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Synthesis and Biophysical Evaluation of 2′,4′-Constrained 2′*O*-Methoxyethyl and 2′,4′-Constrained 2′*O*-Ethyl Nucleic Acid Analogues

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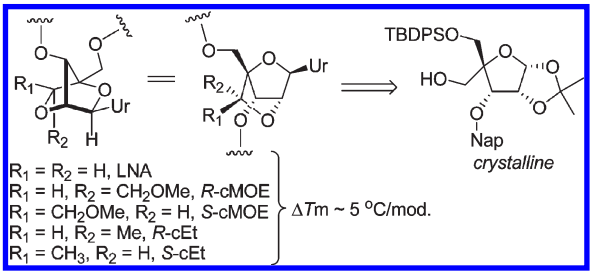
Received December 2, 2009



We have recently shown that combining the structural elements of 2′*O*-methoxyethyl (MOE) and locked nucleic acid (LNA) nucleosides yielded a series of nucleoside modifications (cMOE, 2′,4′-constrained MOE; cEt, 2′,4′-constrained ethyl) that display improved potency over MOE and an improved therapeutic index relative to that of LNA antisense oligonucleotides. In this report we present details regarding the synthesis of the cMOE and cEt nucleoside phosphoramidites and the biophysical evaluation of oligonucleotides containing these nucleoside modifications. The synthesis of the cMOE and cEt nucleoside phosphoramidites was efficiently accomplished starting from inexpensive commercially available diacetone allofuranose. The synthesis features the use of a seldom used 2-naphthylmethyl protecting group that provides crystalline intermediates during the synthesis and can be cleanly deprotected under mild conditions. The synthesis was greatly facilitated by the crystallinity of a key mono-TBDPS-protected diol intermediate. In the case of the cEt nucleosides, the introduction of the methyl group in either configuration was accomplished in a stereoselective manner. Ring closure of the 2′-hydroxyl group onto a secondary mesylate leaving group with clean inversion of stereochemistry was achieved under surprisingly mild conditions. For the *S*-cEt modification, the synthesis of all four (thymine, 5-methylcytosine, adenine, and guanine) nucleobase-modified phosphoramidites was accomplished on a multigram scale. Biophysical evaluation of the cMOE- and cEt-containing oligonucleotides revealed that they possess hybridization and mismatch discrimination attributes similar to those of LNA but greatly improved resistance to exonuclease digestion.

Introduction

Antisense drug discovery technology represents a powerful method to modulate gene expression in animals.[1] The most advanced oligonucleotides in the clinic are second generation antisense oligonucleotides (ASOs), which typically have a central DNA region of 8−14 nucleotides, flanked on the 5′ and 3′ ends with five to two 2′-*O*-methoxyethyl (MOE, **2**) residues.[2] The above "gapmer" design

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DOI: 10.1021/jo902560f Published on Web 02/08/2010

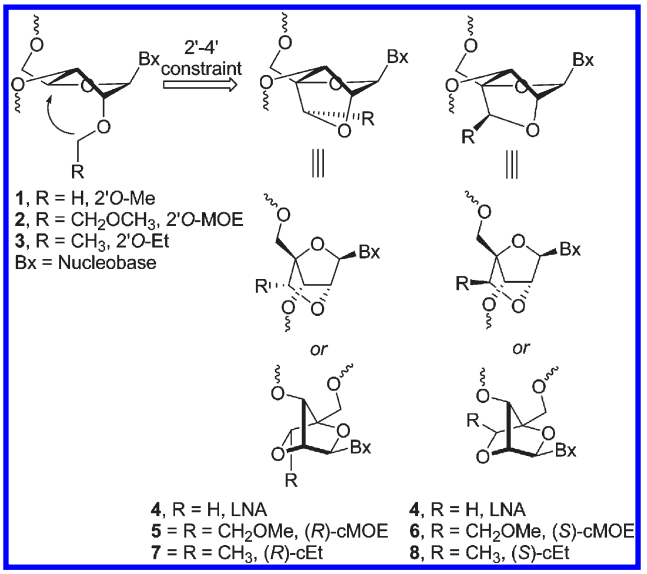

FIGURE 1. Design of constrained methoxyethyl (*R*-cMOE **5** and *S*-cMOE **6**) and constrained ethyl (*R*-cEt **7** and *S*-cEt **8**) nucleic acids.

supports RNase H mediated degradation of target mRNA due to the central DNA region. At the same time, the flanking MOE residues increase hybridization to complementary mRNA and further stabilize the oligonucleotide toward enzymatic degradation. There are currently almost 20 second generation ASOs in human clinical trials for a variety of disease indications including hypercholesteremia, diabetes, and cancer.

We have shown that replacing MOE nucleosides in second generation ASOs with locked nucleic acid (LNA) **4** increases the potency of some ASOs in animals. However, this was sometimes accompanied with an increased risk for hepatotoxicity.[3] We hypothesized that replacing LNA with novel nucleoside monomers that combine the structural elements of MOE and LNA might mitigate the toxicity of LNA while maintaining potency. To this end we designed and prepared nucleoside analogues **5** (*R*-constrained MOE, *R*-cMOE) and **6** (*S*-constrained MOE, *S*-cMOE), **7** (*R*-constrained ethyl, *R*-cEt), and **8** (*S*-constrained ethyl, *S*-cEt) where the 2′*O*-alkyl group was constrained back to the 4′ position of the furanose ring (Figure 1).[4] The 2′−4′ constraint enforces a N-type sugar pucker of the furanose ring and thereby improves hybridization with complementary RNA.[5] Biological evaluation of the cMOE and cEt containing ASOs showed that the *S*-cEt-modified ASOs displayed activity comparable to that of LNA with no elevations in liver transaminase levels after 3 weeks of dosing.[6]

Thus small structural changes on the LNA scaffold appear to have a significant impact on the biological profile of oligonucleotides containing these modifications. It is conceivable that further structure activity relationship studies on

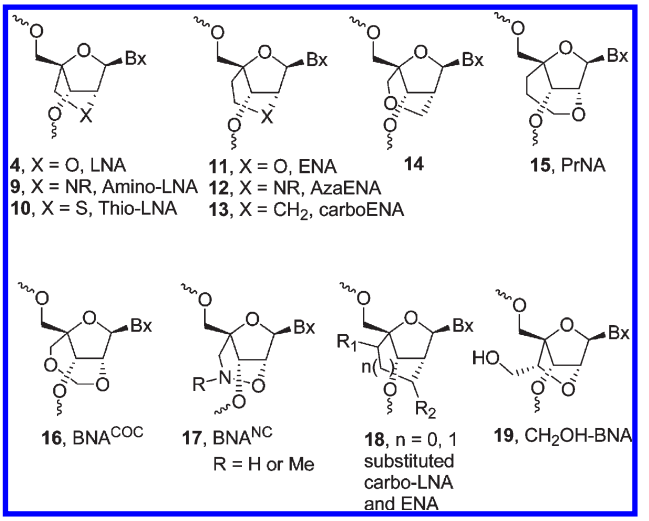

FIGURE 2. Structures of LNA and other related conformationally restricted 2′−4′ bridged nucleic acids (BNA) reported in the literature.

the LNA scaffold will provide additional modifications that have a superior biological profile as compared to that of LNA. As such, since the first report of LNA by Imanishi[7−9] and by Wengel,[10−14] a number of analogues have been reported in the literature (Figure 2). Some of the analogues contain subtle structural changes such as replacing the 2′-oxygen atom with nitrogen or sulfur to provide 2′-thio LNA **10**[15] and 2′-amino LNA **9**,[16,17] respectively. Other analogues differ based on the size and composition of the 2′−4′ bridging substituent. Examples of such analogues include ENA **11**,[18] aza ENA **12**,[19] carbo ENA **13**,[20,21] **14**,[22] PrNA **15**,[18] BNA^{COC} **16**[23] and BNA^{NC} **17**.[24,25] Recently,

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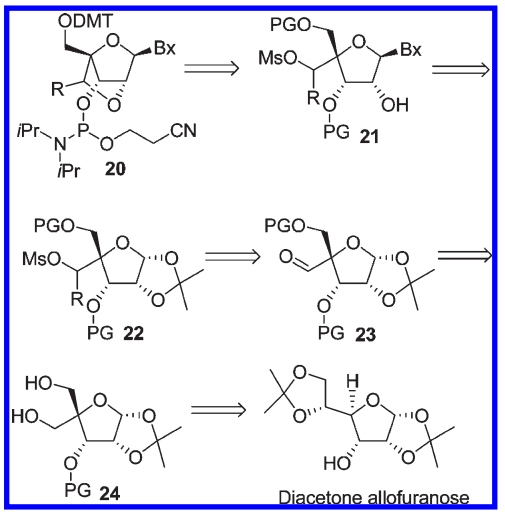


FIGURE 3. Retrosynthetic analysis for cMOE and cEt nucleic acids.

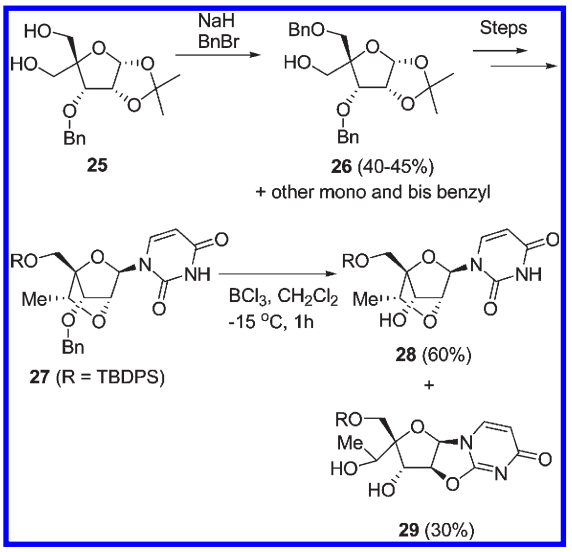
Chattopadhyaya reported the synthesis of substituted carbocyclic LNA and ENA analogues **18** via an intramolecular radical cyclization approach,[26–28] while Nielsen[29] and we[30] reported the synthesis of a (*R*)-6-hydroxymethyl-substituted LNA analogue **19**. With the exception of thio-LNA **10** and BNA^{NC} **17**, all other analogues reduce thermal stability for complementary nucleic acids relative to LNA. A majority of the analogues reported thus far are also significantly more difficult to synthesize, especially in quantities required to carry out meaningful animal experiments, as compared to LNA.

In this report, we describe the detailed synthesis and biophysical characterization of the cMOE- and cEt-modified oligonucleotides. We show that these modifications exhibit similar thermal stability and mismatch discrimination but increased nuclease stability as compared to those of LNA. We also describe a new orthogonal protecting group strategy that greatly facilitates the synthesis of this class of molecules and should find general application for the synthesis of other LNA analogues.

Results and Discussion

The general strategy used to prepare LNA typically involves a cycloetherification reaction between the 2′-hydroxyl and an appropriate leaving group at the 4′ position of the nucleoside furanose ring system.[7,11,13,31] For our purpose, we decided to implement a similar strategy to prepare the cMOE and cEt analogues (Figure 3). However, to the best of our knowledge, there were no reports that described the stereoselective cyclization of the 2′-hydroxyl onto a secondary leaving group required to generate the 6-substituted-2,5-dioxa-bicyclo[2.2.1]heptane ring system present in the cMOE and cEt nucleoside analogues. Access to diastereomerically pure cMOE and cEt nucleoside analogues hinged upon identifying methods to prepare nucleoside **21** in a stereodefined manner. We envisaged a strategy where secondary alcohol **22** could be prepared from aldehyde **23** by the stereoselective addition of an appropriate organometallic reagent. The overall topology of the sugar in **23** and the presence of a neighboring co-ordination site were expected to provide the appropriate elements of stereocontrol during addition of the organometallic reagent. Lastly, it was crucial to identify a robust protecting group strategy that could differentiate the two primary alcohols in **24** on a multigram scale.

SCHEME 1. Problems with 3′*O*-Bn Protecting Group Strategy



At first we chose to differentiate the two primary hydroxyls in diol **25** using the benzyl protecting group strategy that has been utilized for the synthesis of LNA and related molecules in the literature[7,11] (Scheme 1). However, separation of the desired benzyl intermediate **26** from its regioisomers was found to be cumbersome, especially on a large scale. Nonetheless, we were able to elaborate diol **25** to the cyclized nucleoside intermediate **27**.[30] However, removal of the 3′*O*-benzyl protecting group in **27** proved to be difficult and irreproducible. Use of Pd/C under mild reaction conditions resulted in very slow and erratic removal of the benzyl group and often required multiple catalyst changes to drive the reaction to completion. Use of more forcing conditions (ammonium formate, palladium hydroxide) resulted in partial hydrogenolysis of the uridine nucleobase, which could not be easily separated from the desired product **28**.[32] Use of boron trichloride resulted in the formation of significant amounts of the anhydro nucleoside **29**.[30] Presumably, presence of the methyl substituent in **27** results in increased ring strain, which in turn activates the cyclic ether to cleave under strong Lewis acid conditions. To circumvent the problems associated with removal of the 3′*O*-benzyl group, we decided to replace the benzyl group with a 2-naphthylmethyl (abbrev. naphthyl or Nap) protecting group, which possesses similar acid stability as a benzyl group but can be cleaved under mild

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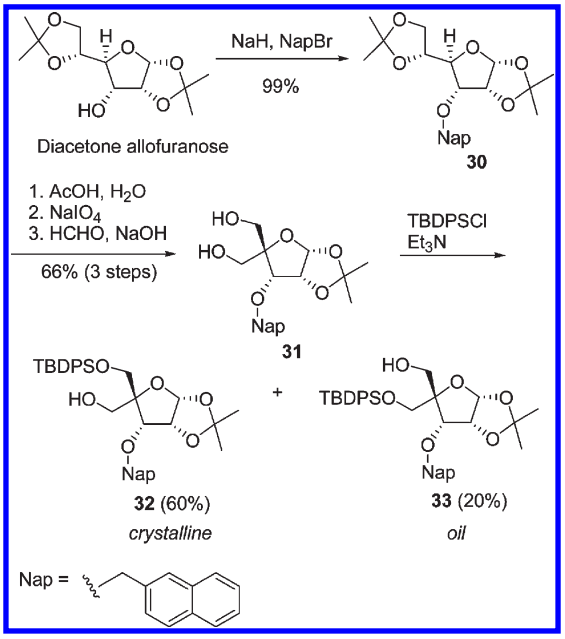
SCHEME 2. Synthesis of Key Mono-TBDPS Diol 32 from Diacetone Allofuranose

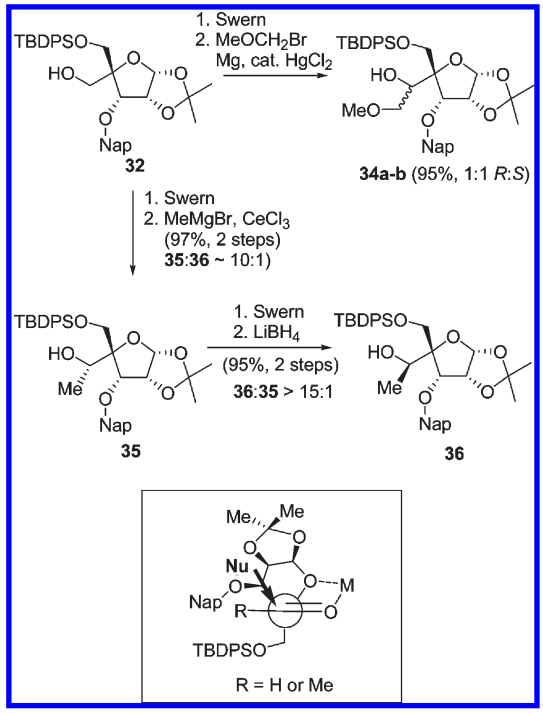


oxidative conditions using 2,3-dichloro-4,5-dicyano quinone (DDQ)[33] or by hydrogenolysis.[34]

Synthesis of the cMOE and cEt nucleosides commenced with protecting the 3′-hydroxyl of commercially available diacetone allofuranose with 2-bromomethyl naphthalene to provide 3′*O*-naphthyl diacetone allofuranose **30** (Scheme 2). The use of the naphthyl protecting group yielded immediate dividends as, unlike 3′*O*-benzyl-protected diacetone allofuranose, **30** could be cleanly precipitated from the reaction mixture in essentially quantitative yield. Selective cleavage of the 5,6-acetonide with aqueous acetic acid, followed by oxidative cleavage of the resulting diol with sodium periodate and subsequent aldol/Cannizzaro reaction[35] provided diol **31** in excellent yield (66% over 3 steps).

Once again, the naphthyl protecting strategy proved advantageous as the diol **31** was cleanly isolated from the reaction mixture by precipitation. The next step in the synthesis involved selective protection of the diol **31**. Exploratory reactions using *tert*-butyldimethylsilyl protection provided a mixture of the mono- and di-TBS-substituted sugars, which could be separated by chromatography. In contrast, use of the TBDPS protecting group provided only a mixture of the two mono-protected sugars **32** (60%) and **33** (20%). No di-TBDPS-protected sugar was formed under the reaction conditions employed, presumably due to the increased steric bulk of the TBDPS group. This proved to be extremely beneficial as it permitted using excess TBDPSCl to drive the reaction to completion. Another unexpected outcome of the TBDPS protecting strategy was that the major and desired product **32** was crystalline (**33** is an oil that slowly solidifies upon refrigeration) and could be isolated from the crude mixture by crystallization.[36]

SCHEME 3. Introduction of Methoxymethyl and Methyl Group for Synthesis of cMOE and cEt Nucleoside Analogues



Armed with a method that allowed for the preparation of the differentiated diol **32** on a kilogram scale, we explored the possibility of introducing the substituent group (methoxymethyl for cMOE and methyl for cEt) in a stereoselective fashion (Scheme 3). Oxidation of the primary alcohol under Swern conditions[37] followed by addition of the methoxymethyl magnesium bromide[38] provided sugars **34a** and **34b** as an inseparable mixture. It was very important to control the temperature during the preparation and use of $MeOCH_2MgBr$. This reagent was found to be unstable at temperatures above −20 °C, and the reaction to form the organometallic reagent did not proceed very efficiently at temperatures below −25 °C. Nonetheless, multigram quantities of alcohols **34a,b** (95% from **32**) could be prepared reproducibly by carefully controlling the reaction conditions for Grignard formation. Even though alcohols **34a,b** could not be separated at this stage we decided to move ahead with the hope of separating the mixture at a later stage in the synthesis.

For the cEt analogues, addition of MeMgBr in the presence of cerium chloride provided a separable mixture of alcohols **35** and **36** in excellent yield and diastereoselectivity (**35**:**36** ~10:1). Since we needed access to both methyl stereoisomers, the major alcohol **35** from the Grignard addition was converted to **36** by oxidation to the corresponding ketone followed by reduction with lithium borohydride (**36**:**35** > 15:1). The absolute stereochemistry of the alcohols **35** and **36** was not established until after cyclization to the

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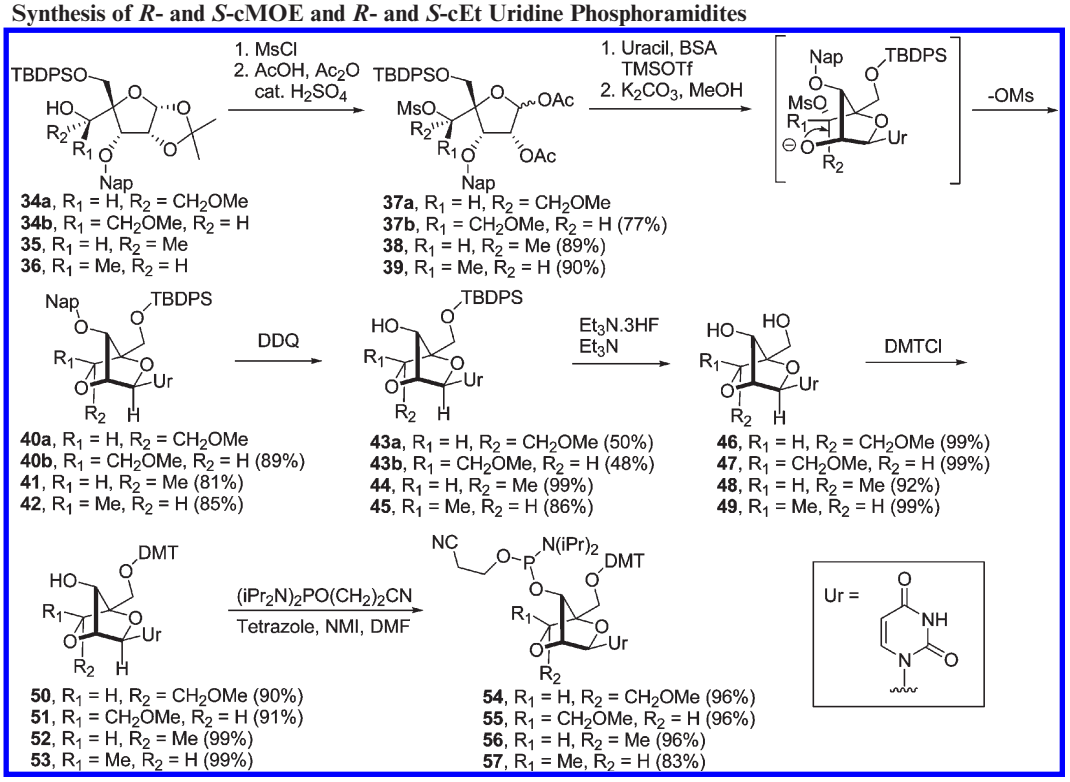
SCHEME 4. Synthesis of *R*- and *S*-cMOE and *R*- and *S*-cEt Uridine Phosphoramidites



R-cEt **41** and *S*-cEt **42** nucleosides, respectively, assuming a S_N2 inversion of the secondary leaving group during the ring-closure reaction. The stereoselectivity observed during addition of the methyl and the hydride group can be explained by means of a chelate-controlled transition state where attack of the nucleophile takes place away from the bulky TBDPS group. The poor stereoselectivity observed during addition of methoxymethylmagnesium bromide could be attributed to the differences in the size, reactivity, and ability to coordinate metal ions for this reagent.

Mesylation of the secondary alcohol in sugar intermediates **34a,b**, **35**, and **36** followed by acetolysis of the 1,2-isopropylidene group provided diacetates **37a,b**, **38**, and **39**, respectively, as an anomeric mixture in good yields (Scheme 4). Installation of the nucleobase in a stereoselective fashion was accomplished by means of a modified Vorbruggen condensation reaction with persilylated uracil.[39−41] The nucleoside mixture was not purified at this stage but treated with potassium carbonate in methanol to deprotect the 2′*O*-acetyl group. To our delight, these conditions resulted in removal of the 2′*O*-acetyl protecting group as well as cyclization onto the secondary mesylate to provide the cyclized nucleosides **40a,b**, **41**, and **42**, respectively, in excellent yields. Treatment of the mixture of *R*- and *S*-cMOE nucleosides **40a,b** with DDQ resulted in clean removal of the 3′*O*-Nap protecting group to provide nucleosides **43a** and **43b** as a separable mixture. The more polar compound was identified to be the *R*-cMOE nucleoside **43a**, while the less polar spot was the *S*-cMOE nucleoside **43b**. Presumably, the methoxymethyl group in the *S*-cMOE nucleoside **43b** is capable of forming an intramolecular H-bond with the liberated 3′-hydroxyl group, causing this nucleoside to be less polar than the *R*-cMOE isomer **43a** (Figure 4). Deprotection of the 3′*O*-Nap group in nucleosides **41** and **42** proceeded smoothly to yield nucleosides **44** and **45**, respectively, in high yield. Removal of the 5′*O*-TBDPS protecting group in nucleosides **43a**, **43b**, **44**, and **45** using buffered triethylamine trihydrofluoride[42] provided nucleosides **46**−**49**, respectively. Further protection of the 5′-hydroxyl group with dimethoxytrityl chloride in pyridine provided nucleosides **50**−**53** in excellent yields (90−99%). A phosphitylation reaction[43,44]

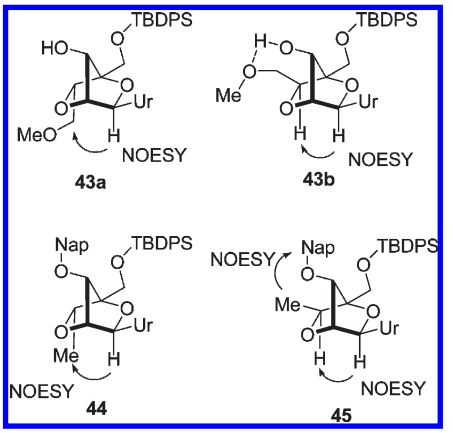


FIGURE 4. Structural elucidation of *R*-cMOE **43a**, *S*-cMOE **43b**, *R*-cEt **44**, and *S*-cEt **45**.

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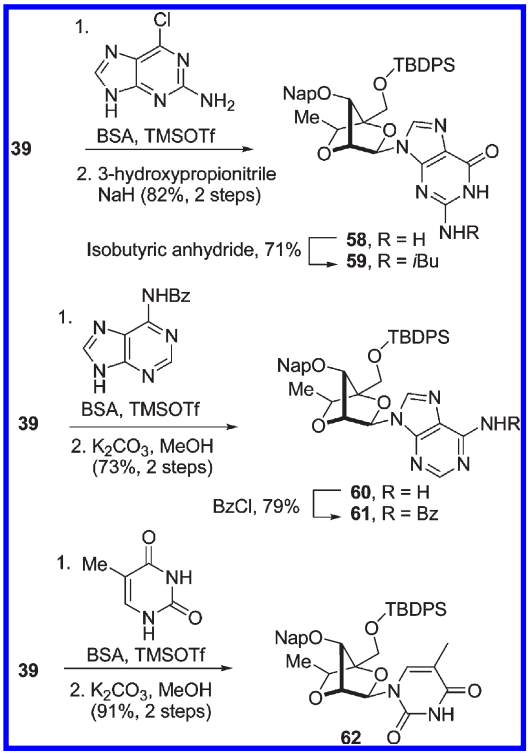
SCHEME 5. Synthesis of Guanine-, Adenine-, and Thymine-Modified *S*-cEt Nucleosides

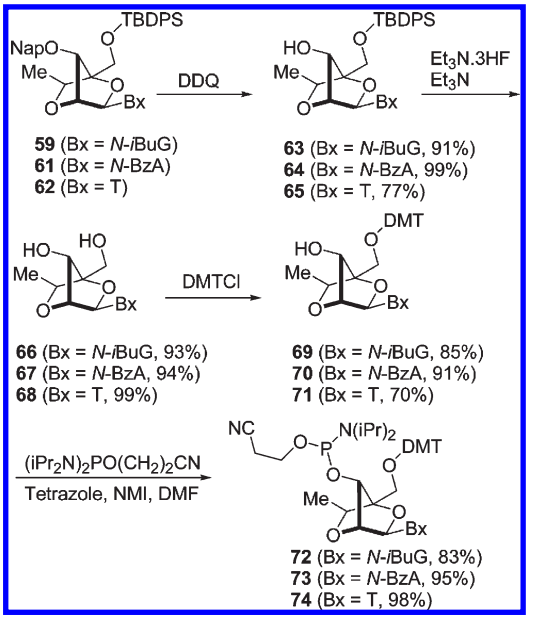


of the 5′*O*-DMT-protected nucleosides provided phosphoramidites **54**−**57**, respectively, in excellent yield after chromatography (83−96%).

The stereochemistry of the methoxymethyl group in the cMOE nucleosides **43a** and **43b** and of the methyl group in the cEt nucleosides **44** and **45** was elucidated by NOESY spectroscopy. For all of the nucleosides, the H1′, H2′, and H3′ proton resonances appear as singlets ($J \approx 0$ Hz), which is consistent with the sugar pucker being locked in the C3′-endo conformation.[7] The stereochemistry of the methoxymethyl groups in **43a** and **43b** was further confirmed by NOESY spectroscopy where a crosspeak between the H1′ and the methylene group of the methoxymethyl substituent was seen for nucleoside **43a** and a crosspeak between the H1′ and H6′ was seen in case of nucleoside **43b**. Similarly, a NOESY crosspeak was seen between the axial methyl group and H1′ for **44** and between the H6′ and H1′ for **45**. In addition, the structure of the fully deprotected *S*-cEt uridine nucleoside **49** was unambiguously assigned by X-ray crystallography (Supporting Information).

To be able to practice antisense drug discovery with a nucleoside modification in a meaningful manner, it is absolutely essential to have access to multigram quantities of the pyrimidine and purine nucleobase-modified phosphoramidites. Moreover, we also wanted to investigate if the Nap protecting strategy was compatible with the synthesis of purine nucleosides. As a result, we prepared the purine (adenine and guanine) and the 5-methyl pyrimidine (thymine and 5-methyl cytosine) phosphoramidites of the *S*-cEt modification for further evaluation in antisense drug discovery applications. Synthesis of the purine phosphoramidites was carried out using slight modifications of the procedures previously described for the preparation of LNA purine analogues

SCHEME 6. Synthesis of Guanine-, Adenine-, and Thymine-Modified *S*-cEt Phosphoramidites

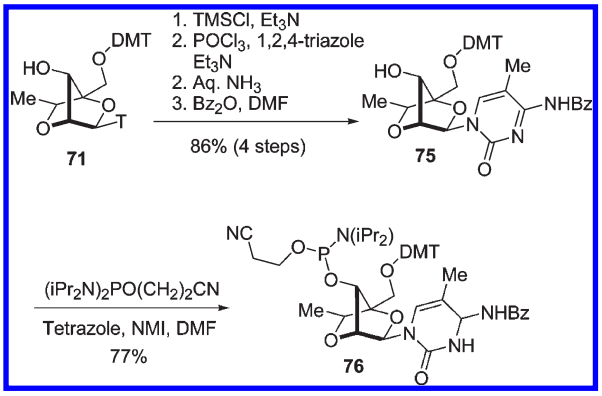


(Scheme 5).[31] A Vorbruggen reaction of silylated 6-chloro-2-aminopurine with diacetate **39** in refluxing dichloroethane provided the nucleoside, which was not purified but treated with the sodium salt of 3-hydroxypropionitrile (2′*O*-acetate deprotection, ring closure, S_NAr displacement of chloro followed by β elimination of acrylonitrile) to provide the cyclized nucleoside **58** in good yield (82% over 2 steps). Protection of the exocyclic amino group as the isobutyryl amide provided nucleoside **59** (71%). Similarly, a Vorbruggen reaction of silylated *N*-benzoyl adenine with diacetate **39** provided the nucleoside, which was not purified but directly subjected to the cyclization conditions (K_2CO_3, MeOH) to provide the cyclized product **60** in good yield (73% from **48**). Because of partial deprotection of the *N*-benzoyl group during the cyclization, the reaction was allowed to continue until the benzoyl group was completely deprotected. The cyclized product **60** was then isolated by crystallization or by silica gel chromatography and reacted with benzoyl chloride to provide the *N*-benzoyl nucleoside **61** (79%). Synthesis of the thymine-modified *S*-cEt nucleoside **62** was accomplished using procedures analogous for the preparation of the *S*-cEt uridine nucleoside **42** (91% over 2 steps).

The glycosylation reaction of activated anomeric sugars using silylated purine heterocycles is known to give mixtures of nucleosides arising from reaction at N7 and N9 positions on the purine heterocycle. Typically, the regioisomers equilibrate to the more stable N9 isomer after prolonged heating. Interestingly, during the glycosylation reaction of acetate **39** with 6-chloro-2-aminopurine and *N*-benzoyl adenine, only a single major N9 isomer was detected after 2 h of reflux in dichloroethane (by LCMS and TLC analysis).

Conversion of nucleosides **59**, **61**, and **62** to the corresponding phosphoramidites **72**, **73**, and **74** was carried out as shown in Scheme 6. Removal of the 3′*O*-naphthyl group using DDQ followed by removal of the 5′*O*-TBDPS protecting group using triethylamine trihydrofluoride yielded nucleosides **66**, **67**, and **68**, respectively, in excellent yield.

SCHEME 7. Synthesis of 5-Methylcytosine-Modified *S*-cEt Phosphoramidite



Reaction with dimethoxytrityl chloride gave the 5′*O*-DMT-protected nucleosides **69** (85%), **70** (91%), and **71** (70%), respectively. A phosphitylation reaction provided the phosphoramidites **72** (83%), **73** (95%), and **74** (98%) in excellent yield.

Lastly, synthesis of the 5-methyl cytosine *S*-cEt amidite **76** was accomplished starting from the 5′*O*-DMT-protected nucleoside **71**. In situ transient protection of the 3′-hydroxyl group as the trimethylsilyl ether, followed by triazolation, displacement with aqueous ammonia and protection of the amino group as the benzoyl amide provided nucleoside **75** in good yield (86% over 3 steps).[45,46] A phosphitylation reaction provided the desired 5-Me-cytosine-modified *S*-cEt phosphoramidite **76** (77%) (Scheme 7).

Evaluation of cMOE- and cEt-Modified Oligonucleotides in Thermal Denaturation Experiments. All of the nucleoside phosphoramidites were incorporated into oligonucleotides using standard automated phosphoramidite chemistry (Supporting Information). The modified oligomers were evaluated in thermal stability and in nuclease resistance assays. We first evaluated the *R*- and *S*-cMOE and *R*- and *S*-cEt modifications in an oligonucleotide sequence that has been previously used to measure thermal stability of LNA-containing oligonucleotides[9] (Table 1). In this sequence the *R*-and *S*-cMOE and *R*- and *S*-cEt oligomers (**A2**−**A5**) showed thermal stability similar to that of LNA oligomer **A7** (ΔT_m ~4.5 °C/mod.), indicating that substitution in either configuration at the 6 position of the 2,5-dioxabicyclo ring system is tolerated and does not interfere with hybridization. This observation is important since it suggests that it should be possible to introduce conjugates and/or fluorescent tags by further derivatization of the 6-substituent group,[29,30] either in the minor groove or along the edges of the major groove (based on the configuration of the substituent) at site-specific locations within an oligonucleotide without disrupting hybridization. Introduction of the 5-methyl substituent (change from uracil to thymine) on the nucleobase in LNA resulted in a slight increase in thermal stability (+0.6 °C/mod. **A7** vs **A8**), whereas the effect of the 5-Me substituent on thermal stability was much more pronounced for the *S*-cEt oligomer **A6** (+1.5 °C/mod. **A5** vs **A6**).

(45) Divakar, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171–1176.

(46) Bhat, V.; Ugarkar, B. G.; Sayeed, V. A.; Grimm, K.; Kosora, N.; Domenico, P. A.; Stocker, E. *Nucleosides, Nucleotides Nucleic Acids* **1989**, *8*, 179–183.

TABLE 1. Thermal Stability Measurements of *R*- and *S*-cMOE and *R*- and *S*-cEt Modifications with RNA Complement

oligomer	modification	sequence (5′−3′)[a]	T_m[b] (°C)	ΔT_m (°C)/mod.
A1	DNA	d(GCGTTTTTTGCT)	45.6	0
A2	*R*-cMOE	d(GCGTT**U**TTTGCT)	50.2	+4.6
A3	*S*-cMOE	d(GCGTT**U**TTTGCT)	50.4	+4.8
A4	*R*-cEt	d(GCGTT**U**TTTGCT)	50.3	+4.7
A5	*S*-cEt	d(GCGTT**U**TTTGCT)	50.1	+4.6
A6	*S*-cEt	d(GCGTT**T**TTTGCT)	51.7	+6.1
A7	LNA	d(GCGTT**U**TTTGCT)	50.1	+4.6
A8	LNA	d(GCGTT**T**TTTGCT)	50.7	+5.1

[a]Sequence and composition of ASOs; base code is A = adenine, T = thymine, G = guanine, C = cytosine, U = uracil; upper case letters indicate deoxynucleotide monomers, and bold letters indicate BNA monomers; all internucleosidic linkages are phosphodiester. [b]T_m values were measured in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 0.1 mM EDTA. Sequence of RNA complement 5′-r(AGCAAAAAACGC)-3′; each T_m value reflects the average of at least three measurements.

We also examined the ability of oligonucleotides **A1**−**A8** to discriminate between mismatched base pairs using RNA complements with a single base mismatch (Table 2) as reported previously by Imanishi.[25] In general, the mismatch discrimination properties of all the modifications evaluated were similar to that observed for the natural DNA/RNA base pairs (**A1**, −4 °C for T-G, −13 °C for T-C, and −14 °C for T-U mismatched pairs). Oligonucleotides **A2**−**A3** (*R*- and *S*-cMOE), **A4** and **A5** (*R*- and *S*-cEt), and **A7** (LNA) containing the uracil nucleobase exhibited excellent mismatch discrimination for the U-C (−14 °C) and U-U (−13 °C) mismatched pairs and good mismatch discrimination (−5 to −6 °C) for the U-G mismatch pair. For oligonucleotides **A6** (S-cEt) and **A8** (LNA) containing the thymine nucleobase, slightly improved mismatch discrimination properties (−6 °C for T-G, −16 °C for T-C, and −14 °C for T-U) were observed.

Nuclease Stability of *R*-cEt, *S*-cEt, LNA, and MOE Oligonucleotides. Oligonucleotides containing the *R*- and *S*-cEt modifications were evaluated in nuclease stability experiments (Figure 5). In one set of experiments, 10-mer poly T DNA oligomers (**A09**−**A13**) with two modified residues at the 3′ end were digested with snake venom phosphodiesterase (SVPD) (Figure 5). Significant increase in stability toward exonuclease digestion was seen for the *R*- and *S*-cEt oligonucleotides **A12** and **A13** as compared to LNA **A11**, MOE, **A10** and DNA **A09** oligonucleotides. For both the *R*-and *S*-cEt oligonucleotides **A12** and **A13**, >70% intact oligomer was seen after 1290 min. In contrast the MOE (**A10**) and LNA (**A11**) oligonucleotides were almost completely degraded at this time point. This result is consistent with recent reports where substitution on the 2′−4′ bridging substituent in the BNA scaffold was reported to increase stability of oligonucleotides toward exonuclease digestion.[25,26]

Conclusion

In conclusion, we have shown that the cMOE/cEt modifications exhibit similar thermal stability and mismatch discrimination but increased nuclease stability as compared to those of LNA. The synthesis features the use of a seldom used 2-naphthyl protecting group that provides crystalline intermediates during the synthesis and can be cleanly

TABLE 2. Mismatch discrimination of DNA, *S*-cEt and LNA versus mismatched RNA complements

		T_m[b] ($\Delta T_m = T_{m(mismatch)} - T_{m(match)}$) (°C)			
oligomer	sequence (5′−3′)[a]	X = A (match)	G	C	U
A1 (DNA)	d(GCGTTTTTTGCT)	45.6	41.5 (−4.1)	32.3 (−13.1)	31.8 (−13.8)
A2 (*R*-cMOE)	d(GCGTTUTTTGCT)	50.2	44.0 (−6.2)	35.3 (−14.9)	37.1 (−13.1)
A3 (*S*-cMOE)	d(GCGTTUTTTGCT)	50.4	44.4 (−6.2)	36.1 (−14.3)	37.2 (−13.2)
A4 (*R*-cEt)	d(GCGTTUTTTGCT)	50.3	43.9 (−6.2)	35.8 (−14.4)	36.3 (−14.0)
A5 (*S*-cEt)	d(GCGTTUTTTGCT)	50.1	45.6 (−4.5)	36.2 (−13.9)	36.5 (−13.6)
A6 (*S*-cEt)	d(GCGTTTTTTGCT)	51.7	45.6 (−5.1)	34.1 (−16.1)	37.2 (−13.5)
A7 (LNA)	d(GCGTTUTTTGCT)	50.1	45.0 (−5.1)	35.2 (−14.9)	37.2 (−12.9)
A8 (LNA)	d(GCGTTTTTTGCT)	50.7	46.0 (−5.7)	35.8 (−16.0)	37.4 (−14.3)

[a]Sequence and composition of ASOs; base code is A = adenine, T = thymine, G = guanine, C = cytosine, U = uracil; upper case letters indicate deoxynucleotide monomers, and bold letters indicate BNA monomers; all internucleosidic linkages are phosphodiester. [b]T_m values were measured in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 0.1 mM EDTA. Sequence of RNA complement 5′-r(AGCAA**X**AAACGC)-3′.

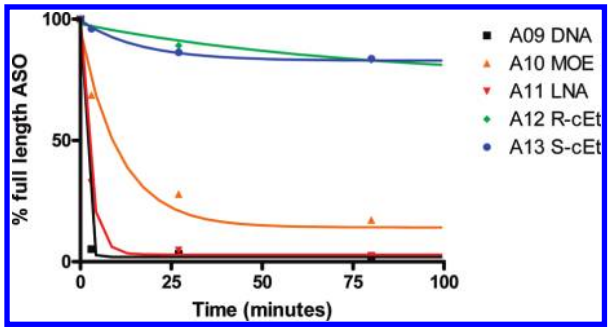


FIGURE 5. *R*- and *S*-cEt-modified oligonucleotides show improved stability to SVPD as compared to LNA, MOE and DNA oligonucleotides. Sequence used for evaluation was TTTTTTTTTTXX; upper case letters denotes deoxynucleotide residues while XX denote modified nucleosides; all internucleosidic linkages are phosphodiester. **A09**, XX = DNA; **A10**, XX = MOE T; **A11**, XX = LNA U; **A12**, XX = *R*-cEt U; **A13**, XX = *S*-cEt U.

deprotected under mild conditions. The new orthogonal protecting group strategy described in this report will greatly facilitate the synthesis of this class of molecules and should find general application for the synthesis of other LNA analogues. With the exception of LNA, the cMOE/cEt analogues are the only class of BNA modifications where the synthesis of the nucleoside phosphoramidites is scaleable enough to permit meaningful evaluation of these modifications in animal experiments. We are currently evaluating the cMOE and cEt modifications for their ability to modulate gene expression using RNaseH and other antisense mechanisms as well as in diagnostic applications. The results from these experiments will be reported in due course.

Experimental Section

4-*C*-Hydroxymethyl-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-α-D-ribofuranose (31). Sodium hydride (29.0 g, 727.0 mmol) was added carefully in portions (3−4 g) over 45 min to a cold (0 °C) solution of commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (135.0 g, 519.0 mmol) and 2-bromomethyl naphthalene (126 g, 570 mmol) in DMF (500 mL). After stirring for 1 h, the reaction was very carefully quenched with H_2O and then poured onto crushed ice, and the white solid was collected by filtration. The solid was resuspended in a biphasic mixture of water and hexanes (500 mL each), and the suspension was stirred vigorously for 45 min. The solid was collected by filtration and then dried under reduced pressure for 16 h to provide 3-*O*-(2-naphthyl)-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose **30** (206 g, 99%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.93−7.78 (m, 4 H), 7.60−7.45 (m, 3 H), 5.74 (d, J = 3.6 Hz, 1 H), 4.94 (d, J = 11.9 Hz, 1 H), 4.77 (d, J = 11.9 Hz, 1 H), 4.66−4.54 (m, 1 H), 4.46−4.30 (m, 1 H), 4.24−4.12 (m, 1 H), 4.08−3.88 (m, 3 H), 1.61 (s, 3 H), 1.38 (br s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.0, 133.2, 133.2, 128.3, 127.9, 127.7, 127.1, 126.2, 126.1, 126.1, 113.0, 109.7, 103.9, 78.2, 78.0, 77.8, 74.9, 72.4, 65.2, 26.9, 26.7, 26.3, 25.2. ESI-MS m/z: $[M + Na]^+$ found 423.1, calcd 423.1886. Anal. Calcd for $C_{23}H_{28}O_6$.0.25 H_2O: C, 68.21; H, 7.09. Found: C, 68.21; H, 6.92.

30 obtained above was dissolved in glacial acetic acid (2.2 L) and H_2O (0.74 L). The reaction was stirred at rt for 16 h after which ∼70% of the solvent was evaporated under reduced pressure on a rotary evaporator. The residue was dissolved in EtOAc (1 L) and the organic layer was washed with water (1 L), saturated sodium bicarbonate, brine, dried (Na_2SO_4) and concentrated to provide crude 3-*O*-(2-naphthyl)-1,2-*O*-isopropylidene-α-D-allofuranose as an oil, which was used without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.90−7.74 (m, 4 H), 7.55−7.41 (m, 3 H), 5.71 (d, J = 3.4 Hz, 1 H), 4.89 (d, J = 11.5 Hz, 1 H), 4.69 (d, J = 11.5 Hz, 1 H), 4.61−4.48 (m, 1 H), 4.12 (dd, J = 3.1 Hz, 8.8, 1 H), 4.06−3.88 (m, 2 H), 3.68 (br s, 2 H), 2.97 (br s, 1 H), 2.81 (br s, 1 H), 1.60 (s, 3 H), 1.34 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 134.4, 133.2, 128.4, 127.9, 127.7, 127.2, 126.3, 126.2, 126.0, 113.2, 104.2, 79.1, 77.4, 77.3, 77.0, 72.3, 71.1, 63.1, 26.8, 26.6. ESI-MS m/z: $[M + Na]^+$ found 383.0, calcd 383.1573.

A solution of sodium periodate (107 g, 500 mmol) in water (3 L) was added to a solution of the crude diol obtained above in 1,4-dioxane (1.5 L). After stirring at rt for 1 h, the reaction was extracted with EtOAc (1.5 L) and the organic layer was sequentially washed with water, brine, dried (Na_2SO_4) and concentrated to provide the crude aldehyde (and hydrate) as an oil, which was used without any further purification.

Sodium hydroxide solution (2N, 600 mL) was added to a cold (0 °C) solution of crude aldehyde from above and formaldehyde (250 mL, 35% aqueous solution) in THF:H_2O (1:1, 1 L) and the mixture was stirred at rt for 72 h. The reaction was extracted with ethyl acetate and the organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated. After roughly 80% of the solvent was removed (a suspension is formed), hexanes (300 mL) was added to the suspension which was allowed to stand for 16 h at rt. The white solid was collected by filtration and resuspended in a mixture of water and hexanes and stirred vigorously for 2−3 h. The solid was collected by filtration and dried under reduced pressure for 16 h to provide diol **31** (124 g, 66% from 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.84 (m, 4 H), 7.50 (m, 3 H), 5.76 (d, J = 3.8 Hz, 1 H), 4.96 (d, J = 11.9 Hz, 1 H), 4.74 (d, J = 11.9 Hz, 1 H), 4.71−4.59 (m, 1 H), 4.26 (d, J = 5.3 Hz, 1 H), 3.96 (br s, 2 H), 3.80 (d, J = 11.9 Hz, 1 H), 3.61 (m, 1 H), 2.40 (m, 1 H), 1.89 (br s, 1 H), 1.66 (s, 3 H), 1.34 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 134.7, 133.2, 133.2, 128.5, 127.9,

127.8, 126.9, 126.4, 126.2, 125.6, 113.6, 104.5, 86.3, 78.5, 78.4, 72.9, 64.3, 63.3, 26.6, 25.9. ESI-MS m/z: $[M + Na]^+$ found 383.1, calcd 383.1573. Anal. Calcd for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.64; H, 6.62.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-α-D-ribofuranose (32) and 4-*C*-(*tert*-Butyldiphenylsilyloxymethyl)-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-α-D-ribofuranose (33). *tert*-Butyldiphenylsilyl chloride (78.2 mL, 305 mmol) was added to a cold (0 °C) solution of diol **31** (100 g, 278 mmol) and triethylamine (42.8 mL, 305 mmol) in dichloromethane (500 mL). After stirring at rt for 4 d, the reaction was diluted with dichloromethane, sequentially washed with 5% HCl, saturated sodium bicarbonate, and brine, dried (Na_2SO_4) and concentrated under reduced pressure to provide a thick oil. Hexanes (150 mL) was added to the oil, and the mixture was sonicated to induce crystallization. After standing for 1 h, the white solid was collected by filtration and washed with 5% ether in hexanes to yield diol **32** (100 g total yield; 80 g first crop, 20 g second crop, 60%) as a white solid. Purification of the residual oil by chromatography (silica gel, eluting with 20% to 40% ethyl acetate in hexanes) provided diol **33** (32.5 g, 20%) as a viscous oil.

32: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.90−7.26 (m, 17 H), 5.81 (d, J = 3.6 Hz, 1 H), 4.97 (d, J = 11.9 Hz, 1 H), 4.77−4.63 (m, 2 H), 4.48 (d, J = 5.3 Hz, 1 H), 3.96−3.63 (m, 4 H), 2.51−2.35 (m, 1 H), 1.67 (s, 3 H), 1.38 (s, 3 H), 0.92 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.6, 135.5, 134.8, 133.2, 133.2, 133.0, 129.7, 129.7, 128.5, 127.9, 127.8, 127.7, 127.7, 126.9, 126.3, 126.2, 125.6, 113.8, 104.5, 87.5, 79.2, 78.0, 72.8, 65.5, 63.3, 27.0, 26.8, 26.3, 19.2. ESI-MS m/z: $[M + Na]^+$ found 621.1, calcd 621.2751. Anal. Calcd for $C_{36}H_{42}O_6Si$: C, 72.21; H, 7.07. Found: C, 72.17; H, 7.06.

33: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.88−7.65 (m, 8 H), 7.52−7.31 (m, 9 H), 5.70 (d, J = 3.8 Hz, 1 H), 4.84 (d, J = 12.2 Hz, 1 H), 4.77 (d, J = 12.1 Hz, 1 H), 4.63−4.51 (m, 1 H), 4.30−4.16 (m, 2 H), 4.16−3.99 (m, 2 H), 3.73−3.57 (m, 1 H), 2.05−1.94 (m, 1 H), 1.25 (br s, 6 H), 1.05 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.9, 135.8, 135.2, 133.3, 133.2, 133.1, 129.6, 128.2, 127.9, 127.7, 127.7, 126.8, 126.1, 126.0, 125.8, 113.2, 104.0, 87.3, 78.9, 77.9, 72.7, 65.2, 64.6, 26.9, 26.4, 25.9, 19.3. ESI-MS m/z: $[M + Na]^+$ found 621.1, calcd 621.2751. Anal. Calcd for $C_{36}H_{42}O_6Si.0.5\ H_2O$: C, 71.20; H, 7.14. Found: C, 71.21; H, 7.12.

5- *O*-(*tert*-Butyldiphenylsilyl)-4-*C*-(1-hydroxy-2-methoxyethyl)-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-α-D-ribofuranose (34a,b). Dimethylsulfoxide (3.5 mL, 50.0 mmol) was added dropwise to a cold (−78 °C) solution of oxalyl chloride (2.2 mL, 25.0 mmol) in dichloromethane (130 mL). After stirring for 30 min, a solution of diol **32** (12 g, 16.7 mmol) in dichloromethane (30 mL) was added to the reaction. The stirring was continued for 45 min at −78 °C, and triethylamine (10.5 mL, 75.0 mmol) was added to the reaction. The reaction was stirred at −78 °C for 15 min, after which the ice bath was removed and the reaction was allowed to gradually warm over 45 min. The reaction was then poured into CH_2Cl_2, and the organic phase was sequentially washed with 5% aqueous HCl, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated under reduced pressure to provide the aldehyde, which was used without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.93 (s, 1 H), 7.94−7.72 (m, 4 H), 7.63−7.26 (m, 13 H), 5.85 (d, J = 3.2 Hz, 1 H), 4.88 (d, J = 12.2, 1 H), 4.81 (d, J = 12.4 Hz, 1 H), 4.72−4.61 (m, 1 H), 4.62−4.52 (m, 1 H), 3.87 (d, J = 11.3 Hz, 1 H), 3.82 (d, J = 11.5 Hz, 1 H), 1.64 (s, 3 H), 1.37 (s, 3 H), 0.92 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 200.2, 135.6, 135.5, 134.5, 133.2, 133.2, 132.8, 132.6, 129.9, 129.8, 128.5, 127.9, 127.8, 127.8, 127.0, 126.3, 126.2, 125.7, 114.2, 105.0, 90.7, 79.1, 78.7, 73.0, 63.2, 26.7, 26.2, 19.2. ESI-MS m/z: $[M + Na]^+$ found 619.1, calcd 619.2594.

A mixture of magnesium turnings (2.5 g, 102.8 mmol) and mercury(II) chloride (93 mg, 0.34 mmol) was covered with dry THF (5 mL), and the reaction was cooled to −20 °C. A few drops of neat methoxymethyl bromide were added to initiate the reaction. After a few minutes, a solution of methoxymethyl bromide (9.33 mL, 102.8 mmol) in THF (12 mL) was added (1 mL every 15 min via a syringe) to the reaction over approximately 3 h. The temperature of the external bath was carefully maintained between −20 and −25 °C during the addition. A small volume of dry THF (5 mL) was added intermittently (over 3 h) to the reaction to facilitate stirring. After the addition of the bromide was complete, the reaction was stirred at −25 °C for 100 min, and a solution of crude aldehyde (from above) in THF (30 mL) was added. After stirring at −20 °C for 45 min, no starting aldehyde was detected by TLC analysis. The reaction was carefully quenched with a solution of saturated ammonium chloride and diluted with ethyl acetate. The organic layer was washed with 5% HCl, a saturated solution of sodium bicarbonate, and brine, dried (Na_2SO_4), and concentrated to provide alcohols **34a,b** (~1:1). The reaction was repeated a second time using identical conditions as described above with similar results. The crude products from both reactions were combined and purified by chromatography (silica gel, eluting with 15% to 30% ethyl acetate in hexanes) to provide alcohols **34a,b** (24.4 g, 95% from **32**) as a viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.90−7.74 (m, 8 H), 7.64−7.44 (m, 14 H), 7.44−7.25 (m, 12 H), 5.95−5.78 (m, 2 H), 5.02 (m, 2 H), 4.75 (m, 2 H), 4.72−4.51 (m, 5 H), 4.36−4.25 (m, 1 H), 3.90 (d, J = 11.3 Hz, 1 H), 3.74 (m, 3 H), 3.62−3.51 (m, 1 H), 3.45 (d, J = 10.9 Hz, 1 H), 3.39−3.20 (m, 4 H), 3.28 (s, 3 H), 3.23 (s, 3 H), 3.13 (s, 1 H), 1.69 (s, 3 H), 1.64 (s, 3 H), 1.39 (s, 6 H), 0.92 (s, 9 H), 0.91 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.5, 135.5, 134.7, 134.4, 133.2, 133.1, 133.0, 133.0, 132.8, 132.7, 129.8, 129.7, 129.6, 128.6, 128.4, 127.9, 127.9, 127.7, 127.6, 127.1, 126.8, 126.3, 126.3, 126.2, 126.1, 125.6, 125.6, 114.4, 113.9, 105.0, 104.8, 89.6, 88.0, 79.5, 79.3, 78.1, 78.1, 73.2, 73.0, 72.8, 72.5, 72.3, 70.8, 64.6, 63.5, 59.0, 58.8, 27.0, 26.9, 26.7, 26.6, 19.1, 19.1. ESI-MS m/z: $[M + Na]^+$ found 665.3, calcd 665.3013.

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-(1*S*-hydroxyethyl)-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-α-D-ribofuranose (35) and 5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-(1*R*-hydroxyethyl)-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)-α-D-ribofuranose (36). Dimethylsulfoxide (10.8 mL, 152.0 mmol) was added dropwise to a cold (−78 °C) solution of oxalyl chloride (6.7 mL, 76.0 mmol) in dichloromethane (400 mL). After stirring for 30 min, a solution of alcohol **32** (34.2 g, 56.4 mmol) in dichloromethane (40 mL) was added to the reaction. The stirring was continued for 45 min at −78 °C and triethylamine (31.4 mL, 224.0 mmol) was added to the reaction. The reaction was stirred at −78 °C for 15 min, after which the ice bath was removed and the reaction was allowed to gradually warm over 45 min. The reaction was diluted with dichloromethane, and the organic phase was sequentially washed with 5% aqueous HCl, saturated sodium bicarbonate, and brine, dried (Na_2SO_4), and concentrated to provide the aldehyde, which was used without any further purification.

A suspension of cerium(III) chloride (9.2 g, 37.5 mmol) in THF (400 mL) was stirred at rt for 60 min. The reaction was cooled in an ice bath, and methyl magnesium bromide (75.0 mL of a 1.0 M solution in THF) was added over 5 min. After stirring at 0 °C for 90 min, the reaction was cooled to −78 °C, and a solution of crude aldehyde from above in THF (75 mL) was added to the reaction. After 3 h at −78 °C, the reaction was gradually warmed to rt and carefully quenched with saturated ammonium chloride. The reaction was diluted with ethyl acetate, and the organic layer was sequentially washed with 5% HCl, saturated sodium bicarbonate, and brine, dried (Na_2SO_4), and concentrated. Purification by column chromatography (silica gel, eluting with 10% to 30% ethyl acetate in hexanes) provided pure alcohol **35** (7.4 g, 21% from **32**) and a mixture of alcohols **35** and **36** (26.3 g, 76% from **32**, **35**:**36** = 10:1) as viscous oils.

35: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.89−7.79 (m, 4 H), 7.65−7.26 (m, 13 H), 5.84 (d, J = 3.6 Hz, 1 H), 5.05 (d, J = 11.5 Hz, 1 H), 4.83−4.53 (m, 4 H), 3.91 (d, J = 11.1 Hz, 1 H), 3.84 (d, J = 11.1 Hz, 1 H), 3.36 (s, 1 H), 1.63 (s, 3 H), 1.39 (s, 3 H), 1.10 (d, J = 6.6 Hz, 3 H), 0.91 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.6, 135.5, 134.4, 133.3, 133.3, 133.2, 133.1, 129.7, 129.7, 128.7, 128.0, 127.8, 127.7, 127.7, 127.2, 126.4, 126.3, 125.7, 113.8, 104.8, 88.6, 79.4, 78.3, 73.0, 68.8, 62.4, 27.1, 26.8, 26.7, 19.2, 16.1. HRMS (ESI-FT), Calcd for $C_{37}H_{44}O_6SiNa$, 635.2799; Found 635.2800. ESI-MS m/z: $[M + Na]^+$ found 635.2. Anal. Calcd for $C_{37}H_{44}O_6Si.1\ H_2O$: C, 70.44; H, 7.35. Found: C, 70.73; H, 7.11.

36: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.88−7.78 (m, 4 H), 7.61−7.27 (m, 13 H), 5.87 (d, J = 3.6 Hz, 1 H), 4.96 (d, J = 12.1 Hz, 1 H), 4.74 (t, 1 H), 4.66 (d, J = 12.1 Hz, 1 H), 4.54 (d, J = 5.3 Hz, 1 H), 4.32−4.18 (m, 1 H), 3.69 (d, J = 10.7 Hz, 1 H), 3.52 (d, J = 10.7 Hz, 1 H), 3.12 (s, 1 H), 1.69 (s, 3 H), 1.39 (s, 3 H), 1.11 (d, J = 6.4 Hz, 3 H), 0.90 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.5, 134.8, 133.2, 133.2, 132.9, 132.8, 129.8, 129.7, 128.4, 127.9, 127.7, 126.9, 126.3, 126.1, 125.7, 114.3, 104.5, 90.4, 79.6, 78.1, 72.8, 67.1, 64.6, 26.9, 26.7, 19.1, 17.0. ESI-MS m/z: $[M + Na]^+$ found 635.2, calcd 635.2907. Anal. Calcd for $C_{37}H_{44}O_6Si.0.5\ H_2O$: C, 71.46; H, 7.29. Found: C, 71.81; H, 7.55.

Preparation of Alcohol 36 Using Lithium Borohydride. Dimethylsulfoxide (37.9 mL, 489.0 mmol) was added dropwise to a cold (−78 °C) solution of oxalyl chloride (21.4 mL, 244.0 mmol) in dichloromethane (800 mL). After stirring for 30 min, a solution of alcohol **35** (100.0 g, 163.0 mmol) in dichloromethane (200 mL) was added to the reaction. The stirring was continued for 45 min at −78 °C, and triethylamine (102.0 mL, 726.0 mmol) was added to the reaction. The reaction was stirred at −78 °C for 15 min, after which the ice bath was removed and the reaction was allowed to gradually warm over 45 min. The reaction was diluted with dichloromethane, and the organic phase was sequentially washed with 10% citric acid solution, saturated sodium bicarbonate, and brine, dried (Na_2SO_4), and concentrated to provide the crude ketone, which was used without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.82−7.27 (m, 17 H), 6.06 (d, J = 4.1 Hz, 1 H), 4.88 (d, J = 12.1 Hz, 1 H), 4.82 (d, J = 4.5 Hz, 1 H), 4.66 (d, J = 12.1 Hz, 1 H), 4.24 (d, J = 5.5 Hz, 1 H), 3.89 (d, J = 10.9 Hz, 1 H), 3.70 (d, J = 10.7 Hz, 1 H), 2.38 (s, 3 H), 1.63 (s, 3 H), 1.43 (s, 3 H), 0.97 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 207.8, 135.5, 134.9, 133.2, 133.0, 132.5, 132.4, 129.9, 129.8, 128.2, 127.9, 127.8, 127.7, 126.6, 126.1, 125.9, 125.7, 114.8, 106.7, 95.9, 80.9, 79.9, 73.4, 67.6, 28.7, 27.5, 26.8, 26.6, 19.1. ESI-MS m/z: $[M + Na]^+$ found 633.1, calcd 633.2751.

A solution of lithium borohydride (122.0 mL of a 2 M solution in THF, 244 mmol) was added dropwise over 30 min to a cold (−78 °C) solution of the ketone (163 mmol) from above in methanol (500 mL). After the addition was complete, the cooling bath was removed, and the reaction was stirred for 2 h. The reaction was then cooled in an ice bath and carefully quenched using saturated NH_4Cl solution and diluted with ethyl acetate. The organic layer was separated and sequentially washed with water, saturated sodium bicarbonate, and brine, dried (Na_2SO_4), and concentrated. Purification by column chromatography (silica gel, eluting with 30% ethyl acetate in hexanes) provided alcohol **36** (97.2 g, 95%, **36**:**35** > 15:1) as a viscous oil.

General Procedure for Mesylation and Acetolysis of 1,2-Acetonide. Methanesulfonyl chloride (1.4 equiv) was added dropwise over 30 min to a cold (0 °C) solution of alcohol (1 equiv), triethylamine (1.8 equiv), and DMAP (0.14 equiv) in dichloromethane (0.5 M). After stirring for 3 to 6 h, the reaction was diluted with chloroform. The organic layer was sequentially washed with 5% HCl, saturated sodium bicarbonate, and brine, dried (Na_2SO_4), and concentrated to provide the mesylate, which was used without any further purification.

Concentrated sulfuric acid (6−10 drops) was added to a solution of crude mesylate (1 equiv) from above, acetic acid, and acetic anhydride (5:1, 0.3 M). After stirring for 3 h at rt, the solvent was removed under reduced pressure, and the residual oil was diluted with ethyl acetate. The organic layer was washed with water, saturated sodium bicarbonate (until pH > 10), and brine, dried (Na_2SO_4), and concentrated. Purification by column chromatography (silica gel, eluting with 20% to 30% ethyl acetate in hexanes) provided an anomeric mixture of diacetates.

1,2-Di-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-4-*C*-(1-methanesulfonyloxy-2-methoxyethyl)-3-*O*-(2-naphthyl)-α-D-ribofuranose (37a,b). Prepared from alcohol **34a,b** (11.0 g, 17.1 mmol) to provide an anomeric mixture of anomeric diacetates **37a,b** (10.0 g, 77%) as a viscous oil. ESI-MS m/z: $[M + Na]^+$ found 787.2, calcd 787.2687. Anal. Calcd for $C_{40}H_{48}O_{11}SSi.1\ H_2O$: C, 61.36; H, 6.44. Found: C, 61.08; H, 6.15.

1,2-Di-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-4-*C*-(1*S*-methanesulfonyloxyethyl)-3-*O*-(2-naphthyl)-α-D-ribofuranose (38). Prepared from alcohol **35** (7.4 g, 12.0 mmol) using the general procedure described above to provide anomeric diacetates **38** (7.7 g, 89%) as a viscous oil. ^{1}H NMR for major anomer (300 MHz, $CDCl_3$) δ: 7.84−7.35 (m, 17 H), 6.22 (s, 1 H), 5.37 (d, J = 5.3 Hz, 1 H), 5.34−5.22 (m, 1 H), 4.77−4.58 (m, 2 H), 4.42 (d, J = 5.5 Hz, 1 H), 3.83 (d, 1 H, J = 10.9 Hz), 3.74 (d, J = 10.9 Hz, 1 H), 2.79 (s, 3 H), 2.11 (s, 3 H), 1.78 (s, 3 H), 1.56 (d, J = 4.1 Hz, 3 H), 1.06 (s, 9 H). ^{13}C NMR for anomeric mixture (75 MHz, $CDCl_3$) δ: 169.7, 169.0, 135.6, 134.4, 133.1, 133.0, 132.6, 130.0, 129.9, 128.4, 128.0, 127.9, 127.8, 127.7, 126.8, 126.3, 126.2, 125.5, 98.2, 88.2, 78.9, 78.8, 74.2, 74.1, 65.6, 38.8, 26.9, 20.9, 20.8, 19.4, 17.0. ESI-MS m/z: $[M + Na]^+$ found 757.1, calcd 757.2581.

1,2-Di-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-4-*C*-(1*R*-methanesulfonyloxyethyl)-3-*O*-(2-naphthyl)-α-D-ribofuranose (39). Prepared from alcohol **36** (16.9 g, 27.6 mmol) using the general procedure described above to provide anomeric diacetates **39** (18.2 g, 90%) as a viscous oil. ^{1}H NMR for major anomer (300 MHz, $CDCl_3$) δ: 7.85−7.41 (m, 17 H), 6.21 (s, 1 H), 5.57−5.45 (m, 1 H), 5.36−5.23 (m, 1 H), 4.76 (d, J = 11.3 Hz, 1 H), 4.70 − 4.60 (m, 1H), 3.92−3.71 (m, 3 H), 3.01 (s, 3 H), 2.14 (s, 3 H), 1.78 (s, 3 H), 1.43 (d, J = 6.6 Hz, 3 H), 1.05 (s, 9 H). ^{13}C NMR for anomeric mixture (75 MHz, $CDCl_3$) δ: 170.6, 169.9, 169.4, 135.7, 135.6, 135.6, 135.5, 135.0, 134.1, 133.2, 132.8, 132.4, 132.2, 130.1, 130.0, 129.9, 128.5, 128.1, 127.9, 127.9, 127.8, 127.8, 127.7, 126.9, 126.4, 126.3, 125.8, 125.5, 125.1, 97.8, 94.1, 90.4, 88.2, 81.7, 81.1, 77.9, 77.7, 74.8, 74.5, 74.2, 73.2, 62.8, 38.6, 38.4, 26.8, 21.3, 20.8, 20.5, 19.3, 19.1, 18.3, 18.1. ESI-MS m/z: $[M + Na]^+$ found 757.1, calcd 757.2581. Anal. Calcd for $C_{39}H_{46}O_{10}SSi.0.25\ H_2O$: C, 63.35; H, 6.33. Found: C, 63.32; H, 6.26.

General Procedure for Vorbruggen Reaction and Ring Closure. *N*,*O*-Bis(trimethylsilyl)acetamide (4 equiv) was added to a suspension of anomeric diacetates (1 equiv) and uracil (2 equiv) in acetonitrile (0.3M). After heating at 40 °C for 15 min to get a clear solution, the reaction was cooled in an ice bath and trimethylsilyltriflate (1.5 equiv) was added. After refluxing for 2 h the reaction was cooled to room temperature and poured into ethyl acetate. The organic layer was washed with half-saturated sodium bicarbonate solution and brine, dried (Na_2SO_4), and concentrated to provide the corresponding nucleoside mixture, which was used without any further purification.

Potassium carbonate (3 equiv) was added to a solution of crude nucleoside from above in methanol (0.1 M). After stirring at room temperature for 16 h, the reaction was concentrated under reduced pressure. The residue was dissolved in ethyl acetate, and the organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated. Purification by column chromatography (silica gel, eluting with 5% to 7.5% acetone in chloroform) provided the cyclized nucleoside.

(1*S*,3*R*,4*R*,6*R*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-6-methoxymethyl-7-(2-naphthyloxy)-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]-heptane and (1*S*,3*R*,4*R*,6*S*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-6-methoxymethyl-7-(2-naphthyloxy)-3-(uracil-1-yl)-2,5-dioxabicyclo-[2.2.1]heptane (40a,b). Prepared from diacetates **37a,b** (10.0 g, 13.0 mmol) using the general procedure described above to provide nucleosides **40a,b** (7.8 g, 89% from **36a,b**) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.58 (br s, 2 H), 7.94−7.27 (m, 36 H), 5.70−5.58 (m, 2 H), 5.48−5.31 (m, 2 H), 4.94−4.58 (m, 5 H), 4.50 (s, 1 H), 4.35−4.26 (m, 1 H), 4.24 (s, 1 H), 4.16−3.92 (m, 8 H), 3.61−3.47 (m, 5 H), 3.26 (s, 3 H), 3.18 (s, 3 H), 1.07 (s, 18 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.8, 149.6, 149.5, 139.1, 139.0, 135.6, 135.5, 135.3, 134.0, 133.8, 133.2, 132.7, 132.2, 130.1, 130.1, 128.5, 128.5, 128.0, 127.9, 127.8, 127.8, 127.1, 126.8, 126.5, 126.4, 126.3, 125.7, 125.6, 101.7, 101.6, 89.2, 89.1, 86.9, 86.7, 82.9, 78.6, 77.2, 76.7, 76.3, 75.9, 72.7, 72.6, 71.0, 70.1, 59.3, 58.9, 58.5, 26.8, 19.3. ESI-MS m/z: $[M + H]^+$ found 679.2, calcd 679.2761.

(1*S*,3*R*,4*R*,6*R*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-6-methyl-7-(2-naphthyloxy)-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (41). Prepared from anomeric diacetates **38** (7.5 g, 10.2 mmol) using the general procedure to provide nucleoside **41** (5.3 g, 81%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.74 (br s, 1 H), 7.91 (d, J = 8.3 Hz, 1 H), 7.87−7.28 (m, 17 H), 5.65 (s, 1 H), 5.43 (d, J = 8.1 Hz, 1 H), 4.83 (d, J = 11.5 Hz, 1 H), 4.75 (d, J = 11.5 Hz, 1 H), 4.49 (s, 1H), 4.38−4.27 (m, 1 H), 4.20 (s, 1 H), 3.93−3.88 (m, 2 H), 1.19 (d, J = 6.4 Hz, 3 H), 1.07 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.9, 149.6, 139.2, 135.6, 135.3, 134.1, 133.2, 132.5, 132.0, 130.2, 130.1, 128.5, 128.0, 127.9, 127.8, 126.9, 126.4, 126.3, 125.7, 101.6, 88.8, 86.7, 76.5, 76.3, 72.6, 58.4, 26.8, 19.3, 13.8. HRMS (ESI-FT), Calcd for $C_{38}H_{41}N_2O_6Si$ 649.2728; found 649.2738. ESI-MS m/z: $[M + H]^+$ found 649.2. Anal. Calcd for $C_{38}H_{40}N_2O_6Si.0.5\ H_2O$: C, 69.38; H, 6.28; N, 4.26. Found: C, 69.01; H, 6.19; N, 4.26.

(1*S*,3*R*,4*R*,6*S*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-6-methyl-7-(2-naphthyloxy)-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (42). Prepared from anomeric diacetates **39** (12.3 g, 16.7 mmol) using the general procedure to provide nucleoside **42** (9.2 g, 85%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.20 (br s, 1 H), 7.84−7.31 (m, 18 H), 5.66 (s, 1 H), 5.43 (d, J = 7.9 Hz, 1 H), 4.82 (d, J = 11.3 Hz, 1 H), 4.72 (s, 1H), 4.71−4.61 (m, 1 H), 4.14−3.97 (m, 4 H), 1.26 (d, J = 6.6 Hz, 3 H), 1.07 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.2, 149.7, 139.1, 135.5, 135.3, 134.0, 133.1, 132.6, 132.1, 130.1, 130.0, 128.4, 127.9, 127.8, 127.7, 126.9, 126.4, 126.3, 125.7, 101.5, 89.3, 87.1, 81.1, 76.7, 76.5, 72.6, 58.7, 26.7, 19.3, 16.3. ESI-MS m/z: $[M + H]^+$ found 649.2, calcd 649.2656. Anal. Calcd for $C_{38}H_{40}N_2O_6Si.0.25\ H_2O$: C, 69.86; H, 6.25; N, 4.29. Found: C, 69.61; H, 6.17; N, 4.22.

General Procedure for Deprotection of 3′*O*-Nap Group Using 2,3-Dichloro-4,5-dicyano Quinone (DDQ). DDQ (2−2.5 equiv) was added to a solution of nucleoside (1 equiv) in dichloromethane and water (20:1, 0.1 M). The biphasic reaction was stirred at room temperature for 16 h, after which the solvent was evaporated under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic layer was sequentially washed with sodium bisulfite, saturated sodium bicarbonate, and brine, dried (Na_2SO_4), and concentrated. Purification by column chromatography (silica gel, eluting 10% to 30% acetone in chloroform) provided the 3′OH nucleoside.

(1*S*,3*R*,4*R*,6*R*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-7-hydroxy-6-methoxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (43a) and (1*S*,3*R*,4*R*,6*S*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-7-hydroxy-6-methoxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]-heptane (43b). Prepared from nucleosides **40a,b** (16.8 g, 24.9 mmol) using the general procedure to provide nucleosides **43a** (6.4 g, 50%, R_f = 0.15, 25% acetone in chloroform) and **43b** (6.7 g, 48%, R_f = 0.22, 25% acetone in chloroform) as crisp foams, respectively.

43a: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.44 (s, 1 H), 7.91 (d, J = 8.3 Hz, 1 H), 7.77−7.64 (m, 4 H), 7.54−7.35 (m, 6 H), 5.58 (s, 1 H), 5.52 (d, J = 8.3 Hz, 1 H), 4.40 (s, 1 H), 4.32 (d, J = 5.1 Hz, 1 H), 4.18 (t, J = 6.3 Hz, 1 H), 4.07 (d, J = 12.2 Hz, 1 H), 3.95 (d, J = 12.4, 1 H), 3.56 (d, J = 6.4 Hz, 2 H), 3.27 (s, 3 H), 3.21−3.13 (m, 1 H), 1.09 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.5, 149.9, 139.2, 135.6, 135.4, 132.7, 132.3, 130.2, 130.1, 128.0, 101.7, 89.2, 86.4, 79.0, 77.9, 71.2, 70.4, 59.2, 58.4, 26.8, 19.3. HRMS (ESI-FT) Calcd for $C_{28}H_{35}N_2O_7Si$, 539.2208; Found 539.2206. ESI-MS m/z: $[M + Na]^+$ found 539.2.

43b: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.20 (br s, 1 H), 7.81−7.61 (m, 4 H), 7.65 (d, J = 8.1 Hz, 1H) 7.55−7.35 (m, 6 H), 5.64 (s, 1 H), 5.60 (d, J = 8.1 Hz, 1 H), 5.07 (d, J = 10.7 Hz, 1 H), 4.43 (s, 1 H), 4.18−3.94 (m, 4 H), 3.66 (dd, J = 11.5, 2.8 Hz, 1 H), 3.52 (d, J = 11.3 Hz, 1 H), 3.35 (s, 3 H), 1.09 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.2, 149.7, 139.0, 135.8, 135.4, 132.7, 132.1, 130.1, 130.1, 128.0, 127.9, 101.8, 90.1, 86.4, 82.8, 80.3, 70.5, 70.1, 59.5, 59.2, 26.7, 19.3. HRMS (ESI-FT) Calcd for $C_{28}H_{35}N_2O_7Si$, 539.2208; Found 539.2209. ESI-MS m/z: $[M + Na]^+$ found 539.2.

(1*S*,3*R*,4*R*,6*R*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-7-hydroxy-6-methyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (44). Prepared from nucleoside **41** (5.3 g, 8.2 mmol) using the general procedure to provide nucleoside **44** (4.15 g, 99%) as a crisp foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.31 (br s, 1 H), 7.92 (d, J = 8.1 Hz, 1 H), 7.77−7.64 (m, 4 H), 7.54−7.35 (m, 6 H), 5.60 (s, 1 H), 5.53 (d, J = 8.1 Hz, 1 H), 4.35 (s, 1 H), 4.30 (d, J = 5.1 Hz, 1 H), 4.25−4.16 (m, 1 H), 3.93−3.86 (m, 2 H), 2.91 (m, 1 H), 1.20 (d, J = 6.2 Hz, 3 H), 1.09 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.4, 149.8, 139.2, 135.6, 135.4, 132.5, 132.2, 130.2, 130.1, 128.0, 101.7, 88.9, 86.3, 79.0, 75.8, 71.3, 58.4, 26.8, 19.3, 13.9. ESI-MS m/z: $[M + H]^+$ found 509.1, calcd 509.2030.

(1*S*,3*R*,4*R*,6*S*,7*S*)-1-(*tert*-Butyldiphenylsilyloxymethyl)-7-hydroxy-6-methyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (45). Prepared from nucleoside **42** (9.2 g, 14.2 mmol) using the general procedure to provide nucleoside **45** (6.2 g, 86%) as a crisp foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.67 (br s, 1 H), 7.80−7.65 (m, 5 H), 7.52−7.36 (m, 6 H), 5.59 (s, 1 H), 5.49 (d, J = 8.1 Hz, 1 H), 4.56 (s, 1 H), 4.22−3.96 (m, 4 H), 3.13 (m, 1 H), 1.28 (d, J = 6.8 Hz, 3 H), 1.09 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.7, 149.9, 139.3, 135.6, 135.4, 132.7, 132.3, 130.1, 130.1, 128.0, 101.6, 89.4, 86.7, 81.1, 79.7, 70.8, 58.9, 26.8, 19.3, 16.3. ESI-MS m/z: $[M + H]^+$ found 509.1, calcd 509.2030.

General Procedure for Removing 5′*O*-TBDPS Protecting Group. Triethylamine trihydrofluoride (6 equiv) was added to a solution of nucleoside (1 equiv) and triethylamine (2.5 equiv) in THF (0.1 M), and the reaction was stirred at rt for 1−2 days. The solvent was removed under reduced pressure, and the thick oil was purified by chromatography (silica gel, eluting with 5% to 12.5% methanol in chloroform) provided the corresponding deprotected nucleoside.

(1*S*,3*R*,4*R*,6*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-6-methoxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (46). Prepared from nucleoside **43a** (6.7 g, 12.0 mmol) using the general procedure described above to provide **46** (3.7 g, 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.36 (s, 1 H), 7.79 (d, J = 8.1 Hz, 1 H), 5.73 (d, J = 4.1 Hz, 1H), 5.64 (d, J = 8.1 Hz, 1 H), 5.40 (s, 1 H), 5.20 (br s, 1 H), 4.10 (s, 1H), 4.02−3.94 (m, 2 H), 3.75 (d, J = 3.8 Hz, 1 H), 3.71−3.54 (m, 2 H), 3.46 (dd, J = 6.4 Hz, 10.0, 1 H), 3.28 (s, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.2, 149.9, 139.0, 100.8, 88.8, 85.8, 78.6, 77.6, 70.5, 69.8, 58.5, 55.2. ESI-MS m/z: $[M + H]^+$ found 301.1, calcd 301.0958. Anal. Calcd for $C_{12}H_{16}N_2O_7.0.11\ CHCl_3$: C, 46.39; H, 5.18; N, 8.93. Found: C, 46.17; H, 5.22; N, 8.77.

(1*S*,3*R*,4*R*,6*S*,7*S*)-7-Hydroxy-1-hydroxymethyl-6-methoxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (47). Prepared from nucleoside **43b** (6.4 g, 12.5 mmol) using the general procedure described above to provide **47** (3.5 g, 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.42 (br s, 1 H), 7.80 (d, J = 8.1 Hz, 1 H), 5.73 (br s, 1 H), 5.68 (d, J = 8.1 Hz, 1 H), 5.47 (s, 1

H), 5.22 (br s, 1 H), 4.28 (s, 1 H), 4.02−3.91 (m, 2 H), 3.83 (br s, 2 H), 3.59 (d, J = 5.5 Hz, 2 H), 3.30 (s, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.3, 150.0, 139.2, 100.8, 89.0, 86.1, 82.8, 79.2, 71.0, 68.9, 58.2, 55.8. ESI-MS m/z: $[M + H]^+$ found 301.1, calcd 301.0958.

(1*S*,3*R*,4*R*,6*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-6-methyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (48). Prepared from nucleoside **44** (4.2 g, 8.2 mmol) using the general procedure described above to provide **48** (2.1 g, 92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.35 (br s, 1 H), 7.81 (d, J = 8.1 Hz, 1 H), 5.71−5.59 (m, 2 H), 5.38 (s, 1 H), 5.16 (t, J = 5.3 Hz, 1 H), 4.21−4.06 (m, 1 H), 4.05 (s, 1H), 3.99 (d, J = 4.1 Hz, 1 H), 3.62 (d, J = 5.1 Hz, 2 H), 1.14 (d, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.1, 149.8, 138.9, 100.6, 88.4, 85.6, 78.4, 75.0, 69.7, 55.1, 13.8. ESI-MS m/z: $[M + H]^+$ found 271.0, calcd 271.0872. Anal. Calcd for $C_{11}H_{14}N_2O_6$.0.5 H_2O: C, 47.31; H, 5.41; N, 10.03. Found: C, 47.01; H, 5.29; N, 9.70.

(1*S*,3*R*,4*R*,6*S*,7*S*)-7-Hydroxy-1-hydroxymethyl-6-methyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (49). Prepared from nucleoside **45** (6.2 g, 12.2 mmol) using the general procedure described above to provide **49** (3.3 g, 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.36 (s, 1 H), 7.73 (d, J = 8.1 Hz, 1 H), 5.62 (d, J = 8.1 Hz, 1 H), 5.55 (d, J = 3.8 Hz, 1 H), 5.38 (s, 1 H), 5.11 (t, J = 5.4 Hz, 1 H), 4.19 (s, 1 H), 3.94 (m, 1 H), 3.86 (d, J = 3.8 Hz, 1 H), 3.80 (d, J = 5.3 Hz, 2 H), 1.22 (d, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.1, 149.9, 139.2, 100.7, 89.0, 86.1, 80.2, 79.2, 69.6, 55.9, 16.2. HRMS (ESI-FT), Calcd for $C_{11}H_{14}N_2O_6Na$, 293.0744; Found 293.0745. ESI-MS m/z: $[M + H]^+$ found 271.0. Anal. Calcd for $C_{11}H_{14}N_2O_6$.0.25 CH_3OH: C, 48.56; H, 5.43; N, 10.07. Found: C, 48.71; H, 5.06; N, 9.92.

General Procedure for 5′*O*-DMT Protection. 4,4′-Dimethoxytrityl chloride (1.5 equiv) was added to a solution of nucleoside (1 equiv) in pyridine (0.2 M). After stirring at rt for 16 h, the reaction was quenched with methanol and diluted with ethyl acetate. The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated. Purification by chromatography (silica gel, eluting with 10% to 20% acetone in dichloromethane) provided the corresponding 5′*O*-DMT-protected nucleoside.

(1*S*,3*R*,4*R*,6*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-6-methoxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]-heptane (50). Prepared from nucleoside **46** (3.7 g, 12.5 mmol) using the general procedure described above to provide **50** (6.7 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.11 (br s, 1 H), 8.01 (d, J = 8.1 Hz, 1 H), 7.45−7.24 (m, 9 H), 6.86 (d, J = 8.5 Hz, 4 H), 5.66−5.51 (m, 2 H), 4.46−4.31 (m, 2 H), 4.23−4.08 (m, 1 H), 3.79 (s, 6 H), 3.70−3.40 (m, 4 H), 3.24 (s, 3 H), 2.69 (d, J = 4.7 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.3, 158.7, 149.7, 144.4, 139.4, 135.3, 130.1, 130.0, 128.0, 127.2, 113.3, 101.8, 88.2, 87.0, 86.4, 78.9, 78.2, 71.6, 70.3, 59.2, 57.4, 55.3. ESI-MS m/z: $[M + Na]^+$ found 625.1, calcd 625.2264.

(1*S*,3*R*,4*R*,6*S*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-6-methoxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]-heptane (51). Prepared from nucleoside **47** (3.5 g, 12.0 mmol) using the general procedure described above to provide **51** (6.5 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.69 (br s, 1 H), 7.70 (d, J = 8.3 Hz, 1 H), 7.50−7.24 (m, 9 H), 6.86 (d, J = 8.7 Hz, 4 H), 5.75−5.60 (m, 2 H), 5.05 (d, J = 10.9 Hz, 1 H), 4.43 (s, 1 H), 4.17 (s, 1 H), 3.97 (d, J = 10.9 Hz, 1 H), 3.80 (s, 6 H), 3.75−3.58 (m, 2 H), 3.41 (m, 2 H), 3.22 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.8, 158.7, 149.6, 144.3, 138.9, 135.5, 135.1, 130.0, 128.0, 128.0, 127.1, 113.3, 113.3, 101.9, 89.2, 86.6, 86.6, 83.1, 80.3, 71.1, 70.0, 59.2, 58.9, 55.3. ESI-MS m/z: $[M + Na]^+$ found 625.1, calcd 625.2264.

(1*S*,3*R*,4*R*,6*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-6-methyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (52). Prepared from nucleoside **48** (2.1 g, 7.5 mmol) using the general procedure described above to provide **52** (4.3 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.03 (br s, 1 H), 8.03 (d, J = 8.1 Hz, 1 H), 7.47−7.25 (m, 9 H), 6.94−6.76 (m, 4 H), 5.69−5.52 (m, 2 H), 4.42−4.27 (m, 2 H), 4.16 (m, 1 H), 3.80 (s, 6 H), 3.51−3.34 (m, 2 H), 2.49 (d, J = 5.7 Hz, 1 H), 1.17 (d, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.3, 158.8, 149.7, 144.4, 139.4, 135.2, 135.2, 130.0, 130.0, 128.0, 128.0, 127.2, 113.3, 101.7, 88.1, 87.0, 86.4, 78.9, 76.0, 71.7, 60.4, 57.5, 55.3, 13.8. 293.0745. HRMS (ESI-FT): calcd for $C_{32}H_{32}N_2O_8Na$ 595.2051; found 595.2048. ESI-MS m/z: $[M + Na]^+$ found 595.1.

(1*S*,3*R*,4*R*,6*S*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-6-methyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (53). Prepared from nucleoside **49** (3.3 g, 12.2 mmol) using the general procedure described above to provide **53** (7.0 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.53−9.17 (br s, 1 H), 7.86 (d, J = 8.1 Hz, 1 H), 7.50−7.26 (m, 9 H), 6.85 (d, J = 8.7 Hz, 4 H), 5.69−5.56 (m, 2 H), 4.58 (s, 1 H), 4.22 (d, J = 5.3 Hz, 1 H), 4.19−4.07 (m, 1 H), 3.78 (s, 6 H), 3.58 (d, J = 10.9 Hz, 1 H), 3.53 (d, J = 11.1 Hz, 1 H), 1.18 (d, J = 6.8 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.5, 158.7, 149.8, 144.5, 139.4, 135.4, 135.2, 130.1, 130.0, 128.0, 127.1, 113.3, 113.2, 101.8, 88.5, 86.8, 86.8, 81.2, 79.6, 71.3, 58.2, 55.2, 16.4. HRMS (ESI-FT): calcd for $C_{32}H_{32}N_2O_8Na$ 595.2051; found 595.2050. ESI-MS m/z: $[M + Na]^+$ found 595.1.

General Procedure for Uridine Phosphoramidite Synthesis. 2-Cyanoethyl tetraisopropylphosphordiamidite (1.5 equiv) was added to a solution of nucleoside (1 equiv), tetrazole (0.8 equiv), and *N*-methylimidazole (0.25 equiv) in DMF (0.2 M). After stirring at room temperature for 8 h the reaction was diluted EtOAc, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Purification by chromatography (silica gel, eluting with 50% to 60% ethyl acetate in hexanes) provided the corresponding phosphoramidite as a white foam.

(1*S*,3*R*,4*R*,6*R*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-6-methoxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (54). Prepared from nucleoside **50** (2.0 g, 3.3 mmol) using the general procedure described above to provide **54** (2.54 g, 96%). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 149.8, 149.5. HRMS (ESI-FT): calcd for $C_{42}H_{52}N_4O_{10}P$ 803.3416; found 803.3421. ESI-MS m/z: $[M + H]^+$ found 803.1. Anal. Calcd for $C_{42}H_{51}N_4O_{10}P$.0.25 H_2O: C, 62.48; H, 6.43; N, 6.94. Found: C, 62.55; H, 6.21; N, 6.85.

(1*S*,3*R*,4*R*,6*S*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-6-methoxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (55). Prepared from nucleoside **51** (2.0 g, 3.3 mmol) using the general procedure described above to provide **55** (2.55 g, 96%). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 150.0, 149.8. HRMS (ESI-FT): calcd for $C_{42}H_{52}N_4O_{10}P$ 803.3416; found 803.3426. ESI-MS m/z: $[M + H]^+$ found 803.2. Anal. Calcd for $C_{42}H_{51}N_4O_{10}P$.0.5 H_2O: C, 62.13; H, 6.46; N, 6.90. Found: C, 62.46; H, 6.18; N, 6.72.

(1*S*,3*R*,4*R*,6*R*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-6-methyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (56). Prepared from nucleoside **52** (2.1 g, 3.7 mmol) using the general procedure described above to provide **56** (2.7 g, 96%). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 149.8, 149.2. HRMS (ESI-FT): calcd for $C_{41}H_{50}N_4O_9P$ 773.3310; found 773.3314. ESI-MS m/z: $[M + H]^+$ found 773.2. Anal. Calcd for $C_{41}H_{49}N_4O_9P$.0.25 H_2O: C, 63.35; H, 6.42; N, 7.21. Found: C, 63.02; H, 6.37; N, 7.06.

(1*S*,3*R*,4*R*,6*S*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-6-methyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (57). Prepared from nucleoside **53** (3.0 g, 5.2 mmol) using the general procedure described above to provide **57** (3.4 g, 83%). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 149.6, 149.4. HRMS (ESI-FT): calcd for $C_{41}H_{50}N_4O_9P$ 773.3310; found 773.3315. ESI-MS m/z: $[M + H]^+$ found 773.2. Anal. Calcd for $C_{41}H_{49}N_4O_9P$.0.5 H_2O: C, 62.99; H, 6.45; N, 7.17. Found: C, 62.77; H, 6.60; N, 7.06.

Acknowledgment. The authors thank Prof. Michael Jung for many useful suggestions.

Supporting Information Available: General experimental procedures for synthesis of **58**−**76**; ^{1}H and ^{13}C NMR spectra and analytical data for all new compounds; ^{31}P NMR spectra for all phosphoramidites; protocols for oligonucleotide synthesis and purification, thermal denaturation, and nuclease stability experiments; analytical data for oligonucleotides and X-ray crystal structure and CIF file for **49**. This material is available free of charge via the Internet at http://pubs.acs.org.