2′-C-Branched Ribonucleosides: Synthesis of the Phosphoramidite Derivatives of 2′-*C*-*β*-Methylcytidine and Their Incorporation into Oligonucleotides

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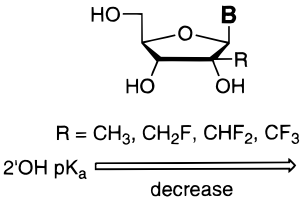
We describe a strategy for the incorporation of a 2′-C-branched ribonucleoside, 2′-*C*-*β*-methylcytidine, into oligonucleotides via solid-phase synthesis using phosphoramidite derivatives. 4-*N*-Benzoyl-2′-*C*-*β*-methylcytidine (**2b**) was synthesized by coupling persilylated 4-*N*-benzoylcytosine with 1,2,3,5-tetra-*O*-benzoyl-2-*C*-*β*-methyl-α-(and *β*)-D-ribofuranose (**1**) in the presence of $SnCl_4$ in acetonitrile, followed by selective deprotection with NaOH in pyridine/methanol. The 3′- and 5′-hydroxyl groups were blocked as a cyclic di-*tert*-butylsilanediyl ether **3** by treatment with di-*tert*-butyldichlorosilane/$AgNO_3$ in DMF. The 2′-hydroxyl group was then protected as a *tert*-butyldimethylsilyl ether **4a** by treatment with *tert*-butylmagnesium chloride followed by addition of *tert*-butyldimethylsilyl trifluoromethanesulfonate in THF. As an alternative to 2′-silyl protection, the corresponding 2′-*O*-tetrahydropyranyl ether **4b** was prepared by treatment of **3** with 4,5-dihydro-*2H*-pyran in the presence of a catalytic amount of 10-camphorsulfonic acid in methylene chloride. The di-*tert*-butylsilanediyl groups of **4a** and **4b** were removed by treatment with pyridinium poly(hydrogen fluoride) to afford **5a** and **5b**, respectively. Protection of the 5′-hydroxyl group as a dimethoxytrityl ether and phosphitylation of the 3′-hydroxyl group by the standard procedure gave the phosphoramidite derivatives **7a** and **7b**. Both **7a** and **7b** could be used to incorporate 2′-*C*-*β*-methylcytidine into oligonucleotides efficiently via standard solid-phase synthesis, but the tetrahydropyranyl group of **7b** was more readily removed from oligonucleotides than the *tert*-butyldimethylsilyl group of **7a**. Oligonucleotides containing 2′-*C*-*β*-methylcytidine undergo base-catalyzed degradation analogous to natural RNA.

Introduction

Nucleoside analogues that contain a 2′-C-branched ribose sugar have been synthetic targets[1] over the past decade because of their potential as chemotherapeutic agents.[1f−l,2] For example, 2′-deoxy-2′-*β*-*C*-methylcytidine exhibits potent cytotoxicity toward several leukemic cell lines.[1h,i,2a] Such analogues might also be used to regulate gene expression by antisense or ribozyme cleavage approaches because modification of the 2′-position increases the nuclease resistance of oligonucleotides,[3] thereby increasing their stability in biological fluids. For ribozyme therapeutics, 2′-C-branched ribonucleosides are of particular interest because specific 2′-hydroxyl groups in ribozymes are essential for catalytic activity.[4]

Some attention has been given to 2′-C-branched ribonucleosides as biochemical probes.[3a,5] For example, phosphorylated forms of 2′-*C*-methyluridine have been studied as substrates for ribonucleotide reductase,[5c] RNA polymerase,[6] and nucleases.[3a] 2′-Branched ribonucleosides also have potential as tools for exploring the structure and function of RNA and ribozymes. Our interest arises from the possibility that a series of nucleoside analogues (Chart 1) in which the 2′-alkyl substituent is methyl, monofluoromethyl, difluoromethyl, or trifluromethyl might allow systematic variation of the pK_a of the 2′-hydroxyl group within the same structural context. For those enzymes and ribozymes that catalyze RNA strand scission by activating the 2′-hydroxyl group for attack at the adjacent 3′-phosphate diester (such as ribonuclease A[7] or the hammerhead and hairpin ribozymes[8]), this series of nucleosides could reveal a linear free energy relation-

Chart 1



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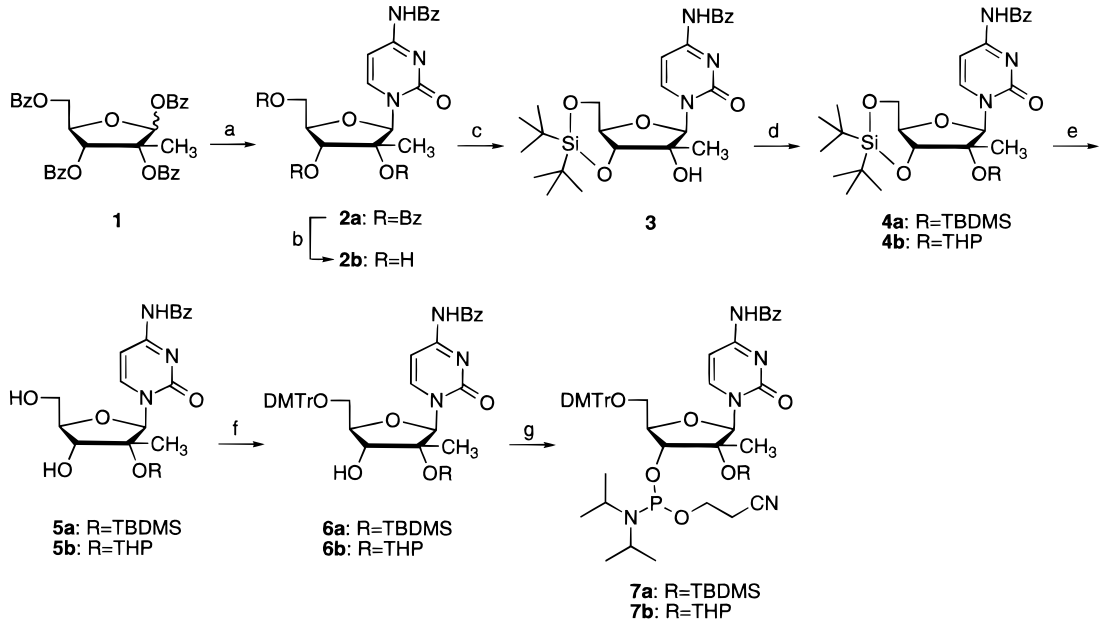
Scheme 1[a]



[a] Conditions: (a) persilylated 4-*N*-benzoylcytosine, $SnCl_4$; (b) NaOH; (c) *t*-Bu_2SiCl_2/$AgNO_3$/Et_3N; (d) for **4a**, (i) *t*-BuMgCl; (ii) *t*-$BuMe_2SiOTf$; for **4b**, 4,5-dihydro-*2H*-pyran/10-camphorsulfonic acid, CH_2Cl_2; (e) HF−pyridine; (f) for **6a**, DMTrCl/$AgNO_3$/pyridine; for **6b**, DMTrCl; (g) *i*-$Pr_2NP(OCH_2CH_2CN)Cl$/1-methylimidazole/*i*-Pr_2NEt.

ship between the catalytic rate and the pK_a of the nucleophile, thereby providing information about the degree of bond-making between the phosphorus and the 2′-OH nucleophile in the transition state.

To employ these nucleoside analogues as ribozyme therapeutics, antisense drugs, or probes for ribozyme mechanism, we must incorporate them into DNA and RNA oligonucleotides. Schmit et al. derivatized 2′-deoxy-2′-C-branched nucleosides as phosphoramidites and incorporated them into DNA by solid-phase synthesis,[9] but there is no method reported for the site specific incorporation of the corresponding ribonucleosides (such as those shown in Chart 1) into oligonucleotides. For phosphoramidite derivatives of the natural nucleosides, the 2′-hydroxyl group is commonly protected as a *tert*-butyldimethylsilyl (TBDMS) ether.[10] Because the tertiary 2′-hydroxyl group of 2′-C-branched ribonucleosides is hindered and therefore more difficult to protect selectively, obtaining suitable phosphoramidite derivatives is more challenging. Herein, we report a solution to this problem and strategies for the incorporation of 2′-*C*-*β*-methylcytidine into oligonucleotides via phosphoramidite chemistry. We also show that, analogous to natural RNA, oligonucleotides containing 2′-*C*-*β*-methylcytidine degrade under basic conditions by a reaction in which the tertiary 2′-hydroxyl group attacks the adjacent 3′-phosphodiester.

Results and Discussion

We prepared 1,2,3,5-tetra-*O*-benzoyl-2-*C*-*β*-methyl-α-(and *β*)-D-ribofuranose (**1**) (Scheme 1) according to the literature[1p−q] from commercially available α-D-ribofura-

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nose 1,3,5-tribenzoate. Even with the steric influence of the β-C2 methyl group, the reaction of **1** with persilylated nucleobases under Vorbruggen conditions still affords β-nucleosides exclusively.[1p−q] The reaction of **1** with persilylated 4-*N*-benzoylcytosine in the presence of $SnCl_4$[11] in acetonitrile at room temperature afforded 2′,3′,5′-tri-*O*-benzoyl-4-*N*-benzoyl-2′-*C*-β-methylcytidine **2a** in 86% yield; reactions carried out at elevated temperature (83 °C) gave much lower yields. Selective deprotection of **2a** with NaOH in pyridine and methanol gave 4-*N*-benzoyl-2′-*C*-β-methylcytidine **2b** in 94% yield. Sodium methoxide or ammonia in methanol removed all four benzoyl groups from **2a**.

Standard procedures for preparation of nucleoside synthons used in solid-phase synthesis of oligonucleotides include protection of the amino groups on the nucleobases as amides, protection of the 5′- and 2′-hydroxyl groups as dimethoxytrityl (DMTr) and TBDMS ethers, respectively, and phosphitylation of the 3′-oxygen to the β-cyanoethyl *N*,*N*-diisopropylphosphoramidite.[10] Although protection of the 5′-hydroxyl group of **2b** as a DMTr ether proceeded well, attempts to protect the 2′-hydroxyl group with *tert*-butyldimethylsilyl chloride (TBDMSCl) either in the presence of imidazole in pyridine or $AgNO_3$ in THF gave exclusively the 3′-*O*-TBDMS ether.[12] We tried to obtain the 2′-isomer by exposing the 3′-isomer to isomerization conditions,[10a] but treatment with imidazole in DMF at room temperature for 1 week afforded only 8% of the 2′-isomer.[14] These results suggested that if a TBDMS group was to be regioselectively installed at the tertiary 2′-hydroxyl group of 2′-*C*-β-methylcytidine, the secondary 3′-hydroxyl group would have to be protected first.

Di-*tert*-butyldichlorosilane appeared to be a useful reagent for this purpose because it simultaneously protects the 3′- and 5′-hydroxyl groups, and the resulting cyclic di-*tert*-butylsilanediyl ether can be cleanly and chemoselectively removed within minutes by exposure to pyridinium poly(hydrogen fluoride) (PPHF)[16] or tributylammonium fluoride[17] without affecting a TBDMS ether moiety. Thus, treatment of **2b** with di-*tert*-butyldichlorosilane in the presence of $AgNO_3$[18] in DMF provided the corresponding 3′,5′-O-(di-*tert*-butylsilanediyl) ether **3** in 95% yield. However, our initial attempts to protect the tertiary 2′-hydroxyl group of **3** as a TBDMS ether were unsuccessful. Even after heating **3** in the presence of either *tert*-butyldimethylsilyl trifluoromethylsulfonate (TBDMSOTf)/2,6-lutidine in methylene chloride[19] or TBDMSCl/$AgNO_3$/pyridine[10c,d] in THF, the starting material was recovered unchanged. Consequently, we tested various bases as possible activators of the 2′-hydroxyl group. It was recently reported that *t*-BuMgCl could be used for oxygen-selective phosphorylation[20] and alkylation[21] of nucleoside derivatives and that potassium hydride (KH)/18-crown-6 could be used for the successful protection of highly hindered hydroxyl groups with TBDMSCl or TBDMSOTf.[22] We found that both *t*-BuMgCl and KH were effective for the silylation of the 2′-hydroxyl group of **3**, but *t*-BuMgCl afforded a higher yield of the desired 2′-*O*-TBDMS product **4a** than KH did. Compound **3** was treated with *t*-BuMgCl for 0.5 h followed by overnight incubation with TBDMSOTf in THF. After workup, purification by silica gel column chromatography afforded **4a** in 64% yield. Selective removal of the di-*tert*-butylsilanediyl group from **4a** with PPHF in THF at 0 °C for 5 min gave **5a** in 82% yield. During protection of the 5′-hydroxyl group of **5a** by overnight treatment with dimethoxytrityl chloride (DMTrCl) in pyridine,[23] some migration of the TBDMS group from the 2′-hydroxyl to the 3′-hydroxyl occurred. To circumvent this problem, we used $AgNO_3$ to accelerate the tritylation reaction.[10d] One hour in the presence of DMTrCl and $AgNO_3$ was sufficient to convert **5a** to **6a**. Under these conditions, no migration of the 2′-*O*-TBDMS group to the 3′-oxygen was observed.

We found that this procedure for silylation of the tertiary 2′-hydroxyl group of 2′-*C*-methylcytidine using *t*-BuMgCl/TBDMSOTf was not effective for silylation of the 2′-hydroxyl group of 2′-*C*-β-trifluoromethyl nucleoside derivatives (Chart 1) because they decomposed under the reaction conditions.[24] We later found that the removal of the TBDMS group from oligonucleotides occurs much more slowly from 2′-*C*-β-methylcytidine residues than from cytidine residues (see below). These observations led us to examine whether other 2′-hydroxyl protecting groups could be used for the solid-phase synthesis of oligonucleotides containing 2′-*C*-β-methylcytidine. The 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl group[25] seemed to be a good alternative for two reasons: it can be introduced with mild acid, conditions under which 2′-*C*-β-trifluoromethyl nucleosides should be stable, and nucleoside phosphoramidites that possess this 2′-protecting group have recently become commercially available. Unfortunately, the reaction of **3** with 1-(2-fluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine under various conditions failed to give any desired product.

We next examined the THP group for the protection of the 2′-hydroxyl of 2′-*C*-β-methylcytidine. This group has been used successfully for solid-phase synthesis of oligoribonucleotides via the phosphoramidite approach,[26] and the conditions necessary for its removal are relatively mild (0.01 N HCl, pH 2; ca. 3−4 h at 20 °C) and do not promote significant migration of the 3′-phosphoryl group to the adjacent 2′-oxygen.[27] Reaction of **3** with excess 4,5-

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(14) Analogously, Reese and Trentham reported that the benzoyl group of 2′-*O*-benzoyl-3′-*C*-methyluridine showed no evidence of isomerization to the neighboring oxygen after storage in pyridine at room temperature for a half-year, whereas those of 2′- and 3′-*O*-benzoyluridine do isomerize under these conditions.[15]

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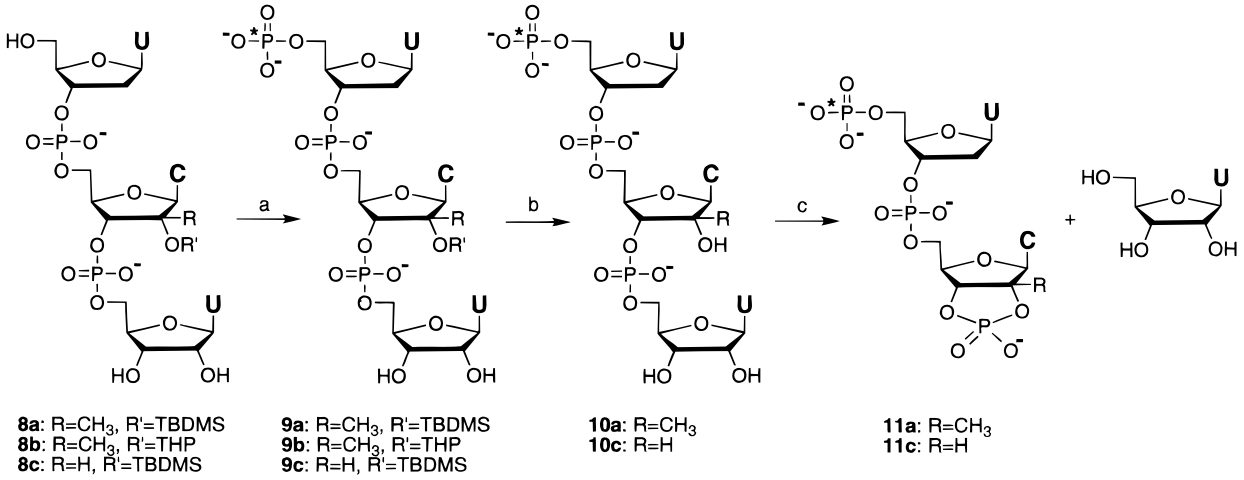
Scheme 2[a]



[a] Conditions: (a) [γ-^{32}P]-ATP, T4 polynucleotide kinase; *P indicates ^{32}P; (b) TBAF or 0.01 N HCl (pH 2.0); (c) 0.1 N NaOH.

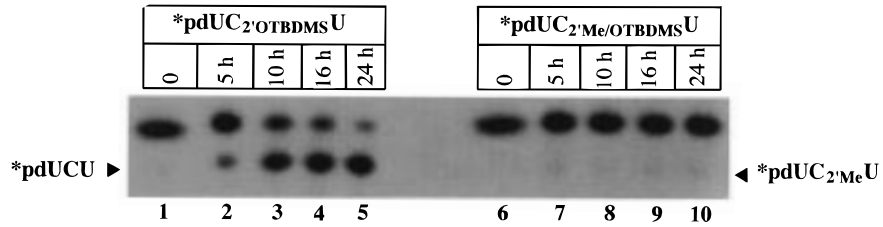


Figure 1. Deprotection of 5′-radiolabeled oligonucleotides with TBAF. TBAF/THF (25 °C) removes the 2′-*O*-TBDMS group from *pdUC$_{2'OTBDMS}$U (**9c**) (lanes 1−5) much more rapidly than from *pdUC$_{2'Me/OTBDMS}$U (**9a**) (lanes 6−10).[28]

dihydro-*2H*-pyran in the presence of a catalytic amount of 10-camphorsulfonic acid in methylene chloride gave **4b** in 95% yield (*p*-toluenesulfonic acid as catalyst gave **4b** in 50% yield) as a mixture of two diastereomers, which could not be separated by silica gel chromatography. However, after desilyation of **4b** by treatment with PPHF, the two diastereomers of **5b** were distinguishable by TLC and were separated by silica gel chromatography to afford equal quantities of each diastereomer in a combined yield of 92%. This strategy for 2′-protection may also be effective for the 2′-(monofluoromethyl)- and the 2′-(trifluoromethyl)ribonucleosides shown in Chart 1.[24]

Overnight treatment of the diastereomeric mixture of **5b** with DMTrCl in pyridine afforded **6b** in 91% yield. Under the standard conditions,[10f] both **6a** and **6b** were phosphitylated with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite to give the corresponding phosphoramidites **7a** and **7b**, which were characterized by HRMS and ^{31}P NMR. The ^{31}P NMR spectrum of **7a** showed two peaks at 151.0 and 150.9 ppm, and as expected, that of **7b** showed four peaks at 152.9, 152.2, 150.3, and 150.0 ppm due to the presence of the chiral THP group.

To determine the suitability of both the 2′-*O*-TBDMS (**7a**) and 2′-*O*-THP (**7b**) ribonucleoside phosphoramidites as building blocks for the incorporation of 2′-*C*-β-methylcytidine into oligonucleotides, we synthesized three trinucleotides (Scheme 2), 2′-deoxyuridylyl(3′−5′)2′-*C*-β-methyl-2′-*O*-(*tert*-butyldimethylsilyl)cytidylyl(3′−5′)uridine (dUC$_{2'Me/OTBDMS}$U, **8a**), 2′-deoxyuridylyl(3′−5′)2′-*C*-β-methyl-2′-*O*-(tetrahydopyranyl)cytidylyl(3′−5′)uridine (dUC$_{2'Me/OTHP}$U, **8b**), and the control trinucleotide 2′-deoxyuridylyl(3′−5′)2′-*O*-(*tert*-butyldimethylsilyl)cytidylyl(3′−5′)uridine (dUC$_{2'OTBDMS}$U, **8c**). During solid-phase synthesis using standard protocols, **7a** and **7b** coupled as efficiently as commercial phosphoramidites, according to the release of the DMTr cation. The trinucleotides **8a**−**c** were 5′-^{32}P-labeled with [γ-^{32}P]-ATP and polynucleotide kinase (PNK) and purified by polyacrylamide gel electrophoresis (PAGE) to give the corresponding 5′-radiolabeled trinucleotides *pdUC$_{2'Me/OTBDMS}$U (**9a**), *pdUC$_{2'Me/OTHP}$U (**9b**), and *pdUC$_{2'OTBDMS}$U (**9c**), respectively (*p indicates ^{32}P phosphate). Deprotection of the 2′-*O*-TBDMS group with tetrabutylammonium fluoride (TBAF) was much slower for 2′-*C*-β-methylcytidine than for normal cytidine. For example, after treatment of *pdUC$_{2'Me/OTBDMS}$U (**9a**) with TBAF in THF for 24 h, none of the desilylated trinucleotide could be detected (Figure 1, lanes 6−10),[28] whereas *pdUC$_{2'OTBDMS}$U (**9c**) was almost completely desilylated to **10c** during this time (Figure 1, lanes 1−5). Sekine et al. recently demonstrated that the 2′-*O*-TBDMS group can be efficiently removed from oligonucleotides under acidic conditions[29] (10% acetic acid or 8% formic acid or 0.01 N HCl). We

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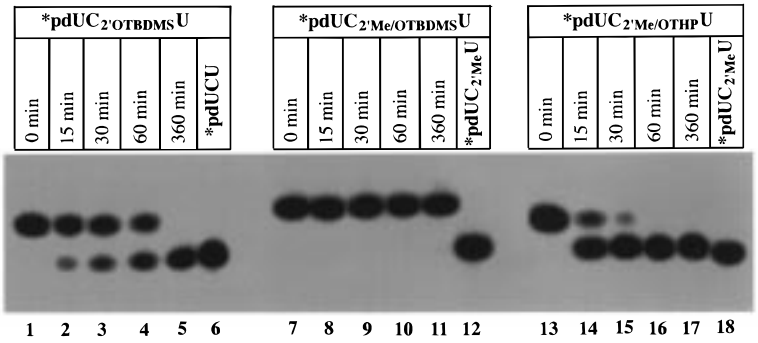

Figure 2. Deprotection of 5′-radiolabled oligonucleotides with acid. Acidic conditions (0.01 N HCl, pH 2; 30 °C) remove the 2′-*O*-TBDMS group from *pdUC$_{2'OTBDMS}$U (**9c**) in less than 6 h (lanes 1−5) and the 2′-*O*-THP group from *pdUC$_{2'Me/OTHP}$U (**9b**) in less than 1 h (lanes 13−17); however, even after 6 h, acidic conditions do not remove the 2′-*O*-TBDMS group from *pdUC$_{2'Me/OTBDMS}$U (**9a**).

also found that 0.01 N HCl readily desilylated **9c** to **10c** within 6 h (Figure 2, lanes 1−5) but failed to desilylate any of *pdUC$_{2'Me/OTBDMS}$U (**9a**) within this time (Figure 2, lanes 7−11). Longer exposure to these conditions (120 h) resulted in decomposition of **9a**. Results obtained using 10% acetic acid or 8% formic acid were similar (data not shown). In contrast, the THP group of trinucleotide dUC$_{2'Me/OTHP}$U (**8b**) was readily removed with 0.01 N HCl (pH 2) at 30 °C to give the corresponding free trinucleotide *pdUC$_{2'Me}$U (**10a**) within 1 h (Figure 2, lanes 14−17), even less time than required for desilyation of *pdUC$_{2'OTBDMS}$U (**9c**) to **10c**. Thus, the 2′-*O*-THP phosphoramidite appears to be a better choice for synthesizing oligonucleotides containing 2′-*C*-*β*-methylcytidine. Additionally, it is compatible with the commercial 2′-*O*-TBDMS phosphoramidites because both the THP and the TBDMS groups are easily removed from synthetic oligonucleotides with 0.01 N HCl.

RNA undergoes base-catalyzed degradation by a pathway in which the 2′-oxygen attacks the adjacent 3′-phosphodiester to give products containing 2′,3′-cyclic phosphate and 5′-hydroxyl termini.[30] We examined whether the tertiary hydroxyl of 2′-*C*-*β*-methylcytidine could participate in an analogous base-catalyzed phos-

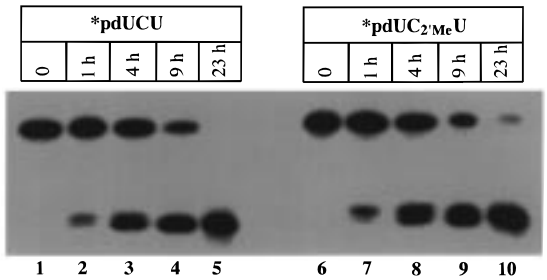

Figure 3. Cleavage of 5′-radiolabeled oligonucleotides with sodium hydroxide. In 0.1 N NaOH (25 °C), *pdUCU (**10c**) (lanes 1−5) and *pdUC$_{2'Me}$U (**10a**) (lanes 6−10) undergo base-catalyzed degradation at similar rates.[30]

photransesterification reaction. When *pdUC$_{2'Me}$U (**10a**) was treated with 0.1 N NaOH, a faster migrating species formed, presumably **11a** containing a 2′,3′-cyclic phosphate (Figure 3, lanes 6−10).[31] This cleavage product forms at a rate similar to the product **11c** from base-promoted cleavage of the trinucleotide *pdUCU (**10c**) (Figure 3, lanes 1−5) and has similar electrophoretic mobility. These results indicate that 2′-*C*-*β*-methylcytidine residues in oligonucleotides undergo base-catalyzed degradation as efficiently as natural RNA even though the 2′-nucleophile is bonded to a more hindered, tertiary carbon center. Thus, ribozymes that cleave RNA by this pathway might be active toward oligonucleotide substrates containing a 2′-C-branched substituent at the cleavage site, raising the possibility that 2′-C-branched ribonucleosides could be used to probe the mechanism of these ribozymes. The results also imply a cis relationship between the 2′-hydroxyl and 3′-hydroxyl groups of the 2′-*C*-*β*-methylcytidine residue, confirming that the methyl group of **1** was installed with the correct stereochemistry.

In conclusion, we have developed a strategy for the synthesis of phosphoramidite derivatives of 2′-*C*-*β*-methylcytidine and their incorporation into oligonucleotides via solid-phase synthesis. Either the TBDMS or the THP group can be used to protect the tertiary 2′-hydroxyl group of 2′-*C*-*β*-methylcytidine. The corresponding phosphoramidites couple efficiently during solid-phase oligo-

(28) We also synthesized both a 2′-*C*-*β*-methylcytidine containing dinucleotide C$_{2'MeOTBDMS}$U using **7a** and a control dinucleotide C$_{2'OTBDMS}$U. Following purification by reversed-phase HPLC, C$_{2'Me/OTBDMS}$U gave the expected molecular ion in the mass spectrum (M^- = 676 *m*/*z*), indicating that **7a** had been correctly installed into the dinucleotide. Removal of the TBDMS group from each dinucleotide was monitored by reversed-phase HPLC. As observed for the trinucleotides (**9a** and **9c**), TBAF/THF removed the TBDMS group more readily from the cytidine residue than from the 2′-*C*-*β*-methylcytidine residue; C$_{2'OTBDMS}$U was completely deprotected in less than 4 h, whereas C$_{2'Me/OTBDMS}$U was only about 70% deprotected after 27 h. Following purification by reversed-phase HPLC, C$_{2'Me}$U gave the expected molecular ion in the mass spectrum (M^- = 562 *m*/*z*), confirming that **7a** can be used to incorporate 2′-*C*-*β*-methylcytidine into oligonucleotides via solid-phase synthesis. The slower desilyation of the trinucleotides (**9a** and **9c**) compared to the dinucleotides may be due to the presence of the 5′-monophosphate group, which could limit their solubility in TBAF/THF. We also synthesized two 17 residue RNA oligonucleotides containing at position 9 either cytidine or 2′-*C*-*β*-methylcytidine. Deprotection was monitored by labeling the 5′-end of the oligonucleotide with ^{32}P phosphate followed by PAGE. After 3 days in TBAF/THF, about 70% of the 2′-*C*-*β*-methylcytidine residue was deprotected. Thus, although TBAF/THF removes the 2′-*O*-TBDMS group from 2′-*C*-*β*-methylcytidine slowly, the desired oligonucleotide can be obtained using the phosphoramidite **7a**.

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(31) If the products of base-promoted cleavage are electrophoresed further in the gel, they resolve into a doublet, suggesting that the 2′,3′-cyclic phosphate at least partially hydrolyzes to a mixture of the 2′- and 3′-monophosphates. Mikhailov et al. reported that under basic conditions 2′-*C*-*β*-methyluridine 2′,3′-cyclic phosphate hydrolyzes nearly as fast as uridine 2′,3′-cyclic phosphate.[5b]

nucleotide synthesis using standard conditions and protocols. Although the THP group adds another chiral center into the phosphoramidite, it is more advantageous than the commonly used TBDMS group because it is more easily introduced and more readily removed from synthetic oligonucleotides. The conditions required for removal of the THP group (0.01 N HCl; 30 °C) also remove the TBDMS group of commercial phosphoramidites, precluding the need for an additional deprotection step. This work represents the first synthesis of oligonucleotides containing a 2′-C-branched ribonucleoside and expands the repertoire of modified nucleic acids for use as antisense molecules or biochemical probes. Our approach should be generally applicable to the incorporation of other 2′-C-branched ribonucleosides (such as those in Chart 1) into oligonucleotides.

Experimental Section

All reagents and anhydrous solvents were purchased from Aldrich; other solvents were from Fisher unless otherwise noted. Melting points are uncorrected. Mass spectra were obtained from the Department of Chemistry, University of California at Riverside. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA. Merck silica gel (9385 grade, 230−400 mesh, 60 Å, Aldrich) was used for column chromatography. Silica gel on glass with fluorescent indicator (Sigma) was used for TLC. Reactions and workup procedures were at room temperature unless otherwise noted.

All nucleotide modifying enzymes were from U. S. Biochemicals. [γ-^{32}P]-Adenosine triphosphate ([γ-^{32}P]ATP) was obtained from New England Nuclear. PAGE was performed using 20% polyacrylamide (acrylamide/bis-acrylamide 29:1, Fisher) with 7.5 M urea (Fisher), 0.1 M Tris (Fisher), 0.1 M boric acid (Fisher), and 2 mM EDTA (Fisher). Gels were preelectrophoresed at constant power (15 W) for 1 h before sample loading. Gel loading buffer contained 8 M urea (Fisher), 50 mM EDTA (pH 8.0, Fisher), 0.02% bromophenol blue (EM Science), and 0.02% xylene cyanol FF (Kodak).

2′,3′,5′-Tri-*O*-benzoyl-4-*N*-benzoyl-2′-*C*-β-methylcytidine (2a). A stirred suspension of 4-*N*-benzoylcytosine (4.42 g, 19.30 mmol) and $(NH_4)_2SO_4$ (50 mg) in freshly distilled 1,1,1,3,3,3-hexamethyldisilazane (150 mL) was heated at reflux overnight under argon. During reflux, the mixture became clear within 1 h. The clear solution was evaporated under vacuum, and dry toluene (60 mL) was added and subsequently distilled off. The crude bis(trimethylsilyl) derivative obtained was dissolved in dry acetonitrile (150 mL), and 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methyl-α-(and β)-D-ribofuranose (**1**)$^{1p-q}$ (5.83 g, 10.05 mmol) was added. Under an argon atmosphere, $SnCl_4$ (2.35 mL, 20.0 mmol) was added in one portion with vigorous stirring and exclusion of moisture. The resultant homogeneous pale yellow solution was stirred overnight. When TLC indicated that **1** was completely consumed, the reaction was quenched carefully by addition of 10% $NaHCO_3$ (100 mL) and stirred for an additional 15 min. The mixture was extracted with methylene chloride, and the organic phase was washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel chromatography eluting with 50% ethyl acetate in hexane to give **2a** (5.81 g, 86%) as a white solid: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 8.67 (s, 1H), 8.07 (d, J = 7.54 Hz, 2H), 8.03 (d, J = 7.55 Hz, 2H), 7.93 (d, J = 6.43 Hz, 1H), 7.84 (d, J = 5.87 Hz, 2H), 7.80 (d, J = 7.35 Hz, 2H), 7.35−7.57 (m, 8H), 7.20 (t, J = 7.28 Hz, 2H), 6.72 (s, 1H), 5.75 (s, 1H), 4.86 (d, J = 3.93 Hz, 2H), 4.64 (q, J = 4.29 Hz, 1H), 1.70 (s, 3H).

4-*N*-Benzoyl-2-*C*-β-methylcytidine (2b). To a solution of **2a** (4.74 g, 7.0 mmol) in a mixture of pyridine (65 mL) and methanol (11.7 mL) was added dropwise ice-cooled aqueous NaOH (2.0 M, 10.6 mL, 21.2 mmol) at −20 °C with stirring. The reaction mixture was stirred at −20 °C to −15 °C for an additional 30 min and then neutralized to pH 7 with Dowex 50WX8-200 resin. The resin was filtered and washed with H_2O/pyridine (4:1, 400 mL). The filtrate was evaporated to dryness, and the residue was purified by silica gel chromatography eluting with 10% methanol in chloroform to give **2b** (2.38 g, 94%) as a white solid: mp 154−155 °C; ^{1}H NMR (500 MHz) ($CDCl_3$) δ 8.60 (d, J = 5.00 Hz, 1H), 8.01 (d, J = 7.50 Hz, 2H), 7.63 (t, J = 7.50 Hz, 1H), 7.52 (t, J = 7.50 Hz, 2H), 7.34 (d, J = 5.00 Hz, 1H), 5.98 (s, 1H), 5.20 (b, 1H), 5.18 (b, 2H), 3.86−3.88 (m, 2H), 3.67−3.74 (m, 2H), 1.01 (s, 1H); ^{13}C NMR (125 MHz) ($CDCl_3$) δ 167.4, 162.8, 154.7, 145.0, 133.2, 132.8, 129.3, 128.5, 96.0, 91.9, 82.3, 78.5, 71.4, 58.8, 19.8; HRMS (FAB) calcd for $C_{17}H_{20}N_3O_6$ $[MH]^+$ 362.1352, found 362.1344.

3′,5′-*O*-(Di-*tert*-butylsilanediyl)-2′-*C*-β-methyl-4-*N*-benzoylcytidine (3). To a solution of **2b** (1.22 g, 3.38 mmol) and $AgNO_3$ (1.27 g, 7.50 mmol) in DMF (30 mL) at 0 °C was added dropwise di-*tert*-butyldichlorosilane (0.79 mL, 3.75 mmol) with vigorous stirring. The mixture was warmed to room temperature and subsequently stirred for 15 min. Triethylamine (1.05 mL, 7.50 mmol) was added, and the mixture was stirred for an additional 5 min. The solvent was evaporated under reduced pressure, and the residue was partitioned between methylene chloride and H_2O. The organic layer was separated, washed with brine, and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by silica gel chromatography eluting with 5% methanol in chloroform to give **3** (1.61 g, 95%) as a white solid: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 8.86 (b, 1H), 7.91 (d, J = 7.50 Hz, 3H), 7.69 (d, J = 6.02 Hz, 1H), 7.62 (t, J = 7.50 Hz, 1H), 7.52 (t, J = 7.50 Hz, 2H), 6.23 (s, 1H), 4.55 (dd, J = 5.02 and 8.50 Hz, 1H), 4.15 (m, 1H), 4.06 (t, J = 9.75 Hz, 1H), 3.56 (d, J = 9.02 Hz, 1H), 2.67 (b, 1H), 1.23 (s, 3H), 1.08 (s, 9H), 1.05 (s, 9H); ^{13}C NMR (125 MHz) ($CDCl_3$) δ 162.5, 143.3, 133.2, 133.0, 129.0, 127.6, 96.8, 93.4, 80.6, 77.7, 74.3, 67.5, 27.3, 27.1, 22.8, 20.4; HRMS (FAB) calcd for $C_{25}H_{36}N_3O_6Si$ $[MH]^+$ 502.2373, found 502.2365.

3′,5′-*O*-(Di-*tert*-butylsilanediyl)-4-*N*-benzoyl-2′-*C*-β-methyl-2′-*O*-(*tert*-butyldimethylsilyl)cytidine (4a). To a solution of **3** (251.5 mg, 0.50 mmol) in THF (5 mL) was added a solution of *t*-BuMgCl in THF (1.0 M, 1.0 mL, 1.0 mmol; Aldrich) at 0 °C under a nitrogen atmosphere. The solution was allowed to warm to room temperature and stirred for 30 min. A solution of TBDMSOTf (230 μL, 1.0 mmol) in THF (2 mL) was added, and the mixture was stirred overnight. When TLC indicated that all the starting material had been consumed, the reaction mixture was carefully quenched with saturated aqueous NH_4Cl (2 mL) and extracted with methylene chloride. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel chromatography eluting with 3% methanol in chloroform to give **4a** (197.6 mg, 64%) as a white foam: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 9.12 (b, 1H), 7.93 (d, J = 7.50 Hz, 2H), 7.71 (d, J = 7.50 Hz, 1H), 7.61 (t, J = 7.50 Hz, 1H), 7.51 (t, J = 7.50 Hz, 2H), 6.22 (s, 1H), 4.55 (m, 1H), 4.16 (m, 1H), 4.07 (t, J = 9.50 Hz, 1H), 3.56 (d, J = 9.00 Hz, 1H), 2.91 (s, 1H), 1.23 (s, 3H), 1.07 (s, 9H), 1.05 (s, 9H), 0.91 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (125 MHz) ($CDCl_3$) δ 160.2, 148.0, 134.7, 130.4, 129.4, 129.1, 96.3, 93.7, 80.1, 77.9, 74.9, 67.1, 27.3, 27.1, 25.6, 22.7, 20.4, −3.6; HRMS (FAB) calcd for $C_{31}H_{50}N_3O_6Si_2$ $[MH]^+$ 616.3238, found 616.3258.

3′,5′-*O*-(Di-*tert*-butylsilanediyl)-4-*N*-benzoyl-2′-*C*-β-methyl-2′-*O*-(tetrahydropyranyl)cytidine (4b). To a solution of **3** (720.0 mg, 1.44 mmol) and 3,4-dihydro-*2H*-pyran (1.31 mL, 14.4 mmol) in dry methylene chloride was added 10-camphorsulfonic acid (150.0 mg). The reaction mixture was stirred overnight, diluted with methylene chloride, washed with 5% $NaHCO_3$, and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel chromatography eluting with 3% methanol in chloroform to give **4b** (798.7 mg, 95%) as a mixture of two diastereomers: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 8.81 (b, 1H), 7.91 (d, J = 7.50 Hz, 2H), 7.68 (d, J = 6.50 Hz, 1H), 7.62 (t, J = 7.50 Hz, 1H), 7.52 (t, J = 7.50 Hz, 2H), 6.58 + 6.10 (s, 1H), 5.59 + 5.48 (s, 1H), 4.56 (dd, J = 9.00, 5.00 Hz, 1H), 4.27−4.37 (m, 1H), 4.07−4.13 (m, 1H), 4.01−4.05 (m, 1H), 3.46−3.64 (m, 2H), 1.52−1.92 (m, 6H), 1.31 (s, 3H), 1.07+1.05 (s, 9H), 1.06 + 1.04 (s, 9H); ^{13}C NMR (125

MHz) ($CDCl_3$) δ 162.1, 143.1, 133.3, 129.1, 127.5, 94.4, 93.3, 92.2, 92.1, 83.0, 82.4, 81.7, 74.2, 67.8, 62.9, 61.6, 60.7, 31.8, 31.5, 30.7, 27.9, 27.8, 27.3, 26.9, 25.4, 25.3, 22.9, 20.3, 18.8, 18.4, 16.9; HRMS (FAB) calcd for $C_{30}H_{44}N_3O_7Si$ $[MH]^+$ 586.2949, found 586.2931.

4-*N*-Benzoyl-2′-*C*-β-methyl-2′-*O*-(*tert*-butyldimethylsilyl)cytidine (5a). HF·pyridine (100 μL, 3.84 mmol; Aldrich) was carefully diluted with pyridine (0.51 mL) and then added dropwise to a solution of **4a** (582.7 mg, 0.95 mmol) in THF (5 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 5 min, and then diluted with pyridine (1.5 mL). Extraction was performed with methylene chloride/H_2O. The organic layer was collected, washed with 5% $NaHCO_3$, and dried over Na_2SO_4. After evaporation of the solvent under reduced pressure, the residue was purified by silica gel chromatography eluting with 8% methanol in chloroform to give **5a** (369.0 mg, 82%) as a white foam: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 8.57 (d, J = 7.50 Hz, 1H), 7.97 (d, J = 7.50 Hz, 2H), 7.63 (d, J = 7.50 Hz, 1H), 7.61 (t, J = 7.50 Hz, 1H), 7.52 (t, J = 7.50 Hz, 2H), 6.18 (s, 1H), 4.07−4.13 (m, 2H), 3.89−3.94 (m, 2H), 1.23 (s, 3H), 0.97 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (125 MHz) ($CDCl_3$) δ 162.5, 156.1, 145.1, 133.3, 132.8, 129.0, 127.6, 97.0, 92.8, 84.2, 78.7, 73.1, 61.2, 25.9, 20.2, 18.5, −5.5; HRMS (FAB) calcd for $C_{23}H_{34}N_3O_6Si$ $[MH]^+$ 476.2217, found 476.2194.

4-*N*-Benzoyl-2′-*C*-β-methyl-2′-*O*-(tetrahydropyranyl)cytidine (5b). HF·pyridine (116 μL, 4.45 mmol; Aldrich) was carefully diluted with pyridine (0.60 mL) and then added dropwise to a solution of **4b** (650.8 mg, 1.11 mmol) in THF (5 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 5 min, and then diluted with pyridine (2 mL). Extraction was performed with methylene chloride/H_2O, and the organic layer was collected, washed with 5% $NaHCO_3$, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure. Chromatography on silica gel, eluting with 8% methanol in chloroform, separated the two diastereomers of **5b** (455.4 mg, 92%). The fast isomer: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 9.01 (s, 1H), 8.65 (d, J = 7.50 Hz, 1H), 7.87 (d, J = 8.00 Hz, 2H), 7.60 (t, J = 7.50 Hz, 1H), 7.49 (t, J = 7.50 Hz, 2H), 6.21 (s, 1H), 5.04 (d, J = 5.00 Hz, 1H), 4.15 (m, 2H), 4.03 (m, 2H), 3.88−3.94 (m, 2H), 3.54 (m, 1H), 3.27 (b, 1H), 1.88 (m, 2H), 1.60 (m, 2H), 1.54 (m, 2H), 1.25 (s, 3H); ^{13}C NMR (125 MHz) ($CDCl_3$) δ 162.3, 145.3, 133.1, 129.0, 127.6, 96.9, 95.3, 91.4, 85.9, 82.6, 72.4, 64.8, 59.6, 31.9, 24.9, 20.9, 15.7; HRMS (FAB) calcd for $C_{22}H_{28}N_3O_7$ $[MH]^+$ 446.1927, found 446.1941. The slow isomer: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 9.08 (b, 1H), 8.63 (b, 1H), 7.87 (d, J = 7.50 Hz, 2H), 7.59 (t, J = 7.02 Hz, 2H), 7.54 (m, 1H), 7.48 (t, J = 7.50 Hz, 2H), 6.75 (b, 1H), 5.30 (b, 1H), 4.17 (d, J = 12.00 Hz, 1H), 4.06 (m, 1H), 4.01 (m, 2H), 3.69 (b, 2H), 3.10 (b, 1H), 1.85 (m, 1H), 1.76 (m, 1H), 1.53−1.65 (m, 4H), 1.25 (s, 3H); ^{13}C NMR (125 MHz) ($CDCl_3$) δ 162.4, 155.7, 145.8, 133.1, 128.9, 127.6, 96.9, 95.7, 84.6, 83.0, 73.1, 64.3, 59.6, 32.0, 27.3, 25.0, 20.6, 16.9.

4-*N*-Benzoyl-5′-*O*-(dimethoxytrityl)-2′-*C*-β-methyl-2′-*O*-(*tert*-butyldimethylsilyl)cytidine (6a). To a solution of **5a** (423.4 mg, 0.89 mmol) in dry THF (10.0 mL) under argon were added anhydrous pyridine (360.5 μL, 4.46 mmol), $AgNO_3$ (151.4 mg, 0.89 mmol), and DMTrCl (138.5 mg, 0.90 mmol) successively with vigorous stirring and exclusion of moisture. The resultant pale-yellow suspension was stirred for 1 h and filtered into a 5% $NaHCO_3$ solution. The filtrate was extracted with chloroform, washed with 5% $NaHCO_3$ and brine, and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by flash chromatography on silica gel eluting with 2% methanol in chloroform to give **6a** (450.2 mg, 65%) as a white foam: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 8.75 (d, 7.50 Hz, 2H), 7.89 (d, J = 6.52 Hz, 2H), 7.60 (t, J = 7.50 Hz, 1H), 7.50 (t, J = 7.50 Hz, 2H), 7.26−7.43 (m, H), 6.90 (d, J = 8.50 Hz, 4H), 6.26 (s, 1H), 4.14 (dd, J = 11.00, 9.50 Hz, 1H), 3.93 (m, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.58−3.67 (m, 2H), 2.29 (d, J = 12.00 Hz, 1H), 1.27 (s, 3H), 0.90 (s, 9H), 0.41 (s, 3H), 0.26 (s, 2H); ^{13}C NMR (125 MHz) ($CDCl_3$) δ 158.7, 143.9, 135.6, 135.2, 133.0, 130.2, 130.1, 129.0, 128.4, 128.1, 127.5, 127.2, 113.3, 91.3, 87.3, 82.7, 82.6, 73.5, 60.5, 55.2, 25.8, 19.5, 18.2, 0.0, −2.5, −3.2; HRMS (FAB) calcd for $C_{44}H_{52}N_3O_8Si$ $[MH]^+$ 778.3824, found 778.3515.

4-*N*-Benzoyl-5′-*O*-(dimethoxytrityl)-2′-*C*-β-methyl-2′-*O*-(tetrahydropyranyl)cytidine (6b). To a solution of the two diastereomers of **5b** (488.9 mg, 1.10 mmol) in dry pyridine (15.0 mL) at 0 °C was added dropwise a solution of dimethoxytrityl chloride (391.4 mg, 1.32 mmol) in pyridine (10.0 mL) over 1 h. The reaction mixture was warmed to room temperature and stirred overnight until TLC indicated that the reaction was complete. Methanol (1.0 mL) was added to quench the reaction, and the mixture was stirred for an additional 15 min and concentrated under reduced pressure. The residue was dissolved in methylene chloride, washed with 5% $NaHCO_3$, water, and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by silica gel chromatography eluting with 2% methanol in chloroform to give **6b** (750.8 mg, 91%) as a slightly yellow foam: ^{1}H NMR (500 MHz) ($CDCl_3$) δ 8.69 (m, 2H), 7.88 (d, J = 7.50 Hz, 2H), 7.59 (m, 1H), 7.49 (m, 2H), 7.43 (m, 2H), 7.28−7.35 (m, 7H), 6.89 (d, J = 9.00 Hz, 4H), 6.81 + 6.21 (s, 1H), 5.34 + 5.04 (m, 1H), 3.96 (m, 1H), 3.83 + 3.84 (s, 6H), 3.69 (m, 1H), 3.59 (m, 1H); ^{13}C NMR (125 MHz) ($CDCl_3$) δ 166.2, 158.6, 145.1, 144.0, 135.6, 135.3, 133.0, 130.2, 130.1, 129.1, 128.9, 128.3, 128.0, 127.8, 127.7, 127.5, 127.2, 127.1, 95.5, 95.2, 91.2, 88.4, 87.2, 87.1, 85.8, 84.5, 82.1, 81.7, 73.8, 73.0, 64.8, 64.3, 60.7, 60.6, 55.2, 32.0, 31.9, 24.9, 24.8, 20.9, 20.5, 17.0, 15.6; HRMS (FAB) calcd for $C_{43}H_{44}N_3O_9$ $[MH]^+$ 748.3234, found 748.3207.

4-*N*-Benzoyl-5′-*O*-(dimethoxytrityl)-2′-*C*-β-methyl-2′-*O*-(*tert*-butyldimethylsilyl)cytidine 3′-*N*,*N*-Diisopropyl(cyanoethyl)phosphoramidite (7a). To a solution of *N*,*N*-diisopropylethylamine (185.9 μL, 1.07 mmol), 1-methylimidazole (21.4 μL, 0.27 mmol), and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (178.0 μL, 0.80 mmol) in dry methylene chloride (5.0 mL) at 0 °C was added dropwise a solution of **6a** (257.7 mg, 0.33 mmol) in methylene chloride (5.0 mL) under an argon atmosphere. The reaction mixture was warmed to room temperature and stirred until the starting material was consumed (∼3 h) as indicated by TLC. 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 phosphoramidite derivative **7a** (271.3 mg, 84%) as a white foam: ^{31}P NMR (500 MHz) ($CDCl_3$) δ 151.0, 150.9; HRMS (FAB) calcd for $C_{53}H_{69}N_5O_9PSi$ $[MH]^+$ 978.4602, found 978.4614.

4-*N*-Benzoyl-5′-*O*-(dimethoxytrityl)-2′-*C*-β-methyl-2′-*O*-(tetrahydropyranyl)cytidine 3′-*N*,*N*-Diisopropyl(cyanoethyl)phosphoramidite (7b). To a solution of **6b** (305.8 mg, 0.41 mmol) in dry methylene chloride (15.0 mL) at 0 °C under an argon atmosphere were added quickly *N*,*N*-diisopropylethylamine (190.9 μL, 1.10 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (182.5 μL, 0.82 mmol), and 1-methylimidazole (22.2 μL, 0.28 mmol). The reaction mixture was then warmed to room temperature and stirred until TLC indicated that the reaction was complete (∼3 h). 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 phosphoramidite derivative **7b** (348.1 mg, 90%) as a slightly yellow foam: ^{31}P NMR (500 MHz) ($CDCl_3$) δ 152.9, 152.2, 150.3, 150.0; HRMS (FAB) calcd for $C_{52}H_{63}N_5O_{10}P$ $[MH]^+$ 948.4313, found 948.4342.

Incorporation of 2′-*C*-β-Methylcytidine into Oligonucleotides by Solid-Phase Synthesis. Oligonucleotides (the trinucleotides **8a**−**c** described in the text and the dinucleotides and 17 residue oligonucleotides described in ref 28) were synthesized on a 1 μmol scale with standard phosphoramidites (Perseptive Biosystems and Glen Research) using a Millipore Expidite nucleic acid synthesis system and standard DNA and RNA protocols. Both phosphoramidites **7a** and **7b** were coupled according to the standard RNA synthesis protocol and gave yields comparable to those of the commercial phosphoramidites. Synthesized oligonucleotides were deprotected at 55 °C for 18−24 h with concentrated aqueous NH_3 and ethanol (2:1) and subsequently evaporated to dryness. Oligonucleotide

10c was desilylated by treatment with 1.0 M TBAF/THF, shaken in the dark for 24 h, neutralized with 0.1 M triethylammonium acetate (TEAAC, pH 6.9), and purified by elution through a NAP-10 gel-filtration column (Pharmacia Biotech.). Overall yields of oligonucleotides containing the 2′-modification were comparable to those for oligonucleotides lacking the modification.

Radiolabeling of Oligonucleotides. Oligonucleotides were 5′-end labeled with [γ-^{32}P]-ATP and PNK. Reactions included [γ-^{32}P]-ATP (30 μCi), PNK (17 units), and oligonucleotide (20 pmol) in a 10 μL volume. After 1 h incubation at 37 °C, the reactions were quenched with gel loading buffer (8 μL), and the components were separated by PAGE. Products were visualized by autoradiography, excised from the gel, and eluted at 4 °C with H_2O. The eluent was applied to a Sep-Pac C18 cartridge (Waters), which was then washed with water (2 mL), 50% aqueous acetonitrile (2 mL), and 75% aqueous acetonitrile (2 mL). Fractions containing oligonucleotide were evaporated to dryness and brought up into water to a concentration of 20 nM as determined by specific radioactivity.

Deprotection of 5′-Radiolabeled Oligonucleotides with TBAF. 5′-Radiolabeled oligonucleotides (1 μL) were evaporated to dryness, taken up into a solution of TBAF in THF (1.0 M, 5 μL), shaken in the dark at room temperature, and neutralized with 0.1 M TEAAC (pH 6.9). Most of the THF was evaporated, and gel loading buffer (8.0 μL) was added. The sample was stored at −78 °C before loading on the gel (see Figure 1).

Deprotection of 5′-Radiolabeled Oligonucleotides with Acids. 5′-Radiolabled oligonucleotides (1 μL) were treated with 10% acetic acid (1 μL), 8% formic acid (1 μL), and 0.01 N HCl (pH 2.0, 1 μL), respectively, at 30 °C. The reactions were quenched with 1X TBE (5 μL) and gel loading buffer (8 μL). The samples were stored at −78 °C before loading on the gel (Figure 2 shows results from deprotection with 0.01 N HCl).

Cleavage of 5′-Radiolabeled Oligonucleotides with Sodium Hydroxide. 5′-Radiolabled oligonucleotides (1 μL) were treated with 0.1 N NaOH (1 μL) at room temperature. The reactions were quenched with 1X TBE (5 μL) and gel loading buffer (8 μL). The sample was stored at −78 °C before loading on the gel (see Figure 3).

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Supporting Information Available: NMR spectra for obtained compounds (23 pages). See any current masthead page for ordering and Internet access information.

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