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Synthesis of 2′-*C*-α-(Hydroxyalkyl) and 2′-*C*-α-Alkylcytidine Phosphoramidites: Analogues for Probing Solvent Interactions with RNA

Nan-Sheng Li* and Joseph A. Piccirilli*

Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biology and Department of Chemistry, University of Chicago, 929 East 57th Street, Chicago, Illinois 60637

nli@uchicago.edu; jpicciri@uchicago.edu

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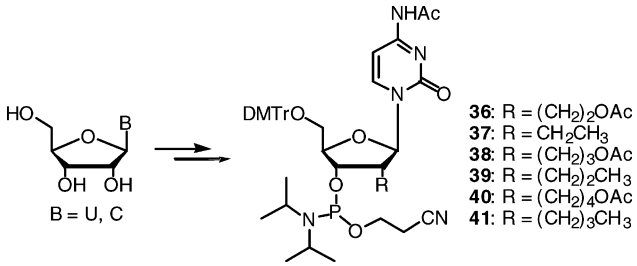

Nucleoside analogues bearing 2′-*C*-α-(hydroxyalkyl) and 2′-*C*-α-alkyl substitutes have numerous applications in RNA chemistry and biology. In particular, they provide a strategy to probe the interaction between the 2′-hydroxyl group of RNA and water. To incorporate these nucleoside analogues into oligonucleotides for studies of the group II intron (Gordon, P. M.; Fong, R.; Deb, S.; Li, N.-S.; Schwans, J. P.; Ye, J.-D.; Piccirilli, J. A. *Chem. Biol.* **2004**, *11*, 237), we synthesized six new phosphoramidite derivatives of 2′-deoxy-2′-*C*-α-(hydroxyalkyl)cytidine (**36**: R = $-(CH_2)_2OH$; **38**: R = $-(CH_2)_3OH$; **40**: R = $-(CH_2)_4OH$) and 2′-deoxy-2′-*C*-α-alkylcytidine (**37**: R = $-CH_2CH_3$; **39**: R = $-(CH_2)_2CH_3$; **41**: R = $-(CH_2)_3CH_3$) from cytidine or uridine via 2′-C-α-allylation, followed by alkene and alcohol transformations. Phosphoramidites **36** and **37** were prepared from cytidine in overall yields of 14% (10 steps) and 7% (11 steps), respectively. Phosphoramidites **38** and **39** were prepared from uridine in overall yields of 30% (10 steps) and 13% (11 steps), respectively. Phosphoramidites **40** and **41** were synthesized from uridine in overall yields of 21% (13 steps) and 25% (14 steps), respectively.

Introduction

Nucleoside analogues bearing 2′-*C*-α-hydroxyalkyl substituents have numerous applications in biomedical science and biophysical and mechanistic chemistry. As components of gene regulatory agents (antisense and siRNAs), they retain the ability to form Watson−Crick base pairs but confer enhanced resistance to nuclease degradation.[1] Within the context of 2′-5′-linked RNA hairpin loops, the 2′-hydroxymethyl substituent lengthens the sugar−phosphate backbone, thereby enhancing thermal stability.[2] Hydroxyalkyl nucleosides also provide valuable precursors to access the corresponding alkyl derivatives[3] or to append amino, thiol, and carboxylate groups to nucleic acids.[4] These functional groups may allow stereocontrolled nucleobase exchange,[5] conjugation of biophysical probes or biological molecules,[6] or introduction of structural constraints.[7]

Nucleotides bearing hydroxyalkyl and alkyl substituents provide a way to reveal the role of water molecules important for nucleic acid function. The substrate specificity of SMUG1, a uracil DNA glycosylase, provides a biological illustration of this approach.[8] (Figure 1). The enzyme deglycosylates uridine residues selectively over thymidine residues because the methyl group displaces an important water molecule in the active site.

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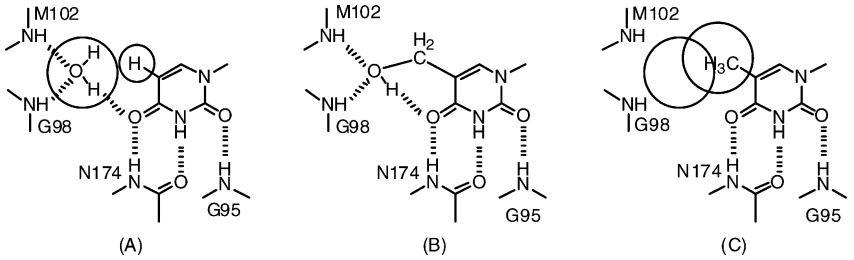

FIGURE 1. Pyrimidine specificity of SMUG1 deglycosylase. Adapted from ref 8 with permission from Elsevier. (A) Uracil makes hydrogen bonds without displacing the active site water molecule. (B) Hydroxymethyluracil makes the same specific hydrogen bonds as uracil. The hydroxymethyl group displaces the water molecule but supplants the lost hydrogen bonds. (C) Thymidine binds less favorably because the methyl group displaces the water molecule but cannot supplant the lost hydrogen bonds.

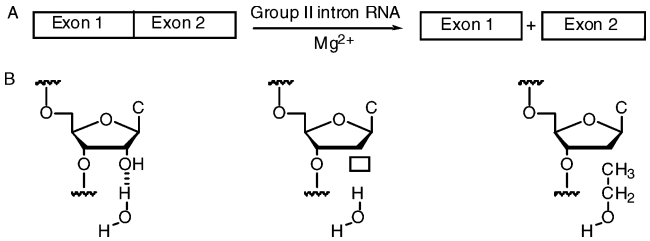

FIGURE 2. (A) SER reaction catalyzed by group II intron RNA. (B) Hypothetical model showing how ethanol could stimulate the reactivity of $E1_{2'H}E2$. Removal of the splice junction 2′-OH might disrupt solvent organization and allowing binding of ethanol.

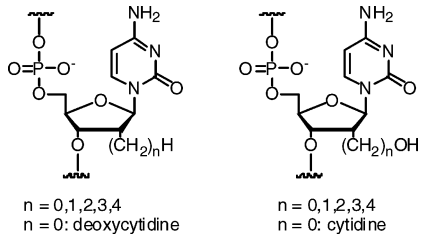

FIGURE 3. RNAs with site-specific 2′-*C*-α-alkyl or 2′-*C*-α-hydroxylalkylcytidine.

A 5-hydoxymethyl substituent supplants this water molecule, allowing SMUG1 to deglycosylate 5-hydroxymethyl uridine residues. Thus, enhanced activity upon hydroxyalkyl substitution (5-hydroxymethyluridine in the case of SMUG1) relative to the corresponding alkyl substitution (thymidine) implicates the localization of an important water molecule in the absence of any substitution (uridine).

The synthetic work described herein was specifically inspired by investigation of the spliced exons reopening (SER) reaction catalyzed by the group II intron RNA. In the SER reaction, an RNA substrate whose sequence corresponds to spliced exons undergoes hydrolysis at the splice junction phosphodiester (Figure 2A).[9] A 2′-deoxycytosine residue at the splice junction reduces the rate of the SER reaction by 10-fold, implying that the 2′-hydroxyl group imparts a functional contribution to catalysis.[10] Using a series of nucleotide analogues that span a range of chemical diversity, we showed that the reaction rate inversely correlates with the substituent hydrophobicity,[11] suggesting that the 2′-hydroxyl group may interact with a water molecule. Consistent with this observation, we observed that ethanol, but not methanol, 2-propanol, or *tert*-butyl alcohol, modestly rescues the deoxynucleotide substrate.[11] Possibly the space vacated by the hydroxyl group allows the ethanol to bind to the ribozyme−substrate complex via hydrophobic interactions with the deoxyribose ring so as to localize or replace an active site water molecule (Figure 2B). This led us to investigate whether we might observe stimulation of the SER reaction by attaching an alcohol moiety covalently to the 2′-carbon of the cytosine residue at the splice junction. We therefore constructed a series of substrates containing hydroxyalkyl groups [$(CH_2)_n$-OH; $n = 1-4$] at the 2′-position (Figure 3). As the SER reaction exhibited sensitivity to the substituent volume, we also synthesized the corresponding deoxygenated substrates containing straight chain alkyl groups [$(CH_2)_n$H; $n = 1-4$] (Figure 3). Together, the analogues allow us to quantitate the effect of extending the hydroxyl group away from the sugar ring in one-carbon steps successively, thereby revealing whether the 2′-hydroxyl group imparts its functional contribution via inductive effects or via space interactions with solvent. The synthesis of 2′-*C*-α-methyl and hydroxymethyl cytidine phosphoramidites was described previously.[12,13] Here, we describe the preparation of the six remaining phosphoramidites ($n = 2-4$).

Results and Discussion

Grotli and Undheim prepared the 2′-*C*-α-allyl-2′-deoxynucleoside analogues of A, C, G, and U via ultraviolet light-induced radical allylation and derivatized them to the corresponding phosphoramidites.[14] Beigelman et al. used an AIBN-initiated radical allylation reaction to obtain the phosphoramidite derivatives of 2′-allyl-2′-deoxycytidine and 2′-allyl-2′-deoxyuridine.[15] As the procedure of Grotli and Undheim required a quartz tube, a 125 W Hg UV lamp, and long photolysis time (ca. 4 days), we opted to use Beigelman et al.'s AIBN-initiated radical allylation procedure for the synthesis of our target phosphoramidites.[15]

1. 2′-*C*-α-(Hydroxyethyl)cytidine and 2′-*C*-α-Ethylcytidine. Cytidine derivatives bearing 2′-ethyl and 2′-hydroxyethyl groups have not been prepared previously, but the corresponding uridine analogues and their phosphoramidites have been prepared. Mesmaeker et al. prepared 2′-*C*-α-ethyldeoxyuridine and the

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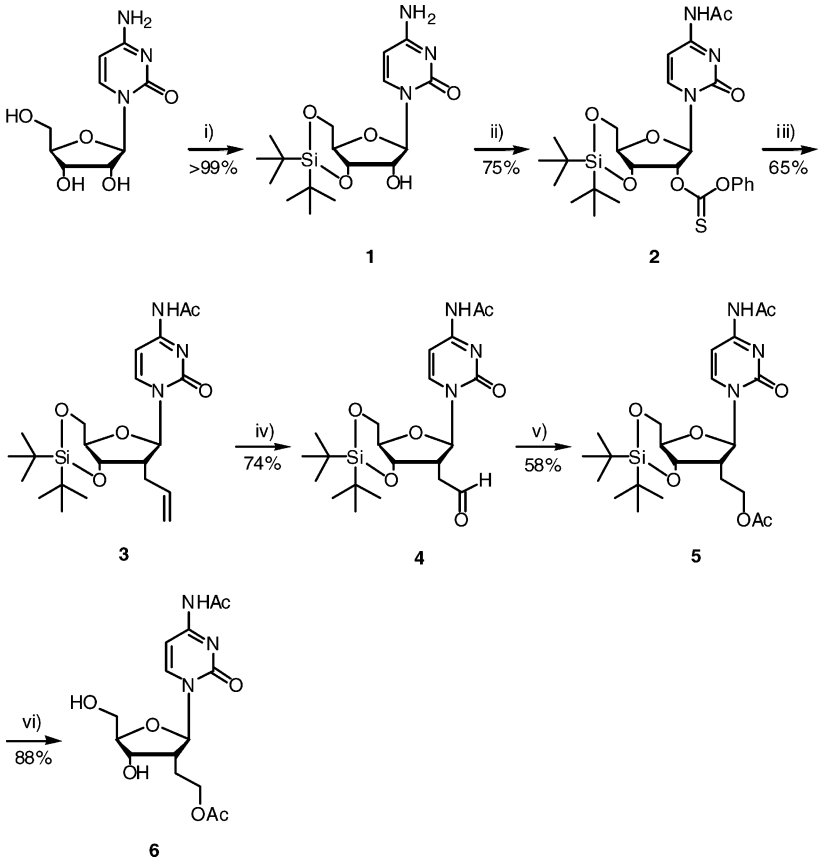
SCHEME 1 [a]



[a] (i) (1) *t*-Bu_2SiCl_2, $AgNO_3$, DMF, 0 °C, 30 min; (2) Et_3N, rt, 5 min; (ii) (1) PhOCSCI, DMAP, CH_2Cl_2, 0 °C to rt, 16 h; (2) AcCl, 0 °C, 1 h; (iii) allyltributylstannane, AlBN, benzene, reflux, 4 h, 65% or toluene, reflux, 6 h, 59%; (iv) $NaIO_4$, OsO_4 (cat), acetone/water (6:1), rt, overnight; (v) (1) $NaBH_4$, MeOH, 0 °C, 30 min; (2) AcCl, DMAP, CH_2Cl_2, 0 °C, 30 min; (vi), Et_3N-3HF, Et_3N, THF, rt, 30 min.

corresponding phosphoramidite from 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine via a reaction sequence that included UV-initiated radical allylation, oxidative cleavage, reduction, and deoxygenation. This reaction sequence gave the phosphoramidite in 10 steps and 7.6% overall yield.[3] Lawrence et al. used the olefin oxidative cleavage product from Mesmaeker's sequence, a 2′-*C*-α-(2-oxyethyl)uridine derivative, to prepare 2′-*C*-α-(hydroxyethyl)deoxyuridine and its phosphoramidite.[16,17] Additionally, Matsuda and co-workers reported access to a 2′-*C*-α-(hydroxyethyl)uridine derivative in low yield from the corresponding 2′-phenylseleno-3′-*O*-vinylsilyluridine derivative by an intramolecular radical reaction.[4]

We accessed 2′-*C*-α-(hydroxylethyl)cytidine and 2′-*C*-α-ethylcytidine from cytidine using reactions analogous to those of Mesmaker et al. and Lawrence et al. for the preparation of the corresponding uridine derivatives. To allow cytidine transformation via this strategy, we chose protecting groups judiciously. We protected the exocyclic amino group as the acetamide for two reasons: (1) we had observed that the 2′-thionoester of both 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)-N^4-benzoylcytidine and 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)cytidine (lacking N^4-protection) resists AIBN-initiated radical 2′-allylation,[15,18] and (2) acetamide protection has been used previously for radical 2′-deoxygenation[19] and radical 2′-allylation (up to 50% yield has been reported using AIBN as initiator).[15] We protected the 3′- and 5′-hydroxyl groups as the di-*tert*-butylsilanediyl ether rather than as the 1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl ether based on the lower cost of the reagent (di-*tert*-butyldichlorosilane) and because its removal occurs under milder conditions.[20,21] To allow incorporation of 2′-hydroxyethylcytidine into oligonucleotides during solid-phase synthesis, we blocked the hydroxyethyl group as the acetate ester, a strategy that has allowed incorporation of 2′-hydroxylmethyluridine into RNA previously.[1] Although nucleobase deprotection conditions that follow solid-phase synthesis remove the acetyl group, residues bearing 2′-hydroxylalkyl groups within RNA exhibit greater stability against base-catalyzed phosphodiester cleavage than do ribonucleotides.[1]

Scheme 1 shows the synthesis of 2′-*C*-α-(acetoxyethyl)-N^4-acetyl-2′-deoxycytidine (**6**) via the 2′-allyl derivative. Using a modified literature procedure, we obtained 3′,5′-*O*-(di-*tert*-butylsilanediyl)cytidine (**1**) in quantitative yield from cytidine and di-*tert*-butyldichlorosilane in the presence of silver nitrate (the reaction was carried out at 0 °C for 30 min rather than at 23 °C for 4 min).[22] This alternative procedure improved the yield by ∼10%. Reaction of **1** with phenyl chlorothionoformate

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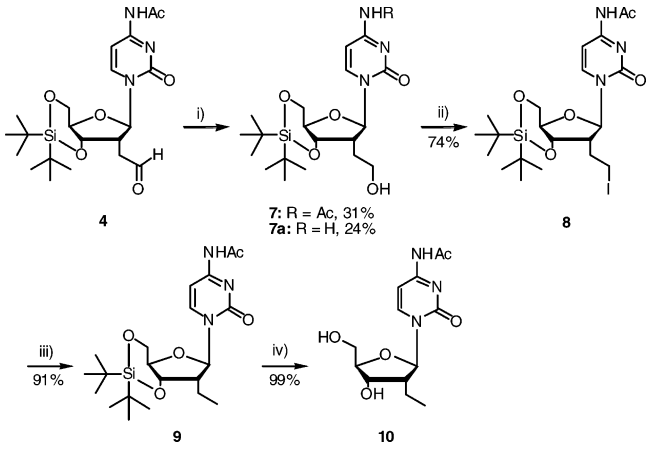
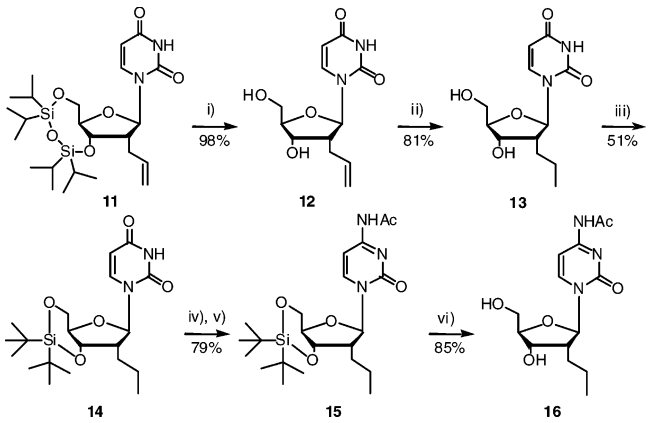
SCHEME 2 [a]



[a] (i) $NaBH_4$, MeOH, 0 °C to rt, 30 min; (ii) I_2, PPH_3, imidazole, benzene/acetonitrile (4:1), 0 °C to rt, 2 h; (iii) *n*-Bu_3SnH/AlBN, benzene, THF, sonication, 0 °C, 1 h; (iv) Et_3N-3HF, Et_3N, THF, rt, 1 h.

SCHEME 3 [a]



[a] (i) TBAF, THF, rt, 10 min; (ii) 10% Pd/C, H_2, MeOH, rt, 24 h; (iii) (1) *t*-Bu_2SiCl_2, $AgNO_3$, DMF, 0 °C, 30 min, (2) Et_3N, rt, 10 min; (iv) (1) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et_3N, CH_3CN, rt, 60 h; (2) NH_4OH, rt, 3 h; (v) CH_3COCl, DMAP, CH_2Cl_2, 0 °C, 30 min; (vi) Et_3N-3HF, Et_3N, THF, rt, 1 h.

gave the 2′-phenoxythiocarbonyl ester, which was then acetylated to give the N^4-acetylcytidine derivative **2** in 75% yield. Radical AIBN-initiated allylation of **2** with allyltributylstannane in refluxing benzene gave the 2′-α-allylcytidine derivative **3** in 65% yield. From refluxing toluene, a slightly lower yield (59%) was obtained. This approach gives superior yields of protected 2′-α-allylcytidine (49% overall yield from cytidine) at less expense than does the literature procedure for preparing the analogous compound with the 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl) protecting group (overall 35% yield from cytidine). Continuing with the synthesis of **6**, osmium tetroxide-catalyzed sodium periodate oxidation converted **3** to the 2′-(2-oxoethyl)cytidine derivative **4** in 74% yield. Sodium borohydride reduction followed by acetylation gave the bisacetyl cytidine derivative **5** in 58% yield. Desilylation with triethylamine−trihydrofluoride complex gave the 2′-*C*-α-(hydroxyethyl)cytidine derivative **6** in 88% yield.

We used intermediate **4** to prepare N^4-acetyl-2′-deoxy-2′-*C*-α-ethylcytidine (**10**) (Scheme 2). Reduction of **4** with sodium borohydride gave the 2′-hydroxyethylcytidine derivative **7** in 31% yield and the corresponding deacetylated product **7a** in 24% yield. Incubation with a mixture of iodine, triphenylphosphine, and imidazole converted **7** to the corresponding 2′-*C*-α-(iodoethyl)cytidine derivative **8** in 74% yield.[23] Ultrasound-initiated radical dehalogenation with tributyltin hydride converted **8** to the 2′-*C*-α-ethylcytidine derivative **9** in 91% yield.[24] Without ultrasound irradiation, the reaction in refluxing toluene gave a ∼1:1 mixture of **7** and **9**, based on TLC analysis. The attempt to convert **7** to **9** with Mesmaeker et al.'s method (tosylation, followed by AIBN-initiated reduction) failed.[3] Subsequent desilylation of **9** with triethylamine−trihydrofluoride complex gave the 2′-*C*-α-ethylcytidine derivative **10** in 99% yield.

2. 2′-*C*-α-Propylcytidine and 2′-*C*-α-(Hydroxypropyl)-cytidine. The susceptibility of the cytosine ring to reduction under hydrogenation conditions precludes preparation of 2′-*C*-α-propylcytidine directly from 2′-*C*-α-allylcytidine.[13,25] Cicero et al. prepared 2′-*C*-α-propylcytidine by transformation of 2′-*C*-α-propyluridine, which was obtained from 2′-*C*-α-allyluridine via palladium-catalyzed hydrogenation.[26] We prepared the starting material, 2′-*C*-α-allyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine, in three steps from uridine[15] and attempted to transform it to N^4-acetyl-2′-*C*-α-propylcytidine as described by Cicero at al. (hydrogenation, desilylation, and amination).[26] Our attempts to hydrogenate 2′-*C*-α-allyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine (treatment of **11** with 10% Pd/C under hydrogen at room temperature for 19 h) gave incomplete hydrogenation and some desilylation, however. We found that the reverse reaction sequence, desilylation[14] followed by palladium-catalyzed hydrogenation, proceeded more smoothly to give 2′-*C*-α-propyluridine (**13**) in 79% yield from **11** (Scheme 3). Simultaneous protection of the 3′ and 5′-hydroxyl groups using di-*tert*-butyldichlorosilane gave the bis-silyl ether **14** in 51% yield.[22] Transformation to the N^4-acetylcytidine derivative **15** was achieved in 79% yield using a two-step reaction sequence: amination using 2,4,6-triisopropylbenzenesulfonyl chloride and ammonium hydroxide[27] followed by acetylation. Desilylation with the triethylamine−trihydrofluoride complex gave N^4-acetyl-2′-*C*-α-propylcytidine (**16**) in 85% yield.

The synthesis of 2′-*C*-α-(hydroxypropyl)cytidine has not been reported, but syntheses for the corresponding uridine and thymidine analogues have been reported. Xi et al. prepared 2′-hydroxypropylthymidine via an intramolecular radical reaction from 2′-phenylseleno-3′-*O*-allyldimethylsilylthymidine.[28] Fehring et al. prepared the 2′-*C*-α-(hydroxypropyl)uridine derivative **17** in 63% yield from **11** by hydroboration using a borane−dimethyl sulfide complex, followed by oxidation with triethylamine-*N*-oxide.[5] We attempted direct hydroboration of the 2′-allylcytidine derivative **3** using 9-BBN and sodium perborate (a generally efficient hydroboration/oxidation sequence[29,30]) but

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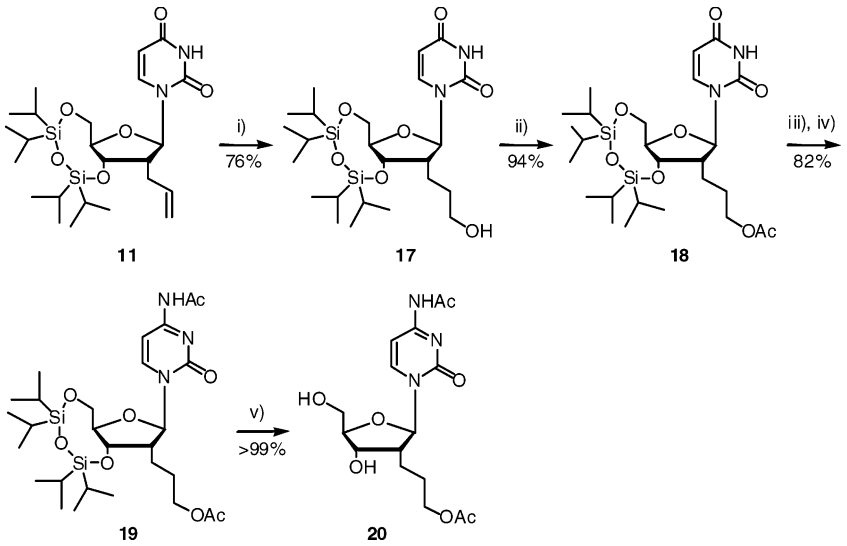
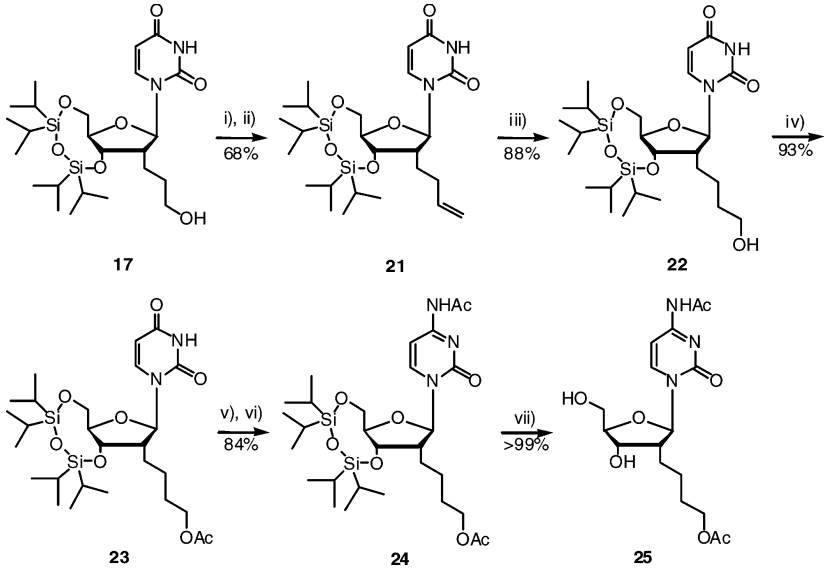
SCHEME 4 [a]



[a] (i) (1) 9-BBN, THF, rt, 4 h; (2) $NaBO_3$-$4H_2O$, rt, 2 h; (ii) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 0 °C to rt, 1 h; (iii) (1) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et_3N, CH_3CN, rt, 48 h; (2) NH_4OH, rt, 3 h; (iv) acetyl chloride, DMAP, CH_2Cl_2, 0 °C to rt, 1 h; (v) Et_3N-3HF, Et_3N, THF, rt, 2 h.

SCHEME 5 [a]



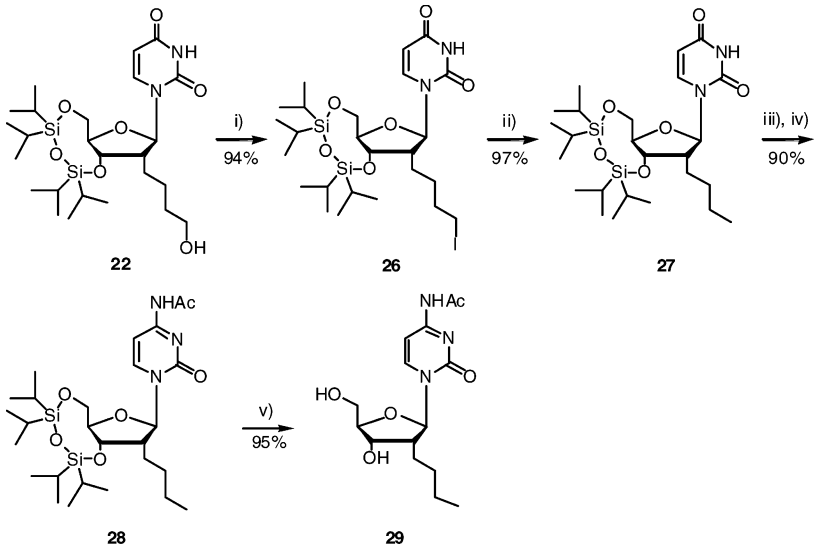
[a] (i) Dess−Martin periodinane, CH_2Cl_2, 0 °C, 3 h; (ii) $Ph_3P^+MeBr^-$, *s*-BuLi, THF, −78 °C to rt, 20 h; (iii) (1) 9-BBN, THF, rt, 2 h, (2) $NaBO_3$-$4H_2O$, rt, 2 h; (iv) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 0 °C to rt, 1 h; (v) (1) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et_3N, CH_3CN, rt, 66 h; (2) NH_4OH, rt, 3 h; (vi) CH_3COCl, DMAP, CH_2Cl_2, 0 °C, 30 min; (vii) Et_3N-3HF, Et_3N, THF, rt, 2 h.

obtained no corresponding 2′-*C*-α-hydroxypropylcytidine derivative. It is possible that the borane reagent reduced the N^4-acetamide.[31] We therefore prepared 2′-*C*-α-acetoxypropyl-N^4-acetylcytidine (**20**) via its uridine analogue (Scheme 4). We improved upon the procedure of Fehring et al. for the preparation of **17** from **11** (76% yield versus 63% yield) by conducting the hydroboration and the oxidation with 9-BBN and sodium perborate, respectively. Protection of the hydroxyl group with acetic anhydride gave 2′-*C*-α-(acetoxypropyl)uridine derivative **18** in 94% yield. Transformation to the N^4-acetylcytidine derivative **19** was achieved in 82% yield by the two-step reaction sequence: amination with 2,4,6-triisopropylbenzenesulfonyl chloride and ammonium hydroxide[27] followed by N^4-acetylation. Subsequent desilylation with the triethylamine−trihydrofluoride complex gave 2′-*C*-α-acetoxypropyl-N^4-acetylcytidine (**20**) in quantitative yield (>99% yield).

3. 2′-*C*-α-(Hydroxybutyl)cytidine and 2′-*C*-α-Butylcytidine. Scheme 5 shows the synthesis of 2′-*C*-α-(acetoxybutyl)-N^4-acetylcytidine (**25**). The 2′-*C*-α-hydroxypropyl chain of **17** was oxidized to the corresponding aldehyde using Dess−Martin periodinane and subsequently extended by one carbon via Wittig olefination to give the 2′-*C*-α-(3-butenyl)uridine derivative **21** in 68% yield. Hydroboration with 9-BBN followed by oxidation with sodium perborate gave the corresponding 2′-*C*-α-(4-hydroxybutyl)uridine **22** in 88% yield. Acetylation of the hydroxyl group with acetic anhydride gave the 2′-*C*-α-(4-acetoxybutyl)uridine derivative **23** (93% yield), which was transformed to the corresponding N^4-acetylcytidine derivative

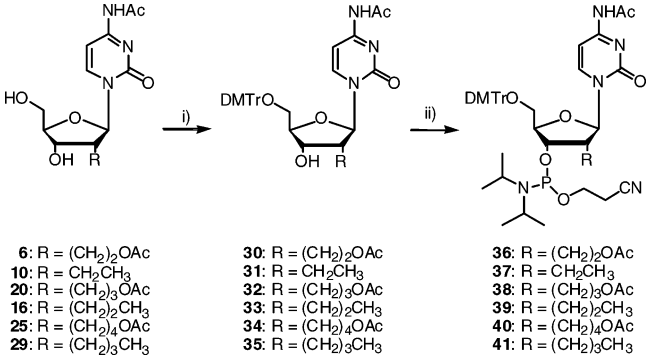
(31) Lane, C. F. *Chem. Rev.* **1976**, *76*, 773.

SCHEME 6 [a]



[a] (i) I_2, PPh_3, imidazole, C_6H_6/CH_3CN (4:1), 0 °C, 45 min; (ii) *n*-Bu_3SnH, AIBN, toluene, sonication, 0 °C, 3 h; (iii) (1) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et_3N, CH_3CN, rt, 53 h, (2) NH_4OH, rt, 3 h; (iv) CH_3COCl, DMAP, CH_2Cl_2, 0 °C, 30 min; (v) Et_3N-3HF, Et_3N, THF, rt, 2 h.

SCHEME 7 [a]



[a] (i) For **30**−**33**, DMTrCl, DMAP, pyridine, rt, 20−27 h, 76−89% yield; for **34** and **35**, DMTrCl, DMAP, $AgNO_3$, pyridine, rt, 48 h, 89−98% yield; (ii) $(i\text{-Pr})_2NP(Cl)OCH_2CH_2CN$, $i\text{-Pr}_2NEt$, 1-methylimidazole, CH_2Cl_2, 0 °C to rt, 1−2 h, TLC indicated quantitative conversion; purity estimated at 95%.

24 in 84% yield. Desilylation with the triethylamine−trihydrofluoride complex gave 2′-*C*-α-(acetoxybutyl)-N^4-acetylcytidine (**25**) in quantitative yield (>99% yield).

Scheme 6 shows the synthesis of N^4-acetyl-2′-*C*-α-butylcytidine (**29**). We first converted 2′-*C*-α-(4-hydroxybutyl)uridine **22** to the 2′-*C*-α-(iodobutyl)uridine derivative **26** in 94% yield using iodine/triphenylphosphine/imidazole at 0 °C for 45 min. Longer reaction times and a higher temperature (room temperature) significantly decreased the yield of **26**, possibly reflecting formation of the triphenylphosphonium iodide salt. Ultrasound irradiation of **26** in the presence of tributyltin hydride induced radical dehalogenation to give 2′-*C*-α-butyluridine derivative **27** in 97% yield. Subsequent transformation to the corresponding N^4-acetylcytidine derivative **28** as described earlier was achieved in 90% yield. Desilylation with the triethylamine−trihydrofluoride complex gave N^4-acetyl-2′-*C*-α-butylcytidine (**29**) in 95% yield.

4. Phosphoramidite Derivatives. Scheme 7 shows the synthesis of the phosphoramidite derivatives from modified cytidines (**6**, **10**, **16**, **20**, **25** and **29**). Treatment of 2′-acetoxyethylcytidine (**6**), 2′-ethylcytidine (**10**), 2′-acetoxypropylcytidine (**20**), and 2′-propylcytidine (**16**) with DMTrCl in the presence of DMAP gave the corresponding 5′-DMTr-protected cytidine derivatives (**30**−**33**) in 76−89% yield. We prepared 5′-*O*-DMTr-2′-acetoxybutylcytidine (**34**) and 5′-*O*-DMTr-2′-butylcytidine (**35**) from 2′-acetoxybutylcytidine (**25**) and 2′-propylcytidine (**29**), in 98 and 89% yield, respectively, by the addition of silver nitrate to accelerate the reaction. Phosphitylation with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite converted **30**−**35** quantitatively to the corresponding phosphoramidite derivatives **36**−**41**. On the basis of the NMR spectra, we estimate the purity of these phosphoramidites to be ≥95%. The small amount of impurity has little or no effect on solid-phase oligonucleotide synthesis. The overall yields of **36**−**41** from cytidine or uridine are summarized in Table 1.

TABLE 1. Synthesis of Phosphoramidites (36−41)

starting material	phosphoramidites	steps	overall yield (%)
cytidine	**36**	10	14
cytidine	**37**	11	7
uridine	**38**	10	30
uridine	**39**	11	13
uridine	**40**	13	21
uridine	**41**	14	25

Summary

Hydroxyalkyl nucleotides serve as valuable analogues for investigating how 2′-hydroxyl groups impart function within RNA molecules. They extend the hydroxyl group away from the ribose ring by successive methylene units. Together with the corresponding alkyl substituents as "deoxynucleotide controls", these analogues provide a means to ascertain whether a 2′-hydroxyl group contributes to function via inductive effects or via through-space interactions with solvent. We have synthesized two series of cytidine phosphoramidites bearing 2′-*C*-α-$(CH_2)_n$OH and $-(CH_2)_n$H substituents, respectively, and have used these phosphoramidites to prepare substrates for the SER reaction catalyzed by the group II intron. The phosphoramidites couple with the same efficiency as commercial phosphoramidites.[11]

Construction of the nucleosides entailed classic alkene and alcohol transformations. We accessed 2′-hydroxyethylcytidine from protected 2′-*C*-α-allylcytidine via oxidative cleavage followed by borohydride reduction. We accessed hydroxypropyl and hydroxybutylcytidine from the corresponding uridine derivatives by amination of the pyrimidine ring. Hydroboration/oxidation of protected 2′-*C*-α-allyluridine provided hydroxypropyluridine. Subsequent oxidation, Wittig olefination, and hydroboration/oxidation gave hydroxybutyluridine. For the alkyl series, we accessed ethylcytidine and butylcytidine from hydroxylethylcytidine and hydroxylbutyluridine, respectively. 2′-Propylcytidine was obtained by direct hydrogenation of 2′-*C*-α-allyluridine, followed by amination.

Several improvements to existing transformations emerge from this work. (1) We developed an alternative, more economical strategy to access protected 2′-allylcytidine, a key intermediate in nucleoside chemistry. (2) We developed an efficient two-step sequence involving mild reaction conditions (iodination followed by ultrasound dehalogenation) to deoxygenate the hydroxyalkyl groups. (3) We improved access to hydroxylpropyluridine using 9-BBN/sodium perborate rather than borane−dimethyl sulfide complex/triethylamine-*N*-oxide for hydroboration/oxidiation of protected 2′-*C*-α-allyluridine. (4) We improved access to propyluridine by removing the TIPS group from 2′-allyluridine before hydrogenation. These results may also allow more facile and efficient synthesis of purine nucleosides bearing 2′-*C*-α-alkyl and hydroxyalkyl substitutents.

Experimental Section

***N*⁴-Acetyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-*O*-(phenoxythiocarbonyl)cytidine (2).** To the solution of 3′,5′-*O*-(di-*tert*-butylsilanediyl)cytidine (**1**)[22] (7.54 g, 19.7 mmol) and 4-(dimethylamino)-pyridine (8.43 g, 69.0 mmol) in dry dichloromethane (150 mL) under argon at 0 °C, phenyl chlorothionoformate (3.18 mL, 23.0 mmol) was added. The reaction mixture was warmed to room temperature and stirred at room temperature for 16 h. TLC showed that no **1** remained in the reaction mixture. The reaction mixture was cooled with an ice bath to 0 °C. Acetyl chloride (1.78 mL, 25.0 mmol) was added, and the mixture was stirred at 0 °C for 1 h. TLC showed that the reaction was complete. The reaction was quenched with methanol (1.0 mL). After being stirred at room temperature for 10 min, the mixture was washed sequentially with water, saturated aqueous $NaHCO_3$, and brine. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 40% hexane in ethyl acetate to give the product as a light yellow foam: 8.25 g (75% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 7.71 (d, 1H, J = 7.6 Hz), 7.51 (d, 1H, J = 7.6 Hz), 7.42 (m, 2H), 7.31 (t, 1H, J = 6.4 Hz), 7.13 (dd, 2H, J = 7.13, 1.0 Hz), 6.07 (d, 1H, J = 5.2 Hz), 5.89 (s, 1H), 4.53 (dd, 1H, J = 4.8, 8.8 Hz), 4.45 (dd, 1H, J = 4.8, 9.2 Hz), 4.16 (m, 1H), 4.08 (m, 1H), 2.30 (s, 3H), 1.09 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 193.7, 171.5, 163.6, 154.4, 153.4, 144.9, 129.5, 126.6, 121.8, 97.4, 92.5, 82.1, 75.2, 75.0, 67.1, 27.2, 27.0, 24.9, 22.7, 20.3; HRMS calcd for $C_{26}H_{35}N_3O_7NaSiS$ [MNa^+] 584.1863, found 584.1894.

***N*⁴-Acetyl-2′-*C*-α-allyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-deoxy cytidine (3). Reaction in Dry Benzene.** A mixture of thionoester **2** (3.18 g, 5.66 mmol) with allyltributylstannane (7.04 mL, 22.7 mmol) in benzene (40 mL) was degassed by bubbling argon through the solution for 1 min. AIBN (483 mg, 2.83 mmol) was added, and the mixture was heated to reflux for 4 h under argon. TLC showed that the reaction was complete. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 30% hexane in ethyl acetate to give the product as a white foam: 1.66 g (65% yield).

Reaction in Dry Toluene. A mixture of **2** (1.586 g, 2.83 mmol) with allyltributylstannane (6.40 mL, 20.6 mmol) in dry toluene (45 mL) was heated to 100 °C under argon. A solution of AIBN (116 mg, 0.71 mmol) in toluene (11 mL) was added slowly over 30 min, followed by refluxing for 6 h. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 30% hexane in ethyl acetate to give the product as a white foam: 746 mg (59% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.15 (brs, 1H), 7.70 (d, 1H, J = 7.6 Hz), 7.46 (d, 1H, J = 7.6 Hz), 6.00 (m, 1H), 5.87 (d, 1H, J = 1.6 Hz), 5.16 (dd, 1H, J = 1.0, 9.0 Hz), 5.08 (m, 1H), 4.50 (dd, 1H, J = 4.4, 8.8 Hz), 4.21 (m, 1H), 4.05−3.95 (m, 2H), 2.70 (m, 1H), 2.49 (m, 1H), 2.30 (s, 3H), 2.35−2.25 (m, 1H), 1.06 (s, 9H), 1.02 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.2, 163.0, 154.7, 143.7, 135.7, 117.0, 96.9, 91.4, 76.03, 76.00, 67.7, 47.4, 31.2, 27.4, 27.1, 24.9, 22.7, 20.4; HRMS calcd for $C_{22}H_{36}N_3O_5Si$ [MH^+] 450.2424, found 450.2432.

***N*⁴-Acetyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-deoxy-2′-*C*-α-(2-oxoethyl)cytidine (4).** To a solution of **3** (411 mg, 0.92 mmol) in a mixed solvent of acetone/water = 6:1 (v/v) (28 mL), osmium tetraoxide (4 wt % in water, 56 μL, 9.2 μmol) was added. After the reaction mixture was stirred at room temperature for 5 min, sodium periodate (413 mg, 1.93 mmol) was added, and the mixture was stirred overnight at room temperature in the dark. TLC showed that the starting material had disappeared. The mixture was diluted with ether, washed with brine, and dried over $MgSO_4$. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 10% hexane in ethyl acetate to give the product as a white foam: 0.308 g (74% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.50 (brs, 1H), 9.80 (brs, 1H), 7.82 (d, 1H, J = 7.4 Hz), 7.50 (d, 1H, J = 7.4 Hz), 5.76 (s, 1H), 4.52 (m, 1H), 4.24 (m, 1H), 4.06 (m, 1H), 3.91 (m, 1H), 3.12 (m, 1H), 2.92 (m, 1H), 2.80 (m, 1H), 2.30 (s, 3H), 1.03 (s, 9H), 1.00 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 199.8, 171.5, 163.1, 154.9, 143.2, 96.9, 91.1, 75.9, 75.1, 67.3, 43.0, 40.8, 27.2, 26.9, 24.8, 22.5, 20.2; HRMS calcd for $C_{21}H_{33}N_3O_6NaSi$ [MNa^+] 474.2036, found 474.2034.

2′-*C*-α-(2-Acetoxyethyl)-*N*⁴-acetyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-deoxycytidine (5). To a solution of **4** (0.177 g, 0.39 mmol) in methanol (3.0 mL) at 0 °C under argon, sodium borohydride (13 mg, 0.35 mmol) was added. The reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was quenched with saturated aqueous ammonium chloride, extracted with ethyl acetate, and dried over $MgSO_4$. The solvent was removed, and the residue was dried under vacuum to give the residue as a white foam. The white foam was dissolved into dry dichloromethane (10 mL). To the resulting solution at 0 °C, 4-(dimethylamino)pyridine (191 mg, 1.56 mmol) and acetyl chloride (55 μL, 0.78 mmol) were added. After the reaction mixture was stirred at 0 °C for 30 min, TLC showed that the reaction was complete. The reaction was quenched with methanol (1.0 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 10% hexane in ethyl acetate to give the product as a white foam: 112 mg (58% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.49 (brs, 1H), 7.77 (d, 1H, J = 7.6 Hz), 7.49 (d, 1H, J = 7.6 Hz), 5.88 (d, 1H, J = 0.75 Hz), 4.52 (dd, 1H, J = 4.9, 9.2 Hz), 4.35 (t, 2H, J = 6.9 Hz), 4.16 (m, 1H), 4.06 (m, 1H), 3.95 (m, 1H), 2.49 (m, 1H), 2.31 (s, 3H), 2.22 (m, 1H), 2.05 (s, 3H), 1.90 (m, 1H), 1.05 (s, 9H), 1.02 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.4, 170.8, 163.0, 154.6, 143.2, 96.8, 91.4, 75.8, 75.7, 67.5, 62.4, 44.7, 27.3, 27.0, 25.9, 24.8, 22.6, 20.9, 20.2; HRMS calcd for $C_{23}H_{38}N_3O_7Si$ [MH^+] 496.2479, found 496.2500.

2′-*C*-α-(2-Acetoxyethyl)-*N*⁴-acetyl-2′-deoxycytidine (6). To a solution of **5** (0.189 g, 0.382 mmol) in THF (10 mL), triethylamine (0.70 mL, 5.0 mmol) and triethylamine−trihydrofluoride (0.31 mL, 1.9 mmol) were added. The mixture was stirred at room temperature for 30 min. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 7.5% methanol in chloroform to give product as a white foam: 0.120 g (88% yield). ^{1}H NMR (CD_3OD/TMS) δ 8.43 (d, 1H, J = 7.6 Hz), 7.41 (dd, 1H, J = 4.4, 7.6 Hz), 6.16 (d, 1H, J = 8.0 Hz), 4.32 (dd, 1H, J = 2.4, 5.6 Hz), 4.13−4.04 (m, 3H), 3.80 (m, 2H), 2.41 (m, 1H), 2.18 (s, 3H), 2.10 (m, 1H), 1.96 (s, 3H), 1.78 (m, 1H); ^{13}C NMR (CD_3OD)

δ 173.0, 172.7, 164.1, 158.2, 146.7, 98.5, 91.1, 89.0, 73.3, 63.8, 63.0, 48.4, 24.7, 24.6, 20.8; HRMS calcd for $C_{15}H_{22}N_3O_7$ [MH^+] 356.1458, found 356.1466.

***N*⁴-Acetyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-deoxy-2′-*C*-α-(2-hydroxyethyl)cytidine (7) and 2′-Deoxy-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-*C*-α-(2-hydroxyethyl)cytidine (7a).** To a solution of **4** (639 mg, 1.42 mmol) in methanol (20 mL) at 0 °C under argon, sodium borohydride (54 mg, 1.42 mmol) was added. The reaction mixture was stirred at 0 °C for 30 min. TLC showed that the reaction was complete. The reaction mixture was quenched with saturated aqueous ammonium chloride, extracted with ethyl acetate, and dried over $MgSO_4$. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with ethyl acetate to give compound **7** as a white foam: 198 mg (31% yield). **7**: ^{1}H NMR ($CDCl_3$/TMS) δ 10.51 (brs, 1H), 7.88 (d, 1H, J = 7.6 Hz), 7.56 (d, 1H, J = 7.6 Hz), 6.01 (s, 1H), 4.60−4.53 (m, 2H), 4.12−3.97 (m, 3H), 3.87 (m, 1H), 2.64 (m, 1H), 2.28 (s, 3H), 2.15 (m, 1H), 1.72 (m, 1H), 1.03 (s, 9H), 1.01 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.4, 163.0, 155.4, 143.0, 97.2, 90.9, 76.3, 75.1, 67.7, 61.0, 46.4, 27.7, 27.3, 27.0, 24.7, 22.6, 20.3; HRMS calcd for $C_{21}H_{35}N_3O_6$-NaSi [MH^+] 476.2193, found 476.2173.

A byproduct, **2′-deoxy-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-*C*-α-(2-hydroxyethyl)cytidine (7a)** (155 mg, 24% yield), was also purified by eluting the column with 7.5% methanol in chloroform. The structure of **7a** was confirmed by ^{1}H and ^{13}C NMR. ^{1}H NMR (CD_3OD/$CDCl_3$/TMS) δ 7.50 (d, 1H, J = 7.5 Hz), 5.93 (d, 1H, J = 7.5 Hz), 5.91 (s, 1H), 4.49 (dd, 1H, J = 4.9, 9.2 Hz), 4.18 (dd, 1H, J = 8.0, 9.2 Hz), 4.05 (m, 1H), 3.94 (m, 1H), 3.87 (m, 1H), 3.82 (m, 1H), 2.53 (m, 1H), 2.16 (m, 1H), 1.70 (m, 1H), 1.05 (s, 9H), 1.02 (s, 9H); ^{13}C NMR (CD_3OD/$CDCl_3$) δ 165.6, 156.2, 139.4, 94.8, 90.4, 75.4, 75.3, 67.2, 60.0, 45.2, 28.2, 26.6, 26.4, 22.1, 19.8. Both **7** and **7a** could be converted to **5** by acetylation (see the description earlier in the Experimental Section for the preparation of **5**).

***N*⁴-Acetyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-deoxy-2′-*C*-α-(2-iodoethyl)cytidine (8).** To a solution of **7** (180 mg, 0.40 mmol) in a solvent mixture of benzene (12 mL) and CH_3CN (3 mL), triphenylphosphine (524 mg, 2.0 mmol) and imidazole (136 mg, 2.0 mmol) were added. The resulting mixture was cooled to 0 °C, and iodine (508 mg, 2.0 mmol) was then added. After the reaction mixture was stirred at room temperature for 3 h, TLC showed that the reaction was complete. The mixture was diluted with dichloromethane and washed with brine. The organic layer was concentrated, and the residue was purified by silica gel chromatography, eluting with 45% ethyl acetate in hexane to give the product as a foam: 167 mg (74% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.62 (brs, 1H), 7.76 (d, 1H, J = 7.6 Hz), 7.47 (d, 1H, J = 7.6 Hz), 5.84 (s, 1H), 4.51 (m, 1H), 4.15 (m, 1H), 4.04 (m, 1H), 3.92 (m, 1H), 3.48 (m, 2H), 2.43 (m, 2H), 2.33 (s, 3H), 2.15 (m, 1H), 1.07 (s, 9H), 1.02 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.5, 163.1, 154.7, 143.1, 97.2, 90.7, 76.0, 75.7, 67.4, 48.8, 31.4, 27.3, 27.0, 24.9, 22.6, 20.3, 3.44; HRMS calcd for $C_{21}H_{35}N_3O_5SiI$ [MH^+] 564.1391, found 564.1411.

***N*⁴-Acetyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-deoxy-2′-*C*-α-ethylcytidine (9).** A solution of **8** (133 mg, 0.236 mmol) and AIBN (16 mg, 0.10 mmol) in a mixed solvent of benzene (10 mL) and THF (10 mL) was cooled in an ice bath (0 °C) under argon. The mixture was irradiated with ultrasound (40 kHz, 100 w) by a Bransonic 2510 R−MT sonicator. *n*-Bu_3SnH (125 μL, 0.47 mmol) was slowly injected into the solution. The reaction was complete in 1 h as monitored by TLC. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 30% hexane in ethyl acetate to give the product as a white foam: 94 mg (91% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.62 (brs, 1H), 7.74 (d, 1H, J = 7.6 Hz), 7.50 (d, 1H, J = 7.6 Hz), 5.91 (d, 1H, J = 1.6 Hz), 4.50 (dd, 1H, J = 4.8, 8.8 Hz), 4.14 (m, 1H), 4.02 (m, 1H), 3.94 (m, 1H), 2.30 (s, 3H), 2.23 (m, 1H), 1.93 (m, 1H), 1.57 (m, 1H), 1.13 (t, 3H, J = 7.2 Hz), 1.06 (s, 9H), 1.02 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.5, 163.0, 154.7, 143.5, 97.0, 91.3, 76.3, 75.8, 67.7, 49.7, 27.3, 27.1, 24.8, 22.7, 20.3, 19.9, 12.2; HRMS calcd for $C_{21}H_{36}N_3O_5Si$ [MH^+] 438.2424, found 438.2413.

***N*⁴-Acetyl-2′-deoxy-2′-*C*-α-ethylcytidine (10).** To a solution of **9** (89 mg, 0.20 mmol) in THF (10 mL), triethylamine−trihydrofluoride (0.17 mL, 1.0 mmol) and triethylamine (0.42 mL, 3.0 mmol) were added. The mixture was stirred at room temperature for 1 h. TLC showed that the reaction was complete. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 7% methanol in chloroform to give the product as a white foam: 60 mg (99% yield). ^{1}H NMR (CD_3OD/TMS) δ 8.42 (d, 1H, J = 7.6 Hz), 7.44 (d, 1H, J = 7.6 Hz), 6.15 (d, 1H, J = 8.4 Hz), 4.31 (dd, 1H, J = 2.0, 5.6 Hz), 4.01 (m, 1H), 3.77 (m, 2H), 2.25−2.12 (m, 1H), 2.17 (s, 3H), 1.74 (m, 1H), 1.32 (m, 1H), 0.94 (t, 3H, J = 7.6 Hz); ^{13}C NMR (CD_3OD) δ 173.1, 164.2, 158.4, 146.8, 98.5, 91.3, 89.1, 73.5, 63.3, 48.4, 24.5, 18.5, 12.4; HRMS calcd for $C_{13}H_{20}N_3O_5$ [MH^+] 298.1403, found 298.1390.

2′-Deoxy-2′-*C*-α-propyluridine (13). To a solution of 2′-*C*-α-allyl-2′-deoxyuridine **12**[14] (442 mg, 1.65 mmol) in methanol (20 mL) under argon, palladium on carbon (10 wt %, 176 mg, 0.165 mmol) was added. The reaction was then carried out under a hydrogen atmosphere with a hydrogen balloon at room temperature for 24 h. The catalyst was removed by filtration, and the solvent was removed by evaporation. The residue was purified by silica gel chromatography, eluting with 2% methanol in ethyl acetate to give 2′-deoxy-2′-*C*-α-propyluridine (**13**)[26] as a white foam: 360 mg (81% yield). ^{13}C NMR (D_2O) δ 165.9, 151.9, 141.7, 102.8, 88.4, 86.8, 72.2, 61.8, 46.9, 25.4, 19.9, 13.3; HRMS calcd for $C_{12}H_{18}N_2O_5Na$ [MNa^+] 293.1113, found 293.1100.

3′,5′-*O*-(Di-*tert*-butylsilanediyl)-2′-deoxy-2′-*C*-α-propyluridine (14). To a solution of **13** (324 mg, 1.20 mmol) in dry DMF (20 mL) at 0 °C, silver nitrate (448 mg, 2.64 mmol) was added, followed by dropwise addition of di-*tert*-butyldichlorosilane (279 μL, 1.32 mmol) with vigorous stirring. After the mixture was stirred at 0 °C for 30 min, triethylamine (368 μL, 2.64 mmol) was added, and the reaction mixture was stirred at room temperature for 10 min. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel chromatography, eluting with 25% ethyl acetate in hexane to give product as a white foam: 252 mg (51% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 9.89 (brs, 1H), 7.24 (d, 1H, J = 8.0 Hz), 5.86 (s, 1H), 5.79 (d, 1H, J = 8.0 Hz), 4.43 (dd, 1H, J = 4.8, 9.2 Hz), 4.17 (m, 1H), 3.96 (m, 1H), 3.80 (m, 1H), 2.27 (m, 1H), 1.80 (m, 1H), 1.53 (m, 1H), 1.40 (m, 2H), 1.10−0.87 (m, 21H); ^{13}C NMR ($CDCl_3$) δ 163.4, 150.0, 139.6, 103.0, 90.1, 76.6, 75.4, 67.4, 47.0, 29.0, 27.3, 27.0, 22.6, 20.5, 20.3, 14.2; HRMS calcd for $C_{20}H_{35}N_2O_5Si$ [MH^+] 411.2315, found 411.2307.

***N*⁴-Acetyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-deoxy-2′-*C*-α-propylcytidine (15) and 2′-Deoxy-2′-*C*-α-propylcytidine (15a).** Triethylamine (0.161 mL, 1.16 mmol) was added to a mixture of **14** (239 mg, 0.58 mmol), 4-(dimethylamino)pyridine (142 mg, 1.16 mmol), and 2,4,6-triisopropylbenzenesulfonyl chloride (351 mg, 1.16 mmol) in dry CH_3CN (15 mL). After the reaction mixture was stirred at room temperature for 60 h, concentrated ammonium hydroxide (28%, 20 mL) was added slowly into the reaction mixture. The mixture was stirred at room temperature for 3 h. The solvent was removed by evaporation, and the aqueous phase was extracted with dichloromethane. The organic layers were combined, washed with brine, and dried over $MgSO_4$. The solvent was removed, and the residue (pale yellow foam) was dried under vacuum overnight.

A portion of the yellow foam (~25%, ~0.145 mmol) was dissolved to THF (5.0 mL) and treated with TBAF (0.30 mL, 1.0 M in THF, 0.30 mmol) at room temperature for 1 h. The solvent was removed, and the residue was dissolved into water (7.0 mL). The resulting solution was washed with chloroform (3 × 5 mL). The aqueous layer was evaporated, and the residue was purified by silica gel chromatography, eluting with 10% methanol in ethyl acetate to give 2′-deoxy-2′-*C*-α-propylcytidine (**15a**):[26] 25 mg (64% yield from **14**). ^{13}C NMR (CD_3OD) δ 167.4, 158.7, 143.1, 96.6,

90.6, 88.8, 74.0, 63.6, 50.4, 27.5, 21.8, 14.6; HRMS calcd for $C_{12}H_{19}N_3O_4Na$ [MNa^+] 292.1273, found 292.1262.

The remaining portion of the yellow foam (~75%, ~0.435 mmol) was dissolved into dry dichloromethane (15 mL) under argon. To the resulting solution at 0 °C, 4-(dimethylamino)pyridine (213 mg, 1.74 mmol) and acetyl chloride (62 μL, 0.87 mmol) were added sequentially. After the reaction mixture was stirred at 0 °C for 30 min, the reaction was quenched with methanol (1.0 mL) and stirred at room temperature for 10 min. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 50% ethyl acetate in hexane to give product **15** as a white foam: 155 mg (79% yield from **14**). ^{1}H NMR ($CDCl_3$/TMS) δ 10.27 (brs, 1H), 7.74 (d, 1H, J = 7.6 Hz), 7.48 (d, 1H, J = 7.6 Hz), 5.89 (s, 1H), 4.50 (dd, 1H, J = 4.4, 9.2 Hz), 4.13 (m, 1H), 4.00 (m, 1H), 3.94 (m, 1H), 2.35−2.25 (m, 1H), 2.28 (s, 3H), 1.84 (m, 1H), 1.66 (m, 1H), 1.40 (m, 2H), 1.07 (s, 9H), 1.02 (s, 9H), 0.95 (t, 3H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 171.2, 162.9, 154.8, 143.5, 96.9, 91.7, 76.2, 75.9, 67.7, 47.8, 28.9, 27.4, 27.1, 24.7, 22.7, 20.7, 20.3, 14.2; HRMS calcd for $C_{22}H_{38}N_3O_5Si$ [MH^+] 452.2581, found 452.2571.

***N*^{4}-Acetyl-2′-deoxy-2′-*C*-α-propylcytidine (16).** To a solution of **15** (153 mg, 0.34 mmol) in THF (10 mL), triethylamine−trihydrofluoride (0.28 mL, 1.7 mmol) and triethylamine (0.70 mL, 5.0 mmol) were added. The reaction was stirred at room temperature for 1 h. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 7% methanol in chloroform to give the product as a white foam: 89 mg (85% yield). ^{1}H NMR (CD_3OD/TMS) δ 8.42 (d, 1H, J = 7.6 Hz), 7.43 (d, 1H, J = 7.6 Hz), 6.15 (d, 1H, J = 8.4 Hz), 4.28 (dd, 1H, J = 2.0, 5.2 Hz), 4.01 (m, 1H), 3.76 (m, 2H), 2.25 (m, 1H), 2.18 (s, 3H), 1.72 (m, 1H), 1.45−1.25 (m, 3H), 0.89 (t, 3H, J = 6.8 Hz); ^{13}C NMR (CD_3OD) δ 173.1, 164.2, 158.5, 146.8, 98.5, 91.4, 89.2, 73.7, 63.3, 51.6, 27.6, 24.5, 21.8, 14.6; HRMS calcd for $C_{14}H_{22}N_3O_5$ [MH^+] 312.1559, found 312.1545.

2′-Deoxy-2′-*C*-α-(3-hydroxypropyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine (17). To a solution of 2′-allyluridine **11** (1.91 g, 3.70 mmol) in THF (30 mL) under argon, 9-BBN (1.81 g, 7.40 mmol) was added. After the reaction mixture was stirred at room temperature for 4 h, sodium perborate tetrahydrate (3.42 g, 22.3 mmol) and water (7.0 mL) were added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was extracted with ethyl acetate, and the organic layers were combined and dried over $MgSO_4$. Following removal of solvent by rotary evaporation, the residue was purified by silica gel chromatography, eluting with 30% hexane in ethyl acetate to give product (**17**)[5] as a white foam: 1.48 g (76% yield). ^{13}C NMR ($CDCl_3$) δ 163.7, 150.9, 139.5, 101.9, 89.4, 82.8, 67.7, 62.5, 59.9, 48.4, 30.3, 21.7, 17.5, 17.4, 17.3, 17.2, 17.03, 16.96, 16.9, 16.8, 13.4, 13.0, 12.9, 12.4; HRMS calcd for $C_{24}H_{44}N_2O_7NaSi_2$ [MNa^+] 551.2585, found 551.2606.

2′-*C*-α-(3-Acetoxypropyl)-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine (18). To a solution of **17** (544 mg, 1.03 mmol) in dichloromethane (10 mL), 4-(dimethylamino)-pyridine (126 mg, 1.03 mmol) and triethylamine (0.72 mL, 5.1 mmol) were added. Under argon, the mixture was cooled to 0 °C, and acetic anhydride (0.12 mL, 1.3 mmol) was added. The ice bath was removed, and the reaction mixture was stirred at room temperature for 1 h. TLC showed that the reaction was complete. The reaction was then quenched with methanol (1.0 mL). After 5 min, the solvent was removed, and the residue was dissolved in chloroform. The chloroform solution was washed sequentially with water and brine and dried over $MgSO_4$. Following removal of solvent by evaporation, the residue was purified by silica gel chromatography, eluting with 50% ethyl acetate in hexane to give product as a white foam: 550 mg (94% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.23 (brs, 1H), 7.84 (d, 1H, J = 8.4 Hz), 5.78 (s, 1H), 5.71 (d, 1H, J = 8.4 Hz), 4.45 (m, 1H), 4.20−4.05 (m, 3H), 3.99 (dd, 1H, J = 2.7, 13.2 Hz), 3.91 (m, 1H), 2.20 (m, 1H), 2.05 (s, 3H), 2.03−1.75 (m, 3H), 1.48 (m, 1H), 1.15−0.90 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 171.0, 164.0, 150.2, 139.5, 101.5, 89.8, 82.8, 68.1, 64.1, 60.1, 48.1, 26.3, 22.1, 21.0, 17.4, 17.3, 17.2, 17.1, 16.9, 16.83, 16.79, 16.7, 13.2, 13.0, 12.8, 12.4; HRMS calcd for $C_{26}H_{46}N_2O_8NaSi_2$ [MNa^+] 593.2690, found 593.2721.

2′-*C*-α-(3-Acetoxypropyl)-*N*^{4}-acetyl-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)cytidine (19). Triethylamine (0.21 mL, 1.5 mmol) was added to a mixture of **18** (441 mg, 0.77 mmol), 4-(dimethylamino)pyridine (183 mg, 1.50 mmol), and 2,4,6-triisopropylbenzenesulfonyl chloride (454 mg, 1.50 mmol) in dry CH_3CN (20 mL). After the reaction mixture was stirred at room temperature for 48 h, concentrated ammonium hydroxide (28%, 30 mL) was slowly added, and the mixture was stirred at room temperature for 3 h. The solvent was removed by evaporation, and the aqueous phase was extracted with chloroform. The organic layers were combined, washed with brine, and dried over $MgSO_4$. The solvent was removed by rotary evaporation, and the residue (pale yellow foam) was dried overnight under vacuum. The pale yellow foam was dissolved in dry dichloromethane (25 mL). To the resulting solution at 0 °C, 4-(dimethylamino)pyridine (376 mg, 3.08 mmol) was added, followed by dropwise addition of acetyl chloride (109 μL, 1.54 mmol). After the reaction mixture was stirred at 0 °C for 30 min, the reaction was quenched with methanol (1.0 mL) and stirred at room temperature for 10 min. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 30% hexane in ethyl acetate to give the product as a white foam: 386 mg (82% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.40 (brs, 1H), 8.33 (d, 1H, J = 7.4 Hz), 7.43 (d, 1H, J = 7.4 Hz), 5.79 (s, 1H), 4.39 (m, 1H), 4.24 (m, 1H), 4.13 (m, 2H), 4.00 (m, 2H), 2.28 (s, 3H), 2.24 (m, 1H), 2.04 (s, 3H), 2.03−1.75 (m, 3H), 1.52 (m, 1H), 1.15−0.90 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 171.13, 171.09, 163.0, 154.9, 144.3, 96.2, 90.2, 82.8, 67.6, 64.3, 59.9, 48.0, 26.6, 24.8, 22.5, 20.9, 17.5, 17.4, 17.3, 17.2, 17.0, 16.9, 16.83, 16.80, 13.3, 13.1, 12.8, 12.5; HRMS calcd for $C_{28}H_{49}N_3O_8NaSi_2$ [MNa^+] 634.2956, found 634.2925.

2′-*C*-α-(3-Acetoxypropyl)-*N*^{4}-acetyl-2′-deoxycytidine (20). To a solution of **19** (376 mg, 0.615 mmol) in THF (15 mL), triethylamine−trihydrofluoride (0.51 mL, 3.1 mmol) and triethylamine (1.39 mL, 10.0 mmol) were added. The reaction was stirred at room temperature for 2 h. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 7.5% methanol in chloroform to give the product as a white foam: 227 mg (>99% yield). ^{1}H NMR (CD_3OD/$CDCl_3$/TMS) δ 8.37 (d, 1H, J = 7.5 Hz), 7.46 (d, 1H, J = 7.5 Hz), 6.07 (d, 1H, J = 7.6 Hz), 4.33 (dd, 1H, J = 2.7, 5.7 Hz), 4.10−4.00 (m, 3H), 3.82 (m, 2H), 2.30 (m, 1H), 2.22 (s, 3H), 2.03 (s, 3H), 1.85−1.65 (m, 3H), 1.45 (m, 1H); ^{13}C NMR (CD_3OD/$CDCl_3$) δ 172.1, 171.7, 162.7, 156.9, 145.9, 97.7, 91.0, 87.6, 71.9, 64.8, 62.2, 50.0, 26.6, 24.5, 21.0, 20.9; HRMS calcd for $C_{16}H_{23}N_3O_7Na$ [MNa^+] 392.1434, found 392.1426.

2′-Deoxy-2′-*C*-α-(3-butenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine (21). Under argon, Dess−Martin periodinane (1.27 g, 3.0 mmol) was dissolved into dry dichloromethane (15 mL) and cooled to 0 °C. 2′-Hydroxypropyluridine **17** (1.06 g, 2.0 mmol) in dichloromethane (20 mL) was then added. After the mixture was stirred at 0 °C for 2 h, TLC showed that the reaction was not yet complete (~5−10% starting material remained). Additional Dess−Martin periodinane (127 mg, 0.30 mmol) was then added, and the reaction mixture was stirred at 0 °C for 1 h. TLC showed that the reaction was complete. The reaction mixture was diluted with ether (50 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL) containing $Na_2S_2O_3 \cdot 5H_2O$ (5.2 g, 21.0 mmol). The aqueous layer was extracted with ether (3 × 15 mL). The combined organic layers were sequentially washed with saturated $NaHCO_3$ and brine and dried over anhydrous $MgSO_4$. The solvent was removed by rotary evaporation, and the residue was dried under vacuum to give the crude aldehyde as a white foam, which was used for the following Wittig reaction without further purification. In a separate flask containing a suspension of triphenylmethylphosphonium bromide (2.86 g, 8.0 mmol) in THF (15 mL) at −78 °C under argon, *s*-butyllithium (6.15 mL, 1.3 M in cyclohexane, 8.0

mmol) was added slowly. The mixture was allowed to warm slowly to −30 °C in 30 min and cooled to −78 °C again. A THF (10 mL) solution of the aldehyde (∼2.0 mmol) was then added slowly under argon. The reaction mixture was allowed to warm to room temperature and stirred for 20 h. The mixture was diluted with dichloromethane (100 mL), and the solution was washed sequentially with water, saturated ammonium chloride, and brine and dried over $MgSO_4$. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in hexane to give the product as a white foam: 0.716 g (68% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.04 (brs, 1H), 7.81 (d, 1H, J = 8.0 Hz), 5.83 (m, 2H), 5.71 (d, 1H, J = 8.0 Hz), 5.06 (d, 1H, J = 16.0 Hz), 4.98 (d, 1H, J = 8.0 Hz), 4.44 (m, 1H), 4.14 (m, 1H), 3.99 (dd, 1H, J = 4.0, 12.0 Hz), 3.90 (m, 1H), 2.42−2.10 (m, 3H), 1.94 (m, 1H), 1.50 (m, 1H), 1.10−0.95 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 164.1, 150.2, 139.7, 138.0, 115.1, 101.6, 89.0, 83.0, 68.4, 60.3, 47.7, 31.1, 24.8, 17.5, 17.33, 17.27, 17.2, 17.0, 16.92, 16.87, 16.8, 13.3, 13.0, 12.9, 12.4; HRMS calcd for $C_{25}H_{45}N_2O_6Si_2$ [MH^+] 525.2816, found 525.2802.

2′-Deoxy-2′-*C*-α-(4-hydroxybutyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine (22). To a solution of **21** (602 mg, 1.15 mmol) in anhydrous THF (20 mL) at 0 °C, 9-BBN (842 mg, 3.45 mmol) was added. After the reaction mixture was stirred at room temperature for 2 h, sodium perborate tetrahydrate (3.19 g, 20.7 mmol) and water (5.0 mL) were added. The mixture was then stirred at room temperature for 2 h. The mixture was extracted with ethyl acetate, and the organic layers were combined and dried over $MgSO_4$. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 35% hexane in ethyl acetate to give the product as a white foam 547 mg (88% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.98 (brs, 1H), 7.96 (d, 1H, J = 8.0 Hz), 5.79 (s, 1H), 5.72 (d, 1H, J = 8.0 Hz), 4.42 (m, 1H), 4.21 (m, 1H), 3.98 (m, 1H), 3.90 (m, 1H), 3.69 (brs, 3H), 2.21 (m, 1H), 1.87 (m, 2H), 1.70−1.30 (m, 4H), 1.11−0.95 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 164.1, 150.9, 139.6, 101.7, 88.9, 82.6, 67.4, 62.2, 59.8, 48.8, 33.0, 25.2, 23.9, 17.4, 17.3, 17.2, 17.1, 16.9, 16.82, 16.78, 16.7, 13.2, 13.0, 12.7, 12.3; HRMS calcd for $C_{25}H_{47}N_2O_7Si_2$ [MH^+] 543.2922, found 543.2909.

2′-*C*-α-(4-Acetoxybutyl)-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine (23). To a solution of 2′-hydroxybutyluridine **22** (174 mg, 0.32 mmol) in dichloromethane (10 mL), 4-(dimethylamino)pyridine (39 mg, 0.32 mmol) and triethylamine (0.22 mL, 1.6 mmol) were added. The mixture was cooled to 0 °C, and acetic anhydride (45 μL, 0.48 mmol) was added. After the reaction mixture was stirred at room temperature for 1 h, the reaction was quenched with methanol (1.0 mL). The solvent was removed by rotary evaporation, and the residue was dissolved into chloroform. The chloroform solution was sequentially washed with water and brine and dried over $MgSO_4$. The solvent was removed, and the product was purified by silica gel chromatography, eluting with 50% ethyl acetate in hexane as a white foam: 174 mg (93% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.21 (brs, 1H), 7.84 (d, 1H, J = 8.0 Hz), 5.77 (d, 1H, J = 1.2 Hz), 5.71 (d, 1H, J = 8.0 Hz), 4.44 (m, 1H), 4.20−4.05 (m, 3H), 3.98 (dd, 1H, J = 2.4, 13.2 Hz), 3.90 (m, 1H), 2.20 (m, 1H), 2.05 (s, 3H), 1.90 (m, 1H), 1.80−1.40 (m, 5H), 1.15−0.90 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 171.1, 164.1, 150.2, 139.6, 101.5, 89.0, 82.9, 68.2, 64.2, 60.2, 48.4, 28.6, 25.4, 23.7, 21.0, 17.4, 17.3, 17.2, 17.1, 16.9, 16.87, 16.83, 16.7, 13.3, 13.0, 12.8, 12.4; HRMS calcd for $C_{27}H_{48}N_2O_8NaSi_2$ [MNa^+] 607.2847, found 607.2839.

2′-*C*-α-(4-Acetoxybutyl)-*N*^{4}-acetyl-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)cytidine (24). Triethylamine (56 μL, 0.40 mmol) was added to a mixture of **23** (85.0 mg, 0.145 mmol), 4-(dimethylamino)pyridine (49 mg, 0.40 mmol), and 2,4,6-triisopropylbenzenesulfonyl chloride (121 mg, 0.40 mmol) in dry CH_3CN (5.0 mL). After the mixture was stirred at room temperature for 66 h, concentrated ammonium hydroxide (28%, 5 mL) was slowly added, and the mixture was stirred at room temperature for 3 h. The solvent was removed by rotary evaporation under reduced pressure, and the aqueous layer was extracted with chloroform. The organic layers were combined, washed with brine, and dried over $MgSO_4$. The solvent was removed, and the residue (pale yellow foam) was dried under vacuum overnight. The pale yellow foam was dissolved into dry dichloromethane (10 mL). To the resulting solution at 0 °C, 4-(dimethylamino)pyridine (71 mg, 0.58 mmol) and acetyl chloride (21 μL, 0.29 mmol) were added. After the reaction mixture was stirred at 0 °C for 30 min, the reaction was quenched with methanol (0.5 mL) and stirred at room temperature for 10 min. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 30% hexane in ethyl acetate to give the product as a white foam: 76 mg (84% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.55 (brs, 1H), 8.33 (d, 1H, J = 7.6 Hz), 7.42 (d, 1H, J = 7.4 Hz), 5.77 (s, 1H), 4.40 (m, 1H), 4.23 (m, 1H), 4.08 (m, 2H), 4.02−3.90 (m, 2H), 2.28 (s, 3H), 2.24 (m, 1H), 2.04 (s, 3H), 1.82−1.42 (m, 6H), 1.15−0.90 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 171.2, 163.1, 154.9, 144.3, 96.2, 90.5, 82.8, 67.6, 64.3, 59.9, 48.2, 28.8, 26.0, 24.8, 24.0, 21.0, 17.5, 17.4, 17.3, 17.2, 17.0, 16.94, 16.86, 16.8, 13.3, 13.1, 12.8, 12.4; HRMS calcd for $C_{29}H_{51}N_3O_8NaSi_2$ [MNa^+] 648.3112, found 648.3144.

2′-*C*-α-(4-Acetoxybutyl)-*N*^{4}-acetyl-2′-deoxycytidine (25). To a solution of **24** (68 mg, 0.11 mmol) in THF (5.0 mL), triethylamine−trihydrofluoride (89 μL, 0.55 mmol) and triethylamine (0.22 mL, 1.6 mmol) were added. The reaction mixture was stirred at room temperature for 2 h. TLC showed that the reaction was complete. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 7.5% methanol in chloroform to give the product as a white foam: 42 mg (>99% yield). ^{1}H NMR (CD_3OD/TMS) δ 8.43 (d, 1H, J = 7.4 Hz), 7.44 (d, 1H, J = 7.4 Hz), 6.15 (d, 1H, J = 8.4 Hz), 4.30 (dd, 1H, J = 1.6, 5.2 Hz), 4.02 (m, 3H), 3.77 (m, 2H), 2.26 (m, 1H), 2.18 (s, 3H), 2.00 (s, 3H), 1.75 (m, 1H), 1.60 (m, 2H), 1.45−1.30 (m, 3H); ^{13}C NMR (CD_3OD) δ 173.1, 164.2, 158.5, 146.8, 98.5, 91.3, 89.1, 73.6, 65.5, 63.3, 51.7, 29.8, 25.1, 25.0, 24.6, 20.8; HRMS calcd for $C_{17}H_{26}N_3O_7$ [MH^+] 384.1771, found 384.1781.

2′-Deoxy-2′-*C*-α-(4-iodobutyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine (26). To a solution of **22** (320 mg, 0.59 mmol), triphenylphosphine (787 mg, 3.0 mmol), and imidazole (204 mg, 3.0 mmol) in a solvent mixture of benzene (32 mL) and CH_3CN (8.0 mL) at 0 °C, iodine (761 mg, 3.0 mmol) was added. The reaction mixture was stirred at 0 °C for 45 min. TLC showed that the reaction was complete. The mixture was oxidized with hydrogen peroxide (50%, 0.5 mL) at room temperature for 10 min. The product was extracted with dichloromethane, washed sequentially with saturated aqueous $NaHCO_3$, saturated aqueous $Na_2S_2O_3$, and brine, and dried over $MgSO_4$. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 30% ethyl acetate in hexane to give the product as a white foam: 0.361 g (94% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 9.56 (brs, 1H), 7.88 (d, 1H, J = 8.1 Hz), 5.80 (d, 1H, J = 1.3 Hz), 5.75 (dd, 1H, J = 1.9, 8.1 Hz), 4.47 (m, 1H), 4.18 (*m*, 1H), 4.02 (dd, 1H, J = 2.7, 13.2 Hz), 3.93 (m, 1H), 3.26 (t, 2H, J = 6.8 Hz), 2.21 (m, 1H), 2.00−1.40 (m, 6H), 1.15−0.95 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 163.7, 150.2, 139.7, 101.6, 89.2, 83.0, 68.2, 60.2, 48.4, 33.6, 28.4, 24.8, 17.5, 17.4, 17.3, 17.2, 17.1, 17.01, 16.96, 16.9, 13.4, 13.1, 12.9, 12.5, 6.6; HRMS calcd for $C_{25}H_{45}N_2O_6NaSi_2I$ [MNa^+] 675.1759, found 675.1758.

2′-Deoxy-2′-*C*-α-butyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)uridine (27). A solution of 2′-α-iodobutyluridine **26** (487 mg, 0.747 mmol) and AIBN (25 mg, 0.15 mmol) in dry toluene (30 mL) under argon was cooled to 0 °C and irradiated with ultrasound (40 kHz, 100 W) by a Bransonic 2510 R-MT sonicator. *n*-Bu_3SnH (387 μL, 1.46 mmol) was slowly injected into the solution, and the reaction was irradiated at 0 °C for 1 h. TLC showed that the reaction was not complete (∼50% conversion). To the mixture, additional AIBN (25 mg, 0.15 mmol) and *n*-Bu_3-SnH (387 μL, 1.46 mmol) were added. After the mixture was irradiated at 0 °C for an additional 2 h, TLC showed that the reaction was complete. The solvent was removed, and the residue was

purified by silica gel chromatography, eluting first with hexane to remove the tin compounds, following with 30% hexane in ethyl acetate to give the product as a white foam: 379 mg (97% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 9.48 (brs, 1H), 7.81 (d, 1H, J = 8.1 Hz), 5.79 (d, 1H, J = 1.9 Hz), 5.70 (d, 1H, J = 8.1 Hz), 4.44 (m, 1H), 4.13 (dd, 1H, J = 2.4, 13.2 Hz), 3.99 (dd, 1H, J = 2.8, 13.2 Hz), 3.90 (m, 1H), 2.14 (m, 1H), 1.86 (m, 1H), 1.57 (m, 1H), 1.50−1.30 (m, 4H), 1.15−0.95 (m, 28H), 0.92 (t, 1H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 163.8, 150.1, 139.8, 101.6, 89.2, 83.1, 68.5, 60.4, 48.5, 29.5, 25.4, 22.8, 17.5, 17.4, 17.3, 17.2, 17.02, 17.96, 16.9, 16.8, 13.9, 13.4, 13.1, 12.9, 12.5; HRMS calcd for $C_{25}H_{47}N_2O_6Si_2$ [MH$^+$] 527.2973, found 527.2977.

***N*^{4}-Acetyl-2′-*C*-α-butyl-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyane-1,3-diyl)cytidine (28).** Triethylamine (185 μL, 1.33 mmol) was added to a mixture of **27** (269 mg, 0.51 mmol), 4-(dimethylamino)pyridine (162 mg, 1.33 mmol), and 2,4,6-triisopropylbenzenesulfonyl chloride (403 mg, 1.33 mmol) in dry CH_3CN (15 mL). After the mixture was stirred at room temperature for 53 h, concentrated ammonium hydroxide (28%, 17.5 mL) was added slowly, and the mixture was stirred at room temperature for 3 h. The solvent was removed by evaporation under vacuum, and the aqueous phase was extracted with chloroform. The organic layers were combined, washed with brine, and dried over $MgSO_4$. The solvent was removed, and the residue (pale yellow powder) was dried under vacuum overnight. The pale yellow solid powder was dissolved into dry dichloromethane (20 mL). To the resulting solution at 0 °C, 4-(dimethylamino)pyridine (125 mg, 1.02 mmol) and acetyl chloride (72.5 μL, 1.02 mmol) were added. After the reaction mixture was stirred at 0 °C for 30 min, the reaction was quenched with methanol (1.0 mL) and stirred at room temperature for 10 min. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 50% hexane in ethyl acetate to give product as a white foam: 260 mg (90% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.87 (brs, 1H), 8.32 (d, 1H, J = 7.5 Hz), 7.43 (d, 1H, J = 7.4 Hz), 5.81 (s, 1H), 4.38 (m, 1H), 4.22 (m, 1H), 3.99 (m, 1H), 3.96 (m, 1H), 2.30 (s, 3H), 2.20 (m, 1H), 1.88 (m, 1H), 1.63 (m, 1H), 1.49 (m, 2H), 1.35 (m, 2H), 1.15−0.80 (m, 31H); ^{13}C NMR ($CDCl_3$) δ 171.5, 163.2, 154.9, 144.3, 96.3, 90.5, 82.9, 67.8, 60.1, 48.3, 29.6, 25.8, 24.7, 22.8, 17.5, 17.4, 17.3, 17.2, 17.0, 16.9, 16.83, 16.80, 13.9, 13.3, 13.1, 12.8, 12.4; HRMS calcd for $C_{27}H_{50}N_3O_6Si_2$ [MH$^+$] 568.3238, found 568.3213.

***N*^{4}-Acetyl-2′-*C*-α-butyl-2′-deoxycytidine (29).** To a solution of **28** (171 mg, 0.30 mmol) in THF (10 mL), triethylamine−trihydrofluoride (245 μL, 1.50 mmol) and triethylamine (0.63 mL, 4.5 mmol) were added. The reaction mixture was stirred at room temperature for 2 h. TLC showed that the reaction was complete. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 6% methanol in chloroform to give the product as a white solid: 93 mg (95% yield). ^{1}H NMR (CD_3OD/TMS) δ 8.39 (d, 1H, J = 7.6 Hz), 7.45 (d, 1H, J = 7.4 Hz), 6.15 (d, 1H, J = 8.6 Hz), 4.34 (m, 1H), 4.10 (m, 1H), 3.79 (m, 2H), 2.35−2.20 (m, 1H), 2.25 (s, 3H), 1.71 (m, 1H), 1.45−1.20 (m, 5H), 0.86 (t, 3H, J = 6.9 Hz); ^{13}C NMR (CD_3OD) δ 174.2, 163.8, 158.3, 146.9, 99.0, 91.2, 88.7, 73.6, 63.0, 51.0, 30.3, 24.8, 24.7, 23.5 14.2; HRMS calcd for $C_{15}H_{24}N_3O_5$ [MH$^+$] 326.1716, found 326.1710.

2′-*C*-α-(2-Acetoxyethyl)-*N*^{4}-acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)cytidine (30). Nucleoside **6** (119 mg, 0.335 mmol) was coevaporated with dry pyridine (2 × 5 mL) and dried under vacuum for 30 min. To a solution of dried **6** in dry pyridine (10 mL), 4-(dimethylamino)pyridine (82 mg, 0.67 mmol) and DMTrCl (227 mg, 0.67 mmol) were added. After the reaction mixture was stirred at room temperature for 15 h, TLC indicated that the reaction was not complete (only ∼10% conversion). To the reaction mixture, additional 4-(dimethylamino)pyridine (82 mg, 0.67 mmol) and DMTrCl (227 mg, 0.67 mmol) were added. The reaction mixture was stirred at room temperature until the reaction was complete (∼5 h). The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 3% methanol in chloroform to give the product as a light yellow foam: 0.167 g (76% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.06 (brs, 1H), 8.19 (d, 1H, J = 7.5 Hz), 7.40 (d, 2H, J = 7.5 Hz), 7.35−7.18 (m, 7H), 7.16 (d, 1H, J = 7.5 Hz), 6.85 (d, 4H, J = 8.8 Hz), 6.35 (d, 1H, J = 7.6 Hz), 4.43 (m, 1H), 4.22 (m, 2H), 4.16−4.05 (m, 1H), 3.78 (s, 6H), 3.47 (m, 2H), 2.56 (brs, 1H), 2.38 (m, 1H), 2.21 (s, 3H), 2.17 (m, 1H), 1.94 (s, 3H), 1.88 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 171.2, 171.0, 162.7, 158.5, 155.9, 144.9, 144.2, 135.2, 135.1, 129.9, 127.9, 127.0, 113.2, 97.3, 89.4, 86.9, 86.0, 72.5, 63.6, 62.9, 55.1, 48.5, 24.7, 23.4, 20.9; HRMS calcd for $C_{36}H_{40}N_3O_9$ [MH$^+$] 658.2765, found 658.2751.

***N*^{4}-Acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-2′-*C*-α-ethylcytidine (31).** Compound **10** (69 mg, 0.23 mmol) was coevaporated with dry pyridine (2 × 3 mL) and redissolved into dry pyridine (10 mL). To the resulting solution, 4-(dimethylamino)pyridine (56 mg, 0.46 mmol) and DMTrCl (156 mg, 0.46 mmol) were added. After the reaction mixture was stirred at room temperature for 15 h, TLC showed that the reaction was not complete. Additional 4-(dimethylamino)pyridine (56 mg, 0.46 mmol) and DMTrCl (156 mg, 0.46 mmol) were added to the reaction mixture. The mixture was stirred at room temperature until TLC indicated that the reaction was complete (∼6 h). The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 3% methanol in chloroform to give the product as a light yellow foam: 0.122 g (89% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.13 (brs, 1H), 8.09 (d, 1H, J = 7.6 Hz), 7.41 (d, 2H, J = 8.8 Hz), 7.35−7.25 (m, 6H), 7.23 (m, 1H), 7.18 (d, 1H, J = 7.6 Hz), 6.85 (d, 4H, J = 8.8 Hz), 6.36 (d, 1H, J = 7.6 Hz), 4.43 (m, 1H), 4.21 (m, 1H), 3.78 (s, 6H), 3.45 (m, 2H), 2.18 (s, 3H), 2.14 (m, 1H), 1.82 (m, 1H), 1.50 (m, 1H), 0.97 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 171.0, 162.6, 158.5, 155.8, 145.0, 144.2, 135.3, 135.1, 130.0, 128.0, 127.9, 127.0, 113.2, 97.2, 89.4, 86.8, 85.8, 72.5, 63.5, 55.1, 53.1, 24.6, 17.2, 12.2; HRMS calcd for $C_{34}H_{38}N_3O_7$ [MH$^+$] 600.2710, found 600.2728.

2′-*C*-α-(3-Acetoxypropyl)-*N*^{4}-acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)cytidine (32). Nucleoside **20** (207 mg, 0.56 mmol) was coevaporated with dry pyridine (2 × 5 mL) and then dissolved into dry pyridine (10 mL). To the resulting pyridine solution, 4-(dimethylamino)pyridine (137 mg, 1.12 mmol) and DMTrCl (383 mg, 1.12 mmol) were added. The reaction mixture was stirred at room temperature for 18 h. TLC indicated that the reaction was not complete (∼90% conversion). Additional DMTrCl (38 mg, 0.11 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for an additional 6 h. The reaction was quenched with MeOH (1.0 mL). The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 3% methanol in chloroform to give the product as a light yellow foam: 323 mg (86% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.05 (brs, 1H), 8.14 (d, 1H, J = 7.6 Hz), 7.40 (d, 2H, J = 7.2 Hz), 7.35−7.20 (m, 7H), 7.18 (d, 1H, J = 7.6 Hz), 6.85 (d, 4H, J = 8.8 Hz), 6.30 (d, 1H, J = 6.8 Hz), 4.42 (m, 1H), 4.19 (m, 1H), 4.04 (m, 2H), 3.79 (s, 6H), 3.47 (m, 2H), 2.25 (m, 1H), 2.19 (s, 3H), 1.99 (s, 3H), 1.85 (m, 1H), 1.74 (m, 2H), 1.50 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 171.2, 170.9, 162.6, 158.6, 155.8, 144.9, 144.1, 135.3, 135.1, 129.9, 128.0, 127.9, 127.0, 113.2, 97.2, 89.5, 86.9, 85.6, 72.1, 64.2, 63.3, 55.1, 50.7, 26.6, 24.6, 20.9, 20.6; HRMS calcd for $C_{37}H_{41}N_3O_9Na$ [MNa$^+$] 694.2741, found 694.2723.

***N*^{4}-Acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-2′-*C*-α-propylcytidine (33).** Nucleoside **16** (86 mg, 0.28 mmol) was coevaporated with dry pyridine (2 × 2 mL) and then dissolved into dry pyridine (10 mL). To the resulting pyridine solution, 4-(dimethylamino)-pyridine (81 mg, 0.66 mmol) and DMTrCl (281 mg, 0.83 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. TLC indicated that the reaction was not complete (∼90% conversion). To the reaction mixture, additional 4-(dimethylamino)-pyridine (38 mg, 0.31 mmol) and DMTrCl (28 mg, 0.088 mmol) were added. The reaction mixture was stirred at room temperature for an additional 3 h. The reaction was quenched with MeOH (1.0 mL). The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 3% methanol in chloroform

to give the product as a light yellow foam: 139 mg (80% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.16 (brs, 1H), 8.11 (d, 1H, J = 7.6 Hz), 7.40 (m, 2H), 7.35−7.18 (m, 7H), 7.16 (d, 1H, J = 7.6 Hz), 6.85 (d, 4H, J = 8.4 Hz), 6.39 (d, 1H, J = 8.0 Hz), 4.41 (m, 1H), 4.20 (m, 1H), 3.79 (s, 6H), 3.50−3.45 (m, 3H), 2.25 (m, 1H), 2.19 (s, 3H), 1.77 (m, 1H), 1.55−1.30 (m, 3H), 0.89 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 171.0, 162.7, 158.6, 155.8, 145.0, 144.2, 135.4, 135.2, 130.0, 128.03, 127.95, 127.1, 113.2, 97.3, 89.4, 86.9, 86.0, 73.0, 63.6, 55.2, 51.2, 26.1, 24.7, 20.8, 14.1; HRMS calcd for $C_{35}H_{39}N_3O_7Na$ [MNa^+] 636.2686, found 636.2684.

2′-*C*-α-(4-Acetoxybutyl)-*N*^{4}-acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)cytidine (34). Nucleoside **25** (42 mg, 0.11 mmol) was coevaporated with dry pyridine and dried under vacuum for 4 h. Under argon, to a solution of dried nucleoside **33** in pyridine (5.0 mL), 4-(dimethylamino)pyridine (53 mg, 0.44 mmol) and DMTrCl (148 mg, 0.44 mmol) were added. After the reaction mixture was stirred at room temperature overnight, TLC indicated that only a small amount of product had formed. To the reaction mixture, silver nitrate (19 mg, 0.11 mmol) was added, and the mixture was stirred at room temperature for 48 h. TLC showed that no starting material remained. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 3% methanol in chloroform to give the product as a light yellow foam: 73 mg (98% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 9.97 (brs, 1H), 8.10 (d, 1H, J = 7.6 Hz), 7.40 (d, 2H, J = 7.2 Hz), 7.35−7.20 (m, 7H), 7.18 (d, 1H, J = 7.6 Hz), 6.85 (d, 4H, J = 8.8 Hz), 6.32 (d, 1H, J = 7.6 Hz), 4.40 (m, 1H), 4.19 (m, 1H), 4.08 (m, 1H), 4.00 (m, 1H), 3.79 (s, 6H), 3.47 (m, 2H), 2.23 (m, 1H), 2.20 (s, 3H), 2.02 (s, 3H), 1.75 (m, 1H), 1.60 (m, 2H), 1.46 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 171.4, 170.9, 162.5, 158.6, 155.8, 145.0 144.2, 135.3, 135.1, 130.0, 128.01, 127.95, 127.1, 113.2, 97.2, 89.4, 86.9, 85.6, 72.5, 64.1, 63.5, 55.2, 51.3, 28.7, 24.7, 24.0, 23.5, 20.9; HRMS calcd for $C_{38}H_{43}N_3O_9Na$ [MNa^+] 708.2897, found 708.2909.

***N*^{4}-Acetyl-2′-*C*-α-butyl-2′-deoxy-5′-*O*-(dimethoxytrityl)cytidine (35).** To a solution of 2′-*C*-α-butylcytidine **29** (88 mg, 0.27 mmol) in dry pyridine (5.0 mL), 4-(dimethylamino)pyridine (132 mg, 1.08 mmol) and DMTrCl (386 mg, 1.08 mmol) were added. After the reaction mixture was stirred at room temperature overnight, TLC indicated that only a small amount of product had formed. To the reaction mixture, silver nitrate (51 mg, 0.30 mmol), DMTrCl (549 mg, 1.62 mmol), and 4-(dimethylamino)pyridine (198 mg, 1.62 mmol) were added. The mixture was stirred at room temperature for 48 h. The reaction was quenched with methanol (1.0 mL). The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 3% methanol in chloroform to give the product as a light yellow foam: 150 mg (89% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 10.09 (brs, 1H), 8.08 (d, 1H, J = 7.6 Hz), 7.40 (m, 2H), 7.35−7.18 (m, 7H), 7.15 (d, 1H, J = 7.6 Hz), 6.84 (d, 4H, J = 8.8 Hz), 6.35 (d, 1H, J = 8.0 Hz), 4.41 (m, 1H), 4.19 (m, 1H), 3.78 (s, 6H), 3.44 (m, 2H), 2.21 (m, 1H), 2.19 (s, 3H), 1.78 (m, 1H), 1.50−1.20 (m, 5H), 0.85 (t, 3H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 171.0, 162.7, 158.6, 155.8, 145.0, 144.2, 135.3, 135.2, 130.0, 128.02, 127.95, 127.1, 113.2, 97.2, 89.4, 86.9, 85.9, 72.8, 63.6, 55.2, 51.3, 29.8, 24.7, 23.6, 22.7, 13.9; HRMS calcd for $C_{36}H_{41}N_3O_7Na$ [MNa^+] 650.2842, found 650.2836.

2′-*C*-α-(2-Acetoxyethyl)-*N*^{4}-acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)cytidine 3′-*N*,*N*-Diisopropyl(cyanoethyl)phosphoramidite (36). Typical procedure for the preparation of phosphoramidites: To a cooled (0 °C) solution of **30** (125 mg, 0.19 mmol) and *N*,*N*-diisopropylethylamine (89 μL, 0.51 mmol) in dry dichloromethane (10 mL) under argon, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (90 mg, 0.38 mmol) and 1-methylimidazole (8.0 μL, 0.10 mmol) were added. The reaction mixture was warmed to room temperature and stirred until TLC indicated that the reaction was complete (1 h). The reaction was then quenched with methanol (0.5 mL). After being stirred at room temperature for 5 min, the reaction mixture was evaporated under reduced pressure, and the residue was purified by silica gel chromatography, eluting with 4% acetone in dichloromethane containing 0.5% triethylamine to give the phosphoramidite derivative as a white foam: 163 mg (100% conversion, ≥95% purity). ^{31}P NMR (CD_3CN) δ 153.7, 152.1; HRMS calcd for $C_{45}H_{56}N_5O_{10}NaP$ [MNa^+] 880.3663, found 880.3644.

***N*^{4}-Acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-2′-*C*-α-ethylcytidine 3′-*N*,*N*-Diisopropyl(cyanoethyl)phosphoramidite (37).** According to the above procedure, the reaction mixture containing **31** (106 mg, 0.177 mmol), *N*,*N*-diisopropylethylamine (82 μL, 0.47 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (83 mg, 0.35 mmol), and 1-methylimidazole (8.0 μL, 0.10 mmol) was stirred at room temperature for 1 h. The phosphoramidite **37** was purified by silica gel chromatography to give a white foam: 141 mg (100% conversion, ≥95% purity). ^{31}P NMR (CD_3CN) δ 153.8, 151.8; HRMS calcd for $C_{43}H_{54}N_5O_8NaP$ [MNa^+] 822.3608, found 822.3597.

2′-*C*-α-(3-Acetoxypropyl)-*N*^{4}-acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)cytidine 3′-*N*,*N*-Diisopropyl(cyanoethyl)phosphoramidite (38). According to the above procedure, a reaction mixture containing **32** (162 mg, 0.241 mmol), *N*,*N*-diisopropylethylamine (113 μL, 0.65 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (107 μL 0.48 mmol), and 1-methylimidazole (12.0 μL, 0.15 mmol) was stirred at room temperature for 2 h. The phosphoramidite **38** was purified by silica gel chromatography to give a white foam: 210 mg (100% conversion, ≥95% purity). ^{31}P NMR (CD_3CN) δ 151.0, 149.2; HRMS calcd for $C_{46}H_{58}N_5O_{10}NaP$ [MNa^+] 894.3819, found 894.3824.

***N*^{4}-Acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-2′-*C*-α-propylcytidine 3′-*N*,*N*-Diisopropyl(cyanoethyl)phosphoramidite (39).** According to the above procedure, a reaction mixture containing **33** (109 mg, 0.178 mmol), *N*,*N*-diisopropylethylamine (78 μL, 0.45 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (84 mg, 0.36 mmol), and 1-methylimidazole (8.0 μL, 0.10 mmol) was stirred at room temperature for 2 h. The phosphoramidite **39** was purified by silica gel chromatography to give a white foam: 145 mg (100% conversion, ≥95% purity). ^{31}P NMR (CD_3CN) δ 151.2, 149.2; HRMS calcd for $C_{44}H_{56}N_5O_8NaP$ [MNa^+] 836.3764, found 836.3749.

2′-*C*-α-(4-Acetoxybutyl)-*N*^{4}-acetyl-2′-deoxy-5′-*O*-(dimethoxytrityl)cytidine 3′-*N*,*N*-Diisopropyl(cyanoethyl)phosphoramidite (40). According to the above typical procedure, a reaction mixture containing **34** (57 mg, 0.083 mmol), *N*,*N*-diisopropylethylamine (32 mg, 44 μL, 0.25 mmol), dry dichloromethane (5.0 mL), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (39 mg, 0.17 mmol), and 1-methylimidazole (4.0 μL, 0.05 mmol) was stirred at room temperature for 1 h. The reaction was quenched with methanol (0.25 mL). The phosphoramidite **40** was purified by silica gel chromatography to give a white foam: 74 mg (100% conversion, ≥95% purity). ^{31}P NMR (CD_3CN) δ 151.3, 149.2; HRMS calcd for $C_{47}H_{60}N_5O_{10}NaP$ [MNa^+] 908.3976, found 908.3965.

***N*^{4}-Acetyl-2′-*C*-α-butyl-2′-deoxy-5′-*O*-(dimethoxytrityl)cytidine 3′-*N*,*N*-Diisopropyl(cyanoethyl)phosphoramidite (41).** According to the above typical procedure, a mixture containing **35** (75 mg, 0.12 mmol), *N*,*N*-diisopropylethylamine (41 mg, 56 μL, 0.32 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (56 mg, 0.24 mmol), and 1-methylimidazole (5.0 μL, 0.06 mmol) in dry dichloromethane (5.0 mL) was stirred at room temperature for 1 h. The reaction was quenched with methanol (0.25 mL). The phosphoramidite **41** was purified to give a white foam: 99 mg (100% conversion, ≥95% purity). ^{31}P NMR (CD_3CN) δ 153.8, 151.6; HRMS calcd for $C_{45}H_{58}N_5O_8NaP$ [MNa^+] 850.3921, found 850.3916.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of new compounds **2**−**10**, **14**−**16**, and **18**−**41**; ^{13}C NMR spectra of known compounds **12**, **13**, **15a**, and **17**; and ^{31}P NMR spectra of phosphoramidites **36**−**41**. This material is available free of charge via the Internet at http://pubs.acs.org.

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