7,8-Dihydropyrido[2,3-*d*]pyrimidin-2-one; a bicyclic cytosine analogue capable of enhanced stabilisation of DNA duplexes.

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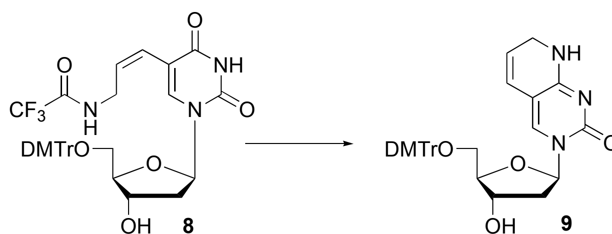
Supplementary Information

Chemical Synthesis

All chemicals were purchased from Aldrich apart from 5-iodo-2'-deoxyuridine purchased from Avocado. *N, N*-Dimethylformamide was obtained as an anhydrous solvent from Aldrich. Other solvents were obtained from Fisher Scientific and used without distillation. Silica gel for column chromatography was obtained from Fluorochem. Thin layer chromatography was carried out on pre-coated Merck Kieselgel 60 F_{254} aluminum backed plates. ^{1}H NMR (250.134MHz) and ^{31}P NMR (101.256 MHz) were run on Bruker AC250 and all chemical shifts are quoted in p.p.m. relative to tetramethylsilane (^{1}H) or 85% phosphoric acid (^{31}P) as an external standard, respectively. All mass spectroscopy were performed by The University of Sheffield Mass Spectrometry Service. Compounds **7** and **8** were synthesised as described previously (Lee *et al., Tetrahedron Lett.,* 2000, **41**, 267 and Brazier *et al. Nucleic Acids Res.,* 2005, **33**, 1362, respectively).



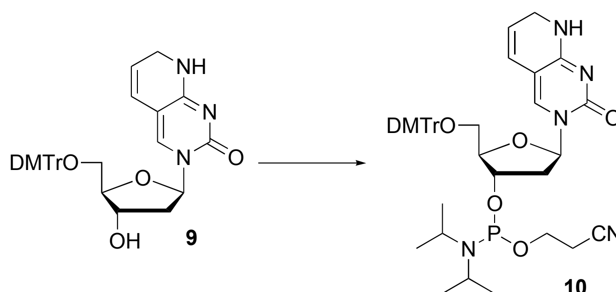
3-(2-Deoxy-5-O-(4,4'-dimethoxytrityl)-ß-D-*erythro*-pentofuranosyl)-7,8-dihydropyrido[2,3-*d*]pyrimidin-2(*3H*)-one (9) To a solution of **8** (1.7 g, 2.49 mmol) in MeOH (20 mL) was added concentrated aqueous ammonia (20 mL) and the reaction mixture stirred overnight at room temp. The solution was then evaporated and the residue purified by silica column chromatography (7 % MeOH in $CHCl_3$ containing and 0.5 % Et_3N) to give a pale yellow foam (1.34 g, 95 %).

R_f (10 % MeOH in DCM) = 0.28

^{1}H NMR δ ($CDCl_3$) 8.24 (1H, bs, N*H*), 7.51 (1H, s, H6), 7.42-7.22 (9H, m, DMTr), 6.85-6.81 (4H, m, DMTr), 6.39 (1H, t, *J*=6.4 Hz, H1'), 5.28 (2H, bs, -C*H*=C*H*-CH_2-), 4.55-4.49 (1H, m, H3'), 4.30 (2H,

bs, -CH=CH-CH_2-), 4.08-4.04 (1H, m, H4'), 3.78 (6H, s, MeO-), 3.45 (1H, dd, *J*=3.4, 10.7 Hz, H5'), 3.33 (1H, dd, *J*=3.4, 10.7 Hz, H5'), 2.60-2.51 (1H, m, H2'), 2.28-2.17 (1H, m, H2')

ES^+MS (m/z) = 590.2260 ($[M+Na]^+$, calc. for $C_{33}H_{33}N_3O_6Na$=590.2267).



3-(2-Deoxy-5-O-(4,4'-dimethoxytrityl)-ß-D-*erythro*-pentofuranosyl)-7,8-dihydropyrido[2,3-*d*]pyrimidin-2(*3H*)-one 3'-[2-cyanoethyl-*N,N'*-diisopropyl)-phosphoramidite] (**10**) To a solution of **9** (1.2 g, 2.11 mmol) in anhydrous dichloromethane (20 mL) was added diisopropylethylamine (3.7 mL, 21.2 mmol) under Ar and the mixture cooled in an ice bath. 2-Cyanoethyl-*N,N'*-diisopropyl chlorophosphoramidite (950 µl, 4.26 mmol) was then added dropwise and the reaction stirred for 2 h at 0 °C. MeOH was added to quench the reaction and the solvent was removed under reduced pressure. The residue was purified by silica column chromatography ($CHCl_3$/EtOAc/Et_3N= 45/45/10) to give a white foam (1.0 g, 62 %).

R_f (5 % MeOH in DCM) = 0.47

^1H NMR δ ($CDCl_3$) = 8.57 (1H, bs, N*H*), 7.56 (0.6H, s, H6), 7.49 (0.4H, s, H6), 7.43-7.25 (9H, m, DMTr), 6.86-6.81 (4H, m, DMTr), 6.42-6.33 (1H, m, H1'), 5.33-5.13 (2H, m, -C*H*=C*H*-CH_2-), 4.68-4.54 (1H, m, H3'), 4.33 (2H, bs, -CH=CH-CH_2-), 4.13-4.07 (1H, m, H4'), 3.85-3.69 (2H, m, -OC$H_2$$CH_2$CN), 3.80 (3H, s, MeO-), 3.79 (3H, s, MeO-), 3.64-3.45 (3H, m, -OCH_2CH_2CN + H5'), 3.33-3.26 (1H, m, H5'), 2.63-2.46 (1H, m, -NC*H*Me_2), 2.61 (1H, t, *J*=6.4 Hz, H2'), 2.38 (1H, t, *J*=6.4 Hz, H2'), 2.31-2.18 (1H, m, -NC*H*Me_2), 1.16-1.13 (12H, m, -NCHMe_2)

^{31}P NMR δ($CDCl_3$) 149.39, 148.72

ES^+MS (m/z) = 768.3544 ($[M+H]^+$, calc. for $C_{42}H_{51}N_5O_7P$=768.3526)

DNA synthesis

All DNA oligomers used in this study were synthesised on Applied Biosystems 394 automated synthesiser. DNA phosphoramidites were purchased from Proligo. Other reagents used in the DNA

synthesis including solid support columns were purchased from Glen Research. Oligonucleotide syntheses were performed DMT-ON using 0.1M solutions of normal phosphoramidites and 0.15M
65 solutions of modified phosphoramidites. Syntheses of natural oligonucleotides were conducted using standard phosphoramidites (Bz-dA, iBu-dG, Bz-dC and dT). Syntheses of olgonucleotides containing modified dU analogues were conducted using base-labile phosphoramidites (Tac-dA, Tac-dG, Tac-dC and dT) with base-labile solid support column (Ac-dC-CPG). Natural oligonucleotides were deprotected at 65°C overnight whilst those containing modified dU analogues and *N4*-acetyl-dC were
70 deprotected at room temp. overnight. All oligonucleotides were purified using reversed phase HPLC (Alltech, ODS C18, 300 x 4.6 mm) using a flow rate of 1 mL/ min with a gradient of 5-50% CH_3CN in 0.1M triethylammonium acetate, pH 7.0 over 30 min. Purified oligonucleotides were then detritylated (20% AcOH, room temp., 1 h, followed by evaporation) then repurified by reversed phase HPLC as above. All oligonucleotides were characterised by MALDI-TOF mass spectra.

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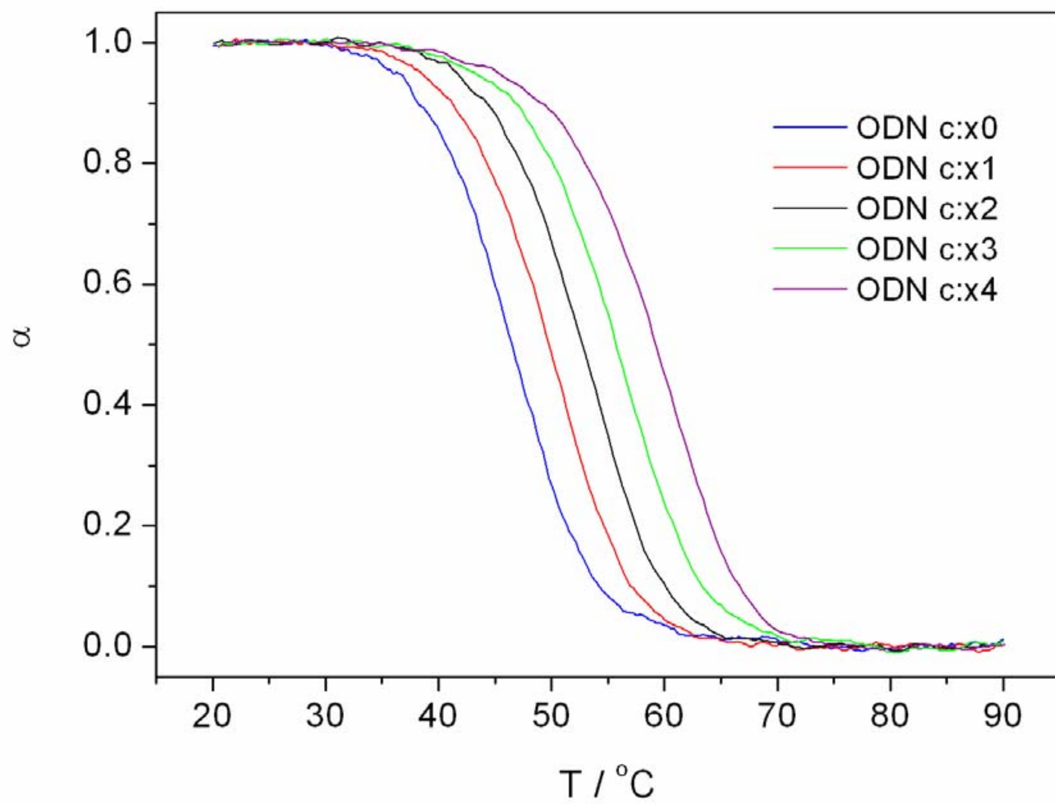
UV melting experiments

UV melting experiments were carried out on a Cary 500 UV / Vis spectrometer with a Cary temperature controller attached. UV melting profiles were obtained using 1 μM of each oligonucleotide strand dissolved in 1 mL of buffer containing 300 mM of sodium chloride, 10 mM of
80 sodium cacodylate and 0.1 mM of Na_2EDTA, pH 7.0. Samples were annealed by heating to 90ºC and cooling to 10ºC at a rate of 1ºC / min. The UV melting curves were measured at 260 nm (in duplicate) with heating the sample from 10ºC – 90ºC at a rate of 1ºC / min.



85 **Supplementary Figure 1**: Fraction of folded duplex α as a function of temperature for ODN c:x0 (blue), ODN c:x1 (red), ODN c:x2 (black), ODN c:x3 (green) and ODN c:x4 (purple).