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One-Step, Nonenzymatic Synthesis of *O*-Acetyl-ADP-ribose and Analogues from NAD and Carboxylates

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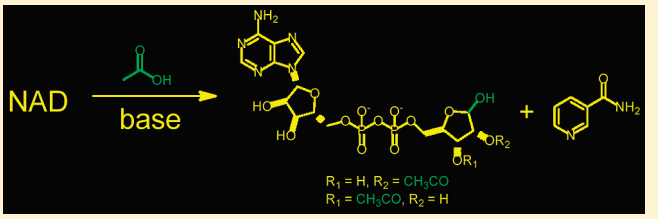
Supporting Information

ABSTRACT: *O*-Acetyl-ADP-ribose (OAADPR) is a metabolite produced from nicotinamide adenine dinucleotide (NAD) as a product of sirtuin-mediated protein deacetylation. We present here a simple, one-step, nonenzymatic synthesis of OAADPR from NAD and sodium acetate in acetic acid. We extended the reaction to other carboxylic acids, demonstrating that the reaction between NAD and nonaqueous carboxylate buffers produces mixtures of the corresponding 2′- and 3′-carboxylic esters.



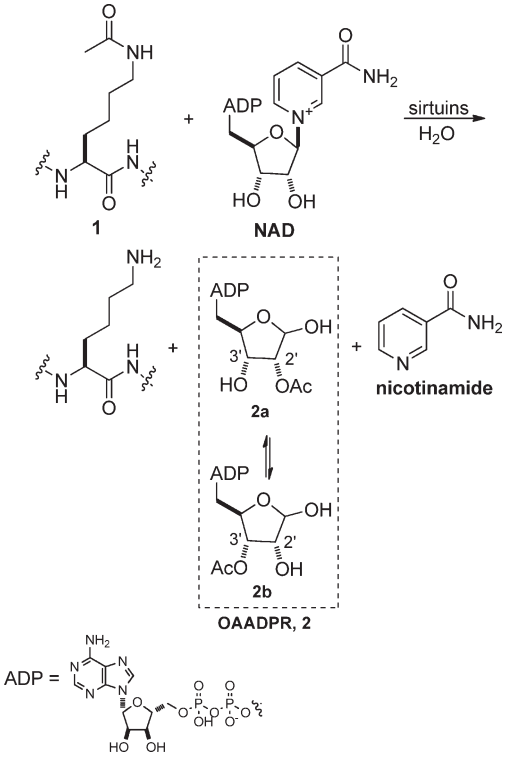
INTRODUCTION

The sirtuins are an evolutionarily ancient class of enzymes present in all organisms from prokaryotes to humans.[1] One demonstrated function of these enzymes is protein deacylation (Scheme 1). Sirtuins remove the acetate from an N_ε-acetyllysine residue (**1**) by transferring the acetate to a molecule of NAD with the displacement of nicotinamide and addition of a water molecule to generate 2′-*O*-acetyl-adenosine diphosphate-ribose (**2a**), which rapidly equilibrates to a mixture of esters (OAADPR, **2**).[2−5] Sirtuins are capable of deacylating many different proteins, but the process is absolutely dependent on NAD, and the common products to all sirtuin-mediated deacylation reactions are nicotinamide and OAADPR (**2**). While nicotinamide is involved in many different biological reactions, the only known biosynthesis of OAADPR (**2**) is via sirtuin-mediated protein deacylation.[6−8]

There is evidence that **2**, presumably as the mixture of 2′- and 3′-isomers, functions as an intracellular second messenger.[9] Microinjection experiments showed that starfish oocyte maturation could be delayed or halted by infusion of **2**.[10] Several intracellular proteins have been shown to bind **2**, and mechanisms for the regulation of gene transcription have been proposed.[11] Additionally, enzymes that catalyze the degradation of **2** are known.[12,13] The identification of additional proteins that interact with **2** is an area of active investigation.[6]

Previously, three groups prepared isolable amounts of OAADPR (**2**). Schramm et al. performed an acyl transfer from an 18 amino acid peptide bearing an N_ε-acetyllysine residue to NAD using a bacterial sirtuin enzyme as the catalyst.[2] Borra and Denu used a similar enzymatic approach to prepare **2**, employing a yeast sirtuin as the catalyst, while Moss used human SIRT1 as the catalyst.[10,12] Comstock and Denu performed the first total synthesis of **2**, using a 12-step sequence that allowed them to prepare a mixture of 2′-*O*-acetyl (**2a**) and 3′-*O*-acetyl (**2b**) isomers, as well as some amide isosteres.[14] Denu and Schramm both showed that regioisomers **2a** and **2b** interconverted in aqueous solution at pH = 7.5, giving a 1:1 ratio of regioisomers at equilibrium.[15] In this work, we present a one-step, inexpensive,

Scheme 1. Sirtuin-Mediated Deacylation of an N_ε-Acetyllysine Residue Produces OAADPR and Nicotinamide



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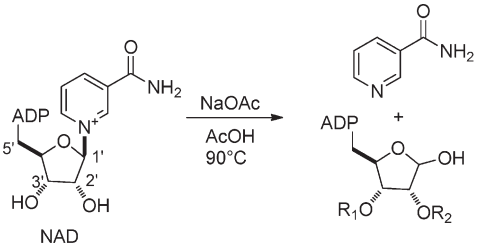
and easily scaled chemical synthesis of **2**. We also demonstrate that the reaction is general for carboxylic acids and NAD, and that it can be used to prepare ester analogues of **2**.

RESULTS AND DISCUSSION

The new preparation is shown in Scheme 2. NAD and an excess amount of sodium acetate were stirred with glacial acetic acid as the solvent. Upon heating, the solids dissolved to give a clear solution. The reactions were monitored by ^{1}H NMR spectroscopy, observing the disappearance of the six aromatic NAD resonances, and the appearance of nicotinamide and new resonances in the region between 4.9 and 5.5 ppm. Typically, the reaction was stopped after 90−95% consumption of NAD in order to avoid side reactions that lowered the product yield on further heating. The reaction was then diluted with ethanol to give a precipitate consisting mainly of 2′-and 3′-*O*-acetyl-ADP-ribose. Mass recoveries were high, and ^{1}H NMR analysis showed the presence of only minor impurities. If desired, the remaining NAD can be removed by chromatography using an aqueous acetate buffer and an amine resin. In our hands, amine-linked silica gel resins were superior to polystyrene-based amine resins for this separation. It is noteworthy that the product-containing fractions (pH = 4.5−5.5) did not undergo any significant spontaneous ester hydrolysis to ADP-ribose when allowed to stand at ambient temperature overnight. The mixture of 2′- and 3′-*O*-acetyl-ADP-ribose isomers obtained from the reaction was identical to that described by Schramm.[2] The final product remained intact in D_2O solution for approximately 1 day at ambient temperature, though ester hydrolysis became noticeable if solutions were kept for more than 1 day. The dry powder was stable for several months when stored in a desiccator at −4 °C.

The reaction also succeeded with other short-chain carboxylic acids. Linear and branched acids from C2 through C7 gave 1:1 mixtures of the corresponding 2′- and 3′-ADP-ribose esters. (Table 1, entries 1−10). We found that separation of C3 or longer chain alkanoic and alkenoic esters **3**-**10** from NAD could be accomplished via reversed-phase HPLC, employing an aqueous TFA/acetonitrile gradient, with no ion chromatography necessary. Lyophilization of the product containing fractions delivered the ADP-ribose esters as white solids, which displayed solution and dry powder stabilities similar to **2**. Additionally, the 2′- and 3′-isomers of several longer chain, and α-branched, carboxylic acids were readily separable under the same conditions. The reaction was also successful with benzoic acid, providing both 2′- and 3′-*O*-benzoyl-ADP-ribose (**12**). Yields with α-branched acids, and benzoic acid, were usually lower than the yields with less hindered acids, implying a steric effect in the reaction. The reaction rate was also noticeably slower with trimethylacetic acid than it was with the other acids. However, since NAD did not completely dissolve in some of the α-branched acids and benzoic acid, even after extended reaction times or at higher temperatures, the lower yields of ADP-ribose esters with these acids was due mainly to poor mixing of the reaction components. When we heated NAD with sodium octanoate in octanoic acid, we observed no reaction (Table 1, entry 12). Since the ionization constants, and the steric environments about the carboxyl groups of hexanoic, heptanoic, and octanoic acids are similar, the downward trend in yield from C-6 to C-8 acids is likely due to the decreasing solubility of NAD in the reaction medium as the chain length increases. Heating NAD with trifluoroacetic acid produced a complicated mixture that gave

Scheme 2. Preparation of 2′-and 3′-OAADPR from NAD and Sodium Acetate



no clearly identifiable products by ^{1}H NMR, and no trifluoroacetate esters were observed (Table 1, entry 13). NAD was completely soluble in this reaction, and monitoring by ^{1}H NMR showed that NAD disappeared and nicotinamide formed as the reaction progressed. The strongly acidic trifluoroacetate medium, combined with the weak nucleophilicity of the trifluoroacetate anion, precluded ADPribose ester formation under the same conditions that were successful for acids with higher pK_a values.

Similar to short-chain monocarboxylates, we found that dicarboxylates, tricarboxylates, and oxygenated carboxylic acids also gave acyl-ADP-ribose products **13**−**17** (Table 1, entries 14−18). With these acids, pyridinium salts gave higher product yields than the sodium salts. The difference in yield was due to two major factors. First, temperatures of about 90 °C were optimal for the formation of ADP-ribose esters, while keeping side reactions to a minimum. The pyridinium salts gave homogeneous melts at 90 °C, while the sodium salt buffer mixtures of citric, succinic, and malic acids melted at significantly higher temperatures. Second, α-hydroxy carboxylate, dicarboxylate, and tricarboxylate pyridinium salts were much more soluble in ethanol than the corresponding sodium salts. This facilitated separation of the ethanol-insoluble ADP-ribose esters from the crude reaction mixtures. α-Oxygenated products proved difficult to isolate, though the products were clearly visible by HPLC/MS and ^{1}H NMR. Following chromatography, α-oxygenated esters were always mixed with large amounts of ADP-ribose. Ester hydrolysis also occurred if water was used as a reaction cosolvent.

When excess carboxylic acid was present, C-1′ substitution occurred much faster than C-5′ substitution or pyrophosphate hydrolysis, consistent with previous findings that showed NAD to undergo nucleophilic substitution at C-1′.[16−19] Acetate substitution via an S_N2 mechanism would provide the α-1′ acetate (Scheme 3). However, we did not observe any significant accumulation of α-1′ acetate, even at lower reaction temperatures.[20] As proposed by Schramm, the α-C-1′ ester is favorably positioned to undergo ester migration to the neighboring C-2′ hydroxyl group, so the α-C-1′ ester undergoes rapid equilibration to the more thermodynamically stable C-2′ and C-3′ isomers.[2] Schuber and Cordes both showed that solvolysis of NAD occurs at C-1′ and bears significant S_N1 character.[21,22] Recent calculations by Lim proposed that NAD C-1′ substitution becomes more S_N2-like as the nucleophile becomes more basic.[23] Our results are consistent with these earlier studies. Acetate anion would be expected to participate in an S_N2 reaction with NAD, providing **2a** and **2b** upon ester equilibration. However, we observed a small amount of the β-1′ ester in every run, indicating

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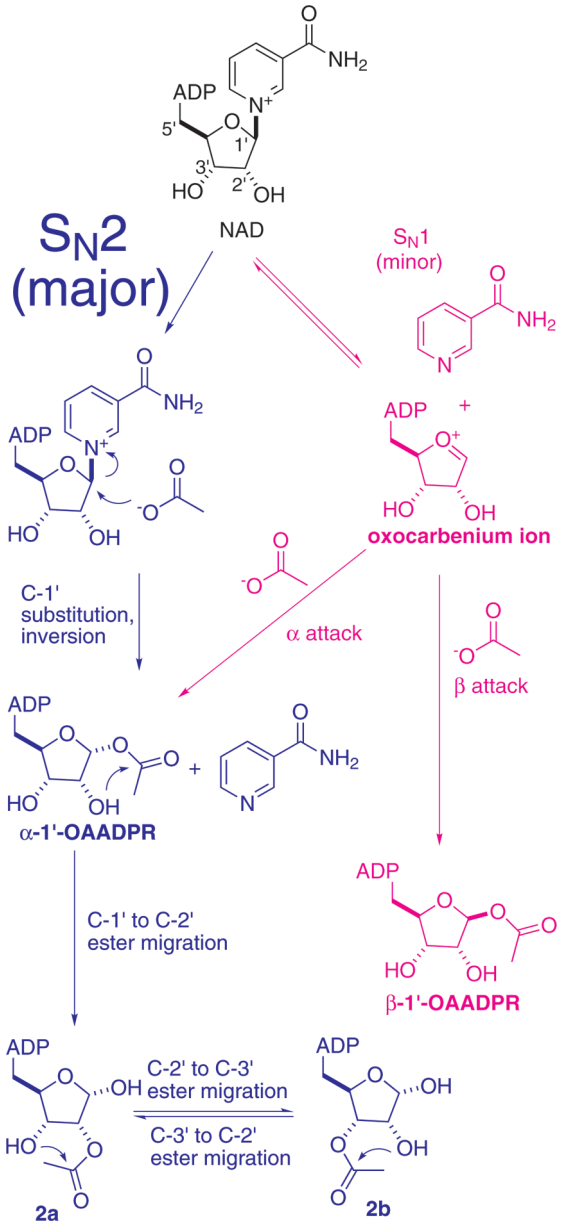
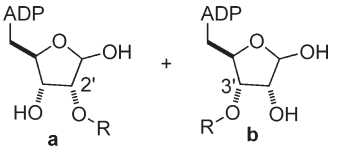
Scheme 3. Proposed Mechanism of the Reaction between NAD and Sodium Acetate in Acetic Acid



that there is some S_N1 character to the reaction.[2,24] The product mixture isolated from the reaction with sodium acetate is identical to that obtained from sirtuin-catalyzed protein deacetylation following equilibration of the 2′- and 3′- isomers.

N_ε-Propionyllysine and N_ε-butyryllysine containing peptides have also been reported to undergo sirtuin-catalyzed deacylation.[25−27] These peptides yield 2′/3′-*O*-propionyl- and 2′/3′-*O*-butyryl-ADP-ribose, which we synthesized from the corresponding carboxylates (Table 1, entries 2 and 3). It is possible that lysine residues N_ε-acylated with an array of carboxylic acids could also serve as substrates for sirtuins. Many other carboxylates commonly present in living cells will react with NAD and yield acyl-ADP-ribose esters. Although *O*-acetyl-ADP-ribose (**2**) is the only 2′/3′-ADP-ribose ester that has been identified in organisms, it is very likely that other esters described here will be found as natural products. Since the reaction appears to be broadly applicable to carboxylates and NAD, it is reasonable to speculate that anionic amino acid residues such as glutamate or aspartate could react with NAD to give ADP-ribose esters. Aspartate and glutamate residues therefore may represent points of attachment for protein ADP-ribosylation.[28]

Table 1. ADP-ribose Esters from NAD and Carboxylates



entry	compd	R =	conditions[a]	yield (%)
1	**2**	acetate	A	36[b,c] (62)[d]
2	**3**	propionate	A	3.2,[e] 3.9[f,g]
3	**4**	*n*-butyrate	A	6.8,[e] 4.8[f]
4	**5**	isobutyrate	A	2.7,[e] 2.0[f]
5	**6**	trimethylacetate	A	0.7,[e] 0.7[f]
6	**7**	*trans*-2-butenoate	A	9.6,[e] 7.3[f]
7	**8**	*n*-pentanoate	A	16,[e] 2.1[f]
8	**9**	3,3-dimethylacrylate	A	9.3,[e] 3.9[f]
9	**10**	*n*-hexanoate	A	8.2,[e] 8.4[f]
10	**11**	*n*-heptanoate	A	0.2,[e] 0.9[f]
11	**12**	benzoate	A	1.7,[e] 6.5[f,h]
12		*n*-octanoate	A	0
13		trifluoroacetate	A	0
14	**13**	succinate	B	18[b,c]
15	**14**	citrate	B	24[b,c,i]
16	**15**	L-malate	B	12[b,c,i]
17	**16**	DL-lactate	B	[j]
18	**17**	glycolate	B	[j]

[a] Reaction conditions: (A) carboxylic acid, Na_2CO_3, NAD, 90 °C; (B) carboxylic acid, pyridine, NAD, 90 °C. [b] Combined yield of 2′- and 3′-isomers. [c] Isolated yield following ion chromatography and desalting. [d] Mass recovery before chromatography, includes 10% NAD. [e] Isolated yield of the 2′-isomer following HPLC purification (C-18 column). [f] Isolated yield of the 3′-isomer following HPLC purification (C-18 column). [g] Ion chromatography was performed before HPLC purification. [h] The β-1′ isomer was also isolated in 0.7% yield. [i] Mixture of carboxylate regioisomers. [j] Product mixture contained mainly ADP-ribose.

The NMR spectral features of ADP-ribose esters in D_2O solution showed that the core resonances are largely conserved across the series. Aliphatic ADP-ribose esters differed only in the resonances specific to the carboxylate residue, with the ADP-ribose region retaining the same ^1H and ^{13}C NMR pattern and spectral assignments. Some downfield shifts (0.1−0.3 ppm) due to anisotropy were present in the ^1H NMR spectra of benzoyl esters **12a** and **12b**. Acrylate analogues **7a**, **7b**, **9a**, and **9b** also showed anisotropic effects, though these were less pronounced than the anisotropic effects observed with the benzoyl esters. Acylation of a ribose OH group caused a downfield shift of approximately 0.6 ppm for the vicinal ring proton, placing these resonances in the region between 4.9 and 5.2 ppm. The resonances for H1′ appeared between 5.1 and 5.5 ppm, roughly 1 ppm upfield of the H1′ resonance in NAD. The concurrent appearance of these signals between 4.9 and 5.5 ppm was

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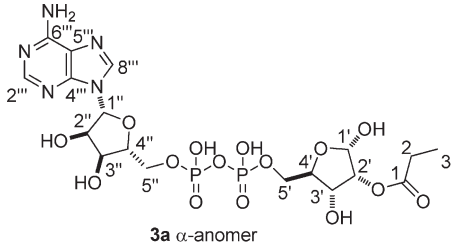
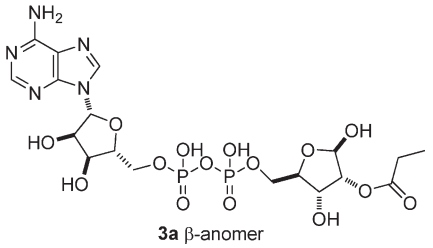
Table 2. ^{1}H and ^{13}C NMR Resonances for 3a



3a α-anomer



3a β-anomer

no.	^{1}H NMR shift (ppm)	multiplicity	integration	coupling constant (Hz)	assignment[a]	^{13}C NMR shift (ppm)
1	8.63	s	1		H8‴ (purine)	143.1
2	8.42	s	1		H2‴ (purine)	145.3
3	6.16	d	1	5.4	H1″	88.6
4	5.53	d	0.34	4.2	H1′β	95.7
5	5.29	d	0.66	1.7	H1′α	99.9
6	5.02	dd	0.34	5.8, 4.2	H2′β	73.4
7	4.96	dd	0.66	5.1, 1.7	H2′α	77.7
8	4.75	m	1		H2″	75.3
9	4.54	m	1		H3″	70.6
10	4.48	dd	0.66	6.2, 5.2	H3′α	69.4
11	4.39	m	1.34		H4″, H3′β	84.7, 69.3
12	4.33−4.00	m	5		H5″, H4′α, H4′β, H5′α, H5′β	65.7, 81.6, 81.7, 66.6, 66.6
13	2.46	m	2		H2 (propyl)	27.7, 27.6
14	1.09	t	3	7.4	H3 (propyl)	8.9, 8.9
15					C1 (propyl)	177.2, 177.2
16					C4‴ (purine)	148.9
17					C5‴ (purine)	119.1
18					C6‴ (purine)	150.5

[a] α or β refer to the 1′-OH stereochemistry and not to the individual proton stereochemistries.

characteristic of ADP-ribose ester formation as the reactions were monitored. Assignment of these resonances, together with COSY data, made determination of the acylation position straightforward. The purine ^{1}H NMR signals were pH sensitive and were broadened or shifted downfield by 0.2 ppm in a few cases. With all ADP-ribose esters, the ^{13}C NMR spectra showed very weak signals for C4′, and C5′, and it was usually necessary to identify these resonances with the aid of HSQC. All of the 2′- and 3′-esters equilibrated in D_2O solution giving 1:1 mixtures similar to acetyl-ADP-ribose **2**. The ^{1}H and ^{13}C NMR assignments for **3** are shown in Tables 2 and 3.

CONCLUSION

We have shown that ADP-ribose esters can be prepared directly from NAD and without the use of enzymes. The product mixtures obtained under abiotic conditions are identical to those isolated from enzyme-mediated preparations. The reaction is broadly applicable to NAD and carboxylic acids, with solubility of NAD in the reaction mixture being the primary limiting factor we encountered. This simple transformation makes ADP-ribose esters readily accessible compounds. Wider availability will greatly facilitate the study of these compounds in enzymatic reactions, and in cellular function.

EXPERIMENTAL SECTION

General Methods. Reagents were obtained from commercial sources and used without further purification. NAD was purchased and used as its inner salt form. ^{1}H NMR spectra were recorded at 300 MHz, and proton-decoupled ^{13}C NMR spectra were recorded at 75 MHz. All ^{1}H NMR and proton-decoupled ^{13}C NMR spectra were run in D_2O using HOD (4.79 ppm) or CH_3OH (^{13}C NMR 49.5 ppm) as an internal reference. Chemical shift data are reported in parts per million. Spectral multiplicity abbreviations are as follows: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet. Integration refers to the number of protons per signal. In cases where a signal can be assigned to one anomer, the integration value may be a fraction of a proton. Coupling constants are reported in hertz. All OH and NH_2 protons were in rapid exchange with the solvent and were not visible in any recorded spectra.

Ion Chromatography. Ion chromatography was performed using an automated medium-pressure liquid chromatography system. Prepacked 4.7 g aminopropyl-linked silica gel columns were used for chromatography. Columns were prewashed several times with elution buffer before use. Chromatograms were monitored by absorbance at 254 nm. Ion chromatography elution buffer compositions were as follows: solution A = 57.25 mL glacial acetic acid diluted to 1000 mL final volume with deionized water (1 M acetic acid), solution B = 114.5 mL glacial acetic acid and 40 g $NaOH_{(s)}$ diluted to 1000 mL final volume with deionized water (1 M sodium acetate, 1 M acetic acid). The column was equilibrated with 90:10 A/B at a rate of 5 mL/min for 15 min. Up to

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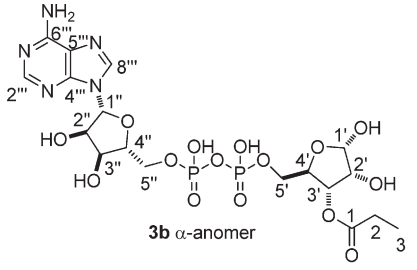
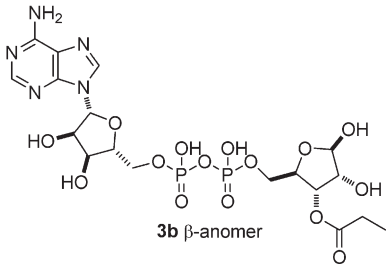
Table 3. ^{1}H and ^{13}C NMR Resonances for 3b



3b α-anomer



3b β-anomer

no.	^{1}H NMR shift (ppm)	multiplicity	integration	coupling constant (Hz)	assignment[a]	^{13}C NMR shift (ppm)
1	8.63	s	1		H8‴ (purine)	143.1
2	8.43	s	1		H2‴ (purine)	145.3
3	6.16	d	1	5.4	H1″	88.6
4	5.40	d	0.5	4.6	H1′β	96.5
5	5.23	d	0.5	3.6	H1′α	101.9
6	5.14	m	1		H3′β, H3′α	73.0, 74.0
7	4.74	m	1		H2″	75.3
8	4.54	t	1	4.4	H3″	70.9
9	4.40	m	1		H4″	84.8
10	4.33	m	1		H4′β,H2′β	81.6, 70.6
11	4.26	m	2.5		H5″, H4′α,	65.8, 80.1
12	4.20	m	0.5		H2′α	74.3
13	4.08	m	2		H5′	66.5, 67.1
14	2.44	m	2		H2 (propyl)	27.7, 27.7
15	1.07	m	3		H3 (propyl)	8.8
16					C1 (propyl)	177.5, 177.2
17					C4‴ (purine)	148.9
18					C5‴ (purine)	119.1
19					C6‴ (purine)	150.5

[a] α and β refer to the 1′-OH stereochemistry and not to the individual proton stereochemistries.

250 mg of crude product mixtures was loaded onto the column in 0.5−1 mL of water; exact concentrations were not determined. Separation gradients were as follows: (gradient 1) 90:10 A/B for 5 min, 90:10 A/B to 50:50 A/B over 5 min, hold at 50% B for 5 min, 50:50 A/B to 100% B over 5 min, then 100% B for 5 min; (gradient 2) 90:10 A/B for 5 min, 90:10 A/B to 100% B over 10 min, then 100% B for 5 min. At the end of a run, the column was again equilibrated with 90:10 A/B, and the next sample could be injected without regeneration. For long-term storage, columns were washed with 50 mL of water, then 50 mL of methanol, capped, and stored in methanol at ambient temperature.

Desalting and Product Isolation. Product-containing fractions were pooled and concentrated in vacuo with a rotary evaporator at or below 25 °C to 1−5 mL final volume. Alternatively, they were frozen and lyophilized until dry and then dissolved in a minimum amount of water. For the first desalting cycle, the aqueous solutions were diluted slowly with denatured alcohol (90:5:5 [v/v/v] ethanol/methanol/2-propanol), to a final concentration of 95:5 (v/v) alcohol/water. The product separated from this mixture as a white solid. The suspension was then centrifuged at 3000*g* and at ambient temperature for 20 min, or until the precipitate settled to the bottom of the tube, and then the supernatant was decanted. The pellet contained the product, along with some sodium acetate. A second desalting cycle was performed as follows. The pellet was dissolved in 1−2 mL of water and diluted slowly with denatured alcohol to a final concentration of 95:5 (v/v) alcohol/water. The suspension was centrifuged as before, and then the supernatant was decanted. The pellet contained the product. The second desalting cycle was repeated for a total of three desalting cycles. Three cycles were sufficient to bring the residual sodium acetate down to trace levels.

After desalting was complete, the pellet was dissolved in 2 mL of water and then concentrated in vacuo at 20 °C. This was repeated to remove any residual alcohol. Finally, the residue was dissolved in water, frozen, and lyophilized to give the product as a solid.

HPLC Purification. Reversed-phase HPLC purification was run using an automated preparative liquid chromatography system equipped with a 30 × 100 mm C-18 functionalized silica gel column. Crude compounds were prepared as solutions in 0.5−3 mL of water for injection. Elution solvents were as follows: solution A, 0.1% (v/v) trifluoroacetic acid in water;solution B, 0.1% (v/v) trifluoroacetic acid in acetonitrile. Samples were eluted using a gradient of A/B specified for each particular compound. Typically, NAD eluted just after the column void volume, followed by the 3′-ADP-ribose ester, and then the 2′-ADP-ribose ester. With some compounds, a peak corresponding to the 1′-ADP-ribose ester could be observed between the 3′- and 2′-peaks. Product-containing fractions were pooled, frozen, and lyophilized to dryness.

*2′- and 3′-O-Acetyl-ADP-ribose (**2**) [(3R,4R,5R)-5-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-2,*

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*4-dihydroxytetrahydrofuran-3-yl Acetate (**2a**)] and [(3S,4R,5R)-2-(((-(((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-methyl)-4,5-dihydroxytetrahydrofuran-3-yl Acetate (**2b**)] (Procedure A).* To 80 mg (0.75 mmol) of sodium carbonate was added 2 mL of glacial acetic acid. The mixture was stirred at ambient temperature until the sodium carbonate had dissolved, and then 250 mg (0.377 mmol) of nicotinamide adenine dinucleotide (NAD) was added. The reaction was heated at 90 °C for 20 min, during which time the NAD completely dissolved. Heating was removed, and then 5 mL of denatured alcohol (90:5:5 [v/v/v] ethanol/methanol/2-propanol) was added slowly to dilute the residual sodium acetate as much as possible before precipitation of the product. The suspension was diluted more rapidly with another 13 mL of denatured alcohol, and then the precipitate was filtered to give a light tan solid. The crude product was purified via ion chromatography (gradient 1) to give 88 mg (36%) of 2′- and 3′-*O*-acetylADP-ribose **2** as the disodium salt. The product eluted at 50%B. **2a**: ^{1}H NMR δ 8.42 (s, 1), 8.08 (s, 1), 6.07 (d, 1, J = 5.5), 5.51 (d, 0.34, J = 4.4), 5.28 (d, 0.66, J = 1.8), 4.99 (dd, 0.34, J = 5.8, 4.1), 4.95 (dd, 0.66, J = 5.1, 1.7), 4.72 (m, 1), 4.52 (m, 1), 4.49 (m, 0.66), 4.40 (m, 1.34), 4.29−3.98 (m, 5), 2.16 (s, 1), 2.15 (s, 2); ^{13}C NMR δ 173.0 (C), 173.0 (C), 155.1 (C), 152.5 (CH), 148.7 (C), 139.6 (CH), 118.2 (C), 99.3 (CH), 95.0 (CH), 86.9 (CH), 83.7 (CH), 81.0 (CH), 80.8 (CH), 77.3 (CH), 74.3 (CH), 72.9 (CH), 70.2 (CH), 68.8 (CH), 68.5 (CH), 66.3 (CH), 65.6 (CH), 65.1 (CH), 20.0 (CH_3). **2b**: ^{1}H NMR δ 8.42 (s, 1), 8.08 (s, 1), 6.07 (d, 1, J = 5.5), 5.38 (d, 0.5, J = 4.5), 5.23 (d, 0.5, J = 3.3), 5.08 (m, 1), 4.72 (m, 1), 4.52 (t, 1, J = 4.2), 4.39 (m, 1), 4.32 (m, 1), 4.23 (m, 2.5), 4.16 (m, 0.5), 4.02 (m, 2), 2.09 (s, 3); ^{13}C NMR δ 173.3 (C), 173.1 (C), 155.1 (C), 152.5 (CH), 148.7 (C), 139.6 (CH), 118.2 (C), 101.2 (CH), 95.0 (CH), 86.9 (CH), 83.7 (CH), 80.9 (CH), 79.5 (CH), 74.3 (CH), 73.6 (CH), 73.5 (CH), 72.5 (CH), 70.2 (CH), 69.9 (CH), 66.3 (CH), 65.6 (CH), 65.1 (CH), 20.1 (CH_3). **2a** and **2b**: MS (ESI$^+$) m/z = 602 (M + H)$^+$; HR-MS (ESI) m/z ([M + H]$^+$) calcd for $C_{17}H_{25}N_5O_{15}P_2$ 602.0901, found 602.0900.

If no ion chromatography was performed, *O*-acetyl-ADP-ribose **2** could be obtained as a mixture with 5−10% NAD. In this case, the remaining sodium acetate was removed by subjecting the product to a second desalting cycle according to the procedure for desalting and product isolation described above. The yield was 62%.

*2′- and 3′-O-Propionyl-ADP-ribose (**3**) [(3R,4R,5R)-5-(((((((-(2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-methyl)-2,4-dihydroxytetrahydrofuran-3-yl Propionate (**3a**)] and [(3S,4R,5R)-2-(((((((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl Propionate (**3b**)].* Prepared from 32 mg (0.30 mmol) of Na_2CO_3, 0.8 mL propionic acid, and 100 mg (0.151 mmol) of NAD according to procedure A. The crude product was purified via ion chromatography (gradient 1), and then by HPLC (95%A, 5% B for 1 min; 5% to 25% B over 12.5 min, 25% to 85% B over 2.5 min) to give 3.6 mg (3.9%) of 3′-*O*-propionyl-ADP-ribose **3b** (t_R = 2.11 min) and 3.0 mg (3.2%) of 2′-*O*-propionyl-ADP-ribose **3a** (t_R = 2.57 min). If ion chromatography was omitted, 11.4 mg (12%) of 2′- and 3′-*O*-propionyl-ADP-ribose **3** was obtained as a white solid following HPLC. t_R = 2.20−2.72 min (two overlapping peaks). **3a**: ^{1}H NMR and ^{13}C NMR (see Table 2). **3b**: ^{1}H NMR and ^{13}C NMR (see Table 3). **3a** and **3b**: MS (ESI$^+$) m/z = 616 (M + H)$^+$; HR-MS (ESI) m/z ([M + H]$^+$) calcd for $C_{18}H_{27}N_5O_{15}P_2$ 616.1057, found 616.1057.

*2′- and 3′-O-n-Butyryl-ADP-ribose (**4**) [(3R,4R,5R)-5-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl Butyrate (**4a**)] and [(3S,4R,5R)-2-(-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)-oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl Butyrate (**4b**)].* Prepared from 32 mg (0.30 mmol) of Na_2CO_3, 0.8 mL of butyric acid, and 100 mg of NAD (procedure A.) The crude product was purified via HPLC (95%A, 5% B for 1 min; 5% to 20% B over 12 min; 20% B for 0.5 min; 20% to 85% B over 2.5 min) to give 4.6 mg (4.8%) of 3′-*O*-butyryl-ADP-ribose **4b** (t_R = 3.61 min) and 6.5 mg (6.8%) of 2′-*O*-butyryl-ADP-ribose **4a** (t_R = 4.75 min), both as white solids. **4a**: ^{1}H NMR δ 8.63 (s, 1), 8.42 (s, 1), 6.16 (d, 1, J = 5.4), 5.53 (d, 0.34, J = 4.2), 5.28 (d, 0.66, J = 1.7), 5.01 (dd, 0.34, J = 5.8, 4.2), 4.96 (dd, 0.66, J = 5.1, 1.7), 4.74 (m, 1), 4.54 (m, 1), 4.48 (dd, 0.66, J = 6.2, 5.2), 4.39 (m, 1.34), 4.34−4.00 (m, 5), 2.43 (m, 2), 1.60 (m, 2), 0.90 (t, 0.9, J = 7.4), 0.89 (t, 2.1, J = 7.4); ^{13}C NMR δ 176.4 (C), 150.2 (C), 148.9 (C), 145.3 (CH), 143.1 (CH), 119.1 (C), 99.9 (CH), 95.7 (CH), 88.6 (CH), 84.7 (CH), 81.7 (CH), 81.6 (CH), 77.7 (CH), 75.3 (CH), 73.5 (CH), 70.8 (CH), 69.4 (CH), 69.3 (CH), 66.6 (CH), 66.5 (CH), 65.7 (CH), 36.1 (CH_2), 18.5 (CH_2), 13.4 (CH_3). **4b**: ^{1}H NMR δ 8.64(s, 1), 8.42 (s, 1), 6.16 (d, 1, J = 5.4), 5.41 (d, 0.5, J = 4.6), 5.23 (d, 0.5, J = 3.6), 5.14 (m, 1), 4.74 (m, 1), 4.54 (t, 1, J = 4.4), 4.41 (m, 1), 4.34 (m, 1), 4.26 (m, 2.5), 4.21 (m, 0.5), 4.09 (m, 2), 2.39 (t, 2, J = 7.3), 1.58 (m, 2), 0.89 (t, 1.5, J = 7.4), 0.89 (t, 1.5 (J = 7.4); ^{13}C NMR δ 176.7 (C), 176.4 (C), 150.5 (C), 148.9 (C), 145.3 (CH), 143.1 (CH), 119.1 (C), 102.0 (CH), 96.6 (CH), 88.6 (CH), 84.9 (CH), 81.6 (CH), 80.3 (CH), 75.4 (CH), 74.3 (CH), 74.0 (CH), 73.0 (CH), 70.9 (CH), 70.6 (CH), 67.3 (CH), 67.2 (CH), 65.7 (CH), 36.1 (CH_2), 36.1 (CH_2), 18.5 (CH_2), 13.4 (CH_3). **4a** and **4b**: MS (ESI$^+$) m/z = 630 (M + H)$^+$; HR-MS (ESI) m/z ([M + H]$^+$) calcd for $C_{19}H_{29}N_5O_{15}P_2$ 630.1214, found 630.1213.

*2′- and 3′-O-Isobutyryl-ADP-ribose (**5**) [(3R,4R,5R)-5-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl Isobutyrate (**5a**)] and [(2R,3S,4R)-2-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl Isobutyrate (**5b**)].* Prepared from 80 mg (0.75 mmol) of Na_2CO_3, 2 mL of isobutyric acid, and 250 mg of NAD (procedure A). The crude product was purified via HPLC (100% A, 0% B to 92% A, 8% B over 1.5 min; 8% to 20% B over 4.75 min; 20% to 95% B over 2 min; step to 95% A, 5% B, hold for 1.75 min) to give 4.7 mg (2.0%) of 3′-*O*-isobutyryl-ADP-ribose **5b** (t_R = 3.27 min) and 6.3 mg (2.7%) of 2′-*O*-isobutyryl-ADP-ribose **5a** (t_R = 3.61 min), both as white solids. **5a**: ^{1}H NMR δ 8.65 (s, 1), 8.43 (s, 1), 6.17 (d, 1, J = 5.3), 5.53 (d, 0.34, J = 4.2), 5.27 (d, 0.66, J = 1.6), 4.98 (dd, 0.34, J = 5.8, 4.2), 4.93 (dd, 0.66, J = 5.1, 1.7), 4.75 (m, 1), 4.54 (m, 1), 4.48 (dd, 0.66 (J = 6.2, 5.2), 4.39 (m, 1.34), 4.34−4.00 (m, 5), 2.67 (m, 1), 1.12 (d, 2, J = 7.1), 1.12 (d, 4, J = 7.1); ^{13}C NMR δ 179.9 (C), 150.5 (C), 148.9 (C), 145.4 (CH), 143.1 (CH), 119.2 (CH), 100.0 (CH), 95.7 (CH), 88.6 (CH), 84.9 (CH), 81.9 (CH), 81.7 (CH), 77.6 (CH), 75.4 (CH), 73.4 (CH), 70.9 (CH), 69.6 (CH), 69.3 (CH), 67.0 (CH), 66.6 (CH), 65.8 (CH), 34.3 (CH), 18.7 (CH_3). **5b**: ^{1}H NMR δ 8.65 (s, 1), 8.43 (s, 1), 6.16 (d, 1, J = 5.4), 5.41 (d, 0.5, J = 4.6), 5.23 (d, 0.5, J = 3.6), 5.10 (m, 1), 4.75 (m, 1), 4.54 (t, 1, J = 4.4), 4.41 (m, 1), 4.34 (m, 1), 4.26 (m, 2.5), 4.21 (m, 0.5), 4.08 (m, 2), 2.65 (m, 1), 1.14 (d, 3, J = 7.1), 1.14 (d, 3, J = 7.1); ^{13}C NMR δ 179.9 (C), 150.5 (C), 148.9 (C), 145.4 (CH), 143.0 (CH), 119.1 (C), 102.0 (CH), 96.6 (CH), 88.6 (CH), 84.9 (CH), 81.9 (CH), 80.3 (CH), 75.4 (CH), 74.3 (CH), 74.1 (CH), 73.1 (CH), 70.9 (CH), 67.1 (CH), 67.0 (CH), 65.7 (CH), 34.3 (CH), 18.7 (CH_3). **5a** and **5b**: MS (ESI$^+$) m/z = 630 (M + H)$^+$; HR-MS (ESI) m/z ([M + H]$^+$) calcd for $C_{19}H_{29}N_5O_{15}P_2$ 630.1214, found 630.1212.

*2′- and 3′-O-Trimethylacetyl-ADP-ribose (**6**) [(3R,4R,5R)-5-(((((((-(2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)-oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl Pivalate (**6a**)] and [(2R,3S,4R)-2-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)-phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl Pivalate*

(**6b**)]. To 80 mg (0.75 mmol) of sodium carbonate and 2.1 g (21 mmol) of trimethylacetic acid was added 8 mL of *N*,*N*-dimethylacetamide. The mixture was stirred until all of the sodium carbonate had dissolved, and then 250 mg (0.376 mmol) of NAD was added. The reaction was heated at 95 °C for 50 min, and then 100 mL of ethanol was added to give a precipitate. The precipitate was filtered and then washed with additional ethanol. The precipitate was purified via HPLC (100% A, 0% B to 92% A, 8% B over 1.5 min; 8% to 20% B over 4.75 min; 20% to 95% B over 2 min; step to 95% A, 5% B, hold for 1.75 min) to give 1.7 mg (0.7%) of 3′-*O*-trimethylacetyl-ADP-ribose **6b** (t_R = 4.17 min) and 1.7 mg (0.7%) of 2′-*O*-trimethylacetyl-ADP-ribose **6a** (t_R = 4.77 min), both as white solids. **6a**: ^{1}H NMR δ 8.66 (s, 1), 8.43 (s, 1), 6.17 (d, 1, *J* = 5.3), 5.52 (d, 0.34, *J* = 4.1), 5.26 (d, 0.66, *J* = 1.6), 4.93 (dd, 0.34, *J* = 5.8, 4.2), 4.91 (dd, 0.66 *J* = 5.1, 1.7), 4.76 (m, 1), 4.55 (m, 1), 4.49 (dd, 0.66, *J* = 6.2, 5.1), 4.40 (m, 1.34), 4.34−4.00 (m, 5), 1.20 (s, 3), 1.19 (s, 6). **6b**: ^{1}H NMR δ 8.64 (s, 1), 8.40 (s, 1), 6.17 (d, 1, *J* = 5.4), 5.42 (d, 0.5, *J* = 4.6), 5.23 (d, 0.5, *J* = 3.6), 5.03 (m, 1), 4.78 (m, 1), 4.54 (m, 1), 4.40 (m, 1), 4.34 (m, 1), 4.24 (m, 2.5), 4.21 (m, 0.5), 4.06 (m, 2), 1.17 (s, 9). **6a** and **6b**: MS (ESI$^+$) *m*/*z* = 644 $(M + H)^+$; HR-MS (ESI) *m*/*z* ($[M + H]^+$) calcd for $C_{20}H_{31}N_5O_{15}P_2$ 644.1370, found 644.1371.

*2′- and 3′-O-(trans-2-Butenoyl)-ADP-ribose (**7**) [(E)-(3R,4R,5R)-5-(-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl But-2-enoate (**7a**)] and [(E)-(2R,3S,4R)-2-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl But-2-enoate (**7b**)].* To 32 mg (0.30 mmol) of sodium carbonate and 700 mg (8.13 mmol) of crotonic acid was added 5 mL of *N*,*N*-dimethylacetamide. The mixture was stirred until all of the sodium carbonate had dissolved, and then 100 mg (0.15 mmol) of NAD was added. The reaction was heated at 95 °C for 15 min, and then 100 mL of ethanol was added to give a precipitate. The precipitate was filtered and then washed with additional ethanol. The residue was purified via HPLC (95% A, 5% B to 75% A, 25% B over 15 min; 25% to 95% B over 5 min) to give 6.9 mg (7.3%) of 3′-*O*-(2-butenoyl)-ADP-ribose **7b** (t_R = 3.16 min) and 9.1 mg (9.6%) of 2′-*O*-(2-butenoyl)-ADP-ribose **7a** (t_R = 3.81 min), both as white solids. **7a**: ^{1}H NMR δ 8.64 (s, 1), 8.42 (s, 1), 7.10 (dq, 1, *J* = 15.6, 6.9), 6.16 (d, 1, *J* = 5.3), 5.95 (dq, 0.34, *J* = 15.6, 1.8), 5.93 (dq, 0.66, *J* = 15.6, 1.8), 5.53 (d, 0.34, *J* = 4.2), 5.30 (d, 0.66, *J* = 1.6), 5.03 (dd, 0.34, *J* = 5.8, 4.2), 4.99 (dd, 0.66, *J* = 5.0, 1.7), 4.76 (m, 1), 4.53 (m, 1), 4.49 (dd, 0.66, *J* = 6.2, 5.1), 4.40 (m, 1.34), 4.34−4.00 (m, 5), 1.89 (dd, 2, *J* = 6.9, 1.8), 1.88 (dd, 1, *J* = 6.9, 1.8); ^{13}C NMR δ 168.3 (C), 150.4 (C), 149.7 (CH), 148.9 (C), 145.3 (CH), 143.0 (C), 121.2 (CH), 119.1 (C), 99.9 (CH), 95.9 (CH), 88.6 (CH), 84.7 (CH), 82.1 (CH), 81.7 (CH), 77.7 (CH), 75.4 (CH), 73.4 (CH), 70.9 (CH), 69.6 (CH), 69.5 (CH), 66.7 (CH), 66.2 (CH), 65.7 (CH), 18.2 (CH_3); MS (ESI$^+$) *m*/*z* = 628 $(M + H)^+$; HR-MS (ESI) *m*/*z* ($[M + H]^+$) calcd for $C_{19}H_{27}N_5O_{15}P_2$ 628.1057, found 628.1057. **7b**: ^{1}H NMR δ 8.65 (s, 1), 8.43 (s, 1), 7.04 (dq, 0.5, *J* = 15.6, 6.9), 7.02 (dq, 0.5, *J* = 15.6, 6.9), 6.17 (d, 0.5, *J* = 5.4), 6.15 (d, 0.5, *J* = 5.4), 5.90 (dq, 0.5, *J* = 15.6, 1.7), 5.88 (dq, 0.5, *J* = 15.6, 1.7), 5.40 (d, 0.5, *J* = 4.5), 5.23 (d, 0.5, *J* = 3.5), 5.13 (m, 1), 4.75 (m, 1), 4.54 (m, 1), 4.39 (m, 1), 4.35 (m, 1), 4.25 (m, 2.5), 4.22 (m, 0.5), 4.07 (m, 2), 1.86 (dd, 1.5, *J* = 6.9, 1.7); ^{13}C NMR δ 168.5 (C), 168.2 (C), 150.5 (C), 149.4 (CH), 149.2 (CH), 149.0 (C), 145.4 (CH), 143.1 (CH), 121.4 (CH), 121.2 (CH), 119.2 (C), 101.9 (CH), 96.5 (CH), 88.7 (CH), 84.8 (CH), 81.5 (CH), 80.3 (CH), 75.3 (CH), 74.4 (CH), 74.0 (CH), 73.0 (CH), 70.9 (CH), 70.7 (CH), 66.5 (CH_2), 65.7 (CH_2), 65.1 (CH_2), 18.2 (CH_3). **7a** and **7b**: MS (ESI$^+$) *m*/*z* = 628 $(M + H)^+$; HR-MS (ESI) *m*/*z* ($[M + H]^+$) calcd for $C_{19}H_{27}N_5O_{15}P_2$ 628.1057, found 628.1057.

*2′- and 3′-n-Pentanoyl-ADP-ribose (**8**) [(3R,4R,5R)-5-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl Pentanoate (**8a**)] and [(2R,3S,4R)-2-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl Pentanoate (**8b**)].* Prepared from 80 mg (0.75 mmol) of Na_2CO_3, 2 mL of valeric acid, and 250 mg (0.376 mmol) of NAD (procedure A). The crude product was purified via HPLC (95% A, 5% B for 1 min; 5% to 75% B over 12 min; 75% to 95% B over 2 min) to give 5.1 mg (2.1%) of 3′-*O*-*n*-pentanoyl-ADP-ribose **8b** (t_R = 4.69 min) and 39.1 mg (16.1%) of 2′-*O*-*n*-pentanoyl-ADP-ribose **8a** (t_R = 5.32 min), both as white solids. **8a**: ^{1}H NMR δ 8.65 (s, 1), 8.43 (s, 1), 6.17 (d, 1, *J* = 5.2), 5.42 (d, 0.34, *J* = 4.2), 5.17 (d, 0.66, *J* = 1.6), 5.00 (dd, 0.34, *J* = 5.7, 4.2), 4.95 (dd, 0.66, *J* = 5.1, 1.6), 4.76 (m, 1), 4.54 (m, 1), 4.48 (dd, 0.66, *J* = 6.4, 5.1), 4.40 (m, 1.34), 4.37−3.97 (m, 5), 2.45 (m, 2), 1.47 (m, 2), 1.31 (m, 2), 0.82 (m, 3). **8b**: ^{1}H NMR δ 8.65 (s, 1), 8.43 (s, 1), 6.17 (d, 1, *J* = 5.2), 5.40 (d, 0.5, *J* = 4.5), 5.22 (d, 0.5, *J* = 3.5), 5.12 (m, 1), 4.75 (m, 1), 4.54 (m, 1), 4.40 (m, 1), 4.33 (m, 1), 4.25 (m, 2.5), 4.20 (dd, 0.5, *J* = 5.2, 3.5), 4.07 (m, 2), 2.41 (t, 2, *J* = 7.4), 1.53 (m, 2), 1.28 (m, 2), 0.84 (t, 3, *J* = 7.3); ^{13}C NMR (both isomers **8a** and **8b**) δ 176.8 (C), 176.6 (C), 150.4 (C), 148.9 (C), 145.4 (CH), 142.9 (CH), 119.0 (C), 101.8 (CH), 99.9 (CH), 96.6 (CH), 95.8 (CH), 88.6 (CH), 84.6 (CH), 81.6 (CH), 80.0 (CH), 77.8 (CH), 75.4 (CH), 74.2 (CH), 73.9 (CH), 73.4 (CH), 72.9 (CH), 70.8 (CH), 70.5 (CH), 69.5 (CH), 69.3 (CH), 67.3 (CH_2), 66.9 (CH_2), 65.9 (CH_2), 33.9 (CH_2), 33.8 (CH_2), 27.1 (CH_2), 27.0 (CH_2), 26.9 (CH_2), 22.2 (CH_2), 22.1 (CH_2), 13.6 (CH_3). **8a** and **8b**: MS (ESI$^+$) *m*/*z* = 644 $(M + H)^+$; HR-MS (ESI) *m*/*z* ($[M + H]^+$) calcd for $C_{20}H_{31}N_5O_{15}P_2$ 644.1370, found 644.1371.

*2′- and 3′-O-(3-Methyl-but-2-enoyl)-ADP-ribose (**9**) [(3R,4R,5R)-5-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl 3-Methylbut-2-enoate (**9a**)] and [(2R,3S,4R)-2-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl 3-Methylbut-2-enoate (**9b**)].* Prepared from 32 mg (0.30 mmol) of sodium carbonate, 700 mg (7.0 mmol) of 3,3-dimethylacrylic acid, 5 mL of *N*,*N*-dimethylacetamide, and 100 mg (0.15 mmol) of NAD according to the same procedure as for **7**. The crude product was purified via HPLC (95% A, 5% B to 75% A, 25% B over 15 min; 25% to 95% B over 5 min) to give 3.9 mg (4.0%) of 3′-*O*-(3-methyl-but-2-enoyl)-ADP-ribose **9b** (t_R = 4.25 min) and 9.3 mg (9.6%) of 2′-*O*-(3-methyl-but-2-enoyl)-ADP-ribose **9a** (t_R = 5.49 min), both as white solids. **9a**: ^{1}H NMR δ 8.65 (s, 0.2), 8.64 (s, 0.4), 8.63 (s, 0.15), 8.62 (s, 0.25), 8.42 (s, 1), 6.15 (m, 1), 5.79 (m, 0.2), 5.76 (m, 0.4), 5.64 (m, 0.15), 5.61 (m, 0.25), 5.53 (d, 0.25, *J* = 4.2), 5.48 (d, 0.15, *J* = 4.8), 5.31 (d, 0.2, *J* = 1.1), 5.29 (d, 0.4, *J* = 1.6), 4.98 (dd, 0.25 (J = 5.8, 4.2), 4.95 (dd, 0.15, *J* = 4.8, 3.1), 4.92 (m, 0.6), 4.74 (m, 1), 4.53 (m, 1), 4.49 (dd, 0.6, *J* = 5.8, 5.1), 4.40 (m, 1.4), 4.34−4.00 (m, 5), 1.89 (dd, 2, *J* = 6.9, 1.8), 1.88 (dd, 1, *J* = 6.9, 1.8), 2.09 (m, 1.8), 2.05 (d, 0.45, *J* = 1.2), 2.02 (d, 0.75, *J* = 1.2), 1.92 (m, 1.25), 1.91 (d, 0.45, *J* = 1.2), 1.86 (m, 1.3); ^{13}C NMR δ 168.1 (C), 162.4 (C), 150.4 (C), 148.9 (C), 145.3 (CH), 143.1 (CH), 119.1 (C), 114.5 (CH), 100.0 (CH), 95.9 (CH), 88.6 (CH), 84.7 (CH), 82.2 (CH), 81.9 (CH), 77.2 (CH), 75.4 (CH), 72.2 (CH), 70.9 (CH), 69.6 (CH), 69.5 (CH), 67.0 (CH_2), 66.7 (CH_2), 66.2 (CH_2), 27.5 (CH_3), 20.6 (CH_3); MS (ESI$^+$) *m*/*z* = 642 $(M + H)^+$; HR-MS (ESI) *m*/*z* ($[M + H]^+$) calcd for $C_{20}H_{29}N_5O_{15}P_2$ 642.1214, found 642.1212. **9b**: ^{1}H NMR δ 8.65 (s, 0.5), 8.64 (s, 0.5), 8.43 (s, 1), 6.16 (d, 0.5, *J* = 5.4), 6.16 (d, 0.5, *J* = 5.4), 5.71 (m, 0.5), 5.69 (m, 0.5), 5.41 (d, 0.5), 5.23 (d, 0.5, *J* = 4.5), 5.23 (d, 0.5, *J* = 3.4), 5.09 (m, 1), 4.75 (m, 1), 4.54 (m, 1), 4.39 (m, 1), 4.35 (dd, 0.5, *J* = 6.2, 4.5), 4.25 (m, 3), 4.22 (dd, 0.5, *J* = 3.4, 5.1); ^{13}C NMR δ 168.2 (C), 162.3 (C), 150.6 (C), 145.4 (CH), 143.1 (CH), 119.1 (C), 114.7 (C), 114.5 (C), 102.0 (CH), 96.6 (CH), 88.7 (CH), 81.6 (CH), 80.7 (CH), 75.4 (CH), 74.4 (CH), 73.4

(CH), 72.4 (CH), 70.9 (CH), 70.6 (CH), 67.1 (CH_2), 66.4 (CH_2), 65.7 (CH_2), 27.5 (CH_3), 20.5 (CH_3), 20.4 (CH_3); MS (ESI^+) m/z = 642 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{20}H_{29}N_5O_{15}P_2$ 642.1214, found 642.1212.

*2′- and 3′-O-n-Hexanoyl-ADP-ribose (**10**) [(3R,4R,5R)-5-(((((((-(2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)-oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl Hexanoate (**10a**)] and [(2R,3S,4R)-2-(((((((((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)-oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl Hexanoate (**10b**)].* To a solution of 80 mg (0.75 mmol) of sodium carbonate dissolved in 2 mL (16 mmol) of *n*-hexanoic acid at 95 °C was added 250 mg (0.376 mmol) of NAD. The reaction was stirred at 95 °C for 10 min and then cooled. The mixture was diluted with 1−2 mL of water and then purified via HPLC (90% A, 10% B for 1 min, 10% B to 95% B over 14 min) to give 20.9 mg (8.4%) of 3′-*O*-*n*-hexanoyl-ADP-ribose **10b** (t_R = 5.01 min) and 20.3 mg (8.2%) of 2′-*O*-*n*-hexanoyl-ADP-ribose **10a** (t_R = 5.67 min), both as white solids. **10a**: ^1H NMR δ 8.66 (s, 1), 8.44 (s, 1), 6.18 (d, 1, J = 5.4), 5.53 (d, 0.34, J = 4.2), 5.28 (d, 0.66, J = 1.7), 5.01 (dd, 0.34, J = 5.8, 4.2), 4.96 (dd, 0.66, J = 5.1, 1.7), 4.77 (m, 1), 4.55 (m, 1), 4.49 (dd, 0.66, J = 6.2, 5.2), 4.40 (m, 1.34), 4.36−3.99 (m, 5), 2.44 (m, 2), 1.60 (m, 2), 1.27 (m, 4), 0.84 (m, 3). **10b**: ^1H NMR δ 8.66 (s, 1), 8.44 (s, 1), 6.18 (d, 1, J = 5.4), 5.41 (d, 0.5, J = 4.6), 5.23 (d, 0.5, J = 3.6), 5.12 (m, 1), 4.75 (m, 1), 4.55 (t, 1, J = 4.4), 4.40 (m, 1), 4.34 (m, 1), 4.25 (m, 2.5), 4.21 (dd, 0.5, J = 5.2, 3.6), 4.07 (m, 2), 2.40 (m, 2), 1.55 (m, 2), 1.26 (m, 4), 0.83 (m, 3); ^{13}C NMR (both isomers **10a** and **10b**) δ 176.8 (C), 176.6 (C), 150.4 (C), 148.8 (C), 145.4 (CH), 143.0 (CH), 119.0 (C), 101.96 (CH), 99.9 (CH), 96.55 (CH), 95.8 (CH), 88.6 (CH), 84.8 (CH), 82.1 (CH), 81.7 (CH), 81.6 (CH), 80.2 (CH), 77.8 (CH), 75.4 (CH), 74.2 (CH), 73.9 (CH), 73.4 (CH), 72.9 (CH), 70.8 (CH), 70.7 (CH), 69.4 (CH), 69.3 (CH), 66.7 (CH_2), 66.4 (CH_2), 65.8 (CH_2), 34.4 (CH_2), 34.2 (CH_2), 31.1 (CH_2), 31.1 (CH_2), 24.6 (CH_2), 24.5 (CH_2), 22.3 (CH_2), 13.8 (CH_3). **10a** and **10b**: MS (ESI^+) m/z = 658 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{21}H_{33}N_5O_{15}P_2$ 658.1527, found 658.1527.

*2′- and 3′-O-n-Heptanoyl-ADP-ribose (**11**) [(3R,4R,5R)-5-(((((((-(2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-methyl)-2,4-dihydroxytetrahydrofuran-3-yl Heptanoate (**11a**)] and [(2R,3S,4R)-2-(((((((((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl Heptanoate (**11b**)].* Prepared from 80 mg (0.75 mmol) of Na_2CO_3, 2 mL (14 mmol) of *n*-heptanoic acid, and 250 mg (0.376 mmol) of NAD according to the same procedure as for **10**. The crude product was purified via HPLC (90% A, 10% B for 1 min, 10% B to 95% B over 14 min) to give 2.4 mg (0.9%) of 3′-*O*-*n*-heptanoyl-ADP-ribose **11b** (t_R = 6.09 min, overlap with 1′-isomer at 6.24 min) and 0.6 mg (0.2%) of 2′-*O*-*n*-heptanoyl-ADP-ribose **11a** (t_R = 6.64 min), both as white solids. **11a**: ^1H NMR δ 8.67 (s, 1), 8.43 (s, 1), 6.19 (d, J = 1), 5.52 (d, 0.34, J = 4.2), 5.28 (d, 0.66, J = 1.7), 4.94 (m, 1), 4.77 (m, 1), 4.55 (m, 1), 4.49 (m, 0.66), 4.40 (m, 1.34), 4.36−4.00 (m, 5), 2.44 (m, 2), 1.59 (m, 2), 1.26 (m, 6), 0.84 (m, 3). **11b**: ^1H NMR δ 8.67 (s, 0.5), 8.66 (s, 0.5), 8.44 (s, 1), 6.18 (d, 0.5, J = 5.4), 6.18 (d, 0.5, J = 5.4), 5.40 (d, (0.5, J = 4.6), 5.22 (d, 0.5, J = 3.6), 5.12 (m, 1), 4.75 (m, 1), 4.55 (m, 1), 4.40 (m, 1), 4.35 (m, 1), 4.25 (m, 2.5), 4.19 (m, 0.5), 4.07 (m, 2), 2.38 (m, 2), 1.53 (m, 2), 1.24 (m, 4), 0.82 (m, 3). **11a** and **11b**: MS (ESI^+) m/z = 672 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{22}H_{35}N_5O_{15}P_2$ 672.1683, found 672.1683.

*1′-, 2′- and 3′-O-Benzoyl-ADP-ribose (**12**) [(2S,3R,4S,5R)-5-(((((((-(2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)-oxy)methyl)-3,4-dihydroxytetrahydrofuran-2-yl Benzoate], [(3R,4R,5R)-5-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)-phosphoryl)oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl Benzoate (**12a**)], and [(2R,3S,4R)-2-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl Benzoate (**12b**)].* To 32 mg (0.30 mmol) of sodium carbonate and 700 mg of benzoic acid was added 5 mL of *N*,*N*-dimethylacetamide. The mixture was stirred at 50 °C until all of the sodium carbonate had dissolved, and then 100 mg (0.15 mmol) of NAD was added, along with 2 mL of water. The reaction was stirred at 95 °C for 20 min, and then 100 mL of ethanol was added to give a precipitate. The precipitate was filtered and then washed with additional ethanol. The residue was purified via HPLC (95% A, 5% B for 1 min; 5% to 25% B over 12.5 min, 25% to 85% B over 2.5 min) to give 6.5 mg (6.5%) of 3′-*O*-benzoyl-ADP-ribose **12b** (t_R = 5.47 min), 0.7 mg (0.7%) of 1′β-*O*-benzoyl-ADP-ribose (t_R = 6.02 min), and 1.7 mg (1.7%) of 2′-*O*-benzoyl-ADP-ribose **12a** (t_R = 7.19 min), as white solids. **12a**: ^1H NMR δ 8.64 (s, 0.4), 8.61 (s, 0.6), 8.33 (s, 0.6), 8.29 (s, 0.4), 7.94 (m, 1), 7.92 (m, 1), 7.63 (m, 1), 7.46 (m, 2), 6.13 (d, 0.4, J = 5.1), 6.12 (d, 0.6, J = 5.1), 5.66 (d, 0.4, J = 4.4), 5.45 (d, 0.6, J = 2.2), 5.17 (dd, 0.6, J = 5.1, 2.2), 5.13 (dd, 0.4, J = 5.6, 4.4), 4.71 (t, 0.6, 5.1), 4.69 (t, 0.4, J = 5.1), 4.59 (dd, 0.6, J = 6.3, 5.1), 4.54 (m, 1.4), 4.40 (m, 1.4), 4.28 (m, 2.6), 4.13 (m, 2); MS (ESI^+) m/z = 664 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{22}H_{27}N_5O_{15}P_2$ 664.1057, found 664.1058. **12b**: ^1H NMR δ 8.63 (s, 0.5), 8.61 (s, 0.5), 8.26 (s, 0.5), 8.26 (s, 0.5), 7.84 (m, 1), 7.81 (m, 1), 7.60 (dt, 1, J = 7.5, 1.0), 7.42 (t, 2, J = 7.5), 6.11 (d, 0.5, J = 5.1), 6.07 (d, 0.5, J = 5.1), 5.48 (d, 0.5, J = 4.7), 5.31 (d, 0.5 (J = 3.6), 5.30 (m, 1), 4.69 (t, 0.5, J = 5.1), 4.67 (t, 0.5, J = 5.1), 4.53 (m, 1.5), 4.47 (m, 1.5), 4.47 (m, 1), 4.42−4.08 (m, 5), 4.33 (m, 0.5); MS (ESI^+) m/z = 664 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{22}H_{27}N_5O_{15}P_2$ 664.1057, found 664.1055. **1′β-*O*-Benzoyl-ADP-ribose**: ^1H NMR δ 8.62 (s, 1), 8.27 (s, 1), 7.89 (dd, 2, J = 8.3, 1.2), 7.64 (m, 1), 7.47 (m, 2), 6.32 (d, 1, J = 4.5), 6.12 (d, 1, J = 5.3), 4.73 (m, 1), 4.52 (dd, 1, J = 5.1, 4.2), 4.45 (dd, 1, J = 6.2, 4.6), 4.41 (m, 2), 4.29 (m, 1, J = 6.2, 3.1), 4.25 (m, 2), 4.11 (m, 2); MS (ESI^+) m/z = 664 $(M + H)^+$.

*2′- and 3′-O-Succinyl-ADP-ribose (**13**) [4-(((3R,4R,5R)-5-(((((((-(2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-methyl)-2,4-dihydroxytetrahydrofuran-3-yl)oxy)-4-oxobutanoic Acid (**13a**)] and [4-(((2R,3S,4R)-2-(((((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl)oxy)-4-oxobutanoic Acid (**13b**)] (Procedure B).* A mixture of 500 mg (4.23 mmol) of succinic acid and 500 μL (6.18 mmol) of pyridine was heated and stirred at 90 °C until the mixture was homogeneous. To the mixture was added 100 mg (0.15 mmol) of NAD, and then the reaction was stirred at 90 °C for 20 min. The heat was removed, and then 10 mL of denatured alcohol was added. The resulting suspension was filtered to give a sticky solid. This was purified via ion chromatography (gradient 1) to give 20 mg (18%) of 2′- and 3′-*O*-succinyl-ADP-ribose trisodium salt **13** as a white powder. The product eluted at 50% B: MS (ESI^+) m/z = 660 $(M + H)^+$. **13a**: ^1H NMR δ 8.49 (s, 1), 8.21 (s, 1), 6.12 (d, 1, J = 5.6), 5.48 (d, 0.34, J = 4.2), 5.27 (d, 0.66, J = 1.5), 4.96 (dd, 0.34, J = 5.4, 4.2), 4.93 (d, 0.66), 4.75 (t, 1, J = 5.6), 4.53 (m, 1), 4.46 (dd, 0.66, J = 6.2, 5.4), 4.39 (m, 1.34), 4.34−4.00 (m, 5), 2.64 (m, 2), 2.45 (m, 2); MS (ESI^+) m/z = 660 $(M + H)^+$. **13b**: ^1H NMR δ 8.49 (s, 1), 8.21 (s, 1), 6.21 (d, 1, J = 5.6), 5.38 (d, 0.5, J = 4.6), 5.23 (d, 0.5, J = 3.6), 5.11 (m, 1), 4.75 (t, 1, J = 5.6), 4.53 (m, 1), 4.39 (m, 1), 4.32 (m, 1), 4.22 (m, 2.5), 4.18 (m, 0.5), 2.64 (m, 2), 2.45 (m, 2). **13a** and **13b**: MS (ESI^+) m/z = 660 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{19}H_{27}N_5O_{17}P_2$ 660.0955, found 660.0956.

*2′- and 3′-O-Citryl-ADP-ribose (**14**) [2-(2-(((3R,4R,5R)-5-(((((((-(2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-*

2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-methyl)-2,4-dihydroxytetrahydrofuran-3-yl)oxy)-2-oxoethyl)-2-hydroxysuccinic Acid], [3-((((3R,4R,5R)-5-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)-phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl)oxy)carbonyl)-3-hydroxypentanedioic Acid], [2-(2-(((2R,3S,4R)-2-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy-(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl)oxy)-2-oxoethyl)-2-hydroxysuccinic Acid], and [3-((((2R,3S,4R)-2-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl)oxy)-carbonyl)-3-hydroxypentanedioic Acid]. Prepared from 400 mg (1.90 mmol) of citric acid monohydrate, 400 μL (4.95 mmol) of pyridine, and 100 mg (0.15 mmol) of NAD (procedure B.) The crude product was purified via ion chromatography (gradient 2) to give 29 mg (24%) of 2′- and 3′-*O*-citryl-ADP-ribose tetrasodium salt **14** as a beige solid. The product eluted at 100% B. The 1-citrate and 4-citrate ADP-ribose esters were both present but could not be separated: ^{1}H NMR (see the Supporting Information); MS (ESI$^+$) m/z = 734 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{21}H_{29}N_5O_{20}P_2$ 734.0957, found 734.0957.

*2′- and 3′-O-Malyl-ADP-ribose (**15**) [(2S)-4-(((3R,4R,5R)-5-(((((((-(2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-methyl)-2,4-dihydroxytetrahydrofuran-3-yl)oxy)-2-hydroxy-4-oxobutanoic Acid], [(3S)-4-(((3R,4R,5R)-5-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy-phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl)oxy)-3-hydroxy-4-oxobutanoic Acid], [(2S)-4-(((2R,-3S,4R)-2-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)-phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl)oxy)-2-hydroxy-4-oxobutanoic Acid], and [(3S)-4-(((2R,3S,4R)-2-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-methyl)-4,5-dihydroxytetrahydrofuran-3-yl)oxy)-3-hydroxy-4-oxobutanoic Acid].* A mixture of 400 mg (2.98 mmol) of L-malic acid and 400 μL (4.95 mmol) of pyridine was heated and stirred at 90 °C until the mixture was homogeneous. To the mixture was added 100 mg (0.15 mmol) of NAD, and then the reaction was stirred at 90 °C for 20 min. The heat was removed, and then 10 mL of denatured alcohol was added. The resulting suspension was centrifuged (3000*g*, 20 °C, 20 min). The supernatant was decanted, and then the pellet was dissolved in 500 μL of water and diluted with 10 mL of denatured alcohol. This suspension was centrifuged again (300 g, 20 °C, 20 min), and then the supernatant was decanted to give the crude product as a solid. This was purified via ion chromatography (gradient 1) to give 13.9 mg (12%) of 2′- and 3′-*O*-malyl-ADP-ribose trisodium salt **15** as a white solid. The product eluted from the column at 50% B, immediately after ADP-ribose. The 1-malate and 4-malate ADP-ribose esters were both present but could not be separated. ^{1}H NMR (see the Supporting Information); MS (ESI$^+$) m/z = 676 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{19}H_{27}N_5O_{18}P_2$ 676.0905, found 676.0906.

*2′- and 3′-O-(2-Hydroxypropionyl)-ADP-ribose (**16**) [(3R,4R,5R)-5-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-2,4-dihydroxytetrahydrofuran-3-yl 2-Hydroxypropanoate] and [(2R,3S,4R)-2-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl 2-Hydroxypropanoate].* Prepared from 300 mL (3.42 mmol) of 85% DL-lactic acid in water, 200 μL (2.98 mmol) of pyridine, and 100 mg of NAD according to the same procedure as for **15**. The crude product was partially purified by ion chromatography (Gradient 1). After desalting, 12 mg of a mixture consisting of mainly ADP-ribose with some 2′- and 3′-*O*-(2-hydroxypropionyl)-ADP-ribose **16** was obtained. The product mixture eluted from the column at 50% B: ^{1}H NMR (see the Supporting Information); MS (ESI$^+$) m/z = 560 (ADP-ribose), 632 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{18}H_{27}N_5O_{16}P_2$ 632.1006, found 632.1006.

*2′- and 3′-O-(Hydroxyacetyl)-ADP-ribose (**17**) [(3R,4R,5R)-5-(((((((-(2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-methyl)-2,4-dihydroxytetrahydrofuran-3-yl 2-Hydroxyacetate] and [(2R,3S,4R)-2-(((((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy-(hydroxy)phosphoryl)oxy)methyl)-4,5-dihydroxytetrahydrofuran-3-yl 2-Hydroxyacetate].* Prepared from 400 mg (5.26 mmol) of glycolic acid, 400 mL (4.95 mmol) of pyridine, and 100 mg (0.15 mmol) of NAD according to the same procedure as for **15**. The crude product was partially purified by ion chromatography (gradient 1). After desalting, 25 mg of a mixture consisting of mainly ADP-ribose with some 2′- and 3′-*O*-(hydroxyacetyl)-ADP-ribose **17** was obtained. The product mixture eluted from the column at 50% B: ^{1}H NMR (see the Supporting Information); MS (ESI$^+$) m/z = 560 (ADP-ribose), 618 $(M + H)^+$; HR-MS (ESI) m/z ($[M + H]^+$) calcd for $C_{17}H_{25}N_5O_{16}P_2$ 618.0850, found 618.0849.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR and ^{13}C assignments for compounds **2** and **4**. ^{1}H NMR, ^{13}C NMR, COSY, and HSQC spectra for compound **3**. ^{1}H NMR spectra for all compounds in Table 1; ^{13}C spectra for compounds **2**−**5** and **7**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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