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Oxy-peptide nucleic acid with a pyrrolidine ring that is configurationally optimized for hybridization with DNA†

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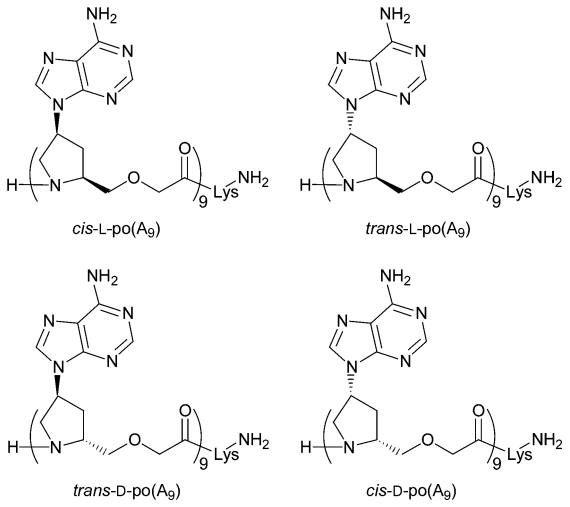
Four stereoisomers of oxy-peptide nucleic acids containing ether linkages in the main chain and conformationally-restricted pyrrolidine rings (pyrrolidine-based oxy-PNA = POPNA) were newly synthesized and investigated for binding to DNA. *cis*-L-POPNA with 9 adenine bases formed the most stable hybrid with dT_9. The POPNA showed high sequence specificity similar to that of the Nielsen-type PNA and sharper melting behavior in hybridization with DNA than the Nielsen-type PNA.

Nielsen's peptide nucleic acid (PNA) has been shown to hybridize with DNAs with higher affinity and fidelity than DNAs.[1] Because of these superior properties, PNA is currently used widely in biological and medicinal research.[2] Since its discovery, a number of PNA analogs have been studied, in order to further improve the affinity to the complementary nucleic acids and the solubility in aqueous media.[3]

Among these attempts, oxy-peptide nucleic acid (OPNA) seems promising because of its improved water solubility and very sharp melting curves in the hybridization with DNAs.[4] The sharp hybridization and the flexible backbone of OPNAs are particularly important for detecting single mismatches in relatively long DNA sequences. To further optimize the structure of OPNA, we have undertaken synthesis of a conformationally restricted version of OPNA (pyrrolidine-based OPNAs = POPNAs) by introducing a pyrrolidine ring. The two chiral centers on a pyrrolidine ring allow four stereoisomers that are named as *cis*-L-POPNA, *trans*-L-POPNA, *cis*-D-POPNA, and *trans*-D-POPNA after the corresponding stereoisomers of 4-hydroxyprolines employed as the starting materials (Scheme 1).

Synthesis of *cis*-L-POPNA has been reported previously.[5] Synthesis and hybridization behavior of the *cis*-D isomer have been reported by Altmann, *et al.*[6] In this study, all four stereoisomeric monomers with an adenine base on the side chain were synthesized and their hybridization behavior compared to find the optimum configuration for hybridization with DNAs.



Scheme 1 Structures of four stereoisomers of POPNAs.

The Fmoc-protected adenine monomer of the POPNA with *cis*-L configuration was synthesized from *trans*-L-4-hydroxyproline through inversion at the 4th position during the attachment of 6-chloropurine by Mitsunobu reaction.[5,7] The monomer of *trans*-L configuration was synthesized through inversions in two consecutive Mitsunobu reactions, first with formic acid and second with 6-chloropurine. Similarly, the Fmoc-protected adenine monomers of *trans*-D and *cis*-D configuration were synthesized from *cis*-D-4-hydroxyproline. As an example, the synthetic route for Fmoc-protected adenine monomer of *cis*-D-POPNA is shown in Fig. 1. The optical purity of each Fmoc-monomer was confirmed by the coincidence of the HPLC profile and NMR spectrum of the *cis*-L/*cis*-D pair and those of the *trans*-L/*trans*-D pair. The optical purity was also checked by HPLC in the presence of chiral shift reagent, NIPF ((*S*)-(−)-1-(2,3-naphthalenedicarboxyimidyl)propionyl fluoride).

The peptides tested for hybridization with DNAs contain 9 adenine monomers with a Lys unit at the C-terminal. The peptide synthesis was carried out by using an Fmoc-NH-SAL-PEG resin (super acid-labile polyethyleneglycol resin, Watanabe Chemicals, Hiroshima, Japan) as the support, with bromo-tris-pyrrolidinophosphonium hexafluorophosphate (PyBrop)/HOBt as the coupling reagents. After cleavage from the resin, the crude POPNA was purified by preparative HPLC (C18 column) to obtain a single peak in analytical HPLC. Each adenine 9-mer was identified by TOF-mass spectroscopy (PerSeptive Biosystems, Voyager DE Pro). CD spectra of the *cis*-L and *cis*-D oligomers showed the same profiles with opposite signs. The same was observed for the *trans*-L and *trans*-D pair. The details of the synthesis and characterization of POPNA monomers and oligomers are described in the Electronic Supplementary Information (ESI).

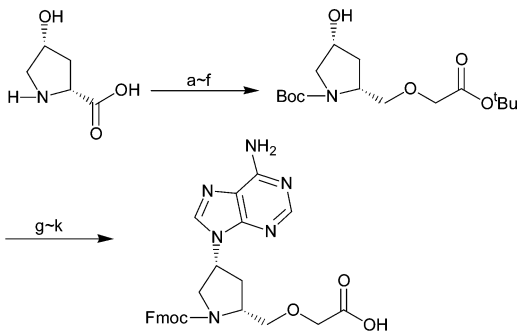


Fig. 1 Synthetic route of Fmoc-protected adenine monomer of *cis*-D-POPNA. a) Boc_2O, $NaHCO_3$, water : dioxane (1 : 1), overnight, DCHA (94.9%); b) EtBr, DMF, overnight (94.0%); c) DHP, pPTs, DCM, 6 h (95.8%); d) $NaBH_4$, EtOH, overnight (84.5%); e) $BrAcO^tBu$, Bu_4NHSO_4, benzene : NaOH aq. (1 : 1) at 5 °C, 6 h (90.9%); f) pPTs, EtOH, 3 h (92.1%); g) formic acid, DEAD, Ph_3P, THF, overnight (80.6%); h) 25% NH_3 aq., MeOH, 6 h (quant.); i) 6-Cl-purine, DEAD, Ph_3P, THF, overnight (crude); j) 25% NH_3 aq., dioxane at 60 °C, 24 h (60.0%); k) 30% HBr/AcOH, Fmoc-ONSu, water : MeCN (1 : 1), overnight (90.0%).

† Electronic supplementary information (ESI) available: details of synthesis and the spectral data of four stereoisomers of POPNA. See http://www.rsc.org/suppdata/cc/b4/b401057d/

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Melting curves of the four stereoisomers of POPNA with 9 adenine bases po(A_9)s, in equimolar mixtures with dT_9 are shown in Fig. 2.‡ All mixtures showed higher melting temperatures (T_ms) than the dA_9–dT_9 pair, *i.e.*, 34.5 °C for *cis*-L-po(A_9)–dT_9, 30.5 °C for *trans*-D-po(A_9)–dT_9, 30.1 °C for *cis*-D-po(A_9)–dT_9 and 22.7 °C for *trans*-L-po(A_9)–dT_9, respectively. The Job plots showed 1 : 1 stoichiometry for all hybrids. The temperature range of 5–95% transition was 18 °C for the *cis*-L-po(A_9)–dT_9 hybrid. This temperature range is narrower than that of the Nielsen-type PNA(A_9)–dT_9 mixture (26 °C). It must be noted that temperature dependences of the absorption of po(A_9)s showed a small jump around 5 °C, indicating some self-structure formation. The self-structure at very low temperature does not affect the melting behavior of the hybrid at higher temperatures (see ESI).

Melting curves of the four po(A_9)s in equimolar mixtures with dT_4XT_4 (X = T, C, A and G) were also measured and the T_m's are collected in Table 1. The A–A and A–G mismatches in the hybrids of *cis*-L-po(A_9) with dT_4XT_4 lowered the T_m by ~20 °C, while an A–C mismatch destabilized the duplex by ~15 °C. These ΔT_m values are about the same as those observed with Nielsen-type PNA(A_9). The large ΔT_m values together with the sharp melting curves of *cis*-L-POPNA are advantageous for the detection of single mutations in the DNA base sequences.

Successful hybridization of all stereoisomers of POPNA is somewhat surprising, since only limited types of peptides with side-chain nucleobases have been reported to hybridize with DNAs. This may be interpreted in terms of flexible main chains that contain ether linkages. Indeed, three of the four stereoisomers of po(A_9) showed similar CD profiles when hybridized with dT_9 (Fig. 3). The *trans*-L-po(A_9)–dT_9 hybrid showed a less intense CD peak, indicating less defined hybrid structure. This may explain the weak sequence specificity of *trans*-L-po(A_9) as found in Table 1. The CD profiles suggest that the helix senses of all hybrids are the same, despite the opposite main-chain chiralities for the D- and L-POPNAs. Flexible polyetheramide main chains may adjust their conformations to the most stable hybrid structure that is close to the dA_9–dT_9 right-handed duplex.

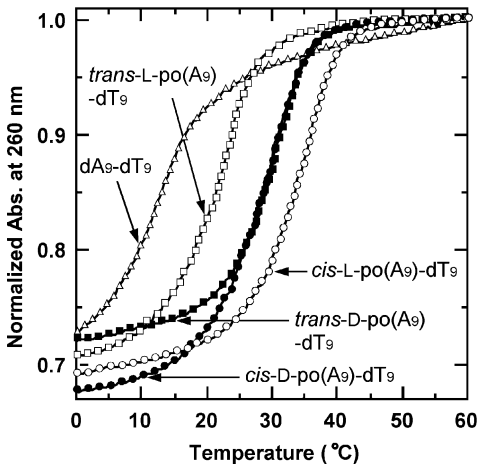

Fig. 2 Temperature dependence of absorption intensity at 260 nm for equimolar mixtures of *cis*-L-po(A_9)–dT_9, *trans*-L-po(A_9)–dT_9, *cis*-D-po(A_9)–dT_9, *trans*-D-po(A_9)–dT_9 and dA_9–dT_9. [po(A_9)] = [dT_9] = 5.0 μM in phosphate buffer.

Table 1 T_ms (°C) of hybrids of various POPNAs with DNAs[a]

	dT_4XT_4			
	T	C	A	G
po(A_9)				
cis-L	34.5	19.0	12.9	15.0
cis-D	30.1	15.5	28.0	13.4
trans-L	22.7	20.4	20.3	20.8
trans-D	30.5	16.8	15.5	16.7

[a] Buffer: 100 mM NaCl, 10 mM NaH_2PO_4 and 0.1 mM EDTA, pH 7.0. T_m evaluated from the midpoint of the transition at [POPNA] = [DNA] = 5.0 μM.

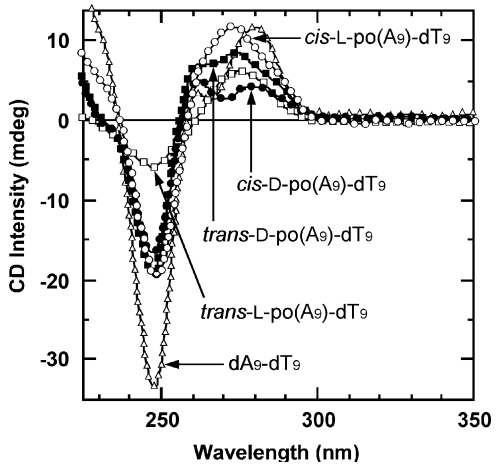

Fig. 3 CD spectra of equimolar mixtures of *cis*-L-po(A_9)–dT_9, *trans*-L-po(A_9)–dT_9, *cis*-D-po(A_9)–dT_9, *trans*-D-po(A_9)–dT_9 and dA_9–dT_9. The spectra were measured at 5 °C after annealing at 60 °C for 15 min. Other conditions are the same as in Fig. 2.

In summary, four stereoisomers of POPNA were synthesized and hybridized with the complementary DNAs. Of the four stereoisomers, the *cis*-L-isomer showed the highest stability when hybridized with DNA, whereas the *trans*-L-isomer showed the lowest stability. The fact that all the stereoisomers of POPNA hybridized with DNA and the hybrids showed a similar CD pattern suggests that POPNA chains are flexible and fit their conformations to essentially the same hybrid structure with the DNA. The *cis*-L-POPNA showed high sequence specificity and a sharper melting curve than the Nielsen-type PNA, at least for A-rich sequences in the POPNA.

Notes and references

‡ UV and CD experiments were conducted in aqueous buffer (100 mM NaCl, 10 mM NaH_2PO_4 and 0.1 mM EDTA, pH 7.0). Each concentration of POPNA and DNA oligomers was 5.0 μM. The UV melting curves were recorded with cooling the solution at 0.5 °C/0.5 min. Essentially the same curves were obtained in the heating process. The observed absorbance has been normalized at 80 °C. The CD spectra were recorded at 5 °C after an annealing process at 60 °C, 15 min.

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