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Synthesis and Properties of the 5-Methyluridine Derivative of 3,4-Dihydro-2*H*-pyran-Bridged Nucleic Acid (DpNA)

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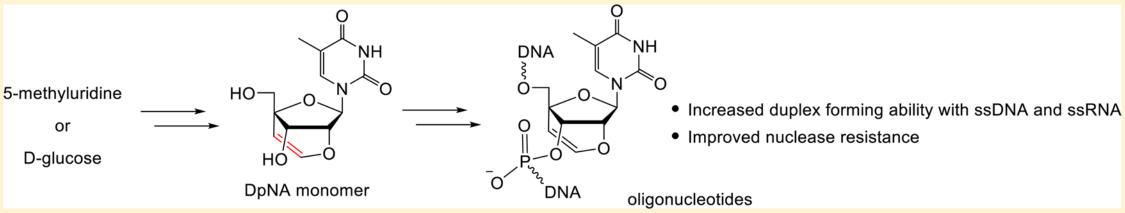
Supporting Information



ABSTRACT: A novel 2′-*O*,4′-*C*-bridged nucleic acid, 3,4-dihydro-2*H*-pyran bridge moiety (DpNA), with a dioxabicyclo[3.2.1]-oct-3-ene ring was designed. Construction of the dihydropyran bridge was achieved by dehydration of a six-membered hemiacetal ring, and the DpNA monomer was synthesized in 10 steps from 5-methyluridine (total yield 9%). The synthesized DpNA monomer was incorporated into oligonucleotides to examine the properties of the modified oligonucleotides. The DpNA-modified oligonucleotides possessed high affinity toward ssRNA and were more resistant to nucleases compared to the corresponding natural oligonucleotide.

INTRODUCTION

Chemically modified oligonucleotides can regulate gene expression, and artificial nucleic acids containing a modified furanose ring have potential as oligonucleotide therapeutics.[1] Among these nucleic acid analogs, the bridged nucleic acid 2′,4′-BNA/LNA (Figure 1A) with a methylene bridge between the 2′-oxygen and 4′-carbon atoms, developed simultaneously by two groups,[2,3] possesses excellent duplex-forming ability with ssRNA, high nuclease resistance, and has been used extensively for *in vivo* application.[1a−c,4]

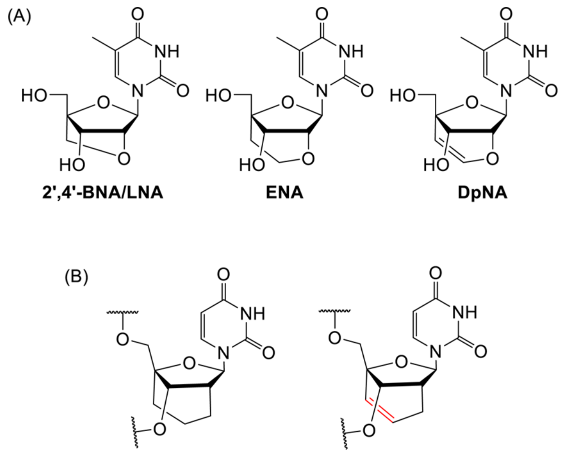


Figure 1. (A) 2′,4′-BNA analogs used in this study. (B) Carbocyclic 2′,4′-BNA analogs.

Therefore, many analogs of 2′,4′-BNA/LNA have been developed to improve the properties.[5] Results have indicated a relation exists between their properties and the size of their bridge structures. The 2′,4′-BNA/LNA with a five-membered bridge had excellent binding affinity for ssRNA and an improved resistance to nucleases. In contrast, a six-membered bridged nucleic acid analog, ENA[5e] (Figure 1A), was strongly nuclease resistant because of the steric hindrance resulting from the large bridge size. The binding affinity of ENA-modified oligonucleotides with ssRNA was greater than that of a natural oligonucleotide; however, it was slightly lower than those modified by 2′,4′-BNA/LNA, probably due to the greater flexibility of the larger six-membered bridge structure. This previous work suggested that replacement of the ethylene bride of ENA with an ethenylene bridge to produce a bridged nucleic acid including a 3,4-dihydro-2*H*-pyran structure (DpNA) (Figure 1A) could reduce the flexibility of the bridge structure while maintaining a large bridge size.

In contrast, Nielsen et al. reported that the binding affinity of 2′,4′-locked nucleic acid with a cyclohexene ring (Figure 1B) was slightly inferior to that of the cyclohexane ring analog, although both analogs contained a carbocyclic bridge structure without a 2′-oxygen atom,[5h] which was considered crucial for

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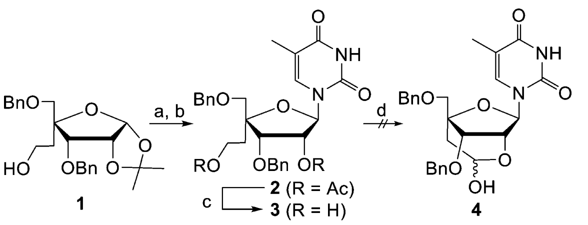
increasing the stability of duplexes with ssRNA. The nuclease resistance of oligonucleotides modified by these carbocyclic analogs was not investigated.

Therefore, the ability of DpNA-modified oligonucleotides to form a complex with ssRNA and the resulting nuclease resistance were of interest. In addition, construction of an enol ether in the bridge moiety of DpNA also is interesting in terms of the synthetic chemistry of nucleic acids. This report describes the synthesis of a 5-methyluridine derivative of DpNA via two different routes and the duplex-forming ability and nuclease resistance of the DpNA-modified oligonucleotides.

■ RESULTS AND DISCUSSION

Synthesis of DpNA. Initially, the synthesis of DpNA was investigated using the 4-*C*-(2-hydroxyethyl)-D-ribose derivative **1**, which was prepared in 11 steps from D-glucose according to a previously reported method.[5e] After deacetonidation and acetylation of compound **1**, treatment of the obtained crude product with silylated thymine, prepared *in situ* from thymine and *N*,*O*-bis(trimethylsilyl)acetamide (BSA), in the presence of TMSOTf, produced the desired 5-methyluridine analog **2** with a *β*-configuration (Scheme 1).[6] Diol **3** was obtained by deacetylation of **2** using aqueous NH_3. Next, to obtain hemiacetal **4**, oxidative cyclization of **3** using 2-iodoxybenzoic acid (IBX) or a combination of TEMPO and bis(acetoxy)-iodobenzene (BAIB) was attempted. However, complex mixtures were produced in all cases, with no compound **4** observed. These results imply that the conformation of the furanose ring of the oxidized compound is not appropriate for cyclization (i.e., hemiacetal formation). Replacement of benzyl protecting groups on the 3′- and 5′-oxygen atoms with cyclized disiloxane protecting groups was expected to restrict the conformation to an N-type that is suitable for intramolecular cyclization between the 2′- and 4′-positions (Figure 2).[7]

Scheme 1[a]



[a]Reagents and conditions: (a) H_2SO_4, Ac_2O, AcOH, rt, 0.5 h; (b) thymine, BSA, TMSOTf, MeCN, reflux, 2 h, reflux, 85% (two steps); (c) 28% NH_3 aq, MeOH, rt, 24 h, quant; (d) IBX, EtOAc, reflux, 2 h or TEMPO, BAIB, CH_2Cl_2, rt, 1 h.

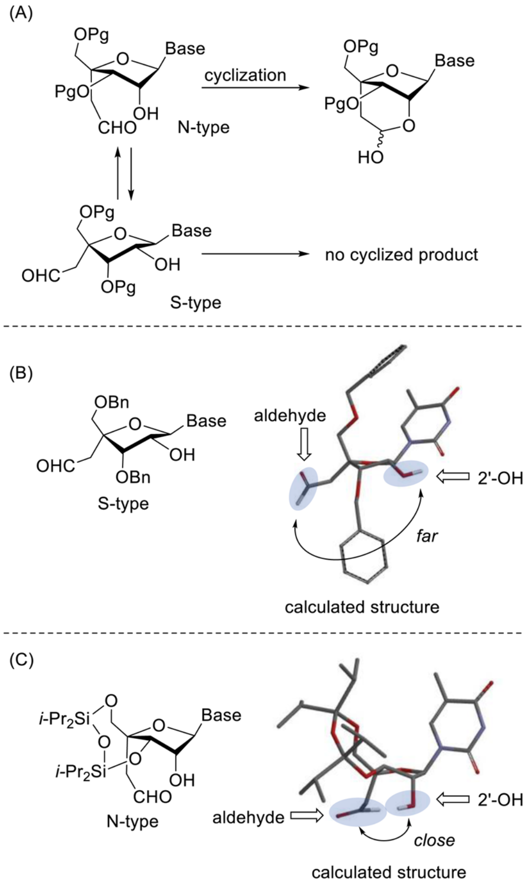
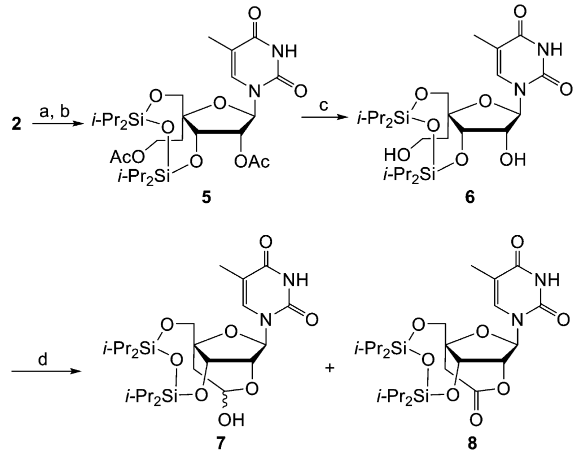


Figure 2. Low-energy conformations of nucleic acids. (A) Conformations of 2′-deoxyribonucleosides. (B) Optimized conformation of 3′,5′-dibenzylated ribonucleoside. (C) Optimized conformation of ribonucleoside cyclized by disiloxane-type protecting group (B3LYP/6-31G*, hydrogen atoms except for 2′-OH and aldehyde are omitted to simplify the optimized structure).

Hydrogenolysis of compound **2** followed by silylation using 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) led to **5**. On exposure to K_2CO_3 in MeOH, **5** was deacetylated to give the desired intermediate **6** (Scheme 2).

Scheme 2[a]



[a]Reagents and conditions: (a) 20 (w/w)% $Pd(OH)_2/C$, H_2, EtOAc, rt, 3 h; (b) $TIPDSCl_2$, imidazole, DMF, rt, 3 h, 68% (two steps); (c) K_2CO_3, MeOH, rt, 24 h, 64%; (d) see Table 1.

Oxidation of diol **6** also was examined (Table 1). As expected, a cyclized product formed in all cases. The PCC oxidation produced desired hemiacetal **7** in 38% yield, although lactone **8** was obtained as the more oxidized product in 29% yield. A reaction system using TEMPO and BAIB increased the yield from **7** to 68% (entry 2). In contrast, Dess−Martin periodinane (DMP) gave an undesirable lactone **8** in high yield (entry 3). The desirable compound **7** was isolated as a single product in the highest yield (77%) when oxidation was performed using IBX,[8] which is less reactive than DMP (entry 4).

Synthesis of DpNA monomer **10** was conducted in two steps from **7** (Scheme 3). Compound **7** was converted to 3,4-

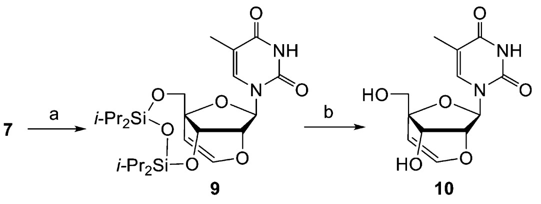
Table 1. Selective Oxidation of Diol 6

entry	reagents	solvent	**7**[e,f]	**8**[e]
1[a]	PCC	CH_2Cl_2	38%	29%
2[b]	TEMPO, BAIB	CH_2Cl_2	68%	10%
3[c]	DMP	CH_2Cl_2	0%	63%
4[d]	IBX	EtOAc	77%	0%

[a] PCC (5 equiv), CH_2Cl_2, rt, 16 h. [b] TEMPO (0.1 equiv), BAIB (1.1 equiv), CH_2Cl_2, rt, 1 h. [c] DMP (3 equiv), CH_2Cl_2, rt, 3 h. [d] IBX (1.2 equiv), EtOAc, reflux, 2 h. [e] Isolated yield. [f] Give two inseparable diastereomers (*dr* = 1:1).

dihydro-2*H*-pyran **9** in 53% yield by dehydration, followed by desilylation with 3HF-TEA, to yield the desired DpNA monomer **10**.

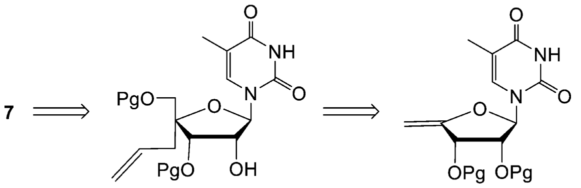
Scheme 3[a]



[a] Reagents and conditions: (a) MsCl, TEA, CH_2Cl_2, rt, 2 h, 53%; (b) 3HF-TEA, THF, rt, 1 h, 91%.

The synthetic efficacy of DpNA could be improved, however, because the synthesis of starting material **1** required 11 steps from D-glucose. Thus, retro-synthetic analysis of DpNA **10** was planned to synthesize intermediate **7** from a 4′-allyl-5-methyluridine analog (Scheme 4). The allyl derivative would be obtained from an *exo*-olefin compound by diastereoselective 4′-allylation of 4′,5′-epoxy nucleoside, which was reported by Haraguchi et al.[9]

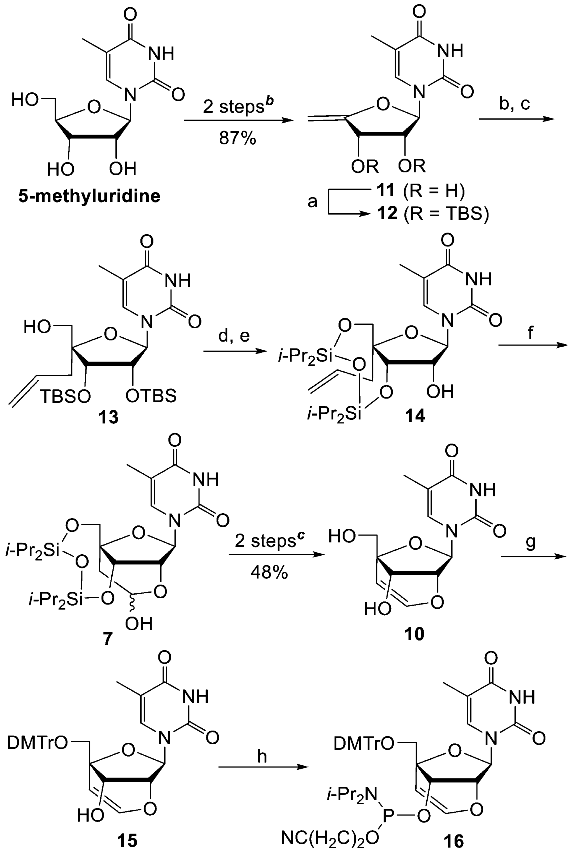
Scheme 4[a]



[a] Pg = protecting group.

The synthesis of DpNA monomer **10** was examined using known *exo*-olefin **11**,[5r] prepared from commercially available 5-methyluridine in 2 steps (Scheme 5). *Exo*-olefin **11** was converted to silyl-protected **12** using TBSCl. Epoxidation of compound **12** was performed using dimethyldioxirane generated *in situ* from acetone and oxone.[10] Then, an allyl group was incorporated at the 4′-position by reaction using $SnCl_4$ and allyltrimethylsilane, to give the desired 4′-C-allyl-5-methyluridine **13** as the sole product. This diastereoselectivity may result from selective β-face epoxidation, caused by masked α-face of the *exo*-olefin, and S_N2-like ring opening of the 4′,5′-epoxide. The 2′- and 3′-TBS groups were removed by TBAF in THF, followed by protection of the 3′- and 5′-hydroxyl groups by $TIPDSCl_2$ to give compound **14** in 63% yield in 2 steps. Finally, the desired intermediate **7** was obtained via oxidative cleavage of the terminal olefin of **14** by Lemieux–Johnson oxidation[11] using K_2OsO_4-$2H_2O$ and $NaIO_4$. The concise synthesis of DpNA monomer **10** from 5-methyluridine was achieved in 10 steps with a total yield of 9%.

Scheme 5[a]



[a] Reagents and conditions: (a) TBSCl, imidazole, DMF, rt, 12 h, 97%; (b) oxone, acetone, $NaHCO_3$, CH_2Cl_2/H_2O, rt, 2 h; (c) allyltrimethylsilane, $SnCl_4$, CH_2Cl_2, −78 °C to rt, 2 h, 63% (two steps); (d) TBAF, THF, rt, 10 h; (e) $TIPDSCl_2$, imidazole, DMF, rt, 10 h, 62% (two steps); (f) K_2OsO_4-$2H_2O$, dioxane/H_2O/pyridine, rt, 2 h, 69% (dr = 1:1). (g) DMTrCl, pyridine, rt, 2 h, quant. (h) i-$Pr_2NP(Cl)O(CH_2)_2CN$, DIPEA, CH_2Cl_2, rt, 2 h, 83%. [b] See ref 6r. [c] See Scheme 3.

Then, phosphoramidite **16** was prepared to obtain a suitable building block for oligonucleotide synthesis. The oligonucleotide synthesis was performed on an automated DNA synthesizer using common phosphoramidite chemistry with a prolonged coupling time (10 min) for the introduction of the DpNA monomer. However, when DpNA monomer **10** was treated with an oxidizing solution containing iodine (commonly used in oligonucleotide synthesis as an oxidant of trivalent phosphite), **10** was decomposed, probably due to reaction between its vinyl ether moiety and iodine. Therefore, 1 M *t*-BuOOH in toluene,[12] instead of an iodine solution, was used for the synthesis of DpNA-modified oligonucleotides. The desired oligonucleotides **18–21** and **29** were obtained successfully without detectable decomposition of DpNA.

Thermal Stability of Duplex Formed by DpNA-Modified Oligonucleotides. The duplex-forming ability of DpNA-modified oligonucleotides **18–21** with ssDNA and ssRNA were evaluated using UV-melting experiments and compared with those of corresponding natural counterparts **17** and ENA-modified oligonucleotides **22–25** (Table 2; see

Table 2. Duplex-Forming Ability of Modified Oligonucleotides 18−25 with ssDNA and ssRNA[a]

	ssDNA	ssRNA
oligonucleotides	T_m (°C)	T_m (°C)
5′-d(GCGTTTTTTGCT)-3′ (**17**)	51	47
5′-d(GCGTT$\underline{\text{T}}$TTTGCT)-3′ (**18**)	52 (+1.0)	51 (+4.0)
5′-d(GCG$\underline{\text{T}}$T$\underline{\text{T}}$T$\underline{\text{T}}$TGCT)-3′ (**19**)	52 (+0.3)	59 (+4.0)
5′-d(GCGT$\underline{\text{TTT}}$TTGCT)-3′ (**20**)	52 (+0.3)	60 (+4.3)
5′-d(GCG$\underline{\text{TTTTTT}}$GCT)-3′ (**21**)	58 (+1.1)	75 (+4.7)
5′-d(GCGTT*T*TTTGCT)-3′ (**22**)	51 (0.0)	52 (+5.0)
5′-d(GCG*T*T*T*T*T*TGCT)-3′ (**23**)	53 (+0.7)	65 (+6.0)
5′-d(GCGT*TTT*TTGCT)-3′ (**24**)	54 (+1.0)	61 (+4.7)
5′-d(GCG*TTTTTT*GCT)-3′ (**25**)	65 (+2.3)	80 (+5.5)

[a] Conditions: 10 mM sodium phosphate buffer (pH 7.2), 100 mM NaCl, and 4 μM of each oligonucleotide. $\underline{\text{T}}$ = DpNA-T. T = ENA-T. The sequences of ssDNA and ssRNA are 5′-d(AGCAAAAAACGC)-3′ and 5′-r(AGCAAAAAACGC)-3′, respectively.

Figure 1A for the structure of ENA). The duplex-forming ability of DpNA-modified oligonucleotides **18−21** showed the same tendency as that of ENA-modified oligonucleotides **22−25**;[5e] duplexes with ssDNA behaved similarly to that of natural oligonucleotide **17**, and duplexes with ssRNA were highly stabilized relative to that of **17**. In addition, DpNA stabilized the duplex with ssRNA by +4.0 to +4.7 °C per modification, which was slightly inferior to ENA (+4.7 to +6.0 °C per modification). For the carbacyclic analog, a cyclohexane-type nucleic acid (+3.5 to +4.5 °C per modification) slightly stabilized the duplex with ssRNA compared to the cyclohexene-type (+2.3 to +4.0 °C per modification) (see Figure 1B for structures).[5h] Furthermore, base-discriminating ability of DpNA was also evaluated using ssRNA including a mismatch site [5′-r(AGCAAAXAACGC)-3′(X = G, C, and U)]. Against ssRNA (X = C and U), the T_m values were 35 and 38 °C, respectively. The duplex with ssRNA (X = G) showed the relatively high T_m value of 46 °C because a T-G wobble base pair is metastable.[13] However, all T_m values of duplexes including single mismatch site were significantly decreased compared to that (51 °C) of full-match duplex. This suggests that DpNA-T should have sufficient ability to discriminate mismatch bases. The sequence-selective stabilization of the ssRNA duplex by DpNA modification could be sufficient for ssRNA targeting technologies.

Interestingly, retention times from reversed-phase HPLC analyses of DpNA-modified oligonucleotides were significantly longer than those of ENA-modified oligonucleotides. For example, **21** with six DpNA modifications had a retention time of 20.2 min [gradient: 7−13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min]. In contrast, the ENA congener **25**[5e] showed a faster retention time (17.8 min), though a less polar gradient system was used [gradient: 5−11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min]. These results indicate that DpNA-modified oligonucleotides were more hydrophobic than ENA-modified oligonucleotide, and this might be because the bridge moiety including planar sp^2 carbons of DpNA rather than that of ENA sticks out far into the minor groove of the duplex formed. As the result of such a structural feature, DpNA modification might disturb the hydrogen-bonding network[14] and could reduce the stability of the duplex with ssRNA compared to ENA modification.

Nuclease Resistance of DpNA. The enzymatic stability of DpNA-modified oligonucleotide **29** was evaluated using 3′-exonuclease (*Crotalus adamanteus* venom phosphodiesterase, CAVP) and compared to that of natural oligonucleotide **26**, the 2′,4′-BNA/LNA-modified oligonucleotide **27**, and ENA-modified oligonucleotide **28**. All of the oligonucleotides used in this study were 10-mers; those bearing modifications were modified singly at the second position from the 3′-end because phosphodiester bond is degraded from 3′-end by CAVP. A comparison of oligonucleotides **26−29** is shown in Figure 3.

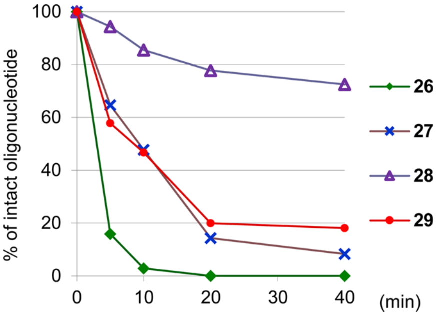


Figure 3. Nuclease degradation experimentsConditions: 0.50 μg/mL *Crotalus adamanteus* venom phosphodiesterase (CAVP), 10 mM $MgCl_2$, 50 mM Tris-HCl (pH 8.0), 7.5 μM each oligonucleotide at 37 °C. Sequence: 5′-TTTTTTTT$\underline{\text{T}}$T-3′ [$\underline{\text{T}}$= thymidine (**26**), 2′,4′-BNA/LNA-T (**27**), ENA-T (**28**), and DpNA-T (**29**)].

Although natural oligonucleotide **26** degraded rapidly, DpNA-modified oligonucleotide **29** showed high resistance against CAVP. However, the nuclease resistance of oligonucleotide **29** was unexpectedly inferior to that of oligonucleotide **28** containing ENA, but similar to that of oligonucleotide **27** containing 2′,4′-BNA/LNA. This result implies ready access of the nuclease to the 3′-phosphate moiety of DpNA compared with that of ENA because the dihydropyran bridge of DpNA adapted a planar structure and was located farther from the 3′-phosphate (Figure 4).

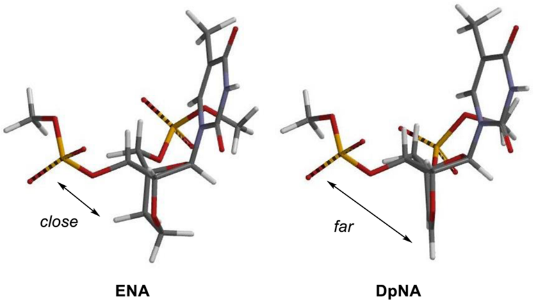


Figure 4. Optimized geometry of ENA and DpNA (B3LYP/6-31+G*).

CONCLUSIONS

The synthesis of a novel 3,4-dihydro-2*H*-pyran-bridged nucleic acid (DpNA) monomer was achieved in 19 steps from D-glucose or in 10 steps from 5-methyluridine. The latter route gave a reasonable total yield of 9%. The DpNA-modified oligonucleotides showed excellent binding affinity with ssRNA and high nuclease resistance compared to natural oligonucleotides, although these properties were inferior to those of the saturated analog, ENA. The enol ether moiety of DpNA is a reactive site, and further modification may enable the synthesis various substituted ENA analogs. Currently, chemical modification of the enol ether moiety is under investigation. Elaboration of the properties of various 2′,4′-BNAs, including the results presented here with DpNA, will continue to contribute to development of an ideal material for nucleic acid-based therapeutics and technology.

■ EXPERIMENTAL SECTION

General Methods. All moisture-sensitive reactions were conducted in well-dried glassware under an N_2 atmosphere. Anhydrous CH_2Cl_2, DMF, MeCN, EtOAc, and pyridine were used as purchased. ^{1}H NMR spectra were recorded at 300 and 400 MHz, ^{13}C HMR were recorded at 75 and 100 MHz, and ^{31}P spectra were recorded at 161 MHz. Chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard, and residual solvents for ^{1}H NMR, and $CHCl_3$ (δ = 77.00 ppm) and methanol (δ = 49.00 ppm) for ^{13}C NMR, and 5% H_3PO_4 (δ = 0 ppm) for ^{31}P NMR. Fast atom bombardment mass spectra (FAB-MS) were recorded in positive-ion mode. For column chromatography, silica gel PSQ 100B was used. The progress of the reaction was monitored by analytical thin-layer chromatography (TLC) on precoated aluminum sheets.

4′-*C*-Acetoxyethyl-2′-*O*-acetyl-3′,5′-di-*O*-benzyl-5-methyluridine (2). Under an N_2 atmosphere, Ac_2O (29 mL, 309 mmol) and H_2SO_4 (1% in AcOH, 8.2 mL, 1.55 mmol) were added to a solution of compound **1**[5e] (12.8 g, 30.9 mmol) in AcOH (60 mL) at 0 °C. The reaction mixture was stirred at room temperature for 0.5 h. The reaction was quenched with sat. $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (15.8 g) obtained was dissolved in anhydrous MeCN (80 mL) under N_2, followed by addition of thymine (3.90 g, 30.9 mmol) and BSA (23 mL, 92.7 mmol) at room temperature. The reaction mixture was refluxed for 1.5 h, then TMSOTf (1.7 mL, 9.3 mmol) was added to the resulting mixture at 0 °C. This reaction mixture was refluxed for 2.5 h. Reaction was then quenched with sat. $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (20.0 g) was purified by column chromatography (silica gel 200 g, *n*-hexane:EtOAc = 1:1) to give compound **2** as a white foam (14.8 g, 85%, 2 steps from **1**). Mp: 37–40 °C. $[\alpha]_D^{23}$ +22.0 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3185, 3063, 3033, 2929, 2871, 1739, 1693, 1496, 1455, 1370, 1233 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.52 (d, *J* = 1.5 Hz, 3H), 1.81–1.91 (m, 1H), 2.02 (s, 3H), 2.11 (s, 3H), 2.18–2.27 (m, 1H), 3.37 (d, *J* = 10.5 Hz, 1H), 3.73 (d, *J* = 10.5 Hz, 1H), 4.17–4.23 (m, 2H), 4.39 (d, *J* = 6.0 Hz, 1H), 4.45 (d, *J* = 11.5 Hz, 2H), 4.51 (d, *J* = 11.5 Hz, 1H), 4.63 (d, *J* = 11.5 Hz, 1H), 5.39 (dd, *J* = 5.0, 6.0 Hz, 1H), 6.19 (d, *J* = 5.0 Hz, 1H), 7.28–7.39 (m, 10H), 7.44, (d, *J* = 1.5 Hz, 1H), 7.93 (brs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.0, 20.8, 21.0, 31.0, 60.0, 72.4, 73.6, 74.3, 74.9, 86.4, 86.5, 111.4, 127.7, 127.9, 128.1, 128.2, 128.5, 128.7, 135.5, 137.0, 137.3, 150.2, 163.5, 170.0, 170.8. MS (FAB): *m*/*z* = 567 [MH^{+}]. HRMS (FAB): calcd for $C_{30}H_{35}N_2O_9$ [MH^{+}] 567.2343, found 567.2355.

4′-*C*-Hydroxyethyl-3′,5′-di-*O*-benzyl-5-methyluridine (3). A 28% aq. NH_3 solution (2.0 mL) was added to **2** (400 mg, 0.706 mmol) in MeOH (10 mL) at room temperature. The reaction mixture was stirred at room temperature for 24 h and then concentrated *in vacuo*. The crude residue (428 mg) was purified by column chromatography (silica gel 250 g, $CHCl_3$:MeOH = 20:1 to 10:1) to give compound **3** as a white foam 349 mg, quant.). Mp: 80–85 °C. $[\alpha]_D^{24}$ −16.9 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3392, 3192, 3063, 3022, 2924, 1693, 1473, 1364, 1270 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ 1.46 (s, 3H), 1.80–1.89 (m, 1H), 2.13–2.22 (m, 1H), 3.52 (d, *J* = 10.0 Hz, 1H), 3.67–3.74 (m, 3H), 4.17 (d, *J* = 6.0 Hz, 1H), 4.45 (t, *J* = 6.0 Hz, 1H), 4.50–4.64 (m, 4H), 5.98 (d, *J* = 6.0 Hz, 1H), 7.27–7.43 (m, 10H), 7.60, (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 12.2, 36.7, 58.5, 74.5, 75.0, 75.0, 75.9, 80.4, 89.3, 111.6, 128.9, 129.0, 129.2, 129.3, 129.4, 129.6, 137.9, 139.3, 139.5, 152.7, 166.3. MS (MALDI): calcd for $C_{26}H_{30}N_2NaO_7$ [MNa^{+}] 505.1951, found 505.1964.

4′-*C*-Acetoxyethyl-2′-*O*-acetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-5-methyluridine (5). Compound **2** (14.0 g, 24.7 mmol) in EtOAc (50 mL) was added to a suspension of 20 w/w% $Pd(OH)_2$ on carbon (8.68 g, 12.4 mmol) in EtOAc (50 mL) at room temperature under N_2. The reaction mixture was stirred at room temperature for 3 h under H_2. The resulting mixture was filtered, and the filtrate concentrated *in vacuo*. The crude residue (10.1 g) was dissolved in anhydrous DMF (100 mL) under N_2, followed by addition of imidazole (8.41 g, 124 mmol) and $TIPDSCl_2$ (7.9 mL, 25 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The reaction then was quenched with sat. $NaHCO_3$ and extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (17.1 g) was purified by column chromatography (silica gel 300 g, *n*-hexane:EtOAc = 2:1) to give compound **5** as a white foam (10.6 g, 68%, 2 steps from **2**). Mp: 61–63 °C. $[\alpha]_D^{23}$ −39.3 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3192, 3038, 2946, 2893, 2869, 1721, 1694, 1465, 1370, 1234 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95–1.12 (m, 28H), 1.92 (d, *J* = 1.0 Hz, 3H), 1.81–1.91 (m, 8H), 3.82 (d, *J* = 12.0 Hz, 1H), 3.92 (d, *J* = 12.0 Hz, 1H), 4.17–4.38 (m, 2H), 4.73 (d, *J* = 7.0 Hz, 1H), 5.56 (dd, *J* = 1.5, 7.0 Hz, 1H), 5.63 (d, *J* = 1.5 Hz, 1H), 7.10, (d, *J* = 1.0 Hz, 1H), 7.99 (brs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.4, 12.5, 12.7, 12.9, 13.1, 16.9, 17.0, 17.0, 17.1, 17.3, 17.3, 20.7, 21.1, 28.3, 59.9, 65.7, 73.1, 75.5, 86.1, 90.1, 111.2, 136.8, 149.7, 163.7, 169.2, 171.0. MS (FAB): *m*/*z* = 629 [MH^{+}]. HRMS (FAB): calcd for $C_{28}H_{49}N_2O_{10}Si_2$ [MH^{+}] 629.2926, found 629.2942.

4′-*C*-(1-Hydroxyethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-5-methyluridine (6). K_2CO_3 (1.14 g, 8.25 mmol) was added to a solution of **5** (10.4 g, 16.5 mmol) in anhydrous MeOH (80 mL) at room temperature. The reaction mixture was stirred at room temperature for 24 h. Silica gel (50 g) was added to the reaction mixture, and this suspension concentrated *in vacuo*. Then the crude residue was purified by column chromatography (silica gel 250 g, $CHCl_3$:MeOH = 20:1 to 7:1) to give compound **6** as a white foam 5.76 g, 64%). Mp: 82–85 °C. $[\alpha]_D^{23}$ −36.1 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3450, 3191, 3063, 2946, 2895, 2868, 1693, 1466, 1388, 1270 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ 1.05–1.15 (m, 28H), 1.86 (d, *J* = 1.0 Hz, 3H), 2.03 (dt, *J* = 7.5, 15.0 Hz, 1H), 2.26 (dt, *J* = 7.5, 15.0 Hz, 1H), 3.75 (t, *J* = 7.5 Hz, 2H), 3.82 (d, *J* = 12.0 Hz, 1H), 3.97 (d, *J* = 12.0 Hz, 1H), 4.32 (dd, *J* = 1.5, 6.5 Hz, 1H), 4.67 (d, *J* = 6.5 Hz, 1H), 5.61 (d, *J* = 1.5 Hz, 1H), 7.49, (d, *J* = 1.0 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 12.4, 13.8, 13.9, 14.2, 14.5, 17.6, 17.7, 17.8, 17.8, 17.9, 18.0, 34.0, 58.3, 67.4, 74.9, 76.5, 87.7, 90.1, 111.2, 136.8, 149.7, 163.7, 169.2, 171.0. MS (FAB): *m*/*z* = 545 [MH^{+}]. HRMS (FAB): calcd for $C_{24}H_{45}N_2O_8Si_2$ [MH^{+}] 545.2714, found 545.2709.

Synthesis of 2′-*O*,4′-*C*-(1-Hydroxyethylene)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-5-methyluridine (7) and 2′-*O*,4′-*C*-(1-Oxoethylene)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-5-methyluridine (8). *PCC Oxidation.* PCC (148 mg, 0.918 mmol) was added to a solution of compound **6** (100 mg, 0.184 mmol) in CH_2Cl_2 (2.0 mL) at room temperature and stirred for 16 h, followed by concentration *in vacuo*. The crude residue (320 mg) was purified by column chromatography (silica gel 3.0 g, *n*-hexane:EtOAc = 2:1) to give compound **7** (38.0 mg, 38%) and compound **8** as white foams (29.1 mg, 29%).

TEMPO Oxidation. Under N_2, BAIB (65.5 mg, 0.203 mmol) and TEMPO (2.9 mg, 0.0184 mmol) were added to a solution of compound **6** (100 mg, 0.184 mmol) in anhydrous CH_2Cl_2 (2.0 mL) at room temperature and stirred for 1 h. The resulting solution was concentrated *in vacuo*, and the crude residue (140 mg) purified by column chromatography (silica gel 3.0 g, *n*-hexane:EtOAc = 2:1) to give compound **7** (68.3 mg, 68%) and compound **8** (10.1 mg, 10%) as white foams.

DMP Oxidation. DMP (234 mg, 0.551 mmol) was added to a solution of compound **6** (100 mg, 0.184 mmol) in CH_2Cl_2 (2.0 mL) at 0 °C. Then, the reaction mixture was stirred at room temperature for 3 h, followed by quenching with sat. $NaHCO_3$/sat. $Na_2S_2O_3$ (1:1) solution, and extraction with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (131 mg) was purified by column chromatography (silica gel 3.0 g, *n*-hexane:EtOAc = 2:1) to give compound **8** as a white foam (62.8 mg, 63%).

IBX Oxidation. Under N_2, IBX (395 mg, 1.41 mmol) was added to a solution of compound **6** (640 mg, 1.17 mmol) in anhydrous EtOAc (10 mL) at room temperature. Then, the reaction mixture was refluxed for 2 h. The resulting mixture was filtered, and the filtrate was concentrated *in vacuo*. The crude residue (828 mg) was purified by column chromatography (silica gel 15 g, *n*-hexane:EtOAc = 1:1) to give compound **7** as a white foam (494 mg, 77%).

Compound 7. Mp: 102−103 °C. IR ν_{max} (KBr): 3384, 3209, 3071, 2946, 2869, 1693, 1466, 1387, 1273 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98−1.12 (m, 28H), 1.78−2.06 (m, 4.5H), 2.16 (dd, *J* = 6.0, 14.0 Hz, 0.5H), 3.61 (d, *J* = 13.0 Hz, 0.5H), 3.66 (d, *J* = 13.0 Hz, 0.5H), 4.01−4.05 (m, 1.5H), 4.13 (d, *J* = 13.0 Hz, 0.5H), 4.35 (d, *J* = 3.0 Hz, 0.5H), 4.41 (d, *J* = 3.0 Hz, 0.5H), 4.88 (d, *J* = 6.0 Hz, 0.5H), 5.08 (brs, 0.5H), 5.44−5.52 (m, 1H), 5.91 (s, 0.5H), 6.39 (s, 0.5H), 7.74 (d, *J* = 1.0 Hz, 0.5H), 7.80 (d, *J* = 1.0 Hz, 0.5H), 9.76 (brs, 0.5H), 9.88 (brs, 0.5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.1, 12.8, 12.9, 13.0, 13.3, 13.4, 16.8, 16.9, 16.9, 17.0, 17.0, 17.1, 17.1, 17.2, 17.2, 17.5, 32.4, 34.9, 61.7, 62.3, 63.7, 64.6, 77.5, 78.3, 83.5, 85.4, 85.9, 87.1, 90.7, 91.4, 109.8, 110.0, 134.8, 135.2, 150.0, 150.4, 164.5. HRMS (MALDI): calcd for $C_{24}H_{42}N_2NaO_8Si_2$ [MNa^+] 565.2377, found 565.2372.

Compound 8. Mp: 149−151 °C. $[\alpha]_D^{24}$ +1.5 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3176, 3032, 2946, 2896, 2869, 1760, 1692, 1465, 1410, 1386, 1368, 1326, 1276 1248, 1231 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03−1.12 (m, 28H), 1.93 (s, 3H), 2.77 (s, 2H), 3.69 (d, *J* = 10.0 Hz, 1H), 4.14 (d, *J* = 10.0 Hz, 1H), 4.37 (d, *J* = 3.5 Hz, 1H), 4.81 (d, *J* = 3.5 Hz, 1H), 5.87 (s, 1H), 7.65 (s, 1H), 9.31 (brs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.3, 12.8, 13.3, 16.8, 16.9, 17.0, 17.1, 17.1, 17.3, 17.4, 37.3, 61.4, 64.7, 80.4, 84.2, 87.8, 110.7, 134.2, 149.8, 163.9, 166.3. HRMS (MALDI): calcd for $C_{24}H_{40}N_2NaO_8Si_2$ [MNa^+] 563.2221, found 563.2215.

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*,4′-*C*-ethynylene-5-methyluridine (9). Under N_2, Et_3N (4.8 mL, 34 mmol) and MsCl (0.58 mL, 7.5 mmol) were added to a solution of compound **7** (3.72 g, 6.85 mmol) in anhydrous CH_2Cl_2 (30 mL) at 0 °C. This reaction mixture was stirred at room temperature for 2 h. The reaction then was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (3.01 g) was purified by column chromatography (silica gel 100 g, *n*-hexane:EtOAc = 3:1 to 2:1) to give compound **9** as a white foam (1.91 g, 53%). Mp: 78−80 °C. $[\alpha]_D^{23}$ +11.9 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3183, 3070, 2945, 2895, 2868, 1694, 1634, 1466, 1389, 1272, 1231, 1217 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00−1.13 (m, 28H), 1.91 (d, *J* = 1.0 Hz, 3H), 3.73 (d, *J* = 13.5 Hz, 1H), 4.04 (d, *J* = 13.5 Hz, 1H), 4.21 (dd, *J* = 1.5, 3.5 Hz, 1H), 4.39 (d, *J* = 3.5 Hz, 1H), 4.68 (dd, *J* = 1.5, 5.5 Hz, 1H), 5.86 (s, 1H), 6.49 (d, *J* = 5.5 Hz, 1H), 7.56, (d, *J* = 1.0 Hz, 1H), 9.21 (brs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.4, 12.5, 12.6, 12.9, 13.5, 16.8, 16.9, 17.0, 17.1, 17.2, 17.3, 17.4, 17.4, 60.2, 61.9, 79.1, 79.3, 88.5, 100.2, 110.5, 134.6, 145.4, 149.8, 163.9. HRMS (MALDI): calcd for $C_{24}H_{40}N_2NaO_7Si_2$ [MNa^+] 547.2272, found 547.2266.

2′-*O*,4′-*C*-Ethynylene-5-methyluridine (10). The 3HF-Et_3N (0.59 mL, 3.6 mmol) was added to a solution of compound **9** (1.91 g, 3.64 mmol) in THF (50 mL) at room temperature and stirred for 2 h. The resulting mixture was concentrated *in vacuo*. The crude residue (2.60 g) was purified by column chromatography (silica gel 60 g, $CHCl_3$:MeOH = 20:1 to 10:1) to give compound **10** as a white foam (935 mg, 91%). Mp: 190−191 °C. $[\alpha]_D^{23}$ +73.7 (c 1.00, CH_3OH). IR ν_{max} (KBr): 3343, 3072, 2941, 2828, 1695, 1471, 1390, 1272, 1213 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ 1.87 (s, 3H), 3.67 (d, *J* = 12.5 Hz, 1H), 3.75 (d, *J* = 12.5 Hz, 1H), 4.21 (dd, *J* = 1.5, 3.5 Hz, 1H), 4.39 (d, *J* = 3.5 Hz, 1H), 4.83 (dd, *J* = 1.5, 5.5 Hz, 1H), 5.84 (s, 1H), 6.49, (d, *J* = 5.5 Hz, 1H), 7.78 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 12.6, 61.3, 62.9, 80.3, 80.8, 89.8, 102.4, 110.9, 137.4, 146.8, 151.9, 166.5. HRMS (MALDI): calcd for $C_{12}H_{14}N_2NaO_6$ [MNa^+] 305.0750, found 305.0734.

2′,3′-*O*-Di-*tert*-butyldimethylsilyl-4′,5′-dehydro-5′-deoxy-5-methyluridine (12). Under N_2, imidazole (1.75 g, 25.6 mmol) and TBSCl (1.93 g, 12.8 mmol) were added to a solution of compound **11**[5r] (12.8 g, 30.9 mmol) in anhydrous DMF (50 mL) at 0 °C. Then, the reaction mixture was stirred at room temperature for 12 h. The reaction was quenched with sat. $NaHCO_3$ and extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (20.0 g) was purified by column chromatography (silica gel 100 g, *n*-hexane:EtOAc = 5:1 to 3:1) to give compound **12** as a white foam (2.65 g, 97%). Mp: 54−58 °C. $[\alpha]_D^{23}$ −16.9 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3190, 3055, 2954, 2930, 2887, 2858, 1693, 1471, 1389, 1362, 1256 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.02 (s, 3H), 0.05 (s, 3H), 0.12 (s, 3H), 0.13 (s, 3H), 0.87 (s, 9H), 0.92 (s, 9H), 1.95 (d, *J* = 1.0 Hz, 3H), 4.23 (dd, *J* = 4.0, 5.5 Hz, 1H), 4.25 (d, *J* = 2.5 Hz, 1H), 4.36 (d, *J* = 4.0 Hz, 1H), 4.53 (d, *J* = 2.5 Hz, 1H), 6.09 (d, *J* = 5.5 Hz, 1H), 6.98 (d, *J* = 1.0 Hz, 1H), 8.88 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.0, −4.7, −4.5, −4.5, 12.4, 17.9, 18.1, 25.6, 25.7, 71.9, 74.9, 86.8, 89.5, 111.6, 135.0, 150.2, 160.5, 163.7. HRMS (MALDI): calcd for $C_{22}H_{40}N_2NaO_5Si_2$ [MNa^+] 491.2373, found 491.2368.

4′-*C*-Allyl-2′,3′-*O*-di-*tert*-butyldimethylsilyl-5-methyluridine (13). Acetone (20 mL) and sat. $NaHCO_3$ (100 mL) were added to a solution of compound **12** (2.50 g, 5.33 mmol) in CH_2Cl_2 (30 mL) at room temperature. Then, oxone (6.56 g, 10.7 mmol) in water (100 mL) was added at 0 °C over 15 min, followed by stirring at room temperature for 2 h. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (2.63 g) was dissolved in anhydrous CH_2Cl_2 (50 mL) under N_2, then allyltrimethylsilane (1.5 mL, 11 mmol) and $SnCl_4$ (0.62 mL, 5.3 mmol) were added at −78 °C. The reaction mixture was stirred at room temperature for 2 h and then was quenched with sat. $NaHCO_3$. This mixture was filtered using Celite, and this filtrate was extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The obtained crude residue (3.88 g) was purified by column chromatography (silica gel 100 g, *n*-hexane:EtOAc = 2:1) to give compound **13** as a white foam (1.78 g, 63%, 2 steps from **12**). Mp: 81−83 °C. $[\alpha]_D^{24}$ −10.7 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3466, 3412, 3184, 3075, 2930, 2898, 2858, 1696, 1471, 1412, 1390, 1362, 1313, 1256 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.01 (s, 3H), 0.06 (s, 3H), 0.12 (s, 3H), 0.12 (s, 3H), 1.91 (d, *J* = 1.5 Hz, 1H), 2.12 (dd, *J* = 8.5, 14.5 Hz, 1H), 2.82 (d, *J* = 6.0, 14.5 Hz, 1H), 3.09 (dd, *J* = 3.0, 8.0 Hz, 1H), 3.46 (dd, *J* = 8.0, 12.5 Hz, 1H), 3.80 (dd, *J* = 3.0, 12.5 Hz, 1H), 4.38 (d, *J* = 5.5 Hz, 1H), 4.61 (dd, *J* = 5.0, 5.5 Hz, 1H), 5.04−5.08 (m, 2H), 5.46, (d, *J* = 5.0 Hz, 1H), 5.82−5.93 (m, 1H), 7.32 (d, *J* = 1.5 Hz, 1H), 9.21 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.6, −4.4, −4.0, 12.3, 17.9, 18.1, 25.9, 26.0, 37.2, 64.9, 72.1, 74.4, 88.0, 94.2, 110.7, 117.8, 133.5, 139.2, 150.4, 163.89. HRMS (MALDI): calcd for $C_{25}H_{46}N_2NaO_6Si_2$ [MNa^+] 549.2792, found 549.2787.

4′-*C*-Allyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-5-methyluridine (14). TBAF (1 M in THF, 5.2 mL, 5.2 mmol) was added to a solution of compound **13** (1.35 g, 2.56 mmol) in THF (15 mL) at room temperature and stirred for 6 h. The resulting mixture was concentrated *in vacuo*. The crude residue (1.55 g) was purified by column chromatography (silica gel 20 g, $CHCl_3$:MeOH = 30:1 to 10:1) to give a crude triol (798 mg). The obtained triol was dissolved in anhydrous DMF (15 mL) under N_2, and then imidazole (523 mg, 7.68 mmol) and $TIPDSCl_2$ (0.78 mL, 2.4 mmol) were added at 0 °C. The reaction mixture was stirred at room temperature for 10 h and then quenched with sat. $NaHCO_3$ and extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (1.45 g) was purified by column chromatography (silica gel 25 g, *n*-hexane:EtOAc = 5:1 to 2:1) to give compound **14** as a white foam (859 mg, 62%, 2 steps from **13**). Mp: 58−60 °C. $[\alpha]_D^{24}$ −42.8 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3434, 3204, 3075, 2946, 2868, 1699, 1466, 1388, 1257 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99−1.18 (m, 28H), 1.91 (d, *J* = 1.5 Hz, 1H), 2.51 (dd, *J* = 8.5, 14.5 Hz, 1H), 2.62 (d, *J* = 7.0, 14.5 Hz, 1H), 3.24 (d, *J* = 1.0 Hz, 1H), 3.80 (d, *J* = 12.0 Hz, 1H), 3.83 (d, *J* = 12.0 Hz, 1H), 4.38 (dt, *J* = 1.0, 7.0 Hz, 1H), 4.73 (d, *J* = 7.0 Hz, 1H), 5.13−5.18 (m, 2H), 5.55, (d, *J* = 1.0 Hz, 1H), 5.82−5.93 (m, 1H), 7.17 (d, *J* = 1.5 Hz, 1H), 8.68 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.3, 12.4, 12.6, 12.7, 13.2, 17.0, 17.1, 17.2, 17.3, 17.3, 17.4, 34.9, 66.0, 73.4, 75.3, 86.8, 94.0, 110.5, 118.5, 132.4, 138.2, 149.8, 164.0. HRMS (MALDI): calcd for $C_{25}H_{44}N_2NaO_7Si_2$ [MNa^+] 563.2585, found 563.2579.

2′-*O*,4′-*C*-(1-Hydroxyethylene)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-5-methyluridine (7). $NaIO_4$ (1.02 g, 4.77 mmol) and K_2OsO_4-$2H_2O$ (58.6 mg, 0.159 mmol) were added to a solution of compound **14** (859 mg, 1.59 mmol) in dioxane/H_2O/pyridine (8:4:1, 13 mL) at room temperature and stirred for 2 h. The reaction then was quenched with sat. $Na_2S_2O_3$ and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (920 mg) was purified by column chromatography (silica gel 20 g, *n*-hexane:EtOAc = 1:1 to 1:2) to give compound **7** as a white foam (595 mg, 69%).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,4′-*C*-ethynylene-5-methyluridine (15). Under N_2, DMTrCl (178 mg, 0.525 mmol) was added to a solution of compound **10** (114 mg, 0.404 mmol) in anhydrous pyridine (5.0 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The reaction then was quenched with sat. $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (325 mg) was purified by column chromatography (silica gel 10 g, $CHCl_3$:MeOH = 30:1 to 15:1) to give compound **15** as a white foam (237 mg, quant.). Mp: 103−106 °C. $[\alpha]_D^{23}$ +7.0 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3325, 3179, 3071, 3006, 2931, 2836, 1693, 1607, 1509, 1464, 1301, 1252, 1217 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.57 (d, J = 1.0 Hz, 3H), 3.01 (d, J = 7.5 Hz, 1H), 3.32 (d, J = 11.0 Hz, 1H), 3.45 (d, J = 11.0 Hz, 1H), 3.78 (s, 3H), 3.78 (s, 3H), 4.47 (d, J = 3.5 Hz, 1H), 4.51−4.54 (m, 1H), 4.77 (dd, J = 1.5, 6.0 Hz, 1H), 5.94 (s, 1H), 6.49, (d, J = 6.0 Hz, 1H), 6.82−7.47 (m, 13H), 7.71 (d, J = 1.0 Hz, 1H), 9.56 (brs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.3, 55.2, 61.2, 62.7, 78.6, 79.4, 86.5, 88.4, 101.7, 110.7, 113.2, 113.2, 123.8, 127.0, 128.0, 130.0, 134.8, 135.2, 136.3, 144.4, 145.6, 149.4, 150.0, 158.5, 164.0. HRMS (MALDI): calcd for $C_{33}H_{32}N_2NaO_8$ [MNa^+] 607.2051, found 607.2054.

3′-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*,4′-*C*-ethynylene-5-methyluridine (16). Under N_2, DIPEA (0.21 mL, 1.18 mmol) and *i*-$Pr_2NP(Cl)O(CH_2)_2CN$ (0.11 mL, 0.472 mmol) were added to a solution of compound **15** (230 mg, 0.393 mmol) in anhydrous CH_2Cl_2 (3.0 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The reaction then was quenched with sat. $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with sat. $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue (312 mg) was purified by column chromatography (silica gel 10 g, *n*-hexane:EtOAc = 1:1 to 1:2) to give compound **16** as a white foam (256 mg, 83%). Mp: 96−100 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.99 (d, J = 6.5 Hz, 2.4H), 1.10−1.18 (m, 9.6H), 1.47 (s, 1.8H), 1.49 (s, 1.2H), 2.35 (t, J = 6.0 Hz, 1.2H), 2.60 (t, J = 6.0 Hz, 0.8H), 3.22−3.26 (m, 1H), 3.46−3.80 (m, 11H), 4.51−4.55 (m, 1H), 4.60−4.66 (m, 1H), 4.70−4.76 (m, 1H), 5.96 (s, 0.4H), 5.97 (s, 0.6H), 6.39−6.42 (m, 1H), 6.81−7.47 (m, 13H), 7.72 (s, 0.4H), 7.76 (s, 0.6H), 8.47 (brs, 0.4H), 8.53 (brs, 0.6H). ^{31}P NMR (161 MHz, $CHCl_3$) δ: 149.0, 149.7. MS (FAB): m/z = 785 [MH^+]. HRMS (FAB): calcd for $C_{42}H_{50}N_4O_9P$ [MH^+], 785.3315, found, 785.3314.

Oligonucleotide Synthesis. Phosphoramidite **16** and ENA phosphramidite[5e] were used for the 0.2 μmol scale synthesis of oligonucleotides on an automated DNA synthesizer using a standard phosphoramidite protocol (DMTr-ON mode). A solution of 1 M *t*-BuOOH in toluene was used as an oxidant of trivalent phosphite.[10] Oligonucleotides **18**−**25** and **29** were prepared by cleavage from CPG supports and deprotection of the nucleobase and phosphate moieties [28% NH_3 aq, rt, 1.5 h (for **29**); 28% NH aq, rt, 1.5 h, then 55 °C, 12 h (for **18**−**25**)]. Removal of ammonia was performed *in vacuo*. Crude compounds **18**−**25** and **29** were purified with Sep-Pak Plus C18 cartridges, followed by reversed-phase HPLC. The compositions of **18**−**25** and **29** were confirmed by MALDI-TOF mass analysis. MALDI-TOF MS data ([M − H]$^-$) for **18**−**25**, **29**: **18**, found 3672.43 (calcd 3672.38); **19**, found 3752.78 (calcd 3752.42); **20**, found 3752.13 (calcd 3752.42); **21**, found 3871.78 (calcd 3872.49); **22**, found 3674.97 (calcd 3674.42); **23**, found 3758.28 (calcd 3758.50); **24**, found 3757.82 (calcd 3758.40); **25**, found 3884.18 (calcd 3884.61); **29**, found 3017.92 (calcd 3018.98).

UV-Melting Experiments. UV-melting experiments were conducted using UV spectrophotometers equipped with a T_m analysis accessory. Oligonucleotides and ssDNA or ssRNA were dissolved in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl to give a final concentration of each strand of 4 μM. The samples were annealed by heating at 100 °C followed by slow cooling to 15 °C. The melting profiles were recorded at 260 nm from 15 to 95 °C at a scan rate of 0.5 °C/min. The two-point average method was employed to obtain the T_m values, and the final values were determined by averaging three independent measurements accurate to within 1 °C.

Enzymatic Degradation Experiments. Enzymatic degradation experiments were conducted using 0.50 μg/mL *Crotalus adamanteus* venom phosphodiesterase (CAVP), 10 mM $MgCl_2$, 50 mM Tris-HCl (pH 8.0), and 7.5 μM each oligonucleotide **26**−**29** at 37 °C. Then, the cleavage reaction was carried out at 37 °C. A portion of each reaction mixture was removed at timed intervals and heated to 90 °C for 2 min to deactivate the phosphodiesterase. Aliquots of the timed samples were analyzed by reversed-phase HPLC under the same gradient with the HPLC analysis of synthesized oligonucleotide **29** [gradient: 7−13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min; flow rate: 1.0 mL/min; column temp.: 50 °C] to evaluate the amount of intact oligonucleotide remaining. The percentage of intact oligonucleotide in each sample was calculated and plotted against the digestion time to obtain degradation curve in time.

Quantum Mechanical Calculations. *Ab initio* quantum mechanical calculations were performed using the Spartan program. Full geometry optimizations (B3LYP/6-31G*) were conducted for compounds **4** and **7** shown in Figure 2. To simplify the calculations, nucleoside 3′,5′-bis(methylphosphate)s of ENA and DpNA shown in Figure 4 were used. Full geometry optimizations (B3LYP/6-31+G*) were conducted.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01425.

> ^1H and ^{13}C NMR spectra of all new compounds (**2**, **3**, **5**−**10**, **12**−**16**), ^1H and ^{31}P NMR spectrum of **16**, HPLC charts, MALDI-TOF mass data of oligonucleotides **18**−**25**, **29**, and *ab initio* quantum mechanical calculation data for Figures 2 and 3 (PDF)

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Notes

The authors declare no competing financial interest.

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