Concise synthesis of novel acyclic analogues of cADPR with an ether chain as the northern moiety†

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Received (in Montpellier, France) 25th October 2009, Accepted 8th December 2009
First published as an Advance Article on the web 5th February 2010
DOI: 10.1039/b9nj00595a

To study the properties of hydrolysates of cyclic adenosine diphosphate ribose (cADPR), a series of novel acyclic analogues of cADPR with an ether chain as the northern moiety and 8-substituted adenine or hypoxanthine as the base moiety were synthesized *via* an N^1 substitution construction, followed by bisphosphorylation, phosphoramidition or pyrophosphorylation. These compounds also provide various precursors for synthesizing cADPR analogues.

Introduction

Cyclic adenosine diphosphate ribose (cADPR, **1**, Fig. 1) is a universal Ca^{2+}-mobilizing second messenger that was first identified in the sea urchin egg system.[1,2] Since its discovery, a significant number of cell systems have been described as utilizing the cADPR/ryanodine receptor (RyR)/Ca^{2+} signalling system to control Ca^{2+}-dependent cellular responses such as fertilization, secretion, contraction, proliferation and so on.[3,4] Moreover, numerous cADPR analogues have been synthesized to further understand its biology. Among these, the majority of structural modifications have been applied to the purine ring and two riboses.[5–11]

A series of novel cADPR mimics, cIDPRE (**2**) and cADPRE (**3**) (Fig. 1), in which the northern ribose[12] (the ribose linked to the N^1 of adenine) was replaced by an ether strand, were reported by Zhang's group.[13–15] Biological data suggested that modification of the northern ribose moiety of cADPR could retain its calcium release activity and increase its membrane permeability. The results revealed that the greater flexibility of the whole molecule, introduced by replacing the northern ribose by an ether strand, continued to allow its fitting into the binding site, and that the functional groups could support a conformation very similar to that of cADPR.[16,17]

cADPR can be hydrolyzed either *in vivo* or *in vitro*.[18,19] Pyrophosphate is one of the easiest linkages to be hydrolyzed. It was reported recently that the pyrophosphate linkage of cADPR could be hydrolyzed by Mn^{2+}-dependent ADP-ribose/CDP-alcohol pyrophosphatase to afford the bisphosphate metabolite.[20] Though modification of the northern ribose by an ether linkage provided relatively stable cADPR analogues,[15] there was no detailed report about the metabolism of these analogues, especially the properties of their metabolites. In this study, a series of acyclic cADPR analogues, which may mimic the hydrolysates of the cyclic pyrophosphate group cleaved in different places, have been designed and synthesized (Fig. 2). It is hypothesized that these acyclic analogues are resistant to both enzymatic and chemical hydrolysis, since they possess a stable N-alkyl linkage instead of the N^1-glycosidic linkage. These compounds provided a mini library for in-depth studies of the nature of the metabolite and suggest further research on the structure activity relationships (SAR) of cADPR analogues. Moreover, they provided various precursors for synthesizing cADPR analogues.

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† This article is part of a themed issue on Biophosphates.

Results and discussion

Synthesis of bisphosphate analogues

The syntheses of 8-substituted analogues **4a–d** are summarized in Scheme 1. The synthetic route adopted for **4a–d** has two main steps: (i) introduction of the 2-(hydroxyethoxy)ethyl chain onto N^1 of the substituted 2′,3′-isopropylidene inosines **6a–c** and **9**, which leads to **7a–d**; (ii) bisphosphorylation of derivatives **7a–d** through phosphorus oxychloride ($POCl_3$), followed by deprotection with aqueous HCOOH to yield the target compounds.

N^1-alkylation of **6a–c** was obtained through the N^1-(2,4-dinitrophenyl)inosine intermediate with 2-(hydroxyethoxy)-ethylamine. 2′,3′-*O*-Isopropylideneinosines were converted into N^1-(2,4-dinitrophenyl) derivatives by a reaction with 2,4-dinitrochlorobenzene and K_2CO_3 in DMF; the latter was

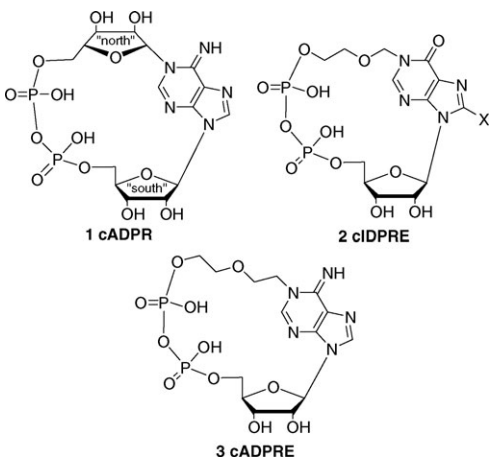


Fig. 1 The structure of cADPR and cADPR analogues.

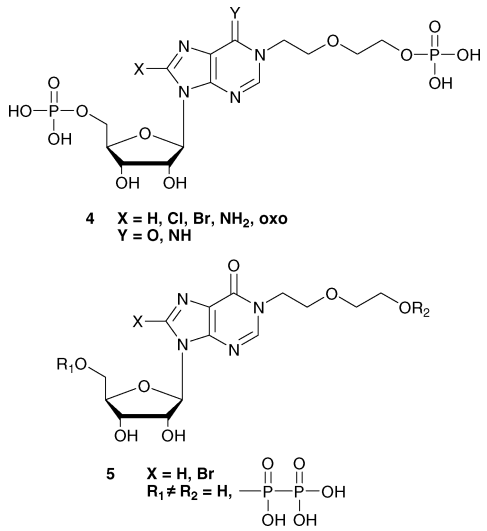
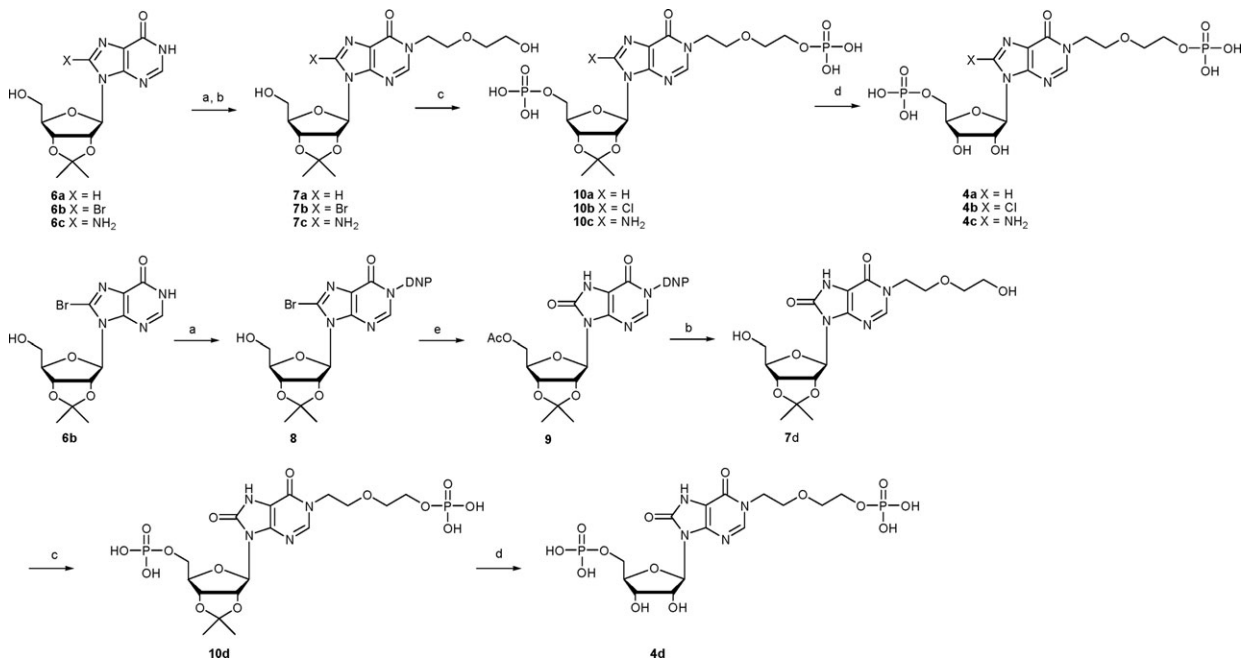


Fig. 2 The general structure of acyclic cADPR analogues.

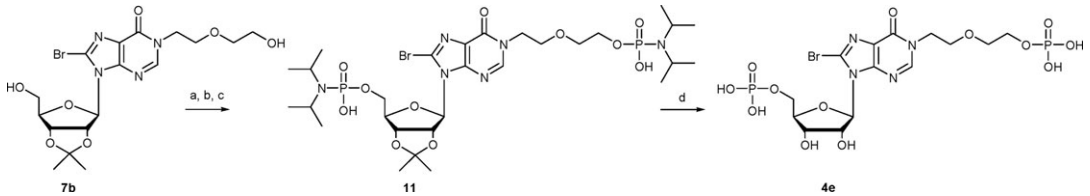
treated with 2-(hydroxyethoxy)ethylamine for 8 h at 50 °C in DMF without separation to afford the N^1-[(5′′-hydroxy)-ethoxyethyl]inosine derivatives **7a–c** in 70–80% yield. **7d** was obtained in a yield of approximately 80% when intermediate **8** was separated and converted into **9** by treatment with NaOAc/AcOH. This was followed by the concomitant aminolysis of the 5′-*O*-acetyl protecting group through stirring with 2-(hydroxyethoxy)ethylamine in DMF under similar conditions to those used in the preparation of **7a–c**. The formation of N^1-alkylated derivatives **7a–d** proceeded through a rearrangement of the pyrimidine ring. Alkylation was induced by nucleophilic attack of the amine at C^2, whose electrophilicity was enhanced by the presence of the 2,4-dinitrophenyl group at the N^1 position. Compounds **7a–d** were bisphosphorylated by $POCl_3$ in pyridine (Py)/H_2O–CH_3CN at 0 °C for 4 h to give **10a–d** in moderate to high yields. It is noteworthy that when 8-Br intermediate **7b** was converted into the bisphosphate by the bisphosphorylation reaction with $POCl_3$, bromine at the 8-position of inosine was simultaneously replaced by chlorine, and none of the expected 8-Br bisphosphate was obtained. By removing the isopropylidene groups in aqueous HCOOH, targets **4a–d** were then obtained in a yield of approximately 90%.

Since attempts to introduce similar 8-Br bisphosphates with corresponding phosphate acids under the above-mentioned conditions failed, we turned to the phosphoramidite strategy as a preferred method in ribonucleic acid (RNA) synthesis.[21] The phosphoramidition of **7b** was achieved with 2-cyanoethyl-*N*,*N*-diisopropylamino-chlorophosphoramidite to afford the expected 8-Br-substituted phosphate, **11**, in high yield (87%) after oxidation of the intermediate phosphite with ^{t}BuOOH and deprotection of the cyanoethyl group with NH_3/MeOH. When the deprotection reaction of **11** by aqueous HCOOH was conducted, it is interesting that debrominated product **4a** (15%) was likewise obtained, in addition to the desired compound **4e** (36%), during the deprotection of the isopropylidene group (Scheme 2).

For the syntheses of analogues substituted at position 8 in the base, a different strategy was adopted for the construction of the N^1 substituted adenosine, which was achieved regiospecifically by condensation reactions of 2-bromo-substituted 5-amino-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide (AICAR) derivatives and 2-(hydroxyethoxy)ethylamine in the presence of a catalytic amount of K_2CO_3 in yields above 90%; this being similar to the synthesis of compound **14a**.[14] Subsequently, the 5′-*O*-*tert*-butyldimethylsilyl (TBDMS) groups in **14a,b** were removed by a tetrabutylammonium fluoride (TBAF) solution to afford **15a** and **15b** in a 95% yield. Compounds **15a,b**, underwent phosphorylation procedures,



Scheme 1 Syntheses of compounds **4a–d**. Reagents and conditions: (a) DNCB (2,4-dinitrochlorobenzene), K_2CO_3, DMF, 80 °C, 2 h; (b) $NH_2(CH_2)_2O(CH_2)_2OH$, DMF, 50 °C, 8 h; (c) $POCl_3$, Py, CH_3CN, H_2O, 0 °C, 4 h; (d) 60% HCOOH/H_2O, rt, 8 h; (e) NaOAc, AcOH, reflux, 6 h.



Scheme 2 Syntheses of compounds **4e**. Reagents and conditions: (a) 2-cyanoethyl-*N*,*N*-diisopropylamino-chlorophosphoramidite, DIPEA, DCM, rt, 30 min; (b) ^{t}BuOOH, rt, 1h; (c) NH_3/MeOH, rt, 8 h; (d) 60% HCOOH/H_2O, rt, 8 h.

leading to bisphosphate derivatives (86–90% yield). After treatment with 60% aqueous HCOOH at rt for 8 h to facilitate the removal of the isopropylidene protecting group, **4f,g** were obtained in approximately 90% yield (Scheme 3).

In order to obtain the 8-oxo-bisphosphate derivative with adenine as the base, compound **15b** was converted into **15c** in a yield of 82% by refluxing with 2-mercaptoethanol in a mixed solvent solution of CH_3CN and water. When **15c** was subjected to phosphorylation by $POCl_3$, the expected conversion of 8-mercaptoethanol to 8-oxo did not take place, though triphosphate derivative **16c** was obtained in 86% yield. By removing the isopropylidene groups in aqueous HCOOH, compound **4h** was obtained in a yield of 89% (Scheme 4).

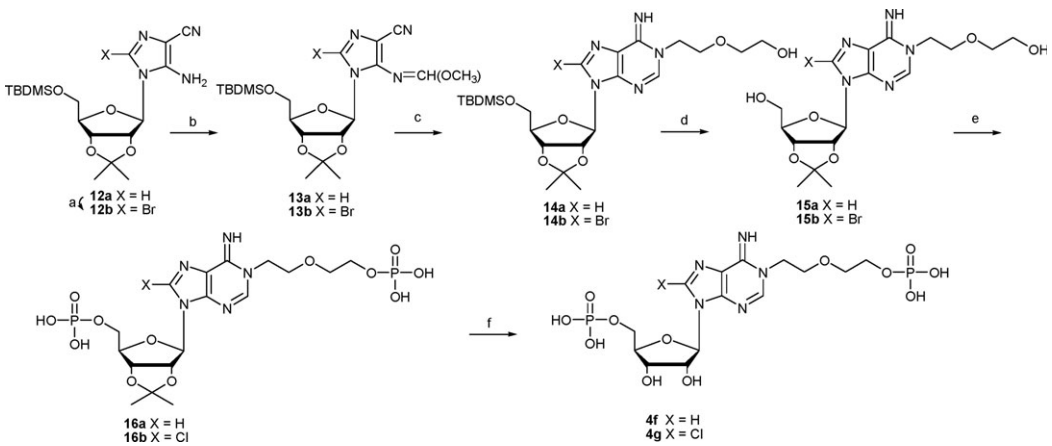
Synthesis of pyrophosphate analogues

For the synthesis of protected pyrophosphate **21a**, the method outlined in Scheme 5 was first attempted. However, compound **18** was not obtained from **17a**. This problem was overcome after modifying the reaction sequence to proceed *via* compound **17b**. Subsequently, compound **18** was converted into **19** using the same reaction conditions as those described earlier. However, the yield of this step was very low (41%) compared to similar reactions (above 80%), although various conditions with different reaction temperatures and times were examined.

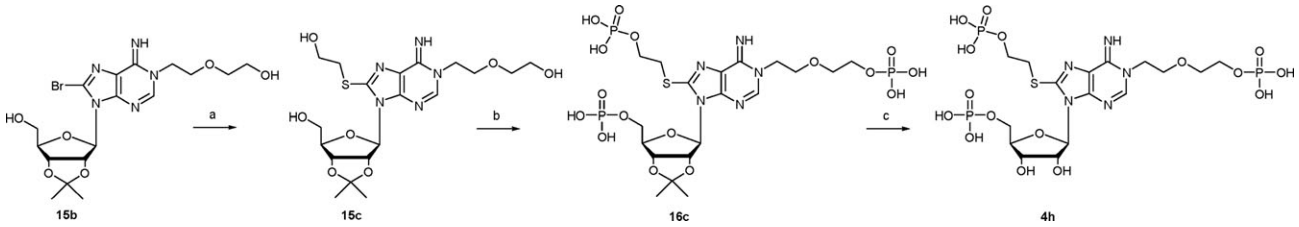
During research to find the optimal conditions for converting compound **19** straight into pyrophosphorylated compound **21a**, it was discovered that the unprotected 5′′-hydroxyl group in **19** indeed affected the pyrophosphorylation, allowing recovery of 80% of the starting material. The rest of the material (20%) decomposed into an inseparable mixture. Meanwhile, when compound **19** was protected with a monomethoxytrityl (MMTr) group using MMTrCl in dry pyridine, affording compound **20a**, the reaction from **20a** to **21a** occurred smoothly in dry CH_3CN. The limitation of this approach was that the active *p*-toluenesulfonyl (Ts) group was introduced too early, reducing the overall yield of precursor **20a** for pyrophosphorylation (yield of 21% from **17b** to **20a**) (Scheme 5).

Evidently, there is a need for a more efficient method to synthesize the precursors. Finally, the synthetic route outlined in Scheme 6 was adopted. Starting from 5′-*O*-TBDMS-2′,3′-*O*-isopropylideneinosine and 5′-*O*-TBDMS-2′,3′-*O*-isopropylidene-8-bromoinosine, with the same procedure as that used in the preparation of **7a**, compounds **22a** and **22b** were obtained in a yield of 80 and 84%, respectively. After the 5′′-hydroxyl group of **22a,b** had been protected with an MMTr group (96 and 92% yield, respectively), it was treated with TBAF in THF to remove the 5′-TBDMS group of **23a,b**. A Ts group was then introduced at the resulting 5′-primary hydroxyl group of **24a,b** *via* the usual method with TsCl/NEt_3/DMAP/DCM to afford precursors **20a,b** (approximately 80% yield over two steps). Although two other steps were required, a significantly improved yield was achieved (64% from **22a** to **20a**, compared to 21% from **17b** to **20a**). Targeted pyrophosphorylated compounds **5a,b** were obtained in almost 90% yield by removing both the MMTr and isopropylidene groups of compounds **21a,b** in one step.

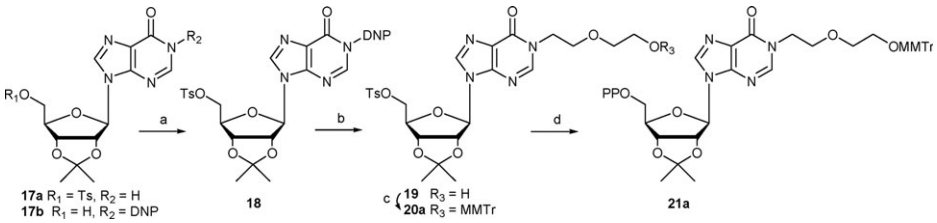
A similar activation approach to the activation of the 5′′-primary hydroxyl groups of **25a,b** by a Ts group was employed for the synthesis of **26a,b** in Scheme 7, which was



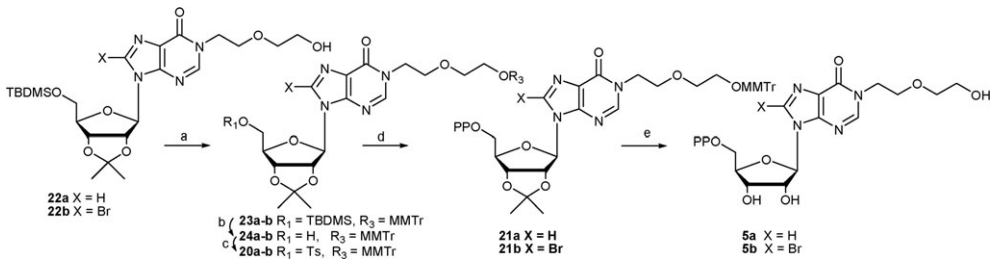
Scheme 3 Syntheses of compounds **4f,g**. Reagents and conditions: (a) NBS, THF, rt, 10 min; (b) $CH(OMe)_3$, CF_3COOH, reflux, 1 h; (c) $NH_2(CH_2)_2O(CH_2)_2OH$, K_2CO_3, DMF, rt, 8 h; (d) TBAF, AcOH, THF, rt, 2 h; (e) $POCl_3$, Py, CH_3CN, H_2O, 0 °C, 4 h; (f) 60% HCOOH/H_2O, rt, 8 h.



Scheme 4 The synthesis of compound **4h**. Reagents and conditions: (a) $HS(CH_2)_2OH$, NEt_3, H_2O, reflux, 2 h; (b) $POCl_3$, Py, CH_3CN, H_2O, 0 °C, 4 h; (c) 60% $HCOOH/H_2O$, rt, 8 h.



Scheme 5 The synthesis of compound **21a**. Reagents and conditions: (a) DNCB, K_2CO_3, DMF, 80 °C, 2 h for **17a**; TsCl, Py, rt, 8 h for **17b**; (b) $NH_2(CH_2)_2O(CH_2)_2OH$, DMF, 50 °C, 8 h; (c) MMTrCl, Py, rt, 8 h; (d) $[(^nBu)_4N]_3P_2O_5OH$, CH_3CN, rt, 16 h.



Scheme 6 Syntheses of compounds **5a,b**. Reagents and conditions: (a) MMTrCl, Py, rt, 8 h; (b) TBAF, AcOH, THF, rt, 2 h; (c) TsCl, NEt_3, DCM, DMAP, rt, 8 h; (d) $[(^nBu)_4N]_3P_2O_5OH$, CH_3CN, rt, 16 h; (e) 60% $HCOOH/H_2O$, rt, 8 h.

converted into pyrophosphates **5c,d** *via* pyrophosphorylation and deprotection. For the pyrophosphorylation of **20a,b** and **26a,b**, it was observed that when the 8-position of inosine was substituted with bromine, the reaction yield was roughly 80% (74% for **20b** and 83% for **26b**). Meanwhile, in the absence of substitution, the yield was approximately 20% (20% for **20a** and 21% for **26a**). The latter yield was close to that reported for the pyrophosphorylation of 5′-*O*-Ts nucleosides.[22] The reason for this difference remains unclear.

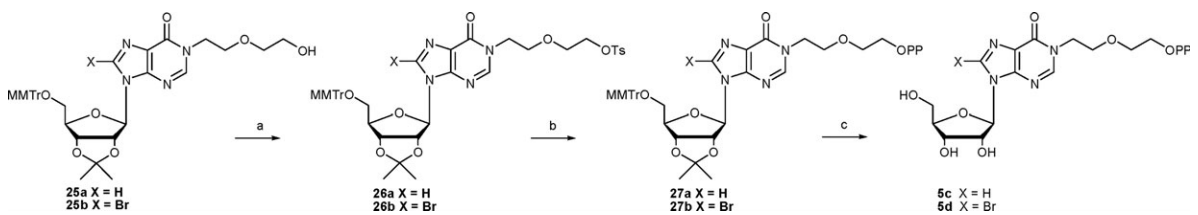
Conclusions

In conclusion, novel acyclic metabolites of cADPR analogues, **4a–g**, with 8-substituted hypoxanthine or adenine as the base moiety, were synthesized *via* N^1 substitution construction reactions, followed by bisphosphorylation or phosphoramidition. Meanwhile, pyrophosphorylated compounds **5a–d** were obtained through pyrophosphorylation. These compounds provide a mini library, not only for the natural study of cADPR metabolites, but also as precursors for the development of syntheses of cADPR analogues.

Experimental

General synthesis methods

Mass spectra were obtained on either VG-ZAB-HS or Bruker APEXTM instruments. High resolution FAB (fast atom bombardment) and HR-ESI (electrospray ionization) mass spectrometry were performed using a Bruker BIFLEXTM III instrument. ^1H and ^{13}C NMR spectra were recorded using a



Scheme 7 Syntheses of compounds **5c,d**. Reagents and conditions: (a) TsCl, NEt_3, DCM, DMAP, rt, 8 h; (b) $[(^nBu)_4N]_3P_2O_5OH$, CH_3CN, rt, 16 h; (c) 60% $HCOOH/H_2O$, rt, 8 h.

JEOL AL300, Bruker AM/DPX 400 or Varian VXR-500 spectrometer with DMSO-d_6 or D_2O as the 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 using a Bruker Avance 300 spectrometer; orthophosphoric acid (85%) was used as an external standard. Purifications on an Alltech preparative C_{18} reversed phase column (2.2 × 25 cm) were conducted through a Gilson HPLC with the buffer system MeCN/TEAB (pH 7.5). Analytical TLC was performed using commercial glass plates coated to a thickness of 0.25 mm with Kieselgel 60 F254 silica and visualized under UV light. Flash chromatography was performed using Qingdao (230–400 mesh) silica under a slight positive pressure of air. Solvents and reagents for anhydrous reactions were dried prior to use by conventional methods.

N^1-Dinitrobenzene-2′,3′-*O*-isopropylidene-8-bromoinosine (8)

A mixture of 2′,3′-*O*-isopropylidene-8-bromoinosine (**6b**) (824 mg, 2.1 mmol), 2,4-dinitrochlorobenzene (647 mg, 3.1 mmol) and K_2CO_3 (442 mg, 3.1 mmol) were suspended in anhydrous DMF (8 mL) and stirred at 80 °C for 2 h. After cooling, the mixture was filtered and the filtrate evaporated to dryness. The residue was purified on a silica gel column eluted with increasing amounts of MeOH in CH_2Cl_2 (from 0 to 5%) to give compound **8** as a pale yellow amorphous solid consisting of a 1 : 1 mixture of atropisomers (918 mg, 79%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.91 (s, 1 H), 8.77, 8.81 (each t, each 0.5 H, J = 2.8 Hz), 8.70, 8.68 (each s, each 0.5 H), 8.18, 8.13 (each d, each 0.5 H, J = 8.8 Hz), 6.10 (t, 1 H, J = 4.8 Hz), 5.66, 5.61 (each dd, each 0.5 H, J = 2.4 and 6.4 Hz), 5.08–4.87 (m, 2 H), 4.17 (dd, 1 H, J = 6.0 and 10.0 Hz), 3.62–3.53 (m, 2 H), 1.57 (s, 3 H) and 1.35 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.8, 148.3 (d), 147.9, 147.6 (d), 145.5 (d), 134.6 (d), 132.6 (d), 129.4 (d), 126.6 (d), 123.7, 120.8 (d), 113.7 (d), 90.7 (d), 87.4 (d), 82.4 (d), 81.3 (d), 61.4 (d), 54.9, 27.1 (d) and 25.2 (d).

N^1-Dinitrobenzene-5′-*O*-acetyl-2′,3′-*O*-isopropylidene-8-oxoinosine (9)

A mixture of **8** (730 mg, 1.3 mmol) and NaOAc (326 mg, 3.9 mmol) was suspended in AcOH (8 mL) and refluxed for 6 h. The mixture was evaporated and the residue was partitioned between H_2O and $CHCl_3$. The organic layer was washed with brine, dried (Na_2SO_4) and evaporated. The residue was purified by silica gel column chromatography (2% MeOH in CH_2Cl_2) to give compound **9** as a pale yellow amorphous solid (685 mg, 99%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.82 (d, 1 H), 8.92 (s, 1 H), 8.80 (d, J = 8.8 Hz, 1 H), 8.58 (s, 1 H), 8.13 (d, J = 8.8 Hz, 1 H), 5.95 (d, J = 3.2 Hz, 1 H), 5.45 (d, J = 6.0 Hz, 1 H), 4.94 (dd, J = 6.0 and 2.4 Hz, 1 H), 4.28 (m, 2 H), 4.17 (m, 1 H), 2.00 (d, 3 H), 1.52 and 1.32 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.6, 170.6, 151.6, 149.9, 148.4, 146.7, 146.0, 144.0, 143.9, 134.9, 134.9, 132.9, 132.8, 129.9, 121.2, 113.8, 113.7, 108.1, 108.1, 86.7, 85.0, 84.8, 82.8, 82.7, 81.8, 64.3, 64.2, 27.5, 27.4, 26.7, 25.6, 21.0 and 21.0. HRMS (ESI, positive) for $C_{21}H_{20}N_6O_{11}$, calc. 533.1263 $[M + 1]^+$; found 533.1256.

Synthesis of 8-substituted N^1-[(5′′-hydroxyl)ethoxyethyl]-2′,3′-*O*- isopropylideneinosines 7a–d

N^1-[(5′′-Hydroxyl)ethoxyethyl]-2′,3′-*O*-isopropylideneinosine (7a). A mixture of **6a** (308 mg, 1 mmol), K_2CO_3 (166 mg, 1.2 mmol) and 2,4-dinitrochlorobenzene (243 mg, 1.2 mmol) was stirred in DMF (3 mL) at 80 °C for 2 h. After the mixture had cooled to room temperature, the insoluble materials were filtered off. The filtrate was evaporated and the residue redissolved in DMF. After 2-(hydroxyethoxy)ethylamine (0.38 mL, 3.8 mmol) had been added, the resulting solution was stirred at 50 °C for 8 h and evaporated. The residue was purified by silica gel column chromatography (4% MeOH in CH_2Cl_2) to give compound **7a** (276 mg, 70%) as a colorless fluffy solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.34, 8.32 (each s, each 1 H), 6.09 (d, J = 2.8 Hz, 1 H), 5.27 (dd, J = 2.8 and 6.0 Hz, 1 H), 5.09 (t, J = 5.6 Hz, 1 H), 4.94 (dd, J = 2.8 and 5.6 Hz, 1 H), 4.57–4.56 (m, 1 H), 4.22 (dd, J = 4.8 and 7.2 Hz, 1 H), 4.18 (t, J = 5.2 Hz, 2 H), 3.66 (t, J = 5.2 Hz, 2 H), 3.54 (t, J = 4.8 Hz, 2 H), 3.48–3.41 (m, 4 H), 1.53 and 1.32 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 155.8, 149.1, 147.1. 139.2, 123.6, 113.1, 89.5, 86.7, 83.8, 81.2, 72.1, 68.9, 67.8, 61.4, 60.1, 45.4, 27.0 and 25.1. HRMS (ESI, positive) for $C_{17}H_{24}N_4O_7$, calc. 397.1718 $[M + 1]^+$; found 397.1708.

7b, **7c** and **7d** were synthesized, respectively, by a procedure similar to that for **7a**.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-2′,3′-*O*-isopropylidene-8-bromoinosine (7b). A pale yellow solid, 71% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.36 (s, 1 H), 6.00 (d, J = 2.4 Hz, 2 H), 5.55 (dd, J = 2.4 and 6.4 Hz, 1 H), 4.97 (dd, J = 4.0 and 6.4 Hz, 1 H), 4.94 (t, J = 6.0 Hz, 1 H), 4.57–4.56 (t, J = 4.8 Hz, 1 H), 4.17–4.11 (m, 3 H), 3.65 (t, J = 4.2 Hz, 1 H), 3.52–3.30 (m, 6 H), 1.53 and 1.31 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 154.6, 149.4, 148.0, 125.5, 124.1, 113.5, 90.6, 87.3, 82.3, 81.3, 72.1, 67.5, 61.3, 60.1, 45.7, 27.1 and 24.2. HRMS (ESI, positive) for $C_{17}H_{23}N_4O_7Br$, calc. 475.0823 $[M + 1]^+$; found 475.0826.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-2′,3′-*O*-isopropylidene-8-aminoinosine (7c). A pale yellow solid, 76% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.09 (s, 1 H), 6.59 (s, 2 H), 5.99 (d, J = 3.6 Hz, 1 H), 5.38 (dd, J = 3.6 and 4.8 Hz, 1 H), 5.35–5.24 (m, 1 H), 4.96–4.92 (m, 1 H), 4.63–4.57 (m, 3 H), 3.66–3.58 (m, 4 H), 3.46–3.42 (m, 2 H), 1.54 and 1.31 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 154.6, 151.0, 145.6, 145.4, 121.3, 113.5, 87.7, 85.0, 81.4, 80.6, 72.1, 67.9, 61.0, 60.1, 45.4, 27.1 and 25.2. HRMS (ESI, negative) for $C_{17}H_{25}N_5O_7$, calc. 410.1681 $[M - 1]^-$; found 410.1677.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-2′,3′-*O*-isopropylidene-8-oxoinosine (7d). A colorless solid, 73% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1 H), 8.23 (s, 1 H), 5.82 (d, J = 2.4 Hz, 1 H), 5.38 (dd, J = 2.4 and 6.4 Hz, 1 H), 4.84 (dd, J = 2.8 and 6.4 Hz, 2 H), 4.56 (br s, 1 H), 4.14 (t, J = 4.2 Hz, 1 H), 4.05–4.01 (m, 1 H), 3.65 (t, J = 4.2 Hz, 2 H), 3.44–3.40 (m, 5 H), 3.29 (br s, 1 H), 1.49 and 1.29 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 151.3, 150.3, 148.0, 143.0, 112. 9, 107.9, 86.9, 86.4, 81.7, 81.7, 72.1, 67.5, 61.6, 60.1, 45.8, 27.1

and 25.2. HRMS (ESI, positive) for $C_{17}H_{24}N_4O_8$, calc. 396.1878 $[M + 1]^+$, found 396.1862.

Synthesis of 8-substituted N^1-(5′′-*O*-phosphonoxyethoxyethyl)-5′-*O*- (phosphoryl)-2′,3′-*O*-isopropylideneinosines 10a–d

***N*^{1}-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-2′,3′-*O*-isopropylideneinosine (10a).** To a mixture of freshly distilled phosphoryl chloride (358 μL, 3.89 mmol), water (23 μL, 2.48 mmol), pyridine (172 μL, 4.24 mmol) and acetonitrile (3 mL), which was maintained at 2 °C with stirring, and **7a** (175 mg, 0.44 mmol) were added. After the mixture had stood for 4 h, ice cooled TEAB (1 M, 10 mL) was added, and the resulting solution stirred for 1 h at 2 °C. After evaporation, the residue was dissolved in 2 mL TEAB (0.05 M, pH 7.5). The solution was purified by a C18 reversed-phase column (2.2 cm × 25 cm) using a linear gradient of 0–60% CH_3CN in TEAB buffer (0.05 M, pH 7.5) within 30 min to give **10a** (205 mg, 84%) as its triethylammonium salt. ^{1}H NMR (400 MHz, D_2O): δ 8.32, 8.31 (each s, each 1 H), 6.22 (d, J = 2.8 Hz, 1 H), 5.34 (dd, J = 2.8 and 6.0 Hz, 1 H) 5.11 (dd, J = 2.0 and 6.0 Hz, 1 H), 4.60 (br s, 1 H), 4.27 (dd, J = 4.8 and 10.0 Hz, 2 H), 3.99 (t, J = 4.0 Hz, 2 H), 3.88 (dd, J = 6.0 and 9.2 Hz, 2 H), 3.83 (t, J = 4.8 Hz, 2 H), 3.66–3.64 (m, 2 H), 1.59 and 1.37 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O): δ 0.64 and 0.16. HRMS (ESI, negative) for $C_{17}H_{26}N_4O_{13}P_2$, calc. 555.0899 $[M - 1]^-$; found 555.08804.

10b, **10c** and **10d** were synthesized, respectively, by a procedure similar to that for **10a**.

***N*^{1}-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-2′,3′-*O*-isopropylidene-8-chloroinosine (10b).** A white solid, 88% yield. ^{1}H NMR (300 MHz, D_2O) δ 8.37 (s, 1 H), 6.24 (s, 1 H), 5.59 (s, 1 H), 5.14 (s, 1 H), 4.33–4.40 (m, 2 H), 3.86–3.56 (m, 9 H), 1.47 and 1.27 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 2.15 and 2.08. HRMS (ESI, negative) for $C_{17}H_{25}ClN_4O_{13}P_2$, calc. 589.0504, $[M - 1]^-$; found 589.0539.

***N*^{1}-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-2′,3′-*O*-isopropylidene-8-aminoinosine (10c).** A white foam, 91% yield. ^{1}H NMR (400 MHz, D_2O): δ 8.17 (s, 1 H), 6.11 (d, J = 4.4 Hz, 1 H), 5.41 (dd, J = 4.4 and 6.4 Hz, 1 H), 5.15 (dd, J = 3.2 and 6.4 Hz, 1 H), 4.49–4.48 (m, 1 H), 4.28–4.19 (m, 2 H), 4.08–4.07 (m, 2 H), 3.90–3.81 (m, 2 H), 3.84–3.81 (m, 2 H), 1.61 and 1.37 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 2.89 and 2.43. HRMS (ESI, negative) for $C_{17}H_{27}N_5O_{13}P_2$, calc. 570.1008 $[M - 1]^-$; found 570.1014.

***N*^{1}-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-2′,3′-*O*-isopropylidene-8-oxoinosine (10d).** A white foam, 76% yield. ^{1}H NMR (400 MHz, D_2O): δ 8.23 (s, 1 H), 6.06 (d, J = 1.2 Hz, 1 H), 5.57 (dd, J = 1.2 and 6.4 Hz, 1 H), 5.09 (dd, J = 3.6 and 6.4 Hz, 1 H), 4.38–4.34 (m, 1 H), 4.24 (dd, J = 4.8 and 12.8 Hz, 2 H), 3.96–3.86 (m, 4 H), 3.81 (t, J = 4.8 Hz, 2 H), 3.65 (t, J = 4.8 Hz, 2 H), 1.56 and 1.36 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O): δ 0.48. HRMS (ESI, negative) for $C_{17}H_{26}N_4O_{14}P_2$, calc. 571.0848 $[M - 1]^-$; found 571.0868.

Synthesis of 8-substituted N^1-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-inosines 4a–d

***N*^{1}-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-inosine (4a).** A solution of **10a** (40 mg, 72 μmol) in 60% HCOOH (5 mL) was stirred for 8 h and then evaporated under reduced pressure. The purification of the residue was performed by HPLC on a C_{18} reversed phase column, eluting with a linear gradient of 0–40% CH_3CN in TEAB buffer (0.05 M, pH 7.5) within 30 min to give target molecule **4a** (33 mg, 90%) as a white foam. ^{1}H NMR (300 MHz, D_2O): δ 8.38 (s, 1 H), 8.24 (s, 1 H), 5.59 (d, J = 5.7 Hz, 1 H), 4.59 (t, J = 5.7 Hz, 1 H), 4.34–4.31 (m, 1 H), 4.19–4.17 (m, 3 H), 3.86–3.85 (m, 2 H), 3.72–3.68 (m, 4 H) and 3.54–3.51 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 7.15 and 7.00. HRMS (ESI, positive) for $C_{14}H_{22}N_4O_{13}P_2$, calc. 517.0731 $[M + 1]^+$; found 517.0731.

4b, **4c** and **4d** were synthesized, respectively, by a procedure similar to that for **4a**.

***N*^{1}-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-8-chloroinosine (4b).** A white foam, 92% yield. ^{1}H NMR (500 MHz, D_2O): δ 8.38 (s, 1 H), 6.11 (d, J = 6.0 Hz, 1 H), 5.25 (dd, J = 6.0 and 5.5 Hz, 1 H), 4.60 (dd, J = 5.5 and 4.5 Hz, 1 H), 4.40 (dd, J = 4.5 and 9.5 Hz, 1 H), 4.25–4.21 (m, 2 H), 4.18–4.10 (m, 2 H), 3.90–3.88 (m, 4 H) and 3.80–3.62 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 2.55 and 2.47. HRMS (ESI, negative) for $C_{14}H_{21}N_4O_{13}P_2Cl$, calc. 549.0196 $[M - 1]^-$; found 549.0178.

***N*^{1}-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-8-aminoinosine (4c).** A white foam, 93% yield. ^{1}H NMR (400 MHz, D_2O): δ 8.12 (s, 1 H), 5.97 (d, J = 4.4 Hz, 1 H), 5.76 (dd, J = 4.4 and 6.4 Hz, 1 H), 5.46 (dd, J = 3.2 and 6.4 Hz, 1 H), 4.80–4.76 (m, 1 H), 4.37–4.35 (m, 2 H), 4.24–4.19 (m, 2 H), 3.94–3.89 (m, 2 H) and 3.59–3.57 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 4.30 and 4.03. HRMS (ESI, negative) for $C_{14}H_{23}N_5O_{13}P_2$, calc. 530.0695 $[M - 1]^-$; found 530.0701.

***N*^{1}-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-8-oxo-inosine (4d).** A white foam, 84% yield. ^{1}H NMR (500 MHz, D_2O): δ 8.29 (s, 1 H), 5.88 (d, J = 5.5 Hz, 1 H), 5.15 (dd, J = 5.5 and 5.0 Hz, 1 H), 4.55 (t, J = 5.5 Hz, 1 H), 4.36–4.26 (m, 2 H), 4.21 (dd, J = 5.5 and 8.0 Hz, 1 H), 4.11–4.07 (m, 1 H), 4.03–3.98 (m, 1 H), 3.93–3.87 (m, 4 H) and 3.72–3.74 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 3.76. HRMS (ESI, negative) for $C_{14}H_{22}N_4O_{14}P_2$, calc. 531.0535 $[M - 1]^-$; found 531.0534.

N^1-5′′-*O*-[(*N*,*N*-diisopropyl)phosphoryl]ethoxyethyl-5′-*O*-[(*N*,*N*-diisopropyl)phosphoryl]-2′,3′-*O*-isopropylidene-8-bromoinosine (11)

Compound **7b** (122 mg, 0.26 mmol) was dissolved in DCM (5 mL). To the solution was added *N*,*N*-diisopropylethylamine (DIEA) (276 μL, 1.56 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylamino-chlorophosphoramidite (136 μL, 1.44 mmol). The mixture was stirred at room temperature for 20 min. After ^{t}BuOOH (150 μL, 60%) had been added and stirred for a further 1 h, the solution was diluted with EtOAc/NEt_3 (20 mL, 20 : 1, v/v), and then washed with 5% $NaHCO_3$ and a

saturated aqueous NaCl solution three times. The organic phase was dried with Na_2SO_4 and evaporated. The residue was resolved in saturated NH_3/MeOH (5 mL) and stirred at 0 °C for 8 h. After evaporation, the residue was purified by silica gel column chromatography (1 : 9 : 90 NEt_3/MeOH/CH_2Cl_2) to afford compound **11** (179 mg, 87%) as a pale yellow solid. ^{1}H NMR (300 MHz, D_2O): δ 8.18 (s, 1 H), 6.17 (s, 1 H), 5.59 (s, 1 H), 5.13 (s, 1 H), 4.30–4.22 (m, 2 H), 4.04–3.99 (m, 1 H), 3.68–3.46 (m, 8 H), 1.44, 1.26 (each s, each 3 H), 0.81 (s, 3 H), 0.79 (s, 3 H), 0.65 (s, 3 H) and 0.63 (s, 3 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 8.98 and 8.35. HRMS (ESI, negative) for $C_{29}H_{51}N_6O_{11}P_2$, calc. 799.2202 $[M - 1]^-$; found 799.2225.

N^1-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-8-bromoinosine (4e)

In a manner similar to that described for the synthesis of **4a**, **4e** was obtained from **11** in a yield of 36%. ^{1}H NMR (400 MHz, D_2O): δ 8.47 (s, 1 H), 6.19 (d, J = 5.7 Hz, 1 H), 4.73 (t, J = 5.7 Hz, 1 H), 4.53–4.51 (m, 1 H), 4.35–4.28 (m, 3 H), 3.86–3.82 (m, 2 H), 3.80–3.72 (m, 4 H) and 3.58–3.50 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O): δ 0.93. HRMS (ESI, negative) for $C_{14}H_{21}N_4O_{13}P_2Br$, calc. 592.9691 $[M - 1]^-$; found 592.9672.

5-Amino-1-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-(isopropylidene)-2-bromo-β-D-ribofuranosyl]imidazole-4-nitrile (12b)

To a solution of **12a**[23] (418 mg, 1.06 mmol) in THF (10 mL) was added NBS (232 mg, 1.27 mmol). The resulting solution was stirred at rt for 10 min and then evaporated under reduced pressure. The residue was purified by silica gel column chromatography (2% MeOH in CH_2Cl_2) to give compound **12b** (452 mg, 90%) as a pale yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.50 (br s, 2 H), 5.83 (d, J = 4.5 Hz, 1 H), 5.03–5.00 (m, 1 H), 4.80–4.77 (m, 1 H), 4.13 (d, J = 3.3 Hz, 1 H), 3.90 (d, J = 3.3 Hz, 2 H), 1.51, 1.31 (each s, each 3 H), 0.89 (9 H) and 0.11 (6 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.7, 115.6, 114.7, 111. 6, 92.4, 90.2, 83.8, 81.0, 79.1, 62.5, 27.2, 25.9, 25.3, 18.3, −5.4 and −5.6. HRMS (ESI, positive) for $C_{18}H_{29}N_4O_4BrSi$, calc. 473.1214 $[M + 1]^+$; found 473.1213.

5-[(Methoxymethylene)amino]-1-[5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-(isopropylidene)-2-bromo-β-D-ribofuranosyl]imidazole-4-nitrile (13b)

A mixture of **12b** (452 mg, 0.96 mmol) and CF_3CO_2H (10 μL) in methyl orthoformate (10 mL) was stirred under reflux for 0.5 h. After evaporation, the residue was purified by silica gel column chromatography (2% MeOH in CH_2Cl_2) to give compound **13b** (463 mg, 94%) as a pale yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.53 (s, 1 H), 5.85 (d, J = 2.7 Hz, 1 H), 5.34–5.32 (m, 1 H), 4.80–4.76 (m, 1 H), 4.05–4.01 (m, 1 H), 3.95 (s, 3 H), 3.74–3.72 (m, 2 H), 1.51, 1.31 (each s, each 3 H), 0.83 (s, 9 H) and 0.00 (s, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.9, 146.9, 118.8, 114.6, 114.2, 100.0, 90.4, 86.2, 81.9, 80.5, 62.9, 55.4, 27.0, 25.7, 25.3, 18.0, −5.4 and −5.5. HRMS (ESI, positive) for $C_{20}H_{31}N_4O_5BrSi$, calc. 515.1314 $[M + 1]^+$; found 515.1295.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene-8-bromoadenosine (14b)

A mixture of **13b** (463 mg, 0.90 mmol), 2-(hydroxyethoxy)-ethylamine (108 μL, 1.08 mmol) and K_2CO_3 (7 mg, 0.05 mmol) in DMF (4 mL) was stirred at room temperature for 8 h. The mixture was evaporated and the residue purified by silica gel column chromatography (3% MeOH in CH_2Cl_2) to give compound **14b** (489 mg, 92%) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.00 (s, 1 H), 7.19 (br s, 1 H), 5.97 (s, 1 H), 5.58 (d, J = 6.0 Hz, 1 H), 4.95 (s, 1 H), 4.61 (s, 1 H), 4.22–4.13 (m, 3 H), 3.77–3.66 (m, 3 H), 3.45–3.44 (m, 4 H), 1.52, 1.32 (each s, each 3 H), 0.88 (s, 9 H) and 0.00 (s, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 151.9, 149.2, 142.1, 123.1, 113.2, 90.6, 87.6, 82.5, 81.3, 72.2, 66.8, 62.9, 60.1, 46.4, 26.9, 25.7, 25.2, 17.9, −5.5 and −5.6. HRMS (ESI, positive) for $C_{23}H_{38}N_5O_6BrSi$, calc. 588.1848 $[M + 1]^+$; found 588.1862.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-2′,3′-*O*-isopropylideneadenosine (15a)

A mixture of **14a** (630 mg, 1.24 mmol), TBAF (1 M in THF, 12.4 mL, 12.4 mmol) and AcOH (45 μL, 0.7 mmol) in THF (15 mL) was stirred at room temperature for 2 h. The mixture was evaporated and the residue purified by silica gel column chromatography (5% MeOH in CH_2Cl_2) to give compound **15a** (464 mg, 95%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.13, 7.98 (each s, each 1 H), 6.00 (d, J = 3.2 Hz, 1 H), 5.23 (dd, J = 3.2 and 6.4 Hz, 1 H), 4.91 (dd J = 2.4 and 6.4 Hz, 1 H), 4.18 (t, J = 5.2 Hz, 3 H), 3.70 (t, J = 5.2 Hz, 2 H), 3.52 (dd, J = 2.0 and 5.2 Hz, 2 H), 3.39–3.42 (m, 4 H), 1.53 and 1.32 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 153.6, 149.0, 140.9, 137.7, 122.7, 113.1, 89.4, 86.5, 83.6, 81.3, 72.2, 66.9, 61.5, 60.1, 57.5, 46.1, 27.0 and 25.2. HRMS (ESI, positive) for $C_{17}H_{25}N_5O_6$, calc. 396.1878 $[M + 1]^+$; found 396.1865.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-2′,3′-*O*-isopropylidene-8-bromoadenosine (15b)

In a manner similar to that described for the synthesis of **10a**, **15b** was synthesized in 95% yield as a pale yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.36 (s, 1 H), 6.01 (d, J = 2.8 Hz, 1 H), 5.67 (dd, J = 4.0 and 6.4 Hz, 1 H), 4.97 (dd, J = 3.6 and 6.4 Hz, 1 H), 4.94 (t, J = 5.6 Hz, 1 H), 4.58–4.55 (m, 1 H), 4.18–4.10 (m, 3 H), 3.67 (t, J = 4.2 Hz, 2 H), 3.52–3.42 (m, 4 H), 1.54 and 1.32 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 154.6, 149.4, 148.1, 125.5, 124.1, 113.5, 90.6, 87.3, 82.3, 81.3, 72.1, 67.6, 61.3, 60.1, 45.7, 27.1 and 25.2. HRMS (ESI, positive) for $C_{17}H_{24}N_5O_6$, calc. 474.0983 $[M + 1]^+$; found 474.0978.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-2′,3′-*O*-isopropylidene-8-ethylthioadenosine (15c)

To a 1.0 mM suspension of **10b** (236 mg, 0.50 mmol) in H_2O/CH_3CN (4 mL/4 mL) were added 3 equiv. of 2-mercapto-ethanol (105 μL, 1.50 mmol) and 10 equiv. of NEt_3 (0.70 mL, 5 mmol). The resulting clear solution was then refluxed for 2 h. Further purification was achieved by silica gel column chromatography (5% MeOH in CH_2Cl_2) to give compound **15c** (193 mg, 82%) as a colorless solid. ^{1}H NMR (400 MHz,

DMSO-d_6): δ 8.17 (s, 1 H), 7.63 (t, J = 5.6 Hz, 1 H), 5.98 (d, J = 2.8 Hz, 1 H), 5.58 (dd, J = 2.8 and 5.6 Hz, 1 H), 5.20 (s, 1 H), 5.04 (s, 1 H), 4.99 (dd, J = 2.8 and 6.4 Hz, 1 H), 4.59 (s, 1 H), 4.18–4.14 (m, 1 H), 3.73–3.54 (m, 6 H), 3.49–3.34 (m, 8 H), 1.54 and 1.32 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 153.0, 151.6, 149.6, 148.0, 119.6, 113.3, 89.7, 86.5, 81.9, 81.5, 72.1, 68.7, 61.6, 60.2, 59.7, 54.9, 35.3, 27.2 and 25.2. HRMS (ESI, positive) for $C_{19}H_{29}N_5O_7S$, calc. 472.1860 $[M + 1]^+$; found 472.1871.

N^1-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-2′,3′-*O*-isopropylideneadenosine (16a)

In a manner similar to that described for the synthesis of **10a**, **16a** was synthesized in 86% yield as a white solid. ^{1}H NMR (400 MHz, D_2O): δ 8.46, 8.45 (each s, each 1 H), 6.28 (d, J = 2.4 Hz, 1 H), 5.39 (dd, J = 2.4 and 6.0 Hz, 1 H), 5.12 (dd, J = 6.0 and 1.6 Hz, 1 H), 4.67–4.65 (m, 1 H), 4.51 (t, J = 4.4 Hz, 2 H), 3.96–3.95 (m, 4 H), 3.67 (t, J = 4.4 Hz, 2 H), 1.59 and 1.38 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O): δ 0.63 and 0.60. HRMS (ESI, negative) for $C_{17}H_{27}N_5O_{12}P_2$, calc. 554.1059 $[M - 1]^-$; found 554.1084.

N^1-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-2′,3′-*O*-isopropylidene-8-chloroadenosine (16b)

In a manner similar to that described for the synthesis of **10a**, **16b** was synthesized in 90% yield as a white solid. ^{1}H NMR (500 MHz, D_2O): δ 8.59 (s, 1 H), 6.42 (d, J = 1.5 Hz, 1 H), 5.84 (dd, J = 3.5 and 1.5 Hz, 1 H), 5.22 (dd, J = 3.5 and 2.5 Hz, 1 H), 4.60 (m, 2 H), 4.41 (m, 1 H), 4.08–4.03 (m, 4 H), 3.84–3.74 (m, 4 H), 1.63 and 1.44 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ −10.05 and −10.22. HRMS (ESI, positive) for $C_{17}H_{26}N_5ClO_{12}P_2$, calc. 590.0814 $[M + 1]^+$; found 590.0823.

N^1-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-2′,3′-*O*-isopropylidene-8-(phosphorylethylthio)adenosine (16c)

In a manner similar to that described for the synthesis of **10a**, except that 14.4 equiv. of $POCl_3$ was used in the synthesis of **13a**, **16c** was synthesized in 86% yield as a white solid. ^{1}H NMR (500 MHz, D_2O): δ 8.22 (s, 1 H), 6.31 (d, J = 3.0 Hz, 1 H), 5.70 (dd, J = 3.0 and 7.0 Hz, 1 H), 5.25 (dd, J = 7.0 and 4.5 Hz, 1 H), 4.44 (dd, J = 5.0 and 11.5 Hz, 1 H), 4.16 (dd, J = 6.5 and 13.5 Hz, 2 H), 4.12–3.98 (m, 4 H), 3.86–3.77 (m, 6 H), 3.61–3.52 (m, 2 H), 1.66 and 1.43 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 3.66, 3.56 and 3.34. HRMS (ESI, negative) for $C_{19}H_{33}N_5O_{16}P_3S$, calc. 710.0705 $[M - 1]^-$; found 710.0702.

N^1-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)adenosine (4f)

In a manner similar to that described for the synthesis of **4a**, **4f** was synthesized in 90% yield as a white solid. ^{1}H NMR (500 MHz, D_2O): δ 8.77, 8.75 (each s, each 1 H), 6.20 (d, J = 5 Hz, 1 H), 4.47 (dd, J = 5.0 and 4.5 Hz, 1 H), 4.58 (t, J = 5.0 Hz, 2 H), 4.39–4.37 (m, 1 H), 4.08–4.00 (m, 4 H), 3.90 (dd, J = 5.0 and 2.0 Hz, 2 H) and 3.72 (dd, J = 5.0 and 4.0 Hz, 2 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 3.88 and −7.32. HRMS (ESI, negative) for $C_{14}H_{22}N_5O_{12}P_2$, calc. 514.0746 $[M - 1]^-$; found 514.0744.

N^1-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-8-chloroadenosine (4g)

In a manner similar to that described for the synthesis of **4a**, **4g** was synthesized in 92% yield as a white solid. ^{1}H NMR (500 MHz, D_2O): δ 8.54 (s, 1 H), 6.15 (d, J = 5.0 Hz, 1 H), 5.26 (m, 1 H), 4.64 (t, J = 5.0 Hz, 1 H), 4.58–4.55 (m, 2 H), 4.31–4.28 (m, 1 H), 4.11–4.07 (m, 1 H), 4.04–4.01 (m, 3 H), 3.92–3.88 (m, 2 H) and 3.75–3.60 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 3.32 and 3.17. HRMS (ESI, negative) for $C_{14}H_{22}N_5ClO_{12}P_2$, calc. 548.0356 $[M - 1]^-$; found 548.0351.

N^1-(5′′-*O*-Phosphonoxyethoxyethyl)-5′-*O*-(phosphoryl)-8-(phosphorylethylthio)adenosine (4h)

In a manner similar to that described for the synthesis of **4a**, **4h** was synthesized in 89% yield as a white solid. ^{1}H NMR (400 MHz, D_2O): δ 8.08 (s, 1 H), 5.99 (d, J = 3.0 Hz, 1 H), 5.07 (t, J = 6.4 Hz, 1 H), 4.41 (dd, J = 4.0 and 6.0 Hz, 1 H), 4.14 (dd, J = 4.4 and 10.4 Hz, 1 H), 4.06–3.98 (m, 4 H), 4.12–3.98 (m, 4 H), 3.85 (dd, J = 6.0 and 9.2 Hz, 2 H), 3.74–3.64 (m, 6 H) and 3.52–3.47 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ 2.04 and 1.87. HRMS (ESI, negative) for $C_{16}H_{28}N_5O_{16}P_3S$, calc. 670.0392 $[M - 1]^-$; found 670.0367.

N^1-Dinitrobenzene-5′-*O*-*para*-toluenesulfonyl-2′,3′-*O*-isopropylideneinosine (18)

A mixture of N^1-dinitrobenzene-2′,3′-*O*-isopropylideneinosine (**17b**) (948 mg, 2.0 mmol), TsCl (457 mg, 2.4 mmol), NEt_3 (336 μL, 2.4 mmol) and DMAP (catalyst) was suspended in anhydrous DCM (10 mL) and stirred at rt for 8 h. After the addition of a few drops of methanol, the mixture was evaporated to dryness and purified on a silica gel column eluted with increasing amounts of MeOH in CH_2Cl_2 (from 0 to 3%) to give compound **18** as a pale yellow amorphous solid consisting of a 1 : 1 mixture of atropisomers (994 mg, 79%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.95 (t, J = 2.0 Hz, 1 H), 8.84–8.79 (m, 1 H), 8.60 (s, 1 H), 8.31, 8.30 (each s, each 0.5 H), 8.11 (t, J = 9.6 Hz, 1 H), 7.71–7.68 (m, 2 H), 7.35 (dd, J = 5.6 and 8.0 Hz, 2 H), 6.22, 6.18 (each d, J = 2.4 Hz, each 0.5 H), 5.35, 5.32 (each dd, J = 2.4 and 6.0 Hz, each 0.5 H), 4.97–4.94 (m, 1 H), 4.37–4.30 (m, 2 H), 4.24–4.21 (m, 1 H), 2.35, 2.34 (each s, each 1.5 H), 1.52 and 1.35 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 155.4, 155.4, 148.3, 147.8, 147.7, 147.6, 147.4, 146.2, 145.7, 145.6, 140.8, 140.6, 135.4, 135.3, 133.0, 132.9, 132.3, 132.2, 130.5, 130.4, 129.9, 129.8, 128.1, 123.8, 121.2, 114.3, 114.2, 89.6, 89.5, 84.2, 84.0, 80.9, 80.8, 70.2, 70.1, 27.3, 27.3, 25.6, 25.7 and 21.5. HRMS (ESI, positive) for $C_{26}H_{24}N_6O_{11}S$, calc. 629.1297 $[M + 1]^+$; found 629.1294.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylideneinosine (22a)

In a manner similar to that described for the synthesis of **7a**, **22a** was synthesized from 5′-*O*-TBDMS-2′,3′-*O*-isopropylideneinosine

in 80% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.32, 8.22 (each s, each 1 H), 6.12 (d, J = 2.0 Hz, 1 H), 5.30 (dd, J = 2.0 and 6.0 Hz, 1 H), 4.90 (dd, J = 2.4 and 6.0 Hz, 1 H), 4.56 (t, J = 5.2 Hz, 1 H), 4.24–4.17 (m, 3 H), 3.74–3.64 (m, 3 H), 3.44–3.41 (m, 4 H), 1.52, 1.32 (each s, each 3 H), 0.79 (s, 9 H) and −0.04 (s, 6 H). HRMS (ESI, positive) for $C_{23}H_{38}N_4O_7Si$, calc. 511.2582 [M + 1]$^+$; found 511.2578.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene-8-bromoinosine (22b)

In a manner similar to that described for the synthesis of **7a**, **22b** was synthesized from 5′-*O*-TBDMS-2′,3′-*O*-isopropylidene-8-bromoinosine in 84% yield as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.37 (s, 1 H), 6.05 (d, J = 2.0 Hz, 1 H), 5.62 (dd, J = 2.0 and 6.0 Hz, 1 H), 4.99 (dd, J = 2.5 and 6.0 Hz, 1 H), 4.56 (d, J = 5.5 Hz, 1 H), 4.20–4.15 (m, 3 H), 3.70–3.62 (m, 4 H), 3.46–3.42 (m, 4 H), 1.54, 1.33 (each s, each 3 H), 0.79 (s, 9 H) and 0.00 (s, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 154.5, 149.3, 147.9, 125.8, 124.1, 113.4, 90.6, 87.6, 82.5, 81.2, 72.1, 67.6, 62.9, 60.1, 45.7, 27.0, 25.6, 25.2, 17.9 and −5.6. Elemental analysis: $C_{23}H_{37}BrN_4O_7Si$ requires C, 46.86; H, 6.33; N, 9.50. Found: C, 47.10; H, 6.38; N, 9.51.

N^1-(5′′-Monomethoxytrityloxyethoxyethyl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylideneinosine (23a)

A mixture of **22a** (820 mg, 1.61 mmol) and MMTrCl (743 mg, 2.41 mmol) in pyridine (10 mL) was stirred at room temperature for 8 h. The mixture was evaporated, and the residue 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 (2% MeOH in CH_2Cl_2) to give compound **23a** (1.21 g, 96%) as a pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.34. 8.27 (each s, each 1 H), 7.34–7.28 (m, 12 H), 6.91–6.87 (m, 2 H), 6.09 (s, 1 H), 5.42 (dd, J = 4.0 and 6.4 Hz, 1 H), 5.16 (dd, J = 4.0 and 6.4 Hz, 1 H), 4.48–4.42 (m, 2 H), 4.35–4.31 (m, 1 H), 4.02–3.99 (m, 3 H), 3.78–3.72 (m, 4 H), 3.36–3.28 (m, 1 H), 3.17–3.12 (m, 1 H), 1.57, 1.34 (each s, each 3 H), 1.04 (s, 9 H) and 0.10 (s, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.0, 154.5, 149.3, 147.2, 145.0, 135.0, 131.5, 129.8, 129.4, 129.0, 127.9, 127.4, 127.3, 126.5, 125.4, 113.4, 113.1, 90.3, 85.3, 85.2, 83.1, 80.4, 69.8, 67.7, 62.6, 61.4, 55.0, 46.0, 27.0, 26.7, 25.0, 21.1 and −5.3. HRMS (ESI, positive) for $C_{43}H_{54}N_4O_8Si$, calc. 783.3789 [M + 1]$^+$; found 783.3772.

N^1-(5′′-Monomethoxytrityloxyethoxyethyl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene-8-bromoinosine (23b)

In a manner similar to that described for the synthesis of **23a**, **23b** was synthesized in 92% yield as a pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (s, 1 H), 7.64–7.48 (m, 12 H), 7.14–7.12 (m, 2 H), 6.29 (s, 1 H), 5.52 (dd, J = 4.0 and 6.4 Hz, 1 H), 5.18 (dd, J = 4.0 and 6.4 Hz, 1 H), 4.62–4.51 (m, 2 H), 4.42–4.38 (m, 1 H), 4.02 (s, 3 H), 3.95–3.80 (m, 4 H), 3.38–3.34 (m, 1 H), 3.28–3.25 (m, 1 H), 1.78, 1.40 (each s, each 3 H), 1.05 (s, 9 H) and 0.15 (s, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.0, 154.5, 149.4, 147.8, 144.2, 135.0, 129.8, 127.9, 127.7, 126.7, 125.9, 124.1, 113.2, 113.1, 90.5, 87.9, 85.4, 82.5, 81.2, 69.7, 67.8, 63.0, 62.7, 55.0, 45.9, 26.9, 25.6, 25.0, 17.9 and −5.6. HRMS (ESI, positive) for $C_{43}H_{53}N_4O_8SiBr$, calc. 861.2894 [M + 1]$^+$; found 861.2872.

N^1-(5′′-Monomethoxytrityloxyethoxyethyl)-2′,3′-*O*-isopropylideneinosine (24a)

In a manner similar to that described for the synthesis of **14a**, **24a** was synthesized in 91% yield as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.39, 8.34 (each s, each 1 H), 7.36–7.20 (m, 12 H), 6.89–6.86 (m, 2 H), 6.09 (d, J = 2.4 Hz, 1 H), 5.15–5.09 (m, 2 H), 4.91–4.88 (m, 1 H), 4.31–4.20 (m, 3 H), 3.74 (s, 3 H), 3.58–3.51 (m, 5 H), 3.05–3.00 (m, 2 H), 1.52 and 1.24 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.1, 155.8, 149.1, 147.0, 144.3, 139.2, 135.0, 129.9, 127.9, 127.8, 126.7, 123.7, 113.1, 113.0, 89.4, 86.8, 85.5, 83.8, 81.2, 69.7, 68.0, 62.8, 61.4, 55.0, 45.5, 26.9 and 25.0. HRMS (ESI, positive) for $C_{37}H_{40}N_4O_8$, calc. 669.2919 [M + 1]$^+$; found 669.2908.

N^1-(5′′-Monomethoxytrityloxyethoxyethyl)-2′,3′-*O*-isopropylidene-8-bromoinosine (24b)

In a manner similar to that described for the synthesis of **14a**, **24b** was synthesized in 87% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.41 (s, 1 H), 7.33–7.17 (m, 12 H), 6.84–6.82 (m, 2 H), 5.95 (d, J = 2.0 Hz, 1 H), 5.14 (dd, J = 2.0 and 6.0 Hz, 1 H), 4.89 (t, J = 6.0 Hz, 1 H), 4.84 (dd, J = 4.0 and 6.0 Hz, 1 H), 4.35–4.30 (m, 1 H), 4.21–4.14 (m, 1 H), 4.08–4.04 (m, 1 H), 3.72 (s, 3 H), 3.62–3.38 (m, 4 H), 3.31 (s, 1 H), 3.07–3.02 (m, 1 H), 2.98–2.94 (m, 1 H), 1.48 and 1.09 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.1, 154.6, 149.5, 147.9, 144.2, 144.2, 135.1, 129.8, 127.9, 127.7, 126.7, 125.6, 124.2, 113.3, 113.1, 90.5, 87.7, 85.4, 82.4, 81.3, 69.7, 67.7, 62.7, 61.4, 55.0, 54.9, 45.9, 27.0 and 25.0. HRMS (ESI, positive) for $C_{37}H_{39}N_4O_8Br$, calc. 747.2024 [M + 1]$^+$; found 747.2037.

N^1-(5′′-Monomethoxytrityloxyethoxyethyl)-5′-*O*-(*para*-toluenesulfonyl)-2′,3′-*O*-isopropylideneinosine (20a)

To a stirred solution of **24a** (110 mg, 0.16 mmol) in DCM (5 mL) were added NEt_3 (0.05 mL, 0.32 mmol), TsCl (50 mg, 0.24 mmol) and DMAP (catalyst). After stirring at rt for 8 h, the mixture was diluted with DCM, washed with brine, dried (Na_2SO_4) and evaporated. The residue was purified by silica gel column chromatography to give compound **20a** (121 mg, 89%) as a pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.38, 8.27 (each s, each 1 H), 7.34–7.18 (m, 12 H), 6.86, 6.84 (each s, each 1 H), 6.12 (d, J = 1.6 Hz, 1 H), 5.17 (dd, J = 2.0 and 6.0 Hz, 1 H), 4.91 (dd, J = 3.6 and 6.0 Hz, 1 H), 4.32–4.06 (m, 5 H), 3.72–3.71 (m, 5 H), 3.56–3.54 (m, 2 H), 3.03–2.98 (m, 2 H), 1.92, 1.50 and 1.23 (each s, each 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.4, 158.6, 156.3, 149.7, 147.4, 144.8, 140.0, 135.6, 130.3, 128.4, 128.2, 127.2, 124.4, 113.9, 113.6, 89.5, 86.7, 85.9, 84.3, 84.0, 81.3, 70.2, 68.4, 64.1, 63.2, 55.5, 46.1, 27.4, 25.5 and 20.9. Elemental analysis: $C_{44}H_{46}N_4O_{10}S$ requires C, 64.22; H, 5.63; N, 6.81. Found: C, 64.09; H, 5.88; N, 7.02.

N^1-(5′′-Monomethoxytrityloxyethoxyethyl)-5′-*O*-(*para*-toluenesulfonyl)-2′,3′-*O*-isopropylidene-8-bromoinosine (20b)

In a manner similar to that described for the synthesis of **20a**, **20b** was synthesized in 85% yield as a pale yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.40 (s, 1 H), 7.32–7.21 (m, 16 H), 6.85–6.84 (m, 2 H), 5.98 (s, 1 H), 5.16 (d, J = 3.3 Hz, 1 H), 4.88–4.78 (m, 1 H), 4.36–4.35 (m, 2 H), 3.70 (s, 3 H), 3.58–3.47 (m, 7 H), 3.15–3.09 (m, 1 H), 2.32 (s, 3 H), 1.54 and 1.32 (each s, each 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.0, 154.5, 149.3, 147.2, 145.0, 144.2, 135.0, 131.5, 129.8, 129.4, 127.9, 127.7, 127.4, 125.6, 124.0, 113.4, 113.1, 90.5, 85.3, 84.0, 80.3, 70.1, 68.4, 62.6, 55.0, 46.0, 26.7, 25.0, 24.8 and 21.1. HRMS (ESI, positive) for $C_{44}H_{45}N_4O_{10}S$, calc. 901.2118 $[M + 1]^+$; found 901.2134.

N^1-(5′′-Monomethoxytrityloxyethoxyethyl)-5′-*O*-pyrophosphate-2′,3′-*O*-isopropylideneinosine (21a)

To a suspension of tris(tetrabutylammonium)hydrogen pyrophosphate (263 mg, 0.60 mmol) in 3 mL of acetonitrile was added dropwise a solution of **20a** (121 mg, 0.15 mmol) in 2 mL of acetonitrile. The reaction mixture was stirred at room temperature for 16 h and the solvent then removed by rotary evaporation. The residue was dissolved in 2 mL of TEAB buffer (1 M, pH 7.5), evaporated to dryness, and partitioned between H_2O and $CHCl_3$. The aqueous layer was washed with $CHCl_3$ (3 × 5 mL) and evaporated. The residue was dissolved in 1 mL of TEAB buffer (0.05M, pH 7.5), applied to a C18 reversed-phase column (2.2 cm × 25 cm) and developed by a linear gradient of 0–80% CH_3CN in TEAB buffer (0.05 M, pH 7.5) within 30 min to give **21a** (29 mg, 20%) as its triethylammonium salt. ^{1}H NMR (500 MHz, D_2O): δ 8.21, 8.10 (each s, each 1 H), 7.28–7.11 (m, 12 H), 6.72, 6.69 (each s, each 1 H), 6.35 (d, J = 7.5 Hz, 1 H), 5.80–5.75 (m, 1 H), 5.20 (dd, J = 2.0 and 4.5 Hz, 1 H), 4.41–4.36 (m, 1 H), 4.23–4.20 (m, 1 H), 4.03–4.00 (m, 2 H), 3.85–3.84 (m, 2 H), 3.80 (s, 3 H), 3.77–3.72 (m, 2 H), 3.63–3.60 (m, 2 H), 1.52 and 1.30 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ −9.89 and −10.07. HRMS (ESI, negative) for $C_{37}H_{42}N_4O_{14}P_2$, calc. 827.2095 $[M - 1]^-$; found 827.2065.

N^1-(5′′-Monomethoxytrityloxyethoxyethyl)-5′-*O*-pyrophosphate-2′,3′-*O*-isopropylidene-8-bromoinosine (21b)

In a manner similar to that described for the synthesis of **21a**, **21b** was synthesized in 74% yield as a pale yellow foam. ^{1}H NMR (400 MHz, D_2O): δ 8.49 (s, 1 H), 7.18–7.06 (m, 12 H), 6.72 (d, J = 8.8 Hz, 2 H), 5.92 (s, 1 H), 5.13 (t, J = 5.5 Hz, 1 H), 4.73–4.70 (m, 1 H), 4.56–4.53 (m, 1 H), 4.35–4.32 (m, 2 H), 3.96–3.94 (m, 2 H), 3.82–3.78 (m, 4 H), 3.75 (s, 3 H), 3.70–3.64 (m, 2 H), 1.54 and 1.31 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O): δ −13.39 and −13.90. HRMS (ESI, negative) for $C_{37}H_{41}N_4O_{14}P_2Br$, calc. 905.1202 $[M - 1]^-$; found 905.1213.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-5′-*O*-pyrophosphateinosine (5a)

In a manner similar to that described for the synthesis of **6a**, **5a** was synthesized in 90% yield as a white foam. ^{1}H NMR (400 MHz, D_2O): δ 8.26, 8.12 (each s, each 1 H), 6.16 (d, J = 6.5 Hz, 1 H), 5.25 (t, J = 5.5 Hz, 1 H), 4.48–4.45 (m, 1 H), 4.33–4.30 (m, 1 H), 4.24–4.18 (m, 2 H), 4.15–4.09 (m, 2 H), 3.85–3.73 (m, 4 H) and 3.70–3.67 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O): δ 3.31 and −7.12. HRMS (ESI, negative) for $C_{14}H_{22}N_4O_{13}P_2$, calc. 515.0586 $[M - 1]^-$; found 515.0586.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-5′-*O*-pyrophosphate-8-bromoinosine (5b)

In a manner similar to that described for the synthesis of **6a**, **5b** was synthesized in 89% yield as a pale yellow foam. ^{1}H NMR (500 MHz, D_2O): δ 8.36 (s, 1 H), 6.23 (d, J = 6.5 Hz, 1 H), 5.15 (t, J = 5.5 Hz, 1 H), 4.58–4.56 (m, 1 H), 4.39–4.34 (m, 1 H), 4.28–4.20 (m, 2 H), 4.00–3.98 (m, 2 H), 3.95–3.87 (m, 4 H) and 3.76–3.72 (m, 2 H); ^{31}P NMR (121.5 MHz, D_2O): δ −10.12 and −10.31. HRMS (ESI, negative) for $C_{14}H_{21}N_4O_{13}P_2Br$, calc. 592.9691 $[M - 1]^-$; found 592.9678.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-5′-*O*-monomethoxytrityl-2′,3′-*O*-isopropylideneinosine (25a)

In a manner similar to that described for the synthesis of **7a**, **25a** was synthesized from 5′-*O*-monomethoxytrityl-2′,3′-*O*-isopropylideneinosine in 72% yield as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21, 8.11 (each s, each 1 H), 7.32–7.15 (m, 12 H), 6.81–6.81 (d, J = 10.2 Hz, 2 H), 6.19 (d, J = 2.0 Hz, 1 H), 5.75 (s, 1 H), 5.34 (dd, J = 2.0 and 6.4 Hz, 1 H), 4.59 (t, J = 4.2 Hz, 1 H), 4.35–4.31 (m, 1 H), 4.23–4.18 (m, 1 H), 4.15–4.11 (m, 1 H), 3.73 (s, 3 H), 3.67–3.64 (m, 2 H), 3.48–3.42 (m, 3 H), 3.27–3.22 (m, 1 H), 3.11–3.08 (m, 1 H), 1.53 and 1.31 (each s, each 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.6, 156.3, 149.3, 147.2, 144.5, 144.4, 140.1, 135.1, 130.4, 128.3, 128.3, 128.2, 128.2, 127.3, 127.2, 124.5, 113.8, 113.6, 89.6, 86.4, 86.0, 84.0, 81.6, 72.6, 68.3, 64.4, 60.6, 55.5, 55.3, 45.9, 27.4 and 25.7. HRMS (ESI, positive) for $C_{37}H_{40}N_4O_8$, calc. 669.2919 $[M + 1]^+$; found 669.2943.

N^1-[(5′′-Hydroxyl)ethoxyethyl]-5′-*O*-monomethoxytrityl-2′,3′-*O*-isopropylidene-8-bromoinosine (25b)

In a manner similar to that described for the synthesis of **7a**, **25b** was synthesized from 5′-*O*-monomethoxytrityloxy-2′,3′-*O*-isopropylidene-8-bromoinosine in 75% yield as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.11 (s, 1 H), 7.27–6.72 (m, 14 H), 6.08 (d, J = 1.2 Hz, 1 H), 5.54 (d, J = 6.0 Hz, 1 H), 4.94 (dd, J = 1.2 and 4.8 Hz, 1 H), 4.63–4.57 (m, 1 H), 4.38–4.34 (m, 1 H), 4.25–4.23 (m, 1 H), 4.01 (s, 3 H), 3.50–3.38 (m, 5 H), 3.23–3.17 (m, 1 H), 3.02–2.98 (m, 1 H), 1.54 and 1.30 (each s, each 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.1, 154.4, 148.9, 147.7, 144.0, 143.9, 134.7, 129.8, 127.8, 127.7, 127.7, 126.8, 126.7, 125.7, 124.0, 113.4, 113.0, 90.6, 86.5, 85.7, 82.6, 81.4, 72.1, 67.6, 63.7, 60.1, 55.0, 45.6, 26.9 and 25.2. HRMS (ESI, positive) for $C_{37}H_{39}BrN_4O_8$, calc. 747.2024 $[M + 1]^+$; found 747.2046.

N^1-[(5′′-*para*-Toluenesulfonyl)ethoxyethyl]-5′-*O*-monomethoxytrityl-2′,3′-*O*-isopropylideneinosine (26a)

In a manner similar to that described for the synthesis of **20a**, **26a** was synthesized in 85% yield as a pale yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23 (s, 1 H), 8.08 (s, 1 H), 7.74–7.72 (m, 2 H), 7.28–7.14 (m, 12 H), 6.82–6.80 (m, 2 H), 6.21 (s, 1 H), 5.34 (d, J = 6.0 Hz, 1 H), 4.92–4.88 (m, 1 H), 4.35–4.30 (m, 1 H), 4.18–4.15 (m, 2 H), 4.08–4.02 (m, 3 H),

3.72 (s, 3 H), 3.61–3.52 (m, 4 H), 3.25–3.22 (m, 1 H), 3.08–3.03 (m, 1 H), 2.38 (s, 3 H), 1.52 and 1.29 (each s, each 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.6, 155.1, 149.5, 147.4, 145.2, 144.6, 134.8, 131.3, 129.1, 128.9, 127.8, 127.5, 127.2, 125.8, 124.6, 113.5, 113.0, 90.8, 85.7, 84.2, 79.8, 71.9, 68.2, 62.9, 55.6, 46.3, 26.8, 25.4, 24.6 and 21.0. HRMS (ESI, positive) for $C_{44}H_{46}N_4O_{10}S$, calc. 823.3007 $[M + 1]^+$; found 823.3009.

N^1-[(5′′-*para*-Toluenesulfonyl)ethoxyethyl]-5′-*O*-monomethoxytrityl-2′,3′-*O*-isopropylidene-8-bromoinosine (26b)

In a manner similar to that described for the synthesis of **20a**, **26b** was synthesized in 85% yield as a pale yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.00 (s, 1 H), 7.74–6.78 (m, 18 H), 6.08 (s, 1 H), 5.54 (d, J = 3.3 Hz, 1 H), 4.98–4.95 (m, 1 H), 4.41–4.32 (m, 1 H), 4.26–4.20 (m, 1 H), 4.03 (s, 3 H), 3.58–3.37 (m, 7 H), 3.18–3.15 (m, 1 H), 3.03–2.99 (m, 1 H), 2.40 (s, 3 H), 1.54 and 1.31 (each s, each 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.9, 155.8, 150.7, 148.2, 146.1, 144.7, 135.3, 131.2, 129.5, 129.3, 127.2, 127.0, 126.8, 125.4, 124.2, 113.6, 113.4, 90.7, 85.7, 83.8, 80.2, 70.6, 68.9, 62.7, 55.8, 46.3, 26.9, 25.6 and 24.6. HRMS (ESI, positive) for $C_{44}H_{45}N_4O_{10}SBr$, calc. 901.2118 $[M + 1]^+$; found 901.2109.

N^1-[(5′′-*O*-Pyrophosphate)ethoxyethyl]-5′-*O*-monomethoxytrityl-2′,3′-*O*-isopropylideneinosine (27a)

In a manner similar to that described for the synthesis of **15a**, **27a** was synthesized in 21% yield as a pale yellow solid. ^{1}H NMR (500 MHz, D_2O): δ 8.23, 8.09 (each s, each 1 H), 7.23–7.05 (m, 12 H), 6.72, 6.67 (each s, each 1 H), 6.32 (d, J = 7.5 Hz, 1 H), 5.78–5.64 (m, 1 H), 5.16 (dd, J = 2.0 and 4.5 Hz, 1 H), 4.9–4.28 (m, 1 H), 4.22–4.16 (m, 1 H), 4.05–4.01 (m, 2 H), 3.88–3.84 (m, 2 H), 3.81 (s, 3 H), 3.79–3.75 (m, 2 H), 3.68–3.66 (m, 2 H), 1.62 and 1.40 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ −9.91 and −10.09. HRMS (ESI, negative) for $C_{37}H_{42}N_4O_{14}P_2$, calc. 827.2095 $[M - 1]^-$; found 827.2071.

N^1-[(5′′-*O*-Pyrophosphate)ethoxyethyl]-5′-*O*-monomethoxytrityl-2′,3′-*O*-isopropylidene-8-bromoinosine (27b)

In a manner similar to that described for the synthesis of **15a**, **27b** was synthesized in 83% yield as a pale yellow solid. ^{1}H NMR (500 MHz, D_2O): δ 8.09 (s, 1 H), 7.32–7.17 (m, 12 H), 6.79, 6.77 (each s, each 1 H), 6.36 (d, J = 8.5 Hz, 1 H), 5.95–5.75 (m, 1 H), 5.16 (dd, J = 2.0 and 4.5 Hz, 1 H), 4.42–4.30 (m, 1 H), 4.24–4.16 (m, 1 H), 4.06–4.00 (m, 2 H), 3.88–3.84 (m, 2 H), 3.81 (s, 3 H), 3.79–3.75 (m, 2 H), 3.68–3.66 (m, 2 H), 1.62 and 1.40 (each s, each 3 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ −10.10 and −10.28. HRMS (ESI, negative) for $C_{37}H_{41}N_4O_{14}P_2Br$, calc. 905.1205 $[M - 1]^-$; found 905.1205.

N^1-[(5′′-*O*-Pyrophosphate)ethoxyethyl]inosine (5c)

In a manner similar to that described for the synthesis of **4a**, **5c** was synthesized in 82% yield as a white solid. ^{1}H NMR (500 MHz, D_2O): δ 8.38, 8.12 (each s, each 1 H), 6.07 (d, J = 6.5 Hz, 1 H), 5.12 (t, J = 5.5 Hz, 1 H), 4.56–4.44 (m, 1 H), 4.37–4.29 (m, 1 H), 4.28–4.16 (m, 2 H), 3.98–3.95 (m, 2 H), 3.90–3.81 (m, 4 H) and 3.62–3.54 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ −9.87 and −10.05. HRMS (ESI, negative) for $C_{14}H_{22}N_4O_{13}P_2$, calc. 515.0586 $[M - 1]^-$; found 515.0612.

N^1-[(5′′-*O*-Pyrophosphate)ethoxyethyl]-8-bromoinosine (5d)

In a manner similar to that described for the synthesis of **4a**, **5d** was synthesized in 91% yield as a white solid. ^{1}H NMR (500 MHz, D_2O): δ 8.38 (s, 1 H), 6.11 (d, J = 6.5 Hz, 1 H), 5.17 (t, J = 5.5 Hz, 1 H), 4.65–4.43 (m, 1 H), 4.39–4.32 (m, 1 H), 4.29–4.21 (m, 2 H), 4.05–3.99 (m, 2 H), 3.93–3.84 (m, 4 H) and 3.78–3.69 (m, 2 H). ^{31}P NMR (121.5 MHz, D_2O, decoupled with H): δ −6.99 and −8.46. HRMS (ESI, negative) for $C_{14}H_{21}N_4O_{13}P_2Br$, calc. 592.9691 $[M - 1]^-$; found 592.9684.

Acknowledgements

This study was supported by the National Natural Sciences Foundation of China (Grant no. 90713005) and the Ministry of Education of China (Grant no. 200800010078).

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