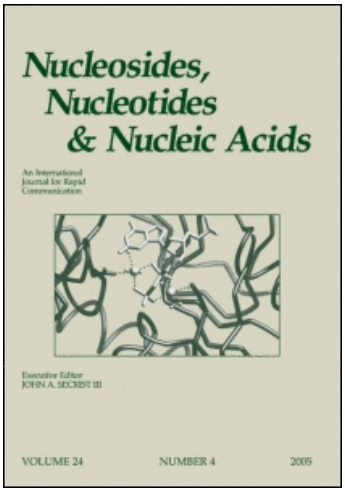

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SYNTHESIS AND BINDING AFFINITY OF A CHIRAL PNA ANALOGUE

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SYNTHESIS AND BINDING AFFINITY OF A CHIRAL PNA ANALOGUE

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ABSTRACT

The synthesis of a chiral peptide nucleic acid (PNA), which is composed of N-aminoethyl-cis-4-nucleobase-L-proline units, was described. The chiral PNA monomers containing all four nucleobases (A, T, C and G) were steroselectively prepared. The x-ray diffraction data from a single crystal confirmed the configuration of a key intermediate. Binding activity of the oligomers with their complementary DNA targets was also investigated.

Synthetic molecules that can bind specifically to a chosen target in a gene sequence are of great interest in medicinal and biotechnological fields. They have shown promise for the development of gene therapeutic agents, diagnostic devices for genetic analysis and as molecular tools for nucleic acid manipulations[1]. PNA[2] is a nucleic acid analogue in which the entire deoxyribose-phosphate backbone in DNA has been replaced by a completely different backbone composed of N-(2-aminoethyl) glycine units (Fig. 1). PNA has shown advantages[3–7] superior to other DNA analogues. In recent years, many applications of PNA have been exploited, especially in a biotechnological context[8–10]. However, as far as developing a drug is concerned, PNA still has some limitations. One is its poor solubility in aqueous media[11]. Another drawback is that a PNA oligomer can bind to a nucleic acid target in both parallel and antiparallel orientations[12], which may lead to lower binding specificity. It is suggested that this kind of orientation independence could be a consequence of the achiral nature of PNA.

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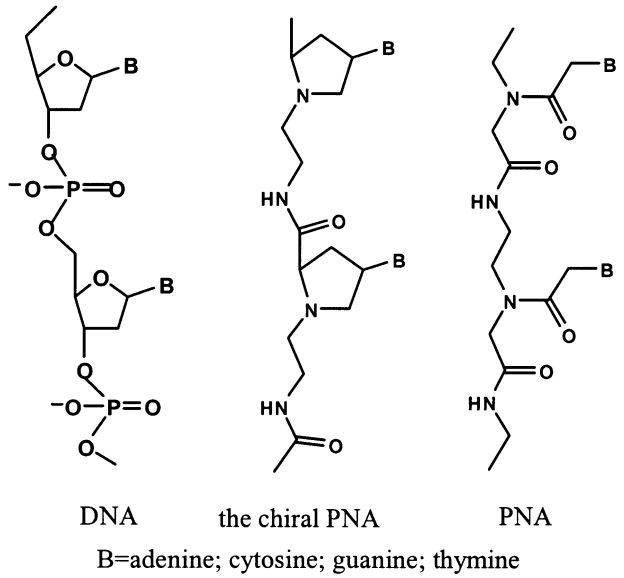


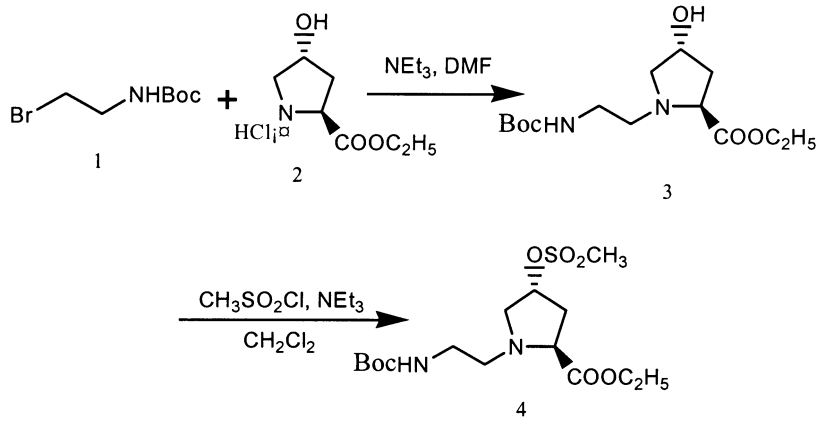
Figure 1. Structure of DNA, PNA and the chiral PNA.

Our research is focused on the design and synthesis a series of chiral PNAs with a partially cyclic backbone to mimic natural nucleic acids. The pyrrolidine ring in proline is a suitable unit for mimicking the ribose moiety in DNA, therefore, chiral PNA is designed to be composed of alternated N-aminoethyl-cis-4-nucleobase-L-proline units (Fig. 1). This chiral PNA not only has a similar dimension and rigidity as natural nucleic acids, but has a tertiary amino structure in each unit, which could be expected to obtain a good solubility and a high affinity for negatively charged nature nucleic acids.

As we were working on this project, D'Costa *et al.*[13] reported a homothymine PNA having the same backbone, but their results showed some differences from ours. In this paper, we described the detailed synthesis of the chiral PNA monomers containing all four natural bases (A, C, T and G) and their oligomerization. Three of the monomers (C, A, G) have not been reported yet. We also studied the crystal structure of compound **5** to confirm its configuration. The data showed that no racemization occurred during the synthesis. The hybridization properties of the chiral PNA oligomers with complementary DNA targets were also discussed.

RESULTS AND DISCUSSION

Since the chiral PNA had a polyamide backbone, the oligomers were assembled by solid phase peptide synthesis (SPPS) with different Boc protected monomers.



Scheme 1.

Synthesis of Monomers

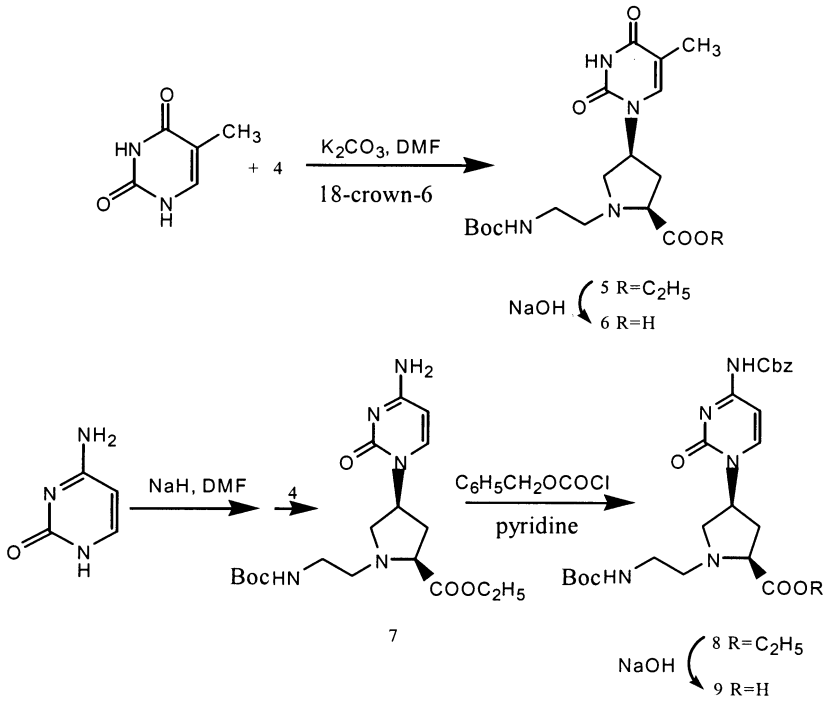
Synthesis of the Key Intermediate N-(2-Boc-amino)ethyl-trans-4-methanesulfonoxyl-L-proline ethyl ester **4**

Compound **4** was the key intermediate in our synthetic pathways. It was prepared as shown in Scheme 1. Alkylation of trans-4-hydroxy-L-proline ethyl ester **2** with N-Boc-aminoethyl bromide[14] gave compound **3** in an overall yield 68%. Then compound **3** was converted to its mesylate derivative **4** in a quantitative yield according to the method described by Borcherding *et al*[15].

Monomers Containing Pyrimidine Bases

Alkylation of thymine with compound **4** was achieved by stirring the mixture of compound **4**, thymine, K_2CO_3 and 18-crown-6 in DMF at 75°C for 36 hours[16]. After extraction and purification by flash chromatography, compound **5** was obtained in a 71% yield. Finally, the ethyl ester was removed by hydrolysis, giving the thymine monomer **6** in a 79% yield (Scheme 2). The cis-sterochemistry of compound **5** was confirmed by a single-crystal x-ray diffraction analysis (Fig. 2).

Alkylation of cytosine with mesylate **4** was carried out under a similar condition reported by Lewis *et al*[17]. Compound **4** reacted with sodium cytosine in anhydrous DMF to give compound **7** as the major product in a 28% yield (Scheme 2). It was reported[16] that direct displacement on an inactive carbon with cytosine may generate N^1-coupled and O^2-coupled isomers. We determined the exact structure of compound **7** by selective DEPT NMR technique[16]. Irradiation H-4′ of compound **7** led to selective enhancement of C-6, C-2 and C-2′ resonance at 143.5, 157.1 and 65.9 ppm,



Scheme 2.

respectively (Fig. 3). The result showed that compound **7** was the N^1-coupled isomer.

The exocyclic amino group of the nucleobase should be protected to prevent chain extension or acetylation on it during the capping procedure in the oligomer synthesis circles[5,18]. Benzyloxycarbonyl(Cbz) was used as the

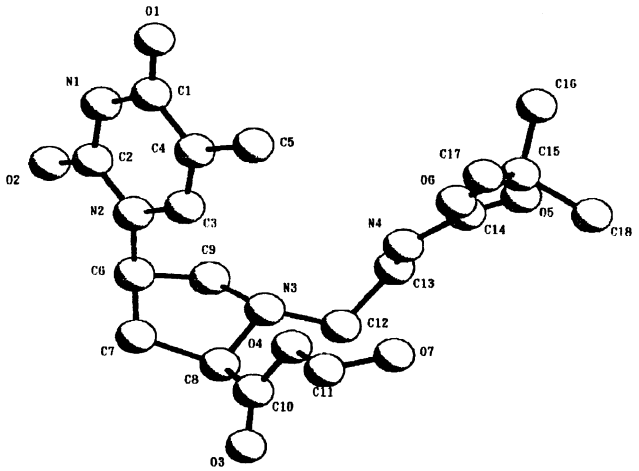
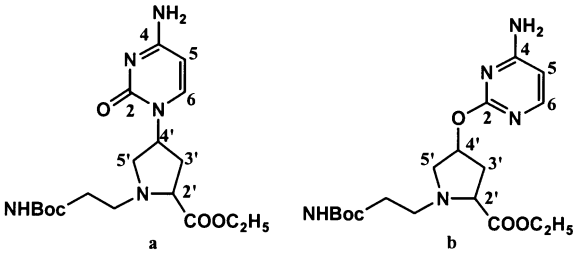
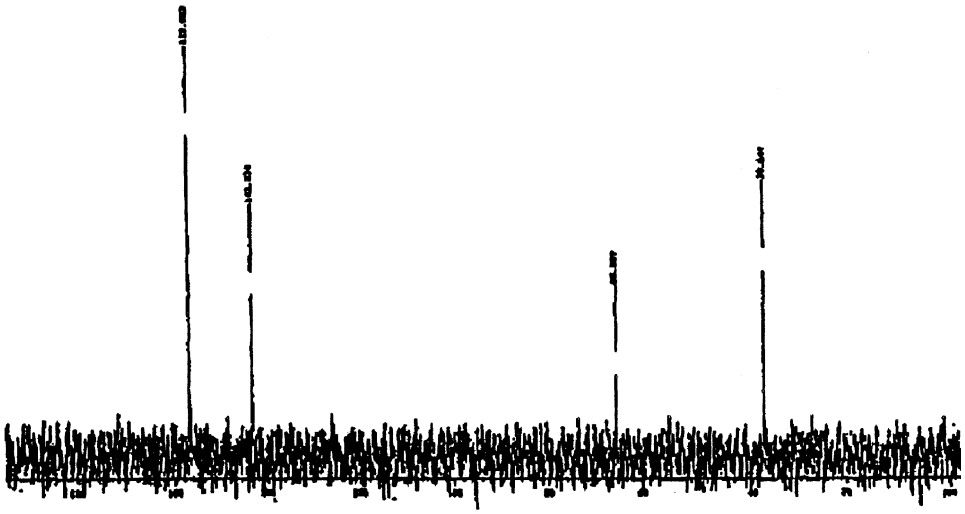


Figure 2. The crystal structure of compound **5** from x-ray diffraction.



Selective DEPT ^{1}H-^{13}C NMR correlations of compound **7**.



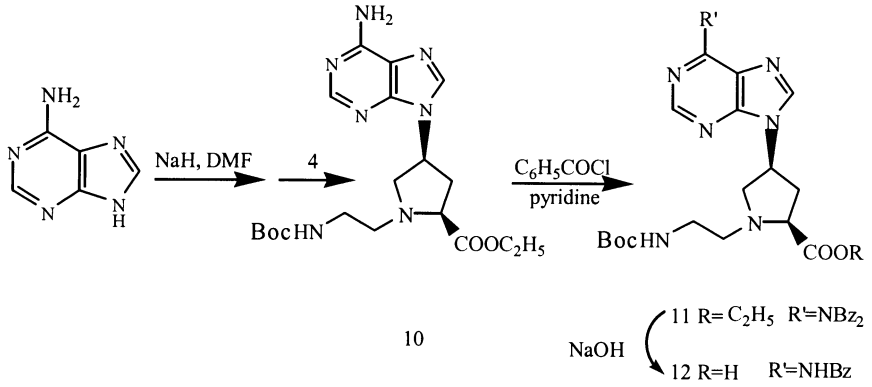
Irradiation at H-4′, using J_{CH} = 4Hz provided the spectrum above.

Figure 3.

protecting group for the exocyclic amino of cytosine. Thus compound **7** reacted with benzyloxycarbonyl chloride (CbzCl) to give compound **8** and hydrolyzed to give the cytosine monomer **9** (Scheme 2).

Monomers Containing Purine Bases

For the synthesis of the adenine monomer, nucleophilic displacement of the mesylate 4 with adenine/NaH in DMF as reported in literature[15] worked well, affording compound **10** in a 24% yield. Attempts to protect the exocyclic amino group of compound **10** with CbzCl failed under a variety of different conditions. We decided to adopt the benzoyl group (Bz), used in conventional DNA chemistry19 as the protecting group. Compound **10** reacted with 4 eq. excess of benzoyl chloride in pyridine to give compound **11** in a 53% yield. Final removal of the ethyl ester by hydrolysis gave the adenine monomer **12** in a 75% yield (Scheme 3).



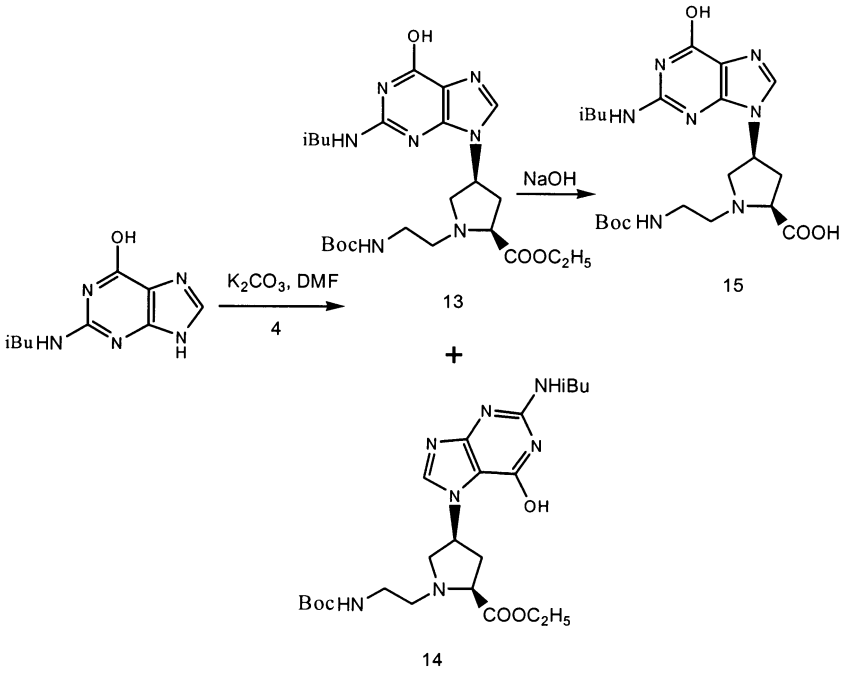
Scheme 3.

In DNA synthesis, two deprotecting methods[19] are available for removing acyl group (including isobutyryl group, acetyl group and benzoyl group). One was treatment with concentrated aqueous ammonia at 55°C for 2 hours. Another was treatment with concentrated aqueous ammonia and 30% methylamine (1:1) at room temperature for 90 min. To test which method was suitable for peptide synthesis, we treated a dipeptide Gly-Trp with the two methods respectively. The result showed that the dipeptide partially degraded in concentrated aqueous ammonia at 55°C for 2 hours, however, no degradation or racemization[20] was observed in aqueous ammonia and methylamine at room temperature for 5 hours. This demonstrated that the latter method was suitable for deprotection in the PNA oligomers synthesis.

Guanine did not react with compound **4** under similar conditions partially because of its poor solubility. We then used N^2-isobutyrylguanine (N^2-iBuG) as the starting material. Direct alkylation[21] N^2-iBuG gave a mixture of two products, the N^9-isomer **13** and N^7-isomer **14**, in 10% and 20% yields respectively. Hydrolysis of compound **13** gave the guanine monomer **15** in a 64% yield (Scheme 4).

Synthesis of Oligomers

Four PNA oligomers were assembled manually in a stepwise fashion using a similar protocol of SPPS described by Dueholm[5]. Lysine was incorporated at the C-terminus in order to suppress self-aggregation and increase solubility in aqueous media[5]. A 4-methylbenzhydrylamine (MBHA) resin was used as the polymeric support. Monomers **6**, **9**, **12** and **15** were coupled using a 1,3-dicyclohexylcarbodiimide(DCC)-coupling protocol. Once all the monomers had been linked one by one, the resin was treated with concentrated aqueous ammonia-methylamine (1:1) at room temperature for 2 hours to remove the protecting groups. After cleavage from the solid



Scheme 4.

support with anhydrous HF, the crude products were purified by sephadex gel filtration and RP-HPLC on a Kromasil C-18 reverse phase column using acetonitrile-water containing 0.1% TFA gradient system to give the pure (>95%, 260 nm) PNA-oligomers. All the oligomers were confirmed by TOF-MS (Table 1). The solubility of these new PNA-oligomers in water is satisfactory (>15 mg/ml for (pT_{10})-lys).

Thermal Transition

The thermal transition curves of the above oligomers were recorded at 260 nm over 6–64°C range (Fig. 4). Neither $p(A_{12})$-lys nor dT_{10} showed any

Table 1. TOF-MS Data of the Chiral PNA-Oligomers

PNA	MW (Calc.)	MW (Found)
$p(A_{12})$-lys	3424.8	3424.0
$p(T_{10})$-lys	2788.0	2788.4
p(TATAAATT)-lys	2294.5	2299.1
p(ATTCCTTCTTCGGGAA)-lys	4415.7	4416.6

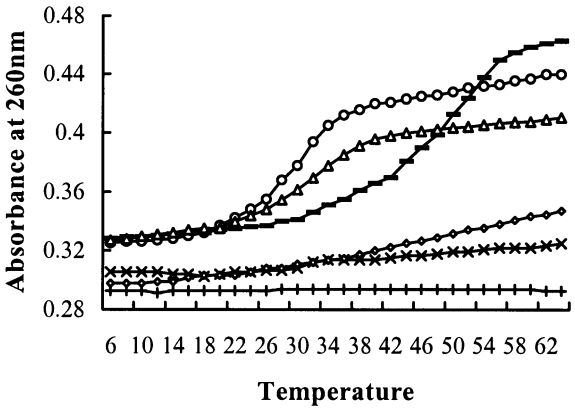


Figure 4. Melting curves (normalized) ◇ for single strand dA_{12}; + for single strand dT_{12}; × for single strand $p(A_{12})$-lys; ○ for complex dA_{12}/dT_{12}; − for complex $p(A_{12})$-lys/dT_{10}; △ for complex $p(A_{12})$-lys/$d(T_4GT_5)$.

clear hyperchromicity, indicating that no self-aggregation occurred. The complex $p(A_{12})$-lys/dT_{10} displayed a well defined single-phased melting profile (Fig. 4), with 40% hypochromicity and a melting temperature(Tm) of 46°C, higher than that of dA_{12}/dT_{12} (Fig. 4, Table 2). It suggested that $p(A_{12})$-lys can bind strongly to dT_{10}. The complex $p(A_{12})$-lys/d(5′-T_4GT_5-3′), containing one mismatched base pair, exhibited a 13°C decrease in Tm (Fig. 4, Table 2), in comparison to fully complementary $p(A_{12})$-lys/dT_{10}. This demonstrated that binding of $p(A_{12})$-lys with complementary DNA was sequence specific.

Table 2. Tm Values of Complexes Between the Chiral PNA and DNA*

Hybrid Complex	Tm (°C)	Hypochromicity (%)
dA_{12}/dT_{12}	28	35.0
$p(A_{12})$-lys/dT_{10}	46	40.7
$p(T_{10})$-lys/dA_{10}	n.d.	9.0
p(TATAAATT)-lys/ d(5′-ATATTTAA-3')	n.d.	5.6
p(TATAAATT)-lys/ d(5′-AATTTATA-3')	n.d.	9.0
$p(A_{12})$-lys/$d(T_4GT_5)$	33	24.6

n.d.: not detectable.
*Experiment condition: Buffer containing 0.1M NaCl, 0.02 M $(CH_3)_2$-AsO_2Na, pH7.0.
Each complex was composed of 1:1 stoichiometry of two component parts.

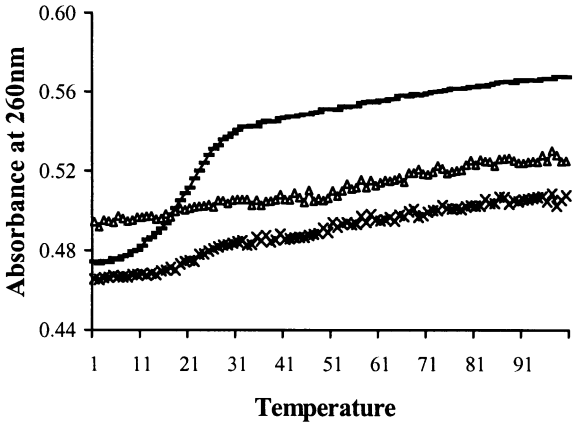


Figure 5. Melting curves. – for complex dA_{10}/dT_{10}; × for complex $dA_{10}/p(T_{10})$-lys; △ for complex $dA_{10}/2[p(T_{10})$-lys].

As $p(T_{10})$-lys had the same backbone as $p(A_{12})$-lys, it was expected to bind with its DNA target, however, no distinct melting transition step was detected for $p(T_{10})$-lys/dA_{10} (both 1:1 and 1:2) mixture over a 1–97°C range (Fig. 5). According to the results of D'Coster *et al.*[13], the Tm of $p(T_{10})$-lys/dA_{10} complex might be too high to be detected. To clarify this, UV-mixing curves were measured at 260 and 280 nm, but no clear transition point at 1:1 or 1:2 (A/T, mole/mole) was observed (Fig. 6). It seemed that $p(T_{10})$-lys did not bind with its complementary DNA target. We also synthesized a shorter, mix-sequenced oligomer p(TATAAATT)-lys and evaluated its hybridization properties. No melting step was observed (melting curves not shown). It demonstrated that thymine on the chiral PNA could not pair with adenine on DNA.

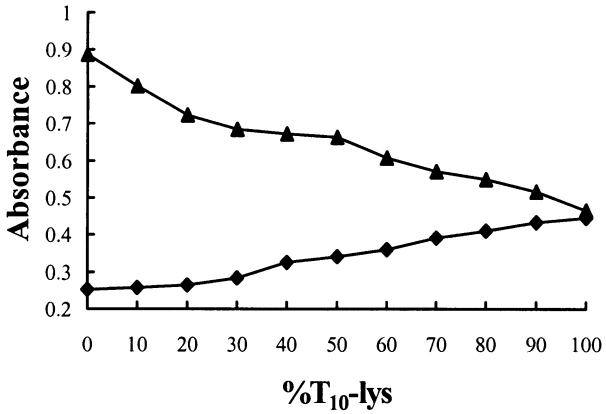


Figure 6. Mixing curve for $dA_{10}/(pT_{10})$-lys ▲ for 260 nm data, ◆ for 280 nm data total conc. = 5 μM, measured at 16°C.

EXPERIMENTAL SECTION

Reagents and solvents were obtained commercially and used without further purification unless indicated. ^{1}H and ^{13}C NMR spectra were measured on JNM-GX 400MNMR spectrometers. Chemical shifts were recorded in ppm relative to tetramethylsilane(TMS). FAB-MS spectra were recorded on a Zabspec mass spectrometer. Melting points were uncorrected.

Chemical Synthesis

N-(2-Boc-amino)ethyl-trans-4-hydroxy-L-proline ethyl ester **(3)**

Triethylamine (32 ml, 0.23 mol) was added dropwise to a stirred solution of compound **1** (24.61 g, 0.11 mol) and compound **2** (19.58 g, 0.10 mol) in anhydrous DMF (300 ml) at room temperature and the reaction mixture was left to stir overnight. The mixture was filtered and evaporated to dryness. The oily residue was dissolved in EtOAc (300 ml) and washed with a saturated solution of $NaHCO_3$ (100 ml) and brine (50 ml). Then the solution was acidified to pH 2 with 10% citric acid (aqueous). The water phase was neutralized to pH 7 with $NaHCO_3$ and extracted with EtOAc (3×150 ml). The combined extracts were dried over anhydrous $MgSO_4$ and then evaporated to dryness *in vacuum*. Compound **3** was obtained as yellow syrup (20.61 g, 68%).

^{1}HNMR($CDCl_3$) δ1.25(t, 3H), 1.41(s, 9H), 2.0–3.6(m, 10H), 4.15(q, 2H), 4.44(m, 1H). 5.26(s, 1H); MS(FAB): m/z 303.2$(M+H)^+$, 247.1$(M\text{-}tBu)^+$, 222.9$(M\text{-}tBuO)^+$, 203.1$(M\text{-}Boc)^+$; Anal. Calcd for $C_{14}H_{26}N_2O_5$: C, 55.16; H, 8.67; N,9.27. Found: C, 55.16; H, 8.85; N, 9.46.

N-(2-Boc-amino)ethyl-trans-4-methanesulfonyloxy-L-proline ethyl ester **(4)**

A solution of compound **3** (18.01 g, 60 mmol) and methanesulfonyl chloride (10.31 g, 90 mmol) in CH_2Cl_2 (500 ml) was cooled in an ice bath. Triethylamine (12.12 g, 120 mmol) was added dropwise. After the addition was complete, the reaction mixture was allowed to come to room temperature. The reaction was judged to be complete as TLC showed the absence of compound **3** (4:1 EtOAc/petroleum ether). The mixture was washed with water (100 ml) and 5% aqueous $NaHCO_3$ (100 ml). The aqueous layers were extracted with CH_2Cl_2(2×50 ml). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give 4 as yellow syrup (23 g, 100%). This material was used immediately without further purification.

^{1}HNMR($CDCl_3$) δ1.29(t, 3H), 1.45(s, 9H), 2.2–4.0(m, 10H), 4.15(q, 2H), 5.24–5.25(m, 2H); MS(FAB) m/z 381.1$(M+H)^+$, 323.1$(M-tBu)^+$, 307.1$(M-tBuO)^+$; Anal. Calcd for $C_{15}H_{28}N_2O_7S$: **C**, 47.36; H, 7.42; N, 7.36. Found: C, 47.11; H, 7.51; N, 7.35.

N-(2-Boc-amino)ethyl-cis-4-(thymin-1-yl)-L-proline ethyl ester **(5)**

A suspension of compound **4** (7.60 g, 20 mmol), thymine (3.78 g, 30 mmol), anhydrous K_2CO_3 (5.53 g, 40 mmol) and 18-crown-6 (10.56 g, 40 mmol) in DMF (250 ml) was stirred at 75°C for 36 hours. The solids in the reaction mixture were filtered and washed thoroughly with EtOAc. The combined filtrate was concentrated and the residue was purified by flash chromatography using 7:3 EtOAc/petroleum ether (60–90°C) as the eluting solvent. Compound **5** was obtained as yellow viscous oil (3.34 g, 40%). Crystallization from EtOAc gave the product as colorless crystals. m.p.:141–143°C.

^{1}HNMR($CDCl_3$) δ1.28(t, 3H), 1.48(s, 9H), 1.92(s, 3H), 1.8–3.4(m, 10H), 4.18(q, 2H), 5.22 (br, 1H), 7.92(s, 1H); 9.0(s, 1H); ^{13}CNMR($CDCl_3$) δ12.61, 14.10, 28.34, 36.66, 38.79, 51.98, 52.63, 58.03, 61.44, 64.80, 79.22, 111.30, 137.80, 151.35, 155.90, 164.02, 173.21; HRMS m/z 411.2234$(M+H)^+$, calcd. for $C_{19}H_{30}N_4O_6+H = 411.2238$; 311.1711$(M-Boc)^+$, calcd. for $C_{14}H_{22}N_4O_4+H = 311.1711$; Anal. Calcd for $C_{19}H_{30}N_4O_6$: C, 55.60; H, 7.37; N, 13.65. Found: C, 55.78; H, 7.46; N, 13.79.

N-(2-Boc-amino)ethyl-cis-4-(cytosin-1-yl)-L-proline ethyl ester **(7)**

Sodium hydride (60% disp., 3.00 g, 75 mmol) was added to a vigorously stirred suspension of cytosine (8.33 g, 75 mmol) in anhydrous DMF (280 ml) at 50°C. After hydrogen production had ceased, compound **4** (9.5 g, 25 mmol) was added and the reaction mixture was left to stir for a week. The solids in the mixture were filtered and washed thoroughly with EtOAc. The combined filtrate was concentrated and the residue was dissolved in EtOAc (100 ml) and filtered again to remove the undissolved solids. The filtrate was purified by flash chromatography using 8:2 EtOAc/EtOH as the eluting solvent. Compound **7** was obtained as white powder (4.0 g, 27.5%). m.p.: 142–144°C.

^{1}HNMR($CDCl_3$) δ1.29(t, 3H), 1.46(s, 9H), 1.9–3.4(m, 10H), 4.21(q, 2H), 5.14(br, 1H), 5.28 (m, 1H), 6.18(d, 1H), 8.22(d, 1H); ^{13}CNMR($CDCl_3$) δ14.85, 29.18, 37.72, 39.75, 53.71, 54.22, 59.28, 61.98, 65.89, 79.86, 95.84, 143.88, 156.75, 156.99, 165.83, 174.08; MS(FAB) m/z 791.5$(2M+H)^+$, 396.3$(M+H)^+$, 322.3$(M-tBuO)^+$, 296.3$(M-Boc)^+$; Anal. Calcd for $C_{18}H_{29}N_5O_5$: C, 54.67; H, 7.39; N, 17.71. Found: C, 54.78; H, 7.38; N, 17.84.

N-(2-Boc-amino)ethyl-cis-4-((N^4-benzoxycarbonyl)cytosine-1-yl)-L-proline ethyl ester **(8)**

A solution of benzoxycarbonyl chloride (3 ml, 17.4 mmol) in anhydrous pyridine (10 ml) was carefully added dropwise to a stirred solution of compound **7** (1.0 g, 2.5 mmol), DMAP (0.08 g, 0.62 mmol) in anhydrous pyridine

(50 ml) at 0°C. The reaction was allowed to warm slowly to room temperature before being left to stir overnight. Subsequently, the reaction was poured into a saturated solution of $NaHCO_3$ (6 ml) and extracted with EtOAc (3 × 50 ml). The combined organic extracts were evaporated to dryness *in vacuum*. The residue was purified by flash chromatography using ethyl acetate as the eluting solvent. Compound **8** was obtained as foam (1.03 g, 77%).

^{1}HNMR($CDCl_3$) δ1.26(t, 3H), 1.43(s, 9H), 1.9–3.4(m, 10H), 4.18(q, 2H), 5.10–5.36 (m, 3H), 7.35(s, 5H); 7.27(d,1H), 8.22(s, 1H); MS(FAB) *m/z* 530.2$(M+H)^+$, 472.1$(M-tBu)^+$, 456.1$(M-tBuO)^+$, 430.1$(M-Boc)^+$; Anal. Calcd. for $C_{26}H_{35}N_5O_7$: C, 58.97; H, 6.66; N, 13.22. Found: C, 58.97; H, 6.87; N, 13.11.

N-(2-Boc-amino)ethyl-cis-4-(adenin-9-yl)-L-proline ethyl ester **(10)**

Sodium hydrid (60%, 1.78 g, and 44.5 mmol) was added to a vigorously stirred suspension of adenine (6.01 g, 44.5 mmol) in anhydrous DMF (100 ml). The reaction mixture was stirred at 55–60°C for 3 hours. Then compound **4** (5.64 g, 14.8 mmol) was added and the reaction mixture was left to stir for 2 days. The solids in the mixture were filtered and washed thoroughly with EtOAc. The filtrate was evaporated to dryness and the resulting residue was purified by flash chromatography using 8:2 EtOAc/EtOH as the eluting solvent. Compound **10** was afforded as viscous oil (1.5 g, 24%).

^{1}HNMR($CDCl_3$) δ1.26(t, 3H), 1.43(s, 9H), 1.8–3.5(m, 10H), 4.19(q, 2H), 5.09(s, 1H), 5.28(m, 1H), 8.29(s, 1H), 8.60(s, 1H); MS(FAB) *m/z* 420.1$(M+H)^+$, 364.1$(M-tBu)^+$, 346.1$(M-tBuO)^+$, 320.1$(M-Boc)^+$; Anal. Calcd for $C_{19}H_{29}N_7O_4$: C, 54.40; H, 6.97; N, 23.37. Found: C, 54.35; H, 6.97; N, 23.20.

N-(2-Boc-amino)ethyl-cis-4-((N^6-dibenzoyl)adenin-9-yl)-L-proline ethyl ester **(11)**

A solution of benzoyl chloride (1.5 ml, 10 mmol) in anhydrous pyridine (5 ml) was carefully added dropwise to a stirred solution of compound 10 (1.05 g, 2.5 mmol) in anhydrous pyridine (5 ml) at 0°C. The reaction was allowed to warm slowly to room temperature before being left to stir overnight. Subsequently, the reaction mixture was poured into a cooled saturated solution of $NaHCO_3$ (20 ml) and extracted with ethyl ether (3 × 20 ml). The combined organic extracts were evaporated to dryness *in vacuum* to give a crude yellow oil which was purified by flash chromatography using 6:4 ethyl acetate/petroleum ether (60–90°C) as the eluting solvent. Compound **11** was obtained as foam (0.7 g, 53%).

^{1}HNMR($CDCl_3$) δ1.20(s, 3H), 1.36(s, 9H), 1.5–3.5(m, 10H), 4.19(q, 2H), 5.09(s, 1H), 5.32(s, 1H), 7.2–7.8(m, 10H), 8.55(s, 1H), 8.70(s, 1H); MS(FAB) m/z 628.1$(M+H)^+$, 524.0$(M-Bz)^+$, 343.9$(Bz_2A)^+$; Anal. Calcd for $C_{33}H_{37}N_7O_6$: C, 63.15; H, 5.94; N, 15.62. Found: C, 62.94; H, 5.90; N, 15.68.

N-(2-Boc-amino)ethyl-cis-4-((N^2-isobutyryl)guanin-9-yl)-L-proline ethyl ester **(13)**

A mixture of N^2-isobutyryl-guanine (2.76 g, 12.5 mmol), K_2CO_3 (1.73 g, 12.5 mmol), and compound **4** (1.90 g, 5.0 mmol) in DMF (30 ml) was stirred at 50°C for 3 days. The solvent was removed under reduced pressure and the residue was purified by flash chromatography with 1:2 acetone/petroleum ether (30–60°C) as the eluting solvent. The more polar fraction were combined and evaporated to give the title compound as foam (0.26 g, 10%).

^{1}HNMR(d_6-DMSO) δ1.10(s, 3H), 1.14(d, 6H), 1.35(s, 9H), 2.23(m, 1H), 2.0–4.4(m, 10H), 4.20(q, 2H), 4.92(d, 1H), 8.18(s, 1H); ^{13}CNMR(d_6-DMSO) δ13.8, 18.7, 28.1, 34.7, 36.2, 38.7, 51.9, 53.3, 58.3, 60.4, 64.4, 77.5, 119.6, 137.9, 147.7, 148.2, 154.9, 155.5, 172.6, 180.0; MS(FAB) m/z 506.3$(M+H)^+$, 406.2$(M-Boc)^+$, 222.1$(iBuG+H)^+$.

General Procedure for Preparing Monomer **6, 9, 12, 15**

In each case, a 2 M aqueous solution of sodium hydroxide (3.0 eq.) was added to a stirred solution of ester **5, 8, 11** or **14** (1 eq.) in methanol at room temperature. The reaction was judged to be complete as TLC showed the absence of the ester. The reaction mixture was then cooled to 0°C and acidified to pH5 with 1 M HCl (aqueous).

N-(2-Boc-amino)ethyl-cis-4-(thymine-1-yl)-L-proline **(6)**

The above procedure was followed using compound **5** (0.41 g, 1 mmol) as starting material. After acidification, the solution was evaporated to dryness and the residue was redissolved in ethanol (20 ml). The solid material was removed by filtration and the filtrate was purified by flash chromatography using 9:1 CH_2Cl_2/MeOH as the eluting solvent to give the product as foam (0.30 g, 79%).

^{1}HNMR(CD_3OD) δ1.43(s, 9H), 1.88(s, 3H), 1.8–4.0(m, 9H), 4.83(s, 1H), 7.67(s, 1H); MS(FAB) m/z 405.0$(M+Na)^+$, 383.0$(M+H)^+$.

N-(2-Boc-amino)ethyl-cis-4-((N^4-benzoxycarbonyl)cytosin-1-yl)-L-proline **(9)**

The above procedure was followed using compound **8** (1.05 g, 2 mmol) as starting material. After acidification, the solution was evaporated to

dryness and the residue was redissolved in methanol (5 ml). The solid material was removed by filtration and the filtrate was purified by flash chromatography using 9:1 CH_2Cl_2/MeOH as the eluting solvent to give the product as foam (0.55 g, 55%).

^{1}HNMR(CD_3OD) δ1.43(s, 9H), 1.9–3.4(m, 9H), 5.10–5.36(m, 4H), 7.35(s, 5H), 7.27(d, 1H), 8.22(s, 1H); MS(FAB) m/z 1002.8$(2M+H)^+$, 524.1$(M+Na)^+$, 502.2$(M+H)^+$.

N-(2-Boc-amino)ethyl-cis-4-((N^6-benzoyl)adenin-9-yl)-L-proline **(12)**

The above procedure was followed using compound **11** (2.62 g, 4.2 mmol) as starting material. After acidification, the solution was evaporated to dryness and the residue was redissolved in methanol (10 ml). The solid material was removed by filtration and the filtrate was purified by flash chromatography using 9:1 CH_2Cl_2/MeOH as the eluting solvent to give the product as foam (1.56 g, 75%).

^{1}HNMR(CD_3OD) δ1.37(s, 9H), 2.0–3.5(m, 9H), 4.91(s, 1H), 5.23(m, 1H), 7.90(s, 1H), 8.20(s, 5H), 8.77(s, 1H); MS(FAB) m/z 518.1$(M+Na)^+$, 496.1$(M+H)^+$, 396.1$(M-Boc)^+$.

N-(2-Boc-amino)ethyl-cis-4-((N^2-isobutyryl)guanin-9-yl)-L-proline **(15)**

The above procedure was followed using compound **13** (0.30 g, 0.59 mmol) as starting material. After acidification, the solution was evaporated to dryness and the residue was redissolved in methanol (2 ml). The solid material was removed by filtration and the filtrate was purified by flash chromatography using 1:1 CH_2Cl_2/MeOH as the eluting solvent to give the product as foam (0.18 g, 64%).

^{1}HNMR(d_3-DMSO) δ1.14(d, 6H), 1.35(s, 9H), 2.23(m, 1H), 2.0–4.4(m, 10H), 4.92(d, 1H), 8.18(s, 1H); ^{13}CNMR(d_3-DMSO) δ18.7, 28.8, 34.7, 37.2, 40.2, 53.4, 54.6, 59.8, 66.0, 80.2, 117.0, 138.6, 152.4, 152.7, 155.2, 158.9, 175.4, 180.0; MS(FAB) m/z 478.2 $(M+H)^+$.

Oligomerization (General Protocol)

The chiral PNA-oligomers were prepared by standard solid-phase peptide synthesis. The syntheses were initiated on a MBHA resin (0.55 mmol/g) from C terminal to N terminal. DCC was used as coupling reagent. The detailed procedure was as follows: 1) Washing the resin with CH_2Cl_2, MeOH, CH_2Cl_2, 2 × 2 min; 2) neutralizing with N,N-diisopropylethylamine (DIEA)/CH_2Cl_2 (8%, v/v), 2 × 5 min; 3) Washing with CH_2Cl_2, MeOH, CH_2Cl_2, 2 × 2 min; 4) Coupling with Boc-lys(2Cl-Cbz)-OH(3 eq.) or

PNA monomer(2 eq.), 1-hydroxybenzotriazole(HOBt)(4 eq.) and DCC(4 eq.), using DMF as solvent, 4–8 hours; 5) Washing with CH_2Cl_2, MeOH, CH_2Cl_2, 2×2 min; 6) Monitoring the coupling using Kaiser test, if negative, go on with next step; Otherwise capping the unreacted amino group with $Ac_2O/DIEA/CH_2Cl_2$ (3:1:30 , v/v/v), 1×20 min, washing with CH_2Cl_2, MeOH, CH_2Cl_2, 2×2 min; 7) Deprotecting Boc with TFA/CH_2Cl_2 (1:1, v/v), 1×5 min, 1×30 min. Steps 1–7 were repeated until the required sequence was obtained.

Stepwise Assembly of H-T_{10}-lys-NH_2

The synthesis was initiated on 100 mg MBHA resin. After the final coupling, the resin was dried under vacuum. The oligomer was cleaved from the support with HF for 1 hour at 0°C. After sufficient washing and lyophilisation, the obtained fluffy solid was purified by sephadex gel filtration: yield 45 mg crude PNA (purity: 44%, according to HPLC at 260 nm). A portion of the sample was purified by RP-HPLC purification to give the pure oligomer for further use. MS(TOF) m/z 2788.4, (calc.2788.0).

Stepwise Assembly of H-A_{12}-lys-NH_2

The synthesis was initiated on 100 mg MBHA resin. After the final coupling, the resin was treated with concentrated aqueous ammonia-methylamine (1:1) at room temperature for 2 hours to remove the protecting group on adenine. After cleavage from the solid support and purification by sephadex gel filtration, 35 mg crude PNA were obtained (purity: 41%, 260 nm). A portion of the sample was purified by RP-HPLC purification to give the pure oligomer for further use. MS(TOF) m/z 3424.0, (calc.3424.8).

Stepwise Assembly of H-TATAAATT-lys-NH_2

The synthesis was initiated on 50 mg MBHA resin. After deprotection, cleavage and sephadex gel filtration, 52 mg crude PNA were obtained (purity: 87%, 260 nm). A portion of the sample was purified by RP-HPLC purification to give the pure oligomer for further use. MS(TOF) m/z 2299.1, (calc.2294.5).

Stepwise Assembly of H-ATT CCT TCT TCG GGA A-lys-NH_2

The synthesis was initiated on 100 mg MBHA resin. After deprotection, cleavage and sephadex gel filtration, 79 mg crude PNA were obtained (purity: 34%, 260 nm). A portion of the sample was purified by RP-HPLC purification to give the pure oligomer for further use. MS(TOF) m/z 4416.6, (calc.4415.7).

Thermal Transition Measurement

UV-melting curves and wavelength scan were recorded using an UV-260 spectrophotometer. The oligomers concentration was determined by measuring the absorbance at 260 nm and assuming the following extinction coefficients: T = 8.5, A = 13.0, C = 7.5, G = 11.7 ml/μmol.cm. The two complementary strands were dissolved in a buffer containing 0.1 M NaCl, 0.02 M sodium cacodylate, pH 7.0. The solution was heated to 95°C for 10 minutes and then cooled slowly to 6°C. Absorbance vs. temperature curve was measured at a heating rate of 0.3°C/min. Tm value was determined as the temperature of the maximum in the first derivative of dissociation curves.

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