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Backbone extended pyrrolidine PNA (*bep*PNA): a chiral PNA for selective RNA recognition

T. Govindaraju and Vaijayanti A. Kumar*

Division of Organic Chemistry (Synthesis), National Chemical Laboratory, Pune 411008, Maharashtra, India

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Abstract—Synthesis of cationic, chiral PNA analogues with an extra atom in the backbone (*bep*PNA) is reported. The (2*S*,4*S*) geometry of the pyrrolidine ring, and an additional carbon atom in the backbone of homopyrimidine-*bep*PNAs resulted in the optimization of the inter-nucleobase distance, such that selective binding to complementary RNA over DNA was observed in the triplex mode. It was evident from circular dichroism studies that oligomers with mixed aminoethylglycyl–bep (*aeg–bep*) repeating units, and also *bep*PNA with homogeneous backbone attained structures quite different from those of *aeg*PNA$_2$:RNA/DNA complexes. The *bep*PNA, when incorporated in a duplex forming mixed purine–pyrimidine sequence, also showed a preference for binding complementary RNA over DNA.

1. Introduction

Synthetic oligonucleotides (Fig. 1, DNA/RNA **I**) have been considered as potential gene targeted therapeutic agents (antisense and antigene).[1] However, the unmodified oligonucleotides are rapidly identified and cleaved by the action of nucleases that hydrolyze the inter-nucleoside phosphodiester linkage of the backbone.[1] Among the known oligonucleotides analogues,[2,3] acyclic *N*-(2-aminoethyl)-glycyl peptide nucleic acids (Fig. 1, *aeg*PNA **II**), are found to be very good mimics of DNA/RNA.[3] Because of the higher thermal stability of PNA:DNA/RNA hybrids and their stability toward proteases and nucleases, PNA has generated interest in medicinal chemistry, having potential for the development as gene targeted drugs and as reagents in molecular biology and diagnostics.[3] The ability of the negatively charged DNA, as well as that of uncharged *aeg*PNA, to cross the cell membrane is poor. Additionally, PNA suffers from drawbacks such as poor aqueous solubility, cell permeability, and ambiguity in binding complementary DNA/RNA in both parallel and antiparallel orientations. To overcome these obstacles and to facilitate their use as antisense therapeutic agents in biological systems, several rational modifications have been reported to-date.[4] Editing at the molecular level of designed backbones is translated in imparting selectivity of binding with DNA or RNA, due to the intrinsic structural differences at duplex/triplex level of the natural nucleic acids.[5] Neutral

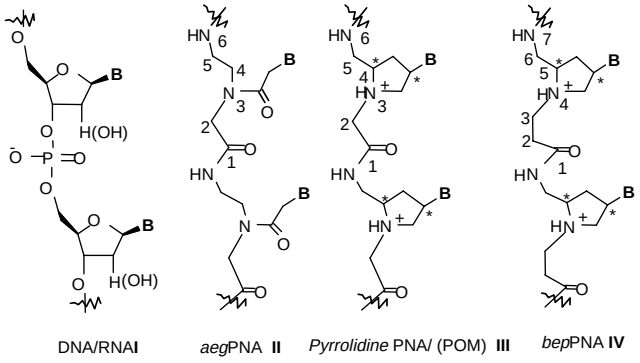

Figure 1. Structure of DNA, PNA, and modified PNAs.

pyrrolidinone and cationic pyrrolidine PNAs (Fig. 1, **III**) belong to this class of modifications. (3*S*,5*R*)-Pyrrolidinone PNA destabilized both DNA and RNA complexes[6] whereas *aepone*-PNA (2*S*,4*S*) stabilized complex with DNA over RNA.[7] A pentameric (2*R*,4*R*) pyrrolidine-amide oligonucleotide mimic (Fig. 1, POM **III**), showed kinetic binding preference to RNA over DNA.[8] Pyrrolidine PNA (Fig. 1, **III**) having one (2*R*,4*S*)-modified unit, showed destabilization with DNA and RNA, but bound strongly when fully modified.[9] (2*S*,4*S*)-Pyrrolidine PNA destabilized complexation with both DNA and RNA.[10] Previous studies on DNA/RNA analogues have shown that the length of the linkage in the repetitive DNA/RNA backbone can be varied from five to seven.[11] An example of the five-bond contracted backbone is TNA, an extraordinary oligonucleotide system introduced by Eschenmoser et al. TNA cross-paired

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* Corresponding author. Tel./fax: +91 20 25893153; e-mail: va.kumar@ncl.res.in

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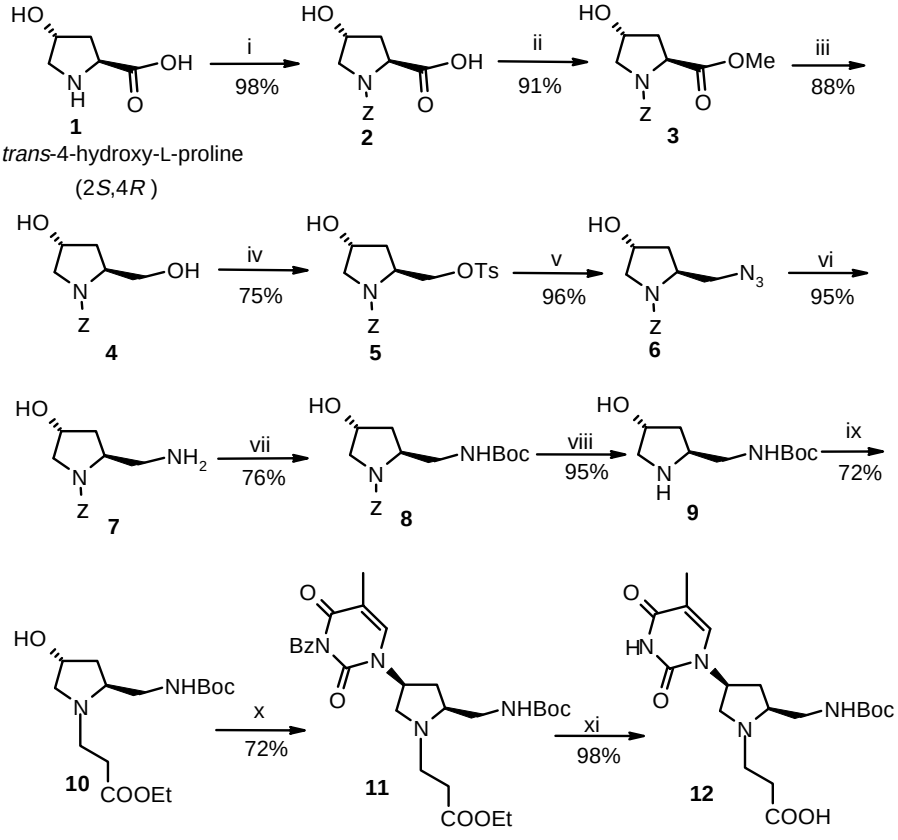
efficiently with complementary DNA and RNA, and showed relatively high affinity to RNA over DNA.[11] Matsuda et al. studied oligonucleotides containing oxetanocin A,[12] an isomer of deoxyadenosine with an oxetane sugar moiety, and a higher analogue. These analogues with extended seven-bond backbones cross-pair with RNA with high affinity compared to DNA. A carbocyclic analogue of the oxetanocin A oligonucleotide system also showed a tendency to form stronger complexes with RNA rather than with deoxyribonucleotide.[13] Many examples in the literature suggest that a five-atom amide leading to a seven-atom repeating backbone may be more useful, because of the reduced conformational flexibility of the amide relative to phosphodiester backbone.[14] This postulate has been supported by X-ray studies. X-ray data confirms that the amide backbone has a trans conformation, and that the distance between neighboring base pairs is not affected by incorporation of a longer backbone.[14a,e] On the contrary, Lowe et al. very effectively accomplished preferential DNA binding by replacing glycine with one-atom extended β-amino acids in his prolyl-glycyl PNA analogues.[15] Our preliminary results on the chimeric phosphate-amide extended backbone revealed that 2*R*,4*R* pyrrolidine–amide chimera were accommodated better in triplex forming sequences.[16]

In view of the above reports, we recently presented preliminary results on the backbone extended pyrrolidine PNA (Fig. 1, *bep*PNA **IV**).[17] In this paper, we present detailed studies on the hybridization properties of *bep*PNA using UV–T_m measurements, gel electrophoretic shift assay, and circular dichroism analysis of the *bep*PNA hybrids that address binding properties in both triplex and duplex modes, using either homopyrimidine or mixed purine–pyrimidine base sequences, respectively.

1.1. Synthesis of *bep*PNA monomer [(2*S*,4*S*)-2-(*tert*-butyloxycarbonylaminomethyl)-4-(thymin-1-yl) pyrolidin-1-yl] propanoic acid

The ring nitrogen of the naturally occurring *trans*-4-hydroxy-L-proline **1** was protected as the carbamate to give compound **2**, which was then converted into its methyl ester **3** on treatment with MeOH/$SOCl_2$.[12] Methyl ester **3** was reduced using LiCl/$NaBH_4$ to yield diol **4** (Scheme 1). Selective tosylation of the primary hydroxy group by controlled dropwise addition of *p*-TsCl (freshly crystallized from chloroform and petroleum ether) in pyridine gave monotosylate **5** (some ditosylate formed was removed by column chromatography). The monotosylate **5** was treated with NaN_3 to obtain azido compound **6**, and selective reduction of azide functionality using Raney Ni in methanol gave free amine **7**. Protection of free amine **7** using $BocN_3$ in DMSO yielded compound **8**. Compound **8** was subjected to hydrogenation using Pd–C catalyst to remove the benzyloxycarbonyl group to get free amine **9**. The free ring nitrogen was subsequently alkylated using ethyl acrylate in methanol as solvent, giving alkylated product **10**.



Scheme 1. Reagents and conditions: (i) Z–Cl (50% toluene soln), Et_3N, $NaHCO_3$, water, rt, 8 h; (ii) $SOCl_2$, Et_3N, MeOH, rt, 7 h; (iii) LiCl, $NaBH_4$, ethanol–THF (4/3), rt, 7 h; (iv) TsCl, pyridine, rt, 7 h; (v) NaN_3, DMF, 70 °C, 8 h; (vi) Raney Ni, H_2, MeOH, 35 psi, rt, 3 h; (vii) $BocN_3$, DMSO, 50 °C, 5 h; (viii) H_2/Pd–C, MeOH, 60 psi, rt, 7 h; (ix) ethyl acrylate, MeOH, rt, 2.5 h; (x) *N*3-benzoylthymine, DEAD, PPh_3, benzene, rt, 4 h; (xi) 2 M aqueous NaOH, rt, 5 h, Dowex-H^+.

No external base was required, as the amine **8** itself acted as a base, and the inclusion of an extra atom in the backbone was easily achieved by using conjugate addition to ethyl acrylate as compared to cumbersome methods in extended sugar-phosphate backbone.[14] Secondary alcohol **10** was converted to protected monomer ethyl ester **11** on treating with *N*3-benzoyl thymine under Mitsunobu conditions.[18] Hydrolysis of ethyl ester **11** and simultaneous deprotection of *N*3 of thymine was achieved using 2 M NaOH in aqueous methanol. The aqueous layer was washed with DCM to remove benzoic acid, and was then neutralized with a cation exchange resin (Dowex-H^+) by careful acidification of the reaction mixture to pH 7.0. The neutral aqueous layer was filtered and concentrated under reduced pressure to yield required monomer [(2*S*,4*S*)-2-(*tert*-butoxycarbonylamino-methyl)-4-(thymin-1-yl) pyrolidin-1-yl] propanoic acid (**12**) in good yield (Scheme 1). All new compounds were characterized by NMR, mass spectroscopy and elemental analysis. One-step conversion of the 4-OH in **10** to the corresponding thymin-1-yl derivative with an inversion of configuration can be achieved under Mitsunobu conditions. As the reactivity of *N*1 and *N*3 of thymine are comparable towards alkylation, this reaction results in both *N*1-alkylated and *N*1,*N*3-dialkylated products. Hence, *N*3 of the thymine was first protected as its benzoyl derivative.[19] The aminoethylglycyl PNA (A/G/C/T) monomers were synthesized following the literature procedure,[20] and these monomers were used for the synthesis of *aeg*PNA T_8 octamer (**13**) and *aeg*PNA **19** mixed decamer for the control studies and to synthesize *bep*PNA–*aeg*PNA chimeras.

1.2. Determination of the pK_a of the pyrrolidine ring nitrogen (*N*1) of the *bep*PNA thymine monomer 12

The *bep*PNA monomer has a tertiary amino group in the pyrrolidine ring that can be protonated. pH titration experiment was carried out with NaOH to determine the approximate pK_a of this nitrogen atom. A plot of pH versus volume of NaOH-added gave three transitions; the first one corresponding to the carboxylic acid, second corresponding to the tertiary ring nitrogen. The third transition could correspond to the free amine group of the deprotected monomer and the N3–H of thymine (Fig. 2). This transition is not very well resolved under the present conditions. The Boc protected monomer **12** was deprotected using trifluoro-acetic acid, and the product titrated against 0.2 M NaOH to find out the approximate pK_a value of pyrrolidine ring nitrogen. The pK_a values for free –COOH was found to be in the range of 3–4 and those of $-NH_2$ and N3–H of thymine overlapped at ~10. The pK_a of the ring nitrogen was found to be ~7.7 (Fig. 2). The pyrrolidine ring nitrogen in the monomer could partially be protonated under physiological conditions (pH). This pK_a (7.7) of the ring nitrogen is higher than any other pyrrolidine PNAs, and can be attributed to the fact that the additional methylene group in the backbone increases the basic character of the ring nitrogen.

1.3. Synthesis of cationic backbone extended pyrrolidine peptide nucleic acids (*bep*PNA) and UV-melting studies of *bep*PNA:DNA/RNA complexes

The modified *cis*-(2*S*,4*S*)-*bep*PNA thymine monomer **12** was incorporated into PNA sequences using Boc-chemistry on L-lysine-derivatized (4-methylbenzhydryl)amine (MBHA) resin as reported before, using HBTU/HOBt/DIEA in DMF as the coupling reagent. Various homo-thymine PNA oligomers (**13**–**18**, Tables 1 and 2) incorporating modified monomers at the middle (**15**), N-terminus (**16**), C-terminus (**14**), at alternative positions (**17**), and through the entire sequence (homo-oligomer **18**) were synthesized in order to study their triplex formation and stability with DNA/RNA. Octamer *aeg*PNA T_8 (**13**) sequence was also synthesized, incorporating *aeg*PNA thymine monomer for the use of control studies. In order to study the duplex formation potential, and in particular DNA/RNA discrimination of the *bep*PNA monomeric units, it was imperative to synthesize mixed sequences, incorporating both purines and pyrimidines. Mixed sequences *aeg*PNA **19** and *bep*PNA **20** were also synthesized by incorporating *aeg*PNA (A/G/C/T) monomers and *bep*PNA (T) monomers (Table 3). The oligomers were cleaved from the resin using a 'low–high TFA–TFMSA' procedure,[21] followed by RP-HPLC purification and characterization by mass spectrometry (LC-TOF-MS). The complementary DNA oligonucleotides **21**, **23**, **24** were synthesized on Applied Biosystems ABI 3900 High Throughput DNA Synthesizer using standard β-cyanoethyl phosphoramidite chemistry. The oligomers were synthesized in the 3′–5′ direction on polystyrene solid support, followed by ammonia treatment.[22] The oligonucleotides were desalted by gel filtration, their purity ascertained by RP-HPLC on a C18 column to be more than 98%, and were used without further purification in the biophysical studies of PNAs. The RNA oligonucleotides **22**, **25**, and **26** were obtained commercially.

The T_m values of homopyrimidine PNAs **13**–**18**, hybridized with complementary DNA and RNA were obtained from

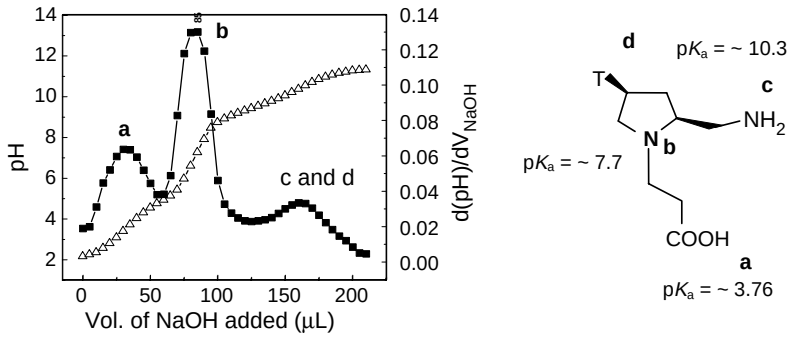


Figure 2. pH titration curve of (2*S*,4*S*)-*bep*PNA monomer (free amine) with NaOH (empty triangles) and change in pH with volume of NaOH (filled squares).

Table 1. HPLC and mass spectral analysis of synthesized PNAs

Entry	PNA	HPLC (RT, in min)	M_W (calcd)	M_W (found)[a]
1	*aeg*PNA **13**	7.536	2274.00 ($C_{96}H_{132}N_{35}O_{32}$)	2275.90
2	*bep*PNA **14**	7.596	2286.00 ($C_{96}H_{132}N_{35}O_{32}$)	2288.00
3	*bep*PNA **15**	7.270	2286.00 ($C_{96}H_{132}N_{35}O_{32}$)	2288.00
4	*bep*PNA **16**	7.359	2286.00 ($C_{96}H_{132}N_{35}O_{32}$)	2288.04
5	*bep*PNA **17**	9.562	2323.00 ($C_{102}H_{144}N_{35}O_{29}$)	2326.06
6	*bep*PNA **18**	8.246	2370.00 ($C_{110}H_{160}N_{35}O_{25}$)	2374.43
7	*aeg*PNA **19**	10.505	2852.00 ($C_{114}H_{147}N_{60}O_{31}$)	2853.00
8	*bep*PNA **20**	7.852	2887.00 ($C_{120}H_{159}N_{60}O_{28}$)	2891.36

[a] LC-TOF-MS, RT=retention time.

Table 2. Melting temperatures (T_m) of PNA_2:DNA/RNA triplexes[a]

No.	Homopyrimidine PNA sequence	DNA	RNA
1	**13**, H-TTTTTTTT-$LysNH_2$	51.5	65.8
2	**14**, H-TTTTTTT**t**-$LysNH_2$	49.0	59.9
3	**15**, H-TTT**t**TTTT-$LysNH_2$	nd	59.2
4	**16**, H-**t**TTTTTTT-$LysNH_2$	53.0	59.0
5	**17**, H-T**t**T**t**T**t**T**t**-$LysNH_2$	nd	84.4
6	**18**, H-**tttttttt**-$LysNH_2$	nd	58.9

[a] T_m=melting temperature (measured in buffer 10 mM sodium phosphate, pH 7.0 with 100 mM NaCl and 0.1 mM EDTA). Measured from 10 to 90 °C at ramp 0.2 °C/min. UV-absorbance measured at 260 nm. All values are an average of three independent experiments and accurate to within ±0.5 °C. DNA **21**=dCGCA_8CGC; RNA **22**=poly rA; nd=not detected.

temperature dependent UV-absorbance data (Fig. 4, Table 2). The UV and CD Job's plots[23] suggest the formation of 2:1 *bep*PNA_2/DNA and *bep*PNA_2/RNA triplexes (Fig. 3) and hence all the complementation studies were performed with 2:1 PNA/DNA stoichiometry. The UV–T_m values were obtained from the first derivatives of the normalized absorbance-temperature plots of the corresponding PNA:DNA complexes (Fig. 4A, Table 2). The C-terminal modified *bep*PNA **14** binds to DNA with slight decrease in T_m ($\Delta T_m=-1$ °C) where as *bep*PNA **16** modified at N-terminal stabilizes the complex ($\Delta T_m=+2$ °C) compare to the control *aeg*PNA **13**. Surprisingly, *bep*PNA **15**, with a modified unit at the center did not show any complexation with DNA. Alternate and homooligomeric *bep*PNAs (**17** and **18**) also did not form

Table 3. UV–T_m (°C) of PNA:DNA/RNA duplexes[a]

Mix PNA sequence	DNA **23**	RNA **25**
19, H-GTAGATCACT-$LysNH_2$	55.0 (40.0)[b]	55.4
20, H-G**t**AGA**t**CAC**t**-$LysNH_2$	nd	81.0 (74)

[a] T_m=melting temperature (measured in buffer 10 mM sodium phosphate, pH 7.0 with 100 mM NaCl and 0.1 mM EDTA). Measured from 10 to 90 °C at ramp 0.2 °C/min. UV-absorbance measured at 260 nm. DNA **23**=5′AGTGATCTAC (ap); DNA **24**, 5′-CATCTAGTGA-3′(p); RNA **25**=5′ AGUGAUCUAC (ap); RNA **26**, 5′-CAUCUAGUGA-3′ (p).

[b] Measured by CD to avoid interference from thermal transitions of single stranded PNAs. nd=not detected. Values in brackets are T_m for parallel duplexes with DNA **24** and RNA **26**.

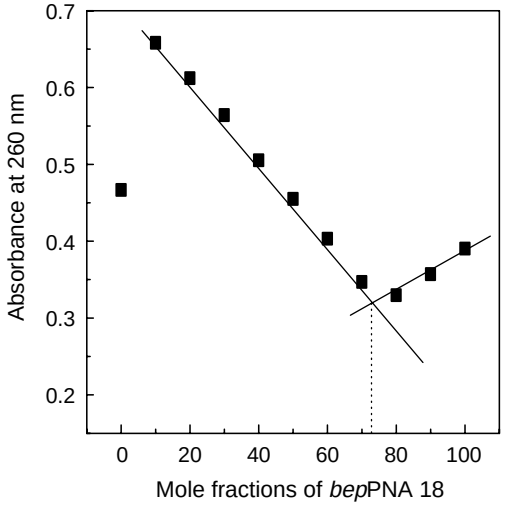


Figure 3. UV–Job's plot for *bep*PNA **18** and the complementary RNA (poly rA) mixtures in the molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 at 260 nm (buffer, 10 mM sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA).

complex with DNA as shown in UV-melting curves. When the complexation studies were performed with RNA, chimeric PNAs with a single *bep*PNA unit were found to bind with approximately same T_m, but slightly lower than that of control *aeg*PNA **13**. *bep*PNA **17**, with alternating *aeg–bep* units exhibiting a very high binding affinity ($\Delta T_m=+4.5$ °C/mod) (Fig. 4B, Table 2). The observed transitions were very sharp with RNA compared to that with DNA. The sequence **18** comprised only of a *bep*PNA backbone also recognized only RNA, but with reduced strength compared to the alternating *aeg–bep*PNA. These results suggest that *bep*PNA monomer in chimeric and homooligomeric PNAs introduced binding selectivity for RNA over DNA. Incorporation of the modified units at the terminals (C-/N-) seems to exert very weak effective preorganized conformation, and allowed binding with DNA as well as RNA. When in the center of the sequence, the induced conformation allows recognition of RNA but that of DNA is suppressed. The high affinity binding of alternating *aeg–bep*PNA **17** with RNA suggests that the alternating *aeg–bep* units are uniformly spaced, such that a balanced optimum conformation may be reached for recognition of RNA. The fully modified backbone in **18** binds to RNA but with reduced strength compared with the alternating sequence **17**. This could be because of over-organization of single strand as suggested for fully modified LNA,[14b,d] or high positive charge concentration of two *bep*-homooligomers in 2:1 binding mode. The 2:1 binding stoichiometry for:**18**:RNA was confirmed by UV–Job's plot (Fig. 3). The charge–charge repulsions could therefore be the possible reason for the observed reduced T_m. To study the RNA binding selectivity of *bep*PNA in duplex formation, purine–pyrimidine-mix *bep*PNA decamer **20** was synthesized incorporating the *bep*PNA-T monomer at three thymine positions in control *aeg*PNA **19**. The UV and CD-thermal denaturation[24] studies of *bep*PNA **20** with DNA and RNA were carried out (Fig. 5 and Supplementary information). Data in Table 3 shows that the reference *aeg*PNA **19** forms ap-duplexes with complementary DNA **23** and RNA **25** with equal stability. Mixed *bep*PNA **20** did

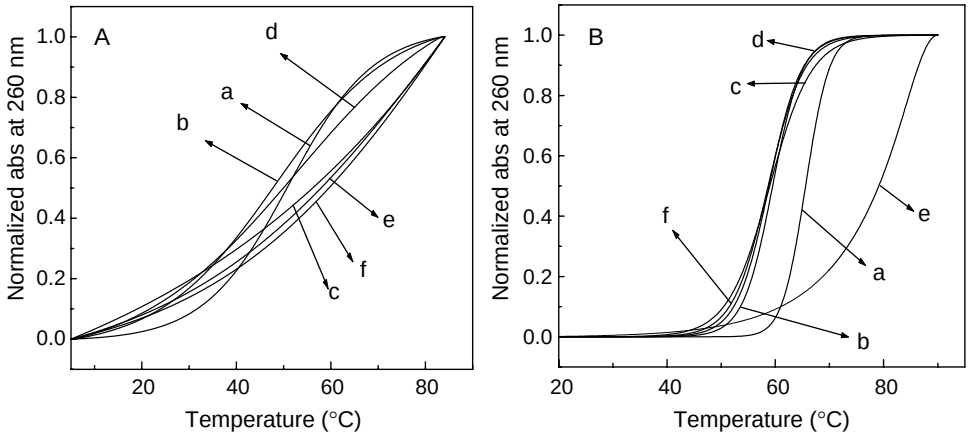


Figure 4. UV–T_m curves of A. (a) *aeg*PNA **13**, (b) *bep*PNA **14**, (c) *bep*PNA **15**, (d) *bep*PNA **16**, (e) *bep*PNA **17** and (f) *bep*PNA **18** with DNA **21**. B. (a) *aeg*PNA **13**, (b) *bep*PNA **14**, (c) *bep*PNA **15**, (d) *bep*PNA **16**, (e) *bep*PNA **17** and (f) *bep*PNA **18** with RNA **22** (buffer, 10 mM sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA).

not bind to DNA (antiparallel and parallel), as there was no transition detected in the UV melting experiment, whereas high affinity binding was observed with RNA (antiparallel, $\Delta T_m = 26\ ^\circ C$). Destabilization of ($\Delta T_m \sim 7\ ^\circ C$) was observed for the parallel *bep*PNA **20**:RNA duplex indicating the preference for antiparallel mode of binding.

1.4. CD spectroscopic studies of *bep*PNA:DNA and *bep*PNA:RNA complexes

Achiral *aeg*PNAs show very weak CD signatures due to the presence of chiral linker amino acid L-lysine. However, PNA:DNA complexes exhibit characteristic CD signatures due to chirality induced by the DNA component. It is known that the formation of PNA_2:DNA triplexes[25] accompanied by the appearance of positive CD bands at 260 and 285 nm that are not present in DNA (Supplementary information). Unlike *aeg*PNA **13**, the single stranded *bep*PNAs (**14**–**18**), showed distinct CD patterns depending on the number and position of modified units present (Supplementary information). Alternating *aeg*–*bep*PNA **17** showed a positive lower intensity bands at 245 nm, high intensity band at 260 nm and negative intensity bands at around 225 and 275 nm region (Fig. 6B, a). The fully modified *bep*PNA homo-oligomer (**18**) gave a CD signature with a maximum intensity positive band at around 235 nm, and a negative intensity band at 265 nm (Fig. 6B, c). Interestingly, the CD signature of the *bep*PNA **18** is more pronounced than that of DNA **21** (Supplementary information). As expected, the C- and N-terminus modified *bep*PNAs **14** and **16** with DNA showed CD signatures similar to the control *aeg*PNA_2:DNA triplex (Fig. 6A, a and c), whereas the CD signature of *bep*PNA **15** with DNA showed broad band from 260–285 nm (Fig. 6A, b) due to weak binding interaction as shown by gel electrophoresis assay. CD patterns of the alternating *aeg*–*bep*PNA **17** and *bep*PNA **18** with DNA **21** (Fig. 6A, d and e) were found to be additive spectra of corresponding CD signals of single stranded *bep*PNA and DNA **21**. Subtraction of the CD spectra of single stranded *bep*PNA **17** and **18** from the CD spectra of [*bep*PNA **17**+DNA **21**] and [*bep*PNA **18**+DNA **21**], respectively, gave the CD spectrum corresponding to single strand DNA **21**(Fig. 6B, b and d). These CD results are in complete agreement with the results obtained by UV measurements and gel shift assay.[17] CD spectra of *bep*PNA–poly rA complexes were recorded to study the structural changes after complexation in comparison with control *aeg*PNA **13**:poly rA complex (Fig. 6C, a). The CD signatures of singly modified *bep*PNAs **14**, **15**, and **16** with poly rA (Fig. 6C, b–d, respectively) gave different pattern compared to the control *aeg*PNA **13**–poly rA that was similar to (*aeg*PNA **13**)$_2$–DNA **21** triplex; CD profile with two characteristic bands at 260 and 285 nm. As expected from the UV–T_m data of *aeg*–*bep*PNA **17** and *bep*PNA **18**, the CD patterns were quite similar to each other as well as to that of control *aeg*PNA **13**–poly rA (Fig. 6C, e and f and a, respectively). The *aeg*–*bep*PNA–poly rA complexation was accompanied by the appearance of strong positive bands at 225 and 265 nm and a low negative intensity band at 245 nm compare to control with slight similarity. However, *bep*PNA **18**–poly rA showed presence of single strand *bep*PNA **18** and strong positive band at 235 nm was also observed. Thus, the CD spectral studies also demonstrated RNA selectivity of *bep*PNAs.

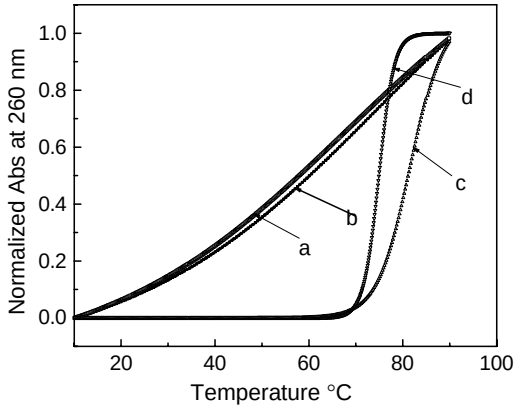


Figure 5. Melting curves of *bep*PNA **20** with A: (a) DNA **23** (antiparallel), (b) DNA **24** (parallel), (c) RNA **25** (antiparallel), and (d) RNA **26** (parallel).

1.5. Electrophoretic gel shift assay

An electrophoretic gel shift experiment was carried out to prove the results obtained from UV-thermal denaturation studies of *bep*PNAs with complementary DNA **21**

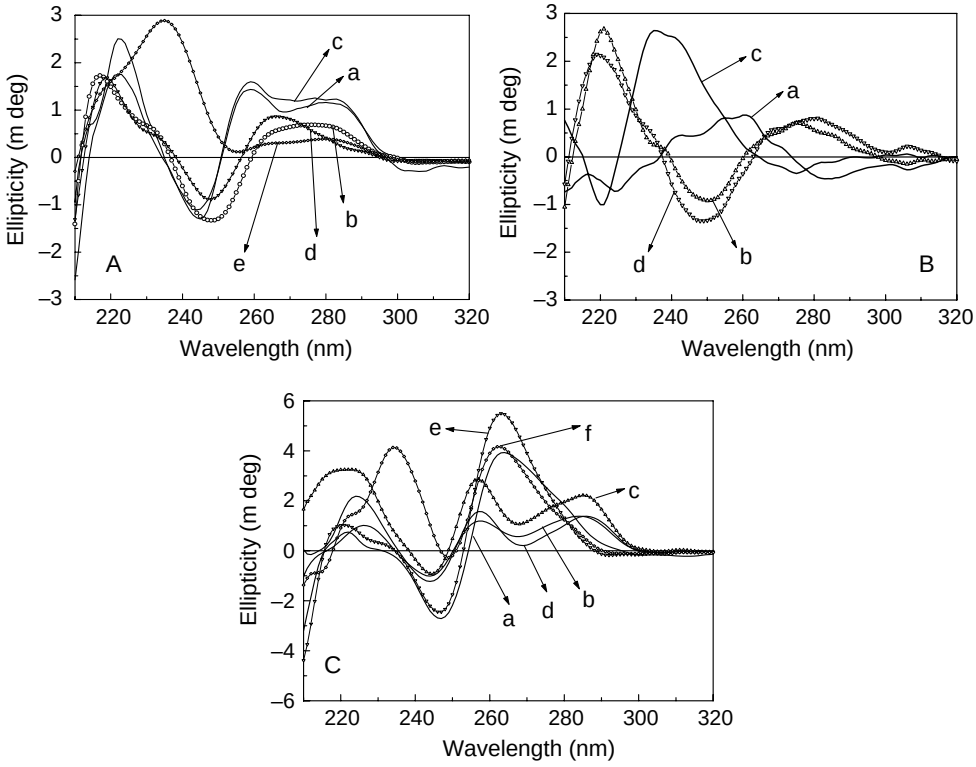


Figure 6. A. CD spectra of PNA_2:DNA (a) *bep*PNA **14**, (b) *bep*PNA **15**, (c) *bep*PNA **16**, (d) *bep*PNA **17** and (e) *bep*PNA **18** with DNA **21**; B. CD spectra of single stranded (a) *bep*PNA **17** and (c) *bep*PNA **18**; subtraction spectra (b) [*bep*PNA **17**:DNA **21**–*bep*PNA **17**] and (d) [*bep*PNA **18**:DNA **21**–*bep*PNA **18**]; C. CD spectra of PNA_2:RNA (a) *aeg*PNA **13**, (b) *bep*PNA **14**, (c) *bep*PNA **15**, (d) *bep*PNA **16**, (e) *bep*PNA **17** and (f) *bep*PNA **18** with RNA **22** (buffer, 10 mM sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA).

(Supplementary information). The various PNAs were individually mixed with DNA **21** in buffer, and subjected to non-denaturing gel electrophoresis[26] at 10 °C. The bands were visualized on a fluorescent TLC background. The formation of a PNA:DNA complex was accompanied by disappearance of the band due to single stranded DNA **21** and appearance of a lower migrating band of complex. The terminally modified *bep*PNAs **14** and **16**, upon mixing with DNA migrated about the same as the control *aeg*PNA **13**:DNA complex (Fig. SI, lane 1) and much lower than that of DNA (Fig. SI, lanes 5 and 7). The *bep*PNA **15** exhibited very weak binding interaction at lower temperature, though it was not seen during UV–T_m thermal denaturation (Fig. SI, lane 6). Under the conditions used, the single stranded PNAs carrying positive charge do not migrate from the well. No complexation was observed in case of alternating *aeg*–*bep*PNA **17** and *bep*PNA **18** with DNA as it can be seen from faster moving band due to unbound single strand DNA and hence clearly support the data obtained from UV–T_m experiments.

2. Summary

We have reported the design and synthesis of a novel class of cationic pyrrolidine PNAs with extended backbone that show improved binding affinity and selectivity towards DNA/RNA recognition. The complementation studies with DNA/RNA reveal that these *bep*PNAs bring in unprecedented RNA binding selectivity in triplex as well as duplex modes. From the application perspective, this chiral, cationic PNA analogue is shown to have very important properties essential for development into a therapeutic drug. Further studies on the mixed purine–pyrimidine sequences with *bep*PNA A/G/C/T units and other diastereomeric *bep*PNAs are underway.

3. Experimental

3.1. General experimental and spectroscopic data

Melting points of samples were determined in open capillary tubes using Buchi Melting point B-540 apparatus and are uncorrected. IR spectra were recorded on an infrared Fourier Transform spectrophotometer using KBr pellets. Column chromatographic separations were performed using silica gel 60–120 mesh, solvent systems gradient EtOAc/pet ether and pure DCM to 3% MeOH/DCM. ^1H and ^{13}C spectra were obtained using Bruker AC-200 (200 MHz) and 500 MHz NMR spectrometers. The chemical shifts are reported in delta (δ) values. The optical rotation values were measured on Bellingham-Stanley Ltd, ADP220 polarimeter. CD spectra were recorded on JASCO-715 Spectropolarimeter. Mass spectra were obtained either by LCMS techniques or by LC-TOF-MS mass spectrometry. Oligomers were characterized by RP-HPLC, C18 column and LC-TOF-MS mass spectrometry.

3.1.1. (2*S*,4*R*)-*N*1-(Benzyloxycarbonyl)-4-hydroxy-2-(hydroxymethyl)-pyrrolidine (4). To an ice cooled solvent mixture of dry THF (175 ml) and absolute ethanol (250 ml) containing $NaBH_4$ (3.192 g, 84.3 mmol) in a three-necked flask, LiCl (3.58 g, 84.3 mmol) was added slowly from a

solid addition funnel over 30 min. The above solution was stirred for 1.0 h and the appearance of a milky solution indicates the formation of $LiBH_4$ in situ. To the above ice-cooled milky solution, (2*S*,4*R*)-*N*1-(benzyloxycarbonyl)-4-hydroxyproline methyl ester (**3**) (9.45 g, 33.75 mmol) dissolved in absolute ethanol (50 ml) was added from a dropping funnel over a period of 30 min under nitrogen atmosphere, and the reaction mixture was stirred over night at rt. Then the pH of the reaction mixture was adjusted to 7.0 by adding saturated NH_4Cl. The solvent mixture was removed under vacuum and the residue was extracted into ethyl acetate (25 ml×3). The organic layer was washed with water, brine solution, dried over anhydrous Na_2SO_4 and concentrated to afford oily product diol **4** (7.5 g, yield 88%, R_f=0.3, ethyl acetate/petroleum ether-1:1). $[\alpha]_D^{20}$ +16.8 (*c* 3.26, CH_2Cl_2); IR (neat) (ν) cm^{-1}. 3410, 3021, 1699, 1470, 1415. ^1H NMR ($CHCl_3$-*d*, 200 MHz); δ: 1.55–1.8 (m, 1H), 1.9–2.2 (m, 1H), 3.25–3.8 (m, 4H), 3.8–4.25 (m, 2H), 4.25–4.5 (br d, 1H), 5.12 (s, 2H), 7.4 (s, 5H). ^{13}C NMR ($CHCl_3$-*d*, 200 MHz); δ: 37.1, 55.4, 58.9, 65.5, 87.2, 89.0, 127.7, 128.3, 136.2, 159.5. Anal. Calcd (%) for $C_{13}H_{17}NO_4$: C, 62.15; H, 10.75; N, 5.57. Found C, 61.83; H, 10.91; N, 5.53; LCMS; 252.07 $[M+1]^+$.

3.1.2. (2*S*,4*R*)-*N*1-(Benzyloxycarbonyl)-4-hydroxy-2-(*p*-toluenesulfonyloxymethyl)-pyrrolidine (5). The diol **4** (7.12 g, 28.36 mmol) was dissolved in dry pyridine (200 ml) and cooled to 0 °C. To this ice cooled solution, freshly crystallized *p*-toluenesulfonyl chloride (5.95 g, 31.2 mmol) in pyridine was added from a dropping funnel over a period of 1.5 h under nitrogen atmosphere. The reaction mixture was stirred for 8 h at rt. Pyridine was removed under reduced pressure and co-evaporated with toluene (twice). The residue was extracted into ethyl acetate (50 ml×2), washed with water, dried over Na_2SO_4 and concentrated to yield crude oily residue. The residue was purfied by column chromatography to get monotosylate **5** as a thick oil. (8.6 g, yield 75%, R_f=0.36, ethyl acetate/petroleum ether-1:1). $[\alpha]_D^{20}$ +27.7 (*c* 4.43, CH_2Cl_2); IR (neat) (ν) cm^{-1}. 3011, 1699,1550, 1500, 1470, 1415. ^1H NMR ($CHCl_3$-*d*, 200 MHz); δ:1.7–2.15 (2H, C2H, C3H), 2.2–2.5 (s, 3H, OCH_3), 3.1–3.6 (m, 2H, C5H_2), 3.7–4.2 (m, 3H, CH_3), 4.25–4.65 (m, 2H, CH_2-OTs), 4.7–5.5 (br d, 4H, C4H, $COOCH_2$, OH), 7.26–7.4 (s, 7H, C_6H_5, two CH-Ts), 7.5–7.8 (m, 2H, two CH-Ts). ^{13}C NMR ($CHCl_3$-*d*, 200 MHz); δ: 21.0, 35.9, 54.5, 54.8, 66.4, 68.7, 69.8, 127.3, 128.0, 129.5, 132.3, 136.0, 144.5, 154.6. Anal. Calcd (%) for $C_{20}H_{23}NO_6S$: C, 59.25; H, 5.67; N, 3.45; S, 7.90. Found C, 58.92; H, 5.77; N, 3.37; S, 7.67; MS LCMS; 405.00 $[M]^+$.

3.1.3. (2*S*,4*R*)-*N*1-(Benzyloxycarbonyl)-4-hydroxy-2-(azidomethyl)-pyrrolidine (6). To the solution of monotosylate **5** (6.0 g, 14.8 mmol) in DMF (50 ml), NaN_3 (7.7 g, 118.4 mmol) was added. The reaction mixture was stirred at 55 °C for 8 h. The solvent was removed under reduced pressure and the residue was extracted into ethyl acetate (25 ml×3). The combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and then concentrated to yield azide **6** as thick oil. (3.9 g, yield 96%, R_f=ethyl acetate/petroleum ether-1:1). $[\alpha]_D^{20}$ +19.3 (*c* 2.15, CH_2Cl_2); IR (neat) (ν) cm^{-1}. 3016, 2360, 2106, 1697, 1419. ^1H NMR ($CHCl_3$-*d*, 200 MHz); δ: 1.95–2.02 (m, 2H, C3H), 3.07–3.85 (m, 4H, 2×C5H, CH_2N_3, OH), 3.97–4.45 (m, 2H, C2H, C4H), 5.09 (s, 2H, OCH_2), 7.31 (s, 5H, C_6H_5). ^{13}C NMR ($CHCl_3$-*d*, 200 MHz); δ: 36.9, 37.5, 51.9, 53.2, 55.2, 54.8, 5, 66.6, 67.0, 68.4, 68.8, 127.2, 127.5, 128.1, 136.2, 162.5; LCMS; 277.00 $[M+1]^+$.

3.1.4. (2*S*,4*R*)-*N*1-(Benzyloxycarbonyl)-4-hydroxy-2-(aminomethyl)-pyrrolidine (7). To a solution of the azide **6** (3.5 g, 12.7 mmol) in methanol (5 ml) taken in hydrogenation flask was added Raney Ni (1.5 ml). The reaction mixture was hydrogenated in a Parr apparatus for 3.5 h at rt and H_2 of pressure 35–40 psi. The catalyst was filtered off and then solvent was removed under reduced pressure to yield a residue of the amine **7** as colorless oil. Yield (3.0 g, 95.0%); this compound was used for the further reaction without any purification.

3.1.5. (2*S*,4*R*)-*N*1-(Benzyloxycarbonyl)-2-(*tert*-butyloxycarbonylaminomethyl)-4-hydroxy pyrrolidine (8). The amine **7** (3.0 g, 12.0 mmol) was taken in DMSO (10 ml), triethylamine (1.58 g, 15.6 mmol) and $BocN_3$ (2.05 g, 14.4 mmol) were added. The reaction mixture was heated to 50 °C for 8 h. The reaction mixture was poured into 150 ml of ice-cold water and the product extracted into ether (20 ml×8). The combined ether layer was washed with water, brine and then concentrated to give Boc protected amine **8** as light yellow oil. (3.2 g, yield 76%, R_f=0.6, ethyl acetate:petroleum ether). $[\alpha]_D^{20}$ −12.7 (*c* 0.7, CH_2Cl_2); IR (neat) (ν) cm^{-1}. 3310, 3121, 1689, 1570. ^1H NMR ($CHCl_3$-*d*, 200 MHz); δ: 1.41 (s, 9H, Boc), 1.6–2.3 (m, 3H, C2H, C3H_2), 3.15–3.75 (m, 4H, C5H_2, CH_2NH), 3.9–4.2 (m, 1H, C4–OH), 4.3–4.5 (m, 1H, C4H), 5.11 (s, 2H, OCH_2), 5.4–5.6 (br d, 1H, carbamate NH), 7.33 (s, 5H, C_6H_5). ^{13}C NMR ($CHCl_3$-*d*, 200 MHz); δ: 28.3, 38.04, 44.1, 54.9, 56.6, 67.0, 69.2, 79.2, 128.4, 136.4, 156.3, 159.6. Anal. Calcd (%) for $C_{18}H_{26}N_2O_5$: C, 61.71; H, 7.42; N, 8.00. Found C, 61.68; H, 7.64; N, 7.78; LCMS; 351.21 $[M+1]^+$.

3.1.6. [(2*S*,4*R*)-2-(*tert*-Butyloxycarbonylaminomethyl)-4-hydroxypyrrolidine (9). To a solution of the ester **8** (3.2 g, 9.5 mmol) in methanol (5 ml) in a hydrogenation flask was added 10% Pd–C (0.32 g). The reaction mixture was hydrogenated in a Parr apparatus for 7 h at rt and H_2 at 60 psi pressure. The catalyst was filtered off and then solvent was removed under reduced pressure to yield a residue of the amine **9** as colorless oil. Yield (1.9 g, 95%); this compound was used for the further reaction without any purification.

3.1.7. Ethyl [(2*S*,4*R*)-2-(*tert*-butyloxycarbonylaminomethyl)-4-hydroxypyrrolidin-1-yl]-propanoate (10). To the cyclic amine **9** (1.9 g, 8.8 mmol) in methanol (20 ml), was added ethyl acrylate (0.97 g, 9.68 mmol) and stirred for 3 h at rt. The reaction mixture was evaporated to dryness and was extracted into ethyl acetate (25 ml×3). The organic layer was dried over Na_2SO_4 and concentrated to give crude residue, which on column chromatography afford ester **10** as thick colorless oil. (2.0 g, yield 72%, R_f=0.6, MeOH/CH_2Cl_2-1:9). $[\alpha]_D^{20}$ −7.7 (*c* 3.23, CH_2Cl_2); IR (neat) (ν) cm^{-1}. 3410, 1730, 1697, 1410. ^1H NMR ($CHCl_3$-*d*, 200 MHz); δ: 1.24 (t, 3H, ester CH_3), 1.41 (s, 9H, Boc), 1.52–1.9 (m, 2H, C3H_2), 1.95–2.6 (m, 5H, –CH_2–CH_2–, C2H), 2.7–3.5 (m, 5H, C5H_2, CH_2NH, OH), 3.7–4.2 (m, 2H, ester CH_2), 4.25–4.4 (m, 1H, C4H), 4.75–5.45 (br d, 1H, NH). ^{13}C NMR ($CHCl_3$-*d*,

200 MHz); δ:13.9, 28.1, 33.7, 37.7, 40.7, 49.1, 60.2, 61.3, 61.6, 69.2, 78.7, 156.3, 172.4. Anal. Calcd (%) for $C_{15}H_{28}N_2O_5$: C, 56.96; H, 8.86; N, 8.86. Found C, 56.65; H, 8.95; N, 8.71; LCMS; 317.00 $[M+1]^+$.

3.1.8. Ethyl [(2*S*,4*S*)-2-(*tert*-butyloxycarbonylaminomethyl)-4-(*N*3-benzoylthymin-1-yl)-pyrrolidin-1-yl]-propanoate (11). To a solution of alcohol **10** (1.5 g, 4.74 mmol), *N*3-benzoylthymine and triphenylphosphine in dry benzene cooled to 4 °C, was added DIAD dropwise by a syringe under nitrogen atmosphere. The reaction mixture was stirred for another 5 h at rt. The reaction mixture was evaporated to dryness and the residue was purified by column chromatography to obtain monomer ethyl ester **11** as foam. (1.8 g, yield 72%, R_f=0.76, MeOH/CH_2Cl_2-1:9). $[\alpha]_D^{20}$ −69.16 (*c* 0.50, CH_2Cl_2); IR (Nujol) (ν) cm^{-1}. 3019, 1730, 1710, 1697, 1550, 1415. ^1H NMR ($CHCl_3$-*d*, 500 MHz); δ_H 1.29 (t, 3H, ester CH_3), 1.41 (s, 9H, Boc), 1.55–1.8 (m, 1H, C3H′), 1.96 (s, 3H, thymine-CH_3), 2.15–2.3 (m, 1H, C3H), 2.35–2.7 (m, 5H, –CH_2–CH_2–, C2H), 3.0–3.2 (m, 3H, C5H′, CH_2NH), 3.25–3.4 (m, 1H, C5H), 3.95–4.15 (m, 2H, ester CH_2), 4.95–5.15 (m, 1H, C4H), 5.2–5.3 (br d, 1H, NH), 7.15–7.25 (m, 2H, Ar), 7.25–7.35 (m, 1H, Ar), 7.35–8.00 (m, 3H, thy CH, Ar). ^{13}C NMR ($CHCl_3$-*d*, 500 MHz); δ_C 12.1, 13.9, 28.0, 33.2, 35.5, 39.5, 47.5, 57.3, 58.5, 60.4, 63.0, 79.0, 110.6, 128.8, 130.0, 131.6, 134.5 and 135.4, 149.6, 156 .0, 162.2, 168.9, 172.2. Anal. Calcd (%) for $C_{27}H_{36}N_4O_7$: C, 61.36; H, 6.81; N, 10.60. Found C, 61.13; H, 6.97; N, 10.43; MS LCMS; 528.01 $[M]^+$, 428.01 $[M-t\text{Boc}]^+$.

3.1.9. [(2*S*,4*S*)-2-(*tert*-Butyloxycarbonylaminomethyl)-4-(thymin-1-yl)-pyrrolidin-1-yl]-propaonic acid (12). The monomer ethyl ester **11** (1.2 g, 2.8 mmol) was dissolved in methanol (6 ml), 2 M NaOH (6 ml) was added and the reaction stirred for 7 h. The aqueous layer was then neutralized with cation exchange resin (Dowex-H^+). The reaction mixture was filtered to remove the resin. The aqueous layer was washed with ethyl acetate to remove benzoic acid. The aqueous layer was concentrated to a residue that on co-evaporation with dichloromethane (10 ml×2) afforded monomer **12** as foam. (1.1 g, yield 98%), mp, 119–121 °C; $[\alpha]_D^{20}$ −78.0 ° (*c* 0.5, CH_2Cl_2); IR (neat) (ν) cm^{-1}. 3016, 1705, 1699. ^1H NMR (D_2O, 500 MHz); δ_H 1.39 (s, 9H, Boc), 1.82 (s, 3H, thy CH_3), 2.1–2.3 (m, 1H, C3H′), 2.45–2.75 (m, 2H, N–CH_2–CH_2–CO), 2.8–2.9 (m, 1H, C3H), 2.95–3.15 (m, 1H, C2H), 3.4–3.8 (m, 5H, COCH_2–CH_2–N, CH_2NH, C5H), 3.95–4.15 (m, 1H, C5H′) 4.8 (m, 1H, C4H), 7.44 (s, 1H, thy CH). ^{13}C NMR (D_2O, 500 MHz); δ_C 11.0, 27.45, 31.9, 32.4, 38.0, 50.3, 56.7, 57.5, 66.8, 81.4, 110.4, 142.6, 157.6, 158.0, 168.5, 177.7. Anal. Calcd (%) for $C_{18}H_{28}N_4O_6$: C, 54.54; H, 7.07; N, 14.14. Found C, 54.23; H, 7.29; N, 13.97; MS LCMS; 397.05 $[M+H]^+$, 297.05 $[M+1-t\text{Boc}]^+$.

3.2. Hydrolysis of the ethyl ester functions of *aeg*PNA monomers (general method)

The ethyl esters were hydrolyzed using 2 M aqueous NaOH in methanol and the resulting acid was neutralized with activated Dowex-H^+ until the pH of the solution was 7.0. The resin was removed by filtration and the filtrate was concentrated to obtain the resulting Boc-protected acids in excellent yield (>85%). In case of cytosine monomer ethyl ester, mild base 0.5 M LiOH was used to avoid deprotection of the exocyclic amine-protecting group.

3.3. Synthesis of PNA oligomers, incorporating *bep*PNA monomers

The modified PNA monomers were built into PNA oligomers using standard procedure on a L-lysine derivatized (4-methylbenzhydryl)amine (MBHA) resin (initial loading 0.25 meq g^{-1}) with HBTU/HOBt/DIEA in DMF/DMSO as a coupling reagent.

3.4. Cleavage of the PNA oligomers from the resin

The PNA oligomers were cleaved from the resin with TFMSA. The oligomrs were purified by RP-HPLC (C18 column) and characterized by LC-TOF-MS mass spectrometry. The overall yields of the raw products were 35–65%. The normal PNAs were prepared similarly as discussed above. The oligomer attached MBHA resin (20 mg) was stirred with thioanisole (40 μl) and 1, 2-ethanedithiol (32 μl) in an ice bath for 10 min. TFA (240 μl) was added, and after equilibration for 10 min, TFMSA (32 μl) was added slowly. The reaction mixture stirred for 2.5 h at rt, filtered and concentrated under vacuum. The product was precipitated with dry ether from methanol and the precipitate was dissolved in water (200 μl) and loaded over Sephadex G25 column. Fractions of 0.5 ml were collected and the presence of oligomer was detected by measuring the absorbance at 260 nm. Fractions containing oligomer were freeze-dried and the purity of the fractions was assessed by analytical RP-HPLC. If the purity is less than 90%, oligomers were purified by preparative HPLC.

3.5. Gel filtration

The crude PNA oligomer obtained after ether precipitation was dissolved in water (200 μl) and loaded on a gel filtration column. This column consisted of G25 Sephadex and had a void volume of 1 ml. The oligomer was eluted with water and ten fractions of 1 ml volume each were collected. The presence of the PNA oligomer was detected by measuring the absorbance at 260 nm. The fractions containing the oligomer were freeze-dried. The purity of the cleaved crude PNA oligomer was determined by RP-HPLC on a C18 column. If the purity of the oligomers found to be above 96%, the oligomers were used as such for experiments without further purification. If the purity was not satisfactory, the oligomers were purified by HPLC.

3.6. HPLC (high performance liquid chromatography) purification of PNA oligomers

Peptide purifications were performed on a Waters DELTA-PAK-RP semi preparative C18 column attached to a Hewlett Packard 1050 HPLC system equipped with an auto sampler and Jasco-UV970 variable-wavelength detector. An isocratic elution method with 10% CH_3CN in 0.1% TFA/H_2O was used with flow rate 1.5 ml/min and the eluent was monitored at 260 nm. The purity of the oligomers was further assessed by RP-C18 analytical HPLC column (25× 0.2 cm, 5 μm) with gradient elution: *A* to 50% *B* in 30 min,

$A=0.1\%$ TFA in H_2O, $B=0.1\%$ TFA in CH_3CN/H_2O 1:1 with flow rate 1 ml/min. The purities of the purified oligomers were found to be $>98\%$.

3.7. LC-TOF-MS

Purity and the integrity of all PNAs synthesized were ascertained by HPLC/LC-TOF-MS mass spectrometry employing electrospray ionization technique. Neat samples were dissolved in methanol and are injected through HPLC system. The TOF (time of flight) detector was used to analyze the molecular ion peaks.

3.8. Binding stoichiometry

Eleven mixtures of PNA:DNA with different ratios to each other such as 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10 and 100:0; all of the same total strand concentration (2 mM) in sodium phosphate buffer (100 mM NaCl, 0.1 mM EDTA, pH 7.0). The samples are heated to 85 °C in water bath for 5 min, allowed to cool to rt and then cooled further in a refrigerator overnight. CD spectra for all the samples were recorded at 10 °C with wavelength range from 350–190 nm with scan speed 100 nm/min, accumulation-8, response time-4 s, band width-1 nm and sensitivity-10 mdeg. CD cell for all the studies was of 10 mm path length. Baseline was subtracted from all the CD spectra.

3.9. UV–T_m measurements

The concentration was calculated on the basis of absorbance from the molar extinction coefficients of the corresponding nucleobases (i.e., T, 8.8 $cm^2/\mu mol$; C, 7.3 $cm^2/\mu mol$; G, 11.7 $cm^2/\mu mol$ and A, 15.4 $cm^2/\mu mol$). The complexes were prepared in 10 mM sodium phosphate buffer, pH 7.0 containing NaCl (100 mM) and EDTA (0.1 mM) and were annealed by keeping the samples at 85 °C for 5 min followed by slow cooling to rt (annealing). Absorbance versus temperature profiles were obtained by monitoring at 260 nm with Perkin-Elmer Lambda 35 UV–vis spectrophotometer scanning from 5 to 85/90 °C at a ramp rate of 0.2 °C per min. The data were processed using Microcal Origin 5.0 and T_m values derived from the first derivative curves.

3.10. Circular dichroism

CD spectra were recorded on a JASCO J-715 spectropolarimeter. The CD spectra of the PNA:DNA complexes and the relevant single strands were recorded in 10 mM sodium phosphate buffer, 100 mM NaCl, 0.1 mM EDTA, pH 7.0. The temperature of the circulating water was kept below the melting temperature of the PNA:DNA complexes, that is, at 10 °C. The CD spectra of the homothymine T_8 single strands were recorded as an accumulation of 10 scans from 320 to 195 nm using a 1 cm cell, a resolution of 0.1 nm, band width of 1.0 nm, sensitivity of 2 mdeg, response 2 s and a scan speed of 50 nm/min for the PNA_2:DNA complexes, spectra were recorded as an accumulation of 8 scans, response of 1 s and a scan speed of 200 nm/min.

3.11. Gel mobility shift assay

The PNAs (**13–18**, Table 2) were individually mixed with DNA **21** in 2:1 ratio (PNA strand, 0.4 mM and DNA **21**, 0.2 mM) in water. The samples were lyophilized to dryness and re-suspended in sodium phosphate buffer (10 mM, pH 7.0, 10 µl) containing EDTA (0.1 mM). The samples were annealed by heating to 85 °C for 5 min followed by slow cooling to rt and refrigeration at 4 °C overnight. To this, 10 µl of 40% sucrose in TBE buffer pH 8.0 was added and the sample was loaded on the gel. Bromophenol blue (BPB) was used as the tracer dye separately in an adjacent well. Gel-electrophoresis was performed on a 15% non-denaturing polyacrylamide gel (acrylamide/bis-acrylamide, 29:1) at constant power supply of 200 V and 10 mA, until the BPB migrated to three-fourth of the gel length. During electrophoresis the temperature was maintained at 10 °C. The spots were visualized through UV shadowing by illuminating the gel placed on a fluorescent silica gel plate, GF_{254} using UV-light.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.12.002. ^1H and ^{13}C NMR Spectra, Mass spectra of selected compounds, HPLC profiles and LC-TOF-MS spectra of bepPNAs and UV–T_m curves, CD curves.

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