

Conformationally Constrained PNA Analogues: Structural Evolution toward DNA/RNA Binding Selectivity

VAIJAYANTI A. KUMAR* AND
KRISHNA N. GANESH*

Division of Organic Synthesis, National Chemical Laboratory,
Pune 411008, India

Received July 28, 2004

ABSTRACT

Since its discovery 12 years ago, aminoethylglycyl peptide nucleic acid (*aeg*-PNA) has emerged as one of the successful DNA mimics for potential therapeutic and diagnostic applications. An important requisite for *in vivo* applications that has received inadequate attention is engineering PNA analogues for able discrimination between DNA and RNA as binding targets. Our approach toward this aim is based on structural preorganization of the backbone to *hybridization-competent* conformations to impart binding selectivity. This strategy has allowed us to design locked PNAs to achieve specific hybridization with DNA or RNA with aims to increase the binding strength without losing the binding specificity. This Account presents results of our rationale in design of different conformationally constrained PNA analogues, their synthesis, and evaluation of hybridization specificities.

Introduction

Peptide nucleic acids I (PNAs) are neutral, achiral DNA mimics that bind to complementary DNA/RNA sequences with high affinity and sequence specificity.¹ In PNA the natural nucleobases are attached via methylenecarbonyl linkers to an uncharged, pseudopeptide backbone composed of repeating *N*-(2-aminoethyl)glycyl units. PNA hybridizes to complementary DNA/RNA sequences via specific base complementation to form duplexes for mixed sequences and triplexes for homopyrimidine/homopurine sequences.² The complexes of PNA with DNA/RNA sequences generally show thermal stabilities higher than the corresponding DNA–DNA/RNA complexes,³ depending on sequence. PNAs and their analogues are resistant to proteases and nucleases. Due to these exceptional properties PNAs have major applications as a tool in molecular biology,⁴ as lead compounds for development of gene-targeted drugs via antisense/antigene technology,⁵ for

diagnostics and biosensors,⁶ and as building blocks for designing PNA supramolecular constructs.⁷

The current limitations in PNA properties (Figure 1) are its poor water solubility and lack of cell permeability coupled with ambiguity in DNA/RNA recognition arising from its equally facile binding in a parallel/antiparallel fashion (II) with the complementary nucleic acid sequences. These limitations are being systematically addressed with rationally modified PNA analogues.^{8,9} The solubility of PNAs was improved through conjugation with cationic ligands such as polyamines,¹⁰ lysine³ at the N/C terminus of the PNA, or guanidinium in backbone¹¹ without compromising the hydrogen-bonding specificity. The cell penetrability has been improved by conjugation with various transfer molecules such as cell-penetrating peptides.¹² The equal binding of PNA in parallel and antiparallel orientations to DNA/RNA reduces its target specificity by a factor of 2, which can have serious implications for therapeutic applications depending on the other gene that gets affected. This issue was addressed by introduction of chirality into achiral PNA backbones to effect orientational selectivity in complementary DNA/RNA binding.¹³ The ambiguity in DNA/RNA binding would lead to recognition of nonspecific target sequences, and achieving PNA specificity in binding is a desirable goal for successful therapeutic applications.

The relatively high binding affinity of PNAs toward natural oligonucleotides is attributed to the lack of electrostatic repulsion between the uncharged PNA backbone and the negatively charged sugar–phosphate backbone of DNA/RNA.¹⁴ The single-stranded form of PNA, being acyclic, is conformationally flexible in its different structural segments (I). Consequently, formation of PNA:DNA/RNA complexes is accompanied by conformational changes in PNA to gain enthalpic advantage by hydrogen-bonding and base-stacking interactions accompanied by an undesirable decrease in entropy.¹⁵ Any further increase in the conformational freedom in *aeg*-PNA through extended backbone^{8,16} or side-chain structures¹⁷ lead to substantial reduction in the stability of the resulting PNA:DNA/RNA complexes.

Conformational Preorganization Strategies

Conformational preorganization by introduction of structural constraints has been successful in DNA analogues such as locked nucleic acids (LNA) in which the prelocked 3'-*endo* sugar conformation, like in DNA:RNA hybrids, favors its binding with complementary DNA/RNA sequences.¹⁸ Other examples include conformationally frozen six-membered cyclohexenyl,¹⁹ hexitol,²⁰ and altritol²¹ nucleic acids. The required conformational preferences of PNA for binding of DNA or RNA are not yet deciphered as well as those in PNA:DNA/RNA complexes.^{22,23} A structural feature of PNA that interferes with hybridization is the presence of *cis* and *trans* rotamers around the

Vaijayanti A. Kumar, born in 1958 at Wai, Maharashtra, received her M.Sc. degree in Organic Chemistry (1978) from the University of Pune followed by Ph.D. degree (1984) from the National Chemical Laboratory, Pune. During her research career at NCL she has developed synthetic routes to drugs and drug intermediates. Since 1988 her research interests are in the synthesis of modified nucleosides, oligonucleotides, and peptide nucleic acids concerning conceptual development and synthesis of conformationally locked, stereodefined PNAs.

Krishna N. Ganesh received his Ph.D. degrees in Chemistry from Delhi University (1977) and the University of Cambridge, U.K., (1980). His main research interests are in modified oligonucleotides and development of recognition-selective peptide nucleic acids for applications in therapeutics and diagnostics.

* To whom correspondence should be addressed. Phone/Fax: 91 20 2589 3153. E-mail addresses: kng@ems.ncl.res.in; vakumar@dalton.ncl.res.in.

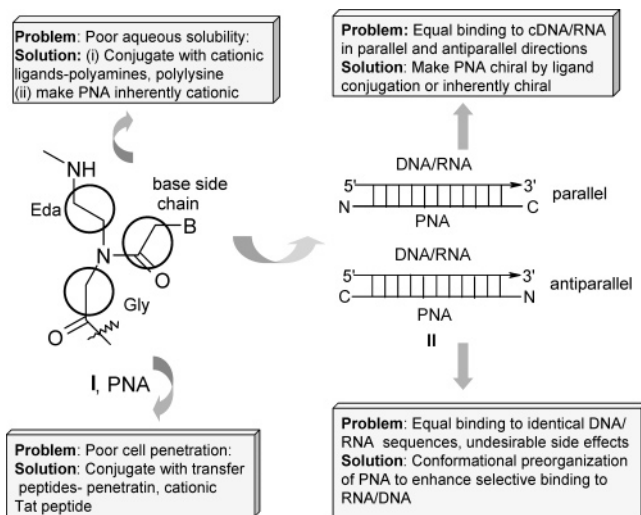


FIGURE 1. Limiting issues for PNA practical applications and conceptual solutions.

tertiary amide linkage in each PNA monomer.²⁴ The high rotation barrier encountered during this interconversion leads to different PNA:DNA/RNA hybridization kinetics in parallel and antiparallel hybrids.²⁵

During the past few years we have been addressing PNA modifications that can potentially preorganize PNA to form PNA:DNA/RNA complexes and achieve selectivity for either DNA or RNA complementation. The strategy is to enrich populations of single-stranded PNAs that have the favorable conformation for binding complementary DNA/RNA. The competently preorganized conformers could trigger a shift in equilibrium toward the desired complex because of the net reduced entropy loss upon complexation provided the enthalpic contributions remain unaffected. Our strategies to conformational preorganization, as shown in Figure 2, are based on introduction of methylene/ethylene groups to bridge the aminoethyl-glycyl backbone and methylenecarbonyl side chain to generate diverse five- or the six-membered nitrogen heterocyclic analogues. The cyclic analogues where the nucleobases are directly attached to the ring have defined nucleobase orientation, overcoming the rotamer problem. It also concomitantly introduces chiral centers, which may impart directional selective binding of PNA with chiral DNA/RNA. This Account presents our systematic efforts so far in rational design and synthesis of conformationally constrained PNA analogues toward evolving PNAs for selective and directional recognition of DNA or RNA.

Prolyl and Pyrrolidinyl PNAs

Aminoprolyl PNA (*ap*-PNA). Introduction of a methylene bridge between the β -carbon atom of the aminoethyl segment and the α' -carbon of the glycine segment of *aeg*-PNA resulted in 4-aminoprolyl PNA (Figure 2, path 1)²⁶ having two chiral centers. All four diastereomeric T-monomers were synthesized from *trans*-4(*R*)-hydroxyproline (Scheme 1, route A) and incorporated into PNA oligomers. None of the homochiral aminoprolyl thymine PNA corresponding to any of the diastereomers bound

to target DNA sequences,²⁷ probably due to high rigidity in the backbone resulting in structural incompatibility. However, incorporation of single chiral *D*-*trans* or *L*-*trans* prolyl PNA monomer into *aeg*-PNA at the N-terminus or within the PNA sequence resulted in higher binding of the target DNA with definite preference for a parallel or an antiparallel mode unlike the unmodified PNA²⁸ (Table 1, entry 1). Efforts directed toward releasing the strain by replacing the backbone amide linker with carbamate²⁹ linkage were not successful.

Aminoethylprolyl PNA (*aep*-PNA). Linking of the α' -carbon atom of the glycyl segment and the β' -carbon atom of the nucleobase linker by a methylene bridge, accompanied by replacement of the side chain carbonyl with a methyl group, leads to *aep*-PNA (Figure 2, path 2).³⁰ The flexibility of the aminoethyl segment of *aeg*-PNA is retained, but that of the glycine and the side chain are constrained via a methylene bridge. The nucleobase is attached directly to the pyrrolidine ring in a stereospecific manner, thus locking its orientation. The presence of a pyrrolidine ring nitrogen with a pK_a of 6.8 in the backbone indicated that it could be partially protonated at physiological pH. The PNA chimeric oligomers (*aep*-PNA) comprised of 4(*S*)-2(*S/R*)-*aep*-T (Scheme 1, route B) and *aeg*-T units showed strong and specific binding properties toward target DNA sequences. The positively charged nitrogen in the backbone did not adversely interfere with the H-bonding molecular recognition process, and the complex with a single mismatch in the target was significantly destabilized similar to the unmodified *aeg*-PNA complexes.³⁰ The stereochemistry at C2 did not exert any significant effect on the binding ability of homothymine sequences. The *aep*-PNA-T₈ oligomer with (2*R*,4*R*) stereochemistry displayed significant stabilization of the complexes with poly rA.³¹ The adenine homooligomer with (2*S*,4*S*) stereochemistry showed improved binding to the target DNA, while the corresponding thymine homooligomer did not show any transition in UV-melting experiments.³²

A single *aep*-PNA unit carrying individual nucleobases adenine, thymine, cytosine, and guanine in a mixed purine/pyrimidine sequence (Table 2) exhibited nucleobase-dependent binding efficiencies and orientation selectivities toward target DNA oligomers.³³ In general, the antiparallel *aep*-PNA:DNA duplexes (Table 1, entry 2) (i) had a higher stability than the corresponding parallel duplexes, barring g4(2*S*,4*S*), and (ii) were more stable than *aeg*-PNA:DNA duplexes, except for c7(2*S* and 2*R*), t6(2*S* and 2*R*), and g4(2*R*) parallel duplexes (Figure 3). This attribute may be a consequence of pyrrolidine ring conformations that depend on the electronegativity of the C4 substituent,³⁴ which varies among the nucleobases: A, G, C, and T. Protonation of the pyrrolidine ring nitrogen (pK_a 6.8) might also influence ring puckering, which alters the conformation of the nucleobase, thereby affecting the stability of the complexes. The initial promise of *aep*-PNA warrants further development of this analogue for useful applications. Toward this end, systematic studies involving

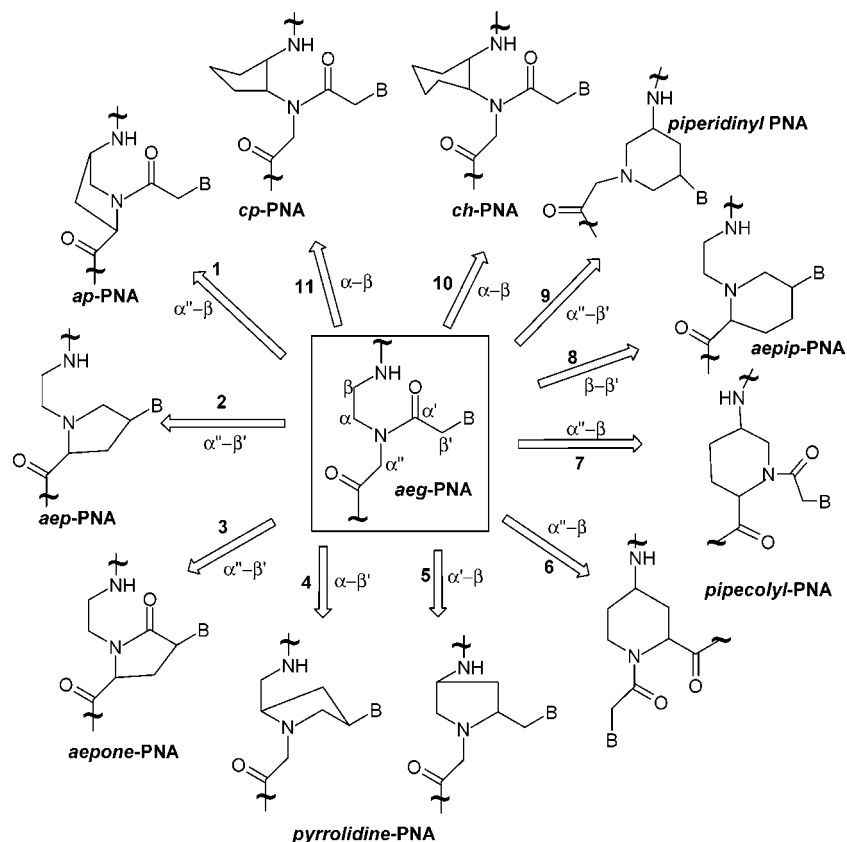


FIGURE 2. Strategies for design of conformationally constrained PNA analogues.

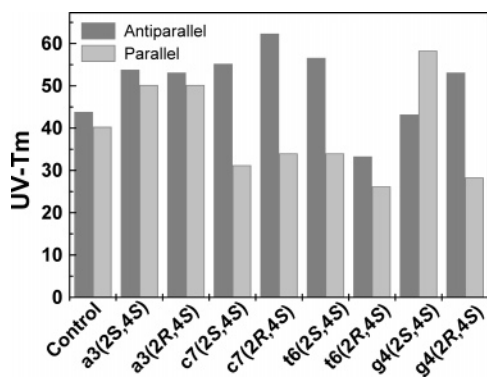


FIGURE 3. UV-Tm values for *aep*-PNA:DNA parallel and antiparallel complexes. The lower case letters on the x-axis refer to the base at the modified backbone unit indicated by the number (see Table 2).

stereoregular mixed purine/pyrimidine sequences are currently underway in our laboratory.

Aminoethylpyrrolidinone PNA (*aepone*-PNA). To reduce the pyrrolidine ring conformational effects seen with *aep*-PNA, an endocyclic carbonyl was installed at C5 in *aep*-PNA. This achieved restoration of the amide character of the ring nitrogen as in *aeg*-PNA (Figure 2, path 3). The pyrrolidine ring nitrogen is not capable of protonation, and the ring puckering effects on base orientations are minimal. The aminoethylprolyl-5-one (*aepone*) thymine monomers were synthesized (Scheme 1, route C)³⁵ and incorporated into *aeg*-PNA-T₈ backbone at different positions. The *aepone*-PNAs showed remarkable stabilization of derived PNA₂:DNA triplexes compared to *aeg*-PNA.³⁶

Interestingly, the *aepone*-PNA oligomers destabilized the corresponding complexes with poly (rA), reversing the binding ability of *aeg*-PNA (RNA > DNA). The homooligomeric *aepone*-PNA T₈ bound to the complementary 12-mer DNA more strongly than to poly rA. These results clearly suggested the possibility of structurally preorganized PNA also leading to discrimination in their efficacy to bind DNA or RNA, which is one of our ultimate goals.

A pyrrolidinone-PNA was reported by Nielsen et al.³⁷ having the same number of main chain backbone atoms but with the N- and C-termini reversed in relation to our *aepone*-PNA. This is equivalent to linking the α -carbon atom of the aminoethyl segment with the β' -carbon of the side chain acetyl linker via a methylene bridge. The hybridization properties of homooligomeric (3*S*,5*R*)-PNA-A₁₀ with complementary DNA and RNA indicated the highest affinity toward rU₁₀, which was slightly lower compared to that of *aeg*-PNA.

Pyrrolidine PNA. Since our first report^{26,28} on the use of proline-based monomers as chiral building blocks for conformationally constrained PNAs several groups have reported interesting variations of this basic theme. The earliest was the gly-pro peptide with nucleobase linked at C4 of proline,³⁸ but the tertiary amide groups as part of main backbone retained the rotameric problem, and the constituted PNA oligomers did not bind to target sequences. The conformational strain in such a backbone was released by replacing the glycine with β -alanine or its constrained version chiral 2-aminocyclopentane or aminopyrrolidine carboxylic acids.³⁹ The PNA oligomers

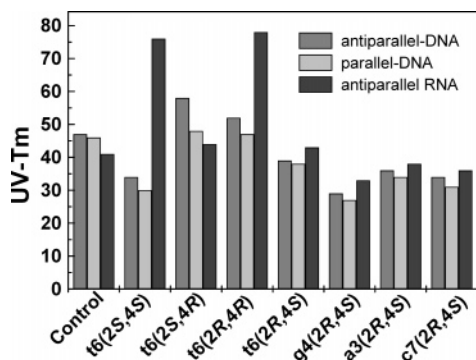


FIGURE 4. UV–T_m values for pyrrolidinyl PNA:DNA/RNA complexes. The lower case letters on the x-axis refer to the base at the modified backbone unit indicated by the number (see Table 2).

with the latter modification showed binding with target DNA. Deletion of the endocyclic carbonyl group in pyrrolidinone PNA³⁷ gave the pyrrolidine PNA (Figure 2, path 4). The derived (2*R*,4*S*) stereomeric homoadenylate chimeric *aeg*-PNA oligomer formed a stable complex with both DNA and RNA.⁴⁰ The (2*R*,4*R*) version of this PNA analogue was shown to bind target DNA and RNA with high affinity and kinetic selectivity toward RNA.⁴¹

We synthesized the (2*S*,4*S*) version of the pyrrolidine PNA analogue (Scheme 1, route **D**), and its thymine oligomer resulted in a decreased binding efficiency with target DNA and RNA sequences (Table 1, entry 4).⁴² We also prepared PNA–DNA dimer from (2*R*,4*R*) pyrrolidine PNA–T, which when placed in PNA–DNA chimera lead to decreased DNA triplex stability. The homologous pyrrolidine PNA–DNA dimer having an extended backbone with a five-atom linker was accommodated well in triplex structures with complementary DNA.⁴³

Introduction of the α' - β -methylene bridge (Figure 2, path 5)⁴⁴ led to another pyrrolidine-PNA in which, unlike the previous analogues, the base is away from the pyrrolidine ring by one carbon. The nucleobase orientation is therefore unrestricted. The (2*R*,4*S*) pyrrolidine-T monomer (Scheme 1, route **E**) when introduced into the middle of the *aeg*-PNA-T₈, bound to the target DNA better than pure *aeg*-PNA-T₈. However, the diastereomeric (2*S*,4*S*) monomer in PNA destabilized the resulting DNA complex as an example of stereochemical discrimination effects in PNA:DNA recognition. When the four diastereomers of this T-monomer were incorporated into a mixed base *aeg*-PNA sequence (Table 2) it was found that the 4*R* modifications stabilized the PNA:DNA duplex with a significant difference in parallel/antiparallel binding compared to *aeg*-PNA:DNA duplexes (Figure 4).⁴⁵ The 4*S* modifications of all four bases destabilized the duplexes without much parallel/antiparallel binding differences as compared to the unmodified hybrids. The enantiomeric pairs (2*S*,4*S*) and (2*R*,4*R*) formed antiparallel complexes with RNA much stronger than that of *aeg*-PNA or other diastereomers. The (2*R*,4*S*) pyrrolidinyl PNA analogues of all four bases lacked the discrimination effects with respect to either parallel/antiparallel or DNA/RNA binding (Table 1, entry 5). Detailed structural studies with this pyrrolidinyl PNA could be very interesting in light of the recently

reported homo-*N*-oligonucleotides that are the first examples of D-sugar nucleic acids having unrestricted glycosyl conformation.⁴⁶

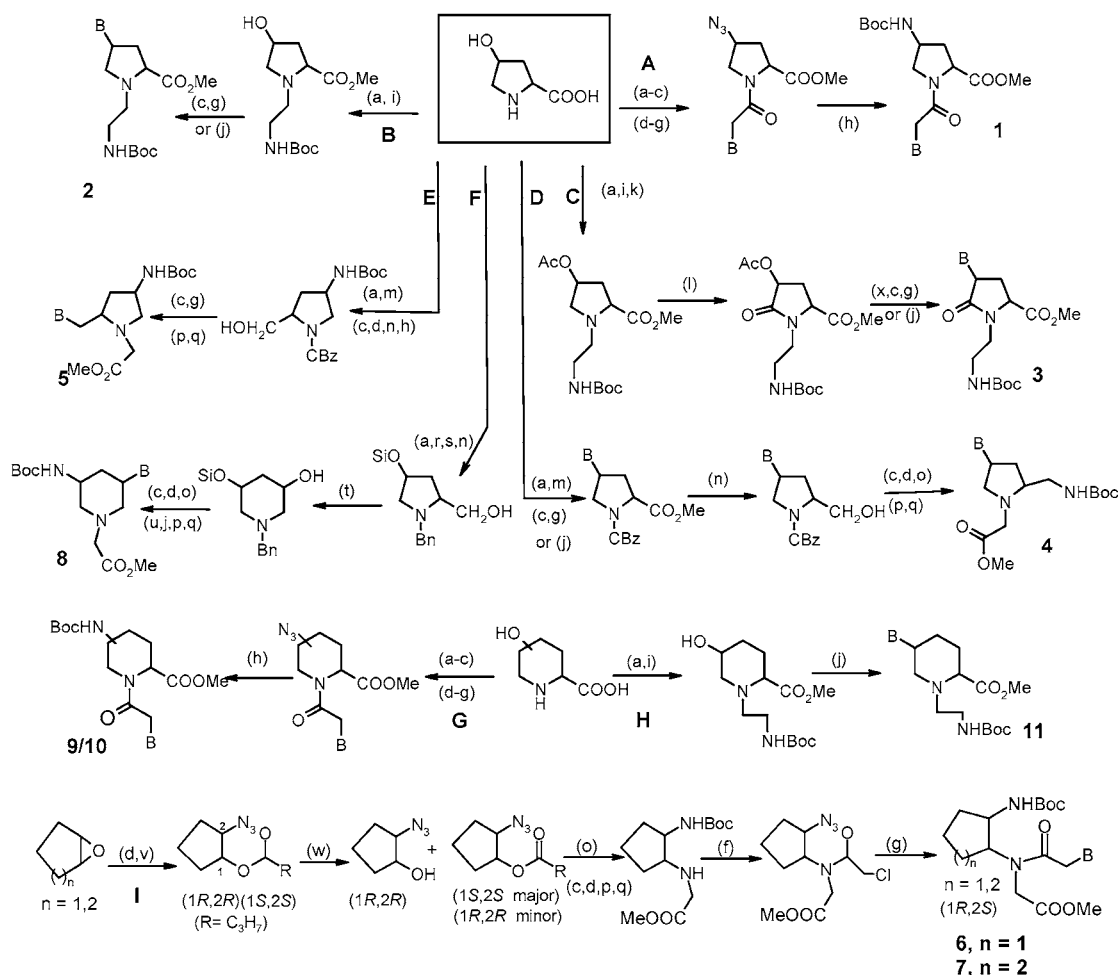
Pipecolic and Piperidinyl PNA

The six-membered ring systems are conformationally more rigid compared to their five-membered counterparts due to high-energy barriers between chair–boat conformations and preferred low-energy equatorial dispositions of substituents. The six-membered rings could thus be frozen in one of the more stable conformers. If the substitution pattern on the ring can afford the right steric fit to bind DNA/RNA, substantial entropic gain would lead to stabilization of the derived complexes. Aminopropylglycyl PNA is an extended form of *aeg*-PNA and binds to target DNA sequence with much less efficiency.¹¹ We introduced a methylene bridge into this extended analogue between the γ -carbon of the aminopropyl segment and the α'' -carbon of the glycol segment to generate a homologue of *ap*-PNA²⁶ (Figure 2, path 6).⁴⁷ This modification was aimed to relieve the constraint caused by the five-membered pyrrolidine by ring expansion. The modified PNA-T₁₀ oligomer (*pip*-PNA) carrying the *trans* (2*S*,4*S*) monomer (Scheme 1, route **G**) impaired triplex formation with cDNA (Table 1, entry 6).

Insertion of an ethylene bridge between the β -carbon atom of the aminoethyl segment and the α'' -carbon of the glycol segment afforded the isomeric analogue 5-aminopipecolyl PNA (Figure 2, path 7). Preliminary results indicated that homothymine mixed-*aeg*PNA sequences form complexes with target DNA oligomers. If the 2,5-substituents remain in the preferred *trans* diequatorial disposition, complexes with the targets may also be stabilized unless the hydrogen-bonding and internucleobase distances are disturbed. The preliminary results indicated that homothymine mixed-*aeg*PNA sequences incorporating the 5-aminopipecolyl unit (Scheme 1, route **G**) form stable complexes with target DNA oligomers (Table 1, entry 6).

Linking the α'' -carbon of the glycol unit with the β' -C of the nucleobase linker by a two-carbon ethylene bridge leads to the homologous analogue of *aep*-PNA,³⁰ namely, *aepip*-PNA (Figure 2, path 8).⁴⁸ This chiral six-membered analogue with (2*S*,5*R*) stereochemistry (Scheme 1, route **H**) upon incorporation into *aeg*-PNA-T₈ homooligomer or into a mixed T/C PNA oligomer at different positions stabilized the corresponding PNA₂:DNA triplexes (Table 1, entry 7). This is interesting since it was suggested earlier⁴⁹ that six-membered piperidines are unlikely to stabilize the PNA:DNA complexes.

Another example of a piperidine-based PNA was introduced by linking the β carbon of the aminoethyl segment with the β' carbon of the nucleobase linker by a methylene bridge which placed the substituents on the ring in 1,3-*trans* positions (Figure 2, path 9).⁵⁰ It was postulated that a backbone diequatorial (*ee*) disposition would place the nucleobase into an axial orientation, and this causes better stacking and hydrogen-bonding interac-

Scheme 1^a

^a Reagents: (a) SOCl_2 , MeOH; (b) BocN_3 , aqueous Na_2CO_3 ; (c) MsCl , DCM, Et_3N ; (d) NaN_3 , DMF; (e) TFA, DCM; (f) ClCH_2COCl , aqueous Na_2CO_3 ; (g) K_2CO_3 , 18crown-6, DMF; (h) H_2 , Pd/C, $(\text{Boc})_2\text{O}$; (i) $\text{BocHNCH}_2\text{CH}_2\text{OMs}$, DIPEA, DMF; (j) DIAD, TPP, B; (k) Ac_2O /pyridine; (l) RuCl_3 , NaIO_4 , water; (m) CBzCl , aqueous Na_2CO_3 ; (n) NaBH_4 , LiCl, EtOH, THF; (o) Ra-Ni , H_2 , $(\text{Boc})_2\text{O}$; (p) H_2 , Pd/C; (q) $\text{BrCH}_2\text{COOMe}$, DIPEA, THF; (r) BnBr , K_2CO_3 , DMF; (s) TBDMSCl , TEA, DMAP, DMF; (t) TFAA, Et_3N , NaOH; (u) TBAF, THF; (v) $(\text{C}_4\text{H}_9\text{CO})_2\text{O}$, TEA, DCM; (w) *P. cepacia* (Lipase), phosphate buffer, pH 7.2; (x) NaOMe/MeOH .

tions with target ODNs as in the case of hexitol nucleic acids.⁵¹ The PNA incorporating the *trans* (3*S*,5*S*)-piperidinyl-T unit (Scheme 1, route F) in *aeg*-PNA-T₈ stabilized the triplex with DNA more than the control PNA. In both the above examples the ring nitrogen is capable of protonation at physiological pH and may add favorable therapeutic features in terms of cellular uptake (Table 1, entry 8).

Carbocyclic PNAs (*ch*-PNA and *cp*-PNA). One of the earliest PNA modifications was to constrain the flexibility in the aminoethyl segment by introducing a cyclohexyl ring (Figure 2, path 10).⁵² The derived *trans*-(1*S*,2*S*)-cyclohexyl PNA oligomer hybridized with complementary DNA as good as the unmodified *aeg*-PNA, while the enantiomeric *trans*-(1*R*,2*R*)-cyclohexyl PNA oligomers lacked such a property. Molecular dynamics simulations performed on model structures of (1*S*,2*S*)- and (1*R*,2*R*)-cyclohexyl PNAs showed the torsion angle β (N-CH₂-CH₂-NHCO) to be close to 180°, corresponding to a *trans* diaxial disposition of the 1,2-substituents. A comparison of the structures of PNA₂:DNA triplex and PNA:DNA duplex derived from X-ray crystallography²² and that of a

PNA:RNA/DNA duplex from NMR²³ studies is shown in Table 3 (entry 1–3). The data suggested that the backbone dihedral angle β could be important in conformationally distinguishing PNA:DNA duplex ($\beta \approx 140^\circ$) from PNA₂:DNA triplex and PNA:RNA duplex ($\beta \approx 65\text{--}70^\circ$). Thus, designs based on structural tuning of the dihedral angle β in PNA analogues could be a rational approach for imparting DNA/RNA binding selectivity. On the basis of this reasoning we favored the use of *cis*-(*a,e*)-(1*S*,2*R*/1*R*,2*S*)-cyclohexyl PNA oligomers (rather than *trans* diaxial) since β would be in the desirable range of 60° (Figure 2, path 10).

Accordingly, we synthesized both enantiomers of *cis*-(1*S*,2*R*/1*R*,2*S*)-cyclohexylthymine PNA monomers (Scheme 1, route I), and the crystal structures showed the dihedral angle β to be -63° for (1*S*,2*R*) and 66° for (1*R*,2*S*) isomers (Table 3, entry 4,5),⁵³ close to those found in the structures of PNA₂:DNA triplex and PNA:RNA duplex. These were incorporated within *aeg*-PNA-T₁₀ oligomers at chosen positions to yield cyclohexyl PNA (*ch*-PNA), and triplex stabilities were measured against target 16-mer DNA, poly dA (40-mer), and poly rA.⁵⁴ In spite of the observed

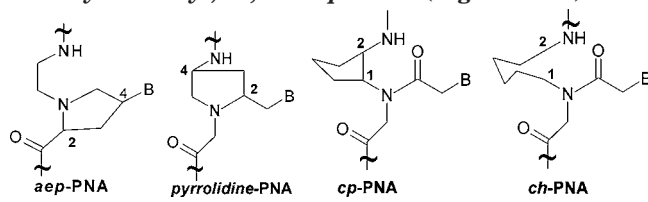
destabilization of the complexes as compared to unmodified *aeg*-PNAs, significant stereodifferentiation was noticed. The *SR* isomer was more destabilizing than the *RS* isomer in *ch*-PNA:DNA complexes. In the case of RNA complexes a reverse trend was observed, with *RS* being more destabilizing than *SR*. Generally, PNA oligomers with this modification exhibited a higher affinity toward RNA compared to DNA (Table 1, entry 9). The stability studies against DNA oligomer carrying a mismatch indicated that although *ch*-PNAs formed less stable complexes, the specificity was slightly better than the unmodified *aeg*-PNAs. In competition binding experiments *ch*-PNAs were preferred to form PNA₂:DNA triplexes over DNA:DNA duplex/triplex structures. The overall results supported the idea that preorganization of the PNA backbone by chemical modifications such that the torsion angle β is restricted to a range found by NMR and crystal structure data would lead to induction of substantial selectivity in DNA/RNA recognition.

To account for the lower binding affinity for triplex formation seen with *ch*-PNA in comparison to unmodified *aeg*-PNA we surmised that despite a favorable β , the substituted cyclohexyl ring is inherently too rigid as it gets locked in either of the two chair conformations that resist further retuning during complex formation. A relatively flexible system would be a cyclopentyl ring (Figure 2, path 11) in which the characteristic *endo-exo* puckering that dictates the pseudoaxial/pseudoequatorial dispositions of substituents may allow better torsional adjustments to attain the necessary hybridization-competent conformations. In an attempt to tune the dihedral angle β in this manner we replaced the cyclohexyl unit with *cis*-(1*S*,2*R*/1*R*,2*S*)-cyclopentyl PNA-T (*cp*-PNA) monomer (Scheme 1, route I) synthesized in optically pure forms.⁵⁵ The crystal structure data of the monomer indicated that the dihedral angle β was around 24° (Table 3, entries 6 and 7), on the low side of the desired value of 60°.

These monomers were incorporated at defined positions within homothymine *aeg*-PNA-T₁₀ to obtain the cyclopentyl PNA (*cp*-PNA) oligomers. The *RS*-*cp*-PNA enantiomer formed higher affinity complexes with DNA as compared to *SR*-*cp*-PNA isomer. For poly rA this was reversed. Like *ch*-PNA, *cp*-PNA also showed higher affinity toward complexation with RNA than DNA. The all-modified homo-oligomers of both enantiomers exhibited significant stabilization of their triplexes with DNA and poly rA (Table 1, entry 10). The *cp*-PNA modification also induced a greater destabilization of the mismatched DNA hybrids than that seen with unmodified *aeg*-PNA, indicating that higher affinity was obtained without sacrificing the base specificity. The rigorousness of complexation of *ch*- and *cp*-PNA oligomers was confirmed jointly by Job's plot, gel electrophoresis, and CD spectral data.^{54,56} While our work was in progress Appella et al.⁵⁷ reported the synthesis and limited studies on monosubstituted *trans*-(1*S*,2*S*)-cyclopentyl PNA that showed only weak stabilization of the derived PNA₂:DNA triplex over that of PNA:RNA duplex.

Table 1. Summary of Modified PNA Properties

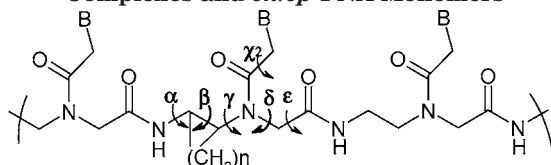
entry	PNA modifications and properties
1	aminoprolyl PNA (<i>ap</i> -PNA) ²⁷ no binding of homochiral oligomers monosubstitution stabilizes PNA:DNA duplexes stereochemistry-dependent parallel/ antiparallel binding preferences
2	aminoethylprolyl PNA (<i>aep</i> -PNA) ^{30,33} cationic, improved solubility (2 <i>R</i> ,4 <i>R</i>)-T stabilizes PNA ₂ :DNA triplexes no effect on C ₂ stereochemistry DNA duplex binding: antiparallel > parallel A/G/C/T-base-dependent binding (Figure 3) single mismatch more destabilizing compared to control
3	aminoethylpyrrolidinone PNA (<i>aepone</i> -PNA) ^{35,36} mono-, di-, tetra-, and all-modified <i>aepone</i> -PNA stabilize PNA ₂ :DNA triplexes destabilize triplexes with poly r(A)
4	pyrrolidine PNA ^{42,43} (2 <i>R</i> ,4 <i>S</i>)-A stabilize both DNA and RNA complexes (2 <i>S</i> ,4 <i>S</i>)-T destabilize DNA and RNA complexes
5	pyrrolidinyl PNA ^{44,45} (2 <i>R</i> ,4 <i>S</i>)-T in T ₈ stabilize PNA:DNA duplex (2 <i>S</i> ,4 <i>S</i>)-T in T ₈ destabilize PNA:DNA duplex (2 <i>S</i> ,4 <i>S</i>) and (2 <i>R</i> ,4 <i>R</i>)-T: RNA duplex > DNA duplex <i>ap</i> duplex is higher binding than parallel duplex (Figure 4)
6	4/5-amino-pipecolyl-PNA ^{47,50} 4-amino-pipecolyl PNA: stabilization of PNA ₂ :DNA triplexes 5-amino-pipecolyl PNA: destabilization of PNA ₂ :DNA triplexes
7	aminoethylpipecolyl-PNA ⁴⁸ marginal stabilization of PNA ₂ :DNA triplexes
8	piperidinyl PNA ⁵⁰ stabilization of PNA ₂ :DNA triplexes
9	cyclohexyl PNA ^{53,54} destabilize PNA ₂ :DNA and PNA ₂ :RNA triplexes stereochemical preferences: <i>SR</i> > <i>RS</i> for DNA, <i>SR</i> < <i>RS</i> for RNA mixed sequences (Figure 5): RNA ≫ DNA
10	cyclopentyl PNA ^{55,56} stabilize PNA ₂ :DNA and PNA ₂ :RNA triplexes stereochemical preferences: <i>SR</i> < <i>RS</i> mixed sequences (Figure 5): stabilize PNA:DNA and PNA:RNA duplexes

Table 2. PNA, DNA, and RNA Sequences for *aep*, *pyrrolidinyl*, *ch*, and *cp* PNAs (Figures 3–5)^a


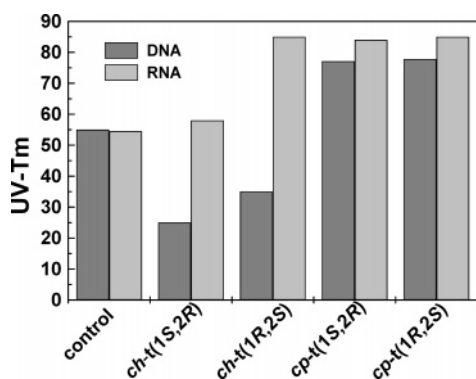
	1	2	3	4	5	6	7	8	9	10	aa		
PNA	H	G	T	A	G	A	T	C	A	C	T	X	
cDNA/RNA (<i>ap</i>)	5'	A	G	T	G	A	T	C	A	T	A	C	3'
cDNA (<i>p</i>)	5'	C	A	T	C	T	A	G	T	G	A	3'	

^a Amino acid, X = β -alanine for *aep*- and pyrrolidine-PNA, L-lysine for *ch*- and *cp*-PNA.

ch-PNA and *cp*-PNA-T monomers of both (1*S*,2*R*) and (1*R*,2*S*) enantiomers were introduced into mixed sequences (Table 2), and their relative *T_m* of hybrids with complementary DNA and RNA were measured. In all the cases PNA:RNA hybrids were more stable than the corresponding PNA:DNA hybrids, and significantly, *cp*-PNA oligomers showed much higher *T_m*'s compared to *ch*-PNA.

Table 3. Dihedral Angles (deg) in PNA:DNA/RNA Complexes and *ch/cp*-PNA MonomersPeptide Nucleic acid (PNA), n=0 *aeg*PNA; n=3, *cp*PNA; n=4, *ch*PNA

entry	compound	α	β	γ	δ	χ_1	χ_2
1	PNA ₂ :DNA ²²	-103	73	70	93	1	-175
2	PNA:DNA ²²	105	141	78	139	-3	151
3	PNA:RNA ²³	170	67	79	84	4	-171
4	<i>ch</i> PNA(1 <i>S</i> ,2 <i>R</i>) ⁵³	128	-63	76	119	1.02	-175
5	<i>ch</i> PNA(1 <i>R</i> ,2 <i>S</i>) ⁵³	-129	66	-78	-119	-0.87	174
6	<i>cp</i> PNA(1 <i>S</i> ,2 <i>R</i>) ⁵⁵	84	-24	86	90	0.89	165
7	<i>cp</i> PNA(1 <i>R</i> ,2 <i>S</i>) ⁵⁵	-84	25	-86	-90	1.2	-165

**FIGURE 5.** Selective DNA/RNA discrimination by cyclohexanyl (*ch*) and cyclopentanyl (*cp*) PNAs. The lower case letters refer to thymines at the modified PNA backbone unit at positions 2, 6, and 10 in Table 2.

Stereochemical differences were expressed better with *ch*-PNA oligomers, the (1*R*,2*S*) isomer having a higher hybrid stability than those derived from the (1*S*,2*R*) isomer. The T_m values suggest that (1*R*,2*S*)-*ch*-PNA, having wide differences in T_m of its DNA and RNA hybrids ($\Delta T_m = 50$ °C), should be a good lead molecule for discriminating DNA and RNA. The designs of *ch*-PNA and *cp*-PNA are the outcome of optimized dihedral angles that constrain the PNA backbone for differential DNA/RNA binding and discrimination via preorganization mediation. The inherently rigid cis-substituted six-membered ring of *ch*-PNAs forbids structural readjustments to bind to DNA (PNA:DNA $\beta \approx 140^\circ$) and prefers binding to RNA (PNA:RNA). The flexible (*SR/RS*)-*cp*-PNA with a relative ease of conformational adjustments in the cis-substituted cyclopentyl system allows reorganization of the ring puckering and binding to both DNA and RNA with high affinity with no selectivity. Further, in *ch/cp*-PNAs the favorable conformational features of the monomer seem to be cooperatively transmitted to the oligomer level even in a chimera. The results on the strong preferences of (*RS*)-*ch*-PNA for binding to RNA over DNA are in consonance with our strategy of adjusting the dihedral angle β through chemical modifications to achieve structure-based selectivity in PNAs.

Summary and Outlook

This Account describes our integrated approach to the design, synthesis, and study of biophysical properties of a variety of conformationally constrained cyclic PNA analogues using the logic of structural preorganization. The work has involved exploration of the chemical, structural, and configurational diversity of the pyrrolidine and piperidine heterocyclic nucleus and expands the repertoire of PNA structures. The rationally designed chiral analogues have been used to address interesting attributes of PNA:DNA/RNA hybridization such as stability, parallel/antiparallel preferences, strand invasion and kinetics of hybridization,⁵⁸ DNA/RNA hybridization selectivity, etc. The design evolution is directed toward developing PNAs to selectively recognize complementary DNA and RNA in the parallel or antiparallel direction, and some success has been obtained with *cp*-PNA and *ch*-PNA. In most cases the higher affinities are obtained without sacrificing the base-pairing specificities since the mismatched complexes are more destabilizing compared to that with unmodified PNA. The next logical step in this endeavor is to extend the work into the biological realm and use some of the successful modifications in cell uptake and toxicity studies and for in-vitro/in-vivo targeting of DNA/RNA in antisense experiments. Modification or conjugation of PNAs with cell-penetrating ligands may also prompt their nuclear entry. Hence, induction of binding specificity for DNA/RNA complement through rational PNA modifications would be highly desirable and will have potential importance for newer applications of PNA in therapeutics such as targeting chromosomal DNA.⁵⁹ The synthetic efforts have also added several new structures to the growing chemo- and stereolibraries of pyrrolidine and piperidine compounds, which may have utility in other areas of drug research.

We acknowledge Baragur Gangamani, Moneesha D'Costa, Meena, Pradeep Pallan, Pallavi Lonkar, Pravin Shirude, Nagendra Sharma, and T. Govindaraju who have primarily contributed to our PNA project over the past 8 years. We thank the Council of Scientific and Industrial Research, Department of Science and Technology, and the Department of Biotechnology, New Delhi, for financial support to the project.

References

- (1) Nielsen, P. E.; Egholm, M.; Buchardt, O. Sequence selective recognition of DNA by strand displacement. *Science* **1991**, *254*, 1497–1500.
- (2) Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Frier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. PNA hybridizes to complementary oligonucleotides obeying the Watson–Crick hydrogen-bonding rules. *Nature* **1993**, *365*, 566–568.
- (3) Nielsen, P. E.; Egholm, M.; Buchardt, O. Peptide nucleic acid (PNA): A DNA mimic with a peptide backbone. *Bioconjugate Chem.* **1994**, *5*, 3–7.
- (4) (a) Nielsen, P. E.; Egholm, M. *Peptide Nucleic Acids. Protocols and Applications*; Horizon Press: Norfolk, 1999. (b) Ray, A.; Norden, B. Peptide nucleic acid: its medical and biotechnological applications and promise for the future. *FASEB J.* **2000**, *14*, 1041–1060.
- (5) Good, L.; Nielsen, P. E. Antisense inhibition of gene expression in bacteria by PNA targeted to mRNA. *Nature (Biotechnology)* **1998**, *16*, 355–358.
- (6) Nielsen, P. E. Applications of peptide nucleic acids. *Curr. Opin. Biotechnol.* **1999**, *10*, 71–76.

- (7) (a) Nielsen, P. E. *Pure Appl. Chem.* **1998**, *70*, 105–110. (b) Corey, D. R. Peptide nucleic acids: expanding the scope of nucleic acid recognition. *Trends Biotechnol.* **1997**, *15*, 224–229.
- (8) (a) Ganesh, K. N.; Nielsen, P. E. Peptide nucleic acids: Analogs and derivatives. *Curr. Org. Chem.* **2000**, *4*, 931–943. (b) Kumar, V. A. Structural pre-organization of peptide nucleic acids: chiral cationic analogs with five- or six- membered ring structures. *Eur. J. Org. Chem.* **2002**, 2021–2032.
- (9) Uhlmann, E.; Breipohl, G.; Will, D. PNA: Synthetic polyamide nucleic acids with unusual binding properties. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2796–2823.
- (10) Gangamani, B. P.; Kumar, V. A.; Ganesh, K. N. Spermine conjugated peptide nucleic acids: UV and fluorescence studies of PNA-DNA hybrids with improved stability. *Biochem. Biophys. Res. Commun.* **1997**, *240*, 778–782.
- (11) Zhou, P.; Wang, M.; Du, L.; Fischer, G. W.; Waggoner, A.; Ly, D. H. Novel binding and efficient cellular uptake of guanidine based peptide nucleic acids. *J. Am. Chem. Soc.* **2003**, *125*, 6878–6879.
- (12) (a) de Koning, M. C.; van der Marcel, G.; Overhand, M. Synthetic developments towards PNA-peptide conjugates. *Curr. Opin. Chem. Biol.* **2003**, *7*, 734–740. (b) Eriksson, M.; Nielsen, P. E.; Good, L. Cell permeabilization and uptake of antisense peptide-peptide nucleic acid into *E. Coli*. *J. Biol. Chem.* **2002**, *277*, 7144–7147.
- (13) Sforza, S.; Corradini, R.; Dossena, A.; Marchelli, R. DNA binding of a D-lysine-based chiral PNA: Direction control and mismatch recognition. *Eur. J. Org. Chem.* **2000**, 2905–2913.
- (14) Nielsen, P. E.; Egholm, M.; Buchardt, O. Peptide nucleic acid (PNA): A DNA mimic with a peptide backbone. *Bioconjugate Chem.* **1994**, *5*, 3–7.
- (15) Tomac, S.; Sarkar, M.; Ratilainen, T.; Wittung, P.; Nielsen, P. E.; Norden, B.; Graslund, A. Ionic effects on the stability and conformation of peptide nucleic acids. *J. Am. Chem. Soc.* **1996**, *118*, 5544–5549.
- (16) Hyrup, B.; Egholm, M.; Nielsen, P. E.; Wittung, P.; Norden, B.; Buchardt, O. Structure activity studies of binding of modified peptide nucleic acids (PNAs) to DNA. *J. Am. Chem. Soc.* **1994**, *116*, 7964–7970.
- (17) Hyrup, B.; Egholm, M.; Buchardt, O.; Nielsen, P. E. A flexible and positively charged PNA analogue with an ethylene-linker to the nucleobase: Synthesis and hybridization properties. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1083–1088 and references therein.
- (18) Wengel, J. Synthesis of 3'-C- and 4'-C-branched oligodeoxynucleotides and the development of locked nucleic acid (LNA). *Acc. Chem. Res.* **1999**, *32*, 301–310.
- (19) Wang, J.; Verbeure, B.; Luyten, I.; Lescrier, E.; Herdewijn, P. Cyclohexane nucleic acids: Serum stable oligonucleotides that activate RNaseH and increase duplex stability with complementary RNA. *J. Am. Chem. Soc.* **2000**, *122*, 8595–8602.
- (20) Lescrier, E.; Esnouf, R.; Schraml, J.; Busson, R.; Heus, H. A.; Hilbers, C. W.; Herdewijn, P. Solution structure of a HNA-RNA hybrid. *Chem. Biol.* **2000**, *7*, 719–731.
- (21) Allart, B.; Khan, K.; Rosemeyer, H.; Schepers, G.; Hendrix, C.; Rothenbacher, K.; Seela, F.; Van Aerschot, A.; Herdewijn, P. D-Altritol Nucleic Acids (ANA): Hybridization properties, stability and initial structural analysis. *Chem. Eur. J.* **1999**, *5*, 2424–2431.
- (22) (a) Betts, L.; Josey, J. A.; Veal, M.; Jordan, S. R. A nucleic acid triple helix formed by a peptide nucleic acid-DNA complex. *Science* **1995**, *270*, 1838–1841. (b) Eriksson, M.; Nielsen, P. E. Solution structure of a peptide nucleic acid-DNA duplex. *Nat. Struct. Biol.* **1996**, *3*, 410–413. (c) Menchise, V.; De Simone, G.; Tedeschi, T.; Corradini, R.; Sforza, S.; Marchelli, R.; Capasso, D.; Saviano, M.; Pedone, C. Insights into peptide nucleic acid structural features: The crystal structure of a D-lysine-based chiral PNA-DNA duplex. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12021–12026.
- (23) (a) Brown, S. C.; Thomson, S. A.; Veal, J. L.; Davis, D. G. NMR solution structure of a peptide nucleic acid complexed with RNA. *Science* **1994**, *265*, 777–780. (b) Leijon, M.; Graslund, A.; Nielsen, P. E.; Buchardt, O.; Norden, B.; Kristensen, S. M.; Eriksson, M. Structural characterization of PNA-DNA duplexes by NMR. Evidence for DNA in a B-like conformation. *Biochemistry* **1994**, *33*, 9820–9825.
- (24) Hollenstein, M.; Leumann, C. J. Synthesis and incorporation into PNA of fluorinated olefinic PNA monomers. *Org. Lett.* **2003**, *5*, 1987–1990.
- (25) Rose, D. J. *Anal. Chem.* **1993**, *65*, 3545–3549.
- (26) Gangamani, B. P.; Kumar, V. A.; Ganesh, K. N. Synthesis of N⁶-(purinyl/pyrimidinyl acetyl)-4-aminoproline diastereomers with potential use in PNA synthesis. *Tetrahedron* **1996**, *52*, 15017–15030.
- (27) Gangamani, B. P.; D'Costa, M.; Kumar, V. A.; Ganesh, K. N. Conformationally restrained chiral PNA conjugates: Synthesis and DNA complementation studies. *Nucleosides Nucleotides* **1999**, *18*, 1409–1011.
- (28) Gangamani, B. P.; Kumar, V. A.; Ganesh, K. N. Chiral analogues of peptide nucleic acids: Synthesis of 4-aminopropyl nucleic acids and DNA complementation studies using UV/CD spectroscopy. *Tetrahedron* **1999**, *55*, 177–192.
- (29) Meena; Kumar V. A. Pyrrolidine carbamate nucleic acids: Synthesis and DNA binding studies. *Bioorg. Med. Chem.* **2003**, *16*, 3393–3399.
- (30) D'Costa, M.; Kumar, V. A.; Ganesh, K. N. Aminoethylpropyl peptide nucleic acids (aepPNA): chiral PNA analogues that form highly stable DNA:aepPNA₂ triplexes. *Org. Lett.* **1999**, *1*, 1513–1516.
- (31) Vilaivan, T.; Khongdeesameor, C.; Harnyuttanokam, P.; Westwell, M. S.; Lowe, G. Synthesis and properties of chiral peptide nucleic acids with a N-aminoethyl-D-proline backbone. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2541–2545.
- (32) Lee, Y.; Jin, T.; Liu, K. Synthesis and binding affinity of a chiral PNA analogue. *Nucleosides, Nucleotides Nucleic Acids* **2001**, *20*, 1705–1721.
- (33) D'Costa, M.; Kumar, V. A.; Ganesh, K. N. Aminoethylpropyl (aep) PNA: Mixed purine/pyrimidine oligomers and binding orientation preferences for PNA:DNA duplex formation. *Org. Lett.* **2001**, *3*, 1281–1284.
- (34) Holmgren, S. K.; Bretscher, L. E.; Taylor, K. M.; Raines, R. T. A hyperstable collagen mimic. *Chem. Biol.* **1999**, *6*, 6370.
- (35) Sharma, N.; Ganesh, K. N. Regioselective oxidation of N-alkyl pyrrolidines to pyrrolidin-5-enes by RuCl₃/NaIO₄. *Tetrahedron Lett.* **2004**, *45*, 1403–1406.
- (36) Sharma, N.; Ganesh, K. N. Expanding the repertoire of pyrrolidyl PNA analogues for DNA/RNA hybridization selectivity: aminoethylpyrrolidinone PNA (aepone-PNA). *Chem. Commun.* **2003**, 2484–2485.
- (37) Puschl, A.; Boesen, T.; Zuccarello, G.; Dahl, O.; Pitsch, S.; Nielsen, P. E. Synthesis of pyrrolidinone PNA: A novel conformationally restricted PNA analogue. *J. Org. Chem.* **2001**, *66*, 707–712.
- (38) Lowe, G.; Vilaivan, T. J. Solid-phase synthesis of novel peptide nucleic acids. *J. Chem. Soc., Perkin Trans. 1* **1997**, 555–561 and references therein.
- (39) Vilaivan, T.; Khongdeesameor, C.; Harnyuttanokam, P.; Lowe, G. Synthesis and properties of novel pyrrolidinyl PNA carrying β-amino acid spacers. *Tetrahedron Lett.* **2001**, *42*, 5533–5536.
- (40) Puschl, A.; Tedeschi, T.; Nielsen, P. E. Unusual RNA and DNA binding properties of a novel pyrrolidine-amide oligonucleotide mimic (POM). *Org. Lett.* **2000**, *2*, 4161–4163.
- (41) Hickman, D. T.; King, P. M.; Cooper, M. A.; Slater, J. M.; Mickelfield, J. Unusual RNA and DNA binding properties of a novel pyrrolidine-amide oligonucleotide mimic (POM). *Chem. Commun.* **2000**, 2251–2252.
- (42) Kumar, V. A.; Pallan, P. S.; Meena; Ganesh, K. N. Pyrrolidine nucleic acids: DNA/PNA oligomers with 2-hydroxy/aminomethyl-4-(thymine-1-yl)pyrrolidine-N-acetic acid. *Org. Lett.* **2001**, *3*, 1269–1272.
- (43) Kumar, V. A.; Meena Pyrrolidine PNA-DNA chimeric oligonucleotides with extended backbone. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1101–1104.
- (44) D'Costa, M.; Kumar, V. A.; Ganesh, K. N. Synthesis of 4(S)-(*N*-Boc-amino)-2(S/R)-(thymine-1-yl)-pyrrolidine-N-1-acetic acid: a novel cyclic PNA with constrained flexibility. *Tetrahedron. Lett.* **2002**, *43*, 883–886.
- (45) Lonkar, P.; Ganesh, K. N.; Kumar, V. A. Chimeric (aeg-pyrrolidine) PNA: Synthesis and stereo-discriminative duplex binding with DNA/RNA. *Org. Biomol. Chem.* **2004**, *2*, 2604–2611.
- (46) Ishiyama, K.; Smyth, G. E.; Ueda, T.; Masutomi, Y.; Ohgi, T.; Yano, J. Homo-N-oligonucleotides (N₁/N₉-C_{1'} Methylene bridge oligonucleotides): Nucleic acids with left-handed helicity. *J. Am. Chem. Soc.* **2004**, *126*, 7476–7485.
- (47) Lonkar, P. S.; Kumar, V. A.; Ganesh, K. N. Constrained flexibility in PNA: DNA binding studies with bridged aminopropylglycyl PNA. *Nucleosides, Nucleotides Nucleic Acids* **2001**, *20*, 1197–1200.
- (48) Shirude, P. S.; Kumar, V. A.; Ganesh, K. N. Chimeric peptide nucleic acids incorporating (2S,5R)-aminoethyl piperidyl units: synthesis and binding studies. *Tetrahedron Lett.* **2004**, *45*, 3085–3088.
- (49) Puschl, A.; Boesen, T.; Tedeschi, T.; Dahl, O.; Nielsen, P. E. Synthesis of (3R,6R) and (3S,6R)-piperidinone PNA. *J. Chem. Soc., Perkin 1* **2001**, 2757–2763.
- (50) Lonkar, P. S.; Kumar, V. A. Design and synthesis of conformationally frozen peptide nucleic acid backbone: chiral piperidine PNA as a hexitol nucleic acid surrogate. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2147–2149.

- (51) Lescrinier, E.; Froeyen, M.; Herdewijn, P. Difference in conformational diversity between nucleic acids with a six-membered 'sugar' unit and natural 'furanose' nucleic acids. *Nucleic Acids Res.* **2003**, *31*, 2975–2989.
- (52) Lagriffoule, P.; Wittung, P.; Eriksson, M.; Jensen, K. K.; Norden, B.; Buchardt, O.; Nielsen, P. E. Peptide nucleic acids (PNAs) with conformationally constrained, chiral cyclohexyl derived backbone. *Chem. Eur. J.* **1997**, *3*, 912–919.
- (53) Govindaraju, T.; Gonnade, R.; Badbhade, M.; Kumar V. A.; Ganesh, K. N. (1*S*,2*R*/1*R*,2*S*)-Aminocyclohexyl glycylyl thymine PNA: Synthesis, monomer crystal structures and DNA/RNA hybridization studies. *Org. Lett.* **2003**, *5*, 3013–3016.
- (54) Govindaraju, T.; Kumar, V. A.; Ganesh, K. N. Synthesis and evaluation of (1*S*,2*R*/1*R*,2*S*) aminocyclohexylglycylyl PNAs as conformationally preorganized PNA analogues for DNA/RNA recognition. *J. Org. Chem.* **2004**, *69*, 1858–1865.
- (55) Govindaraju, T.; Kumar, V. A.; Ganesh, K. N. *cis*-Cyclopentyl PNA (*cp*-PNA) as constrained chiral PNA analogues: stereochemical dependence of DNA/RNA hybridization. *Chem. Commun.* **2004**, 860–861.
- (56) Govindaraju, T.; Kumar, V. A.; Ganesh, K. N. (1*S*,2*R*/1*R*,2*S*)-*cis*-cyclopentyl PNA (*cp*PNA) as constrained PNA analogues: Synthesis and evaluation of *aeg-cp* PNA chimera and stereopreferences in hybridization with DNA/RNA. *J. Org. Chem.* **2004**, *69*, 5725–5734.
- (57) Myers, M. C.; Witschi, M. A.; Larinova, N. V.; Franck, J. M.; Haynes, R. D.; Hara, T.; Grakowski A.; Appella, D. H. A cyclopentane conformational restraint for a peptide nucleic acid: Design, synthesis and improved binding affinity to DNA and RNA. *Org. Lett.* **2003**, *5*, 2695–2698.
- (58) D'Costa, M.; Kumar, V. A.; Ganesh, K. N. N⁷-Guanine as a C⁺ mimic in hairpin *aeg/aep*PNA-DNA triplex: Probing binding selectivity by UV-*T_m* and kinetics by fluorescence-based strand-invasion assay. *J. Org. Chem.* **2003**, *68*, 4439–4445.
- (59) (a) Hunziker, J. On DNA. *Chimia* **2001**, *55*, 1038–1040. (b) Kaihatsu, K.; Janowski, B. A.; Corey, D. R. Recognition of chromosomal DNA by PNAs. *Chem. Biol.* **2004**, 749–758.

AR030277E