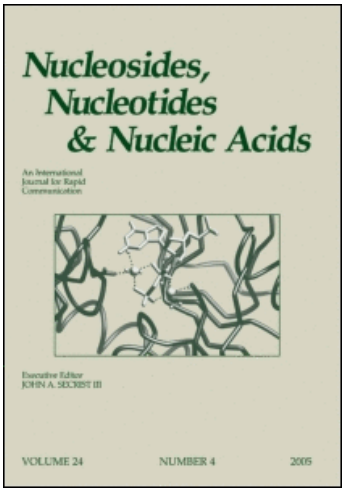

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2′-*O*-DIMETHYLAMINOETHOXYURIDINE AND 5-DIMETHYLAMINOPROPARGYL DEOXYURIDINE FOR AT BASE PAIR RECOGNITION IN TRIPLE HELICES

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□ *The nucleoside analogues 2′-O-dimethylaminoethoxy uridine and 5-dimethylaminopropargyl deoxyuridine have been synthesised and incorporated into oligonucleotides. Their triplex-stabilising properties have been determined in fluorescence melting experiments.*

Keywords Antigene; Triple helices; Triplexes; AT recognition

INTRODUCTION

Triplex-forming oligonucleotides (TFOs) containing 2′-*O*-aminoethoxy-5-aminopropargyluridine (*bis*-amino U) form very stable triplets with AT base pairs in double stranded DNA, making these modified TFOs potentially useful in antigene therapy.[1] The stabilising effect is due to specific interactions between the charged primary amino groups attached to the modified nucleoside and the phosphodiester backbone of the complementary duplex.[2] (The pro-R phosphate group in the case of the 2′-aminoethoxy side-chain.)

One of the drawbacks of the *bis*-amino U modified nucleoside is the requirement for amino protecting groups during solid-phase oligonucleotide synthesis. In certain cases the conditions required to remove these

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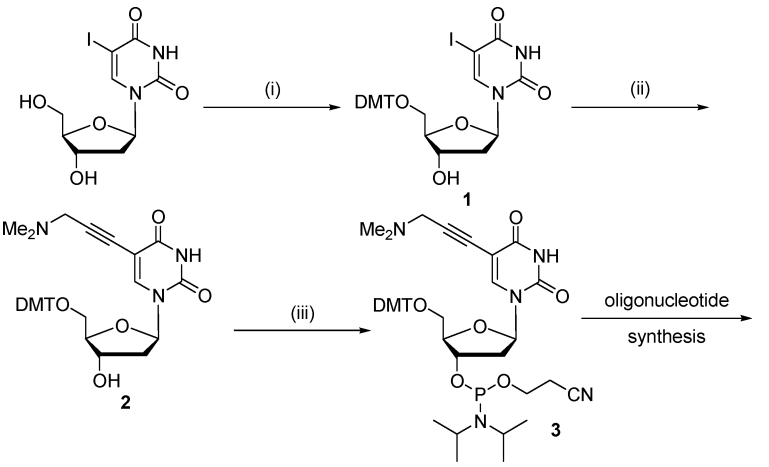


SCHEME 1 Synthesis of 5-dimethylaminopropargyl deoxyuridine. (i) DMTCl, pyridine, rt, 3 hours, 85%; (ii) $(Me)_2NCH_2CCH$, Et_3N, CuI, $Pd^0(PPh_3)_4$, DMF, rt, 16 hours, 84%; (iii) $(^iPr)_2NPCl$ (OCH_2CH_2CN), THF, 2 hr, rt, 72%.

protecting groups (e.g., aqueous methylamine) are too harsh for certain other modifications in the DNA strand (e.g., the cross-linker Psoralen).

In order to circumvent this problem we have prepared phosphoramidites of 5-dimethylaminopropargyl deoxyuridine **3** and 2′-*O*-dimethylaminoethoxyuridine **7**, incorporated them into oligonucleotides and determined their triplex-stabilising properties in fluorescence melting experiments. The synthesis of the two phosphoramidite monomers **3** and **7** is described and results of fluorescence melting experiments are presented.

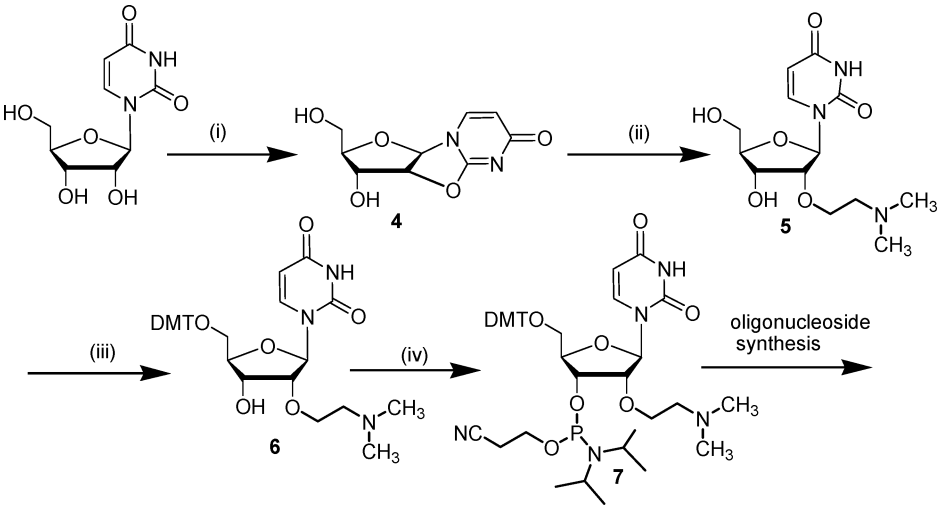
RESULTS AND DISCUSSION

Synthesis of 5-Dimethylaminopropargyl Deoxyuridine

The synthesis of 5-aminopropargyl deoxyuridine has been described previously.[3] A similar strategy was employed for 5-dimethylaminopropargyl deoxyuridine (Scheme 1). Starting from 5-iodo-2′-deoxyuridine, protection of the 5′-hydroxyl group was achieved using dimethoxytrityl chloride in pyridine to give the corresponding 5′-*O*-dimethoxytrityl ether **1**. Compound **1** was then coupled with 3-dimethylaminopropyne under Pd-catalysed cross-coupling conditions[4] to give compound **2**, which was then converted to the corresponding phosphoramidite **3**.

Synthesis of 2′-O-Dimethylaminoethoxyuridine

This synthesis was achieved using published procedure[5,6] (Scheme 2). Uridine was converted into 2,2′-anhydro-1-β-D-arabinofuranosyluracil **4** using phenyl carbonate in the presence of sodium hydrogen carbonate



SCHEME 2 Synthesis of 2′-*O*-dimethylaminoethoxyuridine. (i) $(PhCO)_2O$, $NaHCO_3$, DMF, 100°C, 4 hours, 100%; (ii) Al foil, dimethylaminoethanol, 140°C, 24 hours; (iii) DMTCl, pyridine, rt, 3 hours, 50%; (iv) $[(iPr)_2N]_2P(OCH_2CH_2CN)$, DIHT, DCM, 16 hours, rt, 80%.

in DMF before reaction with the aluminium alkoxide of dimethylaminoethanol to give 2′-dimethylaminoethoxyuridine **5**. Compound **5** was subsequently converted into its 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-phosphoramidite **7**.

Compounds **3** and **7** were incorporated into two different 17-mer TFOs by automated solid-phase DNA synthesis (phosphoramidite method) to give oligonucleotides **TFO1** and **TFO2** respectively. **TFOs 3** and **4** were synthesised for comparative fluorescence melting studies, as well as the unmodified control oligomer. All oligomers were purified by reverse-phase HPLC and characterised by MALDI-TOF mass spectrometry.

5′-**Q**-**X**$\underline{\text{C}}\underline{\text{C}}$T**X**$\underline{\text{C}}T\underline{\text{C}}$**X**T**X**T**X**T$\underline{\text{C}}$**X**T-3′ (TFO)

5′-**F**-GTGTTAGGAAGAGAAAAAAGAACTGGT-HEG

|

CACAATCCTTCTCTTTTTTCTTGACCA-HEG

In the TFOs, $\underline{\text{C}}$ = 5-methyldeoxycytidine and Q = amidohexyldabcyl. In the hairpin duplex F = 6-amidohexylfluorescein, HEG = (hexaethylene) glycol.

Dabcyl-labelled TFOs were hybridized to a complementary hairpin duplex labelled with fluorescein. Results from fluorescence melting experiments are summarised in Table 1.

It has been shown previously that uridine based derivatives such as *bis*-amino U show selective binding affinity towards AT base pairs,[7] and it is envisaged that other uridine based derivatives such as used in this study would also will show similar selectivity towards AT base pairs.

TABLE 1 T_m values determined at pH 6.0 and pH 6.5. Concentration 2.5 μM:0.25 μM (TFO:duplex) in 20 mM sodium acetate with 200 mM NaCl. Modifications in TFOs **3** and **4** have been described previously.[3,8]

TFO	Modification (x)	T_m (pH 6.5)	ΔT_m (pH 6.5)	T_m (pH 6.0)	ΔT_m (pH 6.0)
Unmod	thymidine	38.1	—	45.0	—
1	5-dimethylaminopropargyl dU (DMAPdU)	48.5	10.4	56.5	11.5
2	2′-*O*-dimethylaminoethoxy U (DMAEU)	41.2	3.1	49.2	4.2
3	5-aminopropargyl dU (APdU)	56.2	18.1	65.1	20.1
4	5-propynyl dU (PdU)	46.6	8.5	54.4	9.4

It has been suggested that modification with a propynyl group bearing a charged or an electronegative group at the 5-position of the nucleobase, may lead to an X.AT triplet that is more stable than T.AT.[8] Our results confirm this and show that TFOs with a positive charge attached to a propynyl group in the 5-position of thymine analogues are more stable than those with uncharged propynyl dU, even in the case of the relatively bulky dimethylamino group. It appears that hydrogen bonding is not the sole determinant of stability as the dimethylaminopropargyl substituent enhances triplex stability (although to a lesser extent than aminopropargyl). In future it will be informative to make a comparison between the 2′-*O*-dimethylaminoethoxy uridine analogue in this study and 2′-*O*-aminoethoxy uridine.[9]

In conclusion, two novel nucleoside analogues, each containing a dimethylamino group, have been synthesised and incorporated into triplex-forming oligonucleotides. Fluorescence melting experiments with a complementary hairpin duplex have been carried out. Results show that although the 5-dimethylaminopropargyl group is not as stabilising as the analogous 5-aminopropargyl modification, the charge seems to impart more stability than the uncharged 5-propynyl-derivative.

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