Homopolymeric pyrrolidine-amide oligonucleotide mimics: Fmoc-synthesis and DNA/RNA binding properties†

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By chemically modifying or replacing the backbone of oligonucleotides it is possible to modulate the DNA and RNA recognition properties and fine-tune the physiochemical properties of oligomers. This is important because it challenges our understanding of natural nucleic acid structural and recognition properties and can lead to nucleic acid mimics with a wide range of applications in nucleic acid targeting, analysis or diagnostics. In this paper we describe the solid phase synthesis of pyrrolidine-amide oligonucleotide mimics (POMs) using Fmoc-peptide chemistry. This required the synthesis of adeninyl, cytosinyl, thyminyl and guaninyl pyrrolidine monomers, with Fmoc- and standard acyl-protecting groups on the exocyclic amino groups and nucleobases respectively. These monomers were used to synthesise several thyminyl and adeninyl POM pentamers, with modest coupling efficiency. The pentamers were purified by RP-HPLC, characterised by mass spectrometry and their DNA and RNA binding properties were investigated using UV thermal denaturation/renaturation experiments. This revealed that all the pentamers exhibit strong affinity for complementary nucleic acids. The further evaluation of longer mixed-sequence POMs is described in a second accompanying paper (R. J. Worthington *et al.*, *Org. Biomol. Chem.*, 2006, DOI: 10.1039/b613386j).

Introduction

By carefully evaluating the conformation and recognition properties of synthetic nucleic acid mimics, it is possible to gain a deeper insight into the structure, function and origins of the natural genetic materials.[1,2] Modified nucleic acids which are able to recognize and bind with high affinity and sequence selectivity to RNA or DNA targets can be used to down regulate gene expression for potential therapeutic applications or probe gene function *in vivo*.[3–6] For example, morpholino oligonucleotide mimics **1** (Fig. 1) have been widely used to study embryonic development in zebrafish.[7] Oligonucleotide mimics are also being used as diagnostic agents and as probes for bioanalytical and other applications.[8–10] Most recently, modified nucleic acids have been used as versatile building materials for the self-assembly of nanostructures and devices with defined topologies.[11–14] Amongst the most widely studied and useful nucleic acid mimics are the peptide nucleic acid (PNA) **2**.[5,6,9,15–17] PNA exhibits high affinity for native nucleic acids as well as high *in vivo* stability. However, PNA suffers from low aqueous solubility and limited cellular uptake[5] which has hampered their application *in vivo*.

Previously we introduced cationic pyrrolidine-amide oligonucleotide mimics (POMs) **3** where the DNA nucleobases are retained and the ribose-phosphodiester backbone is replaced with a pyrrolidinyl amide structure **3**.[18,19] The (2*R*,4*R*)-configuration

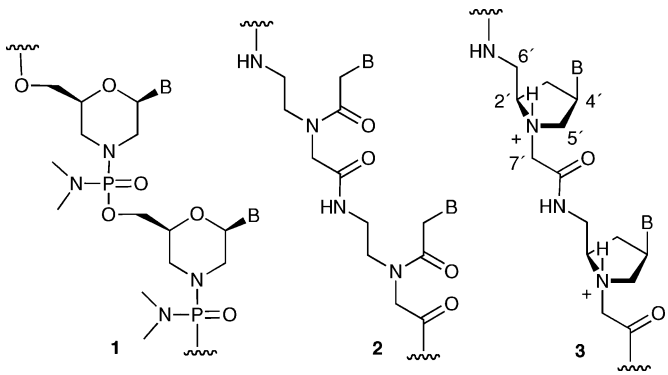

Fig. 1 Structure of morpholino oligonucleotide mimics (morpholinos) **1**, peptide nucleic acid (PNA) **2** and pyrrolidine-amide oligonucleotide mimics (POMs) **3**. B = Nucleobase.

across the pyrrolidine ring renders the POM stereochemically equivalent to the natural nucleic acids and could thus effect the orientational preference for hybridisation with DNA and RNA. In addition, molecular modelling and NMR data suggest that POM adopts a conformation which is similar to the backbone of RNA in an A-type helical conformation. The tertiary amino group of the pyrrolidine units in POM can be protonated at physiological pH which can aid the solubility of the oligomers. Furthermore, homothyminyl and homoadeninyl POM pentamers were shown to have high affinity for complementary DNA and RNA, with interesting kinetic selectivity for RNA over DNA.[18–20] Although these preliminary findings are encouraging, the synthesis and evaluation of longer mixed-sequence oligomers is necessary to fully understand their DNA/RNA hybridisation properties and further explore their potential for hybridisation-based applications.[1–14,21]

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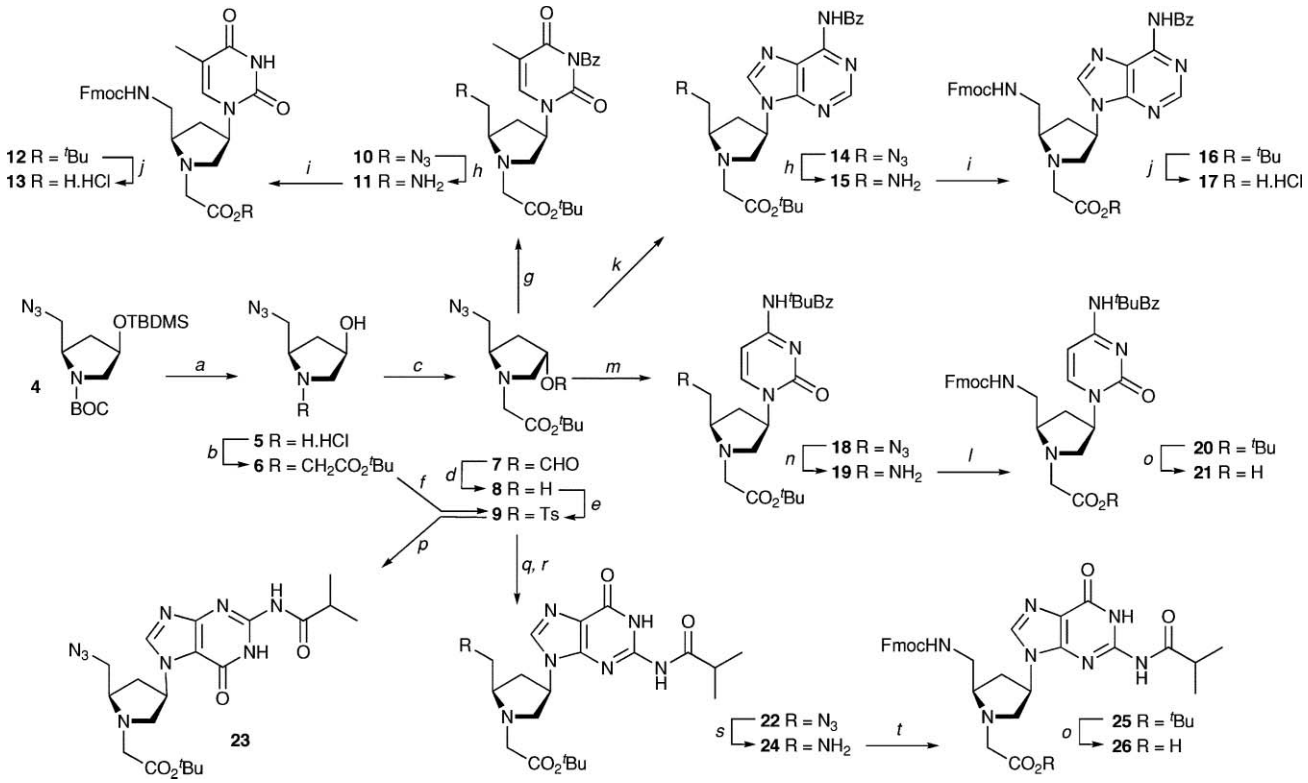
† Electronic supplementary information (ESI) available: additional data, general experimental methods, procedures for the preparation of compounds **5**–**10** and **23** and characterisation of oligomers **27**–**29**, and methods employed in thermal denaturation experiments. See DOI: 10.1039/b613384n



Scheme 1 *Reagents and conditions*: a) 4 M HCl/dioxane, CH_2Cl_2, 0 °C, 15 min, rt, 2 h; b) DIEA, $BrCH_2CO_2{}^tBu$, CH_2Cl_2, 0 °C → rt, 18 h; c) HCO_2H, PPh_3, DIAD, THF, −30 °C → rt, 18 h; d) 0.1% conc. aq. NH_3/CH_3OH, rt, 2 h; e) TsCl, pyridine, 0 °C → rt, 18 h; f) CH_3OTs, PPh_3, DIAD, THF, −10 °C → rt, 18 h; g) N^3-benzoylthymine, PPh_3, DIAD, THF, −25 °C → rt, 18 h; h) sat. H_2S in 60% aq. pyridine, rt, 18 h; i) Fmoc-Cl, DIEA, CH_2Cl_2, 0 °C → rt, 18 h; j) 4 M HCl/dioxane, CH_2Cl_2, rt, 24 h; k) N^6-benzoyladenine, K_2CO_3, 18-crown-6, DMF, 80 °C, 18 h; l) Fmoc-succinimide, 1 : 1 dioxane/10% aq. Na_2CO_3, rt, 4 h; m) N^4-[*p*-(^{t}butyl)benzoyl]cytosine, K_2CO_3, $PhCO_2Na$, 18-crown-6, DMF, 75 °C, 6.5 h; n) PPh_3, H_2O, THF, rt, 48 h; o) 4 M HCl/dioxane, CH_3CN, rt, 18 h; p) 2-*N*-isobutyryl-6-*O*-[(4-nitrophenyl)ethyl]guanine, K_2CO_3, 18-crown-6, DMF, 80 °C, 18 h; q) 2-*N*-isobutyryl-6-*O*-[(4-nitrophenyl)ethyl]guanine, PPh_3, DIAD, $PhCO_2Na$, THF, rt, 18 h; r) DBU, pyridine, rt, 18 h; s) PPh_3, pyridine, rt, 3 h, 25% aq. NH_3, 2 h; t) Fmoc-succinimide, DIEA, CH_2Cl_2, 0 °C, 30 min, rt, 2 h. *Abbreviations*: ^{t}BuBz = *p*-(*tert*-butyl)benzoyl, Bz = benzoyl, Boc = *tert*-butoxycarbonyl, DIAD = diisopropyl azodicarboxylate, DIEA = diisopropylethylamine, Fmoc = fluoren-9-ylmethoxycarbonyl, Ts = tosyl.

Results and discussion

Synthesis of the Fmoc-protected POM monomers

In the preliminary solid phase synthesis of thyminyl and adeninyl POM pentamers we chose the solid phase Fmoc-peptide synthesis protocols.[20] Fmoc chemistry was selected because it employs mild synthetic conditions,[22] allows quantification of the coupling efficiency by UV spectroscopy, following Fmoc deprotection, and is the preferred method for most modern automated synthesisers. In order to utilise this method for the synthesis of mixed-sequence POMs we required a versatile synthetic route to adenine-, cytosine-, thymine- and guanine-derived Fmoc-POM monomers.

Our synthetic strategy involved the synthesis of the azido pyrrolidine **8**, from *trans*-4-hydroxy-L-proline, *via* the known azide **4** (Scheme 1).[20] Accordingly, TBDMS and Boc deprotection of **4** with 4 M HCl gave amine **5** as a crystalline hydrochloride salt in quantitative yield, the structure and absolute stereochemistry of which was confirmed by X-ray crystallography‡ (see ESI†). Subsequent *N*-alkylation of the amine with *t*-butyl bromoacetate afforded *t*-butyl ester **6**. Inversion of the C4-hydroxyl group of **6** using a Mitsunobu reaction followed by ammonolysis of the formyl ester **7** gave the *trans*-alcohol **8** in 80% yield. Introduction of N^3-benzoylthymine[23] using Mitsunobu conditions afforded the (2*R*,4*R*)-pyrrolidine **10** in 77% yield with none of the O^2-isomer, as confirmed by ^{13}C-NMR chemical shifts and comparison with literature compounds.[19,24,25] Azide reduction with hydrogen sulfide in 60% aqueous pyridine gave the amine **11** in quantitative yield. Treatment of the resulting amine with 9-fluorenylmethyl chloroformate (Fmoc-Cl) gave the Fmoc-protected amine **12**. Unexpectedly the N^3-benzoyl protecting group was lost during this reaction, or in the subsequent purification step. However, the N^3 position of thymine does not require protection during solid-phase synthesis. Therefore, the *t*-butyl ester **12** was subject to acidolysis with 4 M HCl in dioxane to reveal the thyminyl Fmoc-acid monomer **13**.

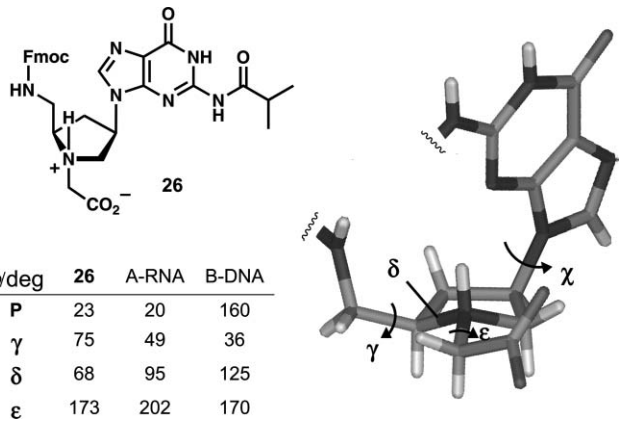
Attempts to introduce N^6-benzoyladenine into the pyrrolidine ring of the *trans*-alcohol **8** under Mitsunobu conditions were

‡ Crystal data for compound **5**: $C_5H_{11}N_4OCl$, M = 178.63, monoclinic, a = 5.1589(2), b = 10.5387(5), c = 7.4162(4) Å, β = 93.901(3)°, V = 402.27(3) Å^3, T = 150(2) K, space group $P2_1$, Z = 2, μ = 0.424 mm^{-1}, reflections collected 4211, independent reflections 2173 [R(int) = 0.0366], R_1 = 0.0372 [$I > 2\sigma(I)$], wR_2 = 0.0692 (all data), Flack parameter 0.07(5). CCDC reference number 624263. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b613384n.

unsuccessful. Therefore, the synthesis of the adeninyl derivative **14** *via* substitution of the tosylate of **9** with N^6-benzoyladenine was investigated. The *trans*-tosylate **9** was synthesised either from *trans*-alcohol **8** with *p*-toluenesulfonyl chloride in pyridine or directly from the *cis*-alcohol **6** with methyl *p*-toluenesulfonate under Mitsunobu conditions.[26] Tosylate displacement by N^6-benzoyladenine in the presence of K_2CO_3 and 18-crown-6 resulted in formation of only the N^9-adeninyl derivative **14** in 37% yield without detection of any N^7 derivative.[27,28] Azide reduction and subsequent Fmoc protection of amine **15** gave adeninyl Fmoc ester **16** with the N^6-benzoyl protecting group intact. Cleavage of the *t*-butyl ester of **16**, as before, afforded the protected adeninyl Fmoc-acid **17**.

In the synthesis of the cytosinyl Fmoc-acid monomer, cytosine was first protected as the N^4-(*t*-butyl)benzamide to aid solubility[29] and then introduced into the pyrrolidine ring *via* substitution of the tosylate from **9** to give the cytosinyl derivative **18** in 46% yield. The Mitsunobu reaction with *trans*-alcohol **8**, and N^4-[*p*-(*tert*-butyl)benzoyl]cytosine, afforded **18** in a superior 71% yield, which was dependent on the presence of sodium benzoate, an additive that has previously been shown to dramatically increase the rates and yields of related reactions.[30,31] In both procedures the N^1-isomer is produced exclusively as determined by ^{13}C-NMR chemical shifts and comparison with literature compounds,[24,27] with none of the O^2-isomer, which is often detected in related reactions. Reduction of the azide using the Staudinger reaction[32] gave amine **19** which was Fmoc-protected to give Fmoc *t*-butyl ester **20** in an overall yield of 31%. The cytosinyl Fmoc-acid **21** was revealed by acidolysis in 92% yield.

We chose to introduce guanine into the pyrrolidine ring as the N^2-isobutyryl-O^6-[2-(*p*-nitrophenyl)ethyl]guanine derivative[33] as this is reported to undergo more regioselective N^9-alkylation.[33] However, the displacement of the tosylate **9** with this guanine derivative gave exclusively the N^7-adduct **23**, missing the O^6-(*p*-nitrophenyl)ethyl group. The N^7-regiochemistry of the product **23** was confirmed through the comparison of ^{13}C-NMR chemical shifts with known compounds.[27,34] Presumably the O^6-protecting group is lost prior to coupling, under the high temperature, basic conditions of the reaction leaving the N^7 the most accessible nucleophilic site. However, under the mild, neutral conditions of the Mitsunobu reaction, N^2-isobutyryl-O^6-[2-(*p*-nitrophenyl)ethyl]guanine and the *trans*-alcohol **8** can be coupled without loss of the O^6-protection. Treatment of the crude adduct with 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) removes the O^6-(*p*-nitrophenyl)ethyl group, revealing the required N^9-adduct **22**. Again, the presence of sodium benzoate in the Mitsunobu reaction was essential in order to attain a reasonable yield of **22** (51%). Reduction of the azide **22** to the amine **24** followed by Fmoc protection gave the Fmoc *t*-butyl ester **25** in 35% yield and acidolysis as before afforded guaninyl Fmoc-acid **26** in 54% yield. The structure and relative stereochemistry of the zwitterion of **26** was determined by X-ray crystallography (Fig. 2).§



ϕ/deg	26	A-RNA	B-DNA
P	23	20	160
γ	75	49	36
δ	68	95	125
ε	173	202	170

Fig. 2 X-Ray crystal structure of the zwitterionic form of guaninyl POM monomer **26** which is shown to exhibit an N1′-*endo*-type conformation with a pseudorotational phase angle (*P*) and backbone torsional angles (γ, δ, and ε) which match most closely the corresponding angles of ribose in typical A-type RNA duplexes, rather than B-type DNA duplexes.[36]

Analysis of the crystal structure of the G-monomer **26** reveals that the pyrrolidine adopts a *trans*-relative configuration about the N-atom and possesses an N1′-*endo* conformation which is described by a pseudorotation phase angle (*P*) of 23° with a maximum torsional angle (ν_{max}) of 45.8°.[35,36] This conformation matches closely the typical C3′-*endo* conformation of ribose in A-type RNA duplexes and agrees very closely with earlier energy-minimised structures of POM pyrrolidine monomer units which were derived from semi-empirical quantum mechanical calculations, along with NMR data.[19] Furthermore, the backbone torsion angles γ, δ and ε more closely match the angles found in typical A-type RNA duplexes, as opposed to B-type DNA duplexes (Fig. 2).[36] This supports the earlier hypothesis that the high affinity of POM pentamers for RNA and DNA strands may in part be due to the pre-organisation of the POM backbone into an A-type helical conformation, which is favoured for the formation of more stable heteroduplexes.

Fmoc-solid phase synthesis of POM homopolymers

In order to test the suitability of the Fmoc approach for the synthesis of POM oligomers we first sought to prepare the POM pentamer Ac-POM(T)$_5$NH$_2$ **27** (Fig. 3) on Rink-amide MBHA resin. Accordingly, POM Fmoc-T amino acid **13** (2 equiv.) was preactivated with 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) (1.9 equiv.), 1-hydroxybenzotriazole (HOBT) (2 equiv.) and DIEA (5 equiv.) and coupling reactions where left to proceeded for 4 h, followed by capping of the unreacted amino groups with acetic anhydride. Each coupling reaction was monitored by the Kaiser test and by UV determination of the dibenzofulvene-piperidine adduct formed during Fmoc deprotection. Following cleavage from the resin with trifluoroacetic acid (TFA) the crude product was analysed by analytical reversed-phase HPLC which revealed a major product, which accounted for *ca.* 80% of the chromatogram (by integration). This approximates to a coupling efficiency of 96% per cycle, which is in agreement with UV quantification of the dibenzofulvene-piperidine adduct. This product was subsequently

§ Crystal data for compound **26**: $C_{31}H_{33}N_7O_6$, $M = 599.64$, orthorhombic, $a = 11.5810(4)$, $b = 14.4762(5)$, $c = 17.7027(7)$ Å, $V = 2967.84(19)$ Å^3, $T = 150(2)$ K, space group $P2_12_12_1$, $Z = 4$, $\mu = 0.096$ mm^{-1}, reflections collected 20 759, independent reflections 2954 [R(int) = 0.12], $R_1 = 0.0450$ [$I > 2\sigma(I)$], $wR_2 = 0.0771$ (all data). CCDC reference number 624249. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b613384n.

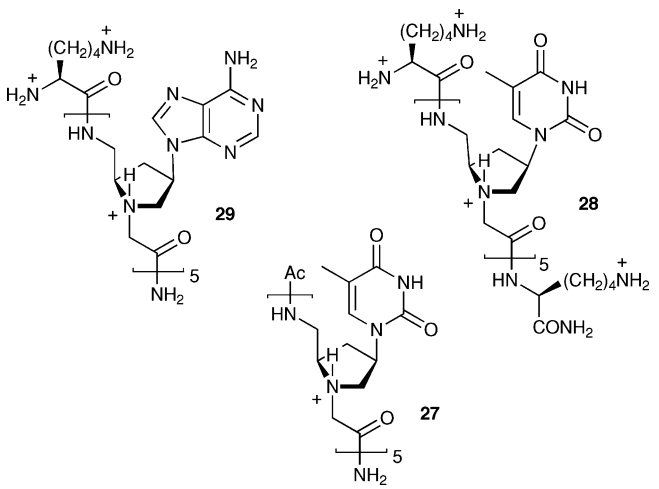


Fig. 3 Structures of POM pentamers Ac-TTTTT-NH_2 **27**, Lys-TTTTT-LysNH_2 **28** and Lys-AAAAA-NH_2 **29**.

purified by semi-preparative HPLC and its identity was confirmed by electrospray ionisation mass spectrometry (ESI-MS).

A pentameric thyminyl POM with *N*- and *C*-lysine termini, Lys-POM$(T)_5$Lys **28**, was similarly prepared using Fmoc-T amino acid **13**. These oligomers were required for DNA/RNA binding studies, which would allow a direct comparison with the corresponding T_5-PNA oligomer, which is available commercially. In the case of T_5-PNA, *N*- and *C*-terminal lysine residues are necessary to increase the aqueous solubility of the PNA and prevent aggregation. Based on UV quantification of the dibenzofulvene-piperidine adducts, and integration of the analytical HPLC chromatogram of the crude product (71% total peak area) following cleavage from the resin, coupling efficiencies in the synthesis of **28** were estimated to be a modest 95% (see ESI†).

Finally, an adeninyl POM pentamer, Lys-POM$(A)_5NH_2$ **29**, was prepared from Fmoc-A^{Bz}-acid **17**. The synthesis proceeded as described above, except that deprotection of the adenine N^6-benzoyl protecting groups was effected using 1 : 1 conc. aq. NH_3 and dioxane at 55 °C for 16 h, prior to cleavage from the resin. The product peak area in this case was only 64%, indicating a low average coupling efficiency of 93% (see Fig. S4 in ESI†). Coupling efficiencies less than 95% are considerably below the level typically obtained for standard peptide chemistry. This indicates that Fmoc protocols would need considerable optimisation if longer mixed-sequence oligomers were to be prepared. Nevertheless, the pentamers prepared in this case (**27**–**29**) were purified by reversed-phase semi-preparative HPLC to greater than 96% purity and the identities of the products were confirmed by ESI-MS.

Nucleic acid binding properties of POM homopolymers

UV thermal denaturation/renaturation experiments were carried out in order to investigate the DNA and RNA binding properties of the POM 5-mers (**27**–**29**) compared with the corresponding PNA 5-mers and DNA 20-mers (Table 1). The Ac-POM$(T)_5NH_2$ pentamer (**27**) showed similar properties as the Phth-T_5-POM reported earlier[18,19] with a denaturation melting temperature (T_m) of *ca.* 8–10 °C per base with poly(rA) which increases slightly at higher ionic strengths. In contrast, $d(T)_{20}$ with poly(rA) exhibits a considerably lower T_m per base of 2.2 °C. Notably, double transitions in the melting curves with Ac-POM$(T)_5NH_2$ (**27**) and poly(rA) were indicative of probable triplex formation. There was also evidence for kinetically selective binding of RNA over DNA. For example, the binding of **27** to $r(A)_{20}$ showed little hysteresis typical of relative fast binding, whereas binding of **27** to $d(A)_{20}$ showed remarkable hysteresis with a >58 °C difference between the T_m extracted from the cooling and heating (melting) curves.

The DNA and RNA binding properties of the POM thyminyl pentamer Lys-POM$(T)_5$Lys (**28**) was similarly investigated (Table 1 and Fig. S5 in ESI†). Following hybridisation with poly(rA) a denaturation T_m of 44.8 °C (T_m/base 9.0 °C) was observed upon heating (melting), with a renaturation T_m of 43.6 °C on cooling. The lack of significant hysteresis between the cooling and heating curves is indicative of relatively fast rates of association/dissociation in this case. The corresponding PNA, Lys-PNA$(T)_5$Lys, exhibited a slightly higher T_m with poly(rA) (T_m/base 10.8 °C). In both cases the T_m values increased with decreasing ionic strength and pH, which is probably in part due to the lysine residues at the *N*- and *C*-termini. Considerable hysteresis was observed in the heating/cooling curves of Lys-POM$(T)_5$LysNH_2 with poly(dA), with denaturation and renaturation T_m values of 65.0 and 25.1 °C respectively. This is indicative of slow rates of association/dissociation and is consistent with the kinetic selectivity of thyminyl POM for RNA over DNA observed previously.[18,19] UV thermal denaturation/renaturation experiments with shorter $r(A)_{20}$ and $d(A)_{20}$ demonstrated similar trends.

The adeninyl POM pentamer, Lys-POM$(A)_5NH_2$ **29**, exhibits considerably higher T_m values for poly(rU) and poly(dT) (T_m/base 10.0 and 14.1 °C respectively) than the corresponding Lys-PNA$(A)_5NH_2$ (Table 1 and Fig. S6 in ESI†). However, the hysteresis between the cooling and heating curves for Lys-POM$(A)_5NH_2$ with poly(dT) (ΔT_m = 14.7 °C) is only slightly higher than the hysteresis observed with poly(rU) (ΔT_m = 6.8 °C). This indicates that the kinetic selectivity for RNA over DNA is less pronounced for the adeninyl POM pentamer **29** compared with the thyminyl pentamers **28**. With target strands $r(U)_{20}$ and $d(T)_{20}$, Lys-POM$(A)_5NH_2$ again exhibits considerably higher T_m values than the corresponding PNA. Finally, Lys-POM$(A)_5NH_2$ can form a stable complex with $r(U)_5$ (T_m of 18.3 °C). On the other hand, neither Lys-PNA$(A)_5NH_2$ nor $d(A)_{20}$ show evidence of hybridisation with $r(U)_5$. These results are consistent with our earlier studies[18,19] and indicate that POMs containing both thymine and adenine bases possess strong nucleic acid binding properties; exhibiting similar and in some cases higher T_m values with DNA/RNA targets than the corresponding PNAs.

Fmoc-solid phase synthesis of mixed-sequence POM

In view of the promising nucleic acid binding properties displayed by POM homo-oligomers, the synthesis of longer, mixed-sequence POM oligomers was investigated using Fmoc-solid phase peptide chemistry. Initially the synthetic effort was focused on the synthesis of POM 10-mer sequence Lys-TTATCATTTC-NH_2 using the Fmoc-monomers **13**, **17** and **21**. However, attempts to optimise the Fmoc chemistry for this sequence failed. UV determination of the dibenzofulvene-piperidine adducts indicated low coupling efficiencies ranging from 70 to 95%. Similarly, analytical

Table 1 UV thermal denaturation and renaturation temperatures T_m values for POM and PNA 5-mers *vs.* complementary nucleic acids

	T_m/°C[a] (% hypochromic[b]/hyperchromic shift[c])						
	$d(T)_{20}$[d]	Lys-PNA$(T)_5$LysNH$_2$		Ac-POM$(T)_5$NH$_2$ **27**		Lys-POM$(T)_5$LysNH$_2$ **28**	
vs.	Heating[d]	Cooling	Heating	Cooling	Heating	Cooling	Heating
Poly(rA)	43.7[a] (31)[c]	53.3[a] (31)[b]	54.1[a] (32)[c]	26.8[a] (33)[b]	51.2[a] (8)[c,e] 37.0 (24)[f]	43.6[a] (28)[b]	44.8[a] (32)[c]
0.22 M,[g] pH 7	47.8 (31)	49.7 (30)	51.2 (31)	26.8 (34)	53.8 (5)[e] 37.4 (30)[f]	40.8 (34)	42.2 (36)
0.62 M,[g] pH7	52.9 (30)	40.8 (29)	45.5 (29)	27.5 (35)	42.2 (36)	34.0 (32)	36.6 (33)
1.20 M, pH 7	54.7 (30)	33.4 (26)	42.2 (26)	26.3 (33)	48.4 (34)	28.5 (28)	34.6 (28)
0.12 M, pH 6	43.7 (30)	57.3 (33)	57.8 (34)	27.2 (36)	56.7 (7)[e] 35.4 (30)[f]	46.9 (38)	48.6 (39)
0.12 M, pH 8	42.7 (30)	50.7 (29)	51.9 (30)	26.6 (30)	37.3 (31)	39.7 (38)	39.7 (39)
Poly(dA)	48.8 (32)	44.2 (25)	48.6 (29)	n.b.[h,i]	n.b.[h,i]	25.1 (17)	54.4 (17) 65.0[j] (28)[j]
$r(A)_{20}$	41.2 (26)	47.2 (27)	47.9 (28)	21.8 (21)	20.5 (22)	37.2 (31)	40.2 (31)
$d(A)_{20}$	47.5 (30)	35.3 (26)	37.4 (27)	22.0 (22)	>80 (12)	21.6 (21)	51.6 (24)
$r(A)_5$	n.d.[k]	18.0 (26)	19.5 (28)	n.d.[k]	n.d.[k]	13.6 (14)	34.5 (15)
r(AAGAA)	n.d.[k]	52.8 (7)	60.6 (8)	n.d.[k]	n.d.[k]		18.2 (14)

	$d(A)_{20}$[d]	Lys-PNA$(A)_5$NH$_2$		Lys-POM$(A)_5$NH$_2$ **29**	
	Heating[d]	Cooling	Heating	Cooling	Heating
Poly(rU)	35 (24)[b]	30.4 (24)[b]	31.0 (25)[c]	43.4 (35)[b]	50.2 (36)[c]
1.20 M,[g] pH 7	60.7 (10)[e] 47.1 (18)[f]	36.4 (24)	36.8 (24)	41.3 (31)	58.8 (13)[e] 47.8 (20)[f]
0.12 M, pH 6	35.7 (26)	31.7 (25)	32.2 (25)	48.4 (42)	53.3 (44)
0.12 M, pH 8	33.2 (25)	28.0 (23)	29.4 (23)	38.8 (42)	47.2 (44)
Poly(dT)	50.1 (31)	47.9 (36)	48.4 (36)	55.6 (28)	70.3 (31)
$r(U)_{20}$	21.3 (26)	24.1 (25)	24.8 (25)	38.8 (42)	46.0 (43)
$d(T)_{20}$	47.5 (31)	34.6 (35)	34.8 (34)	53.0 (22)[e] 31.8 (16)[f]	53.8 (37)
$r(U)_5$	n.b.[h,l]	n.b.[h,l]	n.b[h,l]	<10 (12)	18.3 (11)

[a] All melting experiments were carried out with 42 μM (conc. 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 23 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] DNA/RNA hybridisation is fast so there is no hysteresis, therefore melting and cooling curves are coincident. [e] Probable single strand ↔ duplex transitions. [f] Duplex ↔ triplex transitions. [g] Total salt concentration [K^+]. [h] n.b. = no binding (hyperchromicity) was observed. [i] Melting not evident even after prolonged incubation, possibly due to very slow binding. [j] Lys-POM$(T)_5$LysNH$_2$ was incubated with poly(dA) for 48 h before being subjected to slow thermal denaturation (0.2 °C min^{-1}). [k] n.d. = not determined. [l] Possible transition below 15 °C.

reversed-phase HPLC of the crude oligomer revealed multiple products, whilst MALDI-MS and ESI-MS indicated the formation of probable failure sequences. Similar attempts to optimise the synthesis of shorter test sequences (*e.g.* Lys-TGC-NH$_2$) also resulted in the formation of multiple products and low coupling efficiencies. Several factors could account for this. Firstly, loss of acyl base protecting groups was evident for some monomers (*e.g.* **17** and **26**) after prolonged storage. This suggests that base deprotection is facile and could occur during extended couplings, or during Fmoc deprotection with piperidine, resulting in the formation of branched products. Additionally, the extended coupling times of over 4 h could have caused concomitant Fmoc deprotection, due to the basic coupling conditions, which could have resulted in multiple couplings leading to mixtures of products. In addition, we have previously shown that POM oligomers with *N*-terminal free amino groups will undergo facile cyclisation onto the adjacent amide linkage forming bicyclic lactam side products.[19] Finally, the strong basic conditions (ammonolysis) used to remove the acyl nucleobase protecting groups might also cleave POM amide linkages. Thus, despite the success of Fmoc-solid phase chemistry in the synthesis of mixed-sequence PNA oligomers,[22,37] it appears that this chemistry is incompatible with the synthesis of longer POM mixed sequences.

Conclusion

The synthesis of pyrrolidine monomers with exocyclic Fmoc-protected amino groups and containing thymine, adenine cytosine or guanine acyl-protected nucleobases has been developed. Thyminyl and adeninyl POM pentamers were prepared from the corresponding monomers using typical solid phase Fmoc-peptide synthesis protocols. Despite modest coupling efficiencies it was possible to purify the products by HPLC, allowing subsequent characterisation by mass spectrometry. The pentamers were then shown to hybridise strongly with both DNA and RNA, whilst in some cases exhibiting a noticeable kinetic selectivity for

RNA over DNA. Attempts to optimise the Fmoc-solid phase chemistry for the synthesis of longer, mixed-sequence POMs proved problematic. In light of this an alternative, a more robust strategy utilising Boc-amino backbone and benzyloxycarbonyl (Z)-nucleobase protecting groups was developed. The Boc-Z approach has been used in the successful synthesis of mixed-sequence POMs and will be described in the following paper.[21]

Experimental

(2′*R*,4′*R*)-2′-Aminomethyl-4′-(*N*^{3}-benzoylthymin-1-yl)-*N*1′-(*tert*-butoxycarbonylmethyl)-pyrrolidine (11)

A solution of azide **10** (300 mg, 0.64 mmol) in 60% aqueous pyridine was saturated with H_2S and stirred for 18 h. CH_3OH (6 mL) was added and the mixture was filtered through Celite. Evaporation of eluant under reduced pressure gave the amine **11** in quantitative yield as a pale yellow foam. ν_{max}(KBr)/cm^{-1}: 3392 (NH), 1686 (CO), 1538 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (9H, s, C(CH_3)$_3$), 1.47 (3H, s, CH_3), 1.57–1.66 (1H, m, H_a3′), 2.44–2.55 (1H, m, H_b3′), 2.71–2.75 (1H, m, H2′), 2.78 (1H, dd, *J* 10.9, 7.1 Hz, H_a5′), 3.04–3.13 (2H, m, H_b5′ and H_a6′), 3.20 (1H, d, *J* 17.3 Hz, H_a7′), 3.33 (1H, d, *J* 17.3 Hz, H_b7′), 3.95 (1H, ddd, *J* 14.7, 8.6, 1.5 Hz, H_b6′), 4.95–5.01 (1H, m, H4′), 7.33–7.42 (3H, m, Bz CH), 7.74–7.77 (3H, m, H6 and Bz CH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 12.5 (CH_3), 28.5 (C(*C*H_3)$_3$), 36.4 (C3′), 39.9 (C6′), 51.7 (C4′), 54.7 (C7′), 60.0 (C5′), 64.0 (C2′), 82.5 (*C*(CH_3)$_3$), 111.8 (C5), 127.3 (Bz CH), 129.4 (Bz CH), 132.0 (Bz CH), 136.4 (Bz *ipso*-C), 137.7 (C6), 151.4 (C2), 164.1 (C4), 168.0 (Bz CO), 171.4 (CO_2′Bu); *m*/*z* (ES): 443 ([M + H]$^+$, 100%); HRMS *m*/*z* (ES): 443.2297 ([M + H$^+$], $C_{23}H_{31}N_4O_5$ requires *m*/*z*, 443.2294).

(2′*R*,4′*R*)-2′-[(Fluoren-9-yl-methoxycarbonyl)aminomethyl]-4′-(thymin-1-yl)-*N*1′-(*tert*-butoxycarbonylmethyl)-pyrrolidine (12)

The amine **11** (700 mg, 1.58 mmol) and DIEA (413 µL, 2.37 mmol) were added to a solution of 9-fluorenylmethoxycarbonyl chloride (613 mg, 2.37 mmol) in dry CH_2Cl_2 (8 mL), under N_2. The mixture was stirred for 30 min at 0 °C then at room temperature for 4 h. The solvent was evaporated under reduced pressure and the mixture was purified by column chromatography (3 : 1 EtOAc–hexane) to give the title compound **12** (425 mg, 48%) as a white foam. $[\alpha]_D^{25}$ +20.5° (*c* = 4.0, CH_2Cl_2); Found: C, 66.6; H, 6.6; N, 9.8; $C_{31}H_{36}N_4O_6$ requires: C, 66.4; H, 6.4; N, 10.0%; ν_{max}(KBr)/cm^{-1}: 1693 and 1684 (CO); λ_{max}(CH_3OH)/nm: 265 (ε/dm^{3}mol^{-1}cm^{-1} 2.2 × 10^4); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (9H, s, C(CH_3)$_3$), 1.53–1.60 (1H, m, H_a3′), 1.82 (3H, s, CH_3), 2.39–2.47 (1H, m, H_b3′), 2.65 (1H, m, H2′), 2.72 (1H, dd, *J* 10.5, 7.5 Hz, H_a5′), 3.02–3.07 (2H, m, H_a6′ and H_a7′), 3.15 (1H, d, *J* 10.5 Hz, H_b5′), 3.35 (1H, d, *J* 17.0 Hz, H_b7′), 3.43 (1H, dd, *J* 12.6, 8.6 Hz, H_b6′), 4.10 (1H, t, *J* 7.0 Hz, Fmoc aliphatic CH), 4.24 (1H, dd, *J* 10.6, 7.0 Hz, Fmoc CH_a), 4.34 (1H, dd, *J* 10.6, 7.0 Hz, Fmoc CH_b), 4.95–5.01 (1H, m, H4′), 5.49 (1H, m, carbamate NH), 7.19 (2H, t, *J* 7.5 Hz, Fmoc Ar-H), 7.29 (2H, t, *J* 7.5 Hz, Fmoc Ar-H), 7.49 (2H, t, *J* 7.0 Hz, Fmoc Ar-H), 7.65 (2H, d, *J* 7.5 Hz, Fmoc Ar-H), 7.96 (1H, s, H6), 8.64 (1H, s, H3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 13.1 (CH_3), 28.5 (C(*C*H_3)$_3$), 36.4 (C3′), 41.4 (C6′), 47.6 (Fmoc aliphatic CH), 52.0 (C4′), 54.8 (C7′), 59.6 (C5′), 63.1 (C2′), 67.2 (Fmoc CH_2), 82.3 (*C*(CH_3)$_3$), 111.8 (C5), 120.4 (Fmoc Ar-CH), 125.4 (Fmoc Ar-CH), 127.4 (Fmoc Ar-CH), 128.1 (Fmoc Ar-CH), 138.2 (C6), 141.7 (Fmoc Ar-C), 144.2 (Fmoc Ar-C), 151.7 (C2), 157.3 (Fmoc CO), 164.5 (C4), 170.7 (CO_2′Bu); *m*/*z* (FAB): 561 ([M + H]$^+$, 75%); HRMS *m*/*z* (ES): 561.2716 ([M + H]$^+$, $C_{31}H_{37}N_4O_6$ requires *m*/*z*, 561.2713).

(2′*R*,4′*R*)-2′-[(Fluoren-9-yl-methoxycarbonyl)aminomethyl]-4′-(thymin-1-yl)-*N*1′-(carboxymethyl)-pyrrolidine hydrochloride (13)

tert-Butyl ester **12** (200 mg, 0.35 mmol) was dissolved in dry CH_2Cl_2 (4 mL), under N_2, and treated with 4 M HCl in 1,4-dioxane (6 mL) and allowed to stir at room temperature for 24 h. Solvent was removed under reduced pressure to give a pale brown powder. Recrystallisation from CH_3OH/CH_2Cl_2 gave product **13** (122 mg, 63%) as a white powder. Mp 184–187 °C (decomp., CH_3OH/CH_2Cl_2); $[\alpha]_D^{25}$ −20.0° (*c* = 0.5, CH_3OH); Found: C, 60.1; H 5.5; N, 10.2; Cl, 6.2; $C_{27}H_{29}N_4O_6Cl$ requires: C, 59.9; H, 5.3; N, 10.3; Cl, 6.5%; ν_{max}(KBr)/cm^{-1}: 3130–2724 (OH), 2541 br (R_3NH^+), 1685 (CO); λ_{max}(CH_3OH)/nm: 265 (ε/dm^3 mol^{-1} cm^{-1} 2.5 × 10^4) and 300 (5.8 × 10^3); ^{1}H NMR (400 MHz, CD_3OD): δ 1.89 (3H, s, CH_3), 2.36–2.44 (1H, m, H_a3′), 2.70–2.78 (1H, m, H_b3′), 3.40 (1H, dd, *J* 16.0, 3.0 Hz, H_a5′), 3.66 (1H, dd, *J* 13.0, 9.0 Hz, H_a6′), 3.75–3.80 (1H, m, H2′), 3.89 (1H, dd, *J* 16.0, 3.0 Hz, H_b5′), 3.99 (1H, d, *J* 17.0 Hz, H_a7′), 4.26 (2H, t, *J* 7.0 Hz, Fmoc aliphatic CH), 4.34 (1H, d, *J* 12.5 Hz, H_b6′), 4.44 (2H, d, *J* 7.0 Hz, Fmoc CH_2), 4.63 (1H, d, *J* 17.0 Hz, H_b7′), 4.75–4.81 (1H, m, H4′), 7.32 (2H, t, *J* 7.5 Hz, Fmoc Ar-H), 7.42 (2H, d, *J* 7.5 Hz, Fmoc Ar-H), 7.43 (1H, s, H6), 7.69 (2H, t, *J* 7.5 Hz, Fmoc Ar-H), 7.80 (2H, d, *J* 7.5 Hz, Fmoc Ar-H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 12.6 (CH_3), 32.7 (C3′), 39.0 (C6′), 48.7 (Fmoc aliphatic CH), 54.4 (C7′), 59.8 (C4′), 61.0 (C5′), 68.8 (Fmoc CH_2), 69.9 (C2′), 111.9 (C5), 121.3 (Fmoc Ar-CH), 126.5 (Fmoc Ar-CH), 128.5 (Fmoc Ar-CH), 129.2 (Fmoc Ar-CH), 142.9 (Fmoc Ar-C), 143.6 (C6), 145.5 (Fmoc Ar-C), 153.3 (C2), 160.7 (C4), 166.6 (Fmoc CO), 169.0 (CO_2H); *m*/*z* (FAB): 505 ([M − Cl]$^+$, 100%]; HRMS *m*/*z* (ES): 505.2080 ([M − Cl]$^+$, $C_{27}H_{30}N_4O_6$ requires *m*/*z*, 505.2087).

(2′*R*,4′*R*)-2′-Azidomethyl-4′-(*N*^{6}-benzoyladenin-9-yl)-*N*1′-(*tert*-butoxycarbonylmethyl)-pyrrolidine (14)

A suspension of tosylate **9** (948 mg, 2.31 mmol), *N*^{6}-benzoyladenine (1.38 g, 5.77 mmol), anhydrous K_2CO_3 (798 mg, 5.77 mmol) and 18-crown-6 (231 mg, 0.87 mmol) in dry DMF (12 mL) was stirred under argon at 80 °C. After 18 h, the solvent was evaporated under reduced pressure and saturated KCl (50 mL) was added to the residue. This was extracted with 2 : 1 $CHCl_3$–C_2H_5OH (4 × 50 mL). The organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (5% CH_3OH in EtOAc) to give the *N*^{9}-adeninyl derivative **14** (408 mg, 37%) as a white foam. R_f 0.34 (5% CH_3OH in EtOAc); $[\alpha]_D^{25}$ −81.2° (*c* = 0.5, $CHCl_3$); ν_{max}(KBr)/cm^{-1}: 3410 and 3110 (amide NH), 2101 (N_3), 1732 and 1708 (CO); λ_{max}(CH_3OH)/nm: 282 (ε/dm^3 mol^{-1} cm^{-1} 1.8 × 10^4); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (9H, s, C(CH_3)$_3$), 1.88 (1H, dd, *J* 14.3, 6.8 Hz, H_a3′), 2.59–2.70 (1H, m, H_b3′), 3.00–3.10 (2H, m, H2′ and H_a5′), 3.16–3.22 (2H, m, H_a7′ and H_a6′), 3.39 (1H, dd, *J* 13.1, 4.5 Hz, H_b6′), 3.52–3.61 (2H, m, H_b7′ and H_b5′), 5.11–5.16 (1H, m, H4′), 7.41 (2H, t, *J* 7.1 Hz, Bz CH), 7.50 (1H, t, *J* 7.1 Hz, Bz CH), 7.94 (2H, d, *J* 7.5 Hz, Bz CH), 8.69 (1H, s, H8) 8.70 (1H, s,

H2), 9.28 (1H, s, BzN*H*); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 28.0 (C(CH_3)$_3$), 36.7 (C3′), 51.8 (C4′), 52.8 (C6′), 53.6 (C7′), 58.9 (C5′), 60.6 (C2′), 81.7 (O*C*(CH_3)$_3$), 122.8 (C5), 127.8 (Bz CH), 128.7 (Bz CH), 132.5 (Bz CH), 133.8 (Bz *ipso*-C), 142.3 (C8), 149.2 (C4), 151.6 (C6), 152.3 (C2), 164.6 (Bz CO), 169.2 (CO_2′Bu); *m*/*z* (ES): 500 ([M + Na]$^+$, 10%), 478 ([M + H]$^+$, 100); HRMS *m*/*z* (ES): 478.2324 ([M + H]$^+$, $C_{23}H_{28}N_9O_3$ requires *m*/*z*, 478.2315).

(2′*R*,4′*R*)-2′-Aminomethyl-4′-(*N*^{6}-benzoyladenin-9-yl)-*N*1′-(*tert*-butoxycarbonylmethyl)-pyrrolidine (15)

To a stirred solution of azide **14** (410 mg, 0.86 mmol) in 60% aqueous pyridine (11 mL) was bubbled H_2S gas until saturation and the mixture was left stirring for 18 h at room temperature. Argon was then bubbled through the solution for 30 min and CH_3OH (5.5 mL) was added. Filtration through Celite followed by evaporation under reduced pressure gave the amine **15** (348 mg, 99%) as a pale yellow foam. $[\alpha]_D^{25}$ −5.6° (*c* = 0.25, $CHCl_3$); ν_{max}(KBr)/cm^{-1}: 3325 (NH), 1730 and 1698 (CO), 1613 (NH); λ_{max}(CH_3OH)/nm: 281(ε/dm^3 mol^{-1} cm^{-1} 1.8 × 10^4); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (9H, s, C(CH_3)$_3$), 2.11–2.14 (1H, m, H_a3′), 2.56–2.66 (1H, m, H_b3′), 2.79 (1H, d, H_a5′), 2.93–2.97 (3H, m, H2′ and $H_a$$H_b$6′), 3.12 (1H, d, *J* 17.3 Hz, H_a7′), 3.48–3.54 (2H, m, H_b5′ and H_b7′), 5.14 (1H, br s, H4′), 7.42 (2, t, *J* 7.1 Hz, Bz *m*-H), 7.41 (1H, d, *J* 7.1 Hz, Bz *p*-H), 7.95 (2H, d, *J* 7.1 Hz, Bz *o*-H), 8.68 (1H, s, H2), 8.90 (1H, s, H8); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 28.5 (C(CH_3)$_3$), 36.4 (C3′), 42.2 (C6′), 52.3 (C4′), 54.4 (C7′), 59.9 (C5′), 63.0 (C2′), 81.9 (*C*(CH_3)$_3$), 123.2 (C5), 128.37 (Bz CH), 129.1 (Bz CH), 132.9 (Bz CH), 134.2 (Bz *ipso*-C), 143.4 (C8), 149.5 (C4), 152.2 (C6), 152.4 (C2), 165.4 (PhCO), 170.3 (CO_2′Bu); *m*/*z* (ES): 452 ([M + H]$^+$, 100%); HRMS *m*/*z* (ES): 452.2413 ([M + H$^+$], $C_{23}H_{29}N_7O_3$ requires *m*/*z*, 452.2410).

(2′*R*,4′*R*)-2′-[(Fluoren-9-yl-methoxycarbonyl)aminomethyl]-4′-(*N*^{6}-benzoyladenin-9-yl)-*N*1′-(*tert*-butoxycarbonylmethyl)-pyrrolidine (16)

A solution of amine **15** (240 mg, 0.532 mmol) in a mixture of dioxane (1.5 mL) and 10% aqueous Na_2CO_3 (1.5 mL) was treated portionwise with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide at room temperature. After 4 h, the solution was concentrated under reduced pressure and brine (15 mL) was added. The aqueous phase was extracted with EtOAc (4 × 15 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by column chromatography (0 → 5% CH_3OH in EtOAc) to give the title product **16** (217 mg, 61%) as a white foam. R_f 0.27 (5% CH_3OH/EtOAc); $[\alpha]_D^{25}$ +35.0° (*c* = 0.5, $CHCl_3$); ν_{max}(KBr)/cm^{-1}: 3250 (NH), 1721 (CO); λ_{max}(CH_3OH)/nm: 266 (ε/dm^3 mol^{-1} cm^{-1} 3.6 × 10^4) and 300 (1.7 × 10^4); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (9H, s, C(CH_3)$_3$), 1.90–1.98 (1H, m, H_a3′), 2.58–2.69 (1H, m, H_b3′), 2.91–2.95 (1H, m, H_a5′), 3.01–3.14 (2H, m, H_a6′ and H2′), 3.21 (1H, d, *J* 17.1 Hz, H_a7′), 3.39–3.52 (3H, m, H_b7′, H_b6′ and H_b5′), 4.11 (1H, t, *J* 7.0 Hz, Fmoc aliphatic CH), 4.20 (2H, d, *J* 7.0 Hz, Fmoc CH_2), 5.10–5.17 (1H, m, H4′), 5.47 (1H, m, Fmoc NH), 7.09 (2H, d, *J* 7.7 Hz, Fmoc aromatic CH), 7.15–7.30 (4H, m, Fmoc aromatic CH), 7.35–7.54 (3H, m, Bz CH), 7.63 (2H, d, *J* 7.5 Hz, Fmoc aromatic CH), 7.82 (2H, d, *J* 7.7 Hz, Bz CH), 8.66 (1H, s, H8), 8.72 (1H, s, H2), 8.90 (1H, s, BzN*H*); ^{13}C NMR (75.5 MHz, CD_3CN): δ 27.1 (C(*C*H_3)$_3$), 35.6 (C3′), 40.9 (C6′), 46.8 (Fmoc aliphatic CH), 51.9 (C4′), 53.3 (C7′), 58.3 (C5′), 61.2 (C2′), 65.8 (Fmoc CH_2), 80.6 (*C*(CH_3)$_3$), 119.6 (Fmoc aromatic CH), 123.6 (C5), 124.9 (Fmoc aromatic CH), 126.7 (Bz CH), 127.3 (Fmoc aromatic CH), 127.7 (Bz CH), 128.2 (Fmoc aromatic CH), 132.0 (Bz CH), 133.8 (Bz *ipso*-C), 140.7 (Fmoc aromatic C), 142.6 (C8), 143.8 (Fmoc aromatic C), 149.1 (C4), 150.9 (C2), 151.7 (C6), 156.3 (Fmoc CO), 165.1 (PhCO), 170.1 (CO_2′Bu); *m*/*z* (ES): 696 ([M + Na]$^+$, 20%), 674 ([M + H]$^+$, 100); HRMS *m*/*z* (ES): 674.3087 ([M + H]$^+$, $C_{38}H_{40}N_7O_5$ requires *m*/*z*, 674.3091).

(2′*R*,4′*R*)-2′-[(Fluoren-9-ylmethoxycarbonyl)aminomethyl]-4′-(*N*^{6}-benzoyladenin-9-yl)-*N*1′-(carboxymethyl)-pyrrolidine hydrochloride (17)

To a solution of Fmoc ester **16** (200 mg, 0.29 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added 4 M HCl/dioxane (3 mL) at 0 °C under argon. The solution was stirred 24 h at room temperature and the solvent was removed under reduced pressure to give acid **17** (142 mg, 98%) as a white powder. $[\alpha]_D^{25}$ −21.0° (*c* = 0.5, C_2H_5OH); ν_{max}(KBr)/cm^{-1}: 3390 (NH), 1697 (CO); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.58–2.69 (1H, m, H_a3′), 3.05–3.15 (1H, m, H_b3′), 3.57 (1H, d, *J* 15 Hz, H_a5′) 4.03 (1H, d, *J* 15 Hz, H_b5′), 4.06–4.13 (2H, m, H2′ and Fmoc aliphatic CH), 4.22–4.30 (2H, m, Fmoc CH_a and H_a7′), 4.38–4.44 (1H, m, H_a6′), 4.55–4.67 (2H, m, H_b6′ and Fmoc CH_b), 4.86 (1H, d, *J* 17.0 Hz, H_b7′), 5.85–5.87 (1H, m, H4′), 7.23–7.42 (4H, m, Fmoc aromatic H), 7.61–7.66 (4H, m, 2 Fmoc aromatic H and 2 Bz-H), 7.73–7.82 (3H, m, 2 Fmoc aromatic H and Bz-H), 8.17 (2H, d, *J* 7.5 Hz, Bz-H), 8.79 (1H, s, H8), 9.40 (1H, s, H2); ^{13}C NMR (100.6 MHz, CD_3OD): δ 34.3 (C3′), 39.4 (C6′), 48.5 (Fmoc aliphatic CH), 53.3 (C7′), 55.8 (C4′), 60.8 (C5′), 68.8 (Fmoc CH_2), 69.7 (C2′), 119.6 (Fmoc aromatic CH), 123.6 (C5), 124.9 (Fmoc aromatic CH), 126.7 (Bz CH), 127.3 (Fmoc aromatic CH), 127.7 (Bz CH), 128.2 (Fmoc aromatic CH), 132.0 (Bz CH), 133.8 (Bz *ipso*-C), 140.7 (Fmoc aromatic C), 142.6 (C8), 143.8 (Fmoc aromatic C), 149.1 (C4), 150.9 (C2), 151.7 (C6), 156.3 (Fmoc CO), 165.1 (Bz CO), 170.1 (acid CO); *m*/*z* (ES): 640 ([M + Na − HCl]$^+$, 15%), 618 ([M − Cl]$^+$, 100); HRMS *m*/*z* (ES): 618.2467 ([M − Cl$^+$, $C_{34}H_{32}N_7O_5$ requires *m*/*z*, 618.2465).

(2′*R*,4′*R*)-2′-Azidomethyl-4′-(*N*^{4}-[(4-*tert*-butylbenzoyl)cytosin-1-yl])-*N*1′-(*tert*-butoxycarbonylmethyl)-pyrrolidine (18)

To a stirred solution of DIAD (3.75 mL, 19.05 mmol) and azide **8** (1.92 g, 7.49 mmol) in anhydrous THF (225 mL) under argon, was added, 4-*N*-[*p*-(*tert*-butyl)benzoyl]cytosine (4.065 g, 15.0 mmol) portionwise over 10 min at room temperature. PPh_3 (4.92 g, 18.76 mmol) was then added portionwise and the reaction mixture was left to stir for 5 min followed by portionwise addition of sodium benzoate (2.153 g, 15.0 mmol). After 3 h the solvent was evaporated under reduced pressure and the yellow residue was partitioned between H_2O (1.10 L) and EtOAc (2.20 L). The aqueous layer was extracted with EtOAc (10 × 100 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure to give a yellow foam which was purified by column chromatography (1 : 1 hexane–EtOAc) to give the title product **18** (2.70 g, 71%) as a pale yellow foam. $[\alpha]_D^{25}$ −193.5 (*c* = 1.0, CH_3OH); ν_{max}(KBr)/cm^{-1}: 2100 (N_3), 1734, 1692 and 1663 (CO); λ_{max}(CH_3OH)/nm: 263 (ε/dm^3 mol^{-1} cm^{-1} 2.67 × 10^4) and

305 (1.34 × 10^4); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (9H, s, Bz-C(CH_3)$_3$), 1.42 (9H, s, OC(CH_3)$_3$), 1.68 (1H, dd, *J* 14.7, 6.8 Hz, H_a3′), 2.57–2.67 (1H, m, H_b3′), 2.82 (1H, dd, *J* 11.3, 6.4 Hz, H_a5′), 2.87–2.92 (1H, m, H2′), 3.03 (1H, d, *J* 17.0 Hz, H_a7′), 3.15 (1H, dd, *J* 12.8, 4.1 Hz, H_a6′), 3.37–3.42 (2H, m, H_b5′ and H_b6′), 3.58 (1H, d, *J* 17.0 Hz, H_b7′), 5.10–5.16 (1H, m, H4′), 7.44–7.48 (3H, m, Bz CH and H5), 7.77 (2H, d, *J* 8.3 Hz, Bz CH), 8.59 (1H, d, *J* 7.5 Hz, H6); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 28.5 (ester C(CH_3)$_3$), 31.4 (Bz C(CH_3)$_3$), 35.5 (Bz *C*(CH_3)$_3$), 37.1 (C3′), 53.0 (C6′), 54.5 (C4′), 54.6 (C7′), 58.9 (C5′), 61.8 (C2′), 82.2 (O*C*(CH_3)$_3$), 97.2 (C5), 126.4 (Bz CH), 127.8 (Bz CH), 130.6 (Bz *p*-C), 147.5 (C6), 156.2 (Bz *ipso*-C), 157.3 (C2), 162.1 (C4), 166.7 (Bz CO), 169.6 (CO_2′Bu); *m*/*z* (ES): 532 ([M + Na]$^+$, 40%), 510 ([M + H]$^+$, 100); HRMS *m*/*z* (ES): 510.2829 ([M + H]$^+$, $C_{26}H_{36}N_7O_4$ requires *m*/*z*, 510.2829).

(2′*R*,4′*R*)-2-[(Fluoren-9-ylmethoxycarbonyl)aminomethyl]-4′-(*N*^{4}-[(4-*tert*-butylbenzoyl)cytosin-1-yl])-*N*1′-(*tert*-butoxycarbonylmethyl)-pyrrolidine (20)

A solution of azide **18** (53.8 mg, 0.105 mmol), PPh_3 (69.2 mg, 0.263 mmol) and H_2O (9.5 μL, 0.527 mmol) in THF (0.8 mL) was stirred at room temperature. After 48 h, the solvent was removed under reduced pressure to give the intermediate amine **19** as a pale yellow foam. The crude amine **19** was dissolved in a mixture of dioxane (300 μL) and 10% aqueous Na_2CO_3 (300 μL), treated with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (42.7 mg, 0.126 mmol) portionwise and stirred at room temperature for 4 h. The solution was concentrated under reduced pressure, brine (5 mL) was added and the product was extracted with EtOAc (4 × 5 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The crude product was then purified by column chromatography (0 → 5% CH_3OH in EtOAc) to give the title compound **20** (23 mg, 31%) as a white foam. $[\alpha]_D^{25}$ −37.2° (*c* = 0.25, $CHCl_3$); ν_{max}(KBr)/cm^{-1}: 3258 (NH), 1708 (CO); λ_{max}(CH_3OH)/nm: 264 (ε/dm^3 mol^{-1} cm^{-1} 4.4 × 10^4) and 300 (1.8 × 10^4); ^{1}H NMR (400 MHz, CD_3OD): δ 1.35 (9H, s, Bz-C(CH_3)$_3$), 1.52 (9H, s, OC(CH_3)$_3$), 1.73 (1H, dd, *J* 14.0, 7.0 Hz, H_a3′), 2.55–2.63 (1H, m, H_b3′), 2.80–2.84 (2H, m, H_a5′ and H2′), 3.07 (1H, d, *J* 17.0 Hz, H_a7′), 3.23–3.27 (2H, m, H_aH_b6′), 3.47 (1H, d, *J* 11.0 Hz, H_b5′), 3.75 (1H, d, *J* 17.0 Hz, H_b7′), 4.08–4.14 (1H, t, *J* 7.0 Hz, Fmoc aliphatic CH), 4.22 (1H, dd, *J* 10.5, 7.0 Hz, Fmoc CH_a), 4.30 (1H, dd, *J* 10.5, 7.0 Hz, Fmoc CH_b), 4.95–4.98 (1H, m, H4′), 7.19–7.33 (4H, m, Fmoc aromatic H), 7.49 (2H, d, *J* 8.5 Hz, Bz CH_a), 7.54 (2H, d, *J* 7.0 Hz, Fmoc aromatic H), 7.62 (1H, d, *J* 7.5 Hz, H5), 7.70 (2H, dd, *J* 7.5, 3.0 Hz, Fmoc aromatic H), 7.77 (2H, d, *J* 8.5 Hz, Bz CH_b), 8.86 (1H, d, *J* 7.5 Hz, H6); ^{13}C NMR (75.5 MHz, CD_3OD): δ 28.8 (OC(CH_3)$_3$), 31.8 (Bz-C(CH_3)$_3$), 36.3 (Bz-*C*(CH_3)$_3$), 37.5 (C3′), 42.2 (C6′), 48.5 (Fmoc aliphatic CH), 55.4 (C7′), 56.9 (C4′), 59.1 (C5′), 63.9 (C2′), 68.1 (Fmoc CH_2), 83.0 (O*C*(CH_3)$_3$), 99.0 (C5), 121.2 (Fmoc aromatic CH), 126.4 (Fmoc aromatic CH), 126.6 (Bz CH), 127.0 (Bz CH), 128.4 (Fmoc aromatic CH), 129.0 (Fmoc aromatic CH), 129.4 (Bz *p*-C), 142.9 (Fmoc aromatic C), 145.5 (Fmoc aromatic C), 149.2 (C6), 158.2 (Bz *ipso*-C), 159.0 (C2), 159.4 (C4), 164.5 (Fmoc CO), 169.1 (benzamide CO), 172.5 (CO_2′Bu); *m*/*z* (ES): 728 ([M + Na]$^+$, 10%), 706 ([M + H]$^+$, 100); HRMS *m*/*z* (ES): 706.3611, ([M + H]$^+$, $C_{41}H_{48}N_5O_6$ requires *m*/*z*, 706.3604).

(2′*R*,4′*R*)-2′-[(Fluoren-9-ylmethoxycarbonyl)aminomethyl]-4′-(*N*^{4}-[(4-*tert*-butylbenzoyl)cytosin-1-yl])-*N*1′-carboxylmethyl pyrrolidine hydrochloride (21)

A solution of 4.0 M HCl in dioxane (4.8 mL, 19.2 mmol) was added dropwise to a suspension of *tert*-butyl ester **20** (182 mg, 0.258 mmol) in CH_3CN (5 mL), under N_2. The reaction mixture was stirred at room temperature for 18 h. The solvent was then removed with a stream of argon and the residue was washed with EtOAc (20 mL). Further EtOAc (25 mL) was added and the resulting suspension was subjected to centrifugation (0 °C, 12 000 rpm, 20 min). After removal of the supernatant, the pellets were dried under reduced pressure and the resulting light pink powder was purified by column chromatography (10% CH_3OH in CH_2Cl_2) to give the acid **21** (155 mg, 92%) as a white solid. Mp 170–171 °C (recrystallisation from EtOAc/CH_3OH); $[\alpha]_D^{25}$ −39.4° (*c* = 0.5, CH_3OH); ν_{max} (KBr)/cm^{-1}: 3411 (NH), 1701 and 1647 (CO); λ_{max} (CH_3OH)/nm: 264.0; ^{1}H NMR (400 MHz, CD_3OD): δ 1.36 (9H, s, C(CH_3)$_3$), 2.50–2.53 (1H, m, H_a3′), 2.76–2.80 (1H, m, H_b3′), 3.45 (1H, d, *J* 16.0 Hz, H_a6′), 3.71 (1H, d, *J* 12.0 Hz, H_a5′), 3.83 (1H, m, H2′), 3.93 (1H, d, *J* 16.0 Hz, H_b6′), 4.04 (1H, d, *J* 17.0 Hz, H_a7′), 4.75 (1H, d, *J* 17.0 Hz, H_b7′), 4.97 (1H, m, H4′), 4.26 (1H, t, *J* 7.0 Hz, Fmoc aliphatic CH), 4.38 (1H, dd, *J* 10.5, 7.0 Hz, Fmoc O-CH_a), 4.43 (1H, d, *J* 12.0 Hz, H_b5′), 4.56 (1H, dd, *J* 10.5, 7.0 Hz, Fmoc O-CH_b), 7.26–7.41 (4H, m, Fmoc aromatic CH), 7.59 (2H, d, *J* 7.5 Hz, Bz H and 1H, d, *J* 8.5 Hz, cytosine H5), 7.68 (1H, t, *J* 8.5 Hz, Fmoc aromatic CH), 7.79 (1H, d, *J* 7.5 Hz, Fmoc aromatic CH), 7.92 (2H, d, *J* 7.5 Hz, Bz H), 8.09 (1H, d, *J* 8.5 Hz, cytosine H6); ^{13}C NMR (75.4 MHz, CD_3OD): δ 31.9 (C(*C*H_3)$_3$), 32.8 (C3′), 36.4 (*C*(CH_3)$_3$), 39.6 (C6′), 48.8 (Fmoc aliphatic CH), 55.4 (C7′), 61.0 (C5′), 62.6 (C4′), 68.7 (Fmoc CH_2O), 70.3 (C2′), 99.2 (C5), 121.4 (Fmoc aromatic CH), 126.6 (Fmoc aromatic CH), 126.8 (Bz CH), 127.3 (Bz CH), 128.6 (Fmoc aromatic CH), 129.3 (Fmoc aromatic CH), 129.8 (Bz CH), 131.6 (Bz *ipso*-C), 143.0 (Fmoc aromatic C), 145.4 (Fmoc aromatic C), 145.8 (Fmoc aromatic C), 153.5 (C6), 159.0 (Bz *p*-C), 160.3 (C2), 165.1 (C4), 169.2 (Fmoc CO), 169.3 (Bz CO); *m*/*z* (ES): 650 ([M + H]$^+$, 100%); HRMS *m*/*z* (ES): 650.2995 ([M + H]$^+$, $C_{37}H_{40}N_5O_6$ requires *m*/*z*, 650.2979).

(2′*R*,4′*R*)-2′-Azidomethyl-4′-(*N*^{2}-isobutyrylguanin-9-yl)-*N*1′-(*tert*-butoxycarbonylmethyl)-pyrrolidine (22)

To a stirred solution of DIAD (0.50 mL, 2.54 mmol) and alcohol **8** (256 mg, 1.0 mmol) in anhydrous dioxane (30 mL) was added *N*^{2}-isobutyryl-*O*^{6}-[2-(*p*-nitrophenyl)ethyl]guanine (741 mg, 2.0 mmol) portionwise at room temperature under argon. PPh_3 (656 mg, 2.5 mmol) was added portionwise and the mixture was stirred for 5 min, sodium benzoate (287 mg, 2.0 mmol) was then added portionwise. The reaction mixture was stirred at room temperature under argon for 18 h and the solvent was evaporated under reduced pressure. H_2O (100 mL) was added and the mixture was extracted with EtOAc (4 × 100 mL). The combined organic extracts were then dried over $MgSO_4$. The mixture was then fractionated by column chromatography (5% CH_3OH in EtOAC) and the *O*^{6}-[2-(*p*-nitrophenyl)ethyl]guanine-containing intermediate (R_f = 0.37, 5% CH_3OH in EtOAC) was dissolved in dry pyridine (5 mL), treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (373.9 μL, 2.5 mmol) and stirred at room temperature for 18 h. The solvent

was then evaporated under reduced pressure and purification by column chromatography (10% CH_3OH in EtOAC) gave the guanine derivative **22** as a white solid (234 mg, 51% yield). R_f 0.45 (10% CH_3OH in EtOAC); mp 176 °C; $[\alpha]_D^{25}$ −23.5° ($c = 2.0$, CH_3OH); ν_{max}(KBr)/cm^{-1}: 3112 (NH), 2103 (N_3), 1715, 1682 and 1615 (CO); λ_{max}(CH_3OH)/nm: 261 (ε/dm^3 mol^{-1} cm^{-1} 1.2×10^4) and 280 (8.4×10^3); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (6H, d, J 6.8 Hz, CH(CH_3)$_2$); 1.48 (9H, s, C(CH_3)$_3$), 1.88–1.96 (1H, m, H$_a$3′), 2.52–2.60 (1H, m, H$_b$3′) 2.62–2.73 (1H, m, CH(CH_3)$_2$), 3.07–3.16 (2H, m, H2′ and H$_a$5′), 3.25 (1H, dd, J 12.8, 4.8 Hz, H$_a$6′), 3.27 (1H, d, J 17.0 Hz, H$_a$7′), 3.38 (1H, dd, J 12.8, 5.3 Hz, H$_b$6′), 3.51 (1H, d, J 10.5 Hz, H$_b$5′), 3.60 (1H, d, J 17.0 Hz, H$_b$7′), 4.84–4.89 (1H, m, H4′), 8.18 (1H, s, H8), 8.84 (1H, br s, H1), 11.99 (1H, br s, NH$_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.7 (CH(CH_3)$_2$), 28.9 (C(CH_3)$_3$), 37.2 (CH(CH_3)$_2$), 37.7 (C3′), 52.7 (C4′), 54.0 (C5′), 54.3 (C7′), 59.3 (C6′), 61.3 (C2′), 82.6 (C(CH_3)$_3$), 121.8 (C5), 138.6 (C8), 147.8 (C2), 148.6 (C4), 156.3 (C6), 170.2 (CO$_2$^{t}Bu), 179.0 (isobutyryl CO); m/z (ES): 460.3 ([M + H]$^+$, 100%); HRMS m/z (ES): 460.2424 ([M + H]$^+$, $C_{20}H_{30}N_9O_4$ requires m/z, 460.2421).

(2′*R*,4′*R*)-2′-[(Fluoren-9-yl-methoxycarbonyl)aminomethyl]-4′-(2-*N*-isobutyrylguanin-9-yl)-*N*1′-(*tert*-butoxycarbonylmethyl)-pyrrolidine (25)

To a solution of azide **22** (724 mg, 1.58 mmol) in pyridine (12 mL) was added PPh_3 (2.0 g, 7.62 mmol) and the mixture was stirred at room temperature for 3 h. A solution of 25% aqueous NH_4OH (8 mL) was added and the reaction mixture was stirred at room temperature for 2h. Evaporation of solvents gave a yellow solid residue that was purified by column chromatography over cellulose (6 : 4 hexane–CH_2Cl_2) to give the amine **24** as a yellow solid (670 mg, *ca.* 98%). A solution of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (613 mg, 1.82 mmol) in anhydrous CH_2Cl_2 (8.0 mL) with DIEA (476 μL, 2.73 mmol) was then added to the amine **24** (503 mg, 1.16 mmol) in anhydrous CH_2Cl_2 (16 mL) at 0 °C, under N_2. The solution was stirred at 0 °C for 30 min and at room temperature for 2 h. H_2O (40 mL) was then added and the mixture was extracted with CH_2Cl_2 (2 × 50 mL). The organic extracts were evaporated and purified by silica gel column chromatography (9 : 1 EtOAc–hexane) and then by reversed-phase column chromatography (BondElut® C18, 7 : 3 H_2O–CH_3CN) to isolate the Fmoc-protected guaninyl derivative **25** (269 mg, 35%). R_f 0.58 (4 : 1 EtOAc–CH_3OH); ν_{max} (KBr)/cm^{-1}: 3421 br (NH), 2975 (CH), 1683 br (CO), 1609 (CO); λ_{max}(CH_3OH)/nm: 263.0; mp 118–119 °C (hexane/EtOAc); ^{1}H NMR (300 MHz, CD_3OD): δ 1.21 (6H d, J 7.0 Hz, CH(CH_3)$_2$), 1.49 (9H, s, C(CH_3)$_3$), 1.92 (1H, dd, J 13.5, 4.5 Hz, H$_a$3′), 2.55 (1H, ddd, J 14.0, 8.5, 8.5 Hz, H$_b$3′), 2.68 (1H, m, CH(CH_3)$_2$), 2.93–3.01 (2H, m, H$_a$5′ and H2′), 3.11 (1H, dd, J 14.0, 5.0 Hz, H$_a$6′), 3.19 (1H, d, J 17.0 Hz, H$_a$7′), 3.26 (1H, m, H$_b$5′), 3.58 (1H, d, J 10.5 Hz, H$_b$6′), 3.71 (1H, d, J 17.0 Hz, H$_b$7′), 4.17 (1H, t, J 6.4 Hz, Fmoc aliphatic CH), 4.21 (2H, m, Fmoc O-CH$_2$), 4.94 (1H, m, H4′), 7.23–7.38 (4H, m, Fmoc aromatic CH), 7.55 (1H, d, J 7.0 Hz, Fmoc aromatic CH), 7.60 (1H, d, J 7.5 Hz, Fmoc aromatic CH), 7.76 (2H, d, J 7.5 Hz, Fmoc aromatic CH), 8.48 (1H, br s, H8); ^{13}C NMR (75.4 MHz, CD_3OD): δ 19.68 and 19.73 (CH(CH_3)$_2$), 28.8 (C(CH_3)$_3$), 37.3 (CH(CH_3)$_2$), 37.9 (C3′), 43.3 (C6′), 48.5 (Fmoc aliphatic *C*H), 54.2 (C4′), 55.5 (C7′), 60.1 (C5′), 63.6 (C2′), 68.2 (Fmoc CH$_2$O), 83.0 (*C*(CH$_3$)$_3$), 121.3 (Fmoc aromatic CH), 126.0 (C5), 126.5 (Fmoc aromatic CH), 128.5 (Fmoc aromatic CH), 129.1 (Fmoc aromatic CH), 140.7 (C8), 142.9 (Fmoc aromatic C), 145.6 (Fmoc aromatic C), 145.7 (Fmoc aromatic C), 149.7 (C2), 150.8 (C4), 157.9 (C6), 159.4 (Fmoc CO), 172.5 (*C*O$_2$^{t}Bu), 182.0 (isobutyryl CO); m/z (ES): 656.4 ([M + H]$^+$, 100%); HRMS m/z (ES): 656.3195 ([M + H]$^+$, $C_{35}H_{42}N_7O_6$ requires m/z, 656.3197).

(2′*R*,4′*R*)-2′-[(Fluoren-9-yl-methoxycarbonyl)aminomethyl]-4′-(2-*N*-isobutyrylguanin-9-yl)-*N*1′-carboxylmethyl pyrrolidine hydrochloride (26)

A solution of 4.0 M HCl in dioxane (4.8 mL, 19.2 mmol) was added dropwise to a solution of *tert*-butyl ester **25** (169 mg, 0.258 mg) in CH_3CN (5 mL), under N_2. The reaction mixture was stirred at room temperature for 18 h and the solvent was removed under a stream of argon. The residue was washed with EtOAc (20 mL), resuspended in EtOAc (20 mL) and subjected to centrifugation (0 °C, 12 000 rpm, 20 min). After removal of the supernatent the pellets were dried under reduced pressure and the resulting light pink powder was purified by reversed-phase column chromatography (BondElut® C18, 1 : 1 H_2O–CH_3CN) to give Fmoc-acid **26** (83 mg, 54% yield) as a white powder. Mp 168–169 °C (recrystallisation from ^{i}Pr$_2$O–CH_3OH); Found: C, 57.9; H, 5.8; N, 15.2; $C_{31}H_{33}N_7O_6 \cdot \frac{5}{2}H_2O$ requires: C, 57.8; H, 5.9; N, 15.2%; $[\alpha]_D^{25}$ +61.5° ($c = 0.5$, CH_3OH); ν_{max} (KBr)/cm^{-1}: 3420 (NH), 2974 (CH), 1685 (CO), 1616 (CO); λ_{max}(CH_3OH)/nm: 262.0; ^{1}H NMR (300 MHz, DMF-d$_7$): δ 1.23 (6H, d, J 9.0 Hz, CH(CH_3)$_2$), 2.02 (1H, m, H$_a$3′), 2.67 (1H, ddd, J 13.5, 8.5, 8.5 Hz, H$_b$3′), 2.97 (1H, m, CH(CH$_3$)$_2$), 3.10–3.15 (2H, m, H$_a$5′ and H2′), 3.11 (1H, dd, J 13.5, 5.0 Hz, H$_a$6′), 3.19 (1H, d, J 17.5 Hz, H$_a$7′), 3.46 (1H, m, H$_b$6′), 3.66 (1H, d, J 10.0 Hz, H$_b$5′), 3.92 (1H, d, J 17.5 Hz, H$_b$7′), 4.23–4.30 (3H, m, Fmoc aliphatic CH and Fmoc OCH$_2$), 4.98 (1H m, H4′), 7.33–7.46 (4H, m, Fmoc aromatic CH), 7.70 (1H, d, J 6.0 Hz, Fmoc aromatic CH), 7.72 (1H, d, J 7.0 Hz, Fmoc aromatic CH), 7.93 (2H, d, J 7.0 Hz, Fmoc aromatic CH), 8.43 (1H s, H8), 11.78 (1H, br s, NH), 12.16 (1H, br s, NH); ^{13}C NMR (75.4 MHz, DMF-d$_7$): δ 18.9 (CH(*C*H$_3$)$_2$), 35.8 (*C*H(CH$_3$)$_2$), 37.1 (C3′), 42.8 (C6′), 47.6 (Fmoc aliphatic CH), 52.4 (C4′), 53.4 (C7′), 59.3(C5′), 62.6 (C2′), 66.7 (Fmoc CH$_2$O), 120.5 (Fmoc aromatic CH), 120.9 (C5), 125.8 (Fmoc aromatic CH), 127.6 (Fmoc aromatic CH), 128.1 (Fmoc aromatic CH), 138.5 (C8), 141.6 (Fmoc aromatic C), 144.7 (Fmoc aromatic C), 144.8 (Fmoc aromatic C), 148.6 (C2), 149.0 (C4), 155.7 (C6), 157.3 (Fmoc CO), 172.4 (CO$_2$), 180.9 (isobutyryl CO); m/z (ES): 600.3 ([M + H]$^+$, 100%); HRMS m/z (ES): 600.2585 ([M + H]$^+$, $C_{31}H_{34}N_7O_6$ requires m/z, 600.2571).

Fmoc-solid phase synthesis of POM pentamers Ac-TTTTT-NH$_2$ (27), Lys-TTTTT-LysNH$_2$ (28), Lys-AAAAA-NH$_2$ (29)

Rink amide-methylbenzhydrylamine (MBHA) resin, with a maximum loading of 0.72 mmol g^{-1} (Novabiochem), in a 10 mm diameter solid-phase synthesis vessel, was swelled in CH_2Cl_2 for 30 min after which the solvent was removed through a Buchner flask under reduced pressure. Washing of the resin was carried out three times with DMF and in all cases performed by N_2

agitation for 5 min. Fmoc-protected resin was then treated with freshly prepared 20% (v/v) piperidine in DMF (1.0 mL/25 μmol resin loading, 4 × 2 min). After the specified period of time the reagent was removed and the resin washed exhaustively with DMF. In a separate small vial, DIEA (5 equiv.) was added to Fmoc-POM(T)-OH **13** or Fmoc-POM(A^{Bz})-OH **17** (2 equiv.), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU), (1.9 equiv.) and 1-hydroxybenzotriazole (HOBT) (2 equiv.) in a minimal amount of DMF and the mixture was allowed to activate for 3 min. In the case of lysine, Fmoc-Lys(Boc)-OH (5 equiv.), TBTU (4.9 equiv.), HOBt (5 equiv.) and DIEA (10 equiv.) was used. The resulting mixture was then added to the resin. Coupling was allowed to proceed with N_2 assisted agitation for 4 h. Complete coupling was indicated by a negative Kaiser test. The coupling reagent was removed and the resin washed with DMF. Treatment of the resin with freshly prepared 0.5 M acetic anhydride/0.5 M DIEA in DMF (1 mL per 25 μmol) twice for 15 min after the first and subsequent coupling steps prevents the formation of deletion sequences. The acetylating reagent was removed by vacuum suction and the resin washed in sequence with DMF, CH_2Cl_2, CH_3OH and again with CH_2Cl_2. Deprotection of the resin-bound Fmoc-protected POM oligomer for the next coupling step was accomplished with 20% piperidine in DMF (1 mL per 25 μmol) as described above. The solution from the deprotection step containing the dibenzofulvene-piperidine adduct was collected and the UV absorbance at 290 nm measured to determine the coupling efficiency of each coupling step. Coupling–capping–deprotection sequences were then repeated until the desired oligomer was obtained. POM oligomers were end-capped as an acetamide using 0.5 M acetic anhydride/0.5 M DIEA in DMF or with a single lysine residue. In the case of lysine end-capping, the *N*-terminal Fmoc was deprotected and the resulting free amine was not acetylated. The resin with the attached oligomer was washed sequentially with DMF, CH_2Cl_2, CH_3OH and Et_2O and then dried under reduced pressure. In the case of the Lys-AAAAA-NH_2 **29**, adenine-benzoyl protecting groups were removed from the resin-bound oligomer upon treatment with 1 : 1 concentrated aqueous ammonia in dioxane (0.8 mL) at 55 °C for 16 h. Cleavage of the oligomer from the resin was achieved by treatment with 95% trifluoroacetic acid (TFA)/H_2O for 18 h and the resin was washed several times with fresh 95% TFA (with 5% H_2O). The cleavage mixtures were combined, evaporated under a stream of N_2 and further dried under reduced pressure. Crude POM pentamers were then extracted onto Varian C18 SPE cartridges and eluted using a gradient of 0 → 70% CH_3OH/0.1 M aq. HCl. Fractions containing POM pentamers were evaporated and lyophilised to give a light brown powder. The pentamer was then purified by reversed-phase HPLC on a C18 or C8 column (Phenomenex Luna 5μ C18 or Kromasil 5μ C8; 250 × 10 mm) with a typical increasing gradient of acetonitrile in 0.1% aqueous formic acid. Fractions collected were evaporated and lyophilised to give pure product as a white powder. Product purity was verified by analytical reversed-phase HPLC (Phenomenex Luna 3μ C18 and/or Kromasil 3.5μ C8; 250 × 4.6 mm) and oligomers were characterised by electrospray mass spectrometry (see ESI†).

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