

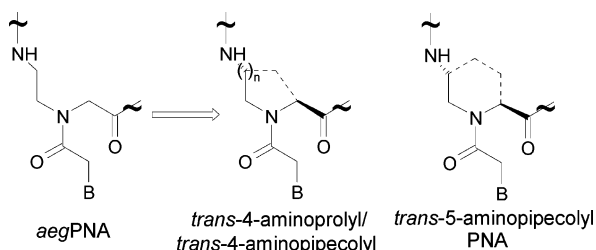
trans-5-Aminopipercolyl-*aeg*PNA Chimera: Design, Synthesis, and Study of Binding Preferences with DNA/RNA in Duplex/Triplex Mode

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The design and synthesis of novel chiral PNA monomer based on *trans*-5-aminopipercolic acid is reported. The *trans* diequatorial disposition of the 1,4 ring substituents in six-membered 5-aminopipercolic acid derivative could be favorable over *trans* 1,3 axial–equatorial disposition in 4-aminopipercolic acid of PNA. Studies on the synthesis of *trans*-4/5-aminopipercolyl PNA-*aeg*PNA chimeras and their binding preferences to DNA/RNA in duplex/triplex modes are described.

Peptide nucleic acids, PNAs, are nucleic acid mimics with a pseudopeptide backbone composed of *N*-(2-aminoethyl)glycine (*aeg*) units (Figure 1, I) to which the nucleobases are attached through methylenecarbonyl linkers.^{1,2} The unique open-chain chemical structure of *aeg*PNA has much potential for refinement considering several available modes for providing charge, chirality, and structural preorganization via chemical bridges between aminoethyl, glycyl, and the nucleobase linker segments of the monomer unit. Most efforts in this direction have been aimed at furthering the potential of PNAs as therapeutic agents with additional requisite properties such as improved water solubility, cellular uptake,³ and discrimination between parallel (*p*) and antiparallel (*ap*) binding modes.⁴ In an earlier study, to understand the structural obligations of *aeg*PNA, structural variations were realized by extending the backbone in either 2-aminoethyl, glycyl, or linker segments of *aeg*PNA.⁵ Aminopropylglycyl PNA *apg*PNA (Figure 1, II)

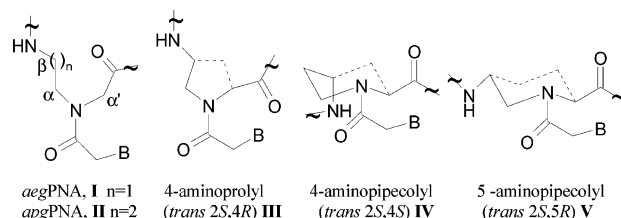


FIGURE 1. *aeg*PNA and its constrained analogues.

was thus synthesized by inserting a methylene group in a 2-aminoethyl segment of I (Figure 1). It was found from UV- T_m studies that the PNA:DNA duplexes or triplexes incorporating a single *apg*PNA monomer were less stable compared to unmodified *aeg*PNA. This decrease in stability for the chimeric *apg*-*aeg*PNA was ascribed to the increase in the degree of conformational freedom in the backbone and consequent large entropy loss during complex formation. Conversely, a favorable structural preorganization of PNA may lead to the desired selective complex formation with the target DNA or RNA, provided the enthalpic contributions are conserved. The structural preorganization approach using additional conformational constraint has been especially successful in the case of DNA analogues such as locked nucleic acids (LNAs),⁶ conformationally frozen hexitol,⁷ cyclohexene,⁸ and altritol nucleic acids.⁹ The stability is conferred because of the RNA-like structures either locked or frozen in 2'-*endo* sugar ring conformations. As the structural preferences of PNA in PNA/DNA or PNA/RNA complexes are not yet as well deciphered as those of DNA–DNA/RNA complexes,¹⁰ there is intense activity in this field to obtain an optimal PNA analogue. Our quest to find correct conformational and configurational preferences for optimal DNA/RNA recognition has previously resulted in very interesting structural modifications¹¹ of PNA that include uncharged 4-aminopropyl (Figure 1, III) and 4-aminopipercolyl (Figure 1, IV) chiral PNA derivatives. These involved introduction of a methylene bridge between the α' carbon atom of the glycyl moiety and the β/γ carbon atom of the aminoethyl/aminopropyl segment of the parent *aeg*/*apg*PNA to generate five-/six-membered pyrrolidine/piperidine rings. The comparative UV- T_m studies after incorporation of these two modifications

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in a triplex forming *aeg*PNAT₁₀ sequence showed that both (2*S*,4*R*) five-¹² and (2*S*,4*S*) six-membered¹³ trans ring conformations caused detrimental effects on the stability of PNA₂:DNA and thus were incapable of adopting the structural bias required for PNA₂:DNA triplex formation.

The primary knowledge of the stability of trans conformations in six-membered rings suggested that a *trans*-5-aminopipercolyl monomer (Figure 1, **V**) could be a better choice because of the stable, favored diequatorial disposition of the backbone. In comparison, the trans axial-equatorial disposition of substituents in either **III** or **IV** (Figure 1) may not be favored. The rationally evolved structure **V** arises from the insertion of an *ethylene bridge* between α' carbon atom of the glycyl moiety and the β carbon atom of the aminoethyl segment of *aeg*PNA **I**. Our recent studies on the conformationally frozen six-membered PNA analogue, a positively charged chimeric piperidine-*aeg*PNAT₈, favored sequence-specific triplex formation with target oligomers.¹⁴

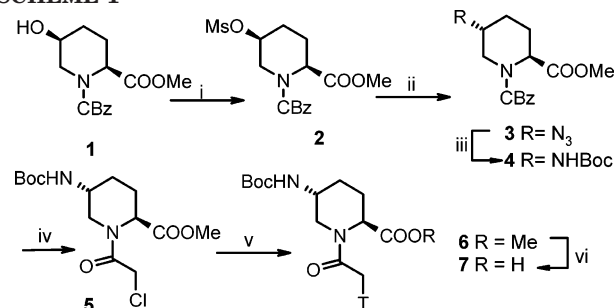
In this note, we present our results on the synthesis and hybridization properties of the designed *trans*-(2*S*,5*R*)-5-aminopipercolyl thyminylnyl PNA monomer, its incorporation into triplex- and duplex-forming oligomers, and their DNA and RNA binding studies in comparison with the *trans*-4-aminopipercolyl PNA analogue reported earlier.¹³

Synthesis of 5-Aminopipercolyl PNA Monomer.

The chiral pipercolyl PNA thymine monomer *trans*-(5*R*)-(N-Boc-amino)-N-(thymine-1-ylacetyl)-(2*S*)-pipercolic acid **7** was synthesized from *cis*-N-benzyloxycarbonyl-(5*S*)-hydroxy-(2*S*)-pipercolic acid methyl ester **1**, which in turn was synthesized from L-glutamic acid¹⁵ (Scheme 1). The 5-hydroxy group was converted to mesylate **2** by reaction with MsCl in dry pyridine followed by S_N2 displacement of (5*S*)-O-mesyl group by treatment with sodium azide to yield (5*R*)-azide **3**. The azide group was selectively reduced to the 5-amino derivative by hydrogenation in the presence of Ra/Ni as a catalyst. The resulting free amine was protected in situ as Boc derivative **4**. Deprotection of the ring nitrogen by hydrogenation over 10% Pd-C and subsequent acylation with chloroacetyl chloride gave compound **5**. N-1 alkylation of thymine was effected by reaction with **5** in the presence of K₂CO₃ in DMF to give N-1 thyminylmethylcarbonyl derivative **6**. The methyl ester in **6** was hydrolyzed using sodium hydroxide in aqueous methanol to give free acid **7**, the required monomer block for building the oligomers. The structural integrity of the PNA monomer was confirmed by NMR and mass spectroscopic analysis.

Synthesis of *aeg*PNA and Pipercolyl-*aeg*PNA Chimeras. The *aeg*PNA oligomers are known to bind DNA/RNA sequences with high specificity and affinity.^{1,2} Incorporation of modified chiral PNA monomer units derived from chiral amino acids in the *aeg*PNA backbone seem to induce conformational preferences that assist the binding of such chimeric PNAs to DNA/RNA.^{11,16} Intro-

SCHEME 1^a



^a Reagents: (i) MsCl, Py, 70%. (ii) NaN₃, DMF, 90%. (iii) H₂, Ra/Ni, (Boc)₂O 90%. (iv) (a) Pd-C/H₂; (b) ClCH₂COCl 60%. (v) Thymine, K₂CO₃, DMF, 73%. (vi) 1 M NaOH, H₂O-MeOH, 90%.

duction of conformationally constrained modified units at predefined positions was therefore undertaken to study their effect on the conformational organization of PNA that could determine the binding preferences. Introduction of the chiral unit at C/N-termini in sequences **8–11** could preferentially induce helicity in PNA oligomers.¹⁷ The propagation of such induced structural bias may be enhanced by the introduction of a second chiral unit as in sequences **12** and **13**.¹⁸ Introduction of the chiral units at adjacent positions or synthesis of homogeneous backbone-modified PNAs was not realized, as the rigidity of the backbone in such PNAs reported earlier did not allow binding to the complementary nucleic acids.^{12,19} The mixed base oligomers **17–19** were synthesized to study the effect of modification in duplex binding mode. The synthesis of PNA oligomers comprising standard *aeg*PNA monomers, 4-aminopipercolyl PNA monomer,¹³ and **7** was carried out using standard solid-phase synthesis protocols.²⁰ The oligomers after the synthesis were cleaved from the support using trifluoroacetic acid-trifluoromethanesulfonic acid²¹ to yield the PNA oligomers carrying β -alanine at their carboxy termini (Table 1 and Table 2). The purity of oligomers was rechecked by RP-HPLC on a C18 column and characterized by MALDI-TOF mass spectrometry (Supporting Information). The PNA sequences used here are either homopyrimidine sequences **8–14** that are known to form PNA₂:DNA triplexes or duplex forming mixed purine-pyrimidine sequences (**17–19**). A Job plot²² confirmed a 2:1 stoichiometry of binding between the PNA strand **13** and DNA **15** (Supporting Information). All DNA/RNA binding studies were performed with 1:2 stoichiometry of DNA and homothyminylnyl pipercolyl-*aeg*PNA. Mixed base sequence complex formation studies were performed using 1:1 PNA:DNA/RNA stoichiometry.

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TABLE 1. UV- T_m Measurements of PNA₂:DNA Triplexes^a

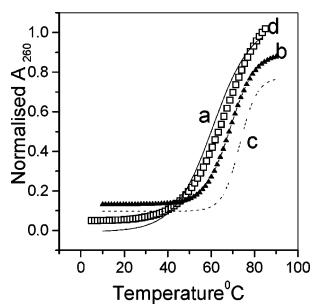
PNA sequences		T_m (°C)
H-(T) ₉ -t-β-ala-OH	(2S,4S) ¹³ 8	55.9
	(2S,5R) 9	61
H-t (T) ₉ -β-ala-OH	(2S,4S) 10	56
	(2S,5R) 11	68
H-(T) ₃ t (T) ₅ tβ-ala-OH	(2S,4S) 12	34
	(2S,5R) 13	78
H-(T) ₁₀ -β-ala-OH	14	67

^a Buffer: 10 mM sodium cacodylate, 100 mM NaCl, 0.01 mM EDTA, pH 7.3. Melting experiments were performed with a PNA:DNA strand concentration of 1 μM each. DNA-**15** 5'-GCAAAAAAAAAACG-3' complementary to PNA **8-14**. DNA-**16** 5'-GCAAAATAAAAACG-3' T represents *aeg*PNA units and t 4/5-aminopipercolyl T = mismatched base in DNA. The values reported here are the average of three independent experiments and are accurate to ±0.5 °C.

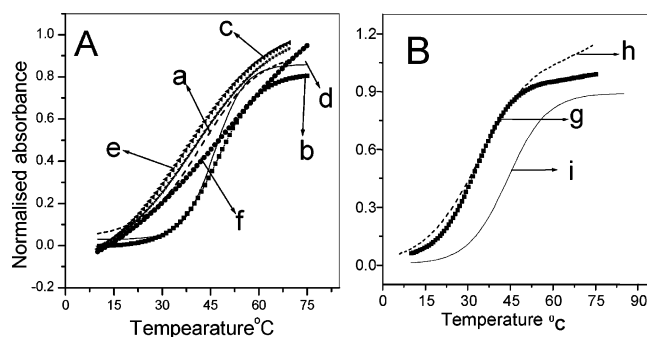
TABLE 2. UV- T_m (°C) Studies of PNA:DNA/RNA Duplexes^a

PNA sequences	DNA		RNA
	<i>ap</i> 20	<i>p</i> 21	<i>ap</i> 22
H-GTA GATCACT-β-ala-OH 17	47	46	43
H-GTAGAt (2S,4S)CACT-β-ala-OH 18	48	37	38
H-GTAGAt (2S,5R)CACT-β-ala-OH 19	43	40	32

^a T, C, G, and A represent *aeg*PNA unit and t represents pipercolyl PNA units, DNA (*ap*) **20** 5'-AGT GAT CTA C-3', DNA (*p*) **21** 5'-CAT CTA GTG A-3', RNA (*ap*) **22** 5'-AGT GAT CTA C-3'.

**FIGURE 2.** UV-melting profile of PNA₂:DNA complexes (a) **9:15**, (b) **11:15**, (c) **13:15**, (d) **14:15**.

Pipercolyl-*aeg*PNA₂:DNA Triplexes. The normalized absorbance versus temperature plots derived from the UV melting data indicated a single transition indicative of the simultaneous dissociation of the two PNA strands from the DNA in the DNA:PNA₂ complex. The results of UV- T_m studies are summarized in Table 1 and shown in Figure 2. The C-terminal 5-aminopipercolyl (2S,5R) unit in PNA **9** as well as the 4-aminopipercolyl (2S,4S) unit in PNA **8** destabilized the resulting complex with DNA ($\Delta T_m = -6$ and -12 °C, respectively) compared to the unmodified *aeg*PNA **14**. The presence of aminopipercolyl units at the N-terminal (2S,4S) unit caused a destabilization of the complex ($\Delta T_m = -11$ °C, PNA **10**), while the (2S,5R) unit had minor effect in altering the stability of its complex with DNA ($\Delta T_m = +1$ °C PNA **11**). Compared to unmodified *aeg*PNA, increasing the number of aminopipercolyl units to two led to favorable complex formation ($\Delta T_m = +11$ °C) for the (2S,5R) unit (PNA **13**) but highly unfavored complex formation ($\Delta T_m = -33$ °C) for the (2S,4S) unit (PNA **12**). Thus, the (2S,5R) unit seems to be quite efficient in binding to complementary DNA in triplex mode. In comparison, both 4-amino pipercolyl and 4-aminopropyl PNA units **13**

**FIGURE 3.** UV-melting profile of (A) PNA:DNA (a) **19:20**, (b) **17:20**, (c) **19:21**, (d) **17:21**, (e) **18:21**, (f) **18:20** and (B) PNA:RNA (g) **19:22**, (h) **18:22**, (i) **17:22**.

in the oligomer were detrimental to the PNA₂-DNA complex stability.

PNA oligomers **13** and **12** with DNA **16** having a single mismatch at the site complementary to the pipercolyl unit caused a decrease in the T_m values of the corresponding triplexes as compared to the fully complementary triplex by 9 and 4 °C, respectively. In comparison, the control PNA₂ **14**:DNA **16** showed a linear increase in absorbance with increasing temperature with no sigmoidal transition.

Pipercolyl-*aeg*PNA:DNA/RNA Duplexes. It was considered necessary to examine the effect of the pipercolyl PNA backbone chirality on the binding orientation in duplexes with mixed purine/pyrimidine sequences. Recently, there is also considerable interest in designing compounds that would show bias for binding either to DNA or RNA.^{11,23} Thus, the 4/5-aminopipercolyl PNA units were introduced in the mixed purine-pyrimidine sequence (**17-19**). All the pipercolyl-*aeg*PNA oligomers formed more stable duplexes in the *ap* mode with complementary DNA. This trend is also observed with *aeg*PNAs tethered with lysine² at the C-terminus. A single (2S,4S) pipercolyl unit destabilized the parallel duplex by 11 °C, whereas the antiparallel duplex is marginally stabilized ($\Delta T_m = +1$ °C). Destabilization was observed for the complexes having 5-aminopipercolyl unit in the *p* mode as well as the *ap* mode (Table 2, Figure 3 A). Both 4-aminopipercolyl (2S,4S) and 5-aminopipercolyl (2S,5R) modified oligomers **18** and **19** hybridized with RNA **22** (Figure 3 B) but with a decrease in T_m compared to control PNA ($\Delta T_m = -5$ and -11 °C). Thus, *trans*-4-aminopipercolyl PNA monomer **IV** that destabilized PNA:RNA and PNA₂:DNA triplexes effected significant *p/ap* orientation selectivity for PNA:DNA duplex formation.

The 1,4 *trans* diequatorial disposition of the substituents in the monomeric form of the *trans*-5-amino pipercolyl derivative could additively effect the conformational bias that stabilized triplex with PNA **13**. The 1,3 *trans* axial-equatorial substitution in the 4-amino pipercolyl derivative resulted in additive destabilization of the triplex with PNA **12**. The duplex formation with either DNA or RNA suffered a set back by introduction of a single modified

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unit of 4/5-amino pipecolyl derivative. although the 4-amino pipecolyl derivative differentiated between *p* versus *ap* orientation of complementary DNA. It is interesting to note that the constrained *apg*PNA analogue, 4-aminopipecolyl (2*S*,4*S*), in **18** reverts the destabilization caused by the open-chain *apg*PNA unit in the same sequence.⁵ Thus, the structural differences in PNA:DNA, PNA:RNA, and PNA₂:DNA and elegant use of substitution pattern of six-membered pipecolic acid derivatives directed the complexation of pipecolic *aeg*PNA with DNA/RNA sequences. These results lead us to believe that a biased preorganized conformation capable of tuning the correct internucleobase geometry could be particularly important when duplex/triplex, *p/ap*, or DNA/RNA selectivity is being addressed.

In summary, the data presented in this note point out the effectiveness of the design that allows *aeg*PNAs to be biased for binding in a triplex mode and an antiparallel duplex mode. The design also enforces superior binding to DNA over RNA. It is likely that PNA oligomers composed exclusively or predominantly of pipecolyl PNA monomers may hybridize better than the chimeric backbone oligomer studied here. More data in terms of sequence and backbone context (number and position of pipecolyl units) are required to fully evaluate the properties of 4/5-aminopipecolyl PNA in terms of producing hybridization-competent conformations. In our recent work using aminoethylpipecolyl PNA, we reported additive properties of the monomeric units in the triplex forming oligomers.^{11c,18} Further work to fully understand these features is currently in progress in our laboratory.

Experimental Section

(2*S*,5*S*)-5-*O*-Methylsulfonyl-*N*-1 (Benzyloxycarbonyl)-pipecolic Acid Methyl Ester 2. To a stirred, cooled solution of compound **1** (1.5 g, 5.1 mmol) in dry pyridine (20 mL) was added methane sulfonyl chloride (0.7 mL, 6.11 mmol) dropwise. Stirring was continued for 2 h at which point TLC indicated complete conversion of starting material. The solvent was removed under vacuum, and the residue was taken in water and extracted with ethyl acetate (2 × 15 mL). The organic layers were pooled, washed with water (2 × 10 mL) followed by brine (1 × 10 mL), and dried over sodium sulfate. Evaporation of the organic layer yielded a crude product that was then purified by silica gel column chromatography (yield, 70%). $[\alpha]_D^{24} -27.5$ (c 0.004, MeOH). ¹H NMR CDCl₃ δ: 7.35(s, 5H), 5.2 (d, 2H), 5–4.75(m, 1H), 4.7–4.3 (m, H₆), 3.7 (m, 3H), 3.1 (m, 3H), 2.5–1.4 (m, 5H). ¹³C NMR CDCl₃ δ: 170, 158,137.1 136.0, 128.4, 128.1, 127.8, 67.8, 52.3, 44.9(C₆), 38.6, 27.9, 20. ESIMS: calcd for C₁₆H₂₁NO₇ S (M⁺) 371, found 389 (M + NH₄⁺).

(2*S*,5*R*)-5-Azido-*N*-1 (Benzyloxycarbonyl)pipecolic Acid Methyl Ester 3. To the compound **2** (0.74 g, 1.99 mmol) in dry DMF was added NaN₃ (0.76 g, 11.6 mmol). The mixture was stirred at 60 °C for 12 h at which point TLC indicated the complete conversion of starting material. DMF was removed under vacuum; water (10 mL) was added to the mixture, and the mixture was extracted with EtOAc (3 × 15 mL). Organic layers were pooled, washed with water (1 × 10 mL) and brine (1 × 10 mL), and concentrated to give a crude product that was then purified by silica gel column chromatography (yield 0.56 g, 90%). $[\alpha]_D^{24} -20$ (c 0.008, MeOH). ¹H NMR CDCl₃ δ: 7.35 (s, 5H), 5.2 (s, 2H), 5.1–4.8 (m, 1H), 4.35–4.1 (m, 1H), 3.9–3.6 (m, 4H), 3.49–3.2 (m, 1H), 2.2–2 (m, 1H), 1.9–1.5 (m, 2H). ESIMS: calcd for C₁₅H₁₈N₄O₄ 318, found 319 (M + 1).

(2*S*,5*R*)-*N*-(*tert*-Butyloxycarbonyl)-*N*-1-(benzyloxycarbonyl) Pipecolic Acid Methyl Ester 4. Compound **3** (0.74 g, 2.32 mmol) was taken in ethyl acetate. To this were added Ra/Ni (0.5 mL) and di-*tert*-butyl dicarbonate (0.55 mL, 2.52 mmol). This mixture was subjected to hydrogenation for 2 h at a

pressure of 40 psi in a Parr hydrogenation apparatus at which point TLC indicated complete disappearance of starting material. The Ra/Ni suspension was removed by filtration through Celite. The filtrate was concentrated to give a crude product that was then purified by column chromatography (yield 0.9 g, 90%). ¹H NMR CDCl₃ δ: 7.3 (s, 5H), 5.15 (s, 2H), 5.0–4.75 (s, 1H), 4.2–4 (m, 1H), 3.7 (s, 4H), 3.35–2.9 (m, 1H), 2.49–1.6 (m, 4H), 1.4 (s, 9H). ¹³C NMR CDCl₃ δ: 171.5, 159.8, 155.2, 136.5, 128.6, 128.1, 127.7, 79.4, 67.6, 52.3, 46.0, 44.2, 28.4, 25.9, 21.1. ESIMS: calcd for C₂₀H₂₈N₂O₆ (M⁺) 392, found 409 (M + 1).

(2*S*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-*N*-1-(chloroacetyl)-pipecolic Acid Methyl Ester 5. Compound **4** (0.61 g, 1.55 mmol) was dissolved in methanol, and 10% Pd–C (90 mg) was then added. This mixture was subjected to hydrogenation at 60 psi for 8 h. The catalyst was filtered off over Celite, and the filtrate was evaporated under vacuum to obtain free amine. Free amine (0.19 g, 0.73 mmol) was taken in 10% Na₂CO₃ solution in dioxane:water (1:1) and cooled in an ice bath. Chloroacetyl chloride (0.16 mL, 1.4 mmol) was added with vigorous stirring. The pH of the reaction was maintained around 9.0 during reaction with addition of solid Na₂CO₃. After 1 h, at which point TLC indicated complete disappearance of starting material, the reaction mixture was concentrated to remove the dioxane. The product was extracted with EtOAc and purified by column chromatography (yield 0.11 g, 60%). $[\alpha]_D^{24} -13$ (c 0.02, MeOH). ¹H NMR CDCl₃ δ: 4.9–4.8 (d, 1H), 4.2–3.8 (m, 2H), 3.7 (s, 3H), 3 (s, 1H), 2.4–2 (m, 2H), 1.9–1.51 (m, 3H), 1.4 (s, 10H). ¹³C NMR CDCl₃ δ: 170.9, 170.4, 167.0, 155.08, 80.34, 79.5, 74.9, 74.6, 52.2, 46.7, 45.3, 44.1, 40.6, 38.6, 28.0, 27.8, 24.8, 20.5. ESIMS: calcd for C₁₄H₂₃N₂O₅Cl (M⁺) 334, found 335 (M + 1).

(2*S*,5*R*)-5-(*tert*-Butyloxycarbonyl)-*N*-1-(thyminyllacetyl)-pipecolic Acid Methyl Ester 6. Compound **5** (0.15 g, 0.49 mmol) was stirred with anhydrous K₂CO₃ (0.186 g, 1.34 mmol) and thymine (0.17 g, 1.34 mmol) in dry DMF at 60 °C for 5 h. When TLC indicated complete disappearance of starting material, DMF was evaporated, and the product was purified by column chromatography (yield = 110 mg, 61%). ¹H NMR CDCl₃ δ: 9.2 (s, 1H), 7.3 (s, 1H), 5.5 (d, 1H), 4.9–4.8 (m, 1H), 4.3–4.2 (m, 1H), 4.1–3.8 (m, 2H), 3.75 (s, 3H), 3.6–3.49 (m, 1H), 2–1.6 (m, 8H), 1.45 (s, 9H). ¹³C NMR CDCl₃ δ: 170.85, 167.13, 164.71, 155.74, 140.94, 52.50, 54.14, 48.18, 46.12, 44.64, 28.33, 25.32, 21, 12.22. ESIMS: calcd for C₁₉H₂₈N₄O₇ (M⁺) 424, found 425(M + 1), 442 (M + NH₄⁺).

(2*S*,5*R*)-5-(*tert*-Butyloxycarbonyl)-*N*-(thyminyllacetyl)-pipecolic Acid 7. Compound **6** (0.12 g, 0.15 mmol) was dissolved in 2 N NaOH in methanol/water (1 mL) and stirred for 20 min. The pH of the solution was adjusted to 2 by addition of cation-exchange resin, which was then filtered off. The filtrate was then evaporated, producing the product as a white foam (0.1 g, 92%). $[\alpha]_D^{24} = -1.25$ (c 0.008, MeOH). ¹H NMR D₂O δ: 7.29 (s, 1H), 5.13 (bs, 1H), 4.9–4.78 (m, 1H), 4.64 (s, 1H), 4.61–4.3 (m, 1H), 3.85–3.73 (m, 1H), 3.70–3.4 (m, 2H), 2.46–1.88 (m, 2H), 1.82 (s, 3H), 1.79–1.65 (m, 2H), 1.38 (s, 9H). ¹³C NMR D₂O δ: 170.62, 169.99, 157.43, 153.63, 143, 47.75, 47.28, 46.5, 29.53, 27.34, 11.10. ESIMS: calcd for C₁₈H₂₆N₄O₇ (M⁺) 410, found 410 (M⁺).

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Supporting Information Available: ¹H and ¹³C NMR spectra of compound **6**; ESIMS for compounds **2**, **6**, and **7**; elemental analysis for compounds **1** and **5**; representative HPLC for oligomers **9**, **11**, **13**, and **17–19**; representative MALDI-TOF spectra for PNAs **8–10**, **11**, **17**, and **18**; Job plot for PNA **13** and DNA **15** showing PNA₂:DNA stoichiometry; representative *T_m* (first derivative) curves for thermal denaturation of the complexes; and gel-shift assay of chimeric pipecolyl-*aeg*PNA:DNA complexes. This material is available free of charge via the Internet at <http://pubs.acs.org>. JO0506884