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Synthesis of scpBNA-^{m}C, -A, and -G Monomers and Evaluation of the Binding Affinities of scpBNA-Modified Oligonucleotides toward Complementary ssRNA and ssDNA

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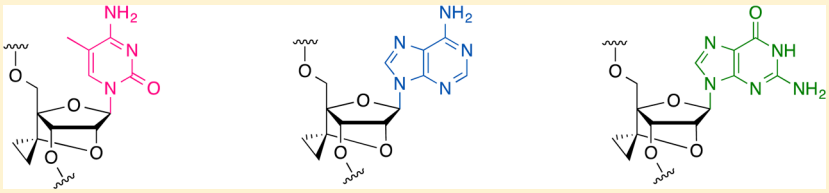
Supporting Information



ABSTRACT: We previously reported the synthesis and evaluation of 2′-*O*,4′-*C*-spirocyclopropylene-bridged nucleic acid (scpBNA) bearing a thymine (T) nucleobase. Oligonucleotides (ONs) modified with scpBNA-T exhibited strong binding affinity to complementary single-stranded RNA (ssRNA) and high enzymatic stability. These biophysical properties suggest that scpBNAs are well suited for use in antisense strategies. Herein, we describe the synthesis of scpBNA monomers bearing 5-methylcytosine (^{m}C), adenine (A), and guanine (G) nucleobases for use in a variety of sequences. The prepared scpBNA monomers were incorporated into ONs at various positions. The scpBNA-modified ONs exhibited excellent duplex-forming ability with the complementary ssRNA comparable to ONs modified with 2′-*O*,4′-*C*-methylene-bridged nucleic acid (2′,4′-BNA/LNA). Moreover, ON modified with scpBNA-^{m}C, -A, and -G showed higher enzymatic stability than the corresponding 2′,4′-BNA/LNA-modified ON. These results demonstrated a promising role for the incorporation of scpBNA monomers into therapeutic antisense ONs.

INTRODUCTION

Antisense oligonucleotides (ONs) inhibit a translational process from mRNA to protein by forming a duplex with complementary target mRNA. Because natural ONs exhibit low binding affinity toward the mRNA and especially show poor resistance against enzymatic degradation, chemically modified ONs are employed in antisense technology.[1] Among modified ONs designed to date, 2′-*O*,4′-*C*-methylene-bridged nucleic acid (2′,4′-BNA/LNA; Figure 1) exhibits excellent binding affinity to single-stranded RNA (ssRNA) and moderate resistance to enzymatic degradation.[2,3] Since the discovery of a promising approach for introducing a bridge structure into the sugar moiety of a nucleoside, many 2′,4′-BNA/LNA analogues have been synthesized and their duplex-forming abilities and enzymatic stabilities have been evaluated.[4,5] We recently synthesized 2′-*O*,4′-*C*-spirocyclopropylene-bridged nucleic acid (scpBNA; Figure 1) bearing a thymine (T) nucleobase.[6] The scpBNA-T-modified ON exhibited high binding affinity toward the complementary ssRNA, similar to that of the corresponding 2′,4′-BNA/LNA-T-modified ON. Moreover, the enzymatic stability of the scpBNA-T-modified ON was superior to that of 2′,4′-BNA/LNA-T-modified ON. These results demonstrated that scpBNA is a promising candidate for use in antisense technology. Here, we report the synthesis of scpBNA monomers containing the other nucleobases, i.e., 5-methylcytosine (^{m}C), adenine (A), and guanine (G) nucleobases, with a view to incorporating these monomers into a variety of sequences.

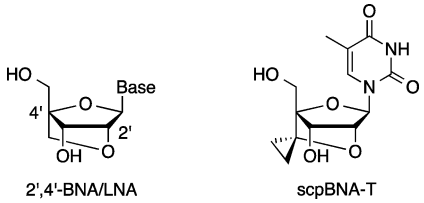


Figure 1. Structures of the 2′,4′-BNA/LNA and scpBNA-T monomers. Base = nucleobase.

RESULTS AND DISCUSSION

Various 5-methylcytidines have been synthesized from the corresponding thymidines by a traditional conversion method in which the 4-carbonyl group of the thymine nucleobase is converted into an amino group under mild conditions.[2a,3b,7] We

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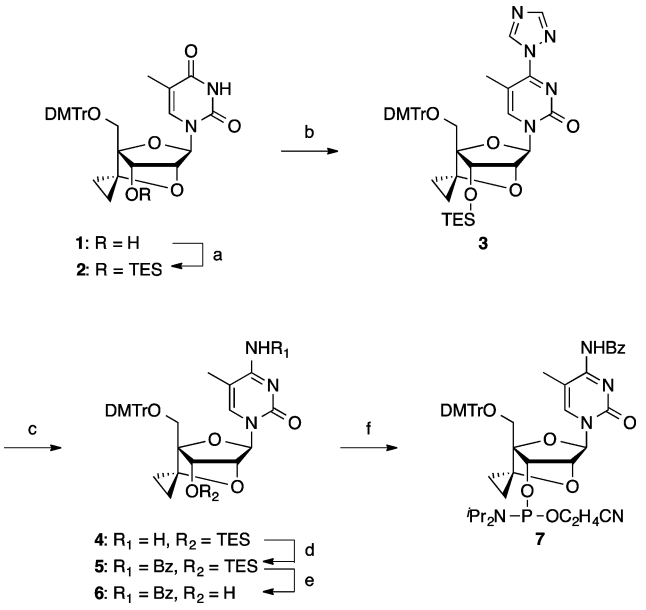
thus expected that the scpBNA-^{m}C monomer could be prepared from **1**,[6] a synthetic intermediate of scpBNA-T monomer (Scheme 1). First, the 3′-hydroxyl group of **1** was

Scheme 1. Synthesis of scpBNA-^{m}C Phosphoramidite 7[a]



[a]Reagents and conditions: (a) TESCl, pyridine, rt, 2 h, 95%; (b) 1,2,4-triazole, $POCl_3$, MeCN, rt, 2 h; (c) NH_3 aq, 1,4-dioxane, rt, 2 h, 98% (two steps); (d) BzCl, pyridine, rt, 3 h, 83%; (e) TBAF, THF, rt, 10 min, 88%; (f) $^{i}Pr_2NP(Cl)OC_2H_4CN$, DIPEA, MeCN, rt, 2 h, 78%.

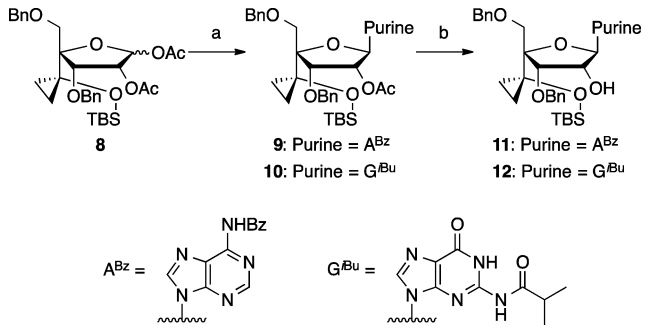
protected by triethylsilylation, and the 4-carbonyl group of **2** was activated using $POCl_3$ and 1,2,4-triazole. Subsequent transformation of the triazole group into an amino group was accomplished by treatment with aq NH_3. After the amino group was protected with BzCl, the TES group was removed with TBAF to afford **6**. Finally, phosphitylation of **6** gave the desired scpBNA-^{m}C phosphoramidite **7** in 78% yield.

The adenosine and guanosine analogues can be easily synthesized by transglycosylation[8] of their thymidine counterparts. However, we have found that bridged nucleic acids are generally unsuitable substrates for transglycosylation. We thus decided to synthesize scpBNA-A and -G monomers from unbridged compound **8**,[6] a common intermediate for scpBNA-T and -^{m}C synthesis (Scheme 2). To our delight, nucleosides **9** and **10**, respectively, containing N^6-benzoyladenine and N^2-isobutyrylguanine, were easily obtained by the Vorbrüggen reaction. The 2′-acetyl groups of **9** and **10** were then removed using general basic conditions to afford **11** and **12**.

In our synthesis of scpBNA-T, the bridge structure was constructed by cyclization from 2,2′-anhydro nucleoside (Figure 2).[6] However, this type of bridge construction is not applicable to **11** and **12**, so our plan was to prepare scpBNA-A and -G monomers via arabino-type nucleosides. The first step of this synthetic approach is an inversion of the 2′-hydroxyl group in **11** and **12**.

Initially, we attempted general inversion methods such as Mitsunobu reaction (for **11**: BzOH, Ph_3P, and DIAD) and typical S_N2 reactions (for the triflated derivative of **11**: NaOBz or NaI), but no arabino-type compounds were obtained. Therefore, we attempted oxidation of the 2′-hydroxyl groups and subsequent stereoselective reduction (Tables 1 and 2).

Scheme 2. Synthesis of the Purine Nucleosides 11 and 12 from Unbridged Compound 8[a]



[a]Reagents and conditions: (a) N^6-benzoyladenine, BSA, TMSOTf, MeCN, 80 °C, 31 h for **9**, 58% or (a) N^2-isobutyrylguanine, BSA, TMSOTf, MeCN, 80 °C, 18 h for **10**, 49%; (b) K_2CO_3, MeOH, 0 °C, 20 min for **11**, 97% or (b) K_2CO_3, MeOH, 0 °C, 1 h for **12**, 95%.

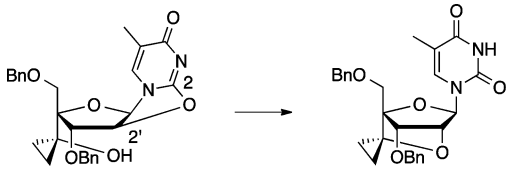
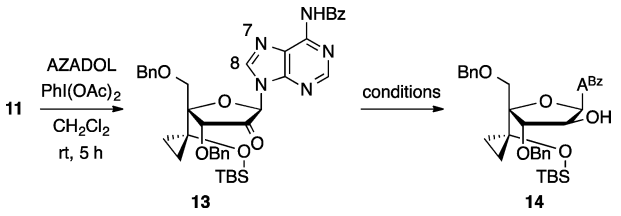


Figure 2. Previous bridge construction method for the synthesis of scpBNA-T.

Table 1. Synthesis of Arabino-Type Adenosine 14 from 11



entry	reagent	solvent	temp (°C)	time (min)	yield (%)	14:11[b]
1	$NaBH_4$	EtOH	0	30	71[a]	1:2.6
2	N-Selectride	THF	0	5	depurination[c]	
3	DIBAL (1 equiv)	THF	0–50	30	dec	
4[d]	DIBAL (5 equiv)	THF	0	30	44[e]	5:1

[a]Yield of a mixture of diastereomers of **14** and **11** over two steps from the starting adenosine **11**. [b]Determined from the ^{1}H NMR spectra. [c]N^6-benzoyladenine was isolated as a major product. [d]Reduction was followed by DDQ oxidization because the 7*N*–8*C* double bond of the adenine nucleobase was reduced with DIBAL. [e]Yield of a mixture of diastereomers **14** and **11** over three steps from the starting material **11**.

Dess–Martin periodinane resulted in incomplete oxidation of **11**, whereas a combination of AZADOL and $PhI(OAc)_2$ converted the 2′-hydroxyl group into ketone more efficiently. $NaBH_4$ reduction of the resulting ketone **13** successfully led to the desired **14** (Table 1, entry 1),[9] but **14** was contaminated with starting **11** as an inseparable isomer (**14**/**11** = 1:2.6). The obtained low stereoselectivity was likely due to the steric bulk of the TBS group of **13** inhibiting access of $NaBH_4$ to the *Re*-face of the 2′-carbonyl group. The bulkier N-Selectride was also tested to obtain additional information about the mechanism controlling stereoselectivity, but its use resulted in depurination (entry 2). The use of 1 equiv of DIBAL resulted in no reaction

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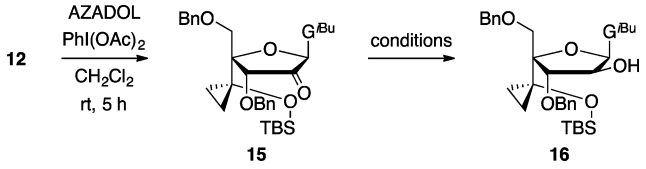
Table 2. Synthesis of Arabino-Type Guanosine 16 from 12



entry	reagent	solvent	temp (°C)	time (min)	yield[a] of **16** (%)	yield[a,b] of **12** (%)
1	DIBAL (5 equiv)	THF	0	20	32	10
2	$NaBH_4$	MeOH/CH_2Cl_2	0	30	31	60

[a]Isolated yield over two steps from the starting guanosine **12**. [b]Compound **12** was obtained by the reduction of ketone **15**.

at 0 °C, and an increase in the reaction temperature to 50 °C resulted in decomposition of the starting material (entry 3). In contrast, the use of 5 equiv of DIBAL at 0 °C afforded **14** with relatively high stereoselectivity (entry 4, **14**/**11** = 5:1). It is possible that transient chelation of DIBAL by the 2′- and 6′-oxygen atoms assisted reduction from the hindered *Re*-face of the 2′-carbonyl to afford **14**.

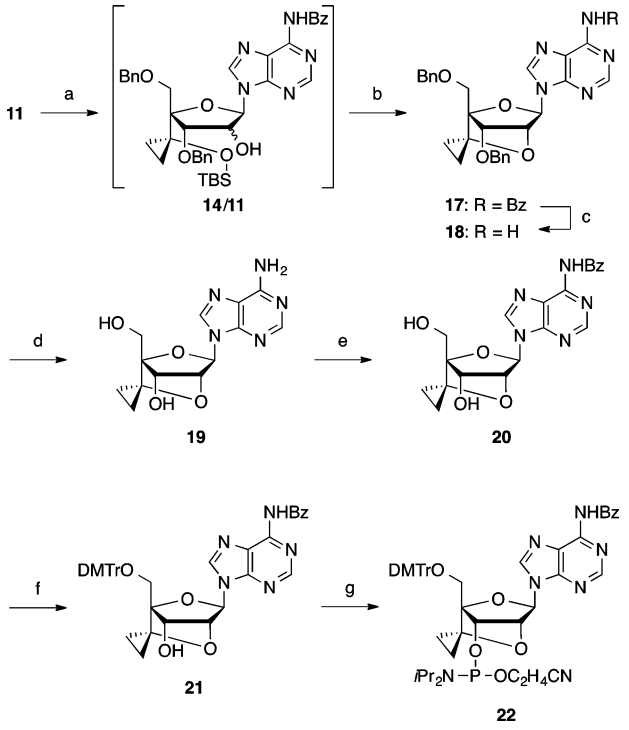
DIBAL reduction of guanosine **12** after AZADOL oxidation preferentially afforded the arabino-type target **16** with a yield of 32% (Table 2, entry 1), but DIBAL reduction also generated many byproducts. In contrast, $NaBH_4$ reduction led to the desired **16** in 31% yield without any side reactions, and **12** was obtained in 60% yield (Table 2, entry 2). Since the recovered **12** is a reusable material, $NaBH_4$ conditions were selected as being optimal for synthesis of the scpBNA-G monomer.

With the optimal reduction conditions in hand, adenosine **11** was rapidly converted into a mixture of **14** and **11** (Scheme 3). The obtained diastereomixture was then treated with Tf_2O under basic conditions, and subsequent reaction with TBAF afforded the bridged nucleoside **17** in 31% yield over five steps.[10] The two benzyl groups of **17** could not be removed using typical hydrogenation conditions but could be removed after deprotection of the N^6-benzoyl group. The resulting amino group of **19** was protected again using benzoyl chloride to furnish scpBNA-A monomer **20**. Finally, scpBNA-A phosphoramidite **22** was obtained by dimethoxytritylation followed by phosphitylation.

The synthesis of scpBNA-G began with $NaBH_4$ reduction of **15** to provide arabino-type **16** (Scheme 4). The 2′-hydroxyl group of **16** was mesylated, and the TBS group was subsequently deprotected to afford **23**. The bridge was constructed by exposing **23** to basic conditions (**24**: 92%).[10] The two benzyl groups of the bridged compound **24** were easily removed by hydrogenolysis, and scpBNA-G monomer **25** was obtained in 70% yield. Similar to the synthesis of scpBNA-A, scpBNA-G phosphoramidite **27** was prepared from **25**.

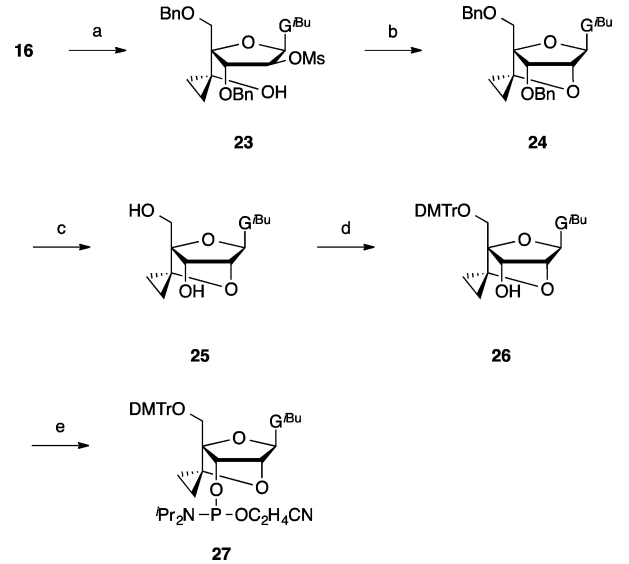
To evaluate the duplex-forming abilities of the scpBNA-modified ONs toward complementary ssRNA or ssDNA, the prepared scpBNA-^{m}C, -A, and -G phosphoramidites, as well as scpBNA-T phosphoramidite, were incorporated into ONs using an automated DNA synthesizer. We chose the phosphatase and tensin homologue (PTEN) sequence.[11] The coupling time was extended to 8 min (0.2 μmol scale) or 10 min (1.0 μmol scale) for incorporation of the scpBNA phosphoramidites, but all other synthetic steps followed the standard phosphoramidite protocol. The synthesized ONs were purified by reversed-phase HPLC (Supporting Information) and identified by MALDI-

Scheme 3. Synthesis of scpBNA-A Phosphoramidite 22[a]



[a]Reagents and conditions: (a) (i) AZADOL, $PhI(OAc)_2$, CH_2Cl_2, rt, 5 h, (ii) DIBAL, THF, 0 °C, 30 min, (iii) DDQ, CH_2Cl_2, rt, 30 min; (b) (i) Tf_2O, DMAP, CH_2Cl_2, rt, 14 h, (ii) TBAF, THF, rt, 30 min, 31% (five steps); (c) $MeNH_2$ aq, THF, rt, 40 min, 90%; (d) HCO_2NH_4, $Pd(OH)_2/C$, EtOH/AcOH, reflux, 6 h, 46%; (e) (i) TMSCl, pyridine, 0 °C, 1 h, (ii) BzCl, rt, 3 h, (iii) NH_3 aq, rt, 1 h, 59% (three steps); (f) DMTrCl, pyridine, rt, 1 h, quant; (g) $^iPr_2NP(Cl)OC_2H_4CN$, DIPEA, MeCN, rt, 3 h, 85%.

Scheme 4. Synthesis of scpBNA-G Phosphoramidite 27[a]



[a]Reagents and conditions: (a) (i) MsCl, Et_3N, CH_2Cl_2, rt, 20 min; (ii) TBAF, THF, rt, 18 h, 57% (two steps); (b) K_2CO_3, DMF, 80 °C, 22 h, 92%; (c) H_2, $Pd(OH)_2/C$, EtOH, rt, 20 h, 70%; (d) DMTrCl, pyridine, rt, 4 h, quant; (e) $^iPr_2NP(Cl)OC_2H_4CN$, DIPEA, MeCN, rt, 3 h, 76%.

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Table 3. T_m Values (°C) of Duplexes formed between ONs and ssRNA or ssDNA[a,b]

ONs		T_m (ΔT_m/mod) (°C)		RNA selectivity
		ssRNA	ssDNA	T_m [ssRNA] − T_m [ssDNA]
5′-d(TCATGGCTGCAGCT)-3′	**ON1**	49	50	−1
5′-d(TCATGGCTGCAGCT)-3′	**ON2a**	59 (+5)	55 (+3)	+4
5′-d(TCATGGCTGCAGCT)-3′	**ON2b**	59 (+5)	56 (+3)	+3
5′-d(TCATGGCTGCAGCT)-3′	**ON3a**	60 (+5)	56 (+3)	+4
5′-d(TCATGGCTGCAGCT)-3′	**ON3b**	61 (+6)	57 (+4)	+4
5′-d(TCATGG^{m}CTG^{m}CAGCT)-3′	**ON4a**	61 (+6)	57 (+4)	+4
5′-d(TCATGG^{m}CTG^{m}CAGCT)-3′	**ON4b**	61 (+6)	58 (+4)	+3
5′-d(TCATGGCTGCAGCT)-3′	**ON5a**	60 (+5)	55 (+3)	+5
5′-d(TCATGGCTGCAGCT)-3′	**ON5b**	61 (+6)	56 (+3)	+5
5′-d(TCATGGCTG^{m}CAGCT)-3′	**ON6a**	73 (+6)	63 (+3)	+10
5′-d(TCATGGCTG^{m}CAGCT)-3′	**ON6b**	73 (+6)	64 (+4)	+9

[a]Conditions: 10 mM phosphate buffer (pH 7.2), 10 mM NaCl, and 4 μM each oligonucleotide. The T_m values reflect the average of three measurements. [b]Target sequences: 5′-r(AGCUGCAGCCAUGA)-3′ (ssRNA), 5′-(AGCTGCAGCCATGA)-3′ (ssDNA). scpBNA: A, G, ^{m}C, and T. 2′,4′-BNA/LNA: *A*, *G*, ^{m}*C*, and *T*. ΔT_m/mod: The change in T_m value (ΔT_m) per modification compared to the unmodified standard strand (**ON1**).

TOF MS analysis (Supporting Information; e.g., the isolated yield of **ON6a** was 8%). A set of 2′,4′-BNA/LNA-modified ONs was also prepared for comparison. The thermal stabilities of the duplexes formed between the modified ONs and the complementary ssRNA or ssDNA were evaluated by UV melting experiments, and the melting temperatures (T_m values) are shown in Table 3.

Similar to the results obtained previously for scpBNA-T-modified ONs,[6] all of the synthesized scpBNA-modified ONs were found to exhibit high binding affinity to the complementary ssRNA (**ON2a**–**6a**: ΔT_m = +5 to +6 °C per modification). The T_m values of the scpBNA-modified ONs were similar to those of the 2′,4′-BNA/LNA-modified ONs (**ON2a**–**6a** versus **ON2b**–**6b**). For example, the duplex formed between scpBNA-A-modified ON (**ON2a**) and ssRNA exhibited a T_m value of 59 °C, which is the same T_m value obtained for the corresponding **ON2b**/ssRNA duplex. **ON6a** containing four different scpBNAs (scpBNA-A, -G, -^{m}C, and -T) also exhibited duplex-forming abilities similar to that of its 2′,4′-BNA/LNA-modified counterpart (**ON6b**). In our previous report,[6] scpBNA-T-modified ONs were shown to exhibit slightly higher RNA selectivity than the corresponding 2′,4′-BNA/LNA-modified ONs. However, the RNA selectivity of **ON2a**–**6a** differed only negligibly from that of **ON2b**–**6b**. This probably means that the sequence of ONs and the position of modifications are important for the beneficial effect of scpBNA on the RNA selectivity.

Next, we set out to investigate the effect of scpBNA-^{m}C, -A, and -G on enzymatic stability. Here, ON modified with scpBNA-^{m}C, -A, and -G (**ON7a**), its natural counterpart (**ON8**), and 2′,4′-BNA/LNA-modified counterpart (**ON7b**) were prepared,[12] and their enzymatic stabilities were evaluated using 3′-exonuclease (*Crotalus admanteus* venom phosphodiesterase, CAVP). As shown in Figure 3, natural **ON8** was degraded within 20 min. Under the same conditions, both chemically modified ONs, **ON7a** and **ON7b**, showed improved resistance against the nuclease. The amount of intact **ON7a** was over 90% after 80 min, whereas intact **ON7b** was 11%. Therefore, scpBNA-modified **ON7a** imparted higher enzymatic stability than 2′,4′-BNA/LNA-modified **ON7b**.

In summary, we have constructed synthetic routes for the synthesis of scpBNA-^{m}C, -A, and -G monomers. In particular, conditions for reducing the 2′-carbonyl groups of **13** and **15** were carefully examined, and stereoselective reductions were

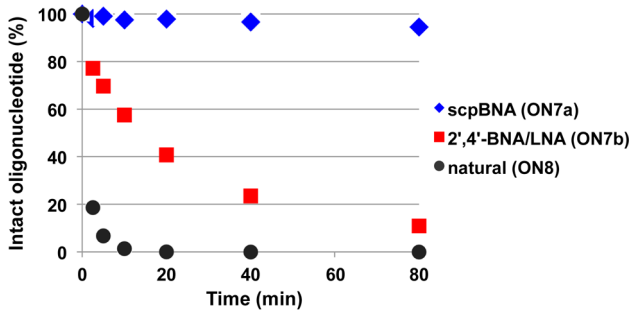

Figure 3. Stability of ONs in the presence of 3′-exonuclease. Conditions: 4 μM ON, 10 mM $MgCl_2$ and 1 μg/mL CAVP in 50 mM Tris-HCl buffer (pH 8.0) at 37 °C. The sequence of the ON was 5′-d(TTTTTGT^{m}CTA)-3′. 5′-d(TTTTTGT^{m}CTA)-3′ (**ON7a**), 5′-d(TTTTT*GT*^{m}*CTA*)-3′ (**ON7b**) and 5′-d(TTTTTGT^{m}CTA)-3′ (**ON8**).

achieved by using 5 equiv of DIBAL (see entry 4 of Table 1 and entry 1 of Table 2), although $NaBH_4$ reduction was selected in the case of scpBNA-G synthesis. Oligonucleotide modified with four different scpBNAs (scpBNA-T, -^{m}C, -A, and -G) was synthesized (**ON6a**: 8% yield), and all scpBNA-modified ONs (**ON2a**–**6a**) were found to show excellent binding affinity to ssRNA, comparable to that of the corresponding 2′,4′-BNA/LNA-modified ONs. In addition, ON modified with scpBNA-^{m}C, -A, and -G (**ON7a**) was more stable against enzymatic degradation than the 2′,4′-BNA/LNA-modified counterpart (**ON7b**). The results presented here demonstrate that scpBNA-T, -^{m}C, -A, and -G hold promise for antisense strategies against many types of diseases.

EXPERIMENTAL SECTION

General Experimental Procedure. Dry dichloromethane, tetrahydrofuran, acetonitrile, and pyridine were used as purchased. **ON1**, **ON2b**–**6b**, and target ONs were synthesized, purified, and identified by GeneDesign, Inc. ^{1}H NMR spectra were recorded at 300 or 500 MHz. ^{13}C NMR spectra were recorded at 75.5 or 125.7 MHz. ^{31}P NMR spectra were recorded at 121.6 MHz. Chemical shift values are expressed in δ values (ppm) relative to internal tetramethylsilane (0.00 ppm), residual $CHCl_3$ (7.26 ppm) or CH_3OH (3.31 ppm) for ^{1}H NMR, and internal tetramethylsilane (0.00 ppm), chloroform-d_1 (77.16 ppm), or methanol-d_4 (49.00 ppm) for ^{13}C NMR and 5% H_3PO_4 (0.00 ppm) as external standard for ^{31}P NMR. Mass spectra of all new compounds and oligonucleotides were measured on MALDI-

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TOF mass spectrometers. For column chromatography, PSQ-100B or FL-100D silica gel was used. For flash column chromatography, PSQ-60B or FL-60D silica gel was used. For preparative thin-layer chromatography, PLC silica gel 60 F_{254} was used.

1-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,4-*C*-spirocyclopropylene-3-*O*-(triethylsilyl)-β-D-ribopentofuranosyl]thymine (2). To a solution of **1**[6] (167 mg, 0.28 mmol) in dry pyridine (2 mL) was added chlorotriethylsilane (0.24 mL, 1.39 mmol) at 0 °C under Ar atmosphere. After the solution was stirred at room temperature for 2 h, saturated aq $NaHCO_3$ was added, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 2:1) to afford **2** (188 mg, 95%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.36−0.64 (m, 8H), 0.74−0.87 (m, 11H), 1.71 (d, *J* = 0.9 Hz, 3H), 3.11 (d, *J* = 10.8 Hz, 1H), 3.15 (d, *J* = 10.5 Hz, 1H), 3.80 (s, 6H), 4.34 (s, 1H), 4.36 (s, 1H), 5.72 (s, 1H), 6.83 (dd, *J* = 2.1, 9.0 Hz, 4H), 7.22−7.34 (m, 7H), 7.44 (dd, *J* = 1.5, 8.4 Hz, 2H), 7.78 (d, *J* = 1.2 Hz, 1H), 8.25 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 4.9, 5.1, 6.7, 9.9, 12.8, 55.4, 57.9, 68.4, 72.5, 79.6, 86.8, 87.5, 88.5, 110.5, 113.4, 113.4, 127.2, 128.1, 128.2, 130.1, 130.2, 135.0, 135.4, 135.5, 144.4, 150.0, 158.8, 164.2; IR (KBr) 3166, 3036, 2954, 2876, 1691, 1509, 1254, 1177, 1054, 835, 734 cm^{-1}; $[\alpha]_D^{24}$ −13.6 (*c* 1.01, MeOH); HRMS (MALDI) calcd for $C_{40}H_{48}N_2O_8NaSi$ $[M + Na]^+$ 735.3072, found 735.3058.

1-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,4-*C*-spirocyclopropylene-3-*O*-(triethylsilyl)-β-D-ribopentofuranosyl]-5-methylcytosine (4). To a solution of **2** (969 mg, 1.36 mmol) and triethylamine (2.79 mL, 20.1 mmol) in dry acetonitrile (15 mL) were added 1,2,4-triazole (1.39 g, 20.1 mmol) and phosphoryl chloride (0.38 mL, 4.03 mmol) at 0 °C. After the mixture was stirred at room temperature for 2 h, saturated aq $NaHCO_3$ was added, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The product **3** (1.06 g) was used immediately for the next reaction without further purification.

To a solution of **3** (1.06 g) in 1,4-dioxane (10 mL) was added aq ammonia (28 wt %, 1.26 mL, 67.0 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the resulting mixture was concentrated. The crude product was purified by column chromatography (SiO_2, $CHCl_3$/MeOH = 30:1) to afford **4** (958 mg, 98%, two steps) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.34−0.64 (m, 8H), 0.74−0.85 (m, 11H), 1.76 (s, 3H), 3.13 (d, *J* = 10.5 Hz, 1H), 3.15 (d, *J* = 10.2 Hz, 1H), 3.80 (s, 6H), 4.33 (s, 1H), 4.47 (s, 1H), 5.81 (s, 1H), 6.84 (dd, *J* = 2.7, 9.3 Hz, 4H), 7.22−7.36 (m, 7H), 7.46 (dd, *J* = 1.5, 8.4 Hz, 2H), 7.86 (s, 1H), 8.20 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 4.9, 5.1, 6.7, 9.8, 13.5, 55.4, 58.0, 68.3, 72.3, 79.6, 86.7, 88.0, 88.1, 102.4, 113.3, 113.4, 127.2, 128.1, 128.2, 130.1, 130.3, 135.4, 135.6, 137.6, 144.5, 156.2, 158.8, 166.3; IR (KBr) 3351, 3085, 2954, 2876, 1661, 1607, 1509, 1253, 1177, 1045, 832, 738 cm^{-1}; $[\alpha]_D^{28}$ −0.4 (*c* 1.00, MeOH); HRMS (MALDI) calcd for $C_{40}H_{49}N_3O_7NaSi$ $[M + Na]^+$ 734.3232, found 734.3238.

***N*^{4}-Benzoyl-1-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-spirocyclopropylene-3-*O*-(triethylsilyl)-β-D-ribopentofuranosyl]-5-methylcytosine (5).** To a solution of **4** (902 mg, 1.27 mmol) in dry pyridine (13 mL) was added benzoyl chloride (0.22 mL, 1.90 mmol) at 0 °C under Ar atmosphere, and the reaction mixture was stirred at room temperature for 3 h. After addition of saturated aq $NaHCO_3$, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 5:1) to afford **5** (861 mg, 83%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.40−0.60 (m, 8H), 0.79−0.87 (m, 11H), 1.91 (d, *J* = 0.9 Hz, 3H), 3.14 (d, *J* = 12.0 Hz, 1H), 3.16 (d, *J* = 10.5 Hz, 1H), 3.81 (s, 6H), 4.36 (s, 1H), 4.42 (s, 1H), 5.77 (s, 1H), 6.83−6.87 (m, 4H), 7.26−7.36 (m, 7H), 7.42−7.54 (m, 5H), 7.96 (d, *J* = 0.9 Hz, 1H), 8.33 (dd, *J* = 1.8, 8.4 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 4.9, 5.1, 6.7, 9.9, 13.9, 55.4, 57.8, 68.4, 72.3, 79.4, 86.8, 87.8, 88.6, 111.6, 113.4, 113.4, 127.2, 128.1, 128.2, 128.3, 130.0, 130.1, 130.2, 132.6, 135.3, 135.4, 136.2, 137.3, 144.5, 147.7, 158.9, 160.0, 179.7; IR (KBr) 3071, 2954, 2875, 1703, 1570, 1509, 1251, 1176, 1051, 832, 735 cm^{-1}; $[\alpha]_D^{25}$ + 47.4 (*c* 1.00, $CHCl_3$); HRMS (MALDI) calcd for $C_{47}H_{53}N_3O_8NaSi$ $[M + Na]^+$ 838.3494, found 838.3497.

***N*^{4}-Benzoyl-1-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl]-5-methylcytosine (6).** To a solution of **5** (701 mg, 0.86 mmol) in tetrahydrofuran (8 mL) was added 1 M tetrabutylammonium fluoride in tetrahydrofuran (2.58 mL, 2.58 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 10 min. After completion of reaction, the reaction mixture was concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 2:1) to afford **6** (528 mg, 88%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.51−0.54 (m, 1H), 0.75−0.94 (m, 3H), 1.92 (s, 3H), 2.03 (d, *J* = 9.9 Hz, 1H), 3.18 (d, *J* = 11.1 Hz, 1H), 3.34 (d, *J* = 10.8 Hz, 1H), 3.81 (s, 6H), 4.32 (d, *J* = 9.9 Hz, 1H), 4.48 (s, 1H), 5.82 (s, 1H), 6.86 (d, *J* = 8.7 Hz, 4H), 7.26−7.56 (m, 12H), 7.83 (s, 1H), 8.32 (d, *J* = 6.6 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 5.3, 9.7, 13.9, 55.4, 57.9, 67.9, 72.6, 79.5, 87.0, 87.1, 88.2, 111.8, 113.5, 127.3, 128.1, 128.2, 128.3, 130.0, 130.2, 130.2, 132.6, 135.3, 135.4, 136.0, 137.2, 144.4, 147.7, 158.8, 159.9, 179.7; IR (KBr) 3068, 2955, 2836, 1702, 1568, 1508, 1251, 1176, 1047, 834, 714 cm^{-1}; $[\alpha]_D^{25}$ +34.5 (*c* 0.99, MeOH); HRMS (MALDI) calcd for $C_{41}H_{39}N_3O_8Na$ $[M + Na]^+$ 724.2639, found 724.2624.

***N*^{4}-Benzoyl-1-[3-*O*-[2-cyanoethoxy(diisopropylamino)-phosphino]-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl]-5-methylcytosine (7).** To a solution of **6** (528 mg, 0.77 mmol) in dry acetonitrile (7 mL) were added *N*,*N*-diisopropylethylamine (0.39 mL, 2.26 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (0.25 mL, 1.13 mmol) at 0 °C under Ar atmosphere. After the solution was stirred at room temperature for 2 h, saturated aq $NaHCO_3$ was added, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, 0.5% triethylamine in *n*-hexane/AcOEt = 2:1) to afford **7** (529 mg, 78%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.39−0.43 (m, 1H), 0.71−0.88 (m, 3H), 0.98 (d, *J* = 6.6 Hz, 3H), 1.07 (d, *J* = 6.6 Hz, 3H), 1.11 (d, *J* = 6.9 Hz, 3H), 1.14 (d, *J* = 6.9 Hz, 3H), 1.86 (s, 3/2H), 1.88 (s, 3/2H), 2.36−2.40 (m, 1H), 2.52−2.57 (m, 1H), 3.17−3.31 (m, 2H), 3.49−3.57 (m, 3H), 3.64−3.77 (m, 1H), 3.81 (s, 3H), 3.81 (s, 3H), 4.40 (d, *J* = 6.6 Hz, 1/2H), 4.44 (d, *J* = 9.0 Hz, 1/2H), 4.65 (s, 1/2H), 4.69 (s, 1/2H), 5.82 (s, 1H), 6.82−6.89 (m, 4H), 7.25−7.52 (m, 12H), 7.88 (s, 1/2H), 7.91 (s, 1/2H), 8.33 (d, *J* = 6.9 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 5.5, 5.6, 9.9, 10.0, 13.8, 14.2, 14.3, 20.3, 20.4, 20.5, 20.5, 21.2, 22.8, 24.5, 24.6, 24.7, 29.1, 32.0, 43.3, 43.3, 43.5, 43.5, 55.4, 55.4, 57.7, 57.8, 58.0, 58.0, 58.2, 58.4, 60.5, 68.5, 72.5, 72.7, 73.1, 73.3, 78.2, 78.3, 78.9, 78.9, 86.9, 86.9, 87.9, 88.2, 88.3, 88.4, 88.5, 111.8, 111.8, 113.4, 113.4, 117.4, 117.5, 127.2, 127.3, 128.1, 128.2, 128.2, 128.2, 128.3, 130.0, 130.2, 130.3, 130.3, 132.5, 132.5, 135.2, 135.3, 135.3, 135.4, 136.1, 137.3, 137.4, 144.3, 144.4, 147.7, 147.8, 158.8, 158.9, 160.0, 160.1, 179.7; ^{31}P NMR (121.6 MHz, $CDCl_3$) δ 148.8, 148.9; HRMS (MALDI) calcd for $C_{50}H_{56}N_5O_9NaP$ $[M + Na]^+$ 924.3708, found 924.3722.

9-[2-*O*-Acetyl-3,5-di-*O*-benzyl-4-*C*-[1-[(*tert*-butyldimethylsilyl)oxy]cyclopropyl]-β-D-ribopentofuranosyl]-*N*^{6}-benzoyladenine (9). Compound **8** was synthesized as reported previously.[6] To a solution of **8** (49.0 mg, 83.8 μmol) in dry acetonitrile (1.5 mL) were added N^6-benzoyladenine (28.0 mg, 117 μmol), *N*,*O*-bis(trimethylsilyl)acetoamide (60.0 μL, 251 μmol), and trimethylsilyl trifluoromethanesulfonate (60.0 μL, 335 μmol) at 0 °C under N_2 atmosphere. After being stirred at 80 °C for 31 h, saturated aq $NaHCO_3$ was added, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2 $CHCl_3$/AcOEt = 7:1) to afford **9** (37.0 mg, 58%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.65−0.88 (m, 3H), 0.75 (s, 9H), 1.08−1.11 (m, 1H), 1.90 (s, 3H), 3.59 (d, *J* = 9.9 Hz, 1H), 4.01 (d, *J* = 9.9 Hz, 1H), 4.45−4.62 (m, 3H), 4.85 (d, *J* = 12.0 Hz, 1H), 5.00 (d, *J* = 11.1 Hz, 1H), 5.90 (dd, *J* =

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J. Org. Chem. 2016, 81, 11000−11008

4.8, 8.4 Hz, 1H), 6.42 (d, J = 8.4 Hz, 1H), 7.30−7.61 (m, 13H), 8.02 (d, J = 7.2 Hz, 2H), 8.51 (s, 1H), 8.78 (s, 1H), 8.99 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −3.4, −3.0, 7.2, 10.7, 18.0, 20.7, 25.8, 58.1, 73.8, 74.0, 75.3, 81.3, 84.7, 88.9, 123.0, 127.5, 127.6, 128.0, 128.3, 128.4, 128.4, 129.0, 132.9, 133.7, 137.1, 138.6, 141.8, 149.4, 152.6, 152.9, 164.8, 170.4; IR (KBr) 3062, 3029, 2952, 2930, 2858, 1747, 1609, 1454, 1240, 1072, 836, 700 cm^{-1}; $[\alpha]_D^{27}$ −48.1 (c 1.02, $CHCl_3$); HRMS (MALDI) calcd for $C_{42}H_{49}N_5O_7NaSi$ $[M + Na]^+$ 786.3294, found 786.3296.

9-[2-*O*-Acetyl-3,5-di-*O*-benzyl-4-*C*-[1-[(*tert*-butyldimethylsilyl)oxy]cyclopropyl]-β-D-ribopentofuranosyl]-*N*^{2}-isobutyrylguanine (10). To a solution of **8** (77.0 mg, 132 μmol) in dry acetonitrile (1.5 mL) were added N^2-isobutyrylguanine (41.0 mg, 184 μmol), *N*,*O*-bis(trimethylsilyl)acetoamide (97.0 μL, 395 μmol), and trimethylsilyl trifluoromethanesulfonate (48.0 μL, 263 μmol) at 0 °C under N_2 atmosphere. After the solution was stirred at 80 °C for 18 h, saturated aq $NaHCO_3$ was added, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by preparative thin-layer chromatography (SiO_2 *n*-hexane/AcOEt/MeOH = 10:2:1) to afford **10** (48.0 mg, 49%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.00 (s, 3H), 0.01 (s, 3H), 0.63−0.79 (m, 12H), 0.99−1.03 (m, 1H), 1.27 (d, J = 7.0 Hz, 6H), 1.93 (s, 3H), 2.53−2.56 (m, 1H), 3.53 (d, J = 10.0 Hz, 1H), 3.98 (d, J = 9.5 Hz, 1H), 4.45 (d, J = 10.5 Hz, 1H), 4.54 (d, J = 11.5 Hz, 1H), 4.54 (d, J = 4.5 Hz, 1H), 4.80 (d, J = 12.0 Hz, 1H), 5.00 (d, J = 10.5 Hz, 1H), 5.82 (dd, J = 4.5, 8.5 Hz, 1H), 6.00 (d, J = 8.5 Hz, 1H), 7.31−7.45 (m, 10H), 7.97 (s, 1H), 8.02 (s, 1H), 11.9 (s, 1H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ −3.4, −3.0, 7.2, 10.6, 18.0, 19.1, 20.7, 25.8, 36.7, 58.0, 73.7, 74.0, 75.3, 77.0, 81.1, 84.2, 88.5, 121.0, 127.7, 128.3, 128.4, 128.4, 128.9, 137.1, 137.5, 138.7, 147.4, 148.8, 155.6, 170.2, 178.0; IR (KBr) 2930, 2858, 1678, 1607, 1254, 1073 cm^{-1}; $[\alpha]_D^{29}$ −33.6 (c 1.10, $CHCl_3$); HRMS (MALDI) calcd for $C_{39}H_{51}N_5O_8NaSi$ $[M + Na]^+$ 768.3399, found 768.3398.

***N*^{6}-Benzoyl-9-[3,5-di-*O*-benzyl-4-*C*-[1-[(*tert*-butyldimethylsilyl)oxy]cyclopropyl]-β-D-ribopentofuranosyl]adenine (11).** To a solution of **9** (2.20 g, 2.88 mmol) in methanol (40 mL) was added potassium carbonate (795 mg, 5.75 mmol) at 0 °C, and the reaction mixture was stirred at the same temperature for 20 min. After addition of water, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 3:2) to afford **11** (2.05 g, 99%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.03 (s, 6H), 0.69−0.84 (m, 3H), 0.79 (s, 9H), 1.06−1.09 (m, 1H), 3.17 (d, J = 10.8 Hz, 1H), 3.57 (d, J = 10.2 Hz, 1H), 4.01 (d, J = 9.6 Hz, 1H), 4.27 (d, J = 5.1 Hz, 1H), 4.51 (d, J = 11.7 Hz, 1H), 4.58 (d, J = 10.5 Hz, 1H), 4.80 (d, J = 12.0 Hz, 1H), 4.97 (m, 1H), 5.24 (d, J = 10.5 Hz, 1H), 5.99 (d, J = 7.5 Hz, 1H), 7.37−7.39 (m, 10H), 7.49−7.63 (m, 3H), 8.02 (d, J = 7.5 Hz, 2H), 8.46 (s, 1H), 8.76 (s, 1H), 8.98 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −3.3, −3.0, 7.3, 10.7, 18.0, 25.8, 58.2, 74.1, 75.8, 76.9, 83.1, 88.0, 88.3, 122.9, 128.0, 128.1, 128.2, 128.3, 128.5, 128.7, 128.9, 128.9, 132.8, 133.8, 137.0, 137.9, 141.6, 149.4, 152.5, 152.7, 164.8; IR (KBr) 3328, 3030, 2929, 1615, 1455, 1256, 1069, 836, 730 cm^{-1}; $[\alpha]_D^{23}$ −62.5 (c 1.02, $CHCl_3$); HRMS (MALDI) calcd for $C_{40}H_{47}N_5O_6NaSi$ $[M + Na]^+$ 744.3188, found 744.3186.

9-[3,5-Di-*O*-benzyl-4-*C*-[1-[(*tert*-butyldimethylsilyl)oxy]-cyclopropyl]-β-D-ribopentofuranosyl]-*N*^{2}-isobutyrylguanine (12). To a solution of **10** (48.0 mg, 65.6 μmol) in methanol (1.5 mL) was added potassium carbonate (27.0 mg, 197 μmol) at 0 °C, and the reaction mixture was stirred at the same temperature for 1 h. After addition of water, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1:2) to afford **12** (44.0 mg, 95%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.03 (s, 6H), 0.68−0.84 (m, 12H), 0.95−1.02 (m, 1H), 1.23 (d, J = 2.1 Hz, 3H), 1.25 (d, J = 1.8 Hz, 3H), 2.48−2.57 (m, 1H), 3.10 (d, J = 11.4 Hz, 1H), 3.51 (d, J = 9.6 Hz, 1H), 3.97 (d, J = 9.6 Hz, 1H), 4.24 (d, J = 5.1 Hz, 1H), 4.48 (d, J = 11.4 Hz, 1H), 4.56 (d, J = 10.5 Hz, 1H), 4.76 (d, J = 12.0 Hz, 1H), 4.86 (m, 1H), 5.21 (d, J = 10.8 Hz, 1H), 5.57 (d, J = 8.1 Hz, 1H), 7.31−7.42 (m, 10H), 8.04 (s, 1H), 8.11 (s, 1H), 11.9 (s, 1H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ −3.3, −2.9, 7.4, 10.7, 18.0, 18.9, 19.1, 25.8, 36.6, 58.0, 74.0, 74.1, 75.9, 76.9, 83.2, 87.9, 88.3, 121.0, 128.1, 128.3, 128.5, 128.6, 128.9, 137.1, 137.3, 138.0, 147.4, 148.5, 155.7, 178.2; IR (KBr) 2931, 2857, 1682, 1606, 1255, 1074 cm^{-1}; $[\alpha]_D^{29}$ −42.3 (c 0.94, $CHCl_3$); HRMS (MALDI) calcd for $C_{37}H_{49}N_5O_7NaSi$ $[M + Na]^+$ 726.3294, found 726.3293.

***N*^{6}-Benzoyl-9-(3,5-di-*O*-benzyl-2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl)adenine (17).** To a solution of **11** (22.0 g, 30.5 mmol) in dichloromethane (300 mL) were added iodobenzene diacetate (11.3 g, 35.1 mmol) and 2-hydroxy-2-azaadamantane (233 mg, 1.50 mmol) at 0 °C. After the solution was stirred at room temperature for 5 h, saturated aq $Na_2S_2O_3$ and saturated aq $NaHCO_3$ were added to the mixture, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude ketone **13** (26.7 g) was used immediately for the next reaction without further purification.

To a solution of the crude ketone **13** (26.7 g) in dry tetrahydrofuran (300 mL) was added 1 M diisobutylaluminum hydride in *n*-hexane (153 mL, 153 mmol) at 0 °C under N_2 atmosphere. After the solution was stirred at the same temperature for 30 min, saturated aq $KNaC_4H_4O_6$ was carefully added at 0 °C, and the resulting mixture was further stirred at room temperature for 1 h. The mixture was then extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to afford 7,8-dihydroadenine compound (21.3 g). The reduced product was used for the next reaction without further purification.

To a solution of the reduced product (21.3 g) in dichloromethane (300 mL) was added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (7.60 g, 33.6 mmol) at 0 °C. After the solution was stirred at room temperature for 30 min, the precipitate was filtered and washed with dichloromethane. The other impurities in the filtrate were removed by addition of activated charcoal. The charcoal was then filtered and washed with dichloromethane, and the resulting filtrate was concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1:1) to afford **14**/**11** (9.10 g) as an inseparable diastereomixture. To analyze the structure of **14**, the following reaction was carried out. To a solution of the diastereomixture (100 mg) in dry pyridine (3 mL) was added methanesulfonyl chloride (1.80 μL, 23.0 μmol) at 0 °C under N_2 atmosphere, and the reaction mixture was stirred at room temperature for 30 min. After addition of saturated aq $NaHCO_3$, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 3:2 to 1:3). As a result, only **11** was mesylated, and **14** (61 mg) was isolated as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.05 (s, 3H), −0.02 (s, 3H), 0.61−1.04 (m, 4H), 0.73 (s, 9H), 3.75 (d, J = 9.9 Hz, 1H), 4.17 (d, J = 9.9 Hz, 1H), 4.27 (s, 1H), 4.28 (dd, J = 3.3, 11.4 Hz, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.75 (s, 1H), 4.85 (d, J = 11.7 Hz, 1H), 5.50 (d, J = 11.7 Hz, 1H), 6.41 (d, J = 3.0 Hz, 1H), 7.29−7.42 (m, 10H), 7.50−7.63 (m, 3H), 8.02 (dd, J = 1.5, 7.2 Hz, 2H), 8.35 (s, 1H), 8.76 (s, 1H), 9.03 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −3.4, −3.1, 8.0, 11.0, 17.9, 25.7, 58.0, 73.5, 74.0, 74.3, 74.8, 86.8, 87.2, 122.5, 127.1, 127.6, 128.0, 128.3, 128.5, 128.8, 128.9, 129.1, 132.7, 133.9, 135.7, 138.0, 142.9, 149.3, 151.6, 152.5, 164.9; IR (KBr) 2950, 2929, 2855, 1612, 1455, 1254, 1101, 706, 510 cm^{-1}; $[\alpha]_D^{28}$ −16.1 (c 1.04, $CHCl_3$); HRMS (MALDI) calcd for $C_{40}H_{47}N_5O_6NaSi$ $[M + Na]^+$ 744.3188, found 744.3198.

To a solution of the diastereomixture (9.00 g, 12.5 mmol) in dry dichloromethane (150 mL) were added 4-(dimethylamino)pyridine (4.58 g, 37.5 mmol) and trifluoromethanesulfonic anhydride (2.70 mL, 16.2 mmol) at 0 °C, and the reaction mixture was stirred at the same temperature for 14 h under N_2 atmosphere. After addition of saturated aq $NaHCO_3$, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product (11.2 g) was used immediately for the next reaction without further purification.

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To a solution of the above product (11.2 g) in tetrahydrofuran (120 mL) was added 1 M tetrabutylammonium fluoride in tetrahydrofuran (37.5 mL, 37.5 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 30 min. After completion of the reaction, the mixture was concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1:1 to 1:3) to afford **17** (5.60 g, 31%, five steps) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.74−0.79 (m, 1H), 0.85−1.03 (m, 3H), 3.56 (d, J = 10.8 Hz, 1H), 3.66 (d, J = 10.8 Hz, 1H), 4.38 (s, 1H), 4.58 (s, 2H), 4.58 (d, J = 12.0 Hz, 1H), 4.66 (d, J = 11.7 Hz, 1H), 4.82 (s, 1H), 6.21 (s, 1H), 7.24−7.38 (m, 10H), 7.52−7.63 (m, 3H), 8.03 (dd, J = 1.2, 6.9 Hz, 2H), 8.26 (s, 1H), 8.76 (s, 1H), 8.93 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 5.8, 10.0, 64.5, 69.1, 72.4, 74.0, 79.2, 86.7, 87.4, 123.7, 127.6, 127.7, 128.0, 128.1, 128.5, 128.7, 129.0, 132.9, 133.6, 137.4, 137.4, 141.1, 149.6, 151.0, 152.8, 164.8; IR (KBr) 3062, 2929, 1610, 1580, 1454, 1248, 1048, 1030, 700 cm^{-1}; $[\alpha]_D^{23}$ −5.4 (*c* 1.00, $CHCl_3$); HRMS (MALDI) calcd for $C_{34}H_{31}N_5O_5Na$ $[M + Na]^+$ 612.2217, found 612.2218.

9-(3,5-Di-*O*-benzyl-2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl)adenine (18). To a solution of **17** (5.60 g, 9.50 mmol) in tetrahydrofuran (100 mL) was added aq methylamine (40 wt %, 15.8 mL, 190 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 40 min. After completion of the reaction, the resulting mixture was concentrated and extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 3:1 to AcOEt only) to afford **18** (4.15 g, 90%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.71−0.78 (m, 1H), 0.85−1.04 (m, 3H), 3.56 (d, J = 11.1 Hz, 1H), 3.66 (d, J = 11.1 Hz, 1H), 4.36 (s, 1H), 4.57(s, 2H), 4.57 (d, J = 11.7 Hz, 1H), 4.66 (d, J = 12.0 Hz, 1H), 4.80 (s, 1H), 5.58 (s, 2H), 6.15 (s, 1H), 7.23−7.39 (m, 10H), 8.00 (s, 1H), 8.33 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 5.8, 9.9, 64.7, 69.0, 72.3, 73.9, 79.2, 86.5, 87.1, 120.1, 127.6, 127.7, 128.0, 128.5, 128.6, 137.4, 137.5, 138.4, 149.0, 153.2, 155.7; IR (KBr) 3317, 3149, 3031, 2871, 1651, 1599, 1471, 1298, 1041, 739, 698 cm^{-1}; $[\alpha]_D^{26}$ −0.3 (*c* 1.02, $CHCl_3$); HRMS (MALDI) calcd for $C_{27}H_{27}N_5O_4Na$ $[M + Na]^+$ 508.1955, found 508.1954.

9-(2-*O*,4-*C*-Spirocyclopropylene-β-D-ribopentofuranosyl)-adenine (19). To a solution of **18** (450 mg, 927 μmol) in ethyl acetate/acetic acid (31 mL, 100:3) were added palladium hydroxide 20% on carbon (97.6 mg) and ammonium formate (3.50 g, 55.6 mmol) at room temperature. After the solution was refluxed for 4 h, palladium hydroxide 20% on carbon (97.6 mg) was added at room temperature, and the mixture was further refluxed for 2 h. The reaction mixture was then filtered and washed by AcOEt. After removal of the solvent, the crude product was purified by column chromatography (SiO_2, $CHCl_3$/MeOH = 10:1) to afford **19** (130 mg, 46%) as white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 0.78−0.96 (m, 4H), 3.63 (d, J = 12.6 Hz, 1H), 3.80 (d, J = 12.6 Hz, 1H), 4.48 (s, 1H), 4.57 (s, 1H), 6.11 (s, 1H), 8.20 (s, 1H), 8.31 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 5.5, 10.0, 57.3, 69.1, 73.0, 81.2, 87.3, 89.6, 120.4, 139.8, 149.6, 153.9, 157.3; IR (KBr) 3345, 3198, 1653, 1602, 1043, 653 cm^{-1}; $[\alpha]_D^{30}$ −49.4 (*c* 0.96, $CHCl_3$); HRMS (MALDI) calcd for $C_{13}H_{16}N_5O_4$ $[M + H]^+$ 306.1197, found 306.1197.

***N*^{6}-Benzoyl-9-(2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl)adenine (20).** To a solution of **19** (1.17 g, 3.83 mmol) in dry pyridine (40 mL) was added chlorotrimethylsilane (0.97 mL, 7.66 mmol) at 0 °C under N_2 atmosphere. After the solution was stirred at the same temperature for 1 h, benzoyl chloride (1.34 mL, 11.5 mmol) was added. The mixture was then stirred at room temperature for 3 h, and to the resulting mixture was added aq ammonia (28 wt %, 18.0 mL, 268 mmol) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated. The crude product was purified by column chromatography (SiO_2, $CHCl_3$/MeOH = 30:1 to 10:1) to afford **20** (925 mg, 59%) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 0.80−0.97 (m, 4H), 3.65 (d, J = 12.6 Hz, 1H), 3.81 (d, J = 12.6 Hz, 1H), 4.51 (s, 1H), 4.68 (s, 1H), 6.23 (s, 1H), 7.55−7.69 (m, 3H), 8.09 (d, J = 7.2 Hz, 2H), 8.60 (s, 1H), 8.73 (s, 1H) ; ^{13}C NMR (75.5 MHz, CD_3OD) δ 5.5, 10.1, 57.3, 69.2, 73.1, 81.1, 87.6, 89.8, 125.4, 129.5, 129.8, 133.9, 134.9, 143.1, 151.1, 152.5, 153.3, 168.1; IR (KBr) 3321, 1615, 1458, 1259, 1043 cm^{-1}; $[\alpha]_D^{25}$ −48.1 (*c* 0.34, CH_3OH); HRMS (MALDI) calcd for $C_{20}H_{19}N_5O_5Na$ $[M + Na]^+$ 432.1278, found 432.1280.

***N*^{6}-Benzoyl-9-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl]adenine (21).** To a solution of **20** (925 mg, 2.26 mmol) in dry pyridine (20 mL) was added 4,4′-dimethoxytrityl chloride (1.15 g, 3.39 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 1 h under N_2 atmosphere. After addition of water, the resulting mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1:1 to AcOEt only) to afford **21** (1.68 g, quant) as a white solid: ^{1}H NMR (300 HMz, $CDCl_3$) δ 0.57−0.65 (m, 1H), 0.85−1.08 (m, 3H), 2.46 (d, J = 9.6 Hz, 1H), 3.23 (d, J = 10.8 Hz, 1H), 3.49 (d, J = 10.8 Hz, 1H), 3.79 (s, 6H), 4.48 (d, J = 9.3 Hz, 1H), 4.72 (s, 1H), 6.28 (s, 1H), 6.85 (d, J = 9.0 Hz, 4H), 7.20−7.65 (m, 12H), 8.03 (d, J = 7.2 Hz, 1H), 8.31 (s, 1H), 8.80 (s, 1H), 9.03 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 5.8, 9.7, 55.4, 59.0, 68.5, 74.2, 79.8, 86.2, 86.9, 87.5, 113.4, 113.4, 123.7, 127.2, 128.0, 128.1, 128.2, 129.0, 130.0, 130.1, 133.0, 133.6, 135.1, 135.5, 140.6, 144.3, 149.6, 151.0, 152.9, 158.7, 158.7, 164.8; IR (KBr) 3271, 3058, 3004, 2836, 1609, 1509, 1455, 1251, 1033, 751 cm^{-1}; $[\alpha]_D^{24}$ −44.4 (*c* 1.00, $CHCl_3$); HRMS (MALDI) calcd for $C_{41}H_{37}N_5O_7Na$ $[M + Na]^+$ 734.2585, found 734.2581.

***N*^{6}-Benzoyl-9-[3-*O*-[2-cyanoethoxy(diisopropylamino)-phosphino]-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl]adenine (22).** To a solution of **21** (800 mg, 1.12 mmol) in dry acetonitrile (7 mL) were added *N*,*N*-diisopropylethylamine (0.59 mL, 3.37 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (0.38 mL, 1.69 mmol) at 0 °C under N_2 atmosphere. After the solution was stirred at room temperature for 3 h, saturated aq $NaHCO_3$ was added, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1:2) to afford **22** (864 mg, 85%) as a white solid: ^{1}H NMR (300 HMz, $CDCl_3$) δ 0.42−0.54 (m, 1H), 0.77−1.04 (m, 3H), 0.91 (d, J = 6.9 Hz, 3H), 0.97 (d, J = 6.9 Hz, 3H), 1.08 (d, J = 6.3 Hz, 3H), 1.10 (d, J = 6.0 Hz, 3H), 2.34 (t, J = 6.3 Hz, 1H), 2.44 (t, J = 6.3 Hz, 1H), 3.17 (d, J = 10.5 Hz, 1/2H), 3.20 (d, J = 10.2 Hz, 1/2H), 3.42−3.75 (m, 5H), 3.79 (s, 3H), 3.80 (s, 3H), 4.54 (d, J = 6.9 Hz, 1/2H), 4.57 (d, J = 8.4 Hz, 1/2H), 4.91 (s, 1/2H), 4.93 (s, 1/2H), 6.30 (s, 1/2H), 6.31 (s, 1/2H), 6.82−6.86 (m, 4H), 7.19−7.37 (m, 7H), 7.45−7.48 (m, 2H), 7.52−7.65 (m, 3H), 8.05 (d, J = 8.4 Hz, 2/2H), 8.05 (d, J = 8.4 Hz, 2/2H), 8.38 (s, 1/2H), 8.41 (s, 1/2H), 8.82 (s, 1/2H), 8.83 (s, 1/2H), 9.10 (s, 1/2H), 9.11 (s, 1/2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 6.1, 6.2, 9.9, 10.1, 20.2, 20.3, 20.4, 24.4, 24.5, 24.5, 24.6, 24.6, 24.7, 43.2, 43.3, 43.4, 55.3, 55.4, 57.9, 58.1, 58.2, 58.5, 58.9, 59.1, 69.1, 73.9, 74.1, 74.5, 74.7, 78.5, 78.5, 79.1, 86.7, 86.8, 87.0, 87.0, 87.7, 87.8, 87.9, 87.9, 113.3, 113.4, 117.4, 117.5, 123.7, 123.8, 127.1, 127.1, 128.0, 128.1, 128.1, 128.2, 129.0, 130.1, 130.1, 130.2, 132.9, 133.8, 135.2, 135.6, 135.6, 140.7, 140.8, 144.3, 144.4, 149.6, 151.0, 151.1, 152.9, 152.9, 158.7, 158.7, 158.7, 164.7; ^{31}P NMR (121.6 MHz, $CDCl_3$) δ 148.9,148.9; HRMS (MALDI) calcd for $C_{50}H_{54}N_7O_8NaP$ $[M + Na]^+$ 934.3664, found 934.3657.

9-[3,5-Di-*O*-benzyl-4-*C*-[1-[(*tert*-butyldimethylsilyl)oxy]-cyclopropyl]-β-D-arabinopentofuranosyl]-*N*^{2}-isobutyrylguanine (16). To a solution of **12** (1.62 g, 2.24 mmol) in dichloromethane (30 mL) were added iodobenzene diacetate (1.08 g, 3.36 mmol) and 2-hydroxy-2-azaadamantane (17.0 mg, 112 μmol) at 0 °C. After being stirred at room temperature for 5 h, saturated aq $Na_2S_2O_3$ and saturated aq $NaHCO_3$ were added to the mixture, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude ketone **15** (1.94 g) was used immediately for the next reaction without further purification.

To a solution of the crude ketone **15** (1.94 g) in methanol/dichloromethane (30 mL, 1:2) was added sodium borohydride (119 mg, 3.14 mmol) at 0 °C. After the solution was stirred at the same

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temperature for 30 min, saturated aq NH_4Cl was added to the mixture, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 2:1−1:1) to afford **16** (492 mg, 31%, two steps) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.03 (s, 3H), 0.00 (s, 3H), 0.65−0.93 (m, 4H), 0.73 (s, 9H), 1.21 (d, J = 5.1 Hz, 3H), 1.24 (d, J = 5.1 Hz, 3H), 2.43−2.57 (m, 1H), 3.73 (d, J = 9.9 Hz, 1H), 4.14 (d, J = 9.9 Hz, 1H), 4.21 (s, 1H), 4.31 (dd, J = 3.0, 11.4 Hz, 1H), 4.56 (d, J = 11.7 Hz, 1H), 4.70 (d, J = 11.7 Hz, 1H), 4.76 (d, J = 11.7 Hz, 1H), 4.81 (d, J = 11.7 Hz, 1H), 5.47 (d, J = 11.1 Hz, 1H), 5.99 (d, J = 3.3 Hz, 1H), 7.29−7.43 (m, 10H), 7.89 (s, 1H), 8.05 (s, 1H), 11.8 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −3.4, −3.0, 8.2, 10.8, 17.9, 18.9, 18.9, 25.7, 36.4, 57.8, 73.6, 73.8, 74.3, 75.0, 87.4, 87.4, 87.5, 120.5, 127.0, 127.5, 128.3, 128.5, 128.8, 129.0, 136.1, 138.2, 138.5, 147.2, 147.4, 155.4, 178.3; IR (KBr) 2928, 2856, 1683, 1609, 1254, 1102 cm^{-1}; $[\alpha]_D^{29}$ −0.67 (*c* 1.05, $CHCl_3$); HRMS (MALDI) calcd for $C_{37}H_{49}N_5O_7NaSi$ $[M + Na]^+$ 726.3294, found 726.3293.

9-(3,5-Di-*O*-benzyl-2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl)-*N*^{2}-isobutyrylguanine (24). To a solution of **16** (1.84 g, 2.61 mmol) in dry dichloromethane (30 mL) were added triethylamine (5.50 mL, 39.2 mmol) and methanesulfonyl chloride (2.02 mL, 26.1 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 20 min under N_2 atmosphere. After addition of saturated aq $NaHCO_3$, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product (2.58 g) was used immediately for the next reaction without further purification.

To a solution of the above product (2.58 g) in tetrahydrofuran (52 mL) was added 1 M tetrabutylammonium fluoride in tetrahydrofuran (7.83 mL, 7.83 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. After addition of saturated aq NH_4Cl, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1:1 to 1:2) to afford **23** (997 mg, 57%, two steps) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.66−0.88 (m, 4H), 1.25 (d, J = 0.9 Hz, 3H), 1.27 (d, J = 0.9 Hz, 3H), 2.57−2.66 (m, 1H), 2.59 (s, 3H), 3.59 (d, J = 10.5 Hz, 1H), 3.68 (s, 1H), 3.90 (d, J = 11.1 Hz, 1H), 4.45 (d, J = 12.0 Hz, 1H), 4.57 (d, J = 11.7 Hz, 1H), 4.64 (d, J = 11.4 Hz, 1H), 4.83 (d, J = 11.4 Hz, 1H), 5.02 (d, J = 6.3 Hz, 1H), 5.60 (t, J = 6.6 Hz, 1H), 6.30 (d, J = 6.6 Hz, 1H), 7.30−7.39 (m, 10H), 8.03 (s, 1H), 8.45 (s, 1H), 12.0 (s, 1H); HRMS (MALDI) calcd for $C_{32}H_{37}N_5O_9NaS$ $[M + Na]^+$ 690.2204, found 690.2200.

To a solution of **23** (915 mg, 1.37 mmol) in *N*,*N*-dimethylformamide (20 mL) was added potassium carbonate (580 mg, 4.20 mmol) at room temperature, and the reaction mixture was stirred at 80 °C for 22 h. After addition of water, the resulting mixture was extracted with Et_2O. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 2:3) to afford **24** (720 mg, 92%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.71−1.02 (m, 4H), 1.27 (d, J = 1.5 Hz, 3H), 1.29 (d, J = 1.5 Hz, 3H), 2.55−2.69 (m, 1H), 3.53 (d, J = 11.4 Hz, 1H), 3.63 (d, J = 10.8 Hz, 1H), 4.24 (s, 1H), 4.45 (s, 1H), 4.53−4.62 (m, 4H), 5.92 (s, 1H), 7.22−7.38 (m, 10H), 7.82 (s, 1H), 8.30 (s, 1H), 12.0 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 5.7, 10.0, 19.1, 36.4, 64.5, 69.0, 72.3, 73.9, 77.5, 78.8, 86.2, 87.1, 121.6, 127.6, 127.6, 128.0, 128.1, 128.5, 128.6, 136.2, 137.3, 137.3, 147.3, 147.9, 155.7, 179.1; IR (KBr) 3152, 3031, 2936, 2875, 1680, 1610, 1558, 1405, 1155, 1048, 737 cm^{-1}; $[\alpha]_D^{29}$ 1.89 (*c* 1.06, $CHCl_3$); HRMS (MALDI) calcd for $C_{31}H_{33}N_5O_6Na$ $[M + Na]^+$ 594.2323, found 594.2324.

***N*^{2}-Isobutyryl-9-(2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl)guanine (25).** To a solution of **24** (600 mg, 1.05 mmol) in ethanol (10 mL) was added palladium hydroxide 20% on carbon (200 mg), and the mixture was stirred at room temperature for 20 h under H_2 atmosphere. After completion of the reaction, the mixture was filtered and washed by AcOEt. The filtrate was concentrated, and the crude product was purified by column chromatography (SiO_2, $CHCl_3$/MeOH = 15:1 to 10:1) to afford **25** (289 mg, 70%) as white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 0.76−1.04 (m, 4H), 1.22 (d, J = 6.9 Hz, 3H), 1.22 (d, J = 6.9 Hz, 3H), 2.63−2.77 (m, 1H), 3.62 (d, J = 12.6 Hz, 1H), 3.79 (d, J = 12.6 Hz, 1H), 4.42 (s, 1H), 4.54 (s, 1H), 5.98 (s, 1H), 8.14 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 5.5, 10.0, 19.3, 37.0, 57.3, 69.1, 72.9, 81.2, 87.3, 89.6, 121.5, 138.4, 149.4, 149.8, 157.3, 181.7; IR (KBr) 3306, 3201, 2977, 2938, 1687, 1609, 1561, 1404, 1044 cm^{-1}; $[\alpha]_D^{30}$ −41.3 (*c* 1.10, CH_3OH); HRMS (MALDI) calcd for $C_{17}H_{21}N_5O_6Na$ $[M + Na]^+$ 414.1384, found 414.1380.

9-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl]-*N*^{2}-isobutyrylguanine (26). To a solution of **25** (210 mg, 537 μmol) in dry pyridine (8 mL) was added 4,4′-dimethoxytrityl chloride (283 mg, 835 μmol) at 0 °C, and the reaction mixture was stirred at room temperature for 4 h under N_2 atmosphere. After addition of water, the resulting mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, $CHCl_3$/MeOH = 50:1 to 20:1) to afford **26** (371 mg, quant) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.55−0.63 (m, 1H), 0.81−0.85 (m, 2H), 1.03−0.95 (m, 1H), 1.22 (d, J = 6.9 Hz, 3H), 1.23 (d, J = 6.9 Hz, 3H), 2.67−2.76 (m, 1H), 3.17 (d, J = 11.1 Hz, 1H), 3.52 (d, J = 11.1 Hz, 1H), 3.62 (d, J = 6.3 Hz, 1H), 3.73 (s, 3H), 3.73 (s, 3H), 4.53 (s, 1H), 4.54 (d, J = 6.3 Hz, 1H), 5.87 (s, 1H), 6.77 (d, J = 9.0 Hz, 4H), 7.12−7.32 (m, 7H), 7.41 (d, J = 7.5 Hz, 2H), 7.91 (s, 1H), 9.64 (s, 1H), 12.1 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 5.8, 9.7, 19.0, 19.1, 36.4, 55.3, 59.3, 68.7, 74.0, 79.9, 86.2, 86.8, 87.3, 113.3, 113.4, 121.4, 127.1, 128.1, 128.1, 130.1, 130.1, 135.2, 135.6, 136.8, 144.4, 147.5, 147.9, 155.8, 158.7, 179.3; IR (KBr) 3131, 2978, 1685, 1607, 1509, 1254, 506 cm^{-1}; $[\alpha]_D^{29}$ −52.0 (*c* 1.04, $CHCl_3$); HRMS (MALDI) calcd for $C_{38}H_{39}N_5O_8Na$ $[M + Na]^+$ 716.2691, found 716.2695.

9-[3-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-spirocyclopropylene-β-D-ribopentofuranosyl]-*N*^{2}-isobutyrylguanine (27). To a solution of **26** (215 mg, 31.0 μmol) in dry acetonitrile (3 mL) were added *N*,*N*-diisopropylethylamine (0.17 mL, 948 μmol) and 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (0.17 mL, 758 μmol) at 0 °C under N_2 atmosphere. After the solution was stirred at room temperature for 3 h, saturated aq $NaHCO_3$ was added, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1:3) to afford **27** (211 mg, 76%) as a white solid: ^{1}H NMR (300 HMz, $CDCl_3$) δ 0.43−0.53 (m, 1H), 0.79−0.84 (m, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 1.07 (d, J = 6.6 Hz, 3H), 1.08 (d, J = 6.9 Hz, 3H), 1.26 (d, J = 6.9 Hz, 3H), 1.26 (d, J = 6.9 Hz, 3H), 2.44−2.63 (m, 3H), 3.09 (d, J = 10.8 Hz, 1/2H), 3.15 (d, J = 11.1 Hz, 1/2H), 3.35−3.72 (m, 5H), 3.79 (s, 3H), 3.80 (s, 3H), 4.36 (d, J = 5.4 Hz, 1/2H), 4.52 (d, J = 7.5 Hz, 1/2H), 4.91 (s, 1/2H), 5.00 (s, 1/2H), 5.99 (s, 1H), 6.81−6.85 (m, 4H), 7.21−7.36 (m, 7H), 7.45 (d, J = 6.9 Hz, 2/2H), 7.45 (d, J = 6.0 Hz, 2/2H), 8.00 (s, 1/2H), 8.02 (s, 1/2H), 8.59 (s, 1/2H), 8.64 (s, 1/2H), 12.0 (s, 1/2H), 12.0 (s, 1/2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 5.9, 6.2, 10.0, 18.9, 19.0, 19.1, 20.2, 20.3, 20.4, 24.1, 24.2, 24.4, 24.5, 24.6, 36.3, 36.4, 43.3, 43.5, 43.5, 43.7, 55.3, 55.3, 57.4, 57.6, 58.4, 58.6, 58.7, 59.2, 69.1, 69.2, 73.5, 73.7, 74.7, 74.9, 78.6, 86.6, 86.7, 87.0, 87.2, 87.5, 87.6, 87.7, 87.7, 113.3, 113.3, 117.7, 117.8, 122.1, 127.1, 128.0, 128.1, 130.0, 130.1, 135.2, 135.3, 135.4, 135.5, 136.1, 136.2, 144.3, 147.2, 147.2, 147.8, 147.8, 155.7, 155.8, 158.7, 178.9, 179.0; ^{31}P NMR (121.6 MHz, $CDCl_3$) δ 145.2,146.9; HRMS (MALDI) calcd for $C_{47}H_{56}N_7O_9NaP$ $[M + Na]^+$ 916.3769, found 916.3775.

Synthesis, Purification, And Characterization of Oligonucleotides. Oligonucleotide synthesis was performed on a 0.2 μmol scale for **ON2a**−**ON6a** and on a 1.0 μmol scale for **ON7a**, **ON7b**, and **ON8** according to the standard phosphoramidite protocol and 5-(ethylthio)-1*H*-tetrazole as the activator. The coupling time of phosphoramidite **7**, **22**, and **27** was prolonged from 32 s to 8 min (for 0.2 μmol scale) and from 40 s to 10 min (for 1.0 μmol scale). The synthesis was carried out in trityl-on mode, and the solid-supported

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J. Org. Chem. 2016, 81, 11000−11008

oligonucleotides were treated with concentrated ammonium hydroxide at 55 °C for 12 h. Compounds **ON2a**–**ON6a** were briefly purified with a Sep-Pak Plus C_{18} cartridge, and **ON7a**, **ON7b**, and **ON8** were purified with a Sep-Pak Plus C_{18} environmental cartridge. The **ON2a**–**ON4a** and **ON6a** were further purified by reversed-phase HPLC with 2.5 μm (10 × 50 mm) columns with a linear gradient of MeCN (8 to 16% over 20 min) in 0.1 M triethylammonium acetate buffer (pH 7.0). The **ON5a** was purified with a linear gradient of MeCN (6 to 12% over 20 min). Compounds **ON7a**, **7b**, and **ON8** were purified with a linear gradient of MeCN (8–16% over 20 min), and fractions including impurities were further purified with a linear gradient of MeCN (6–12% over 20 min). The purity of the oligonucleotides were analyzed by reversed-phase HPLC with 2.5 μm (4.6 × 50 mm) columns and characterized by MALDI-TOF mass spectrometer.

UV Melting Experiments. The UV melting experiments were carried out on UV spectrometers equipped with a T_m analysis accessory. Equimolecular amounts of the target RNA or DNA strand and oligonucleotide were dissolved in buffer (10 mM phosphate buffer at pH 7.2 containing 10 mM NaCl) to give final strand concentration of 4 μM. The samples were annealed by heating at 100 °C followed by slow cooling to room temperature. The melting profile was recorded at 260 nm from 5 to 90 °C at a scan rate of 0.5 °C/min. The T_m value was calculated as the temperature of the half-dissociation of the formed duplexes based on the first derivative of the melting curve.

Enzymatic Stability Evaluations. The sample solutions (130 μL) were prepared by dissolving 4 μM oligonucleotide and 10 mM $MgCl_2$ in 50 mM Tris–HCl buffer (pH 8.0). In each sample solution, 1 μg/mL of CAVP was added, and the cleavage reaction was conducted at 37 °C. A portion of each reaction mixture was taken away at time intervals, and the nuclease was immediately deactivated by heating at 90 °C for 2.5 min. Aliquots of those samples were analyzed by reversed-phase HPLC with 2.5 μm (4.6 × 50 mm) columns to evaluate the amount of remaining intact oligonucleotides. The percentage of intact oligonucleotide in each sample was calculated and plotted against the reaction time.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02036.

^{1}H and ^{13}C NMR spectra of new compounds (**2**, **4**–**7**, **9**–**12**, **14**, **16**–**22**, **24**–**27**), ^{31}P spectrum of new amidites (**7**, **22**, **27**), and HPLC charts and MALDI-TOF mass data of oligonucleotides (PDF)

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Notes

The authors declare no competing financial interest.

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