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Substitution at the 8-position of 3″-deoxy-cyclic ADP-carbocyclic-ribose, a highly potent Ca^{2+}-mobilizing agent, provides partial agonists[1]

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Abstract—We previously showed that 3″-deoxy-cyclic ADP-carbocyclic-ribose (3″-deoxy-cADPcR, **4**) is a stable and highly potent analogue of cyclic ADP-ribose (cADPR, **1**), a Ca^{2+}-mobilizing second messenger. From these results, we designed and synthesized other 3″-modified analogues of cADPcR having a substituent at the 8-position and found that this modification at the 8-position made them partial agonists. Among these compounds, 8-NH_2-3″-deoxy-cADPcR (**10**) was identified as a potent partial agonist with an EC_{50} value of 17 nM.

1. Introduction

Cyclic ADP-ribose (cADPR, **1**, Fig. 1), a naturally occurring metabolite of NAD^+,[2] has been shown to mobilize intracellular Ca^{2+} in various cells, and is now recognized as a second messenger involved in Ca^{2+} signaling.[3] Under neutral conditions, cADPR is in a zwitterionic form with a positive charge around the N1–C6–N^6 moiety of its adenine ring, making the molecule unstable. Since the charged adenine moiety attached to the anomeric carbon of the N1-ribose can be an efficient leaving group, cADPR is readily hydrolyzed at the unstable N1-ribosyl linkage to produce ADP-ribose (ADPR), even in neutral aqueous solution.[4] Under physiological conditions, cADPR is also hydrolyzed at the N1-ribosyl linkage by cADPR hydrolase to give the inactive ADPR.[4]

cADPR analogues can be used to prove the mechanism of Ca^{2+} signaling pathways mediated by cADPR and may be lead structures for the development of clinically useful drugs, since cADPR plays a variety of important roles in physiological processes.[3a,c] The synthesis of

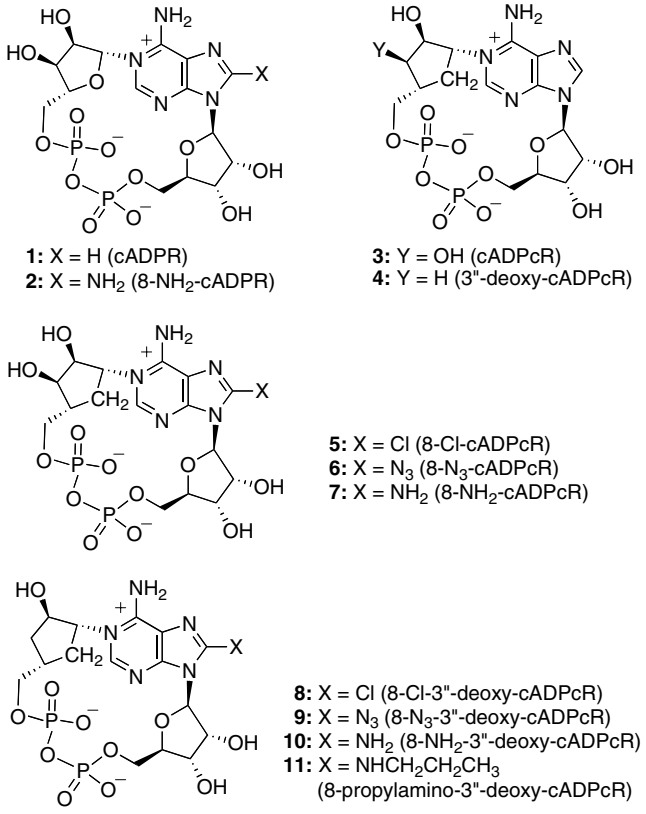

Figure 1. cADPR and its analogues.

Keywords: Calcium; Carbocyclic-ribose; Cyclic ADP-ribose; Partial agonist; Second messenger.
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cADPR analogues has been extensively investigated by enzymatic and chemo-enzymatic methods using ADP-ribosyl cyclase-catalyzed cyclization.[3b–e] For instance, a series of 8-substituted analogues of cADPR were synthesized by the chemo-enzymatic method to show that these analogues were antagonists of cADPR. Among them, 8-NH_2-cADPR (**2**) was identified as the most potent antagonist[5] and has been used effectively as a biological tool.[3] However, the analogues obtained by these methods are limited due to the substrate-specificity of the ADP-ribosyl cyclase.[3d]

We developed an efficient method for forming the backbone 18-membered pyrophosphate ring structure employing phenylthiophosphate-type substrates, which is the key step in the chemical synthesis of cADPR and its analogues.[6] When these substrates were activated by $AgNO_3$ or I_2 in the presence of molecular sieves in pyridine, the corresponding 18-membered pyrophosphate ring products were obtained in high yields.[6b,c] Using this method, we successfully synthesized cyclic ADP-carbocyclic-ribose (cADPcR, **3**),[6c] designed as a stable mimic of cADPR, in which the oxygen atom in the *N*-1-ribose ring of cADPR is replaced by a methylene group. Biological evaluation of cADPcR showed that it actually acts as a biologically and chemically stable equivalent of cADPR.[6c]

While investigating a structure–activity relationship study of cADPcR[7] with a sea urchin egg homogenate assay system, we found 3″-deoxy-cADPcR (**4**) to be the most potent Ca^{2+}-mobilizing compound in the series.[7c] On the other hand, 8-substituted cADPcR analogues were designed and synthesized to possess specifically the properties of both the stable carbocyclic analogue **3** and the 8-substituted cADPR analogues; for example, we expected that 8-NH_2-cADPcR (**7**) might be a chemically and biologically stable potent antagonist of cADPR. However, their biological evaluation disclosed that the 8-substituted cADPcR analogues **5**–**7** were agonists.[7a] Thus, only a subtle change, that is, '–O–' into '–CH_2–', can bring about a dramatic switch from a highly potent antagonist 8-NH_2-cADPcR (**2**) to an almost equipotent agonist 8-NH_2-cADPcR (**7**).

With these results in mind, we were interested in the biological activity of the 8-substituted derivatives of the most potent agonist 3″-deoxy-cADPcR, namely whether these analogues are agonists or antagonists? In this report, we describe the synthesis and Ca^{2+}-mobilizing effect of a series of 3″-deoxy-cADPcR analogues having a substituent at the 8-position, which are the 8-Cl derivative **8**, the 8-N_3 derivative **9**, the 8-NH_2 derivative **10**, and the 8-propylamino derivative **11**, respectively.

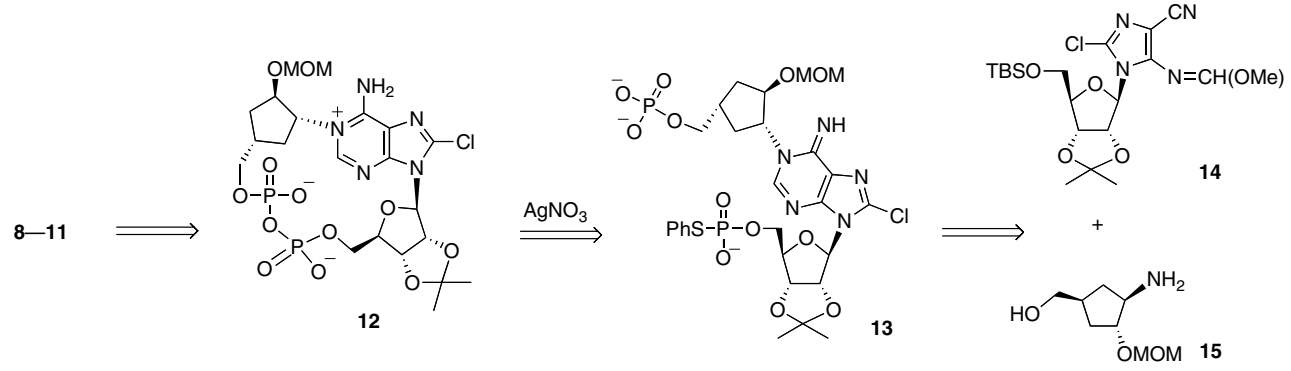
2. Results and discussion

2.1. Synthetic plan

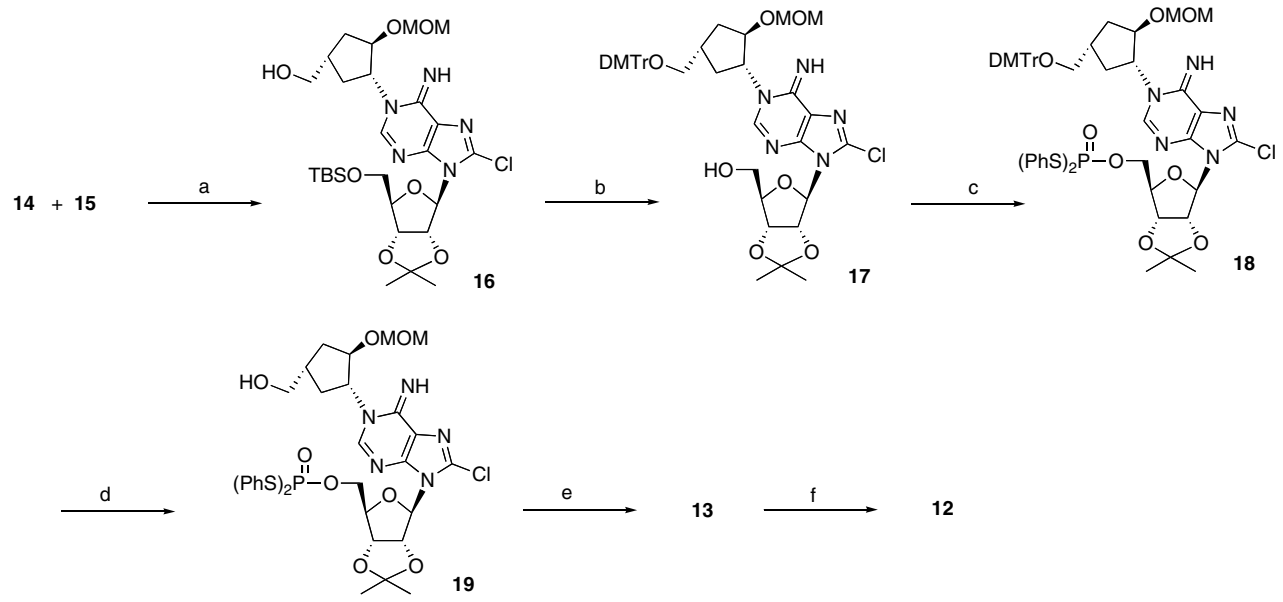
We planned to synthesize the target 8-substituted cADPcR analogues **8**–**11** by the route summarized in Scheme 1, in which the O-protected 8-Cl-3″-deoxy-cADPcR derivative **12** is used as the common intermediate. Acidic deprotection of **12** would readily provide 8-Cl-3″-deoxy-cADPcR (**8**). The other three targets **9**, **10**, and **11** would be obtained via nucleophilic addition–elimination at the 8-position of **12**. The key intramolecular pyrophosphate linkage formation could be achieved according to the method described above: treatment of 8-chloro-5′-phenylthiophosphate substrate **13** with $AgNO_3$/MS 3A as a promoter.[6b,c] The substrate **13** would be derived from the two previously reported units, the 2-chloroimidazole nucleoside derivative **14**[7a] and the optically active carbocyclic amine **15**.[7c]

2.2. Synthesis

The synthesis of the key common intermediate **12** is shown in Scheme 2. When a mixture of **14** and **15** was treated with K_2CO_3 in DMF at room temperature, the desired N1-substituted adenosine derivative **16** was obtained in 90% yield. After protection of the 5″-hydroxyl of **16** with a dimethoxytrityl (DMTr) group, the 5′-*O*-TBS group of the product was removed with TBAF to give **17**. Treatment of **17** with an *S*,*S*′-diphenylphosphorodithioate (PSS)/2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl)/pyridine system[8] gave the 5′-bis(phenylthio)phosphate **18** in 55% yield. After removal of the 5″-*O*-DMTr group of **18**, a phosphoryl group was introduced at the resulting 5″-primary hydroxyl of the resulting **19** with $POCl_3$/$(EtO)_3PO$,[9] followed by treatment of the product with H_3PO_2, Et_3N[10], and *N*-methylmaleimide (NMM)[7a] in pyridine, affording 5′-phenylthiophosphate



Scheme 1.



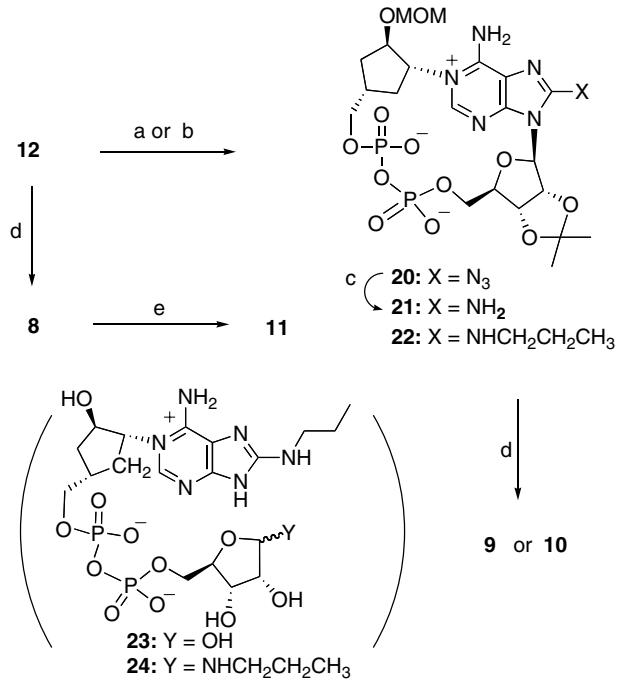
Scheme 2. Reagents and conditions: (a) K_2CO_3, DMF, rt, 90%; (b) 1—DMTrCl, pyridine, rt; 2—TBAF, THF, AcOH, rt, 99%; (c) PSS, TPSCl, py, rt, 55%; (d) aq 60% AcOH, rt, 82%; (e) 1—$POCl_3$, $(EtO)_3PO$, 0 °C; 2—H_3PO_2, Et_3N, NMM, pyridine, 0 °C, rt, 52%; (f) $AgNO_3$, MS 3A, Et_3N, py, rt, 85%.

13 in 52% yield as a triethylammonium salt. When a solution of **13** 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,[6b,c] the desired intramolecular condensation effectively occurred to give the cyclized common intermediate **12** in 85% yield.

All of the target compounds were synthesized from **12** as shown in Scheme 3. Treatment of **12** with aqueous HCO_2H and then NH_4OH furnished 8-Cl-3″-deoxy-cADPcR (**8**). The other three target compounds, **9**, **10**, and **11**, were synthesized via a nucleophilic addition–elimination reaction at the 8-position. Thus, treatment of **12** with LiN_3 or propylamine at room temperature produced the corresponding 8-azido and 8-propylamino derivatives **20** and **22**, respectively. The azido group of **20** was reduced under catalytic hydrogenation conditions with Pd–C to give the 8-amino derivative **21**. Finally, acidic deprotection of **20** and **21** gave the target 8-N_3-3″-deoxy-cADPcR (**9**) and 8-NH_2-3″-deoxy-cADPcR (**10**), respectively. Although similar acidic treatment of **22** did not produce the desired 8-propylamino-3″-deoxy-cADPcR (**11**) but rather the glycosidic bond-cleaved product **23**, the desired **11** was obtained via reaction of the 8-Cl-cADPcR (**8**) and propylamine in 39% yield along with a glycosidic bond-cleaved product **24**.

2.3. Ca^{2+}-mobilizing activity in sea urchin egg homogenate

The Ca^{2+}-mobilizing ability of the newly synthesized 8-substituted 3″-deoxy-cADPcR analogues **8**–**11** was evaluated by fluorometrically monitoring Ca^{2+} with *Hemicentrotus pulcherrimus* sea urchin egg homogenate,[7c,11] and the results are shown in Figure 2 and Table 1. Although the 8-propylamino derivative **11** was completely inactive, the other 8-substituted 3″-deoxy-cADPcR analogues showed Ca^{2+}-mobilizing activity. Figure 2a shows a comparison of dose-dependent Ca^{2+}-release curves of the 8-substituted analogues **8**, **9**, and **10** with those of cADPcR (**3**) and 3″-deoxy-cADPcR (**4**). cADPcR released Ca^{2+} from the homogenate in a dose-dependent manner (EC_{50} = 79 nM) and 3″-deoxy cADPcR (EC_{50} = 17 nM) showed more potent activity than cADPcR with the same maximal activity.



Scheme 3. Reagents and conditions: (a) LiN_3, py, rt, 76% (**20**); (b) propylamine, rt, 74% (**22**); (c) H_2, Pd–C, H_2O, rt, 89%; (d) aq 80% HCO_2H, then 28% NH_4OH, rt, 54% (**8**), 56% (**9**), 43% (**10**); (e) propylamine, rt, 39% (**11**), 24% (**23**).

This is consistent with previous reports.[7c,d] The 8-substituted 3″-deoxy-cADPcR analogues **8**, **9**, and **10** released Ca^{2+} from the homogenate in a dose-dependent manner, but the attainable maximal activity was significantly lower than that of cADPcR; the average maximal level was 14%, 16%, and 21% of the cADPcR for the 8-NH_2 derivative **10**, the 8-N_3 derivative **9**, and the 8-Cl derivative **8**, respectively. The 8-NH_2 derivative **10** was more potent (EC_{50} = 17 nM) than the 8-Cl and the 8-N_3 derivatives (EC_{50} = 0.19 μM (**8**) and 0.49 μM (**9**), respectively). These results suggest that the 8-substituted analogues of 3″-deoxy-cADPcR are partial agonists, in contrast to their full agonistic lead compounds cADPcR and 3″-deoxy-cADPcR.

The cADPR-induced Ca^{2+} release exhibits the property of self-induced desensitization, in which the effect of a subsequent challenge by a normally maximal concentration of cADPR is reduced depending on the concentration of the first cADPR challenge.[3a] Such desensitization for the natural agonist cADPR is also observed with cADPcR,[6c] 3″-deoxy cADPcR,[7c] and some 8-substituted cADPcR analogues.[7a] We therefore tested whether the 8-substituted 3″-deoxy-cADPcR analogues show such desensitization. As shown in Figure 2b, all three compounds cross-desensitized for the Ca^{2+} release of the homogenate with Ca^{2+} release by the maximal concentration of cADPcR (1 μM). The IC_{50} values for the cADPcR-induced Ca^{2+} release were

Table 1. Ca^{2+}-mobilizing activity of 8-substituted 3″-deoxy-cADPcR analogues **8**–**11** in sea urchin egg homogenate

Compound	EC50 (nM)	Maximal activity (%)[a]
cADPcR (**3**)	79	100
3″-Deoxy-cADPcR (**4**)	17	100
8	190	14
9	490	16
10	17	21
11	Inactive	—

[a] Maximal Ca^{2+}-release level relative to that of cADPcR (100%).

44 nM, 0.41 μM, and 1.1 μM for the 8-NH_2 derivative **10**, the 8-Cl derivative **8**, and the 8-N_3 derivative **9**, respectively, and the rank order was well correlated with that of the EC_{50} value (Fig. 2a). These findings provide evidence that these 8-substituted analogues of 3″-deoxy-cADPcR are partial agonists.

The 8-substituted analogues of cADPcR were shown to release Ca^{2+} from *Lytechinus* species sea urchin egg homogenate.[7a] Because deletion of the hydroxyl group at the 3″-position greatly increases the potency for Ca^{2+} release of cADPcR (see Fig. 2a), it would be interesting to compare the Ca^{2+}-release activity of the 8-substituted cADPcR analogues having the 3″-hydroxyl with that of the corresponding 8-substituted analogues lacking the 3″-hydroxyl. Figure 2c shows dose-dependent Ca^{2+} release by the 8-substituted cADPcR analogues **5**, **6**, and **7**, having the 3″ hydroxyl group.

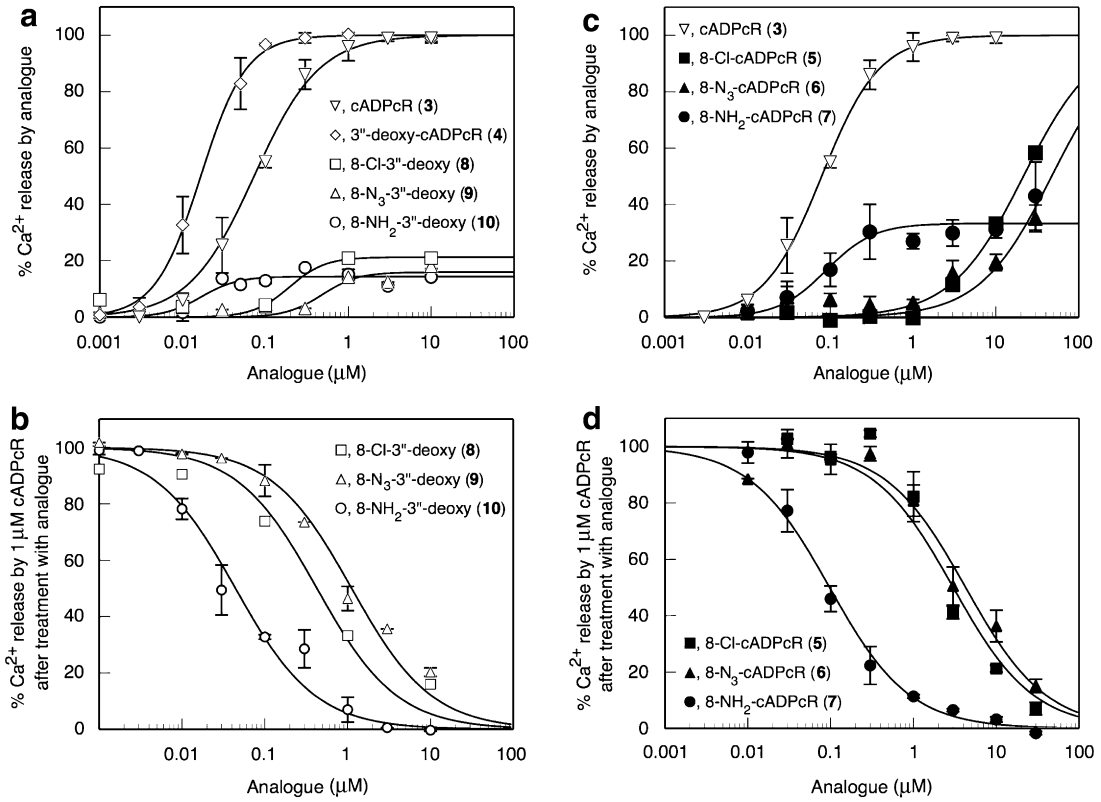


Figure 2. Ca^{2+}-mobilizing activity of compounds in sea urchin egg homogenate. (a and c) dose-dependent Ca^{2+}-mobilizing activity of cADPcR and its analogues; the activity of each compound was expressed as a percent change in fura-2 fluorescence ratio (F340/F380) relative to 1 μM cADPcR. (b and d) dose-dependent desensitization by cADPcR analogues; desensitization of each compound was expressed as a percent inhibition of Ca^{2+} release induced by 1 μM cADPcR after treatment of homogenate with the compound.

8-NH_2-cADPcR (**7**) exhibited a Ca^{2+} release with an apparent saturation of the maximal activity at 33% of the cADPcR, indicating that it is a partial agonist. This is inconsistent with the previous report showing it to be a full agonist.[7a] The reason still remains unclear, but different sea urchin species (*Lytechinus* species in the previous work[7a] vs *Hemicentrotus* species in this study) or assay conditions might cause this difference. The potency for Ca^{2+} release (EC_{50} = 89 nM) was comparable to that of cADPcR (EC_{50} = 79 nM), but significantly lower than that of 8-NH_2-3″-deoxy-cADPcR (EC_{50} = 17 nM).

8-Cl- and 8-N_3-cADPcR (**5** and **6**, respectively) were much less potent and we could not see saturation even at 30 μM. In the previous report, they were shown to be full agonists.[7a] Assuming that the two compounds are full agonists, the EC_{50} values for them would be 21 μM (**5**) and 47 μM (**6**), respectively. This is in marked contrast to the results with the corresponding 3″-deoxy congeners **8** and **9** which showed partial agonist activity with lower EC_{50} values. All three of the analogues **5**, **6**, and **7** showed cross-desensitization with cADPcR (Fig. 2d). 8-NH_2-cADPcR (**7**) was much more potent (IC_{50} = 96 nM) than the 8-Cl (**5**, IC_{50} = 3.0 μM) or the 8-N_3 (**6**, IC_{50} = 4.2 μM) derivatives, and the rank order of desensitization correlated with the potency of Ca^{2+} release.

The present work demonstrated the important role of the 3″-hydroxyl in the structure–activity relationships of cADPcR. It has been reported that 3″-deoxy-cADPcR is more potent than cADPcR (Fig. 2a).[7c] Here, we showed that the 8-substituted 3″-deoxy-cADPcR analogues were more potent (lower EC_{50} values) in Ca^{2+} release from the sea urchin homogenate than the corresponding 8-substituted cADPcR analogues having the 3″-hydroxyl (Figs. 2a and c). This is also the case with dose-dependency for desensitization (Figs. 2b and d). Thus, deletion of the 3″-hydroxyl may increase the affinity for the putative receptor irrespective of modification of the 8-position of adenine ring.

In addition, the 3″-hydroxyl may also affect the agonistic activity. Deletion of the 3″-hydroxyl of the full agonistic 8-Cl- and 8-N_3-cADPcR converted them into partial agonists. Even for 8-NH_2 derivatives which showed partial agonistic activity in spite of the presence or absence the 3″-hydroxyl, the maximal level of Ca^{2+} release was significantly lower in the 3″-deoxy-type compound **10**. These findings suggest that deletion of the 3″-hydroxyl of the N1-ribose may reduce the maximal Ca^{2+} releasing activity of the compounds.

It is known that modification of cADPR at the 8-position of the adenine ring with NH_2, N_3, or halogen substituent converts it from agonist to antagonist.[3] In addition, 7-deaza-cADPR, in which the N7 atom of the adenine ring is replaced by a methine, has been shown to behave as a partial agonist.[12] Thus, there is no doubt that the adenine ring plays an important role in the agonist/antagonist switch of the cADPR and its analogues.[3] On the other hand, 8-substituted cADPcR analogues, in which the oxygen atom in the N1-ribose ring is replaced by a methylene, behaved as full agonists, even when the adenine ring was modified.[7a] Here, we show that deletion of the 3″-hydroxyl of the N1-ribose moiety shifted the compound toward becoming more antagonistic, since a partial agonist is regarded as an intermediate between a full agonist and an antagonist. Thus, the N1-ribose moiety may also be critically important in agonist/antagonist switching of the cADPR activity. Replacement of the oxygen atom with a methylene may shift the compound toward agonistic, and deletion of the 3″-hydroxyl may reverse it.

As described, this study presents substantial results to understand the SAR of cADPR and its analogues at the 8- and the 3″-positions, especially as regards agonist/antagonist switching.

3. Experimental

3.1. General methods

Chemical shifts are reported in ppm downfield from Me_4Si (^1H in $CDCl_3$), HDO (^1H in D_2O), MeCN (^{13}C in D_2O), $CDCl_3$ (^{13}C in $CDCl_3$), 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.

3.2. 8-Chloro-*N*-1-[(1*R*,2*R*,4*S*)-2-(methoxymethyloxy)-4-(hydroxymethyl)cyclopentyl]-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylideneadenosine (16)

A mixture of **14** (370 mg, 0.786 mmol), **15** (176 mg, 1.00 mmol), and K_2CO_3 (5 mg, 39 μmol) in DMF (8 mL) was stirred at room temperature for 16 h, and then the solvent was evaporated. The residue was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 10% MeOH in EtOAc) to give **16** (435 mg, 90%) as a pale yellow foam: ^1H-NMR ($CDCl_3$, 500 MHz) δ 7.65 (s, 1H, H-2), 6.07 (d, 1H, H-1′, $J_{1',2'}$ = 2.2 Hz), 5.53 (dd, 1H, H-2′, $J_{2',1'}$ = 2.2 Hz, $J_{2',3'}$ = 6.4 Hz), 5.00 (m, 2H, H-1″, H-3′), 4.58 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.55 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.50 (m, 1H, H-2″), 4.21 (m, 1H, H-4′), 3.72 (dd, 1H, H-5′a, $J_{5'a,5'b}$ = 10.8 Hz, $J_{5'a,4'}$ = 6.3 Hz), 3.68 (m, 2H, H-5′b, H-5″a), 3.59 (dd, 1H, H-5″b, $J_{5''b,5''a}$ = 10.8 Hz, $J_{5''b,4''}$ = 3.7 Hz), 3.21 (s, 3H, MOM-CH_3), 2.43 (m, 1H, H-4″), 2.32 (m, 2H, H-3″a, H-3″b), 2.20 (m, 1H, H-6″a), 1.85 (m, 1H, H-6″b), 1.59, 1.38 (each s, each 3H, isopropylidene), 0.86 (s, 9H, *tert*-butyl), 0.001, −0.015 (each s, each 3H, Si-CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 152.9, 147.6, 141.4, 135.4, 122.6, 114.3, 96.4, 90.1, 87.5, 83.0, 81.6, 78.5, 68.9, 67.16, 63.0, 55.4, 36.5, 34.3, 28.4, 27.2, 25.9, 25.5, 18.4, −5.4, −5.4; FAB-MS *m*/*z* 614 $(MH)^+$; UV (MeOH) λ_{max} 263 nm; Anal. Calcd for $C_{27}H_{44}ClN_5O_7Si$: C, 52.80; H, 7.22; N, 11.40. Found: C, 52.77; H, 7.17; N, 11.30.

3.3. 8-Chloro-*N*-1-[(1*R*,2*R*,4*S*)-2-(methoxymethyloxy)-4-(dimethoxytrityloxymethyl)cyclopentyl]- 2′,3′- *O*-isopropylideneadenosine (17)

A mixture of **16** (1.51 g, 2.45 mmol) and DMTrCl (2.49 g, 7.35 mmol) in pyridine (25 mL) was stirred at room temperature for 1 h. After addition of MeOH (20 mL), the resulting mixture was evaporated. The residue was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. A mixture of the residue, TBAF (1.0 M in THF, 4.90 mL, 4.90 mmol), and AcOH (140 μL, 2.45 mmol) in THF (15 mL) was stirred at room temperature for 3 h, and then the solvent was evaporated. The residue was purified by column chromatography (SiO_2, 75% EtOAc in hexane) to give **17** (1.94 g, 99%) as a white foam: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.68 (s, 1H, H-2), 6.82–7.43 (m, 13H, DMTr), 6.00 (d, 1H, H-1′, $J_{1',2'} = 5.1$ Hz), 5.22 (m, 1H, H-1″), 5.11 (dd, 1H, H-2′, $J_{2',1'} = 5.1$ Hz, $J_{2',3'} = 5.7$ Hz), 5.01 (dd, 1H, H-3′, $J_{3',2'} = 5.7$ Hz, $J_{3',4'} = 0.9$ Hz), 4.60 (s, 2H, MOM-CH_2), 4.46 (m, 2H, H-2″, H-4′), 3.91 (m, 1H, H-5′a), 3.79 (s, 6H, DMTr-OMe × 2), 3.74 (m, 1H, H-5′b), 3.25 (s, 3H, MOM-CH_3), 3.10 (m, 1H, H-5″a), 3.06 (m, 1H, H-5″b), 2.54 (m, 1H, H-4″), 2.44 (m, 1H, H-6″a), 1.94 (m, 2H, H-3″a, H-3″b), 1.68 (m, 1H, H-6″b), 1.64, 1.37 (each s, each 3H, isopropylidene); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 171.0, 167.7, 158.4, 153.0, 145.9, 145.0, 140.4, 136.3, 134.5, 132.4, 130.8, 130.0, 128.8, 128.1, 127.7, 126.7, 123.1, 114.2, 113.0, 95.6, 92.3, 85.7, 85.5, 83.1, 81.2, 79.6, 68.1, 66.5, 63.1, 60.3, 55.5, 55.2, 38.7, 35.7, 34.3, 33.8, 30.3, 28.9, 27.6, 25.4; HRMS (FAB, positive) calcd for $C_{42}H_{49}ClN_5O_9$ 802.3219 $(MH)^+$, found 802.3214; MeOH λ_{max} 263 nm.

3.4. 8-Chloro-*N*-1-[(1*R*,2*R*,4*S*)-2-(methoxymethyloxy)-4-(dimethoxytrityloxymethyl)cyclopentyl]- 5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine (18)

A mixture of PSS (2.70 g, 7.07 mmol) and TPSCl (1.93 g, 6.36 mmol) in pyridine (23 mL) was stirred at room temperature for 1 h. To the resulting mixture was added **17** (1.89 g, 2.36 mmol), and the mixture was stirred at room temperature for further 90 min. The solvent 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, 75% EtOAc in hexane) to give **18** (1.38 g, 55%) as a white foam: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.66 (s, 1H, H-2), 6.81-7.49 (m, 23H, DMTr, SPh × 2), 6.13 (d, 1H, H-1′, $J_{1',2'} = 1.7$ Hz), 5.46 (dd, 1H, H-2′, $J_{2',1'} = 1.7$ Hz, $J_{2',3'} = 6.3$ Hz), 5.14 (m, 1H, H-1″), 5.04 (dd, 1H, H-3′, $J_{3',2'} = 6.3$ Hz, $J_{3',4'} = 3.1$ Hz), 4.58 (d, 1H, MOM-CH_2, $J = 6.8$ Hz), 4.55 (d, 1H, MOM-CH_2, $J = 6.8$ Hz), 4.50 (m, 1H, H-2″), 4.41 (m, 2H, H-4′, H-5′a), 4.31 (m, 1H, H-5′b), 3.78 (s, 6H, DMTr-OMe × 2), 3.22 (s, 3H, MOM-CH_3), 3.12 (m, 1H, H-5″a), 3.09 (m, 1H, H-5″b), 2.53 (m, 1H, H-4″), 2.38 (m, 1H, H-6″a), 1.94 (m, 2H, H-3″a, H-3″b), 1.74 (m, 1H, H-6″b), 1.61, 1.38 (each s, each 3H, isopropylidene); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 158.4, 153.2, 146.1, 145.1, 141.0, 136.2, 135.3, 135.2, 135.0, 135.0, 134.6, 130.0, 129.5, 129.5, 129.5, 129.3, 128.1, 127.7, 126.6, 126.1, 126.0, 125.9, 125.8, 122.5, 114.5, 113.0, 95.78, 90.1, 85.7, 85.6, 83.6, 81.4, 79.9, 66.4, 66.3, 63.5, 55.5, 55.1, 35.9, 34.46, 33.5, 27.1, 25.3; ^{31}P-NMR ($CDCl_3$, 202 MHz) δ 50.61 (s); FAB-MS *m*/*z* 1066 $(MH)^+$; UV (MeOH) λ_{max} 295 (sh) nm; Anal. Calcd for $C_{54}H_{57}ClN_5O_{10}PS_2$: C, 60.81; H, 5.39; N, 6.57. Found: C, 60.64; H, 5.47; N, 6.31.

3.5. 8-Chloro-*N*-1-[(1*R*,2*R*,4*S*)-2-(methoxymethyloxy)-4-(hydroxymethyl)cyclopentyl]-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine (19)

A solution of **18** (1.32 g, 1.24 mmol) in aqueous 60% AcOH (10 mL) was stirred at room temperature for 1 h and then evaporated. The residue was partitioned between EtOAc and aqueous saturated $NaHCO_3$, and the organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 10% MeOH in EtOAc) to give **19** (775 mg, 82%) as a white foam: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.76 (s, 1H, H-2), 7.30–7.50 (m, 10H, SPh × 2), 6.13 (d, 1H, H-1′, $J_{1',2'} = 1.8$ Hz), 5.48 (dd, 1H, H-2′, $J_{2',1'} = 1.8$ Hz, $J_{2',3'} = 6.3$ Hz), 5.06 (dd, 1H, H-3′, $J_{3',2'} = 6.3$ Hz, $J_{3',4'} = 3.1$ Hz), 4.91 (m, 1H, H-1″), 4.58 (d, 1H, MOM-CH_2, $J = 6.8$ Hz), 4.56 (d, 1H, MOM-CH_2, $J = 6.8$ Hz), 4.51 (m, 1H, H-2″), 4.42 (m, 2H, H-4′, H-5′a), 4.31 (m, 1H, H-5′b), 3.67 (dd, 1H, H-5″a, $J_{5''a,5''b} = 11.0$ Hz, $J_{5''a,4''} = 2.7$ Hz), 3.57 (dd, 1H, H-5″b, $J_{5''b,5''a} = 11.0$ Hz, $J_{5''b,4''} = 2.6$ Hz), 3.21 (s, 3H, MOM-CH_3), 2.41 (m, 1H, H-4″), 2.29 (m, 2H, H-3″a, H-3″b), 2.16 (m, 1H, H-6″a), 1.80 (m, 1H, H-6″b), 1.61, 1.39 (each s, each 3H, isopropylidene); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 152.8, 148.1, 141.3, 135.4, 135.4, 135.1, 135.0, 129.6, 129.6, 129.6, 129.4, 126.0, 126.0, 125.8, 125.7, 122.5, 114.6, 96.4, 90.3, 85.7, 85.7, 83.7, 81.5, 78.8, 66.8, 66.2, 66.2, 55.4, 36.8, 34.1, 28.1, 27.1, 25.4; ^{31}P-NMR ($CDCl_3$, 202 MHz) δ 50.82 (s); FAB-MS *m*/*z* 764 $(MH)^+$; UV (MeOH) λ_{max} 262, 295 (sh) nm; Anal. Calcd for $C_{33}H_{39}ClN_5O_8PS_2$: C, 51.86; H, 5.14; N, 9.16. Found: C, 51.96; H, 5.18; N, 8.88.

3.6. 8-Chloro-*N*-1-[(1*R*,2*R*,4*S*)-2-(methoxymethyloxy)-4-(phosphonoxymethyl)cyclopentyl]-5′- *O*-[(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine (13)

A mixture of $POCl_3$ (186 μL, 2.00 mmol) and **19** (153 mg, 0.200 mmol) in $PO(OEt)_3$ (2.0 mL) was stirred at 0 °C for 2 h. After addition of aqueous saturated $NaHCO_3$ (3 mL), the resulting mixture was stirred at 0 °C for 10 min. To the mixture were added triethylammonium acetate (TEAA) buffer (2.0 M, pH 7.0, 1 mL) and H_2O (5 mL), and the resulting solution was applied to a C_{18} reversed phase column (1.1 × 17 cm). The column was developed using a linear gradient of 0–70% MeCN 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 × 17 cm, eluted with 70% aqueous MeCN). Appropriate fractions were evaporated, and the residue was co-evaporated with pyridine

(2.0 mL × 3). A mixture of the residue, NMM (169 mg, 1.53 mmol), H_3PO_2 (155 μL, 3.05 mmol), and Et_3N (213 μL, 1.53 mmol) was stirred at 0 °C for 4 h under shading. After addition of TEAA buffer (1.0 M, pH 7.0, 2 mL), the resulting mixture was evaporated. The residue was partitioned between EtOAc and H_2O, and the aqueous layer was evaporated. A solution of the residue in H_2O (5 mL) was applied to a C_{18} reversed phase column (1.1 × 17 cm), and the column was developed using a linear gradient of 0–35% MeCN 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 × 17 cm, eluted with 50% aqueous MeCN). Appropriate fractions were evaporated, and the residue was lyophilized to give **13** (89 mg, 52%) as a triethylammonium salt: ^{1}H-NMR (D_2O, 500 MHz) δ 8.76 (s, 1H, H-2), 7.14–7.24 (m, 5H, SPh), 6.39 (br s, 1H, H-1′), 5.78 (d, 1H, H-2′, $J_{2',3'} = 6.3$ Hz), 5.25 (dd, 1H, H-3′, $J_{3',2'} = 6.3$ Hz, $J_{3',4'} = 2.7$ Hz), 4.84 (m, 1H, H-1″), 4.77 (d, 1H, MOM-CH_2, $J = 7.1$ Hz), 4.73 (d, 1H, MOM-CH_2, $J = 7.1$ Hz), 4.68 (m, 2H, H-2″, H-4′), 4.22 (m, 1H, H-5′a), 4.10 (m, 1H, H-5′b), 3.88 (m, 2H, H-5″ × 2), 3.34 (s, 3H, MOM-CH_3), 3.17 (q, 6H, Et_3NH–CH_2 × 3, $J = 7.3$ Hz), 2.66 (m, 1H, H-4″), 2.58 (m, 1H, H-6″a), 2.20 (m, 1H, H-3″a), 2.07 (m, 1H, H-3″b), 1.88 (m, 1H, H-6″b), 1.63, 1.42 (each s, each 3H, isopropylidene), 1.26 (t, 9H, Et_3NH–CH_3 × 3, $J = 7.3$ Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 150.4, 146.7, 145.3, 142.4, 131.4, 130.5, 129.4, 127.8, 118.6, 115.4, 96.8, 91.4, 87.6, 87.6, 83.8, 81.8, 81.7, 68.3, 66.2, 65.9, 56.3, 47.1, 34.8, 34.7, 32.8, 32.0, 26.3, 24.6, 8.7; ^{31}P-NMR (D_2O, 202 MHz) δ 17.31 (s), 1.43 (s); HRMS (FAB, negative) calcd for $C_{27}H_{35}ClN_5O_{12}P_2S$ 750.1167 [$(M - H)^-$], found 750.1141; UV (H_2O) λ_{max} 263 nm.

3.7. 8-Chloro-2″-*O*-methoxymethyl-3″-deoxy-cyclic ADP-carbocyclic-ribose 2′,3′-*O*-acetonide (12)

To a mixture of $AgNO_3$ (412 mg, 2.43 mmol), Et_3N (338 μL, 2.43 mmol), and MS 3A (powder, 500 mg) in pyridine (80 mL), a solution of **13** (99 mg, 0.12 mmol) in pyridine (80 mL) was added slowly over 15 h, using a syringe-pump, at room temperature under shading. The MS 3A was 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, 2 mL), and the resulting solution was evaporated. The residue was partitioned between EtOAc and H_2O, and the aqueous layer was evaporated. A solution of the residue in H_2O (5 mL) was applied to a C_{18} reversed phase column (1.1 × 17 cm), and the column was developed using a linear gradient of 0–25% MeCN 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 × 17 cm, eluted with 40% aqueous MeCN). Appropriate fractions were evaporated, and the residue was lyophilized to give **12** (73 mg, 85%) as a triethylammonium salt: ^{1}H-NMR (D_2O, 500 MHz) δ 9.19 (s, 1H, H-2), 6.40 (br s, 1H, H-1′), 5.63 (d, 1H, H-2′, $J_{2',3'} = 6.2$ Hz), 5.57 (dd, 1H, H-3′, $J_{3',2'} = 6.2$ Hz, $J_{3',4'} = 2.5$ Hz), 4.95 (m, 1H, H-1″), 4.89 (d, 1H, MOM-CH_2, $J = 7.1$ Hz), 4.78 (d, 1H, MOM-CH_2, $J = 7.1$ Hz), 4.58 (m, 1H, H-4′), 4.34 (m, 1H, H-2″), 4.24 (m, 1H, H-5″a), 4.14 (m, 1H, H-5″b), 4.08 (m, 1H, H-5′a), 3.97 (m, 1H, H-5′b), 3.45 (s, 3H, MOM-CH_3), 3.19 (q, 6H, Et_3NH–CH_2 × 3, $J = 7.3$ Hz), 2.97 (m, 1H, H-6″a), 2.81 (m, 1H, H-4″), 2.51 (m, 1H, H-6″b), 2.15 (m, 1H, H-3″a), 2.05 (m, 1H, H-3″b), 1.64, 1.44 (each s, each 3H, isopropylidene), 1.27 (t, 9H, Et_3NH–CH_3 × 3, $J = 7.3$ Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 150.7, 146.9, 145.3, 141.9, 118.8, 115.1, 97.2, 91.7, 87.2, 85.5, 84.8, 82.2, 67.7, 66.5, 64.3, 56.2, 47.1, 37.2, 33.4, 30.3, 26.3, 24.7, 8.6; ^{31}P-NMR (D_2O, 202 MHz) δ −8.86 (d, $J = 11.4$ Hz), −10.08 (d, $J = 11.4$ Hz); HRMS (FAB, positive) calcd for $C_{21}H_{31}ClN_5O_{12}P_2$ 642.1127 (MH^+), found 642.1136; UV (H_2O) λ_{max} 263 nm.

3.8. 8-Chloro-3″-deoxy-cyclic ADP-carbocyclic-ribose (8)

A solution of **12** (19 mg, 25 μmol) in aqueous 80% HCO_2H (1 mL) was stirred at room temperature for 49 h and then evaporated. After co-evaporation of the residue with H_2O (2 mL × 3), aqueous 28% NH_4OH (1 mL) was added, and the mixture was stirred at room temperature for 2 h. The solvent was evaporated, and the residue was co-evaporated with H_2O (2 mL × 3). The resulting residue was dissolved in TEAB buffer (0.1 M, pH 7.0, 786 μL), and the solution was lyophilized to give **8** (8 mg, 54%) as a triethylammonium salt: ^{1}H-NMR (D_2O, 500 MHz, potassium salt) δ 9.09 (s, 1H, H-2), 6.18 (d, 1H, H-1′, $J_{1',2'} = 6.2$ Hz), 5.25 (m, 1H, H-2′), 4.87 (m, 1H, H-1″), 4.67 (m, 1H, H-3′), 4.51 (m, 2H, H-2″, H-5″a), 4.42 (m, 1H, H-4′), 4.20 (m, 1H, H-5″b), 4.11 (m, 1H, H-5′b), 4.04 (m, 1H, H-5″b), 2.91 (m, 1H, H-6″a), 2.79 (m, 1H, H-4″), 2.51 (m, 1H, H-6″b), 2.17 (m, 1H, H-3″a), 1.98 (m, 1H, H-3″b); ^{13}C-NMR (D_2O, 125 MHz) δ 150.9, 147.6, 145.0, 142.5, 119.0, 90.4, 85.5, 78.6, 73.5, 71.0, 68.2, 68.0, 65.2, 37.5, 35.9, 30.7; ^{31}P-NMR (D_2O, 202 MHz) δ −9.72 (d, $J = 11.4$ Hz), −10.62 (d, $J = 11.4$ Hz); HRMS (FAB, positive) calcd for $C_{16}H_{23}ClN_5O_{11}P_2$ 558.0552 (MH^+), found 558.0561; UV (H_2O) λ_{max} 263 nm.

3.9. 8-Azido-2″-*O*-methoxymethyl-3″-deoxy-cyclic ADP-carbocyclic-ribose 2′,3′-*O*-acetonide (20)

A mixture of **12** (46 mg, 61 μmol) and LiN_3 (60 mg, 1.2 mmol) in pyridine (2.0 mL) was stirred at room temperature for 4 days. To the mixture were added TEAA buffer (2.0 M, pH 7.0, 1 mL) and H_2O (5 mL), and the resulting solution was applied to a C_{18} reversed phase column (1.1 × 17 cm). The column was developed using a linear gradient of 0–30% MeCN 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 × 17 cm, eluted with 50% aqueous MeCN). Appropriate fractions were evaporated, and the residue was lyophilized to give **20** (35 mg, 76%) as a triethylammonium salt: ^{1}H-NMR (D_2O, 500 MHz) δ 9.12 (s, 1H, H-2), 6.14 (br s, 1H, H-1′), 5.57 (d, 1H, H-2′, $J_{2',3'} = 6.1$ Hz), 5.50 (d, 1H, H-3′, $J_{3',2'} = 6.1$ Hz), 4.94 (m, 1H, H-1″), 4.90 (d, 1H, MOM-CH_2, $J = 7.2$ Hz), 4.79 (d, 1H, MOM-CH_2,

J = 7.2 Hz), 4.57 (m, 1H, H-4′), 4.41 (m, 1H, H-2″), 4.21 (m, 1H, H-5″a), 4.10 (m, 2H, H-5″b, H-5′a), 3.96 (m, 1H, H-5′b), 3.46 (s, 3H, MOM-CH_3), 3.20 (q, 6H, Et_3NH–$CH_2 \times 3$, J = 7.3 Hz), 2.97 (m, 1H, H-6″a), 2.81 (m, 1H, H-4″), 2.49 (m, 1H, H-6″b), 2.17 (m, 1H, H-3″a), 2.05 (m, 1H, H-3″b), 1.62, 1.43 (each s, each 3H, isopropylidene), 1.28 (t, 9H, Et_3NH–$CH_3 \times 3$, J = 7.3 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 149.5, 149.4, 146.9, 143.9, 118.3, 114.9, 97.1, 90.5, 87.0, 85.4, 84.8, 82.3, 67.6, 66.8, 64.4, 56.2, 47.1, 37.2, 33.5, 30.5, 26.3, 24.7, 8.7; ^{31}P-NMR (D_2O, 202 MHz) δ −9.43 (d, J = 11.4 Hz), −10.77 (d, J = 11.4 Hz); HRMS (FAB, negative) calcd for $C_{21}H_{29}N_8O_{12}P_2$ 647.1386 [$(M - H)^-$], found 647.1389; UV (H_2O) λ_{max} 284 nm.

3.10. 8-Azido-3″-deoxy-cyclic ADP-carbocyclic-ribose (9)

Compound **9** (11 mg, 56%) was obtained from **20** (22 mg, 29 μmol) as described for the synthesis of **8**: ^{1}H-NMR (D_2O, 500 MHz) δ 9.02 (s, 1H, H-2), 5.89 (d, 1H, H-1′, $J_{1',2'} = 6.2$ Hz), 5.17 (m, 1H, H-2′), 4.84 (m, 1H, H-1″), 4.62 (m, 1H, H-3′), 4.49 (m, 1H, H-2″), 4.47 (m, 1H, H-5′a), 4.36 (m, 1H, H-4′), 4.19 (m, 1H, H-5″a), 4.08 (m, 1H, H-5′b), 4.02 (m, 1H, H-5″b), 3.18 (q, 6H, Et_3NH–$CH_2 \times 3$, J = 7.3 Hz), 2.90 (m, 1H, H-6″a), 2.77 (m, 1H, H-4″), 2.52 (m, 1H, H-6″b), 2.16 (m, 1H, H-3″a), 1.97 (m, 1H, H-3″b), 1.26 (t, 9H, Et_3NH–$CH_3 \times 3$, J = 7.3 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 150.1, 149.8, 147.4, 143.9, 118.4, 88.8, 85.3, 78.6, 73.3, 71.0, 68.3, 67.9, 65.2, 47.1, 37.6, 35.9, 30.7, 8.7; ^{31}P-NMR (D_2O, 202 MHz) δ −9.64 (d, J = 11.4 Hz), −10.48 (d, J = 11.4 Hz); HRMS (FAB, positive) calcd for $C_{16}H_{23}N_8O_{11}P_2$ 565.0956 (MH^+), found 565.0968; UV (H_2O) λ_{max} 282 nm.

3.11. 8-Amino-2″-*O*-methoxymethyl-3″-deoxy-cyclic ADP-carbocyclic-ribose 2′,3′-*O*-acetonide (21)

A mixture of **20** (12 mg, 16 μmol) and 10% Pd–C (10 mg) in H_2O (1.0 mL) was stirred under atmospheric pressure of H_2 at room temperature for 1 h. The Pd–C was filtered off with Celite and washed with H_2O, and the combined filtrate and washing were evaporated. To the residue were added TEAA buffer (2.0 M, pH 7.0, 500 μL) and H_2O (3 mL), and the resulting solution was applied to a C_{18} reversed phase column (1.1 × 17 cm). The column was developed using a linear gradient of 0–25% MeCN in TEAA buffer (0.1 M, pH 7.0, 300 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reversed phase column chromatography (1.1 × 17 cm, eluted with 40% aqueous MeCN). Appropriate fractions were evaporated, and the residue was lyophilized to give **21** (10 mg, 89%) as a triethylammonium salt: ^{1}H-NMR (D_2O, 500 MHz) δ 9.06 (s, 1H, H-2), 6.16 (br s, 1H, H-1′), 5.65 (d, 1H, H-2′, $J_{2',3'} = 6.2$ Hz), 5.58 (dd, 1 H, H-3′, $J_{3',2'} = 6.2$ Hz, $J_{3',4'} = 2.3$ Hz), 4.91 (m, 1H, H-1″), 4.88 (d, 1H, MOM-CH_2, J = 7.2 Hz), 4.76 (d, 1H, MOM-CH_2, J = 7.2 Hz), 4.50 (m, 1H, H-4′), 4.29 (m, 1H, H-2″), 4.23 (m, 1H, H-5″a), 4.12 (m, 1H, H-5″b), 4.02 (m, 1H, H-5′a), 3.88 (m, 1H, H-5′b), 3.45 (s, 3H, MOM-CH_3), 3.18 (q, 6H, Et_3NH–$CH_2 \times 3$, J = 7.3 Hz), 2.93 (m, 1H, H-6″a), 2.79 (m, 1H, H-4″), 2.53 (m, 1H, H-6″b), 2.12 (m, 1H, H-3″a), 2.00 (m, 1H, H-3″b), 1.62, 1.43 (each s, each 3H, isopropylidene), 1.26 (t, 9H, Et_3NH–$CH_3 \times 3$, J = 7.3 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 155.3, 147.70, 146.8, 141.9, 118.2, 114.9, 97.2, 89.7, 86.9, 85.8, 84.7, 82.4, 67.5, 66.5, 64.3, 56.2, 47.1, 37.3, 33.1, 30.1, 26.3, 24.7, 8.7; ^{31}P-NMR (D_2O, 202 MHz) δ −9.46 (d, J = 15.3 Hz), −10.61 (d, J = 15.3 Hz); HRMS (FAB, negative) calcd for $C_{21}H_{31}N_6O_{12}P_2$ 621.1481 [$(M - H)^-$], found 621.1462; UV (H_2O) λ_{max} 277 nm.

3.12. 8-Amino-3″-deoxy-cyclic ADP-carbocyclic-ribose (10)

Compound **10** (6.0 mg, 43%) was obtained from **21** (15 mg, 21 μmol) as described for the synthesis of **8**: ^{1}H-NMR (D_2O, 500 MHz) δ 8.88 (s, 1H, H-2), 5.91 (d, 1H, H-1′, $J_{1',2'} = 6.3$ Hz), 5.31 (dd, 1H, H-2′, $J_{2',1'} = 6.3$ Hz, $J_{2',3'} = 4.9$ Hz), 4.82 (m, 1H, H-1″), 4.64 (dd, 1H, H-3′, $J_{3',2'} = 4.9$ Hz, $J_{3',4'} = 2.3$ Hz), 4.49 (m, 2H, H-5′a, H-2″), 4.37 (m, 1H, H-4′), 4.19 (m, 1H, H-5″a), 4.10 (m, 1H, H-5′b), 4.01 (m, 1H, H-5″b), 3.20 (q, 6H, Et_3NH–$CH_2 \times 3$, J = 7.3 Hz), 2.89 (m, 1H, H-6″a), 2.77 (m, 1H, H-4″), 2.53 (m, 1H, H-6″b), 2.16 (m, 1H, H-3″a), 1.96 (m, 1H, H-3″b), 1.28 (t, 9H, Et_3NH–$CH_3 \times 3$, J = 7.3 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 156.0, 148.0, 147.4, 118.3, 88.4, 85.1, 85.0, 78.7, 72.8, 71.0, 68.0, 67.9, 65.2, 47.2, 37.6, 35.9, 30.6, 8.7; ^{31}P-NMR (D_2O, 202 MHz) δ −9.38 (d, J = 11.4 Hz), −10.11 (d, J = 11.4 Hz); HRMS (FAB, positive) calcd for $C_{16}H_{25}N_6O_{11}P_2$ 539.1051 $(MH)^+$, found 539.1052; UV (H_2O) λ_{max} 276 nm.

3.13. 8-Propylmino-2″-*O*-methoxymethyl-3″-deoxy-cyclic ADP-carbocyclic-ribose 2′,3′-*O*-acetonide (22)

A solution of **12** (15 mg, 20 μmol) in propylamine (1.0 mL) was stirred at room temperature for 69 h and then evaporated. To the residue were added TEAA buffer (2.0 M, pH 7.0, 500 μL) and H_2O (3 mL), and the resulting solution was applied to a C_{18} reversed phase column (1.1 × 17 cm). The column was developed using a linear gradient of 0–40% MeCN 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 × 17 cm, eluted with 50% aqueous MeCN). Appropriate fractions were evaporated, and the residue was lyophilized to give **22** (13 mg, 74%) as a triethylammonium salt: ^{1}H-NMR (D_2O, 500 MHz) δ 8.03 (s, 1H, H-2), 6.39 (d, 1H, H-2′, $J_{2',3'} = 5.7$ Hz), 5.87 (s, 1H, H-1′), 5.06 (d, 1H, H-3′, $J_{3',2'} = 5.7$ Hz), 4.85 (d, 1H, MOM-CH_2, J = 7.0 Hz), 4.82 (d, 1H, MOM-CH_2, J = 7.0 Hz), 4.41 (m, 1H, H-4′), 4.37 (m, 1H, H-1″), 4.21 (m, 1H, H-2″), 3.74 (m, 1H, H-5″a), 3.45 (s, 3H, MOM-CH_3), 3.42 (m, 1H, H-5′a), 3.36 (m, 1H, NH–*CH_2*–CH_2CH_3), 3.28 (m, 2H, H-5″b, NH–*CH_2*–CH_2CH_3), 3.19 (q, 12H, Et_3NH–$CH_2 \times 6$, J = 7.3 Hz), 2.93 (m, 1H, H-5′b), 2.51 (m, 1H, H-4″), 2.09 (m, 4H, H-6″a, H-6″b, H-3″a, H-3″b), 1.68 (m, 2H, NH–CH_2–*CH_2*–CH_3), 1.60, 1.51 (each s, each 3H, isopropylidene), 1.27 (t, 18H, Et_3NH–$CH_3 \times 6$, J = 7.3 Hz),

0.97 (t, 3H, NH–CH_2CH_2–CH_3, J = 7.4 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 152.8, 149.6, 148.9, 148.0, 117.1, 113.9, 95.3, 89.9, 87.5, 83.2, 82.3, 69.8, 65.3, 58.0, 55.8, 47.1, 45.2, 36.2, 36.1, 32.6, 31.3, 26.0, 24.6, 22.3, 11.2, 8.7; ^{31}P-NMR (D_2O, 202 MHz) δ -10.00 (d, J = 11.4 Hz), −11.00 (d, J = 11.4 Hz); HRMS (FAB, positive) calcd for $C_{24}H_{39}N_6O_{12}P_2$ 665.2101 $(MH)^+$, found 665.2108; UV (H_2O) λ_{max} 284 nm.

3.14. 8-Propylamino-3″-deoxy-cyclic ADP-carbocyclic-ribose (11)

A solution of **8** (7.0 mg, 12 μmol) in propylamine (1.0 mL) was stirred at room temperature for 11 days and then evaporated. To the residue were added TEAA buffer (2.0 M, pH 7.0, 500 μL) and H_2O (3 mL), and the resulting solution was applied to a C_{18} reversed phase column (1.1 × 17 cm). The column was developed using a linear gradient of 0–25% MeCN in TEAA buffer (0.1 M, pH 7.0, 300 mL). Appropriate fractions for **11** and for **24** were evaporated, respectively. Excess TEAA of each fraction was removed by C_{18} reversed phase column chromatography (1.1 × 17 cm, eluted with 40% aqueous MeCN). The residues were lyophilized to give **11** (4.0 mg, 39%) and **24** (2.0 mg, 24%) as triethylammonium salts, respectively: **11**: ^{1}H-NMR (D_2O, 500 MHz) δ 8.01 (s, 1H, H-2), 5.72 (d, 1H, H-2′, $J_{2',3'}$ = 4.3 Hz), 5.63 (br s, 1H, H-1′), 4.71 (m, 1H, H-3′), 4.39 (m, 1H, H-1″), 4.13 (m, 1H, H-4′), 4.01 (m, 1H, H-2″), 3.80 (m, 1H, H-5′a), 3.73 (m, 1H, H-5″a), 3.64 (m, 1H, H-5′b), 3.44 (m, 1H, H-5″b), 3.33 (m, 1H, NH–CH_2–CH_2CH_3), 3.28 (m, 1H, NH–CH_2–CH_2CH_3), 3.17 (q, 12H, Et_3NH–CH_2 × 6, J = 7.3 Hz), 2.54 (m, 1H, H-4″), 2.13 (m, 1H, H-3″a), 2.01 (m, 1H, H-6″a), 1.98 (m, 1H, H-3″b), 1.95 (m, 1H, H-6″b), 1.68 (m, 2H, NH–CH_2–CH_2–CH_3), 1.25 (t, 18H, Et_3NH–CH_3 × 6, J = 7.3 Hz), 0.96 (t, 3H, NH–CH_2CH_2–CH_3, J = 7.3 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 152.4, 148.8, 147.2, 90.0, 82.7, 77.3, 72.5, 70.3, 69.4, 63.8, 59.8, 47.1, 45.3, 36.0, 34.3, 31.3, 22.1, 11.1, 8.7; ^{31}P-NMR (D_2O, 202 MHz) δ −9.19 (s), −9.37 (s); HRMS (FAB, negative) calcd for $C_{19}H_{29}N_6O_{11}P_2$ 579.1375 $[(M-H)^-]$, found 579.1376; UV (H_2O) λ_{max} 282 nm; **24**: ^{1}H-NMR (D_2O, 500 MHz) δ 8.01 (s, 1H, H-2), 5.98 (d, 1H, H-1′, $J_{1',2'}$ = 7.6 Hz), 4.76 (dd, 1H, H-2′, $J_{2',1'}$ = 7.6 Hz, $J_{2',3'}$ = 5.7 Hz), 4.49 (dd, 1H, H-3′, $J_{3',2'}$ = 5.7 Hz, $J_{3',4'}$ = 2.6 Hz), 4.34 (m, 1H, H-4′), 4.30 (m, 1H, H-5′a), 4.25 (m, 1H, H-1″), 4.21 (m, 1H, H-5′b), 3.88 (m, 1H, H-5″ × 2), 3.45 (m, 1H, H-2″), 3.41 (m, 4H, NH–CH_2–CH_2CH_3 × 2), 3.18 (q, 6H, Et_3NH–CH_2 × 3, J = 7.3 Hz), 2.50 (m, 1H, H-4″), 2.34 (m, 1H, H-3″a), 1.96 (m, 1H, H-6″a), 1.79 (m, 1H, H-6″b), 1.67 (m, 4H, NH–CH_2–CH_2–CH_3 × 2), 1.61 (m, 1H, H-3″b), 1.26 (t, 9H, Et_3NH–CH_3 × 3, J = 7.3 Hz), 0.96 (m, 6H, NH–CH_2CH_2–CH_3 × 2); ^{13}C-NMR (D_2O, 125 MHz) δ 152.4, 151.0, 149.0, 148.3, 116.5, 86.8, 84.6, 74.8, 70.9, 70.4, 69.2, 65.9, 58.3, 47.1, 44.7, 43.1, 35.0, 34.5, 30.6, 22.5, 22.3, 11.1, 11.0, 8.7; ^{31}P-NMR (D_2O, 202 MHz) δ −10.43 (d, J = 19.1 Hz), −11.64 (d, J = 19.1 Hz); FAB-MS m/z 638 $[(M-H)^-]$; UV (H_2O) λ_{max} 283 nm.

3.15. Ca^{2+}-mobilizing activity in sea urchin egg homogenate

The assay was carried out as described previously.[7c]

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References and notes

1. This report constitutes Part 247 of *Nucleosides and Nucleotides*. Part 246: Hirano, S.; Ichikawa, S.; Matsuda, A. Submitted for publication.
2. Clapper, D. L.; Walseth, T. F.; Dargie, P. J.; Lee, H. C. *J. Biol. Chem.* **1987**, *262*, 9561–9568.
3. (a) Lee, H. C. *Physiol. Rev.* **1997**, *77*, 1133–1164; (b) Zhang, F.-J.; Gu, Q.-M.; Sih, C. J. *Bioorg. Med. Chem.* **1999**, *7*, 653–664; (c)*Cyclic ADP-ribose and NAADP: Structures, Metabolism and Functions*; Lee, H. C., Ed.; Kluwer Academic Publishers: Dordrecht, 2002; (d) Shuto, S.; Matsuda, A. *Curr. Med. Chem.* **2004**, *11*, 827–845; (e) Potter, B. V. L.; Walseth, T. F. *Curr. Mol. Med.* **2004**, *4*, 303–311.
4. Lee, H. C.; Aarhus, R. *Biochem. Biophys. Acta* **1993**, *1164*, 68–74.
5. Walseth, T. F.; Lee, H. C. *Biochem. Biophys. Acta* **1993**, *1178*, 235–242.
6. (a) Shuto, S.; Shirato, M.; Sumita, Y.; Ueno, Y.; Matsuda, A. *J. Org. Chem.* **1998**, *63*, 1986–1994; (b) Fukuoka, M.; Shuto, S.; Minakawa, N.; Ueno, Y.; Matsuda, A. *J. Org. Chem.* **2000**, *65*, 5238–5248; (c) Shuto, S.; Fukuoka, M.; Manikowsky, A.; Ueno, Y.; Nakano, T.; Kuroda, R.; Kuroda, H.; Matsuda, A. *J. Am. Chem. Soc.* **2001**, *123*, 8750–8759.
7. (a) Shuto, S.; Fukuoka, M.; Kudoh, T.; Garnham, C.; Galione, A.; Potter, B. V. L.; Matsuda, A. *J. Med. Chem.* **2003**, *46*, 4741–4749; (b) Hashii, M.; Shuto, S.; Fukuoka, M.; Kudoh, T.; Matsuda, A.; Higashida, H. *J. Neurochem.* **2005**, *94*, 316–323; (c) Kudoh, T.; Fukuoka, M.; Ichikawa, S.; Murayama, T.; Ogawa, Y.; Hashii, M.; Higashida, H.; Kunerth, S.; Weber, K.; Guse, A. H.; Potter, B. V. L.; Matsuda, A.; Shuto, S. *J. Am. Chem. Soc.* **2005**, *127*, 8846–8855; (d) Kudoh, T.; Murayama, T.; Ogawa, Y.; Matsuda, A.; Shuto, S. *Nucleosides, Nucleotides, Nucleic Acids* **2006**, *25*, 583–599.
8. Sekine, M.; Nishiyama, S.; Kamimura, T.; Osaki, Y.; Hata, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 850–860.
9. Yoshikawa, M.; Kato, T.; Takenishi, T. *Bull, Chem. Soc. Jpn.* **1969**, *42*, 3505–3508.
10. Hata, T.; Kamimura, T.; Urakami, K.; Kohno, K.; Sekine, M.; Kumagai, I.; Shinozaki, K.; Miura, K. *Chem. Lett.* **1987**, 117–120.
11. Shiwa, S.; Murayama, T.; Ogawa, Y. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2002**, *282*, R727–R737.
12. Bailey, V. C.; Sethi, J. K.; Fortt, S. M.; Galione, A.; Potter, B. V. L. *Chem. Biol.* **1997**, *4*, 51–61.