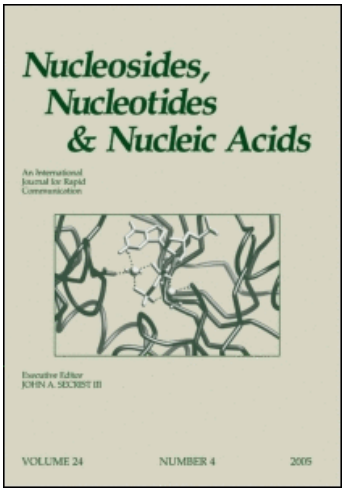

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Terminal Diols as Efficient Substrates for Transglycosylational Activity of NAD Glycohydrolase

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TERMINAL DIOLS AS EFFICIENT SUBSTRATES FOR TRANSGLYCOSYLATIONAL ACTIVITY OF NAD GLYCOHYDROLASE

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ABSTRACT: Linear terminal alkane-diols have been shown to function as more efficient substrates of the transglycosylational activty of NAD+ glycohydrolase (NADase) than the corresponding respective 1-alkanols. A series of eight alkane-diols from ethane-1,2- to nonane-1,9-diols underwent an *O*-ADP-ribosylation in the incubation reaction with NAD/NADase to provide the corresponding ribosylated products. The structural properties of these products were characterized by ^{1}H NMR and MS spectrometries. No substantial double ADP-ribosylation of the two hydroxy functions was observeded which was initially expected in the diols of higher carbon number.

Interestingly mammalian NAD+ glycohydrolase (NADase)[EC 3.2.2.5] reveals ADP-ribosyl transferase-like activity as well as the hydrolase function, depending upon enviromental conditions.[1-3] As the target substrates for the transferase-like activity of NADase, various nitrogen-containing heterocyclic systems have so far been registered.[4-6] In a previous paper,[7] we have reported that the six primary alcohols of methanol to 1-hexanol undergo an ADP-ribosylation and provide the corresponding alkyl β-dinucleotides. Such a new transglycosylational activity of NADase to alcoholic oxygen atoms other than heterocyclic nitrogen atoms has been found out for the first time on that occasion. In connection with those 1-alkanols, our attention was then focused on the corresponding terminal alkanediols. The diols, which have two symmetric hydroxy functions in both terminal positions, are expected to act essentially as more efficient substrate for the NADase

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activity. Furthermore, the diols show increased hydrophilicity, as compared with the corresponding 1-alkanol bearing more than three carbons, and result in a rise of solubility in water. Judging from that the solubility is a secondary important factor for the occurring of effective ADP-ribosylation,[7] diols bearing more than six carbons (heptane-1,7-, octane-1,8-, and nonane-1,9-diols) are also presumed to function as target substrate for the NADase activity , unlike the corresponding 1-alkanols which are slightly soluble or practically insoluble in water. In addition, it is of interest whether or not long carbon-chain terminal diols, which seem to have a relatively loose conformation and hardly to suffer the steric hindrance of ADP-ribosyl group, exhibit a bidentate properties to form a doubly ADP-ribosylated product. It is, therefore, worthy to examine some terminal diols for their functional activities as the target substrate of NADase.

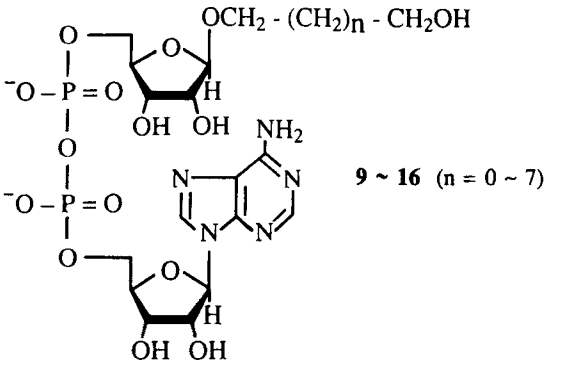
This paper describes the occurrence of efficient *O*-ADP-ribosylation of eight terminal diols (**1–8**) from ethane-1,2- to nonane-1,9-diols in the presence of β-NAD and NADase, and the spectrometric characterisations of the resultant new ADP-ribosylated products (**9–16**).

RESULTS AND DISCUSSION

We have previously shown[7] that, in addition to a nucleophilicity, a solubility in water is also one of the necessary conditions of substrate for ADP-ribosyl transferase-like activity of NADase. An 1-alkanol containing four or more carbons exhibits a decreased solubility in water with increasing carbon number. For this reason, the corresponding alcohols (1-butanol, 1-pentanol, and 1-hexanol) have given the poor yield of ADP-ribosylated products.[7] On the other hand, terminal diols (**3–8**) having the same or more carbon number as that of the corresponding 1-alkanol show an increased hydrophilicity as well as a possibly increased substrate efficiency ascribable to the two functional hydroxy groups, and keep an appreciable solubility (above 100 mM) enough to maintain the enzymatic reaction system.

Thus, each of diols **1–8** (220–300 mM) was incubated with β-NAD in the presence of NADase and during the incubation the progress of reaction was checked by TLC. In all cases, a new spot, other than ones due to the hydrolysis products (ADP-ribose and nicotinamide) of NAD, was developed gradually with the lapse of time to reveal possible occurrence of ADP-ribose transfer. The incubation reaction was ceased with the disappearance of NAD spot. Target products (**9–16**) were isolated in a pure form by

$HOCH_2 - (CH_2)_n - CH_2OH$ **1 ~ 8** (n = 0 ~ 7)



9 ~ 16 (n = 0 ~ 7)

column chromatography on DEAE-Sephadex A-25, and their yield and selected spectrometric data were summarized in TABLE 1. On the whole, isolated yields were fairly to highly good (40 – 64%), and it can be seen that even a seven or eight carbons-containing higher homologue of diols give a more than 60% yield. The negative FAB-MS spectra of products **9–16** exhibited respective [M+ – 1] ion peaks at *m/z* 602, 616, 630, 644, 658, 672, 686 and 700, suggesting that they were mono-ADP-ribosylated products. The whole spectrum of **12** is illustrated in FIG.1 as an example. On the other hand, the ^1H NMR spectra showed appropriate numbers of alkyl-chain protons and two anomeric protons in addition to ten non-exchangeable protons of ADP-ribose moiety, demonstrating the ADP-ribosylated structures of products **9–16.**

As for the ^1H NMR spectral characteristics of the alkyl-chain protons in the ADP-ribosylated products, a geminal coupling (J_{gem} = ca. 10 Hz) was observed for the α-methylene protons (δ 3.35 – 3.51 and δ 3.58 – 3.80) of the respective alkoxy groups and their chemical shifts were located separately by 0.21 – 0.27 ppm with a triplet signal due to terminal hydroxymethyl protons between them. As a typical example, the spectral pattern of **12** was shown in FIG. 2. These observations indicate the nonequivalency of the geminal protons, which can be ascribed to a rotational hindrance of O—CH_2 bond in the alkoxy group by the ribofuranose ring. A signal of newly formed anomeric proton appeared at around δ 4.95 as a singlet or doublet with a small vicinal coupling constant

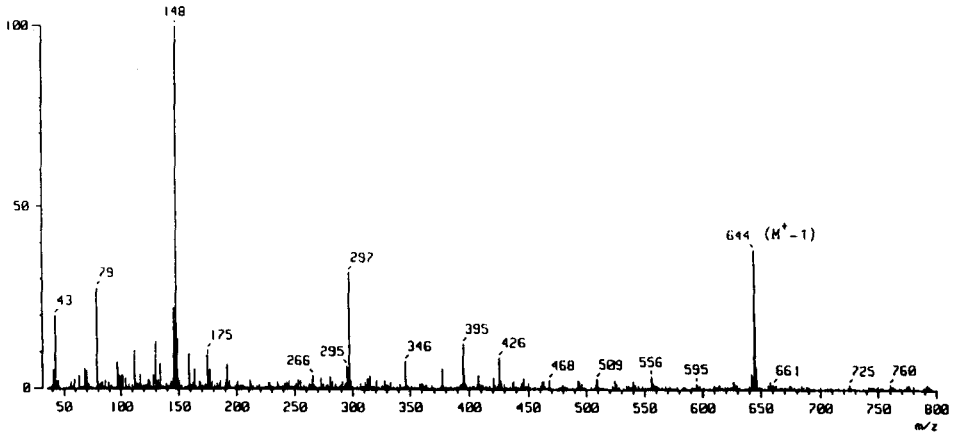


FIG. 1. A FAB-MS spectrum of compound **12**.

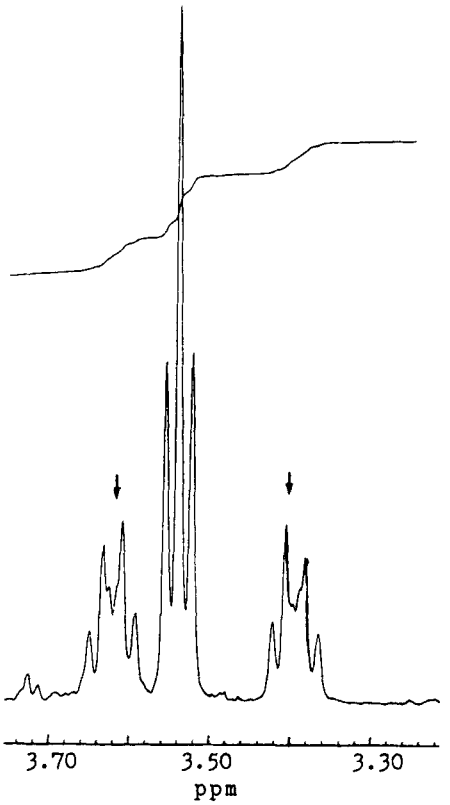


FIG. 2. ^{1}H NMR signal pattern of geminal protons (arrows) of the *O*-methylene moiety in compound **12**.

TABLE 1.
Yields and selected spectrometric data of ADP-ribosylated diols (**9 – 16**).

		MS	^{1}H NMR (δ)		
Entry	Yield (%) [a]	[M$^+$ – 1] (*m/z*)	New anomeric signals [b]	Nonequivalent geminal signals	
9	43	602	4.98	3.78	3.51
10	64	616	4.94	3.75	3.50
11	47	630	4.94	3.65	3.43
12	64	644	4.94	3.61	3.46
13	50	658	4.93	3.59	3.38
14	62	672	4.94	3.58	3.36
15	64	686	4.93	3.58	3.35
16	45	700	4.94	3.58	3.35

a) Based on NAD used. b) J_{vic} = 0.0 – 0.9 Hz.

(≤1.0 Hz) (TABLE 1), demonstrating the dihedral angle of about 90° which implies a *trans* configuration between the anomeric and the vicinal protons in the D-ribofuranoside ring system [8,9] and, in its turn, the β-ribonucleotide structure of the ADP-ribosylated products.

We initially expected the formation of additional di(ADP-ribosyl)ated product (**17**), even if it should be a small amount, particularly in the case of longer carbon-chain diol substrates **6–8**, which were supposed to have a flexible conformation around the remaining terminal hydroxy function. However, no detectable amount of the product **17** other than the mono-*O*-ribosylated diols was isolated in all cases examined. Thus, the mono-ribosylated diols **14–16** isolated in advance were further re-incubated with NAD/NADase, respectively, but no substantial new product corresponding to **17** was formed in these cases as well, indicating that once ADP-ribosylated at one hydroxy group in the diols,

possible stereoconformational alterations around the other terminal hydroxy group occurred to bring about the poor reactivity of the hydroxy function.

Recently, CD38 molecule [10,11] has been noted in connection with NADase activity. This molecule is extensively expressed on mammalian lymphoid cells and shows, besides the hydrolase activity similar to that of tissue NADase, the reguratory function of an intracellular signal transduction.[12,13] When biochemical relationships between tissue NADase and CD38 are elucidated in detail, the physiological significances of NADase can be further explained from the standpoint of cellular metabolism. Efficient NADase-catalysed ADP-ribosylations of terminal diols observed in this study suggest a new role (e.g. a participation in the metabolic pathway of polyol) for NADase in vivo by some possibility.

In conclusion, linear terminal diols were proved to be more effective substrates for the transglycosylational activity of NADase as compared with the corresponding 1-alkanols and to provide stereospecificly hydroxyalkyl β-ribodinucleotides in a good yield. The α-methylene protons of an alkoxy moiety in the enzymatic products were unequivalent irrespective of the carbon-chain length as evidenced by ^{1}H NMR spectrometry. No additional ADP-ribosylation of the other terminal hydroxy function of the products was observed in all cases.

EXPERIMENTAL SECTION

^{1}H NMR spectra were recorded in D_2O with a Bruker MSL-400 spectrometer with sodium [2, 2, 3, 3-d_4] 3-(trimethylsilyl)propionate as internal standard. The abbreviations "s, d, dd, t, dt, quint, sext, br, bs, and bm" denote "singlet, doublet, double doublet, triplet, double triplet, quintet, sextet, broad, broad singlet, and broad multiplet", respectively. FAB-MS (negative) spectra were determined with a JEOL JMX-AX 500 spectrometer. Analytical TLCs were run on precoated silica gel $60F_{254}$ HPTLC plates (Merck, 10 cm x 10 cm) by using 2-propanol/0.2% aqueous ammonia (7 : 3, v/v) as the developing system. Column chromatography was performed on DEAE-Sephadex A-25, with monitoring with an LKB Uvicord II (254 nm). Enzymatic reactions were performed in aqueous solution buffered with 0.1 M tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl/pH 7.2). β-NAD (N-8881, 94% purity) was obtained from Sigma Chemical Co. Each terminal diol was purchased in a state of high purity from Aldrich Chemical Co. NAD^+ glycohydrolase (NADase), containing ca. 0.4 U/ml of activity, was prepared from

fresh porcine brain as a crude particulate enzyme by the method of Zatman et al.,[14] and was used without further purification (U is the activity of NADase which will cleave 1 μmol of β-NAD per min).

Transglycosylational Reactions of Linear Terminal Diols (1–8):

Of ethane-1,2-diol (1): β-NAD (660 mg, 0.99 mmol) and **1** (7.0g, 0.11mol) were incubated with NADase (10 ml, 4U) in Tris–HCl (90 ml, pH 7.2) with continuous stirring at 37 °C for 24 h. During this incubation period, occasional pH adjustments were required because the pH of the incubation system gradually lowered with the progress of the reaction. After TLC had indicated consumption of the NAD, the reaction mixture was treated in a similar manner as described previously,[7] giving a crude white mass. This crude mass was dissolved in water (40 ml) and applied to a column (2.5 cm x 45 cm) of DEAE-Sephadex A-25 (HCO_3^- form). The column was washed with 0.8% (w/w) aqueous NH_4HCO_3 solution (300 ml) to remove any non-adsorbing component and then eluted with a 4% solution of the same hydrogen carbonate salt. The first eluted major component showing a strong UV-absorption peak at 259 nm was the desired product and the second major componeent was ADP-ribose. The corresponding eluate fractions were collected and evaporated to dryness in vacuo to give a white solid mass. The isolated mass was further subjected to rechromatography and appropriate fractions were repeatedly lyophilized to provide **9** (263 mg, 43% yield) as its white ammonium salt, that showed a single spot upon TLC analysis. An analytical sample was obtained by further drying over P_2O_5 in vacuo at 40 °C for 12 h. **9:** ^{1}H NMR (400 MHz, D_2O): δ 8.50 (s, 1H, adenine 8-H), 8.24 (s, 1H, adenine 2-H), 6.13 (d, 1H, J = 5.9 Hz, anomeric/adenine side), 4.98 (s, 1H, anomeric /alkoxy side), 4.77 (t, 1H, J = 5.5 Hz)*, 4.53 (dd, 1H, J = 3.5, 5.1 Hz)*, 4.39 (bs, 1H)*, 4.29 (dd, 1H, J = 4.8, 6.2 Hz)*, 4.22 (bs, 2H)*, 4.11 (m, 2H)*, 4.06 (d, 1H, J = 4.7 Hz)*, 3.98 (sext, 1H, J = 5.7 Hz)* (* eight signals, 10H, two riboses-related), 3.78 (dt, 1H, J = 6.5, 10.0 Hz, –OC**H**H–), 3.68 (t, 2H, J = 6.6 Hz, –C**H**$_2$OH), 3.51 (dt, 1H, J = 6.5, 10.0 Hz, –OCH**H**–). Anal. Calcd. for $C_{17}H_{33}N_7O_{15}P_2 \cdot 2NH_3$: C, 32.03; H, 5.22; N, 15.38. Found: C, 32.23; H, 5.16; N, 15.55.

Of propane-1,3-diol (2): NAD (660 mg, 0.99 mmol) and **2** (6.8 g, 92 mmol) were incubated with NADase (10 ml, 4U) in 0.1 M Tris–HCl (70 ml) at 37 °C with continuous stirring for 24 h. The incubation mixture was worked up as described above and the resulting crude mass was purified by column chromatography in a similar manner

as described above to give **10** (392 mg, 64% yield) as its white ammonium salt. An analytical sample was obtained by drying over P_2O_5 in vacuo at 40 °C for 12 h. **10:** ^{1}H NMR (400 MHz, D_2O): δ 8.51 (s, 1H, adenine 8-H), 8.24 (s, 1H, adenine 2-H), 6.13 (d, 1H, J = 5.9 Hz, anomeric/adenine side), 4.94 (d, 1H, J = 0.9 Hz, anomeric/alkoxy side), 4.77 (t, 1H, J = 5.5 Hz)*, 4.53 (dd, 1H, J = 3.6, 5.0 Hz)*, 4.39 (bs, 1H)*, 4.23 (m, 3H)*, 4.09 (bm, 2H)*, 4.01 (dd, 1H, J = 0.9, 4.7 Hz)*, 3.97 (sext, 1H, J = 5.9 Hz)* (* seven signals, 10H, two riboses-related), 3.75 (dt, 1H, J = 6.4, 10.0 Hz, –OC**H**HCH$_2$–), 3.61 (t, 2H, J = 6.4 Hz, –C**H$_2$**OH), 3.50 (dt, 1H, J = 6.4, 10.0 Hz, –OCH**H**CH$_2$–), 1.76 (quint, 1H, J = 6.4 Hz, –OCHHC**H$_2$**–). Anal. Calcd. for $C_{18}H_{35}N_7O_{15}P_2 \cdot 2NH_3$: C, 33.19; H, 5.42; N, 15.05. Found: C, 33.30; H, 5.39; N, 15.21.

Of butane-1,4-diol (3): A mixture of NAD (660 mg, 0.99 mmol) and **3** (3.2 g, 36 mmol) was incubated with NADase (15 ml, 6U) in Tris–HCl (60 ml) for 20 h. The incubation mixture was worked up as described above and the resulting crude mass was purified by column chromatography in a similar manner as described above to give **11** (297 mg, 47%) as its ammonium salt. An analytical sample was obtained similarly as described above. **11:** ^{1}H NMR (D_2O): δ 8.52 (s, 1H, adenine 8-H), 8.24 (s, 1H, adenine 2-H), 6.13 (d, 1H, J = 5.9 Hz, anomeric/adenine side), 4.94 (d, 1H, J = 0.9 Hz, anomeric /alkoxy side), 4.76 (t, 1H, J = 5.5 Hz)*, 4.54 (dd, 1H, J = 3.6, 4.2 Hz)*, 4.39 (s, 1H)*, 4.23 (br, 3H)*, 4.1 (bm, 2H)*, 4.02 (dd, 1H, J = 0.9, 5.2 Hz)*, 3.95 (quint, 1H, J = 5.5 Hz)* (* 10H, two riboses-related), 3.65 (dt, J = 6.0, 9.7 Hz, –OC**H**HCH$_2$–), 3.55 (t, 2H, J = 6.0 Hz, –CH$_2$C**H$_2$**OH), 3.43 (dt, 1H, J = 6.0, 9.7 Hz, –OCH**H**CH$_2$–), 1.52 (m, 4H, –C**H$_2$**C**H$_2$**CH$_2$OH). Anal. Calcd. for $C_{19}H_{37}N_7O_{15}P_2 \cdot 2NH_3$: C, 34.29; H, 5.60; N, 14.73. Found: C, 34.48; H, 5.49; N, 14.94.

Of pentane-1,5-diol (4): A mixture of NAD (670 mg, 1.01 mmol) and **4** (3.3 g, 32 mmol) was incubated with NADase (15 ml, 6U) in Tris–HCl (70 ml) for 20 h. The reaction mixture was treated similarly as described above and the resultant crude mass was purified by column chromatography in a similar manner as described above to provide **12** (416 mg, 64%) as its ammonium salt. An analytical sample was obtained similarly as described above. **12:** ^{1}H NMR (D_2O): δ 8.51 (s, 1H, adenine 8-H), 8.24 (s, 1H, adenine 2-H), 6.13 (d, 1H, J = 5.8 Hz, anomeric/adenine side), 4.94 (s, 1H, anomeric/alkoxy side), 4.76 (t, 1H, J = 5.5 Hz)*, 4.53 (dd, 1H, J = 3.7 and 4.2 Hz)*, 4.39 (s, 1H)*, 4.23 (bs, 3H)*, 4.09 (br, 2H)*, 4.01 (d, 1H, J = 4.7 Hz)*, 3.96 (sext, 1H, J = 5.5 Hz)* (* 10

H, two riboses-related), 3.61 (dt, 1H, J = 6.6, 9.7 Hz, –OC**H**HCH$_2$–), 3.54 (t, 2H, J = 6.6 Hz, –CH$_2$C**H$_2$**OH), 3.46 (dt, 1H, J = 6.6, 9.7 Hz, –OCH**H**CH$_2$–), 1.47 (quint, 4H, J = 7.0 Hz, –C**H$_2$**CH$_2$C**H$_2$**CH$_2$OH), 1.25 (quint, 2H, J = 7.0 Hz, –CH$_2$C**H$_2$**CH$_2$CH$_2$OH). Anal. Calcd. for $C_{20}H_{39}N_7O_{15}P_2 \cdot 2NH_3$: C, 35.35; H, 5.79; N, 14.43. Found: C, 35.20; H, 5.73; N, 14.64.

Of hexane-1,6-diol (5): NAD (650 mg, 0.98 mmol) and **5** (2.6 g, 22 mmol) were incubated with NADase (10 ml, 4U) in Tris–HCl (65 ml) for 24 h. A crude mass obtained upon a similar treatment of the incubation mixture as described above was purified by column chromatography as described above to provide **13** (325 mg, 50%) as its ammonium salt. An analytical sample was obtained similarly as described above. **13:** ^{1}H NMR (D_2O): δ 8.52 (s, 1H, adenine 8-H), 8.25 (s, 1H, adenine 2-H), 6.13 (d, 1H, J = 5.8 Hz, anomeric/adenine side), 4.93 (s, 1H, anomeric/alkoxy side), 4.76 (t, 1H, J = 5.4 Hz)*, 4.53 (dd, 1H, J = 3.8, 4.6 Hz)*, 4.39 (s, 1H)*, 4.22 (bs, 3H)*, 4.1 (bm, 2H)*, 4.00 (d, 1H, J = 4.7 Hz)*, 3.96 (sext, 1H, J = 5.0 Hz)* (* 10H, two riboses-related), 3.59 (dt, 1H, J = 6.7, 9.8 Hz, –OC**H**HCH$_2$–), 3.54 (t, 2H, J = 6.7 Hz, –CH$_2$C**H$_2$**OH), 3.38 (dt, 1H, J = 6.7, 9.8 Hz, –OCH**H**CH$_2$–), 1.44 [quint, 4H, J = 6.7 Hz, –OCHHC**H$_2$**(CH$_2$)$_2$C**H$_2$**CH$_2$OH], 1.20 (m, 4H, –OCHHCH$_2$C**H$_2$**C**H$_2$**CH$_2$CH$_2$OH). Anal. Calcd. for $C_{21}H_{41}N_7O_{15}P_2 \cdot 2NH_3$: C, 36.37; H, 5.96; N, 14.14. Found: C, 36.33; H, 5.93; N, 14.27.

Of heptane-1,7-diol (6): NAD (762 mg, 1.15 mmol) and **6** (2.1 g, 16 mmol) were incubated with NADase (15 ml, 6U) in Tris–HCl (62 ml) for 24 h. A crude mass obtained upon a similar treatment of the incubation mixture as described above was purified by column chromatography similarly as described above to give **14** (482 mg, 62%) as its ammonium salt. **14:** ^{1}H NMR (D_2O): δ 8.52 (s, 1H, adenine 8-H), 8.24 (s, 1H, adenine 2-H), 6.13 (d, 1H, J = 5.6 Hz, anomeric/adenine side), 4.94 (s, 1H, anomeric/alkoxy side), 4.77 (t, 1H, J = 5.5 Hz)*, 4.54 (t, 1H, J = 4.4 Hz)*, 4.40 (s, 1H)*, 4.23 (s, 3H)*, 4.10 (bm, 2H)*, 4.01 (d, 1H, J = 4.4 Hz)*, 3.97 (sext, 1H, J = 5.5 Hz)* (* 10 H, two riboses-related), 3.58 (dt, 1H, J = 6.7, 9.9 Hz, –OC**H**HCH$_2$–), 3.54 (t, 2H, J = 6.7 Hz, –CH$_2$C**H$_2$**OH), 3.36 (dt, 1H, J = 6.7, 9.9 Hz, –OCH**H**CH$_2$–), 1.47 (quint, 2H, J = 6.7 Hz, –OCHHC**H$_2$**–), 1.40 (br, 2H, –C**H$_2$**CH$_2$OH), 1.24—1.20 (br, 2H, –OCHHCH$_2$C**H$_2$**–), 1.17 [bs, 4H, –C**H$_2$**C**H$_2$**(CH$_2$)$_2$OH]. Anal. Calcd. for

$C_{22}H_{43}N_7O_{15}P_2 \cdot 2NH_3$: C, 37.34; H, 6.13; N, 13.86. Found: C, 37.25; H, 6.09; N, 13.98.

Of octane-1,8-diol (7): A mixture of NAD (777 mg, 1.17 mmol) and **7** (2.4 g, 17 mmol) was incubated with NADase (15 ml, 6U) in Tris–HCl (70 ml) for 28 h. A crude mass obtained upon a similar treatment of the reaction mixture as described above was purified by column chromatography similarly as described above to provide **15** (510 mg, 64%) as its ammonium salt. **15:** ^{1}H NMR (D_2O): δ 8.51 (s, 1H, adenine 8-H), 8.23 (s, 1H, adenine 2-H), 6.13 (d, 1H, J = 5.6 Hz, anomeric/adenine side), 4.93 (d, 1H, J = 0.9 Hz, anomeric/alkoxy side), 4.75 (t, 1H, J = 5.5 Hz)*, 4.53 (t, 1H, J = 4.1 Hz)*, 4.39 (d, 1H, J = 1.2 Hz)*, 4.23 (s, 3H)*, 4.11 (bm, 2H)*, 4.01 (dd, 1H, J = 1.2, 4.6 Hz)*, 3.97 (sext, 1H, J = 5.4 Hz)* (* 10H, two riboses-related), 3.58 (dt, 1H, J = 6.7, 9.9 Hz, –OC**H**HCH$_2$–), 3.55 (t, 2H, J = 6.7 Hz, –C**H$_2$**OH), 3.35 (dt, 1H, J = 6.7, 9.9 Hz, –OCH**H**CH$_2$–), 1.47 (quint, 2H, J = 6.7 Hz, –OCHHC**H$_2$**–), 1.38 (bm, 2H, –CH$_2$C**H$_2$**CH$_2$OH), 1.24—1.16 [br, 4H, –C**H$_2$**(CH$_2$)$_2$C**H$_2$**(CH$_2$)$_2$OH], 1.12 [s, 4H, –CH$_2$C**H$_2$**C**H$_2$**(CH$_2$)$_3$OH]. Anal. Calcd. for $C_{23}H_{45}N_7O_{15}P_2 \cdot 2NH_3$: C, 38.28; H, 6.29; N, 13.59. Found: C, 38.45; H, 6.31; N, 13.46.

Of nonane-1,9-diol (8): A mixture of β-NAD (777 mg, 1.17 mmol) and **8** (2.3 g, 14 mmol) was incubated with NADase (15 ml, 6U) at 37 °C in Tris–HCl (655 ml) for 30 h. A crude mass obtained upon a similar treatment of the incubation mixture as described above was purified by column chromatography on DEAE-Sephadex A-25 in a similar manner as described above to provide **16** (372 mg, 45%) as its ammonium salt. An analytical sample was obtained by further drying over P_2O_5 in vacuo at 40 °C for 12 h. **16:** ^{1}H NMR (D_2O): δ 8.51 (s, 1H, adenine 8-H), 8.22 (s, 1H, adenine 2-H), 6.12 (d, 1H, J = 5.8 Hz, anomeric/adenine side), 4.94 (s, 1H, anomeric/alkoxy side), 4.75 (t, 1H, J = 5.5 Hz)*, 4.53 (t, 1H, J = 4.1 Hz)*, 4.39 (s, 1H)*, 4.23 (s, 3H)*, 4.11 (bm, 2H)*, 4.01 (d, 1H, J = 4.7 Hz)*, 3.98 (sext, 1H, J = 5.3 Hz)* (* 10H, two riboses-related), 3.58 (dt, 1H, J = 6.7, 9.9 Hz, –OC**H**HCH$_2$–), 3.55 (t, 2H, J = 6.6 Hz, –CH$_2$C**H$_2$**OH), 3.35 (dt, 1H, J = 6.7, 9.9 Hz, –OCH**H**CH$_2$–), 1.48 (quint, 2H, J = 6.8 Hz, –OCHHC**H$_2$**–), 1.38 (bm, 2H, –C**H$_2$**CH$_2$OH), 1.25—1.16 [br, 4H, –C**H$_2$**(CH$_2$)$_3$C**H$_2$**(CH$_2$)$_2$OH], 1.10 [s, 6H, –CH$_2$C**H$_2$**C**H$_2$**C**H$_2$**(CH$_2$)$_3$OH]. Anal. Calcd. for $C_{24}H_{47}N_7O_{15}P_2 \cdot 2NH_3$: C, 39.19; H, 6.44; N, 13.33. Found: C, 39.10; H, 6.34; N, 13.57.

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REFERENCES

1) Masmoudi, A.; Mandel, P. *Biochemistry* **1987**, ***26***, 1965–1969.
2) Kim, H.; Jacobson, E. L.; Jacobson, M. K. *Mol. Cell. Biochem.* **1994**, ***138***, 237–243.
3) Nurten, R.; Ustundag, I.; Sayhan, N.; Bermek, E. *Biochem. Biophys. Res. Commun.* **1994**, ***200***, 450–458.
4) Waslter, P.; Kaplan, N. O. *J. Biol. Chem.* **1963**, ***238***, 2823–2830.
5) Tono-oka, S.; Azuma, I. *J. Heterocyclic Chem.* **1989**, ***26***, 339–343.
6) Tono-oka, S.; Azuma, I. *Acta Chem. Scand.* **1994**, ***48***, 780–782.
7) Tono-oka, S.; Azuma, I. *Libigs Ann./Recueil* **1997**, 1823–1826.
8) May Jr., J. A.; Townsend, L. B. *J. Org. Chem.* **1976**, ***41***, 1449–1456.
9) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 5th Ed., John Wiley & Sons, New York, **1991**, pp. 196–197.
10) Shubinsky, G.; Schlesinger, M. *Immunity* **1997**, ***7***, 315–324.
11) Lund, F. A.; Cockayne, D. A.; Randall, T. D.; Solvason, N.; Schuber, F.; Howard, M. C. *Immunol. Rev.* **1998**, ***161***, 79–93.
12) Inageda, K.; Takahashi, K,; Tokita, K.; Nishina, H.; Kanaho, Y.; Kukimoto, I.; Kontani, K.; Hoshino, S.; Katada, T. *J. Biochem. (Tokyo)* **1995**, ***117***, 125–131.
13) De Flora, A.; Guida, L.; Franco, L.; Zocchi, E.; Bruzzone, S.; Bennatti, U.; Damonte, G; Lee, H. C. *J. Biol. Chem.* **1997**, ***272***, 12945–12951.
14) Zatman, L. J.; Kaplan, N. O.; Colowick, S. P.; Ciotti, M. M. *J. Biol. Chem.* **1954**, ***209***, 467–484.

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