Synthesis and Agonist Activity of Cyclic ADP-Ribose Analogues with Substitution of the Northern Ribose by Ether or Alkane Chains

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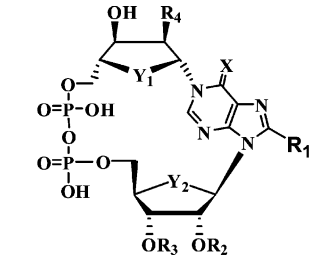
Received March 20, 2006

Novel analogues of cADPR with adenine as base and ether (**10a**) or different alkane chain (**10b**−**d**) substitutions of the northern ribose were synthesized from protected imidazole nucleoside **1** in good yields. The pharmacological activities of cyclic inosine diphosphoribose ether (cIDPRE) and the compounds (**10a**−**d**) were analyzed in intact human Jurkat T-lymphocytes. The results indicate that the analogues **10a**−**d** permeate the plasma membrane and are weak agonists of the cADPR/ryanodine receptor signaling system in intact human Jurkat T cells. They are the first membrane-permeant and biologically active cADPR analogues that contain ether or alkane bridges instead of the northern ribose and retain adenine as its base.

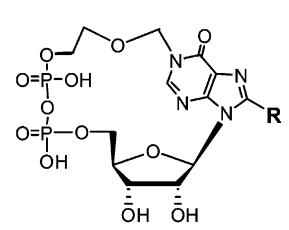
Introduction

Cyclic ADP-ribose (cADPR[a]) is a universal Ca^{2+} mobilizing second messenger first identified in the sea urchin egg system.[1,2] Since its discovery, numerous cell systems have been described to make use of the cADPR/ryanodine receptor (RyR)/Ca^{2+} signaling system to control Ca^{2+}-dependent cellular responses such as fertilization, secretion, contraction, proliferation, and many more. [3,4]

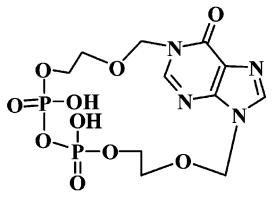
Because of the importance of the cADPR/RyR/Ca^{2+} signaling system in cell regulation, analogues of cADPR have been synthesized (Figure 1) and their biological activity has been tested. The first and still very important analogues were derivatized in the 8-position of adenine, e.g., 8-NH_2-cADPR or 8-Br-cADPR.[5] These compounds turned out to be antagonists of cADPR.[5] In the years following 1993 many more cADPR analogues with modifications in the (i) southern ribose, e.g., 2′-phospho-[6,7] or 3′-OCH_3−,[8] or the (ii) northern ribose, e.g., 2″-NH_2−,[9] or the (iii) pyrophosphate bridge, e.g., a triphosphate bridge in cyclic adenosine triphosphoribose[10] have been synthesized and evaluated for their biological activity. Furthermore, either the southern or the northern ribose was replaced by the corresponding carbocyclic derivatives, termed cyclic aristeromycin diphosphoribose[11] and cyclic adenosine diphosphocarbocyclic ribose (cADPcR).[12] Interestingly, in higher eukaryotic cell types, cADPcR and its analogues were relatively weak in their Ca^{2+} mobilizing activity,[9,13] while the southern ribose carbocyclic substitution was almost identical to cADPR.[9] These results indicated that the polar oxygen in the hemiacetal moiety of the northern ribose might be required for receptor binding of cADPR.



cADPR, X=NH, Y_1=Y_2=O, R_1=R_2=R_3=H, R_4=OH
cIDPR, X=O, Y_1=Y_2=O, R_1=R_2=R_3=H, R_4=OH
8-NH_2-cADPR, X=NH, Y_1=Y_2=O, R_1=NH_2, R_2=R_3=H, R_4=OH
8-Br-cADPR, X=NH, Y_1=Y_2=O, R_1=Br, R_2=R_3=H, R_4=OH
2'-phospho-cADPR, X=NH, Y_1=Y_2=O, R_2=PO_3^{2-}, R_1=R_3=H, R_4=OH
3'-OCH_3-cADPR, X=NH, Y_1=Y_2=O, R_1=R_2=H, R_3=CH_3, R_4=OH
2''-NH_2-cADPR, X=NH, Y_1=Y_2=O, R_1=R_2=R_3=H, R_4=NH_2
cADPcR, X=NH, Y_1=CH_2, Y_2=O, R_1=R_2=R_3=H, R_4=OH
Cyclic Aristeromycin Diphosphoribose
X=NH, Y_1=O, Y_2=CH_2, R_1=R_2=R_3=H, R_4=OH



cIDPRE, R=H
8-Br-cIDPRE, R=Br
8-N_3-cIDPRE, R=N_3
8-NH_2-cIDPRE, R=NH_2
8-Cl-cIDPRE, R=Cl



cIDP-DE

Figure 1. Structures of cADPR analogues.

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[a] Abbreviations: cADPR, cyclic adenosine 5′-diphosphoribose; cIDPR, cyclic inosine 5′-diphosphoribose; cADPcR, cyclic adenosine 5′-diphosphocarboribose; cIDPRE, cyclic inosine diphosphoribose ether; cIDP-DE, cyclic inosine diphosphodiether; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EGTA, ethylene glycol bis(2-aminoethyl ether)tetraacetic acid; ESI, electrospray ionization; FAB, fast atom bombardment; ^{1}H NMR, proton nuclear magnetic resonance; ^{13}C NMR, carbon-13 nuclear magnetic resonance; ^{31}P NMR, phosphorus-31 nuclear magnetic resonance; HMBC, heteronuclear multiple bond correlation; HR-FAB-MS, high-resolution fast atom bombardment mass spectrometry; HR-ESI-MS, high-resolution electrospray ionization mass spectrometry; HPLC, high-performance liquid chromatography; mAb, monoclonal antibody; MMTrCl, monomethoxytrityl chloride; PSS, cyclohexylammonium *S*,*S*-diphenylphosphorodithioate; TOF, time of flight; THF, tetrahydrofuran; Py, pyridine; TPSCl, triisopropylbenzenesulfonyl chloride; TBDMSCl, *tert*-butyldimethylsilyl chloride; TEAA, triethylammonium acetate; TEAB, triethylammonium bicarbonate; TBAF, tetrabutylammonium fluoride; TMS, tetramethyl silicane.

10.1021/jm060320e CCC: $33.50 © 2006 American Chemical Society
Published on Web 08/10/2006

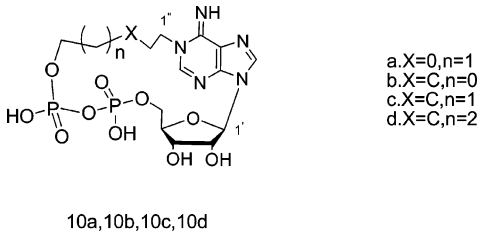


Figure 2. Structures of **10a**−**d**

To initially test this hypothesis, a cADPR analogue with substitution of the northern ribose with an ether strand termed cyclic inosine diphosphoribose ether (cIDPRE, Figure 1) was synthesized; its biological activity was proven in permeabilized and intact higher eukaryotic cells.[14] The biological activity of cIDPRE and some 8-substituted derivatives was fully characterized in permeabilized and intact Jurkat T cells.[15] Importantly, substitution of the base adenine by hypoxanthine in *N*1-cyclic inosine diphosphoribose (*N*1-cIDPR) did not alter the Ca^{2+}-releasing properties compared to cADPR,[16] though *N*7-cIDPR did not release Ca^{2+} in sea urchin egg homogenates.[17] A further simplified cADPR analogue that still retained biological activity is cyclic inosine diphosphodiether (cIDP-DE, Figure 1) in which both the northern and southern ribose were substituted by corresponding ether strands.[18]

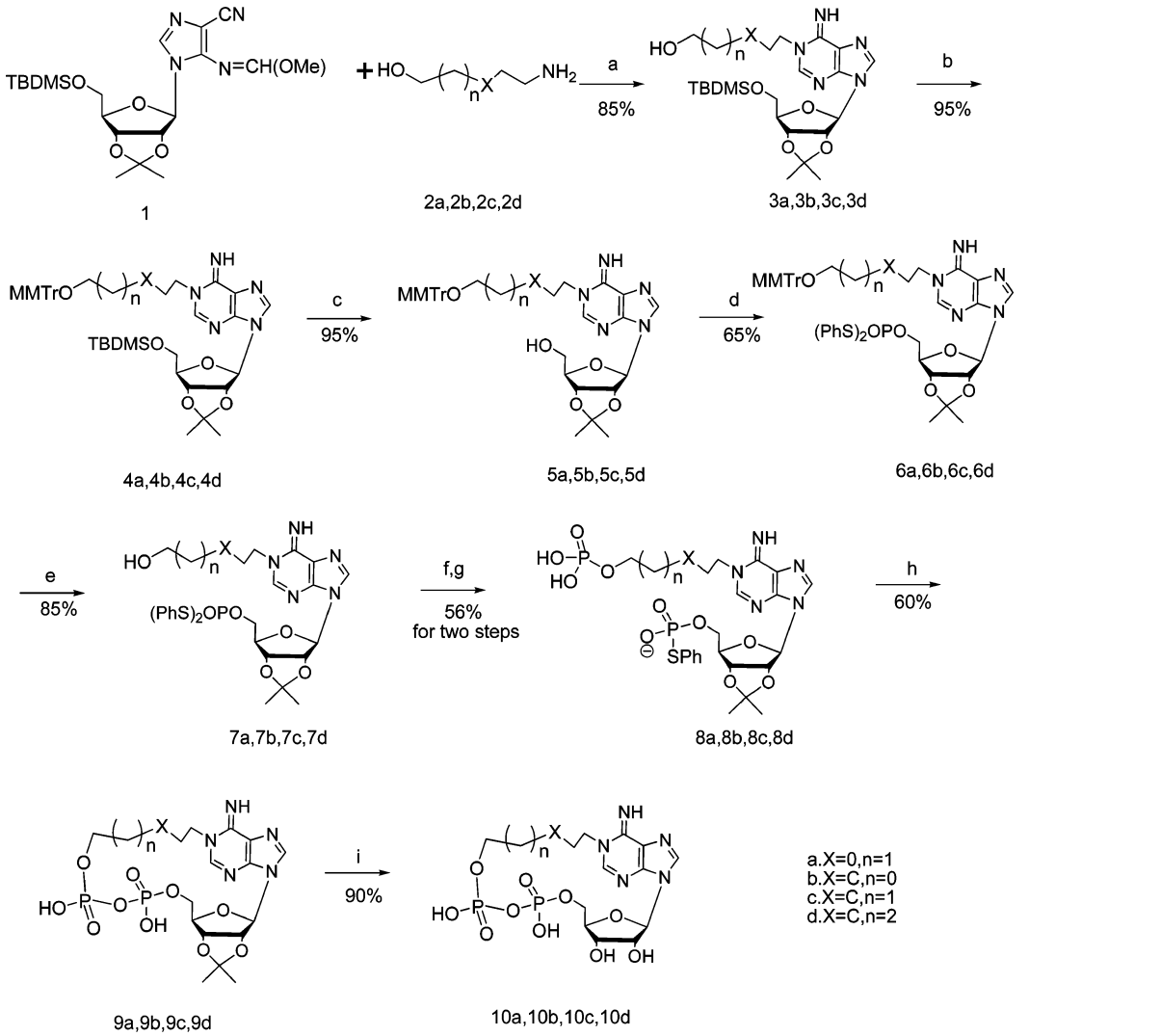
In the present study, novel analogues of cADPR with adenine as base and ether or different alkane chain substitutions of the northern ribose were synthesized and characterized; further, the compounds were tested for their biological activity in Jurkat T-lymphocytes.

For sake of clarity, in this paper the structural positions of compounds **10a**−**d** are numbered as follows: a single prime numbering scheme is used for the position of the N^9-ribosyl moiety, and a double prime numbering scheme is used for the position of the N^1-substitution (Figure 2).

Results and Discussion

Chemistry. The syntheses of compounds **10a**−**d** are summarized in Scheme 1. We reported previously the synthesis of N^1-ether substituted *N*1-cIDPR, cIDPRE.[15] An N^1 substitution was carried out regioselectively on the protected inosine. However, the same strategy cannot be used in the case of adenosine. The N^1 substitution on the adenine moiety is very difficult, and an N^6-substituted adenosine is the main product instead. Blackburn's group provided an efficient method to construct the N^1 substituted adenosine.[19,20] By use of the same

Scheme 1. Syntheses of Compounds **10a**−**d**[a]



[a] Reagents and condictions: (a) K_2CO_3, MeOH, room temp; (b) MMTrCl, Py, room temp; (c) TBAF, THF, room temp; (d) PSS, TPSCl, tetrazole, room temp; (e) 80% AcOH, room temp; (f) $POCl_3$, $PO(OMe)_3$, 0 °C; (g) H_3PO_2, Et_3N, Py, room temp; (h) $AgNO_3$, 3 Å molecular sieves, Py, room temp; (i) 60% HCOOH, room temp.

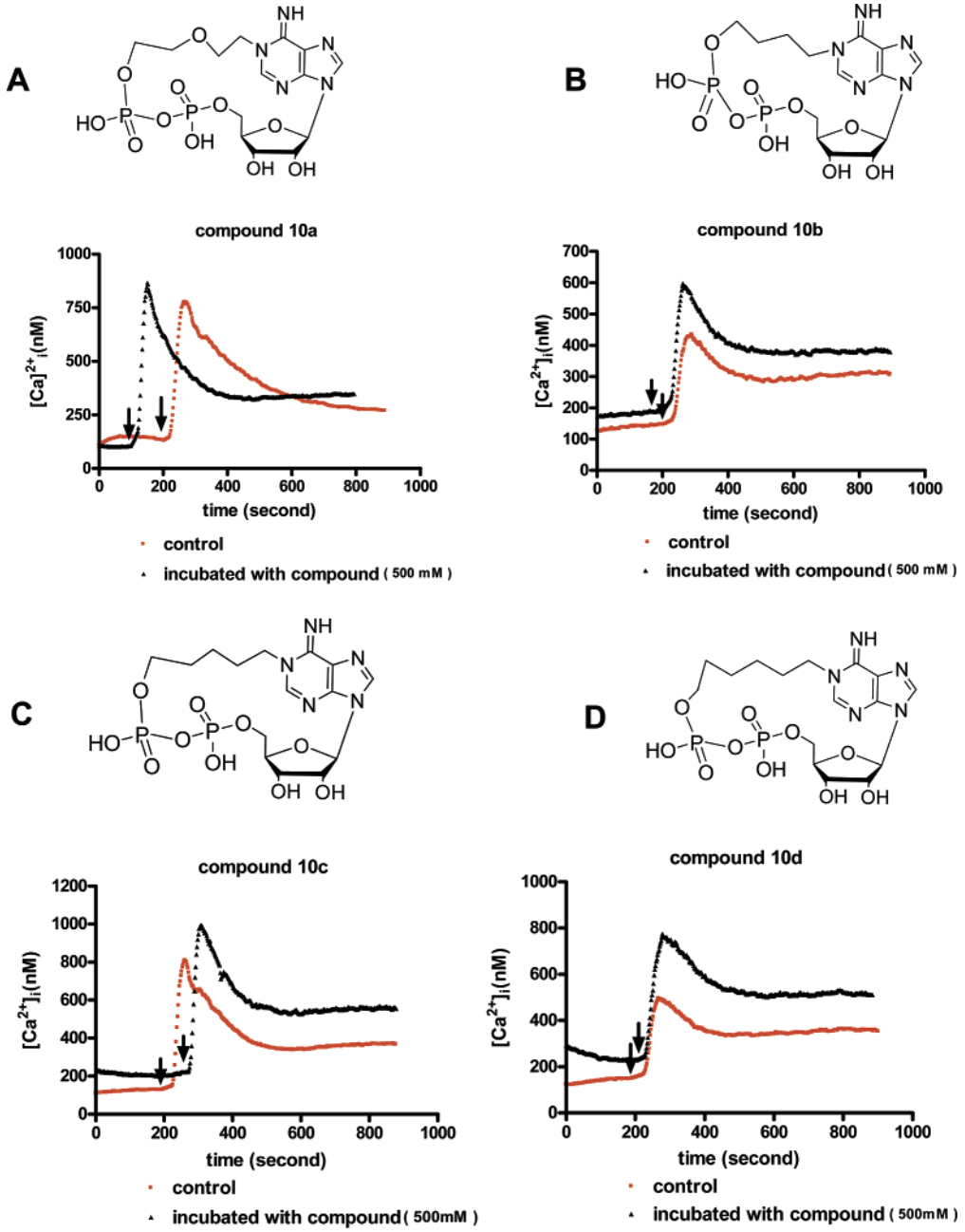


Figure 3. Lack of antagonist effect of compounds **10a**−**d** in intact Jurkat T cells. Jurkat T cells were loaded with Fura 2/AM and analyzed by ratiometric fluorometry on a Hitachi F-2000 flurometer. The cells were preincubated with each compound (500 mM final concentration) for 20 min. OKT3 was added 200 s after the beginning of each measurement, and each experiment was calibrated by the addition of Triton X-100 to obtain a maximal ratio at 900 s and by subsequent addition of EGTA/Tris-base to obtain the minimal ratio.

starting material, 5-[(methoxymethylene)amino]-1-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-*O*-(isopropylidene)-*β*-D-ribofuranosyl]-imidazole-4-nitrile **1**, the formation of N^1-ether or alkane chain substituted adenosines (**3a**−**d**) was achieved regiospecifically by condensation reaction with different amino alcohols (**2a**−**d**) in the presence of a catalytic amount of K_2CO_3 in high yield. The structures **3a**−**d** were confirmed by ^{1}H NMR, ^{13}C NMR, and HMBC. Correlations between H-2 of adenine and C-1″ of the alkane or ether chain on the N^1 position of the intermediate (**3a**−**d**) were observed in the NMR HMBC spectrum. We tried to complete the phosphorylation at the free hydroxyl group of **3a**−**d**; however, it was very difficult to isolate the phosphorylated products in accordance with the report by Shuto in 2001.[12] Therefore, the hydroxyl groups of intermediates **3a**−**d** were protected with MMTrCl, and then the 5′-*tert*-butyldimethylsilyl (TBDMS) groups in **4a**−**d** were removed by TBAF solution. The resulting hydroxyl groups were phosphorylated with cyclohexylammonium *S*,*S*-diphenylphosphorodithioate (PSS) in the presence of triisopropylbenzenesulfonyl choride (TPSCl) and tetrazole in pyridine to give **6a**−**d** in 65% yield. The presence of the bis(phenylthio)phosphoryl group in the molecules was supported by ^{31}P NMR (49.39 ppm, s). Removal of the MMTr group of **6a**−**d** with 80% aqueous AcOH provided **7a**−**d**, respectively. The resulting terminal hydroxyl groups in **7a**−**d** were phosphorylated with the $POCl_3$/$PO(OMe)_3$ system.[21] The phosphorylated products were treated with H_3PO_2 and Et_3N[22] in pyridine to afford directly 5′-phenylthiophosphates **8a**−**d**, the substrates for the intramolecular cyclization reaction. The presence of two different phosphate groups in **8a**−**d** was supported by ^{31}P NMR (see Experiment Section). The intramolecular cyclization of compounds **8a**−**d** was achieved as described in the synthesis of cIDPRE[15] to afford the corre-

sponding cyclic pyrophosphate compounds (**9a**−**d**) in 60% yield. The cyclic pyrophosphate structures of **9a**−**b** were confirmed by the data from HR-ESI-MS, ^{1}H NMR, and ^{31}P NMR. Deprotection of the isopropylidene group of **9a**−**d** was carried out with aqueous HCOOH at room temperature for 8 h to furnish target compounds **10a**−**d.** After repeated HPLC purification, **10a**−**d** were characterized by HR-ESI-MS, ^{1}H NMR, and ^{31}P NMR (see Experiment Section).

Pharmacology. The cADPR/RyR signaling pathway is critically important for T-lymphocyte Ca^{2+} signaling and T cell activation.[4,23,24] Thus, we aimed to pharmacologically characterize the novel analogues in this cell system. Ca^{2+} signaling can be analyzed in living T cells using fluorescent Ca^{2+} indicators, both in cell suspensions[9] and on the single cell level.[25,26] Since cIDPRE has been shown to be membrane-permeant and the novel compounds were expected to be membrane-permeant too, all analyses were carried out with intact cells. To assay the antagonist property of any compound, cells were preincubated and a quasi-physiological stimulation via the T cell receptor/CD3 complex was carried out. To assay the agonist property of any compound, the compound was added to the cell suspension or single cell and any effect on Ca^{2+} signaling was recorded directly.

The pharmacological activities of compounds **10a**−**d** were analyzed in intact human Jurkat T-lymphocytes. Preincubation of **10a**−**d** did not alter Ca^{2+} mobilization evoked by anti-CD3 monoclonal antibody (mAb) OKT3; rather, a small additional stimulatory effect on $[Ca^{2+}]_i$ was observed (Figure 3). Thus, all four compounds appear to have no antagonist effect on the cADPR/RyR signaling system.

Next, the four compounds were analyzed for their potential activity as agonists of the cADPR/RyR signaling pathway. All four compounds **10a**−**d** were added to intact Jurkat T-lymphocytes. At an extracellular concentration of 500 μM, they induced a rapid but weak elevation of $[Ca^{2+}]_i$ (Figure 4). It appears that all compounds are membrane-permeant analogues of cADPR, similar to cIDPRE and cIDP-DE,[15,18] since they act on intact cells. All compounds showed a typical biphasic Ca^{2+} mobilizing kinetics with an initial immediate Ca^{2+} peak and a subsequent plateau phase (Figure 4). The induction of the immediate peak was strongest for compound **10a** at 500 μM, while for the compounds **10b**−**d** a less pronounced initial Ca^{2+} peak was observed. A quantitative comparison with cIDPRE[15] is depicted in Figure 5; while compounds **10b**−**d** produced small Ca^{2+} peak elevations between 10 and 500 μM, there was an increased effect of compound **10a** at 500 μM (Figure 5A). In comparison to cIDPRE, however, even the effect of compound **10a** was approximate 2-fold smaller (Figure 5A). Similar results were also obtained when analyzing the sustained Ca^{2+} signal (Figure 5B); while cIDPRE showed the highest mean activity, all of the new compounds were weaker. Again, as shown in Figure 5A for the Ca^{2+} peak, the effect of compound **10a** was slightly stronger compared to compounds **10b**−**d**.

Since compound **10a** was most effective among the novel compounds, its Ca^{2+} mobilizing effect was also analyzed on the single T cell level by confocal Ca^{2+} imaging experiments. Individual single Jurkat T cells challenged by compound **10a** (500 μM) reacted rapidly with high increases in $[Ca^{2+}]_i$, although the reactivity of some cells was delayed and smaller in amplitude (Figure 6A). The mean value (red curve in Figure 6A) is similar to the kinetics seen in Figure 4 although the initial peak was not as pronounced compared to the cell suspension measurements (see Figure 4, upper panel). Comparing the mean increase in $[Ca^{2+}]_i$ evoked by compound **10a** to a vehicle control (Figure 6B, blue curve) or to a positive control (induction by anti-CD3 mAb OKT3; black curve in Figure 6B) suggests that compound **10a** can substitute for almost the full sustained part of the quasi-physiological activation via the TCR/CD3 complex. However, in the initial phase, activation of RyR by compound **10a** obviously can only induce part of the signal (Figure 6B). Using confocal Ca^{2+} imaging at a faster acquisition rate (approximately one ratio per 150 ms), upon extracellular addition of compound **10a** we demonstrate the induction of localized small Ca^{2+} signals in the vicinity of the cell border but also in many regions

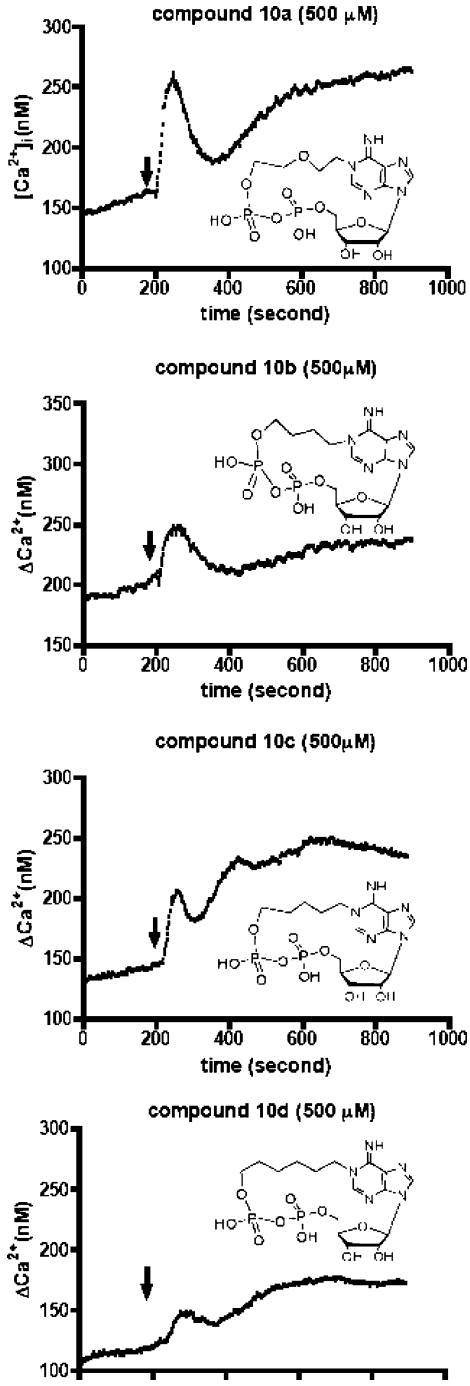


Figure 4. Ca^{2+} mobilization by compounds **10a**−**d** in intact Jurkat T cells. Jurkat T cells were loaded with Fura 2/AM and analyzed by ratiometric fluorometry on a Hitachi F-2000 flurometer. Compounds were added 200 s after the beginning of measurement. Each experiment was calibrated by the addition of Triton X-100 to obtain a maximal ratio at 900 s and by subsequent addition of EGTA/Tris-base to obtain the minimal ratio.

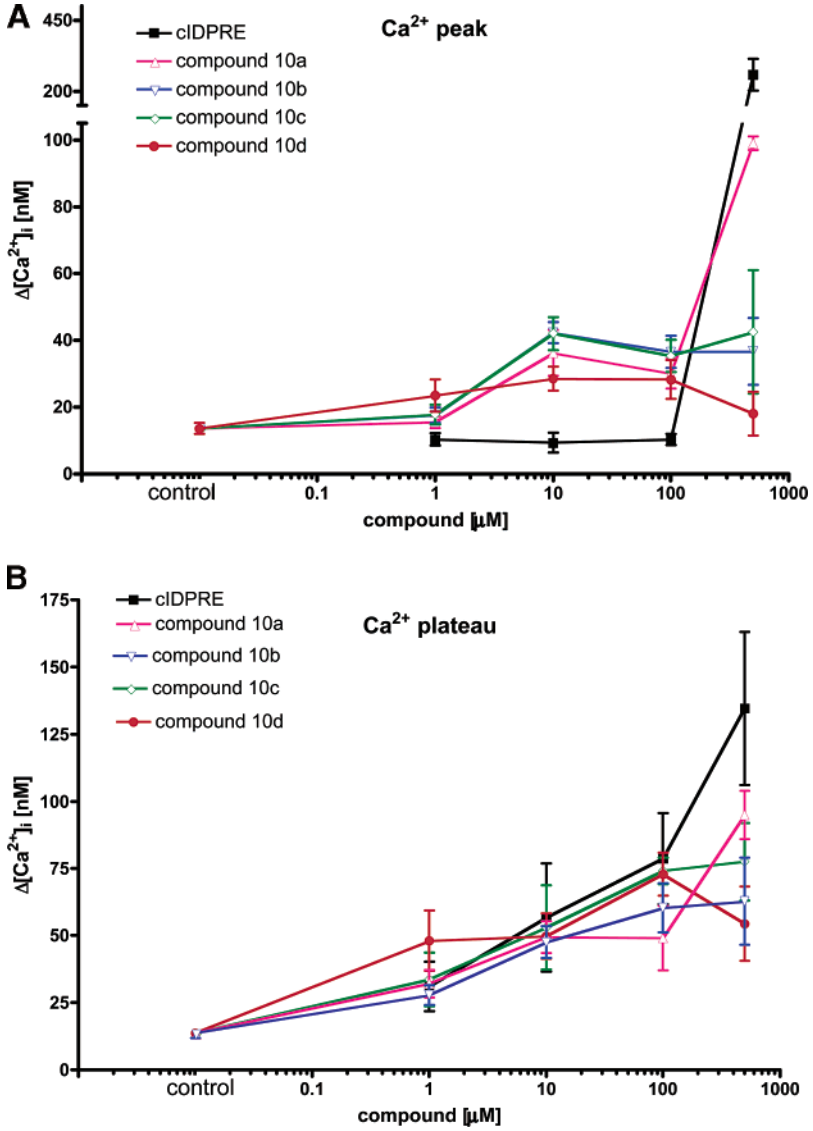


Figure 5. Concentration response relationship of Ca^{2+} mobilization by compounds **10a**−**d** in intact Jurkat T cells. Jurkat T cells were loaded with Fura 2/AM and analyzed by ratiometric fluorometry on a Hitachi F-2000 flurometer. "Ca^{2+} peak" (A) and "Ca^{2+} plateau" (B) relate to the initial Ca^{2+} peak (approximately 150−300 s) and the sustained Ca^{2+} plateau (800 s), respectively. Data are the mean ± SE (n = 2−6).

deep in the cytosol (Figure 6C, time point 39.38 s). The confocal image taken at 39.38 s in Figure 6C (marked and magnified region) also demonstrates the recruitment of neighboring smaller signals. Finally, a global Ca^{2+} signal occurred (Figure 6C).

Taken together, our study demonstrates that the four novel cADPR analogues **10a**−**d** are weak agonists of the cADPR/RyR signaling system. The results also indicate that these four compounds do not display any antagonist activity.

Compared to native cADPR, substitutions on C8 of the adenine ring confer antagonistic activity to the cADPR analogues.[5] Other possibilities to construct cADPR antagonists include modifications at the C3 of the southern ribose.[8] Although the compounds introduced in the current report do not display any modifications at C8 of the adenine ring, we suspected that the replacement of the northern ribose by different types of ether bridges might also result in, at least, some antagonist properties. However, this assumption turned out to be wrong. Thus, the data support the idea that C8 modifications of the adenine base are important constituents of cADPR antagonists. Whether this also applies to analogues in which the northern ribose is replaced by ether bridges similar to the ones used in the current study remains to be demonstrated.

The results presented here confirm that the agonist effects of cADPR analogues **10a**−**d** are retained, although at lower magnitude, even when the northern ribose is replaced by an ether or alkane bridge. Unlike cIDPRE and cIDP-DE, compounds **10a**−**d** are analogues of cADPR in the sense that the base adenine is preserved. They are the first membrane-permeant cADPR analogues that contain ether or alkane bridges instead of the northern ribose and retain adenine as its base. Except for the difference in the base, the ether strand of **10a** contains one CH_2 more than cIDPRE.[15] Even though compound **10a** has one extra carbon, it appears that the oxygen can still bind, possibly because of the increased flexibility provided by the longer chain. The conformation of **10a** may allow a better shift to an ideal binding position to match the receptor binding site. However, when compared to cIDPRE,[15] the Ca^{2+} peak induced by compound **10a** was much less pronounced while a similar effect of **10a** on the sustained Ca^{2+} signal was observed. This result may indicate that there are two independent targets for the cADPR mimics. The initial Ca^{2+} peak evoked by the first target may require a northern ribose closer to the natural molecule, like in cIDPRE,[15] while for the sustained phase the dependency on this part of the molecule is not so strong.

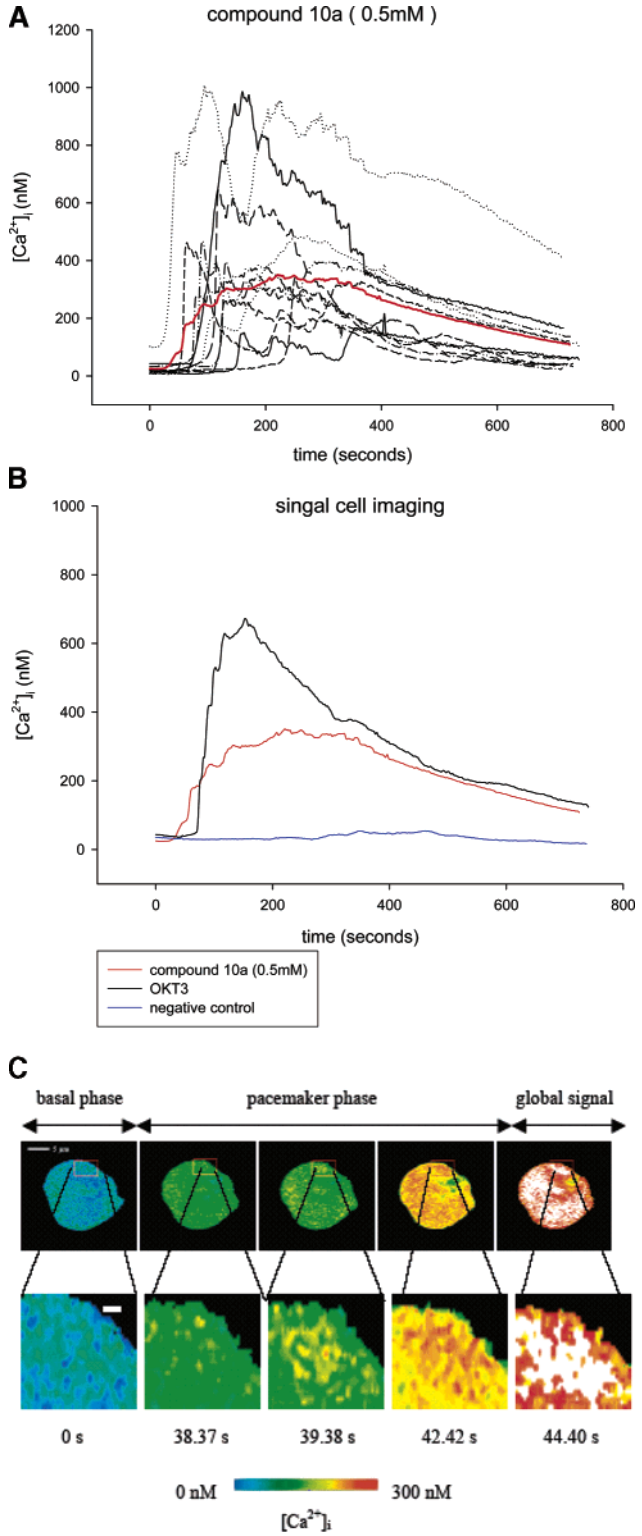


Figure 6. Ca^{2+} mobilization by compound **10a** in single T cells. Jurkat T cells were loaded with Fura-2/AM and analyzed by confocal ratiometric Ca^{2+} imaging as described in "Experimetal Section". Extracellular Ca^{2+} was present at 1 mM throughout the experiments. In panel A the black curves in the diagram represent the behavior of 11 individual Jurkat T cells after the addition of compound **10a** (500 μM) at the time point of 40 s, while the red curve shows the mean value. Panel B shows the mean value curve (red) compared to effects of anti-CD3 mAb OKT3 (black) and vehicle control (blue). In panel C characteristic confocal ratiometric pseudocolor images of a single Jurkat cell and magnifications of a defined subcellular region are shown. Basal phase (before addition of compound **10a** (final concentration of 0.5 mM)), pacemaker phase, and global phase are indicated. The image acquisition rate was approximately 1 ratio image per 150 ms.

According to the results in the intact T cell suspension measurements (Figures 4 and 5), the oxygen of the bridge that connects N^1 of adenosine to the phosphate moiety plays an important role in the binding of the analogue to its target receptor because the biological activities of compounds **10b**−**d** are weaker than that of compound **10a**. The oxygen of the bridge may contribute as a proton acceptor for the formation of a hydrogen bond with a neighboring amino acid residue of the receptor. However, the molecules with the alkane chains still appear to fit into the cADPR receptor binding site, although the oxygen at the right position is required for a better response.

Recently, we have demonstrated that analogues of cADPR containing the base hypoxanthine instead of adenine, e.g., *N*1-cIDPR,[16] 8-Br-cIDPR,[27] cIDPRE,[15] cIDP-DE,[18] are almost not hydrolyzable when incubated with native or recombinant CD38.[28] This is likely due to the amide-like bond between N1 and C1″ of the northern ribose or its substitute, e.g., ether chain. In contrast, the corresponding N1−C1″ bond in cADPR was hydrolyzed by both CD38 and, though to a weaker extent, ADP-ribosyl cyclase from *Aplysia californica*.[28] Although metabolism experiments have not yet been carried out with the novel analogues, a similar behavior as for cADPR can be expected.

Taken together, the first cADPR analogues, in which the northern ribose is replaced by an ether or alkane bridge and the base adenine is retained, were synthesized. The analogues were weak agonists of the cADPR/RyR signaling system in intact human Jurkat T cells but did not show antagonist activity. This series of analogues enlarges our knowledge of the structure−activity relationship of cADPR in the sense that even a replacement of the northern ribose that no longer contains polar components is sufficient for (weak) biological activity.

Experiment Section

Chemistry. Mass spectra were obtained on either VG-ZAB-HS or Bruker APEX. High-resolution FAB (fast atom bombardment) MS and HR-ESI-MS (ESI = electrospray ionization) were performed with Bruker BIFLEX III. ^{1}H NMR and ^{13}C NMR data were recorded with a JEOL AL300 or a Varian VXR-500 spectrometer using DMSO-d_6 or D_2O as solvent. Chemical shifts are reported in parts per million downfield from TMS (^{1}H and ^{13}C). ^{31}P NMR spectra were recorded at room temperature by use of Bruker Avance 300 spectrometer (121.42 MHz); orthophosphoric acid (85%) was used as an external standard. Compounds **10a**−**d** were purified twice on an Alltech preparative C_{18} reversed-phase column (2.2 cm × 25 cm) using a Gilson HPLC buffer system: MeCN/TEAB (pH 7.5) and MeCN/TEAA (pH 7.0).

***N*^{1}-[(5″-Hydroxyl)ethoxyethyl]-5′-*O*-TBDMS-2′,3′-*O*-isopropylideneadenosine 3a.** A mixture of **1** (486 mg, 1.1 mmol), **2a** (144 mg, 1.3 mmol), and K_2CO_3 (8 mg, 0.06 mmol) in MeOH (15 mL) was stirred at room temperature for 4 h. The mixture was evaporated, and the residue was partitioned between H_2O and EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (1:30 MeOH /CH_2Cl_2) to give compound **3a** (477 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.00 (s, 6H, $(CH_3)_2Si$), 0.85 (s, 9H, $(CH_3)_3C$−), 1.38, 1.61 (each s, each 3H, $(CH_3)_2C$), 3.56−3.57 (m, 2H, H-5″), 3.69−3.71 (m, 2H, H-4″), 3.71−3.84 (m, 4H, H-2″, H-5″), 4.18−4.30 (m, 2H, H-1″), 4.35−4.40 (m, 1H, H-4′), 4.90 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.12 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 6.02 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 7.76, 7.82 (each s, each 1H, H-2, H-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.6, 148.1, 141.2, 136.6, 123.7, 114.1, 91.2, 87.0, 85.3, 81.3, 72.7, 68.1, 63.5, 61.6, 47.4, 27.2, 25.9, 25.4, 18.3, −5.6, −5.5. ESI-TOF$^+$-MS: calcd for $C_{23}H_{39}N_5O_6Si$ [$(M + 1)^+$], 510.3; found, 510.3. Anal. ($C_{23}H_{39}N_5O_6Si$) C, H, N.

***N*^{1}-(5″-Monomethoxytrityloxyethoxyethyl)-5′-*O*-TBDMS-2′,3′-*O*-isopropylideneadenosine 4a.** A mixture of **3a** (477 mg, 0.935

mmol) and MMTrCl (577 mg, 1.87 mmol) in pyridine (10 mL) was stirred at room temperature for 8 h. The mixture was evaporated, and the residue was partitioned between H_2O and EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (1:60 MeOH/ CH_2Cl_2) to give compound **4a** (658 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.00 (s, 6H, $(CH_3)_2Si$), 0.86 (s, 9H, $(CH_3)_3C-$), 1.29, 1.58 (each s, each 3H, $(CH_3)_2C$), 3.17−3.21(m, 2H, H-5″), 3.57−3.65 (m, 2H, H-4″), 3.73−3.90 (m, 7H, H-1″, H-2″, OCH_3), 4.13−4.22(m, 1H, H-5′a), 4.30−4.40 (m, 2H, H-4′, H-5′b), 4.85 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.00 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 6.00 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 6.81−7.44 (m, 14H, Ar−H), 7.81, 7.82 (each s, each 1H, H-2, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.5, 152.8, 148.5, 144.5, 135.7, 130.3, 129.2, 128.4, 127.8, 127.1, 126.8, 114.0, 113.2, 113.0, 91.0, 87.1, 86.2, 85.2, 81.2, 70.8, 68.1, 63.5, 63.0, 55.2, 47.7, 27.2, 25.9, 25.3, 18.3, −5.5, −5.4. ESI-TOF$^+$-MS: calcd for $C_{43}H_{55}N_5O_7Si$ [$(M + 1)^+$], 782.4; found, 782.3. Anal. ($C_{43}H_{55}N_5O_7Si$) C, H, N.

***N*^{1}-(5″-Monomethoxytrityloxyethoxyethyl)-2′,3′-*O*-isopropylideneadenosine 5a.** A mixture of **4a** (658 mg, 0.84 mmol), TBAF (1 M in THF, 8.4 mL, 8.4 mmol), and AcOH (266 μL,4.2 mmol) in THF (15 mL) was stirred at room temperature for 2 h. The mixture was evaporated, and the residue was purified by silica gel column chromatography (1:40 MeOH/CH_2Cl_2) to give compound **5a** (533 mg, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28, 1.60 (each s, each 3H, $(CH_3)_2C$), 3.19−3.22(m, 2H, H-5″), 3.59−3.61 (m, 2H, H-4″), 3.72−3.88 (m, 7H, H-5′ , H-2″; OCH_3), 4.11−4.35(m, 2H, H-1″), 4.43−4.44 (m, 1H, H-4′), 4.93 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 4.99 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 5.75 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 6.81−7.42 (m, 14H, Ar−H), 7.43 (s,1H, H-2), 7.78 (s,1H, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.4, 153.9, 148.5, 144.4, 144.3, 140.5, 138.1, 135.7, 130.2, 128.3, 127.7, 126.8, 114.1, 113.1, 93.6, 86.2, 86.0, 83.7, 81.3, 70.8, 67.8, 63.0, 63.0, 55.2, 48.2, 27.5, 25.1. ESI-TOF$^+$-MS: calcd for $C_{37}H_{41}N_5O_7$ [$(M + 1)^+$], 668.3; found, 668.3. Anal. ($C_{37}H_{41}N_5O_7$) C, H, N.

***N*^{1}-(5″-Monomethoxytrityloxyethoxyethyl)-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine 6a.** To a solution of **5a** (533 mg, 0.798 mmol) in pyridine (10 mL) was added TPSCl (483 mg, 1.586 mmol), PSS (909 mg, 2.394 mmol), and tetrazole (168 mg, 2.394 mmol), and the mixture was stirred at room temperature for 12 h. The mixture was evaporated, and the residue was partitioned between H_2O and CH_2Cl_2. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (1:40 MeOH/ CH_2Cl_2) to give compound **6a** (483 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.29, 1.61 (each s, each 3H, $(CH_3)_2C$), 3.20−3.23(m, 2H, H-5″), 3.59−3.62 (m, 2H, H-4″), 3.74−3.91 (m, 7H, H-5′ , H-2″, OCH_3), 4.12−4.35 (m, 2H, H-1″), 4.43−4.45 (m, 1H, H-4′), 4.95 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.00 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 5.76 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 6.81−7.46 (m, 24H, Ar−H), 7.45 (s,1H, H-2), 7.79 (s,1H, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.4, 153.9, 148.5, 144.4, 144.3, 140.5, 138.1, 135.7, 132.5, 130.2, 129.4, 129.1, 128.3, 127.8, 126.8, 125.6, 114.1, 113.1, 93.6, 86.2, 86.0, 83.7, 81.3, 70.8, 67.8, 63.0, 62.9, 55.2, 48.1, 27.5, 25.1. ^{31}P NMR ($CDCl_3$, 81 MHz, decoupled with ^{1}H) δ 49.39 ppm (s). ESI-TOF$^+$-MS: calcd for $C_{49}H_{50}N_5O_8PS_2$ [$(M + 1)^+$], 932.3; found, 932.3. Anal. ($C_{49}H_{50}N_5O_8PS_2$) C, H, N.

***N*^{1}-[(5″-Hydroxyl)ethoxyethyl]-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine 7a.** A solution of **6a** (483 mg, 0.519 mmol) in 80% aqueous AcOH (10 mL) was stirred at room temperature for 8 h. The mixture was evaporated, and the residue was partitioned between aqueous saturated $NaHCO_3$ and EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (1:30 MeOH/CH_2Cl_2) to give compound **7a** (291 mg, 85%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.34, 1.56 (each s, each 3H, $(CH_3)_2C$), 3.42−3.75 (m, 8H, H-5′, H-2″, H-4″, H-5″), 4.37−4.51 (m, 3H, H-1″, OH), 4.59−4.62 (m, 1H, H-4′), 5.05 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.45 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 6.28 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 7.20−7.50 (m, 10H, Ar−H), 7.80 (br s, 1H, NH), 8.25, 8.32 (each s, each 1H, H-2, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.6, 148.1, 141.2, 136.6, 132.5, 129.5, 128.9, 125.6, 123.7, 114.1, 91.2, 87.0, 85.3, 81.3, 72.7, 68.1, 63.5, 61.6, 47.4, 27.2, 25.4. ^{31}P NMR ($CDCl_3$, 81 MHz, decoupled with ^{1}H) δ 49.38 ppm (s). ESI-TOF$^+$-MS: calcd for $C_{29}H_{34}N_5O_7PS_2$ [$(M + 1)^+$], 660.2; found, 660.2. Anal. ($C_{29}H_{34}N_5O_7PS_2$) C, H, N.

***N*^{1}-(5″-Phosphonoxyethoxyethyl)-5′-*O*-[(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine 8a.** $POCl_3$ (282 μL, 3.02 mmol) was added to a solution of **7a** (200 mg, 0.302 mmol) in $PO(OMe)_3$ (3 mL) at 0 °C, and the mixture was stirred at the same temperature for 35 min, then quenched by aqueous saturated $NaHCO_3$ (6 mL). The resulting solution was stirred at 0 °C for 15 min, then concentrated in vacuo. The residue was dissolved in 1 mL of TEAB (0.1 M, pH 7.5).The solution was purified by a C_{18} reversed-phase column (2.2 cm × 25 cm) using a linear gradient of 0−60% CH_3CN in TEAB buffer (0.1 M, pH 7.5). The appropriate fractions were collected and evaporated. The residue was coevaporated with pyridine (5 mL × 3). The residue was mixed with H_3PO_2 (120 μL, 2.4 mmol) and Et_3N (156 μL, 1.1 mmol) in pyridine (3 mL). The mixture was stirred at room temperature in the dark for 12 h, then evaporated in vacuo. The residue was partitioned between H_2O and $CHCl_3$, and the aqueous layer was washed with $CHCl_3$ (5 mL × 3) and evaporated in vacuo. The residue was dissolved in 1 mL of TEAB buffer (0.1 M, pH 7.5), then applied to a C_{18} reversed-phase column (2.2 cm × 25 cm) developed by a linear gradient of 0−60% CH_3CN in TEAB buffer (0.1 M, pH 7.5) within 30 min to give **8a** (110 mg, 56% for two steps) as a triethylammonium salt. ^{1}H NMR (500 MHz, D_2O) δ 1.36, 1.58 (each s, each 3H, $(CH_3)_2C$), 3.50−3.79 (m, 8H, H-5′, H-2″, H-4″, H-5″), 4.40−4.46 (m, 2H, H-1″), 4.59−4.60 (m, 1H, H-4′), 5.12 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.43 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 6.18 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 7.30−7.60 (m, 5H, Ar−H), 8.28, 8.35 (each s, each 1H, H-2, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ 2.06 ppm (s), 17.79 ppm (s). HRMS (ESI-TOF$^-$) calcd for $C_{23}H_{31}N_5O_{11}P_2S$ [$(M - 1)^-$], 646.1138; found, 646.1129.

***N*^{1}-[(5″-*O*-Phosphoryl)ethoxyethyl]-2′,3′-*O*-isopropylidene-5′-*O*-phosphoryladenosine 5′,5″-Cyclicpyrophosphate 9a.** A solution of **8a** (15 mg, 23 μmol) in pyridine (5 mL) was added slowly over 20 h, using a syringe pump, to a mixture of $AgNO_3$ (83 mg, 490 μmol) and 3 Å molecular sieves (2.0 g) in pyridine (50 mL) at room temperature in the dark. The 3 Å molecular sieves were filtered off with Celite and washed with H_2O. The combined filtrate was evaporated, and the residue was partitioned between $CHCl_3$ and H_2O. The aqueous layer was evaporated, and the residue was dissolved in 0.1 M TEAB buffer (1.0 mL), which was applied to a C_{18} reversed-phase column (2.2 cm × 25 cm). The column was developed using a linear gradient of 0−60% CH_3CN in TEAB buffer (0.1 M, pH 7.5) within 30 min to give **9a** (7.4 mg, 60%) as a triethylammonium salt. ^{1}H NMR (500 MHz, D_2O) δ 1.38, 1.61 (each s, each 3H, $(CH_3)_2C$), 3.30−3.80 (m, 8H, H-5′, H-2″, H-4″, H-5″), 4.45−4.48 (m, 2H, H-1″), 4.58−4.59 (m, 1H, H-4′), 5.10 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.45 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 6.15 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 8.30, 8.38 (each s, each 1H, H-2, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ −9.69 ppm (d, $J_{P,P} = 10.0$ Hz), −10.61 ppm (d, $J_{P,P} = 10.0$ Hz). HRMS (ESI-TOF$^-$) calcd for $C_{17}H_{25}N_5O_{11}P_2$ [$(M - 1)^-$], 536.0948; found, 536.0940.

***N*^{1}-[(5″-*O*-Phosphoryl)ethoxyethyl]-5′-*O*-phosphoryladenosine 5′,5″-Cyclicpyrophosphate 10a.** A solution of **9a** (7.4 mg, 13.8 μmol) in 60% HCOOH (5 mL) was stirred for 8 h and then evaporated under reduced pressure. The purification of the residue was performed with the same procedure as for compound **9a** by HPLC on a C_{18} reversed-phase column, eluting with a linear gradient of 0−60% CH_3CN in TEAB buffer (0.1 M, pH 7.5) to give the target molecule **10a** (6.2 mg, 90%). ^{1}H NMR (500 MHz, D_2O) δ 3.20−3.71(m, 8H, H-5′, H-2″, H-4″, H-5″), 4.45−4.48 (m, 2H, H-1″), 4.50−4.60 (m, 1H, H-4′), 5.05 (dd, 1H, $J_{H3',H4'} = 3.0$

Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.40 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.35 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 8.25, 8.58 (each s, each 1H, H-2, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ −9.69 ppm (d, $J_{P,P}$ = 13.4 Hz), −10.38 ppm (d, $J_{P,P}$ =13.4 Hz). HRMS (ESI-TOF$^-$) calcd for $C_{14}H_{21}N_5O_{11}P_2$ [(M − 1)$^-$], 496.0635; found, 496.0629.

***N*^{1}-[(4″-Hydroxyl)butyl]-5′-*O*-TBDMS-2′,3′-*O*-isopropylideneadenosine 3b.** A mixture of **1** (486 mg, 1.1 mmol), **2b** (120 mg, 1.3 mmol), and K_2CO_3 (8 mg, 0.06 mmol) in MeOH (15 mL) was stirred at room temperature for 4 h. The procedure was the same as for the synthesis of **3a** to give **3b** in 82% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.00 (s, 6H, $(CH_3)_2Si$), 0.86 (s, 9H, $(CH_3)_3C-$), 1.39, 1.62 (each s, each 3H, $(CH_3)_2C$), 1.64−1.68 (m, 2H, H-3″), 1.85−1.98 (m, 2H, H-2″), 3.77−3.89 (m, 4H, H-4″ , H-5′), 4.05−4.20 (m, 2H, H-1″), 4.38−4.42 (m, 1H, H-4′), 4.90 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.10 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.02 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 7.72, 7.85 (each s, each 1H, H-2, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.6, 147.1, 141.5, 136.9, 123.7, 114.1, 91.2, 87.0, 85.2, 81.3, 63.5, 61.7, 47.1, 28.1, 27.2, 25.8, 25.7, 25.4, 18.3, −5.5, −5.6. ESI-TOF$^+$-MS: calcd for $C_{23}H_{39}N_5O_5Si$ [(M + 1)$^+$], 494.3; found, 494.3. Anal. ($C_{23}H_{39}N_5O_5Si$) C, H, N.

***N*^{1}-(4″-Monomethoxytrityloxybutyl)-5′-*O*-TBDMS-2′,3′-*O*-isopropylideneadenosine 4b.** A mixture of **3b** (445 mg, 0.9 mmol) and MMTrCl (557 mg, 1.8 mmol) in pyridine (10 mL) was stirred at room temperature for 8 h. The procedure was the same as for the synthesis of **4a** to give **4b** in 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 6H, $(CH_3)_2Si$), 0.82 (s, 9H, $(CH_3)_3C-$), 1.36, 1.59 (each s, each 3H, $(CH_3)_2C$),1.62−1.67 (m, 2H, H-3″), 1.85−1.88(m, 2H, H-2″), 3.05−3.10 (m, 2H, H-4″), 3.73−3.90 (m, 5H, H-5′, OCH_3), 3.92−4.05(m, 2H, H-1″), 4.35−4.37 (m, 1H, H-4′), 4.86 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.07 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.00 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.78−7.41 (m, 14H, Ar−H), 7.60,7.78 (each s, each 1H, H-2, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.2, 153.5, 147.4, 147.3, 144.5, 139.9, 138.3, 138.3, 135.9, 130.2, 128.9, 127.8, 126.8, 125.1, 114.2, 113.1, 93.9, 86.0, 85.6, 83.6, 81.4, 63.2, 62.7, 55.5, 48.7, 27.6, 27.0, 25.7, 25.4, 25.3, 18.3, −5.5, −5.6. ESI-TOF$^+$-MS: calcd for $C_{43}H_{55}N_5O_6Si$ [(M + 1)$^+$], 766.4; found, 766.3. Anal. ($C_{43}H_{55}N_5O_6Si$) C, H, N.

***N*^{1}-(4″-Monomethoxytrityloxybutyl)-2′,3′-*O*-isopropylideneadenosine 5b.** A mixture of **4b** (620 mg, 0.81 mmol), TBAF (1 M in THF, 8.1 mL, 8.1 mmol), and AcOH (260 μL, 4.1 mmol) in THF (20 mL) was stirred at room temperature for 2 h. The procedure was the same as for the synthesis of **5a** to give **5b** in 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37, 1.63 (each s, each 3H, $(CH_3)_2C$), 1.70−1.80 (m, 2H, H-3″), 1.85−1.91 (m, 2H, H-2″), 3.09−3.13 (m, 2H, H-4″), 3.74−3.96 (m, 5H, H-5′ , OCH_3), 3.98−4.03 (m, 2H, H-1″), 4.49−4.50 (m, 1H, H-4′), 4.92 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.10 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 5.95 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.81−7.44 (m, 14H, Ar−H), 7.58 (s, 1H, H-2), 7.63 (s, 1H, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.4, 153.7, 147.4, 147.3, 144.6, 140.3, 138.3, 138.2, 135.9, 130.2, 128.3, 127.8, 126.8, 125.0, 114.1, 113.0, 93.8, 86.1, 85.8, 83.6, 81.4, 63.1, 62.6, 55.2, 48.2, 27.5, 27.0, 25.5, 25.2. ESI-TOF$^+$-MS: calcd for $C_{37}H_{41}N_5O_6$ [(M + 1)$^+$], 652.3; found, 652.2. Anal. ($C_{37}H_{41}N_5O_6$) C, H, N.

***N*^{1}-(4″-Monomethoxytrityloxybutyl)-5′-*O*-[bis(phenylthio)-phosphoryl]-2′,3′-*O*-isopropylideneadenosine 6b.** To a solution of **5b** (475 mg, 0.73 mmol) in pyridine (10 mL) were added TPSCl (444 mg, 1.458 mmol), PSS (831 mg, 2.19 mmol), and tetrazole (154 mg, 2.19 mmol), and the mixture was stirred at room temperature for 12 h. The procedure was the same as for the synthesis of **6a** to give **6b** in 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39, 1.65 (each s, each 3H, $(CH_3)_2C$), 1.73−1.79 (m, 2H, H-3″), 1.87−1.92 (m, 2H, H-2″), 3.08−3.11 (m, 2H, H-4″), 3.72−3.98 (m, 5H, H-5′ , OCH_3), 4.00−4.03 (m, 2H, H-1″), 4.48−4.49 (m, 1H, H-4′), 4.97 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.30 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 5.94 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.81−7.46 (m, 24H, Ar−H), 7.60 (s, 1H, H-2), 7.66 (s, 1H, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.4, 153.7, 147.4, 147.3, 144.6, 140.3, 138.3, 138.2, 135.9, 132.7, 130.2, 129.4, 129.1, 128.3, 127.7, 126.8, 125.6, 125.0, 114.1, 113.0, 93.8, 86.1, 85.8, 83.6, 81.4, 63.1, 62.6, 55.2, 48.2, 27.5, 27.0, 25.5, 25.2. ^{31}P NMR ($CDCl_3$, 81 MHz, decoupled with ^{1}H) δ 49.41 ppm (s). ESI-TOF$^+$-MS: calcd for $C_{49}H_{50}N_5O_7PS_2$ [(M + 1)$^+$], 916.3; found, 916.3. Anal. ($C_{49}H_{50}N_5O_7PS_2$) C, H, N.

***N*^{1}-[(4″-Hydroxyl)butyl]-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine 7b.** A solution of **6b** (435 mg, 0.475 mmol) in 80% aqueous AcOH (10 mL) was stirred at room temperature for 8 h. The procedure was the same as for the synthesis of **7a** to give **7b** in 83% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35, 1.63 (each s, each 3H, $(CH_3)_2C$), 1.76−1.79 (m, 2H, H-3″), 1.89−1.93 (m, 2H, H-2″), 3.05−3.08 (m, 2H, H-4″), 3.82−3.98 (m, 2H, H-5′), 4.04−4.08 (m, 2H, H-1″), 4.49−4.50 (m, 1H, H-4′), 4.89 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.26 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 5.98 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.83−7.43 (m, 10H, Ar−H), 7.63 (s, 1H, H-2), 7.70 (s, 1H, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.6, 147.1, 141.5, 136.9, 132.4, 129.4, 129.1, 125.6, 123.7, 114.1, 91.2, 87.0, 85.2, 81.3, 63.5, 61.7, 47.1, 28.1, 27.2, 25.7, 25.4. ^{31}P NMR ($CDCl_3$, 81 MHz, decoupled with ^{1}H) δ 49.37 ppm (s). ESI-TOF$^+$-MS: calcd for $C_{29}H_{34}N_5O_6PS_2$ [(M + 1)$^+$], 644.2; found, 644.2. Anal. ($C_{29}H_{34}N_5O_6PS_2$) C, H, N.

***N*^{1}-(4″-Phosphonoxybutyl)-5′-*O*-[(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine 8b.** $POCl_3$ (290 μL, 3.11 mmol) was added to a solution of **7b** (200 mg, 0.311 mmol) in $PO(OMe)_3$ (3 mL) at 0 °C, and the mixture was stirred at the same temperature for 35 min. The following procedure was the same as for the synthesis of **8a** to give **8b** in 52% yield. ^{1}H NMR (500 MHz, D_2O) δ 1.35, 1.63 (each s, each 3H, $(CH_3)_2C$), 1.77−1.79 (m, 2H, H-3″), 1.88−1.92 (m, 2H, H-2″), 3.07−3.09 (m, 2H, H-4″), 3.86−3.99 (m, 2H, H-5′), 4.06−4.10 (m, 2H, H-1″), 4.51−4.52 (m, 1H, H-4′), 4.99 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.45 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.02 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.88−7.42 (m, 5H, Ar−H), 8.25 (s, 1H, H-2), 8.38 (s, 1H, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ 2.84 ppm (s), 17.69 ppm (s). HRMS (ESI-TOF$^-$) calcd for $C_{23}H_{31}N_5O_{10}P_2S$ [(M − 1)$^-$], 630.1189; found, 630.1182.

***N*^{1}-[(4″-*O*-Phosphoryl)butyl]-2′,3′-*O*-isopropylidene-5′-*O*-phosphoryladenosine 5′,5″-Cyclicpyrophosphate 9b.** A solution of **8b** (15 mg, 23.8 μmol) in pyridine (5 mL) was added slowly over 20 h, using a syringe pump, to a mixture of $AgNO_3$ (86 mg, 507 μmol) and 3 Å molecular sieves (2.07 g) in pyridine (50 mL) at room temperature in the dark. The procedure was the same as for the synthesis of **9a** to give **9b** in 60% yield. ^{1}H NMR (500 MHz, D_2O) δ 1.39, 1.65 (each s, each 3H, $(CH_3)_2C$), 1.79−1.80 (m, 2H, H-3″), 1.86−1.89 (m, 2H, H-2″), 3.07−3.09 (m, 2H, H-4″), 3.89−3.98 (m, 2H, H-5′), 4.08−4.11 (m, 2H, H-1″), 4.49−4.50 (m, 1H, H-4′), 5.02 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.65 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.10 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 8.27 (s, 1H, H-2), 8.39 (s, 1H, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ −9.65 ppm (d, $J_{P,P}$ = 10.0 Hz), −10.35 ppm (d, $J_{P,P}$ = 10.0 Hz). HRMS (ESI-TOF$^-$) calcd for $C_{17}H_{25}N_5O_{10}P_2$ [(M − 1)$^-$], 520.0999; found, 520.1004.

***N*^{1}-[(4″-*O*-Phosphoryl)butyl]-5′-*O*-phosphoryladenosine 5′,5″-Cyclicpyrophosphate 10b.** A solution of **9b** (7.4 mg, 14.3 μmol) in 60% HCOOH (5 mL) was stirred for 8 h. The procedure was the same as for the synthesis of **10a** to give **10b** in 90% yield. ^{1}H NMR (500 MHz, D_2O) δ 1.77−1.79 (m, 2H, H-3″), 1.87−1.89 (m, 2H, H-2″), 3.05−3.07 (m, 2H, H-4″), 3.90−3.96 (m, 2H, H-5′), 4.12−4.17 (m, 2H, H-1″), 4.52−4.53 (m, 1H, H-4′), 4.95 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.40 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.08 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 8.30 (s, 1H, H-2), 8.38 (s, 1H, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ −9.70 ppm (d, $J_{P,P}$ = 10.0 Hz), −10.35 ppm (d, $J_{P,P}$ = 10.0 Hz). HRMS (ESI-TOF$^-$) calcd for $C_{14}H_{21}N_5O_{10}P_2$ [(M − 1)$^-$], 480.0686; found, 480.0678.

***N*^{1}-[(5″-Hydroxyl)pentyl]-5′-*O*-TBDMS-2′,3′-*O*-isopropylideneadenosine 3c.** A mixture of **1** (486 mg, 1.1 mmol), **2c** (135 mg, 1.3 mmol), and K_2CO_3 (8 mg, 0.06 mmol) in MeOH (15 mL) was stirred at room temperature for 4 h. The procedure was the same

as for the synthesis of **3a** to give **3c** in 84% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.00 (s, 6H, $(CH_3)_2Si$), 0.86 (s, 9H, $(CH_3)_3C-$), 1.39 (s, 3H, isopropyl CH_3), 1.46−1.52 (m, 2H, H-3″), 1.62 (s, 3H, isopropyl CH_3), 1.63−1.67 (m, 2H, H-4″), 1.83−1.89 (m, 2H, H-2″), 3.65−3.67 (m, 2H, H-5″), 3.76−3.86 (m, 2H, H-5′), 4.00−4.10 (m, 2H, H-1″), 4.38−4.40 (m, 1H, H-4′), 4.89 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.11 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.03 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 7.68, 7.82 (each s, each 1H, H-2, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.7, 145.5, 139.4, 134.4, 122.1, 112.3, 89.4, 85.3, 83.5, 79.5, 61.7, 60.3, 46.1, 30.3, 27.9, 26.5, 25.4, 24.1, 23.6, 21.1, 16.5, −7.2, −7.3. ESI-TOF$^+$-MS: calcd for $C_{24}H_{41}N_5O_5Si$ [$(M + 1)^+$], 508.3; found, 508.3. Anal. ($C_{24}H_{41}N_5O_5Si$) C, H, N.

***N*^{1}-(5″-Monomethoxytrityloxypentyl)-5′-*O*-TBDMS-2′,3′-*O*-isopropylideneadenosine 4c.** A mixture of **3c** (469 mg, 0.924 mmol) and MMTrCl (572 mg, 1.848 mmol) in pyridine (10 mL) was stirred at room temperature for 8 h. The procedure was the same as for the synthesis of **4a** to give **4c** in 91% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 6H, $(CH_3)_2Si$), 0.86 (s, 9H, $(CH_3)_3C-$), 1.39 (s, 3H, isopropyl CH_3), 1.45−1.78 (m, 9H, H-2″, H-3″, H-4″, isopropyl CH_3), 3.06 (m, 2H, H-5″), 3.78−3.83 (m, 5H, H-5′, OCH_3), 3.99−4.04 (m, 2H, H-1″), 4.39−4.40 (m, 1H, H-4′), 4.89 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.09 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.03 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.81−7.44 (m, 14H, Ar−H), 7.64, 7.82 (each s, each 1H, H-2, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.3, 153.7, 147.4, 144.6, 141.3, 139.5, 136.3, 130.0, 128.1, 127.7, 124.3, 114.1, 112.8, 93.2, 86.4, 84.2, 81.9, 63.1, 55.5, 49.1, 29.4, 28.3, 27.5, 26.3, 25.2, 23.5, 17.2, −7.1, −7.2. ESI-TOF$^+$-MS: calcd for $C_{44}H_{57}N_5O_6Si$ [$(M + 1)^+$], 780.4; found, 780.3. Anal. ($C_{44}H_{57}N_5O_6Si$) C, H, N.

***N*^{1}-(5″-Monomethoxytrityloxypentyl)-2′,3′-*O*-isopropylideneadenosine 5c.** A mixture of **4c** (656 mg, 0.84 mmol), TBAF (1 M in THF, 8.4 mL, 8.4 mmol), and AcOH (273 μL, 4.3 mmol) in THF (20 mL) was stirred at room temperature for 2 h. The procedure was the same as for the synthesis of **5a** to give **5c** in 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 3H, isopropyl CH_3), 1.45−1.78 (m, 9H, H-2″, H-3″, H-4″, isopropyl CH_3), 3.06 (m, 2H, H-5″), 3.73−3.91 (m, 5H, H-5′, OCH3), 3.95−4.03(m, 2H, H-1″), 4.49−4.50 (m, 1H, H-4′), 4.95 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.05 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 5.78 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.81−7.44 (m, 14H, Ar−H), 7.64, 7.66 (each s, each 1H, H-2, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.3, 153.3, 147.2, 144.7, 141.0, 139.0, 136.0, 130.2, 128.3, 127.7, 124.1, 114.1, 112.9, 93.4, 86.0, 83.9, 81.4, 62.9, 55.1, 48.7, 29.6, 28.4, 27.5, 25.2, 23.3. ESI-TOF$^+$-MS: calcd for $C_{38}H_{43}N_5O_6$ [$(M + 1)^+$], 666.3; found, 666.2. Anal. ($C_{38}H_{43}N_5O_6$) C, H, N.

***N*^{1}-(5″-Monomethoxytrityloxypentyl)-5′-*O*-[bis(phenylthio)-phosphoryl]-2′,3′-*O*-isopropylideneadenosine 6c.** To a solution of **5c** (503 mg, 0.756 mmol) in pyridine (10 mL) were added TPSCl (460 mg, 1.51 mmol), PSS (861 mg, 2.27 mmol), and tetrazole (159 mg, 2.27 mmol). The mixture was stirred at room temperature for 12 h. The procedure was the same as for the synthesis of **6a** to give **6c** in 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (s, 3H, isopropyl CH_3), 1.41−1.72 (m, 9H, H2″, H-3″, H-4″, isopropyl CH_3), 3.11 (m, 2H, H-5″), 3.75−3.95 (m, 5H, H-5′, OCH3), 4.00−4.10(m, 2H, H-1″), 4.51−4.52 (m, 1H, H-4′), 4.89 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.10 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 5.78 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.80−7.56 (m, 24H, Ar−H), 7.56, 7.68 (each s, each 1H, H-2, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.3, 153.5, 147.2, 144.8, 140.9, 138.9, 136.1, 132.5, 130.2, 129.4, 129.1, 128.3, 127.7, 126.3, 124.1, 114.0, 112.9, 93.5, 86.1, 84.0, 81.6, 63.1, 55.1, 48.6, 29.5, 28.5, 27.6, 25.3, 23.4. ^{31}P NMR ($CDCl_3$, 81 MHz, decoupled with ^{1}H) δ 49.45 ppm (s). ESI-TOF$^+$-MS: calcd for $C_{50}H_{52}N_5O_7PS_2$ [$(M + 1)^+$], 930.3; found, 930.3. Anal. ($C_{50}H_{52}N_5O_7PS_2$) C, H, N.

***N*^{1}-[(5″-Hydroxyl)pentyl]-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine 7c**. A solution of **6c** (457 mg, 0.491 mmol) in 80% aqueous AcOH (10 mL) was stirred at room temperature for 8 h. The procedure was the same as for the synthesis of **7a** to give **7c** in 82% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (s, 3H, isopropyl CH_3), 1.48−1.70(m, 9H, H-2″, H-3″, H-4″, isopropyl CH_3), 3.05 (m, 2H, H-5″), 3.85−3.97 (m, 2H, H-5′), 4.05−4.09 (m, 2H, H-1″), 4.52−4.53 (m, 1H, H-4′), 4.90 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.05 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 5.95 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.85−7.42 (m, 10H, Ar−H), 7.65, 7.70 (each s, each 1H, H-2, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.7, 145.5, 139.4, 134.4, 132.7, 129.5, 129.2, 125.7, 122.1, 112.3, 89.4, 85.3, 83.5, 79.5, 61.7, 60.3, 46.1, 30.3, 27.9, 26.5, 25.4, 24.1, 23.6. ^{31}P NMR ($CDCl_3$, 81 MHz, decoupled with ^{1}H) δ 49.50 ppm (s). ESI-TOF$^+$-MS: calcd for $C_{30}H_{36}N_5O_6PS_2$ [$(M + 1)^+$], 658.2; found, 658.2. Anal. ($C_{30}H_{36}N_5O_6PS_2$) C, H, N.

***N*^{1}-(5″-Phosphonoxypentyl)-5′-*O*-[(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine 8c.** $POCl_3$ (283 μL, 3.04 mmol) was added to a solution of **7c** (200 mg, 0.304 mmol) in $PO(OMe)_3$ (3 mL) at 0 °C, and the mixture was stirred at the same temperature for 35 min. The following procedure was the same as for the synthesis of **8a** to give **8c** in 51% yield. ^{1}H NMR (300 MHz, D_2O) δ 1.41 (s, 3H, isopropyl CH_3), 1.52−1.75 (m, 9H, H-2″, H-3″, H-4″, isopropyl CH_3), 3.20 (m, 2H, H-5″), 3.85−4.00 (m, 2H, H-5′), 4.10−4.21 (m, 2H, H-1″), 4.52−4.53 (m, 1H, H-4′), 5.02 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.38 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.05 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 6.90−7.35 (m, 5H, Ar−H), 8.15, 8.32 (each s, each 1H, H-2, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ 3.01 ppm (s), 17.65 ppm (s). HRMS (ESI-TOF$^-$) calcd for $C_{24}H_{33}N_5O_{10}P_2S$ [$(M - 1)^-$], 644.1345; found, 644.1338.

***N*^{1}-[(5″-*O*-Phosphoryl)pentyl]-2′,3′-*O*-isopropylidene-5′-*O*-phosphoryladenosine 5′,5″-Cyclicpyrophosphate 9c.** A solution of **8c** (15 mg, 23.2 μmol) in pyridine (5 mL) was added slowly over 20 h, using a syringe pump, to a mixture of $AgNO_3$ (84 mg, 494 μmol) and 3 Å molecular sieves (2.02 g) in pyridine (50 mL) at room temperature in the dark. The procedure was the same as for the synthesis of **9a** to give **9c** in 59% yield. ^{1}H NMR (300 MHz, D_2O) δ 1.38 (s, 3H, isopropyl CH_3), 1.49−1.72(m, 9H, H-2″, H-3″, H-4″, isopropyl CH_3), 3.25 (m, 2H, H-5″), 3.95−4.15 (m, 2H, H-5′), 4.20−4.32 (m, 2H, H-1″), 4.55 (m, 1H, H-4′), 5.05 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.55 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.10 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 8.25, 8.35 (each s, each 1H, H-2, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with^{1}H) δ −9.99 ppm (d, $J_{P,P}$ = 10.0 Hz), −10.72 ppm (d, $J_{P,P}$ = 10.0 Hz). HRMS (ESI-TOF$^-$) calcd for $C_{18}H_{27}N_5O_{10}P_2$ [$(M - 1)^-$], 534.1155; found, 534.1139.

***N*^{1}-[(5″-*O*-Phosphoryl)pentyl]-5′-*O*-phosphoryladenosine 5′,5″-Cyclicpyrophosphate 10c.** A solution of **9c** (7.3 mg, 13.7 μmol) in 60% HCOOH (5 mL) was stirred for 8 h. The procedure was the same as for the synthesis of **10a** to give **10c** in 89% yield. ^{1}H NMR (300 MHz, D_2O) δ 1.55−1.69 (m, 6H, H-2″, H-3″, H-4″), 3.32 (m, 2H, H-5″), 3.86−4.05 (m, 2H, H-5′), 4.15−4.28 (m, 2H, H-1″), 4.38 (m, 1H, H-4′), 4.98 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.45 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.15 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 8.15, 8.55 (each s, each 1H, H-2, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ −9.72 ppm (d, $J_{P,P}$ = 10.1 Hz), −10.10 ppm (d, $J_{P,P}$ = 10.1 Hz). HRMS (ESI-TOF$^-$) calcd for $C_{15}H_{23}N_5O_{10}P_2$ [$(M - 1)^-$], 494.0842; found, 494.0836.

***N*^{1}-[(6″-Hydroxyl)hexyl]-5′-*O*-TBDMS-2′,3′-*O*-isopropylideneadenosine 3d.** A mixture of **1** (486 mg, 1.1 mmol), **2d** (157 mg, 1.3 mmol), and K_2CO_3 (8 mg, 0.06 mmol) in MeOH (15 mL) was stirred at room temperature for 4 h. The procedure was the same as for the synthesis of **3a** to give **3d** in 83% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.00 (s, 6H, $(CH_3)_2Si$), 0.80 (s, 9H, $(CH_3)_3C-$), 1.31 (s, 3H, isopropyl CH_3), 1.32−1.69 (m, 11H, H-2″, H-3″, H-4″, H-5″, isopropyl CH_3), 3.34−3.38 (m, 2H, H-6″), 3.64−3.73 (m, 2H, H-5′), 3.92−4.08 (m, 2H, H-1″), 4.19−4.20 (m, 1H, H-4′), 4.32 (t, 1H, $J_{H6'',OH}$ = 4.5 Hz, OH), 4.88 (dd, 1H, $J_{H3',H4'}$ = 3.0 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-3′), 5.28 (dd, 1H, $J_{H1',H2'}$ = 2.5 Hz, $J_{H2',H3'}$ = 6.0 Hz, H-2′), 6.04 (d, 1H, $J_{H2',H1'}$ = 2.5 Hz, H-1′), 7.07 (s, br, NH), 8.05,8.06 (each s, each 1H, H-2, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.5, 147.3, 141.2, 136.7, 123.9, 114.1, 91.2, 87.0, 85.3, 81.3, 63.5, 62.5, 47.7, 32.5, 29.7, 28.6, 27.2, 26.3, 25.9, 25.2,

18.3, −5.4, −5.6. ESI-TOF$^+$-MS: calcd for $C_{25}H_{43}N_5O_5Si$ [$(M + 1)^+$], 522.3; found, 522.3. Anal. ($C_{25}H_{43}N_5O_5Si$) C, H, N.

***N*^{1}-(6″-Monomethoxytrityloxyhexyl)-5′-*O*-TBDMS-2′,3′-*O*-isopropylideneadenosine 4d.** A mixture of **3d** (476 mg, 0.9 mmol) and MMTrCl (557 mg, 1.8 mmol) in pyridine (10 mL) was stirred at room temperature for 8 h. The procedure was the same as for the synthesis of **4a** to give **4d** in 89% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 6H, $(CH_3)_2Si$), 0.86 (s, 9H, $(CH_3)_3C-$), 1.39 (s, 3H, isopropyl CH_3), 1.45−1.78(m, 11H, H-2″, H-3″, H-4″, H-5″, isopropyl CH_3), 3.05 (m, 2H, H-6″), 3.78−3.83 (m, 5H, H-5′, OCH_3), 3.99−4.04 (m, 2H, H-1″), 4.39−4.40 (m, 1H, H-4′), 4.89 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.10 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 5.92 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 6.85−7.45 (m, 14H, Ar−H), 7.64, 7.82 (each s, each 1H, H-2, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6, 154.1, 148.2, 145.3, 138.0, 136.3, 129.2, 128.3, 127.5, 126.6, 114.1, 112.9, 94.3, 86.1, 85.8, 83.6, 82.3, 63.5, 63.0, 54.9, 48.3, 28.8, 28.6, 28.0, 27.0, 26.7, 26.1, 25.6, 18.3, −5.4, −5.6. ESI-TOF$^+$-MS: calcd for $C_{45}H_{59}N_5O_6Si$ [$(M + 1)^+$], 795.4; found, 795.5. Anal. ($C_{45}H_{59}N_5O_6Si$) C, H, N.

***N*^{1}-(6″-Monomethoxytrityloxyhexyl)-2′,3′-*O*-isopropylideneadenosine 5d.** A mixture of **4d** (645 mg, 0.80 mmol), TBAF (1 M in THF, 8.0 mL, 8.0 mmol), and AcOH (254 μL, 4.0 mmol) in THF (20 mL) was stirred at room temperature for 2 h. The procedure was the same as for the synthesis of **5a** to give **5d** in 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (s, 3H, isopropyl CH_3), 1.39−1.78(m, 11H, H-2″, H-3″, H-4″, H-5″, isopropyl CH_3), 3.02 (m, 2H, H-6″), 3.74−3.77 (dd, 1H, $J_{H4',H5'a} = 1.5$ Hz, $J_{H5'b,H5'a} = 12.5$ Hz, H-5′a), 3.78 (s, 3H, OCH_3), 3.92−3.95 (dd, 1H, $J_{H4',H5'b} = 1.5$ Hz, $J_{H5'b,H5'b} = 12.5$ Hz, H-5′b), 3.99−4.02(m, 2H, H-1″), 4.48 (m, 1H, H-4′), 4.98 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.12 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 5.79 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 6.81−7.44 (m, 14H, Ar−H), 7.67, 7.71 (each s, each 1H, H-2, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.4, 153.8, 147.3, 144.9, 138.3, 136.2, 130.2, 128.4, 127.7, 126.7, 114.1, 112.9, 93.8, 86.0, 85.8, 83.6, 81.4, 63.2, 63.1, 55.2, 48.3, 28.9, 28.6, 27.6, 26.5, 26.0, 25.2. ESI-TOF$^+$-MS: calcd for $C_{39}H_{45}N_5O_6$ [$(M + 1)^+$], 680.3; found, 680.4. Anal. ($C_{39}H_{45}N_5O_6$) C, H, N.

***N*^{1}-(6″-Monomethoxytrityloxyhexyl)-5′-*O*-[bis(phenylthio)-phosphoryl]-2′,3′-*O*-isopropylideneadenosine 6d.** To a solution of **5d** (489 mg, 0.72 mmol) in pyridine (10 mL) were added TPSCl (437 mg, 1.438 mmol), PSS (820 mg, 2.16 mmol), and tetrazole (152 mg, 2.16 mmol), and the mixture was stirred at room temperature for 12 h. The procedure was the same as for the synthesis of **6a** to give **6d** in 64% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32 (s, 3H, isopropyl CH_3), 1.42−1.79(m, 11H, H-2″, H-3″, H-4″, H-5″, isopropyl CH_3), 3.15 (m, 2H, H-6″), 3.75−3.85 (m, 5H, H-5′, OCH_3), 4.05−4.10 (m, 2H, H-1″), 4.45 (m, 1H, H-4′), 4.86 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.35 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 5.96 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 6.85−7.47 (m, 24H, Ar−H), 7.69, 7.75 (each s, each 1H, H-2, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.3, 154.1, 147.3, 145.2, 138.1, 136.0, 132.2, 129.9, 129.4, 129.1, 128.5, 128.2, 127.0, 125.6, 114.0, 112.6, 93.9, 86.5, 85.8, 83.3, 82.2, 63.6, 63.3, 56.1, 48.2, 29.0, 28.6, 28.2, 26.5, 26.0, 25.4. ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ 49.48 ppm (s). ESI-TOF$^+$-MS: calcd for $C_{51}H_{54}N_5O_7PS_2$ [$(M + 1)^+$], 944.3; found, 944.3. Anal. ($C_{51}H_{54}N_5O_7PS_2$) C, H, N.

***N*^{1}-[(6″-Hydroxyl)hexyl]-5′-*O*-[bis(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine 7d.** A solution of **6d** (435 mg, 0.461 mmol) in 80% aqueous AcOH (10 mL) was stirred at room temperature for 8 h. The procedure was the same as for the synthesis of **7a** to give **7d** in 82% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36 (s, 3H, isopropyl CH_3), 1.38−1.75(m, 11H, H-2″, H-3″, H-4″, H-5″, isopropyl CH_3), 3.05 (m, 2H, H-6″), 3.78−3.86 (m, 2H, H-5′), 3.98−4.06 (m, 2H, H-1″), 4.35 (m, 1H, H-4′), 4.78 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.25 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 5.86 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 6.89−7.43 (m, 10H, Ar−H), 7.70, 7.72 (each s, each 1H, H-2, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.5, 147.4, 141.2, 136.7, 132.4, 129.5, 129.3, 125.8, 123.9, 114.1, 91.3, 87.0, 85.3, 81.4, 63.5, 62.5, 47.7, 32.5, 29.7, 28.7, 27.3, 25.9, 25.3. ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ 49.50 ppm (s) ESI-TOF$^+$-MS: calcd for $C_{31}H_{38}N_5O_6PS_2$ [$(M + 1)^+$], 672.2; found, 672.2. Anal. ($C_{31}H_{38}N_5O_6PS_2$) C, H, N.

***N*^{1}-(6″-Phosphonoxyhexyl)-5′-*O*-[(phenylthio)phosphoryl]-2′,3′-*O*-isopropylideneadenosine 8d.** $POCl_3$ (278 μL, 2.98 mmol) was added to a solution of **7d** (200 mg, 0.298 mmol) in $PO(OMe)_3$ (3 mL) at 0 °C, and the mixture was stirred at the same temperature for 35 min. The following procedure was the same as for the synthesis of **8a** to give **8d** in 54% yield. ^{1}H NMR (500 MHz, D_2O) δ 1.32 (s, 3H, isopropyl CH_3), 1.48−1.80(m, 11H, H-2″, H-3″, H-4″, H-5″, isopropyl CH_3), 3.35 (m, 2H, H-6″), 3.80−3.90 (m, 2H, H-5′), 4.03−4.08(m, 2H, H-1″), 4.55 (m, 1H, H-4′), 4.99 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.35 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 6.01 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 6.90−7.48 (m, 5H, Ar−H), 8.25, 8.35 (each s, each 1H, H-2, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ 2.06 ppm (s), 17.79 ppm (s). HRMS (ESI-TOF$^-$) calcd for $C_{25}H_{35}N_5O_{10}P_2S$ [$(M - 1)^-$], 658.1502; found, 658.1496.

***N*^{1}-[(6″-*O*-Phosphoryl)hexyl]-2′,3′-*O*-isopropylidene-5′-*O*-phosphoryladenosine 5′,5″-Cyclicpyrophosphate 9d.** A solution of **8d** (15 mg, 22.8 μmol) in pyridine (5 mL) was added slowly over 20 h, using a syringe pump, to a mixture of $AgNO_3$ (82 mg, 486 μmol) and 3 Å molecular sieves (1.98 g) in pyridine (50 mL) at room temperature in the dark. The procedure was the same as for the synthesis of **9a** to give **9d** in 60% yield. ^{1}H NMR (500 MHz, D_2O) δ 1.39 (s, 3H, isopropyl CH_3), 1.50−1.79(m, 11H, H-2″, H-3″, H-4″, H-5″, isopropyl CH_3), 3.43 (m, 2H, H-6″), 3.85−3.94 (m, 2H, H-5′), 4.10−4.15(m, 2H, H-1″), 4.58 (m, 1H, H-4′), 5.08 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.50 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 6.02 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 8.25, 8.38 (each s, each 1H, H-2, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ −9.99 ppm (d, $J_{P,P} = 10.0$ Hz), −10.72 ppm (d, $J_{P,P} = 10.0$ Hz). HRMS (ESI-TOF$^-$) calcd for $C_{19}H_{29}N_5O_{10}P_2$ [$(M - 1)^-$], 548.1312; found, 548.1295.

***N*^{1}-[(6″-*O*-Phosphoryl)hexyl]-5′-*O*-phosphoryladenosine 5′,5″-Cyclicpyrophosphate 10d.** A solution of **9d** (7.5 mg, 13.7 μmol) in 60% HCOOH (5 mL) was stirred for 8 h. The procedure was the same as for the synthesis of **10a** to give **10d** in 90% yield. ^{1}H NMR (500 MHz, D_2O) δ 1.48−1.76 (m, 8H, H-2″, H-3″, H-4″, H-5″), 3.48 (m, 2H, H-6″), 3.86−3.96 (m, 2H, H-5′), 4.15−4.18-(m, 2H, H-1″), 4.62 (m, 1H, H-4′), 5.05 (dd, 1H, $J_{H3',H4'} = 3.0$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-3′), 5.45 (dd, 1H, $J_{H1',H2'} = 2.5$ Hz, $J_{H2',H3'} = 6.0$ Hz, H-2′), 6.05 (d, 1H, $J_{H2',H1'} = 2.5$ Hz, H-1′), 8.21, 8.35 (each s, each 1H, H-2, H-8). ^{31}P NMR (D_2O, 81 MHz, decoupled with ^{1}H) δ −9.75 ppm (d, $J_{P,P} = 10.5$ Hz), −9.90 ppm (d, $J_{P,P} = 10.5$ Hz). HRMS (ESI-TOF$^-$) calcd for $C_{16}H_{25}N_5O_{10}P_2$ [$(M - 1)^-$], 508.0999; found, 508.0988.

Pharmacology. Cell Culture. Jurkat T-lymphocytes (clone JMP) were cultured in RPMI 1640 medium supplemented with Glutamax I (2.06 mM), 25 mM HEPES, 110 units/mL penicillin, 110 μg/mL streptomycin, and 7.5% (v/v) newborn calf serum (termed complete medium).

Loading of Cells with Fura2/AM. The cells (1×10^7 cells) were centrifuged (room temperature, 1500 rpm, 5 min), and then the pellet was resuspended in 1 mL of fresh and warm (37 °C) complete medium (see above). The cells were incubated for 5 min at 37 °C. Then Fura 2-AM (4 μL of a 1 mg/mL stock solution) was added, and the cells were incubated for 15 min in the dark. Subsequently another 4 mL of complete medium was added to the cell suspension and the cells were incubated for another 15 min at 37 °C in the dark. Then the cells were washed with Ca^{2+} measurement buffer (140 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 20 mM HEPES, 1 mM NaH_2PO_4, 5.5 mM glucose, pH 7.4) twice, resuspended in 5 mL of Ca^{2+} measurement buffer, and kept in the dark at room temperature until use.

Determination of $[Ca^{2+}]_i$ in Intact Cells. $[Ca^{2+}]_i$ was analyzed in Fura2-loaded Jurkat T-lymphocytes. Changes in Fura2 fluorescence were measured using a Hitachi F-2000 spectrofluorometer operating in ratio mode (alternating excitation at 340 and 380 nm

and emission at 495 nm). To test any antagonist activity of the compounds, the cells in the cuvette (1×10^6 cells) were preincubated with the compound (500 mM) for 20 min before the start of the Ca^{2+} measurement. Anti-CD3 mAb OKT3 (10 μg/mL final concentration) was added to the cell suspension at a time point 200 s after the start of each measurement. To test any agonist effect of the compounds, each compound was added to the cell suspension at the 200 s time point and data were recorded for the next 700 s. To calibrate the Ca^{2+} determinations, Triton X-100 (0.1% (v/v)) and EGTA/Tris (12 mM/90 mM) were added sequentially at the end of each experiment.

Ratiometric Ca^{2+} Imaging. Jurkat T-lymphocytes were loaded with Fura2/AM as described above. Imaging experiments were carried out on thin glass coverslips (0.1 mm) coated with bovine serum albumin (5 mg/mL) and poly-L-lysine (1 mg/mL). Silicon grease was used to seal a small chamber that consisted of a rubber O-ring on the glass coverslip. Then 70 μL of Ca^{2+} measurement buffer (composition see above) and 30 μL of cell suspension were added into the small chamber. The coverslip with cells attached to the bovine serum albumin/poly-L-lysine coating was mounted on the stage of a fluorescence microscope (Leica DM IRB2). An Improvision imaging system (Tübingen, Germany) consisting of a monochromator system (Polychromator IV, TILL Photonics, Graefelfing, Germany) and a gray-scale CCD camera (type C4742-95-12ER; Hamamatsu, Enfield, U.K.; operating in 8-bit mode) built around the Leica microscope at 100-fold magnification was used. Illumination was carried out at 340 and 380 nm alternatively. The acquisition rate was approximately one ratio per second for slow measurements and one ratio per 150 ms for fast measurements. Raw data were stored on a compact disk. Confocal images were obtained after deconvolution using the volume deconvolution module of Openlab software (Improvision). The removal of stray light was set to 0.7. The deconvolved images were used to construct ratio images (340/380). Finally the ratio values were converted into Ca^{2+} concentrations by external calibration. Ratio images were subjected to a median filter (3×3). Data processing was performed using Openlab software, versions 4.0.2, 3.0.8, and 1.7.8 (Improvision).

Acknowledgment. This study was supported by the National Natural Sciences Foundation of China (Grant No. SFCBIC 20320130046 to L.-h.Z. and Grant No. 20472007 to L.Z.), the Ministry of Science and Technology of China (Grant Nos. 2004CB518904 and 2005BA711A04 to L.-h.Z.), the Research Fund for the Doctoral Program of Higher Education (Grant No. 20040001140 to L.Z.), the Deutsche Foschungsgemeinschaft (Grant Nos. GU 360/9-1 and 9-2 to A.H.G.), the Deutsche Akademische Austauschdienst (Grant No. 423/PPP-vrc-sr to A.H.G. and L.-h.Z.), and the Gemeinnützige Hertie-Stiftung (Grant No. 1.01.1/04/010 to A.H.G.).

Supporting Information Available: Elemental analysis data for **3**−**7** and charts of HPLC data for **10a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM060320E