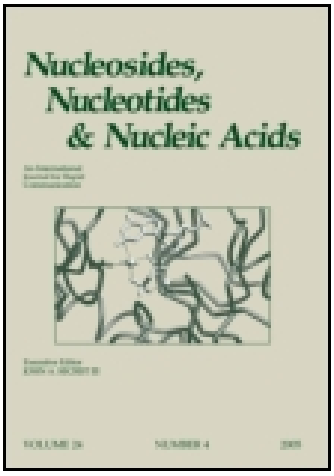

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Synthesis of Methylenebis(Phosphonate) Analogues of 2-, 4-, and 6-Pyridones of Nicotinamide Adenine Dinucleotide

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SYNTHESIS OF METHYLENEBIS(PHOSPHONATE) ANALOGUES OF 2-, 4-, AND 6-PYRIDONES OF NICOTINAMIDE ADENINE DINUCLEOTIDE

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Center for Drug Design, University of Minnesota, Minneapolis, Minnesota, USA

□ *The synthesis of metabolically stable methylenebis(phosphonate) analogues of 2-, 4-, and 6-pyridones of nicotinamide adenine dinucleotide (NAD) is reported. In contrast to natural pyrophosphates, these NAD analogues are able to penetrate the cell membrane and can be used as probes in cellular assays.*

Keywords NAD analogues; IMPDH; NAD kinase

In recent years, intensive studies of nicotinamide adenine dinucleotide (NAD) dependent biological processes have a revealed a variety of functions of this molecule in nature and generated broad interest in evaluation of its potential in biology and medicine. Metabolic pathways leading to the biosynthesis and biodegradation of nicotinamide adenine dinucleotides, NAD and NADP, are under detailed investigation; the focus is on understanding their importance for the well-being of living organisms. The search for NAD-based potential therapeutics is now a fast growing field with numerous drug candidates in clinical studies.[1]

In addition to its role as a co-enzyme in cellular redox reaction, NAD also serves as a substrate for a number of NAD-utilizing enzymes, such as PARP [poly(ADP-ribose) polymerase] and sirtuins (NAD-dependent deacetylases), that release a significant amount of nicotinamide. Nicotinamide is recycled in the cell into NAD or is excreted in urine (almost exclusively) as N-methylnicotinamide. At the end of the 1970s, the 4-pyridone-3-carboxamide ribonucleoside (**1**, Figure 1) had been isolated from the urine of patients with chronic myelogenous leukemia (CML) and proposed as a potential marker of progression of this disease.[2]

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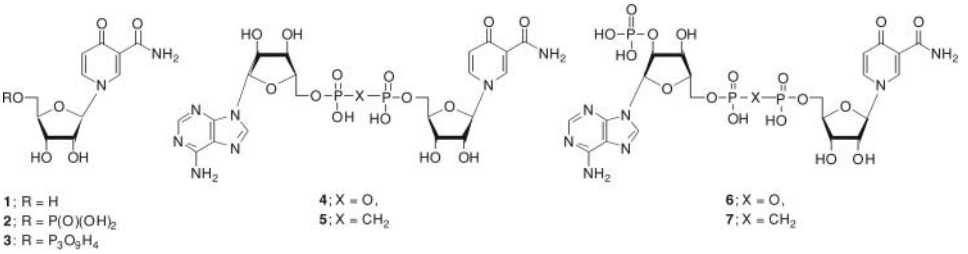


FIGURE 1 4-Pyridone-3-carboxamide riboside, its 5′-triphosphate, and 4-pyridone NAD(P) analogues.

It has been suggested that nucleoside **1** was likely formed due to oxidation of NAD at the 4-position of the pyridine moiety to give the 4-pyridone derivative of NAD **4**. Subsequent cleavage of **4** by cellular phosphodiesterases to the corresponding 5′-monophosphates and dephosphorylation of the monophosphate **2** afforded **1**.[3] Recently, a high concentration (comparable to that of ATP) of 4-pyridone-3-carboxamide-1-β-D-ribonucleoside 5′-triphosphate (**3**) has been reported in erythrocytes of patients with chronic renal failure (CRF).[4] It was also found that mammalian adrenodoxin reductase, involved in steroid and vitamin D biosynthesis, oxidized NADP to give the pyridone **6**.[5,6] The same compound has been reported to be formed as a result of incubation of *Mycobacterium tuberculosis* oxidoreductase FprA with NADP.[7] However, it is not known whether 4-pyridone-NADP has any physiological role.

The synthesis of 2-pyridone-3- and -5-carboxamide ribonucleosides **8** and **11**, respectively (Figure 2), have been earlier reported by the authors[8] and others.[9,10,11] The corresponding 2- and 6-pyridone-NAD analogues (**9** and **12**, respectively) were also prepared by enzymatic and chemical methods.[12] As with 4-pyridone-NAD **4**, these compounds might be of interest as potential donors of the pyridone bases because accumulation of modified pyridone bases in urine of CRF patients is well documented.[13]

Natural pyrophosphate analogues of 2-, 4-, and 6-pyridone-NAD (**9**, **4**, and **12**, X = O) are metabolically unstable, cannot penetrate the cell membrane, and therefore have not been evaluated in cellular assays. In contrast,

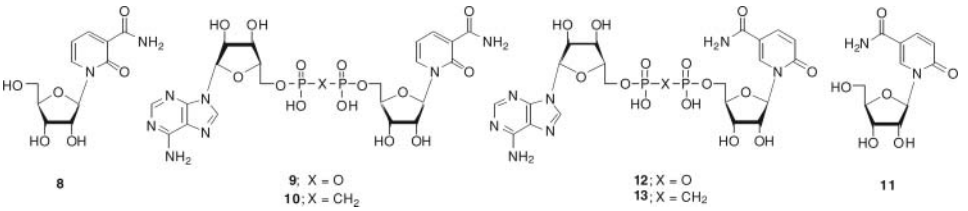
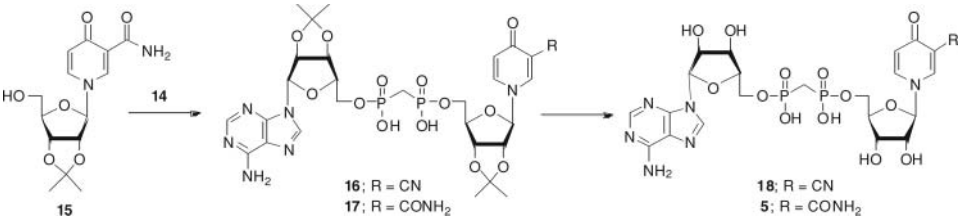


FIGURE 2 2-Pyridone-3-, and 5-carboxamide ribosides and their corresponding NAD analogues.

structurally close methylenebis(phosphonate) analogues of NAD (e.g., benzamide adenine dinucleotide analogue[14]) are resistant to metabolic degradation and are able to enter the cell. Thus, we report herein the synthesis of bis(phosphonate) analogues of 2-, 4-, and 6-pyridone-NAD (**10**, **5**, and **13**, respectively) for evaluation in cellular assays and as potential starting materials for further modification, i.e. phosphorylation. A conversion of **5** to **7** may afford the potent and selective inhibitor of the mycobacterium FprA enzyme.

For synthesis of title compounds we used our standard procedure of N,N-disopropylcarbodiimide (DIC) promoted coupling of 2′, 3′-O-isopropylidene protected pyridone-carboxamide riboside with 2′3′-*O*-isopropylidene-adenosin-5′-yl-methylenebis(phosphonate) (**14**).[15,16]



SCHEME 1 Synthesis of 4-pyridone-NAD analogue.

To our surprise, when the isopropylidene protected 4-pyridone-3-carboxamide nucleoside **15** was used as the starting material (Scheme 1), a mixture of the desired methylenebis-(phosphonate) 4-pyridone-NAD analogue **5** and 4-pyridone-3-nitrile derivative **18** was obtained, which could not be separated on a preparative high performance liquid chromatography (HPLC) column. Our earlier application of the identical coupling procedure for the synthesis of methylenebis(phosphonate) analogue of benzamide adenine dinucleotide (BAD) or tiazofurin adenine dinucleotide (TAD, Figure 3) afforded the desired compounds in high yield and no formation of the corresponding 3-nitrile derivatives was observed.

Although dehydration of carboxyamides to nitriles in the presence of DIC is a known reaction, apparently pyridone carboxyamides are more sensitive to such dehydration than the aromatic benzamide ring of benzamide riboside or thiazole-4-carboxamido group of tiazofurin. Indeed, the

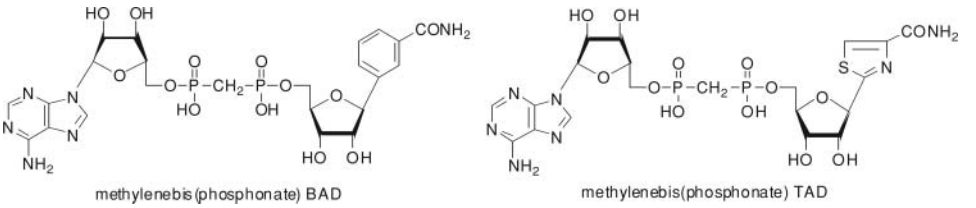
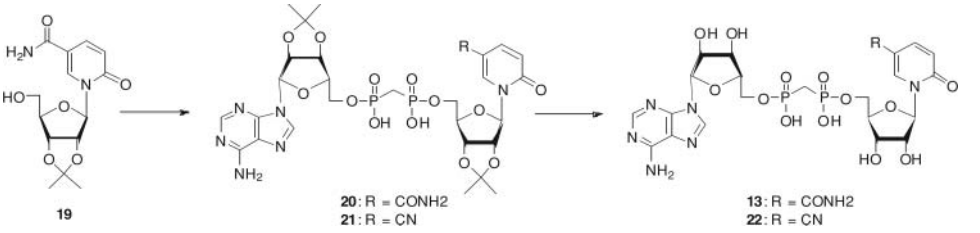


FIGURE 3 Methylenebis(phosphonate) analogues of BAD and TAD.

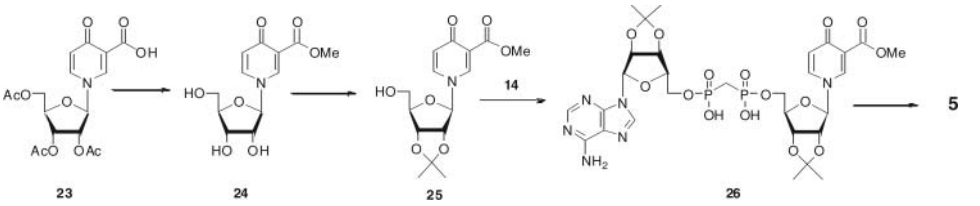
similar DIC coupling of the 2′, 3′-O-isopropylidene protected 2-pyridone-5-carboxamide nucleoside **19** also afforded a mixture of the desired 6-pyridone-NAD **13** and the corresponding nitrile derivative **22** in the ratio of 1:2 (Scheme 2). Fortunately, these compounds were separable on HPLC column and the desired 6-pyridone-NAD **13** was eluted first, followed by the nitrile derivative **22**.



SCHEME 2 Synthesis of 6-pyridone-NAD analogue.

Obviously, DIC coupling of adenosine-5′-methylenebis(phosphonate) **14** with pyridone-3-carboxylic esters ribosides instead of the carboxyamido riboside derivatives would not result in dehydration. In addition, a conversion of esters to amides by treatment with ammonia should be a simple and efficient reaction.

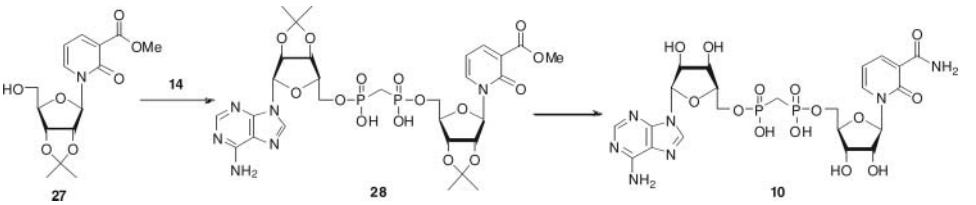
As shown in Scheme 3, we modified our coupling procedure and prepared 4-pyridone NAD **5** using methyl 2′, 3′-O-isopropylidene-4-pyridone-3-carboxylate ribonucleoside **25** as the starting material for DIC coupling with adenosine-5′-methylenebis(phosphonate) **14**. Compound **25** was synthesized from unreported methyl 4-pyridone-3-carboxylate ribonucleoside **24**, which we prepared by Vorbrugen coupling of 4-pyridone-3-carboxylic acid with 1, 2, 3, 5,-tetra-O-acetyl-β-D-ribofuranose to give **23** and its further deacetylation followed by conversion to the methyl ester **24**.



SCHEME 3 Synthesis of 4-pyridone-NAD analogue **5** from methyl ester of 4-pyridone-3-carboxylic acid riboside.

The protected methylenebis(phosphonate) analogue **26** was obtained in good yield. After conversion of **26** into the corresponding carboxyamido derivative by treatment with methanolic ammonia, the isopropylidene protecting groups were removed with formic acid.

In the same manner, coupling of methyl 2′, 3′-O-isopropylidene-2-pyridone-3-carboxylate ribonucleoside **27** with adenosine-5′-methylenebis(phosphonate) **14** followed by treatment with diazometane and deprotection afforded a good yield of the desired 2-pyridone-NAD **10** (Scheme 4).



SCHEME 4 Synthesis of 2-pyridone-NAD analogue.

We evaluated our pyridone-NAD analogues as potential inhibitors of human and mycobacterium IMP-dehydrogenase and NAD kinase. None showed inhibition of IMPDH. Interestingly, 6-pyridone **13** inhibited both human and mycobacterium NAD kinase equally (50% at the concentration of 0.5 mM) but 2-pyridone **10** was found to be 8-fold more potent against the human enzyme (80%) than *Mycobacterium tuberculosis* NAD kinase (9%) at the same concentration.

It was recently found that 4-pyridone-3-carboxamide-adenine dinucleotide phosphate **6** is not only a good ligand and a competitive inhibitor of *Mycobacterium tuberculosis* oxidoreductase FprA ($K_i = 1.2\ \mu M$) but also inhibits *Toxoplasma gondi* FNR enzyme ($K_i = 30\ \mu M$).[6] Thus, our synthetic bis(phosphonate) analogue **5** when converted into the 2′-monophosphate prodrug could be of therapeutic interest.

EXPERIMENTAL

General Methods

All commercial reagents (Sigma-Aldrich, St. Louis, MO, USA; Afla Aesar, War Hill, MA, USA) were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer, Laguna Beach, CA, USA) using two packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2,; two packed columns of molecular sieves were used to dry DMF. Solvents were dispensed under argon. Analytical HPLC was performed on a Varian (Lake Forest, CA, USA) Microsorb column (C18, 5 μ, 4.6 × 250 mm) with a flow rate of 0.5 mL/min whereas preparative HPLC was performed on a Varian Dynamax column (C18, 8 μ, 41.4 × 250 mm) with a flow rate of 40 mL/min. An isocratic or linear gradient of 0.1 M triethylammonium bicarbonate (TEAB) and aqueous MeCN (70%) were used. Flash chromatography was performed using Teledyne ISCO CombiFlash Rf equipped with Teledyne

ISCO RediSep Rf flash column silica cartridges (www.isco.com/combiflash) with the indicated solvent system. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 600 MHz with Me_4Si, DDS, or signals from residual solvent as the internal standard for ^1H and external H_3PO_4 for ^{31}P. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad singlet), and dd (double doublet). Values given for coupling constants are first order. High-resolution mass spectra were recorded on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. All reactions were performed under an inert atmosphere of dry Ar in oven dried (150°C) glassware. The purity of the compounds was ≥95%, and it was determined by HPLC using the above-mentioned analytical column.

4-Pyridone-3-(carboxylic acid)-1-(2, 3, 5-tri-O-acetyl β-D-ribofuranoside) (23)

To the suspension of 4-pyridone-3-carboxylic acid[17] (1.39 g, 10 mmol) and TAR (3.64 g, 11.28 mmol) in dry CH_3CN (25 mL) BSTFA (6.5 ml, 25.7 mmol) was added. The mixture was stirred at room temperature for 1.5 hours. Excess silylating reagent was removed and the residue was dissolved in dry CH_3CN (25 mL). The clear solution was cooled and TMSOTfl (2.1 mL, 11.5 mmol) was added dropwise. The mixture was stirred at room temperature for 6 hours, cooled in an ice, and sat. aq. $NaHCO_3$ was added dropwise to pH 5–6. Solvents were evaporated and the residue was dissolved in water. 1 N HCL was added to adjust pH to 1–2, and the mixture was extracted with CH_2Cl_2. Organic fractions were combined, washed with water, dried over $MgSO_4$, and concentrated to give thick oil, which was purified on silica gel flash column chromatography using CH_2Cl_2:MeOH (9:1). Crude product was crystallized from EtOH to give **23** (3.8 g, 96%). ^1H NMR ($CDCl_3$) δ 15.19 (s, 1H), 8.79 (d, J = 2.17 Hz, 1H), 7.78 (dd, J = 2.24, 7.72 Hz, 2H), 6.77 (d, J = 7.53 Hz, 1H), 5.62 (d, J = 5.07 Hz, 1H), 5.29 (m, 1H), 5.24 (m, 1H), 4.54 (m, 1H), 4.49 (dd, J = 2.40, 12.85, 1H), 4.39 (dd, J = 2.11, 12.85, 1H), 2.26 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H). HRMS $C_{17}H_{18}NO_{10}$ 396.0936 $(M\text{-}H)^-$, found 396.0921.

Methyl 4-pyridone-3-carboxylate-1-β-D-ribofuranoside (24)

Compound **23** was suspended in methanolic ammonia (20 mL of 7 M NH_3), stirred at room temperature for 48 hours, evaporated, dissolved in water, and passed through a column of Dowex 50 W(H^+). The eluents were evaporated, the residue co-evaporated with toluene, dissolved in MeOH, cooled in an ice bath, and treated with TMS-CH_2N_2 until small excess of $TMSCH_2N_2$ was present. When the reaction was completed, AcOH (2 mL) was added and the mixture was stirred at room

temperature for 15 minutes, evaporated and co-evaporated with toluene, EtOH, and finally purified by flash chromatography on RediSep using $CHCl_3$:MeOH (8 : 2) as eluent to give **24** (534 mg 62%). ^{1}H NMR (D_2O) δ 8.71 (d, J = 2.25 Hz, 1H), 7.91 (dd, J = 2.25 Hz, J = 7.64 Hz, 1H), 6.55 (d, J = 7.64 Hz, 1H), 5.52 (d, J = 5.26 Hz, 1H), 4.22 (t, J = 5.18 Hz, 1H), 4.17 (t, J = 4.64 Hz, 1H), 4.12 (m, 1H), 3.80 (dd, J = 2.96 Hz, J = 12.84 Hz, 1H), 3.74 (s, 3H), 3.83 (dd, J = 3.96 Hz, J = 12.84 Hz, 1H). HRMS calcd for $C_{12}H_{16}NO_7$ 286.0921 $(M + H)^+$, found 286.0930.

Methyl 4-pyridone-3-carboxylate -1-(2, 3-O-isopropylidene-β-D-ribofuranoside) (25)

Dimethoxypropane (1 mL, 8.5 mmol) was added to the suspension of **24** (485 mg, 1.7 mmol) in acetone (10 mL), followed by p-TsOH monohydrate (300 mg, 1.7 mmol). The mixture was stirred at room temperature for 3 hours, excess of sat. aq. $NaHCO_3$ was added and the mixture was diluted with water and extracted with CH_2Cl_2. Extracts were combined, dried over $MgSO_4$, filtered, and dried in vaccuo to give **25** as a white foam (233 mg, 42%). ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J = 2.30 Hz, 1H), 7.84 (dd, J = 2.38 Hz, J = 7.82 Hz, 1H), 6.35 (d, J = 7.67 Hz, 1H), 5.40 (d, J = 3.36 Hz, 1H), 4.90 (dd, J = 1.23 Hz, J = 4.70 Hz, 1H), 4.69 (dd, J = 3.33 Hz, J = 5.85 Hz, 1H), 4.57 (bs, 1H), 4.47 (m, 1H), 3.98 (dd, J = 1.76 Hz, J = 11.76 Hz, 1H), 3.84 (dd, J = 1.87 Hz, J = 11.76 Hz, 1H), 3.79 (s, 3H). HRMS calcd for $C_{15}H_{20}NO_7$ 326.1234 $(M + H)^+$, found 336.1242.

4-Pyridone-3-carboxamide -1-β-D-ribofuranoside (1)

Compound **23** (3.8 g, 9.6 mmol) was dissolved in 100 mL of MeOH, cooled in an ice bath, and TMS-CH_2N_2 (2M in diethyl ether) was slowly added until the reaction was complete and slight excess of TMS-CH_2N_2 was present. The mixture was stirred for 15 minutes, 1.5 mL of AcOH was added, and solvents were evaporated and coevaporated with toluene to leave methyl-4-pyridone-3-carboxylate-1-(2, 3, 5, -tri-O-acetyl-β-D-ribofuranoside) as a thick oil, which was used directly in the next step. The crude ester was dissolved 7 M ammonia in MeOH (90 mL) in a pressure vessel. The mixture was stirred for 3 days at room temperature, evaporated, and the residue was purified by flash chromatography on silica gel with CH_2Cl_2 and CH_2Cl_2:MeOH (95:5 and next 8:2) as eluents. A crystallization from MeOH gave **1**, which was washed with acetone and dried (0.875 g, 34%). ^{1}H NMR (D_2O) δ 8.68 (d, J = 2.15 Hz, 1H), 7.93 (dd, J = 2.15 Hz, J = 7.65 Hz, 1H), 6.60 (d, J = 7.71 Hz, 1H), 5.54 (d, J = 5.57 Hz, 1H), 4.22 (t, J = 5.40 Hz, 1H), 4.17 (m, 1H), 4.13 (m, 1H), 3.79 (dd, J = 3.05 Hz, 12.82 Hz, 1H), 3.71 (dd, J = 4.05 Hz, 12.82 Hz, 1H). ^{13}C NMR (D_2O) δ 61.1, 70.2, 75.8, 86.2, 97.2,

118.5, 120.6, 139.0, 143.0, 168.0, 179.4. HRMS $C_{11}H_{15}N_2O_6$ 271.0925 $(M + H)^+$, found 271.0936.

4-Pyridone-3-carboxyamide -1-(2, 3-O-isopropylidene β-D-ribofuranoside) (15)

p-TsOH (190 mg, 1 mmol) was added to the suspension of nucleoside **1** (0.27 g, 1 mmol) in 5 mL of acetone, followed by dimethoxypropane (0.6 mL, 5 mmol). The mixture was stirred at room temperature overnight. Aq. ammonia (2 mL) was added and the whole mixture was evaporated, diluted with water, extracted with AcOEt; extracts were dried with $MgSO_4$, filtered, and evaporated to leave a colorless foam, which was purified by preparative TLC on silica gel with $CHCl_3$:MeOH 9:1 to give **15** (205 mg, 66%) ^{1}H NMR ($CDCl_3$) δ 9.87 (brs, 1H), 8.82 (d, J = 2.25 Hz, 1H), 7.74 (dd, J = 2.25 Hz, J = 7.64 Hz, 1H), 6.58 (d, J = 7.60 Hz, 1H), 5.75 (brs, 2H), 5.51 (m, 1H), 4.94 (m, 1H), 4.72 (m, 1H), 4.49 (m, 1H), 4.04 (dd, J = 1.90 Hz, J = 12.20 Hz, 1H), 3.89 (dd, J = 3.55 Hz, J = 12.20 Hz, 1H), 1.61 (s, 3H), 1.36 (s, 3H). HRMS $C_{14}H_{19}N_2O_6$ 311.1238 $(M + H)^+$, found 311.1230.

4-Pyridone-NAD (5)

To the solution of **14** (88 mg, 0.13 mmol) in dry pyridine (0.7 mL), DIC (80 μL, 0.52 mmol) was added, the mixture was stirred overnight at room temperature., compound **25** (43 mg, 0.13 mmol) was added, and stirring was continued for 70 hours at 65°C. After cooling to room temperature, a mixture of water (0.2 mL) and TEA (0.1 mL) was added, and the reaction was heated for 6 hours at 65°C. When the reaction was complete, the mixture was evaporated and co-evaporated with EtOH, the residue was dissolved in methanolic ammonia (7 M) and stirred at room temperature for 3 days, evaporated and the crude intermediate **26** was dissolved in 50% HCOOH (5 mL) and stirred overnight at room temperature. Solvents were evaporated, and the residue was co-evaporated with a mixture of EtOH and water. The crude product was purified by preparative HPLC with 70% MeCN/0.1 M TEAB (2-100 linear gradient). Fractions containing product were evaporated, the residue was dissolved in water, and then passed through a column of Dowex 50 WX8-200 (Na^+ form). After elution with water, ultraviolet (UV) active fractions were collected, and lyophilized to give **5** (sodium salt) as a white powder (32 mg, 20%). ^{1}H NMR (D_2O) δ 8.35 (d, J = 2.22 Hz, 1H), 8.21 (s, 1H), 8.02 (s, 1H),7.86 (dd, J = 2.22 Hz, J = 7.60 Hz, 1H), 6.39 (d, J = 7.65 Hz, 1H), 5.89 (d, J = 5.20 Hz, 1H), 5.39 (d, J = 5.97 Hz, 1H), 4.55 (t, J = 5.14 Hz, 1H), 4.36 (t, J = 4.82 Hz, 1H), 4.27 (m, 1H), 4.22 (m, 4H), 4.05 (m, 2H), 2.13 (t, J = 19.91 Hz, 2H). ^{31}P NMR (D_2O) δ 18.20 (d, J = 10.45 Hz), 18.18 (d, J = 10.45 Hz). HRMS $C_{22}H_{28}N_7O_{14}P_2$ 676.1175 $(M\text{-}H)^-$, found 676.1134.

The DIC promoted coupling starting from **15** afforded an inseparable mixture of 2′, 3′-O-isopropylidene protected nitrile derivative **16** and carboxyamido analogue **17**. Repeated attempts for HPLC separation of compound **18** and **5** after deisopropylidenetion were also unsuccessful.

2-Pyridone-5-carboxyamide-1-(2, 3-O-isopropylidene β-D-ribofuranoside) (19)

To the suspension of the 2-pyridone-5-carboxyamide-1-β-D-ribofuranoside[8] (0.27 g, 1 mmol) in 5 mL of acetone, p-TsOH (190 mg, 1 mmol) was added, followed by dimethoxypropane (0.6 mL, 5 mmol). The mixture was stirred at room temperature overnight. To the clear solution aq. ammonia was added and the whole mixture evaporated, diluted with water, and extracted with AcOEt; extracts were dried over $MgSO_4$ and evaporated to leave a crude product, which was purified by flash chromatography on silica gel with $CHCl_3$:MeOH 9:1. Fractions containing the desired product were combined, and concentrated in vacuo to give **19** as an oil (250 mg, 81%). ^{1}H NMR ($CDCl_3$) δ 8.38 (d, J = 2.18 Hz, 1H), 7.31 (dd, J = 9.40 Hz, J = 2.18 Hz, 1H), 6.56 (d, J = 9.40 Hz, 1H), 5.83 (d, J = 1.90 Hz, 1H), 5.74 (brs, 2H), 5.07 (m, 1H), 5.00 (m, 1H), 4.42 (m, 1H), 4.01 (dd, J = 1.80 Hz, J = 12.35 Hz, 1H), 3.82 (dd, J = 3.60 Hz, J = 12.35 Hz, 1H), 1.60 (s, 3H), 1.36 (s, 3H). HRMS calcd for $C_{14}H_{19}N_2O_6$ 311.1238 $(M+H)^+$, found 311.1249.

6-Pyridone-NAD (13)

2′,3′-O-Isopropylidene-adenosin-5′-yl-methylenebis(phosphonate) (**14**, 88 mg, 0.13 mmol) and **19** (40.3 mg, 0.13 mmol) were dissolved in dry pyridine (0.7 mL), and to the resulting solution, DIC (80 μL, 0.52 mmol) was added. The resulting mixture was stirred at room temperature for 1 hour and then at 65°C for 69 hours, cooled to room temperature, treated with a mixture of H_2O and TEA (200 μL and 100 μL, respectively) and heated at 65°C for 6 hours. The mixture was evaporated and co-evaporated with EtOH. The residue was dissolved in 80% TFA, stirred vigorously for 3 hours at 0°C, evaporated, co-evaporated with EtOH, and dissolved in a small amount of MeOH. The crude mixture was purified by preparative HPLC with 70% MeCN/0.01M TEAB (5–15 linear gradient) to give two main products. Fractions containing the major product were pooled, evaporated to dryness, co-evaporated with H_2O and EtOH, dissolved in a small amount of H_2O, and passed through a small column of Dowex 50 WX8-200 (Na^+), combined and lyophilized to give **22** as a white powder (20 mg, 22%). ^{1}H NMR (D_2O) δ 8.33 (s, 1H), 8.13 (s, 1H), 8.01 (s, 1H), 7.40 (d, J = 9.29 Hz, 1H), 6.32 (d, J = 9.42 Hz, 1H), 5.86 (d, J = 5.46 Hz, 1H), 5.62 (s, 1H), 4.42 (t, J = 5.48 Hz, 1H), 4.35 (t, J = 4.92 Hz, 1H), 4.21 (m, 1H), 4.14 (m, 2H), 4.05 (m, 4H), 3.97 (m, 1H), 2.14 (t, J = 19.95 Hz, 2H). ^{31}P NMR (D_2O) δ 18.32

(d, $J = 10.49$ Hz), 18.22 (d, $J = 10.49$ Hz). HRMS calcd for $C_{22}H_{26}N_7O_{13}P_2$ 658.1069 $(M\text{-}H)^-$, found 686.1084. Fractions containing the minor product were pooled, evaporated to dryness, co-evaporated with H_2O and EtOH, and converted into the Na^+ salt as described above to give **13** as a white powder (10 mg, 11%). ^1H NMR (D_2O) δ 8.27 (s, 1H), 8.10 (s, 1H), 8.02 (s, 1H), 7.62 (d, $J = 9.05$ Hz, 1H), 6.34 (d, $J = 9.60$ Hz, 1H), 5.82 (d, $J = 5.82$ Hz, 1H), 5.75 (d, $J = 2.48$ Hz, 1H), 4.50 (t, $J = 5.75$ Hz, 1H), 4.31 (t, $J = 4.82$ Hz, 1H), 4.16 (m, 2H), 4.07 (m, 6H), 4.02 (m, 1H), 2.12 (t, $J = 19.95$ Hz, 2H). ^{31}P NMR (D_2O) δ 18.39 (d, $J = 10.84$ Hz), 18.18 (d, $J = 10.84$ Hz). HRMS calcd for $C_{22}H_{28}N_7O_{14}P_2$ 676.1175 $(M\text{-}H)^-$, found 676.1105.

Methyl 2-pyridone-3-carboxylate -1-(2,3-O-isopropylidene β-D-ribofuranoside) (27)

2-Pyridone-3-(carboxylic acid)-1-(β-D-ribofuranoside)[8] (1.2 g, 4.5 mmol) was suspended in MeOH (50 mL) and cooled in an ice bath and TMS-CH_2N_2 (3.37 mL, 6.75 mmol) was added until small excess of reagent was present. The mixture was stirred at room temperature until the reaction was complete. Solvents were evaporated, and the residue dried and used in the next step without further purification. Dried crude methyl ester was suspended in acetone (30 mL) and to this suspension dimethoxypropane (3 mL) was added followed by p-toluenesulphonic acid monohydrate (860 mg, 4.5 mmol). The mixture was stirred at room temperature for 4 hours, neutralized with $NaHCO_3$, concentrated, diluted with water, and extracted with EtOAc. Extracts were combined, washed with H_2O, dried over $MgSO_4$, and finally concentrated to leave semisolid **27** (0.5g, 34%). ^1H NMR ($CDCl_3$) δ 8.18 (dd, $J = 2.20$ Hz, $J = 7.10$ Hz, 1H), 7.69 (dd, $J = 2.10$ Hz, $J = 6.76$ Hz, 1H), 6.30 (t, $J = 7.17$ Hz, 1H), 5.68 (d, $J = 2.23$ Hz, 1H), 5.18 (dd, $J = 2.28$ Hz, $J = 6.40$ Hz, 1H), 5.09 (dd, $J = 3.93$ Hz, $J = 6.40$ Hz, 1H),4.71 (brs, 1H), 4.33 (m, 1H), 3.93 (dd, $J = 2.52$ Hz, $J = 12.20$ Hz, 1H), 3.87 (s, 3H), 3.83 (dd, $J = 3.82$ Hz, $J = 12.20$ Hz, 1H), 1.57 (s, 3H), 1.33 (s, 3H). HRMS calcd for $C_{15}H_{20}NO_7$ 326.1234 $(M + H)^+$, found 326.1246.

2-Pyridone-NAD (10)

Compound **14** (88 mg, 0.13 mmol) was dissolved in dry pyridine (0.7 mL) and DIC (80 μL, 0.52 mmol) was added. To this mixture compound **27** (43 mg, 0.13 mmol) was added and the reaction mixture was heated for 69 hours at 65°C. After the reaction was completed H_2O (200 μL) was added and the mixture was heated for 6 hours. The mixture was evaporated and co-evaporated with EtOH, the residue was dissolved in 7M NH_3 in MeOH, and stirred at room temperature for 3 days. The residue was dissolved in a mixture of HCOOH and water 1:1 (10 mL), and stirred overnight at room temperature, evaporated and purified by preparative HPLC with 70% MeCN/0.01M

TEAB (5–15 linear gradient). Fractions containing the product were collected, evaporated to dryness, co-evaporated with H_2O and EtOH, dissolved in a small amount of H_2O, and passed through a small column of Dowex 50 WX8-200 (Na^+). Fractions containing the desired product were combined and lyophilized to give **10** as a white powder (20 mg, 23%). ^{1}H NMR (D_2O) δ 8.16 (s, 1H), 7.99 (s, 1H), 7.90 (m, 2H), 6.29 (t, J = 7.04 Hz, 1H), 5.78 (m, 2H), 4.33 (t, J = 4.62 Hz, 1H), 4.19 (t, J = 5.05 Hz, 1H), 4.14 (m, 2H), 4.08 (m, 2H), 3.97 (m, 3H), 3.91 (m, 1H), 2.10 (t, J = 20.04 Hz, 2H). ^{31}P NMR (D_2O) δ 18.08 (d, J = 11.06 Hz), 18.06 (d, J = 11.06 Hz). HRMS $C_{22}H_{28}N_7O_{14}P_2$ 676.1175 $(M\text{-}H)^-$, found 676.1112.

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