Supporting Information:

Structural Determinants of A_3 Adenosine Receptor Activation:

Nucleoside Ligands at the Agonist/Antagonist Boundary.

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Preparation of compounds 25 – 35 and 14

(3a*S*,6a*S*,4*R*)-4-Allyl-5-bromo-2,2-dimethyl-3aβ,6aβ-dihydrocyclopenta-l,3-dioxol-4-ol (25). The starting α-bromoenone **24** (2.0 g, 8.6 mmol) was dissolved in 20 mL of dry THF and cooled to -78 °C. Allylmagnesium chloride was added to 100 mL of dry THF and also cooled to -78 °C. The solution containing the starting material was then added to the Grigard solution slowly *via* a cannula. After the addition was completed and additional stirring for 30 min., saturated aqueousNH_4Cl was added while the reaction misture was still at low temperature. Et_2O (200 mL) was then added to the resulting mixture and the phases were separated. The aqueous phase was extracted twice with Et_2O and the combined organics were dried and concentrated. Column chromatography needed to be rapid in order to avoid decomposition of the labile product **25**, and afforded the desired compound as a clear oil in 78% yield. ^{1}H NMR δ 1.38 (s, 3H), 1.44 (s, 3H). 2.40 (ddt, 1H, J = 13.9 Hz. 7.1 Hz, 1.2 Hz), 2.51 (ddt, 1H, J = 13.4 Hz, 7.2 Hz, 1.2 Hz), 3.24 (bs, 1H), 4.45 (d, 1H, J = 5.8 Hz). 4.83 (dd, 1H, J = 5.6 Hz, 1.9 Hz), 5.11-5.20 (m, 2H), 5.51-5.65 (m, 1H), 6.01 (d, 1H, J= 1.8 Hz). ^{13}C NMR δ 26.6 (CH_3), 27.6 (CH_3), 40.9 (CH_2), 79.9 (CH), 81.4 (CH), 81.5 (C), 1l2.5 (C), 119.6 (CH_2), 131.1(CH), 13l.3 (CH), 133.9 (C). HRMS M-CH_3 (obs.) 258.9985, (exp.) 258.9970. MS: m/z (relative) 261 (6),

259 (6), 177 (99), 175 (100), 95 (14), 69 (16), 59 (15). CI ionization: $(M+H)^+$ observed (275 & 277).

Methyl 2,3-*O*-Isopropylidene-4-*C*-(2-oxoethyl)-D-ribofuranosiduronate (27). The vinyl bromide **25** (150 mg, 0.54 mmol) was dissolved in 15 mL of a 20:1 MeOH/pyridine mixture and cooled to -78 °C. Ozone was bubbled until TLC analysis indicated completion (45 min). (The blue coloration indicating saturation of the solution in ozone was not observed, since ozone first converts pyridine into the corresponding yellow colored *N*-oxide: one had to rely on TLC analysis (dipping the TLC plates In dimethyl sulfide) to observe the completion of ozonolysis.) to warm to room temperature before being concentrated. Column chromatography afforded the riboside **27** as an oil, a mixture of anomers (1.8:1); the β anomer is presumably the major component. ^{1}H NMR δ 1.33 (s, 3H, β anomer), 1.38 (s, 3H, α anomer), 1.48 (s, 3H, β anomer), 1,53 (s, 3H, α anomer), 2.98 (ABq, d, 2H, J = 1.8 Hz, β anomer), 3.12 (ABq, 2H, α anomer), 3.75 (s, 3H, α anomer), 3.78 (s, 3H, β anomer), OH (β anomer) buried under signal at 3.77, 3.99 (d, 1H, anomer. J = 12.2 Hz), 4.70-4.73 (m, 1H, α anomer + 1H β anomer), 4.99 (d, 1H, α anomer, J = 5.9 Hz), 5.17 (d, 1H, β anomer, J= 5.8 Hz), 5.38 (dd, 1H, α anomer, J= 12.1 Hz, 3.9 Hz), 4.46 (d, 1H, β anomer, J = 3.2 Hz), 9.73 (d, 1H, α &β anomer, J = 1.8 Hz). ^{13}C NMR δ 24.4 (CH_3), 24.5 (CH_3), 25.7 (CH_3), 45.7 (CH_2), 46.4 (CH_2), 78.6 (CH), 81.5 (CH), 82.3 (CH), 86.3 (CH), 97.3 (CH), 103.2 (CH), quat. C not observed, aldehyde C not observed.

(3a*S*,6a*S*,4*R*)-5-Bromo-4-(2,3-dibromopropyl)-2,2-dimethyl-3β,6aβ-dihudro-cyclopenta-1,3-dioxol-4-ol (26). The vinyl bromide **25** (1.55 g, 5.6 mmol) was dissolved in 50 mL of methylene chloride and cooled to 0 °C. Et_3N (1.57 mL, 11.3 mmol) was added and the mixture was stirred for 20 min. Then bromine (577 mL, 11.3 mmol) was dissolved in 10 mL of methylene chloride and added dropwise to the solution containing the starting material. After the addition was completed and 30 min additional stirring was continued, another solution of bromine (430 mL, 8.4 mmol) in 10 mL of methylene chloride was added dropwise and the resulting mixture was stirred at 0 °C for 30 min. The reaction mixture was quenched by addition of saturated aqueous sodium thiosufate (20 mL). The aqueous phase was reextracted with methylene chloride (100 mL) and the combined organic phases were dried and concentrated. Column chromatography afforded the desired tribromide **26** as an oil in 96% yield as a mixture of two diastereomers (1:1). ^{1}H NMR δ 1.39 (s, 3H), 1.40 (s. 3H), 1.43 (s, 3H), 1.44 (s, 3H), 1.81 (dd, 1H, J = 8.1 Hz, 15,6 Hz), 2.35 (dd, 1H, J = 9.3 Hz, 15.6 Hz), 2.67 (dd, 1H, J = 15.6 Hz, 3.6 Hz). 2.84 (dd, 1H, J = 15.6, 3.6 Hz), 3.34 (bs, 1H), 3.47 (bs, 1H), 3.65-3.89 (m, 4H), 4.03-4.10 (m, 1H), 4.46-4.52 (m, 1H), 4.84 (d, 1H, J = 5.7 Hz), 4.96-5.01 (m, 2H), 5.13 (dd, 1H, J = 5.4 Hz, 2.1 Hz), 6.03 (d, 1H, J = 1.7 Hz), 6.12 (d, 1H, J =1.8 Hz). ^{13}C NMR δ 26.6, 26.7, 27.58, 27.62, 37.5, 38.8, 42.6, 43.0, 46.4, 46.5, 78.3, 80.4, 80.8, 81.3, 81.6, 81.9, 112.9, 113.0, 131.3, 132.5, 132.9, 134.6. HRMS M-CH_3 (obs.) 416.8339, (exp.) 416.8337. MS: m/z (relative) 420 (23), 418 (24), 416 (7), 377 (7), 299 (41), 297(85), 295 (42), 229 (18), 177 (39), 175 (43), 119 (21), 95 (100), 59 (33).

Methyl 4-*C*-(2,3-Dibromopropyl)-2,3-*O*-isopropylidene-D-ribofuranosid-uronate (28). The tribromide **26** (1.60 g, 3.8 mmol) was dissolved in 40 mL of a solution of 5% (vol.) pyridine in methanol and cooled to -78 °C. Ozone was bubbled through the solution. The completion of reaction was monitored by TLC. Then dimethyl sulfide was added; thereby the ozonide was quenched and the yellow pyridine-N-oxide was reduced back to pyridine. The reaction mixture was concentrated and dissolved in 50 mL of methylene chloride and washed with 20 mL of NH_4C1 (sat.) and 20 mL of $CuSO_4$ (sat.), The solvent was removed and residue was purified with chromatography to afford the desired dibromo riboside **28** as an oil in 82% yield, as a mixture of 4 diastereomers. ^1H NMR δ 1.32-1.33 (2 s, 6H), 1,49-1.51 (2s, 6H), 2.39 (dd, 1H, *J*= 15.9 Hz, 9 Hz), 2.54 (dd, 1H, *J*= 8.7 Hz, 15.3 Hz), 2.78 (dd, 1H, *J* = 15.3 Hz, 3.3 Hz), 2.91 (dd, 1H, *J* = 15.6 Hz, 3.6 Hz), 3.77 (s, 3H), 3.79 (s, 3H), 3.62-3.72 (m, 2H), 3.75-3.90 (m, 4H), 3,30-4.37 (m, 2H), 4.63 (dd, 2H, *J* = 12 Hz, 5.7 Hz), 5.02 (d, 1H, *J* = 5,7 Hz), 5.30 (d, 1H, *J*=6 Hz), 5.47-5.50 (m, 2H).

Methyl [Methyl 4-*C*-[2,3-Dibromopropyl)-2,3-*O*-isopropylidene-β-methyl-ribofuranosid)uronate (29). The riboside **28** (3 g, 7.1 mmol) was dissolved in 100 mL of dry methanol and 15 mL of dry acetone and 5 mL of 2,2-dimethoxypropane (DMP) were added. After addition of TsOH (200 mg) the reaction mixture was refluxed for 2 days. After cooling and concentration the crude reaction mixture was dissolved in acetone (25 mL) and DMP (10 mL) and stirred for 12 h at room temperature. Then sodium bicarbonate (powder, 200 mg) was added and the reaction mixture was partitioned between 100 mL of EtOAc and 20 mL of H_2O. The aqueous phase was reextracted twice

with EtOAc (25 mL) and the combined organics were dried and concentrated. Column chromatography afforded the desired β-methyl glycoside **29** as a clear oil in 58% yield and a mixture containing presumably the α anomer, some unprotected starting ribose and materials containing a deprotected acetonide. This complex mixture, upon treatment under the same conditions as above, led to 17% of the desired β-methyl glycoside, thus raising the yield to 75%. ^{1}H NMR δ 1.30 (s, 3H), 1.31 (s, 3H), 1.46 (s, 3H), 1.48 (s, 3H), 2,38 (dd, 1H, J = 15.6 Hz, 7.2 Hz), 2.54 (dd, 1H, J = 15 Hz, 9 Hz), 2.77 (dd, 1H, J = 14.7 Hz, 3 Hz), 2.85 (dd, IH, 15.6 Hz, 5.1 Hz), 3.29 (s, 3H), 3.34 (s, 3H), 3.63 (dd, 1H, J = 10.2 Hz, 7.8 Hz), 3.76-3.87 (m, 3H), 4.27-4.36 (m, 2H), 4.52 (d, 1H, J = 6 3Hz), 4.60 (d, 1H, J = 5.7 Hz), 4.88 (s, 1H), 4.91 (s, 1H), 5.19 (d, 1H, J= 5.7 Hz), 5.34 (d, 1H, J = 6 Hz). ^{13}C NMR δ 24.8, 24.9, 26.1, 37.8, 38.2, 40.8, 45.8, 46.8, 52.37, 52.43, 55.37, 55.43, 80.9, 82.9, 84.7, 85.3, 87.2, 87.3, 108.4, 108.5, 112.8, 172.1, 172.2. HRMS M-CH_3 (obs.) 414.9385, (exp) 414.9392. MS: m/z (relative) 419 (17), 417 (36), 415 (17), 375 (33), 373 (67), 371 (34), 316 (18), 314 136), 127 (64), 115 (19). 113 (100), 87 (18), 85 (69), 75 (18), 73 (21), 69 (19), 59 (87). 57 (20), 55 (23).

Methyl (Methyl 4-*C*-Allyl-2,3-*O*-isopropylidene-β-D-ribofuranosid)uronate (30). The dibromide **29** (1.5 g, 3.47 mmol) was dissolved in dry methanol (50 mL) and activated zinc dust (1 g) was added. The reaction mixture was heated to reflux for 24 h. After cooling to room temperature the mixture was filtered through a pad of Celite and the filtrate was wshed with methanol (2 × 25 mL). The filtrate was concentrated, dissolved in Et_2O and filtered through Celite to remove zinc bromide. Column

chromatography afforded the desired olefin **30** as a clear oil in quantitative yield.. ^{1}H NMR δ 1.32 (s, 3H), 1.46 (s, 3H), 2.58 (dd, 1H, *J* = 13,5 Hz, 8.7 Hz), 2.72 (ddt, 1H, *J*= 13,4 Hz, 6.2 Hz, 1.5 Hz), 3.31 (s, 3H), 3.68 (s, 3H), 4.55 (d, 1H, *J*= 5.7 Hz), 4.87 (s, 1H), 5.04-5.12 (m, 2H), 5.24 (d, 1H, 6 Hz), 5.66-5,80 (m, 1H). ^{13}C NMR δ 24.9, 26.1, 37.8, 51.9, 55.3, 81.0, 84.9, 88.7, 108.4, 112.4, 118,8, 131.5, 172.5. HRMS M-CH_3 (obs.) 257.1028, (exp.) 257.1025.

(2*R*,3*R*,4*S*,5*S*)-3,4-*O*-Isopropylidenedioxy-2-methoxy-7-*N*-(4-methoxybenzyl) -7-aza- l-oxa-6-oxospiro[4.4]nonane (31). The olefin **30** (200 mg, 0.74 mmol) was dissolved in dry methylene chloride (20 mL) and MeOH (1.0 mL) and cooled to -78 °C. Ozone was then bubble*d* to completion and the reaction was quenched with dimethyl sulfide and concentrated. The resulting crude mixture was dissolved in MeOH (20 mL) and cooled to 0 °C. 4-Methoxybenzylamine (100 µl, 0.75 mmol) was added, followed by sodium cyanoborohydride (92 mg, 1.47 mmol). The reaction mixture was then allowed to warm to room temperature and stirred for 12 h before being quenched with saturated NH_4Cl. Extractions with EtOAc followed by drying and concentration of the combined organic phases led to the desired amide **31** as a clear oil in 60% overall yield from **30**. ^{1}H NMR δ 1.31 (s, 3H), 1.44 (s, 3H), 2.00 (ddd, 1H, *J* = 13.5 Hz, 9 Hz, 2.4 Hz), 2.35 (dt, 1H, *J*= 14.1 Hz, 7.8 He), 3.09(td, 1H, *J*= 8.1 Hz, 2.7 Hz), 3.27-3.36 (m, 1H), 3.30 (s, 3H), 3.76 (s, 3H), 4.26 (d, 1H, 14.7 Hz), 4.49 (d, 14.7 Hz), 4.81 (ABq, 2H), 4.97 (s, 1H), 6.81 (d, 2H, *J* = 9.3 Hz), 7.14 (d, *J* = 8.4 Hz). ^{13}C NMR δ 24.9, 26.3, 28.5, 43.5, 46.3, 54.9, 55.2, 81.5, 86.5, 89.7, 108.6, 112.5, 114.0, 128.2, 129.4, 159.1, 172.0.

(2*R*,3*R*,4*S*,5*S*)-3,4-*O*-Isopropylidenedioxy-2-methoxy-7-*N*-(4-methoxy-benzoyl)-7-aza- l-oxa-6-oxospiro[4.4]nonane (33). This compound was obtained as a by-product of the reaction described below, and arises from overoxidation of the benzylic position. ^{1}H NMR δ 1.33 (S, 3H), 1.49 (S, 3H), 2.19 (ddd, 1H, *J* = 13.8 Hz, 6.6 Hz, 3 Hz), 2.43 (dt, 1H, *J*= 14.1 Hz, 8.1 Hz), 3.34 (s, 3H), 3.82 (s, 3H), 3.91-4.02 (m, 2H), 4.75 (d, 1H, *J* = 6 Hz), 4.84 (d, 1H, *J* = 6.3 Hz), 5.02 (s, 1H), 6.86 (d, 2H, *J* = 8.7 Hz), 7.60 (d, 2H, *J* = 9 Hz). ^{13}C NMR δ 24.8 (CH_3), 26.2 (CH_3), 27.6 (CH_2), 43.0 (CH_2), 55.3 (CH_3), 81.0 (CH), 86.3 (CH), 90.7 (C), 109.2 (CH), 112.9 (C), 113.1(CH).

(2*R*,3*R*,4*S*,5*S*)-3,4-*O*-Isopropylidenedioxy-2-methoxy-7-axa-1-oxa-6-oxospiro[4.4] nonane (32). (a) From deprotection of **31**: The deprotection reactions were run following this typical procedure: the starting amide **31** (140 mg, 0.38 mmol) was dissolved in acetonitrile/water mixture (3:1) and ceric ammonium nitrate (4 equiv.) was added in one portion. After 30 min the reaction mixture was poured into a separatory funnel containing EtOAc (10 mL) and a saturated solution of sodium bicarbonate (5 mL). The phases were separated and the aqueous phase was reextracted and the combined organics were dried and concentrated. Column chromatograph afforded the by-product **33** (55% yield) and the desired deprotectd amide **32** (38% yield).

(b) From deprotection of **33**: The amide **33** (160 mg, 0.44 mmol) and 18-crown-6 (20 mg) were dissolved in 5 mL of a solution of 5% K_2CO_3 in methanol and stirred at room temperature for 12 h. Then the reaction was quenched using aqueous NH_4Cl and

after extractions with EtOAc, drying and concentration of the combined organic phases, Column chromatography afforded the desired amide **32** as a clear oil in 95% yield,

^{1}H NMR δ 1.32 (S, 3H), 1.47 (s, 3H), 2.12 (ddd, 1H, *J* = 13.8 Hz, 6.6 Hz, 2.4 Hz), 2,48 (dt, 1H, *J* = 14.1 Hz, 8.7 Hz), 3.27-3.35 (m, 1H), 3.32 (s, 3H), 3.44-3.53 (m, 1H), 4.78 (d, 1H, *J* = 6.3 Hz), 4.84 (d, 1H, *J* = 6.3 Hz), 4.99 (s, 1H), 6.94 (bs, 1H). ^{13}C NMR δ 24.8 (CH_3), 26.2 (CH_3), 30.9 (CH_2), 39.5 (CH_2), 54.9 (CH_3), 81.1(CH), 86 (CH), 112.6 (C), 175.9 (C).

(2*R*,3*R*,4*S*,5*S*)-3,4-Diacetoxy-7-*N*-acetyl-2-methoxy-7-aza-1-oxa-6-oxo-spiro[4.4]nonane (34). The optimal conditions leading to acetonide cleavage involved dissolving **32** in an anhydrous solution of 3% HCl in methanol at room temperature and allowing the mixture to stand for 12 h followed by concentration in *vacuo*. The crude product was dissolved in methylene chloride and treated with acetic anhydride and triethylamine. The triacetate was obtained as a clear oil (55%), a mixture of anomers in a ratio β/α (1.6:1). When distinguishable, the NMR peaks were assigned to the appropriate anomer. ^{1}H NMR δ 2.08 (s, 1H, β), 2.11 (s, 3H, α &β), 2.16 (s, 3H,α), 2.27-2.43 (m, 2H, α &β), 2.51 (s, 3H, α), 2.54 (s, 3H,β), 3.41 (s, 3H, β), 3.43 (s, 3H,α), 3.51-3.65 (m, 1H, α &β), 3.73-3.82 (m, 1H, α &β), 4.95 (s, 1H, β), 5.23 (d, 1H, α, *J* = 4.2 Hz), 5.33 (t, 1H, α, *J* = 6 Hz), 5.37 (d, 1H, β, *J* = 4.2 Hz), 5.46 (d, 1H, α, *J* = 6 Hz), 5.61 (d, 1H, β, *J* = 4.2 Hz).

(2*R*,3*R*,4*S*,5*S*)-3,4-Diacetoxy-7-*N*-acetyl-2-(6'-chloropurin-9'yl)-7-aza-l-oxa-6-oxospiro[4.4]nonane (35). 6-Chloropurine (123 mg, 0.8 mmol) was refluxed for 30 min in hexamethyldisilizane. The excess hexamethyldisilizane was removed in *vacuo* and the residue was dissolved in acetonitrile. Diacetate **34** (50 mg, 0.16 mmol) was dissolved in acetonitrile and the solution containing the silylated 6-chloropurine was added by canula. Trimethylsilyl trifluoromethanesulfonate (0.18 g, 0.15 mL, 0.8 mmol) was added dropwise. The reaction mixture was stirred for 48 h at 65 °C and then quenched with aqueous sodium bicarbonate. The organic phase was separated and the aqueous phase was extracted three times with methylene chloride. After drying the solvent was removed and column chromatography afforded the nucleoside **35** as a yellowish foam in 81% yield. A second fraction (5%) contained a mixture of *N*-9 and *N*-7 isomers.

^{1}H NMR δ 2.00 (s, 3H), 2.24 (s, 3H), 2.19-2.29 (m, 1H), 2.39 (ddd, 1H, *J* = 14.7 Hz, 8.1 Hz, 4.2 Hz), 2.55 (s, 3H), 3.60 (dt, 1H, *J* = 11.7 Hz, 7.2 H2), 3.84 (ddd, 1H, *J*= 11.7 Hz, 8.4 Hz, 4.2Hz), 5.69 (d, 1H, *J*= 4.8 Hz), 6.15 (dd, 1H, *J*= 7.5 Hz, 4.8 Hz), 6.59 (d, 1H, *J*= 7.2 Hz), 8.74 (s, 1H), 8.89 (s, 1H). ^{13}C NMR δ 20.2 (CH_3), 20.4 (CH_3), 24.9 (CH_3), 25.7 (CH_2), 40.9 (CH_2), 71.9 (CH), 74.3 (CH), 84.5 (CH), 89.3 (C), 143.8 (CH), 151.7(C), 152.1 (C), 152.3 (CH), 168.9 (C), 169.2 (C), 170.6 (C), 172.0 (C).

(2*R*,3*R*,4*S*,5*S*)-2-[N^6-3-Iodobenzyl)adenos-9'-yl]-7-aza-1-oxa-6-oxospiro[4.4]-nonan-4,5-diol (14). Compound **35** (50 mg, 0.18 mmol) was treated with ammonia in methanol for 20 min. The reaction mixture was concentrated to dryness and the residue was dissolved in *t*-BuOH. To this solution were added triethylamine (75 mL, 0.54 mmol) and 3-iodobenzylamine hydrochloride (50 mg, 0.18 mmol). The mixture was heated to

reflux for 6 days. Then concentrated and purified with chromatography to afford compound 14 and iodobenzylamine hydrochloride. The latter was removed upon boiling in methanol and decantation of the solution: the target nucleoside has little or no solubility in methanol, and can therefore be conveniently purified. The compound **14** was isolated as a white powder in 87% yield from **35**.

^{1}H NMR (DMSO-d_6) δ 1.96-2.02 (m, 1H), 2.63-2.68 (m, 1H), 3.14-3.22 (m, 2H), 4.06-4.12 (m, 1H), 4.66 (bs, 2H), 4.76-4.80 (m, 1H), 5.62 (d, 1H, J = 3.6 Hz), 5.74 (d, 1H, 3 Hz), 6.07 (d, 1H, 3.9 Hz), 7.10 (t, 1H, J = 4.5 Hz), 7.35 (d, 1H, J = 4.5 Hz), 7.57 (d, 1H, J = 4.8 Hz), 7.72 (s, 1H), 8.17 (s, 1H), 8.22 (s, 1H), 8.88 (bs, 1H), 8.64 (s, 1H). HRMS (FAB) for $C_{19}H_{20}IN_6O_4$ (M+H): calcd 523.0591, found 523.0583.