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Improved synthesis of 2′-amino-2′-deoxyguanosine and its phosphoramidite

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Abstract—2′-Amino-2′-deoxynucleosides and oligonucleotides containing them have proven highly effective for an array of biochemical applications. The guanosine analogue and its phosphoramidite derivatives have been accessed previously from 2′-amino-2′-deoxyuridine by transglycosylation, but with limited overall efficiency and convenience. Using simple modifications of known reaction types, we have developed useful protocols to obtain 2′-amino-2′-deoxyguanosine and two of its phosphoramidite derivatives with greater convenience, fewer steps, and higher yields than reported previously. These phosphoramidites provide effective synthons for the incorporation of 2′-amino-2′-deoxyguanosine into oligonucleotides.

1. Introduction

2′-Amino-2′-deoxynucleosides and oligonucleotides containing them have received much attention in recent years, both as potential therapeutic agents[1–4] and as diagnostic and biochemical probes.[5,6] As components of antisense oligonucleotides,[7–9] they have the potential to improve drug efficacy by imparting resistance to chemical and enzymatic degradation.[10,11] In addition to their therapeutic potential, the distinct physiochemical properties of 2′-amino-2′-deoxynucleosides render them especially powerful tools for exploring RNA structure, function, and dynamics,[12–19] particularly in defining the role and environment of specific 2′-hydroxyl groups within structural and catalytic RNA molecules.[13–18,20]

Our interest in 2′-amino-2′-deoxyguanosine (G_N) emanates from its value in mechanistic investigations of the group I intron, which catalyzes nucleotidyl transfer between an oligonucleotide substrate and guanosine. 2′-Amino-2′-deoxyguanosine provides a direct probe for one of the catalytic metal ions (M_c) at the ribozyme active site,[13] thereby allowing identification of RNA ligands to this metal ion.[21] Additionally, G_N forms an integral component of an atomic mutation cycle designed to explore the role of the 2′-hydroxyl group in hydrogen bonding.[22,23] The literature contains two previous reports of 2′-amino-2′-deoxyguanosine phosphoramidites **A** and **B** (Fig. 1),[24,25] both accessed from uridine derivatives with limited overall efficiency. Recently, Beigelman et al. demonstrated that 2′-phthalimido protection of 2′-aminouridine enhances the stability and coupling yields of the corresponding phosphoramidites relative to 2′-trifluoroacetamido protection.[26] Subsequently, the corresponding 2′-phthalimido phosphoramidites of cytosine and adenosine were prepared.[27] Here, we extend this approach to 2′-amino-2′-deoxyguanosine. The procedures developed offer access to the nucleoside and the phosphoramidite in fewer steps, and in greater yield than reported previously.

2. Results and discussion

We first attempted to introduce a 2′-phthalimido group into the 2′-α position of guanosine by S_N2 displacement of β-triflate from the 3′,5′-*O*-disilyl protected guanosine derivative (**1**) (Fig. 2),[28] analogous to the previously described preparations of 2′-α-phthalimido uridine, cytosine, and adenosine.[27] However, treatment

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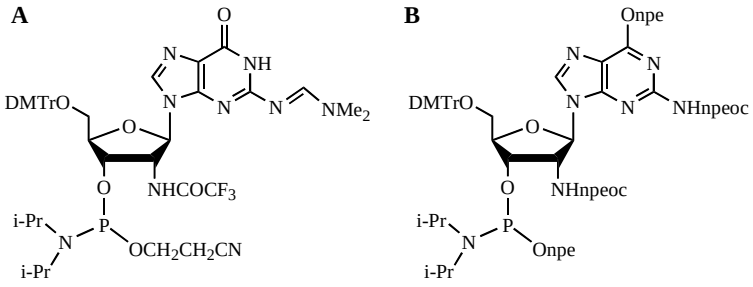


Figure 1. Previously synthesized phosphoramidites for the incorporation of 2′-amino-2′-deoxyguanosine into oligonucleotides. Benseler et al. synthesized **A** in 12% yield from 2′-trifluoroacetamido-2′-deoxyuridine[24] and Greiner synthesized **B** in 9% overall yield from 2′-amino-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine.[25] The abbreviation npeoc represents the [2-(4-nitrophenyl)ethoxy]carbonyl group, and npe represents the 2-(4-nitrophenyl)ethyl group.

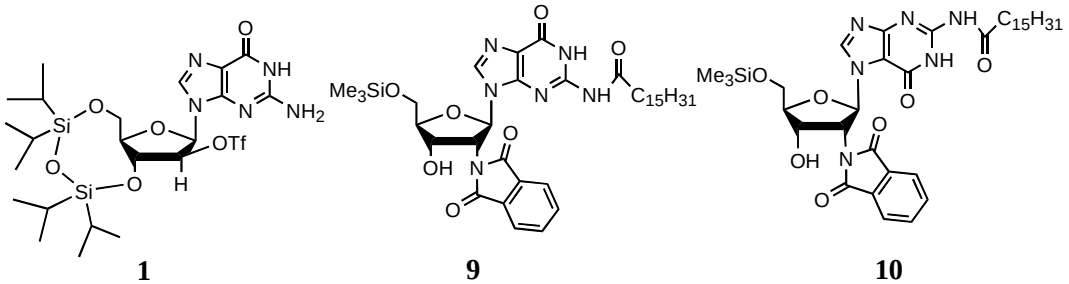


Figure 2. Structures of compounds **1**, **9**, and **10**.

of **1** with phthalimide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave no reaction. Instead, we generated 2′-phthalimidoguanosine by transglycosylation from the known 2′-deoxy-2′-*N*-phthalimidouridine intermediate **4a**,[26] analogous to the approach described by Eckstein et al. for converting 2′-amido-2′-deoxyuridine to the corresponding 2′-amido-2′-deoxyguanosine.[24,29]

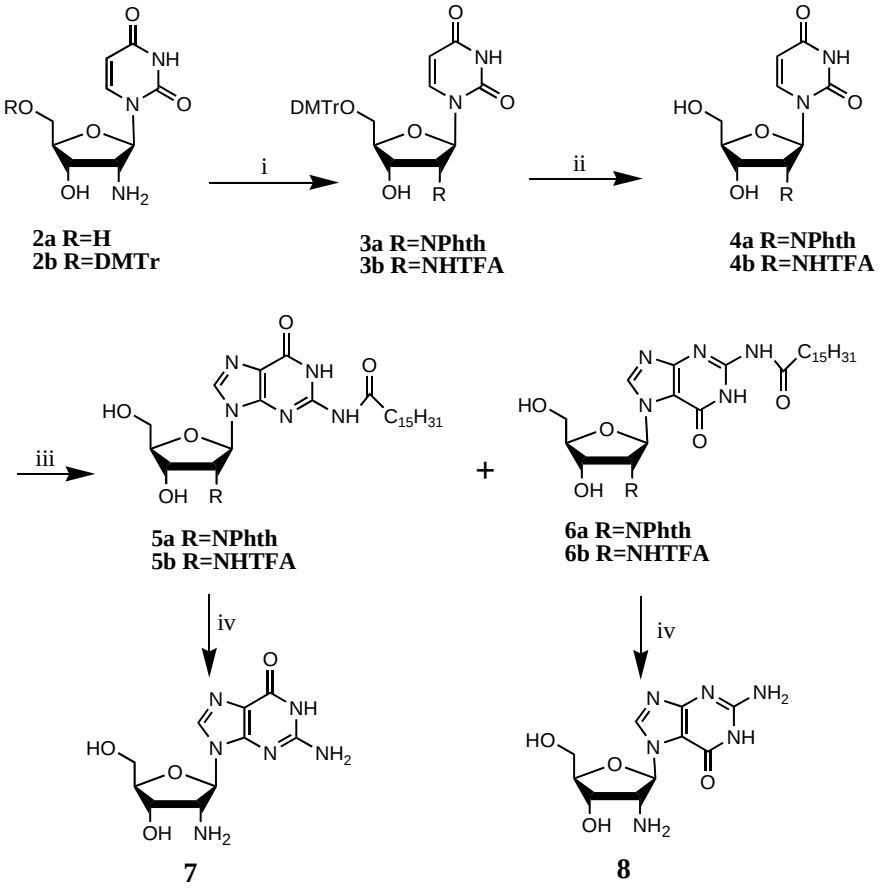
In the reported preparation of **4a**,[26] 2′-amino-2′-deoxyuridine (**2a**) was treated with *N*-carbethoxyphthalimide, followed by 4,4′-dimethoxytrityl chloride (DMTr-Cl) to allow purification as **3a**. Subsequent trichloroacetic acid treatment (TCA, 3% in acetonitrile) gave pure **4a**. Considering that McGee et al. readily prepared 2′-amino-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **(2b)** in 68% yield from commercially available 2,2′-anhydrouridine, we adopted a modified preparation of **4a**, first treating **2b** with *N*-carbethoxyphthalimide to generate **3a**, followed by DMTr removal with 3% TCA (Scheme 1). Overnight treatment of **2b** with 1.2 equiv of *N*-carbethoxyphthalimide and triethylamine (Et_3N) in tetrahydrofuran (THF) produced **3a** and ca. 10% 2′-*N*-3′-*O*-bisphthaloyl by-product.[26] Subsequent treatment of the reaction mixture with methanol (MeOH)/Et_3N converted the by-product quantitatively to **3a**. Detritylation of crude **3a** with 3% TCA generated **4a** in 94% overall yield from **2b**.

2.1. Improved access to 2′-amino-2′-deoxyguanosine and its phosphoramidite

Transglycosylation of **4a** followed by methanolic ammonia treatment according to a literature procedure[29] afforded a mixture of N^9 and N^7-substituted guanosine nucleosides (**7** and **8**) in 80% overall yield (N^9/N^7 = 4:1 as indicated by ^{1}H NMR of the mixture). The high polarity and poor solubility of these products rendered them difficult to purify. To allow easier purification, we isolated the transglycosylation reaction products before methanolic ammonia treatment, thereby retaining the phthaloyl and palmitoyl groups.

Depending on the work-up procedure, different products from the transglycosylation reaction were isolated. If the reaction mixture was cooled to room temperature, quenched with water (ca. 450 equiv), and extracted with dichloromethane (CH_2Cl_2), we isolated mainly the guanosine derivatives **9** and **10** (Fig. 2), each containing a trimethylsilyl group at the 5′-oxygen (as indicated by ^{1}H NMR).[30] Quenching with dilute hydrochloric acid (HCl, 0.1 N, ca. 450 equiv) instead of water converted **9** and **10** to **5a** and **6a**,[31] respectively, which usually were contaminated with unreacted N^2-palmitoylguanine following isolation. If we quenched the reaction with a small amount of HCl (0.1 N, 8.0 equiv), most of the unreacted N^2-palmitoylguanine formed a precipitate, thereby simplifying purification of **5a** and **6a**.

Using the above work-up procedure, the transglycosylation reaction of **4a** with N^2-palmitoylguanine gave **5a** and **6a** in yields of 60% and 15%, respectively. Overnight treatment of **5a** with methanolic ammonia at 55 °C removed both the phthaloyl and palmitoyl groups completely, as estimated by thin-layer chromatography (TLC). Considering that 2′-amino-2′-deoxyguanosine (**7**) dissolves easily in water, we attempted to remove

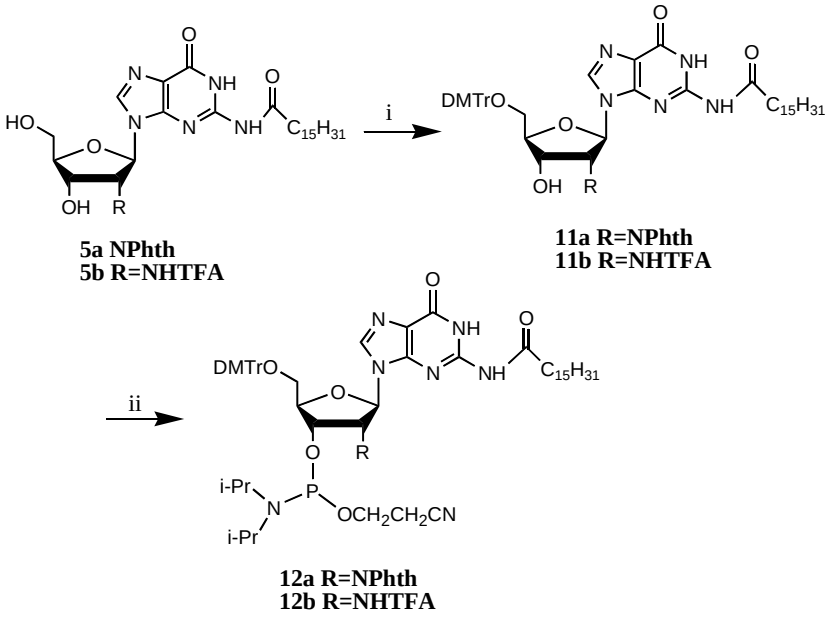


Scheme 1. Reagents and conditions: (i) For **3a**, *N*-carbethoxyphthalimide, THF, Et_3N/MeOH; for **3b**, $CF_3C(O)SEt$, MeOH, 84%. (ii) 3% TCA in CH_3CN; for **4a**, 94% from **2b**; for **4b**, 92% from **3b**. (iii) (a) *N*,*O*-Bis(trimethylsilyl) acetamide, N^2-palmitoylguanine, reflux, 30 min; (b) trimethylsilyl triflate, reflux, 3 h; (c) HCl (0.1 N); for **5a**, 60%; for **6a**, 15%; for **5b**, 70%; for **6b**, 12%. (iv) *n*-Butylamine, ethanol (EtOH), 55 °C, 16 h; for **7** from **5a**, 95%; for **8** from **6a**, 93%.

the palmitoylamide and phthalamide by-products by organic extraction to avoid the two ion-exchange columns needed previously for purification of **7**. After removal of the solvents, the resulting residue was dissolved in water and extracted with CH_2Cl_2. The organic phase contained only palmitoylamide, and the aqueous phase contained **7** and phthalamide. To render the organic extraction more effective, we increased the lipophilicity of the phthalamide using methylamine instead of ammonia to deprotect **5a**. However, the by-product, *N*,*N*′-dimethyl phthalamide, still remained in the aqueous phase with **7**. To increase the lipophilicity further, we deprotected **5a** with ethanolic *n*-butylamine (4:1) at 55 °C. Extraction removed both the *N*-butyl palmitoylamide and *N*,*N*′-di-*n*-butyl-phthalamide by-products to give crude **7**, which was purified further by recrystallization to give pure **7** in 95% yield. We obtained the corresponding 7-(2′-amino-2′-deoxy-β-D-ribofuranosyl)guanine (**8**) from **6a** in the same way. These procedures improve the overall yield of 2′-amino-2′-deoxyguanosine from 24% to 36% starting from uridine and eliminate the need for ion-exchange purification.[29]

In previous reports, complete deprotection of the transglycosylation product to the parent nucleoside preceded conversion to the phosphoramidite, necessitating reprotection of the exocyclic and ribofuranosyl amines.[25,29] In contrast, we transformed the transglycosylation product **5a** directly to the phosphoramidite, assuming the palmitoyl group would offer suitable protection for the exocyclic amino group during solid-phase synthesis. This simple modification allowed access to the 2′-*N*-phthaloyl-2′-deoxyguanosine phosphoramidite **12a** in just two steps (dimethoxytritylation and phosphitylation) following transglycosylation (Scheme 2), resulting in significantly greater yields for phosphoramidite **12a** (41% from **2b**) compared to that for phosphoramidite **A** (12% from **4b**).

We also prepared the corresponding 2′-deoxy-2′-*N*-trifluoroacetyl guanosine phosphoramidite **12b** by a similar route (Schemes 1 and 2), again retaining the palmitoyl group following transglycosylation. We converted **2b** to the corresponding trifluoroacetamide **3b** in 84% yield using *S*-ethyl trifluorothioacetate. TCA-induced removal of the DMTr group afforded 2′-trifluoroacetamido-2′-deoxyuridine **4b** in 92% yield. Transglycosylation of **4b** with N^2-palmitoylguanine gave N^2-palmitoyl-2′-trifluoroacetamido-2′-deoxyguanosine **5b** in 53% yield. We converted **5b** to a suitably protected phosphoramidite using consecutive 5′-dimethoxyltritylation and phosph-



Scheme 2. Reagents and conditions: (i) DMTr-Cl, pyridine, 16 h; for **11a**, 82%; for **11b**, 80%. (ii) 2-Cyanoethyl *N*,*N*-(diisopropylchloro)phosphoramidite, 1-methylimidozale, *N*,*N*-diisopropylethylamine; for **12a**, 88%; for **12b**, 75%.

Table 1. Comparative overview of the syntheses of 2′-amino-2′-deoxyguanosine and its phosphoramidites

Target molecule	Starting material (SM)	Steps	Overall yield (%)	Notes	References
2′-Amino-2′-deoxyguanosine (**7**)	5′-*O*-DMTr-2′-amino-2′-deoxyuridine (**2b**)	4	54	SM prepared from 2,2′-anhydrouridine in 68% yield	Current work
	2′-Azido-2′-deoxyuridine	4	29	SM prepared from uridine in 50% yield; two ion-exchange purifications needed	29
Phosphoramidite **A**	**7**	4	21	SM prepared in 29% yield (see above)	24
Phosphoramidite **B**	5′-*O*-DMTr-2,2′-anhydrouridine	10	6	Many synthetic steps; low overall yield	25
Phosphoramidite **12a**	**2b**	5	41	Purification following glycosylation eliminates synthetic steps	Current work
Phosphoramidite **12b**	**2b**	5	38	Same as for **12a**	Current work

itylation reactions. This approach gives the 2′-TFA protected phosphoramidite **12b** in 38% overall yield from **2b**, whereas the previous method provided 2′-TFA protected phosphoramidite **A** (Fig. 1) in 21% overall yield from **7** (Table 1).

2.2. Oligonucleotide synthesis

We tested the suitability of phosphoramidites **12a** and **12b** for incorporating 2′-aminoguanosine into oligonucleotides of the sequence $CUCG_{2'\text{-}NH_2}A$. Using a 10 min coupling time, we found that **12a** and **12b** coupled with roughly equal efficiency based on the release of DMTr cation (~40% relative to commercial guanosine phosphoramidite), although we made no attempts to optimize this. Apparently, 2′-phthaloyl protection gives no advantage over 2′-TFA protection during the coupling of these phosphoramidites, in contrast to previous observations for the corresponding phosphoramidites of 2′-amino-2′-deoxyuridine.[26] Optimization of coupling conditions may be necessary before any differences become apparent. Coupling yields have not been reported for the previously synthesized 2′-amino-2′-deoxyguanosine phosphoramidites, **A** and **B** (Fig. 1). Following standard oligonucleotide deprotection conditions (55% NH_4OH/EtOH, 24 h; TBAF), the MALDI mass spectrum of $CUCG_{2'\text{-}NH_2}A$ gave the expected peak at 1528 Da, consistent with the calculated molecular weight (1529 Da), and demonstrating full deprotection of 2′-*N*-phthaloyl and palmitoyl groups.

To establish further the integrity of the modified oligonucleotide $CUCG_{2'\text{-}NH_2}A$, we examined its electrophoretic mobility upon exposure to T1 nuclease and the electrophilic reagent, sulfosuccinimidyl-6-(biotinamido) hexanoate (NHS).[16] The oligonucleotides $CUCG_{2'\text{-}NH_2}A$ and CUCGA were 5′-radiolabeled with ^{32}P-phosphate (*) using ^{32}P-ATP and T4 polynucleotide kinase (New England Biolabs) and purified by non-denaturing polyacrylamide gel electrophoresis (PAGE). As expected, the control oligonucleotide, *CUCGA, is cleaved in the presence of ribonuclease T1, which cuts after guanosine residues in single-stranded RNA

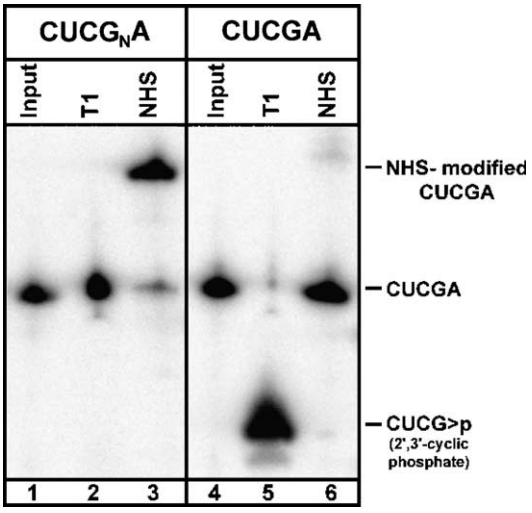


Figure 3. Biochemical reactivity profile of an oligonucleotide containing 2′-amino-2′-deoxyguanosine, CUCG$_N$A. The control oligonucleotide CUCGA (right panel) contains only unmodified ribonucleotides. Both samples were 5′-radiolabeled with ^{32}P-phosphate. Input (lanes 1 and 4) indicates unreacted sample. T_1 (lanes 2 and 5) contains samples incubated with ribonuclease T_1 (37 °C, 20 min); NHS (lanes 3 and 6) contains samples incubated with sulfosuccinimidyl-6-(biotinamido) hexanoate (37 °C, 1 h).

(Fig. 2, lane 5), but *CUCG$_{2'\text{-NH2}}$A resists T1 digestion (Fig. 3, lane 2). Conversely, *CUCG$_{2'\text{-NH2}}$A reacts with NHS as indicated by retarded gel mobility (Fig. 2, lane 3), whereas *CUCGA exhibits no reaction (Fig. 2, lane 6). These results demonstrate the viability of phosphoramidites **12a** and **12b** for the incorporation of 2′-amino-2′-deoxyguanosine into RNA.

3. Conclusions

We have synthesized 9-(N^2-palmitoyl-2′-phthalimido-2′-deoxy-β-D-ribofuranosyl)guanine (**5a**) and 9-(N^2-palmitoyl-2′-trifluoroacetamido-2′-deoxy-β-D-ribofuranosyl)guanine (**5b**) via transglycosylation from 2′-phthalimido and 2′-trifluoroacetamido-2′-deoxyuridine, respectively. Retention of the amine-protecting groups following these transglycosylation reactions allowed direct access to the corresponding phosphoramidites in two steps and simplified access to the nucleoside itself, resulting in greater yields than reported previously (refer to Table 1). Although these phosphoramidites carry the unconventional palmitoyl protecting group, both couple efficiently during solid-phase synthesis and undergo quantitative deprotection afterwards, enabling facile access to oligonucleotides containing 2′-amino-2′-deoxyguanosine.

4. Experimental

4.1. Materials and methods

All reagents and anhydrous solvents were purchased from Aldrich; other solvents were from Fisher unless otherwise noted. All reactions using air-sensitive or moisture-sensitive reagents were carried out under an argon atmosphere. ^{1}H, ^{19}F, and ^{13}C NMR spectra were recorded on Bruker 500 or Bruker 400 MHz NMR spectrometers. ^{1}H chemical shifts are reported in δ (ppm) relative to tetramethylsilane. ^{19}F chemical shifts are reported in δ (ppm) relative to an external standard of trifluoroacetic acid in $CDCl_3$. ^{31}P chemical shifts are reported in δ (ppm) relative to an external standard of 85% aqueous H_3PO_4. High-resolution mass spectra were obtained from the Department of Chemistry, University of California at Riverside. Merck silica gel (9385 grade, 230–400 mesh, 60A, Aldrich) was used for column chromatography. Silica gel on glass with fluorescent indicator (Sigma) was used for TLC.

4.1.1. 5′-*O*-(4,4′-Dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxyuridine (3b). To a solution of 5′-*O*-(4,4′-dimethoxytrityl)-2′-amino-2′-deoxyuridine (**2b**) (531 mg, 0.974 mmol) in methanol (6 mL) was added *S*-ethyl trifluorothioacetate (194 μL, 1.46 mmol, 1.5 equiv). The solution was stirred at room temperature for 1 h and a white solid precipitated. TLC showed quantitative conversion of the starting material. After the mixture 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 **3b** (525 mg, 84%) as a white amorphous solid. Analytical data agree with those previously reported.[11]

^{1}H NMR (400.1 MHz) (CD_3CN) δ: 7.34 (d, J = 8.2 Hz, 1H), 7.17 (d, J = 7.2 Hz, 2H), 7.04 (m, 6H), 7.03 (m, 1H), 6.60 (dd, J = 8.1, 0.8 Hz, 4H), 5.77 (d, J = 7.8 Hz, 1H), 5.14 (d, J = 8.1 Hz, 1H), 4.43 (m, 1H), 4.15 (m, 1H), 3.84 (m, 1H), 3.14 (dd, J = 7.2, 3.6 Hz, 1H), 3.02 (dd, J = 7.9, 2.9 Hz, 1H). ^{13}C NMR (100.6 MHz) (CD_3CN) δ: 162.9, 158.8, 157.3 (q, J = 37.8 Hz), 150.7, 144.7, 140.0, 135.5, 135.3, 132.5, 130.1, 128.0, 127.0, 117.4, 113.2, 108.4, 103.8, 102.2, 86.8, 86.0, 85.1, 70.9, 70.1, 66.8, 63.4, 56.0, 54.9. ^{19}F NMR (376.5 MHz) (CD_3CN) δ: −0.16 ppm. HRMS calcd for $C_{32}H_{30}F_3N_3O_8$, [MNa$^+$] 664.1871 (calcd), 664.1877 (found).

4.1.2. 2′-*N*-Phthalamido-2′-deoxyuridine (4a). To a solution of 2′-amino-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (**2b**) (1.16 g, 2.13 mmol) in THF (30 mL) was added *N*-carbethoxyphthalimide (559 mg, 2.56 mmol, 1.2 equiv). The solution was stirred overnight at room temperature. Et_3N (1 mL) and MeOH (1 mL) were then added. After stirring for another hour, the mixture was concentrated to dryness. The residue was treated with TCA (3% in CH_3CN) for 1 h and MeOH (1 mL) was added. After concentrating the mixture to dryness, the residue was purified by silica gel chromatography, eluting with 5–10% MeOH in CH_2Cl_2, to give **4a** (747 mg, 94%) as a white foam. ^{1}H NMR (500.1 MHz) (CD_3CN) δ: 7.87 (m, 2H), 7.83 (m, 2H), 7.69 (d, J = 8.0 Hz, 1H), 6.64 (d, J = 5.5 Hz, 4H), 5.64 (d, J = 8.5 Hz, 1H), 4.89 (dd, J = 8.0, 5.5 Hz, 1H), 4.44 (dd, J = 8.0, 6.1 Hz, 1H), 4.19 (m, 1H), 3.85 (dd, J = 12.3, 2.6 Hz, 1H), 3.70 (dd, J = 12.3, 3.4 Hz, 1H). ^{13}C NMR (125.8 MHz) (CD_3CN) δ: 168.2, 162.9, 151.0, 134.5,

131.6, 123.1, 117.3, 101.8, 86.2, 85.9, 68.5, 61.0, 56.8. HRMS calcd for $C_{17}H_{15}N_3O_7$, [MH^+] 374.0988 (calcd), 374.0999 (found).

4.1.3. 2′-Trifluoroacetamido-2′-deoxyuridine (4b). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxyuridine (**3b**) (4.10 g, 6.39 mmol) was treated with 3% TCA in CH_3CN for 15 min and then MeOH (1 mL) was added. The reaction mixture was concentrated to dryness. The residue was purified by silica gel chromatography, eluting with 8–12% MeOH in CH_2Cl_2, to give **6** (1.99 g, 92%) as a white foam. Analytical data agree with those previously reported.[29]

^{1}H NMR (500.1 MHz) (CD_3CN) δ: 9.84 (s, 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.84 (d, J = 8.1 Hz, 1H), 6.03 (d, J = 7.9 Hz, 1H), 5.05 (d, J = 8.1 Hz, 1H), 4.55 (m, 1H), 4.33 (m, 1H), 4.08 (m, 1H), 3.72 (m, 2H). ^{13}C NMR (125.8 MHz) (CD_3CN) δ: 163.7, 157.3 (q, J = 37.4 Hz), 151.0, 140.9, 117.3, 115.7 (q, J = 288 Hz), 102.3, 86.7 (d, J = 14.7 Hz), 70.3, 61.6, 55.9, 48.9, 46.6. ^{19}F NMR (470.5 MHz) (CD_3CN) δ: −0.20 ppm. HRMS calcd for $C_{11}H_{12}F_3N_3O_6$, [MH^+] 340.0756 (calcd), 340.0753 (found).

4.1.4. 'One-pot' synthesis from 2b. To a solution of 2′-amino-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (**2b**) (1.12 g, 2.06 mmol) in MeOH (6 mL) was added *S*-ethyl trifluorothioacetate (0.54 mL, 4.12 mmol, 2 equiv). The solution was stirred at room temperature for 1 h and a white solid precipitated. TLC showed that starting material was converted quantitatively to product. The solvent was removed under vacuum to give crude 5′-*O*-(4,4′-dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxyuridine as white foam. This foam was treated with 3% TCA in CH_3CN. After stirring for 15 min, TLC indicated that the intermediate was converted to product completely. MeOH (1 mL) was added, and the reaction mixture was concentrated to dryness. The residue was purified by silica gel chromatography, eluting with 8–12% MeOH in CH_2Cl_2, to give **4b** (0.627 g, 90%) as a white foam.

4.1.5. 9-(N^2-Palmitoyl-2′-phthalimido-2′-deoxy-β-D-ribofuranosyl)guanine (5a) and 7-(N^2-palmitoyl-2′-phthalimido-2′-deoxy-β-D-ribofuranosyl)guanine (6a). 2′-*N*-Phthaloylamido-2′-deoxyuridine (**4a**) (464 mg, 1.25 mmol) was added to a pressure tube (15 mL) containing dry CH_3CN (10 mL) and *N*,*O*-bis(trimethylsilyl) acetamide (2.57 mL, 10.6 mmol, 8.6 equiv) under argon. To this homogeneous solution was added N^2-palmitoylguanine (873 mg, 2.25 mmol, 1.8 equiv). The resulting suspension was heated to reflux for 30 min, and the reaction mixture became a clear solution. Trimethylsilyl triflate (241 μL, 1.59 mmol, 1.3 equiv) was added, and the solution was refluxed for an additional 3 h. The solution was cooled to room temperature, aqueous HCl (0.1 N, 0.1 mL) was added, and a white solid precipitated. The solid was filtered and washed with CH_3CN. The solvent of the combined filtrate was evaporated to dryness, and the residue was purified by silica gel chromatography, eluting with 8–12% MeOH in CH_2Cl_2, to give **5a** (487 mg, 60%) and **6a** (122 mg, 15%) as white solids.

For **5a**: ^{1}H NMR (500.1 MHz) (DMSO-d_6) δ: 12.11 (s, 1H), 11.63 (s, 1H), 8.32 (s, 1H), 7.87 (m, 4H), 6.85 (d, J = 5.9 Hz, 1H), 5.70 (d, J = 4.8 Hz, 1H), 5.18 (dd, J = 7.9, 6.0 Hz, 1H), 4.98 (t, J = 5.5 Hz, 1H), 4.52 (dd, J = 11.7, 6.2 Hz, 1H), 4.15 (m, 1H), 3.72 (m, 1H), 3.56 (m, 1H), 2.45 (t, J = 7.3 Hz, 2H), 1.57 (t, J = 6.6 Hz, 2H), 1.21 (m, 24H), 0.84 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125.8 MHz) (DMSO-d_6) δ: 177.2, 168.9, 155.6, 149.5, 148.9, 139.1, 135.5, 132.2, 124.1, 121.2, 87.1, 82.9, 69.3, 62.4, 57.4, 36.8, 32.2, 29.9, 29.8, 29.7, 29.6, 29.3, 25.2, 23.0, 14.8. HRMS calcd for $C_{34}H_{46}N_6O_7$, [MNa^+] 673.3320 (calcd), 673.3336 (found).

For **6a**: ^{1}H NMR (500.1 MHz) (DMSO-d_6) δ: 12.17 (s, 1H), 11.57 (s, 1H), 8.47 (s, 1H), 7.87 (m, 4H), 6.86 (d, J = 5.3 Hz, 1H), 5.65 (d, J = 5.2 Hz, 1H), 5.01 (dd, J = 9.5, 5.4 Hz, 1H), 4.90 (t, J = 5.6 Hz, 1H), 4.47 (dd, J = 12.4, 6.9 Hz, 1H), 4.17 (m, 1H), 3.74 (m, 1H), 3.58 (m, 1H), 2.40 (t, J = 7.3 Hz, 2H), 1.53 (t, J = 6.7 Hz, 2H), 1.21 (m, 24H), 0.81 (t, J = 6.7 Hz, 3H). ^{13}C NMR (125.8 MHz) (DMSO-d_6) δ: 176.6, 168.3, 158.9, 152.6, 147.8, 135.0, 131.7, 123.6, 110.7, 86.8, 86.0, 68.1, 61.8, 58.2, 36.2, 31.7, 29.4, 29.3, 29.2, 29.1, 29.0, 28.7, 22.5, 14.3. HRMS calcd For $C_{34}H_{46}N_6O_7$, [MNa^+] 673.3320 (calcd), 673.3330 (found).

4.1.6. 9-(N^2-Palmitoyl-2′-trifluoroacetamido-2′-deoxy-β-D-ribofuranosyl)guanine (5b) and 7-(N^2-palmitoyl-2′-trifluoroacetamido-2′-deoxy-β-D-ribofuranosyl)guanine (6b). 2′-Trifluoroacetamido-2′-deoxyuridine**(4b)** (276 mg, 0.813 mmol) was added to a pressure tube (15 mL) containing dry CH_3CN (5 mL) and *N*,*O*-bis(trimethylsilyl) acetamide (1.68 mL, 6.92 mmol, 8.6 equiv) under argon. To this homogeneous solution was added N^2-palmitoylguanine (570 mg, 1.47 mmol, 1.8 equiv). The resulting suspension was heated to reflux for 30 min, and the reaction mixture became a clear solution. Trimethylsilyl triflate (157 μL, 1.04 mmol, 1.3 equiv) was added, and the solution was refluxed for an additional 3 h. The solution was cooled to room temperature, aqueous HCl (0.1 N, 653 μL) was added, and a white solid precipitated. The solid was filtered and washed with CH_3CN. The solvent of the combined filtrate was evaporated to dryness, and the residue was purified by silica gel chromatography, eluting with 8–12% MeOH in CH_2Cl_2, to give **5b** (351 mg, 70%) and **6b** (60.1 mg, 12%) as white solids.

For **5b**: ^{1}H NMR (400.1 MHz) (DMSO-d_6) δ: 12.12 (s, 1H), 11.66 (s, 1H), 9.58 (d, J = 6.5 Hz, 1H), 8.18 (s, 1H), 6.06 (d, J = 8.2 Hz, 1H), 5.82 (d, J = 4.4 Hz, 1H), 5.05 (m, 2H), 4.36 (m, 1H), 3.96 (m, 1H), 3.62 (m, 1H), 3.55 (m, 1H), 2.48 (t, J = 7.4 Hz, 2H), 1.58 (t, J = 6.8 Hz, 2H), 1.22 (m, 24H), 0.85 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125.8 MHz) (DMSO-d_6) δ: 176.8, 157.1 (q, J = 36.5 Hz), 155.2, 149.4, 148.4, 138.3, 120.9, 116.0 (q, J = 288 Hz), 86.9, 84.1, 69.5, 61.6, 55.4, 36.3, 31.6, 31.0, 29.4, 29.3, 29.2, 29.0, 28.7, 24.7, 22.4, 14.3. ^{19}F NMR (376.5 MHz) (DMSO-d_6) δ: 2.25. HRMS calcd for $C_{28}H_{43}F_3N_6O_6$, [MH^+] 617.3274 (calcd), 617.3279 (found).

For **6b**: ^{1}H NMR (400.1 MHz) (DMSO-d_6) δ: 12.22 (br, 1H), 11.58 (br, 1H), 9.47 (br, 1H,), 8.43 (s, 1H), 6.27 (d, J = 7.9 Hz, 1H), 5.82 (br, 1H), 5.03 (t, J = 5.4 Hz, 1H), 4.90 (t, J = 6.6 Hz, 1H), 4.31 (m, 1H), 3.99 (m, 1H), 3.66 (m, 1H), 3.56 (m, 1H), 2.41 (t, J = 7.3 Hz, 2H), 1.55 (t, J = 6.8 Hz, 2H), 1.22 (m, 24H), 0.81 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100.6 MHz) (DMSO-d_6) δ: 176.6, 158.6, 157.0 (q, J = 38.4 Hz), 152.6, 147.8, 144.1, 116.0 (q, J = 288 Hz), 111.2, 87.1, 86.9, 69.5, 61.6, 57.2, 31.7, 29.4, 29.3, 29.2, 29.1, 28.7, 24.8, 22.9, 14.5. ^{19}F NMR (376.5 MHz) (DMSO-d_6) δ: 2.07. HRMS calcd for $C_{28}H_{43}F_3N_6O_6$, [MH$^+$] 617.3274 (calcd), 617.3269 (found).

4.1.7. 9-(2′-Amino-2′-deoxy-β-D-ribofuranosyl)guanine (7). To a pressure tube (15 mL) was added a solution of **5a** (100 mg, 154 μmol) in anhydrous EtOH (4 mL) followed by *n*-butylamine (1 mL). The mixture was heated overnight at 55 °C. The solution was cooled to room temperature, transferred to a flask, and evaporated to dryness. Water (40 mL) was added, and the resulting solution was extracted with CH_2Cl_2 (3 × 40 mL). The aqueous phase was evaporated to dryness, and the resulting solid was rinsed with acetone and recrystallized from water to give **7** (41.1 mg, 95%) as a white solid. ^{1}H NMR (500.1 MHz) (DMSO-d_6): 3.49 (m, 2H), 3.55 (m, 1H), 3.72 (m, 1H), 3.96 (m, 1H), 5.07 (br, 1H), 5.47 (d, J = 5 Hz, 1H), 5.48 (d, J = 8.5 Hz, 1H), 6.54 (br, 2H), 7.90 (s, 1H). ^{13}C NMR (100.6 MHz) (DMSO-d_6) δ: 156.8, 153.8, 151.7, 135.7, 116.8, 87.1, 86.3, 71.6, 61.9, 57.4. δ: HRMS calcd for $C_{10}H_{14}N_6O_4$, [MH]$^+$ 283.1155 (calcd), 283.1165 (found).

4.1.8. 7-(2′-Amino-2′-deoxy-β-D-ribofuranosyl)guanine (8). Using the procedure described for the preparation of **7**, **8** (20.2 mg, 93%) was obtained from **5b** as a white solid. ^{1}H NMR (400.1 MHz) (DMSO-d_6) δ: 8.24 (s, 1H), 6.20 (s, 2H), 5.76 (d, J = 8.0 Hz, 1H), 5.40 (br, 1H), 5.01 (m, 1H), 3.99 (m, 1H), 3.89 (m, 1H), 3.66 (m, 2H), 3.51 (m, 1H). ^{13}C NMR (100.6 MHz) (DMSO-d_6) δ: 161.5, 155.2, 153.6, 143.3, 108.7, 90.9, 87.1, 71.7, 62.5, 59.5. HRMS calcd for $C_{10}H_{14}N_6O_4$, [MH]$^+$ 283.1155 (calcd), 283.1167 (found).

4.1.9. 9-(N^2-Palmitoyl-5′-*O*-trimethylsilyl-2′-phthalimido-2′-deoxy-β-D-ribofuranosyl)guanine (9) and 7-(N^2-palmitoyl-5′-*O*-trimethylsilyl-2′-phthalimido-2′-deoxy-β-D-ribofuranosyl)guanine (10). 2′-*N*-Phthalimido-2′-deoxyuridine (**4a**) (464 mg, 1.25 mmol) was added to a pressure tube (15 mL) containing dry CH_3CN (10 mL) and *N*,*O*-bis(trimethylsilyl) acetamide (2.57 mL, 10.6 mmol, 8.6 equiv) under argon. To this homogeneous solution was added N^2-palmitoylguanine (873 mg, 2.25 mmol, 1.8 equiv). The resulting suspension was heated to reflux for 30 min, and the reaction mixture became a clear solution. Trimethylsilyl triflate (241 μL, 1.59 mmol, 1.3 equiv) was added, and the solution was refluxed for an additional 3 h. After the reaction solution was cooled to room temperature, CH_2Cl_2 (50 mL) and water (50 mL) were added. The aqueous layer was extracted with dichloromethane (50 mL). The organic phases were combined, dried over sodium sulfate, and evaporated to dryness. The residue was purified by silica gel chromatography, eluting with 5–7% MeOH in CH_2Cl_2, to give **9** (361 mg, 40%) and **10** (90.1 mg, 10%) as white solids.

For **9**: ^{1}H NMR (500.1 MHz) ($CDCl_3$) δ: 9.71 (br, 1H), 9.63 (br, 1H), 7.85 (s, 1H), 7.82 (m, 2H), 7.66 (m, 2H), 7.18 (d, J = 7.0 Hz, 1H), 5.31 (t, J = 6.9 Hz, 1H), 4.89 (d, j = 7.6 Hz, 1H), 4.68 (dd, J = 6.4, 3.6 Hz, 1H), 4.29 (m, 1H), 4.02 (d, J = 12.0 Hz, 1H), 3.74 (t, J = 11.2 Hz, 1H), 2.47 (m, 2H), 1.63 (t, J = 6.7 Hz, 2H), 1.25 (m, 24H), 0.85 (t, J = 6.8 Hz, 3H), −0.09 (s, 9H). ^{13}C NMR (125.8 MHz) (CD_3Cl) δ: 175.3, 168.3, 155.3, 147.6, 138.9, 134.3, 131.3, 123.3, 122.1, 99.6, 87.1, 83.8, 70.6, 61.8, 57.9, 37.0, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 24.6, 22.6, 14.0, −0.4. HRMS calcd for $C_{37}H_{54}N_6O_7Si$, [MNa$^+$] 745.3716 (calcd), 745.3724 (found).

For **10**: ^{1}H NMR (500.1 MHz) ($CDCl_3$) δ: 10.18 (br, 1H), 8.16 (s, 1H), 7.82 (m, 2H), 7.72 (m, 2H), 7.15 (d, J = 6.9 Hz, 1H), 5.20 (t, J = 7.3 Hz, 1H), 4.89 (dd, j = 7.5, 4.8 Hz, 1H), 4.40 (d, J = 8.8 Hz, 1H), 4.32 (m, 1H), 4.02 (m, 1H), 3.78 (m, 1H), 2.57 (m, 2H), 1.68 (t, J = 7.1 Hz, 2H), 1.23 (m, 24H), 0.86 (t, J = 7.0 Hz, 3H), −0.09 (s, 9H). ^{13}C NMR (125.8 MHz) (CD_3Cl) δ: 175.8, 168.5, 158.9, 152.7, 147.8, 144.5, 134.3, 131.5, 123.3, 110.8, 87.5, 85.3, 68.8, 61.0, 59.5, 37.1, 31.8, 29.6, 29.5, 29.4, 29.2, 28.9, 24.7, 22.6, 14.0, −0.5. HRMS calcd for $C_{34}H_{46}N_6O_7$, [MNa$^+$] 745.3715 (calcd), 745.3720 (found).

4.1.10. 5′-*O*-(4,4′-Dimethoxytrityl)-N^2-palmitoyl-2′-phthalimido-2′-deoxyguanosine (11a). N^2-Palmitoyl-2′-*N*-phthalimido-2′-deoxyguanosine (**5a**) (400 mg, 0.614 mmol) was dissolved in pyridine (5 mL), and 4,4′-dimethoxytrityl chloride (0.263 g, 0.740 mmol, 1.2 equiv) was added while the solution was stirring. After being stirred overnight at room temperature, the reaction was quenched with MeOH (1 mL), stirred for an additional 5 min, and then evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed consecutively with 5% sodium bicarbonate, water, and brine. The organic layer was dried over sodium sulfate and concentrated. The residue was purified by silica gel chromatography, eluting with 2–5% MeOH in CH_2Cl_2 containing 0.2% Et_3N, to give **11a** (480 mg, 82%) as a pale yellow foam. ^{1}H NMR (CD_2Cl_2): 11.97 (br, 1H), 9.33 (br, 1H), 9.47 (br, 1H), 7.46 (dd, J = 5.4, 3.1 Hz, 2H), 7.36 (m, 2H), 7.18 (m, 2H), 6.89–7.04 (m, 8H), 6.43 (m, 4H), 5.29 (m, 1H), 5.06 (br, 1H), 4.82 (m, 1H), 4.18 (m, 1H), 3.41 (s, 6H), 3.12 (m, 2H), 2.05 (m, 2H), 1.30 (m, 2H), 1.02 (m, 24H), 0.62 (t, J = 6.8 Hz, 3H). ^{13}C NMR (125.8 MHz) (CD_2Cl_2) δ: 175.8, 168.5, 158.5, 155.5, 148.3, 147.5, 145.0, 139.1, 136.1, 135.8, 134.2, 132.4, 131.5, 130.1, 130.0, 129.0, 128.2, 127.4, 127.6, 126.7, 123.3, 121.4, 113.0, 112.9, 86.2, 86.1, 69.7, 64.5, 56.8, 55.2, 55.1, 36.8, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.1, 24.3, 22.7, 13.9. HRMS calcd for $C_{55}H_{64}N_6O_9$, [MNa]$^+$ 975.4624 (calcd), 975.4627 (found).

4.1.11. 5′-*O*-(4,4′-Dimethoxytrityl)-N^2-palmitoyl-2′-trifluoroacetamido-2′-deoxyguanosine (11b). N^2-Palmitoyl-2′-trifluoroacetamido-2′-deoxguanosine (**5b**) (220 mg,

0.375 mmol) was dissolved in pyridine (3 mL), and 4,4′-dimethoxytrityl chloride (450 mg, 0.714 mmol, 1.2 equiv) was added while stirring the solution. 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. 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 4% MeOH in CH_2Cl_2 containing 0.2% Et_3N, to give **11b** (262 mg, 80%) as a white foam. ^1H NMR (500.1 MHz) (CD_2Cl_2) δ: 12.29 (br, 1H), 9.60 (br, 1H), 8.36 (br, 1H), 7.97 (s, 1H), 7.18-7.45 (m, 9H), 6.78 (m, 4H), 6.17 (br, 1H), 5.33 (br, 1H), 4.87 (br, 1H), 4.22 (br, 1H), 3.72 (s, 6H), 3.44 (m, 2H), 2.32 (br, 2H), 1.58 (br, 2H), 1.25 (m, 24H), 0.90 (t, $J = 7$ Hz, 3H). ^{13}C NMR (125.8 MHz) (CD_2Cl_2) δ: 176.0, 167.5, 158.6, 155.8, 149.3, 147.8, 144.6, 138.5, 135.6, 135.4, 134.9, 132.4, 130.8, 130.0, 129.9, 129.0, 128.6, 128.0, 127.9, 127.8, 127.7, 127.6, 127.0, 126.8, 120.3, 115.7 (q, $J = 287$ Hz), 113.0, 86.6, 85.6, 67.8, 63.9, 55.1, 46.3, 38.7, 36.8, 31.8, 30.3, 29.6, 29.5, 29.4, 29.3, 28.9, 24.4, 23.7, 22.9, 22.6, 13.8, 10.7. 8.8. ^{19}F NMR (376.5 MHz) (CD_2Cl_2) δ: 0.36. HRMS calcd for $C_{49}H_{61}F_3N_6O_8$, $[MNa]^+$ 941.4395 (calcd), 941.4366 (found).

4.1.12. 5′-*O*-(4,4′-Dimethoxytrityl)-N^2-palmitoyl-2′-*N*-phthalimido-2′-deoxyguanosine 3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (12a). 5′-*O*-(4,4′-Dimethoxytrityl)-N^2-palmitoyl-2′-trifluoroacetamido-2′-deoxyguanosine (**11a**) (80.0 mg, 83.9 μmol) was dissolved in dry CH_2Cl_2 (5 mL) and 1-methylimidazole (3.40 mg, 41.5 μmol). *N*,*N*-Diisopropylethylamine (78.0 mg, 0.420 mmol) was added to the stirring solution followed by 2-cyanoethyl *N*,*N*-(diisopropylchloro)-phosphoramidite (80.3 mg, 0.336 mmol). After being stirred at room temperature for 1 h, the reaction mixture was concentrated to dryness. The residue was then dissolved in ethyl acetate and washed with 5% aqueous sodium carbonate and brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography, eluting with 10–12% acetone in CH_2Cl_2 containing 0.2% Et_3N, to give **12a** (85.1 mg, 88%) as a white foam. ^{31}P NMR (202.5 MHz) (CD_3CN) 152.0 and 150.2 ppm. HRMS calcd for $C_{64}H_{81}N_8O_{10}P$, $[MNa]^+$1175.5706 (calcd), 1 175.5694 (found).

4.1.13. 5′-*O*-(4,4′-Dimethoxytrityl)-N^2-palmitoyl-2′-trifluoroacetamido-2′-deoxyguanosine 3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (12b). 5′-*O*-(4,4′-Dimethoxytrityl)-N^2-palmitoyl-2′-trifluoroacetamido-2′-deoxyguanosine (**11b**) (65.0 mg, 70.7 μmmol) was dissolved in dry CH_2Cl_2 (5 mL) and 1-methylimidozale (2.90 mg, 35.0 μmol). *N*,*N*-Diisopropylethylamine (67.0 mg, 0.360 mmol) was added to the stirring solution followed by 2-cyanoethyl *N*,*N*-(diisopropylchloro)phosphoramidite (67.0 mg, 283 μmol, 4.0 equiv). After being stirred at room temperature for 1 h, the reaction mixture was concentrated to dryness. The residue was then dissolved in ethyl acetate and 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 **12b** (59.3 mg, 75%) as a colorless oil. ^{31}P NMR (202.5 MHz) (CD_3CN) δ: 150.6 and 150.4 ppm. ^{19}F NMR (470.5 MHz) (CD_3CN) δ: −0.17 and −0.28 ppm. HRMS calcd for $C_{58}H_{78}F_3N_8O_9P$, $[MNa]^+$ 1141.5474 (calcd), 1141.5434 (found).

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30. We assigned the location of the trimethylsilyl group in **9** and **10** by a comparison of ^{1}H NMR and ^{13}C NMR spectra for compounds **5a**, **6a**, **9**, and **10**. The ^{1}H NMR spectrum of **9** contains two broad, D_2O exchangeable peaks at 9.71 and 9.63 ppm corresponding to the NH protons, thereby excluding the possibility that the trimethylsilyl group is bonded to the purine base. The *C*6 carbon resonance occurs at nearly the same ^{13}C chemical shift in compounds **9** (155.3 ppm) and **5a** (155.6 ppm), further suggesting that the trimethylsilyl group does not reside on O^6. The ^{1}H NMR spectrum (CD_3Cl) of **5a** contains a D_2O exchangeable doublet and triplet corresponding to the 3′- and 5′-hydroxyl groups, respectively. The ^{1}H NMR spectrum (CD_3Cl) of **9** contains the doublet but not the triplet, suggesting that the trimethylsilyl group resides on the 5′-oxygen.
31. The structure of **5a** was confirmed by comparison of its ^{1}H NMR spectrum to the literature data (see Ref. 29). The structure of **6a** was assigned on the basis of its ^{1}H NMR and ^{13}C NMR spectra. According to the literature (Chenon, M.-T.; Pugmire, R. J.; Grant, D. M. Panzica, R. P.; Townsend, L. B. *J. Am. Chem. Soc.* **1975**, *97*, 4627; Li, N.-S, Piccirilli, J. A. *Synthesis*, in press), the chemical shift of the purine *C*5 carbon may be used to distinguish the *N*-9 and *N*-7 isomers of guanosine derivatives. For the *N*-9 isomer, the chemical shift of *C*5 usually occurs near 120 ppm (DMSO-d_6), whereas for the *N*-7 isomer, the *C*5 resonance usually occurs near 110 ppm. Consistent with this trend, the *C*5 carbon of **5a** resonates at 121.2 ppm (DMSO-d_6). For **6a**, the *C*5 carbon resonates at 110.7 ppm (DMSO-d_6), consistent with the *N*-7 configuration.