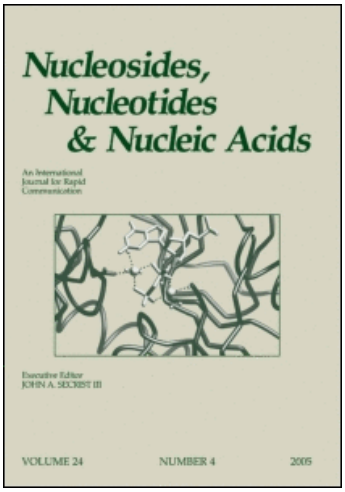

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SYNTHESIS OF ANTHRAQUINONE OLIGONUCLEOTIDES FOR TRIPLEX STABILIZATION

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□ *The synthesis of two anthraquinone phosphoramidites is described. In both cases the anthraquinone moiety is attached via a linker to the 5-position of a uracil base, allowing incorporation at any thymidine position in an oligonucleotide sequence. Anthraquinone-modified oligonucleotides have potential applications as triplex stabilizers and fluorescence quenchers.*

Keywords Anthraquinone; triplex stabilizers; fluorescence quenchers

INTRODUCTION

Triplex-forming oligonucleotides (TFOs),[1] have potential uses as therapeutic agents, since they could regulate gene expression by direct interaction with genomic dsDNA. However, the resulting triplex is usually less stable than the corresponding duplex. In order to improve triplex stability, we have attached substituted anthraquinones to uracil bases in the expectation that intercalation of the anthraquinone moiety will stabilize the triple helix.[2] It also was anticipated that an attached tetramine[3] would further increase triplex stability by interacting with the phosphodiester groups of the DNA. We now report the synthesis of oligonucleotides containing the anthraquinone nucleosides in Figure 1.

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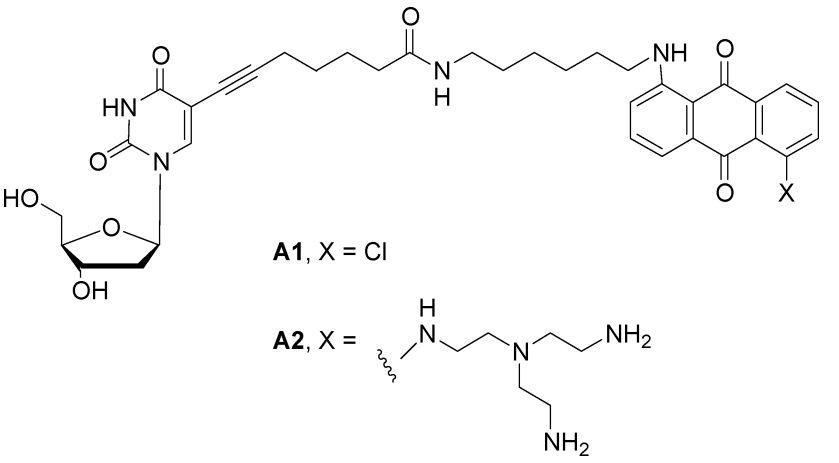
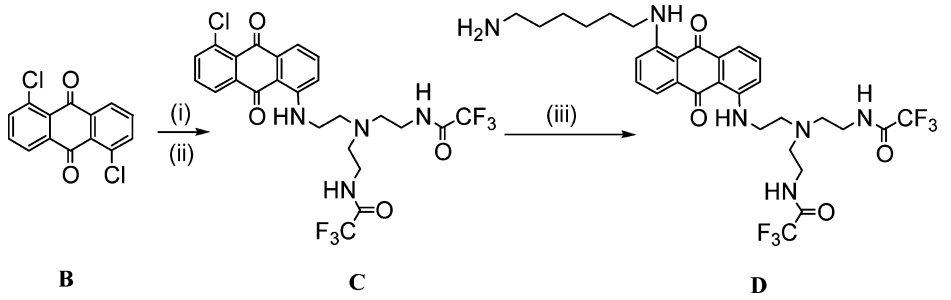


FIGURE 1 Anthraquinone moiety linked to the 5-position of 2′-deoxyuridine.

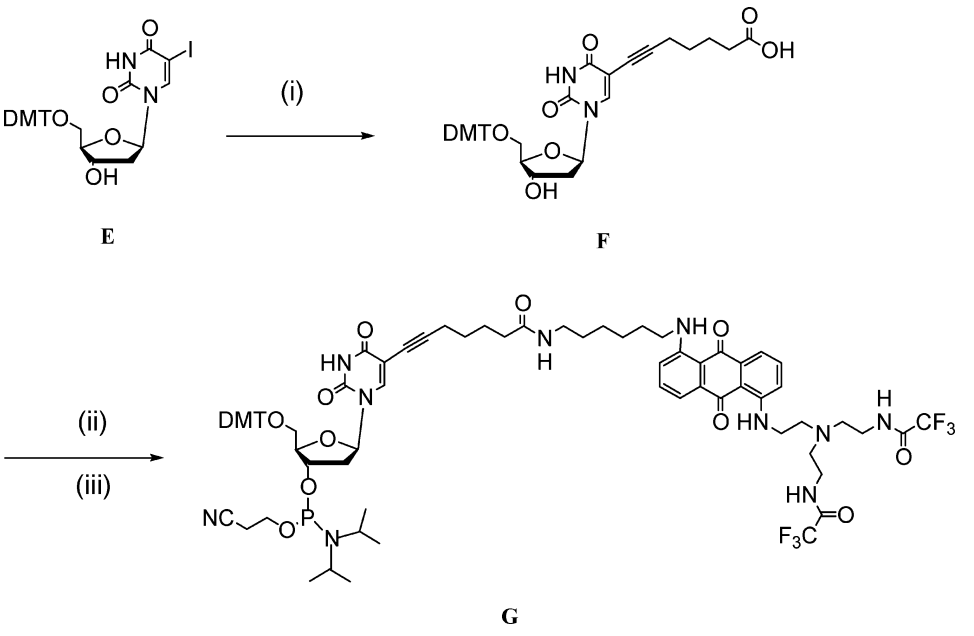
SYNTHESIS

The tetramine, tris(2-aminoethyl)amine and 1,6-diaminohexane were introduced onto the anthraquinone nucleus **B** by sequential nucleophilic substitution reactions (Scheme 1). Firstly the tetramine was added and protected with trifluoroacetyl to give **C**. The trifluoroacetyl protecting groups are compatible with standard DNA synthesis deprotection conditions. After this the diaminohexane was coupled to the remaining nucleophilic site.

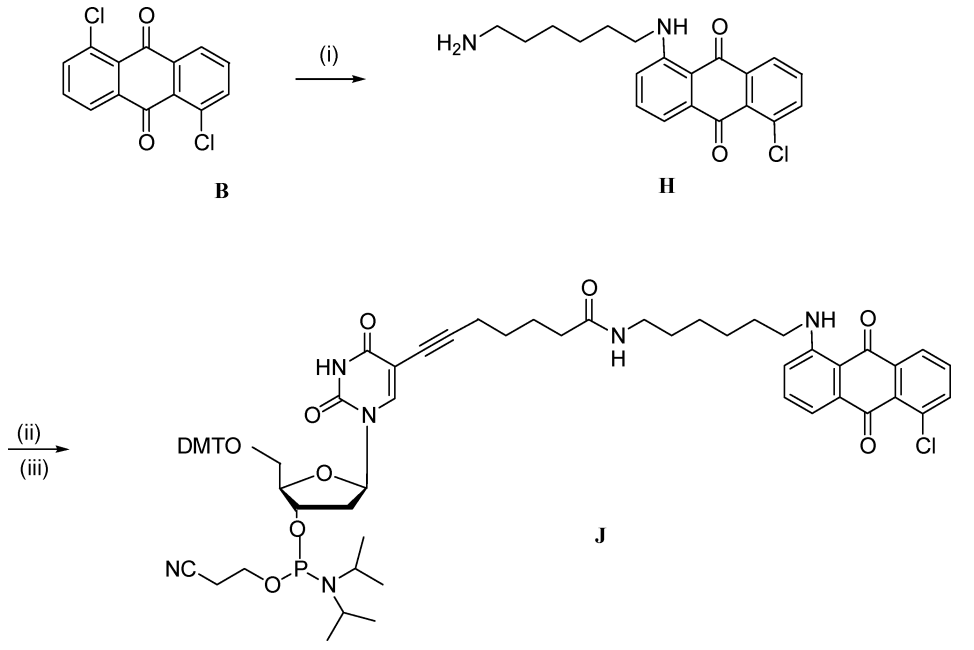
Reaction of 5′-O-DMTr-5-iodo-2′-deoxyuridine **E** with heptynoic acid gave the 5-substituted nucleoside **F** (Scheme 2). The anthraquinone-bearing tetramine **D** was coupled to the carboxylic acid group of the substituted deoxyuridine **F** using EDC and HOBT, then converted into phosphoramidite **G** for incorporation into oligonucleotides.



SCHEME 1 Preparation of anthraquinone-tetramine derivatives. (i) tris(2-aminoethyl)amine (3 eq), toluene, reflux 3 hours; (ii) ethyl trifluoroacetate (10 eq), triethylamine (10 eq), rt 5 hours, combined yield 31%, (iii) 1,6-diaminohexane, xylene, reflux overnight, 40%.



SCHEME 2 Synthesis of uridine bearing anthraquinone-tetramine phosphoramidite. (i) heptynoic acid, triethylamine, CuI, $(Ph_3P)_4Pd$, DMF, 85%; (ii) **D**, EDC, HOBT, DIPEA, DMF, overnight, 65%; (iii) 2-cyanoethyl *bis*-diisopropylphosphoramidite, DIHT, acetonitrile, 68%.



SCHEME 3 Synthesis of chloroanthraquinone bearing 2′-deoxyuridine phosphoramidite. (i) 1,6-diaminohexane (10 eq), xylene, reflux, 63%; (ii) **F**, EDC, HOBT, DIPEA, DMF, 86%; (iii) 2-cyanoethyl *bis*-diisopropylphosphoramidite (1.1 eq), DIHT (0.5 eq), acetonitrile, 88%.

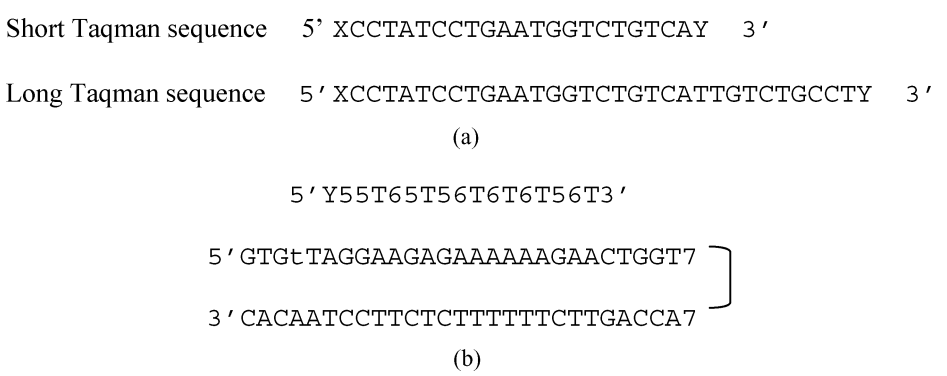


FIGURE 2 Sequences used for fluorescence quencher and fluorescence melting experiments. (a) X = FAM Y = tetramine-anthraquinone dU (**A2**); (b) 5 = 5-methyl dC, 6 = propargylamino-dU, 7 = HEG, t = Fluorescein dT (Glen Research), Y = tetramine-anthraquinone dU (A2).

The synthesis of chloroanthraquinone 2′-deoxyuridine (Figure 1, **A1**), is shown in Scheme 3. Applying a similar strategy, 1,6-diaminohexane was introduced onto the anthraquinone **B**, followed by coupling with the carboxylic function of nucleoside **F** using EDC and HOBT, and was finally converted into phosphoramidite **J**. The coupling and phosphitylation yields were higher than the corresponding tetramine bearing compound **G**.

Oligonucleotides were prepared using phosphoramidite monomers **G** and **J**, and standard phosphoramidite DNA synthesis reagents on an ABI 394 DNA/RNA synthesizer. The coupling time for **G** and **J** was extended from 25 seconds to 6 minutes and deprotection was carried out with concentrated aqueous ammonia at 55°C overnight. Oligonucleotide products were analyzed and purified by reversed-phase HPLC and gel electrophoresis, and characterized by MALDI-TOF MS.

The oligonucleotide bearing anthraquinone-tetramine **A2** was evaluated as a fluorescence quencher.[4] It was labelled at the 3′-end of the Taqman sequences in Figure 2a and used in real-time PCR to monitor the fluorescence changes. The short Taqman sequence was found to be more efficient in terms of cleavage and fluorescence change.

Preliminary fluorescence melting experiments,[5] on TFOs formed by the sequences in Figure 2b, indicate that the anthraquinone-bearing tetramine (**A2**) significantly stabilizes triplexes especially when the modification is at the 5′-end of the TFO. It is significantly more effective than the chloro-version (**A1**).

REFERENCES

1. Felsenfeld, G.; Davies, D.R.; Rich, A. Formation of a three-stranded polynucleotide molecule. *J. Am Chem. Soc.* **1957**, 2023–2024.

2. Huang, H.-S.; Chiu, H.-F.; Yeh, P.-F.; Yuan, C.-L. Structure-based design and synthesis of regioisomeric disubstituted aminoanthraquinone derivatives as potential anticancer agents. *Helv. Chim. Acta* **2004**, 87, 999–1006.
3. Zafrul Azam, A.; Moriguchi, T.; Shinozuka, K. Modified α–β chimeric oligoDNA bearing a multi-conjugate of 2,2-bis(hydroxymethyl) propionic acid-anthraquinone-polyamine exhibited improved and stereo-non-specific triplex-forming ability. *Chem. Commun.* **2006**, 335–337.
4. May, J.P.; Brown, L.J.; van Delft, I.; Thelwell, N.; Harley, K.; Brown, T. Synthesis and evaluation of a new non-fluorescent quencher in fluorogenic oligonucleotide probes for real-time PCR. *Org. Biomol. Chem.* **2005**, 3, 2534–2542.
5. Darby, R.A.J.; Sollogoub, M.; McKeen, C.; Brown, L.; Risitano, A.; Brown, N.; Barton, C.; Brown, T.; Fox, K.R. High throughput measurement of duplex, triplex and quadruplex melting curves using molecular beacons and a LightCycler. *Nucleic Acids Res.* **2002**, 30, e39.