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Synthesis of the Phosphoramidite Derivative of 2′-Deoxy-2′-*C*-*β*-methylcytidine

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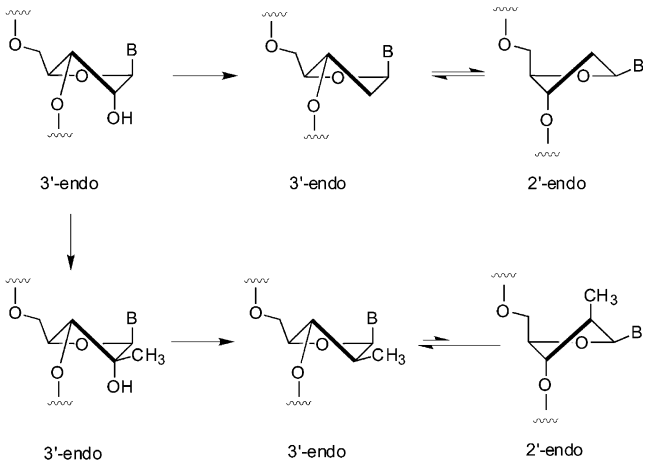
Abstract: 2′-Deoxy-2′-*C*-*β*-methylnucleosides elicit interest as potential therapeutic agents and as analogues for the analysis of nucleic acid structure and function. An efficient route for the synthesis of 2′-deoxy-2′-*C*-methyluridine (**11**), 2′-deoxy-2′-*C*-methylcytidine (**12**), and the phosphoramidite derivative of 2′-deoxy-2′-*C*-*β*-methylcytidine (**10**, 46% overall yield) from 1,2,3,5-tetra-*O*-benzoyl-2-*C*-*β*-methylribofuranose (**1**) is described.

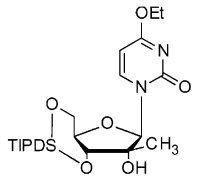
The 2′-hydroxyl group plays an integral role in RNA structure and function, mediating hydrogen bonds, coordinating to metal ions, and providing a scaffold for interactions with water.[1] The locations of residues bearing important 2′-hydroxyl groups therefore provide fundamental clues about the structure and function of an RNA. Deoxynucleotide substitution provides the most common approach by which to locate these residues, as the removal of an important 2′-hydroxyl group has deleterious consequences for RNA function.[2] However, diminished activity from deoxynucleotide substitution cannot be interpreted monolithically as evidence for interactions with the 2′-hydroxyl group because the loss of RNA function could be due to an altered conformational preference of the deoxynucleotide. Ribonucleotides exhibit a preference for the 3′-endo sugar conformation, but deoxyribonucleotides lack this preference, populating the 2′- and 3′-endo conformations equally well (Scheme 1).[3] A molecular framework that allows removal of the 2′-hydroxyl group without a concomitant change in sugar pucker would eliminate this conformational ambiguity and thereby clarify the interpretation of deoxynucleotide substitution experiments. We suggest that 2′-*β*-methylnucleotides offer such a context as they maintain the 3′-endo conformational preference even in the absence of the 2′-hydroxyl group (Scheme 1).[4]

To conduct 2′-deoxynucleotide substitution experiments with RNA using 2′-*β*-methylnucleotides, we must develop the syntheses of the ribo and deoxynucleosides and their phosphoramidites. Here we report an efficient synthesis of 2′-deoxy-2′-*C*-*β*-methylcytidine and the corresponding phosphoramidite for incorporation into oligoribonucleotides. We chose cytidine for its application to our mechanistic work on the group II intron ribozyme[5] and because 2′-deoxy-2′-*C*-*β*-methylcytidine exhibits potent cytotoxicity toward L1210 cells.[6]

SCHEME 1



Two approaches have been described to access 2′-deoxy-2′-methylnucleosides. Cicero et al.[7] and Schmit et al.[4a] used catalytic hydrogenation of a suitably protected 2′-methylene nucleoside.[8] The reduction proceeds quantitatively but suffers from relatively weak diastereoselectivity ($\beta/\alpha = 3:1$; diastereomeric excess 50%). Cicero et al. subsequently used this mixture as the starting material to prepare the phosphoramidite derivative of 2′-deoxy-2′-*C*-*β*-methyl-*N*^{4}-isobutyrylcytidine in six steps with 20% overall yield.[9] In the other approach, Matsuda et al. obtained 2′-deoxy-2′-*C*-*β*-methylcytidine from uridine in 10 steps via synthesis of intermediate **I** followed by 2′-deoxygenation, sequential deblocking and amination.[6] This approach has limited efficiency (9% overall yield from uridine) because **I** forms as a minor product from the Grignard reaction of the corresponding 2′-ketonucleoside with methylmagnesium bromide.[10]



I

After the reports of Cicero and Matsuda, O'kuru established a facile synthesis of 2′-*C*-*β*-methylribonucleo-

(1) Auffinger, P.; Westhof, E. *J. Mol. Biol.* **1997**, *274*, 54 and references therein.
(2) Gesteland, R. F.; Cech, T. R.; Atkins, J. F. *The RNA World*, Second Edition; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 1999.
(3) Saenger, W. *Principles of Nucleic Acid structure*; Springer-Verlag: Berlin, Germany, 1983.
(4) (a) Schmit, C.; Bevierre, M.-O.; Mesmaeker, A. D.; Altmann, K.-H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1969. (b) Cicero, D. O.; Iribarren, A. M.; Bazzo, R. *Appl. Magn. Reson.* **1994**, *7*, 95.
(5) Gordon, P. M.; Sontheimer, E. J.; Piccirilli, J. A. *Biochemistry* **2000**, *39*, 12939.
(6) Matsuda, A.; Takenuki, K.; Sasaki, T.; Ueda, T. *J. Med. Chem.* **1991**, *34*, 234.
(7) Cicero, D. O.; Neuner, P. J. S.; Franzese, O.; D'Onofrio, C.; Iribarren, A. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 861.
(8) Samano, V.; Robins, M. J. *Synthesis* **1991**, 283.
(9) Cicero, D. O.; Gallo, M.; Neuner, P. J.; Iribarren, A. M. *Tetrahedron* **2001**, *57*, 7613.

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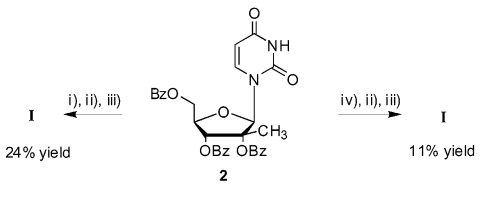
SCHEME 2[a]



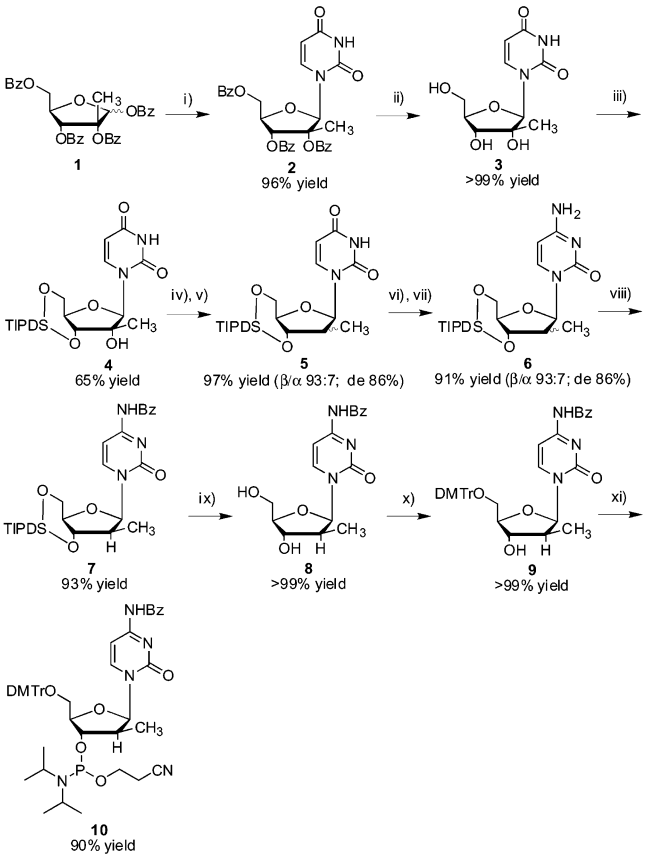
[a] Key: (i) TsCl, K_2CO_3/CH_3CN, 85 °C, 2 h; (ii) 2 N NaOEt/EtOH, reflux, 1 h; (iii) $TIPDSCl_2$, imidazole, DMF, rt, overnight; (iv) $SOCl_2/CHCl_3$/DMF, reflux, 6.5 h.

sides via nucleobase glycosylation with suitably protected 2-*β*-methylribofuranose.[11] We anticipated that this method could help to improve the synthesis of 2′-deoxy-2′-*C*-*β*-methylnucleosides by facilitating access to 2′-*C*-*β*-methylribonucleosides, which may serve as precursors either for conversion to **I** or for direct 2′-deoxygenation. First, we explored the synthesis of intermediate **I** by transformation of 2′,3′,5′-tri-*O*-benzoyl-2′-*C*-*β*-methyluridine (**2**). O'kuru obtained **2** with complete stereoselectivity in 57% yield by reaction of 1,2,3,5-tetra-*O*-benzoyl-2-*C*-*β*-methylribofuranose (**1**) with bis(trimethylsilyl)uracil, prepared in situ from *N*,*O*-bis(trimethylsilyl)acetamide and uracil.[11] We used hexamethyldisilazane[12,13] instead of *N*,*O*-bis-(trimethylsilyl)acetamide and improved the yield of **2** substantially (96%). However, attempts to transform **2** into intermediate **I** gave low yields following reaction sequences using either thionyl chloride[10] or tosyl chloride[14] (Scheme 2).

We then investigated whether suitably protected derivatives of 2′-*C*-*β*-methylcytidine or 2′-*C*-*β*-methyluridine would undergo 2′-deoxygenation directly to give the corresponding 2′-deoxynucleoside. We prepared 2′-*C*-*β*-methyl-N^4-benzoylcytidine and 2′-*C*-*β*-methyluridine as described[11,12] and protected them as 3′,5′-*O*-1,1,3,3-tetraisopropyldisiloxyl ethers. The cytidine derivative failed to undergo 2′-deoxygenation via either the phenoxythiocarbonyl[15] or methyl oxalyl ester[6] using Bu_3SnH in the presence of AIBN. However, deoxygenation of the corresponding uridine analogue proceeded efficiently and stereoselectively (*β*/α = 93:7; diastereomeric excess 86%), thereby enabling facile access to 2′-deoxy-2′-*C*-*β*-methyluridine, 2′-deoxy-2′-*C*-*β*-methylcytidine and the phosphoramidite of 2′-deoxy-2′-*C*-*β*-methylcytidine in high yield and with greater diastereoselectivity than reported previously.

Scheme 3 shows our overall synthesis of the phosphoramidite derivative of 2′-deoxy-2′-*C*-*β*-methylcytidine. Treatment of **2** with saturated ammonia in methanol gave 2′-*C*-*β*-methyluridine (**3**) quantitatively. Protection of the 3′- and 5′-hydroxyl groups of **3** using 1,3-dichloro-1,1,3,3-tetraisopropyldisilane gave 3′,5′-tetraisopropyldisiloxan-1,3-diyl-2′-*C*-*β*-methyluridine (**4**) in 65% yield.

SCHEME 3[a]



[a] Key: (i) bis(trimethylsilyl)uracil, $SnCl_4$, CH_3CN, rt, 20 h; (ii) NH_3, CH_3OH, 0−4 °C, 2 days; (iii) $TIPDSCl_2$, imidazole, DMF, rt, 2 h; (iv) $ClCOCO_2Me$, DMAP, CH_3CN, rt, 1 h; (v) *n*-Bu_3SnH, AIBN, toluene, reflux, 2 h; (vi) TPSCl, DMAP, Et_3N, CH_3CN, rt, 53 h; (vii) NH_4OH, rt, 3 h; (viii) BzCl, DMAP, CH_2Cl_2, rt, 1 h; (ix) Et_3N/3HF, THF, rt, 4 h; (x) DMTrCl, pyridine, rt, overnight; (xi) CIP(NPr-i$_2$)OCH_2CH_2CN, *i*-Pr_2NEt, 1-methylimidazole, CH_2Cl_2, rt, 2 h.

Deoxygenation of **4** via free-radical chemistry gave 3′,5′-tetraisopropyldisiloxan-1,3-diyl-2′-deoxy-2′-*C*-*β*-methyluridine (**5**) in 97% yield with *β*/α selectivity of 93:7 (diastereomeric excess 86%). Reaction of **5** with 2,4,6-triisopropylbenzenesulfonyl chloride[16] followed by treatment with ammonium hydroxide gave the corresponding cytidine derivative (**6**) in 91% yield. Benzoylation of **6** generated **7** in 93% yield and enabled complete removal of the α-isomer by silica gel column chromatography. Removal of the silyl group from **7** with triethylamine trihydrofluoride gave 2′-deoxy-2′-*C*-*β*-methyl-N^4-benzoylcytidine (**8**) quantitatively. Compound **8** was then converted to the phosphoramidite derivative of 2′-deoxy-2′-*C*-*β*-methylcytidine (**10**) in 90% yield.

Deprotection of compounds **5** and **6** gave the free nucleosides 2′-deoxy-2′-*C*-methyluridine (**11**) and 2′-deoxy-2′-*C*-methylcytidine (**12**) in high yields as diastereomeric mixtures (*β*/α ∼93:7). We were unable to separate the diastereomers by silica gel chromatography using as eluent either 10% methanol in ethyl acetate or 20% methanol in chloroform (Scheme 4). We confirmed by

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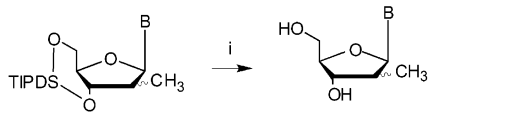
SCHEME 4



5: B = U
6: B = C

11: B = U >99% yield; β/α 93:7
12: B = C 96% yield; β/α 93:7

i. TBAF/THF, rt, 1 h

NOESY NMR spectra of **11** that the major isomer has the 2′-*β*-configuration. We observed a strong NOE between 1′-H (*δ* 6.20) and 2′-H (*δ* 2.52), weaker NOE's from 1′-H (*δ* 6.20) to 2′-*C*-Me (*δ* 0.95) and 4′-H (*δ* 3.72), and no NOE from 1′-H (*δ* 6.20) to either 3′-H (*δ* 3.88) or 5′-H (*δ* 3.78, 3.93). These results suggest that 1′-H and 2′-H reside on the same face of the ribose ring. We also observed a strong NOE between 6-H (*δ* 8.08) and 3′-H (*δ* 3.88) and a weaker NOE between 6-H (*δ* 8.08) and 2′-*C*-Me (*δ* 0.95), consistent with the proposed preference of 2′-deoxy-2′-*C*-*β*-methylnucleosides for the 3′-endo conformation.[4]

In conclusion, 2′-*C*-*β*-methyl-2′-deoxynuclesides may provide a means to eliminate the conformational ambiguity inherent to 2′-deoxynucleoside substitution experiments in functional RNA molecules. As part of our efforts to test this hypothesis, we have established more facile access to the phosphoramidite derivative of 2′-deoxy-2′-*C*-*β*-methylcytidine from a sugar substrate: 1,2,3,5-tetra-*O*-benzoyl-2-*C*-*β*-methylribofuranose. This approach has greater stereoselectivity and a higher overall yield than previously published methods. NOESY experiments support previous arguments based on NMR coupling constants[4] that 2′-deoxy-2′-*C*-*β*-methylnucleosides populate the 3′-endo conformation.

Experimental Section

2′,3′,5′-Tri-*O*-benzoyl-2′-*C*-*β*-methyluridine (2). Under an argon atmosphere, a stirred suspension of uracil (1.12 g, 10.0 mmol) and $(NH_4)_2SO_4$ (25 mg) in 1,1,1,3,3,3-hexamethyldisilazane (25 mL) was heated at reflux until a clear solution formed. The clear solution was concentrated under vacuum, and the residue was dried under high vacuum (<0.1 mmHg) for 2 h. The crude bis(trimethylsilyl)uracil obtained was dissolved in dry acetonitrile (75 mL), and 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methyl-α-(and *β*)-D-ribofuranose (**1**) (2.838 g, 4.89 mmol) was added. Under argon, $SnCl_4$ (1.18 mL, 10.0 mmol) was added in one portion with vigorous stirring. After being stirred at room temperature for 20 h, the reaction mixture was heated to reflux for 1 h. The mixture was cooled to room temperature, and the reaction was carefully quenched with saturated aqueous $NaHCO_3$ (50 mL). The mixture was extracted with ethyl acetate (3 × 50 mL), and the organic layers were combined, washed with brine, and dried over magnesium sulfate. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 50% ethyl acetate in hexane to give product (**2**)[11] as white foam (2.663 g, 96% yield): ^{1}H NMR ($CDCl_3$/TMS) *δ* 9.22 (br s, 1H), 8.08 (m, 4H), 7.89 (d, 2H, *J* = 7.7 Hz), 7.65−7.35 (m, 8H), 7.32−7.18 (m, 2H), 6.54 (s, 1H), 5.79 (d, 1H, *J* = 4.7 Hz), 5.75 (d, 1H, *J* = 8.2 Hz), 4.95−4.70 (m, 2H), 4.64 (m, 1H), 1.74 (s, 3H); ^{13}C NMR ($CDCl_3$) *δ* 166.2, 165.3, 165.2, 163.0, 149.9, 140.8, 133.63, 133.58, 133.5, 129.9, 129.8, 129.6, 129.4, 129.3, 128.5, 128.4, 102.3, 89.5, 84.4, 80.4, 75.4, 63.3, 17.9.

2′-*C*-*β*-Methyluridine (3). A solution of 2′,3′,5′-tri-*O*-benzoyl-2′-*C*-*β*-methyluridine (1.007 g, 1.77 mmol) in methanol (100 mL) was saturated with ammonia gas at 0 °C, and the mixture was stored at 4 °C for 2 days. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with ethyl acetate/methanol 4:1 (v/v), to give the product (**3**)[11] as white powder (0.456 g, 100% yield): ^{1}H NMR (D_2O) *δ* 7.79 (d, 1H, *J* = 8.1 Hz), 5.87 (s, 1H), 5.76 (d, 1H, *J* = 8.1 Hz), 3.90 (m, 2H), 3.74 (m, 2H), 1.07 (s, 3H); ^{13}C NMR (D_2O) *δ* 165.9, 151.4, 141.3, 102.0, 91.4, 81.5, 78.9, 72.1, 59.2, 18.7.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*C*-*β*-methyluridine (4). To a stirred mixture of 2′-*C*-*β*-methyluridine (0.437 g, 1.69 mmol) and imidazole (0.688 g, 10.11 mmol) in DMF (15 mL) under argon was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.637 g, 2.02 mmol). After the mixture was stirred at room temperature for 2 h, water (1.0 mL) was added, and the mixture was concentrated under reduced pressure. The residue was dissolved in chloroform (100 mL), and the solution was washed with water and dried over $MgSO_4$. After solvent was removed, the residue was purified by silica gel chromatography, eluting with 35% ethyl acetate in hexane to give the product as white foam (0.546 g, 65% yield): ^{1}H NMR ($CDCl_3$/TMS) *δ* 10.18 (br s, 1H), 7.80 (d, 1H, *J* = 8.0 Hz), 6.03 (s, 1H), 5.73 (d, 1H, *J* = 8.0 Hz), 4.24 (d, 1H, *J* = 12.0 Hz), 4.12 (m, 1H), 4.02 (m, 1H), 3.98 (m, 1H), 3.37 (s, 1H), 1.25 (s, 3H), 1.15−0.85 (m, 28H); ^{13}C NMR ($CDCl_3$) *δ* 163.6, 150.6, 139.5, 102.2, 90.6, 81.5, 78.8, 72.6, 59.7, 20.3, 17.3, 17.2, 17.1, 17.0, 16.9, 16.8, 16.8, 16.7, 13.6, 12.7, 12.3; HRMS calcd for $C_{22}H_{41}N_2O_7Si_2$ [MH^+] 501.2452, found 501.2439.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-*C*-*β*-methyluridine (5). Methyl chlorooxoacetate (0.132 mL, 1.44 mmol) was added to a solution of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*C*-*β*-methyluridine (0.534 g, 0.96 mmol) and DMAP (0.235 g, 1.92 mmol) in dry acetonitrile (10 mL). The reaction mixture was stirred at room temperature for 1 h under an argon atmosphere, at which time TLC showed that the reaction was complete. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL). The organic phase was dried over magnesium sulfate. After the solvent was removed, the residue (white foam) was dried under vacuum overnight and used directly in the next reaction without further purification. The white foam was dissolved in dry toluene (20 mL). To the resulting solution were added 2,2′-azobisisobutyronitrile (20 mg) and tributyltin hydride (382 *μ*L, 1.44 mmol). The reaction mixture was heated to reflux for 2 h. After the solvent was removed, the residue was purified by silica gel chromatography, eluting with 30% ethyl acetate in hexane to give the product as a white foam (0.502 g, 97% yield) with *β*/α selectivity of 93.4:6.6: ^{1}H NMR ($CDCl_3$/TMS) *δ* 10.43 (br s, 1H), 7.83 (d, 1H, *J* = 8.1 Hz), 6.27 (d, 1H, *J* = 7.2 Hz), 5.73 (d, 1H, *J* = 8.1 Hz), 4.18 (m, 1H), 4.10−3.90 (m, 2H), 3.77 (dd, 1H, *J* = 1.6, 7.8 Hz), 2.70 (m, 1H), 3.37 (s, 1H), 1.25 (s, 3H), 1.15−0.85 (m, 31H); ^{13}C NMR ($CDCl_3$) *δ* 163.8, 150.8, 140.2, 101.7, 85.7, 84.0, 71.9, 59.4, 44.0, 17.3, 17.14, 17.10, 17.0, 16.83, 16.8, 16.7, 13.5, 12.8, 12.7, 12.2, 11.0; HRMS calcd for $C_{22}H_{41}N_2O_6Si_2$ [MH^+] 485.2503, found 485.2491.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-*C*-*β*-methylcytidine (6). Triethylamine (0.14 mL, 1.0 mmol) was added to a mixture of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-*C*-*β*-methyluridine (0.371 g, 0.687 mmol), 2,4,6-triisopropylbenzenesulfonyl chloride (0.606 g, 2.0 mmol), and DMAP (0.244 g, 2.0 mmol) in acetonitrile (20 mL). After the mixture was stirred at room temperature for 53 h, concentrated ammonium hydroxide (28%, 30 mL) was added, and the mixture was stirred at room temperature for 3 h. The solvent was removed, and the aqueous phase was extracted with chloroform (3 × 20 mL). The chloroform solution was washed with brine and dried over magnesium sulfate. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 7.5% ethanol in chloroform to give the product as a white foam (0.337 g, 91% yield): ^{1}H NMR ($CDCl_3$/TMS) *δ* 8.14 (br s, 1H), 7.66 (d, 1H, *J* = 7.4 Hz), 6.47 (br s, 2H), 6.24 (d, 1H, *J* = 7.4 Hz), 5.73 (d, 1H, *J* = 7.47 Hz), 4.06 (m, 1H), 3.93 (m, 1H), 3.86 (m, 1H), 3.65 (m, 1H), 2.55 (m, 1H), 1.10−0.80 (m, 31H); ^{13}C NMR ($CDCl_3$) *δ* 165.7, 156.3, 140.9, 94.6, 85.8, 83.6, 72.5, 59.8, 44.0, 17.3, 17.22, 17.17, 17.1, 16.93, 16.85, 16.8, 13.5, 12.8, 12.7, 12.3, 11.4; HRMS calcd for $C_{22}H_{42}N_3O_5Si_2$ [MH^+] 484.2663, found 484.2667.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-*C*-*β*-methyl-*N*^{4}-benzoylcytidine (7). To the solution of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-*C*-*β*-methylcytidine (0.162 g, 0.3 mmol) in dichloromethane (10 mL) were added DMAP (73 mg, 0.6 mmol) and benzoyl chloride (52 μL, 0.45 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with methanol (0.5 mL), and the mixture was stirred at room temperature for 10 min. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 40% ethyl acetate in hexane to give the product as white foam (0.179 g, 93% yield): ^{1}H NMR ($CDCl_3$/TMS) δ 9.03 (br s, 1H), 8.35 (d, 1H, J = 7.4 Hz), 8.26 (d, 2H, J = 7.4 Hz), 7.93 (t, 1H, J = 7.5 Hz), 7.61 (m, 3H) 6.39 (d, 1H, J = 7.3 Hz), 4.21 (m, 1H), 4.05 (m, 1H), 3.99 (m, 1H), 3.82 (m, 1H), 2.74 (m, 1H), 1.20−0.80 (m, 31H); ^{13}C NMR ($CDCl_3$) δ 162.0, 145.1, 133.0, 128.9, 127.5, 96.3, 86.8, 84.0, 72.1, 59.6, 44.1, 17.4, 17.3, 17.2, 16.94, 16.85, 16.8, 13.6, 12.9, 12.8, 12.3, 11.3; HRMS calcd for $C_{29}H_{45}N_3O_6NaSi_2$ [MNa^+] 610.2745, found 610.2770.

2′-Deoxy-2′-*C*-*β*-methyl-*N*^{4}-benzoylcytidine (8). To a solution of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-*C*-*β*-methyl-*N*^{4}-benzoylcytidine (117 mg, 0.2 mmol) in THF (6 mL) was added Et_3N−3HF (258 mg, 1.6 mmol). The mixture was stirred at room temperature for 4 h. TLC showed that the reaction was complete. The reaction mixture was diluted with pyridine−water (1:4, 10 mL). The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 8% methanol in chloroform to give product as a white solid powder (70 mg, 100% yield): ^{1}H NMR ($CDCl_3$/TMS) δ 8.49 (br s, 1H), 7.86 (d, 1H, J = 7.6 Hz), 7.57 (d, 1H, J = 7.1 Hz), 7.40 (m, 3H), 6.33 (br s, 1H), 4.10−3.85 (m, 4H), 2.70 (m, 1H), 0.89 (d, 3H, J = 6.1 Hz); ^{13}C NMR ($CDCl_3$) δ 169.0, 164.6, 158.1, 147.3, 134.6, 134.1, 129.8, 129.1, 98.1, 89.0, 86.5, 74.3, 60.5, 46.6, 11.7; HRMS calcd for $C_{17}H_{19}N_3O_5Na$ [MNa^+] 368.1222, found 368.1230.

5′-*O*-(Dimethoxytrityl)-2′-deoxy-2′-*C*-*β*-methyl-*N*^{4}-benzoylcytidine (9). Under argon, a solution of 2′-deoxy-2′-*C*-*β*-methyl-*N*^{4}-benzoylcytidine (59 mg, 0.171 mmol) and dimethoxytrityl chloride (64 mg, 0.189 mmol) in dry pyridine (10 mL) was stirred at room temperature overnight. The reaction was quenched with ethanol (0.5 mL) and then evaporated to dryness. The product was purified by silica gel chromatography, eluting with 3% methanol in chloroform to give the product as a pale yellow foam (0.111 g, 100% yield): ^{1}H NMR ($CDCl_3$/TMS) δ 8.82 (br s, 1H), 8.48 (d, 1H, J = 7.5 Hz), 7.72 (d, 2H, J = 7.6 Hz), 7.46 (m, 1H), 7.34 (m, 3H), 7.24 (m, 5H), 7.20−6.98 (m, 5H), 6.77 (m, 3H), 4.02 (m, 1H), 3.84 (m, 1H), 3.70 (s, 6H), 3.55 (dd, 1H, J = 2.4, 11.2 Hz), 3.47 (dd, 1H, J = 2.6, 11.2 Hz), 2.58 (m, 1H), 0.84 (d, 3H, J = 8.6 Hz); ^{13}C NMR ($CDCl_3$) δ 162.1, 158.6, 145.9, 144.0, 135.6, 135.3, 133.0, 130.1, 130.0, 129.1, 128.9, 128.2, 128.1, 128.0, 127.7, 127.4, 127.1, 126.9, 125.2, 96.5, 87.4, 87.1, 83.6, 73.8, 61.0, 55.1, 45.2, 11.4; HRMS calcd for $C_{38}H_{38}N_3O_7$ [MH^+] 648.2710, found 648.2711.

5′-*O*-(Dimethoxytrityl)-2′-deoxy-2′-*C*-*β*-methyl-*N*^{4}-benzoylcytidine 3′-*N*,*N*-diisopropyl(cyanoethyl)phosphoramidite (10). To a solution of 5′-*O*-(dimethoxytrityl)-2′-deoxy-2′-*C*-*β*-methyl-*N*^{4}-benzoylcytidine (**9**) (96 mg, 0.15 mmol) in dry dichloromethane (10 mL) at 0 °C were added quickly *N*,*N*-diisopropylethylamine (69 μL, 0.4 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (67 μL, 0.3 mmol), and 1-methylimidazole (8 μL, 0.1 mmol). The reaction mixture was then warmed to room temperature and stirred until TLC indicated that the reaction was complete. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography on silica gel, eluting with 4% acetone in methylene chloride containing 0.2% triethylamine, to give the corresponding phosphoramidite (0.114 g, 90% yield): ^{31}P NMR ($CDCl_3$) δ 151.8, 149.3; MS (*m*/*z*) 848.3 [MH^+], 303, (DMTr, 100).

2′-Deoxy-2′-*C*-methyluridine (11, *β*/α 93:7). To the solution of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-*C*-*β*-methyluridine (**5**) (100 mg, 0.207 mmol) in THF (5 mL) was added TBAF (1.0 M in THF, 0.4 mL, 0.4 mmol). The reaction mixture was stirred at room temperature for 1 h. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 10% methanol in ethyl acetate to give the product as white solid powder (50 mg, 100% yield). *β*-Isomer: ^{1}H NMR (CD_3OD/TMS) δ 8.08 (d, 1H, J = 8.0 Hz, H-6), 6.20 (d, 1H, J = 7.6 Hz, 1′-H), 5.68 (d, 1H, J = 8.0 Hz, 5-H), 3.93 (dd, 1H, J = 12.4, 2.0 Hz, 5′-H), 3.88 (m, 1H, 3′-H), 3.78 (dd, 1H, J = 12.4, 3.0 Hz, 5′-H), 3.72 (m, 1H, 4′-H), 2.52 (m, 1H, 2′-H), 0.95 (d, 3H, J = 7.0 Hz, 2′-*C*-Me); ^{13}C NMR (CD_3OD) δ 166.1, 152.3, 143.3, 101.9, 87.7, 86.2, 74.2, 60.5, 46.5, 11.5; HRMS calcd for $C_{10}H_{15}N_2O_5$ [MH^+] 243.0981, found 243.0988.

2′-Deoxy-2′-*C*-methylcytidine (12, *β*/α 93:7). To the solution of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-*C*-*β*-methylcytidine (**6**) (54 mg, 0.1 mmol) in THF (3 mL) was added TBAF (1.0 M in THF, 0.2 mL, 0.2 mmol). The reaction mixture was stirred at room temperature for 1 h. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 20% methanol in chloroform to give the product as white solid powder (26 mg, 96% yield). *β*-Isomer: ^{1}H NMR (CD_3OD/TMS) δ 8.06 (d, 1H, J = 7.3 Hz, H-6), 6.24 (d, 1H, J = 7.5 Hz, 1′-H), 5.88 (d, 1H, J = 7.3 Hz, 5-H), 3.92 (dd, 1H, J = 12.5, 2.3 Hz, 5′-H), 3.88−3.65 (m, 3H, 3′-H, 4′-H, 5′-H), 2.52 (m, 1H, 2′-H), 0.88 (d, 3H, J = 7.0 Hz, 2′-*C*-Me); ^{13}C NMR (CD_3OD) δ 167.4, 158.4, 143.5, 95.4, 88.1, 86.0, 74.7, 60.7, 46.6, 11.8; HRMS calcd for $C_{10}H_{16}N_3O_5$ [MH^+] 242.1141, found 242.1137.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of compounds **4**−**12** and NOESY of compound **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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