Supporting information

DNA triplex formation with 5-dimethylaminopropargyl deoxyuridine

David A. Rusling[1], Guomei Peng[2], Natarajan Srinivasan[2], Keith R. Fox[1]* and Tom Brown[2]

[1]*School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, UK*

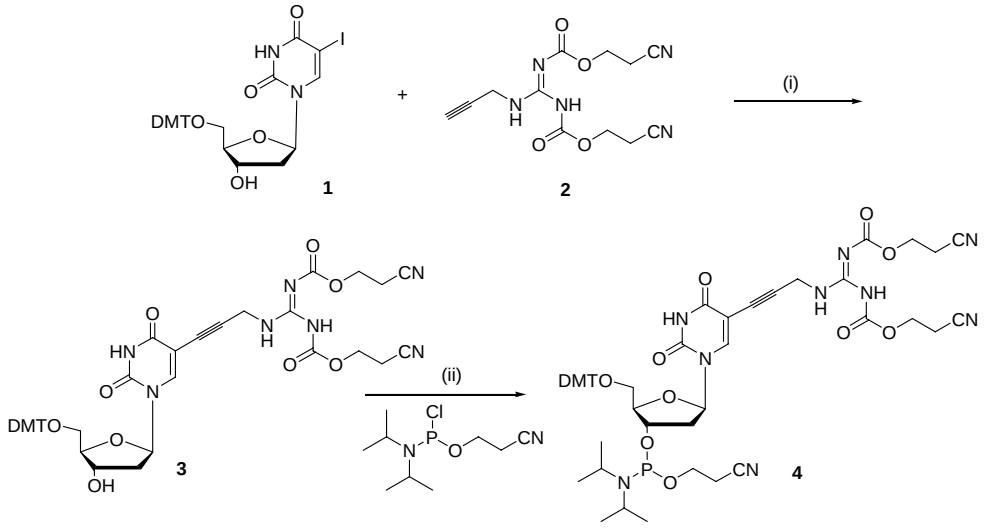
[2]*School of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, UK*

*To whom correspondence should be addressed
Tel: +44 23 8059 4374; Fax: +44 23 8059 4459; Email: k.r.fox@soton.ac.uk

Experimental

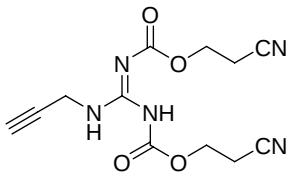
All reagents were purchased from Aldrich, Avocado, Fluka or Link Technologies and used without purification with the exception of the following solvents, which were purified by distillation: THF (over sodium wire and benzophenone), DCM, DIPEA and pyridine (over calcium hydride). All reactions were carried out under an argon atmosphere using oven-dried glassware with purified and distilled solvents. Column chromatography was carried out under argon pressure using Fisher scientific DAVISIL 60A (35-70 micron) silica. Thin layer chromatography was performed using Merck Kieselgel 60 F24 plates (0.22 mm thickness, aluminum backed). ^{1}H NMR spectra were measured at 300 MHz on a Bruker AC300 spectrometer and ^{13}C NMR spectra were measured at 75 MHz. Chemical shifts are given in ppm relative to tetramethylsilane, and *J* values are given in Hz and are correct to within 0.5 Hz. All spectra were internally referenced to the appropriate residual undeuterated solvent signal. Multiplicities of ^{13}C signals were determined using DEPT spectral editing technique. ^{31}P NMR spectra were recorded on a Bruker AV300 spectrometer at 121 MHz and were externally referenced to 85% phosphoric acid in deuterated water. Low-resolution mass spectra were recorded using the electrospray technique on a Fisons VG platform instrument or a Waters ZMD quadrupole mass spectrometer in acetonitrile (HPLC grade). High-resolution mass spectra were recorded in acetonitrile, methanol or water (HPLC grade) using the electrospray technique on a Bruker APEX III FT-ICR mass spectrometer.

Synthesis of 5-guanidylpropargyl-dU phosphoramidite monomer



SCHEME 1: (i) Et_3N/CuI/$(Ph_3P)_4Pd^0$, DMF, rt, 5 h, 75 %; (ii) DIPEA, DCM, rt, 1 h, 83 %.
5'-DMTr 5-iodo dU was synthesized by literature methods.[1]

(*N*,*N*'-bis-cyanoethyloxycarbonylguanidyl)propargylamine (2)



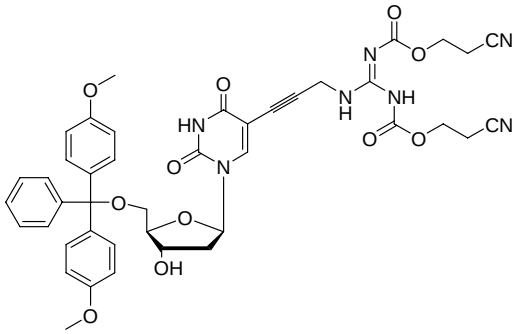
To a solution of propargylamine (0.39 ml, 5.72 mmol) in DCM (10 ml) was added *N*,*N*'-bis-cyanoethyloxycarbonyl-2-methyl-2-thiopseudourea[2] (1.79 g, 6.29 mmol) and diisopropylethylamine (1.2 ml, 6.86 mmol). The reaction mixture was stirred for 2 h before being concentrated *in vacuo*. The crude product was purified by flash column chromatography eluting with a gradient of ethyl acetate in DCM (0-5 %) to give the title compound as colorless solid, which was dried in a vacuum desiccator for 24 h (0.84 g, 51 %).

LRMS(ES^+) m/z: 291.2. $C_{12}H_{13}N_5O_{11}$ requires: 291.10

^{1}HNMR (300 MHz, DMSO-d_6): 11.40 (br s, 1H, NH), 8.56 (br s, 1H, NH), 4.39 (t, 2H, *J* = 6.3 Hz, OCH_2), 4.35 (t, 2H, *J* = 6.6 Hz, OCH_2), 4.12 (dd, 2H, *J* = 4.8, 2.5 Hz, CH_2N), 3.15 (t, 1H, *J* = 2.7 Hz, alkynyl H), 2.96 (t, 2H, *J* = 6.3 Hz, CH_2CN), 2.90 (t, 2H, *J* = 6.3 Hz, CH_2CN).

^{13}CNMR (75 MHz, DMSO-d_6): 162.2, 154.7, 151.9, 118.8, 118.2, 80.1, 73.6, 61.1, 60.1, 30.3, 17.5, 17.4.

5'-*O*-(4,4''-Dimethoxytrityl)-5-[3-*N*-(*N*,*N*'-bis-cyanoethyloxycarbonylguanidylpropynyl)]-2'-deoxyuridine (3)



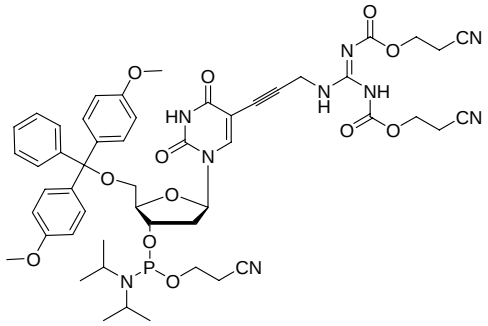
To a solution of 5'-*O*-(4,4'-dimethoxytrityl)-5-iodo-2'-deoxyuridine (**1**) [1] (0.96 g, 1.46 mmol) in anhydrous DMF (10 ml) was added (*N*,*N*'-bis-cyanoethoxycarbonyl-guanidyl)propargylamine (**2**) (0.85 g, 2.93 mmol), anhydrous triethylamine (0.3 ml, 2.19 mmol), copper (I) iodide (0.11 g, 0.58 mmol) and *tetrakis*(triphenylphosphine)$Pd^{(0)}$ (0.34 g, 0.29 mmol). The reaction mixture was covered with aluminium foil and stirred under an argon atmosphere for 5 h before being concentrated *in vacuo*. The residue was purified by silica flash column chromatography (pre-equilibrated with 1% pyridine) eluting with methanol in DCM (0-5 %) to yield the title compound as yellow powder, which was dried in a vacuum desiccator for 24 h (0.9 g, 75 %).

LRMS(ES^+) m/z: 819.5. $C_{42}H_{41}N_7O_{11}$ requires: 819.3

^{1}HNMR (300 MHz, DMSO-d_6): 11.60 (br s, 1H, NH), 11.46 (br s, 1H, NH), 8.50 (br s, 1H, NH), 7.95 (s, 1H, H-6), 7.20-7.42 (m, 9H, Ar-H), 6.90 (d, 4H, J = 9.0 Hz, Ar-H), 6.10 (t, 1H, J = 6.6 Hz, H-1'), 5.32 (d, 1H, J = 4.5 Hz, OH), 4.34 (m, 2H, $C\underline{H}_2NH$), 4.28 (m, 1H, H-3'), 4.19 (m, 4H, CH_2O), 3.92 (m, 1H, H-4'), 3.74 (s, 6H, OCH_3), 3.10-3.28 (m, 2H, H-5'), 2.80-3.10 (m, 4H, CH_2CN), 2.18-2.28 (m, 2H, H-2')

^{13}CNMR(75MHz, DMSO-d_6): 162.2, 161.5, 158.0, 154.5, 152.0, 149.3, 144.7, 143.7, 135.6, 135.3, 129.7, 127.9, 127.6, 126.7, 118.7, 118.2, 113.2, 97.9, 88.1, 85.8, 85.1, 74.8, 70.4, 63.7, 61.2, 60.1, 55.0, 54.9, 39.8, 31.0, 17.5, 17.4

5'-*O*-(4,4''-Dimethoxytrityl)-5-[3-*N*-(*N*,*N*'-bis-cyanoethoxycarbonyl-guanidylpropynyl)]-2'-deoxyuridine 3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (4)



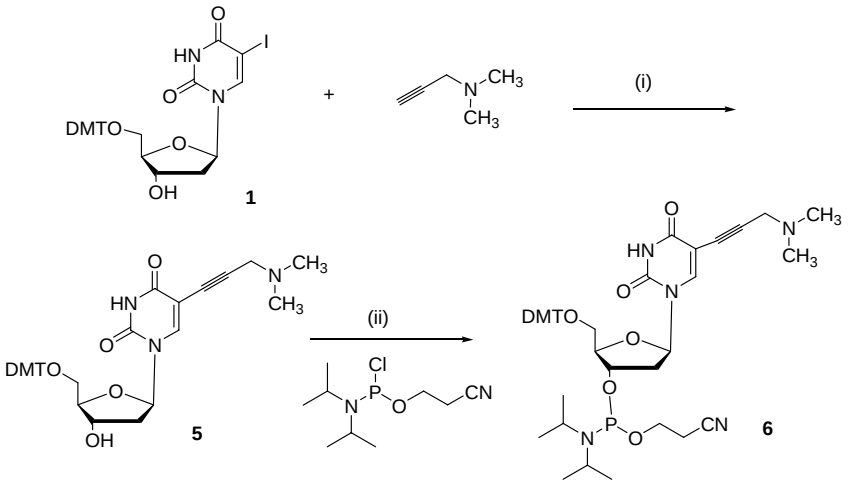
To a solution of 5-(*N*,*N*'-bis-cyanoethoxycarbonylguanidylpropargyl)-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyuridine (**3**) (0.85 g, 1.03 mmol) in dry THF (5 ml) was added diisopropylethylamine (0.45 ml, 2.59 mmol) and 2-cyanoethyl *N'*,*N*-diisopropyl chlorophosphoramidite (0.28 ml, 1.29 mmol). This was stirred for 1 h at room temperature, then DCM (30 ml) was added and the solution was extracted with saturated aqueous KCl (50 ml), dried over Na_2SO_4 and the solvent removed *in vacuo* under an argon atmosphere. The residue was purified by silica flash column chromatography (pre-equilibrated with 1 % pyridine) eluting with ethyl acetate in hexane (4:1) to yield the product as white foam (0.88 g, 83 %).

LRMS(ES^+) m/z: 1019.8. $C_{51}H_{58}N_9O_{12}P$ requires: 1019.4

^{1}HNMR (300 MHz, DMSO-d_6): 11.50 (br s, 2H, NH), 8.50 (m, 1H, NH), 8.03 (s, 1H, H-6), 7.18-7.47 (m, 9H, Ar-H), 6.93 (m, 4H, ArH), 6.14 (m, 1H, H-1'), 4.55 (m, 1H, H-3'), 4.35 (m, 2H, C$\underline{H}_2$NH), 4.21 (m, 4H, CH_2O), 4.13 (m, 1H, H-4'), 3.79 (s, 6H, OCH_3), 3.60 (m, 2H, C$\underline{H}CH_3$), 3.50 (m, 2H, CH_2OP), 3.18-3.36 (m, 2H, H-5'), 2.70-2.90 (m, 4H, CH_2CN), 2.69-2.80 (m, 2H, CH_2CN), 2.40-2.53 (m, 2H, H-2'), 1.03-1.25 (m, 12H, isopropyl-CH_3).

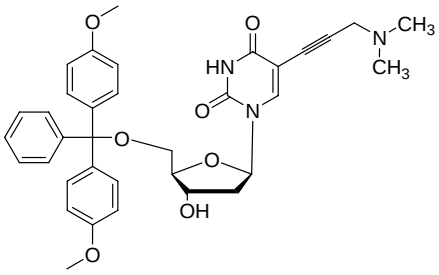
^{31}P NMR (DMSO-d_6): 148.9, 148.5.

Synthesis of 5-dimethylaminopropargyl-dU



SCHEME 2: (i) (i) $Et_3N/CuI/(Ph_3P)_4Pd^0$, DMF, rt, 5 h, 53 %; (ii) DIPEA, DCM, rt, 2 h, 74 %.

5'-O-(4,4''-Dimethoxytrityl)-3-(dimethylaminoprop-1-ynyl)-2'-deoxyuridine (5)



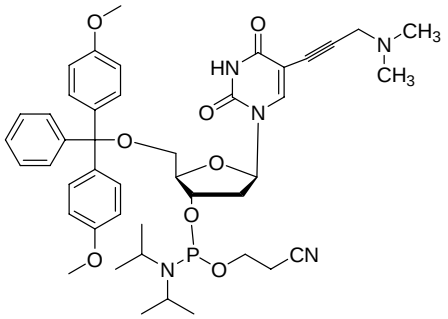
5'-O-(4,4''-Dimethoxytrityl)-5-iodo-2'-deoxyuridine (**1**) [1] (2.0 g, 3.05 mmol) was dissolved in DMF (20 ml) and 3-dimethylamino-1-propyne (0.627 ml, 9.15 mmol), triethylamine (2.58 ml, 18.3 mmol) and copper(I) iodide (0.234 g, 40 mol%) were added. The reaction flask was covered with aluminium foil and stirred at room temperature for 15 min before adding *tetrakis*(triphenylphosphine)Pd^0 (0.34 g, 10 mol%). The reaction was left stirring at room temperature for 5 h then concentrated *in vacuo* to remove DMF. The crude reaction mixture was diluted with ethyl acetate (50 mL) followed by extraction with EDTA sodium salt (5% w/v in H_2O, 50 ml), saturated aqueous KCl and water (30 ml), dried over Na_2SO_4 and evaporated to give a yellow foam (2.2 g). The crude product was purified by silica column chromatography eluting initially with 2 % MeOH in DCM containing 1 % Et_3N followed by 3 % MeOH in DCM containing 1 % Et_3N to give the pure product as a cream coloured solid (0.989 g, 53 %).

LRMS(ES^+) m/z: 611.0. $C_{35}H_{37}N_3O_7$ requires: 611.3

^{1}HNMR (400 MHz, DMSO-d_6): 7.92 (s, 1H, H-6), 7.37 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.29-7.21 (m, 6H, Ar-H), 7.15 (t, 1H, *J* = 7.1 Hz, Ar-H), 6.78 (d, 4H, *J* = 8.8 Hz, Ar-H), 6.25 (t, 1H, *J* = 6.6 Hz, H-1'), 4.43 (br t, 1H, *J* =2.9 Hz, H-3'), 4.00 (d, 1H, *J* = 3.1 Hz, H-4'), 3.73 (s, 6H, OCH_3), 3.35 (dd, 1H, *J* = 10.5 Hz, 3.2 Hz, H-5'), 3.27 (dd, 1H, *J* = 3.7 Hz, 10.5 Hz, H-5'), 3.15 (s, 2H, CH_2NMe_2), 2.41 (ddd, 1H, *J* = 13.4 Hz, 5.7 Hz, 2.9 Hz, H-2'), 2.19 (dt, 1H, *J* = 13.7 Hz, 6.8 Hz, H-2'), 2.04 (s, 6H, $N(CH_3)_2$).

^{13}CNMR(100MHz, DMSO-d_6): 162.3, 159.0, 150.0, 142.3, 136.0, 130.3, 128.3, 128.3, 127.3, 113.6, 100.6, 89.4 , 87.2, 86.6, 85.8, 72.2, 63.9, 55.5, 48.6 , 44.1, 41.7.

5'-O-(4,4''-Dimethoxytrityl)-3-(dimethylaminoprop-1-ynyl)-2'-deoxyuridine-3´-*O*-(2-cyanoethyl-*N*,*N*-diisosopropyl)phosphoramidite (6)



5'-O-(4,4''-Dimethoxytrityl)-3-(dimethylaminoprop-1-ynyl)-2'-deoxyuridine (**5**) (0.5 g, 0.817 mmol) was dissolved in dry THF (15 ml) and degassed using argon for 3 min. DIPEA (0.355 ml, 2.04 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.200 ml, 0.89 mmol) were added and the reaction was stirred for 2 h under argon. The solvent was removed by evaporation *in vacuo* and the residue was partitioning between degassed ethyl acetate (20 ml) and degassed KCl solution (10 ml). The organic layer was dried over Na_2SO_4 and evaporated to give a pale yellow foam. Purification by silica column chromatography (70 % acetone in DCM containing 0.5 % Et_3N) gave a white foam (480 mg, 74 %).

LRMS(ES^+) m/z: 812.0. $C_{44}H_{54}N_5O_8P$ requires: 811.9

^{31}P NMR (DMSO-d_6): 148.2, 148.6

Oligonucleotide synthesis

All oligonucleotides were synthesised on an Applied Biosystems 394 automated DNA/RNA synthesiser using a standard 1.0 μmole phosphoramidite cycle of acid-catalysed detritylation, coupling, capping and iodine oxidation. The 5-aminopropargyl dU monomer was made by the method of Bijapur *et. al.*[1] and 5-propynyl dU phosphoramidite was purchased from Glen Research. Standard A, G, C and T monomers were coupled for 30 s and all other monomers were coupled for 6 min. Stepwise coupling efficiencies were determined by the automated trityl cation conductivity monitoring facility and found to be > 98.0 %. After oligonucleotide assembly the columns were treated with 20% diethylamine in acetonitrile for 20 min to remove cyanoethyl groups from the phosphotriester moieties and prevent addition of acrylonitrile to the N(3)-position of the modified uracil bases. The columns were then washed with acetonitrile then cleavage and deprotection of the oligonucleotides from the solid support was achieved by exposure to concentrated ammonia solution (20 h at room temp). Analysis and purification of oligonucleotides was then carried out by reversed phase HPLC and analysed by MALDI-TOF mass spectrometry using a ThermoBioAnalysis Dynamo MALDI-TOF mass spectrometer in positive ion mode using internal dT_n standards and/or negative mode electrospray on a Fisons VG platform mass spectrometer.

Table 1. MS of dispersed TFOs (5'-Q-XCCTXCTCXTXTXTCXT-3')

Modification	MS	
	Calculated	Measured
Control (X=Thymine)	5534	5535
5-guanidylpropargyl dU (GPdU)	6018	6021
5- aminopropargyl dU (APdU)[1]	5768	5768
5-dimethylaminopropargyl dU (DMAPdU)	5936	5936
5-propynyl dU (PdU)	5678	5679

Table 2. MS of the clustered TFOs (5'-Q-TCCXXCTCTXXXXTCTT-3')

Modifications (X)	MS	
	Calculated	Measured
5-guanidylpropargyl dU (GPdU)	6018	6021
5-aminopropargyl dU (APdU)[1]	5768	5770
5-dimethylaminopropargyl dU (DMAPdU)	5936	5938
5-propynyl dU (PdU)	5678	5680

C = 5MeC Q = DABCYL

References:

[1]. Bijapur, J., Keppler, M.D., Bergqvist, S., Brown, T. and Fox, K.R. *Nucleic Acids Res.*, 1999, **27**, 1802-1809.
[2] Prakash, T. P., Pushi, A., Lesnik, E., Mohan, V., Tereshko, V., Egli, M., Manoharan, M., *Org. Lett*., 2004, **6**, 1971-1974.