

# Synthesis and properties of DNA–PNA chimeric oligomers

Patrick J. Finn, Neil J. Gibson<sup>1</sup>, Rachel Fallon<sup>2</sup>, Alan Hamilton<sup>2</sup> and Tom Brown\*

Department of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, UK, <sup>1</sup>Department of Chemistry, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JJ, UK and <sup>2</sup>Amersham International Plc, Amersham Laboratories, White Lion Road, Amersham, Bucks HP7 9LL, UK

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## ABSTRACT

Adenine, thymine and cytosine PNA monomers have been prepared using 3-amino-1,2-propanediol as a starting material. The benzoyl group was used to protect the exocyclic amines of the heterocyclic bases of A and C PNA monomers and the backbone primary amine was protected with the monomethoxytrityl group. The thymine and cytosine PNA monomers were used in conjunction with standard DNA synthesis monomers to produce chimeric PNA DNA (PDC) oligomers. Ultraviolet melting studies confirmed that these oligomers form stable hybrids with complementary DNA strands and that mismatches in the DNA but more so in the PNA sections lead to duplex destabilisation.

## INTRODUCTION

Since the first report of the synthesis of peptide nucleic acids (PNAs) (1) there have been many reports describing the binding of PNA to DNA and to RNA (2), the biological applications of PNA (3–6) and structural variations of the basic PNA structure (Fig. 1) including the use of elongated (7,8) and chirally modified backbones (9,10). Here we describe the synthesis of PNA monomers which are compatible with solid-phase DNA synthesis and the assembly on solid-phase of DNA–PNA chimeras (Fig. 2) which have potential applications in biology.

PNAs are uncharged at physiological pH and one of the major drawbacks to their use is their poor water solubility. However the lack of backbone charge also gives rise to unusual hybridisation behaviour which is potentially of great value in molecular biology. There are no electrostatic interstrand repulsions when PNAs hybridise with nucleic acids and this leads to higher thermodynamic stability than structurally analogous DNA–DNA or RNA–DNA hybrids (11). In addition, in contrast to DNA–DNA hybrids, PNA–DNA hybrids are stable under low salt conditions and it has been shown that PNA and DNA strands have a faster rate of binding relative to DNA–DNA strands (12).

It is possible that PNA–DNA chimeric oligomers in which both types of monomeric unit are present in a single chain might combine the favourable hybridisation characteristics of PNA with the high water solubility of DNA. Moreover, the structure of such

molecules, if it bears a resemblance to DNA, might be compatible with some enzyme catalysed reactions. Such compounds could therefore be of use in PCR, DNA sequencing, antisense inhibition studies and other technologies.

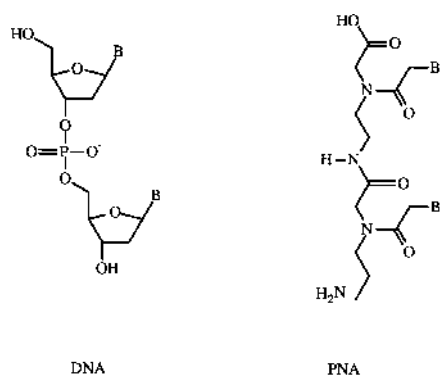
## RESULTS AND DISCUSSION

### Synthesis of PNA monomers

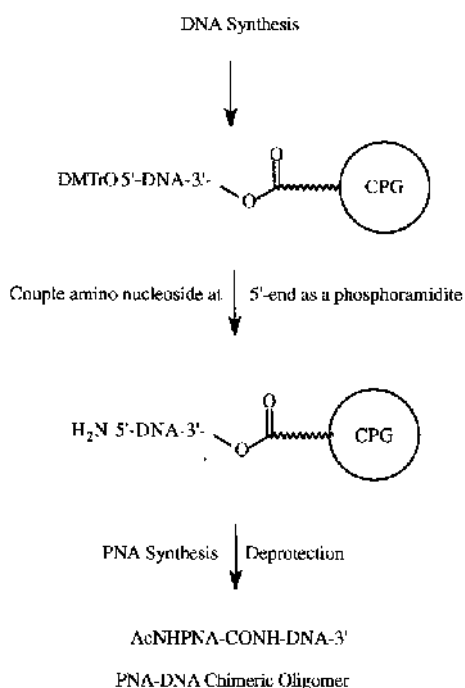
Our strategy to produce such molecules was to assemble a DNA chain on a solid support using standard phosphoramidite coupling chemistry and then to couple the PNA monomers in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Fig. 2). In order to achieve this objective we prepared PNA monomers with the same protection chemistry as conventional DNA phosphoramidites. From the outset it was clear that *t*-butyloxycarbonyl (boc) protection of the PNA N-terminus was not desirable since the TFA used in boc-deprotection is not compatible with oligodeoxynucleotides which depurinate rapidly in acidic media. 9-Fluorenylmethoxycarbonyl (Fmoc) protection was equally unsuitable because its removal with base would lead to cleavage of the oligonucleotide from the solid support. Bergmann *et al.* have shown that these problems can be overcome (13) but in the interests of simplicity and convenience we opted to use the monomethoxytrityl group for N-terminus protection. We adopted a similar approach to that used by Will *et al.* in PNA synthesis (14) and by van der Laan *et al.* (15) and Stetsenko *et al.* (16) in the synthesis of oligo dT and U/dT PNA–DNA hybrids. Benzyloxycarbonyl protection of the exocyclic amino groups of the A and C monomers has been used extensively in PNA synthesis but is not suitable for PNA–DNA chimeras since the hydrogenolysis or acidolysis required to remove this group would add a further deprotection step which could damage the DNA portion of the chimera. Therefore the PNA bases were protected with the benzoyl group, the same as the bases of the corresponding DNA phosphoramidites.

The *N*(2-aminoethyl)glycine backbone of PNA was prepared originally by alkylation of ethylene diamine with chloroacetic acid (17). Related monomers have been made by utilising the Michael addition of mono-boc ethylenediamine to ethyl acrylate (18). We found the most reliable method to be that shown in Scheme 1. Boc protection of 3-aminopropane-1,2-diol was

\* To whom correspondence should be addressed



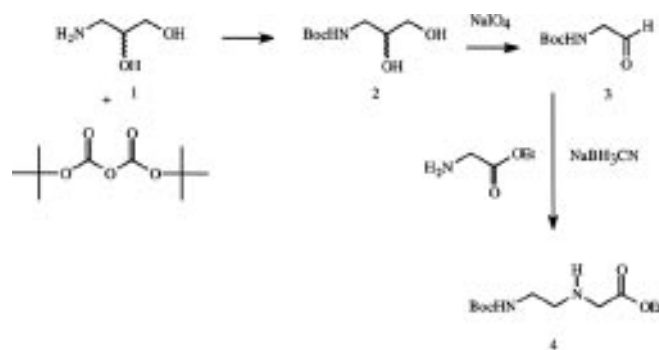
**Figure 1.** The structures of DNA and PNA oligomers.



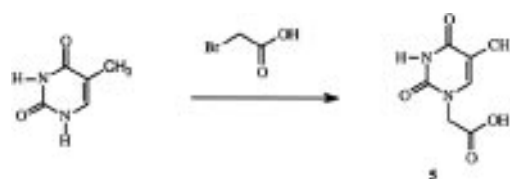
**Figure 2.** Strategy for the preparation of PNA–DNA chimeric molecules.

preferred to monomethoxytrityl protection at this early stage due to the higher stability of this group in subsequent reaction steps. 3-Amino-1,2-propanediol **1** was treated with boc anhydride to produce 3-*t*-butoxycarbonylamino-1,2-propanediol **2** in quantitative yield. Periodate cleavage of the vicinal diol **2** afforded the protected amino aldehyde **3** in high yield, reductive amination of which with sodium cyanoborohydride and ethyl glycinate gave ethyl *N*(2-bocaminoethyl)glycinate **4** in 43% yield. The use of sodium borohydride during the reductive amination was not successful as it resulted in only 24% conversion of **3** to **4** and also led to the formation of several impurities including *t*-butyloxycarbonyl aminoethanol from the reduction of aldehyde **3**.

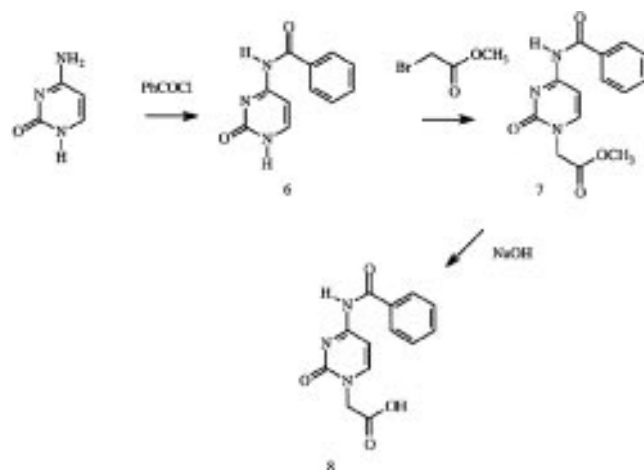
The heterocyclic bases were coupled to an acetic acid moiety for subsequent formation of the PNA monomers as follows: thymine *N*(1)-acetic acid **5** was prepared (Scheme 2) by treating thymine with aqueous bromoacetic acid and sodium hydroxide (**6**). The 4-amino group of cytosine (Scheme 3) was protected by



**Scheme 1.**

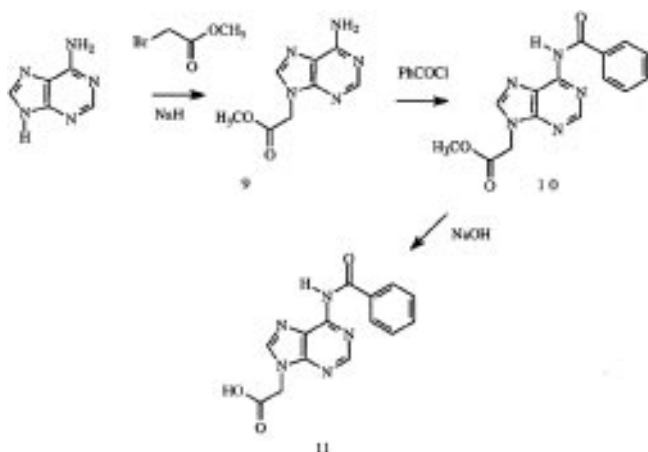


**Scheme 2.**

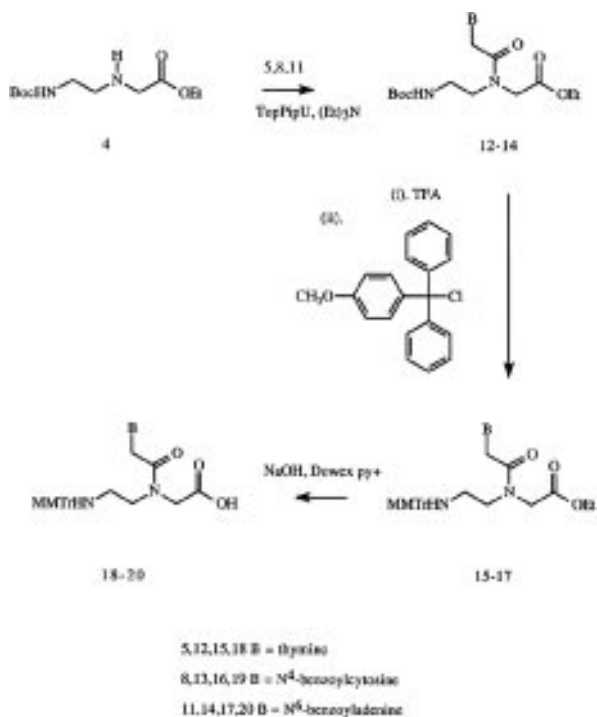


**Scheme 3.**

reaction with benzoyl chloride to give *N*(4)-benzoylcytosine **6** which was alkylated at *N*(1) with methyl bromoacetate in DMF (**6**) to give *N*(4)-benzoylcytosine *N*(1)-acetic acid methyl ester **7**. Selective hydrolysis of **7** with aqueous NaOH at room temperature gave *N*(4)-benzoylcytosine *N*(1)-acetic acid **8**. Adenine was alkylated at *N*(9) by treatment with sodium hydride in DMF followed by addition of methyl bromoacetate to give *N*(9)-adenine acetic acid methyl ester **9** (Scheme 4) according to established procedures (**19**). There was no evidence of alkylation at adenine *N*(7) either by t.l.c. or <sup>1</sup>H NMR analysis. After recrystallisation of ester **9** from H<sub>2</sub>O/EtOH, the 6-amino group was protected by treatment with a slight excess of benzoyl chloride in pyridine to afford the mono-benzoylated amine **10** which was hydrolysed with aqueous sodium hydroxide at room temperature to afford the acid **11**.



Scheme 4.



Scheme 5.

The methodology for adding the heterocyclic bases to the PNA monomeric backbone is shown in Scheme 5. The amine **4** was condensed with acids **5**, **8** and **11** respectively to give the boc protected PNA monomer esters **12-14**. The coupling yields were in the range 30–40% when DCC/HOBT were used to activate the acids. The low yield may be a consequence of the insolubility of the acids in DMF and steric hindrance inhibiting the formation of a tertiary amide. The use of PyBroP (**20**) to activate the acids resulted in ~60% yields of **12-14** while optimum yields of 70–80% were achieved by the use of TopPipU, a recently introduced reagent for the preparation of sterically hindered amides (**21**).

Intermediates **12-14** were deprotected with TFA to give the free amines which were alkylated with monomethoxytrityl chloride to give the MMTr protected amines **15-17**. These were then treated with cold aqueous sodium hydroxide and the acids **18-20** were isolated following neutralisation with pyridinium DOWEX.

### Synthesis of PNA–DNA chimeric oligomers (PDC oligomers)

The PDC oligomers were assembled with the DNA section at the 3′-end and the PNA section at the 5′-end (N-terminus, Fig. 2). In principle this would allow for template directed extension of the DNA chain in subsequent enzymic reactions. Previously a modified PNA thymine monomer bearing a dimethoxytrityl-oxethyl group has been used to couple PNA thymine units to the ‘3′-end’ of DNA (**22**) (the opposite orientation). In our work DNA sections were synthesised using standard phosphoramidite chemistry and the modified nucleoside 5′-(4-methoxytrityl)amino-5′-deoxy-thymidine phosphoramidite (**23**) was added as a linker between PNA and the 5′-end of the DNA chain. The PNA monomers were added using TopPipU as activating agent and deprotection of the monomethoxytrityl terminal amino functions prior to each PNA coupling step was carried out using TCA (3% w/v in dichloromethane). Capping of unreacted amino groups was achieved using acetic anhydride in pyridine. Solution and solid-phase reactions showed that the adenine PNA monomer failed to couple efficiently under these conditions and this is the subject of continuing work. In the present study only cytosine and thymine PNA monomers were used for oligomer synthesis. The chimeric oligomers were cleaved from the solid support and the exocyclic amino protecting groups were removed by heating the resin in concentrated aqueous ammonia at 55°C for 6 h. The crude products were purified by reversed-phase HPLC, desalted by Sephadex gel filtration and analysed by capillary electrophoresis (Figs 3 and 4). This single purification protocol gave oligomers with only minor levels of impurities.

### Ultraviolet melting studies

Ultraviolet melting studies (Table 1; **24**) of the PDC sequences Ac-tttctTGCCAT-3′ and Ac-tttttTGCCAT-3′ (PDC = PNA–DNA chimera, Ac = acetyl on the amino group of the terminal PNA residue, DNA residues in upper case, PNA residues in lower case) with their DNA complements indicate increased stability of the PDC–DNA duplex with respect to the native DNA duplexes (an increase in  $T_m$  of 10.2°C in the former and 7.7°C in the latter case). The inter-strand interaction between the chimera and the complementary DNA strand was shown to occur in a sequence specific manner. In the case of Ac-tttttTGCCAT-3′ a G-t mismatch in the centre of the PNA part of the chimeric strand opposite the DNA strand gave a drop in  $T_m$  of 16.2°C whereas a C-C mismatch in the DNA part of the chimera gave a drop in  $T_m$  of only 2.8°C. This suggests that the duplex is stabilised much more by PNA–DNA interactions than DNA–DNA interactions and that mismatches in the PNA section are very destabilising. This was confirmed with the sequence Ac-tttctTGCCAT-3′ in which a PNA–DNA A.c mismatch caused a massive drop in  $T_m$  of 37.3°C whereas a C-C mismatch involving the DNA portion of the chimera caused a drop in  $T_m$  of only 1.9°C.

### CONCLUSION

We have demonstrated that PNA–DNA chimeric oligomers can be synthesised in a stepwise fashion using PNA monomers which are compatible with DNA synthesis and we have confirmed that the chimeras hybridise specifically to complementary DNA. The PDC oligomers form more stable complexes with complementary DNA than do the equivalent DNA sequences.

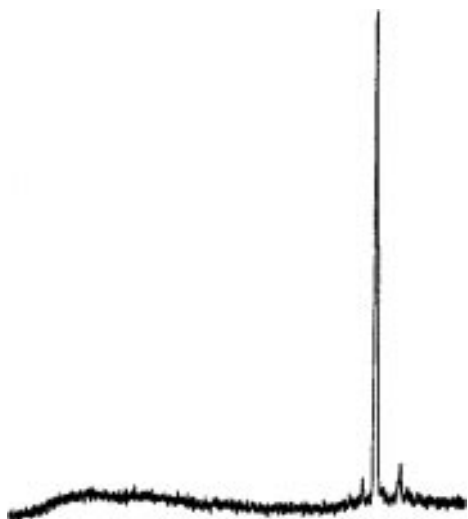


Figure 3. Capillary electropherogram of purified Ac-tttctTGCCAT-3'

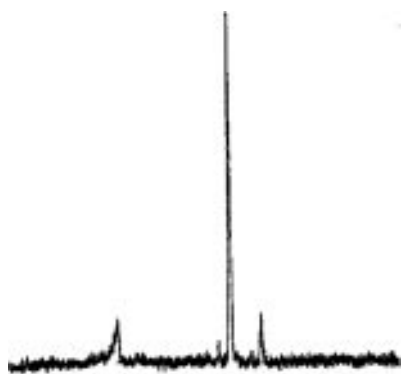


Figure 4. Capillary electropherogram of purified Ac-tttttTGCCAT-3'

## EXPERIMENTAL

All solvents were of analytical grade; dichloromethane (DCM), diethylcyclohexylamine (DECHA) and pyridine were distilled over CaH<sub>2</sub>; methanol (MeOH) was distilled over Mg and I<sub>2</sub>; tetrahydrofuran (THF) was distilled over sodium and benzophenone, *N,N*-dimethylformamide (DMF) was of peptide synthesis grade and DNA synthesis grade acetonitrile was purchased from Perkin-Elmer Ltd. TopPipU was obtained from Novabiochem. All other chemicals were supplied by Aldrich, Sigma or Fluka.

<sup>1</sup>H and <sup>13</sup>C NMR were recorded on Bruker 250AC and Bruker 200WP spectrometers. Positive ion Fast Atom Bombardment (FAB) mass spectra were recorded on a Kratos MS50TC spectrometer using a thioglycerol matrix, chemical ionisation (C.I.) mass spectra were recorded on a VG analytical 70-250 SE Normal Geometry Double Focus Mass Spectrometer and electro spray mass spectra were recorded on a VG Biotech Platform.

Flash chromatography was carried out using silica gel 60 (Fluka). Thin layer chromatography (TLC) was carried out on aluminium sheets, silica 60 F<sub>254</sub>, 0.2 mm layer (Merck) using the following solvent systems; A = *n*-butyl alcohol/acetic acid/H<sub>2</sub>O (3:1:1 v:v),

Table 1. Ultraviolet melting temperatures of PDC-DNA and DNA-DNA duplexes

Hybrid	Mismatch	duplex	T <sub>m</sub> /K
Ac-tttctTGCCAT 3/5' ATGGCAAAGAAA 3'	none	PDC/DNA	328.0
5' TTTCTTTGCCAT 3/5' ATGGCAAAGAAA 3'	none	DNA/DNA	317.8
Ac-tttctTGCCAT 3/5' ATGGCAAAGAAA 3'	A.c	PDC/DNA	290.7
Ac-tttctTGCCAT 3/5' ATGGCAAAGAAA 3'	C.C	PDC/DNA	326.1
Ac-tttctTGCCAT 3/5' ATGGCAAAAAA 3'	none	PDC/DNA	322.9
5' TTTTCTTGCCAT 3/5' ATGGCAAAAAA 3'	none	DNA/DNA	315.2
Ac-tttctTGCCAT 3/5' ATGGCAAAGAAA 3'	G.i	PDC/DNA	306.7
Ac-tttctTGCCAT 3/5' ATGGCAAAAAA 3'	C.C	PDC/DNA	320.1

DNA units in upper case, PNA units in lower case. Ultraviolet melting temperatures (*T<sub>m</sub>*) were determined at 260 nm with a heating rate of 0.5 K/min. Oligonucleotides were dissolved in a buffer consisting of 0.14 M NaCl and 10 mM HEPES adjusted to pH 7.0.

B = DCM/ethyl acetate (1:1 v/v), C = DCM/MeOH (9/1 v/v). Products were visualised using ninhydrin (1% w/v in EtOH) with heating for 5 min, UV irradiation at 254 nm, iodine oxidation or phosphomolybdic acid (10% w/v in water) with heating.

### 3-*t*-Butoxycarbonylamino-1,2-propanediol 2

To an ice-cooled solution of racemic 3-amino-1,2-propanediol (10 g, 0.11 mol) in water (250 ml) was added boc anhydride (25 g, 0.12 mol). The mixture was allowed to warm to room temperature and the pH was maintained at 10.5 by the addition of aqueous NaOH (2N). The solution was concentrated to a paste which was triturated with DCM (500 ml). The suspension was filtered and the organic phase was dried (MgSO<sub>4</sub>) then evaporated to give the diol **2** (20.4 g, 97%) as an oil which solidified upon freezing. *R<sub>f</sub>* 0.75 (A), 0.60 (B), 0.70 (C). δ<sub>H</sub> (CDCl<sub>3</sub>); 1.39 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.15 (m, unresolved, 2H, NCH<sub>2</sub>), 3.52 (m, unresolved, 2H, HOCH<sub>2</sub>), 3.68 (m, unresolved, 1H, CH), 4.75 (s, 2H, 2xOH), 5.52 (t, 1H, J = 5.4 Hz, NH). *m/z* = 192.1232 (M<sup>+</sup> + H) [C<sub>8</sub>H<sub>18</sub>NO<sub>4</sub> requires 192.1236].

### *t*-Butoxycarbonylaminoacetaldehyde 3

To diol **2** (5 g, 26.1 mmol) in water (50 ml) was added NaIO<sub>4</sub> (6.8 g, 1.2 eq.) at room temperature with stirring. After 3 h the mixture was filtered, extracted with DCM (5 × 100 ml) and the combined organic phases were dried (MgSO<sub>4</sub>) and concentrated to yield the aldehyde **3** (3.9 g, 94%) as an oil. *R<sub>f</sub>* 0.70 (A), 0.15 (B), 0.50 (C); δ<sub>H</sub> (CDCl<sub>3</sub>); 1.38 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.98 (s, 2H, CH<sub>2</sub>CHO), 5.33 (b, 1H, NH), 9.56 (s, 1H, CHO). *m/z* (c.i.) = 160.0964 (M<sup>+</sup> + H) [C<sub>7</sub>H<sub>14</sub>NO<sub>3</sub> requires 160.0974].

### Ethyl-*N*-[2-(*t*-butoxycarbonylamino)ethyl] glycinate 4

To *t*-butoxycarbonylaminoacetaldehyde **3** (5.13 g, 32.2 mmol) in MeOH (100 ml) was added ethyl glycinate hydrochloride (11.2 g, 2.5 eq.) and NaBH<sub>3</sub>CN (2.02 g, 1 eq.) with stirring at room temperature. After 16 h the mixture was evaporated, the residue dissolved in water (100 ml) and the pH adjusted to 8.0 by the addition of aqueous NaOH (2 M). The solution was extracted with DCM (5 × 150 ml) and the combined organic fractions were dried (MgSO<sub>4</sub>) and evaporated to give **4** as a colourless oil (3.42 g, 43%)

after flash silica column chromatography (DCM + 0–4% MeOH).  $R_f$  0.55 (A), 0.05 (B), 0.15 (C),  $\delta_H$  (CDCl<sub>3</sub>); 1.22 (t, 3H, J = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.38 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 2.68 (t, 2H, J = 5.8 Hz, BocHNCH<sub>2</sub>), 3.15 (t, 2H, J = 5.8 Hz, CH<sub>2</sub>CH<sub>2</sub>NH), 3.34 (s, 2H, CH<sub>2</sub>CO<sub>2</sub>Et), 4.12 (q, 2H, J = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 5.46 (b, 1H, BocHN).  $m/z$  = 247.1648 (M<sup>+</sup> + H) [C<sub>11</sub>H<sub>23</sub>N<sub>2</sub>O<sub>4</sub> requires 247.1658].

#### Thymine-1-acetic acid 5

To a solution of thymine (10.0 g, 79.3 mmol) and KOH (17.1 g, 3.8 eq.) in water (50 ml) was added dropwise, with stirring, bromoacetic acid (16.5 g, 1.5 eq.) in water (25 ml) at 40°C over 30 min. After 2 h the solution was cooled to room temperature and the pH was adjusted to 5.5 by the addition of conc. HCl. A precipitate was removed after 2 h at –4°C and the pH of the filtrate was adjusted to 2.0 (conc. HCl). The acid **5** (12.5 g, 86%) was collected and dried over P<sub>2</sub>O<sub>5</sub>.  $R_f$  0.10 (A),  $\delta_H$  (d<sub>6</sub>-DMSO) 1.74 (s, 3H, Thy-CH<sub>3</sub>), 4.36 (s, 2H, CH<sub>2</sub>CO<sub>2</sub>H), 7.48 (s, 1H, Thy-H<sub>6</sub>), 11.4 (s, 1H, NH).  $m/z$  (e.i.) = 184.0479 (M<sup>+</sup>) [C<sub>7</sub>H<sub>8</sub>N<sub>2</sub>O<sub>4</sub> requires 184.0484].

#### N(4)-benzoylcytosine 6

Cytosine (10.0 g, 0.09 mol) was suspended in pyridine (750 ml) and benzoyl chloride (21.0 ml, 2 eq.) added dropwise with stirring over 3 h. The reaction mixture was stirred at room temperature for a further 1 h after which the pH was adjusted to 5.0 by the addition of 4 N HCl. The resulting suspension was stirred at room temperature for a further 2 h and the precipitate collected, washed with EtOH (3 × 100 ml) and dried *in vacuo* over P<sub>2</sub>O<sub>5</sub> to give the title amide (12.0 g, 62%). The product was only sparingly soluble in organic solvents and water and therefore no NMR data were obtained.  $m/z$  (f.a.b.) = 216.0778 (M<sup>+</sup> + H) [C<sub>11</sub>H<sub>10</sub>N<sub>3</sub>O<sub>2</sub> requires 216.0773].

#### N(4)-Benzoyl cytosine-N(1)-acetic acid 8

N(4)-benzoylcytosine **6** (5.00 g, 23 mmol) and potassium carbonate (3.20 g, 1 eq.) were suspended in DMF (100 ml) and methyl bromoacetate (2.24 ml, 1 eq.) was added dropwise with stirring over 3 h. Stirring was continued at room temperature for 16 h then the suspension filtered. The filtrate was evaporated to dryness, water (75 ml) and HCl (4N, 2.5 ml) added and the precipitate collected and suspended in water (40 ml). NaOH (2 N, 30 ml) was added and the solution was stirred at room temperature for 30 min then filtered. To the filtrate was added conc. HCl (5 ml), and the precipitate was collected and dried *in vacuo* to afford the title acid (2.80 g, 45%).  $R_f$  0.13 (A), 0.05 (C);  $\delta_H$  (d<sub>6</sub>-DMSO) 4.66 (s, 2H, NCH<sub>2</sub>CO<sub>2</sub>H), 7.40 (d, 1H, J = 7.4 Hz, Cyt-H<sub>5</sub>), 7.51–7.73 (m, unresolved, 3H, *m+p*Ar-H), 8.06 (d, 2H, J = 7.4 Hz, *o*Ar-H), 8.31 (d, 1H, J = 7.4 Hz, Cyt-H<sub>6</sub>), 9.5–10.0 (broad, 1H, CO<sub>2</sub>H).  $m/z$  (f.a.b.) = 274.0837 (M<sup>+</sup> + H) (C<sub>13</sub>H<sub>12</sub>N<sub>3</sub>O<sub>4</sub> requires 274.0827).

#### Adenine-N(9)-acetic acid methyl ester 9

A 60% dispersion of sodium hydride in paraffin, (3.26 g, 0.82 mol) was washed with hexane (3 × 100 ml) then suspended in DMF (250 ml) at 0°C. To this was added adenine (10.0 g, 0.74 mol) slowly with stirring. On completion of addition the reaction mixture was warmed to room temperature and stirred for 2 h before methyl bromoacetate (12.9 ml, 1.8 eq.) was added over 3

h with continual stirring. The reaction mixture was stirred at room temperature for a further 16 h then the solution was concentrated to a paste. Water (100 ml) was added and the precipitate collected and recrystallised from EtOH/water (3:2 v/v, 120 ml), to give the title ester (11.4 g, 75%).  $R_f$  0.55 (A), 0.12 (C).  $\delta_H$  (d<sub>6</sub>-DMSO) 3.68 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 5.09 (s, 2H, CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 7.93 (s, 2H, NH<sub>2</sub>), 8.12 (s, 1H, Ade-H<sub>2</sub>), 8.14 (s, 1H, Ade-H<sub>8</sub>).  $m/z$  (f.a.b.) = 208.0838 (M<sup>+</sup> + H) requires 208.0834).

#### N(6)-Benzoyladenine-N(9)-acetic acid methyl ester 10

Compound **9** (5.00 g, 0.024 mol) was coevaporated with pyridine (3 × 10 ml) then redissolved in pyridine (50 ml). Benzoyl chloride (3.1 ml, 1.1 eq.) in pyridine (50 ml) was added dropwise over 3 h at room temperature and stirring was continued for 16 h. Water (100 ml) was added then the reaction mixture was extracted with DCM (3 × 100 ml). The combined extracts were washed with NaHCO<sub>3</sub> (0.5N, 3 × 100 ml), NaCl (sat., 100 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), then evaporated to an oily residue. Purification by flash column chromatography [SiO<sub>2</sub>, DCM/MeOH (0–6%)] yielded **10** (4.71 g, 64%) as a white solid.  $R_f$  0.61 (A), 0.10 (B), 0.25 (C).  $\delta_H$  (CDCl<sub>3</sub>); 3.76 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 5.04 (s, 2H, CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 7.40–7.56 (m, unresolved, 3H, *m+p*Ar-H), 7.98 (d, 2H, J = 7.8 Hz, *o*Ar-H), 8.07 (s, 1H, Ade-H<sub>8</sub>), 8.71 (s, 1H, Ade-H<sub>2</sub>), 9.50 (bs, 1H, NH).  $m/z$  = 312.1074 (M<sup>+</sup> + H) (C<sub>15</sub>H<sub>14</sub>N<sub>5</sub>O<sub>3</sub> requires 312.1096).

#### N(6)-Benzoyladenine-N(9)-acetic acid 11

Compound **10** (4.41 g, 0.14 mol) was suspended in MeOH (80 ml) at 0°C. NaOH (2N, 80 ml) was added and the reaction mixture stirred at 0°C for 30 min. The solution was washed with DCM (2 × 80 ml) then the pH adjusted to 1 by the addition of conc. HCl (5 ml). The title acid (3.43 g, 72%) was collected and dried over P<sub>2</sub>O<sub>5</sub> under vacuum.  $R_f$  0.35(A), 0.1(C);  $\delta_H$  (d<sub>6</sub>-DMSO); 5.27 (s, 2H, CH<sub>2</sub>CO<sub>2</sub>H), 7.1–7.2 (broad, 1H, CO<sub>2</sub>H), 7.55–7.72 (m, unresolved, 3H, *m+p*ArH), 8.08 (d, 2H, J = 6.8 Hz, *o*ArH), 8.88 (s, 1H, Ade-H<sub>8</sub>), 9.16 (s, 1H, Ade-H<sub>2</sub>).  $m/z$  = 298.0925 (M<sup>+</sup> + H) (C<sub>14</sub>H<sub>12</sub>N<sub>5</sub>O<sub>3</sub> requires 298.0940).

#### N-(*t*-Butoxycarbonylaminoethyl)-N-(thyminyln(1)-acetyl)-glycine ethyl ester 12

Ethyl-N-[2-(*t*-butoxycarbonylamino)ethyl] glycinate **4** (2.75 g, 11 mmol) and thymine-N(1)-acetic acid **5** (2.05 g, 1 eq.) were dissolved in DMF (25 ml) and triethylamine (4.42 ml, 3 eq.) was added. TopPipU (4.2 g, 1.0 eq.) was added and the reaction mixture was stirred at ambient temperature for 16 h. The solvent was removed by evaporation and DCM (250 ml) was added to the residue. The resultant solution was washed with NaHCO<sub>3</sub> (0.5 N, 3 × 100 ml), citric acid (10% w/v, 2 × 100 ml), and saturated NaCl (100 ml), dried (MgSO<sub>4</sub>) then concentrated to an oil which was subjected to flash column chromatography [DCM/MeOH (0–3%)]. The ester **12** (3.2 g, 71%) was isolated as a foam.  $R_f$  0.75 (A), 0.15 (B), 0.33 (C);  $\delta_H$  (CDCl<sub>3</sub>); (two rotational isomers in the ratio 2:1 were detected due to restricted rotation about the tertiary amide bond) 1.25 (t, 3H, J = 7.2 Hz, CH<sub>3</sub>CH<sub>2</sub>), 1.42 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.88 (s, 2.4H, Thy-CH<sub>3</sub>) and 1.89 (s, 0.6H, Thy-CH<sub>3</sub>), 3.25 (t, 0.4H, J = 5.8 Hz, CH<sub>2</sub>CH<sub>2</sub>NCO) and 3.31 (t, 1.6H, J = 5.8 Hz, CH<sub>2</sub>CH<sub>2</sub>NCO), 3.51 (t, 2H, J = 5.8 Hz, CH<sub>2</sub>CH<sub>2</sub>NCO), 4.02 (s, 1.6H, NCH<sub>2</sub>CO<sub>2</sub>Et) and 4.19 (s, 0.4H, NCH<sub>2</sub>CO<sub>2</sub>Et), 4.18 (q, 2H, J = 7.2 Hz, CH<sub>2</sub>CH<sub>3</sub>), 4.41 (s, 0.4H, NCH<sub>2</sub>CON) and 4.56 (s, 1.6H, NCH<sub>2</sub>CON), 5.67 (t, 1H, J = 6.2 Hz, BocHN), 6.95 (s,

0.8H, Thy-H6) and 7.01 (s, 0.2H, Thy-H6), 9.27 (s, 1H, NH). *m/z* (f.a.b.) = 413.2032 ( $M^+ + H$ ) ( $C_{18}H_{29}N_4O_7$  requires 413.2036).

#### ***N*-(*t*-Butoxycarbonylaminoethyl)-*N*-[*N*(4)-benzoylcytosinyl-*N*(1)-acetyl]-glycine ethyl ester 13**

Ethyl-*N*-[2-(*t*-butoxycarbonylamino)ethyl] glycinate **4** and *N*(4)-benzoyl cytosine-*N*(1)-acetic acid **8** were condensed as previously described for amide **12**. The title compound (74%) was isolated as a pale pink foam following flash column chromatography [ $SiO_2$ , DCM/MeOH (0–3%)];  $R_f$  0.33 (B), 0.55 (C);  $\delta_H$  ( $CDCl_3$ ); (two rotational isomers were observed) 1.22 (t, 3H,  $J = 7.2$  Hz,  $CH_2CH_3$ ), 1.40 (s, 9H,  $C(CH_3)_3$ ), 3.29 (m, unresolved, 2H,  $BocHNCH_2CH_2$ ), 3.55 (m, 2H, unresolved,  $BocHNCH_2CH_2$ ), 4.04 (s, 1.6H,  $NCH_2CO_2Et$ ) and 4.29 (s, 0.4H,  $NCH_2CO_2Et$ ), 4.13 (q, 2H,  $J = 7.2$  Hz,  $CH_2CH_3$ ), 4.60 (s, 0.4H,  $NCH_2CON$ ) and 4.80 (s, 1.6H,  $NCH_2CON$ ), 5.71 (t, 1H,  $J = 6.0$  Hz,  $BocHN$ ), 7.21–8.00 (m, unresolved, 7H, Ar-H + Cyt-H5 + Cyt-H6). *m/z* (f.a.b.) = 502.2306 ( $M^+ + H$ ) ( $C_{24}H_{32}N_5O_7$  requires 502.2302).

#### ***N*-(*t*-Butoxycarbonylaminoethyl)-*N*-[*N*(6)-benzoyladenyl-*N*(9)-acetyl]-glycine ethyl ester 14**

Ethyl-*N*-[2-(*t*-butoxycarbonylamino)ethyl] glycinate **4** and *N*(6)-benzoyladenine-*N*(9)-acetic acid **11** were condensed as previously described for amide **12**. The title compound (78%) was isolated as a white solid following flash column chromatography [ $SiO_2$ , DCM/MeOH (0–6%)].  $R_f$  0.85 (A), 0.10 (B), 0.35 (C);  $\delta_H$  ( $CDCl_3$ ); 1.18 (t, 3H,  $J = 7.2$  Hz,  $CH_2CH_3$ ), 1.36 (s, 9H,  $C(CH_3)_3$ ), 3.33 (t, 2H,  $J = 5.5$  Hz,  $BocHNCH_2CH_2$ ), 3.57 (t, 2H,  $J = 5.5$  Hz,  $BocHNCH_2CH_2$ ), 4.01 (s, 1.6H,  $NCH_2CO_2Et$ ) and 4.26 (s, 0.4H,  $NCH_2CO_2Et$ ), 4.11 (q, 2H,  $J = 7.2$  Hz,  $CH_2CH_3$ ), 5.01 (s, 0.4H,  $NCH_2CON$ ) and 5.16 (s, 1.6H,  $NCH_2CON$ ), 5.82 (broad, 1H,  $BocHN$ ), 7.36–7.52 (m, unresolved, 3H, *m+p*Ar-H), 7.98 (d, 2H,  $J = 7.9$  Hz, *o*Ar-H), 8.05 (s, 0.8H, Ade-H2) and 8.10 (s, 0.2H, Ade-H2), 8.64 (s, 0.2H, Ade-H8) and 8.66 (s, 0.8H, Ade-H8). *m/z* = 526.2382 ( $M^+ + H$ ) ( $C_{25}H_{32}N_7O_6$  requires 526.2414).

#### ***N*-(4'-Methoxytritylaminoethyl)-*N*-(thyminyl-*N*(1)-acetyl)-glycine ethyl ester 15**

Boc protected amine **12** (2.00 g, 4.85 mmol) was treated with trifluoroacetic acid (10 ml). After 30 min the reaction mixture was evaporated to dryness *in vacuo*, coevaporated with toluene ( $5 \times 10$  ml) followed by pyridine ( $2 \times 10$  ml) then resuspended in a further volume of pyridine (20 ml). 4'-Methoxytrityl chloride (1.65 g, 1.1 eq.) and 4-dimethylaminopyridine (DMAP) (25 mg) were added and the mixture stirred at ambient temperature for 16 h. Water (30 ml) was added and the mixture was extracted with DCM ( $3 \times 70$  ml). The combined organic phases were dried ( $MgSO_4$ ), then evaporated to an oil. The title amine (2.55 g, 90%) was isolated as a white foam following flash column chromatography [ $SiO_2$ , DCM with MeOH (0–3%)] using silica which had been pre-equilibrated with DCM containing triethylamine (1% v/v) to prevent detritylation.  $R_f$  0.20 (A);  $\delta_H$  ( $CDCl_3$ ); (as with **12**, two rotational isomers were detected) 1.23 (t, 3H,  $J = 7.2$  Hz,  $CH_3CH_2$ ), 1.88 (s, 3H, Thy- $CH_3$ ), 2.38 (t, 2H,  $J = 6.0$  Hz,  $MMTrHNCH_2$ ), 3.57 (t, 2H,  $J = 6.0$  Hz,  $MMTrHNCH_2CH_2$ ), 3.70 (s, 3H,  $OCH_3$ ), 3.85 (s, 1.7H,  $NCH_2CO_2Et$ ) and 4.12 (s, 0.3H,  $NCH_2CO_2Et$ ), 4.00 (q, 2H,  $J = 7.2$  Hz,  $CH_2CH_3$ ), 4.35 (s, 0.3H,  $NCH_2CON$ ) and 4.91 (s, 1.7H,  $NCH_2CON$ ),

6.70–7.40 (m, 15H, ArH + Thy-H6), 9.15 (s, 1H, Thy-H3). *m/z* (f.a.b.) = 585.2732 ( $M^+ + H$ ) ( $C_{33}H_{37}N_4O_6$  requires 585.2713).

#### ***N*-(4'-Methoxytritylaminoethyl)-*N*-[*N*(4)-benzoylcytosinyl-*N*(1)-acetyl]-glycine ethyl ester 16**

Boc protected amine **13** was converted to the corresponding monomethoxytrityl protected amine as described for amine **12**. Following flash column chromatography [ $SiO_2$ , DCM/MeOH (0–4%)] the title amine (81%) was isolated as a colourless foam.  $R_f$  0.22 (B), 0.35 (C);  $\delta_H$  ( $CDCl_3$ ); (two rotational isomers were detected) 1.16 (t, 3H,  $J = 7.1$  Hz,  $CH_3CH_2$ ), 2.31 (t, 2H,  $J = 5.2$  Hz,  $MMTrHNCH_2$ ), 3.60 (m, unresolved, 2H,  $TrHNCH_2CH_2$ ), 3.76 (s, 3H,  $OCH_3$ ), 3.94 (s, 1.4H,  $NCH_2CO_2Et$ ) and 4.32 (s, 0.6H,  $NCH_2CO_2Et$ ), 4.07 (q, 2H,  $J = 7.1$  Hz,  $CH_2CH_3$ ), 4.62 (s, 0.7H,  $NCH_2CON$ ) and 5.18 (s, 1.3H,  $NCH_2CON$ ), 6.75–7.92 (m, 21H, Ar-H + Cyt-H5 + Cyt-H6). *m/z* = (f.a.b.) 674.2979 ( $M^+ + H$ ) ( $C_{39}H_{40}N_5O_6$  requires 674.2978).

#### ***N*-(4-Methoxytritylaminoethyl)-*N*-[*N*(6)-benzoyladenyl-*N*(9)-acetyl]-glycine ethyl ester 17**

Boc protected amine **14** was converted to the corresponding monomethoxytrityl protected amine as described for amine **12**. Following flash column chromatography [ $SiO_2$ , DCM/MeOH (0–2%)] the title amine (83%) was isolated as a colourless foam  $R_f$  0.10 (B), 0.25 (C);  $\delta_H$  ( $CDCl_3$ ); (two rotational isomers were detected) 1.17 (t, 3H,  $J = 7.1$  Hz,  $CH_3CH_2$ ), 2.36 (m, unresolved, 2H,  $TrHNCH_2CH_2$ ), 3.64 (t, 2H,  $J = 5.6$  Hz,  $TrHNCH_2CH_2$ ), 3.75 (s, 3H,  $OCH_3$ ), 3.97 (s, 1.6H,  $NCH_2CO_2Et$ ) and 4.26 (s, 0.4H,  $NCH_2CO_2Et$ ), 4.09 (q, 2H,  $J = 7.1$  Hz,  $CH_2CH_3$ ), 5.02 (s, 0.4H,  $NCH_2CON$ ) and 5.59 (s, 1.6H,  $NCH_2CON$ ), 6.75–7.59 (m, unresolved, 17H, Ar-H), 8.01 (d, 2H,  $J = 6.9$  Hz, *o*Ar-H), 8.14 (s, 1H, Ade-H2), 8.76 (s, 1H, Ade-H8). *m/z* = 698.3046 ( $M^+ + H$ ) ( $C_{40}H_{40}N_7O_5$  requires 698.3091).

#### ***N*-(4'-Methoxytritylaminoethyl)-*N*-(thyminyl-*N*(1)-acetyl)-glycine 18**

The ester **15** (1.34 g, 2.3 mmol) was dissolved in MeOH (60 ml) and NaOH (2 N, 40 ml) added. The mixture was stirred at room temperature for 2 h. then DOWEX (pyridinium form) was added until neutral (wet pH paper). The suspension was filtered and the resin washed with MeOH ( $3 \times 50$  ml). The filtrate was evaporated to dryness to yield acid **18** as a white solid (1.40 g, 96%).  $R_f$  0.05 (B), 0 (C);  $\delta_H$  (d6 DMSO); (two rotational isomers were detected) 1.74 (s, 3H, Thy- $CH_3$ ), 2.10 (m, unresolved, 2H,  $MMTrHNCH_2$ ), 3.64 (m, unresolved, 2H,  $MMTrHNCH_2CH_2$ ), 3.71 (s, 3H,  $OCH_3$ ), 3.85 (s, 1H,  $NCH_2CO_2H$ ) and 4.01 (s, 1H,  $NCH_2CO_2H$ ), 4.47 (s, 1H,  $NCH_2CON$ ) and 4.82 (s, 1H,  $NCH_2CON$ ), 6.80–7.50 (m, 15H, Ar-H + Thy-H6), 8.60 (s, 1H, NH). *m/z* (f.a.b.) = 557.2366 ( $M^+ + H$ ) ( $C_{31}H_{33}N_4O_6$  requires 557.2399).

#### ***N*-(4'-Methoxytritylaminoethyl)-*N*-[*N*(4)-benzoylcytosinyl-*N*(1)-acetyl]-glycine 19**

Ester **16** was hydrolysed as described for ester **15**. The title acid (70%) was obtained as a white solid  $R_f$  0.05 (C);  $\delta_H$  (d6 DMSO) (two rotational isomers were detected) 2.23 (m, unresolved, 2H,  $MMTrHNCH_2$ ), 3.41 (m, unresolved, 2H,  $TrHNCH_2CH_2$ ), 3.76 (s, 3H,  $OCH_3$ ), 3.89 (s, 1H,  $NCH_2CO_2H$ ) and 4.02 (s, 1H,  $NCH_2CO_2H$ ), 4.62 (s, 1H,  $NCH_2CON$ ) and 5.03 (s, 1H,  $NCH_2CON$ ),

6.71–7.99 (m, unresolved, 20H, Ar-H + Cyt-H5 + Cyt-H6).  $m/z$  = (f.a.b.) 646.2671 ( $M^+$  + H) ( $C_{37}H_{36}N_5O_6$  requires 646.2666).

#### *N*-(4-Methoxytritylaminoethyl)-*N*-[*N*(6-benzoyladenyl)-*N*(9-acetyl)-glycine] 20

Ester **17** was hydrolysed as described for ester **15**. The title acid (97%) was obtained as a white solid  $R_f$  0.00 (B), 0.15 (C);  $\delta_H$  (d6 DMSO); (two rotational isomers were detected) 2.18 (m, unresolved, 2H, MMTrHNCH<sub>2</sub>), 3.44 (m, unresolved, 2H, TrHNCH<sub>2</sub>CH<sub>2</sub>), 3.75 (s, 3H, OCH<sub>3</sub>), 3.92 (s, 1.6H, NCH<sub>2</sub>CO<sub>2</sub>H) and 4.03 (s, 0.4H, NCH<sub>2</sub>CO<sub>2</sub>H), 4.83 (s, 0.4H, NCH<sub>2</sub>CON) and 5.32 (s, 1.6H, NCH<sub>2</sub>CON), 6.70–7.63 (m, unresolved, 17H, Ar-H), 8.01 (d, 2H,  $J=6.8$  Hz, *o*Ar-H), 8.17 (s, 1H, Ade-H8), 8.86 (s, 1H, Ade-H2).  $m/z$  (f.a.b.) = 670.2789 ( $M^+$  + H) ( $C_{38}H_{36}N_7O_5$  requires 670.2778).

#### Synthesis of PNA–DNA chimeric oligomers (PDC oligomers)

The DNA sections were synthesised on an ABI 394 DNA/RNA synthesiser on the 1.0  $\mu$ mol scale using standard phosphoramidite chemistry. 5'-(4-Methoxytrityl)-amino-5'-deoxythymidine phosphoramidite (**19**) was added as the final nucleoside to act as a linker between PNA and DNA. The resin was removed from the DNA synthesis column and transferred to a glass sinter funnel used for PNA synthesis. The PNA monomers were added manually with agitation of the resin during each synthetic step by means of a stream of nitrogen. PNA monomer coupling was carried out in DMF/pyridine solution (1:1 v/v, 0.1 M) using diethylcyclohexylamine as base and TopPipU (**20**) as activating group for the acid with a coupling time of 30 min. The *in situ* neutralisation approach was used to inhibit aggregation of the PNA chains and to prevent elimination reactions caused by intramolecular cyclisations of the PNA backbone. Deprotection of the monomethoxytrityl terminal amino function was carried out using TCA (3% w/v, 3  $\times$  2 ml, 10 min) followed by thorough washing with DCM (5  $\times$  1 ml). The resin was filtered then washed with MeOH (5  $\times$  1 ml) then DMF (5  $\times$  1 ml).

Capping of unreacted amino groups was achieved using acetic anhydride/pyridine solution (10% v/v, 1 ml) for 5 min. The resin was washed with DMF (5  $\times$  1 ml) before the cycle was started again as required.

The oligomer was cleaved from the solid support and the exocyclic amino protecting groups removed by heating the resin in concentrated aqueous ammonia (2 ml, 55°C) for 6 h.

#### HPLC purification of PNA–DNA chimeras

The chimeric PDC oligomers were purified by reverse phase HPLC using: A = NH<sub>4</sub>OAc (0.1 M, pH 7.0), B = MeCN (22.5% v/v)/NH<sub>4</sub>OAc (0.1 M, pH 7.0) employing the following gradient: B = 0% 3.5 min, B = 10% 4.5 min, B = 100% 30 min, B = 0% 38 min at a flow rate of 3 ml/min. After evaporation salts were removed by Sephadex gel filtration.

*Chimeric oligomers synthesised.* PDC Ac-tttcttTGCCAT-3', Electrospray mass spec.  $M$  3388.53  $C_{125}H_{161}N_{46}O_{58}P_5$  requires 3390.80 (average mass), 3389.00 (monoisotopic mass). PDC ttttttTGCCAT-3', Electrospray mass spec.  $M$  3405.43,  $C_{126}H_{162}N_{45}O_{59}P_5$  requires 3405.81 (average mass), 3403.97 (monoisotopic mass).

#### UV melting studies of PDC Ac-tttxttTGCCAT 3' (x = t,c)

Ultraviolet melting temperatures ( $T_m$ ) were determined at 260 nm using a Perkin-Elmer Lambda 15 UV spectrometer equipped with a Peltier block and controlled by an IBM PS2 computer. A heating rate of 0.5 K/min was used throughout and the crude data was processed using the PECSS2 software package. The oligonucleotides were dissolved in a buffer consisting of 0.14 M NaCl and 10 mM HEPES adjusted to pH 7.0. All experiments were repeated until three values within 0.5 K of each other were obtained. Molar extinction coefficients used for PNA and DNA bases were as follows: A, 15.4; T, 8.8; G, 11.7; C, 7.3  $\mu$ mol.cm.  $T_m$  values were determined from the maximum of the first derivative of the plot of  $A_{260}$  versus temperature.

See supplementary material available in NAR Online.

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