

Supporting Information  
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## ***Supporting Information***

**Manuscript Title:** Synthesis and evaluation of the biological profile of novel analogs of nucleosides and of potential mimetics of sugar phosphates and nucleotides

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### ***Chemistry***

#### ***General Methods***

NMR spectra were recorded with a BRUKER Avance 400 spectrometer operating at 400.13 MHz for  $^1\text{H}$  or 100.62 MHz for  $^{13}\text{C}$ . Chemical shifts are expressed in parts per million and are reported relative to internal TMS, in the case of  $\text{CDCl}_3$ , or relative to the respective solvent peak as reference. High-resolution mass measurements were performed on an High Resolution QqTOF Impact II mass spectrometer equipped with an ESI ion source (Bruker Daltonics). Spectra were acquired in positive ESI mode with external calibration. Melting points were determined with a Stuart Scientific SMP 3 apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 343 polarimeter. The reactions were monitored by TLC on Merck 60 F<sub>254</sub> silica gel aluminium plates with detection under UV light (254 nm) and/or by spraying with a solution of 10%  $\text{H}_2\text{SO}_4$  in EtOH or with a solution of 0.2% (w/v) cerium(IV) sulphate-5% ammonium molybdate in 6% aq.  $\text{H}_2\text{SO}_4$ . Column chromatography was carried out on silica gel 60 G (0.040–0.063 mm, E. Merck).

#### ***Experimental details and Compounds' data.***

**2-Acetamide-6-chloro-9-(methyl 2,3,4-*O*-acetyl-6-deoxy- $\alpha$ -D-glucopyranosid-6-yl)purine (2):** included in the manuscript [25].

**2-Acetamide-6-hydroxy-9-(methyl 6-deoxy- $\alpha$ -D-glucopyranosid-6-yl)purine (3):**

A solution of 2-acetamide-6-chloro-9-(methyl 2,3,4-*O*-acetyl-6-deoxy- $\alpha$ -D-glucopyranosid-6-yl)purine (**2**, 116 mg, 0.226 mmol) in aq. TFA (80%, 2.5 mL) was stirred at 60 °C for 16 h. After co-evaporation of the solvents with toluene, the crude product was purified by column chromatography (dichloromethane/MeOH, 4:1) to afford the title compound (47 mg, 57%) as a white solid. m.p.: 212.9-214.5 °C.  $[\alpha]_D^{20} = +5$  ( $c = 0.3$ , in CH<sub>3</sub>OH). <sup>1</sup>H NMR (300 MHz, MeOD)  $\delta$  7.96 (s, 1 H, H-8), 4.64 (d, 1 H, H-1',  $J_{1',2'} = 3.7$ ), 4.58 (dd, part A of ABX, H-6'a,  $J_{5',6'a} = 2.4$ ,  $J_{6'a,6'b} = 14.3$ ), 4.24 (dd, part B of ABX, H-6'b,  $J_{5',6'b} = 7.9$ ), 3.77 (ddd, 1 H, H-5'), 3.61 (t, 1 H, H-3'), 3.38 (dd, 1 H, H-2',  $J_{2',3'} = 9.7$ ), 3.14 (dd, 1 H, H-4',  $J_{3',4'} = 9.1$ ,  $J_{4',5'} = 9.8$ ), 3.10 (s, 3 H, OCH<sub>3</sub>), 2.22 (s, 3 H, CH<sub>3</sub>, Ac). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 175.0 (CO, Ac), 157.5 (C-6), 150.7 (C-4), 149.3 (C-2), 142.8 (C-8), 120.4 (C-5), 101.2 (C-1'), 74.8 (C-3'), 73.3, 73.1 (C-2', C-4'), 71.1 (C-5'), 55.4 (OCH<sub>3</sub>), 45.8 (C-6'), 23.9 (CH<sub>3</sub>, Ac). HRMS: calcd for C<sub>14</sub>H<sub>19</sub>N<sub>5</sub>O<sub>7</sub> [ $M + H$ ]<sup>+</sup> 370.1357, found 370.1351.

**Phenyl 2-*O*-acetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-1-thio-6-*O*-triisopropylsilyl-D-mannopyranoside (5):**

To a solution of phenyl 3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-1-thio-D-mannopyranoside (**4**, 0.4 g, 1.04 mmol) in dry pyridine (10 mL) and chloroform (5 mL), under argon, was added DMAP (cat. amount). Then, at 0 °C, TIPSCl (3.11 mmol, 0.66 mL) was added dropwise. The mixture was allowed to warm at room temp. and it was stirred overnight. The reaction mixture was diluted with EtOAc and it was washed with water. The aqueous phase was extracted twice with EtOAc. The combined organic phases were washed with water and dried with MgSO<sub>4</sub>. After filtration and evaporation of the solvent, the residue was dried under vacuum. Then it was dissolved in a mixture of pyridine (16 mL) and acetic anhydride (12 mL). The solution was stirred at room temp. overnight. The solvents were co-evaporated with toluene and the crude was subjected to column chromatography on silica-gel (EtOAc/hexane, 1:9) to yield the title compound (575 mg, 95%) as a colorless oil.  $[\alpha]_D^{20} = +173$  ( $c = 1.0$ , in CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.50-7.44 (m, 2 H, Ph), 7.30-7.19 (m, 3 H, Ph), 5.48 (br. s, 1 H, H-1), 5.26 (br.d, 1 H, H-2), 4.29-4.13 (m, 2 H, H-4, H-5,  $J_{3,4} = J_{4,5} = 9.7$ ), 4.09 (dd, 1 H, H-3,  $J_{3,4} = 9.9$ ,  $J_{2,3} = 2.9$ ), 3.99 (dd, part A of ABX, H-6a,  $J_{5,6a} = 3.5$ ,  $J_{6a,6b} = 11.4$ ), 3.89 (dd, part B of ABX, H-6b,  $J_{5,6b} = 1.5$ ), 3.30, 3.29 (2  $\times$  s, 6 H, OCH<sub>3</sub>), 2.10 (s, 3 H, CH<sub>3</sub>,

OAc), 1.31, 1.30 (2 × s, 6 H, CH<sub>3</sub>), 1.10-0.99 (m, 21 H, 3 × *i*-Pr, TIPS). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 170.8 (CO), 134.5 (Cq, Ph), 131.5, 129.0, 127.5 (CH, Ph), 100.0, 99.9 (2 × Cq, BDA), 86.4 (C-1), 72.7 (C-5), 67.1 (C-3), 62.9 (C-4), 61.6 (C-6), 48.2, 48.1 (2 × OCH<sub>3</sub>, BDA), 21.3 (CH<sub>3</sub>, Ac), 18.1, 18.0 (Me, *i*-Pr, TIPS), 17.8, 17.8 (2 × CH<sub>3</sub>, BDA), 12.1 (CH, *i*-Pr, TIPS). HRMS: calcd for C<sub>29</sub>H<sub>48</sub>O<sub>8</sub>SSi [*M* + Na]<sup>+</sup> 607.2731, found 607.2720; calcd. for [*M* + K]<sup>+</sup> 623.2471, found 623.2461.

**Phenyl 2-*O*-acetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-1-thio-D-mannopyranoside (6):**

To a solution of phenyl 2-*O*-acetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-1-thio-6-*O*-triisopropylsilyl-D-mannopyranoside (**5**, 150 mg, 0.26 mmol) in THF (8 mL), tetrabutylammonium fluoride (TBAF, 1 M in THF, 0.8 mL) was added. The reaction was stirred at room temperature for 1 h. The solution was diluted with dichloromethane and washed with water. The aqueous layers were extracted twice dichloromethane. The combined organic layers were dried with MgSO<sub>4</sub>. After filtration and evaporation of the solvent under vacuum, the residue was purified by column chromatography on silica gel (Hexane/EtOAc, from 3:2 to 1:1) to give the title compound (62 mg, 56%) as a colorless oil.  $[\alpha]_D^{20} = +14$  (c = 1.0, in CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.50-7.44 (m, 2 H, Ph), 7.35-7.26 (m, 2 H, Ph), 5.45 (br.s, 1 H, H-1), 5.31 (br.s, 1 H, H-2), 4.34-4.22 (m, 1 H, H-5), 4.17-4.06 (m, 2 H, H-3, H-4), 3.89-3.75 (m, 2 H, H-6a, H-6b), 3.30, 3.27 (2 s, 2 × CH<sub>3</sub>, OMe), 2.13 (s, CH<sub>3</sub>, OAc), 1.31, 1.29 (2 s, 2 × CH<sub>3</sub>, Me). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 170.6 (CO, Ac), 133.4 (Cq, Ph), 132.5, 129.3, 128.1 (CH, Ph), 100.4, 99.9 (2 × Cq, BDA), 86.9 (C-1), 72.1, 71.8 (C-2, C-5), 66.8, 63.7 (C-3, C-4), 48.2, 48.0 (2 × CH<sub>3</sub>, OMe), 21.3 (CH<sub>3</sub>, OAc), 17.9, 17.7 (2 × CH<sub>3</sub>, BDA). HRMS: calcd for C<sub>20</sub>H<sub>28</sub>O<sub>8</sub>S [*M* + Na]<sup>+</sup> 451.1397, found 451.1405.

**2-Acetamide-6-hydroxy-9-(phenyl 6-deoxy-1-thio-α-D-mannopyranosid-6-yl)purine (7):** To a solution of phenyl 2-*O*-acetyl-3,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-1-thio-D-mannopyranoside (**6**, 52 mg, 0.12 mmol) in tetrahydrofuran (THF, 6 mL) under nitrogen, PPh<sub>3</sub> (156 mg, 0.59 mmol), diethyl azodicarboxylate (DEAD, 0.95 mmol, 0.15 mL) and 2-acetamido-6-chloropurine (115 mg, 0.54 mmol) were sequentially added. The mixture was stirred under reflux for 1 h. The solvent was evaporated and the crude was subjected to column chromatography on silica-gel (EtOAc/hexane, 3:2 to

EtOAc/petroleum ether, 4:1). The obtained white solid was dissolved in aq. TFA (80%, 2.5 mL) and the solution was stirred at 60 °C for 16 h. After co-evaporation of the solvents with toluene, the residue was purified by column chromatography (from dichloromethane to dichloromethane/MeOH, 4:1) to afford the title compound (22 mg, 41%, 2 steps) as a pale yellow oil.  $[\alpha]_D^{20} = +29$  ( $c = 0.5$ , in CH<sub>3</sub>OH). <sup>1</sup>H NMR (300 MHz, MeOD)  $\delta$  7.80 (s, 1 H, H-8), 5.43 (d, 1 H, H-1',  $J_{1',2'} = 1.3$ ), 4.66 (dd, part A of ABX system, H-6'a,  $J_{5',6'a} = 2.0$ ,  $J_{6'a,6'b} = 14.0$ ), 4.38 (td, 1 H, H-5'), 4.20 (dd, part B of ABX system, H-6'b,  $J_{5',6'b} = 9.7$ ), 4.06 (dd, 1 H, H-2',  $J_{2',3'} = 2.8$ ), 3.75-3.62 (m, 2 H, H-3', H-4',  $J_{3',4'} = J_{4',5'} = 9.2$ ), 2.22 (s, 3 H, CH<sub>3</sub>, Ac). <sup>13</sup>C NMR (100 MHz, MeOD)  $\delta$ : 175.1 (CO, Ac), 163.4, 163.0 (C-2, C-6), 150.9 (C-4)\*, 142.7 (C-8), 134.4 (Cq, Ph), 131.7, 129.9, 128.3 (CH, Ph), 119.6 (C-5), 88.9 (C-1'), 73.2 (C-2'), 72.9 (C-3'), 72.4 (C-5'), 70.7 (C-4'), 46.4 (C-6'), 23.9 (CH<sub>3</sub>, Ac). HRMS: calcd for C<sub>19</sub>H<sub>21</sub>N<sub>5</sub>O<sub>6</sub>S [ $M + H$ ]<sup>+</sup> 448.1285, found 448.1294.

***N*-( $\beta$ -D-ribofuranosyl)-*N'*-tosylhydrazide (9):** To a solution of ribose (**8**, 0.5 g, 3.33 mmol) in *N,N*-dimethylformamide (DMF, 3 mL), *p*-toluenesulfonyl hydrazide (0.73 mg, 3.83 mmol) and glacial acetic acid (0.02 mL, 3.5 mmol), was added. The reaction mixture was left at 40 °C, without stirring, for 48 h. The solvent was evaporated under vacuum. Diethyl ether (22 mL) was added to the residue and the mixture was vigorously stirred for 16 h. The mixture was filtered and the white solid was washed with diethyl ether and dichloromethane to yield pure title compound (1.04 g, quant.). m.p.: 162.0-164.0 °C.  $[\alpha]_D^{20} = +35$  ( $c = 0.7$ , in DMSO). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  8.81 (br.d, 1 H, -NHSO<sub>2</sub>-), 7.69 (d, 2 H, H-a,  $J = 7.6$ , Ts), 7.38 (d, 2 H, H-b,  $J = 7.6$ , Ts), 4.98-4.87 (m, 2 H, OH-3, OH-4,  $J_{H-4,OH} = 5.4$ ,  $J_{H-3,OH} = 6.0$ ), 4.62-4.47 (m, 2 H, NH, OH-2,  $J_{NH,H-1} = 10.9$ ,  $J_{NH,NH} = 3.4$ ,  $J_{H-2,OH} = 7.5$ ), 3.92 (d, 1 H, H-1,  $J_{1,2} = 1.2$ ), 3.65 (d, 1 H, H-5a,  $J_{5a,5b} = 11.9$ ,  $J_{H-4,H-5a} = 4.2$ ), 3.57-3.41 (m, 3 H, H-3, H-2, H-4), 3.30 (d, 1 H, H-5b,  $J_{H-4,H-5b} = 2.0$ ), 2.38 (s, 3 H, Me, Ts). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 142.9 (Cq, Ts, b), 136.1 (Cq, Ts, b), 129.4, 127.5 (CH, Ts, a, b), 88.5 (C-1), 70.2 (C-2), 69.0 (C-4), 68.3 (C-3), 65.7 (C-5), 21.0 (CH<sub>3</sub>, Ts). HRMS: calcd for C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>6</sub>S [ $M + H$ ]<sup>+</sup> 319.0958, found 319.0963; calcd for [ $M + Na$ ]<sup>+</sup> 341.0778, found 341.0782.

**1-deoxy-1-(2-tosylhydrazin-1-yl)- $\beta$ -D-glucopyranuronic acid (11):** To a solution of glucuronic acid (**10**, 300 mg, 1.55 mmol) in *N,N*-dimethylformamide (DMF, 2.2 mL), *p*-

toluenesulfonyl hydrazide (289 mg, 1.55 mmol) and glacial acetic acid (9  $\mu$ L, 0.16 mmol), was added. The reaction mixture was left at 40  $^{\circ}$ C, without stirring, for 48 h. The solvent was evaporated under vacuum. Diethyl ether (22 mL) was added to the residue and the mixture was vigorously stirred for 16 h. The mixture was filtered and the white solid was washed with diethyl ether and dichloromethane to yield the pure title compound (376 mg, 67%) as a pale yellow solid. mp: 97.2-99.0  $^{\circ}$ C.  $[\alpha]_D^{20} = +2$  (c = 1, in CH<sub>3</sub>OH). <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  7.78 (d, 2 H, H-a,  $J = 7.9$ , Ts), 7.39 (d, 2 H, H-b,  $J = 7.9$ , Ts), 3.85 (d, 1 H, H-1,  $J_{1,2} = 8.8$ ), 3.70 (d, 1 H, H-5,  $J_{4,5} = 9.7$ ), 3.55-3.43 (m, 2 H, H-2, H-4), 3.39 (t, 1 H, H-3,  $J_{2,3} = J_{3,4} = 8.9$ ), 2.43 (s, 3 H, Me, Ts). <sup>13</sup>C NMR (100 MHz, MeOD)  $\delta$ : 173.4 (CO), 145.2 (Cq, Ts), 137.2 (Cq, Ts), 130.6, 129.0 (CH, Ts), 91.9 (C-1), 77.6, 77.5 (C-3, C-5), 73.2, 71.0 (C-2, C-4), 21.5 (CH<sub>3</sub>, Ts). HRMS: calcd for C<sub>13</sub>H<sub>18</sub>N<sub>2</sub>O<sub>8</sub>S  $[M + Na]^+$  385.0676, found 385.0670

**2,3,4-tri-O-acetyl-N-tosylamino- $\beta$ -D-glucopyranurono-6,1-lactam (12):** A solution of 1-deoxy-1-(2-tosylhydrazin-1-yl)- $\beta$ -D-glucopyranuronic acid (**11**, 22 mg, 0.059 mmol) in pyridine (2.5 mL) and acetic anhydride (2 mL) was stirred at room temp for 30 min. The solvents were co-evaporated with toluene and the crude was purified by column chromatography on silica gel (EtOAc/hexane, 1:1). The obtained residue was dried under vacuum and then dissolved in anhydrous dichloromethane (4 mL), to which *N*-propargylamine (0.131 mmol, 5  $\mu$ L) was added. The solution was stirred overnight at room temp. under nitrogen. After concentration under vacuum, the crude was subjected to column chromatography (EtOAc/hexane, 1:1) to yield the title compound as a colorless oil (12 mg, 38%, 2 steps).  $[\alpha]_D^{20} = -38$  (c = 0.5, in CH<sub>3</sub>OH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.84 (d, 2 H, H-a,  $J = 8.0$ , Ts), 7.35 (d, 2 H, H-b,  $J = 8.1$ , Ts), 5.62 (s, 1 H, H-1), 4.97 (s, 1 H, H-3), 4.92 (s, 1 H, H-2), 4.57 (s, 1 H, H-4), 4.45 (s, 1 H, H-5), 2.45 (s, 3 H, Me, Tol), 2.20 (s, 3 H, CH<sub>3</sub>, Ac), 2.16 (s, 3 H, CH<sub>3</sub>, Ac), 2.07 (s, 3 H, CH<sub>3</sub>, Ac-2). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 169.5, 169.4, 168.1 (3  $\times$  CO, Ac) 167.0 (CO, lactam), 145.8 (Cq, Ts), 132.7 (Cq, Ts), 130.1, 128.7 (CH, Ts), 87.5 (C-1), 73.3 (C-5), 68.9 (C-2), 65.3 (C-4), 63.8 (C-3), 21.8 (CH<sub>3</sub>, Ts), 21.0, 20.9, 20.8 (3  $\times$  CH<sub>3</sub>). HRMS: calcd for C<sub>19</sub>H<sub>22</sub>N<sub>2</sub>O<sub>10</sub>S  $[M + H]^+$  471.1608, found 471.1609

**N-Benzyl- $\alpha,\beta$ -D-glucopyranuronamide (15):** A solution of *N*-benzyl 1,2-*O*-isopropylidene- $\alpha$ -D-glucofuranuronamide (**14**, 500 mg, 1.55 mmol) in aq. TFA (80%,

6.2 mL) was stirred at room temp. for 1.5 h. After co-evaporation of the solvents with toluene, the crude product was purified by column chromatography (from EtOAc/MeOH 1:9 to 4:1) to afford the title compound (430 mg, quantitative, anomeric mixture,  $\alpha/\beta$  ratio, 1:0.60) as a colorless.  $^1\text{H}$  NMR (300 MHz, MeOD)  $\delta$  7.39-7.16 (m, Ph,  $\alpha$ ,  $\beta$ ), 5.22 (d, H-1  $\alpha$ ,  $J_{1,2(\alpha)} = 3.4$ ), 4.58 (d, 1 H, H-1  $\beta$ ,  $J_{1,2(\beta)} = 7.8$ ), 4.52-4.34 (m,  $\text{CH}_2\text{Ph}$ ,  $\alpha$ ,  $\beta$ ), 4.26 (d, 1 H, H-5  $\alpha$ ,  $J_{4,5(\alpha)} = 9.9$ ), 3.85-3.69 (m, H-3  $\alpha$ , H-3  $\beta$ ,  $J_{2,3(\alpha)} = J_{3,4(\alpha)} = 9.3$ ,  $J_{2,3(\beta)} = J_{3,4(\beta)} = 9.6$ ), 3.62-3.38 (m, H-4  $\alpha$ , H-4  $\beta$ , H-2  $\alpha$ , H-5  $\beta$ ), 3.24 (t, 1 H, H-2  $\beta$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 172.9 (CO,  $\alpha$ ), 171.9 (CO,  $\beta$ ), 139.6, 139.5 (Cq, Ph,  $\alpha$ ,  $\beta$ ), 129.4, 128.5, 128.4, 128.2, 128.1 (CH, Ph,  $\alpha$ ,  $\beta$ ), 98.3 (C-1 $\beta$ ), 94.1 (C-1  $\alpha$ ), 77.5 (C-4  $\beta$ ), 76.1 (C-3  $\beta$ ), 75.6 (C-2  $\beta$ ), 74.4 (C-3  $\alpha$ ), 73.9, 73.5, 73.1 (C-2  $\alpha$ , C-4  $\alpha$ , C-5  $\beta$ ), 71.8 (C-5  $\alpha$ ), 43.7, 43.6 ( $\text{CH}_2\text{Ph}$ ,  $\alpha$ ,  $\beta$ ). HRMS: calcd for  $\text{C}_{13}\text{H}_{17}\text{NO}_6$  [ $M + \text{H}$ ] $^+$  284.1129, found 284.1134.

***N*-Benzyl 1-deoxy-1-(2-tosylhydrazin-1-yl)- $\beta$ -D-glucopyranuronamide (16):** To a solution of *N*-benzyl- $\alpha,\beta$ -D-glucopyranuronamide (**15**, 375 mg, 1.32 mmol) in *N,N*-dimethylformamide (DMF, 2 mL), *p*-toluenesulfonyl hydrazide (271 mg, 1.46 mmol, 1.1 equiv.) and glacial acetic acid (8  $\mu\text{L}$ , 0.14 mmol, 0.1 equiv.) was added. The reaction mixture was left at 40  $^\circ\text{C}$  without stirring for 48 h. The solvent was evaporated under vacuum. Diethyl ether (22 mL) was added to the residue and the mixture was vigorously stirred for 24 h. The mixture was filtered and the white solid was washed with diethyl ether, dichloromethane and cold methanol to yield pure title compound (276 mg, 46%) as a white solid. m.p.: 143.4-145.3  $^\circ\text{C}$ .  $[\alpha]_D^{20} = -4$  ( $c = 0.4$ , in MeOH).  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  7.77 (d, 2 H, H-a,  $J = 8.2$ , Ts), 7.41-7.19 (m, 7 H, H-b, Ts, Ph), 4.43 (s, 2 H,  $\text{CH}_2$ , Bn), 3.89 (d, 1 H, H-1,  $J_{1,2} = 7.9$ ), 3.70 (d, 1 H, H-5,  $J_{4,5} = 9.0$ ), 3.52-3.37 (m, 2 H, H-2, H-3, H-4), 2.42 (s, 3 H, Me, Ts).  $^{13}\text{C}$  NMR (100 MHz, MeOD)  $\delta$ : 172.3 (CO), 145.2 (Cq, Ts), 139.6 (Cq, Ts), 137.2 (Cq, Ph), 130.7, 129.6 (CH, Ts), 128.9, 128.6, 128.3 (CH, Ph), 91.8 (C-1), 77.7 (C-3), 76.9 (C-5), 73.6, 71.0 (C-2, C-4), 43.7 (C-7), 21.5 ( $\text{CH}_3$ , Ts). HRMS: calcd for  $\text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_7\text{S}$  [ $M + \text{H}$ ] $^+$  452.1486, found 452.1492.

***N*-(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl-1,2-*O*-isopropylidene- $\alpha$ -D-glucofuranuronamide (18):** To a solution of *N*-propargyl 1,2-*O*-isopropylidene- $\alpha$ -D-glucofuranuronamide (**17**, 100 mg, 0.37 mmol) in dichloromethane (4 mL), benzyl

azide (0.44 mmol, 55  $\mu$ L) and CuI/Amberlyste A21 (0.5 mmol/g, 37 mg) were added. The suspension was stirred overnight for 2 h. The catalyst was filtered off and the solvent was evaporated. The residue was subjected to column chromatography on silica gel (ethyl) to the title compound (130 mg, 87%) as a white solid. m.p.: 164.9-166.8  $^{\circ}$ C.

$[\alpha]_D^{25} = -10$  ( $c = 0.9$ , in MeOH).  $^1\text{H}$  NMR (300 MHz, MeOD)  $\delta$  7.85 (s, 1 H, H-9), 7.41-7.27 (m, 5 H, Ph), 5.84 (d, 1 H, H-1,  $J_{1,2} = 3.6$ ), 5.55 (br. s, 2 H,  $\text{CH}_2\text{Ph}$ ), 4.50 (br. d, 2 H,  $\text{CH}_2$ -7), 4.46 (d, 1 H, H-2,  $J_{1,2} = 3.6$ ), 4.35 (d, 1 H, H-3,  $J_{3,4} = 6.6$ ), 4.22 (dd, 1 H, H-4,  $J_{4,5} = 2.8$ ), 4.17 (d, 1 H, H-5), 1.41 (s, 3 H,  $\text{CH}_3$ , *i*-Pr.), 1.30 (s, 3 H,  $\text{CH}_3$ , *i*-Pr.).  $^{13}\text{C}$  NMR (100 MHz, MeOD)  $\delta$ : 175.0 (CO), 147.0 (C-8), 136.8 (Cq), 130.0, 129.6, 129.1 (CH, Ph), 124.2 (C-9), 112.9 (Cq, *i*-Pr.), 106.4 (C-1), 86.5 (C-2), 82.4 (C-4), 75.7 (C-5), 70.8 (C-3), 55.0 ( $\text{CH}_2\text{Ph}$ ), 35.6 (C-7), 27.1, 26.5 (2 x  $\text{CH}_3$ , *i*-Pr.). HRMS: calcd for  $\text{C}_{19}\text{H}_{24}\text{N}_4\text{O}_6$  [ $M + \text{H}$ ] $^+$  405.1769, found 405.1779.

***N*-(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl- $\alpha,\beta$ -D-glucopyranuronamide (19):** A solution of *N*-(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl-1,2-*O*-isopropylidene- $\alpha$ -D-glucofuranuronamide (**18**, 270 mg, 0.67 mmol) in aq. TFA (80%, 5 mL) was stirred at room temp. for one hour. After co-evaporation of the solvents with toluene, the crude product was purified by column chromatography (from EtOAc to EtOAc/MeOH, 4:1) to afford the title compound (240 mg, quantitative, anomeric mixture,  $\alpha/\beta$  ratio, 1:0.75) as a colorless oil.  $^1\text{H}$  NMR (300 MHz, MeOD)  $\delta$  7.86 (s, 0.75 H, H-9  $\beta$ ), 7.84 (s, 1 H, H-9  $\alpha$ ), 7.41-7.26 (m, 8.75 H, Ph,  $\alpha$ ,  $\beta$ ), 5.55 (br. s, 3.5 H,  $\text{CH}_2\text{Ph}$ ,  $\alpha$ ,  $\beta$ ), 5.16 (d, 1 H, H-1  $\alpha$ ,  $J_{1,2} = 3.6$ ), 4.52 (d, 0.75 H, H-1  $\beta$ ,  $J_{1,2(\beta)} = 7.8$ ), 4.50-4.44 (m, 3.5 H,  $\text{CH}_2$ -7,  $\alpha$ ,  $\beta$ ), 3.78-3.65 (m, 1.75 H, H-3  $\alpha$ , H-5  $\beta$ ), 3.55-3.33 (m, 3.5 H, H-2  $\alpha$ , H-3  $\beta$ , H-4  $\alpha$ , H-4  $\beta$ ), 3.18 (dd, 0.75 H, H-2  $\beta$ ,  $J_{2,3(\beta)} = 9.1$ ).  $^{13}\text{C}$  NMR (100 MHz, MeOD)  $\delta$ : 173.0 (CO,  $\alpha$ ), 171.9 (CO,  $\beta$ ), 136.7 (Cq), 130.0, 129.6, 129.1 (CH, Ph), 124.3 (C-9), 98.4 (C-1  $\beta$ ), 94.2 (C-1  $\alpha$ ), 77.4 (C-2  $\alpha$ ), 76.4 (C-5  $\beta$ ), 75.7 (C-2  $\beta$ ), 74.3 (C-3  $\alpha$ ), 73.9, 73.4, 73.2 (C-3  $\beta$ , C-4  $\alpha$ , C-4  $\beta$ ), 72.0 (C-5  $\alpha$ ), 54.9 ( $\text{CH}_2\text{Ph}$ ,  $\alpha$ ,  $\beta$ ), 35.3, 35.3 (C-7,  $\alpha$ ,  $\beta$ ).

***N*-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl 1-deoxy-1-(2-tosylhydrazin-1-yl)- $\beta$ -D-glucopyranuronamide (20):** included in the manuscript [32].



**Methyl 2,3,4-*O*-acetyl-6-*O*-tosyl- $\alpha$ -D-glucopyranoside (22):** To a solution of methyl 2,3,4-*O*-acetyl- $\alpha$ -D-glucopyranoside (**21**, 400 mg, 1.25 mol) in pyridine (5 mL), tosyl chloride (238 mg, 0.125 mol) was added. The mixture was stirred overnight at room temperature. Then water was added, and the mixture was extracted with dichloromethane. The combined organic layers were washed with water and dried with anhydrous MgSO<sub>4</sub>. After filtration and evaporation of the solvent, the residue was subjected to column chromatography (EtOAc/hexane, 1:2) to afford the title compound (537 mg, 91%) as colorless oil.  $[\alpha]_D^{20} = +135$  ( $c = 1.3$ , in CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  7.78 (d, 2 H, H-a,  $J = 8.2$ , Ts), 7.37 (d, 2 H, H-b, Ts), 5.42 (t, 1 H, H-3), 4.94 (t, 1 H, H-4,  $J_{3,4} = J_{4,5} = 9.8$ ), 4.86 (d, 1 H, H-1,  $J_{1,2} = 3.6$ ), 4.79 (dd, 1 H, H-2,  $J_{2,3} = 10.2$ ), 4.17-4.06 (m, 2 H, CH<sub>2</sub>-6,  $J_{5,6b} = 5.2$ ,  $J_{6a,6b} = 11.3$ ), 3.92 (ddd, 1 H, H-5), 3.35 (s, 3 H, OCH<sub>3</sub>), 2.46 (s, 3 H, Me, Ts), 2.05, 1.99, 1.97 (3  $\times$  s, 3  $\times$  3 H, 3  $\times$  CH<sub>3</sub>, Ac). <sup>13</sup>C NMR (100 MHz, MeOD)  $\delta$ : 169.9, 169.3 (CO), 145.0 (Cq, Ts), 132.4 (Cq, Ts), 129.8, 127.9 (CH, Ts), 96.4 (C-1), 70.4 (C-2), 69.8 (C-3), 68.4 (C-4), 67.6 (C-5), 66.8 (C-6), 55.4 (CH<sub>3</sub>, OMe), 21.5 (CH<sub>3</sub>, Ts), 20.5, 20.5, 20.4 (3  $\times$  CH<sub>3</sub>, Ac). HRMS: calcd for C<sub>20</sub>H<sub>26</sub>O<sub>11</sub>S [ $M + Na$ ]<sup>+</sup> 497.1088, found 497.1082.

**Methyl 6-azido-2,3,4-*O*-acetyl-6-deoxy- $\alpha$ -D-glucopyranoside (23):** To a solution of methyl 2,3,4-*O*-acetyl-6-*O*-tosyl- $\alpha$ -D-glucopyranoside (**22**, 520 mg, 1.1 mol) in *N,N*-dimethylformamide (DMF, 4.5 mL), sodium azide (243 mg, 3.74 mol) was added. The mixture was stirred overnight at 80 °C. Then water was added, and the mixture was extracted with dichloromethane. The combined organic layers were washed with water and dried with anhydrous MgSO<sub>4</sub>. After filtration and evaporation of the solvent, the residue was subjected to column chromatography (EtOAc/hexane, 1:4) to afford the title compound (314 mg, 83%) as a white solid. m.p.: 99.6-101.2 °C.  $[\alpha]_D^{20} = +49$  ( $c = 1.1$ , in CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, MeOD) 5.47 (t, 1 H, H-3), 5.03-4.94 (m, 2 H, H-1, H-4,  $J_{3,4} = J_{4,5} = 10.0$ ), 4.88 (dd, 1 H, H-2,  $J_{1,2} = 3.7$ ,  $J_{2,3} = 10.2$ ), 4.17-4.06 (m, 2 H, CH<sub>2</sub>-6,  $J_{5,6b} = 5.2$ ,  $J_{6a,6b} = 11.3$ ), 3.96 (ddd, 1 H, H-5), 3.45 (s, 3 H, OCH<sub>3</sub>), 3.35 (dd, 1 H, part A of ABX system, H-6a,  $J_{5,6a} = 6.3$ ,  $J_{6a,6b} = 13.2$ ), 3.29 (dd, 1 H, part B of ABX system, H-6b,  $J_{5,6b} = 2.4$ ), 2.08, 2.04, 2.01 (3  $\times$  s, 3  $\times$  3 H, 3  $\times$  CH<sub>3</sub>, Ac). <sup>13</sup>C NMR (100 MHz, MeOD)  $\delta$ : 170.2, 170.2, 169.8 (CO), 96.8 (C-1), 70.9 (C-2), 70.0 (C-3), 69.9 (C-4), 68.7 (C-5), 55.7 (CH<sub>3</sub>, OMe), 51.2 (C-6), 20.8, 20.8, 20.8 (3  $\times$  CH<sub>3</sub>, Ac). HRMS: calcd for C<sub>13</sub>H<sub>19</sub>N<sub>3</sub>O<sub>8</sub> [ $M + Na$ ]<sup>+</sup> 368.1064, found 368.1079.

***N*-[1-(methyl 2,3,4-*O*-acetyl-6-deoxy- $\alpha$ -D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl-1,2-*O*-isopropylidene- $\alpha$ -D-glucofuranuronamide (24):** included in the manuscript [34].

***N*-[1-(methyl 2,3,4-*O*-acetyl-6-deoxy- $\alpha$ -D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl-2,5-di-*O*-acetyl-1,2-*O*-isopropylidene- $\alpha$ -D-glucofuranuronamide (25):** A solution of *N*-[1-(methyl 2,3,4-*O*-acetyl-6-deoxy- $\alpha$ -D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl-1,2-*O*-isopropylidene- $\alpha$ -D-glucofuranuronamide (**24**, 120 mg, 0.195 mmol) in pyridine (3 mL) und acetic anhydride (2.5 mL) was stirred at room temp. for one hour. The solvents were co-evaporated with toluene and the residue was subjected to column chromatography (ethyl acetate/hexane, from 6:1 to 8:1) to give the title compound (135 mg, quantitative) as white crystals. m.p.: 110-111.9 °C.  $[\alpha]_D^{20} = +25$  ( $c = 0.4$ , in CH<sub>3</sub>OH). <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  7.89 (s, 1 H, H-9), 5.98 (d, 1 H, H-1,  $J_{1,2} = 2.7$ ), 5.40 (t, 1 H, H-3',  $J_{2',3'} = J_{3',4'} = 9.6$ ), 5.31 (br. s, 1 H, H-3), 5.03 (d, 1 H, H-5,  $J_{4,5} = 9.5$ ), 4.96-4.79 (m, 2 H, H-1', H-2', H-4',  $J_{1',2'} = 3.7$ ,  $J_{2',3'} = 9.7$ ), 4.68-4.42 (m, H-2, H-4, H-6a, H-6b, H-7a, H-7b), 4.19 (ddd, 1 H, H-5'), 3.16 (s, 3 H, Me), 2.07, 2.07, 2.06, 2.02, 1.98 (5  $\times$  s, 5  $\times$  3 H, 5  $\times$  CH<sub>3</sub>, Ac), 1.52 (s, 3 H, CH<sub>3</sub>, *i*-Pr.), 1.34 (s, 3 H, CH<sub>3</sub>, *i*-Pr.). <sup>13</sup>C NMR (100 MHz, MeOD)  $\delta$ : 171.7, 171.5, 171.3, 171.0, 170.9, 170.5 (6  $\times$  CO, Ac, C-6), 146.6 (C-8), 125.4 (C-9), 113.8 (Cq, *i*-Pr.), 106.7 (C-1), 97.9 (C-1'), 84.2 (C-2), 79.0 (C-4), 76.4 (C-3), 71.9 (C-4'), 71.4, 71.3 (C-2', C-3'), 71.1 (C-5), 68.9 (C-5'), 55.9 (CH<sub>3</sub>, OMe), 51.7 (C-6'), 35.9 (C-7), 27.0, 26.4 (2  $\times$  CH<sub>3</sub>, *i*-Pr.), 20.6, 20.6, 20.5, 20.4, 20.4 (5  $\times$  CH<sub>3</sub>, Ac). HRMS: calcd for C<sub>29</sub>H<sub>40</sub>N<sub>4</sub>O<sub>16</sub> [ $M + H$ ]<sup>+</sup> 701.2512, found 701.2509.

**4-aminomethyl-1-(Methyl 6-deoxy- $\alpha$ -D-glucopyranosid-6-yl)-1*H*-1,2,3-triazole (26):** A solution of *N*-[1-(methyl 2,3,4-*O*-acetyl-6-deoxy- $\alpha$ -D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl-1,2-*O*-isopropylidene- $\alpha$ -D-glucofuranuronamide (**24**, 130 mg, 0.21 mmol) in aq. TFA (80%, 2.5 mL) was stirred at 60 °C for 2 days. After co-evaporation of the solvents with toluene, the crude product was purified by column chromatography (from EtOAc to EtOAc/MeOH, 1:1) to afford the title compound (57 mg, quantitative) as a yellow solid. m.p.: 206.4-208.3 °C.  $[\alpha]_D^{20} = +18$  ( $c = 0.4$ , in CH<sub>3</sub>OH). <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  8.07 (s, 1 H, H-7), 4.82 (dd, 1 H, H-6a,  $J_{5,6a} = 2.0$ ,  $J_{6a,6b} = 14.3$ ), 4.65-

4.52 (m, 2 H, H-1, H-6b,  $J_{1,2} = 3.6$ ,  $J_{5,6b} = 7.8$ ), 4.23 (s, 2 H,  $\text{CH}_2$ -9), 3.87 (ddd, 1 H, H-5,  $J_{4,5} = 9.4$ ), 3.62 (t, 1 H, H-3,  $J_{2,3} = 9.2$ ), 3.33 (dd, 1 H, H-2), 3.17 (s, 3 H, Me), 3.10 (t, 1 H, H-4,  $J_{3,4} = J_{4,5} = 9.4$ ).  $^{13}\text{C}$  NMR (100 MHz, MeOD)  $\delta$ : 141.5 (C-8), 126.7 (C-7), 101.3 (C-1), 74.8 (C-3), 73.3 (C-2), 72.8 (C-4), 71.6 (C-5), 55.7 ( $\text{CH}_3$ ), 52.5 (C-6), 35.6 (C-9). HRMS: calcd for  $\text{C}_{10}\text{H}_{18}\text{N}_4\text{O}_5$  [ $M + \text{H}$ ] $^+$  275.1350, found 275.1351.

***N*-Propargyl 1,2,3,4-tetra-*O*-acetyl- $\alpha,\beta$ -D-glucopyranuronamide (27):** A solution of *N*-propargyl 1,2-*O*-isopropylidene- $\alpha$ -D-glucopyranuronamide (**17**, 355 mg, 1.31 mmol) in aq. TFA (70%, 2.5 mL) was stirred at 40 °C for 30 min. The solvents were co-evaporated with toluene. The residue was dried under vacuum and then it was dissolved in a mixture of pyridine (4 mL) and acetic anhydride (3 mL). The solution was stirred at room temp. for 30 min. The solvents were co-evaporated with toluene and the crude was subjected to column chromatography on silica-gel (AcOEt/hexane, 1:1) to yield the title compound (453 mg, 87%, anomeric mixture  $\alpha/\beta$  ratio, 1:0.5) as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.78 (t, 1 H, NH,  $J = 4.8$ ,  $\alpha$ ), 6.66 (t, 0.5 H, NH,  $J = 4.8$ ,  $\beta$ ), 6.39 (d, 1 H, H-1 $\alpha$ ,  $J_{1,2(\alpha)} = 3.6$ ), 5.79 (d, 0.5 H, H-1 $\beta$ ,  $J_{1,2(\beta)} = 8.0$ ), 5.54 (t, 1 H, H-3 $\alpha$ ,  $J_{2,3(\alpha)} = J_{3,4(\alpha)} = 9.8$ ), 5.32 (t, 0.5 H, H-3 $\beta$ ,  $J_{2,3(\beta)} = J_{3,4(\beta)} = 9.2$ ), 5.28-5.17 (m, 1.5 H, H-4  $\alpha$ , H-4  $\beta$ ), 5.16-5.04 (m, 1.5 H, H-2  $\alpha$ , H-2  $\beta$ ,  $J_{2,3(\alpha)} = 10.1$ ), 4.34 (d, 1 H, H-5 $\alpha$ ,  $J_{4,5(\alpha)} = 10.1$ ), 4.15 (d, 0.5 H, H-5 $\beta$ ,  $J_{4,5(\beta)} = 9.7$ ), 4.12-3.92 (m, 3 H,  $\text{CH}_2$ -7,  $\alpha$ ,  $\beta$ ,  $J_{a,b(\alpha)} = 17.6$ ,  $J_{a,b(\beta)} = 17.6$ ), 2.31-2.27 (m, 1.5 H, C(9)H,  $\alpha$ ,  $\beta$ ), 2.21 (s, 3 H,  $\text{CH}_3$ -Ac,  $\alpha$ ), 2.15 (s, 1.5 H,  $\text{CH}_3$ -Ac,  $\beta$ ), 2.09, 2.08, 2.05, 2.03 (4 s, 13.5 H,  $3 \times \text{CH}_3$ -Ac,  $\alpha$ ,  $3 \times \text{CH}_3$ -Ac,  $\beta$ ).  $^{13}\text{C}$  NMR (100 MHz, MeOD)  $\delta$ : 169.8, 169.8, 169.6, 169.6, 169.3, 168.8, 168.8 ( $4 \times \text{CO}$ , Ac,  $\alpha$ ,  $\beta$ ), 166.4 (CO, amide,  $\alpha$ ), 165.9 (CO, amide,  $\beta$ ), 91.2 (C-1  $\beta$ ), 88.2 (C-1  $\alpha$ ), 78.7 (C-8,  $\alpha$ ,  $\beta$ ), 72.7 (C-5  $\beta$ ), 72.0 (C-9  $\beta$ ), 71.9 (C-9  $\alpha$ ), 71.8 (C-3  $\beta$ ), 70.2 (C-5  $\alpha$ ), 70.0 (C-2  $\beta$ ), 68.9, 68.8, 68.8, 68.7 (C-2  $\alpha$ , C-3  $\alpha$ , C-4  $\alpha$ , C-4  $\beta$ ), 28.8, 28.7 (C-9,  $\alpha$ ,  $\beta$ ), 20.7, 20.6, 20.6, 20.5, 20.5, 20.5, 20.4, 20.3 ( $4 \times \text{CH}_3$ -Ac,  $\alpha$ ,  $\beta$ ). HRMS: calcd for  $\text{C}_{17}\text{H}_{21}\text{NO}_{10}$  [ $M + \text{Na}$ ] $^+$  422.1058, found 422.1063.

***N*-Propargyl-1-(2-acetamido-6-chloro-purin-9-yl)-2,3,4-tri-*O*-acetyl- $\beta$ -D-glucopyranuronamide (28) and *N*-Propargyl-1-(2-acetamido-6-chloro-purin-7-yl)-2,3,4-tri-*O*-acetyl- $\beta$ -D-glucopyranuronamide (29):** To a suspension of 2-acetamido-6-chloropurine (**27**, 40 mg, 0.19 mmol) in anhydrous acetonitrile (1 mL), under  $\text{N}_2$ , was added *N,O*-bis(trimethylsilyl)acetamide (0.1 mL, 0.41 mmol) and the mixture was stirred at room temp. for 20 min. A solution of *N*-propargyl 1,2,3,4-tetra-*O*-acetyl- $\alpha,\beta$ -

D-glucopyranuronamide (55 mg, 0.14 mmol) in anhydrous acetonitrile (1 mL) was added to the previous solution, which was followed by slow addition of trimethylsilyl triflate (0.5 mL, 2.04 mmol). The reaction mixture was stirred under microwave irradiation (150 W, P max = 250 Psi) at 65 °C for 1.5 h. The mixture was then diluted with dichloromethane and neutralized with sat. aq. NaHCO<sub>3</sub> soln. The aq. phase was extracted with dichloromethane (3×), the combined org. phases were dried with MgSO<sub>4</sub>. After filtration and evaporation of the solvent, the residue was subjected to column chromatography on silica-gel (EA/hexane, 5:3, then 4:1, then 9:1) to afford the N<sup>9</sup> nucleoside (**28**, 23 mg, 30%) and its N<sup>7</sup> regioisomer (**29**, 22 mg, 29%) as white crystals.

Data for **28**: m.p.: 122.6-124.2°C.  $[\alpha]_D^{20} = -9$  (c = 0.8, in CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 8.65 (br. s, 1 H, NH), 8.15 (s, 1 H, H-8), 7.07 (t, 1H, NH), 5.85 (d, 1 H, H-1',  $J_{1',2'} = 9.4$ ), 5.75 (t, 1 H, H-2',  $J_{2',3'} = J_{1',2'} = 9.2$ ), 5.50 (t, 1 H, H-3',  $J_{2',3'} = J_{3',4'} = 9.2$ ), 5.42 (t, 1 H, H-4',  $J_{3',4'} \sim J_{4',5'}$ ), 4.34 (d, 1 H, H-5',  $J_{4',5'} = 9.4$ ), 4.09 (ddd, 1 H, H-7'a,  $J_{7'a,7'b} = 17.5$ ,  $J_{7'a,NH} = 5.3$ ,  $J_{7'a,9'} = 2.1$ ), 3.98 (ddd, 1 H, H-7'b,  $J_{7'a,NH} = 5.0$ ,  $J_{7'a,9'} = 2.5$ ), 2.49 (s, 3 H, CH<sub>3</sub>, NHAc), 2.20 (t, 1 H, H-9'), 2.12, 2.05, 1.87 (3 × s, 9 H, 3 × CH<sub>3</sub>, OAc). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100MHz): δ 169.9, 169.6, 169.1 (4 × CO, Ac), 165.5 (CO, amide), 152.6 (C-2 or C-6), 152.5 (C-4), 152.1 (C-2 or C-6), 142.4 (C-8), 128.4 (C-5), 81.3 (C-1'), 79.0 (C-8'), 75.3 (C-5'), 72.1 (C-3'), 71.9 (C-9'), 69.4 (C-2'), 69.0 (C-4'), 29.1 (C-7'), 25.4 (CH<sub>3</sub>, NHAc), 20.8, 20.7, 20.4 (3 × CH<sub>3</sub>, OAc). HRMS: calcd for C<sub>22</sub>H<sub>23</sub>ClN<sub>6</sub>O<sub>9</sub> [*M* + H]<sup>+</sup> 551.1288, found 551.1296.

Data for **29**: m.p.: 110.8 - 112.2 °C.  $[\alpha]_D^{20} = +7$  (c = 1.1, in CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 8.79 (br. s, 1 H, NH), 8.55 (s, 1 H, H-8), 7.38 (t, 1H, NH), 6.23 (br. d, 1 H, H-1'), 5.72 (br. t, 1 H, H-2'), 5.58 (t, 1 H, H-3',  $J_{2',3'} = J_{3',4'} = 9.3$ ), 5.45 (t, 1 H, H-4',  $J_{3',4'} \sim J_{4',5'}$ ), 4.46 (d, 1 H, H-5',  $J_{4',5'} = 9.8$ ), 4.11-3.93 (m, 2 H, CH<sub>2</sub>-7'), 2.52 (s, 3 H, CH<sub>3</sub>, NHAc), 2.20 (br. t, 1 H, H-9'), 2.12, 2.07, 1.89 (3 × s, 9 H, 3 × CH<sub>3</sub>, OAc). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100MHz): δ 170.0, 169.7, 169.3 (4 × CO, Ac), 165.4 (CO, amide), 153.0, 78.9 (C-8'), 74.9 (C-5'), 72.4 (C-3'), 72.1 (C-9'), 70.0 (C-2'), 68.8 (C-4'), 29.1 (C-7'), 25.3 (CH<sub>3</sub>, NHAc), 20.8, 20.7, 20.4 (3 × CH<sub>3</sub>, OAc). HRMS: calcd for C<sub>22</sub>H<sub>23</sub>ClN<sub>6</sub>O<sub>9</sub> [*M* + H]<sup>+</sup> 551.1288, found 551.1289.

***N*-[1-(methyl 2,3,4-*O*-acetyl-6-deoxy- $\alpha$ -D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl-1-(2-acetamido-6-chloro-purin-7-yl)-2,3,4-tri-*O*-acetyl- $\beta$ -D-**

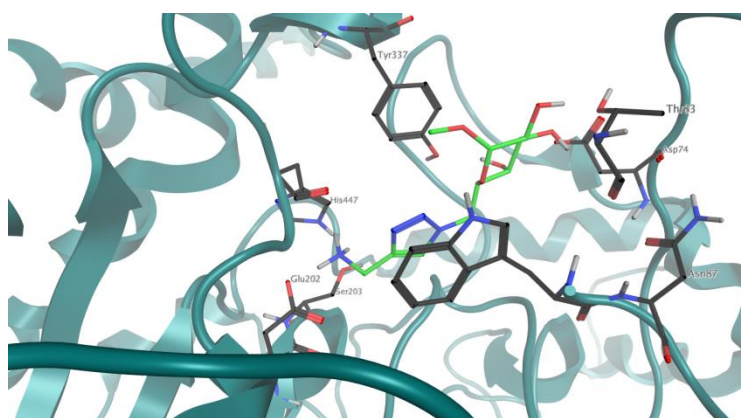
**glucopyranuronamide (30):** To a solution of *N*-propargyl-1-(2-acetamido-6-chloro-purin-7-yl)-2,3,4-tri-*O*-acetyl- $\beta$ -D-glucopyranuronamide (**29**, 20 mg, 36.3  $\mu$ mol) in dichloromethane (3 mL), methyl 2,3,4-*O*-acetyl-6-azido-6-deoxy- $\alpha$ -D-glucopyranoside (**23**, 38  $\mu$ mol, 13 mg) and CuI/Amberlyste A21 (10 mg) were added. The suspension was stirred overnight at room temp. The catalyst was filtered off and the solvent was evaporated. The residue was subjected to column chromatography on silica gel to give triazole-linked disaccharide nucleoside (14 mg, 43%) as yellow crystals. m.p.: 161.3-163.1 °C.  $[\alpha]_D^{20} = +8$  (c = 0.4, in CH<sub>3</sub>OH). <sup>1</sup>H NMR (MeOD, 400 MHz): 8.97 (s, 1 H, H-8), 7.86 (s, 1 H, H-9'), 6.46 (br. d, 1 H, H-1'), 5.81 (t, 1 H, H-2',  $J_{1',2'} \sim J_{2',3'}$ ), 5.69 (t, 1 H, H-3',  $J_{2',3'} = J_{3',4'} = 9.4$ ), 5.46-5.32 (m, 2 H, H-4', H-3''), 4.89 (d, 1 H, H-1''), 4.83 (dd, 1 H, H-2'',  $J_{1'',2''} = 3.6$ ,  $J_{2'',3''} = 10.4$ ), 4.77 (t, 1 H, H-4'',  $J_{3'',4''} = 3.6$ ,  $J_{4'',5''} = 9.8$ ), 4.66-4.44 (m, 4 H, H-5', H-7'a, H-6''a, H-6'b), 4.37 (d, part B of AB system, 1 H, H-7'b,  $J_{7'a,7'b} = 15.2$ ), 4.20 (ddd, 1 H, H-5''), 3.19 (s, 3 H, OCH<sub>3</sub>), 2.31 (s, 3 H, CH<sub>3</sub>, NHAc), 2.06, 2.04, 2.02, 1.97, 1.83 (6  $\times$  s, 18 H, 6  $\times$  CH<sub>3</sub>, OAc). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100MHz):  $\delta$  171.7, 171.6, 171.3, 171.3, 171.1, 170.7 (6  $\times$  CO, Ac), 168.3 (CO, amide), 146.2 (C-8), 125.8 (C-9'), 98.1 (C-1'), 76.1 (C-5'), 73.4 (C-3'), 72.1 (C-4'), 71.7 (C-2'), 71.4 (C-2''), 71.0 (C-3''), 70.4 (C-4''), 68.8 (C-5''), 56.0 (OCH<sub>3</sub>), 51.5 (C-6''), 35.4 (C-7'), 24.6 (CH<sub>3</sub>, NHAc), 20.7, 20.7, 20.6, 20.5, 20.5, 20.1 (6  $\times$  CH<sub>3</sub>, OAc). HRMS: calcd for C<sub>35</sub>H<sub>42</sub>ClN<sub>9</sub>O<sub>17</sub> [*M* + H]<sup>+</sup> 896.2460, found 896.2453.

## Molecular Docking

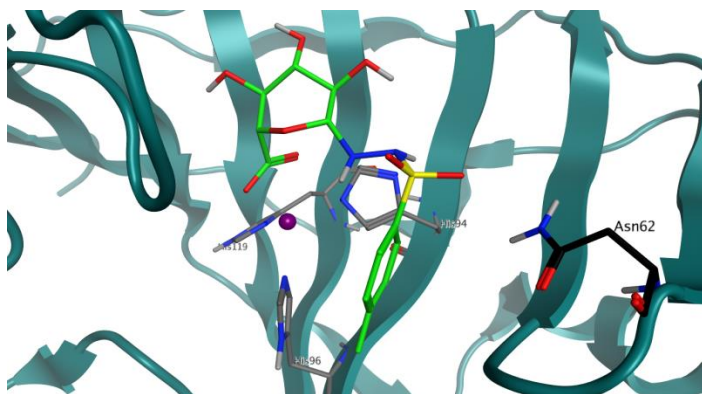
To prepare the enzyme for the docking studies, the co-crystallized inhibitor as well as crystallographic water molecules included in the PDB files were removed. Hydrogen atoms were added and the protonation states were correctly assigned using the Protonate-3D tool within the Molecular Operating Environment (MOE) 2011.13 software package [40] and energy was minimized using MMFF94x force field. Molecular docking studies were then performed using the GoldScore scoring function from GOLD 5.2 software package [38] and each ligand was subjected to 500 docking runs. The 3D structure coordinates of the enzymes were obtained from the Protein Data Bank.

For AChE, PDB code 4BDT with 3.10 Å resolution was used and Trp86 N atom was defined as active site center coordinate. Docking radius was considered 15 Å from the active site center. Docking protocol was validated by the docking of the co-crystallized inhibitor in 4BDT and RMSD value between docked and crystallographic poses was 1.27 Å.

For CA-II, PDB code 2X7T with 1.89 Å resolution was used and  $\text{Zn}^{2+}$  coordinates defined as active-site center. Docking radius was considered 15 Å from the active site center. Docking protocol was validated by the docking of the co-crystallized inhibitor, 2-EtE2bisMATE, and RMSD values between docked and crystallographic pose was 0.8 Å.



**SI Figure 1.** Compound **26** modelled into AChE.



**SI Figure 2.** Compound **11** modelled into CA-II.

## ***Biological Assays***

### *Cholinesterases and Carbonic Anhydrase II Assays*

#### *Spectrophotometer and Chemicals*

A TECAN SpectraFluorPlus working on the kinetic mode and measuring the absorbance at 415 nm was used for the enzymatic studies. Acetylcholinesterase (from *electrophorus electricus*), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide were purchased from Fluka. Butyrylcholinesterase (from equine serum), carbonic anhydrase II (from bovine erythrocytes) as well as 4-nitrophenyl acetate (4-NA) were purchased from Sigma.

#### *Solutions Preparation*

Preparation of 50 mM Tris-HCl buffer solutions: Tris(hydroxymethyl)-aminomethan (606 mg) was dissolved in bidistilled water (100 ml) and adjusted with HCl to a pH of  $8.0 \pm 0.1$  (for AChE, BChE, bCA II). Buffer was freshly prepared and stored in the refrigerator. AChE solution 2.005 U/ml: the enzyme (271 U/mg, 0.037 mg) was dissolved in freshly prepared buffer pH 8.0 (5 ml) containing  $\text{NaN}_3$  (0.98 mg). BChE solution 2.040 U/ml: the enzyme (7.54 U/mg, 1.353 mg) was dissolved in freshly prepared buffer pH 8.0 (5 ml) containing  $\text{NaN}_3$  (0.98 mg). bCA II solution 798 W/A-units/ml: the enzyme ( $\geq 3,000$  W-A units/mg, 0.87 mg) was dissolved in freshly prepared buffer pH 8.0 (5 ml). DTNB solution 3 mM: DTNB (23.8 mg) was dissolved in freshly prepared buffer pH 8.0 (20 ml) containing NaCl (116.8 mg) and  $\text{MgCl}_2$

(38.0 mg). ATChI solution 15 mM: ATChI (43.4 mg) was dissolved in bidistilled water (10 ml). 4-NA solution 6 mM: 4-NA (21.6 mg) was dissolved in methanol (2.2 mg) and bidistilled water (17.8 ml). All solution were stored in eppendorf caps in the refrigerator or freezer, if necessary. The pure compounds were initially dissolved in DMSO, galantamine hydrobromide as standard for AChE and BChE was dissolved in bidistilled water and acetazolamide as standard for bCA II was dissolved in DMSO. The final concentrations for the enzymatic assay were yielded by diluting the stock solution with bidistilled water. No inhibition was detected by residual DMSO (<0.5%).

### *Cholinesterase Assays*

A mixture of the DTNB solution (125  $\mu$ L), enzyme (25  $\mu$ L) and compounds solutions (25  $\mu$ L, 3 different concentrations and once blank water) was prepared and incubated at 30 °C for 20 min. The substrate (25  $\mu$ L, 4 different concentrations) was added to start the enzymatic reaction. The absorbance data (415 nm) was recorded under a controlled temperature of 30 °C for 30 min at 1 min intervals. All measurements were performed as triplicates. The used substrate concentrations in the test were as follows: [ATChI] = 0.9375 mM, 0.625 mM, 0.325 mM, 0.1875 mM. The mode of inhibition as well as  $K_i$  and  $K_i'$  were determined using Lineweaver-Burk plot [44], Dixon plot [45] and Cornish-Bowden plot [46].

### *bCA II Assays*

A mixture of buffer solution pH 8.0 (125  $\mu$ L), enzyme (25  $\mu$ L) and compounds solutions (25  $\mu$ L, 50  $\mu$ M) was prepared and incubated at 37 °C for 20 min. 4-NA (25  $\mu$ L, 0.75  $\mu$ M) was added to start the enzymatic reaction. The absorbance data (415 nm) was recorded under a controlled temperature of 37 °C for 10 min at 1 min intervals. All measurements were performed in duplicate. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges.



### CDK-2/Cyclin E Assays

CDK-2/Cyclin E kinase was produced in Sf9 insect cells via baculoviral infection and purified on a NiNTA column (Qiagen). The kinase reaction was assayed with 1 mg/mL histone H1 in the presence of 15  $\mu$ M ATP for CDK2, 0.05  $\mu$ Ci [ $\gamma$ - $^{33}$ P]ATP and of the test compound in a final volume of 10  $\mu$ L, all in a reaction buffer (60 mM HEPES-NaOH, pH 7.5, 3 mM  $\text{MgCl}_2$ , 3 mM  $\text{MnCl}_2$ , 3  $\mu$ M Na-orthovanadate, 1.2 mM DTT, 2.5  $\mu$ g / 50  $\mu$ l PEG<sub>20,000</sub>). The reaction was stopped by adding 5  $\mu$ L of 3 % aq.  $\text{H}_3\text{PO}_4$ . Aliquots were spotted onto P-81 phosphocellulose (Whatman), washed 3 $\times$  with 0.5 % aq.  $\text{H}_3\text{PO}_4$  and finally air-dried. Kinase inhibition was quantified using a FLA-7000 digital image analyzer (Fujifilm). The concentration of the test compounds required to decrease the CDK activity by 50 % was determined from dose-response curves and designated as  $\text{IC}_{50}$ .

### Cytotoxicity assays

The cytotoxicity of the studied compounds was determined using cell lines of different histological origin. Briefly, cells were assayed with compounds using three-fold dilutions in triplicate. Treatment lasted for 72 h, followed by addition of Calcein AM solution and measurement of the fluorescence of live cells at 485 nm/538 nm (ex/em) with a Fluoroskan Ascent microplate reader (Labsystems). The  $\text{IC}_{50}$  value, that is, drug concentration lethal to 50% of the cells, was calculated from the obtained dose response curves.

All cell lines were maintained in appropriate cultivation medium supplemented with fetal bovine serum, penicillin (100 U/ml) and streptomycin (100  $\mu$ g/ml) and were cultivated at 37  $^{\circ}\text{C}$  in 5%  $\text{CO}_2$ .

## Additional References

[44] Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, 56, 658.

[45] Dixon, M. *Biochem. J.* **1953**, 55, 170.

[46] Cornish-Bowden, A. *Biochem. J.* **1974**, 137, 143.