Supplementary Information

Design of Potential Bisubstrate Inhibitors against *Mycobacterium tuberculosis* (Mtb) 1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase (Dxr)—Evidence of a Novel Binding Mode

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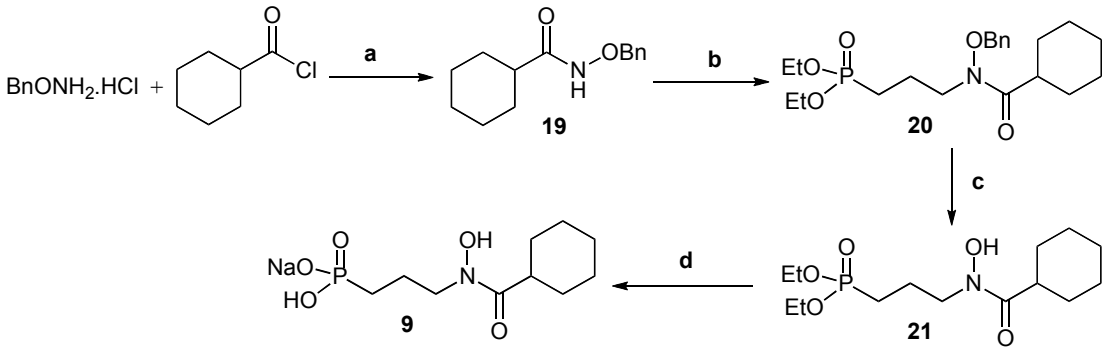
Scheme 3. Synthesis of amide ligand **9**.[a]



[a]*Reagents and conditions:* (a) Et_3N, CH_2Cl_2, 58 %; (b) NaH, NaI, **10**, THF, 70°C, 73 %; (c) H_2, Pd/C, MeOH, 82 %; (d) i: TMSBr, CH_2Cl_2, ii: H_2O, iii: NaOHaq, 74 %.

General. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O on a Varian spectrometer at 200 and 50 MHz, respectively, with TMS or acetone as internal standard. Spin multiplicities are given with the following abbreviations: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quadruplet), qt (quintuplet), m (multiplet). Coupling constants *J* are given in Hertz. Mass spectra were measured in the ESI mode on an LC-MS (Agilent 1100). Thin layer chromatography (TLC) was performed on Merck 60 F_{254} silica gel plates and flash column chromatography was carried out using Merck silica gel 60 (35-70 μm). All reagents were purchase from commercial suppliers and used without further purification. THF and dichloromethane were distilled under argon immediately before use, respectively from sodium/benzophenone and calcium hydride. All air sensitive reactions were carried out under an argon atmosphere. Purity of compounds (>95%) was determined by $^1H/^{13}C$ NMR, LC-DAD-MS and HRMS.

General Procedure for the Deprotection of Ether and Amide Ligands 3-9.

To a solution of diethyl 3-(*N*-(aryloxy)acetamido)propylphosphonate or diethyl 3-(*N*-hydroxy-arylamido)propylphosphonate (1 eq) in CH_2Cl_2 (1.7 mL/mmol of phosphonate) at 0°C was added bromotrimethylsilane (8 eq) dropwise. The reaction mixture was stirred overnight at room temperature. Ethyl bromide and excess silylating agent were removed by rotary evaporation at room temperature. The concentrate was solubilized in dry CH_2Cl_2 and evaporated (×2). H_2O was added to the residue, and the mixture was stirred overnight at room temperature. The solution was filtered (except for products with a low solubility in water) and concentrated *in vacuo* at 50°C. The crude acid was rapidly neutralized with aqueous NaOH (1 eq) and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* at 50°C. No further purification was necessary.

Sodium hydrogen 3-(*N*-(phenethyloxy)acetamido)propylphosphonate (3). Prepared from **13a** (45 mg, 0.126 mmol). Quantitative yield (light yellow solid). ^{1}H NMR (200 MHz, D_2O) δ (24:76 mixture of two conformers) 7.47-7.21 (m, 5H), 4.16 (t, 2H, J = 5.9 Hz), 3.54 (t, 2H, J = 6.3 Hz), 3.09-2.98 (m, 24/100 of 2H), 2.93 (t, 76/100 of 2H, J = 5.9 Hz), 2.02 (s, 24/100 of 3H), 1.92 (s, 76/100 of 3H), 1.79-1.32 (m, 4H). ^{13}C NMR (50 MHz, D_2O) δ 174.7, 138.9, 129.6, 129.4, 127.3, 75.5, 46.1 (d, J = 18.7 Hz), 34.4, 25.4 (d, J = 132.5 Hz), 21.7 (d, J = 40.7 Hz), 19.7. HRMS (ESI^+) *m/z* calcd for $C_{13}H_{20}NNaO_5P$ $[M+H]^+$: 324.0971, found: 324.0972.

Sodium hydrogen 3-(*N*-(3-phenylpropoxy)acetamido)propylphosphonate (4). Prepared from **13b** (50 mg, 0.135 mmol). Yield 88% (light yellow solid). ^{1}H NMR (200 MHz, D_2O) δ (33:67 mixture of two conformers) 7.46-7.26 (m, 5H), 4.13 (t, 33/100 of 2H, J = 6.2 Hz), 3.99 (t, 67/100 of 2H, J = 6.2 Hz), 3.69 (t, 67/100 of 2H, J = 7.0 Hz), 3.40 (t, 33/100 of 2H, J = 7.0 Hz), 2.80 (t,

67/100 of 2H, *J* = 7.6 Hz), 2.79 (t, 33/100 of 2H, *J* = 7.6 Hz), 2.18 (s, 3H), 2.13-1.49 (m, 6H). ^{13}C NMR (50 MHz, D_2O) δ 174.6, 142.3, 129.3, 129.2, 126.8, 74.2, 46.1 (d, *J* = 19.8 Hz), 31.9, 29.4, 25.6 (d, *J* = 133.6 Hz), 21.8 (d, *J* = 29.7 Hz), 19.8. HRMS (ESI$^+$) *m/z* calcd for $C_{14}H_{22}NNaO_5P$ $[M+H]^+$: 338.1128, found: 338.1128.

Sodium hydrogen 3-(*N*-(4-isopropylbenzyloxy)acetamido)propylphosphonate (5). Prepared from **13c** (50 mg, 0.130 mmol). Yield 61% (white solid). ^{1}H NMR (200 MHz, D_2O) δ (75:25 mixture of two conformers) 7.43 (d, 2H, *J* = 8.2 Hz), 7.36 (d, 2H, *J* = 8.2 Hz), 4.92 (s, 2H), 3.83-3.57 (m, 2H), 3.09-2.85 (m, 1H), 2.13 (s, 25/100 of 3H), 2.04 (s, 75/100 of 3H), 1.97-1.72 (m, 2H), 1.62-1.36 (m, 2H), 1.21 (d, 6H, *J* = 6.9 Hz). ^{13}C NMR (50 MHz, D_2O) δ 175.1, 151.6, 131.8, 130.9, 127.6, 76.4, 46.5 (d, *J* = 18.6 Hz), 34.1, 26.3 (d, *J* = 132.9 Hz), 23.8, 22.3 (d, *J* = 36.9 Hz), 20.0. HRMS (ESI$^+$) *m/z* calcd for $C_{15}H_{24}NNaO_5P$ $[M+H]^+$: 352.1284, found: 352.1285.

Sodium hydrogen 3-(*N*-(cyclohexylmethoxy)acetamido)propylphosphonate (6). Prepared from **13d** (50 mg, 0.143 mmol). Quantitative yield (white solid). ^{1}H NMR (200 MHz, D_2O) δ (70:30 mixture of two conformers) 3.86-3.62 (m, 85/100 of 4H), 3.05 (t, 15/100 of 4H, *J* = 7.1 Hz), 2.14 (s, 70/100 of 3H), 2.06 (s, 30/100 of 3H), 2.00-1.42 (m, 9H), 1.39-0.82 (m, 6H). ^{13}C NMR (50 MHz, D_2O) δ 174.6, 80.2, 45.6 (d, *J* = 20.9 Hz), 36.8, 29.8, 26.5, 25.9, 24.8 (d, *J* = 129.7 Hz), 21.4 (d, *J* = 43.8 Hz), 19.8. HRMS (ESI$^+$) *m/z* calcd for $C_{12}H_{24}NNaO_5P$ $[M+H]^+$: 316.1284, found: 316.1285.

Sodium hydrogen 3-(*N*-hydroxy-2-phenylacetamido)propylphosphonate (7). Prepared from **18a** (50 mg, 0.152 mmol). Quantitative yield (white solid). ^{1}H NMR (200 MHz, D_2O) δ (80:20

mixture of two conformers) 7.47-7.23 (m, 5H), 3.85 (s, 80/100 of 2H), 3.79 (s, 20/100 of 2H), 3.68 (t, 80/100 of 2H, J = 6.9 Hz), 3.35 (t, 20/100 of 2H, J = 7.5 Hz), 1.98-1.72 (m, 2H), 1.72-1.46 (m, 2H). ^{13}C NMR (50 MHz, D_2O) δ 174.9, 135.6, 129.9, 129.5, 127.7, 49.3 (d, J = 19.0 Hz), 39.4, 24.8 (d, J = 134.8 Hz), 20.8. HRMS (ESI$^+$) *m/z* calcd for $C_{11}H_{16}NNaO_5P$ $[M+H]^+$: 296.0658, found: 296.0658.

Sodium hydrogen 3-(*N*-hydroxy-4-phenylbutanamido)propylphosphonate (8). Prepared from **18b** (50 mg, 0.140 mmol). Quantitative yield (white solid). ^{1}H NMR (200 MHz, D_2O) δ (58:42 mixture of two conformers) 7.42-7.18 (m, 5H), 3.62 (t, 58/100 of 2H, J = 6.7 Hz), 3.34 (t, 42/100 of 2H, J = 7.3 Hz), 2.64 (t, 2H, J = 7.5 Hz), 2.48 (t, 58/100 of 2H, J = 7.4 Hz), 2.34 (t, 42/100 of 2H, J = 7.4 Hz), 2.06-1.42 (m, 6H). ^{13}C NMR (50 MHz, D_2O) δ 176.8, 142.8, 129.3, 126.8, 49.2 (d, J = 19.0 Hz), 35.2, 31.9, 26.7, 25.1 (d, J = 134.4 Hz), 21.0. HRMS (ESI$^+$) *m/z* calcd for $C_{13}H_{20}NNaO_5P$ $[M+H]^+$: 324.0971, found: 324.0970.

Sodium hydrogen 3-(*N*-hydroxycyclohexanecarboxamido)propylphosphonate (9). Prepared from **21** (52 mg, 0.162 mmol). Yield 74% (light yellow solid). ^{1}H NMR (200 MHz, D_2O) δ (59:41 mixture of two conformers) 3.65 (t, 59/100 of 2H, J = 6.2 Hz), 3.34 (t, 41/100 of 2H, J = 7.5 Hz), 3.12-2.84 (m, 1H), 2.73-2.45 (m, 1H), 2.10-1.45 (m, 8H), 1.44-1.00 (m, 5H). ^{13}C NMR (50 MHz, D_2O) δ 179.9, 49.2 (d, J = 19.0 Hz), 40.2, 29.0, 26.0, 25.8, 25.3 (d, J = 133.3 Hz), 21.0. HRMS (ESI$^+$) *m/z* calcd for $C_{10}H_{20}NNaO_5P$ $[M+H]^+$: 288.0971, found: 288.0971.

***N*-(Benzyloxy)acetamide.**[1] A mixture of *O*-benzylhydroxylamine hydrochloride (10 g, 62.6 mmol), CH_2Cl_2 (200 mL) and triethylamine (10 mL, 68.9 mmol, 1.1 eq) was stirred for 1h at

0°C. Acetyl chloride (5 mL, 68.9 mmol, 1.1 eq) was added at 0°C and the resulting mixture was stirred overnight at room temperature. The organic layer was washed twice with H_2O. The aqueous phase was extracted with dichloromethane. The combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. Flash chromatography (hexane/EtOAc 4/1 then 2/1, then CH_2Cl_2/MeOH 20/1) gave ***N*-(Benzyloxy)acetamide** (8.6 g, 83%) as a light yellow oil, which became a solid after passing in the freezer. ^{1}H NMR (200 MHz, $CDCl_3$) d (mixture of two conformers) 8.63 and 8.22 (2× s, 1H, major and minor), 7.49-7.28 (m, 5H), 4.87 and 4.84 (2× s, 2H, major and minor), 1.99 and 1.85 (2× s, 3H, minor and major). Spectral values are in agreement with the literature.[1] MS (ESI$^+$) *m/z* 166.1 $[M+H]^+$. Mp: 40°C.

Diethyl (3-bromopropyl)phosphonate (10).[2] Triethyl phosphite (10 mL, 58.3 mmol) and 1,3-dibromopropane (18 mL, 174.9 mmol, 3 eq) were heated in a microwave for 20 min under 20% power. Reaction followed by GC/MS. Distillation under reduced pressure using a Kugelrohr allowed to remove the excess of 1,3-dibromopropane and afford the pure phosphonate **10** (13.1 g, 87%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) d 4.20-3.95 (m, 4H), 3.48 (t, 2H, J = 6.3 Hz), 2.23-2.00 (m, 2H), 1.98-1.70 (m, 2H), 1.34 (t, 6H, J = 7.0 Hz). Spectral values are in agreement with the literature.[3] MS (ESI$^+$) *m/z* 259.0 $[M+H]^+$.

Diethyl 3-(*N*-(benzyloxy)acetamido)propylphosphonate (11). To a solution of *N*-(benzyloxy)acetamide (413 mg, 2.5 mmol) in THF (4 mL) at 0°C was added sodium hydride 60% in oil (110 mg, 2.7 mmol, 1.1 eq) in suspension in THF (2 mL). The reaction mixture was allowed to warm to room temperature and **10** (713 mg, 2.7 mmol, 1.1 eq) was added in solution

in THF (2.8 mL). Then, sodium iodide (cat.) was added and the mixture was heated overnight at 70°C. NaH was neutralized with water at 0°C, the organic layer was washed with H_2O and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/MeOH 99/1 to 7/3) to furnish **11** (648 mg, 75%) as a light yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) d (mixture of conformers) *Conformer A (minor):* 7.40-7.22 (m, 5H), 4.92 (s, 2H), 4.18-3.90 (m, 6H), 2.05-1.57 (m, 4H), 1.94 (s, 3H), 1.31 (t, 6H, J = 7.0 Hz). *Conformer B (major):* 7.41-7.32 (m, 5H), 4.82 (s, 2H), 4.07 (qt, 4H, J = 7.0 Hz), 3.71 (t, 2H, J = 6.8 Hz), 2.09 (s, 3H), 2.00-1.63 (m, 4H), 1.30 (t, 6H, J = 7.0 Hz). Spectral values are in agreement with the literature.[4] MS (ESI$^+$) *m/z* 344.1 $[M+H]^+$.

Diethyl 3-(*N*-hydroxyacetamido)propylphosphonate (12). To a solution of **11** (622 mg, 1.8 mmol) in methanol (20 mL) was added 80 mg of Pd/C (10%). The mixture was stirred for 2h at room temperature under hydrogen, filtered over celite and evaporated. Flash chromatography (EtOAc/MeOH 20/1) gave **12** (349 mg, 76%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) d 9.71 (br s, 1H), 4.04 (qt, 4H, J = 7.0 Hz), 3.71 (t, 2H, J = 6.0 Hz), 2.14 (s, 3H), 2.05-1.72 (m, 4H), 1.30 (t, 6H, J = 7.0 Hz). Spectral values are in agreement with the literature.[5] MS (ESI$^+$) *m/z* 254.1 $[M+H]^+$.

General Procedure for the Preparation of the Ether Ligands 13a-d.

To a solution of **12** (1 eq) in THF (3.9 mL/mmol of **12**) at 0°C was added sodium hydride (1.1 eq) in suspension in THF (1.2 mL/mmol of **12**). The reaction mixture was allowed to room temperature and the desired arylbromide (1.1-2.0 eq) was added. Then, the mixture was heated at

70°C for 2-24h. NaH was neutralized with water at 0°C, the organic layer was washed with H_2O and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel (EtOAc/MeOH) gave the expected ether.

Diethyl 3-(*N*-(phenethyloxy)acetamido)propylphosphonate (13a). Reaction time: 1 night, eluant for flash chromatography: EtOAc/MeOH 20/1. Yield 27% (yellow oil), using 50 mg (0.197 mmol) of **12** and 29 mL (0.217 mmol) of (2-bromoethyl)benzene. ^{1}H NMR (200 MHz, $CDCl_3$) d 7.46-7.16 (m, 5H), 4.27-3.94 (m, 6H), 3.77-3.53 (m, 2H), 3.05-2.83 (m, 2H), 2.25-1.54 (m, 4H), 1.97 (s, 3H), 1.32 (t, 6H, J = 7.0 Hz). MS (ESI^+) *m/z* 358.2 $[M+H]^+$.

Diethyl 3-(*N*-(3-phenylpropoxy)acetamido)propylphosphonate (13b). Reaction time: 3h, eluant for flash chromatography: EtOAc/MeOH 20/1. Yield 81% (yellow oil), using 100 mg (0.395 mmol) of **12** and 66 mL (0.434 mmol) of 1-bromo-3-phenylpropane. ^{1}H NMR (200 MHz, $CDCl_3$) d 7.38-7.14 (m, 5H), 4.09 (qt, 4H, J = 7.0 Hz), 3.83 (t, 2H, J = 6.4 Hz), 3.65 (t, 2H, J = 6.8 Hz), 2.73 (t, 2H, J = 7.7 Hz), 2.12 (s, 3H), 2.04-1.60 (m, 6H), 1.32 (t, 6H, J = 7.0 Hz). MS (ESI^+) *m/z* 372.2 $[M+H]^+$.

Diethyl 3-(*N*-(4-isopropylbenzyloxy)acetamido)propylphosphonate (13c). Reaction time: 2h, eluant for flash chromatography: EtOAc/MeOH 30/1 then 20/1 then 7.5/1. Yield 61% (yellow oil), using 150 mg (0.592 mmol) of **12** and 109 mL (0.651 mmol) of 4-isopropylbenzyl bromide. ^{1}H NMR (200 MHz, $CDCl_3$) d 7.47-7.19 (m, 4H), 4.78 (s, 2H), 4.07 (qt, 4H, J = 7.0 Hz), 3.72 (t,

2H, *J* = 6.4 Hz), 3.03-2.82 (m, 1H), 2.10 (s, 3H), 2.06-1.58 (m, 4H), 1.38-1.20 (m, 12H). MS (ESI^+) *m/z* 386.2 $[M+H]^+$.

Diethyl 3-(*N*-(cyclohexylmethoxy)acetamido)propylphosphonate (13d). Reaction time: 24h, eluant for flash chromatography: EtOAc/MeOH 30/1 then 20/1 then 7.5/1. Yield 34% (yellow oil), using 150 mg (0.592 mmol) of **12** and 91 mL (0.651 mmol) of (bromomethyl)cyclohexane. ^1H NMR (200 MHz, $CDCl_3$) d 4.20-3.96 (m, 4H), 3.65 (t, 2H, *J* = 6.9 Hz), 3.60 (d, 2H, *J* = 6.2 Hz), 2.10 (s, 3H), 2.05-1.48 (m, 11H), 1.31 (t, 6H, *J* = 7.1 Hz), 1.26-0.89 (m, 4H). MS (ESI^+) *m/z* 350.2 $[M+H]^+$.

Diethyl 3-(*N*-(benzyloxy)-2-phenylacetamido)propylphosphonate (17a). To a solution of diethyl 3-(benzyloxyamino)propylphosphonate **16** (590 mg, 1.96 mmol) in CH_2Cl_2 (10 mL) at 0°C were added triethylamine (0.33 mL, 2.37 mmol, 1.2 eq) and phenylacetyl chloride (0.36 mL, 2.70 mmol, 1.4 eq). The resulting mixture was stirred overnight at room temperature. The organic layer was washed successively with water, saturated $NaHCO_3$, and water, and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure gave a crude product, which was purified with an Isolera Flash Chromatography System (Hexane/EtOAc 43% Hexane) to give **17a** (411 mg, 50%) as a colorless oil. ^1H NMR (200 MHz, $CDCl_3$) d 7.43-7.34 (m, 5H), 7.32-7.18 (m, 5H), 4.78 (s, 2H), 4.04 (qt, 4H, *J* = 7.0 Hz), 3.74 (t, 2H, *J* = 7.0 Hz), 3.72 (s, 2H), 2.05-1.86 (m, 2H), 1.74-1.57 (m, 2H), 1.29 (t, 6H, *J* = 7.0 Hz). MS (ESI^+) *m/z* 420.1 $[M+H]^+$.

Diethyl 3-(*N*-(benzyloxy)-4-phenylbutanamido)propylphosphonate (17b). To a solution of diethyl 3-(benzyloxyamino)propylphosphonate **16** (616 mg, 2.04 mmol) in CH_2Cl_2 (15 mL) at

0°C were added triethylamine (217 mg, 2.14 mmol, 1.05 eq) and 4-phenylbutyryl chloride (0.4 mL, 2.25 mmol, 1.1 eq). The resulting mixture was stirred overnight at room temperature. The organic layer was washed successively with water (25mL), saturated $NaHCO_3$ (30mL), and water (25mL), and dried over anhydrous magnesium sulfate. Filtration and evaporation of the solvent under reduced pressure gave a crude product, which was purified with an Isolera Flash Chromatography System (EtOAc/MeOH 1-5% MeOH) to give **17b** (690 mg, 75%) as a light yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) d 7.40-7.16 (m, 10H), 4.74 (s, 2H), 4.19-4.00 (m, 4H), 3.72 (t, 2H, *J* = 7.0 Hz), 2.65 (t, 2H, *J* = 7.5 Hz), 2.41 (t, 2H, *J* = 7.4 Hz), 2.04-1.67 (m, 6H), 1.30 (t, 6H, *J* = 7.0 Hz). MS (ESI^+) *m/z* 448.1 $[M+H]^+$.

Diethyl 3-(*N*-hydroxy-2-phenylacetamido)propylphosphonate (18a). To a solution of **17a** (411 mg, 0.98 mmol) in methanol (20 mL) and ethyl acetate (2 mL) was added 10% Pd/C (cat.). The resulting mixture was stirred for 6h at room temperature under hydrogen, filtered over celite and evaporated. Purification with an Isolera Flash Chromatography System (EtOAc/MeOH 5-10% MeOH) gave **18a** (192 mg, 59%) as a light yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) d 9.80 (s, 1H), 7.33-7.21 (m, 5H), 4.19-3.94 (m, 4H), 3.86 (s, 2H), 3.76 (t, 2H, *J* = 5.9 Hz), 2.04-1.72 (m, 4H), 1.30 (t, 6H, *J* = 7.0 Hz). MS (ESI^+) *m/z* 330.1 $[M+H]^+$.

Diethyl 3-(*N*-hydroxy-4-phenylbutanamido)propylphosphonate (18b). To a solution of **17b** (690 mg, 1.54 mmol) in methanol (20 mL) was added 10% Pd/C (cat.). The resulting mixture was stirred overnight at room temperature under hydrogen, filtered over celite and evaporated. Purification with an Isolera Flash Chromatography System (EtOAc/MeOH 1-10% MeOH) gave **18b** (306 mg, 56%) as a light yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) d 9.58 (s, 1H), 7.31-7.18

(m, 5H), 4.19-3.96 (m, 4H), 3.83-3.63 (m, 2H), 2.68 (t, 2H, *J* = 7.6 Hz), 2.57 (t, 2H, *J* = 7.6 Hz), 2.04-1.74 (m, 6H), 1.30 (t, 6H, *J* = 7.0 Hz). MS (ESI^+) *m/z* 358.1 $[M+H]^+$.

***N*-(benzyloxy)cyclohexanecarboxamide (19).** To a solution of *O*-benzylhydroxylamine hydrochloride (260 mg, 1.63 mmol) and triethylamine (0.45 mL, 3.23 mmol, 2 eq) in CH_2Cl_2 (15 mL) at 0°C was added cyclohexanecarbonyl chloride (0.29 mL, 2.17 mmol, 1.3 eq). The resulting mixture was stirred overnight at room temperature. The organic layer was washed successively with water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. Purification by recrystallization gave **19** (220 mg, 58%) as a white solid. ^1H NMR (200 MHz, $CDCl_3$) d 7.82 (s, 1H), 7.38 (s, 5H), 4.90 (s, 2H), 1.78-1.21 (m, 11H). MS (ESI^+) *m/z* 234.1 $[M+H]^+$.

Diethyl 3-(*N*-(benzyloxy)cyclohexanecarboxamido)propylphosphonate (20). A solution of **19** (210 mg, 0.90 mmol) in THF (10 mL) was added dropwise to a suspension of sodium hydride 60% in oil (44 mg, 1.10 mmol, 1.2 eq) in THF (5 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and a solution of **10** (288 mg, 1.11 mmol, 1.2 eq) in THF (5 mL) was added. The resulting mixture was stirred overnight at 70°C. The organic layer was washed successively with water and brine, dried over $MgSO_4$, filtered and evaporated. Flash chromatography using an Isolera system (EtOAc/MeOH 0-10% MeOH) gave **20** (271 mg, 73%) as a yellow oil. ^1H NMR (200 MHz, $CDCl_3$) d 7.38 (s, 5H), 4.81 (s, 2H), 4.07 (qt, 4H, *J* = 7.0 Hz), 3.71 (t, 2H, *J* = 6.8 Hz), 2.62 (t, 1H, *J* = 11.4 Hz), 1.99-1.63 (m, 9H), 1.56-1.07 (m, 5H), 1.30 (t, 6H, *J* = 7.0 Hz). MS (ESI^+) *m/z* 412.1 $[M+H]^+$.

Diethyl 3-(*N*-hydroxycyclohexanecarboxamido)propylphosphonate (21). To a solution of **20** (264 mg, 0.64 mmol) in MeOH (10 mL) was added 10% Pd/C (cat.). The resulting mixture was stirred for 4h at room temperature under hydrogen, filtered over celite and evaporated. Purification with an Isolera Flash Chromatography System (EtOAc/MeOH 1-5% MeOH) gave **21** (169 mg, 82%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) d 9.50 (s, 1H), 4.06 (qt, 4H, J = 7.0 Hz), 3.73 (t, 2H, J = 6.9 Hz), 3.03-2.83 (m, 1H), 2.10-1.68 (m, 9H), 1.50-1.2 (m, 5H), 1.31 (t, 6H, J = 7.0 Hz). MS (ESI^+) *m/z* 322.1 $[M+H]^+$.

Mtb Dxr expression and purification

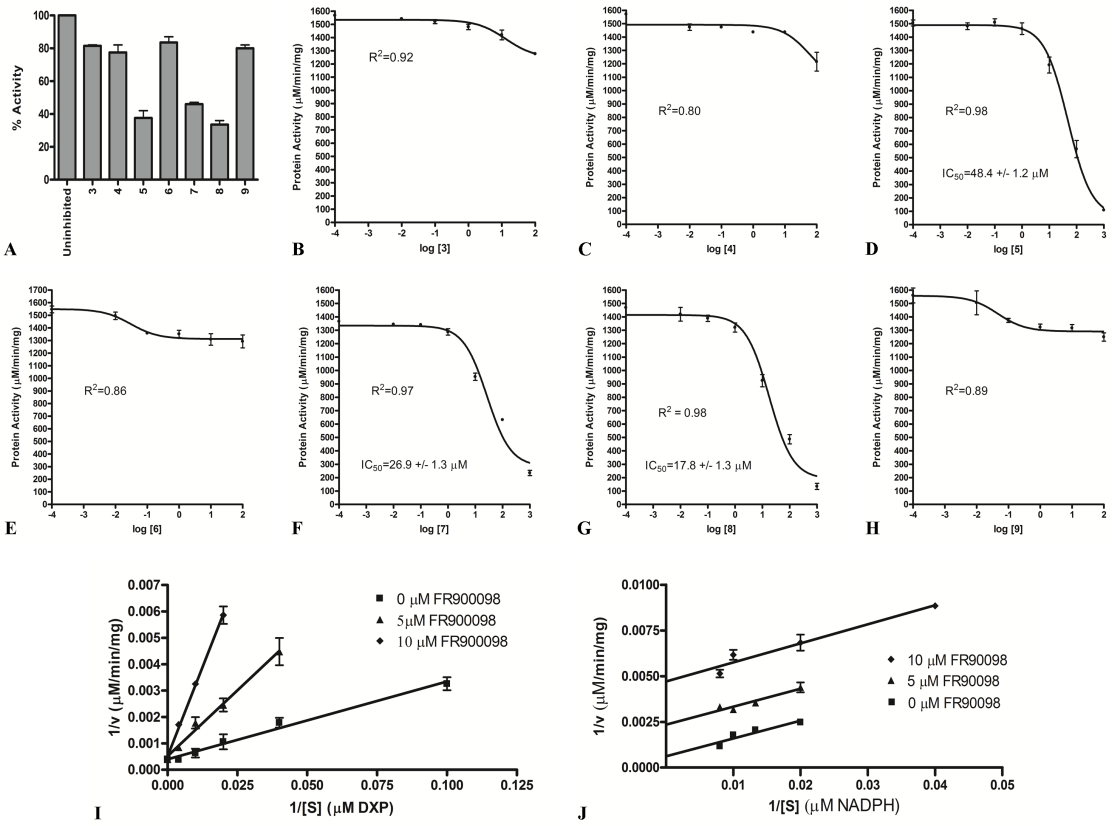
Escherichia coli BL21 (DE3) cells (Life Technologies) were used for recombinant protein expression, cultured in a one liter shake flask at 37 ^oC, 250 rpm, in Luria-Bertani (LB) media containing 100 μg/ml ampicillin. Upon reaching an OD_{600} of 1.1, protein expression was induced by the addition of 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG). After incubation for an additional 18 hours, cells were harvested by centrifugation and stored at -80 ^oC. To purify the His-tagged protein, the cell pellet was thawed then cells were lysed using Lysis Buffer A (100 mM Tris pH 8, .032% lysozyme; 3 mL per mg cell pellet), followed by Lysis Buffer B (0.1 M $CaCl_2$, 0.1 M $MgCl_2$, 0.1 M NaCl, .020 % DNase; 0.3 mL per mg cell pellet). Clarified cell lysate was obtained by centrifugation (48,000 x g, 20 min) then passed through a TALON immobilized metal affinity chromatography column (Clontech Laboratories, Mountain View, CA). The column was washed with 15 column volumes of 1X equilibration buffer (50 mM HEPES pH 7.5, 300 mM NaCl), 10 column volumes of 1X wash buffer (50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM imidazole), 10 column volumes of 2X wash buffer (100 mM HEPES pH 7.5, 600 mM NaCl, 20 mM imidazole), and the His-tagged protein was then eluted with 5

column volumes of 1X elution buffer (150 mM imidazole pH 7.0, 300 mM NaCl). Buffer was exchanged by addition of 0.1 M Tris pH 7.5, 1 mM NaCl, 5 mM DTT while concentrating the protein by ultrafiltration. Protein concentration was determined using the Advanced Protein Assay Reagent (Cytoskeleton, Denver, CO) with γ-globulins (Sigma-Aldrich) as the standard. The protein was visualized via Coomassie stained SDS-PAGE.

Mtb Dxr spectrophotometric assay

Dxr activity was evaluated at 37 ^{o}C by spectrophotometrically monitoring the enzyme catalyzed oxidation of NADPH, as described previously.[6] To determine the mode of inhibition with respect to DXP, the assay was performed with either 150 μM NADPH (for FR900098) or 30 μM NADPH (for compound **8**). The enzyme was preincubated with each inhibitor for 10 minutes (37 ^{o}C) prior to addition of substrate. For the mechanism relative to NADPH, the assays were performed with 47 μM DXP. Here, the enzyme was preincubated with the inhibitor for 10 minutes prior to addition of NADPH, and an additional 5 minutes before addition of DXP. All assays were performed in duplicate. Lineweaver-Burk plots were generated using GraphPad PRISM version 4.00 for Windows (GraphPad Software Inc., San Diego, CA). IC_{50}s were determined via nonlinear regression analysis of standard dose-response plots using GraphPad PRISM.

Inhibitory data for FR900098 and compounds 3-9



Supplementary Figure 1. Kinetic characterization of inhibitor activity. A) Relative inhibition by compounds **3-9** at 100 μM concentration. B) through H) Dose-response plots obtained using the indicated inhibitors. The Lineweaver-Burk plots (I and J) reveal that FR900098 is a competitive inhibitor relative to DXP and an uncompetitive inhibitor with respect to NADPH, indicating that the inhibitor binds to the enzyme only after NADPH is bound.

Molecular Docking. Docking studies were performed using the Glide tool in Schrödinger, suite 2010. The structure of Mtb Dxr protein in complex with fosmidomycin, Mn^{2+} and NADPH was obtained from the Protein Data Bank (PDB code: 2JCZ). Monomer A of the protein was prepared using the protein preparation wizard in Maestro 9.2 with default settings. Water molecules were removed, NADPH and fosmidomycin were extracted from the active site, and Mn^{2+} was kept in place. A grid containing the whole active site was generated with Glide. A

positional constraint of the phosphorus and a CORE constraint were used. Compounds to be docked were built and minimized using Maestro, and molecular docking was performed in extra precision (XP) mode.

Mtb MIC Assay. The assay was performed as described.[7] A stock culture of Mtb H37Rv (ATCC 27294) was grown to OD 0.5 in Middlebrook 7H9 broth (Difco) supplemented with 0.05% Tween-80, 0.2% glycerol, and albumin/NaCl/glucose (ADC) complex. The culture was diluted 1:1000 in 7H9-based medium before aliquoting 50 μL into each well of a 96-well plate. The inhibitors were dissolved in DMSO to make stock solutions of 50 mg/mL. Inhibitors were added to the first row of wells of the 96-well plate with 100 μL of 7H9-based medium. After pipet mixing and use of a multichannel pipet, 50 μL was removed from each well in the first row and added to the second row. 2-Fold dilution in this manner was carried out to give eight dilutions of each inhibitor. The plates were incubated for 2 weeks at 37 °C, and the MIC_{99} values were read macroscopically using an inverted plate reader. Each measurement was made three independent times.

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