Total Synthesis of Cyclic ADP-carbocyclic-ribose, a Stable Mimic of Ca^{2+}-Mobilizing Second Messenger Cyclic ADP-Ribose[1]

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Abstract: The synthesis of cyclic ADP-carbocyclic-ribose (cADPcR, **4**) designed as a stable mimic of cyclic ADP-ribose (cADPR, **1**), a Ca^{2+}-mobilizing second messenger, was achieved using as the key step a condensation reaction with the phenylthiophosphate-type substrate **14** to form an intramolecular pyrophosphate linkage. The *N*-1-carbocyclic-ribosyladenosine derivative **16** was prepared via the condensation between the imidazole nucleoside derivative **17**, prepared from AICA-riboside (**19**), and the readily available optically active carbocyclic amine **18**. Compound **16** was then converted to the corresponding 5″-phosphoryl-5′-phenylthiophosphate derivatives **14**. Treatment of **14** with $AgNO_3$ in the presence of molecular sieves (3 Å) in pyridine at room temperature gave the desired cyclization product **32** in 93% yield, and subsequent acidic treatment provided the target cADPcR (**4**). This represents a general method for synthesizing biologically important cyclic nucleotides of this type. ^{1}H NMR analysis of cADPcR suggested that its conformation in aqueous medium is similar to that of cADPR. cADPcR, unlike cADPR, was stable under neutral and acidic conditions, where under basic conditions, it formed the Dimroth-rearranged N^6-cyclized product **34**. cADPcR was also stable in rat brain membrane homogenate which has cADPR degradation activity. Furthermore, cADPcR was resistant to the hydrolysis by CD38 cADPR hydrolase, while cADPR was rapidly hydrolyzed under the same conditions. When cADPcR was injected into sea urchin eggs, it caused a significant release of Ca^{2+} in the cells, an effect considerably stronger than that of cADPR. Thus, cADPcR was identified as a stable mimic of cADPR.

Introduction

Cyclic ADP-ribose (cADPR, **1**), a naturally occurring metabolite of NAD^+,[2] has been shown to mobilize intracellular Ca^{2+} in various cells, such as sea urchin eggs, pancreatic beta cells, smooth muscle cells, cardiac myocytes, T-lymphocytes, and cerebellar neurons, indicating that it is a general mediator involved in Ca^{2+} signaling.[3] The structure of cADPR had been investigated[4] and was recently confirmed by X-ray crystallographic analysis as shown in Figure 1.[4c]

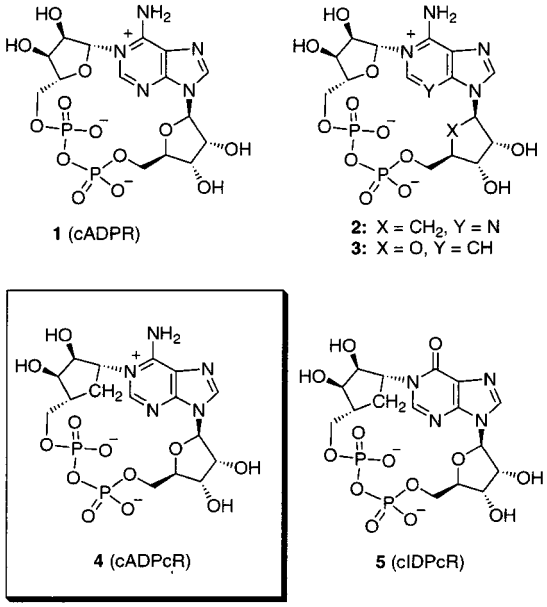

Figure 1. The structures of cADPR (**1**), cADPcR (**4**), and related compounds.

In cells, cADPR is synthesized from NAD^+ by ADP-ribosylcyclase and acts as a second messenger; it is hydrolyzed rapidly by cADPR hydrolase to give inactive ADP-ribose under physiological conditions.[3] cADPR is also known to be readily hydrolyzed nonenzymatically at the unstable *N*-1-glycosidic

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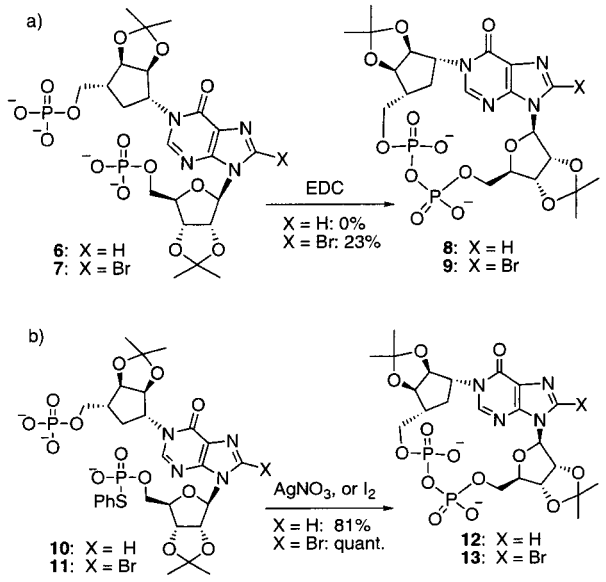
linkage of its adenine moiety to give ADP-ribose, even in neutral aqueous solution.[5] Although intensive studies of cADPR are still needed because of its biological importance, the biological as well as chemical instability of cADPR limits, to some extent, further studies of its physiological role. Therefore, stable analogues of cADPR exhibiting a Ca^{2+}-mobilizing activity in cells similar to that of cADPR are urgently required.

The synthesis of cADPR analogues and their biological effects have been studied extensively.[6,7] Potter and co-workers synthesized cyclic aristeromycin-diphosphate-ribose (**2**) and showed that it acted as a poorly hydrolyzable Ca^{2+}-mobilizing mimic of cADPR.[6j] However, **2** is hydrolyzed by enzymes, although the rate is considerably slower than that of cADPR.[6j] More recently, cyclic 3-deazaadenosine-diphosphate-ribose (**3**) was synthesized by Walseth and co-workers as another analogue resistant to hydrolysis.[6p]

We designed cyclic ADP-carbocyclic-ribose (cADPcR, **4**) and its inosine congener **5** (cIDP-carbocyclic-ribose, cIDPcR) as stable mimics of cADPR,[7] in which the oxygen atom in the *N*-1-ribose ring of cADPR is replaced by a methylene group. The mimics **4** and **5** should be resistant to both enzymatic and chemical hydrolysis, since they have a chemically and biologically stable *N*-alkyl linkage instead of the unstable *N*-1-glycosidic linkage of cADPR. These analogues preserve the functional groups of cADPR except for this ring oxygen and should have a conformation similar to that of cADPR. Therefore, we expect that these analogues would effectively mobilize intracellular Ca^{2+}, as cADPR; therefore, they could be used as pharmacological tools for further study of the mechanism of cADPR-modulated Ca^{2+}-signaling pathways.

cADPR and its analogues, including **2** and **3**, have been synthesized by enzymatic or chemo-enzymatic methods.[6] ADP-ribosyl cyclase from *Aplysia californica* mediates the intramolecular ribosylation of NAD^+ and some modified NAD^+ (prepared chemically or enzymatically) at the *N*-1-position of the purine moiety, to yield cADPR or the corresponding analogues.[6] Although the specificity of ADP-ribosyl cyclase is somewhat loose, the analogues obtained by this method are limited by the substrate specificity of the enzyme.[6o] Furthermore, even though ADP-ribosylcyclase catalyzes the cyclization of the NAD^+ analogues, in some cases the newly formed glycosidic bond is attached to the *N*-7 nitrogen of the purine ring: e.g., the product of the enzymatic reaction of an inosine or guanosine analogue of NAD^+ is not the desired *N*-1-cyclized product, but rather the *N*-7-cyclized product.[6c,g]

Scheme 1. Previous Synthesis of cIDPcR (**8**) and Its 8-Bromo Congener (**9**)



In the chemical synthesis of cADPR and its analogues, the intramolecular condensation to form the pyrophosphate linkage should be the key step; however forming such an intramolecular pyrophosphate linkage has proved difficult for several groups including ours,[6o,7,9] thereby preventing completion of the synthesis of target cADPR analogues. One of the first attempts to prepare cADPR or its analogues by chemical intramolecular condensation was reported by Gu and Sih.[8] They tried to perform a condensation between the two phosphate groups of *N*-1-phosphoribosyl-AMP with EDC, but the yield of cADPR was less than 1%.[8] Later, Fortt and Potter attempted the synthesis of an analogue of cIDPcR. However, the forming of the intramolecular pyrophosphate linkage proved unsuccessful.[9a]

We investigated the intramolecular condensation of the *N*-1-carbocyclic-ribosylinosine bisphosphate derivative **6** which produced none of the desired cyclized product **8**. However, we found that cyclization of the bisphosphate **7** proceeded to give the desired **9** when a bromo group was introduced at the 8-position probably by restricting the conformation of the substrate to a product-like *syn*-form (Scheme 1a).[7a,10] The protecting groups were then removed to complete the synthesis of cIDPcR (**5**).[7a] This was the first total synthesis of a cADPR-related compound, but the over-all yield was low.[7a] More recently, we have developed an efficient method for forming the intramolecular pyrophosphate linkage by activation of phenylthiophosphate type substrates, such as **10** or **11**, with I_2 or $AgNO_3$ in the presence of molecular sieves (3 Å) in pyridine,

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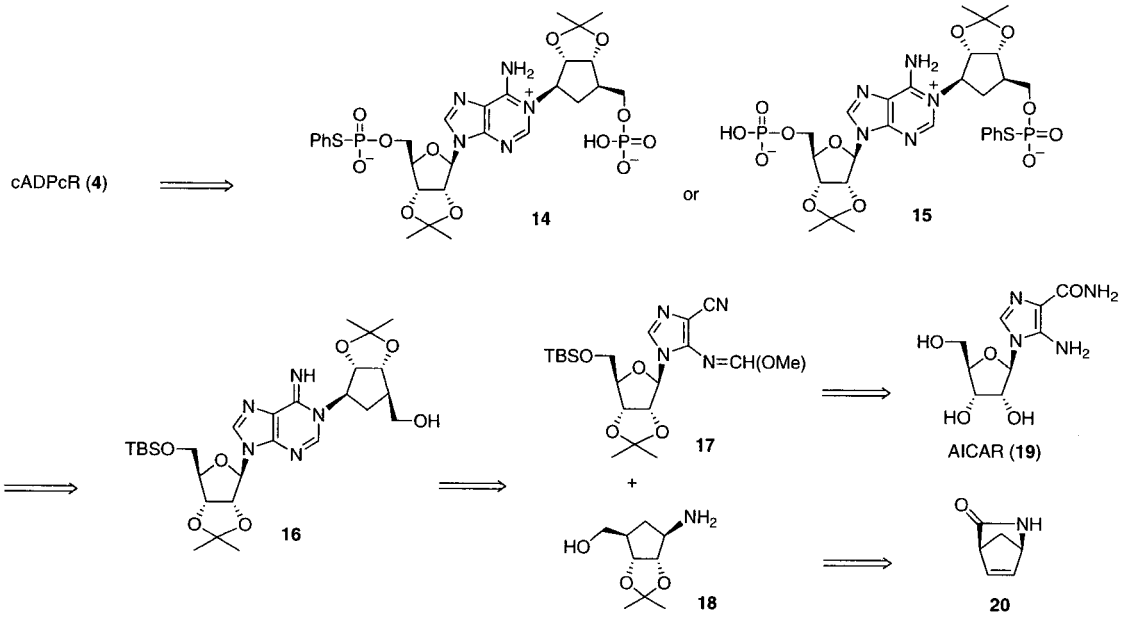
(9) Synthetic approaches to carbocyclic analogues of cADP-ribose have been published by other groups. Although the *N*-1-carbocyclic-inosine and -adenosine structures have been constructed, formation of the intramolecular pyrophosphate linkage has not been achieved. (a) Fortt, S.; Potter, B. V. L. *Tetrahedron Lett*. **1997**, *38*, 5371−5374. (b) Hutchinson, E. J.; Taylor, B. F.; Blackburn, G. M. *J. Chem. Soc., Chem. Commun*. **1997**, 1859−1860.

(10) Although a predominance of *anti*- over *syn*-conformers is well-known for natural nucleosides and their anologues, introducing a bulky substituent, such as a bromo group, into the 8-position of purine nucleosides restricts the conformation in a *syn*-form through steric repulsion with the ribose moiety: Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1983.

Scheme 2. Retrosynthetic Analysis of cADPcR (**4**)



and have successfully synthesized the cIDPcR and its 8-bromo congener (Scheme 1b).[7c,e] This method is very effective for producing the desired cyclization products, even without the conformational restriction of the substrate to a product-like *syn*-form by the introduction of the 8-bromo substituent. The 8-unsubstituted cyclization product **12** and the 8-bromo product **13** were obtained in 81% and quantitative yields from the corresponding substrates **10** and **11**, respectively.

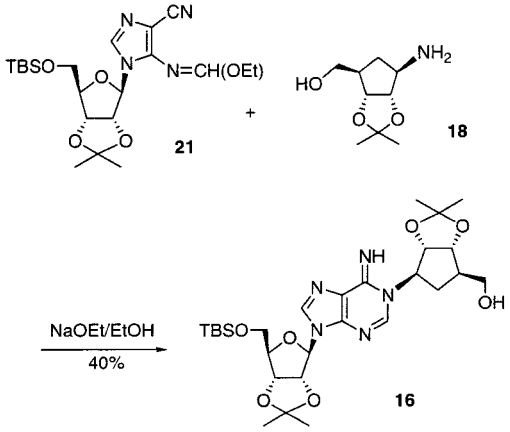
With these encouraging results in mind, we next tried to synthesize cADPcR (**4**). In this report, we describe the synthesis and chemical and biological properties of **4**, a stable mimic of cADPR.

Results and Discussion

Synthetic Plan. As described above, we first tried to synthesize the inosine congener cIDPcR (**5**), since its synthesis was thought to be easier than that of cADPcR (**4**). The charged *N*-1-substituted moiety (pK_a of cADPR is 8.3[4b]), which was expected to be unstable, especially under basic and nucleophilic conditions, might prove to be troublesome. In the synthesis of the target cADPcR, construction of the *N*-1-carbocyclic structure and the condensation between the two phosphate moieties forming an intramolecular pyrophosphate linkage should be the two most important steps. Our plan for the synthesis is shown in Scheme 2. Formation of the intramolecular pyrophosphate linkage was planned to investigate by treating 5′-phenylthiophosphate **14** or its regioisomer 5″- phenylthiophosphate **15** as the substrate with $AgNO_3$ or I_2/molecular sieves (3 Å) as a promoter. The phenylthiophosphates **14** and **15** would be obtained by functional group transformations of *N*-1-carbocyclic-ribosyladenosine derivative **16**. The *N*-1-carbocyclic-ribosyladenosine structure of **16** could be constructed from the imidazole nucleoside derivative **17** and the optically active carbocyclic amine **18**. Compounds **17** and **18** would be readily prepared from 5-aminoimidazole-4-carboxamide riboside (AICAR, **19**) and commercially available (1*R*)-(−)-2-azabicyclo[2.2.1]-hept-5-en-3-one (**20**).[9b]

The key condensation reaction between a phenylthiophosphate and a phosphate forming the pyrophosphate linkage promoted by I_2 or $AgNO_3$ was originally developed by Hata and co-workers.[11] We recently improved the Hata's reaction and used it effectively for the synthesis of cIDPcR as described above.[7c,e]

Scheme 3



Construction of the *N*-1-Carbocyclic-Ribosyl-Adenosine Structure. Development of an efficient method for constructing the *N*-1-carbocyclic-ribosyladenosine structure was essential for completing the synthesis of cADPcR (**4**). Recently, Blackburn and co-workers reported that treatment of imidazole nucleoside **21** and carbocyclic amine **18** under basic conditions provided *N*-1-carbocyclic-ribosyladenosine derivative **16** (Scheme 3).[9b] Although their method was likely to be the most efficient one for the construction of *N*-1-carbocyclic-ribosyladenosine structure known so far, the yield was insufficient (40%).

We attempted to improve their method using the *N*-benzylimidazole derivative **22** as a model substrate, and the results are shown in Scheme 4 and Table 1. In the condensation reaction of Blackburn and co-workers,[9b] it may be that the Dimroth rearrangement[12] of the desired *N*-1-carbocyclic product **16**

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Scheme 4

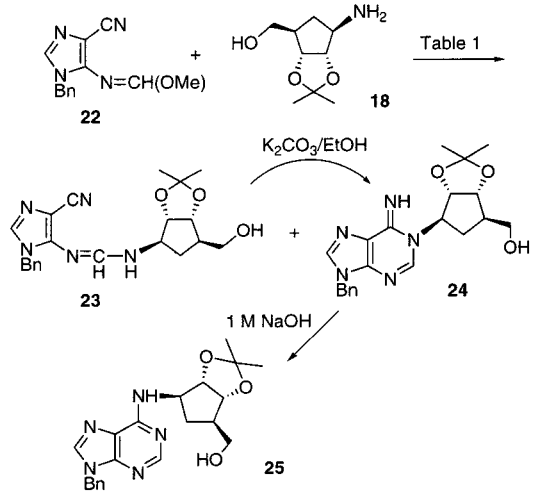


Table 1. Condensation Reactions between **18** and **22**,[a] and Basic Treatments of **23** and **24**.

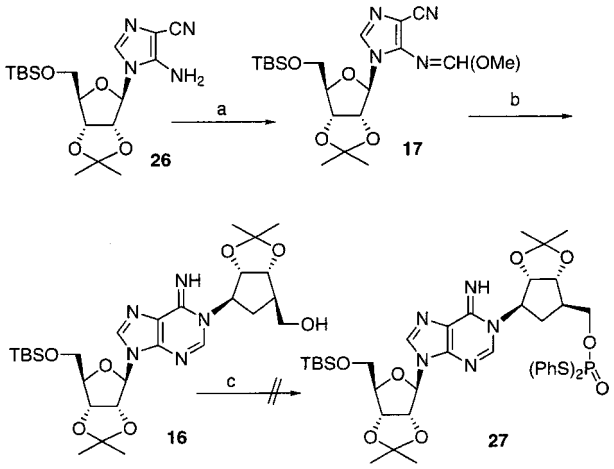
			yield (%)[b]		
entry	substrate	conditions	**23**	**24**	**25**
1	**22** + **18**	CF_3CO_2H/MeCN, reflux	73	trace	-
2	**22** + **18**	MeOH, rt	80	12	-
3	**22** + **18**	EtOH, rt	92	-	-
4	**23**	K_2CO_3/EtOH, rt	-	98	-
5	**22** + **18**	K_2CO_3/MeCN, rt	trace	73	-
6	**22** + **18**	K_2CO_3/MeOH, rt	trace	87	-
7	**24**	1 M NaOH, reflux	-	-	quant

[a] The reactions were performed using a slightly excess (1.1 equiv) of **18** to **22**. [b] Isolated yield.

(Scheme 3) takes place, which produces N^6-alkyladenine products from *N*-1-alkyladenines under basic conditions, thereby decreasing the yield. Consequently, we first treated a mixture of **18** and **22** under acidic (entry 1) or neutral (entries 2 and 3) conditions, and these reactions gave the coupled but not cyclized product **23** in excellent yield. Since compound **23** could be an intermediate for forming the desired *N*-1-carbocyclic product **24**, we examined its pyrimidine ring-closure reaction and found that treatment of **23** under mild basic conditions, namely with K_2CO_3/EtOH at room temperature, quantitatively produced the desired compound **24** (entry 4). The direct treatment of a mixture of **18** and **22** with K_2CO_3 in MeCN or MeOH was next performed to give **24** in 73 and 87% yield (entries 5 and 6). We confirmed that the Dimroth rearrangement of **24**, described above, actually occurred to form the N^6-carbocyclic product **25** quantitatively (Scheme 4), when **24** was treated under basic conditions, such as heating it in 1 M NaOH (entry 7).

Synthesis of cADPcR (4). On the basis of the above results, we next investigated the synthesis of cADPcR (**4**), which is summarized in Schemes 5 and 6. A sugar-protected imidazole nucleoside **26**,[9b] prepared from AICAR (**19**), was heated in $HC(OMe)_3$ under reflux in the presence of a catalytic amount of TFA to give the methoxymethylene derivative **17** quantitatively. Compound **17** was next subjected to the pyrimidine ring-closure reaction with the carbocyclic amine **18** under the conditions described above. When a mixture of **17** and **18** (1.2 equiv) was treated with K_2CO_3 in MeOH at room temperature, the desired ring-closure product **16** was obtained in 83% yield (Scheme 5). The *N*-1-carbocyclic-ribosyladenine structure of **16** was confirmed by its HMBC spectrum; a correlation between the H-2 of the adenine and the C-1″ of the cyclopentane ring was observed. Thus, the desired *N*-1-carbocyclic-ribosyladenosine structure was efficiently constructed from the imidazole nucleoside derivative **17** and the readily available optically active carbocyclic amine **18**. This should be a general method for preparing *N*-1-carbon-substituted adenosine derivatives without the occurrence of the Dimroth rearrangement.

Scheme 5[a]



[a] Reagents and conditions: a) $HC(OMe)_3$, cat. CF_3CO_2H, reflux, quant; b) **18**, K_2CO_3, MeOH, rt, 83%; c) PSS, TPSCI, py, rt.

Introduction of a bis(phenylthio)phosphoryl group at the 5″-position of **16** to produce **27** was attempted (Scheme 5). When **16** was treated with a cyclohexylammonium *S,S*-diphenylphosphorodithioate (PSS)/2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl)/pyridine system,[11] progress of the reaction was observed by TLC. However the product **27** was too unstable to be isolated; the spot likely to be for **27** disappeared during the workup after the reaction. Therefore, we decided to forego preparation of the 5″-phenylthiophosphate-type substrate **15** via **27** and subsequently investigated the introduction of a bis(phenylthio)phosphoryl group at the 5′-positon of the ribose moiety, as shown in Scheme 6. After protection of the 5″-hydroxyl of **16** with a monomethoxytrityl (MMTr) group, the 5′-*O*-TBS group of the product **28** was removed with TBAF to give **29**. Treatment of **29** with PSS/TPSCl in pyridine successfully gave the 5′-bis(phenylthio)phosphate **30** in 66% yield. Removal of the 5″-*O*-MMTr group of **30** with aqueous AcOH gave **31** in 77% yield. An unprotected phosphoryl group was introduced at the resulting 5″-primary hydroxyl of **31** by Yoshikawa's method with $POCl_3/(EtO)_3PO$,[13] followed by treating the product with H_3PO_2 and Et_3N in pyridine,[14] affording 5′-phenylthiophosphate **14**, the substrate for the intramolecular condensation reaction, in 75% yield as a triethylammonium salt after purification by a C_{18} column chromatography.

The intramolecular condensation reaction of **14** under high dilution conditions was next investigated (Scheme 6). When a solution of **14** in pyridine was added slowly, using a syringe pump, to a mixture of a large excess of $AgNO_3$ and Et_3N in the presence of molecular sieves (3 Å) in pyridine at room temperature,[7c,e] the desired cyclization product **32** was obtained in 93% yield as a triethylammonium salt, after purification by C_{18} column chromatography. Although a similar reaction with I_2 instead of $AgNO_3$ as a promoter also produced the cyclization product **32** as the major product, the yield lowered (50%) and several byproducts were observed by HPLC analysis. Nucleo-

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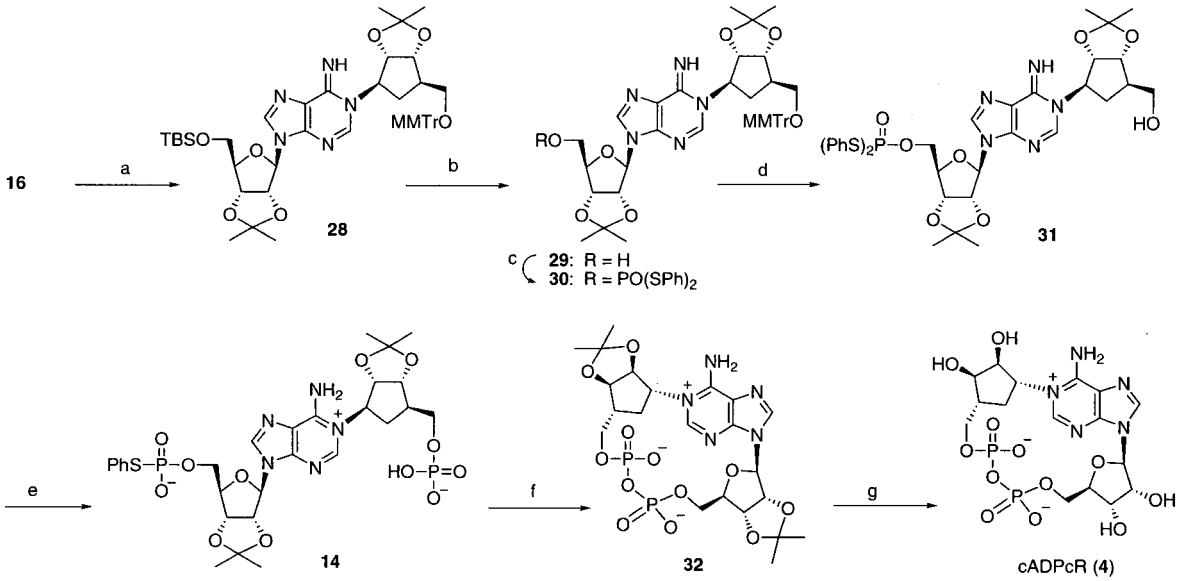
Scheme 6[a]



[a] Reagents and conditions: a) MMTrCl, pyridine, rt, 71%; b) TBAF, THF, AcOH, rt, quant; c) PSS, TPSCl, py, rt, 66%; d) aq 80% AcOH, rt, 77% e) 1) $POCl_3$, $(MeO)_3PO$, rt, 2) H_3PO_2, Et_3N, pyridine, rt, 75% (2 steps); f) $AgNO_3$, MS 3 Å, Et_3N, py, rt, 93%; g) 60% HCO_2H, rt, 88%.

philic attack by iodide ion, formed in the reaction, to the positively charged electron-deficient purine moiety of **32** might occur in this case to form the byproducts. The desired cyclic pyrophosphate structure of **32** was confirmed by the following data: (1) the molecular-ion peak corresponding to **32** was observed at *m*/*z* 618 in the FAB mass spectrum; (2) its ^{31}P NMR spectrum showed two signals at −10.39 and −10.80 ppm, which are typical chemical shifts for a pyrophosphate moiety, with a coupling constant (J = 15.3 Hz) similar to that of cADPR (−9.92 and −10.67 ppm, J = 14.6 Hz).[4e] Finally, the cyclic pyrophosphate **32** was treated with aqueous HCO_2H at room temperature to give the target cADP-carbocyclic-ribose (**4**) in 88% yield.

The results of this study as well as the previous synthesis of cIDPcR (**5**)[7c,e] clearly demonstrate that the strategy using a phenylthiophosphate-type substrate in the key intramolecular condensation reaction forming the pyrophosphate linkage is very efficient for the total syntheses of cADPR related compounds.

The Structure of cADPcR. The structure of cADPcR (**4**) was fully confirmed by ^1H, ^{13}C, and ^{31}P NMR, HMBC, NOE, HR-FAB, and UV spectra. We investigated the conformation of cADPcR and compared it with that of cADPR reported previously.[4c,e] ^1H NMR data of cADPcR in D_2O, together with those of cADPR,[4e] are summarized in S-4 (see Supporting Information). It has been demonstrated that the ribose moiety of the adenosine residue in cADPR adopts a C2′-*endo* conformation by ^1H NMR[4e] and X-ray crystallographic[4c] analyses. Each coupling constant between the ribose protons of the adenosine residue in cADPcR is similar to the corresponding one in cADPR. These similar *J* values between cADPcR and cADPR suggest that the ribose moiety of cADPcR also adopts a C2′-*endo* conformation as cADPR does.[4e]

The NOE experiments on cADPcR demonstrated that the adenosine residue is restricted in a *syn*-form around the glycosidic linkage due to the cyclic structure of the molecule: irradiation of the H-8 produced a strong NOE (14.4%) at the H-1′ as shown in Figure 2. The similar *syn*-conformation of the adenosine moiety of cADPR has been confirmed by X-ray crystallographic[4c] and ^1H NMR[4e] studies. When the ^1H NMR technique is used, conformation of the carbocyclic moiety of cADPcR should be analyzed more easily and more precise than

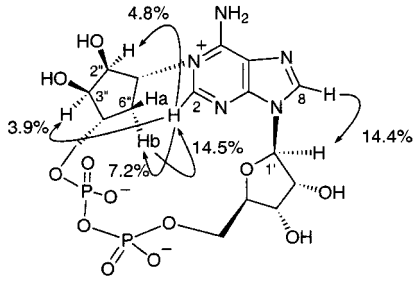


Figure 2. NOE data of cADPcR (**4**) in D_2O.

the corresponding *N*-1-ribose moiety of cADPR, due to the presence of the protons at the 6″-position in cADPcR. Thus, a very strong NOE was observed at the H-2 (14.5%), when the H-6″b was irradiated. Irradiation of the H-2 of cADPcR produced a strong NOE at H-6″b (7.2%) together with moderate ones at both the H-2″ (4.8%) and the H-3″ (3.9%), revealing a C6″-*endo* envelop-like conformation of the carbocyclic moiety, in which the C1″−C2″−C3″−C4″ unit is almost flat. This C6″-*endo* envelop-like conformation is also supported by a large $J_{1'',6''a}$ value (10.2 Hz) and a rather small $J_{1'',6''b}$ value (2.9 Hz). It has been proposed that the *N*-1-ribose moiety of cADPR adopts a C1″−C2″−C3″−C4″ flat conformation in aqueous solution, since the H-2-H-2″ and the H-2-H-3″ correlations with a similar strength were observed in its ROESY spectrum.[4e] On the other hand, the X-ray crystallographic analysis of cADPR showed the C3″-*exo* envelop conformation of the *N*-1-ribose moiety.[4c] The $J_{1'',2''}$, $J_{2'',3''}$, and $J_{3'',4''}$ values of cADPcR obtained in this study are similar to the corresponding *J* values of cADPR.[4e] Thus, it is likely that both the *N*-1-carbocyclic moiety in cADPcR and the *N*-1-ribose moiety in cADPR adopt an analogous C1″−C2″−C3″−C4″ flat/C6″(O1″)-*endo* envelop conformation in aqueous solution.

On the basis of these results, we concluded that the three-dimensional structure of cADPcR in aqueous solution is analogous to that of cADPR, as we had expected.

Chemical Stability of cADPcR. It has been suggested that cADPcR is stable in aqueous media, since the final acidic removal of the isopropylidene groups of **32** was successful in furnishing cADPcR (**4**) in high yield as described above

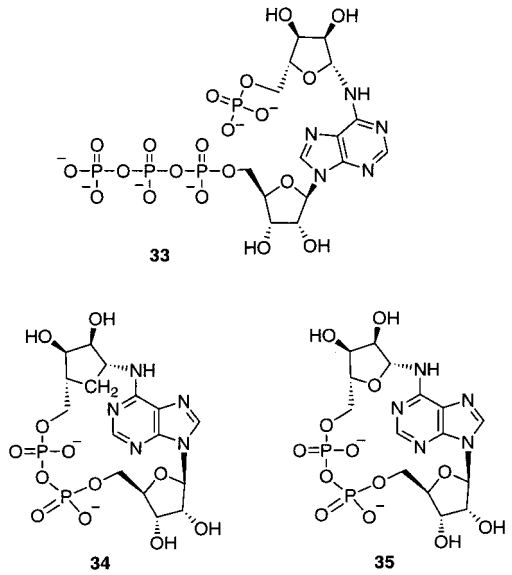


Figure 3. Structures of Dimroth rearrangement product **34** and related compounds.

(Scheme 6). Stability of cADPcR (**4**) and cADPR (**1**) in aqueous solution was examined in detail. The two compounds were treated in acidic (pH 2.0), neutral (pH 7.0), and alkaline (pH 12.0) buffers at 37 °C, and the time courses were analyzed by HPLC. The results are shown in S-1 (see Supporting Information). As we hypothesized, cADPcR is very stable and completely resistant to hydrolysis under acidic and neutral conditions, whereas cADPR rapidly decomposed with a $t_{1/2}$ of 33.8 h at pH 2.0 and 60.5 h at pH 7.0. The product derived from cADPR in neutral and acidic solutions was identified as ADP-ribose by HPLC.

On the other hand, when cADPR and cADPcR were treated under high alkaline conditions (pH12.0), both the compounds decomposed with a $t_{1/2}$ of 36.0 h (cADPR) and 37.1 h (cADPcR). However, HPLC analyses suggested that the reaction course of cADPcR in alkaline solution was different from that of cADPR: only one product was formed from cADPcR by alkaline treatment while the formation of at least three products was observed by HPLC analysis during treatment of cADPR under the same conditions.

The Dimroth Rearrangement of cADPcR: Formation of N^6-cyclic ADP-carbocyclic-Ribose. As described above, when the *N*-1-carbocyclic-ribosyladenine derivative **24** was treated with aqueous NaOH, the Dimroth rearrangement occurred to give the corresponding N^6-carbocyclic product **25** (Scheme 4). Ames and co-workers reported that *N*-1-(phosphoribosyl)-adenosine 5′-triphosphate underwent the Dimroth rearrangement to form the corresponding N^6-phosphoribosyl-product **33** (Figure 3).[12b] Therefore, it would be reasonable to assume that the product of the alkaline treatment of cADPcR (**4**) should be the Dimroth-rearranged N^6-cyclic ADP-carbocyclic-ribose (**34**, Figure 3). We treated cADPcR with aqueous NaOH/KCl buffer (pH 12) at 37 °C for 4 days on a preparative scale. After purification by ion-exchange column chromatography, the N^6-product **34** was obtained in 70% yield as a triethylammonium salt. The structure of **34** was confirmed by the following data: (1) the molecular-ion peak was observed at *m*/*z* 538 in the FAB mass spectrum; (2) two signals around −10 ppm in the ^{31}P NMR spectrum supported the cyclic pyrophosphate structure;[4e] (3) a λ_{max} in the UV spectrum was observed at 271 nm, which is the typical absorption for N^6-alkyladenosine derivatives;[4b] (4) the ^{13}C NMR chemical shift pattern of the base moiety was analogous to that of N^6-methyladenosine derivatives,[15] (5) a correlation of the H-1″ was not observed at the C-2 but at the C-6 in the HMBC spectrum, supporting its N^6-carbocyclic-ribosyl structure.

The structure of cADPR was originally presented as the N^6-cyclic ribosyl compound **35** (Figure 3) by Lee and co-workers,[4a] and later revised as *N*-1-cyclic regioisomer **1** by its X-ray crystallographic,[4c] UV,[4b,d] and ^{1}H NMR[4e] analyses. The N^6-cyclized regioisomer **35** of cADPR has not been synthesized; Jacobson and co-workers reported that the alkaline treatment of cADPR does not result in a Dimroth rearrangement but instead in hydrolysis at the *N*-1-position to afford ADP-ribose because of its unstable *N*-1-glycosidic linkage.[4b] Accordingly, the Dimroth rearranged N^6-carbocyclic product **34**, which corresponds to the carbocyclic congener of **35**, may be of biological importance.

Stability of cADPcR in Rat Brain Extract. The degradation enzymes of cADPR (**1**) are known to be widely distributed in brain extracts of mammals as well as in invertebrates.[16] We investigated whether cADPcR (**4**) is stable in brain extract having cADPR degradation enzymes. Brain extract from rats was prepared according to the previous method.[16] cADPR was first treated with the extract at 37 °C to be rapidly degraded, as shown in S-2 (see Supporting Information). As expected, under the same conditions, cADPcR was almost completely resistant to degradation by the extract. After 120 min of treatment, about 90% of cADPR was degraded, whereas most of cADPcR remained. Thus, it was suggested that cADPcR would be stable under physiological conditions.

Resistance of cADPcR to the CD38 cADPR Hydrolase. The cell-surface antigen CD38, a transmembrane glycoprotein, is known to synthesize cADPR (**1**) from NAD^+.[17] It is also known CD38 catalyzes not only the formation of cADPR but also the hydrolysis of cyclic ADPR.[17] Therefore, we planned to investigate whether cADPcR (**4**) was resistant to the hydrolysis by CD38. The recombinant CD38 fused with thioredoxin (ThioHis-CD38), which involves the extracellular domain of CD38, was expressed in *Escherichia coli* BL21(DE3) and purified according to the previously reported method by Katada and co-workers.[18]

The prepared extracellular domain of CD38 rapidly hydrolyzed cADPR, and ADP-ribose was the sole product observed on HPLC (S-3, see Supporting Information), as previously reported.[18] However, when cADPcR was treated under the same hydrolysis conditions, it was, not surprisingly, completely resistant to the CD38 hydrolase as shown in S-3 (see Supporting Information).

Ca^{2+}-Mobilizing Induced by cADPcR in Sea Urchin Eggs. Since the discovery of cADPR (**1**), sea urchin eggs have been used for bioassay of cADPR and its analogues. We examined whether cADPcR (**4**) could induce the $[Ca^{2+}]_i$ increase when it was injected into the unfertilized eggs of *A. crassispina*. Figure 4 shows representative time courses of the $[Ca^{2+}]_i$ changes induced by cADPR (a) or cADPcR (b). Injection of cADPR at a final concentrations of 30 to 500 nM induced a small increase in $[Ca^{2+}]_i$. Injection of cADPcR at a final concentration of 30

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(17) (a) Howard, M.; Girimaldi, J. C.; Bazan, J. F.; Lund, F. E.; Santos-Argumedo, L.; Parkhouse, R. M. E.; Walseth, T. F.; Lee, H. C. *Science* **1994**, *262*, 1056−1059. (b) Takasawa, S.; Tohgo, A.; Noguchi, N.; Kogura, T.; Nata, K.; Sugimoto, T.; Yonekura, H.; Okamoto, H. *J. Biol. Chem*. **1994**, *268*, 26052−26054.
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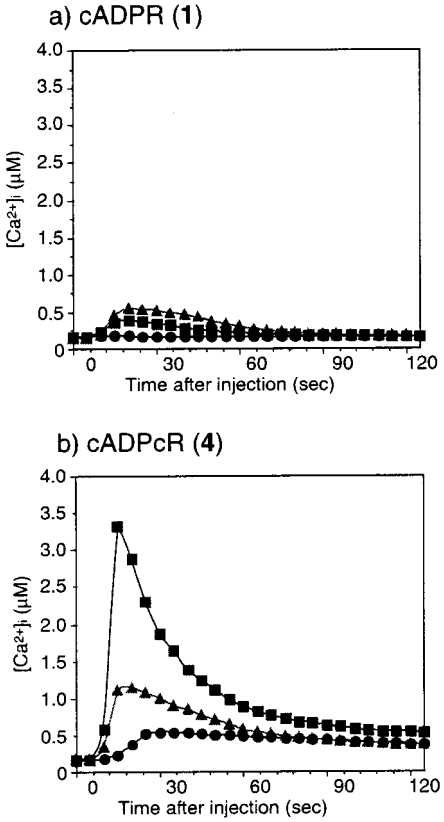

Figure 4. Time courses of the $[Ca^{2+}]_i$ changes induced by cADPR (**1**, a) and cADPcR (**4**, b) injected into eggs of *A. crassispina*: (●) 30 nM, (■) 200 nM, (▲) 500 nM.

nM induced a $[Ca^{2+}]_i$ increase comparable to the peak value of the 500 nM cADPR-induced Ca^{2+} transient. Injection of cADPcR at a final concentration of 200 nM induced the peak value of $[Ca^{2+}]_i$ at about 3.3 μM, which was more than 10 times higher that induced by cADPR. These results clearly show a potent Ca^{2+}-mobilizing activity of cADPcR in comparison to that of cADPR.[19] This difference seems to be caused by the fact that cADPcR is much more biologically stable than cADPR, as described above.[20]

Conclusions

We have successfully synthesized cADPcR (**4**) designed as a stable mimic of cADPR (**1**). The *N*-1-carbocyclic adenosine structure was efficiently constructed via condensation between an imidazole nucleoside derivative **17**, prepared from AICA-riboside (**19**), and a readily available optically active carbocyclic amine **18**. The key intramolecular condensation reaction of the phenylthiophosphate-type substrates **14** proceeded with $AgNO_3$/molecular sieves (3 Å) to give the cyclization product **32** in high yield. This method would be applicable to the synthesis of other cADPR analogues and may be an entry to a general method for synthesizing biologically important cyclic nucleotides of this type. cADPcR was shown to be resistant to chemical and biochemical hydrolysis. It released Ca^{2+} in sea urchin eggs, and the activity was stronger than that of cADPR. These results suggest that cADPcR is a stable mimic of cADPR, as we hypothesized.

Experimental Section

Chemical shifts are reported in ppm downfield from TMS (^{1}H and ^{13}C) or H_3PO_4 (^{31}P). All of the ^{1}H NMR assignments described were in agreement with COSY spectra. Thin-layer chromatography was done on Merck coated plate $60F_{254}$. Silica gel chromatography was done on Merck silica gel 5715. Reactions were carried out under an argon atmosphere.

1-Benzyl-4-cyano-5-[(methoxymethylene)amino]imidazole (22). A mixture of 5-amino-1-benzyl-4-cyanoimidazole[21] (1.03 g 5.22 mmol), TFA (20 μL, 0.26 mmol), and trimethyl orthoformate (2.86 mL, 26.1 mmol) in CH_3CN (30 mL) was stirred at 105 °C for 1 h. The mixture was evaporated, and the residue was purified by column chromatography (SiO_2, 25% acetone in $CHCl_3$) to give **22** (920 mg, 74%) as a pale yellow oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.42 (s, 1 H), 7.88 (s, 1 H), 7.25−7.38 (m, 5 H), 5.12 (s, 2 H), 3.88 (s, 3 H).

1-Benzyl-4-cyano-5-[[(1*R*,2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-hydroxymethyl)cyclopentyl]aminomethyleneamino]imidazole (23; Table 1, entry 3). A mixture of the optically active carbocyclic amine **18** (356 mg, 1.90 mmol) and **22** (415 mg, 1.73 mmol) in EtOH (9 mL) was stirred at room temperature for 23 h. The precipitated solid was filtered, washed by cold EtOH and Et_2O and dried to give **23** (524 mg, 76%) as a white solid. The filtrate was evaporated, and the residue was purified by column chromatography (SiO_2, 5% MeOH in $CHCl_3$) to give further **23** (107 mg, 16%) as a white solid: mp (MeOH) 107−109 °C: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.14 (m, 1 H, NH, exchangeable with D_2O), 8.05 (d, 1 H, N=CH, J = 3.3 Hz), 7.66 (s, 1 H, H-2), 7.24−7.30 (m, 5 H, C_6H_5), 5.04 (s, 2 H, N−CH_2), 4.76 (t, 1 H, 5′-OH, J = 5.0 Hz, exchangeable with D_2O), 4,41 (m, 2 H, H-2′, H-3′), 4.17 (m, 1 H, H-1′), 3.39 (t, 2 H, H-5′, J = 5.8 Hz), 2.19−2.26 (m, 1 H, H-6′b), 2.09 (m, 1 H, H-4′), 1.44−1.51 (m, 1 H, H-6′a), 1.19, 1.37 (each s, each 3 H, isopropyl CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 152.6, 150.0, 137.1, 135.0, 128.5, 127.8, 127.6, 117.9, 110.9, 94.2, 84.8, 81.6, 62.0, 56.8, 46.4, 46.3, 32.5, 27.2, 24.8; HRMS (FAB, positive) calcd for $C_{21}H_{26}N_5O_3$ (MH^+) 396.2035, found 396.2030; UV (MeOH) λ_{max} 289 nm. Anal. Calcd for $C_{21}H_{25}N_5O_3$: C, 63.78; H, 6.37; N, 17.71. Found: C, 63.79; H, 6.44; N, 17.80.

9-Benzyl-6-imino-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-hydroxymethyl)cyclopentyl]purine (24). From **23** (Table 1, entry 4): A mixture of **23** (500 mg, 1.26 mmol) and K_2CO_3 (9.0 mg, 76 μmol) in EtOH (70 mL) was stirred at room temperature for 3 h. The mixture was evaporated, and the residual yellow oil was purified by column chromatography (SiO_2, 14% MeOH in $CHCl_3$) to give **24** (487 mg, 98%) as a white solid. From **18** and **22** (Table 1, entry 6): A mixture of **22** (110 mg, 0.46 mmol), **18** (94 mg, 0.51 mmol), and K_2CO_3 (3.0 mg, 23 μmol) in MeOH (3 mL) was stirred at room temperature for 18 h. The resulting solution was evaporated, and the residue was purified by column chromatography (SiO_2, 12% MeOH in CH_2Cl_2) to give **24** (157 mg, 87%) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.13 (s, 1, H-2), 8.04 (s, 1 H, H-8), 7.35−7.26 (m, 5 H, C_6H_5), 7.05 (br s, 1 H, NH, exchangeable with D_2O), 5.28 (s, 2 H, N−CH_2), 5.14 (m, 1 H, H-1′), 5.06 (t, 1 H, H-2′, J = 6.1 Hz), 4.73 (br s, 1 H, 5′-OH, exchangeable with D_2O), 4.47 (m, 1 H, H-3′), 3.50 (m, 1 H, H-5′b), 3.45 (m, 1 H, H-5′a), 2.03−2.17 (m, 3 H, H-4′, H-6′), 1.20, 1.44 (each s, each 3 H, isopropyl CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 153.9, 146.8, 140.8, 139.1, 136.8, 128.6, 127.6, 127.3, 122.1, 111.9, 82.2, 81.0, 62.3, 61.6, 46.3, 46.0, 33.1, 27.6, 25.3; HRMS (FAB, positive) calcd for $C_{21}H_{26}N_5O_3$ (MH^+) 396.2035, found 396.2039; UV (MeOH) λ_{max} 263 nm, sh 270. Anal. Calcd for $C_{21}H_{25}N_5O_3 \cdot 0.3\ H_2O$: C, 62.92; H, 6.44; N, 17.47. Found: C, 62.92; H, 6.54; N, 17.45.

9-Benzyl-N^6-[[(1*R*,2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-hydroxymethyl]-cyclopentyl]adenine (25; Table 1, entry 7). A suspension of **24** (150 mg, 0.38 mmol) in 1 M NaOH (2 mL) was heated under reflux for 1 h, and then MeOH (1 mL) was added. The resulting solution was extracted with $CHCl_3$ (three times), and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was

(19) Preliminary experiments showed that cADPcR (**4**) also induced $[Ca^{2+}]_i$ increases in patch-clamped NG108-15 neuronal cells: Hashii, M.; Higashida, H. unpublished results.

(20) Although cADPR synthesized from NAD^+ by ADP-ribosylcyclase might be very rapidly hydrolyzed in cells, e.g., within 1 min, because of its role as a second messenger (see ref 3), the reaction conditions of cADPR hydrolysis by the brain extract or CD38 in this study were selected to be suitable for the HPLC assay ($t_{1/2}$ = 15−40 min).

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purified by column chromatography (SiO_2, 5% MeOH in $CHCl_3$) to give **25** (148 mg, 98%) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.26 (s, 1 H, H-8), 8.23 (s, 1 H, H-2), 7.92 (br s, 1 H, NH, exchangeable with D_2O), 7.34−7.24 (m, 5 H, C_6H_5), 5.37 (s, 2 H, N−CH_2), 5.00 (br s, 1 H, 5′-OH, exchangeable with D_2O), 4.60 (m, 1 H, H-1′), 4.54 (dd, 1 H, H-2′, J = 3.2, 5.9 Hz), 4.44 (m, 1 H, H-3′), 3.47 (m, 2 H, H-5′), 2.28 (m, 1 H, H-6′a), 2.13 (m, 1 H, H-4′), 1.53 (m, 1 H, H-6′b), 1.39, 1.19 (each s, each 3 H, isopropyl CH_3); ^{13}C NMR 153.9, 152.5, 148.9, 140.7, 137.1, 128.6, 127.4, 118.9, 110.6, 85.1, 82.0, 62.5, 55.9, 46.4, 46.1, 33.5, 27.2, 24.8; FAB-MS calcd for $C_{21}H_{26}N_5O_3$ (MH^+) 396.2035, found 396.2053; UV (MeOH) λ_{max} 270 nm. Anal. Calcd for $C_{21}H_{25}N_5O_3$: C, 63.78; H, 6.37; N, 17.71. Found: C, 63.81; H, 6.58; N, 17.50.

5-[(Methoxymethylene)amino]-1-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-*O*-(isopropylidene)-β-D-ribofuranosyl]imidazole-4-nitrile (17). Compound **17** (1.35 g, quant) was obtained from 5-amino-1-[5′-*O*-(*tert*-butyldimethylsilyl)-2,3-*O*-(isopropylidene)-β-D-ribofuranosyl]imidazole-4-nitrile (**26**,[9b] 1.18 g, 3.0 mmol) as described for the synthesis of **22** after purification by column chromatography (SiO_2, 25% EtOAc in hexane) as a yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.39 (s, 1 H, N=CH), 7.64 (s, 1 H, H-2), 5.84 (d, 1 H, H-1′, $J_{1',2'}$ = 2.9 Hz), 4.78 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.0, $J_{3',4'}$ = 1.9 Hz), 4.74 (dd, 1 H, H-2′, $J_{2',1'}$ = 2.9, $J_{2',3'}$ = 6.0 Hz), 4.38 (ddd, 1 H, H-4′, $J_{4',3'}$ = 1.9, $J_{4',5'a}$ = 2.5, $J_{4',5'b}$ = 2.9 Hz), 3.95 (s, 3 H, OCH_3), 3.86 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 2.5, $J_{5'a,5'b}$ = 11.5 Hz), 3.77 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 2.9, $J_{5'b,5'a}$ = 11.5 Hz), 1.56, 1.34 (each s, each 3 H, isopropyl CH_3), 0.86 (s, 9H, *tert*-butyl), 0.06, 0.06 (each s, each 3H, dimethyl); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 159.9, 143.4, 133.5, 115.7, 113.8, 99.2, 91.4, 86.5, 86.1, 81.0, 63.5, 54.6, 27.2, 25.8, 25.3, 18.3, −5.5, −5.7; HRMS (FAB, positive) calcd for $C_{20}H_{33}N_4O_5Si$ 437.2220 (MH^+), found 437.2195; UV (MeOH) λ_{max} 273 nm. Anal. Calcd for $C_{20}H_{32}N_4O_5Si$: C, 55.02; H, 7.39; N, 12.83. Found C, 54.86; H, 7.43; N, 12.70.

***N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-(hydroxymethyl)cyclopentyl]-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylideneadenosine (16)**. A mixture of **17** (2.6 g, 6.0 mmol), **18** (1.35 g, 7.2 mmol), and K_2CO_3 (41 mg, 0.3 mmol) in MeOH (60 mL) was stirred at room temperature for 4 h. The mixture was evaporated, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 6% MeOH in EtOAc) to give **16** (2.94 g, 83%) as a white form: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.79 (s, 1 H, H-8), 7.66 (s, 1 H, H-2), 7.19 (br s, 1 H, NH), 5.99 (d, 1 H, H-1′, $J_{1',2'}$ = 2.7 Hz), 5.31 (dd, 1 H, H-2″, $J_{2'',1''}$ = 5.2, $J_{2'',3''}$ = 5.8 Hz), 5.06 (dd, 1 H, H-2′, $J_{2',1'}$ = 2.7, $J_{2',3'}$ = 6.2 Hz), 4.86 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 2.6 Hz), 4.74 (dd, 1 H, H-3″, $J_{3'',2''}$ = 5.8, $J_{3'',4''}$ = 2.7 Hz), 4.57 (ddd, 1 H, H-1″, $J_{1'',2''}$ = 5.2, $J_{1'',6''a}$ = 9.7, $J_{1'',6''b}$ = 9.6 Hz), 4.35 (ddd, 1 H, H-4′, $J_{4',3'}$ = 2.6, $J_{4',5'a}$ = 3.9, $J_{4',5'b}$ = 4.1 Hz), 3.79 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 3.9, $J_{5'a,5'b}$ = 11.2 Hz), 3.79 (dd, 1 H, H-5″a, $J_{5''a,4''}$ = 3.9, $J_{5''a,5''b}$ = 10.8 Hz), 3.74 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 4.1, $J_{5'b,5'a}$ = 11.2 Hz), 3.73 (dd, 1 H, H-5″b, $J_{5''b,4''}$ = 4.1, $J_{5''b,5''a}$ = 10.8 Hz), 2.57 (m, 1 H, H-6″a), 2.52 (m, 1 H, H-4″), 2.45 (m, 1 H, H-6″b), 1.58, 1.53, 1.36, 1.29 (each s, each 3 H, isopropyl CH_3), 0.84 (s, 9 H, *tert*-butyl), 0.03, 0.02 (each s, each 3 H, dimethyl); NOE ($CDCl_3$, 400 MHz) irradiated H-2, observed H-1″ (17.9%); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 153.8, 147.5, 140.7, 137.1, 124.0, 114.1, 111.8, 91.1, 87.0, 85.2, 83.5, 82.3, 81.2, 70.1, 64.6, 63.4, 45.0, 30.5, 28.0, 27.2, 25.8, 25.3, 18.3, −5.5, −5.6; HRMS (FAB, positive) calcd for $C_{28}H_{46}N_5O_7Si$ 592.3166 (MH^+), found 592.3179; UV (MeOH) λ_{max} 261 nm, sh 293 nm. Anal. Calcd for $C_{28}H_{45}N_5O_7Si$: C, 56.83; H, 7.66; N, 11.83. Found C, 56.65; H, 7.56; N, 11.83.

***N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-[(5-monomethoxytrityl)oxymethyl]cyclopentyl]-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylideneadenosine (28)**. A mixture of **16** (1.78 g, 3.0 mmol) and MMTrCl (1.85 g, 6.0 mmol) in pyridine (20 mL) was stirred at room temperature for 1.5 h. The mixture was evaporated, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 60% EtOAc in hexane) to give **28** (1.85 g, 71%) as a white form: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.79 (s, 1 H, H-8), 7.70 (s, 1 H, H-2), 7.44−6.81 (m, 14 H, Ar−H), 6.02 (d, 1 H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.11−5.08 (m, 2 H, H-2′, H-2″), 5.02 (m, 1 H, H-1″), 4.89 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 2.3 Hz), 4.56 (m, 1 H, H-3″), 4.37 (m, 1 H, H-4′), 3.84 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 4.0, $J_{5'a,5'b}$ = 11.2 Hz), 3.79 (s, 3 H, OCH_3), 3.76 (m, 1 H, H-5′b), 3.35 (dd, 1 H, H-5″a, $J_{5''a,4''}$ = 3.2, $J_{5''a,5''b}$ = 8.5 Hz), 3.18 (dd, 1 H, H-5″b, $J_{5''b,4''}$ = 5.4, $J_{5''b,5''a}$ = 8.5 Hz), 2.46−2.39 (m, 3 H, H-4″, H-6″), 1.62, 1.53, 1.39, 1.28 (each s, each 3 H, isopropyl CH_3), 0.88 (s, 9 H, *tert*-butyl), 0.06, 0.05 (each s, each 3 H, dimethyl); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 158.5, 154.2, 146.3, 144.6, 144.5, 140.4, 136.6, 135.7, 130.3, 128.4, 127.7, 126.8, 124.0, 114.1, 113.0, 91.0, 87.0, 86.1, 85.2, 82.8, 81.6, 81.3, 64.2, 63.6, 63.4, 55.2, 45.0, 33.6, 27.7, 27.2, 25.9, 25.3, 18.3, −5.4, −5.5; HRMS (FAB, positive) calcd for $C_{48}H_{62}N_5O_8Si$ 864.4367 (MH^+), found 864.4384; UV (MeOH) λ_{max} 261 nm, sh 297 nm. Anal. Calcd for $C_{48}H_{61}N_5O_8Si$: C, 66.72; H, 7.12; N, 8.10. Found C, 66.65; H, 7.13; N, 7.97.

***N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-[(5-monomethoxytrityl)oxymethyl]cyclopentyl]-2′,3′-*O*-isopropylideneadenosine (29)**. A mixture of **28** (1.74 g, 2.0 mmol), TBAF (1.0 M in THF, 22.6 mL, 22.6 mmol), and AcOH (657 μL, 10.4 mmol) in THF (7 mL) was stirred at room temperature for 1 h. The mixture was evaporated, and the residue was purified by column chromatography (SiO_2, 3.5% MeOH in $CHCl_3$) to give **29** (1.52 g, quant) as a white form: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71 (s, 1 H, H-8), 7.62 (s, 1 H, H-2), 7.44−6.81 (m, 14 H, Ar−H), 5.76 (d, 1 H, H-1′, $J_{1',2'}$ = 3.6 Hz), 5.49 (br s, 1 H, 5′-OH), 5.10 (dd, 1 H, H-2″, $J_{2'',1''}$ = 5.1, $J_{2'',3''}$ = 6.8 Hz), 5.04 (m, 2 H, H-2′, H-3′), 4.97 (m, 1 H, H-1″), 4.53 (dd, 1 H, H-3″, $J_{3'',2''}$ = 5.8, $J_{3'',4''}$ = 2.7 Hz), 4.37 (m, 1 H, H-4′), 3.92 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 1.0, $J_{5'a,5'b}$ = 12.5 Hz), 3.80 (s, 3 H, OCH_3), 3.77 (m, 1 H, H-5′b), 3.34 (dd, 1 H, H-5″a, $J_{5''a,4''}$ = 3.5, $J_{5''a,5''b}$ = 8.8 Hz), 3.17 (dd, 1 H, H-5″b, $J_{5''b,4''}$ = 5.8, $J_{5''b,5''a}$ = 8.8 Hz), 2.42 (m, 3 H, H-4″, H-6″), 1.63, 1.53, 1.37, 1.27 (each s, each 3 H, isopropyl CH_3); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 158.5, 153.8, 146.4, 144.5, 144.5, 139.3, 138.0, 135.7, 130.3, 128.4, 127.7, 126.8, 125.4, 114.2, 113.2, 113.0, 93.8, 86.1, 85.8, 83.6, 82.4, 81.5, 81.3, 64.2, 63.2, 55.2, 44.7, 33.6, 30.8, 27.7, 27.5, 25.3, 25.2; HRMS (FAB, positive) calcd for $C_{42}H_{48}N_5O_8$ 750.3503 (MH^+), found 750.3509; UV (MeOH) λ_{max} 261 nm, sh 300 nm. Anal. Calcd for $C_{42}H_{47}N_5O_8 \cdot 0.5H_2O$: C, 66.48; H, 6.38; N, 9.23. Found C, 66.50; H, 6.44; N, 8.98.

***N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-[(5-monomethoxytrityl)oxymethyl]cyclopentyl]-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine (30).** After stirring a mixture of PSS (2.19 g, 5.7 mmol) and TPSCl (1.74 g, 5.7 mmol) in pyridine (15 mL) at room temperature for 1.0 h, **29** (1.44 g, 1.9 mmol) was added, and the resulting mixture was stirred at room temperature for 1.0 h. The mixture was evaporated, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 2% MeOH in $CHCl_3$) to give **30** (1.27 g, 66%) as a white form: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.66 (s, 1 H, H-8), 7.64 (s, 1 H, H-2), 7.51−6.81 (m, 24 H, Ar−H), 5.97 (d, 1 H, H-1′, $J_{1',2'}$ = 2.4 Hz), 5.28 (br s, 1 H, NH), 5.13 (dd, 1 H, H-2′, $J_{2',1'}$ = 2.4, $J_{2',3'}$ = 6.3 Hz), 5.11 (m, 1 H, H-2″), 4.93 (m, 1 H, H-1″), 4.92 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 2.8 Hz), 4.53 (m, 1 H, H-3″), 4.43−4.36 (m, 3 H, H-4′, H-5′), 3.79 (s, 3 H, OCH_3), 3.79 (m, 1 H, H-5″a), 3.73 (m, 1 H, H-5″b), 2.41 (m, 3 H, H-4″, H-6″), 1.60, 1.52 1.36, 1.25 (each s, each 3 H, isopropyl CH_3); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 158.5, 154.1, 146.6, 144.6, 144.5, 137.3, 135.7, 135.3, 135.2, 135.1, 135.1, 130.3, 129.6, 129.4, 128.4, 127.7, 126.7, 125.9, 124.4, 114.6, 113.0, 90.7, 86.1, 84.9, 84.8, 84.4, 82.6, 81.6, 81.1, 66.4, 66.3, 64.3, 64.1, 55.2, 44.9, 33.5, 27.7, 27.1, 25.3, 25.3; ^{31}P NMR ($CDCl_3$, 202 MHz, decoupled with ^{1}H) δ 51.0; HRMS (FAB, positive) calcd for $C_{54}H_{57}N_5O_9PS_2$ 1014.3335 (MH^+), found 1014.3320; UV (MeOH) λ_{max} 260 nm, sh 296 nm. Anal. Calcd for $C_{54}H_{56}N_5O_9PS_2 \cdot 0.5H_2O$: C, 63.39; H, 5.62; N, 6.84. Found C, 63.32; H, 5.69; N, 6.54.

***N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-(hydroxymethyl)cyclopentyl]-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine (31).** A solution of **30** (1.19 g, 1.17 mmol) in 80% aqueous AcOH (15 mL) was stirred at room temperature for 6 h. The resulting mixture was evaporated, and the residue was partitioned between EtOAc and aqueous saturated $NaHCO_3$. The organic layer was washed with H_2O and then brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 3% MeOH in

$CHCl_3$) to give **31** (665 mg, 77%) as a white form: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.68 (br s, 2 H, H-2, H-8), 7.64−7.22 (m, 10 H, Ar−H), 5.98 (d, 1 H, H-1′, $J_{1',2'}$ = 1.9 Hz), 5.31 (m, 1 H, H-2″), 5.13 (dd, 1 H, H-2′, $J_{2',1'}$ = 1.9, $J_{2',3'}$ = 6.1 Hz), 4.93 (m, 1 H, H-3″), 4.73 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 2.2 Hz), 4.53 (m, 1 H, H-1″), 4.44−4.37 (m, 3 H, H-4′, H-5′), 3.78 (dd, 1 H, H-5″a, $J_{5''a,4''}$ = 5.0, $J_{5''a,5''b}$ = 10.6 Hz), 3.71 (m, 1 H, H-5″b), 2.56 (m, 2 H, H-4″, H-6″a), 2.45 (m, 1 H, H-6″b), 1.60, 1.56 1.36, 1.31 (each s, each 3 H, isopropyl CH_3); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 153.6, 147.6, 140.6, 137.9, 135.3, 135.2, 135.1, 135.0, 129.7, 129.6, 129.6, 129.4, 125.9, 125.8, 124.3, 114.6, 111.8, 90.8, 85.0, 84.9, 84.4, 83.4, 82.3, 81.2, 70.3, 66.4, 66.3, 64.6, 44.9, 30.3, 28.0, 27.1, 25.2; ^{31}P NMR ($CDCl_3$, 202 MHz, decoupled with ^{1}H) δ 51.1; HRMS (FAB, positive) calcd for $C_{34}H_{41}N_5O_8PS_2$ 742.2134 (MH^+), found 742.2128; UV (MeOH) λ_{max} 254 nm, sh 299 nm. Anal. Calcd for $C_{34}H_{40}N_5O_8PS_2 \cdot 0.5H_2O$: C, 54.39; H, 5.50; N, 9.33. Found C, 54.37; H, 5.48; N, 9.44.

***N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-(phosphonoxymethyl)cyclopentyl]-5′-*O*-[(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine (14)**. $POCl_3$ (186 μL, 2.0 mmol) was added to a solution of **31** (148 mg, 0.2 mmol) in $PO(OMe)_3$ (2 mL) at 0 °C, and the mixture was stirred at the same temperature for 20 min. The reaction was quenched by aqueous saturated $NaHCO_3$ (4 mL), and the resulting mixture was stirred at 0 °C for 10 min. To the mixture were added TEAA buffer (2.0 M, 1 mL) and H_2O (4 mL), and then the resulting solution was applied to a C_{18} reversed phase column (1.1 × 18 cm). The column was developed using a linear gradient of 0−60% CH_3CN in TEAA buffer (0.1 M, pH 7.0, 400 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reversed phase column chromatography (1.1 × 18 cm, eluted with 60% aqueous CH_3CN). Appropriate fractions were evaporated, and the residue was coevaporated with pyridine (1 mL × 3). A mixture of the residue, H_3PO_2 (80 μL, 1.6 mmol) and Et_3N (104 μL, 0.75 mmol) in pyridine (2.0 mL) was stirred at 0 °C for 1.0 h and then at room temperature for 16 h in the dark. After TEAA buffer (2.0 M, 1.0 mL) was added, the mixture was evaporated, and the residue was partitioned between EtOAc and H_2O. The aqueous layer was evaporated with TEAA buffer (2.0 M, 1.0 mL), and the residue was dissolved in H_2O (5 mL). The solution was applied to a C_{18} reversed phase column (1.1 × 18 cm), and the column was developed using a linear gradient of 0−40% CH_3CN in TEAA buffer (0.1 M, pH 7.0, 400 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reversed phase column chromatography (1.1 × 18 cm, eluted with 40% aqueous CH_3CN) to give **14** (125 mg, 75%) as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 8.66 (s, 1 H, H-2), 8.41 (s, 1 H, H-8), 7.28−7.11 (m, 5 H, Ar−H), 6.33 (d, 1 H, H-1′, $J_{1',2'}$ = 2.2 Hz), 5.41 (dd, 1 H, H-2′, $J_{2',1'}$ = 2.2, $J_{2',3'}$ = 6.0 Hz), 5.02 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.0, $J_{3',4'}$ = 1.7 Hz), 4.96−4.85 (m, 3 H, H-1″, H-2″, H-3″), 4.75 (m, 1 H, H-4′), 4.23 (m, 1 H, H-5′a), 4.15 (m, 1 H, H-5′b), 4.06 (m, 2 H, H-5″), 3.19 (q, 6 H, $-CH_2N$, J = 7.3 Hz), 2.64 (m, 2 H, H-4″, H-6″a), 2.45 (m, 1 H, H-6″b), 1.66, 1.65, 1.42, 1.42 (each s, each 3 H, isopropyl CH_3), 1.28 (t, 9 H, $C\underline{H}_3CH_2N$, J = 7.3 Hz); ^{13}C NMR (D_2O, 125 MHz) δ 153.8, 148.7, 147.0, 146.5, 135.0, 135.0, 132.3, 131.7, 130.4, 122.1, 118.4, 117.5, 94.3, 89.0, 88.9, 86.8, 86.3, 84.2, 83.2, 68.6, 68.5, 67.9, 67.8, 49.4, 46.2, 46.1, 35.5, 28.7, 27.0, 26.7, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ 1.05 (s), 17.10 (s); HRMS (FAB, negative) calcd for $C_{28}H_{36}N_5O_{12}P_2S$ 728.1556 [(M − H)$^-$], found 728.1576; UV (H_2O) λ_{max} = 260 nm.

Cyclic ADP-carbocyclic-ribose Diacetonide (32). To a mixture of $AgNO_3$ (41 mg, 241 μmol), Et_3N (33 μL, 241 μmol), and molecular sieves (3 Å) (1.0 g) in pyridine (8 mL), was added a solution of **14** (9.5 mg, 11.4 μmol) in pyridine (8 mL) slowly over 15 h, using a syringe-pump, at room temperature in the dark. The molecular sieves were filtered off with Celite and washed with H_2O. To the combined filtrate and washings was added TEAA buffer (2.0 M, pH 7.0, 1 mL), and the resulting solution was evaporated. The residue was partitioned between EtOAc and H_2O, and the aqueous layer was evaporated. The residue was dissolved in 0.1 M TEAA buffer (5 mL), which was applied to a C_{18} reverse phase column (1.1 × 11 cm). The column was developed using a linear gradient of 0−40% CH_3CN in TEAA buffer (0.1 M, pH 7.0, 200 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reverse phase column chromatography (1.1 cm × 11 cm, eluted with 20% aqueous CH_3CN) to give **32** (7.7 mg, 10.5 μmol, 131 OD_{260} units, 93%) as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 8.77 (s, 1 H, H-2), 8.42 (s, 1 H, H-8), 6.40 (br s, 1 H, H-1′), 5.81 (d, 1 H, H-2′, $J_{2',3'}$ = 6.0 Hz), 5.02 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.0, $J_{3',4'}$ = 2.4 Hz), 4.89−4.86 (m, 3 H, H-1″, H-2″, H-3″), 4.61 (m, 1 H, H-4′), 4.20−4.14 (m, 2 H, H-5′), 4.06 (m, 1 H, H-5″a), 3.97 (m, 1 H, H-5″b), 3.20 (q, 6 H, $-CH_2N$, J = 7.3 Hz), 3.14 (m, 1 H, H-6″a), 2.91 (m, 1 H, H-4″), 2.80 (m, 1 H, H-6″b), 1.65, 1.64, 1.46, 1.43 (each s, each 3 H, isopropyl CH_3), 1.28 (t, 9 H, $C\underline{H}_3CH_2N$, J = 7.3 Hz); ^{13}C NMR (D_2O, 125 MHz) δ 153.7, 149.2, 148.0, 122.6, 117.4, 115.2, 94.3, 89.7, 89.2, 89.1, 87.3, 86.3, 84.2, 72.1, 69.2, 67.3, 49.5, 46.9, 46.8, 31.0, 28.9, 28.8, 27.1, 26.9, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ −10.39 (d, J = 15.3 Hz), −10.80 (d, J = 15.3 Hz); HRMS (FAB, negative) calcd for $C_{22}H_{30}N_5O_{12}P_2$ 618.1366 [(M − H)$^-$], found 618.1413; UV (H_2O) λ_{max} = 258 nm.

Cyclic ADP-carbocyclic-ribose (4). A solution of **32** (128 OD_{260} units) in 60% aqueous HCO_2H (1 mL) was stirred at room temperature for 3.5 h. After the solvent was evaporated, H_2O (2 mL × 3) was added to the residue and evaporated. To the residue was added TEAB buffer (0.1 M, 30 μL) and H_2O (2 mL), and the resulting solution was lyophilized to give **4** [115 OD_{260} units, 90%] as a triethylammonium salt: ^{1}H NMR (D_2O, 400 MHz, J value was measured by spin decoupling) δ 9.14 (s, 1 H, H-2), 8.38 (s, 1 H, H-8), 6.06 (d, 1 H, H-1′, $J_{1',2'}$ = 6.3 Hz), 5.14 (dd, 1 H, H-2′, $J_{2',1'}$ = 6.3, $J_{2',3'}$ = 4.4 Hz), 4.95 (ddd, 1 H, H-1″, $J_{1'',2''}$ = 4.4, $J_{1'',6''a}$ = 10.2, $J_{1'',6''b}$ = 2.9 Hz), 4.61 (dd, 1 H, H-3′, $J_{3',2'}$ = 4.4, $J_{3',4'}$ = 2.4 Hz), 4.54 (ddd, 1 H, H-5′a, $J_{5'a,4'}$ = 7.7, $J_{5'a,5'b}$ = 10.2, $J_{5'a,P}$ = 2.4 Hz), 4.41 (dd, 1 H, H-2″, $J_{2'',1''}$ = 4.4, $J_{2'',3''}$ = 3.9 Hz), 4.41 (ddd, 1 H, H-4′, $J_{4',3'}$ = 2.4, $J_{4',5'a}$ = 7.7, $J_{4',5'b}$ = 3.4 Hz), 4.24 (dd, 1 H, H-3″, $J_{3'',2''}$ = 3.9, $J_{3'',4''}$ = 3.9 Hz), 4.19 (m, 2 H, H-5″), 4.11 (ddd, 1 H, H-5′b, $J_{5'b,4'}$ = 3.4, $J_{5'b,5'a}$ = 10.2, $J_{5'b,P}$ = 3.4 Hz), 3.18 (q, 6 H, $-CH_2N$, J = 7.3 Hz), 3.07 (ddd, 1 H, H-6″a, $J_{6''a,1''}$ = 10.2, $J_{6''a,4''}$ = 10.7, $J_{6''a,6''b}$ = 15.1 Hz), 2.55 (m, 1 H, H-4″), 2.41 (ddd, 1 H, H-6″b, $J_{6''b,1''}$ = 2.9, $J_{6''b,4''}$ = 3.4, $J_{6''b,6''a}$ = 15.1 Hz), 1.28 (t, 9 H, $C\underline{H}_3CH_2N$, J = 7.3 Hz); NOE (D_2O, 400 MHz) irradiated H-2, observed H-2″ (4.8%), H-3″ (3.9%), H-6″b (7.2% %), irradiated H-8, observed H-1′ (14.4%), irradiated H-6″b, observed H-2 (14.5%); ^{13}C NMR (D_2O, 125 MHz) δ 154.0 (C-6), 149.2 (C-4), 147.9 (C-8), 147.0 (C-2), 122.8 (C-5), 93.3 (C-1′), 87.7 'C-4′), 81.1 (C-2″), 76.6 (C-3″), 76.2 (C-2′), 73.5 (C-3′), 68.1 (C-5″), 67.5 (C-5′), 66.8 (C-1″), 49.5 ($-CH_2N$), 45.6 (C-4″), 31.1 (C-6″), 11.0 ($C\underline{H}_3CH_2N$); ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ −9.20 (d, J = 11.4 Hz), −10.26 (d, J = 11.4 Hz); HRMS (FAB, negative) calcd for $C_{16}H_{22}N_5O_{12}P_2$ 538.0740[(M − H)$^-$], found 538.0723; UV (H_2O) λ_{max} = 259 nm (ϵ = 11100, based on the total phosphate analysis), UV (pH 11.5, 25 mM phosphate buffer) λ_{max} = 261 nm, sh 291 nm. The potassium salt of **4** was prepared by the successive treatments of the above triethylamomonium salt with Diaion PK-212 resign (H^+ form) and with the same resign (K^+ form).

Chemical Stability of cADPR (1) and cADPcR (4). A buffer (pH 2.0, 50 mM KCl−HCl buffer; pH 7.0, 100 mM TEAA buffer; pH 12.0, 50 mM KCl−NaOH buffer) containing cADPR (free acid, Yamasa, Tokyo, Japan) or cADPcR (potassium salt) (1.0 OD_{260} unit) was incubated at 37 °C. The reaction mixtures (10 μL) were sampled after 2.0, 4.0, 6.0, 8.0, 24, 48, 72 h, and were then added to a TEAA buffer (pH 7.0, 100 mM, 90 μL) at 0 °C. The resulting solutions (50 μL) were analyzed by reverse phase and ion exchange HPLC (TSK-GEL DEAE−2SW, 4.6 mm × 250 mm; 0−300 mM HCO_2NH_4/20% MeCN, 20 min; 260 nm).

The Dimroth Rearrangement of cADPcR (4) Forming N^6-Cyclic ADP-carbocyclic-ribose (34). A solution of **4** (potassium salt, 115 OD_{260} units, 9.3 μmol) in a KCl−NaOH buffer (50 mM, pH 12.0, 4 mL) was stirred at 37 °C for 4 days. After addition of H_2O (90 mL), the pH of the mixture was adjusted about 4.0 with AcOH, and the resulting solution was applied to a DEAE-Sephadex A-25 column (HCO_3^- form, 1.5 cm × 20 cm). The column was developed using a linear gradient of 0.1−0.5 M TEAB buffer (pH 7.8, 400 mL). Appropriate fractions were evaporated and coevaporated with H_2O (three times) to remove excess TEAB. The residue was lyophilized to give **34** (106 OD_{265} units, 6.5 μmol, 70%; the yield was calculated using the molar absorption coefficient of N^6-methyladenosine (ϵ =16300, λ_{max} = 265 nm) [4b] as a triethylammonium salt: ^{1}H NMR (D_2O,

500 MHz) δ 8.51, 8.38 (each s, each 1 H, H-2, H-8), 6.17 (s, 1 H, H-1′), 4.75 (m, 1 H, H-2′), 4.64 (m, 1 H, H-3′), 4.60 (m, 1 H, H-2″), 4.42 (m, 1 H, H-3″), 4.36 (m, 1 H, H-4′), 4.24 (m, 1 H, H-1″), 4.10−3.94 (m, 4 H, H-5′, H-5″), 3.20 (q, 6 H, $-CH_2N$, J = 7.3 Hz), 2.38 (m, 1 H, H-6″a), 2.30 (m, 1 H, H-4″), 2.11 (m, 1 H, H-6″b), 1.28 (t, 9 H, $C\underline{H}_3CH_2N$, J = 7.3 Hz); ^{31}P NMR (D_2O, 202 MHz, decoupled with $^1\underline{H}$) δ −9.25 (m), −9.69 (m); HRMS (FAB, negative) calcd for $C_{16}H_{22}N_5O_{12}P_2$ 538.0740 [$(M - H)^-$], found 538.0752; UV (H_2O) λ_{max} = 271 nm (pH 7.0), UV (pH 11.5, 25 mM phosphate buffer) λ_{max} = 271 nm; ^{13}C NMR (potassium salt, D_2O, 125 MHz) δ 156.6 (C-6), 154.3 (C-2), 151.2 (C-4), 143.4 (C-8), 122.1 (C-5), 93.0 (C-1′), 85.8 (C-4′), 78.8 (C-2″), 76.1 (C-2′), 75.4 (C-3″), 72.1 (C-3′), 68.4 (C-5″), 65.9 (C-5′), 60.1 (C-1″), 44.9 (C-4″), 32.3 (C-6″).

Stabilitiy of cADPR (1) and cADPcR (4) in Membrane Fraction of Rat Brain Extract. Rat brain extract was prepared by a procedure according to the previous method.[16] cADPR (free acid) or cADPcR (potassium salt) (1.0 OD_{260} unit) was preincubated in 8 mM Hepes-Pipes-Tris buffer (pH 7.0, 8 mM, 150 μL) at 37 °C for 5 min. This was added to the solution of the membrane fraction of rat brain extract (100 μL), and the mixture was incubated at 37 °C. The reaction mixture was sampled (25 μL) at every 30 min afterward and diluted with water (175 μL), which was frozen in liquid nitrogen to stop the reaction. After the samples were a centrifuged at 12000 rpm at 4 °C for 15 min, supernatants were filtered using a centrifugal filter at 12000 rpm at 4 °C for 15 min, and the resulting filtrates (50 μL) were analyzed by ion-exchange HPLC (TSK-GEL DEAE−2SW, 4.6 mm × 250 mm; 0−300 mM HCO_2NH_4/20% MeCN, 20 min; 260 nm).

Treatments of cADPR (1) and cADPcR (4) by CD38 cADPR Hydrolase. ThioHis-CD38 cADPR hydrolase was expressed in *Escherichia coli* BL21(DE3) cells and purified from crude cell extract using Ni^{2+}-chelating column (Invitrogen) according to the reported method.[18] cADPR (free acid) or cADPcR (potassium salt) (0.5 OD_{260} unit) was preincbated in Hepes-Pipes-Tris buffer (pH 7.0, 5 mM, 230 μL) at 37 °C for 5 min. To the solution was added CD38 solution (20 μL),[18] and the resulting mixture was incubated at 37 °C. The reaction mixture was sampled (40 μL) at every 15 min, added to water (160 μL), and frozen in liquid nitrogen to stop the reaction. After the samples were centrifuged at 12000 rpm at 4 °C for 15 min, the supernatants were filtered using a centrifugal filter at 12000 rpm at 4 °C for 30 min, and the resulting filtrates (125 μL) were analyzed by ion-exchange HPLC (TSK-GEL DEAE−2SW, 4.6 mm × 250 mm; 0−300 mM HCO_2NH_4/20% MeCN, 20 min; 260 nm).

Measurement of Ca^{2+}-Mobilizing in Sea Urchin Eggs. Eggs of a species of Japanese sea urchin, *Anthocidaris crassispina*, were shed into filtrated seawater (FSW) by injection of 0.5 M KCl into the coelomic cavity, and the shed eggs were washed three times with FSW. The eggs were stripped of their jelly coats by washing twice with Ca^{2+}- and Mg^{2+}-free artificial seawater [CaMgFASW; 520 mM NaCl, 10 mM KCl, 10 mM 3-[4-(2-hydroxyethyl)-1-piperazinyl]-propanesulfonic acid (Epps), titrated to pH 8.2 with tris(hydroxymethyl)aminomethane (Tris), and 2 mM ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA)], then were rinsed twice more with standard artificial seawater (stdASW; 430 mM NaCl, 10 mM KCl, 50 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM Epps, 12.5 mM Tris, pH 8.2) and were finally resuspended in fresh stdASW. Sperm were likewise shed by injecting 0.5 M KCl, collected directly from the genital pores, stored at 4 °C until use, and diluted 5000-fold into stdASW just before use. $[Ca^{2+}]_i$ was measured with a Ca^{2+}-sensitive fluorescent dye, indo-1 (Dojindo, Kumamoto, Japan). Each egg cell was fastened to a poly-L-lysine (M_r = 1000−4000; Sigma) coated glass coverslip at the bottom of 150 μL stdASW in a Lucite-frame chamber, mounted on the stage of a TMD epifluorescence microscope (Nikon, Tokyo, Japan). The dye was pressure-injected into eggs at a final concentration of 100 μM, from a 10 mM stock solution containing 100 mM potassium aspartate and 10 mM Hepes, titrated to pH 7.0 with Tris, according to the method of Hiramoto.[22] For measuring the fluorescence of indo-1, the selected wavelengths were 355 nm (band-pass) at the excitation filter, 380 nm (cutoff) at the dichroic mirror, and simultaneously 405 nm (band-pass) and 485 nm (band-pass) at the emission detectors, which were Hamamatsu R647-01 photomultipliers (Hamamatsu Photonics, Hamamatsu, Japan). Fluorescence from the whole egg was measured, and $[Ca^{2+}]_i$ was calculated from the ratio of 405-nm fluorescence intensity to the corresponding 485-nm intensity, according to the previously reported method.[23] Under the monitoring of $[Ca^{2+}]_i$, cADPR or cADPcR was injected into eggs in the dark. These chemicals were dissolved in injection buffer, were filled in the tip of micropipet, and were pressure-injected ∼1 min after impalement of the micropipet into eggs. Experiments were performed at 20−25 °C.

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Supporting Information Available: Figures showing stability of cADPcR (**4**) and cADPR (**1**) in aqueous media, stability of cADPcR (**4**) and cADPR (**1**) in rat brain extract, and susceptibility of cADPcR (**4**) and cADPR (**1**) to CD38 cADPR hydrolase; ^{1}H NMR data table of cADPR (**1**) and cADPcR (**4**) in D_2O; ^{1}H NMR spectral charts of **22**, **30**, **14**, **32**, and **4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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