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Total Synthesis of a Cyclic Adenosine 5′-Diphosphate Ribose Receptor Agonist

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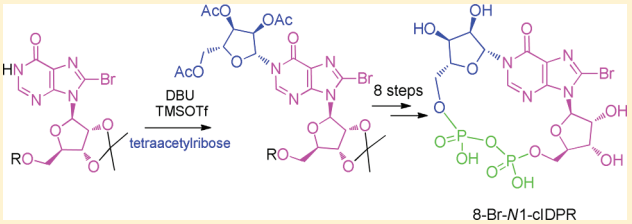
Supporting Information

ABSTRACT: Stable cyclic adenosine 5′-diphosphate ribose (cADPR) analogues are chemical biology tools that can probe the Ca^{2+} release mechanism and structure−activity relationships of this emerging potent second messenger. However, analogues with an intact "northern" ribose have been inaccessible due to the difficulty of generating the sensitive *N*1-ribosyl link. We report the first total synthesis of the membrane permeant, hydrolytically stable, cADPR receptor agonist 8-Br-*N*1-cIDPR via regio- and stereoselective *N*1-ribosylation of protected 8-bromoinosine.



INTRODUCTION

Intracellular Ca^{2+} signaling controls a diverse range of highly regulated cellular processes, from gene transcription and muscle contraction to fertilization, cell proliferation and apoptosis.[1] Cyclic adenosine 5′-diphosphate ribose (cADPR, **1**, Figure 1) is an emerging principal second messenger,[2−5] like the well characterized IP_3, that mobilizes intracellular Ca^{2+}. The cADPR/Ca^{2+} signaling system is active in diverse mammalian cellular systems such as smooth, skeletal and cardiac muscle, acinar cells, as well as in protozoa and plant cells.[5] cADPR is a cyclic dinucleotide that is produced enzymatically from nicotinamide adenine dinucleotide (NAD^+) by ADP-ribosyl cyclases (Figure 1). It is readily hydrolyzed at the labile *N*1 link to give inactive linear adenosine 5′-diphosphoribose in both neutral aqueous solution and under physiological conditions.[6,7] Therefore, the synthesis of stable analogues is particularly important. We have previously reported a chemo-enzymatic route to cyclic inosine 5′-diphosphate ribose (*N*1-cIDPR, **2**) a chemically and biologically stable cADPR analogue.[8] *N*1-cIDPR acts as an agonist with equivalent potency to cADPR in permeablized T-cells. In order to generate this desired *N*1 analogue using the enzyme *Aplysia californica* cyclase, *N*1-cIDPR had to be accessed via its 8-bromo derivative, 8-Br-cIDPR **3**, later shown to be the first membrane permeant agonist of the cADPR receptor.[9,10]

The retention of activity and the chemical and biological stability afforded by the $6NH_2 \rightarrow O$ substitution make the cIDPR scaffold a key template for exploring cADPR structure−activity relationships (SAR) and for structure-based inhibitor design. Indeed, it has been used in cocrystallization studies with native human CD38 to explore the mechanism of cADPR hydrolysis[11] and to engineer potent inhibitors in a rational fashion.[12] However, the chemo-enzymatic route used to prepare these analogues relies on the recognition of an NAD^+ derivative by *Aplysia californica* cyclase and the correct orientation of substrate within the active site to close the 18-membered macrocycle. This limitation is demonstrated by adenine base modifications in NAD^+ that generate biologically inactive, *N*7-cyclized products.[13,14] Total synthetic approaches have included the preparation of analogues with a carbocyclic "northern" ribose (cADPcR[15] and cIDPcR[16,17]), replacement of the "northern" ribose, or both riboses, by an alkyl or ether bridge (cIDPRE and cIDPDE)[18,19] and attaching the "northern" ribose though C-2″.[20] Thus far, all such reported routes have required considerable modification of the "northern" ribose. In Jurkat T-cells, replacement of the "northern" furanose oxygen as in cADPcR or replacement of the entire ribose with an alkyl or ether bridge, gave considerably weaker agonists.[6,18,19,21] These results, and ligand−protein crystal structures obtained using *N*1-cIDPR,[11] both suggest that the "northern" ribose provides key interactions with the binding site. In contrast, more minor changes to the "northern" ribose, such as modification of a single hydroxyl (e.g., 2″-NH_2-cADPR[6]) and changes to the "southern" ribose (e.g., cyclic aristeromycin diphosphoribose[22]) both generated analogues that are equipotent or slightly more active than cADPR and illustrated key SAR features.[6,7] Since the *N*1-ribose motif is the locus of both cADPR formation and degradation it is likely that retaining the complete "northern" ribose motif is crucial for optimal cADPR analogue activity.

A total synthesis that retains an intact "northern" ribose is synthetically challenging, as it requires regio- and stereospecific generation of the sensitive *N*1 glycosidic link. Chemical ribosylation of inosine is limited to basic conditions, under which both the *N*1 and *O*6 positions are nucleophilic.[23] Under

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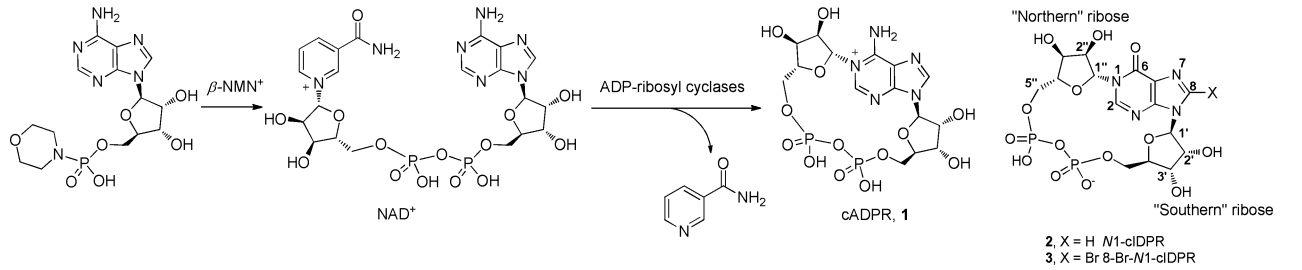
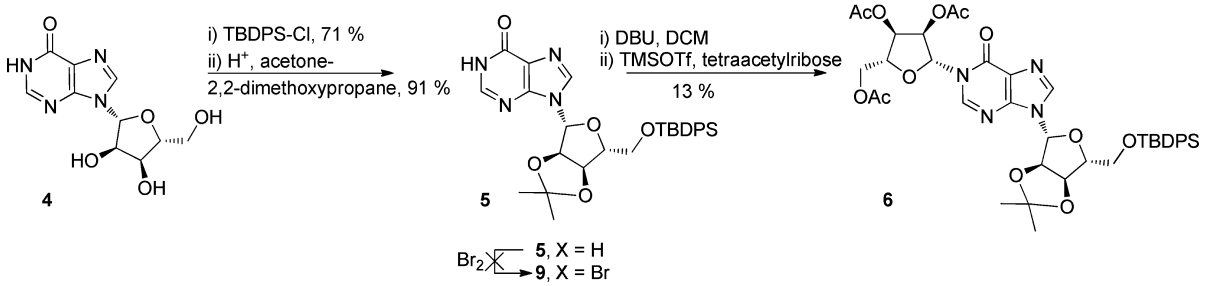


Figure 1. Formation of cADPR **1** by ADP-ribosyl cyclases and the structure of stable analogues cIDPR **2** and 8-Br-cIDPR **3**.

Scheme 1. Application of Modified Vorbrüggen Glycosylation Conditions to Introduce a "Northern" Ribose



Mitsunobu conditions, ribosylation of inosine gave a 5:1 mixture of two products, favoring the undesired *O*6 regioisomer and, under phase transfer conditions, ribosylation of inosine with 2,3,5-tri-*O*-benzoyl-1-bromoribose was also reported to form a mixture of both *N*1 and *O*6 regioisomers.[24] To address this unmet need, we report here the first total synthesis of the cADPR receptor agonist, 8-Br-*N*1-cIDPR **3**, via regio- and stereoselective *N*1-ribosylation of a protected inosine derivative.

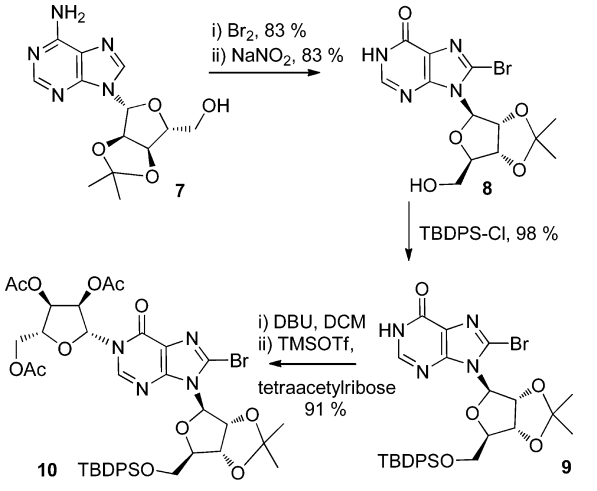
RESULTS AND DISCUSSION

The *N*1 link of previously reported carbocyclic analogues was prepared using substitution of *N*1-2,4-dinitrophenyl purines by alkylamines.[16,17,25] Both a protected *N*1-2,4-dinitrophenylinosine, and the reportedly more reactive *N*1-nitroinosine, were prepared. However, neither of these analogues reacted with a protected ribosylamine. Subsequently, alternative conditions to introduce an intact ribose were sought from the area of nucleoside synthesis. Glycosylation of purine bases at *N*9 to generate a variety of nucleosides is widely reported, and it was hypothesized that these conditions could be exploited to effect a second glycosylation of inosine in the *N*1-position. Therefore, we prepared protected inosine **5** by silylation of the 5′-*O*H, followed by introduction of an 2′,3′-*O*-isopropylidene ketal, Scheme 1.

The reaction of 5 both with a protected α-chlororibofuranose, and under phase transfer glycosylation conditions,[26] gave a complex mixture which included both *N*1 and *O*6 products and unreacted starting material. In all cases, despite utilizing a wide variety of bases and solvents, both *N*1 and *O*6 mixtures were obtained. Therefore, further optimization was not attempted, and we sought alternative conditions that would render only the *N*1 position nucleophilic. We predicted that treatment of **5** under modified Vorbrüggen conditions[27] would effect deprotonation at *N*1 and that under silylating conditions this would generate the *O*6 silyl ether, therefore favoring *N*1, rather than *O*6, alkylation. However, treatment of protected inosine **5** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), followed by trimethylsilyl triflate (TMSOTf) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, afforded a maximum of only 13% of a ribosylated product, **6** (Scheme 1). During their studies into the mechanism of glycosylation for the synthesis of adenosine, Framski et al. reported initial kinetic ribosylation of *N*6-protected adenine at *N*1 under similar conditions.[28] We were encouraged by the observation that, when TMSOTf was added to deprotonated **5** and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose at −78 °C, **6** was formed as a single product upon warming to rt.

Starting from 2′,3′-*O*-isopropylidine adenosine **7**, an 8-bromo substituent was introduced using a solution of bromine in aqueous sodium hydrogen phosphate buffer (Scheme 2).[29] Direct bromination of identically protected inosine **5** was unsuccessful. Subsequent treatment with a large excess of sodium nitrite afforded 2′,3′-*O*-isopropylidene-8-bromoinosine, **8**. After protection of the 5′-*O*H as a silyl ether, the fully protected 8-bromoinosine **9** was subjected to the modified Vorbrüggen conditions described above. This time, a single product could be isolated in 91% yield (**10**, Scheme 2).

Scheme 2. Introduction of 8-Br and Glycosylation



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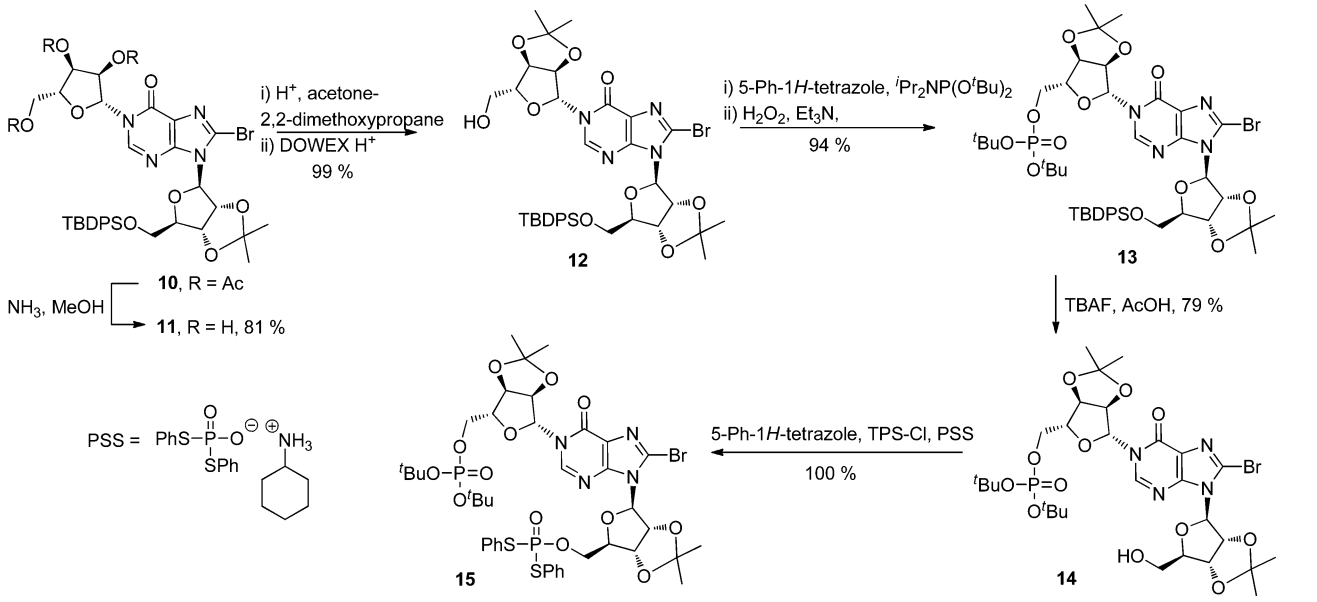
Scheme 3. Introduction of Phosphate Triesters



The regiospecificity of alkylation to generate an *N*1-ribosylated product, rather than the unwanted *O*6-product, was confirmed by 2D-NMR experiments. HMBC interactions were observed between the anomeric H-1″ of the "northern" ribose and the adenine C-2/C-6, and between the adenine H-2 and "northern" ribose C-1″. Furthermore, 1D-NOE irradiation of H-2 excited both H-1″ and H-2″. The *β*-configuration of the newly formed *N*1 link at the "northern" ribose anomeric center was confirmed by the presence of a doublet (J = 4.2 Hz) in the ^{1}H NMR spectrum and an NOE between H-1″ and H-4″, confirming that these two protons lie on the same face of the ribose ring.

In the *chemo-enzymatic* route, substitution at purine C-8 was employed to predispose the linear precursor to cyclize at *N*1, rather than *N*7.[8] Introduction of a bulky C-8 group likely reorientates the purine relative to the "southern" ribose,[30] so that it lies in the *syn*-conformation prior to interaction with the active site, and the *N*1 product is generated. In previous synthetic routes, cyclization was carried out by promoting formation of the pyrophosphate bond, and C-8 substitution was initially employed as it was assumed that a *syn*-orientation would facilitate colocalization of the two phosphate groups.[16] However, it was later demonstrated that while this substitution gave slightly improved yields, it was not necessary for successful formation of the macrocycle.[17] We have demonstrated that, when using modified Vorbrüggen conditions, substitution at C-8 alters the reactivity of inosine for an entirely different reason, unrelated to the conformation of the purine relative to the "southern" ribose. The 8-bromo substitution appears to increase the nucleophilicity of the deprotonated purine sufficiently to attack the acyloxonium ion and generate a glycosylated product. It has not previously been possible to prepare a scaffold containing both intact riboses in a selective manner.

Treatment of **10** with methanolic ammonia effected simultaneous removal of the three acetyl esters from the "northern" ribose, and the resulting triol **11** was stirred with acetone/2,2-dimethoxypropane (4:1 v/v) in the presence of *p*-TsOH, Scheme 3. Surprisingly, initially this generated two distinct products; the desired 2″,3″-*O*-isopropylidene ketal protected **12**, and a higher R_f product **12a** (see Supporting Information), that was isolated by column chromatography and identified as the 2″,3″-*O*-isopropylidene-5″-*O*-(2-methoxypropan-2-yl) derivative. In subsequent reactions, this unwanted side product was converted to **12** by stirring the crude reaction mixture with methanol containing DOWEX H$^+$ resin for 30 min, to selectively cleave the 5″-*O*-hemiacetal before purification.

The isolated "northern" 5″-OH could now be used for introduction of the first phosphate triester. In our hands, phosphorylation of **12** with di(anilino)phosphorochloridate[31] was unreliable. The preparation of the P(V) reagent was low yielding (maximum ∼15%) and the phosphorylation of **12** did not go to completion, leaving an inseparable mixture of unreacted starting material and phosphorylating reagent debris. However, introduction of a *tert*-butyl-protected phosphate triester proceeded in high yield using di-*tert*-butyl *N*,*N*-diisopropylphosphoramidite and 5-phenyl-1*H*-tetrazole followed by oxidation under basic conditions with Et_3N/H_2O_2.[32] We found that oxidation of the intermediate phosphite with mCPBA, or purification of the reaction mixture using silica gel, led to unreliable yields due to partial cleavage of the *tert*-butyl phosphate esters (yields from 17 to 49%). However, when oxidation was carried out using Et_3N/H_2O_2 followed by purification on basified silica with eluting solvents containing 0.5% pyridine, the yield was significantly improved (94%).

The "southern" ribose 5′-*O*H was then revealed by treatment of **13** with TBAF under neutral conditions to afford **14**, and a diphenylphosphorodithioate triester was introduced using cyclohexylammonium *S*,*S*-diphenylphosphorodithioate (PSS)[33] with 5-phenyl-1*H*-tetrazole and 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl) as activating agents.[34] If TPS-Cl was used as the sole activating agent, we observed partial substitution of the 8-bromo substituent by chlorine, which generated an inseparable mixture of the two 8-substituted products (the identity of which was confirmed by mass spectrometry).

The phosphate and phosphorodithioate esters of **15** were sequentially deprotected (Scheme 4). Treatment at 0 °C with 50% aqueous TFA for 4 h effected simultaneous deprotection

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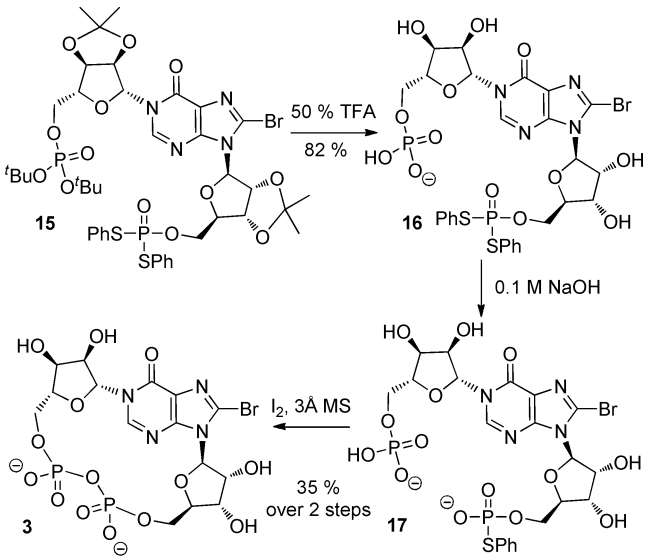
Scheme 4. Deprotection and Intramolecular Cyclization



of the *tert*-butyl phosphate esters and both isopropylidene ketals to reveal the 5″-*O*-phosphomonoester. At higher temperatures, or with longer reaction times, we observed partial cleavage of the *N*9 glycosidic bond.[35] Notably, the inosine *N*1-glycosidic bond was stable to this and all other chemical transformations that were required during the synthesis. Selective basic hydrolysis of one thiophenol group has reportedly been carried out using H_3PO_2.[34] However, we found this reaction difficult to monitor, and the removal of excess reagent during purification was complex.

We sought alternative conditions using a phosphorus-free base, so that the progression of the reaction could be monitored by ^{31}P NMR. Treatment of **16** with 50% 0.1 M NaOH in dioxane effected rapid (15−30 min) selective deprotection to give **17** (Scheme 4). The resulting substrate for intramolecular cyclization did not require any further purification. After neutralizing the solution with 0.1 M HCl, the resulting sodium salt of **17** was converted to the triethylammonium salt. Intramolecular cyclization of **17** was carried out by a modified Hata condensation. A dilute pyridine solution of **17** added over 15 h by syringe pump to a solution of iodine and molecular sieves[17] to promote metaphosphate formation, followed by intramolecular cyclization to generate the pyrophosphate linkage of the target 8-Br-cIDPR. Synthetic 8-Br-cIDPR was identical (by ^1H, ^{13}C, ^{31}P NMR and HPLC) to that prepared by our previous chemo-enzymatic route.[8,9]

In summary, the potent, chemically and biologically stable membrane permeant cADPR receptor agonist 8-Br-cIDPR has been synthesized using a novel total synthetic route. The key feature of this route is the early introduction of an 8-bromo substituent to promote entirely regio- and stereoselective *N*1-glycosylation with an intact "northern" ribose. The resultant *N*1-ribosyl inosine can be sequentially phosphorylated and the product cyclized after appropriate deprotection using the phosphorodithioate method. This procedure is amenable to nucleosides with modifications to the "southern" ribose and will facilitate generation of analogues of cADPR for SAR studies with an intact "northern" ribose that have previously been inaccessible by the chemo-enzymatic approach.

EXPERIMENTAL SECTION

General Experimental Procedures. All reagents and solvents were of commercial quality and were used without further purification, unless described otherwise. Unless otherwise stated, all reactions were carried out under an inert atmosphere of argon. ^1H and ^{13}C chemical shifts (δ) were internally referenced to the residual solvent peak. ^1H and ^{13}C NMR assignments are based on *g*COSY, *g*HMBC, *g*HSQC, and DEPT-135 experiments. Abbreviations for splitting patterns are as follows: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet etc. Coupling constants are given in hertz (Hz). Synthetic phosphates were assayed and quantified by the Ames phosphate test.[36]

5′-O-(tert-Butyldiphenylsilyl)inosine. Triethylamine (518 μL, 3.72 mmol) and TBDPS-Cl (1.06 mL, 4.09 mmol) were added to inosine (500 mg, 1.86 mmol) in DMF (10 mL). After 30 h of stirring at rt, all solvents were evaporated. The crude material was purified by column chromatography on silica gel eluting with DCM/MeOH (9:1 → 4:1 v/v) to afford the title compound (669 mg, 71%): R_f = 0.33 (DCM/MeOH 9:1 v/v); ^1H NMR (270 MHz, DMSO-d_6) δ 8.22 (s, 1H), 8.00 (s, 1H), 7.60 (dd, 4H, *J* = 7.7, 1.4), 7.39−7.34 (m, 6H) (10 × Ar−H), 5.91 (d, 1H, *J* = 4.7, H-1′), 5.62 (d, 1H, *J* = 5.8, −OH, ex), 5.29 (d, 1H, *J* = 5.5, −OH, ex), 4.54 (q, 1H, *J* = 5.0, ex → t, H-2′), 4.30 (q, 1H, *J* = 4.9, ex → t, H-3′), 4.05−4.02 (m, 1H), 3.89 (dd, 1H, *J* = 11.3, 3.4, H-5′a), 3.79 (dd, 1H, *J* = 11.3, 4.7, H-5′b), 0.94 (s, 9H, ^{t}Bu) ppm.

*5′-O-(tert-Butyldiphenylsilyl)-2′,3′-O-isopropylideneinosine (**5**).* *p*-TsOH (28 mg, 0.148 mmol) was added to 5′-*O*-(*tert*-butyldiphenylsilyl)inosine (75 mg, 0.148 mmol) in acetone/2,2-dimethoxypropane (4:1 v/v, 5 mL). After 30 min of stirring at rt, all solvents were removed under reduced pressure, and the resulting material was purified by column chromatography on silica gel eluting with DCM/MeOH (1:0 → 4:1 v/v) to afford the title compound (74 mg, 91%) as a white solid: R_f = 0.85 (DCM/MeOH 9:1 v/v); mp 256−258 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 13.17 (bs, 1H, NH), 8.19 (s, 1H, H-8), 8.07 (s, 1H, H-2), 7.59−7.55 (m, 4H), 7.38−7.28 (m, 6H), 6.10 (d, 1H, *J* = 2.4, H-1′), 5.20 (dd, 1H, *J* = 6.1, 2.4, H-2′), 4.87 (dd, 1H, *J* = 6.1, 2.8, H-3′), 4.40 (ddd, 1H, *J* = 5.2, 4.1, 2.8, H-4′), 3.88 (dd, 1H, *J* = 11.5, 4.1, H-5′a), 3.78 (dd, 1H, *J* = 11.5, 5.2, H-5′b), 1.60 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 0.99 (s, 9H, ^{t}Bu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) 159.4, 148.4, 145.2, 139.0, 135.6 (2C), 135.5 (2C), 132.8, 132.7, 130.0 (2C), 127.83 (2C), 127.78 (2C), 125.4, 114.4, 91.3, 87.1, 84.8, 81.3, 64.0, 27.2, 26.9 (3C), 25.4, 19.2 ppm; HRMS (ESI$^+$) found *m*/*z* [M + H]$^+$ 547.2354, $C_{29}H_{35}N_4O_5Si$ requires 547.2371.

*N1-(2′′,3′′,5′′-Tri-O-acetyl-β-D-ribofuranosyl)-5′-O-(tert-butyldiphenylsilyl)-2′,3′-O-isopropylideneinosine (**6**).* 5′-*O*-TBDPS-2′,3′-*O*-isopropylideneinosine (**5**, 200 mg, 0.366 mmol) was taken up in MeCN (1.0 mL) and DBU (164 μL, 1.098 mmol) added. After 30 min, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (128 mg, 0.402 mmol) was added and the solution cooled to −78 °C. Trimethylsilyl trifluoromethanesulfonate (136 μL, 1.464 mmol) was added dropwise and the solution stirred for a further 45 min before warming to rt. After 1 h, $NaHCO_3$ (satd aq) was added and the crude material extracted into DCM (×3). The combined organic fractions were dried (Na_2SO_4), and solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with DCM/acetone (1:0 → 0:1 v/v) to afford the title compound (41 mg, 13%) as a colorless glass: R_f = 0.74 (DCM:Acetone 3:1 v/v); ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H, H-8), 7.96 (s, 1H, H-2), 7.61 (dd, 2H, *J* = 8.0, 1.5), 7.59 (dd, 2H, *J* = 8.0, 1.5), 7.42−7.30 (m, 6H), 6.37 (d, 1H, *J* = 4.2, H-1′′), 6.09 (d, 1H, *J* = 2.8, H-1′), 5.45−5.43 (m, 2H), 5.07 (dd, 1H, *J* = 6.3, 2.9), 4.86 (dd, 1H, *J* = 6.3, 3.1), 4.40−4.36 (m, 4H), 3.88 (dd, 1H, *J* = 11.0, 4.0, H-5′a), 3.82 (dd, 1H, *J* = 11.0, 4.9, H-5′b), 2.13, 2.12, 2.09 (each s, 3H, 3 × OAc), 1.61 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.03 (s, 9H, ^{t}Bu) ppm; HRMS (ESI$^+$) found *m*/*z* [M + Na]$^+$ 827.2917, $C_{40}H_{48}N_4NaO_{12}Si$ requires 827.2930.

2′,3′-O-Isopropylidene-8-bromoadenosine.[37] Na_2HPO_3 (30 g) was dissolved in Milli-Q (300 mL). The resulting solution was covered in foil and bromine (0.6 mL) added. In a separate flask, 2′,3′-*O*-isopropylidene-adenosine (**7**, 3.05 g) was taken up in dioxane (300 mL) and covered in foil. The bromine solution was decanted into this

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solution, and the resulting solution stirred in the dark for 16 h. The solution was thoroughly extracted with $CHCl_3$, and the combined organic layers washed with $NaHSO_3$ (satd aq), then H_2O, dried ($MgSO_4$) and evaporated to dryness. The residue was crystallized from EtOH to yield the *title compound* (3.19 g, 83%) as needles; ^{1}H (400 MHz, $CDCl_3$) δ 8.26 (s, 1H, H-2), 6.36 (d, 1H, J = 10.8, 5′−OH), 6.10 (d, 1H, J = 5.3, H-1′), 6.01 (bs, 2H, NH_2), 5.28 (dd, 1H, J = 5.7, 5.3, H-2′), 5.08 (dd, 1H, J = 5.7, 1.1, H-3′), 4.53 (d, 1H, J = 1.1, H-4′), 3.97 (d, 1H, J = 13.1, H-5′a), 3.78 (dd, 1H, J = 13.1, 10.8, H-5′b), 1.67 (s, 3H, CH_3), 1.38 (s, 3H, CH_3) ppm; HRMS (ESI$^+$) found m/z [M + H]$^+$ 386.0450, 388.0433; $C_{13}H_{17}N_5O_4{}^{79}$Br requires 386.0458, $C_{13}H_{17}N_5O_4{}^{81}$Br requires 388.0438.

2′,3′-O-Isopropylidene-8-bromoinosine[16] ***(8)***. 2′,3′-*O*-Isopropylidene-8-bromoadenosine (2.00 g, 5.18 mmol) was taken up in acetic acid−water (57.5 mL, 20:3 v/v). $NaNO_2$ (4.29 g, 62.14 mmol) was added in one portion and the resulting solution stirred for 16 h. All solvents were evaporated and the residue taken up in EtOH and evaporated. The residue was partitioned between $CHCl_3$ and H_2O and the organic layer washed with $NaHCO_3$ (satd aq) and then brine, dried ($MgSO_4$), and evaporated to dryness. The residue was crystallized from aqueous EtOH to yield the title compound (1.66 g, 83%) as crystals: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.99 (bs, 1H, NH), 8.34 (s, 1H, H-2), 6.10 (d, 1H, J = 5.5, H-1′), 5.24 (t, 1H, J = 5.5, H-2′), 5.06 (dd, 1H, J = 5.5, 1.9, H-3′), 4.48 (dd, 1H, J = 1.9, 1.6, H-4′), 3.94 (dd, 1H, J = 12.1, 1.6, H-5′a), 3.79 (d, 1H, J = 12.1, H-5′b), 1.66 (s, 3H, CH_3), 1.38 (s, 3H, CH_3) ppm; HRMS (ESI$^+$) found m/z [M + H]$^+$ 387.0311, 389.0289, $C_{13}H_{16}N_4O_5{}^{79}$Br requires 387.0299, $C_{13}H_{16}N_4O_5{}^{81}$Br requires 389.0278.

5′-O-(tert-Butyldiphenylsilyl)-2′,3′-O-isopropylidene-8-bromoinosine[38] ***(9)***. Imidazole (228 mg, 3.36 mmol) and TBDPSCl (435 μL, 1.68 mmol) were added to a solution of 2′,3′-*O*-isopropylidene-8-bromoinosine (**8**, 500 mg, 1.29 mmol) in DMF (10 mL). After 16 h, EtOAc (100 mL) and H_2O (50 mL) were added. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to dryness. The residue was purified by column chromatography on silica gel eluting with DCM/MeOH (1:0 → 1:19 v/v) to afford the title compound (808 mg, 98%) as a white foam: R_f = 0.39 (PE/EtOAc, 1:3 v/v); ^{1}H (400 MHz, $CDCl_3$) δ 13.08 (bs, 1H, NH), 7.91 (s, 1H, H-2), 7.59 (dd, 2H, J = 8.0, 1.4), 7.54 (dd, 2H, J = 8.0, 1.4), 7.40−7.31 (m, 4H), 7.26 (t, 2H, J = 7.3), 6.18 (d, 1H, J = 2.1, H-1′), 5.57 (dd, 1H, J = 6.4, 2.1, H-2′), 5.10 (dd, 1H, J = 6.4, 3.7, H-3′), 4.39 (ddd, 1H, J = 6.5, 5.6, 3.7, H-4′), 3.82 (dd, 1H, J = 11.5, 5.6, H-5′a), 3.74 (dd, 1H, J = 11.5, 6.5, H-5′b), 1.63 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.02 (s, 9H, ^{t}Bu) ppm; HRMS (ESI$^+$) found m/z [M + H]$^+$ 625.1447, 627.1431, $C_{29}H_{34}N_4O_5Si^{79}$Br requires 625.1482, $C_{29}H_{34}N_4O_5Si^{81}$Br requires 627.1461.

N1-(2′′,3′′,5′′-Tri-O-acetyl-β-D-ribofuranosyl)-5′-O-(tert-butyldiphenylsilyl)-2′,3′-O-isopropylidene-8-bromoinosine ***(10)***. 5′-*O*-TBDPS-2′,3′-*O*-isopropylidene-8-bromoinosine (**9**, 160 mg, 0.255 mmol) was taken up in DCM (1.6 mL) and DBU (114 μL, 0.765 mmol) added. After 30 min, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (89 mg, 0.281 mmol) was added and the solution cooled to −78 °C. Trimethylsilyl trifluoromethanesulfonate (185 μL, 1.020 mmol) was added dropwise and the solution stirred for a further 45 min before warming to rt. After 1 h, $NaHCO_3$ (satd aq) was added and the crude material extracted into DCM (×3). The combined organic fractions were dried (Na_2SO_4), and solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with PE/EtOAc (1:0 → 0:1 v/v) to afford the title compound (203 mg, 90%) as a colorless glass: R_f = 0.85 (DCM/acetone 3:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H, H-2), 7.61 (dd, 2H, J = 8.0, 1.4), 7.56 (dd, 2H, J = 8.0, 1.4), 7.42−7.33 (m, 4H), 7.31−7.26 (m, 2H), 6.20 (d, 1H, J = 4.2, H-1′′), 6.17 (d, 1H, J = 2.4, H-1′), 5.45−5.41 (m, 3H, H-2′, H-2′′ and H-3′′), 5.00 (dd, 1H, J = 6.4, 4.3, H-3′), 4.43−4.38 (m, 3H, H-4′′, 2 × H-5′′), 4.36−4.33 (m, 1H, H-4′), 3.90 (dd, 1H, J = 11.0, 5.2, H-5′a), 3.82 (dd, 1H, J = 11.0, 6.6, H-5′b), 2.15, 2.12, 2.04 (each s, 3H, 3 × OAc), 1.64 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.04 (s, 9H, ^{t}Bu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) 170.2, 169.6, 169.4, 154.5, 147.8, 144.3, 135.6, 133.3, 132.9, 129.77, 129.76, 127.7 (2C), 127.5 (2C), 126.2, 124.9, 114.7, 90.9, 88.6, 87.5, 83.3, 81.4, 80.3, 74.3, 70.1, 64.1, 63.0, 27.3, 26.7 (3C), 25.5, 20.6, 20.5, 20.4, 19.2 ppm; HRMS (ESI$^+$) found m/z [M + H]$^+$ 883.2190, 885.2178, $C_{40}H_{48}N_4O_{12}{}^{79}$BrSi requires 883.2216, $C_{40}H_{48}N_4O_{12}{}^{81}$BrSi requires 885.2195.

N1-(β-D-Ribofuranosyl)-5′-O-(tert-butyldiphenylsilyl)-2′,3′-O-isopropylidene-8-bromoinosine ***(11)***. *N*1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-2′,3′-*O*-isopropylidene-5′-*O*-TBDPS-8-bromoinosine (**10**, 500 mg, 0.57 mmol) was taken up in MeOH (5 mL) in a pressure tube. The solution was saturated with NH_3 (g) at 0 °C and then stirred at rt for 12 h. The solvent was evaporated and the residue purified by column chromatography on silica gel eluting with PE/EtOAc (1:0 → 0:1 v/v) to afford the title compound (349 mg, 81%) as a white amorphous solid: R_f = 0.24 (PE:EtOAc 1:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H, H-2), 7.61 (dd, 2H, J = 8.0, 1.4), 7.53 (dd, 2H, J = 8.0, 1.4), 7.41−7.31 (m, 4H), 7.23 (t, 2H, J = 7.4), 6.12 (d, 1H, J = 2.3, H-1′), 5.79 (d, 1H, J = 4.6, H-1′′), 5.43 (dd, 1H, J = 6.4, 2.3, H-2′), 5.00 (dd, 1H, J = 6.4, 3.9, H-3′), 4.49 (t, 1H, J = 4.6, H-2′′), 4.36−4.32 (m, 2H, H-3′′ and H-4′), 4.25−4.22 (m, 1H, H-4′′), 3.91−3.85 (m, 2H, H-5′a and H-5′′a), 3.79−3.73 (m, 2H, H-5′b and H-5′′b), 1.60 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.00 (s, 9H, ^{t}Bu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 148.4, 145.3, 135.6 (2C), 135.5 (2C), 133.5, 132.9, 129.8 (2C), 127.7 (2C), 127.5 (2C), 126.7, 124.8, 114.6, 94.2 91.3, 87.7, 86.4, 83.3, 81.6, 74.7, 70.7, 64.0, 62.0, 27.3, 26.8, 25.5, 19.2 ppm; HRMS (ESI$^+$) found m/z [M + H]$^+$ 757.1877 and 759.1864, $C_{34}H_{42}N_4O_9{}^{79}$BrSi requires 757.1899, $C_{34}H_{42}N_4O_9{}^{81}$BrSi requires 759.1878.

N1-(2′′,3′′-O-Isopropylidene-β-D-ribofuranosyl)-5′-O-(tert-butyldiphenylsilyl)-2′,3′-O-isopropylidene-8-bromoinosine ***(12)***. *p*-TsOH (55 mg, 0.29 mmol) was added to *N*1-(β-D-ribofuranosyl)-5′-*O*-TBDPS-2′,3′-*O*-isopropylidene-8-bromoinosine (**11**, 220 mg, 0.29 mmol) in acetone-2,2-dimethoxypropane (10 mL, 4:1 v/v). After 30 min, DCM and $NaHCO_3$ (satd aq) were added, and the organic layer was dried (Na_2SO_4) and evaporated. The residue was taken up in MeOH (5 mL) and DOWEX H$^+$ resin (50 mg) added to convert any unwanted 5′′-*O*-hemiacetal side product into **12**. After 30 min, the resin was removed by filtration under gravity and the solvent evaporated to obtain the title compound (229 mg, 99%) as a white amorphous solid: R_f = 0.74 (PE/EtOAc 1:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, 2H, J = 7.9, 1.3), 7.57 (s, 1H, H-2), 7.53 (dd, 2H, J = 7.9, 1.3), 7.43−7.32 (m, 4H), 7.23 (t, 2H, J = 7.7), 6.12 (d, 1H, J = 2.2, H-1′), 5.61 (d, 1H, J = 2.9, H-1′′), 5.41 (dd, 1H, J = 6.4, 2.2, H-2′), 5.15 (dd, 1H, J = 6.4, 3.0, H-2′′), 5.07 (dd, 1H, J = 6.4, 3.4, H-3′′), 5.02 (dd, 1H, J = 6.4, 3.9, H-3′), 4.36−4.33 (m, 2H, H-4′ and H-4′′), 3.91 (dd, 1H, J = 12.3, 2.5, H-5′′a), 3.85 (dd, 1H, J = 10.9, 5.6, H-5′a), 3.79 (dd, 1H, J = 12.3, 3.6, H-5′′b), 3.76 (dd, 1H, J = 10.9, 6.4, H-5′b), 1.61 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.01 (s, 9H, ^{t}Bu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.1, 148.1, 146.2, 135.6 (2C), 135.2 (2C), 133.6, 132.8, 129.8, 129.7, 127.7 (2C), 127.5 (2C), 126.8, 125.5, 114.5, 114.3, 96.9, 91.3, 87.87, 87.86, 83.39, 83.36, 81.5, 80.6, 63.9, 62.9, 27.4, 27.3, 26.8, 25.5, 25.3, 19.2 ppm; HRMS (ESI$^+$) found m/z [M + H]$^+$ 797.2186 and 799.2192, and [M + Na]$^+$ 819.1975 and 821.1995, $C_{37}H_{46}N_4O_9{}^{79}$BrSi requires 797.2212, $C_{37}H_{46}N_4O_9{}^{81}$BrSi requires 799.2192, $C_{37}H_{45}N_4O_9{}^{79}$BrSiNa requires 819.2031, $C_{37}H_{45}N_4O_9{}^{81}$BrSiNa requires 821.2011.

N1-[2′′,3′′-O-Isopropylidene-5′′-O-(di-tert-butyl)phosphoryl-β-D-ribofuranosyl]-5′-O-(tert-butyldiphenylsilyl)-2′,3′-O-isopropylidene-8-bromoinosine ***(13)***. 5-Phenyl-1*H*-tetrazole (103 mg, 0.70 mmol) and *N*,*N*-diisopropyldibutylphosphoramidite (166 μL, 0.53 mmol) were added to a solution of *N*1-(2′′,3′′-*O*-isopropylidene-β-D-ribofuranosyl)-5′-*O*-TBDPS-2′,3′-*O*-isopropylidene-8-bromoinosine (**12**, 280 mg, 0.35 mmol) in DCM (2 mL). After 2 h, the solution was cooled to 0 °C, and Et_3N (367 μL, 2.63 mmol) and H_2O_2 (96 μL, 1.09 mmol) were added. The solution was allowed to warm to rt and stirred for a further 2 h, after which time DCM (20 mL) and H_2O were added. The organic layer was washed with $NaHCO_3$ (satd aq) and then brine, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by column chromatography on silica gel eluting with PE/EtOAc (1:0 → 0:1 v/v), where both solvents contained 0.5% v/v pyridine, to afford the title compound (323 mg, 94%) as a colorless glass: R_f = 0.41 (PE/EtOAc 1:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ

7.63 (s, 1H, H-2), 7.60 (dd, 2H, J = 8.0, 1.4), 7.53 (dd, 2H, J = 8.0, 1.4), 7.41−7.31 (m, 4H), 7.23 (t, 2H, J = 7.9), 6.12 (d, 1H, J = 2.2, H-1′), 5.89 (d, 1H, J = 1.8, H-1″), 5.41 (dd, 1H, J = 6.4, 2.2, H-2′), 4.99 (dd, 1H, J = 6.4, 4.1, H-3′), 4.97−4.92 (m, 2H, H-2″ and H-3″), 4.39 (ddd, 1H, J = 5.8, 4.6, 4.2, H-4″), 4.33 (ddd, 1H, J = 6.6, 5.4, 4.1, H-4′), 4.24 (ddd, 1H, J = 11.1, 7.0, 4.6, H-5″a), 4.15 (ddd, 1H, J = 11.1, 7.5, 5.8, H-5″b), 3.84 (dd, 1H, J = 10.9, 5.4, H-5′a), 3.74 (dd, 1H, J = 10.9, 6.6, H-5′b), 1.61 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.45 (s, 9H, ^{t}Bu), 1.44 (s, 9H, ^{t}Bu), 1.36 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.01 (s, 9H, ^{t}Bu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 147.8, 145.7, 135.5 (2C), 135.4 (2C), 133.4, 132.8, 129.74, 129.67, 127.6 (2C), 127.5 (2C), 126.2, 125.2, 114.47, 114.46, 93.7, 91.0, 87.7, 86.5 (d, J = 7.8), 84.7, 83.5, 82.56 (d, J = 7.2), 82.48 (d, J = 7.2), 81.5 (2C), 66.3 (d, J = 6.2), 64.0, 29.8 (3C), 29.7 (3C), 27.2, 27.1, 26.7 (3C), 25.4, 25.3, 19.1 ppm; ^{31}P NMR (162 MHz, ^{1}H decoupled, $CDCl_3$) δ −10.1 ppm; HRMS (ESI$^+$) found m/z [M + Na]$^+$ 1011.2914 and 1013.2935, $C_{45}H_{62}N_4O_{12}{}^{79}$BrSiNaP requires 1011.2947, $C_{45}H_{62}N_4O_{12}{}^{81}$BrSiNaP requires 1013.2926.

*N1-[2′′,3′′-O-Isopropylidene-5′′-O-(di-tert-butyl)-phosphoryl-β-D-ribofuranosyl]-2′,3′-O-isopropylidene-8-bromoinosine (**14**).* Acetic acid (29 μL, 0.51 mmol) and TBAF·3H$_2$O (153 mg, 0.49 mmol) were stirred in DMF (1 mL) for 30 min, after which the solution was cooled to 0 °C and *N*1-(2″,3″-*O*-isopropylidene-5″-*O*-(di-*tert*-butyl)-phosphoryl-β-D-ribofuranosyl)-5′-*O*-TBDPS-2′,3′-*O*-isopropylidene-8-bromoinosine (**13**, 160 mg, 0.16 mmol) in DMF (1.5 mL) added. The resulting solution was allowed to warm to rt and stirred for a further 4 h. The solution was diluted with ether, and $NaHCO_3$ (satd aq) and NH_4Cl (satd aq) were added. The organic layer was separated and the aqueous layer extracted with ether (×3). The combined organic layers were dried (Na_2SO_4) and evaporated to dryness, and the residue was purified by column chromatography on silica gel eluting with DCM/acetone (1:0 → 0:1 v/v) to afford the title compound (96 mg, 79%) as a colorless glass: R_f = 0.73 (DCM/acetone 1:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H, H-2), 6.07 (d, 1H, J = 4.3, H-1′), 5.97 (d, 1H, J = 1.8, H-1″), 5.23 (dd, 1H, J = 6.0, 4.3, H-2′), 5.05 (dd, 1H, J = 6.0, 2.1, H-3′), 5.02 (dd, 1H, J = 6.4, 1.8, H-2″), 4.92 (dd, 1H, J = 6.4, 3.9, H-3″), 4.43−4.38 (m, 2H, H-4′ and H-4″), 4.21 (ddd, 1H, J = 11.2, 6.5, 4.2, H-5″a), 4.12 (ddd, 1H, J = 11.2, 7.2, 5.4, H-5″b), 3.81 (dd, 1H, J = 12.2, 3.3, H-5′a), 3.70−3.68 (m, 1H, H-5′b), 1.61 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.45 (s, 9H, ^{t}Bu), 1.42 (s, 9H, ^{t}Bu), 1.34 (s, 3H, CH_3), 1.32 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 147.7, 145.8, 126.1, 125.7, 114.3, 114.2, 94.4, 92.9, 86.9 (d, J = 7.9), 86.3, 85.1, 83.0, 82.83 (d, J = 8.7), 82.78 (d, J = 8.7), 81.4, 81.1, 66.3 (d, J = 6.3), 62.6, 29.8 (d, 3C, J = 4.2), 29.7 (d, 3C, J = 4.2), 27.4, 27.0, 25.3, 25.2 ppm; ^{31}P NMR (162 MHz, ^{1}H decoupled, $CDCl_3$) δ −10.7 ppm; HRMS (ESI$^+$) found m/z [M + Na]$^+$ 773.1736 and 775.1777, $C_{29}H_{44}N_4O_{12}{}^{79}$BrNaP requires 773.1769, $C_{29}H_{44}N_4O_{12}{}^{81}$BrNaP requires 775.1748.

*N1-[2′′,3′′-O-Isopropylidene-5′′-O-(di-tert-butyl)phosphoryl-β-D-ribofuranosyl]-5′-O-[(diphenylthio)phosphoryl]- 2′,3′-O-isopropylidene-8-bromoinosine (**15**).* *N*1-(2″,3″-*O*-Isopropylidene-5″-*O*-(di-*tert*-butyl)phosphoryl-β-D-ribofuranosyl)-2′,3′-*O*-isopropylidene-8-bromoinosine (**14**, 80 mg, 0.11 mmol) was evaporated from pyridine (3 × 1 mL) and taken up in pyridine (1.5 mL). This solution was added to PSS (122 mg, 0.32 mmol), which had also been evaporated from pyridine (3 × 1 mL). 5-Phenyl-1*H*-tetrazole (47 mg, 0.32 mmol) and TPS-Cl (64 mg, 0.21 mmol) were added, and the solution was stirred at rt for 5 h. DCM and H_2O were added, the organic layer was separated, and the aqueous layer was washed with DCM (×2). The combined organic layer was washed with brine, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by column chromatography on silica gel eluting with PE:EtOAc (1:0 → 0:1 v/v) to afford the title compound (108 mg, 100%) as a white foam: R_f = 0.61 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H, H-2), 7.45−7.25 (m, 10H, Ar-H), 6.16 (d, 1H, J = 2.1, H-1′), 5.98 (d, 1H, J = 1.8, H-1″), 5.46 (dd, 1H, J = 6.4, 2.1, H-2′), 5.09 (dd, 1H, J = 6.4, 3.6, H-3′), 5.00 (dd, 1H, J = 6.5, 1.8, H-2″), 4.90 (dd, 1H, J = 6.5, 4.3, H-3″), 4.45−4.12 (m, 6H, H-4′, H-4″, both H-5′ and both H-5″), 1.60 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.46 (s, 9H, ^{t}Bu), 1.45 (s, 9H, ^{t}Bu), 1.36 (s, 3H, CH_3), 1.28 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 147.9, 146.3, 135.3 (d, 2C, J = 5.2), 135.2 (d, 2C, J = 5.2), 129.69, 129.67, 129.4−129.3 (m, 4C), 126.2, 125.6 (d, J = 6.7), 125.5 (d, J = 6.7), 125.2, 114.8, 114.5, 93.6, 91.0, 86.5 (d, J = 7.8), 85.3 (d, J = 8.4), 84.9, 83.4, 83.1 (d, J = 7.4), 83.0 (d, J = 7.4), 81.2, 80.9, 66.3 (d, J = 6.0), 66.1 (d, J = 8.2), 29.81 (3C), 29.77 (3C), 27.2, 27.1, 25.3, 25.2 ppm; ^{31}P NMR (162 MHz, ^{1}H decoupled, $CDCl_3$) δ 50.5, −10.4 ppm; HRMS (ESI$^+$) found m/z [M + Na]$^+$1037.1563 and 1039.1539, $C_{41}H_{53}N_4O_{13}{}^{79}$BrNaP$_2S_2$ requires 1037.1601, $C_{41}H_{53}N_4O_{13}{}^{81}$BrNaP$_2S_2$ requires 1039.1581.

*N1-[5′′-O-Phosphoryl-β-D-ribofuranosyl]-5′-O-[(diphenylthio)-phosphoryl]-8-bromoinosine (**16**).* *N*1-(2″,3″-*O*-Isopropylidene-(di-*tert*-butyl)phosphoryl-β-D-ribofuranosyl)-5′-*O*[(diphenylthio) phosphoryl]-2′,3′-*O*-isopropylidene-8-bromoinosine (**15**, 150 mg, 0.059 mmol) was stirred in 50% TFA (2 mL) at 0 °C for 4 h. All solvents were evaporated, and the residue was coevaporated with MeOH (×4). The residue was purified by column chromatography on silica gel eluting with EtOAc/MeOH/H_2O (1:0:0 → 4:2:0 → 7:2:1 v/v/v) to afford the title compound (100 mg, 82%) as a colorless glass: R_f = 0.28 (EtOAc/MeOH/H_2O 7:2:1 v/v/v); ^{1}H NMR (500 MHz, MeOD-d_4) δ 8.58 (s, 1H, H-2), 7.44−7.39 (m, 6H), 7.36−7.32 (m, 4H) (10 × Ar−H), 6.33 (d, 1H, J = 3.0, H-1′), 6.24 (d, 1H, J = 1.5, H-1″), 5.72 (dd, 1H, J = 6.5, 1.5, H-2′), 5.21 (dd, 1H, J = 6.0, 3.0, H-3′), 4.95 (dd, 1H, J = 6.5, 3.0, H-3″), 4.90 (dd, 1H, J = 6.5, 3.0, H-2″), 4.45−4.37 (m, 4H), 4.15−4.08 (m, 2H) (H-4′, H-4″, both H-5′ and H-5″), 1.60 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.27 (s, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, MeOD-d_4) δ 156.6, 149.5, 147.8, 136.6 (d, 2C, J = 5.4), 136.4 (d, 2C, J = 5.0), 131.1 (d, J = 2.9), 130.9 (d, J = 2.9), 130.6 (d, 4C, J = 2.3), 130.1 (d, J = 10.5), 126.7, 126.6 (d, J = 5.9), 125.5, 115.6, 115.4, 93.1, 92.0, 87.8 (d, J = 8.1), 86.9 (d, J = 8.3), 86.6, 85.0, 82.5, 82.4, 68.7 (d, J = 9.3), 65.8 (d, J = 5.3), 27.6, 27.4, 25.59, 25.56 ppm; ^{31}P NMR (202 MHz, ^{1}H decoupled, d$_4$-MeOD) δ 51.7, 1.0 ppm; HRMS (ESI$^-$) found m/z [M − H]$^-$ 901.0389 and 903.0376, $C_{33}H_{36}N_4O_{13}{}^{79}BrP_2S_2$ requires 901.0384, $C_{33}H_{36}N_4O_{13}{}^{81}BrP_2S_2$ requires 903.0364.

*N1-(5′′-O-Phosphoryl-β-D-ribofuranosyl)-5′-O-(phenylthio)-phosphoryl-8-bromoinosine (**17**).* *N*1-(5″-*O*-Phosphate-β-D-ribofuranosyl)-5′-*O*-(diphenylthio)phosphoryl-8-bromoinosine (**16**, 20 mg, 0.024 mmol) was taken up in dioxane/H_2O (1 mL, 1:1 v/v). NaOH (100 μL, 1 M) was added and the solution stirred for 30 min at rt before addition of HCl (100 μL, 1 M). The solution was diluted with H_2O and washed with hexane (×3) before evaporation of all solvents to give a colorless glass which was converted to the TEA salt as described below: ^{1}H NMR (500 MHz, D_2O) δ 8.51 (s, 1H, H-2), 7.14 (d, 2H, J = 7.6), 7.09 (t, 1H, J = 7.6), 6.97 (t, 2H, J = 7.6) (5 × Ar−H), 6.10 (d, 1H, J = 3.4, H-1″), 6.05 (d, 1H, J = 5.5, H-1′), 5.59 (t, 1H, J = 5.5, H-2′), 4.57 (t, 1H, J = 3.8, H-3′), 4.31−4.01 (m, 8H) ppm; ^{13}C NMR (125 MHz, D_2O) δ 156.1, 148.8, 145.0, 132.1 (d, 2C, J = 5.3), 129.5 (d, J = 5.2), 128.7, 128.6 (2C), 127.6, 123.7, 90.5, 88.7, 84.2 (d, J = 10.7), 83.1 (d, J = 8.6), 74.7, 70.9, 70.2, 69.2, 65.7 (d, J = 5.7, 63.2 (d, J = 3.9) ppm; ^{31}P NMR (202 MHz, D_2O, ^{1}H-decoupled) δ 17.3, 3.3 ppm; HRMS (ESI$^-$) calcd for $C_{21}H_{24}N_4O_{14}P_2S^{79}Br$ 728.9674 [(M − H)$^-$], found 728.9663; calcd for $C_{21}H_{24}N_4O_{14}P_2S^{81}Br$ 730.9653 [(M − H)$^-$], found 730.9640. Conversion to TEA salt: The Na^+ salt was passed through prewashed DOWEX H^+ resin. Acidic fractions were neutralized with TEAB (2 mL, 1M). All solvents were evaporated and the residue coevaporated with H_2O to remove excess buffer. The colorless glass obtained was used directly for cyclization.

*Cyclic-8-bromoinosine 5′-Diphosphate Ribose[8,9] (8-Br-cIDPR) (**3**).* *N*1-(5″-*O*-Phosphoryl-β-D-ribofuranosyl)-5′-*O*-(phenylthio)-phosphoryl-8-bromoinosine (**17**, 0.024 mmol) was evaporated from pyridine (2 mL, × 2). The residue was taken up in pyridine (10 mL) and added over 15 h to a solution of iodine (140 mg, 0.591 mmol) and 3 Å molecular sieves (0.5 g) in pyridine (20 mL), in the dark. The solution was filtered through Celite and washed with H_2O. After addition of TEAB (2 mL), all solvents were evaporated and the residue partitioned between H_2O and $CHCl_3$. The aqueous layer was washed with $CHCl_3$ and evaporated to dryness. The residue was purified by semipreparative reversed-phase HPLC eluted at 5 mL/min with acetonitrile/0.1 M TEAB (1:0 → 13:7 v/v) over 25 min. Fractions were analyzed by analytical RP-HPLC eluted at 1 mL/min with ion-

pair buffer: 0.17% (m/v) cetrimide and 45% (v/v) phosphate buffer (pH 6.4) in MeOH. Appropriate fractions were collected and evaporated under vacuum to give the title compound (5.3 mg, 35% over two steps): UV (H_2O, pH 7), λ_{max} 255 nm (ε 11,100); ^{1}H NMR (500 MHz, D_2O) δ 8.88 (s, 1H, H-2), 6.06 (d, 1H, J = 6.1, H-1′), 6.01 (s, 1H, H-1″), 5.31 (dd, 1H, J = 6.1, 2.4, H-2′), 4.62 (d, 1H, J = 2.4, H-3′), 4.49 (dd, 1H, J = 10.8, 5.6, H-5′a), 4.40−4.30 (m, 5H), 4.11 (d, 1H, J = 11.9, H-5″a), 4.02 (d, 1H, J = 10.8, H-5′b) ppm; ^{13}C NMR (125 MHz, D_2O) δ 156.7, 149.3, 144.3, 128.5, 123.9, 91.9, 90.8, 84.8 (d, J = 11.0, 83.1 (d, J = 9.4), 75.5, 72.4, 70.5, 67.4, 64.9 (d, J = 3.7), 62.1 (d, J = 3.2) ppm; ^{31}P NMR (202 MHz, D_2O, ^{1}H-decoupled) δ −10.15 (d, J = 12.1), −11.17 (d, J = 12.1) ppm; HRMS (ESI$^-$) calcd for $C_{15}H_{18}N_4O_{14}P_2{}^{79}Br$ 618.9484 [$(M - H)^-$], found 618.9508, and calcd for $C_{15}H_{18}N_4O_{14}P_2{}^{81}Br$ 620.9463 [$(M - H)^-$], found 620.9467.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, and ^{31}P NMR for compounds **3**, **5**, **6**, and **10**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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