SUPPORTING INFORMATION

Structure-activity relationship of adenosine 5′-diphosphoribose at the transient receptor potential melastatin 2 (TRPM2) channel: Rational design of antagonists

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General Experimental. All reagents and solvents were of commercial quality and used without further purification, unless described otherwise. Solvents were either distilled or purchased as SureSeal bottles and stored over molecular sieves. Triethylamine and diisopropylethylamine were dried over potassium hydroxide, distilled and then stored over potassium hydroxide pellets. ADP-ribosyl cyclase was purified from the ovotestis of the opisthobranch mollusk *Aplysia californica*.[1] H_2O was of MilliQ quality. Unless otherwise stated, all reactions were carried out under an inert atmosphere of nitrogen. All ^{1}H, ^{13}C, and ^{31}P NMR spectra of final compounds were collected in D_2O, either on a Bruker machine at 400 MHz (^{1}H), 100 MHz (^{13}C) or 162 MHz (^{31}P), or 500 MHz (^{1}H) or 125MHz (^{13}C). All coupling constants are given in Hertz (Hz). UV spectra were collected in aqueous solution on a Perkin Elmer Lambda EZ 201 or Lambda 3B spectrophotometer. The purity of new tested compounds was determined to be ≥ 95 % by analytical HPLC. HPLC analyses were carried out on a Waters 2695 Alliance module equipped with a Waters 2996 Photodiode Array Detector (210 – 350 nm). The chromatographic system consisted of a Hichrom Guard Column for HPLC and a Phenomenex Synergi 4u MAX-RP 80A column (150 × 4.60 mm), eluted at 1 mL/min with the following ion-pair buffer: 0.17 % (m/v) cetrimide and 45% (v/v) phosphate buffer (pH 6.4) in MeOH. Semi-preparative chromatography was performed on a Waters 2525 system equipped with a Waters 2487 Dual λ Absorbance Detector. The system consisted of a Phenomenex Gemini 5μ C18 (250 × 10 mm) eluted at 5 mL/min with a linear gradient of 0.1 M TEAB in MeCN (95:5 → 35:65 over 30 mins). Preparative chromatography was performed on a Pharmacia Biotech Gradifrac system equipped with a peristaltic P-1 Pump and a fixed wavelength UV-1 Optical Unit (280 nm). The following purification methods were employed: LiChroprep RP-18 equilibrated with 0.05 M TEAB buffer (pH 6.0-6.4), gradient: 0.05 M TEAB buffer against MeCN at 5 mL/min; Q Sepharose washed with H_2O, gradient: 1M TEAB buffer (pH 7.1-7.6) against H_2O at 5 mL/min; Synthetic phosphates were assayed by an adaptation of the Briggs phosphate test. 8-Cl-AMP **87** was purchased from Biolog (Bremen, Germany) and 2′-deoxy-AMP **31** from Sigma-Aldrich.

Experimental Procedures

Adenosine diphosphoribose (ADPR, 1). *β*-NAD^+ (600 mg, 0.91 mmol) and NADase were reacted under general protocol A to leave the ADPR product (TEA salt, 2.6 eq. by ^{1}H NMR) (680 mg, 0.83 mmol, 92%) as a glassy solid which showed ^{1}H (400 MHz, D_2O) δ 8.43 (s, 1H, H-8), 8.17 (s, 1H, H-2), 6.04 (d, 1H, *J* = 6.2, H-1′), 5.21 (d, 0.4H, *J* = 4.3, H-1″), 5.10 (d, 0.6H, *J* = 2.3, H-1″), 4.68 (1H, obscured by HDO peak, H-2′), 4.43-4.45 (m, 1H, H-3′), 4.28-4.32 (br.m, 1H, H-4′) 4.09-4.16 (br.m, 2H, H-5′$_{a/b}$ and H-5′$_{a/b}$) and 3.89-4.07 (m, 5H, H-2″, H-3″, H-4″ and 2 × H-5″$_{a/b}$); ^{31}C (100 MHz, D_2O) δ 155.3 (C-6), 152.3 (C-2), 149.3 (C-4), 140.3 (C-8), 118.8 (C-5), 101.3 ($C_{\alpha/\beta}$-1″), 96.5 ($C_{\alpha/\beta}$-1″), 87.0 (C-1′), 84.1 (C-4′ or $C_{\alpha/\beta}$-4″, d, *J* = 8.9), 81.9 (C-4′ or $C_{\alpha/\beta}$-4″, d, *J* = 8.9), 81.2 (C-4′ or $C_{\alpha/\beta}$-4″, d, *J* = 8.9), 75.3, 74.4 (C-2′), 70.8, 70.6, 70.6, 70.2 66.5 (C-5′ or $C_{\alpha/\beta}$-5″, d, *J* = 5.2), 65.7 (C-5′ or $C_{\alpha/\beta}$-5″, d, *J* = 5.2) and 65.4 (C-5′ or $C_{\alpha/\beta}$-5″, d, *J* = 5.2); ^{31}P (161 MHz, D_2O) δ -10.1 (br.) and -10.3 (br.); HRMS (ES^-) calcd for $C_{15}H_{22}N_5O_{14}P_2$ 558.0644, M^- found 558.0645 and R_T = 11.2 min.

8-Bromo adenosine diphosphoribose (8-Br-ADPR 4). Bromine water (1.0 mL of Br_2 in 25 mL of H_2O) was added slowly to a stirred solution of ADPR **1** (2.6 eq. TEA salts, 230 mg, 0.28 mmol) in NaOAc-AcOH buffer (0.5 M, pH 4.1, 25 mL). The mixture was stirred at rt and the progress of the reaction monitored by HPLC; the bromine water was added until the HPLC confirmed complete reaction. After 4 h the reaction mixture was treated with $Na_2S_2O_3$ (50 mg) to destroy excess bromine, the mixture was then neutralised by the slow addition of NaOH (5 N) and evaporated under reduced pressure to leave a crude product. Purification by reverse-phase (RP-18) column chromatography, eluting with 0-10% MeCN in TEAB (0.05 M) followed by ion-exchange (Q-Sepharose) chromatography eluting with a gradient (0-40%) of TEAB (1.0 M) in MilliQ water followed by reverse-phase (RP-18) column chromatography, eluting with 0-10% MeCN in TEAB (0.05 M) left the 8-Br-ADPR product (TEA salt, 1.7 eq. by ^{1}H NMR) (55 mg, 0.07 mmol, 25%) as a colorless solid which showed ^{1}H (400 MHz, D_2O) δ 8.12 (s, 1H, H-2), 6.02 (d, 1H, *J* = 6.2, H-1′), 5.24 (d, 0.4H, *J* = 4.0, H-1″), 5.21 (t, 1H, *J* = 6.2, H-2′), 5.10 (d, 0.6H, *J* = 2.2, H-1″), 4.52-4.56 (m, 1H, H-2″), 3.88-4.24 (m, 8H, H-3′, H-4′, H-5′, H-3″, H-4″ and 2 × H-5″); ^{13}C (100 MHz, D_2O) δ 154.1, 152.6 (C-2), 150.5, 128.3, 119.4, 101.3 ($C_{\alpha/\beta}$-1″), 96.5 ($C_{\alpha/\beta}$-1″), 89.5 (C-1′), 83.5 (C-4′ or $C_{\alpha/\beta}$-4″, d, *J* = 8.1), 81.9 (C-4′ or $C_{\alpha/\beta}$-4″, d, *J* = 8.1), 81.3 (C-4′ or $C_{\alpha/\beta}$-4″, d, *J* = 8.1), 75.3, 70.9, 70.9, 70.6, 70.2, 69.7, 66.4 (C-5′ or $C_{\alpha/\beta}$-5″, d, *J* = 5.2), 65.4 (C-5′ or $C_{\alpha/\beta}$-5″, d, *J* = 5.2) and 65.3 (C-5′ or $C_{\alpha/\beta}$-5″, d, *J* = 5.2); ^{31}P (162 MHz, D_2O) δ -10.1 (br.); HRMS (ES^-) calcd for $C_{15}H_{21}BrN_5O_{14}P_2$ 635.9749, M^- found 635.9737 and R_T = 14.5 mins.

7-Deaza inosine diphosphoribose (7-deaza IDPR, 14)

7-deaza-IDPR was prepared according to the literature[2]

2′-Deoxy adenosine diphosphoribose (2′-F-ADPR, 16)

2′-F-ADPR was prepared according to the literature[3]

6-Chloro-9-(4-acetoxybutyl)purine 26.[4] A solution of 6-chloropurine **17** (1.6 g, 9.70 mmol), 4-chlorobutylacetate **25** (2.7 mL, 19.4 mmol) and DBU (1.74 mL, 11.6 mmol) in DMF was stirred at 60 °C for 16 h. The solvent was evaporated and the residue was purified by column chromatography on silica gel (DCM-acetone, 7:3 v/v) to afford the desired product as a white solid (1.26 g, 48 %) which showed ^{1}H (270 MHz, d_6-DMSO) δ 8.84 (s, 1H, H-2 or H-8), 8.82 (s, 1H, H-8 or H-2), 4.32 (t, 2H, *J* = 7.0 , CH_2-N), 3.98 (t, 2H, *J* = 6.4, CH2-O), 1.96 (s, 3H, Me), 1.94-1.86 (m, 2H, CH_2) and 1.60-1.52 (m, 2H, CH_2).

9-(4-Hydroxybutyl)adenine 27.[4] Chloro-9-(4-acetoxybutyl)purine **26** (0.7 g, 2.61mmol) was added to a saturated methanolic ammonia solution and it was stirred at 80 °C for 16 h. The solvent was removed in vacuo and the residue was purified by column chromatography on silica gel (DCM-MeOH, 9:1 v/v) to yield the desired product as a white solid (475 mg, 88 %) which showed ^{1}H (270 MHz, d_6-DMSO) δ 8.13 (s, 1H, H-2 or H-8), 8.12 (s, 1H, H-8 or H-2), 7.17 (br.s, 2H, NH_2), 4.43 (t, 1H, *J* = 5.3, OH), 4.13 (t, 2H, *J* = 7.0, CH_2-N), 3.38 (dt, 2H, *J* = 6.4 and *J* = 5.3, CH_2-O), 1.87-1.76 (m, 2H, O-CH_2-CH_2) and 1.41-1.30 (m, 2H, O-CH_2-CH_2).

2′-Deoxynicotinamide adenine 5′-dinucleotide (2′-deoxy-NAD^+, 32)[5]. 2′-Deoxy-AMP free acid **31** (50 mg, 0.151 mmol) was dissolved in dry DMSO (1 mL) and co-evaporated with dry DMF (5 × 2.5 mL). The residue was dissolved in dry DMSO (0.4 mL) to which was added morpholine (68 μL, 0.785 mmol), dipyridyldisulfide (83 mg, 0.377 mmol) and PPh_3 (99 mg, 0.0.377 mmol) at which point the solution turned bright yellow. The reaction was complete within 2 h, as shown by HPLC. Precipitation occurred by addition of a solution of 0.1 M NaI in acetone. It was then filtered, washed with acetone, dried under vacuum and

used in the next step without further purification. Crude 2′-deoxy-AMP morpholidate (50 mg, 0.118 mmol), *β*-NMN^+ (43 mg, 0.130 mmol) and $MgSO_4$ (28 mg, 0.236 mmol) were dissolved in a 0.2 M solution of $MnCl_2$ in formamide (0.88 mL) and stirred at rt for 16 h after which HPLC analysis showed completion of the reaction with a new peak forming at 2.9 min. Precipitation occurred by dropwise addition of MeCN. The precipitate was filtered, dissolved in MilliQ and applied to a RP-18 gradifrac column eluted with a gradient MeCN in 0.05M TEAB. The appropriate fractions were collected and evaporated under reduced pressure. The residue was passed through a small chelex column (Na^+ form). The column was thoroughly washed with MilliQ. The water was then removed to afford the desired product as its sodium salt (50 μmol, 33 %) which showed ^{1}H (270 MHz, D_2O) δ 9.23 (s, 1H, H_N2), 9.02 (d, 1H, $J_{6,5}$ = 6.3, H_N6), 8.70 (dt, 1H, $J_{4,5}$ = 8.0 and $J_{4,6}$ = 1.4, H_N4), 8.25 (s, 1H, H-8), 8.05 (dd, 1H, $J_{5,4}$ = 8.0 and $J_{5,6}$ = 6.3, H_N5), 7.92 (s, 1H, H-2), 6.25 (m, 1H, H-1′), 5.98 (d, 1H, $J_{1'',2''}$ = 5.5, H-1″), 4.72-4.14 (m, 9H, H-ribose), 2.77-2.67 (m, 1H, H-2′a) and 2.48 (ddd, 1H, $J_{2'b,2'a}$ = 13.9, $J_{2'b,1'}$ = 6.6 and $J_{2'b,3'}$ = 3.3, H-2′b); ^{31}P (109 MHz, D2O) δ −10.5 (d, AB system, *J* = 21.2), −10.8 (d, AB system, *J* = 21.2); UV (H_2O, pH 7.2) λ_{max} 250 nm (ε 14400).

2′,5′-Di-*O*-acetyl-3′-bromo-3′-deoxyadenosine 35 To a solution of triethylorthoacetate (7.2 mL, 39.2 mmol) in MeCN (30 mL) was added adenosine **34** (3.5 g, 13.1 mmol) and *p*TsOH (3 g, 15.7 mmol). The mixture was stirred for 2 h at rt, neutralised with a saturated methanolic ammonia solution and evaporated under reduced pressure. Chloroform (20 mL) was added, the solid was filtered and the filtrate was evaporated to leave a white foam which redissolved in dichloroethane (30 mL). Acetyl bromide (3.8 mL, 52.4 mmol) was added and the solution was refluxed for 15 min. Sat. $NaHCO_3$ solution (30 mL) was added, the organic layer was separated and washed with brine (30 mL), dried (Na_2SO_4), filtered and evaporated. The residue was purified with an Isco chromatographic system (DCM-MeOH, 9:1 v/v) to leave the desired product as a white solid (1.6 g, 29 %) which showed ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H, H-2), 8.30 (s, 1H, H-8), 6.19 (d, 1H, $J_{1',2'}$ = 2.1, H-1′), 5.71 (br s, 1H, H-2′), 4.51-4.38 (m, 4H, H-3′, H-4′ and H-5′), 2.12 (s, 3H, CH_3), 2.10 (s, 3H, CH_3) and 2.06 (s, 3H, CH_3); ^{13}C (100 MHz, $CDCl_3$) δ 170.4, 169.2 (2 × C=O), 154.2 (C-6), 150.5 (C-2), 148.8 (C-4), 139.6 (C-8), 118.9 (C-5), 87.9 (C-1′), 82.9 (C-4′), 78.8 (C-2′), 64.8 (C-5′), 49.1 (C-3′), 30.8 and 20.6 (2 × CH_3); HRMS (ES^+) calcd for $C_{14}H_{17}N_5O_5{}^{79}Br$ 414.0408 $(MH)^+$ found 414.0401 and for $C_{14}H_{17}N_5O_5{}^{81}Br$ 416.0387 $(MH)^+$ found 416.0390.

2′,5′-Di-*O*-acetyl-3′-deoxyadenosine 36. The above material (1.6 g, 3.87 mmol) was dissolved in toluene (27 mL) and tri-butyl tin hydride (3.1 mL) and AIBN (55 mg) were added. The mixture was refluxed for 2 h, cooled and evaporated to leave a residue which a purified with an Isco chromatographic system (DCM-MeOH, 9:1 v/v) to afford the title compound as a white solid (1.0 g, 75 %) which showed ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.25 (s, 1H, H-2), 8.13 (s, 1H, H-8), 7.26 (br.s, 2H, NH_2), 6.08 (d, 1H, $J_{1',2'}$ = 1.6, H-1′), 5.67-5.66 (m, 1H, H-2′), 4.51-4.45 (m, 1H, H-4′), 4.26 (dd, 1H, $J_{5'a,5'b}$ = 12.0 and $J_{5'a,4'}$ = 3.1, H-5′a), 4.11 (dd, 1H, $J_{5'b,5'a}$ = 12.0 and $J_{5'b,4'}$ = 5.8, H-5′b), 2.69-2.62 (m, 1H, H-3′a), 2.18 (dd, 1H, $J_{3'b,3'a}$ = 12.6, $J_{3'b,4'}$ = 5.9 and $J_{3'b,2'}$ = 1.4, H-3′b), 2.07 (s, 3H, CH_3) and 1.93 (s, 3H, CH_3); ^{13}C (100 MHz, d_6-DMSO) δ 170.0, 169.8 (2 × C=O), 156.1 (C-6), 152.7 (C-2), 148.8 (C-4), 139.5 (C-8), 118.9 (C-5), 88.4 (C-1′), 77.7 (C-4′), 77.2 (C-2′), 64.4 (C-5′), 32.6 (C-3′), 20.7 and 20.4 (2 × CH_3); HRMS (ES^+) calcd for $C_{14}H_{18}N_5O_5$ 336.1302 $(MH)^+$ found 336.1307.

3′-Deoxyadenosine (Cordycepin)[6] 37 2′,5′-Di-*O*-acetyl 3′-deoxyadenosine **36** (900 mg, 2.68 mmol) was dissolved in a saturated methanolic ammonia solution at 0°C. It was slowly warmed to rt and heated to 60°C for 16 h. The resulting solid was collected by filtration and washed with small portion of MeOH to leave the desired compound as a white solid (606 mg, 90 %) which showed ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.32 (s, 1H, H-2), 8.12 (s, 1H, H-8), 7.21 (br s, 2H, NH_2), 5.84 (d, 1H, $J_{1',2'}$ = 1.6, H-1′), 5.61 (d, 1H, *J* = 3.6, 2′-OH), 5.10 (app. t, 1H, *J* = 5.4, 5′-OH), 4.57-4.55 (m, 1H, H-2′), 4.36-4.30 (m, 1H, H-4′), 3.69-3.47 (m, 2H, H-5′), 2.27-2.20 (m, 1H, H-3′a) and 1.90 (ddd, 1H, $J_{3'b,3'a}$ = 13.0, $J_{3'b,4'}$ = 6.3 and $J_{3'b,2'}$ = 3.3, H-3′b); ^{13}C (100 MHz, d_6-DMSO) δ 156.0 (C-6), 152.4 (C-2), 148.9 (C-4), 139.0 (C-8), 119.0 (C-5), 90.8 (C-1′), 80.6 (C-4′), 74.6 (C-2′), 62.6 (C-5′) and 34.1 (C-3′); HRMS (ES^+) calcd for $C_{10}H_{14}N_5O_3$ 252.1091 $(MH)^+$ found 252.1096.

Bis-(2',5'-*t*Butyldimethylsilyl)-3'-deoxyadenosine 38. To a solution of 3'-deoxyadenosine[6] **37** (150 mg, 0.594 mmol) in DMF (1 mL) at 0 °C was added dropwise a solution of imidazole (242 mg, 3.564 mmol), TBDMSCl (224 mg, 1.487 mmol) and DMAP (11 mg, 0.089 mmol) in DMF (0.5 mL). The reaction mixture

was stirred for 30 mins, diluted with sat. aq. $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 5 mL). The organic layers were dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified on an Isco chromatographic system (Petrol-EtOAc, 1:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H, H-2), 8.26 (s, 1H, H-8), 6.26 (br.s, 2H, NH_2), 5.98 (d, 1H, $J_{1',2'}$ = 1.3, H-1′), 4.63-4.62 (m, 1H, H-2′), 4.55-4.49 (m, 1H, H-4′), 4.06 (dd, 1H, $J_{5'a,5'b}$ = 11.4 and $J_{5'a,4'}$ = 2.7, H-5′a), 3.75 (dd, 1H, $J_{5'b,5'a}$ = 11.4 and $J_{5'b,4'}$ = 2.9, H-5′b), 2.27-2.20 (m, 1H, H-3′a), 1.84 (ddd, 1H, $J_{3'b,3'a}$ = 12.9, $J_{3'b,4'}$ = 5.7 and $J_{3'b,2'}$ = 2.3, H-3′b), 0.86 (s, 18H, 2 × 9H), 0.10 (s, 3H, CH_3), 0.09 (s, 3H, CH_3), 0.08 (s, 3H, CH_3) and 0.05 (s, 3H, CH_3); ^{13}C (100 MHz, $CDCl_3$) δ 155.6 (C-6), 152.7 (C-2), 149.3 (C-4), 138.9 (C-8), 120.0 (C-5), 91.3 (C-1′), 81.1 (C-4′), 77.3 (C-2′), 63.8 (C-5′), 38.8 (C-3′), 26.0, 25.7 (2 × CH_3), 18.8, 17.9 (2 × C), −0.4, −4.7, −5.1, −5.3, −5.4 and −5.6 (6 × CH_3); HRMS (ES^+) calcd for $C_{22}H_{42}N_5O_3Si_2$ 480.2821 $(MH)^+$ found 480.2826.

2'-^{t}Butyldimethylsilyl-3'-deoxyadenosine 39. To a solution of bis-(2',5'-^{t}Butyldimethylsilyl)-3'-deoxyadenosine **38** (0.594 mmol) in THF (7 mL) at 0 °C was added TFA-H_2O (3.5 mL, 1:1 v/v). The reaction mixture was stirred at 0 °C until complete then neutralised with aq. $NaHCO_3$. The solvents were removed under pressure and the residue was purified on an Isco chromatographic system (EtOAc-MeOH, 1:0 → 4:1 v/v) to give a white foam which showed ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H, H-2), 7.76 (s, 1H, H-8), 6.0 (br s, 2H, NH_2), 5.52 (d, 1H, $J_{1',2'}$ = 5.9, H-1′), 4.94 (dt, 1H, $J_{2',1'}$ = 5.9 and $J_{2',3'a}$ = 7.5, H-2′), 4.41 (td, 1H, $J_{4',5'a}$ = 1.6 and $J_{4',3'a}$ = 9.0, H-4′), 3.90 (dd, 1H, $J_{5'a,5'b}$ = 12.8 and $J_{5'a,4'}$ = 1.6, H-5′a), 3.46 (dd, 1H, $J_{5'b,5'a}$ = 12.8 and $J_{5'b,4'}$ = 1.8, H-5′b), 2.46 (ddd, 1H, $J_{3'a,3'b}$ = 12.4, $J_{3'a,2'}$ = 7.3 and $J_{3'a,4'}$ = 3.8, H-3′a), 2.14-2.08 (m, 1H, H-3′b), 0.69 (s, 9H, ^{t}Bu), -0.21 (s, 3H, CH_3), and -0.34 (s, 3H, CH_3); ^{13}C (100 MHz, $CDCl_3$) δ 155.8 (C-6), 152.3 (C-2), 148.6 (C-4), 140.4 (C-8), 113.0 (C-5), 93.5 (C-1′), 80.6 (C-4′), 74.0 (C-2′), 64.8 (C-5′), 34.5 (C-3′), 25.5 (^{t}Bu), 17.7 (C), −5.2 and −5.4 (2 × CH_3); HRMS (ES^+) calcd for $C_{16}H_{28}N_5O_3Si$ 366.1956 $(MH)^+$ found 366.1951.

2'-^{t}Butyldimethylsilyl-5'-*O*-di-^{t}butylphosphoryl-3'-deoxyadenosine 40. To a solution of 2'-^{t}Butyldimethylsilyl-3'-deoxyadenosine **39** (140 mg, 0.38 mmol) in DCM (5 mL) was added Ph-tetrazole (110 mg, 0.77 mmol) and *N,N*-diisopropyl-di-^{t}butylphosphoramidite (180 µmol, 0.57 mmol). The cloudy mixture becomes clear within minutes and it was stirred at rt for 1 h after which it was cooled to 0 °C and triethylamine (200 µmol, 1.425 mmol) and hydrogen peroxide (180 µmol, 2.052 mmol). It was stirred for further 30 mins and sat. aq. $NaHCO_3$ (5 mL) was added. The organic layer was collected, dried (Na_2SO_4), filtered and evaporated to leave a residue which was purified on an Isco chromatographic system containing 0.5% pyridine (DCM-MeOH, 1:0 → 9:1 v/v) to yield the desired product as a glassy solid (115 mg, 69 %) which showed ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H, H-2), 8.16 (s, 1H, H-8), 6.30 (br.s, 2H, NH_2), 5.95 (d, 1H, $J_{1',2'}$ = 1.0, H-1′), 4.73-4.72 (m, 1H, H-2′), 4.65-4.62 (m, 1H, H-4′), 4.29 (ddd, 1H, $J_{5'a,5'b}$ = 11.3, $J_{5'a,4'}$ = 5.2 and $J_{5'a,P'}$ = 3.3, H-5′a), 4.14-4.08 (m, 1H, H-5′b), 2.26-2.19 (m, 1H, H-3′b), 1.95 (ddd, 1H, $J_{3'a,3'b}$ = 13.0, $J_{3'a,2'}$ = 5.8 and $J_{3'a,4'}$ = 2.1, H-3′a), 1.47 (s, 9H, ^{t}Bu), 1.46 (s, 9H, ^{t}Bu), 0.87 (s, 9H, ^{t}Bu), 0.099 (s, 3H, CH_3) and 0.06 (s, 3H, CH_3); ^{13}C (100 MHz, $CDCl_3$) δ 155.6 (C-6), 152.8 (C-2), 149.3 (C-4), 138.6 (C-8), 119.9 (C-5), 92.1 (C-1′), 82.8 (C), 79.0 (C-4′, d, J = 8.9), 76.2 (C-2′), 66.8 (C-5′, d, J = 6.0), 34.5 (C-3′), 29.9 (^{t}Bu), 25.6 (^{t}Bu), 17.8 (C), −4.8 and −5.1 (both CH_3); ^{31}P NMR (162 MHz, $CDCl_3$) δ − 9.99; HRMS (ES^+) calcd for $C_{34}H_{45}N_5O_6PSi$ 558.2877 $(MH)^+$ found 558.2872.

3'-Deoxyadenosine-5'-monophosphate 41. A solution of 2'-^{t}butyldimethylsilyl-5'-*O*-di-^{t}butylphosphoryl-3'-deoxyadenosine **40** (110 mg, 0.197 mmol) in 50 % aqeous TFA solution was stirred 0 °C for 20 h. The volatiles were evaporated under vaccum and the residue was coevaporated with MeOH (3 ×). The residue was washed with water and EtOAc, the aqueous layer was concentrated under reduced pressure to yield the desired monophosphate as a glassy solid (59 mg, 90 %) which showed ^{1}H NMR (400 MHz, D_2O) δ 8.37 (s, 1H, H-2), 8.26 (s, 1H, H-8), 5.97 (d, 1H, $J_{1',2'}$ = 1.0 Hz, H-1′), 4.69-4.59 (m, 2H, H-2′ and H-4′), 4.4.14 (ddd, 1H, $J_{5'a,5'b}$ = 11.7, $J_{5'a,4'}$ = 4.9 and $J_{5'a,P'}$ = 2.0, H-5′a), 3.95-3.89 (m, 1H, H-5′b), 2.28-2.20 (m, 1H, H-3′b) and 2.04 (ddd, 1H, $J_{3'a,3'b}$ = 13.0, $J_{3'a,2'}$ = 6.1 and $J_{3'a,4'}$ = 2.0, H-3′a); ^{13}C (100 MHz, D_2O) δ 162.4 (C-6), 149.6 (C-4), 144.3 (C-2), 141.9 (C-8), 118.3 (C-5), 91.2 (C-1′), 80.3 (C-4′, d, J = 8.4), 75.3 (C-2′), 65.6 (C-5′, d, J = 4.9) and 32.2 (C-3′); ^{31}P NMR (162 MHz, $CDCl_3$) δ − 0.05; HRMS (ES^-) calcd for $C_{10}H_{13}N_5O_6P$ 330.0609 $(M{-}H)^-$ found 330.0611.

Nicotinamide 3'-deoxyadenosine 5'-dinucleotide (3′-deoxy-NAD, 42) 3'-Deoxyadenosine 5'-monophosphate **41** (60 mg, 0.181 mmol) was dissolved in dry DMSO (1 mL) and co-evaporated with dry

DMF (5 × 3 mL). The residue was dissolved in DMSO (300 μL) to which was added morpholine (82 μL, 0.942 mmol), dipyridyldisulfide (99 mg, 0.452 mmol) and triphenylphosphine (118 mg, 0.452 mmol) at which point the solution became bright yellow. It was stirred for 1 h at rt after which HPLC analysis showed completion of the reaction. Precipitation of the product occurred by dropwise addition of a solution of NaI in acetone (0.1M, 15 mL). The resulting precipitate was filtered, washed with acetone and dried. 34mgs of this morpholidate was used in the following step. It was then reacted with *β*-NMN^+ (31 mg, 0.093 mmol) and $MgSO_4$ (20 mg, 0.17 mmol) in a 0.2M solution of $MnCl_2$ in formamide (0.64 mL) at rt for 16 h after which HPLC analysis showed completion of the reaction. Precipitation occurred by dropwise addition of MeCN. The solid obtained was redissolved in MilliQ and treated with Chelex 100 to remove any paramagnetic particles prior to purification by semi-preparative HPLC which afforded the desired dinucleotide (16.1 μmol) which showed ^{1}H (500 MHz, D_2O) δ 9.28 (s, 1H, H_N2), 9.09 (d, 1H, $J_{6,5}$ = 4.8, H_N6), 8.74 (d, 1H, $J_{4,5}$ = 6.6, H_N4), 8.28 (s, 1H, H-8), 8.13-8.10 (m, 1H, H_N5), 8.05 (s, 1H, H-2), 6.0 (d, 1H, $J_{1',2'}$ = 5.0, H-1'), 5.91 (s, 1H, H-1″), 4.48-4.0 (m, 9H, H-2', H-2″, H-3″, H-4', H-4″, 2 × H-5' and 2 × H-5″), 2.34 (dd, 1H, $J_{3'a,3'b}$ = 10.1 and $J_{3'a,4'}$ = 6.8, H-3'a) and 2.15-2.10 (m, 1H, H-3'b); ^{13}C (126 MHz, D_2O) δ 155.2 (C-6), 152.3 (C-2),148.7 (C-4), 145.8 (C_N4), 142.5 (C_N6), 139.8 (C_N2), 133.7 (C_N3), 128.6 (C_N5), 100.0 (C-1″), 90.2 (C-1′), 87.1 (C-4″, d, *J* = 8.5), 79.7 (C-4′, d, *J* = 8.5), 77.6 (C-2″), 75.1 (C-2′), 70.7 (C-3″), 66.7 (C-5″), 64.9 (C-5') and 32.9 (C-3′); ^{31}P (decoupled, 162 MHz, D_2O) δ −11.4 (br.m); HRMS (ES^-) calcd for $C_{21}H_{27}N_7O_{13}P_2$ 647.1142 $(M-H)^-$ found 647.1149. UV (H_2O, pH 7.3) λ_{max} 260 nm (ε 17800).

Nicotinamide adenine trinucleotide (NAT, 45).[7] ADP sodium salt **44** (50 mg, 0.106 mmol) and *β*-NMN^+ (39 mg, 0.116 mmol) were stirred in 1 M $MgCl_2$ (200 μL) and 2 M HEPES (200 μL) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 193 mg, 1.01 mmol) for 24 h at rt. HPLC analysis showed presence of a new peak at R_T = 10.5 mins. The reaction was diluted with MilliQ to bring the conductivity below 300 μS and loaded onto an AG MP-1 ion exchange resin eluted with a gradient of 150 mM TFA. The desired fractions were combined and evaporated to afford the desired trinucleotide as a glassy solid (40.1 μmol, 38 %) which showed ^{1}H (270 MHz, D_2O) δ 9.38 (s, 1H, H_N2), 9.24 (d, 1H, *J* = 5.8, H_N6), 8.87 (d, 1H, *J* = 6.7, H_N4), 8.47 (s, 1H, H-2), 8.29-8.22 (m, 1H, H_N5), 6.13 (d, 1H, $J_{1'',2''}$ = 5.0, H-1″), 6.04 (d, 1H, $J_{1',2'}$ = 5.5, H-1′), 4.69 (app t, 1H, $J_{2',1'} = J_{2',3'}$ = 5.2, H-2′), 4.55-4.46 (m, 4H) and 4.37-4.25 (m, 5H); ^{13}C (68 MHz, D_2O) δ 163.4 (C=O), 162.9 (C-6), 153.1 (C-4), 149.6 (C-8), 146.0 (C_N4), 142.6 (C_N6), 140.0 (C_N2), 133.9 (C_N3), 128.8 (C_N5), 118.6 (C-5), 100.1 (C-1″), 87.4 (C-1′), 87.2 (C-4″ , d, *J* = 8.7), 83.9 (C-4′, d, *J* = 9.3), 77.7 (C-2″), 74.6 (C-2′), 70.8 (C-3′), 70.4 (C-3″), 65.3 (C-5″, d, *J* = 5.0) and 65.0 (C-5′, d, *J* = 6.8); ^{31}P (109 MHz, D_2O) δ –14.4 (d, *J* = 18.8), −10.7 (d, *J* = 18.8) and –21.7–21.9 (m, O-P-O); MS: (ES^-) m/z 678.2 [$(M-H)^-$, 100%]; HRMS (ES^-) calcd for $C_{21}H_{28}N_7O_{17}P_3$ 743.0755 [$(M-H)^-$] found 743.0724; UV (H_2O) λ_{max} 258 nm (ε 20100).

Cyclic adenosine triphosphoribose (cATPR, 46).[7] NAT **45** (40 μmol) was incubated with *Aplysia cyclase* (120 μL) in a 25 mM HEPES buffer (40 mL) at rt. After 3 days, HPLC analysis showed total consumption of starting material. It was applied to a Q-sepharose ion exchange column and eluted with a gradient of 1 M TEAB. The appropriate fractions were collected, evaporated under reduced pressure to afford the cyclic nucleotide as a triethylammonium salt (16 μmol, 39 %) which showed ^{1}H (400 MHz, D_2O) δ 8.77 (s, 1H, H-2), 8.16 (s, 1H, H-8), 5.93 (d, $J_{1'',2''}$ = 4.0, 1H, H-1″), 5.92 (d, $J_{1',2'}$ = 5.5, 1H, H-1′), 5.26 (app. t, $J_{2',1'} = J_{2',3'}$ = 5.1, 1H, H-2′) and 4.69-4.14 (m, 9H); ^{31}P (109 MHz, D_2O) δ –10.5 (br.s) −21.2 (br.s); HRMS (ES^-) calcd for $C_{15}H_{21}N_5O_{16}P_3$ 620.0202 $(M - H)^-$ found 620.0215; UV (H_2O) λ_{max} 259 nm (ε 16000).

1-*O*-Methyl-2,3-*O*-isopropylidene-β-D-ribofuranose 49.[8] To a suspension of D-ribose (1 g, 6.7 mmol) in acetone (5 mL) and MeOH (5 mL) was cautiously added H_2SO_4 (0.5 mL) at which point all the starting material went into solution. The reaction mixture was stirred at room temperature for 48 h after which it was neutralised by addition of solid $NaHCO_3$. The solid was filtered and all solvents evaporated. The residue was purified on an Isco chromatographic system (Petrol-EtOAc, 1:2 v/v) to give the desired product as a colourless oil (1.3 g, 98 %) which showed ^{1}H (400 MHz, $CDCl_3$) δ 4.96 (s, 1H, H-1′), 4.82 (d, 1H, $J_{2',3'}$ = 6.0, H-2′), 4.57 (d, 1H, $J_{3',2'}$ = 6.0, H-3′), 4.41-4.40 (m, 1H, H-4′), 3.68 (dd, 1H, $J_{5'a,5'b}$ = 12.5 and $J_{5'a,4'}$ = 2.3, H-5′a), 3.60 (dd, 1H, $J_{5'b,5'a}$ = 12.5 and $J_{5'b,4'}$ = 3.3, H-5′b), 3.42 (s, 3H, OMe), 1.47 (s, 3H, CH_3) and 1.31 (s, 3H, CH_3).

Diethylsquarate 53.[9] Squaric acid (5 g) was refluxed in EtOH (50 mL) for 3 h after which the solvent was removed under reduced pressure. Another 50 mL of EtOH was added and it was refluxed for another 30 min. This procedure was repeated 3 times to give the desired product as a yellowish liquid (6 g, >99%). ^{1}H (400 MHz, $CDCl_3$) δ 4.72 (q, 4H, J = 7.1, 2 × CH_2) and 1.46 (t, 6H, J = 7.1, 2 × CH_3); ^{13}C (100 MHz, $CDCl_3$) δ 189.2 (2 × C=O), 184.1 (2 × C=C), 70.5 (2 × CH_2) and 15.4 (2 × CH_3).

2′,3′-*O*-Isopropylidene 5′-azido 5′-deoxy adenosine 68.[10] Diphenylphosphoryl azide (2.1 mL, 9.77 mmol) and DBU (2.2 mL, 16.64 mmol) were added dropwise to a suspension of 2′,3′-*O*-isopropylidene adenosine (1.5 g, 4.88 mmol) in dioxane (25 mL) and stirring was continued for 3 h. NaN_3 (1.6 g, 24.4 mmol), TBAI (0.18 g, 0.488 mmol) and 15-crown-5 (96 μL, 0.488 mmol) were then added and the solution was refluxed for 4 h. The solvent was removed under reduced pressure and the residue was purified on an Isco chromatographic system (DCM-MeOH, 9:1 v/v) to yield the desired nucleoside as a pale yellow foam (1.4 g, 88 %) which showed ^{1}H (400 MHz, $CDCl_3$) δ 8.27 (s, 1H, H-8), 7.82 (s, 1H, H-2), 6.02 (d, 1H, $J_{1',2'}$ = 2.2, H-1′), 5.38 (dd, 1H, $J_{2',3'}$ = 6.3 and $J_{2',1'}$ = 2.2, H-2′), 4.97 (dd, 1H, $J_{3',2'}$ = 6.3 and $J_{3',4'}$ = 3.4, H-3′), 4.29 (dt, 1H, $J_{4',3'}$ = 3.4 and $J_{4',5'}$ = 5.5, H-4′), 3.53-3.44 (m, 2H, H-5′), 1.53 (s, 3H, CH_3) and 1.30 (s, 3H, CH_3); ^{13}C (100 MHz, $CDCl_3$) δ 155.6 (C-6), 153.2 (C-8), 149.1 (C-4), 139.8 (C-2), 120.3 (C-5), 114.7 (C), 90.6 (C-1′), 85.6 (C-4′), 84.0 (C-2′), 82.0 (C-3′), 52.3 (C-5′), 27.0 and 23.7 (2 × CH_3); HRMS (ES^+) calcd for $C_{13}H_{17}N_8O_3$ 333.1418 $(MH)^+$ found 333.1386.

2′,3′-*O*-Isopropylidene adenosine mono-sulfonamide 74. Triethylamine (0.27 mL, 1.95 mmol) was added to a solution of 2′,3′-*O*-isopropylidene adenosine **58** (500 mg, 1.63 mmol) in DMA (2 mL) at 0°C and it was stirred for 30 min. Sulfamoyl chloride (5 mL, 3.35 mmol) was added at 0 °C in a dropwise manner over 5 min and the resulting solution was stirred at rt for 16 h after which the solution became cloudy. A small amount a MeOH was added and the solvents were removed under reduced pressure leaving a residue which was purified on an Isco system (EtOAc-MeOH, 8:2 v/v) to yield the desired product as a pale yellow solid (536 mg, 85 %) which showed ^{1}H (400 MHz, d_6-DMSO) δ 8.52 (s, 1H, H-2), 8.42 (s, 1H, H-8), 7.56 (s, 2H, NH_2), 6.27 (d, 1H, $J_{1',2'}$ = 2.3, H-1′), 5.41 (dd, 1H, $J_{2',3'}$ = 6.2 and $J_{2',1'}$ = 2.3, H-2′), 5.04 (dd, 1H, $J_{3',2'}$ = 6.2 and $J_{3',4'}$ = 3.2, H-3′), 4.46-4.43 (m, 1H, H-4′), 4.19 (dd, 1H, $J_{5'a,5'b}$ = 10.9 and $J_{5'a,4'}$ = 4.9, H-5′a), 4.13 (dd, 1H, $J_{5'b,5'a}$ = 10.9 and $J_{5'b,4'}$ = 6.1, H-5′b), 1.54 (s, 3H, CH_3) and 1.32 (s, 3H, CH_3); ^{13}C (100 MHz, d_6-DMSO) δ 151.9 (C-4), 148.0 (C-6), 147.3 (C-8), 141.7 (C-2), 118.9 (C-5), 113.6 (C), 89.6 (C-1′), 83.9 (C-4′), 83.5 (C-2′), 80.9(C-3′), 67.9 (C-5′), 26.9 and 25.1 (2 × CH_3); HRMS (ES^+) calcd for $C_{13}H_{18}N_6O_6S$ 387.1081 $(MH)^+$ found 387.1067.

Salicylic 2′,3′-*O*-isopropylidene adenosine monosulfonamide 75. Salicylic acid (97 mg, 0.699 mmol) and CDI (136 mg, 0.699 mmol) in MeCN (6 mL) were stirred at 60°C for 2 h after which it was cooled to room temperature and a solution of 2′,3′-*O*-isopropylidene adenosine monosulfonamide **74** (90 mg, 0.233 mmol) and DBU (52 μL, 0.349 mmol) in DMF (1.5 mL) was added. The solution was stirred at 60 °C for further 3 h, the solvents were removed under reduced pressure and the residue was purified on an Isco chromatographic system (EtOAc-MeOH, 8:2 v/v) to leave a colorless oil (81 mg, 69 %) which showed ^{1}H (400 MHz, d_4-MeOH) δ 8.37 (s, 1H, H-8), 8.13 (s, 1H, H-2), 7.84 (dd, 1H, J = 7.8 and J = 1.7, CH), 7.28-7.22 (m, 2H, 2 × CH), 6.79-6.72 (m, 1H, CH), 6.19 (d, 1H, $J_{1',2'}$ = 2.9, H-1′), 5.34 (dd, 1H, $J_{2',3'}$ = 6.1 and $J_{2',1'}$ = 2.9, H-2′), 5.04 (dd, 1H, $J_{3',2'}$ = 6.1 and $J_{3',4'}$ = 2.3, H-3′), 4.54-4.52 (m, 1H, H-4′), 4.35 (dd, 1H, $J_{5'a,5'b}$ = 10.9 and $J_{5'a,4'}$ = 4.1, H-5′a), 4.31 (dd, 1H, $J_{5'b,5'a}$ = 10.9 and $J_{5'b,4'}$ = 4.4, H-5′b), 1.54 (s, 3H, CH_3) and 1.31 (s, 3H, CH_3); ^{13}C (100 MHz, d_4-MeOH) δ 162.6 (C=O), 157.3 (C-6), 153.9 (C-8), 150.4 (C-4), 141.7 (C-2), 133.9, 131.7 (2 × CH), 120.5 (C-5), 119.1, 117.2 (2 × CH), 115.4 (C), 92.0 (C-1′), 85.4 (C-4′), 85.7 (C-2′), 83.3 (C-3′), 70.0 (C-5′), 27.4 and 25.5 (2 × CH_3); HRMS (ES^+) calcd for $C_{20}H_{22}N_6O_8S$ 507.1293 $(MH)^+$ found 507.1395.

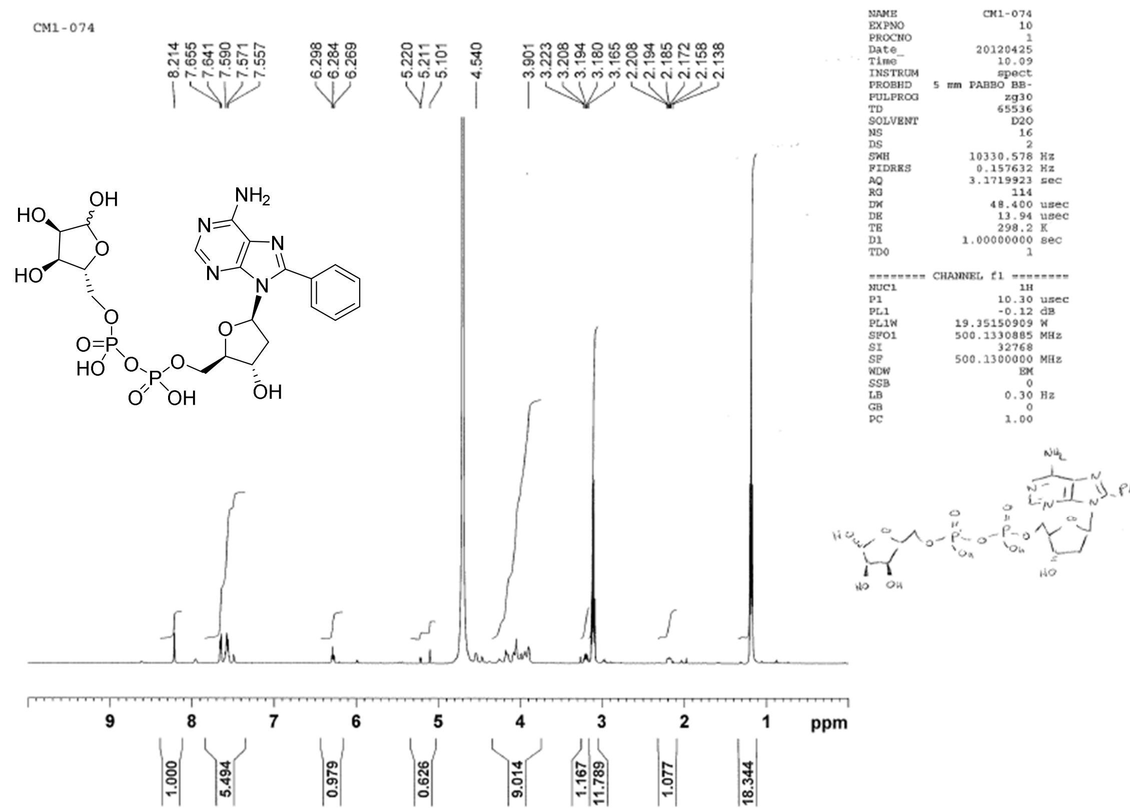
Salicylic Adenosine monosulphonamide (Sal-AMS, 76). Salicylic 2′,3′-*O*-isopropylidene adenosine monosulfonamide **75** (80 mg, 0.158 mmol) was stirred at 0 °C in a 75% aq. TFA solution (10 mL) for 45 min. The solvent was evaporated and co-evaporated with MeOH several times to remove excess TFA. The residue was purified on an Isco chromatographic system (DCM-MeOH, 7:3 v/v) to leave the deprotected sulfonamide as a colourless solid (26.6 mg, 36 %) which showed ^{1}H (400 MHz, d_6-DMSO) δ 8.48 (s, 1H, H-8), 8.17 (s, 1H, H-2), 7.91 (dd, 1H, J = 7.9, 1.7, CH), 7.52 (br.s, 2H, NH_2), 7.29-7.25 (m, 1H, CH), 6.78-6.73 (m, 2H, 2 × CH), 6.07 (d, 1H, $J_{1',2'}$ = 5.6, H-1′), 4.70 (app.t, 1H, $J_{2',3'}$ = $J_{2',1'}$ = 5.6, H-2′) and 4.44-4.30 (m,

4H, H-3′, H-4′ and H-5′); ^{13}C (100 MHz, d_4-MeOH) δ 161.9 (C=O), 156.7 (C-6), 152.9 (C-8), 150.7 (C-4), 141.4 (C-2), 134.4, 131.1 (2 × CH), 120.3 (C-5), 119.3, 117.9 (2 × CH), 89.6 (C-1′), 84.6 (C-4′), 76.0 (C-2′), 72.3 (C-3′) and 69.7 (C-5); HRMS (ES$^+$) calcd for $C_{17}H_{18}N_6O_8S$ 489.0799 (MH)$^+$ found 489.0809.

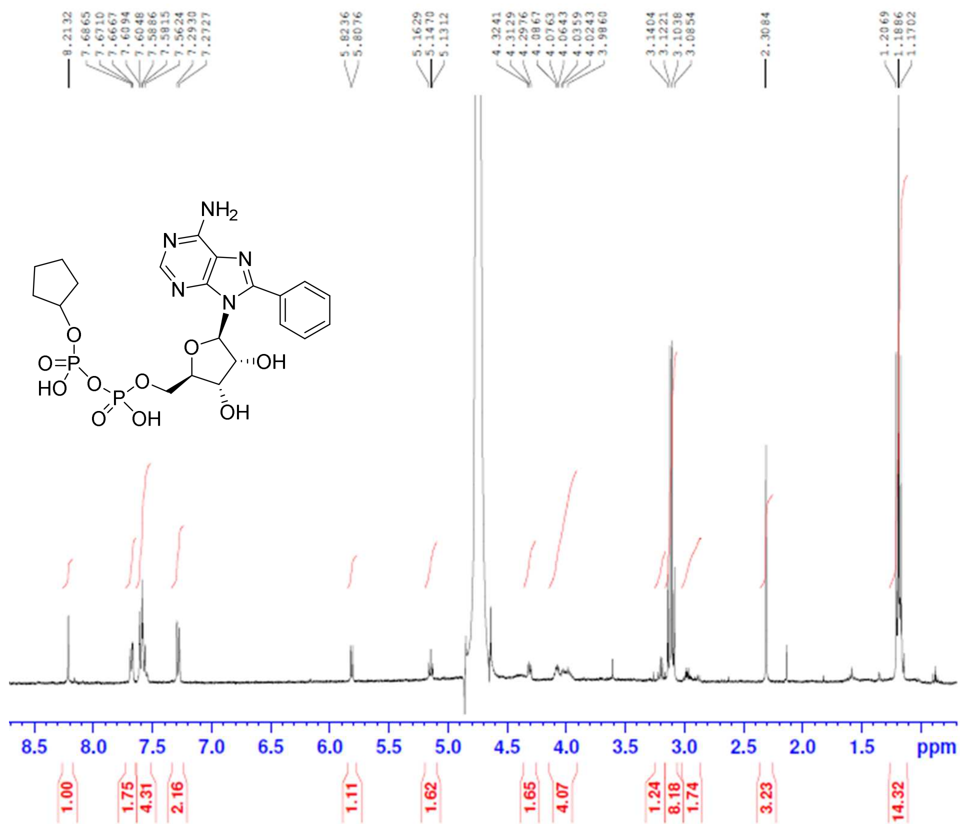
2′,3′-*O*-isopropylidene 8-bromoadenosine 81. To a solution of 2′,3′-*O*-isopropylidene adenosine **58** (5 g, 16.25 mmol) in NaOAc buffer (1 M, pH 4, 800 mL), Br_2 (1.25 mL, 24.5 mmol) was added under vigorous stirring at rt. After 24h, excess bromine was reduced with 4 N $NaHSO_3$ and the solution stirred until decolourised. The product was extracted with DCM, the organic layer dried (Na_2SO_4), filtered and evaporated under reduced pressure. The crude product was purified on an Isco chromatographic system (DCM-MeOH, 9:1 v/v) to yield the title nucleoside as a pale yellow solid (5.6 g, 89 %) which showed ^{1}H (400 MHz, d_6-DMSO) δ 8.12 (s, 1H, H-2), 7.49 (s, 2H, NH2), 6.0 (d, 1H, $J_{1',2'}$ = 2.7, H-1′), 5.72 (s, 1H, 5′-OH), 5.64 (dd, 1H, $J_{2',3'}$ = 6.2 and $J_{2',1'}$ = 2.7, H-2′), 5.01 (dd, 1H, $J_{3',2'}$ = 6.2 and $J_{3',4'}$ = 3.0, H-3′), 4.14 (dd, 1H, $J_{4',5}$′ = 5.8 and $J_{4',3}$′ = 3.0, H-4′), 3.50 (dd, 1H, $J_{5'a,5'b}$ = 11.5 and $J_{5'a,4}$′ = 6.0, H-5′a), 3.41 (dd, 1H, $J_{5'b,5'a}$ = 11.5 and $J_{5'b,4'}$ = 5.8, H-5′b), 1.53 (s, 3H, CH_3) and 1.31 (s, 3H, CH_3); ^{13}C (100 MHz, d_6-DMSO) δ 155.0 (C-6), 152.8 (C-2), 149.7 (C-4), 126.3 (C-8), 119.3 (C-5), 113.2 (C), 90.9 (C-1′), 87.1 (C-4′), 81.9 (C-2′), 81.6 (C-3′), 61.4 (C-5′), 27.1 and 25.2 (2 × CH_3); HRMS (ES$^+$) calcd for $C_{13}H_{17}N_5O_4{}^{79}Br$ 386.0458 (MH)$^+$ found 386.0474 and HRMS (ES$_+$) calcd for $C_{13}H_{17}N_5O_4{}^{81}Br$ 388.0438 (MH)$^+$ found 388.0453.

^{1}H-NMR

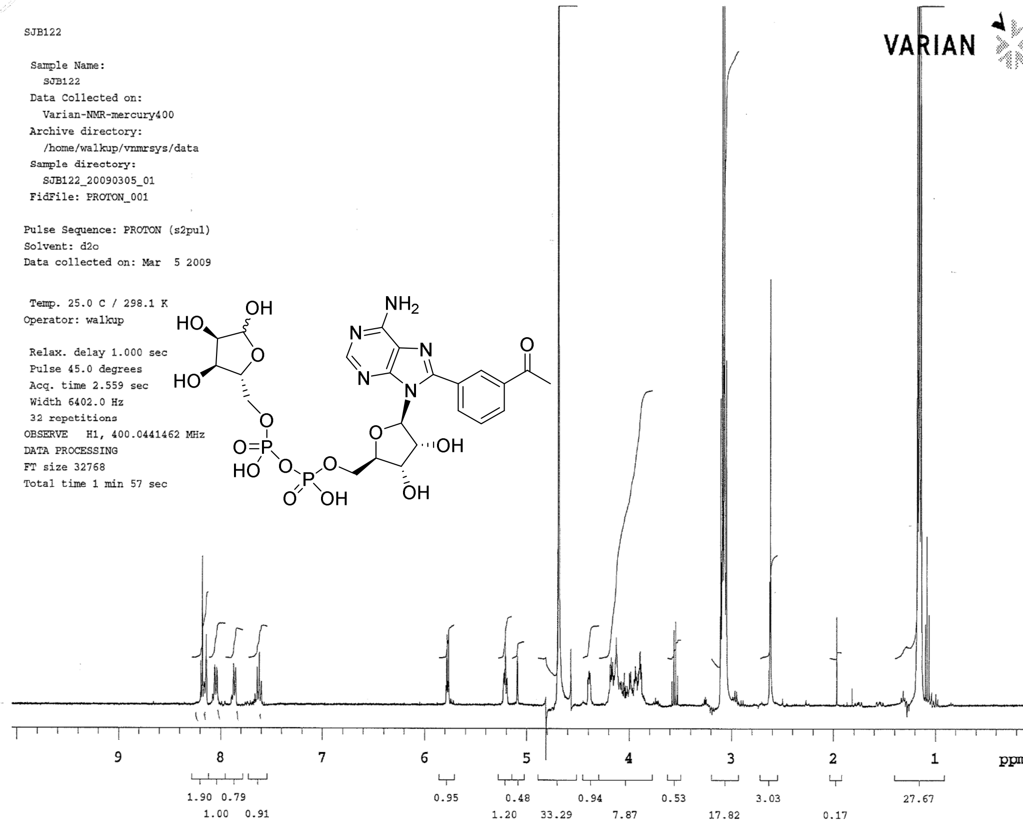
8-Phenyl-2′-deoxy-ADPR (86)

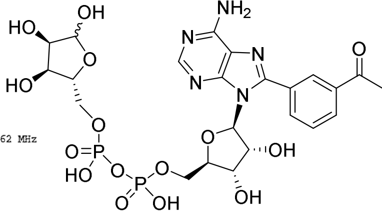


Cyclopentyl-8-phenyl-ADPR (84)

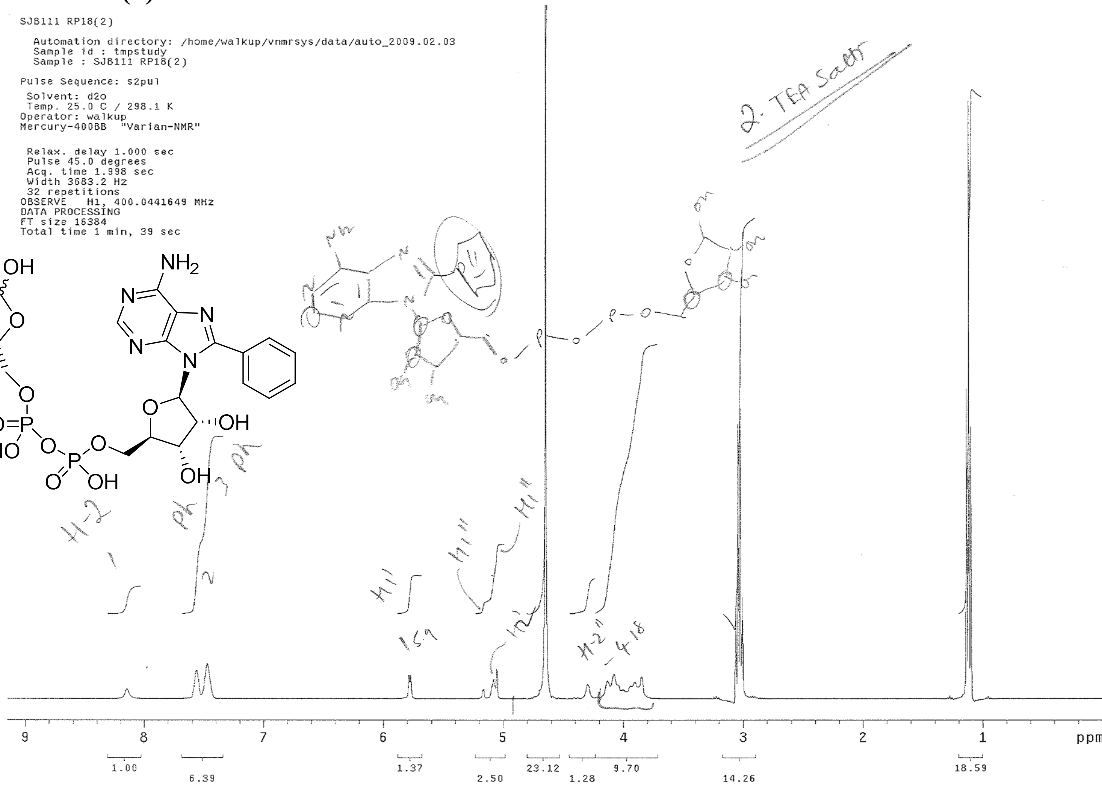


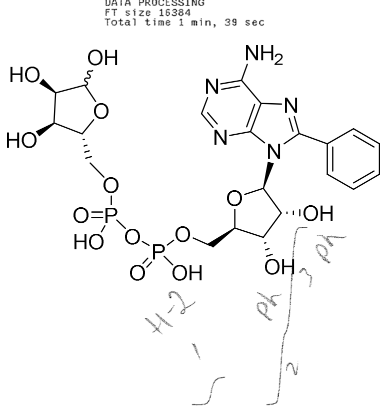
8-(3-Ac-Ph)-ADPR (7)



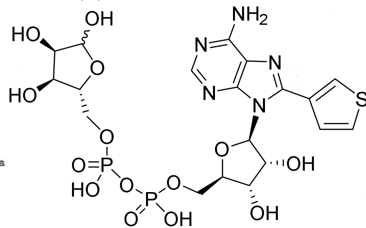


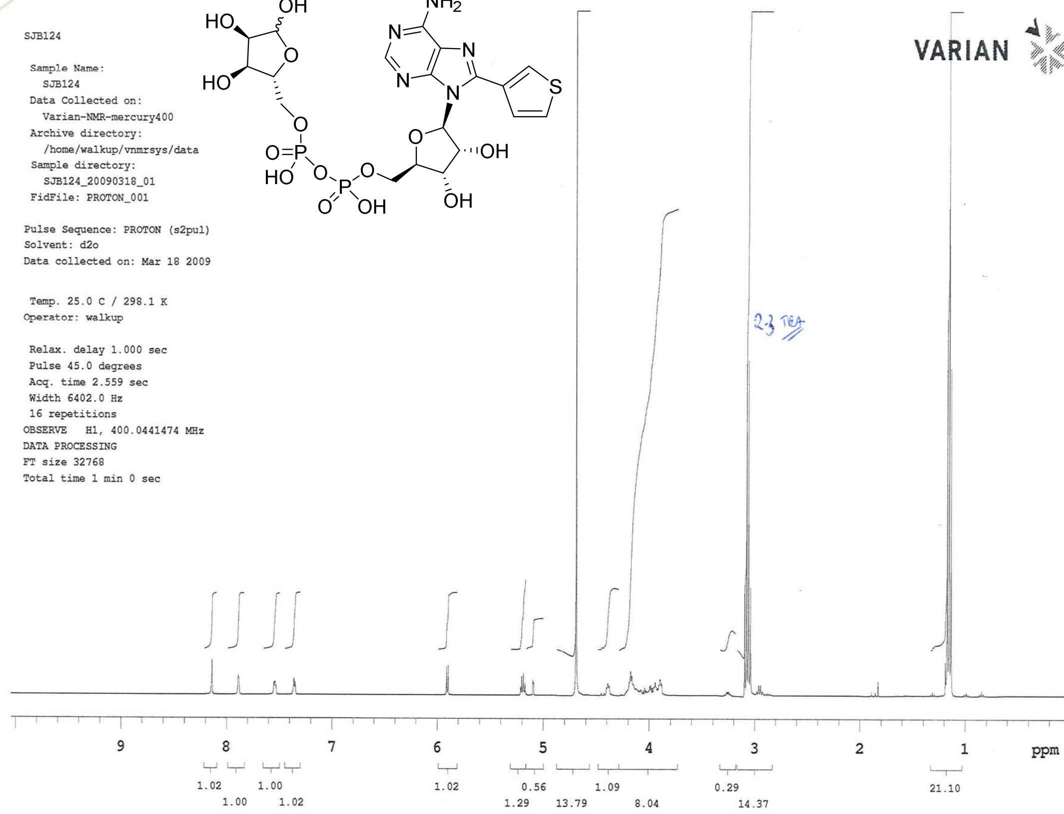
8-Ph-ADPR (5)

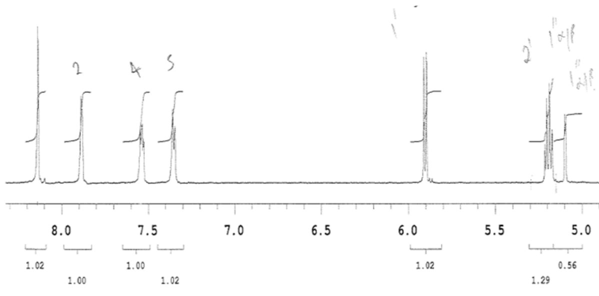




8-Thiophenyl-ADPR (6)







Pharmacology

Measuring $[Ca^{2+}]_i$: Fluorimetric measurement of $[Ca^{2+}]_i$ in HEK293 clones was performed as described previously.[11] In brief, 2.4×10^7 cells were centrifuged (5 min, 500 g, rt) and rinsed once with extracellular buffer (140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM glucose, 0.1% BSA, 15 mM HEPES, pH 7.4) and resuspended in 1 ml of extracellular buffer. Then Fura2/AM (4 µM final concentration) was added, and the cells were incubated for 30 min at 37°C in the dark. Fura2-loaded cells were centrifuged, rinsed again with extracellular buffer and resuspended in extracellular buffer with a cell density of 2×10^6 cells/ml. The cell suspension was divided in 2 ml aliquots and stored in the dark at room temperature until use. Analysis of $[Ca^{2+}]_i$ was carried out by the use of a Hitachi F-2000 spectrofluorometer operating in ratio mode (excitation occurred alternating at 340 and 380 nm every 5s). The emission was recorded at 510 nm. The calculation of $[Ca^{2+}]_i$ was performed as described by Grynkiewicz et al.[12] Measurements were carried out with 2 ml of cell suspension in a quartz cuvette during continuous stirring at room temperature. Cells were stimulated by addition of 300 µM H_2O_2 200s after start of the recording. To calibrate measurements, Triton X-100 (0.1% (v/v)) and EGTA/Tris (8 mM/60 mM) were added successively at the end of each experiment.

Western Blot analysis: TRPM2 expression on the protein level was tested by Western blot of whole cell lysates of wild type HEK293 cells and clonal cell lines derived from transfected HEK293 cells. Proteins from whole cell lysates were separated by SDS-PAGE (7.5%, 5 µg protein per lane) and transferred to PVDF membrane (by tank blotting). Afterwards the membrane was blocked by incubation in 5 % milk powder (w/v) in Tris-buffered saline supplemented with 0.1% Tween 20, pH 7.5 (TBST) overnight at 4°C. TRPM2 was detected by incubation with a primary antibody against human TRPM2 from rabbit (0.02 µg/ml in TBST/2.5% milk powder) for 3.5 h at room temperature and a secondary goat anti-rabbit antibody conjugated with horseradish peroxidase (1:10000 in TBST) for 1 h at room temperature. For detection of the HRP-conjugated secondary antibody the enhanced chemiluminescence system (ECL, GE Life Sciences) was used. To control for non-specific binding, the primary anti-TRPM2 antibody was preincubated with the respective blocking peptide (25-fold excess) at 4°C overnight before addition to the membrane.

Ratiometric Ca^{2+} imaging of human neutrophils: Ratiometric Ca^{2+} imaging was performed as described previously[13] at 40-times magnification with alternating illumination at 340 and 380 nm. Greyscale images with a spatial resolution of 672×510 pixels were recorded at an acquisition rate of 15 ratios per min for 15 min. Exposure times were set at a ratio of 2:1 for the images taken at 340 nm and 380 nm, respectively. Data processing with Openlab Software 4.0.4 involved constructing ratio images (340/380 nm) pixel by pixel, applying a 3×3 pixel median filter and measuring the average ratio value of whole cells. Only cells that did not interact with other cells, did not show elevated ratio prior to stimulation, or stayed within the field of view throughout the measurement were taken into account for data analyses. $[Ca^{2+}]_i$ was calibrated in a separate experiment by subsequent addition of 5 µM ionomycin and 8 mM EGTA (Tris buffered) and calculated using the following equation: $[Ca^{2+}] = K_d\times(R - R_{min})/(R_{max} - R)\times C2/B2$ where R_{min} is the ratio in the absence of Ca^{2+}, R_{max} is the ratio of the Ca^{2+}-saturated indicator, B2 is the minimal fluorescence intensity, C2 is the maximal fluorescence intensity, R is the ratio during the measurement, and K_d is the dissociation constant of Fura-2 (224 nM).

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