Biochemical and Structural Characterization of the Tautomycetin Thioesterase: Analysis of a Stereoselective Polyketide Hydrolase**

*Jamie B. Scaglione, David L. Akey, Rachel Sullivan, Jeffrey D. Kittendorf, Christopher M. Rath, Eung-Soo Kim, Janet L. Smith, and David H. Sherman**

Table of Contents

1. Methods p. 2
2. Crystallography Summary p. 13
3. References p. 14

Expression and Purification of the TMC TE Domain

The TMC TE was amplified from cosmid pTMC2290 using PCR with LIC overhangs and inserted into the vector pMCSG7.[1, 2] Mutants were generated using site-directed mutagenesis. All primers are listed in Supplementary Table 1.

All DNA sequences were confirmed by sequencing.

Primer Name	Primer Sequence	Plasmid(s) Generated
TMCTELICFor	TACTTCCAATCCAATGCC gca cag tcc gac	pDHS4129
TMCTELICRev	TTATCCACTTCCAATGCTA tca ttc ttc cgt ccg	pDHS4129
TMCTESAFor	ggc cgg gca cgc ctc ggg cgg	pDHS4130
TMCTESARev	ccg ccc gag gcg tgc ccg gcc	pDHS4130
TMCTEHRFor	gcg ccc ggc gac cgc ttc acg atc atc	pDHS4131, pDHS4133
TMCTEHRRev	gat gat cgt gaa gcg gtc gcc ggg cgc	pDHS4131, pDHS4133
TMCTESCFor	gcc ggg cac tgc tcg ggc ggc	pDHS4132, pDHS4133
TMCTESCRev	gcc gcc cga gca gtg ccc ggc	pDHS4132, pDHS4133

Supplementary Table 1: Primers used for generation of expression plasmids via ligation independent cloning. All sequences are listed 5' to 3'. Sequences in all capital letters represent the LIC overhangs necessary for insertion into the pMCSG7 vector.

Plasmids encoding TEV protease-cleavable N-terminal His_6-fusion proteins were transformed into *E. coli* BL21(DE3) and grown at 37 °C in TB medium to an OD_{600} of 1.0 in 2-L flasks. The cultures were cooled to 18 °C, and isopropyl β-D-thiogalacto-pyranoside was added to a final concentration of 0.2 mM and grown for 12−16 h with shaking. The cells were harvested by centrifugation and frozen at −20 °C. Selenomethionyl protein was produced in a similar fashion using selenomethionine minimal medium.[3] Cell pellets were thawed to 4 °C and resuspended in 5X volume of lysis buffer (20 mM HEPES, pH 7.4, 300 mM NaCl, 20 mM imidazole, 10% glycerol, and 1mM TCEP) before lysis *via* sonication. Proteins were purified using Ni-NTA (Qiagen) affinity chromatography. The cell lysate was incubated with the resin for 0.5 hr, poured into a column, washed with 3 column volumes of lysis buffer, and eluted with 5 mL of a buffer containing 20 mM HEPES, pH 7.4, 300 mM NaCl, 400 mM imidazole, 10% glycerol, and 1mM TCEP. Fractions were combined, buffer exchanged into storage buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM DTT), concentrated, frozen, and stored at −80 °C. Protein yields were consistently 80 mg L^{-1} of cell culture.

Synthesis of substrates

General Methods. NMR spectra were recorded in $CDCl_3$ at 400 MHz (^1H) or 100 MHz (^{13}C). Optical rotations were determined on a Perkin-Elmer Model 341 polarimeter. Solvents were used either as purchased or dried and purified by standard methodology. Flash chromatography was performed using silica gel (32–63 microns) purchased from Scientific Adsorbents, Atlanta, GA.

(*R*,*E*)-4-ethyl-3-hydroxy-1-((*S*)-4-isopropyl-2-thioxothiazolidin-3-yl)oct-4-en-1-one (2) and **(*S*,*E*)-4-ethyl-3-hydroxy-1-((*R*)-4-isopropyl-2-thioxothiazolidin-3-yl)oct-4-en-1-one (3).** *N*-Ethylpiperidine (0.12 mL, 0.88 mmol) was added dropwise to a suspension of tin(II) trifluoromethanesulfonate (370 mg, 0.88 mmol) in CH_2Cl_2 (5 mL) at -40 ^oC. A solution of the known (*R*) or (*S*)-3-acetyl-4-isopropylthiazolidine-2-thione **1** (150 mg, 0.74 mmol) in CH_2Cl_2 (2 mL) was added, and the reaction stirred at -40 ^oC for 4 h. After addition of trans-2-ethyl-2-hexenal (91 μL, 0.76 mmol) in CH_2Cl_2 at -78 ^oC, the reaction was stirred for 1 h and then water (3 mL) and ether (15 mL) were added. The aqueous layer was extracted with ether (3 x 5 mL) and the combined organic extracts filtered through Celite and concentrated under reduced pressure. Chromatography (25% EtOAc in hexanes) gave either compound **2** or **3** as bright yellow oils (170 mg, 70%). **2:** ^1H NMR δ 5.49-5.45 (1H, t, *J* = 7.4 Hz), 5.17-5.13 (1H, t, *J* = 8.0 Hz), 4.61-4.59 (1H, m), 3.60-3.49 (2H, m), 3.41-3.34 (1H, m), 3.05-3.01 (1H, m), 2.61-2.60 (1H, m), 2.40-2.35 (1H, m), 2.17-2.00 (4H, m), 1.42-1.36 (2H, m), 1.07-0.98 (6H, m), 0.93-0.89 (3H, t, *J* = 7.4); ^{13}C NMR δ 203.0, 173.0, 141.3, 126.7, 71.6, 71.5, 44.7, 30.8, 30.6, 29.4, 22.8, 20.7, 19.1, 17.8, 14.2, 13.9; HRMS (EI) *m/z* [M+Na^+] Calcd for $C_{16}H_{27}NO_2S_2$: 352.1381. Found: 352.1394. The NMR spectra of compound **3** were identical to that of **2**; HRMS (EI) *m/z* [M+Na^+] Calcd for $C_{16}H_{27}NO_2S_2$: 352.1381. Found: 352.1374.

(*R*,*E*)-*S*-2-acetamidoethyl 4-ethyl-3-hydroxyoct-4-enethioate (4) and **(*S*,*E*)-*S*-2-acetamidoethyl 4-ethyl-3-hydroxyoct-4-enethioate (5).** Compound **4** or **5** (160 mg, 0.49 mmol) was dissolved in CH_2Cl_2 (10 mL) and imidazole (95 mg, 1.4 mmol) and N-acetylcysteamine (57 μL, 0.54 mmol) were added. The reaction was stirred at room temperature until it was observed to be complete by TLC (5% MeOH in CH_2Cl_2, ~5 h). At this time the solvent was removed and the residue applied directly to a silica column containing a top layer of copper sulfate impregnated silica to remove any remaining free thiol. Column chromatography (3% MeOH in CH_2Cl_2) yielded compound **4** or **5** as colorless oils (120 mg, 85%). **4:** $[\alpha]_D^{25}$ = +25.4 (c=1, $CHCl_3$); ^1H NMR δ 6.16 (1H, bs), 5.45-5.41 (1H, t, *J* = 7.2 Hz), 4.52-4.49 (1H, m), 3.43-3.38 (2H, m), 3.06-2.98 (2H, m), 2.82-2.71 (3H, m), 2.13-2.03 (1H, m), 2.01-1.96 (3H, m), 1.94 (3H, s), 1.38-1.32 (2H, m), 1.01-0.97 (4H, m), 0.89-0.86 (3H, t, *J* = 7.2

Hz); ^{13}C NMR δ 199.0, 170.5, 141.4, 126.8, 72.5, 50.3, 39.2, 29.3, 28.7, 23.1, 22.7, 20.5, 14.2, 13.8; HRMS (EI) *m/z* [M+Na$^+$] Calcd for $C_{14}H_{25}NO_3S$: 310.1453. Found: 310.1458. **5:** $[\alpha]_D^{25}$= -24.3 (c=1, $CHCl_3$); the NMR spectra of compound **5** were identical to that of **4**; HRMS (EI) *m/z* [M+Na$^+$] Calcd for $C_{14}H_{25}NO_3S$: 310.1453. Found: 310.1440.

(*R*,*E*)-4-ethyl-3-hydroxyoct-4-enoic acid (6). Compound **4** (50 mg, 0.2 mmol) was dissolved in 10% aqueous THF (0.9 mL) and the reaction was cooled to 0 ^{o}C. 30% H_2O_2 (0.6 mL) was added dropwise, followed by LiOH (12 mg, 0.29 mmol), and the reaction was stirred for 0.5 h. The reaction was quenched by addition of 1 N Na_2SO_3 (0.6 mL) and acidified to pH 3 with 5% HCl. The mixture was extracted with EtOAc (2 x 10 mL), washed with brine (3 x 4 mL), dried with Na_2SO_4, and concentrated. Chromatography (2.5% MeOH in CH_2Cl_2 with 1% AcOH) gave carboxylic acid **6** (34 mg, 90%). **6:** ^{1}H NMR δ 6.16 (1H, bs), 5.49-5.46 (1H, t, *J* = 7.4 Hz), 4.52-4.49 (1H, t, *J* = 6.2 Hz), 2.63-2.61 (2H, m), 2.18-2.10 (1H, m), 2.07-2.00 (3H, m), 1.47-1.35 (2H, m), 1.04-1.01 (3H, t, *J* = 7.6 Hz), 0.93-0.89 (3H, t, *J* = 7.4 Hz); ^{13}C NMR δ 177.7, 141.1, 127.0, 71.8, 40.7, 29.4, 22.8, 20.5, 14.2, 13.8; HRMS (EI) *m/z* [M+Na$^+$] Calcd for $C_{10}H_{18}O_3$: 209.1154. Found: 209.1146.

Activity assays

In vitro hydrolysis reactions were set up in a total volume of 100 μL containing 100 mM HEPES buffer at pH = 7.4, substrate at 1 mM, and TE at 5 μM. Reactions were incubated at room temperature overnight. Following incubation, a 4x volume of ice cold acetone was added, the reaction vortexed briefly, and allowed to stand at -20 °C for 1 h. After this hour, the mixture was centrifuged at 4 °C for 30 min to pellet the enzyme and the supernatant was removed and dried. The residue was resuspended in 1:1 acetonitrile:water for LC-MS analysis. A 10 μl sample of this mixture was analyzed by LC-MS with a Shimadzu LCMS-2010EV (Columbia, MD) after separation on an Onyx Monolithic C18 50 x 2.0 mm RP-HPLC column (Phenomenex, Torrance, CA). Samples were desalted online for 5 min with 95% buffer A (5 mM ammonium formate) and 5% buffer B (acetonitrile), followed by gradient elution over 20 min at 0.2 mL/min. The absorbance at 218 nm was monitored and profile mode data was gathered from *m/z* 100-500 utilizing electrospray ionization in scanning ion mode. All masses were in agreement with the predicted hydrolysis products (Figure S1). TMC substrate stereoselectivity was in agreement with the predicted hydroxyl stereochemistry based on the preceding KR sequence (Figure S2).[4]

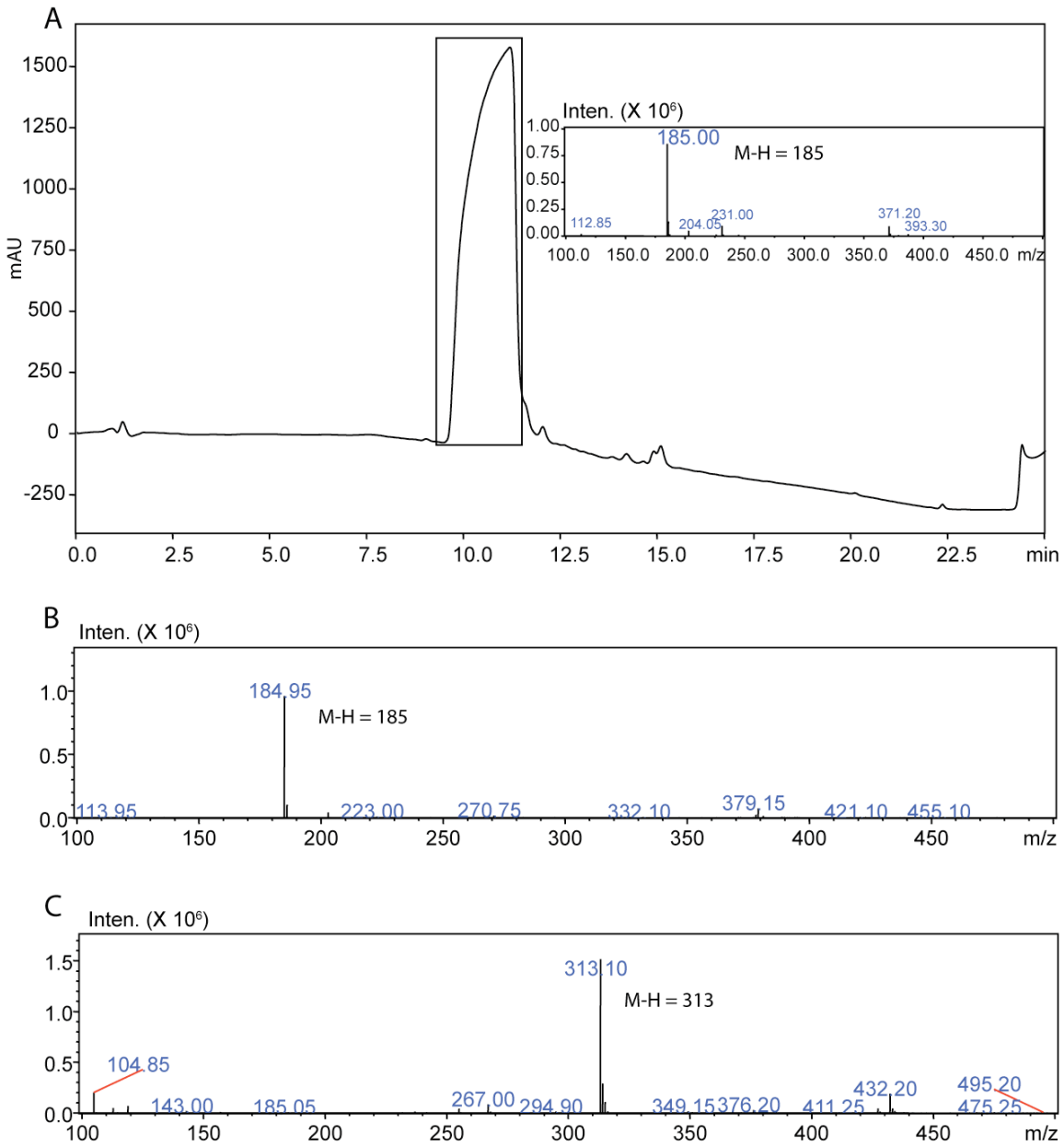

Figure S1. A) LC/MS of the acid standard **6** B) MS of the SNAC **4** hydrolysis product (the MS of SNAC **5** hydrolysis product was identical) C) MS of the pikromycin hexaketide hydrolysis product.

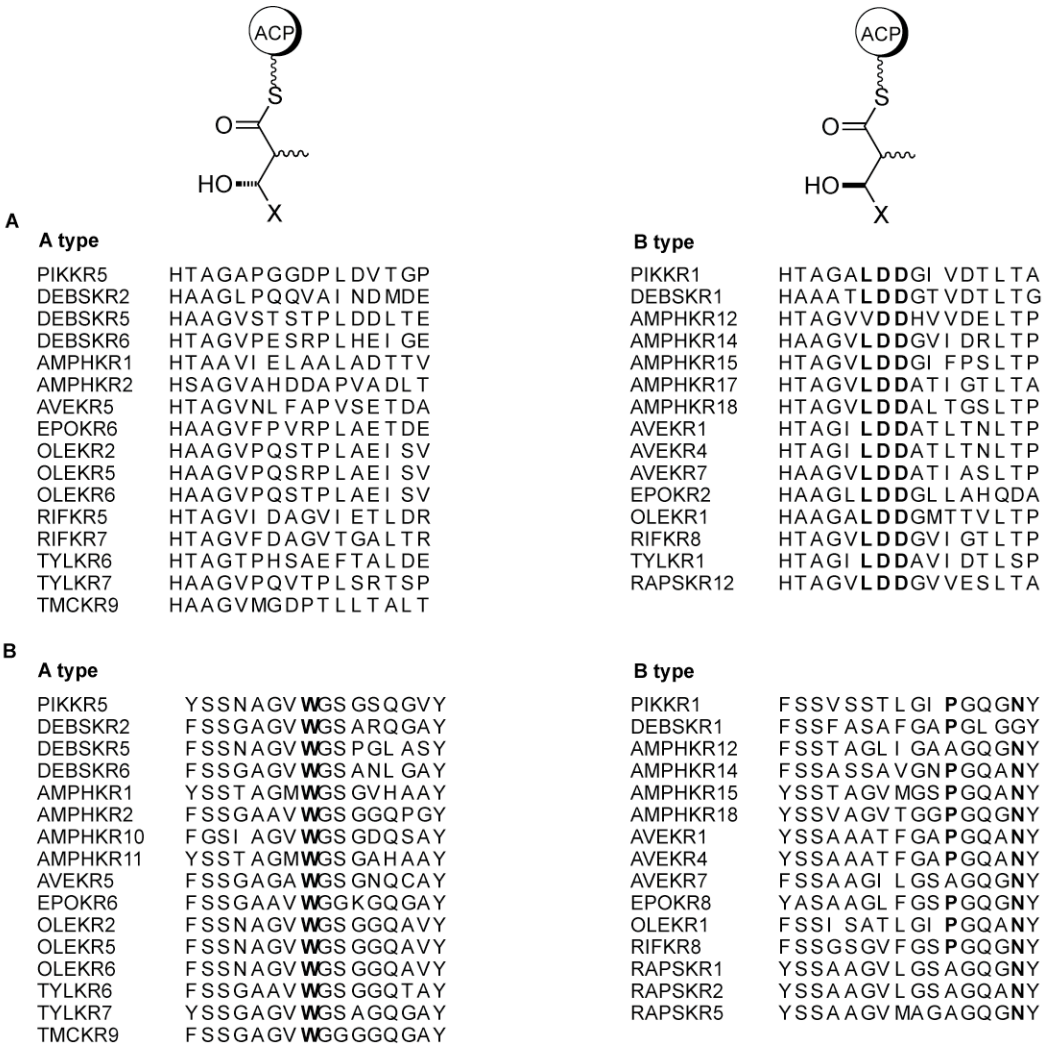

Figure S2. A) Alignment of the residue 88-103 and B) 134-148 regions of various A- and B-type KR domains. The TMC KR9 domain lacks the characteristic LDD and PN motifs of the B-type domains and contains the distinctive W residue of the A-type domains. This stereochemistry is in agreement with biochemical hydrolysis assays. Abbreviations: PIK, pikromycin; DEBS, erythromycin; AMPH, amphotericin; AVE, avermectin; EPO, epothilone; OLE, oleandomycin; RIF, rifamycin, TYL, tylosin; RAPS, rapamycin. Adapted from reference 4.

Kinetic assays

In a 96-well format, 50 μL reactions consisting of 500 nM enzyme and substrate concentrations ranging from 0.2-12.5 mM were quenched at 1, 3, 5, 10, and 20-minute time points with 50 μL isopropanol. ThioGlo® (100 μL at 20 μM) was added to the mixture, and after a 10-minute equilibration the plate was read on a Molecular Devices Spectramax M5 microplate reader with excitation at 379 nm and emission at 513 nm. The rates of hydrolysis were linear over the times analyzed and were corrected for background chemical hydrolysis in the absence of enzyme. All reactions were done in quadruplicate with analysis and fitting done using Prism 5 software (Figure S3).

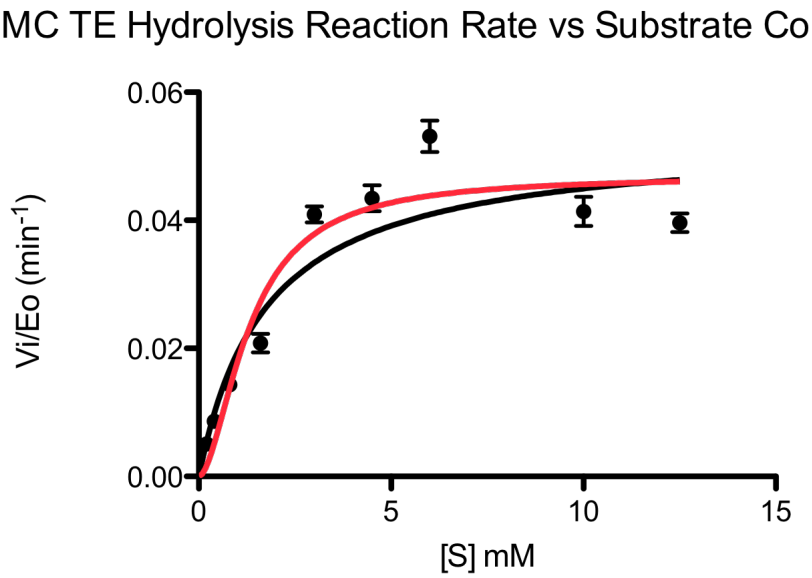

Figure S3. Kinetic analysis of the TMC TE. The black line is the Michaelis-Menten fit with a Vmax of 0.05281 ± 0.003, Km of 1.748 ± 0.36 and R^2 of 0.8553. The red line is the allosteric sigmoidal fit with a Vmax of 0.04697 ± 0.003, Kprime of 1.644 ± 0.34, Hill coefficient of 1.748 ± 0.33 and R^2 of 0.8883.

Labeling/acyltransferase reactions

Labeling reactions were done overnight at room temperature using TE (1 mg/mL, 28 μM) and SNAC **4** or **5** (280 μM). Following the overnight incubation, a 10 μl sample of this mixture was analyzed by LC-MS with a Shimadzu LCMS-2010EV (Columbia, MD) after separation on a PLRP-S 2 × 50 mm, 4000 Å, 8 μm polymeric RP-HPLC column (Varian, Palo Alto, CA) heated to 50°C. Samples were desalted online for 5 min with 95% buffer A (98.9% water, 1% acetonitrile, 0.1% formic acid) and 5% buffer B (1% water, 98.9% acetonitrile, and 0.1% formic acid), followed by gradient elution over 20 min at 0.2 mL/min. The absorbance at 218 nm was monitored and profile mode data was gathered from *m/z* 400–2000 utilizing electrospray ionization in scanning ion mode.

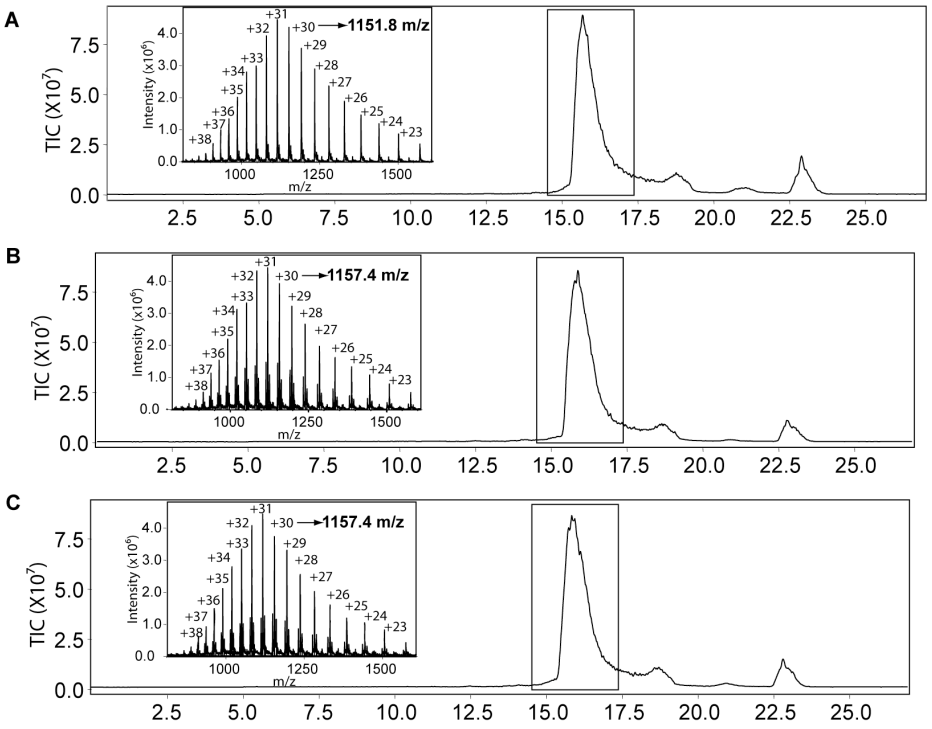

Figure S4. LC/MS analysis of TMC TE covalent modification. Reversed-phase HPLC TIC chromatogram of the overnight incubation of A) wild type TE with SNAC **4**; B) S132C/H255R with SNAC **4**; C) S132C/H255R with SNAC **5**. The inserts are the zoom of the full spectrum, illustrating the increase by 5.6 *m/z* in the +30 charge state of the double mutant, an 168 Da mass increase consistent with covalent loading of SNAC **4** or **5** onto cysteine 132.

For analysis of covalent loading of the double mutant by FTICR-MS, the TMC double mutant was reacted with SNAC **4** as described above. After the overnight incubation, samples were acidified with 1% formic acid. Intact protein samples were desalted with Handee Microspin columns (Pierce) packed with 20 µL of 300 Å polymeric C4 resin (Vydac). Samples were loaded onto the columns and washed with 30 column volumes of 0.1% formic acid prior to elution with 10 column volumes of 50% acetonitrile plus 0.1% formic acid. Intact protein samples were analyzed by an FTICR MS (APEX-Q with Apollo II ion source and actively shielded 7T magnet; Bruker Daltonics). Data was gathered from m/z 500–3,000 utilizing direct infusion electrospray ionization in positive ion mode. Electrospray was conducted at 3,600 V with 24–60 scans per spectrum utilizing 0.5 s external ion accumulation in the hexapole prior to analysis in the FTICR using a loop value of 15. Collision cell pressure was reduced to 2.5e-6 torr. Data was processed (including smoothing and deconvolution) in Data Analysis (Bruker Daltonics). All mass shifts shown were confirmed across all charge states for each ACP present. The most abundant charge state is used for all figures.

For identification of TMC TE double mutant active site and loading with SNAC **4** via FTICR-MS the TMC double mutant (28 µM) was reacted with SNAC **4** (280 µM) in 100 mM HEPES (pH7.5) and 1 mM TCEP. After incubation for overnight at room temperature, 1mg/ml TPCK trypsin (Pierce) was added to a final 1:100 ratio. Samples were incubated at 37 °C overnight. Tryptic peptide samples were desalted with Handee Microspin columns (Pierce) packed with 20 µL of 300 Å polymeric C18 resin (Vydac). Samples were loaded onto the columns and washed with 30 column volumes of 0.1% formic acid prior to elution with 10 column volumes of 50% acetonitrile plus 0.1% formic acid. Tryptic peptide samples were analyzed by an FTICR MS (APEX-Q with Apollo II ion source and actively shielded 7T magnet; Bruker Daltonics). Data was gathered from m/z 200–2,000 utilizing direct infusion electrospray ionization in positive ion mode. Electrospray was conducted at 3,600 V with 12-24 scans per spectra utilizing 1 s external ion accumulation in the hexapole prior to analysis in the FTICR using a loop value of 4. Collision cell pressure was 6.2e-6 torr. CID was performed in the external hexapole at -18 to -30 volts. Data was processed in Data Analysis (Bruker Daltonics) and MIDAS using THRASH (NHMFL Tallahassee Florida).

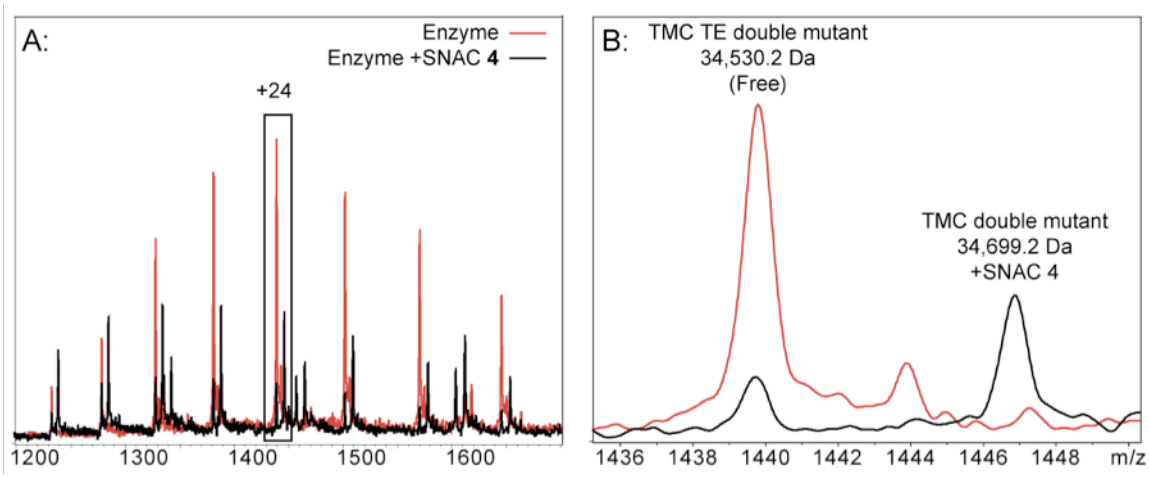

Figure S5. Loading of the TMC double mutant with SNAC 4 via FTICR-MS. Reactions are shown +/- substrate. In the plus substrate reaction, a new peak with a mass shift of 169 Da is observed, corresponding to the loading of SNAC **4** (+169.2 expected). This corresponds to a mass error for the uncalibrated spectrum of -27 ppm.

Table S2: Identification of TMC double mutant active site and loading with SNAC 4 via FTICR-MS. A series of b- and y-ions were identified when the SNAC **4** loaded, tryptic active-site peptide (SLADVVQAEVADGEFALAGH**C**SGGVVAYEVAR) was isolated and subjected to CID MS/MS. This serves to confirm that the mass shift observed in the intact protein spectrum was due to loading of substrate on the enzyme active site.

M mono	ID	dPPM
467.24	b5-H2O	10
584.31	b6	-7.3
566.30	b6-H2O	-6.5
765.39	b8-H2O	-12
894.45	b9-H2O	9
344.22	y3	16
473.27	y4	6
636.32	y5	-0.63
619.30	y5-NH3	9.9
707.37	y6	18
806.44	y7	17
962.53	y9	15

Acyltransferase reactions were performed by two different methods. In Method 1 the TE (1 mg/mL, 28 μM) and SNAC **4** or **5** (280 μM) were incubated overnight over a pH range of 7.5-9 and then excess SNAC removed with Microcon® 3 KDa molecular weight cutoff spin filters. Coenzyme A (100 mM, Sigma) was then added to the filter fraction and allowed to react for 2 or 4 hours at which time the reaction was quenched by enzyme removal with Microcon® 3 KDa molecular weight cutoff spin filters and the products analyzed by LC/MS. No formation of new coenyme A ester was observed with this method. In Method 2 all reaction components (double mutant TE, SNAC **4** or **5**, and free CoA) were mixed together in the same concentrations as Method 1, initiation occurring with enzyme addition and performed over a pH range of 7.5-9. After a 2 hr incubation the reaction was quenched by enzyme removal with Microcon® 3 KDa molecular weight cutoff spin filters and the products analyzed by LC/MS.

For either method, a 10 μL sample of this mixture was analyzed by LC-MS with a Shimadzu LCMS-2010EV (Columbia, MD) after separation on an XBridge 2.1 x 150 mm C18 RP-HPLC column (Waters). Samples were desalted online for 5 min with 100% buffer A (water with 0.05% triethylamine), followed by a 14 minute gradient to 50% buffer A and 50% buffer B (10% water, 90% acetonitrile, with 0.05% triethylamine). The column was then washed by a 1 min gradient to 100% buffer B and a 5 min hold at 100% buffer B, followed by reequilibration of the column by a 1 min gradient to 100% buffer A and a 5 min hold at 100% A. The absorbance at 218 nm was monitored and profile mode data was gathered from *m/z* 100–1000 utilizing electrospray ionization in scanning ion mode.

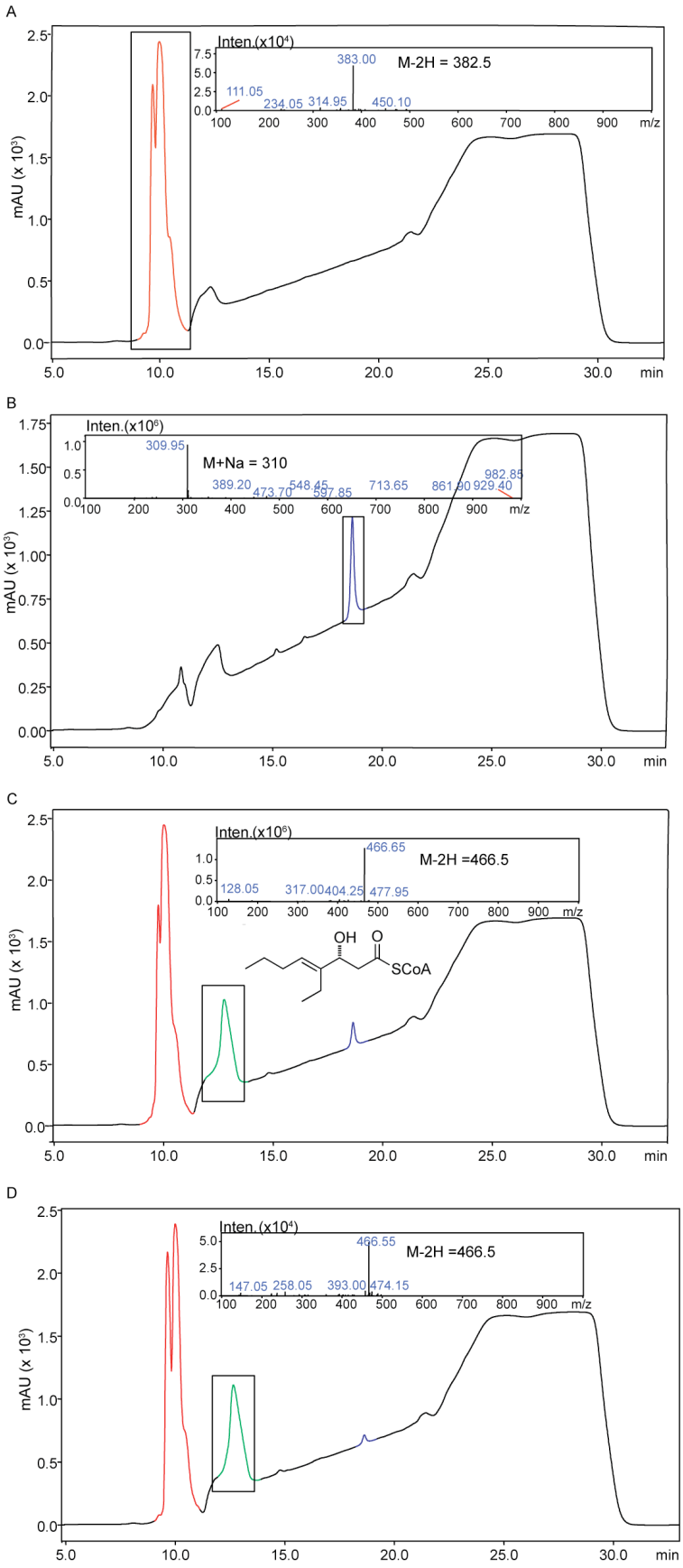

Figure S6. A) LC/MS trace of coenzyme A (CoA) alone; B) LC/MS trace of SNAC **4** alone; C) LC/MS trace of reaction with double mutant TMC TE, CoA, and SNAC **4** resulting in the formation of a new CoA species; D) Same as C without enzyme.

Protein crystallization

The TE was expressed and nickel purified as described above. An additional size exclusion chromatography step was performed on a HiLoad 16/60 Superdex 200 (GE Healthcare) column equilibrated with storage buffer on an Åkta FPLC. Protein concentrations were determined using absorbance at 280 nm and the calculated extinction coefficient 1 A_{280} = 1.0 mg mL^{-1}. TMC TE was crystallized by hanging drop vapor diffusion at 25 ^{o}C. Crystallization drops were set by the addition of protein stock (15-25 mg mL^{-1} TE, 20 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM DTT) to reservoir solution (750 μL of 100-400 mM ammonium formate, 10-40% PEGS 3350) in a ratio of 1:1 (3 μL total) with microseeding of native TMC TE crystals. Crystals were harvested in loops and flash frozen in liquid nitrogen with no cryoprotection step.

Crystallography

X-ray diffraction data were collected at the GM/CA beamline (ID-23D) at the Advanced Photon Source (APS, Argonne National Laboratory). The structure was solved from monoclinic crystals ($P2_1$: a = 53.9 Å, b = 79.7 Å, c = 64.7 Å, b = 104.1°) with two Tmc TE polypeptides forming a biological dimer in the asymmetric unit. Although we expected the structure could be solved by molecular replacement, we chose to use multi-wavelength anomalous diffraction to determine phases. A 2.3 Å three-wavelength MAD data set was recorded from a SeMet TMC TE crystal for structure determination. Data were processed using the HKL2000 package (Supplementary Table 3).[5] Determination of Se atomic positions, experimental phasing, density-modification phase refinement and initial model building were performed using the programs SOLVE and RESOLVE as implemented in PHENIX.[6-8] All eight expected selenium sites were identified resulting in a figure of merit of 0.39 for the SOLVE calculated phases. NCS averaging and solvent flattening in RESOLVE improved the figure of merit to 0.62. A 2.0 Å data set from native crystals was used for final refinement and model building. Model building was carried out with Coot[9] and the model was refined with PHENIX[8] and REFMAC5[10] using translation/libration/screw rigid body motion (TLS) refinement with 6 groups per monomer.[11, 12] Non-crystallographic symmetry restraints were employed in refinement. The final model contains residues 3-280 in both chains with the exception of residues 38-42 in chain A. The structures were validated using MolProbity.[13] SSM[14] was used to calculate structural similarity to the Pik (1MNA) and DEBS (1KEZ) TE domains with a core RMSD of 2.3 Å for both (240 and 245 residues aligned with 30% and 29% identity respecitively). Coordinates and structure factors have been deposited in the protein data bank (PDB id: 3LCR)

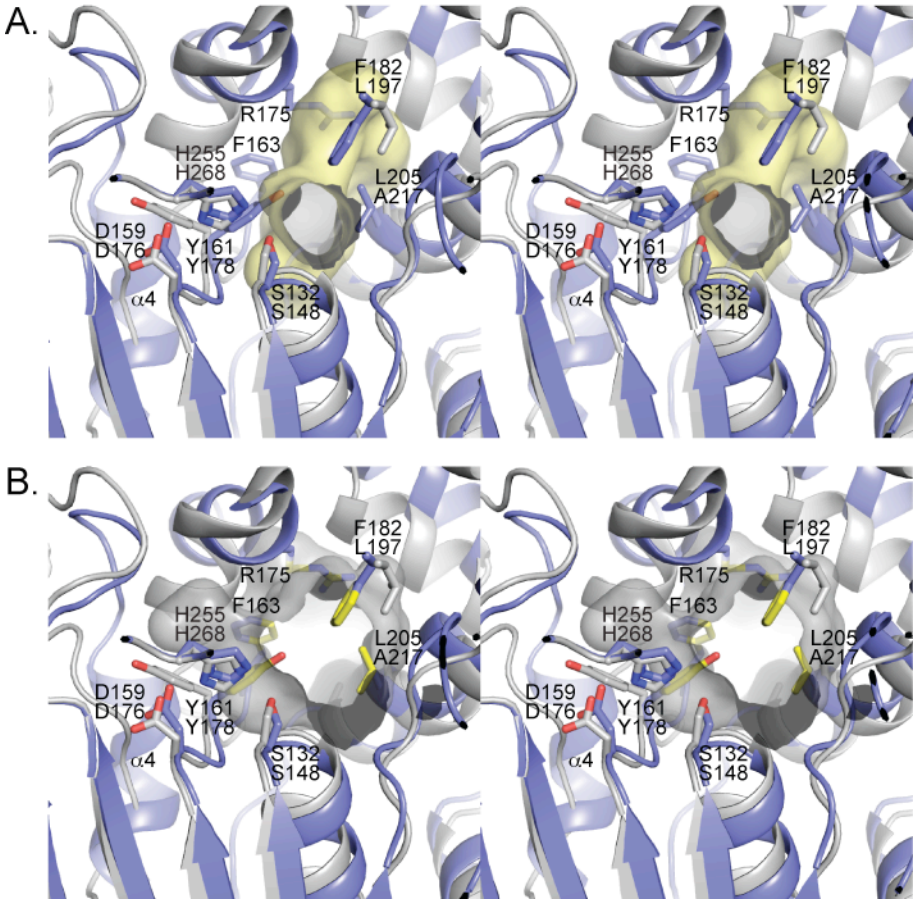

Figure S7. Stereodiagram of active site looking from the entrance (along the pointer in Figure **4A**). **A)** is identical to Figure **4C**. Pik TE (grey) is superposed on TMC TE (blue) with TMC TE substrate channel (yellow surface). The catalytic triad and residues which constrict the active site relative to Pik TE are labeled. Equivalent residues are labeled with the TMC TE designation above the Pik TE designation. Helix a4 is present only in the TMC TE structure. **B)** Is identical to **A)** except that the PikTE substrate tunnel is shown (grey) and C atoms from TMC TE which restrict the TMC TE tunnel with respect to the Pik TE tunnel are colored yellow.

Supplementary Table 3

Crystallographic Summary

	Native	SeMet		
Diffraction Data				
Space group	$P2_1$	$P2_1$		
a, b, c (Å)	54.10 80.18 64.71	53.73 79.61 64.62		
α, β, γ (°)	90 103.76 90	90 104.04 90		
		Peak	Inflection	Remote
Wavelength (Å)	0.97945	0.97937	0.97956	0.96112
Resolution[a] (Å)	2.00 (2.07-2.00)	2.40 (2.49-2.40)	2.30 (2.38-2.30)	2.30 (2.38-2.30)
$\langle I/\sigma_I \rangle$	16.4 (2.3)	20.2 (3.0)	21.0 (3.6)	24.1 (3.9)
R_{symm}	0.059 (0.511)	0.113 (0.617)[b]	0.095 (0.613)[b]	0.092 (0.528)[b]
Completeness	97.5 (85.3)	99.4 (95.1)	99.7 (98.4)	99.2 (95.1)
Avg. Redundancy	1.9 (1.4)	3.8 (3.1)	3.8 (3.2)	3.8 (3.4)
Unique Reflections	36395	20635	23256	23495
Refinement				
Data range (Å)	50.0—2.00			
No. Reflections	34164			
R_{work} / R_{free}[c]	0.180 / 0.235			
RMS Deviations				
Bonds (Å)	0.011			
Angles (°)	1.219			
No. atoms				
Protein	4184			
Water	275			
B-factors (Å^2)				
Protein	53.2			
Water	55.8			
Ramachandran				
Favored	97.6%			
Allowed	2.4%			
Outliers	0%			

[a] Highest resolution shell shown in parentheses.
[b] Anomalous data were included in calculation of R_{symm} values.
[c] 5% percent of data were withheld from refinement for R_{free} data set.

References

[1] S. S. Choi, Y. A. Hur, D. H. Sherman, E. S. Kim, *Microbiology* **2007**, *153*, 1095.
[2] L. Stols, M. Gu, L. Dieckman, R. Raffen, F. R. Collart, M. I. Donnelly, *Protein Expr Purif* **2002**, *25*, 8.
[3] S. A. Guerrero, H. J. Hecht, B. Hofmann, H. Biebl, M. Singh, *Appl Microbiol Biotechnol* **2001**, *56*, 718.
[4] P. Caffrey, *Chembiochem* **2003**, *4*, 654.
[5] W. Minor, M. Cymborowski, Z. Otwinowski, M. Chruszcz, *Acta Crystallogr D Biol Crystallogr* **2006**, *62*, 859.
[6] T. C. Terwilliger, *Methods Enzymol* **2003**, *374*, 22.
[7] T. C. Terwilliger, J. Berendzen, *Acta Crystallogr D Biol Crystallogr* **1999**, *55*, 849.
[8] P. D. Adams, R. W. Grosse-Kunstleve, L. W. Hung, T. R. Ioerger, A. J. McCoy, N. W. Moriarty, R. J. Read, J. C. Sacchettini, N. K. Sauter, T. C. Terwilliger, *Acta Crystallogr D Biol Crystallogr* **2002**, *58*, 1948.
[9] P. Emsley, K. Cowtan, *Acta Crystallogr D Biol Crystallogr* **2004**, *60*, 2126.
[10] *Acta Crystallogr D Biol Crystallogr* **1994**, *50*, 760.
[11] M. D. Winn, G. N. Murshudov, M. Z. Papiz, *Methods Enzymol* **2003**, *374*, 300.
[12] G. N. Murshudov, A. A. Vagin, E. J. Dodson, *Acta Crystallogr D Biol Crystallogr* **1997**, *53*, 240.
[13] I. W. Davis, A. Leaver-Fay, V. B. Chen, J. N. Block, G. J. Kapral, X. Wang, L. W. Murray, W. B. Arendall, 3rd, J. Snoeyink, J. S. Richardson, D. C. Richardson, *Nucleic Acids Res* **2007**, *35*, W375.
[14] E. Krissinel, K. Henrick, *Acta Crystallogr D Biol Crystallogr* **2004**, *60*, 2256.