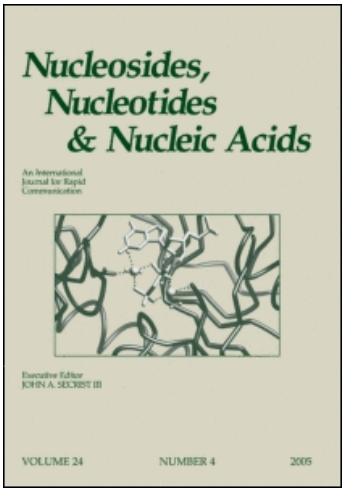

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Synthesis of Methylene-Bridged Analogues of Nicotinamide Riboside, Nicotinamide Mononucleotide and Nicotinamide Adenine Dinucleotide

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SYNTHESIS OF METHYLENE-BRIDGED ANALOGUES OF NICOTINAMIDE RIBOSIDE, NICOTINAMIDE MONONUCLEOTIDE AND NICOTINAMIDE ADENINE DINUCLEOTIDE.[1]

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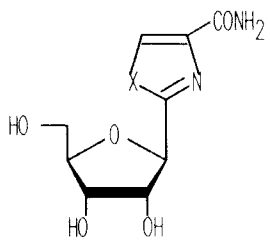
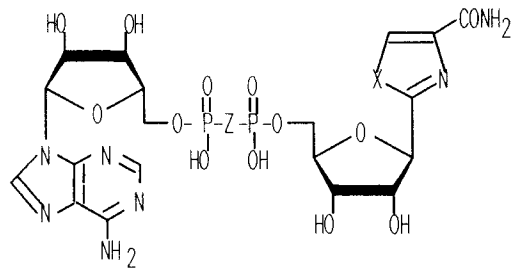
Abstract: 5-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-isopropylidene-3(R)-(nicotinamid-2-ylmethyl)-α-D-ribofuranose (**11a**) and -3(R)-(nicotinamid-6-ylmethyl)-α-D-ribofuranose (**11b**) were prepared by condensation of 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-α-D-*erythro*-3-pentulofuranose (**10**) with lithiated (LDA) 2-methylnicotinamide and 6-methylnicotinamide, respectively, and then deprotected to give 1,2-*O*-isopropylidene-3-(R)-(nicotinamid-2-ylmethyl)-α-D-ribofuranose(**12a**)and1,2-*O*-isopropylidene-3(R)-(nicotinamid-6-ylmethyl)-α-D-ribofuranose (**12b**). Benzoylation as well as phosphorylation of compounds **12** afforded the corresponding 5-*O*-benzoate (**13b**) and 5-*O*-monophosphates (**14a** and **14b**). Treatment of **13b** with CF_3COOH/H_2O caused 1,2-de-*O*-isopropylidenation with simultaneous cyclization to the corresponding methylene-bridged cyclic nucleoside - 3',6-methylene-1-(5-*O*-benzoyl-ß-D-ribofuranose)-3-carboxamidopyridinium trifluoro-acetate (**8b**) - restricted to the "**anti**" conformation. In a similar manner compounds **14a** and **14b** were converted into conformationally restricted 2,3'-methylene-1-(ß-D-ribofuranose)-3-carboxamidopyridinium-5'-monophosphate (**9a** - "**syn**") and 3',6-methylene-1-(ß-D-ribofuranose)-3-carboxamido - pyridinium-5'monophosphate (**9b** - "**anti**") respectively. Coupling of derivatives **12a** and **12b** with the adenosine 5'-methylenediphosphonate (**16**) afforded the corresponding dinucleotides **17.** Upon acidic 1,2-de-*O*-isopropylidenation of **17b**, the conformationally restricted P^1-[6,3'-methylene-1-(ß-D-ribofuranos-5-yl)-3-carboxamidopyridinium]-P^2-(adenosin-5'-yl)methylenediphosphonate **18b** -"**anti**" was formed. Compound **18b** was found to be unstable. Upon addition of water **18b** was converted into the anomeric mixture of acyclic dinucleotides, *i.e.* P^1-[3(R)-nicotinamid-6-ylmethyl-D-ribofuranos-5-yl]-P^2-(adenosin-5'-yl)-methylenediphosphonate (**19b**). In a similar manner, treatment of **17a** with CF_3COOH/H_2O and HPLC purification afforded the corresponding dinucleotide **19a**.

The promising anticancer agents[2], tiazofurin (**1**) and selenazofurin (**2**, FIG. 1) are metabolically converted into NAD analogues, TAD (**3**) and SAD (**4**), which are potent inhibitors of inosine monophosphate dehydrogenase (IMPDH)[3]. TAD and

This paper is dedicated to the 75th birthday of Professor Yoshihisa Mizuno.



1. X = S
2. X = Se



3. TAD, X = S, Z = O
4. SAD, X = Se
5. β-TAD, X = S, Z = CH_2
6. β-SAD, X = Se

FIGURE 1

SAD mimic NAD but are unable to function as the coenzyme. Both compounds **3** and **4** inhibit some other dehydrogenases less effectively.

Enzyme-coenzyme interactions, especially in the case of dehydrogenases, are strongly dependent upon conformation of the bound cofactor. It was found[4] that unusually close intramolecular contact between the positively charged sulfur atom in the aglycone and the negatively charged sugar ring oxygen limits rotation about the C-glycosyl bond in the tiazofurin molecule. In the same manner, rotation around the glycosyl bond in the selenium analogue **2** is restricted[5,6]. Recently we observed[7] the same restriction in the glycosyl bond rotation in phosphodiesterase-resistant analogues of TAD and SAD (**5** and **6**) which contain the methylene group (Z = CH_2) instead of the pyrophosphate oxygen (Z = O). Our crystallographic studies[7] of the complexes with horse liver ADH and **5** and **6** revealed that the adenine moiety of ß-TAD and ß-SAD closely mimics the binding of the adenine part of NAD[8]. We also found that the structure of **5** and **6** in the enzyme-cofactor complex still showed that the close contact between lactol ring oxygen and sulfur or selenium is maintained. Thus, the selenazole (or thiazole) moiety of SAD (or TAD) is incapable of binding in the same "**anti**" conformation as NAD in the nicotinamide pocket of the ADH; it would require an approx. 50° rotation in the glycosidic torsion angle. Since

this is precluded, TAD and SAD do not fit to ADH as well as NAD. Since the crystal structure of the enzyme is not known, the requirements of IMPDH for specific conformation of the cofactor cannot be predicted. Nevertheless, the affinity of compounds **3** - **6** towards IMPDH seems to be much better than that for ADH, since all these analogues are more potent inhibitors for IMPDH than for ADH. This is rather surprising because IMPDH is a B-specific enzyme, *vide infra*, which is expected to bind the cofactor in the "**syn**" rather than "**anti**" conformation.

Enzymatic oxidation-reductions by all known dehydrogenases are stereospecific processes[9], *i.e.* only pro-R hydrogen of the dihydropyridine ring in NADH is transferred by some dehydrogenases (A-specific such as ADH), whereas others (B-specific, for example IMPDH) transfer exclusively the pro-S hydrogen[10]. The X-ray structures of various dehydrogenase-NAD complexes revealed that those enzymes that bind NADH in the "**syn**" conformation (FIG. 2) transfer only the pro-S hydrogen[11], whereas transfer of pro-R hydrogen requires NADH to be bound in the "**anti**" conformation. If conformation of the nicotinamide riboside moiety of NAD analogue is restricted to "**syn**", such NAD analogue may serve as a coenzyme for B-specific but not for A-specific dehydrogenases. Accordingly, "**anti**" analogue would not be able to function for B-specific enzymes. Consequently, the "**syn**" analogues would function as inhibitors only for A-specific enzymes, whereas the "**anti**" analogues would have inhibitory activity against B-specific dehydrogenases because binding of the adenine part of the conformationally restricted NAD analogues should not be affected. In order to test this hypothesis, we synthesized nucleoside and nucleotide analogues (FIG. 3) with restricted "**syn**" and "**anti**" conformation around the glycosyl bond of the nicotinamide riboside moiety.

In our first attempt, we synthesized[12] anhydro nucleosides **7a** and **7b** as close analogues of nicotinamide arabinoside[13] in which conformation is restricted to "**syn**" and "**anti**", respectively. These compounds, however, were too unstable for further conversion into their corresponding NAD analogues. All our attempts at phosphorylation of **7a** and **7b** resulted in the opening of the anhydro linkage of these compounds with simultaneous formation of the corresponding 1-ß-D-arabinofuranosyl -pyridone derivatives.

In this paper we describe the synthesis of conformationally restricted nicotinamide riboside derivatives and nicotinamide mononucleotide analogues (**8** and

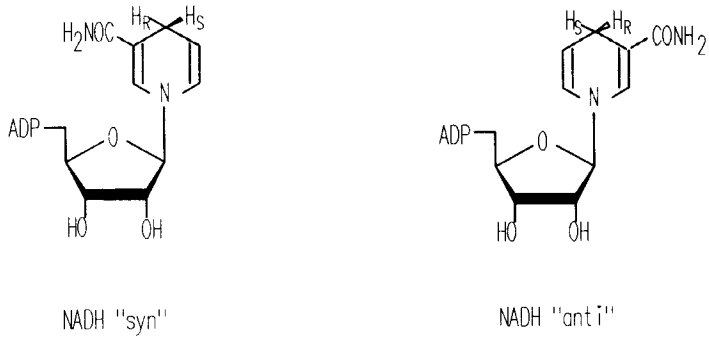


FIGURE 2

9, respectively, FIG. 3), in which the aglycone and sugar moiety are linked through a methylene bridge.

We also describe the synthesis of methylenediphosphonate analogues **19**, which contain nicotinamide moiety linked through a methylene group to the C3' of the ribosyl moiety.

RESULT AND DISCUSSION

As the starting material we used 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-α-D-*erythro*-3-pentulofuranose **10**[14] (SCHEME 1), which upon treatment with 6-methyl-nicotinamide and lithium diisopropylamide (LDA) in THF afforded 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-3(R)-(nicotinamid-6-ylmethyl)-α-D-ribofuranose (**11b**) in crystalline form (mp 186-187°C) in 52% yield. Due to steric hindrance of the 1,2-*O*-isopropylidene group, the attack of carbanion came from the ß-side of sugar **10** (*vide infra*). Because the silyl protecting group of **11b** was not stable during acidic de-*O*-isopropylidenation, it was replaced with benzoyl group to give **13b** in crystalline form (mp 215-218 °C). No benzoylation of 3'-hydroxyl group of **12b** was observed under the reaction conditions. The desired nucleoside **8b**, which is restricted to the "**anti**" conformation, was obtained as trifluoroacetic salt in 90% yield when derivative **13b** was treated with 90% CF_3COOH. ^1H NMR and MS(FAB) were consistent with the structure of **8b**. The resonance signals of H1' and H2' of **8b** appear as singlets, indicating that the dihedral

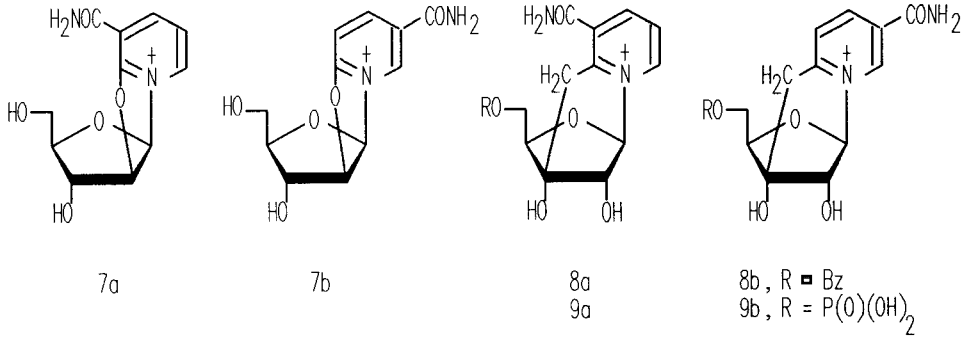
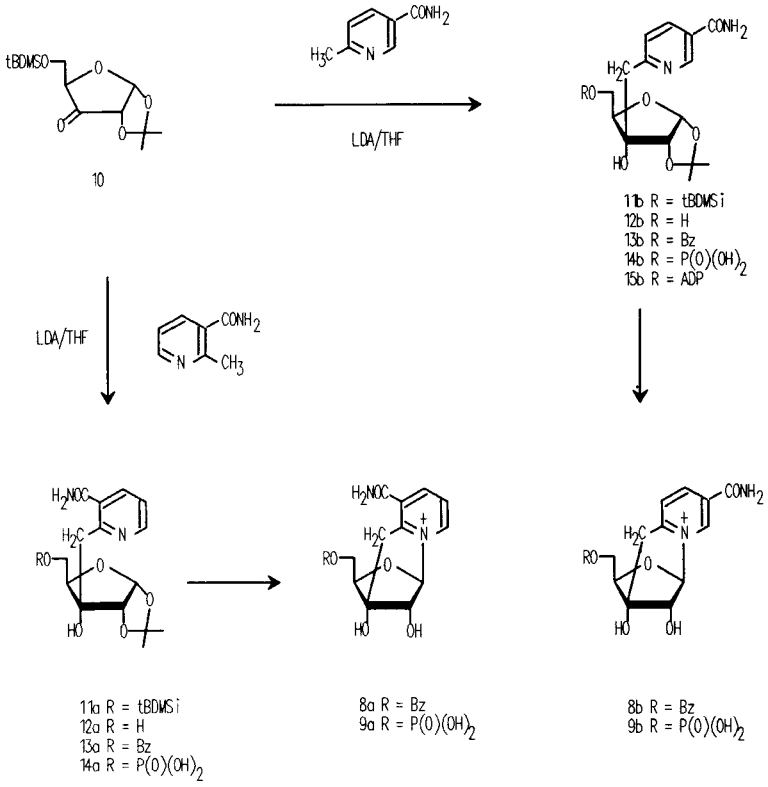


FIGURE 3



SCHEME 1

angle of H1'-C1'-C2'-H2' is close to 85°, and the cyclic nucleoside has the ß-configuration. This also confirmed the ß-configuration of **11**. It is interesting to note that geminal coupling of methylene bridge in **8b** (J=19.4 Hz) is larger than that of **11b-13b** (J=13.6-15.0) which may be due to severe strain in the rigid structure of **8b**.

Our attempted debenzoylation of **8b** was not successful due to instability of the glycosyl bond in basic conditions of the reaction.

It is known that nicotinamide mononucleotide (NMN) is more stable than nicotinamide riboside towards hydrolysis of the glycosyl bond. The conformationally restricted nucleotide **9b** would therefore be more hydrolytically stable than the corresponding nucleoside **8b**. Thus, we synthesized the acyclic nucleotide **14b** by treatment of **12b** with $POCl_3$ in triethylphosphate followed by HPLC purification. The mono-triethylammonium salt of **14b** obtained was converted into free acid by passing a water solution of the salt through a column of Dowex 50 x 8 (H^+ form). Although Dowex 50 x 8 (H^+ form) is used frequently for 2',3'-de-*O*-isopropylidenation, little 1',2'-deprotection was observed and the desired **14b** as a free acid was obtained in very good yield. Subsequent deisopropylidenation of **14b** (free acid) with CF_3COOH/H_2O (9:1, v/v) afforded the desired cyclic 5'-monophosphate **9b** in good yield. The ^{1}NMR and MS(FAB) spectra were consistent with the structure of **9b**. As expected, the negative charge of the 5'-phosphate group of **9b** rendered the compound less susceptible to hydrolysis. We found that the half life of compound **9b** in water was ca. 2 days.

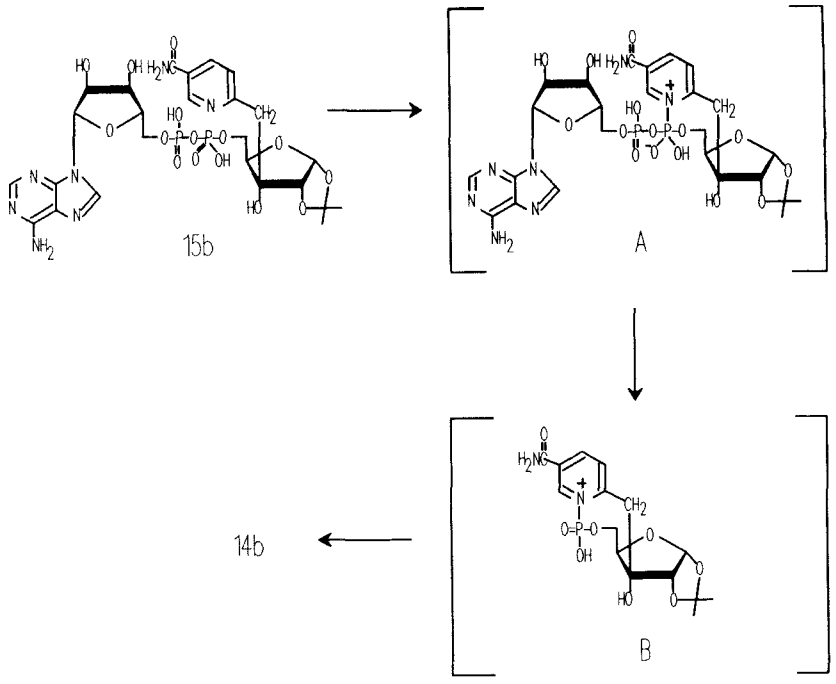
In a similar manner, the corresponding nucleotides **14a** and **9a** were prepared from **10** and 2-methylnicotinamide (prepared from ethyl 2-methylnicotinate).

Further coupling of the conformationally restricted, methylene-bridged 5'-O-monophosphates **9** with AMP to the corresponding NAD analogues failed due to cleavage of the glycosyl bond of **9** in basic reaction conditions (DCC-pyridine or CDI-DMF-n-butylamine). Therefore, we attempted coupling of the acyclic 5-*O*-monophosphate **14b** with AMP to the corresponding pyrophosphate **15b**, which under acidic conditions should undergo deisopropylidenation and cyclization to the desired product. To our surprise, however, the pyrophosphate linkage of dinucleotide **15b** was unstable under acidic conditions. Attempted conversion of the triethylammonium salt of **15b** into free acid with Dowex 50 (H^+ form) resulted in

cleavage of the pyrophosphate bond with formation of AMP and nucleotide **14b** (SCHEME 2). This could be explained by intramolecular assistance of the pyridine nitrogen of **15b** being involved in the cleavage. Computer minimized energy structure for **15b** indicates an extremely close contact between the nitrogen and one of the phosphorus atom (P^1) of the pyrophosphate group. Thus, attack of the nucleophilic nitrogen on the
phosphorus P^1 would result in the formation of pentacovalent intermediate [A], from which AMP would be released to give intermediate [B]. Upon addition of water, intermediate [B] is hydrolyzed into nucleotide **14b**.

In order to prevent such cleavage, we decided to synthesize the methylene diphosphonate derivatives **17**. Thus, the 2',3'-O-isopropylidene adenosine 5'-methylene-diphosphonate **16**[15] was coupled with nucleosides **12a** and **12b** to give the corresponding dinucleotides **17a** and **17b** (SCHEME 3), respectively. Upon treatment with CF_3COOH, compound **17b** cyclized to the desired methylene-bridged NAD analogue **18b** "**anti**". The formation of **18** was evident by the ^1H NMR analysis, *i.e.* the presence of AB system of the methylene-bridged protons at 3.47 and 3.61 ppm with the similar large coupling of 18.8 Hz as was found for cyclic derivatives **8** and **9**. The triplet at 2.41 ppm of the methylenediphosphonate with phosphorus-hydrogen coupling of 20.0 Hz as well as all signals of adenine and nicotinamide moieties also was observed. The cyclic compound **18b** was contaminated with α,ß-anomeric mixture of **19** up to 30% as judged by the presence of a second AB system at 3.43 and 3.50 ppm with smaller coupling constant $J_{A,B}$ = 10.6 Hz and the corresponding signals of adenine and nicotinamide. Dinucleotide **18b** was found to be unstable; upon addition of water compounds **18b** hydrolyzed into the corresponding NAD analogue **19b**, showing all characteristic resonances of **19b** which was first detected as contamination of **18b**. Such instability of methylene bisphosphonate analogues **19** versus the corresponding 5'-monophosphates **9** may be due to poor compensation of the positive charge of **19** by the weakly acidic phosphonic acid moiety of **19**. In contrast, the strongly acidic phosphoric acid moiety of **9** forms zwitterionic structure of **9** which should be less susceptible for the nucleophilic attack by water.

In summary, we prepared conformationally restricted ("**syn**" and "**anti**") analogues of nicotinamide mononucleotide as well as nicotinamide riboside

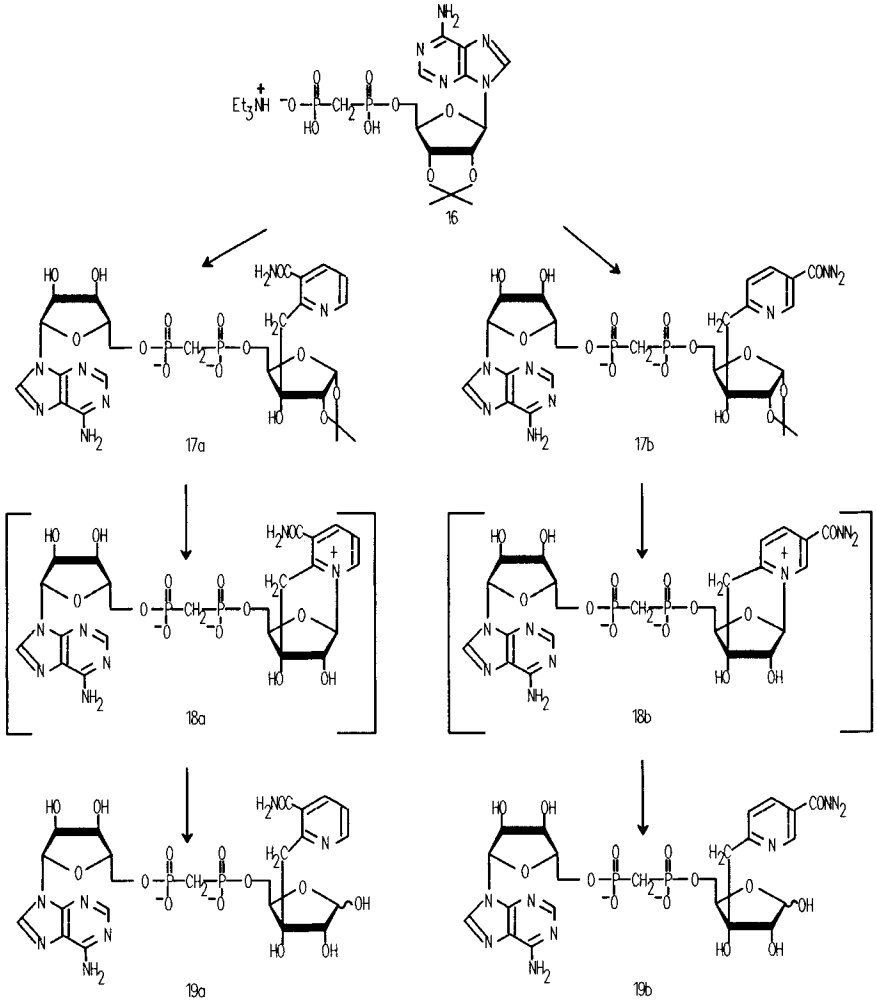


SCHEME 2

analogues, nicotinamide mononucleotide derivatives, and NAD analogues containing nicotinamide moiety linked through a methylene group at C3' of the riboside. Studies of inhibitory activity of these compounds against IMPDH and some cellular dehydrogenases are in progress.

EXPERIMENTAL SECTION

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Column chromatography was performed on silica gel G60 (70-230 mesh, ASTM, Merck). TLC was performed on Analtech Uniplates with short-wavelength UV light for visualization. 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 flow rate of 20 mL/min. of 0.1M TEAB followed by linear gradient of 0.1M TEAB - aq. acetonitrile (70%). Elemental analyses were performed by M-H-W Laboratories,



SCHEME 3

Phoenix, AZ. ^{1}H NMR spectra were recorded on a Bruker AMX-250 and 400 spectrometer with Me_4Si as the internal standard. 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.

1,2-*O*-Isopropylidene-5-*O*-*tert*-butyldimethylsilyl-3(R)-(nicotinamid-2-ylmethyl)-α-D-ribofuranose (11a). Dry ammonia was bubbled through a solution of ethyl 2-methylnicotinate (10 g, 0.06 mmol) in ethanol (250 mL) at 0° C for 2h. The solution was kept at room temperature for 2 days and then heated in a sealed steel cylinder at 80° C overnight. The mixture was concentrated *in vacuo* and the residue was chromatographed on a column of silica gel using $CHCl_3$-EtOH (2%) as the eluent to give unreacted ethyl 2-methylnicotinate (3.1 g) and the desired 2-methylnicotinamide (5.0 g, 61%) as crystalline compound, mp.157-158° C (EtOH) (lit.[16] mp. 158° C). ^1H NMR (Me_2SO-d_6) δ 2.54 (s, 3H, CH_3), 7.26 (dd, 1H, H5, $J_{4,5}$ = 7.6, $J_{5,6}$ = 4.8 Hz), 7.55 and 7.90 (two 1H bs, NH_2), 7.72 (dd, 1H, H4, $J_{4,6}$ = 1.4 Hz), 8.48 (dd, 1H, H6). A 1.5 M solution of lithium diisopropylamide mono(tetrahydrofuran) (LDA) in cyclohexane (6 mmol, 4 mL) was added to a suspension of 2-methylnicotinamide (2 mmol, 272 mg) in THF (15 mL) at -70° C. The mixture was stirred for 45 min. while the temperature was raised to -30° C. Then 1,2-*O*-isopropylidene-5-*O*-*tert*-butyldimethylsilyl-α-D-*erythro*-pentofuranose-3-ulose[14] (**10**, 2 mmol, 630 mg) in THF (5 mL) was added, and the mixture was stirred at -30° C for 1h and neutralized with CH_3COOH. The mixture was concentrated *in vacuo*, the residue was dissolved in EtOAc (100 mL), washed with water (3 x 30 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on a silica gel column using $CHCl_3$ followed by $CHCl_3$ - EtOH (2%) as the eluent to give **11a** (520 mg, 57.6%) as a foam. ^1H NMR ($CDCl_3$) δ 0.05 (s, 6H, 2 x CH_3Si), 0.85 (s, 9H, tBu), 1.25 (s, 3H, iPr), 1.49 (s, 3H, iPr), 2.94, 3.30 (AB system, 2H, 2-CH_2, $^2J_{H,H}$ =14.1 Hz), 3.80 - 3.92 (m, 2H, H5',5", $J_{5',5"}$ = 11.4 Hz), 4.01 (dd, 1H, H4', $J_{4',5'}$ = 3.5 Hz, $J_{4',5"}$ = 6.2 Hz), 4.84 (d, 1H, H2', $J_{1',2'}$ = 3.8 Hz), 5.91 (d, 1H, H1'), 7.23 (dd, 1H, H5, $J_{4,5}$ = 7.8, $J_{5,6}$ = 4.9 Hz), 6.56, 7.50 (two 1H bs, NH_2), 7.87 (dd, 1H, H4, $J_{4,6}$ = 1.5 Hz), 8.57 (dd, 1H, H6). Anal. calcd for: $C_{21}H_{34}N_2O_6Si$: C,57.51; H, 7.82; N, 6.30. Found: C, 57.46; H, 7.95; N, 6.20

1,2-*O*-Isopropylidene-5-*O*-*tert*-butyldimethylsilyl-3(R)-(nicotinamid-6-ylmethyl)-α-D-ribofuranose (11b). A 1.5 M solution of LDA in cycloxehane (6 mmol, 4 mL) was added to a suspension of 6-methylnicotinamide (2 mmol, 272 mg) in THF (15 mL) at -65°C. The temperature was raised to -30° C. The mixture was stirred for 30 min.

and a solution of 1,2-*O*-isopropylidene-5-*O*-*tert*-butyldimethylsilyl-α-D-*erythro*-pentofuranose-3-ulose (**10**, 2 mmol, 630 mg) in THF (5 mL) was added. The mixture was stirred at -30° C for 1h, then at room temperature for 30 min., and neutralized with CH_3COOH. The mixture was concentrated in *vacuo*, the residue was dissolved in EtOAc (100 mL) and washed with water (3 x 20 mL). The organic solution was dried ($MgSO_4$), concentrated in *vacuo*, and the oily residue was crystallized from EtOAc/EtOH to give **11b** (470 mg, 52%), mp. 186-187°C. ^1H NMR ($CDCl_3$) δ 0.1 (s, 6H, 2 x CH_3Si), 0.52 (s, 9H, tBu), 1.27 (s, 3H, iPr), 1.57 (s, 3H, iPr) 2.89, 3.21 (AB system, 2H, 6-CH_2, $J_{H,H}$ = 14.7 Hz), 3.88 (dd, 1H, H5', $J_{4',5'}$ = 6.1 Hz, $J_{5',5''}$ = 11.2 Hz), 3.95 (dd, 1H, H5", $J_{4',5''}$ = 3.8 Hz), 4.04 (dd, 1H, H4'), 4.25 (d, 1H, H2', $J_{1',2'}$ = 3.8 Hz), 5.84 (d, 1H, H1'), 7.31 (d, 1H, H5, $J_{4,5}$ = 8.1 Hz), 8.11 (dd, 1H, H4, $J_{2,4}$ = 2.1 Hz), 8.92 (d, 1H, H2). MS (FAB) m/e 437 $(M-H)^-$, 439 MH^+ Anal. calcd for: $C_{21}H_{34}N_2O_6Si$: C,57.51; H, 7.82; N, 6.30. Found: C,57.58; H,8.00; N, 6.39.

1,2-*O*-Isopropylidene-3(R)-(nicotinamid-2-ylmethyl)-α-D-ribofuranose (12a). Compound **11a** (510 mg, 1.1 mmol) was dissolved in 80% acidic acid, kept at room temperature for 2h, and then refrigerated overnight. The reaction mixture was concentrated in *vacuo*. The residue was coevaporated with toluene (2 x 100 mL), dissolved in water (10 mL), and lyophilized to give **12a** in quantitative yield. ^1H NMR ($CDCl_3$) δ 1.33 (s, 3H, iPr), 1.58 (s, 3H, iPr), 3.04, 3.43 (AB system, 2-CH_2, $^2J_{H,H}$ = 14.2 Hz), 3.86 - 4.00 (m, 2H. H5',H5", $J_{5',5''}$ = 12.2 Hz), 4.09 (t, 1H, H4', $J_{4',5'}$ = 3.8 Hz, $J_{4',5''}$ = 5.5 Hz), 4.86 (d. 1H, H2', $J_{1',2'}$ = 3.7 Hz), 5.99 (d, 1H, H1'), 6.03, 7.10 (two 1H bs, NH_2), 7.30 (dd, 1H, H5, $J_{4,5}$ = 7.8 Hz, $J_{5,6}$ = 4.8 Hz), 7.93 (d, 1H, H4), 8.62 (d, 1H, H6). Anal. calcd for $C_{15}H_{20}N_2O_6$: C, 55.53; H, 6.22; N, 8.64. Found: C, 55.40; H, 6.32; N, 8.60.

1,2-*O*-Isopropylidene-3(R)-(nicotinamid-6-ylmethyl)-α-D-ribofuranose(12b). Method A. Compound **11b** (2.8 g, 6.4 mmol) was dissolved in THF (25 mL) and this solution was treated with 1 M solution of TBAF in THF. The mixture was stirred at room temperature for 20 min. and concentrated in *vacuo*. The residue was then crystallized from $CH_3OH/CHCl_3$ to give **12b** (2.1 g, 98%) as white crystals, mp. 185-186° C. ^1H NMR (Me_2SO-d_6) δ 1.19 (s, 3H, iPr), 1.45 (s, 3H, iPr), 2.72, 2.93 (AB system, 6-CH_2, $J_{H,H}$ = 13.9 Hz), 3.55 (m, 1H, H5', $J_{5',5''}$ = 13.9 Hz), 3.65 (m, 1H, H5"),

3.93 (dd, 1H, H4', $J_{4',5'}$ = 1.9 Hz, $J_{4',5''}$ = 7.9 Hz), 4.33 (d, 1H, H2', $J_{1',2'}$ = 3.8 Hz), 4.80 (t, 1H, OH exchg.), 5.26 (s, 1H, OH exchg.), 5.78 (d, 1H, H1'), 7.48 (d, 1H, H5, $J_{4,5}$ = 8.1), 7.57 (s, 1H, NH_2 exchg.), 8.13 (s, 1H, NH_2 exchg.), 8.15 (dd, 1H, H4, $J_{2,4}$ = 2.2 Hz), 8.95 (d, 1H, H2). Anal. calcd for: $C_{15}H_{20}N_2O_6$: C, 55.53; H, 6.22; N, 8.64. Found: C, 55.34; H, 6.38; N, 8.52.

Method B. Compound **11b** (2.8 g, 6.4 mmol) was dissolved in 80% CH_3COOH (50 mL), the solution was kept at room temperature for 5 h and concentrated in *vacuo*. The residue was coevaporated with toluene and was crystallized as above to give **12b** in 98% yield.

1,2-*O*-Isopropylidene-5-*O*-benzoyl-3(R)-(nicotinamid-2-ylmethyl)-α-D-ribofuranose (13a). Benzoyl chloride (200 μL) was added to an ice-cooled solution of **12a** (100 mg, 0.31 mmol) in pyridine (5 mL). The mixture was kept at room temperature for 4 h. and concentrated in *vacuo*. The residue was coevaporated with toluene (3 x 10 mL), dissolved in $CHCl_3$ (20 mL) washed with water. The organic layer was dried (Na_2SO_4) and concentrated. The residue was chromatographed on a silica gel column using $CHCl_3$ as the eluent to give **13a** as an oil, which crystallized from $CHCl_3/Et_2O$ to give white crystals of **13a** (80 mg, 60%), mp 167-168° C. ^1H NMR ($CDCl_3$) δ 1.30 (s, 3H, iPr), 1.60 (s, 3H, iPr), 3.24, 3.37 (AB system, 2H, 6-CH_2, $J_{A,B}$ = 15.0 Hz), 4.37 (d, 1H, H2', $J_{1',2'}$ = 3.8 Hz), 4.38 (dd, 1H, H4', $J_{4',5'}$ = 6.9 Hz, $J_{4',5''}$ = 3.6 Hz), 4.55 (dd, 1H, H5', $J_{5',5''}$ = 12.0 Hz), 4.70 (dd, 1H, H5''), 5.96 (d, 1H, H1'), 7.37 (dd, 1H, H5, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 5.0 Hz), 7.43 (t, 2H, Bz), 7.55 (bt, 1H, Bz), 8.07 (dd, 2H, Bz), 7.98 (dd, 1H, H5, $J_{5,6}$ = 1.5 Hz), 8.08 (bd, 2H, Bz), 8.73 (d, 1H, H6).

MS (FAB) m/e 427 $(M-H)^-$, 429 MH^+. Anal. Calcd for $C_{22}H_{24}N_2O_7$: C, 61.68; H, 5.68; N, 6.54. Found: C, 61.58; H, 5.75; N, 6.46.

1,2-*O*-Isopropylidene-5-*O*-benzoyl-3(R)-(nicotinamid-6-ylmethyl)-α-D-ribofuranose (13b). Benzoyl chloride (680 μL) was added to an ice-cooled solution of **12b** (648 mg, 2 mmol) in pyridine (12 mL). The mixture was stirred at room temperature overnight and concentrated in *vacuo*. The residue was coevaporated with toluene (3 x 20 mL), dissolved in $CHCl_3$ (50 mL) and the solution was washed with water, saturated $NaHCO_3$ and water, then dried (Na_2So_4) and concentrated. The residue

was crystallized from $CHCl_3/Et_2O$ to give **13b** (580 mg, 68%), mp. 215-218°. ^1H NMR ($CDCl_3$) δ 1.30 (s, 3H, iPr), 1.61 (s, 3H, iPr), 2.97, 3.24 (AB system, 2H, 6-CH_2, $J_{A,B}$ = 14.6 Hz), 4.28 (d, 1H, H2', $J_{1',2'}$ = 3.7 Hz), 4.37 (dd, 1H, H4', $J_{4',5'}$ = 7.8 Hz, $J_{4',5''}$ = 3.5 Hz), 4.48 (dd, 1H, H5', $J_{5',5''}$ = 11.8 Hz), 4.65 (dd, 1H, H5"), 5.90 (d, 1H, H1'), 7.42 (d, 1H, H5, $J_{4,5}$ = 8.0 Hz), 7.45 (t, 2H, Bz), 7.55 (dt, 1H, Bz), 8.07 (dd, 2H, Bz), 8.15 (dd, 1H, H4, $J_{2,4}$ = 1.7 Hz), 8.94 (d, 1H, H2). MS (FAB) m/e 427 $(M-H)^-$, 429 MH^+. Anal. calcd for $C_{22}H_{24}N_2O_7$: C, 61.68; H, 5.68; N, 6.54. Found: C, 61.49; H, 5.85; N, 6.39.

1,2-*O*-Isopropylidene-3(R)-(nicotinamid-2-ylmethyl)-α-D-ribofuranose-5-monophosphate (14a). Compound **12a** (300 mg, 0.93 mmol) was added to a solution of $POCl_3$ (0.3 mL) in triethylphosphate (1.8 mL). The mixture was stirred at room temperature for 4 h , diluted with water (2 mL), and then neutralized with 2 M TEAB. The whole mixture was washed with Et_2O (3 x 30 mL) and $CHCl_3$ (2 x 20 mL) and concentrated in *vacuo*. The residue was chromatographed on a silica gel column with CH_3CN-water (9:1, v/v) to give **14a** (270 mg, 57.5 %) as mono-triethylammonium salt. ^1H NMR (D_2O) δ 1.22 (t, 9H, Et_3N), 1.25 (s, 3H, iPr), 1.52 (s, 3H, iPr), 3.15 (q, 6H, Et_3N), 2.98, 3.32 (AB system, 2H, 2-CH_2, $J_{H,H}$ = 14.6 Hz), 3.85-4.10 (m, 2H, H5', H5", $J_{5',5''}$ = 11.5 Hz, $J_{5',P}$ = 5.4.0 Hz, $J_{5'',P}$ = 5.2 Hz), 4.24 (dd, 1H, H4', $J_{4',5'}$ = 2.7, $J_{4',5''}$ = 7.5 Hz), 4.46 (d, 1H, H2', $J_{1',2'}$ = 3.7 Hz), 5.96 (d, 1H, H1'), 7.44 (dd, 1H, H5, $J_{4,5}$ = 8.1 Hz, $J_{4,6}$ = 1.5 Hz), 7.97 (dd, 1H, H4, $J_{4,5}$ = 8.1 Hz), 8.60 (dd, 1H, H6, $J_{5,6}$ = 5.8 Hz). ^{31}P NMR (D_2O) δ = -1.02. After passing through a column of Dowex 50 x 8 (H^+ form), compound **14a** was obtained as free acid. ^1H NMR (D_2O) δ 1.24 (s, 3H, iPr), 1.52 (s, 3H, iPr), 3.37, 3.40 (AB system, 2H, 2-CH_2, $J_{H,H}$ = 14.8 Hz), 3.75-4.20 (m, 2H, H5', H5"), 4.25 (dd, 1H, H4', $J_{4',5'}$ = 3.6, $J_{4',5''}$ = 6.8 Hz), 4.28 (d, 1H, H2', $J_{1',2'}$ = 3.7 Hz), 5.96 (d, 1H, H1'), 7.94 (dd, 1H, H5, $J_{4,5}$ = 8.1 Hz, $J_{4,6}$ = 1.5 Hz), 8.58 (dd, 1H, H4, $J_{4,5}$ = 8.1 Hz), 8.77 (dd, 1H, H6, $J_{5,6}$ = 5.8 Hz). ^{31}P NMR (D_2O) δ = -0.76. Anal. calcd for $C_{15}H_{21}N_2O_9P$: C, 44,56; H, 5.23; N, 6.93. Found: C, 44,46; H, 5.35; N, 6.80.

1,2-*O*-Isopropylidene-3(R)-(nicotinamid-6-ylmethyl)-α-D-ribofuranose-5-monophosphate (14b). Compound **12b** (300 mg, 0.93 mmol) was treated with $POCl_3$ (0.3 mL) in the manner described above to give **14b** (300 mg, 64%) as mono-

triethylammonium salt. ^{1}H NMR (D_2O) δ 1.31 (t, 9H, Et_3N), 1.34 (s, 3H, iPr), 1.60 (s, 3H, iPr), 3.23 (q, 6H, Et_3N), 3.12, 3.36 (AB system, 2H, 6-CH_2, $J_{A,B}$ = 14.6 Hz), 4.09-4.16 (m, 1H, H5', $J_{5',5''}$ = 11.4 Hz, $J_{5',P}$ = 7.0 Hz), 4.20-4.26 (m, 1H, H5", $J_{5'',P}$ = 5.7Hz), 4.34 (dd, 1H, H4', $J_{4',5'}$ = 7.0, $J_{4',5''}$ = 3.2 Hz), 4.48 (d, 1H, H2', $J_{1',2'}$ = 3.7 Hz), 6.06 (d, 1H, H1'), 7.81 (d,1H, H5, $J_{4,5}$ = 8.3 Hz), 8.43 (dd, 1H, H4, $J_{2,4}$ = 2.2 Hz), 9.00 (d, 1H, H2). ^{31}P NMR (D_2O) δ = -0.8
^{13}C NMR (D_2O) δ 9.2 (Et_3N), 26,3, 26,4 (iPr), 38.2 (6-CH_2), 47.7 (Et_3N), 64.5 (d, C5', $^2J_{P,C}$ = 5.3 Hz), 80.0 (C2'), 81.2 (C3'), 81.5 (d, C4', $^3J_{P,C}$ =8.0 Hz), 104.4 (C1'), 114.6 (iPr), 140.2 (C5), 146.6 (C4), 159 (C2). Compound **14b** (free acid) ^{31}P NMR (D_2O) δ -0.76. Anal. calcd for $C_{15}H_{21}N_2O_9P$: C, 44,56; H, 5.23; N, 6.93. Found: C, 44,44; H, 5.38; N, 6.76.

P^1-[1,2-*O*-Isopropylidene-3(R)-(nicotinamid-6-ylmethyl)-α-D-ribofuranos-5-yl]-P^2-(adenosin-5'-yl)pyrophosphate (15b). Adenosine 5'-monophosphate (110 mg, 0.3 mmol) was activated with cabonyldiimidazole (243 mg, 1.5 mmol)in DMF (1 mL) and coupled with **14b** (120 mg, 0.3 mmol) for 4 days as described by Marquez[17]. The crude product was purified on HPLC column using 0.1 M TEAB followed by linear gradient of 0.1 M TEAB-aq. acetonitrile (70 %) to give **15b** (41 mg, 17.3 %) as triethylammonium salt. ^{1}H NMR (D_2O) δ 1.27 (s , 3H, iPr), 1.31 (t, 9H, Et_3N), 1.45 (s. 3H, iPr), 2.52, 2.74 (AB system, 2H, 6-CH_2, $J_{H,H}$ = 14.0 Hz), 3.20 (q, 6H, Et_3N), 4.00-4.42 [m, 7H, H2'(N), H4'(N), H5'(N), H5"(N), H4'(A), H5'(A), H5"(A)], 4.35 (d, 1H, H2'(N), $J_{1',2'}$ = 3.6 Hz), 4.50 (t, 1H, H3'(A), $J_{2',3'}$ = $J_{3',4'}$ = 5.2 Hz), 4.61 (t, 1H, H2'(A), $J_{1',2'}$ = 5.6 Hz), 5.93 (d, 1H, H1'(N), $J_{1',2'}$ = 3.6 Hz), 6.03 (d, 1H, H1'(A), 7.25 (d, 1H, H5(N), $J_{4,5}$ = 8.2 Hz), 7.98 (dd, 1H, H4(N), $J_{2,4}$ = 2.5 Hz), 8.02, 8.48 (two 1H s, H2(A), H8 (A), 8.70 (d, 1H, H2(N). ^{31}P NMR (D_2O) δ -10.9 (s). This product was dissolved in water (2 mL) and applied on a column of Dowex 50x8 (H^+ form). UV absorbing fractions were collected and lyophilized to give the residue as a white foam. ^{31}P NMR (D_2O), δ -0.8(s). The residue was chromatographed on the HPLC column to give AMP (11 mg) and **14b** (7 mg) identical with original samples.

3',6-methylene-1-(5-*O*-benzoyl-ß-D-ribofuranose)-3-carboxamidpyridinium trifluoroacetate (8b). Compound **13b** (354 mg, 0.8 mmol) was dissolved in a mixture of CF_3COOH/water (9:1, v/v), stirred at room temperature for 1.5 h,and concentrated

in *vacuo*. The residue was coevaporated with water (3 x 10 mL), dissolved in water and lyophilized to give **8b** (300 mg) as a foam. ^{1}H NMR (D_2O) δ 3.62, 3.72 (AB system, 2H, 6-CH_2, $J_{A,B}$ = 19.4 Hz), 4.28 (dd, 1H, H4', $J_{4',5'}$ = 7.9 Hz, $J_{4',5''}$ = 2.3 Hz), 4.37 (s, 1H, H2'), 4.50 (dd, 1H, H5', $J_{5',5''}$ =11.2 Hz), 4.65 (dd, 1H, H5"), 6.16 (s, 1H, H1'), 7.49, (t, 2H, Bz), 7.62 (t, 1H, Bz), 8.04 (d, 1H, H5, $J_{4,5}$ = 8.6 Hz), 8.09 (d, 2H, Bz), 8.82 (d, 1H, H4), 9.60 (s, 1H, H2). MS (FAB) M^+ 371, MH^+, 372.

2,3'-methylene-1-(ß-D-ribofuranose)-3-carboxamidpyridinium-5'-monophosphate (9a). A solution of **14a** (free acid, 100mg, 0.25 mmol) in 90% CF_3COOH (5 mL) was kept at room temperature for 1.5 h and concentrated *in vacuo*. The residue was coevaporated with water (3 x 5 mL) and lyophilized to give **9a** (73 mg) as a foam. ^{1}H NMR (D_2O) δ 3.30, 3.47 (AB system, 2H, 6-CH_2, $J_{A,B}$ = 19.0 Hz), 3.75-4.10 (m, H4', 5', 5"), 4.16 (d, 1H, H2', $J_{1',2'}$ = 2.2 Hz), 5.90 (d, 1H, H1'), 7.81 (t, 1H, H5), 8.30 (dd, 1H, H4, $J_{4,5}$ = 7.7 Hz, $J_{5,6}$ = 1.0 Hz), 8.94 (d, 1H, H6, $J_{5,6}$ = 6.4 Hz). MS (FAB) M^+ 347, MH^+ 348.

3',6-methylene-1-(ß-D-ribofuranose)-3-carboxamidpyridinium-5'-monophosphate (9b). Compound **14b** (100 mg, 0.25 mmol) was treated with 90% CF_3COOH as described above. After lyophilization **9b** (65 mg) was obtained as a white foam. ^{1}H NMR (D_2O) δ 3.51, 3.36 (AB system, 2H, 6-CH_2, $J_{A,B}$ = 19.1 Hz), 3.73-4.12 (m, H4', 5', 5"), 4.20 (s, 1H, H2'), 5.92 (s, 1H, H1'), 7.78 (d, 1H, H5, $J_{4,5}$ = 8.3 Hz), 8.50 (d, 1H, H4), 9.20 (s, 1H, H2). MS(FAB) M^+ 347. MH^+ 348.

P^1-[1,2-*O*-isopropylidene-3(R)-(nicotinamid-2-ylmethyl)-α-D-ribofuranose-5-yl]-P^2-(2',3'-*O*-isopropylideneadenosin-5'-yl)-methylenediphosphonate (17a). Mono triethylammonium salt of 2',3'-*O*-isopropylidneneadenosine-5'-methylene-diphosphonate (**16**)[15] (57 mg, 0.1 mmol) was dissolved in pyridine (0.8 mL) and a solution of DCC (51.5 mg) in pyridine (0.4 mL) was added. The reaction mixture was stirred at room temperature for 24 h and a solution of **12a** (17 mg, 0.05 mmol) in pyridine (0.3 mL) was added. The mixture was kept at 65° C for 12 h and concentrated in *vacuo*. The residue was treated with a 1:1 mixture of ethyl acetate and water; the precipitate of dicyclohexylurea was filtered and the water solution was separated, its pH was adjusted to 9 with Et_3N, kept at room temperature for 24 h,

and lyophilized. The residue was purified by HPLC to give the unreacted **16** (10 mg) and the desired dinucleotide **17a** (26 mg, 59.7%) as the triethylammonium salt. ^{1}H NMR (D_2O), 1.19 (t, 18H, Et_3N), 1.28, [s, 3H, iPr(A)], 1.52 [s, 3H, iPr(A)], 1.45 [s, 3H, iPr(N), 1.67 [s, 3H, iPr(N)], 2.21 (t, 2H, P-CH_2-P, $J_{P,H}$ = 19.8 Hz), 2.47, 3.16 (AB system, 2H, -CH_2-nicotinamide, $J_{A,B}$ = 14.4 Hz), 2.96 (q, 12H, Et_3N), 3.88-4.02 and 4.03-4.17 [two m, 2H, H5', H5" (N)], 4.17-4.27 [m, 3H, H5', H5"(A), H4'(N)], 4.61 [d, 1H, H2'(N), $J_{1',2'}$ = 3.7 Hz), 4.60-4.66 [m, 1H, H4'(A)], 5.24, 5.37 (d of AB system, 2H, H2', H3' (A), $J_{2',3'}$ = 6.3 Hz, $J_{3',4'}$ = 2.4 Hz], 5.90 [d, 1H, H1'(N)], 6.18 [d, 1H, H1'(A), $J_{1',2'}$ = 3.7 Hz], 7.44 [dd, 1H, H6(N), $J_{4,6}$ = 5.0 Hz, $J_{5,6}$ = 7.9 Hz], 7.98 [dd, 1H, H5(N), $J_{4,5}$ = 1.7 Hz], 8.11, 8.52 [two 1H singlets, H2, H8 (A)], 8.55 [dd, 1H, H4(N)]. ^{31}P NMR (D_2O) AB system δ_A = 17.8, J_B = 17.6, $J_{A,B}$ = 10.9 Hz. MS(FAB) M^+ 731, MH^+ 732.

P^1-[1,2-*O*-isopropylidene-3(R)-(nicotinamid-6-ylmethyl)-α-D-ribofuranose-5-yl]-P^2-(2',3'-*O*-isopropylideneadenosin-5'-yl)-methylenediphosphonate (17b). In a similar manner as above, **17b** was obtained. ^{1}H NMR (D_2O), 1.19 (t, 18H, Et_3N), 1.22, [s, 3H, iPr(A)], 1.49 [s, 3H, iPr(A)], 1.41 [s, 3H, iPr(N), 1.64 [s, 3H, iPr(N)], 2.22 (dt, 2H, P-CH_2-P, $J_{P,H}$ = 20.0 Hz, $J_{H,H}$ = 1.48 Hz), 2.58, 2.95 (AB system, 2H, -CH_2-nicotinamide, $J_{A,B}$ = 14.1 Hz), 2.84 (q, 12H, Et_3N), 4.03-4.17 [m, 2H, H5', H5" (N)], 4.15-4.28 [m, 3H, H5', H5"(A), H4'(N)], 4.32 [d, 1H, H2'(N), $J_{1',2'}$ = 3.7 Hz], 4.60-4.66 [m, 1H, H4'(A)], 5.19, 5.21 [AB system, 2H, H2', H3' (A), $J_{A,B}$ = 6.4 Hz], 5.90 [d, 1H, H1'(N)], 6.14 [d, 1H, H1'(A), $J_{1',2'}$ = 2.1 Hz], 7.36 [d, 1H, H3(N), $J_{3,4}$ = 8.2 Hz], 7.96 [dd, 1H, H4(N), $J_{4,6}$ = 2.3 Hz], 8.04, 8.45 [two 1H singlets, H2, H8 (A)], 8.72 [d, 1H, H6(N)]. ^{31}P NMR (D_2O) δ = 17.8 Hz (s). MS(FAB) M^+ 731, MH^+ 732.

P^1-[6,3'-methylene-1-(ß-D-ribofuranos-5-yl)-3-carboxamidpyridinium]-P^2-(adenosin-5'-yl)methylenediphosphonate (18 "anti"). Compound **17b** (10 mg, 0.01 mmol) was dissolved in a mixture of CF_3COOD/D_2O (9:1, v/v) in NMR tube and the proton resonance spectrum was recorded after 15 min. ^{1}H NMR (δ) 1.14 (t, 18H, 2 x Et_3N), 2.07 (s, 12H, 2 x CH_3CO), 2.41 (t, 2H, P-CH_2-P, $J_{P,H}$ = 20.0 Hz), 3.04 (q, 6H, 2 x Et_3N), 3.47, 3.61 (AB system, 2H, -CH_2-nicotinamide, $J_{A,B}$ = 18.8 Hz), 3.91-4.33 [m, 7H, H5'(N), H5"(N), H5'(A), H5"(A), H4'(N), H4'(A), H2'(N)], 5.96-6.06 [m, 2H, H1'(A), H1'(N)], 7.91 [d, 1H, H3(N), $J_{3,4}$ = 8.0 Hz], 8.30, 8.45 [two 1H singets,

H2(A), H8(A)], 8.59 [d, 1H, H4(N)], 8.93 [s, 1H, H6(N)]. In this sample, about 30% of open product **19b** was detected at resonances at δ 3.43, 3.50 (AB system, -CH_2-nicotinamide, $J_{A,B}$ = 10.6 Hz), 8.01 [d, 1H, H3(N), $J_{3,4}$ = 8.0 Hz], 8.64 [d, 1H, H4(N)], 8.93 [s, 1H, H6(N)]

P^1-[3(R)-(nicotinamid-2-ylmethyl)-α,ß-D-ribofuranos-5-yl]-P^2-(adenosin-5'-yl)-methylenediphosphonate (19a). Compound **17a** (20 mg, 0.02 mg) was dissolved in a mixture of CF_3COOH/H_2O (9:1, v/v, 1 mL), kept at room temperature for 0.5 h, and then lyophilized. The residue was dissolved in water (1mL) and the pH of the solution was adjusted to 7 by addition of Et_3N. The ^1H NMR spectrum showed the formation of a α,ß-mixture of **19a** in the ratio of 1.7:1.0 as judged by integration of the anomeric protons. This mixture was purified on HPLC to give **19a** (25 mg) as the mixture of α,ß-anomers in the same ratio. ^{1} H NMR (D_2O), δ, 1.23 (t, 18H, 2 x Et_3N), 2.26 (t 2H, P-CH_2-P, $J_{P,H}$ = 20.0 Hz), 3.12 (q, 14H, -CH_2-nicotinamide, 2 x Et_3N), 3.90-4.30 [m, 6H, H4'(N), H4'(A), H5"(N), H5"(A), H5'(N), H5'(A)], 4.40-4.48 [m, 1H, H2'(N)], 4.50-4.57 [m, 1H, H3'(A)], 4.63-4.70 [m, H2'(A)], 5.15, 5.42 [two 1H doublets in ratio 1.7:1.0, H1'(N), $J_{1',2'}$ = 5.4 Hz, $J_{1',2'}$ = 5.0 Hz), 6.02 [d, 1H, H2'(N), $J_{1',2'}$ = 5.3 Hz], 7.55 [dd, 1H, H5(N), $J_{4,5}$ = 5.0 Hz], 8.02 [d, 1H, H6(N)], 8.12, 8.55 [two 1H singlets, 2H, H2(A), H8(A)], 8.60 [d, 1H, H6(N)]. $_{31}$P NMR δ, 17.55, 17.85 (AB system, $J_{P,P}$ = 11.0 Hz), 17.80, 18.10 (AB system, $J_{P,P}$ = 11.3 Hz). These two signals were in ratio of 1.7:1.0. MS(FAB) M^+ 691, MH^+ 692.

P^1-[3(R)-(nicotinamid-6-ylmethyl)-α,ß-D-ribofuranos-5-yl]-P^2-(adenosin-5'-yl)-methylenediphosphonate (19b). In the same manner as above **19b** was obtained as a mixture of α,ß anomers in ratio of 1.6:1.0. $_1$H NMR (D_2O), δ, 1.24 (t, 18H, 2 x Et_3N), 2.26 (t 2H, P-CH_2-P, $J_{P,H}$ = 20.5 Hz), 2.28 (t, 2H, P-CH_2-P, $J_{P,H}$ = 20.0 Hz), 3.12 (q, 14H, -CH_2-nicotinamide, 2 x Et_3N), 3.94-4.31 [m, 6H, H4'(N), H4'(A), H5"(N), H5"(A), H5'(N), H5'(A)], 4.31-4.40 [m, 1H, H2'(N)], 4.48-4.55 [m, 1H, H3'(A)], 4.61-4.68 [m, H2'(A)], 5.14, 5.41 [two 1H doublets in ratio 1.7:1.0, H1'(N), $J_{1',2'}$ = 5.4 Hz, $J_{1',2'}$ = 5.0 Hz), 6.00, 6.02 [two d, 1H, H2'(N), $J_{1',2'}$ = 5.3 Hz], 7.30 [d, 1H, H3(N), $J_{3,4}$ = 8.0 Hz], 7.91 [d, 1H, H4(N)], 8.07, 8.09 and 8.48, 8.50 (four 1H singlets, 4H, H2(A), H8(A)], 8.62 [s, 1H, H6(N)]. $_{31}$P NMR δ, 17.45, 17.96 (AB

system, $J_{P,P}$ = 11.3 Hz), 17.82, 18.07 (AB system, $J_{P,P}$ = 11.4 Hz). These two signals were in ratio of 1.6:1.0. MS(FAB) M^+ 691, MH^+ 692.

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