

**Bis-Halogen-Anthraniloyl-Substituted Nucleoside 5'-
triphosphates as Potent and Selective Inhibitors of
Bordetella pertussis CyaA**

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Supplemental Information 1

General protocol of mono- and bis-(M)ANT-nucleotide synthesis. (M)ANT-nucleotides were synthesized as described with modifications (). In general, mono- and bis-substituted (M)ANT-nucleotides could be achieved both in an one pot synthesis procedure. The nucleotide (0.33 mmol, 1 eq) was propounded in a small two-neck round flask and dissolved in a minimum amount of water (3 ml). Under continuous stirring a crystalline preparation of the appropriate isatoic anhydride derivative (0.5 mmol, 1.5 eq) was added. After heating to 38 °C the pH-value was adjusted to 8.6 and maintained by titration of 1 N NaOH solution for 2 hours. The reaction mixture was extracted three times by 20 ml chloroform (only for MANT-nucleotides). The aqueous phase was dry-frozen. The received foam showed white to brown color. The crude reaction mixture was purified by preparative reversed phase high pressure liquid chromatography. Especially for the sensitive separation of mono- and Bis-(M)ANT-NTPs from mono- and Bis-(M)ANT-NDPs this purification strategy was required. In case of monophosphate derivatives only size-exclusion chromatography with a long Sephadex® LH-20 column (85 x 2 cm) and subsequently elution with double-distilled water was applied. The desired product could be detected directly by its blue fluorescence in the collection tubes at λ_{ex} of 366 nm and by TLC. After final dry-freezing white to brown solid compounds (purity > 98 %) were

obtained. For all derivatives yields were determined by analytical HPLC measurements of crude reaction mixtures and correlate with the maximal accessible yield. Because of the time consuming and costly preparative HPLC purification separation was stopped after obtaining approximate 5 mg pure compound.

Synthesis of isatoic anhydride precursors. For the propyl (**8**, **9**, **24**, **25**) and acetylated amino (**10**, **11**, **26**, **27**) ANT-nucleotide derivatives the corresponding isatoic anhydrides (**33**, **34**) were easily accessible by nucleophilic substitution with propyl iodide and for acetylation with acetic acid anhydride.

HPLC analysis of (M)ANT-nucleotides. The samples were filtered using a PTFE filter (Chromafil, O-20/15, organic, pore size 0.2 μm ; Machery-Nagel, Düren, Germany). A 10 μL sample was analyzed using a HPLC model 1100 (Agilent Technologies, Waldbronn, Germany) fitted with a C18 analytical column (Phenomenex Luna, particle size 3 μm , 150 x 4.60 mm, Aschaffenburg, Germany) and DAD. Data were analyzed using a HPLC-3D ChemStation Rev. A.10.01 [1635]. Gradient elution was performed with 0.05 M ammonium acetate (solvent A) and acetonitrile (solvent B) at a constant flow rate of 1.0 ml/min. A gradient profile with the following proportions of solvent B was applied [t (min), % B]: [0, 5], [10, 5], [30, 45], [40, 80]. The chromatograms were monitored at 220 nm and 254 nm. In addition, a fluorescence detector was used for the analysis of the fluorescent anthraniloylic compounds at λ_{ex} of 350 nm and λ_{em} of 450 nm.

LC/MS online coupling. All samples were filtered using a PTFE filter and injected into a HPLC model 1100 (Hewlett-Packard, Waldbronn, Germany). The compound to be analyzed was separated by a C18 column (Phenomenex luna, particle size 3 μm , 150 x 2 mm, Aschaffenburg, Germany). A binary eluent mixture consisting of water (10 mM ammonium acetate) (eluent A) and acetonitrile (eluent B) was pumped with a constant flow of 0.3 ml/min. The following gradient profile was used t [min], % B: [0, 5], [10, 5], [30, 45], [40, 80]. The injected volume was 3 μl . The mass of the respective compound was determined using a triple stage mass spectrometer (Finnigan TSQ 7000; Thermo Fisher Scientific, Waltham, MA).

Preparative HPLC. Compound mixtures were dissolved in water (concentration: 30 - 50 mg/ml) and filtered using a PTFE filter. Compounds were separated using a HPLC model 1100 (Agilent Technologies, Waldbronn, Germany) fitted with a C18

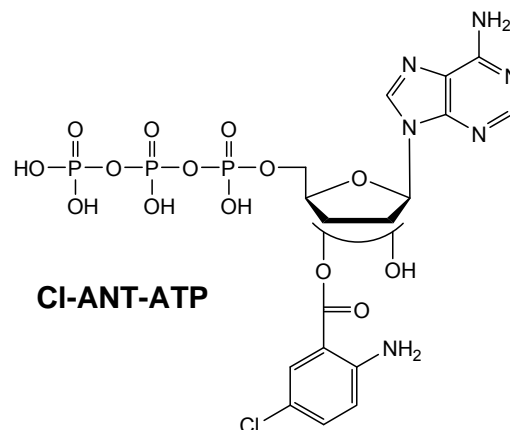
preparative column (Phenomenex Luna, particle size 10 μm , 250 x 21.2 mm). Gradient elution was performed with 0.05 M ammonium acetate (solvent A) and acetonitrile (solvent B) at a constant flow rate of 21 ml/min. Due to the efficiency of separation injection volumes differed from 10 μl to 60 μl for a run. The chromatograms were monitored at 220 nm and 254 nm.

NMR spectrometry. Bruker Avance 400 (^1H : 400.1 MHz, ^{13}C : 100.6 MHz, ^{31}P -NMR: 161.9 MHz, T = 300 K), Bruker Avance 300 (^1H : 300.1 MHz, ^{13}C : 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants are given in Hertz [Hz]. Characterization of the signals: s = singlet, d = doublet, t = triplet, m = multiplet, dd = double doublet, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in ^{13}C -spectra was determined with DEPT-technique (pulse angle: 135 $^\circ$) and given as (+) for CH or CH_3 , (-) for CH_2 and (C_{quat}) for quaternary C. Error of reported values: chemical shift: 0.01 ppm for ^1H -NMR, 0.1 ppm for ^{13}C -NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Spectroscopy. Absorption spectroscopy was performed using a Varian Cary BIO 50 UV/VIS/NIR spectrometer with a 1 cm quartz cuvette (Hellma) and Uvasol solvents (Merck or Baker). IR spectra were recorded by a Bio-Rad FTS 2000 MX FT-IR. Further mass spectrometry measurements with electron ionization technique were applied by a Varian CH-5.

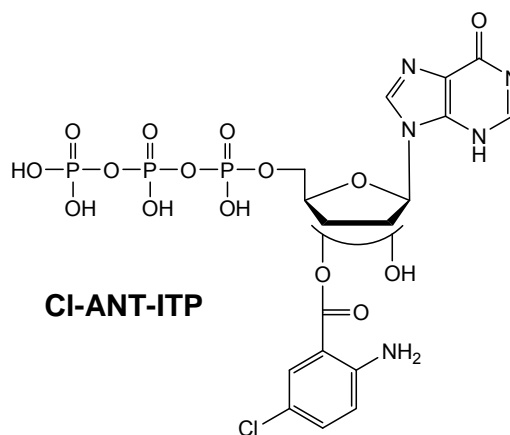
Miscellaneous. Melting Points were determined by a Tottoli micro melting point apparatus and are uncorrected. TLC analyses were conducted on silica gel 60 F-254 with a 0.2 mm layer thickness.

Newly synthesized compounds



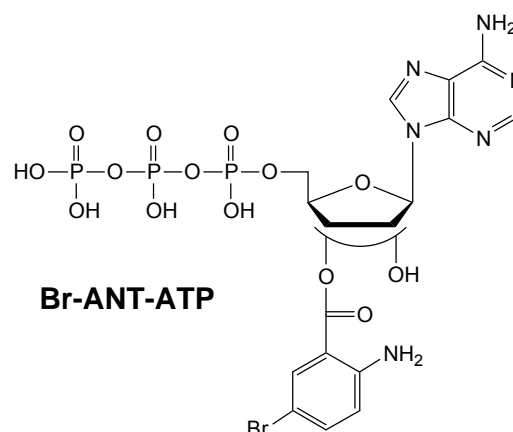
CI-ANT-ATP (2'(3')-O-5-chloroanthraniloyl-adenosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-4(3)-hydroxy-2-[[hydroxy-(hydroxyl-phosphonoxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3(4)-yl]2-amino-5-chlorobenzoate (**4**).

For the procedure see general prescription. 100 mg (0.18 mmol) introduced disodium salt of ATP yielded 61 mg (92 μ mol, 51 %) pure product after purification. R_f = 0.26 (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): R_t = 21.35 min; k = 12.91; LC/MS (ESI, H₂O/CH₃CN): m/z = 678.0 [M+NH₄⁺] (R_t = 18.89 min, 100 %), 660.8 [M+H⁺] (R_t = 18.89 min, 10 %); (-ESI, H₂O/CH₃CN): m/z = 659.1 [M-H] (R_t = 18.89 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 14], [6, 14], [11, 37], [15, 40], [20, 80]): R_t = 10.15 min; UV/Vis (H₂O) λ_{max} (log ϵ) = 255 nm (16,000), 343 nm (3,500); empirical formula: C₁₇H₂₀ClN₆O₁₄P₃; MW = 660.75



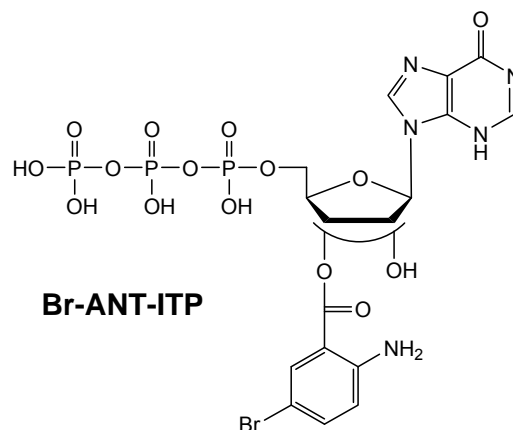
CI-ANT-ITP (2'(3')-O-5-chloroanthraniloyl-inosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-oxo-1H-purin-9-yl)-4(3)-hydroxy-2-[[hydroxy-(hydroxyl-phosphonoxy-phosphoryl)oxyphosphoryl]oxymethyl]oxolan-3(4)-yl]2-amino-5-chlorobenzoate (**5**).

For the procedure see general prescription. 100 mg (0.17 mmol) introduced disodium salt of ITP yielded 47 mg (71 μ mol, 42 %) pure product after purification. $R_f = 0.27$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 20.59$ min, 20.84 min; $k = 13.24$, 13.39; LC/MS (ESI, H₂O/CH₃CN): $m/z = 696.0$ [M+NH₃NH₄⁺] ($R_t = 16.77$ min, 100 %), 679.0 [M+NH₄⁺] ($R_t = 16.77$ min, 90 %), 662.1 [M+H⁺] ($R_t = 16.77$ min, 5 %); (-ESI, H₂O/CH₃CN): $m/z = 660.1$ [M-H⁻] ($R_t = 16.77$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 14], [6, 14], [15, 38], [20, 80]): $R_t = 7.71$ min, 8.44 min; UV/Vis (H₂O) λ_{max} (log ϵ) = 255 nm (14,600), 348 nm (4,500); empirical formula: C₁₇H₁₉ClN₅O₁₅P₃; MW = 661.73



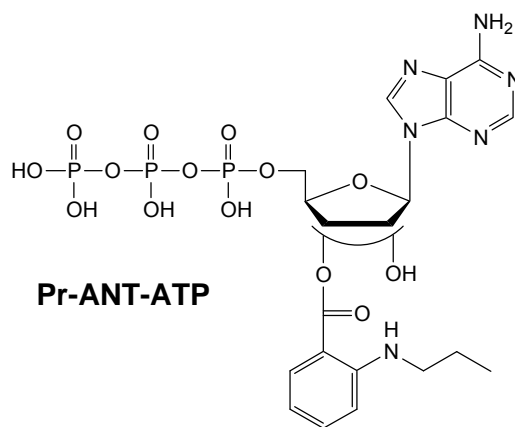
Br-ANT-ATP (2'(3')-O-5-bromoanthraniloyl-adenosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-4(3)-hydroxy-2-[[hydroxy-(hydroxyl-phosphonoxy-phosphoryl)oxyphosphoryl]oxymethyl]oxolan-3(4)-yl]2-amino-5-bromobenzoate (**6**).

For the procedure see general prescription. 100 mg (0.18 mmol) introduced disodium salt of ATP led to 36 mg (50 μ mol, 28 %) pure product after purification. $R_f = 0.24$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 20.24$ min, 20.39 min; $k = 12.18$, 12.28; LC/MS (ESI, H₂O/CH₃CN): $m/z = 724.1$ [M+NH₄⁺] ($R_t = 21.21$ min, 100 %), 741.2 [M+NH₃+NH₄⁺] ($R_t = 21.21$ min, 40 %); (-ESI, H₂O/CH₃CN): $m/z = 705.1$ [M-H⁻] ($R_t = 21.21$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 17], [8, 17], [10, 25], [15, 38], [20, 80]): $R_t = 6.32$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 256 nm (16,400), 332 nm (3,300); empirical formula: C₁₇H₂₀BrN₆O₁₄P₃; MW = 705.20



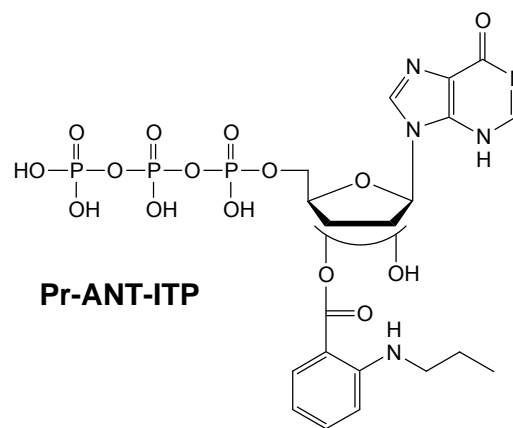
Br-ANT-ITP (2'(3')-O-5-bromoanthraniloyl-inosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-oxo-1H-purin-9-yl)-4(3)-hydroxy-2-[[hydroxy-(hydroxyl-phosphonoxy-phosphoryl)oxyphosphoryl]oxymethyl]oxolan-3(4)-yl]2-amino-5-bromobenzoate (**7**).

For the procedure see general prescription. 100 mg (0.17 mmol) introduced disodium salt of ITP yielded 48 mg (68 μ mol, 40 %) pure product after purification. R_f = 0.25 (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): R_t = 21.16 min, 21.45 min; k = 13.63, 13.83; LC/MS (ESI, H₂O/CH₃CN): m/z = 725.1 [M+NH₄⁺] (R_t = 18.81 min, 100 %), 742.2 [M+NH₃+NH₄⁺] (R_t = 18.81 min, 35 %); (-ESI, H₂O/CH₃CN): m/z = 706.1 [M-H⁻] (R_t = 18.81 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 18], [15, 38], [20, 80]): R_t = 4.27 min, 4.53 min; UV/Vis (H₂O) λ_{max} (log ϵ) = 248 nm (15,000), 328 nm (4,300); empirical formula: C₁₇H₁₉BrN₅O₁₅P₃; MW = 706.18



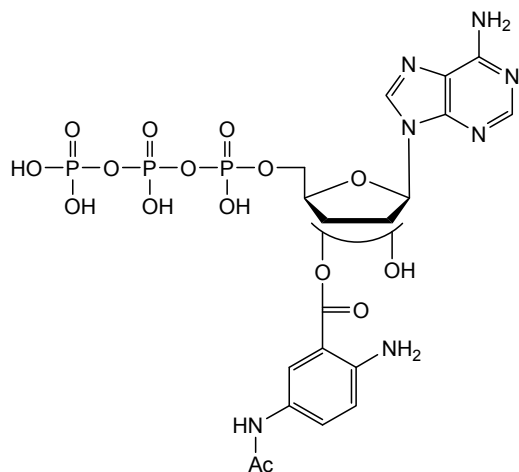
Pr-ANT-ATP (*N*-propyl-2'(3')-O-anthraniloyl-adenosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-4(3)-hydroxy-2-[[hydroxy-(hydroxy-phosphonoxy-phosphoryl)oxyphosphoryl]oxymethyl]oxolan-3(4)-yl]2-propylaminobenzoate (**8**).

For the procedure see general prescription. 100 mg (0.18 mmol) introduced disodium salt of ATP yielded 29 mg (43 μ mol, 24 %) pure product after purification. $R_f = 0.31$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 24.93$ min, 25.67 min; $k = 15.29$, 15.78; LC/MS (ESI, H₂O/CH₃CN): $m/z = 669.0$ [M+H⁺] ($R_t = 23.12$ min, 100 %), 686.0 [M+NH₄⁺] ($R_t = 23.12$ min, 15 %); (-ESI, H₂O/CH₃CN): $m/z = 667.0$ [M-H⁻] ($R_t = 23.12$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 5], [20, 45], [25, 80]): $R_t = 13.33$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 257 nm (17,500), 359 nm (4,600); empirical formula: C₂₀H₂₇N₆O₁₄P₃; MW = 668.38



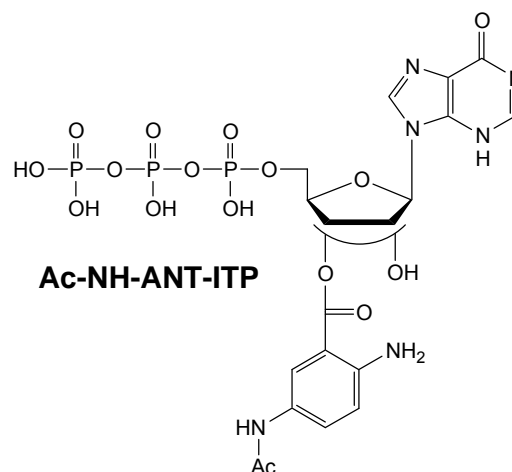
Pr-ANT-ITP (*N*-propyl-2'(3')-*O*-anthraniloyl-inosine-5'-triphosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(6-oxo-1*H*-purin-9-yl)-4(3)-hydroxy-2-[[hydroxy-(hydroxy-phosphonoxy)-phosphoryl]oxyphosphoryl]oxymethyl]oxolan-3(4)-yl]2-propylaminobenzoate (**9**).

For the procedure see general prescription. 100 mg (0.17 mmol) introduced disodium salt of ITP yielded 25 mg (37 μ mol, 22 %) pure product after purification. $R_f = 0.32$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 24.73$ min, 25.16 min; $k = 16.10$, 16.40; LC/MS (ESI, H₂O/CH₃CN): $m/z = 687.0$ [M+NH₄⁺] ($R_t = 22.87$ min, 100 %), 669.8 [M+H⁺] ($R_t = 22.87$ min, 20 %); (-ESI, H₂O/CH₃CN): $m/z = 668.0$ [M-H⁻] ($R_t = 22.87$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 5], [20, 45], [25, 80]): $R_t = 13.12$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 257 nm (17,500), 359 nm (4,600); empirical formula: C₂₀H₂₆N₅O₁₅P₃; MW = 669.37

**Ac-NH-ANT-ATP**

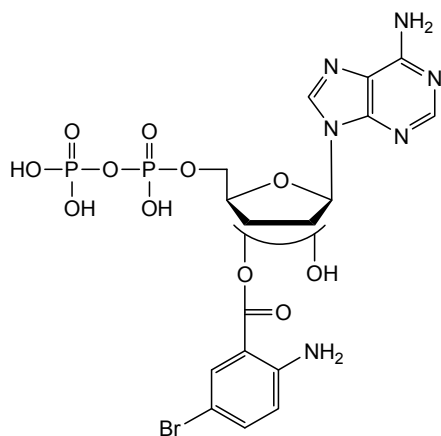
Ac-NH-ANT-ATP (2'(3')-O-5-acetylaminoanthraniloyl-adenosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-4(3)-hydroxy-2-[[hydroxy-(hydroxyl-phosphono-oxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3(4)-yl]2-amino-5-acetylamino-benzoate (**10**).

For the procedure see general prescription. 100 mg (0.18 mmol) introduced disodium salt of ATP yielded 57 mg (84 μ mol, 46 %) pure product after purification. R_f = 0.21 (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): R_t = 8.39 min, 8.75 min; k = 4.48, 4.72; LC/MS (ESI, H₂O/CH₃CN): m/z = 683.9 [M+H⁺] (R_t = 3.34 min, 100 %), 701.0 [M+NH₄⁺] (R_t = 3.34 min, 70 %); (-ESI, H₂O/CH₃CN): m/z = 682.0 [M-H⁻] (R_t = 3.34 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 6], [5, 7], [23, 14], [24, 80], [29, 80]): R_t = 12.06 min; UV/Vis (H₂O) λ_{max} (log ϵ) = 259 nm (17,200), 349 nm (3,100); empirical formula: C₁₉H₂₄N₇O₁₅P₃; MW = 683.35



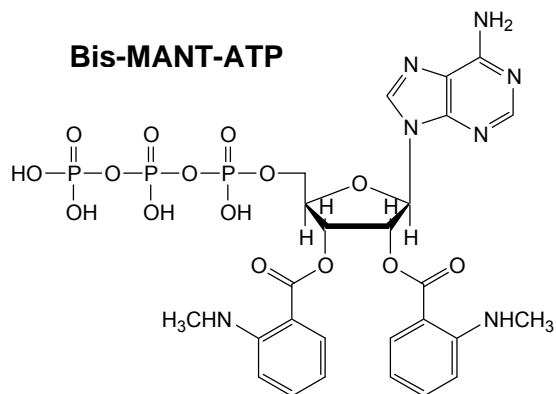
Ac-NH-ANT-ITP (2'(3')-O-5-acetylaminanthraniloyl-inosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-oxo-1H-purin-9-yl)-4(3)-hydroxy-2-[[hydroxy-(hydroxy-phosphonooxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3(4)-yl]2-amino-5-acetylaminobenzoate (**11**).

For the procedure see general prescription. 100 mg (0.17 mmol) introduced disodium salt of ITP yielded 60 mg (88 μ mol, 52 %) pure product after purification. R_f = 0.22 (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): R_t = 7.63 min, 7.99 min; k = 4.28, 4.53; LC/MS (ESI, H₂O/CH₃CN): m/z = 702.0 [M+NH₄⁺] (R_t = 1.91 min, 100 %), 719.0 [M+NH₃+NH₄⁺] (R_t = 1.91 min, 40 %), 684.9 [M+H⁺] (R_t = 1.91 min, 25 %); (-ESI, H₂O/CH₃CN): m/z = 683.0 [M-H⁻] (R_t = 1.91 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 5.8], [11, 5.8], [12, 10], [18, 13], [20, 80]): R_t = 9.50 min; UV/Vis (H₂O) λ_{max} (log ϵ) = 248 nm (11,400; shoulder), 345 nm (2,500); empirical formula: C₁₉H₂₃N₆O₁₆P₃; MW = 684.34



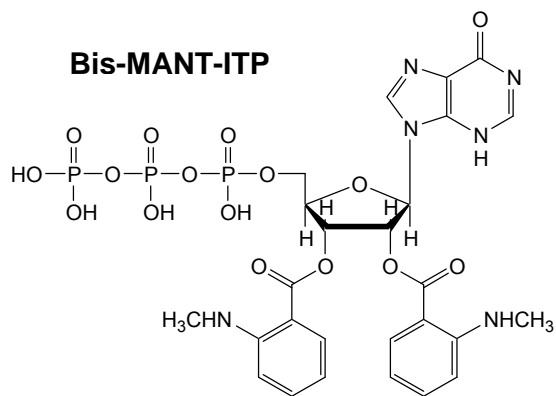
Br-ANT-ADP (2'(3')-O-5-bromoanthraniloyl-adenosine-5'-diphosphate) or [(2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-4(3)-hydroxy-2-[(hydroxy-phosphonooxyphosphoryl)oxymethyl]oxolan-3(4)-yl]2-amino-5-bromobenzoate (**14**).

For the procedure see general prescription. 100 mg (0.18 mmol) introduced disodium salt of ATP yielded 9 mg (14 μmol , 8 %) pure product after purification. $R_f = 0.27$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 20.86$ min, 21.16 min; $k = 12.59$, 12.79; LC/MS (ESI, H₂O/CH₃CN): $m/z = 644.1$ [M+NH₄⁺] ($R_t = 22.09$ min, 100 %), 627.2 [M+H⁺] ($R_t = 22.09$ min, 80 %); (-ESI, H₂O/CH₃CN): $m/z = 625.1$ [M-H⁻] ($R_t = 22.09$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 16], [10, 16], [15, 50], [17, 51.5], [20, 80]): $R_t = 9.94$ min, 10.99 min; UV/Vis (H₂O) λ_{max} (log ϵ) = 255 nm (12,700), 328 nm (3,400); empirical formula: C₁₇H₁₉BrN₆O₁₁P₂; MW = 625.25



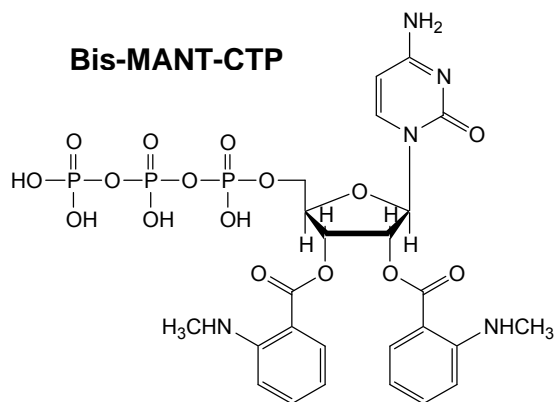
Bis-MANT-ATP (*N*-methyl-2',3'-bis-*O*-anthraniloyl-adenosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-2-[[hydroxy-(hydroxy-phosphonooxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-methylaminobenzoate (**17**).

For the procedure see general prescription. 100 mg (0.18 mmol) introduced disodium salt of ATP led over all purification steps to 21 mg (27 μmol , 15 %) pure product. $R_f = 0.32$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 26.95$ min; $k = 16.61$; LC/MS (ESI, H₂O/CH₃CN): $m/z = 791.2$ [M+NH₄⁺] ($R_t = 28.58$ min, 100 %), 774.2 [M+H⁺] ($R_t = 28.58$ min, 40 %); (-ESI, H₂O/CH₃CN): $m/z = 772.3$ [M-H⁻] ($R_t = 28.58$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 11], [2, 11], [5, 30], [15, 31.5], [18, 80]): $R_t = 9.91$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 255 nm (18,000), 359 nm (7,300); empirical formula: C₂₆H₃₀N₇O₁₅P₃; MW = 773.48



Bis-MANT-ITP (*N*-methyl-2',3'-bis-*O*-anthraniloyl-inosine-5'-triphosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(6-oxo-1*H*-purin-9-yl)-2-[[hydroxy-(hydroxy-phosphonoxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-methylaminobenzoate (**18**).

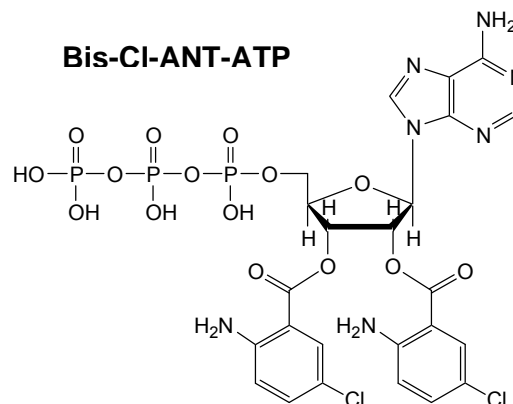
For the procedure see general prescription. 100 mg introduced disodium salt of ITP (0.17 mmol) yielded over all purification steps 18 mg (23 μ mol, 14 %) pure product. R_f = 0.33 (1-propanol:H₂O: NH₃ (32 %) = 2:1:1) HPLC (analytic): R_t = 26.74 min; k = 16.52; LC/MS (ESI, H₂O/CH₃CN): m/z = 792.3 [M+NH₄⁺] (R_t = 28.02 min, 100 %), 775.2 [M+H⁺] (R_t = 28.58 min, 15 %); (-ESI, H₂O/CH₃CN): m/z = 773.3 [M-H] (R_t = 28.08 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 11], [2, 11], [5, 30], [15, 31.5], [18, 80]): R_t = 9.72 min; UV/Vis (H₂O) λ_{max} (log ϵ) = 251 nm (16,400), 358 nm (6,400); empirical formula: C₂₆H₂₉N₆O₁₆P₃; MW = 774.46



Bis-MANT-CTP (*N*-methyl-2',3'-bis-*O*-anthraniloyl-cytosine-5'-triphosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(4-amino-2-oxopyrimidin-1-yl)-2-[[hydroxy-(hydroxy-phosphonoxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-methylaminobenzoate (**19**).

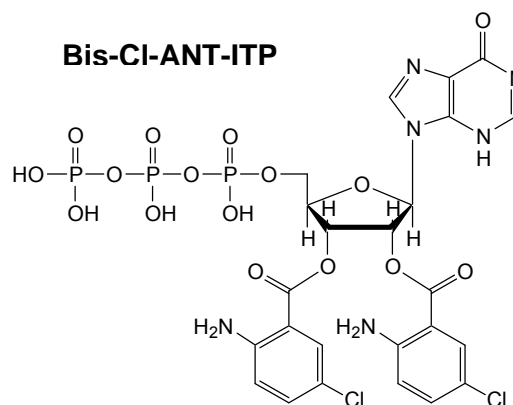
100 mg introduced trisodium salt of CTP (0.18 mmol) yielded 35 mg (46 μ mol, 26 %) pure product. R_f = 0.29 (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic):

$R_t = 26.83$ min; $k = 16.60$; LC/MS (ESI, H_2O/CH_3CN): $m/z = 750.0$ [$M+H^+$]
 ($R_t = 25.37$ min, 100 %), 767.1 [$M+NH_4^+$] ($R_t = 25.37$ min, 10 %); (-ESI, H_2O/CH_3CN):
 $m/z = 748.0$ [$M-H^-$] ($R_t = 25.37$ min, 100 %); HPLC (preparative), gradient (t [min], %
 B: [0, 14], [6, 14], [11, 35], [15, 40], [20, 80]): UV/Vis (H_2O) λ_{max} (log ϵ) = 253 nm
 (14,500), 359 nm (6,600); $R_t = 13.19$ min; empirical formula: $C_{25}H_{30}N_5O_{16}P_3$;
 MW = 749.45



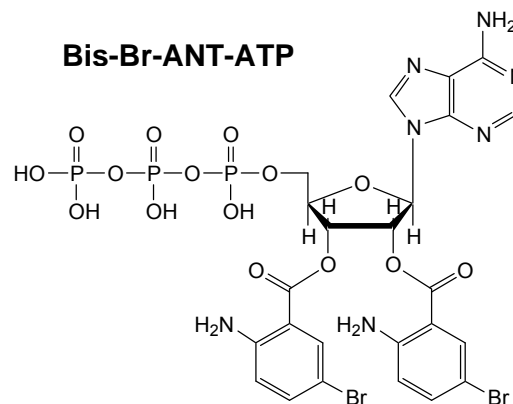
Bis-CI-ANT-ATP (2',3'-bis-*O*-5-chloroanthraniloyl-adenosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-2-[[hydroxy-(hydroxy-phosphonooxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-amino-5-chlorobenzoate (**20**).

For the procedure see general prescription. 100 mg (0.18 mmol) introduced disodium salt of ATP yielded 31 mg (38 μ mol, 21 %) pure product after purification. $R_f = 0.32$ (1-propanol: H_2O : NH_3 (32 %) = 2:1:1). HPLC (analytic): $R_t = 27.79$ min; $k = 17.10$;
 LC/MS (ESI, H_2O/CH_3CN): $m/z = 831.0$ [$M+NH_4^+$] ($R_t = 26.24$ min, 100 %), 814.1
 [$M+H^+$] ($R_t = 26.24$ min, 10 %); (-ESI, H_2O/CH_3CN): $m/z = 812.0$ [$M-H^-$] ($R_t =$
 26.24 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 14], [6, 14], [11,
 37], [15, 40], [20, 80]): $R_t = 13.26$ min; UV/Vis (H_2O) λ_{max} (log ϵ) = 255 nm (16,900),
 350 nm (5,100); empirical formula: $C_{24}H_{24}Cl_2N_7O_{15}P_3$; MW = 814.31



Bis-CI-ANT-ITP (2',3'-bis-*O*-5-chloroanthraniloyl-inosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-oxo-1H-purin-9-yl)-2-[[hydroxy-(hydroxy-phosphonooxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-methyl-5-chloroaminobenzoate (**21**).

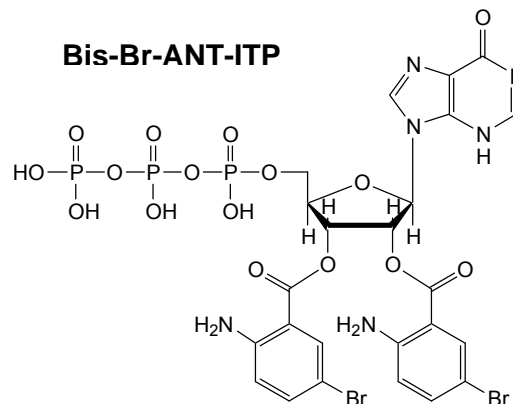
For the procedure see general prescription. 100 mg introduced disodium salt of ITP (0.17 mmol) yielded over all purification steps 30 mg (37 μ mol, 22 %) pure product. R_f = 0.35 (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): R_t = 27.46 min; k = 17.99; LC/MS (ESI, H₂O/CH₃CN): m/z = 849.1 [M+NH₄⁺] (R_t = 25.90 min, 100 %), 832.0 [M+H⁺] (R_t = 25.90 min, 90 %); (-ESI, H₂O/CH₃CN): m/z = 830.0 [M-H] (R_t = 25.90 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 14], [6, 14], [15, 38], [20, 80]): R_t = 15.65 min; UV/Vis (H₂O) λ_{max} (log ϵ) = 249 nm (19,700), 350 nm (6,200); empirical formula: C₂₄H₂₃Cl₂N₆O₁₆P₃; MW = 815.30



Bis-Br-ANT-ATP (2',3'-bis-*O*-5-bromoanthraniloyl-adenosine-5'-triphosphate) or (2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-2-[[hydroxy-(hydroxy-phosphonooxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-amino-5-bromobenzoate (**22**).

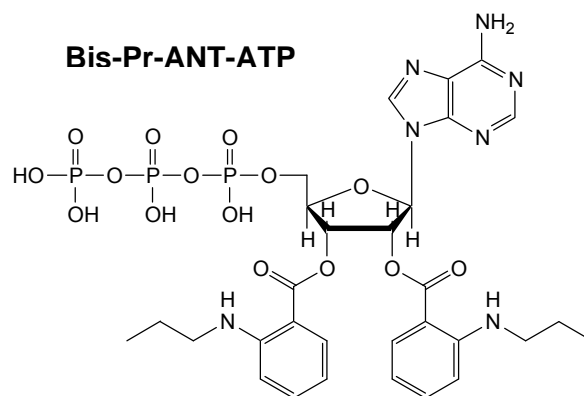
For the procedure see general prescription. 100 mg introduced disodium salt of ATP yielded 18 mg (20 μ mol, 11 %) pure product after purification. R_f = 0.31 (1-

propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): R_t = 26.72 min; k = 16.41; LC/MS (ESI, H₂O/CH₃CN): *m/z* = 920.9 [M+NH₄⁺] (R_t = 26.24 min, 100 %), 904.0 [M+H⁺] (R_t = 26.24 min, 10 %); (-ESI, H₂O/CH₃CN): *m/z* = 901.9 [M-H] (R_t = 26.24 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 17], [8, 17], [10, 25], [15, 38], [20, 80]): R_t = 16.14 min; UV/Vis (H₂O) λ_{max} (log ε) = 255 nm (14,600), 348 nm (4,500); empirical formula: C₂₄H₂₄Br₂N₇O₁₅P₃; MW = 903.21



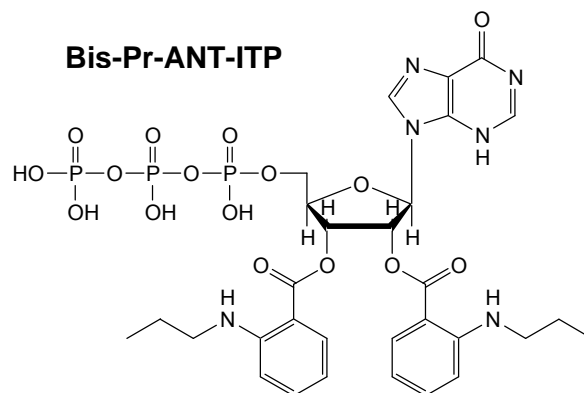
Bis-Br-ANT-ITP (2',3'-bis-O-5-bromoanthraniloyl-inosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-oxo-1H-purin-9-yl)-2-[[hydroxy-(hydroxy-phosphonooxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-methyl-5-bromoaminobenzoate (**23**).

For the procedure see general prescription. 100 mg introduced disodium salt of ITP (0.17 mmol) yielded over all purification steps 32 mg (36 μmol, 21 %) pure product. R_f = 0.33 (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): R_t = 28.16 min; k = 18.47; LC/MS (ESI, H₂O/CH₃CN): *m/z* = 921.9 [M+NH₄⁺] (R_t = 25.89 min, 100 %), 905.0 [M+H⁺] (R_t = 25.89 min, 15 %); (-ESI, H₂O/CH₃CN): *m/z* = 903.0 [M-H] (R_t = 25.89 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 18], [15, 38], [20, 80]): R_t = 12.39 min; UV/Vis (H₂O) λ_{max} (log ε) = 249 nm (19,000), 348 nm (5,600); empirical formula: C₂₄H₂₃Br₂N₆O₁₆P₃; MW = 904.20



Bis-Pr-ANT-ATP (*N*-propyl-2',3'-bis-*O*-anthraniloyl-adenosine-5'-triphosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-2-[[hydroxy-(hydroxy-phosphonoxy)phosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-propylaminobenzoate (**24**).

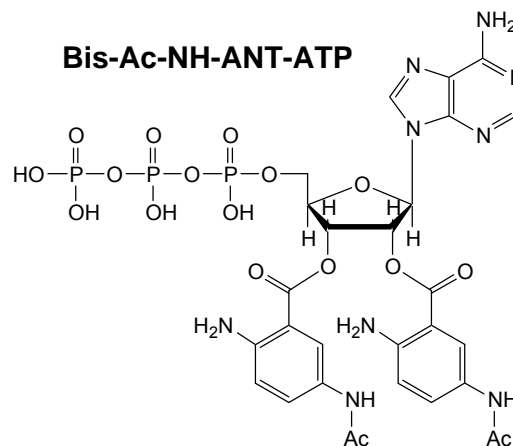
For the procedure see general prescription. 100 mg introduced disodium salt of ATP (0.18 mmol) yielded over all purification steps 21 mg (25 μ mol, 14 %) pure product. R_f = 0.34 (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): R_t = 33.04 min; k = 20.59; LC/MS (ESI, H₂O/CH₃CN): m/z = 830.2 [M+H⁺] (R_t = 30.63 min, 100 %), 847.2 [M+NH₄⁺] (R_t = 30.63 min, 30 %); (-ESI, H₂O/CH₃CN): m/z = 828.1 [M-H] (R_t = 30.63 min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 5], [20, 45], [25, 80]): R_t = 20.14 min; UV/Vis (H₂O) λ_{max} (log ϵ) = 255 nm (16,800), 359 nm (5,800); empirical formula: C₃₀H₃₇N₆O₁₆P₃; MW = 829.58



Bis-Pr-ANT-ITP (*N*-propyl-2',3'-bis-*O*-anthraniloyl-inosine-5'-triphosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(6-oxo-1H-purin-9-yl)-2-[[hydroxy-(hydroxy-phosphonoxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-propylaminobenzoate (**25**).

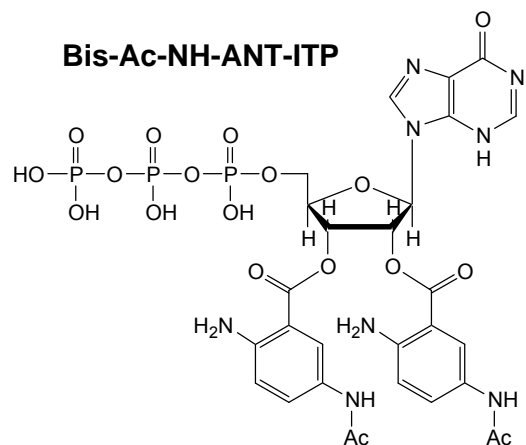
For the procedure see general prescription. 100 mg introduced disodium salt of ITP (0.17 mmol) yielded over all purification steps 15 mg (19 μ mol, 11 %) pure product.

$R_f = 0.35$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 30.57$ min; $k = 20.14$; LC/MS (ESI, H₂O/CH₃CN): $m/z = 848.1$ [M+NH₄⁺] ($R_t = 30.60$ min, 100 %), 831.1 [M+H⁺] ($R_t = 30.60$ min, 20 %); (-ESI, H₂O/CH₃CN): $m/z = 829.1$ [M-H] ($R_t = 30.60$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 5], [20, 45], [25, 80]): $R_t = 19.58$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 255 nm (14,600), 359 nm (5,300); empirical formula: C₃₀H₃₇N₆O₁₆P₃; MW = 830.57



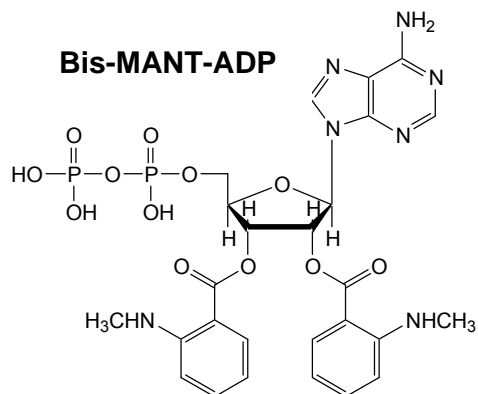
Bis-Ac-NH-ANT-ATP (2',3'-bis-O-5-acetylaminobenzoyl-adenosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-2-[[hydroxy-(hydroxyphosphonoxyphosphoryl)oxyphosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-amino-5-acetylaminobenzoate (**26**).

For the procedure see general prescription. 100 mg introduced disodium salt of ATP (0.18 mmol) yielded over all purification steps 27 mg (32 μ mol, 18 %) pure product. $R_f = 0.27$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 20.05$ min; $k = 12.10$; LC/MS (ESI, H₂O/CH₃CN): $m/z = 860.1$ [M+H⁺] ($R_t = 16.79$ min, 100 %), 877.2 [M+NH₄⁺] ($R_t = 16.79$ min, 30 %); (-ESI, H₂O/CH₃CN): $m/z = 858.1$ [M-H] ($R_t = 16.79$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 6], [5, 7], [23, 14], [24, 80], [29, 80]): $R_t = 21.49$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 261 nm (23,500), 350 nm (5,700); empirical formula: C₂₈H₃₂N₉O₁₇P₃; MW = 859.53



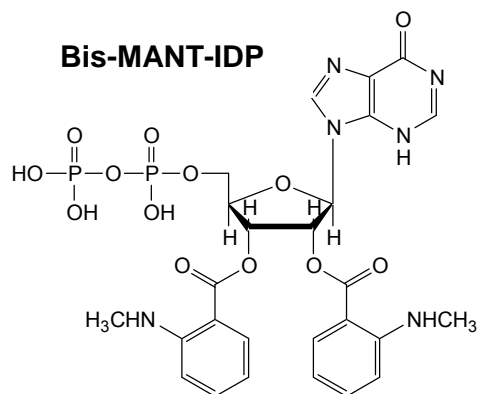
Bis-Ac-NH-ANT-ITP (2',3'-bis-O-5-acetylaminanthraniloyl-inosine-5'-triphosphate) or [(2R,3S,4R,5R)-5-(6-oxo-1H-purin-9-yl)-2-[[hydroxy-(hydroxy-phosphonoxy)-phosphoryl]oxymethyl]oxolan-3,4-bis-yl]2-amino-5-acetylaminobenzoate (**27**).

For the procedure see general prescription. 100 mg introduced trisodium salt of ITP (0.17 mmol) yielded over all purification steps 16 mg (19 μ mol, 11 %) pure product. $R_f = 0.29$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 18.80$ min; $k = 12.00$; LC/MS (ESI, H₂O/CH₃CN): $m/z = 878.1$ [M+NH₄⁺] ($R_t = 8.85$ min, 100 %), 895.1 [M+NH₃+NH₄⁺] ($R_t = 8.85$ min, 35 %), 861.1 [M+H⁺] ($R_t = 8.85$ min, 30 %); (-ESI, H₂O/CH₃CN): $m/z = 859.1$ [M-H⁻] ($R_t = 8.85$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 5.8], [11, 5.8], [12, 10], [18, 13], [20, 80]): $R_t = 18.43$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 259 nm (14,300; shoulder), 348 nm (5,200); empirical formula: C₂₈H₃₁N₈O₁₈P₃; MW = 860.51



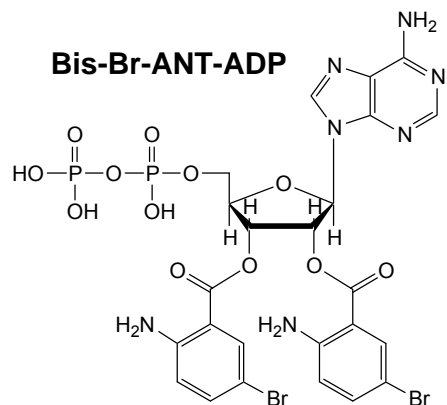
Bis-MANT-ADP (*N*-methyl-2',3'-bis-*O*-anthraniloyl-adenosine-5'-diphosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-2-[(hydroxy-phosphonoxyphosphoryl)oxymethyl]oxolan-3,4-bis-yl]2-methylaminobenzoate (**28**).

For the procedure see general prescription. 100 mg (0.18 mmol) introduced disodium salt of ATP yielded over all purification steps 7 mg (11 μ mol, 6 %) pure product. $R_f = 0.35$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 27.10$ min; $k = 16.78$; LC/MS (ESI, H₂O/CH₃CN): $m/z = 711.3$ [M+NH₄⁺] ($R_t = 28.85$ min, 100 %), 694.3 [M+H⁺] ($R_t = 28.85$ min, 90 %); (-ESI, H₂O/CH₃CN): $m/z = 692.2$ [M-H⁻] ($R_t = 28.83$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 11], [2, 11], [5, 30], [15, 31.5], [18, 80]): $R_t = 11.71$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 255 nm (16,000), 359 nm (6,200); empirical formula: C₂₆H₂₉N₇O₁₂P₂; MW = 693.50



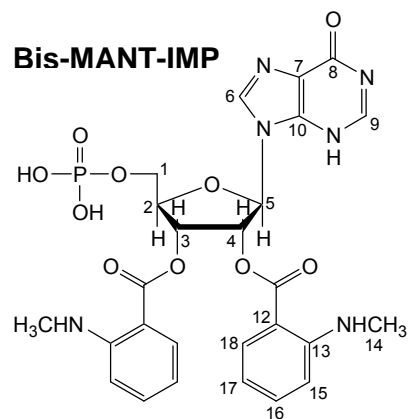
Bis-MANT-IDP (*N*-methyl-2',3'-bis-*O*-anthraniloyl-inosine-5'-diphosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(6-oxo-1*H*-purin-9-yl)-2-[(hydroxy-phosphonoxyphosphoryl)oxymethyl]oxolan-3,4-bis-yl]2-methylaminobenzoate (**29**).

For the procedure see general prescription. 100 mg (0.17 mmol) trisodium salt of ITP yielded 6 mg (9 μ mol, 5 %) pure product. $R_f = 0.36$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 27.48$ min; $k = 16.94$; LC/MS (ESI, H₂O/CH₃CN): $m/z = 712.3$ [M+NH₄⁺] ($R_t = 28.76$ min, 100 %), 695.4 [M+H⁺] ($R_t = 28.76$ min, 10 %); (-ESI, H₂O/CH₃CN): $m/z = 693.3$ [M-H⁻] ($R_t = 28.76$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 11], [2, 11], [5, 30], [15, 31.5], [18, 80]): $R_t = 11.24$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 252 nm (15,800), 359 nm (6,000); empirical formula: C₂₆H₂₈N₇O₁₃P₂; MW = 694.48



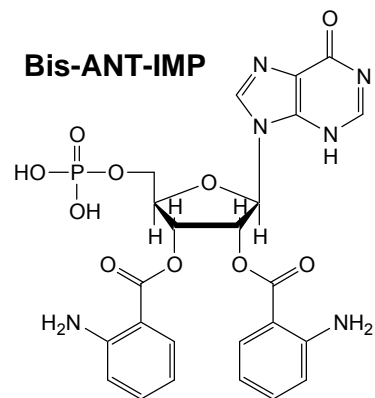
Bis-Br-ANT-ADP (2',3'-bis-*O*-5-bromoanthraniloyl-adenosine-5'-diphosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-2-[(hydroxy-phosphonooxyphosphoryl)oxymethyl]oxolan-3(4)-yl]2-amino-5-bromobenzoate (**30**).

For the procedure see general prescription. 100 mg (0.18 mmol) introduced disodium salt of ATP yielded 6 mg (7 μ mol, 4 %) pure product after purification. $R_f = 0.35$ (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): $R_t = 27.39$ min; $k = 16.85$; LC/MS (ESI, H₂O/CH₃CN): $m/z = 823.9$ [M+H⁺] ($R_t = 27.73$ min, 100 %), 840.9 [M+NH₄⁺] ($R_t = 27.73$ min, 20 %); (-ESI, H₂O/CH₃CN): $m/z = 821.9$ [M-H⁻] ($R_t = 27.73$ min, 100 %); HPLC (preparative), gradient (t [min], % B: [0, 17], [8, 17], [10, 25], [15, 38], [20, 80]): $R_t = 16.68$ min; UV/Vis (H₂O) λ_{max} (log ϵ) = 255 nm (14,100), 348 nm (4,000); empirical formula: C₂₄H₂₃Br₂N₇O₁₂P₂; MW = 823.23



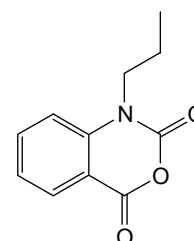
Bis-MANT-IMP (*N*-methyl-2',3'-bis-*O*-anthraniloyl-inosine-5'-monophosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(6-oxo-1*H*-purin-9-yl)-4(3)-hydroxy-2-[phosphonooxymethyl]oxolan-3,4-bis-yl]2-methylaminobenzoate (**31**).

The disodium salt of IMP (100 mg, 0.26 mmol) yielded 80 mg (130 μ mol, 50 %) pure product after size-exclusion chromatography. $R_f = 0.28$ (1-propanol:H₂O:NH₃ (32 % = 2:1:1). HPLC (analytic): $R_t = 28.95$ min, $k = 17.56$; LC/MS (ESI, H₂O/CH₃CN): $m/z = 632.2$ [M+NH₄⁺] ($R_t = 30.28$ min, 100 %); (-ESI, H₂O/CH₃CN): $m/z = 613.2$ [M-H⁻] ($R_t = 30.28$ min, 100 %), 673.2 [M+CH₃COO⁻] ($R_t = 30.28$ min, 70 %); ¹H-NMR (400 MHz, D₂O): $\delta = 2.16$ (s, 3 H, HMBC: 14a-H), 2.25 (s, 3 H, HMBC: 14b-H), 4.09 (s, 2 H, COSY: 1-H), 4.58 (s, 1 H, COSY: 2-H), 5.67 (t, 1 H, COSY, HSQC: 17a-H), 5.75 (d, ³J = 8.35 Hz, 1 H, COSY, HSQC: 15a-H), 5.81 (s, 1 H, COSY, HSQC: 3-H), 5.96 (d, ³J = 8.38 Hz, 1 H, COSY, HSQC: 15b-H), 6.08 (m, 2 H, COSY, HSQC: 4-H, 17b-H), 6.16 (d, ³J = 6.21 Hz, 1 H, COSY, HMBC: 5-H), 6.47 (s, 1 H, COSY: 16a-H), 6.77 (s, 1 H, COSY: 16b-H), 7.22 (d, ³J = 7.52 Hz, 1 H, COSY, HSQC: 18a-H), 7.63 (d, ³J = 7.49 Hz, 1 H, COSY, HSQC: 18b-H), 7.72 (s, 1 H, HSQC: 9-H), 8.51 (s, 1 H, HMBC: 6-H); ¹³C-NMR (100.6 MHz, D₂O): δ [ppm] = 28.6 (1 C, HSQC, HMBC: 14-C), 28.8 (1 C, HSQC, HMBC: 14-C), 64.0 (1 C, HSQC: 1-C), 72.1 (1 C, HSQC: 3-C), 73.8 (1 C, HSQC: 4-C), 84.4 (1 C, HSQC: 2-C), 85.2 (1 C, HSQC, HMBC: 5-C), 107.5 (1 C, HMBC: 12a-C), 108.1 (1 C, HMBC: 12b-C), 110.54 (1 C, HSQC, HMBC: 15a/b-C), 113.7 (1 C, HSQC, HMBC: 17a-C), 114.1 (1 C, HSQC, HMBC: 17b-C), 123.3 (1 C, HMBC: 10-C), 131.0 (1 C, HSQC: 18a-C), 131.3 (1 C, HSQC: 18b-C), 134.9 (2 C, HSQC, HMBC: 16a/b-C), 139.7 (1 C, HMBC: 6-C), 146.0 (1 C, HSQC, HMBC: 9-C), 148.9 (1 C, HMBC: 7-C), 151.7 (1 C, HMBC: 13a-C), 151.8 (1 C, HMBC: 13b-C), 158.1 (1 C, HMBC: 8-C), 166.7 (1 C, HMBC: 11a-C), 167.2 (1 C, HMBC: 11b-C); ³¹P-NMR (161.9 MHz, D₂O): $\delta = 3.3$; UV/Vis (H₂O) λ_{max} (log ϵ) = 250 nm (16,200), 359 nm (6,500); empirical formula: C₂₆H₂₇N₆O₁₀P₃; MW = 614.50



Bis-ANT-IMP (2',3'-bis-*O*-anthraniloyl-inosine-5'-monophosphate) or [(2*R*,3*S*,4*R*,5*R*)-5-(6-oxo-1*H*-purin-9-yl)-2-[phosphonooxymethyl]oxolan-3,4-bis-yl]2-amino-benzoate (**32**).

The disodium salt of IMP (100 mg, 0.26 mmol) yielded 82 mg (140 μ mol, 54 %) pure product after size-exclusion chromatography. R_f = 0.28 (1-propanol:H₂O:NH₃ (32 %) = 2:1:1). HPLC (analytic): R_t = 25.03 min, k = 15.41; LC/MS (ESI, H₂O/CH₃CN): m/z = 604.3 [M+NH₄⁺] (R_t = 26.15 min, 100 %); (-ESI, H₂O/CH₃CN): m/z = 585.3 [M-H⁻] (R_t = 26.15 min, 80 %), 645.3 [M+CH₃COO⁻] (R_t = 26.15 min, 100 %); UV/Vis (H₂O) λ_{max} (log ϵ) = 250 nm (23,600), 333 nm (7,600); empirical formula: C₂₄H₂₃N₆O₁₀P; MW = 586.45

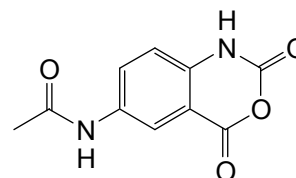


N-propylisatoic anhydride or 1-propyl-1,3-benzoxazine-2,4-dione (**33**).

Isatoic anhydride (1.0 g, 6.1 mmol) was dissolved in a minimum amount of dry dimethylformamide (10 ml) and 177 mg (7.35 mmol, 1.2 eq) sodiumhydrid (60 %) was added stepwise in small portions accompanied by vigorous gas evolution. After five minutes of stirring 1.2 g iodopropane (6.74 mmol, 1.1 eq) were added dropwise to the pacified solution. The reaction mixture was stirred for 24 h and then precipitated in 100 ml cold water to give a white powder. After filtration and washing the product was dried over phosphorpentoxide under vacuum to yield 988 mg (4.8 mmol, 79 %).

¹H-NMR (300 MHz, CDCl₃): δ = 1.04 (t, ³J = 8.1 Hz, 3 H, CH₃), 1.78 (m, 2 H, CH₂), 4.02 (t, ³J = 7.7 Hz, 2 H, CH₂), 7.16 (d, ³J = 8.5 Hz, 1 H, C8-H), 7.28 (ddd, ³J = 7.9 Hz, ³J = 7.4 Hz, ⁴J = 1.0 Hz, 1 H, C6-H), 7.76 (ddd, ³J = 8.6 Hz, ³J = 7.4 Hz., ⁴J = 1.7 Hz, 1 H, C7-H), 8.13 (dd, ³J = 7.9 Hz, ⁴J = 1.7 Hz, 1 H, C5-H); ¹³C-NMR (75.5 MHz, CDCl₃): δ = 11.0 (+), 20.2 (-), 46.4 (-), 111.7 (C_{quat}, phenyl), 114.0 (+), 123.9 (+), 130.9 (+), 137.3 (+), 141.4 (C_{quat}, phenyl), 147.8 (C_{quat}, anhydride), 158.6 (C_{quat}, anhydride); MS (EIMS, 70 eV): m/z = 205.0 [M⁺] (100 %), 161.1 [M⁺-CO₂] (20 %); IR (KBr): [cm⁻¹] = 3094, 2964, 2936, 2875, 1770, 1724, 1603, 1477, 1326,

1022, 766; Mp: 94 °C; UV/Vis (ACN) λ_{\max} (log ϵ) = 245 (8,500), 320 nm (4,000);
empirical formula: C₁₁H₁₁NO₃; MW = 205.21



5-acetylaminoisatoic anhydride or *N*-(2,4-dioxo-1,4-dihydro-benzo[1,3] oxazin-6yl)-acetamide (**34**).

Acetic acid (2 ml) and acetic anhydride (63 mg, 0.61 mmol, 1.1 eq) was propounded in a small flask. Under stirring 5-aminoisatoic anhydride (100 mg, 0.56 mmol) was added and heated to 60 °C for 4 hours. After cooling to room temperature; 10 ml water was added dropwise. The brown colored product was precipitated by ice-cooling followed by filtration and washing with ice-cold water. The precipitate was dried over phosphorpentoxide under vacuum to yield 110 mg (0.5 mmol, 90 %). ¹H-NMR (300 MHz, CDCl₃): δ = 2.05 (s, 3 H, CH₃), 7.10 (d, ³J = 8.8 Hz, 1 H, C8-H), 7.80 (dd, ³J = 8.8 Hz, ⁴J = 2.5 Hz, 1 H, C7-H), 8.24 (d, ⁴J = 2.5 Hz, 1 H, C5-H), 10.17 (s, 1 H, N-H), 11.66 (s, 1 H, N-H); ¹³C-NMR (75.5 MHz, CDCl₃): δ = 23.8 (+), 110.1 (C_{quat}, phenyl), 115.7 (+), 117.5 (+), 128.1 (+), 134.9 (C_{quat}, phenyl), 136.7 (C_{quat}, phenyl), 146.9 (C_{quat}, anhydride), 159.8 (C_{quat}, anhydride), 168.4 (C_{quat}, acetyl); MS (EIMS, 70 eV): m/z = 176.0 [M⁺-CO₂] (100 %), 161.1 [M⁺] (80 %); IR (KBr): [cm⁻¹] = 3117, 2843, 2936, 2735, 1788, 1725, 1645, 1510, 1336, 1264, 911; Mp: 259 °C; UV/Vis (MeOH) λ_{\max} (log ϵ) = 270 nm (15,500), 350 nm (5,600); empirical formula: C₁₀H₈N₂O₄; MW = 220.18