JOC The Journal of Organic Chemistry

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Synthesis and Properties of 7-Deazapurine- and 8-Aza-7-deazapurine-Locked Nucleic Acid Analogues: Effect of the Glycosidic Torsion Angle

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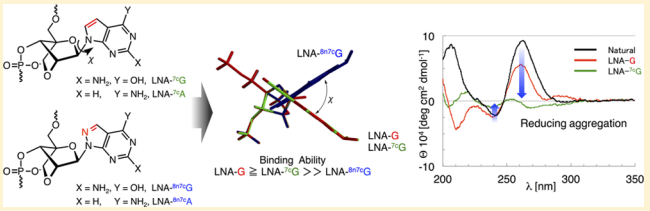
Supporting Information

ABSTRACT: Conformationally restricted nucleoside analogues 2′,4′-BNA/LNA-7-deazaguanine (LNA-^{7c}G) and 2′,4′-BNA/LNA-8-aza-7-deazaguanine (LNA-^{8n7c}G), which avoid extra hydrogen bond formation at the 7-position of the guanine nucleobase, were successfully synthesized and incorporated into oligonucleotides. While the LNA-^{7c}G-containing oligonucleotides show high duplex-forming ability with complementary DNA and RNA similar to LNA-G, the LNA-^{8n7c}G-containing oligonucleotide has lower binding affinity than that of natural 2′-deoxyguanosine. This disparity in thermostability is also observed in 7-deazaadenosine analogues (LNA-^{7c}A, LNA-^{8n7c}A). Thermodynamic parameters and computational chemistry revealed that an inappropriate glycosidic torsion angle χ of 2′,4′-BNA/LNA-8-aza-7-deazapurine analogues destabilizes duplex formation in contrast to 2′,4′-BNA/LNA-7-deazapurine analogues. This result indicates that the nucleobase rotation angle plays an important role in duplex binding affinity. In addition, LNA-^{7c}G-modified oligonucleotide effectively suppresses aggregation even in a guanine-rich sequence.



INTRODUCTION

A variety of artificial nucleic acids, which have diverse properties that natural nucleic acids do not, have been synthesized in the last two decades with the advance of nucleic acid therapeutics and nanotechnologies. In particular, nucleobase and sugar modifications have attracted increasing attention in order to create more useful artificial nucleic acids.

Nucleobase modifications enable us to change the hydrogen-bonding pattern,[1] stacking interactions, or molecular recognition[2] with relative ease. In the 1980s and 1990s, Seela's group developed 7-deaza-type nucleic acids such as 2′-deoxy-8-aza-7-deazaguanosine[3] and 2′-deoxy-7-deazaguanosine.[3b,4] The corresponding oligonucleotides containing the 7-deaza analogues effectively reduced aggregation resulting from Hoogsteen hydrogen bonding at the N7 position of guanine without loss of the ability to form Watson−Crick hydrogen bonding.[5] On the other hand, these modifications affected the hybridization property of oligonucleotides in different manners. The 8-aza-7-deazaguanosine modification slightly increased the stability of DNA duplexes.[3a] In contrast, 7-deazaguanosines had slightly destabilized binding affinity due to the reduced electrostatic density of the aromatic ring system.[4,6] It was also reported that the corresponding adenosine analogues, 8-aza-7-deazaadenosine[7] and 7-deazaadenosine,[5a,8] affected duplex stability in the same manner.[9] More recently, several kinds of 7-substituted 7-deazapurine analogues have been developed and applied to click chemistry,[10] bacterial inhibition,[11] photochemistry,[12] and substrates for polymerases.[13] Because the C7 position of 7-deazapurine is directed into the major groove,[14] substituents at the 7-position of the purine nucleobase should not sterically interfere with duplex formation.[15]

Regarding sugar modifications, numerous kinds of conformationally restricted nucleosides have been developed to date.[16] In particular, 2′,4′-bridged nucleic acid (2′,4′-BNA)[17]/locked nucleic acid (LNA)[18] is one of the most successful examples for giving some of the highest binding affinities with complementary strands. Its outstanding binding affinity is derived from both the reduction of entropic loss because of the preorganized locked structure and the enthalpic gain resulting from strengthening of the nucleobase stacking interactions.[19] Sugar-constrained nucleosides including 2′,4′-BNA/LNA introduced into oligonucleotides have been gathering attention and are being developed as gene modulation drugs against genetic diseases.[16a,b,20] Thus, steady improvement has been made in strategies for the functionalization of oligonucleotides. However, integrated developments have not made as much progress until recently. In the past few years, some promising results have been obtained from the combination of nucleobase and sugar-modified artificial nucleic acids.[21]

Here, in order to investigate whether dual-modified analogues possess multiple effects derived from base and

Received: October 18, 2016
Published: December 13, 2016

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DOI: 10.1021/acs.joc.6b02525
J. Org. Chem. 2017, 82, 25−36

sugar modification, we focused on one successful base and sugar modification, 7-deazapurine nucleobase and 2′,4′-BNA/LNA sugar. In this paper, we report on the synthesis of four different kinds of 7-deazapurine analogues, 2′,4′-BNA/LNA-7-deazaguanine (LNA-^{7c}G), 2′,4′-BNA/LNA-8-aza-7-deazaguanine (LNA-^{8n7c}G), 2′,4′-BNA/LNA-7-deazaadenine (LNA-^{7c}A), and 2′,4′-BNA/LNA-8-aza-7-deazaadenine (LNA-^{8n7c}A) (Figure 1). Additionally, we incorporated them

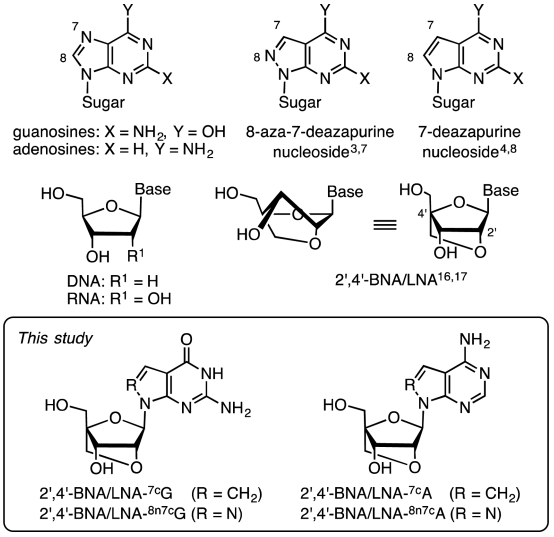


Figure 1. Purine, 7-deazapurine, and 8-aza-7-deazapurine nucleosides, 2′,4′-BNA/LNA, and 2′,4′-BNA/LNA-7-deazapurine analogues studied in this work.

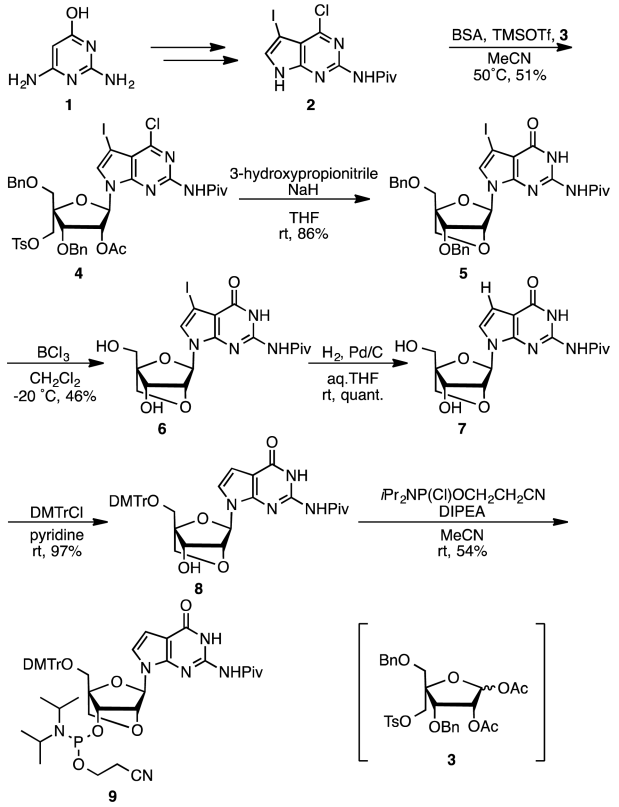
into oligonucleotides and conducted thermal denaturation studies including thermodynamic parameters and quantum chemical calculations to determine synergistic effects between 7-deazapurine nucleobase and 2′,4′-BNA/LNA sugar modifications. Furthermore, we investigated whether LNA-^{7c}G containing oligonucleotides could repress aggregation of oligonucleotides possessing guanine-rich sequences for future applications.

RESULTS AND DISCUSSION

Synthesis of LNA-^{7c}G. LNA-^{7c}G was synthesized as shown in Scheme 1. Regarding the glycosylation of 7-deazapurine derivatives, it was reported that electron-withdrawing substituents at the 7-position of 7-deazaguanine are important.[22] Thus, we chose 7-iodo derivative **2** for the glycosylation. This 7-iodo derivative **2** was synthesized in four steps from compound **1** according to previous reports,[22,23] and the glycosylation with sugar **3**[24] was carried out with BSA and TMSOTf as a Lewis acid in MeCN to give compound **4** in moderate yield. Treatment of **4** with NaH and 3-hydroxypropionitrile resulted in deacetylation, subsequent ring closure of the sugar moiety, and substitution at the 6-position of the nucleobase moiety to give compound **5**. After removal of the 3′-*O*-benzyl group with BCl_3 to afford compound **6**, deiodination by hydrogenolysis afforded the desired 2′,4′-BNA/LNA nucleoside monomer **7**. Tritylation at the primary hydroxy group of **7** with DMTrCl gave compound **8**, and phosphitylation at the secondary hydroxyl group yielded phosphoramidite building block **9**.

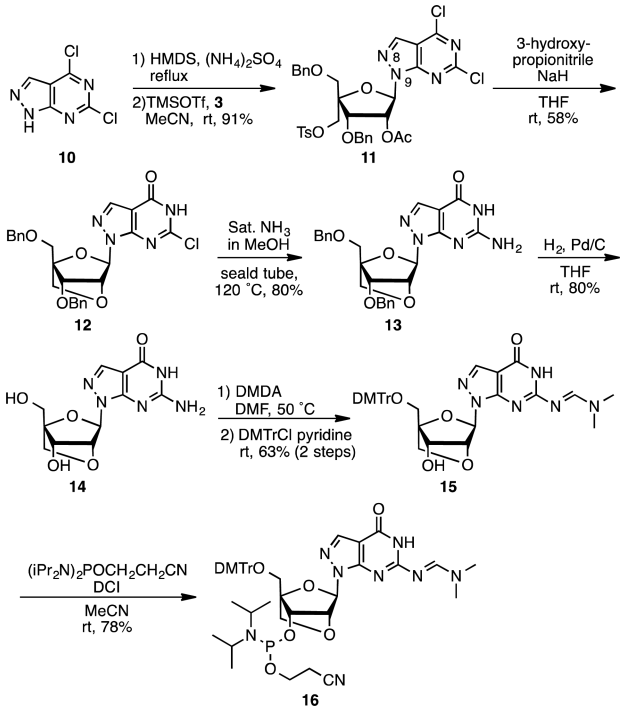
LNA-^{8n7c}G. The synthesis of LNA-^{8n7c}G phosphoramidite is summarized in Scheme 2. Glycosylation of **10**[25] with sugar **3** in

Scheme 1. Synthesis of LNA-^{7c}G Phosphoramidite[a]



[a]Abbreviations: BSA, *N*,*O*-bis(trimethylsilyl)acetamide; TMSOTf, trimethylsilyl trifluoromethanesulfonate; DMTrCl, 4,4′-dimethoxytrityl chloride; DIPEA, *N*,*N*′-diisopropylethylamine.

Scheme 2. Synthesis of LNA-^{8n7c}G Phosphoramidite[a]



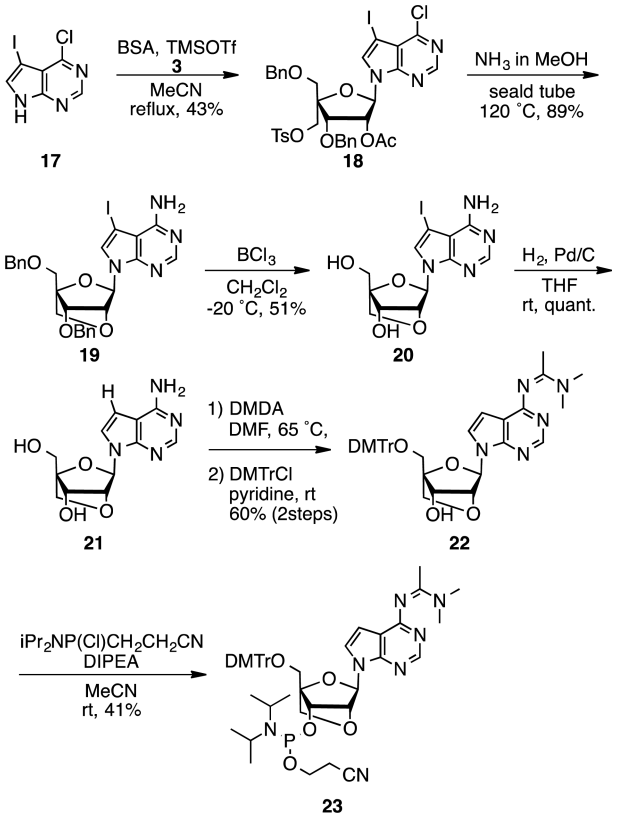
[a]Abbreviations: HMDS, 1,1,1,3,3,3-hexamethyldisilazane; DMDA, *N*,*N*-dimethylacetamide dimethyl acetal; DCI, 1*H*-imidazole-4,5-dicarboxylic acid.

DOI: 10.1021/acs.joc.6b02525
J. Org. Chem. 2017, 82, 25−36

the presence of TMSOTf provided the desired regioisomer **11**. In this synthesis, significant amounts of N8-isomers were observed when an excess amount of Lewis acid was used (4.0 equiv of TMSOTf). On the other hand, regioselective N9 glycosylation was performed by decreasing the amount of TMSOTf (0.5 equiv). The ring-closing reaction of the sugar moiety and substitution of chlorine on the nucleobase were carried out as described above to afford compound **12** in 58% yield. Treatment of compound **12** with methanolic ammonia solution in a sealed tube afforded 2-amino product **13**. The corresponding nucleoside **14** was obtained by removing the 3′- and 5′-benzyl groups by catalytic hydrogenolysis. After amidine protection of the 2-amino group and dimethoxytritylation at the 5′-hydroxyl group of **14**, phosphitylation at the 3′-hydroxyl group of the obtained **15** provided the desired amidite building block **16**.

LNA-^{7c}A. LNA-^{7c}A phosphoramidite was synthesized as shown in Scheme 3. First, we conducted N9 glycosylation of

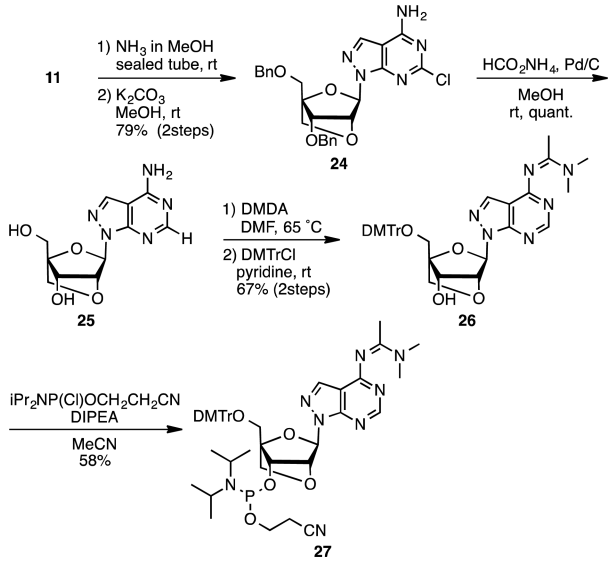
Scheme 3. Synthesis of LNA-^{7c}A Phosphoramidite



compound **17**[26] with sugar **3** in the presence of TMSOTf, and the desired compound **18** was obtained in moderate yield. Ammonolysis at the 6-position, deacetylation, and subsequent ring closure of the sugar by methanolic ammonia in a sealed tube afforded **19**. Nucleoside monomer **21** was obtained by 3′- and 5′-debenzylation with boron trichloride and subsequent deiodination by catalytic hydrogenolysis via **20**. The 6-amino group on the nucleoside **21** was protected as a dimethylacetamidine followed by protection at the 5′-primary hydroxyl group with 4,4′-dimethoxytrityl chloride to give compound **22**. Phosphitylation at the 3′-hydroxyl group produced phosphoramidite building block **23**.

LNA-^{8n7c}A. Gdaniec and Pedersen's groups independently succeeded in synthesizing LNA-^{8n7c}A nucleoside from 8-aza-7-deazaadenine and 8-aza-7-bromo-7-deazaadenine, respectively.[27] We here chose the common intermediate **11** for the LNA-^{8n7c}A phosphoramidite synthesis as shown in Scheme 4.

Scheme 4. Synthesis of LNA-^{8n7c}A Phosphoramidite



First, we attempted to simultaneously conduct ammonolysis at the 6-position of the nucleobase and deacetylation and ring closure at the sugar moiety on compound **11** by treatment with methanolic ammonia. However, a mixture of the desired compound **24** and the corresponding uncyclized compound was obtained. Consequently, we found that treatment of the obtained mixture with methanolic potassium carbonate afforded single compound **24** in 79% yield over two steps. Debenzylation of the 3′- and 5′-hydroxyl groups and simultaneous dechlorination at the 2-position by hydrogenolysis gave nucleoside **25**. Protection at the 2-amino group of the nucleobase and subsequent 4,4′-dimethoxytritylation at the 5′-hydroxyl group afforded **26**. The desired phosphoramidite **27** was finally obtained by phosphitylation at the 3′-hydroxyl group.

ON Synthesis. A variety of modified oligonucleotides (**ON-2**−**4** and **ON-6**−**8**) and natural DNA strands (**ON-1** and **-5**) were synthesized on an automated DNA synthesizer (0.2 μmol scale) (Table 1).The conventional phosphoramidite protocol was used except the coupling time was prolonged for the modified oligonucleotide to 8 min and 5-[3,5-bis-(trifluoromethyl)phenyl]-2*H*-tetrazole was used as an activator. For the oxidation step, an iodine solution (I_2/H_2O/C_6H_4N) was used except in the LNA-^{7c}A elongation; *tert*-butyl hydroperoxide (TBHP) solution[28] was used for LNA-^{7c}A. In the case of LNA-^{7c}G oxidation, iodine oxidation was better than TBHP. The oligonucleotide sequences for thermal denaturation studies were selected as referred to Seela's non-self-complementary strand. The synthesized oligonucleotides were purified by reversed-phase HPLC (RP-HPLC), and each molecular mass was confirmed by MALDI TOF mass spectrometry (mass spectral data and yields are shown in the Supporting Information).

Duplex-Forming Ability. The duplex-forming abilities of the modified oligonucleotides (**ON-3**, **4**, **7**, **8**) with

DOI: 10.1021/acs.joc.6b02525
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Table 1. Sequences of Oligonucleotides Used in this Study[a]

5′-d(AGTATTGACCTA)-3′	**ON-1**
5′-d(AGTATT**G**ACCTA)-3′	**ON-2**
5′-d(AGTATT^{7c}**G**ACCTA)-3′	**ON-3**
5′-d(AGTATT^{8n7c}**G**ACCTA)-3′	**ON-4**
5′-d(AGTATTAACCTA)-3′	**ON-5**
5′-d(AGTATT**A**ACCTA)-3′	**ON-6**
5′-d(AGTATT^{7c}**A**ACCTA)-3′	**ON-7**
5′-d(AGTATT^{8n7c}**A**ACCTA)-3′	**ON-8**
5′-(AGGGGGTGGGAGGGT)-3′	**ON-9**
5′-(A**GGGGG**TGGGA**GGG**T)-3′	**ON-10**
5′-(A**G**^{7c}**GG**^{7c}**GG**TGGGA**G**^{7c}**GG**T)-3′	**ON-11**

[a]Underlined bold characters indicate the 2′,4′-BNA/LNA modified residues. Superscript 7c indicates 7-deaza-, and superscript 8n7c indicates 8-aza-7-deaza-, respectively.

complementary ssDNA and ssRNA were evaluated by UV-melting experiments (T_m measurements) and were compared with the corresponding 2′,4′-BNA/LNA with natural bases (**ON-2** and **ON-6**) and natural DNAs (**ON-1** and **ON-5**). The T_m values are summarized in Table 2. As anticipated, the duplex stability of LNA-^{7c}G-incorporated oligonucleotide (**ON-3**) was increased by almost the same amount as in LNA-G (**ON-2**) compared to that of unmodified oligonucleotide (**ON-1**) (vs ssDNA: +2 °C; ssRNA: +8 °C). **ON-7**, which is modified with LNA-^{7c}A, also forms thermostable duplexes with ssDNA and ssRNA, but its binding affinity is slightly decreased compared to those with natural adenine base (LNA-A). The similarity in T_m shifts of the LNA-^{7c}G and -^{7c}A modifications reflects an additivity effect of thermostabilities between the decreasing stability of the 7-deazapurine nucleobase[11] and increasing stability of the 2′,4′-BNA/LNA sugar moiety. On the other hand, it was found that LNA-^{8n7c}G or -^{8n7c}A introduced into oligonucleotides (**ON-4** and **ON-8**) significantly decreased the binding affinity compared to LNA-G or -A (**ON-2** and **ON-6**) and even natural guanosine and adenosine (−2 to −1 °C/mod). These results were surprising because it is known that 8-aza-7-deazapurine-substituted nucleotides generally stabilize duplexes due to the base−sugar interaction[29] or the different electron density of the aromatic ring system.[30]

Mismatch Recognition of LNA-^{8n7c}G- and -^{7c}G-Modified Oligonucleotides. The binding specificity is also important to discuss in modified oligonucleotides. Because the cause of the low binding affinity of LNA-^{8n7c}G and -^{8n7c}A was possibly the loss of the base-pair recognition property, thermal denaturation experiments of LNA-^{8n7c}G- and -^{7c}G-modified oligonucleotides (**ON-3** and **ON-4**) against three single-mismatch DNA sequences were performed. As indicated in Table 3, all of the base-modified guanine analogues (**ON-1** to **ON-4**) resulted in significant destabilization of the duplex having just a single mismatch. This result indicates that synthetic oligonucleotide analogues maintain sufficient mismatch recognition properties and the cause of the low binding affinity of LNA-^{8n7c}G was not the lack of base-pair formation with complementary strands.

Table 3. T_m Values of Duplexes Formed by Modified Oligonucleotides with Complementary ssDNA Containing a Single-Mismatch Base[a,b]

	T_m ($\Delta T_m = T_{m\,(mismatch)} - T_{m\,(match)}$) (°C)			
ONs	Y = C	= T	= A	= G
natural (**ON-1**)	43	29 (−14)	26 (−17)	19 (−24)
G (**ON-2**)	45	33 (−12)	24 (−21)	21 (−24)
^{7c}**G** (**ON-3**)	44	34 (−10)	24 (−21)	20 (−24)
^{8n7c}**G** (**ON-4**)	41	27 (−14)	26 (−15)	17 (−24)

[a]Modified oligonucleotides, 5′-d(AGTATT**X**ACCTA)-3′; target ssDNA strand, 5′-d(TAGGT**Y**AATACT)-3′. [b]Conditions: 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl; each strand concentration = 4 μM. ΔT_m's are calculated relative to T_m values of full match DNA/DNA duplex.

Thermodynamic Parameters for Duplexes. To examine which factors affect the hybridization ability of four kinds of 2′,4′-BNA/LNA 7-deazapurine analogues, we compared thermodynamic parameters of DNA/RNA duplexes determined by van't Hoff plots. The results are shown in Table 4. The LNA oligonucleotides showed larger or the same $-\Delta G°$ values compared to the DNA duplex in all cases. This stability of 2′,4′-BNA/LNA-guanine-modified oligonucleotides (**ON-2**−**ON-4**) is the result of a smaller $-T\Delta S°$ value. As reported

Table 2. T_m Values of Duplexes Formed by 2′,4′-BNA/LNA-7-deazapurine Analogues with Complementary Sequences[a−c]

		DNA complement			RNA complement		
ODN		T_m (°C)	ΔT_m (°C)	$\Delta\Delta T_m$ (°C)	T_m (°C)	ΔT_m[b] (°C)	$\Delta\Delta T_m$[c] (°C)
G-analogues							
5′-d(AGTATT G ACCTA)-3′	**ON-1**	43			43		
5′-d(AGTATT **G** ACCTA)-3′	**ON-2**	45	+2		51	+8	
5′-d(AGTATT ^{7c}**G** ACCTA)-3′	**ON-3**	44	+1	−1	51	+8	0
5′-d(AGTATT^{8n7c}**G**ACCTA)-3′	**ON-4**	41	−2	−5	46	+3	−5
A-analogues							
5′-d(AGTATT A ACCTA)-3′	**ON-5**	35			35		
5′-d(AGTATT **A** ACCTA)-3′	**ON-6**	39	+4		43	+8	
5′-d(AGTATT ^{7c}**A** ACCTA)-3′	**ON-7**	38	+3	−1	42	+7	0
5′-d(AGTATT^{8n7c}**A**ACCTA)-3′	**ON-8**	34	−1	−5	37	+2	−6

[a]Target strand sequence: DNA complement 5′-d(TAGGTCAATACT)-3′, RNA complement 5′-r(UAGGTCAAUACU)-3 with regard to G analogues. DNA complement 5′-d(TAGGTTAATACT)-3′, RNA complement 5′-r(UAGGTTAAUACU)-3 with regard to A analogues. Thermal denaturation study conditions: 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl; each strand concentration = 4 μM. The number is the average of three independent measurements. [b]ΔT_ms are calculated relative to T_m values of unmodified **ON-1**/DNA and **ON-1**/RNA duplexes with regard to G analogues and A analogues, respectively. [c]$\Delta\Delta T_m$s are calculated relative to T_m values of **ON-5**/DNA and **ON-5**/RNA duplexes.

DOI: 10.1021/acs.joc.6b02525
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Table 4. Thermodynamic Parameters for Duplexes Modified with LNA-G and -A Analogues[a]

ODN		RNA complement			
		$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal K^{-1} mol^{-1})	$T\Delta S°$ (kcal mol^{-1})	$\Delta G°_{310K}$ (kcal mol^{-1})
G-analogues					
5′-d(AGTATT G ACCTA)-3′	**ON-1**	−88.3	−252.1	−78.2	−10.1
5′-d(AGTATT **G** ACCTA)-3′	**ON-2**	−73.9	−187.5	−62.5	−11.3
5′-d(AGTATT ^{7c}**G** ACCTA)-3′	**ON-3**	−81.8	−226.3	−70.2	−11.6
5′-d(AGTATT^{8n7c}**G**ACCTA)-3′	**ON-4**	−68.3	−187.5	−58.2	−10.1
A-analogues					
5′-d(AGTATT A ACCTA)-3′	**ON-5**	−76.1	−220.8	−68.5	−7.6
5′-d(AGTATT **A** ACCTA)-3′	**ON-6**	−96.9	−280.7	−87.1	−9.9
5′-d(AGTATT ^{7c}**A** ACCTA)-3′	**ON-7**	−90.3	−260.8	−80.9	−9.4
5′-d(AGTATT^{8n7c}**A**ACCTA)-3′	**ON-8**	−85.3	−248.8	−77.2	−8.1

[a]Target strand sequence: 5′-r(UAGGTCAAUACU)-3 and 5′-r(UAGGTTAAUACU)-3. Thermal denaturation studies′ conditions: 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl; each strand concentration = 0.9−13.6 μM. These values were determined by van't Hoff plots with six data points.

previously,[31] 2′,4′-BNA/LNA modification stabilizes duplexes in terms of entropic advantage from preorganization. On the other hand, there is a case that a favorable enthalpy compensates for the entropy change in hybridization.[19d] In other words, the main thermodynamic contribution of 2′,4′-BNA/LNA is changeable depending on the sequence.[19b] In this study, the stabilities of 2′,4′-BNA/LNA with adenine (**ON-5** to **ON-8**) and guanine (**ON-1** to **ON-4**) base modifications are a result of a large $-\Delta H°$ value and small $-\Delta S°$ value, respectively, depending on each sequence. From a different point of view, when focusing on thermodynamic parameters among 2′,4′-BNA/LNA oligonucleotides, it was revealed that LNA-^{8n7c}G (**ON-4**) and -^{8n7c}A (**ON-8**) showed the worst $-\Delta H°$ value, respectively. Because the only structural difference of the modified oligonucleotides is the existence of a nitrogen atom at the 8-position of the purine nucleobase, the smaller $-\Delta H°$ values of LNA-^{8n7c}G and -^{8n7c}A would be due to electrostatic repulsion between the nitrogen atom at the 8-position of the nucleobase and O4′ of the 2′,4′-BNA/LNA sugar. We hypothesized that the electrostatic repulsion causes unfavorable rotation of the nucleobase (χ angle), and destabilized hybridization ability appears in terms of the enthalpic disadvantage when the χ angle rotates to the position in which a hydrogen bond can form. On the other hand, LNA-^{8n7c}G and -^{8n7c}A displayed larger $-T\Delta S°$ values than the corresponding LNA analogues. This compensates for the loss of enthalpy.[20b]

Quantum Chemical Calculations. Measurements of the T_m values and thermodynamic parameters indicated that the glycosidic torsion angle χ of LNA is potentially the key factor affecting duplex-forming ability. Because glycosidic torsion angle χ is thought to be rich in flexibility,[32] we performed a precise analysis of χ by quantum chemical calculations (Figure 2). We calculated energy-minimized structures by rotating the dihedral angle χ every 15° and compared their energies. In the case of LNA-G, the calculations indicated that the most favored χ angle is around −165° (anti region), and the χ angles of LNA-^{7c}G are in the same position as well. In contrast, the most favored torsion angle of LNA-^{8n7c}G was calculated to be around −90° (high-anti region),[33] which is a large difference. The calculated electrostatic potential map revealed that condensed negative potentials were observed around O4′, O5′, and N8 of LNA-^{8n7c}G, and electrostatic repulsion of these heteroatoms would contribute to the high-anti conformation (Figure 3). Furthermore, we considered that the restricted N-type (2′-exo, 3′-endo) sugar conformation also contributed to the abnormal glycosidic torsion angle of LNA-^{8n7c}G. Because base rotation and changes in sugar pucker of natural DNA and RNA usually occur concertedly, the energetic disadvantage is moderate.[34] However, the moderation on LNA is hindered due to the restricted sugar conformation. Here, the glycosidic torsion angle of nucleotides in A-form duplexes is generally in the anti range,[35] and this angle is reasonable to accommodate LNA-purine and LNA-7-deazapurine. On the other hand, the glycosidic torsion angle, high-anti, of LNA-8-aza-7-deazapurine

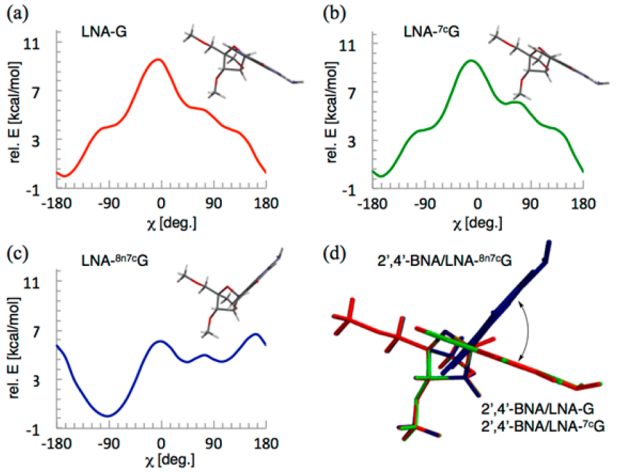

Figure 2. Glycosidic torsion profiles for 2′,4′-BNA/LNA analogues. Profiles are obtained with the Wavefunction Spartan 14. Method: ab initio HF/6-31+G*. The global minimum structure is calculated by comparing 24 local minimized structures for every 15° of glycosidic torsion angle.

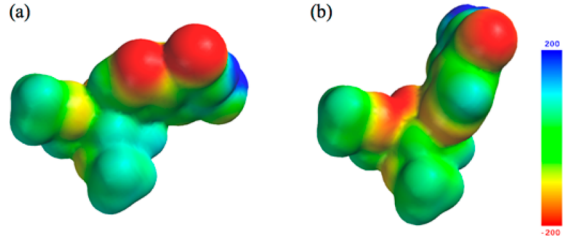

Figure 3. Electrostatic potential maps. (a) LNA-G, (b) LNA-^{8n7c}G. Conditions: Spartan 14, ab initio HF/6-31+G*. Property range: −200 to +200 kJ/mol.

DOI: 10.1021/acs.joc.6b02525
J. Org. Chem. 2017, 82, 25−36

is not suitable in A-form duplexes. Based on these considerations, it was strongly suggested that the nucleobase rotation angle affects the duplex forming ability. Furthermore, the T_m value of LNA-8-aza-7-deazapurine indicates decreased binding affinity toward complementary ssDNA despite the fact that the high-anti conformation is known to be favored in B-form duplexes.[35a,b] This is probably because 2′,4′-BNA/LNA monomers can influence the surrounding duplex helicity by changing the local phosphate backbone geometry[36] and the anti orientation should be the favored angle with complementary ssDNA as well as ssRNA. This consideration is consistent with the result of LNA-8-aza-7-deazapurine showing the same decreasing trend for both ssDNA and ssRNA compared to LNA-purine (see $\Delta\Delta T_m$ values of **ON-4** and **ON-8** in Table 2).

Reducing Aggregation of G-Rich Oligonucleotides by LNA-^{7c}G Modification. Guanosine-rich oligonucleotides cause a variety of problems induced by their aggregation in DNA synthesis, HPLC purification, UV melting experiments, and a variety of applied chemistries. Aggregates are held together by Hoogsteen hydrogen bonding at the N7 of the guanine base, and this inter- or intrastrand hydrogen bonding forms complex higher order structures.[37] It was reported that 7-deaza- and 8-aza-7-deazaguanosine have potential to reduce aggregation because these analogues do not have a hydrogen bonding acceptor at the 7-position.[5a,8] Therefore, we evaluated whether LNA-^{7c}G, which shows high binding affinity via Watson−Crick hydrogen bonding, also retains a potential to suppress higher order structures in G-rich sequences by using HPLC (Figure 4). Considering future applications, we chose a G-rich antisense oligonucleotide that targets a cytosine-rich region of bcL-xL mRNA and prepared unmodified (**ON-9**), 2′,4′-BNA/LNA modified (**ON-10**), and LNA-^{7c}G containing (**ON-11**) oligonucleotides. Generally, retention times of duplexes formed from oligonucleotides or G-quadruplexes are extended, and a broad peak is observed on reversed-phase HPLC.[5b] All oligonucleotides were identified by MALDI-TOF-MS. By HPLC, unmodified oligonucleotide (**ON-9**) showed a broad peak 10 min after injection along with some small peaks. The oligonucleotide containing eight LNA-G units (**ON-10**) showed not only a sharp peak at 10 min but also some broad peaks around 30 min. In contrast, the introduction of three 2′,4′-BLA/LNA-^{7c}G units (**ON-11**) gave only one sharp peak.These results indicated that LNA-^{7c}G suppresses higher order structures by its introduction at three positions of a 15mer oligonucleotide.

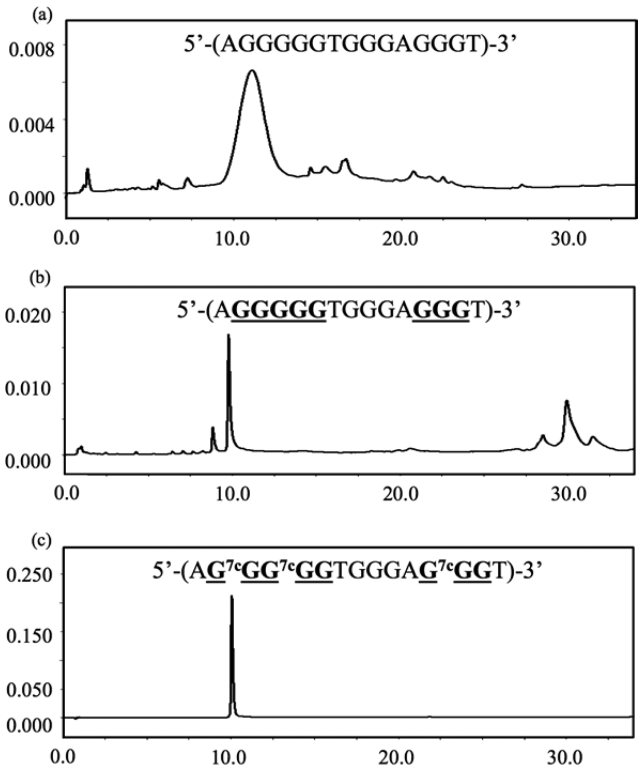

Figure 4. Examination of aggregation by G-rich sequences. (a) **ON-9**, (b) **ON-10**, (c) **ON-11**. Each purified oligonucleotide was analyzed by RP-HPLC with a linear gradient of MeCN (from 6% to 20% over 30 min, 50 °C) in 0.1 M triethylammonium acetate (pH 7.0). *X*- and *Y*-axes indicate time course (min) and voltage variation (mV), respectively.

CD spectra can provide information on a variety of higher order structures including quadruplexes. We examined the CD spectra of **ON-9**−**ON-11**. Each CD spectrum was recorded in the presence of 100 mM KCl, and the results are shown in Figure 5. In the CD spectrum of **ON-9**, positive Cotton bands

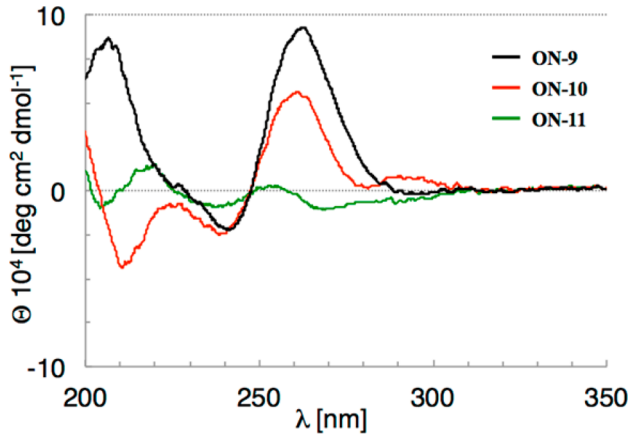

Figure 5. CD spectra of **ON-9** (black line), **ON-10** (red line), and **ON-11** (green line). Conditions: 10 mM sodium phosphate buffer (pH 7.2), 100 mM KCl, 4 μM single strand oligonucleotides; wavelength was measured from 200 to 350 nm.

at 220 and 265 nm as well as a negative band at 240 nm were observed. This spectrum indicates that four oligonucleotide strands form a parallel-type quadruplex.[38] The CD spectrum of **ON-10** containing LNA-G has characteristic positive bands near 260 and 290 nm as well as negative bands near 210 nm and a local maximum at 240 nm. This CD spectrum is not a typical example of the G-quadruplex. It indicated that **ON-10** formed two or more higher order structures such as a mixture of parallel and antiparallel type G-quadruplexes.[38] This result was in good agreement with the HPLC study in Figure 4c because several peaks were observed during the time course. In contrast, significant reduction of positive or Cotton bands is observed, and the characteristic pattern of quadruplex formation is not observed in the case of 2′,4′-BLA/LNA-^{7c}G (**ON-11**). This indicates that no higher order structure can form by itself by introducing 2′,4′-BLA/LNA-^{7c}G to appropriate positions of the G-rich sequence. Previous reports indicated that partly modified LNA oligonucleotides can increase the thermostability of a parallel quadruplex compared to that of DNA.[39] The result obtained here suggests that 2′,4′-BLA/LNA-^{7c}G modification could effectively suppress self-

DOI: 10.1021/acs.joc.6b02525
J. Org. Chem. 2017, 82, 25−36

aggregation even though higher order structures would be stabilized by the locked sugar conformation.

CONCLUSION

We have successfully synthesized 7-deaza types of 2′,4′-BNA/LNA purine analogues, LNA-^{7c}G, -^{8n7c}G, -^{7c}A, and -^{8n7c}A. Glycosylation of all analogues was achieved by the TMSOTf-catalyzed Vorbrüggen method. Regarding the 2′,4′-BNA/LNA-7-deazapurine analogues, each intermediate 7-iodo-^{7c}G and -^{7c}A leaves possibilities for further functionalization of nucleobases with Heck, Stille, Sonogashira, or Suzuki reactions. Thermal denaturation studies revealed that LNA-^{7c}G and -^{7c}A had binding abilities as high as those of LNA-G and -A. On the other hand, LNA-^{8n7c}G and -^{8n7c}A had decreased binding affinity compared with LNA-G. The favored torsion angle χ of LNA-^{8n7c}G and -^{8n7c}A in the nucleosides was different from that of LNA-G and LNA-^{7c}G due to electrostatic repulsion between N8 and O4′. That is the probable reason why LNA-^{8n7c}G and -^{8n7c}A depressed the duplex stability. These results implied that molecular design focusing on glycosidic torsion angle χ could lead to development of a new artificial nucleic acid. Since 8-aza-7-deazapurine modifiers enable us to design poly-G sequences without increasing T_m values, this study will stimulate various research fields such as biochemistry, analytical chemistry, and chemical biology. In addition, LNA-^{7c}G reduces the aggregation of G-rich oligonucleotides without losing high binding affinity to cytosine. LNA-^{7c}G and LNA-^{7c}A could be applied to a variety of nucleic acid chemistries requiring the high binding affinity only to target sequence such as antisense technology.

EXPERIMENTAL SECTION

General Experimental Procedure. Reagents were used as received from commercial suppliers unless otherwise specified. Air- and/or moisture-sensitive experiments were carried out under a N_2 atmosphere. TLC was run on silica gel 60 F_{254} aluminum sheets. Column chromatography was carried out with silica gel.

Physical data were measured as follows: ^{1}H NMR (500, 400, 300 MHz), ^{13}C NMR (126, 101, 76 MHz), and ^{31}P NMR (162 MHz). Chemical shifts are reported in parts per million referenced to internal tetramethylsilane (0.00), residual $CHCl_3$ (7.26), or methanol (3.31) for ^{1}H NMR and chloroform-*d* (77.16) or methanol-d_4 (49.00) for ^{13}C NMR. 85% H_3PO_4 (0.00) as external standard was used for ^{31}P NMR. *J* values are given in hertz (except for ^{13}C NMR experiments of compound **9**, **16**, **23**, and **27**). All observed peaks of **9**, **16**, **23**, and **27** are listed because these compounds are a diastereomixture and every C−P coupling constants makes the assignment difficult.

IR spectra were recorded with samples prepared as KBr tablets on an FT/IR spectrometer. MALDI-TOF mass spectra for all of the oligonucleotides were recorded using a 2,4,6-trihydroxyacetophenone (THAP) matrix.

*9-[2-O-Acetyl-3,5-di-O-benzyl-4-C-(p-toluenesulfonyloxymethyl)-β-D-ribofuranosyl]-6-chloro-7-iodo-2-pivaloylamino-7-deazapurine (**4**).* To a suspension of compound **2** (624 mg, 1.65 mmol) in MeCN (11.6 mL) was added bis(trimethylsilyl)acetamide (BSA) (0.41 mL, 1.66 mmol) at room temperature. After 5 min of stirring, TMSOTf (0.41 mL, 2.27 mmol) was added dropwise, and then the solution was heated at 50 °C. 3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-4-*C*-(*p*-toluenesulfonyloxymethyl)-β-D-ribofuranose (**3**) (2.00 g, 3.30 mmol) was added in three portions (once per 8 h) with a cannula, and the mixture was stirred at 50 °C for 24 h in total. After that, the solution was diluted with CH_2Cl_2, washed with satd $NaHCO_3$, water, and brine, and dried over Na_2SO_4. After concentration, the crude product was purified by column chromatography ($CHCl_3$:methanol/*n*-hexane = 98:2:0.615) and then further purified (*n*-hexane/ethyl acetate = 5:1). The main fraction afforded compound **4** as a white solid (766 mg, 51%). $[\alpha]_D^{18}$ −2.915 (*c* 1.10, $CHCl_3$). UV (MeOH): λ_{max} (ε) = 254 nm (25600), 308 nm (3600). IR ν_{max} (KBr): 3358, 3030, 2965, 2870, 1746, 1714, 1597, 1555, 1513, 1495, 1464, 1420, 1395, 165, 1216, 1176, 1134 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (9H, s), 2.01 (3H, s), 2.40 (3H, s), 3.63 (1H, d, *J* = 12 Hz), 3.69 (1H, d, *J* = 12 Hz), 4.24 (1H, d, *J* = 16 Hz), 4.29 (1H, d, *J* = 16 Hz), 4.42 (1H, d, *J* = 16 Hz), 4.51 (1H, d, *J* = 16 Hz), 5.16 (1H, d, *J* = 8 Hz), 5.80 (1H, dd, *J* = 4, 8 Hz), 6.01 (1H, d, *J* = 4 Hz), 7.19−7.34 (13H, m), 7.75 (2H, d, *J* = 12 Hz), 8.05 (1H, s). NOE H8 (5%) by 2′-irradiation. ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7, 21.6, 27.4, 40.2, 52.5, 69.1, 70.7, 73.5, 74.4, 75.1, 78.4, 85.6, 88.2, 114.0, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 129.8, 132.6, 132.8, 137.4, 137.6, 144.7, 151.1, 151.3, 152.8, 169.9, 174.8. MS (FAB) *m*/*z*: 917 $[M + H]^+$. HRMS (FAB): calcd for $C_{40}H_{43}ClIN_4O_9S$ $[M + H]^+$ 917.1478, found 917.1508.

*9-[(1S,3R,4R,7S)-7-(Benzyloxy)-1-(benzyloxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-7-iodo-N^2-pivaloyl-7-deazaguanine (**5**).* Into a solution of 3-hydroxypropionitrile (30 μL, 0.45 mmol) in dry THF (0.8 mL) was added NaH (60% in mineral oil, 22 mg, 0.55 mmol) at 0 °C, and the solution was stirred for 30 min at room temperature. The mixture was cooled to 0 °C, and a solution of **4** (92 mg, 0.10 mmol) in dry THF (0.8 mL) was added dropwise. After being stirred for an additional 22 h, the resulting mixture was neutralized with aq HCl (1 M)/brine (1:9) and extracted with ethyl acetate. The organic phases were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by column chromatography ($CHCl_3$/acetone = 1:1) to afford **5** (52 mg, 86%) as a milky white solid. $[\alpha]_D^{20}$ −10.9 (*c* 0.93, $CHCl_3$). UV (MeOH): λ_{max} (ε) = 232 nm (29000), 296 nm (23800). IR ν_{max} (KBr): 3129, 2955, 1665, 1610, 1541, 1479, 1417, 1368, 1305, 1251, 1176, 1156, 1100, 1045, 1026 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (9H, s), 3.85 (2H, s), 3.96 (1H, d, *J* = 8 Hz), 4.10 (1H, d, *J* = 8 Hz), 4.15 (1H, s), 4.35 (1H, s), 4.53−4.68 (4H, m), 5.93 (1H, s), 6.99 (1H, s), 7.26−7.40 (10H, m), 7.91 (1H, s), 11.69 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 27.0, 40.2, 54.8, 65.1, 72.4, 72.6, 73.7, 77.3, 77.7, 86.1, 86.7, 106.1, 123.4, 127.5, 127.8, 127.9, 128.2, 128.5, 128.6, 136.8, 137.5, 145.9, 146.3, 156.7, 179.6. MS (FAB) *m*/*z*: 685 $[M + H]^+$. HRMS (FAB): calcd for $C_{31}H_{34}IN_4O_6$ $[M + H]^+$ 685.1518, found 685.1523.

*9-[(1S,3R,4R,7S)-1-(Hydroxymethyl)-7-hydroxy-2,5-dioxabicyclo-[2.2.1]heptan-3-yl]-7-iodo-N^2-pivaloyl-7-deazaguanine (**6**).* Under argon atmosphere, compound **5** (33 mg, 0.048 mmol) was dissolved in dry CH_2Cl_2 (1.2 mL) and cooled to −78 °C with stirring. BCl_3 (1.0 M in CH_2Cl_2, 0.9 mL, 0.9 mmol) was added dropwise. After being stirred for 3 h at the same temperature, the reaction mixture was allowed to reach −20 °C gradually over 4 h. After the reaction was quenched by addition of MeOH at −20 °C, the resulting mixture was warmed to room temperature. The solution was concentrated in vacuo, and the crude product was passed through a pad of SiO_2 gel ($CHCl_3$/MeOH = 9:1) to remove boron compounds and then purified by column chromatography ($CHCl_3$/MeOH = 98:2) to yield **6** (11 mg, 46%) as a white solid. $[\alpha]_D^{19}$ −21.2 (*c* 1.01, MeOH). UV (MeOH): λ_{max} (ε) = 232 nm (14000), 297 nm (11000). IR ν_{max} (KBr): 3332, 2958, 1670, 1608, 1539, 1481, 1422, 1307, 1247, 1205, 1173, 1147, 1045, 1029 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.30 (9H, s), 3.81 (1H, d, *J* = 8 Hz), 3.92 (2H, s), 4.00 (1H, d, *J* = 8 Hz), 4.27 (1H, s), 4.34 (1H, s), 5.98 (1H, s), 7.32 (1H, s). ^{13}C NMR (101 MHz, CD_3OD) δ: 27.0, 41.4, 54.5, 58.2, 71.2, 72.7, 81.6, 87.5, 89.7, 106.4, 125.7, 148.4, 148.7, 159.3, 183.0. MS (FAB) *m*/*z*: 505 $[M + H]^+$. HRMS (FAB): calcd for $C_{17}H_{22}IN_4O_6$ $[M + H]^+$ 505.0579, found 505.0585.

*9-[(1S,3R,4R,7S)-1-(Hydroxymethyl)-7-hydroxy-2,5-dioxabicyclo-[2.2.1]heptan-3-yl]-N^2-pivaloyl-7-deazaguanine (**7**).* To a solution of **6** (11 mg, 0.022 mmol) in THF/water (3:1, 1.5 mL) was added 5% Pd/C (wetted with 55% water, 11 mg) under N_2 atmosphere. The reaction mixture was stirred under H_2 atmosphere at room temperature for 8 h. After filtration, the filtrate was concentrated. The obtained crude mixture was purified by column chromatography ($CHCl_3$/MeOH = 97:3) to give compound **7** (8 mg, 95%) as a white solid. $[\alpha]_D^{22}$ −12.3 (*c* 0.93, MeOH). UV (MeOH): λ_{max} (ε) = 269 nm (9100), 294 nm (9100). IR ν_{max} (KBr): 3325, 2966, 2555, 1683, 1599, 1553, 1417, 1043 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.31 (9H, s), 3.85 (1H, d, *J* = 8 Hz), 3.94 (2H, s), 4.02 (1H, d, *J* = 8 Hz), 4.31 (1H, s), 4.34 (1H, s), 6.03 (1H, s), 6.58 (1H, d, *J* = 4 Hz), 7.22 (1H, d,

DOI: 10.1021/acs.joc.6b02525
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J = 4 Hz). ^{13}C NMR (101 MHz, CD_3OD) δ: 27.0, 41.4, 58.5, 71.5, 72.8, 81.7, 87.3, 89.5, 103.4, 106.0, 120.9, 148.3, 148.6, 160.2, 182.9. MS (FAB) m/z: 379 $[M + H]^+$. HRMS (FAB): calcd for $C_{17}H_{23}N_4O_6$ $[M + H]^+$ 379.1612, found 379.1605.

*9-[(1S,3R,4R,7S)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-N^2-pivaloyl-7-deazaguanine (**8**).* To a stirred solution of compound **7** (40 mg, 0.11 mmol) in dry pyridine (1.1 mL) was added DMTrCl (47 mg, 0.14 mmol), and the reaction mixture was stirred at room temperature for 2 h. After the mixture was cooled to 0 °C, dry MeOH (0.2 mL) was added, and then the mixture was diluted with ethyl acetate. The organic phase was washed with satd $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography ($CHCl_3$/MeOH = 98:2) to afford compound **8** (81 mg, 97%) as a white solid. $[\alpha]_D^{23}$ −21.9 (c 0.53, $CHCl_3$). UV (MeOH): λ_{max} (ε) = 224 nm (26800), 272 nm (12400). IR ν_{max} (KBr): 3218, 2963, 1669, 1606, 15467, 1509, 1431, 1302, 1252, 1177, 1150, 1032 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (9H, s), 2.20 (1H, d, J = 6 Hz), 3.52 (1H, d, J = 11 Hz), 3.60 (1H, d, J = 11 Hz), 3.80 (6H, s), 3.97 (1H, d, J = 8 Hz), 4.02 (1H, d, J = 8 Hz), 4.41 (1H, s), 4.43 (1H, d, J = 6 Hz), 5.99 (1H, s), 6.63 (1H, d, J = 4 Hz), 6.84 (2H, s), 6.86 (2H, s), 7.09 (1H, d, J = 4 Hz), 7.23−7.48 (9H, m), 8.01 (1H, s), 11.79 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 27.0, 40.2, 55.2, 59.2, 71.6, 72.1, 80.3, 86.0, 86.5, 86.7, 103.3, 105.7, 113.2, 118.9, 127.0, 127.9, 128.1, 130.0, 130.0, 135.4, 135.4, 144.3, 145.9, 146.0, 157.7, 158.6, 179.6. MS (FAB) m/z: 680 $[M + H]^+$. HRMS (FAB): calcd for $C_{38}H_{41}N_4O_8$ $[M + H]^+$ 681.2919, found 681.2930.

*2-Cyanoethyl [(1S,3R,4R,7S)-1-(4,4′-Dimethoxytrityloxymethyl)-3-(N^2-pivaloyl-7-deazaguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl]diisopropylphosphoramidite (**9**).* To a solution of compound **8** (80 mg, 0.11 mmol) in dry CH_3CN were added *N*,*N*-diisopropylethylamine (74 μL, 0.42 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (49 μL, 0.24 mmol), and the resultant mixture was stirred at room temperature for 5 h. The reaction mixture was neutralized with satd $NaHCO_3$ and extracted with ethyl acetate. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The obtained crude product was purified by column chromatography (*n*-hexane/ethyl acetate = 3:1 containing 0.5% triethylamine) to give compound **9** (56 mg, 54%) as a white amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.89−0.95 (6H, m), 1.07−1.11 (6H, m), 1.34 (9H, s), 2.38−2.50 (2H, m), 3.41−3.58 (5H, m), 3.68−3.71 (1H, m), 3.79 (3H, s), 3.80 (3H, s), 3.92−4.04 (2H, m), 4.36 (0.4H, d, J = 6 Hz), 4.51 (0.6H, d, J = 9 Hz), 4.63 (0.6H, s), 4.75 (0.4H, s), 6.00 (1H, s), 6.64−6.65 (1H, m), 6.82−6.86 (4H, m), 7.17−7.50 (10H, m), 8.11 (0.6H, s), 8.13 (0.4H, s), 11.80 (1H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 20.1(0), 20.1(6), 20.1(8), 20.2, 24.1, 24.2, 24.3(3), 24.3(5), 24.4, 24.5, 27.0, 40.2, 43.2, 43.3, 43.4, 55.1(9), 55.2(2), 58.0, 58.1, 58.6, 58.7, 58.8, 59.2, 71.4, 71.5, 72.2, 72.7, 72.9, 79.2, 86.3(0), 86.3(5), 86.7, 86.9, 87.1(0), 87.1(4), 103.2, 103.3, 105.9(9), 106.0(2), 113.1, 117.4, 118.7, 118.9, 126.9, 127.0, 127.8, 128.1, 128.2, 130.0(4), 130.0(7), 130.1, 135.4(4), 135.4(7), 135.6, 144.3, 144.4, 145.7, 145.8, 145.9(1), 145.9(4), 157.5(9), 157.6(4), 158.6, 158.6, 179.5, 179.6. ^{31}P NMR (162 MHz, $CDCl_3$) δ: 147.5, 148.0. MS (FAB) m/z: 903 $[M + Na]^+$. HRMS (FAB): calcd for $C_{47}H_{57}N_6NaO_9P$ $[M + Na]^+$ 903.3817, found 903.3825.

*9-[2-O-Acetyl-3,5-di-O-benzyl-4-C-(p-toluenesulfonyloxymethyl)-β-D-ribofuranosyl]-8-aza-2,6-dichloro-7-deazapurine (**11**).* To a suspension of 4,6-dichloro-1*H*-pyrazolo[3,4-*d*]pyrimidine (**10**) (189 mg, 1.0 mmol) in HMDS (1.17 mL, 5.5 mmol) was added $(NH_4)_2SO_4$ (1.1 mg, 85 μmol), and the reaction was refluxed for 1 h. Volatiles were evaporated in vacuo, and the residual solid was dissolved in dried CH_3CN (3.0 mL). To this solution were added compound **3** (599 mg, 1.0 mmol) in dry MeCN (1.5 mL) and TMSOTf (0.1 mL, 0.5 mmol). The reaction mixture was stirred at room temperature for 3 h, satd $NaHCO_3$ was added, and the product was extracted with ethyl acetate. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (*n*-hexane/ethyl acetate = 4:1−2:1) to yield compound **11** (660 mg, 91%) as a white amorphous solid. $[\alpha]_D^{25}$ −21.9 (c 0.51, MeOH). IR ν_{max} (KBr): 3032, 2870, 1750, 1592, 1548 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.04 (3H, s), 2.44 (3H, s), 3.45 (1H, d, J = 10 Hz), 3.55 (1H, d, J = 10 Hz), 4.34 (2H, s), 4.55 (2H, s), 4.86 (1H, d, J = 5 Hz), 5.81 (1H, dd, J = 2, 5 Hz), 6.23 (1H, d, J = 2 Hz), 7.14−7.33 (12H, m), 7.79 (2H, d, J = 8 Hz), 8.00 (1H, s). Stereochemistry on the anomer position was determined by ^{1}H NMR of compound **12**. ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.6, 21.6, 69.1, 70.0, 73.4, 74.2, 74.4, 79.5, 85.1, 86.7, 113.1, 127.5, 127.6, 128.0, 128.0, 128.1, 128.2, 128.4, 129.7, 132.6, 133.8, 137.0, 137.5, 144.8, 154.8, 155.6, 157.2, 169.5. MS (FAB) m/z: 727 $[M + H]^+$. HRMS (FAB): calcd for $C_{34}H_{33}Cl_2N_4O_8S$ $[M + H]^+$ 727.1391, found 727.1396.

*8-Aza-9-[(1S,3R,4R,7S)-7-(benzyloxy)-1-(benzyloxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-2-chloro-4-oxo-7-deazapurine (**12**).* To a stirred solution of 3-hydroxypropionitrile (0.67 mL, 9.86 mmol) in dry THF (17 mL) was added NaH (60% in mineral oil, 0.48 g, 12.0 mmol) at 0 °C, and the mixture was stirred for 30 min at room temperature. The mixture was cooled to 0 °C, and a solution of compound **11** (1.60 g, 2.19 mmol) in dry THF (18 mL) was added dropwise. After being stirred for an additional 40 min, the mixture was neutralized with aq HCl (1 M) and brine (1:9) and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (*n*-hexane/ethyl acetate = 1:1−3:7) to afford **12** (0.731 g, 58%) as a white solid. $[\alpha]_D^{25}$ −73 (c 0.47, MeOH). IR ν_{max} (KBr): 3030, 2948, 2878, 1690, 1580, 1537 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.77 (2H, s), 3.90 (1H, d, J = 8 Hz), 3.98 (1H, d, J = 8 Hz), 4.47 (1H, d, J = 12 Hz), 4.51 (1H, d, J = 12 Hz), 4.64 (1H, d, J = 12 Hz), 4.71 (1H, d, J = 12 Hz), 4.72 (1H, s), 4.94 (1H, s), 6.04 (1H, s), 7.26−7.37 (10H, m), 8.24 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 66.1, 72.5, 73.0, 73.7, 78.2, 79.7, 84.7, 86.5, 104.7, 127.6, 127.7, 127.9, 128.3, 128.4, 136.3, 137.7, 146.9, 152.7, 159.0. MS (FAB) m/z: 495 $[M + H]^+$. HRMS (FAB): calcd for $C_{25}H_{24}ClN_4O_5$ $[M + H]^+$ 495.1430, found 495.1462.

*8-Aza-9-[(1S,3R,4R,7S)-7-(benzyloxy)-1-(benzyloxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-7-deazaguanine (**13**).* Compound **12** (370 mg, 0.749 mmol) was covered with methanolic ammonia (13 mL, saturated −20 °C) in a sealed tube, and the solution was heated at 120 °C for 18 h. After being cooled to 0 °C, the mixture was evaporated to dryness to remove ammonia. The crude mixture was purified by column chromatography ($CHCl_3$/MeOH = 98:2) to give **13** (287 mg, 80%) as colorless needle crystals. Mp: 215−217 °C. $[\alpha]_D^{25}$ −86 (c 0.60, THF). IR ν_{max} (KBr): 3453, 3107, 2947, 2880, 1685, 1605, 1557 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.76 (2H, s), 3.84 (1H, d, J = 8 Hz), 3.97 (1H, d, J = 8 Hz), 4.47 (1H, d, J = 12 Hz), 4.51 (1H, d, J = 12 Hz), 4.61 (1H, d, J = 12 Hz), 4.69 (1H, d, J = 12 Hz), 4.64 (1H, s), 4.94 (1H, s), 5.94 (1H, s), 7.25−7.36 (10H, m), 7.89 (1H, s), 10.68 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 66.0, 71.3, 72.2, 72.6, 77.4, 79.3, 83.7, 85.4, 99.5, 127.5, 127.6, 127.6, 127.6, 128.2, 136.0, 137.9, 138.0, 155.2, 155.9, 157.7. MS (FAB) m/z: 476 $[M + H]^+$. HRMS (FAB): calcd for $C_{25}H_{26}N_5O_5$ $[M + H]^+$ 476.1928, found 476.1921.

*8-Aza-9-[(1S,3R,4R,7S)-7-hydroxy-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-7-deazaguanine (**14**).* To a solution of **13** (456 mg, 0.955 mmol) in THF (24 mL) was added 5% Pd/C (wetted with 55% water, 200 mg) under nitrogen atmosphere. The reaction mixture was stirred under H_2 atmosphere at room temperature for 4 h, filtered, and concentrated. The crude product was purified by column chromatography ($CHCl_3$/MeOH = 9:1) to give compound **14** (215 mg, 80%) as a white solid. IR ν_{max} (KBr): 3398, 2935, 1652, 1560, 1435, 1291 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.87 (2H, s), 3.88 (1H, d, J = 8 Hz), 4.07 (1H, d, J = 8 Hz), 4.27 (1H, s), 5.04 (1H, s), 6.04 (1H, s), 7.85 (1H, s). ^{13}C NMR (76 MHz, CD_3OD) δ: 59.6, 73.1, 82.1, 85.7, 88.7, 101.0, 137.0, 156.6, 157.6, 160.9. MS (FAB) m/z: 296 $[M + H]^+$. HRMS (FAB): calcd for $C_{11}H_{13}N_5O_5$ $[M + H]^+$ 296.0989, found 296.0993.

*8-Aza-9-[(1S,3R,4R,7S)-1-(4,4′-dimethoxytrityloxymethyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-N^2-dimethylformamidine-7-deazaguanine (**15**).* Into a stirred solution of compound **14** (215 mg, 0.729 mmol) in dry DMF (1.8 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (0.97 mL, 7.29 mmol) at room temperature. The reaction mixture was warmed to 50 °C and stirred

DOI: 10.1021/acs.joc.6b02525
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for 3 h. The solution was evaporated to dryness. The crude product (255 mg) was used for the next reaction without further purification. The crude product (255 mg, 0.72 mmol) was dissolved in dry pyridine (7.2 mL), and DMTrCl (320 mg, 0.94 mmol) was added at room temperature. After being stirred for 6 h, the reaction mixture was quenched with dry methanol and concentrated. The crude product was purified by column chromatography ($CHCl_3$/MeOH = 98:2) to yield **15** (323 mg, 68%) as a white powder. $[\alpha]_D^{25}$ −90 (*c* 0.58, $CHCl_3$). IR ν_{max} (KBr): 3300, 2945, 1632, 1426, 1250, 1118 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 2.27 (1H, d, *J* = 5 Hz), 3.12 (3H, s), 3.23 (3H, s), 3.51 (2H, s), 3.78 (3H, s), 4.07 (1H, d, *J* = 8 Hz), 4.16 (1H, d, *J* = 8 Hz), 4.36 (1H, s), 5.31 (1H, d, *J* = 5 Hz), 6.30 (1H, s), 6.80 (4H, d), 7.18−7.43 (9H, m), 8.00 (1H, s), 8.42 (1H, s), 8.76 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 10.9, 22.9, 23.6, 28.8, 30.3, 35.2, 38.6, 41.5, 50.6, 55.1, 60.3, 68.1, 72.4, 73.8, 76.7, 80.8, 83.9, 85.8, 86.2, 102.5, 113.1, 126.7, 127.8, 128.0, 130.0, 135.4(8), 135.5(3), 136.3, 144.4, 155.2, 158.3(9), 158.4(1), 158.8, 159.2. MS (FAB) *m*/*z*: 653 $[M + H]^+$. HRMS (FAB): calcd for $C_{35}H_{37}N_6O_7$ $[M + H]^+$ 653.2718, found 653.2731.

*2-Cyanoethyl [(1S,3R,4R,7S)-1-(4,4′-Dimethoxytrityloxymethyl)-3-(N²-dimethylformamidine-8-aza-7-deazaguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl]diisopropylphosphoramidite (**16**).* To a solution of compound **15** (260 mg, 0.40 mmol) in dry CH_3CN (5.7 mL) were added 4,5-dicyanoimidazole (0.25 M in MeCN, 1.92 mL, 0.48 mmol) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (1.92 mL, 0.48 mmol), and the resultant mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated and diluted with ethyl acetate. The organic phase was washed with satd $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated in vacuo. The obtained crude product was purified by column chromatography (0.5% triethylamine in *n*-hexane/ethyl acetate = 2:1−1:9) to afford a white powder, which was further purified by precipitation with *n*-hexane to give 16 (261 mg, 78%) as a white powder. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.02 (3H, d, *J* = 7 Hz), 1.09−1.17 (9H, m), 2.44 (1.2H, t, *J* = 6 Hz), 2.57−2.61 (0.8H, m), 3.13 (3H, s), 3.25 (3H, s), 3.42−3.58 (6H, s), 3.76−3.86 (6H, m), 4.02−4.07 (1H, m), 4.16−4.21 (1H, m), 4.33 (0.6H, s), 4.45 (0.3H, s), 5.28 (0.4H, d, *J* = 8 Hz), 5.46 (0.6H, d, *J* = 10 Hz), 6.34 (0.4H, s), 6.35 (0.6H, s), 6.77−7.00 (4H, m), 7.16−7.44 (9H, m), 7.94 (0.4H, s), 7.96 (0.6H, s), 8.44 (1H, s), 8.80 (1H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 20.1(0), 20.1(6), 20.2, 20.3, 24.2, 24.3(1), 24.3(7), 24.4, 24.5(0), 24.5(7), 24.6, 35.3, 41.5, 43.2(1), 43.2(7), 43.3, 43.4, 55.1, 55.2, 58.5, 58.6, 58.8, 58.9, 60.2, 60.7, 73.1, 73.3, 73.4, 74.1, 80.0, 80.2, 84.1, 84.2, 85.9(3), 85.9(9), 86.0, 86.1(5), 86.1(9), 86.2, 102.6, 113.0(2), 113.0(4), 117.4(7), 117.5(2), 132.2, 126.7, 127.7, 128.1, 128.2, 130.1, 130.2, 135.6(1), 135.6(4), 135.7(7), 135.8(0), 136.0, 144.6, 144.7, 155.2, 158.3, 158.4, 158.8, 158.9. ^{31}P NMR (162 MHz, $CDCl_3$) δ: 149.5, 149.6. MS (FAB) *m*/*z*: 853 $[M + H]^+$. HRMS (FAB): Calcd for $C_{44}H_{53}N_8O_8P$ $[M + H]^+$: 853.3797, found 853.3811.

*9-[2-O-Acetyl-3,5-di-O-benzyl-4-C-(p-toluenesulfonyloxymethyl)-β-D-ribofuranosyl]-6-chloro-7-iodo-7-deazapurine (**18**).* 7-Iodo-6-chloro-7-deazapurine (**17**) (160 mg, 0.58 mmol) and **3** (380 mg, 0.64 mmol) were dissolved in MeCN (7.3 mL). BSA (0.15 mL, 0.64 mmol) was added, and the mixture was stirred at room temperature for 10 min. To the resulting clear solution was added TMSOTf (0.11 mL, 0.60 mmol) and the solution was refluxed for 2.5 h. After cooling to room temperature, satd aq $NaHCO_3$ was added and the mixture was extracted with ethyl acetate. The organic phase was washed with water and brine, dried over Na_2SO_4, and evaporated in vacuo to give the crude product. The product was purified by column chromatography (*n*-hexane:ethyl acetate =3:1) to give **18** (160 mg, 0.58 mmol) as a milky white powder. $[\alpha]_D^{23}$ −25.5 (*c* 1.10, $CHCl_3$). UV (MeOH): λ_{max} (ε) = 267 nm (2600), 303 nm (2400). IR ν_{max} (KBr): 3122, 3064, 3030, 2871, 1748, 1598, 1576, 1538, 1497, 1445, 1364, 1337, 1307, 1227, 1208, 1190, 1176, 1112, 1019 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.98 (3H, s), 2.40 (3H, s), 3.59 (1H, d, *J* = 10 Hz), 3.67 (1H, d, *J* = 10 Hz), 4.07 (1H, d, *J* = 10 Hz), 4.23 (1H, d, *J* = 10 Hz), 4.49 (1H, d, *J* = 11 Hz), 4.52 (2H, s), 4.46 (1H, d, *J* = 11 Hz), 4.60 (1H, d, *J* = 5 Hz), 5.61 (1H, t, *J* = 5 Hz), 6.30 (1H, d, *J* = 5 Hz), 7.22−7.42 (14H, m), 7.60 (1H, s), 7.71 (1H, s), 7.73 (1H, s), 8.55 (1H, s). NOE H8 (3%) by 2′-irradiation. ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.6, 21.6, 53.0, 68.9, 70.6, 73.8, 74.8, 75.3, 77.2, 77.9, 85.6, 85.7, 117.4, 127.9(5), 128.0(0), 128.1(6), 128.2(0), 128.5, 128.7, 129.8, 132.2(8), 132.3(4), 136.8, 136.9, 145.0, 150.8, 150.9, 152.7, 169.7. MS (FAB) *m*/*z*: 818 $[M + H]^+$. HRMS (FAB): calcd for $C_{35}H_{34}ClIN_3O_8S$ $[M + H]^+$ 818.0794, found 818.0809.

*9-[(1S,3R,4R,7S)-7-(Benzyloxy)-1-(benzyloxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-7-iodo-7-deazaadenine (**19**).* Compound **18** (1.35 g, 1.65 mmol) was dissolved in methanolic ammonia (25 mL, saturated at −20 °C) in a sealed tube, and the solution was heated at 120 °C for 16 h. After being cooled to 0 °C, the mixture was evaporated to dryness. The crude mixture was purified by column chromatography (*n*-hexane/ethyl acetate = 3:1) to afford **19** (860 mg, 89%) as a white solid. $[\alpha]_D^{23}$ −20.6 (*c* 1.05, $CHCl_3$). UV (MeOH): λ_{max} (ε) = 246 nm (10000). IR ν_{max} (KBr): 3454, 3131, 3062, 3030, 2946, 2880, 1628, 1578, 1553, 1496, 1469, 1438, 1364, 1337, 1295, 1254, 1201, 1181, 1137, 1110, 1042 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 3.85 (2H, s), 3.96 (1H, d, *J* = 8 Hz), 4.10 (1H, d, *J* = 8 Hz), 4.18 (1H, s), 4.52 (1H, d, *J* = 12 Hz), 4.61 (1H, d, *J* = 12 Hz), 4.62 (2H, s), 4.62 (1H, d, *J* = 12 Hz), 4.66 (1H, d, *J* = 12 Hz), 5.84 (2H, s), 6.13 (1H, s), 7.23−7.41 (10H, m), 8.24 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 50.1, 65.2, 72.2, 72.5, 73.7, 77.1, 77.4, 86.5, 86.7, 14.5, 125.7, 127.5, 127.7(7), 127.7(9), 128.1, 128.4, 128.6, 137.0, 137.7, 149.1, 152.3, 156.9. MS (FAB) *m*/*z*: 585 $[M + H]^+$. HRMS (FAB): calcd for $C_{26}H_{26}IN_4O_4$ $[M + H]^+$ 585.0993, found 585.0986.

*9-[(1S,3R,4R,7S)-7-Hydroxy-1-(hydroxymethyl)-2,5-dioxabicyclo-[2.2.1]heptan-3-yl]-7-iodo-7-deazaadenine (**20**).* Compound **19** (50 mg, 0.086 mmol) was dissolved in dry CH_2Cl_2 (1.8 mL) under argon atmosphere and cooled to −78 °C with stirring. BCl_3 in *n*-hexane (1.0 M solution, 1.4 mmol, 1.4 mL) was added dropwise. After being stirred for 1 h, the reaction mixture was allowed to reach −40 °C, warmed over 1 h to −20 °C and stirred for 14 h. The reaction was quenched by addition of MeOH at −20 °C, and the resulting mixture was warmed to room temperature. The solution was concentrated in vacuo, and the product was purified by column chromatography ($CHCl_3$/MeOH = 96:4−4:1) to give **20** (18 mg, 0.045 mmol) as a white solid. $[\alpha]_D^{25}$ −50.1 (*c* 0.28, THF/H_2O = 3:1). UV (MeOH): λ_{max} (ε) = 283 nm (9600). IR ν_{max} (KBr): 3462, 3427, 3309, 1637, 1587, 1546, 1474, 1447, 131, 1182, 1030 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.71 (1H, d, *J* = 10 Hz), 3.76 (1H, d, *J* = 4 Hz), 3.78 (1H, d, *J* = 4 Hz), 3.88 (1H, d, *J* = 11 Hz), 4.15 (1H, s), 4.23 (1H, s), 5.11 (1H, t, *J* = 8 Hz), 5.67 (1H, s), 5.95 (1H, s), 6.72 (1H, s), 7.53 (1H, s), 8.11 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 51.5, 56.6, 69.8, 71.3, 79.6, 85.3, 88.2, 103.4, 125.6, 148.8, 152.2, 157.2. MS (FAB) *m*/*z*: 405 $[M + H]^+$. HRMS (FAB): calcd for $C_{12}H_{14}IN_4O_4$ $[M + H]^+$ 405.0054, found 405.0062.

*9-[(1S,3R,4R,7S)-7-Hydroxy-1-(hydroxymethyl)-2,5-dioxabicyclo-[2.2.1]heptan-3-yl]-7-deazaadenine (**21**).* To a stirred solution of compound **20** (16 mg, 0.040 mmol) in THF/water = 3:1 (2.6 mL) was added 5% palladium carbon (wetted with 55% water, 10 mg). The reaction vessel was degassed several times with hydrogen gas, and the mixture was stirred at room temperature under a hydrogen atmosphere for 21 h. The resulting mixture was filtered, and the filtrate was evaporated to dryness. The product was purified by column chromatography ($CHCl_3$/MeOH = 8:1) to give **21** (11 mg, quant) as a white solid. $[\alpha]_D^{25}$ −24.5 (*c* 0.92, MeOH). UV (MeOH): λ_{max} (ε) = 271 nm (8000). IR ν_{max} (KBr): 3360, 2496, 1601, 1562, 1475, 1362, 1304, 1277, 1203, 1137, 1058 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ: 3.88 (1H, d, *J* = 8 Hz), 3.94 (1H, s), 4.03 (1H, d, *J* = 8 Hz), 4.34 (1H, s), 4.35 (1H, s), 6.09 (1H, s), 6.64 (1H, d, *J* = 4 Hz), 7.39 (1H, d, *J* = 4 Hz), 8.09 (1H, s). ^{13}C NMR (101 MHz, CD_3OD) δ: 58.5, 71.7, 72.7, 81.7, 87.3, 89.4, 100.9, 104.8, 122.6, 149.6, 151.4, 158.4. MS (FAB) *m*/*z*: 279 $[M + H]^+$. HRMS (FAB): calcd for $C_{12}H_{15}N_4O_4$ $[M + H]^+$ 279.1088, found 279.1082.

*9-[(1S,3R,4R,7S)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-N⁶-dimethylacetamidine-7-deazaadenine (**22**).* Compound **21** (115 mg, 0.413 mmol) was dissolved in dry DMF (2.0 mL), and *N*,*N*-dimethylacetamide dimethyl acetal was added (0.59 mL, 4.10 mmol) at room temperature. The reaction mixture was warmed to 55 °C and stirred for 3 h. After completion of

DOI: 10.1021/acs.joc.6b02525
J. Org. Chem. 2017, 82, 25−36

the reaction, the solution was concentrated. The obtained crude product (115 mg, 0.413 mmol) was dissolved in dry pyridine (4.0 mL), and DMTrCl (173 mg, 0.573 mmol) was added at room temperature. After being stirred for 2 h, the reaction mixture was cooled to 0 °C and quenched with dry methanol. The solution was extracted with ethyl acetate, washed with satd $NaHCO_3$, water, and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by column chromatography ($CHCl_3$/MeOH = 96:4 containing 0.5% triethylamine) to give **22** (160 mg, 60%) as a white solid. $[\alpha]_D^{24}$ −34.6 (*c* 1.06, $CHCl_3$). UV (MeOH): λ_{max} (ε) = 303 nm (14500). IR ν_{max} (KBr): 3004, 2933, 2836, 1606, 1573, 1543, 1508, 1446, 1396, 1333, 1300, 1251, 1177, 1155, 1059, 1030 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 2.12 (3H, s), 3.14 (6H, s), 3.51 (1H, d, *J* = 11 Hz), 3.57 (1H, d, *J* = 11 Hz), 3.80 (6H, s), 4.00 (2H, s), 4.48 (1H, s), 4.57 (1H, s), 6.26 (1H, s), 6.44 (1H, d, *J* = 4 Hz), 6.84 (2H, s), 6.86 (2H, s), 7.21−7.49 (9H, s), 8.52 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 16.8, 55.2, 59.4, 71.9(8), 72.0(1), 80.2, 86.1, 86.5, 86.6, 100.4, 112.0, 113.2, 122.1, 126.9, 127.9, 128.1, 130.1, 135.5, 135.6, 144.5, 150.3, 151.9, 158.6, 160.0, 161.9. MS (FAB) *m*/*z*: 650 $[M + H]^+$. HRMS (FAB): calcd for $C_{37}H_{40}N_5O_6$ $[M + H]^+$ 650.2973, found 650.29801.

*2-Cyanoethyl [(1S,3R,4R,7S)-1-(4,4′-Dimethoxytrityloxymethyl)-3-(N⁶-dimethylacetamidine-7-deazaguanin-9-yl)-2,5-dioxabicyclo-[2.2.1]heptan-7-yl]diisopropylphosphoramidite (**23**).* To a stirred solution of compound **22** (60.0 mg, 0.092 mmol) in dry MeCN (2 mL) were added *N*,*N*-diisopropylethylamine (47 μL, 0.28 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (31 μL, 0.14 mmol) at room temperature. After being stirred for 4 h, the reaction mixture was neutralized with satd $NaHCO_3$ and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (*n*-hexane/ethyl acetate =1:1 containing 0.5% triethylamine) to afford **23** (35 mg, 41%) as a white powder. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.78−0.93 (12 H, m), 1.05−1.15 (10H, m), 1.18 (14H, s), 2.11 (0.8H, s), 2.12 (2H, s), 2.38−2.56 (3H, m), 3.09 (6H, s), 3.35−3.46 (7H, m), 3.62−3.67 (3H, m), 3.71−3.73 (6H, m), 3.88 (1H, d, *J* = 8 Hz), 3.98 (1H, d, *J* = 8 Hz), 4.37 (0.8H, d, *J* = 7 Hz), 4.39 (0.2H, d, *J* = 7 Hz), 4.64 (0.2H, s), 4.71 (0.8H, s), 6.20 (0.8H, s), 6.21 (0.8H, d, *J* = 4 Hz), 6.43 (0.3H, d, *J* = 4 Hz), 6.68−6.79 (4H, m), 7.23−7.49 (10H, m), 7.61 (0.2H, d, *J* = 4 Hz), 8.46 (0.3H, s), 8.47 (0.7H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 16.6, 16.7, 19.9(8), 20.0(4), 23.7, 24.2, 24.3, 24.4, 24.5, 29.6, 42.6(5), 42.7(3), 43.7, 43.8, 53.9, 54.3, 54.4, 54.9, 55.0, 55.4, 55.5, 56.0, 58.2, 58.4, 59.4, 72.6, 77.1, 78.2, 86.2, 86.3, 86.9, 87.0, 99.7, 99.9, 112.0, 112.5, 113.6, 117.2, 117.3, 121.6, 127.3, 127.4, 127.5, 127.9, 128.0, 128.2, 129.1, 129.7, 129.8, 130.5, 135.5, 135.5(6), 135.6(0), 135.6(5), 135.7(1), 144.4, 144.4(7), 144.5(2), 150.3, 152.0, 152.3, 152.6(7), 152.7(1), 158.4(9), 158.5(2), 158.5(3), 159.8(5), 159.9(1), 161.9(7), 162.0(2). ^{31}P NMR (162 MHz, $CDCl_3$) δ: 148.6, 149.2. MS (FAB) *m*/*z*: 850 $[M + H]^+$. HRMS (FAB): calcd for $C_{46}H_{57}N_7O_7P$ $[M + H]^+$ 850.4052, found 850.4106.

*8-Aza-9-[(1S,3R,4R,7S)-7-(Benzyloxy)-1-(benzyloxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-2-chloro-7-deazaadenine (**24**).* A solution of **11** (627 mg, 0.86 mmol) in methanolic ammonia (30 mL, satd at −20 °C) was stirred at room temperature for 13 h in a sealed tube. The reaction mixture was cooled to 0 °C and moved to a round-bottom flask. The solution was concentrated. To a stirred solution of the obtained crude product in MeOH (10 mL) was added potassium carbonate (65.0 mg, 0.47 mmol) at room temperature, and the mixture was stirred for 2.5 h. After completion of the reaction, the solvent was removed in vacuo, and the crude product was purified by column chromatography (*n*-hexane/ethyl acetate/MeOH = 20:10:3) to give **24** (336 mg, 79%) as a white solid. $[\alpha]_D^{23}$ −68.3 (*c* 1.00, MeOH). UV (MeOH): λ_{max} (ε) = 271 nm (11500). IR ν_{max} (KBr): 3337, 3178, 1647, 1591, 1567, 1454, 1362, 1322, 1251, 1205, 1095, 1034 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ: 3.71 (2H, s), 3.87 (1H, d, *J* = 8 Hz), 3.99 (1H, d, *J* = 8 Hz), 4.43 (2H, s), 4.48 (1H, s), 4.63 (1H, d, *J* = 12 Hz), 4.65 (1H, d, *J* = 12 Hz), 5.00 (1H, s), 6.10 (1H, s), 7.13−7.29 (10H, m), 7.98 (1H, s). ^{13}C NMR (126 MHz, CD_3OD) δ: 48.5, 67.0, 73.3, 73.9, 74.6, 79.4, 80.8, 86.1, 87.6, 101.0, 128.6, 128.8, 129.1, 129.3, 129.3, 135.0, 139.1, 139.2, 156.4, 159.7, 160.2. MS (FAB) *m*/*z*: 494 $[M + H]^+$. HRMS (FAB): calcd for $C_{25}H_{25}ClN_5O_4$ $[M + H]^+$ 494.1590, found 494.1617.

*8-Aza-9-[(1S,3R,4R,7S)-7-hydroxy-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptane-3-yl]-7-deazaadenine (**25**).* To a solution of **24** (690 mg, 1.39 mmol) in MeOH (70 mL) were added ammonium formate (690 mg, 10.9 mmol) and 5% Pd/C (wetted with 55% water, 690 mg). After being refluxed for 20 h, the reaction mixture was filtered, and the filtrate was evaporated. The crude product was purified by column chromatography (ethyl acetate/MeOH = 9:1−4:1) to afford compound **25** (388 mg, quant) as colorless needles. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.88 (2H, s), 3.94 (1H, d, *J* = 6 Hz), 4.10 (1H, d, *J* = 6 Hz), 4.37 (1H, s), 5.10, (1H, s), 6.25 (1H, s), 8.13 (1H, s), 8.20 (1H, s). ^{13}C NMR (76 MHz, DMSO-d_6) δ: 57.8, 71.8, 72.1, 80.2, 83.7, 87.5, 100.0, 133.9, 154.1, 156.4, 158.0. MS (FAB) *m*/*z*: 280 $[M + H]^+$. HRMS (FAB): calcd for $C_{11}H_{13}N_5O_4$ $[M + H]^+$ 280.1040, found 280.1044. Each measurement shows good agreement with published data.[26]

*N²-Dimethylacetamidine-8-aza-9-[(1S,3R,4R,7S)-1-(4,4′-dimethyoxytrityloxymethyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-3-yl]-7-deazaadenine (**26**).* To a stirred solution of **25** (50 mg, 0.018 mmol) in dry DMF was added *N*,*N*-dimethylacetamide dimethyl acetal (0.26 mL, 0.18 mmol). The reaction mixture was stirred at 65 °C for 2 h. After completion of the reaction, the solution was concentrated. To the solution of the obtained crude products in dry pyridine (1.7 mL) was added DMTrCl (76 mg, 0.23 mmol), and the reaction mixture was stirred at room temperature for 2 h. After being cooled to 0 °C, the resulting mixture was quenched with MeOH and extracted with ethyl acetate. The organic layer was washed with satd $NaHCO_3$, water, and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by column chromatography ($CHCl_3$/MeOH = 96:4) to afford compound **26** (70 mg, 60%) as a colorless solid. $[\alpha]_D^{26}$ − 59.1 (*c* 1.07, $CHCl_3$). UV (MeOH): λ_{max} (ε) = 311 nm (23300). IR ν_{max} (KBr): 3004, 2952, 2880, 2836, 1555, 1511, 1443, 1400, 1347, 1276, 1252, 1177, 1152, 1074, 1030 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 2.25 (3H, s), 2.38 (1H, s), 3.16 (3H, s), 3.22 (3H, s), 3.51 (6H, s), 3.77 (1H, s), 4.10 (1H, d, *J* = 8 Hz), 4.15 (1H, d, *J* = 8 Hz), 4.48 (1H, s), 5.25 (1H, s), 6.48 (1H, s), 6.48−6.82 (4H, m), 7.17−7.42 (9H, m), 7.99 (1H, s), 8.60 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 17.1, 55.2, 60.4, 72.3, 74.3, 77.2, 80.7, 84.3, 85.7, 86.3, 108.5, 113.1, 126.8, 127.8, 128.1, 130.0, 134.4, 135.5, 135.6, 144.5, 155.0, 156.3, 158.5, 162.2, 162.6. MS (FAB) *m*/*z*: 651 $[M + H]^+$. HRMS (FAB): calcd for $C_{36}H_{39}N_6O_6$ $[M + H]^+$ 651.2926, found 651.2940.

*2-Cyanoethyl [(1S,3R,4R,7S)-1-(4,4′-dimethoxytrityloxymethyl)-3-(8-aza-N²-dimethylacetamidine-7-deazaguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl]diisopropylphosphoramidite (**27**).* To the solution of **26** (64 mg, 0.098 mmol) in dry MeCN (1.3 mL) were added *N*,*N*-diisopropylethylamine (32 μL, 0.30 mmol) and 2-cyanoethyl-*N*,*N*-diisopropyl-phosphorchloroamidite (51 μL, 0.015 mmol) at 0 °C. After being stirred at room temperature for 2.5 h, the mixture was quenched with satd $NaHCO_3$ and extracted with ethyl acetate. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated to dryness. The crude product was purified by column chromatography ($CHCl_3$/acetone = 10:1 containing 0.5% triethylamine) to give **27** (48 mg, 0.056 mmol) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.03 (3H, d, *J* = 7 Hz), 1.13−1.17 (9H, m), 1.26−1.29 (3H, m), 2.26 (3H, d), 2.45 (1H, t, *J* = 10 Hz), 2.61 (1H, t, *J* = 10 Hz), 2.74−2.78 (1H, m), 3.17 (3H, s), 3.21 (3H, s), 3.40−3.59 (6H, m), 3.76 (3H, s), 3.77 (3H, s), 3.81−3.87 (1H, m), 3.99 (0.5H, d, *J* = 8 Hz), 4.02 (0.5H, d, *J* = 8 Hz), 4.17 (0.5H, d, *J* = 8 Hz), 4.20 (0.5H, d, *J* = 8 Hz), 4.48 (0.5 H, s), 4.56 (0.5H, s), 5.25 (1H, d, *J* = 10 Hz), 5.42 (1H, d, *J* = 10 Hz), 6.52 (1H, s), 6.76−6.80 (4H, m), 7.16−7.43 (9H, m), 7.97 (0.5H, s), 7.99 (0.5H, s), 8.62 (1H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 20.1, 20.1(6), 20.2(5), 20.3, 24.2(5), 24.3(1), 24.3(7), 24.4(2), 24.5, 24.5(7), 24.6(3), 35.3, 41.5, 43.2, 43.2(7), 43.3(1), 43.4, 55.1, 55.2, 58.5, 58.6, 58.8, 58.9, 60.2, 60.7, 73.1, 73.3, 73.4, 74.1, 77.2, 80.0, 80.2, 84.1, 84.2, 85.9, 86.0, 86.0(9), 86.1(5), 86.1(9), 86.2(2), 102.6, 113.0(2), 113.0(4), 117.4(7), 117.5(2), 123.2, 126.7, 127.7, 128.1, 128.2, 130.1, 130.2, 135.6(1), 135.6(4), 135.8, 136.0, 144.6, 144.7, 155.2, 158.3, 158.4, 158.8, 158.9. ^{31}P NMR (162 MHz, $CDCl_3$) δ: 148.7, 148.8. MS

DOI: 10.1021/acs.joc.6b02525
J. Org. Chem. 2017, 82, 25−36

(FAB) m/z: 851 $[M + H]^+$. HRMS (FAB): calcd for $C_{45}H_{56}N_8O_7P$ $[M + H]^+$ 851.4004, found 851.4025.

Oligonucleotides Synthesis. The synthesis of modified ONs was performed on an automated DNA synthesizer on a 0.2 μmol scale using a phosphoramidite-coupling protocol and 5-[3,5-bis-(trifluoromethyl)phenyl]-1*H*-tetrazole as the activator. In the synthesis of **ON-7**, *tert*-butyl hydroperoxide was used as the oxidizer instead of iodine. The concentration of each phosphoramidite was 0.1 M, and the coupling times were prolonged to 8 min. Coupling yields were checked by trityl monitoring. The CPG solid supported ON (DMTr-ON) was treated with concentrated ammonium hydroxide solution at 55 °C for 12 h and then concentrated. The crude ODN was roughly purified and detritylated with a C18 cartridge (360 mg sorbent, 55−105 μm particle size) and then carefully purified by RP-HPLC using C18 2.5 μM column (10 × 50 mm) with a linear gradient of CH_3CN (4−12% over 30 min) in 0.1 M triethylammonium acetate buffer (pH = 7.0). The purity of the ODN was analyzed by RP-HPLC on a C18 2.5 μm column (4.6 × 50 mm), and the ODN was characterized by MALDI-TOF mass spectrometry. The yield of modified ODNs was determined by using the following extinction coefficients. ε_{260} (H_2O): LNA-^{7c}G = 13200; LNA-^{8n7c}G (**14**) = 11200; LNA-^{7c}A (**21**) = 9200; LNA-^{8n7c}A (**25**) = 9500. Regarding to LNA-^{7c}G, UV measurement was performed after cleaving protecting group of compound **7**.

UV-Melting Experiments. UV-melting experiments were performed on UV spectrophotometers equipped with a T_m analysis accessory. Each oligonucleotide and ssDNA or ssRNA was dissolved in 10 mM sodium phosphate buffer (pH 7.2) and 100 mM NaCl to give a final concentration of each strand of 4 μM. The samples were annealed by heating at 100 °C followed by slow cooling to room temperature. The samples were cooled to 5 °C in the scan cell prior to T_m measurements. The melting profiles were recorded at 260 nm from 5 to 90 °C at a scan rate of 0.5 °C/min. The T_m values were calculated as the temperature at which half of the duplex is dissociated to a single strand, based on the first derivative of the melting curve.

CD Spectra. Oligonucleotides were dissolved in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM KCl to give a final concentration of each oligonucleotide of 4 μM. The samples were boiled at 100 °C and then slowly cooled to room temperature. The spectra were measured from 350 to 200 nm at 20 °C, and the molecular ellipticity was calculated by the attached analysis software.

Quantum Mechanical Calculations. Ab initio quantum mechanical calculations were performed by the Spartan program (Spartan'14 for Macintosh, 64-Bit, Wavefunction, Inc.). Theoretical calculations were carried out using HF/6-31G*. To simplify the calculations, the 3′,5′-bismethylated nucleosides of LNA-^{8n7c}G and -^{7c}G were used.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02525.

^{1}H and ^{13}C NMR spectra of new compounds (**4**−**9**, **11**−**16**, **18**−**27**); ^{1}H and ^{31}P spectra of new amidites (**9**, **16**, **23**, **27**); HPLC charts and MALDI-TOF mass data of oligonucleotides (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by JSPS KAKENHI Nos. JP242490070, JP26460144, and JP24790110 and by the Basic Science and Platform Technology Program for Innovative Biological Medicine from the Japan Agency for Medical Research and development, AMED (16am0301004h0003). T.H. acknowledges support from the Program for Leading Graduate Schools for Osaka University: Interdisciplinary Program for Biomedical Sciences (IPBS).

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DOI: 10.1021/acs.joc.6b02525
J. Org. Chem. 2017, 82, 25−36