-Supplementary Information for-

ACTIVE SITE PLASTICITY OF A CRITICAL ENZYME FROM *MYCOBACTERIUM TUBERCULOSIS*

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General

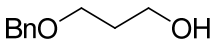
Reactions were carried out under an inert atmosphere of nitrogen unless stated otherwise. Solvents were dried and purified using standard procedures.[1] CuBr●SMe_2 was prepared freshly from CuBr following a published procedure,[2] all other reagents were used as purchased. NMR spectra were recorded on either a Varian UNITY 300 MHz or Varian INOVA 500 MHz NMR spectrometer. ^{1}H and ^{13}C-NMR spectra were referenced to residual protonated solvent or internal tetramethylsilane. ^{31}P-NMR spectra were externally referenced to 85% phosphoric acid. Mass spectrometry was performed on a Bruker maXis 3G mass spectrometer. Optical rotations were measured on a Perkin Elmer 341 Polarimeter. Due to most compounds characterised for this study possessing low rotations at the standard 589 nm wavelength, different wavelengths were chosen to determine the optical rotation, details are given with the analytical data for the respective compounds. Infrared spectra were recorded on a Perkin Elmer Spectrum One IR spectrometer. UV-Visible spectroscopy was carried out on a Varian Cary® 100 Bio UV-Visible spectrometer.

Synthesis

*General procedure for the preparation of bromides (**3**)*

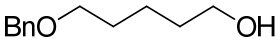
The appropriate neat 1,x-diol (1 eq) was placed in a flask under a stream of nitrogen. Finely powdered KOH (1.2 eq) was added and the suspension heated to about 100°C with stirring, which resulted in some of the KOH being dissolved. Benzyl bromide (1 eq, neat) was added dropwise at a rate which resulted in a controlled reflux. After complete addition, the reaction mixture was heated to reflux for 4 h, cooled to room temperature and diluted with dichloromethane. The organic phase was washed with 1 M HCl and dried over magnesium

sulfate before the solvent was removed under reduced pressure. The crude product was purified by flash chromatography eluting with 30-50% ethyl acetate/PET ether giving the monobenzylated diols **S1a-b**. Yields and analytical data are summarized below.



S1a

40% yield, R_f 0.32 (50% EA/PET ether). ^{1}H-NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.3 - 7.4 (m, 5 H, Ph-H), 4.5 (s, 2 H, Ph-C**H**$_2$-O), 3.8 (t, *J*=5.7 Hz, 2 H, C**H**$_2$OH), 3.7 (t, *J*=5.8 Hz, 2 H, C**H**$_2$OBn), 2.4 (br. s., 1 H, -OH), 1.9 (quin, *J*=5.8 Hz, 2 H, C**H**$_2$CH$_2$OH). ^{13}C-NMR (126 MHz, CHLOROFORM-d) δ 138.1 (**C**-CH$_2$-O), 127.7 (Ar-C), 127.6 (Ar-C), 73.2 (Ph-**C**H$_2$-O), 69.2 (**C**H$_2$OH), 61.7 (**C**H$_2$OBn), 32.1 (**C**H$_2$CH$_2$OH). HRMS [ESI,pos] Calcd for $C_{10}H_{14}O_2Na$ 189.0891, found 189.0889 $[M+Na]^+$. NMR data is in accordance with the literature.[3]

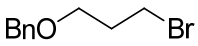


S1b

27% yield, R_f 0.28 (40% EA/PET ether). ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.3 - 7.4 (m, 5 H, Ar-**H**), 4.5 (s, 2 H, Ph-C**H**$_2$), 3.6 (br. t, *J*=6.0, 6.0 Hz, 2 H, C**H**$_2$OH), 3.5 (t, *J*=6.5 Hz, 2 H, BnO-C**H**$_2$), 1.6 - 1.7 (m, 2 H, H2/H4), 1.5 - 1.6 (m, 2 H, H2/H4), 1.4 - 1.5 (m, 2 H, H3). ^{13}C-NMR (126 MHz, CHLOROFORM-d) δ 138.5 (Ar-**C**), 128.3 (Ar-**C**), 127.6 (Ar-**C**), 127.4 (Ar-**C**), 72.8 (Ph-**C**H$_2$, 70.2 (**C**H$_2$OH), 62.6 (BnO-**C**H$_2$), 32.4 (**C**H$_2$CH$_2$OH), 29.4 (BnO_CH$_2$**C**H$_2$), 22.4 (BnOCH$_2$CH$_2$**C**H$_2$). NMR data is in accordance with the literature.[3]

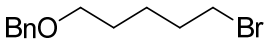
Monobenzylated diols **S1** (1 eq) were dissolved in THF at 0°C and stirred while solid PPh_3 (1.5 eq) and NBS (1.5 eq) were added alternatingly in portions. After complete addition, the mixture was stirred at room temperature for 2 h or until TLC indicated complete conversion of starting material. The solvent was removed under reduced pressure to yield a brown oil,

which was added to rapidly stirring PET ether. The precipitated solids were removed by filtration, the filtrate dried over $MgSO_4$ and concentrated *in vacuo* to yield the crude product. Purification was carried out by flash chromatography eluting with a gradient of 20% to 50% dichloromethane/PET ether giving the bromides **3** with yields and analytical data summarized below.



3a

73% yield, R_f 0.28 (20% DCM/PET ether). ^{1}H-NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.3 - 7.4 (m, 5 H, Ar-**H**), 4.5 (s, 2 H, Ph-C**H$_2$**), 3.6 (t, *J*=6.0 Hz, 2 H, BnOC**H$_2$**), 3.6 (t, *J*=6.5 Hz, 2 H, Br-C**H$_2$**), 2.1 - 2.2 (m, 2 H, BnOCH$_2$C**H$_2$**). ^{13}C-NMR (126 MHz, CHLOROFORM-d) δ 138.1 (Ar-C1), 128.4 (Ar-C), 127.7 (Ar-C), 127.6 (Ar-C), 73.2 (Ph-**C**H$_2$), 69.2 (BnO**C**H$_2$), 61.7 (Br-**C**H$_2$), 32.1 (BnOCH$_2$**C**H$_2$). HRMS [ESI,pos] Calcd for $C_{10}H_{13}OBrNa$ 251.0047, found 251.0053 $[M+Na]^+$. NMR data is in agreement with literature values.[4]



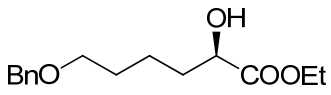
3b

91% yield, R_f 0.49 (10% EA/PET ether). ^{1}H NMR (500 MHz, CHLOROFORM-d) δ 7.28 - 7.43 (m, 5H, Ph-H), 4.49 (s, 2H, Ph-C**H$_2$**), 3.47 (t, *J* = 6.31 Hz, 2H, BnOC**H$_2$**), 3.39 (t, *J* = 6.78 Hz, 2H, Br-C**H$_2$**), 1.80 - 1.95 (m, 2H, BnOCH$_2$C**H$_2$**), 1.57 - 1.73 (m, 2H, BrCH$_2$C**H$_2$**), 1.48 - 1.57 (m, 2H, BnOCH$_2$CH$_2$C**H$_2$**). ^{13}C-NMR (126 MHz, CHLOROFORM-d) δ 138.5 (Ar-C), 128.3 (Ar-C), 127.5 (Ar-C), 127.4 (Ar-C), 72.9 (Ph-**C**H$_2$), 69.9 (Br-**C**H$_2$), 33.6, 32.5, 28.8, 24.9 (4 CH$_2$). NMR data is in agreement with literature values.[5]

Epoxide ring opening. Carried out under argon atmosphere. Magnesium turnings (3.4 eq with respect to bromide) were freshly ground under a stream of argon and suspended in a known volume of dry diethylether under argon. A mixture of the respective bromide **3** (1 eq) and dibromoethane (0.08 eq with respect to bromide) was dissolved in a known volume of

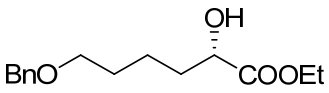
dry diethylether. The total volume of this solution was recorded before dropwise addition to the Mg suspension was started. The speed of addition was chosen so that a gentle reflux of the reaction mixture was maintained. After complete addition, the Grignard reagent solution was stirred for 1 h at room temperature before the solution was titrated against a THF or diethylether solution of salicylaldehyde phenylhydrazone (typically 70-80 mg in 10 mL solvent).[6]

The Grignard solution was added dropwise *via cannula* to a stirred suspension of CuBr●SMe_2 (1 eq with respect to Grignard reagent) which had been pre-cooled to -78°C. After complete addition, the suspension was stirred for one hour, during which most of the solid dissolved and formed an orange/red solution. Ethyl glycidate (0.5 eq with respect to Grignard reagent) dissolved in a small volume of THF was added and the resulting reaction mixture allowed to gradually warm to room temperature over 3-5 hours. The reaction was quenched by the addition of saturated aqueous ammonium chloride solution. The aqueous phase was extracted twice with ether and the combined organic extracts washed with water and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product purified by flash column chromatography eluting with 30% ethyl acetate/PET ether, providing α-hydroxy esters **7** in yields and with analytical data summarized below.



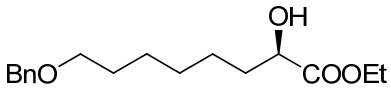
R-**7a**

67% yield from ethyl glycidate, R_f 0.35 (30% EA/PET ether). ^{1}H-NMR (500 MHz, CHLOROFORM-d) δ 7.2 - 7.4 (m, 5H, Ar-H), 4.5 (s, 2H, Ph-C**H$_2$**), 4.2 (q, *J* = 7.2 Hz, 2H, OC**H$_2$**CH_3), 4.16 (ddd, *J* = 4.2, 5.6, 7.4 Hz, 1H, H2), 3.47 (t, *J* = 6.4 Hz, 2H, H6), 2.85 (d, *J* = 5.9 Hz, 1H, -OH), 1.77 - 1.85 (m, 1H, H3a), 1.44 - 1.72 (m, 5H, H3b/H4/H5), 1.25 - 1.31 (m, 3H, OCH_2C**H$_3$**). ^{13}C-NMR (126 MHz, CHLOROFORM-d) δ 175.2 (**C**OOEt), 138.5 (Ar-C), 128.3 (Ar-C), 127.5 (Ar-C), 127.4 (Ar-C), 72.8 (Ph-**C**H_2), 70.3 (C2), 70.1 (C6), 61.6 (O**C**H_2CH_3), 34.1 (C3), 29.4 (C5), 21.5 (C4), 14.1 (OCH_2**C**H_3). HRMS [ESI, pos] Calcd for $C_{15}H_{22}O_4Na$ 289.1410, found 289.1404 $[M+Na]^+$. IR (neat, υ[cm^{-1}]) 3449 (-OH), 2936, 2863 (CH_2), 1735 (C=O). $[\alpha]_{20}$ -5.0° (436 nm, c 3.2, $CHCl_3$).



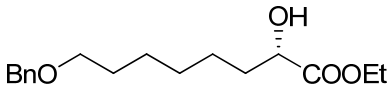
S-**7a**

59% yield from ethyl glycidate. Analytical data are identical to *R*-**7a** above, apart from $[\alpha]_{20}$ +5.4° (436 nm, c 3.44 , $CHCl_3$).



R-**7b**

55% yield from ethyl glycidate, R_f 0.25 (20% EA/PET ether). ^{1}H-NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.2 - 7.4 (m, 5 H, Ph-**H**), 4.5 (s, 2 H, PhC**H$_2$**O), 4.3 (qd, *J*=7.1, 1.3 Hz, 2 H, COOC**H$_2$**CH_3), 4.1 - 4.2 (m, 1 H, H2), 3.5 (t, *J*=6.6 Hz, 2 H, H8), 2.7 (br. s., 1 H, -OH), 1.7 - 1.8 (m, 1 H, H3a), 1.6 - 1.7 (m, 4 H, H4/H7), 1.3 - 1.5 (m, 5 H, H3b/H5/H6), 1.3 (t, *J*=7.0 Hz, 3 H, $COOCH_2C$**H$_3$**). ^{13}C-NMR (126 MHz, CHLOROFORM-d) δ 175.4 (C1), 138.7, 128.3, 127.6, 72.9 (Ph**C**H_2O), 70.4 (C2/C8), 70.4 (C2/C8), 61.6 (COO**C**H_2CH_3), 34.3 (C3), 29.6 , 29.1, 26.0, 24.7 (4 CH_2), 14.2 ($COOCH_2$**C**H_3). HRMS [ESI, pos] Calcd for $C_{17}H_{26}O_4Na$ 317.1723, found 317.1724 $[M+Na]^+$. IR (neat, υ[cm^{-1}]) 3449 (-OH), 3030 (C^{Ar}-H), 2932, 2858 (CH_2), 1735 (C=O). $[\alpha]_{20}$ -5.2° (436 nm, c 2.5, $CHCl_3$).

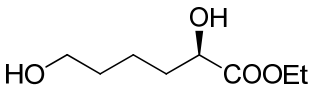


S-**7b**

65% from ethyl glycidate. Analytical data are identical to *R*-**7b** above, apart from $[\alpha]_{20}$ +5.3° (436 nm, c 2.4, $CHCl_3$).

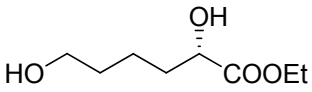
Hydrogenation. To a solution of α-hydroxy esters **7** (1eq) in ethyl acetate was added Pd on charcoal (0.1 eq Pd). The reaction vessel was charged with an atmosphere of hydrogen by means of three freeze-pump-thaw cycles. The reaction mixture was stirred overnight or until

TLC indicated complete conversion of starting material. The catalyst was removed by filtration through a celite pad, the celite pad was washed with ethyl acetate and the combined filtrates concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluting with 80% ethyl acetate/PET ether. Yields and analytical data for the diols **8** obtained are summarized below.



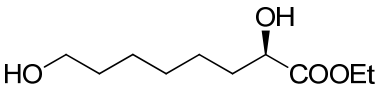
R-**8a**

74% yield, R_f 0.25 (80% EA/PET ether). ^{1}H-NMR (500 MHz, CHLOROFORM-*d*) δ ppm 4.2 (q, *J*=7.2 Hz, 2 H, COOC**H$_2$**CH$_3$), 4.2 (ddd, *J*=7.3, 5.7, 4.2 Hz, 1 H, H2), 3.6 (t, *J*=6.2 Hz, 2 H, H6), 3.2 (d, *J*=5.9 Hz, 1 H, -OH), 2.2 (br. s., 1 H, -OH), 1.7 - 1.9 (m, 1 H, H3a), 1.4 - 1.7 (m, 5 H, H3b/H4/H5), 1.3 (t, *J*=7.2 Hz, 3 H, COOCH$_2$C**H$_3$**). ^{13}C-NMR (126 MHz, CHLOROFORM-d) δ 175.1 (C1), 70.3 (C2), 62.3 (C6), 61.6 (O**C**H$_2$CH$_3$), 33.9 (C3), 32.1 (C5), 21.0 (C4), 14.1 (OCH$_2$**C**H$_3$).HRMS [ESI, pos] Calcd for $C_8H_{16}O_4Na$ 199.0941, found 199.0941 $[M+Na]^+$. IR (neat, υ[cm^{-1}]) 3390 (-OH), 2940, 2869 (CH_2), 1733 (C=O). $[\alpha]_{20}$ -14° (365 nm, c 2.55, $CHCl_3$).



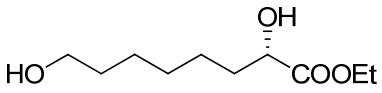
S-**8a**

88% yield. Analytical data are identical to *R*-**8a** above, apart from $[\alpha]_{20}$ +13.7° (365 nm, c 2.91, $CHCl_3$).



R-**8b**

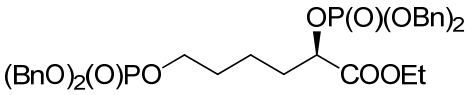
66% yield, R_f 0.42 (80% EA/PET ether). ^{1}H-NMR (500 MHz, CHLOROFORM-d) δ 4.22 (q, *J* = 7.20 Hz, 2H, OC**H$_2$**CH$_3$), 4.11 - 4.18 (m, 1H, H2), 3.61 (t, *J* = 6.60 Hz, 2H, H8), 2.96 (br. s, 1H, -OH), 1.71 - 1.85 (m, 1H, H3a), 1.31 - 1.66 (m, 9H, H3b/H4/H5/H6/H7), 1.28 (t, *J* = 7.20 Hz, 3H, COOCH$_2$C**H$_3$**). ^{13}C-NMR (126 MHz, CHLOROFORM-d) δ 175.3 (C1), 70.3 (C2), 62.7(C8), 61.5 (O**C**H$_2$CH$_3$), 34.2 (C3), 32.5 (C4/C5/C6/C7), 29.0 (C4/C5/C6/C7), 25.5 (C4/C5/C6/C7), 24.6 (C4/C5/C6/C7), 14.1(OCH$_2$**C**H$_3$). HRMS [ESI, pos] Calcd for $C_{10}H_{20}O_4Na$ 227.1254, found 227.1252 $[M+Na]^+$. IR (neat, υ[cm^{-1}]) 3391 (-OH), 2932, 2860 (CH$_2$), 1732 (C=O). $[\alpha]_{20}$ -5.4° (436 nm, c 2.33, $CHCl_3$)



S-**8b**

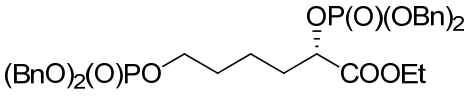
86% yield. Analytical data are identical to *R*-**8b** above, apart from $[\alpha]_{20}$ +5.0° (436 nm, c 2.4, $CHCl_3$).

Phosphorylation. Diols **8** (1 eq) and dibenzyl *N*,*N*-diisopropylphosphoramidite (4 eq) were dissolved in dichloromethane and cooled to 0°C. Solid dicyanoimidazole (4.8 eq) was added in one portion, the reaction mixture allowed to warm to room temperature and stirred for 4 h or until TLC indicated complete conversion of starting material. A solution of *m*-CPBA (6.3 eq) in dichloromethane was dried over $MgSO_4$ before addition to the reaction mixture. The reaction mixture was stirred for 2 h and then quenched by addition of a 10% aqueous sodium thiosulfate solution. The organic phase was separated, washed with 10% aqueous sodium thiosulfate solution, saturated aqueous sodium bicarbonate solution and the combined aqueous phases backextracted with dichloromethane. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 50% ethyl acetate/PET ether as eluent. Yields and analytical data for the phosphorylated diols **9** are summarized below.



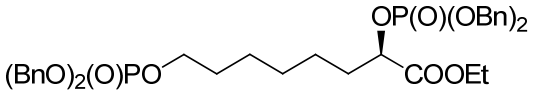
***R*-9a**

80% yield, R_f 0.36 (60% EA/PET ether). ^{1}H-NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.3 - 7.4 (m, 15 H, Ph-H), 4.9 - 5.2 (m, 8 H, BnO-C**H$_2$**), 4.8 (dt, *J*=7.9, 6.1 Hz, 1 H, H2), 4.1 - 4.2 (m, 2 H, H6), 3.9 (q, *J*=6.6 Hz, 2 H, COOC**H$_2$**CH_3), 1.7 - 1.8 (m, 2 H, CH_2), 1.6 (dt, *J*=12.7, 6.5 Hz, 2 H, CH_2), 1.4 (quin, *J*=7.8 Hz, 2 H, CH_2), 1.2 - 1.3 (t, *J*=7.2 Hz 3 H, $COOCH_2C$**H$_3$**). ^{13}C-NMR (126 MHz, CHLOROFORM-*d*) δ ppm 169.8 (d, *J*=3.3 Hz), 135.7 (d, *J*=6.5 Hz), 135.7 (d, *J*=7.4 Hz), 135.6 (d, *J*=7.0 Hz), 128.5 (s), 128.4 (s), 127.9 (s), 127.8 (s), 127.8 (s), 75.2 (d, *J*=5.6 Hz), 69.4 (d, *J*=4.7 Hz), 69.4 (d, *J*=4.7 Hz), 69.1 (d, *J*=5.6 Hz), 67.2 (d, *J*=6.0 Hz), 61.5 (s), 32.3 (d, *J*=6.5 Hz), 29.4 (d, *J*=7.4 Hz), 20.5 (s), 14.0 (s). ^{31}P{^{1}H}-NMR (202 MHz, CHLOROFORM-*d*) δ ppm 0.3 (s, 1P), -0.6 (s, 1P). ^{31}P-NMR (202 MHz, CHLOROFORM-*d*) δ ppm 0.3 (sept, *J*=8 Hz, 1P), -0.6 (sext, *J*=8 Hz, 1P). HRMS [ESI, pos] Calcd for $C_{36}H_{43}O_{10}P_2$ 697.2326, found 697.2340 $[M+H]^+$. IR (neat, υ[cm^{-1}]) 3065, 3035, 2960, 1754 (C=O), 1276 (P=O), 1015 (P-O-C), 735, 697 (monosubstituted aryl). $[\alpha]_{20}$ +4.5 (436 nm, c 2.5, $CHCl_3$).



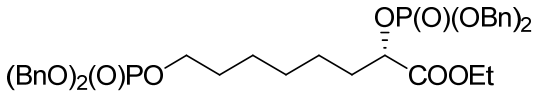
***S*-9a**

87% yield. Analytical data are identical to *R*-**9a** above, apart from $[\alpha]_{20}$ -4.7° (436 nm, c 2.4, $CHCl_3$).



***R*-9b**

80% yield, R_f 0.18 (50% EA/PET ether). ^{1}H-NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.3 - 7.4 (m, 15 H, Ph-H), 5.1 (qd, *J*=11.4, 7.5 Hz, 2 H, POC**H$_2$**Ph), 5.0 - 5.1 (m, 6 H, POC**H$_2$**Ph), 4.7 - 4.8 (m, 1 H, H2), 4.2 (qd, *J*=7.2, 3.5 Hz, 2 H, H8), 4.0 (q, *J*=6.6 Hz, 2 H, COOC**H$_2$**CH$_3$), 1.8 (q, *J*=7.0 Hz, 2 H, H3), 1.5 - 1.6 (m, 2 H, H7), 1.3 (dd, *J*=13.2, 6.6 Hz, 2 H, H4), 1.2 (t, *J*=7.0 Hz and overlapping m, 7 H, COOCH$_2$C**H$_3$**/H5/H6). ^{13}C NMR (126 MHz, CHLOROFORM-*d*) δ ppm 170.0 (d, J^{PC}=3.3 Hz, C1), 135.8 (d, J^{PC}=6.5 Hz, POCH$_2$**C**^{Ph}), 135.7 (d, J^{PC}=7.4 Hz, POCH$_2$**C**^{Ph}), 135.6 (d, J^{PC}=7.0 Hz, POCH$_2$**C**^{Ph}), 128.5 (s, Ar-C), 128.5 (s, Ar-C), 128.4 (s, Ar-C), 128.4 (s, Ar-C), 128.4 (s, Ar-C), 127.9 (s, Ar-C), 127.8 (s, Ar-C), 127.7 (s, Ar-C), 75.5 (d, J^{PC}=5.6 Hz, C2), 69.4 (d, J^{PC}=5.6 Hz Ph**C**H$_2$OP), 69.3 (d, J^{PC}=5.6 Hz, Ph**C**H$_2$OP), 69.1 (d, J^{PC}=5.6 Hz, Ph**C**H$_2$OP), 67.7 (d, J^{PC}=6.0 Hz,C8), 61.4 (s, COO**C**H$_2$CH$_3$), 32.8 (d, J^{PC}=6.0 Hz, C3), 29.9 (d, J^{PC}=7.0 Hz, C7), 28.4 (s, C4/C5/C6), 25.0 (s, C4/C5/C6), 24.3 (s, C4/C5/C6), 14.0 (s, COOCH$_2$**C**H$_3$). ^{31}P{^{1}H}-NMR (202 MHz, CHLOROFORM-*d*) δ ppm 0.3 (br. s., 1 P), -0.6 (s, 1 P). ^{31}P NMR (202 MHz, CHLOROFORM-*d*) δ ppm 0.3 (spt, *J*=6.5 Hz, 1 P), -0.6 (sxt, *J*=6.7 Hz, 1 P). HRMS [ESI,pos] Calcd for $C_{38}H_{46}O_{10}P_2Na$ 747.2458, found 747.2463 $[M+Na]^+$. IR (neat, υ[cm^{-1}]) 3034, 2933, 2860 (CH_2), 1753 (C=O), 1271 (P=O), 1015 (P-O-C), 737, 697 (monosubstituted aryl). $[\alpha]_{20}$ +4.4° (436 nm, c 2.3, $CHCl_3$).

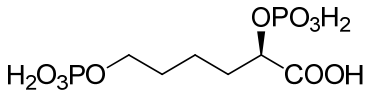


***S*-9b**

79% yield. Analytical data are identical to *R*-**9b** above, apart from $[\alpha]_{20}$ -4.7° (436 nm, c 2.5, $CHCl_3$).

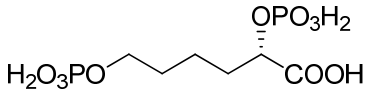
Final deprotection. Fully protected diphosphates **9** (1 eq) were dissolved in ethyl acetate, palladium on charcoal (0.1 eq) added and the flask charged with an atmosphere of hydrogen using three freeze-pump-thaw cycles. The mixture was stirred overnight or until TLC indicated complete conversion of starting material. The catalyst was removed by filtration through a pad of celite, the celite pad was washed with ethyl acetate and methanol. The solvent was evaporated, 1 M KOH added and the reaction mixture stirred vigorously for 2 h.

The reaction mixture was passed down a column of DOWEX 50WX8-100 (H^+-form, approximately 1 cm x 10 cm) and the column washed with water. The combined eluate was neutralized with 1 M KOH and lyophilized. The crude product was purified by anion exchange chromatography on column containing Source Q resin eluting with a gradient of 0-0.6 M ammonium bicarbonate. Fractions containing phosphate were determined using the qualitative Lanzetta assay (see below), pooled and lyophilized. Yields and analytical data of final products are summarized below.



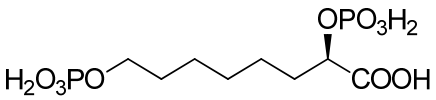
C6R

61% yield. ^{1}H-NMR (500 MHz, DEUTERIUM OXIDE) δ ppm 4.35 (br., 1H, H2), 3.74 (br, 2H, H6), 1.72 (br, 2H, H5), 1.56 (br, 2H, H4), 1.40 (br, 1H, H3a), 1.31 (br, 1H, H3b). ^{13}C-NMR (126 MHz, DEUTERIUM OXIDE) δ ppm 180.2 (d, *J*=4.2 Hz, C1), 76.0 (d, *J*=5.1 Hz, C2), 65.2 (d, *J*=5.6 Hz, C6), 33.4 (d, *J*=4.2 Hz, C4), 30.1 (d, *J*=7.4 Hz, C5), 20.7 (s, C4). ^{31}P{^{1}H}-NMR (202 MHz, DEUTERIUM OXIDE) δ ppm 3.0 (br s, 1P), 1.7 (br s, 1P). ^{31}P-NMR (202 MHz, DEUTERIUM OXIDE) δ ppm 3.0 (br. t, J=6.5 Hz, 1P), 1.7 (d, J=8.8 Hz, 1P). HRMS [ESI,pos] Calcd for $C_6H_{15}O_{10}P_2$ 309.0135, found 309.0127 $[M+H]^+$. $[\alpha]_{20}$ +4.2° (546 nm, c 1.2, H_2O).



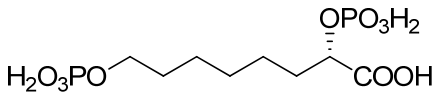
C6S

14% yield. Analytical data are identical to **C6R** above, apart from $[\alpha]_{20}$ -1.9° (546 nm, c 1.3, H_2O).



C8R

5% yield. ^{1}H-NMR (500 MHz, DEUTERIUM OXIDE) δ 4.29 - 4.40 (m, 1H, H2), 3.77 (q, *J* = 6.60 Hz, 2H, H8), 1.69 (br m, 2H, CH_2), 1.55 (br m, 2H, CH_2), 1.21 - 1.41 (m, 6H, 3 CH_2). ^{13}C-NMR (126 MHz, DEUTERIUM OXIDE) δ ppm 179.9 (d, J^{CP}=4.2 Hz, C1), 76.2 (d, J^{CP} =5.6 Hz,C2), 65.6 (d, J^{CP} =5.6 Hz, C8), 33.4 (d, J^{CP} =5.1 Hz, C3), 29.8 (d, J^{CP} =7.0 Hz, C7), 28.3 (s, C4/C5/C6), 24.7 (s, C4/C5/C6), 24.0 (s, C4/C5/C6). ^{31}P{^{1}H} NMR (202 MHz, DEUTERIUM OXIDE) δ ppm 2.2 (s, 1 P), 1.2 (s, 1 P). ^{31}P NMR (202 MHz, DEUTERIUM OXIDE) δ ppm 2.2 (t, *J*=6.5 Hz, 1 P), 1.2 (d, *J*=8.5 Hz, 1 P). HRMS [ESI,neg] Calcd for $C_8H_{17}O_{10}P_2$ 335.0302, found 335.0313 $[M-H]^-$. $[\alpha]_{20}$ +7.9° (436 nm, c 1.0, H_2O). These data are consistent with the data reported for the racemic compound.[7]



C8S

25% yield. Analytical data are identical to **C8R** above, apart from $[\alpha]_{20}$ -4.0° (436 nm, c 1.0, H_2O).

Lanzetta phosphate assay. Lanzetta reagent[8] was prepared fresh as required from the following components: 3 parts 0.045% w/v malachite green in water, 1 part 4.2% w/v ammonium molybdate in 4 M HCl, 0.1 parts 1.5% v/v Triton X-100 in water. The components were mixed in the dark and stirred for 1 h before the solution was filtered through a 0.45 μM syringe filter. For the qualitative detection of phosphate-containing fractions after anion exchange chromatography, a 20 μL sample of each fraction was mixed with 250 μL of Lanzetta reagent and the color change judged by optical inspection. For the quantitative determination of inhibitor concentration, 300 μL of the inhibitor solutions were incubated with 10 μL calf alkaline phosphatase solution (5 units/mL in 4 mM $MgCl_2$) for at least 2 h. To 100 μL of the digested sample was added 700 μL Lanzetta reagent and the absorbance at 630 nm determined after 20 minutes. A calibration curve for the determination of phosphate concentration was obtained from analogous analysis of solutions of appropriate concentrations (6-150 μM) of KH_2PO_4 which had been dried in high vacuum for at least 3 h before use. As a control, a glucose-6-phosphate solution of known concentration was also digested with calf alkaline phosphatase and analyzed.

Enzyme Purification and Assays

DAH7PS from *Mycobacterium tuberculosis* (*Mtu*DAH7PS) was overexpressed and purified as previously described.[9] Cell lysate containing His_6-tagged protein was loaded onto an IMAC column (GE Healthcare). Non-binding contents of the lysate were eluted with a buffer containing a low imidazole concentration (20 mM), then a gradient to high imidazole concentration was applied to elute His_6-tagged MtuDAH7PS. The fractions containing *Mtu*DAH7PS were diluted by half with SEC buffer and tobacco etch virus protease (1 mg) was added to cleave the His-tag. After 1 h at room temperature the solution was loaded onto an IMAC column, this time collecting the flow through containing His-tag cleaved *Mtu*DAH7PS. The solution was concentrated and final purification carried out using a HiLoad 26/60 Superdex™ 200 prep grade column (GE Healthcare) eluting with a buffer containing 10 mM bis-tris propane (BTP) at pH 7.5, 150 mM NaCl, 200 μM tris(2-carboxyethyl)phosphine) (TCEP), 200 μM PEP, 200 μM $MnSO_4$.

Enzymatic activity assays were carried out as previously described.[10] The loss of PEP was monitored at a wavelength of 232 nm. For rate measurements, the enzyme (2 μL, 2.1 mg/mL) was incubated with varying concentrations of PEP (0-35 μM) and inhibitor (0-4.8 μM), $MnSO_4$ (100 μM), with buffer (50 mM BTP pH 7.5, 1 mM TCEP) added to a final volume of 1 mL. The reaction was initiated by the addition of the second substrate E4P (5 μL, giving a cuvette concentration of 135 μM). Initial rates were determined using the slope of a linear least square fit to the UV trace from 0.5 minute after initiation to 1 minute after initiation. Rate measurements were repeated at least twice or until at least two measurements which agreed within 10% standard deviation of each other were recorded.

The rate 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 program Grafit.[11]

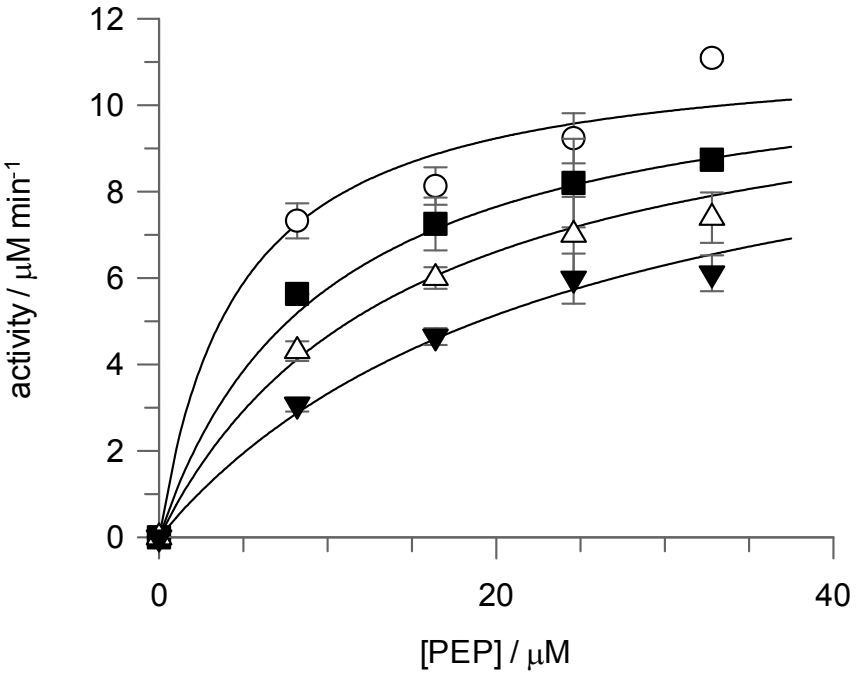


Figure S 1: Enzyme assay results for inhibitor **C6R**, inhibitor concentrations: empty circles: no inhibitor; solid squares: 1.2 μM; empty triangles: 2.4 μM; solid triangles: 4.8 μM. The global fit gave V_{max} 12 ± 0.4 μM min^{-1}, K_m 4.7 ± 1.0 μM, K_i 1.1 ± 0.2 μM.

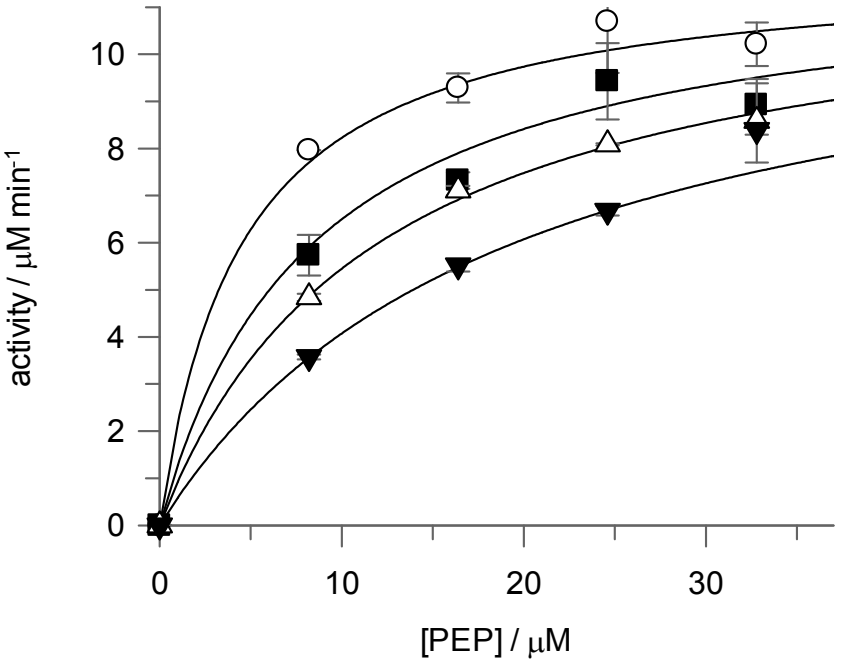


Figure S 2: Enzyme assay results for inhibitor **C6S**, inhibitor concentrations: empty circles: no inhibitor; solid squares: 1.2 μM; empty triangles: 2.3 μM; solid triangles: 4.6 μM. The global fit gave V_{max} 12 ± 0.4 μM min^{-1}, K_m 4.5 ± 0.8 μM, K_i 1.4 ± 0.3 μM.

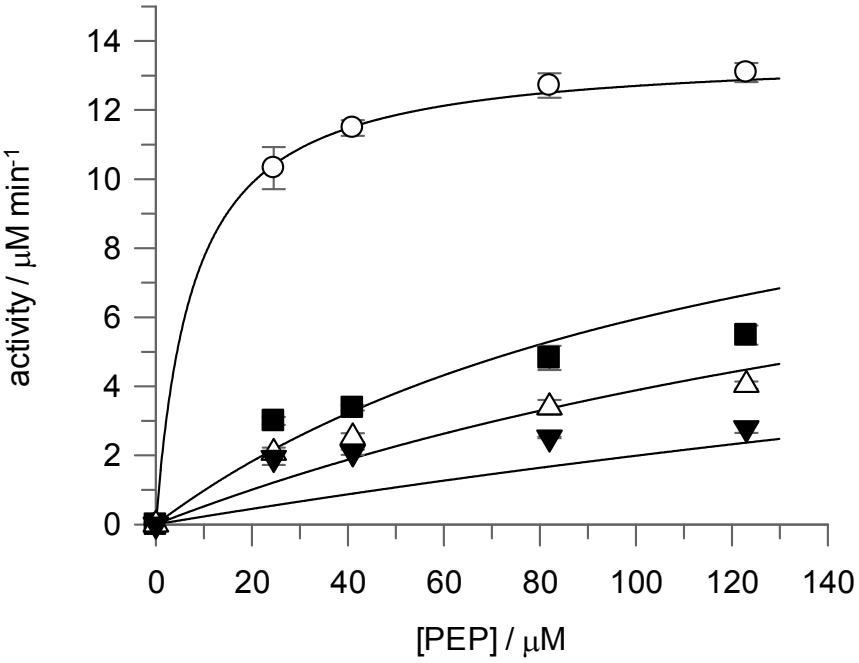


Figure S 3: Enzyme assay results for inhibitor **C8R**, inhibitor concentrations: empty circles: no inhibitor; solid squares: 54 μM; empty triangles: 108 μM; solid triangles: 216 μM. The global fit gave V_{max} 13.7 ± 0.8 μM min^{-1}, K_m 7 ± 3 μM, K_i 3 ± 1 μM.

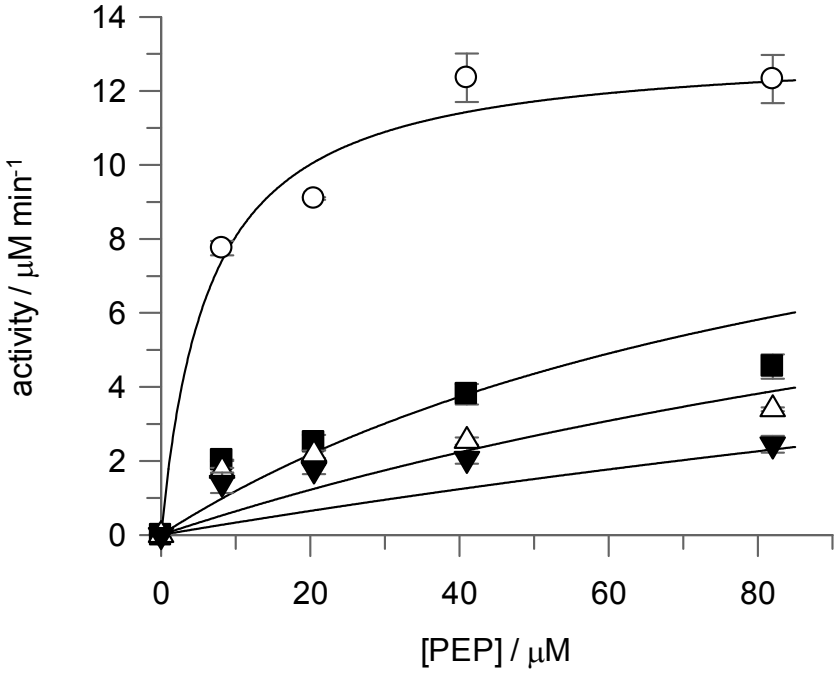


Figure S 4: Enzyme assay results for inhibitor **C8S**, inhibitor concentrations: empty circles: no inhibitor; solid squares: 70 μM; empty triangles: 140 μM; solid triangles: 280 μM. The global fit gave V_{max} 13.2 ± 0.9 μM min^{-1}, K_m 6 ± 2 μM, K_i 5 ± 1 μM.

Modeling procedure

This procedure was modified from a previous report.[10] The structures of the ligands for docking were built using the protein builder in Maestro [12] of the Schrödinger Suite 2011 and then minimized with MacroModel.[13] Ligands were generated as fully deprotonated penta-anions, only the more inhibitory *R*-enantiomers were used for docking studies. 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). An ensemble of low-energy conformers for docking was generated using MCMM Serial Torsional Sampling with a GB/SA water model and the OPLS2005 force field, with 3000 steps for the conformational search with an energy window of 12 kJ/mol for appropriate conformers. The conformational search returned conformers which were largely similar to each other, with similar relative placement of phosphate and carboxylate moieties but varying with respect to the torsional angles along the carbon chain. Three conformers which sampled representative carbon chain torsions were picked from the conformational ensemble and used in the induced fit docking.

The conformation of the active site of *M. tuberculosis* DAH7PS in complex with the C7 inhibitor (pdb code 3PFP) was used as the starting point for the induced fit docking of **C6R** and **C8R** with the Schrödinger Suite 2011 Induced Fit Docking workflow.[14] The active site of chain A of the asymmetric unit was used as the receptor, chain B was removed from the model before docking. The native C7 ligand from the crystal structure (pdb code 3PFP) was defined as the centre of the receptor grid and the box size automatically determined by the Schrödinger software. For the initial docking, the van der Waals radii of the linear intermediate atoms were scaled to 0.8, the receptor atoms were scaled to 1.0. The 20 best solutions from the initial docking were kept. All residues of the protein within a 5 Å distance of the docking pose of the respective ligand molecule 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. Docking solutions which retained the interactions of the 2-phosphoryl carboxylic acid moiety headgroup as observed in the native crystal structure (pdb code 3PFP) were picked. The selection was further refined by elimination of solutions which had unfavourable carbon chain backbone torsions or clashes with protein atoms.

As a benchmark of docking performance, the native C7 ligand was redocked into the receptor. The docking results overlayed with the native binding mode observed in the 3PFP crystal structure with an rmsd of 0.305 Å.

Induced fit docking of the newly synthesised inhibitors **C6R** and **C8R** showed largely unaltered interactions with and identical placement of the 2-phosphoryl carboxylate moiety when compared with the **C7R** enzyme complex crystal structure (Figure S 5, panels **A** and **B**). The docking solutions highlight the importance of the appropriate length carbon chain linker: the docking pose of the **C6R** inhibitor with truncated carbon chain does not allow placement of the 6-phosporyl moiety in the site that was occupied by the 7-phosporyl group in the enzyme-**C7R** complex (Figure S 5, panel **A**). The induced fit docking algorithm did rearrange the mobile KPRS motif in order to optimize enzyme ligand interactions: the positively charged sidechain of Arg135 shifted by 1.7Å (distance between the carbons in the guanidinium groups) when compared to the crystal structure used as the starting point for the induced fit docking (3PLP), which results in a similar distance between this moiety and the terminal phosphate of the ligand in both the modeled and the native crystal structure. Ser136 was also shifted by the induced fit docking protocol, but to a lesser extend, which results in the hydrogen bonding interactions between the backbone N-H and hydroxyl group of Ser136 and the respective ligand being less favourable in the pose for the **C6R** ligand when compared to the values from the **C7**-enzyme complex crystal structure.(Figure S 5, panel **C**, refer to Table S1 for hydrogen bond distances and angles).

Table S1: Hydrogen bond distances and angles from induced fit docking results (**C6R** and **C8R**) and crystal structure (**C7R**).[a]

	d(H-bond1 Ser136-OH-ligand) [Å]	**angle (H-bond1) [°]**	**d(H-bond2 Ser136-NH-ligand [Å]**	**Angle (H-bond2) [°]**
C6R docking	2.9	123.0	3.0	153.0
C7R crystal structure	2.7	130.6	2.8	166.4
C8R docking	3.0	112.7	3.1	122.9

[a]H-bond1: the distance corresponds to the distance between the Ser136-hydroxyl oxygen and the closest phosphate oxygen on the ligand. The angle corresponds to the angle defined by the phosphorous atom, the phosphate oxygen and the Ser136-hydroxyl oxygen making the hydrogen bond. H-bond2: the distance corresponds to the distance between the Ser136-backbone amide nitrogen and the closest phosphate oxygen on the ligand. The angle corresponds to the angle defined by phosphorous atom, the phosphate oxygen and the Ser136-backbone amide nitrogen making the hydrogen bond.

The docking poses obtained from induced fit docking of **C8R** show a preference for the C8-phosphate being placed in the same location in which the corresponding 7-phosphate was found in the **C7**-enzyme complex crystal structure, despite the longer carbon chain of the **C8R** inhibitor (Figure S 5, panel **B**). This results in **C8R** being docked in a collapsed and twisted rather than linear conformation, which might represent a higher energy conformer of the ligand. The twisted conformation of the ligand in turn causes the hydrogen bonding geometry between Ser136 and the C8-phosphate to be less favourable when compared to the C7 ligand (Table S1). This docking solution is the only pose in which H-bond2 is formed between the Ser136 backbone N-H and the alkyl-substituted oxygen on the 8-phosphoryl

group, all other structures show the Ser136 backbone N-H bonded to a charged phosphate oxygen (Figure S 5, panels **D** and **E**).

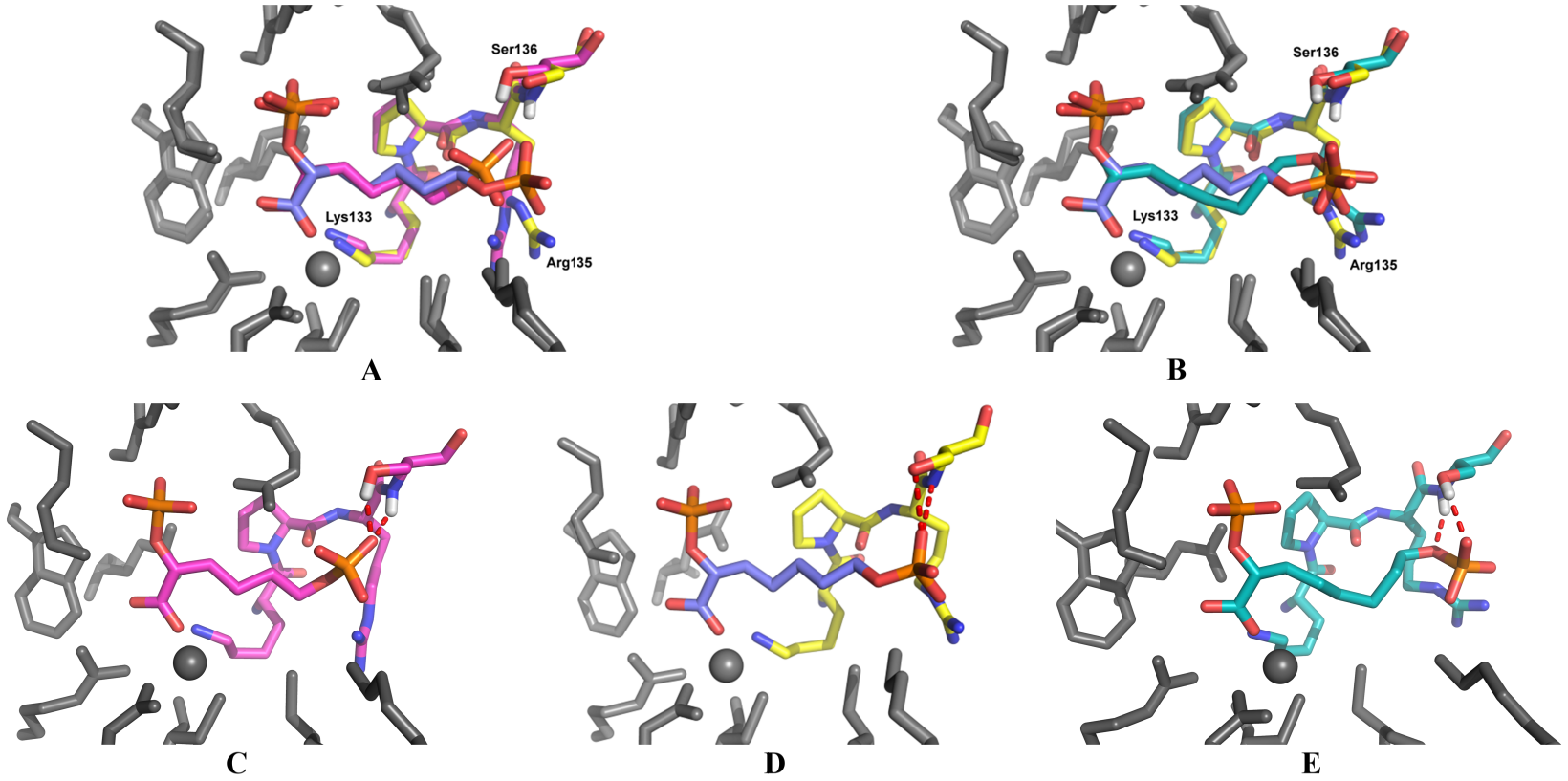


Figure S 5: Comparison of induced fit docking results for **C6R** and **C8R** with the crystal structure of the **C7R**-enzyme complex, Lys133-Pro134-Ar135-Ser136 belonging to the mobile KPRS motif highlighted by colored sticks, all other enzyme residues shown in gray. **A**: superposition of the **C7R**-enzyme complex crystal structure (protein: yellow carbons, **C7R**: blue carbons) and the docking result for inhibitor **C6R** (magenta carbons). **B**: superposition of the **C7R**-enzyme complex crystal structure (protein: yellow carbons, **C7R**: blue carbons) and the docking result for inhibitor **C8R** (cyan carbons). **C**: docking pose of inhibitor **C6R**, hydrogen bonds between the ligand and Ser136 shown as red dashes. **D**: C7R-enzyme complex crystal structure, the hydrogen bonds between the ligand and Ser 136 shown as red dashes. **E**: docking pose of inhibitor **C8R**, hydrogen bonds between the ligand and Ser136 shown as red dashes.

References

1. Armarego, W. L. F.; Chai, C., *Purification of Laboratory Chemicals*. 5th ed.; Butterworth-Heinemann: Oxford, 2003.

2. Matsuo, Y.; Muramatsu, A.; Tahara, K.; Koide, M.; Nakamura, E., *Org. Synth.* 2006, *83*, 80-87.

3. (a) Parmar, D.; Duffy, L. A.; Sadasivam, D. V.; Matsubara, H.; Bradley, P. A.; Flowers, R. A.; Procter, D. J., *J. Am. Chem. Soc.* 2009, *131* (42), 15467-15473; (b) Thompson, A. M.; Delaney, A. M.; Hamby, J. M.; Schroeder, M. C.; Spoon, T. A.; Crean, S. M.; Showalter, H. D. H.; Denny, W. A., *J. Med. Chem.* 2005, *48* (14), 4628-4653.

4. (a) Frankowski, K. J.; Golden, J. E.; Zeng, Y.; Lei, Y.; Aubé, J., *J. Am. Chem. Soc.* 2008, *130* (18), 6018-6024; (b) Ziegler, F. E.; Klein, S. I.; Pati, U. K.; Wang, T. F., *J. Am. Chem. Soc.* 1985, *107* (9), 2730-7.

5. Dai, C.; Narayanam, J. M. R.; Stephenson, C. R. J., *Nature Chem.* 2011, *3* (2), 140-145.

6. Love, B. E.; Jones, E. G., *J. Org. Chem.* 1999, *64* (10), 3755-3756.

7. Harrison, A. N.; Reichau, S.; Parker, E. J., *Bioorg. Med. Chem. Lett.* 2012, *22* (2), 907-911.

8. Lanzetta, P. A.; Alvarez, L. J.; Reinach, P. S.; Candia, O. A., *Anal. Biochem.* 1979, *100* (1), 95-97.

9. Webby, C. J.; Baker, H. M.; Lott, J. S.; Baker, E. N.; Parker, E. J., *J. Mol. Biol.* 2005, *354* (4), 927-939.

10. Reichau, S.; Jiao, W.; Walker, S. R.; Hutton, R. D.; Baker, E. N.; Parker, E. J., *J. Biol. Chem.* 2011, *286* (18), 16197-16207.

11. *GraFit*, Version 5.0.13; Erithacus Software Limited: 2006.

12. *Maestro*, Version 9.2; Schrodinger, LLC: New York, NY, 2011.

13. *MacroModel*, Version 9.9; Schrodinger, LLC: New York, NY, 2011.

14. *Schrodinger Suite 2011 Induced Fit Protocol; Glide version 5.7; Prime version 3.0*, Schrodinger, LLC: New York, NY, 2011.