Syntheses of 5′-Deoxy-5′-*N*-hydroxylaminopyrimidine and Purine Nucleosides: Building Blocks for Novel Antisense Oligonucleosides with Hydroxamate Linkages

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Introduction

Inhibition of gene expression has become an attractive method for treatment of viral disease and cancer. In principle, antisense oligonucleotides target, in a sequence specific manner, a particular gene or mRNA to inhibit the expression of that gene.[1−5] In recent years, chemical modifications of antisense oligonucleotides have resulted in improved solubility, stability toward nucleases, cellular uptake, and appropriate hybridization to target genes or mRNAs.[6] Antisense oligonucleosides have been reported in which the natural phosphodiester backbones have been replaced by synthetic linkages in order to improve the permeation efficiency of antisense agents.[7] In the antisense approach, oligonucleotides have been designed to bind to a given mRNA to inhibit the translation process via duplex formation and/or RNase H-induced degradation of the message. We propose a novel backbone replacement for antisense oligonucleosides with hydroxamates as linkages (Figure 1). Hydroxamic acids effectively chelate Fe(III), which can undergo Fenton chemistry to generate hydroxyl radicals.[8] Therefore, in addition to potential RNase-H cleavage processes, antisense oligonucleosides with hydroxamate linkages might cleave the target mRNA through radical reactions. The retrosynthetic analysis of the hydroxamate-linked oligomers indicated that 5′-deoxy-5′-*N*-hydroxylaminonucleosides **1**−**4**, derived from adenosine **5**, uridine **6**, cytidine **7**, and guanosine **8**, could serve as the common building blocks for these oligomers (Figure 1). Herein we report the syntheses of these useful hydroxamate building blocks.

In addition to the potential antiviral activities of antisense oligonucleosides, nucleoside analogues themselves are a very important class of compounds due to their wide range of biological activities, attracting attention as antitumor,[9] antiviral,[10] and antibiotic agents.[11] Compounds **1**−**4** are modified nucleosides with a novel 5′-C−NO-bond moiety, which might have interesting biological activities. Sanghvi et al. previously reported the syntheses of 5′-*O*-amino-2′-deoxynucleosides.[12] When 2′-deoxynucleosides were reacted with *N*-hydroxyphthalimide via a Mitsunobu reaction in dimethylformamide (DMF), they found that with unprotected nucleosides the Mitsunobu reaction on thymidine and 2′-deoxyadenosine provided regioselective 5′-C−ON-bond formation. We now report formation of the 5′-C−NO-bond of nucleoside analogues employing the Mitsunobu reaction (Figure 1).

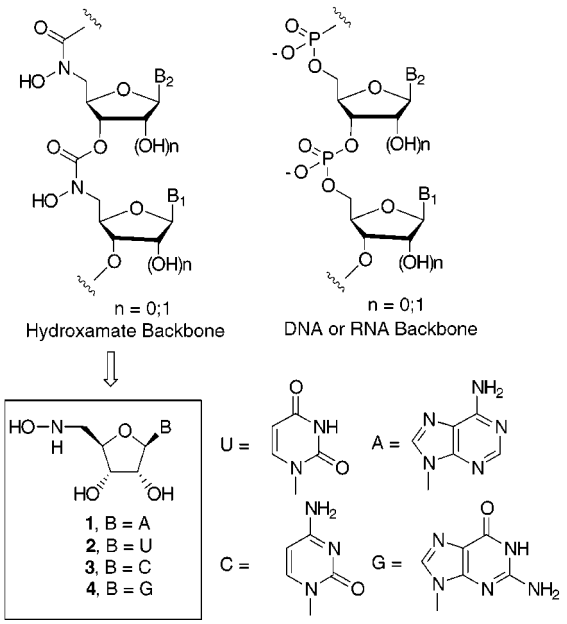

Figure 1.

Results and Discussion

The Mitsunobu reaction[13] of nucleosides with protected hydroxylamines is the key reaction for the planned preparation of 5′-deoxy-5′-*N*-hydroxylaminonucleosides. This reaction is highly dependent upon the pK_a of the hydroxylamine used and the concentration of reaction solutions. Although many different bis-protected hydroxylamines[14] have been used for the Mitsunobu reaction with nucleosides, *N*-(*tert*-butoxycarbonyl)-*O*-(benzyloxycarbonyl)hydroxylamine (BocNHOCbz)[15] was found to be the most effective and high yielding in the study reported here. Treatment of adenosine, **5**, with BocNHOCbz, triphenylphosphine (TPP), and di-*tert*-butyl azodicarboxylate (DBAD) in a mixture of 10:1 tetrahydrofuran

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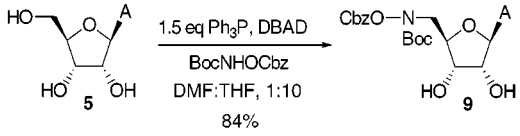
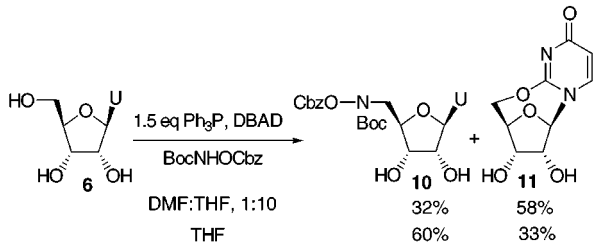
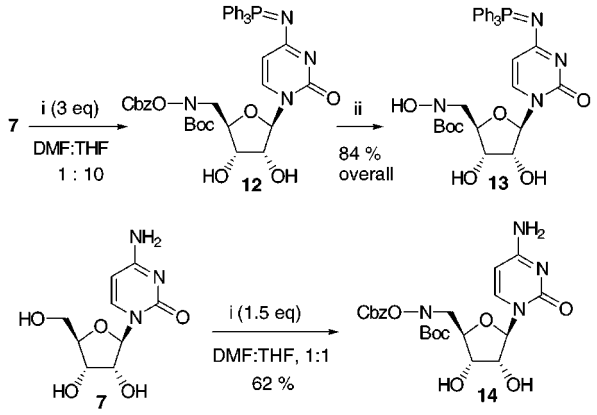
Scheme 1



Scheme 2



Scheme 3



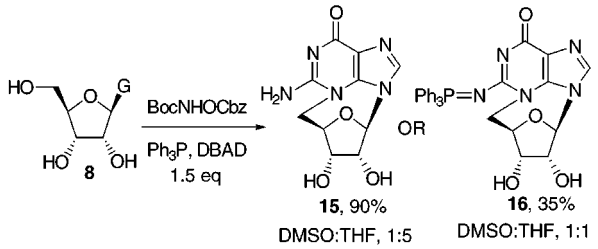
(i) Ph_3P, DBAD, BocNHOCbz, rt; (ii) 10% Pd-C, H_2, MeOH.

(THF)−DMF at room temperature afforded hydroxylamino derivative **9** of adenosine in 84% yield (Scheme 1).

Exposure of uridine, **6**, to similar conditions gave 2,5′-anhydrouridine **11** as the major product. However, when THF was used as the solvent, the desired product **10** was isolated in 60% yield (Scheme 2). It is likely that the poor solubility of uridine in THF resulted in the increased ratio of hydroxylamine to uridine and favored the formation of compound **10** over compound **11** (Scheme 2).

Under similar conditions, cytidine, **7**, gave no reaction. The Mitsunobu reaction on cytidine, **7**, was then repeated under "forcing conditions" with 3 equiv of all reagents to give iminophosphorane **12**. To determine the structure of **12** unambiguously, it was subjected to catalytic hydrogenation to give *N*-hydroxycarbamate **13** in 84% overall yield (Scheme 3). The ^{13}C NMR of compound **13** showed a doublet at δ 103.8 ppm (J^3_{CP} = 25 Hz) for C5, which suggested the formation of the supposed iminophosphorane. The ^{31}P NMR (^1H decoupled) of **13** gave a signal at δ 20.24 ppm, which is consistent with the reported ^{31}P NMR data for other iminophosphoranes.[16,17] Usually, iminophosphoranes are prepared from amines with dihalogen triphenylphosphorane or via the Staudinger reaction of azides and triphenylphosphine.[18] The only examples of making iminophosphoranes under Mitsunobu reaction conditions were when the substrates were amides, sulfonamides, and phosphinamides.[19] Since iminophosphoranes can undergo inter- or intramolecular aza-Wittig reactions and are useful building blocks for the preparation of nitrogen-containing heterocycles,[18] the methodology described here for the formation of cytidine iminophosphorane could be used for constructing other modified nucleosides.[20]

Scheme 4



One possible reason for the failure of cytidine, **7**, to give the desired product may be due to its poor solubility in the 10:1 THF−DMF solvent system used, resulting in a slow reaction with the hydroxylamine. A more polar solvent system was then tested. Indeed, the desired product, **14**, was obtained in 62% yield in 1:1 THF−DMF solution (Scheme 3).

The Mitsunobu reaction of guanosine, **8**, required an alternate, more polar solvent system due to its poor solubility in the variety of solvents utilized. When 1:5 dimethyl sulfoxide (DMSO)−THF was used, the reaction gave cyclized compound **15** in 90% yield (Scheme 4). Increasing the DMSO concentration further to 1:1 DMSO−THF resulted in formation of the corresponding iminophosphorane **16** in 35% yield (Scheme 4).

The formation of cyclic products **15** and **16** indicated that protection of the acidic 6-OH of guanine was necessary. Guanosine, **8**, was treated with *tert*-butyldimethylsilyl chloride (TBSCl) to give compound **17**.[21] Mitsunobu reaction of **17**[22] with benzyl alcohol incorporated a benzyl group at the O^6 position of guanine and afforded product **18** in 59% overall yield. Treatment of **18** with tetrabutylammonium fluoride (TBAF) afforded deprotected triol **19** in 92% yield. The resulting product was then used in the Mitsunobu reaction with BocNHOCbz in THF and the reaction afforded desired product **20** in 91% yield (Scheme 5).

The hydroxylamino derivatives **9**, **10**, **14**, and **20** were deprotected under standard hydrogenolysis conditions to remove the benzyloxycarbonyl (Cbz) group. The resulting compounds, **21**−**24**, were treated with HCl in ethyl acetate to provide target modified nucleosides **1**−**4** in good yields (Scheme 6).

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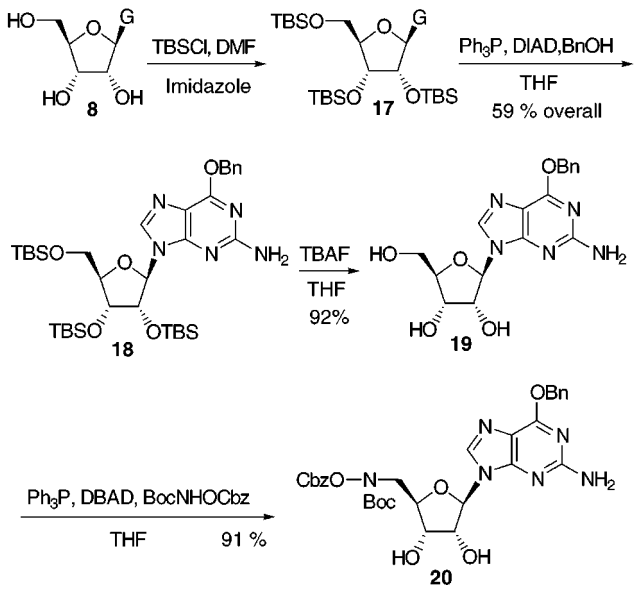
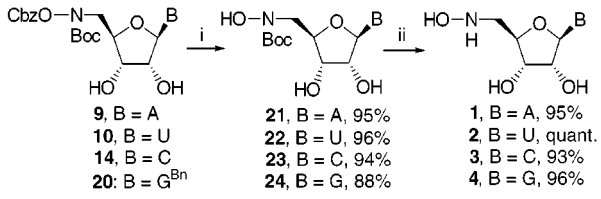
Scheme 5



Scheme 6



(i) 10% Pd-C, H_2, MeOH; (ii) HCl in EtOAc, MeOH.

Conclusions

The methodology described above provides a general and efficient route for the conversion of the 5′-hydroxyl group of a nucleoside into the corresponding 5′-*N*-hydroxylamino-substituted derivatives. To the best of our knowledge, this is the first time that all four natural nucleosides (U, C, A, G) have been transformed to their 5′-*N*-hydroxylamino derivatives, which may be useful as antiviral agents.[23] The formation of iminophosphoranes under Mitsunobu reaction conditions with cytidine and guanosine amines may also be valuable for the syntheses of other base-modified nucleosides. This methodology was also used to synthesize thymidine oligomers with hydroxamate rather than phosphodiester linkages and will be reported in the future.

Experimental Section

General Methods. Melting points (mp) are uncorrected. The NMR spectra were recorded at 300 MHz (^{1}H), 75 MHz (^{13}C), and 121 MHz (^{31}P). ^{13}C and ^{31}P NMR spectra were fully proton decoupled. The chemical shifts are reported relative to TMS or residual solvent and relative to external 85% phosphoric acid (0 ppm) in ^{31}P NMR. Unless otherwise noted, all reactions were performed in septum-capped, oven-dried, and argon-purged flasks. THF was continuously refluxed from sodium benzophenone ketyl and distilled immediately prior to use. DMF and DMSO were dried over 3 Å molecular sieves. The HCl in ethyl acetate solution was generated by passing HCl gas through anhydrous ethyl acetate until saturated. All chemicals and reagents were obtained from Aldrich.

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Experimental Methods A−C Related to Reaction Schemes 1−6. Method A. Mitsunobu Reaction (5 → 9 as a representative example). Adenosine **5** (267 mg, 1 mmol), triphenylphosphine (393 mg, 1.5 mmol), and BocNHOCbz (534 mg, 2 mmol) were suspended in anhydrous DMF (1 mL) and THF (10 mL). At 0 °C, DBAD (345 mg, 1.5 mmol) was added to the reaction mixture. The reaction solution was stirred at room temperature overnight and the solvent was evaporated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 20:1 to 10:1) to give **9** (435 mg, 84%) as a clear oil.

Method B. Hydrogenolysis (9 → 21 as a representative example). A stirred solution of compound **9** (250 mg, 0.484 mmol) in methanol (10 mL) was purged with nitrogen and charged with 10% Pd−C (30 mg). The reaction mixture was stirred under hydrogen at room temperature for 4 h. The mixture was purged with nitrogen to remove hydrogen, then the catalyst was removed by filtration through Celite. The filtrate was evaporated and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 10:1) to give **21** (175 mg, 95%) as a clear oil.

Method C. Deprotection of *tert*-Butoxycarbonyl Group (21 → 1 as a representative example). To a stirred solution of compound **21** (120 mg, 0.314 mmol) in 10% methanol in methylene chloride (3 mL) was added saturated HCl in ethyl acetate solution (2 mL). The mixture was stirred at room temperature for 15 min and was evaporated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 5:1 to 2:1) to give **1** (84 mg, 95%) as a white solid.

5′-Deoxy-5′-*N*-(*N*-*tert*-butoxycarbonyl-*O*-benzyloxycarbonyl)hydroxylaminoadenosine (9). Compound **9** was prepared by method A as a clear oil in 84% yield: ^{1}H NMR (10% CD_3OD in $CDCl_3$) δ 8.22 (s, 1 H), 8.07 (s, 1 H), 7.36−7.29 (m, 5 H), 6.67 (br, 1 H), 5.96 (d, J = 4.2 Hz, 1 H), 5.21 (s, 2 H), 4.59 (t, J = 4.5 Hz, 1 H), 4.38−4.29 (m, 2 H), 4.10−3.94 (m, 2 H), 1.38 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 155.37, 154.78, 154.22, 152.42, 148.84, 139.16, 134.15, 128.74, 128.50, 128.43, 119.17, 88.87, 83.40, 82.21, 74.34, 71.81, 71.11, 52.12, 27.79; IR (neat) 3334, 1787, 1718, 1647, 1602, 1218 cm^{-1}; HRFABMS calcd for $C_{23}H_{29}N_6O_8$ (M + H)$^+$ 517.2047, found 517.2060.

5′-Deoxy-5′-*N*-(*N*-*tert*-butoxycarbonyl-*O*-benzyloxycarbonyl)hydroxylaminouridine (10). Compound **10** was prepared by method A in THF solution as a clear oil in 60% yield: ^{1}H NMR ($CDCl_3$) δ 10.39 (s, 1 H), 7.53 (d, J = 8.1 Hz, 1 H), 7.37−7.31 (m, 5 H), 5.78 (d, J = 3.9 Hz, 1 H), 5.64 (d, J = 8.1 Hz, 1 H), 5.23 (s, 4 H), 4.23 (m, 2 H), 4.08 (m, 1 H), 3.96 (m, 2 H), 1.38 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 163.93, 154.93, 154.15, 151.14, 140.29, 134.14, 128.92, 128.59, 128.55, 102.39, 90.29, 83.58, 81.13, 74.23, 71.21, 70.91, 51.63, 27.82; IR (neat) 3411, 1788, 1695 cm^{-1}; HRFABMS calcd for $C_{22}H_{28}N_3O_{10}$ (M + H)$^+$ 494.1775, found 494.1780.

2,5′-Anhydrouridine (11). Compound **11** was prepared by method A in THF solution as a white solid in 33% yield: mp 200 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.78 (dd, J = 1.5 Hz, 7.5 Hz, 1 H), 6.06 (dd, J = 1.8 Hz, 7.5 Hz, 1 H), 5.50 (s, 1 H), 4.52−4.43 (m, 4 H), 4.12 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 175.85, 160.10, 146.65, 110.62, 102.32, 88.86, 78.40, 76.68, 73.52; IR (KBr) 3496, 3235, 1634, 1601 cm^{-1}; HRFABMS calcd for $C_9H_{11}N_2O_5$ (M + H)$^+$ 227.0668, found 227.0670.

5′-Deoxy-5′-*N*-(*N*-*tert*-butoxycarbonyl)hydroxylamino-4-*N*-triphenylphosphoranylidenecytidine (13). Compound **13** was prepared by methods A and B as a clear oil in 84% overall yield: ^{1}H NMR (10% CD_3OD in $CDCl_3$) δ 7.84−7.77 (m, 6 H), 7.58−7.45 (m, 10 H), 6.16 (d, J = 7.2 Hz, 1 H), 5.58 (d, J = 3.3 Hz, 1 H), 4.21 (q, J = 5.4 Hz, 1 H), 4.08 (m, 2 H), 3.85 (dd, J = 5.7 Hz, 15 Hz, 1 H), 3.71 (dd, J = 4.5 Hz, 15 Hz, 1 H), 1.47 (s, 9 H); ^{13}C NMR (10% CD_3OD in $CDCl_3$) δ 172.04, 171.94, 156.76, 156.51, 139.49, 139.44, 133.22, 133.09, 132.24, 132.20, 128.58, 128.41, 127.85, 126.52, 103.96, 103.62, 92.62, 81.49, 81.25, 74.47, 71.18, 51.82, 28.04; ^{31}P NMR (10% CD_3OD in $CDCl_3$) 20.24; IR (neat) 3249, 1634, 1435, 1112 cm^{-1}; HRFABMS calcd for $C_{32}H_{36}N_4O_7P$ (M + H)$^+$ 619.2322, found 619.2328.

5′-Deoxy-5′-*N*-(*N*-*tert*-butoxycarbonyl-*O*-benzyloxycarbonyl)hydroxylaminocytidine (14). Compound **14** was prepared by method A in a THF (5 mL) and DMF (5 mL) solution as a clear oil in 62% yield: ^{1}H NMR (10% CD_3OD in $CDCl_3$) δ 7.60 (d, J = 7.2 Hz, 1 H), 7.38−7.33 (m, 5 H), 5.79 (d, J = 7.2 Hz, 1 H), 5.72 (d, J = 2.7 Hz, 1 H), 5.25 (s, 2 H), 4.24 (m, 1 H),

4.08 (m, 1 H), 3.94 (m, 3 H), 1.40 (s, 9 H); ^{13}C NMR (10% CD_3OD in $CDCl_3$) δ 165.54, 156.52, 154.86, 154.07, 140.58, 134.02, 128.79, 128.44, 128.39, 94.91, 91.52, 83.46, 80.65, 74.59, 71.10, 70.92, 51.75, 27.64; IR (neat) 3345, 1791, 1652 cm^{-1}; HRFABMS calcd for $C_{22}H_{29}N_4O_9$ (M + H)$^+$ 493.1935, found 493.1944.

3,5′-Anhydroguanosine (15). Compound **15** was prepared by method A in a THF (10 mL) and DMSO (2 mL) solution as a white solid in 90% yield: mp 180 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.72 (s, 1 H), 6.14 (s, 1 H), 4.50 (br, 1 H), 4.42 (d, J = 13.8 Hz, 1 H), 4.18 (m, 1 H), 3.93 (d, J = 13.5 Hz, 1H), 3.87 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 164.27, 153.89, 138.60, 133.15, 121.61, 92.42, 83.13, 75.54, 70.58, 53.38; IR (KBr) 3350, 1634 cm^{-1}; HRFABMS calcd for $C_{10}H_{12}N_5O_4$ (M + H)$^+$ 266.0889, found 266.0891.

3,5′-Anhydro-4-*N*-triphenylphosphoranylideneguanosine (16). Compound **16** was prepared by method A in a THF (5 mL) and DMSO (5 mL) solution as a white solid in 35% yield: mp 180 °C (dec); ^{1}H NMR (10% CD_3OD in $CDCl_3$) δ 7.76−7.69 (m, 6 H), 7.49−7.34 (m, 10 H), 5.95 (s, 1 H), 5.33 (d, J = 12.9 Hz, 1 H), 4.67 (m, 1 H), 4.20 (m, 1 H), 4.07 (dd, J = 2.4 Hz, 14.7 Hz, 1 H), 3.86 (m, 1 H); ^{13}C NMR (10% CD_3OD in $CDCl_3$) δ 165.92, 156.91, 140.06, 133.19, 133.05, 132.64, 132.43, 132.39, 128.59, 128.42, 127.52, 126.15, 121.16, 92.70, 84.48, 75.35, 70.54, 54.25; ^{31}P NMR (10% CD_3OD in $CDCl_3$) 21.87; IR (KBr) 3370, 1597, 1435 cm^{-1}; HRFABMS calcd for $C_{28}H_{25}N_5O_4P$ (M + H)$^+$ 526.1644, found 526.1652.

6-*O*-Benzyl-2′,3′,5′-*O*-tri-*tert*-butyldimethylsilylguanosine (18). Guanosine **8** (1.45 g, 5 mmol) was suspended in dry DMF (30 mL) and treated with imidazole (2.7 g, 40 mmol) and TBSCl (4.5 g, 30 mmol). The reaction mixture was stirred at room temperature overnight. Then DMF was removed under reduced pressure. The residue was diluted with ethyl acetate (100 mL) and was washed with water (3 × 50 mL), saturated aqueous ammonium chloride (NH_4Cl) solution (2 × 50 mL), and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to give a white foam (3.0 g). This white foam was further evacuated overnight under high vacuum and dissolved in anhydrous THF (40 mL). Triphenylphosphine (1.97 g, 7.5 mmol) was added, followed by benzyl alcohol (1.03 mL, 10 mmol). The mixture was cooled to 0 °C and diisopropyl azodicarboxylate (DIAD) (1.48 mL, 7.5 mmol) was added dropwise. The solution then was stirred at room temperature for 4 h and the solvent was removed in vacuo. The residue was purified by flash column chromatography (hexanes/EtOAc = 8:1) to give **18** (2.1 g, 2 steps, 59%) as a white solid: mp 95−97 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (s, 1 H), 7.51−7.48 (m, 2 H), 7.38−7.26 (m, 3 H), 5.96 (d, J = 6.0 Hz, 1 H), 5.55 (s, 2 H), 5.49 (br, 2 H), 4.46 (m, 1 H), 4.28 (m, 1 H), 4.11 (m, 1 H), 3.96 (dd, J = 3.3 Hz, 11.4 Hz, 1 H), 3.78 (dd, J = 2.4 Hz, 11.1 Hz, 1 H), 0.95 (s, 18 H), 0.79 (s, 9 H), 0.16 (s, 3 H), 0.14 (s, 3 H), 0.13 (s, 3 H), 0.125 (s, 3 H), −0.038 (s, 3 H), −0.18 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 160.84, 159.35, 153.91, 137.40, 136.43, 128.25, 128.13, 127.80, 115.46, 87.13, 85.42, 76.57, 72.27, 67.80, 62.71, 26.02, 25.84, 25.61, 18.44, 18.04, 17.88, −4.29, −4.78, −4.80, −5.13, −5.44, −5.50; IR (neat) 3322, 3203, 1635, 1579 cm^{-1}; HRFABMS calcd for $C_{35}H_{62}N_5O_5Si_3$ (M + H)$^+$ 716.4059, found 716.4041.

6-*O*-Benzylguanosine (19). A stirred solution of compound **18** (700 mg, 0.98 mmol) in THF (15 mL) was treated with TBAF (1.28 g, 4.89 mmol). The reaction mixture was stirred at room temperature for 0.5 h and concentrated in vacuo. The residue was redissloved in ethyl acetate (100 mL) and was washed with saturated aqueous NH_4Cl (5 × 50 mL). The ethyl acetate layer was dried over sodium sulfate, filtered, and evaporated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 10:1) to give **19** (334 mg, 92%) as a white solid: mp 86−88 °C; ^{1}H NMR (10% CD_3OD in $CDCl_3$) δ 7.60 (s, 1 H), 7.49−7.45 (m, 2 H), 7.38−7.30 (m, 3 H), 5.70 (d, J = 7.5 Hz, 1 H), 5.59 (d, J = 12.6 Hz, 1 H), 5.44 (br, 1 H), 5.38 (d, J = 12.3 Hz, 1 H), 4.96 (dd, J = 5.4 Hz, 7.5 Hz, 1 H), 4.31 (d, J = 5.1 Hz, 1 H), 4.25 (s, 1 H), 3.92 (dd, J = 2.1 Hz, 12.9 Hz, 1 H), 3.72 (dd, J = 1.2 Hz, 12.6 Hz, 1 H); ^{13}C NMR (10% CD_3OD in $CDCl_3$) δ 160.52, 158.80, 151.62, 139.42, 135.73, 128.26, 127.96, 115.53, 91.02, 86.96, 72.59, 72.22, 68.12, 62.93; IR (KBr) 3357, 1616, 1591 cm^{-1}; HRFABMS calcd for $C_{17}H_{20}N_5O_5$ (M + H)$^+$ 374.1464, found 374.1480.

6-*O*-Benzyl-5′-deoxy-5′-*N*-(*N*-*tert*-butoxycarbonyl-*O*-benzyloxycarbonyl)-hydroxylaminoguanosine (20). Compound **20** was prepared by method A in THF as a clear oil in 91% yield: ^{1}H NMR ($CDCl_3$) δ 7.55 (s, 1 H), 7.52−7.49 (m, 2 H), 7.39−7.28 (m, 8 H), 5.76 (d, J = 5.7 Hz, 1 H), 5.47 (s, 2 H), 5.26 (br, 2 H), 5.17 (s, 2 H), 4.78 (t, J = 5.4 Hz, 1 H), 4.34−4.29 (m, 1 H), 4.23−4.20 (m, 1 H), 4.07−3.91 (m, 2 H), 1.34 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 160.36, 159.19, 155.03, 154.43, 152.56, 138.28, 136.14, 134.17, 128.82, 128.57, 128.47, 128.21, 128.08, 115.16, 90.02, 83.36, 82.04, 73.24, 72.30, 71.13, 68.23, 51.69, 27.81; IR (neat) 3359, 1786, 1717, 1616, 1590, 1259, 1219 cm^{-1}; HRFABMS calcd for $C_{30}H_{35}N_6O_9$ (M + H)$^+$ 623.2466, found 623.2465.

5′-Deoxy-5′-*N*-(*N*-*tert*-butoxycarbonyl)hydroxylaminoadenosine (21). Compound **21** was prepared by method B as a clear oil in 95% yield: ^{1}H NMR (10% CD_3OD in $CDCl_3$) δ 8.21 (s, 1 H), 7.94 (s, 1 H), 5.76 (d, J = 6.0 Hz, 1 H), 4.55 (t, J = 5.7 Hz, 1 H), 4.32−4.25 (m, 2 H), 4.03 (dd, J = 3.3 Hz, 15.3 Hz, 1 H), 3.71 (dd, J = 4.2 Hz, 15.3 Hz, 1 H), 1.44 (s, 9 H); ^{13}C NMR (10% CD_3OD in $CDCl_3$) δ 157.11, 155.45, 152.30, 148.14, 140.12, 119.42, 89.15, 83.18, 81.70, 73.24, 71.28, 52.28, 27.97; IR (neat) 3335, 1652 cm^{-1}; HRFABMS calcd for $C_{15}H_{23}N_6O_6$ (M + H)$^+$ 383.1679, found 383.1666.

5′-Deoxy-5′-*N*-(*N*-*tert*-butoxycarbonyl)hydroxylaminouridine (22). Compound **22** was prepared by method B as a white solid in 96% yield. Mp 179 °C (dec); ^{1}H NMR (10% CD_3OD in $CDCl_3$) δ 7.66 (d, J = 7.8 Hz, 1 H), 5.83 (d, J = 4.5 Hz, 1 H), 5.73 (d, J = 8.1 Hz, 1 H), 4.22−4.18 (m, 2 H), 4.12 (m, 1 H), 3.88−3.76 (m, 2 H), 1.48 (s, 9 H); ^{13}C NMR (10% CD_3OD in $CDCl_3$) δ 164.09, 156.64, 150.42, 140.64, 101.44, 89.75, 81.00, 80.60, 73.15, 70.66, 51.53, 27.23; IR (neat) 3234, 1686 cm^{-1}; HRFABMS calcd for $C_{14}H_{22}N_3O_8$ (M + H)$^+$: 360.1407, found 360.1381.

5′-Deoxy-5′-*N*-(*N*-*tert*-butoxycarbonyl)hydroxylaminocytidine (23). Compound **23** was prepared by method B as a white solid in 94% yield: mp 174 °C (dec); ^{1}H NMR (20% CD_3OD in $CDCl_3$) δ 7.66 (d, J = 7.5 Hz, 1 H), 5.87 (d, J = 7.5 Hz, 1 H), 5.74 (d, J = 3.0 Hz, 1 H), 4.21 (m, 1 H), 4.12 (m, 1 H), 4.04 (m, 1 H), 3.80 (m, 2 H), 1.44 (s, 9 H); ^{13}C NMR (20% CD_3OD in $CDCl_3$) δ 165.51, 156.62, 156.36, 140.82, 94.48, 91.25, 81.34, 80.94, 73.94, 70.64, 51.48, 27.21; IR (KBr) 3344, 1651 cm^{-1}; HRFABMS calcd for $C_{14}H_{23}N_4O_7$ (M + H)$^+$ 359.1567, found 359.1558.

5′-Deoxy-5′-*N*-(*N*-*tert*-butoxycarbonyl)hydroxylaminoguanosine (24). Compound **24** was prepared by method B as a white solid in 88% yield: mp 205 °C (dec); ^{1}H NMR (DMSO-d_6) δ 10.69 (s, 1H), 9.39 (s, 1H), 7.89 (s, 1 H), 6.49 (s, 2 H), 5.68 (d, J = 6.0 Hz, 1 H), 5.47 (d, J = 5.1 Hz, 1 H), 5.21 (br, 1 H), 4.45 (m, 1 H), 4.10−4.05 (m, 2 H), 3.71−3.55 (m, 2 H), 1.33 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 156.77, 155.88, 153.70, 151.33, 135.77, 116.79, 86.35, 80.97, 79.73, 72.98, 71.36, 53.00, 27.95; IR (KBr) 3415, 1694 cm^{-1}; HRFABMS calcd for $C_{15}H_{23}N_6O_7$ (M + H)$^+$ 399.1628, found 399.1610.

5′-Deoxy-5′-*N*-hydroxylaminoadenosine (1). Compound **1** was prepared by method C as a white solid in 95% yield: mp 110 °C (dec); ^{1}H NMR (CD_3OD) δ 8.45 (s, 1 H), 8.39 (s, 1 H), 6.08 (d, J = 5.4 Hz, 1 H), 4.86 (t, J = 5.1 Hz, 1 H), 4.51−4.44 (m, 2 H), 3.87 (dd, J = 9.3 Hz, 13.5 Hz, 1 H), 3.60 (dd, J = 2.7 Hz, 12.9 Hz, 1 H); ^{13}C NMR (CD_3OD) δ 153.64, 150.17, 147.94, 144.20, 121.81, 91.73, 79.86, 74.58, 73.06, 54.11; IR (KBr) 3337, 1684 cm^{-1}; HRFABMS calcd for $C_{10}H_{15}N_6O_4$ (M + H)$^+$ 283.1155, found 283.1148.

5′-Deoxy-5′-*N*-hydroxylaminouridine (2). Compound **2** was prepared by method C as a white solid in quantitative yield: mp 143 °C (dec); ^{1}H NMR (CD_3OD) δ 7.81 (d, J = 8.1 Hz, 1 H), 5.83 (d, J = 5.1 Hz, 1 H), 5.74 (d, J = 8.1 Hz, 1 H), 4.35−4.32 (m, 1 H), 4.19 (m, 2 H), 3.22 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 163.06, 150.76, 141.40, 101.89, 87.94, 81.44, 72.52, 71.25, 55.76; IR (KBr) 3403, 1683 cm^{-1}; HRFABMS calcd for $C_9H_{14}N_3O_6$ (M + H)$^+$ 260.0883, found 260.0840.

5′-Deoxy-5′-*N*-hydroxylaminocytidine (3). Compound **3** was prepared by method C as a clear oil in 93% yield: ^{1}H NMR (CD_3OD) δ 8.06 (d, J = 8.1 Hz, 1 H), 6.21 (d, J = 7.8 Hz, 1 H), 5.77 (d, J = 4.2 Hz, 1 H), 4.45 (dd, J = 3.9 Hz, 5.7 Hz, 1 H), 4.40−4.33 (m, 1 H), 4.18 (t, J = 6.0 Hz, 1 H), 3.73 (dd, J = 9.9 Hz, 13.2 Hz, 1 H), 3.57 (dd, J = 2.4 Hz, 13.2 Hz, 1 H); ^{13}C NMR (CD_3OD) δ 161.29, 148.37, 147.98, 95.50, 95.22, 78.81, 74.13, 72.64, 54.17; IR (neat) 3117, 1679 cm^{-1}; HRFABMS calcd for $C_9H_{15}N_4O_5$ (M + H)$^+$ 259.1042, found 259.1025.

5′-Deoxy-5′-*N*-hydroxylaminoguanosine (4). Compound **4** was prepared by method C as a white solid in 96% yield: mp

154 °C (dec); ^{1}H NMR (CD_3OD) δ 9.28 (s, 1 H), 6.02 (d, J = 4.5 Hz, 1 H), 4.88 (t, J = 4.8 Hz, 1 H), 4.50 (ddd, J = 2.7 Hz, 4.8 Hz, 10.5 Hz, 1 H), 4.44 (t, J = 5.1 Hz, 1 H), 3.97 (dd, J = 10.2 Hz, 13.5 Hz, 1 H), 3.58 (dd, J = 2.4 Hz, 13.5 Hz, 1 H); ^{13}C NMR (CD_3OD) δ 157.24, 154.96, 150.95, 138.02, 109.98, 93.12, 79.78, 74.40, 72.97, 54.12; IR (KBr) 3341, 3129, 3001, 1715, 1644, 1605 cm^{-1}; HRFABMS calcd for $C_{10}H_{15}N_6O_5$ $(M + H)^+$ 299.1104, found 299.1113.

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Supporting Information Available: Copies of the ^{1}H and ^{13}C NMR spectra for compounds **9**−**11**, **13**−**16**, **18**−**24**, and **1**−**4** and the ^{31}P NMR spectra for compounds **13** and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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