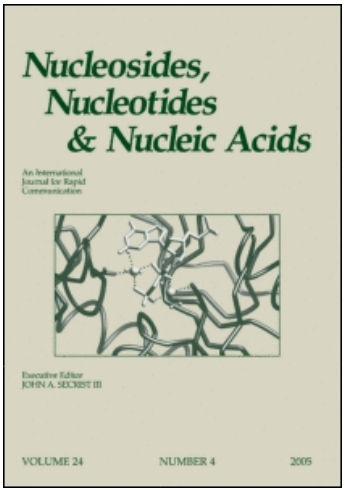

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EXPANSION OF TRIPLEX RECOGNITION CODES BY THE USE OF NOVEL BICYCLIC NUCLEOSIDE DERIVATIVES (WNA)

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▫ *Recently, we have developed new base analogs (WNA) and demonstrated that WNA-T with thymine and WNA-C with cytosine stabilize non-natural antiparallel triplexes with a TA or a CG interrupting site, respectively. However, limitations in recognizable sequences with the WNA-containing TFO were also found. The objective of this study is to search better WNA analogs for expansion of triplex recognition codes to general duplex sequences. In this study, we designed new WNA analogs by systematic modification of the aromatic part and the recognition part. The new WNA analogs with the benzene ring substituted with bromide or cyanide have determined for selective stabilization of triplexes at a TA interrupting site, and general formation of triplexes having a TA interrupting site has been achieved.*

Keywords Triplex DNA, Interrupting Site, Non-Natural Nucleoside, Molecular Recognition, Antigene

INTRODUCTION

The most stable triplexes are formed with homopurine/homopyrimidine sequences, and a pyrimidine base in the purine strand of the duplex interrupts triplex formation.[1] Despite numerous studies, this limitation has remained an unsolved problem. Recently, we have developed new base analogs (WNA)* and

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*Selective formation of stable triplexes including a TA or a CG interrupting site with new bicyclic nucleoside analogs (WNA).[2]

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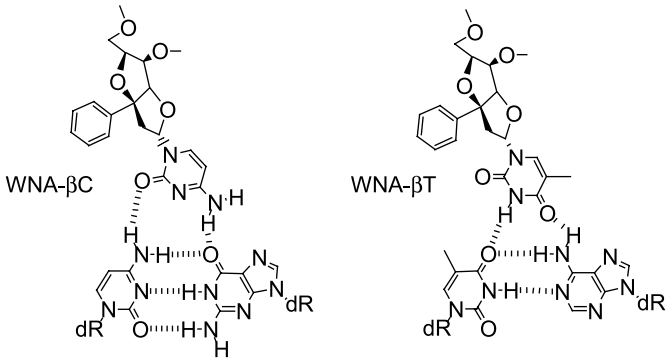

FIGURE 1 The structure of WNA-βT, WNA-βC, and proposed structure of WNA-βT/TA triplet, WNA-βC/CG triplet.

demonstrated that WNA-βT with thymine and WNA-βC with cytosine stabilize non-natural anti parallel triplexes with a TA or a CG interrupting site, respectively.† The WNA has an aromatic ring as a stacking part, a heterocyclic ring as a recognition part and a bicyclic skeleton to hold these components. Complex structures of the WNA-βT with a TA pair and the WNA-βC with a CG pair were hypothesized to include hydrogen bondings at the junction part (Figure 1).† In our continuous efforts for searching nucleoside analogs for expansion of the target duplex sequences for triplex formation, we have further investigated the scope of recognizable sequences by the WNA-containing TFOs.

RESULTS AND DISCUSSION

The previous study has indicated that the benzene part of WNA plays a significant role in triplex stabilization and that the nucleobase part determines base selectivity.† Thus, the new WNA analogs having a variety of aromatic part and 5-substituted pyrimidine nucleoside were designed (Figure 2). They were synthesized by a similar method as reported previously. All the WNA analogues were incorporated into the TFOs with an automated DNA synthesizer by the conventional amidite chemistry. The TFOs were purified by reverse-phase HPLC and treated with 10% aqueous acetic acid. Structure and purity of synthesized TFOs were confirmed by MALDI-TOF MS measurements.

The triplex-forming ability of TFOs containing WNA analogues was investigated by gel shift assay with 15% non-denatured polyacrylamide gel at 10°C using the ^{32}P-labeled TFO as a tracer. The triplex was identified as the slower migration band relative to the TFO, and equilibrium association constants (*K*s) were obtained by quantification of these bands. Table 1 summarizes association

†W-shape nucleic acid (WNA) for selective formation of non-natural anti-parallel triplex including a TA interrupting site.[3]

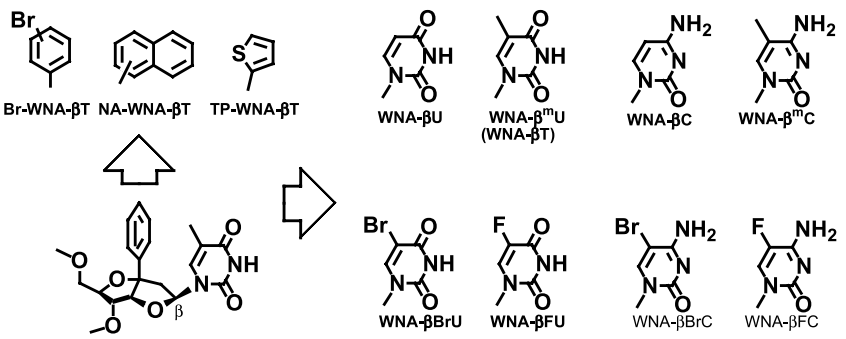

FIGURE 2 The structure of new WNA analogs.

constants of the triplexes with the TFO containing WNA-βT in all neighboring combinations, clearly showing that the TFOs incorporating a WNA-βT stabilize a TA interrupting site selectively only in the cases where the TFO have the sequences of 3′*N*- WNA-βT-G 5′.

Then, we investigated the new WNA analogs to overcome this limitation (Figure 2). As summarized in in Table 2, 5-substituent on the pyrimidine base affects the binding selectivity, although the detailed mechanism is not clear. In contrast, the WNA analogs having substituted benzene (Br-WNA-βT), naphthalene (NA-WNA-βT), and thiophene (TP-WNA-βT) did not show triplex-stabilization effects. These new WNA analogs were evaluated for their stabilization effects toward the target TA interrupting site having different neighboring bases (Table 3). Interestingly, ortho- and meta-substituted Br-WNA-βT stabilize triplexes when they are incorporated into the TFO with the sequence having adenosine at the 5′-side.

TABLE 1 The Association Constants (K_s, 10^9 M^{-1}) of the Triplexes Indicating the Scope and Limitation of the Target Sequence with WNA-βT[a]

	XY			
N-**Z**-*N* *N*-**X**-*N* *N*-**Y**-*N*	TA	AT	CG	GC
- A βT G- - A X G- - T Y C-	**>1.0**	0.042	0.091	0.045
- G βT G- - G X G- - C Y C-	**>1.0**	0.048	0.004	0.058
- A βT A- - A X A - - T Y T -	<0.001	<0.001	<0.001	<0.001
- G βT A - - G X A - - C Y T -	0.04	0.019	0.018	0.02

[a]Triplex formation was done for 12 h at 22°C in the buffer containing 20 mM Tris-HCl, 20 mM $MgCl_2$, 2.5 mM spermidine, and 10% sucrose at pH 7.5. 10 nM TFO containing ^{32}P-labeled one as the tracer and different duplex concentrations ranging from 0 to 100 nM were used. Electrophoresis was done at 10°C with 15% non-denatured polyacrylamide gel, and radioactive bands corresponding to the single strand TFO and those in the triplex were quantified to give the association constants (K_s). K_s = [Triplex]/([duplex][TFO]). βT represents WNA-βT.

TFO 3′ GGAAGG***N*-Z-*N***GAGGAGGGA.
Target 5′ GGGAGGGAGGGAAGG***N*-X-*N***GAGGAGGGAAGC.
duplex 3′ CCCTCCCTCCCTTCC***N*-Y-*N***CTCCTCCCTTCG.

TABLE 2 Equilibrium Association Constants (Ks, 10^9 M^{-1}) of Triplexes[a]

	(X, Y)			
Z	TA	AT	CG	GC
dG	0.004	0.008	0.008	0.086
dA	<0.001	0.074	<0.001	0.047
WNA-βC	<0.001	0.025	0.115	0.047
WNA-β^{m}C	0.021	<0.001	0.018	0.006
WNA-β^{Br}C	<0.001	0.015	0.041	0.029
WNA-β^{F}C	<0.001	0.006	0.048	0.022
WNA-βU	<0.001	<0.001	0.011	0.008
WNA-β^{m}U(T)	0.300	<0.001	0.015	0.082
WNA-β^{Br}U	0.025	0.001	0.040	0.031
WNA-β^{F}U	0.007	0.027	0.074	0.027
*m*Br-WNA-βT	<0.001	<0.001	<0.001	0.01
*o*Br-WNA-βT	0.058	0.029	0.023	0.033
*p*Br-WNA-βT	0.008	<0.001	<0.001	0.018
1-NA-WNA-βT	0.002	0.004	0.002	0.013
2-NA-WNA-βT	0.004	0.004	0.003	0.017
TP-WNA-βT	<0.001	<0.001	<0.001	<0.001

[a]Conditions for evaluation of triplexes are the same as the footnote of Table 1, except that 5 mM $MgCl_2$ was used. Investigated sequences are as follows.

TFO 3′ GGAAGGA-**Z**-GGAGGAGGGA.
Target 5′ GGGAGGGAGGGAAGGA-**X**-GGAGGAGGGAAGC.
duplex 3′ CCCTCCCTCCCTTCCT-**Y**-CCTCCTCCCTTCG.

TABLE 3 The Association Constants (K_s, 10^9 M^{-1}) of the Triplexes Having a TA Interrupting Site[a]

	TFO: $^{3'}$ X–Z–Y $^{5'}$			
Z	A–Z–G	A–Z–A	G–Z–G	G–Z–A
WNA-βT	**0.300**	<0.001	**0.13**	<0.001
*m*Br-WNA-βT	<0.001	<0.001	<0.001	**0.12**
*o*Br-WNA-βT	**0.058**	**0.050**	**0.038**	0.005
*p*Br-WNA-βT	0.008	<0.001	<0.001	<0.001
1-NA-WNA-βT	0.003	<0.001	<0.001	<0.001
2-NA-WNA-βT	<0.001	<0.001	0.006	<0.001
TP-WNA-βT	<0.001	<0.001	<0.001	<0.001

[a]Triplex formation was investigated as described in the footnote to Table 1.

TFO 3′ GGAAGG***X*-Z-*Y***GAGGAGGGA.
Target 5′ GGGAGGGAGGGAAGG***N*-T-*N***GAGGAGGGAAGC.
duplex 3′ CCCTCCCTCCCTTCC***N*-A-*N***CTCCTCCCTTCG.

CONCLUSION

In this study, we have performed systematic investigation to overcome limitation of the WNA analogs in triplex formation, and successively determined the two new analogs of WNA-βT having a bromo-substituted benzene to expand triplex recognition codes to all four combinations.

REFERENCES

1. Soyfer, V.N.; Potaman, V.N. *Triple-Helical Nucleic Acids*; Springer-Verlag: New York, 1996.
2. Sasaki, S.; Yamauchi, H.; Nagatsugi, F.; Takahashi, R.; Taniguchi, Y.; Maeda, M. Selective formation of stable triplexes including a TA or a CG interrupting site with new bicyclic nucleoside analogs (WNA). Tetrahedron Lett. **2001**, *42*, 6915–6918.
3. Sasaki, S.; Taniguchi, Y.; Takahashi, R.; Senko, Y.; Kodama, K.; Nagatsugi, F.; Maeda, M. W-shape nucleic acid (WNA) for selective formation of non-natural anti-parallel triplex including a TA interrupting site. J. Am. Chem. Soc. **2004**, *126*, 516–528.