Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Electronic Supporting Information

CG Base Pair Recognition within DNA Triple Helices by Modified *N*-Methylpyrrolo-dC Nucleosides

Simon R. Gerrard,[1]* Mastoura M. Edrees,[1] Imenne Bouamaied,[2] Keith R. Fox,[3] Tom Brown[1]*

[1]School of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK. E-mail: tb2@soton.ac.uk. [2]ATDBio Ltd, School of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK. [3]School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, UK.

A. ^{1}H NMR (phosphoramidite monomers), ^{13}C NMR and IR data

A.1 General Experimental

Proton and carbon NMR spectra were recorded using either a Bruker AC300 or Bruker DPX400 spectrometer. Assignment was aided by the DEPT spectral editing technique and H-C correlation experiments. See Fig. A1 for compound numbering. IR spectra were recorded on a Satellite FT-IR instrument using a 'Golden Gate' or 'Smart Orbit' adapter, and visualised using Win First-lite or OMNIC software respectively. All absorptions are measured in cm^{-1} and are described as broad (br), weak (w), medium (m), strong (s) or very strong (vs).

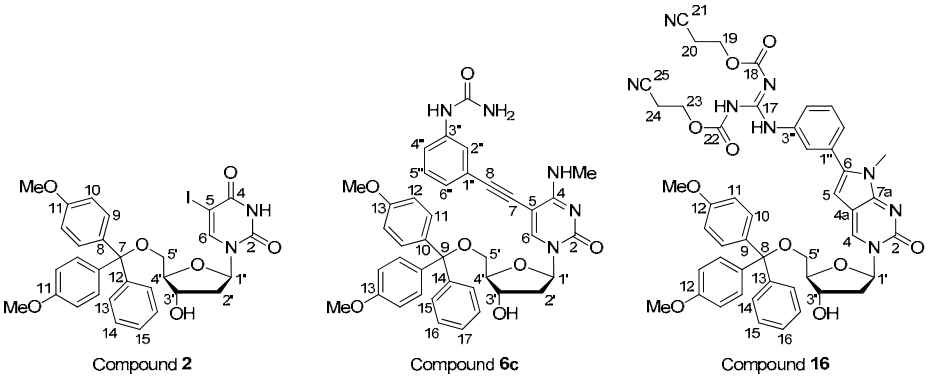

Fig. A1 Example compound numbering (**2**, **6c** and **16**).

A.2 Experimental Data

3'-*O*-Acetyl-5'-*O*-(4,4'-dimethoxytrityl)-5-iodo-2'-deoxyuridine (2).[29]
IR (solid): ν 3056 (w, C–H/N–H), 2933, 2835 (w, C–H), 1683 (br s, C=O), 1606, 1506 (m, ^{Ar}C=C), 1442 (m, C–H), 1379 (w, CH_3), 1244 (vs, C–O), 1174 (s), 1105 (m), 1073 (s), 1027 (s, C–O), 826, 754, 700 (s, ^{Ar}C–H) cm^{-1}; ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.9 (C=OCH_3), 160.5 (C^4), 158.1 (C^{11}–OCH_3), 150.1 (C^2), 144.6 (C^{12}), 144.1 (C^6), 135.3, 135.2 (C^8), 129.7 (C^9), 127.9 (C^{14}), 127.6 (C^{13}), 126.7 (C^{15}), 113.3 (C^{10}), 86.0 (C^7Ar_3), 84.7 ($C^{1'}$), 83.1 ($C^{4'}$), 74.1 ($C^{3'}$), 70.2 (C^5–I), 63.4 ($C^{5'}$), 55.0 (OCH_3), 36.8 ($C^{2'}$), 20.7 (COCH_3).

5'-*O*-(4,4'-Dimethoxytrityl)-5-iodo-4*N*-methyl-2'-deoxycytidine (3).
IR (solid): ν (solid) 3291 (br w, N–H), 3059, 2930, 2835 (w, C–H), 1634 (s, C=O), 1606 (vs, C=O/C=C), 1547 (s, C=O), 1505 (vs, C=C), 1397 (m, O–H), 1284 (s, C–O), 1246 (vs, C–N), 1173 (s), 1092, 1030 (m, C–O), 825, 779 (s, ^{Ar}C=C), 700 (s) cm^{-1}; ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 163.0 (C^4), 159.8 (C^{11}–OCH_3), 155.3 (C^2), 146.8 (C^6), 146.4 (C^{12}), 137.2, 137.1 (C^8), 131.4 (C^9), 129.6 (C^{14}), 129.4 (C^{13}), 128.4 (C^{15}), 115.0 (C^{10}), 87.5 (C^7Ar_3), 87.4 ($C^{4'}$), 87.0 ($C^{1'}$), 72.4 ($C^{3'}$), 65.4 ($C^{5'}$), 59.9 (C^5–I), 56.8 (OCH_3), 42.4 ($C^{2'}$), 30.4 (NHCH_3).

3-Acetamidophenyl acetylene (5a).[30]
IR (solid): ν 3300 (m), 3282 (m, CC–H), 1666 (m), 1605, 1583 (m, ^{Ar}C–H), 1556 (m, C=O), 1481 (m, ^{Ar}C–H), 1425, 1401, 1370, 1308, 1289, 1255 (m), 1015 (w), 880 (m), 786 (m), 721 (m) cm^{-1}; ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.1 (COCH$_3$), 140.1 (C^3), 129.7 (C^5), 126.8 (C^6), 122.5 (C^1), 122.4 (C^2), 120.1 (C^4), 84.0 (C≡CH), 81.0 (C≡CH), 24.6 (COCH$_3$).

3-Ureidophenyl acetylene (5b).[31]
IR (solid): ν 3467 (m), 3329 (m, N–H), 3302 (w, N–H), 3238 (m, N–H), 2100 (w, C≡C), 1649 (vs, C=O), 1586, 1575 (s, ^{Ar}C–H), 1540 (br vs, ^{Ar}C–H), 1473 (s), 1413 (m), 1325 (s), 1284, 1256, 1162 (m), 1120 (br w), 1029 (w), 890 (m, ^{Ar}C–H), 801 (m), 773 (m, ^{Ar}C–H) cm^{-1}; ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 155.9 (CONH$_2$), 140.8 (C^3), 129.0 (C^5), 124.3 (C^6), 121.8 (C^1), 120.5 (C^2), 118.4 (C^4), 83.8 (C≡CH), 80.0 (C≡CH).

5-Phenylethynyl-5'-*O*-(4,4'-dimethoxytrityl)-4*N*-methyl-2'-deoxycytidine (6a).
IR (solid): ν 3307 (br w, O–H, N–H), 2932 (w, C–H), 2835 (w, OCH$_3$, NCH$_3$), 1639 (s, C=O), 1556 (m), 1505 (s, ^{Ar}C=C), 1247 (s, C–O), 1090 (m, C–OH), 1030 (m), 826, 754 (m, ^{Ar}C–H), 725 (m, CH$_2$), 690 (s, ^{Ar}C–H), 583 (m); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 162.5 (C^4), 158.5, 158.5 (C^{13}–OCH$_3$), 154.8 (C^2), 144.5 (C^{14}), 142.7 (C^6), 135.8, 135.6 (C^{10}), 131.4 (C$^{2''}$), 130.0, 129.9 (C^{11}), 128.6 (C$^{4''}$), 128.2 (C$^{3''}$), 128.0 (C^{16}), 127.9 (C^{15}), 126.9 (C^{17}), 122.1 (C$^{1''}$), 113.3, 113.2 (C^{12}), 95.5 (C^8), 91.4 (C^7), 87.0 (C$^{1'}$), 86.8 (C^{9}Ar$_3$), 86.4 (C$^{4'}$), 79.9 (C^5), 72.4 (C$^{2'}$), 63.6 (C$^{5'}$), 55.1 (OCH$_3$), 42.5 (C$^{2'}$), 28.3 (NHCH$_3$).

5-(3-Acetamidophenyl)ethynyl-5'-*O*-(4,4'-dimethoxytrityl)-4*N*-methyl-2'-deoxycytidine (6b).
^{13}C NMR (100 MHz, d_6-DMSO) δ (ppm) 168.4 (COCH$_3$), 161.7 (C^4–NHCH$_3$), 158.0, 158.0 (C^{13}–OCH$_3$), 153.2 (C^2), 144.7 (C^{14}), 143.0 (C^6), 139.1 (C$^{3''}$), 135.6, 135.3 (C^{10}), 129.7, 129.6 (C^{11}), 128.5 (C$^{5''}$), 127.8 (C^{16}), 127.6 (C^{15}), 126.6 (C^{17}), 126.4 (C$^{6''}$), 122.5 (C$^{1''}$), 121.9 (C$^{2''}$), 119.4 (C$^{4''}$), 113.2, 113.1 (C^{12}), 94.3 (C^8), 90.3 (C^7), 85.9 (C$^{4'}$), 85.8 (C^{9}Ar$_3$), 85.6 (C$^{1'}$), 80.4 (C^5), 70.7 (C$^{3'}$), 63.8 (C$^{5'}$), 54.9 (OCH$_3$), 40.8 (C$^{2'}$), 27.8 (NHCH$_3$), 23.9 (COCH$_3$).

5-(3-Aminophenyl)ethynyl-5'-*O*-(4,4'-dimethoxytrityl)-4*N*-methyl-2'-deoxycytidine (9).
IR (solid): ν 3337 (br m, O–H, N–H), 2934, 2835 (w, C–H), 1640 (s, C=O/C=C), 1596 (s, ^{Ar}C–C), 1556 (s), 1505 (vs, C=N), 1444 (m, C–H), 1403 (m), 1352 (m, C–H), 1285 (m, O–H), 1246, 1174 (s), 1086 (br s, C–O), 1029 (br s), 942 (m), 866 (m, C–H), 826 (s, ^{Ar}C–H), 780 (s), 726 (m), 686 (m, ^{Ar}C–H) cm^{-1}; ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 161.7 (C^4–NHCH$_3$), 158.0, 158.0 (C^{13}–OCH$_3$), 153.2 (C^2), 148.3 (C^{14}), 144.6 (C$^{3''}$), 142.5 (C^6), 135.6, 135.2 (C^{10}), 129.7, 129.6 (C^{11}), 128.7 (C$^{5''}$), 127.8 (C^{16}), 127.6 (C^{15}), 126.6 (C^{17}), 122.4 (C$^{1''}$), 119.2 (C$^{6''}$), 116.4 (C$^{2''}$), 114.5 (C$^{4''}$), 113.2, 113.2 (C^{12}), 95.3 (C^8), 90.6 (C^7), 85.9 (C$^{4'}$), 85.8 (C^{9}Ar$_3$), 85.6 (C$^{1'}$), 79.0 (C^5), 70.8 (C$^{3'}$), 63.7 (C$^{5'}$), 54.9 (OCH$_3$), 40.8 (C$^{2'}$), 27.9 (NHCH$_3$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-phenyl-(2,3*H*)-*N*-methylpyrrolo-[2,3-*d*]pyrimidine-2(7*H*)-one (7a).
IR (solid): ν 3338 (br w, OH), 2930 (w, C–H), 2835 (w, OCH$_3$), 1650 (s, C=O), 1606 (m, ^{Ar}C=C), 1556 (m), 1507 (m, ^{Ar}C=C), 1400, 1302 (m), 1246 (s, C–O), 1175 (m), 1093 (m, C–OH), 1030 (s, C–O), 827, 761 (m, ^{Ar}C–H), 699 (s, ^{Ar}C–H) cm^{-1}; ^{13}C NMR (100 MHz, CDCl$_3$) δ (ppm) 158.8 (C^{7a}), 158.6 (C^{12}), 158.6 (C^{12}–OCH$_3$), 154.9 (C^2), 144.2 (C^{13}), 143.2 (C^6), 136.0 (C^4), 135.8 (C^9), 135.5 (C^9), 131.1 (C$^{1''}$), 130.1 (C^{10}), 130.0 (C^{10}), 128.8 (C$^{4''}$), 128.7 (C$^{3''}$), 128.5 (C$^{2''}$), 128.3 (C^{15}), 128.0 (C^{14}), 127.0 (C^{16}), 113.3 (C^{11}), 108.9 (C^{4a}), 99.5 (C^5), 87.7 (C$^{1'}$), 87.0 (C^{8}Ar$_3$), 70.5 (C$^{3'}$), 62.6 (C$^{5'}$), 55.2 (OCH$_3$), 42.7 (C$^{2'}$), 29.7 (NCH$_3$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-(3-acetamidophenyl)-(2,3*H*)-*N*-methyl-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one (7b).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 168.5 (COCH$_3$), 158.5 (C^{7a}), 158.1 (C^{12}–OCH$_3$), 153.4 (C^2), 144.5 (C^{13}), 142.2 (C^6), 139.6 (C$^{3''}$), 136.4 (C^4), 135.4, 135.1 (C^9), 130.9 (C$^{1''}$), 129.8, 129.7 (C^{10}), 129.2 (C$^{5''}$), 127.9 (C^{15}), 127.7 (C^{14}), 126.8 (C^{16}), 122.8 (C$^{6''}$), 119.2 (C$^{4''}$), 118.5 (C$^{2''}$), 113.3 (C^{11}), 107.5 (C^{4a}), 98.9 (C^5), 86.7 (C$^{1'}$), 86.1 (C^{8}Ar$_3$), 85.7 (C$^{4'}$), 69.0 (C$^{3'}$), 62.6 (C$^{5'}$), 55.0 (OCH$_3$), 41.5 (C$^{2'}$), 29.3 (NCH$_3$), 24.0 (COCH$_3$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-(3-ureidophenyl)-(2,3*H*)-*N*-methyl-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one (7c).
IR (solid): ν 3324 (br m, O–H/N–H), 2932, 2836 (w, C–H), 1651 (s, C=O), 1606 (s, C=C), 1556 (vs, C=N), 1507 (s, ^{Ar}C=C), 1479 (s), 1441 (m), 1402 (s), 1338, 1303 (m), 1248 (s, O–H), 1176 (s), 1095, 1032 (s, C–O), 904 (m), 828 (s, C–H), 773, 726 (s), 701 (s, ^{Ar}C–H) cm^{-1}; ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 158.5 (C^{7a}), 158.1 (C^{12}–OCH$_3$), 155.9 (CONH$_2$), 153.5 (C^2), 144.5 (C^{13}), 142.6 (C^6), 140.9 (C$^{3''}$–NHR), 136.2 (C^4), 135.4, 135.1 (C^9), 130.8 (C$^{1''}$), 129.8, 129.7 (C^{10}), 129.1 (C$^{5''}$), 127.9 (C^{15}), 127.7 (C^{14}), 126.8 (C^{16}), 120.9 (C$^{6''}$), 118.1 (C$^{4''}$), 117.4 (C$^{2''}$), 113.3 (C^{11}), 107.6 (C^{4a}), 98.6 (C^5), 86.7 (C$^{1'}$), 86.1 (C^{8}Ar$_3$), 85.7 (C$^{4'}$), 69.0 (C$^{3'}$), 62.6 (C$^{5'}$), 55.0 (OCH$_3$), 41.5 (C$^{2'}$), 29.3 (NCH$_3$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-(3-aminophenyl)-(2,3*H*)-*N*-methyl-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one (10).
IR (solid): ν 3336 (w, O–H/N–H), 3064, 2930, 2835 (w, C–H), 1699 (w), 1651 (s, C=O), 1605 (s, C=C), 1553 (s, C=N), 1507 (s, ^{Ar}C–C), 1476 (s, N–H), 1445, 1398 (s), 1338 (m), 1301 (s), 1246 (vs), 1174 (s), 1095 (s, C–O), 1030 (vs), 969, 914 (m), 827 (s, C–H), 772, 726 (s), 701 (s, Aryl C–H) cm^{-1}; ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 158.5 (C^{7a}), 158.2 (C^{12}–OCH$_3$), 153.5 (C^2), 149.0 (C$^{3''}$–NHR), 144.5 (C^{13}), 143.4 (C^6), 135.9 (C^4), 135.5, 135.2 (C^9), 131.1 (C$^{1''}$), 129.8, 129.7 (C^{10}), 129.3 (C$^{5''}$), 128.0 (C^{15}), 127.8 (C^{14}), 126.9 (C^{16}), 115.6 (C$^{6''}$), 114.3 (C$^{4''}$), 113.3 (C^{11},C$^{2''}$), 107.7 (C^{4a}), 98.0 (C^5), 86.6 (C$^{1'}$), 86.1 (C^{8}Ar$_3$), 85.6 (C$^{4'}$), 69.0 (C$^{3'}$), 62.6 (C$^{5'}$), 55.0 (OCH$_3$), 41.5 (C$^{2'}$), 29.4 (NCH$_3$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-(3-trifluoroacetamidophenyl)-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one (11).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 158.6 (C^{7a}), 158.2 (C^{12}–OCH$_3$), 154.7 (q, $^2J_{CF}$ = 36.9 Hz, COCF$_3$), 153.4 (C^2), 144.5 (C^{13}), 141.6 (C^6), 136.7 (C^4,C$^{3''}$), 135.5, 135.2 (C^9), 131.3 (C$^{1''}$), 129.8, 129.7 (C^{10}), 129.6 (C$^{5''}$), 128.0 (C^{15}), 127.8 (C^{14}), 126.8 (C^{16}), 125.4 (C$^{6''}$), 121.3 (C$^{4''}$), 120.6 (C$^{2''}$), 115.7 (q, $^1J_{CF}$ = 286.7 Hz, COCF$_3$), 113.3 (C^{11}), 107.5 (C^{4a}), 99.3 (C^5), 86.7 (C$^{1'}$), 86.1 (C^{8}Ar$_3$), 85.7 (C$^{4'}$), 68.9 (C$^{3'}$), 62.6 (C$^{5'}$), 55.0 (OCH$_3$), 41.5 (C$^{2'}$), 29.4 (NCH$_3$).

***N,N'-Bis*-[(2-cyanoethoxy)carbonyl]-*S*-methyl-isothiourea (15).[26, 32, 33]**
^{13}C NMR (100 MHz, CDCl$_3$) δ (ppm) 173.7 (NHC^1=N), 160.4 (NC^2=O), 150.8 (NHC$^{2'}$=O), 116.8 (C^{5}N), 116.3 (C$^{5'}$N), 61.0 (C^3), 60.6 (C$^{3'}$), 18.1 (CH$_2$CN), 14.8 (SCH$_3$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-(3-{*N,N'-bis*-[(2-cyanoethoxy)carbonyl]-guanidinyl}phenyl)-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]-pyrimidine-2(7*H*)-one (16).
IR (solid): ν 3271 (br w, O–H/N–H), 2933, 2837 (w, C–H), 2255 (w, C≡N), 1732 (m, C=O), 1650 (s, C=O/C=N), 1622 (s, C=C), 1607, 1555 (s, C=O), 1507 (s, ^{Ar}C–C), 1424, 1398, 1282, 1229, 1175 (s), 1114, 1083 (s, C–O), 1057, 1029 (s), 907 (m), 827 (s, ^{Ar}C–H), 791 (s), 770 (s, ^{Ar}C–H), 727 (s, CH$_2$), 700 (s, ^{Ar}C–H) cm^{-1}; ^{13}C NMR (100 MHz, CDCl$_3$) δ (ppm) 163.5 (C^{17}=N), 159.3 (C^{7a}), 159.1, 158.0 (C^{12}–OCH$_3$), 155.3 (NC^{18}=O), 154.0 (C^2), 153.6 (NHC^{22}=O), 144.8 (C^{13}), 142.7 (C^6), 136.8 (C^4), 136.5 (C$^{3''}$–NHR), 136.1, 135.9 (C^9), 132.3 (C$^{1''}$), 130.6, 130.5 (C^{10}), 129.9 (C$^{5''}$), 128.6 (C^{15}), 128.5 (C^{14}), 127.5 (C^{16}), 126.1 (C$^{6''}$), 123.3 (C$^{2''}$), 123.2 (C$^{4''}$), 117.2 (C^{21}≡N), 116.4 (C^{25}≡N), 113.8 (C^{11}), 109.2 (C^{4a}), 100.4 (C^5), 88.2 (C$^{1'}$), 87.4 (C^{8}Ar$_3$), 86.6 (C$^{4'}$), 70.9 (C$^{3'}$), 63.0 (C$^{5'}$), 61.5 (C^{19}), 60.3 (C^{23}), 55.6 (OCH$_3$), 43.0 (C$^{2'}$), 30.4 (NCH$_3$), 18.4 (C^{20},C^{24}).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-phenyl-(2,3*H*)-*N*-methylpyrrolo-[2,3-*d*]-pyrimidine-2(7*H*)-one-3'-*O*-(2-*O*-cyanoethyl-*N,N*-diisopropyl) phosphoramidite (8a).
^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm) 8.88 and 8.79 (1H, s, H^4), 7.49-7.36 (7H, m, H^{14}, H$^{2'',3'',4''}$), 7.36 and 7.35 (2H, d, J = 8.9 Hz, and J = 9.0 Hz, H^{10}), 7.33 and 7.33 (2H, d, J = 9.0 Hz, H^{10}), 7.31-7.23 (3H, m, H^{15,16}), 6.85 and 6.84 (2H, d, J = 9.0 Hz, H^{11}), 6.83 and 6.82 (2H, d, J = 9.0 Hz, H^{11}), 6.51 and 6.47 (1H, dd, J = 4.9, 6.5 Hz and J = 3.9, 6.9 Hz, H$^{1'}$), 5.43 and 5.36 (1H, s, H^5), 4.80 and 4.73 (1H, td, J = 6.5, 16.7 Hz and J = 5.8, 15.1 Hz, H$^{3'}$), 4.25-4.15 (1H, m, H$^{4'}$), 3.81-3.69 (1H, m, CH$_2$CH$_2$CN), 3.73, 3.76 and 3.75, 3.75 (3H, s, OCH$_3$), 3.69-3.59 (1H, m, CH$_2$CH$_2$CN), 3.60-3.54 and 3.57 (1H, m and dd, J = 2.8, 11.2 Hz, H$^{5'}$), 3.59-3.46 (2H, m, CH(CH$_3$)$_2$), 3.58 (3H, s, NCH$_3$), 3.50 and 3.46 (1H, dd, J = 2.8, 10.6 Hz and J = 2.8, 10.7 Hz, H$^{5'}$), 2.85 and 2.79 (1H, td, J = 6.5, 13.0 Hz and J = 6.5, 12.8 Hz, H$^{2'}$), 2.63 and 2.43 (2H, t, J = 6.3 Hz and J = 6.5 Hz, CH$_2$CH$_2$CN), 2.54-2.36 (1H, m, H$^{2'}$), 1.18 (3H, d, J = 6.7 Hz, CH(CH$_3$)$_2$), 1.17 (6H, d, J = 6.8 Hz, CH(CH$_3$)$_2$), 1.06 (3H, d, J = 6.8 Hz, CH(CH$_3$)$_2$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-(3-acetamidophenyl)-(2,3*H*)-*N*-methyl-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one-3'-*O*-(2-*O*-cyanoethyl-*N,N*-diisopropyl) phosphoramidite (8b).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.08 (1H, s, NHCOCH$_3$), 8.68 and 8.65 (1H, s, H^4), 7.79 (1H, br s, H$^{2''}$), 7.62 (1H, br d, J = 8.0 Hz, H$^{4''}$), 7.42 (1H, t, J = 7.8 Hz, H$^{5''}$), 7.42 and 7.40 (2H, d, J = 8.0 Hz and J = 8.5 Hz, H^{14}), 7.34-7.29 (2H, m, H^{15}), 7.30 and 7.28 (4H, d, J = 9.0 Hz, H^{10}), 7.30-7.23 (1H, m, H^{16}), 7.16 (1H, br d, J = 7.5 Hz, H$^{6''}$), 6.89, 6.88 and 6.87 (2H, 2H and 4H, d, J = 9.0 Hz, J = 8.5 Hz and J = 9.0 Hz, H^{11}), 6.31 and 6.27 (1H, app. t, J = 5.8 Hz, H$^{1'}$), 5.66 and 5.63 (1H, s, H^5), 4.68 and 4.65 (1H, td, J = 5.5, 16.3 Hz and J = 6.0, 16.7 Hz, H$^{3'}$), 4.18 and 4.14 (1H, dd, J = 3.3, 7.8 Hz and J = 3.8, 8.3 Hz, H$^{4'}$), 3.82-3.69 (1H, m, CH$_2$CH$_2$CN), 3.70, 3.70 and 3.69 (3H, 3H and 6H, s, OCH$_3$), 3.70-3.60 (1H, m, CH$_2$CH$_2$CN), 3.62-3.48 (2H, m, CH(CH$_3$)$_2$), 3.47 (3H, s, NCH$_3$), 3.47 and 3.44 (1H, dd, J = 4.5, 11.0 Hz and J = 3.5, 10.5 Hz, H$^{5'}$), 3.43-3.39 and 3.38 (1H, m and dd, J = 3.0, 10.5 Hz, H$^{5'}$), 2.77 and 2.67 (2H, t, J = 6.0 Hz, CH$_2$CH$_2$CN), 2.61 and 2.59 (1H, td, J = 6.9, 13.4 Hz and J = 6.5, 13.1 Hz, H$^{2'}$), 2.35 (1H, td, J = 6.3, 13.1 Hz, H$^{2'}$), 2.08 (3H, s, COCH$_3$), 1.15 (3H, d, J = 7.0 Hz, CH(CH$_3$)$_2$), 1.13 (3H, d, J = 7.0 Hz, CH(CH$_3$)$_2$), 1.12 (3H, d, J = 7.0 Hz, CH(CH$_3$)$_2$), 1.02 (3H, d, J = 7.0 Hz, CH(CH$_3$)$_2$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-6-(3-ureidophenyl)-(2,3*H*)-*N*-methyl-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one-3'-*O*-(2-*O*-cyanoethyl-*N,N*-diisopropyl) phosphoramidite (8c).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.69 (1H, s, NHCONH$_2$), 8.66 and 8.63 (1H, s, H^4), 7.63 (1H, br s, H$^{2''}$), 7.44-7.38 (1H, m, H$^{4''}$), 7.42 and 7.40 (2H, d, J = 8.0 Hz and J = 8.5 Hz, H^{14}), 7.34 (1H, t, J = 7.9 Hz, H$^{5''}$), 7.34-7.24 (3H, m, H^{15},H^{16}), 7.30 and 7.27 (4H, d, J = 8.0 Hz and J = 8.8 Hz, H^{10}), 7.02 (1H, br d, J = 7.5 Hz, H$^{6''}$), 6.89 and 6.87 (2H, d, J = 8.3 Hz and J = 8.6 Hz, H^{11}), 6.89 and 6.87 (2H, d, J = 8.5 Hz, H^{11}), 6.31 and 6.27 (1H, app. t, J = 5.8 Hz, H$^{1'}$), 5.92 (2H, br s, NH$_2$), 5.63 and 5.59 (1H, s, H^5), 4.68 and 4.64 (1H, td, J = 5.4, 16.1 Hz and J = 5.9, 17.9 Hz, H$^{3'}$), 4.17 and 4.13 (1H, dd, J = 3.4, 7.7 Hz and J = 3.8, 8.0 Hz, H$^{4'}$), 3.81-3.68 (1H, m, CH$_2$CH$_2$CN),

3.71, 3.70 and 3.70 (3H, 3H and 6H, s, OCH_3), 3.69-3.61 (1H, m, $C\underline{H}_2CH_2CN$), 3.63-3.49 (2H, m, $C\underline{H}(CH_3)_2$), 3.46 (3H, s, NCH_3), 3.49-3.44 and 3.46-3.41 (1H, m, $H^{5'}$), 3.41 and 3.37 (1H, dd, *J* = 3.5, 11.5 Hz and *J* = 2.9, 10.8 Hz, $H^{5'}$), 2.77 and 2.67 (2H, t, *J* = 5.9 Hz, $CH_2C\underline{H}_2CN$), 2.59 (1H, td, *J* = 6.7, 13.6 Hz, $H^{2'}$), 2.34 (1H, td, *J* = 6.0, 11.9 Hz, $H^{2'}$), 1.15 (3H, d, *J* = 7.3 Hz, $CH(C\underline{H}_3)_2$), 1.13 (3H, d, *J* = 7.3 Hz, $CH(C\underline{H}_3)_2$), 1.12 (3H, d, *J* = 6.8 Hz, $CH(C\underline{H}_3)_2$), 1.02 (3H, d, *J* = 6.8 Hz, $CH(C\underline{H}_3)_2$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-*β*-D-ribofuranosyl]-6-(3-trifluoroacetamidophenyl)-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidine-2(7*H*)-one-3'-*O*-(2-*O*-cyanoethyl-*N,N*-diisopropyl) phosphoramidite (12).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.47 ($N\underline{H}COCF_3$), 8.71 and 8.68 (1H, s, H^4), 7.85 (1H, br s, $H^{2''}$), 7.75 (1H, br dd, *J* = 1.1, 8.2 Hz, $H^{4''}$), 7.54 (1H, t, *J* = 8.0 Hz, $H^{5''}$), 7.42 and 7.40 (2H, d, *J* = 8.3 Hz and *J* = 8.8 Hz, H^{14}), 7.16 (1H, br dd, *J* = 0.9, 7.9 Hz, $H^{6''}$), 7.34-7.28 (2H, m, H^{15}), 7.30 and 7.27 (4H, d, *J* = 8.5 Hz and *J* = 8.8 Hz, H^{10}), 7.28-7.23 (1H, m, H^{16}), 6.89, 6.88 and 6.87 (2H, 2H and 4H, d, *J* = 8.8 Hz, *J* = 9.0 Hz and *J* = 8.5 Hz, H^{11}), 6.30 and 6.26 (1H, dd, *J* = 5.1, 6.4 Hz and *J* = 5.0, 6.5 Hz, $H^{1'}$), 5.69 and 5.65 (1H, s, H^5), 4.68 and 4.64 (1H, td, *J* = 5.3, 10.7 Hz and *J* = 5.6, 11.2 Hz, $H^{3'}$), 4.17 and 4.13 (1H, dd, *J* = 3.5, 7.8 Hz and *J* = 3.8, 8.0 Hz, $H^{4'}$), 3.81-3.68 (1H, m, $C\underline{H}_2CH_2CN$), 3.70, 3.69 and 3.69 (3H, 3H and 6H, s, OCH_3), 3.69-3.59 (1H, m, $C\underline{H}_2CH_2CN$), 3.61-3.47 (2H, m, $C\underline{H}(CH_3)_2$), 3.49 (3H, s, NCH_3), 3.46-3.28 (2H, m, $H^{5'}$), 2.77 and 2.67 (2H, t, *J* = 5.9 Hz, $CH_2C\underline{H}_2CN$), 2.60 and 2.58 (1H, td, *J* = 6.7, 13.3 Hz and *J* = 6.5, 13.2 Hz, $H^{2'}$), 2.35 (1H, td, *J* = 6.0, 12.2 Hz, $H^{2'}$), 1.15 (3H, d, *J* = 6.8 Hz, $CH(C\underline{H}_3)_2$), 1.13 (3H, d, *J* = 6.8 Hz, $CH(C\underline{H}_3)_2$), 1.12 (3H, d, *J* = 6.8 Hz, $CH(C\underline{H}_3)_2$), 1.01 (3H, d, *J* = 6.8 Hz, $CH(C\underline{H}_3)_2$).

3-*N*-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-*β*-D-ribofuranosyl]-6-(3-{*N,N'-bis*-[(2-cyanoethoxy) carbonyl]guanidinyl}phenyl)-(2,3*H*)-*N*-methylpyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one-3'-*O*-(2-*O*-cyanoethyl-*N,N*-diisopropyl) phosphoramidite (17).
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 11.89 (1H, br s, $CON\underline{H}CN$), 10.21 (1H, s, $N\underline{H}^{Ar}$), 8.92 and 8.83 (1H, s, H^4), 7.75 (1H, t, *J* = 1.5 Hz, $H^{2''}$), 7.54 (1H, br d, *J* = 8.7 Hz, $H^{4''}$), 7.47 and 7.44 (2H, d, *J* = 7.7 Hz, and *J* = 8.1 Hz, H^{14}), 7.47-7.40 (1H, m, $H^{5''}$), 7.38-7.26 (3H, m, H^{15}, H^{16}), 7.36 and 7.33 (4H, d, *J* = 8.7 Hz, H^{10}), 7.23 (1H, br d, *J* = 7.6 Hz, $H^{6''}$), 6.85 and 6.84 (2H, d, *J* = 8.8 Hz, H^{11}), 6.83 and 6.83 (2H, d, *J* = 8.8 Hz, H^{11}), 6.50 and 6.46 (1H, dd, *J* = 5.0, 6.8 Hz and *J* = 3.8, 6.8 Hz, $H^{1'}$), 5.44 and 5.37 (1H, s, H^5), 4.85-4.67 (1H, m, $H^{3'}$), 4.49 (2H, t, *J* = 6.3 Hz, H^{24}), 4.30 (2H, t, *J* = 6.6 Hz, H^{20}), 4.23-4.15 (1H, m, $H^{4'}$), 3.90-3.76 and 3.80-3.69 (2H, m, $C\underline{H}_2CH_2CN$), 3.76, 3.75 and 3.76, 3.75 (3H, s, OCH_3), 3.70-3.53 (2H, m, $C\underline{H}(CH_3)_2$), 3.65 and 3.65 (3H, s, NCH_3), 3.59-3.47 (1H, m, $H^{5'}$), 3.56-3.44 (1H, m, $H^{5'}$), 2.87-2.70 (1H, m , $H^{2'}$), 2.84 (2H, t, *J* = 6.2 Hz, H^{23}), 2.72 (2H, t, *J* = 6.6 Hz, H^{19}), 2.63 and 2.43 (2H, t, *J* = 6.3 Hz and *J* = 6.4 Hz, $CH_2C\underline{H}_2CN$), 2.53-2.39 (1H, m, $H^{2'}$), 1.18 (3H, d, *J* = 6.6 Hz, $CH(C\underline{H}_3)_2$), 1.17 (6H, d, *J* = 6.7 Hz, $CH(C\underline{H}_3)_2$), 1.06 (3H, d, *J* = 6.8 Hz, $CH(C\underline{H}_3)_2$).

B. Oligonucleotide Synthesis, Purification, Analysis and Biophysical Studies

B.1 Oligonucleotide Synthesis and Purification

Oligonucleotides were synthesised on an ABI 394 automated DNA/RNA synthesiser using a standard solid-phase 0.2 or 1.0 μmole phosphoramidite synthesis cycle. The cycle comprises acid-catalysed (TCA) detritylation, coupling, capping (Ac_2O) and oxidation (iodine/pyridine/THF). TFOs were synthesised without the capping step. All *β*-cyanoethyl phosphoramidite monomers were dissolved in anhydrous DNA grade acetonitrile, or freshly distilled CH_2Cl_2 to a concentration of 0.1 M immediately before use. Standard A, G, C and T monomers were coupled for 30s and all other monomers for 6 mins. Stepwise coupling efficiencies and overall yields were determined by the automated trityl cation conductivity monitoring function and in all cases were >97.0%. Solid-supports were washed after synthesis with 20% diethylamine/acetonitrile for 20 mins (60 mins for ^{G}P monomer) and dried, prior to cleavage of oligonucleotide from the solid-support and deprotection (conc. aq ammonia, rt or 55 °C, 4-24 hrs). Oligonucleotides were purified by reversed-phase HPLC on a Gilson HPLC system using a Brownlee Aquapore column (C8, 8 × 250 mm, 300Å pore size). The system was controlled by Gilson 7.12 software, and oligonucleotide elution was monitored by UV absorption at 297 nm for non-modified and 310 nm for modified oligonucleotides. The following purification protocol was used: Gradient of acetonitrile in ammonium acetate buffer from 0-50% buffer B over 30 minutes (flow rate: 4 mL/min; buffer A: 0.1 M ammonium acetate, pH 7.0; buffer B: 0.1 M ammonium acetate with 50% acetonitrile, pH 7.0). After HPLC purification, oligonucleotides were desalted by using NAP-10 Sephadex columns (GE Healthcare) according to the manufacturer's instructions.

B.2 Analysis of Oligonucleotides

Oligonucleotides were analysed using electrospray MS and capillary electrophoresis (CE). Mass spectra were recorded using electrospray ionisation (ES) on a Fisons VG platform instrument in HPLC grade water, with tripropylamine to aid ionisation. Data was deconvoluted/reprocessed using the Maximum Entropy facility in MassLynx software version 2.22. CE analysis was conducted using a Beckman Coulter P/ACE™ MDQ Capillary Electrophoresis System, using the 32 Karat Software MDQ UV application, at a concentration of 4 OD/mL. An ssDNA 100-R gel with Tris-Borate-7 M Urea system was used.

B.3 UV Melting

UV melting experiments were performed on a Varian Cary 400 Scan UV-Visible Spectrophotometer in Hellma® SUPRASIL synthetic quartz cuvettes (10 mm pathlength, 1.5 mL sample volume), monitoring at 260 nm, using Cary WinUV Thermal application software. Aqueous solutions of the TFO and duplex strands were mixed in a 5:1 ratio in an Eppendorf tube and lyophilised, before being re-suspended in 1.5 mL of the correct buffer, pH 6.2, 6.6 or 7.0 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA) to afford a 5 μM:1 μM concentration of TFO:duplex. Samples were degassed for 1 minute by sonication then filtered though Kinesis regenerated cellulose syringe filters (0.45 μm, 15 mm). The samples (1.0 mL) were transferred in to cuvettes and subjected to the melt programme alongside a matched cell reference blank. The samples were equilibrated by initial denaturation by heating to 80 °C at 10 °C/min. After holding for 2 mins, the samples were annealed by cooling to 15 °C at 0.5 °C/min and held at 15 °C for 20 mins. Two melting cycles from 15–80–15 °C at 0.5 °C/min with hold time at 80 °C of 2 min and 20 min at 15 °C were carried out, followed by two melting cycles from 15–50–15 °C at 0.25

°C/min. The final anneal from 80 °C at 10 °C/min brought the samples to 20 °C. Average T_m values (Table 1) were derived from the derivatives of the melting curves for each experiment using OriginPro 7.5. Hysteresis (4.7 °C average) was observed using a temperature gradient of 0.5 °C/min. Hysteresis was also observed (3.0 °C average) when a slower temperature gradient of 0.25 °C/min was used, but all trends in melting data for each gradient were identical.

B.4 Fluorescence Melting

Fluorescence melting experiments were conducted on a Roche LightCycler® 1.5 instrument in LightCycler glass capillaries (20 µL volume) using Roche LightCycler Software Version 3.5. The LightCycler has one excitation source (488 nm) and change in fluorescence was monitored at 520 nm. For each run, master solutions (5 µM) of each oligonucleotide were made. For each experiment within that run, 4.5 µL and 45 µL of the appropriate duplex and TFO respectively were lyophilised in 100 µL eppendorf tubes then re-dissolved in 90 µL of the correct buffer, pH 6.2, 6.6, 7.0 or 7.0 with 2 mM spermine.4HCl (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA), to afford a 10:1 ratio of TFO:hairpin duplex (5:0.5 µM). Samples containing only the duplex were used as references within each run. The samples were mixed by vortex then centrifugation, and were degassed in a sonic bath for 1 minute. Aliquots of 20 µL were pipetted into the top of two or three LightCycler tubes per sample, the tubes were capped and centrifugated gently to avoid breakage, then loaded into the carousel and subjected to the desired LC melt programme. The samples were equilibrated by fast heating to 95 °C at 4.0 °C/min and held for 10 min. A stepwise cooling process (1.0 °C step, 0.2 °C/min) to 27 °C and hold at 27 °C for 60 min, annealed the samples. A subsequent stepwise heating/melt process (1.0 °C step, 0.2 °C/min) to 95 °C, from which melt T_m data was derived, and hold for 10 mins was followed by a fast cool to 30 °C (4.0 °C/min). Average T_m values (Table 2) were derived from the negative derivatives of the melting curves for each experiment, using the 'Manual T_m' feature in Roche LightCycler Software Version 3.5. Hysteresis was also observed for fluorescence melting (4.0 °C average).

B.5 Example Melting Curves and derivatives

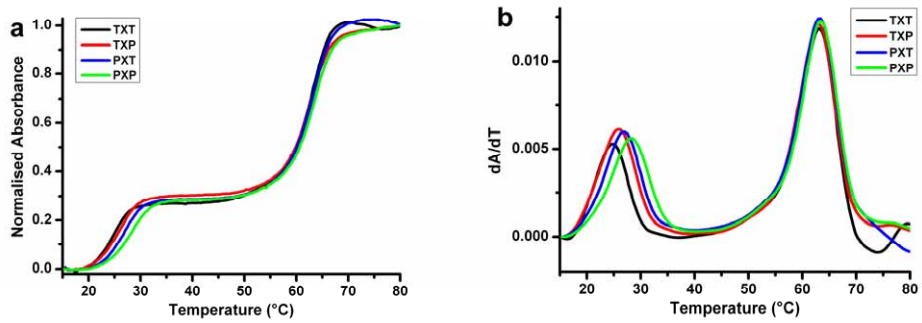

Fig. B1 UV melting curves (**a**) and derivatives (**b**). Experiment: ^{Ph}P (TFO 2,8,14,20, VXW, see ESI, Table C1) against CG at pH 6.6 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA). See Table 1, entries 8-11.

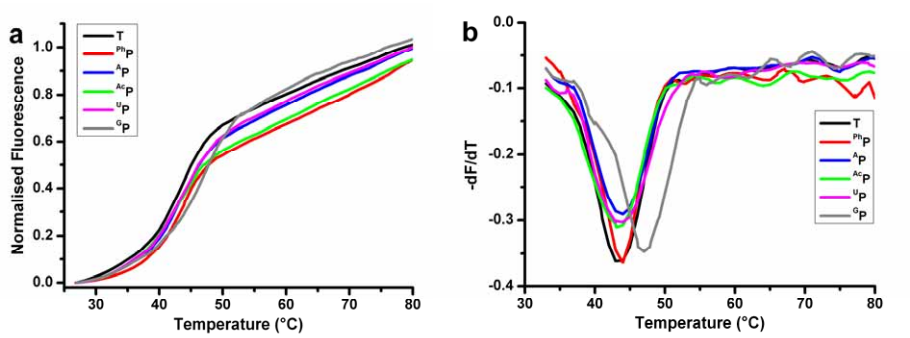

Fig. B2 Fluorescence melting curves (**a**) and negative derivatives (**b**). Experiment: ^{X}P (TFO 25-30, TXT, see ESI, Table C2) against CG at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA). See Table 2, entry 1.

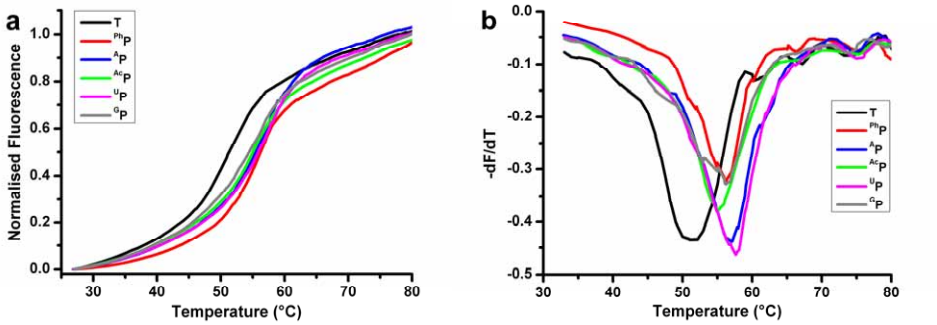

Fig. B3 Fluorescence melting curves (**a**) and negative derivatives (**b**). Experiment: ^{X}P (TFO 43-48, PXP, see ESI, Table C2) against CG at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA). See Table 2, entry 7.

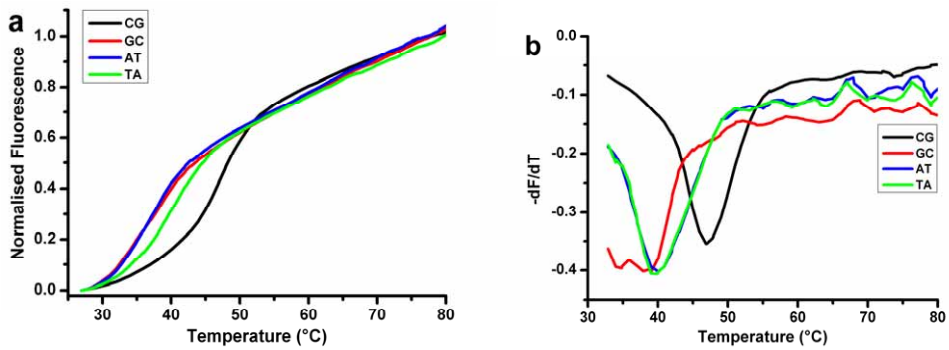

Fig. B4 Fluorescence melting curves (**a**) and derivatives (**b**). Experiment: ^{G}P (TFO 30, TXT, Table C2) against YZ at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA). See Table 2, entries 1-4.

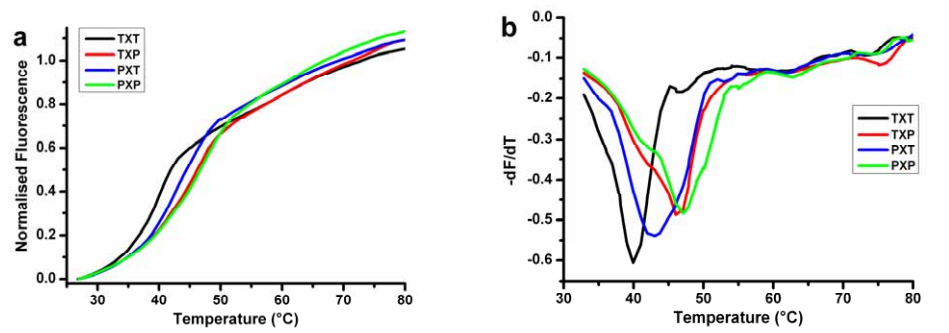

Fig. B5 Fluorescence melting curves (**a**) and derivatives (**b**). Experiment: ^{G}P (TFO 6,12,18,24, VXW, Table C2) against CG at pH 6.6 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA). See Table 2, entries 11,15-17.

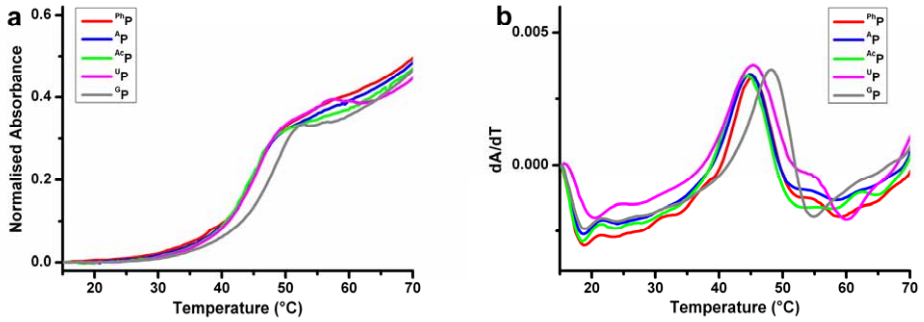

Fig. B6 UV melting curves (**a**) and derivatives (**b**) of fluorescence melting triplex motif. Experiment: ^{X}P (TFO 25-30, TXT, see ESI, Table C2) against CG at pH 6.2 (10 mM sodium phosphate, 200 mM NaCl, 1 mM Na_2EDTA). See Table 3. Compare with Fig. B2.

C. Oligonucleotides

Table C1. Oligonucleotides used in UV melting studies

UV Melting	
*5′-TTTTTM**VXW**MTMTMT* 5′-GCTAAAAAGA**Y**AGAGAGATCG 3′-CGATCTCTCT**Z**TCTTTTTAGC	**YZ** = CG, GC, AT, TA Intermolecular duplexes where Y = base in purine strand and Z = base in pyrimidine strand. The general TFO sequence is shown in italics opposite the target site (underlined) (V = T or P, W = T or P, X = T, ^{Ph}P, ^{A}P, ^{Ac}P, ^{U}P, ^{G}P). M = 5-methyl-2'-deoxycytidine, P = 5-(3-aminoprop-1-ynyl)-2'-deoxyuridine, pdU.
TTTTTMT**X**TMTMTMT	**TFO-1**: **X** = T; **TFO-2**: **X** = ^{Ph}P; **TFO-3**: **X** = ^{A}P; **TFO-4**: **X** = ^{Ac}P; **TFO-5**: **X** = ^{U}P; **TFO-6**: **X** = ^{G}P.
TTTTTMT**X**PMTMTMT	**TFO-7**: **X** = T; **TFO-8**: **X** = ^{Ph}P; **TFO-9**: **X** = ^{A}P; **TFO-10**: **X** = ^{Ac}P; **TFO-11**: **X** = ^{U}P; **TFO-12**: **X** = ^{G}P.
TTTTTMP**X**TMTMTMT	**TFO-13**: **X** = T; **TFO-14**: **X** = ^{Ph}P; **TFO-15**: **X** = ^{A}P; **TFO-16**: **X** = ^{Ac}P; **TFO-17**: **X** = ^{U}P; **TFO-18**: **X** = ^{G}P.
TTTTTMP**X**PMTMTMT	**TFO-19**: **X** = T; **TFO-20**: **X** = ^{Ph}P; **TFO-21**: **X** = ^{A}P; **TFO-22**: **X** = ^{Ac}P; **TFO-23**: **X** = ^{U}P; **TFO-24**: **X** = ^{G}P.

Table C2. Oligonucleotides used in fluorescence melting studies

Fluorescence Melting	
*5′-**Q**-PMMTPM**VXW**TPTPTMPT* 5′-**F**-GTGTTAGGAAGA**Y**AAAAAAGAACTGGT**H** CACAATCCTTCT**Z**TTTTTTCTTGACCA**H**	**YZ** = CG, GC, AT, TA Intramolecular hairpin duplexes where Y = base in homopurine tract strand and Z = base in homo-pyrimidine tract, **Q** = DABCYL (quencher), **F** = FAM (fluorophore), **H** = HEG (hexaethylene glycol spacer). The general TFO sequence is shown in italics opposite the target site (underlined) (V, W = T or P, X = T, ^{Ph}P, ^{A}P, ^{Ac}P, ^{U}P, ^{G}P). M = 5-methyl-2'-deoxycytidine, P = 5-(3-aminoprop-1-ynyl)-2'-deoxyuridine, pdU.
Q-PMMTPMT**X**TTPTPTMPT	**TFO-25**: **X** = T; **TFO-26**: **X** = ^{Ph}P; **TFO-27**: **X** = ^{A}P; **TFO-28**: **X** = ^{Ac}P; **TFO-29**: **X** = ^{U}P; **TFO-30**: **X** = ^{G}P.
Q-PMMTPMT**X**PTPTPTMPT	**TFO-31**: **X** = T; **TFO-32**: **X** = ^{Ph}P; **TFO-33**: **X** = ^{A}P; **TFO-34**: **X** = ^{Ac}P; **TFO-35**: **X** = ^{U}P; **TFO-36**: **X** = ^{G}P.
Q-PMMTPMP**X**TTPTPTMPT	**TFO-37**: **X** = T; **TFO-38**: **X** = ^{Ph}P; **TFO-39**: **X** = ^{A}P; **TFO-40**: **X** = ^{Ac}P; **TFO-41**: **X** = ^{U}P; **TFO-42**: **X** = ^{G}P.
Q-PMMTPMP**X**PTPTPTMPT	**TFO-43**: **X** = T; **TFO-44**: **X** = ^{Ph}P; **TFO-45**: **X** = ^{A}P; **TFO-46**: **X** = ^{Ac}P; **TFO-47**: **X** = ^{U}P; **TFO-48**: **X** = ^{G}P.

Table C3. Triplex-forming Oligonucleotide ESMS Data (UV Melting)

Code	Sequence	Expected Mass	Observed Mass
TFO-1	TTT TTM T**T**T MTM TMT	4496.99	4499.2
TFO-2	TTT TTM T^{Ph}**P**T MTM TMT	4596.13	4600.2
TFO-3	TTT TTM T^{A}**P**T MTM TMT	4611.14	4611.9
TFO-4	TTT TTM T^{Ac}**P**T MTM TMT	4653.18	4653.8
TFO-5	TTT TTM T^{U}**P**T MTM TMT	4654.17	4655.3
TFO-6	TTT TTM T^{G}**P**T MTM TMT	4653.18	4653.9
TFO-7	TTT TTM T**T**P MTM TMT	4536.03	4541.0
TFO-8	TTT TTM T^{Ph}**P**P MTM TMT	4635.16	4638.6
TFO-9	TTT TTM T^{A}**P**P MTM TMT	4650.18	4652.6
TFO-10	TTT TTM T^{Ac}**P**P MTM TMT	4692.21	4696.4
TFO-11	TTT TTM T^{U}**P**P MTM TMT	4693.20	4696.6
TFO-12	TTT TTM T^{G}**P**P MTM TMT	4692.22	4694.4
TFO-13	TTT TTM P**T**T MTM TMT	4536.03	4537.4
TFO-14	TTT TTM P^{Ph}**P**T MTM TMT	4635.16	4639.4
TFO-15	TTT TTM P^{A}**P**T MTM TMT	4650.18	4651.8
TFO-16	TTT TTM P^{Ac}**P**T MTM TMT	4692.21	4693.2
TFO-17	TTT TTM P^{U}**P**T MTM TMT	4693.20	4697.0
TFO-18	TTT TTM P^{G}**P**T MTM TMT	4692.22	4694.1
TFO-19	TTT TTM P**T**P MTM TMT	4575.07	4578.0
TFO-20	TTT TTM P^{Ph}**P**P MTM TMT	4674.20	4677.5
TFO-21	TTT TTM P^{A}**P**P MTM TMT	4689.21	4690.0
TFO-22	TTT TTM P^{Ac}**P**P MTM TMT	4731.25	4733.1
TFO-23	TTT TTM P^{U}**P**P MTM TMT	4732.24	4734.1
TFO-24	TTT TTM P^{G}**P**P MTM TMT	4731.25	4732.2

Table C4. Triplex-forming Oligonucleotide ESMS Data (Fluorescence Melting)

Code	Sequence	Expected Mass	Observed Mass
TFO-25	**Q** PMM TPM T**T**T TPT PTM PT	5731.00	5730.4
TFO-26	**Q** PMM TPM T^{Ph}**P**T TPT PTM PT	5830.13	5831.5
TFO-27	**Q** PMM TPM T^{A}**P**T TPT PTM PT	5845.17	5849.2
TFO-28	**Q** PMM TPM T^{Ac}**P**T TPT PTM PT	5887.04	5889.1
TFO-29	**Q** PMM TPM T^{U}**P**T TPT PTM PT	5888.07	5890.5
TFO-30	**Q** PMM TPM T^{G}**P**T TPT PTM PT	5887.21	5887.8
TFO-31	**Q** PMM TPM T**T**P TPT PTM PT	5770.03	5770.5
TFO-32	**Q** PMM TPM T^{Ph}**P**P TPT PTM PT	5869.17	5870.5
TFO-33	**Q** PMM TPM T^{A}**P**P TPT PTM PT	5884.21	5886.0
TFO-34	**Q** PMM TPM T^{Ac}**P**P TPT PTM PT	5926.11	5929.9
TFO-35	**Q** PMM TPM T^{U}**P**P TPT PTM PT	5927.10	5928.8
TFO-36	**Q** PMM TPM T^{G}**P**P TPT PTM PT	5926.25	5926.1
TFO-37	**Q** PMM TPM P**T**T TPT PTM PT	5770.03	5771.0
TFO-38	**Q** PMM TPM P^{Ph}**P**T TPT PTM PT	5869.17	5870.6
TFO-39	**Q** PMM TPM P^{A}**P**T TPT PTM PT	5884.21	5884.8
TFO-40	**Q** PMM TPM P^{Ac}**P**T TPT PTM PT	5926.11	5929.6
TFO-41	**Q** PMM TPM P^{U}**P**T TPT PTM PT	5927.10	5929.3
TFO-42	**Q** PMM TPM P^{G}**P**T TPT PTM PT	5926.25	5928.6
TFO-43	**Q** PMM TPM P**T**P TPT PTM PT	5809.07	5809.7
TFO-44	**Q** PMM TPM P^{Ph}**P**P TPT PTM PT	5908.20	5910.0
TFO-45	**Q** PMM TPM P^{A}**P**P TPT PTM PT	5923.24	5924.2
TFO-46	**Q** PMM TPM P^{Ac}**P**P TPT PTM PT	5965.14	5966.1
TFO-47	**Q** PMM TPM P^{U}**P**P TPT PTM PT	5966.13	5968.3
TFO-48	**Q** PMM TPM P^{G}**P**P TPT PTM PT	5965.28	5967.5