Structure-Guided Drug Design of 6-Substituted Adenosine Analogs as Potent Inhibitors of *Mycobacterium tuberculosis* Adenosine Kinase

Roberto A. Crespo[†∞], Qun Dang[‡‖], Nian E. Zhou[†], Liam M. Guthrie[+], Thomas C. Snavely[†], Wen Dong[†], Kimberly A. Loesch[†], Takao Suzuki[#], Lanying You[#], Wei Wang[#], Theresa O'Malley[°], Tanya Parish[°], David B. Olsen[‡*] and James C. Sacchettini[†*]

[†]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX, 77843, US.

[+]College of Medicine, Texas A&M University Health Science Center, Bryan, TX, 77807, US.

[‡]Merck Sharp Dohme Corp., West Point PA, 19486.

[#]WuXi AppTec, 288 Fute Zhong Road, Shanghai 200131 China.

[°]TB Discovery Research, Infectious Disease Research Institute, 1616 Eastlake Avenue E, Seattle, WA 98102, USA.

Supporting Information

TABLE OF CONTENTS

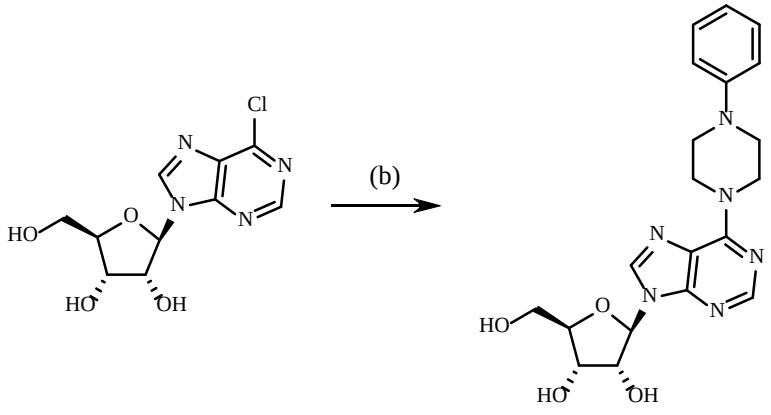
LIST OF FIGURES

LIST OF TABLES

CHEMISTRY

Schemes and reagaents for chemical synthesis of intermediates

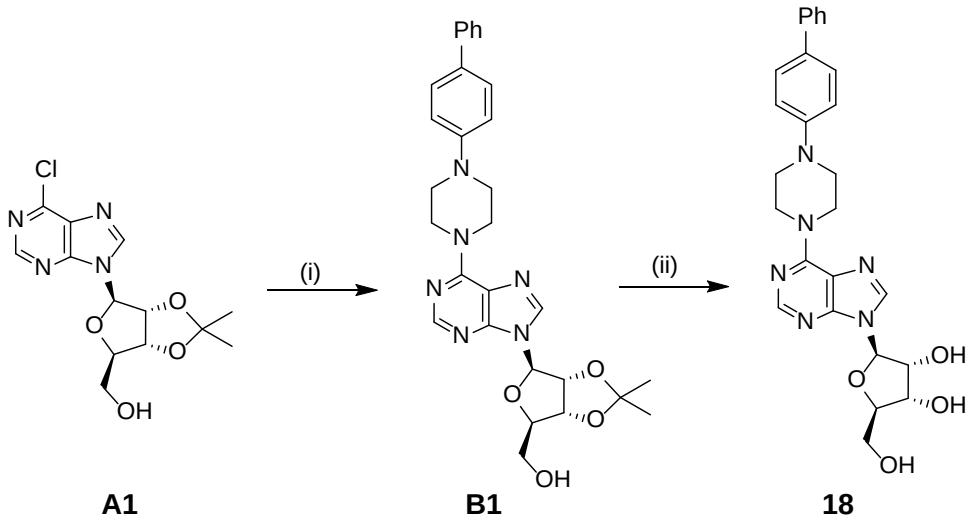
Scheme A0: Synthesis of (2R,3R,4S,5R)-2-(7-(4-([1-phenyl]-4-yl)piperazin-1-yl)-3H-imidazo[4,5-b]pyridin-3-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (7)



A0 **7**

Reagents: (b) 1-([1-phenyl]-4-yl)piperazine, DIEA, EtOH, 70 °C, 17 h.

Scheme A: Synthesis of (2R,3R,4S,5R)-2-(6-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (18)



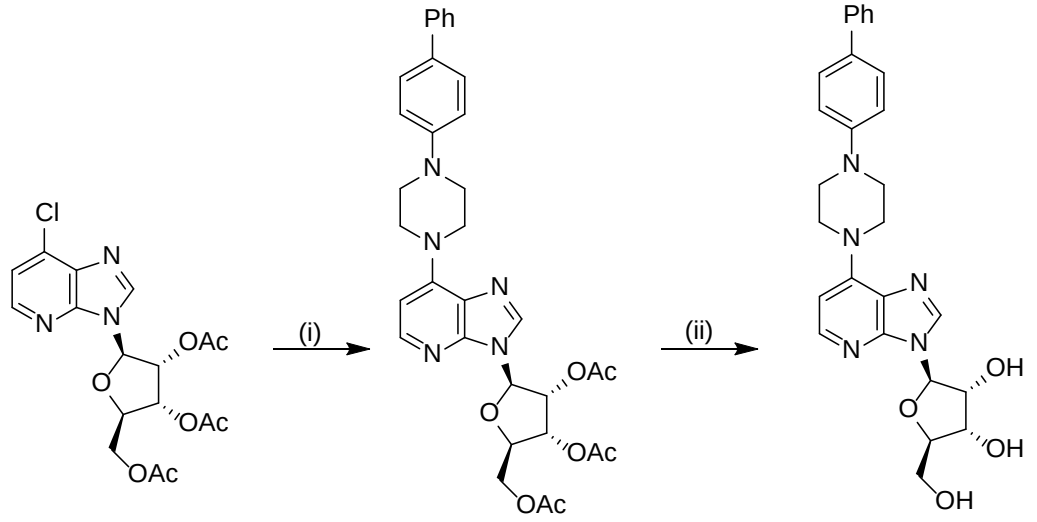
A1 **B1** **18**

Reagents: (i) 1-([1,1'-biphenyl]-4-yl)piperazine hydrochloride, DIEA, EtOH, 80 °C, 17 h; (ii) TFA, THF/H_2O, 15 °C, 2 h.

((3aR,4R,6R,6aR)-6-(6-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol (B1)

To a suspension of 1-([1,1'-biphenyl]-4-yl) piperazine hydrochloride (2.7 g, 8.84 mmol) in anhydrous EtOH (70 mL) were added DIEA (4.28 mL, 24.48 mmol) and ((3aR,4R,6R,6aR)-6-(6-chloro-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol **A1** (2.0 g, 6.12 mmol)[1] at room temperature. After it was stirred at 80 °C for 17 h, the mixture was diluted with EtOH (30 mL), filtered. The resulting precipitate was washed with EtOH (20 mL×3), dried under reduced pressure to afford ((3aR,4R,6R,6aR)-6-(6-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol **B1** (3.1 g, 90% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.44 (s, 1H), 8.31 (s, 1H), 7.61-7.55 (m, 4H), 7.43-7.39 (m, 2H), 7.29-7.27 (m, 1H), 7.09 (d, J = 8.4 Hz, 2H), 6.17 (d, J = 2.0 Hz, 1H), 5.35-5.34 (m, 1H), 5.23 (t, J = 4.8 Hz, 1H), 4.98-4.97 (m, 1H), 4.39 (brs, 4H), 4.24 (s, 1H), 3.59-3.52 (m, 2H), 3.35 (s, 4H), 1.51 (s, 3H), 1.28 (s, 3H). MS (ESI) m/z: 529.4 $[M+H]^+$.

Scheme B: Synthesis of (2R,3R,4S,5R)-2-(7-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-3H-imidazo[4,5-b]pyridin-3-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (8)



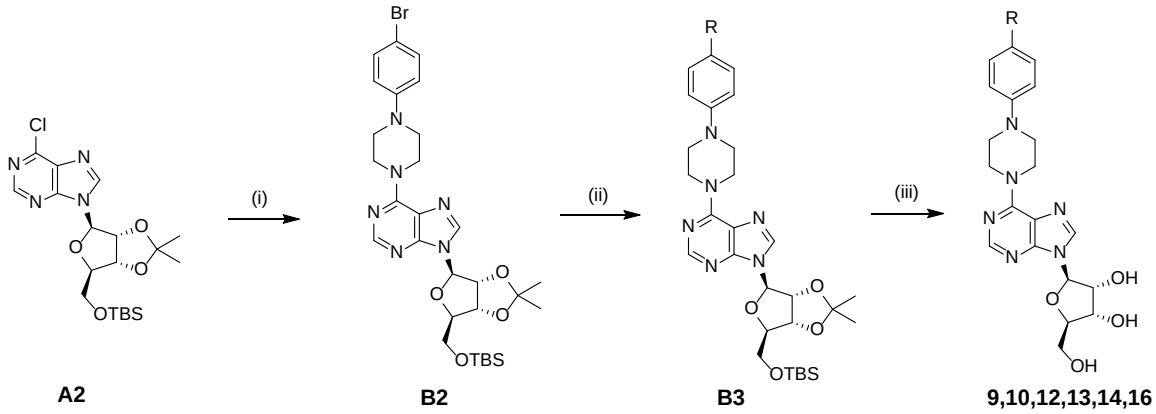
A4 **B4** **8**

Reagents: (i) Cs_2CO_3, 1-([1,1'-biphenyl]-4-yl)piperazine, RuPhos Pd G2, *tert*-Amyl-OH, 100 °C, 17 h; (ii) NH_4OH, MeOH, 15 °C, 24 h.

(2R,3R,4R,5R)-2-(7-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-3H-imidazo[4,5-b]pyridin-3-yl)-5-(acetoxymethyl)tetrahydrofuran-3,4-diyl diacetate (B4)

To the suspension of (2R,3R,4R,5R)-2-(acetoxymethyl)-5-(7-chloro-3H-imidazo[4,5-b]pyridin-3-yl)tetrahydrofuran-3,4-diyl diacetate **A4** (75 mg, 0.17 mmol)[2], Cs_2CO_3 (166 mg, 0.51 mmol) and 1-([1,1'-biphenyl]-4-yl)piperazine (63 mg, 0.25 mmol) in *tert*-amyl alcohol (2 mL) was added RuPhos Pd G2 (13 mg, 0.017 mmol) and it was refluxed for 17 h under N_2 atmosphere. After cooling to room temperature, MeOH (1 mL) was added and it was filtered. The filtrate was concentrated under reduced pressure to afford crude (2R,3R,4R,5R)-2-(7-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-3H-imidazo[4,5-b]pyridin-3-yl)-5-(acetoxymethyl)tetrahydrofuran-3,4-diyl diacetate **B4** (133 mg, 49.6% yield (38.8% purity)) as a yellow solid which was directly used in the next reaction without further purification. MS (ESI) m/z: 488.1, 530.1[M-83&M-125]$^+$.

Scheme C: Synthesis of 9, 10, 12, 13, 14, 16



A2 **B2** **B3** **9,10,12,13,14,16**

Reagents: (i) 1-(4-bromophenyl)piperazine, DIEA, EtOH, 80 °C, 17 h; (ii) $RB(OH)_2$, K_3PO_4, XPhos Pd G2, THF/H_2O, 70 °C, 17 h; (iii) TFA, THF/H_2O, 15–25 °C, 2–17 h.

6-(4-(4-bromophenyl)piperazin-1-yl)-9-((3aR,4R,6R,6aR)-6-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purine (B2)

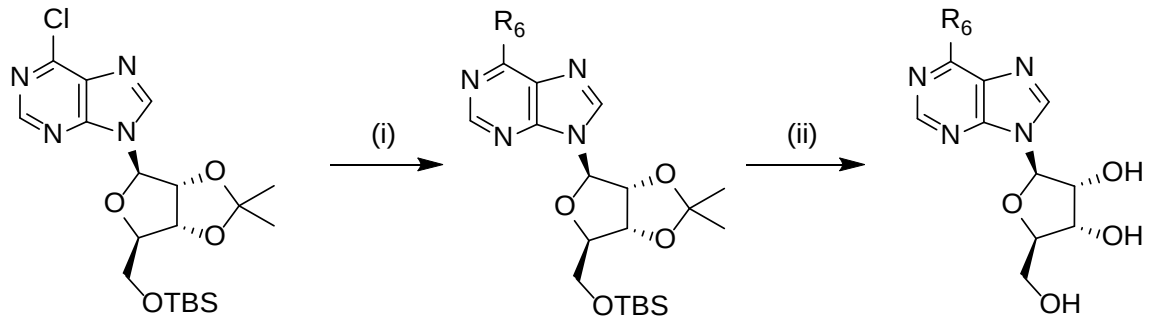
To a suspension of 1-(4-bromophenyl)piperazine (2.7 g, 8.84 mmol) in anhydrous EtOH (70 mL) were added DIEA (4.28 mL, 24.48 mmol) and 9-((3aR,4R,6R,6aR)-6-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-6-chloro-9H-purine **A2** (2.7 g, 6.12 mmol)[25] at room temperature and it was stirred at 80 °C for 17 h. The mixture was diluted with EtOH (30 mL), filtered. The resulting solid was washed with EtOH (20 mL×3), dried under reduced pressure to afford 6-(4-(4-bromophenyl)piperazin-1-yl)-9-((3aR,4R,6R,6aR)-6-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purine **B2** (3.5 g, 90% yield) as a white solid. MS (ESI) m/z: 645.2, 647.2 $[M+H]^+$& 667.2, 669.2 $[M+Na]^+$.

9-((3aR,4R,6R,6aR)-6-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-6-(4-(4-(6-methoxypyridin-3-yl)phenyl)piperazin-1-yl)-9H-purine (B3-1, R=(6-methoxypyridin-3-yl)phenyl)

Into the suspension of (6-methoxypyridin-3-yl)boronic acid (27.5 mg, 0.180 mmol), 6-(4-(4-bromophenyl)piperazin-1-yl)-9-((3aR,4R,6R,6aR)-6-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purine **B2** (97 mg, 0.150 mmol), and

XPhos Pd G2 (11.8 mg, 0.015 mmol) in anhydrous THF (1.5 mL) was added potassium phosphate tribasic (0.300 mL, 0.300 mmol). The mixture was stirred at 70 °C under N_2 for 17 h and then diluted with EtOAc (20 mL), washed with water (10 mL), separated, dried over $MgSO_4$, filtered and concentrated to give the crude product 9-((3aR,4R,6R,6aR)-6-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-6-(4-(4-(6-methoxypyridin-3-yl)phenyl)piperazin-1-yl)-9H-purine **B3-1** (100 mg, 87% yield) as a yellow solid, which was used in next step without further purification. MS (ESI) m/z: 674.3 $[M+H]^+$.

Scheme D: **Synthesis of (2R,3S,4R,5R)-2-(hydroxymethyl)-5-(6-(4-(phenylethynyl)phenyl)-9H-purin-9-yl)tetrahydrofuran-3,4-diol (11) and (2R,3R,4S,5R)-2-(6-([1,1'-biphenyl]-4-ylethynyl)-9H-purin-9-yl)-5-(hydroxymethyl) tetrahydrofuran-3,4-diol (17)**



A2 **C1** **11**

Reagents: (i) 4,4,5,5-tetramethyl-2-(4-(phenylethynyl)phenyl)-1,3,2-dioxaborolane, K_3PO_4, XPhos Pd G2, THF, 70 °C, 17 h or 4-ethynyl-1,1'-biphenyl, Cs_2CO_3, CuI, XPhos Pd G2, CH_3CN, 90 °C, 17 h; (ii) TFA, THF/H_2O, 20 °C, 17 h.

9-((3aR,4R,6R,6aR)-6-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-6-(4-(phenylethynyl)phenyl)-9H-purine (C1-1, R_6=4-(phenylethynyl)phenyl)

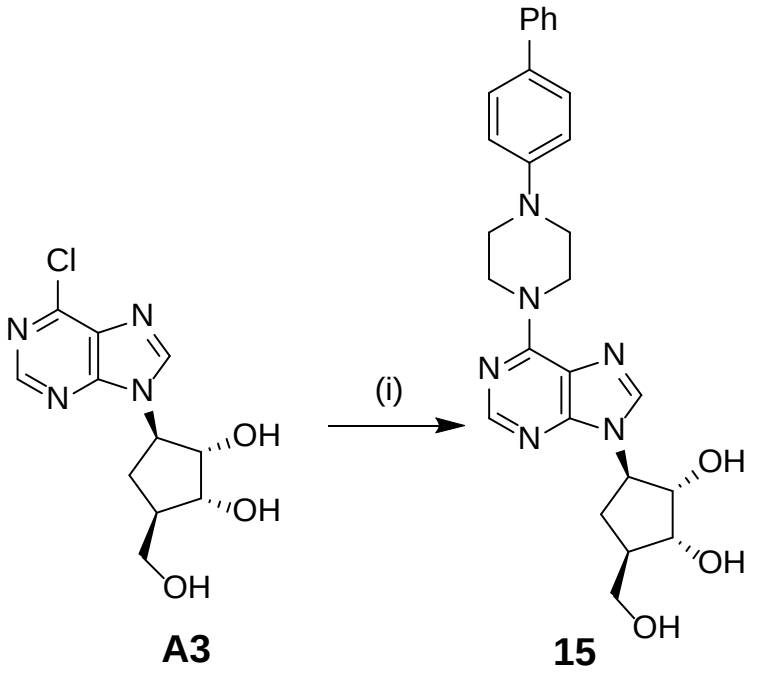
To a suspension of 4,4,5,5-tetramethyl-2-(4-(phenylethynyl)phenyl)-1,3,2-dioxaborolane (260 mg, 0.49 mmol), 9-((3aR,4R,6R,6aR)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-6-chloro-9H-purine **A2** (100 mg, 0.23 mmol)[25] in

aqueous K_3PO_4 (1 M) and THF (2.0 mL) was added XPhos Pd G2 (9 mg, 0.000011 mmol) under N_2 atmosphere and it was stirred at 70 °C for 17 h. The mixture was diluted with water (20 mL), extracted with EtOAc (10 mLx3). The combined organic layers were washed with brine (20 mLx3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give crude 9-((3aR,4R,6R,6aR)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-6-(4-(phenylethynyl)phenyl)-9H-purine **C1-1** (260 mg, 95% yield) as a yellow oil which was directly used in the next reaction without further purification. MS (ESI) m/z: 583.1$[M+H]^+$.

6-([1,1'-biphenyl]-4-ylethynyl)-9-((3aR,4R,6R,6aR)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purine (C1-2, R_6=[1,1'-biphenyl]-4-ylethynyl)

To the suspension of 4-ethynyl-1,1'-biphenyl (58 mg, 0.32 mmol), Cs_2CO_3 (163 mg, 0.50 mmol), CuI (0.950 mg, 4.99 μmol) and the XPhos Pd G2 (10 mg, 0.012 mmol) in anhydrous CH_3CN (1 mL) was added 9-((3aR,4R,6R,6aR)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-6-chloro-9H-purine **A2** (110 mg, 0.25 mmol)[25] under N_2 atmosphere. After it was stirred at 90 °C for 17 h under N_2 atmosphere, the mixture was concentrated and purified by PTLC (SiO_2, Petroleum ether : EtOAc = 3 : 1) to give 6-([1,1'-biphenyl]-4-ylethynyl)-9-((3aR,4R,6R,6aR)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purine **C1-2** (46 mg, 27.2% yield) as a yellow solid. MS (ESI) m/z: 583.1 $[M+H]^+$.

Scheme F: Synthesis of (1R,2S,3R,5R)-3-(6-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-9H-purin-9-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (15)

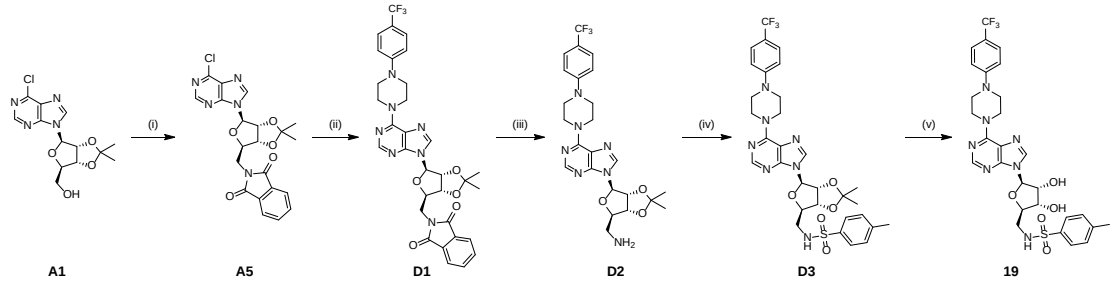


A3 15

Reagents: (i) 1-([1,1'-biphenyl]-4-yl)piperazine, DIEA, EtOH, 70 °C, 17 h.

Synthesis of 19-24

Scheme 1: Synthesis of N-(((2R,3S,4R,5R)-3,4-dihydroxy-5-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl)-4-methylbenzenesulfonamide (19)



Reagents: (i) Phthalimide, DIAD, PPh_3, THF, 25 °C, 17 h; (ii) 1-(4-(trifluoromethyl)phenyl)piperazine, DIEA, EtOH, 60 °C, 17 h; (iii) hydrazine hydrate, EtOH, 90 °C, 17 h; (iv) 4-methylbenzenesulfonyl chloride, DIEA, DCM, 15 °C, 17 h； (v) TFA, THF/H_2O, 15 °C, 17 h.

2-(((3aR,4R,6R,6aR)-6-(6-chloro-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)isoindoline-1,3-dione (A5)

The mixture of triphenylphosphine (482 mg, 1.836 mmol), ((3aR,4R,6R,6aR)-6-(6-chloro-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol **A1** (400 mg, 1.224 mmol), phthalimide (189 mg, 1.285 mmol) in THF (10 mL) and DIAD (0.286 mL, 1.469 mmol) was stirred at 25 °C for 17 h then concentrated and purified by silica gel column chromatography (Petroleum ether : EtOAc =1 : 1) to give 2-(((3aR,4R,6R,6aR)-6-(6-chloro-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)isoindoline-1,3-dione **A5** (600 mg, 95% yield) as a white solid. MS (ESI) m/z: 456.1 $[M+H]^+$.

2-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)isoindoline-1,3-dione (D1)

To the mixture of DIEA (0.607 mL, 3.47 mmol) and 1-(4-(trifluoromethyl)phenyl)piperazine (267 mg, 1.158 mmol) in EtOH (5 mL) was added 2-(((3aR,4R,6R,6aR)-6-(6-chloro-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)isoindoline-1,3-dione **A5** (600 mg, 1.158 mmol). Then the mixture was stirred at 60 °C for 17 h, cooled, filtered to give 2-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)isoindoline-1,3-dione **D1** (650 mg, 82% yield) as yellow solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (s, 3H), 7.77-7.76 (m, 3H), 7.67 (dd, J_1 = 1.6 Hz, J_2 = 4.8 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 6.02 (s, 1H), 6.22-6.10 (m, 1H), 5.50 (dd, J_1 = 3.2 Hz, J_2 = 6.4 Hz, 1H), 5.22 (dd, J_1 = 3.6Hz, J_2 = 6.4 Hz, 1H), 4.55-4.43 (m, 5H), 3.95-4.05 (m, 2H), 3.39 (brs, 4H), 1.56 (s, 3H), 1.33 (s, 3H). MS (ESI) m/z: 650.1 $[M+H]^+$.

((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanamine (D2).

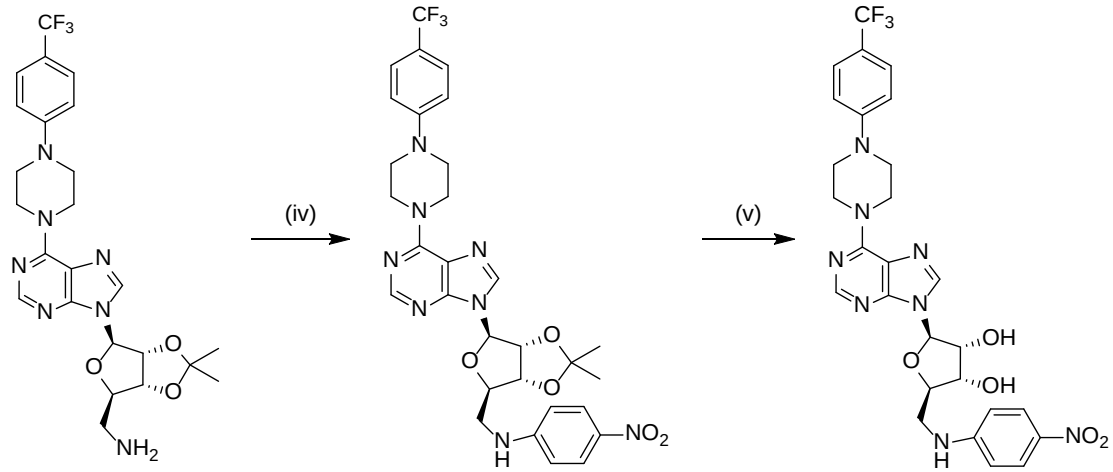
The mixture of 2-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)isoindoline-1,3-dione **D1** (650 mg, 1.0 mmol) and hydrazine hydrate (102 mg, 2.0 mmol) in EtOH (5 mL) was stirred at 90 °C for 17 h, then it was cooled, filtered, and concentrated to give ((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-

purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanamine **F4** (450 mg, 78% yield) as a yellow solid, which was used in next step without further purification. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.33-8.18 (m, 2H), 7.50 (d, J = 8.6 Hz, 2H), 7.17-7.02 (m, 2H), 6.22-6.10 (m, 1H), 5.45 (dd, J_1 = 3.0Hz, J_2 = 6.3 Hz, 1H), 5.02 (dd, J_1 = 3.4Hz, J_2 = 6.3 Hz, 1H), 4.43 (brs, 4H), 4.30-4.19 (m, 1H), 3.52-3.38 (m, 2H), 3.00-2.81 (m, 1H), 1.60 (s, 3H), 1.38 (s, 3H). MS (ESI) m/z: 520.2 $[M+H]^+$.

N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-4-methylbenzenesulfonamide (D3)

To a solution of ((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanamine **D2** (50 mg, 0.096 mmol) and DIEA (0.050 mL, 0.289 mmol) in anhydrous DCM (2 mL) was added 4-methylbenzene-1-sulfonyl chloride (18.35 mg, 0.096 mmol). The mixture was stirred at 15 °C for 17 h, then it was concentrated under reduced pressure to afford N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-4-methylbenzenesulfonamide **D3** (72 mg, 94% yield) as a light yellow oil. The crude product was used in next step without purification. MS (ESI) m/z: 674.3 $[M+H]^+$.

Scheme 2: Synthesis of (2R,3S,4R,5R)-2-(((4-nitrophenyl)amino)methyl)-5-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuran-3,4-diol (20)



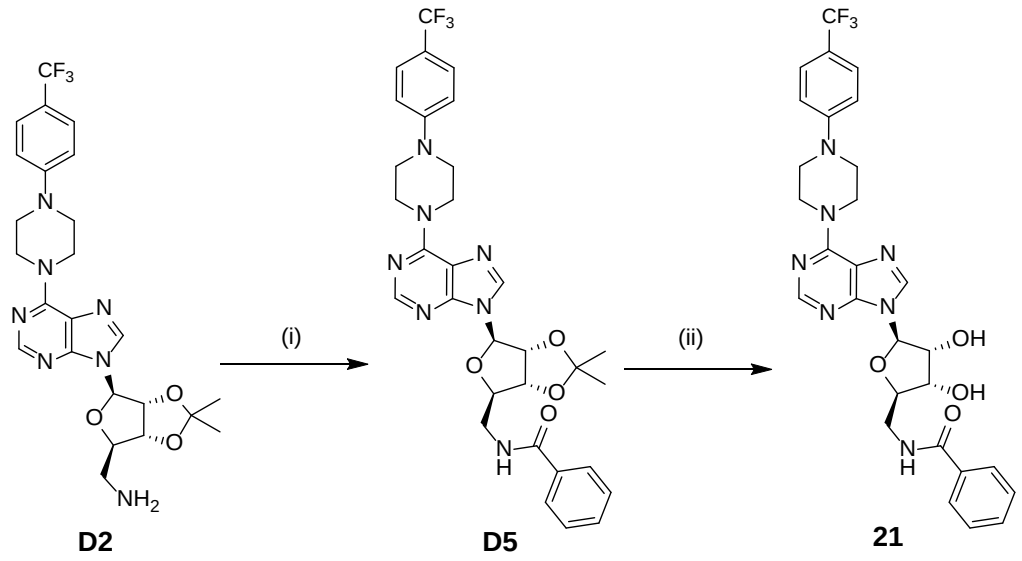
D2 **D4** **20**

Reagents: (i) 1-fluoro-4-nitrobenzene, K_2CO_3, DABCO, DMF, 100 °C, 17 h; (ii) TFA, THF/H_2O, 20 °C, 17 h.

Synthesis of N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-4-nitroaniline (D4)

To a solution of ((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9- yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanamine **D2** (200 mg, 0.385 mmol) and 1-fluoro-4-nitrobenzene (109 mg, 0.770 mmol) in DMF (4 mL) was added K_2CO_3 (106 mg, 0.770 mmol) and DABCO (86 mg, 0.770 mmol). The mixture was stirred at 100 °C for 17 h, then it was diluted with EtOAc (50 mL), washed with water (30 mL), the organic layer was separated and concentrated to give N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4- (trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-4-nitroaniline **F5** (160 mg, 57.7% yield) as a yellow solid, which was used in next step directly without further purification. MS (ESI) m/z: 641.1 $[M+H]^+$.

Scheme 3: Synthesis of N-(((2R,3S,4R,5R)-3,4-dihydroxy-5-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl)benzamide (21)



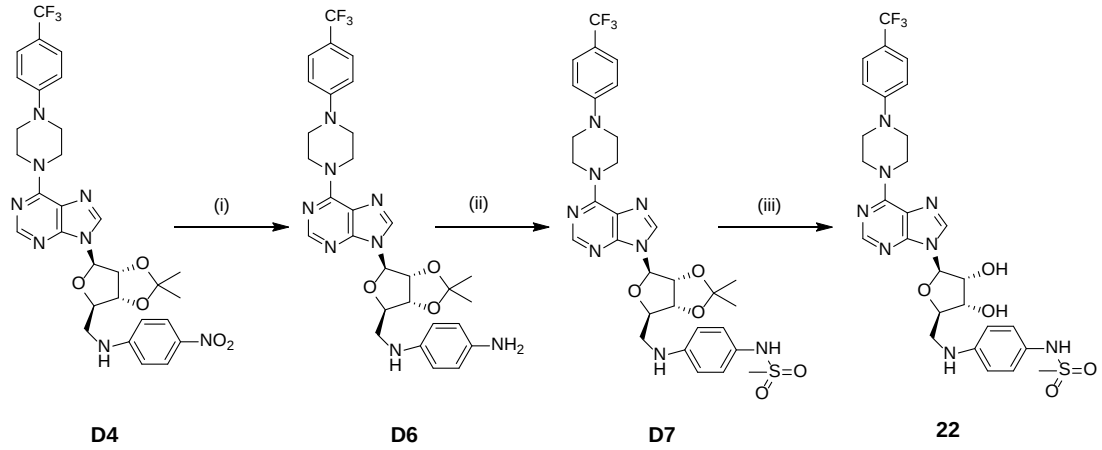
D2 D5 21

Reagents: (i) benzoyl chloride, DIEA, DCM, 15 °C, 17 h; (ii) TFA, THF/H_2O, 15 °C, 17 h.

Synthesis of N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)benzamide (D5)

To a solution of ((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanamine **D2** (50 mg, 0.096 mmol) and DIEA (0.050 mL, 0.289 mmol) in anhydrous DCM (2 mL) was added benzoyl chloride (16.23 mg, 0.115 mmol). The mixture was stirred at 15 °C for 17 h, then it was concentrated under reduced pressure to afford N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)benzamide **D5** (68 mg, 94% yield) as a light yellow oil. The crude product was used in next step without purification. MS (ESI) m/z: 624.3$[M+H]^+$.

Scheme 4: Synthesis of N-(4-((((2R,3S,4R,5R)-3,4-dihydroxy-5-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl)amino)phenyl)methanesulfonamide (22)



Reagents: (i) Pd/C, H_2, EtOH, 20 °C, 2 h; (ii) MsCl, DIEA, DCM, 20 °C, 17 h; (iii) TFA, THF/H_2O, 20 °C, 17 h;

Synthesis of N1-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)benzene-1,4-diamine (D6)

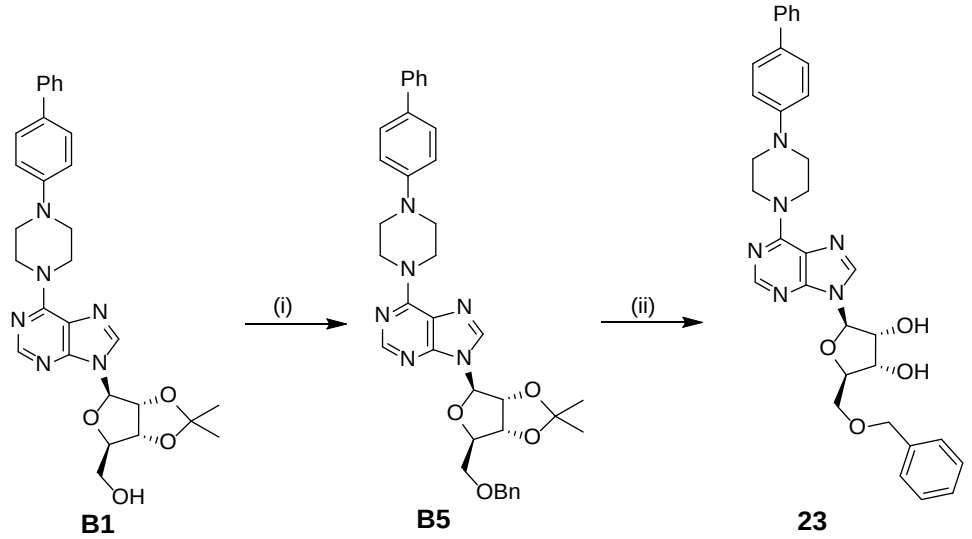
The mixture of N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9- yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-4-nitroaniline **D4** (100 mg, 0.156 mmol) and Pd/C (166 mg, 0.156 mmol) in EtOH (10 mL) was stirred at 20 °C for 2 h under H_2 balloon. The mixture was filtered and the filtrate was concentrated to give N1-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)benzene-1,4-diamine **D6** (100 mg, 86% yield) as a yellow solid, which was used in next step directly. MS (ESI) m/z: 610.9 $[M+H]^+$.

Synthesis of N-(4-((((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)phenyl)methanesulfonamide (D7)

To the solution of DIEA (0.051 mL, 0.295 mmol) and N1-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4- (trifluoromethyl)phenyl)piperazin-

1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)benzene-1,4-diamine **D6** (60 mg, 0.098 mmol) in DCM (5 mL) was added methanesulfonyl chloride (13.51 mg, 0.118 mmol) at 20 °C. The mixture was stirred at 20 °C for 17 h, then it was diluted with DCM (20 mL), washed with water (10 mL), separated, dried over $MgSO_4$, filtered and concentrated to give N-(4-((((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)phenyl)methanesulfonamide **D7** (70 mg, 57.9% yield) as a yellow solid. MS (ESI) m/z: 689.2 $[M+H]^+$.

Scheme 5: Synthesis of (2R,3R,4S,5R)-2-(6-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-9H-purin-9-yl)-5-((benzyloxy)methyl)tetrahydrofuran-3,4-diol (23)



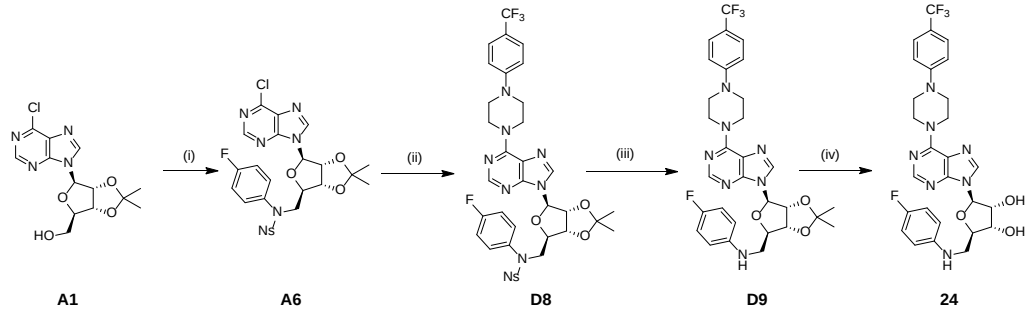
Reagents: (i) benzyl bromide, NaH, THF, 20 °C, 17 h; (ii) HCl in MeOH, 25 °C, 1 h.

Synthesis of 6-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-9-((3aR,4R,6R,6aR)-6-((benzyloxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purine (B5)

To the solution of ((3aR,4R,6R,6aR)-6-(6-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol **B1** (600 mg, 1.135 mmol) in THF (5 mL) was added NaH (54.5 mg, 1.362 mmol) and then benzyl bromide (0.149 mL, 1.249 mmol) at 20 °C. The mixture was stirred at 20 °C for 17 h, then it was diluted with EtOAc (50 mL), washed with water (30 mL) and brine (30 mL), separated, dried over $MgSO_4$, and filtered, concentrated to give 6-(4-([1,1'-biphenyl]-4-yl)piperazin-1-yl)-9-((3aR,4R,6R,6aR)-6-((benzyloxy)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purine **B5** (700 mg,

73.8% yield) as a yellow solid. MS (ESI) m/z: 619.1 $[M+H]^+$.

Scheme 6: Synthesis of (2R,3S,4R,5R)-2-(((4-fluorophenyl)amino)methyl)-5-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuran-3,4-diol (24)



Reagents: (i) N-(4-fluorophenyl)-4-nitrobenzenesulfonamide, DIAD, PPh_3, THF, 25 °C, 17 h; (iii) 1-(4-(trifluoromethyl)phenyl)piperazine, DIEA, EtOH, 70 °C, 17 h; (iv) thiophenol, K_2CO_3, CH_3CN, 40 °C, 17 h; (v) HCl in MeOH, 25 °C, 1 h.

Synthesis of N-(((3aR,4R,6R,6aR)-6-(6-chloro-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-N-(4-fluorophenyl)-4-nitrobenzenesulfonamide (A6)

The mixture of triphenylphosphine (241 mg, 0.918 mmol), ((3aR,4R,6R,6aR)-6-(6-chloro-9H-purin-9-yl)-2,2- dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol **A1** (200 mg, 0.612 mmol) and N-(4-fluorophenyl)-2-nitrobenzenesulfonamide (181 mg, 0.612 mmol) and DIAD (0.143 mL, 0.735 mmol) in THF (10 mL) was stirred at 25 °C for 17 h, then it was concentrated and purified by silica gel column chromatography (Petroleum ether : EtOAc = 1 : 1) to give N-(((3aR,4R,6R,6aR)-6-(6-chloro- 9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-N-(4-fluorophenyl)-2-nitrobenzenesulfonamide **A6** (300 mg, 64.8% yield) as a white solid. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.15 (s, 1H), 7.57-7.50 (m, 2H), 7.40-7.38 (m, 2H), 7.08 (dd, J_1 = 3.6 Hz, J_2 = 8.8 Hz, 2H), 6.88 (t, J = 8.4 Hz, 2H), 6.13 (s, 1H), 5.61 (d, J = 6.4 Hz, 1H), 5.20 (d, J = 4.4 Hz, 1H), 4.40-4.37 (m, 1H), 4.16-4.14 (m, 1H), 3.92-3.83 (m, 1H), 1.58 (s, 3H), 1.41 (s, 3H). MS (ESI) m/z: 605.1 $[M+H]^+$.

Synthesis of N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-N-(4-fluorophenyl)-4-nitrobenzenesulfonamide (D8)

To the solution of 1-(4-(trifluoromethyl)phenyl)piperazine (137 mg, 0.595 mmol) and DIEA (0.087 mL, 0.496 mmol) in EtOH (5 mL) was added N-(((3aR,4R,6R,6aR)-6-(6-chloro-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-N-(4-fluorophenyl)-2-nitrobenzenesulfonamide **A6** (300 mg, 0.496 mmol). The mixture was stirred at 70 °C for 17 h, then it was cooled and concentrated, purified by silica gel column chromatography (Petroleum ether: EtOAc = 2: 1) to give N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-N-(4-fluorophenyl)-2-nitrobenzenesulfonamide **D8** (360 mg, 82% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400MHz,) δ 8.30 (s, 1H), 7.72 (s, 1H), 7.55-7.51 (m, 4H), 7.38 (d, *J* = 2.8 Hz, 2H), 7.02-6.97 (m, 4H), 6.80 (t, *J* = 7.6 Hz, 2H), 6.03 (s, 1H), 6.73-6.62 (m, 2H), 5.60 (d, *J* = 6.4 Hz, 1H), 5.12 (d, *J* = 4.4 Hz, 1H), 4.46-4.40 (m, 5H), 4.22-4.17 (m, 1H), 4.04-3.98 (m, 1H), 3.45-3.42 (m, 4H), 1.57 (s, 3H), 1.40 (s, 3H). MS (ESI) m/z: 798.9 $[M+H]^+$.

Synthesis of N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-4-fluoroaniline (D9)

The mixture of N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin- 9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-N-(4-fluorophenyl)- 2-nitrobenzenesulfonamide **D8** (300 mg, 0.376 mmol), thiophenol (0.077 mL, 0.751 mmol) and K_2CO_3 (208 mg, 1.502 mmol) in CH_3CN (2 mL) was stirred at 40 °C for 17 h, then it was cooled, diluted with DCM (30 mL), washed with water (30 mL) and brine (30 mL), separated, dried over $MgSO_4$, filtered and concentrated to give crude N-(((3aR,4R,6R,6aR)-2,2-dimethyl-6-(6-(4-(4- (trifluoromethyl)phenyl)piperazin-1-yl)-9H-purin-9-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-4-fluoroaniline **D9** (200 mg, 60.7% yield) as a yellow solid. MS (ESI) m/z: 614.3 $[M+H]^+$.

SUPPLEMENTARY FIGURES AND TABLES

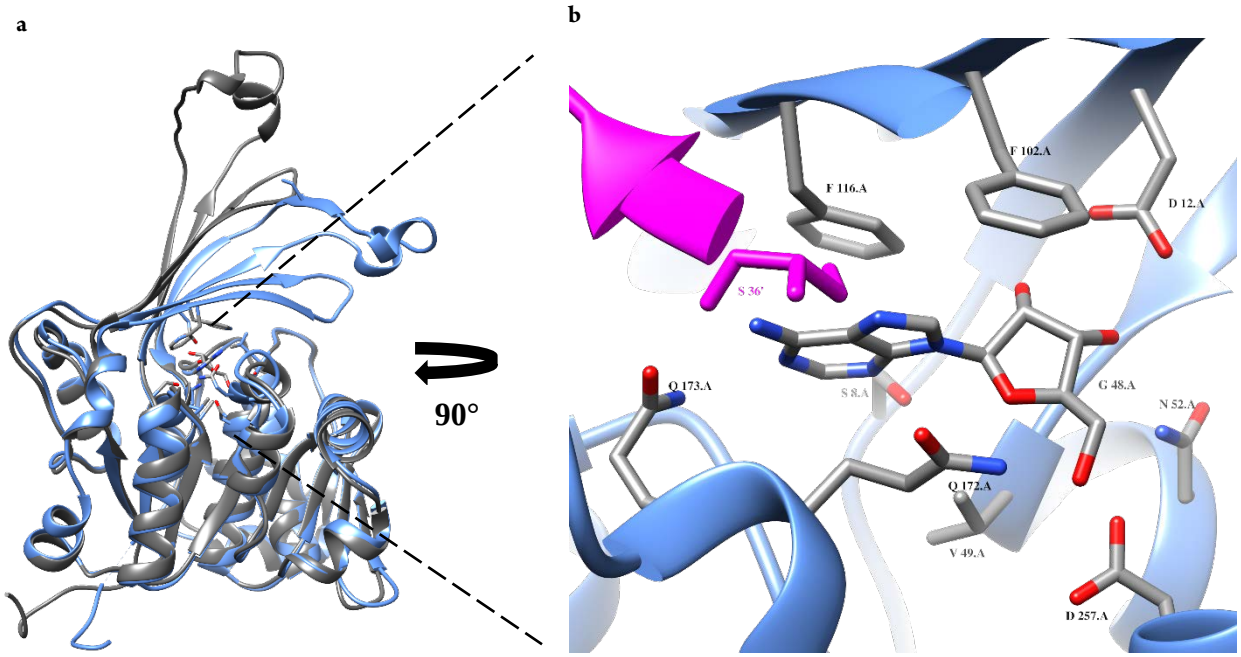


Figure S1. (a) Superimposition of the closed (blue, PDB ID 2PKM) and open conformations (gray, PDB ID 2PKF) of MtbAdoK. Only chain A of the dimer is shown. (b) Adenosine bound to the active site of MtbAdoK. Adenosine and residues involved in binding are shown as sticks and labeled with one-letter code and chain identifier. Chain B residues are colored magenta.

Table S1. Close contacts of the MtbAdoK-**2** complex ≤ 3.5 Å.

Atom	Residue	Distance (Å)
C2	Ser8.A OG	3.17
C2	Ser8.A CB	3.41
N3	Ser8.A OG	2.70
N3	Ser8.A CB	3.38
N6	Gln173.A OE1	3.29
N6	HOH501.B O	3.36
C2’	Asp12.A OD1	3.45
C3’	Asp12.A OD2	3.22
C5’	Asp257.A OD2	3.32
C5’	HOH618.A	3.35
O2’	Asp12.A OD1	2.67
O2’	Gly48.A N	3.08
O2’	Phe102.A CB	3.35
O2’	Ala10.A CB	3.48
O3’	Asp12.A OD2	2.53
O3’	Gly48.A N	2.85
O3’	Asn52.A ND2	3.16
O3’	Asp12.A CG	3.24
O3’	Asp12.A OD1	3.20
O3’	Gly47.A CA	3.43
O4’	Val49.A CG1	3.31
O5’	Asp257.A OD2	2.65
O5’	HOH553.A	2.72
O5’	Asp257.A CG	3.41
IAE	HOH501.B O	2.94
IAE	Ser36.B OG	3.07

Table S2. Crystal data collection and refinement statistics for MtbAdoK-**2**.

Statistic	**MtbAdoK-2**
Data collection	
Space Group	$P4_1$
Cell Dimensions	
a, b, c (Å)	49.0, 49.0, 262.2
α, β, γ (°)	90, 90, 90
Resolution (Å)	34.6 - 2.1 (2.18 -2.10)
R_{merge}	0.07 (0.24)
I/σI	23.3 (7.63)
Completeness %	96.5 (98.49)
Redundancy	7.3
Refinement	
Resolution (Å)	2.1
No. of reflections	34593
R_{work}/R_{free}	0.19/0.22
No. of atoms	
Protein	4676
Ligand	98
Water	259
B factors	
Protein	50.6
Ligand/ion	51.0
Water	50.7
rmsd	
Bond lengths (Å)	0.004
Bond angles (°)	1.01

Table S3. Close contacts of the MtbAdoK-**3** complex ≤ 3.5 Å.

Atom	Residue	Distance (Å)
C2	Ser8.A CB	3.44
C2	Ser8.A OG	3.30
N3	Ser8.A OG	2.76
N3	Ser8.A CB	3.35
N3	Phe116.A CZ	3.48
C4	Phe116.A CE1	3.32
N6	Gln173.A OE1	3.25
C8	Phe102.A CD1	3.47
N9	Phe116.A CE1	3.50
N11	Ser36.B OG	3.00
N11	Phe102.A CE1	3.33
N11	HOH601.A O	3.31
C2’	Asp12.A OD1	3.46
C3’	Asp12.A OD2	3.21
C5’	Asp257.A OD2	3.33
C5’	HOH583.A O	3.43
O2’	Asp12.A OD1	2.70
O2’	Gly48.A N	3.01
O2’	Gly48.A CA	3.41
O3’	Asp12.A OD2	2.39
O3’	Asp12.A CG	3.17
O3’	Gly48.A N	3.02
O3’	Asn52.A ND2	3.23
O3’	Asp12.A OD1	3.20
O4’	Val49.A CG1	3.43
O4’	Gln172.A NE2	3.23
O5’	Asp257.A OD2	2.60
O5’	Gln172.A NE2	2.83
O5’	HOH552.A O	2.79
O5’	Asp257.A CG	3.39

Table S4. Crystal data collection and refinement statistics for MtbAdoK-**3**.

Statistic	**MtbAdoK-3**
Data collection	
Space Group	$P4_1$
Cell Dimensions	
a, b, c (Å)	48.9, 48.9, 262.0
α, β, γ (°)	90, 90, 90
Resolution (Å)	34.5-2.1 (2.17-2.10)
R_{merge}	0.07 (0.24)
I/σI	22.8 (6.85)
Completeness %	98.2 (99.80)
Redundancy	7.3
Refinement	
Resolution (Å)	2.1
No. of reflections	35089
R_{work}/R_{free}	0.18/0.23
No. of atoms	
Protein	4660
Ligand	88
Water	241
B factors	
Protein	46.5
Ligand/ion	41.5
Water	42.3
rmsd	
Bond lengths (Å)	0.01
Bond angles (°)	1.09

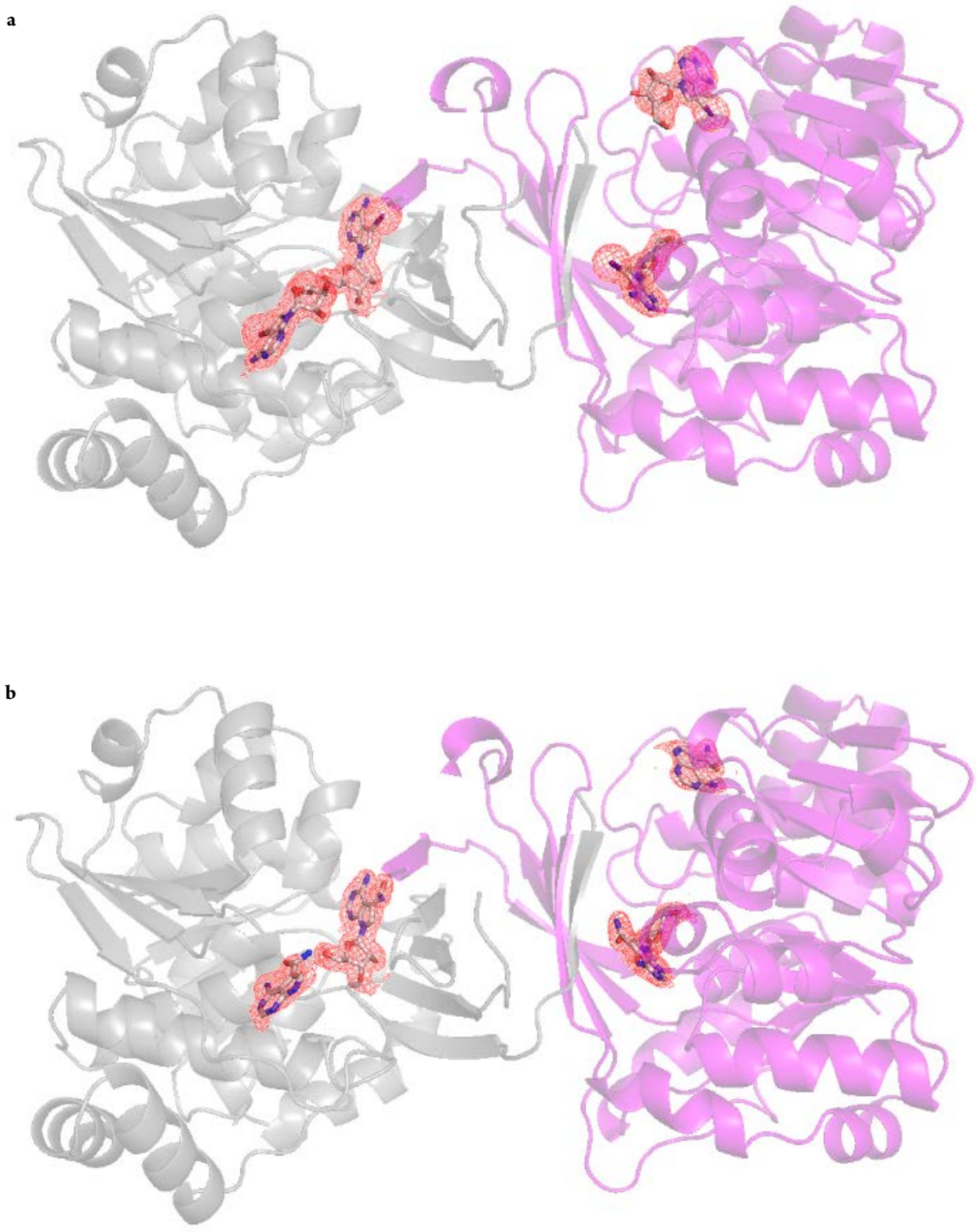


Figure S2. MtbAdoK homodimer embedded with two copies of compound **2** (a) and compound **3** (b) per chain. Chain A is colored gray, and chain B is colored magenta. $2F_o$-F_c maps contoured at 1.6 σ.

Table S5. Close contacts of the MtbAdoK-**1** complex ≤ 3.5 Å. Analyzed from PDB ID 2PKM.

Atom	Residue	Distance (Å)
C2	Ser8.A OG	3.11
N3	Ser8.A OG	2.62
N3	Ser8.A CB	3.47
N3	Phe116.A CE1	3.42
C4	Phe116.A CE1	3.38
C4	Phe116.A CD1	3.39
C5	Gln172.A CB	3.46
C5	Phe116.A CD1	3.39
N1	Gln173.A NE2	2.99
N7	Ser36.B OG	2.65
C8	Phe102.A CE1	3.27
C8	Phe102.A CD1	3.48
C3’	Asp12.A OD2	3.35
C4’	Gln172.A NE2	3.49
O4’	Val49.A CG1	3.22
O4’	Gln172.A NE2	3.26
C5’	Asp257.A OD2	2.87
C5’	Asn52.A ND2	3.31
O5’	Gln172.A NE2	2.62
O5’	Asp257.A OD2	2.69
O5’	Asp257.A CG	3.32
O3’	Asp12.A OD2	2.70
O3’	Gly48.A N	2.99
O3’	Asn52.A ND2	3.01
O3’	Asp12.A CG	3.41
O2’	Gly48.A N	2.82
O2’	Asp12.A OD1	2.76
O2’	Gly48.A CA	3.28
N6	Gln173.A OE1	2.96
N6	Gln172.A O	3.26
N6	Ser36.B OG	3.43

Table S6. Close contacts of the MtbAdoK-**4** complex ≤ 3.5 Å.

Atom	Residue	Distance (Å)
C2	Ser8.A OG	3.43
N3	Ser8.A OG	2.86
N3	Ser8.A CB	3.43
C4	Phe116.A CE1	3.43
C5	Phe116.A CD1	3.41
N7	HOH506.B O	2.87
N7	Phe116.A CD1	3.44
C8	Phe102.A CD1	3.21
C8	Phe102.A CE1	3.40
C3’	Asp12.A OD2	3.27
C5’	Asp257.A OD2	3.24
C5’	HOH575.A O	3.39
O2’	Asp12.A OD1	2.68
O2’	Gly48.A N	3.01
O2’	Phe102.A CB	3.37
O2’	Gly48.A CA	3.46
O3’	Asp12.A OD2	2.47
O3’	Gly48.A N	2.93
O3’	Asp12.A CG	3.24
O3’	Asn52.A ND2	3.18
O3’	Asp12.A OD1	3.27
O4’	Val49.A CG1	3.21
O5’	Asp257.A OD2	2.44
O5’	HOH564.A O	2.78
O5’	Asp257.A CG	3.34
O5’	Gln172.A NE2	3.17
CS	Arg176.A NE	3.41

Table S7. Crystal data collection and refinement statistics for MtbAdoK-**4**.

Statistic	**MtbAdoK-4**
Data collection	
Space Group	$P4_1$
Cell Dimensions	
a, b, c (Å)	49.4, 49.4, 263.9
α, β, γ (°)	90, 90, 90
Resolution (Å)	34.96-2.0 (2.07-2.0)
R_{merge}	0.13 (0.78)
I/σI	14.55 (1.97)
Completeness %	96.0 (97.62)
Redundancy	6.9
Refinement	
Resolution (Å)	2.0
No. of reflections	40839
R_{work}/R_{free}	0.20/0.23
No. of atoms	
Protein	4675
Ligand	80
Water	266
B factors	
Protein	49.0
Ligand/ion	49.6
Water	48.0
rmsd	
Bond lengths (Å)	0.004
Bond angles (°)	1.0

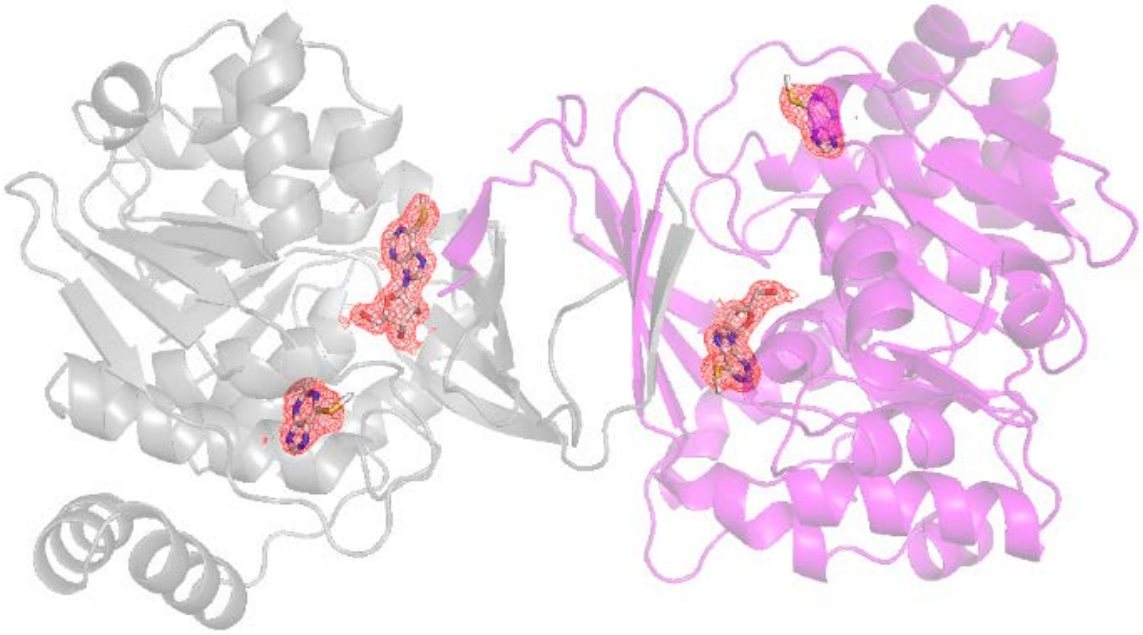


Figure S3. MtbAdoK dimer bound to two copies of compound **4** per chain. Chain A is colored gray, and chain B is colored magenta. $2F_o$-F_c map contoured at 1.6 σ.

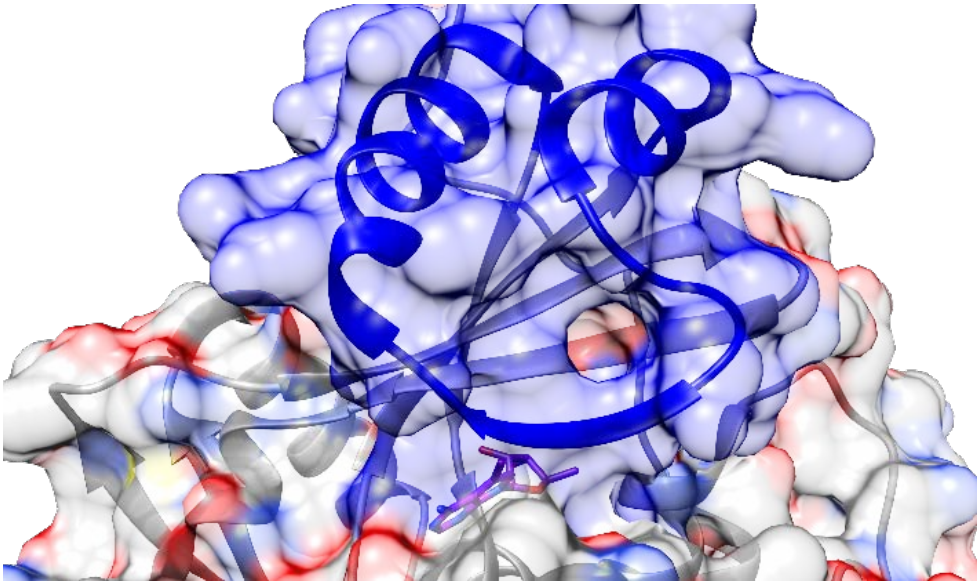


Figure S4. hAdoK-**2** complex (PDB ID 2i6a). **2** (purple) is completely buried within hAdoK due to the latch-like region (blue) of the lid domain formed by residues 23-57.

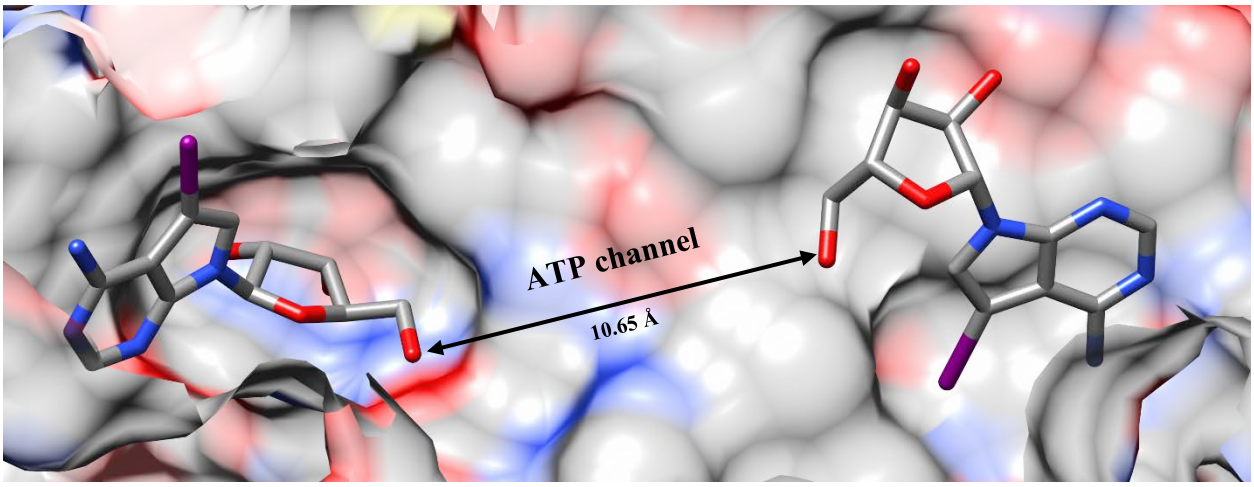


Figure S5. MtbAdoK-**2** structure showing the relative position of the iodotubercidin bound to the active site (left) to that bound to the ATP site (right). The orientation and proximity of the molecules suggest that bisubstrate-like inhibitors might take advantage of both binding sites.

Table S8. Close contacts of the MtbAdoK-**5** complex ≤ 3.5 Å.

Atom	Residue	Distance (Å)
N1	Gln173.A NE2	2.99
N1	Gln173.A CD	3.44
C2	Ser8.A OG	3.40
N3	Ser8.A OG	2.75
N3	Ser8.A CB	3.39
N3	Phe116.A CE2	3.46
C4	Phe116.A CD2	3.36
C4	Phe116.A CE2	3.40
C5	Phe116.A CD2	3.36
N6	Gln173.A OE1	2.94
N7	Ser36.B OG	2.71
N7	Ser36.B CB	3.38
N7	Gln172.A CG	3.46
C8	Phe102.A CE1	3.20
C8	Phe102.A CD1	3.41
C2’	Asp12.A OD1	3.46
C3’	Asp12.A OD2	3.24
C5’	HOH584.A O	3.18
C5’	Asp257.A OD2	3.18
O2’	Gly48.A N	2.92
O2’	Asp12.A OD1	2.71
O2’	Gly48.A CA	3.33
O3’	Asp12.A OD2	2.50
O3’	Gly48.A N	2.98
O3’	Asp12.A CG	3.23
O3’	Asn52.A ND2	3.09
O3’	Asp12.A OD1	3.23
O4’	Val49.A CG1	3.26
N5’	Asp257.A OD2	2.61
N5’	HOH607.A O	2.82
N5’	HOH611.A O	2.85
N5’	Asp257.A CG	3.37
N5’	HOH584.A O	3.02

Table S9. Crystal data collection and refinement statistics for MtbAdoK-**5**.

Statistic	**MtbAdoK-5**
Data collection	
Space Group	$P3_12_1$
Cell Dimensions	
a, b, c (Å)	71.9, 71.9, 110.2
α, β, γ (°)	90, 90, 120
Resolution (Å)	41.2-1.9 (2.02-1.95)
R_{merge}	0.07 (0.16)
I/σI	40.2 (17.16)
Completeness %	99.8 (99.92)
Redundancy	10.7
Refinement	
Resolution (Å)	1.95
No. of reflections	24596
R_{work}/R_{free}	0.18/0.20
No. of atoms	
Protein	2483
Ligand	19
Water	206
B factors	
Protein	34.1
Ligand/ion	17.8
Water	36.0
rmsd	
Bond lengths (Å)	0.009
Bond angles (°)	1.2

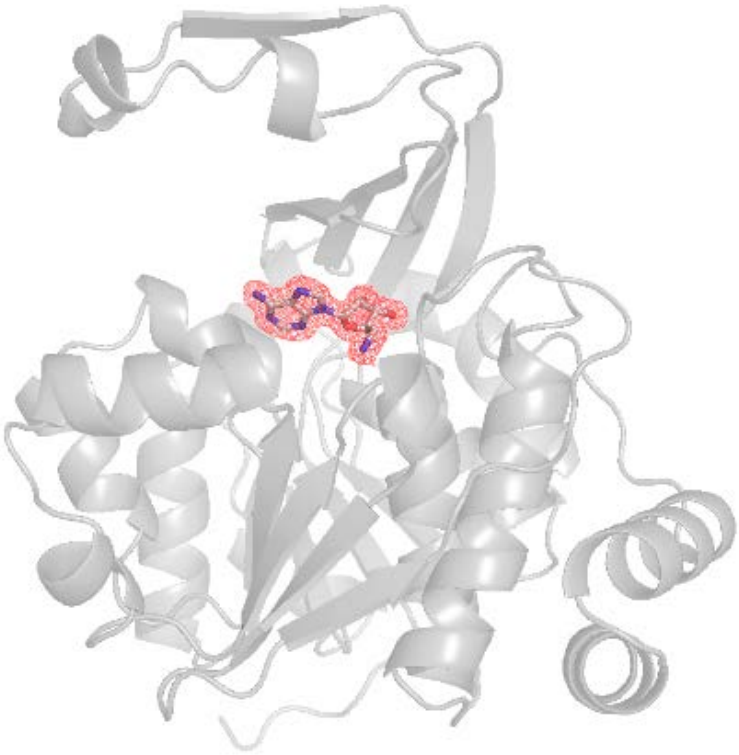


Figure S6. MtbAdoK bound to **5**. Only MtbAdoK monomer observed in asymmetric unit shown. $2F_o$-F_c map contoured at 1.6 σ.

Table S10. Close contacts of the MtbAdoK-**6** complex ≤ 3.5 Å.

Atom	Residue	Distance (Å)
C2	Ser8.A OG	3.47
N3	Ser8.A OG	2.91
N3	Ser8.A CB	3.49
C4	Phe116.A CE1	3.47
C5	Phe116.A CD1	3.47
C8	Phe102.A CD1	3.36
C8	Phe102.A CE1	3.47
N9	HOH563.A O	3.49
C2’	Asp12.A OD1	3.38
C3’	Asp12.A OD2	3.23
C5’	Asp257.A OD2	3.27
C5’	HOH563.A O	3.48
O2’	Asp12.A OD1	2.64
O2’	Gly48.A N	2.96
O2’	Gly48.A CA	3.38
O2’	Ala10.A CB	3.48
O3’	Asp12.A OD2	2.51
O3’	Gly48.A N	2.91
O3’	Asp12.A CG	3.22
O3’	Asn52.A ND2	3.16
O3’	Asp12.A OD1	3.16
O4’	HOH563.A O	2.73
O4’	Val49.A CG1	3.35
O5’	HOH505.A O	2.36
O5’	Asp257.A OD2	2.64
O5’	Asp257.A CG	3.37
O5’	HOH563.A O	3.06
O5’	HOH552.A O	3.30
S21	Arg176.A CD	3.31
C22	Ser36.B OG	3.22
C23	Phe116.A CB	3.45
C23	Ser36.B OG	3.38

Table S11. Crystal data collection and refinement statistics for MtbAdoK-**6**.

Statistic	**MtbAdoK-6**
Data collection	
Space Group	$P2_12_12_1$
Cell Dimensions	
a, b, c (Å)	75.2, 81.9, 158.3
α, β, γ (°)	90, 90, 90
Resolution (Å)	44.38-2.10 (2.17-2.10)
R_{merge}	0.05 (0.31)
I/σI	11.89 (3.43)
Completeness %	99.7 (97.90)
Redundancy	6.6
Refinement	
Resolution (Å)	2.10
No. of reflections	57583
R_{work}/R_{free}	0.18/0.20
No. of atoms	
Protein	5058
Ligand	82
Water	276
B factors	
Protein	39.6
Ligand/ion	42.9
Water	41.6
rmsd	
Bond lengths (Å)	0.008
Bond angles (°)	1.14

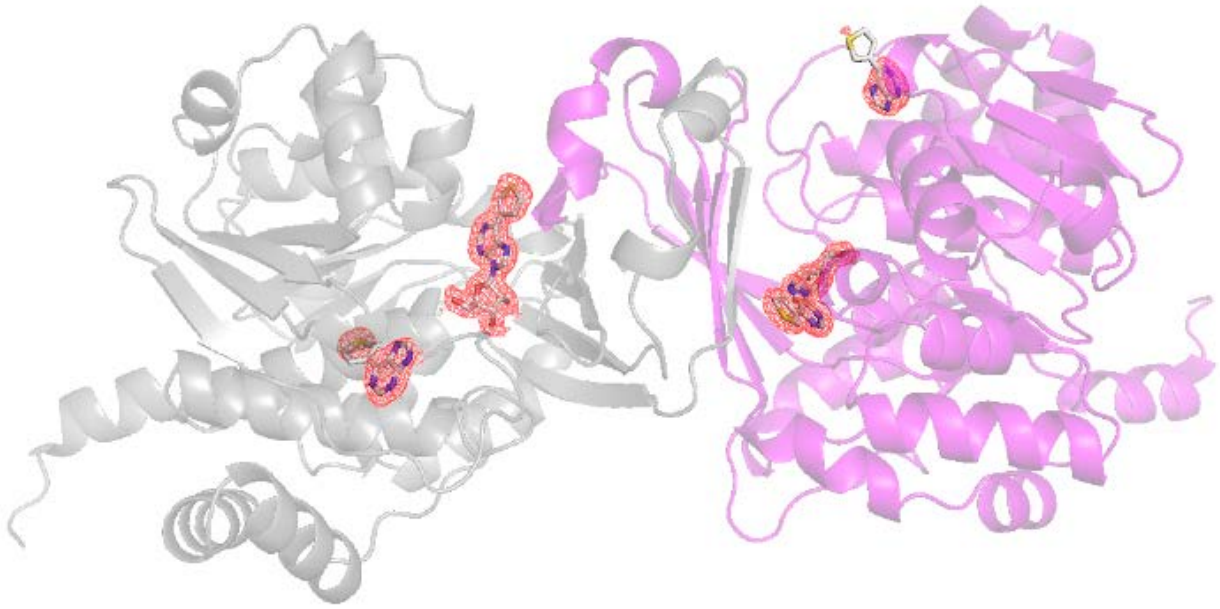


Figure S7. MtbAdoK dimer with two molecules of compound **6** per chain. Chain A is colored gray, and chain B is colored magenta. $2F_o$-F_c maps contoured at 1.6 σ.

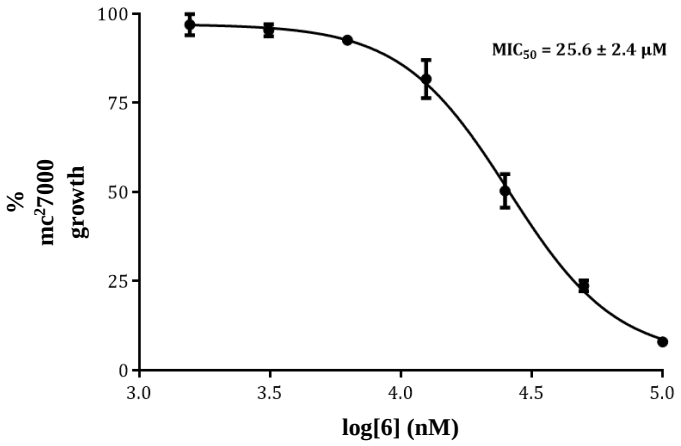


Figure S8. Dose-response curve of compound **6** when tested against mc^{2}7000. Data is normalized to the 0 µM (DMSO) control and the error reported represents the ± SD of 3 experiments.

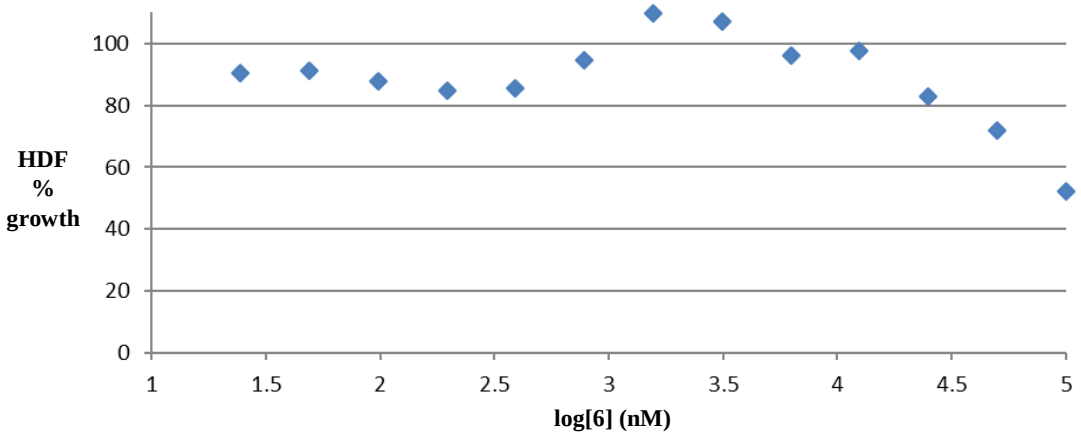


Figure S9. Dose-response curve of compound **6** when tested against HDF cells. Data is normalized to the 0 µM (DMSO) control.

Table S12. Close contacts of the MtbAdoK-**7** complex ≤ 3.5 Å.

Atom	Residue	Distance (Å)
C2	Ser8.A OG	3.37
N3	Ser8.A OG	2.84
N3	Ser8.A CB	3.48
N7	HOH583.B O	2.95
C8	Phe102.A CD1	3.32
C8	Phe102.A CE1	3.48
C8	HOH583.B	3.48
C2’	Asp12.A OD1	3.47
C3’	Asp12.A OD2	3.30
C5’	Asp257.A OD2	3.30
C5’	HOH694.A O	3.49
O2’	Asp12.A OD1	2.68
O2’	Gly48.A N	3.08
O2’	Gly48.A CA	3.45
O2’	Ala10.A CB	3.46
O3’	Asp12.A OD2	2.48
O3’	Gly48.A N	3.02
O3’	Asp12.A CG	3.27
O3’	Asn52.A ND2	3.14
O4’	Gln172.A NE2	3.03
O4’	Val49.A CG1	3.51
O5’	Asp257.A OD2	2.50
O5’	HOH636.A O	2.85
O5’	Asp257.A CG	3.28
O5’	Gln172.A NE2	3.29
O5’	HOH549.A O	3.23
C23	Arg176.A NH2	3.48
C23	Gln173.A OE1	3.37
C26	Gln172.A O	3.14
C27	Gln172.A O	3.27
C29	Ser36.B O	3.34

Table S13. Crystal data collection and refinement statistics for MtbAdoK-**7**.

Statistic	**MtbAdoK-7**
Data collection	
Space Group	$P4_1$
Cell Dimensions	
a, b, c (Å)	50.0, 50.0, 264.6
α, β, γ (°)	90, 90, 90
Resolution (Å)	43.56-1.70 (1.76-1.70)
R_{merge}	0.09 (0.51)
I/σI	11.01 (2.22)
Completeness %	99.8 (98.94)
Redundancy	5.1
Refinement	
Resolution (Å)	1.70
No. of reflections	70903
R_{work}/R_{free}	16.9/20.3
No. of atoms	
Protein	4694
Ligand	78
Water	569
B factors	
Protein	25.8
Ligand/ion	27.4
Water	33.9
rmsd	
Bond lengths (Å)	0.010
Bond angles (°)	1.37

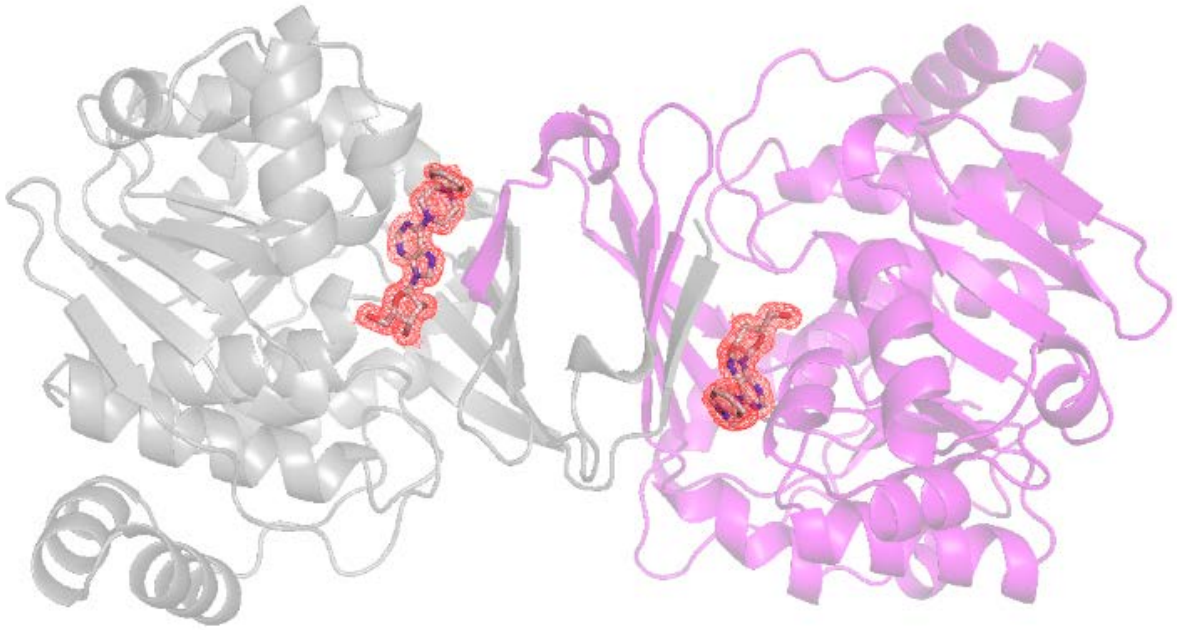


Figure S10. MtbAdoK dimer with two molecules of compound **7** per chain. Chain A is colored gray, and chain B is colored magenta. $2F_o$-F_c maps contoured at 1.6 σ.

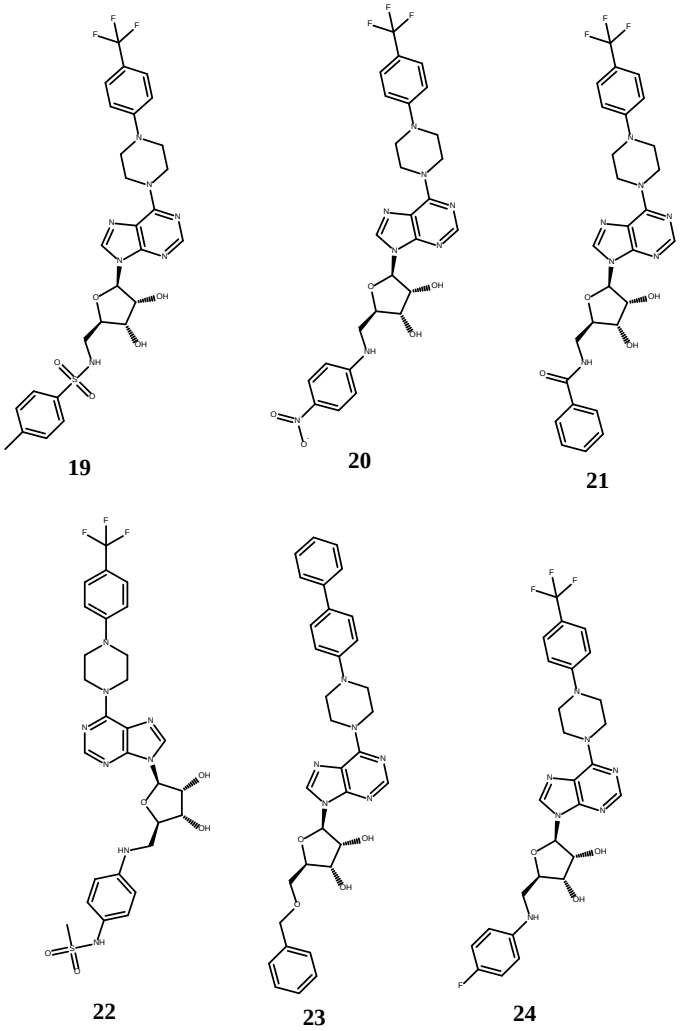


Figure S11. Chemical structures of synthesized adenosine analogs with substitutions at the 5'-position.

Table S14. SAR data for synthesized adenosine analogs with substitutions at the 5'-position.

ID	MtbAdoK IC_{50} (µM)	Mtb EC_{50} (µM)
19	≥ 12.5	> 50.0
20	> 50.0	≥ 50.0
21	> 50.0	> 50.0
22	> 50.0	> 50.0
23	> 50.0	> 50.0
24	> 50.0	> 50.0

Table S15. Close contacts of the MtbAdoK-**17** complex ≤ 3.5 Å.

Atom	Residue	Distance (Å)
N1	Gln173.A NE2	3.21
C2	Ser8.A CB	3.45
C2	Ser8.A OG	3.29
N3	Ser8.A OG	2.73
N3	Ser8.A CB	3.35
C4	Phe116.A CE1	3.39
C5	Phe116.A CD1	3.46
N7	Phe116.A CD1	3.42
C8	Phe102.A CD1	3.40
C2’	Asp12.A OD1	3.30
C3’	Asp12.A OD2	3.35
C5’	Asp257.A OD2	3.45
O2’	Gly48.A N	2.88
O2’	Asp12.A OD1	2.71
O2’	Gly48.A CA	3.26
O3’	Asp12.A OD2	2.46
O3’	Asp12.A CG	3.23
O3’	Gly48.A N	3.05
O3’	Asn52.A ND2	3.17
O4’	Val49.A CG1	3.40
O5’	Asp257.A OD2	2.36
O5’	Asp257.A CG	3.35
O5’	HOH589.A O	3.14
C24	Leu38.B CB	3.47
C25	Leu38.B N	3.43
C26	Ser36.B OG	3.23

Table S16. Crystal data collection and refinement statistics for MtbAdoK-**17**.

Statistic	**MtbAdoK-17**
Data collection	
Space Group	$P4_1$
Cell Dimensions	
a, b, c (Å)	49.9, 49.9, 264.3
α, β, γ (°)	90, 90, 90
Resolution (Å)	35.29-2.23 (2.31-2.23)
R_{merge}	0.06 (0.16)
I/σI	26.43 (10.31)
Completeness %	98.8 (98.29)
Redundancy	6.9
Refinement	
Resolution (Å)	2.23
No. of reflections	30978
R_{work}/R_{free}	0.19/0.23
No. of atoms	
Protein	4675
Ligand	76
Water	207
B factors	
Protein	47.3
Ligand/ion	49.4
Water	42.5
rmsd	
Bond lengths (Å)	0.004
Bond angles (°)	0.99

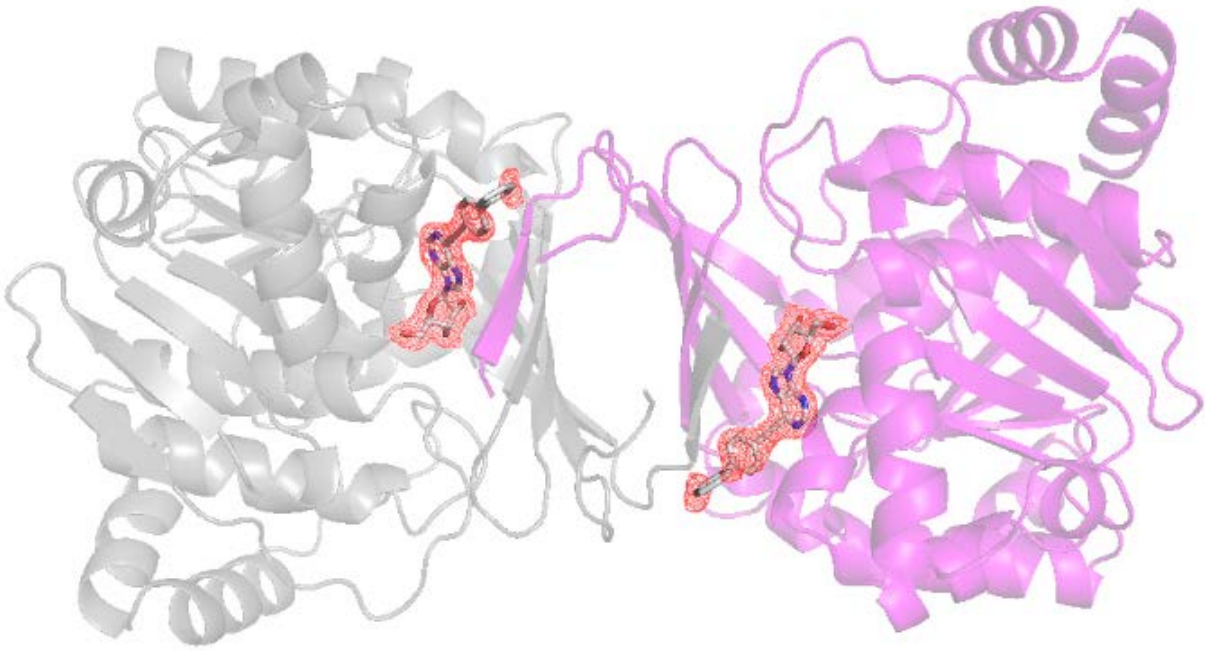


Figure S12. MtbAdoK dimer with two molecules of compound **17** per chain. Chain A is colored gray, and chain B is colored magenta. $2F_o$-F_c maps contoured at 1.6 σ.

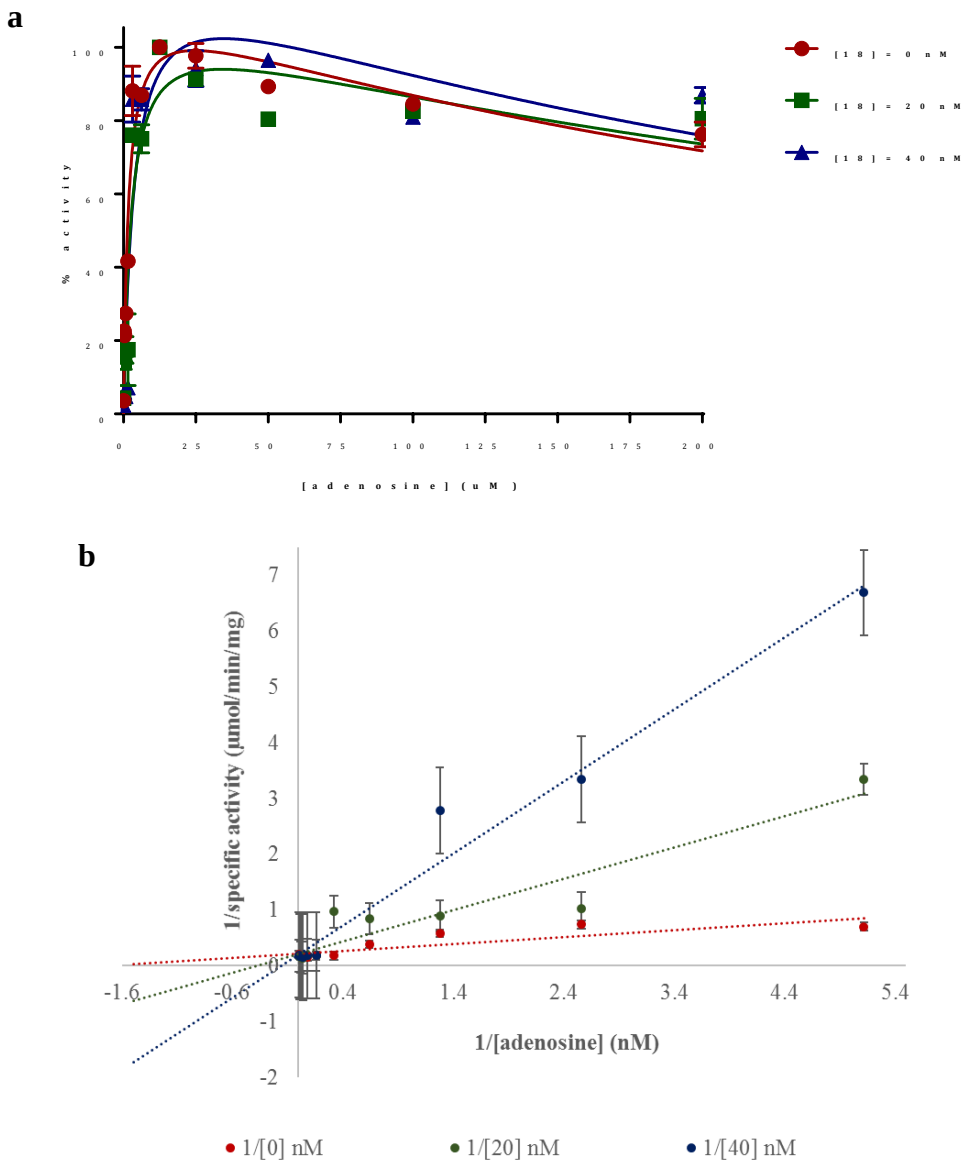


Figure S13. Steady-state kinetics for compound **18**. (a) The initial velocity of MtbAdoK was plotted against increasing concentrations of adenosine in the presence of 0 nM (DMSO-maroon), 20 nM (green) and 40 nM (blue) of **18**. (b) Initial velocity data was transformed to linear analysis to evaluate inhibitor type. In both cases, the error bars represent ± SD of 2 experiments.

Table S17. Steady-state kinetic parameters for MtbAdoK vs. compound **18**.

[18] (nM)	**K_m (µM)**
0.0	1.7 ± 0.02
20.0	2.7 ± 0.6
40.0	3.6 ± 0.02

The error is reported as ± SD of 2 experiments.

Table S18. Mouse Acute Tolerability Studies. *

Compound **18** Acute Tolerability		Mass (g)			
Dose Level $mg \cdot kg^{-1}$	Mouse ID	Start	Day 1	Day 2	Day 3
50	Cp18-10	28.0	28.1	28.0	28.1
	Cp18-11	30.8	30.7	30.5	30.7
	Cp18-12	26.2	26.3	26.4	26.4
	Average	**28.3**	**28.4**	**28.3**	**28.4**
100	Cp18-13	32.2	31.1	30.9	31.3
	Cp18-14	28.6	28.7	28.8	28.8
	Cp18-15	25.0	25.1	25.0	25.2
	Average	**28.6**	**28.3**	**28.2**	**28.4**
200	Cp18-16	30.3	30.3	30.2	30.2
	Cp18-17	25.8	25.6	25.2	25.3
	Cp18-18	26.0	25.9	25.8	25.9
	Average	**27.4**	**27.3**	**27.1**	**27.1**

*Mice (n = 3 per cohort) were administered compound **18** at increasing dose levels by oral gavage for three days. Mass 24 hours after dose administration was recorded. Animals retained mass and demonstrated no adverse effects such as lack of grooming, grimacing, diarrhea, or mortality.

SUPPORTING REFERENCES

1. Zemlicka, J.; Endo, T., O6-(4-Nitrophenyl)inosine and -Guanosine as chromogenic substrates for adenosine deaminase. *Nucleosides and Nucleotides* 1996, *15* (1-3), 619-629.

2. Itoh, T., Sugawara, T., Mizuno, Y, A Novel Synthesis of 1-Deazaadenosine. *Heterocycles* 1982, *17*, 305-309.

3. Katagiri, N.; Matsuhashi, Y.; Kokufuda, H.; Takebayashi, M.; Kaneko, C., A highly efficient synthesis of the antiviral agent (+)-cyclaradine involving the regioselective cleavage of epoxide by neighboring participation. *Tetrahedron Lett* 1997, *38* (11), 1961-1964.