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Synthesis of 2′-*N*-Methylamino-2′-deoxyguanosine and 2′-*N*,*N*-Dimethylamino-2′-deoxyguanosine and Their Incorporation into RNA by Phosphoramidite Chemistry

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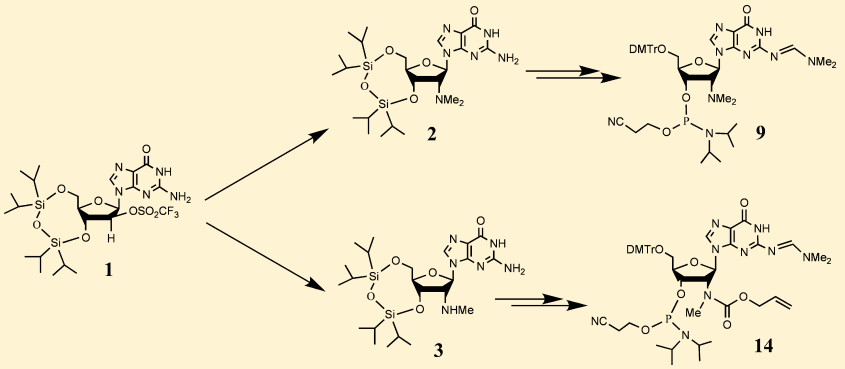
Supporting Information



ABSTRACT: The 2′-hydroxyl groups within RNA contribute in essential ways to RNA structure and function. Previously, we designed an atomic mutation cycle (AMC) that uses ribonucleoside analogues bearing different C-2′-substituents, including $-OCH_3$, $-NH_2$, $-NHMe$, and $-NMe_2$, to identify hydroxyl groups within RNA that donate functionally significant hydrogen bonds. To enable AMC analysis of the nucleophilic guanosine cofactor in the *Tetrahymena* ribozyme reaction and at other guanosines whose 2′-hydroxyl groups impart critical functional contributions, we describe here the syntheses of 2′-methylamino-2′-deoxyguanosine (G_{NHMe}) and 2′-*N*,*N*-dimethylamino-2′-deoxyguanosine (G_{NMe_2}) and their corresponding phosphoramidites. The key step in obtaining the nucleosides involved S_N2 displacement of 2′-β-triflate from an appropriate guanosine derivative by methylamine or dimethylamine. We readily obtained the G_{NMe_2} phosphoramidite and incorporated it into RNA. However, the G_{NHMe} phosphoramidite posed a significantly greater challenge due to lack of a suitable $-$2′-NHMe protecting group. After testing several strategies, we established that allyloxycarbonyl (Alloc) provided suitable protection for 2′-*N*-methylamino group during the phosphoramidite synthesis and the subsequent RNA synthesis. This work enables AMC analysis of guanosine's 2′-hydroxyl group within RNA.

INTRODUCTION

Folded RNAs rely upon their 2′-OH groups to confer stability, often through hydrogen-bond interactions.[1] For many catalytic RNAs, including the group I and II introns, the ribosome, and the spliceosome, multiple 2′-OH groups on the substrates and ribozyme make important energetic contributions to function as 2′-H substitution at these positions results in a substantial loss of activity.[2] However, defining the physiochemical role of these relevant 2′-OH groups poses significant challenges and frequently requires the design and synthesis of chemically modified nucleosides in conjunction with their application in functionally meaningful ways.[3]

To investigate hydrogen-bond donation by 2′-OH groups within RNA, we previously developed atomic mutation cycle (AMC) analysis (Figure 1).[4] This approach requires the synthesis and functional characterization of three analogues bearing modifications at the 2′-position: $-OCH_3$, $-NH_2$, and $-$NHMe. When the energetic penalty for the 2′-OH to 2′-OCH_3 substitution ($\Delta\Delta G_{OH\rightarrow OCH_3}$) exceeds that for the 2′-NH_2 to 2′-$NHCH_3$ substitution ($\Delta\Delta G_{NH_2\rightarrow NHCH_3}$), we attribute the difference to the absence of a hydrogen atom ($\Delta G_{H\ removal}$) on the 2′-OCH_3 analogue and infer that the 2′-hydroxyl group under investigation imparts function by donating a hydrogen bond. A nucleoside bearing a 2′-$N(CH_3)_2$ substitution enables a further test of this conclusion, based on the prediction that the absence of a hydrogen atom on the 2′-amine would engender a greater energetic penalty than the 2′-$NHCH_3$ derivative.

As part of our effort to conduct AMC analysis on the guanosine nucleophile 2′-hydroxyl group in the *Tetrahymena* ribozyme reaction,[5] we herein describe the syntheses of 2′-*N*-methylamino-2′-deoxyguanosine and 2′-*N*,*N*-dimethylamino-2′-

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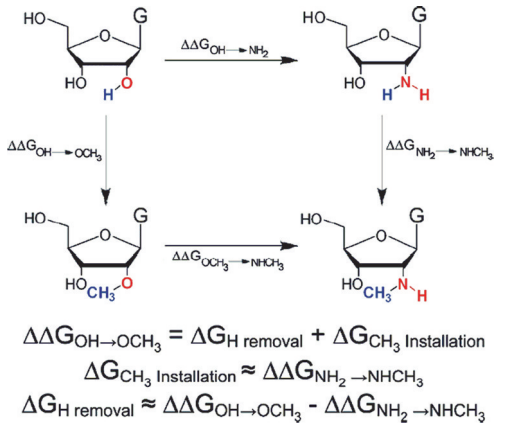


Figure 1. Atomic mutation cycle for analysis of the guanosine nucleophile 2′-hydroxyl.

deoxyguanosine (herein referred to as G_{NHMe} and G_{NMe_2}, respectively) and their incorporation into RNA. The availability of these two novel nucleosides expands the arsenal of modified nucleosides to better understand the role of the RNA's 2′-OH group.

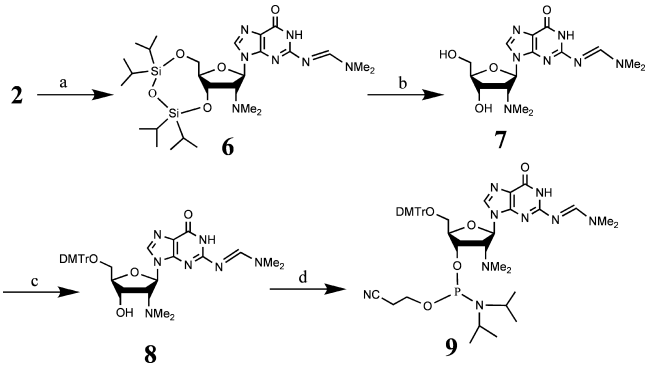
RESULT AND DISCUSSION

1. Syntheses of 2′-*N*,*N*-Dimethylamino-2′-deoxyguanosine (4) and 2′-*N*-Methylamino-2′-deoxyguanosine (5). Unlike 2′-*N*-amino-2′-deoxyguanosine (G_{NH_2}) and its phosphoramidites, whose syntheses and incorporation into oligonucleotides are well documented,[6] there are no reports describing the syntheses of G_{NHMe} and G_{NMe_2} and their phosphoramidites. Since G_{NH_2} has been successfully synthesized from 2′-*N*-trifluoroacetylamido-2′-deoxyuridine by transglycosylation,[6a] we first attempted to synthesize G_{NHMe} in an analogous manner. Thus, 2′-*N*-methyl-*N*-trifluoroacetylamido-2′-deoxyuridine was synthesized from 2′-amino-2′-deoxyuridine by trifluoroacetylation followed by methylation with methyl iodide. However, subsequent transglycosylation with N^2-palmitoylguanine in the presence of BSA and TMSOTf afforded only a byproduct, 2′-*N*-methylamino-2′-deoxyuridine, indicating loss of the 2′-*N*-trifluoroacetyl (TFA) protecting group. Premature deacylation from 2′-*N*-methyl-*N*-trifluoroacetylamido-2′-deoxyuridine but not 2′-*N*-trifluoroacetylamido-2′-deoxyuridine under the same reaction conditions suggests that the presence of the *N*-methyl group increases the liability of the trifluoroacetamide derivative.

As an alternative, we turned to our previously reported strategy to prepare substituted guanosine derivatives by S_N2 reaction from the 2′-*O*-triflate derivative **1**[7] (Scheme 1). Treatment of **1** with dimethylamine (2 M in THF) or methylamine (2 M in THF) overnight at 60 °C in a sealed pressure tube generated the corresponding silyl-protected 2′-*N*,*N*-dimethylamino-2′-deoxyguanosine (**2**) and 2′-*N*-methylamino-2′-deoxyguanosine (**3**) in 55% and 60% yield, respectively. In both cases, we observed byproduct resulting from elimination. Treatment of **2** and **3** with ammonium fluoride in MeOH gave free nucleosides **4** and **5**, respectively, which were purified by reversed-phase column chromatography eluting with water.

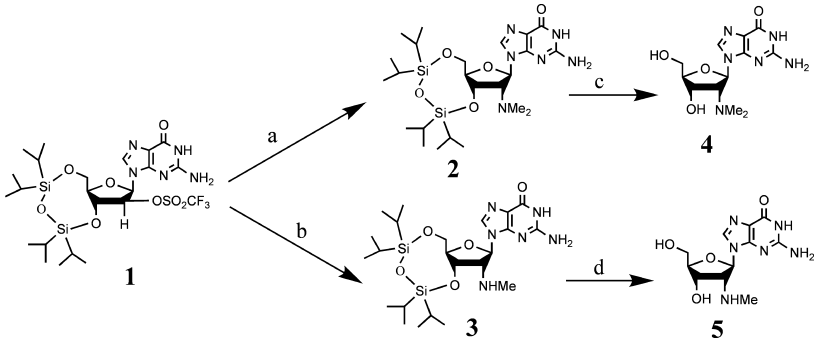
2. Synthesis of the Phosphoramidite of 2′-*N*,*N*-Dimethylamino-2′-deoxyguanosine and Its Incorporation into RNA. We proceeded with synthesis of the phosphoramidite for G_{NMe_2} (**4**) without protection of the tertiary amine (Scheme 2). Treatment of **2** with DMF−DMA in methanol to protect the exocyclic amino group generated intermediate **6** in 88% yield. Removal of the 3′,5′-silyl protecting group with TBAF provided intermediate **7** in 78% yield. 4,4′-Dimethoxyltritylation of **7** gave **8** in 86% yield and

Scheme 2. Synthesis of the Phosphoramidite of 2′-*N*,*N*-Dimethylamino-2′-deoxyguanosine (9)[a]



[a]Key: (a) DMF−DMA, MeOH, 88%; (b) 1.0 M TBAF in THF, 78%; (c) DMTr-Cl, Py, 86%;(d) $(i\text{-Pr})_2NP(Cl)OCH_2CH_2CN$, $(i\text{-Pr})_2NEt/CH_2Cl_2$, 82%.

Scheme 1. Syntheses of 2′-*N*,*N*-Dimethylamino-2′-deoxyguanosine (4) and 2′-*N*-Methylamino-2′-deoxyguanosine (5)[a]



[a]Key: (a) 2 M Me_2NH in THF, 60 °C, 55%; (b) 2 M $MeNH_2$ in THF, 60 °C, 60%; (c) 0.5 M NH_4F in MeOH, 60 °C, 74%; (d) 0.5 M NH_4F in MeOH, 60 °C, 72%.

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subsequent phosphitylation of **8** under standard conditions produced phosphoramidite **9** in 82% yield. Incorporation of **9** into an RNA sequence CUCG$_m$A (G_m = G_{NMe_2}) by solid-phase synthesis occurred as efficiently as that of the commercial guanosine phosphoramidite. After standard deprotection and reversed-phase HPLC purification, the structure of the oligonucleotide was confirmed by MALDI-TOF MS ([M − H]$^-$ = 1554).

3. Synthesis of the Phosphoramidite for 2′-*N*-Methyl-*N*-phenoxyacetylamido-2′-deoxyguanosine and Its Incorporation into an Oligonucleotide (14). In contrast to the phosphoramidite **9**, the synthesis of a phosphoramidite suitable for G_{NHMe} incorporation into RNA presented significant challenges because of the need to establish an appropriate protecting group for the 2′-methylamino group (2′-NHMe). For the synthesis of the phosphoramidite of G_{NH_2}, Eckstein et al. protected the 2′-amino group (2′-NH_2) as a trifluoroacetamide (TFA), which remains stable during phosphoramidite and oligonucleotide synthesis but releases the free amine after oligonucleotide synthesis upon exposure to ethanolic ammonia.[8] Intending to follow the analogous strategy for G_{NHMe}, we generated the trifluoroacetamide derivative of **3** (Figure 2) by treatment with 1-(trifluoroacetyl)imidazole[9] in

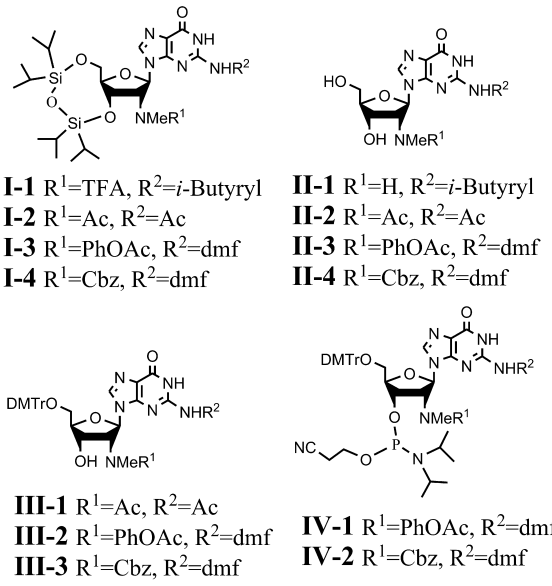

Figure 2. Structures of some intermediates with TFA, Ac, PhOAc, or Cbz as 2′-NHMe protecting group.

pyridine followed by protection of the exocyclic amino group as the isobutyrylamide to give **I-1** in 75% yield as a 1:5 mixture of isomers (*cis*- and *trans*-2′-deoxy-2′-*N*-methylamides).[10] However, attempts to remove the silyl protecting group from **I-1** with TBAF or ammonium fluoride also removed the TFA protecting group to yield only byproduct **II-1** (Figure 2). In contrast, the corresponding derivative of G_{NH_2}, which lacks the methyl group, retains the TFA group under the same desilylation conditions. The greater liability of the *N*-methyltrifluoracetamide parallels our observations from the transglycosylation reaction noted above.

As an alternative protection strategy for the methylamino group, we considered acetylation. Acetylation is less useful for protection of 2′-aminonucleosides because postsynthetic deacetylation from the 2′-amine occurs too slowly.[11] We anticipated that the greater stability of the acetamide relative to the trifluoracetamide could balance the liability conferred by the presence of the methyl group, allowing the *N*-methylacetamide to remain intact during solid-phase synthesis but undergo postsynthetic deacetylation smoothly. To test this hypothesis, we treated **3** with excess acetyl anhydride in pyridine and isolated a bisacetamide **I-2** bearing an acetyl group on both 2′-NHMe and exocyclic amine (Figure 2). TBAF treatment removed the 3′,5′-silyl protecting group readily while retaining the acetyl groups to generate **II-2**. When **II-2** was treated with ammonium hydroxide at 55 °C, the acetyl group on the exocyclic amino group was readily removed within 2 h, but the removal of the acetyl group on the 2′-methylamino required 48 h to reach completion. Subsequent 4,4′-dimethoxytritylation of the 5′-OH afforded **III-1** (Figure 2), but its polar character and poor solubility in dicholoromethane made the corresponding phosphoramidite difficult to isolate. We abandoned this strategy and turned our attention the phenoxyacetyl group (PhOAc).

Two features of PhOAc protection made it seem attractive: (1) the electron-withdrawing character of the phenoxy group causes the PhOAc ester to undergo hydrolysis 50 times faster than the corresponding Ac ester;[12] (2) the large, hydrophobic phenyl ring could reduce the polarity and thus improve the solubility of the DMTr derivative in dichloromethane, thereby facilitating synthesis of the phosphoramidite.

PhOAc has been used to protect the exocyclic amines of adenosine and guanosine in phosphoramidite chemistry[13] but not the 2′-amino group. To test the suitability of PhOAc as a protecting group for the 2′-amine, we first treated **5** with DMF−DMA in methanol to protect the exocyclic amine with dimethylaminomethylene group. Subsequent treatment with phenoxyacetyl chloride in pyridine generated **I-3** (Figure 2). Desilylation with TBAF gave **II-3**. Unlike the *N*-methyltrifluoroacetamide, **II-3**, the 2′-*N*-methylphenoxyacetamide remained intact during this treatment. To test whether the ethanolic ammonia treatment that follows oligonucleotide synthesis would remove the PhOAc, we performed a control reaction by treating **II-3** with ethanolic ammonia at 55 °C. Both dimethylaminomethylene and PhOAc groups were cleanly removed within 4 h to give **5**. Thus, we prepared the corresponding phosphoramidite **IV-1** by treating **II-3** with DMTr-Cl in pyridine to generate **III-2** followed by 3′-*O*-phosphitylation (Figure 2). Under standard coupling conditions, phosphoramidite **IV-1** was incorporated into a RNA sequence CUCGmA as efficiently as commercial guanosine phosphoramidite. After ethanolic ammonia (55 °C, 4 h) and fluoride treatment, we purified the modified oligonucleotide by HPLC. MALDI-TOF MS analysis ([M − H]$^-$ = 1674) indicated that the oligonucleotide retained the PhOAc group, however.

We suspected that loss of the PhOAc group from the nucleoside but not the oligonucleotide upon ethanolic ammonia treatment might reflect participation of the free 3′-OH (Figure 3). Possibly the 3′-OH group attacks the carbonyl directly to form a five-membered-ring tetrahedral intermediate. Subsequent expulsion of the nitrogen would generate the 3′-*O*-acetyl ester, which would undergo deacylation. The absence of a free 3′-OH in the oligonucleotide may allow the PhOAc group to survive ethanolic ammonia treatment. We also attempted to remove the PhOAc group using harsher conditions (stronger base methylamine at 65 °C, 4 h), but

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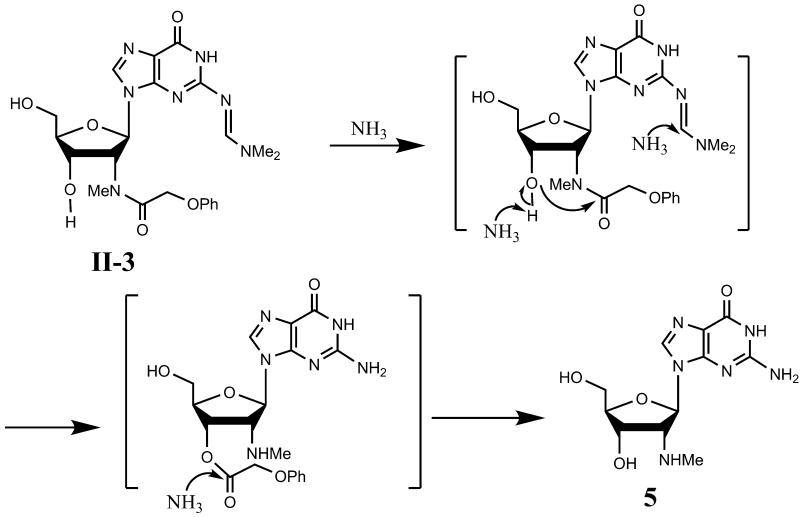
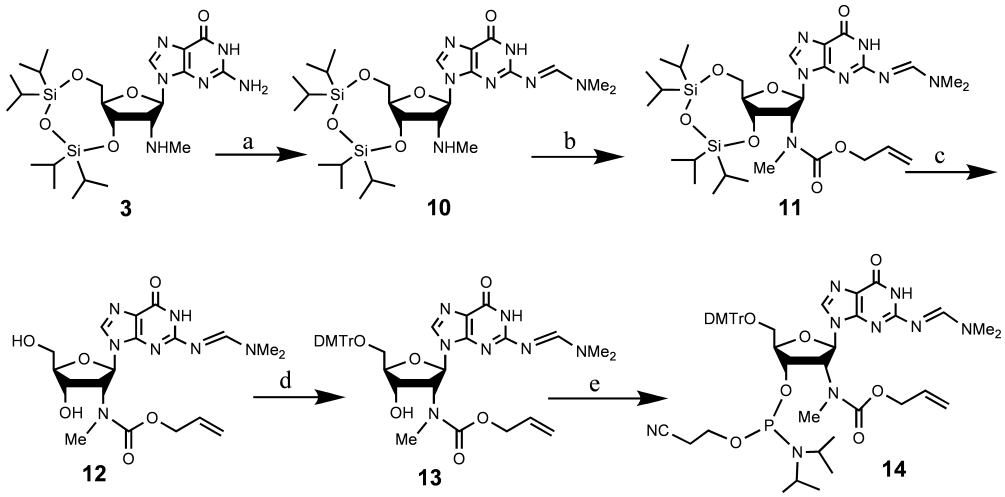


Figure 3. Possible mechanism for removal of PhOAc from **II-3**.

Scheme 3. Synthesis of the 2′-*N*-Methyl-*N*-allyloxycarbonylamido-2′-deoxyguanosine Phosphoramidite (14)[a]



[a]Key: (a) DMF−DMA, MeOH, 88%; (b) allyloxycarbonyloxybenzotriazolyl, *i*-Pr_2NEt, THF, 86%; (c) TBAF, THF, 84%; (d) DMTr-Cl, Py, 81%; (e) $(i\text{-Pr})_2$NP(Cl)OCH_2CH_2CN, $(i\text{-Pr})_2$NEt, 1-methylimidazole, CH_2Cl_2, 78%.

MALDI TOF MS indicated that no desired deprotected oligonucleotide formed.

4. Synthesis of 2′-*N*-Methyl-*N*-benzyloxycarbonylamido- and 2′-*N*-Methyl-*N*-alloxycarbonylamido-2′-deoxyguanosine Phosphoramidites (IV-2 and 14). Having encountered problems with each of the amide protecting groups (TFA, Ac, and PhOAc), we decided to test carbamate protecting groups for 2′-NHMe, which utilize hydrogenation for removal. Both benzyloxycarbonyl (Cbz)[14] and allyloxycarbonyl (Alloc)[15] groups have been used for protection of the exocyclic amines of the nucleobases in phosphoramidites of dA, dG, and dC. Following DNA synthesis and cleavage of the oligonucleotide from the solid support by ammonium hydroxide treatment, the Cbz groups are removed by Pd−C-catalyzed hydrogenation while Alloc groups are removed using a soluble palladium catalyst.

Treatment of **3** with DMF−DMA in methanol gave intermediate **10**, which was converted to **11** and **I-4** by treatment with allyloxycarbonyloxybenzotriazolyl[16] or benzyloxycarbonyl chloride in pyridine, respectively (Scheme 3 and Figure 2). TBAF treatment removed the silyl protecting groups without affecting Cbz or Alloc groups to give **12** and **II-4**, which were converted to the corresponding phosphoramidites **14** and **IV-2**, respectively, using standard tritylation and phosphitylation reactions (Scheme 3 and Figure 2).

During solid-phase synthesis of the RNA sequence $CUCG_mA$ ($G_m = G_{NHMe}$), **14** and **IV-2** coupled under standard conditions as efficiently as commercial guanosine phosphoramidite. However, attempts to remove the Cbz-protecting group from the oligonucleotide by Pd-C-catalyzed hydrogenation failed either with the oligonucleotide attached to the CPG or removed from the CPG (with 2′-TBDMS groups still present or removed).[17]

In contrast to Cbz, removal of Alloc groups on the nucleobases requires only a solution-phase catalyst. For example, Hayakawa et al. reported that tris(dibenzylideneacetone)dipalladium(0)−chloroform complex $[Pd_2(dba)_3{-}CHCl_3]$ catalyzes removal of Alloc protecting groups from CPG-supported DNA oligonucleotides (32−60mer).[18] We treated 5′-$CUCG_mA$-CPG similarly followed by standard ethanolic ammonia and fluoride. MS analysis of the resulting oligonucleotide showed only partial removal of the

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Alloc group (~50%). We tested other conditions and identified two ways to remove the Alloc group more effectively: $Pd(Ph_3P)_4$ in the presence of HCO_2H/Et_3N[19] or $Pd(Ph_3P)_4$ in the presence of AcOH and Bu_3SnH.[20] Both conditions removed the Alloc group completely to allow access to the desired oligonucleotide.[21]

SUMMARY AND IMPLICATIONS

We have synthesized two guanosine analogues, G_{NHMe} and G_{NMe_2}, with the key reaction involving S_N2 displacement of suitably protected 2′-β-triflate derivatives of guanosine with methylamine and dimethylamine, respectively. The tertiary amine of G_{NMe_2} required no protection, allowing straightforward synthesis of the phosphoramidite. The synthesis of the corresponding G_{NHMe} phosphoramidite proved to be more challenging because of the need to identify a suitable protecting group for the 2′-*N*-methylamino group. After testing several strategies, we established that the allyloxycarbonyl moiety provided suitable protection for the 2′-*N*-methylamino group, being easily introduced, stable during the phosphoramidite preparation and subsequent RNA synthesis, and readily removed from the synthetic oligonucleotide by reduction using a soluble palladium catalyst. Both G_{NMe_2} and G_{NHMe} phosphoramidites enabled successful synthesis of oligonucleotides containing these analogues.

The availability of G_{NHMe} and G_{NMe_2}, in conjunction with the other guanosine analogues required for AMC analysis (Figure 1), permits further investigation of functionally important guanosine 2′-hydroxyl groups within structured RNAs. Additionally, G_{NHMe} and G_{NMe_2} may serve as useful analogues to define the functional contribution of hydroxyl groups by application of Quantitative Structure Activity Relationship (QSAR) analysis[2g] or to evaluate the packing density around individual hydroxyl groups.[4a,22]

EXPERIMENTAL SECTION

2′-Dimethylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyguanosine (2). To a pressure tube (35 mL) under argon was added 2′-deoxy-2′-β-triflate-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)guanosine[11] (**1**, 623 mg, 0.95 mmol) and dimethylamine in THF (2 M, 20 mL). The mixture was stirred overnight at 60 °C. After being cooled to rt, the reaction mixture was concentrated to dryness under reduced pressure. The residue was purified by silica gel chromatography, eluting with 5−7% methanol in dichloromethane, to give **2** (287 mg, 55%) as a white solid. ^{1}H NMR (500.1 MHz) (DMSO-d_6) δ: 10.58 (br 1H), 7.78 (s, 1H), 6.38 (br, 2H), 5.84 (d, J = 6.0 Hz, 1H), 4.56 (m, 1H), 3.90 (m, 1H), 3.80 (m, 2H), 3.54 (m, 1H), 2.35 (s, 6H), 0.85−0.97 (m, 28H). ^{13}C NMR (125.8 MHz) (DMSO-d_6) δ: 158.1, 155.3, 152.3, 136.5, 118.0, 85.6, 83.8, 75.0, 69.4, 64.5, 43.9, 18.8, 18.67, 18.65, 18.61, 18.43, 18.41, 18.35, 18.34. HRMS: calcd for $C_{24}H_{45}N_6O_5Si_2$ $[MH]^+$ 553.2990, found 553.2967.

2′-Methylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyguanosine (3). To a pressure tube (35 mL) under argon was added 2′-deoxy-2′-β-triflate-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-guanosine (**1**, 580 mg, 0.88 mmol) and methylamine in THF (2 M, 18 mL). The mixture was stirred overnight at 60 °C. After cooling to rt, the reaction mixture was concentrated to dryness under reduced pressure. The residue was purified by silica gel chromatography, eluting with 5−8% methanol in dichloromethane, to give **3** (284 mg, 60%) as white solid. ^{1}H NMR (500.1 MHz) (DMSO-d_6) δ: 10.55 (br 1H), 7.71 (s, 1H), 6.34 (br, 2H), 5.52 (d, J = 3.5 Hz, 1H), 4.44 (m, 1H), 3.90 (m, 1H), 3.81 (m, 1H), 3.26 (m, 2H), 2.30 (s, 3H), 0.90−0.99 (m, 28H). ^{13}C NMR (125.8 MHz) (DMSO-d_6) δ: 158.1, 155.2, 152.2, 135.9, 118.1, 87.2, 84.2, 71.2, 67.3, 63.2, 36.2, 18.8, 18.64, 18.60, 18.58, 18.4, 18.3, 18.25, 18.24, 14.2, 14.1, 14.0, 13.9. HRMS: calcd for $C_{23}H_{43}N_6O_5Si_2$ $[MH]^+$ 539.2834, found 539.2811.

2′-*N*,*N*-Dimethylamino-2′-deoxyguanosine (4). To a solution of 2′-dimethylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyguanosine (**2**, 110 mg, 0.2 mmol) in MeOH (8 mL) was added ammonium fluoride (0.5 M solution in MeOH, 0.1 mL) under argon. The mixture was heated to 60 °C for 16 h. After removing the solvent under reduced pressure, the residue was coevaporated with deionized water (5 × 30 mL). The residue was purified by C18 reversed-phase column chromatography, eluting with water to give **4** (46 mg, 74%) as a white solid. ^{1}H NMR (500.1 MHz) (DMSO-d_6) δ: 10.75 (br, 1H), 8.08 (s, 1H), 6.60 (br, 2H), 6.05 (d, J = 7.8 Hz, 1H), 5.16 (br, 2H), 4.33 (m, 1H), 3.96 (m, 1H), 3.59 (m, 2H), 3.32 (m, 1H), 2.22 (s, 6H). ^{13}C NMR (125.8 MHz) (DMSO-d_6) δ: 157.1, 154.2, 151.4, 135.8, 116.7, 87.0, 84.0, 72.0, 70.4, 62.2, 43.7. HRMS: calcd for $C_{12}H_{19}N_6O_4$ $[MH]^+$ 311.1468, found 311.1480.

2′-*N*-Methylamino-2′-deoxyguanosine (5). To a solution of 2′-methylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyguanosine (**3**, 82 mg, 0.17 mmol) in MeOH (8 mL) was added ammonium fluoride (0.5 M solution in MeOH, 0.1 mL) under argon. The mixture was heated to 60 °C for 16 h. After removing the solvent under reduced pressure, the residue was coevaporated with deionized water (5 × 30 mL). The residue was purified by C18 reversed-phase column chromatography, eluting with water to give **5** (36.5 mg, 72%) as a white solid. ^{1}H NMR (500.1 MHz) (DMSO-d_6) δ: 10.96 (br, 1H), 7.92 (s, 1H), 6.72 (br, 2H), 5.55 (d, J = 7.5 Hz, 1H), 5.52 (br, 1H), 5.12 (br, 1H), 4.38 (m, 1H), 3.91 (m, 1H), 3.54 (m, 2H), 3.46 (m, 1H), 2.24 (s, 3H). ^{13}C NMR (125.8 MHz) (DMSO-d_6) δ: 157.2, 154.4, 151.7, 135.8, 117.0, 86.9, 85.9, 68.8, 66.1, 62.0, 34.9. HRMS: calcd for $C_{11}H_{17}N_6O_4$, $[MH]^+$ 297.1311, found 297.1302.

2′-Dimethylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*N*^{2}-dimethylaminomethylene-2′-deoxyguanosine (6). To a solution of 2′-dimethylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyguanosine (**2**, 242 mg, 0.44 mmol) in methanol (10 mL) was added DMF-DMA (1 mL). After stirring at rt for 3 h, all the volatile components were removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with 3−5% methanol in dichloromethane, to give **6** (234 mg, 88%) as white solid. ^{1}H NMR (500.1 MHz) ($CDCl_3$) δ: 10.27 (s, 1H), 8.48 (s, 1H), 7.83 (s, 1H), 6.17 (s, 1H), 4.74 (m, 1H), 4.23 (m, 1H), 4.06 (s, 1H), 3.39 (m, 1H), 3.17 (s, 3H), 3.05 (s, 3H), 2.65 (s, 6H), 1.06 (m, 28H). ^{13}C NMR (125.8 MHz) ($CDCl_3$) δ: 158.2, 158.0, 156.9, 149.6, 135.4, 120.2, 84.9, 83.5, 72.4, 70.3, 61.9, 43.3, 41.4, 35.0, 17.4, 17.29, 17.26, 17.22, 17.0, 16.94, 16.88, 13.3, 13.02, 12.96, 12.5. HRMS: calcd for $C_{27}H_{50}N_7O_5Si_2$ $[MH]^+$ 608.3412, found 608.3414.

2′-Dimethylamino-*N*^{2}-dimethylaminomethylene-2′-deoxyguanosine (7). To a solution of 2′-dimethylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*N*^{2}-dimethylaminomethylene-2′-deoxyguanosine (**6**) (220 mg, 0.36 mmol) in THF (10 mL) was added TBAF (1 M, 72 μL), and the mixture was stirred at rt for 15 min. All the volatile components were removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with 0−12% methanol in dichloromethane, to give **7** (103 mg, 78%) as white solid. ^{1}H NMR (500.1 MHz) (MeOD) δ: 8.65 (s, 1H), 8.22 (s, 1H), 6.26 (d, J = 8.5 Hz, 1H), 4.42 (d, J = 5.0 Hz, 1H), 4.10 (t, J = 5.0 Hz, 1H), 3.73 (m, 2H), 3.44 (m, 1H), 3.19 (s, 3H), 3.10 (s, 3H), 2.21 (s, 6H). ^{13}C NMR (125.8 MHz) (MeOD) δ: 158.6, 158.4, 157.9, 150.4, 137.5, 119.0, 87.2, 85.5, 72.2, 71.5, 62.2, 42.9, 40.0, 33.8. HRMS: calcd for $C_{15}H_{24}N_7O_4$, $[MH]^+$ 366.1890, found 366.1899.

2′-Dimethylamino-5′-*O*-4,4′-dimethoxytrityl-*N*^{2}-dimethylaminomethylene-2′-deoxyguanosine (8). 2′-Dimethylamino-*N*^{2}-dimethylaminomethylene-2′-deoxyguanosine (**7**, 88 mg, 0.24 mmol) was dissolved in pyridine (3 mL), and 4,4′-dimethoxytrityl chloride (98 mg, 0.29 mmol, 1.2 equiv) was added while stirring the solution. After being stirred overnight at rt, the reaction mixture was diluted with MeOH (1 mL) and stirred for an additional 5 min. The reaction mixture was concentrated to dryness under vacuum. Water was added to the resulting residue, and the mixture was extracted with CH_2Cl_2. The organic phase was washed consecutively with 5% sodium bicarbonate, water, and brine and then dried over sodium sulfate.

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After the organic phase was concentrated to dryness, the residue was purified by silica gel chromatography, eluting with 4% MeOH in CH_2Cl_2 containing 0.2% Et_3N, to give **8** (143 mg, 86%) as white foam. ^1H NMR (500 MHz) (CD_3CN) δ: 10.05 (br, 1H), 8.65 (s, 1H), 7.76 (s, 1H), 7.51 (d, J = 7.5 Hz, 2H), 7.31−7.46 (m, 7H), 6.91 (m, 4H), 6.27 (d, J = 8.5 Hz, 1H), 4.38 (d, J = 5.0 Hz, 1H), 4.21 (m, 1H), 3.82 (s, 6H), 3.46 (m, 2H), 3.28 (m, 1H), 3.13 (s, 3H), 3.08 (s, 3H), 2.26 (s, 6H). ^{13}C NMR (125.8 MHz) (CD_3CN) δ: 158.6, 158.4, 157.9, 157.6, 150.3, 144.9, 135.9, 135.7, 135.6, 130.0, 127.9, 127.8, 126.8, 119.8, 113.0, 86.3, 84.8, 84.2, 71.7, 71.0, 64.4, 54.8, 43.1, 40.6, 34.3. HRMS: calcd for $C_{36}H_{41}N_7O_6Na$ $[MNa]^+$ 690.3011, found 690.3022.

2′-Dimethylamino-5′-*O*-4,4′-dimethoxytrityl-N^2-dimethylaminomethylene-2′-deoxyguanosine 3′-*O*-(2-Cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (9). 2′-Dimethylamino-5′-*O*-4,4′-dimethoxytrityl-N^2-dimethylaminomethylene-2′-deoxyguanosine (**8**) (120 mg, 0.17 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and 1-methylimidozale (2.90 mg, 35.0 μmol). *N*,*N*-Diisopropylethylamine (126 mg, 0.68 mmol) was added to the stirring solution followed by 2-cyanoethyl *N*,*N*-(diisopropylchloro)phosphoramidite (2.0 equiv). After being stirred at room temperature for 1 h, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 5% $NaHCO_3$ and brine. After dried over sodium sulfate and filtered, the filtrate was concentrated to dryness. The residue was purified by silica gel chromatography, eluting with 12% acetone in CH_2Cl_2 containing 0.2% Et_3N, to give **9** (115 mg, 78%) as a colorless oil. ^1H NMR (500 MHz) (CD_3CN) δ: 10.03 (br, 1H), 8.62 (m, 1H), 7.78 (m, 1H), 7.50 (m, 2H), 7.35−7.40 (m, 7H), 6.91 (m, 4H), 6.36 (m, 1H), 4.92 (m, 1H), 4.01−4.42 (m, 2H), 3.82 (m, 6H), 3.55 (m, 2H), 3.50 (m, 1H), 3.24 (m, 1H), 3.09−3.06 (m, 6H), 2.78 (m, 1H), 2.72 (m, 1H), 2.32 (m, 6H), 1.30 (m, 12H). ^{31}P NMR (202.4 MHz) (CD_3CN) δ: 141.3, 141.5. HRMS: calcd for $C_{45}H_{59}N_9O_7P$ $[MH]^+$ 868.4275, found 868.4268.

2′-Methylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (10). To a solution of 2′-methylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyguanosine (**3**, 264 mg, 0.50 mmol) in methanol (10 mL) was added DMF−DMA (1 mL). After being stirred overnight at rt, all the volatile components were removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with 5% methanol in dichloromethane, to give **10** (234 mg, 88%) as white solid. ^1H NMR (500.1 MHz) ($CDCl_3$) δ: 9.94 (br, 1H), 8.63 (s, 1H), 7.94 (s, 1H), 6.02 (s, 1H), 4.65 (m, 2H), 4.20 (m, 1H), 4.08 (m, 1H), 3.23 (r. 1H), 3.20 (s, 3H), 3.14 (s, 3H), 2.70 (s, 3H), 1.02−1.16 (m, 28H). ^{13}C NMR (125.8 MHz) ($CDCl_3$) δ: 158.0, 158.0, 156.7, 149.2, 135.5, 120.6, 86.9, 82.3, 68.7, 67.8, 60.6, 41.4, 35.1, 17.4, 17.3, 17.2, 17.0, 16.95, 16.88, 16.8, 13.3, 12.91, 12.86, 12.47. HRMS: calcd for $C_{26}H_{47}N_7O_5Si_2$ $[MH]^+$ 594.3272, found 594.3256.

2′-*N*-Methyl-*N*-alloxycarbonyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (11). To a solution of 2′-methylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**10**, 220 mg, 0.37 mmol) in THF (10 mL) was added allyloxycarbonyloxybenzotriazolyl (122 mg, 1.5 equiv) and *N*,*N*-diisopropylethylamine (0.2 mL). The mixture was stirred at rt for 1 h, and MeOH (0.5 mL) was added to quench the reaction. All of the volatile components were removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with 4% methanol in dichloromethane, to give **11** (216 mg, 86%). ^1H NMR (500.1 MHz) ($CDCl_3$) δ: 9.75 (br, 1H), 8.61 (s, 0.75H), 8.50 (s, 0.25H), 8.04 (s, 1H), 6.16 (m, 1H), 5.86 (m, 0.75H), 5.75 (m, 0.25H), 5.42 (m, 0.75H), 5.26 (m, 0.25H), 5.15 (m, 2H), 4.62 (m, 2H), 4.57 (m, 1H), 4.09 (m, 2H), 3.82 (m, 0.25H), 3.80 (m, 0.75), 3.17 (s, 2.25H), 3.12 (s, 0.75H), 3.07 (s, 6H), 0.93−1.03 (m, 28H). HRMS: calcd for $C_{30}H_{51}N_7O_7Si_2$ $[MH]^+$ 678.3467, found 678.3458.

2′-*N*-Methyl-*N*-alloxycarbonyl-N^2-dimethylaminomethylene-2′-deoxyguanosine (12). To a solution of 2′-*N*-methyl-*N*-alloxycarbonyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**11**) (190 mg, 0.28 mmol) in THF (10 mL) was added TBAF (1 M, 0.28 mL), and the mixture was stirred at rt for 15 min. All of the volatile components were removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with 0−10% methanol in dichloromethane, to give **12** (112 mg, 92%) as a white solid. ^1H NMR (500.1 MHz) (DMSO-d_6) δ: 11.35 (br, 1H), 8.55 (s, 1H), 8.03 (s, 1H), 6.31 (m, 1H), 5.85 (br, 1H), 5.78 (br, 1H), 5.20 (m, 1H), 5.14 (m, 2H), 4.44 (m, 2H), 4.34 (m, 1H), 3.97 (m, 1H), 3.62 (m, 2H), 3.34 (m, 1H), 3.14 (s, 3H), 3.01 (s, 6H). ^{13}C NMR (125.8 MHz) (DMSO-d_6) δ: 158.6, 158.5, 157.9, 157.8, 150.1, 149.5, 137.2, 133.5, 120.4, 120.2, 117.3, 116.4, 88.0, 87.5, 87.0, 82.7, 77.1, 71.1, 66.6, 65.9, 62.1, 61.6, 61.2, 49.0, 41.1, 35.0, 32.9, 30.2. ^{13}C NMR (125.8 MHz) ($CDCl_3$) δ: 158.4, 158.0, 157.9, 157.0, 156.5, 156.0, 150.0, 135.8, 134.9, 132.6, 132.2, 120.8, 120.6, 118.1, 117.5, 85.8, 85.2, 83.7, 72.6, 71.9, 66.5, 66.4, 64.6, 64.0, 62.3, 61.1, 41.3, 35.1, 33.2, 32.7, 17.4, 17.3, 17.23, 17.21, 16.90, 16.86, 16.78, 13.21, 13.16, 12.8, 12.5. HRMS: calcd for $C_{18}H_{26}N_7O_6$ $[MH]^+$ 436.1945, found 436.1940.

2′-*N*-Methyl-*N*-alloxycarbonyl-N^2-dimethylaminomethylene-5′-*O*-4,4′-dimethoxytrityl-2′-deoxyguanosine (13). 2′-*N*-Methyl-*N*-alloxycarbonyl-N^2-dimethylaminomethylene-2′-deoxyguanosine (**12**) (100 mg, 0.23 mmol) was coevaporated with anhydrous pyridine (10 mL × 20) and then dissolved in pyridine (8 mL), and 4,4′-dimethoxytrityl chloride (116 mg, 0.34 mmol, 1.5 equiv) was added. After being stirred overnight at room temperature, the reaction mixture was diluted with MeOH (1 mL) and stirred for an additional 5 min. The reaction mixture was concentrated to dryness under vacuum and coevaporated with toluene (20 mL × 2). Water was added to the resulting residue, and the mixture was extracted with CH_2Cl_2. The organic phase was washed consecutively with 5% sodium bicarbonate, water, and brine and then dried over sodium sulfate. After the organic phase was concentrated to dryness, the residue was purified by silica gel chromatography, eluting with 0−3% MeOH in CH_2Cl_2 containing 0.2% Et_3N, to give **13** (137 mg, 81%) as white foam. ^1H NMR (500.1 MHz) (CD_3CN) δ: ^{13}C NMR (125.8 MHz) (CD_3CN) δ: 10.06 (br, 1H), 8.57 (s, 1H), 7.73 (m, 1H), 7.42 (m, 2H), 7.15−7.30 (m, 7H), 6.79 (m, 4H), 6.41 (m, 1H), 5.85 (br, 1H), 4.955.30 (m, 3H), 4.53 (m, 2H), 4.44 (m, 1H), 4.20 (m, 1H), 3.72 (s, 6H), 3.35 (m, 1H), 3.27 (m, 1H), 3.08 (s, 3H), 3.00 (s, 3H), 2.96 (s, 3H). ^{13}C NMR (125.8 MHz) (CD_3CN) δ: 158.6, 158.5, 158.3, 157.9, 157.4, 150.4, 144.9, 135.70, 135.67, 133.2, 130.0, 129.9, 127.9, 127.8, 126.8, 120.1, 116.4, 113.0, 86.2, 85.5, 83.1, 65.9, 64.2, 54.8, 40.6, 34.2, 32.7. HRMS: calcd for $C_{39}H_{44}N_7O_8$ $[MH]^+$ 738.3251, found 738.3232.

2′-*N*-Methyl-*N*-alloxycarbonyl-N^2-dimethylaminomethylene-5′-*O*-4,4′-dimethoxytrityl-2′-deoxyguanosine (2-Cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (14). 2′-*N*-Methyl-*N*-alloxycarbonyl-N^2-dimethylaminomethylene-5′-*O*-4,4′-dimethoxytrityl-2′-deoxyguanosine (**13**, 120 mg, 0.16 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and 1-methylimidazole (2.90 mg, 35.0 μmol). *N*,*N*-Diisopropylethylamine (120 mg, 0.64 mmol) was added to the stirring solution followed by 2-cyanoethyl *N*,*N*-(diisopropylchloro)-phosphoramidite (2.0 equiv). After being stirred at room temperature for 1 h, the reaction mixture was diluted with dichloromethane (50 mL), and the mixture was washed with 5% aqueous sodium carbonate and brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography, eluting with 12% acetone in CH_2Cl_2 containing 0.2% Et_3N, to give **14** (119 mg, 78%) as a colorless oil. ^{31}P NMR (202.4 MHz) (CD_3CN) δ: 149.95 and 149.66, 149.34. HRMS: calcd for $C_{48}H_{60}N_9O_9P$, $[M]^+$ 937.4252, found 937.4288.

2′-*N*-Methyl-*N*-phenoxyacetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (I-3). To a solution of 2′-methylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**10**, 178 mg, 0.30 mmol) in CH_2Cl_2 (10 mL) and pyridine (10 mL) at 0 °C under argon were added phenoxyacetyl chloride (47 μL, 1.1 equiv) and *N*,*N*-diisopropylethylamine (0.2 mL). The mixture was stirred at 0 °C for 1 h, and MeOH (0.5 mL) was added to quench the reaction. All of the volatile components were removed under reduced pressure. The residue was coevaporated with toluene. CH_2Cl_2 was added to the resulting residue, and the mixture was washed consecutively with 5% sodium bicarbonate, water, and brine and then dried over sodium sulfate. After the organic phase was concentrated to dryness, the residue was purified by silica gel

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chromatography, eluting with 0−4% MeOH in CH_2Cl_2, to give **I-3** (164 mg, 75%) as a white foam. ^{1}H NMR (500.1 MHz) ($CDCl_3$) δ: 9.86 (br, 1H), 8.67 (s, 0.75H), 8.40 (s, 0.25H), 7.78 (s, 0.25H), 7.76 (s, 0.75H), 7.30 (m, 2H), 7.25 (m, 1H), 7.00 (m, 1.5H), 6.86 (m, 0.5H), 6.20 (m, 1H), 6.05 (m, 0.75H), 5.25 (m, 0.25H), 4.74−4.80 (m, 3H), 4.25 (m, 1H), 4.18 (m, 1H), 4.05 (m, 0.25H), 3.90 (m, 0.75H), 3.08−3.30 (m, 9H), 1.01−1.06 (m, 28H). ^{13}C NMR (125.8 MHz) ($CDCl_3$) δ: 158.8, 157.9, 157.8, 157.6, 157.4, 157.0, 149.9, 149.6, 136.0, 135.9, 129.6, 129.4, 121.7, 121.5, 120.8, 114.3, 114.0, 86.3, 85.8, 84.6, 84.0, 72.5, 72.3, 68.0, 66.9, 64.5, 63.9, 62.3, 58.8, 41.3, 35.1, 33.0, 32.1, 17.4, 17.3, 17.2, 17.1, 16.97, 16.95, 16.8, 13.21, 13.19, 12.8, 12.5, 12.4. HRMS: calcd for $C_{34}H_{53}N_7O_7Si_2$ $[MH]^+$ 728.3618, found 728.3584.

2′-*N*-Methyl-*N*-phenoxyacetyl-N^2-dimethylaminomethylene-2′-deoxyguanosine (II-3). To a solution of 2′-*N*-methyl-*N*-phenoxyacetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**I-3**) (150 mg, 0.21 mmol) in THF (10 mL) was added TBAF in THF (1.0 M, 0.21 mL), and the mixture was stirred at rt for 15 min. Silica gel (1.0 g) was added, and the volatile components were removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with 0−9% methanol in dichloromethane, to give **II-3** (95 mg, 95%) as a white solid. ^{1}H NMR (500.1 MHz) (CD_3OD) δ: 8.59 (s, 0.75H), 8.40 (s, 0.25H), 8.20 (s, 0.25H), 8.18 (s, 0.75H), 7.16−7.22 (m, 2H), 6.91 (m, 1H), 6.79 (d, J = 8.0 Hz, 1.5H), 6.73 (d, J = 8.5 Hz, 0.5H), 5.75 (m, 0.75H), 5.25 (m, 0.25), 4.85 (m, 2H), 4.75 (m, 1H), 4.62 (m, 1H), 4.22 (m, 1H), 3.83 (m, 2H), 2.98−3.30 (m, 9H). ^{13}C NMR (125.8 MHz) (CD_3OD) δ: 170.6, 169.8, 158.6, 158.1, 157.7, 157.6, 150.3, 150.0, 137.8, 129.1, 128.9, 121.1, 120.9, 119.8, 119.5, 114.1, 114.0, 113.8, 87.6, 87.4, 85.0, 83.4, 71.0, 66.6, 65.9, 64.4, 62.5, 61.9, 61.5, 60.0, 58.0, 40.0, 33.81, 33.75, 32.3, 31.2. HRMS: calcd for $C_{22}H_{27}N_7O_6$ $[MH]^+$ 486.2101, found 486.2092.

2′-*N*-Methyl-*N*-phenoxyacetyl-N^2-dimethylaminomethylene-5′-*O*-4,4′-dimethoxytrityl-2′-deoxyguanosine (III-2). 2′-*N*-Methyl-*N*-phenoxyacetyl-N^2-dimethylaminomethylene-2′-deoxyguanosine (**II-3**) (95 mg, 0.20 mmol) was coevaporated with anhydrous pyridine (10 mL × 20) and then dissolved in pyridine (8 mL), and 4,4′-dimethoxytrityl chloride (99 mg, 0.29 mmol, 1.5 equiv) was added. After being stirred overnight at room temperature, the reaction mixture was diluted with MeOH (1 mL) and stirred for an additional 5 min. The reaction mixture was concentrated to dryness under vacuum and coevaporated with toluene (20 mL × 2). Water was added to the resulting residue, and the mixture was extracted with CH_2Cl_2. The organic phase was washed consecutively with 5% sodium bicarbonate, water, and brine and then dried over sodium sulfate. After the organic phase was concentrated to dryness, the residue was purified by silica gel chromatography, eluting with 0−3% MeOH in CH_2Cl_2 containing 0.2% Et_3N, to give **III-2** (136 mg, 88%) as a white foam. ^{1}H NMR (500.1 MHz) (CD_3CN) δ: 9.91 (br, 1H), 8.54 (s, 0.75H), 8.37 (s, 0.25H), 7.88 (s, 0.25H), 7.76 (s, 0.75H), 7.48 (m, 1.5H), 7.42 (m, 0.5H), 7.22−7.36 (m, 7H), 6.83 (m, 4H), 6.47 (m, 0.75H), 6.42 (m, 0.25H), 5.72 (m, 0.75H), 5.15 (m, 0.25H), 4.82 (s, 1.5H), 4.75 (s, 0.25H), 4.67 (m, 1H), 4.28 (m, 2H), 3.78 (s, 6H), 3.38 (m, 2H), 3.22 (s, 2.25H), 3.18 (s, 0.75H), 2.97−3.01 (m, 6H). HRMS: calcd for $C_{43}H_{45}N_7O_8$ $[MNa]^+$ 810.3222 (calcd), 810.3248 (found).

2′-*N*-Methyl-*N*-phenoxyacetyl-N^2-dimethylaminomethylene-5′-*O*-4,4′-dimethoxytrityl-2′-deoxyguanosine (2-Cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (IV-1). 2′-*N*-Methyl-*N*-phenoxyacetyl-N^2-dimethylaminomethylene-5′-*O*-4,4′-dimethoxytrityl-2′-deoxyguanosine (**III-2**, 120 mg, 0.15 mmol) was dissolved in dry CH_2Cl_2 (5 mL). *N*,*N*-Diisopropylethylamine (104 μL, 0.60 mmol) was added to the stirring solution followed by 2-cyanoethyl *N*,*N*-(diisopropylchloro)phosphoramidite (2.0 equiv). After being stirred at room temperature for 1 h, the reaction mixture was diluted with dichloromethane (50 mL) and the mixture was washed with 5% aqueous sodium carbonate and brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography, eluting with 12% acetone in CH_2Cl_2 containing 0.2% Et_3N, to give **IV-1** (114 mg, 75%) as a colorless oil. ^{31}P NMR (202.4 MHz) (CD_3CN) δ: 152.2, 151.1, 150.51, and 150.46. HRMS: calcd for $C_{52}H_{62}N_9O_9P$ $[MNa]^+$ 1010.4300, found 1010.4344.

2′-*N*-Methyl-*N*-benzyloxycarbonyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (I-4). To a solution of 2′-methylamino-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**10**, 210 mg, 0.35 mmol) in anhydrous THF (10 mL) under argon were added *N*,*N*-diisopropylethylamine (122 μL, 2.0 equiv) and benzyl chloroformate (68 μL, 1.2 equiv). The mixture was stirred at rt for 2 h and MeOH (0.5 mL) was added to quench the reaction. All the volatile components were removed under reduced pressure. CH_2Cl_2 (50 mL) was added to the resulting residue, and the mixture was washed consecutively with 5% sodium bicarbonate, water, brine, and then dried over sodium sulfate. After the organic phase was concentrated to dryness, the residue was purified by silica gel chromatography, eluting with 0−4% MeOH in CH_2Cl_2, to give **I-4** (216 mg, 85%) as a white foam. ^{1}H NMR (500.1 MHz) (CD_3CN) δ: 9.95 (br, 1H), 8.68 (s, 0.63H), 8.35 (s, 0.37H), 7.73 (s, 0.63H), 7.68 (s, 0.37H), 7.25 (m, 5H), 7.01 (s, 0.37H), 6.12 (d, J = 5.5 Hz, 0.63H), 5.56 (m, 1H), 5.05 (m, 2H), 4.61 (m, 1H), 4.06 (s, 1H), 3.84 (m, 2H), 3.08−3.17 (m, 9H), 0.89−1.03 (m, 28H). ^{13}C NMR (125.8 MHz) ($CDCl_3$) δ: 158.0, 157.8, 156.9, 156.3, 149.7, 136.4, 136.0, 128.6, 128.4, 128.2, 127.9, 127.8, 121.1, 98.5, 86.1, 85.3, 84.4, 72.8, 72.1, 67.8, 67.6, 67.4, 64.8,64.1, 61.2, 41.8, 35.8, 33.3, 32.9, 23.6, 17.6, 17.44, 17.40, 17.08, 17.04, 16.9, 13.3, 13.0, 12.6. HRMS: calcd for $C_{34}H_{53}N_7O_7Si_2$ $[MH]^+$ 728.3623, found 728.3609.

2′-*N*-Methyl-*N*-benzyloxycarbonyl-N^2-dimethylaminomethylene-2′-deoxyguanosine (II-4). To a solution of 2′-*N*-methyl-*N*-benzyloxycarbonyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-dimethylaminomethylene-2′-deoxyguanosine (**I-4**) (198 mg, 0.27 mmol) in THF (10 mL) was added TBAF in THF (1 M, 0.27 mL), and the mixture was stirred at rt for 10 min. Silica gel (1.0 g) was added, and volatile components were removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with 0−8% MeOH in CH_2Cl_2, to give **II-4** (126 mg, 96%) as a white solid. ^{1}H NMR (500.1 MHz) (DMSO-d_6) δ: 11.38 (br, 1H), 8.57 (s, 0.63H), 8.42 (s, 0.37H), 8.04 (s, 1H), 7.09 (m, 5H), 6.33 (m, 1H), 5.13 (m, 2H), 5.02 (m, 2H), 4.35 (m, 1H), 4.12 (m, 1H), 3.62 (m, 1H), 3.57 (m, 1H), 2.99−3.34 (m, 9H). ^{13}C NMR (125.8 MHz) (DMSO-d_6) δ: 158.7, 158.1, 158.0, 156.7, 150.3, 137.3, 128.9, 128.3, 127.9, 126.9, 120.5, 88.1, 87.7, 87.2, 77.3, 71.4, 67.0, 66.8, 62.3, 61.8, 61.2, 58.1, 35.15, 33.12, 30.39. HRMS: calcd for $C_{22}H_{27}N_7O_6$ $[MH]^+$ 486.2101, found 486.2092.

2′-*N*-Methyl-*N*-benzyloxycarbonyl -N^2-dimethylaminomethylene-5′-*O*-4,4′-dimethoxytrityl-2′-deoxyguanosine (III-3). 2′-*N*-Methyl-*N*-benzyloxycarbonyl-N^2-dimethylaminomethylene-2′-deoxyguanosine (**II-4**) (120 mg, 0.25 mmol) was coevaporated with anhydrous pyridine (10 mL × 20) and then dissolved in pyridine (8 mL), and 4,4′-dimethoxytrityl chloride (125 mg, 0.37 mmol, 1.5 equiv) was added. After being stirred overnight at room temperature, the reaction mixture was diluted with MeOH (1 mL) and stirred for an additional 5 min. The reaction mixture was concentrated to dryness under vacuum and coevaporated with toluene (20 mL × 2). CH_2Cl_2 was added, and the mixture was washed consecutively with 5% sodium bicarbonate, water, and brine and then dried over sodium sulfate. After the organic phase was concentrated to dryness, the residue was purified by silica gel chromatography, eluting with 0−3% MeOH in CH_2Cl_2 containing 0.2% Et_3N, to give **III-3** (160 mg, 82%) as a white foam. ^{1}H NMR (500.1 MHz) (CD_3CN) δ: ^{13}C NMR (125.8 MHz) (CD_3CN) δ: 9.70 (br, 1H), 8.55 (m, 1H), 7.71 (m, 1H), 7.11−7.42 (m, 14H), 6.78 (m, 4H), 6.38 (m, 1H), 5.09−5.25 (m, 3H), 4.58 (m, 1H), 4.16 (m, 1H), 3.73 (m, 6H), 3.30 (m, 2H), 2.98−3.08 (m, 9H). HRMS: calcd for $C_{43}H_{45}N_7O_8$ $[MNa]^+$ 810.3227, found 810.3216.

2′-*N*-Methyl-*N*-benzyloxycarbonyl-N^2-dimethylaminomethylene-5′-*O*-4,4′-dimethoxytrityl-2′-deoxyguanosine (2-Cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (IV-2). 2′-*N*-Methyl-*N*-benzyloxycarbonyl-N^2-dimethylaminomethylene-5′-*O*-4,4′-dimethoxytrityl-2′-deoxyguanosine (**III-3**, 95 mg, 0.12 mmol) was dissolved in dry CH_2Cl_2 (5 mL). *N*,*N*-Diisopropylethylamine (83 μL, 0.48 mmol) was added to the stirring solution followed by 2-cyanoethyl *N*,*N*-(diisopropylchloro)phosphoramidite (2.0 equiv).

After being stirred at room temperature for 1 h, the reaction mixture was diluted with dichloromethane (50 mL), and the mixture was washed with 5% aqueous sodium carbonate and brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography, eluting with 12% acetone in CH_2Cl_2 containing 0.2% Et_3N, to give **IV-2** (92 mg, 78%) as a colorless oil. ^{31}P NMR (202.4 MHz) (CD_3CN) δ: 150.5, 150.0, 149.8, and 149.5. HRMS: calcd for $C_{52}H_{62}N_9O_9P$ $[MNa]^+$ 1010.4300, found 1010.4321.

RNA Synthesis and Deprotection. RNA oligonucleotide synthesis was performed on an Expedite Nucleic Acid Synthesis System using standard RNA synthesis conditions (scale: 1 μM). Phosphoramidites for A, C, G, U and CPG carriers were obtained from a commercial supplier. The terminal DMTr protecting group was removed from the oligonucleotides by using the DMTr off mode. After oligo synthesis, the resin containing oligos was transferred to a 2 mL vial. For oligo containing G_{NMe_2}modification, the resin was treated with 2.0 mL 3:1 concentrated NH_4OH/EtOH overnight at 55 °C. The supernant was collected and concentrated to dryness using speedvac. The residue was then treated with the mixture of *N*-methylpyrrolidinone, triethylamine, and triethylamine trihydrofluoride (0.3 mL, 6:3:4 v/v/v) at 65 °C for 90 min. After being cooled to rt, the mixture was desalted and purified by HPLC. Its structure was further confirmed by MALDI-TOF MS (SI, $[M - H]^-$ = 1554, $[MNa - 2H]^-$ = 1576). For oligo-containing G_{NHMe} modification, the extra step treatment of resin is needed. the resin was treated with $Pd(Ph_3P)_4$ in the presence of HCO_2H/Et_3N or $Pd(Ph_3P)_4$ in the presence of AcOH and Bu_3SnH and washed with acetone for three time. The rest of the procedure is the same as oligo containing G_{NMe_2}modification. Its structure was also further confirmed by MALDI-TOF MS (SI, $[M - H]^-$ = 1540).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra for compounds **2**−**8**, **10**−**14**, and ^{31}P NMR spectra for compounds **9** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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