Adenosine 5`-Carboxamide-Based Inhibitors of METTL1

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1. Supplementary figures and table

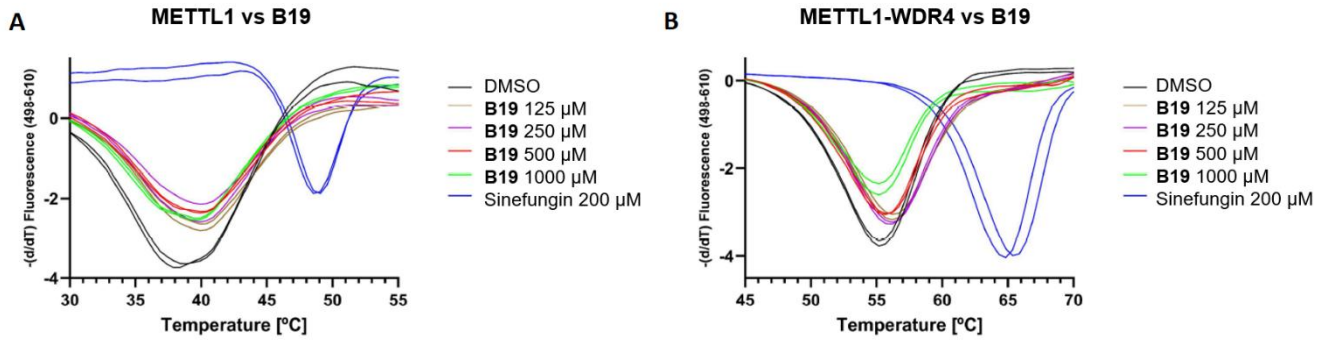


Figure S1. Thermal shift of METTL1 (A) and METTL1-WDR4 (B) in the presence of DMSO control (black) or compound **B19**.

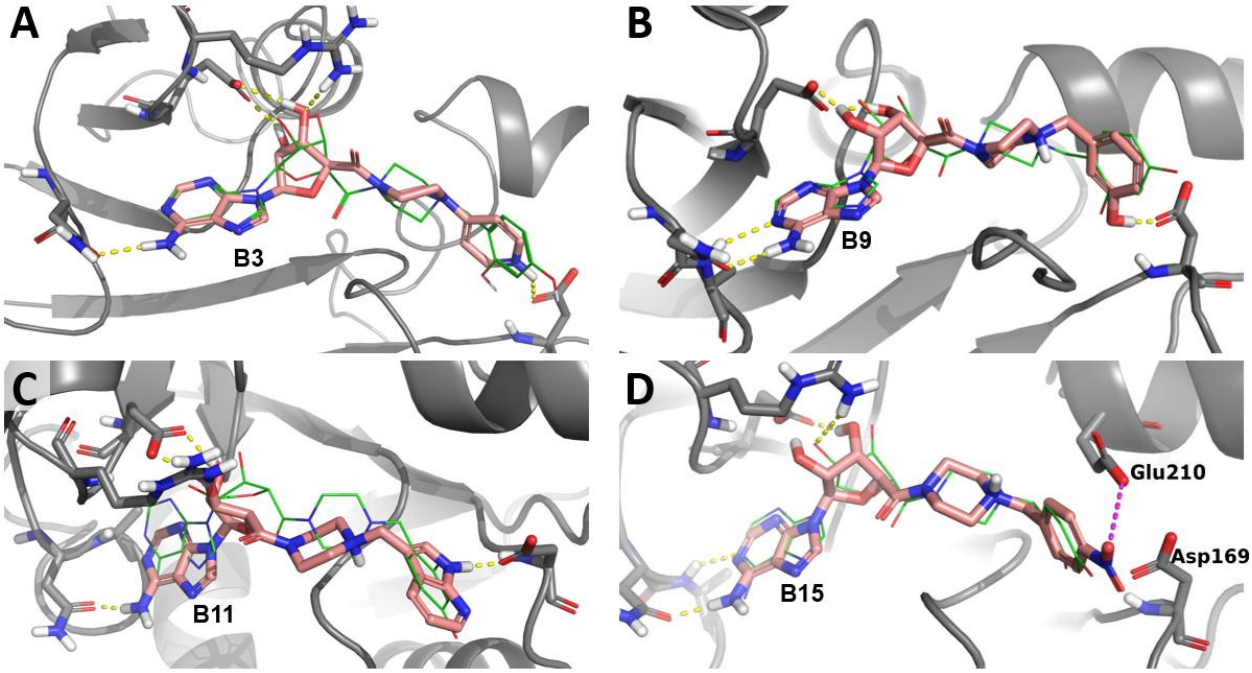
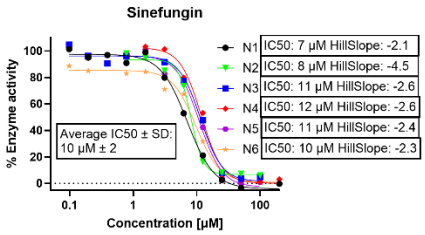
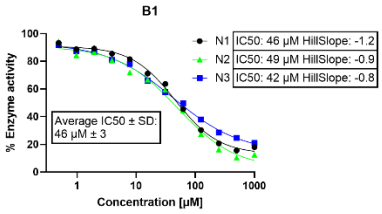
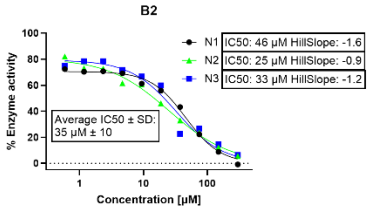


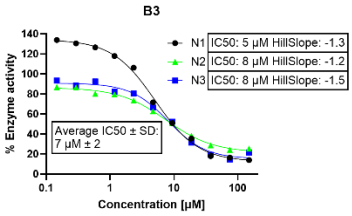
Figure S2. Binding modes of compounds **B3** (A), **B9** (B), **B11** (C), and **B15** (D) in METTL1 (PDB 29GE). **B19** is shown in green; hydrogen bonds are yellow, salt bridges are in magenta.

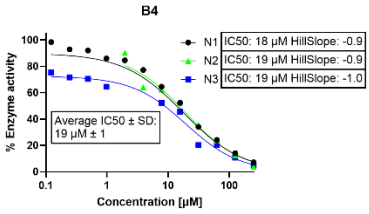
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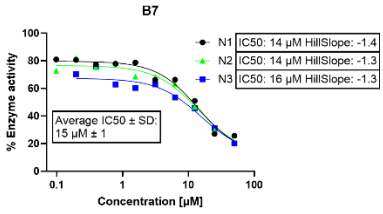


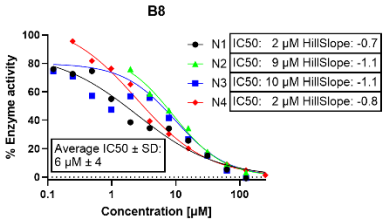


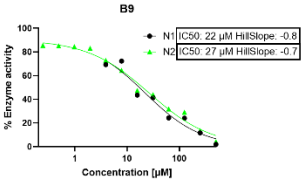


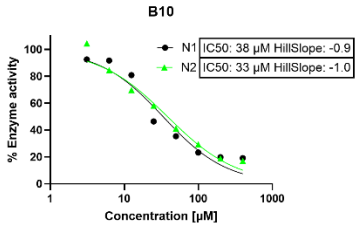


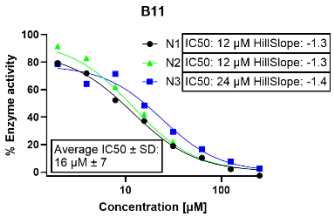


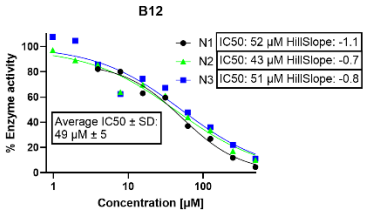


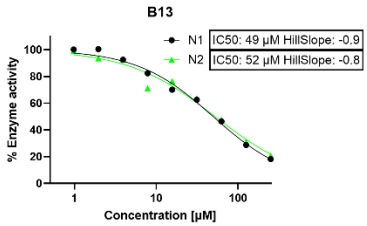


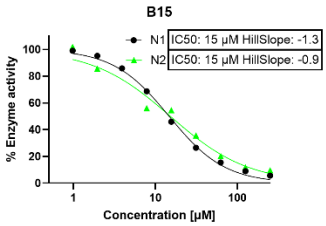


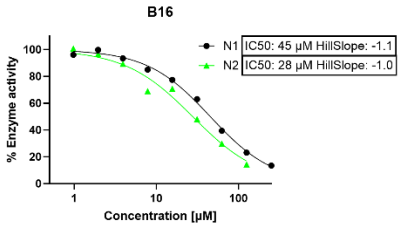


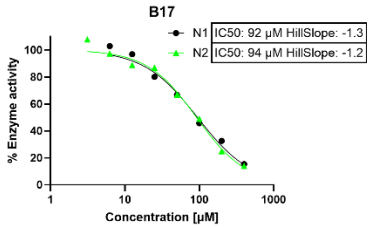


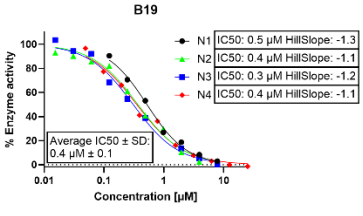


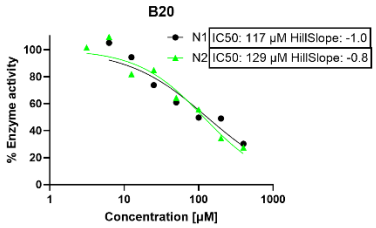


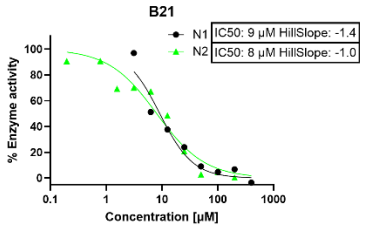












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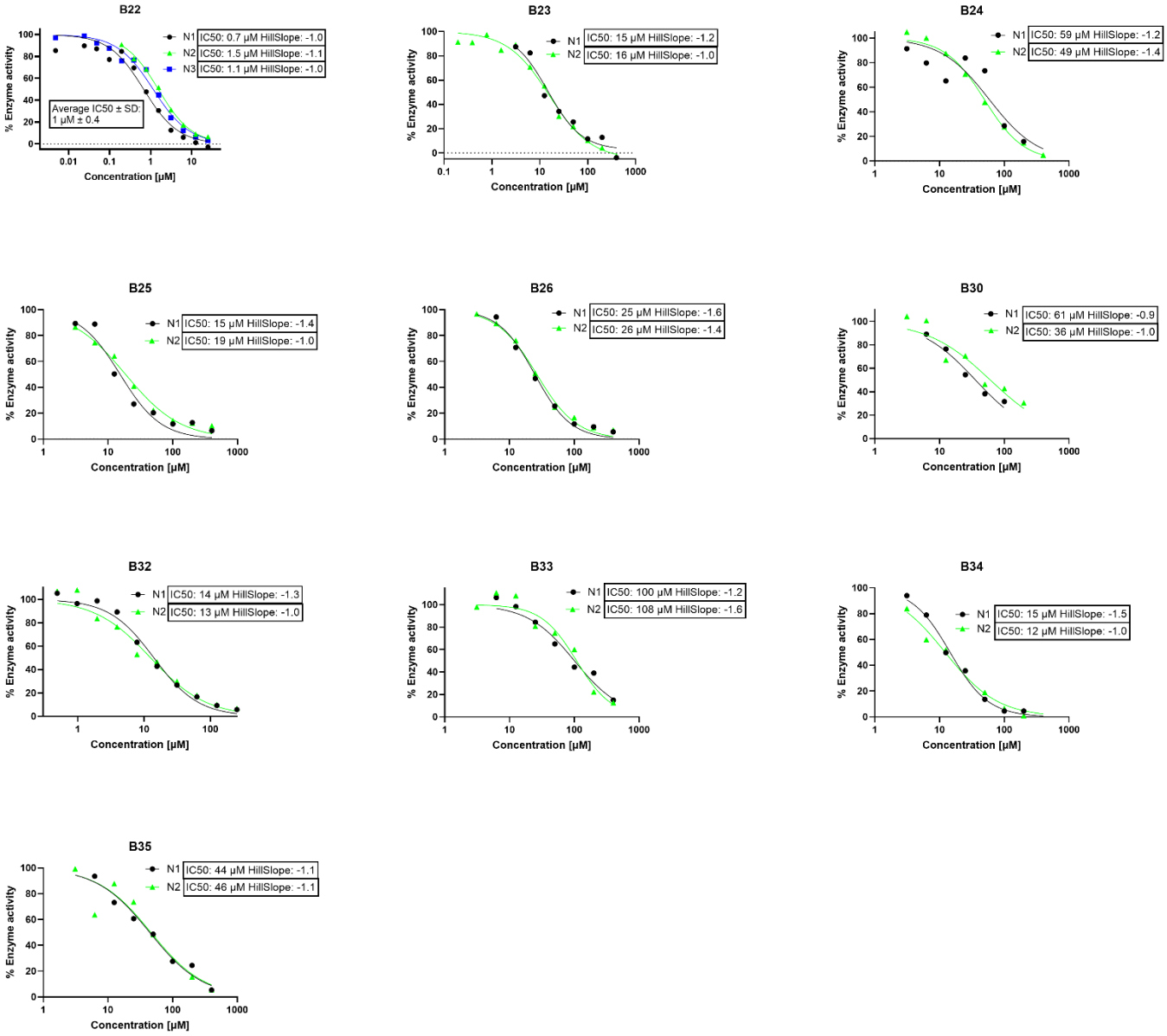


Figure S3. Dose-response curves for METTL1-WDR4 and the main text compounds. Biological duplicates are shown separately. Each biological replicate is the average of two technical replicates.

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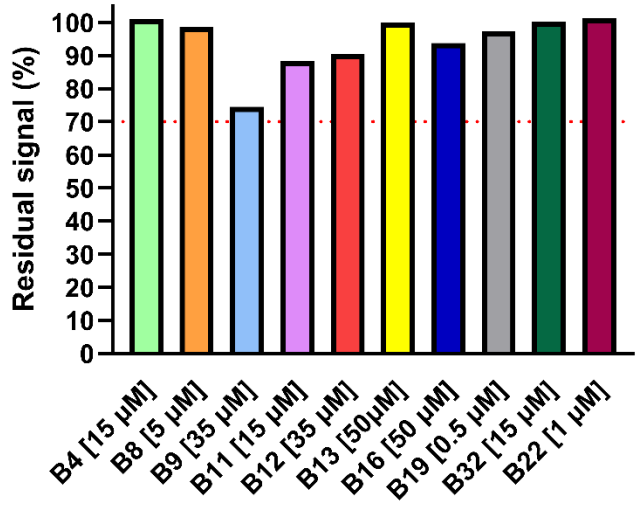


Figure S4. Interference screening of a subset of main text compounds. The compounds that decrease by more than 30 % the maximal emission at a concentration close to the IC_{50} are considered interferents

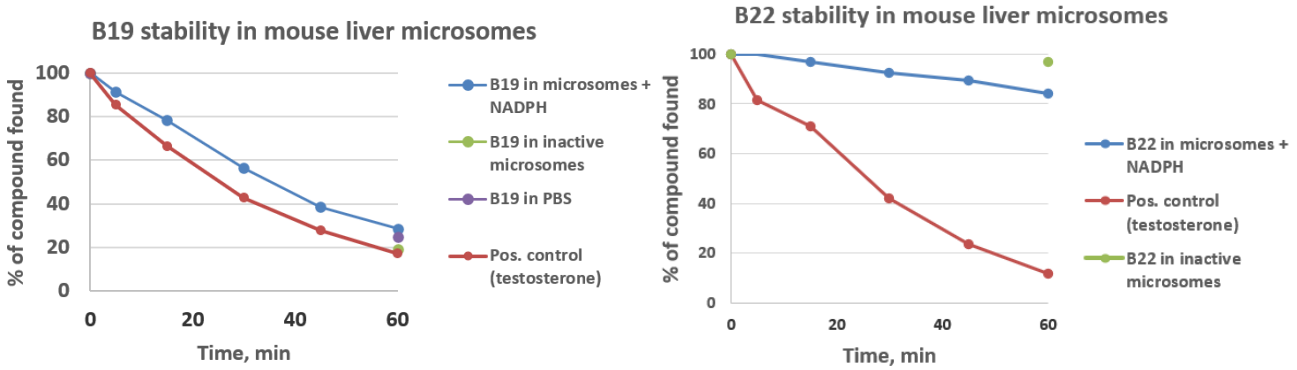


Figure S5. Metabolic stability profiles of **B19** and **B22** in mouse liver microsomes. The remaining percentage of the parent compound is plotted against incubation time (0–60 min).

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Table S1. Data collection and refinement statistics for METTL1 in complex with **B19** (PDB code: 29GE) as generated by phenix.table one. Statistics for the highest-resolution shell are shown in parentheses.

	B19 (OBV617)
Wavelength	
Resolution range	40.52 - 2.889 (3.64 - 2.89)
Space group	P 21 21 2
Unit cell	43.149 154.303 41.999 90 90 90
Total reflections	339593 (80790)
Unique reflections	89264 (33640)
Multiplicity	3.8 (2.4)
Completeness (%)	98.92 (98.88)
Mean I/sigma(I)	0.85 (-0.17)
Wilson B-factor	49.27
R-merge	0.5668 (9.328)
R-meas	0.6516 (11.54)
R-pim	0.3144 (6.644)
CC1/2	0.977 (-0.0176)
CC*	0.994 (-0.189)
Reflections used in refinement	6687 (3252)
Reflections used for R-free	347 (160)
R-work	0.2210 (0.2570)
R-free	0.2879 (0.3348)
CC(work)	
CC(free)	
Number of non-hydrogen atoms	1648
macromolecules	1587
ligands	52
solvent	9
Protein residues	206
Nucleic acid bases	
RMS(bonds)	0.008
RMS(angles)	0.97
Ramachandran favored (%)	97.52
Ramachandran allowed (%)	2.48
Ramachandran outliers (%)	0
Rotamer outliers (%)	3.23
Clashscore	10.46
Average B-factor	43.39
macromolecules	42.87
ligands	60.05
solvent	37.77
Number of TLS groups	

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2. Chemistry

2.1 General information

All chemicals and solvents were obtained from commercial suppliers and utilized as received. Reaction conditions and yields were not optimized. Normal-phase chromatography was performed on Davisil 60 Å 35-70 μm silica, and reverse-phase chromatography was performed using KP-C18-HS SNAP Biotage cartridges on a Biotage Isolera One purification system. Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker 300, 400, or 600 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to the residual solvent signal. High-resolution mass spectrometry (HRMS) analyses were conducted on a Waters Synapt G2-Si TOF mass spectrometer. The purity of the compounds **B1**-**B36** was measured by Waters HPLC with a C18 column under UV detection at 210 nm or 254 nm. All final compounds are ≥ 95 % pure by HPLC analysis unless stated otherwise.

2.2 General procedures

*General procedure **P1** for amide synthesis using HBTU*

To a suspension of carboxylic acid **2** (1 eq) in DMF (0.1-0.2 mmol/mL) was added TEA (2.5 eq), then HBTU (1.1 eq), and after 5 min, an appropriate piperazine derivative (1.3 eq). The reaction mixture was stirred at room temperature for 18 h, then concentrated, and the resulting residue was purified by silica gel chromatography.

*General procedure **P2** for amide synthesis using T3P*

To a suspension of carboxylic acid **2** (1 eq) and an appropriate amine or amine hydrochloride salt (1.1-1.3 eq) in DMF-EtOAc mixture (1:3, 0.1-0.2 mmol/mL) under argon was added TEA (3 eq, when free amine was used; 5 eq when amine HCl or TFA salt was used). The reaction mixture was cooled in the ice bath, then T3P (50 % in EtOAc, 1.5 eq) was added under argon. The reaction mixture was stirred for 5-18 h at room temperature. The reaction mixture was diluted with sat. $NaHCO_3$, extracted with EtOAc (2x), washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography.

*General procedure **P3** for amine synthesis using reductive amination*

A suspension of piperazine derivative **3** (1 eq) in DCE (0.15 mmol/mL) was treated with the appropriate benzaldehyde (1.1 eq) and $Na(OAc)_3BH$ (1.5 eq). The reaction mixture was stirred at room temperature for 16 hours, quenched with saturated $NaHCO_3$, extracted with EtOAc (2x), washed with brine, dried, and evaporated. The residue was purified by silica gel chromatography.

*General procedure **P4** for the deprotection of the acetonide group*

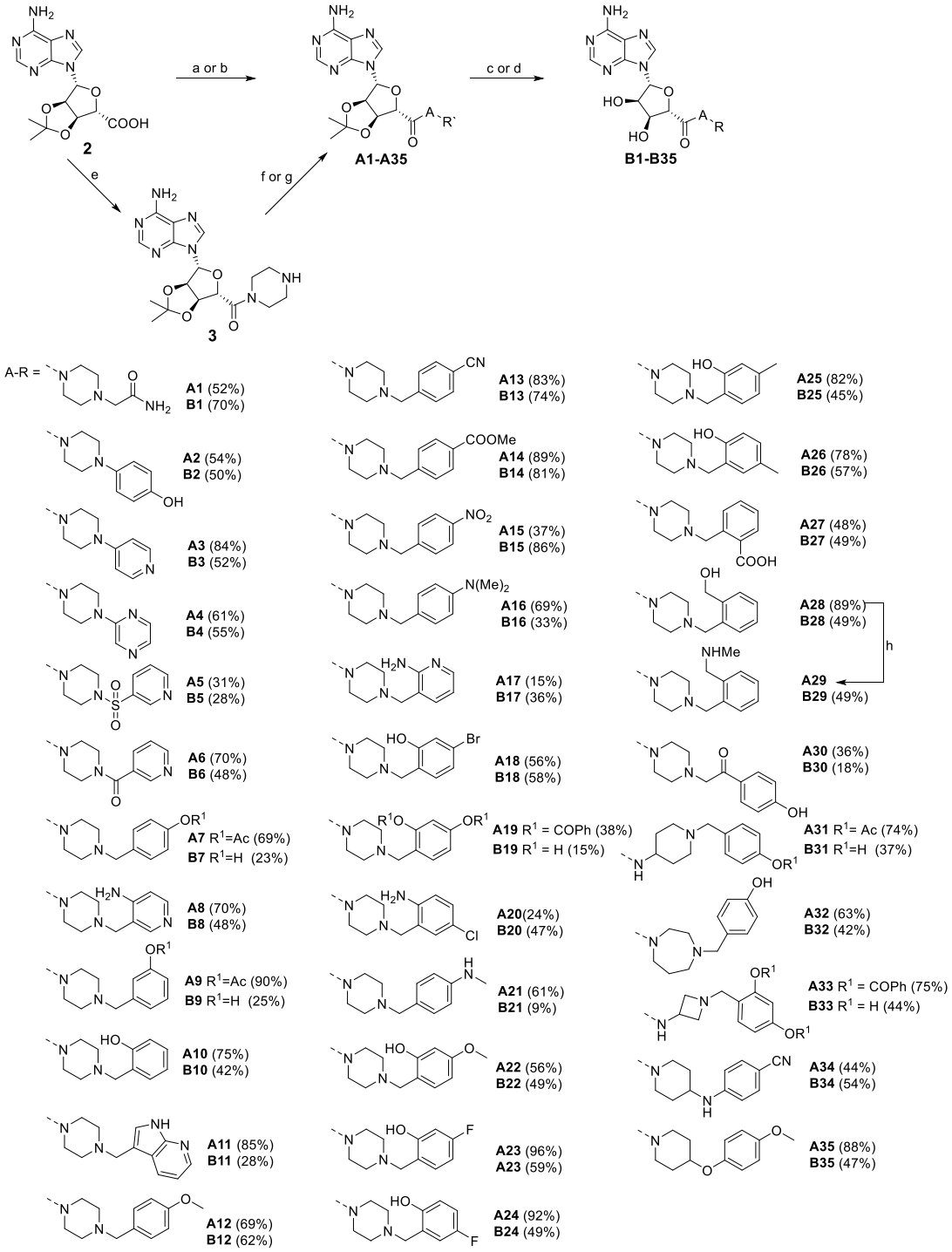
The acetonide-protected adenosine derivative **A** (1 eq) was stirred in 50 % HCOOH (5 mmol/mL) for 6-10 h at 50 °C. The reaction mixture was evaporated, then coevaporated with EtOH. The residue was purified by reverse-phase chromatography.

*General procedure **P5** for the deprotection of the phenol protecting group and acetonide group*

A solution of acetyl-protected (or benzoyl-protected) compound **A** (1 eq) in MeOH (0.3 mmol/1 mL) was degassed by argon bubbling, then K_2CO_3 (0.1 eq) was added, and the mixture was stirred for 40 min for acetyl-protected phenols or 18 h for benzoyl-protected phenol. The mixture was concentrated under reduced pressure. Then 50 % HCOOH (5 mmol/mL) was added to the residue and the reaction mixture was stirred for 6-10 h at 50 °C. The reaction mixture was evaporated, then coevaporated with EtOH. The residue was purified by reverse-phase chromatography.

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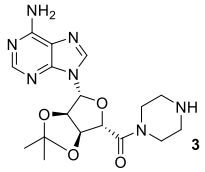
2.3 Synthesis and characterization of the compounds



Scheme S1. Synthesis of Target Compounds **B1-B35**. Reagents and Conditions: (a) appropriate amine, HBTU, TEA, DMF, 18 h, rt; (b) appropriate amine, T3P, TEA, EtOAc-DMF, 6-18 h, rt; (c) 50 % HCOOH, 6-10 h, 50 °C.; (d) *i* K_2CO_3, MeOH; *ii* 50 % HCOOH, 6-10 h, 50 °C.; (e) *i* Cbz-piperazine, T3P, TEA, EtOAc-DMF, 6-18 h, rt *ii* H_2, 10 % Pd/C, MeOH, 4 h, rt; (f) appropriate aldehyde, $Na(OAc)_3BH$, DCE, rt, 16 h; (g) appropriate benzylbromide, TEA, MeCN, 2 h, rt; (h) *i* Dess-Martin reagent, DCM, 18 h, rt; *ii* $MeNH_2HCl$, TEA, $Na(OAc)_3BH$, DCE, 16 h, rt.

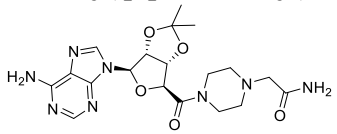
((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(piperazin-1-yl)methanone (3)

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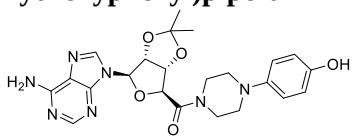
To a suspension of carboxylic acid **2** (1.02 g, 3.17 mmol) and Cbz-piperazine (0.73 µL, 3.81 mmol) in DMF (7 mL) and EtOAc (7 mL) under argon was added TEA (1.11 mL, 7.94 mmol). The reaction mixture was cooled in the ice bath, then T3P (50 % in EtOAc, 2.83 mL, 4.76 mmol) was added under argon. The reaction mixture was stirred for 18 h at room temperature. The reaction mixture was diluted with sat. $NaHCO_3$, extracted with EtOAc (2x), washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by chromatography on silica gel, eluting with 5 % DCM in MeOH to afford Cbz-protected piperazine derivative (1.18 g, 71%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.36 (s, 1H), 7.92 (s, 1H), 7.41 – 7.28 (m, 5H), 6.39 (d, *J* = 1.5 Hz, 1H), 5.69 – 5.54 (m, 3H), 5.41 (dd, *J* = 5.9, 1.5 Hz, 1H), 5.09 (s, 2H), 4.98 (s, 1H), 3.69 – 3.45 (m, 4H), 3.41 – 3.25 (m, 1H), 3.19 (ddd, *J* = 12.4, 8.5, 3.2 Hz, 1H), 2.96 – 2.75 (m, 2H), 1.61 (s, 3H), 1.42 (s, 3H). A stirred suspension of Cbz-protected piperazine derivative (1.18 g, 2.25 mmol) and 10 % Pd/C (0.24 g) was degassed by argon bubbling, then H_2 was bubbled through the reaction mixture for 4 h. The mixture was filtered through Celite, washing with MeOH. The solvent was removed under reduced pressure to afford product (742 mg, 85%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H), 8.09 (s, 1H), 7.28 (s, 2H), 6.23 (d, *J* = 0.8 Hz, 1H), 5.57 – 5.49 (m, 2H), 5.08 (d, *J* = 1.1 Hz, 1H), 3.54 – 3.19 (m, 4H, overlapped with water), 2.70 – 2.58 (m, 2H), 2.34 – 2.23 (m, 2H), 1.53 (s, 3H), 1.37 (s, 3H). The amine group proton was not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.5, 156.0, 152.7, 149.4, 139.2, 118.5, 112.7, 88.8, 83.5, 83.0, 82.2, 45.7, 44.9, 44.5, 41.9, 26.5, 24.9. HRMS (ESI/TOF-Q) m/z: : Calculated for $C_{17}H_{24}N_7O_4$ $[M+H]^+$ 390.1884, found 390.1894.

2-(4-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonyl)piperazin-1-yl)acetamide (A1)



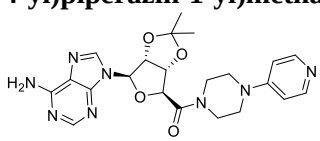
The compound was prepared according to the procedure **P1.** The crude product was purified by chromatography on silica gel eluting with 5 %-10 %-20 % MeOH in DCM to afford the product in 52 % yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1H), 8.06 (s, 1H), 7.29 (s, 2H), 7.14 (s, 1H), 7.08 (s, 1H), 6.22 (s, 1H), 5.58 (s, 2H), 5.08 (s, 1H), 3.54 – 3.45 (m, 1H), 3.40 – 3.25 (m, 1H, overlapped with water), 3.14 – 3.03 (m, 2H), 2.60 (s, 2H), 2.40 – 2.28 (m, 2H), 1.74 – 1.58 (m, 2H), 1.53 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.3, 166.4, 156.1, 152.7, 149.5, 139.2, 118.6, 112.6, 88.7, 83.3, 83.0, 82.2, 60.7, 52.4, 52.1, 44.9, 41.4, 26.5, 24.9. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{19}H_{27}N_8O_5$ $[M+H]^+$ 447.2099, found 447.2114.

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(4-hydroxyphenyl)piperazin-1-yl)methanone (A2)



The compound was prepared according to the procedure **P1.** The crude product was purified by chromatography on silica gel eluting with 5 % MeOH in DCM to afford the product in 54 % yield as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.86 (s, 1H), 8.11 (s, 1H), 8.09 (s, 1H), 7.25 (s, 2H), 6.69 – 6.55 (m, 4H), 6.24 (d, *J* = 0.6 Hz, 1H), 5.60 (dd, *J* = 6.0, 1.3 Hz, 1H), 5.57 (dd, *J* = 6.0, 0.6 Hz, 1H), 5.13 (d, *J* = 1.3 Hz, 1H), 3.60 – 3.50 (m, 1H), 3.46 – 3.36 (m, 2H), 3.27 – 3.16 (m, 1H), 2.91 – 2.76 (m, 2H), 2.42 – 2.33 (m, 1H), 2.32 – 2.23 (m, 1H), 1.55 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ: 166.6, 156.0, 152.7, 151.5, 149.4, 143.8, 139.2, 118.8, 118.6, 115.4, 112.7, 88.8, 83.4, 83.1, 82.3, 50.5, 50.0, 45.2, 41.6, 26.5, 24.9. MS (ESI) *m/z*: Calculated for $C_{23}H_{28}N_7O_5$ $[M+H]^+$ 482.2, found 482.336.

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(pyridin-4-yl)piperazin-1-yl)methanone (A3)

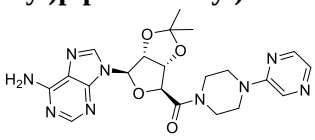


The compound was prepared according to the procedure **P1.** The crude product was purified by chromatography on silica gel eluting with 10 % MeOH in DCM (+ 1% NH_4OH sol.) to afford the product in 84 % yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 – 8.12 (m, 2H), 8.11 (s, 1H), 8.08 (s, 1H), 7.24 (s, 2H), 6.75 – 6.69 (m, 2H), 6.25 (d, *J* = 0.8 Hz, 1H), 5.61 (dd, *J* = 6.0, 1.3 Hz, 1H), 5.59 (dd, *J* = 6.0, 0.8 Hz, 1H), 5.15 (d, *J* = 1.3 Hz, 1H), 3.63 – 3.27 (m, 5H, overlapped with water), 3.25 – 3.14 (m, 1H), 2.83 – 2.74 (m, 1H), 2.67 – 2.56 (m, 1H), 1.55 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.8, 156.0, 154.1, 152.6, 149.4, 139.3, 118.5, 112.7, 108.4,

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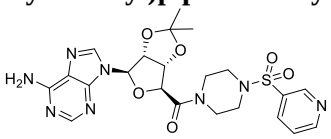
88.8, 83.3, 83.2, 82.2, 45.1, 44.7, 44.2, 40.9, 26.5, 24.9. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{27}N_8O_4$ $[M+H]^+$ 467.2150, found 467.2160.

((3a*S*,4*S*,*6R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(pyrazin-2-yl)piperazin-1-yl)methanone (A4)



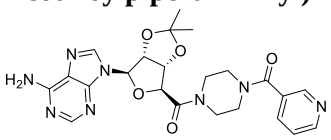
The compound was prepared according to the procedure **P2.** The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in petroleum ether, gradient 15-80% to afford the product in 61 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.03 (dd, *J* = 2.5, 1.5 Hz, 1H), 8.02 (d, *J* = 1.5 Hz, 1H), 7.95 (s, 1H), 7.87 (d, *J* = 2.5 Hz, 1H), 6.40 (d, *J* = 1.4 Hz, 1H), 5.79 (s, 2H), 5.68 (dd, J = 6.0, 1.3 Hz, 1H), 5.44 (dd, *J* = 6.0, 1.4 Hz, 1H), 5.03 (d, *J* = 1.3 Hz, 1H), 3.82 – 3.56 (m, 4H), 3.52 – 3.43 (m, 1H), 3.33 (ddd, *J* = 12.7, 8.6, 3.5 Hz, 1H), 2.98 – 2.85 (m, 2H), 1.62 (s, 3H), 1.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2, 155.5, 154.6, 153.5, 150.2, 141.8, 139.2, 134.0, 131.1, 119.4, 114.1, 90.5, 84.6, 83.8, 82.7, 77.5, 45.2, 44.5, 44.2, 41.7, 26.9, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{26}N_9O_4$ $[M+H]^+$ 468.2102, found 468.2121.

((3a*R*,4*R*,6*S*,6a*S*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(pyridin-3-ylsulfonyl)piperazin-1-yl)methanone (A5)



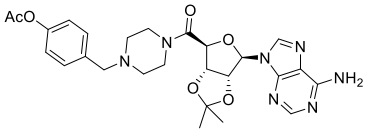
The compound was prepared according to the procedure **P2.** The crude product was purified by chromatography on silica gel eluting with 5 % MeOH in DCM to afford the product in 31 % yield as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 9.70 (dd, *J* = 4.9, 1.6 Hz, 1H), 9.64 (dd, *J* = 2.4, 0.8 Hz, 1H), 8.86 (ddd, *J* = 8.0, 2.4, 1.6 Hz, 1H), 8.83 (s, 1H), 8.82 (s, 1H), 8.49 (ddd, *J* = 8.0, 4.9, 0.8 Hz, 1H), 8.03 (s, 2H), 7.00 (d, *J* = 0.8 Hz, 1H), 6.31 – 6.25 (m, 2H), 5.85 (d, *J* = 1.2 Hz, 1H), 4.33 – 4.16 (s, 5H, overlapped with water), 3.86 – 3.80 (m, 1H), 3.74 (s, 1H), 3.48 – 3.40 (m, 1H), 2.32 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.4, 156.3, 154.5, 153.0, 149.7, 148.0, 139.7, 135.7, 132.2, 125.0, 118.8, 113.2, 89.2, 83.8, 83.5, 82.5, 46.1, 45.8, 45.6, 44.8, 27.0, 25.4. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{27}N_8O_6S$ $[M+H]^+$ 531.1769, found 531.1786.

((3a*R*,4*R*,6*S*,6a*S*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-nicotinoylpiperazin-1-yl)methanone (A6)



The compound was prepared according to the procedure **P2.** The crude product was purified by chromatography on silica gel eluting with 5 % MeOH in DCM to afford the product in 70 % yield as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.66 – 8.61 (m, 1H), 8.56 (d, *J* = 2.1 Hz, 1H), 8.23 (s, 1H), 8.18 (s, 1H), 7.83 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.52 (dd, *J* = 7.9, 5.0 Hz, 1H), 6.37 (s, 1H), 5.71 (d, *J* = 5.9 Hz, 1H), 5.63 (d, *J* = 5.9 Hz, 1H), 5.11 (m, 1H), 3.97 – 3.41 (m, 6H), 2.93 (s, 2H), 2.74 (s, 2H), 1.59 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (101 MHz, MeOH-d_4) δ 169.6, 169.3, 157.4, 154.0, 151.6, 150.9, 148.5, 141.6, 136.9, 133.0, 125.2, 120.0, 114.9, 91.4, 85.7, 85.2, 84.1, 46.6, 46.1, 43.2, 42.7, 27.0, 25.3. **HRMS (ESI/TOF-Q) *m/z*:** Calculated for $C_{23}H_{27}N_8O_5$ $[M+H]^+$ 495.2099, found 495.2103.

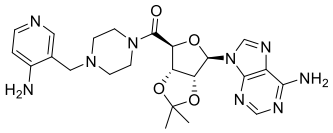
4-((4-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonyl)piperazin-1-yl)methyl)phenyl acetate (A7)



The compound was prepared according to the procedure **P1**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in petroleum ether, gradient 30-100% to afford the product in 69 % yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 8.03 (s, 1H), 7.37 (s, 2H), 7.26 – 7.18 (m, 2H), 7.09 – 7.00 (m, 2H), 6.22 (d, *J* = 0.8 Hz, 1H), 5.67 – 5.58 (m, 2H), 5.06 (d, *J* = 1.0 Hz, 1H), 3.56 – 3.43 (m, 2H), 3.16 – 3.09 (m, 1H), 3.10 (d, *J* = 12.9 Hz, 1H), 3.04 (d, *J* = 12.9 Hz, 1H), 2.93 – 2.83 (m, 1H), 2.30 – 2.20 (m, 2H), 2.25 (s, 3H), 1.52 (s, 3H), 1.43 – 1.27 (m, 2H), 1.37 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 166.4, 156.2, 152.7, 149.7, 149.5, 139.1, 129.8, 121.5, 112.4, 88.6, 83.2, 83.0, 82.2, 61.0, 52.2, 51.7, 45.0, 41.5, 31.3, 28.4, 26.4, 24.8, 20.9. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{26}H_{32}N_7O_6$ $[M+H]^+$ 538.2409, found 538.2426.

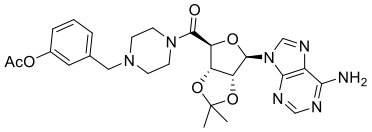
((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-((4-aminopyridin-3-yl)methyl)piperazin-1-yl)methanone (A8)

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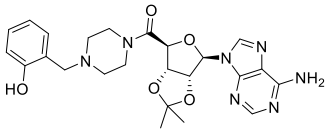
The compound was prepared according to the procedure **P1.** The crude product was purified by chromatography on silica gel eluting with 5 % MeOH in DCM (+1 % NH_4OH sol.) to afford the product in 78 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.11 (d, *J* = 5.5 Hz, 1H), 7.94 (s, 1H), 7.92 (s, 1H), 6.42 (d, *J* = 5.5 Hz, 1H), 6.40 (d, *J* = 1.3 Hz, 1H), 5.92 (s, 2H), 5.68 (dt, *J* = 5.8, 0.8 Hz, 1H), 5.45 (dd, *J* = 5.8, 1.3 Hz, 1H), 5.07 (s, 2H), 4.97 (s, 1H), 3.73 – 3.65 (m, 1H), 3.61 (d, *J* = 13.4 Hz, 1H), 3.25 - 3.19 (m, 1H), 3.24 (d, *J* = 13.1 Hz, 1H), 3.08 (d, *J* = 13.1 Hz, 1H), 3.08 - 2.99 (m, 1H), 2.35 (ddt, *J* = 16.9, 14.8, 4.0 Hz, 2H), 1.60 (m, 3H), 1.57 – 1.43 (m, 2H), 1.42 (s, 3H). 13C NMR (101 MHz, $CDCl_3$) δ 166.8, 155.7, 153.6, 153.1, 150.5, 150.3, 149.8, 139.2, 119.6, 116.1, 113.9, 109.7, 90.4, 84.5, 83.7, 82.7, 58.8, 52.6, 52.0, 45.8, 42.2, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{23}H_{30}N_9O_4$ $[M+H]^+$ 496.2415, found 496.2421.

3-((4-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonyl)piperazin-1-yl)methyl)phenyl acetate (A9)



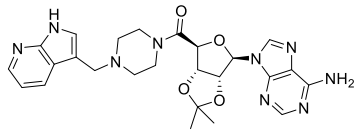
The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in petroleum ether, gradient 15-100 % to afford the product in 90 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.95 (s, 1H), 7.27 (d, *J* = 7.8 Hz, 1H), 7.06 (dt, *J* = 7.8, 1.2 Hz, 1H), 6.99 – 6.97 (m, 1H), 6.95 (ddd, *J* = 8.0, 2.4, 1.0 Hz, 1H), 6.39 (d, *J* = 1.4 Hz, 1H), 5.83 (s, 2H), 5.64 (dd, *J* = 5.9, 0.6 Hz, 1H), 5.42 (dd, *J* = 5.9, 1.4 Hz, 1H), 4.99 (d, *J* = 0.6 Hz, 1H), 3.70 (dt, *J* = 12.9, 3.9 Hz, 1H), 3.64 – 3.56 (m, 1H), 3.29 (ddd, *J* = 13.1, 8.8, 2.9 Hz, 1H), 3.19 (s, 2H), 3.14 (ddd, *J* = 12.7, 9.0, 3.2 Hz, 1H), 2.43 – 2.34 (m, 2H), 2.28 (s, 3H), 1.69 – 1.61 (m, 2H), 1.60 (s, 3H), 1.43 – 1.37 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4, 166.6, 155.5, 153.4, 150.7, 150.3, 139.4, 139.2, 129.2, 126.3, 121.9, 120.4, 119.5, 113.8, 90.5, 84.6, 83.6, 82.7, 76.7, 62.2, 52.8, 52.4, 45.7, 42.1, 26.7, 25.1, 21.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{26}H_{32}N_7O_6$ $[M+H]^+$ 538.2409, found 538.2413.

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(2-hydroxybenzyl)piperazin-1-yl)methanone (A10)



The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in petroleum ether, gradient 15-80 %, then by chromatography on silica gel eluting with 5-10% MeOH in DCM to afford the product in 75% yield as a white solid. 1H NMR (400 MHz, $CDCl_3$) δ 10.13 (s, 1H), 8.41 (s, 1H), 7.92 (s, 1H), 7.17 – 7.12 (m, 1H), 6.88 (dd, *J* = 7.5, 1.7 Hz, 1H), 6.78 – 6.73 (m, 2H), 6.40 (d, *J* = 1.2 Hz, 1H), 5.95 (s, 2H), 5.68 (dd, *J* = 5.9, 1.1 Hz, 1H), 5.44 (dd, *J* = 5.9, 1.2 Hz, 1H), 4.97 (d, *J* = 1.1 Hz, 1H), 3.91 – 3.81 (m, 1H)z, 3.77 – 3.65 (m, 1H), 3.33 (s, 2H), 3.33-3.22 (m, 1H), 3.14 – 2.98 (m, 1H), 2.59 – 2.45 (m, 2H), 1.64 – 1.53 (m, 2H), 1.60 (s, 3H), 1.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 157.3, 155.7, 153.6, 150.5, 139.2, 129.2, 129.0, 120.5, 119.6, 119.6, 116.2, 114.0, 90.4, 84.4, 83.7, 82.7, 61.2, 52.3, 52.0, 45.5, 42.0, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{24}H_{30}N_7O_5$ $[M+H]^+$ 496.2303, found 496.2320.

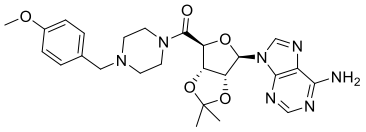
(4-((1*H*-Pyrrolo[2,3-*b*]pyridin-3-yl)methyl)piperazin-1-yl)((3a*S*,4*S*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methanone (A11)



The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in petroleum ether, gradient 50-80% switched to 100 % EtOAc:EtOH (2:1) to afford the product in 85 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.68 (s, 1H), 8.42 (s, 1H), 8.25 (dd, *J* = 4.7, 1.3 Hz, 1H), 7.98 (s, 1H), 7.93 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.09 – 7.06 (m, 1H), 7.00 (dd, *J* = 7.9, 4.7 Hz, 1H), 6.50 (s, 2H), 6.41 (d, *J* = 1.0 Hz, 1H), 5.67 (d, *J* = 5.9 Hz, 1H), 5.45 (dd, *J* = 5.9, 1.0 Hz, 1H), 4.98 (s, 1H), 3.77 – 3.68 (m, 1H), 3.65 – 3.58 (m, 1H), 3.32 (s, 2H), 3.23 (ddd, *J* = 12.7, 9.1, 2.8 Hz, 1H), 3.03 (ddd, *J* = 13.0, 9.4, 3.1 Hz, 1H), 2.47 – 2.34 (m, 2H), 1.60 (s, 3H), 1.58 – 1.51 (m, 2H), 1.41 (s, 3H). 13C NMR (101 MHz, $CDCl_3$) δ 166.7, 156.0, 153.4, 150.4, 149.1, 142.8, 139.2, 128.2, 124.4, 120.3, 119.5, 115.7, 113.9, 110.4, 90.5, 84.6, 83.7, 82.8, 53.7, 52.9, 52.4, 45.9, 42.3, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{30}N_9O_4$ $[M+H]^+$ 520.2415, found 520.2423.

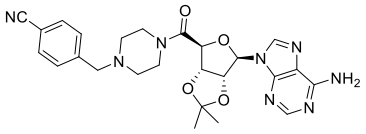
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((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(4-methoxybenzyl)piperazin-1-yl)methanone (A12)



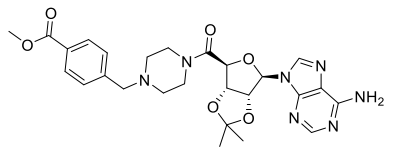
The compound was prepared according to the procedure **P2**. The crude product was purified by chromatography on silica gel eluting with 7 % MeOH in DCM to afford the product in 69 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40 (s, 1H), 7.95 (s, 1H), 7.13 – 7.06 (m, 2H), 6.83 – 6.76 (m, 2H), 6.39 (d, *J* = 1.5 Hz, 1H), 5.87 (br s, 2H), 5.63 (dd, *J* = 5.9, 1.1 Hz, 1H), 5.42 (dd, *J* = 5.9, 1.5 Hz, 1H), 4.99 - 4.97 (m, 1H), 3.77 (s, 3H), 3.72 - 3.64 (m, 1H), 3.63 – 3.54 (m, 1H), 3.33 – 3.23 (m, 1H), 3.18 – 3.06 (m, 3H), 2.42 - 2.31 (m, 2H), 1.60 (s, 5H), 1.44 – 1.38 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.7, 159.0, 155.7, 153.5, 150.5, 139.2, 130.4, 129.6, 119.6, 113.9, 113.8, 90.6, 84.7, 83.6, 82.8, 62.2, 55.4, 52.9, 52.5, 45.8, 42.2, 26.9, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{32}N_7O_5$ $[M+H]^+$ 510.2459, found 510.2475.

4-((4-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonyl)piperazin-1-yl)methyl)benzonitrile (A13)



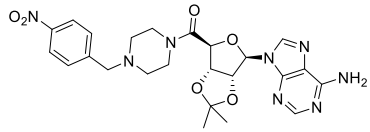
The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 40-100 % to afford the product in 83 % yield as a white solid. 1H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.94 (s, 1H), 7.60 – 7.55 (m, 2H), 7.38 – 7.31 (m, 2H), 6.40 (d, *J* = 1.4 Hz, 1H), 5.77 (s, 2H), 5.66 (dd, *J* = 5.9, 0.6 Hz, 1H), 5.43 (dd, *J* = 5.9, 1.4 Hz, 1H), 4.99 (d, *J* = 0.6 Hz, 1H), 3.78 – 3.70 (m, 1H), 3.66 – 3.59 (m, 1H), 3.32 – 3.26 (m, 1H), 3.24 (d, *J* = 13.9 Hz, 1H), 3.20 (d, *J* = 13.9 Hz, 1H), 3.10 (ddd, *J* = 12.8, 9.2, 3.1 Hz, 1H), 2.41 – 2.33 (m, 2H), 1.65 - 1.57 (m, 2H), 1.61 (s, 3H), 1.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 155.7, 153.4, 150.4, 143.5, 139.3, 132.3, 129.5, 119.4, 118.9, 114.0, 111.3, 90.5, 84.6, 83.7, 82.7, 62.2, 53.0, 52.6, 45.7, 42.1, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{29}N_8O_4$ $[M+H]^+$ 505.2306, found 505.2318.

Methyl 4-((4-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonyl)piperazin-1-yl)methyl)benzoate (A14)



The compound was prepared according to the procedure **P2**. The crude product was purified by chromatography on silica gel eluting with 7% MeOH in DCM to afford the product in 89 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40 (s, 1H), 7.95 (s, 1H), 7.94 – 7.91 (m, 2H), 7.30 – 7.26 (m, 2H), 6.39 (d, *J* = 1.3 Hz, 1H), 5.93 (s, 2H), 5.68 – 5.62 (m, 1H), 5.43 (dd, *J* = 5.9, 1.3 Hz, 1H), 5.00 – 4.94 (m, 1H), 3.89 (s, 3H), 3.76 – 3.65 (m, 1H), 3.65 – 3.56 (m, 1H), 3.32 – 3.24 (m, 1H), 3.22 (s, 2H), 3.14 – 3.06 (m, 1H), 2.41 – 2.32 (m, 2H), 1.66 – 1.56 (m, 5H), 1.42 – 1.39 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.0, 166.7, 155.7, 153.5, 150.5, 143.1, 139.2, 129.7, 129.3, 129.0, 119.6, 113.9, 90.5, 84.7, 83.7, 82.8, 62.4, 53.0, 52.6, 52.2, 45.8, 42.1, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{26}H_{32}N_7O_6$ $[M+H]^+$ 538.2409, found 538.2418.

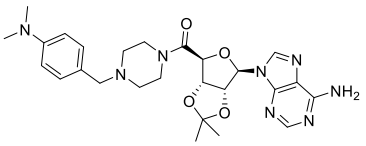
((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(4-nitrobenzyl)piperazin-1-yl)methanone (A15)



The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 50-100 % to afford the product in 37 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.18 – 8.09 (m, 2H), 7.95 (s, 1H), 7.43 – 7.36 (m, 2H), 6.40 (d, *J* = 1.4 Hz, 1H), 5.97 (s, 2H), 5.66 (dd, *J* = 5.9, 1.1 Hz, 1H), 5.43 (dd, *J* = 5.9, 1.4 Hz, 1H), 4.99 (d, *J* = 1.1 Hz, 1H), 3.80 – 3.68 (m, 1H), 3.68 – 3.58 (m, 1H), 3.29 (m, 3H), 3.15 – 3.05 (m, 1H), 2.43 – 2.33 (m, 2H), 1.67 – 1.55 (m, 5H), 1.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 155.6, 153.3, 150.4, 147.3, 145.4, 139.2, 129.4, 123.6, 119.4, 113.9, 90.4, 84.5, 83.6, 82.6, 61.8, 52.9, 52.5, 45.6, 42.0, 26.7, 25.1. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{24}H_{29}N_8O_6$ $[M+H]^+$ 525.2205, found 525.2225.

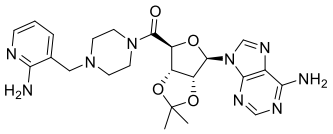
((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(4-(dimethylamino)benzyl)piperazin-1-yl)methanone (A16)

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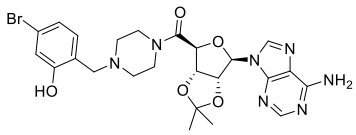
The compound was prepared according to the procedure **P2**. The crude product was purified by chromatography on silica gel eluting with 4% MeOH in DCM to afford the product in 9 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.41 (s, 1H), 7.96 (s, 1H), 7.08 – 7.00 (m, 2H), 6.66 – 6.60 (m, 2H), 6.39 (d, *J* = 1.4 Hz, 1H), 5.90 (s, 2H), 5.62 (dd, *J* = 6.0, 1.2 Hz, 1H), 5.42 (dd, *J* = 6.0, 1.4 Hz, 1H), 5.02 – 4.95 (m, 1H), 3.70 – 3.62 (m, 1H), 3.62 – 3.52 (m, 1H), 3.34 -3.24 (m, 1H), 3.20 – 3.08 (m, 3H), 2.91 (s, 6H), 2.46 – 2.29 (m, 2H), 1.72 – 1.61 (m, 2H), 1.60 (s, 3H), 1.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.7, 155.7, 153.5, 150.4, 150.0, 139.2, 130.2, 125.1, 119.6, 113.9, 112.5, 90.6, 84.8, 83.6, 82.8, 62.4, 52.9, 52.5, 45.8, 42.2, 40.8, 26.9, 25.2. HRMS (ESI/TOF-Q) m/z: Calculated for $C_{26}H_{34}N_8O_4Na$ $[M+Na]^+$ 545.2601, found 545.2618.

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-((2-aminopyridin-3-yl)methyl)piperazin-1-yl)methanone (A17)



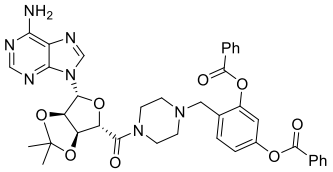
The compound was prepared according to the procedure **P2**. The crude product was purified by chromatography on silica gel eluting with EtOAc: petroleum ether:EtOH (2:1:1), then by chromatography on silica gel eluting 10% MeOH in DCM to afford the product in 15 % yield as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40 (s, 1H), 7.94 (dd, *J* = 5.1, 1.8 Hz, 1H), 7.93 (s, 1H), 7.11 (dd, *J* = 7.2, 1.8 Hz, 1H), 6.53 (dd, *J* = 7.2, 5.1 Hz, 1H), 6.39 (d, *J* = 1.3 Hz, 1H), 6.08 (s, 2H), 5.67 (dd, *J* = 6.1, 1.0 Hz, 1H), 5.48 – 5.39 (m, 3H), 4.97 (s, 1H), 3.80 - 3.72 (m, 1H), 3.68 – 3.58 (m, 1H), 3.26 - 3.16 (m, 1H), 3.13 – 2.94 (m, 3H), 2.39 - 2.29 (m, 2H), 1.59 (s, 3H), 1.52 -1.43 (m, 2H), 1.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.7, 158.4, 155.8, 153.6, 150.5, 147.3, 139.2, 138.2, 119.5, 115.8, 113.9, 113.6, 90.4, 84.5, 83.7, 82.7, 60.8, 52.5, 52.1, 45.7, 42.1, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{23}H_{30}N_9O_4$ $[M+H]^+$ 496.2415, found 496.2418.

((3a*S*,4*S*,6*R*,6aR)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(4-bromo-2-hydroxybenzyl)piperazin-1-yl)methanone (A18)



The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 15-80 % to afford the product in 56 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.39 (s, 1H), 8.41 (s, 1H), 7.91 (s, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 6.88 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.40 (d, *J* = 1.1 Hz, 1H), 5.87 (s, 2H), 5.69 (dd, *J* = 5.8, 1.0 Hz, 1H), 5.45 (dd, *J* = 5.8, 1.1 Hz, 1H), 4.97 (d, *J* = 1.0 Hz, 1H), 3.87 (s, 1H), 3.77 – 3.67 (m, 1H), 3.34 – 3.19 (m, 3H), 3.13 – 2.90 (m, 1H), 2.65 – 2.40 (m, 2H), 1.67 – 1.47 (m, 2 H), 1.60 (s, 3H), 1.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 158.3, 155.7, 153.6, 150.5, 139.3, 130.0, 122.6, 122.5, 119.5, 119.5, 119.5, 114.0, 90.4, 84.4, 83.7, 82.7, 60.7, 52.2, 51.9, 45.4, 41.9, 26.8, 25.2. MS (ESI) *m/z*: Calculated for $C_{24}H_{29}BrN_7O_5$ $[M+H]^+$ 574.1, found 574.2.

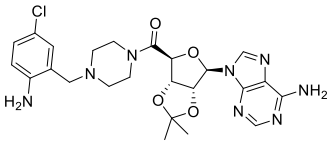
((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(2,4-bis(benzyloxy)benzyl)piperazin-1-yl)methanone (A19)



The compound was prepared according to the procedure **P2.** The crude product was purified by chromatography on silica gel eluting with petroleum ether:EtOAc:EtOH (6:4:1) to afford the product in 38 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.21 – 8.11 (m, 4H), 7.92 (s, 1H), 7.66 – 7.58 (m, 2H), 7.55 – 7.44 (m, 4H), 7.31 – 7.27 (m, 1H), 7.15 – 7.08 (m, 2H), 6.36 (d, *J* = 1.5 Hz, 1H), 5.79 (s, 2H), 5.56 (dd, *J* = 5.9, 1.2 Hz, 1H), 5.38 (dd, *J* = 5.9, 1.5 Hz, 1H), 4.90 (d, *J* = 1.2 Hz, 1H), 3.47 – 3.39 (m, 1H), 3.37 – 3.30 (m, 1H), 3.26 (d, *J* = 13.0 Hz, 1H), 3.19 (d, *J* = 13.0 Hz, 1H), 2.94 – 2.83 (m, 1H), 2.70 (t, *J* = 10.7 Hz, 1H), 2.22 (m, 2H), 1.58 (s, 3H), 1.58 – 1.55 (m, 2H), 1.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 164.9, 155.6, 153.4, 150.9, 150.5, 150.4, 139.2, 133.9, 133.7, 131.4, 130.3, 130.1, 129.8, 129.4, 128.8, 128.7, 127.6, 119.6, 119.3, 117.4, 113.9, 90.6, 84.7, 83.5, 82.7, 58.2, 52.6, 52.3, 45.4, 41.8, 26.9, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{38}H_{38}N_7O_8$ $[M+H]^+$ 720.2776, found 720.2786.

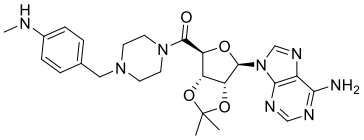
(4-(2-Amino-5-chlorobenzyl)piperazin-1-yl)((3a*S*,4*S*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanone (A20)

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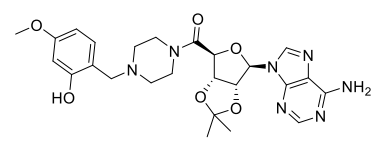
The compound was prepared according to the procedure **P2**. The crude product was purified by chromatography on silica gel eluting with 10% MeOH in DCM to afford the product in 24 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.92 (s, 1H), 7.00 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.85 (d, *J* = 2.5 Hz, 1H), 6.50 (d, *J* = 8.4 Hz, 1H), 6.40 (d, *J* = 1.2 Hz, 1H), 5.92 (s, 2H), 5.68 (dd, *J* = 5.8, 0.8 Hz, 1H), 5.45 (dd, *J* = 5.8, 1.2 Hz, 1H), 4.97 (t, *J* = 0.8 Hz, 1H), 4.50 (s, 2H), 3.74 – 3.68 (m, 1H), 3.64 – 3.58 (m, 1H), 3.22 (ddd, *J* = 12.6, 9.1, 2.9 Hz, 1H), 3.14 (d, *J* = 12.8 Hz, 1H), 3.03 (d, *J* = 12.8 Hz, 1H), 2.99 (s, 1H), 2.38 – 2.29 (m, 2H), 1.60 (s, 3H), 1.57 – 1.43 (m, 2H), 1.43 – 1.40 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 155.7, 153.6, 150.5, 145.3, 139.2, 130.4, 128.4, 122.8, 122.2, 119.6, 116.6, 113.9, 90.4, 84.5, 83.7, 82.7, 61.2, 52.6, 52.1, 45.8, 42.2, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{24}H_{30}ClN_8O_4$ $[M+H]^+$ 529.2073, found 529.2090.

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(4-(methylamino)benzyl)piperazin-1-yl)methanone (A21)



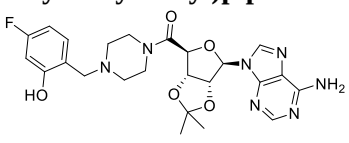
The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 50-100 % to afford the product in 61 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.96 (s, 1H), 7.02 – 6.96 (m, 2H), 6.54 – 6.49 (m, 2H), 6.39 (d, J = 1.4 Hz, 1H), 5.87 (s, 2H), 5.62 (dd, J = 6.0, 1.1 Hz, 1H), 5.41 (dd, J = 6.0, 1.4 Hz, 1H), 4.99 (d, J = 1.1 Hz, 1H), 3.72 – 3.62 (m, 1H), 3.62 – 3.53 (m, 1H), 3.36 – 3.22 (m, 1H), 3.18 – 3.10 (m, 1H), 3.09 (s, 2H), 2.80 (s, 3H), 2.42 – 2.32 (m, 2H), 1.66 – 1.60 (m, 2H), 1.60 (s, 3H), 1.41 (s, 3H). The amine group proton was not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 155.7, 153.4, 150.3, 148.6, 139.1, 130.3, 125.7, 119.5, 113.8, 112.1, 90.5, 84.7, 83.5, 82.6, 62.4, 52.7, 52.3, 45.7, 42.1, 30.8, 26.8, 25.1. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{32}N_8O_4Na$ $[M+Na]^+$ 531.2444, found, 531.2440.

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(2-hydroxy-4-methoxybenzyl)piperazin-1-yl)methanone (A22)



The compound was prepared according to the procedure **P3**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 15-80 % to afford the product in 56 % yield as a white foam. 1H NMR (400 MHz, $CDCl_3$) δ 10.14 (s, 1H), 8.40 (s, 1H), 7.92 (s, 1H), 6.77 (d, *J* = 8.2 Hz, 1H), 6.40 (d, *J* = 1.3 Hz, 1H), 6.35 (d, *J* = 2.6 Hz, 1H), 6.32 (dd, *J* = 8.2, 2.6 Hz, 1H), 5.89 (s, 2H), 5.68 (dd, *J* = 5.9, 1.1 Hz, 1H), 5.44 (dd, *J* = 5.9, 1.3 Hz, 1H), 4.97 (d, *J* = 1.1 Hz, 1H), 3.91 – 3.79 (m, 1H), 3.74 (s, 3H), 3.73 – 3.63 (m, 1H), 3.31-3.26 (m, 1H), 3.29 (s, 2H), 3.13 – 2.97 (m, 1H), 2.58 – 2.47 (m, 2H), 1.69 –1.58 (m, 2H), 1.60 (s, 3H), 1.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 160.8, 158.4, 155.7, 153.6, 150.5, 139.2, 129.5, 119.5, 114.0, 112.8, 105.5, 101.8, 90.4, 84.5, 83.7, 82.7, 60.7, 55.4, 52.2, 51.9, 45.5, 42.0, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{32}N_7O_6$ $[M+H]^+$ 526.2409, found 526.2421.

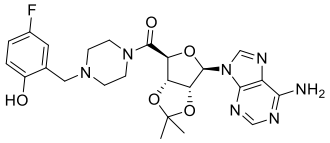
((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(4-fluoro-2-hydroxybenzyl)piperazin-1-yl)methanone (A23)



The compound was prepared according to the procedure **P3**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 15-80 % to afford the product in 96 % yield as a white foam. 1H NMR (400 MHz, $CDCl_3$) δ 10.42 (br s, 1H), 8.40 (s, 1H), 7.92 (s, 1H), 6.80 (dd, *J* = 8.3, 6.4 Hz, 1H), 6.50 – 6.42 (m, 2H), 6.40 (d, *J* = 1.4 Hz, 1H), 6.04 (s, 2H), 5.68 (dd, *J* = 5.9, 1.1 Hz, 1H), 5.45 (dd, *J* = 5.9, 1.4 Hz, 1H), 4.97 (d, *J* = 1.1 Hz, 1H), 3.92 – 3.80 (m, 1H), 3.77 – 3.65 (m, 1H), 3.33-3.25 (m, 1H), 3.29 (s, 2H), 3.14 – 2.95 (m, 1H), 2.59 – 2.43 (m, 2H), 1.65-1.52 (m, 2H), 1.60 (s, 3H), 1.42 (s, 3H). 13C NMR (101 MHz, $CDCl_3$) δ 166.8, 163.6 (d, *J* = 244.7 Hz), 158.7 (d, *J* = 12.4 Hz), 155.8, 153.6, 150.5, 139.2, 129.7 (d, *J* = 10.3 Hz), 119.5, 116.3 (d, *J* = 3.1 Hz), 114.0, 106.3 (d, *J* = 21.7 Hz), 103.8 (d, *J* = 24.3 Hz), 90.4, 84.4, 83.7, 82.7, 60.6, 52.2, 51.9, 45.5, 41.9, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{24}H_{29}FN_7O_5$ $[M+H]^+$ 514.2209, found 514.2219.

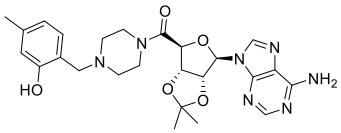
Downloaded from pubs.acs.org/amclct/article-supplement/5250965/pdf/mi6c00203_si_001/ by guest on 06 November 2026

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(5-fluoro-2-hydroxybenzyl)piperazin-1-yl)methanone (A24)



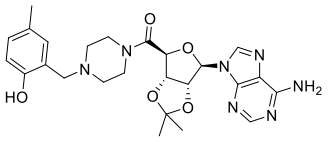
The compound was prepared according to the procedure **P3**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 15-80 % to afford the product in 92 % yield as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.87 (s, 1H), 8.40 (s, 1H), 7.92 (s, 1H), 6.83 (td, J = 8.6, 3.1 Hz, 1H), 6.68 (dd, *J* = 8.9, 4.7 Hz, 1H), 6.61 (dd, *J* = 8.6, 3.1 Hz, 1H), 6.40 (d, *J* = 1.2 Hz, 1H), 6.14 (s, 2H), 5.70 (dd, *J* = 5.9, 1.1 Hz, 1H), 5.45 (dd, *J* = 5.9, 1.2 Hz, 1H), 4.96 (d, *J* = 1.1 Hz, 1H), 3.91 – 3.80 (m, 1H), 3.72 (m, 1H), 3.30 (d, *J* = 13.9 Hz, 1H), 3.29 – 3.23 (m, 1 H), 3.21 (d, *J* = 13.9 Hz, 1H), 3.08 – 2.90 (m, 1H), 2.54 – 2.46 (m, 2H), 1.59 (s, 3H), 1.58-1.54 (m, 2H), 1.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 156.2 (d, *J* = 236.4 Hz), 155.8, 153.6, 153.2 (d, *J* = 2.2 Hz), 150.5, 139.2, 121.4 (d, *J* = 6.9 Hz), 119.5, 116.9 (d, *J* = 7.9 Hz), 115.5 (d, *J* = 8.5 Hz), 115.3 (d, *J* = 9.4 Hz), 114.0, 90.3, 84.3, 83.7, 82.7, 60.8, 52.3, 51.8, 45.4, 41.9, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{24}H_{29}FN_7O_5$ $[M+H]^+$ 514.2209, found 514.2223.

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(2-hydroxy-4-methylbenzyl)piperazin-1-yl)methanone (A25)



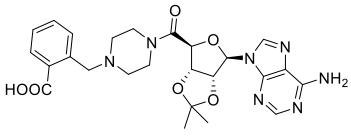
The compound was prepared according to the procedure **P3**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 50-60 % to afford the product in 82 % yield as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.96 (s, 1H), 8.40 (s, 1H), 7.92 (s, 1H), 6.77 (d, *J* = 7.5 Hz, 1H), 6.61 (d, *J* = 1.7 Hz, 1H), 6.57 (dd, *J* = 7.5, 1.7 Hz, 1H), 6.40 (d, *J* = 1.4 Hz, 1H), 5.89 (s, 2H), 5.67 (dd, *J* = 5.9, 1.2 Hz, 1H), 5.44 (dd, *J* = 5.9, 1.4 Hz, 1H), 4.97 (d, *J* = 1.2 Hz, 1H), 3.93 – 3.77 (m, 1H), 3.77 – 3.63 (m, 1H), 3.32 (s, 2H), 3.30 – 3.21 (m, 1H), 3.19 – 2.95 (m, 1H), 2.59 – 2.45 (m, 2H), 2.26 (s, 3H), 1.60 (m, 5H), 1.42 (s, 3H). ^{13}C NMR (100 MHz, CDCl3) δ 166.6, 156.9, 155.5, 153.4, 150.3, 139.2, 139.0, 128.6, 120.2, 119.4, 117.3, 116.6, 113.9, 90.3, 84.3, 83.6, 82.5, 60.8, 52.1, 51.8, 45.4, 41.8, 26.7, 25.0, 21.1. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{32}N_7O_5$ $[M+H]^+$ 510.2459, found 510.2458.

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(2-hydroxy-5-methylbenzyl)piperazin-1-yl)methanone (A26)



The compound was prepared according to the procedure **P3**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 50-60 % to afford the product in 78 % yield as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (s, 1H), 8.40 (s, 1H), 7.93 (s, 1H), 6.94 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.71 – 6.64 (m, 2H), 6.39 (d, *J* = 1.4 Hz, 1H), 5.94 (s, 2H), 5.67 (dd, *J* = 5.9, 1.2 Hz, 1H), 5.43 (dd, *J* = 5.9, 1.4 Hz, 1H), 4.97 (d, *J* = 1.2 Hz, 1H), 3.85 – 3.81 (m, 1H), 3.74 – 3.65 (m, 1H), 3.31 (s, 2H), 3.30 – 3.26 (m, 1H), 3.10 – 3.06 (m, 1H), 2.56 – 2.47 (m, 2H), 2.21 (s, 3H), 1.68 – 1.58 (m, 2H), 1.60 (s, 3H), 1.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 155.6, 154.7, 153.4, 150.3, 139.1, 129.5, 129.3, 128.5, 120.0, 119.4, 115.8, 113.9, 90.3, 84.3, 83.6, 82.5, 61.1, 52.2, 51.8, 45.4, 41.8, 26.7, 25.0, 20.4. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{32}N_7O_5$ $[M+H]^+$ 510.2459, found 510.2472.

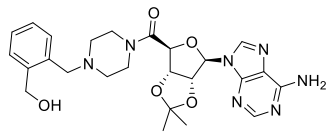
2-((4-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonyl)piperazin-1-yl)methyl)benzoic acid (A27)



The compound was prepared according to the procedure **P3**. The crude product was purified by flash chromatography on silica gel eluting with 10 % MeOH in DCM to afford the product in 48 % yield as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.07 – 8.00 (m, 1H), 7.92 (s, 1H), 7.47 – 7.34 (m, 2H), 7.11 – 7.04 (m, 1H), 6.55 (s, 2H), 6.39 (d, *J* = 1.2 Hz, 1H), 5.70 (d, *J* = 5.8 Hz, 1H), 5.46 (d, *J* = 5.8 Hz, 1H), 4.95 (s, 1H), 4.00 – 3.87 (m, 1H), 3.76 (d, *J* = 13.6 Hz, 1H), 3.37 (s, 2H), 3.29 – 3.17 (m, 1H), 3.02 – 2.84 (m, 1H), 2.57 (t, *J* = 11.3 Hz, 2H), 1.65 –1.52 (m, 2H), 1.59 (s, 3H), 1.41 (s, 3H). The carboxylic acid group proton was not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.6, 166.7, 155.8, 153.3, 150.2, 139.2, 134.0, 133.3, 132.5, 131.8, 131.3, 129.3, 119.1, 114.0, 90.3, 84.0, 83.7, 82.5, 61.2, 50.9, 50.5, 44.5, 40.9, 26.6, 25.0. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{30}N_7O_6$ $[M+H]^+$ 524.2252, found 524.2265.

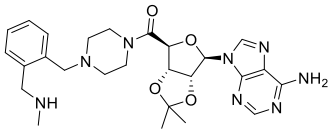
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((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(2-(hydroxymethyl)benzyl)piperazin-1-yl)methanone (A28)



To a solution of piperazine derivative **3** (82 mg, 0.21 mmol, 1 eq) in MeCN (3 mL) was added (2-(bromomethyl)phenyl)methanol (55 mg, 0.27 mmol, 1.3 eq) and TEA (30 µL, 0.22 mmol, 1 eq), and the mixture was stirred for 2 h. The mixture was diluted with EtOAc (20 mL), washed with sat. $NaHCO_3$, water layer was extracted with EtOAc (20 mL). Combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 30-80 % to afford the product (95 mg, 89 %) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.92 (s, 1H), 7.31 – 7.24 (m, 2H), 7.21 (td, *J* = 7.1, 2.5 Hz, 1H), 7.09 – 7.02 (m, 1H), 6.41 (d, *J* = 1.2 Hz, 1H), 6.28 (s, 1H), 6.02 (s, 2H), 5.69 (dd, *J* = 5.9, 1.1 Hz, 1H), 5.45 (dd, *J* = 5.9, 1.2 Hz, 1H), 4.95 (d, *J* = 1.1 Hz, 1H), 4.50 (s, 2H), 3.87 (d, *J* = 13.6 Hz, 1H), 3.68 (d, *J* = 13.6 Hz, 1H), 3.20 (d, *J* = 12.4 Hz, 1H), 3.15 (d, *J* = 12.6 Hz, 1H), 3.13 – 3.07 (m, 1H), 2.90 – 2.79 (m, 1H), 2.52 - 2.43 (m, 2H), 1.59 (s, 3H), 1.46 - 1.39 (m, 2H), 1.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 155.8, 153.6, 150.6, 141.5, 139.3, 135.4, 131.4, 130.2, 128.9, 128.0, 119.5, 113.9, 90.3, 84.3, 83.6, 82.7, 64.9, 61.6, 52.3, 52.0, 45.4, 41.8, 26.8, 25.2. MS (ESI) *m/z*: Calculated for $C_{25}H_{32}N_7O_5$ $[M+H]^+$ 510.2, found 510.5.

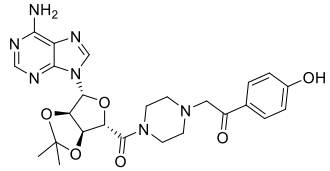
((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)(4-(2-((methylamino)methyl)benzyl)piperazin-1-yl)methanone (A29)



To a solution of benzyl alcohol **A28** (0.19 g, 0.37 mmol, 1 eq) in DCM (5 mL) was added DMP solution in DCM (17 g/ 100 mL, 1.1 mL, 0.44 mmol, 1.2 eq), and the reaction mixture was stirred 18 h at room temperature. The reaction mixture was diluted with DCM (20 mL), quenched by the addition of sodium thiosulfate (10 %, 10 mL) and sat. $NaHCO_3$ (5 mL), the water layer was extracted with DCM. Combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in hexane, gradient 30-80 % to afford the corresponding aldehyde (0.16 g, 86 %) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.29 (s, 1H), 8.41 (s, 1H), 7.95 (s, 1H), 7.82 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.46 (td, *J* = 7.5, 1.5 Hz, 1H), 7.38 (td, *J* = 7.5, 1.3 Hz, 1H), 7.23 (dd, *J* = 7.5, 1.3 Hz, 1H), 6.39 (d, *J* = 1.0 Hz, 1H), 6.06 (s, 2H), 5.64 (d, *J* = 5.8 Hz, 1H), 5.43 (dd, *J* = 5.8, 1.0 Hz, 1H), 4.96 (s, 1H), 3.79 – 3.68 (m, 1H), 3.64 – 3.54 (m, 1H), 3.50 (d, *J* = 13.4 Hz, 1H), 3.44 (d, *J* = 13.4 Hz, 1H), 3.18 (ddd, *J* = 12.7, 9.3, 2.9 Hz, 1H), 2.97 (ddd, *J* = 13.0, 9.5, 3.1 Hz, 1H), 2.44 – 2.33 (m, 2H), 1.59 (s, 3H), 1.56 (s, 2H), 1.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.0, 166.7, 155.8, 153.5, 150.5, 140.1, 139.2, 135.0, 133.4, 130.7, 129.6, 128.2, 119.5, 113.9, 90.5, 84.6, 83.6, 82.8, 59.4, 52.6, 52.2, 45.7, 42.1, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{30}N_7O_5$ $[M+H]^+$ 508.2303, found 508.2311. To a solution of above prepared benzaldehyde (0.16 g, 0.31 mmol, 1 eq) in DCE (3 mL) was added methylamine hydrochloride (28 mg, 0.41 mmol, 1.3 eq) and TEA (48 µL, 0.34 mmol, 1.1 eq), the mixture was stirred for 10 min, then sodium triacetoxyborohydride (91 mg, 0.43 mmol, 1.4 eq) was added in one portion. After stirring for a further 16 h at room temperature, the mixture was quenched with sat. $NaHCO_3$ (10 mL). After stirring for 10 min, the mixture was extracted with EtOAc (x2), washed with water, brine, dried over Na_2SO_4, and concentrated. The residue was purified by chromatography on silica gel, eluent MeOH in DCM (5-10-14 %) (+1% NH_4OH sol.) to obtain the product (45 mg, 27 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.93 (s, 1H), 7.30 – 7.23 (m, 2H), 7.20 (td, *J* = 7.1, 2.1 Hz, 1H), 7.10 (dd, *J* = 7.3, 1.5 Hz, 1H), 6.39 (d, *J* = 1.3 Hz, 1H), 6.00 (s, 2H), 5.66 (dd, *J* = 5.9, 1.1 Hz, 1H), 5.44 (dd, *J* = 5.8, 1.3 Hz, 1H), 4.96 (d, *J* = 1.1 Hz, 1H), 3.81 – 3.76 (m, 2H), 3.75 – 3.69 (m, 1H), 3.66 – 3.55 (m, 1H), 3.32 – 3.11 (m, 4H), 2.97 (t, *J* = 11.5 Hz, 1H), 2.45 – 2.40 (m, 2H), 2.37 (s, 3H), 1.60 (s, 3H), 1.58 – 1.51 (m, 2H), 1.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 155.8, 153.6, 150.5, 139.2, 137.6, 135.7, 131.5, 131.0, 128.3, 127.7, 119.5, 113.9, 90.5, 84.5, 83.7, 82.8, 60.9, 53.3, 52.7, 52.3, 45.7, 42.1, 34.5, 26.8, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{26}H_{35}N_8O_4$ $[M+H]^+$ 523.2776, found 523.2780.

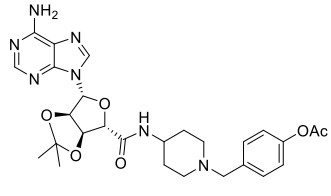
2-(4-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonyl)piperazin-1-yl)-1-(4-hydroxyphenyl)ethan-1-one (A30)

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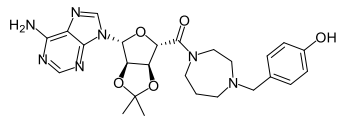
The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (2:1) in petroleum ether, gradient 15-90% to afford the product in 36% yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.00 (s, 1H), 7.80 – 7.74 (m, 2H), 6.78 (m, 2H), 6.45 (s, 2H), 6.40 (s, 1H), 5.68 (d, *J* = 5.8 Hz, 1H), 5.49 (d, *J* = 5.8 Hz, 1H), 5.00 (s, 1H), 3.70 – 3.59 (m, 1H), 3.46 (s, 2H), 3.43 – 3.36 (m, 1H), 3.23 – 3.13 (m, 1H), 2.60 – 2.56 (m, 2H), 1.81 – 1.71 (m, 2H), 1.60 (s, 3H), 1.42 (s, 3H). The phenol hydroxy group proton was not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.1, 166.7, 161.4, 155.5, 153.2, 150.2, 139.2, 130.5, 128.2, 119.1, 115.5, 113.9, 90.6, 84.4, 83.9, 82.8, 63.2, 53.1, 52.6, 45.5, 42.0, 26.7, 25.1. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{30}N_7O_6$, 524.2252, found, 524.2266.

4-((4-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carboxamido)piperidin-1-yl)methyl)phenyl acetate (A31)



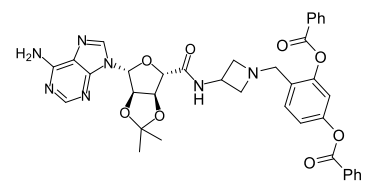
The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in petroleum ether, gradient 50-100% to afford the product in 74% yield as a white solid. 1H NMR (400 MHz, MeOH-d_4) δ 8.22 (s, 1H), 8.16 (s, 1H), 7.32 – 7.25 (m, 2H), 7.07 – 7.01 (m, 2H), 6.35 (d, *J* = 0.9 Hz, 1H), 5.63 (dd, *J* = 6.1, 1.8 Hz, 1H), 5.48 (dd, *J* = 6.1, 0.9 Hz, 1H), 4.64 (d, *J* = 1.8 Hz, 1H), 3.44 (d, *J* = 3.1 Hz, 2H), 3.41 – 3.33 (m, 1H), 2.79 – 2.68 (m, 1H), 2.58 (d, *J* = 11.7 Hz, 1H), 2.26 (s, 3H), 2.08 – 1.99 (m, 1H), 1.98 – 1.89 (m, 1H), 1.72 – 1.63 (m, 1H), 1.57 (s, 3H), 1.45 – 1.38 (m, 3H), 1.35 – 1.27 (m, 1H), 0.99 – 0.78 (m, 2H). ^{13}C NMR (101 MHz, MeOH-d_4) δ 171.2, 171.1, 157.4, 153.9, 151.6, 150.3, 142.6, 135.9, 131.6, 122.6, 120.4, 114.8, 92.4, 88.8, 85.2, 85.2, 63.0, 53.1, 52.9, 47.4, 31.8, 31.1, 27.0, 25.3, 20.9. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{27}H_{34}N_7O_6$ $[M+H]^+$ 552.2565, found 552.2574.

4-((4-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonyl)-1,4-diazepan-1-yl)methyl)phenyl acetate (A32)



To a suspension of carboxylic acid **2** (60 mg, 0.19 mmol, 1 eq) and 4-((1,4λ2-diazepan-1-yl)methyl)phenyl acetate dihydrochloride salt (72 mg, 0.22 eq, 1.2 eq) in DMF-EtOAc mixture (1:3, 2 mL) under argon was added TEA (0.13 mL, 0.93 mmol, 5 eq). The reaction mixture was cooled in the ice bath, then T3P (50% in EtOAc, 0.44 mL, 0.24 mmol, 1.3 eq) was added under argon. The reaction mixture was stirred for 18 h at room temperature. The reaction mixture was diluted with sat. $NaHCO_3$, extracted with EtOAc (2x), washed with brine, dried over Na_2SO_4, and concentrated to afford a crude mixture of acetylated and deacetylated products. The mixture was dissolved in MeOH (2 mL), and K_2CO_3 (2.5 mg, 0.02 mmol, 0.1 eq) was added. The mixture was stirred for 1 h at room temperature, then concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in petroleum ether, gradient 50-100% to afford the product (60 mg, 63 %) as a white solid. Mixture of amide bond cis-trans isomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 0.4H), 8.37 (s, 0.6H), 8.13 (s, 0.6H), 8.11 (s, 0.4H), 7.03 – 6.96 (m, 2H), 6.74 – 6.69 (m, 0.8H), 6.69 – 6.64 (m, 1.2H), 6.43 (d, *J* = 1.5 Hz, 0.6H), 6.40 (d, *J* = 1.7 Hz, 0.4H), 6.21 (br s, 1H), 6.07 (br s, 1H), 5.58 – 5.53 (m, 1H), 5.47 (dd, *J* = 5.8, 1.5 Hz, 0.6H), 5.43 (dd, *J* = 6.0, 1.7 Hz, 0.4H), 5.07 (s, 0.6H), 5.05 (s, 0.4H), 3.73 – 3.12 (m, 7H), 2.71 – 2.44 (m, 2.6H), 2.31 – 2.18 (m, 0.4H), 2.02 – 1.90 (m, 1H), 1.86 – 1.65 (m, 2H), 1.62 (m, 3H), 1.42 (s, 3H). HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{32}N_7O_5$ $[M+H]^+$ 510.2459, found 510.2469.

(3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-*N*-(1-(2,4-bis(benzyloxy)benzyl)azetidin-3-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carboxamide (A33)

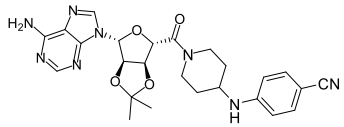


The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in petroleum ether, gradient 15-80% to afford the product in 75 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.21 – 8.16 (m, 4H), 7.81 (s, 1H), 7.68 – 7.61 (m, 2H), 7.58 – 7.46 (m, 4H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.16 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.11 (d, *J* = 2.3 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.10 (d, *J* = 2.2 Hz,

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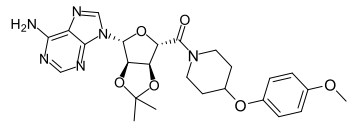
1H), 5.77 (s, 2H), 5.49 (dd, *J* = 6.2, 1.9 Hz, 1H), 5.42 (dd, *J* = 6.2, 2.2 Hz, 1H), 4.67 (d, *J* = 1.9 Hz, 1H), 4.32 – 4.22 (m, 1H), 3.51 – 3.40 (m, 3H), 3.18 (t, *J* = 7.2 Hz, 1H), 2.72 (t, *J* = 6.4 Hz, 1H), 2.06 (t, *J* = 6.6 Hz, 1H), 1.60 (s, 3H), 1.41 – 1.36 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.5, 165.2, 164.7, 155.8, 153.4, 150.3, 149.5, 149.3, 140.0, 133.9, 130.4, 130.3, 129.4, 129.3, 128.8, 128.8, 127.9, 120.3, 119.7, 116.5, 114.3, 91.9, 86.8, 83.9, 83.0, 61.4, 60.8, 57.0, 40.3, 27.0, 25.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{37}H_{36}N_7O_8$ $[M+H]^+$ 706.2620, found 706.2625.

4-((1-((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonyl)piperidin-4-yl)amino)benzonitrile (A34)



The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with 40 % EtOAc:EtOH (3:1) in petroleum ether to afford the product in 44 % yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.95 (s, 1H), 7.35 – 7.30 (m, 2H), 6.49 – 6.44 (m, 2H), 6.44 (s, 1H), 6.21 (s, 2H), 5.99 (s, 1H), 5.77 (d, *J* = 5.8 Hz, 1H), 5.51 (m, 1H), 5.00 (s, 1H), 4.34 – 4.23 (m, 1H), 3.97 – 3.88 (m, 1H), 3.37 – 3.32 (m, 1H), 2.98 – 2.88 (m, 1H), 2.52 – 2.40 (m, 1H), 1.75 (m, 2H), 1.60 (s, 3H), 1.43 (s, 3H), 0.43 – 0.29 (m, 1H), 0.29 – 0.16 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5, 155.7, 153.6, 150.6, 149.7, 139.4, 133.7, 120.3, 119.3, 113.7, 112.5, 98.9, 90.1, 83.9, 83.6, 82.6, 48.6, 44.8, 41.2, 31.9, 31.3, 26.6, 25.0. 14.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{29}N_8O_4$ $[M+H]^+$, 505.2306, found, 505.2299.

((3a*S*,4*S*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)(4-(4-methoxyphenoxy)piperidin-1-yl)methanone (A35)



The compound was prepared according to the procedure **P2**. The crude product was purified by flash chromatography on silica gel eluting with EtOAc:EtOH (3:1) in petroleum ether, gradient 30-50% to afford the product in 88 % yield as a white solid. Mixture of conformers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 0.5H), 8.32 (s, 0.5H), 7.93 (s, 0.5H), 7.92 (s, 0.5H), 6.78 (m, 4H), 6.35 – 6.34 (m, 1H), 5.84 (s, 2H), 5.59 – 5.52 (m, 1H), 5.39 – 5.32 (m, 1H), 5.01 – 4.93 (m, 1H), 4.27 – 4.16 (m, 1H), 3.74 (s, 3H), 3.73 – 3.70 (m, 1H), 3.58 – 3.51 (m, 1H), 3.38 – 3.19 (m, 2H), 1.82 – 1.67 (m, 2H), 1.61 (s, 3H), 1.42 (s, 3H), 1.36 – 1.27 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 155.6, 155.5, 154.3, 154.3, 153.4, 150.2, 139.2, 139.0, 117.9, 117.6, 114.8, 114.7, 113.8, 90.5, 84.8, 83.6, 82.7, 72.6, 71.3, 55.7, 42.8, 41.9, 39.3, 38.3, 31.0, 30.5, 30.2, 29.6, 26.8, 25.1. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{25}H_{31}N_6O_6$ $[M+H]^+$, 511.2300, found, 511.2310.

2-(4-((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carbonyl)piperazin-1-yl)acetamide (B1)

The compound was prepared according to the procedure **P4**. The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 10-50 % to obtain the product (70 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.15 (s, 1H), 7.29 (s, 2H), 7.26 (s, 1H), 7.13 (s, 1H), 6.05 (d, *J* = 6.1 Hz, 1H), 5.72 (s, 2H), 4.87 (d, *J* = 2.7 Hz, 1H), 4.49 (dd, *J* = 6.1, 4.6 Hz, 1H), 4.31 (dd, *J* = 4.6, 2.7 Hz, 1H), 3.59 – 3.49 (m, 4H), 2.86 (s, 2H), 2.47 – 2.35 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.5, 167.5, 156.0, 152.8, 149.8, 139.2, 118.7, 86.9, 80.6, 74.5, 72.7, 60.8, 52.9, 52.4, 44.8, 41.5. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{16}H_{23}N_8O_5$ $[M+H]^+$ 407.1786, found 407.1795. HPLC purity 92 %.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-hydroxyphenyl)piperazin-1-yl)methanone (B2)

The compound was prepared according to the procedure **P4.** The solid residue was washed with MeOH-MeCN mixture, then with diethyl ether, dried *in vacuo* to obtain the product (50 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (s, 1H), 8.57 (s, 1H), 8.15 (s, 1H), 7.28i (s, 2H), 6.82 – 6.75 (m, 2H), 6.69 – 6.62 (m, 2H), 6.07 (d, *J* = 6.1 Hz, 1H), 5.70 (d, *J* = 4.8 Hz, 1H), 5.62 (d, *J* = 6.4 Hz, 1H), 4.92 (d, *J* = 2.7 Hz, 1H), 4.51 (q, *J* = 5.5 Hz, 1H), 4.40 – 4.32 (m, 1H), 3.70 – 3.59 (m, 4H), 3.01 – 2.85 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 156.0, 152.7, 151.4, 149.8, 143.8, 139.2, 118.7, 118.5, 115.5, 86.9, 80.6, 74.5, 72.7, 50.6, 50.2, 44.9, 41.7**.** HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{20}H_{24}N_7O_5$ $[M+H]^+$ 442.1833, found 442.1851.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(pyridin-4-yl)piperazin-1-yl)methanone (B3)

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The compound was prepared according to the procedure **P4**. The solid residue was washed with MeOH-MeCN mixture, then with diethyl ether, dried *in vacuo* to obtain the product formic acid salt (52 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.21 – 8.16 (m, 3H), 8.15 (s, 1H), 7.29 (s, 2H), 6.92 – 6.84 (m, 2H), 6.07 (d, *J* = 6.1 Hz, 1H), 4.93 (d, *J* = 2.5 Hz, 2H), 4.52 (dd, *J* = 6.1, 4.5 Hz, 1H), 4.39 (dd, *J* = 4.5, 2.5 Hz, 1H), 3.72 – 3.57 (m, 4H), 3.51 – 3.42 (m, 2H), 3.42 – 3.33 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.9, 163.5 (HCOOH), 156.0, 154.6, 152.7, 149.8, 148.2, 139.2, 118.7, 108.3, 87.0, 80.6, 74.5, 72.6, 45.4, 45.0, 43.9, 41.0. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{19}H_{23}N_8O_4$ $[M+H]^+$ 427.1837, found 427.1842.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(pyrazin-2-yl)piperazin-1-yl)methanone (B4)

The compound was prepared according to the procedure **P4**. The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 10-80 % to obtain the product (55 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.33 (d, *J* = 1.5 Hz, 1H), 8.15 (s, 1H), 8.09 (t, *J* = 2.6, 1.5 Hz, 1H), 7.86 (d, *J* = 2.6 Hz, 1H), 7.28 (s, 2H), 6.07 (d, *J* = 6.1 Hz, 1H), 5.73 (d, *J* = 4.9 Hz, 1H), 5.65 (d, *J* = 6.3 Hz, 1H), 4.94 (d, *J* = 2.7 Hz, 1H), 4.55 – 4.48 (m, 1H), 4.43 – 4.35 (m, 1H), 3.73 – 3.59 (m, 6H), 3.59 – 3.45 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.9, 156.0, 154.4, 152.8, 149.8, 141.5, 139.2, 132.8, 131.5, 118.7, 87.0, 80.7, 74.5, 72.6, 44.2, 44.0, 43.6, 41.2**.** HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{18}H_{22}N_9O_4$ $[M+H]^+$ 428.1789, found 428.1801

((2*R*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(pyridin-3-ylsulfonyl)piperazin-1-yl)methanone (B5)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (28 %) as a white solid. 1H NMR (300 MHz, DMSO-d_6) δ 8.90 – 8.87 (m, 2H), 8.45 (s, 1H), 8.12 (dt, *J* = 8.1, 2.4, 1.5 Hz, 1H), 8.09 (s, 1H), 7.67 (ddd, *J* = 8.1, 4.8, 0.7 Hz, 1H), 7.27 (s, 2H), 5.98 (d, *J* = 6.2 Hz, 1H), 5.62 (d, *J* = 4.9 Hz, 1H), 5.57 (d, *J* = 6.4 Hz, 1H), 4.80 (d, *J* = 2.7 Hz, 1H), 4.44 (td, *J* = 6.3, 4.5 Hz, 1H), 4.32 – 4.26 (m, 1H), 3.67 – 3.54 (m, 4H), 3.10-3.00 (m, 2H), 2.99 – 2.85 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7, 156.0, 154.0, 152.7, 149.7, 147.7, 139.2, 135.6, 131.7, 124.6, 118.8, 87.0, 80.6, 74.2, 72.4, 45.7, 45.5, 44.2, 40.8. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{19}H_{23}N_8O_6S$ $[M+H]^+$ 491.1456, found 491.1467.

(4-((2*R*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carbonyl)piperazin-1-yl)(pyridin-3-yl)methanone (B6)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (48 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (dd, *J* = 4.9, 1.3 Hz, 1H), 8.64 (d, *J* = 1.7 Hz, 1H), 8.56 (s, 1H), 8.16 (s, 1H), 7.86 (d, *J* = 7.9 Hz, 1H), 7.49 (dd, *J* = 7.9, 4.9 Hz, 1H), 7.30 (s, 2H), 6.06 (d, *J* = 4.0 Hz, 1H), 5.72 (s, 1H), 5.66 (d, *J* = 6.3 Hz, 1H), 4.99 – 4.83 (m, 1H), 4.52 (td, *J* = 6.2, 4.4 Hz, 1H), 4.42 – 4.35 (m, 1H), 3.72 - 3.50 (m, 6H), 3.45 - 3.30 (m, 2H, overlapped with water). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.9, 167.1, 156.0, 152.8, 150.6, 149.8, 147.7, 139.2, 134.9, 131.5, 123.6, 118.8, 87.0, 80.6, 74.4, 72.6, 46.8, 44.8, 41.8, 41.3. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{20}H_{23}N_8O_5$ $[M+H]^+$ 455.1786, found 455.1792.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-hydroxybenzyl)piperazin-1-yl)methanone (B7)

The compound was prepared according to the procedure **P5**. The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 %, then repurified by reverse-phase chromatography, eluent MeCN in water, gradient 0-60 % to obtain the product as a formate salt (20 mg, 21 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H), 8.55 (s, 1H), 8.14 (s, 1H), 7.29 (s, 2H), 7.10 – 7.02 (m, 2H), 6.73 – 6.65 (m, 2H), 6.04 (d, *J* = 6.2 Hz, 1H), 5.67 (d, *J* = 5.0 Hz, 1H), 5.60 (d, *J* = 6.3 Hz, 1H), 4.84 (d, *J* = 2.7 Hz, 1H), 4.53 – 4.44 (m, 1H), 4.36 – 4.26 (m, 1H), 3.52 – 3.41 (m, 4H), 3.34 – 3.29 (m, 2H), 2.33 – 2.22 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.5, 156.4, 156.0, 152.8, 149.8, 139.2, 130.2, 127.7, 118.7, 114.9, 86.9, 80.7, 74.5, 72.7, 61.4, 52.6, 52.0, 44.8, 41.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{26}N_7O_5$ $[M+H]^+$ 456.1990, found 456.2001.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-((4-aminopyridin-3-yl)methyl)piperazin-1-yl)methanone (B8)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in 0.1 % HCOOH, gradient 5-50 % to obtain the product as formic acid salt (66 %)

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as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.20 (s, 1H), 8.15 (s, 1H), 7.96 (d, J = 5.7 Hz, 1H), 7.90 (s, 1H), 7.29 (s, 2H), 6.57 (d, J = 5.7 Hz, 1H), 6.37 (s, 2H), 6.04 (d, J = 6.1 Hz, 1H), 4.86 (d, J = 2.6 Hz, 1H), 4.50 (dd, J = 6.1, 4.5 Hz, 1H), 4.32 (dd, J = 4.5, 2.6 Hz, 1H), 3.60 – 3.55 (m, 4H, overlapped with water), 3.37 (s, 2H), 2.42 – 2.22 (m, 4H). The ribose hydroxy groups protons were not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 164.3 (HCOOH), 156.1, 155.3, 152.8, 149.8, 146.4, 145.5, 139.2, 118.7, 115.9, 109.1, 87.0, 80.7, 74.5, 72.7, 56.8, 52.5, 51.8, 44.8, 41.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{20}H_{26}N_9O_4$ $[M+H]^+$ 456.2102, found 456.2112.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(3-hydroxybenzyl)piperazin-1-yl)methanone (B9)

The compound was prepared according to the procedure **P5**. The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (25 mg, 25 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.56 (s, 1H), 8.15 (s, 1H), 7.29 (s, 2H), 7.09 (t, J = 7.7 Hz, 1H), 6.73 – 6.70 (m, 1H), 6.69 (d, J = 7.5 Hz, 1H), 6.63 (dd, J = 7.9, 1.6 Hz, 1H), 6.05 (d, J = 6.1 Hz, 1H), 5.68 (d, J = 4.9 Hz, 1H), 5.61 (d, J = 6.4 Hz, 1H), 4.86 (d, J = 2.6 Hz, 1H), 4.53 – 4.47 (m, 1H), 4.34 – 4.29 (m, 1H), 3.53 – 3.46 (m, 4H), 3.36 (s, 2H, overlapped with water), 2.39 – 2.23 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.5, 157.3, 156.0, 152.8, 149.8, 139.3, 139.2, 129.2, 119.5, 118.7, 115.6, 114.0, 86.9, 80.7, 74.5, 72.7, 61.8, 52.8, 52.2, 44.9, 41.7. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{26}N_7O_5$ $[M+H]^+$ 456.1990, found 456.2012.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(2-hydroxybenzyl)piperazin-1-yl)methanone (B10)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 5-50 % to obtain the product (42 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (br s, 1H), 8.54 (s, 1H), 8.14 (s, 1H), 7.28 (s, 2H), 7.16 – 7.06 (m, 2H), 6.81 – 6.73 (m, 2H), 6.04 (d, J = 6.1 Hz, 1H), 5.68 (d, J = 4.9 Hz, 1H), 5.62 (d, J = 6.3 Hz, 1H), 4.86 (d, J = 2.7 Hz, 1H), 4.49 (q, J = 5.7 Hz, 1H), 4.35 – 4.30 (m, 1H), 3.65 – 3.30 (m, 6H, overlapped with water), 2.50 – 2.35 (m, 4H). 13C NMR (101 MHz, DMSO-d_6) δ 167.6, 156.4, 156.1, 152.8, 149.8, 139.3, 129.9, 128.4, 122.1, 118.9, 118.8, 115.4, 87.0, 80.7, 74.5, 72.7, 57.7, 52.4, 51.8, 44.7, 41.5. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{26}N_7O_5$ $[M+H]^+$ 456.1990, found 456.1997.

(4-((1*H*-Pyrrolo[2,3-*b*]pyridin-3-yl)methyl)piperazin-1-yl)((2*S*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methanone (B11)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 5-50 % to obtain the product as formic acid salt (28 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (d, J = 2.5 Hz, 1H), 8.54 (s, 1H), 8.19 (dd, J = 4.7, 1.6 Hz, 1H), 8.14 (s, 1H), 8.01 (dd, J = 7.9, 1.6 Hz, 1H), 7.34 (d, J = 2.5 Hz, 1H), 7.28 (s, 2H), 7.03 (dd, J = 7.9, 4.7 Hz, 1H), 6.03 (d, J = 6.2 Hz, 1H), 5.66 (d, J = 4.8 Hz, 1H), 5.59 (d, J = 6.3 Hz, 1H), 4.84 (d, J = 2.6 Hz, 1H), 4.52 – 4.43 (m, 1H), 4.32 – 4.26 (m, 1H), 3.61 (s, 2H), 3.53 – 3.43 (m, 4H), 2.43 – 2.27 (m, 4H). 13C NMR (101 MHz, DMSO-d_6) δ 167.5, 156.0, 152.8, 149.8, 148.7, 142.6, 139.2, 127.3, 125.2, 119.8, 118.7, 115.2, 109.4, 86.9, 80.7, 74.5, 72.7, 52.9, 52.6, 52.1, 44.9, 41.7. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{26}N_9O_4$ $[M+H]^+$ 480.2102, found 480.2108.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-methoxybenzyl)piperazin-1-yl)methanone (B12)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 5-50 % to obtain the product (62 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.56 (s, 1H), 8.15 (s, 1H), 7.29 (br s, 2H), 7.21 – 7.15 (m, 2H), 6.91 – 6.83 (m, 2H), 6.05 (d, J = 6.0 Hz, 1H), 4.85 (d, J = 2.7 Hz, 1H), 4.49 (dd, J = 6.0, 4.6 Hz, 1H), 4.31 (dd, J = 4.6, 2.7 Hz, 1H), 3.73 (s, 3H), 3.55 - 3.24 (m, 6H, overlapped with water), 2.37 – 2.21 (m, 4H). The ribose hydroxy groups protons were not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 167.5, 158.4, 156.0, 152.8, 149.8, 139.2, 130.1, 129.6, 118.7, 113.6, 86.9, 80.7, 74.6, 72.7, 61.2, 55.0, 52.6, 52.0, 44.8, 41.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{28}N_7O_5$ $[M+H]^+$ 470.2152, found 470.2161.

4-((4-((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carbonyl)piperazin-1-yl)methyl)benzonitrile (B13)

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The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (74 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.15 (s, 1H), 7.82 – 7.75 (m, 2H), 7.54 – 7.47 (m, 2H), 7.29 (s, 2H), 6.04 (d, *J* = 6.1 Hz, 1H), 5.68 (d, *J* = 4.9 Hz, 1H), 5.63 (d, *J* = 6.3 Hz, 1H), 4.85 (d, *J* = 2.6 Hz, 1H), 4.50 (td, *J* = 6.2, 4.5 Hz, 1H), 4.32 (td, *J* = 4.7, 2.6 Hz, 1H), 3.54 (s, 2H), 3.54 – 3.46 (m, 4H), 2.42 – 2.26 (m, 4H). 13C NMR (101 MHz, DMSO-d_6) δ 167.6, 156.1, 152.8, 149.8, 144.1, 139.2, 132.3, 129.7, 119.0, 118.7, 109.9, 87.0, 80.7, 74.5, 72.7, 61.1, 52.7, 52.2, 44.9, 41.7. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{25}N_8O_4$ $[M+H]^+$ 465.1993, found 465.1997.

Methyl 4-((4-((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carbonyl)piperazin-1-yl)methyl)benzoate (B14)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (81 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.55 (s, 1H), 8.15 (s, 1H), 7.95 – 7.88 (m, 2H), 7.48 – 7.42 (m, 2H), 7.30 (br s, 2H), 6.05 (d, *J* = 6.1 Hz, 1H), 5.69 (d, *J* = 4.8 Hz, 1H), 5.63 (d, *J* = 6.3 Hz, 1H), 4.86 (d, *J* = 2.7 Hz, 1H), 4.50 (td, *J* = 6.1, 4.5 Hz, 1H), 4.32 (td, *J* = 4.5, 2.7 Hz, 1H), 3.84 (s, 3H), 3.58 - 3.44 (m, 6H), 2.43 – 2.24 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 167.5, 166.2, 156.0, 152.8, 149.8, 143.8, 139.2, 129.2, 129.0, 128.4, 118.7, 86.9, 80.6, 74.5, 72.7, 61.3, 52.8, 52.2, 52.1, 44.8, 41.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{23}H_{28}N_7O_6$ $[M+H]^+$ 498.2096, found 498.2103.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-nitrobenzyl)piperazin-1-yl)methanone (B15)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-80 % to obtain the product (86 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.23 – 8.16 (m, 2H), 8.15 (s, 1H), 7.63 – 7.56 (m, 2H), 7.30 (s, 2H), 6.05 (d, *J* = 6.1 Hz, 1H), 5.98 – 5.50 (m, 2H), 4.86 (d, *J* = 2.6 Hz, 1H), 4.50 (dd, *J* = 6.1, 4.5 Hz, 1H), 4.36 – 4.29 (m, 1H), 3.60 (s, 2H), 3.58 – 3.45 (m, 4H), 2.44 – 2.28 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.0, 156.5, 153.2, 150.3, 147.1, 146.8, 139.6, 130.2, 123.9, 119.2, 87.4, 81.1, 75.0, 73.1, 61.2, 53.2, 52.6, 45.3, 42.1. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{25}N_8O_6$ $[M+H]^+$ 485.1892, found 485.1904.

((2*S*,3*S*,4*R*,*5R)*-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-(dimethylamino)benzyl)piperazin-1-yl)methanone (B16)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (33 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.55 (s, 1H), 8.15 (s, 1H), 7.29 (s, 2H), 7.11 – 7.03 (m, 2H), 6.70 – 6.62 (m, 2H), 6.04 (d, *J* = 6.1 Hz, 1H), 5.67 (d, *J* = 4.9 Hz, 1H), 5.60 (d, *J* = 6.4 Hz, 1H), 4.85 (d, *J* = 2.6 Hz, 1H), 4.49 (q, *J* = 6.1 Hz, 1H), 4.33 – 4.28 (m, 1H), 3.53 - 3.43 (m, 4H), 3.32 (s, overlapped with water, 2H), 2.86 (s, 6H), 2.38 - 2.19 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 167.5, 156.0, 152.8, 149.8, 149.7, 139.2, 129.8, 124.9, 118.7, 112.2, 86.9, 80.6, 74.5, 72.7, 61.4, 52.6, 52.0, 44.8, 41.6, 40.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{23}H_{29}N_8O_4$ $[M-H]^-$ 481.2317, found 481.2324.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-((2-aminopyridin-3-yl)methyl)piperazin-1-yl)methanone (B17)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (36 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.15 (s, 1H), 7.87 (dd, *J* = 5.0, 1.9 Hz, 1H), 7.29 (s, 2H), 7.26 (dd, *J* = 7.2, 1.9 Hz, 1H), 6.50 (dd, *J* = 7.2, 5.0 Hz, 1H), 6.04 (d, *J* = 6.1 Hz, 1H), 5.93 (s, 2H), 5.67 (d, *J* = 5.0 Hz, 1H), 5.62 (d, *J* = 6.4 Hz, 1H), 4.86 (d, *J* = 2.7 Hz, 1H), 4.51 (td, *J* = 6.1, 4.4 Hz, 1H), 4.32 (td, *J* = 4.4, 2.4 Hz, 1H), 3.59 - 3.44 (m, 4H), 3.32 (s, overlapped with water, 2H), 2.41 – 2.19 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 167.5, 158.6, 156.0, 152.8, 149.8, 146.8, 139.2, 137.5, 118.7, 115.2, 112.2, 86.9, 80.6, 74.5, 72.7, 59.4, 52.5, 51.9, 44.8, 41.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{20}H_{26}N_9O_4$ $[M+H]^+$ 456.2102, found 456.2100. HPLC purity 91 %.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-bromo-2-hydroxybenzyl)piperazin-1-yl)methanone (B18)

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The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product as formic acid salt (58 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.14 (s, 1H), 7.29 (s, 2H), 7.10 (d, J = 8.6 Hz, 1H), 6.98 – 6.91 (m, 2H), 6.04 (d, J = 6.1 Hz, 1H), 5.66 (s, 1H), 5.60 (s, 1H), 4.86 (d, J = 2.7 Hz, 1H), 4.49 (t, J = 5.4 Hz, 1H), 4.37 – 4.28 (m, 1H), 3.55 – 3.49 (m, 4H), 3.50 (s, 2H, overlapped with water), 2.46 – 2.32 (m, 4H). The phenol hydroxyl group proton was not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.5, 157.4, 156.0, 152.8, 149.8, 139.2, 131.5, 122.3, 121.7, 120.3, 118.7, 117.9, 86.9, 80.6, 74.5, 72.6, 56.6, 52.4, 51.9, 44.7, 41.5. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{25}BrN_7O_5$ $[M+H]^+$ 534.1095, found 534.1104.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(2,4-dihydroxybenzyl)piperazin-1-yl)methanone (B19)

The compound was prepared according to the procedure **P5**. The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 %, then repurified by reverse-phase chromatography, eluent MeCN in 0.1 % HCOOH, gradient 0-50 % to obtain the product as a formate salt (22 %) as a white foam. 1H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.16 (s, 1H), 8.15 (s, 1H), 7.29 (s, 2H), 6.85 (d, J = 8.1 Hz, 1H), 6.20 (d, J = 2.4 Hz, 1H), 6.17 (dd, J = 8.1, 2.4 Hz, 1H), 6.05 (d, J = 6.1 Hz, 1H), 4.86 (d, J = 2.6 Hz, 1H), 4.49 (dd, J = 6.1, 4.5 Hz, 1H), 4.32 (dd, J = 4.5, 2.6 Hz, 1H), 3.55 – 3.48 (m, 4H), 3.45 (s, 2H), 2.46 – 2.31 (m, 4H). The ribose and phenol hydroxyl groups protons were not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 163.4, 157.7, 157.4, 156.1, 152.8, 149.9, 139.3, 130.6, 118.8, 112.6, 106.2, 102.6, 87.0, 80.7, 74.5, 72.7, 57.4, 52.3, 51.7, 44.7, 41.5. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{26}N_7O_6$ $[M+H]^+$ 472.1939, found 472.1960.

(4-(2-Amino-5-chlorobenzyl)piperazin-1-yl)((2*S*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methanone (B20)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product as formic acid salt (47 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (s, 1H), 8.15 (s, 1H), 7.30 (s, 2H), 7.06 – 6.97 (m, 2H), 6.65 (d, J = 9.0 Hz, 1H), 6.05 (d, J = 6.0 Hz, 1H), 5.74 (d, J = 4.9 Hz, 1H), 5.65 (d, J = 6.4 Hz, 1H), 5.50 (br s, 2H), 4.88 (d, J = 2.7 Hz, 1H), 4.54 – 4.45 (m, 1H), 4.36 – 4.29 (m, 1H), 3.62 – 3.34 (m, 6H, overlapped with water), 2.47 – 2.37 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 156.0, 152.8, 149.8, 146.8, 139.2, 129.8, 128.0, 121.8, 118.9, 118.7, 116.3, 87.0, 80.6, 74.5, 72.7, 58.9, 52.2, 51.6, 44.3, 41.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{26}ClN_8O_4$ $[M+H]^+$ 489.1760, found 489.1763.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-(methylamino)benzyl)piperazin-1-yl)methanone (B21)

The compound was prepared according to the procedure **P4.** The residue was purified by chromatography on silica, eluent aq. 0.5 % NH_4OH in MeCN, gradient 0-60 % to obtain the product (9 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.15 (s, 1H), 7.29 (s, 2H), 7.02 – 6.94 (m, 2H), 6.51 – 6.43 (m, 2H), 6.04 (d, J = 6.1 Hz, 1H), 5.68 (d, J = 4.8 Hz, 1H), 5.61 (d, J = 6.1 Hz, 1H), 5.52 (q, J = 5.0 Hz, 1H), 4.84 (d, J = 2.6 Hz, 1H), 4.49 (q, J = 5.6 Hz, 1H), 4.30 (m, 1H), 3.47 (m, 4H), 3.28 (s, 2H), 2.64 (d, J = 5.0 Hz, 3H), 2.35 – 2.21 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.9, 156.5, 153.2, 150.3, 149.5, 139.6, 130.3, 124.6, 119.2, 111.8, 87.3, 81.1, 75.0, 73.1, 62.2, 53.0, 52.4, 45.3, 42.1, 30.3. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{29}N_8O_4$ $[M+H]^+$, 469.2306, found, 469.2312.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(2-hydroxy-4-methoxybenzyl)piperazin-1-yl)methanone (B22)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (49 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (br s, 1H), 8.57 (s, 1H), 8.16 (s, 1H), 7.31 (s, 2H), 7.02 – 6.95 (m, 1H), 6.34 (m, 2H), 6.06 (d, J = 6.1 Hz, 1H), 5.77 – 5.58 (m, 2H), 4.87 (d, J = 2.6 Hz, 1H), 4.50 (t, J = 5.3 Hz, 1H), 4.36 – 4.30 (m, 1H), 3.68 (s, 3H), 3.59 – 3.48 (m, 6H), 2.46 – 2.31 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 159.6, 157.5, 156.1, 152.8, 149.8, 139.2, 130.5, 118.7, 114.5, 104.5, 101.2, 86.9, 80.6, 74.5, 72.7, 57.4, 54.9, 52.3, 51.7, 44.7, 41.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{28}N_7O_6$ $[M+H]^+$ 486.2096, found 486.2097.

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((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-fluoro-2-hydroxybenzyl)piperazin-1-yl)methanone (B23)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (59 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.15 (s, 1H), 7.30 (s, 2H), 7.19 – 7.09 (m, 1H), 6.60 – 6.54 (m, 2H), 6.05 (d, J = 6.1 Hz, 1H), 5.66 (s, 2H), 4.86 (d, J = 2.6 Hz, 1H), 4.50 (dd, J = 6.1, 4.5 Hz, 1H), 4.32 (dd, J = 4.5, 2.6 Hz, 1H), 3.53 (d, J = 6.2 Hz, 6H), 2.48 – 2.31 (m, 4H). The phenol hydroxyl groups proton was not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. 13C NMR (101 MHz, DMSO-d_6) δ 167.6, 162.1 (d, J_{CF} = 241.7 Hz), 157.8 (d, J_{CF} = 11.6 Hz), 156.0, 152.8, 149.8, 139.2, 131.0 (d, J_{CF} = 10.0 Hz), 118.86 (d, J_{CF} = 2.8 Hz), 118.7, 105.3 (d, J_{CF} = 21.0 Hz), 102.4 (d, J_{CF} = 23.6 Hz), 86.9, 80.6, 74.5, 72.7, 56.9, 52.4, 51.8, 44.7, 41.5. ^{19}F NMR (376 MHz, DMSO-d_6) δ -114.4. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{25}FN_7O_5$ $[M+H]^+$ 474.1896, found 474.1908.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(5-fluoro-2-hydroxybenzyl)piperazin-1-yl)methanone (B24)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (49 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (br s, 1H), 8.56 (s, 1H), 8.15 (s, 1H), 7.30 (s, 2H), 6.98 (dd, J = 9.4, 3.2 Hz, 1H), 6.91 (td, J = 8.6, 3.2 Hz, 1H), 6.75 (dd, J = 8.9, 4.8 Hz, 1H), 6.05 (d, J = 6.1 Hz, 1H), 5.70 (s, 1H), 5.63 (s, 1H), 4.87 (d, J = 2.6 Hz, 1H), 4.49 (t, J = 5.4 Hz, 1H), 4.35 – 4.29 (m, 1H), 3.59 – 3.47 (m, 6H), 2.47 – 2.32 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 156.1, 155.4 (d, J_{CF} = 233.6 Hz),152.8, 152.4 (d, J_{CF} = 1.8 Hz), 149.8, 139.2, 124.3 (d, J_{CF} = 7.0 Hz), 118.7, 116.1 (d, J_{CF} = 7.8 Hz), 115.7 (d, J_{CF} = 23.0 Hz), 114.3 (d, J_{CF} = 22.5 Hz), 86.9, 80.6, 74.5, 72.7, 56.9, 52.5, 52.0, 44.8, 41.6. ^{19}F NMR (376 MHz, DMSO-d_6) δ -125.7. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{25}FN_7O_5$ $[M+H]^+$ 474.1896, found 474.1911.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(2-hydroxy-4-methylbenzyl)piperazin-1-yl)methanone (B25)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (45 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (s, 1H), 8.56 (s, 1H), 8.16 (s, 1H), 7.30 (s, 2H), 6.97 (d, J = 7.6 Hz, 1H), 6.60 – 6.54 (m, 2H), 6.06 (d, J = 6.1 Hz, 1H), 5.72 – 5.68 (m, 1H), 5.63 (m, 1H), 4.87 (d, J = 2.6 Hz, 1H), 4.50 (t, J = 5.4 Hz, 1H), 4.33 (t, J = 3.7 Hz, 1H), 3.60 – 3.49 (m, 6H), 2.49 – 2.31 (m, 4H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.0, 156.7, 156.4, 153.2, 150.2, 139.6, 138.0, 130.0, 120.0, 119.6, 119.1, 116.4, 87.3, 81.0, 74.9, 73.0, 58.0, 52.8, 52.2, 45.1, 42.0, 21.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{28}N_7O_5$ $[M+H]^+$, 470.2146, found, 470.2158. HPLC purity 94%.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(2-hydroxy-5-methylbenzyl)piperazin-1-yl)methanone (B26)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (57 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 8.57 (s, 1H), 8.16 (s, 1H), 7.30 (s, 2H), 6.93 – 6.85 (m, 2H), 6.65 (d, J = 8.1 Hz, 1H), 6.06 (d, J = 6.1 Hz, 1H), 5.70 (s, 1H), 5.63 (d, J = 6.4 Hz, 1H), 4.87 (d, J = 2.6 Hz, 1H), 4.55 – 4.47 (m, 1H), 4.33 (s, 1H), 3.60 – 3.50 (m, 6H), 2.40 (m, 4H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.0, 156.5, 154.6, 153.2, 150.2, 139.6, 130.6, 129.0, 127.6, 122.3, 119.1, 115.6, 87.3, 81.0, 74.9, 73.0, 58.3, 52.8, 52.2, 45.1, 41.9, 20.6**.** HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{28}N_7O_5$ $[M+H]^+$, 470.2146, found, 470.2147. HPLC purity 94 %.

2-((4-((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carbonyl)piperazin-1-yl)methyl)benzoic acid (B27)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (49 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.15 (s, 1H), 7.79 (dd, J = 8.1, 1.5 Hz, 1H), 7.49 (td, J = 7.2, 1.5 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.30 (s, 2H), 6.04 (d, J = 6.1 Hz, 1H), 5.68 (d, J = 4.9 Hz, 1H), 5.63 (d, J = 6.4 Hz, 1H), 4.87 (d, J = 2.7 Hz, 1H), 4.50 (q, J = 5.6 Hz, 1H), 4.38 – 4.30 (m, 1H), 3.83 (s, 2H), 3.69 - 3.42 (m, 4H, overlapped with water),

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2.60 – 2.50 (m, 4H, overlapped with DMSO). The carboxylic acid group proton was not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.2, 167.7, 156.1, 152.8, 149.8, 139.2, 135.3, 134.1, 131.1, 131.0, 130.8, 128.0, 118.8, 87.0, 80.6, 74.4, 72.6, 59.3, 51.3, 50.7, 44.1, 40.9. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{26}N_7O_6$ $[M+H]^+$ 484.1939, found 484.1945.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(2-(hydroxymethyl)benzyl)piperazin-1-yl)methanone (B28)

The acetonide-protected compound **A28** (61 mg, 0.12 mmol) was dissolved in 10 % TFA solution (1 mL) and stirred at 60 °C for 4 h. The reaction mixture was evaporated, then coevaporated with EtOH. The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (22 mg, 49 %) as a white solid. 1H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.15 (s, 1H), 7.40 (d, *J* = 7.4 Hz, 1H), 7.29 (s, 2H), 7.29 – 7.18 (m, 3H), 6.04 (d, *J* = 6.1 Hz, 1H), 5.67 (d, *J* = 5.0 Hz, 1H), 5.61 (d, *J* = 6.3 Hz, 1H), 5.40 (t, *J* = 3.5 Hz, 1H), 4.85 (d, *J* = 2.6 Hz, 1H), 4.58 (d, *J* = 3.5 Hz, 2H), 4.50 (td, *J* = 6.2, 4.5 Hz, 1H), 4.32 (td, *J* = 4.7, 2.6 Hz, 1H), 3.52 – 3.42 (m, 6H), 2.42 – 2.26 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.6, 156.1, 152.8, 149.8, 141.6, 139.2, 135.2, 130.1, 127.8, 127.4, 126.7, 118.7, 87.0, 80.7, 74.5, 72.7, 61.3, 59.6, 52.7, 52.1, 44.8, 41.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{28}N_7O_5$ $[M+H]^+$ 470.2152, found 470.2173.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(2-((methylamino)methyl)benzyl)piperazin-1-yl)methanone (B29)

The acetonide-protected compound **A29** (45 mg, 0.09 mmol) was dissolved in 10 % TFA solution (0.6 mL) and stirred at 40 °C for 2 h. The reaction mixture was evaporated, then coevaporated with EtOH. The residue was purified by reverse-phase chromatography, eluent MeCN in 0.1 % TFA, gradient 0-50 % to obtain the product as a trifluoroacetate salt (30 mg, 49 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (br s, 1H), 8.72 (s, 1H), 8.32 (br s, 1H), 8.19 (s, 1H), 7.62 – 7.37 (m, 4H), 6.08 (d, *J* = 6.0 Hz, 1H), 4.95 (s, 1H), 4.54 – 4.47 (m, 1H), 4.39 – 4.32 (m, 1H), 4.26 (s, 2H), 4.02 – 3.30 (m, 8H, overlapped with water), 2.77 – 2.50 (m, 2H), 2.64 (s, 3H). The ribose hydroxyl and amino groups protons were not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.9, 158.7, 158.4, 152.7, 149.0, 148.3, 141.0, 132.7, 132.5, 131.9, 129.6, 118.6, 87.6, 80.7, 74.7, 72.7, 51.5, 50.8, 49.3, 49.1, 42.9, 32.1. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{23}H_{31}N_8O_4$ $[M+H]^+$ 483.2463, found 483.2484.

2-(4-((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carbonyl)piperazin-1-yl)-1-(4-hydroxyphenyl)ethan-1-one (B30)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-60 % to obtain the product (18 %) as a white solid.
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.57 (s, 1H), 8.15 (s, 1H), 7.91 – 7.81 (m, 2H), 7.29 (s, 2H), 6.87 – 6.78 (m, 2H), 6.05 (d, *J* = 6.1 Hz, 1H), 5.75 – 5.55 (m, 2H), 4.87 (d, *J* = 2.6 Hz, 1H), 4.52 – 4.45 (m, 1H), 4.35 – 4.28 (m, 1H), 3.75 (s, 2H), 3.57 – 3.47 (m, 4H), 2.57 – 2.39 (m, 4H, overlapped with DMSO). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.8, 167.5, 162.1, 156.0, 152.7, 149.8, 139.1, 130.6, 127.4, 118.7, 115.1, 86.8, 80.6, 74.5, 72.6, 62.9, 52.7, 52.2, 44.8, 41.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $[M-H]^-$ $C_{22}H_{24}N_7O_6$, 482.1794, found, 482.1793. HPLC purity 92 %.

(2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxy-*N*-(1-(4-hydroxybenzyl)piperidin-4-yl)tetrahydrofuran-2-carboxamide (B31)

The compound was prepared according to the procedure **P5.** The residue was purified by reverse-phase chromatography, eluent MeCN in 0.1 % HCOOH, gradient 0-50 % to obtain the product as a formate salt (31 mg, 37 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, *J* = 8.1 Hz, 1H), 8.46 (br s, 2H), 8.40 (s, 1H), 8.18 (s, 1H), 7.42 (s, 2H), 7.11 – 7.04 (m, 2H), 6.75 – 6.67 (m, 2H), 5.96 (d, *J* = 7.5 Hz, 1H), 4.59 (dd, *J* = 7.5, 4.6 Hz, 1H), 4.32 (d, *J* = 1.6 Hz, 1H), 4.13 (dd, *J* = 4.6, 1.6 Hz, 1H), 3.69 – 3.59 (m, 1H), 3.33 (s, 2H, overlapped with water), 2.78 (t, *J* = 11.6 Hz, 2H), 2.01 – 1.90 (m, 2H), 1.82 – 1.75 (m, 1H), 1.77 – 1.72 (m, 1H), 1.58 – 1.37 (m, 2H). The ribose hydroxyl and amino groups protons were not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.9, 166.1 (HCOOH), 156.6, 156.3, 152.4, 149.0, 140.8, 129.9, 128.3, 119.6, 115.0, 87.9, 84.6, 73.3, 72.3, 61.7, 51.9, 46.2, 31.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{28}N_7O_5$ $[M+H]^+$ 470.2146, found 470.2152. HPLC purity 94%.

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((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-hydroxybenzyl)-1,4-diazepan-1-yl)methanone (B32)

The compound was prepared according to the procedure **P4.** The residue was purified by reverse-phase chromatography, eluent MeOH in water, gradient 0-50 % to obtain the product (42 %) as a white solid. Mixture of amide isomers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.59 (s, 0.5H), 8.58 (s, 0.5H), 8.15 (s, 1H), 7.29 (s, 2H), 7.11 – 6.98 (m, 2H), 6.75 – 6.63 (m, 2H), 6.07 (d, *J* = 2.6 Hz, 0.5H), 6.05 (d, *J* = 2.6 Hz, 0.5H) 5.64 (s, 2H), 4.85 – 4.79 (m, 1H), 4.59 – 4.49 (m, 1H), 4.35 – 4.28 (m, 1H), 3.68 – 3.41 (m, 6H, overlapped with water), 2.70 – 2.35 (m, 4H, overlapped with DMSO), 1.81 – 1.65 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.9, 168.8, 156.3, 156.0, 152.8, 149.9, 149.8, 139.2, 139.1, 129.8, 129.7, 129.0, 118.7, 118.7, 114.9, 86.9, 86.9, 80.9, 80.7, 74.7, 74.6, 72.9, 72.8, 60.6, 55.2, 54.2, 53.9, 53.2, 46.7, 45.8, 45.3, 44.8, 27.9, 26.6. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{28}N_7O_5$ $[M+H]^+$ 470.2146, found 470.2159.

(2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-*N*-(1-(2,4-dihydroxybenzyl)azetidin-3-yl)-3,4-dihydroxytetrahydrofuran-2-carboxamide (B33)

The compound was prepared according to the procedure **P5**. The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product (19 mg, 44 %) as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (d, *J* = 7.2 Hz, 1H), 8.41 (s, 1H), 8.21 (s, 1H), 7.39 (s, 2H), 6.97 (d, *J* = 8.3 Hz, 1H), 6.31 (d, *J* = 2.3 Hz, 1H), 6.22 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.00 (d, *J* = 7.5 Hz, 1H), 5.78 (s, 1H), 5.61 (s, 1H), 4.66 (dd, J = 7.5, 4.6 Hz, 1H), 4.55 (h, *J* = 7.5 Hz, 1H), 4.34 (d, *J* = 1.6 Hz, 1H), 4.22 (dd, *J* = 4.6, 1.6 Hz, 1H), 4.03 – 3.89 (m, 4H), 3.67 (m, 2H). The phenol hydroxyl groups protons were not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.5, 163.2, 158.9, 157.2, 156.3, 152.6, 149.2, 140.5, 131.7, 119.5, 109.8, 106.5, 102.4, 87.5, 84.2, 73.0, 71.9, 59.5, 59.4, 54.9. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{20}H_{24}N_7O_6$ $[M+H]^+$ 458.1783, found 458.1787. HPLC purity 94 %.

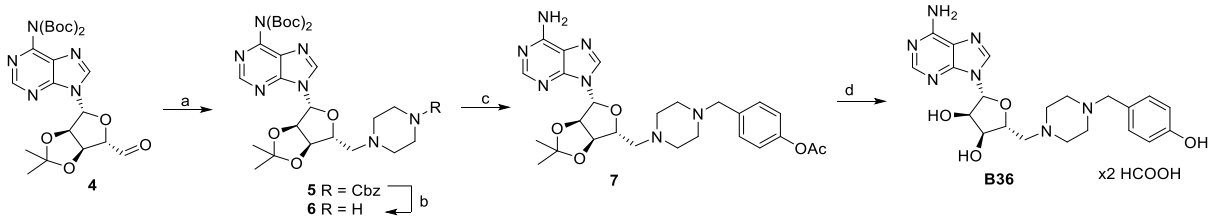
4-((1-((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carbonyl)piperidin-4-yl)amino)benzonitrile (B34)

The compound was prepared according to the procedure **P4**. The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-50 % to obtain the product in 54 % yield as a white solid. Mixture of conformers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 0.5H), 8.57 (s, 0.5H), 8.15 (s, 1H), 7.47 – 7.39 (m, 2H), 7.29 (s, 2H), 6.70 – 6.65 (m, 2H), 6.63 (d, *J* = 7.8 Hz, 0.5H), 6.58 (d, *J* = 7.8 Hz, 0.5H), 6.10 – 6.03 (m, 1H), 5.72 – 5.60 (m, 2H), 4.91 (d, *J* = 2.7 Hz, 0.5H), 4.89 (d, *J* = 2.9 Hz, 0.5H), 4.54 – 4.43 (m, 1H), 4.35 – 4.22 (m, 2H), 3.97 – 3.86 (m, 1H), 3.66 – 3.53 (m, 1H), 3.23 (m, 1H), 2.99 – 2.86 (m, 1H), 2.00 – 1.86 (m, 2H), 1.43 – 1.16 (m, 2H). The amino group proton was not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.9, 156.5, 153.2, 151.6, 150.3, 150.2, 139.6, 139.5, 133.9, 121.1, 119.2, 112.6, 96.1, 96.0, 87.4, 87.3, 81.1, 80.9, 75.1, 75.0, 73.2, 73.0, 48.7, 48.5, 44.0, 43.8, 41.0, 40.8, 40.6, 40.6, 40.4, 40.4, 40.2, 40.2, 40.0, 39.8, 39.5, 39.3, 32.2, 31.5, 31.4. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{25}N_8O_4$, 465.1993, found, 465.1996.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(4-(4-methoxyphenoxy)piperidin-1-yl)methanone (B35)

The compound was prepared according to the procedure **P4**. The residue was purified by reverse-phase chromatography, eluent MeCN in water, gradient 0-70 % to obtain the product in 47 % yield as a white solid. Mixture of conformers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (s, 0.5 H), 8.56 (s, 0.5 H), 8.15 (s, 1H), 7.29 (s, 2H), 6.94 – 6.87 (m, 2H), 6.87 – 6.80 (m, 2H), 6.05 (d, *J* = 6.1 Hz, 1H), 5.74 – 5.67 (m, 1H), 5.66 – 5.59 (m, 1H), 4.89 (d, *J* = 2.7 Hz, 1H), 4.55 – 4.41 (m, 2H), 4.36 – 4.29 (m, 1H), 3.93 – 3.70 (m, 2H), 3.70 – 3.67 (m, 3H), 1.95 – 1.80 (m, 2H), 1.66 – 1.43 (m, 2H). The ribose hydroxyl groups protons were not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.9, 156.4, 154.1, 153.2, 151.0, 150.2, 139.6, 119.1, 117.9, 115.1, 87.3, 81.2, 81.0, 74.9, 73.1, 72.9, 55.8, 42.4, 31.4, 30.7. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{22}H_{27}N_6O_6$, 471.1987, found, 471.2002. HPLC purity 93 %.

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Scheme S2. Synthesis of Target Compound **B36**. Reagents and Conditions: (a) Cbz-piperazine, $Na(OAc)_3BH$, DCM, 18 h, rt, 32 %; (b) H_2, 10 % Pd/C, MeOH, 3 h, rt, 86 %; (c) *i* 4-acetoxybenzaldehyde, $Na(OAc)_3BH$, DCM, 18 h, rt; *ii* TFA in DCM, 2 h, rt, 24 %; (d) *i* K_2CO_3, MeOH; *ii* 50 % HCOOH, 8 %.

4-((4-(((3a*R*,4*R*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)piperazin-1-yl)methyl)phenyl acetate (7)

To a *tert*-butyl (*tert*-butoxycarbonyl)(9-((3a*R*,4*R*,6*S*,6a*S*)-6-formyl-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-6-yl)carbamate (**4**)[1] (0.80 g, 1.35 mmol) solution in DCM (15 mL) was added Cbz-piperazine (0.30 mL, 1.61 mmol, 1.2 eq) and $Na(OAc)_3BH$ (0.40 g, 1.88 mmol, 1.4 eq), and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was quenched with sat. $NaHCO_3$, extracted with EtOAc (2x), washed with brine, dried, and evaporated. The residue was purified by chromatography on silica gel, eluent EtOAc in hexane 1:5 to 1:2 to obtain the bis-Boc-protected product **5** (0.31 g, 32%) and mono-Boc-protected product (0.22 g, 27 %). Bis-Boc protected product **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.23 (s, 1H), 7.39 – 7.30 (m, 5H), 6.12 (d, *J* = 2.1 Hz, 1H), 5.48 (dd, *J* = 6.4, 2.1 Hz, 1H), 5.11 (s, 2H), 4.96 (dd, *J* = 6.4, 3.6 Hz, 1H), 4.47 – 4.38 (m, 1H), 3.50 (t, *J* = 5.1 Hz, 4H), 2.60 (m, 2H), 2.41 (m, 4H), 1.55 (s, 3H), 1.46 (s, 18H), 1.40 (s, 3H). MS (ESI) *m/z*: Calculated for $C_{35}H_{48}N_7O_9$ $[M+H]^+$ 710.3, found 710.9. Bis-Boc-protected adenosine derivative **5** (0.30 g, 0.42 mmol) solution in MeOH (5 mL) was degassed by argon bubbling, then 10% Pd/C (45 mg, 0.1 eq) was added, and H_2 was bubbled through the reaction mixture for 3 h. The mixture was filtered through Celite, washing with MeOH. The solvent was removed under reduced pressure to afford piperazine **6** (0.25 g, 86%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.31 (s, 1H), 6.14 (d, *J* = 2.1 Hz, 1H), 5.47 – 5.44 (m, 1H), 4.95 – 4.88 (m, 1H), 4.47 (m, 1H), 3.41 (t, *J* = 5.1 Hz, 2H), 2.93 – 2.82 (m, 4H), 2.51 – 2.34 (m, 4H), 1.56 (s, 3H), 1.46 (s, 18H), 1.42 – 1.38 (m, 3H). The amino group proton was not observed in the ^{1}H NMR spectrum due to rapid exchange with residual water in the solvent. MS (ESI) *m/z*: Calculated for $C_{27}H_{42}N_7O_7$ $[M+H]^+$ 576.3, found 576.6. Piperazine derivative **6** (0.21 g, 0.36 mmol, 1 eq) was dissolved in DCM (5 mL), then 4-acetoxybenzaldehyde (72 mg, 0.44 mmol, 1.2 eq) was added, followed by $Na(OAc)_3BH$ (0.11 g, 0.51 mmol, 1.4 eq). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was quenched with sat. $NaHCO_3$, extracted with EtOAc (2x), washed with brine, dried, and evaporated to obtain a crude mixture of mono-Boc, bis-Boc, and Boc-deprotected adenosine derivatives. The mixture was dissolved in DCM:TFA (3:1, 4 mL), and the reaction mixture was stirred for 2 h. The solvent was evaporated. The residue was dissolved in DCM, washed with $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by chromatography on silica gel, eluent petroleun ether:EtOAc:EtOH 3:2:1, then by chromatography on silica gel, eluent 10 % MeOH in DCM to afford the product (45 mg, 24 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.36 (s, 1H), 8.02 (s, 1H), 7.33 – 7.28 (m, 2H), 7.04 – 6.98 (m, 2H), 6.06 (d, *J* = 2.1 Hz, 1H), 5.53 (s, 2H), 5.45 (dd, *J* = 6.4, 2.1 Hz, 1H), 4.91 (dd, *J* = 6.4, 3.4 Hz, 1H), 4.43 (td, *J* = 6.6, 3.4 Hz, 1H), 3.47 (s, 2H), 2.59 (d, *J* = 6.6 Hz, 2H), 2.57 – 2.37 (m, 8H), 2.29 (s, 3H), 1.61 (s, 3H), 1.39 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ: 169.7, 155.7, 153.2, 149.8, 149.4, 140.0, 135.8, 130.2, 121.3, 120.4, 114.5, 91.2, 84.8, 84.2, 83.3, 62.4, 60.4, 53.6, 52.9, 27.2, 25.5, 21.2. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{26}H_{34}N_7O_5$ $[M+H]^+$ 524.2621, found 524.2628.

(2*R*,3*R*,4*S*,5*R*)-2-(6-Amino-9*H*-purin-9-yl)-5-((4-(4-hydroxybenzyl)piperazin-1-yl)methyl)tetrahydrofuran-3,4-diol (B36)

The compound was prepared according to the procedure **P5**. The residue was purified by reverse-phase chromatography, eluent MeCN in 0.1 % HCOOH, gradient 0-50 % to obtain the product as a formate (8 %) as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ: 8.26 (s, 1H), 8.21 (s, 1H), 7.26 – 7.17 (m, 2H), 6.84 – 6.77 (m, 2H), 5.96 (d, *J* = 4.2 Hz, 1H), 4.72 (dd, *J* = 5.6, 4.2 Hz, 1H), 4.32 (t, *J* = 5.6 Hz, 1H), 4.20 (q, *J* = 5.6 Hz, 1H), 3.85 (s, 2H), 3.05 – 2.62 (m, 10H). ^{13}C NMR (101 MHz, MeOH-d_4) δ: 159.4, 157.4, 153.9, 150.6, 141.7, 133.1, 131.9, 124.0, 116.6, 90.9, 83.1, 74.5, 73.4, 62.0, 60.5, 52.7, 52.5. HRMS (ESI/TOF-Q) *m/z*: Calculated for $C_{21}H_{28}N_7O_4$ $[M+H]^+$ 442.2203, found 442.2195.

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3. METTL1-WDR4 Enzymatic Methyltransferase Assay

Full-length METTL1-WDR4 was purified as previously reported[2]. The assay was assembled as detailed in Ref[2], with the compound tested as single or multiple dilution points. Interference was evaluated as reported Ref[2]. The dose-response data were analyzed using GraphPad Prism 9.

4. METTL1 Protein Crystallography, Soaking, X-ray Diffraction Experiment and Structure Solution

METTL1$^{\Delta\ 1–31,\ 266–276}$ was purified as described in Ref[2] The METTL1 apo crystal form is obtained by cocrystallization with 8-(4-methylpiperazin-1-yl)-9*H*-purin-6-amine (ChemSpace). The protein at a concentration of 5 mg/mL is mixed with a large excess of crystalline compound to create a saturated solution. The mix is then sonicated and resuspended as described in Ref[2]. The supernatant is mixed in a 1:1 ratio with a solution of 0.1 M phosphate-citrate buffer pH 4.2, 33 % Ethanol, and 1 % PEG 1K (Hampton Research). It is important to note that the buffer is obtained by titrating 0.4 M Sodium phosphate with 0.2 M citric acid to pH 4.2, the final concentration is calculated by summing the individual concentrations of the two components in the mix.

The structure of METTL1 in complex with **B19** was obtained by soaking. A saturated solution of compound in mother liquor was introduced in the crystal-containing drop and the solution incubated overnight. The crystals were collected and flash-frozen in liquid nitrogen following a brief incubation in a cryo-protectant solution composed of 80 % mother liquor and 20 % Glycerol. The X-ray diffraction experiment was performed at the P13 EMBL beamline (DESY, Hamburg). The resulting data were analyzed using AutoPROC, the structures were solved and refined as described in Ref[2].

5. Thermal shift assay

METTL1$^{\Delta\ 1–31,\ 266–276}$ and FL METTL1-WDR4 were tested in thermal shift assay as previously described Ref[2]. In all cases a final concentration of 2 μM was used for the proteins.

6. Stability in microsomes

Mouse liver microsomal stability for compounds **B2** and **B7** was performed by Shanghai ChemPartner using their validated standard protocols. In brief, test compounds (final concentration 1 μM) are incubated with mouse liver microsomes (0.5 mg/mL) in the presence of NADPH. The reaction is stopped at multiple time points with acetonitrile, and the remaining parent compound is quantified by LC–MS/MS.

Mouse liver microsomal stability assay for compounds **B19** and **B22** was performed using pooled male CD-1 mouse liver microsomes (XenoTech). Test compound and microsomes were incubated in phosphate buffer in the presence of NADPH at 37 °C. Reaction mixtures contained 5 μM test compound, 0.5 % DMSO, 0.5 mg/mL microsomal protein, and 1 mM NADPH in 100 mM potassium phosphate buffer (pH 7.4). At 0, 5, 15, 30, 45, and 60 min, aliquots were removed and reactions were terminated by adding ice-cold acetonitrile/methanol (3:1, v/v) containing reserpine as an internal standard. Samples were centrifuged (10 min, 10,000 rpm), and the supernatants were diluted with 0.1% formic acid (1:4, v/v) prior to LC–MS/MS analysis. Testosterone was used as a positive control. Negative control samples were prepared without NADPH and analyzed at 0 and 60 min. Additional stability was assessed in 100 mM PBS under identical incubation conditions, with samples collected at 0 and 60 min. LC–MS/MS analysis was performed on an Acquity UPLC H-Class system coupled to a Xevo TQ-S micro mass spectrometer (ESI positive mode).

7. Stability in PBS

10 mM compound stock solution in DMSO was diluted in PBS buffer pH 7.4 to a final concentration 30 μM. The solution was incubated at 37 °C. The sample aliquots were taken at 6 time points: 0, 1, 2, 4, 6, and 24 h and analyzed immediatly by HPLC at 254 nm.

8. Kinetic solubility

Kinetic solubility assay **B2** and **B7** was performed by Bioduro. A stock solution of compound (50 mM in DMSO) is added to the buffer pH 7.4 to a target concentration of 500 μM. The mixture is shaken at room temperature for 4 h and then filtered. The sample was diluted with a quenching solution and analyzed by LC-MS/MS. Kinetic solubility of compound **B19** and **B22** was assessed in similar conditions, but quantified by HPLC.

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9. Cell Membrane permeability assay

Caco-2 Permeability Assay was performed by Bioduro using 10 μM compound working concentration. The samples were analyzed by LC-MS/MS.

10. Methyltransferase selectivity assay

Methyltransferase HotSpot™ radioisotope-based assay using ^{3}H-AdoMe was performed by Reaction Biology. Compounds were tested in single-dose mode at 20 μM concentrations in duplicate.

11. Computational modeling studies

The receptor (PDB 29GE) was prepared using the Maestro Protein Preparation workflow from the Schrödinger Suite (Schrödinger Release 2024-4: Schrödinger, LLC, New York, NY, 2024). The ligand structures were prepared using LigPrep. Docking experiments were performed using Glide. The docking grid was centered and sized on the crystalized structure **B19**. Docked poses were visualised using PyMOL.

References:

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