Synthesis and Triplex-Forming Ability of 2′,4′-BNAs Bearing Imidazoles as a Nucleobase

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To expand the sequence of double-stranded DNA (dsDNA) targets in a triplex formation, 2′,4′-BNAs (2′-*O*,4′-*C*-methylene bridged nucleic acids) having imidazoles as a nucleobase were synthesized and incorporated into oligonucleotides. Triplex-forming ability of the modified oligonucleotides was evaluated by using melting temperature (T_m) measurements.

Key words methylene bridged nucleic acid (BNA); locked nucleic acid (LNA); triplex; imidazole; oligonucleotide

Triplex-forming oligonucleotide (TFO) is able to hybridize with a double-stranded DNA (dsDNA) target in a sequence-specific manner to form a triplex DNA, and it has attracted great interest because of its applications to gene therapy and diagnosis.[2—4)] There are two fashions in triplex formation. One is a parallel motif triplex, where a TFO consisting of a homopyrimidine sequence hybridizes with a dsDNA target in parallel with a purine strand of the target duplex *via* Hoogsteen hydrogen bond. The other is an antiparallel motif triplex, in which the TFO and the purine strand of the duplex are in an antiparallel orientation. In both triplex motifs, the TFOs are able to hybridize only with a homopurine–homopyrimidine tract in the dsDNA target. This is a severe limitation of the practical use of TFOs. To overcome this problem, extensive research on nucleic acid analogues has so far been carried out.[5—8)] We have focused on the parallel type triplex, and synthesized 2′-*O*,4′-*C*-methylene-bridged nucleic acid (2′,4′-BNA[5,9—13)]/locked nucleic acid (LNA)[14)]) bearing unnatural nucleobases to recognize pyrimidine–purine interruption in the target dsDNA.[15—19)] Recently, it was found that the 2′,4′-BNA bearing oxazole[15,16)] (O^B: Fig. 1) or 2-pyridone[17—19)] as a nucleobase effectively recognized a CG interruption in homopurine–homopyrimidine dsDNA.

Here, we have selected imidazoles as unnatural nucleobases for recognition of pyrimidine–purine interruption. Synthesis of the 2′,4′-BNA monomers bearing imidazoles (I^B and aI^B: Fig. 1) and triplex-forming ability of the oligonucleotide derivatives are described.

Results and Discussion

Synthesis of 2′,4′-BNA Monomers and Their TFO Derivatives As shown in Chart 1, 2′,4′-BNA amidite units bearing imidazole and 2-aminoimidazole were synthesized by using **1**[20)] as the starting material. Coupling reaction of **1** with 2-nitroimidazoles gave **2b**, whereas the reaction of unsubstituted imidazole gave the desired compound **2a** along with an imidazolium salt **2a′**.[21)] Next, **2a** and **2b** were reacted with K_2CO_3 in MeOH to give **3a** and **3b**. Reduction of **3a** and **3b** smoothly proceeded to afford 2′,4′-BNA monomers **4a** and **4b**, respectively. Protection of an amino group in **4b** gave the corresponding *N*,*N*-dimethylformamidine derivative **4b′**. The 5′-hydroxy groups of **4a** and **4b′** were then protected with a dimethoxytrityl (DMTr) group to afford **5a** and **5b**, respectively. The preparation of phosphoramidites **6a** and **6b** was carried out by phosphitilation of **5a** and **5b**, respectively. Incorporation of the obtained amidites **6a** and **6b** into oligonucleotides was successfully achieved by using a standard phosphoramidite method on an automated DNA synthesizer. After purification by reverse-phase HPLC, composition of the oligonucleotides was confirmed by MALDI-TOF-MS.

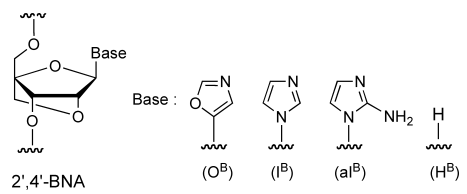
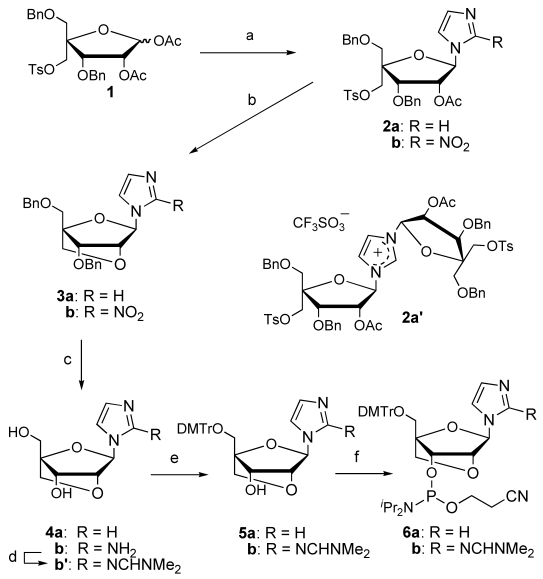


Fig. 1. Structures of the 2′,4′-BNAs Bearing Oxazole (O^B), Imidazole (I^B) and 2-Aminoimidazole (aI^B) as Unnatural Nucleobases and the 2′,4′-BNA without a Nucleobase (H^B)



Reagents: a) 1-trimethylsilylimidazole, trimethylsilyl trifluoromethanesulfonate, 40% (**2a**) and 24% (**2a′**), or *N*,*O*-bis(trimethylsilyl)acetamide, 2-nitro-1-imidazole, trimethylsilyl trifluoromethanesulfonate, 93% (**2b**); b) K_2CO_3, MeOH, 94% (**3a**) and 87% (**3b**); c) 20% $Pd(OH)_2$–C, cyclohexene, EtOH, 78% (**4a**) and 90% (**4b**); d) $Me_2NCH(OMe)_2$, MeOH, 93% (**4b′**); e) DMTrCl, pyridine, 47% (**5a**) and 87% (**5b**); f) $(^iPr_2N)_2POCH_2CH_2CN$, diisopropylammonium tetrazolide, THF–MeCN, 42% (**6a**) and 87% (**6b**).

Chart 1

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TFOs

5′-TTTTTC$\underline{T}$**X**T$\underline{C}$T$\underline{C}$T$\underline{C}$T-3′

X: I^B (**7**), aI^B (**8**), O^B (**9**), H^B (**10**), T (**11**), $\underline{C}$ (**12**) and G (**13**)

dsDNA targets

5′-GCTAAAAAGA**Y**AGAGAGATCG-3′

3′-CGATTTTTCT**Z**TCTCTCTAGC-5′

YZ: CG, GC, TA and AT

Fig. 2. Sequences of the TFOs and the dsDNA Targets Used in This Study

$\underline{C}$ means 5-methylcytidine.

Table 1. T_m Values (°C) for Dissociation of the Triplexes Comprising TFOs **7**—**13** and dsDNA Targets at pH 7.0[a]

TFO	dsDNA target (YZ)			
	CG	GC	TA	AT
7 (I^B)	21	23	25	23
8 (aI^B)	17	33	19	19
9 (O^B)[b]	29	22	27	23
10 (H^B)[c]	24	20	20	16
11 (T)	25	20	17	44
12 ($\underline{C}$)	25	43	16	19
13 (G)	20	23	27	16

a) Conditions: 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM KCl and 10 mM $MgCl_2$. The concentration of triplexes was 1.5 μM. *b*) Ref. 15. *c*) Ref. 18. $\underline{C}$ means 5-methylcytidine.

The sequences of the TFOs used in this study are shown in Fig. 2.

UV Melting Experiments Triplex-forming ability of the TFOs **7**—**13** was evaluated by UV melting experiments under neutral pH conditions (7 mM sodium phosphate buffer (pH 7.0) containing 140 mM KCl and 10 mM $MgCl_2$). The results are summarized in Table 1. The TFO **7** containing I^B showed low affinity with the dsDNA containing CG interruption (T_m=21 °C), while O^B in TFO **9** successfully recognized the CG interruption (T_m=29 °C).[15] This reflects the significant importance of an oxygen atom in the oxazole moiety of O^B for recognition of a CG base pair. One possibility is that the oxygen atom directly makes a hydrogen bond with the 4-amino group of C (Fig. 3), though we previously proposed the interaction between the nitrogen atom of the oxazole and the 4-amino group of C.[15] Although no appropriate hydrogen bonding scheme between the TA base pair and an oxazole or imidazole moiety can be proposed, both I^B and O^B showed moderate affinity with a TA base pair. The T_m values of the triplexes having $I^B \cdot TA$ and $O^B \cdot TA$ triads were 25 °C and 27 °C, respectively, while that of the triplex containing G·TA triad was 27 °C.[22] This moderate affinity of I^B and O^B with the TA base pair might involve shape recognition of the TA base pair by five-membered heteroaromatics. Introduction of a 2-amino group into the imidazole ring of I^B decreased the affinity with the TA base pair (T_m=19 °C), while the triplex containing $aI^B \cdot GC$ triad was found to be stable (T_m=33 °C). It was supposed that the 2-amino group of aI^B interacted with the 6-carbonyl oxygen of G, while the interaction between the 2-amino group of aI^B and the 4-carbonyl oxygen of T was obstructed by steric hindrance of the 5-methyl group of T (Fig. 3).

In conclusion, we have synthesized 2′,4′-BNA monomers bearing imidazole and 2-aminoimidazole as an unnatural nucleobase. UV melting experiments of the corresponding

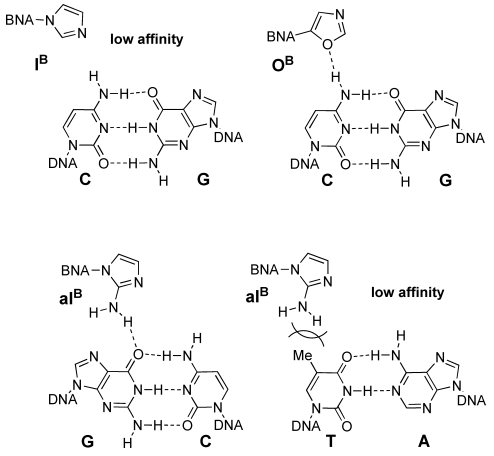

Fig. 3. Plausible Structures of $I^B \cdot CG$, $O^B \cdot CG$, $aI^B \cdot GC$ and $aI^B \cdot TA$ Base Triads

oligonucleotide derivatives revealed that the 2′,4′-BNA with unsubstituted imidazole (I^B) had only low affinity with CG interruption in the homopurine·homopyrimidine dsDNA target, while O^B, which has an oxazole moiety as a nucleobase, successfully interacted with the CG interruption.[15] This result suggests importance of the oxygen atom in O^B for recognition of the CG interrption. On the other hand, I^B showed moderate binding affinity with TA interruption, and the other 2′,4′-BNA analogue aI^B, which has a 2-aminoimidazole moiety, formed a relatively stable $aI^B \cdot GC$ triad. These results would be useful for further development of highly effective TFOs.

Experimental

General All melting points were measured on a Yanagimoto micro melting points apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP-370 instrument. IR spectra were recorded on a JASCO FT/IR-200 spectrometer. ^{1}H-NMR spectra were recorded on a JEOL EX-270 (270 MHz) and ^{31}P-NMR spectrum was recorded on a Varian VXR-200 (^{31}P, 81.0 MHz). Mass spectra of nucleoside analogues were recorded on a JEOL JMS-600 or JMS-700 mass spectrometer. For column chromatography, Fuji Silysia BW-300 (200—400 mesh) and BW-127ZH (100—270 mesh) were used. MALDI-TOF-Mass spectra were recorded on an Applied Biosystems Voeyger®-DE.

1-[2-*O*-Acetyl-3,5-di-*O*-benzyl-4-(*p*-toluenesulfonyloxymethyl)-β-D-ribofuranosyl]imidazole (2a) Under a N_2 atmosphere, 1-trimethylsilylimidazole (0.73 ml, 5.00 mmol) and trimethylsilyl trifluoromethanesulfonate (1.00 ml, 5.53 mmol) were added to a solution of **1**[20] (1.00 g, 1.67 mmol) in anhydrous 1,2-dichloroethane (20 ml) at room temperature and the mixture was stirred at room temperature for 24 h. After addition of a saturated aqueous $NaHCO_3$, the mixture was extracted with AcOEt. Usual work-up and purification by flash column chromatography [$CHCl_3$/Et_2O (5/1)] afforded **2a** (402 mg, 40%) as a colorless oil along with **2a′** (512 mg, 24%) as a colorless prism. **2a**: $[\alpha]_D^{28}$ −30.7° (*c*=1.00, $CHCl_3$). IR ν_{max} (KBr): 1748, 1363, 1229, 1180 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.01 (3H, s), 2.42 (3H, s), 3.54, 3.56 (2H, AB, *J*=10 Hz), 4.16, 4.22 (2H, AB, *J*=11 Hz), 4.46—4.48 (3H, m), 4.48, 4.54 (2H, AB, *J*=12 Hz), 5.28 (1H, dd, *J*=6, 6 Hz), 5.61 (1H, d, *J*=6 Hz), 6.99—7.00 (2H, m), 7.19—7.41 (12H, m), 7.57 (1H, s), 7.72 (2H, d, *J*=8 Hz). Mass (EI): *m/z* 606 (M^+, 2.7), 91 (100). *Anal.* Calcd for $C_{32}H_{34}N_2O_8S \cdot 1/3H_2O$: C, 62.73; H, 5.70; N, 4.57; S, 5.23. Found: C, 62.75; H, 5.74; N, 4.31; S, 5.03. **2a′**: mp 59—61 °C. $[\alpha]_D^{26}$ −4.0° (*c*=0.86, $CHCl_3$). IR ν_{max} (KBr): 1751, 1364, 1268, 1097 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.07 (6H, s), 2.43 (6H, s), 3.40, 3.80 (4H, AB, *J*=11 Hz), 4.03 (2H, d, *J*=11 Hz), 4.42 (2H, d, *J*=12 Hz), 4.33—4.56 (8H, m), 4.69 (2H, d, *J*=6 Hz), 5.51 (2H, dd, *J*=2, 6 Hz), 5.72 (2H, d, *J*=2 Hz), 7.19—7.37 (24H, m), 7.43 (2H, d, *J*=1 Hz), 7.71 (4H, d, *J*=8 Hz), 9.29 (1H, s). Mass (FAB): *m/z* 1145 (M^+), 149 ($CF_3SO_3^-$). *Anal.* Calcd for $C_{61}H_{65}N_2O_{16}S_2 \cdot CF_3SO_3$: C, 57.49; H, 5.06; N, 2.16. Found: C, 57.25; H, 5.04; N, 2.19.

1-[2-*O*-Acetyl-3,5-di-*O*-benzyl-4-(*p*-toluenesulfonyloxymethyl)-β-D-ri-

bofuranosyl]-2-nitroimidazole (2b) Under a N_2 atmosphere, 2-nitroimidazole (277 mg, 2.45 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (0.71 ml, 2.87 mmol) were added to a solution of **1** (1.22 g, 2.04 mmol) in anhydrous 1,2-dichloroethane (20 ml) at room temperature and the mixture was refluxed for 1 h. After the mixture cooled, trimethylsilyl trifluoromethanesulfonate (0.15 ml, 0.83 mmol) was added to the mixture and the mixture was refluxed for 5 h. After addition of a saturated aqueous $NaHCO_3$, the mixture was extracted with AcOEt. Usual work-up and purification by flash column chromatography [*n*-hexane/AcOEt (12/5)] afforded **2b** (1.24 g, 93%) as a white powder. mp 38—41 °C. $[\alpha]_D^{22}$ +2.1° (*c*=1.10, $CHCl_3$). IR ν_{max} (KBr): 1752, 1537, 1478, 1363, 1225, 1181, 1096 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.11 (3H, s), 2.43 (3H, s), 3.42, 3.91 (2H, AB, *J*=11 Hz), 4.12 (1H, d, *J*=12 Hz), 4.24—4.47 (4H, m), 4.44 (1H, d, *J*=6 Hz), 4.54 (1H, d, *J*=12 Hz), 5.40 (1H, dd, *J*=2, 6 Hz), 6.10 (1H, d, *J*=2 Hz), 6.84 (1H, d, *J*=1 Hz), 7.17—7.34 (12H, m), 7.73 (1H, d, *J*=1 Hz), 7.79 (2H, d, *J*=8 Hz). Mass (EI): *m*/*z* 605 (M^+-NO_2, 0.1), 560 (M^+-Bn, 0.1), 91 (100). *Anal.* Calcd for $C_{32}H_{33}N_3O_{10}S$: C, 58.98; H, 5.10; N, 6.45; S, 4.92. Found: C, 58.72; H, 5.19; N, 6.06; S, 4.86.

1-(3,5-Di-*O*-benzyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-imidazole (3a) K_2CO_3 (481 mg, 3.48 mmol) was added to a solution of **2a** (707 mg, 1.16 mmol) in MeOH (15 ml) at room temperature and the mixture was stirred for 16 h. After removal of the solvent, water was added to the residue and the mixture was extracted with AcOEt. Usual work-up and purification by flash column chromatography [$CHCl_3$/AcOEt (3/1)] afforded **3a** (432 mg, 94%) as a colorless oil. $[\alpha]_D^{26}$ −7.5° (*c*=0.68, $CHCl_3$). IR ν_{max} (KBr): 2945, 1492, 1216, 1025 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.81 (2H, s), 3.95, 4.08 (2H, AB, *J*=8 Hz), 4.13 (1H, s), 4.21 (1H, s), 4.56 (2H, s), 4.62 (2H, s), 5.73 (1H, s), 6.91 (1H, s), 7.08 (1H, s), 7.23—7.35 (10H, m), 7.60 (1H, s). Mass (EI): *m*/*z* 392 (M^+, 9.4), 91 (100). *Anal.* Calcd for $C_{23}H_{24}N_2O_4\cdot 1/3H_2O$: C, 69.33; H, 6.24; N, 7.03. Found: C, 69.10; H, 6.14; N, 6.95.

1-(3,5-Di-*O*-benzyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-2-nitroimidazole (3b) Compound **3b** was obtained from **2b** (1.09 g, 1.67 mmol) using the same procedure employed for the preparation of **3a** (pale yellow needles, 639 mg, 87%). mp 126—127 °C. $[\alpha]_D^{23}$ +73.2° (*c*=1.25, $CHCl_3$). IR ν_{max} (KBr): 1536, 1473, 1362, 1052 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.80, 3.84 (2H, AB, *J*=11 Hz), 3.87, 4.06 (2H, AB, *J*=8 Hz), 4.10 (1H, s), 4.40 (1H, s), 4.49, 4.55 (2H, AB, *J*=12 Hz), 4.65, 4.67 (2H, AB, *J*=12 Hz), 6.24 (1H, s), 7.12 (1H, d, *J*=1 Hz), 7.18—7.40 (10H, m), 7.63 (1H, d, *J*=1 Hz). Mass (EI): *m*/*z* 391 (M^+-NO_2, 0.2), 346 (M^+-Bn, 5.0), 91 (100). *Anal.* Calcd for $C_{23}H_{23}N_3O_6$: C, 63.15; H, 5.30; N, 9.61. Found: C, 63.08; H, 5.37; N, 9.43.

1-(2-*O*,4-*C*-Methylene-β-D-ribofuranosyl)imidazole (4a) Twenty percent $Pd(OH)_2$–C (54 mg) and cyclohexene (0.74 ml, 7.31 mmol) were added to a solution of **3a** (57 mg, 0.15 mmol) in EtOH (2 ml) at room temperature and the mixture was refluxed for 1.5 h. After filtration of the solution, silica (0.1 g) was added to the filtrate and the solution was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [$CHCl_3$/MeOH (10/1 to 5/1)] to give **4a** (24 mg, 78%) as a colorless oil. $[\alpha]_D^{22}$ −56.6° (*c*=0.51, CH_3OH). IR ν_{max} (KBr): 3254, 3121, 2961, 1494, 1226, 1051 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 3.90 (2H, s), 3.83, 4.00 (2H, AB, *J*=8 Hz), 4.22 (1H, s), 4.27 (1H, s), 5.78 (1H, s), 7.02 (1H, s), 7.27 (1H, s), 7.87 (1H, s). Mass (EI): *m*/*z* 212 (M^+, 57.7), 69 (100). *Anal.* Calcd for $C_9H_{12}N_2O_4\cdot 1/2H_2O$: C, 48.87; H, 5.92; N, 12.66. Found: C, 48.84; H, 5.77; N, 12.50.

2-Amino-1-(2-*O*,4-*C*-methylene-β-D-ribofuranosyl)imidazole (4b) Compound **4b** was obtained from **3b** (200 mg, 0.46 mmol) using the same procedure employed for the preparation of **4a** (a colorless oil, 93 mg, 90%). $[\alpha]_D^{28}$ −58.2° (*c*=1.08, CH_3OH). IR ν_{max} (KBr): 3418, 1646, 1560 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 3.81, 3.99 (2H, AB, *J*=8 Hz), 3.88 (2H, s), 4.28 (1H, s), 4.32 (1H, s), 5.55 (1H, s), 6.54 (1H, d, *J*=2 Hz), 6.77 (1H, d, *J*=2 Hz). Mass (FAB): *m*/*z* 228 (MH^+). High-resolition Mass (FAB): 228.0981 (MH^+, Calcd for $C_9H_{14}N_3O_4$: 228.0984).

2-[*N*-(Dimethylaminomethylidene)amino]-1-[2-*O*,4-*C*-methylene-β-D-ribofuranosyl]imidazole (4b′) 1,1-Dimethoxytrimethylamine (96 μl, 0.72 mmol) was added to a solution of **4b** (66 mg, 0.29 mmol) in MeOH (1 ml) at room temperature and the mixture was stirred for 11 h. After addition of silica (0.5 g), the solution was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [$CHCl_3$/MeOH (10/1 to 5/1)] to give **4b′** (76 mg, 93%) as a white powder. mp 212—213 °C. $[\alpha]_D^{28}$ −5.2° (*c*=0.97, CH_3OH). IR ν_{max} (KBr): 3447, 3222, 2932, 1638, 1523, 1393, 1041 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 3.03 (3H, s), 3.10 (3H, s), 3.81, 3.99 (2H, AB, *J*=8 Hz), 3.90 (2H, s), 4.21 (1H, s), 4.28 (1H, s), 5.78 (1H, s), 6.64 (1H, d, *J*=2 Hz), 6.99 (1H, d, *J*=2 Hz), 8.18 (1H, s). Mass (FAB): *m*/*z* 283 (MH^+). *Anal.* Calcd for $C_{12}H_{18}N_4O_4\cdot 1/8H_2O$: C, 50.65; H, 6.46; N, 19.69. Found: C, 50.89; H, 6.42; N, 19.39.

1-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl]-imidazole (5a) Under a N_2 atmosphere, DMTrCl (83 mg, 0.24 mmol) was added to a solution of **4a** (40 mg, 0.19 mmol) in anhydrous pyridine (1 ml) at room temperature and stirred for 3.5 h. The reaction was quenched by addition of a saturated aqueous $NaHCO_3$. The mixture was extracted with AcOEt. Usual work-up and purification by silica gel column chromatography [$CHCl_3$/AcOEt/Et_3N (80/4/1)] afforded **5a** (46 mg, 47%) as a white powder. mp 92—94 °C (*n*-hexane/AcOEt). $[\alpha]_D^{24}$ −20.9° (*c*=0.66, $CHCl_3$). IR ν_{max} (KBr): 3135, 2938, 1615, 1508, 1251, 1040 cm^{-1}. ^{1}H-NMR (acetone-d_6) δ: 3.47, 3.57 (2H, AB, *J*=11 Hz), 3.79 (6H, s), 3.94, 3.99 (2H, AB, *J*=8 Hz), 4.31 (1H, s), 4.44 (1H, s), 5.83 (1H, s), 6.87—6.92 (4H, m), 6.98 (1H, s), 7.23—7.53 (10H, m), 7.80 (1H, s). Mass (EI): *m*/*z* 514 (M^+, 6.2), 303 (100). *Anal.* Calcd for $C_{30}H_{30}N_2O_6\cdot 1/3H_2O$: C, 69.22; H, 5.94; N, 5.38. Found: C, 69.39; H, 5.88; N, 5.21.

1-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl]-2-[*N*-(dimethylaminomethylidene)amino]imidazole (5b) Compound **5b** was obtained from **4b′** (68 mg, 0.24 mmol) using the same procedure employed for the preparation of **5a** (a pale yellow powder, 122 mg, 87%). mp 106—110 °C. $[\alpha]_D^{22}$ −4.9° (*c*=0.92, $CHCl_3$). IR ν_{max} (KBr): 1631, 1510, 1251, 1038 cm^{-1}. ^{1}H-NMR (acetone-d_6) δ: 3.02 (3H, s), 3.11 (3H, s), 3.45, 3.54 (2H, AB, *J*=11 Hz), 3.78 (6H, s), 3.88, 3.96 (2H, AB, *J*=8 Hz), 4.22 (1H, s), 4.45 (1H, s), 5.81 (1H, s), 6.62 (1H, d, *J*=2 Hz), 6.87—6.90 (4H, m), 7.03 (1H, d, *J*=2 Hz), 7.23—7.41 (7H, m), 7.52—7.55 (2H, m), 8.40 (1H, s). Mass (EI): *m*/*z* 584 (M^+, 43.3), 303 (100). *Anal.* Calcd for $C_{33}H_{36}N_4O_6\cdot 3/2CH_3OH$: C, 65.49; H, 6.69; N, 8.85. Found: C, 65.74; H, 6.39; N, 8.76.

1-[3-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl]imidazole (6a) Under a N_2 atmosphere, 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (44 μl, 0.14 mmol) was added to a solution of **5a** (60 mg, 0.12 mmol) and diisopropylammonium tetrazolide (14 mg, 81.7 μmol) in anhydrous MeCN/THF (3/1, 2 ml) at room temperature and stirred for 5.5 h. The solvent was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [*n*-hexane/AcOEt/Et_3N (50/50/1)] and precipitated from *n*-hexane/AcOEt to give **6a** (35 mg, 42%) as a colorless oil. ^{31}P-NMR (acetone-d_6) δ: 149.45, 149.66.

1-[3-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl]-2-[*N*-(dimethylaminomethylidene)amino]imidazole (6b) Compound **6b** was obtained from **5b** (55 mg, 94.1 μmol) using the same procedure employed for the preparation of **6a** (a white powder, 64 mg, 87%). mp 66—69 °C. ^{31}P-NMR ($CDCl_3$) δ: 148.66, 148.88.

Synthesis and Purification of TFOs The modified oligonucleotides were synthesized on a 0.2 μmol scale on a Pharmacia Gene Assembler® Plus or on an Applied Biosystems Expedite 8909 according to the standard phosphoramidite protocol. The oligonucleotide supported on CPG, which retains a 5′-terminal DMTr group, was treated with concentrated ammonium hydroxide at 60 °C for 18 h, and the solvents were concentrated. After purification through NENSORB™ PREP, the oligonucleotide was purified by reverse-phase HPLC (ChemcoPak® CHEMCOSORB 300-5C18, 4.6 mm×250 mm) with a 11% MeCN in 0.1 M triethylammonium acetate buffer (pH 7.0). MALDI-TOF-Mass data for TFO **7** $[M-H]^-$: Found 4465.86, Calcd 4466.03; TFO **8** $[M-H]^-$: Found 4480.85, Calcd 4481.04; TFO **9** $[M-H]^-$: Found 4467.24, Calcd 4467.01; TFO **10** $[M-H]^-$: Found 4400.41, Calcd 4399.97.

T_m Measurements UV melting experiments were carried out on a Beckmann DU-650 spectrophotometer equipped with T_m analysis accessory. The UV melting profiles were recorded in 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM KCl and 10 mM $MgCl_2$ at a scan rate of 0.5 °C/min at 260 nm. The final concentration of each oligonucleotide was 1.5 μM. The T_m value was designated as the maximum of the first derivative calculated from the UV melting profile.

References and Notes

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