Notes

Specific Inhibition of Poly(ADP-ribose) Glycohydrolase by Adenosine Diphosphate (Hydroxymethyl)pyrrolidinediol†

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Adenosine diphosphate (hydroxymethyl)pyrrolidinediol (ADP-HPD), an NH analog of ADP-ribose, was chemically synthesized and shown to be a potent and specific inhibitor of poly(ADP-ribose) glycohydrolase. The synthetic starting material was the protected pyrroidine, (2*R*,3*R*,4*S*)-1-(benzyloxycarbonyl)-2-(hydroxymethyl)pyrrolidine-3,4-diol 3,4-*O*-isopropylidene acetal. This starting pyrrolidine was phosphorylated, coupled to adenosine 5′-monophosphate, and deprotected, yielding the title inhibitor ADP-HPD. ADP-HDP was shown to inhibit the activity of poly(ADP-ribose) glycohydrolase by 50% (IC_{50}) at 0.12 μM, a value 1000-times lower than the IC_{50} of the product, ADP-ribose. The NAD glycohydrolase from *Bungarus fasciatus* venom was less sensitive to inhibition by ADP-HPD, exhibiting an IC_{50} of 260 μM. ADP-HPD did not inhibit either poly(ADP-ribose) polymerase or NAD:arginine mono(ADP-ribosyl)-transferase A at inhibitor concentrations up to 1 mM. At low ADP-HPD concentration, inhibition was therefore shown to be highly specific for poly(ADP-ribose) glycohydrolase, the hydrolytic enzyme in the metabolism of ADP-ribose polymers.

The metabolism of ADP-ribose polymers in the cell nucleus is involved with the cellular recovery from DNA damage.[1,2] ADP-ribose polymers are synthesized by poly(ADP-ribose) polymerase, which catalyzes the cleavage of the nicotinamide–ribotide bond of NAD and the subsequent polymerization of the ADP-ribose moiety. ADP-ribose polymers consist of chains of up to 150 ADP-ribose units linked glycosidically through the (1″–2′) ribosyl–ribose bond and covalently attached at the chain terminus to protein through linkage to glutamyl residues (Figure 1). The polymers are thought to facilitate alteration of chromatin structure during DNA repair. Since this alteration must be transient, the efficient degradation of the polymer is necessary to complete the repair cycle. The degradation of ADP-ribose polymers is catalyzed by poly(ADP-ribose) glycohydrolase.[3] This reaction involves hydrolysis of the glycosidic (1″–2′) ribosyl–ribose linkage to release ADP-ribose residues from the polymer chain (Figure 1).

Since the synthesis and degradation of ADP-ribose

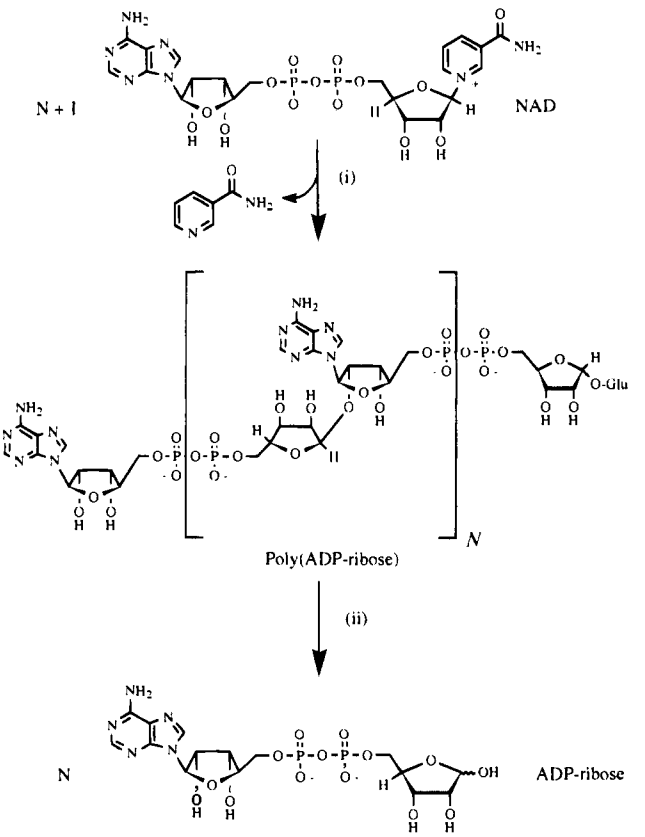

Figure 1. Metabolism of ADP-ribose polymers. ADP-ribose polymers are synthesized from NAD by (i) poly(ADP-ribose) polymerase; ADP-ribose polymers are hydrolyzed to ADP-ribose and an ADP-ribose–protein core by (ii) poly(ADP-ribose) glycohydrolase. The final ADP-ribosyl residue is removed from the protein by another enzyme, ADP-ribosyl protein lyase.

† Portions of this work have been presented in preliminary form before the Paul Mandel International Meeting on ADP-Ribosylation Reactions, Quebec City, Quebec, Canada, May 1991 (Slama, J. T.; Simmons, A. M.; Hassan, M. E.; Aboul-Ela, N.; Jacobson, M. K. In *ADP-Ribosylation Reactions*; Poirier, G. G., Moreau, P., Eds.; Springer-Verlag: New York, 1992; pp 316–320.

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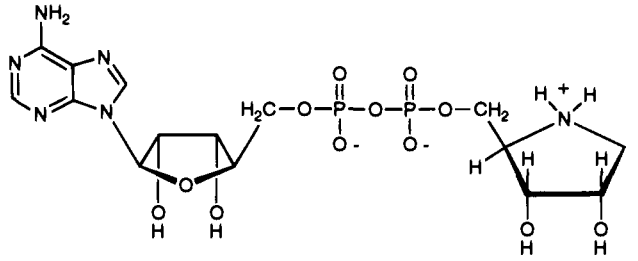
polymers is required for cellular recovery from many kinds of DNA damage,[4] this metabolism represents an attractive target for the development of agents that could chemosensitize cells to radiation or DNA-damaging drugs. Much research has focused on the development and application of poly(ADP-ribose) polymerase inhibitors.[5,6] Although inhibition of poly(ADP-ribose) polymerase appears promising, many of the inhibitors lack specificity as they also inhibit other ADP-ribosyltransferases[7] as well as affect other areas of metabolism unrelated to ADP-ribosyl-transfer reactions.[8-10] The unique structure of ADP-ribose polymers suggests that inhibitors of the poly(ADP-ribose) glycohydrolase could be highly specific. The necessary role for this enzyme in completing the repair cycle suggests further that glycohydrolase inhibitors would also likely act as chemosensitizing agents. At present, specific inhibitors for poly(ADP-ribose) glycohydrolase are not available.

Amino sugar analogs in which NH is substituted for the endocyclic pyranose oxygen atom are often specific and potent inhibitors of glycosidases.[11] This suggested that similarly designed aminoribose analogs might specifically inhibit poly(ADP-ribose) glycohydrolase. We report here that *a*denosine *di*phosphate (*h*ydroxymethyl)*pyrrolidine*diol (**1**, ADP-HPD), an NH analog of ADP-ribose, is a potent and highly specific inhibitor of poly(ADP-ribose) glycohydrolase. ADP-HPD should be useful for studies of enzyme structure in vitro and of enzyme function in vivo.



1, ADP-HPD

Results

Chemistry. The synthesis of the target inhibitor (ADP-HPD, **1**), starting from pyrrolidine **2**,[12] is depicted in Figure 2. Pyrrolidine **2** was phosphorylated using $POCl_3$ in tetrahydrofuran (THF), the acetonide removed, and the resulting phosphate ester **3** isolated chromatographically. Although there is only a single unprotected hydroxyl group available for reaction with the phosphorylating agent in pyrrolidine **2**, there was concern that deprotection during the phosphorylation reaction in buffered THF could produce a mixture of phosphates. The product **3** was therefore carefully characterized by HPLC and microanalysis to establish its purity and by ^{31}P-, ^{14}C-, and ^{1}H-NMR, establishing its structure as **3** (see the Experimental Section). Phosphomonoester **3** was coupled to AMP activated as the phosphoromorpholidate (**5**, Figure 2)[13] and the resulting (*Z*)-ADP-HPD (**4**) purified by anion-exchange chromatography. The protecting group of **4** was removed by hydrogenolysis, producing ADP-HPD, **1**.

Enzymological Testing

Inhibition of Poly(ADP-ribose) Glycohydrolase. ADP-HPD was tested as an inhibitor of poly(ADP-ribose) glycohydrolase isolated from bovine thymus by adding varying concentrations of inhibitor to assay mixtures and measuring the resulting initial rate of ADP-ribose polymer hydrolysis. For comparison, the effect of added ADP-ribose on the rate of enzymatic hydrolysis was also determined. The results, presented in Figure 3, show that when the assay was conducted using a substrate concentration 10 μM in ADP-ribose residues, glycohydrolase activity was inhibited by 50% (IC_{50}) at 0.12 μM ADP-HPD. ADP-ribose was much less effective as an inhibitor with an IC_{50} equal to 120 μM, a value 1000-times higher than the IC_{50} for ADP-HPD. Experiments in which the enzyme and the inhibitor

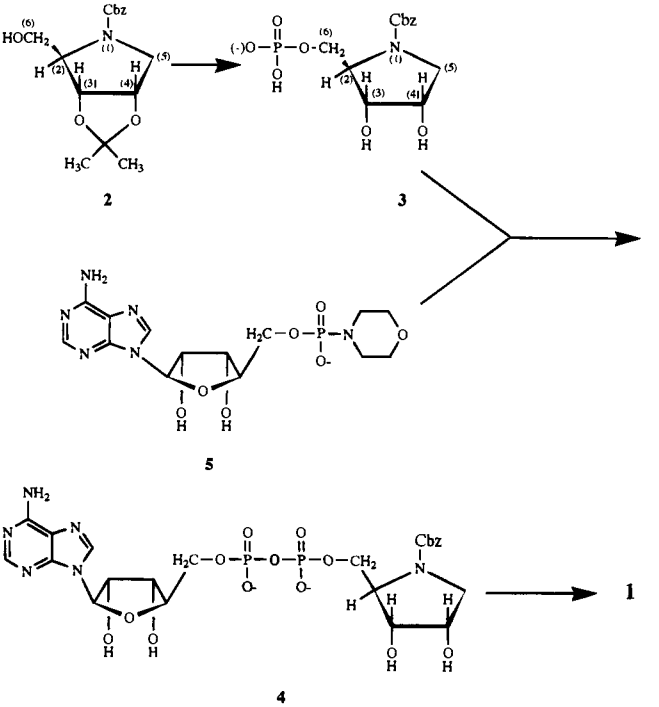


Figure 2. Synthesis of ADP-HPD (**1**) from (2*R*,3*R*,4*S*)-1-(*Z*)-2-(hydroxymethyl)pyrrolidine-3,4-diol 3,4-*O*-isopropylidene acetal, **2**.

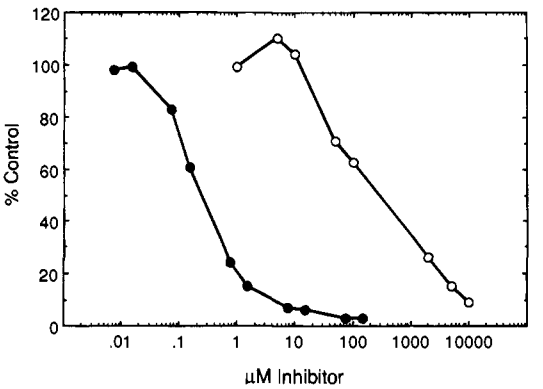


Figure 3. Inhibition of calf thymus poly(ADP-ribose) glycohydrolase by the addition of varying amounts of ADP-HPD (●) or ADP-ribose (○) to the enzyme assay at pH 7.5 in which the substrate [^{32}P]poly(ADP-ribose) was present at a monomer concentration of 10 μM. ADP-ribose which was liberated was separated from the polymer by TLC on poly(ethylenimmine)-impregnated cellulose sheets (0.3 M LiCl–0.9 M acetic acid) and the spot excised and quantitated radiometrically. The assay was otherwise conducted as described in the Experimental Section.

Table 1. Inhibition of ADP-Ribosyltransferases by ADP-HPD

added ADP-HPD (μM)	activity[a] (units per assay)	% control
Poly(ADP-ribose) Polymerase[b]		
0 (control)	0.44	100
100	0.47	106
1000	0.40	90
Mono(ADP-ribosyl)transferase A[c]		
0 (control)	0.06	100
100	0.07	117
1000	0.05	83

[a] The values shown represent the mean values of triplicate assays from two separate experiments. The percent standard deviation of individual measurements was less than 10%. [b] One unit of enzyme incorporates 1 nmol of ADP-ribose into polymer per minute. [c] One unit of enzyme transfers 1 μmol of ADP-ribose to histone per minute.

were incubated together for varying times prior to assay indicated that there was no time dependence of inhibitor potency.

Effect of ADP-HPD on ADP-Ribosyltransferases. The effects of ADP-HPD on two enzymes that catalyze ADP-ribosyl transfer are shown in Table 1. ADP-HPD did not significantly inhibit the activity of bovine thymus poly(ADP-ribose) polymerase at concentrations up to 1 mM. Likewise, ADP-HPD did not inhibit the activity of the NAD:arginine mono(ADP-ribosyl)transferase A significantly at concentrations up to 1 mM. NAD:arginine mono(ADP-ribosyl)transferase A catalyzes the synthesis of (ADP-ribosyl)arginine from NAD and free arginine. This enzyme is one of the classes of enzymes which modify proteins with ADP-ribose monomers.

Effect of ADP-HPD on NAD Glycohydrolases. High concentrations of ADP-HPD inhibited the hydrolysis of NAD to ADP-ribose and nicotinamide catalyzed by the *Bungarus fasciatus* venom NAD glycohydrolase.[14] When the glycohydrolase assay was conducted at pH 7.5 using 50 μM substrate, the IC_{50} for inhibition of the venom enzyme was 260 μM. The effect of the inhibitor on the venom NAD glycohydrolase by ADP-HPD was of interest because this enzyme catalyzes a hydrolytic reaction at an ADP-ribose linkage but possesses an active site capable of accommodating only a single ADP-ribose. A related enzyme, the porcine brain NAD glycohydrolase, was inhibited by less than 5% when assayed under the same conditions and in the presence of 1 mM ADP-HPD.

Discussion

Potent and specific inhibitors for poly(ADP-ribose) glycohydrolase have not heretofore been described despite suggestions that glycohydrolase inhibitors would have unique pharmacological properties.[15,16] In particular, ADP-HPD would be predicted to inhibit the completion of the cycle of DNA repair by preventing the removal of poly(ADP-ribose) from chromatin and therefore inhibiting the recondensation of the chromatin. ADP-HPD might therefore increase the cytotoxicity of DNA-damaging drugs and radiation. Specific inhibition of the glycohydrolase should also be informative in a variety of ongoing studies designed to elucidate the function of ADP-ribose polymer metabolism. ADP-HPD is the first example of a compound possessing the requisite potency and specificity for use in in vitro studies on the function of ADP-ribose polymer metabolism by inhibiting polymer degradation.

ADP-HPD inhibits neither NAD:arginine mono(ADP-ribosyl)transferase A nor poly(ADP-ribose) polymerase. At high concentrations, ADP-HPD inhibits some NAD glycohydrolases. Therefore of the pyridine nucleotide dependant enzymes studied, the analog at low concentrations was specific for poly(ADP-ribose) glycohydrolase, the hydrolytic enzyme in the metabolism of ADP-ribose polymers.

ADP-HPD is a nitrogen-in-the-ring analog of ADP-ribose, the product of the hydrolysis of ADP-ribose polymers catalyzed by poly(ADP-ribose) glycohydrolase. Sugar analogs in which the ring oxygen is replaced by nitrogen are often found to be specific and highly potent glycosidase[11] and nucleosidase inhibitors.[17] In a few cases, the nitrogen-in-the-ring sugars satisfy the criteria required for a transition-state inhibitor and can therefore be considered to be electronic transition-state analogs mimicking the developing positive charge of an oxocarbonium ion intermediate. In other cases, even though the inhibitors are both potent and their mechanism of inhibition competitive, they do not satisfy additional important criteria required of a transition-state analog.[11] Such compounds must exploit other favorable interactions between the enzyme and the inhibitor to produce the observed enhanced binding affinity. Since neither the mechanism of poly(ADP-ribose) glycohydrolase nor the mechanism of its inhibition by ADP-HPD have yet been studied, it is premature to speculate on the basis for the enhanced affinity of ADP-HPD for the glycohydrolase.

The potency and specificity of inhibition by ADP-HPD suggests that it will be useful as a tool for studying ADP-ribose polymer metabolism by selectively inhibiting the glycohydrolase in cell free extracts, in isolated nuclei, and possibly in cultured cells. It is likely that ADP-HPD will serve as a lead structure for developing specific photoaffinity labels and affinity absorbents for the poly(ADP-ribose) glycohydrolase. Further, ADP-HPD will represent a paradigm for designing future inhibitors which will be able to cross cell membranes and therefore to possess activity in vivo. Such compounds will be candidates for chemosensitizing agents which may find utility in cancer chemotherapy.

Experimental Section

Materials. Reagent grade tetrahydrofuran (THF) was dried by distillation over sodium and benzophenone. Anhydrous dimethylformamide (DMF) was purchased from Aldrich (Milwaukee, WI). All other reagents were of the highest available commercial purity.

General Methods. ^{1}H-NMR spectra were determined at 300 MHz at ambient probe temperature, at a concentration of ca. 10 mg/mL. ^{13}C–NMR spectra were determined at 75 MHz. The chemical shifts are reported in ppm referenced to internal dioxane (^{1}H, 3.53 ppm; ^{13}C, 69.41 ppm). The pD of the samples were determined by measuring the pH in 99.8% D_2O using a glass electrode and correcting the reading with the formula $pD = pH_{app} + 0.4$. ^{31}P-NMR spectra were acquired on a Varian Gemini-200 spectrometer at 80.95 MHz using 5 mm tubes. Chemical shifts are referenced to an external standard of 85% aqueous H_3PO_4. To reduce line broadening caused by paramagnetic impurities, samples were extracted with solutions of dithiazone (Eastman Organic Chemicals) in CCl_4 before ^{31}P-NMR spectra were recorded. The reported chemical shifts for second-order AB patterns are calculated from the observed line spacings and coupling constants.

HPLC was performed using a high-pressure gradient system and a fixed wavelength detector operated at 254 nm. Anion-exchange separations were performed using a 10 μM strong

anion exchange column (Alltech, Deerfield, IL; SAX 4.6 × 250 mm) developed isocratically with 250 mM NaH_2PO_4, pH 3.5, at a flow rate of 1 mL/min. Reversed-phase separations were performed with a 3.9 × 300 mm reversed-phase column (C-18 Bondapak, 15–20 μm; Waters Associates, Milford, MA) using an ion-pairing technique. The solvents were (A) 20 mM NaH_2PO_4 and 2 mM tetrabutylammonium H_2PO_4 (pH 6) and (B) 50%, v/v, solvent A and acetonitrile. The separation began using a mobile phase consisting of 90% A and 10% B, the composition of which was changed linearly to 80% A over 5 min, then linearly to 50% A over 10 min, and thereafter isocratically until all compounds of interest had eluted from the column. The flow rate was maintained at 1.5 mL/min throughout the separation. Under these conditions, AMP eluted with a retention time of 5.9 min and diadenosine 5′-pyrophosphate (AppA) eluted with a retention time of 12.5 min.

Fast-atom bombardment mass spectra were acquired on a Finnigan MAT 212 mass spectrometer as described.[18]

(2*R*,3*R*,4*S*)-1-(Benzyloxycarbonyl)-2-(hydroxymethyl)-pyrrolidine-3,4-diol 3,4-*O*-isopropylidene acetal, 2, was synthesized as described by Goli et al.[12]

(2*R*,3*R*,4*S*)-1-(Benzyloxycarbonyl)-2-[(phosphooxy)-methyl]pyrrolidine-3,4-diol, 3. The starting alcohol **2** (617 mg, 2.0 mmol) was dissolved in 5 mL of dry THF, triethylamine (2.52 mL, 1.83 g, 18.1 mmol) was added, and the resulting solution was stirred and cooled using an ice bath. Freshly distilled phosphoryl chloride ($POCl_3$, 6.03 mmol, 0.92 g) was added in a single portion via a syringe. A vigorous reaction ensued, and a large amount of a white precipitate formed. After 1.5 h, TLC (silica gel, *n*-propanol/concentrated ammonia/water, 6:3:1) indicated that all starting material (R_f = 0.73) had been consumed. Several small pieces of ice were added to destroy the excess $POCl_3$. The solvent was removed under reduced pressure and the residue treated with 5 mL of 1 M HCl for 1 h at room temperature and for 18 h at 5 °C to remove the acetonide protecting group. The solvent was removed by evaporation under reduced pressure.

To remove salt, the residue was dissolved in a few milliliters of water and applied to a 50 mL column of Amberchrome CG-7 Ims resin (a reversed-phase type absorbent) (Toso Haas Inc., product 60156). The column was developed by applying 300 mL of water, and 50 mL fractions were collected. The effluent was monitored for salt by measuring conductance and for ester **3** by measuring the absorbance at 260 nm. Salts eluted in the first two bed volumes (100 mL), and product eluted as a broad band into the four subsequent bed volumes (200 mL). Fractions containing the product were combined, and the solvent was removed in vacuo.

The monophosphate **3** was purified by anion-exchange chromatography on a 2.5 × 40 cm column of DE-52 cellulose (Whatman), developed by application of a linear gradient formed between 400 mL of 0.01 M NH_4HCO_3 (pH 7.5) and 400 mL of 0.2 M NH_4HCO_3 (pH 7.5). Fractions (8 mL) were collected, and the absorbance at 260 nm was determined. The single major peak which eluted at the end of the gradient was collected. After repeated lyophilization from water, the monoammonium salt of **3** was isolated as an amorphous residue weighing 0.5 g (70% yield): TLC (silica gel, 2-propanol/concentrated NH_4OH/water, 6:3:1) R_f = 0.35; HPLC (reversed phase, ion pair) retention time = 16.2 min; ^{1}H-NMR (D_2O) δ 3.17–3.32 (m, 1H, CH_2N), 3.35–3.55 (m, 1H, CH_2N), 3.57–3.67 (m, 1H, C*H*N), 3.71–3.82 (m, 1H, CH_2OP), 3.87–3.98 (m, 1H, CH_2OP), 4.07–4.15 (m, 1H, C*H*OH), 4.15–4.21 (m, 1H, CHOH), 4.89–4.97 (m, 2H, CH_2Ph), 7.20–7.24 (m, 5H, Ph*H*); ^{13}C-NMR (D_2O) δ 53.35 & 53.60 (t, C-5), 65.38 & 66.12 (t, C-6), 65.55, 65.80 & 65.91 (d, C-2), 70.37 & 70.63 (t, C*H*Ph), 72.01 & 72.37 (d, *C*HOH), 75.32 & 75.92 (d, *C*HOH), 130.69 & 130.76 (d, Ph), 131.29 (d, Ph), 131.69 (d, Ph), 139.13 (s, Ph), 159.30 & 159.52 (s, carbonyl); ^{31}P-NMR (D_2O, pD 3.8, 25 °C) δ 1.05 (proton decoupled, s; proton undecoupled, t, $^3J_{P-H}$ = 4.4 ± 0.2 Hz); fast-atom bombardment mass spectrum (negative ion) calcd for $C_{13}H_{17}NO_8P$ *m/z* 346, found *m/z* 346 [M^-]. Anal. ($C_{13}H_{17}O_8NP{\cdot}NH_4{\cdot}1/2H_2O$) C, H, N.

***N*-(Benzyloxycarbonyl)-ADP-HPD, 4.** (2*R*,3*R*,4*S*)-1-(*Z*)-2-[(phosphooxy)methyl]pyrrolidine-3,4-diol (**3**) (100 mg, 0.27 mmol) was dissolved in 1 mL of methanol and trioctylamine (118 μL, 0.27 mmol) added. After stirring for 15 min at room temperature, the methanol was removed under reduced pressure and the residue dried three times by adding 2 mL of anhydrous DMF and evaporating the solvent in vacuo. The resulting residue was dissolved in 1 mL of anhydrous DMF, sealed under nitrogen in a vial with a rubber septum cap, and stirred.

Adenosine 5′-monophosphate phosphoromorpholidate (**5**) (291 mg, 0.41 mmol, 50% excess; Sigma) was dried three times by dissolving it in 5 mL of anhydrous pyridine and evaporating the pyridine in vacuo. Finally the material was dissolved in 2 mL of anhydrous pyridine and added to the stirred solution of **3** using a syringe. The resulting solution was stirred at room temperature for 72 h.

The organic solvents were evaporated in vacuo, the residue dissolved in water containing a few percent of ethyl alcohol, and the pH of the solution adjusted to 7.5. The solution was applied to a column of 200 mL of benzylated DEAE-cellulose (Sigma) and the chromatography developed by applying a linear gradient formed between 400 mL of 10 mM NH_4HCO_3 (pH 7.5) and 400 mL of 400 mM NH_4HCO_3 (pH 7.5), followed by an additional 400 mL of the high-ionic strength buffer. Fractions (8 mL each) were collected and assayed for absorbance at 260 nm. The AMP phosphoromorpholidate eluted first followed by 5′-AMP. (*Z*)-ADP-HPD (**4**) eluted from the column near the end of the linear gradient. Peak fractions were combined and lyophilized several times to yield 90 mg (45% yield) of product **4** as the ammonium salt: TLC (silica gel, 2-propanol/ammonia/water, 6:3:1) R_f = 0.60; HPLC (anion exchange, 250 mM KH_2PO_4, pH 3.5) retention time = 31 min; HPLC (reversed phase, ion pair) retention time = 19.1 min; ^{1}H-NMR (D_2O) δ 3.16–3.29 (m, 1H, CH_2N), 3.31–3.49 (m, 1H, CH_2N), 3.56–3.70 (m, 1H), 3.85–4.10 (m, 4H), 4.12–4.26 (m, 3H), 4.29 (br t, *J* = 4.4 Hz, 1H, adenosyl H-3′), 4.49–4.54 (m, 1H, adenosyl H-2′), 4.75–4.94 (m, 2H, CH_2Ph), 5.86–5.93 (m, 1H, adenosyl H-1′), 7.00–7.24 (m, 5H, Ph*H*), 8.06 (s, 1H, adenosyl H-2), 8.36 (s, 1H, adenosyl H-8); ^{31}P-NMR: (D_2O, pD 4.5) δ_a −10.35 and δ_b −10.47 (AB pattern, $^2J_{P'\cdot P}$ = 21.8 ± 0.2 Hz); fast-atom bombardment mass spectrum (positive ion) calcd for $C_{23}H_{30}N_6O_{14}P_2$ *m/z* 676, found *m/z* 677 [M + H]$^+$. Anal. ($C_{23}H_{30}O_{14}N_6P_2{\cdot}1.5NH_3{\cdot}2H_2O$) C, H, N.

Adenosine 5′-Diphosphate (Hydroxymethyl)pyrrolidinediol (ADP-HPD), 1. (*Z*)-ADP-HPD (**4**) (80 mg, 0.113 mmol) was dissolved in 20 mL of methanol and 5 mL of water. Catalyst (5% Pd on C) was added (100 mg), and the hydrogenation was conducted at 30 psi with shaking for 18 h.

The catalyst was removed by filtration, the solvent evaporated, and the product redissolved in water. The product was purified by anion-exchange chromatography using a 50 mL column of benzylated DEAE-cellulose (Sigma), developed with a linear gradient formed between 95 mL of 10 mM NH_4HCO_3 (pH 7.5) and 95 mL 400 mM NH_4HCO_3 (pH 7.5). The single peak which eluted near the end of the gradient was lyophilized to produce 50 mg (0.092 mmol, 82% yield) of a white powder: TLC (silica gel, 2-propanol/ammonia/water, 6:3:1) R_f = 0.37; anion-exchange HPLC (100 mM KH_2PO_4, pH 3.5) single peak with retention time = 4.5 min; HPLC (reversed phase, ion pair) single peak with retention time = 12.5 min; ^{1}H-NMR (D_2O) δ 3.14 (d, *J* = 12.7 Hz, 1H, CH_2N), 3.27 (dd, *J* = 2 & 13 Hz, 1H, CH_2N), 3.50–3.58 (m, 1H, C*H*N), 3.95–4.07 (m, 3H), 4.07–4.23 (m, 4H), 4.29 (t, *J* = 4 Hz, 1H, adenosyl H-3′), 4.55 (t, *J* = 5.3 Hz, 1H, adenosyl H-2′), 5.90 (d, *J* = 5.7 Hz, 1H, adenosyl H-1′), 7.98 (s, 1H, adenosyl H-2), 8.52 (s, 1H, adenosyl H-8); ^{13}C-NMR (D_2O) δ 52.53 (pyrrolidine C-5), 63.27 (d, $^3J_{C\cdots P}$ = 8.2 Hz, pyrrolidine C-2), 65.02 (d, $^2J_{C\cdots P}$ = 4.8 Hz, pyrrolidine C-6), 68.19 (d, $^2J_{C\cdots P}$ = 5.4 Hz, adenosyl C-5′), 72.39 (*C*HOH), 73.13 (*C*HOH), 73.80 (*C*HOH), 77.08 (*C*HOH), 86.53 (d, $^3J_{C\cdots P}$ = 8.7 Hz, adenosyl C-4′), 89.83 (adenosyl C-1′), 121.43 (adenosyl C-5), 142.58 (adenosyl C-8), 151.86 (adenosyl C-4), 155.62 (adenosyl C-2), 158.35 (adenosyl C-6); ^{31}P-NMR: (D_2O, pD 6.2) δ_a −10.35 and δ_b −10.71 (AB pattern, $^2J_{P'\cdot P}$ = 20.9 ± 0.2 Hz); fast-atom bombardment mass spectrum (positive ion) calcd for $C_{15}H_{24}N_6O_{12}P_2$ *m/z* 542, found *m/z* 543 [M + H]$^+$. Anal. ($C_{15}H_{23}O_{12}N_6P_2{\cdot}NH_4{\cdot}2H_2O$) C, H, N.

Poly(ADP-ribose) glycohydrolase from bovine thymus was purified as described by Thomassin et al. (1990).[19] [^{32}P]Poly-

(ADP-ribose) for glycohydrolase assay was synthesized at high specific activity using the procedure described by Menard and Poirier (1987)[20] and purified as described by Thomassin et al. (1990),[19] except that the ethanol precipitation was omitted. Poly(ADP-ribose) glycohydrolase was assayed by measuring the release of [^{32}P]ADP-ribose from [^{32}P]poly(ADP-ribose) (10 μM in ADP-ribose residues) as described previously by Menard and Poirier (1987).[20] One unit of enzyme is the amount that liberates 1 nmol of ADP-ribose/min at 37 °C.

Poly(ADP-ribose) polymerase from bovine thymus was purified to the DNA cellulose step using the procedure of Zahradka and Ebisuzaki (1984).[21] Poly(ADP-ribose) polymerase was assayed by measuring the incorporation of ADP-ribose residues from 100 μM [^{32}P]NAD into polymer as described by Rankin et al. (1989).[6]

NAD:arginine mono(ADP-ribosyl)transferase A was purified from turkey erythrocytes using the procedure of Moss et al. (1980).[22] The transferase was assayed radiometrically by measuring the incorporation of ADP-ribose from [^{32}P]NAD into histones as described by Rankin et al. (1989).[7]

NAD glycohydrolase from *B. fasciatus* venom was purified using the three-step procedure of Yost and Anderson (1981).[14] Porcine brain NAD glycohydrolase was purchased from Sigma Chemical Co. (St. Louis, MO). The NAD glycohydrolases were assayed by measuring the release of [*carbonyl*-^{14}C]nicotinamide from [*carbonyl*-^{14}C]NAD as described.[18] One unit of NADase activity is that quantity of enzyme which catalyzes the hydrolysis of 1 μmol of NAD/min.

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