Enzymatic Synthesis of New Pyridine Nucleosides. Clitidine and Its Amide Derivative[1)]

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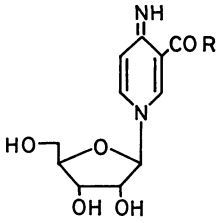
(Received February 28, 1980)

Two β-NAD-analogs were prepared utilizing a base-exchange reaction catalyzed by pig-brain NADase. These analogs were proved to contain a methyl 1,4-dihydro-4-iminonicotinate and a 1,4-dihydro-4-iminonicotinamide moiety. By successive hydrolytic degradations of these analogs, 1,4-dihydro-4-iminopyridine β-ribosides with 3-carboxyl and 3-carbamoyl groups were prepared in good yields. The former compound was identical with clitidine, a toxic pyridine nucleoside recently isolated from a toadstool. The present synthesis confirmed the 1,4-dihydro-4-imino β-riboside structure of clitidine. Preliminary biological tests showed that the 3-carbamoyl compound was more toxic than clitidine, suggesting that the amide derivative of clitidine is an essential toxic substance in the toadstool.

Recently, Konno *et al.*[2)] have isolated a new pyridine nucleoside, clitidine, as a toxic substance from a toadstool, *Clitocybe acromelalga*. Although the structure, 1-(β-D-ribofuranosyl)-1,4-dihydro-4-iminonicotinic acid (**1**), was assigned to the nucleoside by synthesis, no clear evidence has been given for the 1,4-dihydropyridine form and the β-configuration of the glycosidic linkage.

The pharmaceutical properties of clitidine were investigated in detail[3)] and it was pointed out that its toxicity was relatively weak for a toxic substance from a toadstool. We, therefore, had a doubt whether clitidine itself was a major component of the toxic substance or not. It was assumed that the substance might be an amide form (**2**) in the toadstool.



1 R = OH
2 R = NH_2

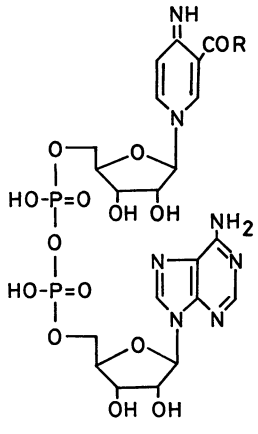
In order to clarify the ambiguous points, we tried to synthesize these pyridine nucleosides by enzyme-catalyzed reactions. Compared with the chemical synthesis by Konno *et al.*,[2)] which consists of six steps involving a laborious process, the enzymatic synthesis can be performed in three steps with high stereospecificity and good yield of product under mild conditions.

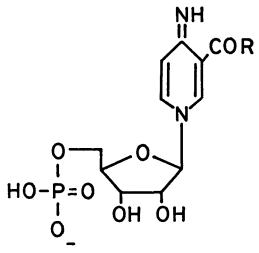
Nicotinamide ribonucleoside[4)] has been prepared by successive enzymatic cleavages of β-NAD. On the other hand, many NAD-analogs[5)] have been prepared by utilizing pig-brain NADase which has β-transglycosidase action along with glycohydrolase activity. These methods were successfully applied to the case of the derivatives of 4-aminonicotinic acid.

The present paper describes details of the preparation of the new NAD-analogs (**4**, **6**), and the degradative synthesis of clitidine (**1**) and its amide derivative (**2**) from the analogs.

Results and Discussion

Preparation of NAD-analogs. When 4-aminonicotinic acid and β-NAD were incubated with pig-brain NADase, only ADP-ribose was produced and no NAD-analog could be detected in which a nicotinamide moiety was replaced by 4-aminonicotinic acid. On the other hand, when methyl 4-aminonicotinate was used as a replacing base, the exchange reaction occurred successfully and yielded the expected analog together with ADP-ribose. These two compounds could be separated and purified by chromatography on DEAE-Sephadex; they provided the ester analog (**4**) and ADP-ribose (**7**) in a ratio of about 2:1. The IR spectrum of **4** showed an ester carbonyl band at 1715 cm^{-1} in place of an amide carbonyl band. The ^{1}H-NMR spectrum also showed an ester methyl group at δ 3.95 (s, 3H) and three protons on the 3,4-disubstituted pyridine ring, together with those of an ADP-ribosyl group (Table 2), indicative of the NAD-analog containing a methyl 4-aminonicotinate moiety.

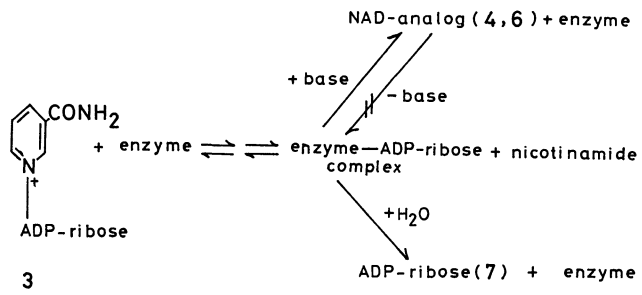




4 R = OCH_3
5 R = OH
6 R = NH_2

10 R = OCH_3
11 R = OH
12 R = NH_2

The formation of a NAD-analog in this base-exchange reaction is considered to proceed in competition with water for an enzyme–ADP-ribose complex



Scheme 1.

(Scheme 1).[6] According to this assumption, a base concentration would have a marked influence on the formation of the analog. In fact, the yield of **4** (based on NAD used) was apparently dependent on the base concentration (Table 1). When NAD was kept at a definite concentration, the yield increased with the concentration of methyl 4-aminonicotinate, becoming almost constant above 25 mM, where the reaction mixture attained a nearly saturated solution of the base. However, the yield was essentially unaffected by the NAD concentrations (Table 1). In the previous communication,[1] the base/NAD in 4:1 ratio has been proved to be useful for this reaction. Accordingly, it was practical to perform the reaction around 7 mM (1 M=1 mol dm^{-3}) NAD and at not less than 25 mM base.

The amide analog (**6**) was also prepared from 4-aminonicotinamide and NAD in a similar manner. In this case, the base concentration could be raised above 45 mM, resulting in a notably higher yield of **6** as compared with that of **4** (Table 1). The ^{1}H-NMR spectrum of **6** showed essentially the same pattern as that of **4** except for the absence of the signal at δ 3.95.

The demethylated analog (**5**) was obtained in 84% yield by treating **4** with 0.2 M NH_4OH at 40 °C for 12 h. In this case, no cleavage of the glycosidic linkage between pyridine and ADP-ribose moieties was observed during the reaction, although NAD was easily cleaved under the same conditions.

The NAD-analogs prepared by Kaplan *et al.*[5] were

Table 1. Effect of the base concentration on the yield of the analogs

Base concn/mM	Yield (%)[a] of analogs			
	4		**6**	
	4 mM NAD	8 mM NAD	4 mM NAD	8 mM NAD
5	23	14	39	32
10	31	40	52	54
15	49	52	65	64
20	55	57	70	73
25	62	63	74	76
30	63	65	76	77
40	—	—	79	78
50	—	—	79	80

a) The yield was estimated on TLC using a thin layer scanner (see Experimental).

all susceptible, more or less, to the hydrolase action of NADase. On the other hand, the new analogs (**4**—**6**) were resistant to this action even if they were incubated with NADase for 24 h. These findings show the irreversible formation of the new analogs at the final step (Scheme 1), suggesting the difference in the character of glycosidic linkage between the two kinds of analogs.

Degradation of NAD-analogs. Nucleosides were prepared *via* mononucleotides by hydrolytic cleavages of the analogs obtained above.

By treatment with phosphodiesterase (PDE, optimum pH 9), followed by chromatographic purification, compounds **5** and **6** gave demethyl and amide mononucleotides (**11**, **12**) in quantitative yield, respectively, along with adenylic acid (**13**).

Compound **4** was found to demethylate slowly during the incubation at pH 9, although methyl 4-aminonicotinate itself remained unchanged under the same conditions. Thus, treatment of **4** with PDE resulted in the formation of a mixture of ester mononucleotide (**10**) and **11** in a ratio of about 1:1. On the other hand, treatment of **4** with pyrophosphatase (optimum pH 7) gave **10** predominantly in 85% yield together with a small amount of **11**. Accordingly, it is preferable to use pyrophosphatase for the preparation of **10**, though the reaction rate is slower than for PDE because of the product inhibition[7] by the adenylic acid formed.

Incubation of **11** with 5′-nucleotidase at pH 9, followed by column chromatography, provided the desired nucleoside (**1**) quantitatively; on crystallization from aqueous ethanol it gave white needles: mp 205—207 °C (dec), $[\alpha]_D^{20}=-53.4°$ (H_2O), λ_{max} 271 nm (log ε 4.32, H_2O). The mixed mp of **1** with authentic clitidine was undepressed, and the spectral properties and TLC mobility were also in good agreement with those of the sample.

In a similar manner, an amide nucleoside (**2**) was obtained in nearly quantitative yield by dephosphorylation of **12**. However, unlike the case of **1**, the purification of **2** could not be achieved by the ion exchange chromatography alone owing to concomitance with inorganic salts. In order to remove the impurities, further repeated chromatography on Sephadex G-10 was required and the nucleoside was purified to give a white crystalline mass: mp 145—147 °C (dec), $[\alpha]_D^{20}=-62.8°$ (H_2O), λ_{max} 272 nm (log ε 4.30, H_2O).

Dephosphorylation of **10** was also attempted at pH 8, where the ester moiety remained unchanged. Although the reaction was observed to proceed successfully on TLC, the ester nucleoside could not be obtained, contrary to our expectation, because the ester was readily hydrolyzed during the purification procedures (pH 7).

Some Chemical Aspects of the Compounds Obtained. In the ^{1}H-NMR spectra (Table 2), two anomeric protons of the new analogs (**4**, **6**) appeared as two clear doublets around δ 5.6 (J=5.0 Hz) and δ 6.0 Hz (J=5.5 Hz), separately. Based on the spectral pattern of the mononucleotides (**10**—**12**) and adenylic acid (**13**), the former doublet was assigned to the anomeric proton of the pyridine side. On the other

Table 2. The ^{1}H-NMR spectra of major products and related compounds[a)]

Compd no.	Chemical shifts/δ[b)]							Coupling constants/Hz[c,d)]	
	py-H_2	py-H_5	py-H_6	py-$C_1'H$	ad-H_2	ad-H_8	ad-$C_1'H$	$J_{2,6}$	$J_{5,6}$
3	9.47 s	8.35 dd	9.33 d	6.1 bd	8.42 s	8.59 s	6.1 bd	0.0	6.0
4	8.75 d	6.90 d	8.13 dd	5.64 d	8.08 s	8.32 s	6.00 d	1.5	7.5
6	8.55 d	6.85 d	8.10 dd	5.60 d	8.02 s	8.35 s	6.00 d	1.5	7.5
9	8.71 s	7.75 d	8.63 d	6.0 bd	8.02 s	8.33 s	6.0 bd	0.0	6.0
11	8.73 d	7.03 d	8.25 dd	5.72 d	—	—	—	1.5	7.5
12	8.80 d	7.10 d	8.30 dd	5.65 d	—	—	—	1.5	7.5
13	—	—	—	—	8.10 s	8.50 s	6.05 d	—	—
1	8.70 d	6.92 d	8.10 dd	5.67 d	—	—	—	1.5	7.5
2	8.75 d	6.97 d	8.18 dd	5.65 d	—	—	—	1.5	7.5

a) The spectra were measured in D_2O at 60 MHz, and the abbreviations "py-H_2 and ad-H_2" refer to the protons at C_2 of pyridine and adenine nuclei, respectively. b) The chemical shifts of the ribose protons fell within δ 3.8—4.5 as a broad peak (**4**, **6**) or two broad singlets (**11**, **12**, **1**, **2**). c) The coupling constants were estimated by first-order approximation. d) $J_{H_1'H_2'(py)}$ and $J_{H_1'H_2'(ad)}$ were 5.0 and 5.5 Hz, respectively, for all compounds except for **3** and **9**.

hand, two anomeric protons of the analogs so far prepared including isonicotinic acid-, and 4-methylnicotinamide analogs (**8**, **9**)[8,9] as well as NAD (**3**), which are known to take a pyridinium form, appeared at *ca.* δ 6.0 as a broad doublet.

The coupling constant between H_5 and H_6 on the pyridine nucleus was estimated to be 7.5 Hz for the new compounds, whereas it was 6.0 Hz for the compounds of the pyridinium form. According to Renault *et al.*,[10] the coupling between H_2 and H_3 on the nucleus of 4-aminoquinoline was 8 Hz for the imino form and 5 Hz for the amino form.

In the UV spectra, compounds **4** and **6** showed a strong absorption maximum at 264 nm in spite of the fact that the pyridine bases and ADP-ribose had the maximum at 252 and 259 nm, respectively. As the analogs were cleaved in turn to the nucleoside, a further bathochromic shift was observed from 264 nm to 271 nm or above. In addition, it was reported previously[11] that 4-aminopyridine exhibited the absorption peak at 242 nm in contrast to that of *N*-methyl-1,4-dihydro-4-iminopyridine at 268 nm.

These observations confirmed the 1,4-dihydro-4-imino β-riboside structure of the compounds prepared here.

Toxic Activity and Stability of Nucleosides. LD_{50}-values of clitidine (**1**) and its amide derivative (**2**) were estimated to be 100 and 16 mg/kg by preliminary tests (mice, intraperitoneal).

The carbamoyl group of **2** proved to be quite susceptible to hydrolysis, based on the results from TLC. When treated with boiling water for 5 h, compound **2** was completely hydrolyzed to yield **1**, and with hot water (70 °C) about 70% of **2** was converted to **1**. On the other hand, 4-aminonicotinamide was quite stable under these conditions. Additionally, compound **2** was completely converted to **1** on standing in 0.1 M NaOH at ambient temperature for 24 h, whereas it remained essentially unchanged on standing in water under the same conditions.

These results suggest the possible existence of the amide nucleoside (**2**) in the toadstool and conversion of **2** into **1** during extraction procedures.

Experimental

All melting points were uncorrected. IR and UV spectra were recorded on a Shimadzu IR-27G and a Hitachi 200-20 spectrophotometers, respectively. ^{1}H-NMR spectra were determined on a Hitachi R-20B (60 MHz) spectrometer in deuterium oxide with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. The abbreviations "s, d, dd, br, bs, and bd" denote "singlet, doublet, double doublet, broad, broad singlet, and broad doublet", respectively Optical rotations were measured with a Union PM 101 polarimeter. All the reactions were monitored by TLC on silica gel $60F_{254}$ plates (Merck, 10×10 cm) developed with 2-propanol–0.3% aqueous ammonia (7:3, v/v). The analyses of the chromatograms obtained were performed by a Shimadzu CS-910 scanner equipped with a chromatopack C-R1A. Column chromatographies[12] were carried out on DEAE-Sephadex A-25 connected to LKB Uvicord II (254 nm), unless stated otherwise. β-NAD (Grade III), phosphodiesterase (EC 3.1.4.1, snake venom, Type II), nucleotide pyrophosphatase (EC 3.6.1.9, snake venom, Type II), and 5'-nucleotidase (EC 3.1.3.5, snake venom, Grade IV) were purchased from Sigma Chemical Co.

Pig-brain NADase. The crude enzyme was prepared by the method of Zatman *et al.*[5a] The colloidal supernatant fluid obtained containing about 0.4 U[13] of NADase activity per ml was used without further purification.

4-Aminonicotinic Acid. Prepared from 3-methylpyridine in four steps, and recrystallized from water: mp 318—320 °C (dec) [lit,[14] 330 °C (dec)].

Methyl 4-Aminonicotinate. Prepared by esterification of the above compound, and recrystallized from water: mp 171—173 °C (lit,[15] 174—176 °C).

4-Aminonicotinamide. Prepared from 3-methylpyridine in six steps, and recrystallized from benzene: mp 227—229 °C (lit,[16] 232—234 °C).

Methyl 1,4-Dihydro-4-iminonicotinate Analog of NAD (4). Methyl 4-aminonicotinate (4.8 mmol, 730 mg) and NAD (1.2 mmol, 800 mg, neutralized with 2 M NaOH to pH 7) were incubated with pig-brain NADase (12 U, 30 ml) in 0.1 M Tris–HCl (pH 7.4, 130 ml) at 37 °C for 8 h. After removal of denatured protein by addition of 70% trichloroacetic acid (2 ml), cold acetone (800 ml) was added to the

clear supernatant to give a yellowish hygroscopic powder (720 mg). The crude product thus obtained was dissolved in water (20 ml) and applied to a column of DEAE-Sephadex (HCO_2^--form) (column size; 2.5 cm × 45 cm). The column was eluted with 300 ml portions of 0.02, 0.2, and 0.5 M HCO_2NH_4 (pH 7), successively. The first major component was eluted with the 0.2 M solution. The eluate (70 ml) showing an UV-absorbing peak at 264 nm was collected and lyophilized repeatedly to give **4** (420 mg) as the ammonium salt. An analytical sample of **4** was obtained by further chromatography and drying over P_2O_5 *in vacuo* for 12 h at 40 °C: $[\alpha]_D^{20} = -26.7°$ (*c* 3.22, H_2O); IR (KBr), 3300—2800, 1715, 1655, 1200, 1100, 1065 cm^{-1}; UV (H_2O), λ_{max} 264 nm (log ε 4.27); ^{1}H-NMR (Table 2). Found: C, 35.92; H, 5.13; N, 16.85%. Calcd for $C_{22}H_{29}N_7O_{15}P_2 \cdot 2NH_3$: C, 36.49; H, 4.83; N, 17.41%.

The second major component was eluted with the 0.5 M solution. The eluate (80 ml) showing an UV-absorbing peak at 259 nm was treated in a similar manner to give the product (190 mg), which was identical with authentic ADP-ribose on spectral comparisons.

*1,4-Dihydro-4-iminonicotinamide Analog of NAD (**6**).* 4-Aminonicotinamide (9.3 mmol, 1270 mg) and NAD (2.2 mmol, 1500 mg) were incubated with NADase (16 U, 40 ml) in 0.1 M Tris–HCl (160 ml) for 7 h. The reaction mixture was treated in a similar manner to that described above to give the crude product (1346 mg), which was dissolved in water (30 ml) and applied to a column (HCO_3^--form). The column was eluted with aqueous NH_4HCO_3 (pH 8) in a similar manner to that described above. The first major component was eluted with the 0.2 M solution. The eluate (80 ml) showing a UV maximum at 264 nm was collected and lyophilized repeatedly to give **6** (1035 mg) as the ammonium salt: $[\alpha]_D^{20} = -32.6°$ (*c* 0.37, H_2O); IR (nujol), 3400—2700, 1675, 1580, 1235 cm^{-1}; UV (H_2O), λ_{max} 264 nm (log ε 4.29); ^{1}H-NMR (Table 2). Found: C, 34.25; H, 4.71; N, 19.62%. Calcd for $C_{21}H_{28}N_8O_{14}P_2 \cdot 2NH_3 \cdot H_2O$: C, 34.52; H, 4.93; N, 19.18%.

*Yield Determination of **4** and **6**.* A series of mixtures (1 ml), containing various amounts of methyl 4-aminonicotinate or 4-aminonicotinamide, 4 or 8 μmol of NAD, and NADase (0.12 U) was incubated for 6 h. The aliquots of the reaction mixture were analyzed by TLC using a thin layer scanner with a chromatopack. On the basis of the chromatograms thus obtained, the ratios of the analogs (**4**, **6**) to ADP-ribose (**7**) were evaluated. This method was based on the fact that **4** or **6** and **7** had nearly the same ε-values at 262 nm. The data shown in Table 1 display the averages of three measurements.

*1,4-Dihydro-4-iminonicotinic Acid Analog of NAD (**5**).* Compound **4** (300 mg) was treated with 0.2 M NH_4OH (3 ml) for 12 h at 40 °C. The residue obtained on evaporation of the resulting solution was purified as described for **6** to give **5** (245 mg) as an ammonium salt: $[\alpha]_D^{20} = -31.5°$ (*c* 0.72, H_2O): UV (H_2O), λ_{max} 263 nm (log ε 4.30); ^{1}H-NMR (δ, D_2O), 4.2—4.6 (10H, br, riboses), 5.60 (1H, d, J=5.0 Hz, py-C$_i'$H), 6.05 (1H, d, J=5.5 Hz, ad-C$_i'$H), 6.80 (1H, d, J=7.5 Hz, py-H$_5$), 8.06 (1H, dd, J=7.5, 1.5 Hz, py-H$_6$), 8.17 (1H, s, ad-H$_2$), 8.43 (1H, s, ad-H$_8$), 8.50 (1H, d, J=1.5 Hz, py-H$_2$). Found: C, 35.02; H, 4.96; N, 15.22%. Calcd for $C_{21}H_{27}N_7O_{15}P_2 \cdot 2NH_3$: C, 35.34; H, 4.63; N, 15.17%.

*1,4-Dihydro-4-iminonicotinic Acid Mononucleotide (**11**).* Compound **5** (539 mg) was incubated with PDE (20 U) in 0.2 M Tris–HCl (pH 9.0, 5 ml) containing $MgCl_2$ (3 mg) at 37 °C for 12 h. In order to reduce the ionic strength, the reaction mixture was diluted with water (250 ml) and applied to a column (HCO_3^--form), followed by successive elution with 200 ml portions of 0.02, 0.2, and 0.5 M NH_4HCO_3 (pH 8). The first major component was eluted with the 0.2 M solution. The eluate (70 ml) showing UV maximum at 268 nm were collected and lyophilized repeatedly to give **11** (260 mg) as the ammonium salt: $[\alpha]_D^{20} = -52.3°$ (*c* 0.65, H_2O); UV (H_2O), λ_{max} 268 nm (log ε 4.31); ^{1}H-NMR (Table 2). Found: C, 35.62; H, 5.07; N, 11.85%. Calcd for $C_{11}H_{15}N_2O_9P \cdot NH_3$; C, 35.97; H, 4.90; N, 11.44%.

Similar treatment of the second major component eluted with the 0.5 M solution gave **13** (255 mg), which was identical with authentic ammonium adenylate on spectral comparisons.

*Methyl 1,4-Dihydro-4-iminonicotinate Mononucleotide (**10**).* Compound **4** (184 mg) was incubated with pyrophosphatase (15 U) in 0.2 M Tris–HCl (pH 7.4, 4 ml) containing $MgCl_2$ (3 mg) for 22 h. The reaction mixture on dilution with water (80 ml) was applied to a column (HCO_2^--form). The column was eluted with aqueous HCO_2NH_4 (pH 7) as described for **11**, followed by lyophilization of the major components eluted with the 0.2 M solution, to give **10** (76 mg) and small amounts of **11** (8 mg). Analytical sample of **10** was obtained by further chromatography and drying over P_2O_5 *in vacuo* for 12 h at 40 °C: $[\alpha]_D^{20} = -50.8°$ (*c* 3.23, H_2O); UV (H_2O), λ_{max} 269 nm (log ε 4.28); ^{1}H-NMR (δ D_2O), 4.00 (3H, s, CO_2CH_3), 4.2 (2H, bs, ribose), 4.45 (3H, bs, ribose), 5.76 (1H, d, J=5.0 Hz, py-C$_i'$H), 7.20 (1H, d, J=7.5 Hz, py-H$_5$), 8.47 (1H, dd, J=7.5, 1.5 Hz, py-H$_6$), 9.03 (1H, d, J=1.5 Hz, py-H$_2$). Found: C, 37.47; H, 5.42; N, 10.88%. Calcd for $C_{12}H_{17}N_2O_9P \cdot NH_3$: C, 37.80; H, 5.25; N, 11.02%.

*1,4-Dihydro-4-iminonicotinamide Mononucleotide (**12**).* Compound **6** (750 mg) was incubated with PDE (20 U) in 0.2 M Tris–HCl (pH 9.0, 6 ml) containing $MgCl_2$ (4 mg) for 16 h. The reaction mixture was treated as described for **11** to give **12** (328 mg) as ammonium salt: $[\alpha]_D^{20} = -55.2°$ (*c* 0.85, H_2O); UV (H_2O), λ_{max} 269 nm (log ε 4.30); ^{1}H-NMR (Table 2). Found: C, 35.71; H, 5.40; N, 15.62%. Calcd for $C_{11}H_{16}N_3O_8P \cdot NH_3$: C, 36.07; H, 5.19; N, 15.30%.

*1,4-Dihydro-5-iminonicotinic Acid Ribonucleoside (**1**).* Compound **11** (260 mg) was incubated with 5′-nucleotidase (40 U) in 0.2 M Tris–HCl (pH 9.2, 3.5 ml) containing $MgCl_2$ (8 mg) for 12 h. The mixture was maintained at pH 9 by occasional addition of 0.2 M NaOH. The precipitated phosphate salt was removed by filtration. The filtrate was then diluted with water (60 ml) and applied to a column (HCO_3^--form), which was eluted with 0.02 M NH_4HCO_3. The eluate (60 ml) having UV absorption was lyophilized to give **1** (192 mg) as a white crystalline mass, which on recrystallization from aqueous ethanol gave an analytical sample as white needles: mp 206—208 °C (dec) (lit,[3] 189—191 °C); $[\alpha]_D^{20} = -56.8°$ (*c* 0.75, H_2O); IR (nujol), 3400—3000, 1665, 1580, 1190 cm^{-1}; UV (H_2O), λ_{max} 271 nm (log ε 4.31); ^{1}H-NMR (Table 2). Found: C, 46.39; H, 5.35; N, 9.55%. Calcd for $C_{11}H_{14}N_2O_6 \cdot H_2O$: C, 45.83; H, 5.60; N, 9.72%.

*1,4-Dihydro-4-iminonicotinamide Ribonucleoside (**2**).* Compound **12** (255 mg) was incubated with 5′-nucleotidase (40 U) in 0.2 M Tris–HCl (pH 9.2, 3.5 ml) containing $MgCl_2$ (8 mg) for 10 h. The mixture was maintained at pH 9 during the reaction by occasional addition of 0.2 M NaOH. The resulting mixture was treated as described for **1**, followed by further repeated chromatography on Sephadex G-10 (column size; 2.5 × 100 cm) to give **2** (188 mg) as a white crystalline mass: mp 145—147 °C (dec); $[\alpha]_D^{20} = -62.8°$ (*c* 0.875, H_2O); IR (nujol), 3350—2700, 1675, 1600, 1290 cm^{-1}; UV (H_2O), λ_{max} 272 nm (log ε 4.30); ^{1}H-NMR (Table

2). Found: C, 43.55; H, 6.13; N, 13.88%. Calcd for $C_{11}H_{15}N_3O_5 \cdot 2H_2O$: C, 43.27; H, 6.26; N, 13.76%.

The authors wish to thank Prof. J. K. Seydel, Forschungsinstitut Borstel, West Germany, for his continuing interest and encouragement. They are also grateful to Prof. I. Azuma and Dr. I. Sekikawa for their helpful dicussions and suggestions during this work.

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