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Preparation of Zwitterionic Ribonucleoside Phosphoramidites for Solid-Phase siRNA Synthesis

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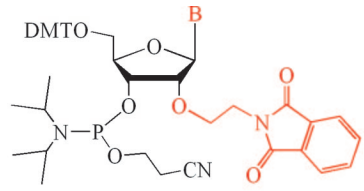

B= Uracil, N^4-Benzoylcytosine, N^6-Benzoyladenine, N^2-Isobutyrylguanine

RNA oligomers, carrying 2′-*O*-modified nucleosides, proved to be extremely useful in different antisense strategies, including RNAi. The 2′-*O*-alkyl modification, carrying an amino functionality, deserves special attention due to its ability to neutralize the negatively charged phosphate backbone, leading to improved physicochemical and pharmaceutical properties of antisense agents. Here, we report a very short, convenient, and straightforward synthesis of phosphoramidites for all four 2′-aminoethyl-modified natural ribonucleosides, where the aminoethyl group is introduced in a single alkylation step.

Introduction

Since its discovery RNAi has already proved to be one of the most powerful tools for investigating gene function and has shown great promise as being therapeutic for diseases, such as cancer and virus infections.[1,2] Despite great hopes of using siRNA for therapeutic purposes, some serious problems exist, such as activation of immune response, low serum stability, and poor in vivo delivery.[3–5] One way to try to overcome these limitations is to design chemically modified siRNAs. Among many possibilities the 2′-*O*-position of the sugar moiety in nucleosides has already proved to be optimal for oligonucleotide modifications in antisense technology.[1,3,6] Alkyl substituents with basic groups, such as amino, in this position enhance the resistance to nucleases,[6–9] and additionally they contribute to the thermal stability of RNA duplexes,[9,10] presumably due to neutralization of the negatively charged phosphate backbone. On the basis of these features, we decided to develop an easy access to the 2′-aminoethyl modification of all four natural ribonucleosides and their appropriate phosphoramidite building blocks for the solid-phase synthesis of oligonucleotides.

Generally, 2′-*O*-aminoethyl nucleosides are synthesized by alkylation of the 2′-OH group with methyl bromoacetate[9–12] or allylvinyl carbonate[12] followed by transformation of the corresponding functional groups to the 2-aminoethyl functionality. In the case of the 2′-*O*-methoxycarbonylmethyl group these steps involve reduction of the ester group to an alcohol, conversion of the latter to tosyl or mesyl derivatives, their replacement with azide, and, finally, reduction of the azide to an amino group, followed by acylation for protection. Conversion of the 2′-*O*-allyl group to aminoethyl includes two additional steps—dihydroxylation of the bond and oxidative cleavage of the 1,2-diol to aldehyde—and further steps are the same as for the ester.

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For pyrimidine nucleosides in the alkylation step only uridine or thymidine with protected 3′- and 5′-hydroxyl groups of the sugar moiety (TIPDS (1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) protection), as well as the N(3)-position of the nucleobase, were utilized.[11] Correspondingly cytidine or 5-methylcytidine derivatives were synthesized from 2′-*O*-trifluoroacetylaminoethyluridine or -thymidine derivatives, transforming the 4-oxo group to triazol-1-yl[12] or amidine-protected amino group.[11] As an alternative to alkylation, the 2′-*O*-aminoethylthymidine derivative was synthesized from 2,2′-anhydrothymidine with the borate of hydroxyethylphthalimide[13] or hydroxyethylphthalimide in the presence of $Ti(O\text{-}i\text{-}Pr)_4$.[14]

In contrast to uridine and thymidine, adenosine was alkylated with methyl bromoacetate without any protection.[9,12] 2′-*O*-Methoxycarbonylmethyladenosine was isolated and purified from the resulting mixture of 2′-, 3′-, and 5′-*O*-isomers by column chromatography.

In the synthesis of 2′-*O*-aminoethylguanosine unprotected 2-aminoadenosine was alkylated with benzyl 2-bromoethyl ether and, after isolation of the 2′-*O*-isomer from the resulting mixture, was enzymatically (with adenosine deaminase) converted to the guanosine derivative. Furthermore, after protection of ribose and nucleobase moieties, the benzyl protecting group was cleaved by hydrogenolysis, yielding the 2′-*O*-hydroxyethyl derivative, which then was converted to the aminoethyl derivative using the steps described above.[12]

The last and, probably, the most versatile reported approach to 2′-*O*-aminoethyl nucleosides includes the preparation of 2′-trifluoroacetylamino(or N-phthalimidino)ethyl-1′,3′,5′-triacetylribose and introduction of the corresponding nucleobase under Vorbrüggen conditions.[15–17] But again, synthesis of the ribose moiety includes the same steps as for uridine or thymidine (TIPDS protection, alkylation with methyl bromoacetate, etc.). It is worth mentioning that in some cases the number of synthetic steps was reduced by replacement of the hydroxy group with a phthalimido functionality under Mitsunobu conditions.[9,10,14–16] The advantage here is that the amino group already is protected (with phthaloyl residue) and could be used further without additional deprotection/protection steps.

In summarizing the syntheses described above, it is clear that for different nucleosides different approaches have to be used, which is a great disadvantage when several 2′-*O*-aminoethyl-modified nucleosides are required to be synthesized. Furthermore, all reported methods are multistep procedures, making these syntheses prolonged and material consuming. Here we report a short and very convenient synthesis of all four building blocks of 2′-*O*-aminoethyl nucleosides useful in the solid-phase synthesis of oligonucleotides.

Results and Discussion

For us, the shortest and most straightforward way to 2′-*O*-aminoethyl nucleosides seemed to be the direct alkylation of the 2′-OH group of nucleosides with XCH_2CH_2NPth (X = leaving group, NPth = *N*-phthalimide) type reagents, in analogy with derivatives containing a longer alkyl chain.[7,18] In that way the desired 2-aminoethyl group could be introduced in a single step. Surprisingly, only in one report did we find a brief note, that attempts to alkylate the 2′-OH group of adenosine with $BrCH_2CH_2NPth$ gave no results.[12] Due to these insufficient data, we decided to study this type of reaction. We chose 3′,5′-TIPDS protected uridine and, as an alkylating agent, the already mentioned $BrCH_2CH_2NPth$. For initial testing of the appropriate conditions we used NaH as a base and carried out the reaction in solvents such as CH_2Cl_2, THF, and DMF. Unfortunately, these attempts were all unsuccessful. Using THF or CH_2Cl_2 at temperatures below 20−25 °C the reaction did not take place, and at higher temperatures (above 25−30 °C) decomposition of the uridine derivative as well as elimination of $BrCH_2CH_2NPth$ to *N*-vinylphthalimide occurred, yielding complex mixtures. Carrying out the reaction in DMF, we observed decomposition of starting materials already at temperatures close to 0 °C. Thus, for further testing of this reaction with other reagents we used only CH_2Cl_2 and THF as solvents and the same base, NaH. Because our attempts to alkylate the 2′-OH group with $BrCH_2CH_2NPth$ failed, we decided to test in this reaction more reactive alkylating agents. For this purpose we synthesized a series of XCH_2CH_2NPth type compounds with increasing reactivity, where X is iodo, mesyl, tosyl, tresyl, and finally the trifluoromethanesulfonyl group.[19,20] Surprisingly to us, only with the last, most active species, the trifluoromethanesulfonyl derivative, did alkylation of 2′-OH took place and the 2′-*O*-phthalimidoethyluridine derivative **2a** was successfully isolated in moderate yield (Scheme 1). The aforementioned phthalimidoethyl triflate[21] is easy to synthesize and purify by column chromatography (see the Experimental Section), and it stays stable at least over 1 year when stored at −20 °C under argon.

Interestingly, carrying out the reaction in THF product **2a** was isolated in a much higher yield (65%), than in CH_2Cl_2 (30%).

Along with the desired compound **2a**, the double-alkylated side product **2a-1** was formed (Scheme 2). In contrast to the yields of the main product **2a**, the yield of compound **2a-1** was higher in CH_2Cl_2 (12%), than in THF (5%).

The same reaction conditions were applied for cytidine, but in that case we used not only 3′,5′-protection on the sugar moiety but also protection on the nucleobase (compound **1b**; Scheme 1). This avoids additional synthetic steps to protect the amino group of cytosine afterward and also improves solubility in THF or CH_2Cl_2. Thus, we successfully synthesized the 2′-*O*-phthalimidoethyl cytidine derivative **2b** (Scheme 1) in the same way as for uridine. The yield of the compound **2b**, as for **2a**, was also higher in THF (60%) than in CH_2Cl_2 (35%). Along with the main product we isolated two side products, **2b-1** and **2b-2** (Scheme 2), but whereas the first species was obtained in THF (5% yield) as well as in CH_2Cl_2 (12% yield), the formation of the second species was observed only in CH_2Cl_2 (11% yield).

Inspired by the successful syntheses of the 2′-*O*-phthalimidouridine and -cytidine derivatives **2a**,**b**, we also attempted to

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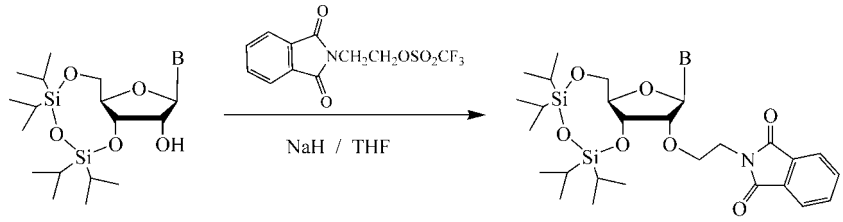
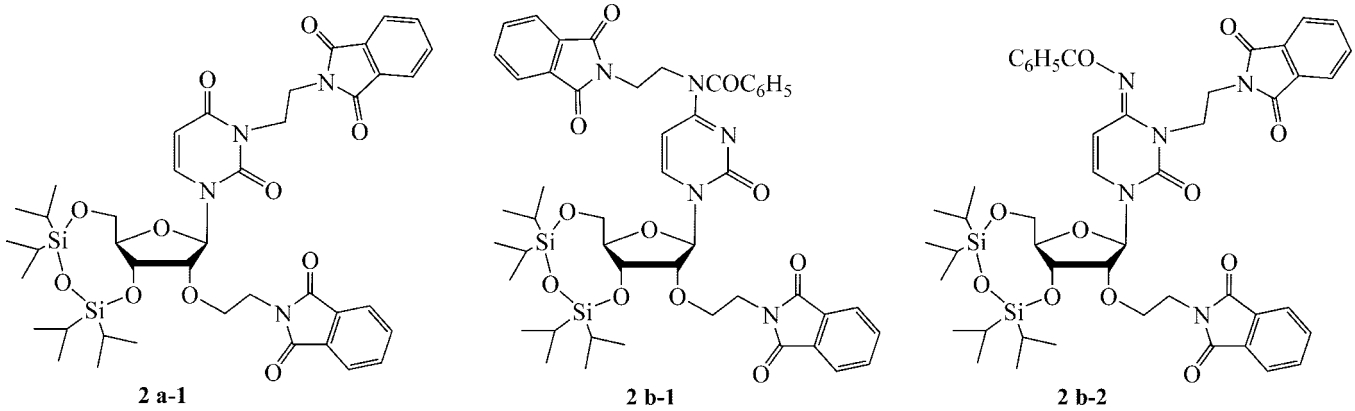
SCHEME 1



B = uracil (**1, 2 a**), N^4-benzoylcytosine (**1, 2 b**), adenine (**1, 2 c**), guanine (**1, 2 d**), N^6,N^6-dibenzoyladenine (**1, 2 e**).

SCHEME 2



synthesize the purinic nucleosides adenosine and guanosine. Here again we used THF as a solvent and NaH as a base, and as in the case for U and C, we used disilyl protection on the sugar moiety and nucleobases were also protected (adenine with benzoyl, guanine with isobutyryl groups). Unfortunately, syntheses of 2′-phthalimidoethyl derivatives of those compounds were unsuccessful. Complex mixtures of products (predominantly alkylated on the base moiety) formed in the reaction of 3′,5′-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine with phthalimidoethyl triflate. When dibenzoyl adenosine **1e** was used, the desired product **2e** was isolated only in 18% yield. Also, in this reaction we observed the formation of 3′,5′-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine, as well as a complex mixture of compounds similar to that mentioned above. In contrast, 3′,5′-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-N^2-isobutyrylguanosine did not react at all.

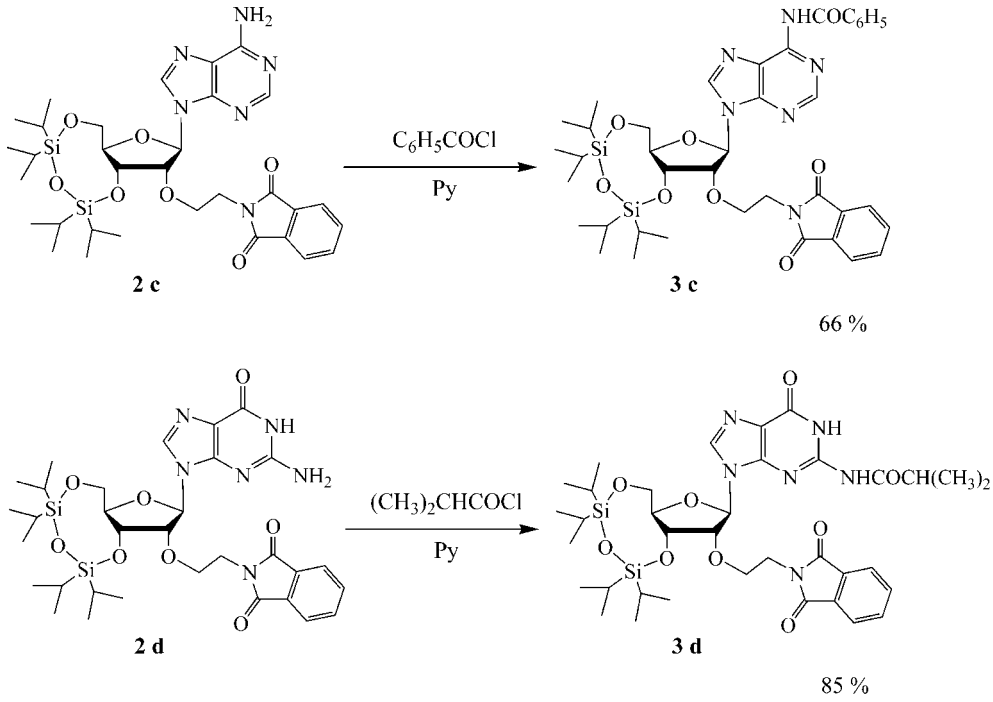
Surprisingly, when these alkylation reactions were performed with A and G having only a protected sugar moiety (compounds **1c**,**d**), the desired 2′-*O*-alkylated products **2c**,**d** were formed and isolated in 65% and 50% yields, respectively, and no double-alkylated products were observed (Scheme 1). Thus, using phthalimidoethyl triflate we successfully synthesized 2′-*O*-phthalimidoethyl derivatives of all four main natural ribonucleosides. Furthermore, we protected nucleobase moieties of compounds **2c**,**d** to the corresponding acyl derivatives **3c**,**d** (Scheme 3). Notably, for adenine along with the monobenzoylated derivative **3c**, the dibenzoylated compound **2e** formed (16%).

Further steps to the 2′-phthalimidoethyl phosphoramidites **6a**−**d** are shown in Scheme 4. TIPDS protection was removed from the derivatives of uridine (**2a**), cytidine (**2b**), and adenosine (**3c**) using TBAF in THF at 0 °C (yields 78% for **4a,** 82% for **4b**, and 97% for **4c**). For deprotection of the guanosine derivative **3d**, due to its low solubility, triethylamine trihydrofluoride in THF at 60 °C was used (82% yield for **4d**). Protection of the 5′-OH group of nucleosides **4a**−**d** with 4,4′-dimethoxytrityl chloride in pyridine yielded the corresponding 5′-DMTr derivatives **5a**−**d** (82−90% yields). Finally, in the last step all four compounds were phosphitylated with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite using 4,5-dicyanoimidazole (DCI) as the activator to obtain the corresponding phosphoramidites **6a**−**d** (80% yields for **6a**−**c**, 72% for **6d**).

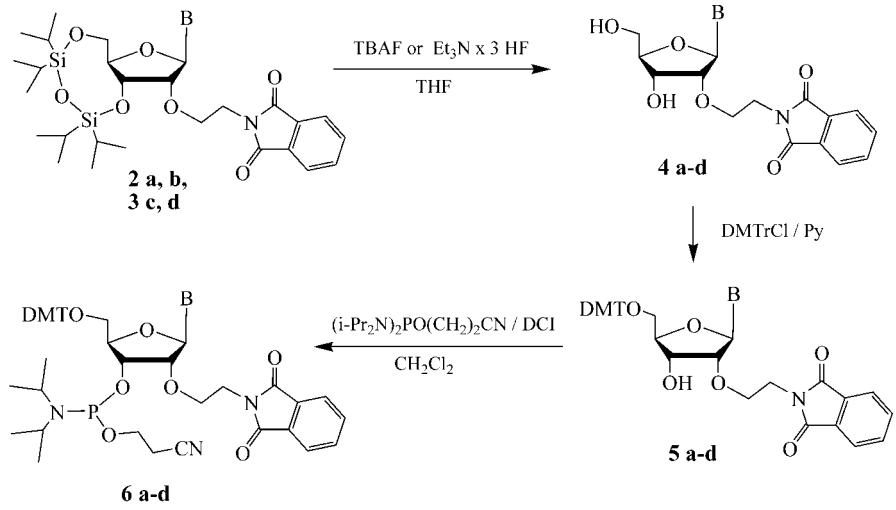
The overall yields were 35% for the final uridine and cytidine derivatives **6a**,**b**, 27% for adenosine **6c**, and 23% for guanosine **6d**, and the synthesis of each phosphoramidite included only five (for U and C) or six steps (for A and G). For comparison, the overall yields in previously reported syntheses of phosphoramidites of 2′-*O*-aminoethyl-modified natural ribonucleosides[12] were at least 2 times lower (17% for U, 9% for C, 12% for A, and 6% for G), while the number of synthetic steps nearly doubled (11−12).

All of the phosphoramidites **6a**−**d** are being used for the syntheses of modified 21−22-mers of siRNAs for testing in RNAi assays, and some of these results have already been published.[9] One should consider that for the preparation of oligonucleotides, containing 2′-aminoethyl-modified nucleosides, full removal of the phthaloyl protection from amino groups requires, after the solid-phase synthesis, prolonged (48 h) treatment with ammonium hydroxide at high temperature (40 °C).

SCHEME 3



SCHEME 4



B = uracil (**2, 4-6a**), N-benzoylcytosine (**2, 4-6b**), N-benzoyladenine (**3, 4-6c**), N-isobutyryl-guanine (**3, 4-6 d**).

Conclusion

We have described here a very short, convenient, and straightforward approach to phosphoramidites of all four 2′-aminoethyl-modified natural ribonucleosides, where the 2′-*O*-phthalimidoethyl group was introduced in a single alkylation step. Also, we show that it is possible to synthesize 2′-modified analogues of U, C, A, and G using the same alkylation procedure for all of them, and there is no greatly observable difference in reactivity.

Experimental Section

Phthalimidoethyl Triflate. This compound was synthesized according to the procedure described in ref 21 with some modifications. A solution of trifluoromethanesulfonic anhydride (5 mL, 8.55 g, 0.03 mol) in 30 mL of CH_2Cl_2 was cooled to 0 °C and a solution of *N*-(2-hydroxyethyl)phthalimide (5 g, 0.0262 mol) and *N*,*N*-diisopropylethylamine (4.48 mL, 3.38 g, 0.0262 mol) in 100 mL of CH_2Cl_2 was added dropwise. The reaction mixture was warmed to room temperature, stirred for 1 h, washed with saturated $NaHCO_3$ (2 × 200 mL), dried over $MgSO_4$, and evaporated at room or lower temperature (heating must be avoided) to dryness. The residue was purified by column chromatography using CH_2Cl_2 as the eluant to yield 7.8 g (92%) of phthalimidoethyl triflate as a grayish solid (yield 60%, mp 79 °C (hexane)[19]). $R_f = 0.6$ (CH_2Cl_2). ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.86 (2H, t, J = 4.9 Hz, CH_2Pth), 4.40 (2H, t, J = 4.9 Hz, OCH_2), 7.82 (4H, m, Pth H_4); ESI-MS: m/z calcd 323.3 $[M]^+$, found 323.5. Anal. Calcd for $C_{11}H_8F_3NO_5S$: C, 40.87; H, 2.49; N, 4.33. Found: C, 41.04; H, 2.67; N, 4.37.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-phthalimidoethyl)uridine (2a). A suspension of NaH (0.72 g, 18 mmol, 60% dispersion in oil) in 15 mL of THF was cooled to −40 °C, and a solution of **1a** (3.65 g, 7.5 mmol) in 25 mL of THF was added dropwise. The mixture was slowly warmed to 0 °C, stirred for 1 h, stirred additionally for 0.5 h at room temperature, and cooled to 0 °C. Then a one-third portion of a solution of phthalimidoethyl triflate (3.39 g, 10.5 mmol) in 10 mL of THF was added dropwise and the reaction mixture was stirred at 0 °C for 1 h. After addition of 20 mL of CH_2Cl_2, the reaction mixture was stirred at 0 °C for 1 h, the rest of the phthalimidoethyl triflate solution in THF was added dropwise over 1 h, and the mixture was further stirred at 0 °C for 3 h. The reaction mixture was neutralized with glacial acetic acid and evaporated to dryness. The residue was dissolved in 100 mL of CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 150 mL) and then with saturated NaCl (1 × 150 mL), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography using 5−30% AcOEt in CH_2Cl_2 as the eluant and collecting fractions with $R_f = 0.7$ (1/1 AcOEt/CH_2Cl_2) to produce 3.22 g (65%) of **2a** as a white foam (30% yield, when the reaction was carried out in CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.67 (1H, q, $J = 7.3$ Hz, Si*CH*CH$_3$), 0.78 (3H, d, $J = 7.3$ Hz, SiCH*CH*$_3$), 0.83−1.01 (24H, m, TIPDS H$_{24}$), 3.74−3.98 (6H, m, 4′,5′-H$_2$, α,β-(CH$_2$)$_2$), 4.05−4.10 (2H, m, 2′,5″-H$_2$), 4.19 (1H, dd, $J = 9.1$, 4.8 Hz, 3′-H), 5.51 (1H, d, $J = 8.1$ Hz, 5-H), 5.54 (1H, s, 1′-H), 7.61 (1H, d, $J = 8.1$ Hz, 6-H), 7.85 (4H, m, Pth H$_4$), 11.36 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.6, 12.13, 12.18, 12.5 (Si*C*HCH$_3$), 16.52, 16.67, 16.73, 16.77, 17.01, 17.06, 17.13, 17.24 (SiCH*C*H$_3$), 37.6 (C-β), 59.5 (C-5′), 67.4 (C-α), 68.3 (C-3′), 80.7 (C-4′), 81.0 (C-2′), 88.8 (C-1′), 100.9 (C-5), 122.9, 131.5, 134.3 (Pth), 139.6 (C-6), 149.9 (C-2), 163.2 (C-4), 167.7 (CO); ESI-MS: *m*/*z* calcd 660.3 [M + H]$^+$, found 660.3. Anal. Calcd for $C_{31}H_{45}N_3O_9Si_2$: C, 56.42; H, 6.87; N, 6.37. Found: C, 56.26; H, 6.68; N, 6.11.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*,*N*^{3}-bis(2-phthalimidoethyl)uridine (2a-1). This compound was isolated from the reaction described above (synthesis of **2a**) collecting fractions with $R_f = 0.9$ (1/2 AcOEt/CH_2Cl_2) to produce 0.31 g (5%) of **2a-1** as a white foam (yield 12%, when reaction was carried out in CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.63 (1H, q, $J = 7.6$ Hz, Si*CH*CH$_3$), 0.78 (3H, d, $J = 7.6$ Hz, SiCH*CH*$_3$), 0.87 (3H, d, $J = 7.6$ Hz, SiCH*CH*$_3$), 0.95−1.03 (21H, m, TIPDS H$_{21}$), 3.69−3.96 (10H, m, 2′,3′,4′,5′-H$_4$, α,β-(CH$_2$)$_2$, β′-CH$_2$, α′-CH), 4.08−4.14 (3H, m, 3′,5″-H$_2$, α′-CH), 5.50 (1H, s, 1′-H), 5.53 (1H, d, $J = 8.1$ Hz, 5-H), 7.62 (1H, d, $J = 8.1$ Hz, 6-H), 7.80 (4H, m, Pth H$_4$), 7.84 (4H, m, Pth H$_4$); ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.6, 12.2, 12.7 (Si*C*HCH$_3$), 16.48, 16.67, 16.79, 17.03, 17.07, 17.14, 17.26 (SiCH*C*H$_3$), 35.2 (C-β′), 37.7 (C-β), 39.1 (C-α′), 59.3 (C-5′), 67.7 (C-α), 67.8 (C-3′), 81.0 (C-2′), 81.7 (C-4′), 88.9 (C-1′), 99.9 (C-5), 122.7, 122.9, 131.4, 131.6, 134.2, 134.4 (Pth), 137.4 (C-6), 149.9 (C-2), 162.0 (C-4), 167.68, 167.75 (CO); ESI-MS: *m*/*z* calcd 832.3 [M]$^+$, found 832.8. Anal. Calcd for $C_{41}H_{52}N_4O_{11}Si_2$: C, 59.11; H, 6.29; N, 6.73. Found: C, 59.45; H, 6.35; N, 6.34.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-phthalimidoethyl)-*N*^{4}-benzoylcytidine (2b). A suspension of NaH (0.72 g, 18 mmol, 60% dispersion in oil) in 25 mL of THF was cooled to −40 °C, and a solution of **1b** (4.42 g, 7.5 mmol) in 25 mL of THF was added dropwise. The mixture was slowly warmed to 0 °C and stirred for 2 h. Then a solution of phthalimidoethyl triflate (3.39 g, 10.5 mmol) in 12 mL of THF was added dropwise in three parts every 1 h to the reaction mixture with stirring at 0 °C. After addition of the last part of the solution of phthalimidoethyl triflate the reaction mixture was stirred for 1 h at 0 °C and cooled to −40 °C and NaH (0.17 g, 4.3 mmol, 60% dispersion in oil) was added. The reaction mixture was slowly warmed to 0 °C, and a solution of phthalimidoethyl triflate (1.37 g, 4.3 mmol) in 5 mL of THF was added dropwise. The reaction mixture was additionally stirred for 2 h at 0 °C, neutralized with glacial acetic acid, and evaporated to dryness. The residue was dissolved in 150 mL of CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 200 mL) and then with saturated NaCl (1 × 200 mL), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography using 5−70% AcOEt in CH_2Cl_2 as eluant and collecting fractions with $R_f = 0.4$ (1/1 AcOEt/CH_2Cl_2) to produce 3.43 g (60%) of **2b** as a white foam (yield 35%, when reaction was carried out in CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.63 (1H, q, $J = 7.3$ Hz, Si*CH*CH$_3$), 0.77 (3H, d, $J = 7.3$ Hz, SiCH*CH*$_3$), 0.83−0.88 (10H, m, TIPDS H$_{10}$), 1.02−1.06 (14H, m, TIPDS H$_{14}$), 3.78−4.09 (7H, m, 2′,4′,5′-H$_3$, α,β-(CH$_2$)$_2$), 4.13 (1H, dd, $J = 9.6$, 4.3 Hz, 3′-H), 4.20 (1H, m(d), 5″-H), 5.68 (1H, s, 1′-H), 7.37 (1H, d, $J = 7.6$ Hz, 5-H), 7.52 (2H, dd, $J_{3,5-4} = 7.8$ Hz, $J_{3-2,5-6} = 7.3$ Hz, Bz H$_2$), 7.64 (1H, m, Bz H$_1$), 7.85 (4H, m, Pth H$_4$), 8.02 (2H, dd, $J_{2-3,6-5} = 7.3$ Hz, $J_{2,6-4} = 1.3$ Hz, Bz H$_2$), 8.12 (1H, d, $J = 7.6$ Hz, 6-H), 11.31 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.6, 12.3, 12.4, 12.5 (Si*C*HCH$_3$), 16.51, 16.67, 16.72, 17.02, 17.08, 17.16, 17.27 (SiCH*C*H$_3$), 37.7 (C-β), 59.3 (C-5′), 67.57 (C-α), 67.59 (C-3′), 81.0 (C-4′), 81.4 (C-2′), 89.1 (C-1′), 95.7 (C-5), 122.9 (Pth), 128.38, 128.42 (Bz), 131.6 (Pth), 132.7, 133.0 (Bzl), 134.2 (Pth), 143.4 (C-6), 154.0 (C-2), 163.2 (C-4), 167.3, 167.7 (CO). ESI-MS: *m*/*z* calcd 762.3 [M]$^+$, found 762.6. Anal. Calcd for $C_{38}H_{50}N_4O_9Si_2$: C, 59.82; H, 6.61; N, 7.31. Found: C, 60.08; H, 6.81; N, 7.13.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*,*N*^{4}-bis(2-phthalimidoethyl)-*N*^{4}-benzoylcytidine (2b-1). This compound was isolated from the reaction described above (synthesis of **2b**), collecting fractions with $R_f = 0.9$ (1/1 AcOEt/CH_2Cl_2) to produce 0.35 g (5%) of **2b-1** as a white foam (yield 12%, when reaction was carried out in CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.62 (1H, q, $J = 7.3$ Hz, Si*CH*CH$_3$), 0.77 (3H, d, $J = 7.3$ Hz, SiCH*CH*$_3$), 0.86 (3H, d, $J = 7.3$ Hz, SiCH*CH*$_3$), 0.94−1.01 (21H, m, TIPDS H$_{21}$), 3.68−3.90 (7H, m, 2′,4′,5′-H$_3$, α,β-(CH$_2$)$_2$), 4.00−4.15 (4H, m, 3′,5″-H$_2$, β′-CH$_2$), 4.31 (1H, m, α′-CH), 4.45 (1H, m, α′-CH), 5.52 (1H, s, 1′-H), 6.38 (1H, d, $J = 8.1$ Hz, 5-H), 7.40 (2H, dd, $J_{3,5-4} = 7.8$ Hz, $J_{3-2,5-6} = 7.3$ Hz, Bz H$_2$), 7.53−7.58 (2H, m, Bz H$_1$, 6-H), 7.74 (4H, m, Pth H$_4$), 7.86 (4H, m, Pth H$_4$), 7.92 (2H, dd, $J_{2-3,6-5} = 7.3$ Hz, $J_{2,6-4} = 1.3$ Hz, Bz H$_2$). ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.5, 12.1, 12.2, 12.6 (Si*C*HCH$_3$), 16.41, 16.59, 16.70, 16.90, 16.99, 17.17 (SiCH*C*H$_3$), 34.8 (C-β′), 37.6 (C-β), 41.0 (C-α′), 59.2 (C-5′), 67.6 (C-α), 67.8 (C-3′), 81.0 (C-2′), 81.5 (C-4′), 88.9 (C-1′), 96.7 (C-5), 122.7, 122.9 (Pth), 128.1, 128.9 (Bz), 131.4, 131.5 (Pth), 132.1 (Bz), 134.1, 134.2 (Pth), 135.8 (Bz), 136.1 (C-6), 149.1 (C-2), 156.7 (C-4), 167.6, 167.8, 175.4 (CO). ESI-MS: *m*/*z* calcd 936.4 [M + H]$^+$, found 936.3. Anal. Calcd for $C_{48}H_{57}N_5O_{11}Si_2$: C, 61.58; H, 6.14; N, 7.48. Found: C, 61.70; H, 6.22; N, 7.40.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*,*N*^{3}-bis(2-phthalimidoethyl)-*N*^{4}-benzoylcytidine (2b-2). This compound was isolated from the reaction described above (synthesis of **2b**), when it was carried out in CH_2Cl_2, collecting fractions with $R_f = 0.7$ (1/1 AcOEt/CH_2Cl_2) to produce 11% of **2b-2** as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.60 (2H, m (q), 2 × Si*CH*CH$_3$), 0.72 and 0.74 (total 6H, each d, $J = 7.1$ Hz, 2 × SiCH*CH*$_3$), 0.81−0.86 (13H, m, TIPDS H$_{13}$ (1 × Si*CH*CH$_3$, 4 × SiCH*CH*$_3$)), 0.94−1.01 (7H, m, TIPDS H$_{21}$ (1 × Si*CH*CH$_3$, 2 × SiCH*CH*$_3$)), 3.74−4.00 (10H, m, 2′,3′,4′,5′-H$_4$, α,β-(CH$_2$)$_2$, β′-CH$_2$), 4.07 (1H, m(d), 5″-H), 4.22 (1H, m, α′-CH), 4.43 (1H, m, α′-CH), 5.47 (1H, s, 1′-H), 5.51 (1H, d, $J = 7.3$ Hz, 5-H), 7.35 (2H, dd, $J_{3,5-4} = 7.8$ Hz, $J_{3-2,\ 5-6} = 7.1$ Hz, Bz H$_2$), 7.41 (2H, d, $J_{2-3,\ 6-5} = 7.1$ Hz, Bz H$_2$), 7.50 (1H, m, Bzl-H$_1$), 7.65 (1H, d, $J = 7.3$ Hz, 6-H), 7.81 (4H, m, Pth H$_4$), 7.86 (4H, m, Pth H$_4$), ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.49, 11.51, 12.1, 12.4 (Si*C*HCH$_3$), 16.38, 16.54, 16.63, 16.70, 16.79, 16.90, 17.10, 17.28 (SiCH*C*H$_3$), 36.6 (C-β′), 37.6 (C-β), 45.4 (C-α′), 59.1 (C-5′), 67.42 (C-α), 67.48 (C-3′), 80.7 (C-2′), 81.2 (C-4′), 88.7 (C-1′), 100.4 (C-5), 122.9, 123.0 (Pth), 128.5 (Bz), 131.49, 131.56 (Pth), 131.74 (Bz), 134.25, 134.29 (Pth), 134.8 (Bzl), 140.6 (C-6), 153.3 (C-2), 166.1 (C-4), 167.7, 167.8, 168.0, 171.2 (CO); ESI-MS: *m*/*z* calcd 936.4 [M +

H]$^+$, found 936.3. Anal. Calcd for $C_{48}H_{57}N_5O_{11}Si_2$: C, 61.58; H, 6.14; N, 7.48. Found: C, 61.83; H, 6.21; N, 7.51.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-phthalimidoethyl)adenosine (2c). A suspension of NaH (0.26 g, 6.5 mmol, 60% dispersion in oil) in 10 mL of THF was cooled to −40 °C, and a solution of **1c** (2.55 g, 5 mmol) in 25 mL of THF was added dropwise. The mixture was slowly warmed to 0 °C and stirred for 1 h, and a solution of phthalimidoethyl triflate (2.1 g, 6.5 mmol) in 12 mL of THF was added dropwise in two parts every 1 h to the reaction mixture with stirring at 0 °C. After addition of the last part of the solution of phthalimidoethyl triflate the reaction mixture was stirred for 2 h at 0 °C and for 0.5 h at room temperature. Then the mixture was cooled to −40 °C, NaH (0.12 g, 3 mmol, 60% dispersion in oil) was added to it, and the mixture was slowly warmed to 0 °C and stirred for 0.5 h. After dropwise addition of a solution of phthalimidoethyl triflate (0.97 g, 3 mmol) in 3 mL of THF the reaction mixture was additionally stirred for 1 h at 0 °C, neutralized with glacial acetic acid, and evaporated to dryness. The residue was dissolved in 100 mL of CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 150 mL) and then with saturated NaCl (1 × 150 mL), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography using 20−100% AcOEt in CH_2Cl_2 and then 0−3% CH_3OH in AcOEt as eluants and collecting fractions with R_f = 0.8 (3% CH_3OH/AcOEt) to produce 2.22 g (65%) of **2c** as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.77 (1H, q, J = 7.1 Hz, Si*CH*CH_3), 0.85 (3H, d, J = 7.1 Hz, SiCH*CH*$_3$), 0.93 (3H, d, J = 7.3 Hz, SiCH*CH*$_3$), 0.96−1.01 (21H, m, TIPDS H_{21}), 3.76−3.89 (4H, m, 4′,5′-H_2, β-CH_2), 3.94 (1H, m, α-CH), 4.00−4.06 (2H, m, 5″-H, α-CH), 4.47 (1H, d, J = 4.8 Hz, 2′-H), 4.90 (1H, dd, J = 8.8, 4.8 Hz, 3′-H), 5.88 (1H, s, 1′-H), 7.30 (2H, s, NH_2), 7.81 (4H, m, Pth H_4), 7.96 (1H, s, 2-H), 8.15 (1H, s, 8-H). ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.8, 12.1, 12.3, 12.6 (Si*CH*CH_3), 16.60, 16.73, 16.83, 16.86, 17.04, 17.09, 17.24 (SiCH*CH*$_3$), 37.9 (C-β), 60.0 (C-5′), 67.7 (C-α), 69.8 (C-3′), 80.4 (C-4′), 81.0 (C-2′), 87.6 (C-1′), 119.2 (C-5), 122.9, 131.4, 134.2 (Pth), 139.2 (C-8), 148.4 (C-4), 152.3 (C-2), 156.1 (C-6), 167.7 (CO). ESI-MS: *m*/*z* calcd 683.3 [M + H]$^+$, found 683.6. Anal. Calcd for $C_{32}H_{46}N_6O_7Si_2$: C, 56.28; H, 6.79; N, 12.31. Found: C, 56.18; H, 6.76; N, 12.44.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-phthalimidoethyl)guanosine (2d). A suspension of NaH (0.48 g, 12 mmol, 60% dispersion in oil) in 45 mL of THF was cooled to −20 °C, and **1d** (2.63 g, 5 mmol) was added. The mixture was slowly warmed to 0 °C, stirred at this temperature for 1 h and then additionally stirred for 0.5 h at room temperature, and cooled to 0 °C. A solution of phthalimidoethyl triflate (2.26 g, 7 mmol) in 5 mL of THF was added dropwise in two parts every 1 h to the reaction mixture with stirring at 0 °C. After addition of the last part of the solution of phthalimidoethyl triflate the reaction mixture was stirred for 2 h at 0 °C and 0.5 h at room temperature. Then the mixture was cooled to −20 °C, NaH (0.12 g, 3 mmol, 60% dispersion in oil) was added to it, and this mixture was slowly warmed to 0 °C and stirred for 0.5 h. After dropwise addition of a solution of phthalimidoethyl triflate (0.97 g, 3 mmol) in 3 mL of THF the reaction mixture was additionally stirred for 2 h at 0 °C, neutralized with glacial acetic acid, and evaporated to dryness. The residue was purified by column chromatography as eluant using 1−15% CH_3OH in AcOEt and collecting fractions with R_f = 0.7 (1/3 CH_3OH/AcOEt). The fractions containing the mixture of the starting material and the product were collected and purified again by column chromatography. The product was crystallized from methanol to produce 1.75 g (50%) of **2d** as a white crystals. Mp: >260 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.71−0.81 (4H, m, TIPDS H_4 (Si*CH*CH_3, SiCH*CH*$_3$)), 0.89−0.96 (10H, m, TIPDS H_{10} (Si*CH*CH_3, 3 × SiCH*CH*$_3$)), 1.00−1.06 (14H, m, TIPDS H_{14} (2 × Si*CH*CH_3, 4 × SiCH*CH*$_3$)), 3.76−3.95 (5H, m, 4′,5′-H_2, α-CH, β-CH_2), 4.00−4.06 (2H, m, 5″-H, α-CH), 4.24 (1H, d, J = 4.8 Hz, 2′-H), 4.38 (1H, dd, J = 8.6, 4.8 Hz, 3′-H), 5.72 (1H, s, 1′-H), 6.45 (2H, s, NH_2), 7.68 (1H, s, 8-H), 7.83 (4H, m, Pth H_4), 10.67 (1H, s, NH); ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.7, 12.2, 12.3, 12.6 (Si*CH*CH_3), 16.54, 16.68, 16.81, 17.04, 17.07, 17.10, 17.23 (SiCH*CH*$_3$), 37.7 (C-β), 59.8 (C-5′), 67.6 (C-α), 69.4 (C-3′), 80.6 (C-4′), 81.0 (C-2′), 86.3 (C-1′), 116.7 (C-5), 122.9, 131.5 (Pth), 133.9 (C-8), 134.3 (Pth), 150.4 (C-2), 153.6 (C-4), 156.6 (C-6), 167.7 (CO); ESI-MS: *m*/*z* calcd 699.3 [M + H]$^+$, found 699.5. Anal. Calcd for $C_{32}H_{46}N_6O_8Si_2$: C, 54.99; H, 6.63; N, 12.02. Found: C, 54.73; H, 6.60; N, 11.95.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-phthalimidoethyl)-*N*^{6},*N*^{6}-dibenzoyladenosine (2e). A suspension of NaH (0.086 g, 2.15 mmol, 60% dispersion in oil) in 3 mL of THF was cooled to −60 °C, and a solution of **1e** (1.03 g, 1.433 mmol) in 12 mL of THF was added dropwise. The mixture was warmed to 0 °C, stirred for 10 min, and cooled to −30 °C. Then a solution of phthalimidoethyl triflate (0.6 g, 1.86 mmol) in 5 mL of THF was added dropwise. The reaction mixture was stirred as it was slowly warmed to 0 °C (in 2 h) and then cooled to −20 °C. Glacial acetic acid (0.02 mL, 0.35 mmol) was added to the reaction mixture, and this mixture was then warmed to room temperature and evaporated to dryness. The residue was dissolved in 50 mL of CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 150 mL) and then with saturated NaCl (1 × 100 mL), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography using 5−20% AcOEt in CH_2Cl_2 as eluant and collecting fractions with R_f = 0.75 (1/9 AcOEt/CH_2Cl_2) to produce 0.23 g (18%) of **2e** as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.75−0.87 (8H, m, TIPDS H_8 (2 × Si*CH*CH_3, 2 × SiCH*CH*$_3$)), 0.92 and 0.94 (6H, m, 2 × SiCH*CH*$_3$), 0.99−1.02 (7H, m, TIPDS H_{14} (2 × Si*CH*CH_3, 4 × SiCH*CH*$_3$)), 3.75−3.92 (4H, m, 4′,5′-H_2, β-CH_2), 3.93−4.08 (3H, m, 5″-H, α-CH_2), 4.71 (1H, d, J = 4.8 Hz, 2′-H), 4.91 (1H, dd, J = 8.9, 4.8 Hz, 3′-H), 6.02 (1H, s, 1′-H), 7.48 (4H, dd, $J_{3,5-4}$ = 7.8 Hz, $J_{3-2,5-6}$ = 7.5 Hz, 2 × Bz H_2), 7.61 (2H, m, 2 × Bz H_1), 7.80 (4H, dd, $J_{2-3,6-5}$ = 7.5 Hz, $J_{2,6-4}$ = 1.3 Hz, 2 × Bz H_2), 7.82 (4H, m, Pth H_4), 8.51 (1H, s, 2-H), 8.64 (1H, s, 8-H). ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.8, 12.2, 12.3, 12.6 (Si*CH*CH_3), 16.59, 16.72, 16.84, 16.88, 16.93, 16.98, 17.08, 17.21 (SiCH*CH*$_3$), 37.7 (C-β), 59.6 (C-5′), 67.1 (C-α), 70.5 (C-5′), 80.0 (C-2′), 80.6 (C-4′), 88.1 (C-1′), 123.0 (Pth), 127.4 (C-5), 128.97, 129.02 (Bz), 131.5 (Pth), 133.37, 133.43 (Bz), 134.3 (Pth), 145.1 (C-8), 150.9 (C-4), 151.4 (C-6), 151.8 (C-2), 167.7, 172.0 (CO). ESI-MS: *m*/*z* calcd 891.3 [M + H]$^+$, found 891.6. Anal. Calcd for $C_{46}H_{54}N_6O_9Si_2$: C, 62.00; H, 6.11; N, 9.43. Found: C, 62.27; H, 6.21; N, 9.33.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-phthalimidoethyl)-*N*^{6}-benzoyladenosine (3c). To a solution of **2c** (2.4 g, 3.51 mmol) in 40 mL of pyridine cooled to 0 °C was added benzoyl chloride (0.41 mL, 0.49 g, 3.51 mmol) dropwise, and the mixture was stirred at 0 °C for 1 h. Then benzoyl chloride (0.41 mL, 0.49 g, 3.51 mmol) was again added dropwise; after this mixture was stirred at 0 °C for 1 h, it was evaporated to dryness. The residue was dissolved in 150 mL of CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 200 mL) and then with saturated NaCl (1 × 200 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography using 5−100% AcOEt in CH_2Cl_2 and then 1−5% CH_3OH in AcOEt as eluants and collecting fractions with R_f = 0.6 (1/1 AcOEt/CH_2Cl_2) to produce 1.81 g (66%) of **3c** as a white foam (also, during purification **2e** (0.5 g (16%)) was isolated). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.80 (1H, m (q), 2 × Si*CH*CH_3), 0.88 (3H, d, J = 7.1 Hz, SiCH*CH*$_3$), 0.94−1.04 (23H, m, TIPDS H_{23}), 3.78−3.90 (4H, m, 4′,5′-H_2, β-CH_2), 3.95−4.09 (3H, m, 5″-H, α-CH_2), 4.62 (1H, d, J = 5.1 Hz, 2′-H), 4.94 (1H, dd, J = 9.1, 5.1 Hz, 3′-H), 6.01 (1H, s, 1′-H), 7.57 (2H, dd, $J_{3,5-4}$ = $J_{3-2,5-6}$ = 7.6 Hz, Bz H_2), 7.67 (1H, m, Bz H_1), 7.81 (4H, m, Pth H_4), 8.07 (2H, d, $J_{2-3,6-5}$ = 7.6 Hz, Bz H_2), 8.49 (1H, s, 8-H), 8.54 (1H, s, 2-H), 11.21 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.8, 12.1, 12.3, 12.6 (Si*CH*CH_3), 16.59, 16.72, 16.83, 16.87, 17.02, 17.06, 17.08, 17.20 (SiCH*CH*$_3$), 37.8 (C-β), 59.8 (C-5′), 67.7 (C-α), 80.49 (C-4′), 80.53 (C-2′), 87.9 (C-1′), 122.9 (Pth), 125.9 (C-5), 128.5 (Bz), 131.4 (Pth),

132.8 (Bz), 134.3 (Pth), 143.3 (C-8), 150.3 (C-4), 151.20 (C-6), 151.26 (C-2), 165.5, 167.7 (CO). ESI-MS: *m/z* calcd 787.3 $[M + H]^+$, found 787.5. Anal. Calcd for $C_{39}H_{50}N_6O_8Si_2$: C, 59.52; H, 6.40; N, 10.68. Found: C, 59.53; H, 6.52; N, 10.56.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-phthalimidoethyl)-*N*²-isobutyrylguanosine (3d). To a solution of **2d** (1.75 g, 2.5 mmol) in 15 mL of pyridine cooled to 0 °C was added isobutyryl chloride (0.66 mL, 0.67 g, 6.25 mmol) dropwise. The mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h and evaporated to dryness. The residue was dissolved in 150 mL CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 250 mL) and then with saturated NaCl (1 × 250 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography using 10−80% AcOEt in CH_2Cl_2 as eluant and collecting fractions with $R_f = 0.75$ (AcOEt) to produce 1.63 g (85%) of **3d** as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.75−0.83 (4H, m, TIPDS H_4 (Si*CH*CH_3, SiCH*CH*$_3$)), 0.91−1.04 (24H, m, TIPDS H_{24}), 1.14 and 1.16 (total 6H, each d, $J = 6.8$ Hz, COCH(CH_3)$_2$), 2.79 (1H, septet, $J = 6.8$ Hz, *CH*CO), 3.75−3.95 (5H, m, 4′,5′-H_2, α-CH, β-CH_2), 4.03−4.08 (2H, m, 5″-H, α-CH), 4.35 (1H, dd, $J = 4.8$, 1 Hz, 2′-H), 4.43 (1H, dd, $J = 8.6$, 4.8 Hz, 3′-H), 5.80 (1H, d, $J = 1$ Hz, 1′-H), 7.81 (4H, m, Pth H_4), 7.99 (1H, s, 8-H), 11.60 (1H, s, NH), 12.09 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.8, 12.2, 12.3, 12.6 (Si*C*HCH$_3$), 16.58, 16.72, 16.84, 16.86, 17.07, 17.12, 17.23 (SiCH*C*H$_3$), 18.83, 18.86 (COCH(*C*H$_3$)$_2$), 34.7 (CO*C*H(CH$_3$)$_2$), 37.6 (C-β), 60.0 (C-5′), 67.6 (C-α), 69.6 (C-3′), 80.8 (C-2′), 81.1 (C-4′), 86.3 (C-1′), 120.2 (C-5), 122.9, 131.4, 134.2 (Pth), 136.4 (C-8), 148.0 (C-2), 148.2 (C-4), 154.7 (C-6), 167.7, 180.1 (CO). ESI-MS: *m/z* calcd 769.3 $[M + H]^+$, found 769.2. Anal. Calcd for $C_{36}H_{52}N_6O_9Si_2$: C, 56.23; H, 6.82; N, 10.93. Found: C, 56.51; H, 7.10; N, 10.65.

2′-*O*-(2-Phthalimidoethyl)uridine (4a). To a solution of **2a** (3.22 g, 4.88 mmol) in 30 mL of THF cooled to 0 °C was added 10.7 mL of 1 M TBAF/THF dropwise. The mixture was stirred at 0 °C for 0.5 h, neutralized with glacial acetic acid, evaporated to dryness, and dried. The residue was purified by column chromatography using 1−5% MeOH in AcOEt as eluant and collecting fractions with $R_f = 0.4$ (3% MeOH/AcOEt). The product was crystallized from ethyl acetate to produce 1.59 g (78%) of **4a** as white crystals. Mp: 151 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.53 (1H, m, 5′-H), 3.62 (1H, m, 5″-H), 3.71−3.83 (4H, m, 4′-H, β-CH_2, α-CH), 3.87 (1H, m, α-CH), 3.96 (1H, dd, $J = 5.1$, 4.8 Hz, 2′-H), 4.08 (1H, m (dd), 3′-H), 5.06 (1H, d, $J = 5.8$ Hz, 3′-OH), 5.10 (1H, t, $J = 5.1$ Hz, 5′-OH), 5.55 (1H, d, $J = 8.1$ Hz, 5-H), 5.77 (1H, d, $J = 5.1$ Hz, 1′-H), 7.83 (4H, br s, Pth H_4), 7.84 (1H, d, $J = 8.1$ Hz, 6-H), 11.22 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 37.2 (C-β), 60.4 (C-5′), 66.4 (C-α), 68.2 (C-3′), 81.0 (C-2′), 84.9 (C-4′), 86.1 (C-1′), 101.7 (C-5), 122.9, 131.5, 134.3 (Pth), 140.2 (C-6), 150.4 (C-2), 163.0 (C-4), 167.7 (CO). ESI-MS: *m/z* calcd 416.1 $[M - H]^-$, found 415.9. Anal. Calcd for $C_{19}H_{19}N_3O_8$: C, 54.68; H, 4.59; N, 10.07. Found: C, 54.85; H, 4.66; N, 10.24.

2′-*O*-(2-Phthalimidoethyl)-N⁴-benzoylcytidine (4b). To a solution of **2b** (3.12 g, 4.1 mmol) in 30 mL of THF cooled to 0 °C was added 9 mL of 1 M TBAF/THF dropwise. The mixture was stirred at 0 °C for 1 h, neutralized with glacial acetic acid, and evaporated to dryness. The residue was purified by column chromatography using 1–12% MeOH in CH_2Cl_2 as eluant and collecting fractions with $R_f = 0.35$ (3% MeOH/AcOEt). The product was crystallized from a methanol/water (6/1) mixture to produce 1.74 g (82%) of **4b** as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.60 (1H, m, 5′-H), 3.77 (1H, m, 5″-H), 3.82−3.87 (3H, m, 4′-H, β-CH_2), 3.91−3.95 (3H, m, 2′-H, α-CH_2), 4.06 (1H, m (dd), 3′-H), 4.97 (1H, d, $J = 6.3$ Hz, 3′-OH), 5.20 (1H, t, $J = 5.1$ Hz, 5′-OH), 5.80 (1H, d, $J = 2.3$ Hz, 1′-H), 7.31 (1H, d, $J = 7.3$ Hz, 5-H), 7.53 (2H, dd, $J_{3,5-4} = 7.8$ Hz, $J_{3-2,5-6} = 7.3$ Hz, Bz H_2), 7.64 (1H, m, Bz H_1), 7.84 (4H, m, Pth H_4), 8.03 (2H, d, $J_{2-3,6-5} = 7.3$ Hz, $J_{2,6-4} = 1.3$ Hz, Bz H_2), 8.50 (1H, d, $J = 7.3$ Hz, 6-H), 11.25 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 37.4 (C-β), 59.3 (C-5′), 66.9 (C-α), 67.3 (C-3′), 82.0 (C-2′), 84.0 (C-4′), 88.4 (C-1′), 95.8 (C-5), 122.9 (Pth), 128.4 (Bz), 131.6 (Pth), 132.7, 133.1 (Bz), 134.2 (Pth), 145.0 (C-6), 154.3 (C-2), 163.1 (C-4), 167.3, 167.8 (CO). ESI-MS: *m/z* calcd 519.2 $[M - H]^-$, found 519.2. Anal. Calcd. for $C_{26}H_{24}N_4O_8$: C, 60.00; H, 4.65; N, 10.76. Found: C, 59.73; H, 4.77; N, 10.52.

2′-*O*-(2-Phthalimidoethyl)-*N*⁶-benzoyladenosine (4c). To a solution of **3c** (1.81 g, 2.3 mmol) in 20 mL of THF cooled to 0 °C was added 5.1 mL of 1 M TBAF/THF dropwise. The mixture was stirred at 0 °C for 0.5 h, neutralized with glacial acetic acid, evaporated to dryness, and dried. The residue was purified by column chromatography using 1−7% MeOH in AcOEt as eluant and collecting fractions with $R_f = 0.3$ (3% MeOH/AcOEt) to produce 1.21 g (97%) of **4c** as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.56 (1H, m, 5′-H), 3.68 (1H, m, 5″-H), 3.73−3.77 (3H, m, α-CH, β-CH_2), 3.93−3.98 (2H, m, 4′-H, α-CH), 4.35 (1H, m (dd), 3′-H), 4.60 (1H, dd, $J = 5.6$, 5.1 Hz, 2′-H), 5.13 (1H, t, $J = 5.6$ Hz, 5′-OH), 5.25 (1H, d, $J = 5.6$ Hz, 3′-OH), 6.07 (1H, d, $J = 5.6$ Hz, 1′-H), 7.59 (2H, dd, $J_{3,5-4} = 7.8$ Hz, $J_{3-2,5-6} = 7.3$ Hz, Bz H_2), 7.68 (1H, m, Bz H_1), 7.75 (4H, m, Pth H_4), 8.09 (2H, dd, $J_{2-3,6-5} = 7.3$ Hz, $J_{2,6-4} = 1.4$ Hz, Bz H_2), 8.61 (1H, s, 2-H), 8.65 (1H, s, 8-H), 11.17 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 37.2 (C-β), 61.0 (C-5′), 66.6 (C-α), 68.8 (C-3′), 81.2 (C-2′), 85.8 (C-1′), 85.9 (C-4′), 122.7 (Pth), 125.5 (C-5), 128.4 (Bz), 131.2 (Pth), 132.4, 133.3 (Bz), 134.1 (Pth), 142.8 (C-8), 150.1 (C-4), 151.4 (C-2), 151.8 (C-6), 165.5, 167.7 (CO). ESI-MS: *m/z* calcd 543.2 $[M - H]^-$, found 543.2. Anal. Calcd for $C_{27}H_{24}N_6O_7$: C, 59.56; H, 4.44; N, 15.43. Found: C, 59.48; H, 4.70; N, 15.20.

2′-*O*-(2-Phthalimidoethyl)-*N*²-isobutyrylguanosine (4d). To a solution of **3d** (1.63 g, 2.12 mmol) in 30 mL of THF was added triethylamine trihydrofluoride (0.28 mL, 0.273 g, 1.7 mmol). The reaction mixture was stirred at 60 °C for 2 h and evaporated to dryness. The residue was purified by column chromatography using 1−20% CH_3OH in AcOEt as eluant and collecting fractions with $R_f = 0.15$ (10% CH_3OH/AcOEt). The product was crystallized from water to produce 0.92 g (82%) of **4d** as a white solid. ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.13 (3H, d, $J = 6.8$ Hz, COCH*CH*$_3$), 1.16 (3H, d, $J = 6.8$ Hz, COCH*CH*$_3$), 2.79 (1H, septet, $J = 6.8$ Hz, *CH*CO), 3.53 (1H, m, 5′-H), 3.60 (1H, m, 5″-H), 3.66−3.73 (3H, m, α-CH, β-CH_2), 3.90−3.93 (2H, m, 4′-H, α-CH), 4.26 (1H, m, 3′-H), 4.39 (1H, dd, $J = 6.6$, 4.8 Hz, 2′-H), 5.06 (1H, t, $J = 5.3$ Hz, 5′-OH), 5.19 (1H, d, $J = 4.5$ Hz, 3′-OH), 5.81 (1H, d, $J = 6.6$ Hz, 1′-H), 7.71 (4H, m, Pth H_4), 8.19 (1H, s, 8-H), 11.55 (1H, s, NH), 11.92 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 18.76, 18.79 (COCH(*C*H$_3$)$_2$), 34.6 (CO*C*H(CH$_3$)$_2$), 37.2 (C-β), 61.1 (C-5′), 66.3 (C-α), 68.8 (C-3′), 81.7 (C-2′), 86.1 (C-4′), 84.4 (C-1′), 119.9 (C-5), 122.5, 131.1, 134.0 (Pth), 137.3 (C-8), 147.8 (C-2), 148.5 (C-4), 154.5 (C-6), 167.5, 179.9 (CO). ESI-MS: *m/z* calcd 527.2 $[M + H]^+$, found 527.2. Anal. Calcd for $C_{24}H_{26}N_6O_8$: C, 54.75; H, 4.98; N, 15.96. Found: C, 54.52; H, 5.12; N, 15.84.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-phthalimidoethyl)uridine (5a). To a solution of **4a** (1.47 g, 3.52 mmol) in 15 mL of pyridine was added 4,4′-dimethoxytrityl chloride (1.43 g, 4.23 mmol). The mixture was stirred at room temperature for 3 h and evaporated to dryness. The residue was dissolved in 75 mL of CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 150 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography using 20−100% AcOEt in CH_2Cl_2 as eluant and collecting fractions with $R_f = 0.35$ (1/1 AcOEt/CH_2Cl_2) to produce 2.19 g (86%) of **5a** as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.20 (1H, dd, $J = 10.6$, 2.5 Hz, 5′-H), 3.27 (1H, dd, $J = 10.6$, 4.3 Hz, 5″-H), 3.76 (6H, s, 2 × OCH_3), 3.80−3.85 (3H, m, α-CH, β-CH_2), 3.88−3.94 (2H, m, 4′-CH, α-CH), 4.03 (1H, dd, $J = 4.8$, 3.8 Hz, 2′-H), 4.18 (1H, m (dd), 3′-H), 5.14 (1H, d, $J = 6.6$ Hz, 3′-OH), 5.24 (1H, d, $J = 8.1$ Hz, 5-H), 5.75 (1H, d, $J = 3.8$ Hz, 1′-H), 6.91 and 6.92 (total 4H, each d, $J = 8.8$ Hz, DMTr H_4), 7.24−7.27 (5H, m, DMTr H_5), 7.31−7.35 (2H, m, DMTr H_2), 7.37−7.40 (2H, m, DMTr H_2), 7.64 (1H, d, $J = 8.1$ Hz, 6-H), 7.84 (4H, m, Pth H_4), 11.30 (1H, s, NH). ^{13}C NMR

(63 MHz, DMSO-d_6): δ 37.2 (C-β), 54.9, 55.0 (OCH_3), 62.7 (C-5′), 66.7 (C-α), 68.5 (C-3′), 80.8 (C-2′), 82.6 (C-4′), 85.8 (DMTr), 87.1 (C-1′), 101.4 (C-5), 113.2 (DMTr), 122.9 (Pth), 126.8, 127.7, 127.9, 129.7 (DMTr), 131.5, 134.3 (Pth), 135.1, 135.3 (DMTr), 140.2 (C-6), 144.6 (DMTr), 150.2 (C-2), 158.1 (DMTr), 163.0 (C-4), 167.8 (CO). ESI-MS: *m/z* calcd 720.2 $[M + H]^+$, found 720.4. Anal. Calcd for $C_{40}H_{37}N_3O_{10}$: C, 66.75; H, 5.18; N, 5.84. Found: C, 66.36; H, 5.39; N, 5.46.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-phthalimidoethyl)-N⁴-benzoylcytidine (5b). To a solution of **4b** (1.89 g, 3.63 mmol) in 20 mL of pyridine was added 4,4′-dimethoxytrityl chloride (1.48 g, 4.36 mmol). The mixture was stirred at room temperature for 3 h and evaporated to dryness. The residue was dissolved in 100 mL of CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 150 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography using 20−100% AcOEt in CH_2Cl_2 and then 1−5% CH_3OH in AcOEt as eluants and collecting fractions with $R_f = 0.6$ (AcOEt) to produce 2.69 g (90%) of **5b** as a yellowish foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.30−3.37 (2H, m, 5′-H_2), 3.77 (6H, s, 2 × OCH_3), 3.87 (2H, t, $J = 5.6$ Hz, β-CH_2), 3.94−4.05 (4H, m, 2′,4′-H_2, α-CH_2), 4.27 (1H, m, 3′-H), 5.07 (1H, d, $J = 7.1$ Hz, 3′-OH), 5.82 (1H, d, $J = 0.8$ Hz, 1′-H), 6.93 (4H, d, $J = 9.1$ Hz, DMTr H_4), 7.15 (1H, d, $J = 7.3$ Hz, 5-H), 7.26−7.30 (5H, m, DMTr H_5), 7.34−7.38 (2H, m, DMTr H_2), 7.41−7.43 (2H, m, DMTr H_2), 7.54 (2H, dd, $J_{3,5-4} = 7.8$ Hz, $J_{3-2,5-6} = 7.3$ Hz, Bz H_2), 7.65 (1H, m, Bz H_1), 7.84 (4H, m, Pth H_4), 8.03 (2H, dd, $J_{2-3,6-5} = 7.3$ Hz, $J_{2,6-4} = 1.3$ Hz, Bz H_2), 8.34 (1H, d, $J = 7.3$ Hz, 6-H), 11.29 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 37.4 (C-β), 54.97, 54.98 (DMTr), 61.5 (C-5′), 67.1 (C-α), 67.7 (C-3′), 81.77 (C-2′), 81.87 (C-4′), 86.0 (DMTr), 88.8 (C-1′), 96.0 (C-5), 113.3 (DMTr), 122.9 (Pth), 126.8, 127.8, 127.9 (DMTr), 128.4 (Bz), 129.6, 129.8 (DMTr), 131.7 (Pth), 132.7, 133.1 (Bz), 134.2 (Pth), 135.2, 135.5 (DMTr), 144.3 (C-6, DMTr), 154.2 (C-2), 158.14, 158.16 (DMTr), 163.2 (C-4), 167.2, 167.9 (CO). ESI-MS: *m/z* calcd 823.3 $[M + H]^+$, found 823.3. Anal. Calcd for $C_{47}H_{42}N_4O_{10}$: C, 68.60; H, 5.14; N, 6.81. Found: C, 68.37; H, 5.36; N, 6.71.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-phthalimidoethyl)-N⁶-benzoyladenosine (5c). To a solution of **4c** (1.21 g, 2.22 mmol) in 15 mL of pyridine was added 4,4′-dimethoxytrityl chloride (0.92 g, 2.7 mmol). The mixture was stirred at room temperature for 4 h and evaporated to dryness. The residue was dissolved in 50 mL CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 100 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography using 10−100% AcOEt in CH_2Cl_2 and then 0.5−2% CH_3OH in AcOEt as eluants and collecting fractions with $R_f = 0.7$ (AcOEt) to produce 1.54 g (82%) of **5c** as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.23 (2H, d, $J = 4.6$ Hz, 5′-H_2), 3.73 (6H, s, 2 × OCH_3), 3.76−3.83 (4H, m, α-CH, β-CH_2), 3.97 (1H, m, α-CH), 4.07 (1H, dd, $J =$ 9.3, 4.6 Hz, 4′-H), 4.46 (1H, m (dd), 3′-H), 4.77 (1H, dd, $J = 5.1$, 4.8 Hz, 2′-H), 5.30 (1H, d, $J = 6.1$ Hz, 3′-OH), 6.08 (1H, d, $J =$ 4.8 Hz, 1′-H), 6.84 and 6.85 (total 4H, each d, $J = 8.9$ Hz, DMTr-H_4), 7.19−7.28 (7H, m, DMTr H_7), 7.36 (2H, m, DMTr H_2), 7.58 (2H, dd, $J_{3,5-4} = 7.8$ Hz, $J_{3-2,5-6} = 7.3$ Hz, Bz H_2), 7.67 (1H, m, Bz H_1), 7.75 (4H, m, Pth H_4), 8.09 (2H, dd, $J_{2-3,6-5} = 7.3$ Hz, $J_{2,6-4} = 1.3$ Hz, Bz H_2), 8.53 (1H, s, 8-H), 8.55 (1H, s, 2-H), 11.18 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 37.4 (C-β), 54.97, 54.98 (DMTr), 63.4 (C-5′), 67.0 (C-α), 69.2 (C-3′), 80.4 (C-2′), 83.5 (C-4′), 85.5 (DMTr), 86.4 (C-1′), 113.1 (DMTr), 122.8 (Pth), 125.7 (C-5), 126.6, 127.6, 127.7 (DMTr), 128.44, 128.49 (Bz), 129.7 (DMTr), 131.3 (Pth), 132.4, 133.4 (Bz), 134.4 (Pth), 135.4, 135.5 (DMTr), 143.2 (C-8), 144.7 (DMTr), 150.3 (C-4), 151.4 (C-2), 151.8 (C-6), 158.0 (DMTr), 165.5, 167.7 (CO). ESI-MS: *m/z* calcd 847.3 $[M + H]^+$, found 847.4. Anal. Calcd for $C_{48}H_{42}N_6O_9$: C, 68.07; H, 5.00; N, 9.92. Found: C, 67.80; H, 5.24; N, 9.67.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-phthalimidoethyl)-N²-isobutyrylguanosine (5d). To a solution of **4d** (0.92 g, 1.75 mmol) in 12 mL of pyridine was added 4,4′-dimethoxytrityl chloride (0.71 g, 2.1 mmol), and the mixture was stirred at room temperature for 4 h. Then 4,4′-dimethoxytrityl chloride (0.071 g, 0.21 mmol) was added once more; the mixture was stirred for an additional 2 h and evaporated to dryness. The residue was dissolved in 75 mL of CH_2Cl_2, and this solution was washed with saturated $NaHCO_3$ (2 × 150 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography using 10−100% AcOEt in CH_2Cl_2 and then 1−7% CH_3OH in AcOEt as eluants and collecting fractions with $R_f = 0.25$ (AcOEt) to produce 1.3 g (90%) of **5d** as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.14 and 1.15 (total 6H, each d, $J = 6.8$ Hz, $COCH(CH_3)_2$), 2.72 (1H, septet, $J = 6.8$ Hz, *CH*CO), 3.15 (1H, dd, $J = 10.6$, 3.3 Hz, 5′-H), 3.26 (1H, dd, $J = 10.6$, 6.1 Hz, 5″-H), 3.74−3.77 (9H, m, α-CH, β-CH_2, 2 × OCH_3,), 3.94 (1H, m, α-CH), 4.01 (1H, m, 4′-H), 4.28 (1H, m (dd), 3′-H), 4.51 (1H, dd, $J = 5.6$, 4.8 Hz, 2′-H), 5.22 (1H, d, $J = 5.6$ Hz, 3′-OH), 5.86 (1H, d, $J = 5.6$ Hz, 1′-H), 6.83 and 6.85 (total 4H, each d, $J = 8.9$ Hz, DMTr H_4), 7.19−7.29 (7H, m, DMTr H_7), 7.35 (2H, m, DMTr H_2), 7.74 (4H, m, Pth H_4), 8.04 (1H, s, 8-H), 11.52 (1H, s, NH), 11.97 (1H, s, NH). ^{13}C NMR (63 MHz, DMSO-d_6): δ 18.78, 18.86 (CO-CH(*C*H_3)$_2$), 34.7 (CO*C*H(CH_3)$_2$), 37.3 (C-β), 54.95, 54.97 (DMTr), 63.9 (C-5′), 66.7 (C-α), 69.2 (C-3′), 81.0 (C-2′), 84.0 (C-4′), 85.1 (C-1′), 85.5 (DMTr), 113.1 (DMTr), 120.3 (C-5), 122.6 (Pth), 126.6, 127.66, 127.74, 129.7 (DMTr), 131.3, 134.1 (Pth), 135.35, 135.43 (DMTr), 137.4 (C-8), 144.7 (DMTr), 147.9 (C-2), 148.5 (C-4), 154.6 (C-6), 158.02, 158.03 (DMTr), 167.7, 180.0 (CO). ESI-MS: *m/z* calcd 829.3 $[M + H]^+$, found 829.4. Anal. Calcd for $C_{45}H_{44}N_6O_{10}$: C, 65.21; H, 5.35; N, 10.14. Found: C, 65.48; H, 5.63; N, 9.88.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-phthalimidoethyl)uridine-3′-*O*-(cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (6a). To a solution of **5a** (0.36 g, 0.5 mmol) in 6 mL of CH_2Cl_2 were added 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.24 mL, 0.226 g, 0.75 mmol) and 4,5-dicyanoimidazole (0.068 g, 0.575 mmol). The reaction mixture was stirred for 3 h at room temperature. Then the mixture was diluted with 60 mL of CH_2Cl_2, washed with saturated $NaHCO_3$ (2 × 150 mL), and then with saturated NaCl (1 × 150 mL), dried over Na_2SO_4, and evaporated to dryness. The mixture of diastereomers was purified by column chromatography using 20−100% AcOEt in CH_2Cl_2 (with 0.1% Et_3N) as eluant and collecting fractions with $R_f = 0.6$ and 0.7 (1/1 AcOEt/CH_2Cl_2) to produce 0.372 g (81%) of **6a** as a white foam. ^{31}P NMR (400 MHz, $CDCl_3$): δ 149.3 and 149.8. ESI-MS: *m/z* calcd for $C_{49}H_{54}N_5O_{11}P$ 920.4 $[M + H]^+$, found 920.5.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-phthalimidoethyl)-N⁴-benzoylcytidine-3′-*O*-(cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (6b). To a solution of **5b** (0.411 g, 0.5 mmol) in 6 mL of CH_2Cl_2 were added 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.24 mL, 0.226 g, 0.75 mmol) and 4,5-dicyanoimidazole (0.068 g, 0.575 mmol). The reaction mixture was stirred for 3 h. Then the mixture was diluted with 60 mL of CH_2Cl_2, washed with saturated $NaHCO_3$ (2 × 150 mL) and then with saturated NaCl (1 × 150 mL), dried over Na_2SO_4, and evaporated to dryness. The mixture of diastereomers was purified by column chromatography using 10−70% AcOEt in CH_2Cl_2 (with 0.1% Et_3N) as eluant and collecting fractions with $R_f = 0.4$ and 0.5 (1/1 AcOEt/CH_2Cl_2) to produce 0.41 g (80%) of **6b** as a white foam. ^{31}P NMR (400 MHz, $CDCl_3$): δ 148.7 and 149.8. ESI-MS: *m/z* calcd for $C_{56}H_{59}N_6O_{11}P$ 1023.4 $[M + H]^+$, found 1023.5.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-phthalimidoethyl)-N⁶-benzoyladenosine-3′-*O*-(cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (6c). To a solution of **5c** (0.423 g, 0.5 mmol) in 5 mL of CH_2Cl_2 were added 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.24 mL, 0.27 g, 0.75 mmol) and 4,5-dicyanoimidazole (0.068 g, 0.575 mmol), and the reaction mixture was stirred for 4 h at room temperature. Then the mixture was diluted with 50 mL of CH_2Cl_2, washed with saturated $NaHCO_3$ (2 × 100 mL), and then with saturated NaCl (1 × 100 mL), dried over Na_2SO_4, and evaporated to dryness. The mixture of diastereomers was purified by column

chromatography using 5−50% AcOEt in CH_2Cl_2 (with 0.1% Et_3N) as eluant and collecting fractions with R_f = 0.55 and 0.7 (1/1 AcOEt/CH_2Cl_2) to produce 0.42 g (80%) of **6c** as a white foam. ^{31}P NMR (400 MHz, acetone-d_6): δ 149.7 and 149.8. ESI-MS: *m*/*z* calcd for $C_{57}H_{59}N_8O_{10}P$ 1047.4 $[M + H]^+$, found 1047.5.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-phthalimidoethyl)-*N*^{2}-isobutyrylguanosine-3′-*O*-(cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (6d). To a solution of **5d** (0.41 g, 0.5 mmol) in 5 mL of CH_2Cl_2 were added 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.24 mL, 0.23 g, 0.75 mmol) and 4,5-dicyanoimidazole (0.068 g, 0.575 mmol), and the reaction mixture was stirred for 3 h at room temperature. Then the mixture was diluted with 30 mL of CH_2Cl_2, washed with saturated $NaHCO_3$ (2 × 100 mL), and then with saturated NaCl (1 × 100 mL), dried over Na_2SO_4, and evaporated to dryness. The mixture of diastereomers was purified by column chromatography using 5−100% AcOEt in CH_2Cl_2 (with 0.1% Et_3N) as eluant and collecting fractions with R_f = 0.5 (AcOEt) to produce 0.37 g (72%) of **6d** as a white foam. ^{31}P NMR (400 MHz, acetone-d_6): δ 149.7 and 150.2. ESI-MS: *m*/*z* calcd for $C_{54}H_{61}N_8O_{11}P$ 1029.4 $[M + H]^+$, found 1029.6.

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Supporting Information Available: Text giving details of the synthesis, purification, and characterization of *N*^{4}-benzoylcytidine, *N*^{6},*N*^{6}-dibenzoyladenosine, and compounds **1a**−**e** and figures giving ^{1}H NMR, ^{13}C NMR, and HSQC NMR spectra of the new compounds **2**−**5** and ^{31}P NMR spectra for phosphoramidites **6a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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