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Chemoenzymatic Synthesis of 7-Deaza Cyclic Adenosine 5′-Diphosphate Ribose Analogues, Membrane-Permeant Modulators of Intracellular Calcium Release

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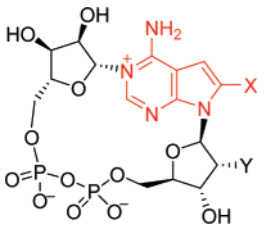

X = Br, Y = OH, 7-Deaza-8-Br-cADPR **3**
X = H, Y = H, 7-Deaza-2′-deoxy-cADPR **4**

An optimized synthetic route to 7-deaza-8-bromo-cyclic adenosine 5′-diphosphate ribose (7-deaza-8-bromo-cADPR **3**), an established cell-permeant, hydrolysis-resistant cyclic adenosine 5′-diphosphate ribose (cADPR) antagonist, is presented. Using NMR analysis, we found that **3** adopted a C-2′ endo conformation in the N9-linked ribose and a syn conformation about the N9-glycosyl linkage, which are similar to that of cADPR. The synthetic route was also employed to produce 7-deaza-2′-deoxy-cADPR **4**, a potential cell-permeant cADPR analogue. **3** and **4** were more stable to chemical hydrolysis, consistent with the observation that 7-deaza-cADPR analogues are more stable than their parent adenosine derivatives. **3** was also found to be stable to enzyme-mediated hydrolysis using CD38 ectoenzyme.

Introduction

Cyclic adenosine 5′-diphosphate ribose (cADPR **1**) is a Ca^{2+}-mobilizing second messenger,[1−3] releasing calcium from intracellular stores in various cell types including sea urchin eggs, pancreatic β cells, and smooth muscle and T-cells. cADPR-induced calcium release is independent of the well-established *myo*-inositol 1,4,5-trisphosphate pathway[4,5] and is regulated by gating the type 2 and/or type 3 ryanodine receptor.[6] In biological systems, cADPR is synthesized from nicotinamide adenine dinucleotide (NAD^+) by *Aplysia* ADP-ribosyl cyclase, an enzyme known to have loose substrate selectivity, thus tolerating structural modifications of its substrates.[7,8] The crystal structure of the enzyme has been studied, and a covalent bond linkage between the enzyme and substrate has been proposed.[9,10] Adenosine 5′-diphosphate ribose (ADPR) analogues have been used to probe mechanistic questions for cyclization and hydrolysis.[11] Numerous derivatives of the biologically important molecule cADPR have now been synthesized, and their

(1) Galione, A.; Lee, H. C.; Busa, W. *Science* **1991**, *253*, 1143−1146.
(2) Koshiyama, H.; Lee, H. C.; Tashjian, A. H. *J. Biol. Chem.* **1991**, *266*, 16985−16988.
(3) Takasawa, S.; Nata, K.; Yonekura, H.; Okamoto, H. *Science* **1993**, *259*, 370−373.
(4) Clapper, D. L.; Walseth, T. F.; Dargie, P. J.; Lee, H. C. *J. Biol. Chem.* **1987**, *262*, 9561−9568.
(5) Dargie, P. J.; Agre, M. C.; Lee, H. C. *Cell Regul.* **1990**, 279−290.
(6) Guse, A. H. *FEBS* **2005**, *272*, 4590−4597.
(7) Lee, H. C.; Aarhus, R. *Cell Regul.* **1991**, *2*, 203−209.
(8) Walseth, T. F.; Lee, H. C. *Biochem. Biophys. Acta* **1993**, *1178*, 235−242.
(9) Prasad, G. S.; McRee, D. E.; Stura, E. A.; Levitt, D. G.; Lee, H. C.; Stout, C. D. *Nat. Struct. Biol.* **1996**, *3*, 957−963.
(10) Love, M. L.; Szebenyi, D. M. E.; Kriksunov, I. A.; Thiel, D. J.; Munshi, C.; Graeff, R.; Lee. H. C.; Hao, Q. *Structure* **2004**, *12*, 477−486.

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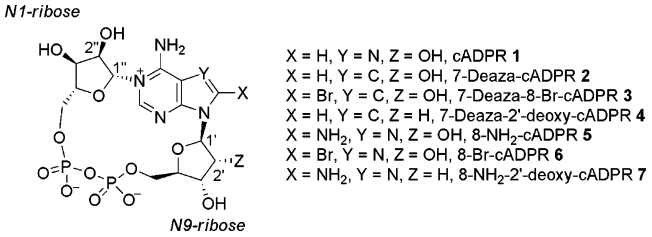


FIGURE 1. Structures and numbering of cADPR and analogues. The N9-linked ribose (also known as "southern") and N1-linked ribose (also known as "northern") are distinguished by adopting prime (′) and double prime (″) symbols, respectively, for sugar carbons shown in this figure.

pharmacological activities have been investigated, although the field is still in its infancy.[12,13] A recent notable example is the nonhydrolyzable cyclic inosine 5′-diphosphate ribose (cIDPR)[14] and its 8-substituted analogues.[15] As a stable analogue, cIDPR was cocrystallized with the ectoenzyme ADP-ribosyl cyclase/hydrolase CD38, providing insight into the binding and catalytic process for the first time using this close analogue of the natural ligand.[16] Conservatively modified carbocyclic analogues of cADPR (e.g., cADPcR) have also been synthesized and investigated biologically.[17−19] Analogues with more radical modifications[20] are also finding useful application.[21]

Among these analogues, deletion of the N7 nitrogen atom of the purine of cADPR, *inter alia*, leads to a class of derivatives that demonstrate hydrolysis-resistant properties, such as 7-deaza-cADPR **2**. **2** (Figure 1) was one of the first 7-deaza-cADPR analogues ever synthesized, and its biological activities were studied.[22] As one of the closest possible analogues of the natural ligand cADPR, 7-deaza-cADPR, however, was found to be a partial agonist relative to cADPR, demonstrating that the N7 position is part of the recognition mechanism for effective calcium release. It is also noteworthy that 7-deaza-cADPR is more stable to heat-induced hydrolysis at the N1 ribosyl linkage and is also a poor substrate for cADPR hydrolase.[22] 8-Position modification is known to convert cADPR from an agonist to an antagonist.[12,13] Thus, 8-amino-cADPR **5** and 8-bromo-cADPR **6** have been reported to demonstrate antagonistic effects in both sea urchin eggs and Jurkat T-cells systems, and moreover, 8-bromo-cADPR **6** was found to be cell permeant.[23]

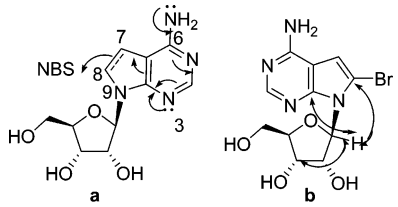


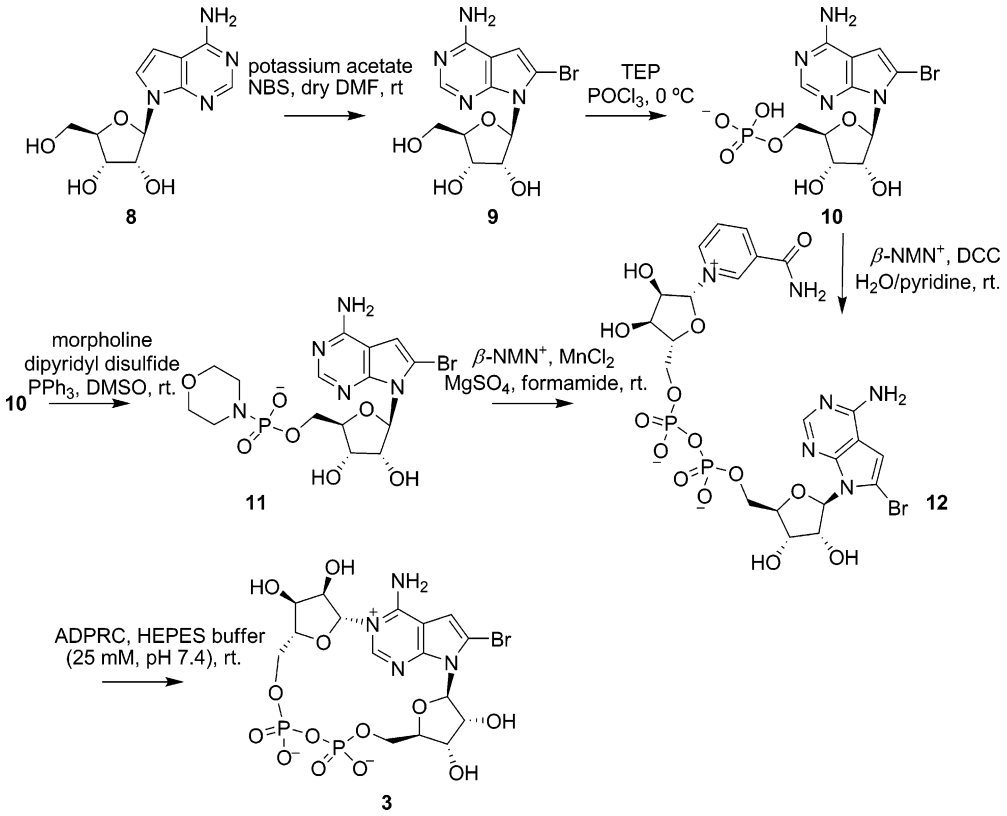
FIGURE 2. (a) Mechanism of 6-amino group directed bromination reaction at the pseudo-8-position (drawing based on the literature[31]). (b) The 8-bromination was determined by gHMBC method, and a coupling between C-1′ proton and the C-8 carbon was observed. 8-Bromo-7-deazaadenosine is proposed to adopt a syn conformation as shown in (b).

With the partial agonistic property of **2** established, it was thus of interest to study the combination of 8-substitution and N7 deletion, leading to the synthesis and biological evaluation of 7-deaza-8-bromo-cADPR **3**.[23,24] 7-Deaza-8-bromo-cADPR exhibited antagonistic activity in sea urchin homogenate, and its potency was higher than that of 8-bromo-cADPR.[23] It was also active in T-lymphocytes and was employed in a seminal study to investigate the signaling by cADPR in T-lymphocyte signal transduction[25] and also to establish a role for the cADPR signaling pathway in long-term synaptic depression in the hippocampus.[26] In addition to antagonistic activity, 7-deaza-8-bromo-cADPR **3** is also more resistant to both chemical and enzymatic hydrolysis, as expected for 7-modified analogues, and importantly is cell permeant. The cell membrane permeability of 7-deaza-8-bromo-cADPR facilitates the administration of this antagonist into intact cells, which usually relies on techniques such as microinjection or patch clamping. Together with these findings, 7-deaza-8-bromo-cADPR has thus become one of the best-known pharmacological tools in this growing area. A preliminary chemo-enzymatic synthetic route to **3** was reported.[24]

The wide applicability of **3** prompted us to better optimize its synthetic route, as the key intermediate 7-deaza-8-bromo-NAD^+ **12** in our earlier synthesis was prepared in a relatively low yield of 28% via a dicyclohexylcarbodiimide (DCC)-mediated condensation of 7-deaza-8-bromo-AMP **10** and β-NMN^+ (Scheme 1).[24] Synthesis of cADPR derivatives in general is a somewhat difficult process. The choice of routes is limited to either a total synthetic methodology[18,20,27] or a chemo-enzymatic method relying on the cyclizing ability of certain analogues of NAD^+ by *Aplysia* ADP-ribosyl cyclase.[28] Although the total synthesis method in principle facilitates preparation of more diverse cADPR compounds, this method has mainly been used most efficiently for cyclic adenosine 5′-diphospho-carbocyclic ribose (cADPcR) analogues, mimics of cADPR in which a methylene group replaces the furan sugar oxygen atom in the N1-linked ribose. Most of the published cADPR analogues have not been synthesized by this method, partially because the N1-ribose linkage is not stable enough to survive in chemical treatments, although this is useful when the N1-linked ribose

(11) Zhang, B.; Muller-Steffner, H.; Schuber, F.; Potter, B. V. L. *Biochemistry* **2007**, *46*, 4100−4109.
(12) Potter, B. V. L.; Walseth, T. F. *Curr. Mol. Med.* **2004**, *4*, 303−311.
(13) Guse, A. H. *Curr. Med. Chem.* **2004**, *11*, 847−855.
(14) Wagner, G. K.; Guse, A. H.; Potter, B. V. L. *J. Org. Chem.* **2005**, *70*, 4810−4819.
(15) Moreau, C.; Wagner, G. K.; Weber, K.; Guse, A. H.; Potter, B. V. L. *J. Med. Chem.* **2006**, *49*, 5162−5176.
(16) Liu, Q.; Kriksunov, I. A.; Moreau, C.; Graeff, R.; Potter, B. V. L.; Lee, H. C.; Hao, Q. *J. Biol. Chem.* **2007**, *282*, 24825−24832.
(17) Shuto, S.; Fukuoka, M.; Kudoh, T.; Garnham, C.; Galione, A.; Potter, B. V. L.; Matsuda, A. *J. Med. Chem.* **2003**, *46*, 4741−47490.
(18) Kudoh, T.; Fukuoka, M.; Ichikawa, S.; Murayama, T.; Ogawa, Y.; Hashii, M.; Higashida, H.; Kunerth, S.; Weber, K.; Guse, A. H.; Potter, B. V. L.; Matsuda, A.; Shuto, S. *J. Am. Chem. Soc.* **2005**, *127*, 8846−8855.
(19) Bailey, V. C.; Fortt, S. M.; Summerhill, R. J.; Galione, A.; Potter, B. V. L. *FEBS Lett.* **1996**, *379*, 227−230.
(20) Gu, X. F.; Yang, Z.; Zhang, L.; Kunerth, S.; Fliegert, R.; Weber, K.; Guse, A. H.; Zhang, L. *J. Med. Chem.* **2004**, *47*, 5674−5682.
(21) Guse, A. H.; Gu, X.; Zhang, L.; Weber, K.; Kramer, E.; Yang, Z.; Jin, H.; Li, Q.; Carrier, L.; Zhang, L. *J. Biol. Chem.* **2005**, *280*, 15952−15959.
(22) Bailey, V. C.; Sethi, J. K.; Fortt, S. M.; Galione, A.; Potter, B. V. L. *Chem. Biol.* **1997**, *4*, 51−61.
(23) Sethi, J. K.; Empson, R. M.; Bailey, V. C.; Potter, B. V. L.; Galione, A. *J. Biol. Chem.* **1997**, *272*, 16358−16363.
(24) Bailey, V. C.; Sethi, J. K.; Galione, A.; Potter, B. V. L. *Chem. Commun.* **1997**, 695−696.
(25) Guse, A. H.; da Silva, C. P.; Berg, I.; Skapenko, A. L.; Weber, K.; Heyer, P.; Hohenegger, M.; Ashamu, G. A.; Schulze-Koops, H.; Potter, B. V. L.; Mayr, G. W. *Nature* **1999**, *398*, 70−73.
(26) Reyes-Harde, M.; Empson, R.; Potter, B. V. L.; Galione, A.; Stanton, P. K. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4061−40660.
(27) Shuto, S.; Matsuda, A. *Curr. Med. Chem.* **2004**, *11*, 827−845.
(28) Ashamu, G. A.; Galione, A.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1995**, 1359−1360.

SCHEME 1. Synthesis of 7-Deaza-8-Br-cADPR[a]



[a] Compounds **3** and **10**−**12** were isolated as triethylammonium salts.

has been deleted.[20] Thus, we report here full details of an optimized chemo-enzymatic approach to compound **3**, in which a morpholine-activated[29] intermediate **11** was introduced, followed by a Lewis acid catalyzed condensation with β-NMN^{+}[30] (Scheme 1) to give the desired intermediate dinucleotide **12** in a high yield over two steps. We also report a synthesis of compound **4**, a potential cell-permeant, hydrolysis-resistant agonist of cADPR-sensitive calcium release, by adaptation of our modified synthetic route, demonstrating the route as a robust, versatile approach for synthesizing cADPR analogues.

Results and Discussion

The synthetic route to compound **3** starts with bromination of commercially available 7-deazaadenosine (tubercidin **8**) (Scheme 1).

Compound **8** was selectively brominated at the 8-position using a combination of *N*-bromosuccinimide (NBS) and DMF,[31] which had earlier been reported to be a mild combination for electrophilic bromination.[32] Purification of the product gave the desired **9**, showing only one NMR singlet at 6.74 ppm, representing H-7, indicating that a bromination had occurred at the 8-position. The occurrence of the 8-bromination was also confirmed by gHMBC (Figure 2b). The 7-deaza-8-bromoadenosine **9** was assumed to adopt a syn conformation around the N9-glycosyl bond to minimize the nonbonded repulsion between the 8-bromo group and the ribose.[33,34] The H-2′ chemical shift was explored as an indicator of the syn/anti conformation. A significant downfield shift of H-2′ for the syn conformer is normally expected, as seen for 8-bromo-7-deazaadenosine **9**, whose H-2′ chemical shift is at δ 5.12 (the chemical shift for the *anti* 7-deazainosine is 4.30 ppm[15]), indicating a syn conformation about the N9-glycosyl linkage. An explanation was proposed to rationalize the 8-position selectivity, in which the reaction was assumed to go through an electrophilic substitution, as the free radical reaction is not likely to happen under the conditions.[31] Under the basic buffer conditions, the 3,6-position nitrogens are not protonated and therefore the lone pair on these nitrogens donates electron density through the conjugated system, facilitating electrophilic attack at C-8, and consequently producing the 8-bromotubercidin as the predominant product, as shown in Figure 2.[31]

Dry 8-bromo-7-deazaadenosine was selectively phosphorylated at the 5′-hydroxyl group by adaptation of a published method.[35] Treatment of compound **9** in TEP with $POCl_3$ and a trace amount of water at 0 °C for 1 h resulted in the formation of a sole product **10** as monitored by HPLC (for conditions, see the Supporting Information). The desired 7-deaza-8-bromo 5′-adenosine monophosphate (7-deaza-8-bromo-AMP) **10** was first purified using reverse-phase chromatography to remove

(29) Prabahar, K. J.; Cole, T. D.; Ferris, J. P. *J. Am. Chem. Soc.* **1994**, *116*, 10914−10920.
(30) Lee, J.; Churchill, H.; Choi, W. B.; Lynch, J. E.; Roberts, F. E.; Volante, R. P.; Reider, P. J. *Chem. Commun.* **1999**, 729−730.
(31) Bergstrom, D. E.; Brattesani, A. J. *Nucleic Acids Res.* **1980**, *8*, 6213−6219.
(32) Mitchell, R. H.; Lai, Y. H.; Williams, R. V. *J. Org. Chem.* **1979**, *44*, 4733−4735.
(33) Ikehara, M.; Uesugi, S.; Yoshida, K. *Biochemistry* **1972**, *11*, 830−836.
(34) Sarma, R. H.; Lee, H. C.; Evans, F. E.; Yathindra, N.; Sundaralingam, M. *J. Am. Chem. Soc.* **1974**, *96*, 7337−7348.
(35) Ikemoto, T.; Haze, A.; Hatano, H.; Kitamoto, Y.; Ishida, M.; Nara, K. *Chem. Pharm. Bull.* **1995**, *43*, 210−215.

any residual inorganic phosphate, which had been otherwise normally eliminated by a yield-reducing charcoal treatment. After further purification by an ion-exchange chromatography to remove remaining starting material, compound **10** was obtained as a triethylammonium salt in 77% yield and in good purity. With 7-deaza-8-bromo-AMP in hand, we now proceeded to synthesize the key intermediate 7-deaza-8-bromo-NAD^+ **12**. 7-Deaza-8-bromo-NAD^+ was first synthesized by us[24] using a DCC-mediated condensation earlier published by Todd et al.[36] A solution of 7-deaza-8-bromo-AMP **10** and nicotinamide 5′-mononucleotide (β-NMN^+) in pyridine water was treated with DCC for 7 days. Purification of the product using ion-exchange chromatography gave the desired dinucleotide **12** as its triethylammonium salt in 28% yield. Although **12** was successfully produced, the yield was relatively low and symmetrical products, for example formed by the coupling of two β-NMN^+ molecules, were also generated. Clearly, as limited by its reaction mechanism, this DCC coupling reaction provides no coupling selectivity and cannot be further optimized to increase the yield of the desired NAD^+ analogue and reduce the occurrence of symmetrical products and is therefore not ideal for synthesizing pyrophosphate **12**. NAD^+ analogues have also been produced from the corresponding ATP analogues using NAD^+ pyrophosphorylase (NADPP).[37] These ATP analogues are often synthesized enzymatically from the corresponding AMP analogues using the combined actions of adenylate kinase and creatine kinase. This, however, is not applicable to all AMP analogues; for example, 8-substituted AMP analogues are not good substrates for adenylate kinase.[38] Clearly, in our case the 8-bromo-7-deaza-AMP is not suitable for this enzymatic reaction. In our earlier report using the Michelson procedure, diphenyl phosphorochloridate was used as the coupling reagent and 7-deaza-NAD^+ was successfully synthesized.[22] However, the coupling yield by this approach was relatively low and a deprotection step was required, which reduced the overall yield and also increased the risk of degrading the pyrophosphate. We required a better route that gives the pyrophosphate in high yield and requires minimum protecting steps.

An approach for synthesizing pyrophosphates was reported by Moffatt and Khorana.[39] This method involved the coupling of a sugar phosphate and a nucleotide phosphoromorpholidate and has been widely used for the synthesis of a large variety of sugar nucleotide derivatives. Later improved,[30] this method was successfully explored for producing NAD^+ analogues in a relatively high yield.[14,15] Compound **12** was thus successfully synthesized by an adaptation of the method of Lee et al.[30] As shown in Scheme 1, the nucleoside monophosphate **10** was first activated using a combination of triphenylphosphine/dipyridyl disulfide and morpholine and the phosphoromopholidate **11** was generated as the sole product in 87% isolated yield. The phosphoromopholidate **11** was then condensed with β-NMN^+ in the presence of a Lewis acid ($MnCl_2$/formamide) to give the desired pyrophosphate **12**, which was then purified by reverse-phase chromatography in 79% yield. The overall yield of this two-step synthesis was 69%. An excess of 1.7 equiv of β-NMN^+ was used in our experiment to completely consume the starting morpholidate. Residual β-NMN^+ was conveniently removed by the reverse-phase column as it had a higher polarity and shorter elution time than the desired compound **12**. The final compound 7-deaza-8-bromo-cADPR **3** was afforded by incubating compound **12**, obtained by either of the two routes, with *Aplysia* cyclase. HPLC analysis indicated efficient cyclization of **12**, at a rate similar to that of NAD^+, demonstrating **12** to be presumably a good substrate for *Aplysia* cyclase. The resulting 7-deaza-8-bromo-cADPR **3** was purified by ion-exchange chromatography to give its triethylammonium salt in 72% yield. It was noteworthy that by HPLC analysis 7-deaza-8-bromo-NAD^+ was clearly over 90% converted into the cyclic analogue, but some material was somehow unavoidably lost during the subsequent purification process. gHMBC data for the cyclic compound **3** were collected and are listed in Table 1. From this 2D spectrum, the connectivity between the C-1′ proton and a quaternary carbon was clearly observed, indicating a substitution at the 8-position. However, the C-1″ proton and C-2/C-6 connectivity was not identified, possibly reflecting a weaker bond linkage between C-1″ and the purine N1. Indeed, as was demonstrated in the X-ray structure of cADPR,[40] the C-1″−N1 bond (1.54 Å) is significantly weaker than the C-1′−N9 bond (1.44 Å). The cyclic structure of compound **3** was firmly identified by the 1D ^1H NMR spectrum, where the nicotinamide signals were clearly lost and two characteristic doublets were observed at 6.12 and 6.05 ppm for both anomeric protons. Thus, a robust optimized route to **3** has now been achieved, and it should allow large amounts of the compound to be synthesized for biological applications.

TABLE 1. Selected $^1H-^{13}C$ Connectivities from the gHMBC Spectrum of 3

H-1′	*H*-C-N-C-8	*H*-C-N-C-4
H-1″	n.d.[a] with C-2	n.d.[a] with C-6

[a] Not detected.

Synthesis of 7-Deaza-2′-deoxy-cADPR. We established earlier that the 8-position unsubstituted compound 7-deaza-cADPR is, interestingly, a partial agonist biologically and is more stable than the parent.[22] By adaptation of the synthetic route for compound **3**, we also synthesized the novel 7-deaza-2′-deoxy-cADPR **4** (Scheme 2) as a potentially more cell-permeant, hydrolysis-resistant partial agonist. With the lack of a 2′-hydroxyl group on the N9-linked ribose, compound **4** should be more lipophilic than its parent and has more potential to be cell permeant. Also, it is likely that this ribose modification in compound **4** should not radically affect any agonist activity for intracellular Ca^{2+} release compared to its ribose analogue **2**, as we showed earlier that 2′-hydroxyl deletion of cADPR in the N9-linked ribose does not decrease activity.[41] An additional hydrolysis-resistant property should also be added to compound **4** by virtue of the 7-deaza modification, and indeed, we demonstrate herein that 7-deaza-2′-deoxy-cADPR **4** is more stable than other 2′-deoxy compounds.

The synthesis of **4** employed compound **15** as the initial building block, which could in principle conveniently be reacted with a range of nucleophilic amines to introduce a variety of N-6-substituted adenosine analogues, including compound **16**

(36) Hughes, N. H.; Kenner, G. W.; Todd, *J. Chem. Soc*. **1957**, 3733−3736.

(37) Wang, L.; Aarhus, R.; Lee, H. C.; Walseth, T. F. *Biochim. Biophys. Acta* **1999**, *1472*, 555−564.

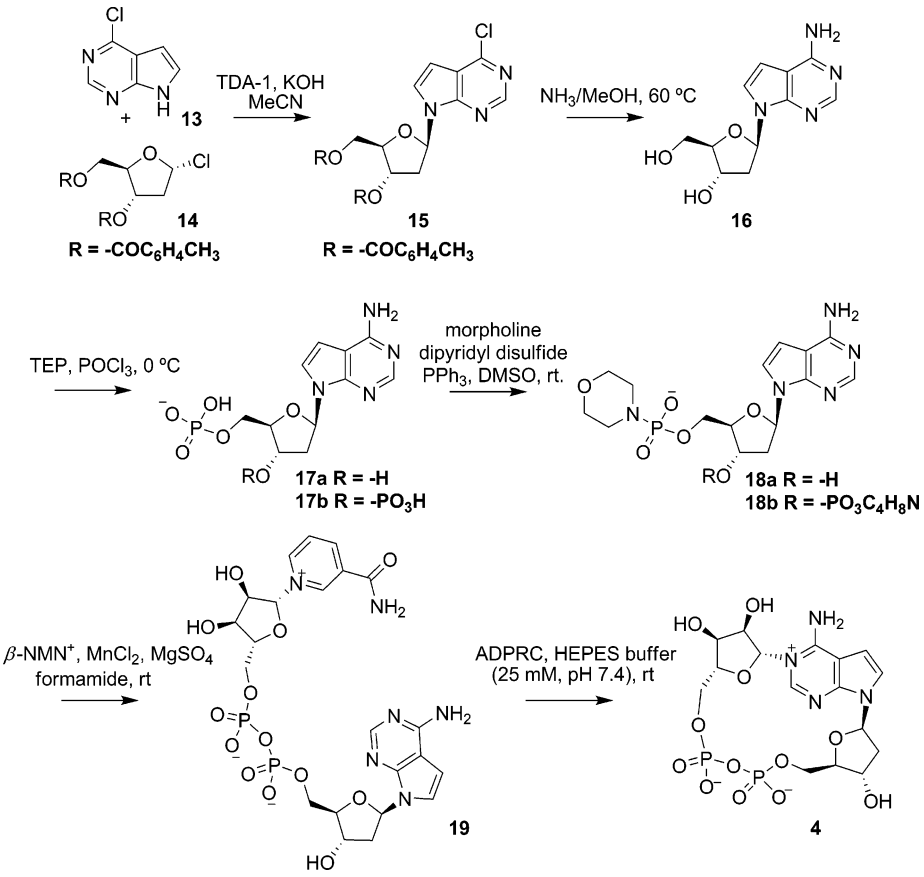
(38) Walseth, T. F.; Aarhus, R.; Gurnack, M. E.; Wong, L.; Breitinger, H. G.; Gee, K. R.; Lee, H. C. *Methods Enzymol*. **1997**, *280*, 294−305.

(39) Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc*. **1961**, *83*, 649−658.

(40) Lee, H. C.; Aarhus, R.; Levitt, D. *Nat. Struct. Biol*. **1994**, *1*, 143−144.

(41) Ashamu, G. A.; Sethi, J. K.; Galione, A.; Potter, B. V. L. *Biochemistry* **1997**, *36*, 9509−9517.

SCHEME 2. Synthesis of 7-Deaza-2′-deoxy-cADPR[a]



[a] Compounds **4** and **17**−**19** were isolated as triethylammonium salts.

as currently desired, formed by a reaction with ammonia. The noncommercial building block **15** was synthesized following a published method,[42] in which **13** (4-chloropyrrolo-[2,3-*d*]-pyrimidine)[43] was condensed with the α-halogenose **14**[44] under phase-transfer conditions using tris-[2-(2-methoxyethoxy)ethyl]-amine (TDA-1) as catalyst. Purification of product using flash column chromatography gave compound **15** solely as the β conformer in 79% yield, showing a characteristic anomeric H-1′ double doublet resonance in the ^{1}H NMR spectrum at 6.80 ppm. 7-Deaza-2′-deoxyadenosine **16** was synthesized by treatment of **15** with methanolic ammonia and was isolated in 53% yield. A similar yield was observed by Seela et al.[42] The introduction of the 5′-monophosphate group was also accomplished using the $POCl_3$/TEP combination, but under anhydrous conditions, as 2′-deoxy compounds were found to be particularly labile to acid-induced hydrolysis of the N9-glycosyl linkage. It was found that phosphorylation of compound **16** proceeded at a rate similar to that of adenosine but that a mixture of products identified as the 5′-monophosphate **17a** and 3′,5′-bisphosphate **17b** was produced, as suggested by the ^{1}H NMR and ^{31}P NMR spectra. HPLC analysis of the reaction mixture, however, deceptively only showed a single peak for products. It is possible that compound **17b** might have an elution time beyond our monitoring time (normally in 20 min). This, however, is highly unlikely as no second peak was notable even after 45 min (for HPLC conditions, see the Supporting Information). It is thus likely that **17a** and **17b** may have the same retention time, since we found that ATP also elutes at the same time as AMP under our HPLC conditions (result not shown). It is not totally clear why phosphorylation of 7-deaza-2′-deoxyadenosine produced two products while phosphorylation of adenosine produced only one product in high yield,[35] but in light of the assumption of Ikemoto et al. that the N7 nitrogen atom might be involved in the formation of a TEP−adenosine complex to activate the 5′-position hydroxyl group, thus facilitating the selective 5′-phosphorylation,[35] we reasoned that the unexpected product **17b** might be produced because of the formation of an unstable 7-deaza-2′-deoxyadenosine−TEP complex. Anhydrous conditions might also contribute to the formation of **17b** as water might be crucial to modulate the reactivity of the phosphorylating reagent, thus maintaining a 5′-selectivity.

In the activation step for coupling, compounds **17a** and **17b** were not separated, since this was deemed to be too difficult, but were used directly to react with the combination of triphenylphosphine, dipyridyl disulfide, and morpholine. Two morpholidates (**18a**/**18b**) were produced, which were now clearly separated by ion-pair HPLC (for conditions, see the Supporting Information), and the retention times of these two molecules were found to be 2.6 and 7.3 min, respectively. Purification using ion-exchange chromatography produced the desired 5′-morpholidate **18a** in 83% isolated yield based on **17a** as its triethylammonium salt. The 5′-morpholidate **18a** was distinguished from the 3′,5′-bisphosphoromorpholidate **18b** by its ^{31}P NMR spectrum in which only one peak was observed at

(42) Seela, F.; Muth, H.-P.; Bindig, U. *Synthesis* **1988**, 670−674.
(43) Tsytovich, A. V.; Shamshin, D. V.; Burkovskii, V. B.; Shvets, V. I. *Russ. J. Bioorg. Chem.* **1995**, *21*, 756−761.
(44) Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777−2780.

TABLE 2. Selected Coupling Constants from 1D ^{1}H NMR of 3 (pH 6.0)

N9-linked ribose	$J_{1',2'}$ 5.9 Hz	$J_{2',3'}$ 5.6 Hz	$J_{3',4'}$ 3.5 Hz	$J_{4',5'a}$ 7.0 Hz	$J_{4',5'b}$ n.d.[a]
N1-linked ribose	$J_{1'',2''}$ 4.1 Hz	$J_{2'',3''}$ 4.6 Hz	$J_{3'',4''}$ 2.3 Hz	n.d.[a]	n.d.[a]

[a] Not detected.

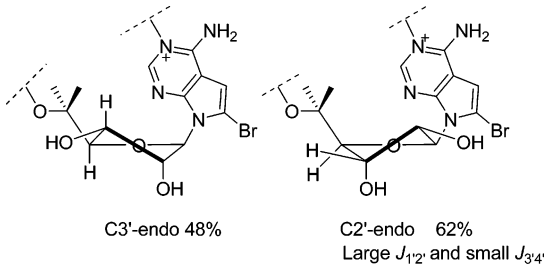


FIGURE 3. C2′-endo/C3′-endo equilibrium for N9-linked ribose of 8-bromo-7-deaza-cADPR. Figure is based on the coupling constants obtained from 1D ^{1}H NMR and gCOSY data.

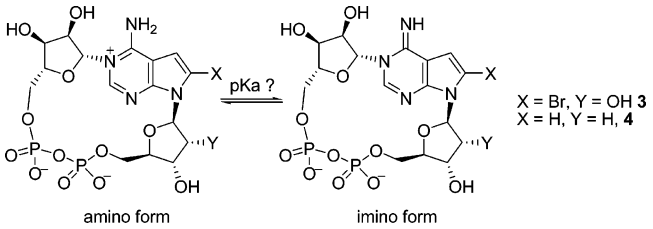


FIGURE 4. Presumed different forms of 7-deaza-cADPR derivatives. At acidic pH, cADPR adopts mainly the amino form, while at basic pH, cADPR adopts the imino form.

8.22 ppm. It was also notable that in the ^{13}C NMR spectrum the C-5′ signal but not C-3′ was split by the phosphate, indicating that the morpholidate is at C-5′ not C-3′. The minor product, the 3′,5′-bisphosphoromorpholidate **18b**, was also isolated (ca 13% based on **16**), the structure being confirmed by the following spectroscopic evidence: in the ^{31}P NMR spectrum, two singlets were observed at 8.05 and 7.77 ppm, indicating the existence of two morpholidates; morpholidates normally have a chemical shift at around 8 ppm; in the ^{1}H NMR spectrum, multiplets at 3.66, 3.45, 3.08, and 2.85 ppm were assigned for the two morpholine moieties. The structure was further confirmed by an LC−MS study where the molecular ion of 574.4 was identified. Evidence was also found in the ^{13}C NMR spectrum, in which the C-3′ resonance was split into a doublet suggesting the direct connection of a phosphate at this position. Phosphoromorpholidate **18a** was then condensed with β-NMN$^+$ in the presence of Lewis acid ($MnCl_2$/formamide), producing the desired 7-deaza-2′-deoxy-NAD$^+$ **19**, which was isolated by reverse-phase chromatography in 60% yield. The final compound 7-deaza-2′-deoxy-cADPR **4** was synthesized by incubation of compound **19** with *Aplysia* cyclase and after ion-exchange chromatography, the product was eluted to give compound **4** in 67% yield as its triethylammonium salt. Preliminary biological evaluation indicated that **4** may have partial agonistic properties in permeabilized cells. Biological data of **4** will be reported elsewhere.

Conformational Study of Compound 3. To date, an important issue that still needs to be answered is why 8-substituted cADPR analogues are antagonists, while cADPR itself is an agonist. Shuto et al. reported that 2″,3″-dideoxydihydro-cADPcR, an inactive compound, adopted a major C3′-endo and high anti conformation in aqueous solution,[18] indicating that the ribose and glycosyl linkage conformations are of crucial importance for the calcium release activities of cADPR analogues. We thus assumed that substitutions such as a bromo group at the 8-position might divert the N9-linked ribose into a C3′-endo conformation, and this in turn may produce the antagonistic effect. To determine the active conformation of compound **3** in aqueous solution, we calculated its ribose C2′-endo/C3′-endo ratio and glycosyl bond conformations. It has been well-established that the C2′-endo/C3′-endo ratio of nucleotide can be mathematically calculated from ^{1}H NMR spectrum by adaptation of the equation [C2′-endo] (%) = [$J_{1',2'}$/($J_{1',2'} + J_{3',4'}$)] × 100.[45,46] Using 1D ^{1}H NMR, 2D *g*COSY, and HMQC, we clearly identified the chemical shift of each proton of compound **3** and calculated the coupling constants of these protons. The coupling constants of protons in the N1-linked ribose and N9-linked ribose are listed in Table 2.

Among these coupling constants, the important $J_{3',4'}$ coupling for the N9-linked ribose could not be easily extracted from the 1D ^{1}H NMR spectrum, since the resonance for H-3′ was largely overlapped by those for H-2″ and H-4″ and therefore the splitting of the signal by H-2′ and H-4′ was not identifiable. Fortunately, H-4′ was clearly separated from all other signals, offering us a good chance to calculate the desired H-3′/4′ coupling constant ($J_{3',4'}$). After decoupling of one of the two H-5′ protons, H-4′ clearly presented a double doublet pattern and the coupling constants ($J_{3',4'}$ and $J_{4',5'a}$) of this double doublet were found to be 3.5 and 7.0 Hz. Further decoupling of the H-3′ multiplet (signal overlaps with H-4″ and H-2″) made H-4′ also a double doublet from which two coupling constants ($J_{4',5'a}$ and $J_{4',5'b}$) were extracted. One of the coupling constants was found to be close to 7.0 Hz (6.2 Hz), and the other was rather small and was not clearly calculated. Altogether, clearly the large coupling constant (7.0 Hz) was derived from the coupling of H-4′ and H-5′a, while the smaller one (3.5 Hz) was produced by the coupling of H-3′ and H-4′. The assignment of $J_{3',4'}$ was confirmed by the finding that the sum of $J_{1',2'}$ and $J_{3',4'}$ is close to 10 Hz.[46] It is noted from Table 2 that the sum of $J_{1'',2''}$ and $J_{3'',4''}$ for the N1-linked ribose is only 6.4, which is smaller than the one for N9-linked ribose. It is known that nucleotides with a charged base (for example, NMN$^+$) compared to those with an uncharged base normally have smaller $J_{1',2'}$ and an unchanged $J_{3',4'}$.[47] Thus, the smaller sum for the N1-linked ribose in our case probably suggests that this ribose is linked with a charged base, and it indicates that under the NMR conditions at pH 6.0 the base is mostly in its "amino form" as would be anticipated (Figure 4).

From the coupling constants listed in Table 2, we find that, in aqueous solution (ca. pH 6.0), 7-deaza-8-bromo-cADPR adopts 62% of the C2′-endo conformation in the N9-linked

(45) Hotoda, H.; Murayama, K.; Miyamoto, S.; Iwata, Y.; Takahashi, M.; Kawase, Y.; Tanzawa, K.; Kaneko, M. *Biochemistry* **1999**, *38*, 9234−9241.

(46) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333−2344.

(47) Birdsall, B.; Birdsall, N. J. M.; Feeney, J.; Thornton, J. *J. Am. Chem. Soc.* **1975**, *97*, 2845−2850.

ribose, which is consistent with the observation that, in the crystal structure, the major puckering mode of cADPR is observed to be C2′-endo.[40] The conformation of the N1-linked ribose was also calculated as 64% C2″-endo. In agreement with our observation, Graham et al.[48] studied three cyclic compounds (cADPR, 2′-OMe-cADPR, and 3′-OMe-cADPR), and all of these compounds demonstrated a dominant C2″-endo conformation in their N1-linked ribose. The conformations of the N9-linked ribose are shown in Figure 3.

Clearly, the C2′-endo/C3′-endo ratio of 7-deaza-8-bromo-cADPR (N9-linked ribose 62% C2′-endo) is close to that of cADPR (N9-linked ribose 64% C2′-endo reported by Shuto et al.[18]). In addition, 7-deaza-8-bromo-cADPR adopts a syn conformation around the N9-glycosyl linkage due to a repulsion of the bulky bromo group by the ribose moiety.[33,34] cADPR also presents a syn conformation around the glycosyl bond as indicated in its crystal structure.[40] The syn conformation of cADPR is characterized by a large downfield shift of the H-2′ proton (+0.59 ppm relative to that of AMP).[49] Compound **3** that has a +1.03 ppm downfield shift relative to that of 7-deaza-AMP also indicates a syn conformation. On the basis of these findings, we therefore assume that, at least for 7-deaza-8-bromo-cADPR **3**, the antagonistic activity is not caused by the C2′-endo/C3′-endo conversion or the syn/anti conformation changes. The issue, however, still remains open since we still do not know what is the biologically active conformation of **3** or other 8-substituted antagonists when they actually bind with their receptor. The reason 7-deaza-8-bromo-cADPR behaves as a antagonist is still not clear, but it is conceivable that the formation of a hydrogen bond by the 8-substitutent with the receptor, or some other perturbing interaction at that site, may be central to the antagonistic effect, consistent with the observation that 8-amino-cADPR is more potent as an antagonist than 8-bromo-cADPR. The amino group is both a hydrogen bond donor and acceptor, whereas the bromo group is only an acceptor and this could also be a key issue.

Hydrolysis Study. 7-Deaza-based cADPR analogues seem to have the benefit of being more hydrolysis resistant chemically. To further clarify this, hydrolysis of both 7-deaza-8-bromo-cADPR **3** and 7-deaza-2′-deoxy cADPR **4** was investigated and compared with 8-bromo-cADPR **6** and the other 2′-deoxy analogue 8-amino-2′-deoxy-cADPR **7**[50] at different pH values and temperatures. These cADPR analogues were thus dissolved in 50 mM phosphate buffer (pH 7.4), 0.2 M HCl solution (pH 0.7), and 0.01 M NaOH solution (pH 12) and were incubated either at room temperature (rt) or at 70 °C. The hydrolysis process was monitored by ion-pair HPLC (for conditions, see the Supporting Information). At room temperature, at pH 7.4 and at pH 12, all cADPR analogues tested were found to be perfectly stable for at least 24 h; under acidic conditions (pH 0.7), 7-deaza-8-bromo-cADPR **3**, 7-deaza-2′-deoxy cADPR **4**, and 8-bromo-cADPR **6** remained largely as cyclic structures for 24 h, while 8-amino-2′-deoxy-cADPR **7** was completely degraded in 2 h. The degradation product of compound **7** was analyzed by HPLC, and a major product was eluted at 2.56 min. It is assumed that compound **7** was degraded at the N9-glycosyl linkage, as we know that 2′-deoxy-cADPR analogues are more acid labile at the N9-ribosyl linkage than their ribose counterparts.[51] The fact that 7-deaza-2′-deoxy-cADPR **4** exhibits much better acid resistance demonstrates that N7 has a crucial influence on stability for the N9-glycosyl linkage, with hydrolysis presumably proceeding by N7 being first protonated in the normal series. Indeed, the N9-glycosyl bond of 7-deaza-cADPR analogues was shown to be perfectly stable in our later studies.

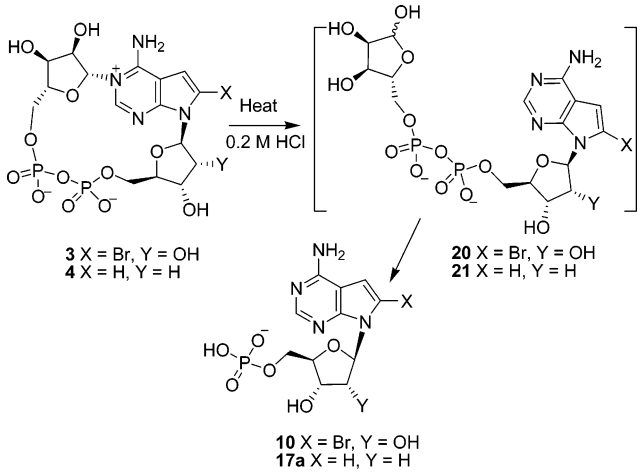


FIGURE 5. Proposed mechanism and products of acid-induced hydrolysis.

TABLE 3. Stability of Cyclic Compounds 3 and 6 after Incubation at 70 °C (pH 7.4)

compound	cyclic form retained after 1 h (%)	cyclic form retained after 4 h (%)
7-deaza-8-Br-cADPR **3**	92	70
8-Br-cADPR **6**	30	0

TABLE 4. Stability of Cyclic Compounds 4 and 7 after Incubation at 70 °C (pH 7.4)

compound	cyclic form retained after 1 h (%)	cyclic form retained after 3 h (%)
7-deaza-2′-deoxy-cADPR **4**	97	88
8-NH_2-2′-deoxy-cADPR **7**	78	49

The hydrolysis of these cADPR analogues was also studied at elevated temperatures. The cADPR analogues (**3**, **4**, **6**, and **7**) were incubated at pH 7.4 and 70 °C, and the amount of cyclic structure retained for each compound was monitored by HPLC (for conditions, see the Supporting Information) and is shown in Tables 3 and 4. From Tables 3 and 4, 7-deaza-cADPR analogues (both **3** and **4**) were found to be more resistant to heat-induced hydrolysis at neutral pH (pH 7.4) compared to those adenine-based cADPR analogues **6** and **7**.

The degradation product of 8-bromo-cADPR **6** was isolated and was identified to be 8-bromo-N9-ADPR by ^{1}H NMR and gHMBC (result not shown). Overnight incubation of 7-deaza compound **3** at 70 °C (pH 7.4) resulted in a complete degradation of this compound, producing mainly a new product that had an HPLC retention time of ca. 15 min. We assume the

(48) Graham, S. M.; Macaya, D. J.; Sengupta, R. N.; Turner, K. B. *Org. Lett.* **2004**, *6*, 233−236.

(49) Wada, T.; Inageda, K.; Aritomo, K.; Tokita, K.; Nishina, H.; Takahashi, K.; Katada, T.; Sekine, M. *Nucleosides Nucleotides* **1995**, *14*, 1301−1314.

(50) Zhang, B.; Wagner, G.; Weber, K.; Garnham, C.; Morgan, A. J.; Galione, A.; Guse, A. H.; Potter, B. V. L. *J. Med. Chem.* **2008**, in press.

(51) Garrett, E. R.; Mehta, P. J. *J. Am. Chem. Soc.* **1972**, *94*, 8532−8540.

new product to be 7-deaza-8-bromo-N9-ADPR **20** (Figure 5) formed by breaking the N1-glycosyl linkage as we have seen for 8-bromo-cADPR. Enzymic CD38 ectoenzyme-mediated hydrolysis of **3** also gave **20** as the sole product, as indicated by HPLC (data not shown). It has been reported by Moreau et al.[52] that, at acidic pH and elevated temperature, the hydrolysis-resistant 8-substituted cIDPR was degraded at both the N9-glycosyl bond and pyrophosphate linkages, producing a novel nucleotide with ribose connected at the N1 position. In light of this, we thus studied the hydrolysis behavior of these 7-deaza-cADPR analogues in both acidic and basic conditions at 70 °C. It was found that these 7-deaza-cADPR analogues degraded much more quickly at both acidic and basic conditions than that at neutral pH. It is assumed that under acidic conditions, cADPR analogues may adopt mainly the "amino form" (Figure 4), thus forming a better leaving group at the N1 position and then facilitating a quick degradation of the molecules. However, this could not explain why the analogues also degraded quickly under basic conditions as they are supposed to adopt the "imino form" (Figure 4). It was proposed that, under basic conditions, an ionized diol oxyanion on the nicotinamide-bearing ribose of NAD^+, by noncovalent stabilization of the oxocarbocation intermediate, may be responsible for the large hydrolysis rate increases observed.[53] Such a mechanism might also stabilize the corresponding oxocarbocation intermediate formed by basic cleavage of the N1-linked ribose linkage of cADPR or an analogue, thus accelerating the hydrolysis process. The final detectable product of both acid- and base-mediated degradation (see the following paragraph) suggested that the pyrophosphate bond was also cleaved under either acidic or basic conditions. It is not surprising that the anhydride pyrophosphate linkage can be hydrolyzed in both acidic and basic conditions and under basic conditions the hydrolysis possibly occurs because of the intramolecular attack of the ionized 3″-OH at the pyrophosphate bond. The pyrophosphate backbone of ADPR was found to be more labile than, for example, reduced diphosphopyridine nucleotide, under basic conditions.[54] The reason for the different reactivity between pyrophosphate bonds towards base-induced hydrolysis is so far not clear.

After being heated at pH 0.7 for 4 h, 7-deaza-8-bromo-cADPR **3** was more than 90% degraded, producing two major products with HPLC elution times of ca. 8.5 and 15 min, respectively. The newly formed product at ca. 15 min is consistent with 7-deaza-8-bromo-N9-ADPR **20**, which is formed via heat-induced hydrolysis (pH 7.4). The product at ca. 8.5 min, however, is most likely to be derived from compound **20** since, after incubating the mixture for a longer period, the peak at ca. 15 min was almost completely consumed and the peak at 8.5 min was found to be significantly increased as the only product. This product is likely to be a monophosphate, as indicated by its elution time under our HPLC conditions (normally 6−10 min for a monophosphate). The ^{31}P NMR spectrum of the hydrolysis mixture showed that the pyrophosphate bond was completely cleaved (the peak at ca. −10 ppm disappeared) and a new peak at 0.02 ppm appeared, indicating the formation of a monophosphate (Supporting Information). A ^1H NMR spectrum of the crude hydrolysis mixture revealed

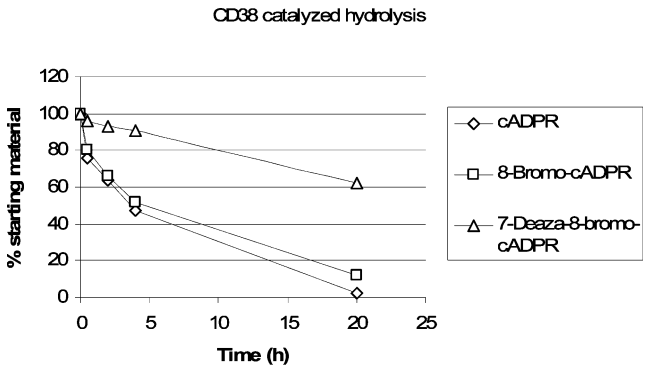


FIGURE 6. CD38-mediated hydrolysis of cADPR analogues. 7-Deaza-8-bromo-cADPR **3** was found to be more stable than 8-bromo-cADPR **6** and cADPR **1**.

a rather downfield H-2′ signal at ca. 5.1 ppm (data not shown). This indicated that the N9-ribosyl linkage was not broken and the downfield shift of H-2′ was caused because the molecule was in its syn comformation due to the nonbonded repulsion by 8-bromo substitutent.[33,34,49] On the basis of this evidence, we thus confirmed that the hydrolysis product found at 8.5 min is indeed 7-deaza-8-bromo-N9-AMP **10**. A mass spectrometry study further confirmed the result, and the expected molecular ions for **10** at ca. 425 and 427 mass units were observed (Supporting Information). Further evidence for the final product being the N9-monophosphate came from the acid-induced hydrolysis of 7-deaza-2′-deoxy-cADPR **4**. After being incubated at 70 °C and pH 0.7 for 1 h, **4** was completely decomposed, resulting in an HPLC profile similar to that earlier described for the acid hydrolysis of **3**. The final product at 6.40 min was identified to be 7-deaza-2′-deoxy-N9-AMP by coeluting with the authentic standard compound **17a** (HPLC trace in Supporting Information). With the above studies, we thus propose that, under acidic conditions, 7-deaza-cADPR derivatives decompose first at the N1-glycosyl bond producing the 7-deaza-N9-ADPR analogues, which in turn are further degraded by pyrophosphate bond cleavage, forming 7-deaza-N9-nucleotides as the final compounds. The proposed order for the acid-induced hydrolysis of 7-deaza-cADPR analogues is shown in Figure 5. Under basic conditions at 70 °C, analogues **3** and **4** also quickly degraded to the same N9-nucleotides as observed earlier. However, no clear order of bond cleavage could be established from the HPLC study in this case. The N9-glycosyl bond was found to be perfectly stable under both acidic and basic conditions.

In addition to chemical stability toward heat-induced hydrolysis, cADPR hydrolase-mediated hydrolysis of 7-deaza-cADPR analogue **3** was also carefully investigated. Ectoenzyme CD38 is a very effective hydrolase.[55] In Figure 6, CD38-catalyzed hydrolysis of 7-deaza-8-bromo-cADPR **3** is directly compared with that of 8-bromo-cADPR **6** and cADPR **1**, showing that **3** retains largely the cyclic form (over 60%) after incubation with CD38 for 20 h in pH 7.5 Tris-HCl buffer, while **1** and **6** are almost totally hydrolyzed. Together with the above chemical stability studies, we can conclude that 7-deaza-cADPR derivatives are indeed more resistant to both chemical and enzymatic hydrolysis and these are attractive features for biological studies. The N9-glycosyl bonds of 7-deaza-cADPR analogues were found to be stable throughout the study.

(52) Moreau, C.; Woodman, T. J.; Potter, B. V. L. *Chem. Commum.* **2006**, 1127−1129.
(53) Johnson, R. W.; Marschner, T. M.; Oppenheimer, N. J. *J. Am. Chem. Soc.* **1988**, *110*, 2257−2263.
(54) Kaplan, N. O.; Colowick, S. P.; Barnes, C. C. *J. Biol. Chem.* **1951**, *191*, 461−472.
(55) Graeff, R.; Munshi, C.; Aarhus, R.; John, M.; Lee, H. C. *J. Biol. Chem.* **2001**, *276*, 12169−12173.

Conclusion

In summary, we present a high-yielding synthesis of the biologically important compound 7-deaza-8-bromo-cADPR **3**, a hydrolysis-resistant, membrane-permeable antagonist of cADPR-induced Ca^{2+} release from intracellular stores. The synthetic route was also extended to facilitate the synthesis of 7-deaza-2′-deoxy-cADPR **4**, a candidate hydrolysis-resistant agonist/partial agonist that may be membrane permeant. The hydrolysis resistance of 7-deaza-cADPR analogues was carefully investigated chemically, and the hydrolysis product is confirmed as a 7-deaza-N9-nucleotide, characteristic of the stable N9-glycosyl linkage of these 7-deaza compounds. 7-Deaza-8-bromo-cADPR **3** was also found to be more stable to enzymatic CD38-mediated hydrolysis compared with cADPR and 8-bromo-cADPR. 7-Deaza-8-bromo-cADPR thus possesses generally attractive properties that should continue to make it an attractive tool with which to explore cADPR-mediated signaling processes.

Experimental Section

CD38-Mediated Hydrolysis of cADPR Analogues. 7-Deaza-8-bromo-cADPR **3**, cADPR **1**, and 8-bromo-cADPR **6** (100 μM) were incubated with CD38[55] (2 μg/mL) in 40 mM Tris-HCl buffer (pH 7.5, total volume 1 mL). The hydrolysis progress was monitored by HPLC at 0, 0.5, 2, 4, and 20 h (for HPLC system, see the Supporting Information).

7-Deaza-8-bromoadenosine (9). To a stirred solution of dry 7-deazaadenosine (tubercidin **8**, 125 mg, 0.47 mmol) and potassium acetate (100 mg, 1.02 mmol) in anhydrous DMF (1.6 mL) was added dropwise a solution of freshly recrystallized NBS (180 mg, 1.02 mmol) in anhydrous DMF (1.2 mL). The reaction mixture immediately turned dark red and was stirred at rt for a further 15 min. The DMF was removed by evaporation in vacuo, and the residue was purified by column chromatography, eluting with a gradient of 0−10% methanol/DCM to give the title compound as a yellow oil (71 mg, 44%). NMR assignment is based on gCOSY, HMQC, and HMBC. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.03 (s, 1H, H-2), 6.74 (s, 1H, H-7), 6.08 (d, $J_{1',2'}$ 7.4 Hz, 1H, H-1′), 5.12 (dd, $J_{2',1'}$ 7.4 Hz, $J_{2',3'}$ 5.3 Hz, 1H, H-2′), 4.35 (dd, $J_{3',2'}$ 5.3 Hz, $J_{3',4'}$ 1.7 Hz, 1H, H-3′), 4.16 (m, 1H, H-4′), 3.87 (dd, $J_{5'a,4'}$ 2.4 Hz, $J_{5'a,5'b}$ 12.6 Hz, 1H, H-5′a), 3.73 (dd, $J_{5'b,4'}$ 2.4 Hz, $J_{5'b,5'a}$ 12.6 Hz, 1H, H-5′b); ^{13}C NMR (CD_3OD, 100.5 MHz) δ 158.1 (C-6), 151.9 (C-2), 150.5 (C-4), 111.5 (C-8), 105.9 (C-5), 103.9 (C-7), 92.2 (C-1′), 88.3 (C-4′), 73.7 (C-2′), 73.1 (C-3′), 64.3 (C-5′); *m*/*z* (ES^+) 345.11, 347.12 $(M + H)^+$; HRMS Calcd for $[M + H]^+$ $C_{11}H_{14}{}^{79}BrN_4O_4^+$ (ES^+), 345.0198; found, 345.0190.

7-Deaza-8-bromoadenosine 5′-Monophosphate (10). A suspension of 7-deaza-8-bromoadenosine **9** (50 mg, 145 μmol) in triethylphosphate (0.8 mL) was heated strongly using a heating gun for 10 min, and the resulting clear solution was cooled to 0 °C in an ice bath. H_2O (4 μL) was then added followed by dropwise addition of $POCl_3$ (70 μL, 750 μmmol). After being stirred for another hour at 0 °C, the reaction was quenched with ice (10 mL) and the excess triethylphosphate was removed by partitioning between aqueous solution and cold ethyl acetate (5 × 20 mL). The aqueous layer was neutralized using 5 M NaOH and then loaded on top of a reverse-phase column, which was eluted with a 0−30% gradient of MeCN against 0.05 M TEAB buffer. Appropriate fractions were combined, and the solvent was evaporated under reduced pressure. Excess TEAB buffer was removed by coevaporating with methanol (3×), and the resulting glassy residue was further purified by ion exchange chromatography using 0−50% gradient of 1 M TEAB/MilliQ water as the eluent. After the appropriate fractions were combined and evaporated, the resulting white solid was coevaporated with methanol (3×) to produce the desired compound **10** as its triethylammonium salt (112 μmol, 77%). HPLC: 9.2 min at 254 nm; ^{1}H NMR (D_2O, 270 MHz) δ 7.87 (s, 1H, H-2), 6.38 (s, 1H, H-7), 5.99 (d, $J_{1'2'}$ 5.4 Hz, 1H, H-1′), 5.03 (m, 1H, H-2′), 4.53 (m, 1H, H-3′), 4.06 (m, 3H, H-4′ and H-5′); ^{13}C NMR (D_2O, 68 MHz) δ 153.2 (C-6), 148.9 (C-4), 148.2 (C-2), 109.9 (C-8), 104.4 (C-7), 103.2 (C-5), 89.2 (C-1′), 82.8 (d, J_{CP} 7.5 Hz, C-4′), 71.4 (C-2′), 69.5 (C-3′), 64.4 (C-5′); ^{31}P NMR (D_2O, 109 MHz) δ 2.35 (s); *m*/*z* (FAB^+) 425.0, 427.0 $(M + H)^+$. HRMS: Calcd for $[M + H]^+$ $C_{11}H_{15}{}^{79}BrN_4O_7P^+$ (ES^+), 424.9862; found, 424.9860.

7-Deaza-8-bromoadenosine 5′-Monophosphate Morpholidate (11). A solution of 7-deaza-8-Br-AMP **10** triethylammonium salt (65 mg, 112 μmol) in anhydrous DMSO (0.5 mL) was coevaporated with anhydrous DMF (3 × 1 mL). The resulting residue was redissolved in anhydrous DMSO (0.5 mL) to give a yellow solution, to which was added in sequence triphenylphosphine (100 mg, 381 μmol), morpholine (65 μL, 744 μmol), and dipyridyl disulfide (84 mg, 381 μmol). After being stirred at rt for three more hours, the reaction was quenched by addition of a solution of NaI in acetone (0.1 M, 10 mL) and the precipitated sodium salt was filtered, washed with acetone, and dried under reduced pressure. Crude **11** was further purified by ion-exchange chromatography, eluting with a gradient of 0−50% 1 M TEAB buffer against MilliQ water. Appropriate fractions were collected and evaporated in vacuo. Excess TEAB was removed by evaporating with methanol (3×) to give compound **11** as a glassy solid in the triethylammomium form. Yield (97 μmol, 87%). HPLC: 3.2 min at 254 nm; ^{1}H NMR (D_2O, 270 MHz) δ 7.93 (s, 1H, H-2), 6.48 (s, 1H, H-7), 6.02 (d, $J_{1',2'}$ 4.7 Hz, 1H, H-1′), 5.21 (m, 1H, H-2′), 4.61 (m, 1H, H-3′), 4.00 (m, 3H, H-4′ and H-5′), 3.36 (m, 4H, 2 × CH_2O), 2.76 (m, 4H, 2 × CH_2N); ^{13}C NMR (D_2O, 68 MHz) δ 155.4 (C-6), 150.9 (C-2), 149.9 (C-4), 109.6 (C-8), 104.2 (C-7), 103.9 (C-5), 89.5 (C-1′), 82.3 (d, J_{CP} 8.7 Hz, C-4′), 71.3 (C-2′), 69.4 (C-3′), 66.9, 66.8 (2 × CH_2O), 64.1 (d, J_{CP} 4.6 Hz, C-5′), 44.7 (2 × CH_2N); ^{31}P NMR (D_2O, 109 MHz) δ 8.33 (s); *m*/*z* (ES^-) 492.05, 494.19 $(M - H)^-$; HRMS calcd for $[M - H]^-$ $C_{15}H_{20}{}^{79}BrN_5O_7P^-$ (ES^-), 492.0284; found, 492.0282.

7-Deaza-8-bromo nicotinamide Adenine Dinucleotide (7-Deaza-8-bromo-NAD$^+$, 12). Method 1. 12 was first synthesized by a Todd carbodiimide method[24] (Supporting information).

Method 2. A suspension of 7-deaza-8-Br-AMP morpholidate TEA salt **11** (52 mg, 80 μmol), β-NMN^+ (zwitterionic form, 45 mg, 135 μmol), and $MgSO_4$ (30 mg, 250 μmol) in $MnCl_2$/formamide solution (0.2 M, 0.95 mL) was stirred at rt for 48 h. HPLC analysis indicated that nearly all the morpholidate had reacted. To the resulting clear yellow solution, MeCN (2 mL) was added dropwise with constant stirring and the precipitated yellow solid was filtered off and washed with acetone. The solid was dissolved in a small amount of MilliQ water and treated with Chelex resin (Na^+ form) for 1 h. The resin was filtered off and washed with MilliQ water, and the filtrated was evaporated. The solid residue was again dissolved in a small amount of 0.05 M TEAB buffer (5−10 mL) and was then purified by a reverse-phase system. Combined fractions were evaporated under vacuum, and the excess TEAB was removed by coevaporating with methanol (3×) to give the desired compound in its triethylammonium form (63 μmol, 79%). HPLC: 3.9 min at 254 nm; ^{1}H NMR (D_2O, 400 MHz) δ 9.16 (s, 1H, H_N-2), 9.00 (d, $J_{6,5}$ 6.4 Hz, 1H, H_N-6), 8.63 (d, $J_{4,5}$ 8.0 Hz, 1H, H_N-4,), 8.05 (dd, $J_{5,4}$ 8.0 Hz, $J_{5,6}$ 6.4 Hz, 1H, H_N-5), 7.87 (s, 1H, H-2), 6.36 (s, 1H, H-7), 5.96 (d, $J_{1',2'}$ 5.6 Hz, 1H, H-1′), 5.91 (d, $J_{1'',2''}$ 4.3 Hz, 1H, H-1″), 5.06 (m, 1H, H-2′), 4.48 (m, 1H, H-3′), 4.42 (m, 1H, H-4″), 4.33−4.11 (m, 7H, ribose-H); ^{13}C NMR (D_2O, 100 MHz) δ 164.8 (CO), 155.2 (C-6), 151.0 (C-2), 149.6 (C-4), 145.2 (C_N-4), 142.1 (C_N-6), 139.5 (C_N-2), 133.2 (C_N-3), 128.5 (C_N-5), 108.8 (C-8), 103.8 (C-7), 103.1 (C-5), 99.9 (C-1″), 88.7 (C-1′), 86.7 (d, J_{CP} 9.2 Hz, C-4″), 82.2 (d, J_{CP} 8.4 Hz, C-4′), 77.5 (C-2″), 70.9 (C-2′), 70.3 (C-3″), 69.0 (C-3′), 65.6 (d, J_{CP} 5.4 Hz, C-5″), 64.7 (d, J_{CP} 5.4 Hz, C-5′); ^{31}P NMR (D_2O, 109 MHz) δ −10.57 (d, J_{PP} 21.1 Hz), −11.02 (d, J_{PP} 21.1 Hz); *m*/*z* (ES^-) 739.31, 741.20 $(M - H)^-$; HRMS Calcd for $[M + H]^+$ $C_{22}H_{28}{}^{79}BrN_6O_{14}P_2^+$ (ES^+), 741.0322; found, 741.0325.

Cyclic 7-Deaza-8-bromoadenosine 5′-Diphosphate Ribose (7-Deaza-8-bromo-cADPR, 3). To a solution of 7-deaza-8-bromo-NAD^+ (**12**, 20 mg, 22 μmol) in HEPES buffer (25 mM, pH 7.4, 52 mL) was added *Aplysia californica* ADP-ribosyl cyclase (100 μL). The resulting clear solution was allowed to stir at rt until HPLC analysis indicated that all the starting pyrophosphate **12** had been consumed. The reaction solution was then diluted with MilliQ water until its conductivity reached an appropriate value (<200 μS/cm) and product was purified by ion-exchange chromatography, eluting with a gradient of 1 M TEAB buffer against MilliQ water. Appropriate fractions were collected, evaporated in vacuo, and coevaporated with methanol (3×) to give the final compound as a glassy solid. Yield (16 μmol, 72%). HPLC: 5.2 min at 254 nm; UV (H_2O) λ_{max} 277 nm (ϵ/dm^3 mol^{-1} cm^{-1} 10 850); ^{1}H NMR (D_2O, 400 MHz) δ 8.84 (s, 1H, H-2), 6.97 (s, 1H, H-7), 6.12 (d, $J_{1',2'}$ 5.9 Hz, 1H, H-1′), 6.05 (d, $J_{1'',2''}$ 4.1 Hz, 1H, H-1″), 5.52 (dd, $J_{2',1'}$ 5.9 Hz, $J_{2',3'}$ 5.7 Hz, 1H, H-2′), 4.66 (m, 2H, H-3′ and H-4″), 4.65 (m, 1H, H-2″), 4.42 (m, 2H, H-3″ and H-5′a), 4.34 (m, 1H, H-5″a), 4.29 (m, 1H, H-4′), 4.12 (m, 1H, H-5″b), 4.04 (m, 1H, H-5′b); ^{13}C NMR (D_2O, 100 MHz) δ 149.4 (C-6), 147.0 (C-4), 139.7 (C-2), 114.2 (C-8), 104.6 (C-7), 104.0 (C-5), 95.0 (C-1″), 90.3 (C-1′), 88.1 (d, J_{CP} 10.7 Hz, C-4″), 84.4 (d, J_{CP} 10.7 Hz, C-4′), 76.8 (C-2″), 72.1 (C-2′), 71.8 (C-3″), 70.2 (C-3′), 64.7 (d, J_{CP} 4.8 Hz, C-5″), 64.0 (J_{CP} 4.7 Hz, C-5′); ^{31}P NMR (D_2O, 109 MHz) δ −10.00 (brs), −10.71 (brs); *m*/*z* (ES^-) 617.25, 619.20 $(M - H)^-$; HRMS Calcd for $[M - H]^-$ $C_{16}H_{20}{}^{79}BrN_4O_{13}P_2^-$ (ES^-), 616.9685; found, 616.9699.

4-Chloro-7-(2-deoxy-3,5-di-*O*-(*p*-toluoyl)-*β*-D-erythropento-furanosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (15). To a fast stirred suspension of powdered potassium hydroxide (81 mg, 1.44 mmol) in dry MeCN (4.9 mL) was added TDA-1 (8.1 μL) under an argon atmosphere. This mixture was stirred at rt for 5 min. Compound **13** (100 mg, 0.66 mmol) was added, and the mixture was again stirred for 5 min. α-Halogenose **14** (259 mg, 0.67 mmol) was then added, and the reaction mixture was stirred for another 20 min until TLC (DCM/ethyl acetate, 12:1) suggested that all the starting material was consumed and a fast moving spot (R_f 0.46) was given. The crude product was evaporated with silica gel, loaded on top of a silica column, and eluted with DCM/ethyl acetate, 20:1. The appropriate fractions were collected and concentrated to dryness under reduced pressure to produce the title compound as a white foam (262 mg, 79%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ 8.62 (s, 1H, H-2), 7.97 (d, $J_{ArH,ArH}$ 8.2 Hz, 2H, 2 × ArCH), 7.91 (d, $J_{ArH,ArH}$ 8.4 Hz, 2H, 2 × ArCH), 7.41 (d, $J_{8,7}$ 3.7 Hz, 1H, H-8), 7.28 (d, $J_{ArH,ArH}$ 8.2 Hz, 2H, 2 × ArCH), 7.25 (d, $J_{ArH,ArH}$ 8.4 Hz, 2H, 2 × ArCH), 6.80 (dd, $J_{1',2b'}$ 8.4 Hz, $J_{1',2a'}$ 5.9 Hz, 1H, H-1′), 6.59 (d, $J_{7,8}$ 3.7 Hz, 1H, H-7), 5.76 (m, 1H, H-3′), 4.64 (m, 3H, H-4′ and H-5′), 2.82 (m, 2H, H-2′), 2.43 (s, 3H, CH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 166.2 (2CO), 151.1 (C-6), 151.1 (C-2), 151.1 (C-4), 144.9 (ArC), 144.4 (ArC), 130.0, 129.7, 129.4 (ArCH), 126.78, 126.5 (ArC), 126.1 (C-8), 118.4, (C-5), 101.0 (C-7), 84.7 (C-1′), 82.6 (C-4′), 75.2 (C-3′), 63.9 (C-5′), 38.1 (C-2′), 21.9 (2CH_3); *m*/*z* (ES^+) 506.45, 508.47 $(M + H)^+$; HRMS Calcd for $[M + H]^+$ $C_{27}H_{25}35ClN_3O_5^+$ (ES^+), 506.1483; found, 506.1474.

7-Deaza-2′-deoxyadenosine (16). Ammonia gas was bubbled into a suspension of compound **15** (260 mg, 0.52 mmol) in methanol (20 mL) at 0 °C for 40 min. The resulting clear saturated methanolic ammonia solution was warmed to rt and then heated at 60 °C in a pressure tube for 4 days. TLC of the reaction mixture showed that a more polar product was formed (R_f 0.23, DCM/methanol, 5:1). After removal of the solvent, the crude product was purified by column chromatography, eluting with DCM/methanol, 6:1, to give the title compound as a colorless oil (70 mg, 53%). ^{1}H NMR ($DMSO_6$, 270 MHz) δ 8.04 (s, 1H, H-2), 7.35 (d, $J_{8,7}$ 3.7 Hz, 1H, H-8), 7.05 (s, 2H, NH_2), 6.57 (d, $J_{7,8}$ 3.7 Hz, 1H, H-7), 6.48 (dd, $J_{1',2b'}$ 6.2 Hz, $J_{1',2a'}$ 5.9 Hz, 1H, H-1′), 5.24 (m, 2H, 3′-OH, 5′-OH), 4.34 (m, 1H, H-3′), 3.81 (m, 1H, H-4′), 3.52 (m, 2H, H-5′), 2.49 (m, 1H, H-2a′), 2.16 (m, 1H, H-2b′); ^{1}H NMR ($DMSO_6$, D_2O shake, 270 MHz) δ 8.02 (s, 1H, H-2), 7.32 (d, $J_{8,7}$ 3.7 Hz, 1H, H-8), 6.57 (d, $J_{7,8}$ 3.7, 1H, H-7), 6.42 (dd, $J_{1',2b'}$ 6.2 Hz, $J_{1',2a'}$ 5.9 Hz, 1H, H-1′), 4.33 (m, 1H, H-3′), 3.83 (m, 1H, H-4′), 3.53 (m, 2H, H-5′), 2.48 (m, 1H, H-2′a), 2.18 (m, 1H, H-2′b); ^{13}C NMR ($DMSO_6$, 68 MHz) δ 158.1 (C-6), 152.2 (C-2), 150.2 (C-4), 122.2 (C-8), 103.5 (C-5), 100.2 (C-7), 87.3 (C-1′), 83.7 (C-4′), 71.6 (C-3′), 62.6 (C-5′), C-2′ is under DMSO peak.; *m*/*z* (ES^+) 251.4 $(M + H)^+$; HRMS Calcd for $[M + H]^+$ $C_{11}H_{15}N_4O_3^+$ (ES^+), 251.1144; found, 251.1144.

7-Deaza-2′-deoxyadenosine 5′-Monophosphate (17a). A suspension of 7-deaza-2′-deoxyadenosine (**16**, 60 mg, 240 μmol, dried in vacuo at 60 °C overnight) in triethylphosphate (0.7 mL) was heated with a heating gun until formation of clear solution, which was then cooled in an ice-salt bath and followed by a dropwise addition of $POCl_3$ (70 μL, 750 μmol). After being stirred in the ice-salt bath for 2 h, the reaction was quenched by the addition of ice (10 mL) and was immediately neutralized to pH 7 with a NaOH solution (5 M). The reaction solution was extracted with cold ethyl acetate (5 × 20 mL), and the aqueous layer was further purified on a reverse column, eluting with 0−30% gradient MeCN against 0.05 M TEAB. Appropriate fractions were evaporated, and the residual white solid was coevaporated with MeOH (3×) to give the crude title compound as a glassy solid (96 mg, 100%). Crude compound contained **17a** (135 μmol, 56%) and **17b** (57 μmol, 23.8%). Yield was calculated based on 1D ^{1}H NMR integration. HPLC: 6.8 min at 254 nm; ^{1}H NMR (D_2O, 270 MHz) δ 7.86 (s, 1H), 7.32 (d, $J_{8,7}$ 3.7 Hz, 1H), 6.43 (d, $J_{7,8}$ 3.7 Hz 1H), 6.39 (m, 1H), 4.53 (m, 1H), 4.01 (m, 1H), 3.74 (m, 2H), 2.57 (m, 1H), 2.31 (m, 1H); ^{31}P NMR (D_2O, 109 MHz) 4.48 (s); **17b** ^{1}H NMR (D_2O, 270 MHz) δ 7.91 (s, 1H), 7.47 (d, $J_{8,7}$ 3.7 Hz, 1H), 6.44 (d, $J_{7,8}$ 3.7 Hz, 1H), 6.43 (m, 1H), 4.69 (m, 1H), other ribose-H overlaps with **17a**; ^{31}P NMR δ (D_2O, 109 MHz) 4.48 (s), 3.95 (s).

7-Deaza-2′-deoxyadenosine 5′-Monophosphate Morpholidate (18a). A solution of 7-deaza-2′-deoxy AMP triethylammonium salt **17a**/**17b** (where **17a** 70 mg, 98 μmol) in anhydrous DMSO (0.4 mL) was coevaporated with anhydrous DMF (3 × 1 mL). The yellow residue was dissolved in anhydrous DMSO (0.4 mL), and to the resulting solution was added dipyridyl disulfide (146 mg, 668 μmol), morpholine (150 μL, 1.72 mmol), and powdered triphenylphosphine (176 mg, 668 μmol) in sequence. The reaction progress was monitored by HPLC, and after 3 h at rt, the starting material had been completely consumed and two new peaks were produced (2.6 min for **18a** and 7.3 min for 3′,5′-bisphosphate morpholidate **18b**). The reaction was then quenched by dropwise addition of a solution of NaI in acetone (0.1 M, 10 mL) under vigorous stirring, and the sodium salt precipitate was filtered, washed with acetone, and dried in vacuo. After further purification by ion-exchange chromatography, eluting with a gradient of 0−50% 1 M TEAB buffer against MQ water, the title compound **18a** was obtained as a white solid (81 μmol, 83%). A small amount of 7-deaza-2′-deoxyadenosine 3′,5′- bisphosphate morpholidate **18b** (10 mg, ca. 13% based on **16**) was also isolated. HPLC: 2.6 min at 254 nm; UV (H_2O) λ_{max} 272.3 nm; ^{1}H NMR (D_2O, 270 MHz) δ 7.96 (s, 1H, H-2), 7.31 (d, $J_{8,7}$ 3.7 Hz, 1H, H-8), 6.47 (d, $J_{7,8}$ 3.7 Hz, 1H, H-7), 6.45 (dd, 1H, overlap with H-7, *J* not given, H-1′), 4.63 (m, 1H, H-3′), 4.09 (m, 1H, H-4′), 3.84 (m, 2H, H-5′), 3.43 (m, 4H, 2 × CH_2O), 2.83 (m, 4H, 2 × CH_2N), 2.67 (m, 1H, H-2′a), 2.45 (m, 1H, H-2′b); ^{13}C NMR (D_2O, 68 MHz) δ 156.6 (C-6), 150.4 (C-2), 148.9 (C-4), 122.1 (C-8), 103.2 (C-5), 100.6 (C-7), 85.1 (d, J_{CP} 9.3 Hz, C-4′), 82.9 (C-1′), 71.3 (C-3′), 66.9, 66.8 (2 × CH_2O), 64.5 (d, J_{CP} 5.6 Hz, C-5′), 44.6 (2 × CH_2N), 38.5 (C-2′); ^{31}P NMR (D_2O, 109 MHz) δ 8.22 (s); *m*/*z* (ES^-) 398.42 $(M - H)^-$; HRMS Calcd for $[M - H]^-$ $C_{15}H_{21}N_5O_6P^-$ (ES^-), 398.1229; found, 398.1226.

7-Deaza-2′-deoxyadenosine 3′,5′-Bisphosphate Morpholidate (18b): HPLC: 7.3 min at 254 nm; UV (H_2O) λ_{max} 271.1 nm; ^{1}H NMR (D_2O, 270 MHz) δ 8.09 (s, 1H, H-2), 7.47 (d, $J_{8,7}$ 3.7 Hz, 1H, H-8), 6.63 (d, $J_{7,8}$ 3.7 Hz, 1H, H-7), 6.59 (dd, 1H, overlap with H-7, *J* not given, H-1′), 4.85 (m, 1H, H-3′), 4.28 (m, 1H, H-4′), 3.89 (m, 2H, H-5′), 3.66 (m, 4H, 5′ morpholidate 2 × CH_2O),

3.45 (m, 4H, 3′ morpholidate 2 × CH_2O), 3.08 (m, 4H, 5′ morpholidate 2 × CH_2N), 2.85 (m, 4H, 3′ morpholidate 2 × CH_2N), 2.71 (m, 1H, H-2′a), 2.59 (m, 1H, H-2′b); ^{13}C NMR (D_2O, 68 MHz) δ 155.4 (C-6), 149.0 (C-4), 148.5 (C-2), 123.0 (C-8), 103.0 (C-5), 101.3 (C-7), 83.4 (dd, J_{CP} 11.5 Hz, J_{CP} 5.6 Hz, C-4′), 83.4 (C-1′), 75.1 (d, J_{CP} 5.6 Hz, C-3′), 67.0 (4 × CH_2O), 64.7 (C-5′), 44.8 (4 × CH_2N), 38.3 (C-2′); ^{31}P NMR (D_2O, 109 MHz) δ 8.05 (s), 7.77 (s); *m*/*z* (ES^-) 547.44 [$(M - H)^-$, 40%]; HRMS Calcd for $(M + H)^+$ $C_{19}H_{31}N_6O_9P_2^+$ (ES^+), 549.1628; found, 549.1630.

7-Deaza-2′-deoxynicotinamide Adenine Dinucleotide (19). A suspension of 7-deaza-2′-deoxy-AMP morpholidate TEA salt (**18a**, 54 μmol), β-NMN^+ (zwitterionic form, 31 mg, 93 μmol), and $MgSO_4$ (20 mg, 167 μmol) in $MnCl_2$/formamide (0.2 M, 0.68 mL) was stirred at rt for 48 h in an argon atmosphere to give a near clear yellow solution. HPLC analysis indicated that all the starting **18a** was reacted. The reaction was quenched by addition of MeCN (2 mL), and the resulting yellow solid was filtered, washed with acetone, and dissolved in a small amount of MilliQ water. The yellow solution was treated with Chelex (sodium form) ion-exchange resin for 1 h to remove any residual Mn^{2+}. The resin was filtered off and washed with MilliQ water (10 mL), and the washings evaporated in vacuo. The residue, containing crude 7-deaza-2′-deoxy-NAD^+ sodium salt, was dissolved in 0.05 M TEAB buffer (5 mL) and was purified using a reverse-phase system, eluting with a gradient of 0−30% MeCN against 0.05 M TEAB buffer. Fractions containing the desired compound were collected and concentrated under reduced pressure. The white residue was coevaporated with MeOH (3×) to give compound **19** as its triethylammonium salt (32 μmol, 60%). HPLC: 3.3 min at 254 nm; ^{1}H NMR (D_2O, 400 MHz) δ 9.25 (s, 1H, H_N-2), 9.05 (brs, 1H, H_N-6), 8.67 (d, $J_{4,5}$ 7.4 Hz, 1H, H_N-4), 8.00 (m, 1H, H_N-5), 7.90 (s, 1H, H-2), 7.36 (brs, 1H, H-8), 6.43 (m, 2H, H-1′ and H-7), 6.06 (d, $J_{1',2'}$ 4.7 Hz, 1H, H-1″), 4.64 (m, 1H, H-3′), 4.52 (m, 1H, H-4″), 4.11 (m, 7H, ribose-H), 2.60 (m, 1H, H-2′a), 2.38 (m, 1H, H-2′b); ^{13}C NMR (D_2O, 68 MHz) δ 154.9 (C-6), 148.6 (C-2), 148.1 (C-4), 145.4 (C_N-4), 142.2 (C_N-6), 140.1 (C_N-2), 132.0 (C_N-3), 128.5 (C-8), 122.8 (C_N-5), 102.5 (C-5), 101.0 (C-7), 100.1 (C-1″), 87.0 (C-4″), 85.2 (C-4′), 83.0 (C-1′), 77.7 (C-2″), 71.6 (C-3″), 70.6 (C-3′), 66.1 (C-5″), 65.0 (C-5′), 38.8 (C-2′); ^{31}P NMR (D_2O, 109 MHz) δ −11.44 (brs); *m*/*z* (ES^-) 645.07; HMRS Calcd for $(M - H)^-$ $C_{22}H_{27}N_6O_{13}P_2^-$ (ES^-), 645.1111; found, 645.1111.

Cyclic 7-Deaza-2′-deoxyadenosine 5′-Diphosphate Ribose (4). To a solution of 7-deaza-2′-deoxy-NAD^+ (**19**, 5 mg, 6 μmol) in HEPES buffer (25 mM, pH 7.4, 16 mL) was added *Aplysia* cyclase (23 μL). The reaction mixture was stirred at rt, and progress was monitored by HPLC. After 1 h, the reaction solution was diluted with MilliQ water (conductivity < 200 μS/cm) and was purified using an ion-exchange system. Evaporation with MeOH gave the final compound as a glassy solid (4 μmol, 67%). HPLC: 4.3 min at 254 nm; UV (H_2O, pH 4.9) λ_{max} 275.8 nm (ϵ/dm^3 mol^{-1} cm^{-1} 10 900); ^{1}H NMR (D_2O, 400 MHz) δ 8.69 (s, 1H, H-2), 7.22 (d, $J_{8,7}$ 3.5 Hz, 1H, H-8), 6.63 (dd, $J_{7,8}$ 3.5 Hz, 1H, H-7), 6.15 (t, $J_{1',2'a}$ = $J_{1',2'b}$ 7.0 Hz, 1H, H-1′), 5.91 (d, $J_{1'',2''}$ 3.9 Hz, 1H, H-1″), 4.71 (m, 1H, H-3′), 4.56 (m, 1H, H-4″), 4.51 (m, 1H, H-2″), 4.40 (m, 1H, H-5′a), 4.30 (m, 1H, H-3″), 4.29 (m, 1H, H-5″a), 4.22 (m, 1H, H-4′), 4.12 (m, 1H, H-5″b), 4.00 (m, 1H, H-5′b), 3.23 (m, 1H, H-2′a), 2.36 (m, 1H, H-2′b); ^{13}C NMR (D_2O, 68 MHz) δ 150.5 (C-6), 146.0 (C-4), 139.2 (C-2), 129.8 (C-8), 104.3 (C-5), 100.7 (C-7), 94.8 (C-1″), 89.5 (C-1′), 87.8 (d, J_{CP} 10.1 Hz, C-4″), 86.4 (d, J_{CP} 10.8 Hz, C-4′), 76.8 (C-2″), 71.6 (C-3′, C-3″), 65.4 (C-5″), 64.6 (C-5′), 37.6 (C-2′); ^{31}P NMR (D_2O, 109 MHz) δ −10.05 (brs), −10.60 (brs); HRMS Calcd for $[M - H]^-$ $C_{16}H_{21}N_4O_{12}P_2^-$ (ES^-), 523.0631; found, 523.0632.

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Supporting Information Available: Todd carbodiimide synthesis of compound **12**; ^{1}H NMR spectra for compounds **3**, **4**, **9**−**12**, and **17**−**19**; ^{31}P NMR and mass spectra for the hydrolysis reaction; HPLC traces for compounds **3**, **4**, **12**, and **19**; and some HPLC evidence for the heat (chemical)-induced degradation of **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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