Enzymatic ADP-ribosylation of Benzotriazoles and Related Triazoles. Difference of Glycosidation Site between Triazoles and Indazoles

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Some benzotriazoles and related triazole were ADP-ribosylated in the presence of NAD and NAD-nucleosidase to give corresponding dinucleotides. The site of ribosylation for these dinucleotides was found exclusively to be N-1 on the basis of their uv and ^{15}N-nmr spectra, contrasting with N-2 for indazole dinucleotides. The origin of this difference in regiochemical specificity between the triazole and diazole systems is discussed.

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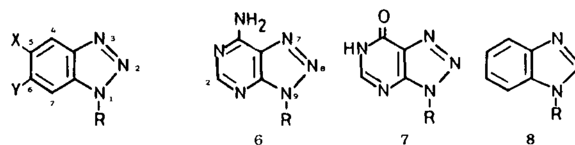
NAD nucleosidase (NADase) [EC 3.2.2.5] derived from mammalian brain is known as an enzyme which catalyses a trans ADP-ribosylation between β-NAD and an appropriate nicotinamide-related pyridine derivative, along with the mere hydrolysis of a quaternary nicotinamide-ribose glycosidic linkage [1-4]. During the course of our extensive studies directed towards the NADase-catalysed reaction, it has been found that the ADP-ribosylation occurrs not only on pyridine bases but also on other bicyclic systems such as indazoles [5,6]. The wide applicability of this enzymatic reaction to many types of nitrogen-containing heterocyclic base can be useful for the efficient and stereo-controlled synthesis of various nucleotides and in its turn the corresponding nucleosides which have some possible biological activities. Thus our attention was then focused on a benzotriazole system containing three ring-nitrogen atoms. It is of deep interest to examine whether the expected enzymatic reaction occurs or not and, if occurs, whether regiospecific or not. In this paper, we describe enzymatic ADP-ribosylations using porcine brain NADase for various benzotriazoles and related triazoles, together with the elucidation of the site of ribosylation comparing with that for indazoles by means of uv and ^{15}N-nmr spectrometries.

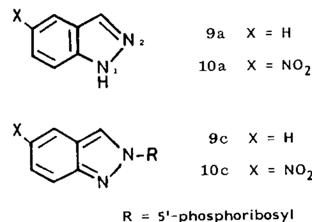
Results and Discussion.

We have previously shown [7], in the preparation of pyridine nucleotides, the necessary conditions of a base nitrogen for undergoing ADP-ribosylation: a sp^2-hybridized ring-nitrogen atom around which no virtual steric hindrance is involved and free from protonation near the optimum pH 7.2 of NADase, and in addition, structural environment that permits neutralization of a quaternary cationic nitrogen once formed by ADP-ribosylation with bond migrations involving a proton release. Benzotriazole (BT) bases **1a-5a** and related bases **6a** and **7a** dealt with here seem suitable for the substrate of the enzymatic transglycosidation reaction: moderate basicity (pKa <6), uncrowded reaction center, passable solubility in water, and suitable structure for bond migrations.



1 X = Y = H
2 X = CH$_3$, Y = H
3 X = Y = CH$_3$
4 X = Cl, Y = H
5 X = NO$_2$, Y = H

a: R = H
b: R = adenosine diphosphoribosyl
c: R = 5'-phosphoribosyl



Thus the NADase-catalysed reaction was carried out with monitoring the process by thin layer chromatography (tlc). A mixture of a substrate base and NAD (*ca* 3:1/molar ratio) was incubated with NADase in Tris-HCl (pH 7.2, 37°). Crude product obtained from the incubation mixture was subjected to column chromatography on anion exchange resins and a desired ADP-ribosylated compound, except for 8-azahypoxanthine (**7a**), was isolated in a good or moderate yield (Table 1). In all cases, only one dinucleotide was produced and no other ADP-ribosylated product was obtained.

Structural characterization by the use of ms- and ^{1}H-nmr spectrometries provided evidences for BT (or 8-azaadenine) adenine dinucleotides of the isolated compounds **1b-6b** apart from the site of ribosylation. In the nmr spectra, four to six low field aromatic signals including two singlets due to H-2 and H-8 of adenine ring, and two doublets falling within δ 5.8-6.4 due to two anomeric protons are characteristic for these dinucleotides (Table 1). The negative fast bombardment (FAB) mass spectra of **1b** (and **3b**) exhibited an intene (M-H)$^+$

Table 1

Yields and ^{1}H-NMR Spectral Data of Dinucleotides Obtained

Compound	Yield [a] %	BT [c] H-4 (H-5)	BT [c] H-7 (H-6)	^{1}H-NMR (δ, ppm) [b] (Deuterium Oxide) Anomeric [d] BT-$C_{1'}$ (AD-$C_{1'}$)	Adenine H-2 (H-8)	Other
1b	81	7.74 d	7.64 d	6.33 d	8.01 s	
		7.39 dd	7.23 dd	5.85 d	8.20 s	
2b	76	7.40 s	7.46 d	6.29 d	8.01 s	2.31 s
		–	7.14 d	5.83 d	8.11 s	(5-CH_3)
3b	36	7.28 s	7.20 s	6.20 d	7.95 s	2.15 s
		–	–	5.74 d	7.98 s	2.12 s
						(5- and 6-CH_3)
4b	43	7.68 d	7.58 d	6.31 d	8.03 s	
		–	7.26 dd	5.85 d	8.12 s	
5b	67	8.59 d	7.76 d	6.39 d	7.87 s	
		–	8.08 dd	5.78 d	8.06 s	
6b	39	–	–	6.25 d	8.05 s	8.08 s
				5.94 d	8.15 s	(H-2 of aza-ad)
8b	20	7.48 d	7.48 d	5.91 d	7.93 s	8.30 s
		7.18 dd	7.14 dd	5.86 d	8.20 s	H-2 of imid)

[a] Based on NAD. [b] Chemical shifts of ten ribose-linked protons fell within δ 4.2-4.8 in all cases. [c] $J_{4,5} = J_{6,7}$ = 8.4-9.0 Hz. [d] $J_{BT\text{-}1',2'}$ = 3.8 Hz for **6b**, 5.9-6.2 Hz for other compounds and $J_{Ad\text{-}1',2'}$ = 5.0-5.2 Hz in all cases.

ion peak at m/z 659 (and 693) and two main fragment peaks at m/z 426 and m/z 410 (and 444) arising from the adenosine diphosphate and the corresponding BT-ribose diphosphate anions, respectively. These observations clearly domonstrate the ADP-ribosylated dinucleotides of compounds **1b-6b**.

The uv spectral aspects of ribosylated BT should reflect the structural change of BT conjugate system that depends on the site of ribosylation. Since the net absorption of BT chromophore in the uv spectra of dinucleotides was concealed behind a strong absorption band (260 nm) of adenine moiety, the dinucleotides were led to BT mononucleotides on cleavage of pyrophosphate linkage with phosphodiesterase I. The respective uv spectra of mononucleotides **1c-6c** thus obtained were compared with those of corresponding free bases **1a-6a** which are known to exist as an N^1-H form both in the solid state and solution [8]. Compounds **1c**, **3c** and **6c** showed quite similar absorption maxima to those of **1a**, **3a** and **6a**, respectively (Table 2). The other mononucleotides showed a considerable hypsochromic shift (4-7 nm) of the first absorption band, accompanying hypo- and in most cases bathochromic shift of the second broad absorption.

Initially we predicted N^2-ribosylation for the bicyclic triazole system, because the N-2 atom is suitable for the aforementioned requirements for undergoing ADP-ribosylation. However, the uv spectral data of **1c-6c** suggest that the ADP-ribosylation occurred at the N-1 atom in the triazole system of **1a-6a**. In order to examine this unexpected trend more clearly, we used ^{15}N-nmr spectrometry that can offer definitive informations on different chemical environments between a ribosylated and an unribosylated nitrogens. The ^{15}N-nmr peaks observed for some of the BT mononucleotides obtained were assigned on the

Table 2

Comparison of UV Spectra between Azole Base and the Corresponding Nucleotide

Compound	λ max nm (log ε) [a]	
1a	259 (3.75)	274 (3.67)
1c	257 (3.81)	275 (3.58)
2a	264 (3.75)	276 (3.73)
2c	257 (3.76)	289 (3.55)
3a	266 (3.75)	280sh (3.72)
3c	267 (3.73)	286sh (3.63)
4a	263 (3.74)	280 (3.73)
4c	259 (3.76)	286 (3.53)
5a	252 (4.09)	306 (3.80)
5c	245 (4.14)	292 (3.74)
6a	273 (4.12)	–
6c	276 (4.13)	–

[a] Measured in 0.01 *M* Tris-HCl (*p*H 7.2).

Table 3

Comparison of Chemical Shifts in ^{15}N-NMR Spectra between Azole Base and the Corresponding Nucleotide

Compound	^{15}N-NMR (δ, ppm) [a] (DMSO-d$_6$) N-1	N-2	N-3	Other [b]
1a	302.8	366.2	302.8	
1c	235.2	377.3	343.0	
2a	318.2	374.1	318.2	
2c	232.9	371.9	334.4	
5a	310.1	387.2	310.1	371.6
5c	234.0	380.5	339.0	368.1
9a	136.0	315.8	–	
9c	283.0	236.4	–	
10a	191.1	324.4	–	373.1
10c	285.4	246.8	–	373.2

[a] Relative to liquid $^{15}NH_3$. [b] Due to NO_2 group.

basis of the following considerations from literature reports [9-11]: 1) Signals for a nitrogen bonded to two other nitrogens occur at particularly low field. 2) Signals for the "pyrrole"-type nitrogens occur at higher field than for the "pyridine"-type nitrogens in locked *N*-substituted system. 3) Signals for two symmetric ring nitrogens either of which bears a proton participating in autotropic rearrangement occur as one peak at an approximately average value of two shifts observed for a "pyrrole"-type and a "pyridine"-type nitrogens.

As shown in Table 3, no great difference in chemical shift of N-2 atom between the BT bases and the corresponding mononucleotides was observed, whereas a common single peak due to both N-1 and N-3 atoms of the triazoles bases came to separate into two resonances (one was shifted to higher field and the other to lower field) upon ribosylation of BT bases, demonstrating the BT nucleotides to be N^1-ribosylated compounds. On the other hand, of the two signals for indazoles bases **9a** and **10a** known to exist as an N^1-*H* form [12], one at higher than δ 200 is justly assigned to N-1 atom and the other one at lower than δ 300 to N-2 atom (Table 3). However, the two resonances were markedly shifted on conversion into corresponding nucleotides **9c** and **10c**. The higher-field one was toward lower field and the other lower-field one toward higher field. These observations show the N^2-ribosylated structure of indazole nucleotides, being compatible with previous reasonings [6] based on their uv spectral data. Thus, the ^{15}N-nmr studies provided conclusive proof that, unlike for indazole bases that suffer N^2-specific glycosidation, the site of ADP-ribosylation for BT bases is exclusively to be N-1 (or N-3) atom. In this connection, BT nucleoside obtained by hydrolysis of **1c** with 5′-nucleotidase agreed with 1-(β-D-ribofuranosyl)benzotriazole, that Revanker and Townsend [13] have previously synthesized by chemical method, in respect of their uv spectra and melting points.

In the ^{1}H-nmr spectra, whereas BT base itself showed two symmetrical two-proton multiplets (δ 7.86 and 7.50), the BT nucleotide **1c** showed four clearly separated aromatic signals (δ 8.04, 7.95, 7.66 and 7.51), indicating the loss of symmetrical property of the BT base upon ribosylation and in its turn the N^1-ribosylated structure of **1c**. In this case, H-4 (δ 8.04) is deshielded *ca* 24 Hz compared with H-7 (δ 7.95), because of its proximity to the "pyridine"-type N-3 atom [14]. Upon conversion of 5-substituted BT bases into nucleotides, similar down-field shift (0.06-0.15 ppm) was also observed for the H-4 the singlet peak due to which is readily distinguishable, whereas virtually not for the H-7. It can, therefore, be concluded that the ribosylation site of 5-substituted BT nucleotides is not to be N-3, but to be N-1 atom.

Then, it is required to elucidate the cause of the regiochemical difference between BTs and indazoles. On examination of the enzymatic reaction for benzimidazole **(8a)** and indole, the former exactly provided ADP-ribosylated compound **(8b)** although not so good yield, whereas the latter did not the corresponding product, demonstrating the necessity of 1,3-diaza structure that can contribute to an autotropic rearrangement, for undergoing the N^1-ribosylation. In this connection, the 8-azahypoxanthine **(7a)**, which was unable to undergo an ADP-ribosylation, was found to show no 1,3-autotropic rearrangement as judged by its ^{15}N-nmr spectrum that exhibited three separated peaks at δ 370.2 (N-2), 336.6 (N-3) and 300.0 (N-1) for the triazole moiety. Although a reason for that no N^2-ribosylated compound was obtained can not be clearly explained, it can be assumed as one possibility that the N^2-ribosyl BT once produced during the initial stage of reaction isomerized readily to the corresponding N^1-ribosyl BT, considering the reports by Katritzky *et al* [14,15] on intermolecular equilibria between 1-BT and 2-BT derivatives where the 1-isomer always predominates. However, if this assumption is accepted, no detectable generation of *N*-ADP-ribosyl **7a** is not reasonably explicable. Further work will be required to elucidate the reaction mechanism of the ADP-ribosylation for the bicyclic triazole systems.

In conclusion, benzotriazoles and a related triazole were subjected to NADase-catalysed transglycosidation reaction to produce regiospecifically corresponding N^1-ADP-ribosylated compounds. These results were contrary to our expectations and contrasted with that indazoles gave exclusively N^2-ribosylated compounds. It can not be unequivocally explained why no N^2-ribosyl product was obtained for the triazole bases, but 1,3-diaza structure capable of autotropic rearrangement was shown to be one of the requirements for the occurrence of N^1-ribosylation.

EXPERIMENTAL

General.

Melting points were determined on a Yamato MP-1 melting point apparatus and are uncorrected. Ultraviolet (uv) spectra were taken in water on a Backman DU-50 spectrophotometer and FAB (negative) mass spectra were determined with a JEOL JMX-DX 300 spectrometer. The ^{1}H-nmr spectra were recorded in deuterium oxide on a Bruker MSL-400 spectrometer (400 MHz) with 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP) as an internal standard and ^{15}N-nmr spectra in dimethyl-d_6 sulfoxide (DMSO-d_6) (200-300 mg/2 ml) containing chromium(III) tris-(acetyl acetonate) (20-30 mg) on the same spectrometer at 40.561 MHz with nitromethane as external standard, with later conversion (conversion factor, 380.23 ppm) to liquid ammonia standard [9]. The abbreviations "s, d, t, q, m, dd, bq and br" denote "singlet, doublet, triplet, quartet, multiplet, double doublet, broad quartet and broad", respectively. The ionic-strength and *p*H measurements were made with an Emuesu OD-35M II conductivity meter and a Hitachi-Horiba F-7DE *p*H meter with a G-202 glass electrode, respectively. Thin layer chromatographies (tlc) were carried out on silica gel $60F_{254}$ HPTLC plates (Merck, 10 cm x 10 cm) using 2-propanol – 0.2% aqueous ammonia (7:3, v/v) as developing system. Column chromatographies were performed on DEAE-Sephadex A-25 (HCO_3^--form) (column size: 2.5 cm x 45 cm) monitored by LKB Uvicord II (254 nm). Enzymatic reactions were carried out in the buffer of tris(hydroxymethyl)aminomethane-hydrochloric acid (Tris-HCl). NAD(Grade III), phosphodiesterase (PDE) I [EC 3.1..4.1] and 5′-nucleotidase [EC 3.1.3.5] were obtained from Sigma Chemical Co., and Benzotriazoles and related triazoles were from Aldrich Chemical Co.

Porcine Brain NADase [EC 3.2.2.5].

The crude particulate enzyme was prepared from fresh porcine brain by the method of Zatam *et al.* [16]. The colloidal homogenate containing *ca* 0.4 U/ml of NADase activity was used without further purification.

General Procedure for the Synthesis of Dinucleotide of Benzotriazoles and Related Azoles.

The substrate base (3-5 mmoles) and β-NAD (1-2 mmoles, neutralized with 0.5% aqueous sodium hydroxide to *p*H 7.2) were incubated with NADase (10-20 ml, 4-8 U) in 0.2 *M* Tris-HCl (50-90 ml, *p*H 7.2) at 37° for 10-30 hours. If the base is slightly soluble in the buffer, the appropriate volume of (5-20 ml) of DMSO was added to raise the solubility. The incubation mixture was treated with 70% trichloroacetic acid (2 ml) and the resulting denatured protein was removed by centrifugation. To the clear supernatant thus obtained, cold aceton (350-500 ml) was added, followed by standing overnight at 4°, to give crude mass. It was dissolved in water (30 ml) and then applied to a column of DEAE Sephadex (HCO_3^--form). The column was washed with 0.7% (w/w) aqueous solution of ammonium bicarbonate (conductivity, 500 μmho) to remove any free base and then eluted with the 4% solution (3 mmho). The first major component, which showed a uv-absorption peak at 260 nm, was ADP-ribose. On further elution, the second major component which showed a uv-absorption peak at 259 nm was obtained. The eluate fractions were collected and evaporated *in vacuo* to give a dinucleotide as ammonium salt. An analytical sample was obtained by drying over diphodphorus pentaoxide *in vacuo* for 12 hours at 40°.

1*H*-Benzotriazole Adenine Dinucleotide (**1b**).

Benzotriazole (0.72 g, 5 mmoles) and NAD (1.43 g, 2.1 mmoles) were incubated with NADase (15 ml, 6 U) in Tris-HCl (80 ml) for 18 hours. A crude mass (1.37 g) obtained from the incubation mixture was subjected to column chromatography to give **1b** (1.16 g, 81%) as white hygroscopic salt; ^{1}H-nmr (See Table 1); ms: m/z 659 [(M-H)$^+$, 100], 426 (80), 410 (80), 346 (40).

Anal. Calcd. for $C_{21}H_{26}N_8O_{13}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, 34.52; H, 4.93; N, 19.17; P, 8.49. Found: C, 34.58; H, 4.85; N, 19.22; P, 8.55.

5-Methyl-1*H*-benzotriazole Adenine Dinucleotide (**2b**).

A mixture of 5-methylbenzotriazole (824 mg, 5.1 mmoles) and NAD (1.52 g, 2.2 mmoles) was incubated with NADase (15 ml, 6 U) in Tris-HCl (80 ml) for 20 hours. The incubation mixture was treated in a similar manner as described above to give **2b** (1.18 g, 76%) as white ammonium salt; ^{1}H-nmr (see Table 1).

Anal. Calcd. for $C_{22}H_{28}N_8O_{13}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, 35.48; H, 5.11; N, 18.82; P, 8.33. Found: C, 35.16; H, 5.25; N, 18.72; P, 8.28.

5,6-Dimethyl-1*H*-benzotriazole Adenine Dinculeotide (**3b**).

A mixture of 5,6-dimethylbenzotriazole (455 mg, 3 mmoles) and NAD (1.05 g, 1.5 mmoles) was incubated with NADase (20 ml, 8 U) in Tris-HCl (70 ml) for 30 hours. The incubation mixture was treated in a similar manner as described above to provide **3b** (374 mg, 36%) as pale tan ammonium salt; ^{1}H-nmr (see Table 1).

Anal. Calcd. for $C_{23}H_{30}N_8O_{13}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, 36.41; H, 5.28; N, 18.47; P, 8.18. Found: C, 36.27; H, 5.32; N, 18.54; P, 8.40.

5-Chloro-1*H*-benzotriazole Adenine Dinucleotide (**4b**).

NAD (1.04 g, 1.5 mmoles) and 5-chlorobenzotriazole (0.72 g, 4.5 mmoles) were incubated with NADase (15 ml, 6 U) for 24 hours. The incubation mixture was treated as described above to provide **4b** (447 gm, 43%) as white ammonium salt; ^{1}H-nmr (see Table 1); ms: m/z 693 [(M-H)$^+$, 100], 444 (90), 426 (100), 408 (35), 346 (55).

Anal. Calcd. for $C_{21}H_{25}N_8O_{13}ClP_2 \cdot 2NH_3 \cdot 2H_2O$: C, 32.96; H, 4.58; N, 18.31; P, 8.11. Found: C, 33.18; H, 4.66; N, 18.29; P, 8.23.

5-Nitro-1*H*-benzotriazole Adenine Dinucleotide (**5b**).

A mixture of 5-nitrobenzotriazole (0.52 g, 3.1 mmoles) and NAD (1.31 g, 1.9 mmoles) was incubated with NADase (10 ml, 4 U) in Tris-HCl (80 ml) for 30 hours. The incubation mixture was treated as described above to give **5b** (0.85 g, 67%) as yellowish ammonium salt; ^{1}H-nmr (see Table 1).

Anal. Calcd. for $C_{21}H_{25}N_9O_{15}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, 32.52; H, 4.52; N, 19.87; P, 8.00. Found: C, 32.62; H, 4.55; N, 19.90; P, 8.15.

8-Aza-9*H*-adenine Adenine Dinucleotide (**6b**).

NAD (0.85 g, 1.3 mmoles) and 8-aza-adenine (0.43 g, 3.1 mmoles) were incubated with NADase (12 ml, 4.8 U) in Tris-HCl (50 ml) containing dimethyl sulfoxide (15 ml) for 30 hours. The incubation mixture was treated as described above to yield **6b** (262 mg, 39%) as white ammonium salt; ^{1}H-nmr (see Table 1).

Anal. Calcd for $C_{19}H_{25}N_{11}O_{13}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, 30.52; H, 4.69; N, 24.36; P, 8.30. Found: C, 30.71; H, 4.73; N, 24.18; P, 8.35.

Benzimidazole Adenine Dinucleotide (**8b**).

Benzimidazole (0.38 g, 3.2 mmoles) and NAD (0.96 g, 1.4 mmoles) were incubated with NADase (10 ml, 4 U) in Tris-HCl (50 ml) containing dimethyl sulfoxide (10 ml) for 25 hours. The incubation mixture was treated as described above to yield **8b** (0.19 g, 20%) as tan ammonium salt; ^{1}H-nmr (see Table 1).

Anal. Calcd. for $C_{22}H_{27}N_7O_{13}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, 36.21; H, 5.08; N, 17.22; P, 8.61. Found: C, 36.39; H, 5.14; N, 17.01; P, 8.72.

1*H*-Benzotriazole Mononucleotide (**1c**).

A mixture of **1b** (0.64 g), PDE-I (0.1 U) and magnesium chloride (3 mg) was incubated in 0.2 *M* Tris-HCl (*p*H 9.0, 5 ml) for 12 hours. During the incubation, occasional *p*H adjustment was required because the *p*H gradually decreased with the progress of reaction. After checking on tlc the complete hydrolysis of the dinucleotide to two mononucleotides, the incubation mixture upon dilution with water (50 ml) to lower its ionic strength below 1 mmho was applied to a column (2.5 cm x 20 cm) of DEAE-Sephadex A-25 (HCO_3^--form). The column was eluted with 4% (w/w) aqueous ammonium bicarbonate (conductivity: 3 mmho) to give, in turn, adenosine monophosphate (AMP, 0.31 g) and **1c** (0.30 g, 96%) with satisfactory separation as ammonium salts. Treatment of **1c** with ethanol-water (6:4, v/v) afforded crystalline plates, mp 182-183° dec; uv (see Table 2); ^{1}H-nmr (deuterium oxide): δ 8.04 (d, 1H, J = 8.8 Hz), 7.95 (d, 1H, J = 8.8 Hz), 6.48 (d, 1H, J = 6.0 Hz), 5.05 (t, 1H, J = 5.2 Hz), 4.65

(t, 1H, J = 4.6 Hz), 4.43 (bq, 1H, J = *ca* 4 Hz), 4.08 (br, 2H); ^{15}N-nmr (see Table 3).

Anal. Calcd. for $C_{11}H_{14}N_3O_7P{\cdot}NH_3$: C, 37.93; H, 4.89; N, 16.09; P, 8.91. Found: C, 37.84; H, 5.03; N, 15.88; P, 8.84.

5-Methyl-1*H*-benzotriazole Mononucleotide (**2c**).

A mixture of **2b** (0.86 g), PDE-I (0.15 U) and magnesium chloride (2 mg) was incubated in 0.2 *M* Tris-HCl (3 ml) for 10 hours. The incubation mixture was treated in a similar manner as described above to provide **2c** (0.42 g, 98%) as ammonium salt; uv (see Table 2); ^{1}H-nmr (deuterium oxide): δ 7.84 (d, 1H, J = 8.8 Hz), 7.80 (s, 1H), 7.52 (d, 1H, J = 8.8 Hz), 6.43 (d, 1H, J = 5.9 Hz), 5.04 (t, 1H, J = 5.5 Hz), 4.62 (dd, 1H, J = 4.8, 5.1 Hz), 4.41 (bq, 1H, J = *ca* 4 Hz), 4.0 (m, 2H), 2.50 (s, 3H); ^{15}N-nmr (see Table 3).

Anal. Calcd. for $C_{12}H_{16}N_3O_7P{\cdot}NH_3{\cdot}H_2O$: C, 37.89; H, 5.53; N, 14.74; P, 8.16. Found: C, 37.81; H, 5.60; N, 15.11; P, 8.24.

5,6-Dimethyl-1*H*-benzotriazole Mononucleotide (**3c**).

A mixture of **3b** (62 mg), PDE-I (0.1 U) and magnesium chloride (1 mg) was incubated in Tris-HCl (1 ml) for 5 hours. The reaction mixture was treated in a similar manner as described above to provide **3c** (30 mg, 99%) as pale tan ammonium salt; uv (see Table 2); ^{1}H-nmr (deuterium oxide): δ 7.73 (s, 1H), 7.63 (s, 1H), 6.38 (d, 1H, J = 5.5 Hz), 5.04 (t, 1H, J = 5.5 Hz), 4.62 (t, 1H, J = 4.8 Hz), 4.40 (q, 1H, J = 4.4 Hz), *ca* 4.0 (br, 2H), 2.41 (s, 3H), 2.36 (s, 3H).

Anal. Calcd. for $C_{13}H_{18}N_3O_7P{\cdot}2NH_3{\cdot}H_2O$: C, 38.05; H, 6.33; N, 17.03; P, 7.54. Found: C, 38.48; H, 6.32; N, 16.58; P, 7.66.

5-Chloro-1*H*-benzotriazole Mononucleotide (**4c**).

A mixture of **4b** (86 mg), PDE-I (0.1 U) and magnesium chloride (2 mg) was incubated in Tris-HCl (1.5 ml) for 5 hours. The reaction mixture was treated similarly as described above to yield **4c** (39 mg, 93%) as white ammonium salt; uv (see Table 2); ^{1}H-nmr (deuterium oxide): δ 8.09 (d, 1H, J = 1.5 Hz), 7.96 (d, 1H, J = 8.8 Hz), 7.64 (dd, 1H, J = 1.5, 8.8 Hz), 6.47 (d, 1H, J = 5.5 Hz), 5.01 (t, 1H, J = 5.5 Hz), 4.63 (t, 1H, J = 4.6 Hz), 4.43 (bd, 1H, J = *ca* 4 Hz), 4.0-4.15 (m, 2H).

Anal. Calcd. for $C_{11}H_{13}N_3O_7ClP{\cdot}NH_3{\cdot}H_2O$: C, 32.96; H, 4.49; N, 13.98; P, 7.74. Found: C, 33.04; H, 4.53; N, 14.12; P, 7.69.

5-Nitro-1*H*-benzotriazole Mononucleotide (**5c**).

A mixture of **5b** (424 mg), PDE-I (0.15 U) and magnesium chloride (2 mg) was incubated in Tris-HCl (3.5 ml) for 10 hours. The reaction mixture was treated similarly as described above to yield **5c** (208 mg, 97%) as yellow ammonium salt; uv (see Table 2); ^{1}H-nmr (deuterium oxide): δ 9.09 (d, 1H, J = 2.1 Hz), 8.44 (dd, 1H, J = 9.1, 2.1 Hz), 8.32 (d, 1H, J = 9.1 Hz), 6.42 (d, 1H, J = 5.1 Hz), 4.80 (t, 1H, J = 5.0 Hz), 4.33 (t, 1H, J = 4.4 Hz), 4.13 (q, 1H, J = 4.0 Hz), 3.66 (dd, 1H, J = 12.1, 3.7 Hz), 3.56 (dd, 1H, J = 12.1, 3.7 Hz): ^{15}N-nmr (see Table 3).

Anal. Calcd. for $C_{11}H_{13}N_4O_9P{\cdot}NH_3$: C, 33.59; H, 4.07; N, 17.81; P, 7.89. Found: C, 33.67; H, 4.13; N, 17.75; P, 8.04.

8-Azaadenosine Monophosphate (**6c**).

A mixture of **6b** (58 mg), PDE-I (0.1 U) and magnesium chloride (1 mg) was incubated in Tris-HCl (1 ml) for 6 hours. The reaction mixture was treated similarly as described above to give **6c** (25 mg, 87%) as white ammonium salt; uv (see Table 2); ^{1}H-nmr (deuterium oxide): δ 8.26 (s, 1H), 6.38 (d, 1H, J = 4.0 Hz), 5.02 (t, 1H, J = 5.5 Hz), 4.63 (t, 1H, J = 4.6 Hz), 4.41 (bq, 1H, J = *ca* 4 Hz), *ca* 4.0 (br, 2H).

Anal. Calcd. for $C_9H_{13}N_6O_7P{\cdot}NH_3{\cdot}2H_2O$: C, 26.93; H, 4.99; N, 24.44; P, 7.73. Found: C, 27.18; H, 5.14; N, 24.09; P, 8.04.

1*H*-Benzotriazole β-Ribonucleoside (**1d**).

A mixture of **1c** (35 mg), 5'-nucleotidase (5 U) and magnesium chloride (4 mg) was incubated in 0.2 *M* Tris-HCl (*p*H 9.2, 1.5 ml) for 8 hours at 37°. The reaction mixture was centrifuged to remove phosphate salt released and resulting supernatant was evaporated to dryness *in vacuo*. The residue was treated with dichloromethane-ethanol (1:1, v/v) to give **1d** (22 mg, 88%), mp 134-135° dec, lit [13] mp 135°; ^{1}H-nmr (deuterium oxide): δ 8.08 (d, 1H, J = 8.4 Hz), 7.99 (d, 1H, J = 8.4 Hz), 7.69 (dd, 1H, J = 7.7, 8.4 Hz), 7.54 (dd, 1H, J = 7.7, 8.4 Hz), 6.50 (d, 1H, J = 5.5 Hz), 5.06 (t, 1H, J = 5.2 Hz), 4.64 (t, 1H, J = 4.6 Hz), 4.43 (bq, 1H, J = *ca* 4 Hz), 4.08 (br, 2H).

Anal. Calcd. for $C_{11}H_{13}N_3O_4$: C, 52.60; H, 5.17; N, 16.73. Found: C, 52.43; H, 5.38; N, 16.59.

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