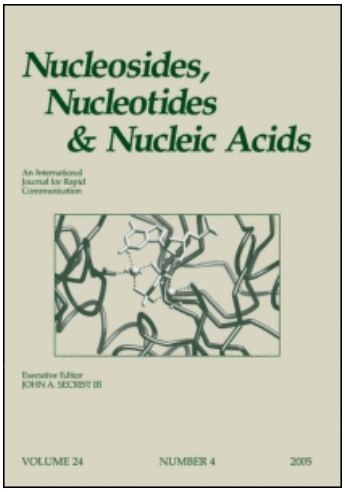

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Use of C5-Diamino-Substituted-Pyridine in Triple Helix Forming Oligonucleotides

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USE OF C5-DIAMINO-SUBSTITUTED-PYRIDINE IN TRIPLE HELIX FORMING OLIGONUCLEOTIDES.

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ABSTRACT : Triple helical structures can be observed between double-stranded nucleic acids and a third strand through the formation of Hoogsteen hydrogen bonds. We report here the synthesis and the preliminary evaluation of oligonucleotides incorporating 5[(N-2-aminoethyl)-3-aminopropynyl]-2'-deoxyuridine **1** as well as its reduced analogue **2**. Synthesis of two phosphoramidites **8** and **9** and the first melting temperature measurements are described.

The antisense strategy is based on the inhibition of gene expression resulting from the complementary hybridization of synthetic oligonucleotides (ODN) to single-stranded RNA targets (1). The efficiency of this method is linked to the high specificity of the interaction between complementary nucleic acid sequences. However, various mRNA structures (hairpins, bulges, pseudo-nots) can reduce or prevent the binding of the antisense ODN to the target sequence (2). To circumvent this difficulty, an alternative strategy was developed where the antisense ODN is designed to interfere directly with a double-stranded region of the mRNA via the formation of a local triple helix (3). The main limitation of this strategy is the weak energy of interaction between the third strand and the double-stranded target. There are a few possible ways to overcome this limitation. One way consists in designing modified nucleic acid bases. In this line C5-propynyl-uridine revealed to be one of the most efficient modification increasing significantly duplex and triplex stability (4). The increase of π-π stacking ability of the heterocycle has been proposed to account for these properties (5). It is also well known that naturally occuring

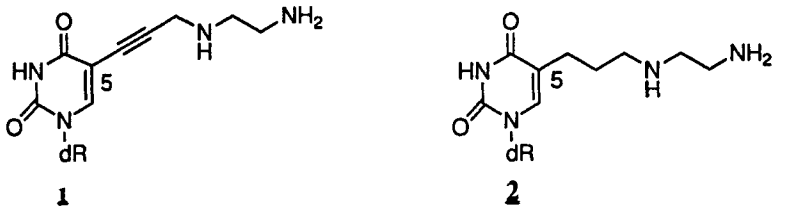
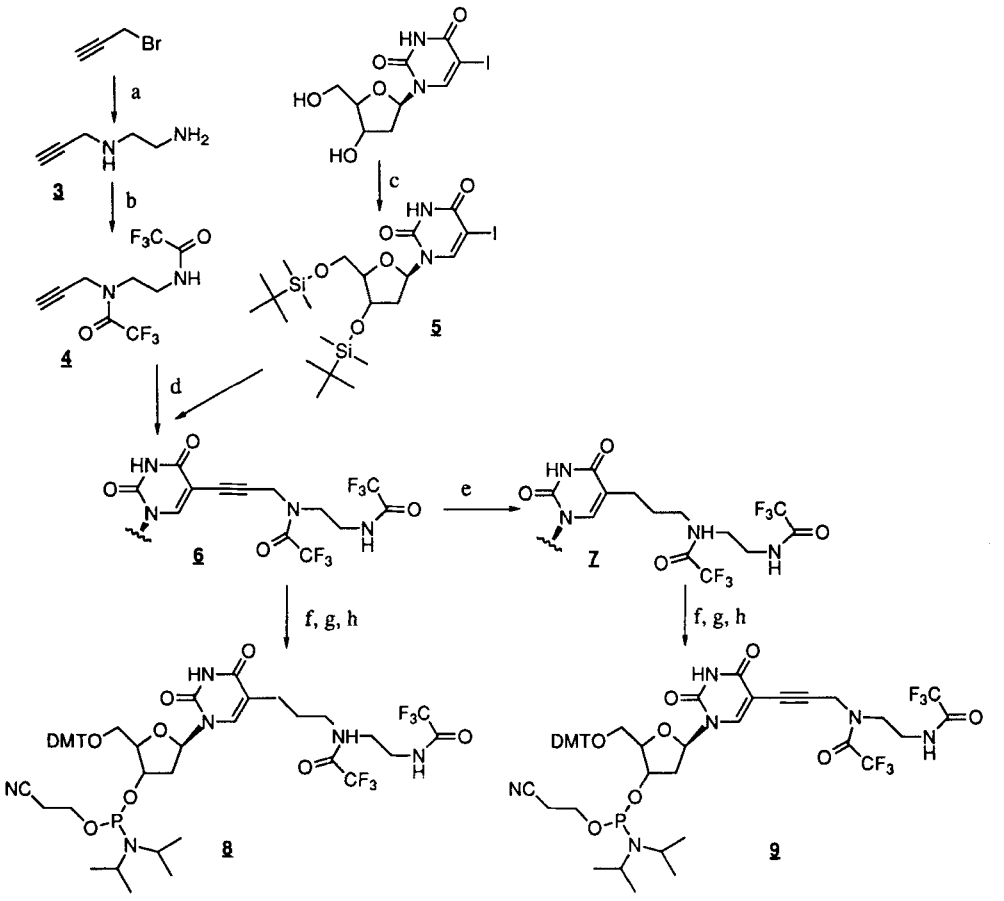


Fig. 1

polyamines such as spermidine and spermine stabilise duplexes and triplexes by reducing the anionic electrostatic repulsion between the phosphate groups (6). We therefore chose to associate these two properties into a single modified base by synthesizing C-5 propynyldiamino substituted uridine. We report here the synthesis and the preliminary evaluation of oligonucleotides incorporating 5[(N-2-aminoethyl)-3-aminopropynyl]-2'-deoxyuridine **1** as well as its reduced analogue **2** for comparison (Fig.1).

The full synthetic pathway is shown in Fig.2.

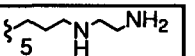
The key step of this synthesis is the direct coupling of alkyne derivatives with 5-iodo-2'-deoxyuridine according to Hobbs (7). The alkynyl arm was elaborated with the commercially available propargylbromide and ethylenediamine. The diamine **3** was purified by distillation. The amine groups were then protected by trifluoroacetic groups introduced via the ethyltrifluoroacetate. **4** was carefully purified by chromatography. The 5-iodo-uridine-2'-deoxy was classically protected by a silyl group using tert-butylchlorodimethylsilane. The nucleoside **5** was then used without any purification. Condensation between **4** and **5** was performed in the presence of triethylamine, CuI and $Pd(PPh_3)_4$. **6** was purified by chromatography (8). At this step, part of **6** was reduced by catalytic hydrogenation under pressure in the presence of 10% Pd/C without any difficulty. **6** and **7** were then treated through the same reaction steps to afford the two phosphoramidites. The nucleosides were easily deprotected with triethylaminetrihydrofluoride. The 5' hydroxyls were classically protected by the acido-labile dimethoxytrityl group, and the 3' hydroxyls were activated by the chlorophosphite derivative. At each reaction steps compounds were purified by chromatography. All these synthetic compounds were checked by mass spectroscopy. ^{1}H NMR and ^{13}C NMR. ^{31}P NMR was used for **8** and **9**.



Reagents and conditions : a) Ethylenediamine (10eq), DBU (1eq), Toluene, RT, overnight, 45% ; b) Ethyltrifluoroacetate (4eq), MeOH, 50°C, 3 days, 76% ; c) ^{t}Butylchlorodimethylsilane (3eq), imidazole (5eq), DMF, RT, 2h, 94% ; d) Et_3N (30eq), $Pd(PPh_3)_4$ (0.01eq), CuI (0.2eq), 40°C, 48h, 76% ; e) 10%Pd/C, MeOH, 5Kg Pressure H_2, 72% ; f) $Et_3N(HF)_3$ (3eq), CH_2Cl_2, RT, overnight, 60% ; g) DMTCl (1.2eq), pyridine, RT, 24h, 56% ; h) DIEA (4eq), 2-cyano-N,N-diisopropylphosphoramido-chloridite (1.5eq), CH_2Cl_2, RT, 1h, 60%.

Fig.2

Table 1.

		X	Thymine			
I	A C C T T T T X T T T T T 5' C G A A A A A A A A A A 3' A C C T T T T T T T T T 3'		34°C	nd	32°C	32°C
II	A C C T T T T T T T T T T 5' C G A A A A A A A A A A 3' A C C T T T X T T T T T 3'		34°C	32°C	34°C	33°C

Buffer : 100mM NaCl, 10mM Mg acetate, 10mM Na cacodylate (pH6).

Oligonucleotide concentration : 0.25µM/(each partner).

nd : not determined.

The two modifications were introduced in DNA sequences I and II (table 1). In I the modification takes place in the duplex target, in II it is in the third strand. We measured a few preliminary melting temperatures and observed a weak destabilisation of duplexes and triplexes. In this context the C-5 propynyl uridine falled to stabilize the triple-stranded structure (9).

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8. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) : 0 (4s, 12H, CH_3Si) ; 0.85 (s, 18H, CH_3Si iPr) ; 2.0-2.3 (2m, 2H, $H_{2'}$) ; 3.8 (m, 7H, $H_{5'}$, $H_{4'}$, H_{5d}, H_{5e}) ; 4.3 (m, 3H, H_3, H_{5c}) ; 6.2 (t, 1H, $H_{1'}$, J =) ; 8.1 (s, 1H, H_6) ; 9.8 (bb, 1H, NH). ^{13}C NMR (50.32 MHz, $CDCl_3$) δ (ppm) : -5.5, -4.9, -4.7 (tBuSi) ; 17.9-18.3 (CHSi) ; 25.6-25.8 (CH_3Si) ; 38.3-39.2 (C_{5d}, C_{5e}) ; 42.1 ($C_{2'}$) ; 46.9 (C_{5c}) ; 62.8 ($C_{5'}$) ; 72.0 ($C_{3'}$) ; 76.6 (C_b) ; 86.0 ($C_{1'}$) ; 87.0 (C_a) ; 88.5 ($C_{4'}$) ; 98.1 (C_5) ; 115.8 (CF_3, q, J = 301 Hz) ; 143.2 (C_6) ; 148.8 (C_2) ; 158.0 (Carbonyl TFA) ; 163.6 (C_4).
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