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Synthesis of the Methyl Analog of 2′-*O*,4′-*C*-Ethylene-Bridged 5-Methyluridine via Intramolecular Radical Cyclization and Properties of the Modified Oligonucleotides

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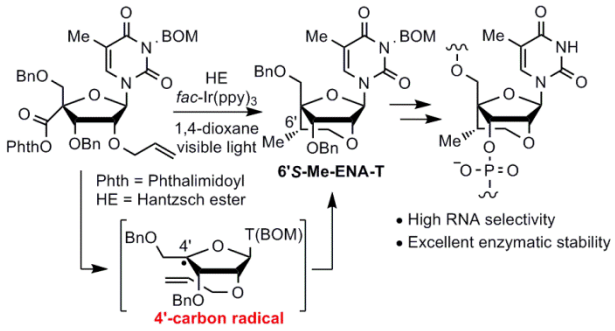


ABSTRACT: The synthesis of 6′*S*-Me-2′-*O*,4′-*C*-ethylene-bridged 5-methyluridine (6′*S*-Me-ENA-T) was achieved using visible light-mediated stereoselective radical cyclization as a key step. This is the first example of a method for constructing a 2′,4′-bridged structure from a 4′-carbon radical intermediate. The 6′*S*-Me-ENA-T monomer was successfully incorporated into oligonucleotides and their properties were examined. The oligonucleotides containing 6′*S*-Me-ENA-T exhibited a highly selective hybridization affinity toward single-stranded RNA and an excellent enzymatic stability,

compared to the corresponding LNA- and ENA-modified oligonucleotides.

INTRODUCTION

Oligonucleotides have recently received much attention as the next-generation drugs with great potential to treat various diseases such a as spinal muscular atrophy,[1] cancer,[2] and other diseases.[3] However, unmodified/natural oligonucleotides are not suitable for use in oligonucleotide therapeutics due to their insufficient hybridization affinity with the target nucleic acids and low resistance against nucleases. To improve these properties of the oligonucleotides, a number of chemical modifications of the nucleobase, sugar and phosphate moieties have been investigated.[4] Among them, bridge modification between the 2′-oxygen atom and the 4′-carbon atom of the ribose ring not only increases the affinity of the oligonucleotides with single-stranded RNA (ssRNA) and/or double-stranded DNA (dsDNA) but also improves their resistance to nuclease degradation; therefore, a variety of bridged nucleic acids have been developed to date.[5] In particular, LNA[6]/2′,4′-BNA,[7] ENA,[8] and *S*-cEt[9] have already been used for clinical trials (Figure 1). The bridge part of these 2′-*O*,4′-*C*-bridged nucleosides is generally synthesized by an intramolecular S_N2 reaction, namely ionic cyclization, in which the 2′-oxygen atom attacks the 4′-carbon atom bearing a leaving group (Scheme 1a). On the

other hand, intramolecular radical cyclization is an attractive approach to construct a bridged structure.[10] For example, 2′-*C*,4′-*C*-bridged nucleic acids like 7′-Me-carba-LNA and 8′-Me-carba-ENA are synthesized using intramolecular cyclization of the 2′-carbon radical (Figure 1 and Scheme 1b).[11] However, this reaction cannot be applied to the 2′-*O*,4′-*C*-bridged nucleosides because the 2′-oxygen atom is removed by the Barton-McCombie deoxygenation, which creates the 2′-carbon radical. In addition, the position where these radical species are generated to construct the 2′,4′-bridged structures via intramolecular radical cyclization has so far been limited to the 2′-carbon atom.[11,12] With this background, we planned to synthesize new 2′-*O*,4′-*C*-bridged nucleoside analogs through the generation of the radical species, via radical cyclization, at different carbon atom positions.

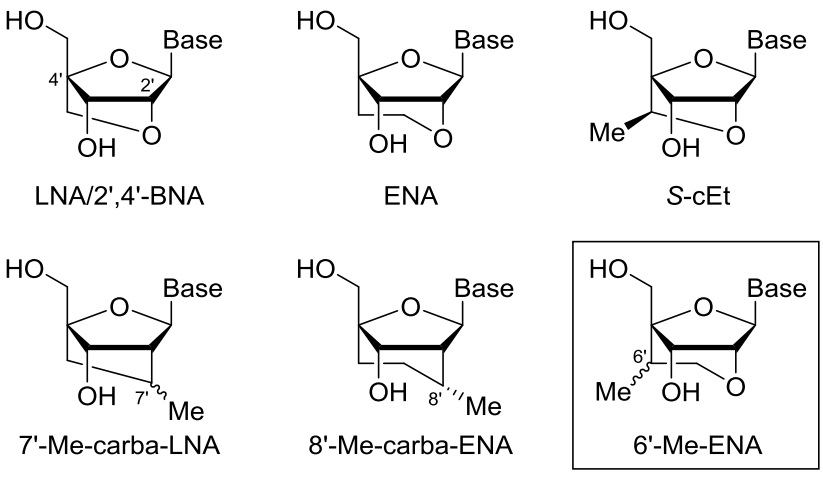
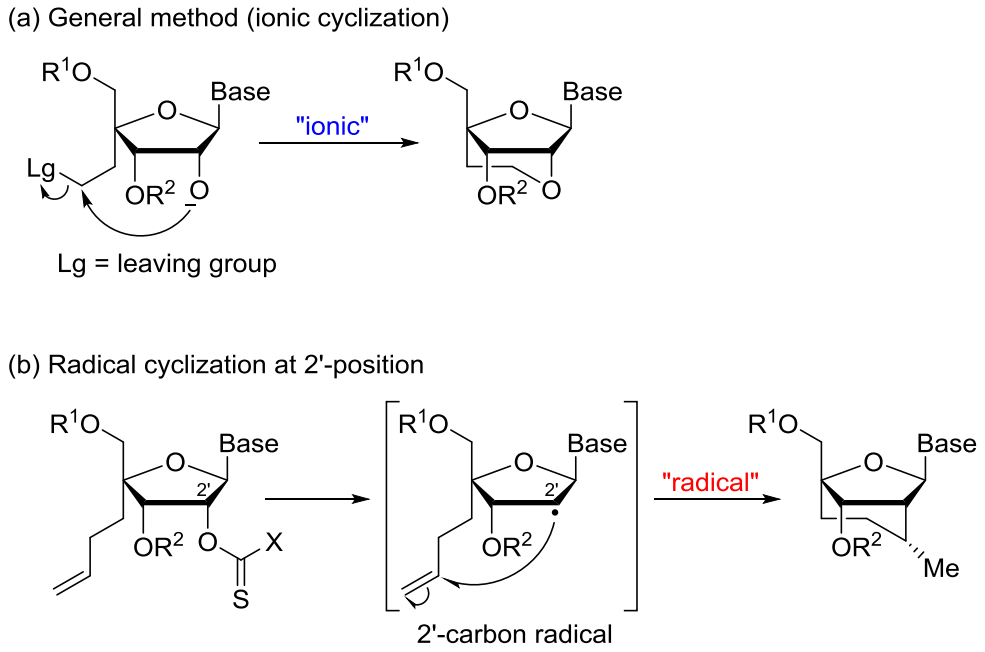


Figure 1. Structures of LNA and ENA analogs

Scheme 1. Strategies for the construction of bridge structures

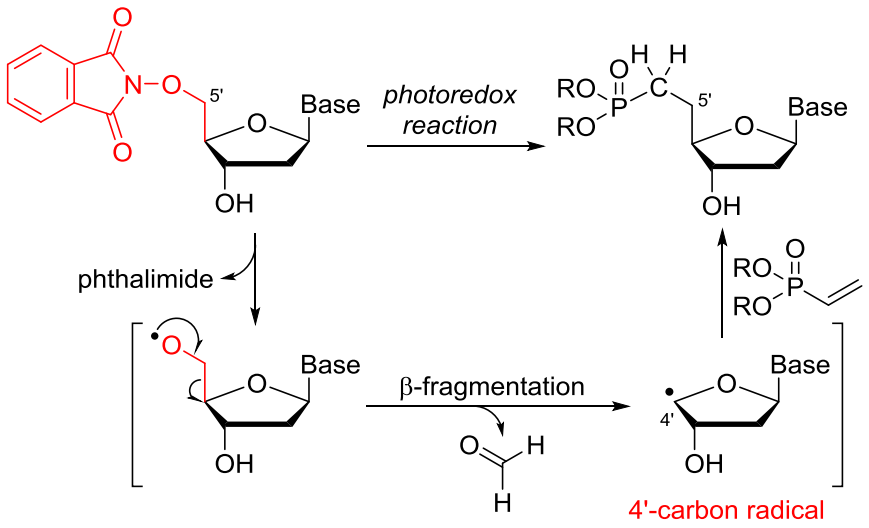


As part of our ongoing research aimed at developing novel methods for the synthesis of useful nucleoside analogs, we had recently reported the concise synthesis of 5′-carba analogs of nucleosides from the 2′-deoxy-5′-*O*-phthalimidonucleosides by the photoredox-catalyzed deformylative 1,4-addition reaction (Scheme 2a).[13] It is known that oligonucleotides containing six-membered ENA exhibit higher nuclease resistance than that containing five-membered LNA, without the loss of hybridization affinity toward the ssRNA.[8] Furthermore, oligonucleotides containing *S*-cEt, which possesses a methyl group at the 6′-position, not only increased the nuclease resistance but also

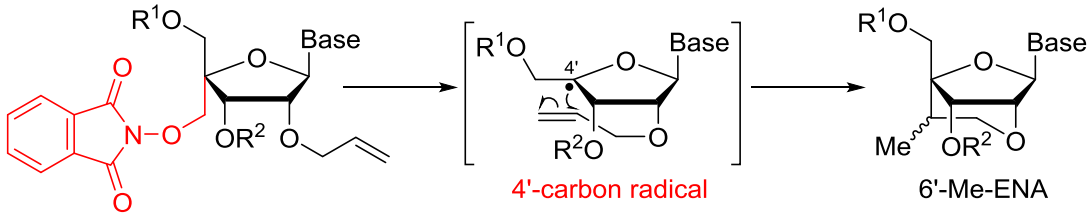
reduced the risk of hepatotoxicity, compared to that containing LNA.[9] These results suggest that 6′-Me-ENA can be expected as highly potential nucleic acids. Herein, we report a novel approach to the synthesis of 6′-Me-ENA-T by the intramolecular radical cyclization through the generation of the 4′-carbon radical. In addition, the hybridization ability and nuclease resistance of the 6′-Me-ENA-modified oligonucleotides were evaluated.

Scheme 2. Strategy for the Synthesis of 6′-Me-ENA via Generation of 4′-Carbon Radical

(a) Previous work:



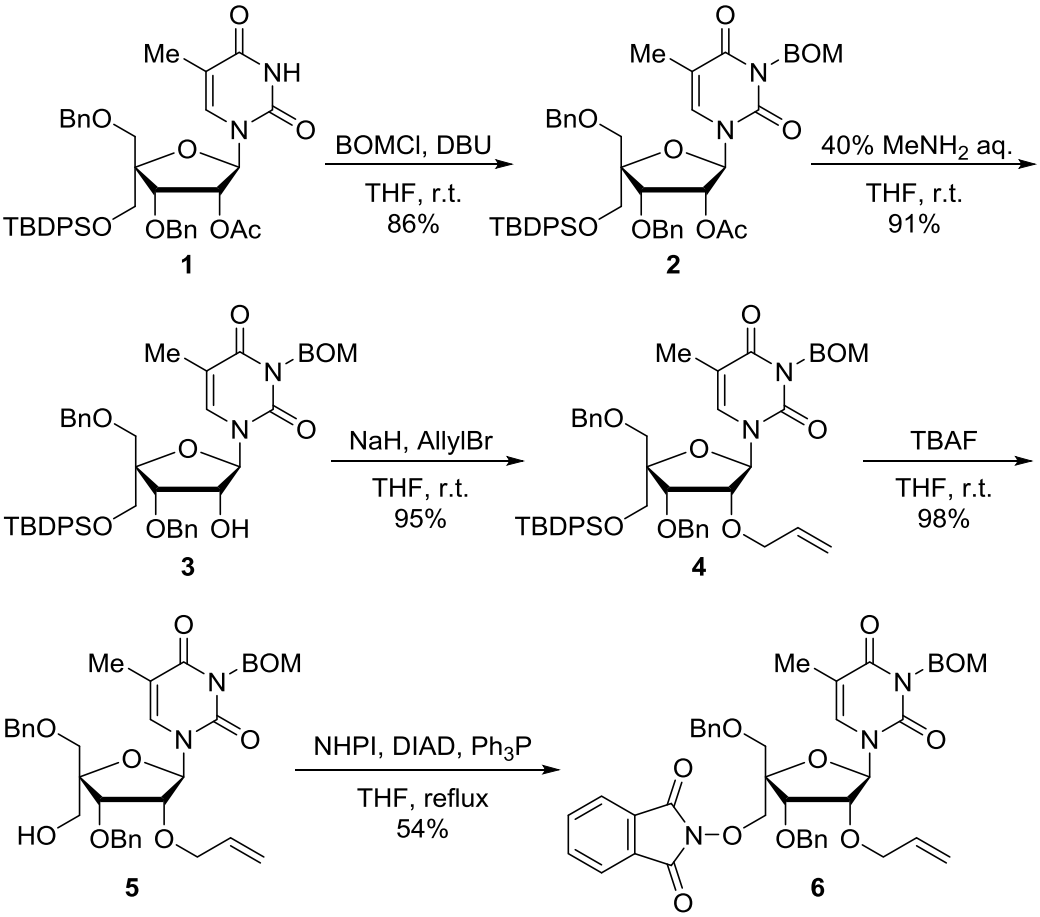
(b) This work:



RESULTS AND DISCUSSION

The known 5-methyluridine derivative **1**[14] was used as the starting material for the synthesis of 6′-Me-ENA-T (Scheme 3). Initially, BOM-protection of the nitrogen atom at the 3-position of **1** followed by the deacetylation of **2** gave **3**, the allylation of which afforded **4** in good yield. The silyl protecting group of **4** was removed using TBAF to give **5**. Subsequently, the obtained alcohol **5** was treated with *N*-hydroxyphthalimide (NHPI) under the Mitsunobu reaction conditions to provide the desired radical precursor **6**.

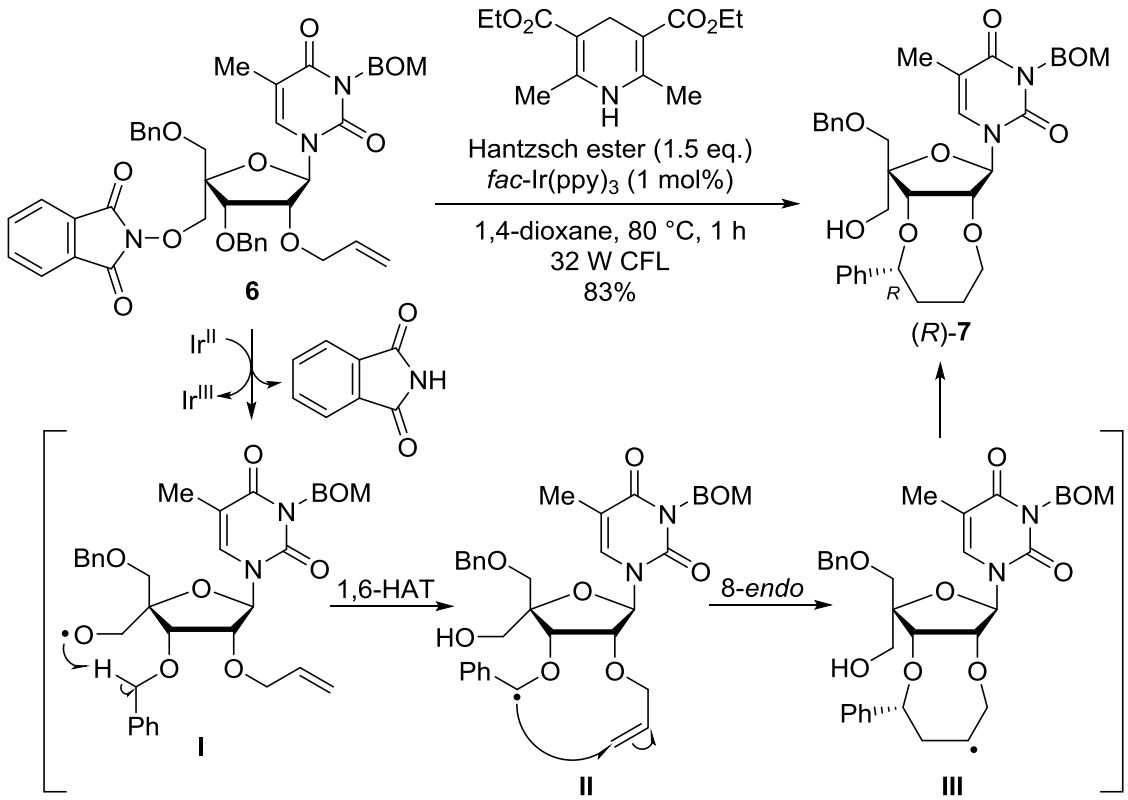
Scheme 3. Synthesis of *N*-Alkoxyphthalimide **6** from **1**



Next, the key radical cyclization was investigated using the visible light-mediated photoredox reaction (Scheme 4). In the presence of *fac*-Ir(ppy)$_3$ as a photocatalyst and Hantzsch ester as a reductant and hydrogen source, *N*-alkoxyphthalimide **6** in 1,4-dioxane was irradiated with a 32 W compact fluorescent lamp (CFL) at 80 °C. Interestingly, the desired six-membered 6′-Me-ENA was not obtained, instead the

compound (*R*)-**7** with an eight-membered ring was obtained as a sole isomer in 83% yield. This result indicates that the intramolecular 1,6-hydrogen atom transfer (1,6-HAT) of the alkoxy radical **I** took precedence over the *β*-fragmentation (for the generation of the 4′-carbon radical), generating the benzyl radical **II**. In general, it is know that 8-*endo* cyclization is fundamentally preferred over 7-*exo* cyclization.[15] Therefore, this radical **II** exclusively underwent the 8-*endo* cyclization to form the intermediate radical **III** and sequential hydrogen atom abstraction led to the eight-membered compound (*R*)-**7**.

Scheme 4. Radical Cyclization of *N*-Alkoxyphthalimide **6**



The stereoselective formation of (*R*)-**7** could be explained as shown in Figure 2. To avoid the steric repulsion between the phenyl group and hydrogen atom at 3′-position in the transition state **IIa**, the radical cyclization would proceed via the transition state **IIb**. The stereochemistry of compound (*R*)-**7** was confirmed by NOESY correlations between 3′-H and 7′-H, 3′-H and 9′-Ha, 7′-H and 9′-Ha (Figures 2 and S1).

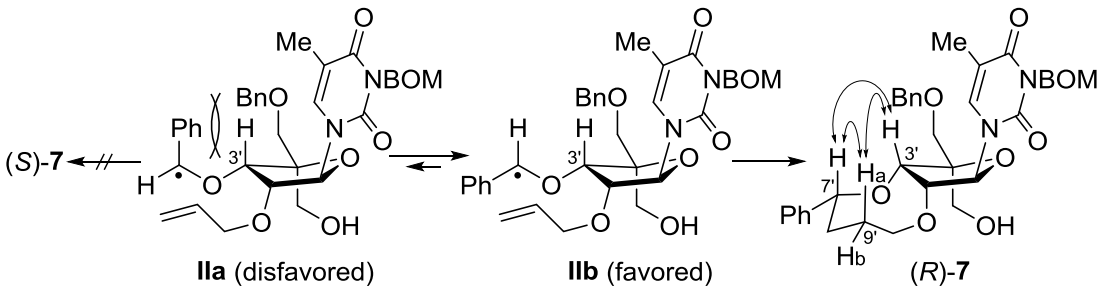
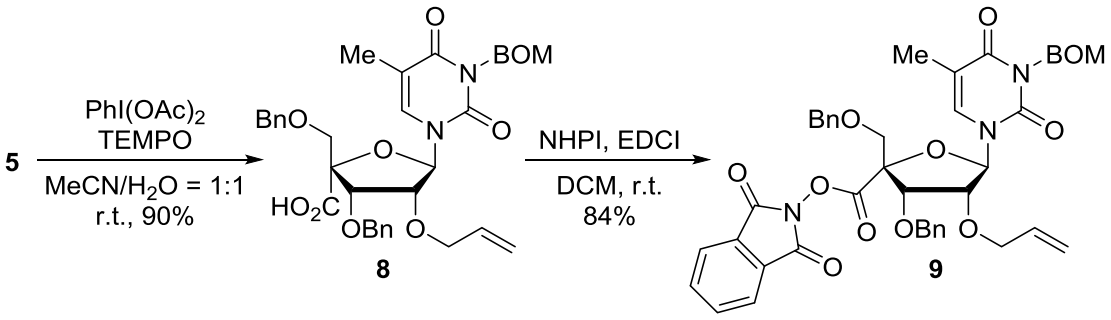


Figure 2. Transition state models of 8-*endo* radical cyclization

As an alternative route for the synthesis of 6′-Me-ENA, we focused on the generation of the 4′-carbon radical from *N*-acyloxyphthalimide by radical decarboxylation, which is much faster than the deformylation in a radical reaction.[16] The radical precursor, *N*-acyloxyphthalimide **9**, was prepared from alcohol **5** in two steps, by its oxidation to carboxylic acid **8** followed by the condensation with NHPI (Scheme 5).

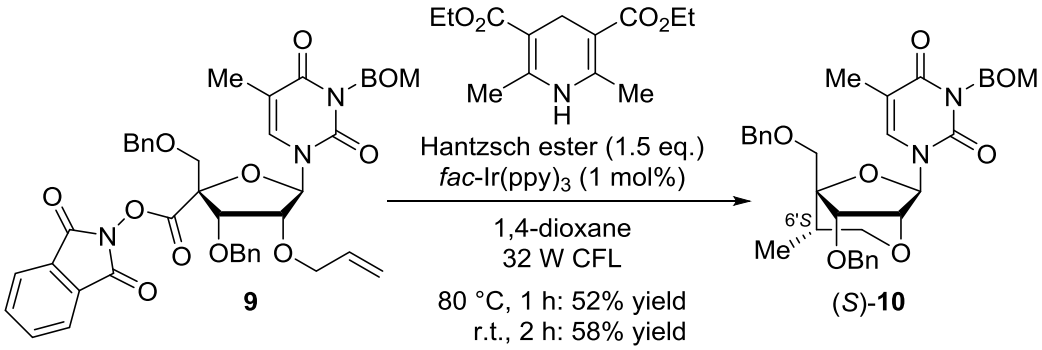
Scheme 5. Synthesis of *N*-Acyloxyphthalimide **9**



The radical cyclization was performed with *N*-acyloxyphthalimide **9,** as the radical

precursor (Scheme 6). When the reaction of **9** was carried out under the same conditions as Scheme 4, the desired radical decarboxylation and 6-*exo* cyclization proceeded smoothly to produce the 6′-Me-ENA-T monomer **10**, with only the (*S*)-configuration at the 6′-position. Decreasing the reaction temperature to room temperature resulted in a slight improvement of the yield. Meanwhile, the reaction without light irradiation at 80 °C resulted in the decomposition of **9** and the desired product was not obtained. Under the classical free radical condition using Bu_3SnH and AIBN, the unreacted **9** was recovered in 52% yield without producing **10**.[17]

Scheme 6. Radical Cyclization of *N*-Acyloxyphthalimide **9**



The observed stereoselectivity could be explained by the six-membered transition states in the 6-*exo* cyclization as depicted in Figure 3. The reaction via conformation **IVa** would be disfavored probably due to the 1,3-diaxial interaction between the olefin

moiety and the 3′-benzyloxy group. Therefore, the reaction should go through the conformation **IVb**, with the less hindered olefin, to give (*S*)-**10** selectively. The stereochemistry of compound (*S*)-**10** was confirmed by NOESY correlations between 1′-H and 7′-Ha, 6′-Me and 7′-Ha (Figures 3 and S2).

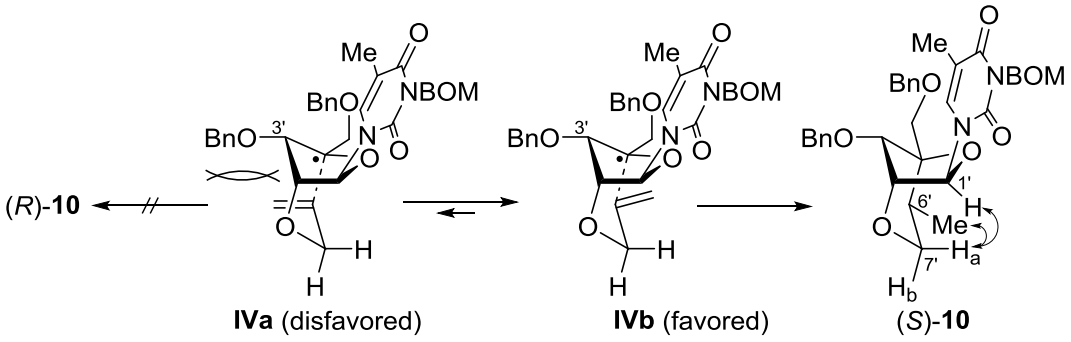
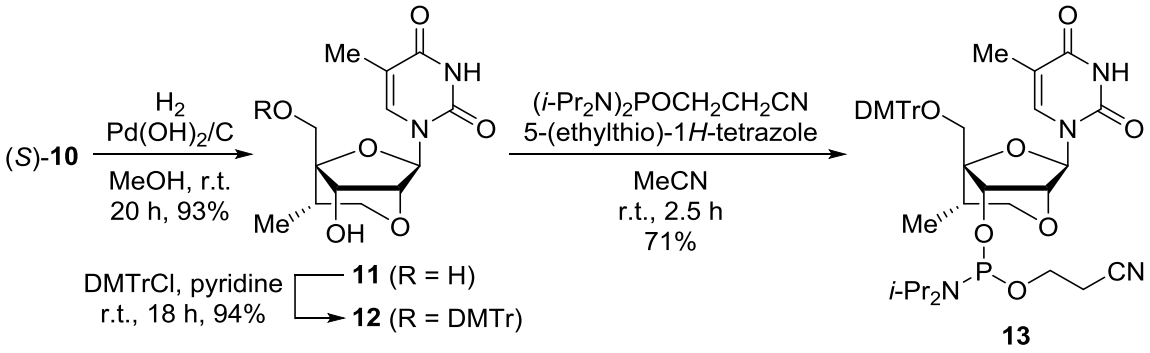


Figure 3. Transition state models of 6-*exo* radical cyclization

For the incorporation of the 6′*S*-Me-ENA-T monomer into oligonucleotides, simultaneous deprotection of the two benzyl and the BOM groups was required. This afforded compound **11** at 93% yield (Scheme 7). Finally, the 4,4′-dimethoxytrityl (DMTr) protection of the primary alcohol group followed by the phosphitylation of the secondary alcohol group gave phosphoramidite **13**. The oligonucleotide synthesis was performed in an automated DNA synthesizer using the common phosphoramidite chemistry. The coupling efficiency for the incorporation of **13** was over 95% (estimated

using the trityl monitor) with a prolonged coupling time from 25 s to 10 min.

Scheme 7. Synthesis of Phosphoramidite **13**



The duplex-forming ability of the 6′*S*-Me-ENA-modified oligonucleotides with the complementary ssRNA and ssDNA was evaluated using the UV-melting experiments and compared with those of the corresponding natural, LNA- and ENA-modified oligonucleotides (Table 1). The melting temperatures (T_ms) of the duplexes of the 6′*S*-Me-ENA-modified oligonucleotides **ON2–4** with the ssRNA were higher than that of the natural oligonucleotide **ON1** and comparable to that of the oligonucleotides containing LNA **ON5–7** and ENA **ON8–10**. As for ssDNA, **ON2–4** showed almost no stabilization or a slight destabilization (ΔT_m/mod. = –3.0 to +0.3 °C), though **ON5–7** and **ON8–10** showed slight stabilization (ΔT_m/mod. = 0 to +2.0 °C). The differences in the T_m values with the ssRNA and ssDNA are summarized in Figure 4. For example, in

comparison with singly modified oligonucleotides, 6′*S*-Me-ENA-modified **ON2** (ΔT_m = +6 °C) displayed larger ΔT_m than LNA-modified **ON5** (ΔT_m = +3 °C) and ENA-modified **ON8** (ΔT_m = +4 °C). These results suggested that the 6′*S*-Me-ENA-modified oligonucleotides could have the ssRNA-selective hybridization ability compared to the corresponding LNA- and ENA-modified oligonucleotides.

Table 1. Duplex-Forming Ability of Modified Oligonucleotides with Complementary ssRNA and ssDNA[a]

	T_m (ΔT_m/mod.) (°C)	
oligonucleotides	ssRNA	ssDNA
d(T<u>C</u>TT<u>C</u>TTTTT<u>C</u>T<u>C</u>T) (**ON1**)	47	46
d(T<u>C</u>TT<u>C</u>TT**X**TT<u>C</u>T<u>C</u>T) (**ON2**)	50 (+3.0)	43 (–3.0)
d(T<u>C</u>TT<u>C</u>T**XXX**T<u>C</u>T<u>C</u>T) (**ON3**)	60 (+4.3)	45 (–0.3)
d(T<u>C</u>TT<u>C</u>**X**T**X**T**X**<u>C</u>T<u>C</u>T) (**ON4**)	63 (+5.3)	47 (+0.3)
d(T<u>C</u>TT<u>C</u>TT**Y**TT<u>C</u>T<u>C</u>T) (**ON5**)	52 (+5.0)	48 (+2.0)
d(T<u>C</u>TT<u>C</u>T**YYY**T<u>C</u>T<u>C</u>T) (**ON6**)	61 (+4.7)	49 (+1.0)
d(T<u>C</u>TT<u>C</u>**Y**T**Y**T**Y**<u>C</u>T<u>C</u>T) (**ON7**)	63 (+5.3)	51 (+1.7)
d(T<u>C</u>TT<u>C</u>TT**Z**TT<u>C</u>T<u>C</u>T) (**ON8**)	51 (+4.0)	46 (0)

d(T<u>C</u>TT<u>C</u>T**ZZZ**T<u>C</u>T<u>C</u>T) (**ON9**)	61 (+4.7)	49 (+1.0)
d(T<u>C</u>TT<u>C</u>**Z**T**Z**T**Z**<u>C</u>T<u>C</u>T) (**ON10**)	64 (+5.7)	48 (+0.7)

[a]Conditions: 10 mM sodium cacodylate buffer (pH 7.4), 100 mM KCl and 2.5 μM of each oligonucleotide. **X** = 6′*S*-Me-ENA-T, **Y** = LNA-T, **Z** = ENA-T, <u>C</u> = 2′-deoxy-5-methylcytidine. The sequence of target RNA and DNA complements are 5′-r(AGAGAAAAAGAAGA)-3′ and 5′-d(AGAGAAAAAGAAGA)-3′. ΔT_m/mod.: the change in the T_m value (ΔT_m) per modification compared with the unmodified oligonucleotide **ON1**.

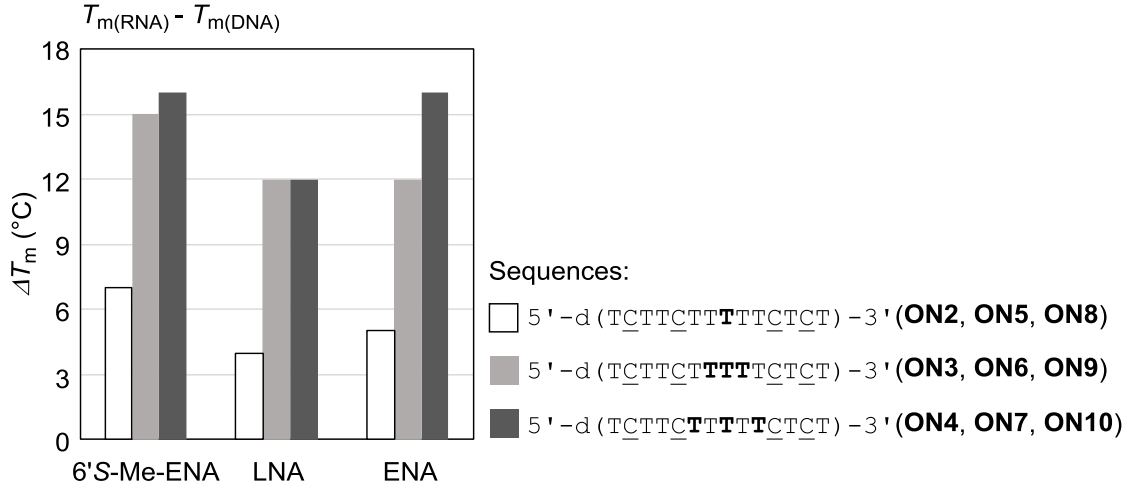


Figure 4. Differences in T_m values with ssRNA and ssDNA. **T** = 6′*S*-Me-ENA, LNA or ENA.

The triplex-forming ability of the 6′*S*-Me-ENA-modified oligonucleotides with dsDNA was also investigated (Figure 5). Although the ΔT_m/mod. values of

oligonucleotides containing the 6′*S*-Me-ENA-T slightly decreased by 0–2 °C compared to those of the oligonucleotides containing LNA and ENA, the 6′*S*-Me-ENA-modified oligonucleotides could sufficiently stabilize the triplex formed with the dsDNA.

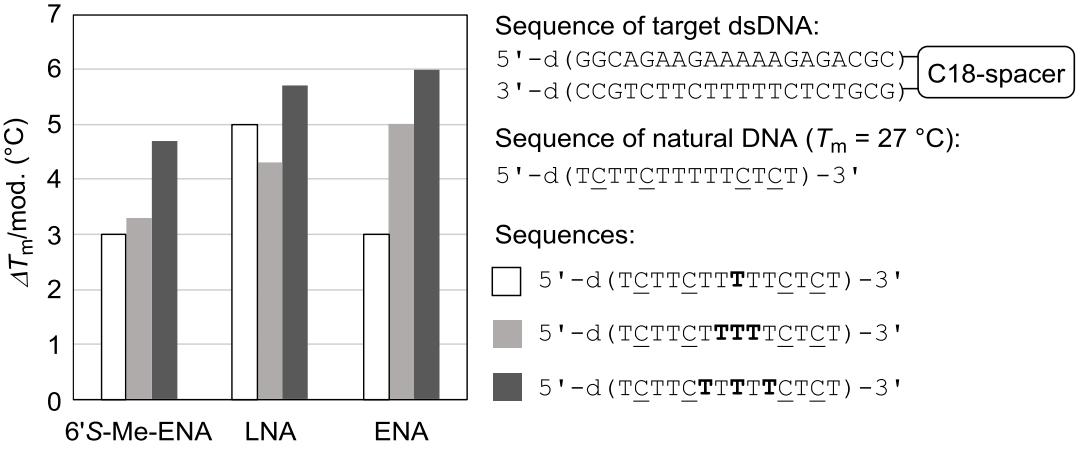


Figure 5. ΔT_m per modification values of the triplex formed by modified oligonucleotides with dsDNA. Conditions: 10 mM sodium cacodylate buffer (pH 7.4), 100 mM KCl, 10 mM $MgCl_2$ and 1.5 μM of each oligonucleotide. **T** = 6′*S*-Me-ENA, LNA or ENA.

Finally, the nuclease resistance of the T10-mer containing the 6′*S*-Me-ENA-T at the second position from the 3′-terminus was evaluated using the 3′-exonuclease (*Crotalus adamanteus* venom phosphodiesterase, CAVP) and compared to those of the natural, LNA- and ENA-modified T10-mers. As shown in Figure 6, the natural and LNA-modified T10-mers (**O11** and **ON13**, respectively) were completely degraded

within 5 and 10 min, respectively. Although the ENA-modified T10-mer **ON14** was more stable than **ON11** and **ON13**, the **ON14** that resisted degradation was less than 5%, after 60 min. In contrast, more than 50% of the 6′*S*-Me-ENA-modified T10-mer **ON12** resisted degradation even after 60 min. This excellent nuclease stability of the 6′*S*-Me-ENA-modified nucleotide was probably due to the steric hindrance of the 6′-methyl group.[18]

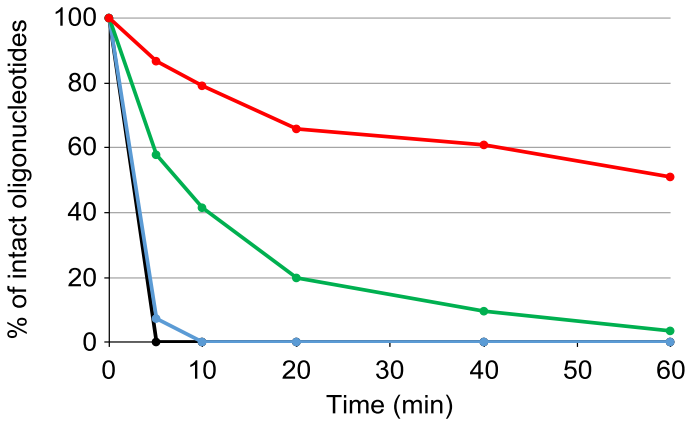


Figure 6. Nuclease degradation experiments. Conditions: 0.1 unit/mL *Crotalus adamanteus* venom phosphodiesterase (CAVP), 10 mM $MgCl_2$, 50 mM Tris-HCl (pH = 8.0), 7.5 μM of each oligonucleotide at 37 °C. Sequence: 5′-d(TTTTTTTT**T**T)-3′, **T** = natural (black, **ON11**), 6′*S*-Me-ENA (red, **ON12**), LNA (blue, **ON13**), ENA (green, **ON14**).

CONCLUSIONS

We developed a novel synthetic method for the construction of a 2′-*O*,4′-*C*-bridged nucleoside, 6′*S*-Me-ENA, by intramolecular 4′-carbon radical cyclization. Although a 4′-carbon radical was not generated from *N*-alkoxyphthalimide because the 1,6-HAT of the alkoxy radical rather than radical deformylation occurred preferentially, *N*-acyloxyphthalimide could successfully generate the 4′-carbon radical, followed by 6-*exo* cyclization to selectively afford the desired 6′*S*-Me-ENA-T monomer. The 6′*S*-Me-ENA-modified oligonucleotides showed selective hybridizing affinity toward ssRNA and high nuclease resistance compared to the LNA- and ENA-modified oligonucleotides. These results suggest that the 6′*S*-Me-ENA can be a useful material for practical applications such as antisense therapy. In addition, we believe that the proposed radical cyclization method can be a powerful tool for synthesis of new nucleic acid analogs and will contribute to development of promising materials of oligonucleotides.

EXPERIMENTAL SECTION

General Information. All moisture-sensitive reactions were conducted in well-dried glassware under an Ar atmosphere. Anhydrous benzene, DCM, 1,4-dioxane, MeCN, MeOH, pyridine and THF were used as purchased. ^{1}H NMR, ^{13}C{^{1}H} NMR, ^{31}P{^{1}H}

NMR spectra were recorded on a Bruker AVANCE III HD 500 equipped with a BBO cryoprobe or Varian MERCURY plus 300. Chemical shift values were reported in ppm, relative to internal tetramethylsilane (δ = 0.00 ppm) or solvent residual signals (δ = 3.31 ppm for CD_3OD) for ^1H NMR, solvent residual signals (δ = 77.0 ppm for $CDCl_3$ and δ = 49.0 ppm for CD_3OD) for $^{13}C\{^1H\}$ NMR, and external 5% H_3PO_4 (δ = 0.00 ppm) for $^{31}P\{^1H\}$ NMR. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. High-resolution mass spectrometry was performed on a Waters SYNAPT G2-Si (Quadrupole/TOF). For column chromatography, silica gel PSQ-60B (Fuji Silysia) was used. The progress of the reaction was monitored by analytical thin-layer chromatography (TLC) on pre-coated aluminum sheets (Silica gel 60 F_{254} by Merck). For HPLC, a JASCO EXTREMA (PU-4180, CO-4060 or CO-4061, UV-4075 and AS-4050) instrument with CHF122SC (ADVANTEC) fraction collector was used. UV-melting experiments were carried out using JASCO V-730 UV/VIS spectrophotometer equipped with a T_m analysis accessory. Synthesis of oligonucleotides was performed on an automated DNA synthesizer (Gene Design nS-8II).

Synthesis of 6′*S*-Me-ENA-T phosphoramidite 13

1-(2-O-Acetyl-4-C-tert-butyldiphenylsilyloxymethyl-3,5-di-O-benzyl-β-D-ribofuranosyl)-3-(benzyloxymethyl)thymine (**2**)

To a solution of **1** (5.63 g, 7.52 mmol) and DBU (2.47 mL, 16.5 mmol) in THF (50 mL), BOMCl (2.08 mL, 15.0 mmol) was added at 0 °C. After being stirred for 27.5 h at room temperature, the reaction mixture was quenched with sat. $NaHCO_3$ aq. and extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane/AcOEt = 2:1) to give compound **2** as a colorless oil (5.61 g, 86%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.62 (dd, *J* = 8.0, 1.0 Hz, 2H), 7.59 (dd, *J* = 8.0, 1.0 Hz, 2H), 7.50 (s, 1H), 7.43-7.20 (m, 21H), 6.16 (d, *J* = 4.5 Hz, 1H), 5.48 and 5.44 (ABq, *J* = 9.5 Hz, 2H), 5.38 (dd, *J* = 6.0, 5.5 Hz, 1H), 4.66 (s, 1H), 4.57-4.50 (m, 4H), 4.41 (d, *J* = 5.5 Hz, 1H), 3.94 and 3.73 (ABq, *J* = 11.0 Hz, 2H), 3.82 and 3.70 (ABq, *J* = 10.0 Hz, 2H), 1.93 (s, 3H), 1.52 (s, 3H), 1.04 (s, 9H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$): δ 170.0, 163.3, 151.2, 138.0, 137.5, 137.3, 135.7, 135.5, 134.5, 133.0, 132.7, 129.9, 129.7, 128.6, 128.4, 128.2, 128.1, 127.9, 127.7, 127.7, 127.7, 127.7, 127.6, 127.5, 110.5, 87.9, 86.5, 77.4, 74.9, 74.5, 73.7, 72.0, 70.5, 63.9, 26.9, 20.6, 19.2, 12.7. IR (ATR) cm^{-1}: 3068, 3030, 2930, 2858, 1747, 1713, 1666, 1453, 1363, 1228. HRMS (ESI-TOF): calcd for $C_{51}H_{56}N_2NaO_9Si$ $[M + Na]^+$ 891.3653, found 891.3661.

3-Benzyloxymethyl-1-(4-C-tert-butyldiphenylsilyloxymethyl-3,5-di-O-benzyl-β-D-ribofur

anosyl)thymine (**3**)

To a solution of **2** (12.5 g, 14.4 mmol) in THF (150 mL), 40% $MeNH_2$ aqueous solution (37 mL, 0.43 mol) was added at room temperature. After being stirred for 1 h, the reaction mixture was concentrated *in vacuo* and diluted with AcOEt. The AcOEt solution was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane/AcOEt = 2:1) to give compound **3** as a colorless foam (10.8 g, 91%). All spectral properties were identical to those reported in the literature.[19]

1-(2-O-Allyl-4-C-tert-butyldiphenylsilyloxymethyl-3,5-di-O-benzyl-β-D-ribofuranosyl)-3-(benzyloxymethyl)thymine (**4**)

To a solution of **3** (3.0 g, 3.63 mmol) in THF (36 mL), NaH (218 mg, 5.45 mmol, 60% dispersion in mineral oil) was added at 0 °C, and the mixture was stirred for 30 min at room temperature. After addition of allyl bromide (0.94 mL, 10.9 mmol) at 0 °C, the reaction mixture was stirred for 1 h. After addition of water, the reaction mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane/AcOEt = 4:1 to 2:1) to give compound **4** as a colorless

oil (2.97 g, 95%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71 (d, *J* = 1.0 Hz, 1H), 7.65 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.62 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.43-7.22 (m, 21H), 5.85 (d, *J* = 3.0 Hz, 1H), 5.75-5.67 (m, 1H), 5.50 and 5.47 (ABq, *J* = 9.5 Hz, 2H), 5.15 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.07 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.68 (s, 2H), 4.67 and 4.43 (ABq, *J* = 12.0 Hz, 2H), 4.58 and 4.52 (ABq, *J* = 11.5 Hz, 2H), 4.26 (d, *J* = 5.5 Hz, 1H), 4.11 (dd, *J* = 13.0, 6.5 Hz, 1H), 4.10 and 3.71 (ABq, *J* = 10.5 Hz, 2H), 4.05 and 3.95 (ABq, *J* = 11.5 Hz, 2H), 4.01 (dd, *J* = 13.0, 6.0 Hz, 1H), 3.93 (dd, *J* = 5.5, 3.0 Hz, 1H), 1.45 (s, 3H), 1.05 (s, 9H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$): δ 163.5, 150.8, 138.0, 137.7, 137.5, 135.8, 135.6, 134.8, 133.8, 133.4, 133.0, 129.7, 129.6, 128.5, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.6, 127.5, 117.9, 109.6, 88.1, 87.9, 80.1, 75.8, 73.6, 72.9, 72.0, 71.0, 71.0, 70.4, 64.6, 26.9, 19.3, 12.5. IR (ATR) cm^{-1}: 3070, 3029, 2929, 2857, 1707, 1661, 1454, 1361, 1265. HRMS (ESI-TOF): calcd for $C_{52}H_{58}N_2NaO_8Si$ $[M + Na]^+$ 889.3860, found 889.3864.

1-(2-O-Allyl-4-C-hydroxymethyl-3,5-di-O-benzyl-β-D-ribofuranosyl)-3-(benzyloxymethyl)thymine (**5**)

To a solution of **4** (2.94 g, 3.39 mmol) in THF (34 mL), TBAF (1 M solution in THF, 6.78 mL, 6.78 mmol) was added at room temperature. After being stirred for 3 h, the reaction mixture was concentrated *in vacuo*. The crude residue was purified by column

chromatography (hexane/AcOEt = 3:2 to 1:2) to give compound **5** as a colorless oil (2.08 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66 (d, *J* = 1.0 Hz, 1H), 7.40-7.20 (m, 15H), 6.10 (d, *J* = 2.5 Hz, 1H), 5.96-5.83 (m, 1H), 5.47 (s, 2H), 5.32 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.21 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.77 and 4.44 (ABq, *J* = 11.5 Hz, 2H), 4.68 (s, 2H), 4.55 and 4.49 (ABq, *J* = 11.5 Hz, 2H), 4.44-4.37 (m, 1H), 4.40 (d, *J* = 6.0 Hz, 1H), 4.19 (dd, *J* = 13.0, 6.0 Hz, 1H), 4.05 (dd, *J* = 6.0, 2.5 Hz, 1H), 3.83-3.70 (m, 4H), 2.71 (br s, 1H), 1.48 (d, *J* = 1.0 Hz, 3H). ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ 163.2, 150.6, 137.8, 137.1, 137.1, 134.5, 133.4, 128.4, 128.4, 128.1, 128.1, 127.9, 127.6, 127.5, 127.5, 127.4, 118.3, 109.7, 89.3, 87.2, 80.3, 75.8, 73.4, 72.8, 71.9, 71.5, 70.5, 70.2, 63.6, 12.4. IR (ATR) cm^{-1}: 3470, 3067, 3030, 2930, 2870, 1707, 1661, 1465, 1453, 1364, 1271. HRMS (ESI-TOF): calcd for $C_{36}H_{40}N_2NaO_8$ $[M + Na]^+$ 651.2682, found 651.2681.

1-[2-O-Allyl-4-C-[O-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)hydroxymethyl]-3,5-di-O-benzyl-β-D-ribofuranosyl]-3-(benzyloxymethyl)thymine (**6**)

To a mixture of **5** (78.7 mg, 0.13 mmol), NHPI (53.1 mg, 0.33 mmol) and Ph_3P (85.4 mg, 0.33 mmol) in THF (2 mL), DIAD (73.8 μL, 0.38 mmol) was added dropwise at 0 °C. After being stirred at reflux for 1.5 h, the reaction mixture was concentrated *in vacuo*. The crude residue was purified by column chromatography ($CHCl_3$/AcOEt =

5:1) to give compound **6** as a colorless oil (52.6 mg, 54%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.81 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.73 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.71 (s, 1H), 7.36-7.23 (m, 15H), 6.13 (d, *J* = 2.5 Hz, 1H), 5.89-5.81 (m, 1H), 5.48 and 5.46 (ABq, *J* = 10.0 Hz, 2H), 5.28 (dd, *J* = 17.0, 1.0 Hz, 1H), 5.16 (d, *J* = 10.5, 1H), 4.77 and 3.96 (ABq, *J* = 10.5 Hz, 2H), 4.71 and 4.47 (ABq, *J* = 11.5 Hz, 2H), 4.67 (s, 2H), 4.64 and 4.60 (ABq, *J* = 11.5 Hz, 2H), 4.43-4.39 (m, 3H), 4.34 (dd, *J* = 13.0, 5.0 Hz, 1H), 4.18 (dd, *J* = 13.0, 6.5 Hz, 1H), 4.06 (dd, *J* = 5.5, 2.5 Hz, 1H), 1.44 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$): δ 163.4, 163.0, 150.8, 138.0, 137.4, 137.3, 134.7, 134.4, 133.8, 128.8, 128.6, 128.4, 128.2, 128.0, 128.0, 127.7, 127.7, 127.6, 123.5, 118.3, 109.7, 89.2, 86.5, 80.0, 78.7, 75.9, 73.7, 72.9, 72.1, 71.5, 70.4, 69.8, 12.5. IR (ATR) cm^{-1}: 3064, 3030, 2926, 2871, 1790, 1732, 1707, 1659, 1453, 1363, 1274. HRMS (ESI-TOF): calcd for $C_{44}H_{43}N_3NaO_{10}$ $[M + Na]^+$ 796.2846, found 796.2846.

(1R,6R,8S,9R,11R)-3-benzyloxymethyl-1-[9-benzyloxymethyl-9-hydroxymethyl-6-phenyl -2,7,10-trioxabicyclo[6.3.0]undecane-11-yl]thymine (**7**)

A solution of **6** (63.0 mg, 0.081 mmol), Hantzsch ester (30.9 mg, 0.12 mmol) and *fac*-Ir(ppy)$_3$ (0.5 mg, 0.81 μmol) in 1,4-dioxane (2 mL) was deaerated by Ar bubbling for 0.5 h. The mixture was stirred and then irradiated with a 32 W CFL at 80 °C. After

stirring for 1 h, the crude solution was concentrated *in vacuo* and purified by flash column chromatography (hexane/AcOEt = 3:1 to 1:1) to give compound **7** as a colorless oil (42.7 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40-7.24 (m, 16H), 6.12 (d, *J* = 5.0 Hz, 1H), 5.51 and 5.47 (ABq, *J* = 10.0 Hz, 2H), 4.71 (s, 2H), 4.58 (s, 2H), 4.52 (dd, *J* = 6.5, 4.0 Hz, 1H), 4.33 (d, *J* = 6.0 Hz, 1H), 4.11-4.06 (m, 2H), 3.77-3.65 (m, 4H), 3.56 (ddd, *J* = 12.0, 6.0, 4.0 Hz, 1H), 2.37 (dd, *J* = 9.0, 6.0 Hz, 1H), 2.10-1.99 (m, 2H), 1.91-1.84 (m, 1H), 1.75-1.67 (m, 1H), 1.73 (d, *J* = 1.0 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$): δ 163.5, 151.1, 142.3, 138.0, 137.5, 135.6, 128.6, 128.5, 128.3, 128.0, 127.7, 127.6, 127.6, 127.5, 125.6, 110.4, 90.8, 88.4, 84.4, 82.7, 80.2, 73.7, 72.4, 72.2, 71.8, 70.6, 63.8, 34.5, 26.2, 12.9. IR (ATR) cm^{-1}: 3495, 3062, 3030, 2926, 2864, 1710, 1666, 1654, 1451, 1363, 1273. HRMS (ESI-TOF): calcd for $C_{36}H_{40}N_2NaO_8$ $[M + Na]^+$ 651.2682, found 651.2686.

1-(2-O-Allyl-4-C-carboxy-3,5-di-O-benzyl-β-D-ribofuranosyl)-3-(benzyloxymethyl)thymine (**8**)

To a solution of **5** (613 mg, 0.97 mmol) in MeCN-H_2O (1:1, 10 mL), $PhI(OAc)_2$ (691 mg, 2.14 mmol) and TEMPO (30.4 mg, 0.19 mmol) were added at room temperature. After being stirred for 21 h, the reaction mixture was quenched with sat. $NaHCO_3$ aq.

and extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography ($CHCl_3$ to $CHCl_3$/MeOH = 20:1) to give compound **8** as a colorless oil (564 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 (s, 1H), 7.34-7.20 (m, 15H), 6.34 (d, *J* = 5.5 Hz, 1H), 5.75-5.68 (m, 1H), 5.43 (s, 2H), 5.12 (d, *J* = 17.5, 1H), 5.10 (d, *J* = 10.5 Hz, 1H), 4.73 and 4.68 (ABq, *J* = 11.5 Hz, 2H), 4.63 (s, 2H), 4.52 and 4.47 (ABq, *J* = 11.5 Hz, 2H), 4.37 (d, *J* = 5.0 Hz, 1H), 4.18 (t, *J* = 5.5 Hz, 1H), 4.05 and 3.78 (ABq, *J* = 10.5 Hz, 2H), 3.98 (dd, *J* = 13.0, 5.5 Hz, 1H), 3.93 (dd, *J* = 12.5, 5.5 Hz, 1H), 1.52 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$): δ 171.8, 163.3, 151.3, 137.8, 137.0, 134.6, 133.5, 128.6, 128.4, 128.2, 128.2, 128.0, 127.7, 127.6, 127.6, 118.6, 110.6, 89.3, 88.1, 79.4, 77.8, 74.3, 73.8, 72.1, 71.7, 71.5, 70.5, 12.6. IR (ATR) cm^{-1}: 3065, 3030, 2926, 2868, 1712, 1668, 1655, 1453, 1363, 1276. HRMS (ESI-TOF): calcd for $C_{36}H_{38}N_2NaO_9$ $[M + Na]^+$ 665.2475, found 665.2474.

1-[2-O-Allyl-4-C-[O-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)carboxy]-3,5-di-O-benzyl -β-D-ribofuranosyl]-3-(benzyloxymethyl)thymine (**9**)

To a solution of **8** (8.2 g, 12.8 mmol) in DCM (100 mL), NHPI (3.12 g, 19.1 mmol) and EDCI·HCl (3.67 g, 19.1 mmol) were added at room temperature. After being stirred

for 4.5 h, the reaction mixture was diluted with water and extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane/AcOEt = 3:1 to 3:2) to give compound **9** as a white foam (8.4 g, 84%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.79 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.45-7.23 (m, 16H), 6.44 (d, *J* = 5.5 Hz, 1H), 5.79 (ddt, *J* = 17.0, 10.5, 5.5 Hz, 1H), 5.49 and 5.47 (ABq, *J* = 9.5 Hz, 2H), 5.18 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.13 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.86 and 4.81 (ABq, *J* = 12.0 Hz, 2H), 4.68 (s, 2H), 4.62 (s, 2H), 4.42 (d, *J* = 4.5 Hz, 1H), 4.20 (dd, *J* = 5.0, 4.5 Hz, 1H), 4.13 and 4.05 (ABq, *J* = 10.5 Hz, 2H), 4.11-4.03 (m, 2H), 1.56 (d, *J* = 1.0 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$): δ 165.7, 163.3, 161.2, 151.0, 137.9, 137.1, 136.7, 134.7, 134.6, 133.8, 128.9, 128.7, 128.3, 128.3, 128.3, 128.3, 127.9, 127.7, 127.6, 124.0, 118.2, 110.5, 88.6, 88.6, 79.3, 77.5, 74.1, 73.8, 72.1, 71.5, 71.2, 70.5, 12.7. IR (ATR) cm^{-1}: 3064, 3032, 2926, 2868, 1788, 1743, 1713, 1670, 1657, 1453, 1362, 1277. HRMS (ESI-TOF): calcd for $C_{44}H_{41}N_3NaO_{11}$ $[M + Na]^+$ 810.2639, found 810.2639.

(1R,2S,5R,6R,8S)-1-[8-benzyloxy-1-(benzyloxymethyl)-2-methyl-4,7-dioxabicyclo[3.2.1] octane-6-yl]-3-(benzyloxymethyl)thymine (**10**)

A suspension of **9** (1.5 g, 1.9 mmol), Hantzsch ester (723 mg, 2.86 mmol) and *fac*-Ir(ppy)$_3$ (12.4 mg, 0.02 mmol) in 1,4-dioxane (19 mL) was deaerated by Ar bubbling for 0.5 h. The mixture was stirred and then irradiated with a 32 W compact fluorescent lamp at room temperature. After stirring for 2 h, a yellow homogeneous solution was obtained. The crude solution was concentrated *in vacuo* and purified by flash column chromatography (hexane/AcOEt = 5:1 to 3:1) to give compound **10** as a white foam (668 mg, 59%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89 (s, 1H), 7.38-7.23 (m, 15H), 6.02 (s, 1H), 5.47 and 5.45 (ABq, *J* = 9.5 Hz, 2H), 4.74 and 4.50 (ABq, *J* = 11.5 Hz, 2H), 4.70 (s, 2H), 4.61 and 4.55 (ABq, *J* = 11.5 Hz, 2H), 4.29 (d, *J* = 3.0 Hz, 1H), 4.02 (d, *J* = 3.0 Hz, 1H), 3.95 and 3.58 (ABq, *J* = 11.0 Hz, 2H), 3.87 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.49 (dd, *J* = 11.5, 11.0 Hz, 1H), 2.54-2.47 (m, 1H), 1.41 (s, 3H), 0.73 (d, *J* = 7.0 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$): δ 163.5, 150.6, 138.0, 137.5, 137.3, 134.3, 128.6, 128.4, 128.3, 128.1, 127.9, 127.7, 127.7, 127.6, 109.4, 86.4, 85.6, 75.5, 73.6, 72.2, 71.8, 71.4, 70.3, 68.1, 66.8, 30.6, 12.5, 9.9. IR (ATR) cm^{-1}: 3065, 3030, 2961, 2926, 2868, 1704, 1659, 1452, 1363, 1278, 1213. HRMS (ESI-TOF): calcd for $C_{35}H_{38}N_2NaO_7$ $[M + Na]^+$ 621.2577, found 621.2574.

(1R,2S,5R,6R,8S)-1-[8-hydroxy-1-hydroxymethyl-2-methyl-4,7-dioxabicyclo[3.2.1]octa

ne-6-yl]thymine (**11**)

Compound **10** (169 mg, 0.28 mmol) was treated in MeOH (5 mL) at room temperature under a hydrogen atmosphere in the presence of $Pd(OH)_2/C$ (170 mg). After stirring for 20 h, the reaction mixture was filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography ($CHCl_3$/MeOH = 10:1) to give compound **11** as a white foam (78.4 mg, 93%). ^{1}H NMR (500 MHz, CD_3OD): δ 8.34 (d, *J* = 1.0 Hz, 1H), 5.96 (s, 1H), 4.16 (d, *J* = 3.0 Hz, 1H), 4.07 (d, *J* = 3.0 Hz, 1H), 3.98 and 3.64 (ABq, *J* = 12.0 Hz, 2H), 3.79 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.46 (dd, *J* = 11.5, 11.5 Hz, 1H), 2.40-2.32 (m, 1H), 1.86 (s, 3H), 0.74 (d, *J* = 6.5 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, CD_3OD): δ 166.7, 152.0, 137.9, 110.2, 87.5, 86.6, 79.9, 67.7, 65.2, 60.4, 31.0, 12.6, 10.0. IR (ATR) cm^{-1}: 3395, 3073, 2963, 2878, 1682, 1659, 1653, 1468, 1274. HRMS (ESI-TOF): calcd for $C_{13}H_{18}N_2NaO_6$ $[M + Na]^+$ 321.1063, found 321.1064.

(1R,2S,5R,6R,8S)-1-[8-hydroxy-1-(4,4'-dimethoxytrityloxymethyl)-2-methyl-4,7-dioxabi cyclo[3.2.1]octane-6-yl]thymine (**12**)

To a solution of **11** (180 mg, 0.60 mmol) in pyridine (6 mL), DMTrCl (306 mg, 0.90 mmol) was added at 0 °C. After being stirred for 18 h at room temperature, the reaction mixture was quenched with sat. $NaHCO_3$ aq. and extracted with AcOEt. The combined

organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane/AcOEt = 1:1 to AcOEt) to give compound **12** as a white foam (339 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.98 (s, 1H), 7.93 (s, 1H), 7.44 (d, *J* = 7.5 Hz, 1H), 7.35-7.22 (m, 7H), 6.85 (d, *J* = 9.0 Hz, 2H), 6.85 (d, *J* = 9.0 Hz, 2H), 6.02 (s, 1H), 4.61 (dd, *J* = 8.0, 3.5 Hz, 1H), 4.30 (d, *J* = 3.5 Hz, 1H), 3.82 (dd, *J* = 12.0, 6.5 Hz, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 3.46 and 3.33 (ABq, *J* = 11.0 Hz, 2H), 3.45 (dd, *J* = 12.0, 10.5 Hz, 1H), 2.93 (d, *J* = 8.0 Hz, 1H), 2.33-2.25 (m, 1H), 1.24 (s, 3H), 0.57 (d, *J* = 6.5 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$): δ 164.0, 158.7, 158.6, 149.9, 144.2, 135.3, 135.2, 135.1, 130.1, 130.0, 128.1, 128.1, 127.1, 113.3, 110.4, 86.9, 86.0, 85.1, 78.5, 66.5, 65.8, 61.4, 55.2, 30.9, 11.8, 9.6. IR (ATR) cm^{-1}: 3412, 3065, 2958, 2931, 2877, 2836, 1686, 1607, 1508, 1464, 1251. HRMS (ESI-TOF): calcd for $C_{34}H_{36}N_2NaO_8$ $[M + Na]^+$ 623.2369, found 623.2371.

(1R,2S,5R,6R,8S)-1-[8-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4'-dimethoxytrityloxymethyl)-2-methyl-4,7-dioxabicyclo[3.2.1]octane-6-yl]thymine (**13**)

To a solution of **12** (207 mg, 0.34 mmol) in MeCN (6 mL), $(i\text{-}Pr_2N)_2POCH_2CH_2CN$ (273 μL, 0.86 mmol) and 5-(ethylthio)-1*H*-tetrazole (98.5 mg, 0.76 mmol) were added at room temperature. After being stirred for 2.5 h, the reaction mixture was quenched

with sat. $NaHCO_3$ aq. and extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane/AcOEt = 1:1) to give compound **13** as a white foam (192 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 and 8.32 (br s, 1H), 8.04 and 8.03 (s, 1H), 7.44-7.41 (m, 2H), 7.36-7.24 (m, 7H), 6.87-6.82 (m, 4H), 6.04 and 6.02 (s, 1H), 4.66 (dd, *J* = 7.0, 3.0 Hz, 0.4H), 4.62 (dd, *J* = 6.5, 3.5 Hz, 0.6H), 4.39 (d, *J* = 3.0 Hz, 0.6H), 4.37 (d, *J* = 3.0 Hz, 0.4H), 3.91-3.41 (m, 13H), 3.22 (d, *J* = 10.5 Hz, 0.4H), 3.17 (d, *J* = 10.5 Hz, 0.6H), 2.69-2.56 (m, 1H), 2.40-2.24 (m, 2H), 1.30-1.04 (s, 15H), 0.59 (d, *J* = 6.5 Hz, 3H). ^{31}P{^{1}H} NMR (202 MHz, $CDCl_3$) δ: 149.3, 148.7. HRMS (ESI-TOF): calcd for $C_{43}H_{53}N_4NaO_9P$ $[M + Na]^+$ 823.3448, found 823.3449.

Synthesis of Oligonucleotides ON2-10 and ON12-14

6′*S*-Me-ENA-T phosphoramidite **13**, LNA-T phosphoramidite, ENA-T phosphoramidite, dT-phosphoramidite (Sigma) and d^mC(Ac)-phosphoramidite (Sigma) were used. Syntheses of oligonucleotides were performed on a 0.2 μmol scale using a standard phosphoramidite protocol (DMTr-ON mode), except for a prolonged coupling time of 10 min for **13** and ENA-T phosphoramidite, 5 min for LNA-T phosphoramidite. Cleavage from the CPG support and removal of the protecting groups were

accomplished by 28% NH_3 aq. at room temperature for 2 h. After removal of ammonia *in vacuo*, the crude oligonucleotides were purified with Sep-Pak® Plus C18 cartridges (Waters), followed by reversed-phase HPLC (Waters XBridge™ Prep Shield RP18 5 μm, 10 × 50 mm) using triethylammonium acetate buffer (0.1 M, pH 7.0) as an ion-pairing mobile phase. The compositions of the oligonucleotides were confirmed by ESI-TOF-MS analysis. The deconvoluted ESI-TOF-MS data [M] for **ON2-10** and **ON12-14**: **ON2**, found 4248.80 (calcd 4248.89); **ON3**, found 4360.70 (calcd 4361.01); **ON4**, found 4360.80 (calcd 4361.01); **ON5**, found 4220.80 (calcd 4220.83); **ON6**, found 4276.80 (calcd 4276.85); **ON7**, found 4276.80 (calcd 4276.85); **ON8**, found 4235.40 (calcd 4234.86); **ON9**, found 4319.50 (calcd 4318.94); **ON10**, found 4319.50 (calcd 4318.94); **ON12**, found 3036.50 (calcd 3036.05); **ON13**, found 3008.40 (calcd 3007.99); **ON14**, found 3022.30 (calcd 3022.02).

UV-Melting Experiments

In the duplex-forming experiment, the synthesized oligonucleotides and ssRNA or ssDNA were dissolved in 10 mM sodium cacodylate buffer (pH 7.4) containing 100 mM KCl to give a final concentration of 2.5 μM, respectively. In the triplex-forming experiment, the synthesized oligonucleotides and hairpin dsDNA were dissolved in 10 mM sodium cacodylate buffer (pH 7.4) containing 100 mM KCl and 10 mM $MgCl_2$ to

give a final concentration of 1.5 μM, respectively. The samples were annealed in boiling water followed by slow cooling to 5 °C. The melting profiles were recorded at 260 nm from 20 °C to 80 °C for ssRNA and ssDNA, and from 10 °C to 90 °C for dsDNA at a scan rate of 0.5 °C/min. The two-point average method was employed to obtain the T_m, and the final values were determined by averaging three independent measurements, which were accurate within a 1 °C range.

Nuclease Degradation Experiments

Enzymatic degradation experiments were carried out using 0.1 unit/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. The amount of intact oligonucleotides was determined by reversed-phase HPLC (Waters XBridge™ Shield RP18 2.5 μm, 4.6 × 50 mm) using triethylammonium acetate buffer (0.1 M, pH 7.0) as an ion-pairing mobile phase.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

NOESY spectra of compounds (*R*)-**7** and (*S*)-**10**, UV-melting data, HPLC charts of

nuclease degradation experiments, ^{1}H, $^{13}C\{^{1}H\}$, and $^{31}P\{^{1}H\}$ NMR spectra for new compounds and HPLC charts of oligonucleotides **ON2-10** and **ON12-14**. (PDF)

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Author Contributions

Y.I. and Y.H. designed the experiments. Y.I., N.T., and T.O. performed experiments. Y.I. and Y.H. co-wrote the paper. Y.H. supervised the project.

Notes

The authors declare no competing financial interest.

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