ELECTRONIC SUPPLEMENTARY INFORMATION

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

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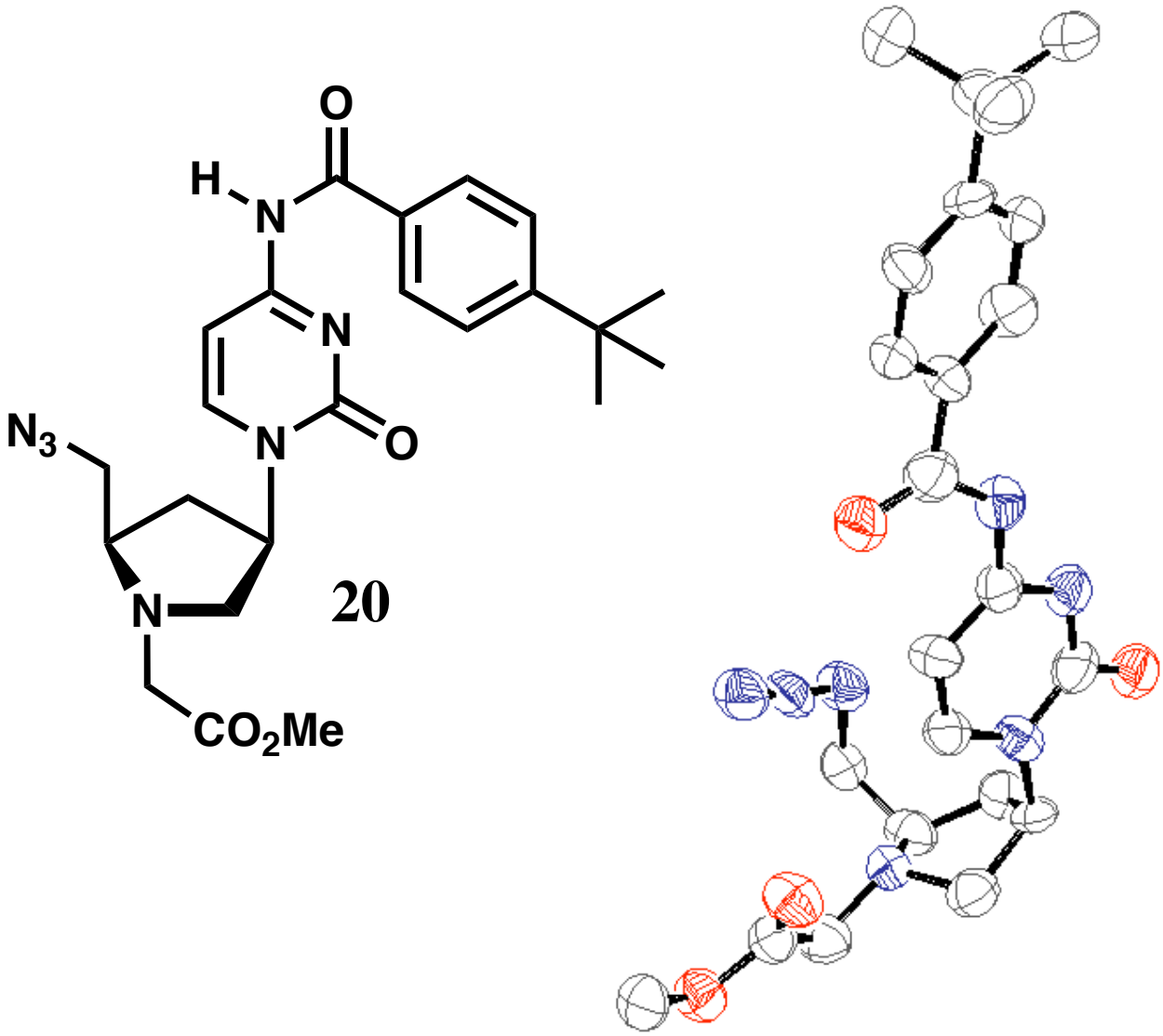

Figure 1. X-ray crystal structure of C-monomer **20**.

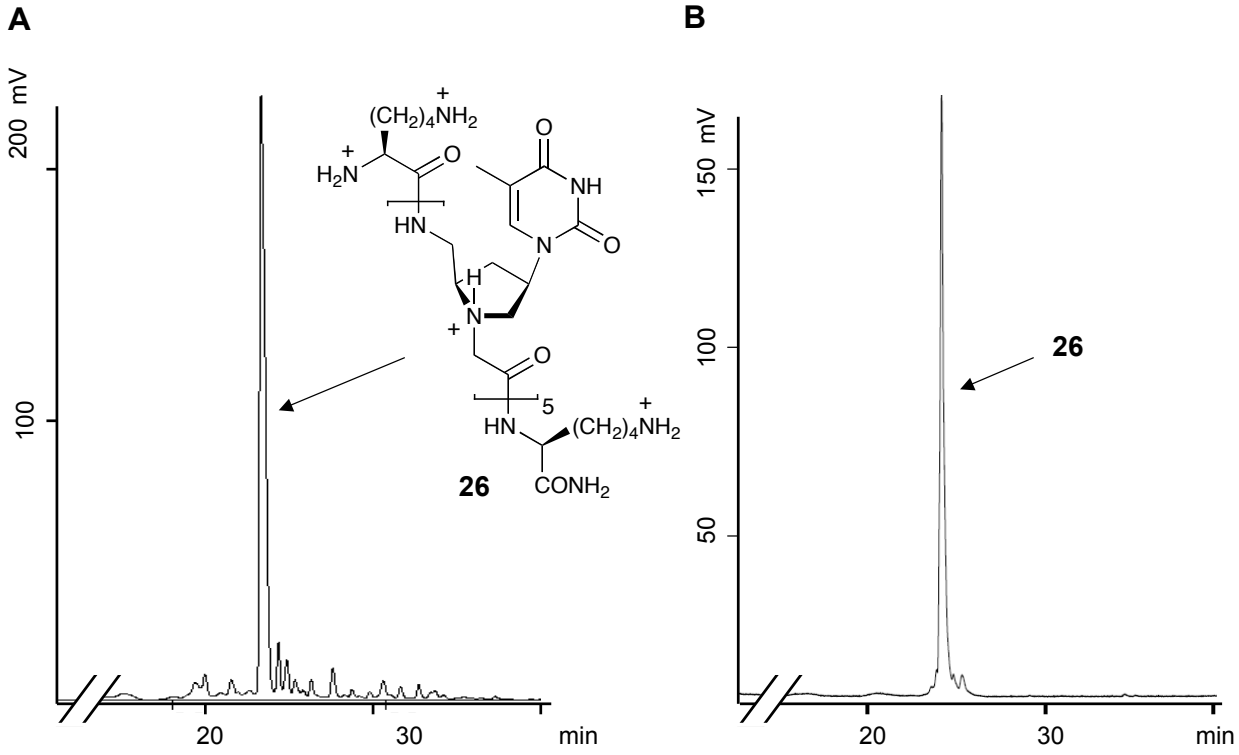

Figure 2. Analytical HPLC chromatographs of crude product Lys-TTTTT-LysNH$_2$ **26**, following the conditions described in the preceding paper[1] (A) Fmoc-synthesis total product peak area was 70%, which corresponds to an average coupling efficiency of 94%. (B) Boc-synthesis total product peak area was 91%, which equates to an average coupling efficiency of > 98%.

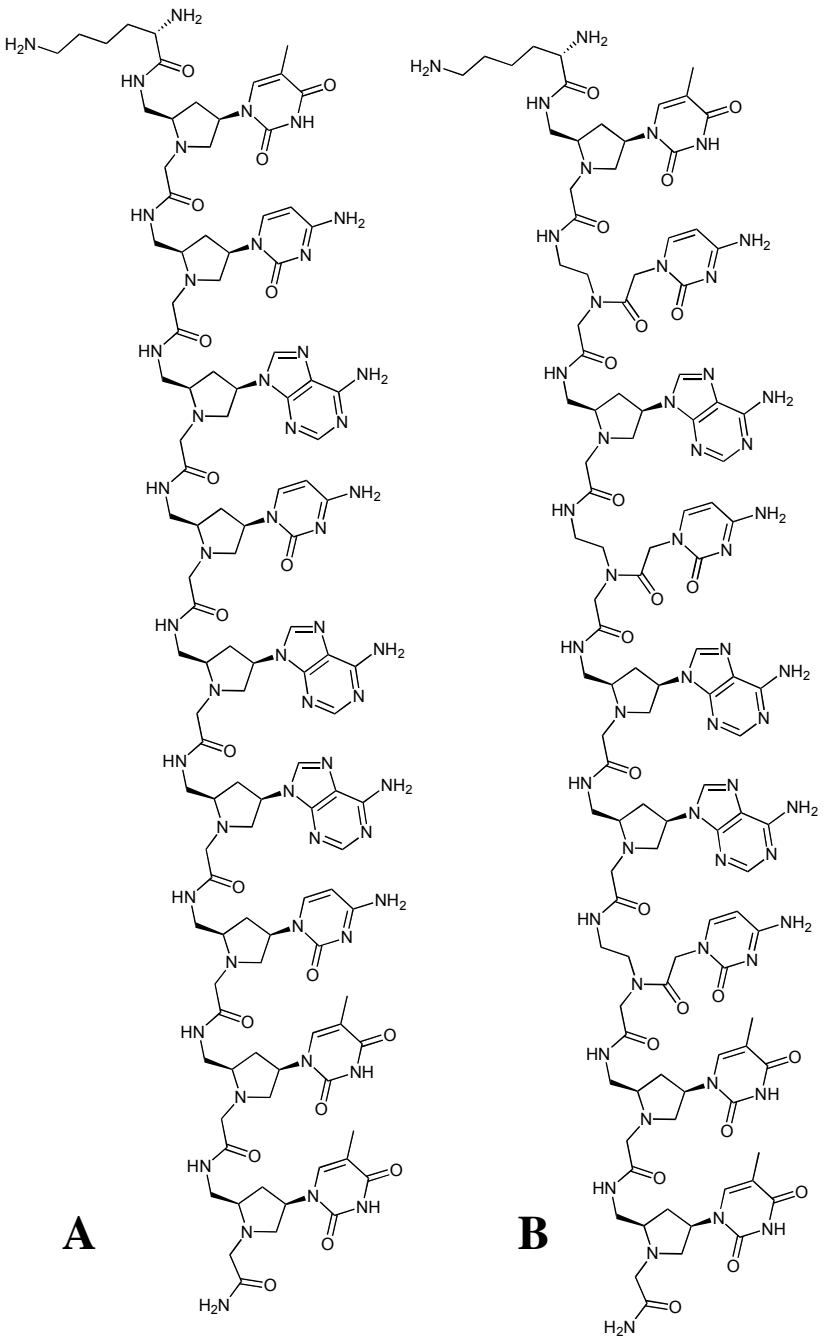

Figure 3. (A) Structure of POM LysTCACAACTT-NH_2. (B) Structure of POM-PNA chimera LysTC*AC*AAC*TT-NH_2

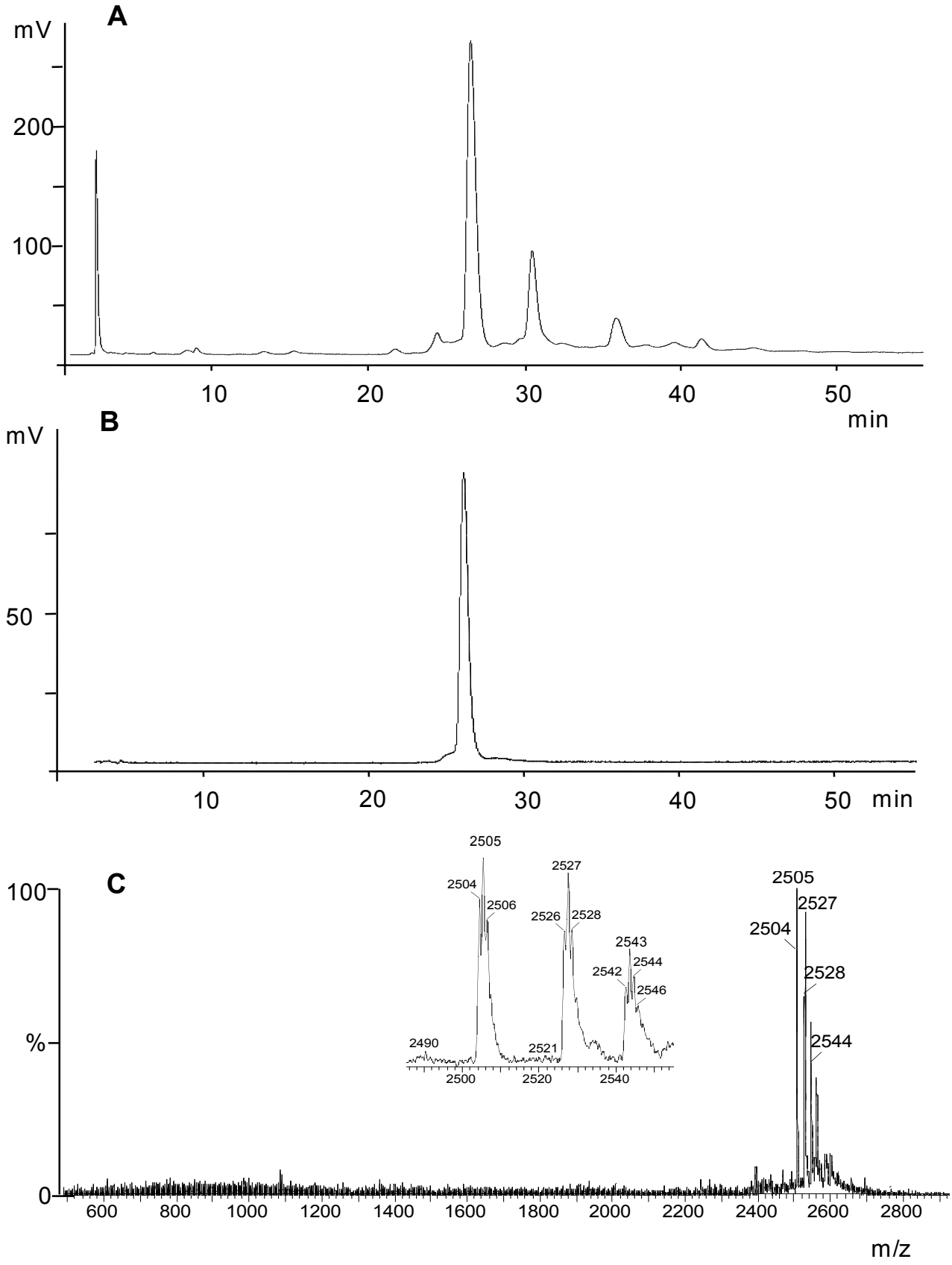

Figure 4. (A) Analytical HPLC chromatograph of the crude POM LysTCACAACTT-NH_2. (B) Analytical HPLC chromatograph of the POM LysTCACAACTT-NH_2 after purification by semi-preparative HPLC. (C) MALDI-MS of the same crude POM LysTCACAACTT-NH_2 with expansion of *m/z* 2505 ($[M+H]^+$ 100%, $C_{111}H_{154}N_{51}O_{19}$ requires *m/z* 2505.3), 2527 ($[M+Na]^+$ 90%, $C_{111}H_{153}N_{51}O_{19}Na$ requires *m/z* 2527.2).

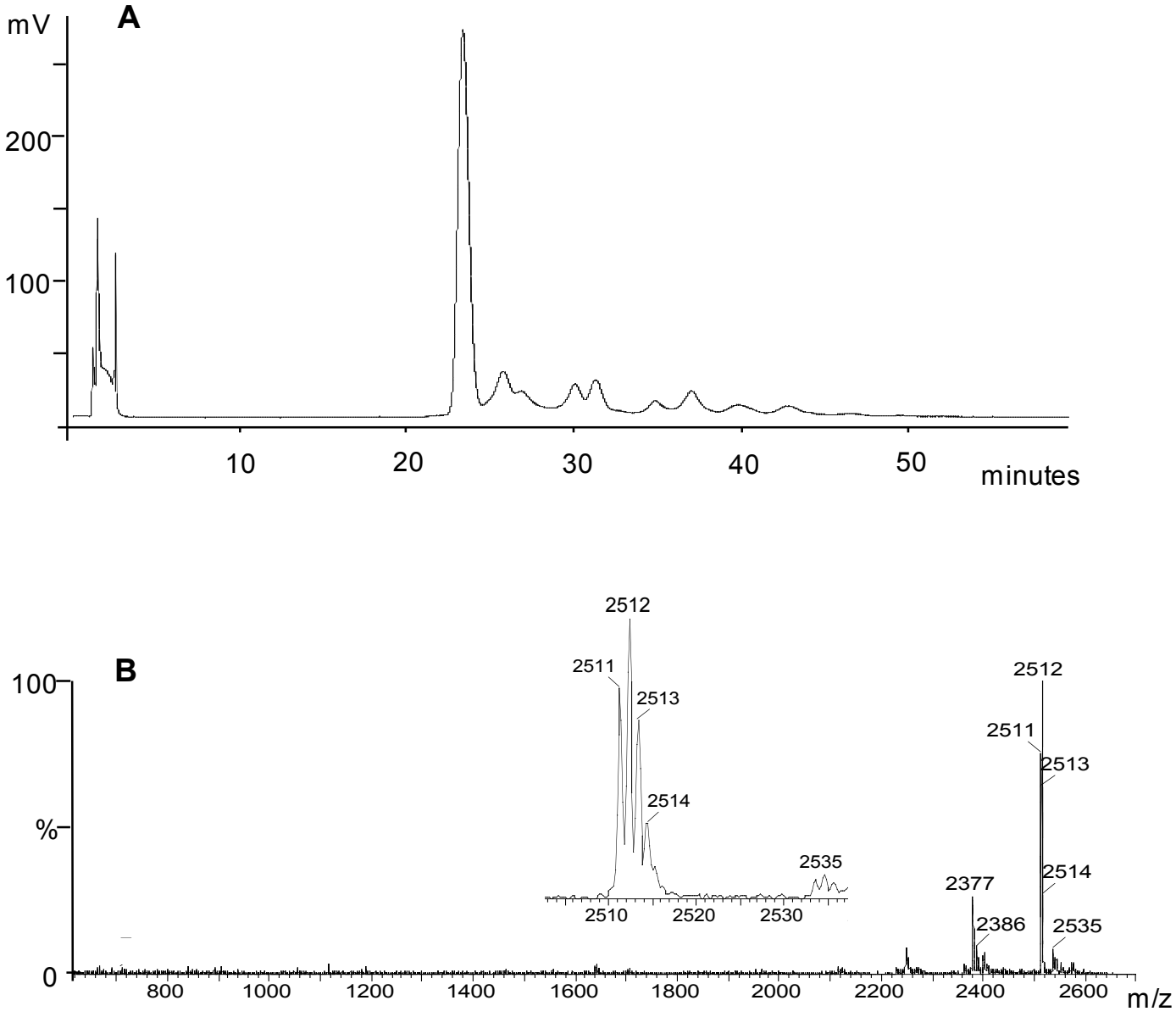

Figure 5. HPLC and MS of POM-PNA chimera (A) Analytical HPLC chromatographs of the crude POM-PNA chimera LysTC*AC*AAC*TT-NH_2. (B) Maldi-MS of the same crude POM-PNA chimera LysTC*AC*AAC*TT-NH_2 with expansion of *m/z* 2512 ($[M+H]^+$ 100%, $C_{108}H_{148}N_{51}O_{22}$ requires *m/z* 2512.2).

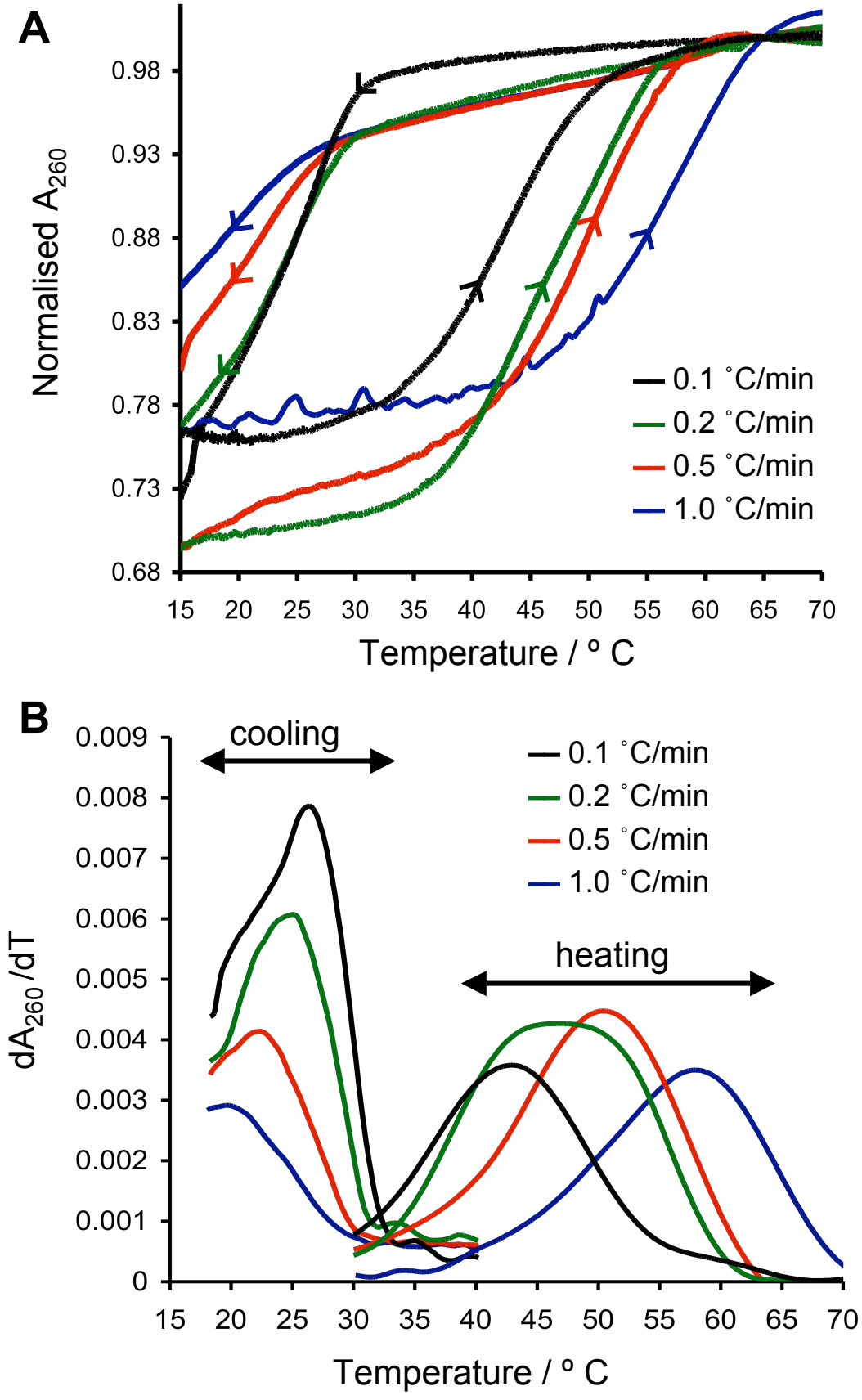

Figure 6. The effects of changing rates of heating and cooling on the UV thermal denaturation curves for POM LysTCACAACTT-NH_2 *vs* antiparallel RNA. (A) UV A_{260} *vs* temperature curves obtained following fast heating at 5 ˚C/min from 23 ˚C to 93 ˚C followed by cooling (down arrows) from 93 ˚C to 15 ˚C and then heating (up arrows) from 15 ˚C to 93 ˚C at 1.0 (black), 0.5 (green), 0.2 (red) or 0.1 (blue) ˚C/min. (A) The first derivatives obtained from slow cooling and slow heating curves with heating/cooling rates of 1.0 (black), 0.5 (green), 0.2 (red) or 0.1 (blue) ˚C/min.

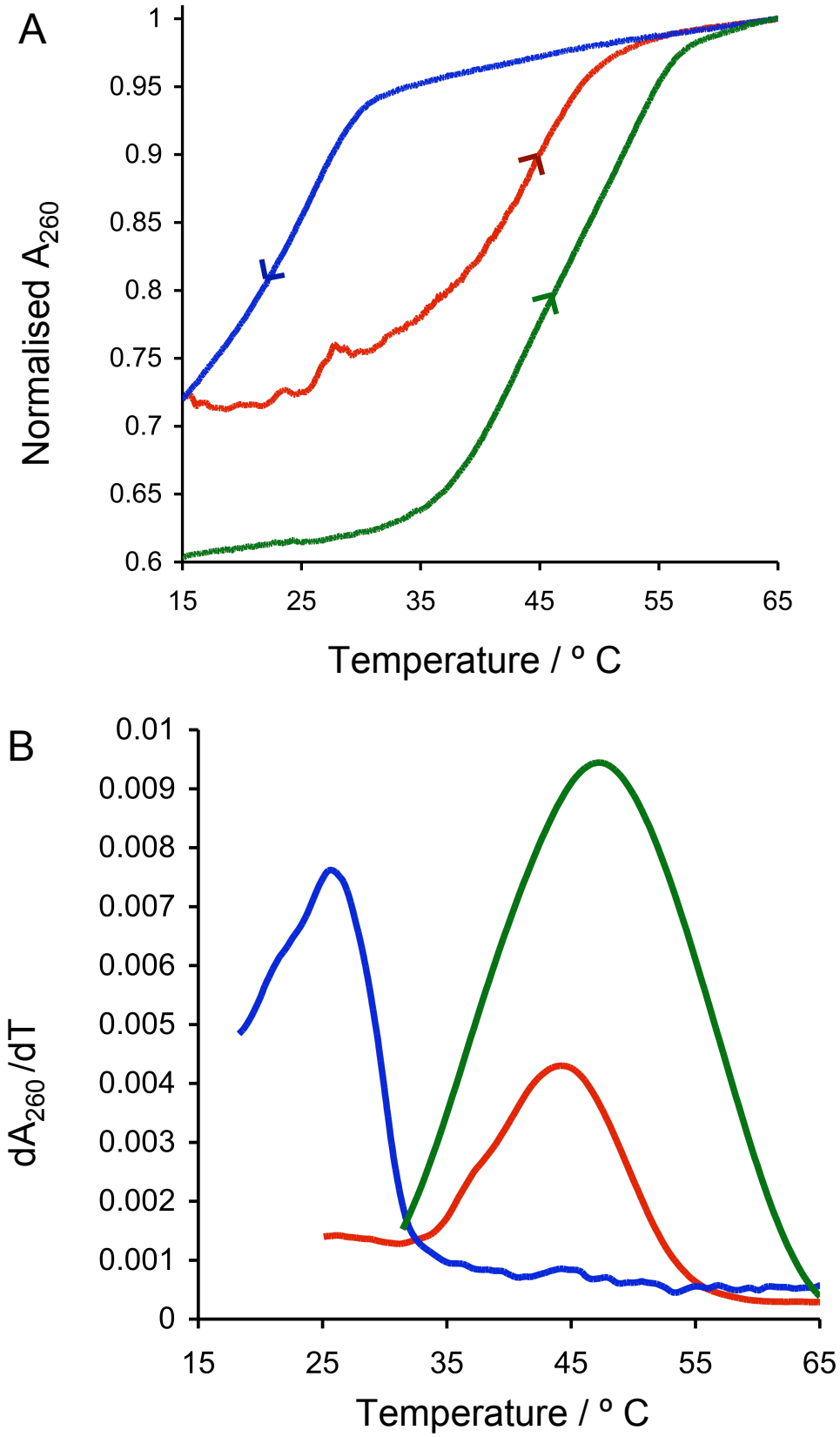

Figure 7. UV thermal denaturation curves and first derivatives for POM LysTCACAACTT-NH_2 *vs* parrallel RNA 5´-AGUGUUGAA-3´. (A) shows the slow cooling (renaturation) curve in blue (0.2 ℃/min), the slow heating (denaturation) curve in red (0.2 ℃/min) and the slow heating (denaturation) curve (0.2 ℃/min) obtained immediately after the POM and parrallel RNA were incubated at room temperature for 12 h is shown in green. (B) The corrsponding first derivatives obtained from slow cooling under standard conditions shown in blue, slow heating under standard conditions shown in red and slow heating following incubation for 12 h at room temperature shown in green.

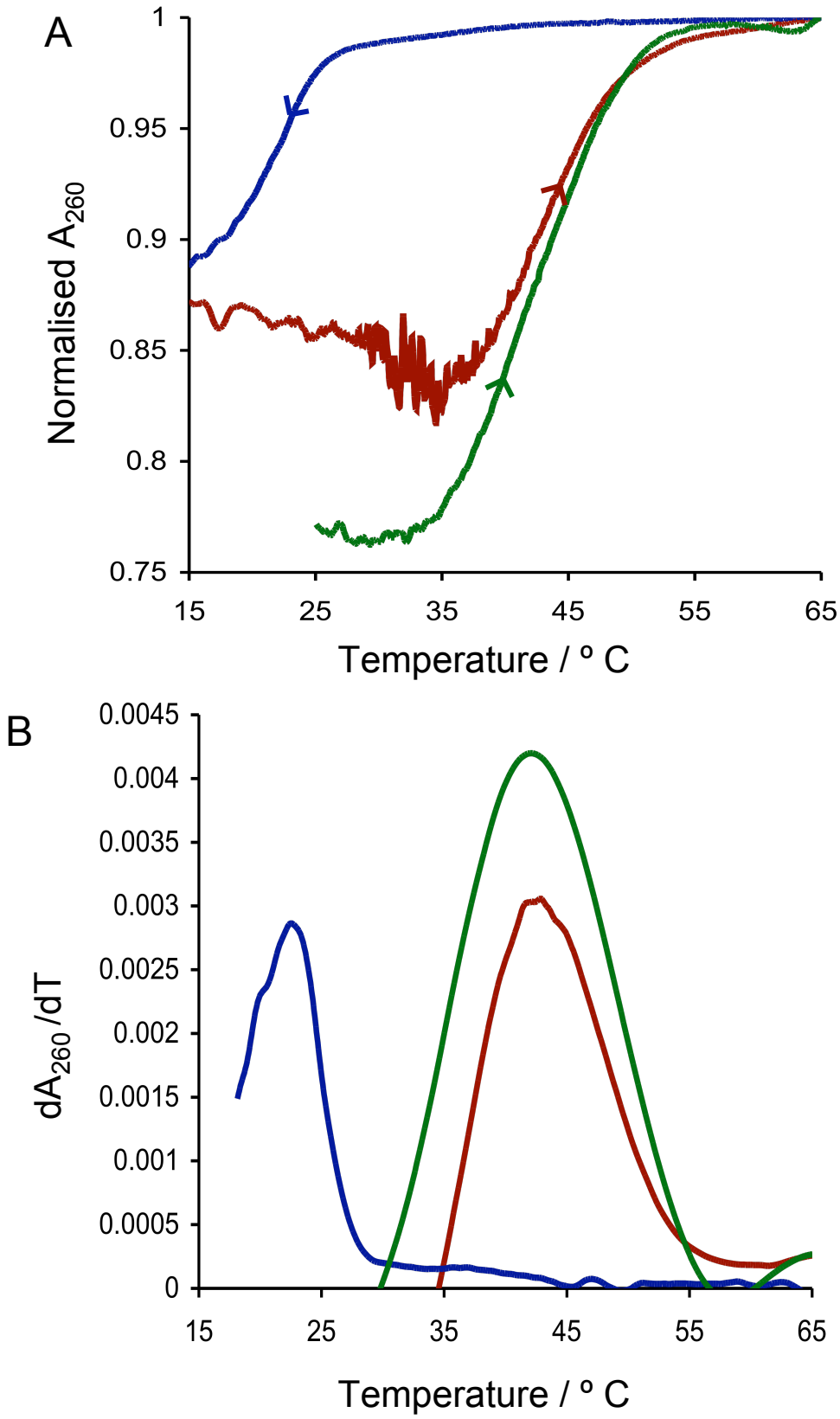

Figure 8. UV thermal denaturation curves and first derivatives for POM LysTCACAACTT-NH_2 *vs* antiparrallel DNA 5´-AAGTTGTGA-3´. (A) shows the slow cooling (renaturation) curve in blue (0.2 ℃/min), the slow heating (denaturation) curve in red (0.2 ℃/min) and the slow heating (denaturation) curve (0.2 ℃/min) obtained immediately after the POM and antiparrallel DNA where incubated at room temperature for 12 h is shown in green. (B) The corrsponding first derivatives obtained from slow cooling under standard conditions shown in blue, slow heating under standard conditions shown in red and slow heating following incubation for 12 h at room temperature shown in green.

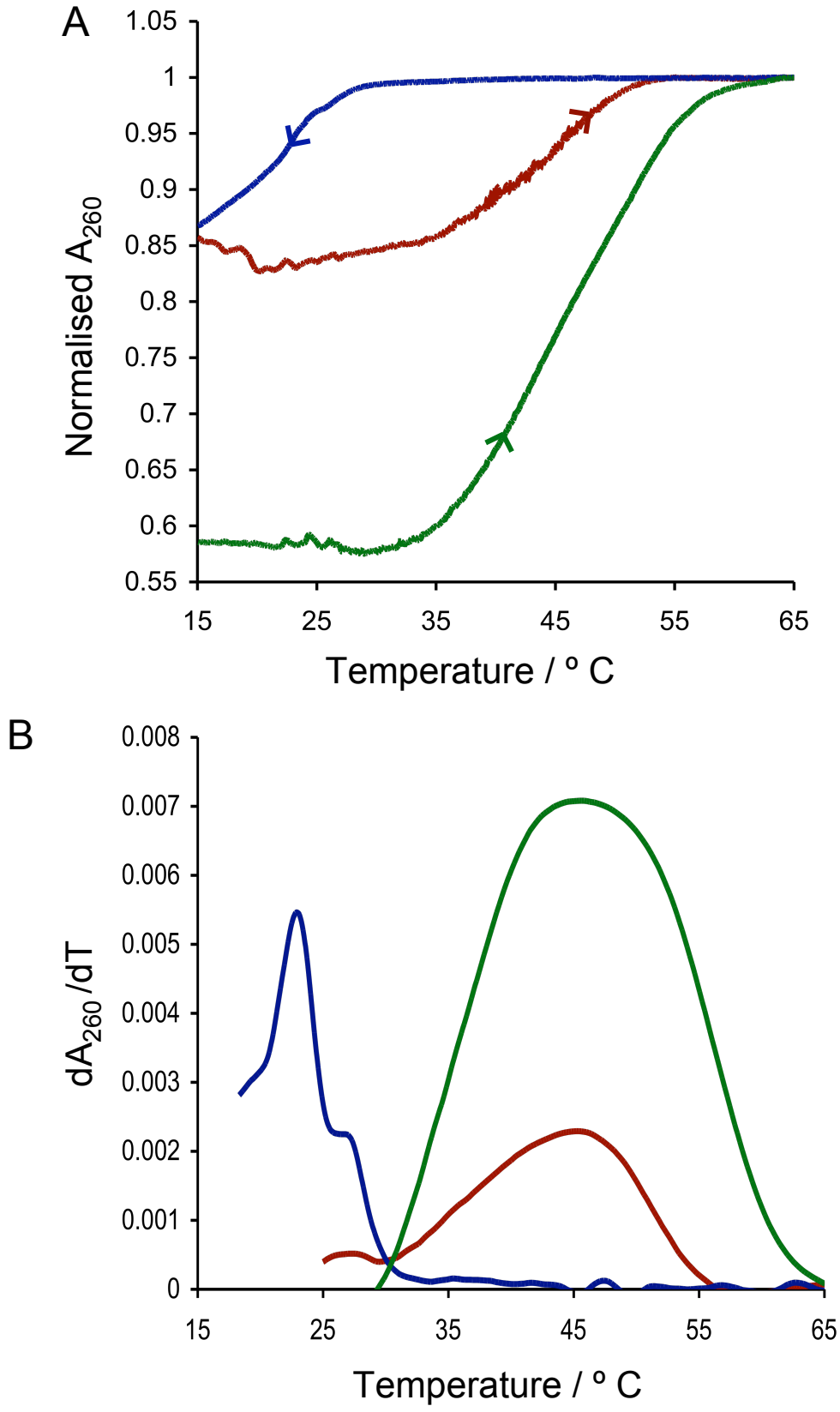

Figure 9. UV thermal denaturation curves and first derivatives for POM LysTCACAACTT-NH_2 *vs* parrallel DNA 5´-AGTGTTGAA-3. (A) shows the slow cooling (renaturation) curve in blue (0.2 ˚C/min), the slow heating (denaturation) curve in red (0.2 ˚C/min) and the slow heating (denaturation) curve (0.2 ˚C/min) obtained immediately after the POM and parrallel DNA were incubated at room temperature for 12 h is shown in green. (B) The corrsponding first derivatives obtained from slow cooling under standard conditions shown in blue, slow heating under standard conditions shown in red and slow heating following incubation for 12 h at room temperature shown in green.

General experimental methods

MALDI mass spectra were obtained on a Micromass Tof Spec 2e using alpha-cyano-4-hydroxycinnamic acid as matrix. All other general experiment methods were as described in the preceding paper.[1]

(2*R*,4*S*)-2-Azidomethyl-4-formyloxy-*N*-methoxycarbonyl methyl)-pyrrolidine (4).

To a solution of alcohol **3** (5.90 g, 22.9 mmol) in anhydrous THF (57 mL) under Ar at –20 ˚C was added anhydrous HCO_2H (1.05 mL, 27.6 mmol) followed by PPh_3 (7.24 g, 27.5 mmol). To the resulting solution was immediately added diisopropylazodicarboxylate (DIAD) (5.46 mL, 27.5 mmol) dropwise at –20 ˚C and the reaction mixture stirred for 1 h. The reaction mixture was allowed to warm to room temperature and stirred under Ar for 18 h. Solvent was removed under reduced pressure, and purification by column chromatography (5% Et_2O in CH_3Ph) gave formyl ester **4** (4.74 g, 71%) as a clear oil. R_f 0.16 (5% Et_2O/CH_3Ph); ν_{max} (KBr)/cm^{-1} 2937 and 2868 (CH), 2102 (N_3), 1720 and 1175 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99-2.06 (2H, m, H_a3 and H_b3), 2.75 (1H, dd, *J* 10.9, 4.0 Hz, H_a5), 3.18-3.29 (2H, m, H_a6 and H2), 3.36-3.46 (2H, m, H_a7 and H_b6), 3.57-3.66 (2H, m, H_b7 and H_b5), 3.70 (3H, s, OCH_3), 5.23-5.31 (1H, m, H4), 8.00 (1H, s, OCHO); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 35.9 (C3), 51.6 (CO_2CH_3), 53.5 (C6), 53.6 (C7), 59.1 (C5), 60.3 (C2), 72.4 (C4), 160.4 (OCHO), 170.9 (CO_2CH_3); *m/z* (ES) 243.2 ($[M+H]^+$, 100%); HRMS *m/z* (ES) 243.1093 ($[M+H]^+$, $C_9H_{15}N_4O_4$ requires *m/z*, 243.1093).

(2*R*,4*S*)-2-Azidomethyl-4-hydroxy-*N*-(methoxycarbonylmethyl)-pyrrolidine (5).

To a stirred solution of formyl ester **4** (7.30 g, 30.1 mmol) in anhydrous CH_3OH (36.5 mL) was added anhydrous K_2CO_3 (8.77 g, 63.5 mmol) and the solution was stirred under N_2 at room temperature. After 2 h, the solvent was evaporated under reduced pressure and the residue was

purified by column chromatography (2:1 EtOAc/hexane) to give alcohol **5** (336 mg, 92%) as a colourless oil. R_f 0.18 (2:1 EtOAc/hexane); ν_{max} (KBr)/cm^{-1} 3408 (OH), 2953 and 2863 (CH), 2101 (N_3), 1739 and 1206 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.74-1.82 (1H, m, H_a3), 1.92-2.02 (1H, m, H_b3), 2.68 (1H, d, *J* 11.1 Hz, H_a5), 3.13 (1H, dd, *J* 11.7, 3.9 Hz, H_a6), 3.28-3.40 (2H, m, H2 and H_b6), 3.48 (1H, dd, *J* 11.1, 4.6 Hz, H_b5), 3.59 (2H, s, H_a7 and H_b7), 3.69 (3H, s, OCH_3), 4.20-4.28 (1H, m, H4); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 39.7 (C3), 51.8 (CO_2CH_3), 52.3 (C7), 54.1 (C6), 59.5 (C2), 61.6 (C5), 70.9 (C4), 173.1 (CO_2CH_3); *m/z* (ES) 215.1 ([M+H]$^+$, 100%); HRMS *m/z* (ES) 215.1136 ([M+H]$^+$, $C_8H_{15}N_4O_3$ requires *m/z*, 215.1144).

(2´*R*,4´*R*)-2´-Azidomethyl-4´-(*N*^{3}-benzoylthymin-1-yl)-*N*1´-(methoxycarbonylmethyl)-pyrrolidine (6). To a stirred solution of alcohol **5** (3.68 g, 17.2 mmol) and DIAD, (8.58 mL, 43.6 mmol) in anhydrous THF (350 mL) under Ar at room temperature was added *N*^{3}-benzoylthymine (7.89 g, 34.3 mmol) portionwise over 15 min. PPh_3 (11.3 g, 42.9 mmol) was added portionwise and the reaction mixture was stirred for 5 min. Sodium benzoate (4.92 g, 34.2 mmol) was added portionwise and the suspension was stirred at room temperature under Ar for 4 h. The solvent was then evaporated under reduced pressure. H_2O (500 mL) was added and the mixture was extracted with EtOAc (3 x 500 mL). The combined organic extracts were dried with $MgSO_4$ and evaporated under reduced pressure to give a yellow foam which was purified by column chromatography (3:7 hexane/EtOAc) to give thyminyl derivative **6** as a colourless waxy solid (5.48 g, 75 %). mp 47-48 °C (hexane/EtOAc); $[\alpha]_D$ –172.0 ° (*c* = 0.5, CH_3OH); ν_{max} (NaCl)/cm^{-1} 2952 (CH), 2101 (N_3), 1744, 1696 and 1653 (CO); λ_{max}(CH_3OH)/nm 253; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.70 (1H ddd, *J* 14.5, 8.0, 3.0 Hz, H_a3´), 1.93 (3H, s, thymine CH_3), 2.49 (1H, ddd, *J* 14.5, 8.0, 8.0, H_b3´), 2.77 (1H, dd, *J* 11.0, 7.0 Hz, H_a5´), 2.86 (1H, m, H2´), 3.14 (1H, d, *J* 17.5 Hz, H_a7´), 3.26 (1H, dd, *J* 13.0, 4.0 Hz, H_a6´),

3.32 (2H, d, *J* 11.0 Hz, H_b5'), 3.50 (1H, dd, *J* 13.0, 4.0 Hz, H_b6'), 3.68 (3H, s, OCH_3), 3.69 (1H, d, *J* 17.5 Hz, H_b7'), 4.96 (1 H, m, H4´), 7.41 (2H, t, *J* 7.5 Hz, BzCH), 7.56 (2H, t, *J* 7.5 Hz, Bz CH), 7.84 (1H, d, *J* 7.5 Hz, Bz CH), 8.10 (1H, br s, H6); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 13.0 (thymine CH_3), 36.6 (C3´), 52.3 (C6´), 52.3 (OCH_3), 52.7 (C4´), 52.9 (C7´), 58.7 (C5´), 61.6 (C2´), 111.4 (C5), 129.5 (Bz CH), 130.8 (Bz CH), 132.1 (Bz C), 135.3 (Bz CH), 138.4 (C6), 150.5 (C2), 163.3 (C4), 169.7 (Bz CO), 170.9 (CO_2CH_3); *m/z* (ES) 427.0 ($[M+H]^+$, 100%); HRMS *m/z* (ES) 427.1728 ($[M+H]^+$, $C_{20}H_{23}N_6O_5$ requires *m/z*, 427.1730).

(2´*R*, 4´*R*)-2´-[(*tert*-Butoxycarbonyl)aminomethyl]-4´-(*N*^{3}-benzoylthymin-1-yl)-*N*1´-(methoxycarbonylmethyl)-pyrrolidine (8). *Method B:* Azide **6** (1.15 g, 2.697 mmol) was dissolved in THF (18.4 mL) at room temperature and a 1 M solution of PMe_3 in THF (3.51 mL, 3.51 mmol) was added. For ten minutes, N_2 effervescence was observed. When this had ceased, Boc-ON (864 mg, 3.51 mmol) was added in small portions and the reaction mixture stirred for 20 minutes. The solvent was removed under reduced pressure, and the crude product adsorbed onto silica (9 g) and purified by column chromatography (gradient elution: 10% *n*-hexane in EtOAc to 100% EtOAc). Carbamate **8** (796 mg, 81%) was obtained as a white foam.

(2´*R*, 4´*R*)-2´-[(*tert*-Butoxycarbonyl)aminomethyl]-4´-thymin-1-yl-*N*1´-(methoxycarbonylmethyl)-pyrrolidine (9). A degassed solution of azide **6** (660 mg, 1.55 mmol), di-*tert*-butyldicarbonate (Boc anhydride) (1.69 g, 7.74 mmol) and 10% Pd/C (660 mg) in EtOAc (22 mL) was hydrogenated at room temperature overnight. The suspension was filtered, evaporated under reduced pressure, and purified by column chromatography (3:2 hexane/EtOAc) to give benzyolated thyminyl derivative **8** (292 mg, 38%) as a colourless waxy solid. Upon further elution with

hexane/EtOAc (1:1) debenzyolated thyminyl derivative **9** (205 mg, 27%) was also isolated as a colourless waxy solid.

Debenzyolated thyminyl derivative **(9)**: mp 48.5-49 °C (Hexane/EtOAc); $[\alpha]_D$ –9.7 (*c* = 1.0, CH_3OH); ν_{max} (KBr)/cm^{-1} 3358 and 3187 (NH), 3054 and 2976 (CH), 1743 (CO); $\lambda_{max}(CH_3OH)$/nm: 272.0; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (9H, s, $C(CH_3)_3$), 1.56 (1H, m, H_a3'), 1.91 (3H, s, thymine CH_3), 2.46 (1H, ddd, *J* 14.5, 8.5, 8.5 Hz, H_b3'), 2.65-2.73 (2H, m, H_a5' and H2′), 3.00 (1H, d, *J* 14.5 Hz, H_a6'), 3.12 (1H, d, *J* 17.5 Hz, H_a7'), 3.19 (1H, d, *J* 11.0 Hz, H_b5'), 3.37 (1H, dd, *J* 14.0, 8.5 Hz, H_b6'), 3.56 (1H d, *J* 17.5 Hz, H_b7'), 3.67 (3H, s, OCH_3), 4.97 (1H, m, H4′), 5.13 (1H broad s, NH), 7.95 (1H, br s, H6); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 13.1 (thymine CH_3), 28.7 ($C(CH_3)_3$), 36.3 (C3′), 40.3 (C6′), 52.1 (OCH_3), 52.3 (C4′), 53.3 (C7′), 59.4 (C5′), 63.1 (C2′), 79.9 ($C(CH_3)_3$), 111.4 (C5), 138.3 (C6), 151.6 (C2), 156.7 ($CO_2{}^tBu$), 164.4 (C4), 171.8 (CO_2CH_3); *m*/*z* (ES) 397 ([M+H]$^+$, 100%); HRMS *m*/*z* (ES) 397.2091 ([M + H]$^+$, $C_{18}H_{29}N_4O_6$ requires *m*/*z*, 397.2087).

The bicyclic lactam **7** was a side product in the reduction of **6** to **8**. This lactam **7** is indeed the major product when reduction was carried out without *in situ* Boc-protection. Characterisation for bicyclic-lactam **(7)** is as follows: R_f 0.20 (9:1 EtOAc/CH_3OH); mp 214-214.5 °C (CH_3CN/H_2O); $[\alpha]_D$ –98.7° (*c* = 0.9, CH_3OH); ν_{max}(KBr)/cm^{-1} 3393 (NH), 1745, 1697 and 1652 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (1H, m, H_a3'), 1.97 (3H, d, *J* 0.75 Hz, C5-CH_3), 2.48-2.56 (1H, m H2′), 2.60 (1H, dd, *J* 8.5, 6.0 Hz, H_a5'), 2.66-2.70 (1H, m, H_b3'), 2.90 (1H, d, *J* 12.5 Hz, H_a7'), 3.14 (1H, d, *J* 8.5 Hz, H_b5'), 3.27 (1H, dd, *J* 8.5, 8.5 Hz, H_a6'), 3.46 (1H, dd, *J* 8.5, 2.5 Hz, H_b6'), 3.68 (1H, d, *J* 12.5 Hz, H_b7'), 5.31 (1H, m, H4′), 6.78 (1H, d, *J* 2.5 Hz, NH), 7.47 (2H, t, *J* 6.0 Hz, Bz H), 7.61-7.65 (2H, m, Bz H and H6), 7.88 (2H, dd, *J* 6.0, 1.0 Hz, Bz H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 13.2 (C5-CH_3),

36.9 (C3´), 46.4 (C6´), 52.8 (C4´), 56.0 (C7´), 58.8 (C2´), 60.1 (C5´), 112.7 (C5), 129.4 (Bz CH), 130.7 (Bz CH), 131.6 (Bz C), 135.4 (Bz CH), 136.9 (C6), 150.1 (C2), 163.0 (C4), 169.3 (Bz CO), 169.4 (CONH); *m/z* (ES) 369.1 ($[M+H]^+$, 100%), 391.2 ($[M+Na]^+$, 5); HRMS *m/z* (ES) 369.1557 ($[M+H]^+$, $C_{19}H_{21}N_4O_4$ requires *m/z*, 369.1555).

(2´*R*, 4´*R*)-2´-(*tert*-Butoxycarbonylamino-methyl)-4´-(thymin-1-yl)-pyrrolidine-1´-yl-acetic acid (10). *Method B*: A solution of debenzoylated thymine derivative **9** (106 mg, 0.204 mmol) in THF (1 mL) was treated with 1 M aqueous NaOH (0.3 mL, 0.3 mmol) and then left stirring for 2 h. More 1 M aqueous NaOH was added (0.3 mL, 0.3 mmol) and the solution was left stirring for a further 30 min. The reaction mixture was worked up and purified as described above to give acid **10** (72 mg, 87%) as a white solid.

(2*R*,4*S*)-2-Azidomethyl-4-(*p*-toluenesulphonyl)oxy-*N*-(methoxycarbonylmethyl)-pyrrolidine (18). *Method A*: To a solution of *trans*-alcohol **5** (1.90 g, 8.87 mmol,) in anhydrous pyridine (23 mL) at 0 ˚C was added *para*-toluenesulphonyl chloride (3.42 g, 17.8 mmol). The solution was stirred for 15 min at 0 ˚C, warmed to room temperature and stirred for 18 h. CH_3OH (14 mL) was added and solvents were removed under reduced pressure. Brine (200 mL) was added to the brown paste, which was extracted with EtOAc (4 x 200 mL). The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure. Purification by column chromatography (2:1 EtOAc/hexane, R_f 0.76) afforded *para*-toluenesulphonate **18** (1.05 g, 61%) as a pale yellow oil.

Method B: To a solution of *cis*-alcohol **3** (500 mg, 2.34 mmol) in anhydrous THF (20 mL) was added triphenylphosphine (735 mg, 2.802 mmol) at –10 °C under N_2. To the suspension was immediately added diethylazodicarboxylate (DEAD) (488 μL, 2.802 mmol). Methyl *para*-toluenesulphonate

(0.522 g, 2.802 mmol) was added dropwise at 0 °C and stirred for 10 min at 0 °C. The suspension was warmed to room temperature and stirred for 18 h under N_2. Evaporation of solvent under reduced pressure and purification by column chromatography (9:1 CH_3Ph/Et_2O), gave *para*-toluenesulphonate **18** (602 mg, 70%) as a pale yellow oil. R_f 0.21 (9:1 CH_3Ph/Et_2O); ν_{max} (KBr)/cm^{-1} 2963 and 2863 (CH), 2102 (N_3), 1743 (CO), 1361 and 1176 (SO_2O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.85-1.95 (1H, m, H_a3), 2.06-2.14 (1H, m, H_b3), 2.44 (3H, s, tosyl CH_3), 2.82 (1H, dd, *J* 11.2, 4.0 Hz, H_a5), 3.16-3.25 (2H, m, H2 and H_a6), 3.32-3.43 (2H, m, H_b6 and H_a7), 3.43-3.52 (1H, m, H_b5), 3.58 (1H, d, *J* 17.0 Hz, H_b7), 3.68 (3H, s, OCH_3), 4.87-4.94 (1H, m, H4), 7.33 (2H, d, *J* 8.2 Hz, tosyl aromatic H), 7.76 (2H, d, *J* 8.2 Hz, tosyl aromatic H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.6 (tosyl CH_3), 36.0 (C3), 51.7 (CO_2CH_3), 53.2 (C6), 54.0 (C7), 59.2 (C5), 60.5 (C2), 79.2 (C4), 127.7 (tosyl *ortho*-CH), 129.9 (tosyl *meta*-CH), 133.5 (tosyl *para*-C), 144.9 (tosyl *ipso*-C), 170.7 (CO_2CH_3); *m/z* (ES) 369.1 ($[M+H]^+$, 100%); HRMS *m/z* (ES) 369.1245 ($[M+H]^+$, $C_{15}H_{21}N_4O_5S$ requires *m/z*, 369.1233).

(2´*R*,4´*R*)-2'-Azidomethyl-4´-[*N*^{4}-benzyloxycarbonyl pyrimidin-2-yloxy]-*N*-(methoxycarbonyl methyl)-pyrrolidine (19). A suspension of tosylate **18** (128 mg, 0.35 mmol), *N*^{4}-benzyloxycarbonylcytosine (128 mg, 0.52 mmol), anhydrous K_2CO_3 (144 mg, 1.04 mmol) and 18-crown-6 (72 mg, 0.27 mmol) in anhydrous DMF (1.28 mL) was stirred over 4 Å molecular sieves at 80 ˚C under N_2. After 4 h, H_2O (100 mL) was added and the mixture was extracted with Et_2O (4 x 100 mL). The combined organic extracts were dried with $MgSO_4$ and evaporated under reduced pressure. Purification of the residue by column chromatography (gradient elution PhMe → 5% Et_2O in CH_3Ph) gave *O*^{2}-adduct **19** (103 mg, 67%) as a pale yellow gum. R_f 0.29 (1:1 Et_2O/CH_3Ph); ν_{max} (KBr)/cm^{-1} 3306 (NH), 2924 and 2854 (CH), 2100 (N_3), 1740 (CO), 1586 (Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.89-1.97 (1H, m, $H_a3´$), 2.46-2.58 (1H, m, $H_b3´$), 3.14-3.29 (2H, m, H2´ and $H_a5´$), 3.29-3.47 (3H, m, $H_b5´$, $H_a6´$and $H_b6´$), 3.60 (2H, s, $H_a7´$ and $H_b7´$), 3.71 (3H, s, OCH_3), 5.22 (2H, s,

benzyl CH_2), 5.34-5.44 (1H, m, H4´), 7.38 (5H, s, benzyl aromatic), 7.44 (1H, s, NH), 7.57 (1H, d, *J* 5.7 Hz, H5), 8.34 (1H, d, *J* 5.7 Hz, H6); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 36.0 (C3´), 51.5 (CO_2CH_3), 52.8 (C6´), 55.2 (C7´), 58.7 (C5´), 60.5 (C2´), 67.8 (Z-CH_2), 76.3 (C4´), 102.4 (C5), 128.3 (*ortho*- and *para*-benzyl CH), 128.7 (*meta*- benzyl CH), 135.1 (*ipso*-Z-C), 152.2 (CO_2Bn), 159.3 (C2), 160.1 (C6), 164.1 (C4), 171.3 (CO_2CH_3); *m*/*z* (ES) 442.2 ([M+H]$^+$, 100%); 464.2 ([M+Na]$^+$, 20%); HRMS *m*/*z* (ES) 442.1858 ([M+H]$^+$, $C_{20}H_{24}N_7O_5$ requires *m*/*z*, 442.1839).

(2´*R*,4´*R*)-2´-Azidomethyl-4´-(*N*^{4}-[para-(*tert*-butyl)benzoyl]cytosin-1-yl)-*N*1´-(methoxycarbonylmethyl)-pyrrolidine (20). A suspension of tosylate **18** (387 mg, 1.05 mmol), *N*^{4}-[*para*-(*tert*-butyl)benzoyl]-cytosine (883 mg, 3.26 mmol), anhydrous K_2CO_3 (232 mg, 1.68 mmol) and 18-crown-6 (91.6 mg, 0.347 mmol) in anhydrous DMF (3.87 mL) was stirred over molecular sieves at 80 °C under N_2. After 4 h, H_2O (100 mL) was added and the mixture was extracted with Et_2O (4 x 100 mL). The organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure. Purification by column chromatography (gradient elution CH_3Ph → 5% Et_2O in CH_3Ph) gave the *N*^{1}-cytosinyl derivative **20** (103 mg, 32%) as white crystals. R_f 0.29 (1:1 Et_2O/CH_3Ph); ν_{max} (KBr)/cm^{-1} 2957 (CH), 2100 (N_3), 1744 and1661 (CO), 1621 (CC), 1555 (CN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (9H, s, $C(CH_3)_3$), 1.72-1.81 (1H, m, H_a3´), 2.71 (1H, ddd, *J* 14.8, 8.8, 8.8 Hz, H_b3´), 2.93 (1H, dd, *J* 11.3, 6.5 Hz, H_a5´), 2.96-3.03 (1H, m, H2´), 3.22-3.29 (2H, m, H_a6´ and H_a7´), 3.44 (1H, br d, *J* 11.3 Hz, H_b5´), 3.51 (1H, dd, *J* 13.0, 4.0 Hz, H_b6´), 3.72-3.81 (4H, m, OCH_3 and H_b7´), 5.17-5.24 (1H, m, H4´), 7.52 (2H, d, *J* 8.5 Hz, *meta* Bz H), 7.61 (1H, br s, H5), 7.84 (2H, d, *J* 7.9 Hz, *ortho* Bz Ar-H), 8.67 (1H, d, *J* 7.5 Hz, H6); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 30.9 ($C(CH_3)_3$), 35.0 ($C(CH_3)_3$), 36.5 (C3´), 51.8 (CO_2CH_3), 52.5 (C6´), 52.7 (C7´), 54.1 (C4´), 58.3 (C5´), 61.0 (C2´), 96.8 (C5), 125.9 (*meta* Bz-CH), 127.4 (*ortho* Bz-CH), 129.9 (*para* Bz-CH), 147.1 (C6), 155.5 (*ipso* Bz-

C), 156.9 (C2), 161.6 (C4), 166.1 (Bz CO), 170.5 (CO_2CH_3); *m/z* (ES) 468.2 ($[M+H]^+$, 100%); HRMS *m/z* (ES) 468.2348 ($[M+H]^+$, $C_{23}H_{30}N_7O_4$ requires *m/z*, 468.2359).

(2´*R*,4´*R*)-2´-(*tert*-Butoxycarbonylaminomethyl)-4´-(N^4-[para-(*tert*-butyl)benzoyl]cytosin-1-yl)-*N*1´-(methoxycarbonylmethyl)-pyrrolidine (22). *Method B*: Azide **20** (1.85 g, 3.957 mmol) was dissolved in THF (29.6 mL) and PMe_3 (1 M in THF, 5.14 mL, 5.14 mmol) was added to the stirred solution. After *ca*. 10 mins stirring, Boc-ON (1.27 g, 5.14 mmol) was added to the mixture and the mixture was stirred for 2 h. The solvent was removed under reduced pressure, leaving a pale yellow solid. H_2O (200 mL) was added and the crude product was extracted with CH_2Cl_2 (3 x 100 mL), the organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by column chromatography (gradient elution: 10% EtOAc in hexane → 100% EtOAc) gave **22** (1.35 g, 63%) as a white foam.

General materials and methods for Boc-solid phase synthesis.

All experiments were carried out in teflon solid-phase synthesis vessels (Kinesis) fitted with frits (porosity grade 3). The reaction mixture, including the resin supports was agitated by rotation using a mixer, rotator (Fisherbrand) and reagents were removed by suction filtration through a Büchner flask. MBHA resin LL (100-200 mesh) (loading of 0.62 mmol/g), N-α-Boc-N-ε-2-chloro-Z-L-lysine and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU) were purchased from Novabiochem. Boc protected cytosine PNA monomers were purchased from Applied Biosystems. Fresh bottles of anhydrous solvents from Acros Organics were used for each oligomer synthesised. All other chemicals used in solid-phase work were obtained at the highest purity grade

from Aldrich Chemical Company or Acros Organics and were used without further purification. Reagents used for the Kaiser test were prepared according to literature.[43]

Thermal Denaturation Experiments

UV melting plots of absorbance versus temperature were measured as described in the previous paper[1] on a Varian Cary 400 Scan UV-visible spectrophotometer. Concentrations of POM oligomers, PNA oligomers and oligonucleotides were measured spectrophotometrically at 80 °C from molar extinction coefficients of oligonucleotides calculated from the literature.[44] Buffers were prepared as double concentrated stock solutions and diluted to the appropriate concentrations during sample preparation. Sterile nuclease, protease and DEPC-free deionised water was used and all appropriate equipment was autoclaved before use. All samples were stored at –20 °C. Oligonucleotides were purchased from Sigma-Genosys, Yorkshire Bioscience or Eurogentec. PNA was purchased from Eurogentec.

Typical melting experiments were carried out as described in the preceding paper[1] with the exception that heating ramps and incubation times were as stated in the text. In brief the standard 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) unless stated otherwise. UV absorbance (A_{260}) was recorded with heating at 5 ˚C/min from 23 ˚C to 93 ˚C, cooling at 0.2 ˚C/min to 15 ˚C and heating at 0.2 ˚C /min to 93 ˚C. The T_m values were obtained from the maxima of first derivative curves calculated from Varian Thermal software using a filter size of 20 (for renaturation curves) and 97 (for denaturation curves) and smoothed every 0.2 °C.

References

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