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Synthesis of a novel bis-amino-modified thymidine monomer for use in DNA triplex stabilisation

Matthieu Sollogoub,[a] Beatriz Dominguez,[a] Keith R. Fox[b] and Tom Brown*[a]

[a] *Department of Chemistry, University of Southampton, Highfield, Southampton, UK SO17 1BJ. E-mail: tb2@soton.ac.uk*

[b] *Division of Biochemistry and Molecular Biology, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, UK SO16 7PX. E-mail: krf1@soton.ac.uk*

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A novel thymidine analogue containing both 5-aminopropargyl† and 2′-aminoethoxy modifications has been synthesised and incorporated into a triplex-forming oligonucleotide; the combination of the two amino groups on the same nucleoside greatly enhances triplex stability.

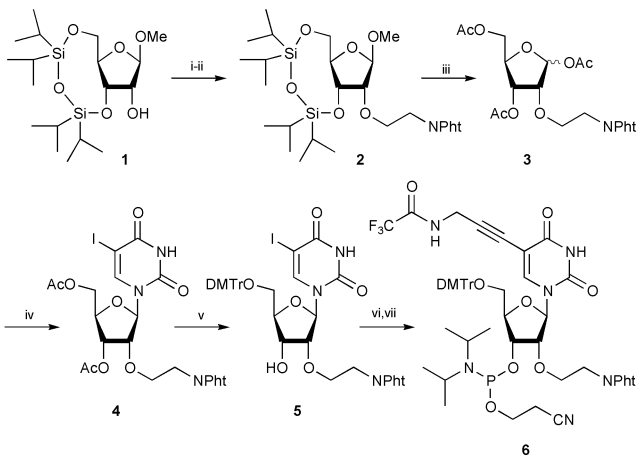
DNA triple-helix formation by sequence-specific triplex-forming oligonucleotides is of considerable interest because of its great potential in a variety of analytical and therapeutic applications, including selective control of gene expression,[1] site-directed mutagenesis,[2] gene repair,[3] *in situ* chromosome labelling and other molecular diagnostics.[4] Although triplex-forming oligonucleotides bind with high specificity, their binding is usually much weaker than that of the underlying DNA duplex. In part this is thought to be due to the charge repulsion resulting from bringing together the three polyanionic DNA strands. Studies by ourselves and others[5] have shown that the introduction of positively charged groups at multiple sites in the backbone, sugar or base can produce stable triplexes, but to date only one such group has been added per nucleoside. Combining two of these modifications in a single nucleoside should produce stable triplexes across a wide range of salt concentrations, particularly under physiological conditions.

To test this hypothesis, we have synthesised a thymidine analogue containing two modifications that individually are known to stabilise triplexes: 5-aminopropargyl[6] and 2′-aminoethoxy.[7] These groups were specifically chosen so that the amines would act at different sites in a cooperative manner. An obvious synthesis for such a compound appears to be alkylation of the 2′-position of the nucleoside as reported for the 2′-aminoethoxy thymidine analogue.[7] However this strategy was not successful for our compound due to side reactions at the 5-position of the pyrimidine. We therefore had to adopt a longer route including a glycosylation step after introduction of the aminoethoxy moiety onto the sugar. The successful synthesis of the doubly modified monomeric thymidine building block **6** is shown in Scheme 1.

Alkylation of the protected β-Me ribofuranoside **1**[8] with methyl bromoacetate in the presence of sodium hydride (74% yield), followed by reduction of the ester by $LiBH_4$ and replacement of the hydroxy group by a phthalimide substituent using Mitsunobu[9] conditions yielded **2** (89% for two steps). Phthalimide is a suitable protecting group for oligonucleotide synthesis because of its stability to the acidic conditions encountered in dimethoxytrityl removal and ease of removal in standard basic conditions at the end of the synthesis. Attempted glycosylations[10] with **2** resulted in ring opening of the sugar. The anomeric position was therefore activated by means of an acetyl group *via* acetolysis with acetic anhydride and a catalytic amount of sulfuric acid in glacial acetic acid. Under these conditions[8] the TIPDS was replaced by acetyls to afford **3** in 95% yield. Triacetate **3** was transformed into the desired nucleoside **4** in 82% yield with silylated 5-iodouracil in the presence of TMSOTf (Vorbrüggen reaction conditions,[11] 82% yield, 2:1 β–α separable mixture). The β-anomer was then deacetylated using NaOMe powder in MeOH and the reaction was quenched by Dowex 50 (pyridinium form) then directly tritylated with freshly recrystallised dry DMTrCl in pyridine to yield **5** (63% for two steps). The aminopropargyl moiety was added protected as the trifluoroacetamide (equally suitable to oligonucleotide synthesis as phthalimide) by a palladium coupling reaction[12] in 89% yield. Conventional phosphitylation of the 3′-hydroxy group in an inert atmosphere using 2-cyanoethoxy(*N*,*N*-diisopropylamino)chlorophosphine afforded the phosphoramidite monomer **6** suitable for oligonucleotide synthesis in 70% yield.

Oligonucleotide syntheses were performed on an ABI 394 automated DNA/RNA synthesiser following standard cycles. The monomer **6** was incorporated in good yield (5 min coupling, > 98.5%) and oligonucleotides were deprotected with concentrated aqueous NH_3 at 55 °C for 5 h. After reversed-phase HPLC purification, the oligonucleotides were desalted (Sephadex G25) and characterised by ESMS.

We synthesised the intramolecular triplex forming oligonucleotide P3 containing a double substitution of the modified monomer **6** and the UV-melting curve was monitored at pH 7 (Table 1). P3 showed an increase in T_m of 22.9 K relative to P2 which contains two aminopropargyl modifications and an increase of 48.7 K relative to unmodified oligonucleotide P1. The general sequence of oligonucleotides P1, P2 and P3 was



Scheme 1 *Reagents and conditions:* (i) $BrCH_2CO_2Me$ (5 eq.), NaH (2.2 eq.), DMF, RT, 3 h, 74 %; (ii) (a) $LiBH_4$ (2 eq.), THF, MeOH, −20 → 0 °C, 1 h; (b) PhtNH (1.05 eq.), PPh_3 (1.05 eq.), DEAD (1.05 eq.), THF, RT, 30 min, 89 %; (iii) Ac_2O, AcOH, H_2SO_4, RT, 3 h, 95%; (iv) 5-iodouracil-bis-TMS (3 eq.), TMSOTf (3 eq.), $Cl(CH_2)_2Cl$, −20 →0 °C, 3 h, 82% (α:β 1:2); (v) (a) MeONa (3 eq.), MeOH, RT, 5 min; (b) DMTrCl (3 eq.), Py, RT, 3 h, 63%; (vi) $HC{\equiv}CCH_2NHCOCF_3$ (3 eq.), CuI (0.25 eq.), Et_3N (5 eq.), $Pd(PPh_3)_4$ (0.1 eq.), DMF, RT, 16 h, 89%; (vii) $(iPr)_2NP(Cl)OCH_2CH_2CN$ (1.5 eq.), DIPEA (2.2 eq.), CH_2Cl_2, RT, 16 h, 70%.

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Table 1 Melting temperatures (T_m) measured at pH 7.0; 50 mM phosphate buffer with 100 mM NaCl : L, octanediol linker; ^{p}U, 5-aminopropargyl modified T; X, bis-amino modified T

	Oligonucleotide	T_m/K
P1	T_6-L_2-T_6-AGTCT-L_2-AGACT-A_6	295
P2	T^{p}UT^{p}UTT-L_2-T_6-AGTCT-L_2-AGACT-A_6	320.8
P3	TXTXTT-L_2-T_6-AGTCT-L_2-AGACT-A_6	343.4

chosen as this motif is known to give clear triplex melting transitions.[6]

Footprinting data from a 9-mer oligonucleotide containing three bis-amino-modified thymidines (XTTXTTCXT) showed a reduction in the intensity of bands within the footprint by 50% at a concentration of 1.0 ± 0.5 μM. This is in contrast to the same sequence with three aminopropargyl T nucleosides which failed to show a footprint at concentrations as high as 50 μM. The footprints were located at a single target site within a 110 base pair fragment indicating that the oligonucleotide retained considerable sequence selectivity.

The above UV-melting data (intramolecular triplexes) and DNase I footprinting experiments (intermolecular triplexes) show that the 5-aminopropargyl/2′-aminoethoxy combination contributes significantly to triplex stabilisation, and is greatly superior to the aminopropargyl substitution alone. Hence the doubly modified nucleotide **6** appears to be a very promising building block for triplex-forming oligonucleotides.

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Notes and references

† The IUPAC name for propargyl is prop-2-ynyl.

1 C. Giovannangeli and C. Helene, *Antisense Nucleic Acid Drug Dev.*, 1997, **7**, 413; C. Giovannangeli, L. Perrouault, C. Escude, N. Thuong and C. Helene, *Biochemistry*, 1996, **35**, 10 539; L. J. Maher, P. B. Dervan and B. Wold, *Biochemistry*, 1992, **31**, 70.
2 G. Wang, D. D. Levy, M. M. Seidman and P. M. Glazer, *Mol. Cell. Biol.*, 1995, **15**, 1759; G. Wang, M. M. Seidman and P. M. Glazer, *Science*, 1996, **271**, 802.
3 S. Broitman, O. Amosova, N. G. Dolinaya and J. R. Fresco, *J. Biol. Chem.*, 1999, **274**, 21 763.
4 M. D. Johnson and J. R. Fresco, *Chromosoma*, 1999, **108**, 181.
5 For a review see: K. R. Fox, *Curr. Med. Chem.*, 2000, **7**, 17.
6 J. Bijapur, M. D. Keppler, S. Bergqvist, T. Brown and K. R. Fox, *Nucleic Acids Res.*, 1999, **27**, 1802.
7 B. Cuenoud, F. Casset, D. Husken, F. Natt, R. M. Wolf, K.-H. Altmann, P. Martin and H. E. Moser, *Angew. Chem., Int. Ed.*, 1998, **37**, 1288.
8 J. P. Schaumberg, G. C. Hokanson and J. C. French, *J. Org. Chem.*, 1985, **50**, 1651.
9 O. Mitsunobu, *Synthesis*, 1981, 1.
10 P. Martin, *Helv. Chem. Acta*, 1996, **79**, 1930.
11 H. Vorbrüggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, 1981, **114**, 1234.
12 S. Takahashi, Y. Kuroyama, K. Sonogashira and N. Hagihara, *Synthesis*, 1980, 627; F. W. Hobbs, *J. Org. Chem.*, 1989, **54**, 3420.