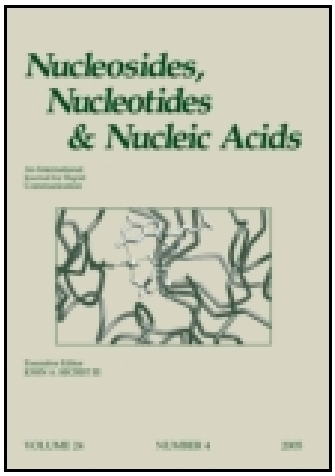


Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Synthesis and Hybridization Properties of Oligonucleotides Containing (2 S ,3 R)-9-(2,3,4-Trihydroxybutyl)Adenine

Yoshihito Ueno [a] , Shinji Ishihara [b] , Yasutomo Ito [b] & Yukio Kitade [c]

[a] Department of Biomolecular Science, Faculty of Engineering, and Center for Emerging Infectious Diseases , Gifu University , Yanagido, Gifu, Japan

[b] Department of Biomolecular Science, Faculty of Engineering , Gifu University , Yanagido, Gifu, Japan

[c] Department of Biomolecular Science, Faculty of Engineering, and Center for Emerging Infectious Diseases, and Center for Advanced Drug Research , Gifu University , Yanagido, Gifu, Japan

Published online: 07 Feb 2007.

To cite this article: Yoshihito Ueno , Shinji Ishihara , Yasutomo Ito & Yukio Kitade (2006) Synthesis and Hybridization Properties of Oligonucleotides Containing (2 S ,3 R)-9-(2,3,4-Trihydroxybutyl)Adenine, Nucleosides, Nucleotides and Nucleic Acids, 25:4-6, 475-487, DOI: 10.1080/15257770600684175

To link to this article: http://dx.doi.org/10.1080/15257770600684175

Nucleosides, Nucleotides, and Nucleic Acids, 25:475–487, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600684175

SYNTHESIS AND HYBRIDIZATION PROPERTIES OF OLIGONUCLEOTIDES CONTAINING (2*S*,3*R*)-9-(2,3,4-TRIHYDROXYBUTYL)ADENINE

Yoshihito Ueno □ *Department of Biomolecular Science, Faculty of Engineering, and Center for Emerging Infectious Diseases, Gifu University, Yanagido, Gifu, Japan*

Shinji Ishihara and Yasutomo Ito □ *Department of Biomolecular Science, Faculty of Engineering, Gifu University, Yanagido, Gifu, Japan*

Yukio Kitade □ *Department of Biomolecular Science, Faculty of Engineering, and Center for Emerging Infectious Diseases, and Center for Advanced Drug Research, Gifu University, Yanagido, Gifu, Japan*

□ *The synthesis and properties of oligonucleotides (ONs) containing 9-(2,3,4-trihydroxybutyl)adenine, $\mathbf{A}^{C2}$ and $\mathbf{A}^{C3}$, are described. The ON containing $\mathbf{A}^{C2}$ involves the 3′ → 4′ and 3′ → 5′ phosphodiester linkages in the strand, whereas that containing $\mathbf{A}^{C3}$ possesses the 3′ → 4′ and 2′ → 5′ phosphodiester linkages. It was found that incorporation of the analogs, $\mathbf{A}^{C2}$ or $\mathbf{A}^{C3}$, into ONs significantly reduces the thermal and thermodynamic stabilities of the ON/DNA duplexes, but does not largely decrease the thermal and thermodynamic stabilities of the ON/RNA duplexes as compared with the case of the ON/DNA duplexes. It was revealed that the base recognition ability of $\mathbf{A}^{C2}$ is greater than that of $\mathbf{A}^{C3}$ in the ON/RNA duplexes.*

Keywords Acyclonucleoside; Oligonucleotides; Thermal stability; Thermodynamic stability

INTRODUCTION

Oligonucleotides (ONs) that are capable of inhibiting cellular processes at the translational level by base-pairing with mRNA are known as antisense ONs.[1] For ONs to be effective as antisense molecules, they need to form stable Watson-Crick hybrids with complementary RNAs and be sufficiently resistant to degradation by ubiquitous nucleases.[2,3] A large number

Received 28 December 2005; accepted 24 January 2006.

This research was supported in part by a Grant-in-Aid for Scientific Research (C) (KAKENHI 16590082) from Japan Society for the Promotion of Science (JSPS).

This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.

Address correspondence to Yukio Kitade, Department of Biomolecular Science, Faculty of Engineering and Center for Advanced Drug Research, Gifu University, Yanagido, Gifu 5011-1193, Japan. E-mail: kitade@biomol.gifu-u.ac.jp

of chemically modified ONs have been synthesized and investigated to improve the binding affinity to complementary RNAs and the stability to nucleases. For instance, it was reported that 2′-*O*,4′-*C*-methyleneribonucleic acid (2′-*O*,4′-*C*-methylene bridged nucleic acid; 2′,4′-BNA), which is a conformationally restricted ON analog consisting of 2′-*O*,4′-*C*-methylene ribonucleosides, forms a thermally and thermodynamically stable duplex with a complementary RNA and is resistant to nucleases.[3] On the other hand, it was also reported that ON analogs containing acyclonucleosides generally destabilize duplexes with complementary DNAs,[4] although thermal and thermodynamic stabilities of duplexes with complementary RNAs have not been examined in detail.

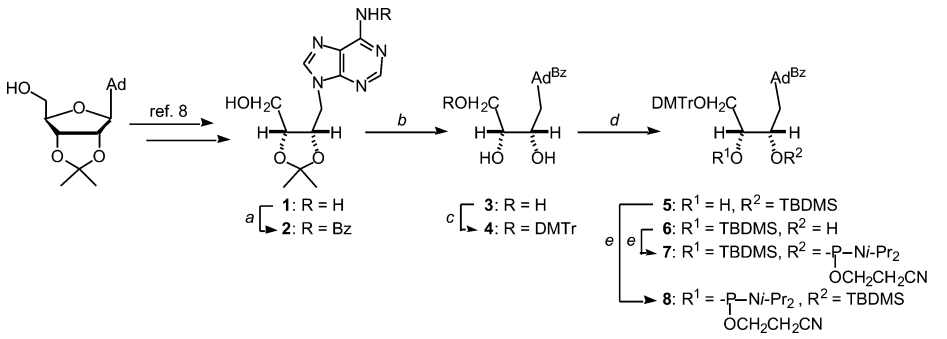
Recently, Zhang et al. showed that an ON composed of a glycerol-phosphate backbone instead of a D-ribose-phosphate backbone, which is an ON analog consisting of acyclonucleosides, forms a thermally stable duplex with a complementary RNA.[5] Furthermore, it was reported that 5′-nor-2′-deoxyoligonucleotide[6] and α-threofuranosyl-(3′→2′)-oligonucleotide,[7] which are ON analogs consisting of two carbons between the phosphates, efficiently cross-pair with complementary RNAs.

Previously, we have reported a facile, synthetic method for the preparation of acyclonucleosides, (2*S*,3*R*)-9-(2,3,4-trihydroxybutyl)purines, via the reductive cleavage of the ribofuranosyl ring of purine nucleosides with diisobutylaluminum hydride (DIBAL-H).[8] Thus, these results and the background information motivated us to investigate the synthesis and properties of ONs containing (2*S*,3*R*)-9-(2,3,4-trihydroxybutyl)adenine (Figure 1).

In this article, we wish to report the synthesis of the ON analogs containing 9-(2,3,4-trihydroxybutyl)adenine. The ON containing $\mathbf{A}^{C2}$ (which is a nucleoside analog consisting of two carbons between the phosphates) involves the 3′→4′ and 3′→5′ phosphodiester linkages, whereas that containing $\mathbf{A}^{C3}$ (which is a nucleoside analog consisting of three carbons between the phosphates) possesses the 3′→4′ and 2′→5′ phosphodiester linkages in the strand. Thermal and thermodynamic stabilities of duplexes with complementary DNAs and RNAs and stabilities of the duplexes containing mismatch base-pairs were examined in detail.

RESULTS AND DISCUSSION

In order to synthesize the ONs by the phosphoramidite method, appropriately protected phosphoramidites of $\mathbf{A}^{C2}$ and $\mathbf{A}^{C3}$ were synthesized. First, 9-[(2′*S*,3′*R*)-4′-hydroxy-2′,3′-isopropylidenedioxybutyl]adenine (**1**), which was prepared by the reported method,[8] was treated with TMSCl in pyridine and then reacted with benzoyl chloride (BzCl) to produce the N^6-Bz derivative **2** (Scheme 1). Treatment of **2** with 80% CH_3CO_2H afforded the triol **3** in 44% yield from **1**. The primary hydroxyl group of **3** was protected with a



SCHEME 1 Structures of the oligonucleotides containing nucleoside analogs.

4,4′-dimethoxytrityl (DMTr) group to give **4** in 94% yield. *O*-DMTr derivative **4** was treated with *tert*-butyldimethylsilyl chloride (TBDMSCl) to afford 2′-*O*-TBDMS and 3′-*O*-TBDMS derivatives, **5** and **6**, in 7 and 35% yields with the recovery of **4** in 20% yield, respectively. The TBDMS derivatives, **5** and **6**, were phosphitylated by a standard procedure[9] to give the corresponding phosphoramidites, **8** and **7**, in 64 and 65% yields, respectively.

ONs containing $\mathbf{A}^{C2}$ or $\mathbf{A}^{C3}$ were synthesized using the phosphoramidites, **8** and **7**, with a DNA/RNA synthesizer.[10] The sequences of the ONs used in this study are shown in Figure 2. The fully protected ONs (1 μmol each) linked to the solid support were treated with concentrated NH_4OH:EtOH

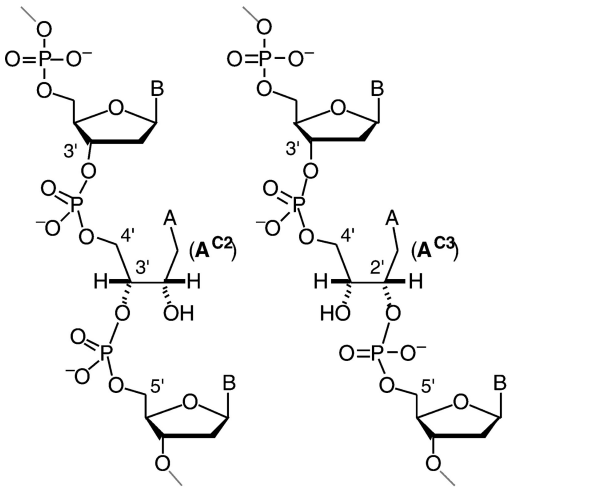


FIGURE 1 Conditions: (a) (1) TMSCl, pyridine, rt; (2) BzCl, pyridine, rt; (b) 80% CH_3CO_2H, 60°C, 44% from **1**; (c) DMTrCl, pyridine, rt, 94%; (d) TBDMSCl, imidazole, DMF, rt, 7% (**5**), 35% (**6**), 20% (**4**); (e) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine, THF, rt, 65% (**7**), 64% (**8**).

No. of ON	Sequence
ON**1**	5'-d(AAGGAAAAGAGGAAAGA)-3'
ON**2**	5'-d(AAGGAAAA$\mathbf{A}^{C2}$GAGGAAAGA)-3'
ON**3**	5'-d(AAGGAAAA$\mathbf{A}^{C3}$GAGGAAAGA)-3'
ON**4**	5'-d($\mathbf{A}^{C2}$AGGAAAAGAGGAAAGA)-3'
ON**5**	5'-d($\mathbf{A}^{C3}$AGGAAAAGAGGAAAGA)-3'
ON**6**	3'-d(TTCCTTTTCTCCTTTCT)-5'
ON**7**	3'-d(TTCCTTTACTCCTTTCT)-5'
ON**8**	3'-d(TTCCTTTGCTCCTTTCT)-5'
ON**9**	3'-d(TTCCTTTCCTCCTTTCT)-5'
ON**10**	3'-r(UUCCUUUUCUCCUUUCU)-5'
ON**11**	3'-r(UUCCUUUACUCCUUUCU)-5'
ON**12**	3'-r(UUCCUUUGCUCCUUUCU)-5'
ON**13**	3'-r(UUCCUUUCCUCCUUUCU)-5'

FIGURE 2 Sequences of oligonucleotides used in this study. Underlined letters indicate the mismatch bases against ONs **1**, **2**, and **3**.

(3:1, v/v) at 55°C for 12 h and then 1 M TBAF/THF at room temperature for 12 h. The released ONs were purified by denaturing 20% polyacrylamide gel electrophoresis to afford deprotected ONs. These ONs were analyzed by MALDI-TOF/MS and the observed molecular weights supported their structures.

The stability of the duplexes formed by these ONs and a complementary DNA (ON**6**) or RNA (ON**10**) was studied by thermal denaturation (Figure 3). Melting temperature (T_ms) andΔT_ms (T_m of duplex containing the analog $-T_m$ of the unmodified duplex) are listed in Table 1. Thermodynamic parameters of these duplexes on duplex formation were determined by calculations based on the slope of a $1/T_m$ versus $\ln(C_T/4)$ plot, where C_T is the total concentration of single strands (Figure 3).[11] The parameters are also summarized in Table 1.

When the DNA (ON**6**) was used as a complementary strand, it was revealed that ON**2** and ON**3** containing $\mathbf{A}^{C2}$ and $\mathbf{A}^{C3}$ in their centers markedly destabilize the duplexes (ΔT_ms $= -5.5$ and -5.4°C, respectively), whereas ON**4** and ON**5** containing the analogs at their 5′-ends did not largely reduce the thermal stabilities of the duplexes (ΔTs $= -0.5$ and -0.5°C, respectively). The $-\Delta G^\circ_{37}$ values of the duplexes, ON**1**:ON**6**, ON**2**:ON**6** containing $\mathbf{A}^{C2}$, and ON**3**:ON**6** containing $\mathbf{A}^{C3}$, were 14.0, 13.2, and 12.2 kcal/mol, respectively. Thus, it was found that incorporation of $\mathbf{A}^{C2}$ or $\mathbf{A}^{C3}$ into the centers of ONs thermodynamically also destabilizes the duplexes with the complementary DNAs. Intriguingly, both the $-\Delta H^\circ$ and $-\Delta S^\circ$ values of

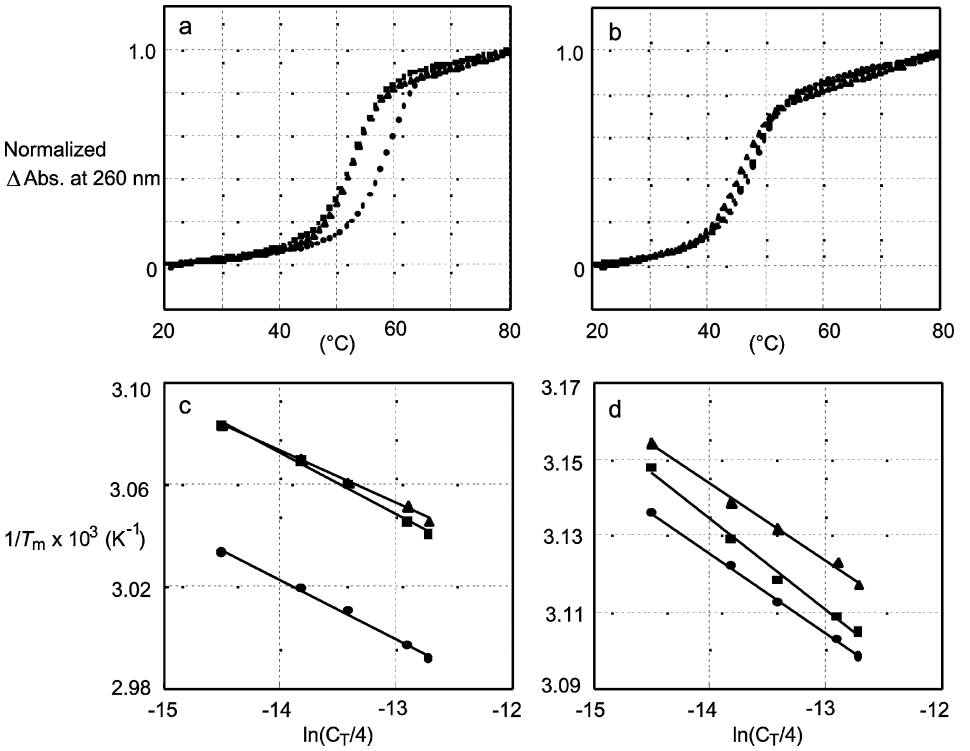


FIGURE 3 Normalized melting curves of ON/DNA duplexes (a) and ON/RNA duplexes (b) at 3 μM duplex concentrations and T_m^{-1} versus ln (C_T) plots of ON/DNA duplexes (c) and ON/RNA duplexes (d). •:ON**1**/ON**6** or ON**1**/ON**10** duplex. ▲: ON**2**/ON**6** or ON**2**/ON**10** duplex. ■: ON**3**/ON**6** or ON**3**/ON**10** duplex.

the duplex between ON**6** and ON**2** containing $\mathbf{A}^{C2}$ ($-\Delta H^\circ = 96.0$ kcal/mol and $-\Delta S^\circ = 267$ cal/K·mol) were greater than those of the unmodified duplex between ON**6** and ON**1** ($-\Delta H^\circ = 85.3$ kcal/mol and $-\Delta S^\circ = 230$ cal/K·mol), while those of the duplex between ON**6** and ON**3** containing $\mathbf{A}^{C3}$ ($-\Delta H^\circ = 81.8$ kcal/mol and $-\Delta S^\circ = 223$ cal/K·mol) were smaller than those of the unmodified duplex. These results indicate that the duplex formation between ON**6** and ON**2** containing $\mathbf{A}^{C2}$ is less favorable in entropy but more favorable in enthalpy than that between ON**6** and ON**1** consisting of the natural nucleosides, whereas that between ON**6** and ON**3** containing $\mathbf{A}^{C3}$ is more favorable in entropy but less favorable in enthalpy than that between ON**6** and ON**1**.

On the other hand, when the RNA (ON**10**) was used as a complementary strand, incorporation of the analogs $\mathbf{A}^{C2}$ and $\mathbf{A}^{C3}$ into ONs did not largely influence the thermal stabilities of the duplexes even when the analogs were introduced into the centers of the strands (ΔT_ms $= -1.7$ and -0.7°C, respectively). The $-\Delta\Delta G^\circ_{37}$ values between the duplexes ON**10**:ON**1** and ON**10**:ON**2** containing $\mathbf{A}^{C2}$, and ON**10**:ON**1** and ON**10**:ON**3** containing

TABLE 1 T_ms and Thermodynamic Parameters[a]

Duplex (base pair)	T_m[b] (°C)	ΔT_m (°C)	$-\Delta H^\circ$ (kcal/mol)	$-\Delta S^\circ$ (cal/K·mol)	$-\Delta G^\circ_{37}$ (kcal/mol)
ON**1**:ON**6** (dA:dT)	58.2	—	85.3	230	14.0
ON**2**:ON**6** ($\mathbf{A}^{C2}$:dT)	52.7	−5.5	96.0	267	13.2
ON**3**:ON**6** ($\mathbf{A}^{C3}$:dT)	52.8	−5.4	81.8	223	12.2
ON**4**:ON**6** ($\mathbf{A}^{C2}$:dT)	57.7	−0.5	87.1	236	14.0
ON**5**:ON**6** ($\mathbf{A}^{C3}$:dT)	57.7	−0.5	86.0	233	13.9
ON**1**:ON**10** (dA:rU)	47.3	—	94.6	268	11.6
ON**2**:ON**10** ($\mathbf{A}^{C2}$:rU)	45.6	−1.7	98.9	283	11.1
ON**3**:ON**10** ($\mathbf{A}^{C3}$:rU)	46.6	−0.7	83.1	233	11.0
ON**4**:ON**10** ($\mathbf{A}^{C2}$:rU)	47.1	−0.2	93.3	264	11.5
ON**5**:ON**10** ($\mathbf{A}^{C3}$:rU)	46.7	−0.6	92.0	260	11.3

[a]Experimental conditions are described in the Experimental section.
[b]T_m values at 2 μM duplex concentrations.

$\mathbf{A}^{C3}$ were 0.5 and 0.6 kcal/mol, respectively. Those values are smaller than those between the corresponding ON/DNA duplexes. Thus, it was found that incorporation of $\mathbf{A}^{C2}$ and$\mathbf{A}^{C3}$ into ONs does not largely reduce the thermal and thermodynamic stabilities of the ON/RNA duplexes as compared with those of the ON/DNA duplexes. Like the case of the ON/DNA duplexes, both the $-\Delta H^\circ$ and $-\Delta S^\circ$ values of the duplex containing $\mathbf{A}^{C2}$ in its center ($-\Delta H^\circ = 98.9$ kcal/mol and $-\Delta S^\circ = 283$ cal/K·mol) were greater than those of the unmodified duplex ($-\Delta H^\circ = 94.6$ kcal/mol and $-\Delta S^\circ = 268$ cal/K·mol), while those of the duplex containing $\mathbf{A}^{C3}$ in its center ($-\Delta H^\circ = 83.1$ kcal/mol and $-\Delta S^\circ = 233$ cal/K·mol) were smaller than those of the unmodified duplex. These results also imply that the duplex formation between ON**10** and ON**2** containing $\mathbf{A}^{C2}$ is less favorable in entropy but more favorable in enthalpy than that between ON**10** and ON**1**, whereas that between ON**10** and ON**3** containing $\mathbf{A}^{C3}$ is more favorable in entropy but less favorable in enthalpy than that between ON**10** and ON**1**.

In order to evaluate base recognition abilities of the analogs, we next examined thermal stabilities of the duplexes containing mismatch base-pairs. The T_m and ΔT_m (T_m of duplex containing mismatch base-pair–T_m of a duplex composed of a match base-pair) values are summarized in Table 2. TheΔT_ms of the duplexes containing $\mathbf{A}^{C2}$ or $\mathbf{A}^{C3}$ were smaller than those of the corresponding duplexes containing dA in both the ON/DNA and ON/RNA duplexes. This implies that the base recognition ability of the

TABLE 2 T_m Values[a]

Duplex (base pair)	T_m[b] (°C)	ΔT_m (°C)	Duplex (base pair)	T_m[b] (°C)	ΔT_m (°C)
ON**1**:ON**6** (dA:dT)	58.7	—	ON**1**:ON**10** (dA:rU)	47.3	—
ON**1**:ON**7** (dA:dA)	49.3	−9.4	ON**1**:ON**11** (dA:rA)	38.6	−8.7
ON**1**:ON**8** (dA:dG)	48.3	−10.4	ON**1**:ON**12** (dA:rG)	41.1	−6.2
ON**1**:ON**9** (dA:dC)	47.8	−10.9	ON**1**:ON**13** (dA:rC)	38.4	−8.9
ON**2**:ON**6** ($\mathbf{A}^{C2}$:dT)	52.7	—	ON**2**:ON**10** ($\mathbf{A}^{C2}$:rU)	45.6	—
ON**2**:ON**7** ($\mathbf{A}^{C2}$:dA)	49.9	−2.8	ON**2**:ON**11** ($\mathbf{A}^{C2}$:rA)	40.4	−5.2
ON**2**:ON**8** ($\mathbf{A}^{C2}$:dG)	51.8	−0.9	ON**2**:ON**12** ($\mathbf{A}^{C2}$:rG)	41.8	−3.8
ON**2**:ON**9** ($\mathbf{A}^{C2}$:dC)	48.8	−3.9	ON**2**:ON**13** ($\mathbf{A}^{C2}$:rC)	42.9	−2.7
ON**3**:ON**6** ($\mathbf{A}^{C3}$:dT)	52.8	—	ON**3**:ON**10** ($\mathbf{A}^{C3}$:rU)	46.6	—
ON**3**:ON**7** ($\mathbf{A}^{C3}$:dA)	49.8	−3.0	ON**3**:ON**11** ($\mathbf{A}^{C3}$:rA)	44.6	−2.0
ON**3**:ON**8** ($\mathbf{A}^{C3}$:dG)	48.8	−4.0	ON**3**:ON**12** ($\mathbf{A}^{C3}$:rG)	45.1	−1.5
ON**3**:ON**9** ($\mathbf{A}^{C3}$:dC)	48.3	−4.5	ON**3**:ON**13** ($\mathbf{A}^{C3}$:rC)	44.6	−2.0

[a] Experimental conditions are described in the Experimental Section.
[b] T_m values at 2 μM duplex concentrations.

analogs is less that that of dA. However, when the ΔT_m values of the duplexes containing $\mathbf{A}^{C2}$ and $\mathbf{A}^{C3}$ were compared, the ΔT_ms of the duplexes containing $\mathbf{A}^{C2}$ were slightly greater than those of the corresponding duplexes containing $\mathbf{A}^{C3}$ in the ON/DNA duplexes, whereas theΔT_ms of the duplexes containing $\mathbf{A}^{C2}$ were smaller than those of the corresponding duplexes containing $\mathbf{A}^{C3}$ in the ON/RNA duplexes. These results indicate that the base recognition ability of the analog $\mathbf{A}^{C2}$ is slightly less than that of $\mathbf{A}^{C3}$ in the ON/DNA duplex, but greater than that of $\mathbf{A}^{C3}$ in the ON/RNA duplex.

To study the global conformation of the duplexes containing $\mathbf{A}^{C2}$ and $\mathbf{A}^{C3}$, we measured the CD spectra of the duplexes. The spectrum of the control duplex between ON**6** and ON**1** showed a positive CD band at 279 nm and a negative band at 245 nm, which were attributable to the B-form duplex (Figure 4). When $\mathbf{A}^{C3}$ was introduced into the duplex, intensity of the positive CD band slightly increased and that of the negative CD band slightly decreased as compared to that of the control duplex. When the analog $\mathbf{A}^{C2}$ was incorporated into the duplex, intensity of the shoulder band around 265 nm slightly increased. This suggests that the conformation of the duplexes is slightly disordered by introducing the analogs, although the global conformation of the duplexes retains the B-like conformation.

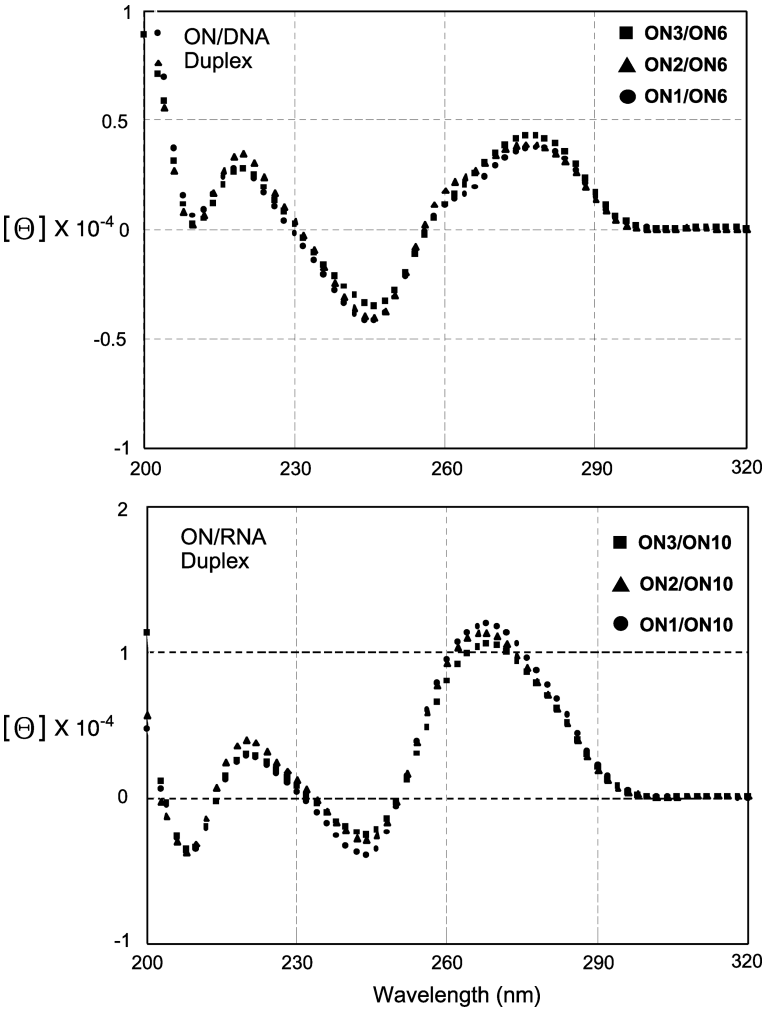


FIGURE 4 CD spectra of unmodified and modified duplexes.

On the other hand, the spectrum of the control duplex between ON**10** and ON**1** revealed a positive CD band at 268 nm and a negative band at 208 nm, which were attributable to the A-form duplex. Although the intensities of the positive CD bands of the duplexes between ON**10** and ON**2** containing $\mathbf{A}^{C2}$ and ON**10** and ON**3** containing $\mathbf{A}^{C3}$ were slightly less than that of the control duplex, those duplexes were found to retain the A-like conformation.

CONCLUSION

In this article, we have demonstrated the synthesis of the ON analogs containing 9-(2,3,4-trihydroxybutyl)adenine. Thermal and thermodynamic

stabilities of duplexes with complementary DNAs and RNAs and stabilities of the duplexes containing mismatch base-pairs were examined in detail. It was found that incorporation of the analogs, $\mathbf{A}^{C2}$ and $\mathbf{A}^{C3}$, into ONs does not largely reduce the thermal and thermodynamic stabilities of the ON/RNA duplexes as compared with those of the ON/DNA duplexes. Furthermore, it turned out that the base recognition ability of the analog $\mathbf{A}^{C2}$ is greater than that of $\mathbf{A}^{C3}$ in the ON/RNA duplex.

EXPERIMENTAL SECTION

General Remarks

NMR spectra were recorded at 400 MHz (^{1}H), at 100 MHz (^{13}C), and at 162 MHz (^{31}P) and are reported in ppm downfield from TMS or 85% H_3PO_4. J values are given in hertz. Mass spectra were obtained by electron ionization (EI) or fast atom bombardment (FAB) method.

*N^6-Benzoyl-9-[(2′S,3′R)-2′,3′,4′-trihydroxybut-1′-yl]adenine (**3**).* A mixture of **1** (8) (701 mg, 2.50 mmol) and TMSCl (1.00 ml, 7.50 mmol) in pyridine (12 ml) was stirred at room temperature. After 2 h, BzCl (0.320 ml, 2.80 mmol) was added to the mixture, and the whole was stirred at room temperature. After 3 h, the mixture was partitioned between EtOAc and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was dissolved in 80% CH_3CO_2H (10 ml), and the mixture was stirred at 60°C. After 23 h, the solvent was evaporated in vacuo, and the resulting residue was purified by column chromatograph (SiO_2, 3–7% MeOH in $CHCl_3$) to give **3** (376 mg, 44% as a white solid): ^{1}H NMR (DMSO-d_6) δ 11.10 (1H, s), 8.71 (1H, s), 8.35 (1H, s), 8.04–7.54 (5H, m), 5.11 (1H, d, J = 6.4), 4.94 (1H, d, J = 4.8), 4.53 (2H, m), 4.16 (1H, m), 3.78 (1H, m), 3.59 (1H, m), 3.38 (2H, m); ^{13}C NMR (DMSO-d_6) δ165.4, 152.7, 151.1, 149.8, 145.6, 133.5, 132.3, 128.4, 128.4, 125.4, 73.5, 69.5, 63.0, 46.8; FAB-HRMS calcd for $C_{16}H_{18}N_5O_4$ (MH^+), 344.1359; found, 344.1372.

*N^6-Benzoyl-9-[(2′S,3′R)-2′,3′-dihydroxy-4′-(4,4′-dimethoxytrityloxy)-but-1′-yl]-adenine (**4**).* A mixture of **3** (888 mg, 2.60 mmol) and DMTrCl (985 mg, 2.90 mmol) in pyridine (13 ml) was stirred at room temperature. After 18 h, DMTrCl (442 mg, 1.30 mmol) was further added to the mixture. After 5 h, the mixture was partitioned between EtOAc and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatograph (SiO_2, 1–2% MeOH in $CHCl_3$) to give **4** (1.58 g, 94%): ^{1}H NMR ($CDCl_3$) δ 9.19 (1H, s), 8.72 (1H, s), 8.01 (1H, s), 8.04–6.75 (18H, m), 4.57 (1H, dd, J = 14.6 and 3.4), 4.50 (1H, s), 4.35 (1H, dd, J = 14.6 and 2.4), 4.29 (1H, s), 3.96 (1H, m), 3.76 (6H, s), 3.51-3.39 (2H, m), 3.04 (1H, m); FAB-HRMS calcd for $C_{37}H_{36}N_5O_6$ (MH^+), 646.2666; found, 646.2653.

*N^6-Benzoyl-9-[(2′S,3′R)-2′-*tert*-butyldimethylsilyloxy-4′-(4,4′-dimethoxytrityloxy)-3′-hydroxy-but-1′-yl]adenine (***5***) and N^6-Benzoyl-9-[(2′S,3′R)-3′-tert-butyldimethylsilyloxy-4′-(4,4′-dimethoxytrityloxy)-2′-hydroxy-but-1′-yl]adenine (***6***).* A mixture of **4** (1.57 g, 2.40 mmol), TBDMSCl (400 mg, 2.60 mmol), and imidazole (372 mg, 5.30 mmol) in DMF (12 ml) was stirred at room temperature. After 9 h, TBDMSCl (224 mg, 2.40 mmol) and imidazole (360 mg, 4.80 mmol) were further added to the mixture. After 14 h, EtOH (2 ml) was added to the mixture, and the whole was stirred at room temperature for 10 min. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatograph (SiO_2, 50–70% EtOAc in hexane) to give **5** (131 mg, 7% as a white solid), **6** (641 mg, 35% as a white solid), and **4** (313 mg, 20%): Physical data of **5**: ^{1}H NMR (DMSO-d_6)δ11.13 (1H, s, 6-NH), 8.70 (1H, s, 8- or 2-H), 8.33 (1H, s, 2- or 8-H), 8.06-6.86 (18H, m, Bz, and DMTr), 5.44 (1H, d, $J=5.2$, 3′-OH), 4.37 (2H, m, 1′H), 4.21 (1H, m, 2′-H), 3.75–3.69 (7H, m, 3′-H and OMe × 2), 3.10 (1H, dd, $J=11.3$ and 5.0, 4′-H), 3.03 (1H, dd, $J=11.3$ and 6.4, 4′-H), 0.57 (9H, s, Si*t*-Bu), −0.30 and −0.54 (each 3H, s, SiMe), the assignments were in agreement with COSY spectrum; FABHRMS calcd for $C_{43}H_{50}N_5O_6Si$ (MH^+), 760.3530; found, 760.3535. Physical data of **6**: ^{1}H NMR (DMSO-d_6) δ 11.12 (1H, s, 6-NH), 8.70 (1H, s, 8- or 2-H), 8.31 (1H, s, 2- or 8-H), 8.05-6.86 (18H, m, Bz and DMTr), 5.21 (1H, d, $J=5.2$, 2′-OH), 4.48 (1H, d, $J=10.0$, m, 1′H), 4.09–4.05 (2H, m, 1′-H and 2′-H), 3.82 (1H, m, 3′-H), 3.72 (6H, s, OMe × 2), 3.19 (1H, dd, $J=9.9$ and 5.2, 4′-H), 3.05 (1H, dd, $J=9.9$ and 5.4, 4′-H), 0.85 (9H, s, Si*t*-Bu), 0.08 and −0.06 (each 3H, s, SiMe), the assignments were in agreement with COSY spectrum; FAB-HRMS calcd for $C_{43}H_{50}N_5O_6Si$ (MH^+), 760.3530; found, 760.3539.

*N^6-Benzoyl-9-[(2′S,3′R)-3′-*tert*-butyldimethylsilyloxy-4′-(4,4′-dimethoxytrityloxy)-but-1′-yl]adenine,2′-O-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoamidite (***7***).* Compound **6** (282 mg, 0.37 mmol) was dissolved in THF (2.0 ml) containing *N*,*N*-diisopropylethylamine (0.38 ml, 2.20 mmol). Chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (0.16 ml, 0.70 mmol) was added to the solution, and the mixture was stirred at room temperature. After 4 h, *N*,*N*-diisopropylethylamine (0.19 ml, 1.10 mmol) and chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (0.08 ml, 0.35 mmol) were further added to the mixture. After 1 h, aqueous $NaHCO_3$ (saturated) and $CHCl_3$ were added to the mixture, and the separated organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatograph (a neutralized SiO_2, EtOAc) to give **7** (234 mg, 65% as a white foam): ^{31}P NMR ($CDCl_3$)δ152.7, 151.5; FAB-HRMS calcd for $_{52}H_{67}N_7O_7PSi$ (MH^+), 960.4609; found, 960.4619.

*N^6-Benzoyl-9-[(2′S,3′R)-2′-tert-butyldimethylsilyloxy-4′-(4,4′-dimethoxytrityloxy)but-1′-yl]adenine, 3′-O-[(2-cyanoethyl)-(N, N-diisopropyl)]-phosphoamidite (**8**).* Compound **5** (127 mg, 0.17 mmol) was phosphitylated as described in the preparation of **7** to give **8** (105 mg, 64% as a white foam): ^{31}P NMR ($CDCl_3$) δ 149.8, 146.8; FAB-HRMS calcd for $C_{52}H_{67}N_7O_7PSi$ (MH^+), 960.4609; found, 960.4612.

Oligonucleotide Synthesis

The synthesis was carried out with an Applied Biosystems DNA/RNA synthesizer (Model Expedite) by the phosphoroamidite method. Deprotection of the bases was performed in concentrated NH_4OH:EtOH (3:1, v/v) at 55°C for 12 h. The 2′-TBDMS groups were removed by 1 M tetrabutylammonium fluoride (TBAF, Aldrich) in THF at room temperature for 12 h. The reaction was quenched with 0.1 M TEAA buffer (pH 7.0) and desalted on a Sep-Pak C18 cartridge. The deprotected ONs were purified by 20% PAGE containing 7 M urea to give the highly purified ONs **2** (11), **3** (12), **4** (5), **5** (11). The yields are indicated in parentheses as OD units at 260 nm starting from 1.0 μmol scale.

MALDI-TOF/MS Analyses of Oligonucleotides

Spectra were obtained with an Applied Biosystems Voyager DE time-of-flight mass spectrometer. ON**2**: calculated mass, 5346.5; observed mass, 5348.8. ON**3**: calculated mass, 5346.5; observed mass, 5346.2. ON**4**: calculated mass, 5346.5; observed mass, 5347.3. ON**5**: calculated mass, 5346.5; observed mass, 5348.9.

Thermal Denaturation Study and CD Spectroscopy

A solution containing the ONs in a buffer composed of 10 mM PIPES (pH 7.0), 100 mM NaCl and 10 mM $MgCl_2$ was heated at 95°C for 3 min, cooled gradually to an appropriate temperature, and then used for the thermal denaturation study. The thermal-induced transition of each mixture was monitored at 260 nm with a Beckman DU 650 spectrophotometer. The sample temperature was increased by 0.5°C/min. CD spectra were measured by a spectropolarimeter. Samples for CD spectroscopy were prepared by the same procedure used in the thermal denaturation study, and spectra were measured at 15°C. The molar ellipticitiy was calculated from the equation $[\theta] = \theta/cL$, where θ is the relative intensity, c the sample concentration, and L the cell path length in centimeters. The extinction coefficients of the ONs were calculated from those of mononucleotides and dinucleotides according to the nearest-neighbor approximation method.[12]

REFERENCES

1. (a) Uhlmann, E.; Peyman, A. Antisense oligonucleotides: A new therapeutic principle. Chemical Reviews **1990**, 90, 544–584. (b) Milligan, J. F.; Matteucci, M. D.; Martin, J. C. Current concepts in antisense drug design. Journal of Medicinal Chemistry **1993**, 36, 1923–1937. (c) Branch, A. D. A good antisense molecule is hard to find. Trends in Biochemical Science **1998**, 23, 45–50. (d) Stein, C. A. Keeping the biotechnology of antisense in context. Nature Biotechnology **1999**, 17, 209.
2. (a) Mesmaeker, A.D.; Altmann, K.-H.; Waldner, A.; Wendeborn, S. Backbone modifications in oligonucleotides and peptides nucleic acid systems. Current Opinion in Structural Biology **1995**, 5, 343–355. (b) Nielsen, P. E.; Egholm, M.; Berg, R.H.; Buchardt, O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. Science **1991**, 254, 1497–1500. (c) Ueno, Y.; Kato, Y.; Okatani, S.; Ishida, N.; Nakanishi, M.; Kitade, Y. Synthesis of antisense oligonucleotides carrying modified 2-5A molecules at their 5′-termini and their properties. Bioconjugate Chemistry **2003**, 14, 690–696. (d) Ito, T.; Ueno, Y.; Komatsu, Y.; Matsuda, A. Synthesis, thermal stability and resistance to enzymatic hydrolysis of the oligonucleotides containing 5-(*N*-aminohexyl)carbamoyl-α-*O*-methyluridines. Nucleic Acids Research **2003**, 31, 2514–2523. (e) Kanazaki, M.; Ueno, Y.; Shuto, S.; Matsuda, A. Highly nuclease-resistant phosphodiester-type oligodeoxynucleotides containing 4′α-*C*-aminoalkylthymidines form thermally stable duplexes with DNA and RNA. A candidate for potent antisense molecules. Journal of the American Chemical Society **2000**, 122, 2422–2432. (f) Haginoya, N.; Ono, A.; Nomura, Y.; Ueno, Y.; Matsuda, A. Synthesis of oligodeoxyribonucleotides containing 5-(*N*-aminoalkyl)carbamoyl-2′-deoxyuridines by a new postsynthetic modification method and their thermal stability and nuclease-resistance properties. Bioconjugate Chemistry **1997**, 8, 271–280.
3. (a) Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. Stability and structural features of the duplexes containing nucleoside analogs with a fixed N-type conformation, 2′-*O*,4′-*C*-methyleneribonucleosides. Tetrahedron Letters **1998**, 39, 5401–5404. (b) Koshkin, A.A.; Nielsen, P.; Meldgaard, M.; Rajwanshi, V.K.; Singh, S.K.; Wengel, J. LNA (locked nucleic acid): An RNA mimic forming exceedingly stable LNA:LNA duplexes. Journal of the American Chemical Society **1998**, 120, 13252–13253.
4. (a) Schneider, K.C.; Benner, S.A. Oligonucleotides containing flexible nucleoside analogs. Journal of the American Chemical Society **1990**, 112, 453-455. (b) Augustyns, K.; Van Aerschot, A.; Van Schepdael, A.; Urbanke, C.; Herdewijn, P. Influence of the incorporation of (*S*)-9-(3,4-dihydroxybutyl)adenine on the enzymic stability and base-pairing properties of oligodeoxynucleotides. Nucleic Acids Research **1991**, 19, 2587–2593. (c) Azymah, M.; Chavis, C.; Lucas, M.; Morvan, F.; Imbach, J.-L. Isotactic glycero oligothymidylate. A convenient preparation of (*R*)- and (*S*)-1′,2′-*seco*-2′-*nor*-thymidine. Nucleosides & Nucleotides **1992**, 11, 1241–1255. (d) Nielsen, P.; Kirpekar, F.; Wengel, J. Incorporation of (*R*)- and (*S*)-3′,4′-*seco*-thymidine into oligodeoxynucleotides: Hybridization properties and enzymic stability. Nucleic Acids Research **1994**, 22, 703–710. (e) Nielsen, P.; Dreiøe, L.H.; Wengel, J. Synthesis and evaluation of oligodeoxynucleotides containing acyclic nucleosides: Introduction of three novel analogs and a summary. Bioorganic and Medicinal Chemistry **1995**, 3, 19–28. (f) Kværnø, L.; Kumar, R.; Dahl, B.M.; Olsen, C.E.; Wengel, J. Synthesis of abasic locked nucleic acid and two seco-LNA derivatives and evaluation of their hybridization properties compared with their more flexible DNA counterparts. Journal of Organic Chemistry **2000**, 65, 5167–5176. (g) Merle, Y.; Bonneil, E.; Merle, L.; Sági, J.; Szemzö, A. Acyclic oligonucleotide analogues. International Journal of Biological Macromolecules **1995**, 17, 239–246. (h) Obika, S.; Takashima, Y.; Matsumoto, Y.; Shimoyama, A.; Koishihara, Y.; Ohsugi, Y.; Doi, T.; Imanishi, T. Properties of novel oligonucleotide analogues containing an acyclic nucleosides and a carbamate linkage. Bioorganic & Medicinal Chemistry Letters **1996**, 6, 1357–1360. (i) Peng, L.; Roth, H.-J. Synthesis and properties of 2′-deoxy-1′,2′-seco-D-ribosyl (5′→3′)oligonucleotides (=1′,2′-seco-DNA) containing adenine and thymine. Helvetica Chimica Acta **1997**, 80, 1494–1512. (j) Benhida, R.; Devys, M.; J.-L. Fourrey, Lecubin, F.; Sun, J.-S. Incorporation of serinol derived acyclic nucleoside analogues into oligonucleotides: influence on duplex and triplex formation. Tetrahedron Letters **1998**, 39, 6167-6170. (k) Rana, V.S.; Kumar, V.A.; Ganesh, K.N. Oligonucleotides with (*N*-thymin-1-ylacetyl)-1-arylserinol backbone: chiral acyclic analogs with restricted conformational flexibility. Tetrahedron **2001**, 57, 1311–1321.
5. Zhang, L.; Peritz, A.; Meggers, E. A simple glycol nucleic acid. Journal of the American Chemical Society **2005**, 127, 4174–4175.

6. Koga, M; Tamai, K.; Ueda, M.; Uchida, T.; Yamamuro, T.; Suzuki, T.; Saeki, T. Structure and properties of carbocyclic 5′-*nor* oligodeoxynucleotides. Nucleic Acids Research, Suppl. **2001**, 1, 19–20.
7. Schöning, K.-U.; Scholz, P.; Guntha, S.; Wu, X.; Krishnamurthy, R.; Eschenmoser, A. Chemical etiology of nucleic acid structure: the α-threofuranosyl-(3′ → 2′) oligonucleotide system. Science **2000**, 290, 1347–1351.
8. (a) Kitade, Y.; Hirota, K.; Maki, Y. Reductive cleavage of the ribose moiety in purine nucleosides using diisobutylaluminum hydride: a new method for the preparation of acyclonucleosides. Tetrahedron Letters **1993**, 34, 4835–4836. (b) Hirota, K.; Monguchi, Y.; Sajiki, H.; Sako, M.; Kitade, Y. Novel synthesis of purine acyclonucleosides possessing a chiral 9-hydroxyalkyl group by sugar modification of 9-D-ribitylpurines. Perkin Transactions 1, **1998**, 941–946.
9. Beaucage, S.L.; Caruthers, M.H. Deoxynucleoside phosphoramidites. A new class of key intermediates for deoxypolynucleotide synthesis. Tetrahedron Letters **1981**, 22, 1859–1862.
10. Gait, M.J., Ed. *Oligonucleotides Synthesis: A Practical Approach.* IRL Press, Oxford, U.K., 1984.
11. Marky, L.A.; Breslauer, K.L. Calculating thermodynamic data for transitions of any molecularity from equilibrium melting curves. Biopolymer **1987**, 26, 1601–1620.
12. Puglisi, J.D.; Tinoco, I., Jr. Absorbance melting curves of RNA, in *Methods in Enzymology.* Dahlberg, J.E.; Abelson, J.N., Eds.; Academic Press, San Diego 1989, 180, 304–325.