Supporting Information for:

A Cyclopentane Conformational Restraint for a Peptide Nucleic Acid: Design, Asymmetric Synthesis, and Improved Binding Affinity to DNA and RNA

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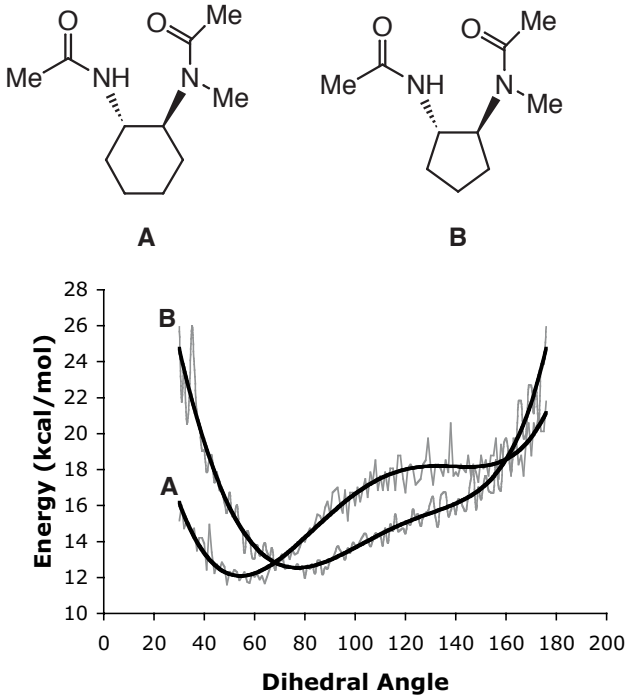
Computational Methods.

Molecular mechanics calculations were used to evaluate modified PNA monomers. Using the program Tinker with the MM3 force field[1,2,3] and the GB/SA aqueous solvation model,[4] the conformations and associated relative energies of candidate molecules were examined. In order to avoid lengthy computational time, each calculation examines the conformations of a simplified model of a PNA monomer. For each molecule, the relative energies associated with the dihedral angle between the two nitrogens were calculated by examining the various populations in a standard 1500 K molecular dynamics simulation, where E=(-0.0019871 kcal/(K*mol)) * 1500 K * ln(population over 1° range). For each molecule, these studies provide an indication of the potential energy over a range of dihedral angles. The first computational study examined a PNA monomer in which the ethylenediamine portion of a PNA monomer was incorporated into a cyclohexane ring (Figure 1). Cyclohexyl-PNA has been synthesized previously by Nielsen and co-workers.[5] The binding of cyclohexyl-PNA to complementary DNA and RNA is about the same or weaker than regular PNA, depending on the base sequence. Our computational studies provide some insight into this result. The minimum energy conformations for our model has dihedral angles of 50-65° between the two nitrogen (which is in accord with placing the two subtituents in equatorial positions on the ring). The potential energy well for this molecule is also very steep. The observation that cyclohexyl-PNA binds to complementary RNA and DNA in an unchanged or diminished capacity relative to unsubstituted PNA is understandable from our model. While the cyclohexane ring imparts a large degree of conformational rigidity to a PNA, at the same time it rigidifies the PNA to a range of dihedral angles that are not optimal for binding and as a result the overall binding affinity either does not change or decreases. We conclude from Nielsen's work and our computational model that the dihedral angles for cyclohexyl-PNA are inappropriate to promote binding to RNA or DNA.

Figure 1. Molecular modeling of PNA monomers **A** and **B**. The graph shows the relative energies of the dihedral angle between the nitrogens on the ring. Raw data is shown in gray. The dark lines represent 5th order polynomials that have been fit to each data set.



We considered alternatives to a cyclohexyl ring that would afford PNAs with more appropriate dihedral angles for oligonucleotide binding. In this regard, we examined a cyclopentane-derived PNA monomer. Our computational results indicated that the cyclopentane ring should have dihedral angles that impart correct dihedral angles to cyclopentyl PNA so that it can bind to RNA. The lowest energy conformations for the cyclopentane model have dihedral angles of 70-90°. These values overlap very nicely with the observed dihedral angles in a PNA-RNA duplex. Compared to cyclohexane, cyclopentane rings are known to adopt several low energy conformations.[6] This difference is reflected in the significantly broader potential energy well from our cyclohexane vs. cyclopentane calculations. A published crystal structure of a *trans*-1,2-disubstituted cyclopentane possesses a dihedral angle of 70° between the substituents,[7] reinforcing that our calculations are in accord with a low-energy conformation for such molecules.

Synthesis.

General Considerations: Melting points (m.p.) were obtained on a Fisher-Johns Melting Point Apparatus and are uncorrected. Optical rotations were measured on an Optical Activity AA-100 Automatic digital polarimeter using sodium light (D line, 589.3 nm) and are reported in degrees; concentrations (c) are reported in g / 100 mL. Proton nuclear magnetic resonances (**^{1}H NMR**) were recorded in deuterated solvents on a Mercury 400 (400 MHz) and iNOVA 500 (500 MHz) spectrometers. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (δ 0.00) If

tetramethylsilane was not present in the deuterated solvent, the residual protio solvent is referenced ($CDCl_3$, δ 7.27; $C\underline{D}_3OD$, δ 3.30; DMSO-d_6, δ 2.50). AB quartets were solved according to the following equations: $C = 1/2(\nu_1 + \nu_4) = 1/2(\nu_2 + \nu_3)$; $\Delta\nu = [(\nu_1 + \nu_4)(\nu_2 - \nu_3)]^{1/2}$; $\nu_A = C + (\Delta\nu_1 / 2)$; $\nu_B = C - (\Delta\nu / 2)$. ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), or AB (AB quartet). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m) or broad (br). Coupling constants are reported in Hertz (Hz). Gas chromatography (**GC**) data were obtained using a Varian CP-3800 Gas Chromatograph. HPLC data were obtained using a Varian ProStar HPLC. Mass spectra (**MS**) were obtained using a Hewlett Packard 6890 GC/MSD. Electrospray mass spectra (**ESI-MS**) were obtained using a Micromass Quattro II Triple Quadrupole HPLC/MS/MS Mass Spectrometer. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were distilled from sodium and benzophenone prior to use. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride prior to use. All reactions were performed in oven dry glassware under a positive pressure of nitrogen unless otherwise noted. Analytical thin layer chromatography (TLC) was carried out on Sorbent Technologies TLC plates precoated with silica gel (250 μm layer thickness). Flash column chromatography was performed on EM science silica gel 60 (230-400 mesh). Solvent mixtures used for TLC and flash column chromatography are reported in v/v ratios. Unless otherwise noted, all other commercially available reagents and solvents were purchased from Aldrich and used without further purification. HATU was purchased from Applied Biosystems, and ethyl glyoxlate/toluene solution was purchased from Fluka.

Abbreviations: (TMP), tetramethylpiperidine; (TEMPO), 2,2,6,6-tetramethylpiperidinyl-1-oxy; (TEA), triethylamine; (DIEA), diisopropylethylamine; (HATU), N,N,N',N'-Tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate.

(-)-Dimenthyl-(1*S*,2*S*)-cyclopentane-1,2-dicarboxylate (2) was prepared using a modification of the procedure reported by Yamamoto and co-workers.[8] Two separate oven dry 1 L RBFs were charged with dimenthylsuccinate (**1**) (70 g, 0.177 mol) and 1,3-propaneditosylate (68 g, 0.177 mol), respectively. Dry THF (800 mL) was added to each 1 L RBF and cooled to 0 °C. Dry THF (800 mL) was added to an oven dry 3 L 3-neck RBF and cooled to 0 °C. TMP (75 mL, 0.444 mol) was added to the 3 L reaction vessel using an oven-dried addition funnel. The addition funnel was then washed with dry THF (10 mL) and replaced with a septum. Next, n-BuLi [248 mL, 0.444 mol, 1.79 M (calculated from titration)[9]] was transferred via cannula to the reaction vessel and the solution was stirred for 45 min at 0 °C. The resulting solution of LiTMP was cooled with a dry ice-IPA bath and stirred for 30 min. Next, the cooled solution of **1** was transferred via cannula to the reaction vessel over a 1 hour period. The resulting dienolate solution was stirred for an additional 30 min. (NOTE: The solution was stirred vigorously and kept at -78 °C throughout the entire reaction.) Next, the 1,3-propaneditosylate solution was added via cannula to the reaction vessel over a 3 hour period. The reaction solution was allowed to stir 3 hours after the addition of 1,3-propaneditosylate and a GC of the solution was taken at 1 hour intervals. The diasteroselectivity of **2** was found to be 84% by GC analysis after 3 hours of stirring. Teraphaldicarboxaldehyde (8 g, 60 mmol) in dry

THF (250 mL) was then added via cannula to the reaction vessel. The dialdehyde reacted with most of the remaining dianion while stirring over a 4 hour period. The reaction was finally quenched with 2 N HCl (400 mL) and transferred to a 2 L separatory funnel. The upper organic layer was removed and the remaining aqueous solution was extracted with Et_2O (4 x 150 mL). All organic layers were combined and washed with sat. solutions of $NaHCO_3$ (2 x 150 mL) and NaCl (2 x 150 mL). The organic layer was separated, dried over Na_2SO_4, filtered via suction filtration, and concentrated on a rotary evaporator to give a thick yellow sticky oil (110 g). The mixture contained all quenched by-products as well as **2**. The yellow mixture was split in two equal portions and each portion was eluted (10:1 hexane : ethyl acetate, 1.4 L) through a 600 mL glass fritted funnel filled with 400 mL of silica gel. The resulting solutions were concentrated to give 60 g of a crude solid. The product was purified by silica gel chromatography eluting with 18:1 hexane : diethyl ether to afford a mixture of diastereomers as a clear oil. The (*S,S*) diastereomer of **2** selectively crystallized from the mixture of diasteromers in 10:1 hexane : diethylether to give 32 g (42%) of **2** in >99% de, as clear crystals: **m.p.** = 62-64 ^oC; **^{1}H NMR** ($CDCl_3$-*d*, 500 MHz): δ 4.60 (dt, *J* = 10.8, 4.5 Hz, 2H, menthyl-OCOC<u>H</u>), 3.00 (m, 2H, cyclopentane-C<u>H</u>), 0.83 (m, 12H, HC(C<u>H</u>$_3$)$_2$), 0.68 (d, *J* = 7.0 Hz, 6H, HCC<u>H</u>$_3$), 0.70-2.05 (complex, 24H); $[\alpha]_D^{23.5}$ +33.2^o (*c* 1.0, $CHCl_3$). A crystal structure of **2** was obtained (see associated CIF file for details). GC conditions to determine the de of **2** employed a DB-WAX column ramping from 80 ^{o}C to 240 ^{o}C at a rate of 5 ^{o}C / min, and holding for 15 min.

(-)-(1*S*,2*S*)-cyclopentane-1,2-dicarboxylic acid (3) was prepared from **2** in two steps, a reduction followed by an oxidation. **Reduction:** (-)-(*S,S*)-cyclopentanedicarboxylate menthylester **(2)** (4.3 g, 10 mmol) was weighed into an oven dried 100 mL RBF. Dry THF (50 mL) was added to dissolve **2**. In a separate oven dried 250 mL RBF, dry THF (100 mL) was added followed by $LiAlH_4$ (1.1 g, 30 mmol). The resulting solution was cooled in an ice bath to 0 ^{o}C. The solution of **2** was transferred via cannula to the $LiAlH_4$ solution over a 10 minute period . Additional THF (10 mL) was added to rinse the inside of the flask, and then transferred to the reaction solution. The ice bath was removed and the resulting solution was stirred for 5 hours while warming to rt. Saturated, aqueous Na_2SO_4 (20 mL) was added dropwise until H_2 evolution stopped, then the remaining Na_2SO_4 solution was added gradually. The lithium salts were filtered by suction filtration and the solution concentrated on a rotary evaporator to yield a mixture of diol and (-)-menthol as a clear oil. **Oxidation:** The mixture of diol and (-)-menthol were oxidized following the procedure of Zhao and co-workers.[10] Sodium phosphate buffer (150 mL, 0.6 M, pH 6.7) and MeCN (200 mL) were added to the mixture of diol and (-)-menthol, in a 500 mL RBF. TEMPO (650 mg, 4.2 mmol) was added, and the resulting solution heated to 35 ^{o}C. Next 20% of a $NaClO_2$ solution (9.15 g 80%, 80 mmols in 25 mL of H_2O) and 20% of a NaClO solution (0.8 mL 4.0 % in 25 mL of H_2O) were added concurrently via syringe to give a rust red solution. The remaining chlorite and hypochlorite solutions were added over 30 min. (NOTE! Do not mix the solutions prior to addition.) The resulting solution was vigorously stirred at 35 ^{o}C for 9 hours. H_2O (100 mL) was added to the reaction, followed by 2 N NaOH until the solution reached a pH of 10. The mixture was poured into an ice cold Na_2SO_3 solution (12.0 g in 150 mL of H_2O), maintained at <20 ^{o}C with an ice bath, and stirred for 30 min. The solution was

transferred to a separatory funnel and extracted with Et_2O (3 x 150 mL) to remove TEMPO and impurities. The solution was acidified to pH 1 with 3 N HCl and extracted with Et_2O (5 x 150 mL). The organic layers were combined, washed with H_2O (1 x 100 mL), dried over sodium sulfate, filtered by suction filtration, and concentrated on a rotary evaporator to obtain 1.33 g of an off white solid. The solid was washed with $CHCl_3$ (8 mL), collected by suction filtration, and dried under vacuum to give 1.30 g (83% yield) of **4** as a white solid: **m.p.** = 187-189 °C; **^{1}H NMR** (DMSO-d_6, 500 MHz): δ 12.16 (s, 1H, COOH), 2.80 (m, 2H, HOOCCH), 1.94 (m, 2H, CH_2CH_2), 1.65 (m, 4H, $CHCH_2$); $[\alpha]_D^{23.5}$ +77.4° (*c* 1.0, MeOH). Spectroscopic data were consistent with literature data for this compound.[11,12]

(-)-(1*S*,2*S*)-di(*tert*-butoxycarbonylamino)cyclopentane (4) was prepared using a modification of the procedure reported by Aitken and co-workers.[13] (-)-(*S,S*)-cyclopentanedicarboxylic acid **3** (1.30 g, 8.20 mmol) was weighed into an oven dry 100 mL RBF, dissolved in THF (60 mL), and cooled to 0 °C. TEA (5.24 mL, 32.8 mmol) and EtOCOCl (3.60 mL, 32.8 mmol) were added and the suspension stirred for 20 min. Next, an aqueous solution of NaN_3 (3.66 g, 49.2 mmol in 15 ml of H_2O) was added via syringe to the reaction flask. The reaction mixture was stirred vigorously for 20 min, after which time the ice bath was removed, and the mixture stirred an additional 10 min. The solution was transferred to a separatory funnel, washing the reaction flask with H_2O (15 mL) and EtOAc (2 x 15 mL), and extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered via suction filtration, and concentrated in an oven dry 250 mL RBF (to an oil of approximately 3 mL). (NOTE! Do not concentrate the solution to dryness.) Benzene (80 mL) was added to the oil via syringe and the reaction flask was attached to a reflux condenser. The solution was heated under reflux at 95 °C for 45 min to promote the double Curtius rearrangement. After refluxing, benzene was removed under reduced pressure, the RBF was reattached to the condenser, and 2-methyl-2-propanol (40 mL, excess) added via syringe to the bis-isocyanate. The reaction solution was then heated under reflux for 15 hours. After 15 hours, the solution was allowed to cool to 50 °C and excess 2-methyl-2-propanol was removed on a rotoray evaporator. The resulting off white solid was dried under vacuum to give 2.00 g (81 %) of **4**: **m.p.** = 172-173 °C; **^{1}H NMR** ($CDCl_3$-*d*, 500 MHz): δ 4.88 (br s, 2H, NH), 3.63 (br s, 2H, carbamate-CH), 2.11 (m, 2H, CH_2), 1.68 (m, 2H, CH_2), 1.44 (s, 18H, *t*-butyl-CH_3), 1.35 (m, 2H, CH_2). Spectroscopic data were consistent with the literature data for this compound.[13]

(-)-(1*S*,2*S*)-*N*-Mono(*tert*-butoxycarbonyl) cyclopentanediamine (5). (-)-(1*S*,2*S*)-di(*tert*-butoxycarbonylamino)cyclopentane **4** (740 mg, 2.46 mmol) was weighed into an oven dry 100 mL RBF and Et_2O (57 mL) was added. **4** partially dissolved upon adding Et_2O. Next trifluoroacetic acid (3.8 mL, 49 mmol) was added and **4** completely dissolved. After stirring the solution for 18 hours, the solvents were blown off with a steady stream of $N_{2\,(g)}$, over 1 hour. The remaining viscous oil was transferred to a separatory funnel with H_2O (50 mL) and Et_2O (50 mL). The acidic mixture (pH 0) was extracted with Et_2O (3 x 50 mL) to remove all starting material **4**. Next, the pH of the acidic aqueous layer was adjusted to a pH of 14 and extracted with Et_2O (4 x 50 mL). The combined organic layers from the pH 14 extraction were dried over Na_2SO_4, filtered via suction

filtration, and concentrated on a rotary evaporator. The resulting oil was placed under vacuum to give 99.3 mg (20%) of **5** as an oil: **^{1}H NMR** ($CDCl_3$-*d*, 500 MHz): δ 4.51 (br s, 1H, carbamate-NH), 3.51 (br m, 1H, carbamate-CH), 2.99 (m, 1H, H_2NCH), 2.13 (m, 1H, cyclopentane), 1.97 (m, 1H, cyclopentane), 1.84 (m, 1H, cyclopentane), 1.68 (m, 2H, cyclopentane), 1.45 (s, 9H, *t*-butyl-CH_3), 1.36 (m, 2H, cyclopentane).

Distillation procedure for ethyl glyoxylate: To an oven-dried 25 mL round bottom flask fitted with a magnetic stirring bar and a short path distillation apparatus was added ethyl glyoxylate/toluene solution (10 mL). The distillation pot was warmed to 110 °C for 1 h, then to 140-150 °C to remove most of the toluene (head temp 110-118 °C). The distillation pot was warmed to 160-170 °C and the remaining ethyl glyoxylate/toluene was collected (head temp 120-130 °C). ^{1}H NMR indicates the distilled glyoxylate solution to be typically a 8:1 mixture of ethyl glyoxylate:toluene. After ^{1}H NMR analysis of the appropriate fraction, the 10 mL round bottom flask containing the fraction was attached to a reflux condenser and heated to 110 °C for 30 min. The 8:1 mixture of ethyl glyoxylate:toluene was now ready for use to prepare **6**.

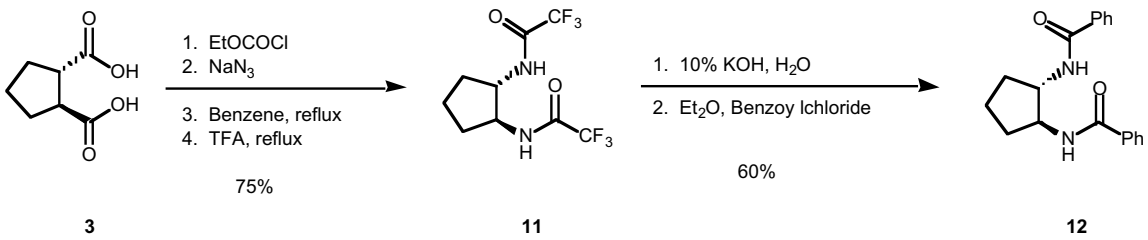
N-[(2s)-Boc-aminocyclopen-(1s)-yl]-glycine ethyl ester (6). (-)-(1*S*,2*S*)-*N*-Mono(*tert*-butoxycarbonyl) cyclopentanediamine **5** (156 mg, 0.78 mmol) was concentrated in a 50 mL RBF and dried under vacuum overnight prior to being used in the condensation reaction. Once **5** was dry, EtOAc (10 mL) was added to the reaction flask. Ethylglyoxylate as a 8:1 mixture in toluene (80 μL, 0.78 mmol) was added directly to the reaction solution and stirred for 15 hours. Next, the solution was transferred to a Parr reaction vessel, washing the reaction flask with EtOAc (2 mL), and 10% Pd/C (25 mg) was added. The reaction vessel was placed on the Parr, purged three times with $H_{2\,(g)}$, and set to shake under 30 psi for 10 hours. Once the imine reduction was complete, the reaction solution was filtered through a 50 mL glass fritted funnel, containing 40 mL of celite, concentrated on a rotary evaporator, and dried under vacuum to obtain a light orange oil. The product was purified by silica gel chromatography, eluting with 100% EtOAc. The fractions containing the desired product, with an Rf of 0.4, were combined, concentrated, and dried under vacuum to give 192 mg (86 % yield) of **6** as a clear viscous oil: **^{1}H NMR** (DMSO-d_6, 500 MHz): δ 6.81 (d, *J* = 8.0 Hz, 2H, carbamate-NH), 4.07 (q, *J* = 6.5 Hz, 2H, OCH_2CH_3), 3.46 (m, 1H, carbamate-CH), 3.31 (m, 2H, $HNCH_2$), 2.76 (m, 2H, HNCH), 1.98 (br s, 1H, NH), 1.84 (m, 1H, cyclopentane), 1.77 (m, 1H, cyclopentane), 1.53 (m, 2H, cyclopentane), 1.37 (s, 9H, *t*-butyl-CH_3), 1.26 (m, 1H, cyclopentane), 1.18 (t, *J* = 6.5 Hz, 3H, OCH_2CH_3); **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 172.21, 155.29, 77.36, 64.14, 59.80, 56.96, 48.68, 30.71, 30.60, 28.20, 21.27, 14.05.

N-[(2s)-Boc-aminocyclopen-(1S)-yl]-N-(thymin-1-ylacetyl)-glycine ethyl ester (7). For 12 hours prior to the reaction, $N_{2(g)}$ was bubbled through DMF. N-[(2*S*)-Boc-aminocyclopen-(1*S*)-yl]-glycine ethyl ester (**6**) (102 mg, 0.36 mmol) was weighed into an oven dry 50 mL RBF and DMF (12 mL) added. Next HATU (220 mg, 0.58 mmol) and thymine acetic acid (131 mg, 0.71 mmol) were added to the reaction mixture. The reaction flask was cooled to 0 °C via an icebath and DIEA (186 μL, 1.07 mmol) was added slowly over a 30 min period. The reaction was allowed to warm to rt gradually over 8 hours. The reaction solution was stirred for a total of 24 hours then transferred to

a separatory funnel, washing the reaction flask with EtOAc (50 mL). Next, the organic mixture was washed with a sequence of aqueous solutions: sat NaCl (50 mL), sat $NaHCO_3$ (2 x 50 mL), and sat NaCl (50 mL). The layers were separated and the combined aqueous washes were extracted with EtOAc (4 x 80 mL). All organic aliquots were combined, dried over Na_2SO_4, filtered via suction filtration and concentrated on a rotary evaporator to a viscous yellow oil. The product was purified by silica gel chromatography, eluting with 19:1 MeOH/CH_2Cl_2. The fractions containing the desired product, with an Rf of 0.3, were combined, concentrated and dried under vacuum to give 129 mg (80 % yield) of **7** as solid white foam: **m.p.** = 87-89 °C; **^{1}H NMR** (DMSO-d_6, 500 MHz): Major Rotamer δ 11.28 (s, 1H, imide NH), 7.16 (s, 1H, H_3CCCH), 6.94 (d, J = 7.5 Hz, 1H, carbamate-NH), 4.81 (AB, J = 17 Hz, 1H, thymine-CH_2), 4.62 (AB, J = 17 Hz, 1H, thymine-CH_2), 4.04 (q, J = 7.0 Hz, 2H, CH_3CH_2O), 3.89 (m, 2H, COOEtCH_2), 1.75 (s, 3H, thymine-CH_3), 1.36 (s, 9H, *t*-butyl-CH_3), 1.2-2.0 (m, 8H, cyclopentane-CH_2), 1.16 (t, J = 7.0 Hz, 3H, CH_3CH_2O); Minor Rotamer δ 6.73 (m, 1H, carbamate-NH); **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 169.01, 167.27, 164.37, 155.36, 150.97, 141.73, 108.23, 77.88, 61.11, 60.20, 52.66, 47.84, 43.80, 28.25, 28.16, 25.92, 18.78, 14.00, 11.94.

***N*-[(2*S*)-Boc-aminocyclopent-(1*S*)-yl]-*N*-(thymin-1-ylacetyl)-glycine** (**8**) was prepared employing similar conditions to the procedure of Nielsen and co-workers.[14] *N*-[(2*S*)-Boc-aminocyclopent-(1*S*)-yl]-*N*-(thymin-1-ylacetyl)-glycine ethyl ester (**7**) (440 mg, 0.97 mmols) was weighed into a 100 mL RBF and dissolved in THF (15 mL). The solution was cooled in an ice bath to 0 °C. Next, a 1 N aqueous solution of LiOH♦H_2O (549 mg, 13.0 mmol) was prepared and added dropwise, via syringe, to the reaction solution over a 5 min period. The icebath was removed and the solution stirred for 5 hours. The basic solution was transferred to a separatory funnel, washing the reaction flask with H_2O (20 mL) and Et_2O (20 ml), and extracted with Et_2O (3 x 40 mL). The remaining aqueous layer was acidified to pH 1 with 3 N HCl and extracted with EtOAc (5 x 50 mL). The organic layers were combined, dried over Na_2SO_4, and filtered by suction filtration. The solution was concentrated on a rotary evaporator and dried under vacuum to give 350 mg (85% yield) of 11 as a brittle white solid: Decompostion occurs at 140 °C; **^{1}H NMR** (DMSO-d_6, 500 MHz): Major Rotamer δ 12.39 (br s, 1H, COOH), 11.29 (s, 1H, imide NH), 7.16 (s, 1H, H_3CCCH), 6.95 (d, J = 8.0 Hz, 1H, carbamate-NH), 4.82 (AB, J = 17 Hz, 1H, thymine-CH_2), 4.60 (AB, J = 17 Hz, 1H, thymine-CH_2), 3.94 (AB, J = 17 Hz, 1H, $COOHCH_2$), 3.68 (AB, J = 17 Hz, 1H, $COOHCH_2$), 1.75 (s, 3H, thymine-CH_3), 1.36 (s, 9H, *t*-butyl-CH_3), 1.2-2.0 (m, 8H, cyclopentane-CH_2); Minor Rotamer δ 11.26 (s, 1H, imide-NH), 6.76 (d, J = 8.0 Hz, 1H, carbamate-NH); **ESI-MS** *m/z* 423.2 ($M-H^+$).

Procedure to determine ee of 3:



3 **11** **12**

Analyzed by Chiral HPLC

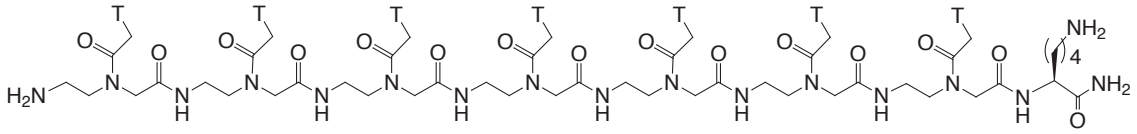
(-)-(1*S*,2*S*)-bis(trifluoroacetamide)cyclopentane (11) was prepared by a modification of the procedure reported by Pfister and Wymann.[15] (-)-(*S*,*S*)-cyclopentanedicarboxylic acid **3** (3.15 g, 0.02 mmol) was weighed into an oven dry 100 mL RBF, dissolved in THF (80 mL), and cooled to 0 °C. TEA (11.15 mL, 80.0 mmol) and EtOCOCl (7.65 mL, 80.0 mmol) were added and the suspension stirred for 20 min. Next, an aqueous solution of NaN_3 (7.8 g, 120.0 mmol in 31 ml of H_2O) was added via syringe to the reaction flask. The reaction mixture was stirred vigorously for 20 min, after which time the ice bath was removed, and the mixture stirred an additional 10 min. The solution was transferred to a separatory funnel, washing the reaction flask with H_2O (30 mL) and EtOAc (2 x 30 mL), and extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered via suction filtration, and concentrated in an oven dry 250 mL RBF (to an oil of approximately 3 mL). (NOTE! Do not concentrate the solution to dryness.[5]) Benzene (105 mL) was added to the oil via syringe and the reaction flask was attached to a reflux condenser. The solution was heated under reflux at 95 °C for 45 min to promote the double Curtius rearrangement. Next, the solution was cooled to 40 °C and trifluoroacetic acid (15.4 mL, 200 mmol) was added directly to the reaction solution. The reaction solution was then heated under reflux. After 15 hours, the solution was allowed to cool to rt. and sat. $NaHCO_3$ (30 mL) was added dropwise to the reaction flask. After the evolution of all CO_2, EtOAc (50 mL) was added and the resulting mixture was transferred to a separatory funnel. The organic layer was washed with sat. $NaHCO_3$ (4 x 60 mL), the layers were separated, and the resulting aqueous layer was extracted with EtOAc (4 x 75 mL). All organic aliquots were combined, dried over Na_2SO_4, filtered via suction filtration, and concentrated on a rotary evaporator to obtain 4.04 g of a yellowish solid. The solid was washed with $CHCl_3$ (2 x 20 mL), collected by suction filtration, and dried under vacuum to give 3.2 g (55% yield) of **11** as an off white solid: **m.p.** = 200-201 °C; **^{1}H NMR** (DMSO-d_6, 500 MHz): δ 9.49 (br d, J = 5.5 Hz, 2H, NH), 4.18(m, 2H, HNCH), 1.95(m, 2H, cyclopentane), 1.69(m, 2H, cyclopentane), 1.60(m, 2H, cyclopentane).

(-)-(1*S*,2*S*)-dibenzamidocyclopentane (12). (-)-(1*S*,2*S*)-bis(trifluoroacetamide)cyclopentane **11** (100 mg, 0.38 mmol) was weighed into a 100 mL RBF. A solution of 10% aqueous KOH (1.06 g, 18.9 mmol) was prepared and added directly to **11**. The mixture was heated to 65 °C for 2 hours then allowed to cool rt. Next, the solution was cooled to 0 °C via an icebath and Et_2O (18 mL) was added. The

solution was stirred vigorously as benzoyl chloride (1.76 mL, 15.2 mmol, 40 equiv) was added over a 5 min period. After stirring 15 min, **12** precipated as a solid and was separated from the solution via suction filtration. The remaining solid was washed with EtOAc (4 x 25 mL), to remove unreacted benzoyl chloride, and dried under vacuum to give 77.5 mg (58 % yield) of **12** as a white solid: **m.p.** = 273-274 °C. **^{1}H NMR** (DMSO-d_6, 500 MHz): δ 8.45 (br d, *J* = 7.0 Hz, 2H, NH), 7.79 (d, *J* = 7.5 Hz, 2H, o-CH), 7.49 (t, *J* = 7.5 Hz, 1H, p-CH), 7.43 (t, *J* = 7.5 Hz, 2H, m-CH), 74.36 (m, 2H, HNCH), 2.03 (m, 2H, cyclopentane), 1.73 (m, 2H, cyclopentane), 1.60 (m, 2H, cyclopentane). Enantiomers were separated on a (*S,S)*-ULMO column using 9:1 hexanes : isopropanol at 1.5 mL / min.

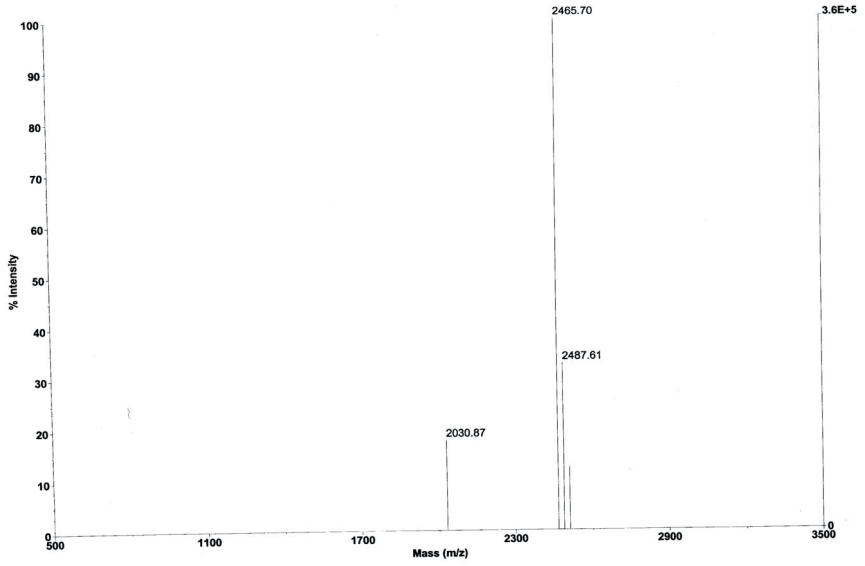
PNA Purification and Characterization.

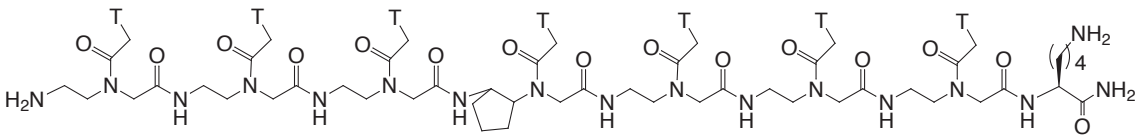
All peptide nucleic acid (PNA) oligomers were purified on reverse-phase HPLC with UV detection at 215nm. Both MetaChem Polaris C18 (d=21.2mm, l=250mm, 10 microns) and VYDEK C18 (d=10mm, l=250mm, 5 microns) semi-prep columns were utilized, eluting with 0.05% TFA in water (Solution A) and 0.05% TFA in acetonitrile (Solution B). An elution gradient of 100% A to 100% B over 60 minutes at flow rate 5.05mL/min for MetaChem column and 2.2mL/min for VYDAC column was used. PNAs were characterized by mass spectroscopy, using a PerSeptive Biosystems Voyager DE MALDI-TOF system with α-cyano-4 hydroxy cinnamic acid matrix. Rennin substrate tetradecapeptide and adrenocorticotropic hormone (fragment 18-39) were used as internal mass standards. Mass spectra were acquired using a N_2 laser (337 nm wavelength, 5ns pulse), with an average of 100 shots per sample. All PNA oligomers gave molecular ions consistent with the final product.



MW = 2007, MW + Na^+ (2030) observed

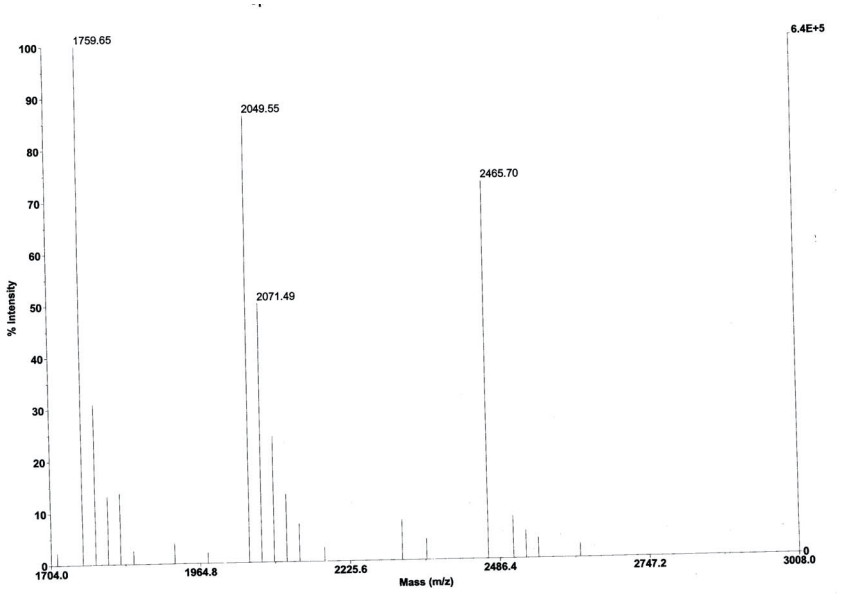
PNA 1





MW = 2047, M+2 observed, M+1+Na$^+$ observed

PNA 2



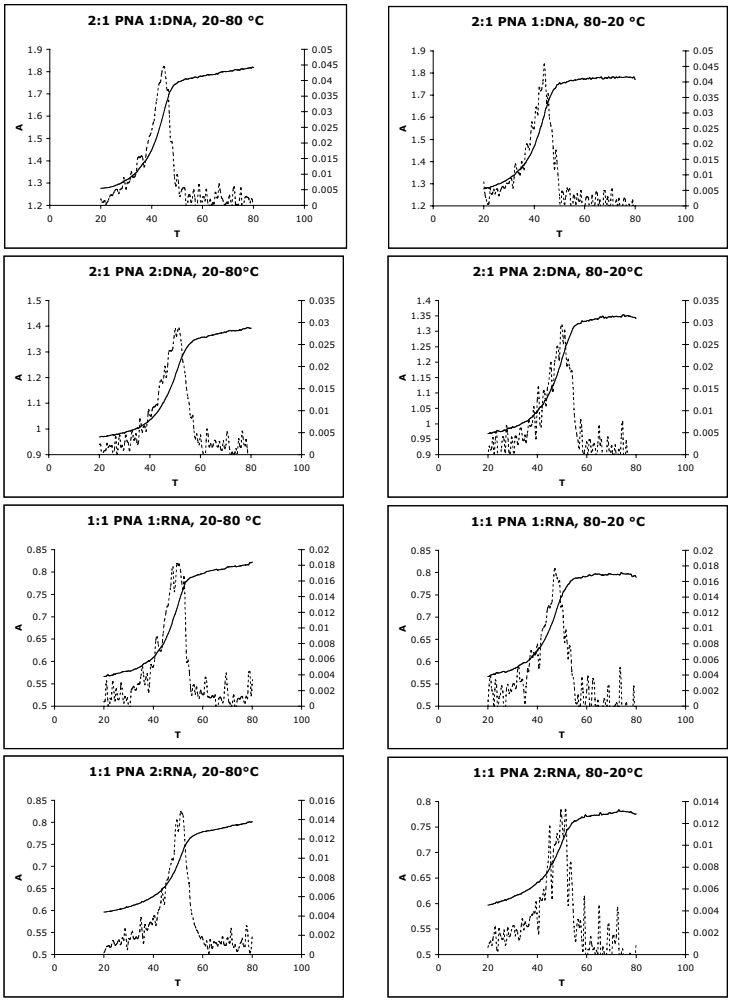
Melting Curves.

Oligonucleotides were purchased from Integrated DNA Technologies, Inc. (IDT) and were desalted prior to use. Concentrations of oligonucleotide and PNA solutions were determined by UV absorption at 260 nm on a Cary-50 Bio UV-Visible spectrometer using extinction coefficients based on the nearest-neighbors method.[16] Extinction coefficients for oligonucleotides were reported by the supplier (IDT).

Solutions of 1:1 and 1:2 oligonucleotide:PNA were prepared in pH=7.0 buffer consisting of 10 mM sodium phosphate, 0.1 mM EDTA, and 150 mM NaCl. Duplex/Triplex concentrations were varied from 5 μM to 15 μM. Samples were annealed by heating to 95°C for 3 minutes and cooling to 25°C over 3 hours. The solutions were degassed under vacuum for 3 minutes prior to melting analysis. Thermal denaturation profiles (absorbance vs temperature) of the hybrids were measured at 260 nm with a diode array UV/VIS spectrophotometer equipped with a Peltier temperature controller that is interfaced to a personal computer. For the temperature range 20°-80°C, 121 measurement points were taken every 0.5°C, with an equilibration time of 60 s for each measurement point. A heating and a cooling profile were recorded for each complex. Using these

conditions hysteresis between the heating and cooling curves was minimized (see data below). The melting temperature (T_m) was determined from the first derivative of each of the heating and cooling curves and reported in the Table as the average of these two temperatures. For each sample, there was minimal variation in the shape of the curves and calculated T_m values between the heating and melting curves; indicating that there is minimal hysteresis.

Melting temperature data: For each graph, the melting curve and first derivative are plotted. PNA numbers refer to the structures above. Heating and cooling profiles are presented side-by-side for a single sample. The table displays the calculated T_m values for each curve.



PNA:Oligonucleotide	T_m (20 – 80 °C)	T_m (80 – 20 °C)	Average T_m
2:1 PNA1:DNA	44.8	44.0	44.4
2:1 PNA2:DNA	50.8	49.8	50.3
1:1 PNA1:RNA	49.5	47.0	48.3
1:1 PNA2:RNA	51.3	51.5	51.4

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