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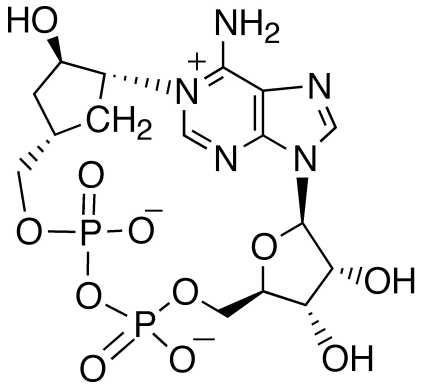
J. Am. Chem. Soc., **2005**, 127 (24), 8846-8855• DOI: 10.1021/ja050732x • Publication Date (Web): 26 May 2005



3"-deoxy-cADPcR

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Synthesis of Stable and Cell-type Selective Analogues of Cyclic ADP−Ribose, a Ca^{2+}-Mobilizing Second Messenger. Structure−Activity Relationship of the N1-Ribose Moiety[1]

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Abstract: We previously developed cyclic ADP−carbocyclic ribose (cADPcR, **2**) as a stable mimic of cyclic ADP−ribose (cADPR, **1**), a Ca^{2+}-mobilizing second messenger. A series of the N1-ribose modified cADPcR analogues, designed as novel stable mimics of cADPR, which were the 2″-deoxy analogue **3**, the 3″-deoxy analogue **4**, the 3″-deoxy-2″-*O*-(methoxymethyl) analogue **5**, the 3″-*O*-methyl analogue **6**, the 2″,3″-dideoxy analogue **7**, and the 2″,3″-dideoxydidehydro analogue **8**, were successfully synthesized using the key intramolecular condensation reaction with phenylthiophosphate-type substrates. We investigated the conformations of these analogues and of cADPR and found that steric repulsion between both the adenine and N9-ribose moieties and between the adenine and N1-ribose moieties was a determinant of the conformation. The Ca^{2+}-mobilizing effects were evaluated systematically using three different biological systems, i.e., sea urchin eggs, NG108-15 neuronal cells, and Jurkat T-lymphocytes. The relative potency of Ca^{2+}-mobilization by these cADPR analogues varies depending on the cell-type used: e.g., 3″-deoxy-cADPcR (**4**) > cADPcR (**2**) > cADPR (**1**) in sea urchin eggs; cADPR (**1**) ≫ cADPcR (**2**) ≈ 3″-deoxy-cADPcR (**4**) in T-cells; and cADPcR (**2**) > cADPR (**1**) > 3″-deoxy-cADPcR (**4**) in neuronal cells, respectively. These indicated that the target proteins and/or the mechanism of action of cADPR in sea urchin eggs, T-cells, and neuronal cells are different. Thus, this study represents an entry to cell-type selective cADPR analogues, which can be used as biological tools and/or novel drug leads.

Introduction

Cyclic ADP−ribose (cADPR, **1**, Figure 1), a naturally occurring metabolite of NAD^+ originally reported by Lee and co-workers,[2] has been shown to mobilize intracellular Ca^{2+} in various cells, such as sea urchin eggs, pancreatic β-cells, smooth muscles, cardiac muscles, T-lymphocytes, and cerebellar neurons, indicating that it is a general mediator involved in Ca^{2+} signaling.[3]

cADPR has the characteristic 18-membered cyclic structure consisting of an adenine, two (N1- and N9-) riboses, and a pyrophosphate, in which the two primary hydroxy groups of the riboses are linked by a pyrophosphate unit.[4] Under neutral conditions, cADPR is in a zwitterionic form with a positive charge around the N(1)−C(6)−N^6 moiety (pK_a = 8.3), making the molecule extremely unstable. The charged adenine moiety attached to the anomeric carbon of the N1-ribose can be a very efficient leaving group. Accordingly, cADPR is readily hydrolyzed at the unstable N1-ribosyl linkage of its adenine moiety

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(1) This report constitutes Part 234 of Nucleosides and Nucleotides. For Part 233: Murata, S.; Ichikawa, S.; Matsuda, A. *Tetrahedron*, in press.

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10.1021/ja050732x CCC: $30.25

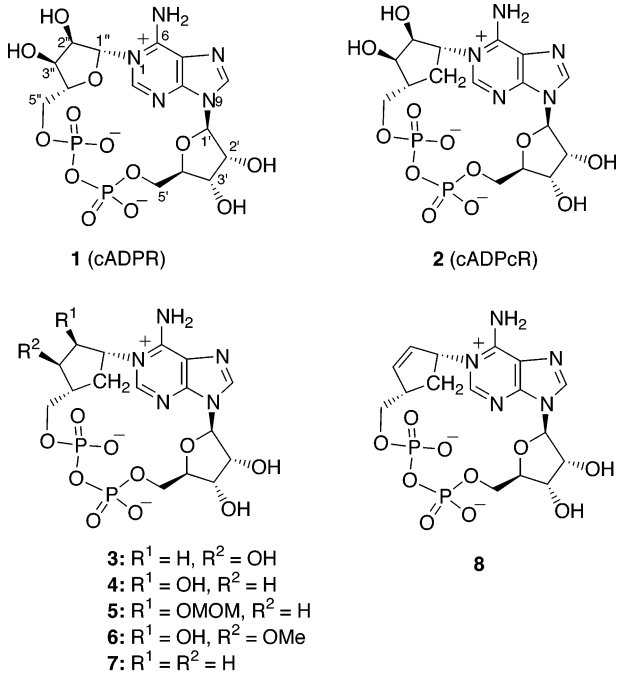

Figure 1. cADPR (**1**), cADPcR (**2**), and the N1-ribose-modified cADPcR analogues **3**−**8**.

to produce ADP−ribose (ADPR), even in neutral aqueous solution.[5] Under physiological conditions, cADPR is also hydrolyzed at the N1-ribosyl linkage by cADPR hydrolase to give the inactive ADPR.[5]

cADPR analogues can be used in investigating the mechanism of cADPR-mediated Ca^{2+} signaling pathways. Agonists or antagonists of cADPR are also expected to be lead structures for the development of drugs, since cADPR has been shown to play important physiological roles, such as insulin release from β-cells.[6] Therefore, the synthesis of cADPR analogues has been extensively investigated.[7−10] However, because of the unique structure and instability of cADPR, chemical synthesis of cADPR and its analogues has proved to be rather difficult.[7a−e] Consequently cADPR analogues have been synthesized predominantly by enzymatic and chemoenzymatic methods using ADP−ribosyl cyclase-catalyzed cyclization under mild neutral conditions.[8] By this method, cADPR analogues modified in the purine ring and the N9-ribose moieties have been prepared from the corresponding β-nicotinamide dinucleotide-type precursors.[8] Although evaluation of these analogues has identified biologically useful analogues, the analogues obtained by these methods are limited due to the substrate specificity of the ADP−ribosyl cyclase.[10a,b]

In recent years, on the other hand, methods for the chemical synthesis of cADPR analogues have been extensively studied, and useful cADPR analogues, which could not be prepared by the enzymatic and chemoenzymatic methods, have been synthesized.[7,9,10] In the synthesis of cADPR and its analogues, construction of the large 18-membered ring structure is the key step, and we recently developed an efficient method for forming the 18-membered ring employing phenylthiophosphate-type substrates.[7d−f] When these substrates were activated by $AgNO_3$ or I_2 in the presence of molecular sieves in pyridine, the corresponding 18-membered ring products were obtained in high yields. Using this method, we accomplished the total synthesis of cyclic ADP−carbocyclic ribose (cADPcR, **2**),[7f] designed as a stable mimic of cADPR, in which the oxygen atom in the N1-ribose ring of cADPR is replaced by a methylene group. We showed (1) that the mimic is actually resistant to both enzymatic and chemical hydrolysis, since it has a chemically and biologically stable *N*-alkyl linkage instead of the unstable N1-glycosidic linkage of cADPR, (2) that the mimic has a conformation similar to that of cADPR, and (3) that the mimic, like cADPR, effectively mobilizes intracellular Ca^{2+} in sea urchin eggs.[7f]

As other second messengers, cADPR functions generally in many organs,[3] and accordingly, cADPR analogues can be nonselectively active irrespective of cell-types, which may be undesirable in their use as a biological tool or a drug lead. Thus, with a biologically and chemically stable cADPcR with potent Ca^{2+}-mobilizing activity in hand, we focused our attention on the development of analogues with cell-type selectivity based on the structure of cADPcR.

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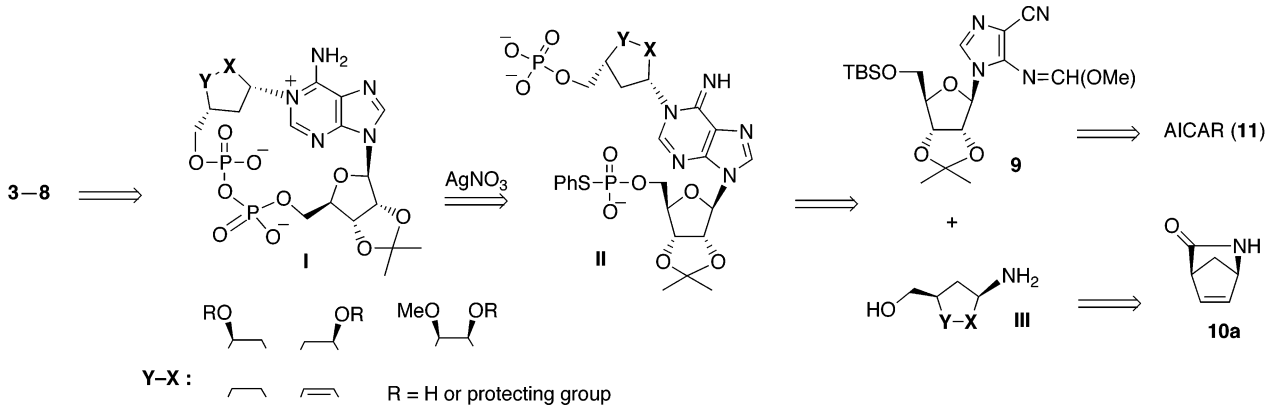
Scheme 1



The previous studies of enzymatically or chemoenzymatically synthesized cADPR analogues have disclosed the structure−activity relationship (SAR) for the N9-ribose and adenine moieties,[8,10] at least to some extent. Nevertheless, the SAR of the N1-ribose moiety remains virtually unknown, since N1-ribose-modified analogues are difficult to access by the chemoenzymatic and enzymatic route.[7g] In the enzymatic and the chemoenzymatic synthesis, preparation of sugar-modified NAD-type precursors is crucial. Furthermore, even if these sugar-modified NAD analogues are provided, the cyclase may still not catalyze their desired cyclization.[10a,b] Therefore, a chemical synthetic method is essential for providing these biologically important N1-ribose-modified analogues.

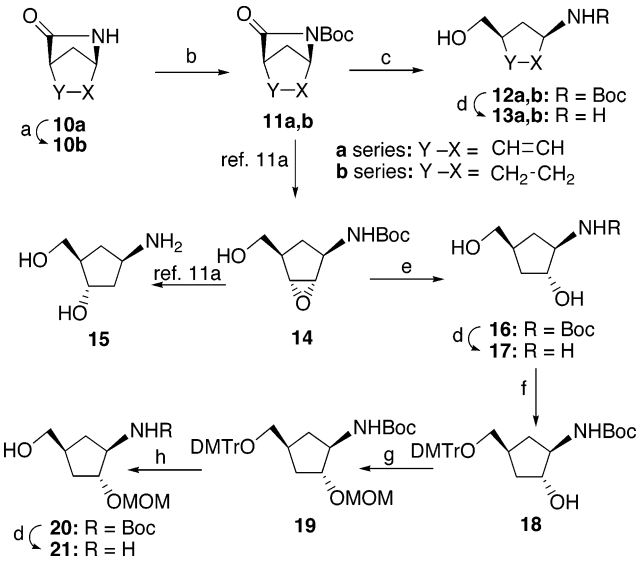
These results and considerations prompted us to synthesize the N1-ribose-modified analogues to clarify the SAR and also to develop compounds of further biological importance. Therefore, we designed a series of N1-ribose-modified analogues of cADPcR, namely, the 2″-deoxy analogue **3**, the 3″-deoxy analogue **4**, the 3″-deoxy-2″-*O*-(methoxymethyl) (MOM) analogue **5**, the 3″-*O*-methyl (Me) analogue **6**, the 2″,3″-dideoxy analogue **7**, and the 2″,3″-dideoxydidehydro analogue **8**, the structures of which are shown in Figure 1. We describe here the synthesis and SAR of these N1-ribose-modified analogues. In this study, the Ca^{2+}-mobilizing effects of the compounds were evaluated systematically using three different biological systems, i.e., sea urchin eggs, Jurkat T-lymphocytes, and NG108-15 neuronal cells, to determine if the target proteins and/or the mechanism of action of cADPR in these three-types of cells were different.

Results and Discussion

Synthetic Plan. As described above, we recently developed an efficient chemical synthetic method for cADPR analogues,[7d−f] which we[7g,h] and other groups[9] have used in the synthesis of a variety of cADPR analogues. Since it was unlikely that the target N1-ribose-modified cADPcR analogues **3**−**8** would be accessible by the chemoenzymatic method, we planned to synthesize them by a route based on the total synthetic method, as summarized in Scheme 1.

The chiral carbocyclic amines **III**, composing the modified N1-ribose moiety in the targets **3**−**8**, could probably be prepared from commercially available (1*R*)−(−)-2-azabicyclo[2.2.1]hept-5-en-3-one (**10a**). From these carbocyclic amines **III** and the known imidazole nucleoside derivative **9**,[7f] the 5′-phenylthiophosphate-type substrates **II** for the key intramolecular condensation could be prepared. Treatment of **II** with $AgNO_3$/MS 3A as a promoter[7d−f] was expected to form the cyclized products **I**, and subsequent acidic treatment for deprotection would furnish the target N1-ribose-modified analogues **3**−**8**.

Scheme 2 [a]



[a] Reagents and conditions: (a) H_2, Pd−C, MeOH, rt, quant.; (b) Boc_2O, DMAP, MeCN, rt, 88% (**11a**), quant. (**11b**); (c) $NaBH_4$, MeOH, 0 °C, 95% (**12a**), 97% (**12b**); (d) H_2O, reflux, quant. (**13a**, **b**, **17**, **21**); (e) Red-Al, CH_2Cl_2, 0 °C, 49%; (f) DMTrCl, py, rt, 87%; (g) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 96%; (h) 50% aq. AcOH, 83%.

Preparation of the Chiral Carbocyclic Amines. The carbocyclic amine units **13a**,**b**, **15**, and **21** were synthesized from the optically active bicyclic lactam **10a**, as summarized in Scheme 2. Catalytic hydrogenation of **10a** gave the corresponding saturated lactam **10b**. *N*-Boc protection of **10a** and **10b** followed by reductive ring cleavage with $NaBH_4$/MeOH afforded **12a** and **12b**. Removal of the Boc group by heating **12a**,**b** in H_2O gave the units **13a**,**b**, respectively.

Preparation of the 2-deoxy unit **15** and the 3-deoxy unit **17** was attempted via the 2,3-epoxide **14** according to the previously reported method[11a] with a slight modification. Following the reported reaction conditions for the regioselective reductive cleavage at the 3-position of **14** with Red-Al using toluene as the reaction solvent,[11a] we could not obtain the 3-deoxy product

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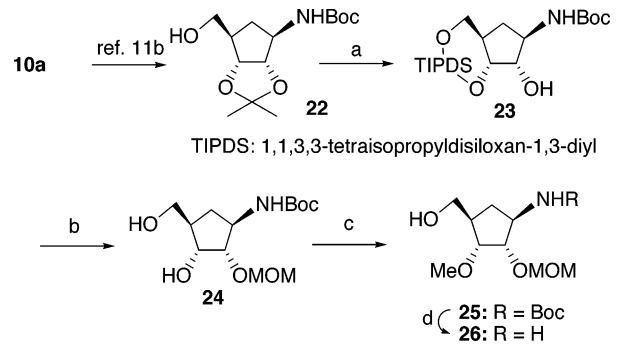
Scheme 3 [a]



[a] Reagents and conditions: (a) (1) 80% aq. AcOH, rt and (2) $TIPDSCl_2$, py, rt, 67%; (b) (1) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt and (2) TBAF, AcOH, THF, rt, 88%; (c) (1) DMTrCl, py, rt, (2) MeI, NaH, THF, 0 °C, and (3) 60% aq AcOH, rt, 84%; (d) H_2O, reflux, quant.

16, probably due to the insolubility of **14** in toluene. However, when CH_2Cl_2 was used as the solvent in the Red-Al reduction, the desired **16** was isolated in 49% yield along with the 2-deoxy regioisomer (14%). Removal of the Boc group of **16** gave the 3-deoxy carbocyclic unit **17**.

The 3-deoxy-2-*O*-MOM unit **21** was prepared from **16**. Dimethoxytrityl (DMTr) protection of the primary hydroxyl of **16**, methoxymethylation of the 2-hydroxy, and removal of the DMTr group followed by removal of the Boc group gave **21**.

The known carbocyclic amine **22**, prepared from **10a** by a literature procedure,[11b] was converted to the 3-*O*-Me-2-*O*-MOM unit **26** in several reaction steps, as shown in Scheme 3.

Synthesis of the cADPcR Analogues. The 2″,3″-dideoxy-type cADPcR analogues **7** and **8** were successfully synthesized from the carbocyclic amine **13a** or **13b** and the imidazole nucleoside **9**, as shown in Scheme 4.

The 2″,3″-dideoxy-N1-carbocyclic ribosyl derivatives **27a**,**b** were obtained in high yield by the treatment of a mixture of **9** and either amine **13a** or amine **13b** with K_2CO_3 in MeOH at room temperature. The 5″-hydroxy group of **27a** or **27b** was protected with a monomethoxytrityl (MMTr) group, and the 5′-*O*-TBS group of the product **28a** or **28b** was removed with TBAF to give **29a** or **29b**. Treatment of **29a** or **29b** with an *S*,*S*′-diphenylphosphorodithioate (PSS)/2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl)/pyridine system[12] gave the 5′-bis(phenylthio)phosphate **30a** or **30b**, respectively. The 5″-*O*-MMTr group of **30a** or **30b** was removed, and a phosphoryl group was introduced at the resulting 5″-primary hydroxyl of **31a** or **31b** by Yoshikawa's method with $POCl_3/(EtO)_3PO$,[13] followed by treatment of the product with H_3PO_2 and Et_3N[14] in the presence of *N*-methylmaleimide (NMM) in pyridine,[7f] to afford the corresponding 5′-phenylthiophosphate **32a** or **32b**, respectively, the substrate of the key intramolecular condensation reaction. When a solution of **32a** in pyridine was added slowly to a mixture of a large excess of $AgNO_3$ and Et_3N in the presence of MS 3A in pyridine at room temperature,[7c,e,f] the desired cyclization product **33a** was obtained in 84% yield. Similarly, the other substrate **32b** was condensed to give the cyclization product **33b** in 81% yield. Finally, removal of the isopropylidene groups of **33a** and **33b** with aqueous HCO_2H furnished 2″,3″-dideoxy-cADPcR (**7**) and its 2″,3″-unsaturated analogue **8**, respectively.

Using similar routes, we synthesized the other N1-ribose-modified analogues of cADPcR, namely, the 2″-deoxy analogue **3**, the 3″-deoxy analogue **4**, the 3″-deoxy-2″-*O*-MOM analogue **5**, and the 3″-*O*-Me analogue **6** from the corresponding carbocyclic amines **15**, **21**, or **26** and the imidazole nucleoside **9**, as summarized in Scheme 5.

Ca^{2+}-Mobilizing Activity in Sea Urchin Egg Homogenate. We tested the Ca^{2+}-mobilizing ability of the newly synthesized analogues as well as cADPR (**1**) and cADPcR (**2**) by fluorometrically monitoring Ca^{2+} with *H. pulcherrimus* sea urchin egg homogenate.[15] cADPR released Ca^{2+} from the homogenate in a dose-dependent manner, with an EC_{50} value of 210 nM, and cADPcR showed more potent activity (EC_{50} = 79 nM) than cADPR, where the maximal Ca^{2+}-mobilizing activity of cADPcR was almost equal to that of cADPR (Figure 2A). These results are in accordance with previous reports that cADPcR acts as a potent activator of the cADPR-induced Ca^{2+} release in sea urchin eggs of *A. crassispina* or *L. pictus*.[7f,h]

The dose−response curves of the N1-ribose-modified analogues are shown in Figures 2A,B. All these compounds mobilized Ca^{2+} with the same maximal activity as cADPR and cADPcR. Deletion of the 2″-hydroxy group (**3**) resulted in a marked reduction of potency (EC_{50} = 0.61 μM). However, to our surprise, deletion of the 3″-hydroxy group (**4**) greatly potentiated the Ca^{2+}-mobilizing ability (EC_{50} = 14 nM), which was about 15-fold and 6-fold more potent than natural cADPR and cADPcR, respectively. These results clearly demonstrate that the two hydroxy groups play different roles in the ligand−receptor recognition: the 2″-hydroxy group enhances the affinity of cADPR for the target biomolecule, whereas the 3″-hydroxy group reduces it. It also shows that the 3″-hydroxy group itself has no direct effect on the ligand-target biomolecule recognition, because **7** (EC_{50} = 0.73 μM) showed potency similar to **3**.

We next tested analogues with modifications of the hydroxy groups (Figure 2B) in our continuing investigation into the importance of these hydroxy groups. Methylation of the 3″-hydroxy group (**6**) resulted in a greater than 10-fold reduction of potency (EC_{50} = 0.88 μM) compared with cADPcR. 3″-Deoxy-2″-*O*-MOM-cADPcR (**5**) was more than 400-fold less potent (EC_{50} = 5.7 μM) than 3″-deoxy cADPcR. Thus, replacement of the hydroxy groups with a methyl or a larger substituent reduced the affinity for the receptor.

Introduction of an unsaturated bond between the 2″ and 3″ carbons (**8**) dramatically reduced the Ca^{2+}-mobilizing activity (EC_{50} > 20 μM) compared with **7** without the unsaturated bond. These results suggest that the conformation of the ribose moiety may be crucial in determining the affinity for the receptor.

Ca^{2+}-Mobilizing Activity in T-cells. The pharmacological activity of the cADPcR derivatives was analyzed in permeabilized and intact human Jurkat T-lymphocytes,[16,17] as de-

(12) Sekine, M.; Nishiyama, S.; Kamimura, T.; Osaki, Y.; Hata, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 850−860.
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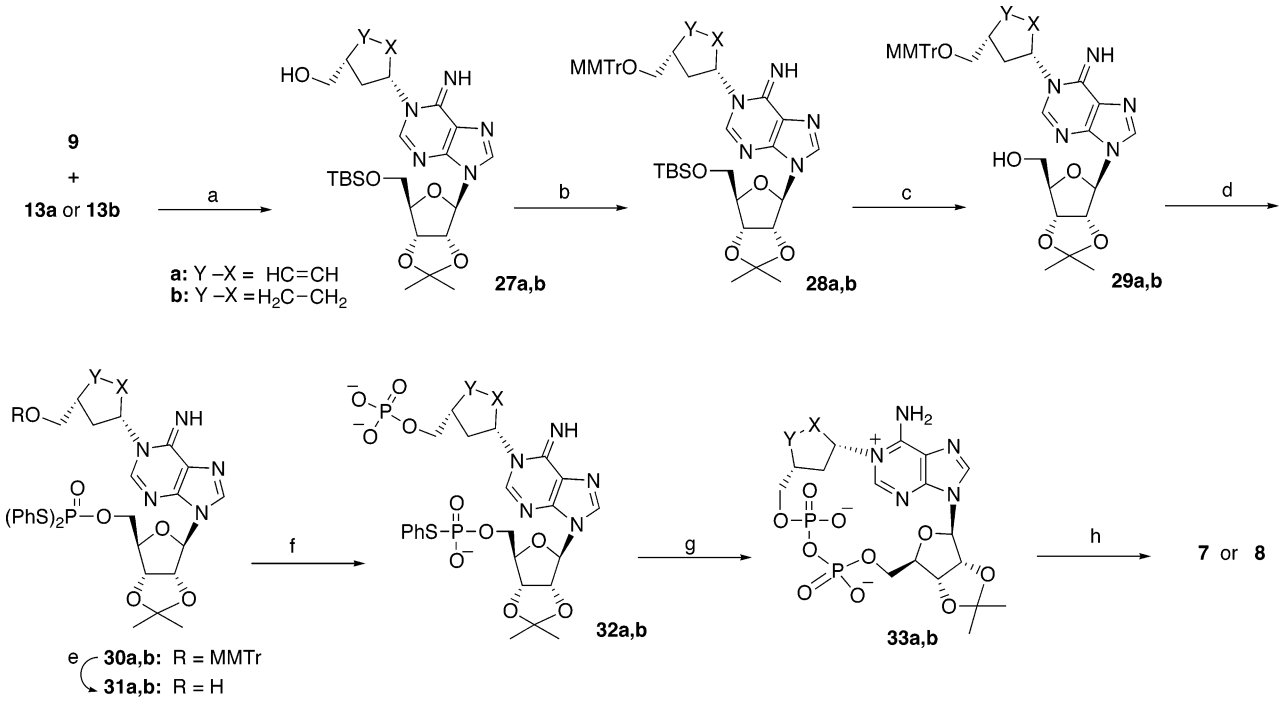
(14) Hata, T.; Kamimura, T.; Urakami, K.; Kohno, K.; Sekine, M.; Kumagai, I.; Shinozaki, K.; Miura, K. *Chem. Lett.* **1987**, 117−120.
(15) Shiwa, M.; Murayama, T.; Ogawa, Y. *Am. J. Physiol.*: *Regul. Integr. Comp. Physiol.* **2002**, *282*, R727−R737.
(16) (a) Guse, A. H.; Roth, E.; Emmrich, F. *Biochem. J.* **1993**, *291*, 447−451. (b) Guse, A. H.; Berg, I.; da Silva, C. P.; Potter, B. V. L.; Mayr, G. W. *J. Biol. Chem.* **1997**, *272*, 8546−8550. (c) Schwarzmann, N.; Kunerth, S.; Weber, K.; Mayr, G. W.; Guse, A. H. *J. Biol. Chem.* **2002**, *277*, 50636−50642.
(17) Guse, A. H.; Berg, I.; da Silva, C. P.; Potter, B. V. L.; Mayr, G. W. *J. Biol. Chem.* **1997**, *272*, 8546−8550.

Scheme 4 [a]



[a] Reagents and conditions: (a) K_2CO_3, MeOH, rt, 82% (**27a**), 85% (**27b**); (b) MMTrCl, pyridine, rt, 84% (**28a**), 97% (**28b**); (c) TBAF, AcOH, THF, rt, 97% (**29a**), quant. (**29b**); (d) PSS, TPSCl, py, rt, 54% (**30a**), 69% (**30b**); (e) aq 80% AcOH, rt, 77% (**31a**), 85% (**31b**); (f) (1) $POCl_3$, $(EtO)_3PO$, 0 °C and (2) H_3PO_2, Et_3N, NMM, pyridine, 0 °C, 64% (**32a**), 75% (**32b**); (g) $AgNO_3$, MS 3A, Et_3N, py, rt, 84% (**33a**), 81% (**33b**); (h) aq 60% HCO_2H, rt, quant. (**7**, **8**)

scribed previously.[18] Addition of cADPR, used here as a positive control, immediately released Ca^{2+} from permeabilized cells (Figure 3A). Replacement of cADPR by one of the six cADPcR analogues resulted in weaker Ca^{2+} release (Figure 3A,B). Compounds **3**, **7**, and **8** were similar in their efficacy to release Ca^{2+}, while **4**, **6**, and **5** only had a small effect on Ca^{2+} release (Figure 3B). It is interesting that **4**, the most potent one in the above sea urchin system, showed only a slight effect in T-cells.

Because of its significant Ca^{2+}-mobilizing activity in permeabilized cells and its enhanced hydrophobic nature as compared to cADPR, **8** was also tested for its ability to induce Ca^{2+} signaling in intact Jurkat T cells (Figure 3C,D). Addition of 1 mM of **8** (extracellular concentration) to intact cells resulted in a somewhat delayed but marked increase in the free cytosolic and nuclear Ca^{2+} concentration ($[Ca^{2+}]_I$) as measured by single-cell Ca^{2+} imaging (Figure 3C). The variability between individual cells both in amplitude and in delay of signal onset reflects the natural behavior of these T cells; it can also be observed when a physiological stimulus is used.

One of the underlying mechanisms of Ca^{2+} signaling is the development of local subcellular pacemaker signals into global Ca^{2+} signals. Using confocal Ca^{2+} imaging at the subcellular level, we were able to obtain the induction of localized small Ca^{2+} signals close to the cell border (Figure 3D; image at 132.6 s; arrow) by extracellular addition of **8**. With time isolated neighboring Ca^{2+} signals merged into larger signals suggesting recruitment of additional Ca^{2+} release microdomains (Figure 3D; image at 136.3 s). Finally, individual signals merged into the global signal (Figure 3D; image at 137.9 s). These results demonstrate that the two hydroxy groups of the N1-ribose moiety are not essential for the Ca^{2+}-mobilizing activity of cADPR in T-cells.

The SAR on the N1-ribose moiety is significantly different in the two evaluation systems: in sea urchin egg homogenate, **4** > **2** > **1** > **3** ≈ **7** ≈ **6** > **5** > **8**; in T-cells, **1** > **3** ≈ **7** ≈ **8** > **4** ≈ **6** ≈ **5**.

Facilitating Effect on Ca^{2+}-Mobilization in Neuronal Cells. The effect of compounds on the depolarization-induced cytosolic Ca^{2+} elevation in NG108-15 neuronal cells was next investigated. We selected **4** and **8**, which were typically active in the sea urchin egg and the T-cell systems, respectively.

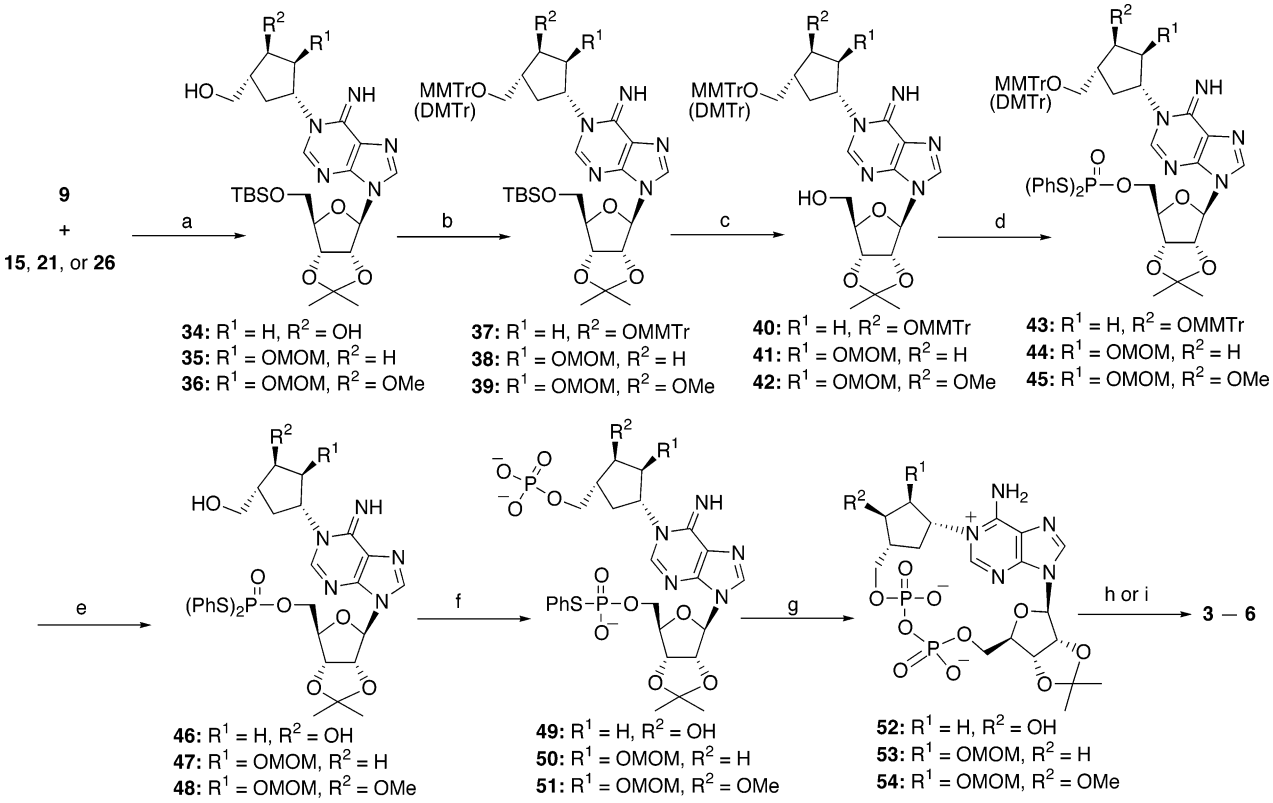
The membrane of the NG108-15 mouse neuroblastoma x rat glioma hybrid cells was sealed and disrupted with patch pipets and voltage-clamped. cADPcR and the two analogues **4** and **8** did not evoke any apparent changes in $[Ca^{2+}]_i$ at the resting membrane potential of around −40 mV immediately after injection, as in the case of cADPR.[19a]

We examined the effect of **2** and the analogues on depolarization-induced $[Ca^{2+}]_i$ elevation and found membrane depolarization from −40 to −20 mV in control cells evoked a slight $[Ca^{2+}]_i$ increase, with an average peak value of 179 ± 16% ($n = 5$) of the pre-depolarization level. This increase is due to Ca^{2+} influx by opening voltage-activated Ca^{2+} channels (VACCs) by depolarization and subsequent Ca^{2+} release by Ca^{2+}-induced Ca^{2+} release (CICR) mechanism (Figure 4A). In cells injected with 10 μM cADPR, the depolarization-induced $[Ca^{2+}]_i$ increase

(18) (a) Kunerth, S.; Mayr, G. W.; Koch-Nolte, F.; Guse, A. H. *Cell. Signalling* **2003**, *15*, 783−792. (b) Kunerth, S.; Langhorst, M. F.; Schwarzmann, N.; Gu, X.; Huang, L.; Yang, Z.; Zhang, L.; Mills, S. J.; Zhang, L.-H.; Potter, B. V. L.; Guse, A. H. *J. Cell Sci.* **2004**, *117*, 2141−2149.

(19) (a) Hashii, M.; Minabe, Y.; Higashida H. *Biochem. J.* **2000**, *345*, 207−215. (b) Higashida, H.; Hashii, M.; Yokoyama, S.; Hoshi, N.; Asai, K.; Kato, T. *J. Neurochem.* **2001**, *76*, 321−331.

Scheme 5 [a]



[a] Reagents and conditions: (a) K_2CO_3, MeOH, rt, 85% (**34**), 59% (**35**), 78% (**36**); (b) MMTrCl or DMTrCl, pyridine, rt, 94% (**37**), 97% (**38**), 95% (**39**); (c) TBAF, AcOH, THF, rt, 98% (**40**), quant. (**41**), quant. (**42**); (d) PSS, TPSCl, py, rt, 87% (**43**), 79% (**44**), 69% (**45**); (e) aq 60 or 80% AcOH, rt, 57% (**46**), 81% (**47**), 75% (**48**); (f) (1) $POCl_3$, $(EtO)_3PO$, 0 °C and (2) H_3PO_2, Et_3N, NMM, pyridine, 0 °C, 22% (**49**), 70% (**50**), 63% (**51**); (g) $AgNO_3$, MS 3A, Et_3N, py, rt, 31% (**52**), 87% (**53**), 82% (**54**); (h) aq 60% HCO_2H, rt, quant. (**3**), quant. (**5**); (i) aq 80% HCO_2H, then 28% NH_4OH, rt, 87% (**4**), 56% (**6**)

was amplified, as reported previously.[19a] The average $[Ca^{2+}]_i$ level at the peak point was increased to 274 ± 15% ($n = 3$) of the control level significantly than that of noninfused cells ($p < 0.01$, Figure 4C).

The same protocol was applied to NG108-15 cells injected with cADPcR and its analogues. Figure 4A shows three typical traces of the depolarization-induced $[Ca^{2+}]_i$ increases in cells injected with **2**, **4**, and **8** at a concentration of 10 μM. cADPcR facilitated depolarization-induced $[Ca^{2+}]_i$ increases, and the peak $[Ca^{2+}]_i$ level was 306 ± 17% ($n = 5$) of the pre-depolarization level, which is the strongest of the three compounds and also more potent than natural cADPR. 3″-Deoxy-cADPcR and 2″,3″-dideoxydidehydro-cADPcR facilitated depolarization-induced $[Ca^{2+}]_i$ increases to 238 ± 11% ($n = 7$) and 217 ± 23% ($n = 3$) of the pre-depolarization level, respectively. These responses were less effective than that in cADPcR-injected cells.

Dose−response relationships were compared between the N1-ribose-modified analogues, as shown in Figure 4C. cADPcR significantly potentiated depolarization-induced $[Ca^{2+}]_i$ increases at concentrations ranging from 100 nM to 10 μM. In sharp contrast, **4** and **8** had little or no apparent potentiating effects at concentrations below 1 μM. At the maximum dose tested (10 μM), 3″-deoxy-cADPcR showed a significant increase than the noninjected level. 2″,3″-Dideoxydidehydro-cADPcR also showed a slight potentiating effect, though it was insignificant. Our results in NG108-15 cells indicate that cADPcR and its two analogues act as agonists. However, cADPcR is the most potent ligand in NG 108-15 neuronal cells.

The most important finding in the current biological experiments proved to be the difference in sensitivity of these analogues in the three different assay systems. This may be due to the utilization of cADPR in the CICR system and may also reflect the different properties of the ryanodine receptors (RyRs) in these cells.[20]

Conformational Analysis. The three-dimensional structure of biologically active compounds in aqueous solution is very important from the viewpoint of the bioactive conformation. The conformations of **4** and **8**, which were typically active and inactive in sea urchin eggs, respectively, were constructed by molecular dynamics calculations with a simulated annealing method based on the NOE constraints of the intramolecular proton pairs measured in D_2O.[21] The structures of **1** and **2** were also obtained by the same method. The structures obtained by the calculations based on their observed NOE in the NOESY spectra (see Figure S1 in Supporting Information) are shown in Figure 5. The calculated structures of cADPR (Figure 5A) and cADPcR (Figure 5B) were analogous, each adopting a 2′-*endo* form in the N9-ribose moiety and a *syn*-form around the N9-glycosyl linkage, which was in accord with the previously reported results on the conformation of cADPR.[4] 3″-Deoxy-cADPcR prefers a similar 2′-*endo*/*syn* conformation (Figure 5C). However, the calculated structure of **8** assumes a

(20) (a) Noguchi, N.; Takasawa, S.; Nata, K.; Tohgo, A.; Kato, I.; Ikehata, F.; Yonekura, H.; Okamoto, H. *J. Biol. Chem.* **1997**, *272*, 3133−3136. (b) Wang, Y. X.; Zheng, Y. M.; Mei, Q. B.; Wang, Q. S.; Collier, M. L.; Fleischer, S.; Xin, H. B.; Kotlikoff, M. I. *Am. J. Physiol.*: *Cell. Physiol.* **2004**, *286*, C538−C546.

(21) Calculations were performed using SYBYL ver. 6.5 software (Tripos. Inc.).

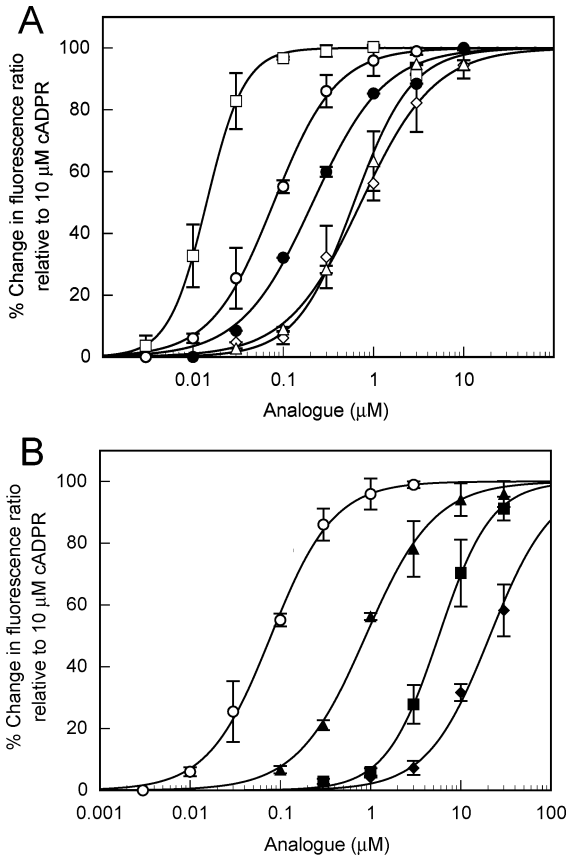


Figure 2. Ca^{2+}-mobilizing activity of compounds in sea urchin egg homogenate. The Ca^{2+}-mobilizing activity of each compound was expressed as a percent change in ratio of fura-2 fluorescence (F340/F380) relative to that of 10 μM cADPR: (A) dose-dependent activity of cADPR (**1**, filled circles), cADPcR (**2**, open circles), 2″-deoxy-cADPcR (**3**, open triangles), 3″-deoxy-cADPcR (**4**, open squares), and 2″,3″-dideoxy-cADPR (**7**, open diamonds); (B) dose-dependent activity of **2** (open circles), 3″-*O*-Me-cADPcR (**6**, filled triangles), 3″-deoxy-2″-*O*-MOM-cADPcR (**5**, filled squares), and 2″,3″-dideoxydidehydro-cADPR (**8**, filled diamonds). Data are meant to be ±SEM of three to six experiments.

3′-*endo* form in the N9-ribose moiety and a *high-anti* form around the N9-glycosyl linkage, which is demonstrably different from those of the other three compounds (Figure 5D).

The ^{1}H NMR data measured in D_2O supported the conformation of the N9-ribose moiety obtained by the above calculations. The furanose ring of nucleosides generally is in equilibrium between a C2′-*endo* and a C3′-*endo* form, and their ratio is calculated by the equation [C2′-*endo*] (%) = $[J_{1',2'}/(J_{1',2'} + J_{3',4'})] \times 100$.[22] From the *J* values summarized in Figure 5, [C2′-*endo*] for **1**, **2**, and **4** were 64, 72, 70%, respectively, where [C2′-*endo*] for **8** was only 19%.

It is known that nucleosides prefer a conformation around the glycoside linkage that avoids steric repulsion between the base and sugar moieties. Therefore, in cADPR and its analogues, the most stable conformation is that in which steric repulsion between the adenine moiety and the N9- and N1-ribose moieties, particularly between the adenine H-8 and the N9-ribose H-2′ and also between the adenine H-2 and the N1-ribose H-2″, is minimal. Because of the absence of the sp^3-hydrogens on the tetrahedral 2″- and 3″-carbons in **8**, steric repulsion between the carbocyclic ring and the adenine H-2 would be significantly smaller than those in the other three compounds. This may be why **8** prefers a characteristic 3′-*endo*/*high-anti* conformation. It is interesting to note that the structure of the N1-ribose moiety changes the conformation of the N9-ribose into the 3′-*endo* form in **8**.

With regard to the N1-ribose moiety, the previous study suggests that **1** and **2** adopt an similar C1″−C2″−C3″−C4″ flat/C6″ (O1″)-*endo* envelope conformation in aqueous solution.[7f] Both **4** and **8** might also prefer a C6″-*endo* envelope-like conformation, since their $J_{1'',6''a}$, $J_{1'',6''b}$, $J_{4'',6''a}$, and $J_{4'',6''b}$ values (see Experimental Section) were similar to those of cADPcR.[7f] Therefore, these modification at the 2″- and 3″-postitions might not affect the conformation of the carbocyclic ring so much.

Discussion. As described above, the N1-ribose-modified analogues have been successfully synthesized, which clearly demonstrates that the strategy of using a phenylthiophosphate-type substrate in the key intramolecular condensation reaction forming the pyrophosphate linkage is effective for total syntheses of cADPR-related compounds.

The conformations of cADPR and its analogues are important in their interaction with the target biomolecules. We therefore analyzed the conformation of the compounds and showed that steric repulsion between both the adenine and N9-ribose moieties and between the adenine and N1-ribose moieties, particularly between the adenine H-8 and the N9-ribose H-2′ and also between the adenine H-2 and the N1-ribose H-2″, can be a determinant of the conformation of cADPR and its analogues. Interestingly, we found that the structural modification of the N1-ribose moiety can change the conformation of the N9-ribose into the unusual 3′-*endo* form in **8**, presumably due to steric demand.

We initially evaluated the Ca^{2+}-mobilizing effect of the newly synthesized compounds with sea urchin egg homogenate, which would be effective in understanding the SAR, since to date most of the cADPR analogues have been evaluated with this system. As a result, **4** was identified as the most potent agonist in this series of compounds. The Ca^{2+}-mobilizing effect of 3″-deoxy-cADPcR, which is more potent than that of **2**, seems to result from its higher binding affinity for the target protein, since both compounds would be similarly stable in the evaluation system.[23] Although proteins involved in the metabolism and function of **1** constitute potential targets for pharmacological intervention, these proteins are poorly defined. Because of the characteristic stability and high affinity of 3″-deoxy-cADPcR, it may be an effective tool for the identification of target proteins, especially in sea urchin eggs.

While there are considerable similarities in the recognition of cADPR analogues by proteins of lower and higher eukaryotes, i.e., sea urchin and mammalian cells, it has also been demonstrated that there are some differences in the recognition by the proteins between sea urchin and mammalian cells. For example, although cADPR 2′-phosphate mobilizes Ca^{2+} similarly to cADPR in rat brain microsomes[24a] and T-cells,[24b] it is inactive in sea urchin eggs,[24c] and 2′-deoxy-cADPR is active in sea urchin eggs but not in T-cells.[10c] On the other hand, cADPR analogues having selectivity between mammalian cell types, i.e.,

(22) (a) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1974**, *95*, 2333−2344. (b) Hotoda, H.; Murayama, K.; Miyamoto, S.; Iwata, Y.; Takahashi, M.; Kawase, Y.; Tanzawa, K.; Kaneko, M. *Biochemistry* **1999**, *38*, 9234−9241.

(23) 3″-Deoxy-cADPcR (**4**) was shown to be as stable as cADPcR (**2**): no degradation of **4** and **2** was observed after their incubation in 50% human serum for 3 days, whereas cADPR (**1**) was unstable with a $t_{1/2}$ value of 60 h under the same conditions.

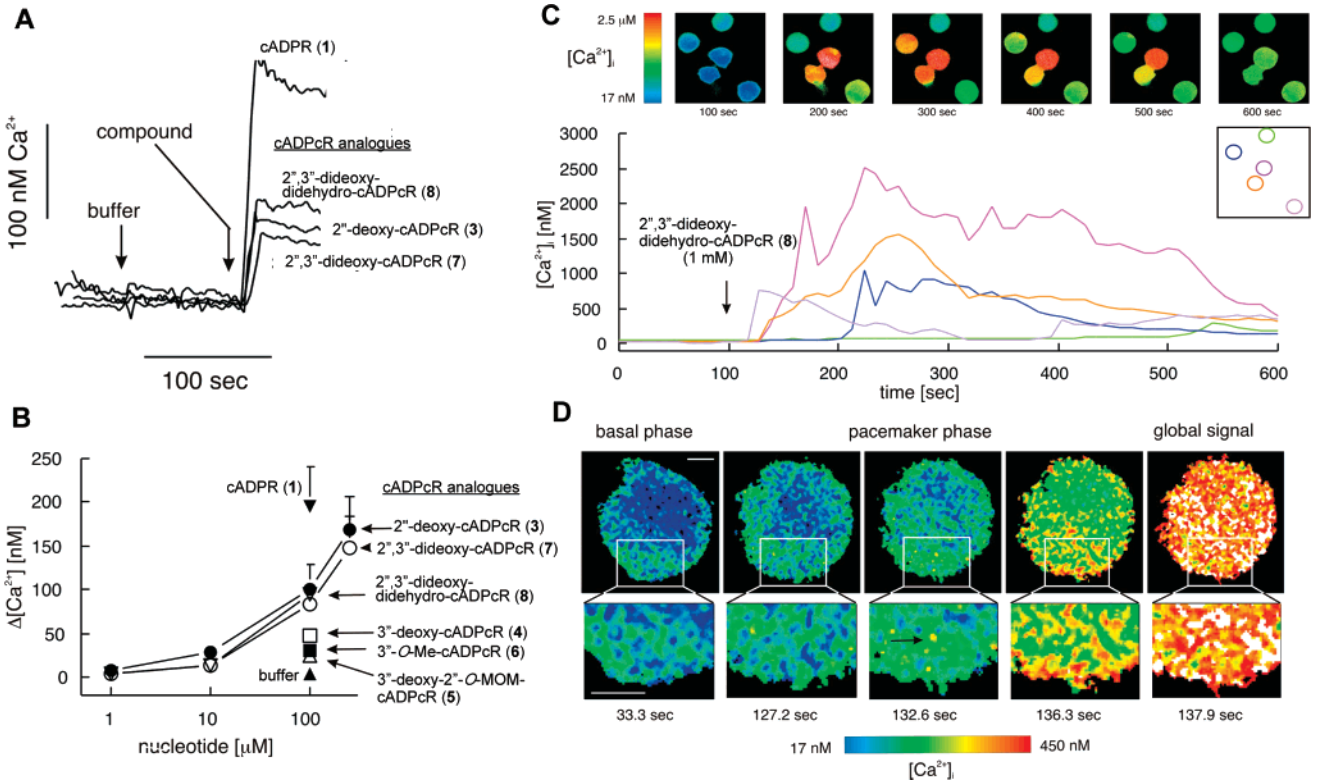


Figure 3. Effect of compounds on Ca^{2+} signaling in T cells. (A, B) Cells were permeabilized, and vehicle (buffer) and cADPcR analogues or cADPR (100 μM) were added as indicated. Combined data (mean ± SEM; $n \geq 3$) for all analogues tested are displayed in B. Error bars are only partially visible because the values are smaller than the symbol size. (C, D) Intact Jurkat T cells were loaded with fura 2/AM, and $[Ca^{2+}]_i$ was determined fluorimetrically by conventional (C) or confocal (D) Ca^{2+} imaging on the single-cell level. (C) 2″,3″-Dideoxydidehydro-cADPcR (**8**) was added as indicated (final concentration, 1 mM), a representative field with five cells displayed. Changes in $[Ca^{2+}]_i$ are shown as pseudocolor images (upper panel) and by quantification of the mean $[Ca^{2+}]_i$ in each single cell (lower panel). Color encoding is as indicated to the right. (D) Characteristic confocal ratiometric pseudocolor images of a single Jurkat cell and magnifications of a defined subcellular region are shown. Basal phase (before addition of 1 mM 2″,3″-dideoxydidehydro-cADPcR [final concentration]), pacemaker phase, and global phase are indicated. White bar corresponds to 3 μm. In total 25 cells were analyzed.

the compounds selectively active in some mammalian cells but inactive in other mammalian cells, have not been reported. The cell-type-selective cADPR analogues would be useful tools to investigate the target biomolecules as well as the Ca^{2+}-mobilizing mechanisms. Development of such cADPR analogues may be feasible, since the target proteins of cADPR may be different depending on cells, tissues, and/or organs. It is therefore an important discovery that **2** and **4**, while virtually inactive in T-cells, were found to be active not only in sea urchin eggs but also in neuronal cells. One possible explanation for such heterogeneity is that the recognition system of cADPR is different between the cell systems. For example, a 140 kDa protein was reported to be a candidate for the putative "cADPR-receptor" in sea urchin eggs,[8c] whereas the plausible target was FKBP12.6 in islet cells[20a] and smooth muscle cells.[20b] It may also be that the target channel or regulatory mechanism of Ca^{2+} release by cADPR differs according to cell types. Whereas type 2 RyR is shown as the cADPR-gated Ca^{2+} release channel in islet cells[20a] and smooth muscle cells,[20b] the release channel is reported to be type 3 RyR in T cells.[18b] The heterogeneity might arise from the different roles of the cADPR−Ca^{2+} release system in cell types through evolution of eukaryotes. cADPR acts as the second messenger for Ca^{2+} waves at the fertilization of sea urchin eggs. In T cells, cADPR plays important roles in the generation of pacemaker Ca^{2+} signals.[18b] On the other hand, cADPR acts as an endogenous mediator for bidirectional coupling between RyRs and VACCs in NG108-15 neuronal cells; cADPR does not act as a primary ligand to open RyRs, but rather it sustainedly amplifies the depolarization-induced $[Ca^{2+}]_i$ increase via RyRs and also facilitates Ca^{2+} influx through VACCs.[19]

As described above, this study shows that the relative potency of Ca^{2+}-mobilization by the cADPR analogues can be changed depending on the cells type: i.e., 3″-deoxy-cADPcR > cADPcR > cADPR in sea urchin eggs, cADPR > cADPcR ≈ 3″-deoxy-cADPcR in T-cells,[25] and cADPcR > cADPR > 3″-deoxy-cADPcR in neuronal cells, respectively. These results with the N1-ribose-modified analogues confirmed that the target proteins and/or the mechanism of action of cADPR in sea urchin eggs, T-cells, and neuronal cells are actually different, as suggested by previous biological studies.[18−20]

Conclusion. A series of N1-ribose-modified cADPcR analogues, designed as novel stable mimics of cADPR, were successfully synthesized using the key intramolecular condensation reaction with phenylthiophosphate-type substrates. Conformational analysis of these analogues suggested that steric repulsion between both the adenine and N9-ribose moieties and the adenine and N1-ribose moieties can be a determinant of

(24) (a) Vu, C. Q.; Lu, P. J.; Chen, C. S.; Jacobson, M. K. *J. Biol. Chem.* **1996**, *271*, 4747−4754. (b) Guse, A. H.; de Silva, C. P.; Weber, K.; Armah, C. N.; Ashamu, G. A.; Schulze, C.; Potter, B. V. L.; Mayr, G. W.; Hilz, H.; *Eur. J. Biochem.* **1997**, *245*, 411−417. (c) Aarhus, R.; Graeff, R. M.; Dickey, D. M.; Walseth, T. F.; Lee, H. C. *J. Biol. Chem.* **1995**, *270*, 30327−30333.

(25) In the Jurkat T-cell system, cADPR (**1**) was roughly 3-fold more potent in inducing calcium release than cADPcR (**2**) at 100 μM (see ref 7g), indicating that 3′-deoxy-cADPcR (**4**) was slightly less effective than cADPcR.

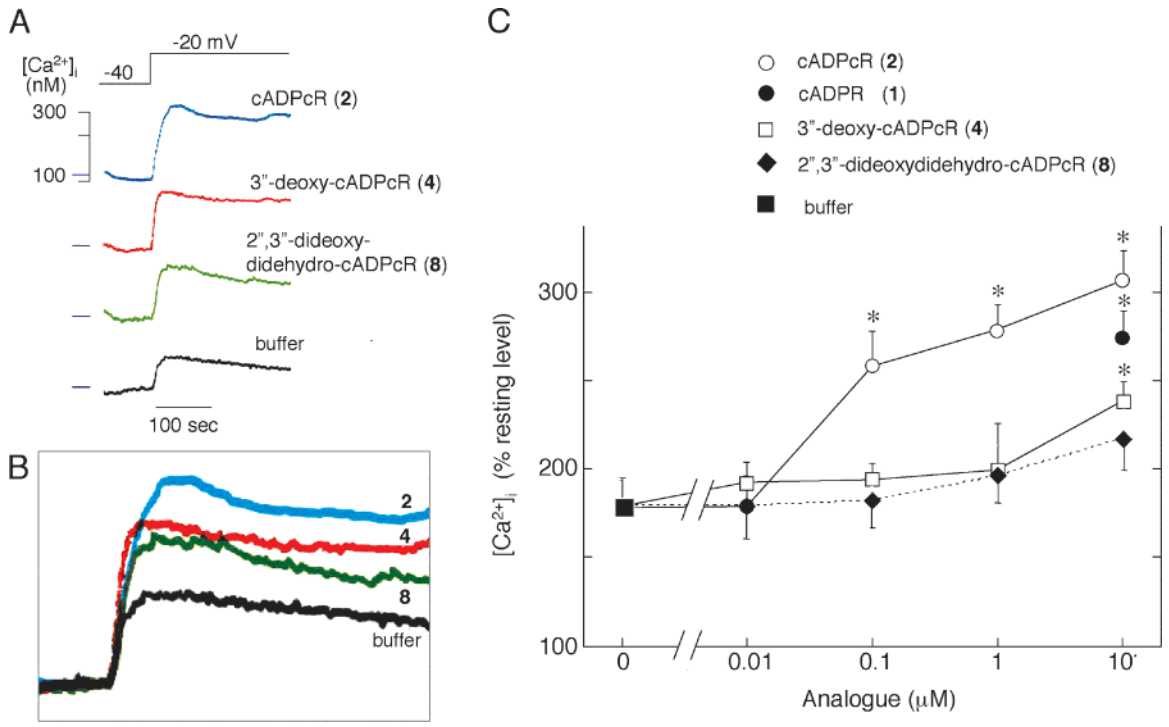


Figure 4. Effects of compounds on membrane depolarization-evoked $[Ca^{2+}]_i$ increases in patch-clamped NG108-15 cells. (A) Traces show $[Ca^{2+}]_i$ changes in fura 2-loaded cells. At about 100 s before the beginning of each trace, cell membranes were ruptured with pipets filled with 10 μM of cADPcR (**2**), 3′′-deoxy-cADPcR (**4**), or 2′′,3′′-dideoxydidehydro-cADPcR (**8**) or without compound. Then membranes were voltage-clamped at −40 mV, followed by depolarization to −20 mV. (B) The four traces in A were superimposed to compare the responses. (C) The graph shows dose-dependent activity of **2**, **4** and **8** in NG108-15 cells. A filled circle indicates activity of cADPR (**1**) at 10 μmol. Symbols indicate $[Ca^{2+}]_i$ levels at peak after depolarization to −20 mV, represented by the percentage of $[Ca^{2+}]_i$ at −40 mV. Each symbol is meant to be the ±SEM of three to seven experiments. Asterisks indicate values significantly different from a drug-free control value (a filled square) ($P < 0.05$).

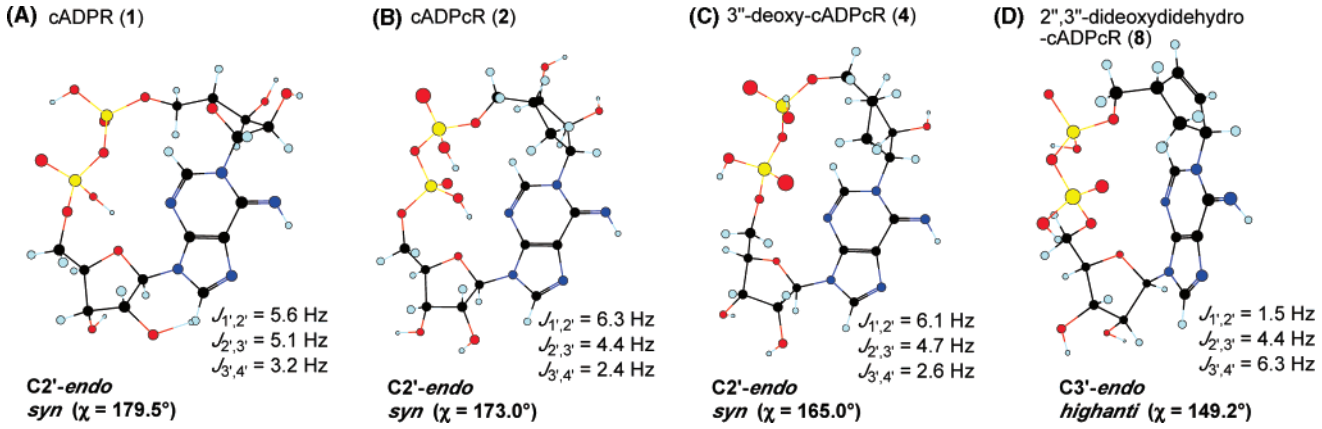


Figure 5. Calculated structures of cADPR (**1**, A), cADPcR (**2**, B), 3′′-deoxy-cADPcR (**4**, C), and 2′′,3′′-dideoxydidehydro-cADPR (**8**, D) by molecular dynamics calculations with a simulated annealing method based on the NOE data in D_2O.

the conformation of cADPR and its analogues. Evaluations of Ca^{2+}-mobilizing potencies using three different biological systems, i.e., sea urchin eggs, NG108-15 neuronal cells, and Jurkat T-lymphocytes showed that the relative potency of Ca^{2+}-mobilization by the compounds changes depending on the kind of cells used and that the target proteins and/or the mechanism of action of cADPR in these various cells may be different. Thus, this study can be an entry to developing cell-type selective cADPR analogues, which may be useful as biological tools and/or drug leads.

Experimental Section

Chemical shifts are reported in parts per million downfield from Me_4Si (^1H), MeCN (^{13}C), or H_3PO_4 (^{31}P). All of the ^1H 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.

2′′,3′′-Dideoxy-2′′,3′′-didehydro-cyclic ADP−Carbocyclic Ribose (8). A solution of **33a** (see Supporting Information, 59 mg, 0.091 mmol) in aqueous 60% HCO_2H (1 mL) was stirred at room temperature for 9.5 h and then evaporated. After coevaporation with H_2O (2 mL × 3), the resulting residue was dissolved in TEAB buffer (0.1 M, pH 7.0, 800 μL), and the solution was lyophilized to give **8** (59 mg, quant.) as a triethylammonium salt: ^1H NMR (D_2O, 400 MHz, J values were determined by spin decoupling) δ 8.57 (s, 1H, H-2), 8.34 (s, 1H, H-8), 6.51 (dd, 1H, H-2′′, $J_{2'',1''} = 2.4$, $J_{2'',3''} = 5.4$ Hz), 6.12 (d, 1H, H-1′, $J_{1',2'} = 1.5$ Hz), 6.06 (dd, 1H, H-3′′, $J_{3'',2''} = 5.4$, $J_{3'',4''} = 2.7$ Hz), 5.51 (ddd, 1H, H-1′′, $J_{1'',2''} = 2.4$, $J_{1'',6''a} = 7.8$, $J_{1'',6''b} = 1.5$ Hz), 5.06

(dd, 1H, H-2′, $J_{2',1'} = 1.5$, $J_{2',3'} = 4.4$ Hz), 4.52 (dd, 1H, H-3′, $J_{3',2'} = 4.4$, $J_{3',4'} = 6.3$ Hz), 4.32 (m, 1H, H-4′), 4.13 (d, 1H, H-5′a, $J_{5'a,5'b} = 11.2$ Hz), 3.93 (m, 2H, H-5″ × 2), 3.86 (d, 1H, H-5′b, $J_{5'b,5'a} = 11.2$ Hz), 3.25 (m, 1H, H-4″), 3.20 (q, 6H, Et_3NH-CH_2 × 3, $J = 7.3$ Hz), 2.94 (ddd, 1H, H-6″a, $J_{6''a,6''b} = 15.2$, $J_{6''a,1''} = 7.8$, $J_{6''a,4''} = 9.5$ Hz), 2.07 (ddd, 1H, H-6″b, $J_{6''b,6''a} = 15.2$, $J_{6''b,4''} = 2.0$, $J_{6''b,1''} = 1.5$ Hz), 1.28 (t, 9H, Et_3NH-CH_3 × 3, $J = 7.3$ Hz); ^{13}C NMR (D_2O, 68 MHz) δ 150.37, 147.73, 144.62, 143.54, 141.86, 126.98, 120.15, 90.80, 83.71, 83.56, 74.26, 69.85, 67.16, 67.09, 65.54, 63.85, 47.05, 46.24, 46.10, 32.08, 8.65; ^{31}P NMR (D_2O, 108 MHz) δ −8.94, −10.80, $J = 14.3$ Hz; HRMS (FAB, positive) calcd for $C_{16}H_{22}N_5O_{10}P_2$ 506.0842 (MH^+), found 506.0803; UV (H_2O) λ_{max} = 259 nm.

2″,3″-Dideoxy-cyclic ADP−Carbocyclic Ribose (7). Compound **7** (44 mg, quant.) was obtained from **33b** (see Supporting Information, 48 mg, 73 μmol) as described for the synthesis of **8**: ^{1}H NMR (D_2O, 400 MHz, *J* values were determined by spin decoupling) δ 9.04 (s, 1H, H-2), 8.37 (s, 1H, H-8), 6.07 (d, 1H, H-1′, $J_{1',2'} = 5.4$ Hz), 5.17 (dd, 1H, H-2′, $J_{2',1'} = 5.4$, $J_{2',3'} = 4.4$ Hz), 4.99 (m, 1H, H-1″), 4.63 (dd, 1H, H-3′, $J_{3',2'} = 4.4$, $J_{3',4'} = 2.4$ Hz), 4.39 (m, 1H, H-4′), 4.36 (m, 1H, H-5′a), 4.12 (m, 2H, H-5″ × 2), 3.97 (m, 1H, H-5′b), 3.19 (q, 6H, Et_3NH-CH_2 × 3, $J = 7.3$ Hz), 2.66 (m, 2H, H-6″a, H-4″), 2.46 (m, 1H, H-6″b), 2.30 (m, 1H, H-2″a), 2.15 (m, 1H, H-2″b), 1.95 (m, 1H, H-3″a), 1.82 (m, 1H, H-3″b), 1.27 (t, 9H, Et_3NH-CH_3 × 3, $J = 7.3$ Hz); ^{13}C NMR (D_2O, 68 MHz) δ 155.727, 145.987, 142.358, 140.615, 123.221, 90.535, 85.036, 84.874, 73.572, 71.254, 67.337, 65.270, 58.586, 46.403, 39.143, 38.999, 33.644, 31.830, 26.726, 9.566; ^{31}P NMR (D_2O, 108 MHz) δ −8.98 (d, $J = 13.4$ Hz), −10.23 (d, $J = 13.4$ Hz); HRMS (FAB, positive) calcd for $C_{16}H_{24}N_5O_{10}P_2$ 508.0998 (MH^+), found 508.1006; UV (H_2O) λ_{max} 259.

2″-Deoxy-Cyclic ADP-Carbocyclic Ribose (3). Compound **3** (4.1 mg, quant.) was obtained from **52** (see Supporting Information, 4.5 mg, 6.8 μmol) as described for the synthesis of **8**: ^{1}H NMR (D_2O, 400 MHz, *J* value was determined by spin decoupling) δ 9.07 (s, 1H, H-2 or H-8), 8.38 (s, 1H, H-2 or H-8), 6.08 (d, 1H, H-1′, $J_{1',2'} = 5.9$ Hz), 5.18 (dd, 1H, H-2′, $J_{2',1'} = 5.9$, $J_{2',3'} = 4.9$ Hz), 5.08 (m, 1H, H-1″), 4.64 (dd, 1H, H-3′, $J_{3',2'} = 4.9$, $J_{3',4'} = 3.0$ Hz), 4.44 (m, 3H, H-4′, H-5′a, H-5″a), 4.17 (m, 3H, H-3″, H-5′b, H-5″b), 2.96 (dd, 1H, H-6″a, $J_{6''a,6''b} = 17.2$, $J_{6''a,4''} = 11.2$ Hz), 2.49 (ddd, 1H, H-6″b, $J_{6''b,6''a} = 17.2$, $J_{6''b,1''} = 8.4$, $J_{6''b,4''} = 3.6$ Hz), 2.35 (m, 3H, H-4″, H-2″ × 2); ^{13}C NMR (D_2O, 125 MHz) δ 144.61, 90.75, 85.19, 73.70, 72.08, 71.09, 65.23, 64.68, 59.11, 46.16, 40.31, 30.22; ^{31}P NMR (D_2O, 202 MHz) δ −9.18, −10.39, $J = 11.4$ Hz; HRMS (FAB, positive) calcd for $C_{16}H_{24}N_5O_{11}P_2$ 524.0948 (MH^+), found 524.0932; UV (H_2O) λ_{max} 259 nm.

2″-*O*-(Methoxymethyl)-3″-deoxy-cyclic ADP−Carbocyclic Ribose (5). Compound **5** (13 mg, quant.) was obtained from **53** (see Supporting Information, 13 mg, 18 μmol) as described for the synthesis of **8**: ^{1}H NMR (D_2O, 500 MHz) δ 9.18 (s, 1H, H-2 or H-8), 8.41 (s, 1H, H-2 or H-8), 6.08 (d, 1H, H-1′, $J_{1',2'} = 5.3$ Hz), 5.14 (dd, 1H, H-2′, $J_{2',1'} = 5.3$, $J_{2',3'} = 4.8$ Hz), 4.98 (m, 1H, H-1″), 4.88 (d, 1H, MOM-CH_2, $J = 7.2$ Hz), 4.77 (d, 1H, MOM-CH_2, $J = 7.2$ Hz), 4.63 (dd, 1H, H-3′, $J_{3',2'} = 4.8$, $J_{3',4'} = 2.2$ Hz), 4.52 (m, 1H, H-5′a), 4.43 (m, 2H, H-4′, H-2″), 4.22 (m, 1H, H-5″a), 4.13 (m, 1H, H-5′b), 4.10 (m, 1H, H-5″b), 3.43 (s, 3H, MOM-CH_3), 3.20 (q, 6H, Et_3NH-CH_2 × 3, $J = 7.3$ Hz), 2.98 (m, 1H, H-6″a), 2.81 (m, 1H, H-4″), 2.50 (m, 1H, H-6″b), 2.19 (m, 1H, H-3″a), 2.06 (m, 1H, H-3″b), 1.28 (t, 9H, Et_3NH-CH_3 × 3, $J = 7.3$ Hz); ^{13}C NMR (D_2O, 125 MHz) δ 151.45, 147.02, 145.60, 120.51, 97.15, 90.90, 86.02, 85.48, 85.39, 73.84, 71.19, 67.53, 66.34, 65.30, 56.19, 47.15, 37.25, 37.18, 33.72, 30.72, 8.68; ^{31}P NMR (D_2O, 202 MHz) δ −9.48 (s), −10.45 (s); HRMS (FAB, positive) calcd for $C_{18}H_{28}N_5O_{12}P_2$ 568.1210 (MH^+), found 568.1212; UV (H_2O) λ_{max} 260 nm.

3″-Deoxy-cyclic ADP−Carbocyclic Ribose (4). Compound **4** (22 mg, 87%) was obtained from **53** (see Supporting Information, 29 mg, 41 μmol) as described for the synthesis of **8**, using aqueous 80% HCO_2H instead of aqueous 60% HCO_2H: ^{1}H NMR (D_2O, 400 MHz, *J* values were determined by spin decoupling) δ 9.05 (s, 1H, H-2), 8.39 (s, 1H, H-8), 6.08 (d, 1H, H-1′, $J_{1',2'} = 6.1$ Hz), 5.19 (dd, 1H, H-2′, $J_{2',1'} = 6.1$, $J_{2',3'} = 4.7$ Hz), 4.86 (dd, 1H, H-1″, $J_{1'',2''} = 2.9$, $J_{1'',6''a} = 9.3$ Hz), 4.64 (dd, 1H, H-3′, $J_{3',2'} = 4.7$, $J_{3',4'} = 2.6$ Hz), 4.54 (ddd, 1H, H-2″, $J_{2'',1''} = 2.9$, $J_{2'',3''a} = 5.6$, $J_{2'',3''b} = 5.4$ Hz), 4.51 (m, 1H, H-5′a), 4.42 (m, 1H, H-4′), 4.20 (dd, 1H, H-5″a, $J_{5''a,5''b} = 10.3$, $J_{5''a,4''} = 2.9$ Hz), 4.13 (m, 1H, H-5′b), 4.02 (dd, 1H, H-5″b, $J_{5''b,5''a} = 10.3$, $J_{5''b,4''} = 3.9$ Hz), 2.93 (ddd, 1H, H-6″a, $J_{6''a,6''b} = 16.6$, $J_{6''a,1''} = 9.3$, $J_{6''a,4''} = 10.0$ Hz), 2.80 (dddddd, 1H, H-4″, $J_{4'',3''a} = 6.1$, $J_{4'',3''b} = 7.8$, $J_{4'',5''a} = 2.9$, $J_{4'',5''b} = 3.9$, $J_{4'',6''a} = 10.0$, $J_{4'',6''b} = 2.2$ Hz), 2.50 (dd, 1H, H-6″b, $J_{6''b,6''a} = 16.6$, $J_{6''b,4''} = 2.2$ Hz), 2.17 (ddd, 1H, H-3″a, $J_{3''a,3''b} = 14.2$, $J_{3''a,2''} = 5.6$, $J_{3''a,4''} = 6.1$ Hz), 1.99 (ddd, 1H, H-3″b, $J_{3''b,3''a} = 14.2$, $J_{3''b,2''} = 5.4$, $J_{3''b,4''} = 7.8$ Hz); ^{13}C NMR (D_2O, 125 MHz) δ 151.86, 146.68, 145.36, 120.66, 90.88, 85.36, 85.27, 78.35, 73.68, 71.16, 67.81, 65.26, 37.53, 37.45, 35.66, 30.75; ^{31}P NMR (D_2O, 202 MHz) δ −9.44 (d, $J = 11.4$ Hz), −10.42 (d, $J = 11.4$ Hz); HRMS (FAB, positive) calcd for $C_{16}H_{24}N_5O_{11}P_2$ 524.0948 (MH^+), found 524.0977; UV (H_2O) λ_{max} 260 nm.

2″-*O*-(Methoxymethyl)-3″-*O*-methyl-cyclic ADP−Carbocyclic Ribose (6). Compound **6** (7.6 mg, 56%) was obtained from **54** (see Supporting Informatin, 12 mg, 16 μmol) as described for the synthesis of **7**, using aqueous 80% HCO_2H instead of aqueous 60% HCO_2H: ^{1}H NMR (D_2O, 400 MHz, *J* value was determined by spin decoupling) δ 9.04 (s, 1H, H-2), 8.31 (s, 1H, H-8), 6.04 (d, 1H, H-1′, $J_{1',2'} = 6.3$ Hz), 5.14 (dd, 1H, H-2′, $J_{2',1'} = 6.3$, $J_{2',3'} = 4.9$ Hz), 4.98 (ddd, 1H, H-1″, $J_{1'',2''} = 3.9$, $J_{1'',6''a} = 10.2$, $J_{1'',6''b} = 2.9$ Hz), 4.63 (dd, 1H, H-3′, $J_{3',2'} = 4.9$, $J_{3',4'} = 2.4$ Hz), 4.53 (m, 2H, H-2″, H-5′a), 4.41 (m, 1H, H-4′), 4.20 (m, 2H, H-5″ × 2), 4.14 (m, 1H, H-5′b), 3.92 (dd, 1H, H-3″, $J_{3'',2''} = 4.4$, $J_{3'',4''} = 5.9$ Hz), 3.43 (s, 3H, Me), 2.99 (ddd, 1H, H-6″a, $J_{6''a,6''b} = 16.6$, $J_{6''a,1''} = 10.2$, $J_{6''a,4''} = 11.2$ Hz), 2.65 (br s, 1H, H-4″), 2.37 (ddd, 1H, H-6″b, $J_{6''b,6''a} = 16.6$, $J_{6''b,4''} = 3.9$, $J_{6''b,1''} = 2.9$ Hz); ^{13}C NMR (D_2O, 125 MHz) δ 152.81, 145.02, 144.46, 121.18, 90.89, 85.37, 85.28, 83.19, 76.27, 73.84, 71.18, 65.62, 65.30, 63.55, 57.88, 40.97, 40.90, 28.19; ^{31}P NMR (D_2O, 202 MHz) δ −9.41 (d, $J = 11.4$ Hz), −10.53 (d, $J = 11.4$ Hz); HRMS (FAB, negative) calcd for $C_{17}H_{24}N_5O_{12}P_2$ 552.0897 [$(M-H)^-$], found 552.0903; UV (H_2O) λ_{max} 260 nm.

Acknowledgment. This investigation was supported by a Grant-in-Aid for Scientific Research on "Exploitation of Multielement Cyclic Molecules" (S.S.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and a Grant-in-Aid for Creative Scientific Research (Grant 13NP0401, A.M.) from the Japan Society for the Promotion of Science. We thank the Wellcome Trust for a Project Grant (055709 to B.V.L.P.) and a Biomedical Research Collaboration Grant (068065 to B.V.L.P. and A.H.G.). This study was also partially supported by the Deutsche Forschungsgemeinschaft (Grant GU 360/0-1 and 9-2 to A.H.G.), Werner-Otto-Stiftung (to A.H.G.), and Gemeinnützige Hertie-Stiftung (Grant 1.01.1/04/010 to A.H.G.). We also thank Mr. Kohei Nozawa for his kind suggestion on the NOE-based calculations.

Supporting Information Available: Experimental procedures for the synthesis of compounds other than the final compounds **3**−**8**, biological evaluations, and calculations and Figure S1 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA050732X