Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Acyclic L-Threoninol Nucleic Acid (L-*a*TNA) with Suitable Structural Rigidity Cross-pairs with DNA and RNA

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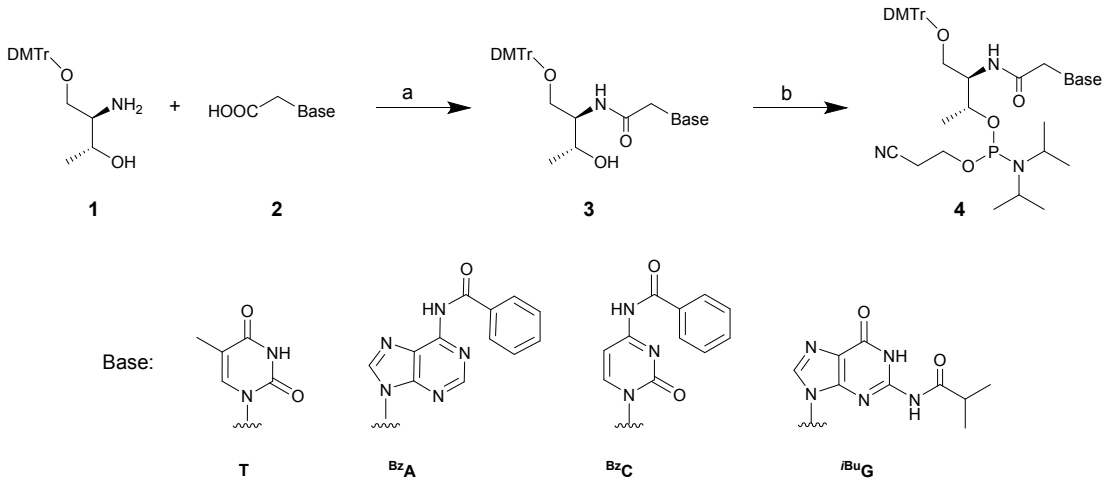
Experimental Section

Materials: Reagents for DNA synthesis and Poly-Pak II cartridges were purchased from Glen Research. Other reagents for the synthesis of phosphoramidite monomers were purchased from Tokyo Kasei Co., Ltd., Wako Pure Chemical Industries, Ltd., and Aldrich. Native DNA and RNA were purchased from Integrated DNA Technologies.

Synthesis of L-*a*TNA phosphoramidite:



Scheme S1. Synthesis of the phosphoramidite monomers of L-*a*TNA. Reagents and conditions: a) DMT-MM, Et_3N, DMF, r.t., 2 h; b) 2-cyanoethyl diisopropylchlorophosphoramidite, Et_3N, dry CH_2Cl_2, 0 °C, 30 min.

General procedures of the synthesis of compound 3: To a stirred solution of **2**[1-3] (1.2 eq.), triethylamine (5.0 eq.) and **1**[4] (1.0 eq.) in DMF were added DMT-MM (1.5 eq.) and the mixture was stirred for 2 h. Then $CHCl_3$ was added, and the mixture was washed with saturated aqueous solution of $NaHCO_3$ and that of NaCl. The solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography using chloroform and methanol as eluent (3% triethylamine was added). After column chromatography, products were collected by re-precipitation using ether and hexane.

Obtained amounts for compound **3** containing indicated bases were:

Compound 3 containing T: **1.85 g, 3.22 mmol (87.0%)**
Compound 3 containing ^{Bz}A: **1.90 g, 2.77 mmol (74.9%)**
Compound 3 containing ^{Bz}C: **2.04 g, 3.08 mmol (83.2%)**
Compound 3 containing ^{iBu}G: **2.26 g, 3.38 mmol (91.4%)**

General procedures for the synthesis of phosphoramidite monomers (compound 4): In dry dichloromethane under nitrogen, compound **3** (1.0 eq.) and triethylamine (5.0 eq.) were reacted with 2-cyanoethyl diisopropylchlorophosphoramidite (2.0 eq.) at 0 ^{o}C. After 30 min, the reaction mixture was diluted with excess ethyl acetate and washed with saturated aqueous solutions of $NaHCO_3$ and of NaCl. The solvent was removed by evaporation, and the crude mixture was subjected to silica gel column chromatography to afford compound **4**. Chloroform and acetone were used as eluent (3% triethylamine was added) for **4** containing $^{i\mathrm{Bu}}$G. Hexane, ethyl acetate and triethylamine were used for the others. After column chromatography, products were re-precipitated twice from hexane. Before the synthesis of L-*a*TNA on a DNA synthesizer, **4** were dried by co-evaporation with dry acetonitrile.

Obtained amounts for compound **4** were shown below.

Compound 4 containing T: **1.37 g, 1.77 mmol (67.8 %)**
Compound 4 containing ^{Bz}A: **1.10 g, 1.24 mmol (56.9 %)**
Compound 4 containing ^{Bz}C: **1.02 g, 1.18 mmol (52.2 %)**
Compound 4 containing $^{i\mathrm{Bu}}$G: **1.59 g, 1.83 mmol (68.0 %)**

Characterization of L-*a*TNA phosphoramidites: All synthesized L-*a*TNA phosphoramidites and synthetic intermediates were enantiomers of D-*a*TNA, which we previously reported.[5]

Synthesis and purification of oligonucleotides: All L-*a*TNAs (**L-*a*TN8a**, **L-*a*TN8b**, **L-*a*TN8c**, **L-*a*TN15a**, **and L-*a*T15b**) were synthesized using an ABI 3400 DNA/RNA synthesizer using phosphoramidite chemistry as described in a previous report.[5] Synthesized oligonucleotides were purified using Poly-Pak cartridges and reversed-phase HPLC (Merck LiChrospher 100 RP-18(e) column). After purification, synthesized oligonucleotides were characterized by MALDI-TOF MS.

[1] L. Christensen, H. F. Hansen, T. Koch, P. E. Nielsen, Inhibition of PNA triplex formation by N4-benzoylated cytosine. *Nucleic Acids Res.* 1998, **26**, 2735.

[2] Z. Timár, L. Kovács, G. Kovács, Z. Schmél, Fmoc/acyl protecting groups in the synthesis of polyamide (peptide) nucleic acid monomers. *J. Chem. Soc. Perkin Trans.* 2000, **1**, 19.

[3] T. Kofoed, H. F. Hansen, H. Ørum, T. Koch, PNA synthesis using a novel Boc/acyl protecting group strategy. *J. Peptide Sci.* 2001, **7**, 402.

[4] Y. Hara, et al., Coherent Quenching of a Fluorophore for the Design of a Highly Sensitive In-Stem Molecular Beacon. *Angew. Chem. Int. Ed*. 2010, **49**, 5502.

[5] H. Asanuma, T. Toda, K. Murayama, X. Liang and H. Kashida, Unexpectedly Stable Artificial Duplex from Flexible Acyclic Threoninol. *J. Am. Chem. Soc.*, 2010, **132**, 14702.

Results of MALDI-TOF MS:

L-*a*TN8a: Obsd. m/z 2641 (Calcd. for [**L-*a*TN8a**+H^+]: m/z 2641)

L-*a*TN8b: Obsd. m/z 2640 (Calcd. for [**L-*a*TN8b**+H^+]: m/z 2641)

L-*a*TN8c: Obsd. m/z 2641 (Calcd. for [**L-*a*TN8c**+H^+]: m/z 2641)

L-*a*TN15a: Obsd. m/z 4992 (Calcd. for [**L-*a*TN15a**+H^+]: m/z 4991)

L-*a*TN15b: Obsd. m/z 5018 (Calcd. for [**L-*a*TN15b**+H^+]: m/z 5018)

Melting-temperature measurements: Melting curves of all nucleic acid duplexes were obtained using a Shimadzu UV-1800 by measuring the change in absorbance at 260 nm versus temperature. The temperature ramp was 0.5 ^oC min^{-1}. The melting temperature (T_m) was determined from the maximum in the first derivative of the melting curve. Both the heating and cooling curves were measured, and the T_m measurements obtained agreed within 2.0 ^oC. The solution conditions were (unless otherwise noted) 100 mM NaCl, 10 mM phosphate buffer (pH 7.0), 2.0 μM oligonucleotide.

CD measurements: CD spectra were measured using a JASCO model J-820 instrument equipped with a programmable temperature controller. The sample solutions contained 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of duplex was 4.0 μM and experiments were performed in a 10-mm quartz cell.

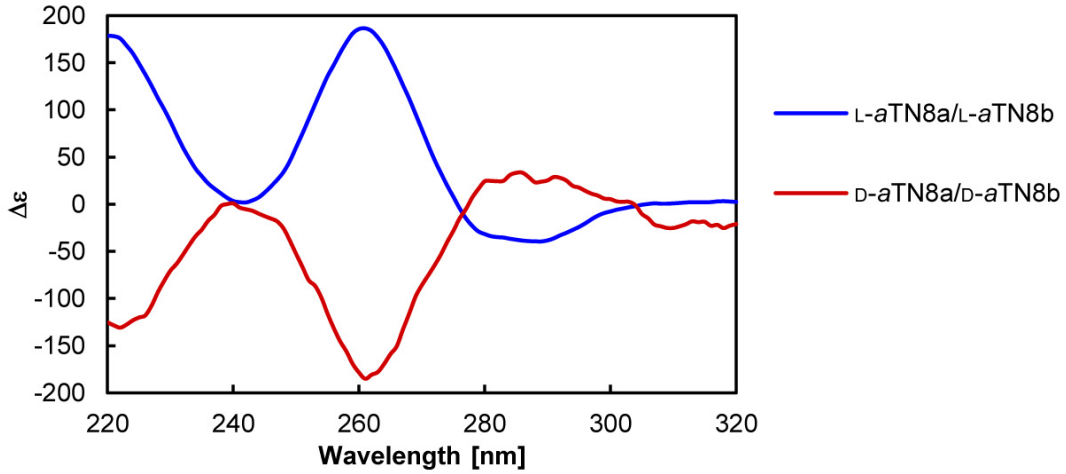


Fig. S1. CD spectra of D-*a*TNA/D-*a*TNA[1] and L-*a*TNA/L-*a*TNA**.**

Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0), 20 °C. The concentration of **D-TN8a**/**D-TN8b** was 5.0 μM, and experiments were performed in a 1-mm cell. The concentration of **L-TN8a**/**L-TN8b** was 4.0 μM, and experiments were performed in a 10-mm quartz cell.

L-*a*TN8a: 1'-TGACTACG-3'
L-*a*TN8b: 3'-ACTGATGC-1'
D-*a*TN8a: 1'-TGACTACG-3'
D-*a*TN8b: 3'-ACTGATGC-1'

[1] K. Murayama, Y. Tanaka, T. Toda, H. Kashida and H. Asanuma, *Chem. Eur. J.*, 2013, **19**, 14151-14158.

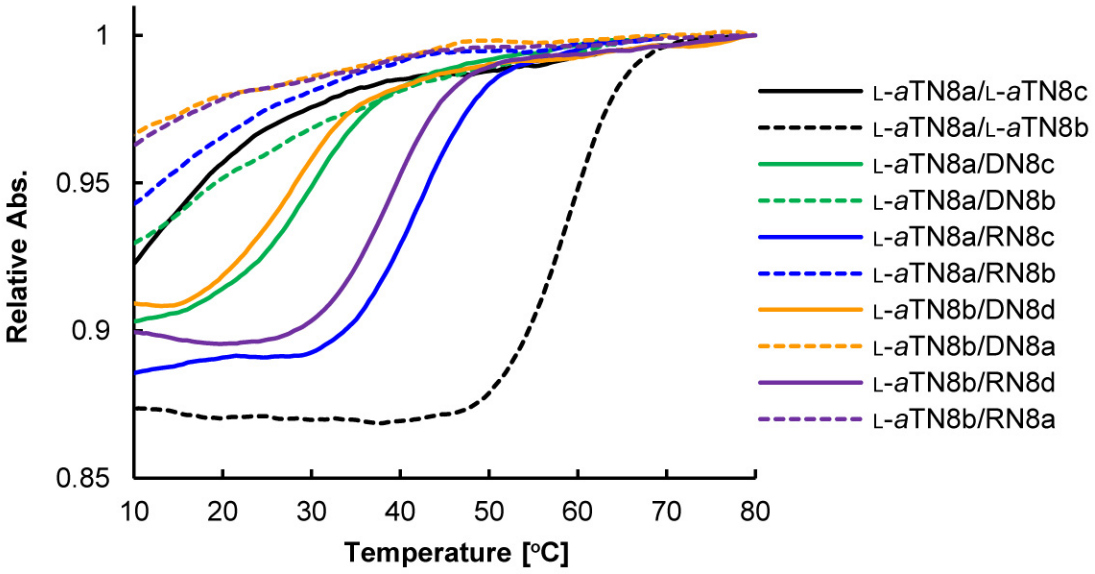


Fig. S2. Melting profiles of 8-base-pair L-*a*TNA homo- and hetero-duplexes.
Conditions: 2.0 μM oligonucleotide, 100 mM NaCl, 10 mM phosphate buffer (pH 7.0).

L-*a*TN8a:	1'-TGACTACG-3'	L-*a*TN8b:	3'-ACTGATGC-1'
DN8a:	5'-TGACTACG-3'	DN8b:	3'-ACTGATGC-5'
RN8a:	5'-UGACUACG-3'	RN8b:	3'-ACUGAUGC-5'
L-*a*TN8c:	1'-ACTGATGC-3'		
DN8c:	5'-ACTGATGC-3'	DN8d:	3'-TGACTACG-5'
RN8c:	5'-ACUGAUGC-3'	RN8d:	3'-UGACUACG-5'

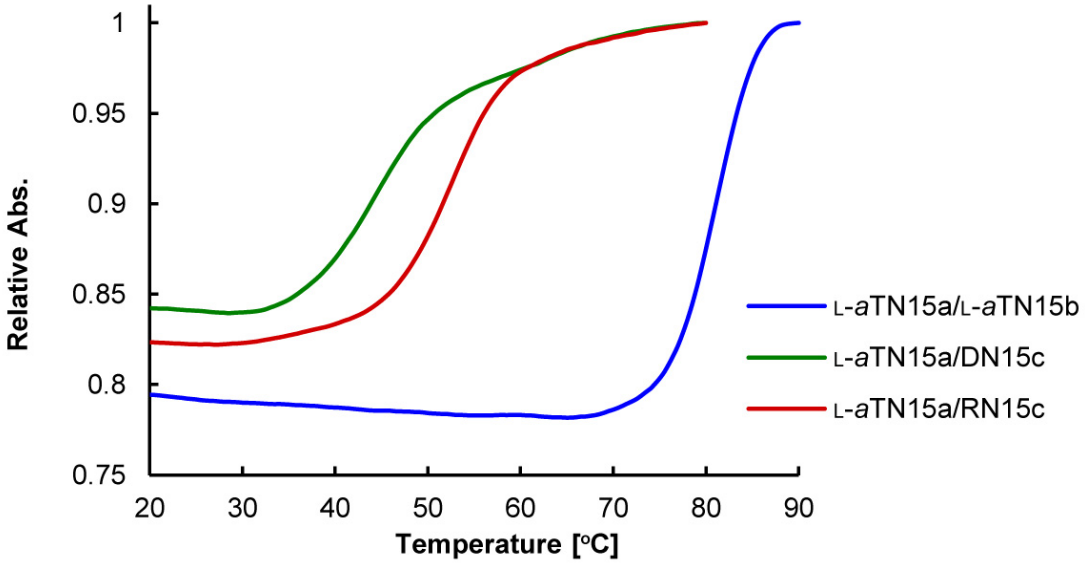


Fig. S3. Melting profiles of 15-base-pair L-*a*TNA homo- and hetero-duplexes.
Conditions: 2.0 μM oligonucleotide, 100 mM NaCl, 10 mM phosphate buffer (pH 7.0).

L-*a*TN15a: 1'-GTGTAATAACAACAT-3'
L-*a*TN15b: 3'-CACATTATTGTTGTA-5'
DN15c: 5'-CACATTATTGTTGTA-3'
RN15c: 5'-CACAUUAUUGUUGUA-3'

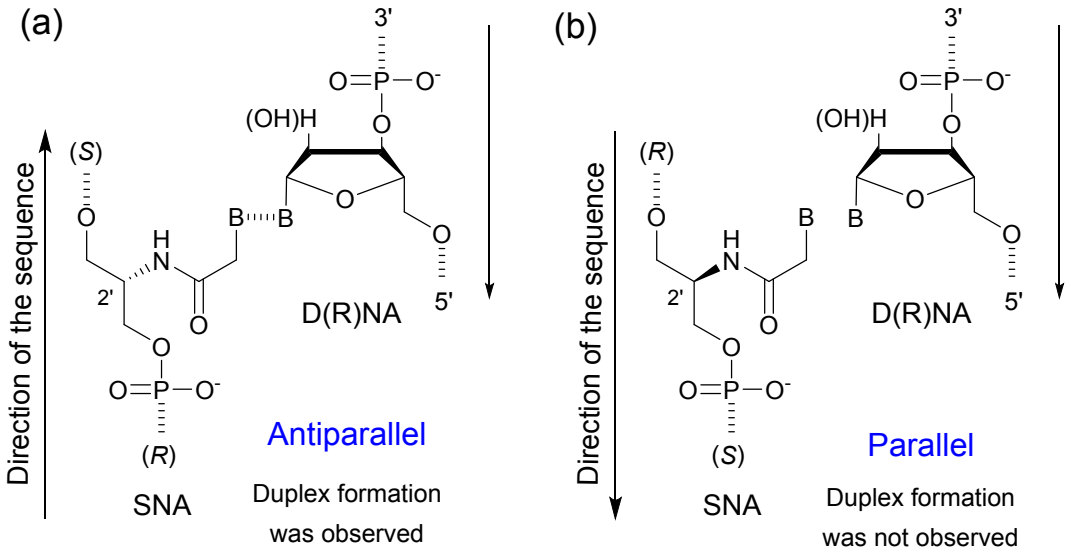


Fig. S4. Relationship between the direction of the strand synthesis and configuration of 2'-amide bond of SNA. (a) SNA/D(R)NA in an antiparallel manner. (b) SNA/D(R)NA in an parallel manner.