Chemical Synthesis of Benzamide Adenine Dinucleotide: Inhibition of Inosine Monophosphate Dehydrogenase (Types I and II)[1]

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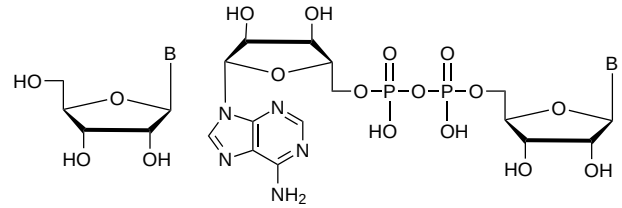
Treatment of 3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)benzamide (**6**) with $POCl_3$ in $(EtO)_3$-PO afforded only little phosphorylation product (**8**, 5%), but the major product was 5′-chlorobenzamide riboside (**7**, 85%). Reaction of **6** with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite followed by 2-cyanoethanol/tetrazole treatment and oxidation with *tert*-butyl peroxide gave a 1:1 mixture of the desired 5′-*O*-bis(2-cyanoethyl) phosphate **9** and the chloro derivative **7**. This mixture was treated with methanolic ammonia and partitioned between $CHCl_3$ and water. The 2′,3′-*O*-isopropylidenebenzamide mononucleotide (**8**) was obtained in 21.2% overall yield from the aqueous layer. Compound **8** was then converted into the corresponding imidazolide **11b** which, upon coupling with 2′,3′-*O*-acetonide of AMP, afforded the acetonide of benzamide adenine dinucleotide (**15**) in 94% yield together with small amounts of symmetrical pyrophosphates P^1,P^2-bis(2′,3′-*O*-isopropylideneadenosin-5′-yl)pyrophosphate (**13**, 3%) and P^1,P^2-bis(2′,3′-*O*-isopropylidene-3-(carbamoylphenyl)-5′-ribosyl)pyrophosphate (**14**, 2%). Deprotection of **15** with Dowex 50/H^+ in water afforded the desired benzamide adenine dinucleotide (BAD) in 93% yield. BAD inhibits inosine monophosphate dehydrogenase type I (IC_{50} = 0.78 μM) and type II (IC_{50} = 0.88 μM) with same degree of potency.

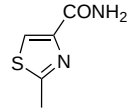
Introduction

IMP-dehydrogenase (IMPDH), the enzyme which catalyzes the NAD-dependent conversion of inosine 5′-monophosphate (IMP) to guanosine 5′-monophosphate (GMP) is positioned at the branch point in the *de novo* synthesis of guanine nucleotides.[2] Inhibition of IMPDH has been shown to have antiviral,[3,4] immunosuppressive,[5,6] antiparasitic,[7,8] and anticancer effects.[9,10] The level of IMPDH activity was found to be much greater in several tumors as compared to the level in normal tissues.[11,12] It has been recently discovered[13] that human IMPDH exists as two isoforms, types I and II. The two distinct cDNAs have been characterized, and the two isoforms are found to be regulated differently.[13−16] Type I is constitutively expressed and is the preponderant isoform in normal cells, while type II is selectively up-regulated in neoplastic and replicating cells and emerges as the dominant species. Thus, the selective inhibition of type II IMPDH is expected to provide significant *therapeutic advantage* by eliminating or reducing potential toxicity caused by inhibition of the type I isoform.[13−16]

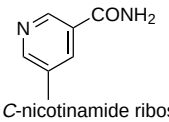
Tiazofurin, 2-(β-D-ribofuranosyl)thiazole-4-carboxamide (**2**, Figure 1), is a potent inhibitor of IMPDH. This oncolytic C-nucleoside requires a unique metabolic activation. It is phosphorylated by adenosine kinase or other kinase(s) to the 5′-monophosphate[17] and then converted by NAD-pyrophosphorylase to the NAD analogue, thiazole-4-carboxamide adenine dinucleotide (TAD).[18] TAD has been synthesized[18,19] and was found to be much more potent inhibitor of IMPDH than tiazofurin 5′-monophosphate.



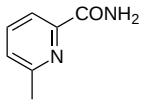
1, nicotinamide riboside — NAD



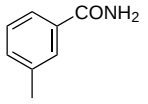
2, tiazofurin — TAD



3, *C*-nicotinamide riboside — C-NAD



4, *C*-picolinamide riboside — C-PAD



5, benzamide riboside — BAD

Figure 1.

We have reported[20−22] the synthesis of the *closest structural analogues of nicotinamide riboside (**1**), i.e.*

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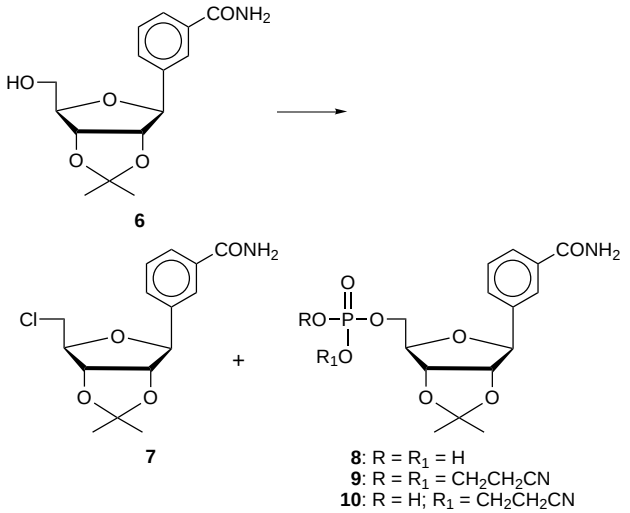
5-β-D-ribofuranosylnicotinamide (**3**) and 6-β-D-ribofuranosylpicolinamide (**4**), the C-nucleoside isosteres of **1**. In contrast to our expectation *these compounds showed only weak inhibitory activity* against L1210, P-815, HL-60, CCRF-CEM, MOLT/4F, and MT-4 cell lines.[20−23] However, when our C-nucleosides **3** and **4** were converted into their corresponding NAD analogues, we found[24] that C-NAD (IC_{50} = 7 μM) and C-PAD (IC_{50} = 26 μM) were relatively effective competitive inhibitors of IMPDH. Interestingly, C-NAD caused extremely potent inhibition of horse liver alcohol dehydrogenase (ADH, K_i = 1.1 nM), whereas C-PAD exhibited a much less potent effect against ADH (K_i = 20 μM). Crystallographic studies[25,26] later showed that the specificity and affinity of C-NAD for ADH are due to coordination of the zinc cation at the ADH catalytic site by the C-NAD pyridine nitrogen. Displacement of the pyridine nitrogen to the opposite side of the ring, as in C-PAD, removes the specificity for ADH.[25,26]

More recently, the synthesis of 3-β-D-ribofuranosylbenzamide (**5**, benzamide riboside) was published.[27] This compound showed *potent toxicity* (at nanomolar concentration) toward S49.1 lymphoma cells[27] and leukemia K562 cells.[28] It was found that, in K562 cells, nucleoside **5** is anabolized to an NAD analogue, benzamide adenine dinucleotide (BAD). K562 cells were incubated with tritium-labeled adenosine and benzamide riboside or tiazofurin. The cells were centrifuged, washed, homogenized, and analyzed on HPLC. BAD was produced in 2−3-fold higher quantities than TAD.[29] Such "biologically prepared" BAD was later used to study inhibition of lactate, glutamate, and malate dehydrogenases and was found to be a better general dehydrogenase inhibitor than TAD.[30] Finally, BAD was prepared enzymatically (in a minute amount using NAD-pyrophosphorylase and ATP) and was shown to inhibit IMPDH (K_i = 0.118 μM).[29]

We became interested in comparing the inhibitory effects of our NAD analogues with those of BAD and TAD as part of our on-going program to design and synthesize selective inhibitors of IMPDH, particularly against type II. Since the enzymic preparation of BAD is inefficient and laborious, we confronted the *first* chemical synthesis of BAD. Our initial attempts using established methods were unsuccessful. Phosphorylation of the 2′,3′-O isopropylidenebenzamide riboside (**6**, Scheme 1) with $POCl_3$ in $(MeO)_3PO$ followed by acetonide cleavage was reported to give the desired 5′-mononucleotide in moderate yield.[27] In our hands, however, such a phosphorylation procedure afforded only little phosphorylated product (**8**, 5%). The major product was 5′-chlorobenzamide riboside (**7**, 85%), although similar reaction with 2′,3′-*O*-isopropylideneadenosine gave the 2′,3′-*O*-acetonide of AMP (**12**, Scheme 2) in good yield. Therefore, for the synthesis of benzamide mononucleotide, we used the phosphitylation procedure. Reaction of **6** with 2-cyanoethyl *N*,*N*-diisopropylchlorophosporamidite followed by 2-cyanoethanol/tetrazole treatment and oxidation with *tert*-butyl peroxide gave a 1:1 mixture of the desired 5′-*O*-bis(2-cyanoethyl) phosphate **9** and the chloro derivative **7**. This mixture was then treated with methanolic ammonia to give a 1:1 mixture of **7** and 5′-*O*-(2-cyanoethyl) phosphate derivative **10**. After concentration *in vacuo* the mixture was partitioned between water and $CHCl_3$.

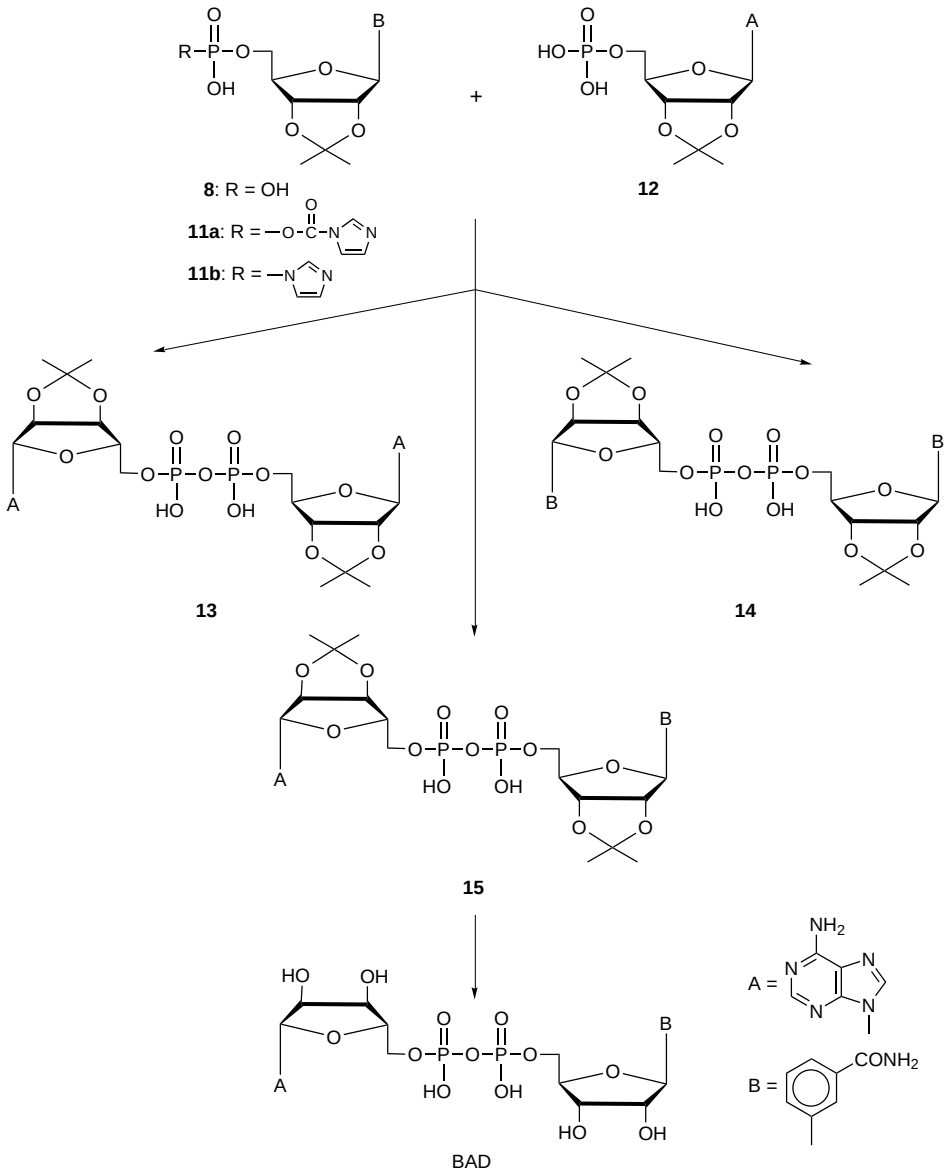
Scheme 1



The 5′-chlorobenzamide riboside (**7**) was recovered from the organic layer, and derivative **10** was obtained from the water solution. The second cyanoethyl group was removed from **10** by prolonged treatment with methanolic ammonia, which required heating at 55 °C for 18 h to furnish the desired 2′,3′-*O*-isopropylidenebenzamide mononucleotide (**8**) in 21.2% overall yield after HPLC purification.

Our recently improved procedure[31] was used for efficient coupling of **10** with 2′,3′-acetonide of AMP (**12**, Scheme 2). We found that preparation of *nucleotide imidazolides* prior to the reaction with the corresponding nucleotides gave much better results than commonly used *in situ* generation of the nucleotide imidazolides. Thus, we prepared the imidazolide **11b** as the triethylammonium salt and coupled it with nucleotide **12** in DMF-d_7. After 8 days, when the singlet of **11b** vanished (^{31}P NMR), the reaction was quenched by addition of water and the mixture was lyophilized. The residue was chromatographed on HPLC column to give the acetonide of BAD (**15**) as the major product (94.5%), together with small amounts of symmetrical pyrophosphates P^1,P^2-bis(2′,3′-*O*-isopropylideneadenosin-5-yl)pyrophosphate (**13**, 3%) and P^1,P^2-bis(2′,3′-*O*-isopropylidene-3-(carbamoylphenyl)-5′-ribosyl)pyrophosphate (**14**, 2%). Treatment of **15** with an excess of Dowex 50/H^+ in water afforded the desired BAD in 93% yield as the free acid analyzed as a single peak on HPLC. The ^{1}H NMR spectrum of our synthetic BAD was identical to that of BAD obtained enzymatically,[29] except that our sample lacked the singlet of unidentified impurity observed at δ 3.72 in the enzymatically prepared sample. Also, our sample showed the resonance for the anomeric proton of benzamide ribose at δ 4.75 (d, $J_{1',2'}$ = 7.1 Hz), which was obscured by the water signal in the enzymatic sample. The assignments of some of the sugar absorbances of the enzymatically prepared BAD were not correct. In particular, the most up-field shifted signal at δ 4.03 (dd) is not the H3′ of adenosine but rather the double doublet of the H2′ of the benzamide ribose (coupling constants $J_{1',2'}$ = 7.2 Hz and $J_{2',3'}$ = 5.3 Hz, see experimental part). The identical coupling constants and a similar, unusual, upfield shift of the H2′ were observed in the ^{1}H NMR spectrum of the benzamide riboside.[29] In acetonide-protected derivatives **6**, **7**, **10**, **14**, and **15**, the resonance signal of benzamide

Scheme 2



ribose H2′ is always present at higher field (0.1−0.3 ppm) than is the H3′ signal. This may be explained by the strong diamagnetic shielding of the H2′ by the aromatic benzamide aglycon.

BAD obtained in the form of a free acid (as a white powder after lyophilization) was found to be unstable when kept at room temperature. In two months 50% of the compound was hydrolyzed into AMP and the 5′-monophosphate of benzamide riboside. When the same sample was dissolved in water, no such decomposition was observed. Furthermore, the sodium or triethylammonium salt of BAD were stable as solids.

We examined the inhibitory effect of BAD and TAD against IMP dehydrogenase type I and type II. The IC_{50}'s of these compounds for each isoform were measured in the presence of 100 μM NAD, 50 μM IMP, 100 mM Tris-HCl, 100 mM KCl, 3 mM EDTA, and 25 nM enzyme at pH 8.0, following the formation of NADH at 340 nm. TAD was equally potent against IMPDH type I (IC_{50} = 0.094 μM) and type II (IC_{50} = 0.101 μM). BAD was more potent than C-NAD and C-PAD but it was 8 times less potent than TAD with equal affinity toward IMPDH type I (IC_{50} = 0.787 μM) and type II (IC_{50} = 0.884). This is in contrast to previously reported result showing equal activity of TAD and BAD.[29] These results demonstrate that *neither TAD nor BAD exhibit selectivity toward IMPDH type II*, the target isozyme predominant in neoplastic cells.[32]

Experimental Section

General Methods. HPLC was performed on a Dynamax-60A C18-83-221-C column with flow rate of 5 mL/min or Dynamax-300A C18-83-243-C column with a flow rate of 20 mL/min of 0.1 M Et_3N H_2CO_3 (TEAB) followed by a linear gradient of 0.1 M TEAB−aqueous MeCN (70%). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Nuclear magnetic resonance spectra were recorded on a JEOL Eclipse 270 and Bruker AMX-400 spectrometer with Me_4Si or DDS as the internal standard for ^1H and external H_3PO_4 for ^{31}P. Chemical shifts are reported in ppm (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), and dd (double doublet). Values given for coupling constants are first order.

Phosphorylation of 3-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)benzamide. (A) Procedure with $POCl_3$. To a cold solution of **6**[27] (58.6 mg, 0.2 mmol) in $(EtO)_3PO$ (0.5 mL) was added a mixture $(EtO)_3PO$ (0.5 mL), water (0.6 μL), and $P(O)Cl_3$ (100 mg, 0.65 mmol, 60 μL). The mixture was kept at 5−10 °C for 24 h and then added dropwise into a solution of 2 M TEAB (1 mL) in water (20 mL). Extraction with EtOAc

(2 × 10 mL) and concentration of the aqueous layer *in vacuo* gave the residue that was further purified by HPLC. After lyophilization the 5′-monophosphate **8** (5 mg, 5%) was obtained as mono (triethylammonium salt). The organic layer was concentrated, and the residue was chromatographed on a silica gel column to give 3-(5-chloro-5-deoxy-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)benzamide (**7**) (53 mg, 85%): ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, iPr), 1.59 (s, 3H, iPr), 3.75 (dd, 1H, H5′, $J_{4',5'} = 4.2$ Hz, $J_{5',5''} = 12.3$ Hz), 3.94 (dd, 1H, H5″, $J_{4',5''} = 2.7$ Hz), 4.15−4.16 (m, 1H, H4′), 4.63 (pseudo t, 1H, H2′), 4.70 (bs, 1H, $CONH_2$), 4.76 (dd, 1H, H3′, $J_{2',3'} = 6.8$ Hz, $J_{3',4'} = 4.1$ Hz), 4.85 (d, 1H, H1′, $J_{1',2'} = 5.5$ Hz), 6.50 (bs, 1H, $CONH_2$), 7.36 (pseudo t, 1H, H5), 7.47 (d, 1H, H4, $J_{4,5} = 7.7$ Hz), 7.78 (d, 1H, H6, $J_{5,6} = 7.7$ Hz), 7.99 (s, 1H, H2); MS (CI) 311 (M^+). Anal. ($C_{15}H_{18}NO_4Cl$) C,H,N.

(B) Phosphitylation Procedure. To a mixture of **6** (87 mg, 0.3 mmol) and iPr_2NEt (75 mg, 0.58 mmol) in dry CH_2Cl_2 (3 mL) was added 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (91.5 mg, 0.38 mmol). After 30 min at room temperature the starting nucleoside **6** disappeared (TLC, $CHCl_3$−EtOH, 9:1). The ^{31}P NMR analysis showed the presence of the unreacted excess of $(iPr)_2NP(Cl)OCH_2CH_2CN$ at δ 184.8 and a 1:1 mixture of the desired product at δ 153.4 and H-phosphonate [$(iPr)_2N(H)P(O)OCH_2CH_2CN$] at δ 18.9 (d, J_{P-H} = 640 Hz, collapsed into a singlet upon decoupling), formed from the starting phosphitilating reagent as a result of chlorination of **6**. The reaction mixture was diluted with CH_2Cl_2 (3 mL) and washed with an ice-cold 10% solution of $NaHCO_3$ (5 mL), saturated NaCl (3 × 5 mL), and water (10 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*, and the residue was dissolved in CH_3CN (1 mL). TLC analysis ($CHCl_3$−EtOH, 9:1) showed the presence of the faster migrating 5′-chloro derivative **7** and the slower migrating 5′-O-phosphitilated nucleoside **6**. These compounds were not separated but treated with *β*-cyanoethanol (60 μL) and a solution of 1*H*-tetrazole (42 mg, 0.6 mmol) in CH_3CN (1.2 mL). After 30 min *tert*-butyl hydroperoxide (100 μL) was added, and the reaction mixture was stirred for 1 h and concentrated *in vacuo*. The residue was dissolved in saturated methanolic ammonia (4 mL) and kept in a refrigerator overnight. The reaction mixture was concentrated, and the residue was dissolved in a mixture of $CHCl_3$ (5 mL) and water (5 mL). The water layer was separated and washed with $CHCl_3$ (2 × 5 mL). The organic layers were combined and concentrated, and the residue was chromatographed on a silica gel column using $CHCl_3$−EtOH, 19:1, as the eluent to give **7** (37.5 mg, 40%) identical with that obtained in method A. The water solution was concentrated *in vacuo* to give crude **10** (70 mg, 53%). An analytical sample of **10** was obtained as the triethylammonium salt after purification by HPLC: ^{1}H NMR ($CDCl_3$) δ 1.23 [t, 9H, ($CH_3CH_2)_3N$], 1.34 (s, 3H, iPr), 1.63 (s, 3H, iPr), 2.67 (t, 2H, OCH_2CH_2CN), 2.97 [q, 6H, ($CH_3CH_2)_3N$], 4.02−4.11 (m, 3H, H5′, OCH_2CH_2CN), 4.26 (dd, 1H, H5″, $J_{4',5''} = 3.2$ Hz, $J_{5',5''}$ = 11.3 Hz), 4.32−433 (m, 1H, H4′), 4.58 (pseudo t, 1H, H2′), 4.88 (dd, 1H H3′, $J_{2',3'} = 6.2$ Hz, $J_{3',4'} = 3.0$ Hz), 4.98 (d, 1H, H1′, $J_{1'',2''} = 4.9$ Hz), 6.02 (bs, 1H, $CONH_2$), 7.37−7.43 (m, 2H, H4,5), 7.96 (d, 1H, H6, $J_{5,6} = 7.2$ Hz), 8.23 (s, 1H, H2), 8.61 (bs, 1H, $CONH_2$); ^{31}P NMR ($CDCl_3$) δ −0.31. Crude **10** was dissolved in saturated methanolic ammonia (5 mL) and heated in a steel cylinder at 55 °C for 18 h. The reaction mixture was concentrated, and the residue was chromatographed by HPLC to give **10** (56 mg, 40%) as mono(triethylammonium salt): ^{1}H NMR (D_2O) δ 1.27 (t, 9H, Et_3N), 1.42 (s, 3H, iPr), 1.67 (s, 3H, iPr), 3.19 (q, 6H, Et_3N), 4.08−4.11 (m, 2H, H5′,5″), 4.42−4.43 (m, 1H, H4′), 4.78 (pseudo t, 1H, H2′), 4.98 (dd, 1H, H3′, $J_{2',3'} = 6.5$ Hz, $J_{3',4'} = 3.5$ Hz), 5.02 (d, 1H, H1′, $J_{1',2'} = 5.4$ Hz), 7.57 (pseudo t, H5), 7.67 (d, 1H, H4, $J_{4,5} = 7.8$ Hz), 7.81 (d, 1H, H6, $J_{5,6} = 7.8$ Hz), 7.86 (s, 1H, H2); ^{31}P NMR (D_2O) δ 4.09. Anal. ($C_{15}H_{20}NO_8P\cdot Et_3N\cdot 2H_2O$) C, H, N. A small amount of unreacted **10** (8 mg, 5%) was also eluted from the column.

Phosphorylation of 2′,3′-*O*-Isopropylideneadenosine. To a cold solution of 2′,3′-*O*-isopropylideneadenosine (153 mg, 0.5 mmol) in $(EtO)_3PO$ (1.25 mL) was added a mixture of $(EtO)_3PO$ (1.25 mL), water (1.5 μL), and $P(O)Cl_3$ (250 mg, 1.65 mmol, 150 μL). The mixture was kept at 5−10 °C for 24 h and then added dropwise into a solution of 2 M TEAB (1 mL) in water (20 mL). Extraction with EtOAc (2 × 10 mL) and concentration of the aqueous layer *in vacuo* gave the residue that was further purified by HPLC. After lyophilization the 5′-monophosphate **12** (214 mg, 88%) was obtained as a mono-(triethylammonium salt): ^{1}H NMR (D_2O) δ 1.30 (t, 9H, Et_3N), 1.47 (s, 3H, iPr), 1.69 (s, 3H, iPr), 3.22 (q, 6H, Et_3N), 4.07−4.09 (m, 2H, H5′,5″), 4.72−4.73 (m, 1H, H4″), 5.21 (dd, 1H, H3′, $J_{2',3'} = 6.0$ Hz, $J_{3',4'} = 1.9$ Hz), 5.44 (dd, 1H, H2′, $J_{1'2'} = 3.0$ Hz), 6.31 (d, 1H, H1′), 8.32, 8.47 (two 1H singlets, H2, H8). Anal. ($C_{13}H_{18}N_5O_7P\cdot Et_3N\cdot 3H_2O$) C, H, N.

3-(2,3-*O*-Isopropylidene-*β*-D-ribofuranosyl)benzamide 5-Phosphoimidazolide (11b) and Its Reaction with 2,3-*O*-Isopropylideneadenosine 5′-Monophosphate. Nucleotide **8** (20 mg, 0.042 mmol as mono(triethylammonium salt)) was dissolved in DMF-d_7 (0.5 mL) followed by addition of CDI (9 mg, 0.056 mmol), and the reaction was monitored by ^{31}P NMR. After 45 min the resonance signal of **8** (δ 1.80) diminished, and the new signal of the anhydride **11a** emerged at δ −6.45, which diminished in time with simultaneous formation of the resonance of the imidazolide derivative **11b** (δ −8.04). After 2.5 h, the formation of imidazolide **11b** was completed, the mono(triethylammonium salt) of **12** (31 mg, 0.063 mmol) was added, and the mixture was kept at room temperature for 8 days. ^{31}P NMR analysis showed the presence of the imidazolide **11b** resonance (8.0%), excess of **12** (δ 2.53), and a group of signals at δ 8.52−9.55. Finally, the reaction mixture was heated at 55 °C until the signal of **11b** completly disappeared (15 h). The reaction mixture was then lyophilized, and the residue was subjected to HPLC to give nucleotide **12** (t_R = 40.9 min, 9 mg), P^1,P^2-bis(2′,3′-*O*-isopropylideneadenosin-5′-yl)pyrophosphate (**13**) as the bis-(triethylammonium salt) [t_R = 49.9 min, 1.5 mg; ^{1}H NMR (D_2O) δ 1.25 (t, 18H, Et_3N), 1.43 (s, 6H, iPr), 1.67 (s, 6H, iPr), 3.12 (q, 12H, Et_3N), 4.19−4.21 (m, 4H, 5′,5″), 4.57−4.58 (m, 2H, H4′), 5.15 (dd, 2H, H3′, $J_{2',3'} = 6.0$ Hz, $J_{3',4'} = 5.4$ Hz, $J_{3',4'}$ = 2.2 Hz), 5.22 (dd, 2H, H2′, $J_{1',2'} = 3.7$ Hz), 6.06 (d, 2H, H1′, $J_{1',2'} = 3.7$ Hz), 8.05, 8.15 (two 2H singlets, H2, H8); ^{31}P NMR (D_2O) δ -9.61], and the desired P^1-[3-(2,3-*O*-isopropylidene-*β*-D-ribofuranos-5-yl)carbamoylphenyl]-P^2-(2′,3′-*O*-isopropylideneadenosin-5′-yl)pyrophosphate (**15**) also as the bis(triethylammonium salt) (t_R = 53.5 min, 37.5 mg, 94.5%): ^{1}H NMR (D_2O) δ 1.15 (t, 18H, Et_3N), 1.33 [s, 3H, iPr (B)], 1.42 [s, 3H, iPr (B)], 1.61 [s, 3H, iPr (A)], 1.65 [s, 3H, iPr (A)], 2.90 (q, 12H, Et_3N), 4.10−4.17 [m, 4H, H5′,5″(A),H5′,5′(B)], 4.24−4.26 [m, 2H, H4′(B)], 4.55−4.58 [m, 2H, H4′(A), H2′(B)], 4.75 [d, 1H, H1′(B), $J_{1',2'} = 5.6$ Hz], 4.85 [dd, 1H, H3′(B), $J_{2',3'} = 6.6$ Hz, $J_{3',4'} = 3.8$ Hz], 5.17 [dd, 2H, H3′(A), $J_{3',4'} = 2.1$ Hz, $J_{2',3'} = 6.1$ Hz], 5.21 [dd, 2H, H2′(A), $J_{1',2'} = 3.6$ Hz), 6.08 [d, 2H, H1′(A), $J_{1',2'} = 3.6$ Hz], 7.34 [pseudo t, 1H, H5(B)], 7.42 [d, 1H, H4(B), $J_{4,5} = 7.7$ Hz], 7.58 [d, 1H, H6(B), $J_{5,6} = 7.7$ Hz], 7.62 [s, 1H, H2(B)], 8.07, 8.29 [two 1H singlets, H2(A), H8(A)]; ^{31}P NMR (D_2O) δ −9.44 (P^1), −9.85 (P^2, AB system, $J_{P,P} = 21.6$ Hz), and P^1,P^2-bis[3-(2,3-*O*-isopropylidene-*β*-D-ribofuranos-5-yl)carbamoylphenyl]pyrophosphate (**14**) (t_R = 56.6 min, 1 mg): ^{1}H NMR (D_2O) δ 1.27 (t, 18H, Et_3N), 1.36 (s, 6H, iPr), 1.63 (s, 6H, iPr), 3.20 (q, 12H, Et_3N), 4.16−4.17 (m, 4H, H5′,5″), 4.35−4.36 (m, 2H, H4′), 4.60 (pseudo t, 1H, H2′), 4.81 (d, 2H, H1′, $J_{1',2'} = 5.6$ Hz), 4.92 (dd, 2H, H3′, $J_{2',3'} = 6.6$ Hz, $J_{3',4'} = 3.6$ Hz), 7.49 (pseudo t, 2H, H5), 7.58 (d, 2H, H4, $J_{4,5} = 7.7$ Hz), 7.72 (d, 2H, H6, $J_{5,6} = 7.7$ Hz), 7.76 (s, 2H, H2); ^{31}P NMR (D_2O) δ −9.64.

Compound **15** was de-*O*-isopropylidenated by treatment with Dowex 50-X8 (H^+) in water, which requires 14 h, and purified by passage through a Dowex 50-X8 (H^+) column to give BAD (26 mg, 93%): ^{1}H NMR (D_2) δ 4.07 [dd, 1H, H2′(B), $J_{1',2'} = 7.2$ Hz, $J_{2',3'} = 5.3$ Hz], 4.15−4.26 [m, 6H, H3′(B), H4′-(B), H5′,5″(A), H5′,5″(B)], 4.35 [m, 1H, H4′(A)], 4.44 [pseudo t, 1H, H3′(A)], 4.59 [pseudo t, 1H, H2′(A)], 4.75 [d, 1H, H1′-(B), $J_{1',2'} = 7.2$ Hz], 6.01 [d, 1H, H1′(A), $J_{1',2'} = 5.3$ Hz], 7.41 (t, 1H, H5, $J = 7.7$ Hz), 7.57 (d, 1H, H4, $J = 7.7$ Hz), 7.66 (d, 1H, H6), 7.73 [s, 1H H2(B)], 8.31, 8.55 [two 1H singlets, H2, H8-(A)]; ^{31}P NMR (D_2O), AB system, δ 9.36 P^1, 9.59 P^2, $J_{P,P} = 21.2$ Hz. MS was identical with that reported earlier.[29]

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