Synthesis of Enzymatically and Chemically Non-hydrolyzable Analogues of Dinucleoside Triphosphates Ap_3A and Gp_3G

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Dinucleoside polyphosphates are ubiquitous compounds tightly involved in the regulation of a number of key biological processes. Hydrolysis-resistant analogues of Ap_3A and Gp_3G, two important members of that family of nucleotides, have been synthesized. P^1,P^2:P^2,P^3-Bis-methylene diadenosine and diguanosine triphosphates were prepared from *O,O*-dialkyl methaneselenophosphonates using an original methodology. Whereas the 2-fold addition of the methanephosphonate anion to the activated phosphorus species cannot be performed, multiple condensation of lithiated methaneselenophosphonate with electrophilic trivalent phosphorus compounds is revealed to be very effective. A one-pot condensation/esterification/oxidation sequence involving *O,O*-dialkyl methaneselenophosphonates provides a highly efficient route to the PCH_2PCH_2P backbone. This new development in selenophosphonate chemistry offers a great potential for further regioselective functionalization of polyphosphate mimics.

Introduction

Dinucleoside polyphosphates (DNPs, Np_nN') constitute a family of nucleotides containing two nucleoside moieties (N, N′) joined through their 5′-position by a linear polyphosphate chain (p_n) incorporating up to 7 (n) phosphorus atoms. The first natural members of this group, diguanosine tri- (Gp_3G) and tetraphosphate (Gp_4G), were discovered in 1963 in brine shrimp eggs.[1,2] The existence of DNPs incorporating adenosine was demonstrated shortly afterward with the discovery of diadenosine tetraphosphate (Ap_4A).[3] Since then, such compounds have been detected in nearly all prokaryotic and eukaryotic cells examined thus far.

Many efforts have been devoted to the identification of the metabolic pathways involving DNPs,[4] and some of these compounds have been synthesized in vivo or in vitro using enzymes (aminoacyl-tRNA synthetases, acyl-CoA synthetases, Ap_4A phosphorylases, guanylyl transferases, luciferases, DNA ligases).[5−8] These nucleotides are believed to be involved in a number of intra- and extracellular processes including DNA replication and repair, response to metabolic stress, regulation of ion channels, cardiovascular modulation, platelet disaggregation, synaptic transmission, activation of glycogen breakdown and phospholipase D, regulation of neutrophil function, and stimulation of cell proliferation.[4,9] More recently some DNPs have assumed vital significance as ligands for the tumor suppressor protein Fhit.[10−12] However a number of working hypotheses, mostly based on indirect evidence, are no longer supported by experimental results.[9,13] Therefore, it remains unclear whether these compounds have important signaling and regulatory functions, or are only the inevitable and potentially toxic byproducts of metabolism that must be eliminated before their accumulation leads to the inhibition of essential biological processes. Though it is likely that some of the proposed activities simply reflect the ability of DNPs to behave as structural analogues of nucleotides, there is increasingly compelling evidence that true functions also exist. First, though the concentrations and binding affinities of DNPs are such that they would be

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unlikely to have any true physiological effect when compared to nucleotides, their half-life (in the order of several minutes) is considerably longer than that of nucleotides;[14] second, there is evidence for DNP-specific receptors that respond poorly to mononucleotides.[15−17] The difficulty here is to determine which species are biologically important. The characterization of the enzymes involved in the synthesis and degradation of DNPs and their mechanisms of action is then of the upmost interest for understanding the biological role of these compounds.

A major problem in assessing the true functions of DNPs is their great lability, and this probably explains difficulties experienced in reproducing observations. Many functions of nucleotides in cellular chemistry have been explored using nucleotide analogues stable to hydrolysis. Such compounds possess intriguing possibilities for metabolic regulation or perturbation and are essential to probe biological pathways. Herein we describe the synthesis of enzymatically and chemically stable analogues of Ap_3A and Gp_3G. Their biological evaluation should contribute to a better understanding of diadenosine and diguanosine triphosphates functions in living cells.

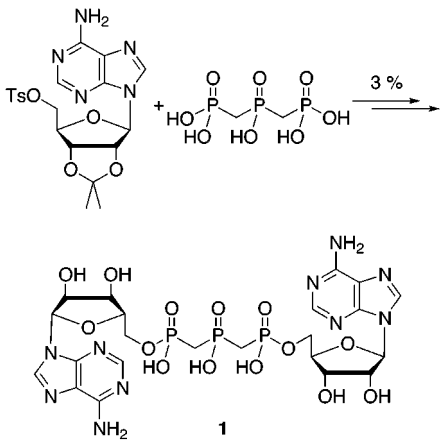
Results and Discussion

Several analogues of DNPs have been described in the literature.[18] By now, chemists most essentially focused on Ap_4A and very few publications report the synthesis of other dinucleoside polyphosphates mimics (Ap_3A,[19] Ap_5A,[20] Ap_nT,[21] and Ap_nG[22,23] derivatives). All the different analogues of DNPs described so far are only partially resistant to hydrolysis as one pyrophosphate group at least has been preserved in the structure. The only exception was reported by Blackburn et al. who described the P^1,P^2:P^2,P^3-bis-methylenediadenosine triphosphate **1**[19] which was prepared by the reaction between 2′,3′-*O*-isopropylidene adenosine 5′-tosylate and bis(dihydroxyphosphinomethyl)phosphinic acid in 3% yield (Scheme 1).

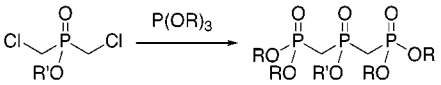
To prepare practical quantities of the P^1,P^2:P^2,P^3-bis-methylene analogues of Ap_3A and Gp_3G, we developed a new and promising methodology based on *O,O*-dialkyl selenophosphonate chemistry.

Many strategies have been developed for the synthesis of methylene analogues of diphosphate;[24−34] however,

Scheme 1



Scheme 2



only a very small number have been directed toward the preparation of bis-methylene analogues of triphosphate.[35−38] The latter are exclusively based on a double Michaelis−Arbuzov reaction between a bis-chloromethylphosphinate and a phosphorus triester (Scheme 2). The introduction of one or two nucleoside moieties into the polyphosphate analogue backbone[19,39,40] requires the saponification of all 5-alkyl esters prior to coupling, since the partial regioselective hydrolysis of these compounds is expected to be very difficult and has not been previously described. Recently, the use of tribenzyl phosphite in the Michaelis−Arbuzov reaction under particular experimental conditions[38,41] offered an interesting alternative to the strategy developed by Shermergorn[35] and Maier[36] (Scheme 3). The resulting pentabenzyl ester **2** can be selectively converted into tetrabenzyl ester **3**[42] prior to functionalization with nucleosides and final hydrogenolysis affording analogues of nucleoside triphosphates.[43−47] However, the Michaelis−Arbuzov reaction

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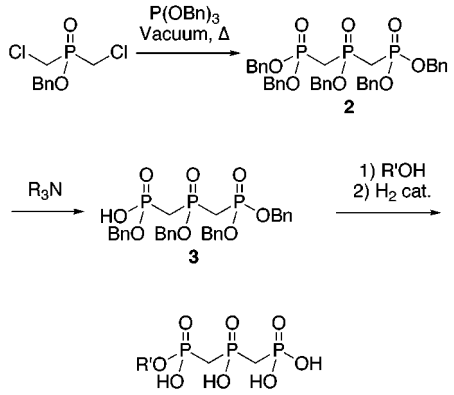
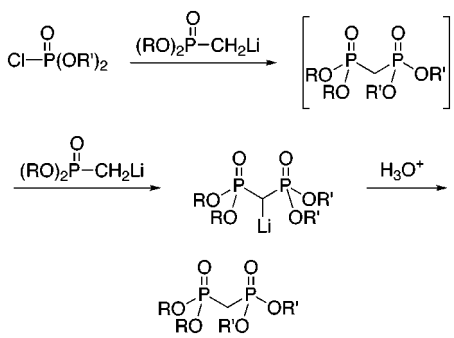
Scheme 3



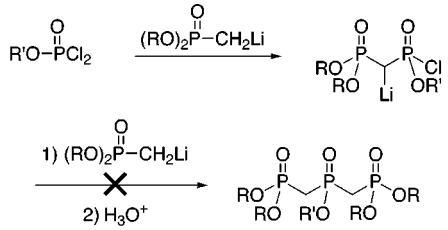
Scheme 4



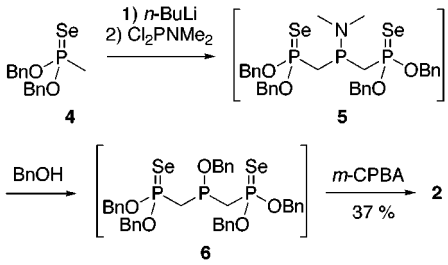
with benzyl phosphite appears to be highly sensitive and poorly reproducible if the benzyl chloride formed in situ is not efficiently removed from the reaction mixture. To overcome that difficulty we became interested in the chemistry of the methanephosphonate anion.

A major route in the preparation of methylene bisphosphonates involves the reaction between the lithiated anion of a dialkyl methanephosphonate and a chlorophosphate[48−54] (Scheme 4). A 2-fold excess of anion or base is required to bring the reaction to completion because the hydrogen atoms of the methylene bridge between the phosphoryl moieties are much more acidic than the ones of the starting phosphonate. Deprotonation rapidly occurs in the reaction medium so that the intermediate bisphosphonate is never observed during the reaction. The negative charge developed nearby the electrophilic phosphorus atom prevents any attack of a second carbanionic reagent and so does not allow access to triphosphate analogues (Scheme 5).

Scheme 5



Scheme 6



To tentatively extend that methodology to the preparation of a bis-methylene analogue of triphosphate it is thus necessary to lower the basicity of the anion to be condensed with the electrophilic phosphorus species. The high basicity of the methanephosphonate anion results from the formation of a stable complex between Li^+ and the P═O group.[55] The partial neutralization of the charge of the lithium cation through coordination with the oxygen atom of the P═O group weakens the Coulombic interaction between Li^+ and the negative carbon atom which increases the reactivity and the basicity of the latter. Considering that lower elements in group VI do not form stable complexes with lithium,[56] we investigated the coupling of methaneselenophosphonate anions with electrophilic phosphorus species. We found that such anions, which are less basic but also less nucleophilic, do not condense with electrophilic P^V species (dialkyl chlorophosphates or phosphorus oxychloride). By contrast, P^{III} activated compounds readily react with the lithiated anion of methaneselenophosphonates and multiple substitutions can occur. We took advantage of this reactivity to prepare compound **2** in 37% overall yield (Scheme 6). Dibenzyl methaneselenophosphonate **4** prepared from methyl dichlorophosphine was treated with *n*-butyllithium, and the resulting anion was condensed with *N*,*N*-dimethylphosphonamidous dichloride.[57,58] The intermediate aminophosphine **5** was transformed into the corresponding benzyl phosphonite **6** by displacement with benzyl alcohol. Finally, the P^{III} center and the two selenophosphonates were oxidized with *m*-CPBA to yield **2**. The reaction sequence was carried out in a one-pot procedure. It is noteworthy that this reaction can be reproducibly performed on the milligram or gram scale, by contrast with the route involving a double Michaelis−Arbuzov reaction and where the absence of solvent is not compatible with small quantities of reagents.

The preparation of dinucleoside triphosphates analogues starting from **2**, however, proved to be lengthy and difficult because the simultaneous deprotection of both phosphonate moieties lacks selectivity (Scheme 7).

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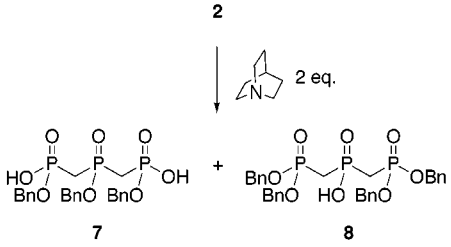
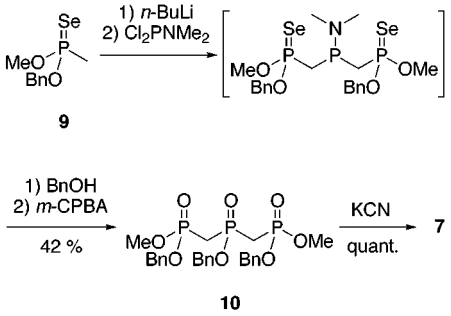
Scheme 7



Scheme 8



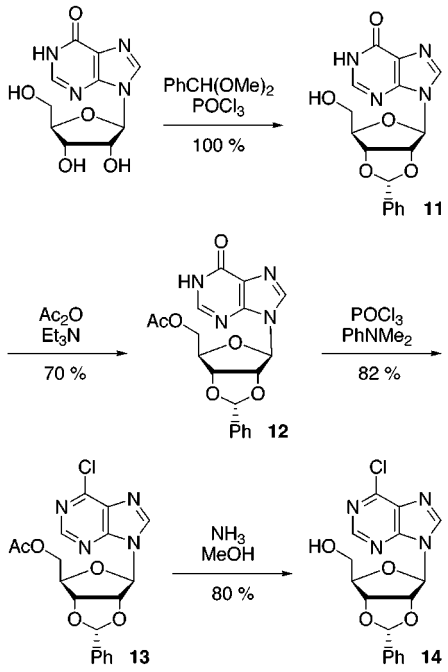
When **2** is treated with 2 equiv of quinuclidine, the expected bis-phosphonic acid **7** is always obtained as a mixture with phosphinic acid **8** (20%). Due to their high polarity these two compounds could not be separated by flash chromatography over silica gel. Interestingly, no isomer of these compounds nor other deprotected compounds could be detected in the crude reaction mixture even when the reaction was conducted in the presence of a large excess of quinuclidine or DABCO. These results clearly indicate that, in our experimental conditions, the removal of one benzyl group disallows further deprotection on the same phosphorus atom or on an adjacent one, most likely for stereoelectronic reasons. As a consequence, if the first deprotection occurs on the phosphinate, compound **8** is obtained and does not react further whatever the experiment duration and the excess of tertiary amine used. On the other hand, if deprotection occurs first on one phosphonate group, another reaction is possible on the second phosphonate, but not on the adjacent phosphinate, leading to **7**.

As diacid **7** could not be prepared with enough purity from **2**, we decided to introduce different protecting groups on the phosphonate and phosphinate moieties in order to get additional flexibility and selectivity in our synthesis (Scheme 8).

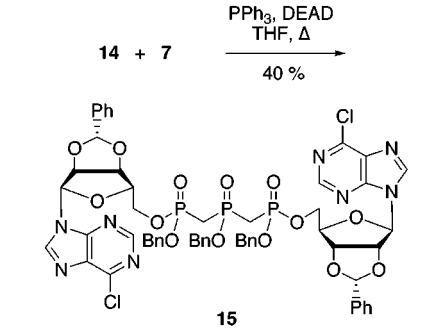
Condensation of the mixed methyl benzyl methaneselenophosphonate **9** with *N*,*N*-dimethylphosphonamidous dichloride, transesterification with benzyl alcohol and oxidation afforded dimethyl ester **10** in 42% yield. The latter compound was then unambiguously and quantitatively transformed into **7** using an excess of potassium cyanide in DMF.

Compound **7** is a key intermediate in our synthesis of analogues of dinucleoside triphosphates. A double condensation with adequately protected nucleosides will provide us with fully protected Np_3N analogues. To perform the final deprotection steps of both nucleosides and phosphonylated moieties using a one-pot procedure, we carefully selected nucleoside protections that can be removed by hydrogenolysis. In the case of the Ap_3A analogue target, we prepared 2′,3′-*O*-benzylidene-6-chloroadenosine **14**[59] starting from inosine (Scheme 9). Diacid **7** was then condensed with **14** under modified conditions of the Mitsunobu reaction to afford **15** in 40% yield (Scheme 10).

Scheme 9



Scheme 10



The 6-chloroadenosine derivative was chosen as a precursor of adenosine in order to avoid the well-documented cyclization side-reaction occurring with purine nucleosides during the Mitsunobu reaction or any 5′-hydroxyl group activation[60] (Scheme 11).

However, the subsequent displacement of the 6-chloro substituents in **15** with sodium azide failed, and we only obtained highly polar degradation products instead of the expected bis-azido compound **17**. We overcame that difficulty by carrying out the chlorine substitution[61] in **14** prior to condensation with **7** (Scheme 12).

The Mitsunobu reactions between **7** and nucleosides **14** and **16** were conducted in refluxing THF to avoid another side-reaction leading to *N*-alkylation of DEAD (Scheme 13). These conditions were previously established for imidophosphorylation of nucleosides.[62] It is

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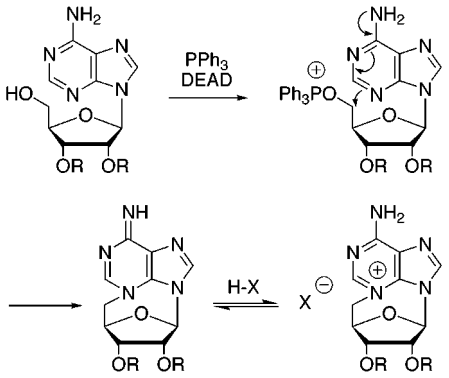
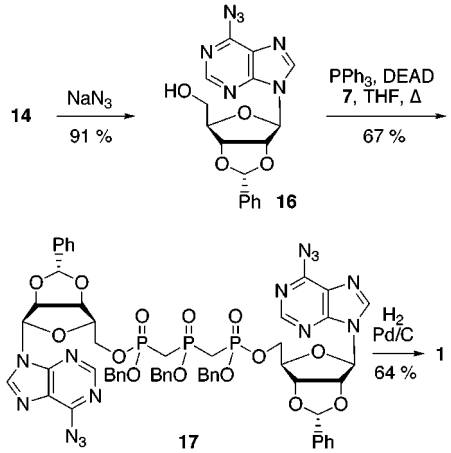
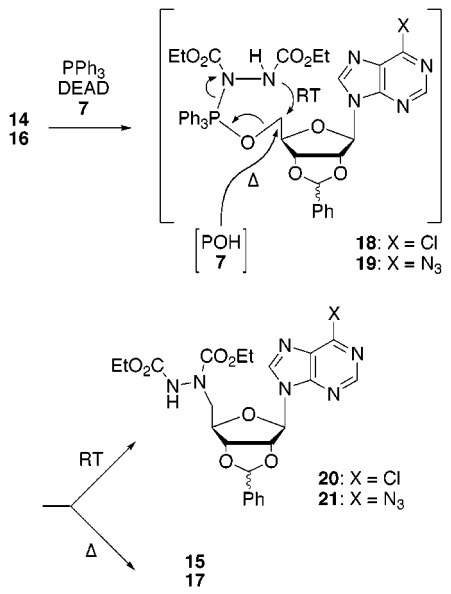
Scheme 11



Scheme 12



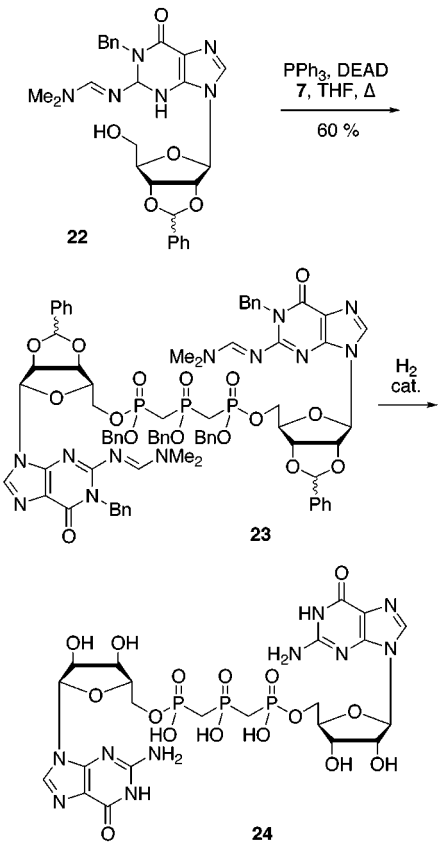
Scheme 13



likely that compound **20** and **21** result from the intra- rather than intermolecular rearrangement of the intermediate species **18** and **19** through a 5-center cyclic mechanism. Increasing the temperature therefore favors the intermolecular issue of the reaction, i.e., phosphonylation of the nucleosides into **15** and **17**.

Finally the catalytic hydrogenolysis of **17** allowed the one-step removal of the seven protecting groups in the

Scheme 14



molecule and afforded the target compound **1** in 64% yield without purification. That compound proved to be more than 96% pure by analytical HPLC.

The diguanosine derivative was prepared using a similar strategy (Scheme 14). To prevent the formation of an N^3-5′-cycloguanosine derivative upon activation of the 5′-hydroxyl group, the guanine moiety was protected according to Vincent et al.[63] The bis-phosphonic acid **7** was esterified with protected nucleoside **22** to afford **23** in 60% yield.

Subsequent hydrogenolysis of **23** required harsher conditions than for **17** due to the presence of the dimethylformamidine and *N*-benzyl protecting groups.[64] Compound **24** was obtained in a mixture with two other major compounds. That mixture results from partial anomerization of the two sugar moieties in the molecule, which is likely owing to the acidification of the reaction mixture occurring in the course of the debenzylation of the phosphonic and phosphinic acids. Running the final hydrogenolysis in the presence of 3 equiv of sodium hydrogenocarbonate markedly reduced the ratio of the unwanted isomers. However, purification of **24** by preparative HPLC was revealed to be necessary to obtain a highly pure compound.

Conclusion

Dinucleoside polyphosphates are intriguing compounds, and many questions remain unanswered concerning their properties and functions in biological

(62) Vincent, S.; Grenier, S.; Valleix, A.; Salesse, C.; Lebeau, L.; Mioskowski, C. *J. Org. Chem.* **1998**, *63*, 7244−7257.

(63) Vincent, S.; Mioskowski, C.; Lebeau, L. *Nucleosides Nucleotides* **1999**, *18*, 2127−2139.

(64) Vincent, S.; Mioskowski, C.; Lebeau, L. *J. Org. Chem.* **1999**, *64*, 991−997.

systems. This may be owing to the high lability of these compounds, and stable analogues are required in order to increase understanding of this field. To prepare stable analogues of dinucleoside triphosphates, we have developed an original strategy involving new advances in the chemistry of selenophosphonates. Thus different bis(dialkoxyphosphinomethyl)phosphinic acid esters were prepared. The results obtained offer a very powerful alternative to the only described synthetic route to the PCH_2PCH_2P motif and involving a double Michaelis–Arbuzov reaction. These variously protected triphosphate analogues served as key building blocks in the synthesis of $ApCH_2pCH_2pA$ and $GpCH_2pCH_2pG$, two non-hydrolyzable analogues of Ap_3A and Gp_3G. The title compounds **1** and **24** were prepared in 43% and 37% yield, respectively. Biological evaluation is currently underway and will be reported in due course.

Experimental Section

General. ^1H, ^{13}C, ^{31}P, and ^{77}Se NMR chemical shifts δ are reported in ppm relative to their standard reference (^1H: $CHCl_3$ at 7.27 ppm, HDO at 4.63 ppm, CD_2HOD at 3.31 ppm; ^{13}C: $CDCl_3$ at 77.0 ppm, CD_3OD at 49.0 ppm; ^{31}P: H_3PO_4 external at 0.00 ppm; ^{77}Se: $PhSe_2Ph$ in $CHCl_3$ external at 465.2 ppm). IR spectra were recorded in wavenumbers (cm^{-1}). Mass spectra (MS) were recorded at chemical ionization (CI) or in the electrospray (ES) mode. Mass data are reported in mass units (*m*/*z*). Analytical HPLC studies were carried out in the isocratic mode using a reversed phase column (Zorbax SB C_{18}, 250 × 4.6 mm, 5 μm; flow rate 1 mL/min at 30 °C) and a photodiode array detector (LKB 2410, detection at 255 nm). Preparative HPLC was carried out in the isocratic mode (Zorbax SB C_{18}, 250 × 21.2 mm, 7 μm; flow rate 5 mL/min for 3 min then 20 mL/min at 25 °C). Aqueous triethylamine was acidified to pH 7.3 by using CO_2. Abbreviations: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; sat., satellite.

(α,*β*: *β*,*γ*-Bis-methylene)diadenosine Triphosphate Trisodium Salt 1. Compound **17** (162 mg, 129 μmol) and Pd/C 10% (160 mg) in THF/*t*-BuOH/H_2O 3:3:2 (15 mL) were vigorously stirred for 18 h at 50 °C under hydrogen pressure (12.5 bar). The reaction mixture was filtered over a Celite pad and the filtrate reduced in vacuo. The crude residue was dissolved in water (5 mL) and washed with ether (4 × 5 mL) and dichloromethane (4 × 5 mL), and the aqueous phase was adjusted to pH 8 with NaOH 0.5 mM and lyophilized. Compound **1** was obtained as a white hygroscopic powder (97 mg, 64 **%**). TLC (RP-18) R_f 0.40 (H_2O/CH_3CN 8:2). Anal. HPLC (aqueous Et_3N 125 mmol, pH 7.3/CH_3CN 97:3) t_R 28.1 min. F° > 300 °C (decomposition). ^1H NMR (D_2O, 200 MHz) δ 8.16 (s, 2H); 7.81 (s, 2H); 5.78 (d, J = 4.6 Hz, 2H); 4.47 (dd, J = 4.9, 4.6 Hz, 2H); 4.33 (dd, J = 4.9, 4.4 Hz, 2H); 4.23–4.12 (m, 2H); 4.10–3.95 (m, 4H); 2.19 (t, J = 18.2 Hz, 4H). ^{13}C NMR (D_2O, 50 MHz) δ 155.4; 152.9; 148.7; 139.9; 118.5; 87.6; 83.9 (d, J = 8.0 Hz); 74.9; 70.5; 63.7; 31.6 (dd, J = 126.3, 82.1 Hz). ^{31}P NMR (D_2O, 81 MHz) δ 27.07 (t, J = 10.4 Hz, 1P); 19.49 (d, J = 10.4 Hz, 2P). IR (KBr) ν 3700–2500; 1650; 1477; 1424; 1331; 1205; 1091.

Bis(*O*,*O*′-dibenzyl phosphonomethyl)phosphinic Acid Benzyl Ester 2. *n*-Butyllithium (1.6 M in hexane, 250 μL, 400 μmol) was added dropwise to *O*,*O*′-dibenzyl methaneselenophosphonate **4** (136 mg, 400 μmol) in anhydrous THF (3 mL) at −78 °C. The mixture was stirred for 2 min before *N*,*N*-dimethylphosphonamidous dichloride[57] (29 mg, 200 μmol) in THF (1 mL) was added. The resulting solution was stirred for 15 min at −78 °C and for 1 h at room temperature, and the solvent was removed under reduced pressure. Ether (5 mL) was added to the crude residue, the precipitate was filtered off under argon atmosphere, and the filtrate was evaporated to yield crude bis(*O*,*O*′-dibenzyl selenophosphonomethyl) *N*,*N*-dimethylaminophosphine **5** as a colorless oil. TLC R_f 0.55 (C_6H_{14}/Et_2O 7:3). ^1H NMR ($CDCl_3$, 200 MHz) δ 7.42–7.24 (m, 20H); 5.00 (AB part of ABMX syst., J_{AB} = 12.2 Hz, J_{AX} = 12.2 Hz, J_{BX} = 10.2 Hz, J_{AM} = 4.0 Hz, J_{BM} = 4.0 Hz, $\Delta\nu$ = 26 Hz, 8H); 2.44 (d, J = 9.3 Hz, 6H). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 99.81 (dd, J = 843.0, 62.1 Hz, 2P); 38.91 (t, J = 62.1 Hz, 1P). Anhydrous toluene (2 mL) and benzyl alcohol (83 μL, 800 μmol) were added, and the solution was refluxed for 2 h. The solvent was removed in vacuo to yield the crude intermediate bis(*O*,*O*′-dibenzyl selenophosphonomethyl)benzyl phosphinite **6** as a colorless oil. TLC R_f 0.55 (C_6H_{14}/Et_2O 7:3). ^1H NMR ($CDCl_3$, 200 MHz) δ 7.43–7.28 (m, 25H); 5.20–4.81 (m, 10H); 3.02–2.46 (m, 4H). The previous residue was solubilized in dichloromethane (4 mL), and a solution of *m*-CPBA (218 mg, 884 μmol) in dichloromethane (2 mL) was added dropwise at −20 °C. The mixture was stirred for 30 min at −20 °C and for 1 h at room temperature. The red precipitate of selenium formed was filtered off. The filtrate was treated with aqueous $Na_2S_2O_5$ and neutralized with aqueous $NaHCO_3$. The resulting mixture was extracted with ethyl acetate (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and reduced in vacuo. The crude residue was chromatographed over silica gel (AcOEt/MeOH 10:0 to 9:1) to yield **2** (53 mg, 37%) as a white solid. TLC R_f 0.5 (AcOEt). F° = 49–50 °C. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.44–7.24 (m, 25H); 5.11 (d, J = 8.6 Hz, 2H); 5.06 (AB part of ABX syst., J_{AB} = 11.4 Hz, J_{AX} = 9.6 Hz, J_{BX} = 7.7 Hz, $\Delta\nu$ = 40.0 Hz, 4H); 4.99 (d, J = 8.6 Hz, 4H); 2.85 (dd, J = 20.5, 18.4 Hz, 4H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 135.8 (d, J = 3.7 Hz); 135.7 (d, J = 3.6 Hz); 127.8–126.7 (m); 67.9 (d, J = 6.1 Hz); 67.7 (d, J = 6.2 Hz); 66.8 (d, J = 6.5 Hz); 28.7 (dd, J = 132.5, 88.0 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 39.05 (t, J = 4.4 Hz, 1P); 21.49 (d, J = 4.4 Hz, 2P). MS (CI/NH_3) *m*/*z* 722 $[M + NH_4]^+$. IR (film) ν 3034; 2954; 2894; 1380; 1250; 998.

Methaneselenophosphonic Acid *O*,*O*′-Dibenzyl Ester 4. Benzyl alcohol (8.9 mL, 85.5 mmol) and triethylamine (11.9 mL, 85.5 mmol) in anhydrous toluene (80 mL) were added dropwise to dichloromethylphosphine (tech. 90%, 5.0 g, 38.5 mmol) at −78 °C. The reaction mixture was stirred for 20 min at −78 °C and for another 20 min period at room temperature before powdered selenium (4.1 g, 51.3 mmol) was added. The heterogeneous solution was refluxed for 3 h, cooled to room temperature, and diluted with ether (200 mL). The precipitate was removed by filtration. The filtrate was reduced under vacuum, and the crude residue was purified by flash chromatography over silica gel (C_6H_{14}/Et_2O 95/5 to 90/10) to yield **4** (12.6 g, 96 %) as a white solid. TLC R_f 0.65 (C_6H_{14}/Et_2O 7/3). F° = 42–43 °C. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.43–7.29 (m, 10H); 5.02 (AB part of ABX syst., J_{AB} = 12.4 Hz, J_{AX} = 12.4 Hz, J_{BX} = 10.1 Hz, $\Delta\nu$ = 24 Hz, 4H); 2.00 (d, J = 14.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 135.1 (d, J = 7.9 Hz); 128.6; 128.4; 128.2; 69.0 (d, J = 6.5 Hz); 25.2 (d, J = 101.8 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 101.81 (s; sat.: d, J = 848 Hz). ^{77}Se–NMR ($CDCl_3$, 57 MHz) δ −268.91 (d, J = 848 Hz). MS (CI/NH_3) *m*/*z* 341 $[M + H]^+$; 358 $[M + NH_4]^+$. IR (film) ν 3035; 2946; 2881; 1456; 996; 900.

Bis(*O*-benzyl phosphonomethyl)phosphinic Acid Benzyl Ester 7. Dimethyl ester **10** (319 mg, 577 μmol) and potassium cyanide (94.0 mg, 1.44 mmol) in anhydrous DMF (6 mL) were stirred at 70–80 °C for 5 h. DMF was removed under vacuum, and the residue was solubilized in methanol/water 7:1 (20 mL) and treated in a batch for 15 h with an ion-exchange resin (Dowex 50 × 8, H^+ form) at room temperature. The resin was filtered off and the filtrate reduced in vacuo to yield diacid **7** (301 mg, 99%) as a white hygroscopic solid. F° 79–80 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.34–7.23 (m, 15H); 5.05 (AB part of ABX syst., J_{AB} = 12.1 Hz, J_{AX} = 6.4 Hz, J_{BX} = 6.8 Hz, $\Delta\nu$ = 4.5 Hz, 4H); 5.04 (d, J = 7.9 Hz, 2H); 2.91 (AB part of ABX_2 syst., J_{AB} = 18.5 Hz, J_{AX} = 18.5 Hz, J_{BX} = 18.5 Hz, $\Delta\nu$ = 18.0 Hz, 4H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 136.4 (d, J = 7.5 Hz); 136.1 (d, J = 7.0 Hz); 128.9; 128.8; 128.7; 128.4; 128.3; 68.0 (d, J = 5.3 Hz); 67.7 (d, J = 5.3 Hz); 29.2 (dd, J = 132.2, 88.6 Hz). ^{31}P NMR ($CDCl_3$, 81 MHz) δ 41.93 (t, J = 5.5 Hz, 1P); 18.88 (d, J = 5.5 Hz, 2P). IR (film) ν 3200–2300; 1718; 1422; 1265; 1017.

Methaneselenophosphonic Acid *O*-Benzyl-*O*′-Methyl Ester 9. Anhydrous triethylamine (9.2 mL, 65.6 mmol) was added to dichloromethylphosphine (tech. 90%, 5.9 mL, 59.0

mmol) in anhydrous toluene (650 mL) at −78 °C. After 5 min, benzyl alcohol (6.8 mL, 65.6 mmol) in toluene (300 mL) was slowly added over a 4 h period. The reaction mixture was stirred at 60 °C for 2 h, and the temperature was lowered again down to −78 °C. Then another portion of triethylamine (9.2 mL, 65.6 mmol) was added followed by a slow addition of anhydrous methanol (2.7 mL, 65.6 mmol) in toluene (300 mL) over 4 h. The mixture was stirred for 30 min at −78 °C and 30 min at room temperature, and powdered selenium (5.7 g, 72.2 mmol) was added. The suspension was refluxed for 3 h, cooled to room temperature, and filtered over a Celite pad. The filtrate was reduced under vacuum, and the crude residue was purified by flash chromatography over silica gel (C_6H_{14}/Et_2O 95/5) to yield **9** (11.4 g, 73 %) as a slightly yellow oil. TLC R_f 0.50 (C_6H_{14}/Et_2O 7:3). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.43−7.35 (m, 5H); 5.13 (AB part of ABX syst., J_{AB} = 12.5 Hz, J_{AX} = 12.8 Hz, J_{BX} = 10.8 Hz, $\Delta\nu$ = 9.0 Hz, 2H); 3.61 (d, J = 14.7 Hz, 3H); 1.99 (d, J = 14.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 136.1 (d, J = 6.9 Hz); 128.6; 128.5; 128.2; 69.2 (d, J = 5.7 Hz); 53.7 (d, J = 6.5 Hz); 24.5 (d, J = 101.7 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 103.38 (s; sat.: d, J = 846 Hz). MS (CI/NH_3) m/z 263 $[M + H]^+$; 280 $[M + NH_4]^+$. IR (film) ν 2989; 2944; 1456; 1050; 1008; 899.

Bis(*O*-benzyl-*O*′-methyl phosphonomethyl)phosphinic Acid Benzyl Ester 10. Selenophosphonate **9** (5.63 g, 21.4 mmol) in anhydrous THF (90 mL) was treated dropwise with *n*-BuLi (1.6 M in hexane, 13.4 mL, 21.4 mmol) at −78 °C. The solution was stirred for 2 min, and *N*,*N*-dimethylphosphonamidous dichloride[57] (1.56 g, 10.7 mmol) in THF (20 mL) was added. The reaction mixture was stirred for 1 h at −78 °C, for 1 h more at room temperature, and the solvent was removed under vacuum. Toluene (40 mL) was added to the crude residue followed by benzyl alcohol (5.6 mL, 53.4 mmol) and 1*H*-tetrazole (750 mg, 10.7 mmol). The solution was refluxed for 30 min, and then cooled to −30 °C and *m*-CPBA (15.8 g, 64.0 mmol) in toluene (130 mL) was added dropwise. After 45 min at −30 °C and 1 h at room temperature, the red precipitate that formed was removed by filtration. The filtrate was treated with aqueous $Na_2S_2O_3$, and the resulting solution was neutralized with aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and reduced under vacuum. The crude residue was purified by chromatography over silica gel (AcOEt/MeOH 10:0 to 8:2) to yield **10** (2.5 g, 42%) as a glassy solid (*dl* and *meso* forms). TLC R_f 0.45 (AcOEt/MeOH 95:5). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40 (m, 15H); 5.18−4.97 (m, 6H); 3.72, 3.71, 3.64 and 3.63 (4d, J = 11.3 Hz, 6H); 2.86, 2.85 and 2.83 (3t, J = 19.6 Hz, 4H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 135.7 and 135.6 (2d, J = 3.8, 6.5 Hz); 128.4; 128.3; 128.0; 127.9; 127.8; 68.1, 68.0 and 67.8 (3d, J = 6.1 Hz); 66.9 (d, J = 6.5 Hz); 53.0; 52.9 and 52.7 (3d, J = 6.5, 6.1, 6.5 Hz); 27.9 (dd, J = 132.6, 88.4 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 39.64 and 39.42 (2t, J = 3.9, 4.1 Hz, 0.5P); 39.56 (t, J = 4.1 Hz, 0.5P); 22.40 (d, J = 3.9 Hz, 0.5P); 22.38, 22.37 and 22.33 (3d, J = 4.1 Hz, 1.5P). MS (CI/NH_3) m/z 570 $[M + NH_4]^+$. IR (film) ν 2956; 2899; 1456; 1251; 1186; 1016.

2′,3′-*O*-(1*R*)-Benzylideneinosine 11. Freshly distilled phosphorus oxychloride (5.2 mL, 55.6 mmol) was added dropwise at 0 °C to a solution of inosine (7.46 g, 27.8 mmol) and benzaldehyde dimethyl acetal (20.9 mL, 139.0 mmol) in anhydrous acetonitrile (210 mL). The initial suspension was stirred for 1 h at 0 °C and for 2 h at room temperature. The resulting solution was poured into iced saturated $NaHCO_3$ solution (600 mL) and stirred for 1.5 h at 0 °C. The precipitate was collected by filtration and washed with ether to yield **11** (9.91 g, 100%) as a white powder. TLC R_f 0.35 (AcOEt/EtOH 8:2). F° = 254−255 °C. ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.34 (s, 1H); 8.09 (s, 1H); 7.58−7.45 (m, 5H); 6.26 (d, J = 3.0 Hz, 1H); 6.01 (s, 1H); 5.40 (dd, J = 5.0, 3.0 Hz, 1H); 5.12 (dd, J = 5.0, 2.5 Hz, 1H); 4.46−4.38 (m, 1H); 3.73−3.61 (m, 2H). ^{13}C NMR (DMSO-d_6, 50 MHz) δ 156.4; 147.8; 146.0; 138.8; 136.1; 129.7; 128.4; 126.8; 124.4; 106.6; 89.6; 86.5; 84.4; 82.6; 61.5. MS (CI/NH_3) m/z 357 $[M + H]^+$. IR (film) ν 3500−2500; 1700; 1589; 1550; 1508; 1419; 1214; 1098; 974.

5′-*O*-Acetyl-2′,3′-*O*-(1*R*)-benzylideneinosine 12. Triethylamine (4.7 mL, 33.5 mmol), 4-DMAP (0.34 g, 2.8 mmol), and acetic anhydride (2.9 mL, 30.7 mmol) were added to a suspension of compound **11** (9.90 g, 27.8 mmol) in anhydrous acetonitrile (200 mL). The reaction mixture was stirred for 1.5 h at room temperature and the resulting clear solution reduced in vacuo. The residual oil was poured into ether/dichloromethane 9:1 (800 mL), and the precipitate that formed was collected by filtration and washed with ether to yield **12** (7.70 g, 70%) as a white powder. TLC R_f 0.65 (CH_2Cl_2/EtOH 9:1). F° = 170−171 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 10.06 (s broad, 1H); 8.33 (s, 1H); 7.95 (s, 1H); 7.56−7.40 (m, 5H); 6.27 (d, J = 1.9 Hz, 1H); 6.06 (s, 1H); 5.55 (dd, J = 6.6, 1.9 Hz, 1H); 5.13 (dd, J = 6.6, 3.2 Hz, 1H); 4.72−4.62 (m, 1H); 4.38 (AB part of ABX syst., J_{AB} = 12.0 Hz, J_{AX} = 6.1 Hz, J_{BX} = 4.4 Hz, $\Delta\nu$ = 11.2 Hz, 2H); 2.01 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.9; 156.5; 147.7; 146.0; 139.0; 135.9; 129.8; 128.4; 126.9; 124.4; 106.9; 89.1; 84.3; 83.6; 82.0; 63.6; 20.3. MS (CI/NH_3) m/z 399 $[M + H]^+$; 416 $[M + NH_4]^+$. IR (film) ν 3370; 3066; 2990; 2900; 1745; 1689; 1588; 1547; 1513; 1460; 1374; 1229; 1093.

6-Chloro-9-[5′-*O*-acetyl-2′,3′-*O*-(1*R*)-benzylidene-β-D-ribofuranosyl]purine 13. Protected nucleoside **12** (7.33 g, 18.4 mmol) was added to a mixture of freshly distilled phosphorus oxychloride (48 mL) and *N*,*N*-dimethylaniline (2.35 mL, 18.4 mmol). The mixture was refluxed for 2 min and then the temperature quickly reduced to 0 °C. Phosphorus oxychloride was removed under vacuum, and the crude residue was poured into iced saturated $NaHCO_3$ solution (400 mL) and stirred for 30 min at 0 °C. The solution was extracted with dichloromethane, and the organic layer was dried over $MgSO_4$, reduced under vacuum, and purified by silica gel chromatography (Et_2O/C_6H_{14} 8:2 to 10:0) to yield **13** (6.32 g, 82%) as a white powder. TLC R_f 0.45 (Et_2O). F° = 112−114 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.78 (s, 1H); 8.26 (s, 1H); 7.56−7.39 (m, 5H); 6.32 (d, J = 2.1 Hz, 1H); 6.06 (s, 1H); 5.64 (dd, J = 6.5, 2.1 Hz, 1H); 5.16 (dd, J = 6.5, 3.1 Hz, 1H); 4.73−4.66 (m, 1H); 4.31 (AB part of ABX syst., J_{AB} = 20.0 Hz, J_{AX} = 6.0 Hz, J_{BX} = 4.4 Hz, $\Delta\nu$ = 12.0 Hz, 2H); 1.97 (s, 3H). ^{13}C NMR (D_2O, 50 MHz) δ 169.9; 152.0; 151.5; 150.8; 150.7; 144.2; 135.4; 131.4; 130.0; 128.5; 126.5; 108.0; 90.9; 84.6; 84.5; 82.3; 63.5; 20.4. MS (CI/NH_3) m/z 418 $[M + H]^+$; 435 $[M + NH_4]^+$. IR (film) ν 1744; 1592; 1561; 1403; 1227; 1095.

6-Chloro-9-[2′,3′-*O*-(1*R*)-benzylidene-β-D-ribofuranosyl]-purine 14. Compound **13** (4.00 g, 9.6 mmol) was stirred for 3 h in methanolic ammonia (70 mL) at room temperature. The solution was reduced in vacuo and the residue chromatographed over silica gel. Compound **14** (2.91 g, 80%) was obtained as a white powder. TLC R_f 0.60 (AcOEt). F° = 190−191 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.79 (s, 1H); 8.22 (s, 1H); 7.60−7.46 (m, 5H); 6.12 (d, J = 4.6 Hz, 1H); 6.09 (s, 1H); 5.51 (dd, J = 6.2, 4.6 Hz, 1H); 5.23 (dd, J = 6.2, 1.3 Hz, 1H); 4.73 (m, 1H); 3.93 (m, 2H). ^{13}C NMR (DMSO-d_6, 50 MHz) δ 151.6; 151.3; 149.3; 149.2; 145.7; 136.0; 131.4; 129.7; 128.3; 126.8, 106.4; 90.3; 87.1; 84.3; 82.6; 61.3. MS (CI/NH_3) m/z 375 $[M + H]^+$. IR (KBr) ν 3400; 3069; 2926; 2876; 1593; 1565; 1493; 1434; 1406; 1339; 1201; 1111; 1072.

***P*^{1},*P*^{3}-Bis-[2′,3′-*O*-(1*R*)-benzylidene-1′-(6-chloropurin-9-yl)-β-D-ribofuranos-5′-yl]-α,β,γ-tribenzyl α: β,β: γ-Bis-methylene Triphosphate 15.** Diethyl azodicarboxylate (108 μL, 687 μmol) was added to diacid **7** (60 mg, 114 μmol), protected nucleoside **14** (86 mg, 229 μmol), and triphenylphosphine (180 mg, 687 μmol) in refluxing anhydrous THF (2 mL). After 2.5 h the solvent was removed and the residue purified by silica gel chromatography (Et_2O/AcOEt/MeOH 10:0:0 to 0:7:3) to yield **15** (56 mg, 40%) as a yellowish solid (mixture of four diastereomers). TLC R_f 0.55 (AcOEt/MeOH 9:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.75−8.41 (m, 4H); 7.62−7.17 (m, 25H); 6.45−6.26 (m, 2H); 6.10−5.94 (m, 2H); 5.65−4.92 (m, 10H); 4.73−4.51 (m, 2H); 4.49−4.07 (m, 4H); 3.09−2.49 (m, 4H).

6-Azido-9-[2′,3′-*O*-(1*R*)-benzylidene-β-D-ribofuranosyl]-purine 16. Compound **14** (1.00 g, 2.67 mmol) and sodium azide (1.73 g, 2.67 mmol) in anhydrous DMF (20 mL) were stirred at 75 °C for 1 h. DMF was removed under reduced pressure and the residue purified by chromatography over silica gel (AcOEt/C_6H_{14} 7:3 to 10:0) to yield **16** (923 mg, 91%) as a white powder. TLC R_f 0.50 (AcOEt). F° = 158−159 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 9.86 (s, 1H); 8.82 (s, 1H); 7.61−7.42 (m, 5H);

6.60 (d, J = 2.6 Hz, 1H); 6.05 (s, 1H); 5.51 (dd, J = 6.4, 2.6 Hz, 1H); 5.17 (dd, J = 6.4, 2.3 Hz, 1H); 4.61 (ddd, J = 3.5, 3.4, 2.3 Hz, 1H); 3.82 (AB part of ABX syst., J_{AB} = 11.9 Hz, J_{AX} = 3.4 Hz, J_{BX} = 3.5 Hz, $\Delta\nu$ = 4.5 Hz, 2H). ^{13}C NMR (DMSO-d_6, 50 MHz) δ 145.3; 142.7; 141.5; 136.0; 135.8; 129.7; 128.3; 126.8; 120.4; 106.5; 90.6; 87.0; 84.8; 82.7; 61.3. MS (CI/NH_3) m/z 382 $[M + H]^+$. IR (KBr) ν 3405; 3121; 2941; 2884; 2115; 1640; 1483; 1372; 1274; 1110; 1055; 978.

***P*^{1},*P*^{3}-Bis[1′-(6-azidopurin-9-yl)-2′,3′-*O*-(1*R*)-benzylidene-*β*-D-ribofuranos-5′-yl)-α,*β*,*γ*-tribenzyl α:*β*,*β*:*γ*-Bis-methylene Triphosphate 17.** Compound **17** (164 mg, 67%) was obtained as a yellow powder (mixture of four diastereomers) starting from **16** and **7** and following the same procedure as described for **15**. TLC R_f 0.40 (AcOEt). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.52−8.22 (m, 4H); 7.60−7.07 (m, 25H); 6.53−6.15 (m, 2H); 6.10−5.81 (m, 2H); 5.68−4.75 (m, 10H); 4.71−4.48 (m, 2H); 4.45−4.00 (m, 4H); 3.79−2.41 (m, 4H). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 38.93−38.00 (m, 1P); 22.92−21.03 (m, 2P). MS (ES) m/z 1273 $[M - H + Na]^+$.

6-Chloro-9-{5′-deoxy-5′[*N*,*N*′-bis(diethylcarboxy)hydrazino]}-2′,3′-*O*-(1*R*)-benzylidene-*β*-D-ribofuranosyl]purine 20. This compound (51 mg, 33%) was obtained as a yellow powder following the same procedure as described for **15** except the experiment was carried out at room temperature. TLC R_f 0.35 (Et_2O). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.82 (s, 1H); 8.24 (s, 1H); 7.60−7.43 (m, 5H); 6.27−6.14 (m, 1H); 6.06 (s, 1H); 5.72−5.61 (m, 1H); 5.22 (dd, J = 6.7, 3.0 Hz, 1H); 4.72−4.62 (m, 1H); 4.12 (q, J = 7.1 Hz, 4H); 4.01−3.70 (m, 2H); 1.22 (t, J = 7.1 Hz, 6H). MS (CI/NH_3) m/z 534 $[M + H]^+$.

***N*^{1}-Benzyl-2′,3′-*O*-benzylidene-*N*^{2}-dimethylaminomethyleneguanosine 22.** 5′-Acetyl-N^1-benzyl-2′,3′-*O*-benzylidene-N^2-dimethylaminomethyleneguanosine[63] (573 mg, 1.0 mmol) was stirred for 3 h in methanolic ammonia (15 mL) at 0 °C. The solution was reduced in vacuo, added with toluene (10 mL), and reduced again. Compound **22** (530 mg, 100%) was obtained as a yellow powder and was used without purification (mixture of two diastereomers). TLC R_f 0.45 (AcOEt/MeOH 9:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.39 and 8.36 (2s, 1H); 7.72 and 7.67 (2s, 1H); 7.58−7.16 (m, 10H); 6.24 and 6.07 (2s, 1H); 5.97 and 5.96 (2d, J = 4.2 Hz, 1H); 5.53 (s, 2H); 5.41−5.32 (m, 1H); 5.28−5.14 (m, 1H); 4.59−4.44 (m, 1H); 4.02−3.77 (m, 2H); 3.17 and 3.16 (2s, 3H); 3.10 and 3.09 (2s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.0; 157.8 and 157.7; 157.4; 147.3; 138.2; 137.7 and 137.6; 136.2 and 136.0; 129.8 and 129.6; 128.5 and 128.4; 128.0; 126.8; 126.5 and 126.4; 120.8 and 118.1; 107.5 and 104.3; 91.7 and 90.4; 85.2 and 83.9; 84.9 and 83.6; 83.2 and 80.3; 62.7 and 62.4; 45.5; 41.1; 35.2. MS (CI/NH_3) m/z 517 $[M + H]^+$. IR (film) ν 3326; 3064; 2930; 1682; 1629; 1531; 1494; 1455; 1071.

***P*^{1},*P*^{3}-Bis(*N*^{1}-benzyl-2′,3′-*O*-benzylidene-*N*^{2}-dimethylaminomethyleneguanosine)-α,*β*,*γ*-tribenzyl-α:*β*,*β*:*γ*-bismethylene Triphosphate 23.** Compound **23** (208 mg, 60%) was obtained as a yellow powder (mixture of 16 diastereomers) starting from **22** and **7**, following the same procedure as described for **15**. TLC R_f 0.40 (AcOEt/EtOH 9:1). F° = 101−102 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.51−8.42 (m, 1H); 7.62−7.11 (m, 37H); 6.28−5.95 (m, 4H); 5.62−4.85 (m, 14H); 4.62−3.92 (m, 6H); 3.23−2.45 (m, 16H). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 39.26−38.20 (m, 1P); 22.99−20.42 (m, 2P). MS (ES) m/z 1543 $[M - H + Na]^+$. IR (film) ν 2955; 2924; 1686; 1629; 1493; 1381; 1249; 999.

(α,*β*:*β*,*γ*-Bis-methylene)diguanosine Triphosphate Bistriethylammonium Salt 24. Compound **23** (39 mg, 26 μmol), Pd/C 10% (79 mg), $Pd(OH)_2$/C 20% (79 mg), and $NaHCO_3$ (7 mg, 78 μmol) in THF/*t*-BuOH/H_2O 1:1:1 (9 mL) were vigorously stirred for 68 h at 50 °C under hydrogen pressure (12.5 bar). The reaction mixture was filtered (Millipore, Millex-FG, 0.22 μm) and the filtrate reduced in vacuo. The crude residue was purified by preparative HPLC to yield compound **24** (22 mg, 37%) as a white hygroscopic powder. TLC (RP-18) R_f 0.40 (H_2O/CH_3CN 8:2). Anal. HPLC (aqueous Et_3N 125 mmol, pH 7.3/CH_3CN 97:3) t_R 9.1 min. Prep. HPLC (aqueous Et_3N 125 mmol, pH 7.3/CH_3CN 97:3) t_R 18.8 min. F° > 300 °C (decomposition). ^{1}H NMR (D_2O, 300 MHz) δ 7.98 (s, 2H); 5.72 (d, J = 5.5 Hz, 2H); 4.63 (dd, J = 5.5, 4.9 Hz, 2H); 4.39 (dd, J = 4.9, 4.3 Hz, 2H); 4.22−4.15 (m, 2H); 4.09−3.92 (m, 4H); 3.07 and 2.94 (2q, J = 7.3 Hz, 12H); 2.22 (t, J = 18.3 Hz, 4H); 1.15 (t, J = 7.3 Hz, 18H). ^{13}C NMR (D_2O, 50 MHz) δ 158.6; 153.8; 151.5; 137.8; 115.9; 87.3; 83.8 (d, J = 9.7 Hz); 73.7; 70.3; 63.4 (d, J = 5.9 Hz); 46.8; 42.3; 31.1 (dd, J = 123.0, 86.5 Hz); 10.6; 8.3. ^{31}P NMR (D_2O, 121 MHz) δ 27.86 (t, J = 10.0 Hz, 1P); 18.76 (d, J = 10.0 Hz, 2P). IR (KBr) ν 3700−2500; 1650; 1480; 1331; 1200; 1099; 1035.

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