Supporting Information

Freeman *et al.* 10.1073/pnas.0804500105

SI Text

***p*-Methoxybenzyl 2-Hydroxycarbapenams 4a, 4b.** The general procedure of Shibuya (29, refer to the main text) was followed. Sodium borohydride (29 mg, 0.77 mmol) was added to a −78°C solution of *p*-methoxybenzyl 2-oxo-carbapenam **3** (809 mg, 2.79 mmol) (27, 28) in 1:1 THF, methanol (10 ml). After 30 min, the reaction was quenched with acetic acid (65 μl) and slowly diluted with ethyl acetate (65 ml). The solution was warmed to room temperature and washed three times with 10 ml of water. The organic layer was dried with brine and sodium sulfate and then concentrated. The resulting light green oil (800 mg) was a 10:1 mixture of **4a** and **4b** determined from the vicinal coupling constant observed at the C3 proton ($^3J_{trans}$ = 2.0 Hz, $^3J_{cis}$ = 5.2 Hz) and correlation with the literature. The mixture could be used without purification or the diastereomers could be separated on a column of silica gel, 30–100% ethyl acetate in hexanes eluted 519 mg (1.78 mmol, 68%) of white solid **4a**, 57 mg of a mixture, and 123 mg (0.42 mmol, 15%) of white solid **4b**.

***p*-Methyoxybenzyl (2S)-Hydroxycarbapenam 4a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 (dt, *J* = 10.8, 3.6 Hz, 1H), 2.18 (ddd, *J* = 10.8, 8.0, 7.6 Hz, 1H), 2.93 (dd, *J* = 16.0, 2.4 Hz, 1H), 3.26 (dd, *J* = 16.0, 5.2 Hz, 1H), 3.78 (s, 3H), 3.88 (br m, 1H), 4.48 (d, *J* = 2.0 Hz, 1H), 4.65 (br m, 1H), 5.05 (s, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 36.53, 44.94, 51.50,55.16, 66.90, 69.81, 79.98, 113.87, 127.16, 130.05, 159.64, 169.09, 178.64.

***p*-Methyoxybenzyl (2R)-Hydroxycarbapenam 4b.** ^{1}H NMR(400 MHz, $CDCl_3$) δ 1.58 (ddd, *J* = 13.6, 9.2, 8.4 Hz, 1H), 2.31 (ddd, *J* = 13.6, 5.2, 1.6 Hz, 1H), 2.62 (dd, *J* = 15.6, 1.6 Hz, 1H), 3.31 (dd, *J* = 15.6, 4.8 Hz, 1H), 3.79 (s, 3H), 4.11 (br m, 1H), 4.50 (d, *J* = 5.2 Hz, 1H), 4.93 (ddd, *J* = 4.4 Hz, 1H), 5.13 (ABq, *J* = 12.0 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 7.29 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 40.22, 42.45, 52.20, 55.22, 65.36, 66.99, 79.29, 113.96, 127.31, 130.14, 159.73, 168.91, 176.42.

***p*-Methoxybenzyl Carbapenem 5**. A solution of 2-hydroxycarbapenams **4a**/**4b** (457 mg, 1.57 mmol) in methylene chloride (10 ml) was treated with mesyl chloride (183 μl, 2.35 mmol) and Et_3N (1.10 ml, 7.85 mmol). After 30 min, the mixture was diluted with ethyl acetate (50 ml) and washed with three times with 10 ml of water. The organic layer was dried with brine and sodium sulfate and then concentrated. The resulting yellow oil was purified by using a plug of silica gel, 50% ethyl acetate in hexane-eluted carbapenem **5** (350 mg, 1.28 mmol, 81%), clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.73 (ddd, *J* = 19.2, 8.0, 2.0 Hz, 1H), 2.91 (ddd, *J* = 19.2, 9.6, 3.2 Hz, 1H), 2.95 (dd, *J* = 16.4, 2.8 Hz, 1H), 3.47 (dd, *J* = 16.4, 5.6 Hz, 1H), 3.78 (s, 3H) 4.25 (sym m, 1H), 5.20 (ABq, *J* = 16.4 Hz, 2H), 6.48 (t, *J* = 2.8 Hz, 1H), 6.88 (d, *J* = 8.8 Hz, 2H), 7.34 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 36.31, 45.45, 51.10, 55.21, 66.78, 113.89, 127.58, 130.01, 131.87, 135.61, 159.66, 159.85, 176.71.

Addition of Panetheine Acetonide 9 to *p*-Methoxybenzyl Carbapenem 5. The procedure of Bateson was used (31). To a solution of *p*-methoxybenzyl carbapenem **5** (175 mg, 0.64 mmol) in DMF (1 ml) was added pantetheine acetonide 9 (224 mg, 0.70 mmol) (30) and Et_3N (133 μl, 0.96 mmol). After 2 h, the solution was diluted with saturated $NaHCO_3$ (20 ml) and extracted three times with 5 ml o f ethyl acetate. The combined organic layers were dried with brine and sodium sulfate and then concentrated. The resulting yellow oil (408 mg) was a 2:1:1 mixture of thioethers **6-8** determined by correlation of the ^{1}H NMR chemical shift of the C3 proton with the literature [(2*S*,3*R*)-**6** 4.37; (2*R*,3*R*)-**7** 4.71; (2*R*,3*S*)-**8** 4.09]. The diastereomers were separated by HPLC [Phenomonex Luna 5μ silica (2) 100A 250 × 10 mm 5μ, 3% methanol in ethyl acetate mobile phase, observed at 265 nm, retention time (min): pantetheine acetonide **9**, 13.4; **6**, 15.2; **7**, 17.7, **8**, 25.2].

***(2S,3R,5R) p*-Methoxybenzyl-2-(2-(3-(2,2,5,5-Tetramethyl-1,3-Dioxane-6-Carboxamido) Propanamido)Ethylthio))Carbapenam (6)**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (s, 3H), 1.04 (s, 3H), 1.42 (s, 3H), 1.46 (s, 3H), 1.62 (ddd, *J* = 14.4, 6.8 Hz, 1H), 2.43 (t, *J* = 6.4 Hz, 2H), 2.60 (sym m, 2H), 2.74 (ddd, *J* = 14.4, 7.6, 1.6 Hz, 1H), 2.82 (dd, *J* = 16.0, 2.0 Hz, 1H), 3.26 (d, *J* = 11.6 Hz, 1H), 3.30 (dd, *J* = 16.0, 5.2 Hz, 1H), 3.35 (m, 2H), 3.50 (m, 2H), 3.67 (d, *J* = 11.6 Hz, 1H), 3.72 (ddd, *J* = 7.6, 6.8, 5.2 Hz, 1H), 3.81 (s, 3H), 3.89 (sym m, 1H), 4.07 (s, 1H), 4.37 (d, *J* = 5.2 Hz, 1H), 5.12 (ABq, *J* = 12.0 Hz, 2H), 6.23 (br t, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 7.03 (br t, 1H), 7.30 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 18.68, 18.88, 22.11, 29.45, 32.22, 32.94, 34.77, 35.89, 38.32, 44.37, 51.96, 52.47, 55.28, 66.29, 67.33, 71.41, 77.13, 99.03, 114.03, 127.16, 130.25, 155.50, 160.50, 169.77, 170.03, 171.16, 175.46.

***(2R,3R,5R) p*-Methoxybenzyl-2-(2-(3-(2,2,5,5-Tetramethyl-1,3-Dioxane-6-Carboxamido) Propanamido)Ethylthio))Carbapenam (7)**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (s, 3H), 1.03 (s, 3H), 1.42 (s, 3H), 1.46 (s, 3H), 2.20 (m, 2H), 2.42 (t, *J* = 6.0 Hz, 2H), 2.61 (m, 1H), 2.73 (m, 1H), 2.75 (dd, *J* = 13.2, 2.8 Hz, 1H), 3.26 (d, *J* = 11.6 Hz, 1H), 3.32 (m, 2H), 3.35 (dd, *J* = 13.2, 5.6 Hz, 1H), 3.50 (m, 2H), 3.55 (ddd, *J* = ≈7.2 Hz, 1H), 3.68 (d, *J* = 11.6 Hz, 1H), 3.81 (s, 3H), 4.07 (s, 1H), 4.09 (m, 1H), 4.71 (d, *J* = 7.2 Hz, 1H), 5.12 (s, 2H), 6.30 (br t, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 7.03 (br t, 1H), 7.30 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 18.69, 18.89, 22.12, 29.45, 32.95, 33.06, 34.77, 35.95, 36.02, 38.51, 44.45, 49.06, 51.97, 55.28, 65.35, 67.01, 71.41, 77.14, 99.05, 113.99, 127.50, 130.36, 159.86, 168.35, 170.11, 171.07, 176.11

***(2R,3S,5R) p*-Methoxybenzyl-2-(2-(3-(2,2,5,5-Tetramethyl-1,3-Dioxane-6-Carboxamido) Propanamido)Ethylthio))Carbapenam (8)**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (s, 3H), 1.03 (s, 3H), 1.42 (s, 3H), 1.46 (s, 3H), 1.90 (ddd, *J* = ≈11.6 Hz, 1H), 2.29 (ddd, *J* = 12.4, 5.2 Hz, 1H), 2.42 (t, *J* = 6.4 Hz, 2H), 2.58 (sym m, 1H), 2.71 (sym m, 1H), 2.82 (dd, *J* = 16.0, 2.4 Hz, 1H), 3.10 (dd, *J* = 16.0, 4.4 Hz, 1H), 3.28 (d, *J* = 11.6 Hz, 1H), 3.30 (m, 2H), 3.50 (m, 2H), 3.68 (d, *J* = 11.6 Hz, 1H), 3.72 (m, 2H), 3.82 (s, 3H), 4.07 (s, 1H), 4.09 (d, *J* = 7.6 Hz, 1H), 5.14 (ABq, *J* = 16.0 Hz, 2H), 6.31 (br t, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 7.01 (br t, 1H), 7.35 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 18.66, 18.87, 22.10, 29.45, 32.93, 33.0, 34.79, 35.98, 36.05, 38.78, 42.21, 51.79, 52.16, 55.25, 64.59, 67.42, 71.38, 77.13, 99.05, 113.91, 127.03, 130.64, 159.84, 167.88, 170.15, 171.11, 172.33.

(2S,3R,5R)* Potassium-2-(2-(3-(2,2,5,5-Tetramethyl-1,3-Dioxane-6-Carboxamido) Propanamido)Ethylthio))Carbapenam (1)**. The general procedure of Lee was adopted (32). A solution of of protected pantethenyl carbapenam ***6 (17 mg, 0.028 mmol) in methylene chloride (450 μl) was covered with nitrogen and cooled to 0°C. TFA (220 μl) and anisole (44 μl) were added. After 15 min, the solution was concentrated, and the residue was taken up in benzene (1 ml) and concentrated again. The residue was triturated with two times with 1 ml of ether to give a white solid. This

was taken up in water (10 ml) and 1 equivalent of $KHCO_3$ was added. The solution was washed two times with 3 ml of ethyl acetate and lyophilized to give the desired product as a white solid.

(2S,3R,5R) **Potassium-2-(2-(3-(2,4-Dihydroxy-3,3-Dimethylbutamido-)Propanamido) Ethylthio)Carbapenam 1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (s, 3H), 0.94 (s, 3H), 1.86 (ddd, J = 13.6, 9.6 Hz, 1H), 2.53 (t, J = 6.4 Hz, 2H), 2.80 (ddd, J = 13.6, 6.8 Hz, 1H), 2.90–3.05 (m, 3H), 3.40–3.58 (m, 7H), 3.77 (ddd, J = ≈7.2 Hz, 1H), 4.01 (s, 1H), 4.15 (sym m, 1H), 4.19 (d, J = 6.0 Hz, 1H).

(2R,3R,5R) **Potassium-2-(2-(3-(2,4-Dihydroxy-3,3-Dimethylbutamido-)Propanamido) Ethylthio))Carbapenam 2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (s, 3H), 0.95 (s, 3H), 2.29 (m, 1H), 2.53 (t, J = 6.4 Hz, 2H), 2.88 (m, 2H), 2.98 (m, 1H) 3.38–3.62 (m, 7H), 3.98 (br m, 1H), 4.02 (s, 1H), 4.38 (br m, 1H), 4.61 (d, J = 5.6 Hz, 1H).

29. Shibuya M, Kubota S (1981) Synthesis of 1,1-dimethylcarba-2-penem derivatives via a Dieckmann-type cyclization. *Tetrahedron Lett* 22:3611–3614.
27. Reider PJ, Grabowski EJ (1982) Total synthesis of thienamycin—A new approach from aspartic-acid. *Tetrahedron Lett* 23:2293–2296.
28. Ueda Y, Roberge G, Vinet V (1984) A simple method of preparing trimethylsilyl-enol and tert-butyldimethylsilyl-enol ethers of alpha-diazoacetoacetates and their use in the synthesis of a chiral precursor to thienamycin analogs. *Can J Chem* 62:2936–2940.
31. Bateson JH, Hickling RI, Smale TC, Southgate R (1990) Olivanic acid analogs. 6. Biomimetic synthesis of (+/−)-Ps-5, (+/−)-6-Epi-Ps-5, and (+/−)-Benzyl Mm22381. *J Chem Soc Perk T* 1:1793–1801.
30. Patil G (1995) Patent Cooperation Treaty Appl WO95/11893 (May 4, 1995).

A.

Enzyme		Proposed cleavage site	
		▼	
DmpA	242	QSQLQER**GS**IIVVLATDLPI	261
BapA-PS	231	KVGVPGM**GS**IVITIATDAPL	250
BapA	271	AGKPQDK**NS**LLIVIATDAPL	290
NylC	259	PPVTEAG**NT**TISAIVTNVRM	278
M.tur.	225	GAFNTPF**NT**TIGVIACDAAL	244
M.lep.	236	KSPLSAL**NT**TIGVVATDATL	255
ThnT	274	AGGAATL**NT**TLAVVATDATL	293
		▲	

B.

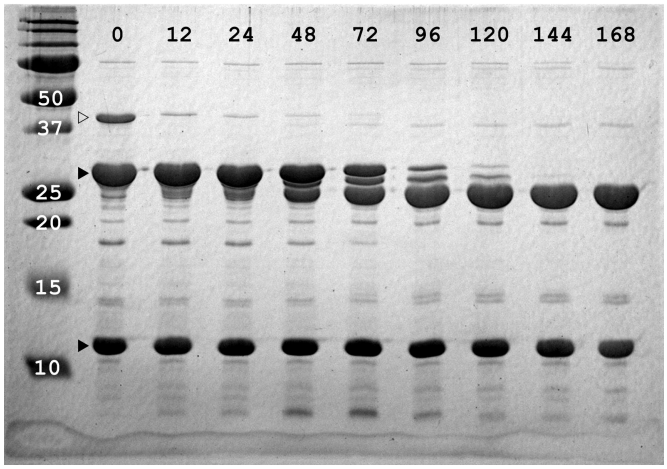

Fig. 1. The autocatalytic cleavage site of ThnT. (*A*) Sequence alignment of probable intramolecular cleavage sites, indicated with solid arrows, of members from the DmpA/OAT superfamily. Corresponding amino acid numbers flank the appropriate sequences. Proteins aligned: DmpA (GenBank accession no. CAA66259) from *Ochrobactrum anthropi*; BapA-PS (GenBank accession no. BAE02664) from *Pseudomonas* sp. MCI3434; BapA (GenBank accession no. AAX93858) from *Sphingosinicella xenopeptidilytica*; NylC (GenBank accession no. BAA05088) from *Flavobacterium* sp. KI723T1; M.tur. (GenBank accession no. CAA98097) from *Mycobacterium tuberculosis* H37Rv; M.lep. (GenBank accession no. AAA50889) from *Mycobacterium leprae*; ThnT (GenBank accession no. CAD18988) from *Streptomyces cattleya*. (*B*) SDS/PAGE (15%) of ThnT autocatalytic cleavage time course at room temperature. Open arrow indicates full-length, unprocessed Nhis-ThnT (41,321.08 Da), and solid arrows indicate predicted cleavage products of 29,229.4 Da and 12,109.7 Da, respectively. Lane 1: Bio-Rad Precision Plus Protein Standards (molecular masses of pertinent standards labeled on the gel); lanes 2–10: corresponding cleavage time points denoted in hrs. Total protein (6.7 μg) loaded per lane.

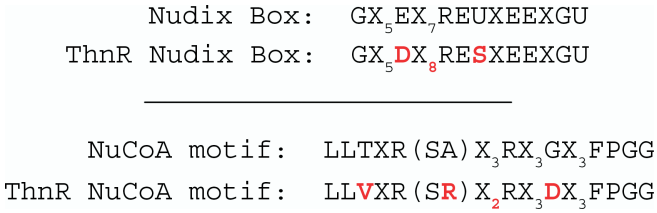

Fig. S2. Nudix box and NuCoA motifs of CoA pyrophosphatases compared with ThnR sequence. X represents any amino acid, and U denotes a bulky hydrophobic amino acid, usually isoleucine, leucine, or valine. Differences in ThnR motifs are highlighted in red.

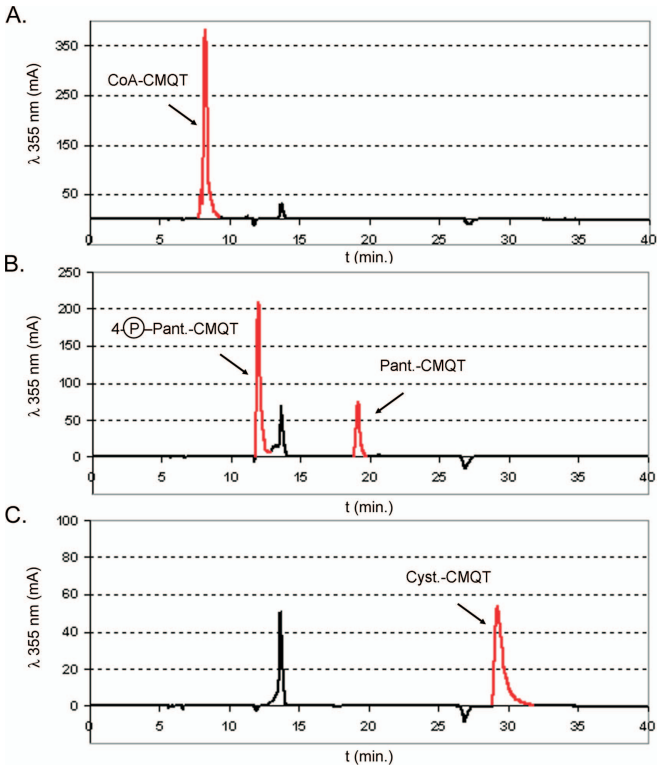

Fig. S3. Enzymatic reactions of substrates prederivatized with CMQT run for 3 h at 37°C. (*A*) ThnR with derivatized CoA. (*B*) ThnH with derivatized 4-phosphopantetheine. (*C*) ThnT with derivatized pantetheine. ThnR was unable to accept CMQT-CoA as a substrate, whereas ThnH minimally produced CMQT-pantetheine from CMQT-4-phosphopantetheine. Only ThnT was able to efficiently accept and cleave a thiol-derivatized substrate. The retention times of each derivatized standard were: CoA-CMQT, 8.2 min; 4-phosphopantetheine-CMQT, 11.7 min; pantetheine-CMQT, 18.9 min; and cysteamine-CMQT at 29.3 min.

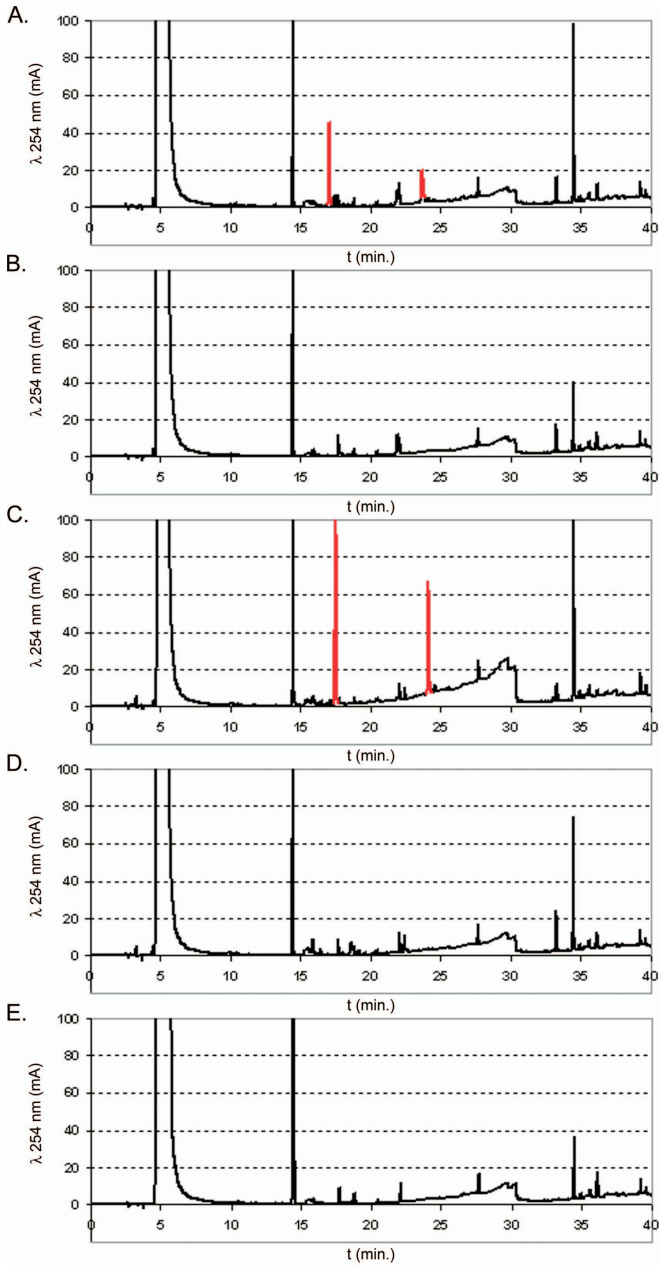

Fig. S4. HPLC analyses of ThnT reactions with *cis*- and *trans*-pantetheinyl-carbapenams. Reactions (200 μl) containing 2 μM ThnT and 2.5 mM substrate in 100 mM Tris (pH 7.5) were run for 1–3 h at 37°C. Aliquots (100 μl) were derivatized with 100 mg/ml (wt/vol) dansyl-Cl and 100 ml 0.1 M $NaHCO_3$ and incubated for 1 h at ambient temperature. The resulting samples were then 0.2-μm filtered, and 30 μl was loaded onto a Phenomenex Luna 5 μm Phenyl-Hexyl 100 Å (250 $\times$ 10.0 mm) column. A flow rate of 1.0 ml/min. was used with solvents acetonitrile (solvent A) and dH_2O with 0.1% (vol/vol) TFA (solvent B). Monitoring at 254 nm, a method of 95% solvent B from 0–5 min, 50% solvent B at 25 min, 5% solvent B from 35–45 min, and 95% solvent B from 50–60 min was used for optimum separation of all analytes. The *trans*-cysteaminyl-carbapenam eluted from the column at 24.1 min with the corresponding β-lactam hydrolyzed product at 17.5 min, whereas the *cis*-cysteaminyl-carbapenam eluted at 23.7 min and its hydrolyzed product at 17.0 min. (*A*) ThnT reaction with the *cis*-pantetheinyl-carbapenam. (*B*) *Cis*-pantetheinyl-carbapenam without enzyme. (*C*) ThnT reaction with the *trans*-pantetheinyl-carbapenam. (*D*) *Trans*-pantetheinyl-carbapenam without enzyme. (*E*) ThnT reaction without substrate. Cysteamine-containing products are highlighted in red.

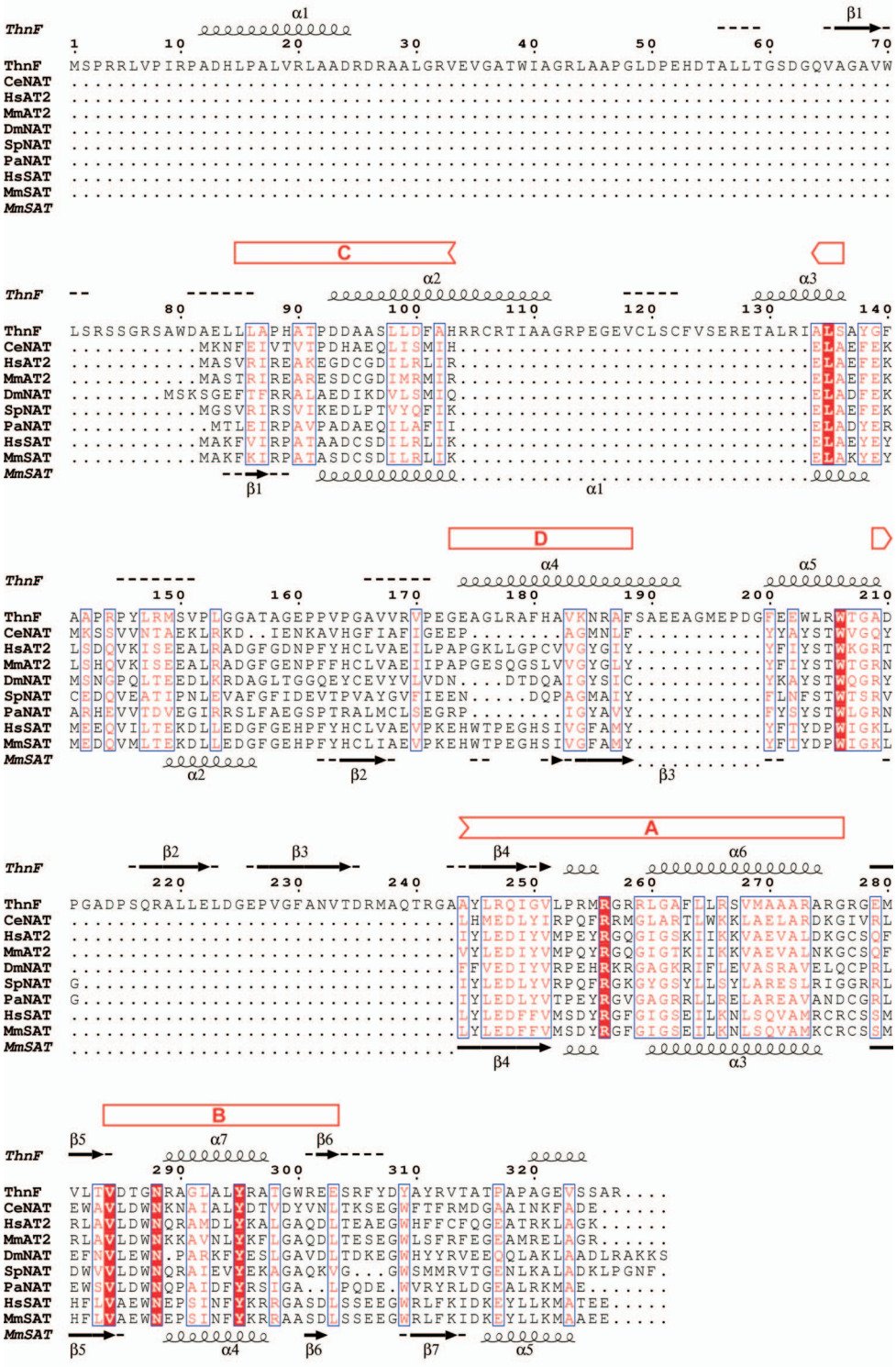

Fig. S5. Sequence alignment of ThnF with other representatives of the GNAT-acetyltransferase superfamily. Figure adapted from Abo-Dalo *et al.* (1) Predicted secondary structures of ThnF and MmSAT lie above and below the sequence alignment, respectively. GNAT-acetyltransferase conserved domains A–D are marked as boxes above the alignment. In the ThnF sequence, domains A and B are the only conserved domains that bear any resemblance to the other acetyltransferase sequences, although strong similarity is not observed in any region of the protein. This is further supported by similarities of secondary structure prediction only in the C-terminal region of ThnF. Proteins aligned: ThnF (GenBank accession no. CAD18974) from *Streptomyces cattleya*; CeNAT (GenBank accession no. NP_505978) from *Caenorhabditis elegans*; HsAT2 (GenBank accession no. NP_597998) from *Homo sapiens*; MmAT2 (GenBank accession no. NP_081267) from *Mus musculus*; DmNAT (GenBank accession no. NP_650430) from *Drosophila melanogaster*; SpNAT (GenBank accession no. NP_593494) from *Schizosaccharomyces pombe* 972h-; PaNAT (GenBank accession no. NP_249169) from *Pseudomonas aeruginosa* PAO1; HsSAT (GenBank accession no. CAA78509) from *Homo sapiens*; MmSAT (GenBank accession no. Q01612) from *Mesocricetus auratus*.

1. Abo-Dalo B, Ndjonka D, Pinnen F, Liebau E, Luersen K (2004) A novel member of the GCN5-related *N*-acetyltransferase superfamily from *Caenorhabditis elegans* preferentially catalyses the *N*-acetylation of thialysine [*S*-(2-aminoethyl)-L-cysteine]. *Biochem J* 384:129–137.

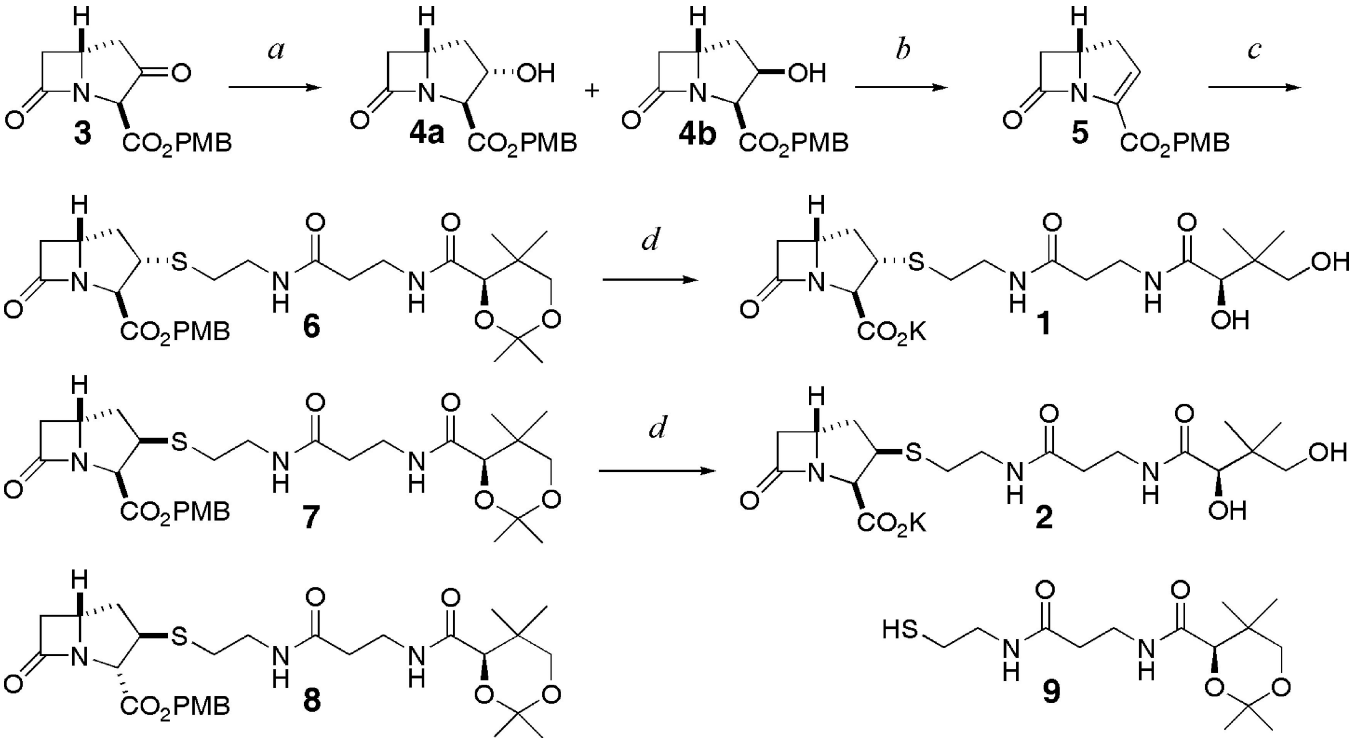

Fig. S6. Synthesis of 2-pantetheinyl carbapenams. Reagents: $NaBH_4$, THF/MeOH, −78°C, 80%, (**4a**:**4b**; 10:1 ratio) (*a*); Et_3N, MsCl, CH_2Cl_2, 80% (*b*); DMF, Et_3N, **9**, 86%, (6:7:8; 2:1:1 ratio) (*c*); CH_2Cl_2, TFA, anisole, 0°C, 90% (*d*).

Table S1. DNA primers used in this study

Primer name	Sequence
ThnTexp-codon-F	5′-CAAGACATATGGACCCGGAAGCTGGTTCTCGTGCTC-3′
F-thnT-exp	5′-GAGTATCCATATGGACCCCGAGGCCGG-3′
R-thnT-exp	5′-CCGGAATTCTCAGGGACGCGCGTACAG-3′
thnT-codon-F	5′-TATGGACCCGGAAGCTGGTTCTCGTGCTCGTGGTCTGAAAGGTGTTCACCGG-3′
thnT-codon-R	5′-GTGAACACCTTTCAGACCACGAGCACGAGAACCAGCTTCCGGGTCCA-3′
ThnR-F	5′-CAAGACATATGGCGGACGGCCCCGGTGC-3′
ThnR-R	5′-GCCAAGCTTTCAGCGACCGAAACCGCCGC-3′
ThnR-chis-R	5′-CACTATCTCGAGGCGACCGAAACCGCCGCC-3′
ThnR-codon-F	5′-CAAGACATATGGCTGACGGTCCGGGCGCTT-3′
ThnR-link-F	5′-TATGGCTGACGGTCCGGGCGCTTACGCTGATCCGGTTGACCTGGAT-3′
ThnR-link-R	5′-CCAGGTCAACCGGATCAGCGTAAGCGCCCGGACCGTCAGCCA-3′
thnH-opt-1F	5′-GCCACTGAGCACCAACTACGCATATGGTTTGC-3′
thnH-opt-2F	5′-GCAGCATCCTCACCTGCTGGAGCACTGCTTGTAGATTGGG-3′
thnH-opt-3F	5′-GGGGAGTTTTGACGCAGCCGTTCTACGCGGGGCATTGCGGA-3′
thnH-opt-4F	5′-ATGGGCAGCGCGTGACGGAGTGGATGCAGACGCTTTTCAC-3′
thnH-opt-5F	5′-GCCTTACTGGCTCGCCATCTGGGCCCGGGCGCGCCGGGTG-3′
thnH-opt-6F	5′-GCGCGGCCGCGAGTGTATTTCACCGTGTCGAACGTGGCGA-3′
thnH-opt-7F	5′-GGTGCCAGTGGCGGAACTCGAAGTCACCCTGGCGGAATCT-3′
thnH-opt-8F	5′-TTGCGGCGTCCGGACGGTACCGGGCCCCCCGCCGAGGGTT-3′
thnH-opt-9F	5′-TGATTCAACGCATGTTTCAGCCTTTCACAATGGCCGGCGC-3′
thnH-opt-10F	5′-CATGGTTGAACTCGTACGTCGTGTTCGTGCCTCTGGTGCT-3′
thnH-opt-11F	5′-GCCGTGGCACTGCTGAGCATTCATGGGGTCACACTTATG-3′
thnH-opt-12F	5′-ATCGTACCGGTTGGGACGGCCTGTTTGATGAGGTGGTCAT-3′
thnH-opt-13F	5′-CTCGTGCGAGGTTGGGATGCGGAAACCAGAACCGGAAATC-3′
thnH-opt-14F	5′-TACCGCTATACCGCGCGCCGTTTAGGCGTAGCACCACGCC-3′
thnH-opt-15F	5′-GCTGTGTCTTCCTGGATGATTTAGGCCGCAACGTCCGTGC-3′
thnH-opt-16F	5′-GGCGGCTGCCGTGGGGATGACTGCTGTGCAGCATACGTCG-3′
thnH-opt-17F	5′-GTGGAAGAAAGTTCCCGGGAGCTTGCGCGCTTCTTTGATG-3′
thnH-opt-18F	5′-TTAGCCCGCTGCCGGCCGGTCGCTAAAAGCTTGGTGTAGT-3′
thnH-opt-1R	5′-GGCACTTCGCTCACTACACCTTCGAATTAGCG-3′
thnH-opt-2R	5′-ACCGGCCGGCAGCGGGCTAACATCAAAGAAGCGCGCAAGC-3′
thnH-opt-3R	5′-TCCCGGGAACTTTCTTCCACCGACGTATGCTGCACAGCAG-3′
thnH-opt-4R	5′-TCATCCCCACGGCAGCCGCCGCACGGACGTTGCGGCCTAA-3′
thnH-opt-5R	5′-ATCATCCAGGAAGACACAGCGGCGTGGTGCTACGCCTAAA-3′
thnH-opt-6R	5′-CGGCGCGCGGTATAGCGGTAGATTTCCGGTTCTGGTTTCC-3′
thnH-opt-7R	5′-GCATCCCAACCTCGCACGAGATGACCACCTCATCAAACAG-3′
thnH-opt-8R	5′-GCCGTCCCAACCGGTACGATCATAAGTGTGACCCCATGAA-3′
thnH-opt-9R	5′-TTGCTCAGCAGTGCCACGGCAGCACCAGAGGCACGAACAC-3′
thnH-opt-10R	5′-GACGTACGAGTTCAACCATGGCGCCGGCCATTGTGAAAGG-3′
thnH-opt-11R	5′-CTGAAACATGCGTTGAATCAAACCCTCGGCGGGGGGCCCG-3′
thnH-opt-12R	5′-GTACCGTCCGGACGCCGCAAAGATTCCGCCAGGGTGACTT-3′
thnH-opt-13R	5′-CGAGTTCCGCCACTGGCACCTCGCCACGTTCGACACGGTG-3′
thnH-opt-14R	5′-AAATACACTCGCGGCCGCGCCACCCGGCGCGCCCGGGCCC-3′
thnH-opt-15R	5′-AGATGGCGAGCCAGTAAGGCGTGAAAAGCGTCTGCATCCA-3′
thnH-opt-16R	5′-CTCCGTCACGCGCTGCCCATTCCGCAATGCCCGCGTAGAA-3′
thnH-opt-17R	5′-CGGCTGCGTCAAAACTCCCCCCCAATCTACAAGCAGTGCT-3′
thnH-opt-18R	5′-CCAGCAGGTGAGGATGCTGCGCAAACCATATGCGTAGTTG-3′
thnH-codon-F	5′-CAAGACATATGGTTTGCGCAGCATCCTCACCT-3′
thnH-codon-R	5′-GCCAAGCTTTTAGCGACCGGCCGG-3′
thnH-codon-chis-R	5′-CACTAACTCGAGGCGACCGGCCGGC-3′
ThnF-F	5′-CAAGACATATGAGCCCACGGCGGCTGGTCCC-3′
ThnF-R	5′-GCCAAGCTTTCATCGGGCGGAGCTGACCTCC-3′
ThnF-chis-R	5′-CACTATCTCGAGTCGGGCGGAGCTGACCTCCCC-3′