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(1*S*,2*R*/1*R*,2*S*)-*cis*-Cyclopentyl PNAs (*cp*PNAs) as Constrained PNA Analogues: Synthesis and Evaluation of *aeg-cp*PNA Chimera and Stereopreferences in Hybridization with DNA/RNA

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Conformationally constrained chiral PNA analogues were designed on the basis of stereospecific imposition of a 1,2-*c*is-cyclopentyl moiety on an aminoethyl segment of *aeg*PNA. It is known that the cyclopentane ring is a relatively flexible system in which the characteristic puckering dictates the pseudoaxial/pseudoequatorial dispositions of substituents. Hence, favorable torsional adjustments are possible to attain the necessary hybridization-competent conformations when the moiety is imposed on the conventional PNA backbone. The synthesis of the enantiomerically pure 1,2-*cis*-cyclopentyl PNA monomers (**10a** and **10b**) was achieved by stereoselective enzymatic hydrolysis of a key intermediate ester **2**. The chiral (1*S*,2*R*/1*R*,2*S*)-aminocyclopentylglycyl thymine monomers were incorporated into PNA oligomers at defined positions and through the entire sequence. Hybridization studies with complementary DNA and RNA sequences using UV−T_m measurements indicate that *aeg-cp*PNA chimera form thermally more stable complexes than *aeg*PNA with stereochemistry-dependent selective binding of cDNA/RNA. Differential gel shift retardation was observed on hybridization of *aeg-cp*PNAs with complementary DNA.

Introduction

In the area of modified nucleic acids and oligonucleotide analogues, several chemical modifications have been reported to date for applications in antisense therapeutics.[1] Among these, peptide nucleic acids (PNAs), composed of *N*-(2-aminoethyl)glycine units to which natural nucleobases are attached via a methylenecarbonyl linker (Figure 1, PNA **II**), are found to mimic many of the oligonucleotide properties.[2] PNA binds to complementary DNA and RNA to form duplexes via Watson−Crick base pairs and triplexes through Watson−Crick base pair and Hoogsteen hydrogen bonding, and PNA−DNA/RNA hybrids exhibit thermal stability higher than that of analogous DNA:DNA and DNA:RNA complexes.[3] Because of this attractive feature and stability to proteases and nucleases, PNAs are of great interest in medicinal chemistry,[4] with potential for the development as gene-targeted drugs (antigene and antisense) and as reagents in molecular biology and diagnostics.[5] However, PNA suffers from drawbacks such as poor aqueous solubility, cell permeability, and ambiguity in directional binding of the complementary DNA/RNA, in both parallel and antiparallel orientations. Attempts to address these shortcomings have led to several structural

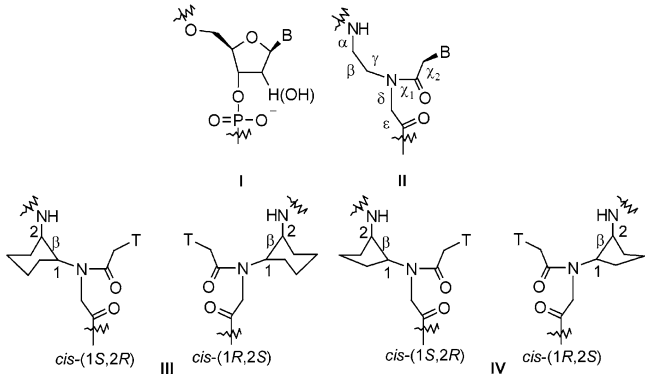

FIGURE 1. Structures of **I** DNA (RNA), **II** *aeg*PNA, **III** *ch*PNA, and **IV** *cp*PNA.

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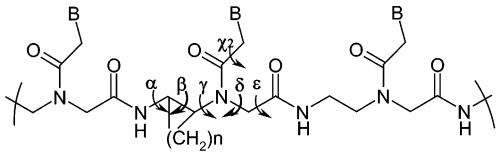
modifications of PNA.[6] The open-chain acyclic PNA backbone is conformationally flexible, and an effective way to preorganize the PNA strand for attaining hybridization competent conformation is to conformationally rigidify the backbone by introducing bulky substituents or ring structures into the backbone. Such approaches providing a conformational lock as in locked nucleic acids (LNAs) by Wengel et al.[7] or freezing the conformations of six-membered rings as in hexitol nucleic acids (HNAs)[8] and carbocyclic nucleic acids such as cylohexanyl (CNA)[9] and cyclohexenyl (CeNA)[10] nucleic acids by Herdewijn et al. have resulted in elegant designs of preorganized, hybridization-competent structures with remarkable success in oligonucleotide series. The pioneering work of Eschenmoser et al.[11,12] employing pyranosyl (*p*-RNA) and threofuranosyl (TNA) nucleic acids has shed light on the important conformational differences and the hybridization properties of five- and six-membered rings in a nucleic acid backbone. Deriving inspiration from such successes, we[13] and others[14] have employed cyclic PNA monomers based on a five-membered pyrrolidine or six-membered pipecolic acid nucleus as part of a PNA backbone to introduce conformational constraint, with varied success in terms of DNA/RNA recognition. In one of the earliest approaches, the six-membered carbocyclic *trans*-(1*S*,2*S*/1*R*,2*R*)-cyclohexyl ring was imposed on the ethylenediamine segment of the backbone[15] but was ineffective in hybridization to the complementary DNA. An analysis of the X-ray structural data of the PNA_2:DNA triplex[16a] and the PNA:DNA duplex[16b] and NMR data of the PNA:RNA duplex[16c] suggested to us that the dihedral angle β (Table 1) could be a key factor for the rational design of a preorganized PNA structure. The preferred values for β in the PNA_2:DNA triplex and the PNA:RNA duplex are in the range 65−70°, while that for the PNA:DNA duplex is about ∼140°, suggesting that it may be possible to impart DNA/RNA binding selectivity if β is restricted to 65−70° through suitable chemical modifications.

TABLE 1. Dihedral Angles (deg) in PNA and PNA:DNA/RNA Complexes



Peptide Nucleic acid (PNA), n=0 *aeg*PNA; n=3, *cp*PNA; n=4,*ch*PNA

compound	α	β	γ	δ	χ_1	χ_2
PNA_2:DNA[9a]	−103	73	70	93	1	−175
PNA:DNA[9b]	105	141	78	139	−3	151
PNA:RNA[9c]	170	67	79	84	4	−171
*ch*PNA(1*S*,2*R*)[a]	128	−63	76	119	1.02	−175
*ch*PNA(1*R*,2*S*)[a]	−129	66	−78	−119	−0.87	174
*cp*PNA(1*S*,2*R*)[a]	84	−24	86	90	0.89	165
*cp*PNA(1*R*,2*S*)[a]	−84	25	−86	−90	1.2	−165

[a] Dihedral angles from monomer crystal structures: *ch*PNA,[17] *cp*PNA.[19]

With such a rationale, we recently reported[17] *cis*-cyclohexyl PNA (*ch*PNA) in which the axial−equatorial disposition of cis-1,2 substituents with β in the range 63−66° (opposite in sign for the two enantiomers 1*S*,2*R* and 1*R*,2*S*) showed a stereochemistry-dependent preferential binding of the derived PNA-T oligomers to RNA as compared to DNA. This was interesting in comparison to the earlier report on trans-1,2-diaxial-substituted cyclohexyl system in which the dihedral angle β is 180° and hence unsuitable for forming hybrids with either DNA or RNA in agreement with X-ray data. However, a slightly lower binding affinity for triplex formation was seen with our *ch*PNA in comparison to unmodified *aeg*PNA.[17] We surmised that despite a favorable β, the substituted cyclohexyl ring is inherently too rigid as it gets locked up in either of the chair conformations. A relatively flexible system would be a cyclopentyl ring in which the characteristic interconvertible *endo*−*exo* puckering that dictates the pseudoaxial/pseudoequatorial dispositions of substituents[18] 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 recently reported preliminary studies of *cis*-(1*S*,2*R*/1*R*,2*S*)-cyclopentyl PNA-T (*cp*PNA)-incorporated homothymine *aeg*PNA chimera that showed stereochemistry-dependent, sequence-specific triplex formation with complementary DNA and RNA with a high mismatch discrimination.[19] The crystal structures of both the enantiomeric monomers supported the dihedral angle rationale. While our work was in progress, Appella et al.[20] reported the synthesis and limited studies on monosub-

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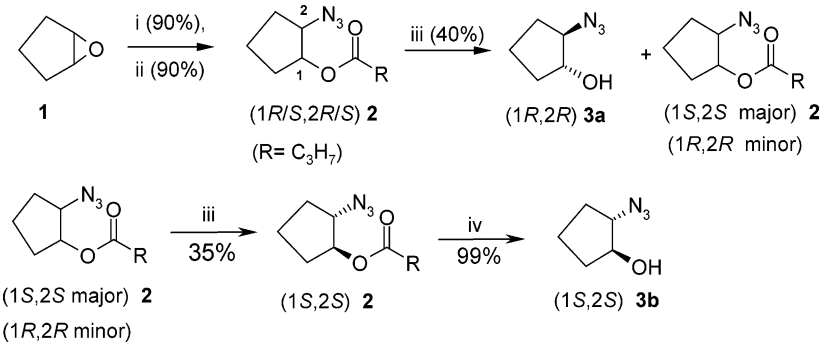
SCHEME 1[a]



[a] Reagents and conditions: (i) NaN_3, NH_4Cl, ethanol/water (2:8) reflux, 16 h. (ii) *n*-Butyric anhydride, dry pyridine, DMAP (cat), rt, 16 h. (iii) *P. cepacia* (lipase), phosphate buffer, pH 7.2, 1.5 h. (iv) NaOMe in MeOH.

stituted *trans*-(1*S*,2*S*)-cyclopentyl PNA that showed only weak stabilization of the derived PNA_2:DNA triplex over that of PNA:RNA duplex.

In this paper we present full results on the chemoenzymatic stereospecific synthesis of *cis*-(1*S*,2*R*/1*R*,2*S*)-*cp*PNA thymine monomers in their enantiomerically pure form, positionwise incorporation into homothymine *aeg*PNA oligomers, and DNA/RNA hybridization studies using UV−T_m measurements, gel electrophoretic shift assay, and circular dichroism analysis of the *cp*PNA hybrids. Electrophoretic gel shift assay shows differential retardation as a result of hybridization of *cp*PNA sequences with complementary DNA sequences whereas mismatched hybrids showed weaker and incomplete complexation. Interesting preferences are seen in DNA/RNA hybridization that are related to the stereochemistry of chiral *cp*PNA units.

Results and Discussion

Synthesis of *cis*-(1*S*,2*R*)- and (1*R*,2*S*)-Aminocyclopentylglycyl Thymine Monomers. The synthesis of title compounds was achieved starting from a mixture of (+)- and (−)-*trans*-2-azido cyclopentanoate **2** using lipases well-known in the literature.[21] The butyrate ester of racemic *trans*-2-azido cyclopentanol was synthesized by oxirane ring opening of the commercially available *meso*-epoxide **1** with sodium azide[22] followed by esterification with butyric anhydride (Scheme 1). The racemic butyrate ester **2** was resolved by enzymatic enantioselective hydrolysis using the lipase from *Pseudomonas cepacia* (Amano-PS)[23] in sodium phosphate buffer (pH = 7.2), which proceeded with 40% conversion, followed by chromatography, to afford the optically pure (1*R*,2*R*)-azido alcohol **3a**. *P. cepacia* lipase (Amano-PS) was found to be very effective for the resolution of *racemic-trans*-cyclohexanaote, but lipase from *Candida cylinderacea* was totally ineffective as reported.[17] We found that the Amano-PS also gave a slightly better enantiomeric purity and yield for the azido alcohols **3a** and **3b** than the reported ones under identical reaction conditions and in less reaction time. Further enzymatic treatment of the mixture of minor (1*R*,2*R*)- and major (1*S*,2*S*)-butyrate esters **2** for the second time followed by column purification and subsequent methanolysis of the (1*S*,2*S*)-**2** ester using NaOMe in methanol gave pure (1*S*,2*S*)-**3b** in 35% overall yield. The enantiopurity of both (1*R*,2*R*)-**3a** and (1*S*,2*S*)-**3b** isomers was confirmed by comparison with the known values of optical rotations reported in the literature[21] and by ^{1}H NMR using the chiral chemical shift reagent Eu(*hfc*)$_3$. The reduction of the azide function in (1*R*,2*R*)-**3a** by hydrogenation using Adam's catalyst[24] and in situ *t*-Boc protection of the resulting amine function yielded the Boc-protected amino alcohol (1*R*,2*R*)-**4** (Scheme 2). An attempt to convert alcohol **4** to corresponding mesylate (1*R*,2*R*)-**5** using mesyl chloride in pyridine with DMAP as a catalyst was unsuccessful. The mesylate **5** was obtained by using triethylamine (2.5 equiv) in dry dichloromethane at room temperature in less than 30 min. The resulting mesylate **5** is very unstable to acidic conditions and decomposed on silica gel during purification and hence was used for further reaction without any purification. The mesylate was treated with NaN_3 in dry DMF to yield the azide (1*S*,2*R*)-**6**, the reaction accompanied by inversion of configuration at C1.

The azide (1*S*,2*R*)-**6** was hydrogenated using Adam's catalyst to give the amine (1*S*,2*R*)-**7**, which without further purification was alkylated with ethyl bromoacetate in the presence of KF−Celite. This resulted in the monosubstituted *cis*-1,2-diamine (1*S*,2*R*)-**8**, which on acylation with chloroacetyl chloride gave the compound (1*S*,2*R*)-**9**. The condensation of (1*S*,2*R*)-**9** with thymine in the presence of K_2CO_3 in DMF yielded the desired (1*S*,2*R*)-aminocyclopentyl (thymin-1-yl-acetyl)glycyl PNA monomer ethyl ester **10a**, which on hydrolysis with 0.5 M LiOH in aqueous THF gave the free acid monomer (1*S*,2*R*)-**11a**. The synthesis of the other enantiomer (1*R*,2*S*)-**11b** was accomplished starting from alcohol (1*S*,2*S*)-**3b** following the same steps as described above. It should be pointed out that our synthetic route involving resolution steps permits synthesis of both cis enantiomers, while the route described for the corresponding trans analogues[13] allows access to only one of the enantiomers. All new compounds were characterized by ^{1}H and ^{13}C NMR and mass spectral data.

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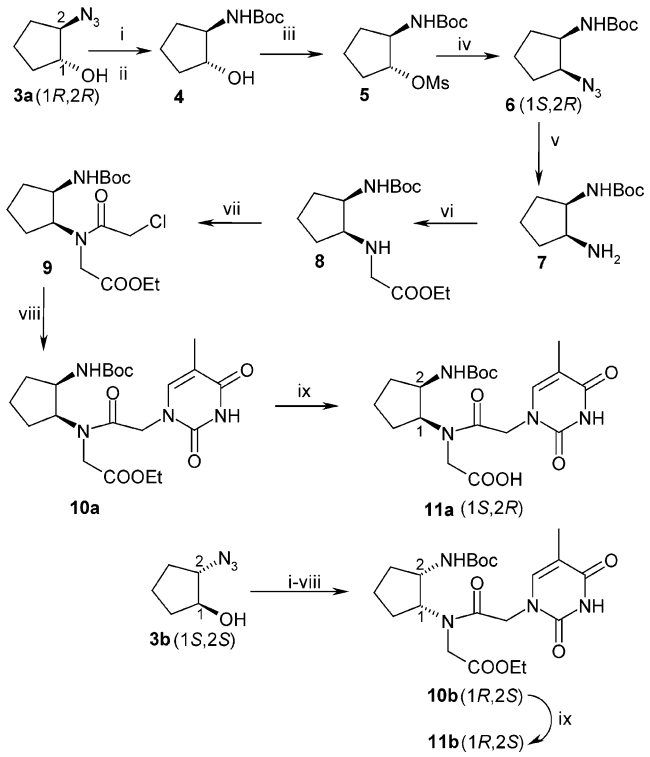
SCHEME 2[a]



[a] Reagent and conditions. (i) PtO_2, dry EtOAc, H_2, 35−40 psi, rt, 3.5 h. (ii) $(Boc)_2O$. (iii) MsCl, dry triethylamine, rt, 0.5 h. (iv) NaN_3, dry DMF, 70 °C, 5 h. (v) PtO_2, MeOH, H_2, 35−40 psi, rt, 3.5 h. (vi) $BrCH_2COOEt$, KF−Celite, dry CH_3CN, 65 °C, 4 h. (vii) $ClCH_2COCl$, Na_2CO_3, Dioxan/H_2O (1:1), 0 °C, 5 min. (viii) Thymine, K_2CO_3 dry DMF, 65 °C, 4 h. (ix) 0.5 M LiOH, aq THF, 0.5 h.

The enantiomeric purity of both alcohols (1*R*,2*R*)-**3a** and (1*S*,2*S*)-**3b** was confirmed from ^{1}H NMR of corresponding *O*-acetyl derivatives utilizing the chiral chemical shift reagent (+)-tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium(III) [Eu(*hfc*)$_3$] (8.0mol %),[25] and the ee for both **3a** and **3b** was found to be >98% (for data, see Supporting Information). The torsion angles β as deduced from the X-ray crystal structures[19] for (1*S*,2*R*)-**10a** and (1*R*,2*S*)-**10b** are −24 and +25° (Supporting Information), respectively, which are much less than that found in PNA_2:DNA and PNA:RNA complexes and *cis*-cyclohexyl systems and possess opposite signs, as expected for the enantiomeric pairs. In solution, the tertiary amide bond in PNA is known to exist as a rotameric mixture.[9] In the present structures, the amide bond is trans with carbonyl pointing toward the C-terminus, similar to that previously seen in cyanuryl PNA monomer[26] and to that seen in the recently reported crystal structure of the D-lysine-based chiral PNA:DNA duplex.[27] The crystal structure data also shows pseudoaxial−psuedoequatorial dispositions for the cis-1,2-substituents in (1*S*,2*R*/1*R*,2*S*)-cyclopentyl PNA monomers, with the bulky substituent carrying the nucleobase directed into a pseudoequatorial position. The CD of the monomers **10a** and **10b** gave the mirror-image CD signature as expected for the enantiomeric pairs (Supporting Information).

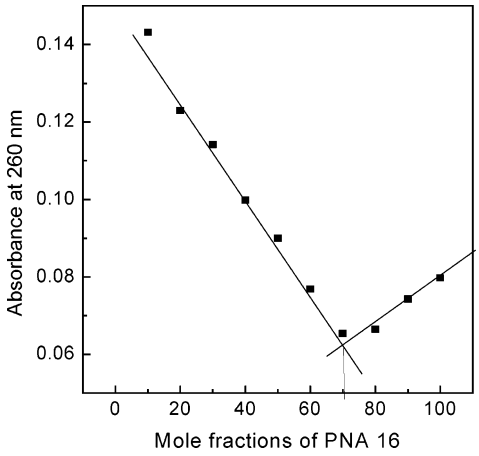


FIGURE 2. UV absorbance (at 260 nm) of mixtures of PNA **16** and the complementary DNA **I** in the relative molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, and 100:0 (buffer, 10 mM sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA).

Synthesis of *cp*PNA Oligomers. The modified *cis*-(1*S*,2*R*/1*R*,2*S*)-aminocyclopentylglycyl thymine monomers **11a** and **11b** were 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 homothymine PNA oligomers (**12**−**20**, Table 1) incorporating modified monomers at the middle (**14** and **17**), N-terminus (**15** and **18**), and C-terminus (**13** and **16**) and over the entire sequence (homooligomers **19** and **20**) were synthesized. The oligomers were cleaved from the resin using "low−high TFA-TFMSA" procedure[28] followed by RP-HPLC purification and characterized by mass spectrometry (MALDI-TOF).

UV−T_m Studies of *aeg-cp*PNA−DNA/RNA Complexes. The hybridization of various *cp*PNAs with complementary DNA **I** sequence was studied by temperature-dependent UV absorbance experiments. The stoichiometry for *aeg-cp*PNA:DNA complexation was established by Job's plot[29] of UV absorbance data at 260 nm and found to be 2:1 (Figure 2). The thermal stabilities (T_m) of *aeg-cp*PNA_2:DNA triplexes (Table 2, Figure 3) were obtained for various *cp*PNAs with complementary DNA **I** and that having a single mismatch (DNA **II**) and poly rA to study the relative compatibility of the imposed stereochemistry constraints with deoxyribonucleic and ribonucleic acids. In general, the PNA_2:DNA triplexes were found to be more stable for *cp*PNAs containing (1*S*,2*R*)- and (1*R*,2*S*)-cyclopentyl thymine units as compared to unmodified PNA. The *cp*PNA oligomers **14** and **17** having modifications in the center of the sequence showed contrasting binding stabilities as (1*S*,2*R*)-*cp*PNA **14** destabilized the complex with DNA **I** (ΔT_m = −23 °C), whereas (1*R*,2*S*)-

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TABLE 2. UV−T_m of *cp*PNA:DNA/RNA Triplexes[a]

entry	PNA	DNA **I**	DNA **II** (ΔT_m)[b]	poly rA
1	*aeg*PNA **12**, H-TTTTTTTT-LysNH$_2$	45.0	34.5 (10.5)	62.0
2	*cp*PNA **13**, H-TTTTTTT**t$_{SR}$**-LysNH$_2$	51.0	27.8 (23.2)	73.5
3	*cp*PNA **14**, H-TTT**t$_{SR}$**TTTT-LysNH$_2$	22.0	11.0 (11.0)	76.0
4	*cp*PNA **15**, H-**t$_{SR}$**TTTTTT-LysNH$_2$	44.5	26.4 (18.1)	66.0
5	*cp*PNA **16**, H-TTTTTTT**t$_{RS}$**-LysNH$_2$	55.0	28.0 (27.0)	>85.0
6	*cp*PNA **17**, H-TTT**t$_{RS}$**TTTT-LysNH$_2$	62.0	32.0 (30.0)	61.0
7	*cp*PNA **18**, H-**t$_{RS}$**TTTTTT-LysNH$_2$	48.7	27.8 (20.9)	69.0
8	*cp*PNA **19**, H-(**t$_{SR}$**)$_8$-LysNH$_2$	66.6	nd	>85.0
9	*cp*PNA **20**, H-(**t$_{RS}$**)$_8$-LysNH$_2$	72.0	nd	>85.0

[a] All values are an average of three independent experiments and accurate to within ±0.5°. DNA **I**, d(CGCAAAAAAAACGC); DNA **II**, d(CGCAAAACAAACGC). Buffer: sodium phosphate (10 mM), pH 7.0 with 100 mM NaCl and 0.1 mM EDTA; nd, transition not detected. [b] Mismatch destabilization.

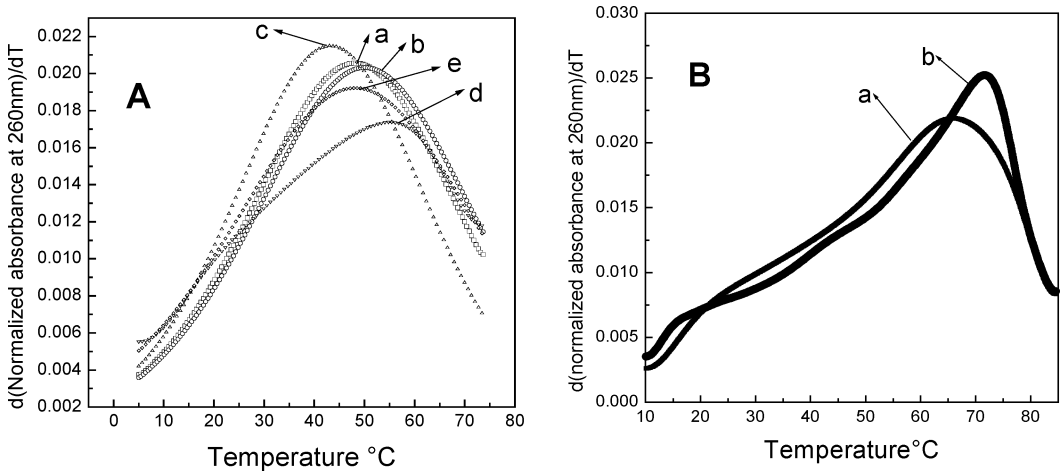

FIGURE 3. Derivative curves of melting profiles of (A) (a) *aeg*PNA **12**, (b) *cp*PNA **13**, (c) *cp*PNA **15**, (d) *cp*PNA **16**, (e) *cp*PNA **18** and (B) (a) *cp*PNA **19** and (b) *cp*PNA **20** with DNA **I**.

*cp*PNA **17** stabilized the complex (ΔT = +17 °C) in comparison to the control PNA **12**. Thus a remarkable T_m difference of 40 °C was noticed between the two enantiomeric PNAs **14** and **17** in binding the complementary DNA. In the case of *aeg-cp*PNAs (1*S*,2*R*)-**13** and (1*R*,2*S*)-**16** having single C-terminus modifications, both stabilized their complexes with DNA (ΔT = +6 and +10 °C, respectively) as compared to *aeg*PNA **12**, with a mutual difference of only +4 °C between themselves. The N-terminus modified *aeg-cp*PNAs (1*S*,2*R*)-**15** and (1*R*,2*S*)-**18** showed T_m values almost comparable to the unmodified PNA but lower compared to respective C-terminus-modified PNAs.

Significantly, in contrast to singly modified analogues, the all-modified homooligomeric, homochiral *cp*PNAs (1*S*,2*R*)-**19** and (1*R*,2*S*)-**20** exhibited high thermal stabilities (ΔT_m = +21 and +27 °C, respectively) compared to the unmodified PNA **12**. The PNA sequence with one modification in the center with (1*S*,2*R*)-stereochemistry, **14**, largely destabilized the complex with DNA **I**. This could be caused by the introduction of unfavored conformational discontinuity at the modification site. However, in the fully modified oligomer **19** with the same stereochemistry, the stability is regained perhaps due to a uniform conformational change over the entire backbone, without any sharp discontinuities. Such a behavior is not unusual and has been observed by Nielsen et al in the case of the pyrrolidine PNA.[14d] The singly and fully modified sequences with the other and probably favorable stereochemistry (1*R*,2*S*) in PNA sequences **17** and **20** show better binding affinity toward DNA **I**, suggesting that the effect of increased T_m could be additive (1.4 °C per modification) from a single favorable modification to fully modified oligomer sequences.

The enhanced thermal stabilities observed for *aeg-cp*PNA and *cp*PNA complexes with DNA is not at the cost of losing base pairing specificities. This is borne by data for their complexation with DNA **II** [dA$_4$*C*A$_3$] that carries a single mismatch at the middle position. The mismatch ΔT_m for control *aeg*PNA **12** was −10.5°, while that for all *cp*PNAs were higher, except for (1*S*,2*R*)-**14**, which was as good. The mismatch destabilization was highest (−30°) for (1*R*,2*S*)-**17** with modification at the center. Other modified PNAs exhibited intermediate degrees of mismatch destabilization in the range 18−27°. The lower mismatch tolerance and higher binding affinities to matched complementary DNA **I** sequence reflects the sequence specific recognition of *cp*PNAs. The fully modified oligomers **19** and **20** did not show any binding with mismatch DNA **II**. Complete inhibition of hybridization with fully modified sequences *cp*PNA **19** and **20** with DNA **II** having a mismatch in the center of the sequence could be a result of mismatched hydrogen bonding in the center, in turn effecting steric incompatibility of a fully modified sequence for DNA binding. The open-chain PNAs in other sequences do not impose the additional steric incompatibility and permit complex formation with DNA **II**, albeit reduced stability. In general, the (1*R*,2*S*)-cyclopentyl modified PNAs were

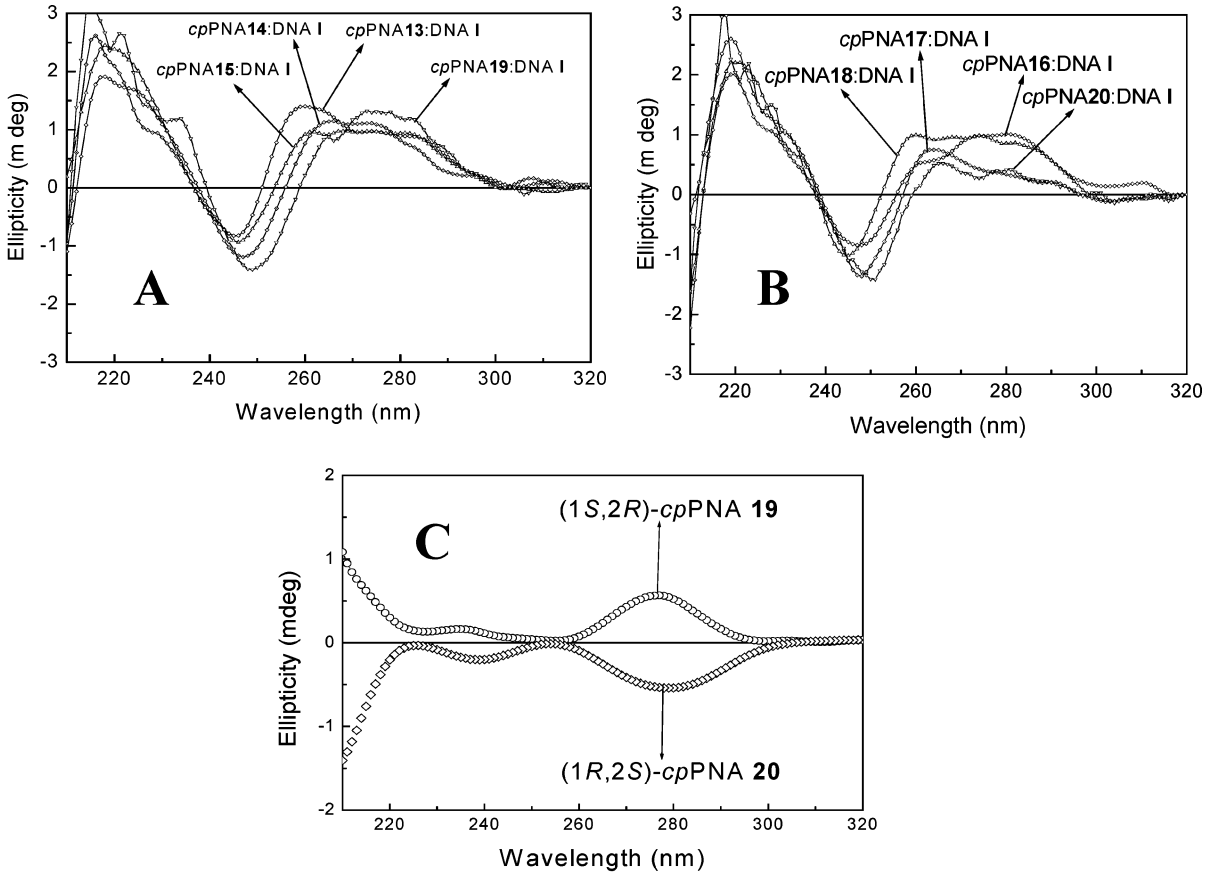

FIGURE 4. A: CD spectra of complexes (*cp*PNA **13**)$_2$:DNA **I**, (*cp*PNA **14**)$_2$:DNA **I**, (*cp*PNA **15**)$_2$:DNA **I**, and (*cp*PNA **19**)$_2$:DNA **I**. B: CD spectra of complexes (*cp*PNA **16**)$_2$:DNA **I**, (*cp*PNA **17**)$_2$:DNA **I**, (*cp*PNA **18**)$_2$:DNA **I**, and (*cp*PNA **20**)$_2$:DNA **I**. C: CD spectra of *cp*PNA **19** (SS), *cp*PNA **20** (SS) (10 mM sodium phosphate buffer, pH = 7, 100 mM NaCl, 0.1 m M EDTA, 20 °C).

better in binding and mismatch differentiation in complexing with complementary DNA.

In the case of *aeg-cp*PNA complexation with poly rA, the presence of either *S*,*R* or *R*,*S* isomers at C/N terminus or internally, in general, stabilized the complexes compared to *aeg*PNA. The *cp*PNA oligomers **14** and **17**, modified at the center showed a reverse selectivity in binding to RNA with (*S*,*R*)-**14** > (*R*,*S*)-**17** by 15° as compared to binding with DNA **I**, where (*R*,*S*)-**17** > (*S*,*R*)-**14** by 40°. Unprecedented stabilization was observed for homooligomeric, homochiral (*S*,*R*)-**18** and (*R*,*S*)-**19** *cp*PNAs binding with poly rA, with no melting seen up to 85 °C. The overall pattern of T_m values suggests that *cp*PNAs have a preference for binding to RNA over DNA, though this needs to be verified with mixed-base oligoribonucleotides.

CD Spectroscopic Studies of *cp*PNA:DNA Complexes. Being achiral, *aeg*PNAs show very weak CD signatures due to the presence of L-lysine at the C-termius. 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[30] is accompanied by appearance of positive CD bands at 258 and 285 nm that are not present in DNA. The presence of chiral centers in cyclopentyl PNAs should further influence the CD patterns of the derived PNA:DNA triplexes. Figure 4 shows the CD spectra of complexes of *cp*PNAs **13**−**20** with DNA **I**. The single stranded cyclopentyl PNAs (**13**−**18**) show weak CD bands in the 250−290 nm region (Supporting Information), and all *cp*PNA:DNA triplexes show the expected positive bands at 258 and 285 nm. The relative ratios of 258/285 nm are different for the cyclopentyl PNA:DNA triplexes as compared to the control PNA:DNA triplex. These results imply that the cyclopentyl modifications alter base stacking patterns in PNA:DNA complexes. Systematic changes in the CD spectral features also occur in continuous variation mixing of *cp*PNA and DNA components to generate Job's plot (Supporting Information) for determining the binding ratio, which corresponded to 2:1 for the cyclopentyl PNA:DNA complexes. Fully modified single-stranded *cp*PNAs containing (1*S*,2*R*)- or (1*R*,2*S*)-cyclopentyl thymines show weak but distinct CD spectra (Figure 4C), which are near mirror images of each other and similar to that of monomers.

Electrophoretic Gel Shift Assay and Competitive Binding Experiments. Electrophoretic gel experiment[17b,31] was used to establish the binding of modified PNAs to the complementary DNA **I** and mismatch DNA **II** (Figure 5). The various PNAs were individually mixed with DNA **I** in buffer (for conditions, see caption of Figure 5) and subjected to nondenaturing gel electrophoresis at

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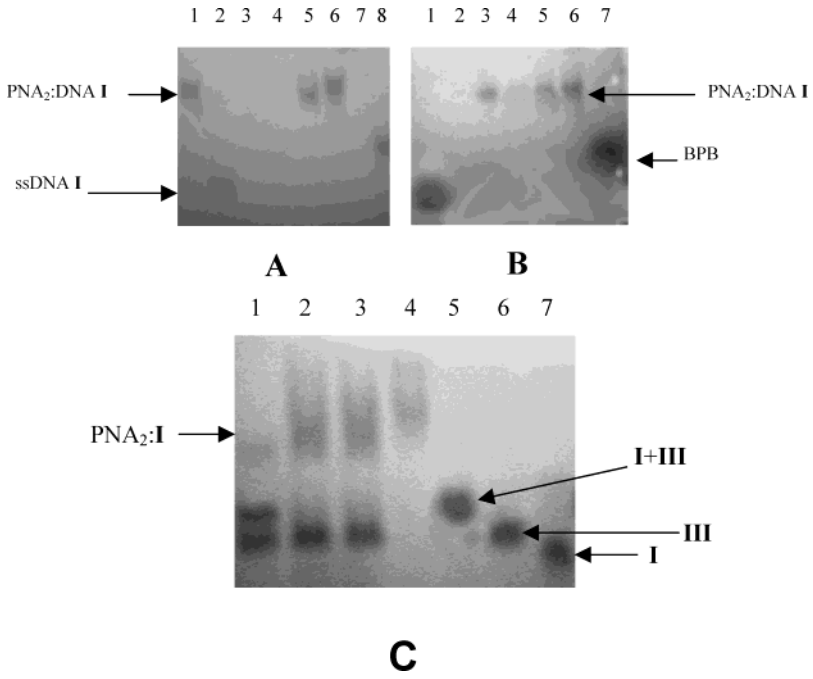

FIGURE 5. PNA:DNA Complexation. A: lane 1, (*aeg*PNA **12**)$_2$ + DNA **I**; lane 2, ssDNA **I**; lane 3, ss*cp*PNA **12**; lane 4, ss*cp*PNA **14**; lane 5, (*cp*PNA **14**)$_2$:DNA **I**; lane 6, (*cp*PNA **17**)$_2$ + DNA **I**; lane 7, ss*cp*PNA **17**; lane 8, marker bromophenol blue (BPB): **B**, lane 1, ssDNA **I**; lane 2, ss*cp*PNA **13**; lane 3, (*cp*PNA **13**)$_2$ + DNA **I**; lane 4, (*cp*PNA **15**)$_2$ + DNA **I**; lane 5, (*cp*PNA **16**)$_2$ + DNA **I**; lane 6, (*cp*PNA **18**)$_2$ + DNA **I**; lane 7, BPB. C: Competition binding experiments. Lane 1, DNA **I** + DNA **III** + (*cp*PNA **14**)$_2$; lane 2, DNA **I** + DNA **III** + (*cp*PNA **15**)$_2$; lane 3, DNA **I** + DNA **III** + (*cp*PNA **17**)$_2$; lane 4, DNA **I** + (*cp*PNA **17**)$_2$; lane 5, DNA duplex (**I** + **III**); lane 6, ssDNA **III**; lane 7, ssDNA **I**. Buffer, 0.5 X TBE, pH 8.0; DNA **I** = CGCA$_8$CGC, DNA **III** = GCGT$_8$GCG, BPB = bromophenol blue.

10 °C, and the bands were visualized on a fluorescent TLC background. The formation of PNA$_2$:DNA complexes was accompanied by the disappearance of the band due to single-strand DNA **I** and appearance of a lower migrating band of the complex. The singly modified PNAs **13**−**18** upon mixing with DNA **I** migrated about the same as the unmodified PNA **12**:DNA **I** complex and much lower than that of DNA **I** (Figures 5A and 5B), clearly showing successful formation of *cp*PNA$_2$:DNA **I** triplexes. Under the conditions, the single-stranded PNAs do not migrate from the well. The gel retardation experiments for singly modified *aeg-cp*PNAs with mismatch DNA **II** showed weaker and incomplete complexation, as seen by the presence of faster moving band unbound DNA **II** (Supporting Information). Competition binding experiments were carried out by adding *aeg-cp*PNAs **15** or **17** separately to the DNA duplex (**I**:**III**) followed by annealing and gel electrophoresis. Due to a higher stabilization of *aeg-cp*PNA:DNA **I** complexes compared with the DNA: DNA duplex, the added PNA binds to the complementary DNA **I** in the DNA duplex, concomitantly releasing the DNA strand **III** (Figure 5C). This is clearly seen in the gel electrophoresis by the appearance of lower moving bands due to the *aeg-cp*PNA:DNA **I** complex (Figure 5C, lanes 2 and 3), a faster moving band due to the released DNA strand **III**, and the disappearance of the DNA (**I**:**III**) duplex band (as in lane 5). When the experiment is performed with *aeg-cp*PNA**14**:DNA **I** complex that has a lower stability than DNA duplex, the competition binding is ineffective, as seen by a weak band due to the PNA:DNA complex and in efficient dissociation of DNA duplex **I**:**III**, which is still persistent (Figure 5C, lane 1). Thus, the modified *aeg-cp*PNAs are capable of successfully competing for binding with the complementary DNA **I** strand in a DNA duplex, just as in unmodified PNA.

Comparison of *cis*-Cyclohexyl (*ch*PNA) and *cis*-Cyclopentyl (*cp*PNA) PNAs: Affinity vs Selectivity. *ch*PNA and *cp*PNA are the result of the designs based on optimizing dihedral angle β to constrain PNA backbone for optimal DNA/RNA binding and discrimination via preorganization-mediated entropy gain. The UV melting temperatures of the *ch*PNAs with complementary DNA and RNA suggested a stereochemistry-dependent discrimination, but the thermal stability of resultant cDNA/RNA complexes was reduced compared to *aeg*PNA. The cyclohexyl ring although has the dihedral β in the requisite range of 65−75° has the probable drawback that the ring gets locked into one of the chair conformations, which may induce unfavorable conformation in the resultant PNA oligomer. To improve the binding affinity and enhance the thermal stability of the complexes without losing the discrimination and stereospecificity, *cp*PNAs were designed on the basis of the fact that the cyclopentyl ring is less rigid than the cyclohexyl ring, and unlike the cyclohexyl ring, it has inherent puckering flexibility with a low-energy barrier for transitions. This may allow such systems to induce a favorable conformation toward cRNA binding, inspite of the dihedral angle β being much less (∼25°) than the cyclohexyl system (∼65°). Thus, these offer a balance between the complete rigidity of cyclohexyl systems and the flexibility of open-chain PNA backbone to fine-tune the dihedral angle β in modified PNA oligomers. *cp*PNAs showed a higher binding affinity to cDNA/RNA with stereochemistry-dependent DNA/RNA discrimination. The binding of *cis*-cyclopentyl modifications reported here appear to be

superior to the *trans*-cyclopentyl analogues reported recently.[13] The stereoselective binding was reflected in their lowering T_m values with mismatch cDNA (ΔT = −18 to 30 °C), which is more than that observed with *ch*PNAs. The relative ease of conformational adjustments in *cis*-cyclopentyl system seems to have significant consequences on the hybridization ability of *cp*PNA oligomers despite the less dihedral angle compared to *ch*PNAs.

When biochemical recognitions are mediated by steric fit, higher binding affinity is accompanied by higher recognition specificities (positive correlation), which is the case for most enzyme−substrate interactions. A negative correlation is intrinsic to the nucleation-zipping model, which governs the sequence recognition between complementary nucleic acid strands.[32] Strategies to improve the affinity of oligonucleotide analogues along with a corresponding increase in sequence fidelity could involve enhancing both hydrogen bonding interactions (to have an enthalpic advantage) and steric fitting (to impart an entropic advantage). In our previous studies that involved rational chemical modifications to induce preorganization of a single-stranded PNA backbone to match that of the DNA/RNA hybrids, higher affinities are mostly accompanied by a higher discrimination of single mismatches. In modified analogues with somewhat rigid structural features, incorrect hydrogen bonding recognition of DNA sequences with a single-base mismatch would amplify destabilization effects due to steric interruption in the zipping mechanism. The observed differences in DNA affinity between cyclohexyl and cyclopentyl PNAs may arise from differences in relative contributions of the above factors, and delineating these may help achieve optimal fine-tuning of PNA chemical modifications to achieve balanced affinity/selectivity toward target sequences.

Conclusion

To introduce PNA binding selectivity between DNA and RNA and higher affinity, we have synthesized *cp*PNAs incorporating optically pure *cis*-(1*S*,2*R*)- and *cis*-(1*R*,2*S*)-aminocyclopentylglycyl thymine monomers. These monomers were built into PNA oligomers by solid-phase peptide synthesis; UV melting temperatures of the *cp*PNAs with complementary and mismatched DNA and RNA indicated that *cp*PNAs having a (1*S*,2*R*)-cyclopentyl unit have a higher binding affinity to RNA than DNA, and PNA oligomers with (1*R*,2*S*)-cyclopentyl units showed relatively higher affinity toward DNA. The stereopreferences in *cp*PNA binding of DNA/RNA is accompanied by overall enhanced binding affinities compared to *aeg*-PNA, unlike *ch*PNA where stereochemistry-dependent discrimination of DNA/RNA was observed but with lower T_m values. *cis*-*cp*PNAs show high mismatch discrimination compared to *aeg*PNAs, revealing base-specific recognition. The torsional angle β in a 1,2-disubstituted *cis*-cyclopentyl system is less than that in *ch*PNA but seems to have significant consequences on the hybridization ability of *cp*PNA oligomers. The higher binding of cyclopentyl PNAs is perhaps a consequence of the relative ease of conformational adjustments in a cyclopentyl ring as compared to rigid locking of cyclohexane systems. The favorable conformational features of the monomers are cooperatively transmitted to the oligomeric level. The results of *cis*-*cp*PNAs presented here support the idea of improving the stability and DNA/RNA binding selectivity via rational conformational tuning of classical PNA to achieve preorganization. Further studies on sequence-dependent and RNA/DNA discriminatory effects of *cp*PNA in mixed base *cp*PNA sequences and thermodynamic studies to delineate entropic and enthalpic contributions are in progress.

Experimental Section

Enzymatic Resolution of Racemic Butyrate (1*R*/*S*,2*R*/*S*)-2 with *P. cepacia*. To a solution of enzyme *P. cepacia* (500 mg) in phosphate buffer (150 mL, pH = 7.2) was added racemic butyrate (1*R*/*S*,2*R*/*S*)-**2** (10 g). The mixture was stirred vigorously for 1.5 h, which accomplished 40% conversion for alcohol (−) (1*R*,2*R*)-**3a**. The enzyme was separated by filtration, and the filtrate was extracted into DCM. The solvent was evaporated, and the column chromatographic separation of the residue afforded chiral pure alcohol (1*R*,2*R*)-**3a** and optically enriched butyrate (1*S*,2*S*)-**2**. The optically enriched butyrate (*1S*,*2S*)-**2** was subjected to second enzyme hydrolysis as described above to yield the optically pure butyrate (1*S*,2*S*)-**2** after column separation. From butyrate (1*S*,2*S*)-**2**, alcohol (1*S*,2*S*)-**3b** was obtained by methanolysis with a catalytic amount of NaOMe in MeOH in 35% overall yield.

The enantiomeric purity of **3a** and **3b** was confirmed by their optical rotations: (*1R*,*2R*)-**3a** $[\alpha]_{20}^{D}$ −84.0° (lit.[13] −78.0°, *c* 1.5, CH_2Cl_2); (1*S*,2*S*)-**3b**; $[\alpha]_{20}^{D}$ +84.0° (lit.[13] +84.1°, *c* 1.6, CH_2Cl_2).

(1*R*,2*R*)-2-(*N*-*t*-Boc-Amino)-cyclopentanol (1*R*,2*R*)-4. To a solution of (1*R*,2*R*)-2-azido cyclopentanol (3.5 g, 27.55 mmol) in dry ethyl acetate (10 mL) placed in a hydrogenation flask was added di-*tert*-butyl dicarbonate (7.21 g, 33.06 mmol) and Adams catalyst (2 mol %). The mixture was hydrogenated in a Parr apparatus (rt, 35−40 psi, 3.5 h). The catalyst was filtered, and the solvent in the filtrate was evaporated under reduced pressure; the residue was purified by column chromatography (EtOAc/petroleum ether) to afford a white solid of the alcohol (1*R*,2*R*)-**4**: yield 4.6 g, 83%; mp 87.0 °C; $[\alpha]^{20}_{D}$ +21.0° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 1.00−1.45 (10H, m, 4-CH, *t*-Boc), 1.45−1.8 (3H, m, 4-CH, 3-CH_2), 1.8−2.15 (2H, m, 5-CH_2), 3.4−3.7(1H, m, 2-CH), 3.8−4.0 (1H, m, 1-CH), 4.1−4.4 (1H, bd, −OH), 4.6−5.0 (1H, bd, carbamate NH); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz) δ_C 20.2, 27.9, 29.5, 31.5, 59.5, 78.2, 78.9, 156.5. Anal. Calcd (%) for $C_{10}H_{19}NO_3$: C, 59.7; H, 9.45; N, 6.96. Found: C, 59.63; H, 9.81; N, 6.86. LCMS: 202.05 [M + H].

(1*S*,2*S*)-2-(*N*-*t*-Boc-Amino)-cyclopentanol (1*S*,2*S*)-4. This was prepared from the azido alcohol (1*S*,2*S*)-**3b** using a procedure similar to the one described for the alcohol (1*R*,2*R*)-**4**: mp 87.0 °C; $[\alpha]^{20}_{D}$ −21.0° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 1.00−1.45 (10H, m, 4-CH, *t*-Boc), 1.45−1.8 (3H, m, 4-CH, 3-CH_2), 1.8−2.15 (2H, m, 5-CH_2), 3.4−3.7 (1H, m, 2-CH), 3.8−4.0 (1H, m, 1-CH), 4.1−4.4 (1H, bd, −OH), 4.6−5.0 (1H, bd, carbamate NH); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz): δ_C 20.2, 27.9, 29.5, 31.5, 59.5, 78.2, 78.9, 156.5. Anal. Calcd (%) for $C_{10}H_{19}NO_3$: C, 59.7; H, 9.45; N, 6.96. Found: C, 59.67; H, 9.89; N, 6.80. LCMS: 202.05 [M + H].

(1*R*,2*R*)-2-(*N*-*t*-Boc-Amino)-cyclopentan-1-methyl Sulfonate (1*R*,2*R*)-5. To a stirred solution of alcohol (1*R*,2*R*)-**4** (4.3 g, 21.39 mmol) and triethylamine (6.49 g, 64.17 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C under nitrogen was added methanesulfonyl chloride (3.92 g, 34.22 mmol) over a period of 15 min. The mixture was stirred for another 20 min at room temperature, and the solvent was evaporated under reduced

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pressure. The residue was extracted with CH_2Cl_2, washed successively with $KHSO_4$ solution, water, and brine, and stored over Na_2SO_4. The organic layer was concentrated to afford mesylate (1*R*,2*R*)-**5**. The yield was 5.6 g, 93%, which was used without purification for further reaction.

(1*S*,2*S*)-2-(*N*-*t*-Boc-Amino)-cyclopentan-1-methyl Sulfonate (1*S*,2*S*)-5. The compound (1*S*,2*S*)-**5** prepared from the alcohol (1*S*,2*S*)-**4** using a procedure similar to the one described for the mesylate (1*R*,2*R*)-**5**, and the crude product was used for the further reaction without any purification.

(1*S*,2*R*)-2-(*N*-*t*-Boc-Amino)-1-azidocyclopentane (1*S*,2*R*)-6. A stirred mixture of the mesylate (1*R*,2*R*)-**5** (5.0 g, 17.92 mmol) and NaN_3 (9.3 g, 0.143 mol) in DMF (25 mL) under nitrogen was heated at 68−70 °C for 5 h. After the mixture was cooled, the solvent was evaporated under reduced pressure and the residue was extracted with EtOAc (25 mL x 2) and stored over Na_2SO_4. The organic layer was removed under reduced pressure, and the crude product was purified by column chromatography (EtOAc/petroleum ether) to afford a white solid of azide (1*S*,2*R*)-**6**: yield (3.65 g, 91.5%); mp 81.0 °C; IR ν (cm^{-1}) (KBr) 3442.7, 3014.53, 2995.24, 2113.84, 1706.88 cm^{-1}; $[\alpha]_D^{20}$ + 116° (*c* 1.0, CH_2Cl_2); ^1H NMR ($CHCl_3$-*d*, 200 MHz); δ_H 1.0−1.45 (11H, m, 4-CH_2, *t*-Boc), 1.65−2.0 (4H, m, 5-CH_2, 3-CH_2), 3.7−4.1 (2H, m, 1-CH, 2-CH), 4.6−5.0 (1H, bd, carbamate NH); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz) δ_C 19.8, 28.2, 28.7, 29.0, 54.7, 64.1, 79.4, 155.3. Anal. Calcd (%) for $C_{10}H_{18}N_4O_2$: C, 53.09; H, 7.96; N, 24.77. Found: C, 52.55; H, 7.9; N, 24.8. MS (FAB+): 227 (35%) [M + 1], 171 (100%) [M + 1 − N_3], 127 (15%) [M + 1 − *t*Boc].

(1*R*,2*S*)-2-(*N*-*t*-Boc-Amino)-1-azidocyclopentane (1*R*,2*S*)-6. A procedure similar to the one described for (1*S*,2*R*)-**6** was used to prepare (1*R*,2*S*)-**6** from (1*S*,2*S*)-**5**: mp 81.0 °C; IR ν (cm^{-1}) (KBr) 3442.7, 3014.53, 2995.24, 2113.84, 1706.88 cm^{-1}; $[\alpha]_D^{20}$ −116° (*c* 1.0, CH_2Cl_2); ^1H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 1.0−1.45 (11H, m, 4-CH_2, *t*-Boc), 1.65−2.0 (4H, m, 5-CH_2, 3-CH_2), 3.7−4.1 (2H, m, 1-CH, 2-CH), 4.6−5.0 (1H, bd, carbamate NH); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz) δ_C 19.8, 28.2, 28.7, 29.0, 54.7, 64.1, 79.4, 155.3. Anal. Calcd (%) for $C_{10}H_{18}N_4O_2$: C, 53.09; H, 7.96; N, 24.77. Found: C, 52.55; H, 7.98; N, 24.71. MS (FAB+) 227 (35%) [M + 1], 171 (100%) [M + 1 − N_3], 127 (15%) [M + 1 − *t*Boc].

(1*S*,2*R*)-2-(*N*-*t*-Boc-Amino)-1-aminocyclopentane(1*S*,2*R*)-7. To a solution of the azide (1*S*,2*R*)-**6** (3.0 g, 13.27 mmol) in methanol (5 mL) taken in hydrogenation flask was added Adam's catalyst (2 mol %). The reaction mixture was hydrogenated in a Parr apparatus for 3.5 h at room temperature and a H_2 pressure of 35−40 psi. The catalyst was filtered off, and the solvent was removed under reduced pressure to yield a residue of the amine (1*S*,2*R*)-**7** as a colorless oil. The yield was 2.6 g, 98.0%; this compound was used for further reaction without any purification.

(1*R*,2*S*)-2-(*N*-*t*-Boc)-1-Aminocyclopentane (1*R*,2*S*)-7. The amine (1*R*,2*S*)-**7** was obtained on hydrogenation of the azide (1*R*,2*S*)-**6** following the same procedure described for the synthesis of the amine (1*S*,2*R*)-**7**.

***N*-[(2*R*)-*t*-Boc-Aminocyclopent-(1*S*)-yl]-glycine Ethyl Ester (1*S*,2*R*)-8.** To a stirred mixture of amine (1*S*,2*R*)-**7** (2.5 g, 12.5 mmol) and freshly prepared KF−Celite (4.35 g, 37.5 mmol) in dry acetonitrile (150 mL) was added ethyl bromoacetate (1.87 g, 11.25 mmol) dropwise for 30 min at room temperature under a nitrogen atmosphere, and the mixture was heated to 65 °C. After 3.0 h, the Celite was filtered off and the solvent in the filtrate was evaporated under reduced pressure, which on column chromatographic purification (EtOAc) afforded the ethyl ester (1*S*,2*R*)-**8** as a colorless oil: yield 2.81 g, 78.5%; $[\alpha]_D^{20}$ −32.69° (*c* 1.0, CH_2Cl_2); ^1H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 0.62−2.1 (18H, m, 4-CH_2, 3-CH_2, 5-CH_2, *t*-Boc, ester-CH_3), 2.7−3.1 (1H, bd, 2-CH), 3.1−3.4 (2H, s, $-CH_2-C=O$), 3.5−3.9 (1H, bd, 1-CH), 3.9−4.2 (2H, q, ester-CH_2), 4.9−5.6 (2H, bd, NH and carbamate-NH); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz) δ_C 13.5, 19.9, 27.8, 29.3, 30.2, 48.6, 52.5, 59.5, 60.1, 78.4, 155.5, 171.2. Anal. Calcd (%) for $C_{14}H_{26}N_2O_4$: C, 58.74; H, 9.09; N, 9.79. Found: C, 58.85; H, 8.66; N, 9.50. LCMS: 287.05 [M + H], 187 [M + H − *t*Boc].

***N*-[(2*S*)-*t*-Boc-Aminocyclopent-(1*R*)-yl]-glycine Ethyl Ester (1*R*,2*S*)-8.** A procedure similar to the one described for the ethyl ester (1*S*,2*R*)-**8** afforded (1*R*,2*S*)-**8** starting from the amine (1*R*,2*S*)-**7**: $[\alpha]_D^{20}$ +32.69° (*c* 1.0, CH_2Cl_2); ^1H NMR ($CHCl_3$-*d*, 200 MHz); δ_H 0.62−2.1 (18H, m, 4-CH_2, 3-CH_2, 5-CH_2, *t*-Boc, ester-CH_3), 2.7−3.1 (1H, bd, 2-CH), 3.1−3.4 (2H, s, $-CH_2-C=O$), 3.5−3.9 (1H, bd, 1-CH), 3.9−4.2 (2H, q, ester-CH_2), 4.9−5.6 (2H, bd, NH and carbamate-NH); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz) δ_C 13.5, 19.9, 27.8, 29.3, 30.2, 48.6, 52.5, 59.5, 60.1, 78.4, 155.51, 171.2. Anal. Calcd (%) for $C_{14}H_{26}N_2O_4$: C, 58.74; H, 9.09; N, 9.79. Found: C, 58.87; H, 8.71; N, 9.72. LCMS; 287.05 [M + H], 187.05 [M + H − *t*Boc].

***N*-[(2*R*)-*t*-Boc-Aminocyclopentan-(1*S*)-yl]-*N*-(chloroacetyl)-glycine Ethyl Ester (1*S*,2*R*)-9.** To a stirred solution of the amine (1*R*,2*S*)-**8** (2.5 g, 8.7 mmol) in 10% Na_2CO_3 (65 mL) and 1,4-dioxan (65 mL) cooled to 0 °C was added chloroacetyl chloride (4.93 g, 43.7 mmol) in two additions. After 30 min, the dioxan was removed under reduced pressure and the residue was extracted into EtOAc (2 × 50 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (MeOH/CH_2Cl_2) affording chloro compound (1*S*,2*R*)-**9** as a white solid: yield 2.4 g, 76%; mp 131.0 °C; $[\alpha]_D^{20}$ +55.0° (*c* 1.0, CH_2Cl_2); ^1H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.0−2.4 (18H, m, $3CH_2$ cycl, *t*Boc, ester CH_3), 3.5−4.5 (8H, m, 1-CH, 2-CH, N−CH_2, $-COCH_2Cl$, $-COOCH_2$), 5.5−6.0 (1H, bd, carbamate NH); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 13.7, 20.8, 27.8, 28.0, 30.5, 41.1, 46.05, 53.3, 58.8, 61.0, 78.2, 155.5, 167.6, 169.5. Anal. Calcd (%) for $C_{16}H_{27}N_2O_5Cl$: C, 52.89; H, 7.43; N, 7.71; Cl, 9.77. Found: C, 52.61; H, 7.91; N, 7.58; Cl, 9.72. MS LCMS: 363.05 [M + H], 263.05 [M + 1 − *t*Boc].

***N*-[(2*S*)-*t*-Boc-Aminocyclopentan-(1*R*)-yl]-*N*-(chloroacetyl)-glycine Ethyl Ester (1*R*,2*S*)-9.** This compound was prepared from the amine (1*S*,2*R*)-**8** following the procedure described for the chloro compound (*1S*,*2R*)-**9**: mp 131.0 °C; $[\alpha]_D^{20}$ −55.0° (*c* 1.0, CH_2Cl_2); ^1H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.0−2.4 (18H, m, $3CH_2$ cycl, *t*Boc, ester CH_3), 3.5−4.5 (8H, m, 1-CH, 2-CH, N−CH_2, $-COCH_2Cl$, $-COOCH_2$), 5.5−6.0 (1H, bd, carbamate NH); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 13.7, 20.8, 27.8, 28.0, 30.5, 41.1, 46.0, 53.3, 58.8, 61.0, 78.2, 155.5, 167.6, 169.5; Anal.Calcd (%) for $C_{16}H_{27}N_2O_5Cl$: C, 52.89; H, 7.43; N, 7.71; Cl, 9.77; Found: C, 52.73; H, 7.97; N, 7.61; Cl, 9.75: MS LCMS; 363.05 [M+H], 263.05 [M+1-*t*Boc].

***N*-[(2*R*)-*t*-Boc-Aminocyclopent-(1*S*)-yl]-*N*-(thymin-1-acetyl)-glycine Ethyl Ester (1*S*,2*R*)-10a.** A mixture of chloro compound (1*S*,2*R*)-**9a** (2.0 g, 5.52 mmol), thymine (0.70 g, 5.52 mmol), and anhydrous K_2CO_3 (0.91 g, 6.62 mmol) in dry DMF (10 mL) under nitrogen was heated with stirring at 65 °C for 4.0 h. After cooling, the solvent was removed under reduced pressure to leave a residue, which was extracted into DCM (2 × 25 mL) and dried over Na_2SO_4. The solvent was evaporated, and the crude compound was purified by column chromatography (MeOH/DCM) to afford a white solid of thymine monomer ethyl ester (1*S*,2*R*)-**10a**: yield 1.8 g, 72.2%; mp 211.0−215.0 °C; $[\alpha]_D^{20}$ +32.64° (*c* 0.52, CH_2Cl_2); ^1H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.11−1.2 (3H. t, ester CH_3), 1.26−1.4 (10H, *t*Boc, 4-CH), 1.4−1.53 (1H, m, 4-CH), 1.6−1.7 (1H,m, 5-CH), 1.7−1.78 (1H, m, 3-CH),1.81(3H, s, thymine CH_3), 2.02−2.15 (2H, 5-CH), 3.6−4.5 (7H, m, 2-CH, N−CH_2, $-COCH_2$, $-COOCH_2$), 5.0−5.2 (1H, d, 1-CH), 5.4−5.6 (1H, bd, carbamate NH), 7.04 (1H, s, thymine $-CH=C-$), 9.4−9.6 (1H, bd, thymine NH); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 12.0, 13.8, 21.3, 28.1, 28.3, 31.1, 46.0, 47.8, 52.4, 58.8, 61.2, 79.8, 110.0, 141.2, 151.1, 155.7, 164.3, 167.3, 169.3. Anal. Calcd (%) for $C_{21}H_{32}N_4O_7$: C, 55.75; H, 7.07; N, 12.38. Found: C, 55.63; H, 7.45; N, 12.08. MS (FAB$^+$): 453 [M + 1] (42%), 353 (100%) [M + 1 − *t*Boc].

***N*-[(2*S*)-*t*-Boc-Aminocyclopent-(1*R*)-yl]-*N*-(thymin-1-acetyl)-glycine Ethyl Ester (1*R*,2*S*)-10b.** A procedure similar to the one described for the monomer (1*S*,2*R*)-**10** afforded

(1*R*,2*S*)-**10b** from (1*R*,2*S*)-**9b**: mp 211.0−215.0 °C. $[\alpha]_D^{20}$ −32.64° (*c* 0.52, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.11−1.2 (3H. t, ester CH_3), 1.26−1.4 (10H, *t*Boc, 4-CH), 1.4−1.53 (1H, m, 4-CH), 1.6−1.7 (1H,m, 5-CH), 1.7−1.78 (1H, m, 3-CH),1.81(3H, s, thymine CH_3), 2.02−2.15 (2H, 5-CH), 3.6−4.5 (7H, m, 2-CH, N−CH_2, −$COCH_2$, −$COOCH_2$), 5.0−5.2 (1H, d, 1-CH), 5.4−5.6 (1H, bd, carbamate NH), 7.04 (1H, s, thymine −CH═C−), 9.4−9.6 (1H, bd, thymine NH); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 12.0, 13.8, 21.3, 28.1, 28.3, 31.1, 46.0, 47.8, 52.4, 58.8, 61.2, 79.8, 110.0, 141.2, 151.1, 155.7, 164.3, 167.3, 169.3. Anal.Calcd (%) for $C_{21}H_{32}N_4O_7$: C, 55.75; H, 7.07; N, 12.38. Found: C, 55.69; H, 7.37; N, 12.16. MS (FAB^+): 453 [M + 1] (42%), 353 (100%) [M + 1 − *t*Boc].

***N*-[(2*R*)-*t*-Boc-Aminocyclopent-(1*S*)-yl]-*N*-(thymin-1-acetyl)-glycine (1*S*,2*R*)-11a.** To the monomer ester (1S,2*R*)-**10a** (1.0 g, 2.283 mmol) suspended in THF (10 mL) was added a solution of 0.5 M LiOH (10 mL, 5.1 mmol), and the mixture was stirred for 30 min. The mixture was washed with EtOAc (2 × 10 mL). The aqueous layer was acidified to pH 3 and extracted with EtOAc (4 × 20 mL). The EtOAc layer was dried over sodium sulfate and evaporated under reduced pressure to afford monomer (1*S*,2*R*)-**11a** as a white solid: yield 0.92 g, 97.8%; mp 139.0−141.0 °C; $[\alpha]_D^{20}$ −17.0° (*c* 1.0, CH_2Cl_2); ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 1.0−2.0 (18 H, thymine CH_3, Boc, 3CH_2), 3.5−3.75 (m, 1H), 3.85−4.25 (m, 3H), 4.3−4.7 (m, 1H), 4.8−5.1 (bd, 1H), 6.9−7.15 (bd, 1H), 7.29 (s, 1H), 11.28 (s, 1H); ^{13}C NMR (DMSO-d_6, 200 MHz) δ_C 12.0, 21.8, 28.6, 28.31, 30.0, 38.4, 45.8, 48.1, 50.54, 59.0, 78.5, 108.4, 141.9, 151.4, 156.0, 164.6, 167.9, 171.2. Anal. Calcd (%) for $C_{19}H_{28}N_4O_7$: C, 53.77; H, 6.60; N, 13.20. Found: C, 53.57; H, 6.91; N, 13.11. MS (FAB^+): 425 [M+1] (6%), 325 (100%) [M + 1 − *t*Boc].

***N*-[(2*S*)-*t*-Boc-Aminocyclopent-(1*R*)-yl]-*N*-(thymin-1-acetyl)-glycine (1*R*,2*S*)-11b.** To the monomer ester (1S,2*R*)-**10b** (1.0 g, 2.283 mmol) suspended in THF (10 mL) was added a solution of 0.5 M LiOH (10 mL, 5.1 mmol), and the mixture was stirred for 30 min. The mixture was washed with EtOAc (2 × 10 mL). The aqueous layer was acidified to pH 3 and extracted with EtOAc (4 × 20 mL). The EtOAc layer was dried over sodium sulfate and evaporated under reduced pressure to afford monomer (1*S*,2*R*)-**11b** as a white solid: yield 0.92 g, 97.8%; mp 139.0−141.0 °C; $[\alpha]_D^{20}$ +17.0° (*c* 1.0, CH_2Cl_2); ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 1.0−2.0 (18 H, thymine CH_3, Boc, 3CH_2), 3.5−3.75 (m, 1H), 3.85−4.25 (m, 3H), 4.3−4.7 (m, 1H), 4.8−5.1 (bd, 1H), 6.9−7.15 (bd, 1H), 7.29 (s, 1H), 11.28 (s, 1H); ^{13}C NMR (DMSO-d_6, 200 MHz) δ_C 12.0, 21.8, 28.6, 28.3, 30.0, 38.4, 45.8, 48.1, 50.5, 59.0, 78.5, 108.4, 141.9, 151.4, 156.0, 164.6, 167.9, 171.2. Anal. Calcd (%) for $C_{19}H_{28}N_4O_7$: C, 53.77; H, 6.60; N, 13.20. Found: C, 53.49; H, 6.93; N, 13.17. MS (FAB^+): 425 [M + 1] (6%), 325 (100%) [M + 1 − *t*Boc].

Enantiomeric Purity from ^{1}H NMR Using a Chiral Chemical Shift Reagent. The enantiomeric purity of alcohols (1*R*,2*R*)-**3a** and (1*S*,2*S*)-**3b** was confirmed from ^{1}H NMR measurement of their acetyl derivatives utilizing a chiral chemical shift reagent, (+)-tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium (III) [Eu(hfc)$_3$] (8.0 mol %). Examination of the ^{1}H NMR spectrum of the acetyl derivatives of racemic (1*R*/*S*,2*R*/*S*)-**3** shows the splitting in the methyl carbon signal into a clear doublet. But in the corresponding spectra of acetyl derivatives of (1*R*,2*R*)-**3a** and (1*S*,2*S*)-**3b**, the above-mentioned signal was a singlet and corresponded to a respective signal in the spectrum of their racemate. Integration of the methyl signal (doublet) in the racemate and that of the pure (1*R*,2*R*)-**3a** and (1*S*,2*S*)-**3b** (singlet) shows that the enatiomeric excess will be >98% for both isomers (see Supporting Information)

Synthesis of PNA-Oligomers Incorporating *cis*-(1*S*,2*R*)- and (1*R*,2*S*)-Aminocyclopentyl 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 mequiv g^{-1}) with HBTU/HOBt/DIEA in DMF/DMSO as a coupling reagent. The PNA oligomers were cleaved from the resin with TFMSA. The oligomers were purified by RP HPLC (C18 column) and characterized by MALDI-TOF mass spectrometry. The overall yields of the raw products were 35−65%. The normal PNAs were prepared as described in the literature.

MALDI-TOF. PNA **12**: Calcd for $C_{94}H_{126}N_{34}O_{34}$, 2274.0; found, 2279.0 $[M + 5H]^+$. PNA **13**: Calcd for $C_{97}H_{130}N_{34}O_{34}$, 2314.0; found, 2320.0 $[M + 6H]^+$. PNA **14**: Calcd for $C_{97}H_{130}N_{34}O_{34}$, 2314.0; found, 2321.0 $[M + 7H]^+$. PNA **15**: Calcd for $C_{97}H_{130}N_{34}O_{34}$, 2314.0; found, 2321.0 $[M + 7H]^+$. PNA **16**: Calcd for $C_{97}H_{130}N_{34}O_{34}$, 2314.0; found, 2316.0 $[M + 2H]^+$. PNA **17**: Calcd for $C_{97}H_{130}N_{34}O_{34}$, 2314.0; found, 2321.0 $[M + 7H]^+$. PNA **18**: Calcd for $C_{97}H_{130}N_{34}O_{34}$, 2314.0; found, 2321.0 $[M + 7H]^+$. PNA **19**: Calcd for $C_{118}H_{158}N_{34}O_{34}$, 2594.0; found, 2596.0 $[M + 2H]^+$. PNA **20**: Calcd for $C_{118}H_{158}N_{34}O_{34}$, 2594.0; found 2596.0 $[M + 2H]^+$.

UV−T_m Measurements. The concentration of PNA and DNA was calculated on the basis of absorbance from the molar extinction coefficients of the corresponding nucleobases. 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 95 °C for 5 min followed by slow cooling to room temperature. The samples were cooled by keeping at 4 °C for overnight. 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 minute. The data were processed using Microcal Origin 5.0 and T_m values derived from the derivative curves.

Electrophoretic Gel Mobility Shift Assay. The PNAs (**12**−**18**, Table 1) were individually mixed with DNA (**I** or **II**) in a 2:1 ratio (PNA strand, 0.4 mM, and DNA **II**, 0.2 mM or **I**) in water. The samples were lyophilized to dryness and resuspended in sodium phosphate buffer (10 mM, pH 7.0, 10μL) 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 was added 10 μL of 40% sucrose in TBE buffer pH 8.0, 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% nondenaturing polyacrylamide gel (acrylamide/bisacrylamide 29:1) at constant power supply of 200 V and 10 mA, until the BPB migrated to three-fourths 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, F_{254}, using UV light.

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Supporting Information Available: General experimental procedures; enantiopurity of alcohols **3a** and **3b**; ^{1}H NMR, ^{13}C NMR, and mass spectra of compounds **4**−**6** and **8**−**11**; IR spectrum of (1*S*,2*R*)-**6**, CD curves for *cp*PNA monomers (1*S*,2*R*)-**10a** and (1*R*,2*S*)-**10b**; HPLC, MALDI-TOF, and characterization data table for PNAs **12**−**20**; CD spectra of PNA **12**, DNA **I**, single-strand *cp*PNAs, and complex PNA **12**:DNA **I**; CD Job's plots for PNAs **14** and **16**; UV Job's plot for PNA **14**; UV−melting curves for complexes of PNAs **12**−**20** with DNA **I** [d(CGCA$_8$CGC)], DNA **II** [d(CGCA$_4$*C*A$_3$CGC], and poly rA; gel shift assay for the complexation of PNAs **13**−**15**, **17**, and **18** with DNA **II**; X-ray crystal structures of *cp*PNA monomer esters **10a**, and **10b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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