, 1H), 3.09 (dd, *J* = 15.4, 5.5 Hz, 1H), 2.66 (dd, *J* = 15.3, 2.7 Hz, 1H), 2.52-2.62 (m, 1H), 2.10-2.24 (m, 1H), 0.96 (s, 9H), 0.25 (s, 3H), 0.22 (s, 3H) ppm.



S9

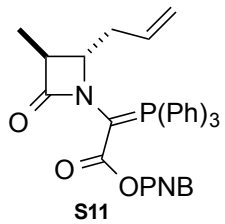
(±)-(3*S*,4*S*)-4-Allyl-1-dimethyl-*t*-butylsilyl-3-methylazetidin-2-one (S9). The procedure was carried out as described previously (9). A solution of **S8** (7.0 g, 31.1 mmol) in tetrahydrofuran (77.8 mL) was added to lithium diisopropylamine (2 M, 34.21 mL, 68.4 mmol) in tetrahydrofuran (155 mL) at -78°C under a nitrogen atmosphere. After stirring for 30 min at -78 °C, methyl iodide (19.4 mL, 44.14g, 310.9 mmol) was added and the reaction mixture was stirred at -78 °C for 30 min before it was allowed to warm to room temperature for 1 h. Acetic acid (3.9 mL) was added to the solution, and the reaction mixture was concentrated *in vacuo*. The resulting oil was dissolved in ethyl acetate (155 mL) and washed with brine (85.5 mL), dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The resulting oil was purified by silica gel chromatography (diethyl ether:hexanes, 1:9) to provide **S9** as a light yellow oil (7.13 g, 96%). TLC (ethyl acetate:hexanes, 2:8 v/v): R_f = 0.72; IR 2940, 2860, 1725, and, 1640 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.65-5.80 (m, 1H), 4.91-5.21 (m, 2H), 3.15 (ddd, *J* = 9.0, 4.0, 3.0 Hz, 1H), 2.77 (qd, *J* = 8.0, 3.0 Hz, 1H), 1.9-2.6 (m, 2H), 1.21 (d, *J* = 8.0 Hz), 0.91 (s, 9H), 0.21 (s, 3H), 0.20 (s, 3H) ppm.



S10

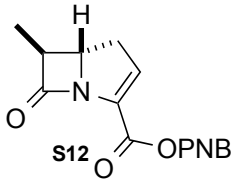
(±)-(3*S*,4*S*)-4-Allyl-3-methylazetidin-2-one (S10). The procedure was carried out as described previously (9). Acetic acid (5.11 mL, 89.4 mmol) followed by tetra-*n*-butylammonium fluoride (13.2 g, 41.72 mmol) was added to a solution of **S9** (7.13 g, 29.8 mmol) in anhydrous tetrahydrofuran (150 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, diluted with ethyl acetate (500 mL) and washed with water (2 × 140 mL) and brine (150 mL). The organic layer was dried with

anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The resulting oil was purified by silica gel chromatography (ethyl acetate:hexanes, 3:7 to 4:6) to provide **S10** as a colorless oil (1.18 g, 70%). TLC (ethyl acetate:hexanes, 2:3 v/v): R_f = 0.36; IR 3420, 1755, and, 1640 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.5 (br s, 1H), 5.8 (m, 1H), 5.15-5.25 (m, 1H), 4.95-5.1 (m, 1H), 3.30 (td, *J* = 6.0, 2.0 Hz, 1H), 2.80 (qd, *J* = 7.0, 2.0, 1H), 2.36 (td, *J* = 6.0, 1.0 Hz, 2H), 1.30 (d, *J* =7.0 Hz, 3H), ppm.



S11

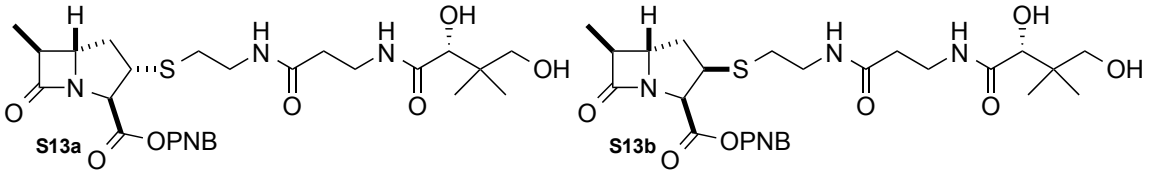
***p*-Nitrobenzyl (±)-2-(2-allyl-3-methyl-4-oxoazetidin-1-yl)-2-(triphenyl-λ$_5$-phosphanylidene)acetate (S11).** The procedure was carried out as described previously (9). *p*-Nitrobenzyl glyoxylate hydrate (6.03 g, 26.5 mmol), triethylamine (432.7 µL, 3.08 mmol), and 4.9 g 3Å molecular sieves were added sequentially to a solution of **S10** (3.32 g, 26.5 mmol) in anhydrous tetrahydrofuran (93.2 mL) and stirred at room temperature for 16 h. The reaction mixture was filtered through Celite, washed with ethyl acetate, and concentrated *in vacuo*. The crude residue was dissolved in anhydrous tetrahydrofuran (82.2 mL), cooled to -15 °C to which was then added 2,6-lutidine (5.85 mL, 50.4 mmol) followed by thionyl chloride (2.70 mL, 37.1 mmol) dropwise. The reaction mixture was stirred for 1 h at -15 °C. The solution was filtered through Celite, and the filtrate was washed with ethyl acetate and concentrated *in vacuo*. The crude oil was dissolved in 1,4-dioxane (85.1 mL) and triphenylphosphine (9.02 g, 34.4 mmol) and 2,6-lutidine (3.38 mL, 39.2 mmol) were added. The resulting mixture was stirred at room temperature for 16 h, diluted with ethyl acetate (858.7 mL), and washed with water (244.4 mL) and brine (244.4 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The resulting brown oil was purified by silica gel chromatography (ethyl acetate:hexanes, 1:1 to 7:3) to yield **S11** as an orange foam (3.07g, 20 % yield). The product was characterized by subsequent transformation to the respective bicyclic β-lactams (*vide infra*). TLC (diethyl ether:ethyl acetate, 1:1 v/v): R_f = 0.52.



S12

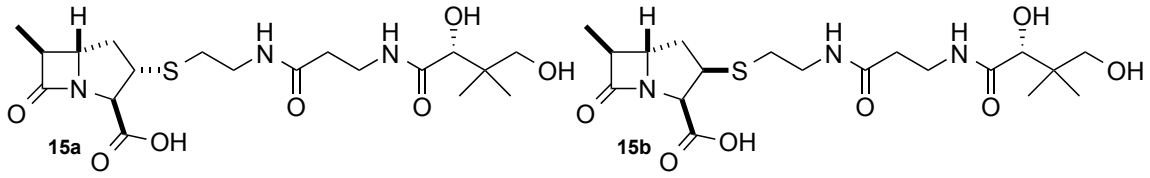
***p*-Nitrobenzyl (±)-(5*S*,6*S*)-6-methylcarbapenem-3-carboxylate (S12).** The procedure was carried out as described previously (9). Trifluoroacetic acid (7.17

mL) was added to a solution of **S11** (3.07 g, 5.3 mmol) in dichloromethane (117 mL) at 0 °C. The reaction mixture was cooled to -78 °C and ozone was bubbled through the solution until a blue color persisted. The solution was stirred for 5 min at -78 °C, then a stream of nitrogen was bubbled through the reaction mixture until the color changed from blue to yellow. Dimethyl sulfide (7.83 mL) was added to the solution at 0 °C and the solution was stirred for 1 h. The reaction mixture was diluted with dichloromethane (70 mL) and washed with saturated sodium bicarbonate (2 × 200 mL) and brine (201 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude brown oil was purified by silica gel chromatography (ethyl acetate:hexanes, 3:7 to 6:4) to give a diastereomeric mixture of the *cis* and *trans* methyl isomers (1.24 g, 78%). Repeated purification gave the desired *trans* methyl isomer **S12** (0.70 g, 44%). TLC (ethyl acetate:hexanes, 4:6 v/v): R_f = 0.45 **epi-S12**, 0.37 **S12**; IR 2970, 1778, 1730, 1608, 1521, and, 1348 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.6 Hz, 2H), 7.62 (d, *J* = 8.3 Hz, 2H), 6.54 (t, *J* = 2.8 Hz, 1H), 5.45, 5.28 (ABq, J_{AB} = 16, 2H), 3.98 (ddd, *J* = 10.3, 7.8, 2.8 Hz, 1H), 3.23 (dq, *J* = 7.5, 2.8 Hz, 1H), 2.97 (ddd, *J* = 19.5, 9.7, 3.1 Hz, 1H), 2.80 (ddd, *J* = 19.4, 7.8, 2.5 Hz, 1H), 1.45 (d, *J* = 7.3 Hz, 3H), ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 180.1, 160.0, 147.6, 142.7, 134.5, 132.3, 128.1, 123.7, 65.3, 59.1, 54.5, 36.0, 13.9 ppm; **HRMS** *m/z*: $[M+H]^+$ calcd. for $C_{15}H_{14}N_2O_5$ 303.0981; found 303.0978.



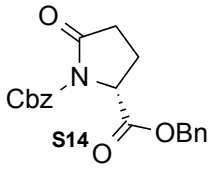
***p*-Nitrobenzyl (±)-(3*R*,5*S*,6*S*)-6-methyl-2-pantetheinyl-carbapenam-3-carboxylate (S13).** Pantetheine acetal (1.1 g, 3.45 mmol) in tetrahydrofuran (3.5 mL) was added 1 M HCl and stirred at room temperature for 30 min or until determined to be complete by TLC. The reaction mixture was then neutralized by the addition of aqueous NaOH (3.5 mL, ~1 M) followed by triethylamine (297.5 µL). This was added to *p*-nitrobenzyl **S12** (0.70 g, 2.3 mmol) in acetonitrile (9.4 mL). After stirring for 20 min at room temperature, the reaction mixture was diluted with ethyl acetate (125 mL) and washed with saturated ammonium chloride (16 mL) and brine (35 mL). The aqueous layer was removed and extracted with ethyl acetate (40 mL). The combined organic layers were dried with anhydrous sodium sulfate, filtered, and concentrated in *vacuo*. The product was purified by silica gel chromatography (ethanol:chloroform, 1:9) to give **S13** as inseparable C2 stereoisomers (560 mg, 42%). ^{1}H NMR analysis of the crude mixture revealed the C2 diastereomers in a ratio of 4:1. TLC (ethanol:chloroform, 1:9 v/v): major isomer **S13a** R_f = 0.36, minor isomer **S13b** R_f = 0.32; IR 3408, 2967, 2856, 1772, 1749, 1661, 1643, 1525, 1442, and, 1346 cm^{-1}; **S13a: ^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.24 (d, *J* = 8.6 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.36 (bt, *J* = 5.6 Hz, 1H), 6.44 (bt, *J* = 5.4 Hz, 1H), 5.28 (s, 2H), 4.45 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.92-4.08 (m, 2H), 3.74-3.82 (m, 1H),

3.20-3.60 (m, 6H), 3.12 (dq, *J* = 7.5, 2.1 Hz, 1H), 2.40-2.80 (m, 5H), 1.68-1.78 (m, 1H), 1.40 (d, *J* = 7.6 Hz, 3H), 1.00 (s, 3H), 0.91 (s, 3H) ppm; **S13b: ^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.24 (d, *J* = 8.6 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.36 (bt, *J* = 5.6 Hz, 1H), 6.44 (bt, *J* = 5.4 Hz, 1H), 5.28 (s, 2H), 4.79 (dd, *J* = 7.1, 3.8 Hz, 1H), 3.92-4.08 (m, 2H), 3.74-3.82 (m, 1H), 3.20-3.60 (m, 6H), 3.01 (dq, *J* = 7.3, 2.8 Hz, 1H), 2.40-2.80 (m, 4H), 2.24-2.34 (m, 1H), 2.06-2.19 (m, 1H), 1.39 (d, *J* = 7.3 Hz, 3H), 1.00 (s, 3H), 0.91 (s, 3H) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.2, 173.9, 171.8, 169.4, 147.8, 142.2, 128.4, 123.8, 70.7, 65.8, 65.3, 60.1, 53.1, 51.7, 39.2, 38.5, 37.3, 35.7, 31.9, 31.8, 21.3, 21.0, 20.4, 13.8 ppm; **HRMS** *m/z*: $[M+H]^+$ calcd. for $C_{26}H_{36}N_4O_9S$ 581.2281; found 581.2277.

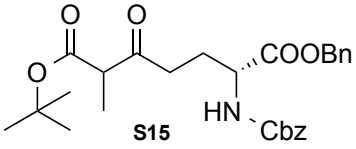


Potassium (±)-(3*R*,5*S*,6*S*)-6-methyl-2-pantetheinyl-carbapenam-3-carboxylate (15). Water (4.9 mL) containing potassium bicarbonate (74.5 mg, 0.74 mmol) was added to a solution of **S13** (360.0 mg, 0.62 mmol) in tetrahydrofuran (10.4 mL) in a pressure flask. 10% palladium on carbon (40 mg) was carefully added to the solution. The flask was fitted to a Parr apparatus to which hydrogen was added (40 PSI) and shaken for 2 h. The catalyst was removed by filtration of the reaction mixture through Celite and the filtered solids were washed with water (6 mL) followed by ethyl acetate (30 mL). The aqueous layer was separated from the filtrate and washed with ethyl acetate (25 mL). The washed aqueous layer was filtered through a 25 mm 0.2 μm nylon syringe filter and lyophilized. The resulting solids were dissolved and desalted by Diaion-HP resin chromatography (water). Fractions that contained **15** (visualized with potassium permanganate) that were free of salt (visualized by silver nitrate precipitation) were lyophilized to produce **15** (239.6 mg, 80%) as a white solid. ^{1}H NMR analysis of the crude mixture revealed the ratio of C2 diastereomers in a ratio of 7:3 (**15a**:**15b**). **15a: ^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.21 (d, *J* = 4.3 Hz, 1H), 4.00 (s, 1H), 3.75-3.95 (m, 2H), 3.35-3.60 (m, 6H), 3.18 (qd, *J* = 7.3, 1.1 Hz, 1H), 2.70-2.90 (m, 2H), 2.59 (dt, *J* = 13.9, 7.1 Hz, 1H), 2.52 (t, *J* = 6.4 Hz, 2H), 1.76 (dt, *J* = 13.9, 6.1 Hz, 1H), 1.36 (d, *J* = 7.6 Hz, 3H), 0.93 (s, 3H), 0.89 (s, 3H) ppm; **15b: ^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.52 (d, *J* = 6.6 Hz, 1H), 4.00 (s, 1H), 3.70 (bt, *J* = 5.6 Hz, 2H), 3.35-3.60 (m, 6H), 3.07 (qd, *J* = 6.6, 1.1 Hz, 1H), 2.70-2.90 (m, 2H), 2.52 (t, *J* = 6.4 Hz, 2H), 2.25-2.35 (m, 1H), 2.08-2.18 (m, 1H), 1.36 (d, *J* = 7.6 Hz, 3H), 0.93 (s, 3H), 0.89 (s, 3H) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 184.5, 177.1, 175.7, 174.5, 76.4, 69.2, 69.0, 60.6, 53.1, 52.6, 39.2, 39.1, 36.5, 36.1, 35.9, 31.5, 21.2, 19.8, 13.5 ppm; **HRMS** *m/z*: $[M+H]^+$ calcd. for $C_{19}H_{31}N_3O_7S$ 446.1956; found 446.1977.

Compounds 8, 9, 13, 14, 16:

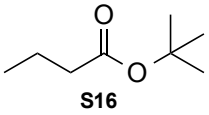


Benzyl (2*R*)-1-carboxybenzyl-5-oxopyrrolidine-2-carboxylate (S14). Diisopropylethylamine (54 mL, 310 mmol was added to D-pyroglutamic acid (20 g, 155 mmol, TCI America/AK Scientific) in dichloromethane (500 mL). Benzyl bromide (22 ml, 185 mmol) was added and the solution was refluxed for 4h. The reaction mixture was concentrated *in vacuo* and the crude oil was redissolved in ethyl acetate (400 ml). The cloudy mixture was filtered through silica on top of Celite and the silica/Celite plug was washed twice with ethyl acetate (200 mL). The eluate was concentrated *in vacuo* and redissolved in tetrahydrofuran (600 mL) to which was added lithium bis(trimethylsilyl)amide (154.5 mL, 1.0 M, 155 mmol) at -78 ˚C and stirred for 10 min. Benzyl chloroformate (30.8 mL, 202 mmol) was added and the reaction mixture was allowed to stir for 10 min. The reaction was quenched with aqueous ammonium chloride (400 mL) and the solution was warmed to room temperature and the phases were separated. The aqueous layer was extracted 5× with ethyl acetate (400 mL) and the combined organic fractions were washed with brine, dried with anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The solid crude product was recrystallized from hot ethyl acetate/hexanes to give **S14** as white crystals (34.4 g, 63% yield). **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 7.36-7.28 (m, 10H), 5.22 (d, *J* = 1.3 Hz, 2H), 5.13 (s, 2H), 4.72 (dd, *J* = 9.4, 2.7 Hz, 1H), 2.68-2.58 (m, 1H), 2.50 (ddd, *J* = 17.6, 9.3, 3.3 Hz, 1H), 2.34 (ddt, *J* = 13.4, 10.4, 9.4 Hz, 1H), 2.09-2.02 (m, 1H) ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 172.9, 170.9, 150.9, 135.10, 135.08, 128.80, 128.73, 128.69, 128.56, 128.41, 128.26, 68.5, 67.5, 58.9, 31.1, 21.9 ppm. **HRMS** (FAB) *m/z*: $[M+H]^+$ calcd. for $C_{20}H_{19}NO_5$ 354.13415; found 354.13342.



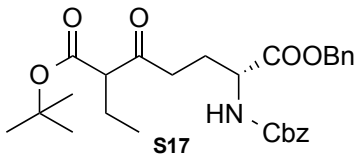
7-Benzyl 1-*tert*-butyl (6*R*)-6-(benzyloxycarbonylamino)-2-methyl-3-oxoheptanedioate (S15). This synthesis was adapted from the procedures of Ohta *et al.* (10). A solution of lithium diisopropylamide in tetrahydrofuran (17 mL, 2.0 M, 33.6 mmol) was added to a solution of *tert*-butyl propionate (4.26 ml, 28 mmol) in tetrahydrofuran (300 mL) at -78 ˚C and allowed to stir for 30 min. A solution of **S14** in tetrahydrofuran (40 mL) was added slowly to the reaction mixture. After stirring for 30-60 min at -78 ˚C, the reaction was quenched with saturated ammonium chloride and the phases were separated. The aqueous layer was extracted three times with dichloromethane (50 mL). The combined organic layers were washed with brine, dried with anhydrous magnesium sulfate, and

concentrated *in vacuo*. The crude oil was purified by silica gel chromatography using 25% ethyl acetate in hexanes to give **S15** (9.88 g, 72%) as a colorless oil, which solidified overnight at room temperature. TLC (ethyl acetate:hexanes, 2:3 v/v): R_f = 0.75; **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 7.35 (s, 10H), 5.30-5.46 (m, 1H), 5.17 (s, 2H), 5.09 (s, 2H), 4.31-4.47 (m, 1H), 3.34-3.36 (m, 1H), 2.47-2.73 (m, 2H), 1.94-2.22 (m, 2H), 1.42 (s, 9H), 1.23 (bd, *J* = 6.8 Hz, 3H) ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 205.2, 171.8, 169.6, 156.0, 136.2, 135.3, 128.74, 128.62, 128.44, 128.29, 128.20, 82.0, 67.4, 67.2, 53.9, 53.6, 37.3, 28.0, 26.4, 12.8 ppm; **HRMS** (FAB) *m/z*: $[M\text{-}OH]^+$ calcd. for $C_{27}H_{33}NO_7$ 466.2230; found 466.2207.



S16

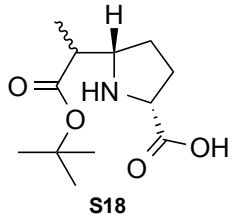
***tert*-Butyl butyrate (S16).** Conditions were modeled after a similar reaction (http://orgprepdaily.wordpress.com/2007/07/25/2-chloro-5-iodobenzoic-acid-tert-butyl-ester/. [Accessed 09 April 15]). Butyryl chloride (20 mL, 191 mmol) in tetrahydrofuran (40 mL) was added to a slurry of tetrahydrofuran (400 mL) and potassium *tert*-butoxide (23.4 g, 209 mmol) at 0 ˚C. Mixture was stirred for 90 min and then warmed to room temperature. Silica gel (76 g, 1.3 mol) and distilled water (1.14 mL, 62.6 mmol) were added and allowed to stir 20 min. Slurry was filtered through silica gel and concentrated. A yellow oil (18.1 g, 66%) was isolated. The crude product was purified by vacuum distillation (heating at 35-40 ˚C, and collected over liquid nitrogen) to give a clear oil. **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 2.18 (t, *J* = 7.4 Hz, 2H), 1.61 (sext, *J* = 7.4 Hz, 2H), 1.44 (s, 9H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 173.3, 80.0, 37.6, 28.2, 18.7, 13.7 ppm. The product data matched previous characterization (11).



S17

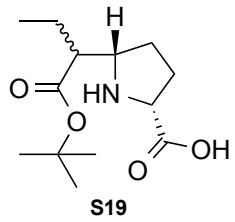
7-Benzyl 1-*tert*-butyl (6*R*)-6-(benzyloxycarbonylamino)-2-ethyl-3-oxoheptanedioate (S17). Reaction was conducted similarly to that for compound **S15**, but utilized **S16**. The oil was purified by silica gel chromatography using ethyl acetate/hexanes (3:20) to give the titled product (56%) as an oil, which solidified to a white solid. TLC (ethyl acetate:hexanes, 2:3 v/v): R_f = 0.69; **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 7.34 (bs, 10H), 5.40-5.34 (m, 1H), 5.16 (s, 2H), 5.09 (s, 2H), 4.42-4.36 (m, 1H), 3.21-3.16 (m, 1H), 2.64-2.48 (m, 2H), 2.20-1.90 (m, 2H), 1.81-1.73 (m, 2H), 1.42 (s, 9H), 0.89-0.85 (m, 3H) ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 204.7, 171.87, 171.85, 168.9, 156.0, 136.2, 135.3, 128.75, 128.62, 128.43, 128.29, 128.19, 82.01, 81.97,

67.4, 67.2, 61.7, 53.57, 53.51, 37.71, 37.61, 28.0, 26.44, 26.31, 21.6, 12.0 ppm; **HRMS** (FAB) *m/z*: $[M+H]^+$ calcd. for $C_{28}H_{35}NO_7$ 498.24918; found 498.24837.



S18

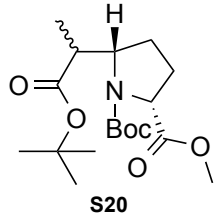
(2*R*,5*S*)-5-(1-(*tert*-Butoxy)-1-oxopropan-2-yl)pyrrolidine-2-carboxylic acid (S18). This synthesis was adapted from the procedures of Ohta *et al.* (10). Palladium on carbon (0.4 g, 8%w/w) was carefully added to a solution of **S15** (5 g, 10 mmol) in acetic acid (1 mL) and ethanol (40 mL) in a bomb flask. The reaction mixture was pressurized to 50 psi with hydrogen gas in a Parr-shaker apparatus and shaken for 16 h. The reaction mixture was then filtered over Celite and concentrated *in vacuo*. The as-isolated product is a thick foam/oil (near quantitative yield). TLC (methanol:acetonitrile, 3:7 v/v): R_f = 0.19; **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 4.05 (t, *J* = 6.8 Hz, 1H), 3.77-3.59 (m, 1H), 2.85-2.74 (m, 1H), 2.30-2.21 (m, 2H), 2.12-2.00 (m, 1H), 1.66-1.57 (m, 1H), 1.46 (s, 9H), 1.33 (d, *J* = 7.1 Hz, 2H), 1.27 (d, *J* = 7.3 Hz, 1H) ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 173.56, 173.52, 172.6, 172.0, 82.33, 82.23, 62.3, 61.4, 60.8, 60.4, 42.5, 42.3, 29.2, 29.0, 28.3, 28.04, 28.01, 27.7, 15.4, 14.4 ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_{12}H_{21}NO_4$ 244.1549; found 244.1551.



S19

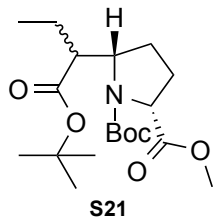
(2*R*,5*S*)-5-(1-(*tert*-Butoxy)-1-oxobutan-2-yl)pyrrolidine-2-carboxylic acid (S19). Reaction with compound **S17** was conducted as described above for **S18**. The product is a foam (near quantitative yield), which solidified. TLC (methanol:acetonitrile, 3:7 v/v): R_f = 0.18; **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 4.08 (t, *J* = 6.1 Hz, 1H), 3.82-3.63 (m, 1H), 2.64-2.53 (m, 1H), 2.31-2.20 (m, 2H), 2.05-2.00 (m, 1H), 1.86-1.69 (m, 2H), 1.69-1.57 (m, 1H), 1.51-1.43 (m, 9H), 0.97 (t, *J* = 7.2 Hz, 3H) ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 173.50, 173.47, 172.4, 171.5, 82.7, 82.2, 61.3, 60.76, 60.57, 50.7, 49.4, 29.06, 28.88, 28.3, 28.1, 23.7, 23.4, 11.61, 11.47 ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_{13}H_{23}NO_4$ 258.1705; found 258.1699.

*The following protections (**S20-S23**) result in mixtures of diastereomers/rotamers, so NMR analyses were done at 50 °C.*



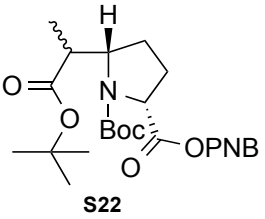
S20

Methyl (2*R*,5*S*)-1-(*tert*-butyloxycarbonyl)-5-(1-(*tert*-butoxy)-1-oxopropan-2-yl)pyrrolidine-2-carboxylate (S20). This synthesis was adapted from the procedures of Ohta *et al.* (10). Potassium carbonate (3.49 g, 25 mmol) and sodium hydroxide (0.49 g, 12.5 mmol) were added to a solution of **S18** (2.05 g, 8.4 mmol) in water (25 mL) at 0 ˚C. A solution of di-*tert*-butyl dicarbonate (3.67g, 17 mmol) in tetrahydrofuran (33.8 mL) was added to the mixture at 0 ˚C over 15 min. The reaction mixture was stirred for 1 h at 0 ˚C and then allowed to warm to room temperature and stirred for 16 h. The reaction mixture was concentrated *in vacuo,* washed with diethyl ether, and acidified to pH = 2 with potassium bisulfate. The solution was then extracted twice with ethyl acetate and the combined organic layers were washed with brine, dried with anhydrous magnesium sulfate, and concentrated *in vacuo*. The crude residue was dissolved in dimethylformamide (84 mL) to which cesium carbonate (3.43 g, 10.5 mmol) was added and allowed to stir for 10 min. Methyl iodide (1.05 mL, 16.9 mmol) was added to the reaction mixture dropwise and the solution was stirred for 16 h. The reaction was quenched with saturated ammonium chloride, the phases separated, and the aqueous layer was extracted twice with diethyl ether. The combined organic fractions were dried with anhydrous magnesium sulfate and concentrated *in vacuo*. The crude oil was purified by silica gel chromatography using ethyl acetate:hexanes (1:3) to give **S20** (2.39 g, 79%) as an oil, which became a waxy solid overnight. TLC (ethyl acetate:hexanes, 7:13 v/v): R_f = 0.77; (major isomer) **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 4.20 (dd, *J* = 19.9, 8.4 Hz, 1H), 4.03 (app t, *J* = 8.2 Hz, 1H), 3.65 (s, 3H), 2.39-2.10 (m, 2H), 1.92-1.60 (m, 3H), 1.40 (s, 9H), 1.36 (s, 9H), 1.16 (d, *J* = 7.0 Hz, 3H) ppm; (minor isomer) **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 4.24-4.16 (m, 1H), 4.13-4.08 (m, 1H), 3.65 (s, 3H), 2.39-2.10 (m, 2H), 1.92-1.60 (m, 3H), 1.40 (s, 9H), 1.36 (s, 9H), 1.03 (d, *J* = 7.2 Hz, 3H) ppm; (major isomer) **^{13}C NMR** (101 MHz; $CDCl_3$): δ 173.5, 173.0, 153.6, 79.6, 79.1, 59.3, 59.1, 51.5, 44.7, 27.7, 27.4, 25.3, 14.7 ppm; (minor isomer) **^{13}C NMR** (101 MHz; $CDCl_3$): δ 173.1, 172.8, 153.6, 79.4, 79.1, 59.8, 59.3, 51.3, 44.7, 27.7, 27.4, 23.3, 9.5 ppm; **HRMS** (FAB) *m/z*: $[M+H]^+$ calcd. for $C_{18}H_{31}NO_6$ 358.22296; found 358.22304.



S21

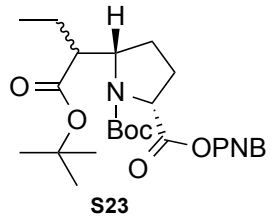
Methyl (2*S*,5*S*)-1-(*tert*-butyloxycarbonyl)-5-(1-(*tert*-butoxy)-1-oxobutan-2-yl)pyrrolidine-2-carboxylate (S21). Reaction with compound **S19** was conducted as described above. The crude oil was purified by silica gel chromatography using 20% ethyl acetate in hexanes to give **S21** (73%) as an oil. TLC (ethyl acetate:hexanes, 1:4 v/v): R_f = 0.52; (major isomer) **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 4.20 (dd, *J* = 16.3, 7.9 Hz, 1H), 4.00 (m, 1H), 3.64 (s, 3H), 2.30-2.22 (m, 2H), 1.90-1.52 (m, 5H), 1.41 (s, 9H), 1.36 (s, 9H), 0.84 (t, *J* = 7.4 Hz, 3H) ppm; (minor isomer) **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 4.18 (dd, *J* = 16.1, 7.6 Hz, 1H), 4.00 (m, 1H), 3.64 (s, 3H), 2.15-2.07 (m, 1H), 1.90-1.52 (m, 6H), 1.40 (s, 9H), 1.36 (s, 9H), 0.83 (t, *J* = 7.3 Hz, 3H) ppm; (major isomer) **^{13}C NMR** (101 MHz; $CDCl_3$): δ 173.0, 172.7, 153.5, 79.7, 79.1, 59.3, 59.0, 52.4, 51.4, 28.2, 27.6, 27.5, 25.4, 22.5, 11.5 ppm; (minor isomer) **^{13}C NMR** (101 MHz; $CDCl_3$): δ 172.4, 172.3, 153.5, 79.4, 59.8, 59.0, 52.4, 51.3, 28.2, 27.6, 27.5, 25.5, 22.5, 12.3 ppm; **HRMS** (FAB) *m/z*: $[M+H]^+$ calcd. for $C_{19}H_{33}NO_6$ 372.23727; found 372.23770.



S22

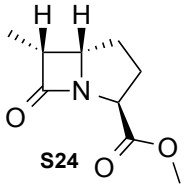
***p*-Nitrobenzyl (2*R*,5*S*)-1-(*tert*-butyloxycarbonyl)-5-(1-(*tert*-butoxy)-1-oxopropan-2-yl)pyrrolidine-2-carboxylate (S22).** The Boc protection of **S18** was conducted as described above for **S20**. The residue (0.2 g, 0.58 mmol) was redissolved in dimethylformamide (6 mL) and diisopropylethylamine (0.22 ml, 1.3 mmol) followed by the addition of *p*-nitrobenzyl bromide (0.14 g, 0.64 mmol) in dimethylformamide (3 mL). Solution was stirred 80 min. Saturated ammonium chloride was added to the solution and was extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated. Silica gel chromatography (ethyl acetate/hexanes, 1:4) was used to purify the oil (0.227 g, 65% over both steps). TLC (ethyl acetate:hexanes, 1:4 v/v): R_f = 0.21; (major isomer) **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 8.22 (d, *J* = 8.7 Hz, 2H), 7.64 (d, *J* = 8.7 Hz, 2H), 5.34, 5.23 (ABq, J_{AB} = 14 Hz, 2H), 4.31 (q, *J* = 8.0 Hz, 1H), 4.07-4.02 (m, 1H), 2.39-2.28 (m, 2H), 1.98-1.84 (m, 2H), 1.64 (q, *J* = 5.7 Hz, 1H), 1.39 (s, (H), 1.33 (s, 9H), 1.11 (d, *J* = 7.0 Hz, 3H) ppm; (minor isomer) **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 8.22 (d, *J* = 8.7 Hz, 2H), 7.64 (d, *J* = 8.7 Hz, 2H), 5.34, 5.23 (ABq, J_{AB} = 14 Hz, 2H), 4.31 (q, *J* = 8.0 Hz, 1H), 4.12 (dt, *J* = 8.0, 4.2 Hz, 1H), 2.39-2.28 (m, 1H), 2.21-2.14 (m, 1H), 1.98-1.84 (m, 2H), 1.79-1.73 (m, 1H), 1.39 (s, (H), 1.33 (s, 9H), 0.99 (d, *J* = 7.2 Hz, 3H) ppm; (major

isomer) **^{13}C NMR** (101 MHz; $CDCl_3$): δ 173.3, 172.1, 153.6, 147.0, 143.4, 128.3, 123.1, 79.5, 79.2, 64.5, 59.4, 59.1, 44.7, 28.8, 27.6, 27.4, 25.0, 14.7 ppm; (minor isomer) **^{13}C NMR** (101 MHz; $CDCl_3$): δ 173.3, 172.7, 153.6, 147.0, 143.4, 128.3, 123.1, 79.3, 79.2, 64.5, 59.9, 59.4, 44.7, 28.8, 27.6, 27.4, 25.0, 9.4 ppm; **HRMS** (FAB) *m/z*: $[M+H]^+$ calcd. for $C_{24}H_{34}N_2O_8$ 479.23934; found 479.23938.



S23

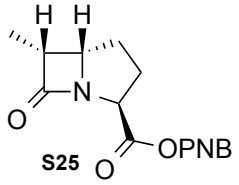
***p*-Nitrobenzyl (2*R*,5*S*)-1-(*tert*-butyloxycarbonyl)-5-(1-(*tert*-butoxy)-1-oxobutan-2-yl)pyrrolidine-2-carboxylate (S23).** Reaction with **S19** was conducted as described above. Multiple rounds of silica gel chromatography (ethyl acetate:hexanes, 1:19 to 3:7) were used to purify the product as an oil (51%). TLC (ethyl acetate:hexanes, 1:4 v/v): R_f = 0.35; (major isomer) **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 8.22 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.5 Hz, 2H), 5.34, 5.22 (ABq, J_{AB} = 13.6 Hz, 2H), 4.34-4.27 (m, 1H), 4.05-3.99 (m, 1H), 2.25-2.14 (m, 2H), 1.91-1.83 (m, 2H), 1.67-1.60 (m, 2H), 1.57-1.49 (m, 1H), 1.39 (s, (H), 1.34 (s, 9H), 0.74 (t, *J* = 8.2 Hz, 3H) ppm; (minor isomer) **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 8.22 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.5 Hz, 2H), 5.28 (s, 2H), 4.34-4.27 (m, 1H), 4.05-3.99 (m, 1H), 2.35-2.29 (m, 1H), 1.91-1.83 (m, 3H), 1.67-1.60 (m, 2H), 1.57-1.49 (m, 1H), 1.39 (s, (H), 1.34 (s, 9H), 0.77 (t, *J* = 8.1 Hz, 3H) ppm; (major isomer) **^{13}C NMR** (101 MHz; $CDCl_3$): δ 172.6, 172.1, 153.4, 147.1, 143.4, 128.4, 123.2, 79.8, 79.3, 64.5, 59.5, 59.1, 52.4, 28.3, 27.6, 27.5, 25.5, 22.5, 11.4 ppm; (minor isomer) **^{13}C NMR** (101 MHz; $CDCl_3$): δ 172.2, 172.1, 153.7, 147.1, 143.4, 128.4, 123.2, 79.4, 79.3, 64.5, 60.0, 59.1, 52.4, 28.3, 27.6, 27.5, 25.5, 22.5, 12.2 ppm; **HRMS** (FAB) *m/z*: $[M+H]^+$ calcd. for $C_{25}H_{36}N_2O_8$ 493.25499; found 493.25445.



S24

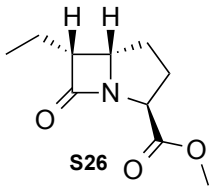
Methyl (3*S*,5*S*,6*R*)-6-methylcarbapenam-3-carboxylate (S24). This synthesis was adapted from the procedures of Ohta *et al.* (10). 2,6-Lutidine (1.44 mL, 12 mmol) and trimethylsilyl triflate (1.82 mL, 10 mmol) were added slowly to a solution of **S20** (1.2 g, 3.3 mmol) in dichloromethane (35 mL) at 0 ˚C and the mixture stirred for 2 h. A solution of hydrochloric acid in dioxane (7.65 mL, 4M, 31 mmol) was added at 0 ˚C and stirred for 10 min. The reaction mixture was concentrated *in vacuo* to yield dark red oil. The crude oil was dissolved in

dichloromethane (500 mL) to which diisopropylethylamine (2.63 mL, 15 mmol) followed by a solution of *N,N'*-dicyclohexylcarbodiimide (0.9 g, 4.4 mmol) in dichloromethane (10 mL) were added at 0 ˚C. The mixture was allowed to slowly warm to ambient temperature while stirring over 23 h. The reaction mixture was concentrated *in vacuo* and dissolved in dichloromethane and filtered through Celite. The eluate was washed 3 × with a cold solution of acetic acid in water (5%), dried with anhydrous sodium sulfate, and concentrated *in vacuo*. The crude oil was purified by silica gel chromatography using ethyl acetate:hexanes (1:2) to give a clear oil (0.452 g, 73%). TLC (ethyl acetate:hexanes, 2:3 v/v): R_f = 0.36. 1,8-Diazabicyclo[5.4.0]undec-7-ene (1.13 mL, 7.6 mmol) was added to a solution of the mixture of diastereomers (1.38 g, 7.5 mmol) in acetonitrile (150 mL) and stirred at 50 ˚C for 5 d. The reaction mixture was concentrated *in vacuo* to give a brown oil. The crude product was purified from its diastereomer by silica gel chromatography using ethyl acetate:hexanes (3:17) to give the titled compound (eluted first) as an oil (0.59 g, 43%). TLC (ethyl acetate:hexanes, 2:3 v/v): R_f = 0.52; **^{1}H-NMR** (300 MHz; $CDCl_3$): δ 4.35 (t, *J* = 7.3 Hz, 1H), 3.96 (app q, *J* = 6.3 Hz, 1H), 3.73 (s, 3H), 3.50 (qd, *J* = 7.7, 5.5 Hz, 1H), 2.45-2.35 (m, 1H), 2.29-2.17 (m, 1H), 2.06-1.96 (m, 1H), 1.63 (app. ddt, *J* = 13.1, 9.0, 7.8 Hz, 1H), 1.13 (d, *J* = 7.7 Hz, 3H) ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 181.1, 172.0, 58.6, 57.7, 52.5, 45.3, 34.6, 25.2, 9.3 ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_9H_{13}NO_3$ 184.0974; found 184.0966.



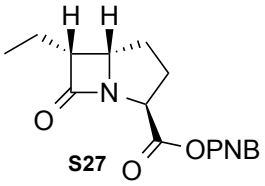
S25

***p*-Nitrobenzyl (3*S*,5*S*,6*R*)-6-methylcarbapenam-3-carboxylate (S25)**. The coupling reaction was conducted as above using **S22**, 63% yield overall. TLC (ethyl acetate:hexanes, 2:3 v/v): R_f = 0.31; **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 8.23 (d, *J* = 8.8 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 5.25 (s, 2H), 4.43 (t, *J* = 7.3 Hz, 1H), 3.97 (app. dd, *J* = 12.9, 6.5 Hz, 1H), 3.52 (qd, *J* = 7.7, 5.6 Hz, 1H), 2.49-2.41 (m, 1H), 2.29-2.20 (m, 1H), 2.07-1.99 (m, 1H), 1.71-1.63 (m, 1H), 1.15 (d, *J* = 7.7 Hz, 3H) ppm; **HRMS** (FAB) *m/z*: $[M+H]^+$ calcd. for $C_{15}H_{16}N_2O_5$ 305.11375; found 305.11333. The product data matched previous characterization (12).



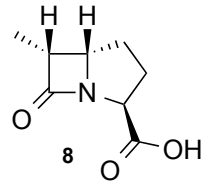
S26

Methyl (3*S*,5*S*,6*R*)-6-ethylcarbapenam-3-carboxylate (26). The coupling reaction was conducted as above using **S21,** 35% yield overall. TLC (ethyl acetate:hexanes, 2:3 v/v): R_f = 0.61; **^{1}H-NMR** (300 MHz; $CDCl_3$): δ 4.34 (t, *J* = 7.5 Hz, 1H), 3.95 (app dt, *J* = 7.5, 5.9 Hz, 1H), 3.73 (s, 3H), 3.33 (ddd, *J* = 9.6, 7.4, 5.4 Hz, 1H), 2.48-2.37 (m, 1H), 2.29-2.17 (m, 1H), 2.09-1.99 (m, 1H), 1.74-1.40 (m, 3H), 0.98 (t, *J* = 7.4 Hz, 3H) ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 180.3, 172.1, 58.4, 57.2, 52.52, 52.49, 34.6, 25.3, 18.4, 12.0 ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_{10}H_{15}NO_3$ 198.1130; found 198.1126.



S27

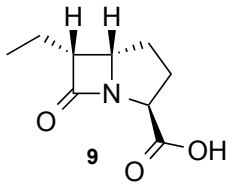
***p*-Nitrobenzyl (3*S*,5*S*,6*R*)-6-ethylcarbapenam-3-carboxylate (S27).** The coupling reaction was conducted as above using **S23,** 29% yield overall. TLC (ethyl acetate:hexanes, 2:3 v/v): R_f = 0.38; **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 8.23 (d, *J* = 8.7 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 5.25 (s, 2H), 4.41 (t, *J* = 7.5 Hz, 1H), 3.97-3.92 (m, 1H), 3.35 (ddd, *J* = 9.6, 7.4, 5.4 Hz, 1H), 2.52-2.39 (m, 1H), 2.31-2.17 (m, 1H), 2.13-1.99 (m, 1H), 1.75-1.57 (m, 2H), 1.55-1.42 (m, 1H), 0.99 (t, *J* = 7.4 Hz, 3H) ppm; **HRMS** (FAB) *m/z*: $[M+H]^+$ calcd. for $C_{16}H_{18}N_2O_5$ 319.12940; found 319.12849. The product data matched previous characterization (12).



8

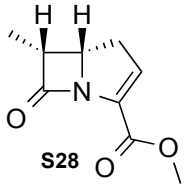
Potassium (3*S*,5*S*,6*R*)-6-methylcarbapenam-3-carboxylate (8). S25 (60 mg) was dissolved in tetrahydrofuran (4 mL) and potassium phosphate buffer (2 mL, 0.5M, pH 7.0). 10% palladium on carbon (1:1 w/w with compound **S25**) was added and placed under hydrogen (40 psi) for 1 h with shaking. The mixture was filtered through Celite and washed with water and diethyl ether. The aqueous solution was washed with diethyl ether, filtered (0.2 μm, nylon), and lyophilized. The resulting powder was desalted by passage through HP-20 diaion resin. **^{1}H-NMR** (400 MHz; D_2O): δ 4.14 (t, *J* = 7.8 Hz, 1H), 3.95 (dt, *J* = 8.1, 5.6 Hz, 1H), 3.50 (qd, *J* = 7.7, 5.1 Hz,

1H), 2.56-2.48 (m, 1H), 2.14-2.05 (m, 1H), 2.01-1.94 (m, 1H), 1.67-1.57 (m, 1H), 1.11 (d, *J* = 7.8 Hz, 3H) ppm; **HRMS** (ESI) *m/z*: [M-H]$^-$ calcd. for $C_8H_{11}NO_3$ 168.0661; found 168.0648. The product data matched previous characterization (12).



9

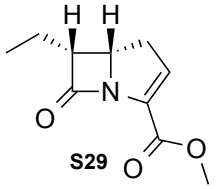
Potassium (3*S*,5*S*,6*R*)-6-ethylcarbapenam-3-carboxylate (9). S27 was deprotected in a similar fashion to the C6-methyl analog **S25**). **^{1}H-NMR** (400 MHz; D_2O): δ 4.12 (t, *J* = 7.8 Hz, 1H), 3.96-3.91 (m, 1H), 3.38-3.32 (m, 1H), 2.58-2.50 (m, 1H), 2.11-1.96 (m, 2H), 1.65-1.47 (m, 3H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; **^{13}C NMR** (101 MHz; D2O): δ 184.8, 180.4, 61.6, 57.9, 51.2, 36.1, 25.6, 18.3, 11.6 ppm; **HRMS** (ESI) *m/z*: [M-H]$^-$ calcd. for $C_9H_{13}NO_3$ 182.0817; found 182.0804.



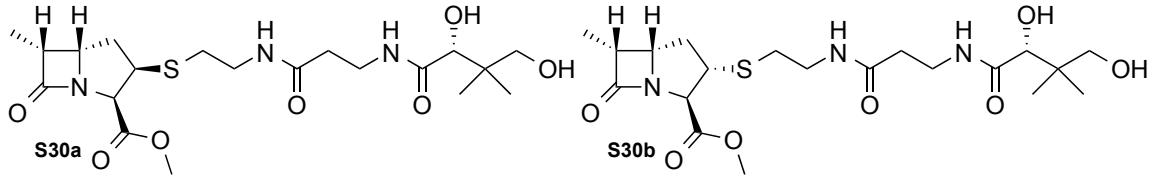
S28

Methyl (5*S*,6*R*)-6-methylcarbapenem-3-carboxylate (S28). This synthesis was adapted from the procedures of Ohta *et al.* (10). A solution of lithium hexamethyldisilazane in tetrahydrofuran (3.576 mL, 1M, 3.58 mmol) was added to **S24** (301 mg, 1.643 mMol) in tetrahydrofuran (16.4 mL) at -78 ˚C and stirred for 5 min. Phenylselenium bromide (1.16 g, 4.93 mmol) was added at -78 ˚C and allowed to warm to room temperature over 1h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (20 mL), brine (20 mL), dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude residue was run through a silica plug with 20% ethyl acetate in hexanes to remove excess phenyl selenium bromide and then concentrated. The resulting oil (257mg, 0.760 mmol) was dissolved in dichloromethane (41 mL) to which was then added a solution of *m*-chloroperbenzoic acid (131 mg, 0.760 mmol) in dichloromethane (5 mL) at -30˚C. The reaction was stirred for 15 min at -30˚C before it was quenched with triethylamine (141 µL, 1.01 mmol) and diluted with dichloromethane (150 mL). The reaction mixture was washed twice with saturated ammonium bicarbonate (50 mL), brine (50 mL), dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography using ethyl acetate:hexanes (3:7) to give **S28** (52 mg, 17%) as a yellow oil. TLC (ethyl acetate:hexanes, 3:7 v/v): R_f = 0.32; **^{1}H-NMR** (300 MHz; $CDCl_3$): δ 6.52 (t, *J* = 2.8 Hz, 1H), 4.37 (ddd, *J* = 10.0, 8.7, 6.2 Hz, 1H), 3.83 (s, 3H), 3.74-3.66 (m, 1H), 2.76 (app. qdd, *J* = 19.4, 9.4, 2.8 Hz, 2H), 1.27 (d, *J* = 7.7 Hz, 3H)

ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 180.8, 161.3, 135.9, 132.4, 56.1, 52.5, 47.4, 30.65, 9.9 ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_9H_{11}NO_3$ 182.0817; found 182.0809.

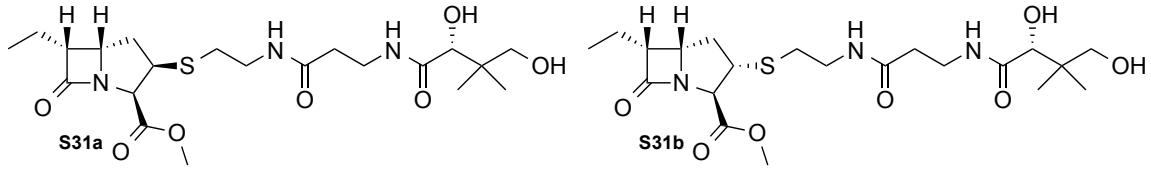


Methyl (5*S*,6*R*)-6-ethylcarbapenem-3-carboxylate (S29). ***S26*** was desaturated in a similar fashion to the C6-methyl analog (**S24**) to give **S29** (40.8 mg, 9.4%). TLC (ethyl acetate:hexanes, 3:7 v/v): R_f = 0.60; **^{1}H-NMR** (400 MHz; $CDCl_3$): δ 6.51 (t, *J* = 2.6 Hz, 1H), 4.35 (ddd, *J* = 9.9, 8.9, 6.1 Hz, 1H), 3.82 (s, 3H), 3.53 (dt, *J* = 9.6, 6.6, 1H), 2.82-2.68 (m, 2H), 1.89-1.78 (m, 1H), 1.65-1.54 (m, 1H), 1.00 (t, *J* = 7.4 Hz, 3H) ppm; **^{13}C NMR** (101 MHz; $CDCl_3$): δ 180.1, 161.3, 135.7, 132.3, 55.7, 54.3, 52.5, 30.9, 18.9, 11.9 ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_{10}H_{13}NO_3$ 196.0974; found 196.0971.

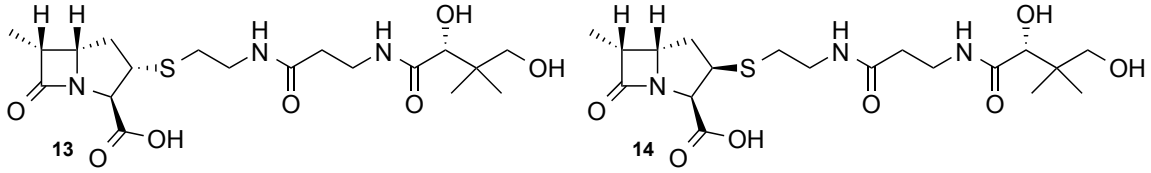


Methyl (3*R*,5*S*,6*R*)-6-methyl-2-pantetheinyl-carbapenam-3-carboxylate (S30). Hydrochloric acid (471 µL, 1M) was added to pantetheine acetonide (157 mg, 0.495 mmol, as described elsewhere(6)) in tetrahydrofuran (691 µL) and stirred for 30 min. Sodium hydroxide (471 µL, 1M) was then added to neutralize the reaction mixture. The product pantetheine diol was directly added to **S28** (64 mg, 0.353 mmol) in acetonitrile (960 µL) to which was added triethylamine (25 µL, 0.177 mmol) and stirred for 30 min at ambient temperature. The reaction mixture was diluted with ethyl acetate (15 mL), washed with saturated ammonium chloride (4 mL), and brine (10 mL). The combined aqueous layers were extracted with ethyl acetate (15 mL) and the combined organic fractions were dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo.* The crude oil was purified by silica gel chromatography using ethanol:chloroform (1:9) to give **S30** (16 mg) as an inseparable mixture of C2 diastereomers in a ratio of 7:5 (**S30a**:**S30b**) by ^{1}H-NMR. TLC (ethanol:chloroform, 1:9 v/v): R_f = 0.25; **S30a: ^{1}H-NMR** (400 MHz; MeOD): δ 4.67 (d, *J* = 7.2 Hz, 1H), 4.2-4.14 (m, 2H), 3.89 (s, 1H), 3.74 (s, 3H), 3.58-3.53 (m, 3H), 3.50, 3.36 (ABq, J_{AB} = 14.4 Hz, 2H), 2.84-2.65 (m, 4H), 2.43 (t, *J* = 6.5 Hz, 2H), 2.28

(ddd, *J* = 14.0, 8.2, 3.5 Hz, 1H), 1.97 (ddd, *J* = 13.8, 10.1, 8.3 Hz, 1H), 1.15 (d, *J* = 7.8, 3H), 0.92 (s, 6H) ppm; **S30b: ^{1}H-NMR** (400 MHz; MeOD): δ 4.17 (d, *J* = 6.1 Hz, 1H), 4.2-4.14 (m, 1H) 3.99 (ddd, *J* = 8.0, 6.5, 5.4 Hz, 1H), 3.85 (s, 1H), 3.76 (s, 3H), 3.58-3.53 (m, 3H), 3.43, 3.29 (ABq, J_{AB} = 11.2 Hz, 2H), 2.84-2.65 (m, 6H), 2.43 (t, *J* = 5.7 Hz, 2H), 1.19 (d, *J* = 7.7, 3H), 0.92 (s, 6H) ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_{20}H_{33}N_3O_7S$ 460.2117; found 460.2112.

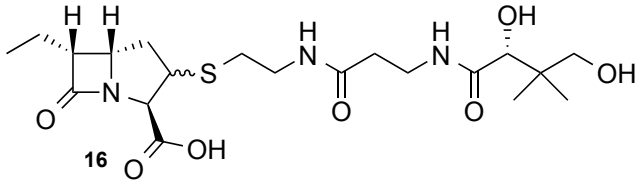


Methyl (3*R*,5*S*,6*R*)-6-ethyl-2-pantetheinyl-carbapenam-3-carboxylate (S31). The carbapenem **S29** was reacted with pantetheine in a similar fashion to the C6-methyl analogue (carbapenem **S28**) to give **S31**(12 mg, 13%) as an inseparable mixture of C2 diastereomers in a nearly equal ratio by ^{1}H-NMR. TLC (ethanol:chloroform, 1:9 v/v): R_f = 0.45; **S31a: ^{1}H-NMR** (400 MHz; MeOD): δ 4.60 (d, *J* = 7.3 Hz, 1H), 4.15-3.98 (m, 1H), 3.83-3.78 (m, 1H), 3.81 (s, 1H), 3.71 (s, 3H), 3.48-3.26 (m, 4H), 3.43, 3.29 (ABq, J_{AB} = 10.8 Hz, 2H), 2.72 (dt, *J* = 13.0, 6.2, 1H), 2.64 (dt, J = 12.9, 6.5, 1H), 2.36 (t, *J* = 5.5 Hz, 2H), 2.31-2.13 (m, 2H), 1.61-1.41 (m, 2H), 0.94 (t, *J* = 7.8 Hz, 3H), 0.85 (s, 6H) ppm; **S31b: ^{1}H-NMR** (400 MHz; MeOD): δ 4.08 (d, *J* = 6.5 Hz, 1H), 4.15-3.98 (m, 1H), 3.94-3.86 (m, 1H), 3.82 (s, 1H), 3.67 (s, 3H), 3.49-3.60 (m, 1H), 3.48-3.26 (m, 4H), 3.43, 3.29 (ABq, J_{AB} = 10.8 Hz, 2H), 3.08-2.79 (m, 2H), 2.55-2.39 (m, 1H), 2.36 (t, *J* = 5.5 Hz, 2H), 1.61-1.41 (m, 2H), 0.96 (t, *J* = 7.5 Hz, 3H), 0.85 (s, 6H) ppm; **S31a and S31b: ^{13}C NMR** (101 MHz; MeOD): δ 177.0, 175.2, 174.9, 173.1, 172.5, 80.5, 78.3, 71.4, 68.8, 68.4, 67.3, 67.2, 59.2, 55.4, 55.3, 54.6, 54.5, 54.2, 54.1, 53.4, 53.3, 41.4, 37.5, 37.4, 37.3, 34.1, 33.1, 22.4, 22.0, 20.7 20.3, 13.3, 13.0 ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_{21}H_{35}N_3O_7S$ 474.2274; found 474.2267.



Ammonium (3*R*,5*S*,6*R*)-6-methyl-2-pantetheinyl-carbapenam-3-carboxylate (13, 14). A solution of lithium hydroxide in water (1M, 20 µL) was added to **S30** (8 mg, 0.0176 mmol) in tetrahydrofuran (500 µL) at 0 ˚C and stirred for 20 min, at which time TLC monitoring revealed incomplete reaction. A second batch of lithium hydroxide solution (1M, 20 µL) was then added and after 30 min at 0 ˚C the reaction was quenched with K_2HPO_4 (10 mM, pH = 7.0, 500 µL). The mixture was concentrated *in vacuo* to remove the organic solvent, and immediately injected onto

a preparatory reverse-phase HPLC (Phenomonex Luna C18(2) 250 x 21.2 mm 10 micron 100 Å, 10:90 5 mM NH_4HCO_3 in water : acetonitrile, 5 mL/min) for purification. The C2-C3 "cis" diastereomer **14** eluted at 11 min and the C2-C3 "trans" diastereomer **13** eluted at 13 min, allowing for enrichment of each diastereomer. The compounds were collected and lyophilized to give carbapenams **13** (1.27 mg, 16%) and **14** (0.63 mg, 8%) as white powders. **13: ^{1}H-NMR** (400 MHz; D_2O): δ 4.06 (d, *J* = 5.4 Hz, 1H), 4.08-4.04 (m, 1H), 3.99 (s, 1H), 3.82-3.75 (m, 1H), 3.61 (dd, *J* = 8.2, 5.3 Hz, 1H), 3.53-3.38 (m, 4H), 3.58, 3.34 (ABq, J_{AB} = 11.0 Hz, 2H), 2.86 (td, *J* = 13.3, 6.5 Hz, 1H), 2.78 (td, *J* = 13.0, 6.3 Hz, 1H), 2.51 (t, *J* = 6.3, 2H), 2.45 (dt, *J* = 13.9, 6.8 Hz, 1H), 1.7 (dt, *J* = 14.3, 7.2 Hz, 1H), 1.20 (d, *J* = 7.9 Hz, 3H), 0.93 (s, 3H), 0.89 (s, 3H) ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_{19}H_{31}N_3O_7S$ 446.1961; found 446.1957; **14: ^{1}H-NMR** (400 MHz; D_2O): δ 4.34 (d, *J* = 7.3 Hz, 1H), 4.10 (dd, *J* = 13.5, 6.2, 1H), 3.90 (s, 1H), 3.71 (s, 1H), 3.67 (dd, *J* = 12.3, 6.4 Hz, 1H), 3.56 (dd, *J* = 11.9, 4.7 Hz, 1H), 3.56-3.34 (m, 3H), 3.48, 3.24 (ABq, J_{AB} = 11.6 Hz, 2H), 2.41 (t, *J* = 6.6, 2H), 2.09 (dt, *J* = 13.5, 6.8 Hz, 1H), 2.00 (dt, *J* = 13.4, 6.7 Hz, 1H), 1.04 (d, *J* = 7.7 Hz, 3H), 0.83 (s, 3H), 0.80 (s, 3H) ppm; **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_{19}H_{31}N_3O_7S$ 446.1961; found 446.1952.



Analytical Standard:

Sodium (3*R*,5*S*,6*R*)-6-ethyl-2-pantetheinyl-carbapenam-3-carboxylate (16). An analytical amount of the protected methyl ester **S31** was dissolved in 1:1 acetonitrile:H_2O. The solution was adjusted to pH ≥ 10 with NaOH (~1 M) and allowed to react at room temperature for 10 min. The solution was then neutralized to pH = 7 with HCl (~1 M) and analyzed by UPLC-MS. **HRMS** (ESI) *m/z*: $[M+H]^+$ calcd. for $C_{20}H_{33}N_3O_7S$ 460.2117; found 460.2112. See Fig. 3*D*.

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Supporting Figures:

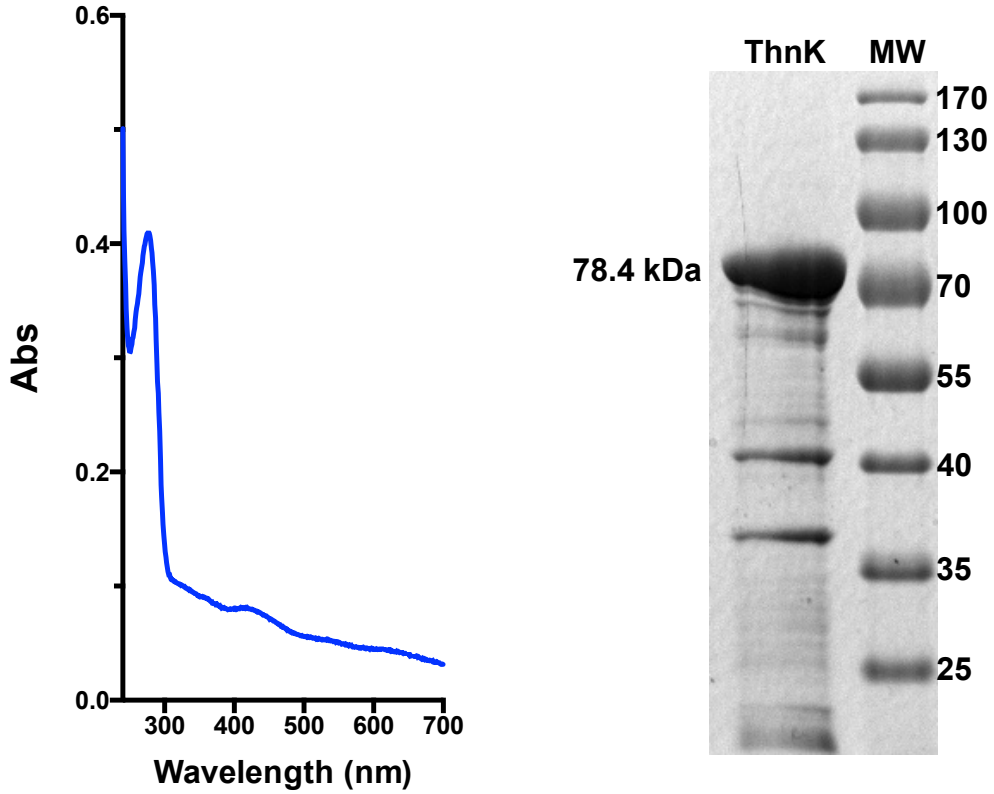

Fig. S1. UV-Visible spectrum and SDS PAGE of ThnK**.** The UV-visible spectrum of ThnK shows a 420 nm shoulder, indicative of a bound iron sulfur cluster.

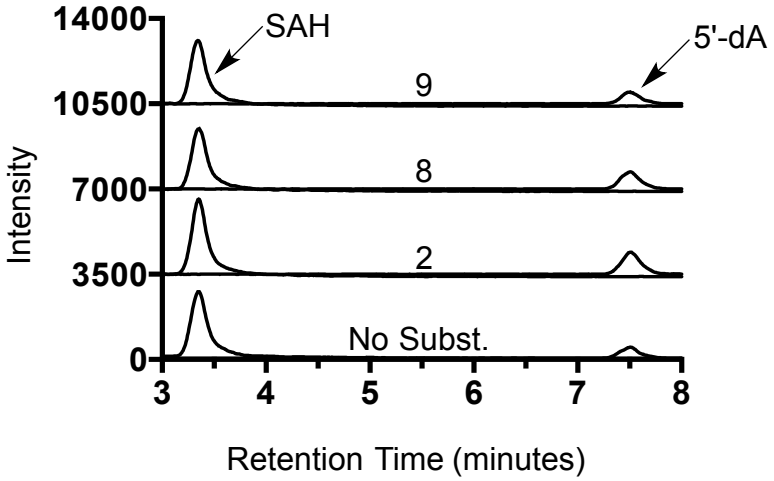

Fig. S2. Detection of SAM-derived products of ThnK with compounds **2, 8,** and **9.** LC-MS/MS was used to detect SAM-derived coproducts, SAH (385.4 -> 136 *m/z*) and 5'-dA (252.1 -> 136 *m/z*). The extracted-ion chromatograms (EICs) for the transitions of SAH and 5'-dA are overlaid for each reaction. The reactions with compounds **2**, **8**, and **9** show about the same level of turnover as the reaction without substrate. Reactions were run for approximately 2 hours at room temperature.

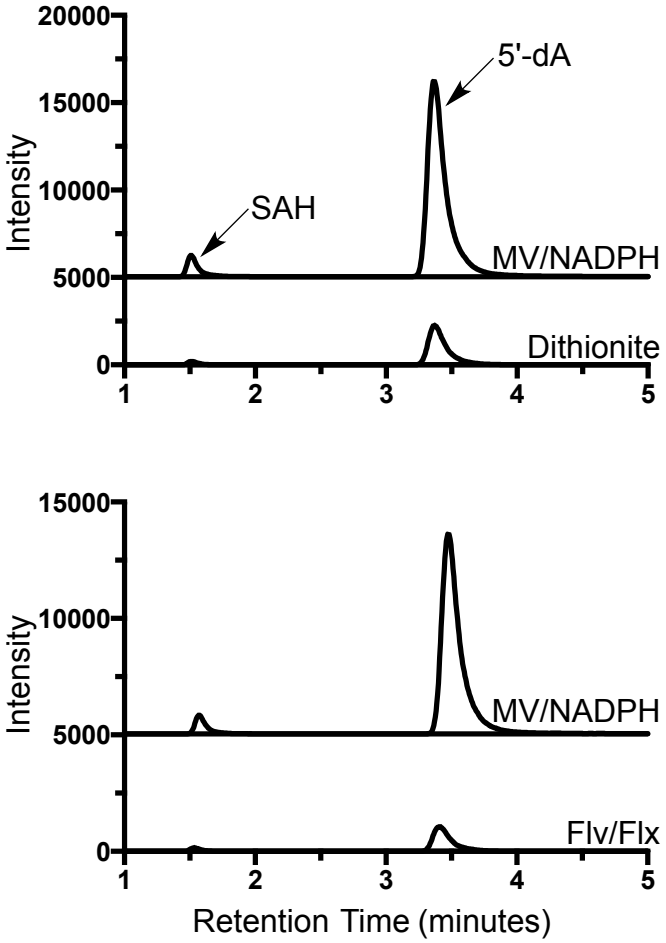

Fig. S3. LC-MS/MS comparison of enzymatic turnover using different iron-sulfur cluster reductants. The extracted-ion chromatograms (EICs) for the transitions of SAH (385.4 -> 136 *m/z*) and 5'-dA (252.1 -> 136 *m/z*) are overlaid for each reaction. Methyl viologen with NADPH gave higher turnover than dithionite or flavodoxin with flavodoxin reductase and NADPH. Reactions were run for approximately 2 hours at room temperature with substrate **11**.

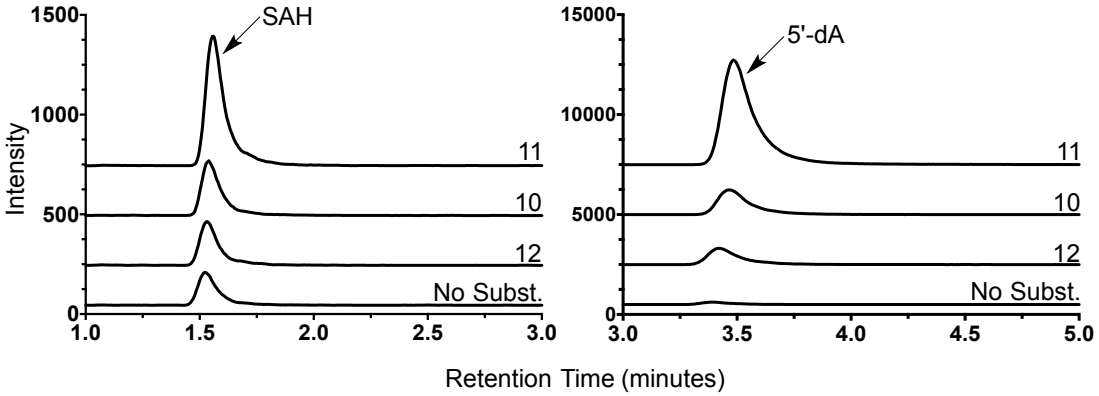

Fig. S4. Reproducibilty of SAM-derived products of ThnK. LC-MS/MS was used to detect SAM-derived coproducts, SAH (385.4 -> 136 *m/z*) and 5'-dA (252.1 -> 136 *m/z*). The extracted-ion chromatograms (EICs) for the transitions of SAH and 5'-dA are overlaid for each reaction. The reaction with compound **11** gave the highest levels of these SAM-derived coproducts. Reactions with ThnK (~80 μM) were run for 1.5 hours at room temperature and included 1 mM HOCbl, though added cobalamin was determined not to be required for activity.

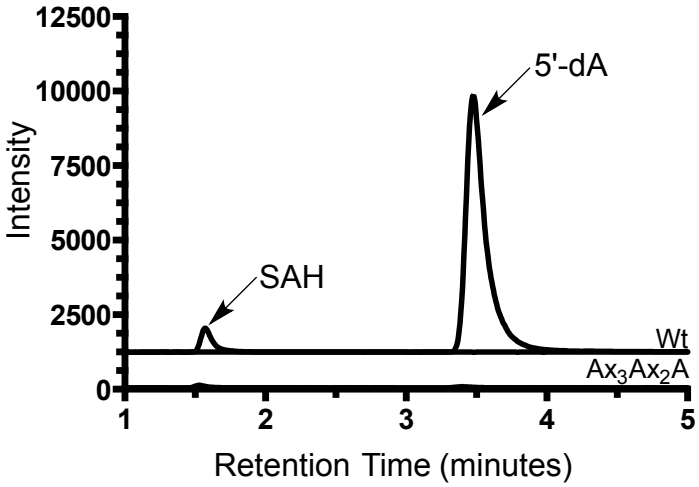

Fig. S5. Fe/S cluster required for ThnK activity. LC-MS/MS was used to detect SAM-derived coproducts, SAH (385.4 -> 136 *m/z*) and 5'-dA (252.1 -> 136 *m/z*). The extracted-ion chromatograms (EICs) for the transitions of SAH and 5'-dA are overlaid for each reaction. Alteration of the Cx_3Cx_2C motif to Ax_3Ax_2A resulted in little SAH production and no 5'-dA production. Reactions were run for approximately 2 hours at room temperature with substrate **11**.

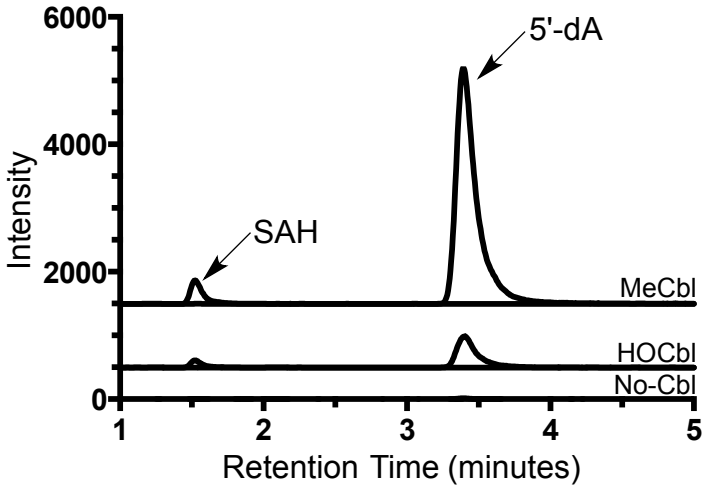

Fig. S6. Cobalamin required for ThnK activity. LC-MS/MS was used to detect SAM-derived coproducts, SAH (385.4 -> 136 *m/z*) and 5'-dA (252.1 -> 136 *m/z*). The extracted-ion chromatograms (EICs) for the transitions of SAH and 5'-dA are overlaid for each reaction. ThnK, expressed in and isolated from *E. coli* cultured in the absence of cobalamin, was either given no cobalamin, HOCbl, or MeCbl. Added cobalamin was able to rescue ThnK activity with MeCbl yielding higher levels of SAH and 5'-dA than HOCbl. Reactions were run for 1 hour and 10 minutes at room temperature with substrate **11**.

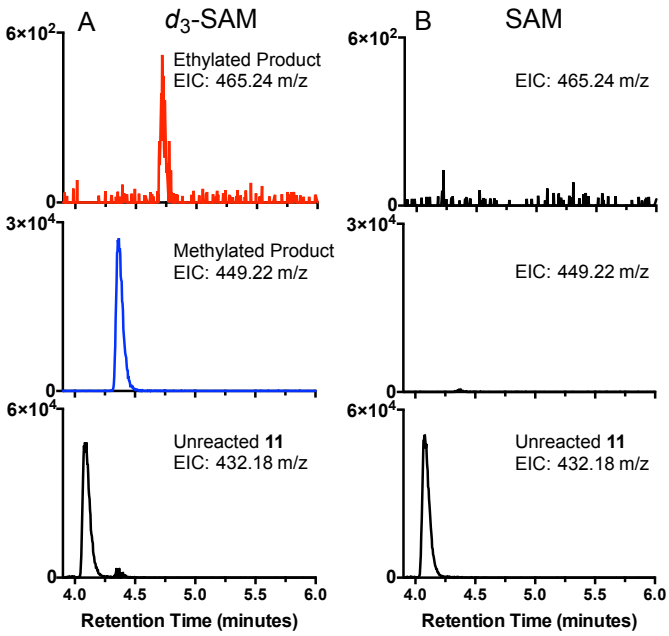

Fig. S7. Product detection of ThnK assays with d_3-SAM.
(*A*) UPLC-HRMS detection of carbapenams from ThnK reaction with compound **11** and d_3-SAM. The bottom, middle, and top traces show the extracted-ion chromatograms (EICs, m/z ± 0.05) for unreacted substrate (432.18 m/z), methylated product (449.22 m/z), and ethylated product (465.24 m/z), respectively. The middle and top traces show a +3 m/z and a +5 m/z shift, respectively, from Fig 3*A*. (*B*) UPLC-HRMS detection of carbapenams from ThnK reaction with compound **11** and natural abundance SAM. The shifted masses observed in *A* are not present in *B*.

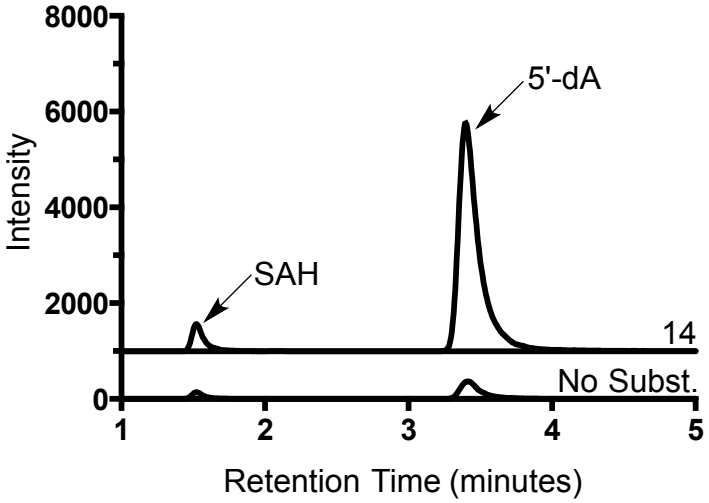

Fig. S8. Detection of SAM-derived products of ThnK with compound **14**. LC-MS/MS was used to detect SAM-derived coproducts, SAH (385.4 -> 136 *m/z*) and 5'-dA (252.1 -> 136 *m/z*). The extracted-ion chromatograms (EICs) for the transitions of SAH and 5'-dA are overlaid for each reaction. The reaction with compound **14** shows increased turnover compared to the no substrate control. Reactions were run for 2 hours and ten minutes at room temperature.

Table S1. LC-MS mass fragments for detection of SAH and 5'd-A			
	Parent Ion	Product Ion 1	Product Ion 2
SAH	385.4	136	134
5'-dA	252.1	136	119
Tyrosine	182.0	165	123

Table S1. Detection of SAH and 5'-dA was performed using electrospray ionization in the positive mode (ESI+) with multiple reaction monitoring (MRM). The masses used for the detection of SAH and 5'-dA are shown. The mass transition from the parent ion to product ion 1 was used for quantification.