Syntheses and Calcium-Mobilizing Evaluations of N^1-Glycosyl-Substituted Stable Mimics of Cyclic ADP-Ribose

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Cyclic ADP-ribose (cADPR) is not only a potent endogenous calcium modulator but also a second messenger. However, studies on the mechanism of cADPR action were limited due to its instability and lack of available structural modifications in the N^1-glyosyl unit of cADPR. In the present work, a series of N^1-glycosyl mimics with different configurational glycosyls or an ether strand were designed and synthesized mimicking the furanose ring. S_N2 substitutions were carried out between the protected inosine and glycosyl triflates to form the N^1-glycosylinosine derivatives, accompanied with some O^6-glycosyl-substituted as side products. The intramolecular cyclization was followed the strategy described by Matsuda et al. It was found that the 8-unsubstituted substrate could also be used to construct the intramolecular cyclic pyrophosphate. The activities of N^1-glycosyl-substituted cADPR mimics were evaluated by induced Ca^{2+} release in rat brain microsomes and HeLa cells. It was found that the configuration of the N^1-glycosyl moiety in cADPR is not a critical structural factor for retaining the activity of mobilizing Ca^{2+} release. More interestingly, the N^1-acyclic analogue **6** exhibited strong activity by inducing Ca^{2+} release in both rat brain microsomes and HeLa cells. It constitutes a useful tool for further studies.

Introduction

Ryanodine receptors, together with IP_3 receptors, represent a major pathway of Ca^{2+}-release from intracellular stores and have been shown to be involved in many physiological and pathological processes.[1] Cyclic ADP-ribose (cADPR, **1**), a novel cyclic nucleotide known since 1987,[2] has attracted worldwide attention in recent years because of its potent calcium-mobilizing activities in many systems, especially as a potential second messenger in cellular Ca^{2+} homeostasis.[3]

Due to their biological importance, many analogues with modifications at the adenosine unit have been synthesized from NAD^+ analogues using ADP-ribosyl cyclase and served as valuable research tools in the elucidation of the mechanism of cADPR action.[4] However, the analogues that can be obtained by enzymatic and chemoenzymatic methods are limited by the inherent substrate specificity of the enzyme. In fact, there are few analogues modified at the N^1-ribosyl moiety of cADPR in contrast to many adenosine-modified cADPR analogues prepared by an enzymatic method. Furthermore, in some cases, the newly formed glycosylic bond is attached to the N-7 of the purine ring instead of the desired N-1 position.[5] In addition, cADPR can be readily hydrolyzed at the unstable N^1-glycosylic linkage to give ADP-ribose.[6] Therefore, to build up a structure−activity profile for the Ca^{2+}-releasing ability of cADPR, stable

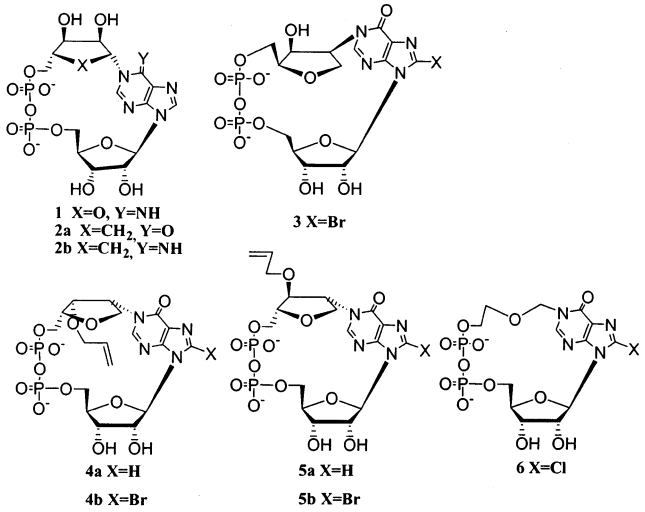

Figure 1. Structures of cADPR and its analogues.

N^1-substituted cADPR mimics are urgently needed and have to be made by means of chemical synthesis.[7]

Recently, Matsuda and co-workers first described the synthesis of cyclic IDP-carbocyclic-ribose (cIDP-carboribose, **2a**), in which the 4′-oxygen[8] in the N^1-ribosyl moiety is substituted by a methylene group.[9] In the present study, we designed a novel class of stable cIDPR mimics (**3**, **4a**, **4b**, **5a**, **5b**, **6**), in which the N^1-ribosyl moiety is replaced by different configurational glycosyl and the N^1-glycosyl linkage is shifted from N^1-$C_{1''}$ to the N^1-$C_{2''}$ position of furanose in order to improve the stabilization (Figure 1).[10] The 3′′-*O*-allyl and 8-bromo modifications have been included due to their anticipated increased cell permeability.[11] Furthermore, to explore the role of N^1-glycosyl linkage in structural

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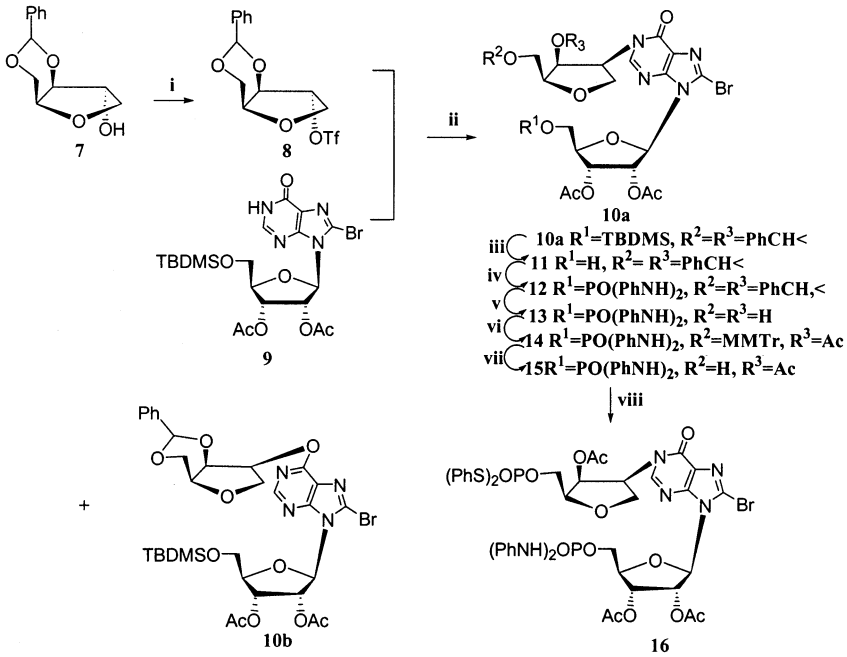
Scheme 1[a]



[a] Reagents and conditions: (i) $(CF_3SO_2)_2O$, Py, CH_2Cl_2, −15 °C; (ii) K_2CO_3, 18-crown-6, THF, 45 °C; (iii) TBAF, THF, rt; (iv) $(PhNH)_2POCl$, tetrazole, Py, rt; (v) 80% AcOH, 70 °C; (vi) a) MMTrCl, Py, rt; b) Ac_2O, rt; (vii) 5% Cl_3CCOOH, CH_2Cl_2, rt; (viii). PSS, TPSCl, Py, tetrazole.

modifications, an N^1-acyclic analogue has been synthesized in this study. These new cIDPR analogues will provide a greater understanding of ryanodine receptor function and could lead to novel therapeutic agents.

Results and Discussion

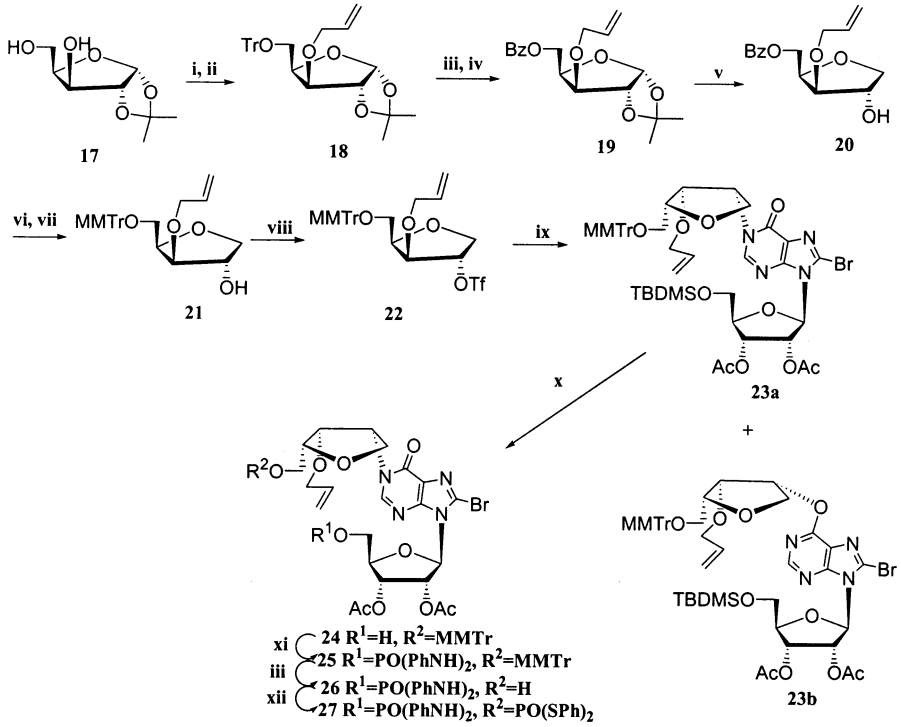
Chemistry: (1) The Syntheses of *N*1-Glycosyl-Substituted Inosine Bisphosphate Derivatives. The key steps for the synthesis of cIDPR analogues involve substitution at N-1 position of inosine and intramolecular cyclization of corresponding bisphosphate. Starting with protected L-xylitol **7**,[12] triflate **8** was prepared by the known method.[9] **8** was reacted with protected 8-bromoinosine **9** in the presence of K_2CO_3 and 18-crown-6 to give the key intermediate, 5′-*O*-TBDMS-2′,3′-di-*O*-acetyl-*N*1-(2″-deoxy-1″,4″-anhydro-3″,5″-*O*-benzylidene-L-lyxitol-2″-yl)-8-bromoinosine **10a** in 28% yield, accompanied by O^6-substituted derivative **10b** in 8% yield. (Scheme 1) After treatment of TBAF in THF, the 5′-hydroxyl derivative **11** was obtained in high yield and **11** was phosphorylated by $(PhNH)_2POCl$ and tetrazole in pyridine to afford **12** in 89% yield. The structure of **12** was identified by X-ray crystallographic analysis.[13] The 3″,5″-*O*-benzylidene group of **12** was hydrolyzed and an attempt to direct coupling of **13** with cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate (PSS) failed. Thus, compound **13** was selectively protected by reaction with *p*-anisylchlorodiphenylmethane (MMTrCl) and then followed by acetylation to give the intermediate **14** in 70% overall yield. Deprotection of **14** was carried out by treatment with 5% Cl_3CCOOH (TCA) in CH_2Cl_2 to produce the 5″-hydroxyl derivative **15**. The resulting 5″-primary hydroxyl of the lyxitol moiety was phosphorylated with PSS, triisopropylbenzenesulfonyl chloride (TPSCl), and tetrazole in pyridine to afford **16** in 91% yield.

Employing the same strategy, the other two N^1-glycosyl inosine derivatives, **27** and **39,** were also prepared from corresponding sugar. Starting from **17**, 5-*O*-benzoyl-3-*O*-allyl-1,2-*O*-isopropylidene-α-D-xylose **19** was attained in a 65% yield over four steps using the same method (Scheme 2).[14] To obtain compound **20**, deprotection of 1,2-*O*-isopropylidene furanoside **19** could be completed using the Et_3SiH/TMSOTf system in CH_2Cl_2.[15] However, the yield was only moderate even under conditions such as prolonging reaction time and use of 4-fold excess of catalyst. Alternatively, the use of Et_3SiH/BF_3-Et_2O in CH_2Cl_2 led to **20** in excellent yield.[16] Compound **20** was converted into **21** by debenzoylation and subsequent reaction with MMTrCl in 79% overall yield. By means of a similar method, the key N^1-glycosyl-substituted inosine derivative **23a** was prepared by S_N2 substitution with triflate **22** in 34% yield, accompanied by the O^6-substituted compound **23b** in 6.8% yield. The bisphosphate compound **27** was obtained in four steps from **23a**. The stereochemistry at C-2″ was as expected according to the NOESY of compound **26**, in which there is a cross-peak between H-5a″ and H-2.

From the same starting material **17**, compound **28** can be obtained by known procedures and a conventional route was chosen to synthesize protected 1,4-anhydro-D-ribitol **31** from **28** (Scheme 3).[14,16,17] Reaction of **31** with trifluoromethanesulfonic anhydride gave triflate **32**, which was directly used for substitution with protected inosine **9** without isolation at room temperature. The N^1-glycosyl inosine derivative **33a** was formed in 26.8% yield, together with the O^6-substituted compound **33b** in 21.5% yield. The corresponding bisphosphate **39** was prepared according to a similar sequence described in Scheme 2. The configuration of C-2″ was also assigned from the NOESY spectrum of compound **33a**, in which a significant NOE was observed between the H-2 and H-5a″.

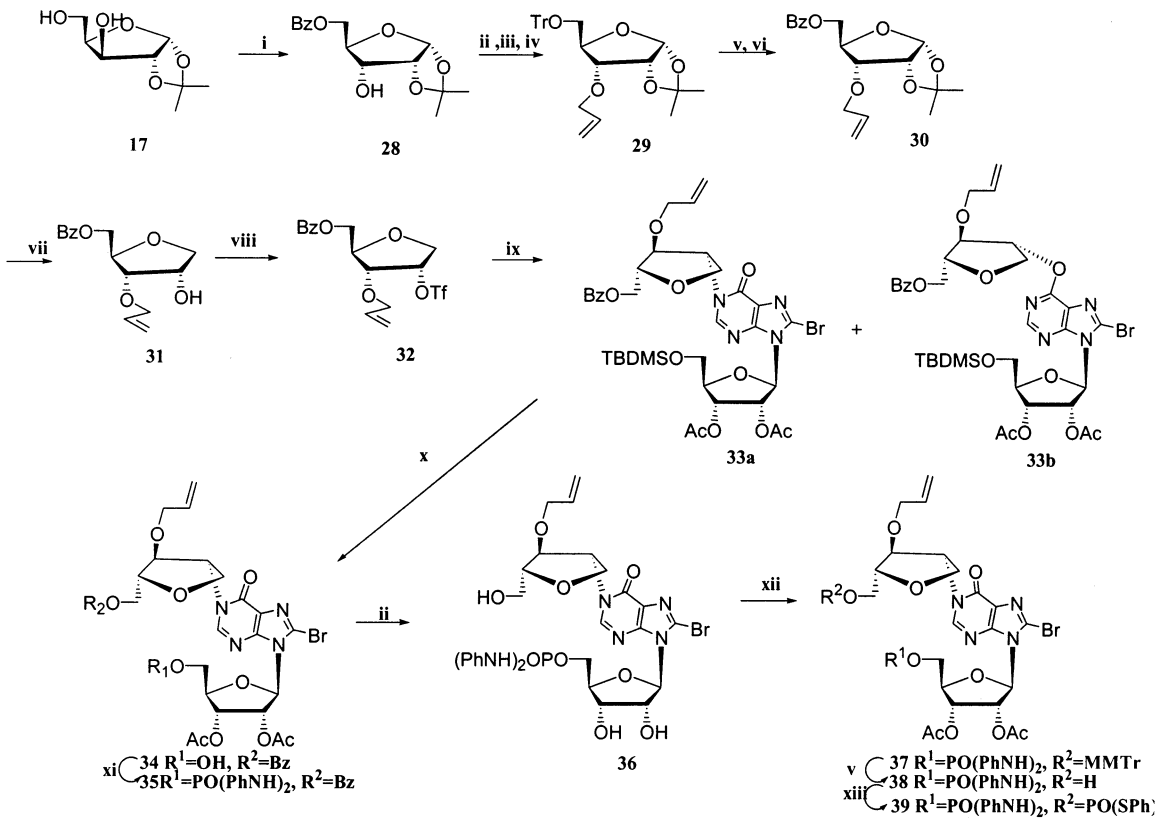
The synthesis of the N^1-acyclic sugar analogue **41** is depicted in Scheme 4. The reaction of protected inosine **40**[9] with excess 2-chloromethoxyethyl acetate afforded

Scheme 2[a]



[a] Reagents and conditions: (i) TrCl, Py, rt; (ii) 80% NaH, allyl bromide, DMF; (iii) 5% Cl_3CCOOH, CH_2Cl_2, rt; (iv) BzCl, Py, rt; (v) Et_3SiH, BF_3Et_2O, CH_2Cl_2, rt; (vi) CH_3ONa, CH_3OH, rt; (vii) MMTrCl, Py, rt; (viii) $(CF_3SO_2)_2O$, Py, −15 °C; (ix) **9**, K_2CO_3, 18-crown-6, THF; (x) TBAF, THF, rt; (xi) $(PhNH)_2POCl$, tetrazole, Py; (xii) PSS, TPSCl, tetrazole, Py, rt.

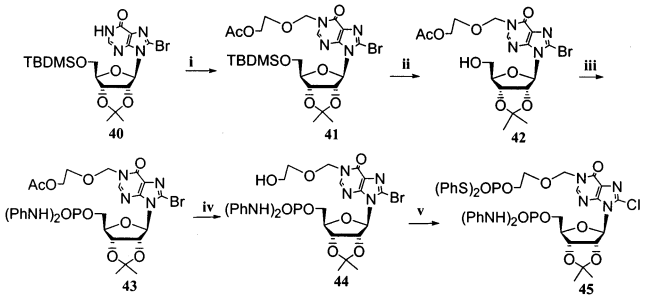
Scheme 3[a]



[a] Reagents and conditions: (i) Reference 17; (ii) CH_3ONa, CH_3OH, rt; (iii) TrCl, Py, rt; (iv) 80% NaH, allyl bromide, DMF; (v) 5% Cl_3CCOOH, CH_2Cl_2, rt; (vi). BzCl, Py, rt; (vii). Et_3SiH, BF_3Et_2O, CH_2Cl_2, rt; (viii) $(CF_3SO_2)_2O$, Py, −15 °C; (ix) **9**, K_2CO_3, 18-crown-6, THF; (x) TBAF, THF, rt; (xi) $(PhNH)_2POCl$, tetrazole, Py; (xii) (a) MMTrCl, Py, rt; (b) Ac_2O, rt; (xiii) PSS, TPSCl, tetrazole, Py, rt.

the desired N^1-acyclic compound **41** regioselectively in 82% yield using DBU as base.[18] The bisphosphate **45** was produced as described for compounds **16**, **27**, and **39**. However, MALDI-TOF MS of **45** showed that the

Scheme 4[a]



[a] Reagents and conditions: (i) DBU, $ClCH_2OCH_2CH_2OAc$, DCM, rt; (ii) TBAF, THF, rt; (iii) $(PhNH)_2POCl$, tetrazole, Py; (iv) CH_3ONa, CH_3OH, rt; (v) PSS, TPSCl, tetrazole, Py, rt.

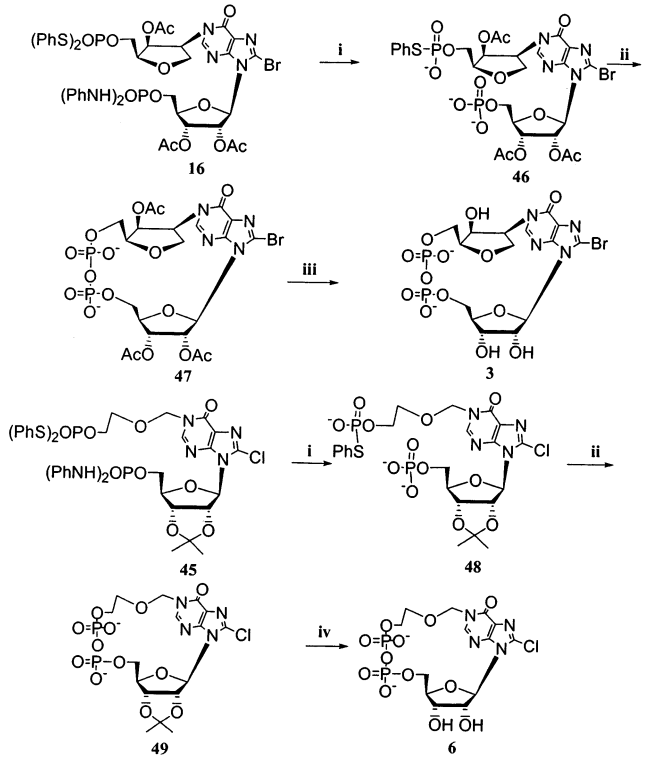
bromine at the 8-position of inosine had been replaced by chlorine during the phosphorylation. Further investigation indicated that the excess of TPSCl in that reaction caused the substitution of bromine at the 8-position of inosine.

(2) The Intramolecular Cyclization. Matsuda's group developed an effective strategy for the cyclization of cADPR analogues. They found that introducing a bulky group into the 8-position of purine nucleosides could restrict the conformation to a syn-form in which the two phosphate moieties are near each other and facilitate intramolecular condensation in the presence of EDC.[9] Using this method, Capua et al. reported that they failed to obtain the N^1-glucosyl substituted cADPR analogue due to the absence of the bulky group at the 8-position.[19] Matsuda et al. also reported that the cyclization was completed by I_2/MS 3 Å as a promoter in pyridine in quantitative yield,[20] and they synthesized cyclic ADP-carbocyclic-ribose by using a similar method.[21] We followed the Matsuda strategy to synthesize the N^1-glycosyl substituted mimics (**3**, **4a**, **4b**, **5a**, **5b**, and **6**).

Treatment of **16** with isoamyl nitrite in a mixed solvent of pyridine−AcOH−Ac_2O and H_3PO_2 gave **46**, the cyclic precursor for the intramolecular condensation, in 60.7% yield as a triethylammonium salt. The intramolecular cyclization was performed by adding a solution of **46** slowly over 20h, using a syringe pump, to a large excess of promoter (I_2/MS 3 Å) in pyridine at room temperature. The cyclic product **47** was purified as its triethylammonium salt by HPLC in 28% yield. The structure of **47** was identified by HR FABMS and NMR. The corresponding peak $[M - 2 \times (acetyl)]^+$ was observed at 644.9647 *m/z* in a HR FAB spectrum, which was consistent with ^{1}H NMR results. Further support for the cyclic product comes from the ^{31}P NMR at −9.37 and −10.16 ppm that are typical shifts for a pyrophosphate moiety.[22] Deprotection of **47** gave the target molecule **3** in 27.9% yield (Scheme 5). Similarly, the desired cyclic product **49** was also obtained as a triethylammonium salt after purification by HPLC in 42.6% yield. The cyclic structure was characterized by HR FABMS and ^{31}P NMR. Deprotection of cyclic product **49** provided the target molecule **6** in 44.6% yield in 60% HCOOH solution. [18]

The selective deprotection of bisphosphate **27** was carried out under a similar condition as above. The phenylthio substrate **50** was obtained in 70% yield. An intramolecular condensation was carried out by promotion of I_2 and 3 Å MS to construct the cyclic pyrophosphate linkage from **50**. Interestingly, a debrominated cyclic product **51a** was obtained in addition to the normal product **51b** during the cyclization. Deacetylation of **51a** and **51b** gave the targets **4a** and **4b**. Their structures were characterized by the ^{1}H NMR, ^{31}P NMR, and HR FAB mass spectra (Scheme 6). Debromination was also observed in the selective deprotection of phosphorodithioate derivative **39,** affording the 8-bromo-substituted **52b** accompanied by 8-unsubstituted derivative **52a**. The debromination was investigated under various conditions. The result showed that the ratio of H_3PO_2 and triethylamine was the key condition and that increased H_3PO_2 can lead to the abnormal debromination or other side reactions. On the basis of the same method, we synthesized two cyclic products **53a** and **53b** starting from precursors **52a**, **52b**, respectively. Deacetylation gave the desired targets **5a** and **5b,** which were characterized by the ^{1}H NMR, ^{31}P NMR, and HR FAB mass spectra.

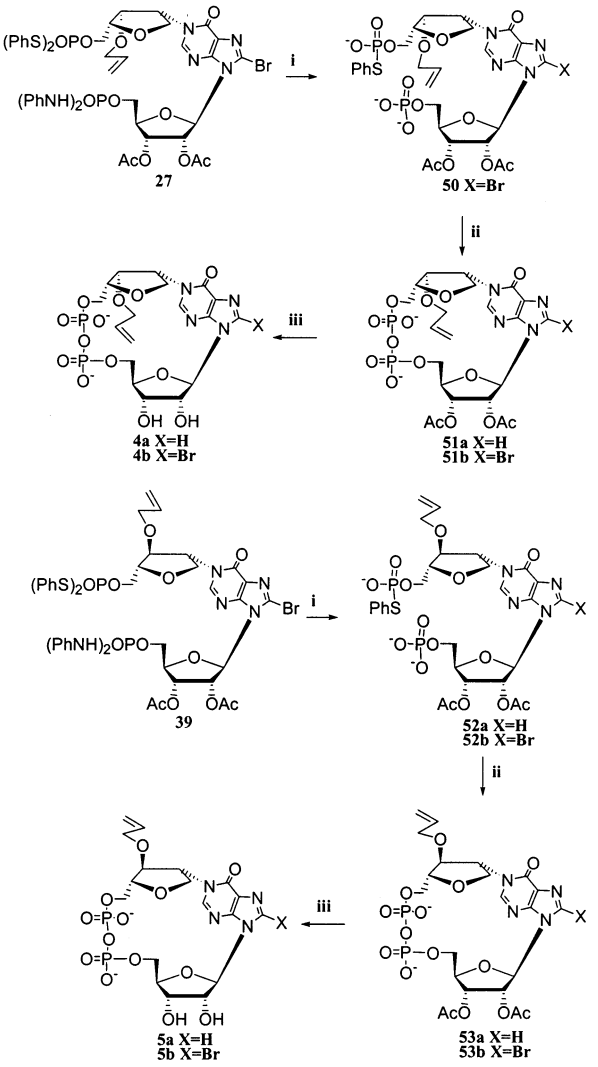
Scheme 5[a]



[a] Reagents and conditions: (i) (a) isoamyl nitrite, Py:AcOH:$(AcO)_2O$ (2:1:1); (b) anhydrous H_3PO_2, Py, Et_3N; (ii) I_2, 3 Å MS, Py, rt; (iii) CH_3ONa, CH_3OH, rt. (iv) 60% HCOOH, rt.

Calcium Release Assay. A series of cADPR analogues with substitutions at the 8-position of the adenine ring have been synthesized and found to be antagonists of cADPR.[23] The order of potency is 8-amino > 8-azido ≫ 8-Br > 8-I-cADPR. In particular, 8-amino-cADPR has been shown to be effective not only in sea urchin egg microsomes but also intact sea urchin eggs[24] as well as intact and permeabilized mammalian cells.[25,26] Additionally, several agonists of cADPR have also been synthesized from NAD^+ analogues. For example, 2′-deoxy-cADPR and cADPR-2′-phosphate are similar to cADPR in inducing Ca^{2+} release, however, 3′-*O*-methyl or 3′-deoxy-cADPR has not the same activity. It was suggested that the 3′-OH was essential for the calcium releasing activity.[27]

Scheme 6[a]



[a] Reagents and conditions: (i) (a) isoamyl nitrite, Py:AcOH: $(AcO)_2O$ (2:1:1); (b) anhydrous H_3PO_2, Py, Et_3N; (ii) I_2, 3 Å MS, Py, rt; (iii) CH_3ONa, CH_3OH, rt.

The first non-hydrolysable mimic of cADPR is cyclic aristeromycin-diphosphate-ribose, which had a similar calcium release profile to that of cADPR.[28] Another agonist of cADPR, 3-deaza-cADPR, is 70-fold more potent than cADPR.[29] A further structural modification of cADPR includes the pyrophosphate moiety, which has been replaced by triphosphate[30] or 1-(2-nitrophenyl)-ethyl pyrophosphate.[31] The cyclic adenosine triphosphate ribose is more potent in inducing calcium release. However, little emphasis has placed on the Ca^{2+}-release activities of cADPR analogues modified at the N^1-ribosyl unit. Recently, Shuto et al. reported that the stable mimic of cADPR, cADPcR (**2b**), caused a significant release of Ca^{2+} in sea urchin eggs.[21]

Rat brain microsomes were one of the first mammalian cell preparations in which cADPR and IP_3 were shown to trigger Ca^{2+} release independently of each other.[32] The six novel cyclic nucleotide analogues (**3**, **4a**, **4b**, **5a**, **5b**, and **6**) were tested for their abilities to release calcium from rat brain microsomes and compared to authentic cADPR by using a confocal laser-scanning microscope (CLSM). The dilution and all experiments were conducted at 17 °C. The cross-membrane property was tested with HeLa cells through

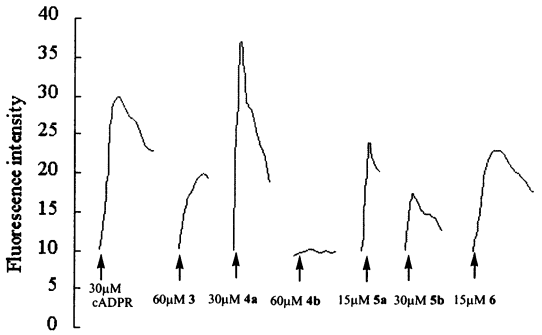


Figure 2. Ca^{2+} release induced by cADPR, **3**, **4a**, **4b**, **5a**, **5b**, and **6** in rat brain microsomes measured fluorometrically with Fluo-3. Microsomes were loaded with Fluo-3 and then assayed as described in the Experimental Section. The data shown are typical curves for at least two experiments carried out in duplicate using different microsomal preparations.

a similar method. It is known that fluo-3 has a higher affinity for Ca^{2+} than other divalent cations.[33] The increase in fluorescence intensity indicates more complex formation between fluorescence dye and Ca^{2+}. In this study, the changes in the fluorescence intensity were used to monitor the changes in free Ca^{2+} concentrations in rat brain microsomes and intact HeLa cells. The data shown are typical curves for at least two experiments carried out in duplicate using different rat brain microsomes and HeLa cells preparations. The concentrations of samples are measured according to the extinction coefficients of their UV spectra.

Figure 2 describes the fluo-3 fluorescence changes when cADPR or the synthetic sample (**3**, **4a**, **4b**, **5a**, **5b**, and **6**) was added into the microsome homogenates. cADPR or the synthetic samples induced Ca^{2+} release from brain microsomes in a concentration-determination with a threshold concentration of less than 15 μM under these experimental conditions. Ca^{2+} was soon resequestered into the microsomes (<400 s). An abrupt fluorescence increase was observed when compounds **5a** or N^1-acyclic mimic **6** in 1−5 μL aliquot was added to 100 μL of rat brain microsomes at 15 μM, respectively, and then the fluorescence signals quickly declined after about 30 s. The other synthetic compounds (**4a**, **4b**, **3**, **5b**) and cADPR are not capable of stimulating obvious fluo 3 fluorescence increase at the same concentration (15 μM). At a concentration of 30 μM, compounds **4a**, **5b**, and cADPR can initiate similar fluorescence spikes while the amplitude of the fluorescence spike induced by compound **4a** is higher than cADPR and **5b**. A low fluorescence spike was triggered by compound **3** at 60 μM. **4b** was not able to induce fluorescence increase even at a concentration of 60 μM. According to the changes in fluo-3 fluorescence, we can compare the relative Ca^{2+} release activities of these compounds in rat brain microsomes homogenates under these experimental conditions. Synthetic compounds **4a**, **5a**, and **6** have more potent activity in inducing $[Ca^{2+}]_i$ increase than authentic cADPR. It is evident that the 8-nonsubstituted analogues (**4a**, **5a**) have more potent calcium modulating activity than the corresponding 8-bromo cADPR mimics (**4b**, **5b**). It seems that the configuration of N^1-glycosyl of cADPR is not a critical structural factor for retaining the activity of induced Ca^{2+} release.

Lee has reviewed 23 different cellular systems responsive to cADPR.[34] The widespread responses to

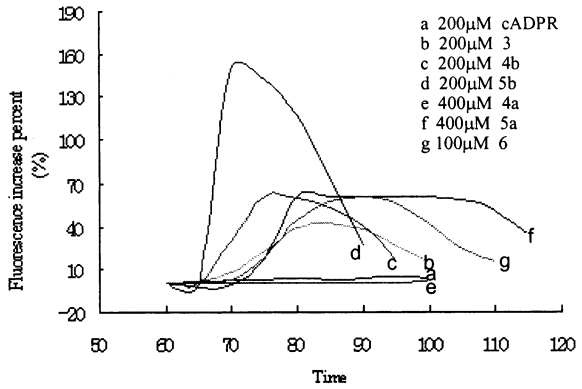


Figure 3. Effects of cADPR, and different N^1-glycosyl substituted cADPR analogues (**3**, **4a**, **4b**, **5a**, **5b**, and **6**) on intracellular calcium levels of intact HeLa cells. Cells were loaded with Fluo-3 and then assayed as described in the Experimental Section. The data shown are typical curves for at least two experiments carried out in duplicate using different HeLa cell preparations.

cADPR among species indicate it regulates a general Ca^{2+}-releasing mechanism in cells. To date, cell preparations include intact cells accessed by microinjection, patch clamping or detergent permeabilization, and isolated organelles. To determine the cross-membrane property of the analogues, intact cells were chosen to evaluate the Ca^{2+} release. In human HeLa cells transfected with CD 38, a lymphocyte antigen, cADPR is shown to play a role in regulating the cell doubling time.[35] It would be interesting to know if the cellular Ca^{2+} level correlates with the cADPR level in HeLa cells. Our results are shown in Figure 3. cADPR (200 μM) exhibited no Ca^{2+} -releasing activity, presumably because it cannot cross the cell membrane. However, when challenged with 200 μM **3**, **4b**, and **5b**, the $[Ca^{2+}]_i$ of about one-half recorded cells promptly displayed a sharp increase in fluorescence intensity which soon declined. Acyclic compound **6** causes a $[Ca^{2+}]_i$ increase at a concentration of 100 μM. The 8-unsubstituted analogue **5a** was not able to provoke calcium release at the same concentration and only elicited calcium release in less than 20% of cells at concentrations as high as 400 μM while the 8-unsubstituted mimic **4a** exhibited no Ca^{2+} release activity even at 400 μM concentration.

These results are consistent with the findings described above that mimics with different configurations at the N^1-glycosyl moiety retained the activities of induced Ca^{2+} release and 8-substituted mimics (**4b**, **5b**) facilitate the permeability of cell membrane. More interestingly, the N^1-acyclic analogue **6** exhibited a strong potency to induce Ca^{2+} release in both of rat brain microsomes and intact HeLa cells. The data obtained from the experiments on HeLa cells showed that the extracellular cADPR mimics can increase the level of Ca^{2+} in intact cells, but it is still not well understood if the cellular Ca^{2+} level correlates with the level of cADPR mimics in cells or the cellular Ca^{2+} level is elevated from other unknown mechanisms. Although the mechanism of their activity is not clear, the N^1-glycosyl substituted mimics will be used as tools for further investigation.

In conclusion, a series of N^1-glycosyl modified mimics of cADPR have been synthesized. The N^1-glycosyl mimics were designed with different configurations or an acyclic analogue mimicking the furanose ring. S_N2 substitution was carried out between the protected inosine and glycosyl triflates to form the N^1-glycosyl inosine derivatives, accompanied with some O^6-glycosyl substituted as side products. The intramolecular cyclization was based on the strategy described by Matsuda et al. It was found that successful cyclization depended on the reaction conditions, and that the 8-unsubstituted substrate can also be used to construct the intramolecular cyclic pyrophosphate. The activities of N^1-glycosyl-substituted cADPR mimics were evaluated by induced Ca^{2+} release in rat brain microsomes and HeLa cells. It was found that the configuration of N^1-glycosyl moiety in cADPR is not a critical structural factor for retaining the activity of induced Ca^{2+} release. More interestingly, the N^1-acyclic sugar analogue **6** exhibited strong activity by induced Ca^{2+} release in both rat brain microsomes and HeLa cells. These analogues should prove to be useful tools for further studies on the mechanism of cADPR action.

Experimental Section

Chemistry. Anhydrous H_3PO_2 was prepared by coevaporating 50% aqueous H_3PO_2 with dry pyridine until the total weight was not reduced further. All solvents were dried and distilled prior to use. Unless otherwise noted, materials were obtained from commercial suppliers and were used as provided. Thin-layer chromatography was performed on silica gel GF-254 (Qing-Dao Chemical Co., China) plates. Column chromatography was done on silica gel (200−300 mesh; Qing-Dao Chemical Co.) Melting points were determined on an XT-4A melting point apparatus and are uncorrected. Optical rotation was determined with Perkin-Elmer 243B polarimeter. UV spectra were recorded on a Varian DMS200 UV−visible spectrophotometer. Mass spectra were obtained on either VG-ZAB−HS or Bruker APEX. High-resolution FAB (fast atom bombardment) mass and HR EIMS (electrospray ionization) were performed with Bruker BIFLEX III. ^{1}H NMR and ^{13}C NMR were recorded with a JEOL AL300 or a Varian VXR-500 spectrometer using $CDCl_3$, DMSO-d_6, or D_2O as a 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. Evaporations were carried out under reduced pressure with a bath temperature <30 °C. Compounds (**3, 4a, 4b, 5a, 5b**, and **6**) can be purified on C18 reverse phase column by two different developing buffer systems: MeCN/TEAA (pH 7.0) or MeCN/TEAB (pH 7.0).

8-Bromo-N^1-(1″,4″-anhydro-2″-deoxy-3″,5″-*O*-benzylidene-L-lyxitol-2″-yl)-5′-*O*-TBDMS-2′,3′-di-*O*-acetyl-inosine (10a) and 8-Bromo-O^6-(1″,4″-anhydro-2″-deoxy-3″,5″-*O*-benzylidene-L-lyxitol-2″-yl)-5′-*O*-TBDMS-2′,3′-di-*O*-acetyl-inosine (10b). Trifluoromethansulfonic anhydride (0.22 mL, 0.13 mmol) was added dropwise at −15 °C to a solution of compound **7** (220 mg, 1 mmol) in dry CH_2Cl_2 (5 mL) and pyridine (0.5 mL). The mixture was stirred for 1 h and poured into 2.5 mL of cool saturated $NaHCO_3$ solution. The organic phase was separated and the aqueous layer extracted with CH_2Cl_2 (5 mL × 2). Combined organic phase was dried over $MgSO_4$, and solvent was concentrated to give triflate **8**, which was used for the next reaction without purification. A mixture of **9** (544 mg, 1 mmol), K_2CO_3 (278 mg, 2 mmol), and catalytic amount 18-Crown-6 in anhydrous THF (6 mL) was refluxed for 1 h. After cooling to room temperature, triflate **8** was added and the resulting mixture stirred for 24 h at 45 °C. After removal of the solvent, the residue was purified by column chromatography (SiO_2, petroleum ether−EtOAc) to afford **10a** as a white solid (209 mg, 27.9%) and **10b** a solid (60 mg, 8%). **10a**: mp 106−108 °C. $[\alpha]^{25}_D$ −89.4 (*c* 0.71, CH_3OH), UV λ_{max}^{MeOH} 208.2 (3.7), 232.4 (4.04), FAB-MS-

(*m*/*z*): 749 [(M + 1)$^+$]. ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.01, 0.01(each s, each 3H, $(CH_3)_2Si$), 0.82 (9H, $(CH_3)_3C$), 2.02, 2.13 (each s, each 3H, 2 × AcO), 3.80 (dd, 1H, $J_{5'b,5'a}$ = 11.1 Hz, $J_{5'b,4'}$ = 4.8 Hz, $H_{5'b}$), 3.90 (dd, 1H, $J_{5'a,5'b}$ = 11.1 Hz, $J_{5'a,4'}$ = 4.5 Hz, $H_{5'a}$), 3.97 (m, 1H, $H_{4''}$), 4.06−4.22 (m, 2H, $H_{4'}$, $H_{1''}$), 4.27−4.34 (m, 2H, $H_{5'}$,$H_{5''}$), 4.42−4.47 (m, 1H, $H_{1''}$), 4.60 (m, 1H, $H_{3''}$), 5.50 (s, 1H, PhCH), 5.70 (t, 1H, *J* = 5.4 Hz, $H_{3'}$), 5.97−6.05 (m, 2H, $H_{1'}$, $H_{2''}$), 6.27 (t, 1H, *J* = 5.7 Hz, $H_{2'}$), 7.37 (m, 5H, C_6H_5), 8.37 (s, 1H, H_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.4, −5.5, 18.3, 20.3, 20.6, 25.7, 54.3, 62.3, 67.4, 69.8, 70.3, 71.6, 73.8, 74.2, 83.1, 88.0, 99.1, 124.6, 125.7, 126.2, 128.5, 129.1, 136.9, 146.8, 148.1, 155.5, 169.1, 169.4. Anal. ($C_{32}H_{41}BrN_4O_{10}Si$) C, H, N. **10b**: mp 80−81 °C. **[α]**$^{25}_D$ −51.9 (*c* 0.9, CH_3OH), UV λ_{max}^{MeOH} 210.7 (4.16), 256.8(4.17); MALDI-TOF (*m*/*z*): 772 [(M + Na)$^+$], 788 [(M + K)$^+$]. ^{1}H NMR (500 MHz, $CDCl_3$) δ −0.04, −0.03, (each s, each 3H, $(CH_3)_2Si$), 0.83 (s, 9H, $(CH_3)_3C$), 2.06, 2.14 (each s, each 3H, 2 × AcO), 3.84 (dd, 1H, *J* = 4.5, 11.0 Hz, $H_{5'a}$), 3.95 (dd, 1H, *J* = 5.0 Hz, 11 Hz, $H_{5'b}$), 4.02 (m, 1H, $H_{4''}$), 4.15 (dd, *J* = 1.5, 13 Hz, $H_{1''a}$), 4.23 (dd, *J* = 5.0, 10 Hz, $H_{4'}$), 4.38 (m, 1H, $H_{1b''}$), 4.43 (m, 1H, $H_{5''a}$), 4.49 (m, 1H, $H_{5''b}$), 4.96 (dd, 1H, *J* = 2.0, 5.0 Hz, $H_{3''}$), 5.69 (, dt, *J* = 4.0, 9.0, 9.0 Hz, $H_{2''}$), 5.86 (t, 1H, *J* = 5.5 Hz, $H_{3'}$), 6.10 (d, 1H, *J* = 5.5 Hz, $H_{1'}$), 6.50 (t, *J* = 5.5 Hz, $H_{2'}$), 7.30 (m, 3H, Ar H), 7.45 (m, 2H, Ar H), 8.48 (s, 1H, H_2). ^{13}C NMR (75 MHz, DMSO) δ 11.26, 20.41, 20.61, 25.75, 62.42, 67.86, 69.09, 70.58, 71.54, 72.72, 73.86, 83.02, 88.31, 99.01, 126.32, 128.09, 128.86, 130.32, 130.42, 137.63, 151.92, 152.90, 158.64, 169.56, 169.37. Anal. ($C_{32}H_{41}BrN_4O_{10}Si$) C, H, N.

8-Bromo-*N*^{1}-(1″,4″-anhydro-2″-deoxy-3″,5″-*O*-benzylidene-L-lyxitol-2″-yl)- 2′,3′-di-*O*-acetyl-inosine (11). A mixture of **10a** (143 mg, 0.19 mmol) and TBAF (1 M in THF, 0.38 mL, 0.38 mmol) in THF (2 mL) was stirred for 6 h at room temperature under neutral conditions. The resulting mixture was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to give **11** (109 mg, 90%): mp 126−128 °C. **[α]**$^{25}_D$ −8.3 (*c* 0.46, CH_3OH); UV λ_{max}^{MeOH} 203.6 (4.41), 233.1 (4.06); MALDI-TOF (*m*/*z*): 635 [(M + 1)$^+$], 657[(M + Na)$^+$], 673 [(M + K)$^+$]. ^{1}H NMR (300 MHz, DMSO) δ 2.04, 2.09 (each s, each 3H, 2 × AcO), 3.57−3.74 (m, 2H, 2 × $H_{5''}$), 3.94 (m, 1H, $H_{4''}$), 4.07−4.24 (m, 4H, $H_{1''a}$, 2 × $H_{5'}$, $H_{4'}$), 4.45 (m, 1H, $H_{1''b}$), 4.68 (m, 1H, $H_{3''}$), 5.13 (t, *J* = 5.7 Hz, exchangeable, 5′-OH), 5.60 (s, 1H, PhCH<), 5.62 (t, 1H, *J* = 5.7 Hz, $H_{3'}$), 5.74-(m, 1H, $H_{2''}$), 6.02 (d, 1H, *J* = 5.7 Hz, $H_{1'}$), 6.09 (t, 1H, *J* = 5.7 Hz, $H_{2'}$), 7.29 (m, 5H, C_6H_5), 8.43(s, 1H, H_2). ^{13}C NMR (75 MHz, DMSO) δ 20.23, 20.44, 55.46, 60.83, 66.76, 69.24, 70.10, 71.50, 73.10, 73.50, 83.06, 88.04, 98.07, 123.71, 126.00, 128.15, 128.82, 137.91, 147.73, 148.00, 154.80, 169.41, 169.55. Anal. ($C_{26}H_{27}BrN_4O_{10}$) C, H, N.

8-Bromo-*N*^{1}-(1″,4″-anhydro-2″-deoxy-3″,5″-*O*-benzylidene-L-lyxitol-2″-yl)-5′-*O*-(dianilinophosphoryl)-2′,3′-di-*O*-acetyl-inosine (12). To solution of **11** (66 mg, 0.104 mmol) and tetrazole (36 mg, 0.52 mmol) in anhydrous pyridine (2 mL) was added $(PhNH)_2POCl$ (139 mg, 0.52 mmol) at room temperature, and the mixture was stirred for 48 h. The solvent was evaporated to dryness, and the residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to afford **12** as a white solid (80 mg, 89%). The crystal needles were obtained from 95% ethanol: mp 146−148°. **[α]**$^{25}_D$ −47.2 (*c* 0.33, CH_3OH); UV λ_{max}^{MeOH} 235.6 (4.00); MALDI-TOF (*m*/*z*): 887 [(M + Na)$^+$], 903 [(M + K)$^+$]. ^{1}H NMR (500 MHz, DMSO), δ 2.08, 2.09 (each s, each 3H, 2 × AcO), 3.88 (m, 1H, $H_{4''}$), 4.09 (t, *J* = 10.5 Hz, 1H, $H_{1''}$), 4.17−4.26 (m, 4H, $H_{1''}$, $H_{5'}$, 2$H_{5''}$), 4.33 (m, 1H, $H_{5'}$), 4.44 (dt, $J_{4',5'a}$ = 6.5 Hz, $J_{4',5'b}$ = 3.5 Hz, $J_{4',3'}$ = 6.5 Hz, 1H, $H_{4'}$), 4.63 (dd, $J_{3'',4''}$ = 5 Hz, $J_{3'',2''}$ = 2.0 Hz, 1H, $H_{3''}$), 5.58 (s, 1H, PhCH), 5.72 (m, 1H, $H_{2''}$), 5.80 (t, *J* = 6.5 Hz, 1H, $H_{3'}$), 6.03 (dd, $J_{1',2'}$ = 4.0 Hz, 1H, $H_{1'}$), 6.05 (m, 1H, $H_{2'}$), 7.33−6.7 (m, 15H, Ar H), 8.06 (d, $J_{P,H}$ = 3.0 Hz, 1H, NH) 8.04 (d, $J_{P,H}$ = 3.5 Hz, 1H, NH), 8.22 (s, 1H, H_2). Anal.($C_{38}H_{38}BrN_6O_{11}P$) C, H, N.

8-Bromo-*N*^{1}-(1″,4″-anhydro-2″-deoxy-L-lyxitol-2″-yl)-5′-*O*-dianilinophosphoryl-2′,3′-di-*O*-acetyl-inosine (13). The solution of **12** (160 mg, 0.185 mmol) in 80% HOAc (2 mL) was stirred for 4 h at 70 °C, and then the mixture was evaporated in vacuo. The resulting residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to afford **13** (106 mg, 73.7%) as a solid: mp 148−150 °C. UV λ_{max}^{MeOH} 202.5 (4.28), 251.2 (4.27); MALDI-TOF (*m*/*z*): 799 [(M + Na)$^+$], 815 [(M + K)$^+$]. ^{1}H NMR (300 MHz, DMSO) δ 2.07(s, 6H, 2 × AcO), 3.56−3.67 (m, 2H, 2 × $H_{5'}$), 3.81−3.97 (m, 2H, 2 × $H_{5''}$), 4.18 (m, 1H, $H_{4''}$), 4.22 (m, 1H, $H_{3''}$), 4.28−4.41 (m, 3H, $H_{4'}$, 2 × $H_{1''}$), 4.60 (t, 1H, *J* = 5.4 Hz, $H_{3'}$), 5.32 (m, 1H, *J* = 5.4 Hz, $H_{2''}$), 5.42 (m, 1H, 3″-OH, exchangeable), 5.72 (m, 1H, exchangeable, 5″-OH), 6.01−6.02 (m, 2H, $H_{1'}$, $H_{2'}$), 6.74−7.04 (m, 10H, Ar H), 8.02, 8.03 (each d, each 1H, $J_{P,H}$ = 3.3 Hz, exchangeable, NH × 2), 8.01 (s, 1H, H_2). ^{13}C NMR (75 MHz, DMSO) δ 20.6, 55.4, 59.9, 63.7, 69.1, 69.7, 72., 80.3, 80.4, 83.9, 88.7, 114.2, 114.3, 117.2, 117.5, 117.6, 120.6, 120.7, 124.1, 125.6, 128.9, 147.8, 148.0, 169.7, 169.9. Anal. ($C_{31}H_{34}BrN_6O_{11}P$) C, H, N.

8-Bromo-*N*^{1}-(5″-*O*-MMTr-1″,4″-anhydro-3″-*O*-acetyl-2″-deoxy-L-lyxitol-2″-yl)-5′-*O*-dianilinophosphoryl-2′,3′-di-*O*-acetyl-inosine (14). The mixture of **13** (413 mg, 0.53 mmol) and MMTrCl (245 mg, 0.795 mmol) in anhydrous pyridine (5 mL) was stirred for 48 h at room temperature, and then acetic anhydride (270 mg, 2.65 mmol) was added to the resulting mixture. The mixture was stirred overnight at room temperature, and the solvent was concentrated in vacuo. CH_2Cl_2 (30 mL) and water (15 mL) were added, and the resulting mixture was partitioned. The organic layer was separated and washed in sequence with 1 M H_2SO_4 (10 mL), saturated $NaHCO_3$ solution (10 mL), and brine (10 mL) and dried ($MgSO_4$). The solvent was concentrated and the residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to give **14** (406 mg, 70%) as a white solid: mp 145−147 °C. **[α]**$^{25}_D$ −4.3 (*c* 0.58, CH_3OH); UV λ_{max}^{MeOH} 205.6 (4.7), 232.8 (4.53); MALDI-TOF (*m*/*z*): 1113 [(M + Na)$^+$], 1129 [(M + K)$^+$]. ^{1}H NMR (300 MHz, DMSO) δ 1.50, 2.03, 2.10 (each s, each 3H, 3 × AcO), 3.00 (m, 1H, $H_{5a'}$), 3.26 (m, 1H, $H_{5'b}$), 3.74 (s, 3H, CH_3O), 4.11−4.44 (m, 6H, 2 × $H_{5''}$, 2 × $H_{1''}$, $H_{4'}$, $H_{4''}$), 5.34 (m, 1H, $H_{3''}$), 5.73 (m, 1H, $H_{3'}$), 5.76 (m, 1H, $H_{2''}$), 5.98 (d, 1H, *J* = 5.4 Hz, $H_{1'}$), 6.12 (m, 1H, $H_{2'}$), 6.7−7.35 (m, 24H, Ar H), 7.84 (s, 1H, H_2), 8.05, 8.06 (each d, each 1H, 2H, $J_{P,H}$ = 5.4 Hz, exchangeable, NH × 2). ^{13}C NMR (75 MHz, DMSO) δ 19.52, 20.17, 20.27, 53.59, 55.06, 60.63, 63.36, 69.03, 69.65, 71.14, 79.41, 80.35, 85.82, 88.07, 113.25, 117.24, 120.37, 123.46, 125.92, 126.99, 127.89, 128.53, 128.58, 129.94, 134.69, 140.69, 140.91, 140.97, 143.83, 144.05, 146.69, 147.29, 154.14, 158.27, 168.40, 169.30, 169.38. Anal. ($C_{53}H_{52}BrN_6O_{13}P$) C, H, N.

8-Bromo-*N*^{1}-(1″,4″-anhydro-3″-*O*-acetyl-2″-deoxy-L-lyxitol-2″-yl)-5′-*O*-dianilinophosphoryl-2′,3′-di-*O*-acetyl-inosine (15). The solution of **14** (380 mg, 0.35 mmol) in 5% (w/v) TCA (6 mL) was stirred for 30 min and then poured into cool saturated $NaHCO_3$ solution (5 mL). The organic phase was separated and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to give compound **15** (190 mg, 66.8%): mp 137−139 °C. **[α]**$^{25}_D$ −5.6 (*c* 0.36, CH_3OH), UV λ_{max}^{MeOH} 203.1 (4.29), 232.4 (4.01) MALDI-TOF (*m*/*z*): 841 [(M + Na)$^+$], 857 [(M + K)$^+$]. ^{1}H NMR (300 MHz, DMSO) δ 2.01, 2.03, 2.08 (each s, each 3H, 3 × AcO), 3.92−4.01 (m, 2H, 2 × $H_{5'}$), 4.07−4.43 (m, 7H, 2 × $H_{5''}$, 2 × $H_{1''}$, $H_{4'}$, $H_{4''}$), 5.43 (m, 1H, $H_{3'}$), 5.56 (m, 1H, $H_{2''}$), 5.76 (m, 1H, exchangeable, 5″-OH), 6.02 (m, 2H, $H_{1'}$, $H_{2'}$), 6.74−7.05 (m, 10H, Ar H), 8.02−8.06 (m, 3H, 2 × NH, exchangeable, H_2). ^{13}C NMR (75 MHz, DMSO) δ 20.62, 21.08, 55.14, 55.39, 63.39, 63.65, 69.30, 69.46, 69.63, 72.39, 80.27, 80.40, 88.82, 117.60, 117.66, 121.05, 124.19, 125.95, 129.05, 140.94, 141.00, 147.92, 155.28, 170.05, 170.25, 171.10. Anal. ($C_{33}H_{36}BrN_6O_{12}P$) C, H, N.

8-Bromo-*N*^{1}-(1″,4″-anhydro-5″-*O*-[bis-(phenylthio)phosphoryl]-3″-*O*-acetyl-2″-deoxy-L-lyxitol-2″-yl)-5′-*O*-dianilinophosphoryl-2′,3′-di-*O*-acetyl-inosine (16). To a solution of **15** (150 mg, 0.183 mmol), PSS (244 mg, 0.64 mmol), and tetrazole (44 mg, 0.64 mmol) in anhydrous pyridine (2 mL) was added TPSC (110 mg, 0.37 mmol). The resulting mixture was stirred for 72 h, and then the solvent was evaporated. The residue was partitioned between CH_2Cl_2 (20 mL) and water (10 mL), and the organic phase was washed with aqueous saturated $NaHCO_3$ (10 mL) and brine (10 mL), dried (Na_2SO_4),

and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to give compound **16** (188 mg, 95%): mp 111−113 °C. **[α]**$^{25}_D$ −57.1 (*c* 0.80, CH_3OH), UV λ_{max}^{MeOH} 203.8 (4.54), 232.4 (4.54); MALDI-TOF (*m*/*z*): 1105 [(M + Na)$^+$], 1121 [(M + K)$^+$]. ^{1}H NMR (300 MHz, DMSO) δ 1.95, 2.01, 2.08 (each s, each 3H, 3 × AcO), 3.96−4.09 (m, 3H, 2 × $H_{5'}$, $H_{4'}$), 4.20−4.24 (m, 2H, 2 × $H_{5''}$), 4.30−4.37 (m, 2H, 2 × $H_{1''}$), 4.45 (m, 1H, $H_{4''}$), 5.38 (m, 1H, $H_{3''}$), 5.71−5.76 (m, 2H, $H_{3'}$, $H_{2''}$), 6.02 (d, 1H, *J* = 4.5 Hz, $H_{1'}$), 6.09 (m, 1H, $H_{2'}$), 6.72−7.42 (m, 20H, Ar H), 7.88 (s, 1H, H_2), 8.05 (m, 2H, exchangeable, 2 × NH). ^{13}C NMR (75 MHz, DMSO) *δ*: 20.02, 20.22, 20.58, 53.96, 61.81, 63.55, 68.31, 69.45, 71.63, 76.59, 78.64, 80.29, 80.40, 88.00,117.18, 117.26, 117.34, 120.37, 124.18, 124.96, 125.62, 128.51, 128.59, 129.52, 129.62, 129.87, 135.07, 135.15, 135.23, 140.88, 140.97, 146.78, 147.29, 154.54, 169.22, 169.28, 169.87. ^{31}P NMR (121 MHz, DMSO) δ 3.93(s), 51.26(s). Anal. ($C_{45}H_{45}BrN_6O_{13}P_2S_2$) C, H, N.

5-*O*-Benzoyl-3-*O*-allyl-1,4-anhydro-D-xylitol (20). To a solution of **19** (6.4 g, 19.2 mmol) in 75 mL of anhydrous CH_2Cl_2 were added 28.4 mL of Et_3SiH (192 mmol) and 22.6 mL of BF_3·Et_2O solution at −5 °C under nitrogen atmosphere. After the mixture was stirred at room temperature overnight, 100 mL saturated $NaHCO_3$ solution was slowly added to the mixture to destroy the excess of BF_3−Et_2O at 0 °C. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (70 mL × 2). Combined organic phase was dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified using silica gel column chromatography (petroleum ether−EtOAc) to yield white solid **20** (4.8 g, 90%). FAB-MS (*m*/*z*): 278 [M + 1]. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.57 (dd, 1H, *J* = 1.5 Hz, 12 Hz, H_{1b}), 3.88 (m, 1H H_2), 4.00 (m, 2H, 2 × H_5), 4.18 (m, 1H, H_3), 4.31 (m, 2H, OCH_a, H_4), 4.38 (m, 1H, H_{1a}), 4.45 (dd, 1H, *J* = 4.2, 10.8, OCH_b), 5.12−5.28 (m, 2H, CH_2═), 5.29 (d, 1H, *J* = 2.4 Hz, 2-OH exchangeable), 5.89 (m, 1H, ═CH), 7.55 (m, 2H, Ar H), 7.67 (m, 1H, Ar H), 7.97 (m, 2H, Ar H). ^{13}C NMR (75 MHz, DMSO): δ 63.5, 70.05, 73.3, 77.26, 84.13, 116.54, 128.78, 129.17, 129.63, 133.39, 134.96, 165.63. Anal. ($C_{15}H_{15}O_5$) C, H, N.

5-*O*-MMTr-3-*O*-allyl-1,4-anhydro-D-xylitol (21). Compound **20** (4.8 g, 17.2 mmol) was dissolved in a mixture of methanol (50 mL) and sodium methoxide(1.39 g, 25.8 mmol) and stirred overnight. The mixture was neutralized with HOAc and concentrated to dryness in vacuo. The resulting residue was coevaporated with dry pyridine (3.5 mL) and used in the next reaction without further purification. To a solution of crude compound in anhydrous pyridine (20 mL) was added MMTrCl (8.0 g, 25.8 mmol) under nitrogen atmosphere. The mixture was stirred for 48 h and evaporated to dryness. The residue was applied to a silica gel column and eluted (petroleum ether−EtOAc) to yield **21** (6.05 g, 78.8%). FAB-MS(*m*/*z*): 446 [M +]. ^{1}H NMR (300 MHz, DMSO):δ 3.09(m, 2H, 2 × H_5), 3.49 (m, 1H, H_{1a}), 3.74 (s, 3H, CH_3O), 3.86 (m, 4H, H_{1b}, H_2, H_3, OCH_a), 4.14 (m, 2H, H_4, OCH_b), 5.06 (m, 2H, CH_2═), 5.20 (m, 1H, exchangeable, 2-OH), 5.70 (m, 1H, −CH═), 6.89−7.39 (m, 14H, Ar H). Anal. ($C_{28}H_{30}O_5$) C, H, N.

8-Bromo-*N*¹-(5″-*O*-MMTr-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl)-5′-*O*-TBDMS-2′,3′-di-*O*-acetyl-inosine (23a) and 8-Bromo-*O*⁶-(5″-*O*-MMTr-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl)-5′-*O*-TBDMS-2′,3′-di-*O*-acetyl-inosine (23b). Compound **23a** (solid, 426 mg, 34%) and the corresponding O^6-regioisomer **23b** (syrup, 85 mg, 6.8%) were obtained from 700 mg of **9** (1.29 mmol) as described for the preparation of **10**, with **22** instead of **8**. **23a**: mp 88−91 °C. **[α]**$^{25}_D$ −1.15 (*c* 0.35, CH_3OH); UV λ_{max}^{MeOH} 206.9 (4.66), 232.8 (4.53); MALDI-TOF (*m*/*z*): 995 [(M + Na)$^+$], 1011 [(M + K)$^+$]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.05, 0.06 (each 3H, each 3H, $(CH_3)_2Si$), 0.90 (s, 9H, $(CH_3)_3C$), 2.09, 2.15, (each 3H, each s, 2 × AcO), 3.65,3.77 (m, 2H, 2 × $H_{5'}$), 3.81 (s, 3H, CH_3O), 3.82−4.08 (m, 6H,2 × $H_{1''}$, OCH_2, $H_{4'}$, $H_{5''a}$), 4.17 (dd, 1H, *J* = 1.8 Hz, 6.6 Hz, $H_{5''b}$), 4.23 (m, 1H, $H_{4''}$), 4.42 (dd, 1H, *J* = 3.3 Hz, 3.9 Hz, $H_{3''}$), 5.05 (m, 2H, CH_2═), 5.53 (m, 1H, CH═), 5.72 (t, 1H, *J* = 3.3 Hz, $H_{3'}$), 5.88 (m, 1H, $H_{2''}$), 6.06 (d, 1H, *J* = 3.3 Hz, $H_{1'}$), 6.29 (t, 1H, $H_{2'}$, *J* = 3.3 Hz), 7.01−7.48 (m, 14H, Ar H), 8.27 (s, 1H, H_2). ^{13}C NMR (125 MHz, DMSO) δ −5.48, −5.4, 18.31, 20.4, 20.56, 25.81, 53.86, 55.22, 61.13, 62.84, 70.57, 71.51, 71.77, 77.66, 78.57, 82.18, 83.02, 88.28, 113.17, 118.6, 124.4, 126.15, 126.93, 127.14, 127.81, 127.86, 128.36, 129.18, 130.24, 132.52, 139.18, 147.06, 147.15, 148.11, 155.41, 158.64, 169.31, 169.54. Anal. ($C_{48}H_{57}BrN_4O_{11}Si$) C, H, N. **23b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: −0.02, −0.03 (each s, each 3H, $(CH_3)_2$-Si), 0.833 (s, 9H, 3 × CH_3), 2.04, 2.12 (each s, 2 × AcO), 3.52 (m, 2H, 2 × $H_{5'}$), 3.75 (s, 3H, OCH_3), 3.8−4.0 (m, 7H, OCH_2, $2H_{1''}$, $H_{5''a}$, $H_{4'}$, $H_{4''}$), 4.20 (m, 2H, $H_{3''}$, $H_{5''b}$), 4.56 (m, 1H, $H_{3'}$), 5.10 (m, 2H, CH_2═), 5.69 (m, 1H, CH═), 5.84 (m, 1H, $H_{2''}$), 6.10 (d, 1H, *J* = 4.8 Hz, $H_{1'}$), 6.50 (m, 1H, $H_{2'}$), 6.80−7.45 (m, 14H, Ar H), 8.45(s, 1H, H_2). HRMS (ESI, positive) for $C_{48}H_{57}BrN_4O_{11}SiNa$: Calcd, 995.2868 [(M + Na)$^+$]; Found, 955.2855.

8-Bromo-*N*¹-(5″-*O*-MMTr-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl)-2′,3′-di-*O*-acetyl-inosine (24). Starting from **23a** (430 mg, 0.44 mmol) with the same procedure shown in the preparation of **11**, **24** (310 mg) was obtained as a white solid (86%): mp 75−77 °C. **[α]**$^{25}_D$ +0.033 (*c* 3.15, CH_3OH); UV λ_{max}^{MeOH} 204.2 (4.45); MALDI-TOF (*m*/*z*): 881 [(M + Na)$^+$], 897 [(M + K)$^+$]. ^{1}H NMR (500 MHz, DMSO) δ 2.04, 2.12 (each s, each 3H, 2 × AcO), 3.08 (dd, 1H, *J* = 5.5, 9.5 Hz, $H_{5'a}$), 3.31 (m, 1H, $H_{5'b}$), 3.39 (m, 1H, OCH_a), 3.38−3.64 (m, 2H, OCH_b, $H_{5''a}$), 3.69 (m, 1H, $H_{5''b}$), 3.98 (s, 1H, CH_3O), 4.02 (m, 1H, $H_{1''a}$), 4.17−4.20 (m, 3H, $H_{4''}$, $H_{4'}$, $H_{3''}$), 4.25 (m, 1H, $H_{1''b}$), 4.78 (m, 2H, CH_2═), 5.07 (t, *J* = 5.5 Hz, exchangeable, 5′-OH), 5.26 (m, 1H, CH═), 5.56 (t, *J* = 5.5, $H_{3'}$), 5.66 (m, 1H, $H_{2''}$), 6.02 (d, *J* = 5.5 Hz, $H_{1'}$), 6.09 (t, 1H, *J* = 5.5 Hz, $H_{2'}$), 6.09−6.92 (m, 2H, Ar H), 7.26−7.43 (m, 12H, Ar H), 8.093 (s, 1H, H_2). ^{13}C NMR (125 MHz, DMSO) δ 20.22, 20.38, 54.22, 55.05, 59.77, 60.90, 61.61, 69.52, 70.13, 71.41, 71.90, 77.54, 80.89, 83.07, 85.89, 87.90, 113.23, 116.88, 123.60, 125.85, 126.92, 127.94, 129.97, 133.41, 134.95, 144.04, 144.36, 147.17, 147.63, 154.72, 158.22, 162.31, 169.43, 169.49. Anal. ($C_{42}H_{43}BrN_4O_{11}$) C, H, N.

8-Bromo-*N*¹-(5″-*O*-MMTr-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl)-5′-*O*-(dianilinophosphoryl)-2′,3′-di-*O*-acetyl-inosine (25). Starting from **24** (150 mg, 0.175 mmol) with the same procedure shown in the preparation of **12**, 180 mg of **25** was obtained as a white solid (95%): mp 218−220 °C. **[α]**$^{25}_D$ +4.26 (*c* 0.63, CH_3OH); UV λ_{max}^{MeOH} 204.6 (5.03), 235.9 (5.23); MALDI-TOF (*m*/*z*): 1112 [(M + Na + 1)$^+$], 1128 [(M + K + 1)$^+$]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.05, 2.07 (each s, each 3H, 2 × AcO), 3.07 (dd, 1H, *J* = 3.3, 5.4 Hz, $H_{5'a}$), 3.32 (m, 2H, $H_{5'b}$, CH_aO), 3.51 (dd, 1H, *J* = 3.3 Hz, 12.5 Hz, CH_bO), 3.72 (s, 3H, CH_3O), 4.04−4.12 (m, 2H, $H_{1''a}$, $H_{3''}$), 4.21−4.28 (m, 3H, $H_{5''a}$, $H_{4'}$, $H_{4''}$), 4.32 (m, 1H, $H_{5''b}$), 4.41 (dd, 1H, *J* = 6.5, 10.5 Hz, $H_{1''b}$), 4.72 (m, 2H, CH_2═), 5.20 (m, 1H, >CH═), 5.64 (m, 2H, $H_{2''}$, $H_{3'}$), 5.97 (d, 1H, *J* = 4.0, 6.0 Hz, $H_{2'}$), 6.02 (d, *J* = 4.0 Hz, $H_{1'}$), 6.77−7.43 (m, 24H, Ar H), 8.03 (s, 1H, H_2), 8.11 (t, 2H, $J_{P,H}$ = 10 Hz, 2 × NH, exchangeable).

8-Bromo-*N*¹-(1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl)-5′-*O*-(dianilinophosphoryl)-2′,3′-di-*O*-acetyl-inosine (26). Starting from **25** (210 mg, 0.193 mmol) with the same procedure shown in the preparation of **15**, 125 mg of **26** was obtained as a white solid (79.6%): mp 116−118 °C. **[α]**$^{25}_D$ +20.8 (*c* 0.59, CH_3OH); UV λ_{max}^{MeOH} 204.3 (4.33), 231.6 (4.36); MALDI-TOF (*m*/*z*): 839 [(M + Na)$^+$], 855 [(M + K)$^+$]. ^{1}H NMR (500 MHz, DMSO) δ 2.07, 2.07 (each s, each 3H, 2 × AcO), 3.41 (dd, 1H, *J* = 5.5, 13 Hz, $H_{1''a}$), 3.60−3.65 (m, 2H, 2 × $H_{5'}$), 3.76 (dd, 1H, *J* = 5.0, 13 Hz, $H_{1''b}$), 3.88 (dd, 1H, *J* = 5.0, 11 Hz, $H_{4'}$), 3.93 (dd, 1H, *J* = 8.0, 10 Hz, CH_aO), 4.08 (m, 1H, $H_{5a''}$), 4.17 (dd, 1H, *J* = 4.5, 10 Hz, $H_{5b''}$), 4.2 (dd, 1H, *J* = 5.0, 6.0 Hz, $H_{3''}$), 4.35 (m, 1H, CH_bO), 4.42 (m, 1H, $H_{4''}$), 4.77 (bs, 1H, exchangeable, 5″-OH), 5.41 (m, 1H, CH═), 5.66 (m, 2H, $H_{2''}$, $H_{3'}$), 6.02 (m, 2H, $H_{1'}$, $H_{2'}$), 6.78−7.15 (m, 10H, Ar H), 8.13 (s, 1H, H_2), 8.12 (t, 2H, $J_{P,H}$ = 10 Hz, exchangeable NH × 2). Anal. ($C_{34}H_{38}BrN_6O_{11}P$) C, H, N.

8-Bromo-*N*¹-[5″-*O*-[bis-(phenylthio)phosphoryl]-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl]-5′-*O*-(dianilinophosphoryl)-2′,3′-di-*O*-acetyl-inosine (27). Starting from **26** (200 mg, 0.29 mmol) with the same procedure shown in the preparation of **16**, 255 mg of **27** was obtained as a white solid (96.3%): mp 88−90 °C. **[α]**$^{25}_D$ +22.3 (*c* 0.45, CH_3OH); UV λ_{max}^{MeOH} 207.1 (4.86), 229.4 (4.69); MALDI-TOF (*m*/*z*): 1103

[(M + Na)$^+$], 1116 [(M + K)$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.04, 2.04 (each s, each 3H, 2 × AcO), 3.29 (dd, 1H, J = 4.8, 12.6 Hz, H$_{1''a}$), 3.67 (dd, 1H, J = 5.1, 12.6 Hz, H$_{1''b}$), 3.95−4.13 (m, 4H, OCH$_2$, 2 × H$_{5''}$), 4.22−4.45 (m, 5H, 2 × H$_{5'}$, H$_{4'}$, H$_{4''}$, H$_{3''}$), 4.8 (m, 2H, CH$_2$═), 5.34 (m, 1H, CH═), 5.63−5.67 (m, 2H, H$_{2''}$, H$_{3'}$), 5.98−6.01 (m, 2H, H$_{1'}$, H$_{2'}$), 6.81−7.55 (m, 20H, Ar H), 8.11 (s, 1H, H$_2$), 8.11−8.14 (m, 2H, exchangeable, NH × 2). ^{13}C NMR (75 MHz, DMSO) δ 20.21, 54.05, 63.96, 65.96, 69.69, 69.92, 71.62, 71.82, 77.15, 80.03, 88.08, 117.02, 117.03, 120.43, 123.49, 125.48, 125.58, 125.67, 128.74, 129.75, 129.87, 133.12, 135.09, 135.14, 141.00, 147.70, 147.87, 154.71, 169.29, 169.42. ^{31}P NMR δ: 4.01 (s), 50.45 (s). Anal. ($C_{46}H_{47}BrN_6O_{12}P_2S_2$) C, H, N.

5-*O*-Benzoyl-3-*O*-allyl-1,4-anhydro-D-ribitol (31). Starting from **30** (4.9 g, 14.7 mmol) with the same procedure shown in the preparation of **20**, 3.4 g of **31** was obtained as pale yellow syrup (83%). FAB-MS (m/z): 279 [M + H]$^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.63 (dd, 1H, J = 2.7, 9.3 Hz, H$_{5a}$), 3.79 (dd, 1H, J = 4.8, 7.2 Hz, H$_4$), 3.92 (dd, 1H, J = 4.5, 9.3 Hz, H$_{5b}$), 3.40−4.07 (m, 2H, 2 × H$_1$), 4.13−4.30 (m, 3H, OCH$_a$, H$_2$, H$_3$), 4.43 (dd, 1H, J = 3.0 Hz, 8.7 Hz, OCH$_b$), 4.10 (d, 1H, J = 4.8 Hz, 2-OH, exchangeable), 5.09−5.29 (m, 2H, CH$_2$═), 5.88 (m, 1H, CH═), 7.49−7.97 (m, 5H, Ar H). ^{13}C NMR (75 MHz, DMSO) δ 64.94, 68.73, 70.26, 73.02, 77.47, 79.60, 116.61, 128.76, 129.17, 129.55, 133.40, 135.16, 165.57. Anal. ($C_{15}H_{18}O_5$) C, H, N.

8-Bromo-*N*^{1}-(5″-*O*-benzoyl-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl)-5′-*O*-TBDMS-2′,3′-di-*O*-acetyl-inosine (33a) and 8-bromo-*O*^{6}-(5″-*O*-benzoyl-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl)-5′-*O*-TBDMS-2′,3′-di-*O*-acetyl-inosine (33b). Starting from **31** (1.62 g, 5.8 mmol), with the same procedure shown in the preparation of **10**, **33a** (1.25 g, 26.8%) was obtained as white solid, accompanied by the O^6-regioisomer **33b** (1 g, 21.5%). **33a**: mp 106−107 °C. **[α]**$^{25}_D$ +19.7 (c 0.21, CH$_3$OH); UV λ_{max}^{MeOH} 209.7 (4.41), 250.7 (4.00); MALDI-TOF (m/z): 827 [(M + Na)$^+$], 843 [(M + K)$^+$]. ^{1}H NMR (500 MHz, CDCl$_3$) δ −0.01,0.01 (each s, each 3H, (CH$_3$)$_2$Si), 0.85 (s, 9H, (CH$_3$)$_3$C), 2.08, 2.15 (each s, each 3H, 2 × AcO), 3.75 (dd, 1H, J = 5.0, 11.5 Hz, H$_{5'a}$), 3.85 (dd, 1H, J = 5.0, 11.5 Hz, H$_{5'b}$), 3.96 (m, 1H, H$_{4'}$), 4.09 (dd, 1H, J = 6.5, 13 Hz, OCH$_a$), 4.18−4.23 (m, 1H, H$_{4''}$, H$_{3''}$), 4.23−4.35 (m, 2H, 2 × H$_{1''}$), 4.40 (dd, 1H, J = 5.0, 13 Hz, OCH$_b$), 4.52 (dd, 1H, J = 6.0, 12 Hz H$_{5''a}$), 4.56 (dd, 1H, J = 4.0, 12 Hz, H$_{5''b}$), 5.13−5.27 (m, 2H, CH$_2$═), 5.47 (m, 1H, H$_{2''}$), 5.68 (t, 1H, J = 5.5 Hz, H$_{3'}$), 6.82 (m, 1H, CH═), 6.05 (d, 1H, J = 5.5 Hz, H$_{1'}$), 6.27 (t, 1H, J = 5.5 Hz, H$_{2'}$), 7.39 (t, 2H, J = 9.0 Hz, Ar H), 7.54 (m, 1H, Ar H), 7.94 (m, 2H, Ar H), 8.33 (s, 1H, H$_2$). ^{13}C NMR (75 MHz, CDCl$_3$) δ −5.45, −3.70, 20.58, 20.77, 20.41, 25.62, 25.76, 59.58, 62.39, 63.97, 70.37, 71.60,71.66, 71.79, 82.88, 83.30, 87.00, 88.10, 118.37, 128.45, 129.42, 129.60, 133.23, 133.43, 133.49, 144.79, 145.27, 148.04, 154.07, 166.12, 169.50, 169.54. Anal. ($C_{35}H_{45}BrN_4O_{11}Si$) C, H, N. **33b**: mp 49 °C. **[α]**$^{25}_D$ −34.2 (c 0.87, CH$_3$OH); UV λ_{max}^{MeOH} 202.3 (4.37), 257.9 (4.10). ^{1}H NMR (300 MHz, CDCl$_3$) δ: −0.01,0.03 (each s, each 3H, (CH$_3$)$_2$-Si), 0.831 (s, 9H, (CH$_3$)$_3$C), 2.06, 2.13 (each s, each 3H, 2 × AcO), 3.85 (dd, 1H, J = 5.1, 11.1 Hz, H$_{5a'}$), 3.96 (dd, 1H, J = 4.8, 11.1 Hz, H$_{5b'}$), 4.13−4.27 (m, 7H, 2 × H$_{1''}$, H$_{4'}$, H$_{4''}$, H$_{3''}$, OCH$_2$), 4.50 (m, 2H, 2 × H$_{5''}$), 5.24 (m, 2H, CH$_2$═), 5.68 (m, 1H, H$_{2''}$), 5.81 (m, 1H, H$_{3'}$), 5.89 (m, 1H, CH═), 6.10 (d, 1H, J = 4.8 Hz, H$_{1'}$), 6.48 (dd, 1H, J = 4.8, 12 Hz, H$_{2'}$), 7.41 (m, 3H, Ar H), 8.03 (m, 2H, Ar H), 8.47 (s, 1H, H$_2$). ^{13}C NMR (75 MHz, CDCl$_3$) δ: −5.57, −5.59, 18.26, 20.39, 20.55, 25.73, 62.37, 64.35, 70.58, 71.26, 71.61, 72.01, 76.58, 77.00, 77.43, 81.19, 81.85, 82.99, 84.07,88.38, 117.77, 122.19, 128.32, 129.32, 129.74, 130.38, 132.95, 133.81, 151.83, 152.93, 158.36, 166.31, 169.49. Anal. $C_{35}H_{45}BrN_4O_{11}Si$) C, H, N.

8-Bromo-*N*^{1}-(5″-*O*-Benzoyl-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl) −2′,3′-di-*O*-acetyl-inosine (34). Starting from **33a** (1.16 g, 1.4 mmol) with the same procedure shown in the preparation of **11**, 0.9 g of **34** was obtained as a white solid (90%): mp 89−91 °C. **[α]**$^{25}_D$ +8.1 (c 1.22, CH$_3$OH); UV λ_{max}^{MeOH} 203.3 (4.34), 257.5 (4.02); MALDI-TOF (m/z): 713 [(M + Na)$^+$]. ^{1}H NMR (300 MHz, DMSO) δ 2.30, 2.12 (each s, each 3H, 2 × AcO), 3.51 (m, 1H, H$_{5'a}$), 3.66 (m, 1H, H$_{5'b}$), 4.15−4.20 (m, 4H, OCH$_2$, 2 × H$_{5''}$), 4.32−4.34 (m, 2H, H$_{4'}$, H$_{4''}$), 4.52−4.59 (m, 2H, 2 × H$_{1''}$), 5.04−5.18 (m, 2H, CH$_2$═), 5.21−5.23 (m, 2H, H$_{3'}$, H$_{3''}$), 5.55 (t, 1H, J = 5.7 Hz, 5′-OH, exchangeable), 5.84 (m, 1H, CH═), 6.02 (d, 1H, J = 5.4 Hz, H$_{1'}$), 6.08 (t, 1H, J = 5.4 Hz, H$_{2'}$), 7.48 (t, 2H, J = 4.5 Hz, Ar H), 7.64 (t, 2H, J = 7.5 Hz, Ar H), 7.91 (d, 1H, J = 7.2 Hz, Ar H), 8.39 (s, 1H, H$_2$). Anal. ($C_{29}H_{31}BrN_4O_{11}$) C, H, N.

8-Bromo-*N*^{1}-(5″-*O*-Benzoyl-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl)-5′-*O*-(dianilinophosphoryl)-2′,3′-di-*O*-acetyl-inosine (35). Starting from **34** (0.8 g, 1.16 mmol) with the same procedure shown in the preparation of **12**, 1 g of **35** was obtained as a white solid (93%): mp 110−112 °C. **[α]**$^{25}_D$ +17.8 (c 0.39, CH$_3$OH); UV λ_{max}^{MeOH} 203.3 (5.07), 229.9 (5.01); MALDI-TOF (m/z): 943 [(M + Na)$^+$], 959 [(M + K)$^+$]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.07, 2.08 (each s, each 3H, 2 × AcO), 3.97 (dd, 1H, J = 4.5,11.5 Hz, H$_{5'a}$), 4.10−4.18 (m, 4H, OCH$_2$, H$_{5'b}$, H$_{1''a}$), 4.25−4.31 (m, 2H, 2 × H$_{5''}$), 4.43 (m, 1H, H$_{4'}$), 4.48−4.58 (m, 2H, H$_{4''}$, H$_{3''}$), 4.50 (dd, 1H, J = 4.0, 12 Hz, H$_{1''b}$), 5.02−5.15 (m, 2H, CH$_2$═), 5.20 (m, 1H, H$_{2''}$), 5.64 (m, 1H, H$_{3'}$), 5.79 (m, 1H, CH═), 6.04−6.05 (m, 2H, H$_{1'}$, H$_{2'}$), 6.78 (m, 2H, Ar H), 6.97−7.11 (m, 8H, Ar H), 7.49 (m, 2H, Ar H), 7.64 (m, 1H, Ar H), 7.93 (m, 2H, Ar H), 8.07 (t, 2H, $J_{P,H}$ = 6.0 Hz, exchangeable, 2 × NH), 8.03 (s, 1H, H$_2$). Anal. ($C_{41}H_{42}BrN_6O_{12}P$) C, H, N.

8-Bromo-*N*^{1}-(1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl)-5′-*O*-(dianilinophosphoryl)-inosine (36). To solution of **35** (0.9 g, 0.98 mmol) in methanol (10 mL) was added CH$_3$ONa (190 mg, 3.5 mmol), and the reaction mixture was stirred overnight. The resulting mixture was evaporated under reduced pressure and purified by silica gel column chromatography (CH$_3$OH−CH$_2$Cl$_2$) to yield white solid **36** (676 mg, 95%): mp 130−132 °C. MALDI-TOF (m/z): 755 [(M + Na)$^+$]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.58 (m, 1H, H$_{5'a}$), 3.68 (m, 1H, H$_{5'b}$), 3.77 (dd, 1H, J = 4.0, 8.5 Hz, H$_{5''a}$), 3.99 (dd, 1H, J = 6.0, 13.5 Hz, OCH$_a$), 4.06−4.32 (m, 8H, OCH$_b$, 2 × H$_{1''}$, H$_{4'}$, H$_{4''}$, H$_{3''}$, H$_{3'}$, H$_{5''b}$), 4.97 (m, 1H, exchangeable, 3′-OH), 5.08−5.11 (m, 2H, H$_{2'}$, H$_{2''}$), 5.17−5.25 (m, 2H, CH$_2$═), 5.47 (d, J = 5.0 Hz, exchangeable, 2′-OH), 5.63 (m, exchangeable, 5″-OH), 5.82−5.87 (m, 2H, CH═, H$_{1'}$), 6.74−7.15 (m, 10H, Ar H), 8.04, 8.09 (each d, 2H, $J_{P,H}$ = 10 Hz, exchangeable, 2 × NH), 8.37 (s, 1H, H$_2$). Anal. ($C_{30}H_{34}BrN_6O_9P$) C, H, N.

8-Bromo-*N*^{1}-(5″-*O*-MMTr-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl)-5′-*O*-(dianilinophosphoryl)-2′,3′-di-*O*-acetyl-inosine (37). Starting from **36** (600 mg, 0.82 mmol) with the same procedure shown in the preparation of **14**, 520 mg of **37** was obtained as a white solid (58.3%). MALDI-TOF (m/z): 1111 [(M + Na)$^+$], 1127 [(M + K)$^+$]. ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.05, 2.08 (each s, each 3H, 2 × AcO), 3.58 (m, 1H, H$_{5'a}$), 3.75 (s, 3H, CH$_3$O), 3.82−4.00 (m, 3H, H$_{5'b}$, 2 × H$_{5''}$), 4.09−4.20 (m, 6H, OCH$_2$, 2 × H$_{1''}$, H$_{4'}$, H$_{4''}$), 4.57 (m, 1H, H$_{3''}$), 5.12−5.30 (m, 3H, CH$_2$═, H$_{2''}$), 5.73−5.84 (m, 2H, H$_{3'}$, CH═), 6.05 (d, 1H, J = 5.4 Hz, H$_{1'}$), 6.36 (t, 1H, J = 5.4 Hz, H$_{2'}$), 6.69−7.24 (m, 12H, Ar H), 8.54 (s, 1H, H$_2$). Anal. ($C_{54}H_{54}BrN_6O_{12}P$) C, H, N.

8-Bromo-*N*^{1}-(1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl)-5′-*O*-(dianilinophosphoryl)-2′,3′-di-*O*-acetyl-inosine (38). Starting from **37** (480 mg, 0.44 mmol) with the same procedure shown in the preparation of **15**, 180 mg of **38** was obtained as a white solid (50%) and 80 mg **37** was recovered: mp 119−121 °C. **[α]**$^{25}_D$ +13.0 (c 0.33 CH$_3$OH); MALDI-TOF (m/z): 839 [(M + Na)$^+$], 855 [(M + K)$^+$]. ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.07, 2.10 (each s, each 3H, 2 × AcO), 3.35 (bs, 1H, OH, exchangeable), 3.59 (dd, 1H, J = 2.4, 12.3 Hz, H$_{5'a}$), 3.81−3.87 (m, 3H, 2 × H$_{5''}$, H$_{5'b}$), 4.10−4.23 (m, 2H, OCH$_2$), 4.30−4.44 (m, 4H, 2 × H$_{1''}$, H$_{4'}$, H$_{4''}$), 4.63 (m, 1H, H$_{3''}$), 5.10−5.31 (m, 3H, CH$_2$═, H$_{2''}$), 5.72−5.88 (m, 2H, CH═, H$_{3'}$), 6.08 (d, 1H, J = 5.4, H$_{1'}$), 6.38 (t, 1H, J = 5.4 Hz, H$_{2'}$), 6.71−7.24 (m,12H, Ar H, 2 × NH), 8.55 (1, 1H, H$_2$). Anal. ($C_{34}H_{38}BrN_6O_{11}P$) C, H, N.

8-Bromo-*N*^{1}-[5″-*O*-[bis-(phenylthio)phosphoryl]-1″,4″-anhydro-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl]-5′-*O*-(dianilinophosphoryl)-2′,3′-di-*O*-acetyl-inosine (39). Starting from **38** (150 mg, 0.183 mmol) with the same procedure shown in the preparation of **16**, 190 mg of **39** was obtained as a white solid (96%). **[α]**$^{25}_D$ +45.6 (c 0.17, CH$_3$OH); UV λ_{max}^{MeOH} 236.1

(4.00); MALDI-TOF (*m*/*z*):1104 [(M + Na + 1)⁺], 1120 [(M + K + 1)⁺]. ¹H NMR (300 MHz, $CDCl_3$) *δ*: 2.03, 2.05 (each s, each 3H, 2 × AcO), 3.92 (m, 1H, $H_{5'a}$), 3.94−4.25 (m, 7H, $H_{5'b}$, 2 × $H_{5''}$, OCH_2, $H_{3''}$, $H_{4''}$), 4.42 (m, 3H, 2 × $H_{1''}$, $H_{4'}$), 5.03−5.11 (m, 2H, CH_2═), 5.16 (m, 1H, $H_{3'}$), 5.61 (m, 1H, $H_{2''}$), 5.81 (m, 1H, CH═), 6.02 (m, 2H, $H_{1'}$, $H_{2'}$), 6.74−7.56 (m, 20H, AromH), 8.06 (m, 2H, exchangeable, 2 × NH), 8.24 (s, 1H, H_2). ³¹P NMR (121 MHz, $CDCl_3$) *δ* 3.05 (s), 49.91(s). Anal. ($C_{46}H_{47}BrN_6O_{12}PS_2$) C, H, N.

8-Bromo-*N*¹-[(2-acetoxyethoxy)-methyl]-5′-*O*-TBDMS-2′,3′-*O*-isopropylidene-inosine (41). To the mixture of **40** (500 mg, 1 mmol) and DBU (0.76 g, 0.5 mmol) in CH_2Cl_2 (10 mL) was added $ClCH_2OCH_2CH_2OAc$ (1.52 g, 10 mmol) at 0 °C. After stirring for 30 min, the solvent was evaporated in vacuo and the residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to give compound **41** (605 mg, 82%). ¹H NMR (300 MHz, DMSO) *δ* −0.01, 0.00 (each s, each 3H, $(CH_3)_2Si$), 0.88 (s, 9H, $(CH_3)_3C$), 1.44, 1.63 (each s, each 3H, $(CH_3)_2C$), 2.09 (s, 3H, AcO), 3.60−3.85 (m, 4H, OCH_2, 2 × $H_{5'}$), 3.42−4.27 (m, 3H, $H_{4'}$, CH_2OAc), 5.09 (dd, 1H, *J* = 3.6, 6.3 Hz, $H_{3'}$), 5.57 (dd, 2H, *J* = 10.5,17.1, OCH_2N), 5.71 (m, 1H, $H_{2'}$), 6.15 (d, *J* = 1.8 Hz), 8.61 (s, 1H, H_2).

8-Bromo-*N*¹-[(2-acetoxyethoxy)-methyl]-2′,3′-*O*-isopropylidene-inosine (42). Starting from **41** (143 mg, 0.23 mmol) with the same procedure shown in the preparation of **11**, 119 mg of **42** was obtained as a white solid (90%). ¹H NMR (300 MHz, DMSO) *δ* 1.24, 1.33 (each s, each 3H, $(CH_3)_2C$), 1.98 (s, 3H, AcO), 3.76 (m, 2H, 2 × $H_{5'}$), 3.78 (m, 2H, OCH_2), 4.14 (m, 3H, CH_2OAc, $H_{4'}$), 4.96 (m, 2H, $H_{3'}$, OH, exchangeable), 5.01 (m, 2H, OCH_2N), 5.56 (dd, 1H, *J* = 2.1, 6.3 Hz, $H_{2'}$), 6.05 (d, 1H, 2.1 Hz), 8.55 (s, 1H, H_2).

8-Bromo-*N*¹-[(2-acetoxyethoxy)-methyl]-5′-*O*-dianilinophosphoryl-2′,3′-*O*-isopropylidene-inosine (43). Starting from **42** (66 mg, 0.135 mmol) with the same procedure shown in the preparation of **12**, 84 mg of **43** was obtained as a white solid (84.5%). UV λ_{max}^{MeOH} 203.8 (4.16), 256.8 (4.17) MALDI-TOF (*m*/*z*): 755 [(M + Na)⁺], 771 [(M + K)⁺]. ¹H NMR (300 MHz, DMSO) *δ* 1.20, 1.53 (each s, each 3H, CH_3 × 2), 1.96 (s, 3H, AcO), 3.74 (t, 2H, *J* = 4.5 Hz, 2 × $H_{5'}$), 4.10 (t, 2H, *J* = 4.5 Hz, OCH_2), 4.23 (m, 2H, OCH_2OAc), 4.37 (m, 1H, $H_{4'}$), 5.08 (dd, 1H, *J* = 3.6, 6.0 Hz, $H_{3'}$), 5.33 (m, 1H, $H_{2'}$), 5.48 (m, OCH_2N), 6.05 (d, 1H, *J* = 1.8 Hz, $H_{1'}$), 6.73−7.12 (m, 10H, Ar H), 8.02, 8.03 (each d, each1H, $J_{P,H}$ = 9.9 Hz, exchangeable, NH × 2), 8.32 (s, 1H, H_2). ¹³C NMR (75 MHz, DMSO) *δ* 20.62, 25.18, 26.96, 62.91, 64.15, 67.22, 75.06, 80.88, 82.87, 85.51, 90.58, 113.81, 117.21, 120.41, 124.29, 125.58, 128.62, 128.77, 140.93, 141.07, 147.94, 149.10, 154.70, 170.32. HRMS (ESI, positive) for $C_{30}H_{35}BrN_6O_9P$: Calcd, 733.1381 [(M + 1)⁺]; Found, 733.1383.

8-Chloro-*N*¹-[[[bis(phenylthio)phosphoryl]oxy-ethoxy]-methyl]-5′-*O*-(dianilinophosphoryl)-2′,3′-*O*-isopropylideneinosine (45). To the mixture of **43** (73 mg, 0.1 mmol) in 2.5 mL of methanol was added CH_3ONa (10 mg, 0.19 mmol), and the resulting solution was stirred overnight. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to give 50 mg of **44**, which was dissolved in anhydrous pyridine (3 mL) and then were added TPSCl (70 mg, 0.232 mmol), PSS (44 mg, 0.116 mmol), and tetrazole (8 mg, 0.116 mmol). The resulting mixture was stirred for 24 h at room temperature. After removal of the solvent, the residue was partitioned between CH_2Cl_2 (20 mL) and water (10 mL), and the organic phase was washed with aqueous saturated $NaHCO_3$ (10 mL) and brine (10 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to give compound **45**[18] (44 mg, 48.4%). [α]$^{25}_D$ +1.46 (*c* 0.27, CH_3OH), UV λ_{max}^{MeOH} 202.5 (4.63).

8-Bromo-*N*¹-[5″-*O*-(phenylthio)phosphoryl-2″-deoxy-3″-*O*-acetyl-L-lyxitol-2″-yl]-5′-*O*-phosphoryl-2′,3′-di-*O*-acetyl-inosine (46). A mixture of **16** (140 mg, 0.129 mmol) and isoamyl nitrite (261 μL, 1.94 mmol) in pyridine−AcOH−Ac_2O (2:1:1, v/v, 4 mL) was stirred at room temperature for 8 h. After the reaction mixture was evaporated (at <30 °C), the residue was dissolved in a mixture of H_3PO_2 solution in pyridine (254 mg, ca. 2.69 mmol), Et_3N (179 μL, 1.29 mmol), and pyridine (3 mL), and the resulting solution was stirred for 11 h at room temperature. After the solvent was evaporated to dryness under reduced pressure, the residue was partitioned between $CHCl_3$ and H_2O. The aqueous layer was evaporated (<30 °C) with pyridine (5 mL), and the residue was dissolved in TEAA (0.1 M, pH 7.0, 10 mL). The solution was purified by a C18 reverse phase column (1.8 × 15 cm), and the column was developed using a linear gradient of 0−40% CH_3CN in TEAA buffer (0.1 M, pH 7.0, 600 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C18 reverse phase column chromatography (1.8 × 13 cm, eluted was 20% aqueous CH_3CN) to give **46** (80 mg, yield 60.7%) as a triethylammonium salt. [α]$^{25}_D$ −41.6 (*c* 0.69, H_2O); UV $\lambda_{max}^{H_2O}$ 243.3 (4.30). ¹H NMR (500 MHz, D_2O) *δ* 2.01, 2.02, 2.09 (each s, each 3H, 3 × CH_3), 3.84 (m, 1H, $H_{5'a}$), 3.97 (m, 1H, $H_{5'b}$), 4.09 (m, 1H, $H_{4''}$), 4.21 (m, 2H, $H_{4'}$, $H_{1''a}$), 4.34−4.41 (m, 3H, $H_{1''b}$, 2 × $H_{5''}$), 5.26 (m, 1H, $H_{3''}$), 5.64−5.74 (m, 3H, $H_{2'}$, $H_{3'}$, $H_{2''}$), 6.11 (d, 1H, *J* = 3.0 Hz, $H_{1'}$), 6.96−7.05 (m, 5H, Ar H), 8.23 (s, 1H, H_2). ³¹P NMR (D_2O 121 MHz, decoupled with ¹H) *δ* 1.08 (s), 15.39 (s). HRMS (FAB, negative) for $C_{27}H_{30}N_4O_{16}P_2SBr$: Calcd, 839.0041 [(M − 1)⁻]; Found, 839.0049.

***N*¹-[5″-*O*-Phosphoryl-2″-deoxy-3″-*O*-acetyl-L-lyxitol-2″-yl]-8-bromo-5′-*O*-phosphoryl-2′,3′-di-*O*-acetyl-inosine 5′,5″-cyclicpyrophosphate (47).** A solution of **46** (32 mg, 31.3 μm) in pyridine (10 mL) was added slowly over 20 h, using a syringe pump, to a mixture of I_2 (170 mg, 646 μmol) and MS 3 Å (2 g), in pyridine (40 mL) at room temperature in the dark. The MS 3 Å was 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 TEAA buffer (5.0 mL), which was applied to C18 reverse phase column (1.5 × 11 cm). The column was developed using a linear gradient of 0−40% CH_3CN in TEAA buffer (0.1 M, pH 7.0, 300 mL). Appropriate fractions were evaporated under reduced pressure, and excess of TEAA was removed by C18 reversed phase column chromatography (1.5 × 11 cm, eluted with 20% aqueous CH_3CN) to afford **47** (8 mg, yield 28.1%) as a triethylammonium salt. ¹H NMR (500 MHz, D_2O) *δ* 2.02, 2.05, 2.11 (each s, each 3H, 3 × AcO), 3.89 (m, 2H, 2 × $H_{5'}$), 4.02 (t, 1H, *J* = 10 Hz, $H_{4''}$), 4.18 (m, 1H, $H_{5''a}$), 4.24 (t, 1H, *J* = 10 Hz, $H_{1''a}$), 4.38 (m, 1H, $H_{5''b}$), 4.46 (m, 1H, $H_{1''b}$), 4.76 (m, 1H, $H_{3''}$), 5.18 (m, 1H, $H_{3'}$), 6.25 (s, 1H, $H_{1'}$), 6.34 (m,1H, $H_{2''}$), 6.39 (d, 1H, 5.0 Hz, $H_{2'}$), 8.20 (s, 1H, H_2). ³¹P NMR (D_2O 121 MHz, decoupled with ¹H) *δ* −7.9, −10.1. HRMS (FAB, negative) for $C_{17}H_{20}N_4O_{14}P_2Br$ Calcd, 644.9640 [(M − 1)⁻]; Found, 644.9647 (removal of two acetyl group).

***N*¹-[5″-*O*-Phosphoryl-2″-deoxy-L-lyxitol-2″-yl]-8-bromo-5′-*O*-phosphoryl-inosine 5′, 5″-cyclicpyrophosphate (3).** To the solution of compound **47** (75 OD_{254}) in methanol (5 mL) were added CH_3ONa (5 mg) and the mixture was stirred for 3 h at room temperature. The solvent was evaporated in vacuo and the residue was dissolved in 0.1 M TEAA buffer (5.0 mL), which was applied to C18 reverse phase column (1.5 × 11 cm). The column was developed using a linear gradient of 10−40% CH_3CN in TEAA buffer (0.1 M, pH 7.0, 200 mL). Appropriate fractions were evaporated under reduced pressure, and excess of TEAA was removed by C18 reverse phase column chromatography (1.5 × 11 cm, eluted with 20% aqueous CH_3CN) to afford (48 OD_{254}, yield 48%) as a triethylammonium salt. [α]$^{25}_D$ −39.6 (*c* 0.114, H_2O); UV $\lambda_{max}^{H_2O}$ 205.5 (4.20), 250.5 (3.71). ¹H NMR (500 MHz, D_2O) *δ*: 4.00 (m, 2H, 2 × $H_{5'}$), 4.06 (m, 1H, $H_{4''}$), 4.16 (m, 1H, *J* = 5.0, 10 Hz, $H_{5''a}$), 4.24 (m, 1H, $H_{1''a}$), 4.32 (m, 1H, $H_{5''b}$), 4.45 (m, 1H, $H_{1''b}$), 4.55 (m, 2H, $H_{3''}$, $H_{4'}$), 4.86 (dd, 1H, *J* = 2.0, 5.0 Hz, $H_{3'}$), 5.19 (t, 1H, *J* = 5.0 Hz, $H_{2'}$), 5.76 (m, 1H, $H_{2''}$), 5.98 (d, 1H, *J* = 5.0 Hz, $H_{1'}$), 8.27 (s, 1H, H_2). HRMS (FAB, negative) for $C_{15}H_{18}N_4O_{13}P_2Br$: 602.9534 [(M − 1)⁻]; Found, 602.9536.

8-Chloro-*N*¹-[[[2-(phenylthio)phosphoryl]oxy-ethoxy]-methyl]-5′-*O*-phosphoryl-2′,3′-*O*-isopropylideneinosine (48). Starting from **45** (120 mg, 0.132 mmol) with the same procedure shown in the preparation of **46**, 75 mg of **48** was obtained as a triethylammonium salt (67%). [α]$^{25}_D$ +0.54 (*c*

0.18, H_2O); UV $\lambda_{max}^{H_2O}$ 246.0 (4.00). ^{1}H NMR (300 MHz, D_2O) δ 1.50,1.76 (each s, each 3H, $(CH_3)_2C$), 3.77−3.83 (m, 4H, $POCH_2$-, 2 × $H_{5'}$), 3.97 (m, 2H, OCH_2), 4.32 (m, 1H, $H_{4'}$), 5.11 (m, 1H, $H_{3'}$), 5.24 (d, 1H, J = 10.8 Hz, $-NCH_aO$), 5.38 (m, 1H, $H_{2'}$), 5.68 (d,1H, J = 10.8 Hz, $-NCH_bO$), 6.13 (d, 1H, $H_{1'}$), 7.08−7.25 (m, 5H, Ar H), 8.25 (s, 1H, H_2). ^{31}P NMR (D_2O 121 MHz, decoupled with ^{1}H) δ 18.68 (s), 2.80 (s). HRMS (FAB, nagative) for $C_{22}H_{27}N_4O_{12}P_2SCl$: Calcd, 667.0437 [(M − 1)$^-$]; Found 667.0433.

***N*^{1}-[(2-*O*-Phosphoryl-ethoxy)-methyl]-8-chloro-5′-*O*-phosphoryl-2′,3′-*O*-isopropylidene-inosine 5′,5″-cyclicpyrophosphate (49).** Starting from **41** (26 mg, 0.23 mmol) with the same procedure shown in the preparation of **47**, 12 mg of **49** was obtained as a triethylammonium salt (46.2%). [18]

***N*^{1}-[(2-*O*-Phosphoryl-ethoxy)-methyl]-8-chloro-5′-*O*-phosphoryl-inosine 5′, 5″-cyclicpyrophosphate (6).** The mixture of 49 (150 OD_{254}) in 60% HCOOH (3 mL) was stirred for 5 h and then evaporated under reduced pressure. The purification of the residue was performed at the same procedure as above by C18 reverse column to give the target molecule **6**. [18] (67 OD_{254}, 44.6%) **[**α**]**$^{25}_D$ +18.7(*c* 0.21, H_2O); UV $\lambda_{max}^{H_2O}$ 201.6 (4.05). ^{1}H NMR (500 MHz, D_2O) δ 3.69 (3H, m, $H_{5'a,}$ $-OCH_2$), 3.80 (2H, m, $POCH_2O$), 3.92 (1H, dd, J = 3.0 Hz, 9.0 Hz, $H_{5'b}$), 4.18 (2H, m, CH_2), 4.64 (1H, m, $H_{4'}$), 4.75 (1H, m, $H_{3'}$), 5.18 (1H, d, J = 11 Hz, $-OCH_aN$), 5.52 (1H, t, J = 5.0 Hz, $H_{2'}$), 5.85 (1H, d, J = 11 Hz, $-OCH_bN$), 5.98(1H, d, J = 5.0 Hz $H_{1'}$).^{13}P NMR δ_P (D_2O 121 MHz, decoupled with ^{1}H): −10.44, −10.79. HRMS (FAB$^-$) for $C_{13}H_{16}N_4O_{12}P_2Cl$: 516.9934 [(M−H)$^-$]; Found 516.9925.

***N*^{1}-[5″-*O*-(Phenylthio)phosphoryl-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl]-5′-*O*-phosphoryl-2′, 3′-di-*O*-acetyl-inosine (50).** Starting from **27** (68 mg, 62.9 μm) with the same procedure shown in the preparation of **46**, 45 mg of **50** was obtained as a white solid (70%). ^{1}H NMR (300 MHz, D_2O) δ 1.94, 1.11 (each s, each 3H, 2 × CH_3), 3.27 (m, 1H, $H_{5'a}$), 3.68 (m, 1H, $H_{5'b}$), 3.94−4.18 (m, 8H, 2 × $H_{1''}$, OCH_2, $H_{4''}$, 2 × $H_{5''}$, $H_{4'}$), 4.37 (m, 1H, $H_{3''}$), 4.64 (m, 2H, CH_2═), 5.19 (m, 1H, CH═), 5.58 (m, 2H, $H_{2''}$, $H_{3'}$), 5.92 (m, 1H, $H_{1'}$), 6.17 (m, 1H, $H_{2'}$), 7.20−7.45 (m, 5H, C_6H_5), 8.13 (s, 1H, H_2). ^{31}P NMR (D_2O 121 MHz, decoupled with ^{1}H) δ 5.39, 18.93. HRMS (FAB, negative) for ($C_{28}H_{32}N_4O_{15}SP_2Br$): 837.0249 [(M − 1)$^-$]; Found 836.0267.

***N*^{1}-[5″-*O*-Phosphoryl-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl]-5′-*O*-phosphoryl-2′,3′-di-*O*-acetyl-inosine 5′,5″-cyclicpyrophosphate (51a) and *N*^{1}-[5″-*O*-Phosphoryl-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl]-8-bromo-5′-*O*-phosphoryl-2′,3′-di-*O*-acetyl-inosine 5′,5″-cyclicpyrophosphate (51b).** Starting from **50** (41 mg, 40 μm) with the same procedure shown in the preparation of **47**, 4 mg of **51a** was obtained (12%), accompanied by 12 mg of **51b** (33%) as triethylammonium salts. **51a**: ^{1}H NMR (500 MHz, D_2O) δ 1.89,2.04 (each s, each 3H, 2 × CH_3), 3.67 (dd, 1H, J = 4.5, 13.5 Hz, $H_{5'a}$), 3.75 (1H, m, OCH_a), 3.83 (1H, dd, J = 5.5, 13.5 Hz, $H_{5'b}$), 3.94 (1H, m, OCH_b), 4.00 (dd, 1H, J = 5.5, 12 Hz, $H_{5''a}$), 4.07 (m, 1H, $H_{1''a}$), 4.22 (m, 1H, $H_{4''}$), 4.31 (m, 2H, $H_{1''b}$, $H_{4'}$), 4.40 (m, 1H, $H_{3''}$), 4.61 (t, 1H, J = 9.0 Hz, $H_{3''}$), 4.79 (m, 1H, CH_2═), 5.45 (m, 1H, CH═), 5.52 (1H, m, $H_{2''}$), 5.67 (m, 1H, $H_{3'}$), 6.11 (d, J = 6.0 Hz, $H_{1'}$), 6.28 (m, 1H, $H_{2'}$), 7.95 (s, 1H, H_8), 8.62 (s, 1H, H_2). ^{31}P NMR (D_2O 121 MHz, decoupled with ^{1}H) δ −8.64, −9.50. HRMS (FAB, negative) for $C_{22}H_{27}N_4O_{15}P_2$: Calcd, 649.0953 [(M − 1)$^-$]; Found, 649.0968. **51b**: ^{1}H NMR (300 MHz, D_2O) δ2.04,2.92 (each s, each 3H, 2 × AcO), 3.72−4.39 (m, 11H, 2 × $H_{5'}$, 2 × $H_{5''}$, $H_{4''}$, CH_2, 2 × $H_{1''}$, $H_{4'}$, $H_{3''}$), 4.61 (2H, m, CH_2═), 5.50 (m, 2H, $H_{2''}$, CH═), 5.71 (m, $H_{3'}$, 1H,), 6.16 (d, J = 5.4 Hz, $H_{1'}$), 6.29 (t, 1H, J = 5.4 Hz, $H_{2'}$), 8.60 (s, 1H, H_2). HRMS (FAB, negative) for $C_{22}H_{26}N_4O_{15}P_2Br$: Calcd, 727.0059 [(M − 1)$^-$]; Found, 727.0067.

***N*^{1}-[5″-*O*-phosphoryl-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl]-5′-*O*-phosphoryl-inosine 5′,5″-cyclicpyrophosphate (4a) and *N*^{1}-[5″-*O*-Phosphoryl-2″-deoxy-3″-*O*-allyl-D-lyxitol-2″-yl]-8-bromo-5′-*O*-phosphoryl-inosine 5′,5″-cyclicpyrophosphate (4b).** Starting from **51a** (60 OD_{254}) and **51b** (40 OD_{254}) with the same procedure shown in the preparation of **3**, **4a** (28 OD_{254}, 46.7%) and **4b** (15 OD_{254}, 37.5%) were obtained as triethylammonium salts. **4a**: **[**α**]**$^{25}_D$ +18.9. (*c* 0.20, H_2O), UV $\lambda_{max}^{H_2O}$ 206.4 (4.02), 251.5 (3.62). ^{1}H NMR (500 MHz, D_2O) δ: 3.63 (1H, m, OCH_a), 3.69 (dd, 1H, J = 5.0, 13 Hz, $H_{5'a}$), 3.86 (m, 2H, OCH_b, $H_{5'b}$), 4.05 (m, 3H, 2 × $H_{5''}$, $H_{4''}$), 4.24−4.35 (m, 4H, 2 × $H_{1''}$, $H_{4'}$, $H_{3''}$,), 4.50 (m, 1H, $H_{2''}$), 4.82 (m, 2H, CH_2═), 5.36 (t, 1H J = 5.5 Hz, $H_{3'}$), 5.55 (m, 1H, CH═), 5.59 (m, 1H, $H_{2'}$), 5.84 (d, 1H, J = 5.5 Hz, $H_{1'}$), 8.05 (s, 1H, H_8), 8.66 (s, 1H, H_2). ^{31}P NMR (D_2O 121 MHz, decoupled with ^{1}H) δ −9.93, −10.83. HRMS (FAB, negative) for $C_{18}H_{23}N_4O_{13}SP_2$: Calcd, 565.0742 [(M − 1)$^-$]; Found, 565.0742. **4b**: **[**α**]**$^{25}_D$ +34.9 (*c* 0.18, H_2O), UV $\lambda_{max}^{H_2O}$ 207 (4.23), 256.2 (3.93). ^{1}H NMR (300 MHz, D_2O) δ 3.69 (dd, 1H, J = 5.0, 13 Hz, $H_{5'a}$), 3.82 (m, 2H, OCH_2), 3.87 (dd, 1H, J = 5.5, 13 Hz, $H_{5'b}$), 4.02 (m, 3H, $2H_{5''}$, $H_{4'}$), 4.22 (m, 3H, $H_{4''}$ $H_{3'}$, $H_{1''}$), 4.34 (m, 1H, $H_{1''}$), 4.50 (m, 1H, $H_{3''}$), 4.79 (m, 2H, CH_2═), 5.49 (m, 1H, $H_{2''}$), 5.53 (m, 1H, CH═), 5.57 (1H, m, $H_{2'}$), 5.98 (d, 1H, 6.5 Hz). ^{31}P NMR (D_2O 224 MHz, decoupled with ^{1}H) δ: −10.11, −10.95; HRMS (FAB, negative) for $C_{18}H_{22}N_4O_{13}P_2Br$: Calcd, 642.9847 [(M − 1)$^-$]; Found, 642.9849.

***N*^{1}-[5″-*O*-(Phenylthio)phosphoryl-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl]-5′-*O*-Phosphoryl-2′,3′-di-*O*-acetyl-inosine (52a) and 8-Bromo-*N*-1-[5″-*O*-(phenylthio)phosphoryl- 2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl]-5′-*O*-phosphoryl-2′,3′-di-*O*-acetyl-inosine (52b).** Starting from **39** (100 mg, 92.6 μm) with the same procedure shown in the preparation of **46**, only the ratio between H_3PO_2 and Et_3N (3:1), **52a** (25 mg, 28.7%) and **52b** (27 mg, 28.7%) were obtained as triethylammonium salts. **52a**: **[**α**]**$^{25}_D$ 11.6 (*c* 0.36, H_2O); UV $\lambda_{max}^{H_2O}$ 202.5 (4.39), 244.2 (3.97). ^{1}H NMR (300 MHz, D_2O) δ: 1.83,2.01 (each s, each 3H, 2 × AcO), 3.48 (m, 1H, $H_{5'a}$), 3.69 (m, 1H, $H_{5'b}$), 3.86−4.13 (m, 8H, OCH_2, 2 × $H_{5''}$, $H_{4'}$, 2 × $H_{1''}$, $H_{4''}$), 4.42 (m, 1H, $H_{3''}$), 4.93 (m, 2H, CH_2═), 5.03 (m, 1H, $H_{2''}$), 5.46 (m, 1H, $H_{3'}$), 5.63 (m, 1H, $H_{2'}$), 5.69 (m, 1H, CH═), 6.18 (d, 1H, J = 6.0 Hz, $H_{1'}$), 6.75−6.83 (m, 3H, Ar H), 7.10−7.13 (m, 2H, Ar H), 8.12 (s, 1H, H_2), 8.36 (s, 1H, H_8). ^{31}P NMR (D_2O 121 MHz, decoupled with ^{1}H) δ: 1.80 (s), 18.02 (s). HRMS (FAB, negative) Calcd, $C_{28}H_{33}N_4O_{15}SP_2$ 759.1144 [(M − 1)$^-$], Found, 759.1144. **52b**: **[**α**]**$^{25}_D$ +18.2 (*c* 0.4, H_2O); UV $\lambda_{max}^{H_2O}$ 248.2 (4.06). ^{1}H NMR (300 MHz, D_2O) δ: 1.97, 2.05 (each s, each 3H, 2 × AcO), 3.75 (dd, 1H, m, $H_{5'a,}$), 3.83 (dd, 1H, J = 5.7, 13.6 Hz, $H_{5'b}$), 3.95−4.10 (m, 6H, OCH_2, 2 × $H_{5''}$, $H_{4'}$, $H_{1''a}$), 4.16 (m, 2H, $H_{1''b}$, $H_{4''}$), 4.42 (m, 1H, $H_{3''}$), 5.07 (m, 2H, CH_2═), 5.13 (m, 1H, $H_{2''}$), 5.62 (m, 1H, $H_{3'}$), 5.72 (m, 1H, CH═), 5.95 (dd, 1H, J = 4.5, 6.6 Hz, $H_{2'}$), 6.21 (d, 1H, J = 4.5 Hz, $H_{1'}$), 6.96 (m, 3H, Ar H), 7.30 (m, 2H, Ar H), 8.23 (s, 1H, H_2). ^{31}P NMR (D_2O 121 MHz, decoupled with ^{1}H) δ: 4.47 (s), 17.46 (s). HRMS (FAB, negative) for $C_{28}H_{32}N_4O_{15}SP_2Br$: Calcd, 837.0249 [(M − 1)$^-$]; Found, 837.0232.

***N*^{1}-[5″-*O*-Phosphoryl-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl]-5′-*O*-phosphoryl-2′,3′-di-*O*-acetyl-inosine 5′, 5″-cyclicpyrophosphate (53a).** Starting from **52a** (15 mg, 15.9 μmol) with the same procedure shown in the preparation of **47**, 8 mg of **53a** was obtained as a white solid (60.6%). ^{1}H NMR (500 MHz, D_2O) δ: 1.91,2.04 (each s, each 3H, 2 × AcO), 3.90 (dd, 1H, J = 5.0, 12 Hz, $H_{5'a}$), 3.95−4.03 (m, 3H, $H_{5'b}$, 2 × $H_{5''}$), 4.07 (m, 2H, $H_{4'}$, $H_{4''}$), 4.10−4.22 (m, 2H, OCH_2), 4.32 (m, 2H, 2 × $H_{1''}$), 4.41 (1H, m, $H_{3''}$), 5.06−5.19 (m, 2H, CH_2═), 5.31 (m, 1H, $H_{2''}$), 5.71 (dd, J = 5.5, 10.5 Hz, 1H, $H_{3'}$), 5.82 (m, 1H, CH═), 6.12 (d, 1H, J = 5.5 Hz, $H_{1'}$), 6.26 (t, 1H, J = 5.5 Hz, $H_{2'}$), 7.97 (s, 1H, H_8), 8.46 (s, 1H, H_2). ^{31}P NMR (D_2O 121 MHz, decoupled with ^{1}H); δ: −8.07, −9.43. HRMS (FAB, negative) for $C_{22}H_{27}N_4O_{15}P_2$: Calcd, 649.0953 [(M − 1)$^-$]; Found, 649.0951.

***N*^{1}-[5″-*O*-Phosphoryl-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl]-5′-*O*-phosphoryl-inosine 5′,5″-cyclicpyrophosphate (5a).** Starting from **53a** (89 OD_{254}) with the same procedure shown in the preparation of **3**, **5a** (33 OD_{254}, 37%) was obtained as triethylammonium salt. **[**α**]**$^{25}_D$ 28.3 (*c* 0.22, H_2O), UV $\lambda_{max}^{H_2O}$ 207.1 (4.18), 251.2 (3.75). ^{1}H NMR (500 MHz, D_2O) δ: 3.96 (m, 2H, 2 × $H_{5'}$), 4.05 (m, 2H, 2 × $H_{5''}$), 4.16 (m, 2H, $H_{4'}$, $H_{4''}$), 4.26 (m, 2H, OCH_2, $H_{1''a}$), 4.32 (m, 1H, $H_{1''b}$), 4.38 (m, 1H, $H_{3''}$), 4.56 (m, 1H, $H_{2''}$), 5.11−5.28 (m, 2H, OCH_2═), 5.32 (t, 1H, J = 5.5 Hz, $H_{3'}$), 5.38 (m, 1H, $H_{2'}$), 5.86 (m, 1H, CH═), 5.88 (d, 1H, J = 5.5, $H_{1'}$), 8.07 (s, 1H, H_8), 8.49(s, 1H, H_2). ^{31}P NMR (D_2O 121 MHz, decoupled with ^{1}H) δ: −8.07, −9.43. HRMS (FAB,

negative) for $C_{18}H_{23}N_4O_{13}P_2$: Calcd, 565.0742 [(M − 1)$^-$]; Found, 565.0743.

***N*^{1}-[5″-*O*-Phosphoryl-2″-deoxy-3″-*O*-allyl-D-ribitol-2″-yl]-8-bromo-5′-*O*-phosphoryl-inosine 5′,5″-cyclicpyrophosphate (5b).** Starting from **52b** (15 mg, 14.7 μm) with the same procedure shown in the preparation of **47**, compound **53b** was obtained, accompanied by minor of **53a** as a triethylammonium salt. Compound **53b** was directly used to deacetylation without removal of excess of TEAA. After neutralizing the mixture, the residue was evaporated to dryness in vacuo and purified by C18 reverse column to give the target product **5b**: $[\alpha]^{25}_D$ +18.7 (*c* 0.13, H_2O), **UV** $\lambda_{max}^{H_2O}$ 206.4 (4.23), 253.1 (3.48). ^{1}H NMR (500 MHz, D_2O) δ: 3.95 (m, 2H, 2 × $H_{5'}$), 4.05 (m, 2H, 2 × $H_{5''}$), 4.13−4.32 (m, 7H, 2 × $H_{1''}$, OCH_2, $H_{4''}$, $H_{4'}$, $H_{3''}$), 4.64 (m, 1H, $H_{2''}$), 5.11−5.34 (m, 2H, CH_2═), 5.35 (m, 1H, $H_{3'}$), 5.42 (t, J = 5.5 Hz, $H_{2'}$), 5.83 (m, 1H, CH═), 6.00 (d, 1H, J = 5.5 Hz, $H_{1'}$), 8.46 (s, 1H, H_2).^{31}P NMR (D_2O 121 MHz, decoupled with ^{1}H) δ: −8.27 (d, J = 12 Hz), −9.53 (d, J = 12 Hz). HRMS (FAB, negative) for $C_{18}H_{22}N_4O_{13}P_2Br$: Calcd, 642.9847 [(M − 1)$^-$]; Found, 642.9858.

Biological Activity Assays. (1) Preparations of rat brain microsomes homogenates and HeLa cells culture. Microsomes were prepared from Sprague−Dawley rat brain described previously.[32] The homogenate medium was 250 mM *N*-methylglucamine, 250mM potassium gluconate, 20 mM HEPES, 1 mM $MgCl_2$, 100 μg/mL soybean trypsin inhibitor, 20 μg/mL aprotinin, and 25 μg/mL leupeptin (pH 7.2). A 10% (w/v) homogenate was prepared using a glass homogenizer. The homogenate was centrifuged at 1000*g* for 5 min and the resultant supernatant was centrifuged at 8000*g* for further 10 min. The supernatant was further centrifuged at 100000*g* for 40 min to sediment the microsomes fraction. The microsomes fraction was resuspended in homogenate medium plus 1 mM ATP, 10 mM phosphocreatine, 10 units/mL creatine phosphokinase, 1 μg/mL oligomycin, 1 μg/mL antimycin A, and 1 mM sodium azide (pH 7.2) plus 3 μM Fluo3 to a final protein concentration of 1 mg/mL for the release experiment.

Aliquots (10−20 mL) of HeLa Cells from RPMI 1640 medium were centrifuged at low speed for 5 min at 25 °C, and the supernatant was removed. The packed cells were resuspended in experimental solution (ES) consisting of 125 mM NaCl, 1.2 mM K_2HPO_4, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, 6 mM Glucose, 25 mM HEPES (pH 7.4). The suspension at a density of 2 × 10^5 cells/mL was planted in a 35 mm rat tail collagen-coated dishes (NUNC, DMEM) for 18 h. The packed cells were incubated in ES containing 20 μM Fluo-3/AM (Molecular Probe)) at 37 °C in dark for 30 min. The cells were then washed twice and then incubated for additional 30 min in a dye-free-HEPES solution to complete deesterification.

(2) Ca^{2+}-Release Assays. The experiment was preformed at 17 °C using 500 μL of cell suspension and microsomes homogenate after loading fluorescence indicator on CLSM (Lecia TCS-NT, Germany). Free $[Ca^{2+}]_i$ variation was measured by monitoring fluorescence at excitation and emission wavelengths of 490 and 535 nm, respectively.

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Appendix

Abbreviations: cADPR, cyclic adenosine 5′-diphosphate ribose; cIDPR, cyclic inosine 5′-diphosphate ribose; cADPcR, cyclic adenosine 5′-diphosphate carboribose; NAD^+, nicotinamide adenosine dinucleotide phosphate; ^{1}H NMR, proton nuclear magnetic resonance; ^{13}C NMR, carbon-13 nuclear magnetic resonance; NOESY, nuclear overhauser effect spectroscopy; FAB MS, fast atom bombardment mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; ESI, electrospray ionization; TOF, time-of-flight; HR, high resolution; CLSM, confocal laser-scanning microscope; HPLC, high performance liquid chromatography; MS, mass spectrometry; IP_3, inositol triphosphate, 3 Å MS, 3 Å molecular sieve, EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; TsCl, tosyl chloride; Py, pyridine; PDC, pyridinium dichromate; TPSCl, triisopropylbenzenesulfonyl chloride; MMTrCl, *p*-anisylchlorodiphenylmethane; DCM, dichloromethane; TCA, trichloroacetic acid; PSS, cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate; TBDMS, *tert*-butyldimethylsilyl; TEAA, triethylammonium acetate; TEAB, triethylammonium bicarbonate; TBAF, tetrabutylammonium fluoride; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; TrCl, triphenylmethyl chloride.

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