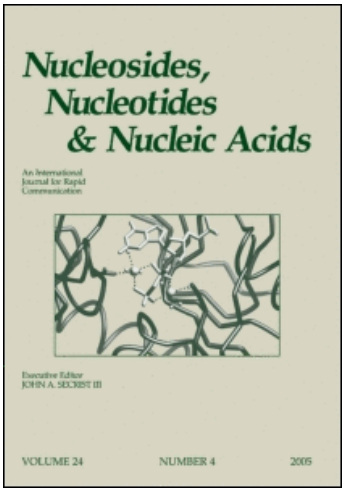

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Synthesis And Properties Of Oligonucleotides Containing A Cholesterol Thymidine Monomer

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SYNTHESIS AND PROPERTIES OF OLIGONUCLEOTIDES CONTAINING A CHOLESTEROL THYMIDINE MONOMER

Adeline Durand and Tom Brown □ *School of Chemistry, University of Southampton, Highfield, Southampton, UK*

□ *Highly selective base-pair recognition makes DNA a suitable building block for orderly self-assembled structures. For some applications in nanotechnology DNA complexes need to be fixed onto surfaces. To fulfil this requirement on lipid membranes we have synthesised a thymidine monomer modified with a cholesterol moiety. Solution studies show that the melting temperature (T_m) of the duplex, with adjacent cholesterols on each strand, is much higher than that of the unmodified duplex.*

Keywords Cholesterol; nonotechnology; DNA; fluorescence melting; self-assembly

INTRODUCTION

Double helical DNA is an attractive molecule for the design of nano-networks due to its inherent rigidity and highly selective base pairing.[1–3] Indeed, complexes formed by simple oligonucleotides (ODNs) have been shown to self-assemble into orderly double-stranded structures on solid surfaces.[4–7] In this context we have an interest in assembling ODN arrays on soft lipid membranes.[8] To this end, we have synthesized a cholesterol phosphoramidite monomer, which can be incorporated into DNA sequences at thymidine sites. ODNs modified with cholesterol have previously been synthesized for various purposes.[9–13] For example Hook et al. used a cholesterol, attached at either the 3′- or 5′-end of the ODN, to tether them to supported lipid bilayers.[14,15] For our purposes it is important to be able to pin the nano-network to the lipid surface at any desired locus. Therefore, we designed a phosphoramidite monomer, Chol-dT (Figure 1, A), which can be positioned at any thymidine site within the synthetic ODN, without disrupting base pairing (Figure 1, B). The monomer is functionalized with

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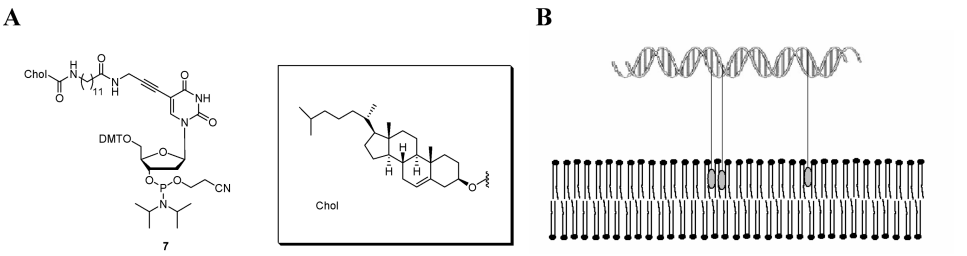
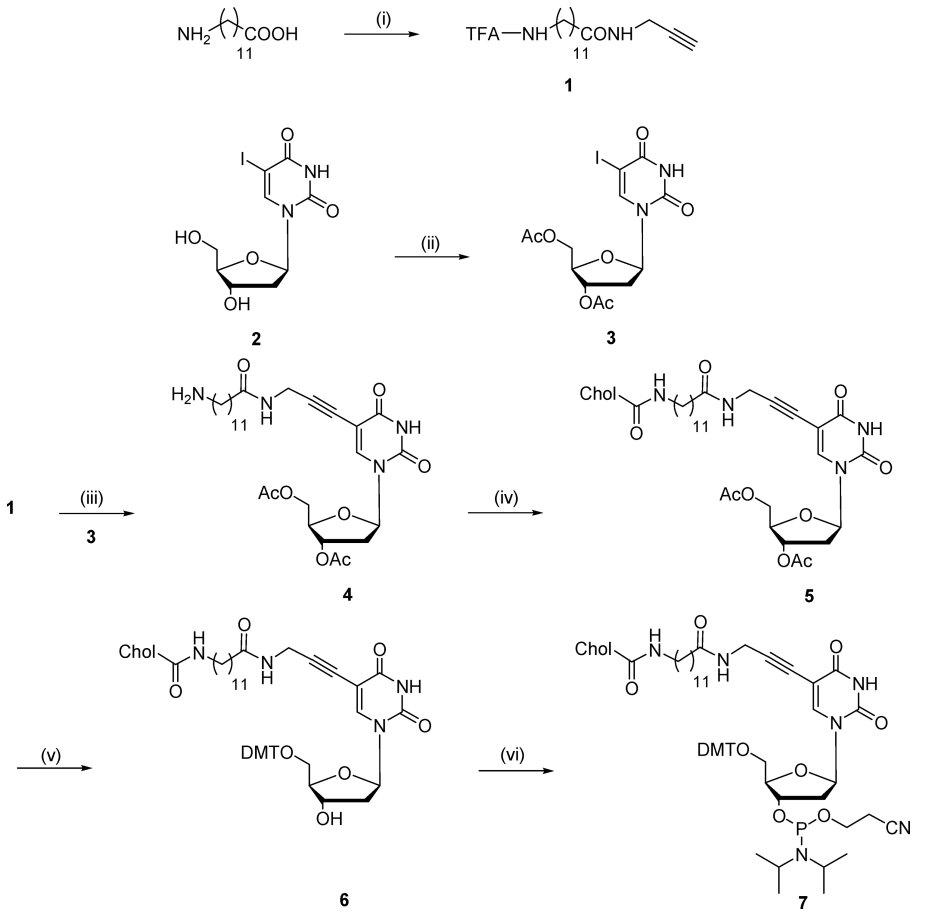


FIGURE 1 (A) Structure of the phosphoramidite monomer Chol-T. (B) Duplex anchored to a lipid bilayer via cholesterol.



SCHEME 1 (i) a) Ethyl trifluoroacetate (1.5 eq), TEA (3 eq), MeOH, 22.5 h, rt; b) propargylamine (1 eq), EDC (1.3 eq), DCM, 24 h, rt, 79% overall; (ii) Ac_2O (3 eq), pyridine, rt, 16.5 h, quant.; (iii) a) Ethanolic methylamine (70 eq), 55°C, 20 h; b) deprotected amine (2.2 eq), CuI (0.4 eq), $(Ph_3P)_4Pd$ (0.2 eq), TEA (40 eq), DMF, rt, 30 h, dark, 73% overall; (iv) Cholesteryl chloroformate (1.2 eq), DIPEA (1.2 eq), DCM and DMF, 2 h at 0°C and 2 h at rt, 32%; (v) a) K_2CO_3 0.4M in MeOH:H_2O 4:1(6 eq), 1.75 h, rt; b) 4,4′-dimethoxytritylchloride (3 eq), pyridine, DCM, rt, 4.5 h, 45% overall; (vi) 2-cyanoethyl-*N*,*N*-diisopropyl chlorophosphine (1.1 eq), DIPEA (2.5 eq), THF, rt, 0.5 h, 82%.

cholesterol and a hydrophobic spacer unit, which forms a bridge to the polar anionic phosphate backbone of the DNA chain.

RESULTS AND DISCUSSIONS

The synthetic route to the Chol-dT monomer is outlined in Scheme 1. Firstly, the commercially available 12-aminododecanoic acid was protected with trifluoroacetyl and coupled with propargylamine to give the spacer **1** in 79% overall yield. 5-iodo-2′-deoxyuridine was acetylated to give 3 quantitatively and spacer 1 was deprotected and coupled via a Sonogashira reaction to give the protected thymidine **4**. The free amino group of compound **4** was then coupled with cholesteryl chloroformate in 32% yield. The acetyl protection groups were removed by treating with potassium carbonate in a mixture of methanol and water and dimethoxytritylation of the resultant free nucleoside afforded **6**, which was converted to its 2-cyanoethyl-*N*,*N*-diisopropyl phosphoramidite **7** in 82% yield. Monomer **7** was incorporated into the 18-mer ODNs **8** [5′-ACCAAGGTGCTA**T**CGTCG-3′, **T** = Chol-dT] and its complementary strand **9** [5′-CGACGA**T**AGCACCTTGGT-3′, **T** = Chol-dT] by automated solid-phase ODN synthesis. All ODNs were purified by reversed-phase HPLC.

To evaluate the hybridization property of the Chol-dT fluorescence, melting experiments[16] were conducted using **8**, **9**, and their respective unmodified ODNs **8′** and **9′** (Figure 2).

The melting temperature (T_m) of the duplex **8**/**9**, was higher than that of the natural duplex **8′**/**9′** (+2.4°C) and much higher than that of **8′**/**9** and **8**/**9′** (+8.1°C and +8.8°C respectively) (Table 1).

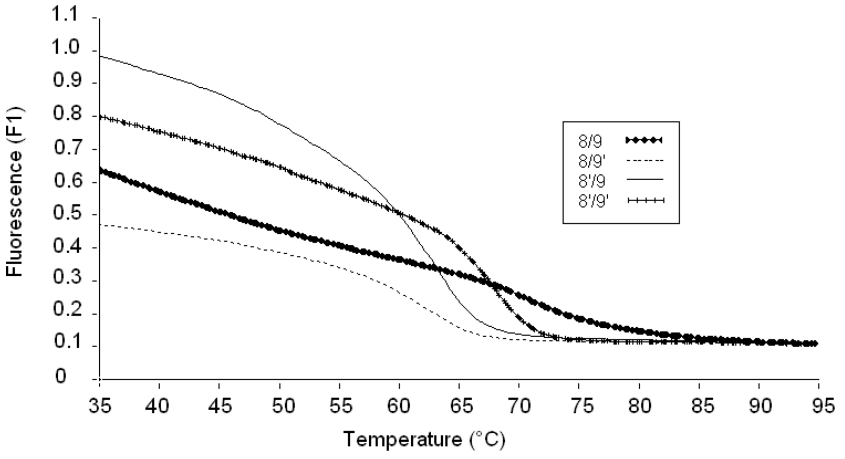


FIGURE 2 Fluorescence melting curves at pH 7.0 (10 mM sodium phosphate buffer containing 200 mM NaCl and 1 mM Na_2EDTA). The concentration of each ODN was 0.5 μM.

TABLE 1 Fluorescence melting curves at pH 7.0 (10 mM sodium phosphate buffer containing 200 mM NaCl and 1 mM Na_2EDTA). Each Tm is an average of two separate runs

	buffer with NaCl 200 mM			
ODN	8′/9′	8′/9	8/9′	8/9
Tm (°C)	67.6	61.9	61.2	70

These results clearly illustrate a destabilization of the duplex with a cholesterol moiety on one DNA strand, but stabilization when cholesterol is present on both strands. We are planning further melting studies of the duplex under various conditions.

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SUPPLEMENTARY MATERIAL

^{1}H NMR spectra were measured at 400 MHz on a Bruker DPX400 spectrometer. ^{13}C NMR spectra were measured at 100 MHz on a Bruker DPX400 spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane, and *J* values are given in Hz and are correct to within 0.5 Hz. Multiplicities of ^{13}C signals were determined using DEPT spectral editing technique and are described as s (quaternary carbon), d (CH), t (CH_2) and q (CH_3). Assignment was also aided by COSY (^{1}H-^{1}H) and HMQC experiments. ^{31}P NMR spectra were recorded on a Bruker AC300 spectrometer at 121 MHz and were externally referenced to 85 % phosphoric acid in D_2O. Low-resolution mass spectra were recorded using electrospray technique on a Fisons VG platform instrument in acetonitrile or a Waters ZMD quadrupole mass spectrometer in methanol. High-resolution mass spectra were recorded using electrospray technique on a Bruker APEX III FT-ICR mass spectrometer in acetonitrile or methanol. Infrared spectra were recorded on a Satellite FT-IR using a Golden Gate adapter and WIN FIRST-lite software or using a Smart Orbit adapter and OMNIC software. Absorptions are described as strong (s), medium (m) and weak (w). Melting points were measured on a Gallenkamp electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed by Medac Ltd using CE-440 and Carlo Erba elemental analysers and the results were within ± 0.50 of the calculated values. All reactions were carried out under inert atmosphere. Dichloromethane, pyridine, DIPEA and triethylamine were dried over CaH_2, methanol was dried over iodine and magnesium and THF over sodium and benzophenone.

12-(2,2,2-trifluoroacetamido)-N-(prop-2-ynyl)dodecanamide (1). 12-Aminododecanoic acid (5.0 g, 23.22 mmol) was dissolved in MeOH (55 mL) and triethylamine (10 mL) was added. The solution was stirred at rt and ethyltrifluoroacetate (4.14 mL, 34.83 mmol) was added. The reaction was complete after 22.5 hours. The solvents were removed *in vacuo* to give a pale yellow solid which was then redissolved in distilled CH_2Cl_2 (100 mL). EDC (7.14 g, 37.26 mmol) and propargylamine (1.96 mL, 28.66 mmol) were added to the solution which was stirred at rt for 24 hours. The reaction was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution, then saturated KCl solution and dried (Na_2SO_4), filtered and the solvents removed *in vacuo* to give a pale yellow solid. The crude mixture was purified by silica gel column chromatography (6:4 EtOAc:hexane) to give **1** as a colourless solid (6.35 g, 79% overall): mp 91–92°C; ν_{max} (neat)/cm^{-1} 3315 (m, NH), 3290 (m, NH), 2918 (m, CH_2), 2851 (w, CH_2), 1697 (s, CO), 1644 (s, CO), 1570 (w), 1531 (m), 1470 (m, CH_2), 1448 (w, CH_2), 1416 (w), 1373 (w), 1207 (s, CF_3), 1179 (s, CF_3), 719 (s); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.43 (s, 1H), 5.65 (s, 1H), 4.04 (dd, $J = 5.0$, 2.5 Hz, 2H), 3.35 (q, $J = 6.9$ Hz, 2H), 2.22 (t, $J = 2.5$ Hz, 1H), 2.18 (t, $J = 7.5$ Hz, 2H), 1.59

(m, 4H), 1.28 (m, 14H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 172.8, 157.3, 79.8, 71.6, 40.1, 36.6, 29.4, 29.4, 29.3, 29.3, 29.1 29.0, 26.7, 25.6; LRMS [ES$^+$]: m/z 349 (M+H$^+$, 80%), 371 (M+Na$^+$, 100%); HRMS [ES$^+$]: m/z found 349.2093 (M+H)$^+$ $C_{17}H_{28}F_3N_2O_2$ requires 349.2098; Anal calculated: C, 58.61; H, 7.81; N, 8.04. Found: C, 58.78; H, 7.84; N, 7.95.

3′,5′-Di-O-acetyl-5-iodo-2′-deoxyuridine (3). 5-Iodo-2′-deoxyuridine (500 mg, 1.41 mmol) was dissolved in pyridine (3 mL) and acetic anhydride (40 μL, 4.24 mmol) was added. The reaction was stirred under argon for 16.5 hours at rt, then the pyridine was removed and co-evaporated with toluene *in vacuo*. The crude mixture was purified by column chromatography (6:4 EtOAc:hexane) to give **3** as a colourless solid (610 mg, 99%): mp 156–157°C; ν_{max}(neat)/cm^{-1}3236 (m, NH), 1736 (s, CO), 1673 (s, CO), 1362 (s, CH), 1235 (s, C-O), 1194 (s, C-O), 1106 (s, C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H9.20 (s, 1H), 7.96 (s, 1H), 6.28 (dd, J = 8.3, 5.6 Hz, 1H), 5.23 (dt, J = 6.0, 2.0 Hz, 1H), 4.40 (dd,J = 12.6, 3.0 Hz, 1H), 4.33 (dd,J = 12.6, 3.0 Hz, 1H), 4.29 (dd, J = 6.0, 3.0 Hz, 1H), 2.54 (ddd, J = 14.1, 5.5, 2.0, 1H), 2.19 (m, 1H), 2.20 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.7, 170.5, 160.0, 150.2, 144.1, 85.8, 83.0, 74.4, 69.3, 64.2, 38.6, 21.4, 21.2; LRMS [ES$^+$]: m/z461 (M+Na$^+$, 20%); HRMS [ES$^+$]: m/z found 460.9823 (M+Na)$^+$ $C_{13}H_{15}IN_2O_7Na$ requires 460.9816; Anal calculated: C, 35.63; H, 3.45; N, 6.39. Found: C, 35.60; H, 3.38; N, 6.31.

5-(12-amino-N-(prop-2-ynyl)dodecanamido)-3′,5′-di-O-acetyl-2′-deoxyuridine (4). (1.0 g, 2.87 mmol) was diluted in ethanolic methylamine 33% (25 mL, 201 mmol) and the reaction mixture was stirred at rt for 24 hours. The solvent was removed *in vacuo* and dried overnight under high vacuum to give a white solid (929 mg), which was used without further purification. Compound **3** (193 mg, 0.44 mmol) was dissolved in DMF (2 mL) to which the deprotected amine (244 mg, 0.97 mmol), triethylamine (2.46 mL, 17.62 mmol) and CuI (34 mg, 0.18 mmol) were added. The solution was stirred in the dark, at rt, under argon for 10 min before adding tetrakis(triphenylphosphine)palladium (102 mg, 0.88 mmol). The reaction had gone to completion after 30 hours. The solvents were removed *in vacuo*, the residue dissolved in a mixture of CH_2Cl_2 and MeOH, filtered on celite and the solvents were removed *in vacuo*. A methanolic solution of the crude material was pre-adsorbed onto silica and purified by column chromatography (8:2 EtOAc:hexane) to give **4** as brown syrup (238 mg, 96%): ν_{max}(neat)/cm^{-1} 3254 (w, NH), 2925 (m, CH_2), 2844 (w),1710 (s, CO), 1651 (s, CO), 1456 (m), 1362 (m, CH), 1228 (s, C–O), 1101 (m), 1023 (m), 977 (C–O), 776 (m), 603 (m), 564 (m); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H7.93 (s, 1H), 6.18 (dd, J = 7.7, 6.2 Hz, 1H), 5.24 (dt, J = 6.6, 2.6 Hz, 1H), 4.26–4.37 (m, 3H), 4.10 (s, 2H), 2.89 (m, 2H), 2.47 (ddd, J = 14.6, 6.2, 2.7 Hz, 1H), 2.15–2.23 (m, 1H), 2.17 (m, 2H), 2.14 (s, 3H), 2.09 (s, 3H), 1.62 (m, 4H), 1.31 (m, 14H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 177.0, 173.2, 145.6,

101.6, 88.4, 85.2, 76.7, 66.1, 42.0, 39.6, 38.0, 31.6, 31.5, 31.5, 31.3, 31.3, 29.7, 28.5, 27.9, 22.1, 21.9; LRMS [ES$^+$]: m/z 563 (M+H$^+$, 30%); HRMS [ES$^+$]: m/z found 563.3066 (M+H)$^+$ $C_{28}H_{43}N_4O_8$ requires 563.3075.

5-(12-Cholesteryloxycarbonylamino-N-(prop-2-ynyl)dodecanamido)-3′,5′-di-O-acetyl-2′-deoxyuridine (5). A solution of cholesteryl chloroformate (133 mg, 0.30 mmol) in distilled CH_2Cl_2 (0.6 mL) was added to a stirred solution of **4** (139 mg, 0.25 mmol) in DMF (1 mL) with DIPEA (50 μL, 0.30 mmol). The solution was stirred at 0°C, under an argon atmosphere for 2 hours and then at rt for a further 2 hours. The solvents were removed *in vacuo* and the residue was dissolved in CH_2Cl_2, washed with citric acid 10%, saturated KCl solution, dried (Na_2SO_4), filtered and the solvents removed *in vacuo*. The crude mixture was purified by column chromatography (EtOAc:hexane 3:2 to 4:1) to give **5** as a yellow solid (77 mg, 33%): mp 132°C; ν_{max}(neat)/cm^{-1} 3273 (w, NH), 2924 (m, CH_2), 2845 (m, CH_3), 2358 (w, C-C alkyne), 1695 (s, CO), 1628 (s), 1542 (m), 1459 (m, CH_2), 1361 (m, CH_3), 1224 (s, C-O), 1039 (m), 953 (m, C-O), 776 w), 604 (w); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H9.06 (s, 1H), 7.80 (s, 1H), 6.26 (dd, J = 7.5, 6.0 Hz, 1H), 6.06 (br s, 1H), 5.36 (m, 1H), 5.23 (dt, J = 6.5, 3.0 Hz, 1H), 4.64 (br s, 1H), 4.48 (br s, 1H), 4.36 (m, 2H), 4.28 (dd, J = 6.5, 3.0 Hz, 1H), 4.23 (d, J = 5.02 Hz, 2H), 3.13 (m, 2H), 2.54 (ddd, J = 14.1, 6.0, 2.5 Hz, 1H), 2.20–2.25 (m, 3H), 2.16 (s, 3H), 2.11 (s, 3H), 1.47 (m, 4H), 1.25 (m, 14H), 1.07–2.56 (br m, 43H); ^{13}C NMR (100MHz, $CDCl_3$): δ_C 173.0, 170.4, 149.1, 142.3, 140.0, 122.6, 100.1, 85.8, 82.8, 74.1, 63.8, 56.8, 56.3, 50.2, 42.5, 39.9, 39.7, 38.7, 38.4, 37.1, 36.7, 36.6, 36.3, 35.09, 32.0, 32.0, 30.1, 30.0, 29.8, 29.6, 29.5, 29.4, 29.4, 29.4, 28.3, 28.1, 26.9, 25.6, 24.4, 24.0, 22.9, 22.7, 21.2, 21.0, 20.1, 19.5, 18.9, 12.0; LRMS [ES$^+$]: m/z 997 (M+Na$^+$, 100%); HRMS [ES$^+$]: m/z found 997.6244 (M+Na)$^+$ $C_{56}H_{86}N_4O_{10}Na$ requires 997.6236; Anal calculated: C, 68.96; H, 8.89; N, 5.74. Found: C, 69.29; H, 9.01; N, 5.51.

5-(12-Cholesteryloxycarbonylamino-N-(prop-2-ynyl)dodecanamido)-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyuridine (6). Compound **5** (800 mg, 0.82 mmol) was diluted in potassium carbonate solution at 0.4 mol.L^{-1} in MeOH:H_2O 4:1 (13 mL, 5.20 mmol). The reaction mixture was stirred at rt for 1.75 hours then diluted in CH_2Cl_2. DOWEX 50WX8-400 (pyridinium form) was added and the solution was stirred until pH = 7, filtered and the solvents were evaporated and co-evaporated with pyridine *in vacuo* to give a solid (891 mg) which was used without further purification. This compound (653 mg, 0.73 mmol) was dissolved in CH_2Cl_2 (5 mL) to which 3 mL of pyridine was added followed by 4,4′-dimethoxytrityl chloride (298 mg, 0.88 mmol) in small portions. The reaction was stirred for 5 hours and 5 mL of MeOH was added to the solution. The solvents were evaporated and co-evaporated with toluene *in vacuo* and the crude mixture was purified by column chromatography (EtOAc:hexane 4:1, 1% pyridine) to give **6** as a yellow solid (358 mg, 45%): mp 104°C; ν_{max}(neat)/cm^{-1} 3346 (w,

NH), 3056 (w, CH Ar), 2925 (m, CH_2), 2844 (m, CH_3), 2357 (w, C–C alkyne), 1687 (s, CO), 1601 (m), 1503 (s, C=C Ar), 1446 (s, CH_2), 1283 (s), 1242 (s, C–O), 1172 (s), 1172 (s), 1086 (m), 1025 (s, C–O), 825 (m), 723 (m), 694 (m), 576 (s); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H9.40 (s, 1H), 8.19 (s, 1H), 6.83–7.44 (m, 13H), 6.32 (br t, J = 6.5 Hz, 1H), 6.18 (br s, 1H), 5.53 (br s, 1H), 5.37 (br s, 1H), 4.83 (br s, 1H), 4.69 (br s, 1H), 4.11 (s, 1H), 3.87 (d, J = 5.5 Hz, 2H), 3.81 (s, 3H), 3.79 (s, 3H), 3.43 (d, J = 9 Hz, 1H), 3.33 (d, J = 8 Hz, 1H), 3.13 (br s, 2H), 2.51 (m, 1H), 2.22–2.36 (m, 3H), 1.52 (m, 4H), 1.26 (m, 14H), 0.97–6.16 (br m, 50H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 207.0, 172.7, 158.8 , 147.5, 144. 7, 143.3, 140.0, 135.7, 135.6, 130.2, 130.2, 130.1, 129.3, 129.2 128.3, 128.2, 128.1, 127.9, 127.2, 122.6, 113.3, 86.3, 85.9, 81.6, 56.8, 56.3, 56.3, 55.4, 55.4, 55.3, 54.0, 50.2, 42.5, 41.7, 41.1, 41.0, 40.9, 39.9, 39.7, 38.7, 38.2, 37.1, 36.7, 36.4, 36.4, 36.3, 36.3, 35.9, 32.0, 31.0, 30.1, 30.0, 29.6, 29.6, 29.5, 29.4, 28.4, 28.3, 28.1, 26.9, 26.9, 25.7, 25.6, 24.4, 24.0, 24.0, 22.9, 22.7, 21.2, 19.5, 18.9, 12.0; LRMS [ES$^+$]: m/z 1216 (M+Na$^+$, 40%); Anal calculated: C, 73.46; H, 8.44; N, 4.69. Found: C, 73.16; H, 8.46; N, 4.59.

5-(12-Cholesteryloxycarbonylamino-N-(prop-2-ynyl)dodecanamido)-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyuridine-3′-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (7). 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphine (61 μL, 0.28 mmol) was added dropwise to a degassed solution of **6** (298 mg, 0.25 mmol) in THF (1.5 mL) with DIPEA (109 μL, 0.62 mmol). After stirring at rt for 0.5 hour, the solution was transferred *via* cannula under argon to a separating funnel containing degassed ethyl acetate. The mixture was washed with saturated KCl solution, dried (Na_2SO_4) and the solvent was removed *in vacuo*. Purification by column chromatography under argon pressure (EtOAc: Hexane 4:1, 0.5% pyridine) gave **7** as a colourless air sensitive foam (284 mg, 82%): ^{1}H NMR (400 MHz, d_6-DMSO): δ_H11.61 (br s, 1H), 7.92 (br s, 1H), 6.86–7.40 (m, 14H), 6.96 (br s, 1H), 6.88 (dd, J = 13.6, 7.0 Hz, 1H), 5.31(br s, 1H), 4.29 (br t, J = 11 Hz, 1H), 4.02 (s, 1H), 3.93 (d, J = 5.0 Hz, 1H), 3.90 (d, J = 5.0 Hz, 1H),3.74 (s, 3H), 3.73 (s, 3H), 3.62 (m, 2H), 3.52 (m, 2H), 3.19 (d, J = 7.5 Hz, 2H), 3.14 (d, J = 7.5 Hz, 2H), 2.92 (q, J = 6.5 Hz, 2H), 2.75 and 2.64 (t, J = 5.8 Hz, 2H), 2.40 (m, 1H), 2.23 (m, 1H), 1.30–1.51 (m, 4H), 1.21 (m, 14H), 0.64–2.59 (br m, 59H); ^{31}P NMR (162 MHz, d_6-DMSO)δ_p148.5, 148.9; LRMS [ES$^+$]: m/z 1216 (M+Na$^+$, 40%).

Oligonucleotide Synthesis

All oligonucleotides were synthesised on an Applied Biosystems 394 automated DNA/RNA synthesiser using a standard 0.2 μmole phosphoramidite cycle of acid-catalysed detritylation, coupling, capping and iodine

oxidation. Stepwise coupling efficiencies and overall yields were determined by the automated trityl cation conductivity monitoring facility and were >98.0%. Chol-T phosphoramidite monomer was dissolved in anhydrous CH_2Cl_2 (100 mg.mL^{-1}) coupled for 360 seconds rather than the standard 25 seconds. Standard A, G, C and T DNA phosphoramidites, solid supports and additional reagents were purchased from Link Technologies or Applied Biosystems Ltd. Cleavage of the oligonucleotides from the solid support was achieved by exposure to concentrated ammonia solution (30 min at rt) followed by heating in conc. aqueous ammonia, 55°C for 5 hours in a sealed tube.

Purification of oligonucleotides was carried out by reversed phase HPLC on a Gilson system using an ABI Aquapore column (C8), 8 mm × 250 mm, pore size 300 Å. The system was controlled by Gilson 7.12 software and the following protocol was used: Run time 30 mins, flow rate 4 mL per min, binary system, gradient: Time in mins (% buffer B);0 (0); 3(0); 5(20); 21 (100); 25(100); 27 (0); 30(0). Buffers for unmodified oligonucleotides: Elution buffer A: 0.1 M ammonium acetate, pH 7.0, buffer B: 0.1 M ammonium acetate with 25% acetonitrile pH 7.0. Buffers for modified oligonucleotides: Elution buffer A: 0.1 M ammonium acetate, pH 7.0, buffer B: 0.1 M ammonium acetate with 60% acetonitrile pH 7.0. Elution of oligonucleotides was monitored by ultraviolet absorption at 295 nm. After HPLC purification oligonucleotides were desalted using disposable NAP 10 Sephadex columns (Pharmacia), aliquoted into eppendorf tubes and stored at –20°C. Purified oligonucleotides containing the synthesised monomer were then analysed by MALDI-TOF MS using a ThermoBioAnalysis Dynamo MALDI-TOF mass spectrometer in positive ion mode using internal T_n standards.[1]

ODN Code	Sequence d(5′-3′)	Molecular ion expected(M+H$^+$)	Molecular ion found(M+H$^+$)
8	ACCAAGGTGCTA**T**CGTCG	6149.6	6149.6
9	CGACGA**T**AGCACCTTGGT	6149.6	6150.7

T = Chol dT.

Fluorescence melting experiments

A 5 μM stock solution of each oligonucleotide was prepared in distilled water and the experiments were performed using a Roche LightCycler in a volume of 20 μL containing 0.5 μM of each strand, and the appropriate

concentration of NaCl (200 mM, 400 mM or 600 mM NaCl) with 10 mM sodium phosphate, 1 mM Na_2EDTA at pH = 7.0 and 2 μL of SyberGreen (1:1000 v:v). The complexes were denaturated by heating to 95°C at a rate of 20°C/sec and were maintained at this temperature for 5 mins before cooling to 30°C at 0.1°C/sec. Samples were then held at 30°C for 5 mins before melting again by heating to 95°C at 0.1°C/sec. Data were collecting during annealing and melting steps and compared. The lightcycler has one excitation source (488 nm) and the changes in fluorescence emission were measured at 520 nm. Each experiment was performed in duplicate.

REFERENCE

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