Mixed-sequence pyrrolidine-amide oligonucleotide mimics: Boc(Z) synthesis and DNA/RNA binding properties†

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Received 14th September 2006, Accepted 9th November 2006
First published as an Advance Article on the web 1st December 2006
DOI: 10.1039/b613386j

Pyrrolidine-amide oligonucleotide mimics (POMs) exhibit promising properties for potential applications, including *in vivo* DNA and RNA targeting, diagnostics and bioanalysis. Before POMs can be evaluated in these applications it is first necessary to synthesise and establish the properties of fully modified oligomers, with biologically relevant mixed sequences. Accordingly, Boc-Z-protected thyminyl, adeninyl and cytosinyl POM monomers were prepared and used in the first successful solid phase synthesis of a mixed sequence POM, Lys-TCACAACTT-NH_2. UV thermal denaturation studies revealed that the POM oligomer is capable of hybridising with sequence selectivity to both complementary parallel and antiparallel RNA and DNA strands. Whilst the duplex melting temperatures (T_m) were higher than the corresponding duplexes formed with isosequential PNA, DNA and RNA oligomers the rates of association/dissociation of the mixed sequence POM with DNA/RNA targets were noticeably slower.

Introduction

Pyrrolidine-amide oligonucleotide mimics (POMs) **1** (Fig. 1) are stereochemical and conformational mimics of natural nucleic acids.[1–5] Owing to protonation of the pyrrolidine ring, POMs are positively charged, which aids solubility and could increase affinity for target DNA and RNA. Previously we have shown that a series of thyminyl and adeninyl POM oligomers can form stable complexes with complementary DNA and RNA and in some cases possess a noticeable kinetic selectivity for RNA over DNA.[1–5] A number of related pyrrolidine-containing oligonucleotide mimics, or peptide nucleic acid analogues, have similarly been developed.[6–16] Several of these pyrrolidine-containing mimics also exhibit promising nucleic acid recognition and other physiochemical properties, which might be useful for a wide range of applications including *in vivo* DNA and RNA targeting,[17–19] diagnostics and bioanalysis,[20,21] or programmed assembly of nanostructures.[22] Despite this, most of the results compiled so far,[1–12,14,15] with the exception of a few studies,[13,16] are based on the properties of chimeric oligomers containing a mix of PNA and pyrrolidine monomer units or fully modified pyrrolidinyl-homopolymers (*e.g.* poly-T oligomers), which are not useful in real, practical applications.[17–22] Therefore, in order to realise the potential of POM and the other pyrrolidine-containing nucleic acid mimics it is neccessary to systematically evaluate the properties of a variety of fully modified mixed sequence oligomers, which would be more relevant for biological and analytical purposes.[17–22]

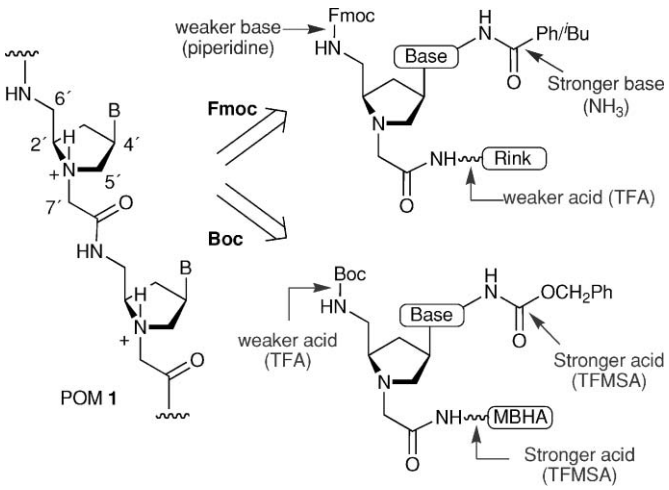

Fig. 1 Structure of pyrrolidine-amide oligonucleotide mimics (POMs) **1**. The Fmoc approach, described in the preceding paper,[1] used monomers containing Fmoc-backbone amino protecting groups, which are cleavable with piperidine and standard acyl-protected bases (C, A and G), which are cleaved with ammonia. A polystyrene resin functionalised with the Rink amide linker allows oligomers to be cleaved by trifluoroacetic acid (TFA). In this paper, the Boc-Z strategy is employed which utilises monomers with Boc-backbone amino protecting groups, which are cleavable with TFA, along with benzyloxycarbonyl(Z)-protected bases (C, A and G) that are stable to TFA, but can be removed with trifluoromethanesulfonic acid (TFMSA). A polystyrene resin functionalised with methylbenzhydrylamine (MBHA) groups is used as the solid support, in this case, allowing oligomers to be cleaved with TFMSA, but not TFA.

In the preceding paper[1] we described the synthesis of Fmoc-protected POM monomers, with standard acyl-protected nucleobases (Fig. 1). Using these monomers we were able to prepare short homopolymers, using standard Fmoc-peptide chemistry. However, the Fmoc approach failed to deliver longer mixed-sequence POM oligomers, despite the fact that similar chemistry

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† Electronic supplementary information (ESI) available: additional data, general experimental methods for monomer and solid phase synthesis and characterisation of compounds not presented in the main experimental section, and methods employed in thermal denaturation experiments. See DOI: 10.1039/b613386j

had been used for the efficient synthesis of PNA.[23,24] Prior to the development of the Fmoc strategy for PNA synthesis, Boc-solid phase chemistry, with benzyloxycarbonyl (Z)-nucleobase protecting groups was most widely used.[24–26] The Boc-Z strategy has a number of advantages over Fmoc chemistry,[27] including the stability of the protecting groups to the coupling conditions, which reduces the occurrence of multiple couplings. Also, Boc deprotection with trifluoroacetic acid (TFA) is fast and TFA can help prevent aggregation of the growing oligomers.[27] Additionally, the resulting *N*-terminal amino group remains protonated after deprotection, which can reduce side reactions that involve cyclisation of terminal free amino groups onto adjacent amide linkages. Such intramolecular cyclisation reactions can result in six-membered lactam side products in both PNA[23,27] and POM[3] synthesis, which are equivalent to diketopiperazine formation occurring during standard peptide synthesis. In this paper we demonstrate the first successful synthesis of longer mixed-sequence POMs, using the Boc-Z strategy, which has enabled the DNA and RNA hybridisation properties of a more relevant POM sequence to be evaluated.

Results and discussion

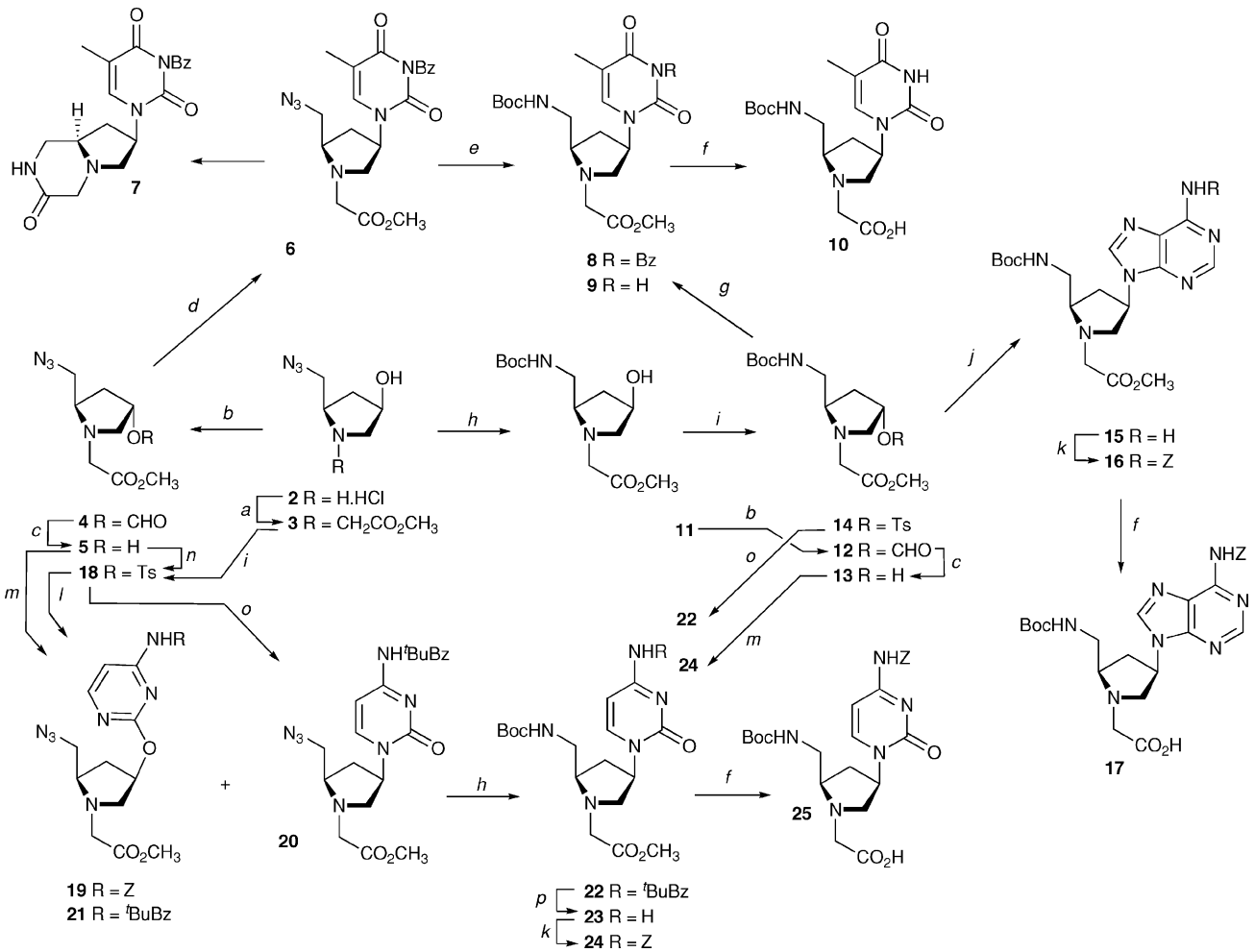
Synthesis of the Boc-Z-protected POM monomers

In order to investigate the alternative Boc-Z approach for the synthesis of POM mixed sequences, the appropriately protected POM monomers were first prepared (Scheme 1). Accordingly the amine HCl salt **2**, used in the synthesis of the Fmoc-protected POM monomers,[1] was alkylated with methyl bromoacetate to give methyl ester **3**. The methyl ester, as opposed to the *tert*-butyl ester used previously,[1] allows for subsequent deprotection by mild basic hydrolysis to give the required acid functionality without jeopardising the Boc- and Z-protecting groups. The *cis*-alcohol **3** was transformed to the *trans*-formyl ester **4** using Mitsunobu's conditions. Cleavage of the formyl ester of **4** to give *trans*-alcohol **5** was achieved with potassium carbonate in anhydrous methanol. Introduction of N^3-benzoylthymine onto the pyrrolidine ring of **5**, was carried out under Mitsunobu conditions, to give the (2*R*,4*R*) protected thymine derivative **6** in a yield of 75%, which depends upon the presence of the additive sodium benzoate.[28,29] Azide reduction and isolation of the resulting amine was not possible under the conditions used previously in the synthesis of Fmoc monomers,[1] due to the facile formation of a bicyclic lactam **7**, resulting from intramolecular attack of the amine on the methyl ester. It was therefore necessary to carry out azide reduction with *in situ* Boc protection. This was achieved, by the hydrogenation of azide **6** over 10% Pd-C in the presence of di-*tert*-butyldicarbonate (Boc-anhydride) to afford protected thyminyl derivative **8**, along with the de-benzoylated thyminyl derivative **9**, in a combined yield of 65%. Alternatively it was subsequently found that the azide **6** could be reduced more efficiently using trimethylphosphine in the presence of 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON)[30] to give **8** as the major product in 81% yield. Saponification of both **8** and **9**, followed by neutralization of the reaction mixture with HCl resulted in Boc thyminyl acid **10** in 73 and 87% yields from **8** and **9** respectively.

In addition to this route, the possibility of introducing the nucleobase, after azide reduction and Boc protection, was also investigated. Accordingly, azide **3** was treated with trimethylphosphine and Boc-ON[30] to afford Boc-protected amine **11** in 51% yield. The *cis*-alcohol **11** was then inverted, as before, to give *trans*-alcohol **13** *via* the formyl ester **12**. The *cis*-alcohol **11** was also inverted with concomitant tosylation to give **14**, with methyl *p*-toluenesulfonate under Mitsunobu's conditions.[31] Introduction of N^3-benzoylthymine into the pyrrolidine ring of the *trans*-alcohol **13** under standard Mitsunobu conditions afforded the N^3-benzoylthymine derivative **8**, in 70% yield, which was hydrolysed to the required thyminyl Boc-acid **10**. The advantage of this approach is that potentially all the nucleobases could be introduced in the penultimate step, *via* the *trans*-alcohol **13** or the tosylate **14** leading to a more convergent synthesis of the required POM monomers. With this in mind N^6-Z-adenine was used to displace the tosylate of **14** in the presence of K_2CO_3 and 18-crown-6 resulting in the adeninyl derivative **15** in 40% yield. The regiochemistry of the product **15** was established through comparison of ^{13}C-NMR chemical shifts with known N^7- and N^9-alkylated adeninyl derivatives from the literature.[32,33] During this transformation the N^6-Z-protecting group was lost, but was subsequently replaced by treating **15** with freshly prepared 1-(benzyloxycarbonyl)-3-ethylimidazolium tetrafluoroborate ('Rapoport's reagent')[34] to afford **16** in 50% yield. Saponification and neutralization as before revealed the Boc-Z adeninyl acid **17** in 75% yield.

Initially the synthesis of the required cytosinyl monomer **25** was investigated *via* the *trans*-alcohol **5**. However, attempts to introduce N^4-Z-cytosine into the pyrrolidine **5** by Mitsunobu chemistry gave none of the desired N^1-adduct and instead gave the O^2-adduct **19** in a very low yield. Displacement of the tosylate **18** with N^4-Z-cytosine also gave exclusively the O^2-adduct **19** in 67% yield. Conversely, treatment of tosylate **18** with N^4-[*p*-(*tert*-butyl)benzoyl]cytosine gave the desired N^1-adduct **20** in a modest 32% yield, along with a minor amount of the O^2-adduct **21**. As before, the regiochemistry was assigned through comparison of ^{13}C-NMR chemical shifts with known compounds,[32,35] and in the case of the N^1-adduct **20** the structure and the relative stereochemistry was ultimately confirmed by X-ray crystallography (Fig. 2).‡ The regiochemical outcome of these reactions is probably a reflection of the different electronic properties of the N^4-protecting groups. For example, the more electron withdrawing *p*-(*tert*-butyl)benzoyl protecting group presumably decreases the delocalisation of the lone pair of electrons from the N^4-amino group onto the O^2-atom of the carbonyl group and, as a result, predominately N^1-alkylation would be favoured. To complete the synthesis, the azide **20** was reduced to the amine with *in situ* Boc-protection, using trimethylphosphine and Boc-ON[30] to give **22** in 63% yield. Subsequently it was found that **22** could be prepared more efficiently *via* substituting the tosylate of **14** with N^4-[*p*-(*tert*-butyl)benzoyl]cytosine in 46% yield. Removal of the N^4-[*p*-(*tert*-butyl)benzoyl]cytosine protecting group of **22** with sodium methoxide in methanol resulted in the cytosine derivative **23** which was treated with freshly prepared 'Rapoport's reagent'[34] to give the Z-protected cytosinyl monomer **24** in 92% yield over the two

‡ Crystal data for compound **20**: $C_{23}H_{29}N_7O_4$, CH_4O, H_2O, $M = 517.59$, orthorhombic, $a = 7.7564(9)$, $b = 13.3948(9)$, $c = 25.589(3)$ Å, $V = 2658.6(5)$ Å^3, $T = 150(2)$ K, space group $P2_12_12_1$, $Z = 4$, $\mu = 0.095$ mm^{-1}, reflections collected 7393, independent reflections 3846 [R(int) = 0.1129], $R_1 = 0.0956$ [$I > 2\sigma(I)$], $wR_2 = 0.3014$ (all data). CCDC reference number 624250. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b613386j.



Scheme 1 *Reagents and conditions*: a) DIEA, $BrCH_2CO_2CH_3$, CH_2Cl_2, 0 °C → rt, 18 h; b) HCO_2H, PPh_3, DIAD, THF, −30 °C → rt, 18 h; c) $NaOCH_3$ in CH_3OH or K_2CO_3 in CH_3OH, rt, 2 h; d) N^3-benzoylthymine, PPh_3, DIAD, $PhCO_2Na$, THF, rt, 18 h; e) 10% Pd-C, H_2, Boc anhydride, EtOAc, rt, 18 h or PMe_3, 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON); f) 1M aq NaOH, THF, rt 4 h, then 0.1M HCl; g) N^3-benzoylthymine, PPh_3, DIAD, THF, rt, 18 h; h) PMe_3, Boc-ON, THF, −20 °C → rt, 1 h; i) CH_3OTs, PPh_3, DIAD, THF, −20 °C → rt, 20 h; j) N^6-benzyloxycarbonyladenine, K_2CO_3, 18-crown-6, DMF, 80 °C, 4 h; k) 1-(benzyloxycarbonyl)-3-ethylimidazolium tetrafluoroborate ('Rapoport's reagent'), CH_2Cl_2, rt, 18 h; l) N^4-benzyloxycarbonylcytosine, K_2CO_3, 18-crown-6, DMF, 75 °C, 16 h; m) N^4-benzyloxycarbonylcytosine, PPh_3, DIAD, THF, −25 °C → rt, 18 h; n) tosyl chloride, pyridine, 0 °C → rt, 18 h; o) N^4-[*p*-(*tert*-butyl)benzoyl]cytosine, K_2CO_3, 18-crown-6, DMF, 75 °C, 16 h; p) $NaOCH_3$, CH_3OH, rt, 6 h. *Abbreviations*: ^{t}BuBz = *p*-(*tert*-butyl)benzoyl, Bz = benzoyl, Boc = *tert*-butoxycarbonyl, DIAD = diisopropyl azodicarboxylate, DIEA = diisopropylethylamine, Ts = tosyl.

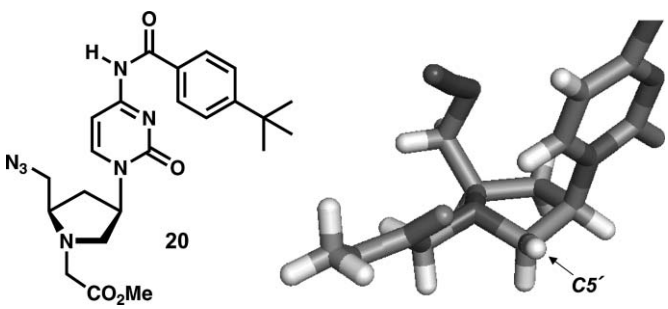


Fig. 2 X-Ray crystal structure of the cytosinyl POM free amine **20** which is shown to exhibit a C5′-*exo*-type conformation with a pseudorotational phase angle ($P = -27°$) and backbone torsional angles ($\gamma = 63°$, $\delta = 82°$ and $\varepsilon = 152°$) that correspond more closely with the torsional angles of ribose in typical A-type RNA duplexes, rather than B-type DNA duplexes.[39]

steps. Saponification of **24** then revealed Boc-Z cytosinyl acid **25** in 90% yield. Despite this, we later found that Boc-Z cytosinyl monomer **24** could be prepared directly from the *trans*-alcohol **13** which was coupled with N^4-benzyloxycarbonylcytosine under Mitsunobu's conditions to give the N^1-cytosinyl product **24** in 29% yield, with the regiochemistry again confirmed by ^{13}C NMR.[32,35] It is interesting to note that the regioselectivity in this case is opposite to that observed in the reaction between the azido *trans*-alcohol **5** and N^4-benzyloxycarbonylcytosine, which gave the O^2-adduct **19**. This may be due to participation of the Boc amino group of **13**, which could potentially form an H-bond with the more electronegative O^2-atom of the cytosinyl carbonyl group, in the transition state, which may direct nucleophilic attack *via* the N^1-atom of the ambident nucleophile.

The availability of the X-ray crystal structure of the cytosinyl POM monomer **20** (Fig. 2) gave the opportunity to consider the conformation of the POM pyrrolidine ring as the free amine.

Owing to nearest neighbour interactions[36,37] it is not anticipated that every pyrrolidine ring in a POM oligomer will be protonated simultaneously at physiological pH. It is therefore useful to consider the conformation of the POM monomer units in both the protonated and the free amine forms. Analysis of the structure of **20** reveals an overall C5′-*exo* conformation which is described by a pseudorotation phase angle (P) of −27°.[38,39] This conformation is equivalent to a nucleotide C2′-*exo* conformation which most closely matches the conformation of ribose in A-type RNA duplexes. The backbone torsion angles ($\gamma = 63°$, $\delta = 82°$ and $\varepsilon = 152°$) are also close to the corresponding angles found in A-type RNA duplexes.[39] This suggests that the protonation state of the pyrrolidine ring in the POM oligomers will have little effect on the conformation of oligomers, and both states possess the prerequisite conformation for hybridisation with RNA and DNA. The fact that our earlier studies[1–5] reveal that changes in pH do affect UV thermal denaturation temperature (T_m) values for complexes with DNA and RNA is therefore most likely due to electrostatic as opposed to conformational effects.

Boc-solid phase synthesis of Lys-POM(T)$_5$-Lys

In order to establish if Boc-solid phase chemistry is more efficient than the Fmoc approach for the synthesis of POMs, the previously prepared Lys-POM(T)$_5$-Lys **26**[1] (see ESI†) was re-synthesised from monomer **10** by the standard Boc-PNA synthetic protocol.[26] The pentamer was prepared on methylbenzhydrylamine low loading (MBHA LL) functionalised resin which was adjusted at the first coupling of *N*-α-Boc-*N*-ε-2-chloro-Z-L-lysine to give a loading of 0.12 mmol g^{-1} (*ca.* 20% of the maximum loading of the resin). POM Boc-T amino acid **10** (2 equiv.) was then preactivated with 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU) (1.9 equiv.) and diisopropylethylamine (DIEA) (2.2 equiv.) prior to subsequent coupling reactions, which proceeded for 2 h and were monitored by the Kaiser test. Acetyl capping of any unreacted oligomer was carried out followed by Boc deprotection and repeated coupling. Cleavage from the resin was effected by the 'low-high' TFMSA method[26] to give Lys-POM(T)$_5$-Lys **26** in a yield of 95.8% as determined by analytical C18 HPLC (see ESI†). This equates to an average coupling efficiency of 99.2% and is superior to the modest average coupling efficiency of *ca.* 95% which was obtained for the same synthesis of Lys-POM(T)$_5$-Lys **26** using the Fmoc protocol.

Boc-solid phase synthesis of mixed-sequence POMs

The synthesis of Lys-TCACAACTT-NH$_2$ was achieved following a similar protocol to that used in the synthesis of the thyminyl pentamer. However, it was necessary to use 5 equiv. of the POM Boc amino acid for each coupling (along with 4.9 equiv. HBTU, and 5.5 equiv. DIEA) and a monomer concentration of 0.1 M. In addition, it was shown to be advantageous to employ double couplings when extending from *N*-terminal adeninyl residues. The POM mixed sequence was synthesised in a yield of 61% as determined by analytical C18 HPLC (see ESI†), equating to an average coupling efficiency of 95.2%. These coupling efficiencies are respectable compared with the efficiencies typically resulting from peptide or PNA synthesis. The POM-PNA chimera Lys-TC*AC*AAC*TT-NH$_2$ (where C* corresponds to a PNA cytosinyl monomer) was synthesised *via* the same method. The yield for the synthesis of the chimera was determined by analytical C18 HPLC as 68.7%, corresponding to an average coupling efficiency of 96.3%. A number of additional biologically relevant mixed-sequence POM oligomers have now been successfully synthesised using this approach, which will be published separately.

Nucleic acid binding properties of POM Lys-TCACAACTT-NH$_2$

In order to investigate the RNA and DNA hybridisation properties of the mixed-sequence POM, UV thermal denaturation/renaturation experiments were carried out with POM Lys-TCACAACTT-NH$_2$ and complementary RNA and DNA oligonucleotides. Initial experiments were carried out under close to physiological conditions using a 10 mM K_2HPO_4 buffer solution, adjusted to a salt concentration of 0.12 M K^+ and pH 7.0. Under these conditions POM Lys-TCACAACTT-NH$_2$ hybridises with complementary antiparallel RNA 5′-AAGUUGUGA-3′ exhibiting a melting temperature (T_m) of 46 °C with a significant hyperchromic shift of 24% (Fig. 3 and Table 1). Antiparallel, in this case, is defined as the *N*-terminal (Lys-capped) end of the POM

Table 1 T_m values for POM Lys-TCACAACTT-NH$_2$ *vs.* complementary nucleic acids

	T_m/°C[a] (% hypochromic[b]/hyperchromic shift[c])			
	POM Lys-TCACAACTT-NH$_2$		DNA 5′-TCACAACTT-3′	PNA Lys-TCACAACTT-NH$_2$
	Cooling	Heating	Heating[c]	Heating
Antiparallel RNA 5′-AAGUUGUGA-3′	25[a] (25)[b]	46[a] (24)[c] 47[d] (30)	25[a] (9)	38[a] (15)[c]
Parallel RNA 5′-AGUGUUGAA-3′	26 (29)	44 (29) 47[d] (39)	n.t.[e]	24[a] (12)[c]
Antiparallel DNA 5′-AAGTTGTGA-3′	23 (10)	43 (15) 42[d] (24)	32 (14)	35[a] (8)[c]
Parallel DNA 5′-AGTGTTGAA-3	20 (12)	45 (16) 45[d] (42)	n.t.	n.t.

[a] Melting experiments were carried out at a concentration of 42 μM (in bases) of each strand in 10 mM K_2HPO_4, 0.12 M K^+, pH 7.0 (total volume 1.0 cm^3). UV absorbance (A_{260}) was recorded with heating at 5 °C min^{-1} from 15 to 93 °C, cooling at 0.2 °C min^{-1} to 15 °C and heating at 0.2 °C min^{-1} to 93 °C. The T_m was determined from the first derivative of the slow heating and cooling curve. [b] Hypochromic shifts are indicated in parentheses and were calculated as follows: $[A(93\ °C) - A(15\ °C)] \times 100/A(93\ °C)$. [c] Hyperchromic shifts are indicated in parentheses and were calculated as for the hypochromic shifts. [d] POM Lys-TCACAACTT-NH$_2$ was incubated with the oligonucleotide for 12 h before being subjected to slow thermal denaturation (0.2 °C min^{-1}). [e] n.t. = no transition observed

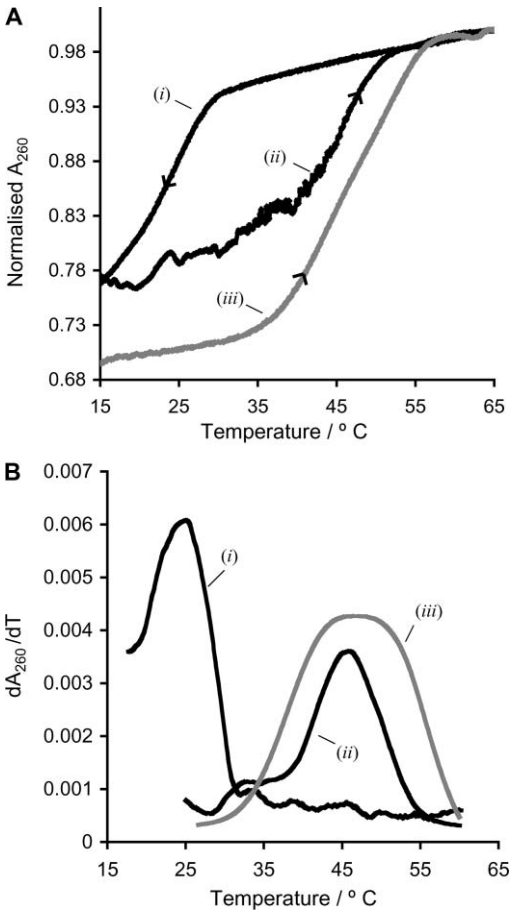


Fig. 3 UV thermal denaturation curves and first derivatives for POM Lys-TCACAACTT-NH_2 *vs.* antiparallel RNA. (A) (*i*) shows the slow cooling (renaturation) curve (0.2 °C min^{-1}), (*ii*) the slow heating (denaturation) curve (0.2 °C min^{-1}) and (*iii*) the slow heating (denaturation) curve (0.2 °C min^{-1}) obtained immediately after the POM and RNA were incubated at room temperature for 12 h is shown in grey. Control experiments show that the fluctuations in the slow heating curves (curve *ii*) are entirely due to the sample and not the instrumentation and therefore remain uncorrected. These fluctuations may be due to slow, incomplete hybridisation given that the same sample gives a very smooth melting curve following incubation for 12h (curve *iii*). (B) (*i*) The corresponding first derivatives obtained from slow cooling under standard conditions, (*ii*) slow heating under standard conditions and (*iii*) slow heating following incubation for 12 h at room temperature shown in grey.

hybridising with the 3′-end of the RNA target. In comparison, the isosequential DNA:RNA heteroduplex melts with a T_m of 25 °C whilst the PNA Lys-TCACAACTT-NH_2:RNA heteroduplex exhibits a T_m of 38 °C. Noticeable hysteresis was observed between the cooling and heating curves for these UV melting experiments with POM Lys-TCACAACTT-NH_2 and the antiparallel RNA (Fig. 3). As a consequence, the T_m extracted from the denaturation (cooling curve) was 25 °C, which is considerably lower than the T_m extracted from the melting curve ($\Delta T_m = -21$ °C). This indicates that the rate of heating/cooling employed (0.2 °C min^{-1}) is faster than the rate of association/dissociation of the POM and RNA, such that a true equilibrium is not attained. In contrast, the isosequential DNA:RNA and PNA:RNA UV thermal denaturation and renaturation shows little or no hysteresis, under the same conditions.

To explore this apparently slow hybridisation event, the POM Lys-TCACAACTT-NH_2 and antiparallel RNA target were incubated for 12 h at room temperature, prior to melting at 0.2 °C min^{-1}. Whilst the T_m extracted from this melting curve was similar the hyperchromic shift was noticeably larger (30%) (Fig. 3), compared with the standard heating/cooling ramps initially employed. The larger hyperchromic shift is most likely due to the extended incubation time (12 h), allowing a greater portion of POM and RNA strands to anneal and is also indicative of slow hybridisation. In addition to this a series of UV thermal denaturation and renaturation curves were generated for the POM and antiparallel RNA at decreasing rates of heating and cooling from 1.0 to 0.1 °C min^{-1} (see ESI†). From this it can been seen that whilst the T_m values extracted from the melting curves decrease from 58 to 43 °C, whilst the T_m extracted from the cooling curves increase from 20 to 26 °C, as the cooling rate is changed from 1.0 to 0.1 °C min^{-1}. By extrapolation, this suggests that the true thermodynamic T_m would lie within the range 30–40 °C (Fig. 4). Unexpectedly, we also found that POM Lys-TCACAACTT-NH_2 can hybridise to complementary parallel RNA 5′-AGUGUUGAA-3′ with a denaturation T_m of 44 °C under the same conditions. Again, significant hysteresis is evident, indicating slow rates of association/dissociation (see Table 1 and ESI† for more data).

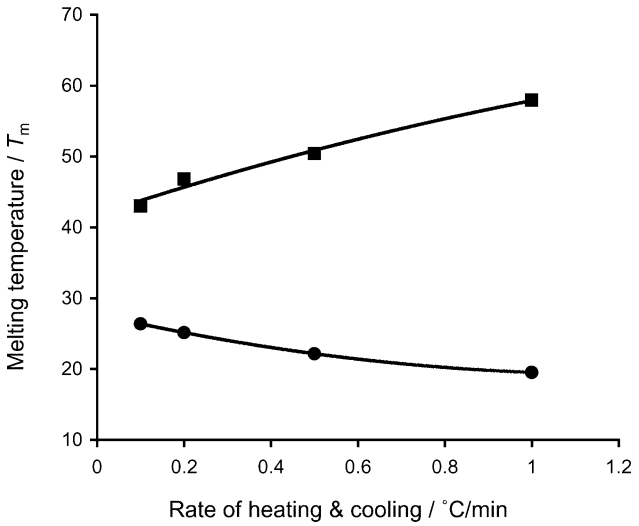


Fig. 4 Thermal denaturation (■) and renaturation (●) transition temperatures (T_m) for POM Lys-TCACAACTT-NH_2 *vs.* antiparallel RNA as a function of rates of heating (■) and cooling (●), as extracted from the first derivatives shown in the ESI† (Fig. S6B). As the rates of heating and cooling are reduced the T_m values extracted from renaturation and denaturation approach one another, allowing the true thermodynamic T_m to be extrapolated to be within the range of *ca.* 30–40 °C.

Preliminary UV thermal denaturation experiments also show that POM Lys-TCACAACTT-NH_2 hybridises with complementary antiparallel DNA 5′-AAGTTGTGA-3′ with a T_m (heating) of 43 °C (Table 1) under close to physiological conditions (0.12 M K^+ and pH 7.0). In comparison the isosequential DNA duplex has a T_m of 32 °C, whereas the PNA Lys-TCACAACTT-NH_2 antiparallel DNA duplex exhibits a T_m of 35 °C under the same conditions. Hysteresis is again evident between the heating and cooling curves for POM Lys-TCACAACTT-NH_2 with antiparallel DNA ($\Delta T_m = -20$ °C); similarly, the denaturation T_m values

decrease, whilst the renaturation T_m values increase as the rate of cooling is progressively decreased. In addition to this it was also evident that POM Lys-TCACAACTT-NH_2 hybridises with complementary parallel DNA 5′-AGUGUUGAA-3′ exhibiting a similar T_m of 45 °C with noticeable hysteresis. Finally we explored the sequence selectivity of DNA hybridisation with POM Lys-TCACAACTT-NH_2 using a number of mismatched antiparallel DNA oligomers. For example, the UV thermal denaturation curve for the hybridisation of POM Lys-TCACAACTT-NH_2 to DNA 5′-AAGTTCTGA-3′ (containing a single CC mismatch underlined) resulted in a noncooperative (near linear) hyperchromic shift from which it was not possible to define a transition melting point (T_m) (Fig. 5). UV thermal denaturation experiments for POM Lys-TCACAACTT-NH_2 and a double mismatch antiparallel DNA sequence, 5′-AAGGTATGA-3′ again showed a noncooperative transition, from which a T_m could not be determined.

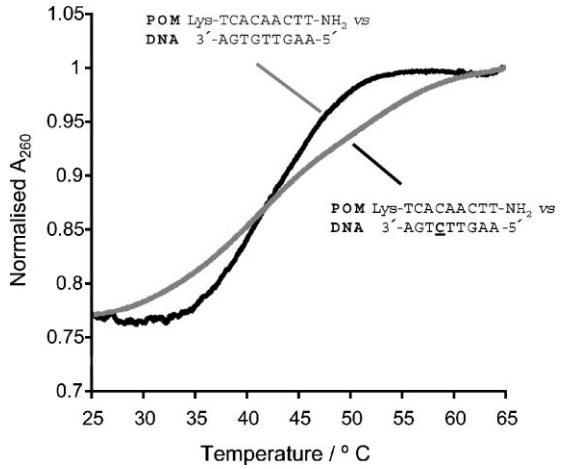


Fig. 5 Thermal denaturation curves for POM Lys-TCACAACTT-NH_2 *vs.* antiparallel DNA (5′-AAGTTGTGA-3′) and antiparallel DNA containing a single CC-mismatch underlined (5′-AAGTTCTGA-3′). Strands were incubated for 12 h at room temperature prior to slow melting (0.2 °C min^{-1}).

Overall, these preliminary results indicate that the mixed-sequence POM is able to hybridise sequence-specifically to both complementary antiparallel and parallel DNA and RNA targets to form duplexes with roughly equal stability. In all cases there is hysteresis between heating and cooling curves, which is indicative of slow rates of association and dissociation. This is in contrast to our earlier studies,[1–5] where we found that homopolymers hybridise slowly with DNA, but faster with RNA. Whilst there are many possible reasons for the slow rates of hybridisation of POM, compared with PNA or natural nucleic acids, one possible cause could be the increased rigidity in the POM backbone. This may result in the formation of a stable secondary structure in the single-stranded POMs, which have conformations that do not facilitate hybridisation. In this case the rate-determining step in binding to DNA and RNA may be the conformational reorganisation of the POM backbone into a more linear structure which enables base pairing to take place. To explore this possibility we prepared a POM/PNA chimera Lys-TC*AC*AAC*TT-NH_2, which contains three PNA cytosinyl units (C*). It is expected that the PNA monomers would be more flexible than the POM monomers and might therefore disrupt the formation of secondary structures, resulting in a more random coil in the single-stranded state, which might facilitate faster hybridisation. Despite this, the POM/PNA chimera did not show any evidence of hybridisation with complementary parallel and antiparallel RNA or DNA in UV thermal denaturation/renaturation experiments. Whilst this is not informative for rationalising the slow rates of hybridisation of POM with DNA or RNA, it does demonstrate how the behaviour of a chimera cannot be used to understand the properties of uniformly modified oligomers *per se*. For example, many studies with PNA analogues, including pyrrolidine-modified PNAs, are based on the effects of inserting one or more new modified monomers within a PNA strand resulting in chimeras.[15] Typically, if the T_m of the chimera is higher than the standard unmodified PNA the modification is viewed as potentially useful, whilst modifications that reduce the T_m are discarded.[15] Clearly, the results presented here show the obvious danger of basing any conclusions on the chimeric approach. Indeed, in the absence of any other information about PNA, if we were to follow the logic described above, we would conclude that PNA is not a potentially useful nucleic acid mimic, because insertion of PNA monomers into a POM abolishes its ability to hybridise with DNA and RNA. Moreover, the results presented here and earlier[1–5] also show that whilst studying the DNA and RNA properties of fully modified homopolymers is better than using chimeras, it too can lead to conclusions which are not necessarily general. Indeed, it is well known that DNA homopolymers, for example, behave differently to random, mixed-sequence nucleic acids.[40,41] Thus if any nucleic acid mimic is to be considered for potential DNA and RNA hybridisation-based applications, a variety of fully modified mixed sequences must necessarily be evaluated. To this end we are currently investigating the biophysical properties of other mixed-sequence POMs, which will be the subject of future publications.

Conclusion

Boc-solid phase chemistry, with benzyloxycarbonyl (Z)-nucleobase protecting groups, has been used in the early synthesis of PNA oligomers. To investigate whether this approach can be applied to the synthesis of pyrrolidine-amide oligonucleotide mimics, Boc-Z-protected thyminyl, adeninyl and cytosinyl monomers have been prepared. Solid phase synthesis of POM oligomers using the Boc-Z approach proved much more efficient than the Fmoc approach which had been investigated earlier.[1] Indeed, the Boc-Z chemistry enabled the synthesis of the first longer mixed-sequence POM Lys-TCACAACTT-NH_2 to be completed, in an overall yield of 61%, with an average coupling efficiency of 95%. UV thermal denaturation studies with this more biologically relevant POM oligomer and complementary DNA and RNA targets were then performed. This revealed that the POM Lys-TCACAACTT-NH_2 is capable of hybridising with sequence specificity to both complementary parallel and antiparallel RNA and DNA strands. The hysteresis between the heating (denaturation) and cooling (renaturation) curves in these UV melting experiments indicated that the rates of association/dissociation of the mixed-sequence POM with DNA/RNA targets are slow compared with hybridisation of isosequential PNA, DNA and RNA oligomers. Interestingly, the introduction of PNA monomers into the POM mixed-sequence strand, to form a chimera, completely abolishes the hybridisation with DNA and RNA. Currently, several more mixed-sequence

POMs, including structural variants, which are designed for duplex and triplex formation, are being evaluated in order to assess the generality of these findings.

Experimental

(2*R*,4*R*)-2-Azidomethyl-4-hydroxy-*N*-(methoxycarbonylmethyl)-pyrrolidine (3)

To a stirred suspension of pyrrolidine HCl salt **2**[1] (11.5 g, 64.1 mmol) in anhydrous CH_2Cl_2 (80 mL), under N_2, was added diisopropylethylamine (DIEA) (25.7 mL, 147.5 mmol) at 0 °C until dissolution. Methyl bromoacetate (7.72 mL, 83.3 mmol) was added dropwise at 0 °C and the reaction mixture stirred for a further 30 min at this temperature followed by 18 h at room temperature. Solvent was subsequently removed under reduced pressure. Purification by column chromatography (gradient elution using 3 : 1 hexane–EtOAc → 1 : 1 hexane–EtOAc,) gave methyl ester **3** (10.9 g, 79%) as a pale yellow oil. R_f 0.37 (3 : 1 EtOAc–hexane); $[a]_D^{25}$ +36.5° (c = 1.1, $CHCl_3$); ν_{max} (KBr)/cm^{-1}: 3386 (OH), 2955 and 2858 (CH), 2101 (N_3), 1740 and 1204 (CO); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.67–1.74 (1H, m, H_a3), 2.32–2.42 (1H, m, H_b3), 2.67 (1H, d, J 8.2 Hz, OH), 2.95 (1H, dd, J 9.7, 3.9 Hz, H_a5), 3.09 (1H, dd, J 9.7, 0.7 Hz, H_b5), 3.14–3.20 (1H, m, H2), 3.37 (1H, dd, J 12.3, 4.3 Hz, H_a6), 3.49 (1H, dd, J 12.3, 4.3 Hz, H_b6), 3.52 (1H, d, J 17.4 Hz, H_a7), 3.60 (1H, d, J 17.4 Hz, H_b7), 3.71 (3H, s, OCH_3), 4.23–4.30 (1H, m, H4); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 38.6 (C3), 51.5 (CO_2CH_3), 52.5 (C7), 54.7 (C6), 59.9 (C2), 62.0 (C5), 70.6 (C4), 171.3 (CO_2CH_3); m/z (ES): 215.1 ([M + H]$^+$, 100%), 237.1 ([M + Na]$^+$, 60%); HRMS m/z (ES): 215.1138 ([M + H]$^+$, $C_8H_{15}N_4O_3$ requires m/z, 215.1144).

(2*R*,4*R*)-2-[(*tert*-Butoxycarbonyl)aminomethyl]-4-hydroxy-*N*1-(methoxycarbonylmethyl)-pyrrolidine (11)

To a solution of azide **3** (110.3 mg, 0.52 mmol) in THF (1.5 mL) was added PMe_3 (1 M solution in THF, 0.60 mL, 0.60 mmol) followed by 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) (155 mg, 0.63 mmol). The reaction mixture was stirred at room temperature for 1 h. Solvent was removed under reduced pressure and the crude product was purified by flash chromatography (EtOAc) to afford Boc-protected amine **11** (52.6 mg, 53%) as a pale yellow oil. R_f 0.2 (EtOAc); $[a]_D^{25}$ + 64.7° (c = 2, CH_3OH); ν_{max}(KBr)/cm^{-1}: 3381 br (OH), 1743 and 1692 (CO); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (9H, s, $C(CH_3)_3$), 1.66 (1H, dd, J 14.1, 5.4 Hz, H_a3'), 2.28 (1H, ddd, J 14.1, 9.1, 6.5, Hz, H_b3'), 2.72 (1H, dd, J 9.6, 3.7 Hz, H_a5'), 2.89–2.91 (1H, m, H2′), 3.06–3.13 (2H, m, H_a6' and H_b5'), 3.28–3.33 (1H, m, H_b6'), 3.31 (1H, d, J 17.2 Hz, H_a7'), 3.50 (1H, d, J 17.2 Hz, H_b7'), 3.69 (3H, s, OCH_3), 4.26 (1H, br s, H4), 5.44 (1H, br s, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 28.4 ($C(CH_3)_3$), 38.0 (C3′), 41.4 (C6′), 51.7 (CO_2CH_3), 53.0 (C7′), 61.1 (C2′), 62.5 (C5′), 70.1 (C4′), 79.1 ($C(CH_3)_3$), 156.6 ($CO_2{}^t$Bu), 171.6 (CO_2CH_3); m/z (ES): 289 ([M + H]$^+$ 100%); HRMS m/z (ES): 289.1756 ([M + H]$^+$, $C_{13}H_{25}O_5N_2$ requires m/z, 289.1758).

(2*R*,4*S*)-2-[(*tert*-Butoxycarbonyl)aminomethyl]-4-formyloxy-*N*-(methoxycarbonylmethyl)-pyrrolidine (12)

To a solution of alcohol **11** (2.11g, 7.32 mmol) in anhydrous THF (35 mL) was added PPh_3 (2.49 g, 9.49 mmol) and anhydrous HCO_2H (0.36 mL, 9.54 mmol). The solution was cooled to −20 °C and DIAD (1.90 mL, 9.65 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred under N_2 for 16 h. Solvent was removed under reduced pressure and flash chromatography (2 : 1 hexane–EtOAc) afforded formyl ester **12** (1.71 g, 74%) as a pale yellow oil. R_f 0.2 (2 : 1 hexane–EtOAc); $[a]_D^{25}$ +63.0° (c = 1.0, CH_3OH); ν_{max}(KBr;)/cm^{-1}: 3397 (NH), 1719 (CO); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (9H, s, $C(CH_3)_3$), 1.97–2.04 (2H, m, H_aH_b3), 2.67 (1H, dd, J 10.8, 3.1 Hz, H_a5), 3.03–3.12 (2H, m, H2, H_a6), 3.35 (1H, d, J 17.2 Hz, H_a7), 3.37–3.41 (1H, m, H_b6), 3.54 (1H,d, J 17.2 Hz, H_b7), 3.67 (1H, dd, J 10.8, 6.0 Hz H_b5), 3.72 (3H, s, OCH_3), 5.09, (1H, br s, NH), 5.26 (1H, br s, H4), 8.00 (1H, s, CHO); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 28.3 ($C(CH_3)_3$), 34. 9 (C3), 40.5 (C6), 51.7 (O-CH_3), 53.8 (C7), 59.7 (C5), 61.0 (C2), 72.5 (C4), 79.2 ($C(CH_3)_3$), 156.2 ($CO_2{}^t$Bu), 160.5 (CHO), 171.3 (CO_2Me); m/z (ES): 399 ([M + Na]$^+$ 100%), 317 ([M + H]$^+$ 20%); HRMS m/z (ES): 317.1706 ([M + H]$^+$, $C_{13}H_{25}O_5N_2$ requires m/z, 317.1707).

(2*R*,4*S*)-2-[(*tert*-Butoxycarbonyl)aminomethyl]-4-hydroxy-*N*1-(methoxycarbonylmethyl)-pyrrolidine (13)

To a solution of formyl ester **12** (750 mg, 2.37 mmol) in anhydrous CH_3OH (5 mL) was added anhydrous sodium methoxide (20 mg, 0.37 mmol). The reaction mixture was stirred under N_2 at room temperature for 1.5 h. Solvent was removed under reduced pressure and column chromatography (EtOAc) afforded alcohol **13** (597 mg, 87%) as a pale yellow oil. R_f 0.2 (EtOAc); $[a]_D^{25}$ +49.6° (c = 2.0, CH_3OH); ν_{max}(KBr)/cm^{-1}: 3405br (OH), 1735 and 1688 (CO); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (9H, s, $C(CH_3)_3$), 1.78 (1H, ddd, J 13.2, 9.7, 5.2 Hz H_a3), 1.91 (1H, dd, J 13.2, 6.4 Hz, H_b3), 2.66 (1H, d, J 11.1 Hz, H_a5), 3.01 (1H, ddd, J 13.6, 4.2, 3.6, Hz, H_a6), 3.20–3.36 (2H, m, H2 and H_b6), 3.46 (1H, d, J 11.1 Hz, H_b5), 3.49 (1H, d, J 17.9 Hz, H_a7), 3.56 (1H, d, J 17.9 Hz, H_b7), 3.71 (3H, s, OCH_3), 4.27 (1H, br s, H4), 4.96, (1H, br s, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 28.4 ($C(CH_3)_3$), 29.7 (C3), 39.0 (C6), 51.8 (CO_2CH_3), 52.5 (C7), 60.1 (C2), 62.0 (C5), 70.9 (C4) 79.2 ($C(CH_3)_3$), 156.3 ($CO_2{}^t$Bu), 172.9 (CO_2CH_3); m/z (ES): 289 ([M + H]$^+$ 50%); HRMS m/z (ES): 289.1768 ([M + H]$^+$, $C_{13}H_{25}O_5N_2$ required m/z, 289.1763).

(2*R*,4*S*)-2-[(*tert*-Butoxycarbonyl)aminomethyl]-4-(toluene-4-sulfonyloxy)-*N*1-(methoxycarbonylmethyl)-pyrrolidine (14)

To a solution of alcohol **11** (1.50 g, 5.22 mmol) in anhydrous THF (50 mL) at −20 °C was added DIAD (1.24 mL, 6.30 mmol) and methyl *p*-toluenesulfonate (0.94 mL, 6.23 mmol) followed by PPh_3 (1.64 g, 6.25 mmol) portionwise under N_2. The reaction mixture was allowed to warm to room temperature and stirred under N_2 for 20 h. Solvent was removed under reduced pressure and the crude product purified by flash chromatography (1 : 1 Et_2O–CH_3Ph) to afford *p*-toluenesulfonate **14** (1.35 g, 59%) as a pale yellow oil. R_f 0.4 (1 : 1 Et_2O–CH_3Ph); $[a]_D^{25}$ +24.4° (c = 2.0, CH_3OH); ν_{max}(KBr)/cm^{-1}: 3400 (NH), 1750 and 1712 (CO), 1361 and 1186 (SO_2O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (9H, s, $C(CH_3)_3$), 1.84 (1H, ddd, J 15.1, 14.7, 7.8 Hz, H_a3'), 1.97 (1H, dd, J 14.7, 5.1 Hz, H_b3'), 2.45 (3H, s, tosyl CH_3), 2.75 (1H, dd, J 11.3, 3.7 Hz, H_a5'), 2.97 (1H, ddd, J 14.0, 3.6, 3.3 Hz, H_a6'), 3.01–3.06 (1H, m, H2′), 3.22–3.36 (1H, m, H_b6'), 3.29 (1H, d, J 17.0 Hz, H_a7'),

3.48 (1H, d, *J* 17.0 Hz, H_b7'), 3.51 (1H, dd, *J* 11.3, 5.7 Hz, H_b5'), 3.70 (3H, s, OCH_3), 4.87 (1H, br s, H4′), 5.05 (1H, br s, NH), 7.34 (2H, d, *J* 8.2 Hz, tosyl aromatic), 7.76 (2H, d, *J* 8.2 Hz, tosyl aromatic); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 21.7 (tosyl CH_3), 28.3 ($C(CH_3)_3$), 35.1 (C3′), 40.5 (C6′), 51.8 (CO_2CH_3), 54.2 (C7′), 59.9 (C5′), 61.3 (C2′), 79.3 ($C(CH_3)_3$), 79.6 (C4′), 127.7 (tosyl aromatic CH), 129.9 (tosyl aromatic CH), 133.5 (tosyl *p*-C), 144.9 (tosyl *ipso*-C), 156.2 ($CO_2{}^t$Bu), 171.4 (CO_2CH_3); *m*/*z* (ES): 443 ([M + H]$^+$ 100%); HRMS *m*/*z* (ES): 443.1857 ([M + H]$^+$, $C_{20}H_{31}O_7N_2S$ requires *m*/*z*, 443.1852).

(2′*R*,4′*R*)-2′-[(*tert*-Butoxycarbonyl)aminomethyl]-4′-(N^3-benzoylthymin-1-yl)-*N*1′-(methoxycarbonylmethyl)-pyrrolidine (8)

To a solution of alcohol **13** (574 mg, 1.99 mmol) in anhydrous THF (7.5 mL) was added N^3-benzoylthymine[42] (555 mg, 2.41 mmol), and PPh_3 (628 mg, 2.39 mmol) under N_2. The mixture was cooled to −20 °C and DIAD (0.54 mL, 2.74 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. Solvent was removed under reduced pressure and flash chromatography (1 : 1 hexane–EtOAc, R_f 0.3) afforded thyminyl derivative **8** (681 mg, 68%) as a white foam. Mp 75–77 °C (hexane/EtOAc); $[\alpha]_D^{25}$ −25.07 (*c* = 0.5, CH_3OH); ν_{max} (NaCl)/cm^{-1}: 3376 (NH), 2978 (CH), 1745, 1698 and 1653 (CO); λ_{max}(CH_3OH)/nm 253.0; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (s, 9 H, $C(CH_3)_3$), 1.70 (1H, m, H_a3'), 1.89 (3H, s, thymine CH_3), 2.43 (1H, ddd, *J* 15.0, 8.0, 8.0 Hz, H_b3'), 2.55–2.75 (2H, m, H_a5' and H2′), 2.99 (1H d, *J* 14.5 Hz, H_a6'), 3.08 (1H, d, *J* 17.5 Hz, H_a7'), 3.19 (1H, d, *J* 11.0 Hz, H_b5'), 3.33 (1H, dd, *J* 14.5, 8.5 Hz, H_b6'), 3.49 (1H, d, *J* 17.5 Hz, H_b7'), 3.63 (3H, s, OCH_3), 4.93 (1H, m, H4′), 7.34 (2H, t, *J* 7.5 Hz, Bz CH), 7.49 (2H, t, *J* 7.5 Hz, Bz CH), 7.76 (1H, d, *J* 8.0 Hz, Bz CH), 8.02 (1H, s, H6); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 13.2 (thymine CH_3), 28.7 ($C(CH_3)_3$), 36.4 (C3′), 40.3 (C6′), 52.3 (OCH_3), 52.4 (C4′), 53.2 (C7′), 59.5 (C5′), 63.2 (C2′), 80.0 ($C(CH_3)_3$), 111.5 (C5), 129.5 (Bz CH), 130.8 (Bz CH), 132.1 (Bz C), 135.3 (Bz CH), 138.2 (C6), 150.4 (C2), 156.7 ($CO_2{}^t$Bu), 163.2 (C4), 169.6 (Bz CO), 171.7 (CO_2CH_3); *m*/*z* (ES): 501.1 ([M + H]$^+$, 80%); HRMS *m*/*z* (ES): 501.2344 ([M + H]$^+$, $C_{25}H_{33}N_4O_7$ requires *m*/*z*, 501.2349).

(2′*R*,4′*R*)-2′-(*tert*-Butoxycarbonylaminomethyl)-4′-(thymin-1-yl)-pyrrolidine-1′-yl-acetic acid (10)

To a solution of benzoyl-protected derivative **8** (650 mg, 1.30 mmol) in THF (5 mL) was added 1 M aqueous NaOH (3.25 mL, 3.25 mmol). The mixture was stirred at room temperature for 3 h then a second portion of 1 M aqueous NaOH (3.25 mL, 3.25 mmol) was added to the reaction mixture which was stirred for a further 3 h. THF was removed under a stream of nitrogen and the pH of the remaining aqueous solution was adjusted to 7 by addition of 0.1 M aqueous HCl. Water was removed under reduced pressure and the resulting white residue was submitted to column chromatography (EtOAc–CH_3OH 7 : 3) followed by reversed-phase chromatography (BondElut® C18, H_2O–CH_3CN 9 : 1) to afford acid **10** (361 mg, 73%) as a white solid. Mp 185–186 °C (H_2O); $[\alpha]_D^{25}$ +38.9° (*c* = 0.5, CH_3OH); ν_{max}(KBr)/cm^{-1}: 3406 (NH), 2979 (CH), 1693 (CO); λ_{max}(CH_3OH)/nm: 271.0; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (9H, s, $C(CH_3)_3$), 1.53 (1H, m, H_a3'), 1.97 (3H, s, thymine CH_3), 2.44 (1H, ddd, *J* 14.0, 9.0, 8.5, Hz, H_b3'), 2.51–2.59 (2H, m, $H_a5'$and H2′), 2.70 (1H, d, *J* 14.5 Hz, H_a6'), 3.08 (1H, dd, *J* 14.5, 3.0 Hz, H_a7'), 3.26 (1H, d, *J* 11.5 Hz, H_b5'), 3.33 (1H, d, *J* 14.5 Hz, H_b6), 3.45 (1H, d, *J* 14.5 Hz, H_b7'), 4.83–4.95 (1H, m, H4′), 8.30 (1H, s, H6);^{13}C NMR (75.4 MHz, $CDCl_3$): δ 13.2 (thymine CH_3), 29.1 ($C(CH_3)_3$), 37.1 (C3′), 40.4 (C6′), 54.0 (C4′), 59.5 (C7′), 60.5 (C5′), 65.3 (C2′), 80.1 ($C(CH_3)_3$), 111.8 (C5), 141.5 (C6), 154.1 (C2), 159.5 ($CO_2{}^t$Bu), 168.0 (C4), 179.5, (CO_2H); *m*/*z* (ES): 405 ([M + Na]$^+$, 20%), 383.1 ([M + H]$^+$, 45), 327.1 ([M − ^{t}Bu]$^+$, 100); HRMS *m*/*z* (ES): 383.1922 ([M + H]$^+$, $C_{17}H_{27}N_4O_6$ require *m*/*z*, 383.1931).

(2′*R*,4′*R*)-2′-[(*tert*-Butoxycarbonyl)aminomethyl]-4′-(adenin-9-yl)-*N*1′-(methoxycarbonylmethyl)-pyrrolidine (15)

To a solution of *p*-toluenesulfonate **14** (270 mg, 0.61 mmol) in anhydrous DMF (4.5 mL) was added N^6-benzyloxycarbonyladenine (280 mg, 1.04 mmol), K_2CO_3 (144 mg, 1.04 mmol) and 18-crown-6 (81 mg, 0.31 mmol). The reaction mixture was stirred at 80 °C under N_2 for 4 h. H_2O (250 mL) was added to the reaction mixture and the mixture was extracted with EtOAc (5 × 250 mL). The organic fractions were combined and dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The crude product was purified by flash chromatography (3% CH_3OH in $CHCl_3$) to afford adeninyl derivative **15** (111 mg, 45%) as a white foam. R_f 0.1 (3% CH_3OH in $CHCl_3$); mp 52–54 °C ($CHCl_3$); $[\alpha]_D^{25}$ +65.9° (*c* = 2.0, CH_3OH); ν_{max}(KBr)/cm^{-1}: 3327 and 3183 (NH), 1743 and 1696 (CO); λ_{max}(CH_3OH)/nm: 261.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (9H, s, $C(CH_3)_3$), 1.92 (1H, ddd, *J* 14.3, 7.0, 2.5 Hz, H_a3'), 2.63 (1H, ddd, *J* 14.3, 8.6, 8.4 Hz, H_b3'), 2.94–2.98 (1H, m, H2′), 3.02 (1H, dd, *J* 10.4, 6.3 Hz, H_a5'), 3.11 (1H, d, *J* 14.4 Hz, H_a6), 3.30 (1H, d, *J* 17.4 Hz, H_a7'), 3.36 (1H, dd, *J* 14.4, 8.3 Hz, H_b6'), 3.54 (1H, d, *J* 10.4 Hz, H_b5'), 3.67 (1H, d, *J* 17.4 Hz, H_b7'), 3.72 (3H, s, OCH_3), 5.06 (1H, br s, H4), 5.34 (1H, br s, NH), 6.22 (2H, br s, NH_2), 8.29 (1H, s, H2), 8.41 (1H, s, H8); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 28.2 ($C(CH_3)_3$), 36.1 (C3′), 40.4 (C6′), 51.7 (CO_2CH_3), 51.8 (C2′), 52.8 (C7′), 59.4 (C5′), 62.0 (C4′), 79.3 ($C(CH_3)_3$), 119.4 (C5), 139.4 (C8), 149.5 (C4), 152.4 (C2), 155.4 (C6), 156.2 ($CO_2{}^t$Bu), 171.2 (CO_2CH_3); *m*/*z* (ES): 406 ([M + H]$^+$ 100%); HRMS *m*/*z* (ES): 406.2194 ([M + H]$^+$, $C_{18}H_{28}O_4N_7$ requires *m*/*z*, 406.2197).

(2′*R*,4′*R*)-2′-(*tert*-Butoxycarbonylaminomethyl)-4′-(N^6-benzyloxycarbonyladenin-9-yl)-*N*1′-(methoxycarbonylmethyl)-pyrrolidine (16)

To a freshly prepared solution of 1-(benzyloxycarbonyl)-3-ethylimidazolium tetrafluoroborate ('Rapoport's reagent')[34] (1.49 g, 4.7 mmol) in anhydrous CH_2Cl_2 (6 mL) was added adenine derivative **15** (200 mg, 0.49 mmol) and the reaction mixture stirred at room temperature under N_2 for 18 h. Saturated aqueous $NaHCO_3$ (10 mL) was then added to the reaction mixture and the aqueous solution was extracted with CH_2Cl_2 (4 × 25 mL). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (2% CH_3OH in $CHCl_3$) to afford the benzyloxycarbonyl-protected product **16** (158 mg, 60%) as a white foam. R_f 0.1 (2% CH_3OH in $CHCl_3$); mp 58–60 °C ($CHCl_3$); $[\alpha]_D^{25}$ +58.0° (*c* = 1.0, CH_3OH); ν_{max}(KBr)/cm^{-1}: 3373 (NH), 1747 and 1708 (CO), 1610 (aromatic C=C); λ_{max}(CH_3OH)/nm: 271.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (9H, s, $C(CH_3)_3$); 1.86 (1H,

dd, *J* 16.7, 7.2 Hz, H_a3'), 2.61 (1H, ddd, J 16.7, 14.3, 8.6 Hz, H_b3'), 2.87–2.93 (1H, m, H2′), 2.97–3.05 (2H, m, H_a5' and H_a6'), 3.26 (1H, d, *J* 17.4 Hz, H_a7'), 3.33 (1H, dd, *J* 15.3, 8.5 Hz, H_b6'), 3.47 (1H, d, *J* 10.5 Hz, H_b5'), 3.60 (1H, d, *J* 17.4 Hz, H_b7'), 3.69 (3H, s, OCH_3), 4.94 (1H, br s, H4′), 5.09 (1H, br s, NH), 5.24 (2H, s, benzyl CH_2), 7.28–7.34 (3H, m, benzyl aromatic), 7.39 (2H, d, *J* 6.6 Hz, benzyl aromatic), 8.02 (1H, s, H2), 8.68 (1H, s, H8); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 28.1 (C(CH_3)$_3$), 36.0 (C3′), 40.5 (C7′), 41.7 (C2′), 51.9 (C6′), 52.7 (CO_2CH_3), 59.2 (C5′), 61.9 (C4′), 67.5 (benzyl CH_2), 79.3 (C(CH_3)$_3$), 121.9 (C5), 128.4 (benzyl aromatic CH), 129.1 (benzyl CH), 129.2 (benzyl CH), 135.5 (benzyl *ipso*-C), 141.8 (C8), 149.3 (C4), 151.1 (C6), 152.4 (C2), 156.1 (CO_2′Bu), 156.2 (CO_2Bn), 171.1 (CO_2CH_3); *m*/*z* (ES): 540 ([M + H]$^+$ 100%); HRMS *m*/*z* (ES): 540.2567 ([M + H]$^+$, $C_{26}H_{34}O_6N_7$ requires *m*/*z*, 540.2565).

(2′*R*,4′*R*)-2′-(*tert*-Butoxycarbonylaminomethyl)-4′-(*N*^{6}-benzyloxycarbonyladenin-9-yl)-pyrrolidine-1-yl-acetic acid (17)

To a solution of methyl ester **16** (30.6 mg, 0.057 mmol) in THF (360 μL) was added a 1 M aqueous solution of NaOH (62 μL, 0.062 mmol). The reaction mixture was stirred at room temperature for 4 h. 1 M aqueous NaOH (28 μL, 0.028 mmol) was added and the reaction mixture stirred for a further 2 h at room temperature. THF was then removed under a stream of N_2 and the pH of the resulting aqueous solution was adjusted to 7 by dropwise addition of aqueous HCl (0.02 M). H_2O was then removed under reduced pressure and the crude product was purified by reversed-phase (C18) column chromatography (H_2O → CH_3CN) to afford Boc-protected acid monomer **17** (21.3 mg, 72%) as a white powder. Mp 120–121 °C (H_2O); $[a]_D^{25}$ +58.8° (*c* = 0.5, CH_3OH); ν_{max}(KBr)/cm^{-1}: 3370 (NH), 1746 (CO); λ_{max}(CH_3OH)/nm: 269.0; ^{1}H NMR (400MHz, CD_3OD): δ 1.13 (9H, s, C(CH_3)$_3$), 1.74 (1H, dd, *J* 14.1, 6.6 Hz, H_a3'), 2.62 (1H, ddd, *J* 14.1, 8.8, 8.4 Hz, H_b3'), 2.71 (1H, dd, *J* 8.4, 6.6 Hz, H2′), 2.79 (1H, dd, *J* 10.8, 5.7 Hz, H_a5'), 2.85 (1H, d, *J* 14.9 Hz, H_a7'), 3.06 (1H, dd, *J* 14.5, 2.6 Hz, H_a6'), 3.35 (1H, br s, H_b6'), 3.51 (1H, d, *J* 14.9 Hz, H_b7'), 3.58 (1H, d, *J* 10.8 Hz, H_b5'), 5.08–5.11 (1H, m, H4′), 5.31 (2H, s, benzyl CH_2), 7.31–7.40 (3H, m, benzyl aromatic), 7.47 (2H, d *J* 7.1 Hz, benzyl aromatic), 8.57 (1H, s, H2), 9.06 (1H, s, H8); ^{13}C NMR (100.6 MHz, CD_3OD): δ 28.5 (C(CH_3)$_3$), 37.0 (C3′), 40.2 (C6′), 53.7 (C2′), 58.8 (C7′), 60.5 (C5′), 64.5 (C4′), 68.4 (benzyl CH_2), 79.6 (C(CH_3)$_3$), 122.9 (C5), 129.3 (benzyl aromatic CH), 129.4 (benzyl aromatic CH), 129.6 (benzyl aromatic CH), 137.5 (benzyl *ipso*-C), 144.9 (C8), 150.6 (C4), 152.4 (C6), 152.8 (C2), 153.5 (CO_2′Bu), 158.8 (CO_2Bn), 179.1 (CO_2H); *m*/*z* (ES): 524 ([M − H]$^-$ 100%); HRMS (ES): 548.2224 ([M + Na]$^+$, $C_{25}H_{31}O_6N_7Na$ requires *m*/*z*, 548.2228).

(2′*R*,4′*R*)-2′-(*tert*-Butoxycarbonylaminomethyl)-4′-(*N*^{4}-[para-(*tert*-butyl)benzoyl]cytosin-1-yl)-*N*1′-(methoxycarbonylmethyl)-pyrrolidine (22)

A suspension of tosylate **14** (120 mg, 0.271 mmol), N^4-[*p*-(*tert*-butyl)benzoyl]cytosine (186 mg, 0.67 mmol), K_2CO_3 (190 mg, 1.38 mmol) and 18-crown-6 (27 mg, 0.102 mmol) in anhydrous DMF (1.4 mL) was stirred at 75 °C under N_2 for 18 h. The solvent was removed under reduced pressure and brine (5 mL) was added to the resulting brown paste. The mixture was extracted with EtOAc (4 × 10 mL) and the combined organic extracts were dried over $MgSO_4$, and then evaporated under reduced pressure. Purification by column chromatography (5% CH_3OH in EtOAc) afforded product **22** (68 mg, 46%) as a white foam R_f 0.20 (EtOAc); mp 99–101 °C (from $CHCl_3$, decomp.); $[a]_D^{25}$ −36.2° (*c* = 0.89, $CHCl_3$); ν_{max} (KBr)/cm^{-1}: 3269 (NH), 1749 and 1699 (CO); λ_{max} ($CHCl_3$)/nm 266; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (9H, s, Bz-C(CH_3)$_3$), 1.35 and 1.38 (9H, 2 × s, Boc C(CH_3)$_3$), 2.35–2.48 (1H, m, H_a3'), 2.48–2.92 (3H, m, H_a5', H_b3' and H2′), 2.95–3.25 (2H, m, H_a7' and H_a6'), 3.28–3.93 (6H, m, OCH_3, H_b7', H_b6' and H_b5'), 4.78 and 5.13 (1H, 2 × br s, H4′ rotamers), 7.24–7.53 (3H, m, H5 and *m*-Bz H rotamers), 7.61 and 7.72 (1H, 2 × br s, BocNH rotamers), 7.83–7.97 (2H, m, *o*-Bz H rotamers), 8.64 (1H, d, *J* 6.9 Hz, H6), 11.29 (1H, br s, BzNH); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 28.0 and 28.2 (Bz C(CH_3)$_3$ rotamers), 31.0 and 31.1 (Boc C(CH_3)$_3$ rotamers), 34.8 (Bz C(CH_3)$_3$), 35.0 (C3′), 40.0 (C6′), 51.6 and 51.9 (CO_2CH_3 rotamers), 53.3 (C7′), 55.3 (C4′), 56.5 (C5′), 62.3 (C2′), 79.3 and 79.4 (Boc C(CH_3)$_3$ rotamers), 96.8 (C5), 124.8 and 125.8 (Bz CH rotamers), 126.9 (Bz CH), 127.3 and 127.7 (Bz CH rotamers), 128.3 and 128.6 (Bz CH rotamers), 130.0 and 131.1 (Bz *p*-C rotamers), 147.4 (C6), 155.0 (Boc CO), 155.8 and 156.2 (C2 rotamers), 156.8 and 157.0 (Bz *ipso*-C rotamers), 162.9 (C4), 167.9 (Bz CO), 171.0 (CO_2CH_3); *m*/*z* (ES): 542.3 ([M + H]$^+$, 100%); HRMS *m*/*z* (ES): 542.2977 ([M + H]$^+$, $C_{28}H_{40}N_5O_6$ requires *m*/*z*, 542.2979).

(2′*R*,4′*R*)-2′-(*tert*-Butoxycarbonylaminomethyl)-4′-cytosin-1-yl-*N*1′-(methoxycarbonylmethyl)-pyrrolidine (23)

$NaOCH_3$ (35.6 mg, 0.659 mmol) was dissolved in anhydrous CH_3OH under a stream of N_2 (3.40 mL) with the aid of sonication. This solution was cooled to 0 °C, before being added to *tert*-butyl benzoyl-protected cytosine derivative **22** (340 mg, 0.628 mmol). The mixture was stirred at room temperature under N_2 for 10 h and solvent was removed under reduced pressure. Purification by column chromatography (gradient elution: 1 : 1 EtOAc–hexane → EtOAc → 1:4 CH_3OH–EtOAc) gave give cytosine derivative **23** (211 mg, 88%) as a white foam. R_f 0.13 (5% EtOH in CH_2Cl_2); $[a]_D^{25}$ −21.1° (*c* = 0.35, $CHCl_3$); ν_{max} (KBr)/cm^{-1}: 3334, 3210 (NH), 1747, 1700, 1653 (CO); λ_{max} (CH_3OH)/nm 277.0; ^{1}H NMR (300 MHz, CD_3OD): δ 1.32 (9H, s, C(CH_3)$_3$), 1.43–1.56 (1H, m, H_a3'), 2.38–2.54 (1H, m, H_b3'), 2.60–2.73 (2H, m, H2′ and H_a5'), 2.99–3.15 (3H, m, H_a7', $H_a6'$and H_b6'), 3.25–3.32 (1H, m, H_b5'), 3.65 (3H, s, OCH_3), 3.74 (1H, d, *J* 17.4 Hz, H_b7'), 4.85–4.93 (1H, m, H4′), 5.84 (1H, d, *J* 7.4 Hz, H5), 6.48 (1H, br t, *J* 5.5 Hz, BocNH), 8.32 (1H, d, *J* 7.4 Hz, H6); ^{13}C NMR (75 MHz, CD_3OD): δ 28.8 (C(CH_3)$_3$), 37.5 (C3′), 41.2 (C6′), 52.3 (CO_2CH_3), 53.9 (C7′), 54.9 (C4′), 59.3 (C5′), 63.8 (C2′), 80.0 (C(CH_3)$_3$), 96.1 (C5), 145.0 (C6), 158.6 (Boc CO), 158.9 (C2), 167.2 (C4), 173.3 (CO_2CH_3); *m*/*z* (ES): 282.2 ([M − CO_2′Bu + H]$^+$, 100%); 382.2 ([M + H]$^+$, 80%); 402.2 ([M + Na]$^+$, 80%); HRMS *m*/*z* (ES): 382.2084 ([M + H]$^+$, $C_{17}H_{28}N_5O_5$ requires *m*/*z*, 382.2090).

(2′*R*,4′*R*)-2′-(*tert*-Butoxycarbonylaminomethyl)-4′-(*N*^{4}-benzyloxycarbonylcytosin-1-yl)-*N*-(methoxycarbonylmethyl)-pyrrolidine (24)

Method A. *N*-(Benzyloxycarbonyl)imidazole (923 mg, 4.56 mmol) was dissolved in anhydrous CH_2Cl_2 (922 μL) under a stream of N_2. The stirred solution was cooled to 0 °C before

$Et_3O{\cdot}BF_4$ (2.74 mL 1 M solution in anhydrous CH_2Cl_2, 2.74 mmol) was added. After 7.5 h stirring under N_2 the cytosine derivative **23** (174 mg, 456 μmol) in CH_2Cl_2 (174 μL) was transferred into the reaction mixture which was stirred for a further 14.5 h. The reaction mixture was cooled to 0 °C and saturated $NaHCO_3$ (9.2 mL) was added. The mixture was partitioned between CH_2Cl_2 (30 mL) and H_2O (30 mL) and the organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure. Purification by column chromatography (gradient elution: 1 : 1 EtOAc–hexane → EtOAc → 1 : 4 CH_3OH–EtOAc) gave Z-protected cytosine derivative **24** (216 mg, 92%) as a white foam.

Method B. To a solution of alcohol **13** (650 mg, 2.25 mmol) in anhydrous THF (8.5 mL) under N_2 was added N^4-benzyloxycarbonylcytosine (670 mg, 2.73 mmol) and PPh_3 (715 mg, 2.73 mmol). The reaction mixture was cooled to −20 °C and DIAD (610 μL, 3.10 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred under N_2 for 18 h. Solvent was removed under reduced pressure and the residue was purified by flash chromatography, as described above, to give the Z-protected cytosine derivative **24** (340 mg, 29%) as a white foam. R_f 0.28 (5% EtOH/EtOAc); $[\alpha]_D^{25}$ −42.7° ($c = 0.88$, $CHCl_3$); ν_{max} (KBr)/cm^{-1}: 3249, 1622 and 1505 (NH), 2984, 2930 and 2844 (CH), 1747 and 1696 (CO), 1225 and 992 (NCOO); λ_{max} (CH_3OH)/nm 239.0; ^{1}H NMR (300 MHz, CH_3OD): δ 1.28 (9H, s, $C(CH_3)_3$), 1.47–1.61 (1H, m, H_a3'), 2.42–2.59 (1H, m, H_b3'), 2.63–2.79 (2H, m, H_a5' and H2′), 3.04–3.15 (3H, m, H_a6', H_b6' and H_a7'), 3.36 (1H, br d, J 11.3 Hz, H_b5'), 3.67 (3H, s, CO_2CH_3), 3.77 (1H, d, J 17.4 Hz, H_b7'), 4.83–4.92 (1H, m, H4′), 5.17 (2H, AB q, J 12.4 Hz, Bn CH_2), 7.18–7.46 (6H, m, Bn aromatic H and H5), 8.76 (1H, d, J 7.5 Hz, H6); ^{13}C NMR (75 MHz, CD_3OD): δ 28.7 ($C(CH_3)_3$), 37.1 (C3′), 40.8 (C6′), 52.3 (CO_2CH_3), 53.6 (C7′), 56.1 (C4′), 58.8 (C5′), 63.6 (C2′), 68.5 (Cbz CH_2), 80.0 ($C(CH_3)_3$), 96.9 (C5), 129.3 (*o*- and *p*-Z-CH), 129.6 (*m*-Z-CH), 137.3 (*ipso*-Z-C), 148.6 (C6 cytosine), 154.6 (Z-CO), 158.5 (Boc CO), 158.5 (C2), 164.1 (C4), 173.3 (CO_2CH_3); m/z (ES): 516.2 ([M + H]$^+$, 100%); HRMS m/z (ES): 516.2450 ([M + H]$^+$, $C_{25}H_{34}N_5O_7$ requires m/z, 516.2458).

(2′*R*,4′*R*)-2′-(*tert*-Butoxycarbonylaminomethyl)-4′-(*N*^{4}-benzyloxycarbonylcytosin-1-yl)-*N*1′-(methoxycarbonylmethyl)-pyrrolidine-1-yl-acetic acid (25)

Methyl ester **24** (216 mg, 419 μmol) was dissolved in THF (864 μL) and 1 M NaOH aq. (440 μL, 440 μmol) was added to the stirred solution at room temperature. After 6 h, the reaction was cooled to 0 °C and 0.1 M HCl aq. (4.40 mL) was added to neutralize the mixture. The solvent was evaporated under reduced pressure and the resulting white powder was purified by silica gel column chromatography (gradient elution: $CHCl_3$ → 1 : 4 CH_3OH–$CHCl_3$) followed by C18 reversed-phase column chromatography (Varian BondElut, 10 g cartridge, gradient elution H_2O → MeOH) to give the cytosinyl Boc-acid **25** (190 mg, 90%) as a white powder powder. R_f 0.29 (30% CH_3OH in $CHCl_3$); mp 134 °C (from H_2O, decomp.); $[\alpha]_D^{25}$ −146.4° ($c = 0.87$, CH_3OH); λ_{max} (CH_3OH)/nm 239 (ε/dm^3 mol^{-1} cm^{-1} 2.67 × 10^4); ^{1}H NMR (400 MHz, CD_3OD): δ 1.24 (9H, s, $C(CH_3)_3$), 1.59–1.66 (1H, m, H_a3'), 2.46–2.56 (1H, m, H_b3'), 2.71–2.81 (2H, m, H2′ and H_a5'), 2.90 (1H, d, J 15.8 Hz, H_a7'), 3.06 (1H, d, J 12.3 Hz, H_a6'), 3.30 (1H, d, J 14.6 Hz, H_b6'), 3.46 (1H, d, J 11.4 Hz, H_b5'), 3.55 (1H, d, J 15.8 Hz, H_b7'), 4.75–4.87 (1H, m, H4′), 5.12 (1H, d, J 12.4 Hz, Cbz CH_2), 5.18 (1H, d, J 12.4 Hz, Cbz CH_2), 7.22–7.37 (6H, m, benzyl aromatic CH and H5), 8.62 (1H, d, J 6.4 Hz, H6); ^{13}C NMR (100.6 MHz, CD_3OD): δ 28.7 ($C(CH_3)_3$), 36.4 (C3′), 41.1 (C6′), 54.9 (C7′), 58.6 (C4′), 60.1 (C5′), 65.5 (C2′), 68.6 (Cbz CH_2), 80.3 ($C(CH_3)_3$), 97.4 (C5), 129.4 (Z Ar-CH), 129.5 (Z Ar-CH), 129.6 (*m*-Z-CH), 137.1 (*ipso*-Z-C), 150.2 (C6 cytosine), 154.3 (Z-CO), 158.2 (C2), 158.8 (Boc CO), 164.3 (C4), 178.5 (CO_2H); m/z (ES): 500 ([M − H]$^-$, 100%); HRMS m/z (ES): 524.2118 ([M + Na]$^+$, $C_{24}H_{32}N_5O_7Na$ requires m/z, 524.2121).

Solid phase synthesis of POM oligomers

Into a 1 mL solid phase synthesis vessel was weighed MBHA LL resin (100–200 mesh) (5 equiv. relative to the first monomer loaded). The resin was then washed three times with DMF and three times with CH_2Cl_2 (all washings use 1 mL per 25 μmol resin loading, with rotation of reaction vessel for 30 s each time unless stated otherwise). The resin was swelled overnight in CH_2Cl_2, and then the solvent was removed by vacuum suction. The resin was washed three times with DMF, once with 5% piperidine/DMF (with 4 min agitation) and three times with DMF–CH_2Cl_2 (1 : 1). In a separate small vial, monomers Boc-POM(T)-OH **10**, Boc-POM(A^z)-OH **17**, Boc-POM(C^z)-OH **25**, Boc-PNA(C^z)-OH or *N*-α-Boc-*N*-ε-2-chloro-Z-L-lysine (1 equiv.), HBTU (0.95 equiv.) and diisopropylethylamine (DIEA) (1.1 equiv.) in DMF–pyridine (3 : 1) (total monomer concentration of 0.1 M) were allowed to activate for 3 min. The mixture was then added to the resin. Coupling was allowed to proceed with agitation for 6 h for the first coupling. The coupling mixture was removed and the resin washed two times with DMF before a freshly prepared acetic anhydride–collidine–DMF (1 : 1 : 8, v/v/v) (1 mL per 25 μmol) mixture was added with agitation for 15 min. The acetylating reagent was removed by vacuum suction and the resin washed with DMF (three times), complete reaction was indicated by negative Kaiser test. The resin was then washed with 5% piperidine/DMF (once for 4 min) and DMF–CH_2Cl_2 (1 : 1) (three times).

Deprotection of the resin-bound Boc-protected POM oligomer was accomplished using TFA–*m*-cresol (95 : 5, v/v) (1 mL per 25 μmol) four times for 4 min each. The resin was washed with DMF–CH_2Cl_2 (1 : 1) (three times) and successful deprotection was indicated by a positive Kaiser test. The resin was then washed with pyridine (two times). Subsequent coupling employed monomers **10**, **17**, **25** or Boc-PNA(C^z)-OH (5 equiv.), HBTU (2.38 equiv.), DIEA (2.25 equiv.) and coupling times of 2 h. A second coupling onto adenine residues using monomer **10**, **17**, **25** or Boc-PNA(C^z)-OH (2.5 equiv.), HBTU (2.38 equiv.), DIEA (2.75 equiv.) and coupling times of 1 h was carried out. In the case of the terminal lysine residue, *N*-α-Boc-*N*-ε-2-chloro-Z-L-lysine (6 equiv.), HBTU (5.7 equiv.), and DIEA (6.6 equiv.) were used. Capping after subsequent couplings was carried out for 5 min. The coupling–capping–deprotection sequence was repeated until the desired oligomer was obtained. Deprotection of Z-protected nucleobases and cleavage of the oligomer from the resin was achieved using trifluoromethanesulfonic acid (TFMSA) *via* the 'low–high TFMSA' method.[26] During 'low TFMSA' treatment

the resin was treated with a solution of (TFA–DMS–*m*-cresol, 1 : 3 : 1, v/v/v) and a solution of (TFA–TFMSA, 9 : 1, v/v) (each 1 mL per 20 μmol resin loading) each separately cooled to 0 °C before being added to the resin, followed by agitation for 1 h. The cleavage mixture was removed by vacuum suction. 'High TFMSA' treatment was carried out by addition of a solution of TFMSA–TFA–*m*-cresol (1 : 8 : 1 v/v/v) (1 mL per 10 μmol resin loading) cooled to 0 °C before being added to the resin and agitated for 1 h. The cleavage mixture was removed by vacuum suction.

The cleavage solutions were separately concentrated under a stream of nitrogen to a volume of *ca.* 50 μL and the oligomer was precipitated from the cleavage mixtures by addition of a ten-fold excess of anhydrous Et_2O. The mixture was subject to centrifugation (10 min, 12 000 rpm at 4 °C) and the resulting pellet was redissolved in formic acid and diluted again with anhydrous Et_2O. The centrifugation process was repeated a further three times. After the final time the pellets were dissolved in water and lyophilised to give crude POM oligomers as off-white powders. The oligomers were then purified by semi-preparative reversed-phase HPLC on a C18 column (Phenomenex Gemini 5 μ C18, 250 × 10 mm) with a typical gradient of 0–10% acetonitrile with 0.1% HCO_2H–0.1% aqueous HCO_2H. Fractions collected were evaporated and lyophilised to give pure product as a white powder. Product purity was verified by analytical reversed-phase HPLC (Phenomenex Gemini 5 μ C18, 150 × 4.6 mm) and oligomers were characterised by MALDI-TOF mass spectrometry.

POM Lys-TCACAACTT-NH_2

Retention time on analytical HPLC was 26 min, using a Phenomenex Gemini 5 μ C18 150 × 4.6 mm analytical column. Solvent A was H_2O with 0.1% HCO_2H and solvent B was acetonitrile with 0.1% HCO_2H. The flow rate was 1 mL min^{-1} with 100% A for 9 min followed by a gradient from 100% A changing to 90% A with 10% B over 52 min. *m/z* MALDI-TOF MS: 2505 ($[M + H]^+$ 100%, $C_{111}H_{154}N_{51}O_{19}$ requires *m/z*, 2505.3), 2527 ($[M + Na]^+$ 90%, $C_{111}H_{153}N_{51}O_{19}Na$ requires *m/z*, 2527.2); 2543 ($[M + K]^+$ 70%, $C_{111}H_{153}N_{51}O_{19}K$ requires *m/z*, 2543.2).

POM-PNA chimera Lys-TC*AC*AAC*TT-NH_2

Retention time on analytical HPLC was 24 min, using a Phenomenex Gemini 5 μ C18 150 × 4.6 mm analytical column. Solvent A was H_2O with 0.1% HCO_2H and solvent B was acetonitrile with 0.1% HCO_2H. The flow rate was 1 mL min^{-1} with 100% A for 9 min followed by a gradient from 100% A changing to 90% A with 10% B over 52 min. *m/z* MALDI-TOF MS: 2512 ($[M + H]^+$ 100%, $C_{108}H_{148}N_{51}O_{22}$ requires *m/z*, 2512.2)

Acknowledgements

This work was funded by the BBSRC (research grant 36/B15998) and EPSRC (partial PhD studentships to R.J.W. and A.O.). We also thank Dr Robin Pritchard for X-ray crystallography and the EPSRC National Mass Spectrometry Centre (University of Wales, at Swansea).

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