ELECTRONIC SUPPLEMENTARY INFORMATION

Fmoc-synthesis and DNA/RNA binding properties of homopolymeric pyrrolidine-amide oligonucleotide mimics.

T. H. Samuel Tan, Roberta J. Worthington, Robin G. Pritchard, Jordi Morral and Jason Micklefield*

School of Chemistry and Manchester Interdisciplinary Biocentre,

The University of Manchester, 131 Princess Street, Manchester, M1 7DN

*Address correspondence to: jason.micklefield@manchester.ac.uk

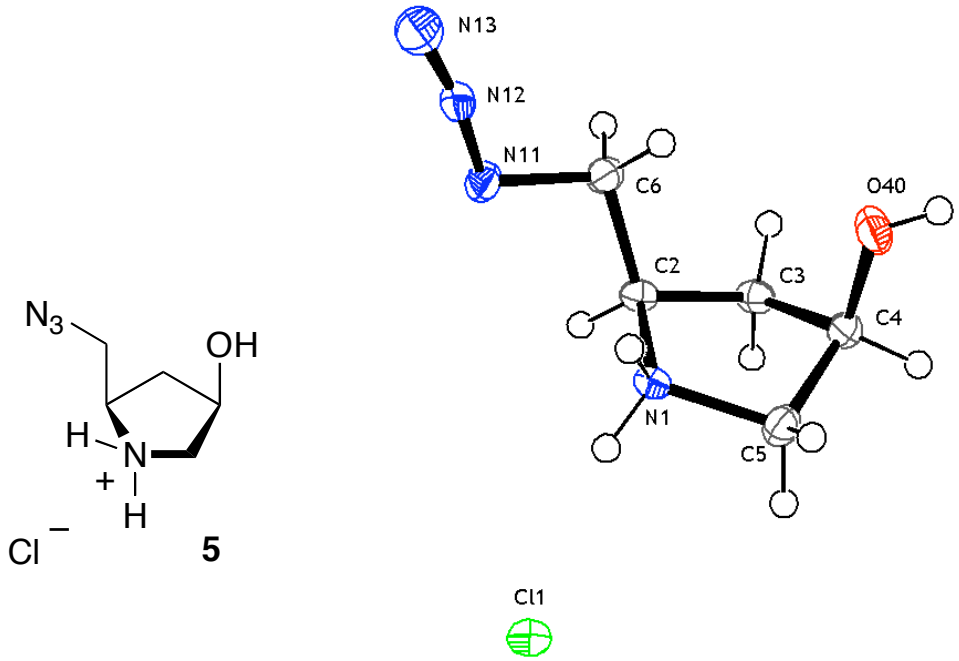

Figure 1. X-ray crystal structure of azide **5**

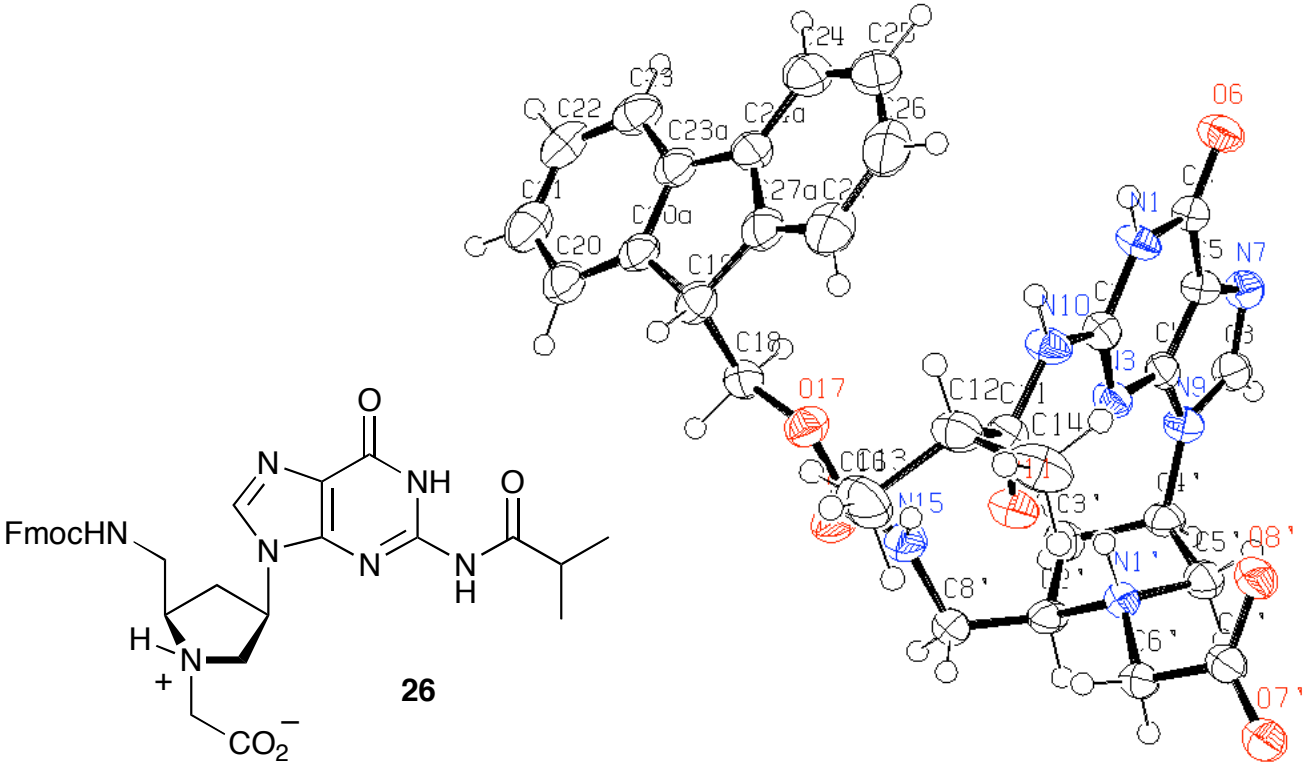

Figure 2. X-ray crystal structure of G-monomer (zwitterion) **26**

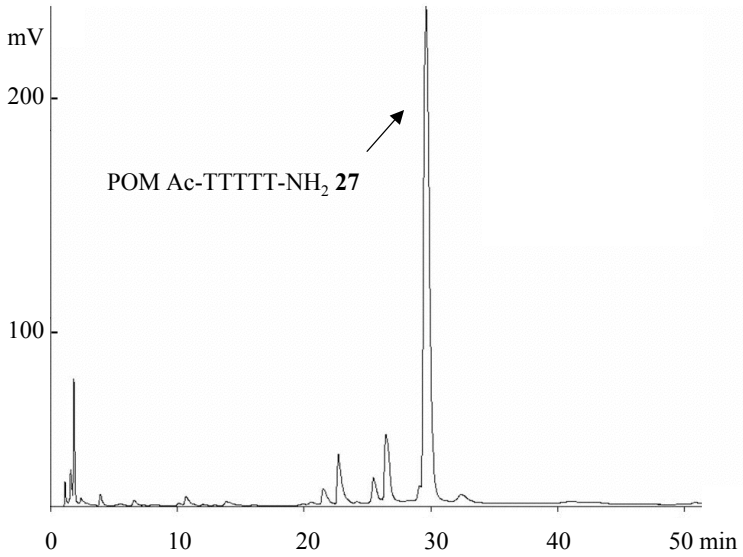

Figure 3. Analytical HPLC of crude POM Ac-TTTTT-NH_2 **27.**

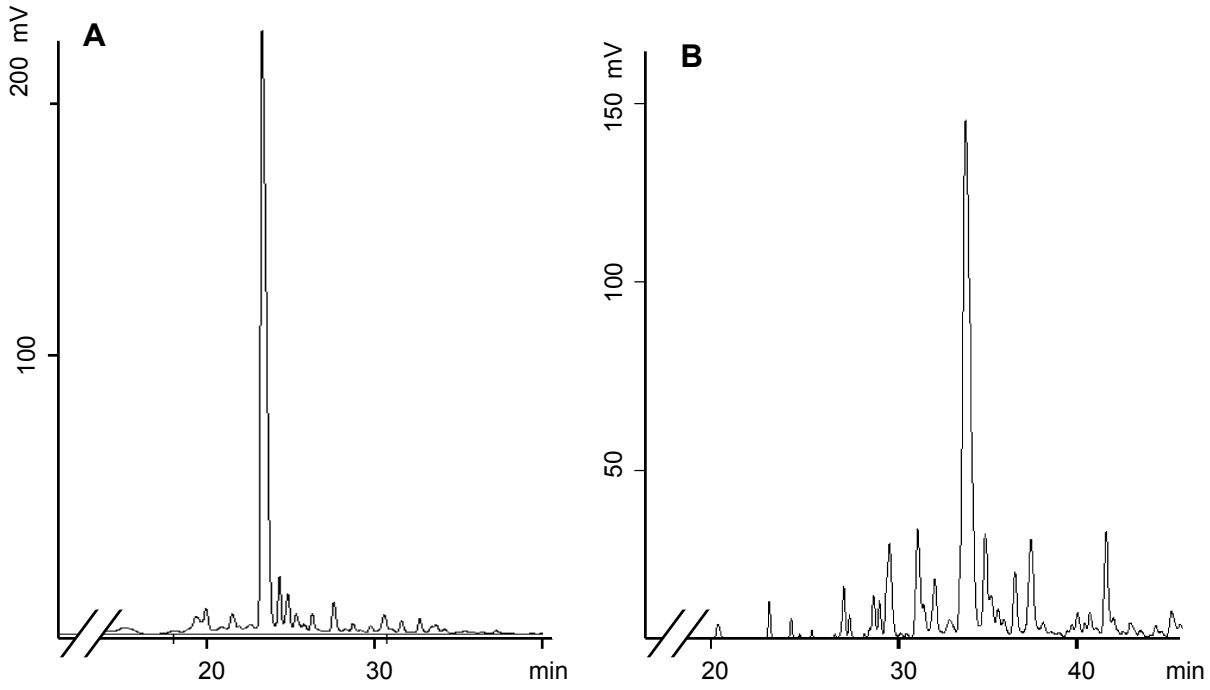

Figure 4. (A) Analytical reverse phase (C8) HPLC chromatogram of crude POM Lys-TTTTT-LysNH_2 **28**. Total peak area of the product **28** was 71% and the coupling efficiency was *ca*. 95%. (B) Analytical reverse phase (C18) HPLC chromatogram of crude POM Lys-AAAAA-NH_2 **29**. Total peak area of the product **29** was 64 % and the coupling efficiency was *ca*. 93%.

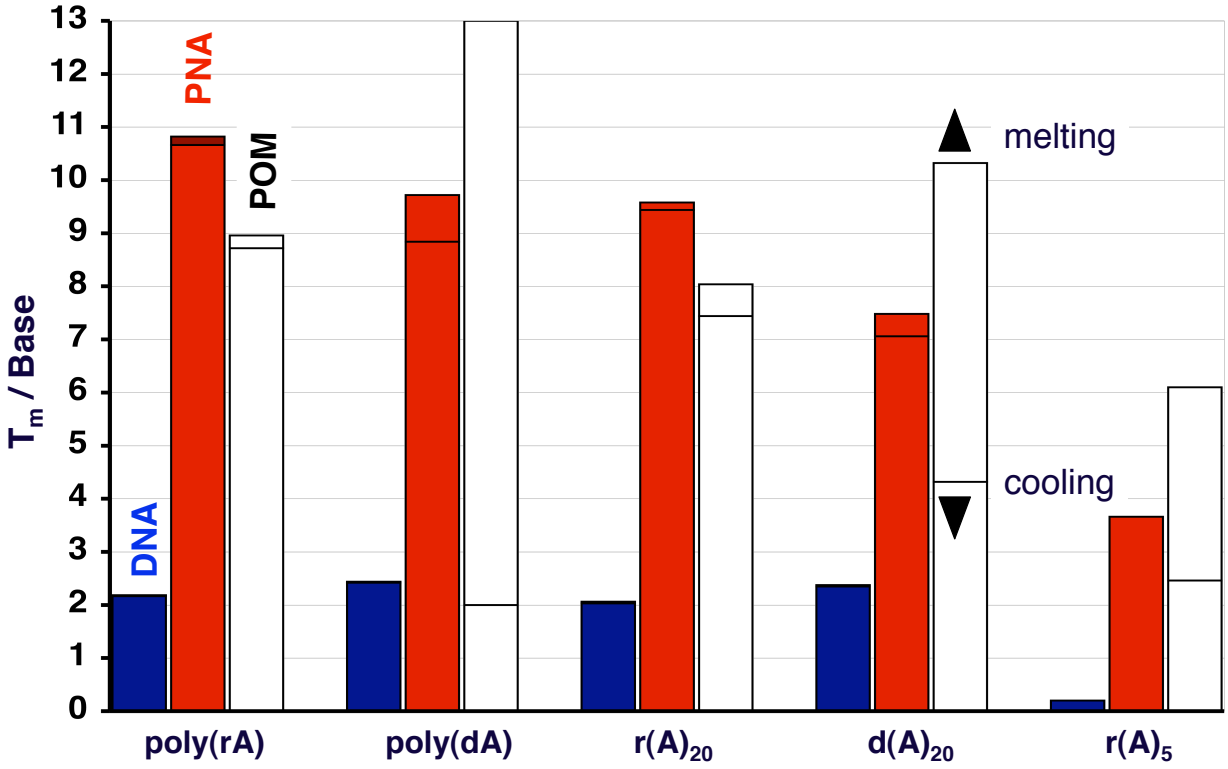

Figure 5. UV thermal denaturation and renaturation temperatures per thymine base (T_m/Base) for $d(T)_{20}$, PNA and POM Lys-TTTTT-LysNH_2 **28** *vs*. RNA & DNA, pH 7.0 & 0.12M K^+. T_ms/Base extracted from the heating (melting) curve are represented by the top of the bar, whilst T_ms extracted from cooling curves are represented by the line within the bar, unless both are coincident.

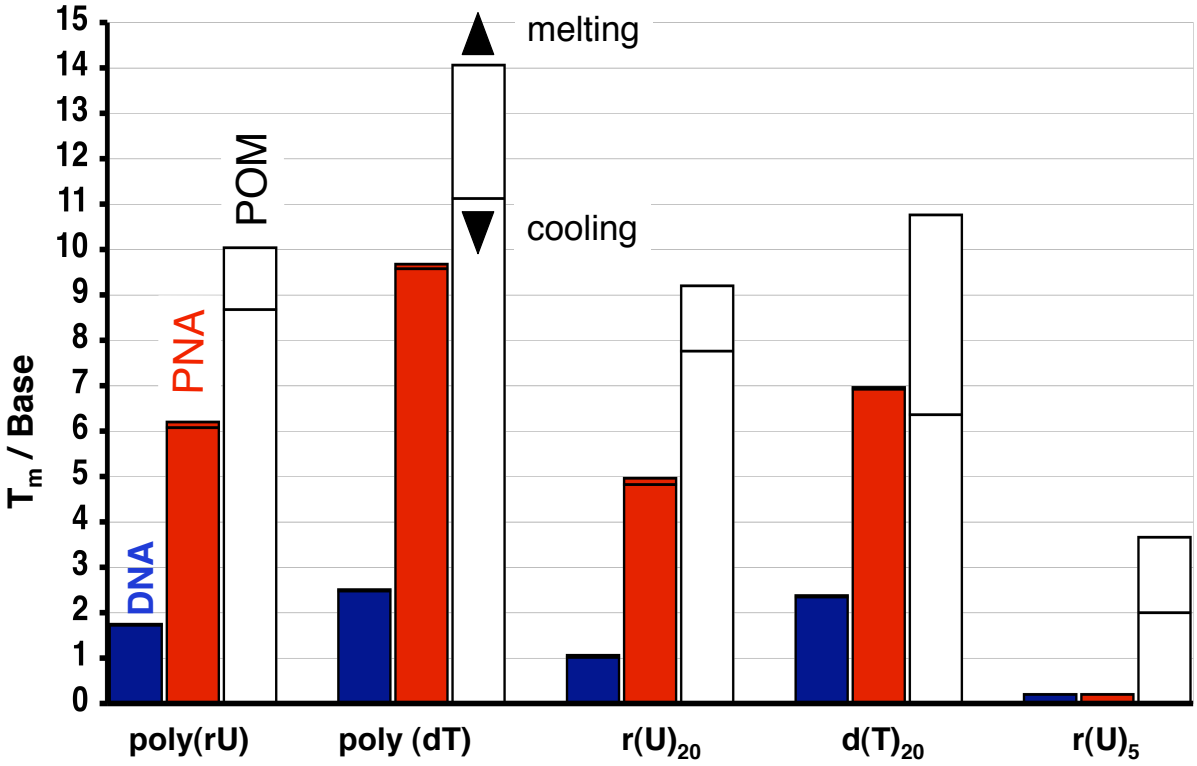

Figure 6. UV thermal denaturation and renaturation temperatures per adenine base (T_m/Base) for $d(A)_{20}$, PNA and POM Lys-AAAAA-NH_2 **29** *vs*. RNA & DNA, pH 7.0 & 0.12M K^+. T_ms/Base extracted from the melting curve are represented by the top of the bar, whilst T_ms extracted from cooling curves are represented by the line within the bar, unless both are coincident.

General experimental methods

NMR spectra were recorded on a Bruker DPX 300 operating at 300 MHz (^{1}H) and 75.5 MHz (^{13}C) or a Bruker DPX 400 operating at 400 MHz (^{1}H) and 100.6 MHz (^{13}C). Chemical shifts in ^{1}H and ^{13}C NMR spectra are expressed in ppm relative to tetramethylsilane and were internally referenced to the residual solvent signal. Chemical-shift assignments for ^{1}H and ^{13}C spectra were assisted with COSY, DEPT, HMQC and HMBC experiments. The splitting patterns for NMR spectra are designated as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), ddd (doublet of doublet of doublets), m (multiplet) and br (broad). Mass spectra were obtained using fast atom bombardment (FAB) on a VG 7AB-E Hybrid (*m*-$NO_2C_6H_4CH_2OH$ as matrix) or by electrospray (ES) on a MassLynx orthogonal accelerated-TOF mass spectrometer with samples introduced from a Waters 7240 sample injector. Low resolution and accurate masses obtained from EPSRC National Mass Spectrometry Centre (Swansea University) were performed on a Micromass Quattro II and Finnigan MAT900 respectively. Infrared spectra were recorded on a Nicolet Nexus 670 FT-IR spectrometer with samples prepared as a thin film on KBr discs or as Nujol mulls. UV measurements were carried out on a Varian Cary 400 spectrometer with cell transport accessories with samples. Molar extinction coefficients (ε) were calculated from Beer- Lambert law from a sample solution of known concentration. Elemental analyses were performed on a Carlo Erba 1108 elemental analyser. Optical rotations were measured at 25 °C with an Optical Activity AA-1000 polarimeter. Melting points were determined with an Electrothermal capillary apparatus and are uncorrected. X-ray crystallographic analysis was collected on a Nonius κCCD diffractometer. Thin layer chromatography was performed on Fluka silica gel (60 F254) coated on aluminium plates. TLC plates were visualised by UV (254 nm) and/or developed using potassium permanganate, vanillin, anisaldehyde, ninhydrin or Dragendorff stains. Flash column chromatography was performed on silica gel LC 60A purchased from Fluorochem

Limited. Chemicals were purchased from Aldrich Chemical Company, Lancaster Synthesis Ltd and Acros Organics and were used without further purification unless otherwise noted. Solvents were purified and dried where necessary according to the literature. THF was distilled from sodium with benzophenone as indicator under argon. Dichloromethane was distilled from CaH_2. Formic acid was distilled over phthalic anhydride for 6 h under argon. Deionised water was used throughout. Reactions requiring anhydrous conditions were carried out in flame-dried glassware under a positive pressure of nitrogen or argon.

(2*R*,4*R*)-2-Azidomethyl-4-hydroxy-pyrrolidine hydrochloride (5).

To a solution of azide **4** (2.0 g, 5.61 mmol) in anhydrous CH_2Cl_2 (6.0 mL), under argon, was added 4 M HCl/dioxane (6.0 mL) at 0 °C and stirred for 15 min. The solution was warmed to room temperature and stirred for 2 h. The resulting suspension was evaporated under reduced pressure, redissolved in CH_3OH (10 mL) and filtered through a pad of activated charcoal. Evaporation of the filtrate and recrystallisation from CH_3OH gave the desired amine HCl salt **5** (992 mg, 99%) as white crystals. mp 116-119°C (CH_3OH); Found: C, 33.8; H, 6.2; N, 31.4, Cl, 19.9; $C_5H_{11}N_4OCl$ requires: C, 33.6; H, 6.1; N, 31.3, Cl, 19.8%; $[\alpha]_D$ –41.7 ° (*c* = 1.0, CH_3OH); ν_{max}(KBr)/cm^{-1} 3349 (OH), 2946 (OH), 2745 ($R_2NH_2^+$), 2099 (N_3), 1598 ($R_2NH_2^+$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.66-1.72 (1H, m, H_a3), 2.30-2.37 (1H, m, H_b3), 3.15 (1H, dd, *J* 11.8, 1.7 Hz, H_a5), 3.25 (1H, dd, *J* 11.8, 4.8 Hz, H_b5), 3.70-3.83 (2H, m, H2 and H_a6), 3.93 (1H, m, H_b6), 4.46 (1H, d, *J* 3.3 Hz, H4), 5.68 (1H, d, *J* 3.3 Hz, OH), 9.80 (1H, br s, NH_2); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 36.6 (C3), 52.2 (C5), 52.5 (C6), 57.3 (C2), 68.7 (C4); *m*/*z* (ES^-) 217/215/213 ([M+Cl]$^-$, 10/50/80 %], 179/177 ([M-H]$^-$, 100/25); *m*/*z* (ES^+) 143 ([M-Cl]$^+$, 100); HRMS *m*/*z* (ES^+) 143.0934, ([M-Cl]$^+$, $C_5H_{11}N_4O$ requires *m*/*z* 143.0933).

(2*R*,4*R*)-2-Azidomethyl-4-hydroxy-*N*-(*tert*-butoxycarbonylmethyl)-pyrrolidine (6).

To a suspension of pyrrolidine HCl salt **5** (1.29 g, 7.22 mmol) in anhydrous CH_2Cl_2 (9 mL), under argon, was added diisopropylethylamine (DIEA) (2.9 mL, 16.6 mmol) at 0 °C until dissolution. *tert*-Butylbromoacetate (1.51 mL, 9.39 mmol) was added dropwise at 0 °C and stirred for a further 30 min. The solution was stirred at room temperature for 18 h and solvent was evaporated under reduced pressure. Purification by column chromatography (5% CH_3OH in CH_2Cl_2) gave the pure product **6** (1.73 g, 94%) as a clear oil. R_f 0.37 (5% CH_3OH in CH_2Cl_2); $[\alpha]_D$ +18.8° (c = 0.25, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3404 (OH), 2100 (N_3), 1731 and 1693 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (9H, s, $C(CH_3)_3$), 1.58-1.66 (1H, m, H_a3), 2.28 (1H, ddd, J 15.4, 9.8, 6.0 Hz, H_b3), 2.86 (1H, dd, J 9.8, 4.1 Hz, H_a5), 3.00-3.10 (2H, m, H_b5 and H2), 3.27-3.44 (4H, m, H_aH_b6 and H_aH_b7), 4.18-4.21 (1H, m, H4); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.8 ($C(CH_3)_3$), 39.1 (C3), 53.7 (C7), 55.1 (C6), 60.2 (C2), 62.3 (C5), 71.0 (C4), 81.7 ($C(CH_3)_3$), 170.6 ($CO_2{}^t$Bu); m/z (ES) 279 ([M+Na]$^+$, 95%), 257 ([M+H]$^+$, 100); HRMS m/z (ES) 257.1614 ([M+H]$^+$, $C_{11}H_{21}N_4O_3$ requires m/z, 257.1614).

(2*R*,4*S*)-2-Azidomethyl-4-formyloxy-*N*-(*tert*-butoxycarbonylmethyl)-pyrrolidine (7).

To a solution of alcohol **6** (519 mg, 2.02 mmol) in anhydrous THF (5 mL) under argon at –20 °C was added dry HCO_2H (92 μL, 2.43 mmol) followed by solid PPh_3 (637 mg, 2.42 mmol). To the resulting solution was immediately added diisopropylazodicarboxylate (DIAD) (480 μL, 2.43 mmol) dropwise at –30 °C and stirred for 1 h. The solution was warmed to room temperature and stirred for 18 h. Solvent was evaporated under reduced pressure and purification by column chromatography (5% EtOAc in $CHCl_3$) gave the pure product **7** (464 mg, 80%) as a clear oil. R_f 0.32 (5% EtOAc/$CHCl_3$); $[\alpha]_D$ +14.0° (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (9H, s, $C(CH_3)_3$), 2.03-2.08 (2H, m, H_aH_b3), 2.79-2.84 (1H, dd, J 10.9, 4.1 Hz, H_a5), 3.25-3.33 (3H, m, H_aH_b6 and H2), 3.38-3.53 (2H, m, H_aH_b7), 3.65(1H, dd, J

10.9, 6.0 Hz, H_b5), 5.28-5.33 (1H, m, H4), 8.03 (1H, s, HCO_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.8 ($C(CH_3)_3$), 36.4 (C3), 53.9 (C7), 54.7 (C6), 59.3 (C2), 60.5 (C5), 72.8 (C4), 81.8 ($C(CH_3)_3$), 160.9 (HCO_2), 170.1 ($CO_2{}^tBu$); *m/z* (ES) 285 ($[M+H]^+$, 20%).

(2*R*,4*S*)-2-Azidomethyl-4-hydroxy-*N*-(*tert*-butoxycarbonylmethyl)-pyrrolidine (8).

To a solution of formyl ester **7** (407 mg, 1.43 mmol) in CH_3OH (25 mL) was added concentrated aqueous ammonia (25 μL) and the mixture was stirred at room temperature. After 2 h, the solvent was evaporated under reduced pressure and purification by column chromatography (2:1 → 1:2 hexane/EtOAc) gave the product **8** (336 mg, 92%) as a clear oil R_f 0.20 (1:2 hexane/EtOAc); $[\alpha]_D$ +23.4° (*c* = 0.5, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3584 and 3386 (OH), 2099 (N_3), 1732 (CO); ^1H NMR (400 MHz, $CDCl_3$) δ 1.40 (9H, s, $C(CH_3)_3$), 1.72 (1H, ddd, *J* 13.1, 9.3, 4.5 Hz, H_a3), 1.95 (1H, dd, *J* 13.1, 5.8 Hz, H_b3), 2.70 (1H, d, *J* 11.1 Hz, H_a5), 3.06-3.11 (1H, m, H2), 3.32-3.36 (2H, m, H_aH_b6), 3.39-3.50 (3H, m, H_b5 and H_aH_b7), 4.15- 4.19 (1H, m, H4); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 28.5 ($C(CH_3)_3$), 40.4 (C3), 52.9 (C7), 54.6 (C6), 59.7 (C2), 61.8 (C5), 71.7 (C4), 82.7 ($C(CH_3)_3$), 172.9 ($CO_2{}^tBu$); *m/z* (FAB) 279 ($[M+Na]^+$, 25%), 257 ($[M+H]^+$, 50); HRMS *m/z* (ES) 257.1613 ($[M+H]^+$, $C_{11}H_{21}N_4O_3$ requires *m/z*, 257.1614).

(2*R*,4*S*)-2-Azidomethyl-4-(*p*-toluenesulfonyl)oxy-*N*-(*tert*-butoxycarbonylmethyl)-pyrrolidine (9). *Method 1*: To a solution of azide **8** (1.71 g, 6.60 mmol) in anhydrous pyridine (17.0 mL), under N_2, was added *p*-toluenesulphonyl chloride (2.54 g, 13.3 mmol) at 0 °C. The solution was stirred for 15 min at 0 °C and then allowed to warm to room temperature. After 18 h stirring at room temperature CH_3OH (10 mL) was added and solvents were evaporated under reduced pressure. Brine (150 mL) was added to the brown residue and the mixture was extracted with EtOAc (4 × 150 mL). The combined organic extracts were dried over $MgSO_4$

and evaporated under reduced pressure. Purification by column chromatography (3:1 hexane/EtOAc, R_f 0.30) afforded *p*-toluenesulphonate **9** derivative (2.36 g, 86 %) as a colourless oil.

Method 2: To a solution of alcohol **6** (103 mg, 0.40 mmol) in anhydrous THF (4 mL), under N_2, was added PPh_3 (126 mg, 0.48 mmol) at –10 °C, under N_2. To the suspension was immediately added DIAD (95 μL, 0.48 mmol) and stirred for 5 min. Methyl *p*-toluenesulphonate (73 μL, 0.48 mmol) was added dropwise at 0 °C and stirred for a further 10 min at 0 °C. The suspension was warmed to room temperature and stirred for 18 h. Evaporation of the solvent under reduced pressure, followed by purification by column chromatography (5% EtOAc in $CHCl_3$, R_f 0.50) gave the title product **9** (103 mg, 66%) as a colourless oil. R_f 0.50 (5% EtOAc in $CHCl_3$); $[\alpha]_D$ + 5.4° (*c* = 1.5, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2100 (N_3), 1731 (CO), 1366 and 1176 (-SO_2O-); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (9H, s, C(CH_3)$_3$), 1.80-1.87 (1H, m, H_a3), 2.01-2.07 (1H, m, H_b3), 2.38 (3H, s, tosyl CH_3), 2.76 (1H, dd, *J* 11.1, 4.3 Hz, H_a5), 3.12-3.16 (2H, m, H2 and H_a6), 3.20 (1H, d, *J* 16.9 Hz, H_a7), 3.26-3.31 (1H, m, H_b6), 3.36-3.43 (2H, m, H_b7 and H_b5), 4.81-4.87 (1H, m, H4), 7.28 (2H, d, *J* 8.4 Hz, tosyl H), 7.71 (2H, d, *J* 8.4 Hz, tosyl H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 22.1 (tosyl CH_3), 28.5 (C(CH_3)$_3$), 36.5 (C3), 53.7 (C7), 55.1 (C6), 59.4 (C5), 60.7 (C2), 79.7 (C4), 81.9 (*C*(CH_3)$_3$), 128.2 (tosyl CH), 130.4 (tosyl CH), 134.0 (tosyl *p*-C), 145.4 (tosyl *ipso*-C), 170.0 (CO_2^{t}Bu); *m/z* (FAB) 433 ([M+Na]$^+$, 30%), 411 ([M+H]$^+$, 100%); HRMS *m/z* (ES) 411.1699 ([M+H]$^+$, $C_{18}H_{26}N_4O_5S$ requires *m/z*, 411.1701).

(2´*R*,4´*R*)-2´-Azidomethyl-4´-(*N*^{3}-benzoylthymin-1-yl)-*N*1´-(*tert*-butoxycarbonylmethyl)-pyrrolidine (10). To alcohol **8** (1.7 g, 6.60 mmol) and *N*^{3}- benzoylthymine (1.84 g, 7.90 mmol) in dry THF (25 mL), under N_2 at –25 °C, was added PPh_3 (2.08 g, 7.96 mmol) followed immediately by the dropwise addition of DIAD (1.8 mL, 8.62 mmol) over 15 min.

The suspension was stirred at –25 °C for 30 min and a further 18 h at room temperature. The resulting solution was evaporated under reduced pressure and the crude product was purified by column chromatography (5% EtOAc in CH_2Cl_2) to give the title compound **10** (2.39 g, 77%) as a white foam. R_f 0.25 (5% EtOAc in CH_2Cl_2); $[\alpha]_D$ +34.6° (c = 2.0, $CHCl_3$); Found: C, 59.1; H 5.9; N, 17.7; $C_{23}H_{28}N_6O_5$ requires: C, 58.9; H, 5.9; N, 17.9%; ν_{max}(KBr)/cm^{-1} 3064 (C=C), 2101 (N_3), 1745, 1697 and 1654 (CO); λ_{max}(CH_3OH)/nm 253 (ε/$dm^3mol^{-1}cm^{-1}$ 9600); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (9H, s, ester $C(CH_3)_3$), 1.78-1.84 (1H, m, H_a3´), 2.03 (3H, s, CH_3), 2.56-2.64 (1H, m, H_b3´), 2.87 (1H, dd, J 11.0, 7.0 Hz, H_a5´), 2.92-2.96 (1H, m, H2´), 3.11 (1H, d, J 17.0 Hz, H_a7´), 3.26 (1H, dd, J 13.0, 3.5 Hz, H_a6´), 3.46 (2H, d, J 11.0 Hz, H_b5´), 3.62 (1H, dd, J 13.0, 3.5 Hz, H_b6´), 3.67 (1H, d, J 17.0 Hz, H_b7´), 5.07-5.11 (1H, m, H4´), 7.51 (2H, t, J 7.5 Hz, BzCH), 7.66 (2H, t, J 7.5 Hz, Bz CH), 7.94 (1H, d, J 7.0 Hz, Bz CH), 8.19 (1H, s, H6); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.0 (CH_3), 28.5 ($C(CH_3)_3$), 36.6 (C3´), 52.2 (C6´), 52.6 (C4´), 54.1 (C7´), 58.8 (C5´), 61.8 (C2´), 82.1 ($C(CH_3)_3$), 111.4 (C5), 129.4 (Bz CH), 130.8 (Bz CH), 132.1 (Bz C), 135.2 (CH), 138.3 (C6), 150.5 (C2), 163.2 (C4), 169.5 (Bz CO), 169.6 ($CO_2{}^t$Bu); m/z (ES) 491 ([M+Na]$^+$, 10%), 469 ([M+H]$^+$, 100); HRMS m/z (ES) 469.2195 ([M + H]$^+$, $C_{23}H_{29}N_6O_5$ requires m/z, 469.2199).

(2´*R*,4´*R*)-2´-(Azidomethyl)-4´-[*N*^{2}-isobutyrylguanin-7-yl]-*N*1´-(*tert*-butoxycarbonylmethyl)-pyrrolidine (23). A suspension of tosylate **9** (100 mg, 0.25 mmol), N^2-isobutyryl-O^6-[2-(*p*-nitrophenyl)ethyl]guanine (137.5 mg, 0.62 mmol), anhydrous K_2CO_3 (175 mg, 1.25 mmol) and 18-crown-6 (50 mg, 0.19 mmol) in DMF (1.2 mL) was stirred at 70 °C under argon for 48 h. The reaction mixture was concentrated under reduced pressure. Brine (5 mL) was then added and the mixture was extracted with EtOAc (4 × 5 mL) dried over $MgSO_4$ and then evaporated under reduced pressure. The residue was then purified by column chromatography (10% CH_3OH in $CHCl_3$) to give N^7-guaninyl derivative **23** (29.5 mg, 26%) as

white crystals. R_f 0.42 (10% CH_3OH in $CHCl_3$); mp 58 °C; ^{1}H NMR (300 MHz, $CDCl_3$); $[\alpha]_D$ –13.4° (c = 2.0, CH_3OH); ν_{max}(KBr)/cm^{-1} 3108 (NH), 2098 (N_3), 1736, 1681 and 1614 (CO); λ_{max}(CH_3OH)/nm 221 (ε/dm^3 mol^{-1} cm^{-1} 1.1 x 10^4) and 267 (7.4 × 10^3); δ 1.24 (6H, d, J 8 Hz, isobutyryl $(CH_3)_2$), 1.49 (9H, s, $C(CH_3)_3$), 1.90-1.95 (1H, m, H_a3´), 2.67-2.77 (1H, m, H_b3´) 2.84-2.95 (1H, m, isobutyl CH), 3.03-3.09 (2H, m, H2´ and H_a5´), 3.21 (1H, d, J 17.3 Hz, Ha7´), 3.19 (1H, m, H_a6´), 3.38 (1H, m, H_b6´), 3.61-3.67 (2H, m, H_b7´ and H_b5´), 5.41-5.48 (1H, m, H4´), 8.53 (1H, s, H8), 9.91 (1H, br s, H1), 12.24 (1H, br s, NH_2); ^{13}C NMR (300 MHz, CDCl3) δ 19.8 ($CH(CH_3)_2$), 28.9 ($C(CH_3)_3$), 36.8 ($CH(CH_3)_2$), 38.4 (C3´), 53.8 (C5´), 54.8 (C7´), 55.7 (C2´), 59.7 (C6´), 61.6 (C4´), 82.5 ($C(CH_3)_3$), 112.3 (C5), 142.9 (C8), 148.1 (C2), 154.1 (C4), 157.6 (C6), 170.1 ($CO_2{}^t$Bu), 180.2 (isobutyryl CO); *m/z* (ES) 460 ([M+H]$^+$, 100%); HRMS *m/z* (ES) 460.2420 ([M+H]$^+$, $C_{20}H_{30}N_9O_4$ requires *m/z*, 460.2421).

POM Ac-TTTTT-NH$_2$ (27).

Retention time on analytical HPLC was 29 min, using a Phenomenex Luna 3µ C18 250 × 4.6 mm analytical column. Solvent A was H_2O with 0.1 % HCO_2H and solvent B was acetonitrile. The flow rate was 1 mLmin^{-1} with 100 % A for 5 min followed by a gradient from 100 % A changing to 95 % A with 5 % B over 30 min. *m/z* (ES-MS) 460.9 ([M+3H]$^{3+}$ 100%, $C_{62}H_{88}N_{21}O_{16}{}^{3+}$ requires *m/z*, 460.9); 356.8 ([M+2H+2Na]$^{4+}$ 90%, $C_{62}H_{87}N_{21}O_{16}Na_2{}^{4+}$ requires *m/z*, 356.2), 345.9 ([M+4H]$^{4+}$ 75%, $C_{62}H_{89}N_{21}O_{16}{}^{4+}$ requires *m/z*, 345.9), 690.8 ([M+2H]$^{2+}$ 15%, $C_{62}H_{87}N_{21}O_{16}{}^{2+}$ requires *m/z*, 690.8), 701.8 ([M+H+Na]$^{2+}$ 10%, $C_{62}H_{86}N_{21}O_{16}Na^{2+}$ requires *m/z*, 701.8); 712.8 ([M+2Na]$^{2+}$ 8%, $C_{62}H_{85}N_{21}O_{16}Na_2{}^{2+}$ requires *m/z*, 712.8).

POM Lys-TTTTT-LysNH$_2$ (28).

Retention time on analytical HPLC was 24 min (Figure 4A), using a Kromasil 3.5 µ, 250 x 4.6 mm, C8-reverse phase column. Solvent A was H_2O with 0.1 % HCO_2H and solvent B was

acetonitrile with. The flow rate was 1 mLmin^{-1} with100 % A for 5 min followed by a gradient from 100 % A changing to 95 % A with 5 % B over 30 min. *m/z* (ES-MS) 409.7 ([M+2H+2Na]$^{4+}$ 100%, $C_{72}H_{109}N_{25}O_{17}Na_2^{4+}$ requires *m/z*, 410.4), 532.3 ([M+3H]$^{3+}$ 50%, $C_{72}H_{110}N_{25}O_{17}^{3+}$ requires *m/z*, 532.3), 797.9 ([M+2H]$^{2+}$ 12%, $C_{72}H_{109}N_{25}O_{17}^{2+}$ requires *m/z*, 797.9), 808.9 ([M+H+Na]$^{2+}$ 10%, $C_{72}H_{108}N_{25}O_{17}Na^{2+}$ requires *m/z*, 808.9).

POM Lys-AAAAA-NH$_2$ (29).

Retention time on analytical HPLC was 34 min (Figure 4B), using a Kromasil 3.5 μ, 250 x 4.6 mm, C8-reverse phase column. Solvent A was H_2O with 0.1 % HCO_2H and solvent B was acetonitrile. The flow rate was 1 mLmin^{-1} with 100 % A for 5 min followed by a gradient from 100 % A changing to 70 % A with 30 % B over 30 min, then changing to 30 % A with 70 % B over the next 20 min. *m/z* (ES-MS) 378.7 ([M+4H]$^{4+}$ 100%, $C_{66}H_{94}N_{38}O_6^{4+}$ requires *m/z*, 378.7), 324.4 ([M+H+3Na+K]$^{5+}$ 100%, $C_{66}H_{91}N_{38}O_6Na_3K^{5+}$ requires *m/z*, 323.9), 504.6 ([M+3H]$^{3+}$ 43%, $C_{66}H_{93}N_{38}O_6^{3+}$ requires *m/z*, 504.609), 756.8 ([M+2H]$^{2+}$ 12%, $C_{66}H_{92}N_{38}O_6^{2+}$ requires *m/z*, 756.4), 778.8 ([M+2Na]$^{2+}$ 5%, $C_{66}H_{90}N_{38}O_6Na_2^{2+}$ requires *m/z*, 778.40).

Thermal Denaturation Experiments

UV melting plots of absorbance versus temperature were measured at 260 nm on a Varian Cary 400 Scan UV-visible spectrophotometer fitted with a 6 × 6 Peltier thermostable multicell holder connected to a temperature controller module. Experiments were performed in double beam mode and controlled by an interfaced Dell OptiPlex GX150 computer. Denaturation experiments were performed in 10 mm path length 4 mm path width self-masking semi-micro quartz cells fitted with a Teflon stopper. Temperature was monitored using temperature probes attached to reference cells by probe holders. Concentrations of POM oligomers, oligonucleotides and polynucleotides were measured spectrophotometrically at 80 °C from

known molar extinction coefficients of nucleotidyl units (15000 $M^{-1}cm^{-1}$ for dA, rA and POM(A); 8500 $M^{-1}cm^{-1}$ for dT and POM(T); 7600 $M^{-1}cm^{-1}$ for rC; 12160 $M^{-1}cm^{-1}$ for rG; 10210 $M^{-1}cm^{-1}$ for rU and 63180 $M^{-1}cm^{-1}$ for rA_2GA_2). Buffers were prepared as double concentrated stock solutions and diluted to the appropriate concentrations during sample preparation. Typically, to K_2HPO_4 (2.0 mmol) and KCl (24, 44, 124 or 240 mmol) in a standard 100 mL volumetric flask was added sterile deionised water up to three-quarters of the final volume. The pH was adjusted using 1 M HCl or 1 M KOH and addition of water to the final mark afforded the desired 20 mM phosphate buffer. All appropriate equipment was autoclaved before use. Sterile nuclease, protease and DEPC-free deionised water was used throughout. All samples were stored at –20 °C. Polynucleotides were purchased from Sigma. Short chain RNA oligonucleotides $r(A)_{20}$, $r(U)_{20}$, $r(A)_5$ and $r(U)_5$ were purchased from Transgenomic (Cruachem). Short chain DNA oligonucleotides $d(A)_{20}$ and $d(T)_{20}$ were obtained from Sigma Genosys. $LysPNA(T)_5LysNH_2$ oligomer was purchased from Oswel Research Products Ltd.

Procedure for typical UV melting experiment.

In a typical melting experiment, equimolar amount in bases of each oligomer were added to 500 μL of buffer solution. Water was added to give a final volume of 1 mL and the solution was gently shaken. A final concentration of 42 μM in bases was used throughout unless otherwise noted. Each thermal denaturation experiment consists of 3 ramps and an averaging time of 1 s was used throughout. Data was collected every 1 °C for the first ramp and 0.1 °C for subsequent parts of the experiment. Samples were initially heated at a rate of 5 ˚C/min to 93 °C to dissociate all strands. After 1 min, samples were cooled at 0.2 °C/min to 15 ˚C and after a holding time of 1 min were heated at 0.2 ˚C/min to 93 ˚C. Experiments requiring incubation were prepared following the described protocol and were left to stand at room

temperature for 48 h. Melts were carried out at 0.2 ˚C/min from 15 to 93 ˚C. All melting experiments were performed at least twice. All T_m values were obtained from the maxima of first derivative curves that were calculated from Varian Thermal software using a filter size of 97 and smoothed at every 0.3 °C.