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Enzymic Synthesis and Biochemical Activity of Various Indazole Adenine Dinucleotides[1)]

Shuichi TONO-OKA,* Yukiko TONE, Victor E. MARQUEZ,† David A. COONEY,† Isao SEKIKAWA, and Ichiro AZUMA

Section of Chemistry, Institute of Immunological Science, Hokkaido University, Kita-ku, Sapporo 060
†Laboratory of Medicinal Chemistry and Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, U.S.A.

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Each of 5- or 6-amino-, acetamido-, hydroxy-, methoxy-, and chloroindazoles (including an unsubstituted one) and β NAD were subjected to an NADase-catalyzed base-exchange reaction to produce a corresponding title compound with a 41—76% yield. A difficulty, due to the poor solubility in water of indazole bases, was overcome by the addition of DMSO (~20%) without a remarkable decrease in NADase activity. In most cases, the obtained dinucleotides were ascertained to be N^2-ribosylated compounds. From 5- and 6-aminoindazoles, however, N^1-ribosylated dinucleotide was also obtained as a minor product. In some of the N^2-ribosylated dinucleotides, an unusual tautomerism was suggested to occur on the benzene ring of an indazole moiety. Finally, the synthesized title compounds were examined for inhibition activity against NAD-linked inosine monophosphate dehydrogenase. Four compounds among them were markedly effective at a 10^{-3} M concentration.

Interestingly, porcine brain NADase catalyses an exchange reaction between the nicotinamide moiety of β NAD and the structurally related pyridine compound, besides the mere hydrolysis of the quaternary pyridinium linkage.[2-4)] Modifying this principle, we have previously prepared various new NAD analogs containing 3,4-disubstituted 1,4-dihydropyridine moiety.[5-7)] Moreover, the necessary conditions for the concerned replacing base for participating in the reaction have been discussed.[7)] Such NAD analogs can be useful intermediates leading to the naturally occurring pyridine nucleosides quantitatively.[6)] As for the biological aspect, some of the analogs have been observed to show significant immunological activities *in vitro*.[8)]

From the viewpoint of applying the enzymic synthesis to other nitrogen-containing heterocycle nucleotides, our interest has initially been focused on readily preparable indazole bases. Since no indazole adenine dinucleotide has been synthesized, chemically or enzymicly, this application of an exchange reaction to the indazole bases can be of great significance. On the other hand, in view of the NAD analogs, the dinucleotides are expected to show a possible inhibition activity against an NAD-linked dehydrogenase system, particularly inosine monophosphate (IMP) dehydrogenase which is a target enzyme for many antitumor agents.[9,10)] In this paper, we give a full account of the enzymic synthesis and characterization of new indazole adenine dinucleotides arising from 5- or 6-amino-, acetamido-, hydroxy-, methoxy-, and chloroindazoles (as well as an unsubstituted one), together with their biochemical activities against IMP dehydrogenase.

Results and Discussion

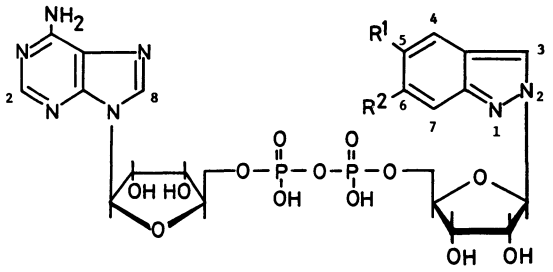
Preparation of Indazole Dinucleotides. When a replacing base shows a strong basicity ($pK_a \gtrsim 9$), no exchange reaction occurs because the concerned nitrogen atom is blocked by a protonation under the optimum conditions of NADase (pH 7.2).[7)] The indazole bases now used, however, are relatively weak in basicity ($pK_a \sim 5$)[11)] and have no substituent on the pyrazole ring which would cause a steric hindrance at the reaction site. Thus, the occurrence of a base exchange was expected. The only problem in carrying out the reaction was the poor solubility in water of the indazole compounds. The base concentration proved to be an important factor in promoting the formation of a new dinucleotide.[6)] Fortunately, the indazole bases were very soluble in DMSO, one of the typical aprotic solvents. Were it not for an extreme influence on the NADase activity, therefore, the addition of DMSO seemed favorable in view of enhancing base nucleophilicity as well as improving base concentration. In fact, no significant decrease in the activity was observed, even if DMSO (~20%) was added to get a suitable base concentration (20 mM††). The problem of base solubility in the reaction system was, thus, overcome.

Each indazole base and NAD were incubated with NADase at 37 °C in DMSO-containing Tris-HCl (pH 7.2) until the spot of NAD disappeared in TLC. After incubation, the rusulting mixture contained four major components: An unchanged indazole base, the NAD-derived nicotinamide, the desired product, and ADP-ribose. The mixture (after the removal of the enzyme, Tris-HCl, and DMSO) was subjected to column chromatography on DEAE-Sephadex to provide the desired dinucleotide in 41—76% yield (Table 1). Its purity was checked by TLC in two different develo-

†† 1 M=1 mol dm^{-3}.

ping systems and, if necessary, the chromatography was repeated.

Considering the two nitrogen atoms which form the indazole skeleton, though probably different in reactivity, the formation of two distinct dinucleotides can be anticipated in an enzymic reaction. In most cases, however, single dinucleotide was produced with no detectable amount of an alternative compound. As an exceptional case, a minor product was brought about from 5- and 6-aminoindazoles in *ca.* 10% yield besides the major one.



1: $R^1=R^2=H$
2: $R^1=NH_2$, $R^2=H$
3: $R^1=H$, $R^2=NH_2$
4: $R^1=NHAc$, $R^2=H$
5: $R^1=H$, $R^2=NHAc$
6: $R^1=OH$, $R^2=H$
7: $R^1=H$, $R^2=OH$
8: $R^1=OCH_3$, $R^2=H$
9: $R^1=H$, $R^2=OCH_3$
10: $R^1=Cl$, $R^2=H$
11: $R^1=H$, $R^2=Cl$

So far as we know, there has been no report concerning halogen-containing NAD analogs. Accordingly, an exchange reaction was also tried for the preparation of dinucleotides originating from 5-, 6-, and 4-chloroindazoles. As a result, we obtained the corresponding dinucleotides (**10**—**12**) in reasonable yield (Table 1), indicative of the availability of this method for halogen-containing bases as well.

Structural Characterization of Indazole Dinucleotides. In order to obtain structural information regarding the new compounds, we first compared their ^{1}H-NMR spectra with those of NAD and the corresponding indazole base. Relevant signals falling within δ 6.5—8.3 indicated the presence of indazole and adenine moieties (Table 1). In all cases, two anomeric protons appeared at δ 5.8—6.0 as clearly separate doublets, and ribose-linked ten protons at δ 4.2—4.8 overlapping one another. In addition, the phosphorus content was quantitatively analyzed to show 2 mol per molecule of the compound. These observations ascertained the indazole adenine dinucleotide of the products obtained.

By means of UV spectroscopy, we next examined the site of ribosylation in the indazole moiety of the dinucleotides. In the UV spectra, the absorption maxima of indazole chromophore was wrapped up with the adenine-derived strong absorption band at 260 nm, so that the dinucleotide (**1**) was led to the indazole mononucleotide (**15**) on cleavage of pyrophosphate linkage with phosphodiesterase (PDE), followed by chromatographic isolation. The UV spectrum of **15** was compared with those of 1- and 2-methylindazoles, because ribosylation or methylation was expected to produce similar inductive effects. Compound **15** closely resembles 2-methylindazole in its absorption curve, but differs markedly from 1-methylindazole (Fig. 1). Indazole mononucleotides formed from other dinucleotides also exhibited a similar

Table 1. Yields and ^{1}H-NMR spectral data of indazole adenine dinucleotides

Compd No.	Yield [b] %	Chemical shifts (δ, D_2O)[a]								
		Indazole[c]					Adenine		Anomeric	
		H_3	H_4	H_5	H_6	H_7	H_2	H_8	ad-C_1' [d]	ind-C_1' [e]
1	54	8.29 s	7.53 d	7.19 dd	6.93 dd	7.40 d	7.97 s	8.24 s	5.99 d	5.87 d
2	41	8.17 s	n.o.[f]	—	6.89 d	7.32 d	7.95 s	8.10 s	5.94 d	5.86 d
3	43	8.21 s	7.42 d	6.62 d	—	n.o.[f]	7.97 s	8.16 s	5.91 d	5.87 d
4	72	8.21 s	7.53 d	—	7.00 d	7.31 d	7.90 s	8.12 s	5.98 d	5.79 d
5	47	8.24 s	7.41 d	6.70 d	—	7.51 s	7.90 s	8.06 s	5.98 d	5.79 d
6	73	8.13 s	6.75 d	—	6.80 dd	7.30 d	7.96 s	8.08 s	5.95 d	5.87 d
7	76	8.18 s	7.40 d	6.54 d	—	n.o.[f]	7.98 s	8.13 s	5.93 d	5.86 d
8	62	8.16 s	6.78 s	—	6.75 d	7.28 d	7.94 s	8.12 s	5.99 d	5.86 d
9	53	8.22 s	7.39 d	6.51 dd	—	6.66 d	7.99 s	8.13 s	5.95 d	5.86 d
10	62	8.23 s	7.42 d	—	7.01 dd	7.32 d	7.98 s	8.14 s	6.01 d	5.87 d
11	48	8.31 s	7.47 d	6.82 dd	—	7.30 d	7.99 s	8.09 s	6.01 d	5.83 d
12	48	8.27 s	—	7.09 dd	6.88 dd	7.28 dd	7.87 s	8.13 s	5.99 d	5.84 d
13	12	8.26 s	7.20 s	—	7.10 d	7.35 d	7.96 s	8.16 s	5.98 d	5.84 d
14	9	8.29 s	7.47 d	6.76 d	—	7.05 s	7.99 s	8.20 s	6.00 d	5.86 d

a) Chemical shifts of ribose-linked ten protons fell within δ 4.2—4.8 in all cases. b) Based on NAD used. c) Acetamido methyl protons of **4** and **5** appeared at δ 2.16 and 2.19, respectively, and methoxy methyl protons of **8** and **9** were observed at δ 3.76 and 3.81, respectively. d) J=4.2—4.4 Hz. e) J=4.0—5.4 Hz. f) Not observed.

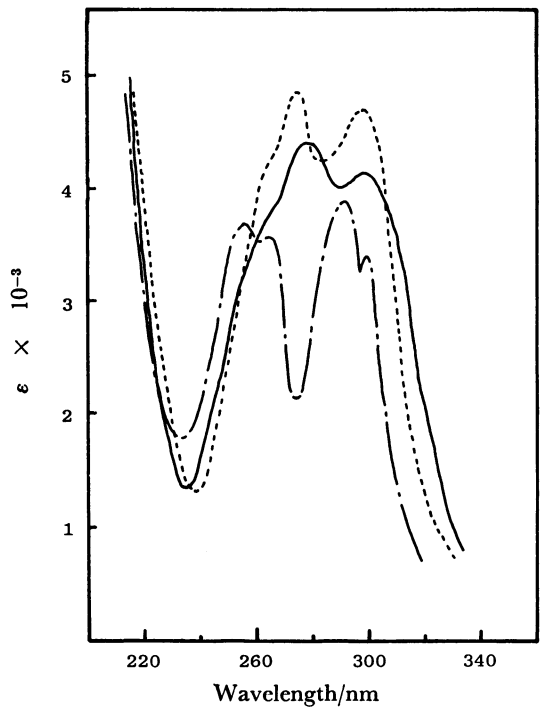


Fig. 1. UV spectral comparison of indazole mononucleotide (**15**) with 1- and 2-methylindazoles. (——): **15**; (— — —): 1-methylindazole; (-----): 2-methyl indazole.

bathochromic shift with somewhat hyperchromic effect. These observations indicate the quinonoid structure of mononucleotides, and consequently that the site of ribosylation is at the N^2-atom.

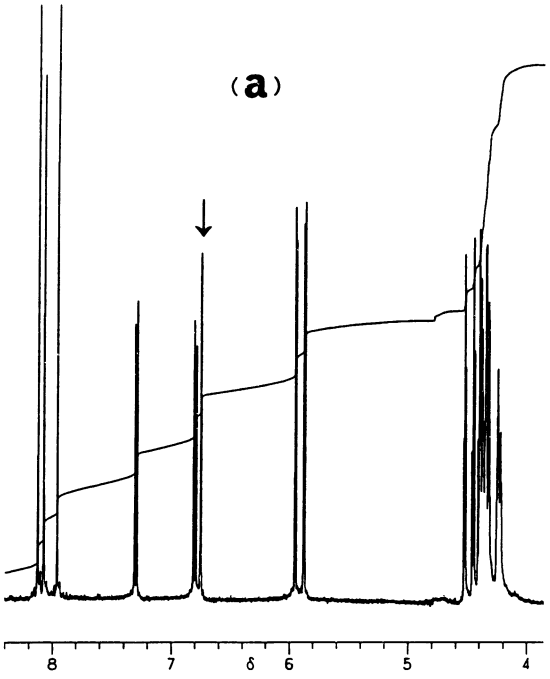
By ^{1}H-NMR spectroscopy, this reasoning could not be taken to a more definite conclusion. In order to gain further evidence, some appropriate N^1-acetylated indazoles (unsubstituted, 5-, and 6-acetamido ones) were submitted to the exchange reaction. Dinucleotides identical to **1**, **4**, and **5**, respectively, on ^{1}H-NMR spectral comparisons were produced in low yield. Upon incubation of the N^1-acetylated bases with NADase alone, however, no appreciable deacetylation occurred with N^1-acetyl group. These results strongly support the following conclusion: ADP-ribosylation occurred initially at the N^2-atom to form a 2-quaternary transient intermediate. Subsequently, the elimination of the N^1-acetyl group was accelerated by a strong electron-withdrawing effect of the quaternary nitrogen atom upon double-bond migrations, resulting in the quinonoid form of the indazole moiety.

Incidentally, the aforementioned minor products obtained with 5- and 6-aminoindazoles proved to be N^1-ribosylated dinucleotides (**13** and **14**) since their mononucleotides depicted a similar UV spectrum to that of the corresponding original base. Compounds **13** and **14** would arise from a certain proportion of the tautomeric 2*H*-indazole forms of the aminoindazoles although its existence has not been established.

The ^{1}H-NMR spectra in D_2O of compounds **2**, **3**, and **7** had no observable signal due to the aromatic proton (H_4 or H_7) adjacent to the amino or hydroxyl group. On the other hand, the corresponding signal was present as a singlet in compounds **4**, **5**, and **9** where the amino and hydroxyl substituents were blocked with acetyl and methyl groups, respectively (Table 1). Moreover, a signal was observed for the original amino- or hydroxyindazole, compound **13** and **14**. These facts suggest the occurrence of an unusual tautomerism on the benzene ring of the indazole moiety in **2**, **3**, and **7** which is probably dependent upon N^2-ribosylation. It is of interest to note that a signal due to H_4 appeared for compound **6**, whereas that due to H_7 disappeared for compound **7** under the same conditions (Fig. 2).

The assignment of the two doublets due to the anomeric protons remains unsettled. Thus, the respective coupling constants were estimated for the dinucleotides by a second-order approximation. As a consequence, the upfield signals were J=4.0—5.4 Hz, whereas the downfield ones were J=4.2—4.4 Hz. It can be expected that the dihedral angle between H–C_2'–C_1' and C_2'–C_1'–H in the ribose moiety of indazole side is somewhat changed by the difference in conformation of various kinds of the indazole bases. On the contrary, one can presume that the angle in the adenine side is little changed since the ribose moiety therein is under the influence of the same adenine base. Considering that the $J_{1',2'}$-value is reflected by the variation of the dihedral angle,[12] the upfield doublet can be assigned to the anomeric proton of indazole side, whereas the downfield one to that of adenine side (Table 1). In this connection, it is necessary to note that although respective chemical shifts and $J_{1',2'}$-values of a couple of mononucleotides were compared with each other, there was no unambiguous distinction between them in all cases.

Biochemical Activity of Indazole Dinucleotides against IMP Dehydrogenase. NAD-linked IMP dehydrogenase is known as one of the key rate-controlling enzymes of nucleic acid biosynthesis,[13] and many compounds which inhibit the enzyme have a more or less antitumor activity.[14,15] It is, therefore, of interest whether or not the indazole dinucleotides prepared here show an inhibitory effect on the dehydrogenase system. Some of those dinucleotides were examined for their ability to inhibit a partially purified IMP dehydrogenase from P388 leukemic cells. Among them, compounds **1**, **2**, **3**, and **6** showed an inhibition by 33 to 75% at a 10^{-3} M concentration (Table 2). At a 10^{-4} M concentration, however, none of them showed a significant level of inhibition, except for **2** which showed a 14% inhibition. Further investigations to correlate these results with actual features of cell proliferation are now in progress.



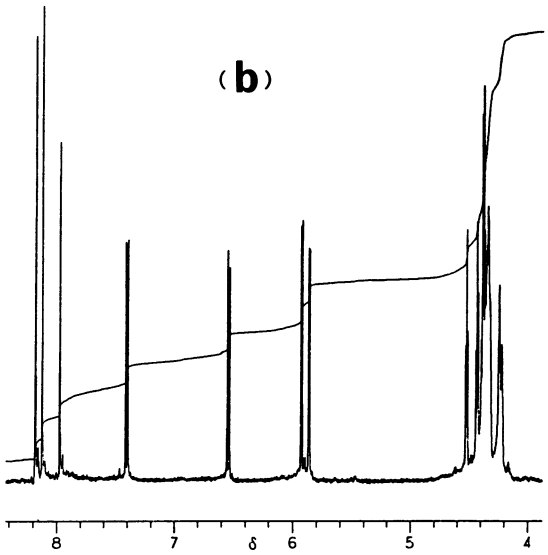


Fig. 2. 500 MHz ^{1}H NMR spectra of **6** (a) and **7** (b) in D_2O. (a) A signal assignable to H_4 is existent (an arrow). (b) A signal assignable to H_7 is nonexistent.

TABLE 2. INHIBITION ACTIVITY OF SOME OF INDAZOLE ADENINE DINUCLEOTIDES AGAINST IMP DEHYDROGENASE

Compd	Inhibition of IMP Dehydrogenase/%[a)]
1	33
2	75
3	55
4	0
5	0
6	75
7	5

a) At a 10^{-3} M concn of each compd.

Experimental

General. Melting points were measured with a Yamato MP-1 melting-point apparatus and are uncorrected. UV spectra were recorded on a Hitachi 200-20 spectrophotometer. ^{1}H-NMR spectra were determined on a JEOL GX-500 or a Varian XL-200 spectrometer in deuterium oxide with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal standard. The abbreviations "s, d, and dd" denote "singlet, doublet, and double doublet," respectively. Optical rotations were measured on a Union PM-101 digital polarimeter. The ionic-strength and pH measurements were made with a Emuesu CD-35M II conductivity meter and a Radiometer PHM-26 pH meter equipped with a G-202 glass electrode, respectively. TLC was carried out on silica gel $60F_{254}$ HPTLC plates (Merck, 10 cm×10 cm) using 2-propanol–0.2% aq ammonia (7:3, v/v) and/or 1-propanol–0.3% aq acetic acid (3:2, v/v) as developing system. Column chromatographies were carried out on DEAE-Sephadex A-25 (HCO_3^--form) (column size: 2.5 cm×45 cm) monitored by LKB Uvicord II (254 nm). Phosphorus was determined colorimetrically by the method of Bartlett.[16] 1-Methyl- and 2-methylindazoles were obtained by the decarboxylation of respective 3-carboxylic acids.[17] β NAD (Grade III) and phosphodiesterase (PDE) (EC 3.1.4.1) were purchased from Sigma Chemical Co. Indazole, 5-, and 6-aminoindazoles were obtained from Aldrich Chemical Co.

Porcine Brain NADase (EC 3.2.2.5). The Crude particulate enzyme was prepared from a fresh porcine brain by the method of Zatman *et al.*[18] The colloidal supernatant fluid containing *ca.* 0.4 U[19]/ml of NADase activity was used without further purification.

N^1-*Acetylindazole.* Prepared by acetylation of indazole with acetic anhydride, and recrystallized from petroleum ether: Mp 44—46 °C (lit,[20] 42—43 °C).

N^1-*Acetyl-5-acetamidoindazole.* Prepared by acetylation of 5-aminoindazole with acetic anhydride, and recrystallized from benzene: Mp 167—169 °C. Found: C, 60.74; H, 5.12; N, 19.26%. Calcd for $C_{11}H_{11}N_3O_2$: C, 60.83; H, 5.07; N, 19.35%.

5-Acetamidoindazole. Prepared by the partial hydrolysis of the above compound with methanol–0.3% aq ammonia (60 °C, 3h), and recrystallized from methanol: Mp 203—205 °C. Found: C, 61.68; H, 5.16; N, 24.07%. Calcd for $C_9H_9N_3O$: C, 61.71; H, 5.14; N, 23.99%.

N^1-*Acetyl-6-acetamidoindazole.* Prepared by the acetylation of 6-aminoindazole with acetic anhydride, and recrystallized from benzene: Mp 187—189 °C. Found: C, 60.78; H, 5.06; N, 19.31%. Calcd for $C_{11}H_{11}N_3O_2$: C, 60.83; H, 5.07; N, 19.35%.

6-Acetamidoindazole. Prepared by the partial hydrolysis of the above compound, and recrystallized from methanol: Mp 253—255 °C. Found: C, 61.75; H, 5.18; N, 23.94%. Calcd for $C_9H_9N_3O$: C, 61.71; H, 5.14; N, 23.99%.

5-Hydroxyindazole. Prepared from 5-aminoindazole by the method of Davies,[21] and recrystallized from water: Mp 187—189 °C (lit,[21] 186 °C).

6-Hydroxyindazole. Prepared from 6-aminoindazole, and recrystallized from water: Mp 212—214 °C (lit,[21] 216 °C).

5-Methoxyindazole. Prepared from 4-methoxy-2-

methylaniline by diazotization followed by intramolecular cyclization, and recrystallized from ethanol-petroleum benzine: Mp 168—170 °C (lit,[22] 168—169 °C).

6-Methoxyindazole. Prepared from 6-hydroxyindazole by *O*-methylation with dimethyl sulfate, and recrystallized from petroleum benzine: Mp 125—127 °C (lit,[23] 124 °C).

5-Chloroindazole. Prepared from 2-amino-5-chlorotoluene, and recrystallized from benzene: Mp 122—124 °C (lit,[20] 118—120 °C).

6-Chloroindazole. Prepared from 2-amino-4-chlorotoluene, and recrystallized from benzene: Mp 175—177 °C (lit,[20] 175—177 °C).

4-Chloroindazole. Prepared from 2-amino-6-chlorotoluene, and recrystallized from benzene: Mp 158—160 °C (lit,[20] 155—157 °C).

Inhibition Activity against IMP Dehydrogenase. The assay was performed by the radiometric technique as reported previously:[24] NAD (1 mM), [2-^{3}H]IMP (0.075 mM), and each dinucleotide (1 mM or 0.1 mM) were incubated with P388 leukemic cell IMP dehydrogenase at 37 °C for 10 min, and then the reaction was terminated by 1 min of heating at 95 °C. The ^3H_2O arising from the oxidation of [2-^{3}H]IMP *via* [^{3}H] NAD was isolated by quantitative microdistillation and its tritium content was measured with a scintillation counter.

2H-Indazole Adenine Dinucleotide (1). Indazole (370 mg, 3.1 mmol) and NAD (780 mg, 1.1 mmol, neutralized to pH 7 with 0.2 M NaOH) were incubated with NADase (20 ml, 8 U) in DMSO (15 ml)-containing 0.1 M Tris-HCl (65 ml, pH 7.2) at 37 °C for 5 h. The incubation mixture was treated with 70% trichloroacetic acid (2 ml) and the denatured protein was removed by centrifugation. To the clear supernatant thus obtained, cold acetone (500 ml) was added, followed by standing overnight at 4 °C, to give white hygroscopic mass (708 mg). It was dissolved in water (30 ml) and then applied to a column (2.5 cm×25 cm) of DEAE-Sephadex (HCO_3^--form). The column was washed with 0.05 M NH_4HCO_3 (300 ml) to remove any free bases and then eluted with 0.4 M NH_4HCO_3 (pH 7.8). The first major component eluted, which showed a UV-absorption peak at 260 nm, was ADP-ribose. On further elution, the second major component was obtained and showed a UV-absorption peak at 264 nm. The eluate (200 ml) was immediately collected and lyophilized repeatedly to give **1** (375 mg) as ammonium salt. An analytical sample of **1** was obtained by drying over P_2O_5 *in vacuo* for 12 h at 40 °C: $[\alpha]_D^{23}$ −28.8° (*c* 0.59, H_2O); UV (H_2O) 264 (log ε 4.33) and *ca.* 293 nm (sh, log ε 3.78); ^{1}H-NMR (Table 1). Found: C, 34.17; H, 5.31; N, 16.76%. Calcd for $C_{22}H_{27}N_7O_{13}P_2 \cdot 2NH_3 \cdot 4H_2O$: C, 34.53; H, 5.36; N, 16.48%.

5-Amino-1H-indazole- and 5-Amino-2H-indazole Adenine Dinucleotides (13 and 2). 5-Aminoindazole (420 mg, 3.2 mmol) and NAD (620 mg, 0.9 mmol) were incubated with NADase (15 ml, 6 U) in DMSO (12 ml)-containing 0.1 M Tris-HCl (60 ml) for 5 h. The incubation mixture was treated in a similar manner as described above to give a pale-brown crude product (557 mg). It was dissolved in water (30 ml) and the water-insoluble part was removed by centrifugation. The clear supernatant was applied to a column (2.5 cm×40 cm) of DEAE-Sephadex. The column was then eluted with aq NH_4HCO_3, as described above. The eluate (60 ml) of the first major component posterior to the elution of ADP-ribose was immediately collected and lyophilized repeatedly to provide **13** (74 mg): $[\alpha]_D^{23}$ −33.0° (*c* 2.35, H_2O); UV (H_2O) 244 (log ε 4.31) and *ca.* 315 nm (log ε 3.55); ^{1}H-NMR (Table 1). Found: C, 35.39; H, 4.76; N, 16.81%. Calcd for $C_{22}H_{28}N_8O_{13}P_2 \cdot NH_3 \cdot 3H_2O$: C, 35.46; H, 4.97; N, 16.93%. After the elution of **13**, the second major component was eluted and showed a UV-absorption peak at 261 nm. The eluate (150 ml) was collected and lyophilized repeatedly to provide **2** (261 mg): $[\alpha]_D^{23}$ −38.7° (*c* 0.85, H_2O); UV (H_2O) 261 (log ε 4.27) and 330 nm (log ε 3.65); ^{1}H-NMR (Table 1). Found: C, 35.19; H, 4.90; N, 17.16%. Calcd for $C_{22}H_{28}N_8O_{13}P_2 \cdot NH_3 \cdot 3H_2O$: C, 35.46; H, 4.97; N, 16.93%.

6-Amino-1H-indazole- and 6-Amino-2H-indazole Adenine Dinucleotides (14 and 3). 6-Aminoindazole (390 mg, 2.8 mmol) and NAD (630 mg, 1.1 mmol) were incubated with NADase (15 ml, 6 U) in DMSO (15 ml)-containing Tris-HCl (70 ml) for 5 h. The pale brown crude mass (550 mg) obtained was separated by the chromatographic procedures as described for **13** to give **14** (55 mg) firstly: $[\alpha]_D^{23}$ −37.4° (*c* 0.19, H_2O); UV (H_2O) *ca.* 235 (sh, log ε 4.32), *ca.* 260 (log ε 4.21), and 296 nm (log ε 3.88); ^{1}H-NMR (Table 1). Found: C, 35.68; H, 5.09; N, 17.22%. Calcd for $C_{22}H_{28}N_8O_{13}P_2 \cdot NH_3 \cdot 3H_2O$: C, 35.46; H, 4.97; N, 16.93%. The chromatography was further continued as described for **2** to give **3** (276 mg) secondly: $[\alpha]_D^{23}$ −45.3° (*c* 0.19, H_2O); UV (H_2O) 263 (log ε 4.27) and 296 nm (log ε 3.92); ^{1}H-NMR (Table 1). Found: C, 35.39; H, 4.67; N, 16.67%. Calcd for $C_{22}H_{28}N_8O_{13}P_2 \cdot NH_3 \cdot 3H_2O$: C, 35.46; H, 4.97; N, 16.93%.

5-Acetamido-2H-indazole Adenine Dinucleotide (4). 5-Acetamidoindazole (540 mg, 3.0 mmol) and NAD (650 mg, 1.0 mmol) were incubated with NADase (15 ml, 6 U) in DMSO (5 ml)-containing Tris-HCl (60 ml) for 10 h. Chromatographic purification of the crude mass (600 mg) obtained, followed by repeated lyophilization, provided **4** (501 mg) as ammonium salt. An analytical sample of **4** was given by drying over P_2O_5 *in vacuo* for 12 h at 40 °C: $[\alpha]_D^{23}$ −31.2° (*c* 0.92, H_2O); UV (H_2O) 240 (log ε 4.38), *ca.* 260 (sh, log ε 4.22), and 310 nm (log ε 3.68); ^{1}H-NMR (Table 1). Found: C, 36.05; H, 5.70; N, 17.58%. Calcd for $C_{24}H_{30}N_8O_{14}P_2 \cdot 2NH_3 \cdot 3H_2O$: C, 35.85; H, 5.97; N, 17.43%.

6-Acetamido-2H-indazole Adenine Dinucleotide (5). 6-Acetamidoindazole (400 mg, 2.2 mmol) and NAD (600 mg, 0.9 mmol) were incubated with NADase (10 ml, 4 U) in DMSO (15 ml)-containing Tris-HCl (50 ml) for 12 h. The crude mass (545 mg) obtained was treated as described above to provide **5** (301 mg): $[\alpha]_D^{23}$ −20.6° (*c* 0.57, H_2O); UV (H_2O) 239 (log ε 4.32), *ca.* 265 (sh, log ε 4.13), and 297 nm (log ε 3.84); ^{1}H-NMR (Table 1). Found: C, 35.53; H, 5.78; N, 17.54%. Calcd for $C_{24}H_{30}N_8O_{14}P_2 \cdot 2NH_3 \cdot 3H_2O$: C, 35.85; H, 5.97; N, 17.43%.

Exchange Reaction with N^1-Acetyl Derivative of Indazole, 5-, and 6-Acetamidoindazoles. NAD (225 mg) and each of *N*^{1}-acetyl derivatives of indazole, 5-, and 6-acetamidoindazoles were incubated with NADase (5 ml, 2 U) in DMSO (5 ml)-containing 0.1 M Tris-HCl (30 ml) at 37 °C for 5—10 h. The, thus obtained, three dinucleotides (30—40 mg) were proved to be identical with **1**, **4**, and **5** from ^{1}H-NMR spectral comparisons, respectively. In all cases, no other detectable amount of dinucleotide was produced except for ADP-ribose.

5-Hydroxy-2H-indazole Adenine Dinucleotide (6).

NAD (958 mg, 1.4 mmol) and 5-hydroxyindazole (630 mg, 4.7 mmol) were incubated with NADase (10 ml, 4 U) in DMSO (5 ml)-containing Tris-HCl (50 ml) for 15 h. The reaction mixture was treated as previously described to provide a pale-brown mass of **6** (699 mg): $[\alpha]_D^{23}$ −38.3° (*c* 1.15, H_2O); UV (H_2O) 263 (log ε 4.33) and 319 nm (log ε 3.73); ^{1}H-NMR (Table 1). Found: C, 35.83; H, 5.01; N, 16.54%. Calcd for $C_{22}H_{27}N_7O_{14}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, 35.42; H, 4.96; N, 16.90%.

6-Hydroxy-2H-indazole Adenine Dinucleotide (7). NAD (984 mg, 1.5 mmol) and 6-hydroxyindazole (625 mg, 4.7 mmol) were incubated with NADase (12 ml, 4.8 U) in DMSO (5 ml)-containing Tris-HCl (55 ml) for 12 h. The incubation mixture was treated as previously described to provide a pale-brown hygroscopic mass of **7** (764 mg): $[\alpha]_D^{23}$ −43.0° (*c*, 0.55, H_2O); UV (H_2O) 264 (log ε 4.30) and *ca.* 292 nm (sh, log ε 4.06); ^{1}H-NMR (Table 1). Found: C, 37.11; H, 4.82; N, 17.30%. Calcd for $C_{22}H_{27}N_7O_{14}P_2 \cdot 2NH_3 \cdot 1/2\,H_2O$: C, 36.75; H, 4.73; N, 17.54%.

5-Methoxy-2H-indazole Adenine Dinucleotide (8). A mixture of NAD (810 mg, 1.2 mmol), 5-methoxyindazole (420 mg, 2.9 mmol), and NADase (10 ml,4 U) was incubated in DMSO (8 ml)-containing Tris-HCl (70 ml) for 11 h. The incubation mixture was treated as previously described to give **8** (520 mg): $[\alpha]_D^{23}$ −25.5° (*c* 1.0, H_2O); UV (H_2O) 261 (log ε 4.27) and 310 nm (log ε 3.66); ^{1}H-NMR (Table 1). Found: C, 37.45; H, 4.92; N, 16.70%. Calcd for $C_{23}H_{29}N_7O_{14}P_2 \cdot 2NH_3 \cdot H_2O$: C, 37.27; H, 5.00; N, 17.01%.

6-Methoxy-2H-indazole Adenine Dinucleotide (9). A mixture of NAD (823 mg, 1.2 mmol), 6-methoxyindazole (325 mg, 2.2 mmol), and NADase (15 ml, 6 U) was incubated in DMSO (10 ml)-containing Tris-HCl (55 ml) for 15 h. The incubation mixture was treated as previously described to give a pale-yellow mass of **9** (452 mg): $[\alpha]_D^{23}$ −32.9° (*c* 0.78, H_2O); UV (H_2O) 266 (log ε 4.30) and *ca.* 290 nm (sh, log ε 4.04); ^{1}H-NMR (Table 1). Found: C, 37.22; H, 5.07; N, 16.68%. Calcd for $C_{23}H_{29}N_7O_{14}P_2 \cdot 2NH_3 \cdot H_2O$: 37.27; H, 5.00; N, 17.01%.

5-Chloro-2H-indazole Adenine Dinucleotide (10). A mixture of 5-chloroindazole (206 mg), NAD (750 mg), and NADase (10 ml) was incubated with DMSO (10 ml)-containing Tris-HCl (55 ml) for 10 h. The incubation mixture was treated as previously described to provide **10** (468 mg): $[\alpha]_D^{23}$ −22.1° (*c* 1.15, H_2O); UV (H_2O) 261 (log ε 4.18) and 305 nm (log ε 3.67); ^{1}H-NMR (Table 1). Found: C, 35.51; H, 4.57; N 16.64%. Calcd for $C_{22}H_{26}ClN_7O_{13}P_2 \cdot 2NH_3 \cdot H_2O$: C, 35.44; H, 4.56; N, 16.91%.

6-Chloro-2H-indaole Adenine Dinucleotide (11). A mixture of 6-Chloroindazole (310 mg), NAD (660 mg), and NADase (10 ml) was incubated in DMSO (8 ml)-containing Tris-HCl (50 ml) for 12 h. The reaction mixture was treated as previously described yielding a white hygroscopic mass of **11** (347 mg): $[\alpha]_D^{23}$ −33.1° (*c* 2.7, H_2O); UV (H_2O) 263 (log ε 4.17), *ca.* 300 (log ε 3.77), and *ca.* 310 nm (log ε 3.48); ^{1}H-NMR (Table 1). Found: C, 34.97; H, 4.62; N, 16.74%. Calcd for $C_{22}H_{26}ClN_7O_{13}P_2 \cdot 2NH_3 \cdot H_2O$: C, 35.44; H, 4.56; N, 16.91%.

4-Chloro-2H-indazole Adenine Dinucleotide (12). A mixture of NAD (660 mg), 4-chloroindazole (310 mg), and NADase (10 ml) was, similarly, incubated as described above. The incubated mixture was treated as previously described to give **12** (342 mg): $[\alpha]_D^{23}$ −35.6° (*c* 1.45, H_2O); UV (H_2O) 262 (log ε 4.19), 299 (log ε 3.74), and *ca.* 310 nm (sh, log ε 3.61); ^{1}H-NMR (Table 1). Found: C, 34.80; H, 4.66; N, 16.49%. Calcd for $C_{22}H_{26}ClN_7O_{13}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, 34.60; H, 4.72; N, 16.51%.

Hydrolytic Cleavage of Dinucleotides to Mononucleotides. Compound **1** (120 mg) was incubated with PDE (0.4 U) in 0.2 M Tris-HCl (pH 8.8, 2 ml) containing 0.2 ml of 0.2 M $MgCl_2$ at 37 °C for 6 h. The reaction mixture, upon dilution with water (30 ml), was applied to a column (2.5 cm×15 cm) of DEAE-Sephadex. The column was eluted with 0.3 M NH_4HCO_3 to provide, in turn, AMP (54 mg) and 2*H*-indazole mononucleotide (**15**) (52 mg), separately. Spectral data regarding **15** were as follows: UV (H_2O) 276 (log ε 3.63) and 295 nm (log ε 3.56); ^{1}H-NMR (δ, D_2O) 8.64 (1H, s), 7.82 (1H, d, *J*=8.8 Hz), 7.68 (1H, d, *J*=8.8 Hz), 7.43 (1H, dd, *J*=8.8, 7.2 Hz), 7.18 (1H, dd, *J*=8.8, 7.2 Hz), 6.12 (1H, d, *J*=5.6 Hz), and 4.2—4.7 (5H, ribose). 5-Amino-2*H*-indazole mononucleotide (10 mg) was likewise obtained from compound **2** (20 mg), and its spectral data were as follows: UV (H_2O) 269 (log ε 3.72), 279 (log ε 3.70), and 330 nm (log ε 3.65); ^{1}H-NMR (δ, D_2O) 8.39 (1H, s), 7.57 (1H, d, *J*=9.0 Hz), 7.07 (1H, d, *J*=9.0 Hz), 6.04 (1H, d, *J*=5.4 Hz), and 4.1—4.7 (5H, ribose). The spectral data of 5-amino-1*H*-indazole mononucleotide (3.2 mg) obtained from compound **13** (8 mg) were as follows: UV (H_2O) 235 (log ε 4.17), *ca.* 270 (sh, log ε 3.65), and 311 nm (log ε 3.67); ^{1}H-NMR (δ, D_2O) 8.57 (1H, s), 7.54 (1H, s), 7.33 (1H, d, *J*=9.2 Hz), 7.22 (1H, d, *J*=9.2 Hz), 6.09 (1H, d, *J*=5.3 Hz), 4.1—4.7 (5H, ribose). Other mononucleotides also gave satisfactory spectral data compatible with the conclusion described in the text.

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