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Synthesis and Evaluation of (1*S*,2*R*/1*R*,2*S*)-Aminocyclohexylglycyl PNAs as Conformationally Preorganized PNA Analogues for DNA/RNA Recognition

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Conformationally constrained *cis*-aminocyclohexylglycyl PNAs have been designed on the basis of stereospecific imposition of 1,2-*cis*-cyclohexyl moieties on the aminoethyl segment of aminoethylglycyl PNA (*aeg*PNA). The introduction of the *cis*-cyclohexyl ring may allow the restriction of the torsion angle β in the ethylenediamine segment to 60−70° that is prevalent in PNA_2:DNA and PNA:RNA complexes. The synthesis of the optically pure monomers (**10a** and **10b**) is achieved by stereoselective enzymatic hydrolysis of an intermediate ester **2**. The chiral PNA oligomers were synthesized with (1*S*,2*R*/1*R*,2*S*)-aminocyclohexylglycyl thymine monomers in the center and N-terminus of *aeg*PNA. Differential gel shift retardation with one or more units of modified monomer units was observed as a result of hybridization of PNA sequences with complementary DNA sequences. Hybridization studies with complementary DNA and RNA sequences using UV−T_m measurements indicate that PNA with (1*S*,2*R*)-cyclohexyl stereochemistry enhances selective binding with RNA over DNA as compared to control *aeg*PNA and PNA with the other (1*R*,2*S*) isomer.

Introduction

Peptide nucleic acids (PNA **II**) are DNA/RNA (**I**) mimics in which the natural nucleobases are attached to an uncharged, pseudopeptide backbone composed of *N*-(2-aminoethyl)glycine units *via* methylene carbonyl linkers (Figure 1).[1] PNA hybridizes to complementary DNA/RNA sequences via duplex formation for mixed sequences (W−C base pairing) and triplex formation for homopyrimidine or homopurine sequences involving both W−C and Hoogsteen base pairing. The complexes of PNA with DNA/RNA have consistently shown higher thermal stabilities as compared to the corresponding DNA−DNA and DNA−RNA complexes.[2] Due to these exceptional properties and their resistance to proteases and nucleases, PNAs are ideal lead candidates for development as new therapeutic agents in medicinal chemistry via antigene or antisense approaches[3] and also find useful applications in molecular biology and diagnostics.[4]

The current limitations in PNA properties are its poor water solubility and lack of cell permeability coupled with ambiguity in DNA/RNA recognition arising from its equally facile binding in parallel/antiparallel fashion with the complementary nucleic acid sequences. These limitations are now being systematically addressed with modified PNA analogues.[1b] Major improvements in DNA/RNA recognition selectivity were anticipated by pre-organization of PNA structure *via* conformational constraint strategies.[5] The approaches include introduction of chirality in the achiral PNA backbone to influence the orientation of cDNA/RNA binding[1b] and designing cyclic analogues that preorganize the PNA structure for attaining hybridization competent conformation and hence entropically drive the complex formation.[5] Most of the cyclic PNA analogues have been constructed by insertion of a five-membered pyrrolidine ring in to the backbone.[6] In one of the earliest approaches, the six-membered *trans*-(1*S*,2*S*/1*R*,2*R*)-cyclohexyl ring was imposed on the PNA backbone,[7] which exhibited destabilization of the derived

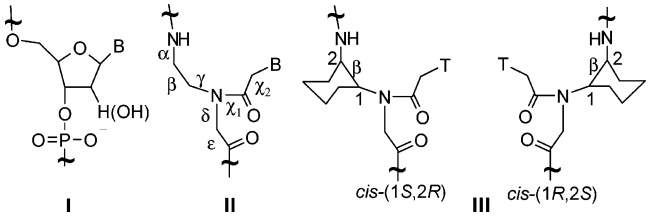

FIGURE 1. Structures of **I** DNA(RNA), **II** PNA, and **III** chiral *cis*-(1*S*,2*R*)- and (1*R*,2*S*)-aminocyclohexyl PNAs.

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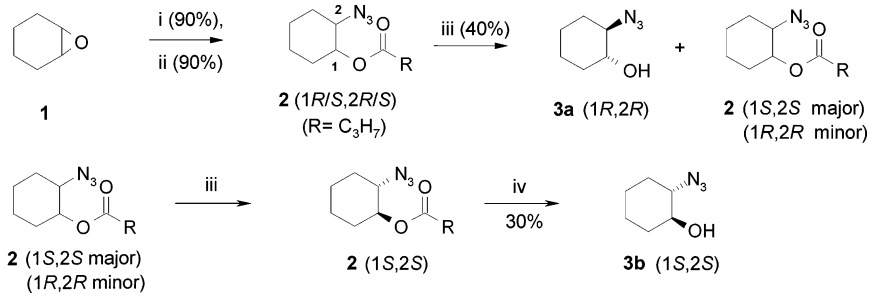
SCHEME 1[a]



[a] Reagents and conditions: (i) NaN_3, NH_4Cl, aq ethanol, reflux, 18 h; (ii) *n*-butyric anhydride, dry pyridine, DMAP (cat.), rt, 16 h; (iii) *Pseudomonas cepacia* (lipase), phosphate buffer, pH 7.2, 2.5 h; (iv) NaOMe in MeOH.

DNA complexes. In this analogue, the *trans*-(1,2-diaxial) disposition of the substituents caused the torsion angle β to be ~180°. This geometry is far from the conformation required for PNA strand in PNA_2:DNA triplexes (β ~73°)[8a] and PNA:DNA duplex (β ~141°)[8b] as inferred from the reported X-ray structures or PNA:RNA (β ~65−70°)[8c] duplex computed from NMR studies.[8] Recently, two approaches have emerged that involve the tuning of the torsion angle β in PNA backbone by superimposition of *cis*-(1*R*,2*S*/1*S*,2*R*)-cyclohexyl[9] and *trans*-(1*S*,2*S*)-cyclopentyl ring systems.[10] In this paper, we elaborate on the synthesis and DNA/RNA hybridization studies on *cis*-(1*S*,2*R*/1*R*,2*S*)-cyclohexyl PNAs that we reported in a recent communication.[9] The homothymine oligomer incorporated with this modification shows stereochemistry-dependent, sequence-specific triplex formation with complementary DNA and RNA sequences. Electrophoretic gel shift assay is presented to show differential retardation as a result of hybridization of PNA sequences carrying one or more units of modified monomer with the complementary DNA sequences. Hybridization study with complementary DNA and RNA sequences using UV−T_m measurements to study the DNA/ RNA binding selectivity and comparative mismatch tolerance is also reported.

Results and Discussion

Synthesis of *cis*-(1*S*,2*R*)- and (1*R*,2*S*)-Aminocyclohexylthymine Monomers and the PNA Oligomers. The synthesis of the title compounds was achieved starting from a mixture of (+)- and (−)-*trans*-2-azidocyclohexanoate **2** using lipases well-known in the literature.[11] The butyrate ester of racemic *trans*-2-azidocyclohexanol was synthesized by the oxirane ring opening of the commercially available *meso*-epoxide **1** with sodium azide[12] 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) in sodium phosphate buffer which proceeded with 40% conversion, followed by chromatography, to obtain the optically pure (1*R*,2*R*)-azido alcohol **3a**. The earlier reported procedure for this resolution step employed the lipase from *Candida cylindracea* but was found to be less effective as compared to Amano-PS. After a comparative resolution of racemic butyrate **2** with both the enzymes, we found that the *P. cepacia* lipase (Amano-PS) gave a better enantiomeric purity of the azido alcohol than the lipase from *C. cylindracea* 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 **2** (1*S*,2*S*) ester using NaOMe in methanol gave pure (1*S*,2*S*)-**3b** in 30% overall yield. The enantiopurity of both (1*R*,2*R*)-**3a** and (1*S*,2*S*)-**3b** isomers was confirmed by comparing with known values for the optical rotations reported in the literature[11] and by ^{13}C 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[13] and *in situ* *t*-Boc protection of the resulting amine function yielded the Boc-protected amino alcohol (1*R*,2*R*)-**4** (Scheme 2). Other hydrogenation catalysts such as Raney Ni and 10% Pd/C were also effective at catalyzing the reduction of azide, but the product obtained required extensive purification due to the resulting side products and consequently lower yields. The alcohol **4** was then converted to corresponding mesylate (1*R*,2*R*)-**5**. The mesylate was treated with NaN_3 in dry DMF to yield the azide (1*S*,2*R*)-**6** with inversion of configuration at C1. This was confirmed from the crystal structures of mesylate (1*R*,2*R*)-**5** and azide (1*S*,2*R*)-**6** (Supporting Information). 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 bromoac-

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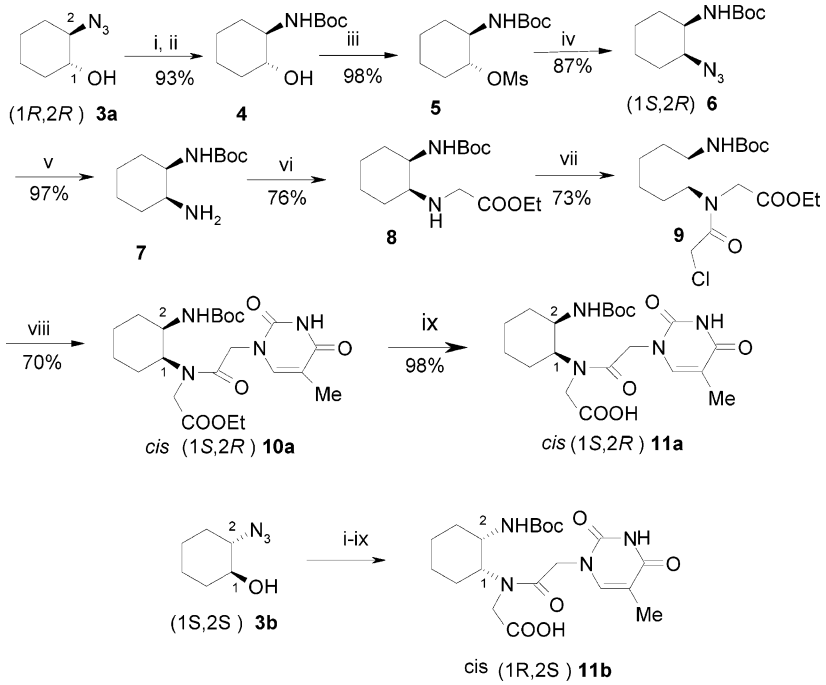
SCHEME 2[a]



[a] Reagent and conditions (i) PtO_2, dry EtOAc, H_2, 35−40 psi, rt, 3.5 h; (ii) $(Boc)_2O$ (iii) MsCl, Dry Pyridine, DMAP, 0−5 °C, 5 h; (iv) NaN_3, dry DMF, 72 °C, 18 h; (v) PtO_2, MeOH, H_2, 35−40 psi, rt, 3.5 h; (vi) $BrCH_2COOEt$, KF−Celite, dry CH_3CN, rt, 4 h; (vii) $ClCH_2COCl$, Na_2CO_3, dioxane/H_2O (1:1), 0 °C, 30 min; (viii) thymine, K_2CO_3 dry DMF, 60−65°C, 3.5 h; (ix) 0.5 M LiOH, aq THF, 0.5 h.

etate in the presence of KF−Celite. This resulted in the monosubstituted cis-1,2-diamines (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*)-aminocyclohexyl(thymin-1-yl-acetyl)glycyl PNA monomer ethyl ester (1*S*,2*R*)-**10a**, which on hydrolysis using 0.5 M LiOH in aqueous THF gave the monomer **11a**. All attempts to couple thymine acetic acid and the amine **8** using coupling reagents such as DCC, HBTU, and TBTU were unsuccessful. This may be attributed to the presence of the bulky *tert*-butyloxycarbonyl (Boc) group in the adjacent position. This is seen in the crystal structures of the final monomer esters **10a** and **10b** where the thymine base attached to the main backbone via methylene carbonyl linker is away from both Boc group and the glycine in chain (Supporting Information). All new compounds were characterized by ^1H and ^{13}C NMR and mass spectral data. The synthesis of the other enantiomer (1*R*,2*S*)-**11b** monomer was accomplished starting from (1*S*,2*S*)-**3b** following same steps as described above.

The enantiomeric purity of alcohols (1*R*,2*R*)-**3a** and (1*S*,2*S*)-**3b** was confirmed from ^{13}C NMR studies of their acetyl derivatives utilizing the chiral chemical shift reagent, (+)-tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium (III) [Eu(*hfc*)$_3$] (7.7 mol %)[14] (Supporting Information). The torsion angles β as deduced from the X-ray crystal structure[9] structures for **10a** (1*S*,2*R*) and **10b** (1*R*,2*S*) are −63° and +66°, respectively, which are closer in magnitude to that found in PNA_2:DNA and PNA:RNA complexes, with difference in their relative signs as expected for the enantiomeric pairs. In solution, the tertiary amide bond in PNA is known to exist as a rotameric mixture.[8] In the present structures, the amide bond is *trans* with carbonyl pointing toward the C-terminus, similar to that previously observed by us for cyanuryl monomer[15] and to that seen in the recently reported crystal structure of the D-lysine-based chiral PNA−DNA duplex.[16] The crystal structure data also show axial−equatorial dispositions for the *cis*-disubstituted (1*S*,2*R*/1*R*,2*S*)-cyclohexyl PNA monomers, with the bulky substituent carrying the nucleobase directed into an equatorial position.

The modified *cis*-(1*S*,2*R*/1*R*,2*S*)-aminocyclohexylglycylthymine monomers **11a** and **11b** were incorporated into PNA oligomers using Boc chemistry on L-lysine-derivatized (4-methylbenzhydryl)amine (MBHA) resin as reported before.[9] Various homothymine PNA decamers (**13**−**16**, Table 1) incorporating one or two *cis*-(1*S*,2*R*/1*R*,2*S*)-aminocyclohexylglycylthymine PNA monomers in different positions were synthesized. The oligomers were cleaved from the resin using TFMSA procedure[17] followed by RP HPLC purification and characterized by mass spectrometry (MALDI-TOF).

UV−T_m Studies of PNA−DNA/RNA Complexes. The hybridization of modified PNAs with complementary DNA **I** sequences were studied by temperature-dependent UV absorbance experiments. The stoichiometry for

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TABLE 1. UV−Melting Temperatures (T_m Values)[a] of PNA_2/DNA Complexes

PNA	DNA **I**[b]	DNA **II**[b]	poly rA	poly dA
12, H-TTTTTTTTTT-Lys-NH_2	69.2	58.0 (−11.2)[c]	>80.0	72.4
13, H-TTTTT$_{SR}$TTTTT-Lys-NH_2	41.1	26.1 (−15.0)	77.2	61.5 (−15.7)[d]
14, H-TTTTT$_{RS}$TTTTT-Lys-NH_2	45.0	29.1 (−16.1)	70.7	63.5 (−7.2)
15, H-T$_{SR}$TTTT$_{SR}$TTTTT-Lys-NH_2	34.4	23.3 (−11.0)	64.4	53.7 (−10.7)
16, H-T$_{RS}$TTTT$_{RS}$TTTTT-Lys-NH_2	37.5	25.7 (−12.0)	58.6	53.5 (−5.1)

[a] T_m = melting temperature (measured in the buffer 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH = 7.0), PNA_2:DNA complexes. [b] **I** = **5′**d(CGCA_{10}CGC); **II** **5′**d(CGCA_4CA_5CGC). [b] Values in parentheses indicate the difference in T_m as a result of the mismatch in DNA sequence. [c] Values in parentheses indicate difference in binding with RNA over DNA. The values reported here are the average of three independent experiments and are accurate to ±0.5 °C.

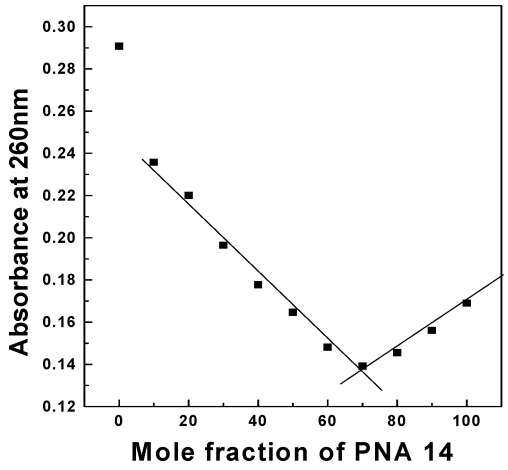

FIGURE 2. UV absorbance (at 260 nm) of mixtures of PNA **14** and the complementary DNA **I** in the relative molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, and 100:0 (buffer, 10 mM sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA).

PNA:DNA complexation as established by UV absorbance mixing data at 260 nm (Job plot)[18] was a 2:1 ratio (Figure 2).

The thermal stabilities (T_m) of PNA_2:DNA triplexes (Table 1, Figure 3) were obtained for different PNA modifications with both complementary DNA **I** and DNA **II** having a single mismatch. The corresponding complexes with poly rA and poly dA were also studied for relative compatibility of the imposed stereochemistry with ribo- and deoxyribonucleic acids. In general, the PNA_2:DNA triplexes were found to be less stable for PNAs containing enantiomeric (1*S*,2*R*)- or (1*R*,2*S*)-modified cyclohexyl units, as compared to the unmodified PNA. The PNA oligomers **13** and **14** having a modification in the center of the sequence destabilized the complexes with DNA **I** (ΔT_m = −28 and −24 °C, respectively) in comparison to the control PNA **12**. In the case of PNA oligomers **15** and **16** having two modified units (at N-terminal and center), a further decrease in T_m (ΔT_m = −35 and −32 °C) was observed. PNAs **14** and **16** modified with (1*R*,2*S*) isomer were slightly better binding with DNA **I** than the PNAs **13** and **15** with enantiomeric (1*S*,2*R*) isomer. The comparative binding study was also conducted for complexation with poly-rA. For appropriate comparison of DNA and RNA complex stabilities, UV experiments were done with poly dA, which exhibited a higher T_m than the DNA oligonucleotide **I**. The complexes of the modified oligomers with poly rA exhibited decreased T_m, indicating destabilization as compared to the unmodified PNA **12**, but the magnitudes were relatively small for either poly-rA or poly dA (ΔT_m ∼7° per modification) than observed for the complexes with DNA oligonucleotides. The most interesting observation is that the stereochemistry of the monomeric units is able to direct the DNA verses RNA binding selectivity; thus, the (1*S*,2*R*)-PNAs **13** and **15** with one and two modifications show better binding to poly-rA compared to the corresponding (1*R*,2*S*)-PNAs **14** and **16**. The situation is reverse for binding with DNA—the (1*R*,2*S*)-PNAs **14** and **16** bind DNA better compared to (1*S*,2*R*)-PNAs **13** and **15**. In general, all PNA oligomers showed a slightly lower binding with poly dA than with poly-rA. The observed transitions are sharp for modified PNA complexes with poly rA and poly dA as compared to complexes of unmodified PNA **12**. Introduction of the second modified unit at the N-terminus actually reduced the overall stability of complexes both with dA_{10}, poly dA, and poly-rA as well as the differential RNA over DNA binding selectivity.

The thermal stabilities of PNA complexes with DNA **II** [d(A_4CA_5] containing a single mismatch at the middle modification site were also measured. The ΔT_m for control PNA **12** was −11° while that for modified PNAs **13** and **14** were −15° when a single modified unit is present. With the second modified unit this difference also reduced to −11° with a lowering of T_m. These results indicated that the cyclohexyl modified PNAs retain the ability of PNAs of sequence specific recognition and binding to cDNA allowing discrimination against mismatch sequences.

CD Spectroscopic Studies of PNA:DNA Complexes. PNA is nonchiral and does not show any significant CD signatures. However, PNA:DNA complexes exhibit characteristic CD signatures due to chirality induced by DNA component. It is known that formation of PNA triplexes is accompanied by the appearance of positive CD bands at 258 and 285 nm that are not present in DNA. The 258 nm band is slightly sharper and of higher intensity than 285 nm. The presence of chiral centers in cyclohexyl PNAs should further influence the CD patterns of the derived PNA:DNA triplexes. Figure 4 shows the CD spectra of individual PNAs **13**−**15**, DNA **I**, and the PNA:DNA complexes. The single-stranded cyclohexyl PNAs (**13**−**15**) show weak CD bands in the 250−290 nm region that are absent in unmodified PNA **12** (Supporting Information). The CD spectra of complexes of PNA **13**, PNA **14**, PNA **15**, and PNA **16** with d(A_{10}) are nearly identical (Figure 4A,B) and are similar to the CD spectrum of the unmodified (PNA**12**)$_2$-d(A_{10})

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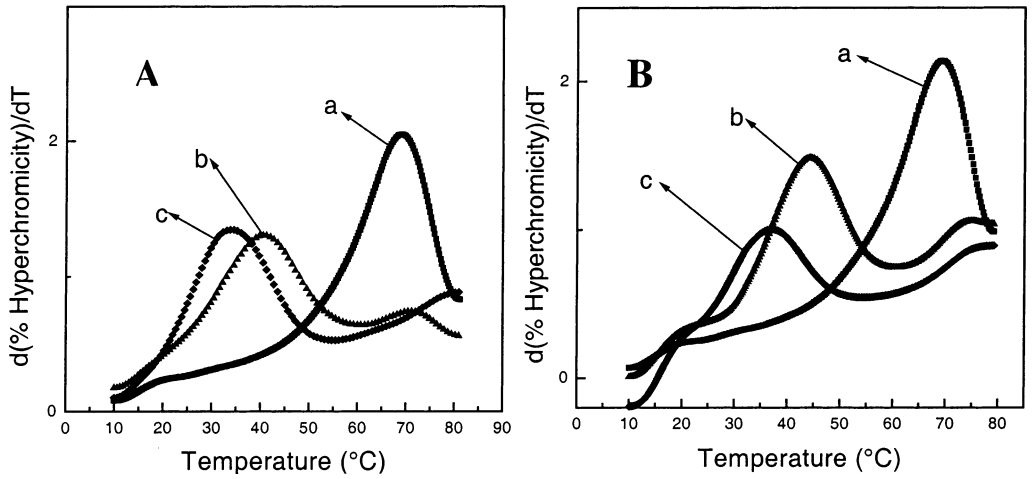

FIGURE 3. First derivative UV−T_m Plots of PNA_2:DNA **I** complexes (A) for the (1*S*,2*R*)-PNA, (a) PNA **12**, (b) PNA **13**, (d) PNA **15** and (B) (1*R*,2*S*) modification, (a) PNA **12**, (c) PNA **14**, (e) PNA **16**.

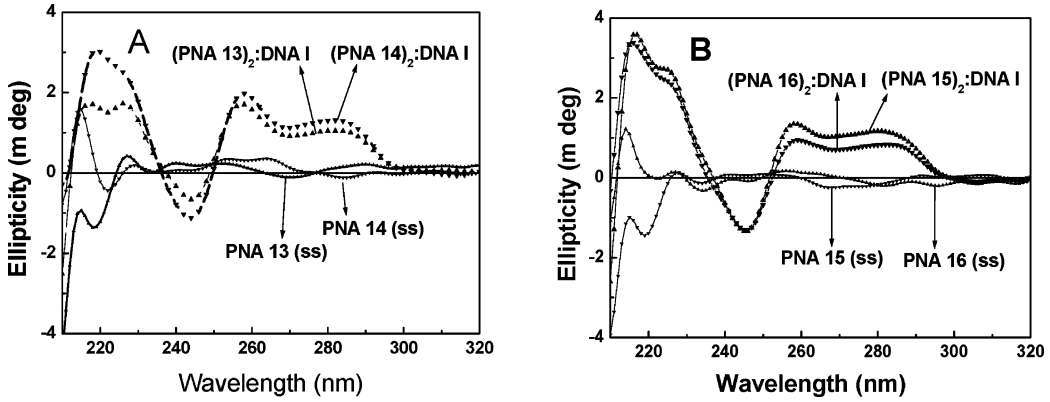

FIGURE 4. (A) CD spectra of PNA **13** (SS), PNA **14** (SS), and complexes (PNA **13**)$_2$:DNA **I** and (PNA **14**)$_2$:DNA **I**. (B) CD spectra of PNA **15** (SS), PNA **16** (SS), and complexes (PNA **15**)$_2$:DNA **I** and (PNA **16**)$_2$:DNA **I** (10 mM sodium phosphate buffer, pH = 7, 100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

triplex.[19] All PNA:DNA triplexes show the expected positive bands at 258 and 285 nm, with intensity of the former higher than that of latter. Interestingly the relative intensity ratios of 258/285 nm bands are different for the cyclohexyl PNA:DNA triplexes as compared to the control PNA:DNA triplex. The 285 nm band increased in intensity with the degree of modification, irrespective of the nature of the modification (*RS*/*SR*). These results suggest that the cyclohexyl modifications significantly alter the base stacking in the modified PNA:DNA complexes. The systematic changes in the CD spectral features upon variable stoichiometric mixing of PNA and DNA components can be used to generate a Job plot, which also indicated a binding ratio of 2:1 for the cyclohexyl PNA:DNA complexes, confirming the formation of triplexes (Supporting Information).

Gel Shift Assay and Competition Binding Experiments. Electrophoretic gel shift assay[20] was used to establish the binding of different PNAs to the complementary DNA **I**. The PNAs modified with one or two units of *SR*/*RS* cyclohexyl units and the control PNA **12** were individually treated with oligonucleotide **I** (see Figure 5 legend for experimental conditions), and the complexations were monitored by nondenaturing gel electrophoresis at 10 °C. The spots were visualized on a fluorescent TLC background, and the results are shown in Figure 5. The formation of PNA_2:DNA complexes was accompanied by the disappearance of the single strand DNA **I** and appearance of a lower migrating band due to PNA:DNA complexes. The single modified PNA_2:DNA complexes migrated about the same as the unmodified PNA:DNA complex but lower than that of DNA **I** (Figure 5A, lanes 6−8), while the doubly modified PNA:DNA complexes showed more retardation (Figure 5A, about the same as the unmodified PNA:DNA complex but lower than that of DNA **I** (Figure 5A, lanes 6−8), while the doubly modified PNA:DNA complexes showed more retardation (Figure 5A, lanes 1 and 2). The different mobility retardation shifts of cyclohexyl PNA:DNA complexes may arise from the increased molecular weight upon formation of the complexes, a rigid geometry imposed by the cyclohexyl ring on the derived complexes or from an enhanced hydrophobicity due to cyclohexyl rings. Under the electrophoretic conditions employed, the single stranded PNAs that carry a net positive charge did not move out of the well (Figure 5A, lanes 3 and 5).

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(20) Sambrook, J.; Fritsch, E. F.; Maniatis, T. In *Molecular Cloning: A Laboratory Manual*, *2*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.

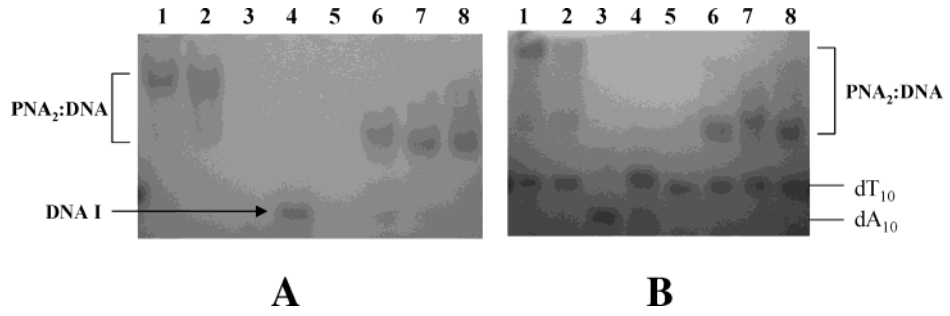

FIGURE 5. PNA/DNA complexation. (A) Gel shift assay: lane 1, PNA **15** + DNA **I**; lane 2, PNA **16** + DNA **I**; lane 3, ssPNA-**12** (aeg-T_{10}); lane 4, DNA **I**; lane 5, ssPNA **13**; lane 6, PNA **13** + DNA **I**; lane 7, PNA **14** + DNA **I**; lane 8, PNA **12** (aeg-T_{10}) + DNA **I**. (B) Competition binding experiments: lane 1, DNA **I** + DNA **III** + PNA **15**; lane 2, DNA **I** + DNA **III** + PNA **16**; lane 3, ssDNA **I**; lane 4, DNA duplex (**I** + **III**); lane 5, ssDNA **III**; lane 6, DNA I + DNA **III** + PNA **13**; lane 3, ssDNA **I**; lane 4, DNA duplex (**I** + **III**); lane 5, ssDNA **III**; lane 6, DNA **I** + DNA **III** + PNA **13**; lane 7, DNA **I** + DNA **III** + PNA **14**; lane 8, DNA **I** + DNA **III** + PNA **12**; DNA **I** = $CGCA_{10}CGC$ (**dA_{10}**), DNA **III** = $GCGT_{10}GCG$ (dT10). 0.5X TBE pH 8.0.

The results of competition binding experiments carried out by adding PNAs to the DNA duplex (**I/III**) followed by annealing and gel electrophoresis is shown in Figure 5B. Due to a higher binding affinity of PNA:DNA complexes compared with DNA:DNA complexes, the added PNA binds to the complementary DNA **I** in the DNA duplex, releasing the DNA strand **III**. This is clearly seen in the gel electrophoresis in which the DNA duplex band (Figure 5B, lane 4) is converted into a PNA:DNA complex seen as a retarded band and the released DNA strand (Figure 5B, lane 6−8) seen as a faster moving band. The results indicate that the modified PNAs are as good as the unmodified PNA in successfully competing for binding with the complementary DNA strand. The systematic changes in the CD spectral features upon variable stoichiometric mixing of PNA and DNA components can be used to generate a Job's plot which also indicated a binding ratio of 2:1 for the cyclohexyl PNA:DNA complexes, confirming the formation of triplexes. An isobestic point seen at 262 nm in the CD spectra (Supporting Information) clearly confirmed the CD changes to arise as a consequence of complex formation.

Conclusion

On the basis of a rational design, we have synthesized the (1*S*,2*R*)- and (1*R*,2*S*)-aminocyclohexylglycyl PNA monomers, in which the torsion angle β is restricted to 60−70° by virtue of a *cis* axial−equitorial disposition of the backbone substutuents. The designed monomers were built into PNA oligomers by solid-phase synthesis using Boc protection strategy. The UV melting temperatures of the modified oligomers with the complemetary/mismatched DNA and RNA were measured. The imposed stereochemistry of the PNA oligomer having the (1*S*,2*R*) isomer preferred to bind RNA and the oligomer having the (1*R*,2*S*) isomer showed higher affinity toward DNA in homothymine oligomers, leading to a stereodiscrimination in recognition of DNA and RNA. The gel shift experiments indicated that the modifications retain the competitive binding attributes of PNA toward DNA duplex. The preorganization of the PNA backbone by chemical modifications such that the torsion angle β is restricted in a range found by NMR and crystal structure data may lead to the induction of substantial selectivity (discrimination) in DNA/RNA recognition. On the basis of this design, work is in progress to examine the effects in mixed sequences that form PNA:DNA/RNA duplexes and explore other ring structures to further improve the binding affinity via entropic gains and impart efficient DNA/RNA selectivity.

Experimental Section

Enzymatic Resolution of Racemic alcohols (1*R/S*,2*R/S*) 3 with Lipase *P. cepacia*. To a solution of lipase Amano-PS (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 2.5 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 chirally pure alcohol (1*R*,2*R*)-**3a** and the optically enriched butyrate (1*S*,2*S*)-**2**. The later was again subjected to enzyme hydrolysis as described to yield the optically pure butyrate (1*S*,2*S*)-**2** after purification. From this the alcohol (1*S*,2*S*)-**3b** was obtained by methanolysis with catalytic amount of NaOMe in MeOH in 30% overall yield. The enantiomeric purity was confirmed by comparison with known values of the optical rotations of alcohols (1*R*,2*R*)-**3a** and (1*S*,2*S*)-**3b** from the literature [for alcohol (1*R*,2*R*)-**3a**: $[\alpha]^{20}_{D}$ = −69.33 (lit.[8] $[\alpha]^{20}_{D}$ −66.9, *c* 1.5, CH_2Cl_2); alcohol (1*S*,2*S*)-**3b**: $[\alpha]^{20}_{D}$ = +68.7 (lit.[8] $[\alpha]^{20}_{D}$ +66.3, *c* 1.6, CH_2Cl_2)].

(1*R*,2*R*)-2-(*N-tert*-Butyloxycarbonylamino)cyclohexanol [(1*R*,2*R*)-4]. To a solution of (1*R*,2*R*)-2-azidocyclohexanol (5.6 g, 39.7 mmol) in dry ethyl acetate (10 mL) placed in a hydrogenation flask were added di-*tert*-butyl dicarbonate (10.4 g, 47.6 mmol) and Adams catalyst (2 mol %). The mixture was hydrogenated in Parr apparatus (rt, 35−40 psi 3 h). The catalyst was filtered, solvent in the filtrate was evaporated under reduced pressure, and the residue was purified by column chromatography (EtOAc/petroleum ether) to afford a white solid of the alcohol (1*R*,2*R*)-**4**: yield (7.9 g, 92.6%); mp 111 °C; $[\alpha]^{20}_{D}$ +5.0 (*c* 0.6, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.04−1.34 (4H, m), 1.42 (9H, s), 1.61−1.73 (2H, m), 1.89−2.06 (2H, m) 3.2−3.36 (2H, m) 2.8−3.5 (1H, bd), 4.0−5.0 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 23.9, 24.6, 28.3, 31.7, 34.0, 56.5, 75.2,79.9, 160.0; MS (FAB$^+$) 216 [M + 1] (50), 116 (30) [M + 1 − Boc]. Anal. Calcd for $C_{11}H_{21}NO_3$: C, 61.39; H, 9.76; N, 6.51. Found: C, 61.17; H, 9.71; N, 6.33;

(1*S*,2*S*)-2-(*N-tert*-butyloxycarbonylamino)cyclohexanol [(1*S*,2*S*)- 4]. This compound 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 111 °C; $[\alpha]^{20}_{D}$ −6.6 (*c* 0.6, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.04−1.34 (4H, m), 1.42 (9H, s), 1.61−1.73 (2H, m), 1.89−2.06 (2H, m), 3.2−3.36 (2H, m), 2.8−3.5 (1H, bd), 4.0−5.0 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 23.9, 24.6, 28.3, 31.7, 34.0, 56.5, 75.2, 79.9, 60.0; MS (FAB$^+$) 216 [M + 1] (50), 116 (30) [M + 1 −

Boc]. Anal. Calcd for $C_{11}H_{21}NO_3$: C, 61.39; H, 9.76; N, 6.51. Found C, 61.56; H, 10.01; N, 6.40.

(1*R*,2*R*)-2-(*N*-*tert*-Butyloxycarbonylamino)cyclohexane-1-methyl Sulfonate [(1*R*,2*R*)-5]. To a stirred solution of alcohol (1*R*,2*R*)-**4** (6.0 g, 27.9 mmol) and DMAP (0.05 g) in dry pyridine (100 mL) at 0 °C under nitrogen was added methanesulfonyl chloride (4.16 g, 36.3 mmol) over a period of 20 min. The mixture was stirred for 1 h and then refrigerated overnight. Pyridine was evaporated under reduced pressure, and the residue was purified by column chromatography (EtOAc/petroleum ether) affording mesylate (1*R*,2*R*)-**5** as a white crystalline solid: yield (8.0 g, 97.9%); mp 121 °C; $[\alpha]^{20}_D$ −10.67 (*c* 1.5, CH_2Cl_2): ^{1}H NMR ($CHCl_3$-*d*, 500 MHz); δ_H 1.15−1.34 (3H, m), 1.41 (9H, s), 1.52−1.86 (3H, m), 1.92−2.09 (1H, m), 2.1−2.2 (1H, m), 3.0 (3H, s), 3.45−3.65 (1H, bd), 4.3−4.5 (1H, bd), 4.6−4.8 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 23.5, 23.7, 28.2, 31.6, 32.0, 38.3, 51.8, 79.3, 82.4, 155.2; MS (FAB^+) 294 [M + 1] (5), 194 (85) [M + 1 − Boc]. Anal. Calcd for $C_{12}H_{23}NO_5S$: C, 49.0; H, 7.80; N, 4.77; S, 10.92. Found: C, 49.30; H, 7.86; N, 4.50; S, 10.55.

(1*S*,2*S*)-2-(*N*-*tert*-Butyloxycarbonylamino)cyclohexan-1-methyl Sulfonate [(1*S*,2*S*)-5]. The compound (1*S*,2*S*)-**5** was 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**: mp 121 °C; $[\alpha]^{20}_D$ +13.33 (*c* 1.5, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.15−1.34 (3H, m), 1.41 (9H, s), 1.52−1.86 (3H, m), 1.92−2.09 (1H, m), 2.1−2.2 (1H, m), 3.0 (3H, s), 3.45−3.65 (1H, bd), 4.3−4.5 (1H, bd), 4.6−4.8 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 23.5, 23.7, 28.2, 31.6, 32.0, 38.3, 51.8, 79.3, 82.4, 155.2; MS (FAB^+) 294 [M + 1] (5), 194 (85) [M + 1 − Boc]. Anal. Calcd for $C_{12}H_{23}NO_5S$: C, 49.0; H, 7.80; N, 4.77; S, 10.92. Found: C, 49.21; H, 7.81; N, 4.56; S, 10.67.

(1*S*,2*R*)-2-(*N*-*tert*-Butyloxycarbonylamino)-1-azidocyclohexane [(1*S*,2*R*)-6]. A stirred mixture of the mesylate (1*R*,2*R*)-**5** (8.0 g, 33.33 mmol) and NaN_3 (17.3 g, 0.26 mol) in DMF (80 mL) under nitrogen was heated at 65−70 °C for 12 h. After cooling, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (EtOAc/petroleum ether) to afford a white solid of azide (1*S*,2*R*)-**6**: yield (5.7 g, 87%); mp 69−70 °C; IR, ν (cm^{-1}) (KBr) 3359, 2954, 2104, 1720, 1681, 1367, 1319, 1166 cm^{-1}; $[\alpha]^{20}_D$ +102 (*c* 1.5, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.21−1.35 (1H, m), 1.35−1.51 (12H), 1.52−1.64 (2H, m), 1.65−1.77 (1H, m), 1.89−2.01 (1H, m), 3.5−3.65 (1H, bd), 3.8−4.0 (1H, bd), 4.56−4.8 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 19.6, 24.2, 27.5, 28.2, 28.6, 51.0, 61.5, 79.4, 154.9; MS (FAB+) 241 (35), [M + 1], 141 (15) [M + 1 − Boc]. Anal. Calcd for $C_{11}H_{20}N_4O_2$: C, 55.00; H, 8.33; N, 23.33. Found: C, 55.18; H, 8.00; N, 23.14.

(1*R*,2*S*)-2-(*N*-*tert*-Butyloxycarbonylamino)-1-azidocyclohexane [(1*R*,2*S*)-6]. A procedure similar to the one described for (1*S*,2*R*)-**6** was used to prepare (*1R*,*2S*)-**6** from (1*S*,2*S*)-**5**: mp 69−70 °C; IR, ν (cm^{-1}) (KBr) 3359, 2954, 2104, 1720, 1681, 1367, 1319, 1166 cm^{-1}; $[\alpha]^{20}_D$ −105.33 (*c* 1.5, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.21−1.35 (1H, m), 1.35−1.51 (12H), 1.52−1.64 (2H, m), 1.65−1.77 (1H, m), 1.89−2.01 (1H, m), 3.5−3.65 (1H, bd), 3.8−4.0 (1H, bd), 4.56−4.8 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 19.6, 24.2, 27.5, 28.2, 28.6, 51.0, 61.5, 79.4, 154.9; MS (FAB+) 241 (35) [M + 1], 141 (15) [M + 1 − Boc]. Anal. Calcd for $C_{11}H_{20}N_4O_2$: C, 55.00; H, 8.33; N, 23.33. Found: C, 55.11; H, 8.07; N, 23.21.

(1*S*,2*R*)-2-(*N*-*tert*-Butyloxycarbonylamino)-1-aminocyclohexane [(1*S*,2*R*)-7]. To a solution of the azide (1*S*,2*R*)-**6** (4.0 g, 16.7 mmol) in methanol (5 mL) taken in a hydrogenation flask was added Adam's catalyst (2 mol %). The reaction mixture was hydrogenated in a Parr apparatus for 3 h at rt and H_2 of pressure 35−40 psi. The catalyst was filtered off, and then solvent was removed under reduced pressure to yield a residue of the amine (1*S*,2*R*)-**7** as a colorless oil, yield (3.45 g, 96.9%). This compound was used for the further reaction without any purification.

(1*R*,2*S*)-2-(*N*-*tert*-Butyloxycarbonylamino)-1-aminocyclohexane [(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 as described for the synthesis of the amine (1*S*,2*R*)-**7**.

***N*-[(2*R*)-*tert*-Butyloxycarbonylaminocyclohex-(1*S*)-yl]-glycine Ethyl Ester [(1*S*,2*R*)]-8.** To a stirred mixture of amine (1*S*,2*R*)-**7** (3.4 g, 15.9 mmol) and freshly prepare KF−Celite (5.52 g, 47.66 mmol) in dry acetonitrile (150 mL) was added ethyl bromoacetate (2.38 g, 14.3 mmol) dropwise for 30 min at rt under nitrogen atmosphere. After 3.5 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 (3.63 g, 76.6%); $[\alpha]^{20}_D$ −9.15 (*c* 1.42, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 1.24 (3H, t), 1.41 (s), 1.6−1.85 (2H, m), 2.0−2.35 (2H, m), 2.35−2.7 (3H, m), 2.75−3.52 (5H, m), 3.67 (1H, bd), 4.0−4.5 (2H, m), 4.9−5.75 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz) δ_C 13.9, 28.1, 33.74, 37.7, 40.7, 49.1, 60.2, 61.3, 61.6, 69.2, 78.7, 156.3, 172.4; MS (FAB^+) 301 [M + 1] (100), 201 (12) [M + 1 − Boc].

***N*-[(2*S*)-*tert*-Butyloxycarbonylaminocyclohex-(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**: $[\alpha]^{20}_D$ +10.56 (*c* 1.42, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 1.24 (3H, *t*), 1.41 (*s*), 1.6−1.85 (2H, m), 2.0−2.35 (2H, m), 2.35−2.7 (3H, m), 2.75−3.52 (5H, m), 3.67 (1H, bd), 4.0−4.5 (2H, m), 4.9−5.75 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz) δ_C 13.9, 28.1, 33.7, 37.7, 40.7, 49.1, 60.2, 61.3, 61, 69.2, 78.7, 156.3, 172.4; MS (FAB^+) 301 [M + 1] (100), 201 (12) [M + 1 − Boc].

***N*-[(2*R*)-*tert*-Butyloxycarbonylaminocyclohex-(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** (3.6 g, 12 mmol) in 10% Na_2CO_3 (90 mL) and 1,4-dioxane (90 mL) cooled to 0 °C was added chloroacetyl chloride (6.78 g, 60 mmol) in two additions. After 30 min, the dioxane was removed under reduced pressure and the residue was extracted into EtOAc (2 × 100 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 (3.3 g, 73%); mp 65−68 °C; $[\alpha]^{20}_D$ −89.54 (*c* 2.2, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 1.0−2.0 (20H, m), 3.65−4.5 (8H, m), 4.7−5.2 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz) δ_C 13.9, 19.8, 23.7, 25.2, 28.2, 30.9, 41.4, 45.8, 49.0, 57.0, 61.0, 79.5, 155.5, 167.0, 169.0; MS LCMS 377 [M + 1], 277 [M + 1 − Boc].

***N*-[(2*S*)-*tert*-Butyloxycarbonylaminoaminocyclohex-(1*R*)-yl]-*N*-(chloroacetyl)glycine Ethyl Ester [(1*R*,2*S*)-9.** This compound was prepared from the amine (1*R*,2*S*)-**8** following the procedure described for the chloro compound (1*S*,2*R*)-**9**: mp 65−68 °C; $[\alpha]^{20}_D$ + 91.36 (*c* 2.2, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 1.0−2.0 (20H, m), 3.65−4.5 (8H, m), 4.7−5.2 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 200 MHz) δ_C 13.9, 19.8, 23.7, 25.2, 28.2, 30.9, 41.4, 45.8, 49.0, 57.0, 61.0, 79.5, 155.5, 167.0, 169.0; MS LCMS 377 [M + 1], 277 [M + 1 − *t*Boc].

***N*-[(2*R*)-*tert*-Butyloxycarbonylaminocyclohex-(1*S*)-yl]-*N*-(thymin-1-acetyl)glycine Ethyl Ester [(1*S*,2*R*)-10a].** A mixture of chloro compound (1*S*,2*R*)-**9** (1.5 g, 4.0 mmol), thymine (0.55 g, 4.38 mmol), and anhydrous K_2CO_3 (0.66 g, 4.8 mmol) in dry DMF (10 mL) under nitrogen was heated with stirring at 65 °C for 3.5 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 (1.3 g, 70.2%); mp 115−119 °C; $[\alpha]^{20}_D$ −85.33 (*c* 1.5, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.17−1.8 (17H), 1.82 (3H, s), 3.5−5.0 (8H, m), 5.0−5.5 (1H, bd), 7.1 (1H, s), 8.3−8.5 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 12.0, 14.1, 19.8, 23.5, 25.0, 28.1, 30.1, 36.6, 45.3, 47.5, 55.6, 61.0,79.4, 111.0,

141.1, 151.0, 155.5, 164.4, 167.4, 169.3; MS (FAB^+) 467 [M + 1] (10), 367 (100) [M + 1 − *t*Boc]. Anal. Calcd for $C_{22}H_{34}N_4O_7$: C, 56.65; H, 7.29; N, 12.01. Found: C, 56.81; H, 7.31; N, 11.97.

***N*-[(2*S*)-*tert*-Butyloxycarbonylaminocyclohex-(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*)-**10a** afforded (1*R*,2*S*)-**10b** from (1*R*,2*S*)-**9**: mp 115−119 °C; $[\alpha]^{20}_D$ +86.67 (*c* 1.5, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 500 MHz) δ_H 1.17−1.8 (17H), 1.82 (3H, s), 3.5−5.0 (8H, m), 5.0−5.5 (1H, bd), 7.1 (1H, s), 8.5−8.5 (1H, bd); ^{13}C NMR ($CHCl_3$-*d*, 500 MHz) δ_C 12.0, 14.1, 19.8, 23.5, 25.0, 28.1, 30.1, 36.6, 45.3, 47.5, 55.6, 61.0,79.4, 111.0, 141.1, 151.0, 155.5, 164.4, 167.4, 169.3; MS (FAB^+) 467 [M + 1] (10), 367 (100) [M + 1 − *t*Boc]. Anal. Calcd for $C_{22}H_{34}N_4O_7$: C, 56.60; H, 7.22; N, 12.23. Found: C, 56.81; H, 7.31; N, 11.97.

***N*-[(2*R*)-*tert*-Butyloxycarbonylaminocyclohex-(1*S*)-yl]-*N*-(thymin-1-acetyl)glycine [(1*S*,2*R*)-11a].** To the monomer ester (1*S*,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 (3 × 50 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 172−177 °C; $[\alpha]^{20}_D$ −107 (*c* 1.06, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 0.5−1.8 (17H, m, *t*Boc, 4CH_2), 1.88 (3H, s, thymine CH_3), 3.3−5.5 (7H, m, N-CH_2, acyl CH_2, 1-CH, 2-CH, carbamate NH), 7.18 (1H, s, thymine-H), 10.0−11.0 (2H, bd, thymine NH and −COOH); ^{13}C NMR (DMSO-d_6, 200 MHz) δ_C 12.2, 19.7, 23.1, 25.5, 28.6, 31.0, 47.7, 55.7, 78.1, 108.5, 142.3, 151.4, 156.1, 164.7, 167.7, 174.0. MS (FAB^+) 439 [M + 1] (6), 339 (100) [M + 1 − *t*Boc]. Anal. Calcd for $C_{20}H_{30}N_4O_7$: C, 54.78; H, 6.84; N, 12.77. Found: C, 54.61; H, 7.01; N, 12.61.

***N*-[(2*S*)-*tert*-Butyloxycarbonylaminocyclohex-(1*R*)-yl]-*N*-(thymin-1-acetyl)glycine [(1*R*,2*S*)-11b].** To the monomer ester (1*R*,2*S*)-**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 (3 × 50 mL). The EtOAc layer was dried over sodium sulfate and evaporated under reduced pressure to afford monomer (1*R*,2*S*)-**11a** as a white solid: Yield 0.92 g (97.8%); mp 172−177 °C; $[\alpha]^{20}_D$ +110 (*c* 1.06, CH_2Cl_2); ^{1}H NMR ($CHCl_3$-*d*, 200 MHz) δ_H 0.5−1.8 (17H, m, t-Boc, 4CH_2), 1.88 (3H, s, thymine CH_3), 3.3−5.5 (7H, m, N-CH_2, acyl CH_2, 1-CH, 2-CH, carbamate NH), 7.18 (1H, s, thymine-H), 10.0−11.0 (2H, bd, thymine NH and −COOH); ^{13}C NMR (DMSO-d_6, 200 MHz) δ_C 12.2, 19.7, 23.1, 25.5, 28.6, 31.0, 47.7, 55.7, 78.1, 108.5, 142.3, 151.4, 156.1, 164.7, 167.7, 174.0; MS (FAB^+) 439 [M + 1] (6), 339 (100) [M + 1 − Boc]. Anal. Calcd for $C_{20}H_{30}N_4O_7$: C, 54.78; H, 6.84; N, 12.77. Found: C, 54.67; H, 7.13; N, 12.8.

Synthesis of PNA-Oligomers Incorporating *cis*-(1*S*,2*R*)- and (1*R*,2*S*)-Aminocyclohexyl PNA Monomers. The modified PNA monomers were built into PNA oligomers using standard procedures on an 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 $C_{116}H_{154}N_{42}O_{42}$ 2806.0, found 2808.0 $[M + 2H]^+$; PNA **13**, calcd for $C_{120}H_{160}N_{42}O_{42}$ 2860.0, found 2862.0 $[M + 2H]^+$; PNA **14**, calcd for $C_{120}H_{160}N_{42}O_{42}$ 2860.0, found 2861.0 $[M + H]^+$; PNA **15**, calcd for $C_{124}H_{166}N_{42}O_{42}$ 2915.0; found 2917.0 $[M + 2H]^+$; PNA **16**, calcd for $C_{124}H_{166}N_{42}O_{42}$ 2915.0, found 2916.0 $[M + H]^+$.

***T*$_m$ Measurements.** The concentration 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 85 °C for 5 min followed by slow cooling to room temperature. Absorbance versus temperature profiles were obtained by monitoring at 260 nm with a UV−VIS spectrophotometer scanning from 5 to 85 °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.

Gel Mobility Shift Assay. The PNAs (**12**−**16**, Table 1) were individually mixed with DNA (**I** or **III**) in 2:1 ratio (PNA strand, 0.4 mM and DNA **I** or **III**, 0.2 mM) in water. The samples were lyophilized to dryness and re-suspended in sodium phosphate buffer (10 mM, pH 7.0, 10 μL) containing EDTA (0.1 mM). The samples were annealed by heating to 85 °C for 5 min followed by slow cooling to rt and refrigeration at 4 °C overnight. To this, 10 μL of 40% sucrose in TBE buffer pH 8.0 was added and the sample was loaded on the gel. Bromophenol blue (BPB) was used as the tracer dye separately in an adjacent well. Gel electrophoresis was performed on a 15% nondenaturing polyacrylamide gel (acrylamide/bis-acrylamide, 29:1) at constant power supply of 200 V and 10 mA, until the BPB migrated to three-fourth of the gel length. During electrophoresis the temperature was maintained at 10 °C. The spots were visualized through UV shadowing by illuminating the gel placed on a fluorescent silica gel plate, F_{254} using UV light.

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Supporting Information Available: General experimental procedures; ^{1}H NMR, ^{13}C NMR, and mass spectra of compounds **4**−**6** and **8**−**11**. Enantiopurity data for alcohols **3a** and **3b**. HPLC profiles, mass spectra (MALDI-TOF), and characterization data table for PNA **12**, PNA **13**, PNA **14**, PNA **15**, and PNA **16**. CD spectra of (a) PNA **12**, (b) DNA **I**, and (c) complex PNA **12**: DNA **I** and CD Job's plot. Melting curves of PNA **12**, PNA **13**, PNA **14**, PNA **15**, PNA **16** with d(A_{10}), d[A_5CA_4], poly dA, and poly rA, and their corresponding derivatives. X-ray cryatal structures of (a) monomer esters **10a**, **10b**, and cyanuryl PNA monomer and (b) compounds **5a**,**b** and **6a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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