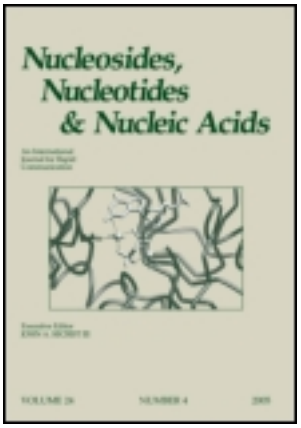

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PROPERTIES OF OLIGONUCLEOTIDE WITH PHENYL-SUBSTITUTED CARBOCYCLIC NUCLEOSIDE ANALOGS FOR THE FORMATION OF DUPLEX AND TRIPLEX DNA

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□ *(1S,3S,4R)-1-Phenyl-1-thymidyl-3-hydroxy-4-hydroxymethylcyclopentane (**10**) and their analogs were synthesized, incorporated into the oligodeoxynucleotides, and their properties were evaluated for the formation of duplex and triplex DNA. The known chiral cyclopentanone derivative was converted into the corresponding ketimine sulfonamide derivative, which was subjected to a stereoselective PhLi addition. The formed sulfonamide was hydrolyzed to afford the primary amino group, on which the thymine moiety was built. The benzyl protecting groups were removed to form the nucleoside analog having a phenyl group and the thymine unit at the 1′ position of a carbocyclic skeleton (**10**). In the estimation of the oligodeoxynucleotides incorporating **10** for duplex and triplex formation, the carbocyclic nucleoside analog **10** did not show the stabilizing effect for duplex formation; on the other hand, it stabilized the triplex. Therefore, the skeleton of the phenyl-substituted carbocyclic nucleoside analog **10** may be a platform for the formation of stable triplex DNA.*

Keywords Oligonucleotides; carbocyclic nucleoside analog; duplex DNA; triplex DNA; asymmetric synthesis

INTRODUCTION

Carbocyclic nucleoside analogs have attracted interest because of their potential biological activity. Also, a number of studies describe the properties and the applications of oligonucleotides incorporating carbocyclic

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nucleoside analogs. For example, 2′-deoxyaristeromycin (dAr) is used as a nucleoside analog that is resistant to DNA glycosylases. The 3D structure of an 11-mer DNA duplex containing a single dAr-T base pair at the center part was determined by NMR spectroscopy and MD simulations to be in a right-handed conformation in solution.[1] It was also reported that the modified oligonucleotides consisted of a carbocyclic analog formed a triplex DNA with uridine oligoribonucleotide under physiological conditions.[2] In a series of our studies on non-natural type oligonucleotides for the formation of the duplex and triplex, we became interested in carbocyclic nucleoside analogs.

Triplex DNA has been investigated as a biological tool such as for inhibition[3] or activation[4] of gene expression, and gene recombination,[5] etc. In an antiparallel triplex DNA, the purine-rich triplex forming oligonucleotide (TFOs) binds the homopurine strand of the homopurine-homopyrimidine duplex by two reverse Hoogsteen hydrogen bonds (G/G-C and A/A-T) within the major groove of the duplex DNA in a sequence-specific manner.[6] A pyrimidine nucleotide insertion into the purine strand causes destabilization of the triplex. Previously, we developed nucleoside analogs (WNA: W-shaped nucleoside analogs) having a nucleobase as a recognition part and an aromatic ring as a stacking part.[7,8] It was demonstrated that the WNA-βT and WNA-βC exhibited selective recognition of a T-A or a C-G interrupting site, respectively (Figure 1). However, it turned out in the subsequent study that the recognition of the interrupting sites is dependent on the neighboring nucleotides around the WNA analogs in the TFOs.[9] This sequence dependency problem has been partially overcome

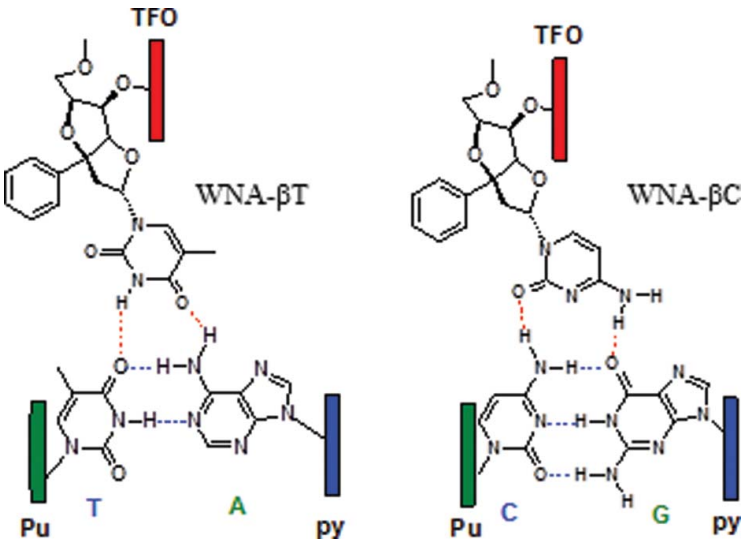


FIGURE 1 Speculated recognition model of WNA-βT/T-A and WNA-βC/C-G combinations (Color figure available online).

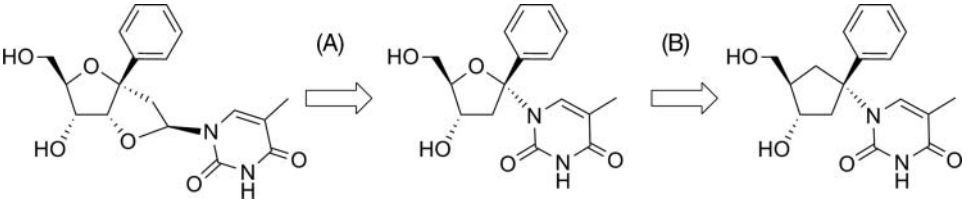


FIGURE 2 Structure modification of WNA to 1′-*β*-phenyl-*α*-thymidine **(A)**. Structure modification of 1′-*β*-phenyl-*α*-thymidine to 1-*p*henyl-1-*t*hymidyl-3-hydroxy-4-hydroxymethyl-*c*yclopentane (**10**) **(B)**.

by selecting modified WNA analogs depending on the TFO sequence.[10] In an attempt to develop new nucleoside analogs that may be generally used in the TFO, we designed analogs having both the phenyl and the nucleobase parts at the 1′-position of the sugar part (Figure 2A). However, it turned out that the 1′-phenyl-substituted ribonucleoside analogs were unstable under acidic condition and not suitable for incorporation into ODNs.[11] Thus, in this study, we focused on the carbocyclic nucleoside analogs as the more stable 1,1′-disubstituted one (Figure 2B). Figure 3 illustrates the expected triplex structure and the recognition mode of 1-phenyl-1-thymidyl-3-hydroxy-4-hydroxymethyl-cyclopentane (PTC) regarding the phenyl and thymine moieties. As it is incorporated in oligodeoxynucleotides, it should be obtained in an optically pure form. Here, we describe the synthesis of **10**, its incorporation into the oligodeoxynucleotides and evaluation of their properties for duplex and triplex DNA.

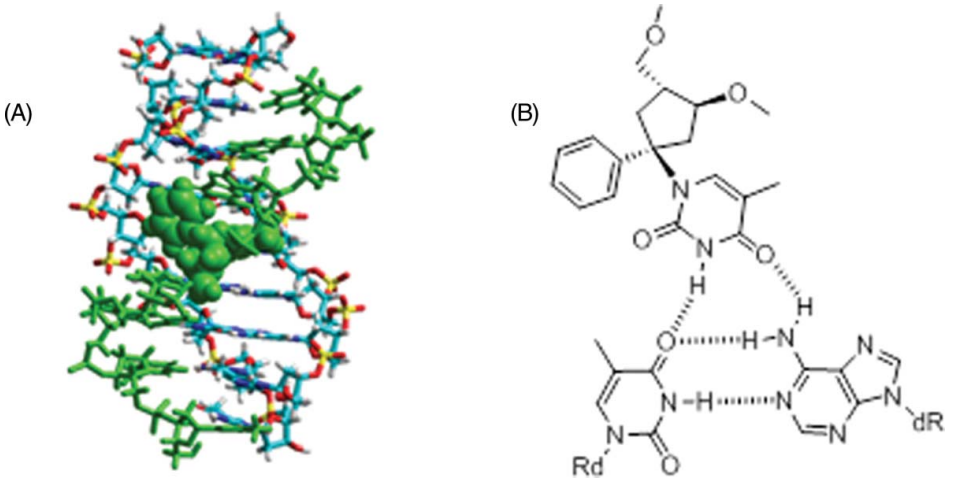
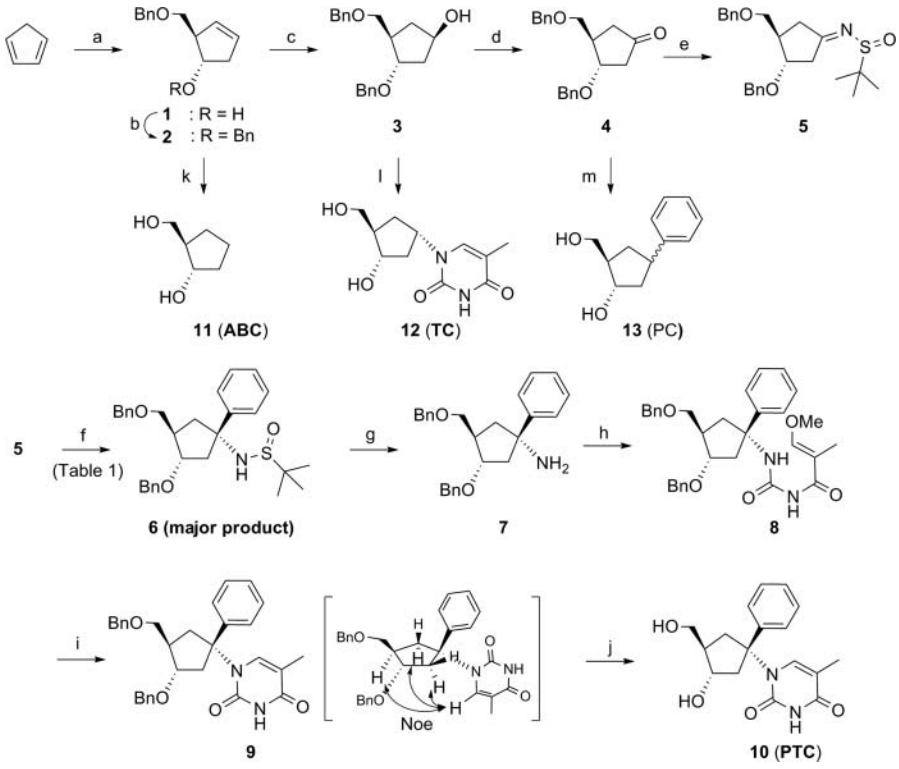


FIGURE 3 The predicted structure of triplex formation with TFO having **10**, which is depicted in the Space-Filling Model in the TFO shown in green color (**A**). An expected base-triplet between **10** and a TA base pair (**B**) (Color figure available online).

RESULTS AND DISCUSSION

Synthesis of (1*S*,3*S*,4*R*)-1-Phenyl-1-Thymidyl-3-Hydroxy-4-Hydroxymethylcyclopentane (10)

The synthesis of **10** was started with chiral (1*S*,2*R*)-2-benzyloxymethylcyclopent-3-enol **1**, which was obtained in more than 96% e.e. by a sequence of reactions according to the reported procedure involving the alkylation of cyclopentadiene using benzylchloromethyl ether and NaH and the subsequent asymmetric hydroboration with (-)-diisopinocamphenylborane[12] (Scheme 1). Next, **1** was benzylated to give **2** in 90% yield, followed by hydroboration of **2** with 9-BBN to produce



SCHEME 1 (1*S*,3*S*,4*R*)-1-Phenyl-1-thymidyl-3-hydroxy-4-hydroxymethylcyclopentane. (a) (1) NaH, THF, 0°C, 1 hour; (2) benzylchloromethylether, THF, –60°C, 2 hours; (3) –$(Ipc)_2BH$, THF, –60°C, 1 hour then 0°C, 16 hours; (4) 3 M NaOH, 30% H_2O_2, 0°C, 12 hours (25% for four steps). (b) benzyl bromide, Nah, DMF, 2 hours, r.t. (90%). (c) (1) 9-BBN, THF, r.t., 12 hours; (2) 3 M NaOH, 30% H_2O_2, 0°C, 12 hours (79% for two steps). (d) PCC, CH_2Cl_2, Celite, 12 hours, r.t. (81%). (e) R(+)-2-methyl-2-propane-sulfinamide, $Ti(OEt)_4$, THF, 70°C, 4 hours (94%). (f) PhLi, $AlMe_3$, toluene,-78°C, 3 hours (46%). (g) 4M HCL in dioxane, MeOH, r.t., 1 hour (83%). (h) 3-Methoxy-2-methyl-acryloyl isocyanate, dry benzene, r.t., 1 hour (81%) (i) 1 M aq. H_2SO_4, DMF, 80–100°C, 5 hour (60%). (j) H_2, $Pd(OH)_2/C$, MeOH, 2M HCl (cat.) (98%). (k) H_2, $Pd(OH)_2/C$, MeOH, HCl (cat), r.t., 1 hour (56%). (l) (1)N^3-benzoyl thymine, PPh_3, DIAD, CH_3CN, –40°C to r.t. 16 hours. (2) 1% NaOH in MeOH, r.t., 16 hours (3) $Pd(OH)_2/C$, H_2, MeOH, HCl (cat.), r.t., 1 hour.(m) (1) PhLi, THF, –78°C, 3 hour; (2) H_2, $Pd(OH)_2/C$, EtOAc, 20 min, r.t.

TABLE 1 1,2-addition of PhLi to tert-butane sulfinylketimine derivative

Entry	Reagent	Catalyst	Solvent	Temperature	Time (hour)	Yield
1	PhLi (1.1 eq.)	Me_3Al (1.1 eq.)	Toluene	–78°C→0°C[a]	11	23%[b]
2	PhLi (3 eq.)	Me_3Al (1.1 eq.)	Toluene	–78°C→0°C[a]	11	46%[b]
3	PhLi (3 eq.)	CsF (1.1 eq.)	Toluene	–78°C→0°C[a]	11	Trace[c]
4	PhLi (3 eq.)	CsF + Me_3Al [d]	Toluene	–78°C→0°C[a]	11	n. d.[e]
5	PhLi (3 eq.)	CuI (1.1 eq.)	Toluene	–78°C→0°C[a]	11	Trace[c]
6	PhLi (3 eq.)	CuI + Me_3Al [d]	Toluene	–78°C→0°C[a]	11	n. d.[f]
7	PhLi (3 eq.)	–	THF	–78°C→0°C[g]	4.5	Trace[h]
8	PhLi (2 eq.)	$BF_3.Et_2O$ (2 eq.)	THF	–78°C	19	n. d.[j]

[a]The reaction mixture was stirred at –78°C for 3 hours and warmed up gradually to 0°C and stirred at that temperature for more 1 hour. [b]The target compound was obtained as two isomers in the ratio (9:1). [c]As indicated by TLC. [d]Each is a 1.1 equivalent. [e]Same TLC result as entry 2. [f]TLC showed that the target amount is less than entry 2. [g]The reaction was stirred at –78°C for 2.5 hours, then allowed to warm gradually to 0°C within 2 hours. [h]TLC shows that the target compound was obtained as a trace, while the by-product was the major part. [j]TLC shows that the reaction was complicated and the desired product was obtained in low yield.

the known compound **3** as the major product in 79% yield in 2 steps.[13] The compound **3** was oxidized with PCC to afford the cyclopentanone **4** in 81% yield. The control compounds (**11**, **12**, and **13**) were synthesized from **1**, **3**, and **4**, respectively. In order to build the thymine moiety of **10**, the compound **7** was designed as the intermediate. In order to obtain the primary amine at the quaternary carbon, we adopted the method involving 1,2-addition of the phenyl group to the ketimine **5**.

For the addition of nucleophiles to ketimine, selection of the proper protecting group of the ketimine was a key issue, and the sulfonamide group was used in this study. Thus, a THF solution of the ketone **4** was refluxed with tetraethylorthotitanate and R-(+)-2-methyl-2-propane sulfonamide to afford the desired ketimine **5** in total yield 94%.[14] The crude compound **5** was subjected to flash chromatography in order to avoid hydrolysis on a silica gel column, and the purified **5** was kept under argon at –30°C. The addition of PhLi to **5** was investigated under different conditions, and the results are summarized in Table 1.

When a toluene solution of the ketimine **5** was reacted with trimethylaluminum and phenyllithium at –78°C, the desired product **6** was obtained in 23% yield as two isomers in the ratio of 9:1 (Table 1, entry 1). The two isomers were easily separated by column chromatography, but it was not possible to determine their stereochemistry by 2D 1H-NMR Noesy measurements. The yield was increased to 46% when the amount of PhLi was increased to 3 equivalents (Table 1, entry 2). The other additives, CsF, CuI, or $BF_3.Et_2O$ did not produce a better result than the conditions of entry **2**. The *tert*-butylsulfinyl group of the major isomer of **6** was easily cleaved by 4 M HCl in dioxane and methanol at room temperature[14a] to afford the primary amine **7** in 83% yield (Scheme 1). The stereochemistry at the 1'-position was not determined at this step. A benzene solution of the primary amine **7** was

reacted with freshly prepared 3-methoxy-2-methyl-acryloyl isocyanate[15] to afford compound **8** in 81% yield. Compound **8** was subjected to cyclization by refluxing with 15 eq. of 1 M aqueous H_2SO_4 in DMSO for 5 hours to afford compound **9** in 60% yield. The stereochemistry was determined as shown in **9** by 2D ^{1}H-NMR NOESY measurement. The benzyl protecting groups of **9** were removed by hydrogenolysis with H_2-$Pd(OH)_2/C$ in the presence of HCl as a catalyst to afford the desired 1′-phenyl substituted carbocyclic thymidine **10** (**PTC**) in 98% yield.

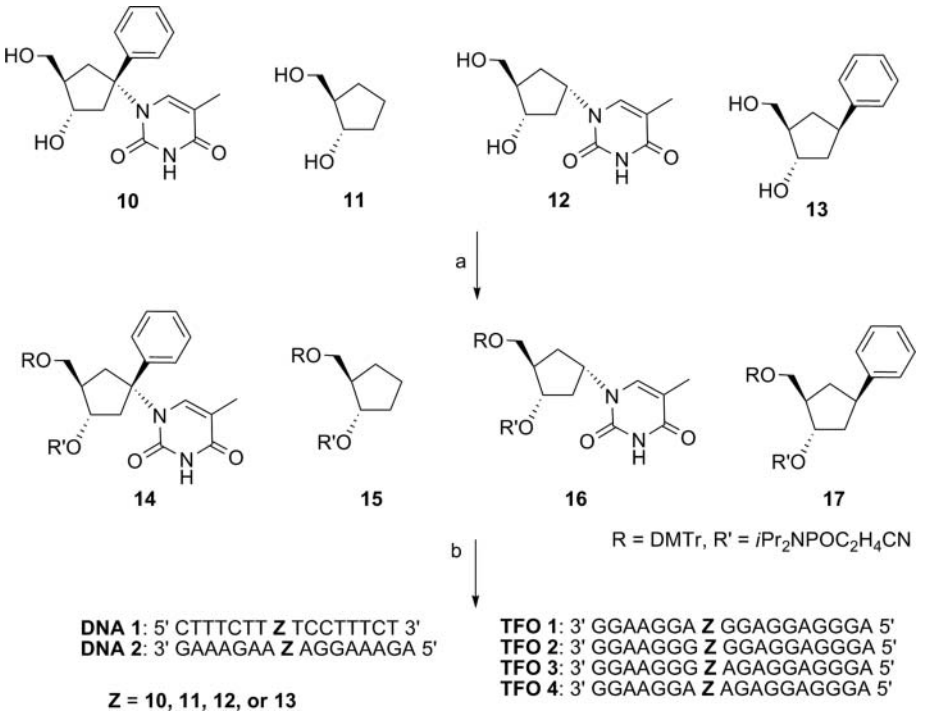
In order to evaluate the effect of both the phenyl and the thymine moieties at the 1′-position of the compound **10**, we synthesized the control compounds **11** (**ABC**), **12** (**TC**), and **13** (**PC**) (Scheme 1). The carbocyclic abasic control **11** was obtained in 56% yield from the optically active compound **1** upon treatment with hydrogen gas in the presence of $Pd(OH)_2/C$ and 2 M aqueous HCl as a catalyst, and its spectral data were comparable to the reported data.[16] The compound **12** was synthesized from the compound **3** according to the reported procedure, and its spectral data were comparable to the reported data.[13] The cyclopentanone compound **4** was reacted with phenyllithium at –78°C to afford the alcohol as a mixture of two isomers. Two isomers were not separated by column chromatography. A solution of the racemic mixture in ethyl acetate was reacted with H_2 gas in the presence of $Pd(OH)_2/C$ and 2 M aq. HCl to afford the compound **13** together with its α-isomer in the ratio (2:1) as confirmed by ^{1}H-NMR. Because these two isomers could not be separated by column chromatography, the mixture was used for the next reaction without separation.

Synthesis of Oligonucleotides

The diol compounds **10**, **11**, **12**, and **13** were transformed to the corresponding 5′-dimethoxytrityl (DMTr)-protected 3′-phosphoramidite precursors **14**, **15**, **16**, and **17**, respectively[10d] (Scheme 2). The DMTr-protected **13** was separated from its α-isomer by column chromatography, and its stereochemistry was determined by proton 2D-NMR spectra. The β-isomer was converted to the corresponding amidite precursor **17**. These DMTr-protected 3′-phosphoramidite precursors were incorporated into ODNs by automated DNA synthesizer by the conventional method. After purification of these ODNs by reverse-phase HPLC, the structures of the synthesized ODNs were confirmed by the MALDI-TOF mass measurement. DNA **1** and **2** were used to evaluate the duplex formations, and TFO (**1–4**) was used to evaluate the ability for triplex formations (Scheme 2).

Measurement of UV Melting Temperature (T_m Values)

The T_m values of DNA **1** and **2** having the carbocyclic compound or 2′-deoxy-thymidine were measured using the DNA with complementary sequences having dC, dT, dA, and dG at the opposite site for *Z* and are



SCHEME 2 Sequences of DNA **1–2** and TOF **1–4** incorporating **10,** and the control compounds **11**, **12**, and **13**. (a) (1) DMTrCl, dry pyridine, r.t., 1.5 hours; (2) $^{i}Pr_2NP(Cl)OCH_2CH_2CN$, DIPEA, CH_2Cl_2, 0°C, 1 hour. (b) (1) DNA synthesizer (2) 28% NH_3 aq., 55°C, 6 hour. (3) HPLC purification (4) 10% aq. Acetic acid, r.t., 30 min.

summarized in Figure 4. It was clearly shown that these carbocyclic compounds did not show the stabilizing effect for the duplex formation. These results indicated that the stereochemistry of the thymine base is not suitable and that the phenyl moiety has no stabilizing effect for the formation of the

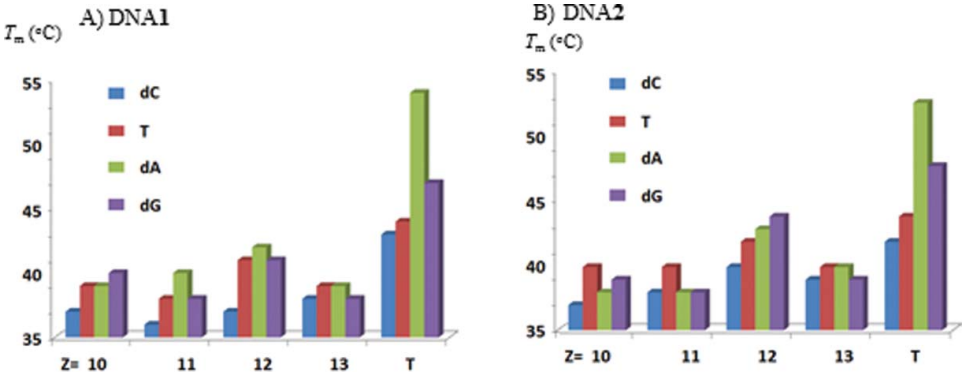


FIGURE 4 UV-melting temperature of the duplex formed with DNA **1** (A) or DNA **2** (B). The nucleotide at the complementary position to *Z* is shown in color bars. Melting profiles were measured using 2 μM each of the DNA strand in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl and 10 mM $MgCl_2$ at the scan rate of 1.0°C/min at 260 nm (Color figure available online).

duplex DNA. Compared to the T_m values of the mismatched duplexes with $Z = T$, all carbocyclic nucleoside analogs destabilized the duplex. This may be attributed to the difference in the sugar puckering and base configuration of carbocyclic nucleoside analogs.[17]

Evaluation of Triplex Formation by Gel-Shift Assay

TFO (**1–4**) was used to evaluate the ability for triplex formation by gel-shift assay and the equilibrium association constants (*Ks*) for the formation of triplex formation were calculated from the radioactive intensity of the bands according to the procedure described in our previous report.[8,9] All association constants (*Ks*) are summarized in Figure 5.

In the case of TFO **1**, **10** produced relatively high stability for the C-G, T-A, and GC sites with small selectivity (Figure 5A). Either **11** or **12** did not afford stable triplexes. Interestingly, highly stable triplex was formed with **13**. In contrast, highly stable triplexes were formed only with TFO **2** ($Z = \mathbf{13}$) with selectivity for a GC site (Figure 5B). In the sequence of TFO **3**, **10**, **11**, and **12** showed nonselective stabilizing effects except for a TA interrupting site (Figure 5C). Selective stabilization for the CG and TA interrupting site was found with **13**. No stable triplexes were formed with TFO **4** (Figure 5D).

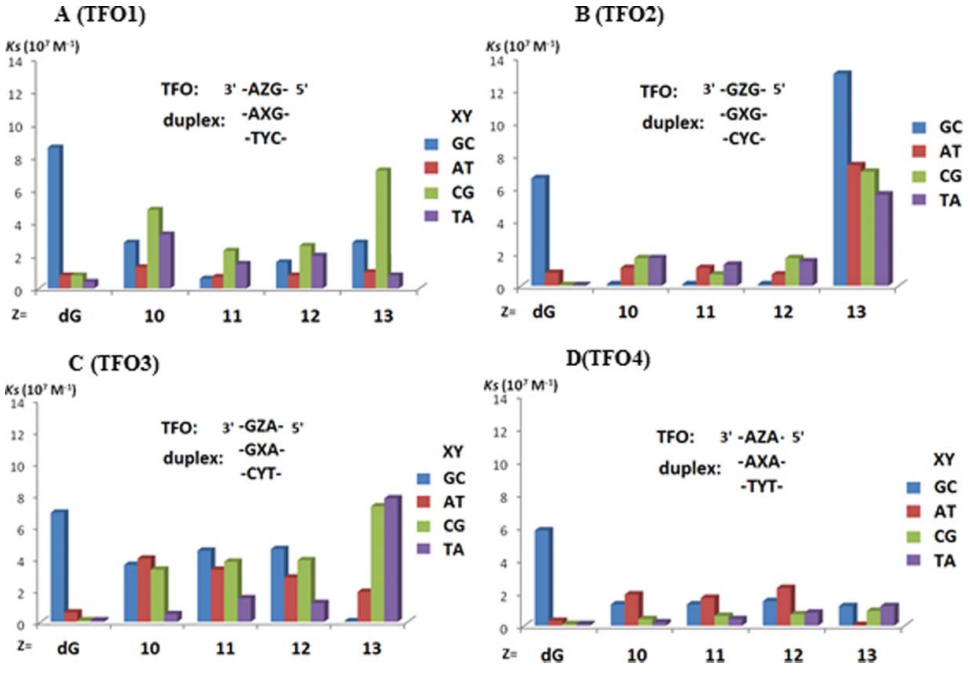


FIGURE 5 Equilibrium association constants (*Ks*) of TFOs containing carbocyclic nucleoside analogs (Color figure available online).

As **13** provided the highest stabilizing effect in TFO **1–3**, the phenyl ring of **13** might function as a hydrophobic and/or stacking unit, whereas the thymine unit of **10** cancelled its stabilizing effect probably due to the steric repulsion. In our previous study with the W-shaped nucleoside analogs (WNA), a strong sequence dependency was observed. Although the origin of sequence dependency could not be analyzed in detail, the room for the formation of the base triplet in the antiparallel triplex seems to be restricted and its shape to be determined by the surrounding bases. This study showed that the replacement of O-4 by the CH_2 group might produce a more hindered room for the base triplet.

CONCLUSIONS

This study has shown that the carbocyclic nucleoside analog is a potential candidate as a new platform structure for the synthesis of non-natural analogs for the formation of the triple helix DNA. Considering that the phenyl-substituted carbocyclic compound **13** showed a relatively high stabilizing effect and that the additional thymine substitution of **10** diminished the stabilizing effect, a proper heterocycle might provide selective interaction with a T-A or a C-G interrupting site.

EXPERIMENTAL

Melting points are uncorrected. The ^{1}H NMR (400, 500 MHz) and ^{13}C NMR (100, 125 MHz) spectra were recorded with Varian UNITY-400 and INOVA-500 spectrometers, respectively. IR spectra were obtained using a PerkinElmer FTIR-SpectrumOne. ESI-Mass spectra (ESI-MS) were taken in either positive or negative mode using methanol and formic acid or methanol only as a solvent respectively. High-resolution mass spectra were recorded on an Applied Biosystems Mariner System 5299 spectrometer. Column chromatography was carried out on 60N or FL60D silica gel. Thin layer chromatography (TLC) was performed on precoated silica gel 60F_{245} plates. Reactions below –40°C were carried out using an Eyela low temperature pairstirrer PSL-1800. DNA was synthesized by a DNA/RNA synthesizer (Applied Biosystems 394 or Nihon Techno Service NTS-H6 DNA/RNA synthesizer) and purified by HPLC. The HPLC column was a Nacalai Tesque COSMOSIL 10 × 250 mm 5C_{18}-MS-II). HPLC buffers were prepared from high quality reagents and filtered through Milipore, Millicup®-LH. All DNA samples were freeze-dried. MALDI-TOF mass spectra were obtained by a Microflex-KS Bruker Daltonios. UV spectra and *Tm* values were determined by a Beckman Coulter DU 800 spectrophotometer linked to Beckman Coulter high performance temperature controller. Fluorescence spectra were obtained with a Jasco FP-750 spectrofluorometer linked to Jasco ETC-272T

temperature controller. CD spectra were measured with a Jasco J-720 W spectropolarimeter.

(1*S*,2*R*)-1-Benzyloxy-2-Benzyloxymethylcyclopent-4-one (4)

In a dry flask, Celite (1.5 g) was suspended in dichloromethane (6 mL) at room temperature. In to the above suspension, PCC (1.034 g, 4.8 mmol) was added to afford an orange suspension. A solution of **3**[13] (azeotropically dried with dry dichloromethane three times, 500 mg, 1.6 mmol) in dry dichloromethane (2 mL) was added into the above mixture, and the reaction mixture was stirred at room temperature for 12 hours to afford a deep brown suspension. The reaction mixture was diluted with diethyl ether (30 mL) and filtered through a bad of Celite and silica gel. The filtrate was evaporated under vacuum to afford a crude pale yellow oil, which was purified by column chromatography (60 N silica gel and hexane/ ethyl acetate 3:1) to afford **4** as a pure pale yellow oil (81% yield). IR (cm^{-1}) 3030, 2858, 1744, 1496, 1454. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.35–7.24 (10 H, m), 4.55–4.47 (4 H, m), 4.14 (1H, m), 3.55 (1 H, dd, $J = 4.8$ Hz), 3.50–3.44 (1 H, m), 2.68–2.52 (3H, m), 2.32 (1H, dd, $J = 4.8$ and 4.4 Hz), 2.18 (1H, dd, $J = 6.0$ and 6.4 Hz). ^{13}C-NMR (125.68 MHz, $CDCl_3$) 215.9, 138.0, 128.4-127.5, 78.0, 73.2, 71.3, 70.4, 44.4, 42.4, 40.0. ESI-HRMS (m/z) calcd. for $C_{20}H_{22}O_3$ $[M+H]^+$ 311.1642, found 311.1622.

(1*S*,2*R*)-1-Benzyloxy-2-Benzyloxymethylcyclopent-4-*N*-*tert*-Butanesulfinyl Imine (5)

The ketone (**4**) (133 mg, 0.429 mmol) was azeotropically dried with dry acetonitrile three times (3 × 3 mL) and then dissolved in 0.6 mL dry THF under argon. Into the above solution, a solution of tetraethylorthotitanate (208 μL, 0.943 mmol, >95% W) in 1.2 mL dry THF was added dropwise at room temperature. To this reaction mixture, *R*-(+)-2-methyl-2-propane sulfonamide (64 mg, 0.514 mmol) was added and the reaction mixture was immediately heated at 70°C for 4 hours. After cooling to room temperature, the mixture was added rapidly into a volume of brine (1.75 mL) to form a white solid. The mixture was filtered through a Celite pad and the collected paste was washed with ethyl acetate. The filtrate was transferred to a separating funnel and washed with brine (15 mL). The separated brine was extracted with ethylacetate (30 mL). The combined organic solvents were dried over anhydrous sodium sulfate and evaporated under vacuum to afford a crude dark yellow oil, which was purified by flash column chromatography (60 N silica gel, hexane/ ethylacetate 4:1→3:1→2:1) to afford the target as a yellow oil (166 mg, 94% yield). The target should be eluted within 15 minutes as it slowly hydrolyzes on silica gel. IR (cm^{-1}) 2917.0, 2861.0, 1637.5, 1496.2, 1454.1, 1361.3. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.33–7.24 (m, 10H), 4.45-4.42

(m, 4H), 4.06–4.11 (m, 1H), 3.50–3.23 (m, 3H), 2.89–2.80 (m, 1H), 2.68 (m, 1H), 2.58–2.46 (m, 1H), 1.20 (s, 9H). ESI-MS for $C_{24}H_{31}NO_3S$ $[M+H]^+$ calcd. 414.21 found 414.24.

(1*S*,2*R*)-1-Benzyloxy-2-Benzyloxymethyl-4-Phenylcyclopent-4-*N*-*tert*-butanesulfinyl Amine (6)

In a very dry flask, compound **(5)** (30 mg, 0.0726 mmol) was dried azeotropically in acetonitrile (3 × 2 mL), then dissolved in 0.3 mL dry toluene under argon atmosphere and cooled to –78°C. Trimethylaluminum (2M solution in toluene, 40 μL, 0.0799 mmol) was then added and the reaction mixture was stirred at –78°C for 30 minutes. Phenyllithium (1.09 M/L in cyclohexane/diethyl ether, 200 μL, 0.2179 mmol) was added dropwise, and the reaction mixture was stirred at –78°C for 3 hours, then allowed to warm gradually to 0°C over 7 hours. After stirring for 1 hour at 0°C, TLC indicated the disappearance of the starting material. The mixture was quenched by the slow addition of a saturated aqueous solution of sodium sulfate until gas evolution stopped, then warmed to room temperature, filtered and diluted with ethyl acetate (10 mL). The organic layer was separated and washed with water (10 mL) and brine (10 mL), dried over sodium sulfate, and evaporated to afford a crude yellowish brown oil. The crude product was purified by short flash column chromatography (FL60D silica gel, hexane/ ethyl acetate 3:1) to afford the target as a yellowish brown oil (46% total yield for 2 isomers in the ratio 9:1).

Physical Data for the Major Isomer

IR (cm^{-1}) 3265.3, 3029.0, 2959.6, 2862.0, 1743.9, 1603.1, 1496.0, 1453.8. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.58–7.08 (15 H, m, aromatic), 5.22 (1H, s), 4.51 (2H, d, $J = 3.4$ Hz), 4.47 (2H, d, $J = 12.0$ Hz), 4.05 (1H, d, $J = 4.5$ Hz), 3.60 (1H, dd, $J = 4.5$ and 3.4 Hz), 3.44–3.36 (1H, m), 3.12–2.86 (2H, m), 2.40 (1H, d, $J = 14.8$ Hz), 2.03 (1H, t, $J = 10.8$ Hz), 1.9 (1H, dd, $J = 6.4$ and 6.8 Hz), 0.97 (9H, s). ^{13}C-NMR (125.68 MHz, $CDCl_3$) 144.5, 138.5, 138.5, 128.4, 128.3, 128.3, 128.3, 128.3, 128.36, 128.0, 127.8, 127.5, 127.5, 127.5, 127.5, 127.2, 126.9, 126.7, 82.9, 73.0, 72.0, 70.8, 68.9, 55.6, 48.0, 44.8, 39.3, 22.7, 22.5, 22.3. ESI-HRMS (m/z) for $C_{30}H_{37}NO_3S$ $[M+H]^+$ calcd. 492.2567 found 492.2595.

Physical Data for the Minor Isomer

IR (cm^{-1}) 2918.7, 2859.4, 1604.0, 1495.7, 1474.0, 1454.3, 1361.6. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.36–7.08 (m, 15 H), 6.34 (1H, NH), 4.49 (s, 4H), 3.51–3.40 (m, 4 H), 3.38–3.10 (m, 2 H), 2.86-2.43 (m, 2H), 1.21 (s, 9H). $C_{30}H_{37}NO_3S$ (M.W. 491.25). ESI-MS for $C_{30}H_{37}NO_3S$ $[M+H]^+$ calcd. 492.25 found 492.31.

(1*S*,2*R*)-1-Benzyloxy-2-Benzyloxymethyl-4-Phenylcyclopent-4-Amine (7)

The major isomer of compound **(6)** (13 mg, 0.0264 mmol) was dissolved in methanol (0.25 mL), then 4 M HCl in dioxane (0.132 mmol, 35 μL) was added and the reaction mixture was stirred at room temperature for 30 minutes until TLC indicated the disappearance of all the starting material. The reaction mixture was diluted with methanol (5 mL) and neutralized with solid sodium bicarbonate until effervescence stopped. The mixture was filtered and diluted with ethyl acetate (10 mL). The separated organic layer was washed with water (5 mL), dried over anhydrous sodium sulfate and evaporated to afford a crude yellow oil (10 mg). The crude product was purified by flash column chromatography (FL60D silica gel, chloroform/methanol 50:1→40:1) to afford the desired amine as a yellowish brown oil (83% yield). IR (cm^{-1}) 3059.9, 3028.4, 2924.5, 2854.1, 1602.4, 1495.3, 1453.2. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.47–7.16 (15H, m, aromatic), 4.56 (2H, d, $J = 4.4$ Hz), 4.49 (2H, s), 4.01–3.99 (1H, m), 3.48 (1H, d, $J = 6.5$ Hz), 3.43 (1H, d, $J = 6.5$ Hz), 2.85–2.76 (1H, m), 2.53–2.29 (3H, br m), 2.25 (1H, d, $J = 13.6$ Hz), 2.17 (1H, dd, $J = 6.5$ Hz), 1.88 (1H, dd, $J = 10.4$ Hz). ESI-HRMS (m/z) for $C_{26}H_{29}NO_2$ $[M+H]^+$ calcd. 388.2271 found 388.2270.

The Acyclic Intermediate (8)

Freshly prepared 3-methoxy-2-methyl-acryloyl isocyanate (121.82 mg, 0.864 mmol) was added to a solution of the amine (**7**) (85 mg, 0.217 mmol) in dry benzene (2.2 mL) under argon, and the reaction mixture was stirred at room temperature for 45 minutes. The solvents were evaporated to afford a crude brown oil (257 mg), which was purified by column chromatography (60N silica gel, hexane ethyl acetate 4:1→3:1→2.5:1) to produce **8** as a pale brown oil (81% yield). IR (cm^{-1}) 3242.5, 2917.2, 2850.2, 1689.3, 1660.9, 1616.9, 1642.1, 1494.4, 1453.1. ^{1}H-NMR (400 MHz, $CDCl_3$) 9.35 (1H, s, NH), 7.49–7.15 (17 H, m), 4.59 (1H, s), 4.48 (3H, m), 4.01-3.91 (1H, m), 3.78 (3H, s), 3.55–3.47 (2H, m), 2.78–2.68 (3H, m), 2.21 (1H, dd, $J = 7.4$ and 7.4 Hz), 1.96 (1H, t), 1.68 (3H, s, CH_3). ^{13}C-NMR (125.68 MHz, $CDCl_3$) 168.8, 158.4, 152.3, 144.5, 138.5, 138.5, 128.3, 128.3, 128.1, 128.1, 128.1, 127.6, 127.6, 127.5, 127.2, 126.6, 125.7, 125.7, 124.5, 110.7, 81.5, 73.1, 71.6, 71.1, 65.0, 61.4, 45.9, 45.0, 40.8, 8.7. ESI-HRMS for $C_{32}H_{36}N_2O_5$ $[M+H]^+$ calcd. 529.2697 found 529.2713.

The Benzyl Protected Derivative (9)

The acyclic intermediate (**8**) (7 mg, 0.0132 mmol) was dissolved in 1M, aq. H_2SO_4 (198 μL) and the mixture was heated at 100°C. DMSO was then added dropwise until a clear yellow solution was obtained. The reaction mixture was further heated at the same temperature for 5 hours. After cooling

to room temperature, the reaction mixture was neutralized by the addition of solid sodium bicarbonate until effervescence stopped. The mixture was filtered and the collected solid was washed with ethyl acetate. The combined filtrates were washed with water (10 mL), and the separated water was extracted with ethyl acetate (2 × 20 mL). The combined organic solvents were dried over sodium sulfate and evaporated to afford a crude brown oil, which was purified by column chromatography (FL60D silica gel, hexane/ethyl acetate 4:1→3:1→2:1→3:2) to produce **9** as a colorless oil (4 mg, 60% yield). IR (cm^{-1}) 3026.1, 2924.6, 1684.5, 1453.8, 1364.9. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.77 (1H, s), 7.61 (1H, s), 7.32–7.19 (15H, m), 4.47 (2H, s,), 4.46 (2H, s), 4.09–3.98 (1H, m), 3.46 (2H, d, $J = 5.2$ Hz), 2.96–2.93 (1H, m), 2.87 (1H, m), 2.51 (1H, dd, $J = 7.2$ Hz, 8.0 Hz), 2.48–2.46 (1H, m), 2.27 (1H, dd, 10.6 Hz), 1.86 (3H, s). ^{13}C-NMR (125.68 MHz, $CDCl_3$) 163.7, 150.1, 143.2, 138.8, 138.1, 128.4–125.7, 110.5, 80.5), 73.4, 73.3, 71.5, 71.2, 45.5, 43.2, 39.8, 12.7. ESI-HRMS for $C_{31}H_{32}N_2O_4$ $[M+H]^+$ calcd. 497.2435 found 497.2478.

(1*R*,3*S*,4*R*)-1-Phenyl-1-Thymidyl-3-Hydroxy-4-Hydroxymethylcyclopentane (10)

$Pd(OH)_2/C$ (22 mg) was added to a solution of **9** (22 mg, 0.443 mmol) in methanol (6 mL). The flask was purged with hydrogen gas and the mixture was stirred for 20 minutes at room temperature after the addition of two drops of 2 M aqueous HCl. The reaction mixture was filtered through a basic alumina pad to remove $Pd(OH)_2/C$ and HCl, and the pad was eluted with 10 mL methanol. The combined methanol was evaporated to afford a colorless crude oil, which was purified by flash column chromatography (FL60D silica gel, HPLC grade chloroform/methanol 80:1→50:1→30:1→20:1→8:1) to afford the desired material as a pure colorless oil (14.4 mg, 98% yield). $[\alpha]_D^{25} + 16.4^\circ$ (c 0.45, EtOH). IR (cm^{-1}) 3373.1, 3029.8, 2926.7, 1666.4, 1496.3, 1369.1. ^{1}H-NMR (400 MHz, CD_3OD) 7.95 (1H, s), 7.32–7.18 (5H, m), 4.063 (1H, q, $J = 6.0$ Hz), 3.67 (1H, dd, $J =$ 5.0 and 5.0 Hz), 3.57 (1H, ddd, $J = 7.2$, 6.8, and 6.8 Hz), 2.81 (1H, dd, J = 8.8 and 8.0 Hz), 2.75–2.67 (2H, m), 2.34–2.28 (1H, m), 2.20–2.11 (1H, m), 1.95 (3H, s). ^{13}C-NMR (125.68 MHz, CD_3OD) 166.8, 152.3, 145.5, 141.1, 129.4-126.2, 109.8, 73.9, 73.6, 63.9), 50.1, 47.3, 41.2), 12.1. ESI-HRMS for $C_{17}H_{20}N_2O_4$ $[M+Na]^+$ calcd. 339.1315 found 339.1317.

(1*RS*,3*S*,4*R*)-3-Hydroxy-4-Hydroxymethyl-1-Phenylcyclopentane (13)

To a solution of the ketone **4** (100 mg, 0.322 mmol, dried by co-evaporation three times with acetonitrile) in anhydrous THF (1 mL) at –78°C, PhLi (1.14 M/L, 422 μL, 0.483 mmol) was added dropwise, and the

reaction mixture was stirred at the same temperature for 3 hours. After warming to room temperature, the reaction mixture was quenched with saturated aqueous ammonium chloride (5 mL) and extracted with ethyl acetate (2 × 30 mL). The combined organic solvents were washed with water (30 mL) and brine (30 mL), dried over anhydrous sodium sulfate, and evaporated to yield a crude brownish yellow oil, which was purified by column chromatography (60 N silica gel, hexane/ethyl acetate 5:1) to afford the benzyl protected derivative of **13** as an oil. 71 mg (57% total yield of two isomers). IR (cm^{-1}) 3441, 3040, 2923, 2854, 1495, 1452, 1361. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.51–7.46 (2 H, m), 7.34–7.20 (13 H, m), 4.58–4.44 (4 H, m), 4.13 (1 H, d, $J = 5.4$ Hz), 3.52 (1 H, dd, $J = 5.4$ Hz), 3.40–3.36 (1 H, m), 2.82–2.78 (1H, m), 2.39–2.33 (1H, m), 2.27 (1H, d, $J = 14.4$ Hz), 2.15 (1H, dd, $J =$ 5.4 and 6.0 Hz), 1.86 (1H, dd, $J = 8.8$ and 8.4 Hz), 1.23 (1 H, s). ESI-HRMS (m/z) calcd. for $C_{26}H_{28}O_3$ $[M+K]^+$ 427.1670, found 427.1715.

The above oil (10 mg, 0.025 mmol) was dissolved in EtOAc (0.5 mL), followed by the addition of $Pd(OH)_2/C$ (20%, 5 mg) and one drop of 2 M aqueous HCl. The reaction flask was purged with hydrogen gas, and the mixture was stirred at room temperature for one hour. The reaction mixture was filtered through a basic alumina pad to remove $Pd(OH)_2/C$ and HCl. The filtrate was dried over sodium sulfate and evaporated to afford a crude yellowish brown oil, which was purified by column chromatography (60 N silica gel, chloroform/methanol 40:1→30:1) to afford **13** as a colorless oil (mixture of 2 isomers, 1:1.75 as determined by H^1-NMR) and the total yield was 83%. IR (cm^{-1}) 3342.5, 3025.3, 2932.9, 2869.5, 1601.7, 1494.0, 1449.1, 1350.8. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.29–7.15 (5H, m, aromatic protons), 4.14–4.12 (1H, m), 3.78–3.76 (1H, m), 3.64–3.59 (1H, m), 3.59–3.06 (3H, m), 2.43–2.23 (1H, m), 2.20 (1H, br s), 1.96–1.81 (1H, m), 1.79–1.76 (2H, m). ESI-HRMS for $C_{12}H_{16}O_2$ $[M+Na]^+$ calcd. 215.1042 found 215.1016.

Synthesis of DMTr-Protected Amidite Precursor 14

To a solution of compound **10** (24 mg, 0.075 mmol, dried azeotropically with acetonitrile) in dry pyridine (0.34 mL), was added 4,4-dimethoxytrityl chloride (39 mg, 0.114 mmol) under argon atmosphere. The reaction was stirred at room temperature for 1.5 hours. The reaction was quenched with saturated aqueous sodium bicarbonate (5 mL), extracted with chloroform (10 mL). The organic layer was dried over anhydrous sodium sulfate. The organic layer was evaporated to yield a crude yellow oil, which was purified by column chromatography (60 N silica gel, chloroform/methanol 100:1 and changed gradually to 20:1 containing 0.5% pyridine) to afford the DMTr-protected derivative of **10** as an oil in 87% yield as a colorless oil. IR (cm^{-1}) 3032.9, 2927.6, 2868.0, 1686.2, 1625.7, 1607.3, 1552.1, 1508.7, 1445.7. ^{1}H-NMR (400 MHz, $CDCl_3$) 8.02 (1H, bs), 7.58 (1H, s), 7.42-7.12 (14H, m,

aromatic), 6.80 (4H, d, $J = 8.2$ Hz), 4.10 (1H, q, $J = 6.2$ Hz), 3.77 (6H, s, 2× OCH_3), 3.38 (1H, m), 3.18–3.05 (1H, m), 2.74–2.64 (2H, m), 2.55–2.40 (2H, m), 2.35–2.22 (2H, m), 1.96 (3H, s). ESI-MS for $C_{38}H_{38}N_2O_6$ $[M–H]^-$ calcd 617.26 found 617.27

The above oil (21 mg, 0.034 mmol, dried azeotripically with acetonitrile three times) was dissolved in dry dichloromethane (0.43 mL) containing *N,N*-diisoproopylethylamine (34 μL, 0.204 mmol), followed by the addition of 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (46 μL, 0.204 mmol) at 0°C. The reaction mixture was stirred at 0°C for 1 hour. The reaction mixture was quenched with saturated aqueous sodium bicarbonate (5 mL) and extracted with ethyl acetate (10 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated to yield a crude yellow oil, which was purified by flash column chromatography (FL60D silica gel, HPLC grade hexane/ ethylacetate 1:2) to afford **14** in 83% yield as pale yellow oil. IR (cm^{-1}) 2966.9, 2930.4, 1868.6, 1607.9, 1508.7, 1463.8, 1446.7, 1364.4. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.84 (1H, bs), 7.71–7.10 (15H, m), 6.80–6.71 (4H, m), 4.41–4.26 (1H, m), 3.77 (3H, s), 3.76 (3H, s), 3.73–3.46 (4H, m), 3.28–3.20 (1H, m), 3.18–3.13 (1H, m), 3.08–2.92 (1H, m), 2.90–2.78 (1H, m), 2.74–2.62 (1H, m), 2.58 (1H, t, $J = 11.2$ Hz), 2.52–2.44 (1H, m), 2.44–2.29 (2H, m), 2.02 (1.5H, s), 1.96 (1.5H, s), 1.32–1.22 (4H, m), 1.20–1.04 (6H, m), 0.98 (2H, d, $J = 6.8$ Hz). ^{31}P-NMR (161.9 MHz, $CDCl_3$) δ 150.3, 148.9. ESI-MS for $C_{47}H_{55}N4O_7P$ $[M+H]^+$ calcd. 819.38 found 819.42.

Synthesis of DMTr-Protected Amidite Precursor 15

To a solution of **11** (50 mg, 0.431 mmol, dried azeotropically three times with dry pyridine) in dry pyridine (1.93 mL), was added 4,4-dimethoxytrityl chloride (219 mg, 0.646 mmol) under argon atmosphere. The reaction mixture was stirred at room temperature for 45 minutes and quenched with saturated aqueous sodium bicarbonate (5 mL). The mixture was extracted with chloroform (10 mL), and the organic layer was dried over anhydrous sodium sulfate, evaporated to yield a crude yellow oil, which was purified by column chromatography (60 N silica gel, chloroform/methanol 100:1 containing 0.5% pyridine) to afford the DMTr-protected derivative of 11 in 90% yield as a colorless oil. IR (cm^{-1}) 3439.3, 2955.5, 2835.2, 1607.4, 1582.9, 1508.1, 1463.1, 1445.3. ^{1}H-NMR (500 MHz, $CDCl_3$) 7.44–7.19 (9H, m, arom.), 8.81 (4H, d, $J = 8.8$ Hz), 3.92 (1H, q, $J = 7.2$ Hz), 3.77 (6H, s, 2× OCH_3), 3.29 (1H, dd, $J = 5.2$ and 5.2 Hz), 2.94 (1H, t, $J = 9.0$ Hz), 2.49 (1H, bs, OH), 2.11–2.02 (1H, m), 1.94–1.84 (1H, m), 1.82–1.64 (2H, m), 1.61–1.46 (3H, m). ESI-MS for $C_{27}H_{30}O_4$ $[M+Na]^+$ calcd. 441.20 found 441.19

To the above oil (106 mg, 0.253 mmol, dried azeotropically with acetonitrile three times) in dry dichloromethane (3.2 mL) containing

N,N-diisopropylethylamine (265 μL, 1.521 mmol) was added 2-cyanoethyl-*N,N*-diisoprpylchlorophosphoramidite (339 μL, 1.521 mmol) at 0°C, and the reaction mixture was stirred at the same temperature for 1 hour. The reaction mixture was quenched with saturated aqueous sodium bicarbonate (5 mL), extracted with ethyl acetate (10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to yield a crude yellow oil, which was purified by flash column chromatography (FL60D silica gel, HPLC grade hexane/ ethyl acetate 4:1) to afford **15** in 80% yield as a pale yellow oil. IR (cm^{-1}) 2964.0, 1607.6, 1508.5. ^{1}H-NMR (500 MHz, $CDCl_3$) 7.46–7.10 (9H, m), 6.81–6.77 (4H, m), 4.15–4.08 (1H, m), 3.77 (6H, s), 3.71–3.51 (4H, m), 3.09–3.02 (1H, m), 3.00–2.89 (1H, m), 2.56 (0.5H, t, $J = 6.6$ Hz), 2.49–2.36 (1.5H, m), 2.24–2.15 (1H, m), 2.02–1.91 (1H, m), 1.78–1.55 (4H, m), 1.42–1.88 (1H, m), 1.17–1.08 (10H, m), 1.07–1.03 (2H, d, $J = 6.6$ Hz). ^{31}P-NMR (161.9 MHz, $CDCl_3$) δ 148.0, 147.4. ESI-MS for $C_{36}H_{47}N_2O_5P$ $[M+H]^+$ calcd 619.32 found 619.38.

Synthesis of DMTr-Protected Amidite Precursor 16

To a solution of compound **12** (24 mg, 0.099 mmol, dried azeotropically three times with dry pyridine) in dry pyridine (0.45 mL), was added 4,4-dimethoxytrityl chloride (51 mg, 0.149 mmol) under argon atmosphere. The reaction mixture was stirred at room temperature for 1 hour and quenched with saturated aqueous sodium bicarbonate (5 mL). The mixture was extracted with chloroform (10 mL), and the organic layer was dried over anhydrous sodium sulfate, evaporated to yield a crude yellow oil, which was purified by column chromatography (60 N silica gel, chloroform/methanol 100:1 and changed gradually to 10:1 containing 0.5% pyridine) to afford the DMTr-protected **12** in 93% yield as a colorless oil. IR (cm^{-1}) 3418.3, 3191.6, 3054.0, 2907.6, 1679.7, 1607.6, 1580.6, 1508.7, 1464.6. ^{1}H-NMR (400 MHz, $CDCl_3$) 8.18 (1H, bs), 7.34–7.14 (10H, m), 6.77 (4H, d, $J = 7.6$ Hz), 4.80 (1H, quin., $J = 7.6$ Hz), 4.14–4.06 (1H, m), 3.73 (6H, s), 3.19–3.13 (1H, m), 3.00–2.88 (1H, m), 2.70 (1H, bs), 2.38–2.26 (2H, m), 1.87 (3H, s), 1.91–1.83 (1H, m), 1.83–1.76 (1H, m), 1.76–1.66 (1H, m). ESI-MS for $C_{32}H_{34}N_2O_6$ $[M+Na]^+$ calcd. 565.23 found 565.21.

To the above oil (50 mg, 0.092 mmol, dried azeotropically with acetonitrile three times) in dry dichloromethane (1.16 mL) containing *N,N*-diisopropylethylamine (97 μL, 0.553 mmol), was added 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (123 μL, 0.553 mmol) at 0°C, and the reaction mixture was stirred at the same temperature for 1 hour. The reaction mixture was quenched with saturated aqueous sodium bicarbonate (5 mL), and extracted with ethyl acetate (10 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated to yield a crude yellow oil, which was purified by flash column chromatography (FL60D silica gel, HPLC grade hexane/ethylacetate 1:2) to afford **16** in 64% yield as a

colorless oil. IR (cm^{-1}) 2967.3, 2200.0, 1783.0, 1608.0, 1508.9, 1464.6. ^{1}H-NMR (400 MHz, $CDCl_3$) 8.16 (1H, bs, NH), 7.47 (0.5H, s), 7.44 (0.5 H, s), 7.40-7.01 (9H, m), 6.82–6.79 (4H, m), 5.12–5.03 (1H, m), 4.43–4.26 (1H, m), 4.15–4.07 (1H, m), 3.92–3.79 (1H, m), 3.775 (3H, s), 3.773 (3H, s), 3.76–3.44 (5H, m), 3.09–2.94 (1H, m), 2.86–2.29 (3H, m), 2.03 (1.5H, s), 1.93 (1.5H, s), 1.86–1.52 (2H, m), 1.31–1.10 (12H, m). ^{31}P-NMR (161.9 MHz, $CDCl_3$) δ 149.4, 148.7. ESI-MS for $C_{41}H_{51}N_4O_7P$ $[M+H]^+$ calcd. 743.35 found 743.38.

Synthesis of DMTr-Protected Amidite Precursor 17

To a solution of **13** (38 mg, 0.197 mmol, dried azeotropically three times with dry pyridine) in dry pyridine (0.88 mL), was added 4,4-dimethoxytrityl chloride (100 mg, 0.296 mmol) under argon atmosphere, and the reaction mixture was stirred at room temperature for 1 h, then quenched with saturated aqueous sodium bicarbonate (5 mL). The mixture was extracted with chloroform (10 mL), and the organic layer was dried over anhydrous sodium sulfate, evaporated to yield a crude yellow oil, which was purified by open column chromatography (FL60D silica gel, hexane/ethylacetate 4:1) to produce the DMTr protected derivative of **13** as a mixture of two isomers is 89% yield. The isomers were separated by repetition of column chromatography to produce the α-isomer as a major isomer and the β-isomer as a minor isomer. The stereochemistry was determined by 2D NMR COSY and NOESY.

Physical Data for α-Phenyl Isomer

IR (cm^{-1}) 3452.2, 3060.9, 3026.6, 2931.3, 2862.20, 2835.5, 1607.0, 1582.4, 1508.1, 1462.0, 1445.3. ^{1}H-NMR (400 MHz, CD_3OD) 7.44 (2H, d, aromatic, $J = 8.0$ Hz), 7.32–7.01 (12H, m), 6.84 (4H, d, $J = 8.8$ Hz), 4.03 (1H, q, $J =$ 6.4 Hz), 3.76 (6H, s), 3.22 (1H, dd, $J = 5.4$, 5.4 Hz), 3.12–308 (1H, m), 3.00 (1H, quin, $J = 8.8$ Hz), 2.31 (1H, dd, $J = 6.4$ and 6.4 Hz), 2.27–2.14 (1H, m), 1.99–1.91 (2H, m), 1.67 (1H, ddd, $J = 8.4$, 8.8, and 8.4 Hz). ^{13}C-NMR (125.68 MHz, CD_3OD) 160.0-108.1, 87.2, 76.5, 66.2, 55.7, 48.9, 44.5, 42.7, 37.1. ESI-MS for $C_{33}H_{34}O_4$ $[M+Na]^+$ calcd. 517.23 found 517.35.

Physical Data for β-Phenyl Isomer

IR (cm^{-1}) 3352.0, 3023.8, 2924.8, 2853.5, 1607.1, 1582.2, 1508.1, 1446.2. NMR (400 MHz, CD_3OD) 7.43 (2H, d, aromatic, $J = 7.6$ Hz), 7.32–7.10 (12H, m, aromatic), 6.83 (4H, d, $J = 8.4$ Hz), 4.17–4.07 (1H, m), 3.76 (6H, s, 2× CH_3O), 3.38–3.08 (3H, m), 2.35–2.19 (2H, m), 2.02–1.91 (2H, m), 1.81 (1H, ddd, $J = 6.4$, 6.4, 6.8 Hz). ESI-MS for $C_{33}H_{34}O_4$ $[M+K]^+$ calcd. 533.20 found 533.38.

To a solution of the β-isomer (30 mg, 0.06 mmol, dried azeotropically with acetonitrile three times) in dry dichloromethane (0.75 mL) containing

TABLE 2 MALDI TOF-MS analysis $[M-H]^-$ of synthesized, modified (ODNs) incorporating compound **10-13**.

DNA(Z) TFO(Z)	Nucleotide for **Z**	Single Stranded DNA	Calcd	Found
DNA **1(10)**	**10**	5′ CTT TCT TZT CCT TTC T 3′	4800.60	4800.78
DNA **2(10)**	**10**	3′ GAA AGA AZA GGA AAGA 5′	5090.85	5091.85
TFO**1(10)**	**10**	3′ GGA AGG AZG GAG GAG GGA 5′	5812.91	5810.09
TFO **2(10)**	**10**	3′ GGA AGG GZG GAG GAG GGA 5′	5828.90	5828.16
TFO**3(10)**	**10**	3′ GGA AGG GZA GAG GAG GGA 5′	5812.91	5811.34
TFO**4(10)**	**10**	3′ GGA AGG AZA GAG GAG GGA 5′	5796.92	5793.00
DNA **1(11)**	**11**	5′ CTT TCT TZT CCT TTC T 3′	4600.60	4598.30
DNA **2(11)**	**11**	3′ GAA AGA AZA GGA AAGA 5′	4890.85	4887.94
TFO**1(11)**	**11**	3′ GGA AGG AZG GAG GAG GGA 5′	5612.91	5613.12
TFO **2(11)**	**11**	3′ GGA AGG GZG GAG GAG GGA 5′	5628.90	5627.31
TFO**3(11)**	**11**	3′ GGA AGG GZA GAG GAG GGA 5′	5612.91	5610.56
TFO**4(11)**	**11**	3′ GGA AGG AZA GAG GAG GGA 5′	5596.92	5597.40
DNA **1(12)**	**12**	5′ CTT TCT TZT CCT TTC T 3′	4724.60	4721.01
DNA **2(12)**	**12**	3′ GAA AGA AZA GGA AAGA 5′	5014.85	5010.12
TFO**1(12)**	**12**	3′ GGA AGG AZG GAG GAG GGA 5′	5736.91	5734.19
TFO **2(12)**	**12**	3′ GGA AGG GZG GAG GAG GGA 5′	5752.90	5747.22
TFO**3(12)**	**12**	3′ GGA AGG GZA GAG GAG GGA 5′	5736.91	5733.85
TFO**4(12)**	**12**	3′ GGA AGG AZA GAG GAG GGA 5′	5720.92	5717.10
DNA **1(13)**	**13**	5′ CTT TCT TZT CCT TTC T 3′	4677.72	4677.90
DNA **2(13)**	**13**	3′ GAA AGA AZA GGA AAGA 5′	4967.97	4965.36
TFO**1(13)**	**13**	3′ GGA AGG AZG GAG GAG GGA 5′	5690.03	5690.87
TFO **2(13)**	**13**	3′ GGA AGG GZG GAG GAG GGA 5′	5706.02	5705.62
TFO**3(13)**	**13**	3′ GGA AGG GZA GAG GAG GGA 5′	5690.03	5686.17
TFO**4(13)**	**13**	3′ GGA AGG AZA GAG GAG GGA 5′	5674.04	5674.54

N,N-diisopropylethylamine (63 μL, 0.364 mmol), was added 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (81 μL, 0.364 mmol) at 0°C, and the reaction mixture was stirred at the same temperature for 1 hour. The reaction mixture was quenched with saturated aqueous sodium bicarbonate (5 mL), and extracted with ethyl acetate (10 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated, and purified by flash column chromatography (FL60D silica gel, HPLC grade hexane/ethyl acetate 4:1) to afford **17** in 69% yield as a colorless oil. IR (cm^{-1}) 2963.5, 2929.0, 1607.4, 1508.3. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.49–7.40 (2H, m, aromatic), 7.32–7.16 (12H, m, aromatic), 6.81–6.72 (4H, m, aromatic), 4.32–4.25 (1H, m), 3.77 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.72–3.51 (3H, m), 3.37–3.36 (1H, m), 3.19–3.16 (1H, m), 3.14–3.03 (1H, m), 2.56 (1H, t, J = 6.8 Hz), 2.49–2.21 (3H, m), 2.17-2.01 (1H, m), 1.88–1.80 (1H, m), 1.55–1.39 (2H, m), 1.23–0.85 (12H, m). ^{31}P-NMR (161.9 MHz, $CDCl_3$) δ 148.20, 147.48. ESI-MS for $C_{42}H_{51}N_2O_5P$ $[M+H]^+$ calcd 695.36 found 695.24.

Synthesis and Purification of ODN

All oligonucleotides were synthesized by the standard DNA synthesis procedures. The synthesized ODNs were cleaved from the CPG column in

28% ammonium solution at 55°C for 5 hours and were purified by HPLC (Nacalai Tesque COSMOSIL C18-ARII) using a linear gradient (A: 0.1 M TEAA buffer, B: CH_3CN, B conc. 10% to 40%/20 min). The DMTr-group was cleaved in 10% aqueous acetic acid at room temperature and the resulting DMTr-OH was removed by washing with ether. The purities and structures of the synthesized TFOs and labeled-ODNs were confirmed by MALDI-TOF Mass measurement (Table 2).

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