DNA duplexes stabilized by modified monomer residues: synthesis and stability

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The synthesis of a series of 2′-deoxyuridine analogues modified at the 5-position and a series of 2′-deoxypurines modified at the 8-position is described. Ultraviolet melting studies have been used to assess the stability of DNA duplexes 12- and 8-residues in length that contain these modifications. Of those modified residues which have been incorporated into oligonucleotides, 5-(prop-1-ynyl)-2′-deoxyuridine is found to impart the greatest increase in stability on the DNA duplexes studied.

Thermodynamic analyses show that the reason for this increase in stability arises from a decrease in enthalpy which is related to the DNA base stacking process. We suggest that the degree of stabilisation imparted by the propyne moiety may be sequence dependent.

Introduction

The current need for the development of new therapeutic agents capable of diminishing the incidence and the effects of both viral- and genetically-based illnesses is one which is fuelling research amongst a class of what can quite rightly be described as 'molecular engineers'. The widespread occurrence of diseases caused primarily through retrovirus invasion of a host, such as HIV, has encouraged the development of an ever increasing variety of potential drugs based on both the antisense and the antigene strategy.[1,2] In both instances part of the key problem in using oligonucleotides as drugs is their susceptibility to cleavage by exonucleases. Related to this is the relative ease with which such materials might form stable duplex (in the case of antisense) or triplex (in the case of antigene) structures with target DNA and RNA sequences thereby curtailing aberrant transcriptional or translational processes. Investigations into factors which influence double helix stability are conducted by means of structural modifications introduced chemically into nucleotides. In particular the modification of pyrimidine bases at the 5-position to include a hydrophobic moiety has provoked a large amount of interest.[3–5]

The addition of a methyl group to the C-5 position of cytidine and uridine (*i.e.* producing thymidine) has been shown to increase significantly duplex stability.[3] Froehler *et al.*[4,5] took this further by adding a propynyl group at the C-5 position of both 2′-deoxyuridine and 2′-deoxycytosine. As a result they found the melting temperature of the investigated oligonucleotides to increase by 1.4 K per base modification for propynyl modified 2′-deoxyuridine and 1.5 K for propynyl modified 2′-deoxycytidine for two DNA 15-mer oligonucleotides hybridized to RNA. The two reasons suggested to explain this increase in melting temperature were firstly that the hydrophobicity of the propyne group could displace highly ordered water molecules in the major groove thereby increasing the entropy of the system and secondly that the π-system of the propyne group could be expected to overlap with the π-systems of the bases above and below it within the same strand. In the former case and according to the second law of thermodynamics, the free energy of the duplex would decrease thereby increasing duplex stability. In the latter case the anticipated result would be a decrease in enthalpy and hence a decrease in the free energy.

Many other functional groups have been substituted at the 5-position of pyrimidines but this has mainly been related to the search for potent antiviral nucleosides including a range of alkanes, alkenes and alkynes.[6,7] However, of the functionalities added to the 5-position of 2′-deoxyuridine, very few have been incorporated into oligonucleotides and the subsequent duplex stability measured. Sagi *et al.* followed up this research by incorporating a range of 5-alkynyl and 5-alkyl-2′-deoxypyrimidines into oligonucleotides.[8] UV-melting studies showed that the alkynyl modifications were shown to impart greater duplex stability when compared with the alkane modifications, with propyne being optimal.

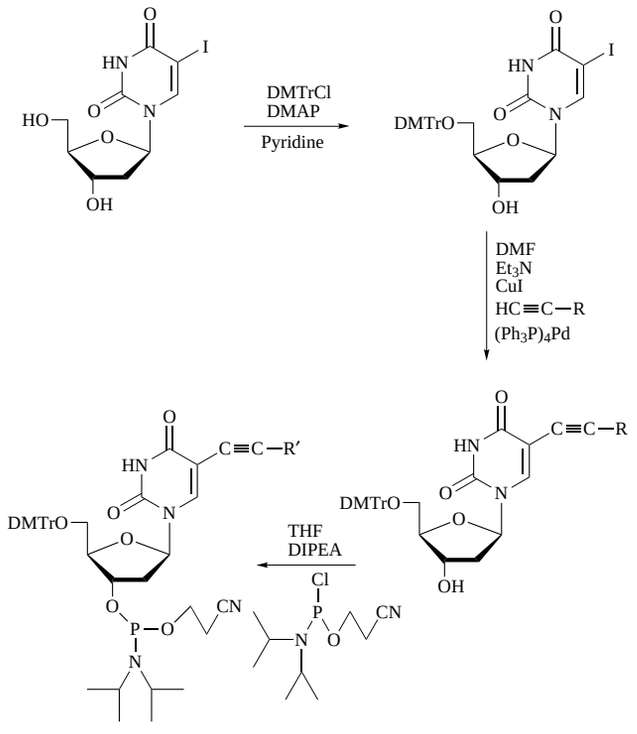
Other chemical modifications with varying electronic properties have been added to the 5-position of 2′-deoxyuridine. Among these are the aminopropyne[9] and the cyano[10,11] groups, although these have not been further investigated by incorporation into oligonucleotides. By the same token the 8-position of 2′-deoxypurines also presents itself as being potentially suitable for modification in the same manner as that of the pyrimidines. Previously, as for the pyrimidines, modifications at this position have mostly been conducted in a search for potent antivirals.[12–15] However, Seela *et al.*[16] have reported that the introduction of small substituents such as bromo, chloro or methyl to the 7-position of 7-deaza-2′-deoxyadenosine increased the duplex stability compared to oligonucleotides containing 2′-deoxyadenosine. Following their promising results Seela *et al.*[17] synthesised 7-(prop-1-ynyl)-7-deaza-2′-deoxyadenosine. When incorporated into an oligonucleotide this compound affected the stability of the resulting duplex, which increased relative to duplexes containing 7-methyl or 7-halo-7-deaza-2′-deoxyadenosine.

Here we describe the synthesis of a variety of 5-modified-2′-deoxyuridine monomers and 8-(prop-1-ynyl)-2′-deoxypurine monomers suitable for incorporation into oligonucleotides *via* solid phase synthesis. The results of ultraviolet melting studies on duplexes containing these modifications are reported and compared. The information obtained from the thermodynamic studies provides an explanation for the observed changes in modified duplex stabilites.

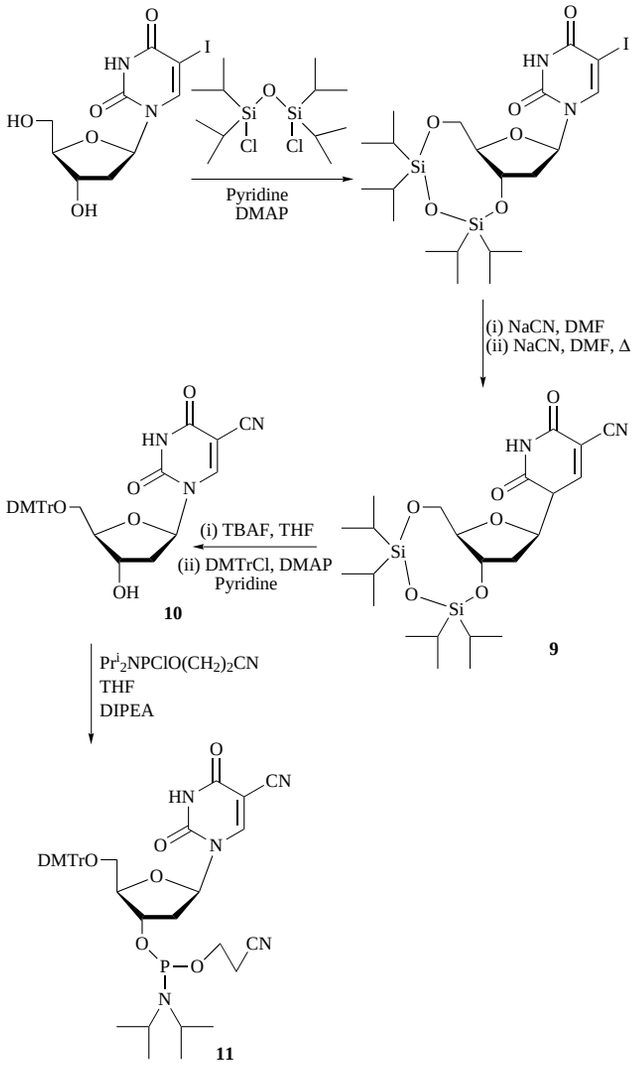
Results and discussion

5-Alkynyl-2′-deoxyuridines were incorporated into oligodeoxyribonucleotides using solid phase synthesis.[18] The monomer phosphoramidites were synthesised according to the synthetic routes detailed in Schemes 1–4.

The monomers produced by these procedures were each incorporated separately into the basic 12 base pair oligonucleotide 5′-d(CGC TTC TTC CTG)-3′ using solid phase



Scheme 1 General synthetic pathway to the acetylenic modified 2′-deoxyuridines



Scheme 2 Synthetic pathway to the 5-cyano-2′-deoxyuridine monomer

procedures. The sequence chosen had previously been used to test the effect of the incorporation of a 5-bromo-2′-deoxyuridine monomer residue on DNA duplex stability.[19] The DNA is part of the 28-residue sequence known as SREV,[20] which is known to be particularly effective therapeutically as an antisense anti-HIV oligodeoxyribonucleotide. Thymidine bases were replaced by the 5-modified deoxyuridines for UV melting studies. Solid phase synthesis of the 12-mer was carried out by incorporating phosphodiester linkages rather than the more common phosphorothioate linkages usually associated with antisense oligonucleotides. The stepwise coupling yield was over 98% for each monomer addition. Routine synthesis, deprotection and purification of all oligonucleotides were carried out according to the methods detailed in the Experimental section. The oligonucleotide containing monomer **6** required further deprotection to remove the silyl group.

Each oligonucleotide produced a major peak on the HPLC chromatogram several minutes after the native oligonucleotide. This confirmed the increased hydrophobicity of the modified oligonucleotide. The retention times were dependent upon the nature of the modification carried out at the 5-position. Thus the hexyne modified oligonucleotide had a longer retention time than that containing the propyne modification. After HPLC purification and desalting the oligonucleotides were analysed by capillary zone electrophoresis (CZE).

Two monomers presented problems during solid phase oligonucleotide synthesis. 5-[3-(Trimethylsilyl)prop-1-ynyl]-2′-deoxyuridine phosphoramidite **2** produced an HPLC chromatogram indicating that the modified base was unstable to the conditions used for solid phase synthesis. In this instance the average stepwise yield for each step of the synthesis of the 12-mer oligonucleotide was greater than 98%. This usually indicates that the next base in the sequence is being added to more than one reactive site on the solid support. In this instance oligomers of varying length and structure were being produced. The only chemical difference between this monomer and those successfully used was the presence of a trimethylsilyl group. It was concluded that this group is lost during solid phase synthesis resulting in a mixture of products as observed by HPLC.

The 6-cyano-2′-deoxyuridine phosphoramidite **11** also proved to be unstable under the conditions of solid phase synthesis which was expected due to the presence of the electron withdrawing cyano group at the 6-position. This enhances the susceptibility of the 5-position to nucleophilic attack from ammonia and iodine.

Enzymatic degradation of modified oligonucleotides

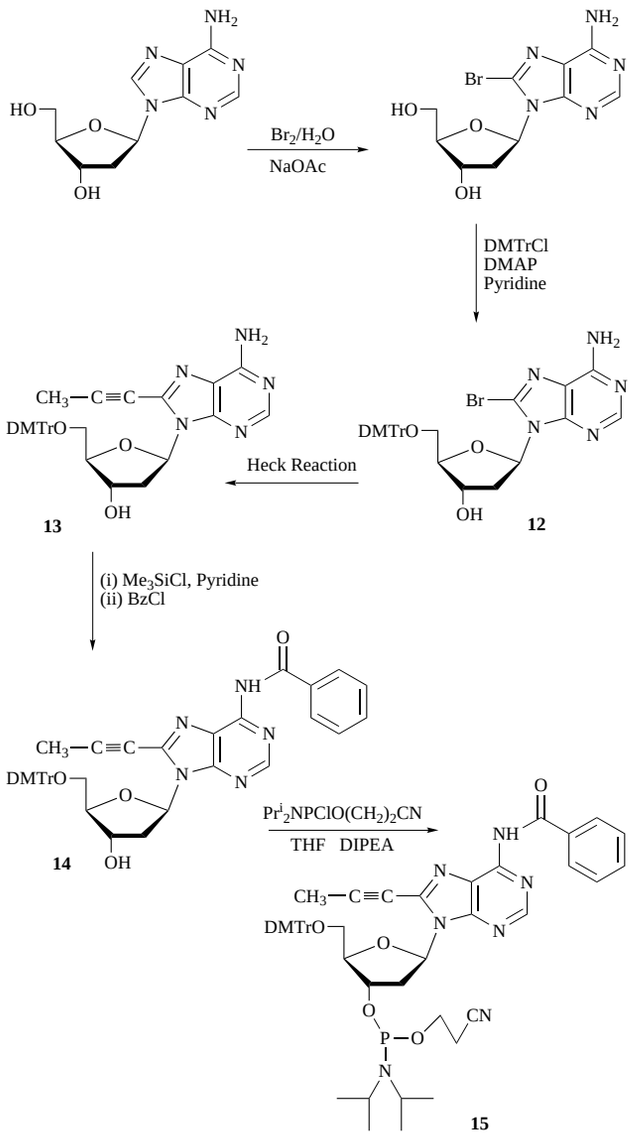
The incorporation of modified bases into oligonucleotides was confirmed by enzymatic digest according to the procedures outlined in the Experimental section. Analysis of the resulting mixture of nucleosides by reversed-phase HPLC allowed identification of the bases present. Nucleosides eluted off the column in the order 2′-deoxycytosine (characteristically broad peak), 2′-deoxyguanosine, undigested oligonucleotide and finally the modified nucleoside. The retention time of the modified nucleoside depended upon the modification at the 5-position.

Oligonucleotides containing 8-(prop-1-ynyl)-2′-deoxypurines

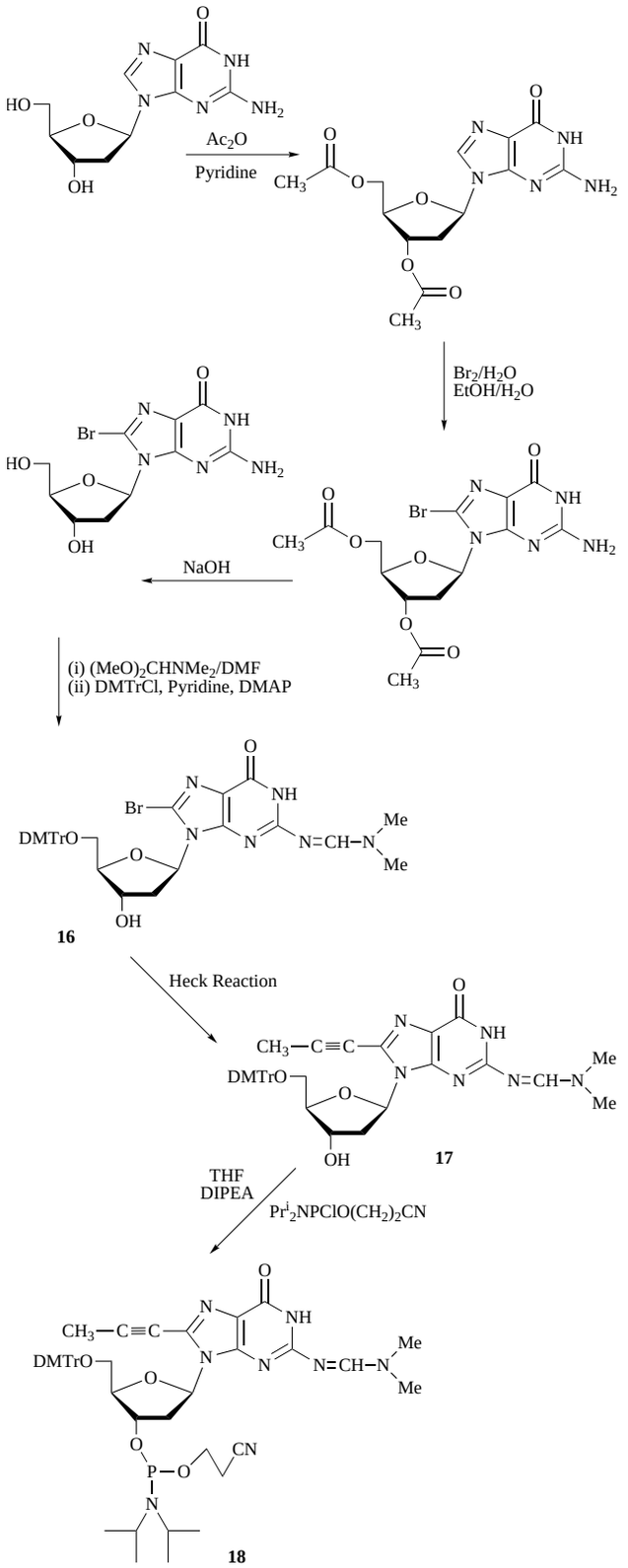
The 12-mer sequence used to test the stabilizing effect of the 5-modified nucleosides only contained one purine base, guanosine. A different sequence was produced to test both modified purine bases. The sequence chosen, 5′-d(CGT AAC GT)-3′, has previously been used to test the duplex stabilizing effect of 2,6-diaminopurine.[21] The guanine and adenine nucleobases

Table 1 Summary of UV melting data for the 12-mer oligonucleotides based on the sequence d(CGC TTC TTC CTG) where every T is replaced by residues modified at the 5-position

Modification	T_m/K	ΔT_m/Base (K)
Native duplex	323.6 ± 0.36	—
Ethyne	326.4 ± 0.31	0.56
Propyne	329.0 ± 0.075	1.08
Hexyne	325.8 ± 0.28	0.44
Prop-2-yn-1-ol	NA	—
Cyano	313.7 ± 0.34	−2.0



Scheme 3 Synthetic pathway to the 8-propynyl-2′-deoxyadenosine monomer



Scheme 4 Synthetic pathway to the 8-propynyl-2′-deoxyguanosine monomer

were substituted for their respective 8-(prop-1-ynyl) modified nucleobases. As a comparison the 8-mer was also synthesized with thymidine substituted by 5-(prop-1-ynyl)-2′-deoxyuridine. Oligonucleotide synthesis, purification and analysis was carried out according to experimental procedures.

Ultraviolet melting studies

To ensure consistency, all melting curves were determined for the same concentration of nucleic acids. A summary of the UV melting data collected for the 12-mer oligonucleotide duplexes containing 5-modified-2′-deoxyuridines is detailed in Table 1.

These results show that the propyne modification imparts the greatest change in stability upon the duplex with an increase of 1.08 K per base modification. The oligonucleotide containing the prop-2-ynyl alcohol failed to produce a distinctive melting curve but instead gave a sloping baseline indicating a constant rate of dissociation from duplex to single strands.

The most destabilizing modification was the addition of the cyano group to the 5-position which leads to a reduction in melting temperature of 2.0 K per base modification. This moiety is strongly electron withdrawing, a feature regarded as the main reason for the observed decrease in duplex stability. The effect can be explained by the fact that electron density is

Table 2 UV melting temperatures for the modified 8-mer duplexes based on the sequence d(CGT AAC GT)

Modification	T_m/K
Native duplex	302.3 ± 0.60
8-Prop-1-ynyl-dA	<283
8-Prop-1-ynyl-dG	<283
5-Prop-1-ynyl-dU	305.5 ± 0.48

reduced at the heterocyclic imino group of deoxyuridine thus reducing the electron donating capability of the imine. A large reduction in the pK_a of the imine could lead to its becoming negatively charged at pH 7.0 which in turn would lead to a reduction in the strength of the hydrogen bonds formed between the modified base and 2′-deoxyadenosine. If the hydrogen bond strength of this base pair decreases, the effect would be a drop in melting temperature, as we observe.

The melting temperatures for the 8-mer duplexes modified by the inclusion of 8-(prop-1-ynyl)-2′-deoxyadenosine, 8-(prop-1-ynyl)-2′-deoxyguanosine and 5-(prop-1-ynyl)-2′-deoxyuridine are summarized in Table 2.

As Table 2 suggests, both of the propynyl modified purines provided a large destabilizing effect on the DNA duplex. The melting temperatures were below the accurate limit of the Peltier block used for analysis. However, estimates of just below 10 °C were made for these melting temperatures. This destabilization is most certainly due to the propyne moiety clashing sterically with the sugar phosphate backbone of the duplex thereby imposing a large strain upon the structure and thus favouring single stranded oligonucleotides.

By way of contrast, the oligonucleotide containing the 5-(prop-1-ynyl)-2′-deoxyuridine showed an increase in melting temperature relative to the native duplex. This increase was measured as 1.6 K per base modification compared to 1.08 K for the native 12-mer. The implication is that the degree of stabilization due to the inclusion of a 5-(prop-1-ynyl)-2′-deoxyuridine base is both sequence and length dependent, an important consideration in terms of antisense targeting.

Thermodynamic parameters of 12-mer duplexes

The thermodynamic parameters relating to the oligonucleotides under study have been calculated from the relationship between the melting temperature and the solution concentration according to eqn. (1),[22] where T_m is the melting temperature, R is a

$$1/T_m = (R/\Delta H^\ominus)\mathrm{In}(C/N) + \Delta S^\ominus/\Delta H^\ominus \qquad (1)$$

constant, $\Delta H^\ominus$ is the change in enthalpy, $\Delta S^\ominus$ is the change in entropy, C is the total concentration of oligonucleotide and N is either 1 for self complementary oligonucleotides or 4 for non-self complementary oligonucleotides. By measuring melting temperatures over a range of concentrations the thermodynamic parameters can be calculated. A plot of $1/T_m$ *vs.* In(C/N) yields $R/\Delta H^\ominus$ from the slope and $\Delta S^\ominus/\Delta H^\ominus$ from the intercept at effectively infinite dilution. The effect on the melting temperature of varying the solution concentration of the oligonucleotide containing 5-(prop-1-ynyl)-2′-deoxyuridine is shown in Fig. 1.

It follows that the enthalpy, $\Delta H^\ominus = -127.32$ kcal mol^{-1} and the entropy $\Delta S^\ominus = -360.89$ cal. mol^{-1} K^{-1}. The duplex free energy is derived by application of eqn. (2) thereby providing a measure of the stability of the duplex.

$$\Delta G^\ominus = \Delta H^\ominus - T\Delta S^\ominus \qquad (2)$$

The results are compared with those previously obtained for 5-bromo-2′-deoxyuridine and the native DNA. The thermodynamic parameters calculated for all three duplex structures under discussion are shown in Table 3.

These results indicate that when a bromine atom is substituted at the 5-position of 2′-deoxyuridine the resulting duplex is more stable than that of the native duplex. Furthermore there is a very large increase in the stability of the duplex when 5-(prop-1-ynyl)-2′-deoxyuridine is incorporated.

Table 3 Comparison of the thermodynamic parameters for the three 12-mer duplex structures analysed

Oligonucleotide Modification	$\Delta H^\ominus$/kcal mol^{-1}	$\Delta S^\ominus$/cal mol^{-1} K^{-1}	$\Delta G^\ominus$/kcal mol^{-1}
Me-dU (Native)	−87.0 ± 10.9	−255.1 ± 2.1	−11.0 ± 1.1
Br-dU	−85.6 ± 10.7	−249.9 ± 2.0	−11.2 ± 1.1
Prop-dU	−127.3 ± 16.0	−360.9 ± 2.9	−19.8 ± 2.0

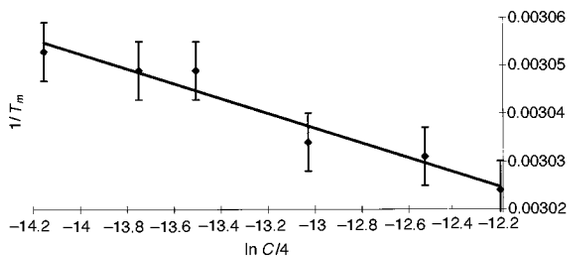


Fig. 1 Plot of $1/T_m$ against In$C/4$ for the propynyl modified duplex

The thermodynamic results show that when a bromine atom is substituted at the 5-position of 2′-deoxyuridine the increase in melting temperature compared to the native 2′-deoxyuridine is due to an increase in entropy. Entropy factors provide the dominant stabilizing influence. The enthalpy is also seen to increase. This tends to have an opposing, unfavourable effect which implies that the base stacking process is not as favoured as for the native strand. This effect is attributed to the bulky bromine atom causing structural disruption by steric interference. This disruption also displaces highly ordered water molecules, commonly found in the major groove. The result is a substantial increase in the entropy of the system which counteracts the increase in enthalpy. Overall a drop in the free energy and hence an increase in duplex stability results.

Comparison of the thermodynamics of the oligonucleotide containing the 5-(prop-1-ynyl)-2′-deoxyuridine bases and the native strand containing 2′-deoxyuridine leads to a different conclusion. In this instance the entropy decreases which would ordinarily result in an increase in the free energy thereby decreasing duplex stability. However, in this instance the free energy decreases markedly causing a significant increase in duplex stability. This can be attributed to the large decrease in enthalpy which is dependent upon the base stacking process. The propyne group has a π-system capable of enhancing the π–π interaction between the modified base and the base either above or below it, leading to a decrease in energy for the base stacking process. This is due in part to the increased electronic interactions highlighted by the change in enthalpy. With changes in the interaction between the bases occurring, the overall duplex stability is expected to alter. In such an instance the water molecules normally found in the major groove would be expected to become more ordered thus decreasing the entropy. These results support the hypothesis that the changes observed in the stability of these duplex structures is due to an increased π-overlap between the modified base and that of the base above or below. Further evidence for this increased π-overlap was found by studying the NMR data of various duplex analogues which contained 5-(prop-1-ynyl)-2′-deoxyuridine residues. Initial results show an interaction of the propyne group with only the base on the 5′-side of the oligonucleotide. This may explain why different degrees of stability have been observed per base modification for different sequences. Comprehensive structural studies are underway and will be reported in the future.

Experimental

General preparations

Solvents were of laboratory grade, except those used for the extraction and chromatography of phosphoramidites, which were of HPLC grade. Reverse osmosis-purified water was used during oligonucleotide synthesis, analysis and purification. Dichloromethane was distilled over CaH_2; tetrahydrofuran was distilled from sodium–benzophenone; pyridine was distilled from CaH_2; triethylamine and diisopropylethylamine were dried over CaH_2; anhydrous *N*,*N*-dimethylformamide was purchased from Aldrich and anhydrous acetonitrile was purchased from Applied Biosystems Ltd. (ABI). 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite was synthesized by the procedure of Koster.[23] 4,4′-Dimethoxytrityl chloride was purchased from Courtaulds and propyne gas was purchased from Argo International. All other chemicals were supplied by Aldrich, Sigma or Fluka. CHN analysis was carried out on a Perkin-Elmer 2400 elemental analyser. All samples were kept in a drying pistol at 60 °C over P_2O_5 for at least 48 hours prior to analysis. Positive ion Fast Atom Bombardment (FAB) mass spectra were recorded on a Kratos MS50 TC spectrometer using a 3-nitrobenzyl alcohol or thioglycerol–acetonitrile matrix. IR spectra were recorded on a Bio Rad FTS-7 fourier transform spectrometer controlled by a Bio Rad SPC 3200 microcomputer using KBr plates. UV spectra were recorded on a Perkin-Elmer Lambda 15 ultraviolet-visible spectrometer using PECSS2 software and the various buffer mixtures as listed for each compound scanned. Flash chromatography was carried out using silica gel 60 (Merck). Thin layer chromatography (TLC) was carried out on aluminium sheets, silica gel 60 F_{254}, 0.2 mm layer (Merck) using the following solvent systems: (A) hexane–ethyl acetate (1:1, v/v); (B) dichloromethane–methanol (9:1, v/v); (C) ethyl acetate–methanol–ammonia (5:1:1, v/v); (D) ethyl acetate; (E) dichloromethane–methanol (8:2, v/v). Products were visualized on TLC by UV absorption at 264 nm, spraying with a solution of 4-methoxybenzaldehyde–acetic acid–sulfuric acid–ethanol (5:1:1:50, v/v/v/v) or exposure to conc. hydrochloric acid for compounds containing a DMTr group.

NMR. Standard ^{1}H NMR spectra were recorded on Bruker WP-200 and Bruker AC-250 NMR spectrometers operating at proton resonance frequencies of 200.13 MHz and 250.13 MHz respectively. Where necessary ^{1}H NMR spectra were recorded at 360.13 MHz using a Bruker WM-360 spectrometer. ^{13}C NMR spectra were recorded on a Bruker WP-200 spectrometer operating at a ^{13}C resonance frequency of 50.32 MHz, a Bruker AC-250 spectrometer operating at a ^{13}C resonance frequency of 62.90 MHz and a Bruker WM-360 spectrometer operating at a ^{13}C resonance frequency of 90.556 MHz. ^{31}P NMR spectra were recorded on a JEOL FX90Q spectrometer. *J* values are given in Hz.

Chemical synthesis

General procedure 1: addition of alkynes *via* the Heck[7,24–26] reaction. To a degassed solution of the desired nucleoside (2 mmol) in anhydrous DMF (15 ml) was added successively copper(I) iodide (0.4 mmol), anhydrous triethylamine (7 ml), the alkyne (6 mmol) and tetrakis(triphenylphosphine)-palladium(0) (0.2 mmol) and the mixture left to stir. After 4 hours the solvent was removed *in vacuo* and the residue dissolved in ethyl acetate (200 ml), washed with saturated aqueous KCl (50 ml), 5% EDTA (50 ml) and saturated aqueous KCl (50 ml), dried (Na_2SO_4) then reduced to an oil.

General procedure 2: phosphoramidite monomer creation for DNA synthesis. The relevant alcohol (1 mmol) was co-evaporated three times with anhydrous THF (5 ml) before dissolving in anhydrous THF (5 ml). Anhydrous diisopropylethylamine (4 mmol) was added with stirring under argon. 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (1.1 mmol) was added dropwise and the mixture left to stir for 1 hour. Ethyl acetate (50 ml) was added, the organic layer washed with saturated aqueous KCl (50 ml), dried (Na_2SO_4) and the solvent removed *in vacuo* to leave an oil which was purified by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with 100% ethyl acetate.

Basic starting materials. The basic starting material used to obtain 5-modified 2′-deoxyuridines was 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine.[27] Commercially available 5-iodo-2′-deoxyuridine was dissolved in anhydrous pyridine and a catalytic amount of dimethylaminopyridine (DMAP) and 4,4′-dimethoxytrityl chloride added slowly. Purification by wet flash column chromatography yielded the product 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine as a white solid in 80% yield.

5-[3-(Trimethylsilyl)prop-1-ynyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 1

5-Iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine[27] and 3-(trimethylsilyl)prop-1-yne were combined according to general procedure 1. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–10%) yielded **1** as a yellow foam (1.038 g, 1.62 mmol, 71%) (Found: C, 67.49; H, 6.72; N, 4.05. $C_{36}H_{40}N_2O_7Si$ requires C, 67.49; H, 6.29; N, 4.37%); R_f (B) 0.55, (C) 0.27; $\delta_H(CDCl_3)$ 2.63 (9 H, s, 3 × CH_3), 1.48 (2 H, s, CH_2), 2.15–2.26 (1 H, m, 2′H), 2.40–2.49 (1 H, m, 2″H), 3.33 (2 H, d, *J* 4.0, 5′Hs), 3.77 (6 H, s, 2 × CH_3O), 4.02–4.06 (1 H, m, 4′H), 4.42–4.46 (1 H, m, 3′H), 6.27 (1 H, dd, *J* 6.0 + 7.5, 1′H), 6.70–6.84 (4 H, m, DMTr), 7.16–7.44 (10 H, m, 3′OH + DMTr), 7.78 (1 H, s, 6H) and 8.93 (1 H, br s, NH); $\delta_C(CDCl_3)$ 7.91 (CH_3), 25.40 (CH_2), 40.98 (CH_2), 55.02 (CH_3), 67.75 (CH_2), 72.09 (CH), 85.29 (CH), 86.18 (C), 86.64 (CH), 93.47 (C), 101.83 (C), 112.94 (CH), 113.08 (CH), 126.85 (CH), 127.63 (CH), 127.78 (CH), 128.97 (CH), 129.82 (CH), 135.46 (C), 139.30 (C), 140.21 (CH), 144.36 (C), 149.54 (C), 158.32 (C) and 162.08 (C); λ_{max}(DCM–MeOH 9:1)/nm 283; *m*/*z* (FAB) 641.268 28 [(M + H)$^+$ calc. for $C_{36}H_{41}N_2O_7Si$: 641.268 29].

5-[3-(Trimethylsilyl)prop-1-ynyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) 2

Preparation was carried out by phosphitylation of 5-[3-(trimethylsilyl)prop-1-ynyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **1** according to general procedure 2 and purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with 100% ethyl acetate which yielded **2** as a pale yellow foam (536 mg, 0.637 mmol, 82%), R_f (B) 0.85; δ_P[$(CD_3)_2CO$ internal standard (int.) in DCM] 149.09 (d).

5-(Hex-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 3

5-Iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine[27] and hex-1-yne were combined according to general procedure 1. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–5%) yielded **3** as a tan-coloured foam (1.214 g, 1.94 mmol, 75%) (Found: C, 70.68; H, 5.18; N, 4.51. $C_{36}H_{38}N_2O_7$ requires C, 70.74; H, 6.27; N, 4.59%); R_f (B) 0.61, (C) 0.61; $\delta_H(CDCl_3)$ 0.71–0.78 (3 H, m, CH_3), 1.11–1.25 (4 H, m, 2 × CH_2), 2.04–2.09 (2 H, m, CH_2), 2.22–2.30 (1 H, m, 2′H), 2.43–2.51 (1 H, m, 2″H), 3.27–3.42 (2 H, m, 5′Hs), 3.77 (6 H, s, 2 × CH_3O), 4.05–4.09 (1 H, m, 4′H), 4.41 (2 H, br s, NH + 3′OH), 4.49–4.51 (1 H, m, 3′H), 6.32 (1 H, dd, *J* 6.0 + 7.5, 1′H), 6.79–6.85 (4 H, m, DMTr), 7.16–7.44 (9 H, m, DMTr) and 7.98 (1 H, s, 6H); $\delta_C(CDCl_3)$ 13.32 (CH_3), 18.91 (CH_2), 21.81 (CH_2), 30.11 (CH_2), 41.24 (CH_2), 54.97 (CH_3), 63.41 (CH_2), 70.49 (C), 71.96 (CH), 85.44 (CH), 86.44 (CH), 86.65 (C), 95.07 (C), 100.95 (C), 113.02 (CH), 126.62 (CH), 127.73 (CH), 129.76 (CH), 135.43 (C), 141.35 (CH), 144.30 (C), 149.72 (C), 158.25 (C) and 162.56 (C); λ_{max}(DCM–MeOH 9:1)/nm 285; *m*/*z* (FAB) 611.275 74 [(M + H)$^+$ calc. for $C_{36}H_{39}N_2O_7$: 611.275 71].

5-(Hex-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) 4

5-(Hex-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **3** was treated according to general procedure 2. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with 100% ethyl acetate yielded **4** as a pale brown foam (502 mg, 620 mmol, 76%), R_f (B) 0.63 + 0.73 (diastereomers); δ_P[$(CD_3)_2CO$ int. in DCM] 149.09 (d).

5-[3-(*tert*-Butyldimethylsilyloxy)prop-1-ynyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 5

Preparation was carried out from 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine[27] and required the use of the protected prop-2-ynyl alcohol, *tert*-butyldimethyl(prop-2-ynyloxy)-silane.[28] General procedure 2 followed by product purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–5%) yielded **5** as a brown foam (0.893 g, 1.28 mmol, 65%) (Found: C, 66.64; H, 7.05; N, 3.80. $C_{39}H_{46}N_2O_8Si$ requires C, 66.96; H, 6.63; N, 4.01%); R_f (B) 0.57, (C) 0.63; δ_H($CDCl_3$) 0.03–0.08 (6 H, m, 2 × CH_3), 0.82–0.89 (9 H, m, 3 × CH_3), 2.17–2.20 (1 H, m, 2′H), 2.47–2.61 (1 H, m, 2″H), 3.26–3.49 (2 H, m, 5′Hs), 3.74 (6 H, s, 2 × CH_3O), 4.09–4.10 (1 H, m, 4′H), 4.23 (2 H, s, 3Hs), 4.54 (1 H, m, 3′H), 5.89–6.05 (2 H, br s, 3′OH + NH), 6.30 (1 H, dd, 1′H), 6.78–6.83 (4 H, m, DMTr), 7.13–7.43 (9 H, m, DMTr) and 7.91 (1 H, s, 6H); δ_C($CDCl_3$) 25.57 (CH_3), 25.62 (CH_3), 41.21 (CH_2), 51.85 (CH_2), 54.98 (CH_3), 63.49 (CH_2), 71.75 (CH), 75.59 (C), 85.51 (CH), 86.21 (CH), 86.60 (C), 91.91 (C), 99.69 (C), 113.05 (CH), 126.64 (CH), 127.76 (CH), 127.82 (CH), 129.80 (CH), 135.28 (C), 135.44 (C), 142.21 (CH), 144.36 (C), 150.08 (C), 158.30 (C), 158.43 (C) and 162.63 (C); *m*/*z* (FAB) 699.310 21 [$(M + H)^+$ calc. for $C_{39}H_{47}N_2O_8Si$: 699.310 21].

5-[3-(*tert*-Butyldimethylsilyloxy)prop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) 6

This compound was prepared from 5-[3-(*tert*-butyldimethylsilyloxy)prop-1-ynyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **5** according to general procedure 2. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with 100% ethyl acetate yielded **6** as a yellow foam (440 mg, 0.489 mmol, 67%), R_f (B) 0.79; δ_P[$(CD_3)_2CO$ int. in DCM] 147.81 (s).

5-Trimethylsilylethynyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 7

This compound was prepared from 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine[27] and trimethylsilylacetylene according to general procedure 1. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–5%) yielded **7** as a pale yellow foam (2.365 g, 3.78 mmol, 83%), R_f (C) 0.42; δ_H($CDCl_3$) −0.01 (9 H, t, ^2J 7.0, 3 × CH_3), 2.15–2.21 (1 H, m, 2′H), 2.48–2.55 (1 H, m, 2″H), 3.24–3.41 (2 H, m, 5′H), 3.75 (6 H, s, 2 × CH_3O), 4.12–4.13 (1 H, m, 4′H), 4.44–4.46 (1 H, m, 3′H), 6.00–6.38 (2 H, br s, 3′OH + NH), 6.29 (1 H, dd, 1′H), 6.80–6.86 (4 H, m, DMTr), 7.15–7.45 (9 H, m, DMTr) and 8.03 (1 H, s, 6H); δ_C($CDCl_3$) −0.56 (CH_3), 41.25 (CH_2), 55.00 (CH_3), 63.37 (CH_2), 72.11 (CH), 85.70 (CH), 86.49 (CH), 94.69 (C), 99.40 (C), 100.36 (C), 113.12 (CH), 126.70 (CH), 127.72 (CH), 127.84 (CH), 129.78 (CH), 135.37 (C), 142.56 (CH), 144.27 (C), 149.39 (C), 158.29 (C) and 161.66 (C); *m*/*z* (FAB) 627.248 44 [$(M + H)^+$ calc. for $C_{35}H_{39}N_2O_7Si$: 627.252 66].

5-Ethynyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) 8

This compound was prepared from 5-ethynyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine[29] according to general procedure 2. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with 100% ethyl acetate yielded **8** as a pale yellow foam (440 mg, 5.83 mmol, 66%), R_f (D) 0.72; δ_P[$(CD_3)_2CO$ int. in DCM] 149.56 (d); *m*/*z* (FAB) 755.321 72 [$(M + H)^+$ calc. for $C_{41}H_{49}N_4O_8P$: 755.320 978].

5-Cyano-5′,3′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine 9

To a solution of 5-iodo-5′,3′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine[30] (2.5 g, 4.195 mmol) in DMF (25 ml) was added sodium cyanide (226 mg, 4.61 mmol, 1.1 equiv.) and the mixture left to stir for 24 hours at room temperature after which the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate (250 ml), washed with saturated aqueous KCl (3 × 100 ml), dried (Na_2SO_4) and then reduced to a yellow foam [crude 6-cyano-5′,3′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine] which was subsequently dissolved in DMF (25 ml). Sodium cyanide (226 mg, 4.61 mol, 1.1 equiv.) was added, the mixture heated to 80 °C and left to stir for 18 hours after which TLC showed the reaction to be complete. The solvent was removed *in vacuo* and the residue dissolved in ethyl acetate (250 ml). The organic phase was washed with saturated aqueous KCl (3 × 100 ml), dried (Na_2SO_4) and purified by wet flash column chromatography eluting with methanol in dichloromethane (0–5%) to give the product as a yellow foam (1.367 g, 2.76 mmol, 64%), R_f (C) 0.73; δ_H($CDCl_3$) 0.87–1.29 (28 H, m, diisopropyl Hs), 2.25–2.33 (1 H, m, 2′H), 2.46–2.58 (1 H, m, 2″H), 3.76–3.81 (1 H, m, 4′H), 3.97 (1 H, dd, 5′H), 4.15 (1 H, dd, 5″H), 4.32–4.42 (1 H, m, 3′H), 5.94 (1 H, d, *J* 6.0, 1′H), 7.25 (1 H, br s, NH) and 8.32 (1 H, s, 6H); δ_C($CDCl_3$) 12.23–13.27 (CH), 16.63–17.24 (CH_3), 39.56 (CH_2), 59.24 (CH_2), 66.12 (CH), 85.37 (CH), 85.52 (CH), 89.56 (C), 112.91 (CN), 147.26 (CH), 149.23 (C) and 160.77 (C); ν_{max}(Nujol)/cm^{-1} 2233 (m, CN); *m*/*z* (FAB) 496.227 58 [$(M + H)^+$ calc. for $C_{22}H_{38}N_3O_6Si_2$: 496.229 92].

6-Cyano-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 10

5-Iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine[27] (1.0 g, 1.52 mmol) was dissolved in DMF (10 ml) and sodium cyanide (112 mg, 2.29 mmol, 1.5 equiv.) added with stirring. After 24 hours TLC showed the reaction to be complete and the solvent was removed *in vacuo* before the residue was dissolved in ethyl acetate (100 ml). The organic phase was then washed with saturated aqueous KCl (3 × 50 ml), dried (Na_2SO_4) and reduced to a yellow oil which was purified by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–4%) to yield **10** as a white foam (444 mg, 799 mmol, 53%), R_f (C) 0.56; δ_H($CDCl_3$) 2.18–2.29 (1 H, m, 2′H), 2.40–2.53 (1 H, m, 2″H), 3.28 (1 H, dd, 5′H), 3.61 (1 H, dd, 5″H), 3.72 (6 H, s, 2 × CH_3O), 3.66–3.92 (1 H, m, 4′H), 4.27–4.35 (1 H, m, 3′H), 6.19 (1 H, s, 5H), 6.61 (1 H, t, *J* 7.5, 1′H), 6.72–6.81 (4 H, m, DMTr) and 7.10–7.44 (11 H, m, DMTr 9Hs + 3′OH + NH); δ_C($CDCl_3$) 38.81 (CH_2), 54.90 (CH_3), 63.73 (CH_2), 71.49 (CH), 83.42 (CH), 84.46 (CH), 85.97 (C), 112.16 (C), 112.74 (CH), 114.92 (CH), 123.91 (C), 126.41 (CH), 127.46 (CH), 127.96 (CH), 129.80 (CH), 129.87 (CH), 135.64 (C), 135.87 (C), 144.51 (C), 154.13 (C), 158.07 (C) and 167.86 (C); ν_{max}(Nujol)/cm^{-1} 2233 (m, CN); λ_{max}(DCM–MeOH 9 : 1)/nm 282; *m*/*z* (FAB) 556.209 57 [$(M + H)^+$ calc. for $C_{31}H_{30}N_3O_7$ 556.208 38].

6-Cyano-5′-*O*-(4,4′-dimethoxytrityl-2′-deoxyuridine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) 11

This compound was prepared from 6-cyano-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **10** according to general procedure 2. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with 100% ethyl acetate yielded **11** as a clear oil (89 mg, 0.118 mmol, 24%), R_f (B) 0.67; δ_P[$(CD_3)_2CO$ int. in DCM] 148.45 (s).

8-Bromo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 12

8-Bromo-2′-deoxyadenosine[31] (2.5 g, 7.58 mmol) was co-evaporated three times with anhydrous pyridine (20 ml) before dissolving in anhydrous pyridine (25 ml) and 4-dimethylaminopyridine (185 mg, 1.52 mmol, 0.2 equiv.) was added. To this was added portionwise dimethoxytrityl chloride (3.08 g, 9.10 mmol, 1.2 equiv.) over 3 hours and the mixture left to stir for a further 1 hour. The reaction was quenched with methanol (20 ml) and the solvents removed *in vacuo*. The residue was dissolved in ethyl acetate (250 ml) and then washed with saturated aqueous KCl (3 × 100 ml), dried (Na_2SO_4) and the solvent removed. The residue was purified by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–6%) to give the product as a yellow foam (3.135 g, 4.96 mmol, 65%) (Found: C, 59.25; H, 5.56; N, 11.05. $C_{31}H_{30}BrN_5O_5$ requires C, 58.85; H, 4.78; N, 11.08%); R_f (B) 0.37, (C) 0.69; $\delta_H(CDCl_3)$ 2.28–2.38 (1 H, m, 2′H), 3.38–3.40 (2 H, m, 5′Hs), 3.52–3.63 (1 H, m, 2″H), 3.71 (6 H, s, 2 × CH_3O), 4.15–4.21 (1 H, m, 4′H), 4.48 (1 H, br s, 3′OH), 4.90–4.96 (1 H, m, 3′H), 6.39 (3 H, t + br s, *J* 7.0, 1′H + NH_2), 6.70–6.74 (4 H, m, DMTr), 7.08–7.37 (9 H, m, DMTr) and 8.03 (1 H, s, 4H); $\delta_C(CDCl_3)$ 36.56 (CH_2), 54.95 (CH_3), 63.52 (CH_2), 72.03 (CH), 85.89 (C), 85.94 (CH), 86.07 (CH), 112.75 (CH), 120.02 (C), 126.46 (CH), 127.45 (CH), 127.57 (C), 127.89 (CH), 129.78 (CH), 129.80 (CH), 135.74 (C), 135.78 (C), 144.54 (C), 150.45 (C), 152.25 (CH), 154.14 (C) and 158.11 (C); *m*/*z* (FAB) 632.154 40 [$(M + H)^+$ calc. for $C_{31}H_{31}BrN_5O_5$: 632.150 86].

8-(Prop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 13

This compound was prepared from 8-bromo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine **12** and propyne according to general procedure 1. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–5%) gave the product as a yellow foam (915 mg, 1.55 mmol, 98%), R_f (B) 0.43, (C) 0.66; $\delta_H(CDCl_3)$ 2.01 (3 H, s, CH_3), 2.27–2.34 (1 H, m, 2′H), 3.39–3.49 (3 H, m, 2″H + 5′Hs), 3.70 (3 H, s, CH_3O), 3.71 (3 H, s, CH_3O), 4.19–4.21 (1 H, m, 4′H), 4.27–4.52 (1 H, br s, 3′OH), 4.84–4.87 (1 H, m, 3′H), 6.34 (2 H, br s, NH_2), 6.55 (1 H, t, *J* 7.0, 1′H), 6.68–6.73 (4 H, m, DMTr), 7.11–7.40 (9 H, m, DMTr) and 8.02 (1 H, s, 2H); $\delta_C(CDCl_3)$ 9.73 (CH_3), 36.86 (CH_2), 54.93 (CH_3), 63.90 (CH_2), 69.01 (C), 72.61 (CH), 84.80 (CH), 85.90 (C), 86.10 (CH), 94.15 (C), 112.72 (CH), 119.27 (C), 126.43 (CH), 127.43 (CH), 127.91 (CH), 129.78 (CH), 129.80 (CH), 135.11 (C), 135.76 (C), 135.84 (C), 144.59 (C), 148.88 (C), 152.74 (C), 154.86 (C) and 158.11 (C); λ_{max}(DCM–MeOH 9:1)/cm^{-1} 284; *m*/*z* (FAB) 592.255 10 [$(M + H)^+$ calc. for $C_{34}H_{36}N_5O_5$: 592.255 99].

***N*(6)-Benzoyl-8-(prop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 14**

8-(Prop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine **13** (500 mg, 0.846 mmol) was co-evaporated with anhydrous pyridine (3 × 5 ml) before dissolving in anhydrous pyridine (5 ml) and trimethylsilyl chloride (460 mg, 0.54 ml, 4.23 mmol, 5 equiv.) was added with stirring. After 15 minutes the mixture was cooled to 0 °C and benzoyl chloride (595 mg, 0.49 ml, 4.23 mmol, 5 equiv.) was added. The mixture was left to warm to room temperature whilst stirring for 2 hours then quenched with methanol (20 ml) and the solvent removed *in vacuo*. The residue was dissolved in ammonia-saturated methanol (20 ml) and left for 1 hour before being reduced and dissolved in ethyl acetate (100 ml). The organic phase was washed with saturated aqueous KCl (3 × 50 ml), dried (Na_2SO_4) and reduced to an oil. The oil was purified by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–5%) to give the product as a yellow foam (342 mg, 0.493 mmol, 58%), R_f (B) 0.54; $\delta_H(CDCl_3)$ 2.08 (3 H, s, CH_3), 2.36–2.37 (1 H, m, 2′H), 3.40–3.50 (3 H, m, 2″H + 5′Hs), 3.71 (3 H, s, CH_3O), 3.72 (3 H, s, CH_3O), 4.18–4.23 (1 H, m, 4′H), 4.84–4.92 (1 H, m, 3′H), 6.58 (1 H, t, *J* 7.0, 1′H), 6.68–6.74 (4 H, m, DMTr), 7.13–7.80 (12 H, m, 9 × DMTr + 3 × benzyl), 8.14 (2 H, d, *J* 7.0, benzyl), 8.53 (1 H, s, 2H) and 9.31 (1 H, br s, NH); $\delta_C(CDCl_3)$ 9.72 (CH_3), 36.82 (CH_2), 54.98 (CH_3), 63.90 (CH_2), 68.74 (C), 72.75 (CH), 85.20 (CH), 85.96 (C), 86.35 (CH), 96.22 (C), 112.73 (CH), 122.35 (C), 127.48 (CH), 127.64 (CH), 127.94 (CH), 128.57 (CH), 129.68 (CH), 129.83 (CH), 131.73 (CH), 135.50 (C), 135.80 (C), 137.63 (C), 144.49 (C), 148.71 (C), 150.38 (CH), 152.11 (C), 158.16 (C) and 164.42 (C); *m*/*z* (FAB) 696.282 61 [$(M + H)^+$ calc. for $C_{41}H_{38}N_5O_6$: 696.282 21].

***N*(6)-Benzoyl-8-(prop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) 15**

This compound was prepared from *N*(6)-benzoyl-8-(prop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine **14** according to general procedure 2. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with 100% ethyl acetate yielded **15** as a cream foam (315 mg, 0.352 mmol, 88%), R_f (B) 0.74; δ_P[$(CD_3)_2CO$ int. in DCM] 148.82 (s).

***N*(2)-Dimethylaminomethylene-8-bromo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyguanosine 16**

8-Bromo-2′-deoxyguanosine[32] (150 mg, 0.34 mmol) was co-evaporated from anhydrous pyridine (3 × 5 ml), then dissolved in anhydrous pyridine (5 ml) and dimethylformamide dimethylacetal (619 mg, 0.69 ml, 5.20 mmol, 12 equiv.) added. After stirring at 40 °C for 1 hour TLC showed the reaction to be complete and the solvents were removed *in vacuo*. The residue was co-evaporated from anhydrous pyridine (3 × 5 ml) before being redissolved in anhydrous pyridine (5 ml) and 4-dimethylaminopyridine (5.3 mg, 0.04 mmol, 0.2 equiv.) added. To the stirred mixture was added portionwise dimethoxytrityl chloride (176 mg, 0.520 mmol, 1.2 equiv.) over 3 hours and the mixture was left to stir for a further 1 hour. The reaction was quenched with methanol (10 ml) and the solvents removed *in vacuo*. The residue was dissolved in ethyl acetate (50 ml), washed with saturated aqueous KCl (3 × 20 ml), dried (Na_2SO_4), the solvent removed and the residue purified by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–5%) to give the product as a white foam (80 mg, 0.114 mg, 26%), R_f (B) 0.56, (C) 0.45; $\delta_H(CDCl_3)$ 2.30–2.41 (1 H, m, 2′H), 2.98 (3 H, s, CH_3), 3.02 (3 H, s, CH_3), 3.15–3.33 (2 H, m, 2″H + 5′H), 3.44–3.57 (1 H, m, 5′H), 3.75 (6 H, s, 2 × CH_3O), 3.98–4.04 (1 H, m, 4′H), 4.75–4.82 (1 H, m, 3′H), 6.36 (1 H, dd, *J* 6.0 + 7.5, 1′H), 6.73–6.77 (4 H, m, DMTr), 7.13–7.38 (9 H, m, DMTr) and 8.13 (1 H, s, formamidine H); $\delta_C(CDCl_3)$ 35.06 (CH_3), 37.63 (CH_2), 41.28 (CH_3), 55.11 (CH_3), 63.93 (CH_2), 72.41 (CH), 84.14 (CH), 84.92 (CH), 86.23 (C), 112.96 (CH), 120.69 (C), 122.07 (C), 126.74 (CH), 127.70 (CH), 127.95 (CH), 129.84 (CH), 129.87 (CH), 135.67 (C), 135.67 (C), 144.54 (C), 151.06 (C), 156.10 (C), 156.43 (C), 157.80 (CH) and 158.35 (C); *m*/*z* (FAB) 703.188 05 [$(M + H)^+$ calc. for $C_{34}H_{36}BrN_6O_6$: 703.187 97].

***N*(2)-Dimethylaminomethylene-8-(prop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyguanosine 17**

This compound was prepared from *N*(2)-dimethylaminomethylene-8-bromo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyguanosine **16** and propyne according to general procedure 1. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with methanol in dichloromethane (0–5%) gave the product as a pale yellow foam (51 mg, 0.77 mmol, 77%), R_f (C) 0.48; $\delta_H(CDCl_3)$ 2.06 (3 H, s, CH_3), 2.33–2.47 (1 H, m, 2′H), 3.01 (3 H, s, CH_3), 3.05 (3 H, s, CH_3), 3.16–3.32 (2 H, m, 2″H + 5′H), 3.40–3.46 (1 H, m, 5″H), 3.72 (6 H, s, 2 × CH_3O), 4.04–4.08 (1 H, m, 4′H), 4.74–4.83 (1 H, m, 3′H), 6.35 (1 H, dd, *J* 6.0 + 7.5, 1′H), 6.70–6.74 (4 H, m, DMTr),

7.12–7.37 (9 H, m, DMTr) and 8.33 (1 H, s, formamidine H); $\delta_C(CDCl_3)$ 9.84 (CH_3), 35.02 (CH_3), 37.73 (CH_2), 41.30 (CH_3), 55.06 (CH_3), 63.94 (CH_2), 68.51 (C), 71.94 (CH), 84.29 (CH), 85.10 (CH), 86.03 (C), 96.61 (C), 112.86 (CH), 120.49 (C), 126.61 (CH), 127.60 (CH), 127.96 (CH), 129.82 (CH), 129.86 (CH), 134.65 (C), 135.70 (C), 135.73 (C), 144.57 (C), 151.12 (C), 156.14 (C), 156.73 (C), 157.90 (CH) and 158.23 (C); *m*/*z* (FAB) 663.293 87 [$(M + H)^+$ calc. for $C_{37}H_{39}N_6O_6$: 663.293 11].

N(2)-Dimethylaminomethylene-8-(prop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyguanosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) 18

This compound was prepared from *N*(2)-dimethylaminomethylene-8-(prop-1-ynyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyguanosine **17** according to general procedure 2. Purification by wet flash column chromatography (silica pre-equilibrated with 1% Et_3N) eluting with 100% ethyl acetate gave a white foam (41.1 mg, 0.048 mmol, 79%), R_f (B) 0.87; $\delta_P[(CD_3)_2CO$ int. in DCM] 149.32 (d).

Oligonucleotide synthesis

Oligonucleotide synthesis was performed on an Applied Biosystems 394 DNA synthesizer. All DNA synthesis reagents and cyanoethylphosphoramidite monomers were supplied by Applied Biosystems. The phosphoramidite monomers were used at a concentration of 0.12 M in anhydrous dichloromethane. Normal DNA synthesis cycles were modified by the introduction of additional steps for modified monomers. Thus two 10 second and one 25 second deliveries of anhydrous dichloromethane were added to the synthesis column after the coupling step together with a 6 second reverse argon flush added after each dichloromethane wash and a 3 second argon purge added after all three dichloromethane washes and reverse argon flushes. In addition, the coupling wait time was prolonged to 5 minutes in order to increase coupling yields.

Deprotection of oligonucleotides containing monomer 6. This was carried out by dissolving the crude oligonucleotide in 1.5 ml of 1.1 M TBAF in THF solution and the mixture left overnight. After evaporation the tetrabutylammonium counter ion was exchanged for ammonium by passing an aqueous solution (1 ml) of the oligonucleotide through a Dowex (NH_4^+) column. Purification was then carried out as normal according to the procedure detailed below.

Oligonucleotide analysis and purification

Reversed-phase HPLC analysis and purification were carried out on a Gilson model 306 HPLC system using ABI Brownlee Aquapore Octyl reversed phase columns. Oligonucleotides were desalted on a Sephadex G-25 column (35 cm × 2 cm) using a 10 ml min^{-1} flow rate.

Enzyme digests

The oligonucleotide (10 OD) to be digested was dissolved in buffer (0.5 ml, 1 M NaCl, 50 mM tris, pH = 8.8) and phosphodiesterase I (type VII from crotalus atrox venom, 5 μl = 0.05 units) added and alkaline phosphatase (type VII from bovine intestinal mucosa, 1 μl = 0.15 units) added. The digest was incubated for 48 hours at 37 °C and analysed by HPLC at 260 nm. The peaks arising from modified bases were collected and examined by UV to ensure they were modified.

Ultraviolet melting studies

The ultraviolet melting temperatures of all oligonucleotides containing modified bases hybridized to a normal DNA template were measured. The melting temperature of the control unmodified duplex was also determined in each case. Each melting curve was measured in triplicate at 260 nm on a Perkin-Elmer Lambda 15 ultraviolet spectrometer equipped with a Peltier Block and controlled by an IBM PS2 microcomputer. Where thermodynamic parameters were calculated the melting temperatures were measured at 6 points over a 20-fold concentration range. A heating rate of 0.9 K per minute was used throughout and the crude data were collected and processed using the PECSS-2 software package. The oligonucleotides were dissolved in a buffer consisting of aqueous sodium chloride (0.1 M), sodium dihydrogen orthophosphate (0.01 M) and EDTA (1 mM) which had been adjusted to pH 7.0 by the addition of sodium hydroxide. The melting temperatures of the modified oligonucleotides were collected at duplex concentrations of 13.08 μM for purine modifications and 9.74 μM for pyrimidine modifications.

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