Transition state analogs enhanced by fragment-based structural analysis: Bacterial methylthioadenosine nucleosidases

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Experimental Procedures.

MTAN structural analysis. Crystal structures of bacterial MTANs were from protein data bank files (PDB) by searching "methylthioadenosine nucleosidase" and "adenosylhomocysteine nucleosidase" and "aminodeoxyfutalosine nucleosidase". The structures were analyzed using PyMOL.

Table S1: Summary of MTAN structures for Protein Data Bank (PDB).

No.	PDB code	Mutations	Organisms	Ligand bound
1	5CCD	D198N	*Helicobacter pylori*	S-ADENOSYL-L-HOMOCYSTEINE
2	5CCE	WT	*Helicobacter pylori*	(2S)-2-AMINO-4-({[(2S,3S,4R,5S)-3,4,5-TRIHYDROXYTETRAHYDROFURAN-2-YL]METHYL}SULFANYL)BUTANOIC ACID
				ADENINE
3	5JPC	WT	*Helicobacter pylori*	(1S)-1-(7-AMINO-1H-PYRAZOLO[4,3-D]PYRIMIDIN-3-YL)-1,4-ANHYDRO-D-RIBITOL
4	5K1Z	WT	*Helicobacter pylori*	(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-{[(4-CHLOROPHENYL)SULFANYL]METHYL}PYRROLIDIN-3-OL
5	5KB3	WT	*Helicobacter pylori*	(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-{[(4-CHLOROPHENYL)SULFANYL]METHYL}PYRROLIDIN-3-OL
				MAGNESIUM ION
6	4WKN	WT	*Helicobacter pylori*	(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-[(METHYLSULFANYL)METHYL]PYRROLIDIN-3-OL

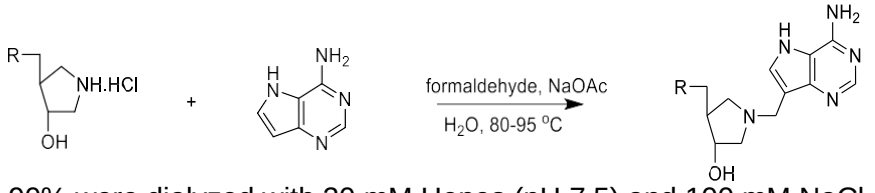
7	4WKO	WT	*Helicobacter pylori*	(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-{[(4-HYDROXYBUTYL)SULFANYL]METHYL}PYRROLIDIN-3-OL
8	4WKP	WT	*Helicobacter pylori*	(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-(2-{[2-(2-HYDROXYETHOXY)ETHYL]SULFANYL}ETHYL)PYRROLIDIN-3-OL
				SULFATE ION
9	4YNB	WT	*Helicobacter pylori*	GLYCEROL
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-[(PYRAZIN-2-YLSULFANYL)METHYL]PYRROLIDIN-3-OL
				DI(HYDROXYETHYL)ETHER
10	4YO8	WT	*Helicobacter pylori*	ZINC ION
				{[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL](HEXYL)AMINO}METHANOL
11	4OY3	D198N	*Helicobacter pylori*	CHLORIDE ION
				S-ADENOSYL-L-HOMOCYSTEINE
12	4P54	D198N	*Helicobacter pylori*	CHLORIDE ION; 5'-DEOXY-5'-METHYLTHIOADENOSINE
13	4OJT	WT	*Helicobacter pylori*	(2S)-2-AMINO-4-({[(2S,3S,4R,5S)-3,4,5-TRIHYDROXYTETRAHYDROFURAN-2-YL]METHYL}SULFANYL)BUTANOIC ACID
				ADENINE
14	4BMX	WT	*Helicobacter pylori*	2-AMINO-2-HYDROXYMETHYL-PROPANE-1,3-DIOL
				ADENINE
15	4BMY	D199A	*Helicobacter pylori*	SULFATE ION
16	4BMZ	D199N	*Helicobacter pylori*	5'-DEOXY-5'-METHYLTHIOADENOSINE
17	4BN0	E14Q	*Helicobacter pylori*	N/A
18	4FFS	WT	*Helicobacter pylori*	CHLORIDE ION
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-[(BUTYLSULFANYL)METHYL]PYRROLIDIN-3-OL
19	3NM4	WT	*Helicobacter pylori*	1,2-ETHANEDIOL
				2-AMINO-2-HYDROXYMETHYL-PROPANE-1,3-DIOL
20	3NM5	WT	*Helicobacter pylori*	(1S)-1-(7-AMINO-1H-PYRAZOLO[4,3-D]PYRIMIDIN-3-YL)-1,4-ANHYDRO-D-RIBITOL
21	3NM6	WT	*Helicobacter pylori*	1,2-ETHANEDIOL
				2-AMINO-2-HYDROXYMETHYL-PROPANE-1,3-DIOL
				ADENINE
22	4YML	WT	*Escherichia coli*	PHOSPHATE ION
				(3S,4R)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-[(METHYLSULFANYL)METHYL]PYRROLIDIN-3-OL
23	4WKC	WT	*Escherichia coli*	TETRAETHYLENE GLYCOL
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-[(BUTYLSULFANYL)METHYL]PYRROLIDIN-3-OL
24	3DF9	WT	*Escherichia coli*	(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-[(BENZYLSULFANYL)METHYL]PYRROLIDIN-3-OL
25	1Z5N	E12Q	*Escherichia coli*	5-S-METHYL-5-THIO-ALPHA-D-RIBOFURANOSE
				ADENINE

26	1Z5O	D197N	*Escherichia coli*	5'-DEOXY-5'-METHYLTHIOADENOSINE
27	1Z5P	WT	*Escherichia coli*	3,6,9,12,15,18,21,24-OCTAOXAHEXACOSAN-1-OL
				GLYCEROL
				ISOPROPYL ALCOHOL
28	1Y6Q	WT	*Escherichia coli*	CHLORIDE ION
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-[(METHYLSULFANYL)METHYL]PYRROLIDIN-3-OL
29	1Y6R	WT	*Escherichia coli*	(3S,4R)-2-(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)-5-[(METHYLSULFANYL)METHYL]PYRROLIDINE-3,4-DIOL
30	1NC1	WT	*Escherichia coli*	2-(4-AMINO-PYRROLO[2,3-D]PYRIMIDIN-7-YL)-5-METHYLSULFANYLMETHYL-TETRAHYDRO-FURAN-3,4-DIOL
31	1NC3	WT	*Escherichia coli*	(1S)-1-(7-AMINO-1H-PYRAZOLO[4,3-D]PYRIMIDIN-3-YL)-1,4-ANHYDRO-D-RIBITOL
32	1JYS	WT	*Escherichia coli*	ADENINE
33	6AYM	WT	*Campylobacter jejuni*	1,2-ETHANEDIOL
34	6AYO	WT	*Campylobacter jejuni*	(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-PROPYLPYRROLIDIN-3-OL
				1,2-ETHANEDIOL
				2-[BIS-(2-HYDROXY-ETHYL)-AMINO]-2-HYDROXYMETHYL-PROPANE-1,3-DIOL
35	6AYQ	WT	*Campylobacter jejuni*	GLYCEROL
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-[(METHYLSULFANYL)METHYL]PYRROLIDIN-3-OL
36	6AYR	WT	*Campylobacter jejuni*	1,2-ETHANEDIOL
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-[(BUTYLSULFANYL)METHYL]PYRROLIDIN-3-OL
37	6AYS	WT	*Campylobacter jejuni*	1,2-ETHANEDIOL
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-[(HEXYLSULFANYL)METHYL]PYRROLIDIN-3-OL
38	6AYT	WT	*Campylobacter jejuni*	1,2-ETHANEDIOL
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN- 7-YL)METHYL]-4-[(PYRAZIN-2-YLSULFANYL)METHYL]PYRROLIDIN-3-OL
39	6IF8	WT	*Aeromonas hydrophila*	ADENINE
40	5B7G	WT	*Aeromonas hydrophila*	GLYCEROL；
				ADENINE
41	5B7N	WT	*Aeromonas hydrophila*	GLYCEROL；
				S-ADENOSYL-L-HOMOCYSTEINE
42	5B7P	WT	*Aeromonas hydrophila*	5'-DEOXY-5'-METHYLTHIOADENOSINE
43	5B7Q	WT	*Aeromonas hydrophila*	5'-DEOXYADENOSINE
44	4F1W	WT	*Salmonella enterica*	1,2-ETHANEDIOL；
				TETRAETHYLENE GLYCOL；
				TRIETHYLENE GLYCOL；
				DI(HYDROXYETHYL)ETHER；
				ADENINE

45	4F2P	WT	*Salmonella enterica*	ACETATE ION;
				GLYCEROL;
				1,2-ETHANEDIOL;
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-({[2-(2-HYDROXYETHOXY)ETHYL]SULFANYL}METHYL)PYRROLIDIN-3-OL
46	4F2W	WT	*Salmonella enterica*	CHLORIDE ION;
				1,2-ETHANEDIOL;
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-[(METHYLSULFANYL)METHYL]PYRROLIDIN-3-OL
47	4F3C	WT	*Salmonella enterica*	1,2-ETHANEDIOL;
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-[(BUTYLSULFANYL)METHYL]PYRROLIDIN-3-OL
48	4F3K	WT	*Salmonella enterica*	{(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-HYDROXYPYRROLIDIN-3-YL}-L-
				METHIONINE;
				1,2-ETHANEDIOL
49	3LGS	WT	*Arabidopsis thaliana*	1,2-ETHANEDIOL;
				S-ADENOSYL-L-HOMOCYSTEINE; ADENINE
50	2QSU	WT	*Arabidopsis thaliana*	N/A
51	2QTG	WT	*Arabidopsis thaliana*	2-(4-AMINO-PYRROLO[2,3-D]PYRIMIDIN-7-YL)-5-METHYLSULFANYLMETHYL-TETRAHYDRO-FURAN-3,4-DIOL;
				1,2-ETHANEDIOL
52	2QTT	WT	*Arabidopsis thaliana*	(1S)-1-(7-AMINO-1H-PYRAZOLO[4,3-D]PYRIMIDIN-3-YL)-1,4-ANHYDRO-D-RIBITOL;
				1,2-ETHANEDIOL;
				ADENINE
53	2H8G	WT	*Arabidopsis thaliana*	ADENINE
54	4WKB	WT	*Vibrio cholerae*	(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-[(METHYLSULFANYL)METHYL]PYRROLIDIN-3-OL
55	4X24	A113P/V153I/R158G	*Vibrio cholerae*	(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-[(METHYLSULFANYL)METHYL]PYRROLIDIN-3-OL;
				TRIETHYLENE GLYCOL
56	3DP9	WT	*Vibrio cholerae*	IODIDE ION;
				(3R,4S)-1-[(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)METHYL]-4-[(BUTYLSULFANYL)METHYL]PYRROLIDIN-3-OL
57	3MMS	A23T/V39A/V64D/A184T	*Streptococcus pneumoniae*	GLYCEROL;
				9H-PURINE-6,8-DIAMINE
58	1ZOS	WT	*Streptococcus pneumoniae*	(3S,4R)-2-(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)-5-[(METHYLSULFANYL)METHYL]PYRROLIDINE-3,4-DIOL
59	4GMH	WT	*Staphylococcus aureus*	ACETATE ION
60	4KN5	WT	*Weissella paramesenteroides*	GLYCEROL
				1,2-ETHANEDIOL
61	4G41	WT	*Streptococcus*	5'-DEOXY-5'-METHYLTHIOADENOSINE

			pyogenes	
62	5DK6	WT	*Colwellia psychrerythraea*	GLYCINE
				ADENINE
63	3EEI	WT	*Neisseria meningitidis*	(3S,4R)-2-(4-AMINO-5H-PYRROLO[3,2-D]PYRIMIDIN-7-YL)-5-[(METHYLSULFANYL)METHYL]PYRROLIDINE-3,4-DIOL
64	4QEZ	WT	*Bacillus anthracis*	2-AMINO-2-HYDROXYMETHYL-PROPANE-1,3-DIOL
				ADENINE
65	4PR3	WT	*Brucella melitensis*	PHOSPHATE ION
				GLYCEROL
				ADENINE
66	4JWT	WT	*Sulfurimonas denitrificans*	1,2-ETHANEDIOL
				ADENINE
67	4L0M	WT	*Borreliella burgdorferi*	ADENINE
68	3BL6	WT	*Staphylococcus aureus*	(1S)-1-(7-AMINO-1H-PYRAZOLO[4,3-D]PYRIMIDIN-3-YL)-1,4-ANHYDRO-D-RIBITOL
69	5UE1	WT	*Aliivibrio fischeri*	CHLORIDE ION
				CALCIUM ION
				1,2-ETHANEDIOL
				9-DEAZAADENINE
				2-AMINO-2-HYDROXYMETHYL-PROPANE-1,3-DIOL
70	4G89	WT	*Klebsiella pneumoniae*	SULFATE ION;
				S-ADENOSYL-L-HOMOCYSTEINE;
				ADENINE
71	4QAQ	WT	*Chlamydia trachomatis*	SULFATE ION
72	4QFB	WT	*Chlamydia trachomatis*	N/A
73	4QAR	WT	*Chlamydia trachomatis*	SULFATE ION
				ADENINE
74	4QAT	D161N	*Chlamydia trachomatis*	5'-DEOXY-5'-METHYLTHIOADENOSINE
75	4JOS	WT	*Francisella philomiragia*	SODIUM ION
				1,2-ETHANEDIOL
				ADENINE

Protein purification. *Hp*MTAN and *Sa*MTAN were purified using the same protocol as previously described.[1,2] Briefly, *Hp*MTAN in pET28, bearing an N-terminal 6 x His tag was chemically transformed into BL21 (DE3) cells that were grown overnight on a kanamycin-containing (50 mg/mL) nutrient agar plate. Subsequently, single colonies were picked to grow in LB broth media at 37 ^{0}C until the O.D. at 600 nm reached 0.6. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to reach a final concentration of 0.5 mM, and cells were incubated for an additional 4 hours before harvesting by centrifugation. The cell pellet was suspended in cold buffer containing 20 mM Hepes (pH 7.5), 100 mM NaCl, 0.1 mM PMSF, 50 µg/mL of lysozyme and a few crystals of DNAse I and RNAse A. Resuspended cells were stirred for ~ 30 min on ice, then treated with sonication (6 intervals, 1 min/interval) followed by centrifugation at 14,000 rpm, at 4 ^{o}C, for 1hr. Additional PMSF was added twice with intervals of ~ 30 min. The supernatant of centrifugation was loaded onto a Co-NTA column preequilibrated with 20 mM Hepes (pH 7.5) and 100 mM NaCl. Using gradually increased concentrations of imidazole (buffered to pH 7.5), *Hp*MTAN was eluted from the column and fractions with purity



over 90% were dialyzed with 20 mM Hepes (pH 7.5) and 100 mM NaCl.

Inhibitor synthesis and analysis. Synthesis of inhibitors has been described previously.[3] Briefly, the condensation reactions of an imidazo-[1,2-a]-pyridine derivative with a pyrrolidine nucleoside were performed using the Mannich reaction, shown below, where R are fragment derivatives. The structure and purity of the synthesized inhibitors were originally evaluated using NMR, HPLC, and MS, and the samples here were reanalyzed by the same methods to assure purity.[3,4] All inhibitors were found to be >98% as originally described, by the methods below.

High-Performance Liquid Chromatography Analysis (HPLC) analysis. HPLC of all inhibitors was performed prior use to assay to assure purity. An Agilent (Santa Clara, Ca) Eclipse XDB-C18 column with an Agilent 1100A HPLC system was used. The column was maintained at 37°C for the duration of HPLC. Elution was performed with a flow rate of 1mL/min using a solvent gradient of 5-95% ethanol with 0.1% v/v formic acid (solvent B), in a water background with 0.1% v/v formic acid (solvent A). Each inhibitor was solubilized in HPLC grade MeOH (Sigma-Aldrich, St Louis, MO) to be approximately 1mM in concentration. With a 15 µL injection volume, the elution took place over 15 minutes from 5% solvent B to 95% solvent B monitoring at 274nm, with an additional 5 minutes wash at 95% solvent B. Compound 7 had an additional 5 min wash with 95% solvent B due to hydrophobicity. Peaks were analyzed and collected. This peak sample was then run on mass spectrometry for confirmation of the identity of the peak.

Mass Spectrometry (MS) analysis. MS was performed on the collected eluted compound from HPLC testing mentioned above. MS was performed using a Thermo Finnigan LCQ Deca (Waltham, MA). The MS was performed using 20 µL of the inhibitors previously prepared for HPLC. The injection speed was set to 8 µL/s. The MS monitored positive ions with a 10 Hz channel sample rate, under atmospheric pressure and was ionized using a corona discharge needle in the nebulizer of the instrument. The detected peaks were analyzed using Xcalibur software (Thermo-Fisher, Waltham, MA). MTDIA and compounds 2-6 were analyzed using positive mode and compound 7 was analyzed using negative mode.

NMR analysis. 0.5 - 1 mg of each inhibitor was dissolved in 750µL of CD3OD (VWR, Radnor, Pa.). ^{1}H-NMR (400 MHz) was performed using a JEOL ECS NMR Spectrometer (Tokyo, Japan) and data was analyzed using Delta 5.2.1 Software (JEOL). For each experiment, 20,000 scans were used with an X_offset of 7.

Enzymatic assay. At 25 ^{o}C or 37 ^{o}C, assay solutions contained 50 mM Hepes (pH 7.5), 100 mM NaCl, 0.87 mM MTA, catalase (5 units/mL) and xanthine oxidase (0.5 unit/mL). Reactions were started by adding *Hp*MTAN or *Sa*MTAN to reach a final concentration of 5 nM and monitored by following the increase of absorption at 295 nM. Reaction kinetics were measured at six different inhibitor concentrations (ranging from 0 to 50,000 nM) for the transition state analogue inhibitors. A reaction without enzyme added served as the control to be subtracted during data analysis. The assays were monitored for 2 hours to analyze for slow-onset binding. Inhibitors with slow-onset binding have a two-step inhibition chronologically. Initial inhibition (K_i) occurs

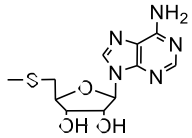
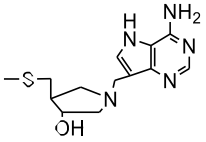
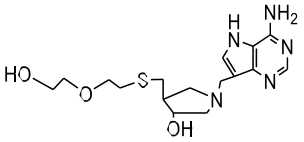
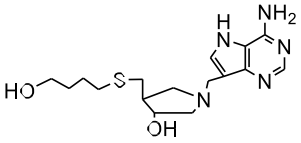
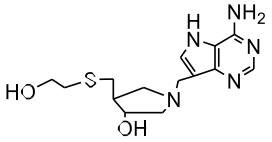
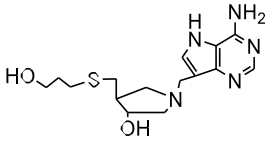
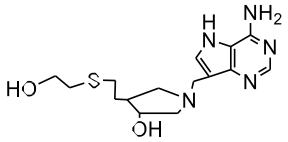
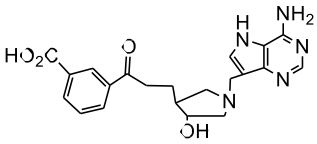
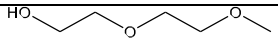
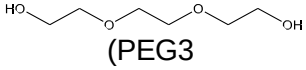
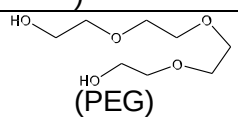
by rapid binding followed by the slow onset of more potent inhibition, resulting in a decreased but constant rate (K_i*) due to slow-onset binding. For PEG2.5, PEG3 and PEG4, a direct assay was performed by monitoring the absorption decrease at 275 nm for conversion of MTA to adenine. Assay solutions contained 50 mM Hepes (pH 7.5), 100 mM NaCl, 0.09 mM MTA, and PEG2.5 or PEG3 or PEG4 (0 – 300 mM).

Kinetics were analyzed using KaleidaGraph (Synergy Software) by fitting data to the following equation,

$v/v_0 = (K_m + [S])/ (K_m + [S] + (K_m [I])/ K_i)$

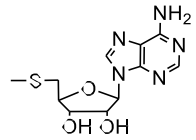
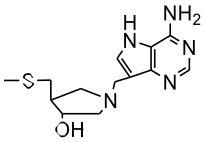
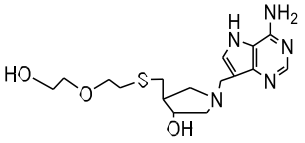
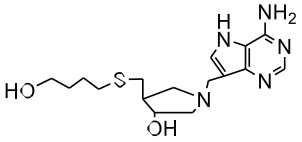
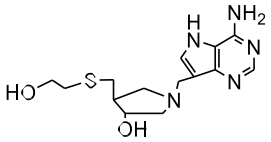
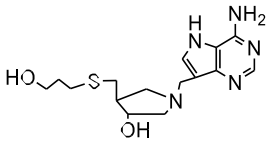
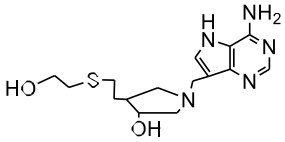
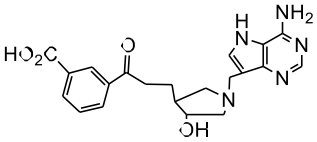
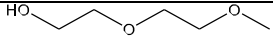
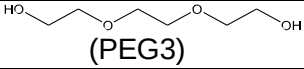
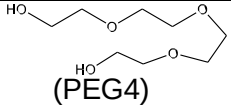
Here, v_0 and v are the measured steady-state kinetics in the absence and presence of an inhibitor, respectively; [S] and [I] are the concentrations of MTA and a given inhibitor, respectively; K_m is the Michaelis-Menten constant for MTA.

Table S2: K_m, K_i an K_i^* for *Hp*MTAN at 25 °C and 37 °C.

Inhibitor (*Hp*MTAN)	***K_i* @ 25°C (nM)**	***K_i^** @ *25°C (nM)***	***K_i* @ 37°C (nM)**
 (substrate)	0.6 x 10^3 ± 0.3 x 10^3 [a] (K_m)		4.1 x10^3 ± 0.5 x10^3 (K_m)
	0.19 ± 0.03[a]	0.089 ± 0.019[a]	1.25 ± 0.16
	0.96 ± 0.16[a]	0.015 ± 0.004[a]	0.09 ± 0.01
	0.34 ± 0.07[a]	0.11 ± 0.04[a]	0.79 ± 0.08
	0.43 ± 0.12[a]	0.04 ± 0.01[a]	0.74 ± 0.21
	0.89 ± 0.13[a]	0.10 ± 0.01[a]	0.31 ± 0.07
	0.26 ± 0.03[a]	0.05 ± 0.01[a]	0.28 ± 0.03
	4.2 ± 1.2[a]	N/A[a]	1.08 ± 0.15
Fragment	***K_i* @ 25°C (mM)**	***K_i^** @ *25°C (nM)***	***K_i* @ 37°C (mM)**
 (PEG2.5)	16±3	N/A	> 100
 (PEG3)	2.3 ± 0.2	N/A	13 ± 1
 (PEG)	7.1 ± 3.5	N/A	54 ±19

[a]These are reported values of K_m, K_i or K_i^* from references.[1,3,4]

Table S3: K_m, K_i and K_i^* for *Sa*MTAN at 25 °C and 37 °C.

Inhibitor (*Sa*MTAN)	**K_i @ 25°C (nM)**	**K_i^* @ 25°C (nM)**	**K_i @ 37°C (nM)**
 (substrate)	0.9 x 10^3 ± 0.3 x10^3 [a] (K_m)		5.1 x 10^3 ± 1.3 x10^3 (K_m)
	0.16 ± 0.03	0.054 ± 0.001	7.8 ± 0.1
	0.078 ± 0.015	0.0058 ± 0.0009	0.096 ± 0.016
	0.17 ± 0.02	0.013 ± 0.002	0.51 ± 0.11
	0.077 ± 0.017	0.014 ± 0.002	0.58 ± 0.11
	0.097 ± 0.017	0.022 ± 0.003	0.11 ± 0.034
	0.19 ± 0.06	0.055 ± 0.001	18 ± 5.3
	2.3 ± 0.3	0.47 ± 0.14	> 20
Fragment	**K_i @ 25°C (mM)**	**K_i^* @ 25°C (nM)**	**K_i @ 37°C (mM)**
 (PEG2.5)	6.2 ± 0.8	N/A	> 100
 (PEG3)	2.6 ± 0.5	N/A	27 ± 8
 (PEG4)	2.8 ± 0.3	N/A	41 ± 9

[a] These are reported values of K_m from references.[5]

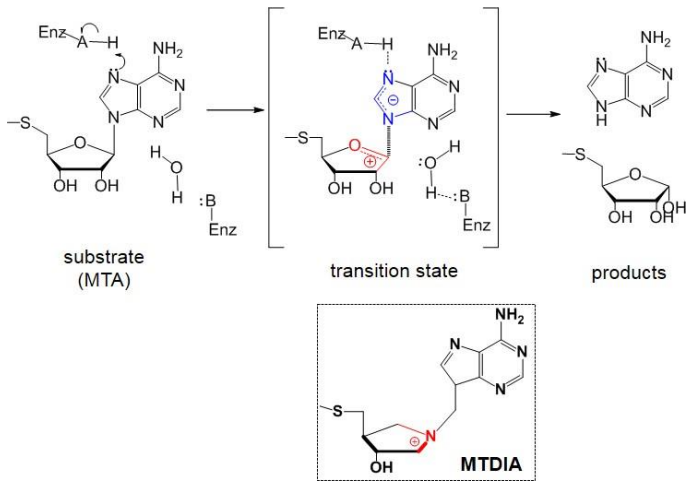


Figure S1: Transition state of MTAN reaction and lead inhibitor MTDIA.

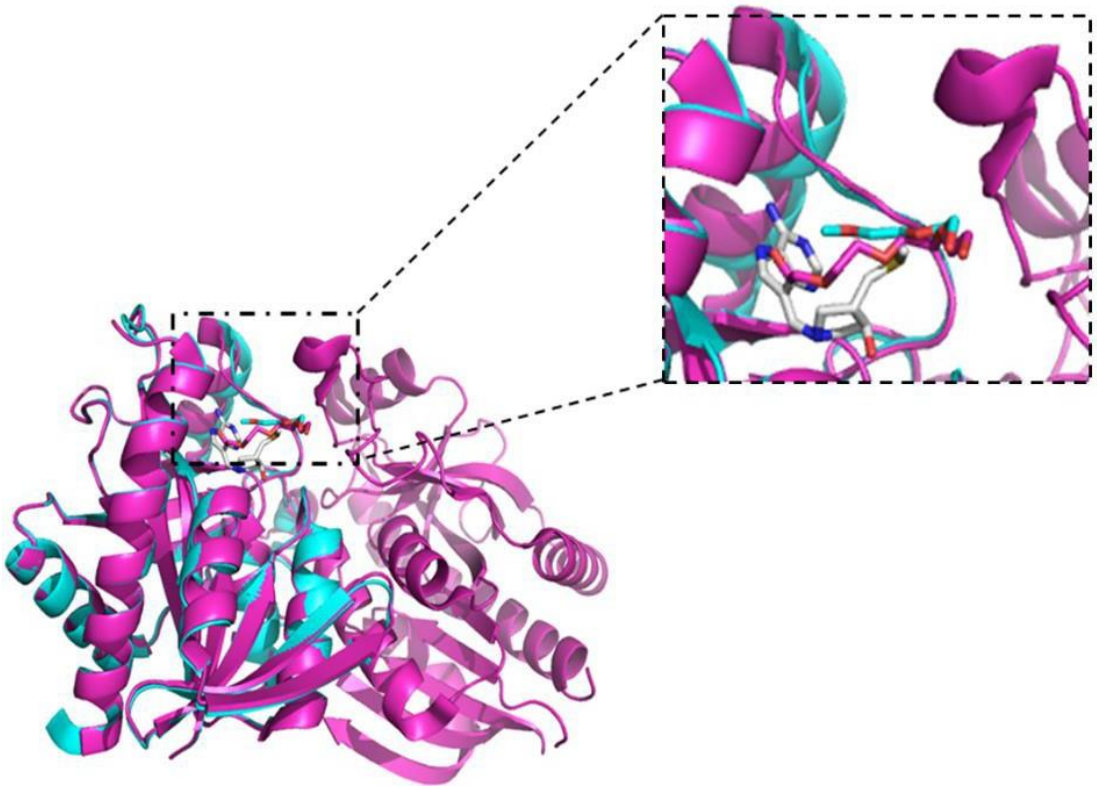


Figure S2: Structural alignment of PEG2.5 bound *Ec*MTAN (PDB file 1Z5P) and PEG3 bound *Se*MTAN (PDB file 4F1W). *Ec*MTAN bound with PEG2.5 is in cyan; *Se*MTAN bound with PEG3 is in magenta. MTDIA was modeled into the active site of *Se*MTAN in grey.

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3. Wang, S., Cameron, S. A., Clinch, K., Evans, G. B., Wu, Z., Schramm, V. L., and Tyler, P. C. (2015) New Antibiotic Candidates against *Helicobacter pylori*, *J Am Chem Soc* 137, 14275-14280.
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