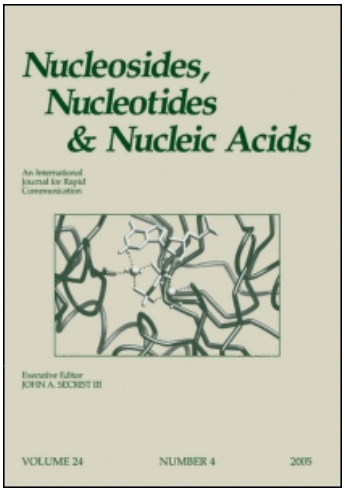

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SYNTHESIS OF 3′-AMINO-3′-DEOXYGUANOSINE AND 3′-AMINO-3′-DEOXYXYLOGUANOSINE MONOPHOSPHATE HEPDIRECT PRODRUGS FROM GUANOSINE

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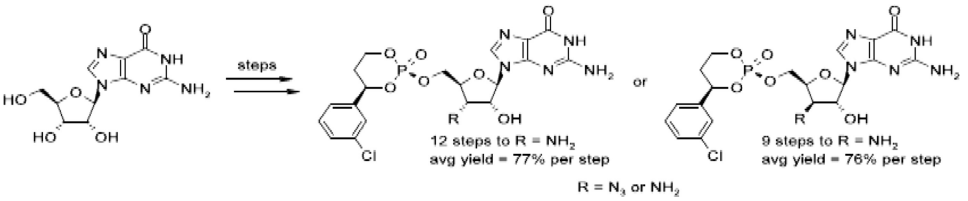

□ *The synthesis of 3′-amino-3′-deoxyguanosine and 3′-amino-3′-deoxyxyloguanosine monophosphate HepDirect prodrugs from guanosine is reported. Initial incorporation of* N,N-*dibenzylformamidino protection of the C2-amino of guanosine masked the reactivity of that group and simplified purification of subsequent analogues. The first key intermediate, 9-(2,5-bis-*O-tert-*butyldimethylsilyl-β-*D*-ribofuranosyl)-2-*N-(N,N-*dibenzylformamidino)guanine (**3a**), was prepared in 60% yield after recycling of the undesired 3′,5′-bis-*O-*protected byproduct (**4a**) by simple equilibration in methanol to a mixture of the two bis-*O-*protected compounds. Thus, protected, the 3′-position was manipulated to form the 3′-deoxyribo- or 3′-deoxyxylo-3′-azido derivatives (**9** or **16**, respectively). Further selective manipulations provided the* cis-*5′-monophosphate (3-chlorophenyl)-1,3-propanyl diester prodrugs (HepDirect prodrugs), **15** and **21**. These HepDirect prodrugs were demonstrated to activate to their respective NTPs in rat hepatocytes.*

Keywords HepDirect prodrugs; 3′-amino-3′-deoxyguanosines; NTPs

INTRODUCTION

Early research into the synthesis of 3′-amino-nucleosides was driven by the discovery of 9-(3-deoxy-3-((4-methoxy-L-phenylalanyl)amino)-β-D-ribofuranosyl)-6-(dimethylamino)purine, commonly known as puromycin,

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Supporting Information Available: ^{1}H NMR for all new compounds.
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and its antibiotic properties.[1] Further research elaborated the series with analogues having antibacterial, anticancer, and other properties of potential medicinal benefit.[2] Later, 3′-azido-3′-deoxynucleosides, as exemplified by 3′-azido-3′-deoxythymidine or AZT, propelled research in antiviral applications on into modern times.[3] More recently groups have been interested in the synthesis of 3′-amino-3′-deoxynucleosides and their derivatives as ligands for purine receptors for a variety of disease applications.[4–5] Still other work has focused particularly on the preparation of 3′-azido-3′-deoxyguanosine, 3′-azido-3′-deoxyxyloguanosine, 3′-amino-3′-deoxyguanosine, their 2′,3′-dideoxy analogues and their 5′-phosphorylated derivatives for use as antivirals,[6] anticancer agents,[7] for the preparation of backbone modified oligonucleotides,[8] and as bioorganic tool molecules.[9]

Our interest in 3′-deoxy-3′-modified guanosine analogues originates in their potential hepatitis C antiviral applications. Among the natural nucleotides, ribose modified guanosine 5′-triphosphate (GTP) analogues are the most potent RdRp inhibitors.[10] When considering the reported structural model of binding of known 2′-*C*-methyl-nucleoside triphosphate inhibitors to RdRp, the 3′-position of the inhibitor NTP is exposed to the solvent surface.[11] Thus, producing a series of 3′-deoxyguanosines derivatized at the 3′-position in order to search for adventitious binding elements was chosen for exploration as HCV inhibitor NTP precursors.

3′-Deoxy-3′-modified nucleoside analogues derive their activity when converted to their respective nucleoside 5′-triphosphates (NTPs), which act as polymerase inhibitors or chain terminators to growing viral DNA or RNA.[12] However, if the nucleoside is an inefficient substrate for the intracellular kinase cascade required for conversion to NTP, it will be ineffective as an antiviral agent. The *cis*-5′-monophosphate (3-chlorophenyl)-1,3-propanyl diester prodrug (HepDirect prodrug)[13] has been developed as part of a kinase-bypass strategy to deliver NMP molecules specifically to hepatocytes, which is especially relevant in the search for anti-HCV drugs. Hence, in order to target nucleosides which can be converted to the active NTP selectively in the target tissue (i.e., the liver), our synthetic strategy required the incorporation of this phosphate triester group at the 5′-position in addition to manipulation of the 3′-site. As a consequence, mild and selective reactions were employed to differentiate the two positions and prepare the desired derivatives directly from guanosine.

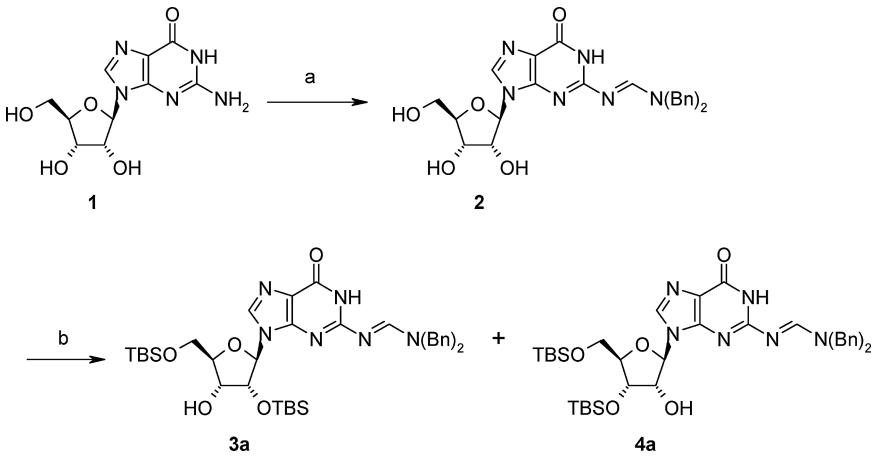
The identification of potential HCV inhibitors is restricted by the limited number of in vitro assays available for this target. The most direct path involves first testing for inhibition of RdRp by the NTP, followed by confirmation by testing the nucleoside in the cellular replicon model of HCV inhibition. It is possible for the NTP to be a good RdRp inhibitor but its nucleoside to be inactive in the replicon model because of a lack

of cellular penetration or the previously mentioned inefficient conversion to NTP by the cellular kinases. Alternatively, undesired metabolism of the nucleoside within the replicon model Huh-7 cell may further diminish potential activity.[14] Hence application of the NMP-SATE prodrug strategy was found useful for establishing promising nucleosides as active in this important assay.[14] While HepDirect prodrugs have been validated as clinical candidates where SATE prodrugs have not,[13b] the HepDirect prodrugs cannot be tested in the replicon assay because Huh-7 cells lack sufficient CYP3A4 to metabolize this prodrug to the NMP. Moreover, this report only describes the preparation of HepDirect prodrugs. An assessment of the activation of these HepDirect prodrugs to their NTP derivatives in hepatocytes is also reported. It is envisioned that the chemistry would apply equally well to the preparation of their respective SATE prodrugs making them available for replicon testing.

CHEMISTRY

For guanosine, amino- and azido-ribose modified derivatives have been prepared by one of the following methods: (1) detailed synthetic manipulation of guanosine;[6a,b,8,9b,c] (2) glycosylation of an acylated guanine with a previously elaborated ribose molecule;[6c,7a,9a] and (3) transglycosylation of an acylated guanine with an available ribose-modified nucleoside.[7b] For the current work, the first approach was taken and due to the reactivity of the 5′-phosphate triester prodrug, that is, HepDirect prodrug group, strong hydrolytic, hydrogenolytic, and nucleophilic reactions were avoided. Hence, the azide group was used to introduce the primary amino group. The reaction sequence begins with the previously reported N^2-*N*,*N*-dibenzylformamidino-protected guanosine **2**[15] (Scheme 1). This hydrophobic N^2-protecting group served to render the extremely polar guanosine nucleoside and derivatives into more chromatographically manageable forms as well as remove the C2-NH_2 active hydrogens as a source of potential side reactions during subsequent reactions. A recent report[9c] and our own experience, have determined that because of purification difficulties it is not practical to prepare the desired 2′,5′-bis-*O*-*t*-butyldimethylsilyl-protected (*O*-TBS-protected) guanosine analogue from a similar N^2-*N*,*N*-dimethylformamidino-protected derivative.

The protection of nucleoside 2′- and 5′-hydroxy groups with TBS groups, although well precedented[16] is known to proceed with only marginal selectivity for guanosine analogues.[16b,c] After standard reaction conditions, the mixture of 2′,5′-bis-*O*-TBS product **3a** and 3′,5′-bis-*O*-TBS-product **4a** obtained was separated using standard silica gel chromatography. Consistent with literature precedent,[1i,16b,17] it was observed that **3a** and **4a** could be equilibrated by simple mixing in methanol at room temperature.

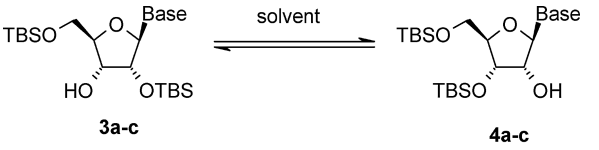


SCHEME 1 a) $HC(OMe)_2$, Bn_2NH, ACN, 80°C (70%, see ref.[13]); b) TBS-Cl, imidazole, DMF (60% **3a** after recycling).

Since this was a concern with regards to structural integrity in the next reaction, the isomerization was investigated in various solvents with the results presented in Table 1. In methanol, equilibration of **3a** to **4a** was complete after 22 hours at 22°C or 1 hour at 80°C to provide a **3a**/**4a** mixture of 58:42 or 54:46, respectively (entries 2 and 3). Confirmation of the equilibration was demonstrated by isomerizing pure **4a** into a 52:48 **3a**/**4a** mixture (methanol, 1 hour, 80°C, entry 11). Evaluation of other solvents revealed that isomerization occurred in DMSO (1 hour, 80°C), acetonitrile (1 hour, 80°C), pyridine (17 hours, 80°C), and THF (17 hours, 80°C), (entries 5–8). However, the isomer **3a** was stable in DMSO (22 hours, 22°C), toluene (17 hours, 80°C), and dichloromethane 17 hours, 22°C (entries 4, 9, and 10) thus indicating these solvents are compatible with our planned chemistry. In fact, all aprotic solvents tested at 22°C did not induce significant isomerization of **3a** to **4a** (not listed). The equilibration was further demonstrated to be general for nucleosides since adenosine and uridine derivatives **3b**, **3c**, and **4c** also were converted to isomeric mixtures in methanol (1 hour, 80°C), (entries 12–14). The equilibration process (methanol, 22 hours, 22°C) was used to advantage by recycling unwanted isomer **4a** obtained during the preparation of **3a** into a mixture of **3a** and **4a**. Performing the equilibration/chromatography recycling procedure three times resulted in a 60% overall yield of **3a** from **2** on a practical research scale (43 g **3a** prepared).

Scheme 2 describes how a double inversion of the 3′-position was applied to the synthesis of the 3′-amino-3′-deoxyribofuranosyl analogue **15**. Certain reactions within this approach, or variations thereof, have been applied to functionalization of the 2′- and 3′-positions of analogues of guanine,[6a,b,9b,18] 2,6-diaminopurine,[18a,19] and adenine[1i,5,9b,18b] based

TABLE 1 Solvent and temperature effect on 2′- to 3′-*O*-TBS isomerization[a]



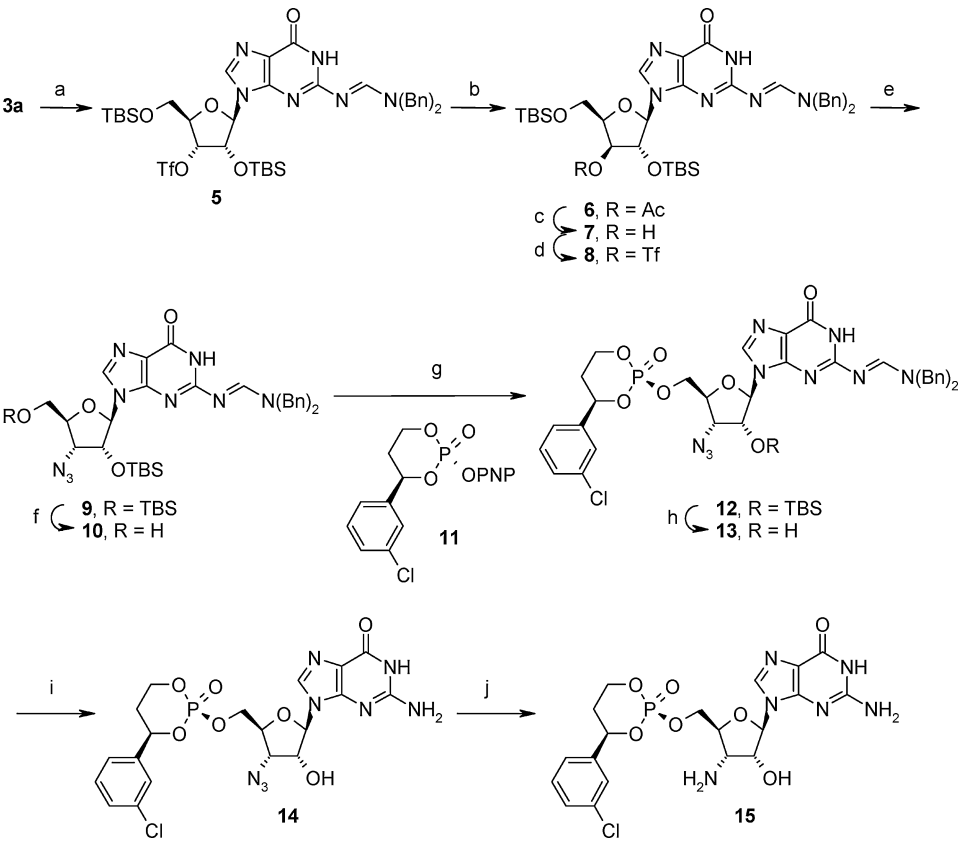
3a-c **4a-c**

a, Base = 2-*N*-(*N*,*N*-dibenzylformamidino)guanine
b, Base = adenine
c, Base = uracil

Entry	Cmpd	Solvent	Time	Temp	2′,5′-bis-*O*-TBS (%Y)[a]	3′,5′-bis-*O*-TBS (%Y)[a]
1	**3a**	MeOH	4 h	22 C	**3a** (75)	**4a** (25)
2	**3a**	MeOH	22 h	22°C	**3a** (58)	**4a** (42)
3	**3a**	MeOH	1 h	80°C	**3a** (54)	**4a** (46)
4	**3a**	DMSO	22 h	22°C	**3a** (99)	**4a** (1)
5	**3a**	DMSO	1 h	80°C	**3a** (57)	**4a** (43)
6	**3a**	CH_3CN	1 h	80°C	**3a** (65)	**4a** (35)
7	**3a**	Pyridine	17 h	80°C	**3a** (57)	**4a** (43)
8	**3a**	THF	17 h	80°C	**3a** (72)	**4a** (28)
9	**3a**	toluene	17 h	80°C	**3a** (>99)	**4a** (<1)
10	**3a**	CH_2Cl_2	17 h	22°C	**3a** (>99)	**4a** (<1)
11	**4a**	MeOH	1 h	80°C	**3a** (52)	**4a** (48)
12	**3b**	MeOH	1 h	80°C	**3b** (51)	**4b** (49)
13	**3c**	MeOH	1 h	80°C	**3c** (62)	**4c** (38)
14	**4c**	MeOH	1 h	80°C	**3c** (62)	**4c** (38)

[a]Percentage of yield calculated from the mixture by HPLC peak area with compound retention times verified by comparison with authentic samples.

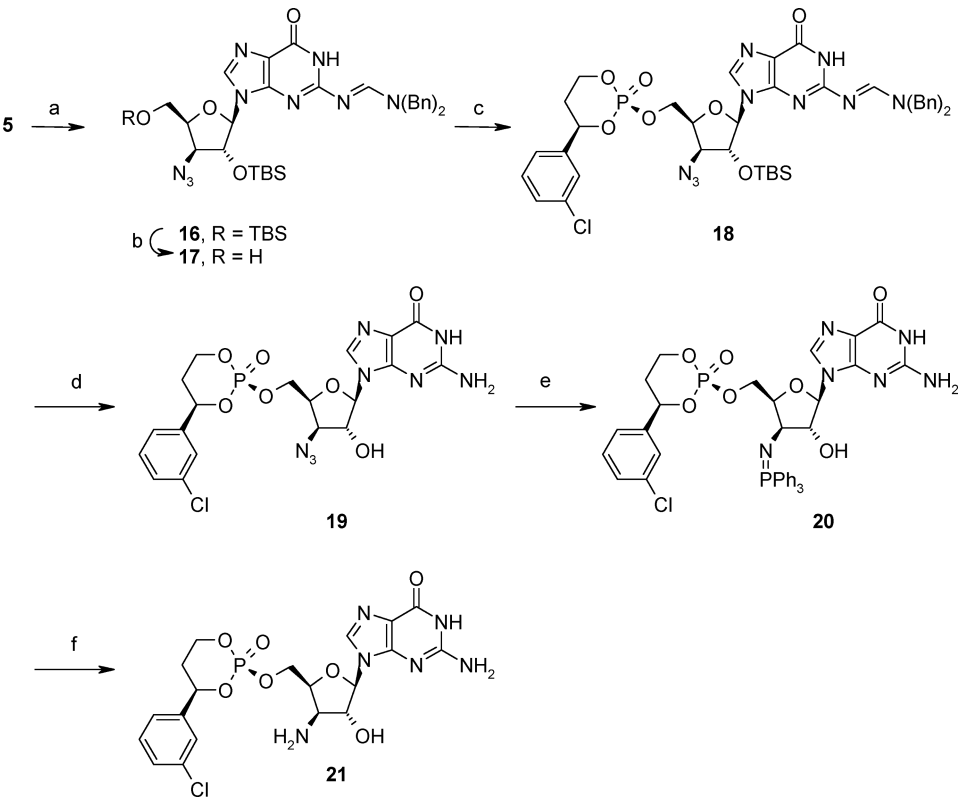
nucleosides. Preparation of triflate **5**, inversion with cesium acetate to **6** and methanolysis to the xylofuranosyl derivative **7** all proceeded satisfactorily. However, formation of triflate **8** was sensitive to time, temperature and reagents. This is in contrast to the preparation of close analogues: ribofuranosyl triflate **5** and a reported xyloadenosine 3′-*O*-triflate.[18b] An optimal yield of 31% of **8** was realized only when trifluoromethanesulfonic anhydride was used and the reaction was processed with starting material remaining in the reaction mixture (29% **7** recovered). The low yield is perhaps not surprising since previous reports have described a low yield of triflate from an arabinoguanosine analogue,[18c] as well as the formation of guanine nucleoside O^6-triflate as a product depending on reaction conditions.[18a,b] Alternate approaches have been applied for the inversion of the 3′-position of xyloguanosine analogues,[6a,b,9b] but this method was satisfactory for our purposes.[20] Displacement of the triflate in **8** with lithium azide to form **9** followed by selective 5′-*O*-desilylation provided **10**. Deprotonation of the 5′-hydroxy group with *t*-BuMgCl followed by phosphorylation with reagent **11**[13a,13f,21] provided the HepDirect prodrug **12** in 93% yield. Sequential



SCHEME 2 Synthesis of ribofuranosyl analogue. a) TfCl, DMAP, DCM (80%); b) AcOH, Cs_2CO_3, DMF (67%); c) MeONa, MeOH (84%); d) Tf_2O, DMAP, DCM (31%, and 29% recovered **7**); e) LiN_3, DMF (93%); f) 70% TFA in water (68%); g) *t*-BuMgCl, THF; **11** (93%); h) Et_4NF, DMF (85%); i) 50% TFA in water (107%); j) PPh_3, NH_3, MeOH, pyridine (95%).

deprotection of the TBS and amidine protecting groups provided azide **14** in high yield. Application of the Staudinger reduction[22] to the azide provided the target amine **15** in 95% yield. Overall, from guanosine (**1**), the preparation of ribo-analogue **15** required 12 steps, with an average yield of 77% for each step.

Preparation of the 3′-amino-3′-deoxyxylofuranosyl analogue **21** followed a similar route and is described in Scheme 3. Displacement of the triflate functionality in **5** with lithium azide produced xylo-analogue **16** which was selectively deprotected to **17**. Synthesis of the phosphate prodrug **18** followed by simultaneous deprotection of the amidine and TBS groups with 50% trifluoroacetic acid in water provided the xylo-azide **19**. Unlike compound **14**, the Staudinger reduction of azide **19** was best performed stepwise with isolation of the intermediate triphenylphosphoranylidene **20** prior to ammoniolysis to the target amine **21**. Overall, from guanosine (**1**),



SCHEME 3 Synthesis of xylofuranosyl analogue. a) LiN_3, DMF (100%); b) 70% TFA in water (75%); c) *t*-BuMgCl, THF; **11** (65%); d) 50% TFA in water (77%); e) PPh_3, pyridine (72%); f) NH_4OH, MeOH (87%).

the preparation of xylo-analogue **21** required 9 steps, with an average yield of 76% for each step.

These HepDirect prodrugs were evaluated for activation to derivative NTPs in the reported rat hepatocyte model.[23] The ribo-azide and -amine **14** and **15** at 500 μM produced 64.8 and 7.5 nmol/g NTP, respectively, after a 2 hours incubation. The xylo-azide and -amine **19** and **21** at 250 μM produced 58.1 and 36.1 nmol/g NTP, respectively, after a 2 hours incubation. Since the parent compounds **14**, **15**, **19,** and **21** are activated in rat hepatocytes to their respective NTPs, it is likely their planned amine derivatives will also activate.

These routes provide both the ribo- and xylo-epimers of 3′-amino-3′-deoxyguanosine as well as corresponding azido-analogues. Liver targeting of their potentially bioactive NTPs is possible since they may be derived from in vivo metabolic transformations of the installed HepDirect prodrug of the 5′-phosphates. Further extensions of this methodology are envisioned which involve the generation of additional analogues through standard

amine derivatization reactions, that have been reported previously for other nucleoside systems.[1h-i,4a,c,24] This synthesis demonstrated that the traditionally difficult guanosine nucleoside system can be manipulated selectively in the 2′-, 3′-, and 5′-*O*- as well as the N^2-positions with appropriate protection/deprotection strategies to produce 3′-deoxy-GMP prodrugs ready for 3′-amino derivatization. To study this series in the replicon cellular model of HCV inhibition, another GMP prodrug such as SATE[14] would be necessary and this synthesis should readily extend to the preparation of those analogues.

EXPERIMENTAL

Glassware for moisture-sensitive reactions was flame-dried and cooled to room temperature in a desiccator. All reactions were carried out under an atmosphere of nitrogen. For the reactions, room temperature was 22°C. Anhydrous solvents were purchased and stored over 4Å molecular sieves. Flash chromatography was performed on 230–400 mesh EM Science silica gel 60. ^{1}H NMR were obtained in DMSO-d_6 at 200 MHz and spectra were recorded in units δ with $CD_2HS(O)CD_3$ (δ 2.504) as the reference line internal standard. Certain OH and NH proton chemical shifts were confirmed by D_2O exchange as indicated. Analytical HPLC was conducted with three serially connected Chromolith SpeedRODs RP-18e, 100 × 4.6 mm. Solvent A = HPLC grade acetonitrile; Solvent B = 20 mM ammonium phosphate buffer (pH 6.1, 0.018 M $NH_4H_2PO_4$/0.002 M $(NH_4)_2HPO_4$) with 5% acetonitrile. The UV detector was set to 255 nm and the flow rate was 4 mL/minutes. Gradient program: minutes (%B), 0 (100), 10 (60), 10.1 (100), 12 (100). Mass spectral data were obtained by an LCMS operating in the positive ion mode. C, H, N microanalyses were performed by Robertson Microlit Laboratories, Inc. (Madison, NJ, USA).

9-(2,5-Bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (3a). A mixture of 2-*N*-(*N*,*N*-dibenzylformamidino)-9-(β-D-ribofuranosyl)guanine[15] (**2**) (49.08 g, 100.1 mmol), *t*-butylchlorodimethylsilane (45.25 g, 300.2 mmol), and imidazole (23.85 g, 350.2 mmol) in 800 mL DMF was stirred at room temperature for 6 hours. Then it was diluted with 600 mL of EtOAc and extracted with 500 mL water, 500 mL brine, dried ($MgSO_4$) and evaporated to provide 120 g of residue. This was subjected to chromatography through a 1200 g SiO_2 column eluting with 3:2:1 EtOAc/hexanes/CH_2Cl_2 to provide the 7.73 g of the title compound (**3a**) as an amorphous solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ −0.13 (s, 3H), −0.04 (s, 3H), 0.08 (s, 3H), 0.10 (s, 3H), 0.76 (s, 9H), 0.90 (s, 9H), 3.8–4.2 (m, 4H), 4.41 (t, 1H, J = 6 Hz), 4.58–4.63 (m, 5H), 5.16 (d, 1H, J = 6 Hz, 3′-OH, D_2O exchanged), 5.90 (d, 1H, J = 5 Hz), 7.26–7.41 (m, 10H), 8.04 (s, 1H), 8.95 (s, 1H), 11.60

(s, 1H, NH, D_2O exchanged). Anal. Calcd for $C_{37}H_{54}N_6O_5Si_2$ (719.1):%C, 61.81;%H, 7.57;%N, 11.69. Found:%C, 62.18;%H, 7.88;%N, 11.63. Further elution with 10:3:3 EtOAc/hexanes/CH_2Cl_2 eluted a mixture of the title compound (**3a**) and 9-(3,5-bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (**4a**). Finally elution with 12:1:3 EtOAc/hexanes/CH_2Cl_2 provided 5.0 g compound **4a** as an amorphous solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.02 (s, 3H), 0.03 (s, 3H), 0.12 (s, 6H), 0.86 (s, 9H), 0.89 (s, 9H), 3.3–3.9 (m, 4H), 4.27 (m, 1H), 4.5–4.7 (m, 5H), 5.46 (d, 1H, J = 6 Hz, 2′-OH, D_2O exchanged), 5.83 (d, 1H, J = 6 Hz), 7.3–7.4 (m, 10H), 8.06 (s, 1H), 8.96 (s, 1H), 11.60 (s, 1H, NH, D_2O exchanged). Anal. Calcd for $C_{37}H_{54}N_6O_5Si_2 \bullet 0.5H_2O$ (728.1):%C, 61.04;%H, 7.61;%N, 11.54. Found:%C, 61.05;%H, 7.89;%N, 11.45. The impure fractions were combined and rechromatographed in the same manner to provide an additional 8.12 g of title compound (**3a**). Impure fractions and isolated isomer **4a** were combined and stirred in methanol for 16 hours at room temperature. This mixture was evaporated and subjected to chromatographic purification as before to provide an additional 7.93 g of title compound (**3a**). The remaining **3a**/**4a** mixture isolated was subjected to this isomerization/chromatographic purification process 2 times further to provide an additional 19.36 g of title compound. Total yield of the title compound (**3a**) was 43.14 g (60%).

Studies on the Isomerization of 9-(2,5-Bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (3a) into 9-(3,5-Bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (4a). A solution of compound 3a (20 mg, 0.028 mmol) in 0.4 mL of the specified solvent was stirred in a sealed vial at the specified temperature. After the specified time, 0.020 mL was removed and diluted with 1.5 mL of methanol and evaluated by HPLC to determine the isomeric ratio. The same method was used to study the equilibration of 4a to 3a; 3b to 4b; 3c to 4c; and 4c to 3c.

9-(2,5-Bis-*O*-*tert*-butyldimethylsilyl-3-*O*-trifluoromethylsulfonyl-β-D-ribofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (5). To a mixture of compound **3a** (25 g, 34.8 mmol) and DMAP (12.7 g, 104.3 mmol) in 350 mL CH_2Cl_2 at 0°C was added slowly over a period of 30 minutes trifluoromethanesulfonylchloride (4.1 mL, 38.3 mmol) and stirring continued at 0°C for 30 minutes. Then the mixture was partitioned with water, the organic layer separated, washed with water, brine, dried ($MgSO_4$) and evaporated. The residue was dissolved in 60 mL of EtOAc, diluted with 20 mL of CH_2Cl_2 and 20 mL of hexanes and subjected to chromatography on a column containing 400 g SiO_2 and eluted with 10:3:3 EtOAc/hexanes/CH_2Cl_2 to provide 23.7 g (80%) of the title compound (**5**) as an amorphous white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ −0.41 (s, 3H), −0.02 (s, 3H), 0.03 (s, 3H), 0.10 (s, 3H), 0.73 (s, 9H), 0.84 (s, 9H), 3.8–4.0 (m, 2H), 4.36 (t, 1H, J = 6 Hz), 4.5–4.7 (m, 4H), 5.3–5.4 (m, 1H),

5.51 (d, 1H, $J = 5$ Hz), 5.94 (d, 1H, $J = 8$ Hz), 7.27–7.41 (m, 10H), 8.08 (s, 1H), 8.96 (s, 1H), 11.68 (s, 1H).

9-(3-*O*-Acetyl-2,5-bis-*O*-*tert*-butyldimethylsilyl-D-xylofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (6). A mixture of acetic acid (3.2 mL, 56.4 mmol) and Cs_2CO_3 (18.4 g, 56.4 mmol) in 140 mL DMF was heated to 80°C for 1 hour, cooled to 0°C and then compound **5** (24.0 g, 28.2 mmol) as a solution in 140 mL DMF cooled to 0°C was added via cannula needle. The mixture was stirred at 0°C for 1 hour and then diluted with EtOAc and washed with water, brine, dried ($MgSO_4$) and evaporated. The residue was subjected to chromatography on SiO_2 and eluted with 3:2:1 EtOAc/hexanes/CH_2Cl_2 followed by 10:3:3 to provide 14.38 g (67%) of the title compound (**6**) as an amorphous white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ −0.12 (s, 3H), 0.04 (s, 3H), 0.07 (s, 6H), 0.77 (s, 9H), 0.87 (s, 9H), 2.01 (s, 3H), 3.83 (dd, 1H, $J = 9$, 5 Hz), 3.92 (dd, 1H, $J = 9$, 5 Hz), 4.37 (dd, 1H, $J = 6$, 2 Hz), 4.56 (s, 2H), 4.6–4.7 (m, 3H), 5.21 (dd, 1H, $J = 5$, 2 Hz), 5.93 (d, 1H, $J = 4$ Hz), 7.2–7.4 (m, 10H), 7.98 (s, 1H), 8.98 (s, 1H), 11.64 (s, 1H). Anal. Calcd for $C_{39}H_{56}N_6O_6Si_2$ (761.1):%C, 61.55;%H, 7.42;%N, 11.04. Found:%C, 61.32;%H, 7.74;%N, 11.20.

9-(2,5-Bis-*O*-*tert*-butyldimethylsilyl-β-D-xylofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (7). A 0.5 M solution of sodium methoxide in methanol was prepared by dissolving sodium metal (1.20 g, 50 mmol) in 100 mL of methanol. To a solution of compound **6** (14.38 g, 18.9 mmol) in 100 mL methanol cooled to 0°C was added 75 mL of the 0.5 M solution of sodium methoxide (37.5 mmol) and the resulting mixture stirred at 0°C for 30 minutes and then poured onto 400 mL of 0.2 N aqueous NH_4Cl. The resulting white solid was collected by filtration, dissolved in 400 mL EtOAc, washed with brine, dried ($MgSO_4$), and evaporated to provide 11.44 g (84%) of the title compound (**7**) as an amorphous white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ −0.02 (s, 3H), 0.02 (s, 3H), 0.04 (s, 6H), 0.80 (s, 9H), 0.87 (s, 9H), 3.8–4.3 (m, 4H), 4.55 (s, 2H), 4.62 (s, 2H), 5.71 (d, 1H, $J = 4$ Hz, 3′-OH, D_2O exchanged), 5.87 (d, 1H, $J = 2$ Hz), 7.2–7.4 (m, 10H), 7.96 (s, 1H), 8.99 (s, 1H), 11.61 (s, 1H, NH, D_2O exchanged). Anal. Calcd for $C_{37}H_{54}N_6O_5Si_2$ (719.1):%C, 61.81;%H, 7.57;%N, 11.69. Found:%C, 61.41;%H, 7.98;%N, 11.63.

9-(2,5-Bis-*O*-*tert*-butyldimethylsilyl-3-*O*-trifluoromethylsulfonyl-β-D-xylofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (8). Trifluoromethanesulfonic anhydride (1.30 mL, 7.65 mmol), was added to a solution of compound **7** (5.00 g, 6.95 mmol) and DMAP (2.55 g, 20.9 mmol) in 70 mL CH_2Cl_2 at −40°C and then the solution was stirred at 0°C for 1 hour. It was diluted with CH_2Cl_2 and washed with aqueous 2.5% acetic acid, water, aqueous saturated $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated to provide 6 g of crude product. This was adsorbed onto 18 g of SiO_2 from CH_2Cl_2 and subjected to chromatography through a column of 200 g of SiO_2 eluting with 3:2:1 EtOAc/hexane/CH_2Cl_2 to provide 1.7 g (31%)

of the title compound (**8**) as an amorphous solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ -0.22 (s, 3H), -0.13 (s, 3H), 0.11 (s, 6H), 0.73 (s, 9H), 0.91 (s, 9H), 3.8–4.0 (m, 2H), 4.32 (m, 1H), 4.4–4.7 (m, 4H), 4.86 (dd, 2H, J = 8, 4 Hz), 5.92 (d, 1H, J = 8 Hz), 7.2–7.4 (m, 10H), 7.93 (s, 1H), 8.99 (s, 1H), 11.70 (s, 1H). Anal. Calcd for $C_{38}H_{53}F_3N_6O_7SSi_2$ (851.1):%C, 53.63;%H, 6.28;%N, 9.87. Found:%C, 53.27;%H, 6.51;%N, 9.95. Continued elution with 12:1:3 EtOAc/hexane/CH_2Cl_2 provided 1.45 g (29%) of recovered starting material (**7**).

9-(3-Azido-2,5-bis-*O*-*tert*-butyldimethylsilyl-3-deoxy-β-D-ribofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (9). A mixture of compound **8** (4.40 g, 5.17 mmol) and LiN_3 (791 mg, 15.5 mmol) in 52 mL of DMF was stirred at 0°C for 3 hours and then diluted with EtOAc and washed with water, brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography eluting with 3:2:1 EtOAc/hexane/CH_2Cl_2 to provide 3.56 g (93%) of the title compound (**9**) as an amorphous solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ -0.20 (s, 3H), 0.01 (s, 3H), 0.08 (s, 6H), 0.78 (s, 9H), 0.89 (s, 9H), 3.8–4.1 (m, 3H), 4.32 (t, 1H, J = 5 Hz), 4.59 (s, 2H), 4.63 (s, 2H), 4.87 (t, 1H, J = 5 Hz), 5.89 (d, 1H, J = 5 Hz), 7.2–7.4 (m, 10H), 8.02 (s, 1H), 8.97 (s, 1H), 11.61 (s, 1H). Anal. Calcd for $C_{37}H_{53}N_9O_4Si_2$ (744.1):%C, 59.73;%H, 7.18;%N, 16.94. Found:%C, 59.35;%H, 7.46;%N, 16.48.

9-(3-Azido-2-*O*-*tert*-butyldimethylsilyl-3-deoxy-β-D-ribofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (10). A solution of compound **9** (2.65 g, 3.56 mmol) and 15 mL of 70% TFA in water was stirred for 45 minutes at room temperatur. Then it was poured onto a mixture of 250 mL saturated aqueous $NaHCO_3$ and 250 mL EtOAc and stirred vigorously for 15 minutes at room temperature. The organic layer was separated and washed with saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated. The residue was subjected to chromatography eluting with 10:1 EtOAc/hexane and then EtOAc to provide 1.53 g (68%) of the title compound (**10**) as an amorphous solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ -0.22 (s, 3H), -0.03 (s, 3H), 0.77 (s, 9H), 3.5–3.8 (m, 2H), 3.9–4.0 (m, 1H), 4.38 (dd, 1H, J = 5, 4 Hz), 4.57 (s, 2H), 4.62 (s, 2H), 5.04 (t, 1H, J = 5 Hz), 5.35 (t, 1H, J = 6 Hz, 5′-OH, D_2O exchanged), 5.86 (d, 1H, J = 6 Hz), 7.2–7.4 (m, 10H), 8.13 (s, 1H), 8.94 (s, 1H), 11.64 (s, 1H, NH, D_2O exchanged). Anal. Calcd for $C_{31}H_{39}N_9O_4Si{\cdot}H_2O$ (647.8):%C, 57.48;%H, 6.38;%N, 19.46. Found:%C, 58.10;%H, 6.54;%N, 18.83.

9-(3-Azido-2-*O*-*tert*-butyldimethylsilyl-*cis*-5-*O*-(4-(3-chlorophenyl)-1,3-dioxa-2-oxophosphorinan-2-yl)-3-deoxy-β-D-ribofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (12). To a solution of compound 10 (1.65 g, 2.62 mmol) in 52 mL of THF at −40°C was added 6.3 mL of a 1 M solution of *t*-BuMgCl in THF (6.29 mmol) and the mixture stirred for 15 minutes at −40°C. Then *trans*-4-(3-chlorophenyl)-2-(4-nitrophenoxy)-2-oxo-1,3,2-dioxaphosphorinane (**11**)[13a] (1.45 g, 3.93 mmol) was added as a solid

and the mixture stirred at 0°C for 1 hour. Then it was stirred at room temperature for 16 hours, diluted with 1 M aqueous NH_4Cl and extracted into EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was dissolved in EtOAc and loaded onto a column of SiO_2 and eluted with EtOAc and then 100:1 EtOAc/methanol to provide 2.1 g (93%) of the title compound (**12**) as an amorphous solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ −0.23 and −0.21 (s each, 3H),[21] −0.03 and −0.01 (s each, 3H), 0.76 and 0.77 (s each, 9H), 2.0–2.2 (m, 2H), 4.1–4.2 (m, 1H), 4.2–4.6 (m, 7H), 5.11 (q, 1H, J = 6 Hz), 5.6–5.8 (m, 1H), 5.91 (d, 1H, J = 6 Hz), 7.2–7.5 (m, 14H), 8.04 (s, 1H), 8.96 (s, 1H), 11.62 and 11.63 (s each, 1H). Anal. Calcd for $C_{40}H_{47}ClN_9O_7PSi$ (860.4):%C, 55.84;%H, 5.51;%N, 14.65. Found:%C, 55.69;%H, 5.62;%N, 14.32.

9-(3-Azido-*cis*-5-*O*-(4-(3-chlorophenyl)-1,3-dioxa-2-oxophosphorinan-2-yl)-3-deoxy-β-D-ribofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (13). A mixture of compound 12 (1.00 g, 1.16 mmol) and tetraethylammonium fluoride in 12 mL of DMF was stirred for 2 hours at room temperature. Then it was diluted with EtOAc and washed with water, brine, dried ($MgSO_4$), and evaporated. The residue was adsorbed to SiO_2 from a solution in CH_2Cl_2/methanol and then subjected to chromatography on SiO_2 eluting with 20:1 CH_2Cl_2/methanol followed by 10:1 to provide 733 mg (85%) of the title compound (**13**) as an amorphous solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.0–2.2 (m, 2H), 4.12 (q, 1H, J = 4 Hz), 4.2–4.7 (m, 6H), 4.9–5.1 (m, 1H), 5.6–5.8 (m, 1H), 5.89 (d, 1H, J = 6 Hz), 6.39 (br s, 1H, 2′-OH, D_2O exchanged), 7.2–7.5 (m, 14H), 8.05 (s, 1H), 9.01 (s, 1H), 11.60 (s, 1H, NH, D_2O exchanged). Anal. Calcd for $C_{34}H_{33}ClN_9O_7P$ (746.1):%C, 54.73;%H, 4.46;%N, 16.90. Found:%C, 54.48;%H, 4.67;%N, 16.93.

9-(3-Azido-*cis*-5-*O*-(4-(3-chlorophenyl)-1,3-dioxa-2-oxophosphorinan-2-yl)-3-deoxy-β-D-ribofuranosyl)guanine (14). A mixture of compound **13** (733 mg, 0.98 mmol) in 10 mL of 50% aqueous TFA was stirred at room temperature for 16 hours and then carefully added in a dropwise manner to 75 mL of saturated aqueous $NaHCO_3$ with rapid stirring. The resulting white solid was collected and dried at (room temperature) rt/0.1 mm vacuum to provide 566 mg (107%) of the title compound (**14**) as a white amorphous solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.1–2.3 (m, 2H), 4.0–4.1 (m, 1H), 4.2–4.6 (m, 4H), 4.8–4.9 (m, 1H), 5.6–5.7 (m, 1H), 5.74 (d, 1H, J = 5 Hz), 6.33 (d, 1H, J = 5 Hz, 2′-OH, D_2O exchanged), 6.55 (br s, 2H, NH_2, D_2O exchanged), 7.2–7.5 (m, 4H), 7.86 and 7.87 (s each, 1H), 10.70 (s, 1H, NH, D_2O exchanged); MS calcd for $C_{19}H_{20}ClN_8O_7P$: M+1 = 539.1; M+1 found: 539.7.

9-(3-Amino-*cis*-5-*O*-(4-(3-chlorophenyl)-1,3-dioxa-2-oxophosphorinan-2-yl)-3-deoxy-β-D-ribofuranosyl)guanine (15). A mixture of compound **14** (303 mg, 0.56 mmol) triphenylphosphine (438 mg, 1.67 mmol), 2.5 mL of 6 M NH_3 in methanol and 2.5 mL of pyridine was stirred at 50°C for 1.5

hours. The solvent was evaporated and the residue suspended in EtOAc. An equal volume of ether was added and the resulting white solid was collected by filtration and dried at rt/0.1 mm vacuum to provide 265 mg (95%) of the title compound (**15**) as an amorphous white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.0–2.2 (m, 2H), 3.4–3.5 (m, 1H), 3.8–3.9 (m, 1H), 4.1–4.6 (m, 5H), 5.6–5.7 (m, 1H), 5.77 (d, 1H, J = 1 Hz), 6.48 (br s, 2H, NH_2, D_2O exchanged), 7.2–7.5 (m, 4H), 7.79 and 7.80 (s each, 1H); MS calcd for $C_{19}H_{22}ClN_6O_7P$: M+1 = 513.1; M+1 found: 513.2. Anal. Calcd for $C_{19}H_{22}ClN_6O_7P\bullet1.5H_2O$ (539.9):%C, 42.27;%H, 4.67;%N, 15.57. Found:%C, 41.90;%H, 4.49;%N, 16.01.

9-(3-Azido-2,5-bis-*O*-*tert*-butyldimethylsilyl-3-deoxy-β-D-xylofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (16). A mixture of **5** (6.36 g, 7.47 mmol), lithium azide (1.90 g, 37.4 mmol), and DMF (100 mL) was stirred at room temperature for 3 hours. The reaction mixture was partitioned between ethyl acetate (300 mL) and saturated $NaHCO_3$ (150 mL). The layers were separated and the organic layer was washed with saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography (SiO_2, 10:3:3 EtOAc/Hexane/CH_2Cl_2) which provided 5.78 g (100%) of the title compound (**16**) as a light yellow foam: ^{1}H NMR (200 MHz, DMSO-d_6) δ -0.30 (s, 3H), −0.05 (s, 3H), 0.08 (d, 6H, J = 3 Hz), 0.75 (s, 9H), 0.89 (s, 9H), 3.8–3.9 (m, 2H), 4.3–4.4 (m, 1H), 4.4–4.8 (m, 7H), 5.84 (d, 1H, J = 6 Hz), 7.2–7.5 (m, 10H), 8.02 (s, 1H), 8.98 (s, 1H), 11.63 (s, 1H); MS calcd for $C_{37}H_{53}N_9O_4Si_2$: M+1 = 744.4. M+1 found: 745.1. Anal. calcd. for $C_{37}H_{53}N_9O_4Si_2$ (744.06): C, 59.73; H, 7.18; N, 16.94. Found: C, 59.83; H, 7.19; N, 16.38.

9-(3-Azido-2-*O*-*tert*-butyldimethylsilyl-3-deoxy-β-D-xylofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (17). A solution of **16** (4.99 g, 6.70 mmol) in 75 mL of 70% trifluoroacetic acid/water was stirred at room temperature for 10 minutes. The reaction mixture was poured into a beaker containing saturated $NaHCO_3$ (300 mL) and ethyl acetate (300 mL) with stirring. The layers were separated and the organic layer was washed with saturated $NaHCO_3$ (3×300 mL), dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography (SiO_2, 20:1 EtOAc/MeOH) which provided 3.16 g (75%) of the title compound (**17**) as an amorphous white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ -0.26 (s, 3H), -0.01 (s, 3H), 0.77 (s, 9H), 3.6–3.8 (m, 2H), 4.2–4.3 (m, 1H), 4.4–4.5 (m, 1H), 4.56 (s, 2H), 4.62 (s, 2H), 4.8–4.9 (m, 1H), 5.19 (m, 2H), 5.83 (d, 1H, J = 6 Hz), 7.2–7.5 (m, 10H), 8.10 (s, 1H), 8.97 (s, 1H), 11.66 (s, 1H); MS calcd for $C_{31}H_{39}N_9O_4Si$: M+1 = 630.3. M+1 found: 630.3.

9-(3-Azido-2-*O*-*tert*-butyldimethylsilyl-*cis*-5-*O*-(4-(3-chlorophenyl)-1,3-dioxa-2-oxophosphorinan-2-yl)-3-deoxy-β-D-xylofuranosyl)-2-*N*-(*N*,*N*-dibenzylformamidino)guanine (18). A solution of 17 (1.23 g, 1.95 mmol) in THF (100 mL) was cooled to −40°C and a 1 M solution of t-butylmagnesium chloride (in THF) (4.68 mL, 4.68 mmol) was added dropwise over 10

minutes. The reaction mixture was stirred for an additional 15 minutes. at −40°C. Then *trans*-4-(3-chlorophenyl)-2-(4-nitrophenoxy)-2-oxo-1,3,2-dioxaphosphorinane (11) (1.08 g, 2.93 mmol) was added as a solid and the mixture stirred at −40°C for 15 minutes. The reaction mixture was warmed to room temperature and stirred for 16 hours, diluted with EtOAc (200 mL), washed with 2 N NH_4Cl (200 mL), saturated $NaHCO_3$ (100 mL), brine (100 mL), dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography (SiO_2, gradient of 2–5% MeOH/CH_2Cl_2) which provided 1.09 g (65%) of the title compound (18) as an amorphous light yellow solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ −0.26 (s, 3H), −0.02 (s, 3H), 0.75 (s, 9H), 2.1–2.3 (m, 2H), 3.3–3.5 (m, 2H), 4.3–4.5 (m, 2H), 4.57 (s, 2H), 4.5–4.6 (m, 3H), 4.8–5.0 (m, 1H), 4.6–4.7 (m, 1H), 5.89 (d, 1H, J = 6 Hz), 7.2–7.5 (m, 14H), 8.06 (s, 1H), 8.97 (s, 1H), 11.67 (s, 1H).

9-(3-Azido-*cis*-5-*O*-(4-(3-chlorophenyl)-1,3-dioxa-2-oxophosphorinan-2-yl)-3-deoxy-β-D-xylofuranosyl)guanine (19). A solution of **18** (763 mg, 0.877 mmol) in a 1:1 mixture of trifluoroacetic acid/water (5 mL) was stirred at room temperature for 16 hours. The reaction mixture was concentrated and azeotroped with MeOH and acetonitrile. The residue was adsorbed onto SiO_2 and subjected to chromatography (SiO_2, gradient of 5–10% MeOH/CH_2Cl_2) which provided 370 mg (77%) of the title compound (**19**) as an amorphous white solid.: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.1–2.3 (m, 2H), 3.4–3.5 (m, 2H), 4.31 (d, 2H, J = 6 Hz), 4.4–4.6 (m, 2H), 4.6–4.7 (m, 1H), 5.67 (d, 1H, J = 6 Hz), 5.6–5.7 (m, 1H), 6.34 (d, 1H, J = 6 Hz), 6.56 (br s, 2H), 7.3–7.5 (m, 4H), 7.87 (s, 1H), 10.72 (s, 1H); MS calcd for $C_{19}H_{20}ClN_8O_7P$ M+1 = 539.1. M+1 found: 539.0.

9-(*cis*-5-*O*-(4-(3-Chlorophenyl)-1,3-dioxa-2-oxophosphorinan-2-yl)-3-deoxy-3-(triphenylphosphoranylidene)amino-β-D-xylofuranosyl)guanine (20). To a solution of **19** (458 mg, 0.850 mmol) in pyridine (20 mL) was added triphenylphosphine (668 mg, 2.55 mmol) and the solution was stirred at room temperature for 1 hour. The reaction mixture was concentrated, adsorbed onto SiO_2 and subjected to chromatography (SiO_2, 3:10:0.05 MeOH/CH_2Cl_2/conc. NH_4OH) to provide 470 mg (72%) of the title compound (20) as an amorphous white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.0–2.2 (m, 2H), 3.7–3.9 (m, 2H), 4.2–4.3 (m, 2H), 4.3–4.4 (m, 1H), 4.4–4.6 (m, 1H), 4.8–5.0 (m, 1H), 5.55 (d, 1H, J = 8 Hz), 5.6–5.8 (m, 1H), 6.2–6.3 (m, 1H), 6.45 (br s, 2H), 7.2–7.5 (m, 4H), 7.6–8.0 (m, 16H), 10.80 (s, 1H); MS calcd for $C_{37}H_{35}ClN_6O_7P_2$: M+1 = 773.2. M+1 found: 773.5.

9-(3-Amino-*cis*-5-*O*-(4-(3-chlorophenyl)-1,3-dioxa-2-oxophosphorinan-2-yl)-β-D-xylofuranosyl)guanine (21). A solution of **20** (335 mg, 0.443 mmol) in a 1:1 mixture of concentrated NH_4OH in MeOH (100 mL) was stirred at room temperature for 8 hours. The reaction mixture was concentrated, adsorbed onto SiO_2, and subjected to chromatography (SiO_2, 3:10:0.05 MeOH/CH_2Cl_2/conc. NH_4OH) to provide 194 mg (87%) of the title

compound (**21**) as a amorphous white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.1–2.3 (m, 2H), 3.4–3.5 (m, 2H), 4.1–4.2 (m, 2H), 4.2–4.4 (m, 3H), 5.61 (d, 1H, J = 5 Hz), 5.69 (d, 1H, J = 12 Hz), 5.80 (d, 1H, J = 5 Hz), 6.49 (br s, 2H), 7.3–7.6 (m, 4H), 7.98 (s, 1H), 10.66 (br s, 1H); MS calcd for $C_{19}H_{22}ClN_6O_7P$: M+1 = 513.1; M+1 found: 513.3. Anal. Calcd. for $C_{19}H_{22}ClN_6O_7P\bullet0.25H_2O$ (517.4): C, 44.38; H, 4.38; N, 16.24. Found: C, 44.09; H, 5.13; N, 15.38.

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