SUPPORTING INFORMATION

Bisubstrate Inhibitors of Biotin Protein Ligase from *Mycobacterium tuberculosis* Resistant to Cyclo-nucleoside Formation

Ce Shi,[1] Divya Tiwari,[2] Daniel J. Wilson,[1] Christopher L. Seiler,[3] Dirk Schnappinger,[2] Courtney C. Aldrich[1,3]

[1]Center for Drug Design, University of Minnesota, Minneapolis, MN, 55455, USA

[2]Department of Microbiology and Immunology, Weill Cornell Medical College, New York, NY, 10065, USA

[3]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, 55455, USA

Table of Contents

I. Chemical synthesis procedures

General methods: All chemical reactions were performed under an inert atmosphere of dry Ar or N_2 in oven-dried (150 °C) glassware. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 MHz or a Varian 400 MHz spectrometer. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26 ppm) or methanol (3.31 ppm), and carbon chemical shifts are reported using an internal standard of residual chloroform (77.0 ppm) or methanol (49.1 ppm). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad), coupling constant, integration. High resolution mass spectra were obtained on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. TLC analyses were performed on TLC silica gel 60F254 from EMD Chemical Inc., and were visualized with UV light, iodine chamber, 10% sulfuric acid or 10% PMA solution. Optical rotations values were obtained on a Rudoldf Autopol III Polarimeter. Purifications were performed by flash chromatography on silica gel (Dynamic Adsorbents, 60A).

Materials: Chemicals and solvents were purchased from Sigma-Aldrich Company or Acros Organic Fischer Company, and were used as received. An anhydrous solvent dispensing system (J. C. Meyer) using 2 packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2 while 2 packed columns of molecular sieves were used to dry DMF and the solvents were dispensed under argon. Compound **14**,[1] **19**[2] and **18**[3] were synthesized according to the reported procedures.

6-Amino-N^6,N^6-bis(*tert*-butoxycarbonyl)-9-[5,6-dideoxy-6-(ethoxysulfonyl)-2,3-*O*-isopropylidene-*β*-D-*ribo*-hex-5-enofuranosyl]-9*H*-purine (17). To a solution of **14** (250 mg, 0.50 mmol, 1.0 equiv) in CH_2Cl_2 (20 mL) at 25 °C was added Dess-Martin Periodinane (318 mg, 0.75 mmol, 1.5 equiv) portionwise over 10 min. After stirring for 1 h, TLC and MS indicated full conversion of the starting material. The reaction was cooled in an ice bath and quenched by addition of aqueous 1 M $Na_2S_2O_3$ (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The mixture was stirred for 30 min at 25 °C and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with H_2O (25 mL) and saturated aqueous NaCl (25 mL). The organic extract was dried, filtered, and concentrated to afford the crude aldehyde, which was used directly in the next step without further purification.

To a solution of *tert*-butyl (diphenylphosphoryl)methylsulfonylcarbamate (217 mg, 0.55 mmol, 1.1 equiv) in DMF–THF (1:1, 5.0 mL) at -78 °C, was added a solution of LiHMDS (1 M in THF, 1.1 mL, 2.2 equiv) dropwise over 15 min. The solution was stirred for 30 min, and the crude aldehyde from the previous step in THF (4 mL) was cannulated into the reaction. The mixture was allowed to warm to 25 °C and stirred for 15 h. The solvent was then removed *in vacuo* and the residue was re-suspended in H_2O (20 mL). An aqueous 1 M HCl solution was used to adjust the pH to 3–4, and the suspension was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with H_2O (20 mL), saturated aqueous NaCl (20 mL), dried ($MgSO_4$), and concentrated to afford the crude product. Purification by flash column chromatography on silica gel (5:95 EtOAc–Hexane→50:50 EtOAc–hexane, linear gradient) afforded the title compound (153 mg, 45% over 2 steps) as a colorless oil: R_f = 0.35 (50:50 EtOAc–

hexane); $[\alpha]_D^{23}$ +82 (*c* 1.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.35 (s, 3H), 1.44 (s, 9H), 1.49 (s, 18H), 1.60 (s, 3H), 4.92 (q, *J* = 3.0 Hz, 1H), 5.32 (q, *J* = 3.0 Hz, 1H), 5.72 (d, *J* = 6.0 Hz, 1H), 6.20 (br s, 1H), 6.40 (dd, *J* = 15.0, 2.4 Hz, 1H), 6.83 (dd, *J* =15.0, 3.0 Hz, 1H), 8.12 (s, 1H), 8.83 (s, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 25.1, 26.7, 27.8, 27.9, 83.3, 83.8, 84.3, 84.4, 85.2, 90.8, 114.6, 128.6, 129.1, 141.7, 144.2, 149.5, 150.7, 150.8, 152.38, 152.41; HRMS (ESI–) calcd for $C_{29}H_{41}N_6O_{11}S$ $[M - H]^-$ 681.2554, found 681.2560 (error 0.9 ppm).

***C*-(2′,3′-*O*-Isopropylidene-5′-adenosyl)methansulfonamide (18).** To a solution of **17** (35 mg, 0.05 mmol, 1.0 equiv) in MeOH (5 mL) under nitrogen was added 10 wt% Pd/C (5.3 mg, 10 mol%) in one portion. The atmosphere was then replaced by H_2 (1 atm). The mixture was stirred vigorously for 24 h whereupont MS indicated complete conversion of starting material. The mixture was filtered over Celite and the filtrate was concentrated to afford the crude Boc-protected sulfonamide intermediate, which was used directly in the next step without further purification.

To a solution of the Boc-protected sulfonamide intermediate from the previous step in CH_2Cl_2 (2 mL) at 0 °C was added TFA (0.5 mL). The solution was stirred at 0 °C for 2 h and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (CH_2Cl_2→10:90 MeOH–EtOAc, linear gradient) afforded the title compound (16.7 mg, 87% over 2 steps) as a colorless oil: R_f = 0.25 (10:90 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ –0.9 (*c* 0.7, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.38 (s, 3H), 1.59 (s, 3H), 2.23 (q, *J* = 8.4 Hz, 2H), 3.08–3.14 (m, 2H), 4,30 (q, *J* = 3.6 Hz, 1H), 4.98 (dd, *J* = 6.6, 3.6 Hz, 1H), 5.50 (dd, *J* = 6.6, 2.4 Hz, 1H), 6.17 (d, *J* = 2.4 Hz, 1H), 8.26 (s, 1H), 8.30 (s, 1H); ^{13}C NMR

($CDCl_3$, 150 MHz) δ 25.7, 27.6, 29.2, 52.4, 85.3, 85.4, 86.3, 91.4, 116.0, 120.8, 142.5, 150.3, 152.8, 156.7; HRMS (APCI+) calcd for $C_{14}H_{21}N_6O_5S$ $[M + H]^+$ 385.1300, found 385.1263 (error 9.6 ppm).

***N*-D-Biotinoyl-*C*-(2′,3′-*O*-isopropylidene-5′-adenosyl)methansulfonamide triethylammonium salt (10).** To a solution of **18** (12 mg, 0.030 mmol, 1.0 equiv) and D-(+)-biotin *N*-hydroxysuccinimide ester (19 mg, 0.06 mmol, 2.0 equiv) in DMF (1 mL) at 0 °C was added Cs_2CO_3 (27 mg, 0.09 mmol, 3.0 equiv) in one portion. The mixture was gradually warmed to 25 °C and stirred for 15 h. The reaction was concentrated *in vacuo* and re-suspended in 1:9 MeOH–CH_2Cl_2 (20 mL). Filtration over Celite and concentration *in vacuo* afforded the crude biotinylated intermediate (33 mg), which was used directly in the next step without further purification.

The crude biotinylated intermediate (33 mg) prepared above was dissolved in 1:1 TFA–H_2O (1 mL) at 0 °C and the solution was stirred for 60 min. The reaction was quenched by addition of NEt_3 (0.5 mL) and concentrated *in vacuo*. The crude product was purified by preparative reverse-phase HPLC with a Phenomenex Gemini 10 μm C18 110A (250 × 21.2 mm) column at a flow rate of at 30 mL/min for 20 min and an isocratic elution of 13:87 MeOH–10 mM triethylammonium bicarbonate (pH 7.0). The retention time of the product was 13.5 min and the appropriate fractions were pooled and lyophilized to afford the title compound (60% over 2 steps): $[\alpha]_D^{23}$ +23.5 (*c* 0.4, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.28 (t, J = 7.2 Hz, 9H), 1.41–1.45 (m, 2H), 1.55–1.64 (m, 3H), 1.71–1.75 (m, 1H), 2.15–2.25 (m, 2H), 2.18 (t, J = 7.2 Hz, 2H), 2.68 (d, J = 13.2 Hz, 1H), 2.89 (dd, J = 13.2, 4.8 Hz, 1H), 3.14 (q, J = 7.2 Hz, 6H), 3.15–3.19 (m, 1H), 3.24–3.31 (m, 1H), 3.41–3.47 (m, 1H), 4.08 (dt, J = 8.4, 4.8 Hz, 1H), 4.27 (t, J = 6.0 Hz,

1H), 4.29 (dd, J = 7.2, 4.8 Hz, 1H), 4.45 (dd, J = 7.8, 4.8 Hz, 1H), 4.71 (t, J = 4.8 Hz, 1H), 5.95 (d, J = 4.8 Hz, 1H), 8.21 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 9.5, 27.4, 29.5, 29.7, 30.0, 39.9, 41.3, 48.0, 57.1, 61.7, 63.4, 71.9, 74.9, 75.4, 84.3, 90.6, 120.8, 141.6, 150.8, 154.1, 157.5, 166.3, 182.6; HRMS (ESI–) calcd for $C_{21}H_{29}N_8O_7S_2$ $[M - H]^-$ 569.1601, found 569.1606 (error 0.9 ppm).

1-[5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-*O*-isopropylidene-*β*-D-ribofuranosyl]-5-iodo-1*H*-imidazole-4-carbonitrile (20). To a solution of **19** (2.4 g, 10 mmol, 1.0 equiv) in acetone (100 mL) at 25 °C was added *p*-toluenesulfonic acid monohydrate (3.8 g, 20 mmol, 2.0 equiv) and 2,2-dimethoxypropane (24.5 mL, 200 mmol, 20 equiv). The solution was stirred at 25 °C for 15 h, and quenched by the addition of solid $NaHCO_3$ (2.52 g, 30 mmol, 3.0 equiv). The resulting suspension was stirred for an additional 30 min, then filtered. The filtrate was concentrated and the crude solid partitioned between EtOAc (200 mL) and saturated aqueous $NaHCO_3$ (50 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with saturated aqueous NaCl, dried ($MgSO_4$) and concentrated to afford 5-amino-1-(2′,3′-*O*-isopropylidene-*β*-D-ribofuranosyl)-1*H*-imidazole-4-carbonitrile (2.8 g). The crude product was used directly in the next step without further purification.

To a solution of 5-amino-1-(2′,3′-*O*-isopropylidene-*β*-D-ribofuranosyl)-1*H*-imidazole-4-carbonitrile prepared above (2.8 g, 10 mmol, 1.0 equiv) in DMF (40 mL) at 0 °C was added imidazole (1.6 g, 24 mmol, 2.4 equiv) and *tert*-butyldimethylsilyl chloride (1.8 g, 12 mmol, 1.2 equiv). The solution was stirred at 0 °C for 30 min and at 25 °C for 6 h before concentration *in vacuo*. The crude material was re-dissolved in $CHCl_3$ (200 mL) and washed with 5% aqueous $NaHCO_3$ (50 mL). The organic layer was dried over

$MgSO_4$ and concentrated to afford 5-amino-1-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene-*β*-D-ribofuranosyl]-1*H*-imidazole-4-carbonitrile (3.5 g). The crude product was used directly in the next step without further purification.

To a solution of 5-amino-1-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene-*β*-D-ribofuranosyl]-1*H*-imidazole-4-carbonitrile (3.5 g, 8.9 mmol, 1.0 equiv) in CH_2I_2 (10 mL) at 90 °C was added isoamylnitrite (4.7 mL, 35.6 mmol, 4.0 equiv) in $CHCl_3$ (10 mL) dropwise over 20 min. The reaction was stirred for 1 h at 90 °C, and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (5:95 EtOAc–hexane→50:50 EtOAc–hexane, linear gradient) afforded the title compound (2.9 g, 55% over 3 steps) as a yellow foam: R_f = 0.40 (50:50 EtOAc–hexane); $[\alpha]_D^{23}$ –52.0 (*c* 0.2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.89 (s, 3H), 0.90 (s, 3H), 0.88 (s, 9H), 1.38 (s, 3H), 1.63 (s, 3H), 3.81 (dd, *J* = 12.0, 2.4 Hz, 1H), 3.92 (dd, *J* = 12.0, 2.4 Hz, 1H), 4.40–4.45 (m, 1H), 4.70 (dd, *J* = 6.6, 2.4 Hz, 1H), 4.83 (dd, *J* = 6.6, 2.4 Hz, 1H), 5.81 (d, *J* = 2.4 Hz, 1H), 8.06 (s, 1H); ^{13}C NMR ($CDCl_3$, 150 Hz) δ –5.7, –5.5, 18.3, 25.3, 25.8, 27.2, 63.3, 78.5, 80.8, 86.3, 86.8, 95.0, 114.2, 114.3, 123.1, 139.8; HRMS (APCI+) calcd for $C_{18}H_{29}IN_3O_4Si$ $[M + H]^+$ 506.0967, found 506.0972 (error 1.0 ppm).

1-[5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-*O*-isopropylidene-*β*-D-ribofuranosyl]-5-trimethylsilylethynyl-1*H*-imidazole-4-carbonitrile (21). To a solution of **20** (250 mg, 0.5 mmol, 1.0 equiv) in CH_3CN (2 mL) at 25 °C was added $PdCl_2(PhCN)_2$ (9.3 mg, 0.025 mmol, 5% equiv) and the solution was purged with argon for 10 min. Trimethylsilylacetylene (0.21 mL, 1,5 mmol, 3.0 equiv) and NEt_3 (0.19 mL, 1.5 mmol, 3.0 equiv) were then added sequentially. The solution was heated at 100 °C for 6 h. The

solution was concentrated *in vacuo*. Purification by flash column chromatography on silica gel (5:95 EtOAc–hexane→30:70 EtOAc–hexane, linear gradient) afforded the title compound (166 mg, 70%) as a yellow foam: R_f = 0.25 (20:80 EtOAc–hexane); $[\alpha]_D^{23}$ –11.7 (*c* 2.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.05 (s, 3H), 0.06 (s, 3H), 0.28 (s, 9H), 0.86 (s, 9H), 1.36 (s, 3H), 1.58 (s, 3H), 3.79 (dd, *J* = 11.4 , 3.0 Hz, 1H), 3.90 (dd, *J* = 11.4, 3.0 Hz, 1H), 4.46–4.47 (m, 1H), 4.79–4.80 (m, 2H), 5.89 (d, *J* = 1.8 Hz, 1H), 7.83 (s, 1H); ^{13}C NMR ($CDCl_3$, 150 Hz) δ –5.7, –5.6, –0.7, 18.2, 25.2, 25.8, 27.2, 63.5, 81.1, 86.5, 87.4, 87.8, 93.8, 110.6, 113.6, 113.8, 118.9, 121.6, 136.5; HRMS (ESI+) calcd for $C_{23}H_{38}N_3O_4Si_2$ $[M + H]^+$ 476.2395, found 476.2405 (error 2.1 ppm).

4-Amino-1-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene-*β*-D-ribofuranosyl]-1*H*-imidazo[4,5-*c*]pyridine (22). A 7 N NH_3 methanolic solution (3 mL, 21 mmol, 84 equiv) was added to **21** (120 mg, 0.25 mmol, 1.0 equiv) and the solution was heated at 110 °C for 15 h in a sealed tube. The reaction was cooled to 25 °C and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (CH_2Cl_2→7:93 MeOH–CH_2Cl_2, linear gradient) afforded the title compound (82 mg, 77%) as a yellow foam: R_f = 0.55 (10:90 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ –35.2 (*c* 0.25, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.03 (s, 3H), 0.04 (s, 3H), 0.84 (s, 9H), 1.38 (s, 3H), 1.63 (s, 3H), 3.82 (dd, *J* = 11.4, 3.0 Hz, 1H), 3.92 (dd, *J* = 11.4, 3.0 Hz, 1H), 4.45 (d, *J* = 1.8 Hz, 1H), 4.81 (t, *J* = 3.0 Hz, 1H), 4.87 (t, *J* = 2.7 Hz, 1H), 5.91 (d, *J* = 3.6 Hz, 1H), 6.90 (d, *J* = 6.0 Hz, 1H), 7.80 (d, *J* = 5.4 Hz, 1H), 8.04 (s, 1H); ^{13}C NMR ($CDCl_3$, 150 Hz) δ –5.7, –5.5, 18.3, 25.3, 25.8, 27.3, 63.3, 81.1, 85.3, 86.3, 93.1, 98.4, 114.6, 127.7, 137.5, 138.9, 139.2, 151.3; HRMS (ESI+) calcd for $C_{23}H_{38}N_4O_4Si$ $[M + H]^+$ 421.2266, found 421.2283 (error 4.0 ppm).

4-Amino-1-(*β*-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (23). To a solution of **22** (29.4 mg, 0.07 mmol, 1 equiv) in DMF (2 mL) at 25 °C was added KO*t*Bu (25 mg, 0.21 mmol, 3 equiv). The mixture was stirred at 25 °C for 1 h then concentrated *in vacuo*. The resulting crude material was redissovled in 1:9 MeOH–CH_2Cl_2 (20 mL), filtered over Celite and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (CH_2Cl_2→1:9 MeOH–CH_2Cl_2, linear gradient) afforded the title compound (16.8 mg, 78%) as a yellow foam: R_f = 0.15 (10:90 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ –48 (*c* 0.3, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.39 (s, 3H), 1.63 (s, 3H), 3.71–3.77 (m, 2H), 4.37 (q, *J* = 3.6 Hz, 1H), 4.97 (dd, *J* = 6.6, 2.4 Hz, 1H), 5.04 (dd, *J* = 6.0, 3.6 Hz, 1H), 6.05 (d, *J* = 3.6 Hz, 1H), 6.99 (d, *J* = 6.0 Hz, 1H), 7.71 (d, *J* = 5.4 Hz, 1H), 8.36 (s, 1H); ^{13}C NMR (CD_3OD, 150 Hz) δ 25.6, 27.6, 63.0, 82.8, 86.1, 88.0, 93.7, 99.5, 115.9, 128.2, 139.5, 140.4, 141.7, 153.2; HRMS (ESI+) calcd for $C_{14}H_{19}N_4O_4$ $[M + H]^+$ 307.1401, found 307.1407 (error 2.0 ppm).

4-Amino-1-(5′-*O*-sulfamoyl-*β*-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (24). To a solution of **23** (35 mg, 0.11 mmol, 1.0 equiv) in DME (2 mL) at 0 °C was added NaH (60 wt% in mineral oil, 17.6 mg, 0.44 mmol, 4.0 equiv). The suspension was stirred at 0 °C for 30 min. Next, a stock solution of sulfamoyl chloride in THF (2 mL, 0.11 M, 2.0 equiv) was added. The reaction was gradually warmed to 25 °C and stirred for 15 h. The mixture was concentrated *in vacuo* and redissolved in 10:90 MeOH–CH_2Cl_2 (20 mL). The suspension was filtered over Celite and the filtrate was concentrated *in vacuo*. Purification by flash column chromatography on silica gel (CH_2Cl_2→10:90 MeOH–CH_2Cl_2, linear gradient) afforded the title compound (34 mg, 80%) as a yellow foam: R_f = 0.15 (10:90 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ –56 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ

1.40 (s, 3H),1.64 (s, 3H), 4.31–4.33 (m, 2H), 4.58 (q, J = 2.8 Hz, 1H), 5.02 (dd, J = 6.4, 3.6 Hz, 1H), 5.10 (dd, J = 6.4, 3.6 Hz, 1H), 6.09 (d, J = 3.6 Hz, 1H), 6.99 (d, J = 6.0 Hz, 1H), 7.73 (d, J = 6.4 Hz, 1H), 8.28 (s, 1H); ^{13}C NMR (CD_3OD, 100 Hz) δ 25.6, 27.6, 69.8, 82.4, 84.7, 85.6, 93.6, 99.5, 116.4, 127.2, 139.4, 141.0, 141.5, 153.3; HRMS (ESI+) calcd for $C_{14}H_{20}N_5O_6S$ $[M + H]^+$ 386.1129, found 386.1145 (error 4.1 ppm).

4-Amino-1-{5′-*O*-[*N*-(D-biotinoyl)sulfamoyl]-*β*-D-ribofuranosyl}-1*H*-imidazo[4,5-*c*]pyridine triethylammonium salt (11). The title compound was prepared from **24** (34.5 mg, 0.1 mmol) analogously to **10** and purified by preparative reverse-phase HPLC with a Phenomenex Gemini 10 μm C18 110A (250 × 21.2 mm) column at a flow rate of 18 mL/min using a linear gradient of 10→40% MeOH–10 mM triethylammonium bicarbonate (pH 7.0) over 15 min followed by 40% MeOH–10 mM triethylammonium bicarbonate (pH 7.0) for 5 min. The retention time of the product was 12.5 min and the appropriate fractions were pooled and lyophilized to afford the title compound (43.5 mg, 65% over 2 steps): $[\alpha]_D^{23}$ +16 (*c* 0.3, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.28 (t, J = 6.6 Hz, 9H), 1.41–1.45 (m, 2H), 1.52–1.56 (m, 1H), 1.62–1.72 (m, 3H), 2.22 (t, J = 7.2 Hz, 2H), 2.64 (d, J = 12.6 Hz, 1H), 2.85 (dd, J = 12.0, 5.4 Hz, 1H), 3.09–3.17 (m, 7H), 4.22–4.25 (m, 1H), 4.31–4.36 (m, 4H), 4.40–4.43 (m, 1H), 4.53 (t, J = 6.0 Hz, 1H), 5.89 (d, J = 7.2 Hz, 1H), 7.11 (d, J = 5.4 Hz, 1H), 7.72 (d, J = 6.6 Hz, 1H), 8.44 (s, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 9.5, 27.4, 29.6, 30.0, 40.1, 41.2, 48.0, 57.1, 61.7, 63.4, 69.3, 72.4, 76.1, 85.3, 90.9, 111.6, 128.1, 139.9, 140.1, 142.5, 153.0, 166.3, 183.2; HRMS (ESI–) calcd for $C_{21}H_{28}N_7O_8S_2$ $[M - H]^-$ 570.1446, found 570.1450 (error 0.7 ppm).

(P/M)–5-(2-Aminophenyl)-1-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene-*β*-D-ribofuranosyl]-1*H*-imidazole-4-carbonitrile (25). To a suspension of $Pd(OAc)_2$ (1.1 mg, 0.0050 mmol, 0.05 equiv) and PPh_3 (3.9 mg, 0.015 mmol, 0.15 equiv) in DME (2 mL) were sequentially added **20** (51 mg, 0.10 mmol, 1.0 equiv), 2-aminophenylboronic acid hydrochloride salt (26 mg, 0.15 mmol, 1.5 equiv) and 0.3 M aqueous Na_2CO_3 (1.0 mL, 0.30 mmol, 3.0 equiv) at 25 °C. The reaction was heated at 90 °C for 5 h and cooled down to 25 °C. TLC and MS analysis indicated the complete consumption of **20**. The reaction mixture was partitioned between EtOAc (50 mL) and H_2O (10 mL). The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (25 mL), saturated aqueous NaCl (25 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (10:90 EtOAc–hexane→40:60 EtOAc–hexane, linear gradient) afforded the title compound (35.3 mg, 75%) as a yellow foam, which was a 2:1 mixture of atropisomers: R_f = 0.20 (50:50 EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.11 (d, J = 2.4 Hz, 2H), 0.13 (d, J = 4.8 Hz, 4H), 0.92 (s, 3H), 0.9 (s, 6H), 1.27 (s, 2H), 1.29 (s, 1H), 1.33 (s, 2H), 1.36 (s, 1H), 3.77–3.81 (m, 1.33H), 3.87–3.91 (m, 0.67H), 4.22–4.23 (m, 0.33 H), 4.28–4.30 (m, 0.67H), 4.58–4.60 (m, 1H), 4.70–4.72 (m, 1H), 4.75–4.77 (m, 1H), 5.48 (d, J = 2.4 Hz, 0.33H), 5.58 (d, J = 4.0 Hz, 0.67H), 6.77–6.85 (m, 2H), 7.09–7.26 (m, 1H), 7.25–7.29 (m, 1H), 7.98 (s, 0.33H), 8.06 (s, 0.67H); LRMS (ESI+) 471.2.

4-Amino-1-(2′,3′-*O*-isopropylidene-*β*-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]quinoline (26). To a solution of **25** (34 mg, 0.07 mmol, 1 equiv) in MeOH (1 mL) at 25 °C was added NaOMe (12 mg, 0.21 mmol, 3 equiv). The reaction was heated at reflux for 15 h. The mixture was cooled to room temperature and concentrated *in vacuo.* The

residue was redissolved in 1:9 MeOH–CH_2Cl_2 (20 mL), filtered over Celite and the filtrate was concentrated. Purification by flash column chromatography on silica gel (CH_2Cl_2→1:9 MeOH–CH_2Cl_2, linear gradient) afforded the title compound (23.6 mg, 92% over 2 steps) as a yellow foam. R_f = 0.25 (1:9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ +37.6 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.39 (s, 3H), 1.70 (s, 3H), 3.87 (dd, *J* = 12, 6.0 Hz, 1H), 4.05 (dd, *J* = 12, 6.0 Hz, 1H), 4.57 (d, *J* = 1.8 Hz, 1H), 5.06–5.08 (m, 2H), 6.52 (d, *J* = 1.8 Hz, 1H), 7.23 (t, *J* = 7.2 Hz, 1H), 7.29 (t, *J* = 7.2 Hz, 1H), 7.60 (d, *J* = 6.0 Hz, 1H), 8.09 (d, *J* = 6.0 Hz, 1H), 8.39 (s, 1H); ^{13}C NMR ($CDCl_3$, 150 Hz) δ 25.3, 27.2, 61.8, 81.2, 85.4, 87.7, 87.8, 93.1, 114.3, 121.0, 121.4, 122.6, 125.3, 127.5, 127.8, 132.8, 139.2, 150.9; HRMS (ESI+) calcd for $C_{18}H_{21}N_4O_4$ $[M + H]^+$ 357.1557, found 357.1559 (error 0.6 ppm).

4-Amino-1-(2′,3′-*O*-isopropylidene-5′-*O*-sulfamoyl-*β*-D-ribofuranosyl)-1*H*-imidazo [4,5-*c*]quinoline (27). To a solution of **26** (25 mg, 0.07 mmol, 1.0 equiv) in DMA (1 mL) at 0 °C was added a 0.35 M stock solution of sulfamoyl chloride in CH_3CN (0.50 mL, 0.18 mmol, 2.5 equiv) over 5 min. The solution was stirred at 0 °C for 2 h, then quenched by the addition of NEt_3 (0.2 mL) and MeOH (0.2 mL). The mixture was diluted with EtOAc (50 mL), washed with 5% aqueous $NaHCO_3$ (2 × 25 mL), saturated aqueous NaCl (2 × 25 mL), then dried ($MgSO_4$) and concentrated *in vacuo* to afford the title compound (24 mg, 88%) as yellow foam: R_f = 0.15 (1:9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ +54 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.41 (s, 3H), 1.69 (s, 3H), 4.30–4.31 (m, 2H), 4.67 (t, *J* = 3.6 Hz, 1H), 5.09 (dd, *J* = 6.0, 3.3 Hz, 1H), 5.29 (dd, *J* = 6.6, 2.4 Hz, 1H), 6.65 (d, *J* = 1.8 Hz, 1H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 8.47 (s, 1H); ^{13}C NMR ($CDCl_3$, 150 Hz) δ 25.6,

27.5, 69.6, 82.4, 85.9, 86.2, 94.1, 116.0, 116.2, 123.2, 123.7, 126.8, 129.1, 129.2, 134.5, 140.8, 145.9, 153.2; HRMS (ESI+) calcd for $C_{18}H_{22}N_5O_6S$ $[M + H]^+$ 436.1285, found 436.1276 (error 2.1 ppm).

4-Amino-1-{5′-*O*-[*N*-(D-biotinoyl)sulfamoyl]-*β*-D-ribofuranosyl}-1*H*-imidazo[4,5-*c*]quinoline triethylamonium salt (12). The title compound was prepared from **27** (39.5 mg, 0.100 mmol) analogously to **10** and purified by preparative reverse-phase HPLC with a Varian Dynamax Microsorb 100-8 C18 column (250 × 41.4 mm) at a flow rate of 35 mL/min using a linear gradient of 10→48% MeOH–10 mM triethylammonium bicarbonate (pH 7.0) over 10 min followed by 48% MeOH–10 mM triethylammonium bicarbonate (pH 7.0) for 10 min. The retention time of the product was 18.2 min and the appropriate fractions were pooled and lyophilized to afford the title compound (62% over 2 steps): $[\alpha]_D^{23}$ +76 (*c* 0.05, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 0.99 (t, J = 6.6 Hz, 9 H), 1.18–1.33 (m, 2H), 1.35–1.50 (m, 1H), 1.54–1.65 (m, 3H), 2.07–2.18 (m, 2H), 2.50 (d, J = 12.6 Hz, 1H), 2.85 (dd, J = 12.6, 5.4 Hz, 1H), 2.82 (q, J = 6.6 Hz, 9 H), 2.92–2.99 (m, 1H), 4.09–4.15 (m, 1H), 4.24–4.29 (m, 2H), 4.30-4.35 (m, 3H), 4.61 (t, J =12.0 Hz, 1H), 6.38 (d, J = 4.8 Hz, 1H), 7.29 (t, J = 8.4 Hz, 1H), 7.45 (t, J = 8.4 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 8.60 (s, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 13.9, 25.2, 28.0, 28.2, 37.8, 40.0, 47.2, 55.4, 57.9, 59.1, 61.0, 69.7, 74.3, 82.3, 89.9, 113.7, 116.8, 121.9, 122.7, 127.3, 128.1, 129.6, 132.9, 140.3, 150.9, 162.3, 184.3; HRMS (ESI–) calcd for $C_{25}H_{30}N_7O_8S_2$ $[M - H]^-$ 620.1603, found 620.1608 (error 0.8 ppm).

2-(3,3-Dimethyl-1-butyn-1-yl)adenosine (29). To a stirring solution of 2-iodoadenosine **28** (590 mg, 1.5 mmol, 1.0 equiv), CuI (14 mg, 0.075 mmol, 0.05 equiv),

$Pd(PPh_3)_2Cl_2$ (105 mg, 0.15 mmol, 0.10 equiv) in DMF (10.5 mL) was added NEt_3 (0.25 mL, 1.8 mmol, 1.2 equiv) and 3,3-dimethyl-1-butyne (0.22 mL, 1.8 mmol, 1.2 equiv). The reaction was stirred at 88 °C for 16 h. ESI-MS indicated complete conversion and the reaction was concentrated *in vacuo*. The residue was redissolved in $CHCl_3$ (20 mL) and solid potassium sodium tartrate hydrate was added. The suspension was filtered through a Celite pad and washed with $CHCl_3$ (3 × 5 mL). The filtrate was concentrated *in vacuo*. Purification by silica gel column chromatography (CH_2Cl_2→10:90 MeOH–CH_2Cl_2) afforded the title compound (401 mg, 77%) as a light yellow solid: R_f = 0.13 (10:90 MeOH–CH_2Cl_2); $[\alpha]_D^{24}$ –47.3 (*c* 0.2, MeOH); mp 143–145° C; ^{1}H NMR (CD_3OD, 600 MHz) δ 1.37 (s, 9H), 3.78 (d, *J* = 12.3 Hz, 1H), 3.93 (d, *J* = 13.0 Hz, 1H), 4.17–4.20 (m, 1H), 4.32–4.35 (m, 1H), 4.69 (t, *J* = 5.3 Hz, 1H), 5.97 (d, *J* = 5.8 Hz, 1H), 8.35 (s, 1H); ^{13}C NMR (CD_3OD, 150 MHz) *δ* 28.9, 31.0, 63.5, 72.6, 75.8, 79.8, 88.2, 91.2, 96.5, 120.3, 142.5, 147.9, 150.4, 157.3 (missing 1 carbon); HRMS (ESI+) calcd for $C_{16}H_{22}N_5O_4$ $[M + H]^+$ 348.1666, found 348.1676 (error 2.9 ppm).

2-(3,3-Dimethyl-1-butyn-1-yl)-2′,3′-*O*-isopropylideneadenosine (30). To a solution of **29** (310 mg, 0.89 mmol, 1.0 equiv), 2,2-dimethoxypropane (0.53 mL, 4.4 mmol, 4.9 equiv) in acetone (10.5 mL) was added *p*-toluenesulfonic acid monohydrate (188 mg, 0.97 mmol, 1.1 equiv). The reaction was stirred at 23° C for 16 h and quenched with solid $NaHCO_3$. The resulting suspension was stirred for an additional 30 min, then filtered, and the filtrate was concentrated. Purification by silica gel column chromatography (75:25 hexane–EtOAc→25:75 hexane–EtOAc) afforded the title compound (293 mg, 85%) as a light yellow foam: R_f = 0.6 (10:90 MeOH–CH_2Cl_2); $[\alpha]_D^{24}$–53.7° (*c* 0.2, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.36 (s, 9H), 1.39 (s, 3H),

1.63 (s, 3H), 3.74 (dd, J = 12.0, 3.5 Hz, 1H), 3.81 (dd, J = 12, 3.5 Hz, 1H), 4.36–4.39 (m, 1H), 5.04 (dd, J = 5.9, 1.8 Hz, 1H), 5.20–5.23 (m, 1H), 6.15 (d, J = 3.5 Hz, 1H), 8.35 (s, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 25.7, 27.8, 28.9, 31.0, 63.8, 79.9, 83.0, 85.5, 86.1, 92.7, 96.4, 111.6, 115.5, 120.0, 142.3, 148.2, 150.5; HRMS (ESI+) calcd $C_{19}H_{26}N_5O_4$ $[M + H]^+$ 388.1979, found 388.2013 (error 8.8 ppm).

2-(3,3-Dimethyl-1-butyn-1-yl)-2′,3′-*O*-isopropylidene-5′-*O*-(sulfamoyl)adenosine (31). To a solution of **30** (146 mg, 0.38 mmol, 1.0 equiv) and Et_3N (0.16 mL, 1.1 mmol, 3.0 equiv) in DMF (5.0 mL) at 0 °C was added sulfamoyl chloride (130 mg, 1.1 mmol, 3.0 equiv). The reaction was stirred for 2 h at 0 °C, then allowed to warm to 25 °C and stirred for 16 h. TLC and MS indicated complete conversion and the reaction was concentrated *in vacuo*. Purification by silica gel column chromatography (CH_2Cl_2→8:92 MeOH–CH_2Cl_2) afforded the title compound (141 mg, 80%) as a light yellow foam: R_f = 0.4 (10:90 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = –18.4 (c 1.4, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) 1.36 (s, 9H), 1.40 (s, 3H), 1.62 (s, 3H), 4.29–4.35 (m, 2H), 4.51–4.54 (m, 1H), 5.10 (dd, J = 6.0, 3.0 Hz, 1H), 5.34 (dd, J = 5.4, 2.4 Hz, 1H), 6.24 (d, J = 2.4 Hz, 1H), 8.29 (s, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 25.7, 27.6, 28.9, 31.0, 70.0, 80.0, 82.9, 85.62, 85.64, 91.4, 96.3, 115.9, 119.6, 141.8, 148.3, 150.7, 157.1; HRMS (ESI+) calcd $C_{19}H_{27}N_6O_6S$ $[M + H]^+$ 467.1707, found 467.1712 (error 1.1 ppm).

5′-*O*-[*N*-(D-Biotinoyl)sulfamoyl]-2-(3,3-dimethyl-butyn-1-yl)adenosine triethylammonium salt (13). The title compound was prepared from **31** (42.6 mg, 0.1 mmol) analogously to **10** and purified by preparative reverse-phase HPLC with a Phenomenex Gemini 10 μm C18 110A (250 × 21.2 mm) column at a flow rate of 30 mL/min and an isocratic elution of 15% MeOH–10 mM triethylammonium bicarbonate

(pH 7.0) over 15 min. The retention time of the product was 10.5 min and the appropriate fractions were pooled and lyophilized to afford the title compound (65% over 2 steps): $[\alpha]_D^{23}$ +17 (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.28 (t, J = 7.2 Hz, 9H), 1.35 (s, 9H), 1.40–1.43 (m, 2H), 1.54–1.58 (m, 1H), 1.61–1.64 (m, 2H), 1.65–1.74 (m, 1H), 2.21 (t, J = 6.6 Hz, 2H), 2.65 (d, J =12.6 Hz, 1H), 2.87 (dd, J = 12.6, 4.8 Hz, 1H), 3.10–3.12 (m, 1H), 3.15 (q, J = 7.2 Hz, 6H), 4.25–4.30 (m, 3H), 4.32–4.35 (m, 1H), 4.38 (t, J = 4.2 Hz, 1H), 4.45 (dd, J = 7.8, 4.8 Hz, 1H), 4.56 (t, J = 4.8 Hz, 1H), 6.08 (d, J = 5.4 Hz, 1H), 8.52 (s, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 9.5, 27.4, 28.9, 29.6, 29.9, 31.1, 40.0, 41.2, 48.0, 57.1, 61.7, 63.4, 69.2, 72.2, 76.6, 80.1, 84.6, 89.3, 96.0, 119.5, 141.7, 148.2, 151.2, 157.1, 166.3, 183.2; HRMS (ESI–) calcd for $C_{26}H_{35}N_8O_8S_2[M - H]^-$ 651.2025, found 651.2020 (error 0.8 ppm).

II. General description of ITC experiments

ITC experiments were as essentially as previously described.[4] Briefly, all experiments were performed on a Microcal VP-ITC microcalorimeter (GE Healthcare) thermostated to 20 °C using ITC buffer (10 mM Tris pH 7.5, 200 mM KCl, 2.5 mM $MgCl_2$). The nominal concentration of BirA was determined using the protein's extinction coefficient and the active concentration was determined by titrating the enzyme (20 μM) with biotin (150 μM) and adjusting the enzyme concentration *in silico* until an *n* of 1 was achieved. Once the precise active concentration of BirA was calculated 5 μM of the enzyme was titrated with 75 μM of each compound. For **10**, **11** and **12**, the quantity $c = K_A M_t(0)$, in which $M_t(0)$ is the total concentration of enzyme in the experiment, was greater than 1000. Therefore, displacement ITC experiments were performed using biotin as a competitive ligand. For **10** and **11** we employed 10 μM BirA and 250 μM biotin and for **12** we used 20 μM BirA and 500 μM biotin in the cell. The inhibitor concentrations used in the syringe for the competitive experiments were 75 μM for **10** and **11** and 200 μM for **12**. Initial analyses were carried out using the Origin software package included with the instrument. For competitive experiments the association constant was determined using equation 1:

$$K_A = K_A^{app}(1 + K_A^B[B]) \quad (1)$$

where $[B]$ is the concentration of biotin and K_A^B is the association constant of biotin (1.08 x 10^6 M^{-1}). The thermodynamic parameters were then calculated using equation 2:

$$\Delta G = -RT\ln K = \Delta H - T\Delta S \quad (2)$$

where R = 1.98 cal $mol^{-1}K^{-1}$, T is the absolute temperature, and ΔH is the value determined from the direct titration experiment.

III. Antitubercular activity assay

The MIC **9–13** were determined for *Mycobacterium tuberculosis* H37Rv by a broth microdilution assay using a 2-fold dilution from 50 µM down to 0.1 µM employing a 20 mM stock solution. Aliquots of GAST medium with 0.5% DMSO containing two-fold serial dilutions of compounds were inoculated with logphase *Mycobacterium tuberculosis* H37Rv to a final OD_{600} of 0.002 and incubated at 37 °C for 10–14 days in presence of CO_2. The MIC was defined as the lowest concentration of compound that prevented growth, as determined by measuring the endpoint OD_{600} values.

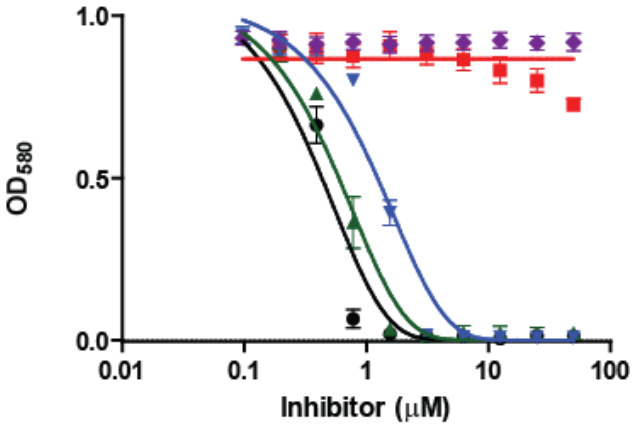

Figure S1: A two-fold serial dilution of inhibitors namely **9**(●), **10**(▼), **11**(▲), **12**(■), and **13**(◆) was prepared and their activity tested by inhibition of growth as measured by optical density. Graph indicates the normalized growth calculated as the OD_{600} at a particular drug concentration divided by the OD_{600} in the absence of drug. Error bars indicate standard error of the mean (SEM) from 8 measurements in two independent experiments.

IV. References

(1) Ikeuchi, H.; Meyer, M. E.; Ding, Y.; Hiratake, J.; Richards, N. G. J. A critical electrostatic interaction mediates inhibitor recognition by human asparagine synthetase. *Bioorg. Med. Chem.* **2009**, *17*, 6641–6650.

(2) (a) Shaw, E. A convenient preparation of 5-amino-4-imidazolecarboxamide riboside. Ring opening of N^1-*p*-toluenesulfonyl-inosine. *J. Am. Chem. Sci.* **1959**, *81*, 6021–6022. (b) Minakawa, N.; Takeda, T.; Sasaki, T.; Matsuda, A.; Ueda, T. Nucleosides and nucleotides. 96. Synthesis and antitumor activity of 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) and its derivatives. *J. Med. Chem.* **1991**, *34*, 778–786.

(3) Matsuda, A.; Shinozaki, M.; Yamaguchi, T.; Homma, H.; Nomoto, R.; Miyasaka, T.; Watanabe, Y.; Abiru, T. Nucleosides and nucleotides. 103. 2-alkynyladenosines: a novel class of selective adenosine A2 receptor agonists with potent antihypertensive effects. *J. Med. Chem.* **1992**, *35*, 241–252.

(4) Duckworth, B. P.; Geders, T. W.; Tiwari, D.; Boshoff, H. I.; Sibbald, P. A.; Barry, C. E. III; Schnappinger, D.; Finzel, B. C.; Aldrich, C. C. Bisubstrate adenylation inhibitors of biotin protein ligase from *Mycobacterium tuberculosis*. *Chem. Biol*. **2011**, *18*, 1432–1441.