

## Electronic Supplementary Information (ESI)

### Stereospecific Backbone Methylation of Pyrrolidine-amide Oligonucleotide Mimics (POM)

Anila I. Khan, T. H. Samuel Tan and Jason Micklefield\*

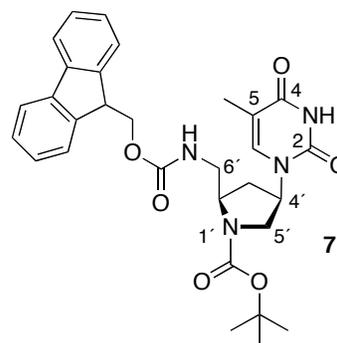
School of Chemistry, The University of Manchester, Faraday Building, Sackville Street, PO Box 88, Manchester M60 1QD, UK.

Email: [jason.micklefield@manchester.ac.uk](mailto:jason.micklefield@manchester.ac.uk)

**(1) Synthesis of MePOM monomers:** For general synthetic experimental details see ref 5b.

#### (2'R, 4'R)-[2'-(Fluoren-9-yl-methoxycarbonyl)aminomethyl]-4'-(thymine-1-yl)-N-(tert-butoxycarbonyl)-pyrrolidine (7).

A solution of phthalimide derivative **6**<sup>5b</sup> (6.73 g, 14.77 mmol) in 40% aqueous methylamine was heated to 50 °C for 2 h and the resulting yellow solution was evaporated under reduced pressure and the resulting residue was further dried under high vacuum. The crude amine was dissolved in dioxane (100 mL) and 10% (w/v) aqueous sodium carbonate (125 mL) and cooled to 0 °C. A solution of 9-fluorenylmethoxy chloroformate (4.60 g, 17.78 mmol) in dioxane (25 mL) was then added to the crude amine and the resulting suspension was stirred for 18 h at room temperature. The dioxane was evaporated under reduced pressure and brine (150 mL) was then added to the aqueous solution, which was extracted with EtOAc (5 x 150 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered and evaporated under reduced pressure. Purification of the crude product by column chromatography (5% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>; R<sub>f</sub> 0.26) gave the title product **7** (6.56 g, 81%) as a pale yellow foam.

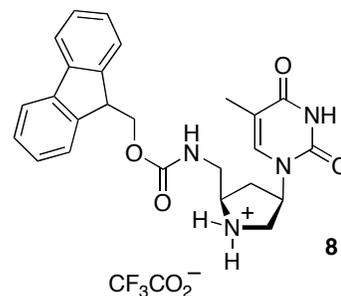


<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.49 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.88 (3H, s, CH<sub>3</sub>), 1.99-2.05 (1H, m, H<sub>a</sub>3'), 2.43-2.48 (1H, m, H<sub>b</sub>3'), 3.21-3.27 (1H, m, H<sub>a</sub>5'), 3.51 (1H, br s, H<sub>a</sub>H<sub>b</sub>6'), 4.01-4.04 (2H, m, H<sub>b</sub>5' and H2'), 4.23 (1H, t, J 7.0 Hz, Fmoc aliphatic CH), 4.38-4.45 (2H, m, Fmoc CH<sub>2</sub>), 4.95-5.02 (1H, m, H4'), 7.04 (1H, s, H6), 7.31 (2H, t, J 7.0 Hz, Fmoc Ar-H), 7.40 (2H, t, J 7.0 Hz, Fmoc Ar-H), 7.60 (2H, d, J 7.5 Hz, Fmoc Ar-H), 7.76 (2H, d, J 7.0 Hz, Fmoc Ar-H), 9.29 (1H, s, H3); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>) δ 12.9 (CH<sub>3</sub>), 28.8 (Boc C(CH<sub>3</sub>)<sub>3</sub>), 33.7 (C3'), 45.1 (C6'), 47.6 (Fmoc aliphatic CH), 49.6 (C5'), 52.7 (C4'), 56.5 (C2'), 67.2 (Fmoc CH<sub>2</sub>), 81.4 (Boc C(CH<sub>3</sub>)<sub>3</sub>), 112.1 (C5), 120.4, 125.3, 127.4 & 128.1 (all Fmoc Ar-CH), 136.4 (C6), 141.7 & 144.2 (both Fmoc Ar-C), 151.5 (C2), 155.1 (Boc CO), 157.4 (Fmoc CO), 163.9 (C4); m/z (FAB<sup>+</sup>) 569 ([M+Na]<sup>+</sup>, 20%), 547

( $[M+H]^+$ , 25), 447 (100%); HRMS (ESI<sup>+</sup>)  $m/z$   $[M+H]^+$  547.2552, calc'd for C<sub>30</sub>H<sub>35</sub>N<sub>4</sub>O<sub>6</sub> 547.2557;  $\nu_{\max}$ (KBr)/cm<sup>-1</sup> 1690 (CO);  $\lambda_{\max}$ (CH<sub>3</sub>OH)/nm 265 ( $\epsilon$ /dm<sup>-3</sup>mol<sup>-1</sup>cm<sup>-1</sup> 2.1 x 10<sup>4</sup>) and 300 (4.3 x 10<sup>3</sup>);  $[\alpha]_D + 18.0^\circ$  ( $c = 1.0$ , CH<sub>2</sub>Cl<sub>2</sub>).

**(2'R, 4'R)-[2'-(Fluoren-9-yl-methoxycarbonyl)aminomethyl]-4'-(thymine-1-yl)-pyrrolidinium trifluoroacetate (8).**

To pyrrolidine **7** (500 mg, 0.91 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (7.2 mL) was added trifluoroacetic acid (1.8 mL) and the mixture was stirred for 4 h at room temperature. The solution was then evaporated under a stream of N<sub>2</sub> and resulting oil was triturated with ether. The precipitate was filtered and dried *in vacuo* to give trifluoroacetate salt **8** (500 mg, 98%) as a white powder.

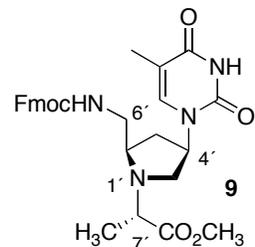


mp 130-132 °C (CF<sub>3</sub>CO<sub>2</sub>H/ether); Found: C, 57.7; H, 4.8; N, 10.0; F, 9.9; Calc'd for C<sub>27</sub>H<sub>27</sub>N<sub>4</sub>O<sub>6</sub>F<sub>3</sub>: C, 57.8; H, 4.8; N, 10.0; F, 10.1%; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.78 (3H, s, CH<sub>3</sub>), 1.84-1.95 (1H, m, H<sub>a</sub>3'), 2.35-2.44 (1H, m, H<sub>b</sub>3'), 3.39-3.47 (4H, m, H<sub>a</sub>H<sub>b</sub>5' and H<sub>a</sub>H<sub>b</sub>6'), 3.66 (1H, br s, H<sub>2</sub>'), 4.24 (1H, t,  $J$  6.5 Hz, Fmoc aliphatic CH), 4.39 (2H, d,  $J$  6.5 Hz, Fmoc CH<sub>2</sub>), 5.02-5.07 (1H, m, H<sub>4</sub>'), 7.29-7.43 (4H, m, Fmoc Ar-H), 7.61-7.70 (3H, m, H<sub>6</sub> and Fmoc Ar-H), 7.88 (2H, d,  $J$  7.0 Hz, Fmoc Ar-H), 8.64 (1H, br s, NH<sub>a</sub>1'), 9.62 (1H, br s, NH<sub>b</sub>1'), 11.46 (1H, s, H<sub>3</sub>); <sup>13</sup>C NMR (75.5 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  12.4 (CH<sub>3</sub>), 32.7 (C3'), 41.1 (C6'), 47.0 (Fmoc aliphatic CH), 48.0 (C5'), 54.9 (C4'), 59.6 (C2'), 66.0 (Fmoc CH<sub>2</sub>), 109.8 (C5), 120.5, 125.5, 127.4 and 128.0 (all Fmoc Ar-CH), 139.5 (C6), 141.1 & 144.2 (Fmoc Ar-C), 151.4 (C2), 157.0 (Fmoc CO), 164.2 (C4);  $m/z$  (FAB<sup>+</sup>): 447 ( $[M-CF_3COO]^-$ , 85%), 251 (20), 179 (100); HRMS (ESI<sup>+</sup>)  $m/z$  (M-CF<sub>3</sub>COO<sup>-</sup>)<sup>+</sup> 447.2028, calc'd for C<sub>27</sub>H<sub>28</sub>N<sub>4</sub>O<sub>6</sub>F<sub>3</sub> 447.2032;  $\nu_{\max}$ (KBr)/cm<sup>-1</sup> 2799 (NH<sub>2</sub><sup>+</sup>), 1685 (CO), 761, 743 and 722 (CF);  $\lambda_{\max}$ (CH<sub>3</sub>OH)/nm 265 ( $\epsilon$ /dm<sup>3</sup>mol<sup>-1</sup>cm<sup>-1</sup> 2.1 x 10<sup>4</sup>) and 300 (5.0 x 10<sup>3</sup>);  $[\alpha]_D -15.8^\circ$  ( $c = 1.0$ , CH<sub>3</sub>OH).

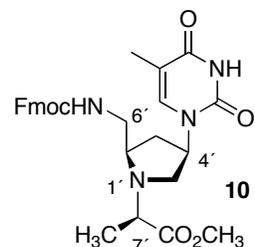
**(2'R, 4'R, 7'S)- & (2'R, 4'R, 7'R)- [2'-(fluoren-9-yl-methoxycarbonyl)aminomethyl]-4'-(thymine-1-yl)-pyrrolidiny-N-[( $\alpha$ -methyl)acetic acid methyl ester] (9) & (10).**

Diisopropylethylamine (DIPEA) (1.3 mL, 7.74 mmol) was added to a suspension of TFA salt **8** (1.74 g, 3.26 mmol) in anhydrous DMF (25 mL) at 0 °C and the mixture was stirred for 30 min under N<sub>2</sub>. Additional DIPEA (1.1 mL, 6.19 mmol) was added followed by racemic methyl 2-bromopropionate (0.5 mL, 4.64 mmol). The reaction mixture was left stirring for further for 18 h and then evaporated under reduced pressure. Purification by column chromatography (3:1 EtOAc/hexane) gave the (7'S)-diastereoisomers **9** (795 mg, 46%) and (7'R)-diastereoisomers **10** (693 mg, 42%) as white foams. This reaction was repeated, as above, with **8** (50 mg, 89.2  $\mu$ mol) and (2R)-methyl 2-bromopropionate<sup>10</sup> (19.0  $\mu$ L, 140.8  $\mu$ mol) to give the 7'S- diastereoisomers as the major product (24.7 mg, 52%). Similarly a repeat reaction with **8** (50 mg, 89.2  $\mu$ mol) and (2S)-methyl 2-bromopropionate<sup>10</sup> (19.0  $\mu$ L, 140.8  $\mu$ mol) gave the (7'R)-diastereoisomer **10** (23.3 mg, 49%). In both cases a small amount (5-7 %) of the opposite diastereoisomer was isolated.

**(7'S)-diastereoisomers (9):**  $R_f = 0.28$  (3:1 EtOAc/hexane); mp 90–93 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.22 (3H, d,  $J$  7.0 Hz,  $\text{C7}'\text{-CH}_3$ ), 1.55-1.65 (1H, m,  $\text{H}_a3'$ ), 1.82 (3H, s,  $\text{CH}_3$  Thymine), 2.33-2.41 (1H, m,  $\text{H}_b3'$ ), 2.85-2.90 (1H, m,  $\text{H}_a5'$ ), 2.84 (1H, br d,  $J$  10.9 Hz,  $\text{H}_a5'$ ), 2.95-3.01 (1H, m,  $\text{H}_a6'$ ), 3.03-3.06 (1H, m,  $\text{H}_b6'$ ), 3.38-3.44 (1H, m,  $\text{H2}'$ ), 3.67 (3H, s,  $\text{OCH}_3$ ), 3.64-3.70 (1H, m,  $\text{H7}'$ ), 4.11 (1H, t,  $J$  7.3 Hz, Fmoc aliphatic CH), 4.25-4.31 (1H, m, Fmoc  $\text{CH}_a\text{H}_b$ ), 4.32-4.34 (1H, m, Fmoc  $\text{CH}_a\text{H}_b$ ), 4.89 (1H, br s,  $\text{H4}'$ ), 5.25 (1H, br s, carbamate NH), 7.21 (2H, t,  $J$  7.3 Hz, Fmoc Ar-H), 7.31 (2H, t,  $J$  7.3 Hz, Fmoc Ar-H), 7.48 (2H, t,  $J$  6.6 Hz, Fmoc Ar-H), 7.66 (2H, d,  $J$  7.6 Hz, Fmoc Ar-H), 7.92 (1H, s,  $\text{H6}$ ), 9.04 (1H, s,  $\text{H3}$ );  $^{13}\text{C}$  NMR (100.6 MHz,  $\text{CDCl}_3$ )  $\delta$  10.9 ( $\text{C7}'\text{-CH}_3$ ), 13.0 (Thymine  $\text{CH}_3$ ), 36.4 ( $\text{C3}'$ ), 41.9 ( $\text{C6}'$ ), 47.6 (Fmoc aliphatic CH), 52.2 ( $\text{C4}'$ ), 52.3 ( $\text{C5}'$ ), 52.7 ( $\text{C2}'$ ), 55.6 ( $\text{OCH}_3$ ), 59.5 ( $\text{C7}'$ ), 67.2 (Fmoc  $\text{CH}_2$ ), 111.4 ( $\text{C5}$ ), 120.4, 125.4, 127.4 & 128.1 (all Fmoc Ar-CH), 138.3 ( $\text{C6}$ ), 141.7 & 144.2 (both Fmoc Ar-C), 151.6 ( $\text{C2}$ ), 157.2 (Fmoc CO), 164.3 ( $\text{C4}$ ), 173.5 ( $\text{CO}_2\text{CH}_3$ );  $m/z$  (ESI $^+$ ) 533 ( $[\text{M}+\text{H}]^+$ , 80%), 555 ( $[\text{M}+\text{Na}]^+$ , 100%); HRMS (ESI $^+$ )  $m/z$   $[\text{M}+\text{Na}]^+$  555.2212, calc'd for  $\text{C}_{29}\text{H}_{32}\text{N}_4\text{O}_6\text{Na}$  555.2214;  $[\alpha]_D = -10.6^\circ$  ( $c = 2.0$ ,  $\text{CH}_3\text{Cl}$ ).



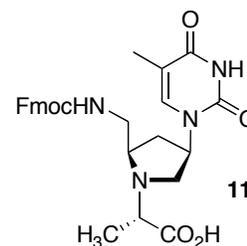
**(7'R)-diastereoisomers (10):**  $R_f = 0.31$  (3:1 EtOAc/hexane); mp 79–81 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.43 (3H, d,  $J$  7.0 Hz,  $\text{C7}'\text{-CH}_3$ ), 1.65-1.74 (1H, m,  $\text{H}_a3'$ ), 1.90 (3H, s,  $\text{CH}_3$  Thymine), 2.48-2.53 (1H, m,  $\text{H}_b3'$ ), 3.03-3.08 (2H, m,  $\text{H}_a5'$  and  $\text{H2}'$ ), 3.23-3.27 (1H, m,  $\text{H}_b5'$ ), 3.52 (2H, s,  $\text{H6}'$ ), 3.74 (3H, s,  $\text{OCH}_3$ ), 3.77-3.86 (1H, m,  $\text{H7}'$ ), 4.23 (1H, t,  $J$  7.0 Hz, Fmoc aliphatic CH), 4.45 (1H, dd,  $J$  10.5, 7.0 Hz Fmoc  $\text{CH}_a\text{H}_b$ ), 4.47 (1H, dd,  $J$  10.5, 7.0 Hz Fmoc  $\text{CH}_a\text{H}_b$ ), 5.01-5.10 (1H, m,  $\text{H4}'$ ), 5.33 (1H, s, carbamate NH), 7.31-7.35 (2H, m, Fmoc Ar-H), 7.43 (2H, t,  $J$  7.0 Hz, Fmoc Ar-H), 7.59 (2H, t,  $J$  7.0 Hz, Fmoc Ar-H), 7.44 (1H, s,  $\text{H6}$ ), 7.79 (2H, d,  $J$  7.5 Hz, Fmoc Ar-H), 8.39 (1H, s,  $\text{H3}$ );  $^{13}\text{C}$  NMR (100.6 MHz,  $\text{CDCl}_3$ )  $\delta$  13.1 (Thymine  $\text{CH}_3$ ), 17.0 ( $\text{C7}'\text{-CH}_3$ ), 36.1 ( $\text{C3}'$ ), 42.0 ( $\text{C6}'$ ), 47.7 (Fmoc aliphatic CH), 51.9 ( $\text{C4}'$ ), 52.0 ( $\text{C2}'$ ), 53.0 ( $\text{C5}'$ ), 55.5 ( $\text{OCH}_3$ ), 59.7 ( $\text{C7}'$ ), 67.1 (Fmoc  $\text{CH}_2$ ), 111.7 ( $\text{C5}$ ), 120.4, 125.3, 127.4 & 128.1 (all Fmoc Ar-CH), 137.5 ( $\text{C6}$ ), 141.7 & 144.2 (both Fmoc Ar-C), 151.4 ( $\text{C2}$ ), 157.1 (Fmoc CO), 164.0 ( $\text{C4}$ ), 173.2 ( $\text{CO}_2\text{CH}_3$ );  $m/z$  (ESI $^+$ ) 533 ( $[\text{M}+\text{H}]^+$ , 60%), 555 ( $[\text{M}+\text{Na}]^+$ , 100%); HRMS (ESI $^+$ )  $m/z$   $[\text{M}+\text{Na}]^+$  555.2214, calc'd for  $\text{C}_{29}\text{H}_{32}\text{N}_4\text{O}_6\text{Na}$  555.2214;  $[\alpha]_D = +11.2^\circ$  ( $c = 2.0$ ,  $\text{CH}_3\text{Cl}$ ).



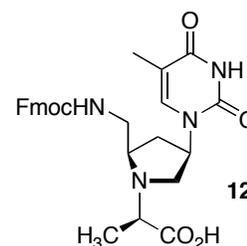
**(2'R, 4'R, 7'S)- & (2'R, 4'R, 7'R)-[2'-(fluoren-9-yl-methoxycarbonyl)aminomethyl]-4'-(thymine-1-yl)-pyrrolidinyl-N-[( $\alpha$ -methyl)acetic acid] (11) & (12).**

2M aqueous HCl (18.0 mL) was added to the (7'S)-methyl ester **9** (500 mg, 0.94 mmol) in dioxane (85 mL) and the resulting mixture was heated under reflux for 18 h. The solution was evaporated under reduced pressure and the crude acid was purified on a reverse-phase (C18) bond-elute column (Varian) eluting with 3:1  $\text{CH}_3\text{OH}/\text{H}_2\text{O}$  to give the (7'S)-acid **11** (0.38 g, 78%) as a white powder. The (7'R)-methyl ester **10** (420 mg, 0.79 mmol) was similarly hydrolysed to give (7'R)-acid **12** (0.33 g, 82%) as a white powder.

**(7'S)-diastereoisomers (11):**  $R_f = 0.29$  (C18-reverse-phase, 3:1 CH<sub>3</sub>OH/H<sub>2</sub>O); mp 168–170 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.16 (3H, d, *J* 6.9 Hz, C7'-CH<sub>3</sub>), 1.56-1.62 (1H, m, H<sub>a</sub>3'), 1.76 (3H, s, CH<sub>3</sub> Thymine), 2.33 (1H, ddd, *J* 6.4, 8.3, 14.6 Hz, H<sub>b</sub>3'), 2.88-2.99 (4H, m, H5', H<sub>a</sub>6' and H7'), 3.16-3.21 (1H, m, H<sub>b</sub>6'), 3.83 (1H, dd, *J* 6.4, 13.6 Hz, H2') 4.16-4.32 (3H, m, Fmoc aliphatic CH & Fmoc CH<sub>2</sub>), 4.70 (1H, br s, H4'), 7.29-7.34 (2H, m, Fmoc Ar-H), 7.41 (2H, t, *J* 7.3 Hz, Fmoc Ar-H), 7.66 (2H, d, *J* 5.9 Hz, Fmoc Ar-H), 7.88 (2H, d, *J* 7.5 Hz, Fmoc Ar-H), 8.17 (1H, s, H6), 11.2 (1H, s, CO<sub>2</sub>H); <sup>13</sup>C NMR (100.6 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  9.7 (C7'-CH<sub>3</sub>), 12.7 (CH<sub>3</sub> Thymine), 36.3 (C3'), 42.9 (C6'), 47.1 (Fmoc aliphatic CH), 51.1 (C5'), 52.4 (C4'), 54.7 (C2'), 58.5 (C7'), 65.7 (Fmoc CH<sub>2</sub>), 108.7 (C5), 120.5, 125.4, 127.4 & 128.0 (Fmoc Ar-CH), 139.0 (C6), 141.1 & 144.2 (Fmoc Ar-C), 151.4 (C2), 156.8 (Fmoc CO), 164.3 (C4), 175.2 (CO<sub>2</sub>H); (ESI<sup>+</sup>) 541 ([M-HCl+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) *m/z* ([M-HCl+Na]<sup>+</sup>) 541.2065, calc'd for C<sub>28</sub>H<sub>30</sub>N<sub>4</sub>O<sub>6</sub>Na 541.2058;  $\nu_{\max}$ (nujol)/cm<sup>-1</sup> 3734 and 3853 (OH), 1683 (CO) and 1653 (CO);  $[\alpha]_D = -5.9^\circ$  (*c* = 0.5, CH<sub>3</sub>OH).



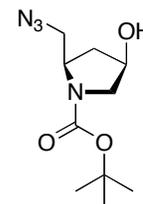
**(7'R)-diastereoisomers (12):**  $R_f = 0.30$  (C18-reverse-phase, 3:1 CH<sub>3</sub>OH/H<sub>2</sub>O); mp 139–144 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.28 (3H, d, *J* 7.0 Hz, C7'-CH<sub>3</sub>), 1.69 (1H, ddd, *J* 6.5, 7.0, 13.8 Hz, H<sub>a</sub>3'), 1.77 (3H, s, CH<sub>3</sub> Thymine), 2.28 (1H, ddd, *J* 6.5, 7.4, 13.8 Hz, H<sub>b</sub>3'), 3.0-3.22 (5H, m, H5', H6' and CH propionate), 3.72 (1H, dd, *J* 6.5, 13.8 Hz, H2') 4.20 (1H, t, *J* 6.7 Hz, Fmoc aliphatic CH), 4.26-4.30 (2H, m, Fmoc CH<sub>2</sub>), 4.85-4.89 (1H, m, H4'), 7.31-7.35 (2H, m, Fmoc Ar-H), 7.41 (2H, t, *J* 7.3 Hz, Fmoc Ar-H), 7.68 (2H, dd, *J* 12.5, 6.3 Hz, Fmoc Ar-H), 7.76 (1H, s, H6), 7.88 (2H, d, *J* 7.5 Hz, Fmoc Ar-H), 11.2 (1H, s, CO<sub>2</sub>H); <sup>13</sup>C NMR (100.6 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  12.8 (CH<sub>3</sub> Thymine), 17.2 (C7'-CH<sub>3</sub>), 35.6 (C3'), 42.2 (C6'), 47.1 (Fmoc aliphatic CH), 51.7 (C4' and C5'), 55.2 (C2'), 59.7 (C7'), 65.7 (Fmoc CH<sub>2</sub>), 109.2 (C5), 120.5, 125.5, 127.4 & 128.0 (all Fmoc Ar-CH), 138.1 (C6), 141.1 & 144.2 (both Fmoc Ar-C), 151.3 (C2), 156.9 (Fmoc CO), 164.2 (C4), 174.4 (CO<sub>2</sub>H); *m/z* (ESI<sup>+</sup>) 519 ([M-HCl]<sup>+</sup>, 100%); HRMS (ES<sup>+</sup>) *m/z* ([M - HCl]<sup>+</sup>) 519.2243, calculated for C<sub>28</sub>H<sub>31</sub>N<sub>4</sub>O<sub>6</sub> 519.2244;  $\nu_{\max}$ (nujol)/cm<sup>-1</sup> 3750 and 3853 (OH), 1684 (CO) and 1653 (CO);  $[\alpha]_D = +5.3^\circ$  (*c* = 0.5, CH<sub>3</sub>OH).



**(2'R, 4'S)-2'-Azidomethyl-N-(tert-butoxycarbonyl)-4'-(para-toluenesulfonyl)-pyrrolidine 14.**

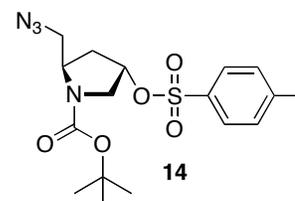
A solution of tetrabutylammonium fluoride (TBAF) 1 M in THF (63.1 mL, 63.1 mmol) was added to **13** (15.0 g, 42.1 mmol) in THF (357 mL) and the mixture was stirred for 18 h at room temperature under N<sub>2</sub>. The solvent was then evaporated under reduced pressure and brine (50 mL) was added to the mixture, which was extracted with ethyl acetate (6 x 50 mL). The organic extract was dried (MgSO<sub>4</sub>) filtered, evaporated under reduced pressure, and then purified by column chromatography (5% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>,  $R_f = 0.27$ ) to give (2'R, 4'R)-2'-azidomethyl-N-(tert-butoxycarbonyl)-4'-hydroxy-pyrrolidine (9.98 g, 98%) as a white powder.

mp 52–55 °C; Found: C, 49.3; H, 7.7; N, 22.6%; calc'd for C<sub>10</sub>H<sub>18</sub>N<sub>4</sub>O<sub>5</sub>: C, 49.6; H, 7.5; N, 23.1%; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.45 (9H, s, (C(CH<sub>3</sub>)<sub>3</sub>), 1.75-1.80 (1H, m, H<sub>a3</sub>), 2.06-2.18 (1H, m, H<sub>b3</sub>), 3.09-3.13 (1H, m, H<sub>a5</sub>), 3.49 (1H, dd, *J* 11.3, 5.2 Hz, H<sub>b5</sub>) 3.54-3.55 (2H, m, H<sub>6</sub>), 3.82-3.92 (1H, m, H<sub>2</sub>), 4.22-4.29 (1H, m, H<sub>4</sub>), 5.11 (1H, d, *J* 3.0 Hz, OH); <sup>13</sup>C NMR (75.5 MHz; DMSO-*d*<sub>6</sub>) δ 28.5 (C(CH<sub>3</sub>)<sub>3</sub>), 36.4 & 37.2 (C<sub>3</sub> rotamer), 53.0 (CH<sub>2</sub>N<sub>3</sub>), 54.0 & 54.9 (C<sub>5</sub> rotamer), 55.1 & 56.2 (C<sub>2</sub> rotamer), 68.4 & 69.0 (C<sub>4</sub> rotamer), 79.2 (C(CH<sub>3</sub>)<sub>3</sub>), 154.1 (CO<sub>2</sub><sup>t</sup>Bu); *m/z* (ESI<sup>+</sup>) 265 [M+Na]<sup>+</sup>; HRMS (ESI<sup>+</sup>) *m/z* [M+H]<sup>+</sup> 243.1457, calc'd for C<sub>10</sub>H<sub>19</sub>N<sub>4</sub>O<sub>3</sub> 243.1452; ν<sub>max</sub>(neat)/cm<sup>-1</sup> 3427 (OH), 2100 (CH<sub>2</sub>N<sub>3</sub>), 1734 and 1673 (CO); [α]<sub>D</sub> = +28.3° (*c* = 1.0, CHCl<sub>3</sub>).



Triphenylphosphine (13.0 g, 49.5 mmol) was added to a solution of the above alcohol (10.0 g, 41.3 mmol) in dry THF (130 mL) under N<sub>2</sub>. The solution was cooled to -15 °C and DIAD (9.8 mL, 49.5 mmol) was added drop-wise until a suspension formed. Methyl *p*-toluenesulfonate (9.61 g, 51.6 mmol) was next added and the mixture was stirred 0 °C under N<sub>2</sub> for 10 min, warmed to room temperature and then stirred for a further 18 h at room temperature. Evaporation of solvent, under reduced pressure, and purification of the by column chromatography (5% diethyl ether in toluene, *R*<sub>f</sub> = 0.28) gave the product tosylate **14** (11.78 g, 60%) as a white crystalline solid.

mp 64–66 °C; Found: C, 51.2; H, 6.1; N, 13.8; S, 8.1%; calc'd for C<sub>17</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub>S: C, 51.5; H, 6.1; N, 14.1; S, 8.1%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.39 & 1.46 (9H, s, (C(CH<sub>3</sub>)<sub>3</sub> rotamers), 2.04-2.08 (1H, m, H<sub>a3</sub>), 2.13-2.18 (1H, m, H<sub>b3</sub>), 2.47 (3H, s, Ar-CH<sub>3</sub>), 3.36 (1H, dd, *J* 9.9, 13.0 Hz, H<sub>a5</sub>), 3.58 (1H, m, H<sub>b5</sub>), 3.74 (1H, dd, *J* 4.5, 12.5 Hz, H<sub>a6</sub>), 3.85 (1H, dd, *J* 4.5, 12.5 Hz, H<sub>b6</sub>), 3.97-4.03 (1H, m, H<sub>2</sub>), 5.07-5.12 (1H, m, H<sub>4</sub>), 7.54 (2H, d, *J* 7.4 Hz, tosyl aromatic H), 7.85 (2H, d, *J* 7.7 Hz, tosyl aromatic H); <sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>) δ 21.4 (Ts CH<sub>3</sub>), 28.2 (C(CH<sub>3</sub>)<sub>3</sub>), 34.8 & 35.7 (C<sub>3</sub> rotamers), 51.7 & 52.5 (C<sub>6</sub> rotamers), 52.7 & 52.9 (C<sub>5</sub>), 55.2 (C<sub>2</sub>), 79.7 & 80.1 (C<sub>4</sub> rotamers), 80.7 & 80.2 (C(CH<sub>3</sub>)<sub>3</sub> rotamers), 127.8 & 130.7 (tosyl aromatic CH), 133.5 (tosyl *p*-C), 145.5 (tosyl *ipso*-C), 153.5 & 153.8 (Boc CO rotamers); *m/z* (ESI<sup>+</sup>) 419 [M+Na]<sup>+</sup>; HRMS (ESI<sup>+</sup>) *m/z* [M+Na]<sup>+</sup> 419.1356, calc'd for C<sub>17</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub>NaS 419.1360; ν<sub>max</sub>(neat)/cm<sup>-1</sup> 2106 (CH<sub>2</sub>N<sub>3</sub>), 1695 (CO), 1399 and 1175 (-SO<sub>2</sub>-O-); [α]<sub>D</sub> = +16.3° (*c* = 1.0, CHCl<sub>3</sub>).

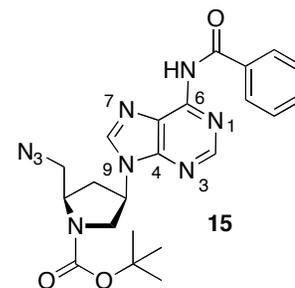


### (2'*R*, 4'*R*)-2'-Azidomethyl-4'-(*N*<sup>6</sup>-benzoyladenine-9-yl)-*N*-(*tert*-butoxycarbonyl)-pyrrolidine (**15**).

*N*<sup>6</sup>-benzoyladenine (17.4 g, 72.9 mmol), anhydrous K<sub>2</sub>CO<sub>3</sub> (10.0 g, 72.9 mmol) and 18-crown-6 ether (2.9 g, 10.7 mmol) were added to a solution of tosylate **14** (11.6 g, 29.2 mmol) in anhydrous DMF (140 mL). The resulting suspension was stirred for 18 h at 80 °C under N<sub>2</sub>. The solution was allowed to cool to room temperature and concentrated under reduced pressure. Saturated aqueous KCl (100 mL) was added to the mixture was extracted with 2:1 CHCl<sub>3</sub>/C<sub>2</sub>H<sub>5</sub>OH (4 x 100 mL). The combined organic extract were dried over

MgSO<sub>4</sub>, evaporated under reduced pressure and purified by column chromatography (5% CH<sub>3</sub>OH/EtOAc, *R<sub>f</sub>* = 0.29) to give the product **15** (7.93 g, 59%) as a white foam.

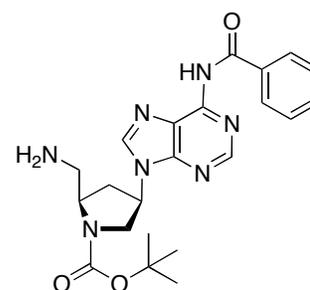
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.49 (9H, s, (C(CH<sub>3</sub>)<sub>3</sub>), 1.90-2.01 (1H, m, H<sub>a</sub>3'), 2.59-2.72 (1H, m, H<sub>b</sub>3'), 3.36-3.42 (1H, m, H<sub>a</sub>5'), 3.60-3.62 (1H, m, H<sub>a</sub>6'), 3.65 (1H, dd, *J* 4.5, 11.7 Hz, H<sub>b</sub>6'), 4.06-4.14 (1H, m, H<sub>b</sub>5'), 4.18-4.28 (1H, m, H<sub>2</sub>'), 4.99-5.08 (1H, m, H<sub>4</sub>'), 7.46 (2H, t, *J* 7.8 Hz, benzoyl CH), 7.55 (1H, t, *J* 7.4 Hz, benzoyl CH), 7.96 (2H, d, *J* 7.4 Hz, benzoyl CH), 8.07 (1H, s, H<sub>8</sub>), 8.73 (1H, s, H<sub>2</sub>), 9.14 (1H, s, N<sub>6</sub>-H); <sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>) δ 28.8 (C(CH<sub>3</sub>)<sub>3</sub>), 34.7 & 36.9 (C3' rotamers), 51.3 & 51.9 (C6' rotamers), 52.0 (C4'), 53.7 (C5'), 55.9 (C2'), 81.4 & 81.6 (C(CH<sub>3</sub>)<sub>3</sub>), 123.8 (C5), 128.3, 129.2 and 133.2 (all benzoyl CH), 133.9 (benzoyl *ipso*-C), 141.2 (C8), 150.1 (C4), 152.3 (C6), 152.8 (C2), 154.2 (benzoyl CO), 165.5 (Boc CO); *m/z* (ESI<sup>+</sup>) 463 [M+H]<sup>+</sup>; HRMS (ESI<sup>+</sup>) *m/z* [M+H]<sup>+</sup> 464.2147, calculated for C<sub>22</sub>H<sub>26</sub>N<sub>9</sub>O<sub>3</sub> 464.2153; *v*<sub>max</sub>(nujol)/cm<sup>-1</sup> 2104 (CH<sub>2</sub>N<sub>3</sub>), 1698 (CO); [α]<sub>D</sub> = -76.4° (*c* = 0.5, CHCl<sub>3</sub>).



#### (2'*R*, 4'*R*)-4'-(*N*<sup>6</sup>-benzoyladenin-1-yl)-*N*-(*tert*-Butoxycarbonyl)-2'-[(fluoren-9-yl-methoxycarbonyl)aminomethyl]-pyrrolidine **16**

H<sub>2</sub>S gas was bubbled through a solution of the azide derivative **15** (7.2 g, 15.4 mmol) dissolved in 60% aqueous pyridine (190 mL) until saturation. The resulting solution was sealed and stirred for 18 h. The reaction was then quenched with methanol, filtered through celite and evaporated under reduced pressure to give crude intermediate (2'*R*, 4'*R*)-2'-aminomethyl-*N*-(*tert*-butoxycarbonyl)-4'-(*N*<sup>6</sup>-benzoyladenin-9-yl)-pyrrolidine, as a yellow foam.

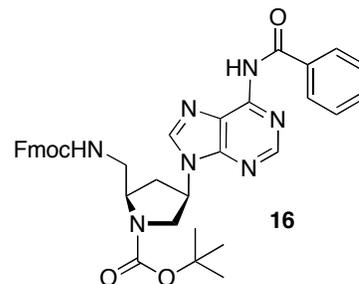
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.45 (9H, s, (C(CH<sub>3</sub>)<sub>3</sub>), 2.51-2.63 (1H, m, H<sub>a</sub>3'), 2.68 (1H, ddd, *J* 5.3, 7.0, 12.4 Hz, H<sub>b</sub>3'), 2.85-2.90 (1H, m, H<sub>a</sub>5'), 2.91-3.09 (1H, m, H<sub>a</sub>6'), 3.49-3.66 (1H, m, H<sub>b</sub>6'), 3.91-4.0 (1H, m, H<sub>b</sub>5'), 4.16-4.23 (1H, m, H<sub>2</sub>'), 4.99-5.08 (1H, m, H<sub>4</sub>'), 7.55 (2H, t, *J* 7.8 Hz, benzoyl CH), 7.65 (1H, t, *J* 7.3 Hz, benzoyl CH), 8.06 (2H, d, *J* 7.3 Hz, benzoyl CH), 8.70 (1H, s, H<sub>8</sub>), 8.76 (1H, s, H<sub>2</sub>); <sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>) δ 28.8 (C(CH<sub>3</sub>)<sub>3</sub>), 34.7 (C3'), 51.6 (C6'), 52.2 (C4'), 57.2 (C5'), 63.9 (C2'), 81.1 (C(CH<sub>3</sub>)<sub>3</sub>), 124.2 (C5), 128.4, 129.2 & 133.1 (all benzoyl CH), 134.0 (benzoyl *ipso*-C), 136.4 (C8), 150.2 (C4), 152.4 (C6), 152.6 (C2), 165.6 (benzoyl CO), 166.7 (Boc CO); *m/z* (ESI<sup>+</sup>) 438 [M+H]<sup>+</sup>; HRMS (ESI<sup>+</sup>) *m/z* [M+H]<sup>+</sup> 438.2245, calculated for C<sub>22</sub>H<sub>28</sub>N<sub>7</sub>O<sub>3</sub> 438.2248; *v*<sub>max</sub> (neat)/cm<sup>-1</sup> 3853 (NH), 1698 (CO), 1558 (NH); [α]<sub>D</sub> = +23.9° (*c* = 0.5, CHCl<sub>3</sub>).



*N*-(9-fluoromethyloxycarbonyloxy) succinimide (6.3 g, 15.4 mmol) was added portion-wise to a solution of the crude amine in 1:1 dioxane/10% aqueous Na<sub>2</sub>CO<sub>3</sub> (87 mL). The suspension was stirred at room temperature for 4

h. Evaporation of the solvent, under reduced pressure, and purification by column chromatography (3:1 EtO<sub>2</sub>Ac/hexane,  $R_f=0.23$ ) gave the product **16** (7.30 g, 85% over two steps from **15**) as a white foam.

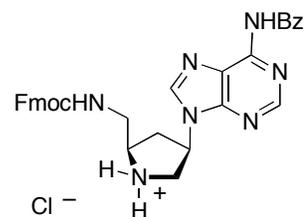
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.44 (9H, s, (C(CH<sub>3</sub>)<sub>3</sub>), 2.36-2.46 (1H, m, H<sub>a</sub>3'), 2.59-2.72 (1H, m, H<sub>b</sub>3'), 3.19-3.20 (1H, m, H<sub>a</sub>5'), 3.48-3.53 (1H, m, H<sub>a</sub>6'), 3.61-3.66 (1H, m, H<sub>2</sub>' and H<sub>b</sub>6'), 3.94-4.0 (2H, m, H<sub>b</sub>5' and Fmoc aliphatic CH), 4.22 (2H, t,  $J$  6.6 Hz, Fmoc CH<sub>2</sub>), 4.29-4.39 (1H, m, H<sub>4</sub>'), 5.01-5.11 (1H, m, Fmoc NH), 7.29 (2H, t,  $J$  7.5 Hz, Fmoc aromatic CH), 7.38 (2H, d,  $J$  7.5 Hz, Fmoc aromatic CH), 7.07 (2H, t,  $J$  7.5 Hz, Fmoc aromatic CH), 7.67 (3H, dd,  $J$  12.2, 7.4 Hz benzoyl CH), 7.88 (2H, d,  $J$  7.5 Hz, Fmoc aromatic CH), 8.06 (2H, d,  $J$  7.4, benzoyl CH), 8.33 (1H, s, H<sub>8</sub>), 8.56 (1H, s, H<sub>2</sub>), 8.73 (1H, s, N<sub>6</sub>H); <sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  28.4 (C(CH<sub>3</sub>)<sub>3</sub>), 34.5 (C3'), 43.6 (C6'), 47.1 (Fmoc aliphatic CH), 49.8 (C5'), 52.1 (C4'), 56.0 (C2'), 65.6 (Fmoc CH<sub>2</sub>), 79.5 (C(CH<sub>3</sub>)<sub>3</sub>), 120.4 (C5), 125.5 (Fmoc aromatic CH), 126.2 (benzoyl CH), 127.4 (Fmoc aromatic CH), 127.9 (benzoyl CH), 128.8 (Fmoc aromatic CH), 132.9 (benzoyl CH), 133.7 (benzoyl *ipso*-C), 141.1 (Fmoc aromatic C), 143.5 (C<sub>8</sub>), 144.2 (Fmoc aromatic C), 150.7 (C<sub>4</sub>), 151.7 (C<sub>6</sub>), 152.8 (C<sub>2</sub>), 153.9 (Fmoc CO), 156.8 (benzoyl CO), 166.0 (Boc CO);  $m/z$  (ESI<sup>+</sup>) 682 [M+Na]<sup>+</sup>; HRMS (ESI<sup>+</sup>)  $m/z$  [M+Na]<sup>+</sup> 682.2748, calculated for C<sub>37</sub>H<sub>37</sub>N<sub>7</sub>O<sub>5</sub>Na 682.2748;  $\nu_{\max}$ (nujol)/cm<sup>-1</sup> 3335 (NH), 1699 (CO), 1558 (NH);  $[\alpha]_D = +42.0^\circ$  ( $c = 0.5$ , CHCl<sub>3</sub>).



**(2'R, 4'R)-4'-(N<sup>6</sup>-benzoyladenine-1-yl)-2'-[(fluoren-9-yl-methoxycarbonyl)aminomethyl]-pyrrolidinyl-N-[( $\alpha$ -methyl)acetic acid methyl ester] (**17**).**

4 M HCl/dioxane (100 mL) was added to a solution of **16** (7.3 g, 13.1 mmol) in dry DCM (80 mL) and stirred for 15 min at 0 °C under N<sub>2</sub>. The solution was allowed to warm to room temperature and stirred for a further 4 h. The solvent was evaporated under reduced pressure and the crude product redissolved in methanol (50 mL) and then filtered through a pad of activated charcoal. The filtrate was and evaporated to give (2'R, 4'R)-4'-(N<sup>6</sup>-benzoyladenine-1-yl)-2'-[(fluoren-9-yl-methoxycarbonyl)aminomethyl]-pyrrolidinium chloride salt (5.86 g, 98%) as a white powder.

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  2.33-2.41 (1H, m, H<sub>a</sub>3'), 2.76-2.80 (1H, m, H<sub>b</sub>3'), 3.42-3.63 (3H, m, H<sub>5</sub>' and H<sub>a</sub>6'), 3.74-3.98 (3H, m, H<sub>2</sub>', H<sub>b</sub>6' and Fmoc aliphatic CH), 4.13-4.25 (2H, m, Fmoc CH<sub>2</sub>), 4.30-4.34 (1H, m, H<sub>4</sub>'), 5.62-5.67 (1H, m, Fmoc NH), 7.30-7.33 (2H, m, Fmoc aromatic CH), 7.36-7.42 (2H, m, Fmoc aromatic CH), 7.56 (2H, t,  $J$  7.6 Hz, Fmoc aromatic CH), 7.65-7.74 (3H, m, benzoyl CH), 7.88 (2H, d,  $J$  7.6 Hz, Fmoc aromatic CH), 8.10 (2H, d,  $J$  7.4, benzoyl CH), 8.93 (1H, s, H<sub>8</sub>), 9.76 (1H, s, H<sub>2</sub>), 10.0 (1H, s, benzamide NH), 10.5 (NH); <sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  34.3 (C3'), 41.0 (C6'), 47.0 (Fmoc aliphatic CH), 48.1 (C5'), 52.9 (C4'), 59.1 (C2'), 66.1 (Fmoc CH<sub>2</sub>), 120.5 (C5), 125.6 (Fmoc aromatic CH), 127.4



(benzoyl CH), 128.0 (Fmoc aromatic CH), 128.9 (benzoyl CH), 129.2 (Fmoc aromatic CH), 132.7 (benzoyl CH), 133.5 (benzoyl *ipso*-C), 141.1 (Fmoc aromatic C), 143.8 (C8), 144.1 (Fmoc aromatic C), 148.8 (C4), 151.6 (C6), 152.0 (C2), 156.8 (Fmoc CO), 167.0 (benzoyl CO); *m/z* (ESI<sup>+</sup>) 582 ([M-HCl+Na]<sup>+</sup>, 100%) 560 ([M-Cl]<sup>+</sup>, 80%); HRMS (ESI<sup>+</sup>) *m/z* [M-Cl]<sup>+</sup> 560.2408, calculated for C<sub>32</sub>H<sub>30</sub>N<sub>7</sub>O<sub>3</sub> 560.2405.

DIPEA (1.8 mL, 10.0 mmol) was added to a solution of the intermediate pyrrolidinium chloride salt (3.0 g, 5.04 mmol) in anhydrous DMF (3 mL) and the mixture was stirred for 30 min at 0 °C under N<sub>2</sub>. Further DIPEA (2.1 mL, 12.6 mmol) was added, followed by (2*R*)-methyl 2-bromopropionate (0.8 mL, 7.56 mmol). The mixture was allowed to warm to room temperature and left stirring for 18 h at room temperature under N<sub>2</sub>. The solvent was then evaporated, under reduced pressure, and residue was purified by column chromatography (3:1 EtOAc/hexane, *R<sub>f</sub>* = 0.28) to give the product **17** (1.65 g, 51%) as a white foam.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.43 (3H, d, *J* 7.0 Hz, C7'-CH<sub>3</sub> propionate),

2.15-2.23 (1H, m, H<sub>a</sub>3'), 2.62 (1H, ddd, *J* 6.5, 8.0, 14.6 Hz, H<sub>b</sub>3'), 3.25-3.32

(3H, m, H<sub>a</sub>5', H<sub>a</sub>6' & H2'), 3.37 (1H, br d, *J* 10.0 Hz, H<sub>b</sub>5'), 3.41-3.46 (1H, m,

H<sub>b</sub>6'), 3.51 (1H, t, *J* 6.5 Hz, Fmoc aliphatic CH), 3.74 (3H, s, OCH<sub>3</sub>), 3.84

(1H, q, *J* 7.0 Hz, H7'), 4.21 (2H, t, *J* 7.0 Hz, Fmoc Ar-H), 4.31-4.36 (1H, m,

Fmoc CH<sub>a</sub>H<sub>b</sub>), 4.37-4.43 (1H, m, Fmoc CH<sub>a</sub>H<sub>b</sub>), 5.19 (1H, br s, H4'), 5.72

(1H, br s, Fmoc NH), 7.23-7.29 (2H, m, Fmoc aromatic CH), 7.35 (2H, t, *J* 7.0

Hz, Fmoc aromatic CH), 7.46 (2H, d, *J* 7.0 Hz, Fmoc aromatic CH), 7.53-7.59

(3H, m, benzoyl CH), 7.72 (2H, d, *J* 7.0, benzoyl CH), 7.97 (2H, d, *J* 7.5 Hz, Fmoc aromatic CH), 8.36 (1H, s,

H8), 8.75 (1H, s, H2), 9.33 (1H, s, N6H); <sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>) δ 14.6 (C7'-CH<sub>3</sub>), 36.1 (C3'), 42.1

(C6'), 47.6 (Fmoc aliphatic CH), 51.9 (OCH<sub>3</sub>), 52.7 (C4'), 53.6 (C5'), 55.6 (C7'-), 59.7 (C2'), 67.1 (Fmoc CH<sub>2</sub>),

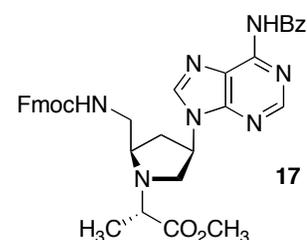
120.3 (C5), 125.4 (Fmoc aromatic CH), 127.4 (benzoyl CH), 128.1 (Fmoc aromatic CH), 128.7 (benzoyl CH),

129.1 (Fmoc aromatic CH), 133.1 (benzoyl CH), 134.0 (benzoyl *ipso*-C), 141.6 (Fmoc aromatic C), 142.2 (C8),

144.3 (Fmoc aromatic C), 149.9 (C4), 152.2 (C6), 157.0 (C2), 157.3 (Fmoc CO), 165.2 (benzoyl CO), 173.6

(propionate CO); *m/z* (ES) 646 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) *m/z* [M+H]<sup>+</sup> 646.2776, calc'd for C<sub>36</sub>H<sub>36</sub>N<sub>7</sub>O<sub>5</sub>

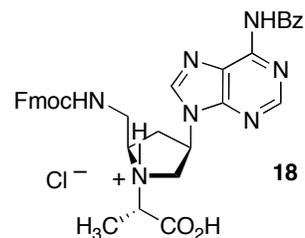
646.2772; *ν*<sub>max</sub>(nujol)/cm<sup>-1</sup> 3066 (NH), 1699 (CO), 1521 (NH); [α]<sub>D</sub> = -29.4° (*c* = 0.5, CHCl<sub>3</sub>).



**(2'*R*, 4'*R*)-4'-(*N*<sup>6</sup>-benzoyladenin-1-yl)-2'-[(fluoren-9-yl-methoxycarbonyl)aminomethyl]-pyrrolidinyl-*N*-[(α-methyl)acetic acid] hydrochloride (**18**).**

2M aqueous HCl (50.0 mL) were added to a solution of **17** (1.5 g, 2.32 mmol) in dioxane (200 mL) and the resulting mixture was heated under reflux for 18 h. The solvent was evaporated under reduced pressure and residue was dried under high vacuum and redissolved in anhydrous pyridine (5.0 mL). To this mixture, benzoyl chloride (0.3 mL, 2.56 mmol) was added dropwise and the mixture was heated to reflux for 2 h under N<sub>2</sub>. The resulting suspension was cooled to room temperature and the solvent evaporated under reduced pressure. Purification was achieved by reverse phase C18 bond elute column (3:1 MeOH/H<sub>2</sub>O, *R<sub>f</sub>* = 0.30) which gave **18** (270 mg, 19 %) as a white powder.

mp 117–119 °C; <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 1.92 (3H, d, *J* 7.0 Hz, C7'-CH<sub>3</sub>), 2.41-2.59 (1H, m, H<sub>a</sub>3'), 2.92-3.09 (1H, m, H<sub>b</sub>3'), 3.66-3.81 (4H, m, H5', H<sub>a</sub>6' and H7') 3.97-4.15 (3H, m, H<sub>a</sub>6', H2', Fmoc aliphatic CH and Fmoc Ar-H), 4.40 (2H, m, Fmoc CH<sub>2</sub>), 4.62-4.69 (1H, m, H4'), 5.74 (1H, br s, Fmoc NH), 7.44-7.47 (2H, m, Fmoc aromatic CH), 7.62 (2H, t, *J* 7.0 Hz, Fmoc aromatic CH), 7.72 (2H, d, *J* 7.0 Hz, Fmoc aromatic CH), 7.78 (3H, m, benzoyl CH), 7.95 (2H, d, *J* 7.0, benzoyl CH), 8.16 (2H, d, *J* 7.5 Hz, Fmoc aromatic CH), 8.54 (1H, s, H8), 8.61 (1H, s, H2), 9.34 (1H, s, benzamide NH); <sup>13</sup>C NMR (75.5 MHz, CD<sub>3</sub>OD) δ 22.5 (C7'-CH<sub>3</sub>), 41.4 (C3'), 42.4 (C6'), 48.9 (Fmoc aliphatic CH), 51.2 (C4'), 53.8 (H7'), 57.2 (C5'), 63.3 (C2'), 68.7 (Fmoc CH<sub>2</sub>), 121.4 (C5), 126.6 (Fmoc aromatic CH), 128.6 (benzoyl CH), 129.3 (Fmoc aromatic CH), 130.3 (benzoyl CH), 130.4 (Fmoc aromatic CH), 133.1 (benzoyl CH), 135.4(benzoyl *ipso*-C), 141.6 (Fmoc aromatic C), 143.0 (C8), 145.5 (Fmoc aromatic C), 146.1 (C4), 152.2 (C6), 156.5 (C2), 160.3 (Fmoc CO), 168.6 (benzoyl CO), 172.8 (CO<sub>2</sub>CH<sub>3</sub>); *m/z* (ESI<sup>+</sup>) 654 ([M+Na]<sup>+</sup>, 80%); HRMS (ESI<sup>+</sup>) *m/z* [M+H]<sup>+</sup> 632.2614, calc'd for C<sub>35</sub>H<sub>34</sub>N<sub>7</sub>O<sub>5</sub> 632.2616; ν<sub>max</sub>(nujol)/cm<sup>-1</sup> 3734 (OH), 3402 (NH), 1683 (CO), 1521 (NH); [α]<sub>D</sub> = -12.5° (*c* = 0.5, CHCl<sub>3</sub>).



## (2) Solid Phase Synthesis of MePOM oligomers

Solid-phase synthesis was carried out in a 10 mm glass vessel consisting of a sintered glass tube fitted with a T-junction Teflon stopcock. The N<sub>2</sub> gas is introduced *via* the T-junction. The reagents were removed by suction filtration through a Buchner flask. Rink-amide Novagel resin (max loading 0.60 mmol/g), Fmoc-Lys(Boc)-COOH, TBTU and HOBt were purchased from Novabiochem. DMF (peptide synthesis grade), piperidine and acetic anhydride were purchased from Aldrich Chemical Company.

Rink-amide Novagel® resin (typically 50 mg, 0.03mmol based on a maximum loading of 0.60 mmol/g) was swelled in CH<sub>2</sub>Cl<sub>2</sub> (*ca.* 3 – 4 mL) for 30 min, then washed three times with DMF. In a separate vial, DIPEA (1.0 molar equiv.) was added to a solution of Fmoc-Lys(Boc)-OH or the adeninyl MePOM monomer **18** (0.2 molar equiv. relative to the maximum loading of the resin), TBTU (0.19 molar equiv.), HOBt (0.2 molar equiv.), in a minimal amount of DMF, allowing 3 min of pre-activation before adding to the resin. Coupling was allowed to proceed with N<sub>2</sub> agitation for 4 h. complete coupling was indicated by negative Kaiser test (orange beads). The coupling reagents were removed and the resin was washed with DMF. Capping was then carried out with 0.5 M acetic anhydride/0.5 M DIPEA in DMF (2 x 1 mL, 15 min each) and then the resin was washed in the following sequence: DMF, CH<sub>2</sub>Cl<sub>2</sub>, CH<sub>3</sub>OH, CH<sub>2</sub>Cl<sub>2</sub> and DMF (each solvent 2 times). Fmoc deprotection was achieved with 20% (v/v) piperidine in DMF (4 x 1.0 mL, 2 min) and coupling efficiencies were calculated by UV determination of the dibenzofulvene-piperidine adduct after Fmoc deprotection. The resin is then washed exhaustively with DMF and gave a positive Kaiser test (blue beads). In a separate vial, DIPEA (5 equiv) was added to the thyminyl or adeninyl monomers **11**, **12** or **18** (2 molar equiv relative to the first monomer coupled to the resin), TBTU (1.9 equiv), HOBt (2 equiv) in minimal amount of DMF and pre-activated for 3 min. The mixture was added to the resin and agitated with N<sub>2</sub> for 4 h. Capping, Fmoc-deprotection, coupling steps were

repeated as above. The resin bound oligomers were washed sequentially with DMF, CH<sub>2</sub>Cl<sub>2</sub>, CH<sub>3</sub>OH and Et<sub>2</sub>O and then cleaved from the resin with 90% TFA in H<sub>2</sub>O for 18 h after which, the resin was washed a few times with freshly prepared 90% TFA in H<sub>2</sub>O. The cleavage mixtures were combined, evaporated under a stream of N<sub>2</sub> and further dried *in vacuo*.

The crude pentamers were analysed by analytical reverse-phase C18 HPLC on a Phenomex Luna 3  $\mu$  C<sub>18</sub>(2) 250  $\times$  4.6 mm column (Figure S1) and then similarly purified by semi-preparative reverse-phase HPLC on a Phenomenex Luna 5 $\mu$  C<sub>18</sub>(2) 250  $\times$  10 mm column. The purity of pentamers was *ca.* 85-95% as determined by repeat analytical HPLC analysis. MePOM oligomers were analysed by Electrospray Ionisation (ESI) MS on a MassLynx oa-TOF mass spectrometer (Table S1). Samples were introduced from a Waters 7240 sample injector in (1:1) in H<sub>2</sub>O/CH<sub>3</sub>CN with 0.1% formic acid.

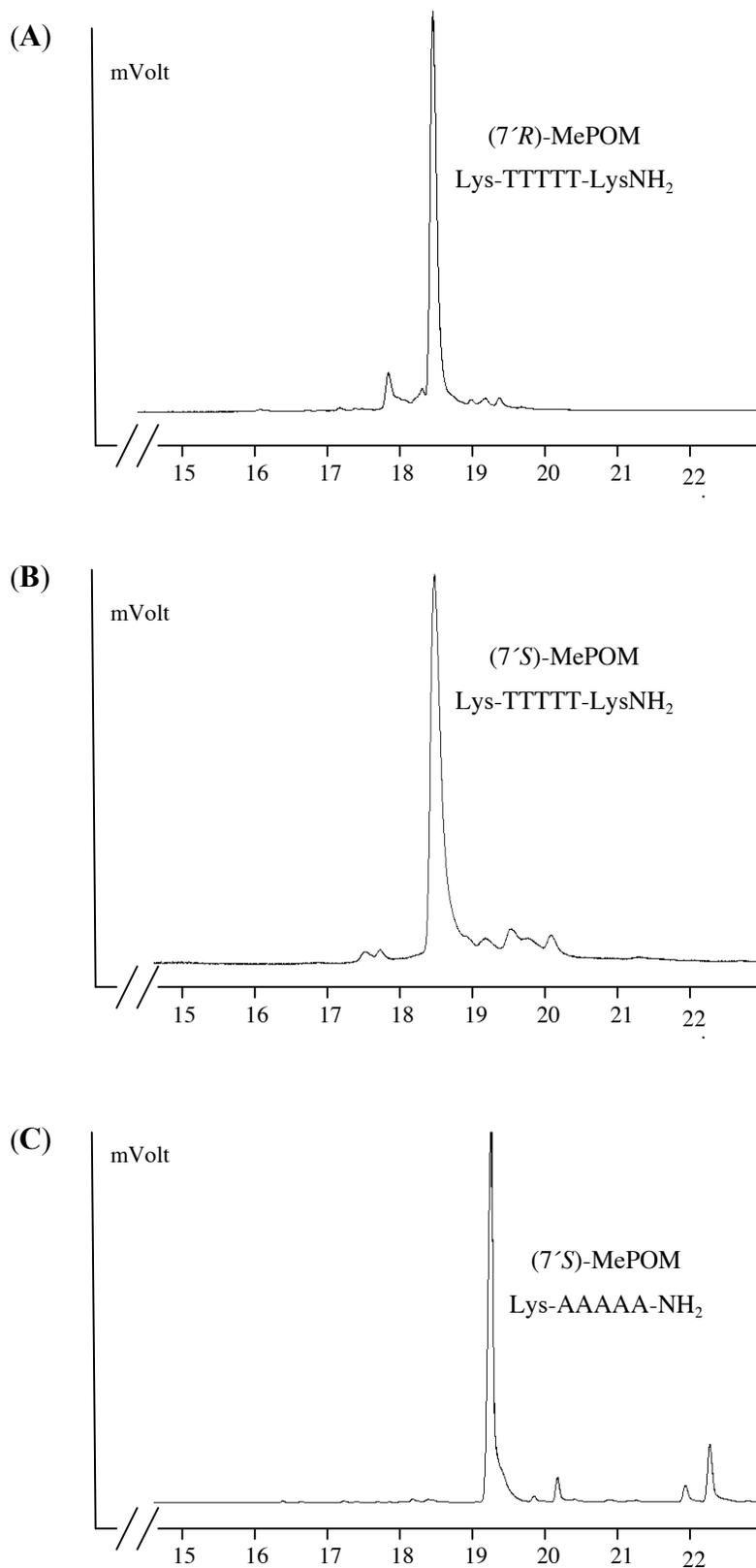
### **(3) UV thermal Denaturation-renaturation experiments.**

Polynucleotides were purchased from Sigma, short chain DNA were purchased from Sigma Genosys and short chain RNA were purchased from Yorkshire Bioscience, UK. PNA oligomers were purchased from Oswel Research products Ltd, UK.

UV thermal melting experiments were performed on a Varian Cary 400 scan UV-visible spectrophotometer fitted with a 6  $\times$  6 Peltier thermostable multicell holder connected to a temperature controller module, which was controlled by an interfaced Dell OptiPlex GX150 computer. Melting experiments were performed in a 10 mm path length 4 mm path width self-masking semi-micro quartz cells fitted with Teflon stoppers. The melting curves were measured at an absorbance of 260 nm versus temperature. The temperature was monitored using temperature probes attached to the reference cells by the probe holders. The concentration of MePOM oligomers and RNA and DNA were measured spectrophotometrically at 80  $^{\circ}$ C from the known molar extinction coefficients.

The buffer solution was prepared with sterile nuclease, protease and DEPC-free deionised water as double concentrated stock solutions (20 mM K<sub>2</sub>HPO<sub>4</sub>) and was diluted to the appropriate concentrations during sample preparation. The pH was adjusted using 1 M HCl or 1 M KOH and then was topped up with water to the final mark. Stock solutions were also prepared with varying the concentration of KCl (44, 124 and 240 mmol). In all melting experiments, an equimolar amount of bases of each oligomer were added to give a solution, which was 42  $\mu$ M (conc. in bases) for each strand. The solution was then mixed by purging with N<sub>2</sub>.

The thermal denaturation-renaturation experiments were performed in three ramps. In the first ramp (fast heating) the samples were heated at a rate of 5  $^{\circ}$ C/min to 93  $^{\circ}$ C to dissociate all strands and held for 1 min, with data collected every 1  $^{\circ}$ C. In the the second ramp (slow cooling/annealing) the samples were cooled at 0.2  $^{\circ}$ C/min to 15  $^{\circ}$ C and held for 1 min, with data collected every 0.1  $^{\circ}$ C. In the third ramp (slow heating/denaturation) the samples were heated at 0.2  $^{\circ}$ C/min to 93  $^{\circ}$ C. The melting experiments were performed at least twice to ensure reproducibility. The  $T_m$  values were obtained from the maxima of first derivative curves that were calculated from Varian thermal software.

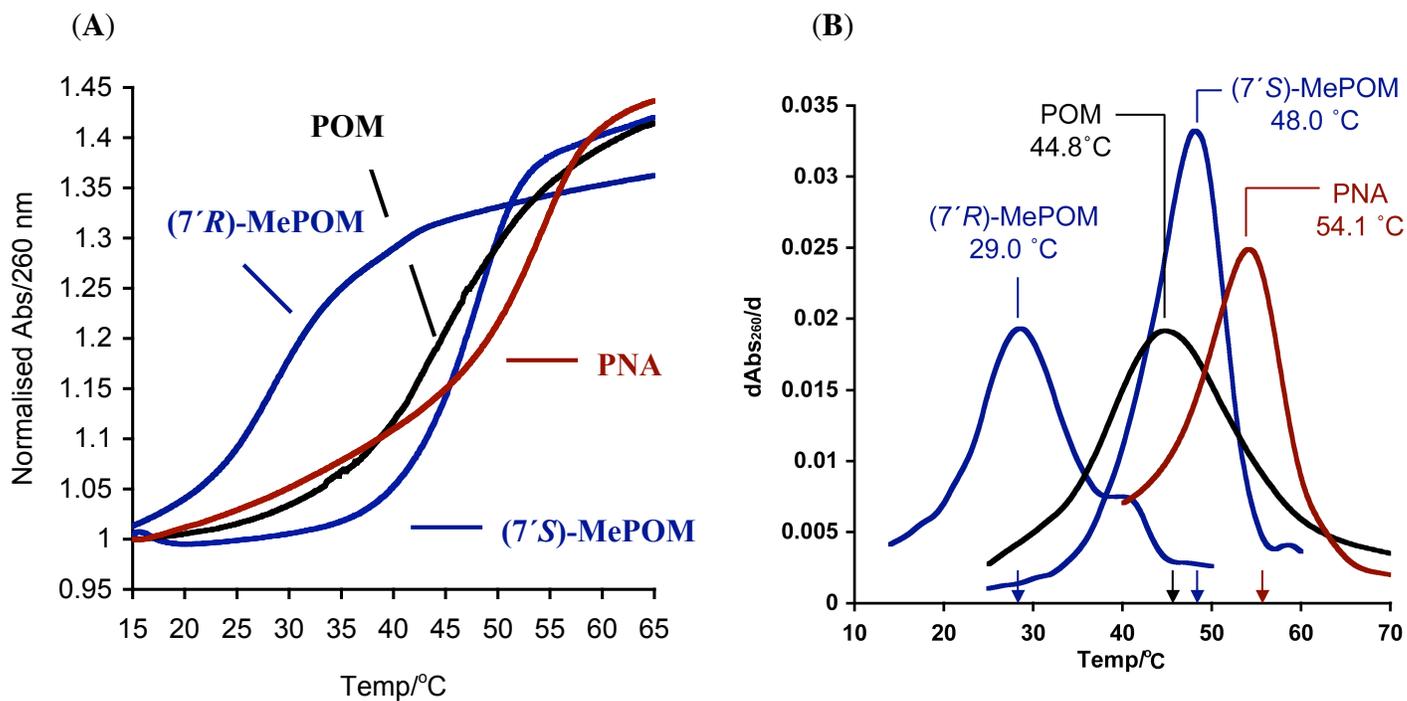


**Figure S1.** C18 reversed-phase HPLC of the crude MePOM pentamers using an analytical (250 × 4.6 mm) column. Solvent A is 0.1% Formic acid/H<sub>2</sub>O and solvent B is 0.1% Formic acid/CH<sub>3</sub>CN. The following gradient 100:0 (A:B) hold 10 min then 100:0 to 70:30 (A:B) from 10-40 min gave (A) Lys-(7'R)-MePOM(T)<sub>5</sub>LysNH<sub>2</sub> with a retention time of 18.7 min and (B) Lys-(7'S)-MePOM(T)<sub>5</sub>LysNH<sub>2</sub> with a retention time of 18.5 min. A gradient of 100:0 (A:B) hold for 10 min then 100:0 to 50:50 (A:B) from 10-40 min gave (C) Lys-(7'S)-MePOM(A)<sub>5</sub>NH<sub>2</sub> with a retention time of 19.2 min.

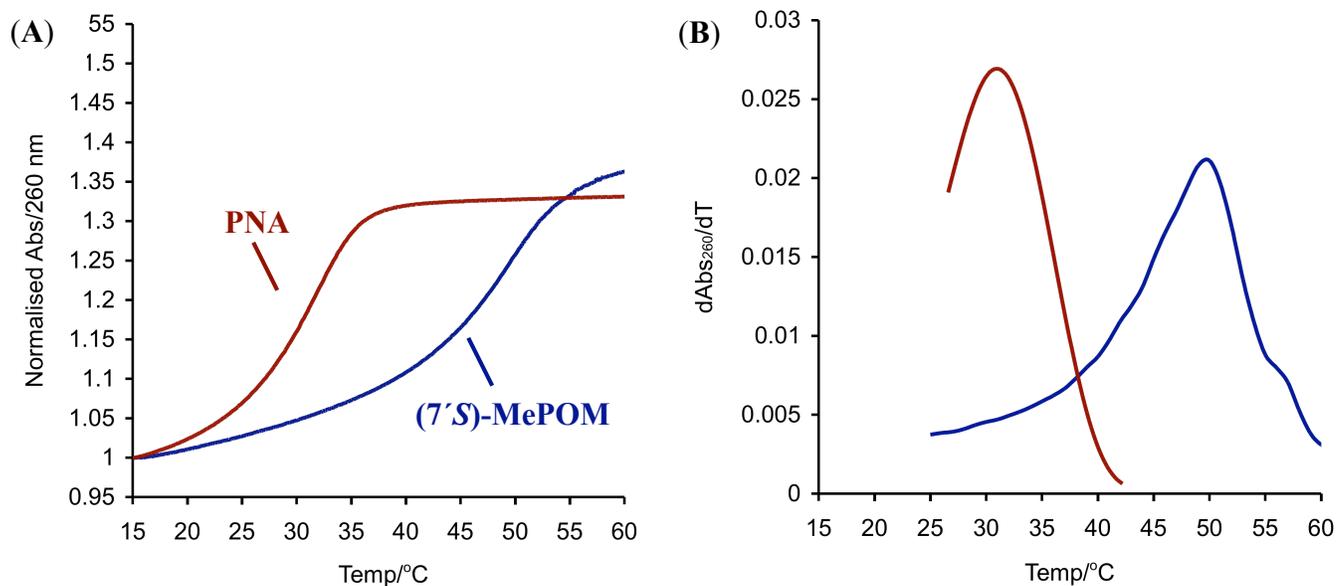
**Table S1.** Mass to charge ratios observed by ES-MS for MePOM oligomers.

POM	Observed $m/z$	Required $m/z$	Corresponding peaks
	832.9	832.9	$[M + 2H]^{2+}$
<b>(7'R)- MePOM</b>	852.1	852.0	$[M + H + K]^{2+}$
<b>Lys-TTTTT-LysNH<sub>2</sub></b>	555.8	555.6	$[M + 3H]^{3+}$
$C_{77}H_{111}N_{25}O_{17}$	417.0	417.0	$[M + 4H]^{4+}$
MW: 1663.91	427.4	427.9	$[M + 2H + 2Na]^{4+}$
	358.5	358.9	$[M + 4Na + K]^{5+}$
	350.1	350.2	$[M + 2H + 2Na + K]^{5+}$
	832.9	832.9	$[M + 2H]^{2+}$
<b>(7'S)- MePOM</b>	852.4	852.0	$[M + H + K]^{2+}$
<b>Lys-TTTTT-LysNH<sub>2</sub></b>	555.7	555.6	$[M + 3H]^{3+}$
$C_{77}H_{111}N_{25}O_{17}$	427.6	427.9	$[M + 2H + 2Na]^{4+}$
MW: 1663.91	358.5	358.9	$[M + 4Na + K]^{5+}$
	350.1	350.2	$[M + 2H + 2Na + K]^{5+}$
	803.2	802.6	$[M + H + Na]^{2+}$
<b>(7'S)- MePOM</b>	810.5	810.6	$[M + H + K]^{2+}$
<b>Lys-AAAAA-NH<sub>2</sub></b>	535.9	535.4	$[M + 2H + Na]^{3+}$
$C_{71}H_{100}N_{38}O_6$	421.8	421.0	$[M + H + Na + 2K]^{4+}$
MW: 1581.80	321.5	321.1	$[M + 4H + Na]^{5+}$
	338.2	338.2	$[M + H + 3Na + K]^{5+}$

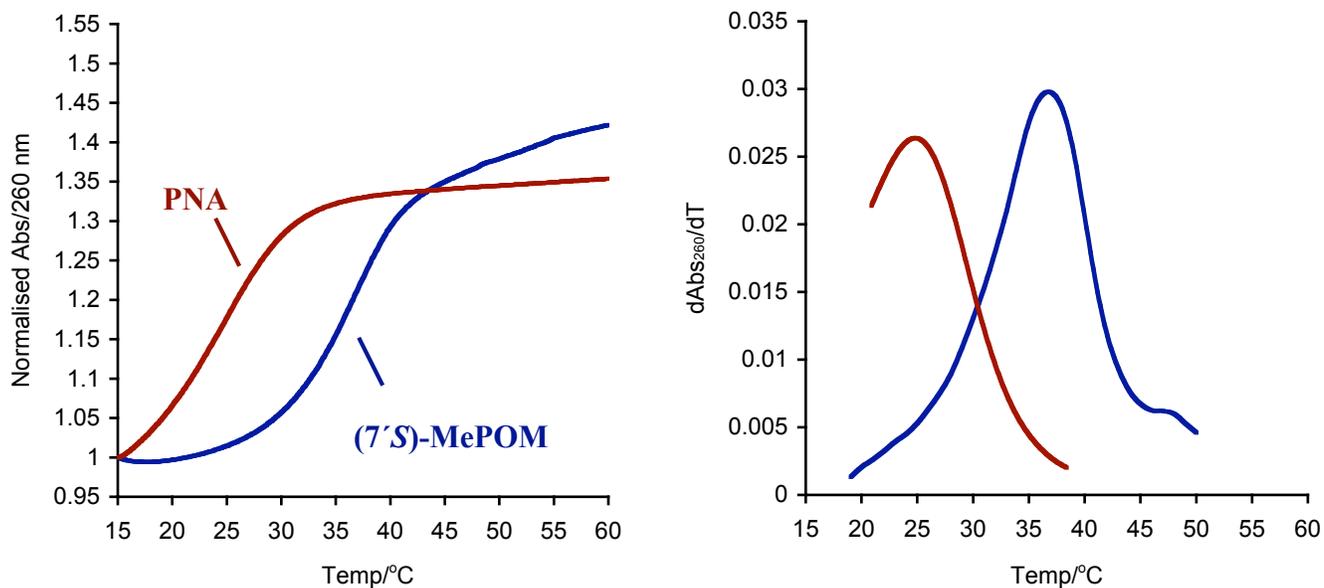
ESI spectra of all POM oligomers show multiply charged quasi-molecular ions (e.g.  $[M+zH]^{z+}$ ) and exhibit the expected molecular ion charged state distribution. The reason for observing this distribution pattern is because of the relatively little fragmentation caused by the electrospray ionisation technique. The width of the distribution and most abundant multiply charged state is usually at about half the value of the highest charge state [R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga, H. R. Udseth, *Anal. Chem.* **1990**, *62*, 882-899]. That is to say the total number of charged state possible for LysMePOM(T)<sub>5</sub>LysNH<sub>2</sub> is 8+ and thus the most abundant charged state for this oligomer should be about 4+ ( $m/z$   $[M+2H+2Na]^{4+}$ : 427.9). The most abundant charged states observed for POM oligomers by ES-MS are hence in good agreement with the total number of sites possible for protonation under acidic conditions. <sup>13</sup>C/<sup>12</sup>C isotopic relationships of the quasi-molecular ions were also in agreement with the assignments. For example doubly, triply and quadruply charged ions showed 0.5, 0.33 and 0.25 amu separation of ion peaks which further supports the identity of this oligomer.



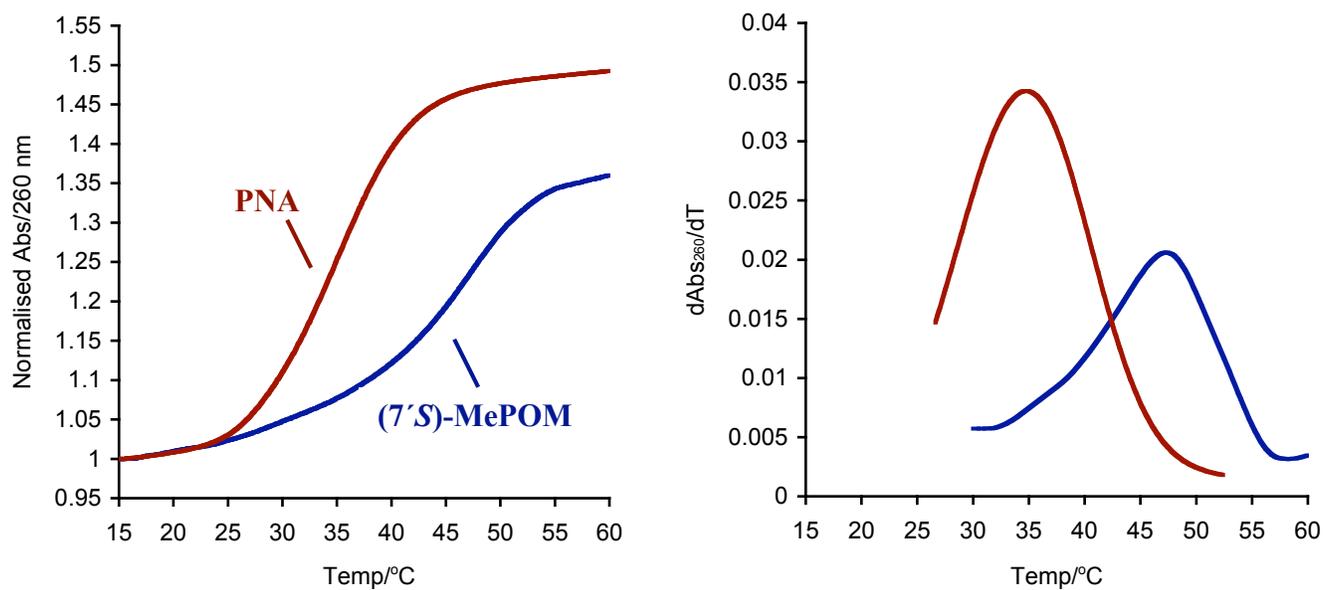
**Figure S2.** (A) Slow UV denaturation (heating curves) and (B) first derivatives of the slow heating curves for PNA, POM (7'R)- and (7'S)-MePOM Lys-TTTTT-LysNH<sub>2</sub> vs. poly(rA) at 0.12 M K<sup>+</sup>, pH 7.0. For first derivatives see Figure 2 in the manuscript.



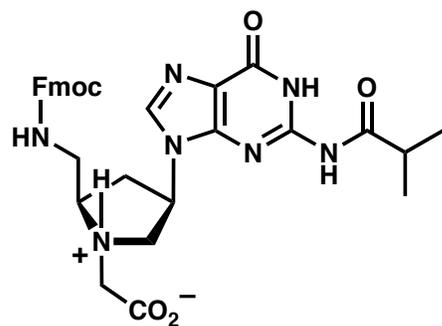
**Figure S3.** (A) Slow UV denaturation (heating curves) and (B) first derivatives of the slow heating curves for PNA and (7'S)-MePOM Lys-AAAAA-NH<sub>2</sub> vs. poly(rU) at 0.12 M K<sup>+</sup>, pH 7.0.



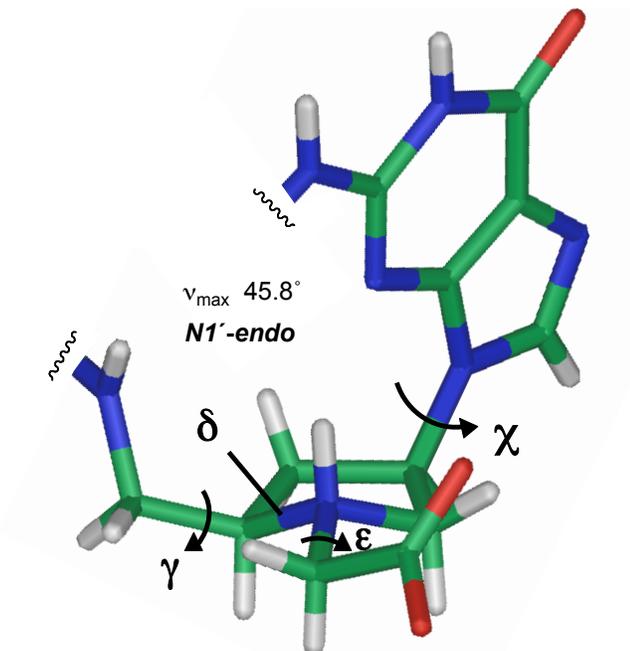
**Figure S4.** (A) Slow UV denaturation (heating curves) and (B) first derivatives of the slow heating curves for PNA and (7'S)-MePOM Lys-AAAAA-NH<sub>2</sub> vs. r(U)<sub>20</sub> at 0.12 M K<sup>+</sup>, pH 7.0.



**Figure S5** (A) Slow UV denaturation (heating curves) and (B) first derivatives of the slow heating curves for PNA and (7'S)-MePOM Lys-AAAAA-NH<sub>2</sub> vs. d(T)<sub>20</sub> at 0.12 M K<sup>+</sup>, pH 7.0.



$\phi$ /deg	POM	A-RNA	B-DNA
<b>P</b>	23	20	<b>160</b>
$\gamma$	75	49	36
$\delta$	68	95	<b>125</b>
$\epsilon$	173	202	170



**Figure S6.** An X-ray crystal structure of a protected G-POM monomer [Morral, J.; Micklefield, J. *unpublished work*], shows a *trans*-relative configuration about the pyrrolidine *N*-atom and the *N1'*-endo conformation which is consistent with our earlier modelling and NMR results. The pseudorotation phase angle *P* and backbone torsion angles  $\gamma$ ,  $\delta$  and  $\epsilon$ , of the POM monomer, match the corresponding torsion angles of a nucleotide in a typical A-type RNA duplex more closely than those of a nucleotide unit in a B-type DNA duplex.