<u>**SUPPLEMENTAL DATA**</u>

POTENT INHIBITORS OF A SHIKIMATE PATHWAY ENZYME FROM *MYCOBACTERIUM TUBERCULOSIS*: COMBINING MECHANISM- AND MODELING-BASED DESIGN

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Running title: Potent active site inhibitors for *M. tuberculosis* DAH7PS

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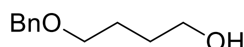
SYNTHESIS AND CHARACTERIZATION OF COMPOUNDS

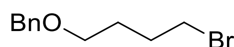
Compound **6** (1) and compounds **12-14** (2) were synthesized according to literature procedures and had analytical data consistent with those reported.

Preparation of 4-benzyloxy-1-butanol



Silver(I) oxide was prepared freshly as follows: NaOH (7.99 g, 0.20 mol) was dissolved in water (200 mL) and heated to 80-90°C. This solution was added to a solution of $AgNO_3$ (34 g, 0.20 mol) in water (200 mL) at 80-90°C. The hot suspension was filtered to collect the dark brown precipitate that formed. The precipitate was washed with hot water (200 mL) and ethanol (200 mL) before it was dried for 4 h under high vacuum while heated with an oil bath at 100°C. The silver oxide obtained from this procedure (18.74 g, 0.08 mol) was added to a solution of 1,4-butanediol (4.47 g, 0.05 mol) and benzyl bromide (10.2 g, 7.1 mL, 0.06 mol) in dichloromethane. The mixture was stirred at room temperature for 10 h, then filtered through a plug of celite. The celite was washed with dichloromethane and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluted with a gradient of ethylacetate/PET ether (20 % v/v to 50 % v/v) solvent to yield 5.35 g (59 %) of the desired product. ^{1}H NMR (500 MHz, CHLOROFORM-d) δ 7.28 - 7.39 (m, 5H), 4.52 (s, 2H), 3.60 (t, J = 6.2 Hz, 2H), 3.51 (t, J = 6.1 Hz, 2H), 3.13 (br. s., 1H), 1.61 - 1.74 (m, 4H). ^{13}C NMR (126 MHz, CHLOROFORM-d) δ 138.0, 128.2, 127.5, 127.4, 72.8, 70.1, 62.2, 29.6, 26.3. HRMS[ESI,pos] Calcd for $C_{11}H_{16}O_2Na$ 203.1043 found 203.1047 $[M+Na]^+$.

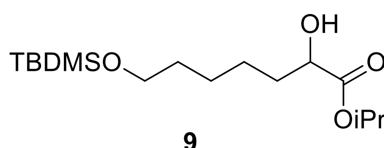
Preparation of 4-benzyloxy-1-bromobutane



Triphenylphosphine (5.58 g, 0.02 mol) and 4-benzyloxy1-butanol (2.513 g, 0.014 mol) were dissolved in dichloromethane (25 mL) and cooled to 0°C. *N*-bromosuccinimide (3.730 g, 0.021 mol) was added in batches with stirring. The reaction mixture was stirred at 0°C for 1.5 h, was the allowed to room temperature and stirred for an additional 3 h. The solvent was evaporated under reduced pressure and the resulting brown viscous oil was triturated with PET ether (3x50 mL). The combined PET ether extracts were evaporated under reduced pressure to yield a colorless oil. The crude product was purified by flash column chromatography on silica gel (10% ethyl acetate/PET ether) to yield 2.6 g (78%) of the desired bromide. ^{1}H NMR (500 MHz, CHLOROFORM-d) δ 7.28 - 7.40 (m, 5H), 4.53

(s, 2H), 3.53 (t, *J* = 6.2 Hz, 2H), 3.46 (t, *J* = 6.8 Hz, 2H), 1.97 - 2.04 (m, 2H), 1.75 - 1.82 (m, 2H). ^{13}C NMR (126 MHz, CHLOROFORM-d) δ 138.3, 128.3, 127.5, 127.5, 72.8, 69.2, 33.7, 29.6, 28.3. HRMS[ESI,pos] Calcd for $C_{11}H_{15}OBrNa$ 265.0198 found 265.0204 $[M+Na]^+$.

Preparation of propan-2-yl 7-[(tert-butyldimethylsilyl)oxy]-2-hydroxyheptanoate (9)



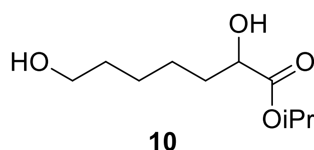
Alcohol **6** (2.65 g, 12.1 mmol) in dichloromethane solution (5 mL) was added to a suspension of Dess-Martin Periodinane (5.65 g, 13.3 mmol) in dichloromethane (20 mL). The solution was stirred for 1 h, after which complete consumption of starting material 6 was indicated by TLC analysis. The reaction mixture was poured into ether (100 mL) and aqueous 1 M sodium hydroxide solution was added (200 mL) until all the white precipitate had dissolved. The organic phase was separated, the aqueous phase was extracted with ether (2x120 mL) and the combined organic extracts washed with 1 M aqueous NaOH (50 mL) and brine (100 mL). The organic phase was dried over sodium sulphate, filtered and the solvent evaporated under reduced pressure to give 1.945 g of a pale yellow oil. This crude aldehyde **7** was used in the next step without further purification.

18 mL of a freshly prepared potassium isopropoxide solution (potassium (810 mg, 20.7 mmol) dissolved in 20 mL isopropyl alcohol) was added to a solution of isopropyl-2,2-dichloroacetate (3.1 g, 18 mmol) in a mixture of isopropanol (5 mL) and ether (10 mL) at 0°C. The crude aldehyde **7** (1.95 g, 6.30 mmol) was added and the mixture stirred at 0°C for 3 h, during which the solution turned dark orange. Ether (50 mL) and water (25 mL) were added and the solution was stirred until the precipitate dissolved. The organic phase was separated, the aqueous phase was extracted with ether (100 mL) and the combined organic extracts dried over sodium sulphate. After filtration, the solvent was evaporated under reduced pressure to yield 3.4 g of crude chloroepoxide 8 which was used immediately in the next step without further purification.

Chloroepoxide **8** was dissolved in isopropanol (20 mL) and sodium cyanoborohydride (587 mg, 9.3 mmol) was added as a solid. The reaction mixture was heated to reflux for 19 h and then quenched by the addition of saturated aqueous ammonium chloride solution (10 mL). Volatile components were removed by evaporation under reduced pressure, the residue was taken up in dichloromethane and water added until all the precipitate dissolved. The organic phase was separated and the aqueous phase was extracted with dichloromethane (200 and 150 mL). The combined organic extracts were dried with brine and sodium sulphate and the solvent evaporated under reduced pressure. Flash

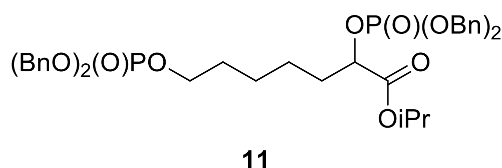
column chromatography on silica gel (eluent 20% ethyl acetate/PET ether) afforded 1.6 g (42% yield from alcohol **6**) of the desired α-hydroxyester **9** as a colorless oil. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 5.10 (spt, *J* = 6.3 Hz, 1H), 4.13 (ddd, *J* = 4.4, 5.8, 7.1 Hz, 1H), 3.60 (t, *J* = 6.5 Hz, 2H), 2.78 (d, *J* = 5.9 Hz, 1H), 1.70 - 1.85 (m, 1H), 1.33 - 1.62 (m, 7H), 1.29 (d, *J* = 2.6 Hz, 3H), 1.27 (d, *J* = 2.9 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 174.9, 70.4, 69.4, 63.1, 34.4, 32.7, 26.0, 25.6, 24.5, 21.8, 18.3, -5.3. HRMS[ESI,pos] Calcd for $C_{16}H_{34}NaO_4Si$ 341.2119, found 341.2122 $[M+Na]^+$.

Synthesis of propan-2-yl 2,7-dihydroxyheptanoate (10)



α-Hydroxyester **9** (342 mg, 1.07 mmol) was dissolved in THF (10 mL) and a 1 M solution of tetrabutylammoniumfluoride in THF (3.6 mL, 3.6 mmol) added. The reaction mixture was stirred at room temperature for 3 h after which volatile compounds were removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel (eluent 50% ethyl acetate/PET ether) to afford 123 mg (56%) of the desired dihydroxyester **10** as a colorless oil. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 5.03 (spt, *J* = 6.3 Hz, 1H), 4.07 (dd, *J* = 4.1, 7.3 Hz, 1H), 3.56 (t, *J* = 6.5 Hz, 2H), 2.96 (br. s., 2H), 1.31 - 1.78 (m, 8H), 1.23 (d, *J* = 2.6 Hz, 3H), 1.20 (d, *J* = 2.6 Hz, 3H). ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 174.7, 70.2, 69.1, 62.3, 34.1, 32.3, 25.3, 24.3, 21.6, 21.6. HRMS [ESI, pos] Calcd for $C_{10}H_{21}O_4$ 205.1440, found 205.1433 $[M+H]^+$.

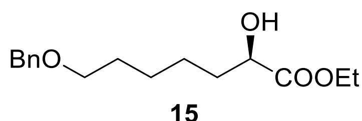
Preparation of propan-2-yl 2,7-di{[bis(benzyloxy)phosphoryl]oxy}heptanoate (11)



1H-Tetrazole (300 mg, 4.28 mmol) was added to a solution of dihydroxyester **10** (123 mg, 0.60 mmol) and dibenzyl *N*,*N*-diisopropylphosphoramidite (810 µL, 787 mg, 2.30 mmol) in dichloromethane (7 mL). After stirring the reaction mixture for 3 h at room temperature, it was cooled to 0°C and *m*-chloroperbenzoic acid (633 mg, 3.70 mmol) was added. After 2 h, the reaction mixture was allowed to warm to room temperature and after an additional hour the reaction was quenched by

addition of 10% w/v aqueous sodium thiosulfate solution (15 mL). The organic phase was separated, washed with 10% w/v aqueous sodium thiosulfate solution (2x15 mL), saturated aqueous sodium bicarbonate solution (2x15 mL) and dried with brine (15 mL) and sodium sulfate. Filtration and removal of the solvent under reduced pressure afforded the crude product as an oil. Flash column chromatography on silica gel (eluent 1% methanol/dichloromethane) yielded 218 mg (50%) of the desired product **11** as a pale yellow oil. ^{1}H NMR (500 MHz, CHLOROFORM-d) δ 7.28 - 7.40 (m, 20H), 4.98 - 5.19 (m, 8H), 4.74 (td, *J* = 6.1, 7.9 Hz, 1H), 3.94 (apparent q, *J* = 6.6 Hz, 2H), 1.73 - 1.80 (m, 2H), 1.50 - 1.58 (m, 2H), 1.24 - 1.39 (m, 4H), 1.23 (d, *J* = 6.2 Hz, 5H). ^{13}C NMR (126 MHz, CHLOROFORM-d) δ 169.4 (d, J^{PC}=3.3 Hz), 135.9, 135.8, 135.7, 135.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 127.9, 127.9, 127.8, 75.5 (d, J^{PC}=6.1 Hz), 69.4, 69.3, 69.3, 69.2, 69.1, 67.6 (d, J^{PC}= 6.1 Hz), 32.7 (d, J^{PC}=6.2 Hz), 29.9 (d, J^{PC}=7.2 Hz), 24.9, 24.0, 21.6. ^{31}P NMR (121 MHz, CHLOROFORM-*d*) δ ppm -2.8 (app spt, *J*=7.9 Hz, 1 P), -3.6 (app sxt, *J*=7.8 Hz, 1 P). HRMS[ESI,pos] Calcd for $C_{38}H_{46}O_{10}P_2Na$ 747.2464, found 747.2448 $[M+Na]^+$.

Synthesis of ethyl (2*R*)-7-(benzyloxy)-2-hydroxyheptanoate (*R*)-15

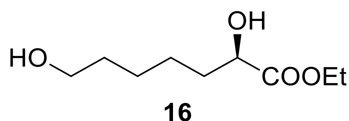


Cuprous bromide dimethylsulfide complex was prepared following the procedure the described by Nakamura *et al.*(3) Cuprous bromide dimethylsulfide complex was recrystallized fresh before each use from a solution in dimethylsulfide by addition of PET ether. The resulting white solid was dried in a stream of nitrogen for 3-5 h.

Magnesium turnings (185 mg, 7.60 mmol) were ground under a stream of nitrogen and then suspended in THF (4 mL). After addition of 1,2-dibromoethane (15.0 µL, 32.7 mg, 0.17 mmol) the solution was heated to reflux for 10 minutes. The solvent was discarded, the activated magnesium turnings were washed with fresh THF (4 mL) which was also discarded. The turnings were suspended in fresh THF (4 mL) and 4-benzyloxy-1-bromobutane (738 mg, 3.00 mmol) in THF solution (2 mL) was added. The reaction mixture was heated to reflux for 1 h, during which a green solution formed. The excess magnesium was left to settle and the Grignard solution transferred dropwise *via cannula* to a flask containing a suspension of cuprous bromide dimethyl sulfide complex (626 mg, 3.00 mmol) in THF cooled to -78°C. The solution was stirred at -78°C for 20 minutes before (*R*)-ethylglycidate ((*R*)-**14**, 174 mg, 1.50 mmol) was added in THF solution (2 mL). The mixture was stirred at -78°C for 2 h, quenched at that temperature by the addition of saturated aqueous ammonium chloride solution (15 mL) and allowed to warm to room temperature. The mixture was transferred to a separating

funnel and water was added until all precipitate had dissolved. The organic phase was separated and the aqueous phase extracted three times with ether (2x50 mL and 100 mL). The combined organic phases were washed with brine (2x40 mL), dried over sodium sulphate, filtered and evaporated. The crude product was purified by flash column chromatography on silica gel (20% ethyl acetate/PET ether) to yield 133 mg (32%) of α-hydroxyester (*R*)-**15** as a colorless oil. The preparation of (*S*)-**15** on a 6.6 mmol scale (referring to *(S)*-ethylglycidate) following a similar procedure yielded 483 mg (26%) of the desired product. Spectral data are identical to those reported for (*R*)-**15** with the exception of optical rotation (see below). ^{1}H NMR (500 MHz, CHLOROFORM-d) δ 7.28 - 7.39 (m, 5H), 4.48 - 4.53 (m, 2H), 4.24 (q, *J* = 7.3 Hz, 2H), 4.17 (dd, *J* = 4.2, 7.5 Hz, 1H), 3.48 (t, *J* = 6.4 Hz, 2H), 1.76 - 1.84 (m, 1H), 1.59 - 1.70 (m, 3H), 1.37 - 1.52 (m, 4H), 1.30 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, CHLOROFORM-d) δ 175.3, 138.6, 128.3, 127.6, 127.5, 72.9, 70.3, 70.2, 61.6, 34.3, 29.6, 25.9, 24.6, 14.2. HRMS[ESI,pos] Calcd for $C_{16}H_{24}O_4Na$ 303.1566, found 303.1567 $[M+Na]^+$. $[\alpha]_D^{20}$ (*R*)-**15** -1.4 (c 2.3, $CHCl_3$), (*S*)-**15** +0.9 (c 2.1, $CHCl_3$).

Preparation of ethyl-*(2R)*-2,7-dihydroxyheptanoate (*R*)-16



α-hydroxyester (*R*)-**15** (600 mg, 2.10 mmol) was dissolved in ethyl acetate and 10% Palladium on charcoal (257 mg, 0.24 mmol Pd) was added. The reaction flask was purged with hydrogen gas with three freeze-pump-thaw cycles, then stirred at room temperature for 3 days.[1] The reaction mixture was filtered through a celite pad and the celite washed with ethyl acetate (50 mL). The solvent was evaporated under reduced pressure to give a yellow oil. The crude product was purified by flash column chromatography on silica gel (60% ethyl acetate/PET ether) to yield the desired dihydroxyester (*R*)-**16** as a pale yellow oil (265 mg, 66%).^{1}H NMR (300 MHz, CHLOROFORM-d) δ 4.17 (q, *J* = 7.0 Hz, 2H), 4.11 (dd, *J* = 4.3, 7.5 Hz, 1H), 3.55 (t, *J* = 6.5 Hz, 2H,), 3.31 (br. s, 1H), 2.64 (br. s, 1H), , 1.29 - 1.79 (m, 8H), 1.23 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 175.1, 70.2, 62.3, 61.4, 34.1, 32.2, 25.2, 24.4, 14.0. HRMS[ESI,pos] Calcd for $C_9H_{18}O_4Na$ 213.1097, found 231.1096 $[M+Na]^+$. $[\alpha]_D^{20}$ (*R*)-**16** +0.8 (c 1.8, $CHCl_3$), (*S*)-**16** -0.8 (c 2.3, $CHCl_3$).

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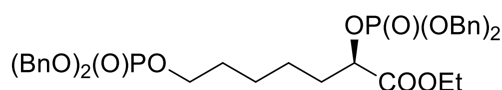
[1] This long reaction time is exceptional, typical reaction times are 12-15 h. Reactions were monitored by TLC, in this particular example full conversion had not occurred overnight so that hydrogenolysis was continued.

Preparation of ethyl-(2*R*)-2,7-di{[bis(benzyloxy)phosphoryl]oxy}heptanoate (*R*)/(*S*)-17



17

(*R*)/(*S*)-**17** were synthesized from (*R*)/(*S*)-**17** in an analogous procedure to the synthesis of **11**. (*R*)- and (*S*)-**17** could be obtained in 64% and 74% yield respectively. Spectral data for the two enantiomers were identical except for optical rotation, the data for (*R*)-**17** is listed below. ^{1}H NMR (500 MHz, CHLOROFORM-d) δ 7.29 - 7.39 (m, 20H), 4.99 - 5.18 (m, 8H), 4.77 (td, *J*= 5.9, 8.0 Hz, 1H), 4.18 (m, 2H), 3.94 (q, *J*= 6.6 Hz, 2H), 1.77 (q, *J* = 6.7 Hz, 2H), 1.54 (m, 2H), 1.27 - 1.37 (m, 4H), 1.25 (t, *J*= 7.2 Hz, 3H). ^{13}C NMR (126 MHz, CHLOROFORM-d) δ 169.9 (d, J^{PC}=3.3 Hz), 135.9, 135.82, 135.80, 135.73, 135.70, 135.6, 128.51, 128.49, 128.47, 128.46, 128.42, 127.90, 127.86, 127.8, 75.4 (d, J^{PC}=5.4 Hz), 69.4 (m), 69.1 (m), 67.6 (d, J^{PC}=6.0 Hz), 61.5, 32.7 (d, J^{PC}=6.4 Hz), 29.9 (d, J^{PC}=6.9 Hz), 24.9, 24.1, 14.1. ^{31}P NMR (121 MHz, CHLOROFORM-d) δ -0.4 (sept, *J*=7.9 Hz, 1P, P-2), -1.24 (sext, *J*=7.8 Hz, 1P, P-7). $[\alpha]_D^{20}$ (*R*)-**17** +2.9° (c 2.2, $CHCl_3$), (*S*)-**17** -2.9° (c 2.2, $CHCl_3$). HRMS[ESI,pos] Calcd for $C_{37}H_{45}O_{10}P_2$ 711.2488, found 711.2478 $[M+H]^+$.

INHIBITION STUDIES

Assay conditions are described in the main manuscript. Initial rates were obtained by a linear least square fit of the initial rate data. Each rate measurement is the average of at least duplicate measurement with less than 10% standard deviation. Rate measurements were repeated until at least two rates were found to agree within 10% standard deviation. The negative rates obtained from consumption of PEP were converted into progress rates using Beer's Law and the extinction coefficient of PEP ε=2800 L mol^{-1} cm^{-1}(30°C, 232 nm) (4). Error bars in the graphs (Figure S1 and Figure S2) show the standard deviation of rates used to obtain average rates. The kinetic data obtained was globally fitted to the equation

$$v = \frac{V_{max} \times [S]}{K_m \times \left(1 + \frac{[I]}{K_i}\right) + [S]}$$

using the computer program GraFit®.(5) V_{max} represents the maximum progress rate, [S] represents the concentration of the substrate PEP, K_m represents the Michaelis-constant for PEP, [I] represents the concentration of inhibitor present and K_i represents the inhibition constant with respect to PEP. Grafit® data and fits were replotted using ORIGIN®(6) to allow inclusion of error bars.

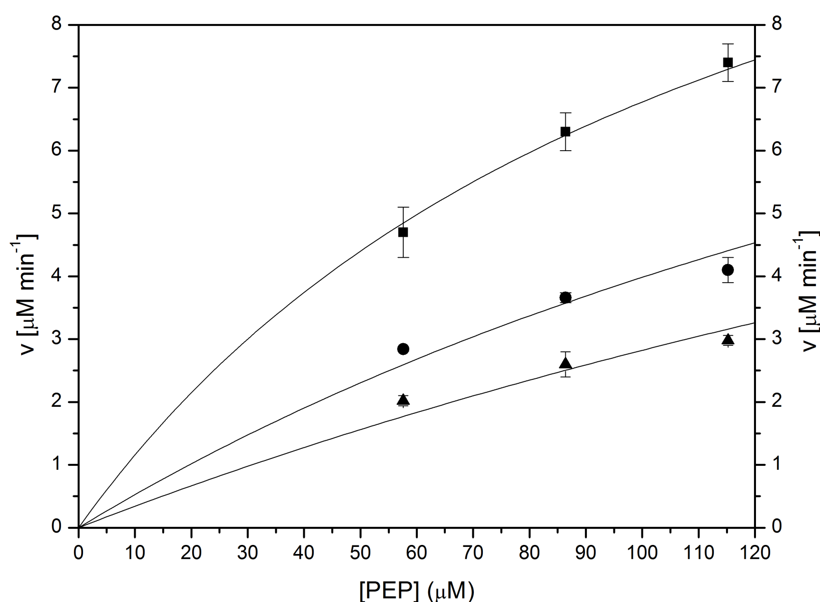


Figure S1: Results of Inhibition assays with *R*-**4**, represented as Michaelis-Menten-Plot. Inhibitor concentrations: squares – no inhibitor; circles [*R*-**4**]= 470 nM; triangles – [*R*-**4**]=940 nM. Error bars are standard deviations of averaged rates, [PEP] has an error of ±10%. Solid lines represent the global fit obtained using all assay measurements (21 data points in total) in Grafit® to a model of competitive inhibition, giving V_{max}=15 ± 2 μM min^{-1}, K_m=120 ± 30 μM , K_i= 360 ± 50 nM.

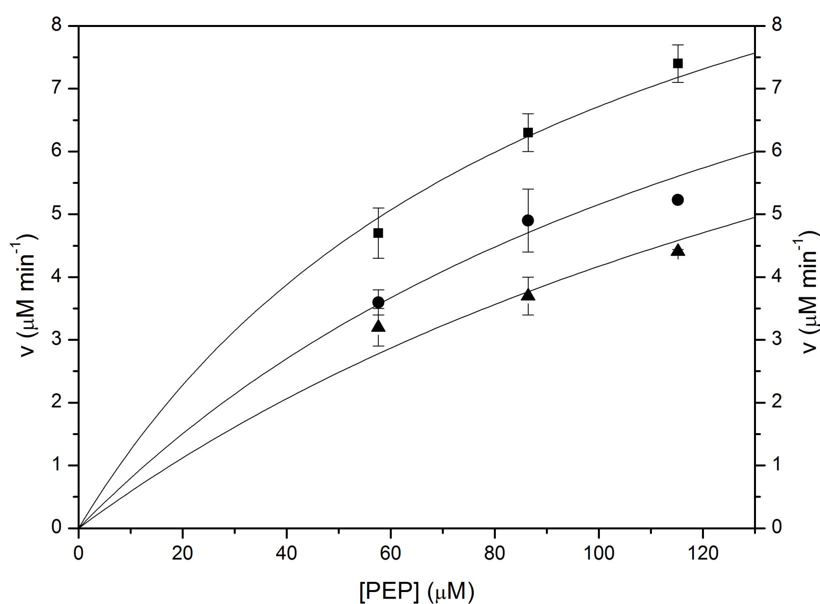


Figure S2: Results of Inhibition assays with *S*-**4**, represented as Michaelis-Menten-Plot. Inhibitor concentrations: squares – no inhibitor; circles [*S*-**4**]=390 nM; triangles – [*S*-**4**]=780 nM. Error bars are standard deviations of averaged rates. [PEP] has an error of ±10%. Solid lines represent the global fit obtained using all assay measurements (21 data points in total) in Grafit® to a model of competitive inhibition, in Grafit®, giving V_{max}=13 ± 2 μM min^{-1}, K_m=100 ± 30 μM , K_i= 620 ± 110 nM.

DETAILED INHIBITOR MODELING PROCEDURE AND RESULTS

The structures of both possible diastereoisomers of the linear intermediate and the designed inhibitor **4** were built using the protein builder in Maestro (7) of the Schrödinger Suite 2006 and then minimized with MacroModel (8). The Polak-Ribiere Conjugate Gradient (PRCG) minimization method was used with up to 5000 iterations and a gradient convergence threshold of δ=0.05 kJ/(mol*Å). A conformational search was then carried out for each isomer in order to generate an ensemble of low-energy conformers to find a suitable starting conformation for the later docking calculation. Conformational searches were conducted with the MCMM Serial Torsional Sampling method and were run with a GB/SA water model, using the OPLS2005 force field, with 3000 steps for the conformational search and an energy window of 12.0 kJ/mol for collecting appropriate conformers. All the conformers from the conformational search were clustered by consideration of torsional RMS deviation of the backbones, and the representatives were selected based on the clustering statistics, which were then used in the docking calculations as the starting conformation for each epimer of the linear intermediate.

The modeling of the linear intermediate epimers into the active site of *Mtu*DAH7PS was carried out with the Schrödinger Suite 2006 Induced Fit Docking protocol (9). The active site in chain A of the unliganded crystal structure of *Mtu*DAH7PS was used as the receptor. The center of the receptor grid was defined as the centroid of residues 87, 126, 133, 134, 135, 136, 137, 248, 250, 280, 282, 283, 284, 306, 337, 366, 369, 380, 382, 409, 411, 440, and 441 as defined in the wild type *Mtu*DAH7PS crystal structure (PDB-code 2B7O) (10). For the initial docking, the van der Waals radii of the linear intermediate atoms were scaled to 0.8. The 20 best solutions from the initial docking were kept. All residues of the protein within a 5 Å distance of the respective linear molecule solution were refined. The ligands were re-docked, with a van der Waals radius scale of 0.8, to the top 20 newly generated protein structures if the energy was within 30 kcal/mol of the best protein structure.

The (*R*)- and (*S*)-isomers of inhibitor **4** were modeled into *Mtu*DAH7PS by docking flexible inhibitors into the rigid receptor with the "intermediate adapted" conformation of the active site. This grid is generated based on the best pose from induced fit modeling of the linear intermediate molecule, from which the active site conformation, especially the residues in close proximity to the modeled linear intermediate molecule (within 5 Å), have adopted different conformations from the original crystal structure (2B7O), to optimize interactions with the modeled linear intermediate. Therefore, this active site conformation can be considered as "intermediate-adapted" and can be used in modeling of inhibitors that are designed to mimic the binding mode of the linear intermediate molecule. The inhibitor docking was conducted in Glide (11) with OPLS2005 force field and extra precision (XP) mode. 90000 poses per ligand were kept for initial docking, and scoring window for keeping initial poses was 5000. The best 1000 poses per ligand were kept for energy minimisation with a distance

dependent dielectric constant of 2 and a maximum of 5000 conjugate gradient steps. Ten poses per ligand were saved for evaluation.

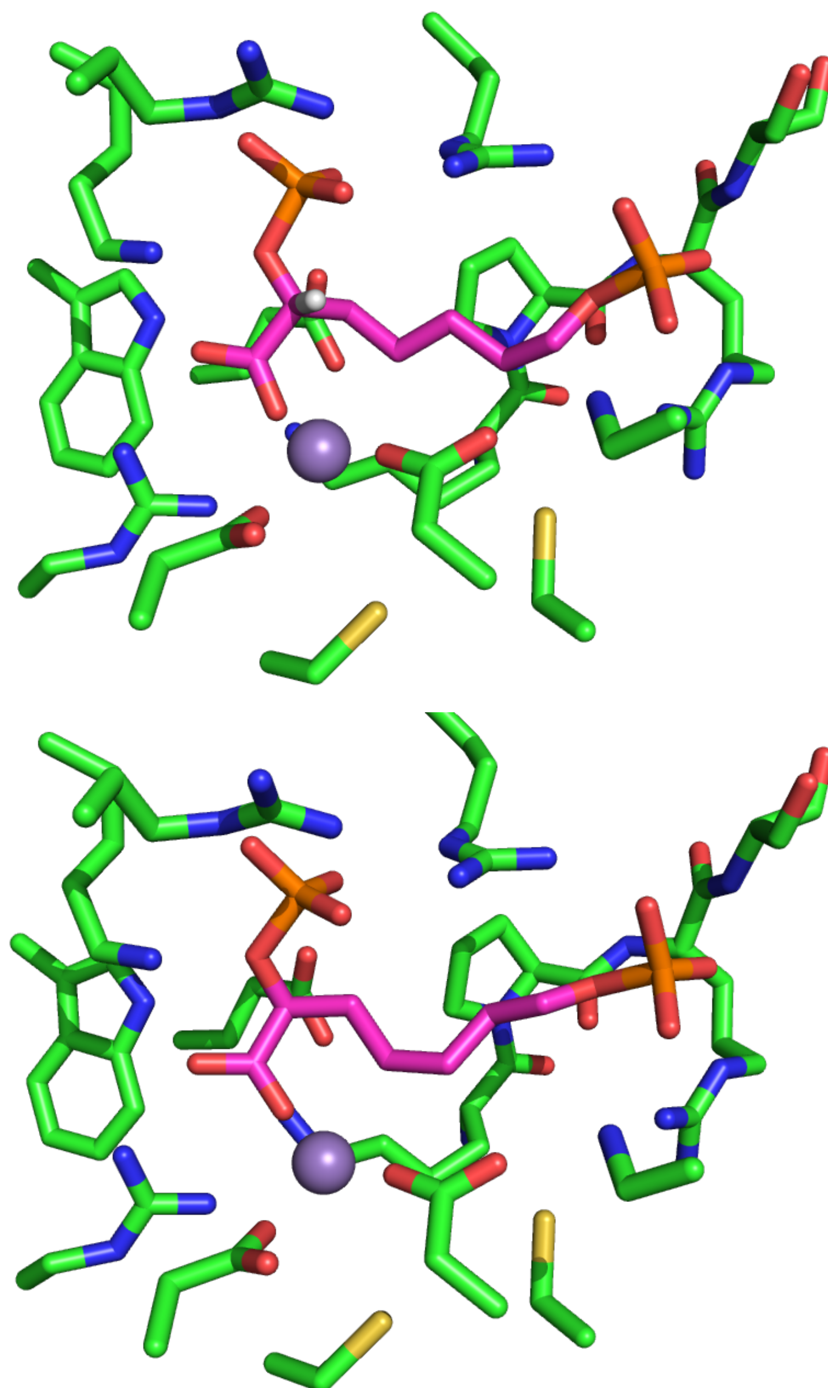


Figure S3: Best pose of inhibitors (magenta carbons) (*R*)-**4** (top) and (*S*)-**4** (bottom) when modeled into the intermediate adapted conformation of *Mtu*DAH7PS (carbon: green, nitrogen: blue, oxygen: red, sulfur: yellow, manganese: gray sphere). All hydrogen atoms except for the C2 hydrogen of **4** highlighting the inhibitor configuration were omitted for clarity, the C2 hydrogen of (*S*)-**4** points away from the viewer into the plane. Enzyme conformation and inhibitor binding closely resemble the experimentally determined structure.

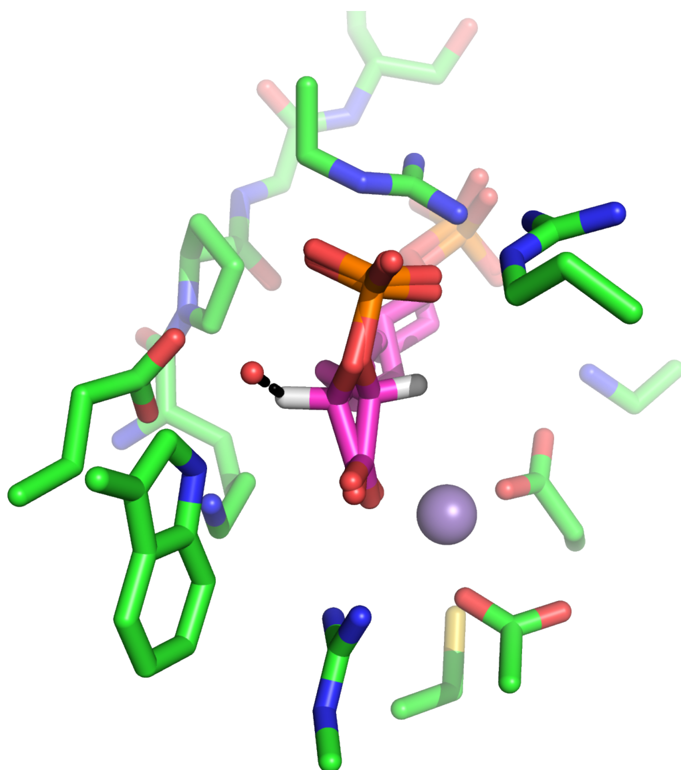


Figure S4: Side-on view (Figure S3 rotated approximately 90° around vertical axis) of the best poses of inhibitors (*R*)-**4** and (*S*)-**4** (magenta carbons) when modeled into the intermediate adapted conformation of *Mtu*DAH7PS. This view illustrates the hydrogen pointing towards ((*S*)-**4**) and away from ((*R*)-**4**) Glu248 and Trp280 in the foreground on the left. This potential steric clash of (*S*)-**4** with a water molecule (red sphere) coordinated to these residues can be used to rationalize the slightly lower inhibitory potency of (*S*)-**4**. The distance between H2 of (*S*)-**4** and the water molecule (black dashed line) is only 1.7 Å.

REFERENCES

1. McDougal, P. G., Rico, J. G., Oh, Y. I. & Condon, B. D. (1986) *J. Org. Chem.* **51**, 3388-3390.
2. Petit, Y. & Larcheveque, M. (1998) *Org. Synth.* **75**, 37-44.
3. Matsuo, Y., Muramatsu, A., Tahara, K., Koide, M. & Nakamura, E. (2006) *Org. Synth.* **83**, 80-87.
4. Schoner, R. & Herrmann, K. M. (1976) *Journal of Biological Chemistry* **251**, 5440-5447.
5. GraFit, Version 5.0.13, Erithacus Software Limited, 2006.
6. Origin Pro 8.1 SR3, v8.1.34.90, OriginLab Corporation, 2010.
7. Maestro, Version 7.5, Schrodinger, LLC, 2005.
8. MacroModel, Version 9.1, Schrodinger, LLC, 2005.
9. Schrodinger Suite 2006 Induced Fit Protocol; Glide version 4.0; Prime version 1.5, Schrodinger, LLC, 2005.
10. Webby, C. J., Baker, H. M., Lott, J. S., Baker, E. N. & Parker, E. J. (2005) *J. Mol. Biol.* **354**, 927-939.
11. Glide, Version 4.0, Schrodinger, LLC, 2005.