Convergent Synthesis and Unexpected Ca^{2+}-Mobilizing Activity of 8-Substituted Analogues of Cyclic ADP-Carbocyclic-Ribose, a Stable Mimic of the Ca^{2+}-Mobilizing Second Messenger Cyclic ADP-Ribose

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Cyclic ADP-carbocyclic-ribose (cADPcR, **2**) is a biologically and chemically stable equivalent of cyclic ADP-ribose (cADPR, **1**), a Ca^{2+}-mobilizing second messenger. In this study, a series of 8-substituted analogues of cADPcR, namely the 8-chloro analogue **6** (8-Cl-cADPcR), the 8-azido analogue **7** (8-N_3-cADPcR), the 8-amino analogue **8** (8-NH_2-cADPcR), and the 8-phenylthio analogue **9** (8-SPh-cADPcR), were designed as effective pharmacological tools for studies on cADPR-modulated Ca^{2+} signaling pathways. These target compounds were synthesized by a convergent route via 8-Cl-cADPcR bisacetonide (**14**) as the common intermediate, in which a method for forming the intramolecular pyrophosphate linkage by activation of the phenylthiophosphate type substrate **15** with $AgNO_3$ to produce **14** was used as the key step. The carbocyclic analogues were tested for activity in the sea urchin egg homogenate system. Compounds were assessed for their calcium-mobilizing effects and their ability to cross-desensitize with calcium release induced by a normally maximal concentration of cADPR, as well as cADPR antagonism of cADPR-evoked calcium release. While cADPcR was 3−4 times more potent than cADPR, the 8-substituted analogues were less efficacious, with 8-SPh-cADPcR largely acting as a competitive antagonist. Most surprisingly, given that 8-N_3-cADPR and 8-NH_2-cADPR are known as potent antagonists, 8-N_3-cADPcR and 8-NH_2-cADPcR were full agonists, but ca. 80 and 2 times less potent than cADPR, respectively. These data contribute to developing structure−activity relationships for the interaction of cADPR with its receptor.

Introduction

Considerable attention has been focused on cyclic ADP-ribose (cADPR, **1**), an intracellular Ca^{2+}-mobilizing adenine nucleotide.[2] cADPR has been shown to mobilize intracellular Ca^{2+} in various cells, such as sea urchin eggs, pancreatic beta cells, smooth muscles, cardiac muscles, T-lymphocytes, and cerebella neurons, indicating that it is a general mediator involved in Ca^{2+} signaling.[3] The structure of cADPR has been investigated[4] and was confirmed by X-ray crystallographic analysis as shown in Figure 1.[4c]

In cells, cADPR is synthesized from NAD^+ by ADP-ribosyl cyclases and acts as a second messenger; it is hydrolyzed rapidly by cADPR hydrolases to give the non-Ca^{2+} mobilizing metabolite ADP-ribose under physiological conditions.[3] cADPR is also known to be readily hydrolyzed nonenzymatically at the unstable *N*-1-glycosidic linkage of its adenine moiety to give ADP-ribose, even in neutral aqueous solution.[5] Consequently, the biological as well as chemical instability of cADPR limits, to some extent, further studies of its physiological role. Therefore, stable analogues of cADPR exhibiting a Ca^{2+}-mobilizing activity in cells similar to that of cADPR are very useful in biological studies.

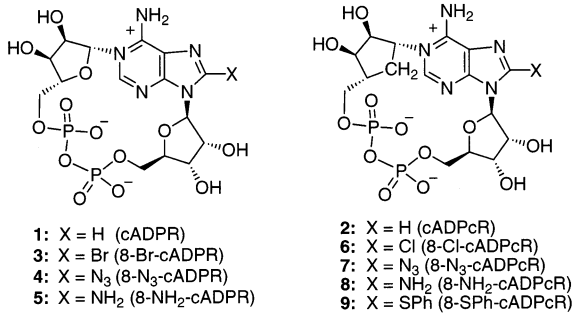

Figure 1. Structures of cADPR (**1**), cADPcR (**2**), and their 8-substituted analogues.

We designed cyclic ADP-carbocyclic-ribose (cADPcR, **2**) as a stable mimic of cADPR,[6] in which the oxygen atom in the *N*-1-ribose ring of cADPR is replaced by a methylene group. We hypothesized that (1) the mimic should be resistant to both enzymatic and chemical hydrolysis, since it has a chemically and biologically stable *N*-alkyl linkage instead of the unstable *N*-1-glycosidic linkage of cADPR, and that (2) the mimic, like cADPR, would effectively mobilize intracellular Ca^{2+} since it preserves all of the functional groups of cADPR except for the ring oxygen of the *N*-1-linked ribose and should have a conformation similar to that of cADPR. We recently accomplished the total synthesis of the mimic **2**, showing that it is actually chemically and biologically stable.[6f] The preliminary evaluation of **2** injected into sea urchin eggs suggested that it seems to

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have very potent Ca^{2+}-mobilizing activity.[6f] Therefore, studies on the mechanism of cADPR-modulated Ca^{2+} signaling pathways using the mimic **2** are now in progress.

cADPR and its analogues have been synthesized by enzymatic or chemoenzymatic methods using ADP-ribosyl cyclase from *Aplysia californica*, which mediates the intramolecular ribosylation of NAD^+ and some modified NAD^+ (prepared chemically or enzymatically) at the *N*-1-position of the purine moiety, to yield cADPR or the corresponding analogues.[7] These studies disclosed that some 8-substituted analogues of cADPR, such as 8-Br-cADPR (**3**), 8-N_3-cADPR (**4**), or 8-NH_2-cADPR (**5**), are antagonists of cADPR at its intracellular receptor,[7a] and these analogues have been effectively used as pharmacological tools for the studies on cADPR-modulated Ca^{2+} signaling pathways.[3]

These findings prompted us to synthesize the 8-modified analogues of cADPcR, which were expected to possess the properties of both the carbocyclic analogue **2** and the 8-substituted cADPR analogues. For example, 8-NH_2-cADPcR (**8**) was expected to be a chemically and biologically stable potent antagonist of cADPR, and 8-N_3-cADPcR (**7**) might be used as an efficient photoaffinity labeling probe for cADPR-related proteins.

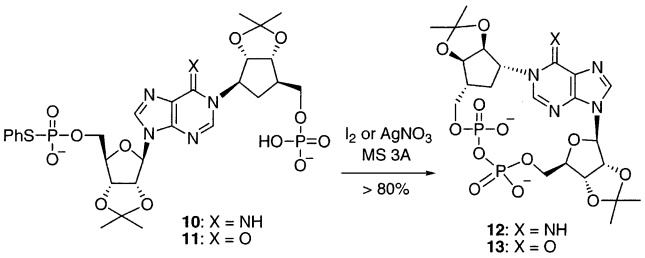
In this report, we describe the convergent synthesis of a series of 8-substituted analogues of cADPcR, namely the 8-chloro analogue **6** (8-Cl-cADPcR), the 8-azido analogue **7** (8-N_3-cADPcR), the 8-amino analogue **8** (8-NH_2-cADPcR), and the 8-phenylthio analogue **9** (8-SPh-cADPcR) (Figure 1), and their effects on Ca^{2+}-mobilization in the sea urchin homogenate system. The detailed Ca^{2+}-mobilizing activity of cADPcR is also described. The carbocyclic analogues were tested for activity in sea urchin egg homogenate, a robust assay for calcium mobilization, and the system in which the calcium mobilizing properties of cADPR were first discovered.[4a]

Results and Discussion

Synthetic Plan. Most of the previous cADPR analogues have been synthesized by enzymatic or chemoenzymatic methods with ADP-ribosyl cyclase from *A. californica* as described above.[7] Although the specificity of ADP-ribosyl cyclase is somewhat loose, the analogues obtained by this method are limited by the substrate specificity of the enzyme.[7p]

On the other hand, in the chemical synthesis of cADPR and its analogues, the key intramolecular condensation step to form the pyrophosphate linkage has proved difficult, preventing completion of the chemical synthesis of the target cADPR analogues.[8] However, in recent years, we have developed an efficient method for forming the intramolecular pyrophosphate linkage by activation of phenylthiophosphate type substrates, such as **10** or **11**, with I_2 or $AgNO_3$ in the presence of molecular sieves (MS) 3A in pyridine, where the cyclization products **12** and **13** were obtained in 93% and 81% yield, respectively (Scheme 1).[6] Using this method we have successfully synthesized cADPcR (**2**) and its inosine congener.[6e,f] This represents a general method for synthesizing these types of biologically important cyclic nucleotides and particularly those chemically modified in the *N*-1-linked ribose moiety, which are not expected to be accessible by the chemoenzymatic route.[6g]

Scheme 1

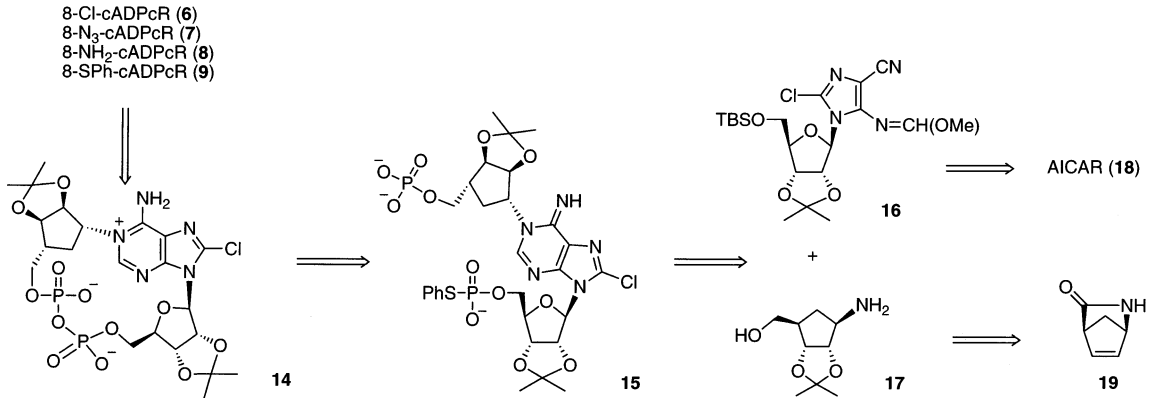


Encouraged by these results, we decided to synthesize the target 8-substituted cADPcR analogues **6**−**9** by a convergent route, as summarized in Scheme 2, using 8-Cl-cADPcR bis-acetonide (**14**) as the common intermediate. Acidic deprotection of **14** would readily provide 8-Cl-cADPcR (**6**). The other three targets **7**, **8**, and **9** would be obtained by nucleophilic addition−elimination reaction at the 8-position of **14** followed by deprotection. The intramolecular pyrophosphate linkage could be constructed according to the method described above: treatment of 8-chloro-5′-phenylthiophosphate substrate **15** with $AgNO_3$/MS 3A as a promoter[6c,e,f] was expected to give the cyclized **14**. The substrate **15** would be provided from the 2-chloroimidazole nucleoside derivative **16** and the optically active carbocyclic amine **17**, which could be prepared from 5-aminoimidazole-4-carboxamide riboside (AICAR, **18**) and commercially available (1*R*)-(−)-2-azabicyclo[2.2.1]hept-5-en-3-one (**19**).[8c]

Construction of the *N*-1-Carbocyclic-Ribosyladenosine Structure. An efficient method for constructing the *N*-1-carbocyclic-ribosyladenosine structure was essential for completing the synthesis of the targets. We previously found that treatment of the imidazole nucleoside **22** and the chiral carbocyclic amine **17** under mild basic conditions with K_2CO_3 provided the *N*-1-carbocyclic-ribosyladenosine derivative **23** in high yield (Scheme 3).[6f] Thus, construction of the 8-chloro-*N*-1-carbocyclic-ribosyl derivative **24** was investigated according to a similar procedure. A chloro group was introduced at the imidazole 2-position of the known imidazole nucleoside **20**[8c] by treatment with *N*-chlorosuccinimide (NCS) in THF to give **21**. The 2-chloroimidazole nucleoside **21** was heated in $HC(OMe)_3$ under reflux in the presence of a catalytic amount of TFA to give the methoxymethylene derivative **16**, which was next subjected to the pyrimidine ring-closure reaction. When a mixture of **16** and **17** (1.4 equiv) was treated with K_2CO_3 in DMF at room temperature, the ring-closure reaction proceeded to give the desired product **24** in 89% yield. The *N*-1-carbocyclic-ribosyladenine structure of **24** was confirmed by an NOE experiment; a correlation (19.6%) between the H-2 of the adenine and the C-1″ of the cyclopentane ring was observed.

Synthesis of 8-Cl-cADPcR. 8-Cl-cADPcR (**6**) was successfully synthesized from the *N*-1-carbocyclic-ribosyl-adenosine derivative **24**, as shown in Scheme 4. After protection of the 5″-hydroxyl of **24** with a monomethoxytrityl (MMTr) group, the 5′-*O*-TBS group of the product **25** was removed with TBAF to give **26**. Treatment of **26** with an *S*,*S*′-diphenylphosphorodithioate (PSS)/2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl)/pyridine system[9] gave the 5′-bis(phenylthio)-phosphate **27** in 67% yield. After removal of the 5″-*O*-

Scheme 2



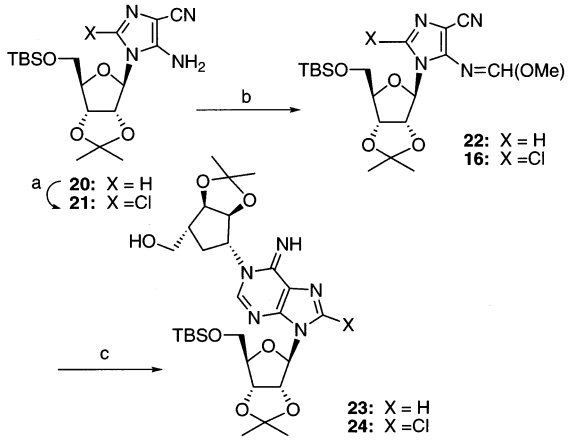
MMTr group of **27**, a phosphoryl group was introduced at the resulting 5″-primary hydroxyl of **28** using Yoshikawa's method with $POCl_3/(EtO)_3PO$,[10] followed by treatment of the product with H_3PO_2 and Et_3N[11] in the presence of *N*-methylmaleimide (NMM) in pyridine,[12] affording 5′-phenylthiophosphate **15** in 47% yield as the triethylammonium salt. When a solution of **15** 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,[6c,e,f] the desired cyclization product **14** was obtained in 88% yield. Finally, removal of the isopropylidene groups of **14** was carried out with aqueous HCO_2H to furnish 8-Cl-cADPcR (**6**).

Synthesis of the 8-Azido-, 8-Amino-, and 8-Phenylthio-cADPcR Analogues. The other three 8-substituted cADPcR analogues, **7**, **8**, and **9**, were synthesized from the isopropylidene-protected 8-Cl-cADPcR **14** via a nucleophilic addition−elimination reaction at the 8-position. Thus, treatment of **14** with LiN_3 or PhSH in pyridine at room temperature produced the corresponding 8-azido and 8-phenylthio derivatives **29** and **31** in excellent yields (Scheme 5). The azido group of **29** was readily reduced under usual catalytic hydrogenation conditions with Pd−C to give the 8-amino derivative **30**. The isopropylidene groups of **29**, **30**, and **31** were removed with aqueous HCO_2H to provide the target compounds, 8-N_3-cADPcR (**7**), 8-NH_2-cADPcR (**8**), and 8-SPh-cADPcR (**9**), respectively.

The results of this study as well as the previous syntheses of cADPcR (**2**)[7f] and its inosine congener[7c,e] clearly demonstrate that the strategy using a phenylthiophosphate-type substrate in the key intramolecular condensation reaction forming the pyrophosphate linkage is very efficient for the total syntheses of cADPR-related compounds.[13]

Biology. In the sea urchin egg homogenate, cADPR (**1**) mobilizes sequestered Ca^{2+} from microsomal vesicles in a concentration-dependent fashion (Figure 2a).[14] Such Ca^{2+} release exhibits the property of self-induced desensitization,[15] in that a subsequent challenge by a normally maximum concentration of cADPR (250 nM) is reduced depending on the initial concentration of the first cADPR challenge. We demonstrated the biological properties of cADPcR (**2**) by showing that it was a potent Ca^{2+}-mobilizing molecule and that after sequestration of mobilized calcium it cross-desensitized with calcium release by the subsequent addition of a normally maximal concentration of cADPR (250 nM) in a concentration-dependent manner as for cADPR (Figure 2b).

Scheme 3[a]



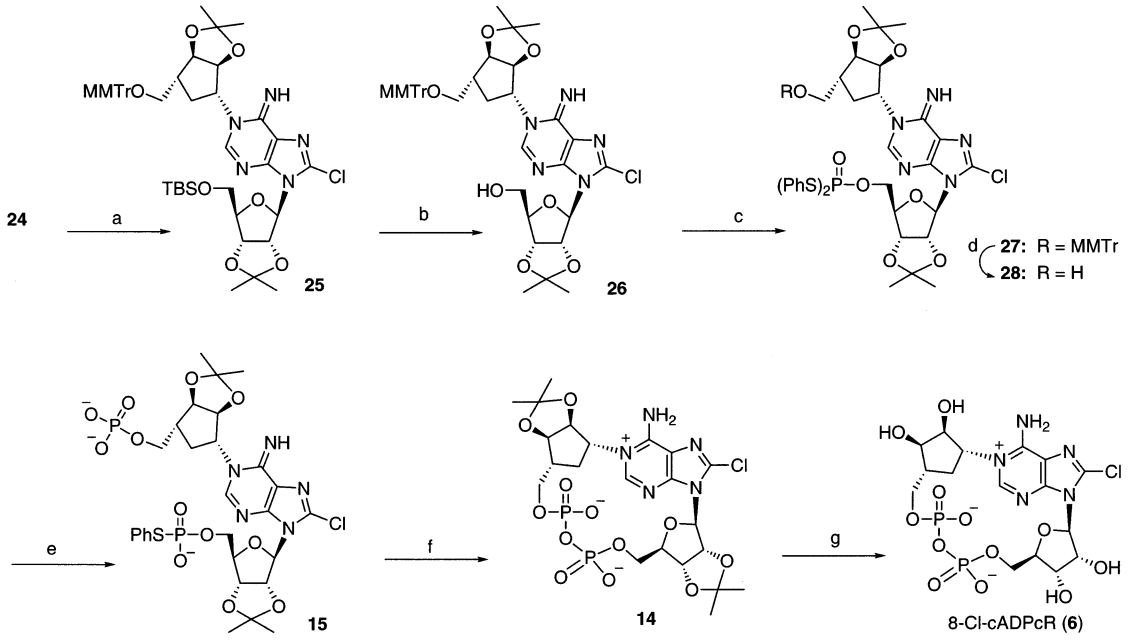
[a] Reagents and conditions: (a) NCS, THF, rt, 84%; (b) $HC(OMe)_3$, cat. CF_3CO_2H, reflux, 95% (**16**), quant % (**22**); (c)**17**, K_2CO_3, MeOH or DMF, rt, 83% (23), 89% (**24**).

Interestingly, cADPcR, with an EC_{50} value of 14.6 nM, was ca. 3−4 times more potent than cADPR (EC_{50} = 50 nM). These qualitative data, showing that cADPcR is an even more potent Ca^{2+}-mobilizing agent than cADPR, are in accord with the preliminary results by the injection of the compounds into sea urchin eggs.[6f]

Next, the effects of a series of 8-substituted carbocyclic analogues of cADPR were examined. Surprisingly, both 8-N_3-cADPcR (**7**) and 8-NH_2-cADPcR (**8**) were full agonists, with EC_{50} values of 3.9 μM (Figure 2c) and 80 nM, respectively (Figure 2d), and were therefore ca. 80 and 2 times less potent than cADPR in mobilizing intracellular Ca^{2+}. This is in contrast to the effects of the corresponding non-carbocyclic analogues, 8-N_3-cADPR and 8-NH_2-cADPR, which are both antagonists.[7a] In addition, the 8-substituted carbocyclic analogues **7** and **8** also exhibited the property of cross-desensitization with cADPR-induced Ca^{2+} release.

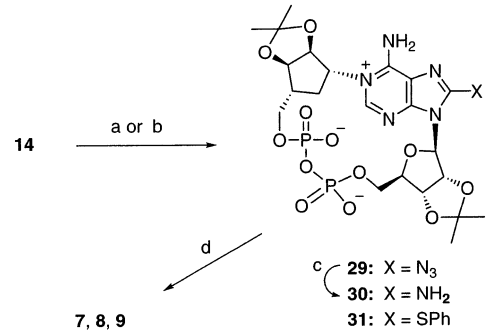
Further 8-substituted analogues were assessed. 8-Cl-cADPcR (**6**) mobilized Ca^{2+} with a steep concentration-dependence (Figure 2e) with an EC_{50} of 19 μM, i.e., ca. 400-fold less potent than cADPR. This compound, however, did not fully cross-desensitize with cADPR for reasons that are not immediately apparent. Increasing the size and hydrophobicity of the 8-substituted group in 8-SPh-cADPcR (**9**) dramatically altered its pharmacological properties. This compound had minimal Ca^{2+} mobilizing efficacy, while at high concentrations it behaved as a competitive cADPR antagonist (Figure 2f).

Scheme 4[a]



[a] Reagents and conditions: (a) MMTrCl, pyridine, rt, 79%; (b) TBAF, THF, AcOH, rt, 88%; (c) PSS, TPSCl, py, rt, 67%; (d) aq 80% AcOH, rt, 85%; (e) 1) $POCl_3$, $(EtO)_3PO$,0 °C, (2) H_3PO_2, Et_3N, NMM, pyridine, rt, 47%; (g) $AgNO_3$, MS 3A, Et_3N, py, rt, 88%; (h) aq 60% HCO_2H, rt, 98%.

Scheme 5[a]



[a] Reagents and conditions: (a) PhSH, py, rt, 86% (**29**); (b) LiN_3, py, 50 °C, 81%; (c) H_2, Pd-C, H_2O, rt, 67%; (d) aq 60% HCO_2H, rt, 97% (7), 83% (**8**), 88% (**9**).

cADPcR is a highly potent agonist in sea urchin homogenates and indeed even more potent than cADPR itself. This is due either to lack of hydrolysis during the assay, or perhaps to a more favorable interaction of the modified *N*-1-attached "northern ribose"[6g] with the receptor, which would be rather surprising given that a potential H-bond acceptor has been deleted.

The simple expectation for the activity of the 8-substituted analogues reported here was that they would retain significantly the activity of their parent 8-substituted cADPR analogues, perhaps with some modulation of Ca^{2+}-mobilizing potency, but that the nature of the activity agonist vs antagonist would not be affected. If we leave aside the activity of the 8-phenylthio analogue **9**, which only begins to be apparent at high concentrations, it is very striking that all three of the other analogues **6**, **7**, and **8** that possess the 8-substitutions, previously shown to confer antagonist behavior to various degrees in the cADPR series, become agonists as their cADPcR counterparts. Nowhere is this more apparent than with 8-NH_2-cADPcR (**8**), which has a potency very close to cADPR itself. It is only possible to speculate at this time as to the reasons behind this, but clearly deletion of the "northern" ribofuranosyl oxygen atom removes a structural motif in an extremely sensitive area that determines the fundamental activity. Interestingly, when cADPcR (**2**) and its 8-amino derivative **8** were examined for Ca^{2+}-mobilizing activity in another well characterized biological system, Jurkat T-lymphocytes, both compounds were found to exhibit weak agonism, being about 5−10-fold less potent than cADPR.[6g] Thus, in a mammalian cell system, cADPcR behaves, in terms of potency, differently to in the invertebrate sea urchin system. However, 8-NH_2-cADPcR, while weak, does also exhibit agonist rather than antagonist properties, which is in qualitative agreement with the data reported here. No further interpretation on these relative aspects can be entered into at the present time, except insofar as it is known that these two receptors exhibit different recognition characteristics toward some cADPR analogues,[3h] pointing to not surprising potentially wider differences in protein structure and function.[3] It is worth also noting that recently diverse analogues have been synthesized with more radical changes in the adenine ring and also in the "northern" ribose than those reported here.[13] Interestingly, some of these compounds, although substituted at the 8-position, like most of the compounds reported here, also appear in intact HeLa cells and rat brain microsomes to exhibit agonist rather than antagonist properties.[13b,c]

Although the factors that control agonist/antagonist behavior in cADPR are not well understood, the present work provides new insight into them in analogues particularly close in structure to cADPR itself. It seems unlikely however that one unique motif is all pervading in this respect and more so that a global conformation is adopted in either mode, that may be perturbed by point changes in the molecule, for example by disruption of hydrogen bonds or changes in the conformation of the

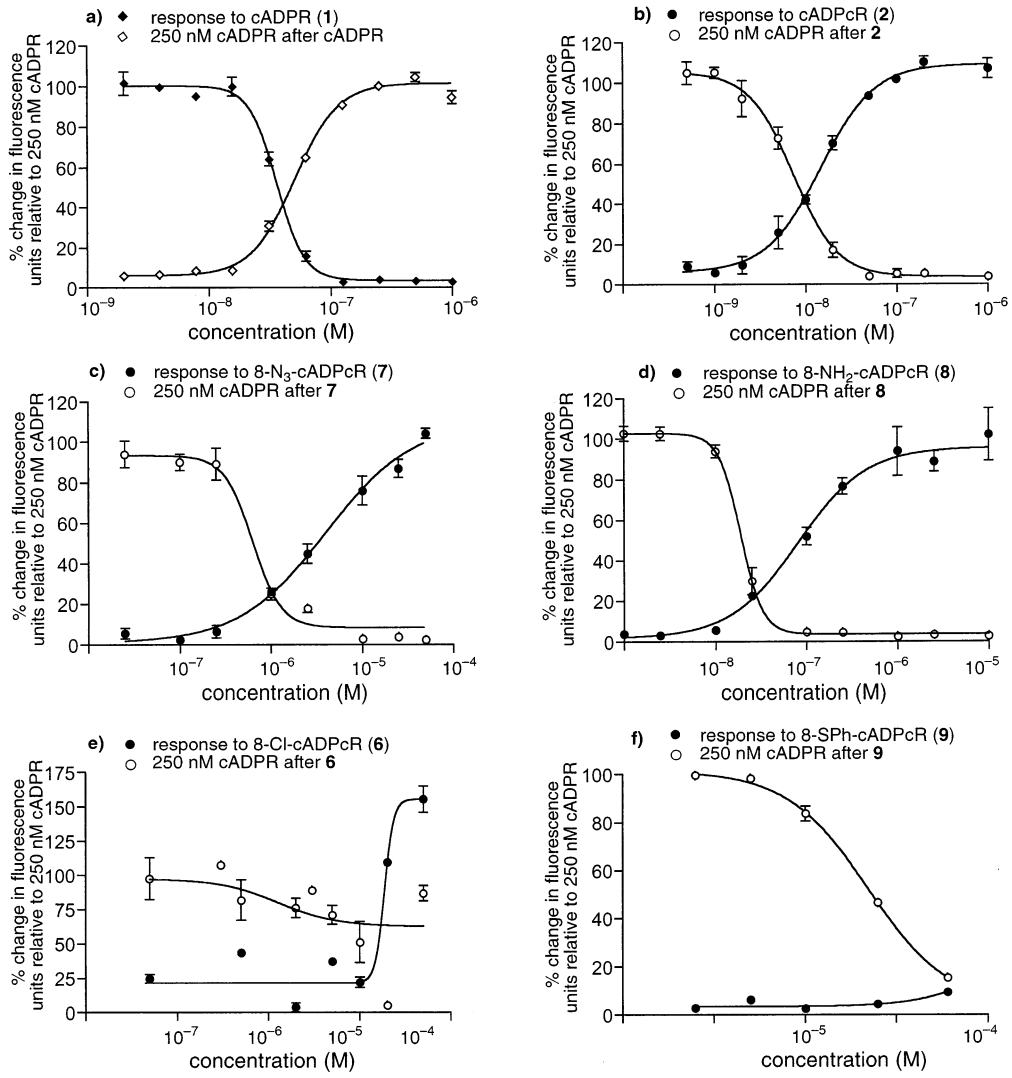


Figure 2. Ca^{2+} release by cADPR (**1**) and its carbocyclic analogues in the sea urchin egg homogenate: (a) cADPcR (**1**), (b) cADPcR (**2**), (c) 8-N_3-cADPcR (**7**), (d) 3-NH_2-cADPcR (**8**), (e) 8-Cl-cADPcR (**6**), and (f) 8-SPh-cADPcR (**9**).

ribose groups. The previous preliminary ^{1}H NMR analysis suggested that an overall change in the conformation of cADPcR compared with natural cADPR is unlikely.[6f] Further detailed studies to clarify the precise conformation of the molecules are required, particularly in relation to the biological activity. There is no doubt that this antagonist/agonist switch induced by a rather conservative carbocycle introduction is a novel effect that will stimulate further work toward this goal.

Conclusion. A series of 8-substituted analogues of cADPcR was synthesized by a convergent route via 8-Cl-cADPcR bis-acetonide as the common intermediate, in which a method for forming the intramolecular pyrophosphate linkage by activation of the phenylthiophosphate moiety with $AgNO_3$ was used as the key step. We have characterized quantitatively the more potent Ca^{2+}-mobilizing activity of cADPcR than the natural second messenger cADPR. Additionally, to contribute toward the emerging SAR of cADPR, we have evaluated the 8-substituted analogues, most of which possess nonadditive and unexpected biological activity as agonists rather than antagonists. One, in particular, by means of a single substitution illustrates a dramatic switch from a highly potent antagonist to an almost equipotent agonist. Elucidation of the structural basis for this effect will be of significant future interest.

Experimental Section

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

2-Chloro-5-amino-1-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-*O*-(isopropylidene)-*β*-D-ribofuranosyl]imidazole-4-nitrile (21). A mixture of **20** (5.9 g, 15.0 mmol) and NCS (2.4 g, 18.0 mmol) in THF (150 mL) was stirred at room temperature for 10.5 h. After addition of saturated aqueous $NaHCO_3$ (10 mL), the resulting solution was stirred at room temperature for 10 min and then 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, 20% AcOEt in hexane) to give **21** (5.4 g, 84%) as a yellow solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.84 (d, 1 H, H-1′, $J_{1',2'} = 4.0$ Hz), 5.04 (s, 2 H, NH_2), 4.97 (dd, 1 H, H-2′, $J_{2',1'} = 4.0$, $J_{2',3'} = 6.8$ Hz), 4.89 (dd, 1 H, H-3′, $J_{3',2'} = 6.8$, $J_{3',4'} = 4.3$ Hz), 4.14 (ddd, 1 H, H-4′, $J_{4',3'} = 4.3$, $J_{4',5'a} = 1.7$, $J_{4',5'b} = 0.9$ Hz), 4.02 (dd, 1 H, H-5′a, $J_{5'a,4'} = 1.7$, $J_{5'a,5'b} = 11.7$ Hz), 3.90 (dd, 1 H, H-5′b, $J_{5'b,4'} = 0.9$, $J_{5'b,5'a} = 11.7$ Hz), 1.59, 1.36 (each s, each 3 H, isopropyl CH_3),

0.93 (s, 9H, *tert*-butyl), 0.14, 0.13 (each s, each 3H, dimethyl); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 147.3, 125.6, 115.6, 114.6, 93.5, 93.5, 90.6, 84.5, 82.1, 78.8, 62.4, 27.3, 26.0, 25.4, 18.7, −5.4; HRMS (FAB, positive) calcd for $C_{18}H_{30}ClN_4O_4Si$ 429.1725 (MH^+), found 429.1683; UV (MeOH) λ_{max} 250 nm. Anal. ($C_{18}H_{29}ClN_4O_4Si$) C, H, Cl, N.

2-Chloro-5-(methoxymethyleneamino)-1-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-*O*-(isopropylidene)-*β*-D-ribofuranosyl]imidazole-4-nitrile (16). A mixture of **21** (4.7 g, 11 mmol) and TFA (43 μL, 550 μmol) in $(MeO)_3CH$ (23.5 mL, 220 mmol) was stirred at 50 °C for 10 min. The mixture was evaporated, and the residue was purified by column chromatography (SiO_2, 20% AcOEt in hexane) to give **16** (4.9 g, 95%) as a yellow solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.34 (s, 1 H, N=CH), 5.94 (d, 1 H, H-1′, $J_{1',2'}$ = 3.2 Hz), 5.23 (dd, 1 H, H-2′, $J_{2',1'}$ = 3.2, $J_{2',3'}$ = 6.8 Hz), 4.79 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.8, $J_{3',4'}$ = 5.1 Hz), 4.05 (m, 1 H, H-4′), 3.98 (s, 3 H, OCH_3), 3.83−3.76 (m, 2 H, H-5′), 1.58, 1.36 (each s, each 3 H, isopropyl CH_3), 0.89 (s, 9 H, *tert*-butyl), 0.06, 0.05 (each s, each 3 H, dimethyl); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 160.9, 145.7, 130.5, 115.3, 114.4, 98.9, 89.0, 85.7, 82.4, 80.3, 62.8, 55.2, 27.3, 25.9, 25.5, 18.4, −5.4; HRMS (FAB, positive) calcd for $C_{20}H_{32}ClN_4O_5Si$ 471.1830 (MH^+), found 471.1805; UV (MeOH) λ_{max} 277 nm. Anal.($C_{20}H_{31}ClN_4O_5Si$ $C_{18}H_{29}ClN_4O_4Si$) C, H, Cl, N.

8-Chloro-*N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-(hydroxymethyl)cyclopentyl]-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylideneadenosine (24). A mixture of **16** (4.7 g, 10 mmol), **17** (2.7 g, 14 mmol), and K_2CO_3 (20 mg, 0.15 mmol) in DMF (65 mL) was stirred at room temperature for 20 h and then 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, 90% AcOEt in hexane) to give **24** (5.6 g, 89%) as a white foam: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.61 (s, 1 H, H-2), 7.17 (br s, 1 H, NH), 6.07 (d, 1 H, H-1′, $J_{1',2'}$ = 2.0 Hz), 5.48 (dd, 1 H, H-2′, $J_{2',1'}$ = 2.0, $J_{2',3'}$ = 6.3 Hz), 5.32 (dd, 1 H, H-2″, $J_{2'',1''}$ = 5.2, $J_{2'',3''}$ = 5.8 Hz), 4.99 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 3.8 Hz), 4.74 (dd, 1 H, H-3″, $J_{3'',2''}$ = 5.8, $J_{3'',4''}$ = 2.8 Hz), 4.59 (br s, 1 H, 5′-OH), 4.52 (ddd, 1 H, H-1″, $J_{1'',2''}$ = 5.2, $J_{1'',6''a}$ = 9.8, $J_{1'',6''b}$ = 9.7 Hz), 4.20 (ddd, 1 H, H-4′, $J_{4',3'}$ = 3.8, $J_{4',5'a}$ = 6.1, $J_{4',5'b}$ = 6.1 Hz), 3.80 (dd, 1 H, H-5″a, $J_{5''a,4''}$ = 3.8, $J_{5''a,5''b}$ = 10.8 Hz), 3.74−3.68 (m, 3 H, H-5′, H-5″b), 2.63 (m, 1 H, H-6″a), 2.54 (m, 1 H, H-4″), 2.45 (m, 1 H, H-6″b), 1.59, 1.56, 1.37, 1.31 (each s, each 3 H, isopropyl CH_3), 0.85 (s, 9 H, *tert*-butyl), 0.00, −0.02 (each s, each 3 H, dimethyl); NOE ($CDCl_3$, 400 MHz) irradiated H-2, observed H-1″ (19.6%); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 152.5, 147.4, 141.5, 135.6, 122.8, 114.3, 111.9, 90.0, 87.6, 83.5, 83.2, 82.0, 81.5, 71.0, 64.7, 63.0, 44.9, 30.2, 28.1, 27.2, 25.9, 25.4, 25.3, 18.4, −5.3, −5.4; HRMS (FAB, positive) calcd for $C_{28}H_{45}ClN_5O_7Si$ 626.2777 (MH^+), found 626.2747; UV (MeOH) λ_{max} 263 nm, sh 300 nm (ϵ = 14860 at λ_{max} = 263 nm (pH 2.0)); Anal. ($C_{28}H_{44}ClN_5O_7Si$) C, H, Cl, N.

8-Chloro-*N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-[(5-monomethoxytrityl)oxymethyl]cyclopentyl]-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylideneadenosine (25). A mixture of **24** (5.5 g, 8.8 mmol) and MMTrCl (5.4 g, 17.6 mmol) in pyridine (50 mL) was stirred at room temperature for 1.5 h and then 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, 30% AcOEt in hexane) to give **25** (6.2 g, 79%) as a yellow foam: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.63 (s, 1 H, H-2), 7.45−6.82 (m, 15 H, NH, Ar−H), 6.08 (d, 1 H, H-1′, $J_{1',2'}$ = 2.0 Hz), 5.52 (dd, 1 H, H-2′, $J_{2',1'}$ = 2.0, $J_{2',3'}$ = 6.3 Hz), 5.13 (dd, 1 H, H-2″, $J_{2'',1''}$ = 4.8, $J_{2'',3''}$ = 6.8 Hz), 5.01 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 3.6 Hz), 4.89 (m, 1 H, H-1″), 4.56 (dd, 1 H, H-3″, $J_{3'',2''}$ = 6.8, $J_{3'',4''}$ = 6.8 Hz), 4.22 (ddd, 1 H, H-4′, $J_{4',3'}$ = 3.6, $J_{4',5'a}$ = 6.5, $J_{4',5'b}$ = 6.1 Hz), 3.79 (s, 3 H, OCH_3), 3.73 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 6.5, $J_{5'a,5'b}$ = 10.7 Hz), 3.70 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 6.1, $J_{5'b,5'a}$ = 10.7 Hz), 3.34 (dd, 1 H, H-5″a, $J_{5''a,4''}$ = 4.5, $J_{5''a,5''b}$ = 9.0 Hz), 3.19 (dd, 1 H, H-5″b, $J_{5''b,4''}$ = 6.5, $J_{5''b,5''a}$ = 9.0 Hz), 2.52 (m, 1 H, H-6″a), 2.42−2.38 (m, 2 H, H-4″, H-6″b), 1.60, 1.53, 1.39, 1.28 (each s, each 3 H, isopropyl CH_3), 0.88 (s, 9 H, *tert*-butyl), 0.02, 0.01 (each s, each 3 H, dimethyl); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 158.5, 152.8, 146.4, 144.6, 144.5, 141.1, 135.7, 135.0, 130.4, 128.4, 127.7, 126.8, 122.8, 114.2, 113.1, 113.0, 90.0, 87.6, 86.2, 83.1, 82.4, 81.7, 64.8, 64.2, 63.0, 55.2, 44.9, 33.2, 27.7, 27.2, 25.9, 25.4, 25.3, 18.4, −5.3, −5.4; HRMS (FAB, positive) calcd for $C_{48}H_{61}ClN_5O_8Si$ 898.3978 (MH^+), found 898.3979; UV (MeOH) λ_{max} 263 nm, sh 301 nm. Anal. ($C_{48}H_{60}ClN_5O_8Si$) C, H, Cl, N.

8-Chloro-*N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-[(5-monomethoxytrityl)oxymethyl]cyclopentyl]-2′,3′-*O*-isopropylideneadenosine (26). A mixture of **25** (6.1 g, 6.8 mmol), TBAF (1.0 M in THF, 15 mL, 15 mmol), and AcOH (440 μL, 6.9 mmol) in THF (10 mL) was stirred at room temperature for 1 h and then evaporated. The residue was purified by column chromatography (SiO_2, 60% AcOEt in hexane) to give **26** (4.7 g, 88%) as a yellow foam: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.69 (s, 1 H, H-2), 7.44−6.81 (m, 15 H, NH, Ar−H), 6.00 (d, 1 H, H-1′, $J_{1',2'}$ = 5.0 Hz), 5.28 (m, 1 H, 5′-OH), 5.11−5.09 (m, 2 H, H-2′, H-2″), 5.02 (dd, 1 H, H-3′, $J_{3',2'}$ = 5.8, $J_{3',4'}$ = 1.0 Hz), 4.91 (m, 1 H, H-1″), 4.53 (m, 1 H, H-3″), 4.45 (m, 1 H, H-4′), 3.91 (m, 1 H, H-5′a), 3.79 (s, 3 H, OCH_3), 3.75 (m, 1 H, H-5′b), 3.34 (dd, 1 H, H-5″a, $J_{5''a,4''}$ = 3.5, $J_{5''a,5''b}$ = 8.8 Hz), 3.18 (dd, 1 H, H-5″b, $J_{5''b,4''}$ = 5.6, $J_{5''b,5''a}$ = 8.8 Hz), 2.45−2.38 (m, 3 H, H-4″, H-6″), 1.64, 1.52, 1.38, 1.27 (each s, each 3 H, isopropyl CH_3); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 158.5, 152.5, 146.7, 144.5, 144.5, 140.3, 135.7, 134.5, 130.3, 128.4, 127.7, 126.8, 123.4, 114.2, 113.3, 113.0, 92.2, 86.1, 85.5, 83.1, 82.2, 81.5, 81.2, 64.8, 64.2, 63.1, 55.2, 44.7, 33.4, 27.7, 27.5, 25.3, 25.3; HRMS (FAB, positive) calcd for $C_{42}H_{47}ClN_5O_8$ 784.3113 (MH^+), found 784.3090; UV (MeOH) λ_{max} 264 nm, sh 307 nm. Anal. ($C_{42}H_{46}ClN_5O_8$) C, H, Cl, N.

8-Chloro-*N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-[(5-monomethoxytrityl)oxymethyl]cyclopentyl]-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine (27). After stirring a mixture of PSS (7.6 g, 20 mmol) and TPSCl (6.1 g, 20 mmol) in pyridine (40 mL) at room temperature for 2 h, **26** (4.7 g, 5.9 mmol) was added, and the resulting mixture was stirred at room temperature for further 2 h. The mixture was evaporated, and the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated, and the residue was purified by column chromatography (SiO_2, 60% AcOEt in hexane) to give **27** (4.1 g, 67%) as a yellow foam: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.66 (s, 1 H, H-2), 7.48−6.81 (m, 25 H, NH, Ar−H), 6.13 (d, 1 H, H-1′, $J_{1',2'}$ = 1.3 Hz), 5.44 (dd, 1 H, H-2′, $J_{2',1'}$ = 1.3, $J_{2',3'}$ = 6.3 Hz), 5.09 (m, 1 H, H-2″), 5.08 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.3, $J_{3,4'}$ = 3.4 Hz), 4.92 (m, 1 H, H-1″), 4.50 (m, 1 H, H-3″), 4.42−4.33 (m, 3 H, H-4′, H-5′), 3.78 (s, 3 H, OCH_3), 3.33 (m, 1 H, H-5″a), 3.16 (dd, 1 H, H-5″b, $J_{5''b,4''}$ = 4.0, $J_{5''b,5''a}$ = 8.6 Hz), 2.40 (m, 3 H, H-4″, H-6″), 1.61, 1.52 1.38, 1.22 (each s, each 3 H, isopropyl CH_3); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 158.5, 152.6, 146.7, 144.5, 144.5, 135.6, 135.3, 135.3, 135.1, 135.0, 130.3, 129.5, 129.3, 128.4, 127.7, 126.8, 125.9, 114.6, 113.2, 113.0, 90.1, 86.2, 85.7, 85.6, 83.7, 82.5, 81.6, 81.3, 66.2, 66.1, 64.8, 64.2, 55.2, 44.8, 33.4, 27.7, 27.1, 25.3; ^{31}P NMR ($CDCl_3$, 202 MHz, decoupled with ^{1}H) δ 50.9; HRMS (FAB, positive) calcd for $C_{54}H_{56}ClN_5O_9PS_2$ 1048.2945 (MH^+), found 1048.2950; UV (MeOH) λ_{max} 254 nm, sh 305 nm. Anal. ($C_{54}H_{55}ClN_5O_9PS_2$) C, H, Cl, N.

8-Chloro-*N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-(hydroxymethyl)cyclopentyl]-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine (28). A solution of **27** (3.9 g, 3.7 mmol) in 80% aqueous AcOH (40 mL) was stirred at room temperature for 6 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 then with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 3% MeOH in $CHCl_3$) to give **28** (2.4 g, 85%) as a white foam: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.66 (br s, 1 H, H-2), 7.49−7.27 (m, 11 H, NH, Ar−H), 6.13 (d, 1 H, H-1′, $J_{1',2'}$ = 1.0 Hz), 5.43 (dd, 1 H, H-2′, $J_{2',1'}$ = 1.0, $J_{2',3'}$ = 6.2 Hz), 5.25 (dd, 1 H, H-2″, $J_{2'',1''}$ = 5.4, $J_{2'',3''}$ = 5.5 Hz), 5.07 (dd, 1 H, H-3′, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 3.0

Hz), 4.68 (dd, 1 H, H-3″, $J_{3'',2''} = 5.5$, $J_{3'',4''} = 1.7$ Hz), 4.54 (m, 1 H, H-1″), 4.41−4.31 (m, 3 H, H-4′, H-5′), 3.73 (dd, 1 H, H-5″a, $J_{5''a,4''} = 3.7$, $J_{5''a,5''b} = 10.8$ Hz), 3.69 (dd, 1 H, H-5″b, $J_{5''b,4''} = 2.6$, $J_{5''b,5''a} = 10.8$ Hz), 2.53 (m, 2 H, H-4″, H-6″a), 2.40 (m, 1 H, H-6″b), 1.61, 1.55 1.38, 1.30 (each s, each 3 H, isopropyl CH_3); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 152.5, 147.7, 141.3, 135.3, 135.3, 135.2, 135.0, 135.0, 130.2, 129.6, 129.6, 129.5, 129.5, 129.3, 129.3, 128.0, 126.0, 125.9, 125.7, 122.7, 114.5, 111.8, 90.2, 86.8, 85.7, 83.8, 83.3, 82.3, 81.4, 70.4, 66.2, 66.1, 64.5, 44.8, 30.1, 28.0, 27.1, 25.2; ^{31}P NMR ($CDCl_3$, 202 MHz, decoupled with ^{1}H) δ 51.1; HRMS (FAB, positive) calcd for $C_{34}H_{40}ClN_5O_8PS_2$ 776.1744 (MH^+), found 776.1733; UV (MeOH) λ_{max} 256 nm, sh 304 nm. Anal. ($C_{34}H_{39}ClN_5O_8PS_2$) C, H, Cl, N.

8-Chloro-*N*-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-(phosphonoxymethyl)cyclopentyl]-5′-*O*-[(phenylthio)-phosphoryl]-2′,3′-*O*-isopropylideneadenosine (15). A mixture of $POCl_3$ (93 μL, 1.0 mmol) and **28** (78 mg, 0.10 mmol) in $PO(OMe)_3$ (2.0 mL) was stirred at 0 °C for 2 h. After addition of aqueous saturated $NaHCO_3$ (3.0 mL), the resulting mixture was stirred at 0 °C for 10 min. To the mixture was added triethylammonium acetate (TEAA, 2.0 M, pH 7.0, 1.0 mL) buffer and H_2O (4.0 mL), and the resulting solution was applied to a C_{18} reversed phase column (1.1 × 11 cm). The column was developed using a linear gradient of 0−65% 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 × 11 cm, eluted with 60% aqueous MeCN). Appropriate fractions were evaporated, and the residue was coevaporated with pyridine (1.0 mL x 3). A mixture of the residue, NMM (68 mg, 0.6 mmol), H_3PO_2 (62 μL, 1.2 mmol), and Et_3N (85 μL, 0.60 mmol) was stirred at room temperature for 3.5 h. After addition of TEAA buffer (2.0 M, pH 7.0, 0.5 mL), the resulting mixture was evaporated. The residue was partitioned between EtOAc and H_2O, and the aqueous layer diluted with TEAA buffer (2.0 M, pH 7.0, 0.5 mL) was evaporated. A solution of the residue in H_2O (5.0 mL) was applied to a C_{18} reversed phase column (1.1 × 11 cm), and 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 × 18 cm, eluted with 40% aqueous MeCN) to give **15** (41 mg, 47%) as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 8.67 (s, 1 H, H-2), 7.19−7.10 (m, 5 H, Ar−H), 6.37 (s, 1 H, H-1′), 5.78 (d, 1 H, H-2′, $J_{2',3'} = 6.1$ Hz), 5.23 (dd, 1 H, H-3′, $J_{3',2'} = 6.1$, $J_{3',4'} = 2.5$ Hz), 4.91−4.82 (m, 3 H, H-1″, H-2″, H-3″), 4.65 (m, 1 H, H-4′), 4.21 (m, 1 H, H-5′a), 4.06 (m, 1 H, H-5′b), 4.00 (m, 2 H, H-5″), 3.14 (q, 6 H, $-CH_2N$, $J = 7.3$ Hz), 2.59−2.54 (m, 2 H, H-4″, H-6″a), 2.39 (m, 1 H, H-6″b), 1.62, 1.61, 1.40, 1.40 (each s, each 3 H, isopropyl CH_3), 1.23 (t, 9 H, CH_3CH_2N, $J = 7.3$ Hz); ^{13}C NMR (D_2O, 125 MHz) δ 152.7, 149.1, 147.4, 144.8, 133.7, 133.7, 132.7, 131.7, 130.1, 120.8, 118.5, 117.7, 93.9, 89.9, 89.8, 86.4, 86.0, 83.9, 83.2, 68.1, 67.9, 49.4, 46.2, 46.2, 35.5, 28.7, 38.6, 26.9, 26.8, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ 1.08 (s), 17.2 (s); HRMS (FAB, negative) calcd for $C_{28}H_{35}ClN_5O_{12}P_2S$ 762.1167 [$(M - H)^-$], found 762.1165; UV (H_2O) $\lambda_{max} = 263$ nm.

8-Chloro-cyclic ADP-carbocyclic-ribose Diacetonide (14). To a mixture of $AgNO_3$ (34 mg, 200 μmol), Et_3N (27 μL, 200 μmol), and MS 3A (820 mg) in pyridine (7 mL) was added a solution of **14** (8.1 mg, 9.4 μmol) in pyridine (7 mL) slowly over 15 h, using a syringe-pump, at room temperature in the dark. 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, 1 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 TEAA buffer (0.1 M, pH 7.0, 5 mL) was applied to a C_{18} reverse phase column (1.1 × 11 cm), and the column was developed using a linear gradient of 0−40% MeCN in TEAA buffer (0.1 M, pH 7.0, 200 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reverse phase column chromatography (1.1 × 11 cm, eluted with 20% aqueous MeCN) to give **14** [123.5 OD_{263} units ($\epsilon = 14860$), 88%, calculated using $\epsilon = 14860$ at $\lambda_{max} = 263$ nm of **24** (pH 2.0)] as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 8.75 (s, 1 H, H-2), 6.39 (br s, 1 H, H-1′), 5.86 (d, 1 H, H-2′, $J_{2',3'} = 5.6$ Hz), 5.47 (dd, 1 H, H-3′, $J_{3',2'} = 5.6$, $J_{3',4'} = 2.3$ Hz), 4.83−4.81 (m, 3 H, H-1″, H-2″, H-3″), 4.55 (m, 1 H, H-4′), 4.15 (m, 1 H, H-5″a), 4.04 (m, 2 H, H-5′a, H-5″b), 3.87 (m, 1 H, H-5′b), 3.16 (q, 6 H, $-CH_2N$, $J = 7.3$ Hz), 3.12 (m, 1 H, H-6″a), 2.87 (m, 1 H, H-4″), 2.75 (m, 1 H, H-6″b), 1.60, 1.59, 1.42, 1.38 (each s, each 3 H, isopropyl CH_3), 1.23 (t, 9 H, CH_3CH_2N, $J = 7.3$ Hz); ^{13}C NMR (D_2O, 125 MHz) δ 152.8, 150.0, 146.6, 144.8, 121.1, 117.3, 115.2, 113.0, 93.7, 89.9, 89.8, 89.6, 87.5, 86.1, 84.2, 72.6, 69.3, 67.1, 49.5, 46.7, 46.6, 30.9, 28.9, 28.8, 27.2, 26.9, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ −10.56 (d, $J = 16.5$ Hz), −10.71 (d, $J = 16.5$ Hz); HRMS (FAB, negative) calcd for $C_{22}H_{29}ClN_5O_{12}P_2$ 652.0976 [$(M - H)^-$], found 652.0963; UV (H_2O) $\lambda_{max} = 263$ nm.

8-Chloro-cyclic ADP-carbocyclic-ribose (6). A solution of **14** (98.3 OD_{263} units) in 60% aqueous HCO_2H (1 mL) was stirred at room temperature for 3.5 h and then evaporated. After the residue was coevaporation with H_2O (2 mL × 3), the resulting residue was dissolved in TEAB buffer (0.1 M, pH 7.0, 30 μL) and H_2O (2 mL), and the solution was lyophilized to give **6** (96.8 OD_{263} units, 98%) as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 9.13 (s, 1 H, H-2), 6.13 (d, 1 H, H-1′, $J_{1',2'} = 6.3$ Hz), 5.16 (dd, 1 H, H-2′, $J_{2',1'} = 6.3$, $J_{2',3'} = 4.8$ Hz), 4.94 (m, 1 H, H-1″), 4.62 (dd, 1 H, H-3′, $J_{3',2'} = 4.8$, $J_{3',4'} = 2.4$ Hz), 4.51 (m, 1 H, H-5′a), 4.38−4.35 (m, 2 H, H-2″, H-4′), 4.21 (dd, 1 H, H-3″, $J_{3'',2''} = 4.4$, $J_{3'',4''} = 4.4$ Hz), 4.17 (m, 2 H, H-5″), 4.07 (m, 1 H, H-5′b), 3.16 (q, 6 H, $-CH_2N$, $J = 7.3$ Hz), 3.02 (m, 1 H, H-6″a), 2.51 (m, 1 H, H-4″), 2.37 (m, 1 H, H-6″b), 1.23 (t, 9 H, CH_3CH_2N, $J = 7.3$ Hz); ^{13}C NMR (potassium salt, D_2O, 125 MHz) δ 151.8, 147.6, 145.8, 142.5, 119.7, 91.0, 86.1, 79.6, 74.7, 74.2, 71.5, 66.3, 65.7, 64.7, 43.7, 29.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ -9.29 (d, $J = 11.4$ Hz), −10.31 (d, $J = 11.4$ Hz); HRMS (FAB, negative) calcd for $C_{16}H_{21}ClN_5O_{12}P_2$ 572.0350 [$(M - H)^-$], found 572.0359; UV (H_2O) $\lambda_{max} = 263$ nm. The absolute amount of **6** was calculated using $\epsilon = 14860$ at $\lambda_{max} = 263$ nm of **24** (pH 2.0)

8-Azido-cyclic ADP-carbocyclic-ribose Diacetonide (29). A mixture of **14** (9.0 mg, 12 μmol) and LiN_3 (23 mg, 48 μmol) in pyridine (5.0 mL) was stirred at 50 °C for 4 days. After addition of TEAA buffer (2.0 M, pH 7.0, 0.5 mL), the resulting solution was evaporated, and the residue was partitioned between AcOEt and H_2O. The aqueous layer was evaporated, and a solution of the residue in H_2O (5.0 mL) was applied to a C_{18} reverse phase column (1.1 × 12 cm). The column was developed using a linear gradient of 0−40% MeCN in TEAA buffer (0.1 M, pH 7.0, 300 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reverse phase column chromatography (1.1 × 11 cm, eluted with 30% aqueous Me_3CN) to give **29** (7.4 mg, 81%) as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 8.71 (s, 1 H, H-2), 6.16 (d, 1 H, H-1′, $J_{1',2'} = 1.2$ Hz), 5.81 (dd, 1 H, H-2′, $J_{2',1'} = 1.2$, $J_{2',3'} = 5.9$ Hz), 5.45 (dd, 1 H, H-3′, $J_{3',2'} = 5.9$, $J_{3',4'} = 2.2$ Hz), 4.89−4.85 (m, 3 H, H-1″, H-2″, H-3″), 4.58 (m, 1 H, H-4′), 4.17 (m, 1 H, H-5″a), 4.08 (m, 2 H, H-5′a, H-5″b), 3.94 (m, 1 H, H-5′b), 3.20 (q, 6 H, CH_2N, $J = 7.3$ Hz), 3.14 (m, 1 H, H-6″a), 2.91 (m, 1 H, H-4″), 2.76 (m, 1 H, H-6″b), 1.63, 1.63, 1.45, 1.43 (each s, each 3 H, isopropyl CH_3), 1.28 (t, 9 H, CH_3CH_2N, $J = 7.3$ Hz); ^{13}C NMR (D2O, 125 MHz) δ 152.1, 151.8, 149.6, 145.6, 120.6, 117.2, 115.2, 92.4, 89.6, 89.4, 87.4, 86.0, 84.2, 72.4, 69.2, 67.2, 49.5, 46.8, 31.1, 28.9, 28.8, 27.1, 26.9, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with 1H) δ −10.57 (d, $J = 15.3$ Hz), −10.69 (d, $J = 15.3$ Hz); HRMS (FAB, negative) calcd for $C_{22}H_{29}N_8O_{12}P_2$ 659.1380 [$(M - H)^-$], found 659.1345; UV (H_2O) $\lambda_{max} = 284$ nm.

8-Amino-cyclic ADP-carbocyclic-ribose Diacetonide (30). A mixture of **29** (181 OD_{284} units, 9.9 μmol) and 10% Pd−C (1.1 mg) in H_2O (1.0 mL) was stirred under atmospheric pressure of H_2 at 50 °C for 30 min. The Pd−C was filtered off with Celite and washed with H_2O, and the combined filtrate and washing was evaporated to give **30** (139 OD_{277} units, 87%) as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 8.60 (s, 1 H, H-2), 6.16 (s, 1 H, H-1′), 5.93 (d, 1 H, H-2′, $J_{2',3'} = 5.7$

Hz), 5.48 (d, 1 H, H-3′, $J_{3',2'}$ = 5.7 Hz), 4.83 (m, 3 H, H-1″, H-2″, H-3″), 4.55 (m, 1 H, H-4′), 4.18 (m, 1 H, H-5″a), 4.04 (m, 2 H, H-5′a, H-5″b), 3.88 (m, 1 H, H-5′b), 3.20 (q, 6 H, $-CH_2N$, J = 7.3 Hz), 3.13 (m, 1 H, H-6″a), 2.91 (m, 1 H, H-4″), 2.79 (m, 1 H, H-6″b), 1.64, 1.63, 1.47, 1.42 (each s, each 3 H, isopropyl CH_3), 1.28 (t, 9 H, CH_3CH_2N, J = 7.3 Hz); ^{13}C NMR (D_2O, 125 MHz) δ 157.9, 149.6, 117.1, 115.2, 91.9, 89.8, 89.3, 87.3, 85.9, 84.3, 72.2, 69.0, 67.1, 49.5, 46.5, 30.8, 28.9, 28.7, 27.1, 26.9, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ -10.38 (d, J = 15.3 Hz), −10.64 (d, J = 15.3 Hz); HRMS (FAB, negative) calcd for $C_{22}H_{31}N_6O_{12}P_2$ 633.1475 [$(M - H)^-$], found 633.1480; UV (H_2O) λ_{max} = 277 nm.

8-Phenylthio-cyclic ADP-carbocyclic-ribose Diacetonide (31). A mixture of **14** (9.0 mg, 12 μmol) and PhSH (49 μL, 48 μmol) in pyridine (1.0 mL) was stirred at room temperature for 1.5 h. After addition of TEAA buffer (2.0 M, pH 7.0, 0.5 mL), the resulting solution was evaporated. The residue was partitioned between AcOEt and H_2O, and the aqueous layer was evaporated. A solution of the residue in H_2O (5.0 mL) was applied to a C_{18} reverse phase column (1.1 × 11 cm), and the column was developed using a linear gradient of 0−50% MeCN in TEAA buffer (0.1 M, pH 7.0, 200 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reverse phase column chromatography (1.1 × 11 cm, eluted with 30% aqueous MeCN) to give **31** (8.5 mg, 119 OD_{286} units, 86%) as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 8.73 (s, 1 H, H-2), 7.66−7.51 (m, 5 H, Ar−H), 6.41 (d, 1 H, H-1′, $J_{1',2'}$ = 1.2 Hz), 5.91 (dd, 1 H, H-2′, $J_{2',1'}$ = 1.2, $J_{2',3'}$ = 5.9 Hz), 5.48 (dd, 1 H, H-3′, $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 2.6 Hz), 4.83−4.78 (m, 3 H, H-1″, H-2″, H-3″), 4.57 (m, 1 H, H-4′), 4.19 (m, 1 H, H-5″a), 4.12−4.08 (m, 2 H, H-5′a, H-5″b), 3.91 (m, 1 H, H-5′b), 3.20 (q, 6 H, CH_2N, J = 7.3 Hz), 3.13 (m, 1 H, H-6″a), 2.91 (m, 1 H, H-4″), 2.78 (m, 1 H, H-6″b), 1.64, 1.61, 1.46, 1.41 (each s, each 3 H, isopropyl CH_3), 1.28 (t, 9 H, CH_3CH_2N, J = 7.3 Hz); ^{13}C NMR (D_2O, 125 MHz) δ 156.7, 152.2, 151.0, 145.9, 136.8, 133.1, 133.0, 129.9, 122.3, 117.3, 115.2, 113.0, 93.4, 89.7, 89.5, 89.5, 87.5, 85.9, 84.1, 72.5, 69.3, 67.2, 49.5, 46.6, 46.6, 30.9, 28.9, 27.2, 26.9, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ -10.78 (d, J = 15.3 Hz), −10.94 (d, J = 15.3 Hz); HRMS (FAB, negative) calcd for $C_{28}H_{34}N_5O_{12}P_2S$ 726.1400[$(M - H)^-$], found 724.1427; UV (H_2O) λ_{max} = 286 nm.

8-Azido-cyclic ADP-carbocyclic-ribose (7). Compound **7** (63.3 OD_{283} units, 97%) was obtained from **29** (65.0 OD_{282} units) as described for the synthesis of **6** as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 9.13 (s, 1 H, H-2), 5.93 (d, 1 H, H-1′, $J_{1',2'}$ = 6.2 Hz), 5.17 (dd, 1 H, H-2′, $J_{2',1'}$ = 6.2, $J_{2',3'}$ = 5.7 Hz), 4.99 (m, 1 H, H-1″), 4.66 (m, 1 H, H-3′), 4.55 (m, 1 H, H-5′a), 4.41 (m, 2 H, H-2″, H-4′), 4.27 (m, 1 H, H-3″), 4.22 (m, 2 H, H-5″), 4.12 (m, 1 H, H-5′b), 3.22 (m, 6 H, CH_2N), 3.09 (m, 1 H, H-6″a), 2.58 (m, 1 H, H-4″), 2.46 (m, 1 H, H-6″b), 1.30 (m, 9 H, CH_3CH_2N); ^{13}C NMR (D_2O, 125 MHz) δ 152.4, 149.6, 146.4, 139.6, 120.7, 91.2, 87.6, 81.5, 74.6, 75.8, 73.4, 68.1, 67.6, 67.1, 49.5, 45.7, 30.9, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ -9.30 (d, J = 11.4 Hz), −10.26 (d, J = 11.4 Hz); HRMS (FAB, negative) calcd for $C_{16}H_{21}N_8O_{12}P_2$ 579.0754 [$(M - H)^-$], found 579.0792; UV (H_2O) λ_{max} = 283 nm. The absolute amount of **7** was calculated using ϵ = 18215 at λ_{max} = 281 nm of 8-azido-cADPR (**4**).[7a]

8-Amino-cyclic ADP-carbocyclic-ribose (8). Compound **8** (19.8 OD_{277} units, 83%) was obtained from **30** (23.9 OD_{277} units) as described for the synthesis of **6** as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 8.96 (s, 1 H, H-2), 5.90 (d, 1 H, H-1′, $J_{1',2'}$ = 6.3 Hz), 5.24 (dd, 1 H, H-2′, $J_{2',1'}$ = 6.3, $J_{2',3'}$ = 5.7 Hz), 4.92 (m, 1 H, H-1″), 4.63 (m, 1 H, H-3′), 4.52 (m, 1 H, H-5′a), 4.36 (m, 2 H, H-2″, H-4′), 4.23−4.19 (m, 3 H, H-3″, H-5″), 4.09 (m, 1 H, H-5′b), 3.19 (q, 6 H, CH_2N, J = 7.3 Hz), 3.03 (m, 1 H, H-6″a), 2.54 (m, 1 H, H-4″), 2.43 (m, 1 H, H-6″b), 1.27 (t, 9 H, CH_3CH_2N, J = 7.3 Hz); ^{13}C NMR (D_2O, 125 MHz) δ 158.3, 150.4, 149.6, 144.3, 120.6, 90.8, 87.4, 81.5, 76.5, 75.4, 73.4, 68.1, 67.6, 66.8, 49.5, 45.6, 30.8, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ -9.32 (d, J = 11.4 Hz), −10.19 (d, J = 11.4 Hz); HRMS (FAB, negative) calcd for $C_{16}H_{23}N_6O_{12}P_2$ 553.0849 [$(M - H)^-$], found 553.0839; UV (H_2O) λ_{max} = 277 nm (pH 7.0). The absolute amount of **6** was calculated using ϵ = 16000 at λ_{max} = 274 nm of 8-amino-cADPR (**5**).[7a]

8-Phenylthio-cyclic ADP-carbocyclic-ribose (9). A solution of **31** (117 OD_{286} units) in aqueous 60% HCO_2H (1.0 mL) was stirred at room temperature for 1.5 h and then evaporated. A solution of the residue in TEAA buffer (0.1 M, pH 7.0, 5.0 mL) was applied to a C_{18} reverse phase column (1.1 × 15 cm), and the column was developed using a linear gradient of 0−35% MeCN in TEAA buffer (0.1 M, pH 7.0, 200 mL). Appropriate fractions were evaporated under the reduced pressure, and excess TEAA was removed by C_{18} reverse phase column chromatography (1.1 × 11 cm, eluted with 30% aqueous MeCN) to give **9** (104 OD_{286} units, 89%) as a triethylammonium salt: ^{1}H NMR (D_2O, 500 MHz) δ 9.11 (s, 1 H, H-2), 7.67−7.40 (m, 5 H, Ar−H), 6.21 (d, 1 H, H-1′, $J_{1',2'}$ = 6.4 Hz), 5.20 (dd, 1 H, H-2′, $J_{2',1'}$ = 6.4, $J_{2',3'}$ = 5.9 Hz), 4.92 (m, 1 H, H-1″), 4.64 (m, 1 H, H-3′), 4.56 (m, 1 H, H-5′a), 4.41 (m, 1 H, H-4′), 4.36 (m, 1 H, H-2″), 4.22 (m, 1 H, H-3″), 4.19 (m, 2 H, H-5″), 4.11 (m, 1 H, H-5′b), 3.20 (q, 6 H, CH_2N, J = 7.3 Hz), 3.04 (m, 1 H, H-6″a), 2.55 (m, 1 H, H-4″), 2.40 (m, 1 H, H-6″b), 1.27 (t, 9 H, CH_3CH_2N, J = 7.3 Hz); ^{13}C NMR (D_2O, 125 MHz) δ 157.1, 152.5, 150.8, 146.7, 136.9, 133.1, 133.0, 130.0, 122.5, 92.7, 87.8, 81.6, 76.7, 76.0, 73.4, 68.2, 67.5, 66.9, 49.5, 45.7, 31.0, 11.0; ^{31}P NMR (D_2O, 202 MHz, decoupled with ^{1}H) δ -9.27 (d, J = 11.4 Hz), −10.24 (d, J = 11.4 Hz); HRMS (FAB, negative) calcd for $C_{22}H_{26}N_5O_{12}P_2S$ 646.0774 [$(M - H)^-$], found 646.0762; UV (H_2O) λ_{max} = 285 nm. The absolute amount of **9** was calculated using ϵ = 15265 at λ_{max} = 285 nm based on the total phosphate analysis using KH_2PO_4 as a standard.

Biological Assay. Sea urchin eggs from *Lytechinus pictus* (Marinus, Long Beach, CA) were obtained by intracoelomic injection of 0.5 M KCl, shed into artificial seawater (in mM, NaCl 435, $MgCl_2$ 40, $MgSO_4$ 15, $CaCl_2$ 11, KCl 10, $NaHCO_3$ 2.5, EDTA 1, and [pH 8]), dejellied by passing through 90 μm nylon mesh, and then washed twice by centrifugation. Homogenates of sea urchin eggs were prepared as described previously.[2] Briefly, eggs were disrupted in an intracellular-like medium consisting of 250 mM potassium gluconate, 250 mM *N*-methylglucamine, 20 mM HEPES, and 1 mM $MgCl_2$, [pH 7.2] supplemented with 1 mM ATP, 10 U/mL creatine kinase, and 10 mM phosphocreatine and protease inhibitors. Ca^{2+} concentrations were measured with fluo-3 (3 μM) at 17 °C, using 500 μL of continuously stirred homogenate in a fluorimeter (Perkin-Elmer LS-50B) at 506 nm excitation and 526 nm emission.

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Supporting Information Available: HPLC charts of the final compounds **6**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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