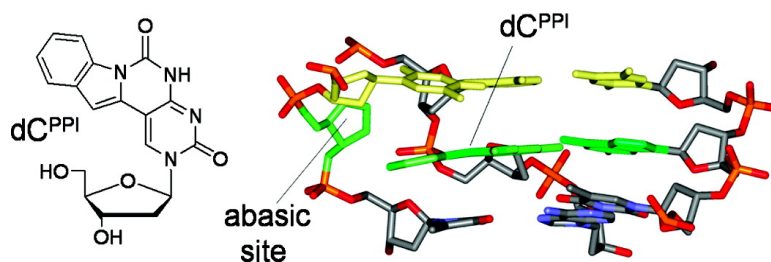


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## New Nucleotide Pairs for Stable DNA Triplexes Stabilized by Stacking Interaction

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Triple-helical structures, which represent a class of major supramolecular structures of DNA, have various applications, including fabrication of DNA nanostructures.<sup>1</sup> Recently, many artificial nucleic acids that can improve the stability of triple-helical DNAs have been reported.<sup>2</sup> In typical parallel DNA triplexes that comprise natural-type nucleotides, only two types of Hoogsteen base pairs, T•A and C+•G, can be used as stable base pairs between triplex-forming oligonucleotides (TFO) and DNA duplexes. Consequently, the TFO should have homopyrimidine sequences, and the DNA duplexes should have homopurine–homopyrimidine sequences. Therefore, if we can develop new artificial base pairs, which can replace the Hoogsteen base pairs without the loss of DNA triplex stability, the variety of nucleotide sequences capable of forming DNA triplexes would increase. Such an idea was first proposed by Ts'o et al.,<sup>3</sup> they designed various artificial nucleobases such as deoxypseudouridine that could form hydrogen bonds in the major groove with cytosine.

In this communication, we describe a possibility of new nucleotide pairs applicable to DNA triplex. We designed oligonucleotide duplexes incorporating 5-aryl deoxycytidine derivatives (dC<sup>5Ar</sup>s), **1a–c**, dC<sup>PPP</sup>, and dC<sup>PPI</sup> (Figure 1). The cyclic derivatives, dC<sup>PPP</sup> and dC<sup>PPI</sup>, having an expanded aromatic area, have recently been reported by us.<sup>4</sup> The aromatic region at the 5-position of these cytidine derivatives is expected to protrude into the major groove. As pairing partners in TFO, which recognize these aromatic groups, we selected an abasic residue ( $\phi$ ) and a propylene linker (C3) that has a space (Figure 2). This space can accommodate an aromatic group due to the absence of the nucleobase. In duplex formation, the recognition of the abasic site by artificial nucleosides that have an aromatic ring has been reported by other research groups.<sup>5</sup> The strategy shown in this communication is an example of the recognition of aromatic groups in the major groove by TFO incorporating  $\phi$  or C3.

The synthesis of **1a–c**, dC<sup>PPP</sup>, and dC<sup>PPI</sup>, and their phosphoramidite derivatives is shown in Scheme S1. Hairpin oligodeoxynucleotides, such as **OL-1a** (X = dC), **-1b** (dC<sup>5Ph</sup>), **-1c** (dC<sup>5Th</sup>), **-1d** (dC<sup>5Fur</sup>), **-1e** (dC<sup>PPP</sup>), and **-1f** (dC<sup>PPI</sup>) were synthesized, using these phosphoramidite units. In addition, triplex-forming oligodeoxynucleotides, **TFO-1a** (Y =  $\phi$ ), **TFO-1b** (T), **TFO-1c** (dA), **TFO-1d** (dC), **TFO-1e** (dG), and **TFO-1f** (C3) were also synthesized, as shown in Figure 2.

First, we studied the triplex formation between **OL-1a** to **-1f** and **TFO-1a** to **-1e** to clarify the interaction between 5-aryl-dC and  $\phi$ . The thermal stabilities of the triplexes are summarized in Table 1. In all cases, the UV melting curves showed typical two-step transitions, as shown in Figure S1 giving lower  $T_m$  values at around 16–56 °C and higher  $T_m$  values at around 80–86 °C, which corresponded to the melting patterns of triplexes and hairpin duplexes, respectively. As a result of the triplex formation of **TFO-1a** (Y =  $\phi$ )/**OL-1a** (X = dC) and **TFO-1a** (Y =  $\phi$ )/**OL-1b** (X =

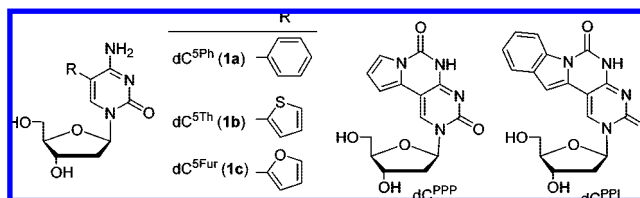


Figure 1. Structure of 5-aryl-dC derivatives, dC<sup>PPP</sup> and dC<sup>PPI</sup>.

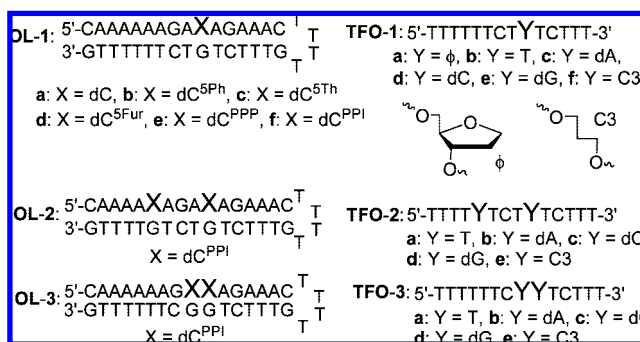


Figure 2. Sequences of hairpin oligonucleotides (**OL-1**, **-2**, and **-3**) and TFOs.

Table 1. Melting Temperature<sup>a</sup> (°C) and Selectivity (in Parentheses) of Modified Triplexes in 50 mM Sodium Cacodylate, 500 mM NaCl, 10 mM MgCl<sub>2</sub> (pH 5.4)

	<b>OL-1a</b> X = dC	<b>-1b</b> dC <sup>5Ph</sup>	<b>-1c</b> dC <sup>5Th</sup>	<b>-1d</b> dC <sup>5Fur</sup>	<b>-1e</b> dC <sup>PPP</sup>	<b>-1f</b> dC <sup>PPI</sup>
<b>TFO-1a</b>	22	28	32	36	41	52
Y = $\phi$	(–)	(3 <sup>b</sup> )	(2 <sup>b</sup> )	(2 <sup>b</sup> )	(2 <sup>b</sup> )	(3 <sup>b</sup> )
<b>-1b</b>	27	21	<u>30</u>	<u>34</u>	<u>39</u>	48
dT						
<b>-1c</b>	20	16	27	31	38	45
dA						
<b>-1d</b>	<u>25</u>	23	<u>30</u>	<u>34</u>	39	<u>49</u>
dC						
<b>-1e</b>	22	<u>25</u>	26	<u>34</u>	39	45
dG						
<b>-1f</b>	21	30	35	38	44	56
C3		(5 <sup>b</sup> )	(5 <sup>b</sup> )	(4 <sup>b</sup> )	(5 <sup>b</sup> )	(7 <sup>b</sup> )

<sup>a</sup> Averages of at least three independent experiments. The standard deviation is  $\pm 0.6$  °C. <sup>b</sup> The selectivity of X– $\phi$  or X–C3 pair calculated by  $T_m$  of X– $\phi$  or X–C3 minus the  $T_m$  of the most stable X–N pair indicated by underlining, where N represents the natural type bases such as T, dA, dG, and dC.

dC<sup>5Ph</sup>), it was revealed that the introduction of a phenyl ring of dC<sup>5Ph</sup> increased the  $T_m$  value from 22 to 28 °C of the triplex with **TFO-1a** incorporating  $\phi$ . This result indicated that  $\phi$  accommodated the phenyl ring, resulting in the increased stability of the triplex probably due to the stacking interactions between the phenyl ring

**Table 2.** Stacking Surface Areas ( $\text{\AA}^2$ ) between  $\text{dC}^{5\text{Ar}}$ s and Neighboring Bases

X	Y	stacking surface areas	$T_m^a$ ( $^\circ\text{C}$ )
dC	$\phi$	23.4	22
$\text{dC}^{5\text{Ph}}$	$\phi$	42.0	28
$\text{dC}^{5\text{Th}}$	$\phi$	50.9	32
$\text{dC}^{5\text{Fur}}$	$\phi$	56.3	36
$\text{dC}^{\text{PPP}}$	$\phi$	67.3	41
$\text{dC}^{\text{PPP}}$	C3	73.0	44
$\text{dC}^{\text{PPI}}$	$\phi$	84.7	52
$\text{dC}^{\text{PPI}}$	C3	92.8	56

<sup>a</sup> These  $T_m$  values are also shown in Table 1.

and the bases flanking  $\phi$ . In addition, comparison of the  $T_m$  of the triplexes of **TFO-1a** with **OL-1b**, **-1c**, **-1d**, **-1e**, and **-1f** revealed that the stability decreased in the order of **OL-1f** ( $X = \text{dC}^{\text{PPI}}$ ,  $52^\circ\text{C}$ ) > **OL-1e** ( $\text{dC}^{\text{PPP}}$ ,  $41^\circ\text{C}$ ) > **OL-1d** ( $\text{dC}^{5\text{Fur}}$ ,  $36^\circ\text{C}$ ) > **OL-1c** ( $\text{dC}^{5\text{Th}}$ ,  $32^\circ\text{C}$ ) > **OL-1b** ( $\text{dC}^{5\text{Ph}}$ ,  $28^\circ\text{C}$ ). As shown in Table 2, the stability trend was in correlation with the stacking surface area between the aromatic ring and the nucleobases.

Next, we evaluated the selectivity of the  $\text{dC}^{5\text{Ar}}$ s toward  $\phi$  over the canonical bases. As shown in the column of **OL-1a** in Table 1, the unmodified dC bound most strongly to T with a  $T_m$  value of  $27^\circ\text{C}$ . This stability might be explained by the hydrogen bond between the amino group of the cytosine and the O4 or O2 of the carbonyl group of the thymine. In contrast, the modified triplexes incorporating **OL-1b** to **-1f** gave the highest  $T_m$  values unexceptionally when the modified dC bound to  $\phi$ , but the selectivities were at most  $3^\circ\text{C}$ .

Because **OL-1e** ( $X = \text{dC}^{\text{PPP}}$ ) and **-1f** ( $\text{dC}^{\text{PPI}}$ ) showed rather high  $T_m$  values toward **TFO-1a** ( $Y = \phi$ ), we tried to change the structure of  $\phi$  to the one that had higher affinity for  $\text{dC}^{5\text{Ar}}$ s. As shown in the bottom row of Table 1, **TFO-1f** incorporating C3 showed higher affinity toward **OL-1b**, **1c**, **1d**, **1e**, **1f** of 30, 35, 38, 44, and  $56^\circ\text{C}$ , respectively, compared with  $\phi$  probably due to the reduced steric crush between the ribose moiety and the aromatic ring. As a result, the selectivity increased significantly to  $4\text{--}7^\circ\text{C}$  when paired with C3 instead of  $\phi$ . The stability of the triplexes shown in Table 1 was in correlation with the stacking surface areas between the  $\text{dC}^{5\text{Ar}}$ s and the adjacent natural bases derived from the MD simulated triplex structures, as shown in Table 2 and Figure S2. When the melting temperatures of the triplexes consisting of **OL-1f** were measured in the absence of  $\text{Mg}^{2+}$ , the  $T_m$  of the triplexes decreased by  $5\text{--}8^\circ\text{C}$ . Even under this condition, triplex **OL-1f/TFO-1f** showed the highest stability and selectivity of  $6^\circ\text{C}$  toward **OL-1f/TFO-1d** (Table S2).

Finally, we measured the  $T_m$  of the triplexes using the hairpin oligonucleotides incorporating two  $\text{dC}^{\text{PPI}}$  residues either nonconsecutively (**OL-2**) or consecutively (**OL-3**). As shown in Table 3, **OL-2** still formed stable triplexes with a  $T_m$  value of  $63^\circ\text{C}$  retaining the selectivity toward the TFO incorporating C3. However, when the  $\text{dC}^{\text{PPI}}$  residues were incorporated consecutively, as in the case of **OL-3**, the stability of the triplexes was reduced drastically probably because **TFO-3** incorporating the consecutive C3 residues are too flexible and the selectivity was lost.

In summary, it was concluded that, when the  $\text{dC}^{5\text{Ar}}$ s paired with C3 in TFO, the stability of the triplexes increased. The recognition of the C3 with the  $\text{dC}^{5\text{Ar}}$ s was selective because of the stacking interactions between the aromatic part and the nucleobases flanking

**Table 3.** Melting Temperature ( $^\circ\text{C}$ ) of the Triplexes Incorporating Two  $\text{dC}^{\text{PPI}}$ -Y Pairs at pH 5.4

	TFO-				
	2a Y = T	2b dA	2c dC	2d dG	2e C3
<b>OL-2</b>	52	44	53	46	63
	TFO-				
	3a Y = T	3b dA	3c dC	3d dG	3e C3
<b>OL-3</b>	19	19	20	28	22

the C3 site. At present, the selectivity of  $\text{dC}^{\text{PPI}}$  toward C3 is as large as  $7^\circ\text{C}$ . The results shown in this study indicate the potential utility of new nucleotide triplets stabilized by stacking interaction. Such nucleotide triplets might be useful for applications such as DNA nanotechnologies utilizing DNA triplexes. Norden and co-workers reported that DNA nanostructures consisting of branched duplexes recognized the complementary TFOs at specific duplex sequences to construct the addressable DNA nanostructure system.<sup>6</sup> Currently, the sequences of such TFOs are limited to homopyrimidine sequences which can form Hoogsteen base pairs with the homopurine sequences. If the  $\text{dC}^{\text{PPI}}$ -C3 pair was incorporated into this system, the number of addresses could be increased and the sequences could be designed more freely. The application of the new nucleotide triplets to such DNA nanotechnologies is underway and will be reported elsewhere.

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**Supporting Information Available:** The procedures for the synthesis,  $T_m$  analyses and the computation, and the NMR charts of new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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