Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron
Tetrahedron 60 (2004) 2235–2246

Synthesis of novel analogs of aromatic peptide nucleic acids (APNAs) with modified conformational and electrostatic properties

Lee D. Fader,[a] Eddie L. Myers[a] and Youla S. Tsantrizos[a,b,*]

[a]*Department of Chemistry, McGill University, Montreal, Quebec, Canada H3A 2K6*
[b]*Department of Chemistry, Boehringer Ingelheim (Canada) Ltd, 2100 Cunard Street, Laval, Quebec, Canada H7S 2G5*

Received 5 November 2003; revised 13 January 2004; accepted 13 January 2004

Abstract—Aromatic peptide nucleic acid analogs having an *N*-(2-aminobenzyl)glycine backbone (APNA **1**) were previously identified as promising new leads for the design of polyaromatic DNA mimics. Structural modifications of **1**, which lock the aromatic backbone into a unique conformation, while maintaining the same space distribution between the nucleobases as in **1**, were investigated. The electrostatic potential of the aromatic backbone was also modified in an attempt to improve the solubility of these compounds in aqueous media and to evaluate how the quadrapole of the aromatic backbone may influence the biophysical properties of the APNA oligomers. PNA hexamers containing a single monomer insert of each new APNA monomer were used to explore the hybridization properties of these analogs with poly rA and poly dA. Preliminary results indicated that these modifications do not seriously alter the molecular recognition properties of APNAs towards DNA and RNA.

1. Introduction

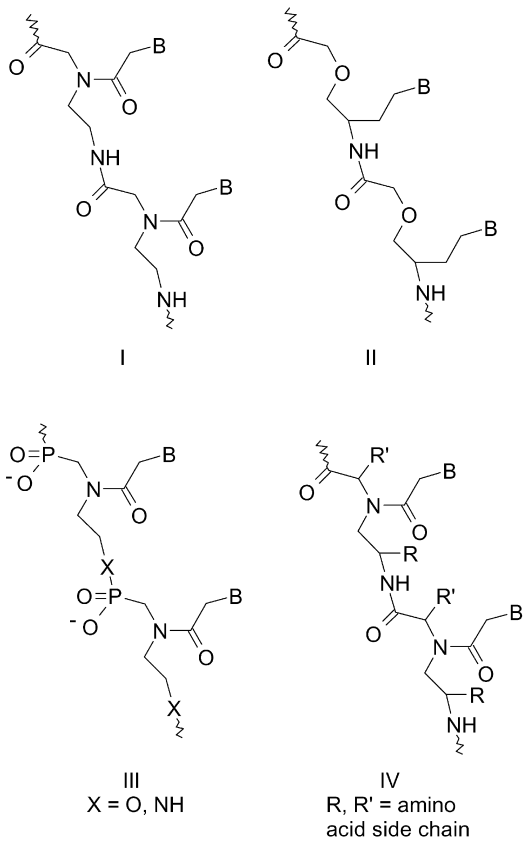
Peptide nucleic acids (PNAs, **I**) are mimics of natural oligonucleotides where the phosphate/deoxyribose backbone has been replaced by repeating 2-aminoethylglycine units and the nucleic acid base is attached to the backbone via an acetate linker.[1] These molecules exhibit several unique and unexpected molecular recognition properties, including enhanced hybridization affinity for DNA and RNA, in both the antiparallel and parallel binding modes,[2] and formation of invasion duplexes or triplexes when targeted to double stranded DNA[3] or RNA.[4] Another unique property of PNAs is the monophasic thermal denaturation profile of triplex structures which form between polypyrimidine PNAs and polypurine complementary strands, which indicate exclusive triplex to a random coil dissociation of the complex.[5] Although PNAs hold many promises as potential therapeutic agents[6] and as diagnostic tools,[7] this class of oligonucleotide analogs suffer from poor solubility in aqueous media and poor cell membrane permeability.[8]

In an attempt to improve the solubility of PNAs in biological fluids, a number of structural modifications have been reported which have incorporated charged or hydrophilic functional groups along the backbone. Promising examples of this type include the oxy-PNAs (**II**),[9] the phosphono-PNAs (**III**)[10] and various substituted forms of the original 2-aminoethylglycine backbone (**IV**).[11] In addition, a significant amount of effort has been devoted to developing analogs and conjugates with good cell membrane permeability. Although some types of cells, including nerve cells[12] and various strains of *Escherichia coli*,[13] appear to be permeable to PNAs, most mammalian cells are fairly impermeable to these molecules. Efforts to overcome this problem include covalent cross-linking of the oligomer to nuclear internalizing peptides and structural modifications.[14] In a recent example, Meir and co-workers[15] reported on the biological properties of a PNA-peptide conjugate using the cyclic peptide octreotate, a mimic of somatostatin.[16] In vivo data in rat suggested that this conjugate was selectively recognized and internalized by somatostatin-receptors, which are usually widely displayed on the cell membranes of cancer cells.[15] A more general approach for transporting PNA oligomers across cell membranes could be by chemical modification. This approach was recently exemplified by Zhou and co-workers,[17] who demonstrated that a polycationic, guanidine-based backbone (i.e. **IV**, $R'=-(CH_2)_3NH(C{=}NH)NH_2$, R=H) could dramatically alter the uptake and localization of the PNA oligomer in the cell nucleus of

Keywords: Novel aromatic peptide nucleic acid monomers; PNA/APNA triplexes with DNA and RNA.
* Corresponding author. Address: Department of Chemistry, Boehringer Ingelheim (Canada) Ltd, 2100 Cunard Street, Laval Québec, Canada H7S 2G5. Tel.: +1-450-682-4640; fax: +1-450-682-4189;
e-mail address: ytsantrizos@lav.boehringer-ingelheim.com

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.01.026

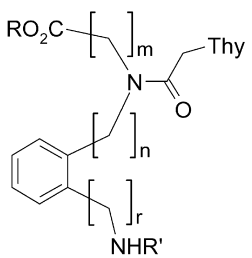
cultured HCT116 cells, possibly by involvement of a TAT transduction pathway.[17,18]



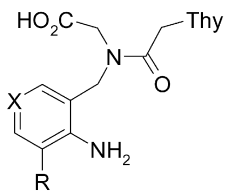
During our previous investigations in this field,[19,20] we focused our attention on the design of novel peptide nucleic acid analogs having an aromatic moiety as an integral part of their backbone; we termed these derivatives as aromatic peptide nucleic acids (APNAs).[19,20] Preliminary hybridization experiments with DNA and RNA identified monomer **1** as the most promising lead structure from this class of analogs.[20] Unfortunately, the poor solubility observed with homopolymers of **1** prevented an in-depth evaluation of these analogs as potential antisense agents or as ligands for nucleic acid processing enzymes.[20b] In this study, we describe the synthesis and some preliminary hybridization properties of four new derivatives of **1** which were designed in order to (a) lock the aromatic backbone into a conformationally unique structure, while maintaining the same space distribution between the nucleobases and (b) modulate the electrostatic potential of the aromatic backbone of **1** in an attempt to improve the solubility of its homopolymers in aqueous media.

2. Results and discussion

Structural pre-organization of a synthetic oligomer into a conformation that closely resembles its DNA- or RNA-bound conformation is expected to increase the thermal stability of the complex (duplex or triplex) it would form with its complementary natural oligonucleotide. In recent years, a number of rigidified PNA analogs have been described in the literature, aiming to address the conformational flexibility of the original PNA analogs.[21] The tertiary amide moiety of the PNA monomer unit that tethers the nucleic acid base to the backbone has received much attention as a target for these modifications. Early molecular modeling investigations suggested that the carbonyl oxygen of the tertiary amide might participate in an intramolecular hydrogen bond, helping to preorganize PNA oligomers for duplex formation.[22] Nielsen[23] and Leumann[24] further examined the importance of this carbonyl moiety and suggested that the amide geometry and/or its electrostatic interaction with adjacent residues contribute to the molecular recognition of PNAs for natural oligonucleotides. In addition, NMR and X-ray crystallographic data have independently shown that in the bound conformation the amide bond favors the rotamer, which orients the carbonyl oxygen toward the C-terminal of the peptide (formally the Z(O) geometry) in PNA/DNA,[25] PNA/RNA,[26] as well as the PNA/PNA[27] complexes. However, the two rotamers of the amide are in rapid equilibrium in the free state of a single stranded PNA oligomer.[25a] Based on this literature precedence, we focused our attention on replacing the 2-aminobenzyl glycyl backbone of the APNA monomer **1** with a conformationally more rigid backbone such as the *N*-phenyl-*N*-alkyl amide derivatives **2** and **3**. In most cases, *N*-phenyl-*N*-alkyl amides would be expected to adopt exclusively the E(O) geometry, with the plane of the phenyl moiety oriented perpendicularly to the plane of the amide bond.[28] Consequently, the *N*-alkyl unit of monomers **2** and **3** would be expected to extend in the C-terminal direction, having an amide conformation analogous to the Z(O) rotamer preferred by PNAs for binding to natural oligonucleotides in either a duplex or triplex structure.[25–27] However, these new analogs were designed so as to maintain the space distribution between nucleobases the same as in the lead structure **1** (total of a 6-bond spacing between units, 5 σ-bonds and 1 π-bond) in homopolymers of APNA. Furthermore, modifications to the electrostatic character of the 2-aminobenzyl ring of the lead structure **1** by the incorporation of an anionic carboxylate substituent or possibly a cationic pyridyl backbone (**4** and **5**, respectively), was expected to improve the solubility of APNAs in aqueous media, and possibly alter their binding properties.



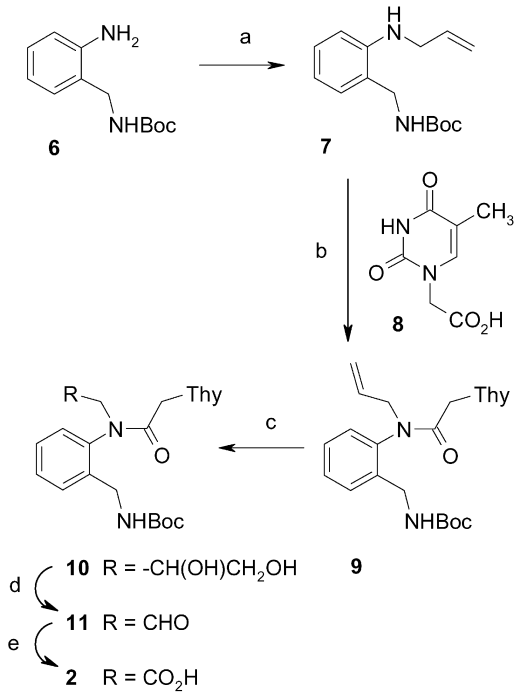
1 m = n = 1, r = 0
2 m = 1, n = 0, r = 1
3 m = 2, n = 0, r = 0



1 R = H, X = CH
4 R = CO_2H, X = CH
5 R = H, X = N

2.1. Synthesis of APNA monomers

The synthesis of the APNA monomer **2** was accomplished as outlined in Scheme 1. The previously reported mono-Boc protected aromatic diamine **6**[20b] was reacted with allyl bromide in the presence of DIPEA to give the secondary

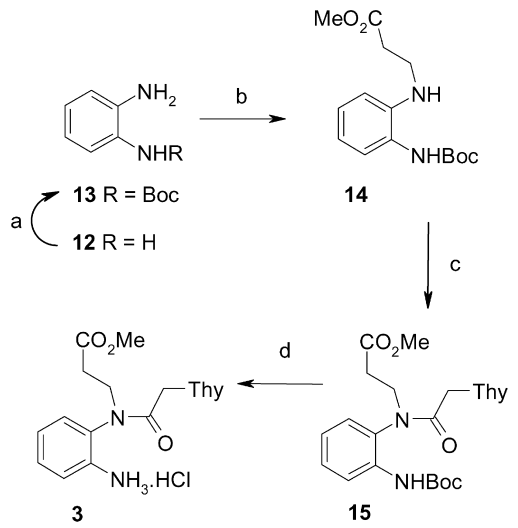


Scheme 1. Synthesis of APNA monomer **2**. Conditions: (a) $BrCH_2CHCH_2$, DIPEA, DMF 76%; (b) **8**, EDC, DMF, 89%; (c) OsO_4, NMO, *t*BuOH/H_2O/THF, 86%; (d) $NaIO_4$, THF/H_2O, >98%; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH/THF/H_2O, 60%.

aniline **7** in good yield. The thymine derivative **8** was then attached to the backbone via EDC mediated amide bond formation to give the amide intermediate **9**. The ^1H NMR spectrum of this compound revealed the presence of non-equivalent geminal protons for each methylene group, confirming that amide **9** was intrinsically chiral. This observation suggested that the plane of the phenyl moiety was oriented perpendicularly to the plane of the amide bond, thus leading to a chiral molecule. Importantly, only one resonance was observed for each non-equivalent proton, suggesting that the amide rotamer equilibrium favored only one isomer. Upon dihydroxylation of the terminal olefin of **9** with OsO_4 a diastereomeric mixture of the corresponding diol (**10**) was formed. This diol was subsequently oxidatively cleaved to the aldehyde **11** which was in turn oxidized to the desired APNA monomer **2**, having a free carboxylic acid moiety (Scheme 1).

The synthesis of the APNA monomer **3** was accomplished using the protocol illustrated in Scheme 2. Commercially available diamine **12** was first selectively protected as the mono *N*-Boc carbamate **13** in good yield.[29] Under neutral conditions, alkylation of **13** with methyl 3-bromoproprionate could only be achieved at high temperature, giving the secondary aniline **14** in a very modest yield. The thymine acetate derivative **8** was then coupled to **14** to obtain the fully protected APNA monomer **15**, which was then deprotected with HCl/dioxane to the free aniline momomer **3**.

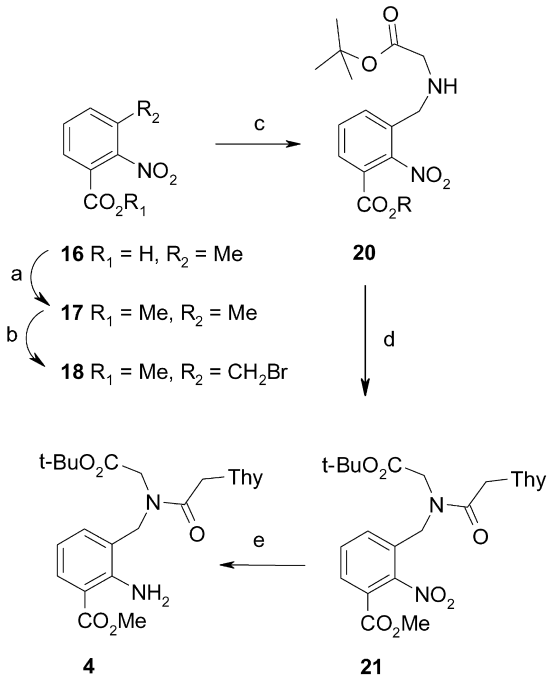
The synthesis of monomer **4** was achieved following a scheme similar to that previously described for the preparation of **1** (Scheme 3).[20a] 3-Methyl-2-nitrobenzoic acid was converted to the ester **17** under acidic conditions in



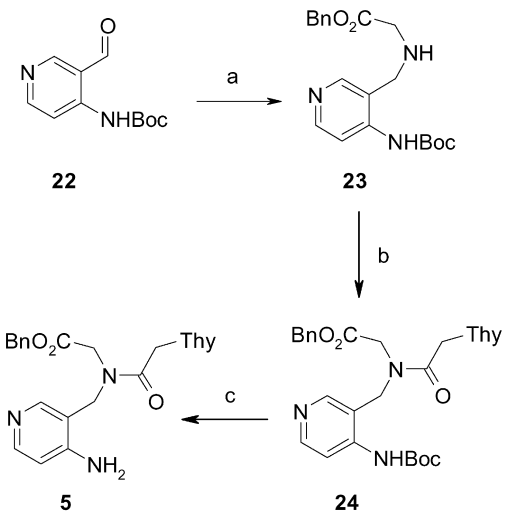
Scheme 2. Synthesis of APNA monomer **3**. Conditions: (a) Boc_2O, THF, 75%; (b) $BrCH_2CH_2CO_2Me$, DMF, Δ, 36%; (c) Compound **8** EDC, DMF, 49%; (d) HCl/dioxane, quant.

methanol which was then brominated to give the benzylic bromide **18** in modest yield. The backbone fragment **20** was obtained in nearly quantitative yield after alkylation of glycine *t*-butyl ester (**19**) with the bromide **18** under basic conditions (Scheme 3). The thymine acetate **8** was then coupled to the backbone moiety and the nitro group of the amide intermediate **21** was reduced under catalytic hydrogenation conditions to give the differentially protected diester monomer **4** in good overall yield from the acid **16**.

For the synthesis of the pyridyl analog **5**, the aldehyde intermediate **22** was first prepared by *ortho* lithiation of the Boc protected 4-amino pyridine, followed by formylation



Scheme 3. Synthesis of APNA monomer **4**. Conditions: (a) MeOH, H_2SO_4, Δ, 89–100%; (b) NBS, Bz_2O_2, Δ, 49%; (c) $NH_2CH_2CO_2tBu$ (**19**), DIPEA, DMF, 98%; (d) Compound **8**, EDC, DMF, 93%; (e) HCO_2H, DIPEA, Pd/C, DMF, 89%.



Scheme 4. Synthesis of APNA monomer **5**. Conditions: (a) $TsOH{\cdot}H_2NCH_2CO_2Bn$, $NaBH_3CN$, EtOH, 43%; (b) Compound **8**, EDC, DMF, 73%; (c) HCl, 4 M in 1,4-dioxqane, quant.

with DMF as previously described.[30] Reductive alkylation of the aldehyde with the benzyl ester of glycine gave the backbone intermediate **23** in modest yield (Scheme 4). Coupling of **23** with the thymine derivative **8** in the usual manner, followed by removal of the carbamate protecting group under acidic conditions, gave monomer **5** in good overall yield from aldehyde **22**.

2.2. Conformational analysis of the *N*-phenyl APNA monomers

The preferred conformation of each new APNA monomer was investigated by NMR. A mixture of two thermodynamically stable rotamers of each compound was clearly evident in both their ^1H and ^{13}C NMR spectra of monomers **1**, **4** and **5**. For each APNA monomer, as well as each key intermediate leading to its synthesis, variable temperature (VT) ^1H NMR was used to confirm that the two sets of resonances observed at RT were due to the presence of rotamers and not due to the presence of undesired impurities in the samples. Rapid equilibrium (on the NMR time scale) between the two rotamers of the tertiary amides, and consequent coalescence of the two sets of resonances into one set, was usually observed at temperatures greater than 90–100 °C (e.g. compound **25**, Fig. 1(b)).

As we had anticipated, however, only one set of resonances was observed for each non-equivalent proton and carbon (in the ^1H and ^{13}C NMR spectra acquired at RT) of the APNA monomers **2** and **3**, strongly suggesting that these compounds adopted predominantly only one conformation. Similar NMR data were also obtained for all of the synthetic precursors of these analogs (e.g. compound **9**, Scheme 1, Fig. 1(a)). Unfortunately, due to the extensive overlap of the aromatic resonances in the ^1H NMR spectra of monomers **2** and **3**, detailed investigations of these analogs by NMR experiments proved to be very challenging. In order to gain some insight into the conformation of these compounds, the model compound **26** (the adenine analog of the synthetic intermediate **9**, Scheme 1), was subsequently used for structural studies. It should be noted that all of the ^1H NMR resonances of compound **26** could be unambiguous assigned and VT ^1H NMR experiments did not reveal any significant changes in these signals.[31] Chemical shift assignments for both H_a and H_b were confirmed by the NOE correlations observed (Fig. 2), which included a strong NOE between both H_a and H_b and H8 of the adenine base. Strong NOE was also observed between H_a and aromatic H_c proton but not between H_b and H_c. Furthermore, a significant difference in chemical shifts was observed for the two diastereotopic protons H_a and H_b. The complete set of NMR data from compound **26** were consistent with a mixture of atropisomers which preferentially adopted the desired E(O) rotamer conformation (Fig. 2). Based on this data and literature precedence on other *N*-phenyl-*N*-alkyl amides,[28] it is reasonable to assume that the single conformation observed for the pyrimidine analogs **2** and **3** is also that of the E(O) rotamer.

2.3. Comparison of the hybridization properties of the APNA units 1–5 in a PNA/APNA chimera

We previously observed that the coupling of PNA monomers to a polymer-bound *ortho*-substituted aniline

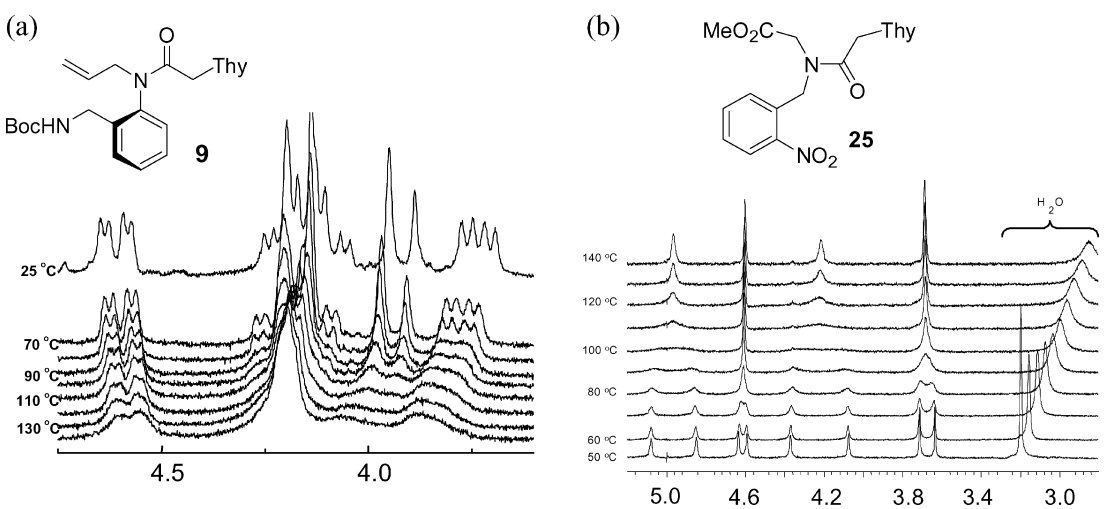


Figure 1. Temperature dependence of selected regions of the 270 MHz ^1H NMR spectra of intermediates **9** and **25**.

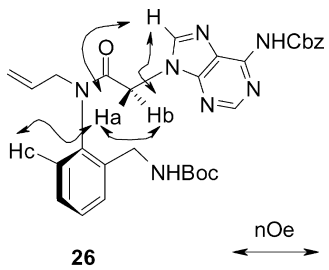
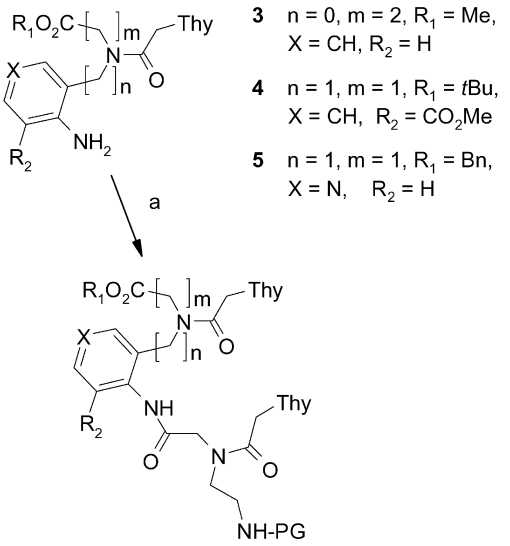


Figure 2. Key NOE correlations from NOESY spectra of amide **26**.

monomer (**1**) was problematic, resulting in poor coupling yields and difficult separations of the desired final products from the shorted oligomers often formed as side products. This problem was overcome by using PNA/APNA dimers as the building blocks in our protocol for the solid-phase synthesis. Based on this protocol, single inserts of monomers **3**–**5** were incorporated into PNA-T_6 oligomers via the dimer building blocks **30**–**32** and previously established solid-phase chemistry (Scheme 5).[20a,32] In contrast, a PNA monomer could be efficiently coupled to a resin-bound APNA monomer **2**, presumably due to the superior nucleophilicity and lower steric hindrance of the benzylic amine N-terminal derived from **2** vs. the aniline nitrogen derived from monomers **1**, **3**–**5**.

In order to gain some insight into the binding properties of the APNA units **2**–**5**, the hybridization stability of triplex structures formed between each of the Lys-PNA_2-APNA-PNA_3-R chimeras (Table 1, oligomers **35**–**38**) and poly(rA) or poly(dA) were examined by UV-thermal melting (T_m) experiments and compared to those of the control PNA hexamer **33** and the previously described APNA/PNA chimera **34**.[20a,33] In all cases, introduction of one APNA unit into the PNA hexamer led to destabilization of the triplexes relative to the PNA control (Table 1). As previously reported, a destabilization of approximately 13 °C (or ~6.5 °C per APNA monomer of the triplex) was observed for hexamer **34** as compared to the unmodified PNA hexamer **33**.[20a] Incorporation of monomer **2** into the PNA oligomers (hexamer **35**) resulted in only a slight decrease of T_m value for the triplex with RNA (ΔT_m=−3 °C) compared to hexamer **34**, whereas a more significant difference was observed with DNA (ΔT_m=−6 °C). Interestingly, no binding was observed between chimera **36** (with an insert of monomer **3**) and poly dA or poly rA. Perhaps this was to be expected, since



3 n = 0, m = 2, R_1 = Me, X = CH, R_2 = H

4 n = 1, m = 1, R_1 = *t*Bu, X = CH, R_2 = CO_2Me

5 n = 1, m = 1, R_1 = Bn, X = N, R_2 = H

27 n = 0, m = 2, R_1 = Me, X = CH, R_2 = H, PG = Boc

28 n = 1, m = 1, R_1 = *t*Bu, X = CH, R_2 = CO_2Me, PG = Fmoc

29 n = 1, m = 1, R_1 = Bn, X = N, R_2 = H, PG = Fmoc

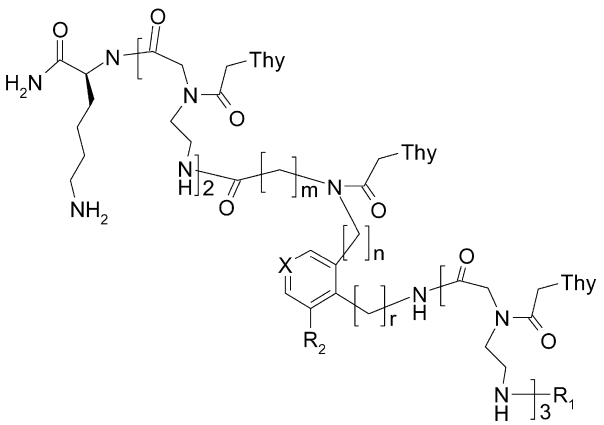
30 n = 0, m = 2, R_1 = H, X = CH, R_2 = H, PG = Boc

31 n = 1, m = 1, R_1 = H, X = CH, R_2 = CO_2Me, PG = Fmoc

32 n = 1, m = 1, R_1 = H, X = N, R_2 = H, PG = Fmoc

Scheme 5. Synthesis of APNA/PNA dimers 29–31. Conditions: (a) PG-PNA-Thy-OH, coupling conditions, see Section 4; (b) conversion of ester to carboxylic acid, see Section 4.

Table 1. Results of thermal denaturation experiments[a] for complexes of hexamers **33**–**38** with poly rA and poly dA



	m	n	r	X	R_2	R_1
34	1	1	0	CH	H	Ac
35	1	0	1	CH	H	Ac
36	2	0	0	CH	H	Ac
37	1	1	0	CH	CO_2H	Ac
38	1	1	0	N	H	H

Monomer insert	Hexamer	Complex with poly rA (T_m, °C)	Complex with poly dA (T_m, °C)
PNA	**33**	65	56
1	**34**	50	45
2	**35**	47	39
3	**36**	n.o.	n.o.
4	**37**	48	42
5	**38**	47	39

n.o.—a clear sigmoidal transition was not observed.

[a] Melting temperatures of triplexes were determined on a Cary 3 UV–vis spectrophotometer. All T_m solutions were 150 mM in NaCl, 10 mM in NaH_2PO_4, 1 mM in EDTA, 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. T_m values are the maxima of the first derivative plots obtained from each melting curve.

incorporation of monomer **3** into the PNA oligomer (hexamer **36**), significantly alters the space distribution between adjacent nucleic acid bases, in particular at the junction points between the APNA and PNA units, presumably leading to a dramatic destabilization of any complex that could potentially form with DNA or RNA.

Since insertion of **1** into PNA gave the PNA/APNA chimera with the best hybridization properties, structural modifications which would increase the solubility of oligomers derived from **1** in aqueous media were subsequently investigated. The binding properties of chimeras **37** and **38** composed in part of APNA units **4** and **5**, respectively, were initially explored by evaluating the thermal stability of the triplexes formed by these hexamers and poly dA or poly rA (Table 1). In both cases, a slightly detrimental effect on the RNA recognition properties of the APNA/PNA chimeras (ΔT_m=~2 °C) were observed, and a more significant destabilization was observed for the triplexes formed with DNA (ΔT_m=3–6 °C). Nonetheless, APNA homopolymers composed of monomers **4** and **5** are expected to exhibit better solubility in aqueous buffer than the corresponding homopolymers of monomer **1**.[34] Therefore, these new analogs represent two potential new lead structures, with improved solubility properties that could facilitate our future studies into the structural and biological properties of aromatic peptide nucleic acids.[34]

3. Conclusions

Previously, we reported that insertion of monomer **1** into a PNA oligomer was well tolerated in both triplex and duplex structures, allowing for selective Watson-Crick and/or Höogsteen base pairing recognition and hybridization with complementary DNA or RNA strands.[20] In this report, we have used monomer **1** as a lead structure for further optimization of the 2-aminobenzyl glycyl backbone. Conformationally pre-organized monomers having the amide bond in the preferred E(O) conformation (purine **26** and most likely pyrimidine **2**) and analogs with a modified electrostatic potential along the backbone[34] (analogs **4** and **5**) were synthesized. PNA/APNA chimeras containing a monomer unit of **2**, **4** or **5** (oligomers **35**, **37** and **38**, respectively) were prepared and their hybridization properties with DNA and RNA were evaluated. Based on the preliminary data, these structural alterations appear to be well tolerated, leading to only a minor decrease in the thermal stability of the triplexes formed with DNA or RNA. However, these minor effects may not accurately reflect the hybridization properties of the corresponding APNA homopolymers.[17,20b,24b,35] Thus, the synthesis and evaluation of the biophysical properties of homopolymers composed of **2**, **4** or **5** are in progress and will be reported in the near future. The more polar compounds **4** and **5** are of special interest, since their homopolymers are expected to be fairly soluble in aqueous media.[34] These oligomers may be valuable tools in exploring possible inter-residue $\pi-\pi$ interactions,[36] such as those observed in protein folding,[37] DNA duplex structures[38] or protein/nucleic acid interactions.[39] Plausible dipole/quadrapole interactions (i.e. π-cation or X–H–π hydrogen bonds)[40] along the backbone of these oligomers may also contribute to the pre-organizing forces that favor helix formation, or binding to proteins, and will be explored. Ultimately, our ability to affect the biophysical properties of synthetic oligomers by modifying their structure and electrostatic potential is critical to our endeavour towards achieving a better understanding of the factors, which dictate molecular recognition between synthetic oligomers and natural oligonucleotides.

4. Experimental

4.1. General methods

Solvents were purchased from Fischer Scientific and purified as follows: THF was distilled from sodium/benzophenone ketyl; CH_2Cl_2 distilled from P_2O_5 or CaH_2; DMF treated with KOH overnight at RT, then vacuum distilled from CaO or BaO and stored over activated 4 Å molecular sieves; MeCN distilled from CaH_2; pyridine distilled from CaH_2. HATU was purchased from PerSeptive Biosystems Ltd. Hexane used for chromatography was reagent grade *n*-hexane that contained small amounts of hexane isomers. 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. HPLC solvents were HPLC grade and were filtered through 0.45 μm filters (Supelco, Bellefonte, PA) prior to use. Analytical HPLC analysis was carried out using one of the following sets of conditions: Condition A: HP Hypersil ODS-C_{18} reversed phase column (4.6×250 mm, 5 μm), flow rate 1.2 mL/min, linear gradient from 100% A to 60% A/40% (v/v) B in 40 min, UV monitored at λ=266 and 254 nm, at 55 °C. Condition B: Waters Symmetry® C18 reversed phase column (4.6×150 mm, 5 μm), flow rate 1.2 mL/min, linear gradient from 95% A/5% B to 70% A/30% (v/v) B in 55 min, UV monitored at λ=266 and 254 nm, at 55 °C. Condition C: Waters Symmetry® C18 reversed phase column (4.6×150 mm, 5 μm), flow rate 1.2 mL/min, linear gradient from 95% A/5% B to 100% (v/v) B in 18 min, UV monitored at λ=266 and 254 nm, at 55 °C. Semipreparative HPLC was carried out using the following set of conditions: HP Zorbax® C18 reversed phase column (9.4×250 mm, 5 μm), flow rate 4.2 mL/min, linear gradient from 95% A/5% B to 70% A/30% (v/v) B in 55 min, UV monitored at λ=266 and 254 nm, at 55 °C. All compounds were purified to >95% homogeneity as determined by C18 reversed phase analytical HPLC using the conditions described above.

Deuterated NMR solvents were purchased from Isotec Inc. (Miamisburg, OH). NMR spectra were obtained at ambient 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 signals are denoted as major (ma.) and minor (mi.). All ^{1}H NMR spectra were recorded on Varian Mercury (300 or 400 MHz) or JEOL (270 MHz) Spectrometers. ^{13}C NMR spectra were recorded

on a JEOL spectrometer (67.7 MHz) or Varian Mercury spectrometers (75 MHz).

4.1.1. Synthesis of {[2-(*tert*-butoxycarbonylamino-methyl)-phenyl]-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetyl]-amino}-acetic acid (2). A solution of $NaClO_2$ (310 mg, 3.44 mmol) and NaH_2PO_4 (257 mg, 2.14 mmol) in H_2O (1 mL) was added to a solution of aldehyde **12** (184 mg, 0.43 mmol) dissolved in a 1:8:4 mixture of 2-methyl-2-butene/tBuOH/THF (5.5 mL). After complete consumption of the aldehyde as determined by TLC, the reaction was partitioned between aqueous NaOH (5 mL, 0.5 M) and EtOAc (5 mL). The aqueous phase was then acidified to pH=3 by addition of HCl (3 M) and then extracted with EtOAc (3×5 mL). The organic layer was then dried over anhydrous $MgSO_4$ and concentrated to a white foam. Residual H_2O and acetic acid were removed by suspending the product in toluene and concentrating to dryness (3×). Yield: 60%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.37 (s, 9H), 1.71 (m, 3H), 3.81–4.63 (m, 6H), 7.13–7.55 (m, 5H), 11.30 (br, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 11.9, 28.2, 48.3, 50.4, 78.1, 107.8, 125.1, 128.0, 128.7, 128.8, 129.1, 129.2, 137.8, 138.0, 141.7, 150.6, 155.7, 164.1, 166.6.

ES^+ MS *m*/*z*: 469.2 $(M+Na)^+$; ES^- MS *m*/*z*: 445.2 $(M-H)^-$.

4.1.2. Synthesis of 2-amino-3-({*tert*-butoxycarbonyl-methyl-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetyl]-amino}-methyl)-benzoic acid methyl ester (4). To a solution of compound **21** (578 mg, 1.16 mmol) dissolved in DMF (5 mL) was added Pd/C (75 mg) followed by DIPEA (670 μL, 3.85 mmol) and HCO_2H (120 μL, 3.50 mmol). After stirring for 2.5 h, the reaction was diluted with EtOH (10×) and filtered through celite. The filtrate was then evaporated to dryness and the residue purified by flash column chromatography (2–5% (v/v) MeOH/CH_2Cl_2) to give 480 mg of aniline **5** (89%). ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers as determined by VT ^{1}H NMR experiments) δ: 1.25 (ma.) and 1.37 (mi.) (s, 9H), 1.74 (mi.) and 1.76 (ma.) (s, 3H), 3.77 (ma.) and 3.80 (mi.) (s, 3H), 3.88 (mi.) and 4.19 (ma.) (s, 2H), 4.44 (ma.) and 4.51 (mi.) (s, 2H), 4.56 (br, 2H), 6.50–6.70 (m, 3H), 7.22–7.40 (m, 2H), 7.69–7.74 (m, 1H), 11.28 (mi.) and 11.31 (ma.) (br, 1H). ^{13}C NMR (67.7 MHz, acetone-d_6) δ: 12.4, 27.8, 28.1, 47.8, 48.0, 48.5, 48.9, 49.3, 52.1, 81.4, 82.2, 108.5, 108.7, 109.7, 114.8, 115.6, 121.4, 130.6, 131.3, 132.5, 136.2, 142.5, 142.8, 149.2, 150.0, 151.5, 164.9, 167.9, 168.5, 169.2.

ES^+ MS *m*/*z*: 483.1 $(M+Na)^+$; ES^- MS *m*/*z*: 459.0 $(M-H)^-$.

4.1.3. Synthesis of (2-allylamino-benzyl)-carbamic acid *tert*-butyl ester (7). To a solution of aniline **6** (9.6 g, 43 mmol) in anhydrous DMF (420 mL) was added DIPEA (3.7 mL, 21 mmol) and allyl bromide (25.8 g, 21 mmol). The reaction was stirred overnight under N_2 and then concentrated to an oil. The residue was then taken up in EtOAc (400 mL) and washed with H_2O (3×300 mL). The organic layer was dried over Na_2SO_4 (anhydrous) and the solvent removed under reduced pressure. The product was obtained as an off-white solid after silica gel chromatography (10% (v/v) EtOAc/hexanes) in 76% yield. ^{1}H NMR (300 MHz, $CDCl_3$). δ: 1.44 (s, 9H), 3.83 (m, 2H), 4.27 (d, 2H, *J*=6.6 Hz), 4.82 (br, 1H), 5.18 (m, 1H), 5.29 (m, 1H), 5.90–6.03 (m, 1H), 6.67–6.72 (m, 2H), 7.06 (d, 1H, *J*=6.3 Hz), 7.21 (t, 1H, *J*=7.7 Hz). ^{13}C NMR (67.7 MHz, DMSO-d_6) δ: 14.4, 22.5, 28.7, 31.4, 45.8, 78.5, 110.4, 115.7, 115.9, 123.9, 128.4, 136.4, 146.2, 156.7.

ES^+ MS *m*/*z*: 263.1 $(M+H)^+$; ES^- MS *m*/*z*: 261.2 $(M-H)^-$.

4.1.4. Synthesis of (2-{allyl-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetyl]-amino}-benzyl)-carbamic acid *tert*-butyl ester (9). Secondary aniline **7** (600 mg, 2.29 mmol) was dissolved in anhydrous DMF (2.3 mL). To the solution was added carboxylic acid **8** (843 mg, 4.58 mmol) followed by EDC (875 mg, 4.58 mmol). The reaction was stirred for 5–7 h under N_2 and then concentrated to a yellow oil. The residue was partitioned between EtOAc (8 mL) and sat. aq. $NaHCO_3$ (8 mL). The organic layer was further extracted with sat. aq. $NaHCO_3$ (1×8 mL) and H_2O (1×8 mL). The organic layer was concentrated to give a yellowish solid and the product was purified by dissolution in a minimal amount of EtOAc, followed by precipitation by slow addition of hexanes. Yield: 89% (white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.38 (s, 9H), 1.72 (s, 3H), 3.72 (dd, 1H, *J*=14.4, 7.2 Hz), 3.91 (d, 1H, *J*=16.8 Hz), 4.07 (dd, 1H, *J*=16.0, 6.0 Hz), 4.14–4.22 (m, 2H), 4.60 (dd, 1H, *J*=14.4, 5.6 Hz), 5.06–5.11 (m, 4H), 5.74–5.85 (m, 1H), 7.20–7.59 (m, 5H), 11.29 (br, 1H). ^{13}C NMR (67.7 MHz, DMSO-d_6) δ: 12.3, 28.7, 49.2, 51.7, 78.7, 108.4, 119.0, 128.6, 129.3, 129.7, 130.0, 133.2, 137.9, 138.7, 142.5, 151.4, 156.5, 164.8, 166.6.

ES^+ MS *m*/*z*: 429.3 $(M+H)^+$; ES^- MS *m*/*z*: 427.2 $(M-H)^-$.

4.1.5. Synthesis of (2-{(2,3-dihydroxy-propyl)-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetyl]-amino}-benzyl)-carbamic acid *tert*-butyl ester (10). Compound **9** (261 mg, 0.61 mmol) was dissolved in 1:1:1 H_2O/THF/tBuOH (1.8 mL). To the solution was added OsO_4 (583 μL, 0.052 M in benzene) followed by NMO (80 mg, 0.67 mmol) and the reaction was stirred for 10 h. The reaction was then quenched by addition of a 1:1 mixture of saturated Na_2SO_3/sat. aq. $NaHCO_3$ (2 mL for every 1 mL of reaction mixture). This mixture was stirred for 30–60 min and then extracted with EtOAc (1×8 mL). The desired tertiary amide **10** was then purified by silica gel chromatography using a gradient from 0 to 10% (v/v) MeOH/CH_2Cl_2. Yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.39 (s, 9H), 1.72 (s, 3H), 3.27–4.76 (m, 6H), 7.16–7.58 (m, 5H), 11.26 (s, 1H). ^{13}C NMR (67.7 MHz, $CDCl_3$) δ: 11.9, 28.5, (40.2, 40.5), 50.4, (52.7, 53.6), (64.9, 65.1), (70.4, 70.5), 80.0, (110.3, 110.4), 129.2, (129.7, 129.8), (130.2, 130.3), 130.4, (138.7, 138.7), 139.7, (143.3, 143.3), 152.6, 158.0, 166.4, (169.0, 169.4).

ES^+ MS *m*/*z*: 463.3 $(M+H)^+$; ES^- MS *m*/*z*: 461.2 $(M-H)^-$.

4.1.6. Synthesis of {2-[[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetyl]-(2-oxo-ethyl)-amino]-benzyl}-carbamic acid *tert*-butyl ester (11). An aqueous

solution of $NaIO_4$ (1.8 mL, 0.25 M) was added to a solution of diol **10** (200 mg, 0.43 mmol) dissolved in THF (0.8 mL) and the reaction was carefully monitored by TLC. Upon complete consumption of the starting material, the reaction was filtered and the product was extracted from the reaction mixture with EtOAc (3×5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, concentrated to dryness and the resulting white foam was used immediately in the next step. Yield (crude): >98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.38 (s, 9H), 1.72 (s, 3H), 4.04–4.34 (m, 4H), 4.68 (d, 1H, J=18.0 Hz), 5.16 (d, 1H, J=6.8 Hz), 7.34–7.56 (m, 5H), 9.51 (s, 1H).

ES$^+$ MS m/z: 431.2 $(M+H)^+$; ES$^-$ MS m/z: 429.2 $(M-H)^-$.

4.1.7. Synthesis of 3-(2-*tert*-butoxycarbonylamino-phenylamino)-propionic acid methyl ester (14). A flask containing mono-Boc protected diamine **13** (711 mg, 3.4 mmol) was charged with anhydrous DMF (750 μL) and methyl 3-bromoproprionate (230 mg, 1.4 mmol). The mixture was rapidly (over ~5 min) heated to ~110–130 °C and then allowed to cool to RT with stirring over 1 h. The reaction was then evaporated to dryness and the mixture purified by silica gel chromatography (gradient of 25–50% (v/v) EtOAc/hexanes as eluant) to give the desired secondary aniline **14** in 36% yields (in addition, Boc-deprotected backbone and some unreacted starting aniline **13** were also recovered). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.52 (s, 9H), 2.66 (t, 2H, J=6.6 Hz), 3.44 (t, 2H, J=6.3 Hz), 3.72 (s, 3H), 6.76–6.83 (m, 2H), 7.08 (dt, 1H, J=7.5, 1.8 Hz), 7.37 (d, 1H, J=7.8 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 28.3, 33.8, 39.9, 51.8, 80.4, 113.1, 118.7, 124.9, 125.2, 126.2, 141.2, 154.1, 172.8.

ES$^+$ MS m/z: 295.2 $(M+H)^+$; ES$^-$ MS m/z: 293.3 $(M-H)^-$.

4.1.8. Synthesis of 3-{(2-*tert*-butoxycarbonylamino-phenyl)-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetyl]-amino}-propionic acid methyl ester (15). Secondary aniline **14** (40 mg, 0.136 mmol) and thymine derivative **8** (50 mg, 0.27 mmol) were dissolved in anhydrous DMF (500 μL). To the solution was added EDC (52 mg, 0.27 mmol) and the reaction was stirred overnight under N_2. The reaction mixture was diluted with EtOAc (5 mL) and extracted with sat. aq. $NaHCO_3$ (2×5 mL) and H_2O (1×5 mL). The organic layer was dried over $MgSO_4$ and then concentrated to dryness. The desired amide **15** was isolated as a white foam after purification by silica gel chromatography (EtOAc as eluant) in 49% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.53 (s, 9H), 1.91 (m, 3H), 2.48–2.56 (m, 1H), 2.60–2.67 (m, 1H), 3.66 (sw, 3H), 3.83–3.90 (m, 1H), 4.06–4.14 (m, 3H), 6.94 (m, 1H), 7.15–7.22 (m, 2H), 7.41–7.45 (m, 1H), 8.00 (br, 1H), 8.05 (d, 1H, J=7.2 Hz). ^{13}C NMR (67.7 MHz, DMSO-d_6) δ: 12.2, 28.2, 32.1, 44.6, 49.5, 52.0, 81.2, 110.4, 123.4, 124.7, 129.4, 130.0, 130.1, 135.7, 141.3, 151.3, 153.3, 164.8, 167.4, 172.0.

ES$^+$ MS m/z: 461.4 $(M+H)^+$; ES$^-$ MS m/z: 459.2 $(M-H)^-$.

4.1.9. Synthesis of 3-methyl-2-nitrobenzoic acid methyl ester (17). Commercially available 3-methyl-2-nitrobenzoic acid **16** (52.0 g, 287 mmol) was dissolved in 500 mL reagent grade methanol and concentrated H_2SO_4 (3 mL) was added to the solution. This mixture was heated to reflux for 24 h, cooled to RT and concentrated to 1:10 the original volume on a rotary evaporator. The mixture was diluted with EtOAc (500 mL) and washed with sat. aq. $NaHCO_3$ (2×500 mL) and H_2O (1×500 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness to give the title compound **17** in 89% yield as a pale orange solid. ^{1}H NMR (270 MHz, ($CDCl_3$) δ: 2.34 (s, 3H), 3.89 (s, 3H), 7.42–7.49 (m, 2H), 7.84 (d, 1H, J=6.0 Hz), 7.98. ^{13}C NMR (67.7 MHz, $CDCl_3$) δ: 17.3, 53.1, 123.3, 128.9, 130.1, 130.7, 135.8, 150.8, 164.1.

4.1.10. Synthesis of 3-bromomethyl-2-nitro-benzoic acid methyl ester (18). 3-Methyl-2-nitrobenzoic acid methyl ester **17** (1.00 g, 5.13 mmol) and *N*-bromosuccinimde (1.00 g, 5.64 mmol) were suspended in anhydrous CCl_4 (50 mL) and the mixture heated to reflux. The reflux condenser was then removed, benzoyl peroxide (319 mg, 1.28 mmol) quickly added and the condenser reattached. The reaction was maintained at reflux until no further progress was detected (TLC), at which time the reaction was cooled to RT. The solution was concentrated to near dryness and the resulting residue taken up in EtOAc (50 mL) and washed with a 1:1 mixture of sat. aq. $NaHCO_3$ and saturated $Na_2S_2O_3$ (50 mL) followed by H_2O (50 mL). The organic layer was dried over Na_2SO_4 and concentrated to an orange oil. The residue was purified by silica gel chromatography (elution with 5% (v/v) EtOAc/hexanes until the starting material had been collected, and then elution with 20% (v/v) EtOAc/hexanes) to give 689 mg of the bromide **18** (49%). ^{1}H NMR (400 MHz, acetone-d_6) δ: 3.89 (s, 3H), 4.43 (s, 2H), 7.56 (t, 1H, J=6.7 Hz), 7.72 (d, 1H, J=7.1 Hz), 7.94 (d, 1H, J=7.9 Hz). ^{13}C NMR (67.7 MHz, $CDCl_3$) δ: 25.8, 53.5, 124.5, 130.5, 131.0, 131.4, 135.5, 149.9, 163.6.

4.1.11. Synthesis of 3-[(*tert*-butoxycarbonylmethyl-amino)-methyl]-2-nitro-benzoic acid methyl ester (20). To a solution of glycine, *tert*-butyl ester **19** (1.02 g, 7.54 mmol) and DIPEA (0.44 mL, 2.5 mmol) in anhydrous DMF (20 mL) was added bromide **18** (689 mg, 2.51 mmol). The reaction was stirred for 3 h, after which the reaction had gone to completion. The solution was diluted with EtOAc (100 mL) and extracted with H_2O (60 mL) and sat. aq. $NaHCO_3$ (aqueous, 3×60 mL). The organic layer was dried over Na_2SO_4 and concentrated to a pale yellow oil, which was pure amine **20** by ^{1}H NMR. Yield >98%. ^{1}H NMR (300 MHz, acetone-d_6) δ: 1.45 (s, 9H), 3.25 (s, 2H), 3.86 (s, 2H), 3.87 (s, 3H), 7.71 (t, 1H, J=7.6 Hz), 7.93 (dd, 1H, J=8.0, 1.6 Hz), 7.97 (dd, 1H, J=7.6, 1.6 Hz). ^{13}C NMR (67.7 MHz, acetone-d_6) δ: 28.0, 48.4, 51.4, 53.2, 81.1, 124.4, 130.3, 131.4, 134.2, 135.4, 150.6, 164.9, 171.9.

ES$^+$ MS m/z: 324.9 $(M+H)^+$; ES$^-$ MS m/z: 323.2 $(M-H)^-$.

4.1.12. Synthesis of 3-({*tert*-butoxycarbonylmethyl-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetyl]-amino}-methyl)-2-nitro-benzoic acid methyl ester (21). Amine **20** (501 mg, 1.54 mmol), thymine derivative **8** (311 mg, 1.69 mmol) and EDC (311 mg, 1.63 mmol) were dissolved in anhydrous DMF (3 mL) and the reaction was stirred overnight. The mixture was then

diluted with EtOAc (15 mL) and extracted with H_2O (15 mL) and sat. aq. $NaHCO_3$ (3×15 mL). The organic layer was then dried over Na_2SO_4 and concentrated to dryness to give 705 mg (93%) of the desired amide **21** in an analytically pure form. ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers as determined by VT ^{1}H NMR experiments) δ: 1.37 (mi.) and 1.40 (ma.) (s, 9H), 1.74 (s, 3H), 3.83 (ma.) and 3.84 (mi.) (s, 3H), 3.87 (mi.) and 4.23 (ma.) (s, 2H), 4.50 (ma.) and 4.61 (mi.) (s, 2H), 4.57 (ma.) and 4.71 (mi.) (s, 2H), 7.34 (ma.) and 7.40 (mi.) (s, 1H), 7.70–7.96 (m, 3H), 11.30 (br, 1H). ^{13}C NMR (67.7 MHz, acetone-d_6) δ: 12.3, 28.1, 28.1, 46.8, 47.4, 48.8, 48.9, 49.2, 50.4, 53.3, 53.4, 82.1, 83.1, 109.8, 109.9, 124.6, 125.1, 130.4, 130.8, 131.0, 131.2, 131.8, 132.4, 133.7, 134.6, 142.5, 142.6, 150.2, 152.0, 164.5, 164.6, 164.9, 168.4, 168.7, 168.8, 169.5.

ES$^+$ MS *m/z*: 513.0 $(M+Na)^+$; ES$^-$ MS *m/z*: 489.1 $(M-H)^-$.

4.1.13. Synthesis of (2-*tert*-butoxycarbonylamino-benzyl-amino)-acetic acid benzyl ester (23). The benzyl ester of glycine TsOH (7.84 g, 23.22 mmol) was dissolved in warm ethanol (10 mL). NaOAc (1.59 g, 19.35 mmol) was added followed by aldehyde **22** (2.15 g, 9.67 mmol). The suspension was stirred for 30 min and this was followed by addition of $NaBH_3CN$ (0.37 g, 5.81 mmol). After a period of 7 h the solvent was evaporated and the residue partitioned between water (50 mL) and EtOAc (50 mL). The organic layer was washed with water (3×30 mL), sat. aq. $NaHCO_3$ (2×30 mL) and brine (2×30 mL). The organics were then dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (eluent: Hex-EtOAc/Hex, 1:1) to provide the required amine **23** as an oil (1.56 g, 43% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.45 (s, 9H), 3.06 (b, 1H), 3.42 (s, 2H), 3.75 (s, 2H), 5.14 (s, 2H), 7.37–7.33 (m, 5H), 7.89 (d, 1H, J=5.5 Hz), 8.18 (s, 1H), 8.32 (d, 1H, J=5.5 Hz), 9.90 (b, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.9, 48.1, 48.6, 65.6, 80.2, 112.1, 121.2, 128.0, 128.1, 128.4, 136.0, 145.7, 149.6, 149.8, 152.1, 171.7.

4.1.14. Synthesis of {(2-*tert*-butoxycarbonylamino-benzyl)-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetyl]-amino}-acetic acid benzyl ester (24). Backbone **23** (0.29 g, 0.79 mmol) and thymin-1-yl acetic acid **8** (0.31 g, 1.69 mmol) were dissolved in DMF (5 mL) and EDC (0.31 g, 1.62 mmol) was added to the solution. The reaction was stirred overnight at RT and then the solvent was evaporated and the resulting residue was partitioned between water (100 mL) and EtOAc (100 mL). The organics were then washed with sat. aq. $NaHCO_3$ (100 mL) and brine (100 mL), dried over Na_2SO_4 and evaporated to dryness, which provided monomer **24** as a pure white solid (0.33 g, 78% yield). ^{1}H NMR (300 MHz, DMSO-d_6, mixture of rotamers as determined by VT ^{1}H NMR experiments) δ: 1.40 (ma.) and 1.47 (mi.), (s, 9H), 1.72 (mi.) and 1.73 (ma.), (s, 3H), 4.10–4.78 (m, 6H), 5.03 (ma.) and 5.11 (mi.), (s, 2H), 7.22–7.43 (m, 6H), 7.62 (mi.) and 7.88 (ma.), (d, 1H, J=6 Hz), 8.33–8.40 (m, 2H), 9.07 (mi.) and 9.10 (ma.), (s, 1H), 11.31 (mi.) and 11.39 (ma.), (b, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 27.7, 28.0, 45.0, 48.0, 48.0, 66.7, 80.1, 108.2, 112.7, 119.6, 128.1, 128.2, 128.5, 135.3, 141.8, 144.6, 150.0, 151.0, 151.0, 152.2, 164.4, 168.7, 169.4.

ES$^+$ MS *m/z*: 538.3 $(M+H)^+$; ES$^-$ MS *m/z*: 536.2 $(M-H)^-$.

4.1.15. Synthesis of Boc-protected, methyl ester APNA/PNA dimer 27. Thymine derivative **15** (302 mg, 0.66 mmol) was treated with HCl in dioxane (20 M equiv.) at RT. After complete conversion of the starting material (as determined by HPLC), the solution was evaporated to dryness to give the aniline **3** as an off-white powder, which was used immediately without purification. To the flask containing aniline **3** was added Boc-PNA-Thy-OH (300 mg, 0.79 mmol), HATU (298 mg, 0.79 mmol) and HOAt (106 mg, 0.79 mmol). The flask was placed under N_2, cooled to 0 °C and charged with a solution of collidine (490 μL, 3.7 mmol) in anh. DMF (4 mL). The reaction was allowed to warm to RT and stir overnight under N_2. The reaction was diluted with EtOAc (12 mL) and extracted with sat. aq. $NaHCO_3$ (2×12 mL) and H_2O (1×12 mL). The organic layer was dried over $MgSO_4$ and concentrated to an orange residue. The product was purified by silica gel chromatography (0–10% (v/v) MeOH/CH_2Cl_2) giving dimer **27** in 47% yield. ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers as determined by VT ^{1}H NMR experiments) δ: 1.42–1.44 (m, 9H), 1.85–1.91 (m, 6H), 2.41–2.69 (m, 2H), 3.27–4.85 (m, 12H), 5.86 (br, 1H), 6.89–7.52 (m, 6H), 8.22–8.24 (ma.) and 8.37–8.39 (mi.) (m, 1H), 9.27 (ma.) and 9.57 (mi.) (s, 1H), 9.39 (ma.) and 9.86 (mi.) (br, 2H).

4.1.16. Synthesis of Fmoc protected, *tert*-butyl ester APNA/PNA dimer 28. Aniline **4** (180 mg, 0.391 mmol), Fmoc-PNA-Thy-OH (525 mg, 1.03 mmol) and EDC (190 mg, 1.00 mmol) were placed under an N_2 atmosphere in a flame dried round bottom flask equipped with a magnetic stirring bar. The reaction vessel was charged with anhydrous DMF (1.3 mL) and the reaction was stirred at RT for 30 h. The reaction was then partitioned between sat. aq. $NaHCO_3$ (5 mL) and EtOAc (5 mL). The organic layer was then washed with sat. aq. $NaHCO_3$ (2×5 mL), dried over $NaSO_4$ and concentrated to dryness. The residue was then purified by flash column chromatography (2–8% (v/v) MeOH/$CHCl_3$) to give the desired amide **28** in 30% yield (112 mg, 0.118 mmol). HPLC: Conditions B, retention time: 16.4 min, peak area: 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.33–1.35 (m, 9H), 1.70–1.74 (m, 6H), 3.13–3.49 (m, 4H), 3.71–3.76 (m, 3H), 3.82–4.69 (m, 13H), 7.21–7.74 (m, 11H), 7.86, (d, 2H, J=7.2 Hz), 9.64, 9.71, 9.84, 9.97 (s, 1H), 11.25–11.29 (m, 2H).

ES$^+$ MS *m/z*: 971.3 $(M+Na)^+$; ES$^-$ MS *m/z*: 947.3 $(M-H)^-$.

4.1.17. Synthesis of Boc-protected, benzyl ester APNA/PNA dimer 29. Boc-protected monomer **24** (0.42 g, 0.78 mmol) was dissolved in 4 M HCl in dioxane (15 mL) and was stirred for 45 min, after which TLC showed complete conversion of starting material into aniline **5**. The solvent was evaporated and the resulting residue was dissolved in DMF (3 mL). Fmoc-PNA-Thy-OH (0.44 g, 0.86 mmol), HATU (0.33 g, 0.86 mmol) and DIPEA (0.50 mL, 2.81 mmol) were added sequentially to the solution of compound **5** and the resulting solution was allowed to stir overnight at RT. The solvent was evaporated and the resulting residue was dissolved in a minimum amount of 10% (v/v) MeOH/CH_2Cl_2. EtOAc was added and

the resulting precipitate was collected by filtration. The residue was purified by flash chromatography (eluent: CH_2Cl_2/MeOH, 19:1) giving **29** as a white solid (0.32 g, 45% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.70–1.73 (m, 6H), 3.90–4.80 (m, 17H), 5.02–5.10 (m, 2H), 7.20–7.50 (m, 12H), 7.60–8.10 (m, 4H), 8.38–8.49 (m, 2H), 9.71–9.95 (m, 2H), 11.27–11.35 (m, 2H).

ES$^+$ MS *m*/*z*: 926.4 $(M+H)^+$; ES$^-$ MS *m*/*z*: 924.2 $(M-H)^-$.

4.1.18. Synthesis of Boc-protected, free acid APNA/PNA dimer 30. A solution of aqueous LiOH (4 M equiv., 0.75 M) was added to a solution of dimer **27** (142 mg, 0.19 mmol) dissolved in THF (1 mL). After 20 min of stirring, the reaction was diluted with H_2O (5 mL) and acidified to pH=3 by dropwise addition of HCl (3 M). The product was then extracted into 5% (v/v) MeOH/CH_2Cl_2 (5×10 mL) and the organic layer dried over $MgSO_4$ and concentrated to give dimer **30** as a glassy white solid (97%). ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers as determined by VT ^{1}H NMR experiments) δ: 1.34 (mi.) and 1.37 (ma.) (s, 9H), 1.67–1.69 (m, 6H), 2.36–2.53 (m, 2H), 3.02–3.48 (m, 6H), 3.85–4.74 (m, 6H), 6.76 (mi.) and 6.98 (ma.) (m, 1H), 7.17–7.49 (m, 6H) 7.83–7.85 (m, 1H), 9.45 (ma.) and 9.88 (mi.) (s, 1H), 11.28–11.31 (m, 2H).

4.1.19. Synthesis of Fmoc protected, free acid APNA/PNA dimer 31. Dimer **28** (112 mg, 0.118 mmol) was dissolved in 50% (v/v) TFA/CH_2Cl_2 (precooled to 0 °C). The reaction was stirred for 5.5 h while slowly warming to RT. The reaction was then diluted with $CHCl_3$ and evaporated to dryness. The residue was then suspended in PhMe (10 mL) and evaporated to dryness three times providing pure carboxylic acid **31**. Yield: >99%. HPLC: Conditions B, retention time 14.5 min, peak area 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.70–1.74 (m, 6H), 3.13–3.47 (m, 4H), 3.70–3.76 (m, 3H), 3.88–4.69 (m, 13H), 7.12–7.74 (m, 11H), 7.87 (d, 2H, *J*=7.2 Hz), 9.68, 9.74, 9.87, 9.99 (s, 1H), 11.25–11.29 (m, 2H).

ES$^+$ MS *m*/*z*: 893.3 (M−H)+; ES$^-$ MS *m*/*z*: 891.3 $(M-H)^-$.

4.1.20. Synthesis of Boc-protected, free acid APNA/PNA dimer 32. Benzyl ester **29** (0.30 g, 0.32 mmol) was dissolved in DMF (5 mL). Pd/C (10% by weight) (0.06 g) was added and the resulting suspension was placed under H_2 (atmospheric pressure). The suspension was allowed to stir for 4 h, after which TLC showed complete conversion of starting material. The suspension was filtered through Celite and the filtrate evaporated to give carboxylic acid **32** as an off-white solid (0.26 g, 99% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.70–1.74 (m, 6H), 3.93–4.70 (m, 17H), 7.24–8.50 (m, 13H), 9.90–10.20 (m, 2H), 11.29 (s, 2H).

ES$^+$ MS *m*/*z*: 836.3. $(M+H)^+$; ES$^-$ MS *m*/*z*: 834.3. $(M-H)^-$.

4.2. Synthesis of APNA/PNA chimeras 35–38

The APNA/PNA chimeras **36**–**37** were synthesized on a MBHA resin by solid phase peptide synthesis as previously described.[20a] Chimera **35** was synthesized using exactly the same procedure as for PNA control **33**,[20a] except monomer **2** was used in place of the third PNA monomer in the synthetic sequence. In the case of chimera **37**, an ester hydrolysis step was required after the solid phase synthesis in order to convert the ester moiety attached to the benzene ring of the APNA residue to the desired carboxylic acid. This was done in the following way: after the final cleavage and ether precipitation of the methyl ester derivative of chimera **37** (16 μmol scale synthesis), HPLC analysis of the crude pellet (37.7 mg) indicated the desired compound was 63% pure and UV analysis indicated a total crude yield of A_{266}=540.5 OD units (65%). The crude product (7.6 mg) dissolved in H_2O (200 μL) was treated with 32 μL of 1 M LiOH (aq.) and the saponification was followed by HPLC. After 360 min, the reaction was quenched by addition of one drop of AcOH and the resulting mixture immediately separated by preparative HPLC. Fractions containing pure chimera **37** were combined and lyophilized to give pure product as an amorphous white powder in 49% yield from the crude ester. Chimera **38** was synthesized on a trityl resin using dimer **32** and Fmoc-PNA-Thy-OH following established procedures.[41,42]

4.2.1. Hexamer 35. Conditions A, retention time=22.1 min (peak area 99%). FAB$^+$ MS *m*/*z*: 1848 $(M+H)^+$.

4.2.2. Hexamer 36. Conditions A, retention time=24.5 min (peak area 92%). FAB$^+$ MS *m*/*z*: 1848 $(M+H)^+$.

4.2.3. Hexamer 37. Conditions A, retention time=20.8 min (peak area 100%). ES$^+$ MS *m*/*z*: 1890.8 $(M+H)^+$, 965.7, $(M+2H)^{2+}$.

4.2.4. Hexamer 38. Conditions B, retention time=22.0 min (peak area 94%). ES$^+$ MS *m*/*z*: 1828.9 $(M+Na)^+$, 915.2, $(M+2Na)^{2+}$.

4.3. Thermal denaturation (T_m) experiments

DNA (poly dA) and RNA (poly rA) oligomers were purchased from Sigma-Aldrich Canada Ltd (Oakville, Ontario). The concentrations of the oligonucleotide solutions (poly dA, poly rA, PNA and PNA/APNA chimeras) were estimated using the appropriate molar extinction coefficients of nucleotides, calculated according to literature protocols: ε_{260} M^{-1} cm^{-1}; A 15,340; T 8700.[43] Job plots were used to confirm a 2:1 stoichiometry of binding between the synthetic oligomers and either DNA or RNA.[33]

Acknowledgements

This work was supported by grants to Y. S. T. from the Natural Sciences and Engineering Research Council of Canada and Boehringer Ingelheim (Canada) Ltd. We are grateful to Dr. Araz Jakalian, from the Computational Research Group of Boehringer Ingelheim (Canada) Ltd, for the ab initio minimizations and calculations of the backbone electrostatic potential surfaces of the APNA monomers **1**, **4** and **5**. L. D. F. thanks NSERC and the J. W. McConnell Foundation for post-graduate fellowships. E. L. M. thanks the Alexander McFee Trust Fund for a post-graduate fellowship.

References and notes

1. Reviews see: (a) Nielsen, P. E. *Acc. Chem. Res.* **1999**, *32*, 624–630. (b) Nielsen, P. E. *Curr. Opin. Struct. Biol.* **1999**, *9*, 353–357.
2. Hyrup, B.; Egholm, M.; Nielson, P. E.; Wittung, P.; Nordén, B.; Buchardt, O. *J. Am. Chem. Soc.* **1994**, *116*, 7964–7970.
3. (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1500. (b) Nielsen, P. E.; Christensen, L. *J. Am. Chem. Soc.* **1996**, *118*, 2287–2288.
4. Dias, N.; Senamaud-Beaufort, C.; le Forestier, E.; Auvin, C.; Helene, C.; Saison-Behmoaras, T. E. *J. Mol. Biol.* **2002**, *320*, 489–501.
5. Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895–1897.
6. Hanvey, J. C.; Peffer, N. J.; Bisi, J. E.; Thomson, S. A.; Cadila, R.; Josey, J. A.; Ricca, D. J.; Hassman, C. F.; Bonham, M. A.; Au, K. G.; Carter, S. G.; Bruckenstein, D. A.; Boyd, A. L.; Noble, S. A.; Babiss, L. E. *Science* **1992**, *258*, 1481–1485.
7. (a) Lansdorp, P. M.; Verwoerd, N. P.; van de Rijke, F. M.; Dragowska, V.; Little, M.-T.; Dirks, R. W.; Raap, A. K.; Tanke, H. J. *Hum. Mol. Genet.* **1996**, *5*, 685–691. (b) Orum, H.; Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O.; Stanely, C. *Nucleic Acids Res.* **1993**, *21*, 5332–5336. (c) Weiler, J.; Gausepohl, H.; Hauser, N.; Jensen, O. N.; Hoheisel, J. D. *Nucleic Acids Res.* **1997**, *25*, 2792–2799. (d) Griffin, T.; Tang, W.; Smith, L. M. *Nat. Biotechnol.* **1997**, *15*, 1368–1372.
8. Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 2796–2823.
9. (a) Kuwahara, M.; Arimitsu, M.; Shigeyasu, M.; Saeki, N.; Sisido, M. *J. Am. Chem. Soc.* **2001**, *123*, 4653–4658. (b) Kuwahara, M.; Arimitsu, M.; Sisido, M. *J. Am. Chem. Soc.* **1999**, *121*, 256–257.
10. (a) Peyman, A.; Uhlmann, E.; Wagner, K.; Augustin, S.; Weiser, C.; Will, D. W.; Breipohl, G. *Angew. Chem. Int. Ed.* **1997**, *36*, 2809–2812. (b) Efimov, V. A.; Choob, M. V.; Buryakova, A. A.; Kalinkina, A. L.; Chakhmakhcheva, O. G. *Nucleic Acids Res.* **1998**, *26*, 566–575.
11. (a) Sforza, S.; Haaima, G.; Marchelli, R.; Nielsen, P. E. *Eur. J. Org. Chem.* **1999**, 197–204. (b) Haaima, G.; Lohse, A.; Buchardt, O.; Nielsen, P. E. *Angew. Chem. Int. Ed.* **1996**, *35*, 1939–1941. (c) Dueholm, K. L.; Petersen, K. H.; Jensen, D. K.; Egholm, M.; Nielsen, P. E.; Buchardt, O. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1077–1080.
12. (a) Aldrian-Herrada, G.; Desarmenien, M. G.; Orcel, H.; Boissin-Agasse, L.; Mery, J.; Brugidou, J.; Rabie, A. *Nucleic Acids Res.* **1998**, *26*, 4910–4916. (b) Tyler, B. M.; McCormick, D. J.; Hoshall, C. V.; Douglas, C. L.; Jansen, K.; Lacy, B. W.; Cusack, B.; Richelson, E. *FEBS Lett.* **1998**, *421*, 280–284. (c) Rezaei, K.; Xu, I. S.; Wu, W.-P.; Shi, T.-J.; Soomets, U.; Land, T.; Xu, X.-J.; Wiesenfeld-Hallin, Z.; Hokfelt, T.; Bartfai, T.; Langel, U. *NeuroReport* **2001**, *12*, 317–320.
13. (a) Good, L.; Nielsen, P. E. *Nat. Biotechnol.* **1998**, *16*, 355–358. (b) Good, L.; Nielsen, P. E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2073–2076.
14. (a) Bickel, U.; Yoshikawa, T.; Landaw, E. M.; Faull, K. F.; Pardrige, W. M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2618–2622. (b) Pardridge, W. M.; Boado, R. J.; Kang, Y.-S. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5592–5596.
15. Mier, W.; Eritja, R.; Mohammed, A.; Haberkorn, U.; Eisenhut, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 1968–1971.
16. Bauer, W.; Briner, U.; Doepfner, W.; Haller, R.; Hugnuenin, R.; Marbach, P.; Petcher, T. J.; Pless, J. *Life Sci.* **1982**, *31*, 1133–1140.
17. Zhou, P.; Wang, M.; Du, L.; Fisher, G. W.; Waggoner, A.; Ly, D. H. *J. Am. Chem. Soc.* **2003**, *125*, 6878–6879.
18. Wender, P. A.; Mitchell, D. J.; Pattabiraman, K.; Pelkey, E. T.; Steinman, L.; Rothbard, J. B. *Proc. Natl. Acad. Sci.* **2000**, *97*, 13003–13008.
19. Tsantrizos, Y. S.; Lunetta, J. F.; Boyd, M.; Fader, L. D.; Wilson, M.-C. *J. Org. Chem.* **1997**, *62*, 5451–5457.
20. (a) Fader, L. D.; Boyd, M.; Tsantrizos, Y. S. *J. Org. Chem.* **2001**, *66*, 3372–3379. (b) Fader, L. D.; Tsantrizos, Y. S. *Org. Lett.* **2002**, *4*, 63–66.
21. (a) Puschl, A.; Boesen, T.; Zuccarello, G.; Dahl, O.; Pitsch, S.; Nielsen, P. E. *J. Org. Chem.* **2001**, *66*, 707–712. (b) Pueschl, A.; Tedeschi, T.; Nielsen, P. E. *Org. Lett.* **2000**, *2*, 4161–4163. (c) D'Costa, M.; Kumar, V.; Ganesh, K. N. *Org. Lett.* **2001**, *3*, 1269–1272. (d) Vilaivan, T.; Suparpprom, C.; Harnyuttanakorn, P.; Lowe, G. *Tetrahedron Lett.* **2001**, *42*, 5533–5536. (e) Sforza, S.; Haaima, G.; Marchelli, R.; Nielsen, P. E. *Eur. J. Org. Chem.* **1999**, 197–204.
22. (a) Almarsson, O.; Bruice, T. C.; Kerr, J.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7518–7522. (b) Almarsson, O.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9542–9546.
23. Hyrup, B.; Egholm, M.; Buchardt, O.; Nielsen, P. E. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1083–1088.
24. (a) Schutz, R.; Cantin, M.; Roberts, C.; Greiner, B.; Uhlmann, E.; Leumann, C. *Angew. Chem. Int. Ed.* **2000**, *39*, 1250–1253. (b) Hollenstein, M.; Leumann, C. *J. Org. Lett.* **2003**, *5*, 1987–1990.
25. (a) Leijon, A.; Graslund, A.; Nielsen, P. E.; Buchardt, O.; Norden, B.; Kristensen, M.; Eriksson, M. *Biochemistry* **1994**, *33*, 9820–9825. (b) Betts, L.; Josey, J. A.; Veal, J. M.; Jordan, S. R. *Science* **1995**, *270*, 1838–1841.
26. Brown, S. C.; Thomson, S. A.; Veal, J. M.; Davis, D. G. *Science* **1994**, *265*, 777–780.
27. Rasmussen, H.; Kastrup, J. S.; Nielsen, J. N.; Nielsen, J. M.; Nielsen, P. E. *Nat. Struct. Biol.* **1997**, *4*, 98–101.
28. Manea, V. P.; Wilson, K. J.; Cable, J. R. *J. Am. Chem. Soc.* **1997**, 2033–2039, and references cited therein.
29. Seto, C. T.; Mathias, J. P.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 1321–1329.
30. Venuti, M. C.; Stephenson, R. A.; Alvarez, R.; Bruno, J. J.; Strosberg, A. M. *J. Med. Chem.* **1988**, *31*, 2136–2145.
31. Compound **26** was prepared by coupling the known adenine derivative $Ad(Cbz)CH_2CO_2H$ to the backbone moiety **7** using the same protocol as for the coupling of the Thy-acetic acid **8** with **7** (Scheme 1). Analytical data of compound **26**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44 (s, 9H), 3.92 (dd, 1H, J=14.4, 7.2 Hz), 4.25–4.62 (m, 4H), 4.91 (d, 1H, J=16.8 Hz), 5.12 (m, 1H), 5.18 (m, 1H), 5.30 (s, 2H), 5.77–5.93 (m, 1H), 6.64 (br, 1H), 7.22–7.53 (m, 4H), 7.71 (d, 1H, J=7.2 Hz), 8.29 (br, 1H), 8.79 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.5, 14.6, 21.3, 28.6, 49.1, 50.7, 60.3, 80.1, 108.4, 118.7, 125.8, 126.2, 129.6, 130.8, 133.2, 133.2, 135.9, 142.5, 151.6, 154.0, 164.9, 167.2, 170.8. ES^+ MS *m/z*: 572.3 $(M+H)^+$; ES^- MS *m/z*: 570.3 $(M-H)^-$.
32. (a) Christensen, L.; Fitzpatrick, R.; Gildea, B.; Petersen, K. H.; Hansen, H. F.; Koch, T.; Egholm, M.; Buchardt, O.; Nielsen, P. E.; Coull, J.; Berg, R. H. *J. Pept. Sci.* **1995**, *1*, 175–183. (b) Koch, T.; Hansen, H. F.; Andersen, P.; Larsen, T.; Batz, H. G.; Otteson, K.; Oerum, H. *J. Pept. Res.* **1997**, *49*, 80–88.

33. Job plots were used to confirm the 2:1 stoichiometry of the complexes formed as previously described: (a) Ref. 19. (b) Job, P. *Ann. Chim. (Paris)* **1928**, *9*, 113–203.
34. Ab inition minimization (at the HF/6-31G** level of theory) was carried out on model compounds corresponding to the core backbone structure of monomers **1**, **4** and **5**, respectively. These calculations provided unambiguous evidence of the order of polarity of these analogs (most polar **5**≫**4**>**1** least polar); a significant increase in polarity is expected to correlate with an increase in the solubility of that compound in aqueous media. In addition, we observed the anticipated differences in the retention time for each compound on a C18 reversed phase HPLC column. Finally, although the exact solubility of each compound was not determined, qualitatively it was clearly apparent that the new APNA monomers **4** and **5** were more soluble in polar solvents than the first generation analog **1**.
35. (a) Haaima, G.; Lohse, A.; Buchardt, O.; Nielsen, P. E. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1939–1941. (b) Püschl, A.; Sforza, S.; Haaima, G.; Dahl, O.; Nielsen, P. E. *Tetrahedron Lett.* **1998**, *39*, 4707–4710.
36. For a recent review on the importance of aromatic interactions in molecular recognition, including in a biological context see: Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210–1250.
37. Burley, S. K.; Petsko, G. A. *Adv. Protein Chem.* **1988**, *39*, 125–189.
38. For a recent review on this topic, see: Kool, E. T. *Ann. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 1–22.
39. For reviews on the RNA recognition of proteins involved in RNA processing, see: (a) Varani, G.; Nagai, K. *Ann. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 407–445. (b) Krecic, A. M.; Swanson, M. S. *Curr. Opin. Cell Biol.* **1999**, *11*, 363–371.
40. For a recent review on π-cation interactions in small molecule-biomolecule recognition see: (a) Zacharias, N.; Dougherty, D. A. *Trends Pharmacol. Sci.* **2002**, *23*, 281–287. (b) See also Ref. 34.
41. For synthesis of PNA on a Trityl-based resin see: Thomson, S. A.; Josey, J. A.; Cadilla, R.; Gaul, M. D.; Hassman, C. F.; Luzzio, M. J.; Pipe, A. J.; Reed, K. L.; Ricca, D. J.; Wiethe, R. W.; Noble, S. A. *Tetrahedron* **1995**, *51*, 6179–6194.
42. Modifications to the synthesis cycles for Fmoc-synthesis of PNA oligomers were as previously described. See Ref. 20b.
43. Gray, D. M.; Hung, S.-H.; Johnson, K. H. *Methods in enzymology*; Academic: New York, 1995; Vol. 246. pp 19–34.