Synthesis of Enzymatically Stable Analogues of GDP for Binding Studies with Transducin, the G-Protein of the Visual Photoreceptor

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The synthesis of five enzymatically stable analogues of guanosine diphosphate (GDP) has been carried out. The pyrophosphate moiety was mimicked in turn by the malonate, the acetophosphonate, the phosphonoacetate, the methylene-bis-phosphonate, and the imidodiphosphate groups. All the compounds were prepared via the synthesis of a transient fully protected nucleoside diphosphate analogue, and the final deprotection step was achieved by catalytic hydrogenolysis. The biological properties of the compounds have been evaluated toward transducin, the G-protein of the visual photoreceptor. Three guanosine imidodiphosphate derivatives bearing a linker at different positions on the sugar and on the base were then prepared and evaluated, giving some insight into the GDP binding site of transducin.

Introduction

Cells communicate with each other by a variety of signal molecules that are detected by specific receptors on the plasma membrane of the responding cell. Hundreds of cell-surface receptors employ G-proteins to initiate intracellular signaling chain reactions.[1−6] These G-proteins are heterotrimeric assemblies (G$\alpha\beta\gamma$) and are under the control of both GDP and GTP.[7,8]

One of the best characterized heterotrimeric G-protein-coupled pathways is the visual cascade of the rod cell.[9,10] In retinal rod outer segment, conversion of the light signal into an electrical signal involves in series rhodopsin (the photon receptor), transducin (the retinal guanine nucleotide-binding protein), and a specific phosphodiesterase (PDE, the effector) hydrolyzing the cytosolic cGMP.[11,12] Recently, structural data at near atomic resolution were obtained from crystals of a modified heterotrimeric transducin and independent subunits.[13−17] These remarkable results, however, do not clarify every phenomenon involved in the visual cascade, and the way the receptor activates the effector still remains unknown. Therefore, many efforts are directed toward structural analysis of intermediate complexes between the different protagonists of the visual cascade. In particular, our group is interested in the G$\alpha\beta\gamma$−GDP−rhodopsin complex and aim to study its structure by electron crystallography.[18,19]

Electron crystallography was initially developed for structural analysis of membrane proteins, and performance of the technique (minute amount of biological material needed, no molecular weight limitation for the protein to be studied, resolution reached...) rapidly extended to the structural analysis of water soluble proteins.[20−22] The key point of the technique consists of the concentration and organization into periodic two-dimensional (2D) arrays of the investigated macromolecule, mostly at the air−water interface. This can be simply achieved by using Coulombic interactions between the protein and charged lipids spread at the interface.[23−26]

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Nevertheless, that approach is hardly successful as it is usually difficult to select a discrete number of orientations of the protein when binding onto the lipid film. This process mainly results in obtaining noncrystalline 2D close-packing of proteins. By selecting unique and unambiguous interactions between proteins and the lipid layer, it is possible to increase the number of successful results in 2D crystallization experiments. Such unique interactions can be achieved by anchoring a specific ligand of the protein to the lipid molecule.[20,27−32]

In particular, for the structural analysis of G-proteins, we were led to investigate the possible ways to immobilize GDP on lipid supports. Due to the relative poor stability of the pyrophosphate bridge in nucleotides, our efforts were consecutively directed in two directions: (i) synthesis of a GDP analogue stable to enzymatic hydrolysis and recognized by transducin; (ii) functionalization of that GDP analogue (for further immobilization purpose) in a manner preserving its affinity for the protein.

Results and Discussion

Synthesis of Nonhydrolyzable Analogues of GDP. The lack of stability in nucleoside polyphosphates results from the presence of pyrophosphate bridges in the structure. The pyrophosphate moiety is highly sensitive to both enzymatic and chemical hydrolysis and is prone to dismutation reactions.[33,34] It is thus of outmost interest to use stable analogues of nucleotides in biological experiments.[35] There are very few reports in the literature on the synthesis of GDP analogues. The bisthiophosphate analogue of GDP (GDP$\alpha S\beta S$) has been described by Ludwig and Eckstein a few years ago.[36] Guanosine phosphonoacetate (compound **3**, Figure 1) has been claimed in an early patent by Heimer and Nussbaum in 1978 and was obtained via phosphorylation of an appropriate protected nucleoside with phosphonoacetic acid ethyl ester.[37] Last, the synthesis of guanosine imidodiphosphate (compound **5**) has been described via the reaction of guanosine with trichloro[(dichlorophosphoryl)imido]phosphorane, followed by careful hydrolysis and tricky purification.[38] We prepared five different GDP

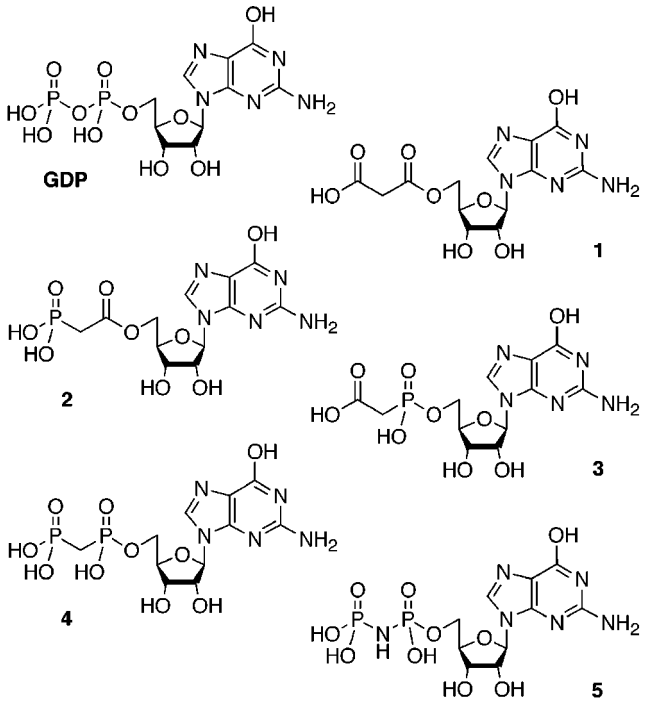

Figure 1.

analogues (including compounds **3** and **5**) that could be recognized and bound by transducin for further functionalization and immobilization experiments (Figure 1). In compounds **1**−**4**, the oxygen atom in the pyrophosphate bridge is replaced with a methylene group and, for compounds **1**−**3**, one or both phosphate group(s) is (are) replaced with carboxylate(s). The rationale for these structural modifications is based on documented biological properties of such diphosphate analogues of other nucleosides.[35,39−41] In compound **5**, an imidodiphosphate moiety stands for the pyrophosphate group. The imidodiphosphate group has been widely used to mimic pyrophosphates in nucleosides triphosphates,[35,42−44] and the resulting nucleotide analogues are usually well recognized by their target enzymes, but are generally poor substrates.

Compounds **1** and **2** were conveniently prepared by acylation of 2′,3′-*O*-benzylidene guanosine **6**[45,46] with the corresponding carboxylic acids, and further debenzylation by catalytic hydrogenolysis (Figure 2). Compounds **3**−**5** could not be obtained by following the same reaction sequence as phosphonic acid monoesters and essentially remain unreacted in the presence of carbodiimides and alcohols. Only rare examples of such coupling reactions are reported in the literature.[47−50] Though phosphonic acid monoesters are poor phosphonylating agents toward

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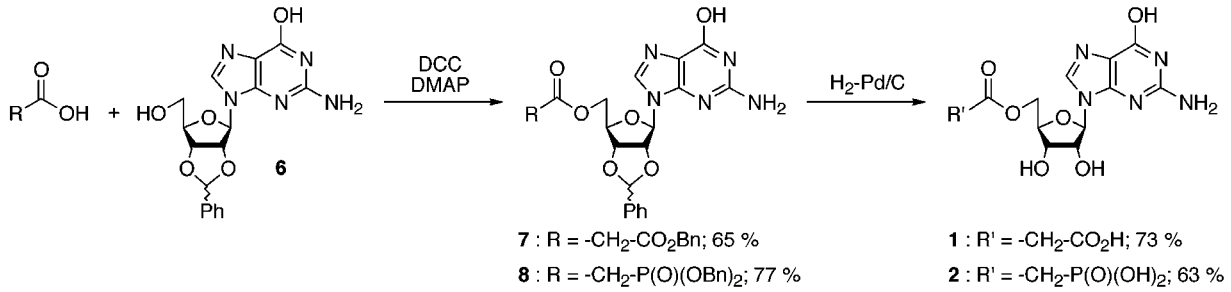

Figure 2.

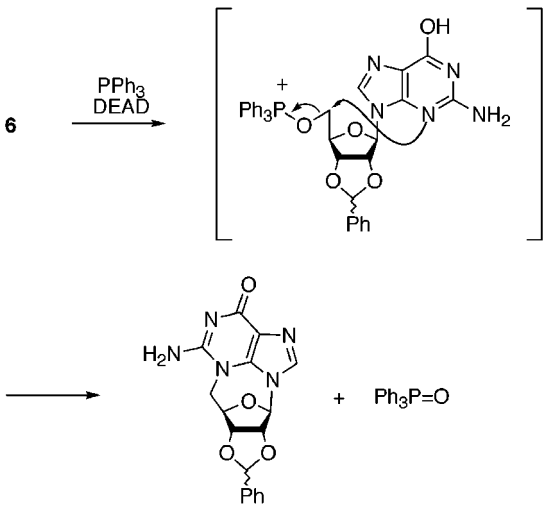

Figure 3.

alcohols, high yield phosphonylations can be performed using the Mitsunobu reaction.[51−54] However, the activation of 2′,3′-*O*-benzylidene guanosine **6** as an alkoxyphosphonium species straightforwardly provides a cycloguanosine product resulting from intramolecular displacement of the nucleofugal group on C^5 by N^3 (Figure 3).[55−57] That particular reactivity of purine nucleosides in the Mitsunobu reaction has been documented in an early work by Mitsunobu and co-workers on phosphorylation of nucleosides.[58] The intramolecular cyclization reaction can be efficiently circumvented in the case of adenosine derivatives by adequate masking of the exocyclic amino group on adenine resulting in a decreased nucleophilicity of N^3.[51] To address the same problem in the case of guanosine derivatives, we realized a series of phosphonylation reactions involving different guanine-protected compounds (Figure 4). Compounds **9**−**12** do not sufficiently deactivate N^3 to prevent the intramolecular cyclization reaction upon activation of the 5′-hydroxy

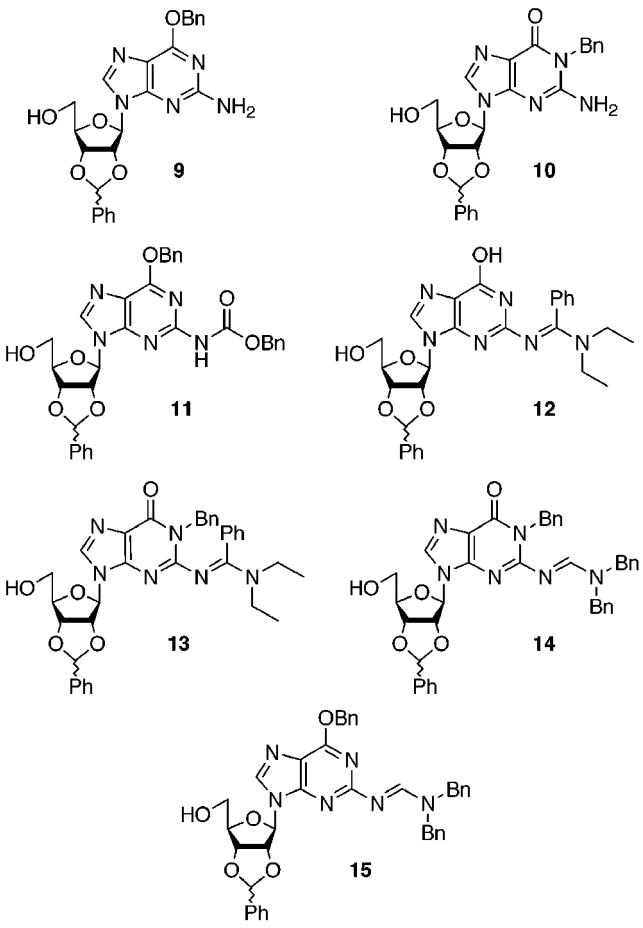

Figure 4.

group as an alkoxyphosphonium species. We concluded that as long as there is a labile hydrogen atom on the base, the cyclization side-reaction can proceed. This is argued considering the tautomeric forms of the substituted bases, as one of them always displays the acidic proton on the N^3 position, which indicates the high nucleophilicity of that nitrogen atom, at least in the intramolecular reaction. So, introducing a divalent protective group on the exocyclic amine of guanine together with an alkyl substituent at O^6 or N^1 is expected to definitely prevent the formation of cycloguanosine. This proved to be the case as compounds **13**, **14**, and **15** could be phosphonylated with good to excellent yields (vide infra).

Guanosine alkylation at O^6 is well documented and is used in many transformation sequences of that nucleoside. It can be regioselectively achieved via a Mitsunobu reaction on sugar-protected guanosine derivatives, with the exocyclic amine either masked[59−66] or unmasked.[67−70]

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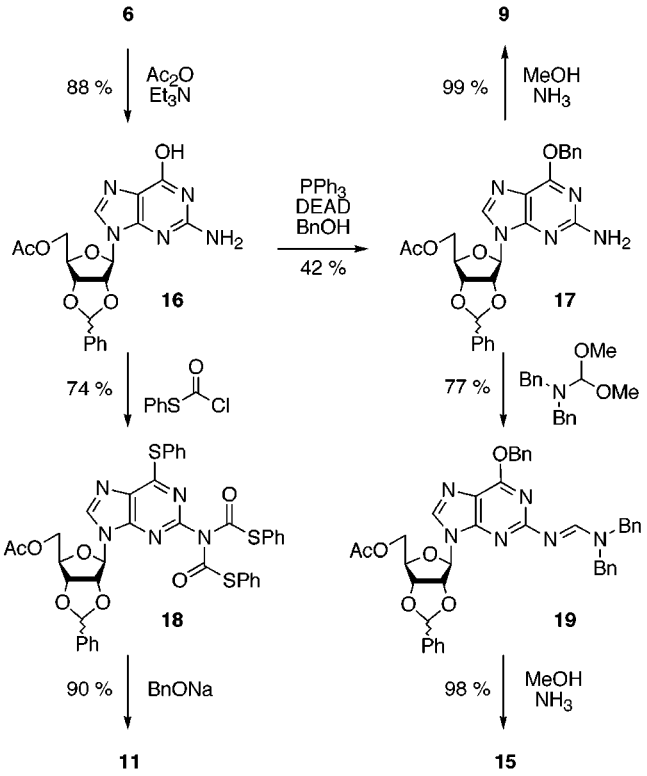

Figure 5.

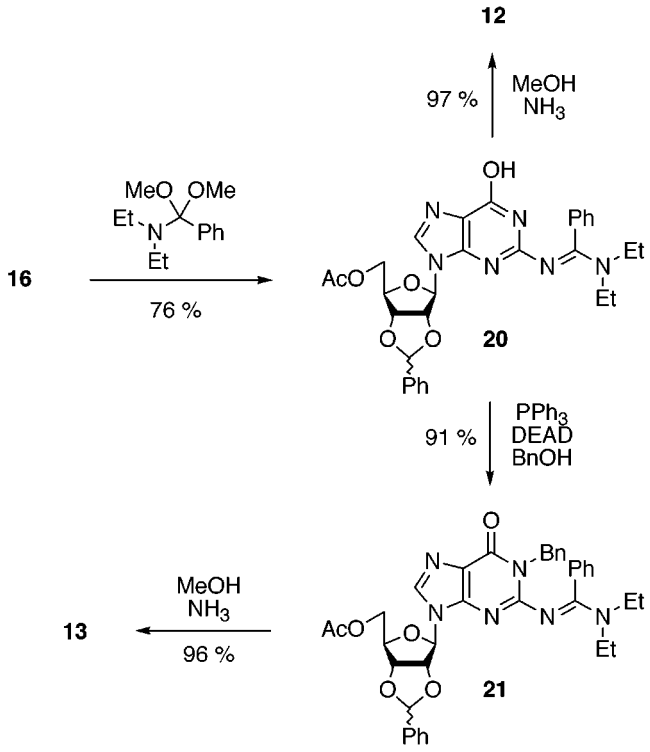

Figure 6.

Thus O^6-protected benzylidene guanosine **9** was obtained in three steps from **6** (Figure 5). The sequence involved acetylation of the 5′-hydroxy group prior to alkylation of **16** at O^6 to yield compound **17**. Removal of the 5′-acetyl protective group from **17** by methanolic ammonia produced the O^6-benzyl nucleoside **9**. When compound **16** was treated with phenyl chlorothioformate, it provided the fully protected nucleoside **18**[71,72] that was directly transformed into 2′,3′-*O*-benzylidene N^2-benzyloxycarbonyl guanosine **11** upon treatment with sodium benzylate. That triple transformation was achieved with 90% yield. Introduction of the *N*,*N*-dibenzyl formamidine protection[73] at the N^2 position in **17** produced the fully protected guanosine derivative **19**, and removal of the 5′-protective group was achieved in methanolic ammonia to yield compound **15**.

N,*N*-Diethyl benzamidine compounds **12** and **13** were prepared by reacting the sugar-protected guanosine derivative **16** with *N*,*N*-diethyl benzamide diethyl acetal[73] to give the intermediate compound **20** (Figure 6). Compound **12** was obtained from **20** via treatment with methanolic ammonia as described above. Attempted introduction of an O^6-benzyl group on **20** using the Mitsunobu reaction invariably failed and almost quantitatively led to compound **21** resulting from N^1-alkylation as assessed by X-ray crystallography.[74] This observation appears especially interesting as all reported alkylation of guanine nucleosides through the Mitsunobu reaction exclusively produced the O^6-alkylated compounds.[59−70] All of the examples described in the literature refer either to guanine or N^2-acyl guanine derivatives. Thus, this unprecedented regioselectivity of guanine alkylation is clearly due to the amidine protection at N^2 which provides a straightforward and very efficient access to N^1-alkylated guanosine derivatives when compared to those previously described in the literature.[75−81] Compound **13** resulted from treatment of **21** with methanolic ammonia.

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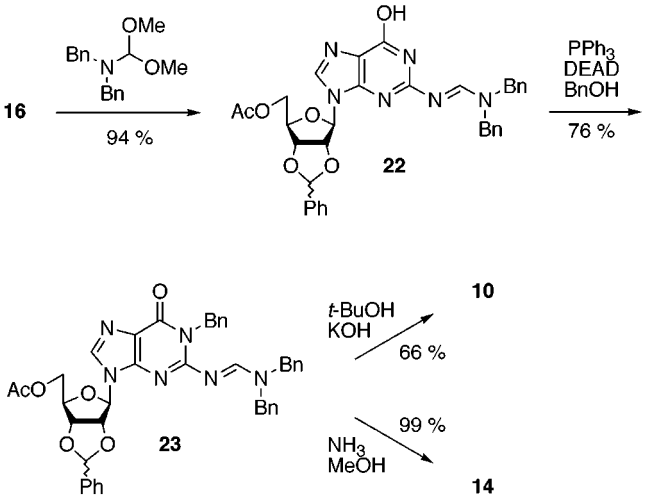

Figure 7.

The N^1-benzyl derivatives of guanosine **10** and **14** were prepared by reacting **16** with *N*,*N*-dibenzyl formamide dimethyl acetal[73] to yield **22** that was further regioselectively alkylated at N^1 via a Mitsunobu reaction to produce compound **23** (Figure 7). Treatment of this compound with potassium hydroxide yielded nucleoside **10**, whereas **14** was obtained through standard treatment with methanolic ammonia.

When compounds **13**−**15** were treated with triphenylphosphine, diethyl azodicarboxylate, and a phosphonic acid monoester, the corresponding phosphonic acid diesters were obtained with satisfactory yields (i.e. 60−80%). However, all attempts for the removal of the benzamidine protective group on **13** either by hydrogenolysis or hydrolysis failed.[73] This is presumably due to the high conjugation energy of the amidine double bond with the phenyl ring and the heterocycle. Considering the overall yields for the syntheses of compounds **14** (Figure 7) and **15** (Figure 5), the subsequent phosphorylation reactions with diphosphate analogue building blocks were realized with the N^1-benzyl nucleoside **14** (Figure 8). Interestingly, when the phosphorylation reactions were run at room temperature, product **30** resulting from *N*-alkylation of the reduced form of DEAD was obtained as the major product, once more due to the above-mentioned poor nucleophilicity of phosphonic acid monoesters (Figure 9). Neither the use of other phosphine compounds (PBu_3, $(4\text{-Cl-Ph})_3P$[53]) nor other azo derivatives (DIAD, ADDP[82−84]) prevented such a side reaction. Finally, the best results were obtained when conducting the reaction with PPh_3 and DEAD at 60 °C. Hydrogenolysis of compounds **25**, **27**, and **29** was achieved using a mixture of Pd/C and Pearlman catalyst[85] at pH 8.5, and compounds **3**−**5** were obtained as their ammonium or triethylammonium salt.

Synthesis of Functionalized Derivatives of Guanosine Imidodiphosphate. Binding experiments of compounds **1**−**5** with transducin showed that only the imidodiphosphate compound **5** was satisfactorily recog-

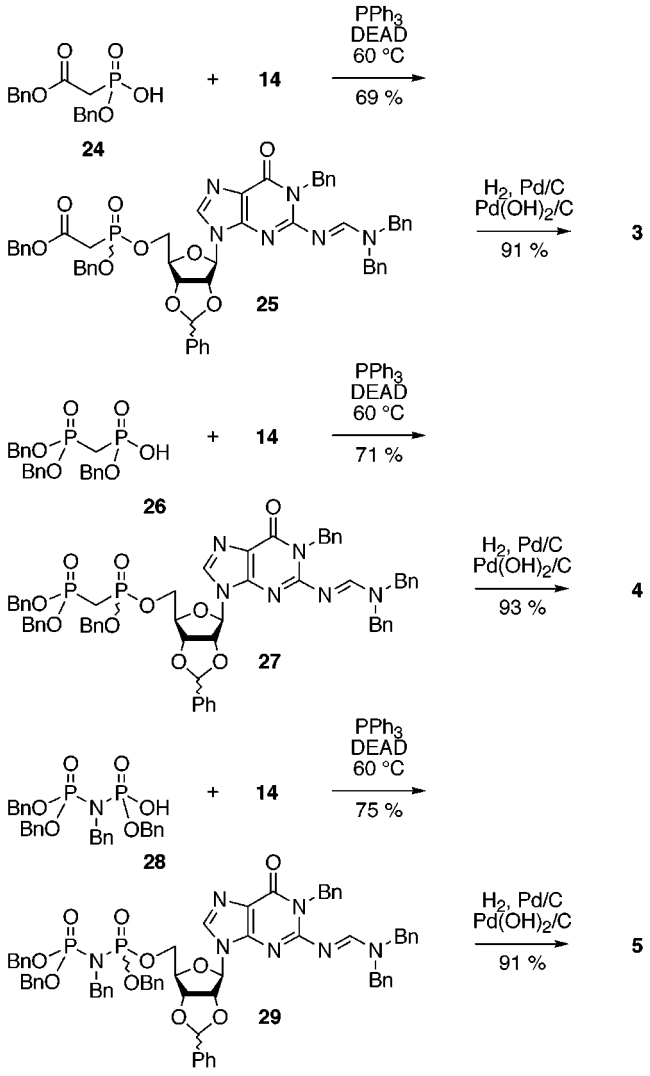

Figure 8.

nized by the protein (vide infra). Consequently, we modified our previous synthetic schemes so as to introduce a substituent at different positions on this GDP analogue. This was achieved in order to determine which ligand modification if any is allowed for further immobilization of transducin.

Functionalization at N^1 was realized starting from compound **22** (Figure 10). Instead of alkylating N^1 with activated benzyl alcohol in the Mitsunobu reaction conditions as described in Figure 7, we used alcohol **31** with an aim to selectively reduce the azide group into amine for the eventual coupling to a lipid matrix. After hydrolysis of the 5′-acetate of **32**, compound **33** was phosphorylated into **34** and fully deprotected by catalytic hydrogenolysis to produce the first functionalized GDP analogue **35**. Functionalization at O^6 was achieved using a different reaction sequence (Figure 11). Compound **22** was first sulfonated at O^6 to yield **36** according to a previously described procedure,[86] and the sulfonate was successively displaced by quinuclidine and azido alcohol **37** to produce the protected nucleoside **38**. Deacetylation, subsequent phosphorylation of **39** into **40**, and hydrogenolysis led to the second functionalized GDP analogue **41**.

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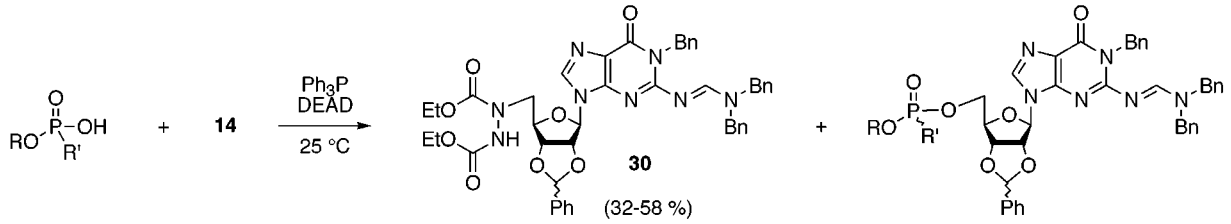

Figure 9.

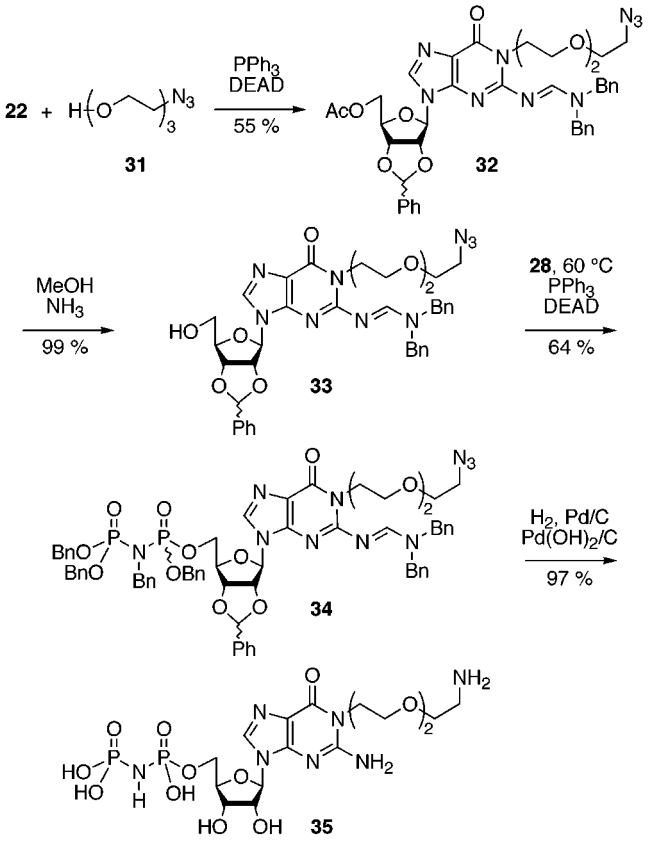

Figure 10.

A third functionalized GDP analogue bearing a substituent on the sugar moiety has been prepared (Figure 12). The synthetic route to **42** originated from the previously reported reactivity of the 2′-hydroxy group of nucleosides toward electrophiles.[87−90] Ikehara and coworkers realized the selective alkylation of N^2-isobutyryl guanosine at the 2′-position on the sugar moiety with 29% yield by reacting this compound with *o*-nitrobenzyl bromide and sodium hydride (Figure 13).[90] Surprisingly the treatment of N^2-(*N*,*N*-dibenzyl formamidine) guanosine **43** with a stoichiometric amount of benzyl bromide and NaH produced nearly quantitatively compound **44** resulting from the N^1-benzylation of the base, leaving the 2′-hydroxy group unreacted (Figure 14). This result is in contrast with the data of Ikehara and once again accounts for the unusual reactivity of guanosine protected at N^2 as an amidine. When compound **43** was treated

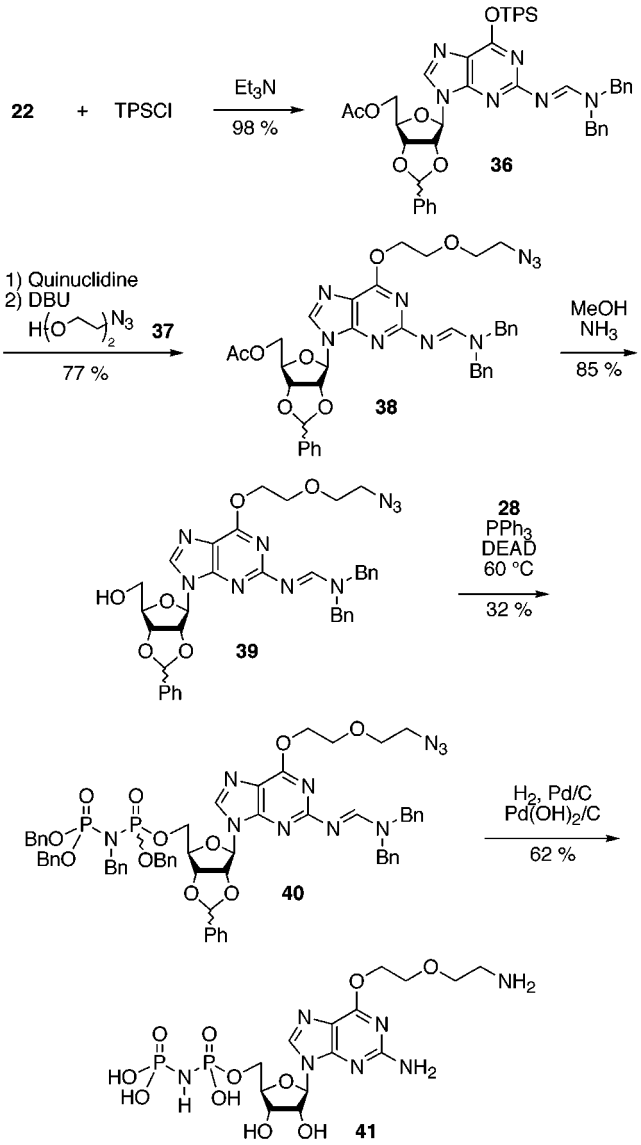

Figure 11.

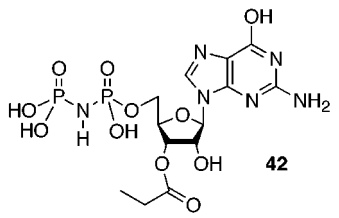

Figure 12.

with 2 equiv of sodium hydride and benzyl bromide, the 1-benzyl-2′-*O*-benzyl compound **45** was obtained with 54% yield. Direct regioselective phosphorylation of **45** with **28** yielded the 3′-hydroxy compound **46** that was

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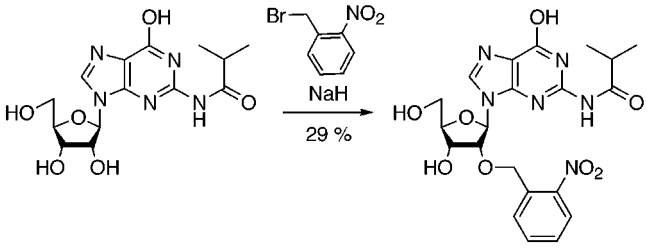

Figure 13.

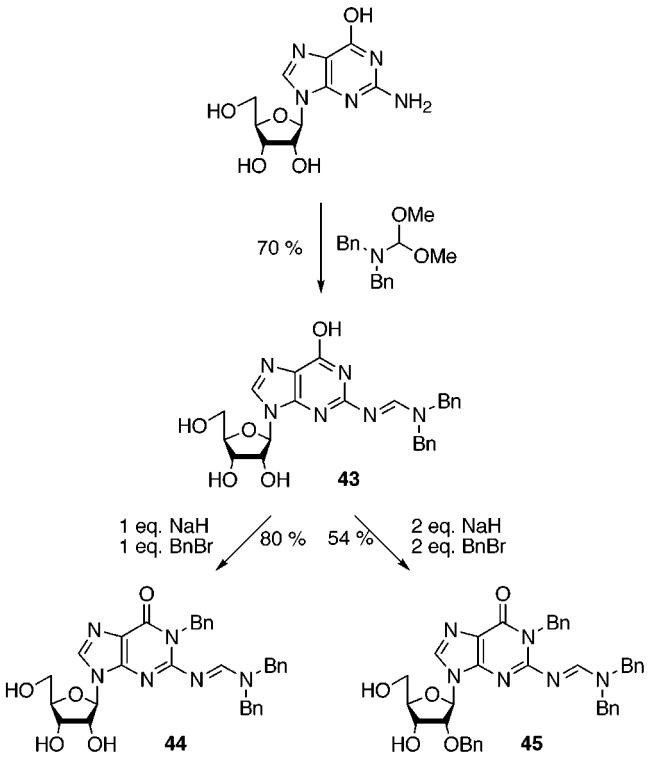

Figure 14.

further acylated with propanoyl chloride to produce the masked nucleotide **47** (Figure 15). Hydrogenolysis of compound **47** led to a mixture of the two regioisomers **42** and **48**, resulting from partial migration of the acyl moiety from the 3′- to the 2′-position.

Finally, we investigated the synthesis of a GDP analogue functionalized at the nitrogen atom bridging the two phosphorus atoms (Figure 16). The synthetic route to compound **49** is similar to those described above except for the use of the diphosphate building block **52** (Figure 17). Attempts to completely debenzylate compound **53** to obtain **49** invariably failed, resulting in an important hydrolysis of the imidodiphosphate moiety and formation of a mixture of guanosine monophosphate and guanosine phosphoramide. This result highlights the fact that if imidodiphosphate nucleosides are more resistant to enzymatic hydrolysis than the natural corresponding nucleotides, they remain much sensitive to chemical hydrolysis and have to be handled and stored with care.[38,91−93]

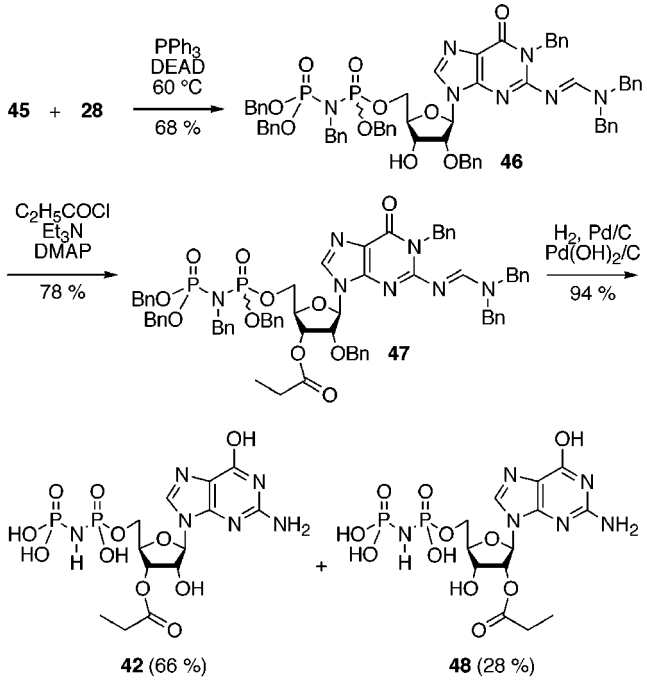

Figure 15.

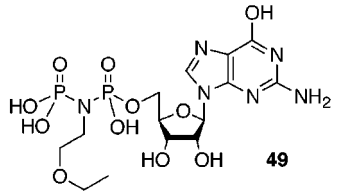

Figure 16.

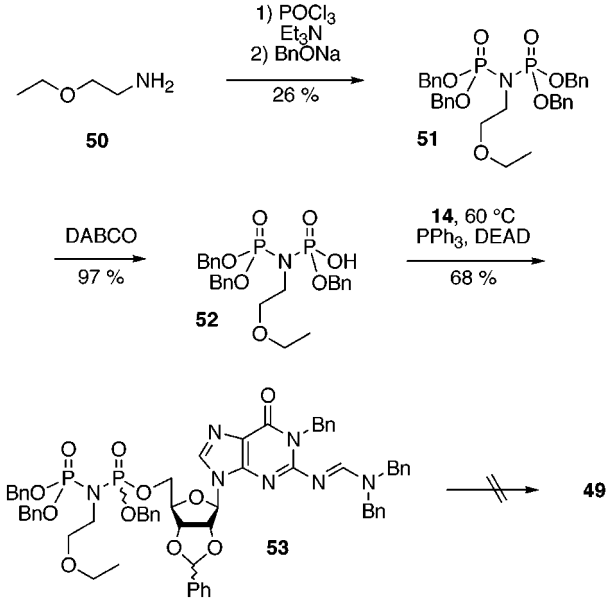

Figure 17.

Binding to Transducin. To our knowledge, up to now only two GDP analogues have been tested toward transducin (GDPαS and GDPβS).[94] Nevertheless, as they appear to be a substrate for many enzymes, their use meets some limitations.[95]

Binding experiments were realized with compounds **1**−**5**, using membranes of retinal rod outer segments.

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Assays involve the binding of a radioactive nucleotide (^{3}H-GDP), and detailed experimental procedures will be described elsewhere.[96] Compounds **1**−**4** could not displace the radioactive nucleotide even with a 10^4-fold excess. Only guanosine imidodiphosphate (compound **5**) could efficiently compete with ^{3}H-GDP for binding to transducin, and thus was selected for further chemical transformations. Whatever the structural modification tested (on the base at N^1: compound **35**, or O^6: compound **41**; or on the sugar moiety: compounds **42** and **48**), it definitely prevents any recognition of the compound by the enzyme. This is markedly in opposition with the results obtained with other G-proteins of similar structure that exhibit a higher tolerance for modified nucleotides.[97−101] Further analysis is underway.

Conclusion

The synthesis of 5 analogues of GDP stable to enzymatic hydrolysis has been carried out. The carboxylic esters **1** and **2** were obtained using the DCC/DMAP methodology for the coupling to the nucleoside of a precursor of the diphosphate moiety mimicry. The phosphonic and phosphoramidic esters **3**−**5** were prepared using an alternate procedure for the phosphorylation of the guanosine moiety, involving a Mitsunobu reaction and requiring the complete protection of the purine base. Only compound **5** could bind to transducin, the G-protein of the visual photoreceptor. Four derivatives of **5** bearing a linker at different positions were synthesized. None of these compounds was recognized by the protein, indicating that transducin is especially highly restrictive and specific at the GDP binding site, in contrast to numerous other heterotrimeric G-proteins of closely related structure.

Experimental Section

General. ^{1}H, ^{13}C, and ^{31}P NMR chemical shifts δ are reported in ppm relative to an internal reference resulting from incomplete deuteration of the NMR solvent (^{1}H: $CHCl_3$ at 7.27 ppm, HDO at 4.63 ppm, CD_2HOD at 3.31 ppm, and DMSO-d_5 at 2.50 ppm; ^{13}C: $CDCl_3$ at 77.0 ppm, CD_3OD at 49.0 ppm, DMSO-d_6 at 39.5 ppm; ^{31}P: H_3PO_4 at 0.00 ppm). IR spectra were recorded in wavenumbers (cm^{-1}). Mass spectra (MS) were recorded at chemical ionization (CI, NH_3). High-resolution mass spectra (HRMS) were recorded in the negative electrospray mode. Mass data are reported in mass units (*m*/*z*). Analytical HPLC studies were carried out in the isocratic mode using a reversed-phase analytical column (Zorbax SBC18, 250 × 4.6 mm, 5 μm) equipped with a photodiode array detector (detection at 267 nm). Elutions were conducted at 25 °C with a 1 mL/min flow rate.

9-(5′-*O*-(Malonyl)-*β*-D-ribofuranosyl)guanine (1). A solution of triethylammonium carbonate in water (0.3 mL, 0.2 M) is added to compound **7** (60 mg) in THF/*t*-BuOH/H_2O (1/8/4, 15 mL) until pH 8.5. Pd/C (10%, 280 mg) and $Pd(OH)_2$/C (20%, 257 mg) are added, and the mixture is stirred under hydrogen pressure (6 atm) for 24 h. Catalysts are removed by centrifugation on a Millipore membrane (Millex GV, 0.22 μM, *solvent resistant*), and solvents are evaporated in vacuo. The aqueous residue is lyophilized to dryness to yield the triethylammonium salt of **1** as a white powder (38 mg, 73%). ^{1}H NMR (D_2O, 200 MHz) δ 7.76 (s, 1H); 5.68 (d, J = 4.8 Hz, 1H); 4.62−4.54 (m, 1H); 4.32−4.14 (m, 4H); 2.96 (q, J = 9.2 Hz, 6H); 1.02 (t, J = 9.2 Hz, 9H). ^{13}C NMR (D_2O, 75 MHz) δ 170.75; 158.71; 154.03; 151.88; 137.42; 116.50; 87.49; 81.98; 73.54; 70.18; 64.14; 46.76; 39.39; 8.37. IR (KBr) ν 3104; 2626; 1691; 1598; 1364. HRMS: calcd for $C_{13}H_{14}N_5O_8$ 368.0842, found 368.0822 [M − H]$^-$. HPLC (H_2O/TFA 1000/1) t_R 40.0 min.

9-(5′-*O*-(Phosphonoacetyl)-*β*-D-ribofuranosyl)guanine (2). Compound **2** is obtained as its bis-triethylammonium salt from **8** following the same procedure as for **1** (white powder, 71 mg, 63%). ^{1}H NMR (D_2O, 200 MHz) δ 7.71 (s, 1H); 5.62 (d, J = 4.8 Hz, 1H); 4.53−4.46 (m, 1H); 4.28−4.23 (m, 1H); 4.18−4.04 (m, 3H); 2.96 (q, J = 9.2 Hz, 12H); 2.43 (d, J = 19.4 Hz, 2H); 1.02 (t, J = 9.2 Hz, 19H). ^{13}C NMR (D_2O, 50 MHz) δ 172.57; 163.20; 158.31; 151.79; 136.29; 117.21; 87.08; 81.97; 73.39; 70.39; 63.98; 46.61; 38.08 (d, J = 106.5 Hz); 8.43. ^{31}P NMR (D_2O, 121 MHz, [Et_3N] = 0.5 M) δ 11.49. IR (KBr) ν 3134; 1691; 1270; 1122; 1033. HRMS: calcd for $C_{12}H_{15}N_5O_9P$ 404.0607, found 404.0630 [M − H]$^-$. HPLC (H_2O/TFA 1000/1) t_R 62.5 min.

9-{5′-*O*-[(Carboxymethyl)hydroxyphosphinyl]-*β*-D-ribofuranosyl}guanine (3). Compound **3** is obtained as its bis-triethylammonium salt from **25** following the same procedure as for **1** (white powder, 43 mg, 91%). ^{1}H NMR (D_2O, 300 MHz) δ 8.00 (s, 1H); 5.74 (d, J = 6.0 Hz, 1H); 4.70−4.52 (m, 1H); 4.32 (dd, J = 3.0, 4.6 Hz, 1H); 4.15 (m, 1H); 3.99 (m, 2H); 2.96 (q, J = 9.2 Hz, 12H); 2.73 (d, J = 21.7 Hz, 2H); 1.02 (t, J = 9.2 Hz, 18H). ^{13}C NMR (D_2O, 75 MHz) δ 174.27; 158.99; 154.03; 151.88; 137.70; 116.22; 86.79; 84.13 (d, J = 7.8 Hz); 73.81; 70.58; 64.00 (d, J = 5.3 Hz); 46.78; 37.01 (d, J = 121.4 Hz); 8.34. ^{31}P NMR (D_2O, 121 MHz, [Et_3N] = 0.5 M) δ 18.20. IR (KBr) ν 3422; 3135; 1696; 1398; 1172; 1036. HRMS: calcd for $C_{12}H_{15}N_5O_9P$ 404.0607, found 404.0633 [M − H]$^-$. HPLC (H_2O/TFA 1000/1) t_R 40.7 min.

9-[5′-*O*-(Methylenebisphosphonate)-*β*-D-ribofuranosyl]-guanine (4). Compound **4** is obtained as its tris-ammonium salt from **27** following a similar procedure to that described for **1**, except that triethylammonium carbonate is replaced with ammonium carbonate (white powder, 53 mg, 93%). ^{1}H NMR (D_2O, 300 MHz) δ 8.05 (s, 1H); 5.84 (d, J = 5.5 Hz, 1H); 4.43 (dd, J = 4.0, 4.4 Hz, 1H); 4.31−3.95 (m, 4H); 2.08 (t, J = 18.9 Hz, 2H). ^{13}C NMR (D_2O, 75 MHz) δ 159.31; 154.95; 152.51; 136.78; 115.30; 87.82; 84.75; 74.57; 71.29; 64.34; 47.64; 25.90 (t, J = 47.1 Hz); 9.22. ^{31}P NMR (D_2O, 121 MHz, [Et_3N] = 0.5 M) δ 19.72 (b, 1P); 15.73 (b, 1P). IR (KBr) ν 3134; 1686; 1406; 1254; 1079. HRMS: calcd for $C_{11}H_{16}N_5O_{10}P_2$ 440.0372, found 440.0387 [M − H]$^-$. HPLC (H_2O/CH_3CN/TFA 980/20/1) t_R 11.4 min.

9-[5′-*O*-(Imidodiphosphate)-*β*-D-ribofuranosyl]guanine (5). Compound **5** is obtained as its tris-triethylammonium salt from **29** following the same procedure as for **1** (white powder, 75 mg, 91%). ^{1}H NMR (D_2O, 300 MHz, [Et_3N] = 0.5 M) δ 8.00 (s, 1H); 5.83 (d, J = 5.4 Hz, 1H); 4.60 (dd, J = 5.1, 5.4 Hz, 1H); 4.53 (dd, J = 4.6, 5.1 Hz, 1H); 4.23 (m, 1H); 4.15−3.98 (m, 2H). ^{13}C NMR (D_2O, 75 MHz, [Et_3N] = 0.5 M) δ 158.15; 154.38; 152.97; 137.35; 117.58; 87.66; 84.66; 74.15; 70.20; 64.06; 46.78; 10.64. ^{31}P NMR (D_2O, 121 MHz, [Et_3N] = 0.5 M) δ 3.73 (d, J = 3.6 Hz, 1P); −0.09 (d, J = 3.6 Hz, 1P). IR (KBr) ν 3130; 2351; 1682; 1455; 1064. HRMS: calcd for $C_{10}H_{15}N_6O_{10}P_2$ 441.0325, found 441.0329 [M − H]$^-$. HPLC (ammonium carbonate 0.5 M, pH 7.0/CH_3CN 99/1) t_R 4.8 min.

9-[5′-*O*-(*O*-Benzylmalonyl)-2′,3′-benzylidene-*β*-D-ribofuranosyl]guanine (7). 2′,3′-*O*-Benzylidene guanosine **6**[45,46] (mixture of 2 diastereomers, 90 mg, 0.24 mmol), malonic acid monobenzyl ester[102] (81 mg, 0.42 mmol), and DCC (104 mg, 0.50 mmol) are sonicated in THF (5 mL) for 2 h at room temperature. The crude reaction mixture is filtered, reduced

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in vacuo, and purified by chromatography on silica gel (AcOEt/EtOH: 100/0 to 60/40) to yield **7** as a white powder (mixture of two diastereomers, 85 mg, 65%). ^{1}H NMR ($CDCl_3$/CD_3OD: 9/1, 200 MHz) δ 7.57 and 7.55 (2s, 1H); 7.42−7.15 (m, 10H); 6.01 and 5.85 (2s, 1H); 5.98 and 5.93 (2d, J = 1.8 Hz, 1H); 5.24 (dt, J = 1.8, 7.7 Hz, 1H); 5.11−4.99 (m, 3H); 4.64−4.35 (m, 2H); 4.23−4.11 (m, 1H). ^{13}C NMR (CD_3OD/$CDCl_3$: 3/1, 50 MHz) δ 167.27; 167.24; 158.69; 154.31; 151.37; 137.61; 136.19; 135.66; 130.71; 130.43; 129.01; 128.74; 127.33; 127.25; 117.91; 108.25 and 104.76; 90.51; 85.81 and 85.23; 84.35 and 84.18; 82.93 and 82.05; 67.93; 65.31; 61.13. IR ($CHCl_3$) ν 2926; 2372; 1670 1090. MS (CI/NH_3): 548 $[M + H]^+$. Anal. Calcd for $C_{27}H_{25}N_5O_8$: C, 59.22; H, 4.60; N, 12.79. Found: C, 59.01; H, 4.50; N, 12.74.

9-(5′-*O*-Dibenzylphosphonoacetyl-2′,3′-*O*-benzylidene-β-D-ribofuranosyl)guanine (8). Compound **8** is obtained as a white powder (mixture of two diastereomers, 163 mg, 77%), starting from **6** and α-dibenzylphosphonoacetic acid,[103] and following the same procedure as for **7**. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.70 and 7.64 (2s, 1H); 7.50−7.15 (m, 15H); 6.03 and 5.98 (2s, 1H); 5.35−5.27 (m, 1H); 5.11−4.94 (m, 6H); 4.56−4.25 (m, 2H); 3.06 (d, J = 21.4 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 157.81; 153.28; 150.36; 136.76; 135.21 and 135.14; 135.10 and 135.01; 129.70; 129.59; 128.36; 128.29; 128.17; 127.64; 126.42; 126.34; 117.18; 107.31 and 103.90; 89.65; 84.93 and 84.35; 83.48 and 82.33; 82.13 and 81.40; 68.15 (d, J = 6.5 Hz); 64.38; 33.42 (d, J = 132.1 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 21.50. IR ($CHCl_3$) ν 3420; 2927; 1682; 1260; 995. MS (CI/NH_3): 674 $[M + H]^+$. Anal. Calcd for $C_{33}H_{32}N_5O_9P$: C, 58.84; H, 4.79; N, 10.40. Found: C, 58.80; H, 4.43; N, 10.44.

6-*O*-Benzyl-9-(2′,3′-*O*-benzylidene-β-D-ribofuranosyl)-guanine (9). Compound **17** (100 mg, 0.20 mmol) is stirred in methanolic ammonia (3 mL) for 1 h at 0 °C. The crude mixture is evaporated to dryness to yield **9** as a white powder (mixture of two diastereomers, 91 mg, 99%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.70 and 7.61 (2s, 1H); 7.61−7.15 (m, 10H); 6.29 and 6.05 (2s, 1H); 5.95 and 5.90 (2d, J = 4.5 Hz, 1H); 5.56 (s, 2H); 5.34 (m, 2H); 5.18 (m, 2H); 4.64 and 4.52 (2m, 1H); 3.98 (t, J = 12.1 Hz, 1H); 3.81 (t, J = 9.2 Hz, 1H). ^{13}C NMR ($CDCl_3$/CD_3OD: 2/1, 50 MHz) δ 161.21; 159.03; 152.84; 138.79 and 138.52; 136.17; 135.84 and 135.66; 132.82 and 132.64; 129.73 and 129.50; 128.23; 127.97; 127.08; 126.35 and 126.15; 125.51 and 125.21; 116.14 and 115.05; 107.47 and 104.42; 92.85 and 90.98; 85.31 and 85.12; 83.58 and 83.27; 82.75 and 80.26; 68.10; 62.92 and 62.59. IR ($CHCl_3$) ν 3364; 2929; 1612; 1257; 1093. MS (CI/NH_3): 462 $[M + H]^+$. Anal. Calcd for $C_{24}H_{23}N_5O_5$: C, 62.46; H, 5.03; N, 15.18. Found: C, 62.17; H, 4.98; N, 15.00.

1-Benzyl-9-(2′,3′-*O*-benzylidene-β-D-ribofuranosyl)guanine (10). Compound **23** (46 mg, 0.06 mmol) and K_2CO_3 (48 mg, 0.35 mmol) in methanol (2 mL) are stirred for 2 h at room temperature. The solvent is removed under reduced pressure, and the residue is purified by preparative TLC (AcOEt/EtOH: 9/1) to yield **10** as a white powder (mixture of two diastereomers, 20 mg, 66%). ^{1}H NMR (CD_3OD, 200 MHz) δ 7.99 and 7.97 (2s, 1H); 7.55−7.15 (m, 10H); 6.20 and 6.17 (2d, J = 2.2 Hz, 1H); 6.18 and 6.01 (2s, 1H); 5.49 and 5.42 (2dd, J = 2.2, 6.6 Hz, 1H); 5.31 (s, 2H); 5.22 and 5.16 (2dd, J = 4.0, 6.6 Hz, 1H); 4.47 and 4.36 (2m, 1H); 3.79 (m, 2H). ^{13}C NMR (CD_3OD, 50 MHz) δ 158.91; 155.61; 150.57 and 150.50; 138.92; 137.74 and 137.59; 136.79; 130.85 and 130.71; 129.90 and 129.76; 129.43 and 129.36; 129.20 and 128.58; 128.07 and 127.94; 127.58; 116.87; 108.77 and 105.25; 91.54 and 90.87; 88.53 and 86.77; 86.31 and 85.21; 84.09 and 82.47; 63.44; 45.33. IR (KBr) ν 3332; 2923; 1690; 1630; 1584; 1535; 1092. MS (CI/NH_3): 462 $[M + H]^+$. Anal. Calcd for $C_{24}H_{23}N_5O_5$: C, 62.46; H, 5.03; N, 15.18. Found: C, 62.31; H, 4.98; N, 14.88.

6-*O*-Benzyl-2-*N*-(*O*-benzylcarbamoyl)-9-(2′,3′-*O*-benzylidene-β-D-ribofuranosyl)guanine (11). Benzyl alcohol (9.4 mL, 90.4 mmol) is slowly added to a suspension of sodium hydride (60% in oil, 3.60 g, 86.4 mmol) in THF (90 mL) at 0 °C. The mixture is stirred at room temperature for 1 h before it is dropwise added to compound **18** (7.6 g, 10.2 mmol) in THF (80 mL) at 0 °C. The solution is stirred for 2 h at that temperature and then is treated with a saturated NH_4Cl aqueous solution. THF is removed in vacuo, and the residue is extracted with chloroform. The organic layer is dried over $MgSO_4$, concentrated, and purified by silica gel chromatography (Et_2O/*n*-C_6H_{14}: 4/1) to yield **11** as a white powder (mixture of two diastereomers, 5.48 g, 90%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 789 and 7.83 (2s, 1H); 7.71−7.15 (m, 15H); 6.23 and 6.05 (2s, 1H); 6.09 and 6.06 (2d, J = 2.6 Hz, 1H); 5.76−5.20 (m, 6H); 4.60 (m, 1H); 3.90 (m, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 181.65; 160.22; 151.61; 151.23; 140.78; 135.82; 135.59; 135.41; 129.25 and 129.07; 127.97; 127.92; 127.85; 127.71; 127.62; 127.59; 127.57; 126.21 and 126.07; 117.80 and 117.71; 106.97 and 103.82; 90.38 and 89.78; 86.87 and 85.57; 84.27 and 83.55; 82.01 and 80.73; 68.25; 66.64; 61.78. IR ($CHCl_3$) ν 3289; 3016; 1757; 1607; 1219; 1095. MS (CI/NH_3): 596 $[M + H]^+$. Anal. Calcd for $C_{32}H_{29}N_5O_7$: C, 64.53; H, 4.91; N, 11.76. Found: C, 64.70; H, 4.99; N, 11.91.

2-*N*-(*N*′,*N*′-Diethylbenzamidine)-9-(2′,3′-*O*-benzylidene-β-D-ribofuranosyl)guanine (12). Compound **12** is obtained as a white solid (mixture of two diastereomers, 1.03 g, 97%), starting from **20** and following the same procedure as for **9**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.54−7.11 (m, 11H); 6.19 and 6.01 (2s, 1H); 5.68 and 5.65 (2d, J = 3.0 Hz, 1H); 5.08 and 5.03 (2dd, J = 1.8, 4.3 Hz, 1H); 4.59 and 4.52 (m, 1H); 4.45−4.40 (m, 1H); 3.91−3.86 (m, 2H); 3.77−3.72 (m, 1H); 3.53−3.38 (m, 1H); 3.29−3.09 (m, 2H); 1.38−1.30 (m, 3H); 1.15−1.06 (m, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.27 and 164.15; 158.48; 157.18; 149.08 and 149.02; 137.94 and 137.74; 136.85; 133.93; 133.26 and 133.21; 129.82; 129.64; 129.58; 128.55; 128.47; 126.41 and 126.23; 107.49 and 104.38; 92.49 and 91.03; 85.45 and 85.13; 83.37 and 83.01; 82.33 and 80.29; 63.30 and 63.03; 44.43 and 42.45; 14.21 and 12.23. IR ($CHCl_3$) ν 3178; 2934; 1681; 1525; 1459; 1104. MS (CI/NH_3): 531 $[M + H]^+$. Anal. Calcd for $C_{28}H_{30}N_6O_5$: C, 63.38; H, 5.70; N, 15.84. Found: C, 63.36; H, 5.51; N, 15.66.

1-Benzyl-2-*N*-(*N*′,*N*′-diethylbenzamidine)-9-(2′,3′-*O*-benzylidene-β-D-ribofuranosyl)guanine (13). Compound **13** is obtained as a white solid (mixture of two diastereomers, 1.20 g, 96%), starting from **21** and following the same procedure as for **9**. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.70−7.61 (m, 1H); 7.54−7.17 (m, 13H); 7.09 (t, J = 7.7 Hz, 1H); 6.86 (t, J = 7.7 Hz, 1H); 6.18 and 5.98 (2s, 1H); 5.91 and 5.85 (2d, J = 3.2 Hz, 1H); 5.63 (dd, J = 4.4, 7.3 Hz, 1H); 6.15−4.98 (m, 2H); 4.49−4.30 (m, 2H); 4.10 and 4.03 (2q, J = 6.8 Hz, 1H); 3.79 (AB part of ABX syst, $\Delta\delta$ = 58.7 Hz, J_{AB} = 12.4 Hz, J_{AX} = 4.0 Hz, J_{BX} = 3.0 Hz, 2H); 3.46 and 3.43 (2q, J = 6.8 Hz, 1H); 1.42−1.32 (m, 3H); 1.03 and 1.00 (2t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 163.44; 158.06; 156.77; 146.82; 138.10; 137.76 and 137.61; 136.70; 135.82; 132.70; 132.56; 132.00; 131.79; 129.07; 129.44; 129.25; 129.16; 128.73; 128.49; 128.42; 128.34; 128.20; 127.22; 126.32; 126.05; 120.30; 107.36 and 104.20; 92.12 and 90.76; 85.27 and 84.94; 83.31 and 82.59; 81.97 and 80.28; 63.20 and 62.97; 45.75; 44.09; 42.26; 14.22; 12.72. IR ($CHCl_3$) ν 3306; 2934; 1691; 1519; 1295; 1115; 732. MS (CI/NH_3): 621 $[M + H]^+$. Anal. Calcd for $C_{35}H_{36}N_6O_5$: C, 67.72; H, 5.85; N, 13.54. Found: C, 67.80; H, 6.03; N, 13.80.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(2′,3′-*O*-benzylidene-β-D-ribofuranosyl)guanine (14). Compound **14** is obtained as a white solid (mixture of two diastereomers, 0.41 g, 99%), starting from **23** and following the same procedure as for **9**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.72 and 8.69 (2s, 1H); 7.73 and 7.67 (2s, 1H); 7.60−7.15 (m, 20H); 6.19 and 6.03 (2s, 1H); 5.98 and 5.95 (2d, J = 4.4 Hz, 1H); 5.52 (AB syst, $\Delta\delta$ = 135.0 Hz, J_{AB} = 14.6 Hz, 2H); 5.48 and 5.42 (2dd, J = 2.2, 4.4 Hz, 1H); 5.15 (m, 1H); 4.75−4.55 (m, 2H); 4.52−4.35 (m, 3H); 4.00 (m, 1H); 3.77 (m, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.21; 158.16 and 157.97; 157.55; 147.20; 137.94; 136.24; 135.92; 134.85; 134.71; 129.80; 129.60; 128.90; 128.73; 128.50; 128.41; 128.15; 127.81; 127.51; 126.76; 128.48; 126.33; 120.95; 107.50 and 104.26; 91.88 and 90.52; 85.28 and 83.93; 85.08 and 83.64; 83.34 and 80.37; 62.65 and 62.32; 54.83; 48.02 and 47.92; 45.56. IR ($CHCl_3$) ν 3306; 2924; 1687; 1612;

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1493; 1094. MS (CI/NH_3): 669 $[M + H]^+$. Anal. Calcd for $C_{39}H_{36}N_6O_5$: C, 70.04; H, 5.43; N, 12.57. Found: C, 70.04; H, 5.49; N, 12.66.

6-*O*-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(2′,3′-*O*-benzylidene-*β*-D-ribofuranosyl)guanine (15). Compound **15** is obtained as a white solid (mixture of two diastereomers, 1.87 g, 98%), starting from **19** and following the same procedure as for **9**. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.90 and 8.89 (2s, 1H); 7.87 and 7.77 (2s, 1H); 7.61−7.21 (m, 20H); 6.31 and 6.06 (2s, 1H); 6.07 and 6.00 (2d, J = 4.4 Hz, 1H); 5.65 (s, 2H); 5.46 (dd, J = 4.8, 6.2 Hz, 1H); 5.27−5.21 (m, 1H); 4.93 and 4.86 (2d, J = 5.1 Hz, 1H); 4.71−4.68 (m, 1H); 4.64 and 4.53 (2d, J = 1.6 Hz, 1H); 4.41 (m, 2H); 4.08−4.01 (m, 1H); 3.87−3.75 (m, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 162.50 and 162.39; 160.60; 159.16 and 159.10; 152.63 and 152.46; 140.27 and 139.92; 136.63 and 136.52; 136.05 and 136.00; 135.53; 129.84; 129.56; 128.92; 128.86; 128.57; 128.46; 128.37; 128.17; 127.94; 127.59; 126.90; 126.49; 126.23; 119.33 and 119.13; 107.44 and 104.61; 93.46 and 91.38; 85.78 and 85.49; 83.79 and 82.77; 80.41; 68.28; 63.34 and 62.99; 54.39; 48.00. IR ($CHCl_3$) ν 3273; 2928; 1592; 1377; 1215; 1071. MS (CI/NH_3): 669 $[M + H]^+$. Anal. Calcd for $C_{39}H_{36}N_6O_5$: C, 70.04; H, 5.43; N, 12.57. Found: C, 70.26; H, 5.55; N, 12.59.

9-(5′-*O*-Acetyl-2′,3′-*O*-benzylidene-*β*-D-ribofuranosyl)-guanine (16). 2′,3′-*O*-Benzylidene guanosine **6**[45,46] (3.53 g, 12.2 mmol), 4-DMAP (0.10 g, 0.90 mmol), and triethylamine (2.90 mL, 20.80 mmol) in acetonitrile (120 mL) are treated dropwise at 0 °C with acetic anhydride (1.60 mL, 16.90 mmol). The mixture is stirred at room temperature for 1 h before it is reduced in vacuo. The residue is washed twice with water and recrystallized in EtOH/H_2O (8/2) to yield **16** as a white powder (mixture of two diastereomers, 4.42 g, 88%). ^1H NMR (DMSO-d_6, 200 MHz) δ 7.88 (s, 1H); 7.67−7.15 (m, 5H); 6.57 (s, 2H); 6.11 (m, 2H); 5.39 (m, 2H); 4.23 (m, 3H); 1.99 and 1.98 (2s, 3H). ^{13}C NMR (DMSO-d_6/$CDCl_3$: 1/2, 50 MHz) δ 170.45; 156.98; 153.86; 149.94; 136.34; 135.16; 129.83; 128.48 and 128.38; 126.91; 117.04; 106.86 and 104.05; 88.41; 84.75 and 82.89; 84.01 and 82.06; 82.04 and 80.97; 64.07 and 64.04; 20.49. IR (KBr) ν 3260; 2978; 1675; 1316; 1094. MS (CI/NH_3): 414 $[M + H]^+$. Anal. Calcd for $C_{19}H_{19}N_5O_6$: C, 55.20; H, 4.64; N, 16.94. Found: C, 55.10; H, 4.62; N, 16.80.

6-*O*-Benzyl-9-(5′-*O*-acetyl-2′,3′-*O*-benzylidene-*β*-D-ribofuranosyl)guanine (17). Diethyl azodicarboxylate (420 μL, 2.68 mmol) is added to a mixture of compound **16** (550 mg, 1.33 mmol), triphenylphosphine (700 mg, 2.70 mmol), and benzyl alcohol (280 μL, 2.73 mmol) in THF (10 mL) at room temperature. The solution is stirred for 2 h and concentrated under reduced pressure, and the crude residue is purified by chromatography on silica gel (Et_2O/n-C_6H_{14}: 1/1 to 1/0). Compound **17** is obtained as a white powder (mixture of two diastereomers, 281 mg, 42%). ^1H NMR ($CDCl_3$, 200 MHz) δ 7.69 and 7.68 (2s, 1H); 7.71−7.15 (m, 10H); 6.16 and 6.09 (2d, J = 1.8 Hz, 1H); 6.16 and 5.99 (2s, 1H); 5.56 (s, 2H); 5.49 (m, 2H); 5.17 (m, 3H); 4.62 (m, 2H); 4.12 (m, 1H); 2.06 and 2.04 (2s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.31; 160.84; 159.09; 152.82; 138.23 and 138.11; 136.04 and 135.67; 135.50 and 135.38; 129.68; 129.55; 128.22; 128.07; 127.89; 127.68; 127.26; 115.76; 107.31 and 104.05; 89.74 and 89.67; 84.80 and 84.56; 83.37 and 82.61; 82.32 and 81.68; 67.75; 63.61 and 63.43; 20.38. MS (CI/NH_3): 504 $[M + H]^+$. Anal. Calcd for $C_{26}H_{25}N_5O_6$: C, 62.02; H, 5.01; N, 13.91. Found: C, 62.20; H, 5.09; N, 14.16.

6-Thiophenyl-2-(*N*,*N*-dithiophenylcarboxy)-9-(5′-*O*-acetyl-2′,3′-*O*-benzylidene-*β*-D-ribofuranosyl)guanine (18). Compound **16** (6.0 g, 14.5 mmol) in pyridine (60 mL) is treated with phenyl chlorothioformate (50.0 g, 145.0 mmol) at 0 °C in the dark. The mixture is stirred for 5 h at room temperature, and then it is poured on iced water (200 mL), stirred for 30 min, and extracted with chloroform. The organic layer is dried over $MgSO_4$ and reduced in vacuo, and the residue is purified by chromatography on silica gel (Et_2O/n-C_6H_{14}: 1/1 to 1/0) to yield **18** as a pale yellow powder (mixture of two diastereomers, 7.9 g, 74%). ^1H NMR ($CDCl_3$, 200 MHz) δ 8.33 (s, 1H); 7.69−7.27 (m, 20H); 6.34 and 6.28 (2d, J = 1.8 Hz, 1H); 6.22 and 6.08 (2s, 1H); 5.66 and 5.60 (2dd, J = 1.7, 6.4 Hz, 1H); 5.25 and 5.14 (2dd, J = 3.3, 6.4 Hz, 1H); 4.73−4.58 (m, 1H); 4.41−4.22 (m, 2H); 1.98 and 1.96 (2s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.11; 169.76 and 169.63; 168.81; 163.09; 149.73; 149.12; 144.05; 135.69 and 135.52; 135.15; 131.10; 131.05; 129.81; 129.67; 129.45; 128.98; 128.38; 128.33; 127.31; 126.54; 126.39; 123.46; 107.74 and 104.74; 90.36; 84.66 and 83.51; 84.71 and 83.14; 82.25 and 81.56; 63.66 and 63.54; 20.28. IR ($CHCl_3$) ν 3425; 1745; 1680; 1563; 1369; 1196. MS (CI/NH_3): 778 $[M + H]^+$. Anal. Calcd for $C_{39}H_{31}N_5O_7S_3$: C, 60.22; H, 4.02; N, 9.00. Found: C, 60.65; H, 4.27; N, 9.16.

6-*O*-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(2′,3′-*O*-benzylidene-5′-*O*-acetyl-*β*-D-ribofuranosyl)guanine (19). Compound **19** is obtained as a white solid (mixture of two diastereomers, 0.53 g, 77%), starting from **17** and following the same procedure as described for **22**. ^1H NMR ($CDCl_3$, 300 MHz) δ 9.00 and 8.99 (2s, 1H); 7.88 and 7.87 (2s, 1H); 7.56−7.22 (m, 20H); 6.34 and 6.32 (2d, J = 1.9 Hz, 1H); 6.19 and 6.04 (2s, 1H); 5.66 (s, 2H); 5.64 and 5.62 (dt, J = 1.9, 6.4 Hz, 0.5H); 5.53 (dd, J = 1.9, 6.8 Hz, 0.5H); 5.22−5.15 (m, 1H); 4.91 (AB syst, $\Delta\delta$ = 13.0 Hz, J_{AB} = 6.8 Hz, 1H); 4.73 (AB syst, $\Delta\delta$ = 14.4 Hz, J_{AB} = 3.0 Hz, 1H); 4.62−4.56 and 4.53−4.47 (2m, 1H); 4.43−4.38 (m, 2H); 4.34−4.26 (m, 2H); 2.02 and 1.99 (2s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.41; 162.54; 160.46; 159.23; 153.33 and 153.16; 139.45 and 139.12; 136.65; 135.95; 135.62; 135.57; 129.95; 129.84; 128.91; 128.63; 128.47; 128.36; 128.18; 127.94; 127.87; 127.78; 127.66; 118.34; 107.90 and 104.39; 89.53 and 88.94; 85.06 and 84.26; 83.73 and 82.22; 82.11 and 81.25; 68.21; 63.95 and 63.84; 54.38; 48.05; 20.64. IR (KBr) ν 2927; 1743; 1376; 1228; 1090. MS (CI/NH_3): 710 $[M + 1]^+$. Anal. Calcd for $C_{41}H_{38}N_6O_6$: C, 69.28; H, 5.39; N, 11.82. Found: C, 69.31; H, 5.45; N, 11.83.

2-*N*-(*N*′,*N*′-Diethylbenzamidine)-9-(5′-*O*-acetyl-2′,3′-*O*-benzylidene-*β*-D-ribofuranosyl)guanine (20). *N*,*N*-Diethyl benzamide (5.04 g, 28.4 mmol) is added to triethyloxonium tetrafluoroborate (5.40 g, 30.5 mmol) in anhydrous ether (10 mL) at 0 °C. The reaction mixture is stirred at room temperature for 1 h before sodium ethylate (2.26 g, 33.3 mmol) in ethanol (15 mL) is added. The solution is stirred for 3 h and filtered, and the filtrate is reduced in vacuo. The crude *N*,*N*-diethyl benzamide diethyl acetal is then added to compound **16** (2.35 g, 5.7 mmol) in anhydrous DMF (25 mL). The reaction mixture is stirred at room temperature for 16 h, reduced in vacuo, and purified by chromatography on silica gel (CH_2Cl_2/EtOH: 100/0 to 95/5) to yield **20** as a white solid (mixture of two diastereomers, 2.48 g, 76%). ^1H NMR ($CDCl_3$, 200 MHz) δ 7.80−7.15 (m, 11H); 6.21 and 6.14 (2d, J = 2.2 Hz, 1H); 6.06 and 6.00 (2s, 1H); 4.86 and 4.80 (2dd, J = 2.2, 6.8 Hz, 1H); 4.68 (m, 1H); 4.35 (m, 2H); 4.08 (m, 1H); 3.78 (m, 1H); 3.50 (m, 1H); 3.18 (m, 2H); 1.97 and 1.96 (2s, 3H); 1.37 (m, 3H); 1.11 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.75 and 169.69; 163.43 and 163.32; 158.53; 156.15; 149.09; 136.97 and 136.78; 135.42; 133.25 and 133.17; 129.38; 128.78; 127.97; 127.27; 127.09; 126.17; 126.04; 119.69; 107.03 and 103.43; 89.38; 84.18 and 82.58; 83.77 and 82.04; 81.96 and 81.28; 63.40 and 63.28; 43.77; 41.77; 20.04; 13.71; 11.76. IR ($CHCl_3$) ν 3067; 2935; 1743; 1681; 1640; 1223; 1104. MS (CI/NH_3): 573 $[M + H]^+$. Anal. Calcd for $C_{30}H_{32}N_6O_6$: C, 62.92; H, 5.64; N, 14.67. Found: C, 62.79; H, 5.60; N, 14.61.

1-Benzyl-2-*N*-(*N*′,*N*′-diethylbenzamidine)-9-(5′-*O*-acetyl-2′,3′-*O*-benzylidene-*β*-D-ribofuranosyl)guanine (21). Compound **21** is obtained as a white solid (mixture of two diastereomers, 1.16 g, 91%), from **20** and following the same procedure as for **17**. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.80−7.15 (m, 16H); 6.21 and 6.14 (2m, 1H); 6.05 and 6.01 (2s, 2H); 5.30 (m, 1H); 4.92 (m, 1H); 4.75−3.80 (m, 5H); 3.55 (m, 1H); 3.14 (m, 2H); 1.98 and 1.97 (2s, 3H); 1.31 (m, 3H); 1.06 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.91 and 169.85; 163.16 and 163.11; 157.91; 155.87; 146.88 and 146.82; 135.55; 138.20; 137.08 and 136.77; 135.55; 133.18; 132.90; 132.80; 131.76; 131.57; 131.11; 129.59; 129.52; 128.82; 128.19; 128.02; 127.93; 127.28; 127.03; 126.85; 126.29; 126.14; 119.62 and 119.54; 107.18 and 103.62; 89.84 and 89.74; 84.26 and 83.89; 82.61 and 82.36; 81.66; 63.60 and 63.44; 45.32; 43.78; 41.83; 20.23; 13.96; 12.64. MS (CI/NH_3): 693 $[M + H]^+$. Anal. Calcd for $C_{37}H_{38}N_6O_6$: C, 67.05; H, 5.78; N, 12.68. Found: C, 66.82; H, 5.70; N, 12.51.

2-*N*-(*N*′,*N*′-Dibenzylformamidine)-9-(5′-*O*-acetyl-2′,3′-*O*-benzylidene-*β*-D-ribofuranosyl)guanine (22). Freshly distilled dibenzylamine (7.5 g, 38.1 mmol) and *N*,*N*-dimethylformamide dimethylacetal (1.7 mL, 12.8 mmol) are refluxed in acetonitrile (15 mL) for 24 h. Anhydrous toluene (15 mL) is added, and the solution is reduced in vacuo. That operation is repeated twice before the crude residue in anhydrous THF (10 mL) is added to compound **16** (1.73 g, 4.2 mmol) in THF (20 mL). The resulting solution is stirred for 16 h at room temperature. THF is removed in vacuo, and the residue is chromatographed on silica gel (AcOEt/EtOH: 100/0 to 95/5) to yield compound **22** (mixture of two diastereomers, 2.45 g, 94%) as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.94 and 8.93 (2s, 1H); 7.73 (s, 1H); 7.60−7.15 (m, 15H); 6.24 and 6.17 (2d, J = 2.5 Hz, 1H); 6.19 and 6.04 (2s, 1H); 5.45 and 5.41 (2dd, J = 2.5, 6.6 Hz, 1H); 5.07−4.97 (m, 1H); 4.71 and 4.69 (2s, 2H); 4.65−4.39 (m, 2H); 4.47 (s, 2H); 4.28−4.18 (m, 1H); 2.10 and 2.01 (2s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.45; 158.27; 157.77; 156.86; 149.47; 136.96 and 136.86; 135.44 and 134.08; 134.71 and 134.65; 130.01; 129.87; 129.08; 128.79; 128.56; 128.50; 127.99; 127.84; 126.63; 126.48; 121.26; 107.83 and 104.37; 89.58; 85.25 and 83.88; 83.86 and 82.36; 82.28 and 81.38; 63.88 and 63.77; 54.87; 48.45; 20.63. IR (KBr) ν 2928; 1740; 1684; 1616; 1527; 1358; 1218. MS (CI/NH_3): 621 [M + H]$^+$. Anal. Calcd for $C_{34}H_{32}N_6O_6$: C, 65.79; H, 5.20; N, 13.54. Found: C, 66.00; H, 5.39; N, 13.69.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(5′-*O*-acetyl-2′,3′-*O*-benzylidene-*β*-D-ribofuranosyl)guanine (23). Compound **23** is obtained as a white solid (mixture of two diastereomers, 456 mg, 76%), from **22** and following the same procedure as for **17**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.84 and 8.81 (2s, 1H); 7.73 and 7.72 (2s, 1H); 7.60−7.15 (m, 20H); 6.23 and 6.17 (2d, J = 2.3 Hz, 1H); 6.19 and 6.03 (2s, 1H); 5.57 (AB syst, $\Delta\delta$ = 9.0 Hz, J_{AB} = 14.3 Hz, 2H); 5.48 and 5.42 (2dd, J = 2.3, 6.4 Hz, 1H); 5.03 and 4.98 (2dd, J = 4.8, 6.4 Hz, 1H); 4.66 (s, 2H); 4.62 and 4.53 (2q, J = 4.5 Hz, 1H); 4.43−4.37 (m, 3H); 4.25 (dd, J = 6.0, 11.7 Hz, 1H); 2.00 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.41; 157.99; 157.79 and 157.69; 157.13; 147.27; 138.14; 136.82 and 136.62; 135.55; 135.47 and 134.89; 134.78 and 134.71; 132.09 and 131.89; 129.96 and 129.81; 129.06; 128.77; 128.46; 128.24; 128.13; 127.92; 127.61; 127.56; 126.71; 126.60; 126.42; 120.80; 107.83; 104.38; 89.49; 85.26 and 83.81; 83.73 and 82.34; 82.16 and 81.35; 63.91 and 63.80; 54.21; 48.47; 45.44; 20.59. IR ($CHCl_3$) ν 2929; 1743; 1689; 1611; 1220; 1093. MS (CI/NH_3): 711 [M + H]$^+$. Anal. Calcd for $C_{41}H_{38}N_6O_6$: C, 69.28; H, 5.39; N, 11.82. Found: C, 69.38; H, 5.44; N, 12.01.

(Benzyloxyhydroxyphosphoryl)acetic Acid Benzyl Ester (24). α-Dibenzylphosphonoacetic acid[103] (5.45 g, 17.0 mmol), DCC (5.14 g, 25.0 mmol), and benzyl alcohol (2.0 mL, 19.0 mmol) are stirred at room temperature in dichloromethane (60 mL) for 6 h. The reaction mixture is filtered, and the filtrate is washed with 5% HCl. The organic layer is dried over $MgSO_4$, reduced in vacuo, and purified by chromatography on silica gel (Et_2O/*n*-C_6H_{14}: 3/7 to 10/0) to yield the intermediate (dibenzyloxyphosphoryl)acetic acid benzyl ester (5.30 g, 76%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.40−7.20 (m, 15H); 5.13 (s, 2H); 5.04 (m, 4H); 3.04 (d, J = 21.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.33 (d, J = 5.7 Hz); 135.72 (d, J = 5.8 Hz); 135.06; 128.39 (b); 128.29 (b); 127.87; 67.95 (d, J = 5.8 Hz); 57.28; 34.58 (d, J = 134.4 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 20.86 (s). IR ($CHCl_3$) ν 3065; 3033; 2948; 1734; 1456; 1381; 1270; 1215; 1115; 997. MS (CI/NH_3): 411 [M + H]$^+$.The latter compound (1.20 g, 2.9 mmol) is refluxed in toluene (15 mL) with DABCO (0.36 g, 3.2 mmol) for 4 h. The solution is reduced in vacuo, and the residue is gently stirred with Dowex 50 × 8 resin (H^+ form) in water/methanol (9/1, 100 mL) for 12 h. The ion-exchange resin is filtered off, and the filtrate is reduced in vacuo and dried by azeotropic distillation with toluene to yield **24** (0.90 g, 97%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.40−7.20 (10H); 5.14 (s, 2H); 5.07 (d, J = 7.9 Hz, 2H); 3.05 (d, J = 21.6 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.47; 135.84 (d, J = 5.8 Hz); 135.22; 128.3 (b); 127.80 (b); 67.67 (d, J = 5.8 Hz); 57.34; 34.59 (d, J = 137.9 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 22.69 (s). IR ($CHCl_3$) ν 3500; 2941; 1738; 1272; 1016. MS (CI/NH_3): 338 [M + NH_4]$^+$. Anal. Calcd for $C_{16}H_{17}O_5P$: C, 60.00; H, 5.35. Found: C, 60.43; H, 5.54.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-[2′,3′-*O*-benzylidene-5′-*O*-(*O*-benzylacetyl(benzyloxy)phosphoryl)-*β*-D-ribofuranosyl]guanine (25). Compounds **14** (100 mg, 0.15 mmol) and **24** (96 mg, 0.30 mmol) and DEAD (72 μL, 0.37 mmol) are dissolved in anhydrous THF (2 mL). The solution is warmed to 60 °C, and triphenylphosphine (79 mg, 0.30 mmol) in solution in anhydrous THF (0.6 mL) is added in one portion. The reaction mixture is stirred for 2 h at that temperature, reduced in vacuo, and chromatographed on silica gel (AcOEt/EtOH: 100/0 to 95/5) to yield **25** (mixture of four diastereomers, 101 mg, 69%) as a colorless glassy solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.80 and 8.53 (2s, 1H); 7.80, 7.78, 7.77 and 7.74 (4s, 1H); 7.60−7.15 (m, 30H); 6.22, 6.19 and 6.12 (3d, J = 2.6 Hz, 1H); 6.15, 6.14, 5.97 and 5.94 (4s, 1H); 5.55 (AB syst, $\Delta\delta$ = 9.0 Hz, J_{AB} = 14.7 Hz, 2H); 5.38, 5.30 and 5.21 (3dd, J = 2.6, 6.4 Hz, 1H); 5.15−4.85 (m, 5H); 4.75−4.10 (m, 7H); 2.99, 2.98 and 2.95 (3d, J = 21.4 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.25 and 165.14; 158.05; 157.28; 147.52; 138.22; 136.75; 136.65; 135.01; 129.86; 129.07; 128.66; 128.46; 128.32; 128.17; 127.94; 127.74; 127.62; 126.74; 126.62; 120.47; 107.72 and 104.41; 89.14, 88.98 and 88.79; 85.09 and 84.93; 83.83, 83.71 and 82.64; 81.92 and 80.65; 68.51 and 67.52; 65.22; 54.69; 48.41; 45.50; 34.24 (d, J = 135.8 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 21.72 (s, 0.25P), 21.70 (s, 0.25P), 21.67 (s, 0.25P) and 21.64 (s, 0.25P). IR ($CHCl_3$) ν 2924; 1733; 1689; 1612; 1493; 1455; 1267; 1073; 1016; 999. Anal. Calcd for $C_{55}H_{51}N_6O_9P$: C, 68.03; H, 5.30; N, 8.65. Found: C, 68.00; H, 5.19; N, 8.58.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-{2′,3′-*O*-benzylidene-5′-*O*-[*P*,*P*′,*P*′-tribenzyl-methylenebis(phosphonate)]-*β*-D-ribofuranosyl}guanine (27). Compound **27** is obtained as a white solid (mixture of four diastereomers, 116 mg, 71%), starting from **14** and **26**[104,105] and following the same procedure as for **25**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.87, 8.86, 8.85 and 8.84 (4s, 1H); 7.95, 7.91, 7.86 and 7.83 (4s, 1H); 7.60−7.15 (m, 35 H); 6.24, 6.18, 6.15 and 6.11 (4d, J = 2.6 Hz, 1H); 6.13, 6.11, 5.95 and 5.92 (4s, 1H); 5.53 (AB syst, $\Delta\delta$ = 12.0 Hz, J_{AB} = 14.3 Hz, 2H); 5.17 (m, 2H); 5.40−5.14 (m, 2H); 5.08−4.91 (m, 6H); 4.61−4.06 (m, 7H); 2.46, 2.45, 2.42 and 2.37 (4t, J = 21.1 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.09 and 158.00; 157.22; 147.63 and 147.52; 136.83 and 136.66; 135.65; 135.59; 135.50; 135.01; 134.90; 133.45; 132.12; 131.92; 131.86; 1311.40; 129.79; 129.03; 128.78; 128.54; 128.46; 128.29; 128.08; 127.88; 127.71; 127.59; 126.70; 126.61; 126.49; 120.46, 120.37 and 120.26; 107.67 and 104.32; 88.98, 88.75, 88.66 and 88.52; 85.14, 84.91, 84.22 and 84.05; 83.84, 83.73, 82.75 and 83.66; 82.00, 81.76, 80.67 and 80.58; 68.51, 68.39, 68.28 and 68.16; 65.33 and 65.22; 54.65; 48.32; 45.47; 25.83 (t, J = 137.2 Hz). ^{131}P NMR ($CDCl_3$, 121 MHz) δ 20.99 (AB syst, $\Delta\delta$ = 76.8 Hz, J_{AB} = 6.1 Hz, 0.5P); 20.98 (AB syst, $\Delta\delta$ = 76.8 Hz, J_{AB} = 6.1 Hz, 0.5P); 20.95 (AB syst, $\Delta\delta$ = 78.0 Hz, J_{AB} = 5.8 Hz, 0.5P); 20.91 (AB syst, $\Delta\delta$ = 77.4 Hz, J_{AB} = 6.2 Hz, 0.5P). IR ($CHCl_3$) ν 3063; 2925; 1689; 1612; 1493; 1259; 1066; 998. Anal. Calcd for $C_{61}H_{58}N_6O_{10}P_2$: C, 66.78; H, 5.33; N, 7.66. Found: C, 67.02; H, 5.47; N, 7.81.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-{2′,3′-*O*-benzylidene-5′-*O*-[*N*-benzyl-(*P*,*P*′,*P*′-tribenzylimidodiphosphate)]-*β*-D-ribofuranosyl}guanine (29). Compound **29** is obtained as a glassy solid (mixture of four diastereomers, 83 mg, 75%), starting from **14** and **28**,[105] and following the same procedure as for **25**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.86 and 8.83 (2s, 1H); 7.80, 7.77, 7.74, and 7.72 (4s, 1H); 7.60−7.15 (m, 40H); 6.16 and 6.15 (2d, J = 1.9 Hz, 0.5H), 6.08 and 6.07 (2d, J = 3.0 Hz, 0.5H); 6.09, 5.89 and 5.86 (3s, 1H); 5.55 (AB syst, $\Delta\delta$ = 10.5 Hz, J_{AB} = 13.9 Hz, 2H); 5.17 (m, 1H); 5.05−4.31 (m, 14H); 4.25−3.95 (m, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.07; 158.06; 157.20; 147.54; 138.25 and 137.84; 136.52;

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135.67; 135.55; 135.41; 135.28; 135.03; 134.98; 134.90; 129.86; 129.80; 129.02; 128.76; 128.31; 128.13; 128.01; 127.96; 127.90; 127.75; 127.64; 126.70; 126.61; 126.49; 120.50; 107.66, 104.36 and 104.31; 88.65 and 88.45; 84.87 and 84.72; 83.70, 83.42 and 82.24; 82.10, 81.98 and 80.61; 69.19; 69.07; 68.98 and 68.88; 66.13 and 66.04; 54.62; 50.81; 48.32; 45.46. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 4.71 (AB syst, $\Delta\delta = 65.3$ Hz, $J_{AB} = 20.4$ Hz, 0.5P); 4.67 (AB syst, $\Delta\delta = 67.8$ Hz, $J_{AB} = 20.4$ Hz, 0.5P); 4.66 (AB syst, $\Delta\delta = 54.4$ Hz, $J_{AB} = 20.7$ Hz, 0.5P); 4.56 (AB syst, $\Delta\delta = 59.9$ Hz, $J_{AB} = 20.4$ Hz, 0.5P). IR ($CHCl_3$) ν 2926; 1691; 1612; 1493; 1275; 1016. Anal. Calcd for $C_{67}H_{63}N_7O_{10}P_2$: C, 67.72; H, 5.35; N, 8.25. Found: C, 67.58; H, 5.29; N, 8.23.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-[2′,3′-*O*-benzylidene-5′-*N*-(*N*,*N*′-diethylcarboxydiaza)-β-D-ribofuranosyl]guanine (30). Compound **30** is a side product obtained as a white solid in the different coupling reactions (mixture of two diastereomers, 32−58%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.85 (s, 1H); 7.64 and 7.62 (2s, 1H); 7.60−7.15 (m, 20H); 6.15 and 5.98 (2s, 1H); 6.09 and 6.04 (2d, $J = 4.4$ Hz, 1H); 5.55 (AB syst, $\Delta\delta = 16.5$ Hz, $J_{AB} = 14.2$ Hz, 2H); 4.92 (m, 1H); 4.80−4.40 (m, 6H); 4.35−3.98 (m, 6H); 1.18 (m, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.03; 158.02; 157.22; 156.78 and 156.64; 147.24; 138.14; 137.57; 135.55; 134.96; 132.15; 131.95; 129.84; 129.05; 128.79; 128.59; 128.48; 128.28; 128.16; 128.01; 127.96; 127.91; 127.75; 127.64; 126.75; 126.60; 126.52; 126.45; 120.98; 107.50 and 104.32; 89.18; 83.61; 83.06 and 81.97; 80.63; 62.73 and 62.06; 54.63; 51.33; 48.44; 45.54; 14.31. IR (KBr) ν 3237; 2981; 1694; 1612; 1493; 1092. MS (CI/NH_3): 828 $[M + H]^+$. Anal. Calcd for $C_{45}H_{46}N_8O_8$: C, 65.36; H, 5.61; N, 13.55. Found: C, 65.41; H, 5.67; N, 13.60.

1-{2-[2-(2-Azidoethoxy)ethoxy]ethyl}-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(5′-*O*-acetyl-2′,3′-*O*-benzylidene-β-D-ribofuranosyl)guanine (32). Compound **32** is obtained as a white solid (mixture of two diastereomers, 603 mg, 55%), starting from **22** and **31**,[106] and following the same procedure as for **17**, except that triphenylphosphine and DEAD are mixed together before they are added to **22** and **31**. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.91 and 8.88 (2s, 1H); 7.70 (s, 1H); 7.60−7.15 (m, 15H); 6.23 and 6.14 (2d, $J = 6.4$ Hz, 1H); 6.19 and 6.03 (2s, 1H); 5.47 and 5.42 (2dd, $J = 2.3$, 6.4 Hz, 1H); 5.03 (2dd, $J = 3.2$, 6.4 Hz, 1H); 4.71 (s, 2H); 4.60 (t, $J = 6.3$ Hz, 2H); 4.62−4.36 (m, 2H); 4.48 (s, 2H); 4.28−4.19 (m, 1H); 3.74 (t, $J = 6.3$ Hz, 2H); 3.69−3.54 (m, 6H); 3.28 (t, $J = 5.2$ Hz, 2H); 2.00 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.34; 157.73; 157.64; 156.97; 147.04; 136.75 and 136.54; 135.48 and 135.40; 135.10; 134.68; 129.90; 129.74; 129.02; 128.77; 128.40; 128.07; 127.89; 127.67; 126.54; 126.36; 120.70; 107.72 and 104.27; 89.50; 85.16; 83.72; 82.34; 82.18 and 81.36; 70.37; 70.01; 69.71; 68.32; 63.89; 54.85; 50.43; 48.56; 41.16; 20.53. IR ($CHCl_3$) ν 2923; 2106; 1743; 1690; 1613; 1493; 1223; 1097. MS (CI/NH_3): 779 $[M + H]^+$. Anal. Calcd for $C_{40}H_{43}N_9O_8$: C, 61.77; H, 5.58; N, 16.21. Found: C, 61.29; H, 5.31; N, 15.84.

1-{2-[2-(2-Azidoethoxy)ethoxy]ethyl}-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(2′,3′-*O*-benzylidene-β-D-ribofuranosyl)guanine (33). Compound **33** is obtained as a white solid (mixture of two diastereomers, 1.33 g, 99%), starting from **32** and following the same procedure as for **9**. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.82 and 8.79 (2s, 1H); 7.77 and 7.72 (2s, 1H); 7.60−7.15 (m, 15H); 6.25 and 6.08 (2s, 1H); 6.23 and 6.14 (2d, $J = 2.4$ Hz, 1H); 5.42 (m, 1H); 5.20 (m, 1H); 4.77−4.45 (m, 7H); 4.08−3.97 (m, 1H); 3.85−3.52 (m, 9H); 3.27 (t, $J = 4.9$ Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.13; 157.64; 157.24; 146.97; 137.61; 136.08 and 135.80; 134.99; 134.56; 129.61; 129.43; 128.64; 128.24; 128.05; 127.95; 127.84; 127.68; 126.67; 126.39; 125.03; 120.55; 107.28 and 103.98; 91.42 and 90.12; 85.27 and 84.96; 83.84 and 82.71; 83.14 and 80.25; 70.21; 69.96; 69.61; 68.11; 62.41; 62.08; 54.88; 50.30; 48.04; 41.19. IR ($CHCl_3$) ν 3338; 2923; 2106; 1688; 1613; 1494; 1097. MS (CI/NH_3): 736 $[M + H]^+$. Anal. Calcd for $C_{38}H_{41}N_9O_7$: C, 62.03; H, 5.62; N, 17.13. Found: C, 62.14; H, 5.65; N, 17.20.

1-{2-[2-(2-Azidoethoxy)ethoxy]ethyl}-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-{2′,3′-*O*-benzylidene-5′-*O*-[*N*-benzyl-(*P*,*P*′,*P*′-tribenzylimidodiphosphate)]-β-D-ribofuranosyl}-guanine (34). Compound **34** is obtained as a glassy solid (mixture of four diastereomers, 121 mg, 97%), starting from **33** and **28**,[105] and following the same procedure as for **25**, except that triphenylphosphine and DEAD are mixed together before they are added to **28** and **33**. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.93 and 8.91 (2s, 1H); 7.80, 7.75, 7.72, and 7.68 (4s, 1H); 7.60−7.15 (m, 35H); 6.16 and 6.08 (2m, 1H); 6.10, 6.06, 5.90, and 5.87 (4s, 1H); 5.17 (m, 1H); 5.13−4.43 (m, 15H); 4.68 (s, 2H); 4.32 (m, 1H); 4.19−4.02 (m, 1H); 3.72 (t, $J = 6.4$ Hz, 2H); 3.64−3.45 (m, 6H); 3.27 (t, $J = 5.0$ Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.07; 157.89; 157.11; 147.54 and 147.48; 137.84; 136.46; 135.73; 135.56; 135.42; 135.30; 134.93; 129.81; 129.06; 128.86; 128.72; 128.46; 128.31; 128.23; 128.08; 127.97; 127.88; 127.74; 126.61; 126.50; 120.55; 107.76 and 104.30; 88.47; 83.70 and 83.44; 82.31 and 82.14; 81.99 and 80.67; 70.50; 70.13; 69.84; 68.97 (b); 68.42; 66.14; 54.88; 50.83; 50.58; 48.50; 41.25. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 4.78 (AB syst, $\Delta\delta = 65.3$ Hz, $J_{AB} = 20.1$ Hz, 0.5P); 4.76 (AB syst, $\Delta\delta = 65.3$ Hz, $J_{AB} = 20.1$ Hz, 0.5P); 4.63 (AB syst, $\Delta\delta = 64.1$ Hz, $J_{AB} = 20.7$ Hz, 0.5P); 4.60 (AB syst, $\Delta\delta = 61.7$ Hz, $J_{AB} = 20.1$ Hz, 0.5P). IR (KBr) ν 2926; 2105; 1692; 1611; 1493; 1454; 1273; 1216. Anal. Calcd for $C_{66}H_{68}N_{10}O_{12}P_2$: C, 67.15; H, 5.46; N, 11.16. Found: C, 63.29; H, 5.49; N, 11.22.

1-{2-[2-(2-Azidoethoxy)ethoxy]ethyl}-9-[5′-*O*-(imidodiphosphate)-β-D-ribofuranosyl]guanine (35). Compound **35** is obtained as its bis-triethylammonium salt (white powder, 25 mg, 97%), starting from **34** and following the same procedure as for **1**. ^{1}H NMR (D_2O, 300 MHz) δ 8.04 (s, 1H); 5.83 (d, $J = 6.6$ Hz, 1H); 4.40 (m, 1H); 4.25−3.95 (m, 5H); 3.81 (t, $J = 5.1$ Hz, 2H); 3.65−3.45 (m, 6H); 3.11 (q, $J = 7.3$ Hz, 12H); 3.01 (t, $J = 4.4$ Hz, 2H); 1.19 (t, $J = 7.3$ Hz, 18H). ^{13}C NMR (D_2O/CD_3OD: 9/1, 75 MHz) δ 159.37; 149.84; 138.15; 116.53; 86.93; 84.24; 73.63; 70.64; 70.18; 69.76; 68.06; 66.46; 64.46; 46.80; 42.37; 39.18. ^{31}P NMR (D_2O, 121 MHz, [Et_3N] = 0.5 M) δ 3.73 (d, $J = 2.4$ Hz, 1P), −0.11 (d, $J = 2.4$ Hz, 1P). IR (KBr) ν 3338; 3216; 2947; 2602; 2493; 1694; 1537; 1198; 1037. HRMS: calcd for $C_{16}H_{28}N_7O_{12}P_2$ 572.1271, found 572.1260 $[M - H]^-$. HPLC (ammonium carbonate 0.5 M, pH 7.0/CH_3CN 99/1) t_R 14.2 min.

6-*O*-(2,4,6-Triisopropylbenzenesulfonyl)-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(2′,3′-*O*-benzylidene-5′-*O*-acetyl-β-D-ribofuranosyl)guanine (36). 2,4,6-Triisopropylbenzenesulfonyl chloride (TPSCl) (710 mg, 3.00 mmol) is added at 0 °C to a solution containing compound **22** (720 g, 1.16 mmol), 4-DMAP (35 mg, 0.29 mmol), triethylamine (0.6 mL, 4.30 mmol), and CH_2Cl_2 (5 mL). The reaction mixture is refluxed for 30 min. Then it is washed with aqueous $NaHCO_3$, and the organic layer is dried over $MgSO_4$ and reduced in vacuo. The crude residue is purified by chromatography on silica gel (CH_2Cl_2/Et_2O: 1/0 to 0/1) to yield compound **36** as a white solid (mixture of two diastereomers, 1.011 g, 98%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.74 and 8.73 (2s, 1H); 8.02 and 8.00 (2s, 1H); 7.60−7.15 (m, 17H); 6.35 and 6.34 (2d, $J = 2.2$ Hz, 1H); 6.18 and 6.04 (2s, 1H); 5.60 and 5.48 (2dd, $J = 2.2$, 4.9 Hz, 1H); 5.17 (td, $J = 2.5$, 4.5 Hz, 1H); 4.85 and 4.81 (2d, $J = 4.0$ Hz, 1H); 4.66−4.28 (m, 6H); 4.53 (h, $J = 6.9$ Hz, 2H); 2.83 (h, $J = 6.9$ Hz, 1H); 2.04 and 2.00 (2s, 3H); 1.28 (d, $J = 6.9$ Hz, 12H); 1.19 (d, $J = 6.8$ Hz, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.25; 162.12; 159.82; 156.12 and 156.05; 154.31; 153.93; 150.67; 141.64 and 141.28; 135.43 and 135.17; 131.88; 129.80; 128.86; 128.57; 128.41; 128.20; 127.68; 126.60; 126.54; 123.71; 119.91 and 119.83; 107.96 and 104.42; 89.54 and 88.75; 84.86 and 83.64; 84.16 and 81.99; 81.98 and 80.92; 63.81; 54.41; 47.65; 34.08; 29.80; 24.57; 23.35; 20.60. IR ($CHCl_3$) ν 2959; 2869; 1745; 1596; 1381; 1198; 1073. MS (CI/NH_3): 887 $[M + H]^+$. Anal. Calcd for $C_{49}H_{54}N_6O_8S$: C, 66.34; H, 6.14; N, 9.47. Found: C, 66.12; H, 6.02; N, 9.39.

6-*O*-[2-(2-Azidoethoxy)ethyl]-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(2′,3′-*O*-benzylidene-5′-*O*-acetyl-β-D-ribofuranosyl)guanine (38). Quinuclidine (74 mg, 0.66 mmol) and compound **37** (527 mg, 0.59 mmol) in anhydrous dichloromethane (4 mL) are stirred at room temperature for 2 h. DBU (100 μL, 0.67 mmol) and (2-azidoethoxy)ethanol **37**[106] in dichloromethane (2 mL) are added, and the reaction mixture

(106) Lebeau, L.; Olland, S.; Oudet, P.; Mioskowski, C. *Chem. Phys. Lipids* **1992**, *62*, 93−103.

is stirred for 12 h, reduced in vacuo, and chromatographed (Et_2O/*n*-C_6H_{14}: 6/4 to 10/0) to yield **38** as a white solid (330 mg, 77%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.04 (s, 1H); 7.88 and 7.86 (2s, 1H); 7.60−7.15 (m, 15H); 6.32 and 6.28 (2d, J = 2.5 Hz, 1H); 6.14 and 5.98 (2s, 1H); 5.59 and 5.50 (2dd, J = 2.5, 5.0 Hz, 1H); 5.17 (m, 1H); 4.92−4.18 (m, 9H); 3.91 (t, J = 5.0 Hz, 2H); 3.32 (t, J = 5.0 Hz, 2H); 1.94 and 1.96 (2s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.07; 162.24; 160.25; 158.81; 153.00 and 152.86; 139.37 and 139.11; 135.71; 135.41; 135.33; 129.67; 129.56; 128.63; 128.34; 128.23; 128.17; 127.88; 127.50; 127.36; 126.44; 126.35; 117.92; 107.52 and 104.03; 89.27 and 88.72; 84.77 and 83.38; 84.01 and 82.02; 81.88 and 81.01; 69.87 and 68.94; 65.56; 63.69 and 63.57; 54.07; 50.37; 47.74; 20.37. IR ($CHCl_3$) ν 2934; 2104; 1742; 1591; 1377; 1230; 1072. MS (CI/NH_3): 734 $[M + H]^+$. Anal. Calcd for $C_{38}H_{39}N_9O_7$: C, 62.20; H, 5.36; N, 17.18. Found: C, 62.65; H, 5.63; N, 17.38.

6-*O*-[2-(2-Azidoethoxy)ethyl]-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(2′,3′-*O*-benzylidene-*β*-D-ribofuranosyl)guanine (39). Compound **39** is obtained as a white solid (273 mg, 92%), starting from **38** and following the same procedure as for **9**. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.97 and 8.96 (2s, 1H); 7.90 and 7.79 (2s, 1H); 7.80−7.15 (m, 15H); 6.09 and 6.01 (2d, J = 4.8 Hz, 1H); 6.29 and 6.04 (2s, 1H); 5.47 and 5.40 (2dd, J = 4.8, 6.2 Hz, 1H); 6.29 and 6.04 (2s, 1H); 5.47 and 5.40 (2dd, J = 4.8, 6.2 Hz, 1H); 5.23 (2d, J = 6.2 Hz, 1H); 5.01−4.35 (m, 5H); 4.29 and 4.04 (2s, 2H); 4.08−3.75 (m, 2H); 3.96 (t, J = 5.1 Hz, 2H); 3.76 (t, J = 4.9 Hz, 2H); 3.39 (t, J = 4.9 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 162.77 and 162.63; 160.97 and 160.95; 159.22 and 159.16; 152.81 and 152.67; 140.50 and 140.15; 136.86; 136.20; 135.75; 129.76; 129.03; 128.76; 128.69; 128.49; 128.33; 128.15; 127.79; 127.31; 126.43; 119.02 and 118.79; 107.35 and 104.43; 93.14 and 90.97; 85.60 and 85.43; 83.73 and 83.58; 82.80 and 80.35; 70.07; 69.06; 65.73; 63.14 and 62.76; 54.30; 50.55; 47.89. IR ($CHCl_3$) ν 3277; 2922; 2104; 1592; 1377; 1239; 1072. MS (CI/NH_3): 692 $[M + H]^+$. Anal. Calcd for $C_{36}H_{37}N_9O_6$: C, 62.51; H, 5.39; N, 18.22. Found: C, 62.50; H, 5.36; N, 18.22.

6-*O*-[2-(2-Azidoethoxy)ethyl]-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-{2′,3′-*O*-benzylidene-5′-*O*-[*N*-benzyl-(*P*,*P*′,*P*′-tribenzylimidodiphosphate)]-*β*-D-ribofuranosyl}guanine (40). Compound **40** is obtained as a white solid (mixture of four diastereomers, 53 mg, 32%), starting from **39** and **28**,[105] and following the same procedure as for **34**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.10, 9.09, 9.08, and 9.07 (4s, 1H); 7.99, 7.98, 7.94, and 7.91 (4s, 1H); 7.74−7.71 (m, 2H); 7.56−7.20 (m, 33H); 6.36, 6.35, 6.32, and 6.31 (4d, J = 3.6 Hz, 1H); 6.09, 6.08, 5.90, and 5.86 (4s, 1H); 5.23−5.12 (m, 1H); 5.05−4.76 (m, 9H); 4.56 (t, J = 20.5 Hz, 2H); 4.40 and 4.39 (2s, 2H); 4.32 (t, J = 6.6 Hz, 2H); 4.28−4.05 (m, 3H); 3.97 (t, J = 5.4 Hz, 2H); 3.77 (t, J = 4.9 Hz, 2H); 3.38 (t, J = 4.9 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 162.48; 160.45; 159.27; 153.61 and 153.44; 139.00; 138.82; 137.87; 135.88; 135.62; 135.48; 135.33; 133.40; 132.13; 131.92; 131.35; 130.02; 129.81; 129.73; 128.86; 128.78; 128.57; 128.43; 128.00; 127.74; 127.59; 126.67; 126.61; 117.86 and 117.74; 107.90, 107.74, 107.90 and 107.81; 88.55, 88.52, 87.25 and 87.16; 84.59 and 83.64; 84.44 and 81.82; 81.67 and 79.98; 70.36; 69.66; 69.23 and 69.14; 69.02 and 68.94; 66.57, 66.31, 66.20 and 66.13; 65.80; 54.30; 50.81; 50.78; 47.89. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 5.90 (m, 1P); 5.07 (m, 1P). IR ($CHCl_3$) ν 2926; 2104; 1706; 1595; 1252; 1014. Anal. Calcd for $C_{64}H_{64}N_{10}O_{11}P_2$: C, 63.46; H, 5.33; N, 11.56. Found: C, 63.64; H, 5.40; N, 11.62.

6-*O*-[2-(2-Aminoethoxy)ethyl]-9-[5′-*O*-(imidodiphosphate)-*β*-D-ribofuranosyl]guanine (41). Compound **41** is obtained as its bis-triethylammonium salt from **40** following the same procedure as for **1** (white powder, 15 mg, 62%). ^{1}H NMR (D_2O, 200 MHz) δ 8.04 (s, 1H); 5.83 (d, J = 6.6 Hz, 1H); 4.68 (m, 1H); 4.46 (m, 1H); 4.26 (m, 1H); 4.10−3.77 (m, 8H); 3.11 (q, J = 7.5 Hz, 12H); 2.97 (m, 2H); 1.18 (t, J = 7.5 Hz, 18H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.00; 157.26; 152.92; 129.79; 116.71; 87.80; 84.97; 74.72; 71.58; 69.18; 66.95; 66.46; 64.82; 39.17. ^{31}P NMR (D_2O, 121 MHz, [Et_3N] = 0.5 M) δ 4.57 (s, 1P); −0.06 (s, 1P). HRMS: calcd for $C_{14}H_{24}N_7O_{11}P_2$ 528.1009, found 528.1031 $[M - H]^-$. HPLC (ammonium carbonate 0.5 M, pH 7.0/CH_3CN 99/1) t_R 12.6 min.

9-[5′-*O*-(Imidodiphosphate)-3′-*O*-propionyl-*β*-D-ribofuranosyl]guanine (42). Compound **42** is obtained as its tris-ammonium salt from **41** following the same procedure as for **1**, but replacing triethylamine with ammonium carbonate (white powder, 23 mg, 94%). Analytical sample contains 30% of the 2′-propionyl isomer **48**, resulting from partial isomerization of **42**. ^{1}H NMR (D_2O, 300 MHz) δ 8.20 (s, 1H); 6.03 (d, J = 4.1 Hz, 0.3H); 5.90 (d, J = 7.2 Hz, 0.7H); 5.56 (dd, J = 4.1, 5.1 Hz, 0.3H); 5.41 (d, J = 5.1 Hz, 0.7H); 4.98 (dd, J = 5.1, 7.2 Hz, 0.7H); 4.70−4.62 (m, 0.7H); 4.43 (m, 0.7H); 4.29 (m, 0.3H); 4.19−4.05 (m, 2H); 2.47 (q, J = 7.2 Hz, 1.4H); 2.41 (q, J = 7.2 Hz, 0.6H); 1.09 (t, J = 7.2 Hz, 2.1H); 1.01 (t, J = 7.2 Hz, 0.9H). ^{31}P NMR (D_2O, pH = 7.0, 121 MHz) δ 1.36 (s, 1P); 0.19 (s, 1P). HRMS: calcd for $C_{13}H_{19}N_6O_{11}P_2$ 497.0587, found 497.0577 $[M - H]^-$. HPLC (ammonium carbonate 0.5 M, pH 7.0) t_R 3.1 min (**42**), 6.6 min (**48**).

2-*N*-(*N*′,*N*′-Dibenzylformamidine)-9-(*β*-D-ribofuranosyl)-guanine (43). Freshly distilled dibenzylamine (7.5 g, 38.1 mmol) and *N*,*N*-dimethylformamide dimethylacetal (1.7 mL, 12.8 mmol) are refluxed in acetonitrile (15 mL) for 24 h. Anhydrous toluene (15 mL) is added, and the solution is reduced in vacuo. That operation is repeated twice before the crude residue in anhydrous acetonitrile (10 mL) is added to guanosine (1.19 g, 4.2 mmol) in acetonitrile (20 mL). The resulting solution is stirred for 24 h at 45 °C and poured in Et_2O (65 mL). The precipitate is collected by filtration, and the filtrate is reduced in vacuo and once again poured into Et_2O (65 mL). The solids are combined and purified by chromatography on silica gel (CH_2Cl_2/EtOH: 99/1 to 80/20) to yield **43** as a white powder (1.44 g, 70%). ^{1}H NMR (CD_3OD, 200 MHz) δ 9.05 (s, 1H); 8.10 (s, 1H); 7.40−7.20 (m, 10H); 5.99 (d, J = 5.9 Hz, 1H); 4.73 (dd, J = 5.5, 5.9 Hz, 1H); 4.68 (s, 2H); 4.53 (s, 2H); 4.37 (dd, J = 3.3, 5.5 Hz, 1H); 4.14 (m, 1H); 3.82 (AB part of ABX syst, $\Delta\delta$ = 13.0 Hz, J_{AB} = 12.2 Hz, J_{AX} = 2.7 Hz, J_{BX} = 2.7 Hz, 2H). ^{13}C NMR (CD_3OD, 50 MHz) δ 160.75; 160.29; 159.34; 151.80; 139.84; 136.99; 136.79; 130.26; 130.03; 129.68; 129.59; 129.10; 121.74; 90.50; 87.58; 75.91; 72.51; 63.32; 56.13; 49.15. IR ($CHCl_3$) ν 3282; 2922; 1691; 1618; 1536; 1360; 1193. MS (CI/NH_3): 491 $[M + H]^+$. Anal. Calcd for $C_{25}H_{26}N_6O_5$: C, 61.21; H, 5.35; N, 17.13. Found: C, 61.29; H, 5.37; N, 17.17.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(*β*-D-ribofuranosyl)guanine (44). Sodium hydride (60% in oil, 183 mg, 4.5 mmol) is added to compound **43** (1.74 g, 3.6 mmol) in anhydrous DMF (35 mL) at −10 °C. The reaction mixture is stirred for 2 h at that temperature. Then benzyl bromide (620 mg, 3.6 mmol) in DMF (5 mL) is added dropwise in 20 min, and the solution is stirred for 12 h at −10 °C. Aqueous NH_4Cl (1 mL) is added, the mixture is reduced in vacuo, and the residue is purified by chromatography on silica gel (CH_2Cl_2/EtOH: 99/1 to 90/10) to yield **44** as a white powder (1.69 g, 81%). ^{1}H NMR ($CDCl_3$,/CD_3OD: 1/3, 200 MHz) δ 8.81 (s, 1H); 7.94 (s, 1H); 7.40−7.10 (m, 15H); 5.91 (d, J = 6.4 Hz, 1H); 5.47 (s, 2H); 4.74 (dd, J = 5.3, 6.4 Hz, 1H); 4.57 (s, 2H); 4.45 (s, 2H); 4.34 (dd, J = 3.0, 5.3 Hz, 1H); 4.16 (m, 1H); 3.79 (AB part of ABX syst, $\Delta\delta$ = 15.0 Hz, J_{AB} = 12.4 Hz, J_{AX} = 2.6 Hz, J_{BX} = 2.6 Hz, 2H). ^{13}C NMR (CD_3OD, 50 MHz) δ 159.11; 159.10; 158.28; 148.66; 139.16; 138.39; 135.61; 135.47; 129.53; 129.33; 128.99; 128.85; 128.69; 128.39; 127.72; 127.49; 120.69; 89.95; 86.88; 74.85; 71.82; 62.69; 55.49; 48.52; 43.35. IR ($CHCl_3$) ν 3384; 1674; 1611; 1492; 1118. MS (CI/NH_3): 581 $[M + H]^+$. Anal. Calcd for $C_{32}H_{33}N_6O_5$: C, 66.08; H, 5.72; N, 14.45. Found: C, 66.20; H, 5.74; N, 14.79.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-(2′-*O*-benzyl-*β*-D-ribofuranosyl)guanine (45). Sodium hydride (60% in oil, 39 mg, 0.98 mmol) is added to compound **43** (201 mg, 0.41 mmol) in anhydrous DMF (8 mL) at −15 °C. The reaction mixture is stirred for 3 h at that temperature. Then benzyl bromide (144 mg, 0.84 mmol) in DMF (3 mL) is added dropwise, and the solution is stirred for 16 h at −15 °C. Aqueous NH_4Cl (1 mL) is added, the mixture is reduced in vacuo, and the residue is purified by chromatography on silica gel (CH_2Cl_2/EtOH: 99/1 to 95/5) to yield **45** as a white solid (148 mg, 54%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.08 (s, 1H); 7.67 (s, 1H); 7.40−6.76, (m, 20H); 5.81 (d, J = 7.3 Hz, 1H);

5.41 (AB syst, $\Delta\delta$ = 10.7 Hz, J_{AB} = 14.3 Hz, 2H); 4.70 (dd, J = 4.9, 7.3 Hz, 1H); 4.57 (m, 3H); 4.40−4.19 (m, 5H); 3.69 (AB part of ABX syst, $\Delta\delta$ = 27.0 Hz, J_{AB} = 12.8 Hz, J_{AX} = 2.6 Hz, J_{BX} = 2.6 Hz, 2H). ^{13}C NMR ($CD_3OD/CDCl_3$: 3/1, 50 MHz) δ 158.69; 158.51; 157.98; 147.67; 139.39; 138.42; 137.22; 135.42; 129.52; 129.38; 129.04; 128.79; 128.60; 128.38; 127.89; 127.70; 121.61; 88.72; 87.70; 78.54; 72.77; 63.19; 55.32; 48.60; 46.26. IR ($CHCl_3$) ν 3306; 1682; 1614; 1495; 1091. MS (CI/NH_3): 671 $[M + H]^+$. Anal. Calcd for $C_{39}H_{38}N_6O_5$: C, 69.83; H, 5.71; N, 12.53. Found: C, 70.19; H, 5.80; N, 12.73.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-{2′-*O*-benzyl-5′-*O*-[*N*-benzyl-(*P*,*P*′,*P*′-tribenzyl-imidodiphosphate)]-*β*-D-ribofuranosyl}guanine (46). Compound **46** is obtained as a white solid (mixture of two diastereomers, 76 mg, 68%), starting from **45** and **28**,[105] and following the same procedure as for **25**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.81 and 8.80 (2s, 1H); 7.80 and 7.74 (2s, 1H); 7.60−7.10 (m, 40H); 6.03 (d, J = 2.6, Hz, 0.5H); 6.02 (d, J = 4.1 Hz, 0.5H); 5.58 (s, 2H); 5.07−4.77 (m, 6H); 4.68−4.51 (m, 6H); 4.20−4.06 (m, 7H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.10; 157.86; 157.06; 147.60; 138.42; 137.82; 136.75; 136.68; 136.24 and 136.00; 135.45; 135.06; 134.85; 132.15; 132.02; 131.92; 129.03; 128.80; 128.69; 128.50; 128.39; 128.28; 128.16; 127.96; 127.77; 127.69; 126.74; 126.11; 120.47; 92.91; 82.01 and 81.70 (2d, J = 7.9 Hz); 81.00 and 80.43; 73.04; 69.95 and 69.75; 69.20; 69.19; 69.18; 69.13; 66.38 and 66.05 (2d, J = 5.6 Hz); 54.65; 50.87; 48.26; 45.43. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 5.15 (AB syst, $\Delta\delta$ = 103.5 Hz, J_{AB} = 22.2 Hz, 1P); 5.13 (AB syst, $\Delta\delta$ = 61.7 Hz, J_{AB} = 20.7 Hz, 1P). IR ($CHCl_3$) ν 2930; 1686; 1610; 1495; 1270; 1021. Anal. Calcd for $C_{67}H_{65}N_7O_{10}P_2$: C, 67.61; H, 5.51; N, 8.24. Found: C, 67.73; H, 5.58; N, 8.28.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-{2′-*O*-benzyl-3′-*O*-propionyl-5′-*O*-[*N*-benzyl-(*P*,*P*′,*P*′-tribenzylimidodiphosphate)]-*β*-D-ribofuranosyl}guanine (47). Propanoyl chloride (15 μL, 172 μmol) is added to alcohol **46** (70 mg, 59 μmol), triethylamine (25 μL, 180 μmol), and 4-DMAP (5 mg, 82 μmol) in dichloromethane (3 mL) at room temperature. The reaction mixture is refluxed for 3 h and reduced in vacuo, and the crude residue is chromatographed on silica gel (CH_2Cl_2/Me_2CO: 95/5 to 80/20) to yield **47** as a white solid (mixture of two diastereomers, 57 mg, 78%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.85 and 8.84 (2s, 1H); 7.66 and 7.61 (2s, 1H); 7.60−7.10 (m, 40H); 5.80 and 5.79 (2d, J = 4.4 Hz, 1H); 5.52−5.35 (m, 3H); 5.01−4.66 (m, 7H); 4.58−4.06 (m, 11H); 2.30−2.19 (m, 2H); 1.02 and 1.01 (2t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.16; 158.19; 158.10; 156.53; 157.07; 147.74; 138.36; 137.89; 137.82; 137.38 and 137.19; 136.83; 136.78; 135.62; 135.49; 135.24; 135.15; 132.16; 132.02; 131.93; 129.02; 128.78; 128.71; 128.57; 128.43; 128.29; 128.15; 128.02; 127.92; 127.92; 127.87; 127.69; 127.62; 126.75; 120.79; 86.85 and 86.84; 79.99 and 79.98; 78.32; 73.33; 71.46 and 71.12; 69.23; 69.15; 68.96; 68.89; 66.48 and 66.39; 54.39; 50.80; 48.24; 45.49; 27.12; 8.85. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 4.65 (AB syst, $\Delta\delta$ = 81.7 Hz, J_{AB} = 21.1 Hz, 1P); 4.57 (AB syst, $\Delta\delta$ = 37.5 Hz, J_{AB} = 20.7 Hz, 1P). IR ($CHCl_3$) ν 2941; 1741; 1693; 1610; 1495; 1271; 1019. Anal. Calcd for $C_{70}H_{69}N_7O_{11}P_2$: C, 67.46; H, 5.58; N, 7.87. Found: C, 67.01; H, 5.36; N, 7.77.

***N*-(2-Ethoxyethyl)-*P*,*P*′,*P*′-tetrabenzylimidodiphosphate (51).** A solution of 2-ethoxyethylamine (792 mg, 4.7 mmol) in THF (15 mL) is added dropwise over 4 h to phosphorus oxychloride (0.90 mL, 9.6 mmol) and anhydrous triethylamine (1.40 mL, 10.1 mmol) in THF (15 mL) at −78 °C. The reaction mixture is stirred at room temperature for 30 min before it is filtered on Celite. The filtrate is cooled to −78 °C, and sodium benzylate [prepared from benzyl alcohol (2.020 g, 18.7 mmol) and sodium hydride (60% in oil, 750 mg, 18.7 mmol)] in THF (80 mL) are added. The solution is stirred for 1 h at −78 °C and then for 1 h at room temperature. Aqueous NH_4Cl is added, and the solution is extracted with AcOEt. The organic layer is dried over $MgSO_4$, reduced in vacuo, and purified by chromatography on silica gel (Et_2O/n-C_6H_{14}: 75.25 to 100/0) to yield **51** as a slightly yellow oil (726 mg, 26%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35−7.20 (m, 20H); 5.03 (m, 8H); 3.59 (m, 4H); 3.40 (q, J = 7.1 Hz, 2H); 1.09 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 135.71 (t, J = 3.4 Hz); 128.33; 128.20; 127.78; 69.00; 68.81, 66.13; 46.47; 14.97. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 4.69 (s). IR ($CHCl_3$) ν 2977; 1456; 1272; 1014. MS (CI/NH_3): 627 $[M + NH_4]^+$; 610 $[M + H]^+$. Anal. Calcd for $C_{32}H_{37}NO_7P_2$: C, 63.05; H, 6.12; N, 2.30. Found: C, 62.81; H, 5.99; N, 2.18.

***N*-(2-Ethoxyethyl)-*P*,*P*′,*P*′-tribenzylimidodiphosphoric Acid (52).** Compound **52** is obtained as a yellow oil (616 mg, 97%), starting from **51** and following the procedure used to produce **24** from (dibenzyloxyphosphoryl)acetic acid benzyl ester. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40−7.15 (m, 15H); 5.05 (m, 6H); 3.60 (t, J = 6.0 Hz, 2H); 3.47 (m, 2H); 3.33 (q, J = 6.9 Hz, 2H); 1.09 (t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.73 (b); 135.63 (b); 128.37; 128.24; 127.86; 127.53; 68.97; 68.91; 68.72; 67.97; 66.25; 45.58; 14.91. ^{31}P NMR (CD_3OD, 121 MHz) δ 5.85 (d, J = 23.0 Hz, 1P); 3.11 (d, J = 23.0 Hz, 1P). IR ($CHCl_3$) ν 2971; 1253; 1025. MS (CI/NH_3): 537 $[M + NH_4]^+$. Anal. Calcd for $C_{25}H_{31}NO_7P_2$: C, 57.80; H, 6.02; N, 2.70. Found: C, 57.87; H, 6.03; N, 2.86.

1-Benzyl-2-*N*-(*N*′,*N*′-dibenzylformamidine)-9-{2′,3′-*O*-benzylidene-5′-*O*-[*N*-(2-ethoxyethyl)-(*P*,*P*′,*P*′-tribenzylimidodiphosphate)]-*β*-D-ribofuranosyl}guanine (53). Compound **53** is obtained as a white solid (mixture of four diastereomers, 106 mg, 68%), starting from **14** and **52** and following the same procedure as for **25**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.85 and 8.83 (2s, 1H); 7.85, 7.84, 7.80, and 7.72 (4s, 1H); 7.60−7.15 (m, 35H); 6.21−5.88 (m, 2H); 5.63−5.48 (m, 3H); 5.34−5.23 (m, 1H); 5.18−4.98 (m, 7H); 4.65−4.58 (m, 2H); 4.54−3.93 (m, 5H); 3.65−3.25 (m, 6H); 1.15 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 162.21; 158.05 (b); 157.12 (b); 147.53 and 147.23; 136.60 and 136.46; 138.25; 137.55; 136.60 and 136.46; 135.68 (b); 135.00 (b); 131.92; 131.85; 129.79; 129.71; 128.96; 128.72; 128.43; 128.37; 128.22; 128.07; 127.86; 127.82; 127.70; 127.57; 126.63; 126.57; 126.44; 120.96, 120.42; 120.38 and 120.30; 107.69, 107.64, 104.34 and 104.27; 88.80 and 88.53; 83.59, 83.44, 83.05 and 82.35; 82.23, 82.08; 80.73 and 77.63; 69.30; 69.23; 69.17; 69.09; 69.02; 68.95; 68.88; 66.21; 62.96 and 62.63; 54.60; 48.34 and 48.29; 46.67; 45.51 and 45.42; 14.98. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 4.85 (AB syst, $\Delta\delta$ = 59.9 Hz, J_{AB} = 21.1 Hz, 0.5P); 4.84 (AB syst, $\Delta\delta$ = 59.3 Hz, J_{AB} = 21.1 Hz, 0.5P); 4.81 (AB syst, $\Delta\delta$ = 58.7 Hz, J_{AB} = 20.7 Hz, 0.5P); 4.78 (AB syst, $\Delta\delta$ = 56.7 Hz, J_{AB} = 20.7 Hz, 0.5P). IR ($CHCl_3$) ν 2927; 1730; 1691; 1608; 1489; 1269; 1212; 1063. Anal. Calcd for $C_{64}H_{65}N_7O_{11}P_2$: C, 65.69; H, 5.60; N, 8.38. Found: C, 65.84; H, 5.66; N, 8.40.

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