Backbone Modifications of Aromatic Peptide Nucleic Acid (APNA) Monomers and Their Hybridization Properties with DNA and RNA

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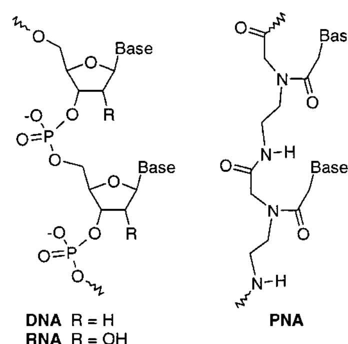
Aromatic peptide nucleic acid (APNA) monomers containing *N*-(2-aminobenzyl)-glycine, *N*-(2-aminobenzyl)-(*R*)- or -(*S*)-alanine, and *N*-(2-aminobenzyl)-β-alanine moieties as part of their backbone were synthesized. These novel analogues were incorporated as a single "point mutation" in PNA hexamers, and their physicochemical properties were investigated by UV thermal denaturation and CD experiments. Destabilization in triplex formation between the PNA-APNA chimeras and complementary DNA or RNA oligomers was observed, as compared to the PNA control. The APNA monomer composed of the *N*-(2-aminobenzyl)-glycine backbone led to the smallest decrease in the thermal stability of the triplexes formed with DNA and RNA, while maintaining selectivity for base-pairing recognition. Since the PNA-APNA chimeras are more lipophilic than the corresponding PNA homopolymers, these oligomers may also exhibit better cell membrane permeability properties.

Introduction

Over the past 20 years, the preparation and properties of modified oligonucleotides (ODNs) have been extensively investigated, as their potential as therapeutic agents is increasingly recognized.[1] Peptide nucleic acids (PNAs) are DNA analogues having the phosphodiester/deoxyribose backbone completely replaced by *N*-(2-aminoethyl)-glycine units to which the nucleobases are attached via acetate linkers.[2] These molecules have emerged as a unique class of oligonucleotide mimics with ever-increasing potential as antisense/antigene agents and as biomedical diagnostic tools. PNAs bind to DNA and RNA in a sequence specific manner and with remarkably higher thermal stability relative to all other synthetic or natural analogues of ODNs.[3] Moreover, PNAs are stable toward degradation by both protease and nuclease enzymes, thus possessing many of the critical requirements for oligomers used in antisense/antigene drug development.[4]

Although the microinjection of PNA oligomers into cells has been shown to induce transcription and translation arrest,[5] their therapeutic utility is seriously compromised by their poor cell membrane permeability. In an effort to overcome this limitation, PNA conjugates of nuclear internalizing peptides were recently explored. For example, biotinylated PNA conjugates linked to the monoclonal antibody OX26 were shown to undergo in vivo receptor-mediated transcytosis through the blood-brain barrier, thus effectively transporting the PNA oligomers into brain cells.[6] PNAs have also been transported into cells as unmodified PNA-DNA duplexes encapsulated into cationic lipid micelles. This latter approach has been used to effectively inhibit telomerase activity in DU145 prostate-derived tumor cells.[7] Although such studies are encouraging, a more direct approach involves modifications of the PNA structure so as to increase their inherent cell membrane permeability without significantly compromising their superior hybridization properties or stability against enzymatic degradation.



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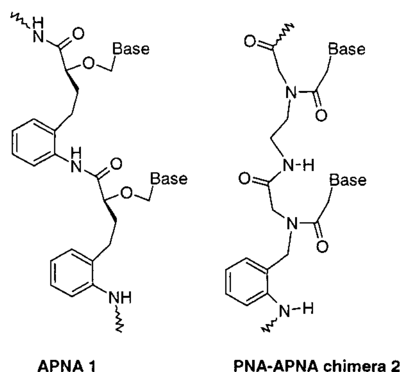
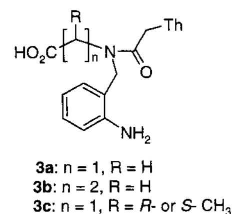
In 1997, we reported the design and synthesis of the first peptide nucleic acid analogue having an aromatic moiety as an integral part of its backbone, which we termed *aromatic peptide nucleic acids* (APNA).[8] The synthesis of a thymine derivative of (*S*)-2-hydroxy-4-(2-aminophenyl)butanoic acid was achieved in high enantiomeric purity and was shown to be a useful building block for the production of this novel class of APNA oligomers (**1**). At approximately the same time, oligonucleotide analogues having an *N*-phenylacetamide backbone,[9] as well as acyclic oligomers composed of 2-amino-1-phenyl-1,3-propanediol phosphoramidite monomers,[10] were described in the literature. All of these reports suggested that the introduction of an aromatic substituent in the backbone of a modified oligonucleotide does not seriously hamper the formation of a duplex or triplex structure with DNA or RNA.



More recently, PNA-APNA chimeras of the general structure **2** were investigated by our group and shown to form stable duplex structures with complementary DNA and RNA strands.[11] In addition, both APNA analogues **1** and **2** were designed in order to investigate possible π-stacking or dipole–quadrupole interactions along the backbone of the APNA oligomers and the possible stabilizing effect on a duplex or triplex structure.[12,13] Although it is widely believed that the major stabilizing forces of double and triple helices are the π-stacking interactions between adjacent base pairs,[14] the study of such interactions within the backbone of oligonucleotide analogues is completely unprecedented and therefore may be of considerable interest.[15] Furthermore, Kool and co-workers have recently demonstrated that DNA shape complemetarity may play as important a role in recognition and catalysis associated with DNA polymerase enzymes as base-base hydrogen bonding.[16] Thus, in addition to applications in antisense/antigene chemotherapy, APNA chimeras of natural ODNs or synthetic analogues could also be of potential value as enzyme inhibitors of virally encoded DNA or RNA processing enzymes.

Our ongoing research in the area of peptide nucleic acids has recently led to the development of a new class of APNA monomers (**3a**–**c**), which we incorporated into



the solid-phase synthesis of APNA-PNA chimeras. We developed an efficient synthetic protocol that is amenable to the parallel or combinatorial preparation of numerous APNA building blocks with a high degree of structural diversity. As in the case of the original PNA analogues, the nucleobases are attached to the backbone via an acetate linker, therefore conserving the three-bond distance between the base and the backbone of the oligomer. However, since these molecules incorporate aromatic rings directly into the backbone of each monomer unit, they are fundamentally different from the PNAs, having at least three bonds of the backbone coplanar. Although the optimum distance between nucleobases along the backbone of PNAs corresponds to six σ bonds, oligonucleotides having a distance of seven σ bonds between units have also been shown to form stable duplexes with natural ODNs.[17] Thus, one of our goals is to identify the optimum length and backbone substitution required in order to achieve maximum stability of the complexes formed between the APNAs and natural oligonucleotides, via either Watson–Crick or Höogsteen base pairing. In this paper, we report our synthetic protocol for the preparation of APNA monomers having the general structure **3** and the relative hybridization properties of four key analogues (**3a**–**c**).

Results and Discussion

Synthesis of APNA Monomers. Our initial efforts in the structure optimization of the APNA monomers focused on establishing the optimum backbone space between adjacent nucleobases in a Lys-PNA_2-APNA-PNA_3-Ac chimera. Synthesis of the required APNA monomers was initiated with reductive alkylation of the appropriate amino esters with commercially available 2-nitrobenzaldehyde using sodium triacetoxyborohydride as the reducing agent (Scheme 1).[18] Following workup of

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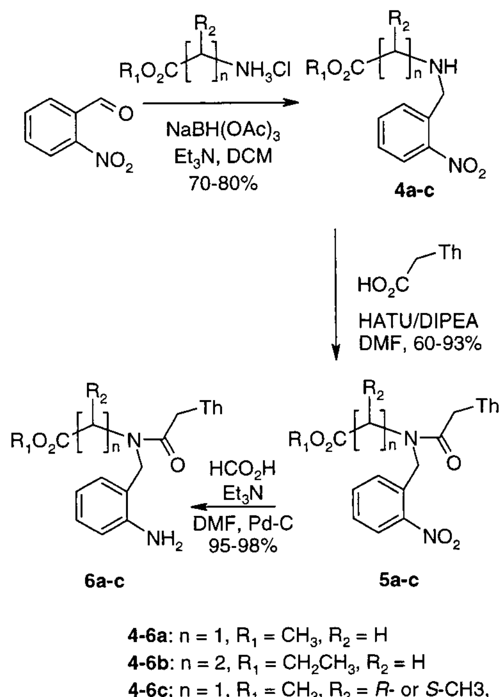
Scheme 1. Synthesis of APNA Monomers 6a−c

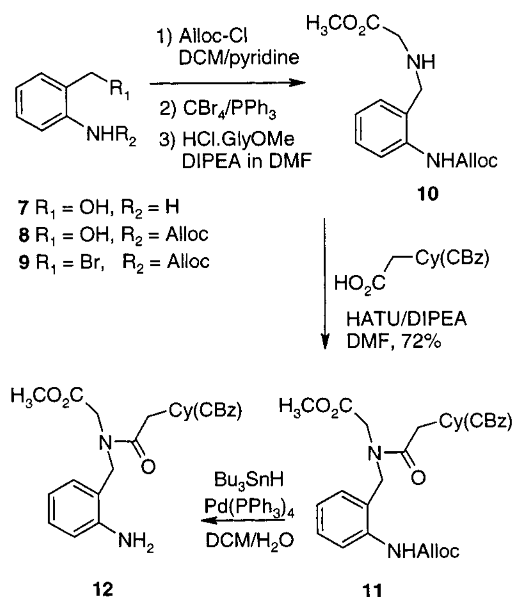


each reaction mixture, the secondary amines **4a**, **4b**, and (*R*)-**4c** or (*S*)-**4c** were precipitated as the corresponding HCl salts, in good yield and high purity as determined by NMR. The amines were then coupled to thymidyl-1-acetic acid,[19] using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) as the coupling reagent, to give the desired tertiary amides in good to excellent yields (60% for the (*R*)- or (*S*)-alanine, 85% and 93% for the glycine and β-alanine analogues, respectively). In all cases, the products were isolated after trituration of the reaction mixture with ethyl acetate/hexane giving the pure thymine derivatives **5a−c**. The subsequent catalytic hydrogenation of the nitro moiety was carried out using palladium on charcoal with triethylammonium formate, leading to the isolation of the aniline intermediates **6a−c** in nearly quantitative yields and excellent purity. [None of the synthetic steps shown in Scheme 1 require purification of the products by chromatography.]

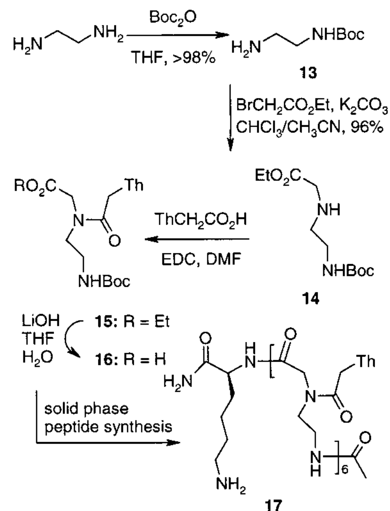
APNA monomers containing cytosine were synthesized following a slightly modified protocol (Scheme 2). Aminobenzyl alcohol **7** was first converted to the allyl carbamate derivative **8**,[20] and the hydroxy moiety was subsequently converted to the bromide to obtain compound **9**. Displacement of the bromide with glycine methyl ester gave intermediate **10** in high yield, which was then coupled with the Cbz-protected cytosine in the presence of HATU under basic conditions. Deprotection of the aniline moiety of **11** was efficiently achieved using Bu_3SnH and catalytic amounts of $Pd(Ph_3)_4$,[21] giving the required methyl ester of the APNA cytosine monomer **12** in >80% yield after chromatography (Scheme 2).

Synthesis of APNA-PNA Hexamers. To evaluate the hybridization properties of these novel monomers **3a−c**,

Scheme 2. Synthesis of Cbz-Protected APNA-Cy Monomer 12



Scheme 3. Synthesis of PNA-Th6 (17)



PNA oligomers were synthesized having a single APNA substitution in the middle of a hexamer strand. The thymine PNA monomers were prepared using a modified literature procedure (Scheme 3).[22] Ethylenediamine was mono-Boc protected by reaction with limiting amounts of di-*tert*-butyl dicarbonate to give pure compound **13** in >98% yield. Compound **13** was further reacted with a limiting amount of ethyl bromoacetate in the presence of K_2CO_3, giving the PNA backbone **14** in 96% yield.[22] Excess amine **13** was required in this reaction in order to avoid dialkylation; however, the unreacted starting material was usually recovered quantitatively. Thymidyl-1-acetic acid was then coupled to secondary amine **14**, giving fully protected thymine monomer **15**, which was hydrolyzed to the free carboxylic acid **16**. Compound **16** was used as such in the automated, solid-phase synthesis of the PNA homothymine hexamer **17**, as well as the

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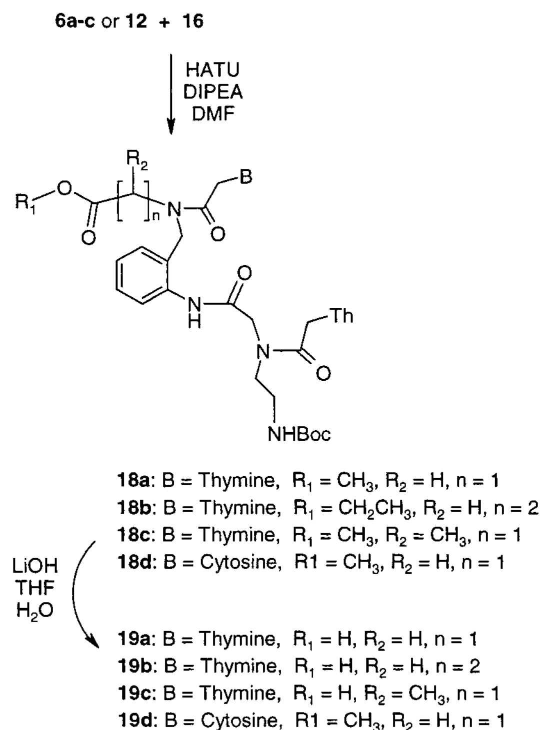
synthesis of the PNA-APNA chimeras **20**−**24**, following a slightly modified protocol from that originally developed by Nielsen and co-workers. [23]

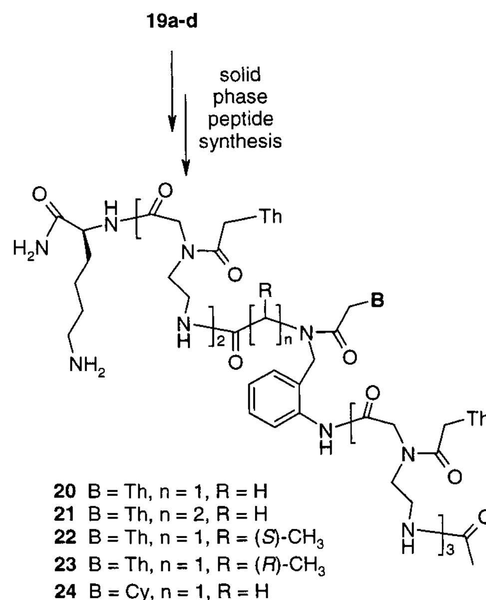
The solid-phase peptide synthesis was carried out on a methylbenzhydrylamine (MBHA) resin using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as the coupling reagent in the presence of diethylcyclohexylamine (DECA) and in a solvent mixture of pyridine/DMF. After each step, unreacted free amino residues were capped by treatment of the resin with Ac_2O in the presence of pyridine. Capping of the *N*-terminus of the hexapeptide is essential in order to avoid intramolecular *N*-acyl transfer of the last acetyl-nucleobase moiety.[23b] The Boc protecting group was hydrolyzed by treatment of the peptide with 25% TFA in dichloromethane, and the progress of both the coupling and deprotection steps was monitored using the Kaiser test.[24] A lysine residue was included at the *C*-terminus of each peptide in order to prevent aggregation of the polymer-bound oligomer during its synthesis on the solid support.[2b] Cleavage from the resin was achieved by treatment of the polymer-bound peptide with trifluromethanesulfonic acid (TFMSA) in TFA. The crude peptides were first precipitated from the TFA solution by diluting the mixture with anhydrous diethyl ether (20-fold dilution) and then purified by preparative C18 reversed-phase HPLC.

The *ortho*-substituted anilines (e.g., **3a**−**c**) were found to react sluggishly during peptide synthesis and under the above conditions, even when an excess of free acid monomer and coupling reagents were used. Furthermore, free aniline moieties cannot be detected using the Kaiser test. Therefore, a different method for monitoring the coupling cycles of each APNA unit had to be explored. We decided to incorporate all of the APNA monomers into the synthetic scheme as the APNA-PNA dimers **19a**−**d**. These were easily synthesized in solution by the coupling of monomers **6a**−**c** and **12** with the PNA monomer **16** using HATU/DIPEA (Scheme 4). The ester moieties of intermediates **18a**−**d** were hydrolyzed to give the free acids **19a**−**d**, which were used directly in the solid-phase synthesis of the Lys-PNA_2-APNA-PNA_3-Ac hexamers (**20**−**24**) following the same protocol as in the PNA control (Scheme 5). After cleavage of the hexamers from the solid support, HPLC analysis of the crude material indicated the presence of the desired PNA-APNA chimeras in >65% of the total sample. However, oligomers **20**−**24** were isolated in approximately 30−50% yield after HPLC purification.

Scheme 4. Synthesis of APNA-PNA Dimers



Scheme 5. PNA-APNA Chimeras



Hybridization Studies of APNA-PNA Hexamers. To gain some insight into the physicochemical properties of each APNA monomer, we compared the hybridization properties of hexamers **20**−**24** with poly(rA) and poly(dA) to those of the control PNA-T_6 (**17**). In all cases, the stability of triplex formation was evaluated by UV−thermal melting (T_m) experiments at nearly physiological conditions (pH = 7.1, 150mM NaCl, 10mM NaH_2PO_4, 1mM EDTA). Hexamers were mixed in a 2:1 ratio (T_2/A) based on the expectation that each PNA strand (or APNA-PNA chimera) would bind to the DNA or RNA strand to form PNA_2/DNA or PNA_2/RNA triplexes.[25] The circular dichroism (CD) spectrum of the complex formed between the Lys-PNA_2-APNA-PNA_3-Ac chimera **20** and poly(rA) at a 2:1 molar ratio was very similar to that observed for the triplex of the control PNA-T_6 (**17**) with poly(rA).[25] Similar CD spectra were also observed for the complexes formed between the PNA-T_6 and poly(dA), as well as chimera **20** and poly(dA), strongly suggesting that the incorporation of a single monomer of structure **3a** into the PNA homopolymer did not alter the known hybridization characteristics of these oligomers.[25] The melting

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Table 1. Results of Thermal Denaturation Experiments[a] for Complexes of Hexamers 17 and 20–24 with poly(rA) and poly(dA)

compound	T_m (°C) complex with poly(rA)	complex with poly(dA)
17	64	56
20	50	44
21	26	<5
22	30	26
23	39	30
24	17	<5

[a] Melting temperatures of triplexes were determined on a Cary 3 UV–vis spectrophotometer.[31] All T_m solutions were 150 mM in NaCl, 10 mM in NaH_2PO_4, 1 mM in EDTA, and the pH was adjusted to 7.1. The solutions were heated to 90 °C for 10 min, then cooled slowly to 4 °C and stored at that temperature for at least 1 h. The melting curves were recorded by heating the solutions from 5 to 90 °C in steps of 0.5 °C/min.

temperatures (T_m) of the complexes studies are summarized in Table 1. The PNA-T_6 control strand (**17**) gave T_m values of 65 and 56 °C when hybridized to poly(rA) and poly(dA), respectively (Table 1), consistent with those reported previously for triplexes of PNAs with natural oligonucleotides.[2b,25,26] Destabilization was observed upon insertion of one APNA unit into the hexamer, resulting in a significant decrease in the T_m values observed for the complexes formed between the Lys-PNA_2-APNA-PNA_3-Ac chimeras and poly(rA) or poly(dA) as compared to those of the control (Table 1). Increasing the backbone distance between adjacent nucleobases as in the case of the β-alanine APNA analogue (chimera **21**) had the most detrimental effect in the stability of the complex formed with poly(rA) [hybridization was not observed between **21** and poly(dA)], whereas the glycine APNA analogue (chimera **20**) corresponded to the most favorable backbone modification in this series of compounds (Table 1). In addition, a pronounced hysteresis of the melting behavior of the triplexes was observed with all of the PNA-APNA chimeras with either poly(rA) or poly(dA), indicating a very slow rate of triplex formation, typical of PNAs.[27]

Backbone substitutions at Cα of the amino acid moiety in the APNA backbone was also detrimental, although methyl substitution of the *R* configuration (chimera **23**) was slightly better than the *S* (chimera **22**). The destabilizing effects observed due to backbone substitution of the APNA analogues are far more significant than those previously reported for Cα-substituted analogues of PNAs. However, a direct comparison with previous studies of substituted PNAs cannot be made, since our investigation was focused on the formation of triplexes, whereas previous reports examined the effects of backbone substitutions in the formation of duplexes.[28] In all cases, the known selectivity of PNAs for recognition of RNA over DNA was also observed with the PNA-APNA chimeras (Table 1).[26] This is particularly important for DNA-PNA chimeras and their applications in antisense technology, since a key approach to halting the flow of genetic information is through activation of RNase H and degradation of the target RNA molecules.[29]

Once the APNA monomer derived from glycine (**3a**) was identified as the most promising lead structure in this series, it was important to confirm that the decrease in the melting temperatures observed with chimera **20** ($\Delta T_m = -15$ and -11 °C for hybridization with poly(rA) and poly(dA), respectively), as compared to the PNA control, was not due to lack of base-pairing between the APNA thymine residue and its complementary adenosine residue.[30] Thus, we prepared hexamer **24** containing a cytosine residue at the APNA position and studied the stability of the triplexes formed with poly(rA) and poly(dA). A far greater destabilizing effect was observed due to the presence of a cytosine-adenosine mismatch than that resulting by the incorporation of an APNA unit having a complementary base (Table 1), strongly suggesting that the APNA unit of hexamer **20** is indeed participating in Watson–Crick or Höogsteen base-pairing with the complementary nucleobase of poly(rA) and poly(dA).

In summary, we have described the synthesis of a new class of aromatic peptide nucleic acid monomers with potential biomedical applications. The synthetic methodology developed can provide APNA monomers rapidly and in high yields and purity. Furthermore, our synthetic scheme is amenable to combinatorial or parallel synthesis of larger libraries of APNA analogues. Incorporation of these monomers into the automated, solid-phase synthesis of APNA-PNA chimeras allows for rapid evaluation of their hybridization properties, as well as modulation of the physicochemical properties of the resulting oligomers. Further optimization of the chemical structure and hybridization properties of novel APNA-PNA chimeras and investigation of their ability to penetrate cell membranes in vivo is currently in progress.

Experimental Section

General Methods. Solvents were purchased from Fischer Scientific and purified as follows. THF was distilled from sodium/benzophenone ketyl; CH_2Cl_2 was distilled from P_2O_5 or CaH_2; DMF was treated with KOH overnight at room temperature and then vacuum distilled from CaO or BaO and stored over activated 4 Å molecular sieves; MeCN was distilled from CaH_2; and pyridine was distilled from CaH_2. HPLC solvents were HPLC grade and were filtered through 0.45 μm filters (Supelco, Bellefonte, PA) prior to use. HATU was purchased from PerSeptive Biosystems Ltd., and MBHA resin was purchased from Nova Biochem Ltd. All other starting materials and reagents were purchased from Sigma/Aldrich Canada and were used without further purification, except for DIPEA and Et_3N, which were refluxed over CaH_2 and then distilled and stored over activated 4 Å molecular sieves. Thin-layer chromatography was carried out on aluminum-backed silica gel 60 F_{254} plates (EM Science, Germany) using the solvent systems indicated. Reversed phase thin-layer chromatography was carried out on glass-backed RP-18 F_{254}s plates (EM Science, Germany) using the solvent systems indicated. Deuterated NMR solvents were purchased from Isotec Inc. (Miamisburg, OH). NMR spectra were obtained at ambient

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(31) (a) A variability of ±1 °C was observed in the T_m values measured for different samples of the same oligomers; this may be due to some variability in the purity of the commercial poly(rA) and poly(dA) samples. (b) At a slower heating rate of 0.2 °C/min, all T_m values measured were on the average 2–4 °C lower, as would be expected.

temperature unless otherwise indicated. ^{1}H and ^{13}C NMR chemical shifts are quoted in ppm and are referenced to the internal deuterated solvent. Mixtures of rotamers were often observed by NMR; in those cases the ratio is indicated and the signals are denoted as major (*ma*) and minor (*mi*). Some NMR spectra were also recorded at high temperatures in order to confirm the presence of rotamers. All ^{1}H NMR spectra were recorded on a Varian Mercury (400 MHz) or a Unity Inova (300 MHz) spectrometer. ^{13}C NMR spectra were recorded on a JEOL (67.5 MHz) or a Varian Mercury spectrometer (100 MHz).

Synthesis of Methyl *N*-(2-Nitrobenzyl)glycinate (4a). 2-Nitrobenzaldehyde (5.70 g, 38 mmol), methyl glycinate hydrochloride (4.30 g, 34 mmol), and triethylamine (5.0 mL, 38 mmol) were dissolved in dry CH_2Cl_2 (160 mL). This mixture was stirred under N_2 for 30 min, and then $NaBH(OAc)_3$ was added. The reaction was stirred for 5 h and then quenched by the addition of H_2O (30 mL), and the aqueous layer was discarded. The crude product was extracted into 0.1 M HCl (100 mL), and the water layer was back-extracted with CH_2Cl_2 (2 × 300 mL). The pH was adjusted to 4 by dropwise addition of NaOH (1 M), and the product was extracted into CH_2Cl_2 (4 × 500 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated to dryness, giving the title compound as an orange oil in 68% yield and in high purity. For more convenient storage and handling of the material, compound **4a** was converted quantitatively to its hydrochloride salt by dissolution of the oil in 1:1 Et_2O/EtOAc (60 mL) followed by addition of HCl (1.5 equiv, 2 M HCl in Et_2O). The white precipitate was collected by filtration and dried in vacuo: TLC R_f (60% hex in EtOAc) 0.246; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.41 (bs, 1H), 3.43 (s, 2H), 3.69 (s, 3H), 4.07 (s, 2H), 7.40 (dt, 1H, J = 8.0 and 1.4 Hz), 7.53−7.62 (m, 2H), 7.93 (d, 1H, J = 8.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 50.27, 50.36, 51.94, 124.91, 128.23, 131.06, 133.31, 135.12, 149.17, 172.70. HCl salt of **4a**: ^{1}H NMR (300 MHz, CD_3OD) δ 3.86 (s, 3H), 4.14 (s, 2H), 4.58 (s, 2H), 7.74−7.89 (m, 3H), 8.29 (dd, 1H, J = 8.4 and 1.0 Hz); FAB$^+$ HRMS (glycerol/KCl) *m/z* found 225.0875 (M + H)$^+$, calcd for $(C_{10}H_{13}N_2O_4)^+$ 225.0876.

Synthesis of 4b and 4c. These analogues were synthesized using the same procedure as that described for **4a**. Amine **4b**: yield 70%; TLC R_f (free amine, 1:1 hex/EtOAc) = 0.182; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25, (t, 3H, J = 6.9 Hz), 3.02 (t, 2H, J = 5.9 Hz), 3.36, (t, 2H, J = 6.4 Hz), 4.17, (q, 2H, J = 7.0 Hz), 4.37, (s, 1H), 7.63, (dt, 1H, J = 7.6 and 1.2 Hz), 7.76 (dt, 1H, J = 7.6 and 1.8 Hz), 8.01 (dd, 1H, J = 6.4 and 1.2 Hz), 8.16 (dd, 1H, J = 7.0 and 1.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.7, 30.8, 43.3, 47.6, 61.2, 125.9, 127.9, 131.3, 134.0, 134.9, 149.0, 170.6; FAB$^+$ HRMS (glycerol) *m/z* found 253.1188 (M + H)$^+$, calcd for $(C_{12}H_{17}N_2O_4)^+$ 253.1188. Amine (*R*)-**4c** and (*S*)-**4c**: yields in both cases 70−75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (d, 3H, J = 6.9 Hz), 3.25 (q, 1H, J = 6.9 Hz), 3.68 (s, 3H), 3.95 (d, 1H, J = 15 Hz), 4.07 (d, 1H, J = 15 Hz), 7.39, (dt, 1H, J = 8.6 and 1.6 Hz), 7.55 (dt, 1H, J = 7.8 and 1.2 Hz), 7.60 (dd, 1H, J = 7.8 and 1.2 Hz), 7.92 (dd, 1H, J = 7.8 and 1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.3, 49.1, 52.1, 56.6, 125.0, 128.3, 131.3, 133.4, 135.3, 149.3, 176.0; FAB$^+$ HRMS (glycerol/KCl) *m/z* found 239.1032 (M + H)$^+$, calcd for $(C_{11}H_{15}N_2O_4)^+$ 239.1032.

Synthesis of Methyl *N*-(2-Nitrobenzyl)-*N*-(thymin-1-ylacetyl)glycinate (5a). Thymidyl acetic acid (1.50 g, 8.1 mmol) and HATU (3.09 g, 8.1 mmol) were dissolved in anhydrous DMF (20 mL) under N_2 and cooled to 0 °C. To this solution, DIPEA (1.5 mL, 8.6 mmol) was added, and the reaction mixture was stirred for 10 min at 0 °C. Compound **4a** (1.69 g, 7.5 mmol) was than added as a solution in anhydrous DMF (10 mL), and the reaction was allowed to warm up to room temperature and stir overnight. The reaction mixture was then concentrated to ~5 mL, and EtOAc (50 mL) was added. This solution was washed with water (3 × 15 mL), and the organic layer was concentrated to about 10 mL and cooled to 0 °C. The precipitate formed was collected by filtration and washed once with 10% hexanes in EtOAc giving 2.50 g of compound **5a** (85% yield) in high purity. For large-scale synthesis of **5a** (i.e. >20 g) it was far more cost-effective to use a DCC/pentafluorophenol preactivation protocol; however, this procedure gave a lower yield of **5a** (~70%). Compound **5a**: TLC R_f (EtOAc) = 0.288; ^{1}H NMR (300 MHz, DMSO-d_6, mixture of rotamers in 1:1 ratio) δ 1.74 and 1.75 (s, 3H), 3.62 and 3.69 (s, 3H), 4.05 and 4.36 (s, 2H), 4.58 and 4.83 (s, 2H), 4.64 and 5.06 (s, 2H), 7.39−7.44 (m, 1.5H), 7.53 (dt, 0.5H, J = 7.0 and 1.2 Hz), 7.58−7.62 (m, 1H), 7.70 and 7.80 (dt, 1H, J = 7.0 and 1.2 Hz), 8.05 and 8.16 (dd, 1H, J = 7.0 and 1.2 Hz), 11.28 and 11.31 (s, 1H), coalescence of each pair of rotamer resonances was achieved at ~90 °C; ^{13}C NMR (67.5 MHz, DMSO-d_6, 120 °C) δ 12.0, 48.6, 49.4, 52.4, 109.0, 125.2, 129.1, 129.6, 132.3, 134.2, 142.2, 149.1, 151.5, 164.6, 169.0, 169.6; FAB$^+$ MS (glycerol) *m/z* 391 (M + H)$^+$.

Synthesis of Compounds 5b and 5c. Analogues **5b** and **5c** were synthesized using the same procedure as that described for compound **5a**. Compound **5b**: yield 93%; TLC R_f (EtOAc) = 0.226; ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotomers in 1:1.7 ratio) δ 1.14 (*mi*) and 1.15 (*ma*) (t, 3H, J = 7.0 Hz), 1.73 (bs, 3H), 2.55 (*mi*) and 2.71 (*ma*) (t, 2H, J = 8.1 Hz), 3.45 (*mi*) and 3.62 (*ma*) (t, 2H, J = 8.1 Hz), 3.99 (*mi*) and 4.01 (*ma*) (q, 2H, J = 7.8 Hz), 4.45 (*mi*) and 4.75 (*ma*) (s, 2H), 4.81 (*ma*) and 5.01 (*mi*) (s, 2H), 7.31−7.37 (m, 1.6H), 7.44 (d, 0.4H, J = 7.6 Hz), 7.53 (*ma*) and 7.60 (*mi*) (t, 1H, J = 7.2 Hz), 7.69 (*ma*) and 7.81 (*mi*) (t, 1H, J = 7.6 Hz), 8.06 (*ma*) and 8.17 (*mi*) (d, 1H, J = 8.0 Hz), 11.25 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.4, 12.5, 14.5, 32.5, 33.2, 34.2, 35.4, 43.2, 43.4, 46.7, 48.6, 48.7, 49.0, 49.7, 60.6, 60.7, 108.5, 108.6, 125.4, 125.9, 128.0, 128.3, 128.7, 129.1, 133.2, 133.3, 134.3, 135.0, 142.7, 148.2, 148.6, 151.7, 151.9, 165.3, 168.4, 171.6, 171.8; FAB$^+$ MS (glycerol) *m/z* 419 (M + H)$^+$. Compounds (*R*)-**5c** and (*S*)-**5c**: yields for both compounds ~80%; TLC R_f (EtOAc) = 0.350; ^{1}H NMR (300 MHz, DMSO-d_6, 120 °C) δ 1.37 (d, 3H, J = 7.2 Hz), 1.79 (d, 3H, J = 1.2 Hz), 3.64 (s, 3H), 4.48−4.87 (overlapping broad signals, 4H), 5.05 (d, 1H, J = 18 Hz), 6.67.28 (d, 1H, J = 1.2 Hz), 7.53−7.59 (m, 2H), 7.73 (bd, 1H, J = 5.4 Hz), 8.06 (d, 1H, J = 8.1 Hz), 10.70 (bs, 1H); ^{13}C NMR (67.5 MHz, DMSO-d_6, 130 °C) δ 11.9, 15.3, 46.5, 49.1, 52.4, 55.7, 108.9, 125.1, 129.0, 129.5, 133.0, 134.0, 142.3, 148.7, 151.5, 164.6, 167.0, 171.5; FAB$^+$ MS (glycerol) *m/z* 405 (M + H)$^+$.

Synthesis of Methyl *N*-(2-Aminobenzyl)-*N*-(thymin-1-ylacetyl)glycinate (6a). Compound **5a** (434 mg, 1.1 mmol) was dissolved in anhydrous DMF (3 mL), and Pd−C (65 mg) was added. Triethylamine (0.6 mL, 4.3 mmol) and formic acid (0.13 mL, 3.3 mmol) were added, and the reaction mixture was stirred at room temperature for 4 h. EtOH (10 mL) was added, and the mixture was filtered through Celite and concentrated to dryness. The resulting white solid was dried in vacuo to giving 388 mg (97% yield) of compound **6a**, which was determined to be pure by ^{1}H NMR: TLC R_f (EtOAc) = 0.213; ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers in 1:1.5 ratio) δ 1.75 (*mi*) and 1.76 (*ma*) (d, 3H, J = 1.2 Hz), 3.59 (*mi*) and 3.62 (*ma*) (s, 3H), 3.97 (*mi*) and 4.20 (*ma*) (s, 2H), 4.37 (*ma*) and 4.50 (*mi*) (s, 2H), 4.56 (*ma*) and 4.61 (*mi*) (s, 2H), 5.01 (*mi*) and 5.06 (*ma*) (bs, 2H), 6.47 (dt, 0.6H, J = 6.3 and 1.2 Hz), 6.60 (m, 0.9H, J = 7.0 and 1.2 Hz), 6.69 (dd, 0.5H, J = 7.6 and 1.2 Hz), 6.96−7.01 (m, 2H), 7.35 (*ma*) and 7.40 (*mi*) (d, 1H, J = 1.2 Hz), 11.29 (*mi*) and 11.32 (*ma*) (bs, 1H); ^{13}C NMR (67.5 MHz, DMSO-d_6, 130 °C) δ 12.0, 48.0, 48.6, 52.2, 109.0, 116.3, 117.2, 119.5, 129.0, 130.0, 142.3, 147.1, 151.5, 164.6, 168.7, 169.6; FAB$^+$ HRMS (glycerol) *m/z* found 361.1512 (M + H)$^+$, calcd for $(C_{17}H_{21}N_4O_5)^+$ 361.1511.

Synthesis of Compounds 6b and 6c. These analogues were synthesized using the same procedure as for compound **6a**. Aniline **6b**: yield 80%; TLC R_f (EtOAc) = 0.130; ^{1}H NMR (300 MHz, DMSO-d_6, mixture of rotamers in 1:2.3 ratio) δ 1.14 (*mi*) and 1.18 (*ma*) (t, 3H, J = 7.1 Hz), 1.74 (*mi*) and 1.75 (*ma*) (bs, 3H), 2.49 (*mi*) and 2.69 (*ma*) (t, 2H, J = 7.5 Hz), 3.42 (*mi*) and 3.48 (*ma*) (t, 2H, J = 7.5 Hz), 4.00 (*mi*) and 4.07 (*ma*) (q, 2H, J = 7.1 Hz), 4.37 (*ma*) and 4.42 (*mi*) (s, 2H), 4.49 (*mi*) and 4.67 (*ma*) (s, 2H), 4.99 (*mi*) and 5.05 (*ma*) (s, 2H), 6.49 (t, 0.6H, J = 6.9 Hz), 6.57−6.61 (m, 1H), 6.67 (d, 0.4 H, J = 7.8 Hz), 6.90−7.03 (m, 2H), 7.39 (*mi*) and 7.41 (*ma*) (bs, 1H), 11.28 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.9, 14.0, 32.1, 32.20, 40.91, 42.3, 44.7, 46.5, 48.1, 48.2, 60.0, 60.2, 107.9, 108.0, 114.7,

115.3, 115.7, 116.6, 119.0, 119.3, 126.2, 127.9, 128.4, 129.8, 142.3, 142.4, 146.0, 146.6, 151.1, 164.5, 167.3, 171.0, 171.1; FAB$^+$ HRMS (glycerol) *m/z* found 389.1825 (M + H)$^+$, calcd for $(C_{19}H_{25}N_4O_5)^+$ 389.1826. Aniline (*R*)-**6c** and (*S*)-**6c**: yield 80%; R_f (EtOAc) = 0.40; ^{1}H NMR (300 MHz, DMSO-d_6, 120 °C) δ 1.34 (d, 3H, J = 6.9 Hz), 1.80 (bs, 3H), 3.60 (s, 3H), 4.41–4.64 (m, 5H), 6.62 (dd, 1H, J = 7.2 Hz), 6.72 (d, 1H, J = 7.8 Hz), 7.01 (dd, 1H, J = 7.5 Hz), 7.12 (d, 1H, 7.5 Hz), 7.25 (bs, 1H), 10.70 (s, 1H); FAB$^+$ HRMS (glycerol) *m/z* found 375.1668 (M + H)$^+$, calcd for $(C_{18}H_{23}N_4O_5)^+$ 375.1667.

Synthesis of Allyl Carbamate Derivative 8. 2-Aminobenzyl alcohol (**7**, 1.04 g, 8.5 mmol) was dissolved in 1:1 DCM/pyridine (20 mL), and alloc-Cl (1.12 g, 9.3 mmol) was added dropwise via syringe over a period of 15 min. After 1.5 h the reaction mixture was diluted with DCM (200 mL) and extracted with H_2O (100 mL). The organic layer was then dried over anhydrous $MgSO_4$ and concentrated to dryness. Flash column chromatography, using 12% EtOAc in hexanes as the eluent, provided 1.36 g of pure carbamate **8** (77% yield) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (bd, 1H, J = 11.7 Hz), 4.65 (ddd, 2H, J = 5.1 and 1.8 Hz), 4.67 (s, 2H), 5.26 (dm, 1H, J = 10.5 Hz), 5.36 (dm, 1H, J = 17.1 Hz), 5.91–6.04 (m, 1H), 7.03 (ddd, 1H, J = 7.0 and 1.2 Hz), 7.15 (dd, 1H, J = 7.0 and 1.2 Hz), 7.31 (ddd, 1H, J = 8.2 and 1.8 Hz), 7.90 (bd, 1H, J = 8.1 Hz), 7.97 (bs, 1H).

Synthesis of Bromide Derivative 9. Carbamate **8** (1.35 g, 3.7 mmol) was dissolved in anhydrous THF (50 mL). CBr_4 (2.2 g, 6.5 mmol) followed by PPh_3 (1.70 g, 6.5 mmol) were added, and the reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, the solvent was evaporated to dryness, and crude product was redissolved in EtOAc (20 mL). The precipitated ($OPPh_3$) was removed by filtration, and the filtrate was concentrated to dryness. The residue was the purified by flash column chromatography, using 5% EtOAc in hexanes as the eluent, to give bromide **9** as a fluffy white solid (920 mg, 58% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.50 (s, 2H), 4.68 (dt, 2H, J = 5.6 and 1.6 Hz), 5.27 (dm, 1H, J = 10.8 Hz), 5.39 (dm, 1H, J = 17.2 Hz), 5.95–6.05 (m, 1H), 6.88 (bs, 1H), 7.11 (ddd, 1H, J = 7.1 and 1.2 Hz), 7.28 (dd, 1H, J = 8.0 and 1.6 Hz), 7.34 (ddd, 1H, J = 7.8 and 1.6 Hz), 7.83 (bd, 1H, J = 7.2 Hz).

Synthesis of Methylamine Intermediate 10. To a solution of bromide **9** (268 mg, 1 mmol) in anhydrous DMF (3 mL) were added HCl·GlyOMe (500 mg, 3 mmol) and DIPEA (1.0 mL, 6.0 mmol). The reaction mixture was stirred overnight at room temperature under N_2. The crude product was then partitioned between H_2O (12 mL) and EtOAc (25 mL). The organic layer was washed once with H_2O (12 mL), dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by flash column chromatography, using 10% EtOAc in hexanes as the eluent, to give 293 mg of pure amine **10** (92% yield) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.46 (s, 2H), 3.75 (s, 3H), 3.90 (s, 2H), 4.67 (ddd, 2H, J = 6.0 and 0.8 Hz), 5.24 (dm, 1H, J = 10.4 Hz), 5.38 (dm, 1H, J = 17.2 Hz), 5.94–6.04 (m, 1H), 7.03 (ddd, 1H, J = 7.2 and 0.8 Hz), 7.18 (d, 1H, J = 7.2 Hz), 7.31 (ddd, 1H, J = 7.8 and 1.6 Hz), 7.94 (bd, 1H, J = 8.0 Hz), 9.31 (bs, 1H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 28.2, 50.1, 52.2, 65.5, 81.5, 117.6, 120.2, 122.6, 126.3, 128.6, 129.7, 133.0, 138.9, 153.8, 171.1.

Synthesis of Protected APNA Cytosine Monomer 11. Amine **10** (328 mg, 1.2 mmol), $Cy^{Cbz}CH_2CO_2H$ (466 mg, 1.5 mmol) and HATU (583 mg, 1.5 mmol) were suspended in anhydrous DMF (7 mL) under N_2 at 0 °C. To the stirred suspension was added DIPEA (0.54 mL, 3.1 mmol), and after stirring at 0 °C for 10 min the reaction mixture was allowed to warm up to room temperature and stir for an additional 8 h. The crude product was diluted with EtOAc (100 mL) and washed with H_2O (1 × 75 mL), 5% $NaHCO_3$ (1 × 75 mL), and H_2O (1 × 75 mL). The organic layer was then dried over anhydrous $MgSO_4$ and concentrated to dryness. Pure monomer **11** was obtained after flash column chromatography, using pure EtOAc as the eluent, as a white foam (642 mg, 72% yield): ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotamers in 1:3.5 ratio) δ 3.69 (bs, 3H), 4.00 (*mi*) and 4.26 (*ma*) (bs, 2H), 4.59–4.85 (m, 6H), 5.19–5.37 (m, 4H), 5.85–6.02 (m, 1H), 6.98–7.03 (m, 1H), 7.10–7.12 (m, 1H), 7.22–7.39 (m, 7H), 7.53 (bs, 1H), 7.70 (bd, 1H, J = 6.9 Hz), 8.04 (bd, 1H, J = 6.9 Hz), 8.34 (bs, 1H); ES$^+$ MS *m/z* 564 (M + H)$^+$; ES$^-$ MS *m/z* 562 (M − H)$^-$.

Synthesis of APNA Cytosine Monomer 12. Allyl carbamate **11** (164 mg, 0.3 mmol) was dissolved in DCM (6 mL) and H_2O (100 μL), and Bu_3SnH (81 μL, 0.3 mmol) followed by $Pd(PPh_3)_4$ (16 mg, 5mol %) were added. After 15 min the reaction mixture was concentrated to an oil, which was then purified by flash column chromatography using a solvent gradient of 0–5% MeOH in $CHCl_3$ as the eluent. Pure aniline **12** was isolated as a glassy solid (140 mg, 91%): ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotamers in 1:1.3 ratio) δ 3.58 (*mi*) and 3.62 (*ma*) (s, 3H), 3.96 (*mi*) and 4.23 (*ma*) (s, 2H), 4.37 (*ma*) and 4.51 (*mi*) (s, 2H), 4.70 (*ma*) and 4.77 (*mi*) (s, 2H), 5.01 (*mi*) and 5.02 (*ma*) (s, 2H), 5.17 (*mi*) and 5.18 (*ma*) (s, 2H), 6.47 (ddd, 0.6H, J = 7.2 and 1.2 Hz), 6.56–6.60 (m, 0.9H), 6.69 (d, 0.5H, J = 7.6 Hz), 6.95–7.03 (m, 3H), 7.33–7.40 (m, 5H), 7.94–7.99 (m, 1H), 10.74 (bs, 1H); ES$^+$ MS *m/z* 480 (M + H)$^+$; ES$^-$ MS *m/z* 478 (M − H)$^-$.

Synthesis of [APNA(gly)-Th]-[PNA-Th] Protected Dimer (18a). Free acid **16** (830 mg, 2.2 mmol), aniline **6a** (773 mg, 2.2 mmol), and HATU (828 mg, 2.2 mmol) were placed in a flask under N_2, and anhydrous DMF (5 mL) was added. The mixture was cooled to 0 °C, and a solution of DIPEA (0.11 mL, 10% solution in anhydrous DMF, 4.3 mmol) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stir for 6 h before quenching with H_2O (15 mL). A mixture of 10% MeOH in $CHCl_3$ (50 mL) was added, and the organic layer was washed with 5% $NaHCO_3$ (2 × 15 mL) and brine (1 × 15 mL), dried over anhydrous $MgSO_4$, and concentrated to dryness. The crude product was purified by flash column chromatography, using a solvent gradient of 0–8% MeOH in $CHCl_3$ as the eluent to give 1.56 g (79% yield) of pure dimer **18a**: TLC R_f (8% MeOH in $CHCl_3$) = 0.211; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36–1.38 (bs, 9H), 1.74 (bs, 6H), 3.06–3.44 (m, 4H), 3.59–3.62 (bs, 3H), 3.97–4.68 (m, 8H), 6.74–7.68 (m, 6H), 9.55–9.86 (m, 1H), 11.29–11.33 (m, 2H); FAB$^+$ HRMS (glycerol/KCl) *m/z* found 765.260910 (M + K)$^+$, calcd for $(C_{32}H_{40}N_8O_{11}K)^+$ 765.261013.

Synthesis of Dimers 18b–d. Dimers **18b**, **18c**, and **18d** were synthesized using the same procedure as for dimer **18a**. Dimer **18b**: yield 71%; TLC R_f (12% MeOH in EtOAc) = 0.121; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09–1.19 (m, 3H), 1.35–1.37 (m, 9H), 1.74 (bs, 6H), 2.41–2.71 (m, 2H), 3.00–3.52 (m, 6H), 3.93–4.73 (m, 10H), 6.74–7.66 (m, 7H), 9.57–9.90 (m, 1H), 11.27–11.30, (m, 2H); ES$^+$ MS *m/z* 755 (M + H)$^+$; ES$^-$ MS *m/z* 753 (M − H)$^-$. Dimer (*R*)-**18c** and (*S*)-**18c**: yield 70%; ^{1}H NMR (300 MHz, DMSO-d_6, 140 °C) δ 1.34 (d, 3H, J = 7.2 Hz), 1.42 (s, 9H), 1.79 (m, 6H), 3.25 (q, 2H, J = 6.3 Hz), 3.22–3.55 (m, 2H), 3.60 (s, 3H), 4.22 (s, 2H), 4.44–4.68 (m, 7H), 6.32 (bs, 1H), 7.23–7.46 (m, 6H), 9.26 (bs, 1H), 10.63, (bs, 2H); ES$^+$ MS *m/z* 764 (M + Na)$^+$. Dimer **18d**: yield 69%; TLC R_f (5% MeOH in $CHCl_3$) = 0.19; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35–1.42 (m, 9H), 1.83–1.88 (m, 3H), 3.26–3.75 (m, 7H), 4.02–4.79 (m, 10H), 5.19–5.43 (m, 2H), 6.97–8.47 (m, 13H), 8.81–9.03 (m, 1H), 9.50–9.85 (m, 1H), 10.68 (bs, 1H); ES$^+$ MS *m/z* 832 (M + H)$^+$; ES$^-$ MS *m/z* 830 (M − H)$^-$.

Synthesis of [APNA(gly)Th]-[PNA-Th] Free Acid Dimer 19a. To a solution of ester **18a** (1.97 g, 2.7 mmol) in THF (20 mL) was added aqueous LiOH (10 mL, 0.8M, 8.0 mmol), and the reaction was stirred at room temperature for 45 min. The mixture was then diluted with H_2O (30 mL), cooled to 0 °C, and acidified to pH 3 by the dropwise addition of aqueous HCl (0.1 M). The solution was then extracted with 10% MeOH in $CHCl_3$ (3 × 100 mL), and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated to give dimer **19a** as a white foam in 88% yield (1.70 g) and in high purity: RP-TLC R_f (80% MeOH in H_2O) = 0.1; ^{1}H NMR (300 MHz, DMSO-d_6, mixture of four rotamers*)* δ 1.34–1.36 (m, 9H), 1.71–1.75 (m, 6H), 3.02–3.48 (m, 4H), 3.82–4.69 (m, 10H), 5.19 (s, 2H), 5.31 (*ma*) and 5.35 (*mi*) (s, 2H), 6.70–7.70 (m, 6H), 9.57–9.68 (m, 1H), 10.66 (bs, 1H), 11.26–11.31 (m, 2H); FAB$^+$ HRMS (glycerol/NaCl) *m/z* found 735.2714 (M + Na)$^+$, calcd for $(C_{32}H_{40}N_8O_{11} + Na)^+$ 735.2712.

Synthesis of Dimer 19b and 19c. The free acids of dimers **19b** and **19c** were synthesized using the same procedure as for **19a**. Free acid **19b**: yield 90%; ^{1}H NMR (400 MHz, DMSO-d_6) 1.35 and 1.37 (m, 9H), 1.73 and 1.75 (m, 6H), 2.36−2.65 (m, 2H), 3.03−3.61 (m, 6H), 4.04−4.73 (m, 10H), 6.74−7.71 (m, 6H), 9.64−9.97 (m, 1H), 11.29−11.32, (m, 2H), 12.26 (br, 1H); ES$^+$ MS m/z 727 (M + H)$^+$; ES$^-$ MS m/z 725 (M − H)$^-$. Free acid (*R*)-**19c** and (*S*)-**19c**: yield 97%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17−1.25 (m, 3H), 1.36−1.37 (m, 9H), 1.73 (br, 6H), 3.06−3.45 (m, 4H), 4.04−4.77 (m, 9H), 6.57−7.59 (m, 6H), 9.64−9.90 (m, 1H), 11.28−11.32 (m, 1H); FAB$^+$ HRMS (glycerol/KCl) m/z found 765.261013 (M + K)$^+$, calcd for ($C_{33}H_{42}N_8O_{11}$ + K)$^+$ 765.260910. Free acid **19d**: yield 88%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34−1.36 (m, 9H), 1.71−1.75 (m, 3H), 3.02−3.48 (m, 4H), 3.86−4.83 (m, 10H), 5.18 (s, 2H), 6.70−7.92 (m, 13H), 9.54−9.85 (m, 1H), 10.79 (bs, 1H), 11.30 (m, 1H); ES$^+$ MS m/z 846 (M + H)$^+$; ES$^-$ MS m/z 844 (M − H)$^-$.

Solid-Phase Synthesis of Oligomers. Oligomers **17** and **20**−**24** (0.02−0.03 mmol) were synthesized on MBHA resin with a loading capacity of 0.19−0.40 mmol/g, using the following protocol.

(1) Derivatization of MBHA Resin (per 100 mg of dry resin). The dry MBHA·HCl resin was suspended in DCM (5 mL) for 24 h. The resin was washed with 5% DIPEA in DMF (2 × 3 mL, 10 min each time). Boc-Lys(2-Cl-Cbz)-OH (5 equiv) was cross-linked to the resin using HATU (4.8 equiv) as the coupling agent in the presence of DIPEA (10 equiv) until a negative kaizer test was observed (∼3 h). The resin was treated with a mixture of Ac_2O/pyridine/DCM (1:25:25 ratio, 3 mL) for 10 min and then washed with DCM (2 × 30 s, 3 mL), DMF (2 × 30 s, 3 mL) and DCM (2 × 30 s, 3 mL).

(2) Coupling of Monomer or Dimer Fragments (chain elongation). The resin-bound, Boc-protected amino moiety was treated with 35% TFA in DCM (3 mL for 2 min and 3 mL for 40 min), followed by washing with DCM (4 × 30 s, 3 mL), DMF (3 × 30 s, 3 mL), DCM (3 × 30 s, 3 mL), and DMF (3 × 30 s, 3 mL). Deprotection was monitored by the Kaizer test using a small aliquot of resin-bound peptide (∼1 mg). The free acid was first preactivated by reacting with HBTU/DECA in a 3:2.85:6 molar ratio (acid/HBTU/DECA) in a solvent mixture of 1:1 DMF/pyridine for 1 min. This latter solution was then transferred to the reaction vessel containing the resin to give a final concentration 0.07 M with respect to the free acid. The coupling was allowed to proceed until a negative kaiser test was observed (∼0.5−4 h). The resin was washed with DMF (3 × 30 s, 3 mL), DCM (3 × 30 s, 3 mL), DMF (3 × 30 s, 3 mL), and DCM (3 × 30 s, 3 mL). The resin was finally capped by treating with Ac_2O/pyridine/DCM (1:1:2, 3 mL) for 5 min and washed with DCM (4 × 30 s, 3 mL), DMF (2 × 30 s, 3 mL), and DCM (2 × 30 s, 3 mL).

(3) Cleavage of Oligomers from the Resin. The resin was dried in vacuo for at least 12 h. The dry resin was then washed with TFA (2 × 1 min) and then subjected to a precooled (0 °C) solution of TFMSA/thioanisole/TFA (1:1:8) and mixed for 1 h while coming to room temperature. The resin was then removed by filtration, and the filtrate was diluted with anhydrous Et_2O (20×). The resulting mixture was then centrifuged to give a white pellet. The ethereal supernatant was decanted off, fresh Et_2O was added, and the pellet was broken up and resuspended. After centrifugation the supernatant was decanted off, and the pellet was dried in vacuo. The oligomers were then purified by reversed-phase HPLC and analyzed by mass spectrometry. HPLC conditions A: C_{18} Vydac reversed-phase column (4.6 × 125 mm, 5 μm), flow rate 1.5 mL/min, linear gradient from 5% aqueous CH_3CN to 100% CH_3CN (all solvents contained 0.06% TFA) in 35 min, UV monitored at $\lambda = 260$ nm, room temperature. HPLC conditions B: HP Zorbax RX-C_{18} reversed-phase column (9.4 × 250 mm, 5 μm), flow rate 4.2 mL/min, linear gradient from 100% H_2O to 30% aqueous CH_3CN (all solvents contained 0.06% TFA) in 55 min, UV monitored at $\lambda = 260$ nm, at 55 °C.

Hexamer 17. HPLC conditions A; retention time = 8.1 min (peak area 100%); ES$^+$ MS m/z 1807 (M + H)$^+$, 1830 (M + Na)$^+$; ES$^-$ MS m/z 1805 (M − H)$^-$, 1828 (M + Na − H)$^-$.

Hexamer 20. HPLC conditions A; retention time = 9.6 min (peak area 100%); ES$^+$ MS m/z 1846.6 (M + H)$^+$; ES$^-$ MS m/z 1844.6 (M − H)$^-$.

Hexamer 21. HPLC conditions B; retention time = 24.8 min (peak area 100%); ES$^+$ MS m/z 1861 (M + H)$^+$; ES$^-$ MS m/z 1859 (M − H)$^-$.

Hexamer 22. HPLC conditions A; retention time = 9.8 min (peak area 100%); ES$^+$ MS m/z 1861 (M + H)$^+$; ES$^-$ MS m/z 1859 (M − H)$^-$.

Hexamer 23. HPLC conditions A; retention time = 9.7 min (peak area 100%); ES$^+$ MS m/z 1861 (M + H)$^+$; ES$^-$ MS m/z 1859 (M − H)$^-$.

Hexamer 24. HPLC conditions B; retention time = 24.3 min (peak area 100%); ES$^+$ MS m/z 1856 (M + Na)$^+$; ES$^-$ MS m/z 1854 (M + Na − H)$^-$.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra for key compounds monomers **6a**−**c** and **12** and dimers **18a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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