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Oligonucleotides Covalently Linked to an Acridine at Artificial Abasic Site: Influence of Linker Length and the Base-Sequence

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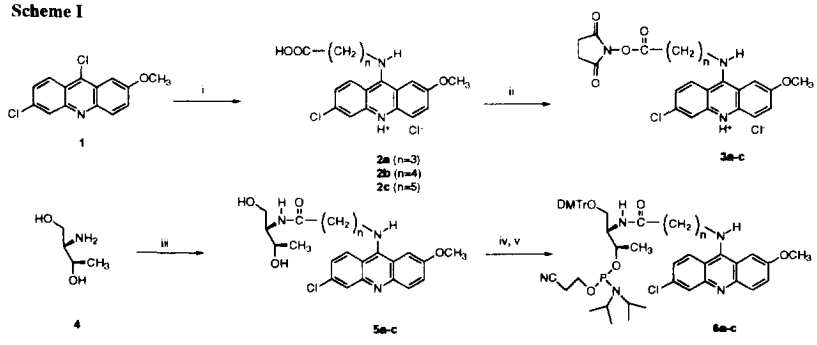
Abstracts: *Oligonucleotides covalently linked to an 9-amino-6-chloro-3-methoxyacridine via from a trimethylene to pentamethylene linker were prepared using novel L-threoninol backbone phosphoramidites. Although all of the modified oligonucleotides could bind to the complementary oligonucleotides, the behavior of intercalation of the acridine ring was strongly affected by linker length and the base-sequence.*

Oligonucleotides covalently linked to functional molecules have been attracting current interest because of their widespread biological usage such as in antisence[1] and DNA probes.[2] Moreover, it has also been demonstrated that the DNA helix involving a stacked aromatic column can serve as an efficient medium through which electron transfer from donor to acceptor occurs.[3,4] Thus, the methodology to incorporate the functional molecules at any desired site (or sites) of the oligonucleotide and to exactly anchor those into the DNA helix should be established not only for improvement of biological efficiency and selectivity but also for clear understanding of such electron transfer mechanism.

In the previous design of a handle connecting a dye to oligonucleotides, we have employed stereochemically defined L-threoninol **4** to mimic the spacing of the traditional ribose moiety and to suppress the appearance of several optical isomers in the incorporated DNA.[5] The acridine-oligonucleotides conjugate has been prepared by postsynthetic attachment of acridine-succinimidyl ester **3c** to an amino group of L-threoninol incorporated

Scheme I



Reagents and conditions: i) 4-Aminobutylic acid for **2a**, 5-aminovaleric acid for **2b**, 6-aminocaproic acid for **2c**, phenol, 100°C; ii) *N*-hydroxysuccinimide, EDC, DMF; iii) **3a-c**, triethylamine, MeOH; iv) DMTrCl, pyridine; v) $(i\text{-}Pr_2N)_2POEtCN$, tetrazole, CH_3CN.

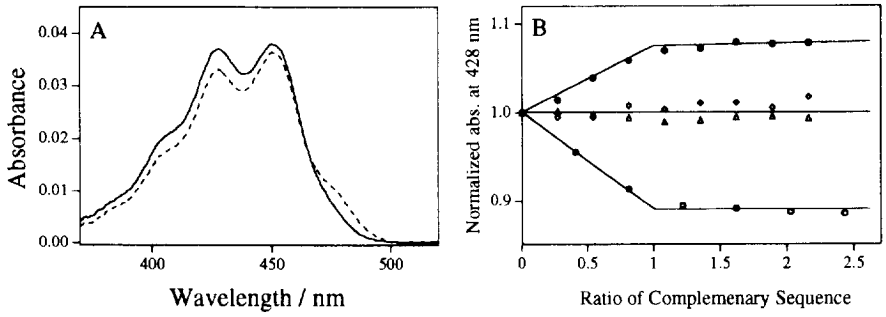


Figure 1. (A) : Absorption spectra of acridine moiety of **Ic** in single (solid line) and double (dashed line) stranded DNA formed between **Ic** and the complementary DNA **II**. (B) : Changes of absorbance of **Ia-c** at 428 nm in addition of complementary DNA. **Ia** (Δ), **Ib** (◊), **Ic** (o) with **II**, **Ic** with **III** (•). Measurements were carried out at 0 °C in 10 mM phosphate buffer/150 mM sodium chloride, pH 7.2.

into oligonucleotides. For these systems, it is well known that the length of the linker chain conjugating oligonucleotide and intercalater strongly affects the stability of double-stranded DNA and the style of intercalation.[6-8] In this postsynthetic procedure[5], however, we have encountered some difficulty: (i) The acridine-succinimide reagent **3a, b** is only slightly soluble in water, (ii) the protocol for syntheses and purification of the DNA is complicated and much time-consuming, and (iii) it is impossible to multilabel DNA with other dye-succinimide esters.[9] Hence it is of obvious importance to develop phosphoramidites that can be conveniently used in DNA synthesis to directly label and modify oligonucleotides. For the fulfillment of such requirement, we would like to report here the synthesis of novel phosphoramidites from an L-threoninol backbone and the behavior of acridine intercalated into double-stranded DNA.

The acridine-amidites **7a-c** were prepared according to Scheme I. We started from commercially available **1** (Aldrich Co.), which was derived to the succinimidyl ester **3c** in line with the previous report[5] and **3a, b** were synthesized in the same manner. The stereochemically pure 'amino-diol' L-threoninol **4** was prepared by reduction of natural amino acid, L-threonine.[8,10] Compounds **3a-c** were then allowed to react with small excess of **4**/ triethylamine (1:1) in methanol. After the solvent was removed, the gummy residue including **5b, c** was purified by silica gel column chromatography eluted with chloroform/methanol (85:15, v/v) containing 1% triethylamine. Compounds **5b** and **5c** were obtained in the forms containing two equivalent of triethylamine. Compound **5a**

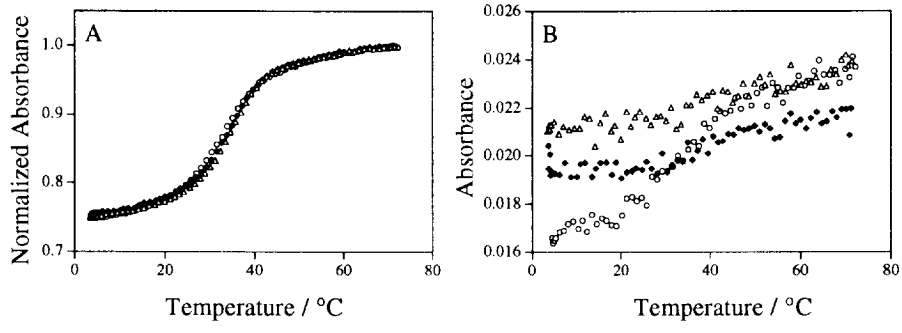


Figure 2. Melting curves measured at 260 nm (A) and 428 nm (B) for complexes of **Ia** (Δ), **Ib** (♦) and **Ic** (o) with **II**. The concentration of duplexes was 2.5 μM. The buffer was the same as that used in Figure 1.

was only slightly soluble in the ordinary organic solvent, thus **5a** was filtered and washed several times with DMF and chloroform, and used without further purification. Subsequent protection of the primary alcohol with dimethoxytrityl chloride in dry pyridine and phosphitylation of the secondary alcohol with 2-cyanoethoxy-bis(diisopropylamino)cyanoethoxyphosphine in dry acetonitrile gave phosphoramidites **6a-c** as yellowish foam.[11] The overall yields from **1** were 32% (**6a**), 31% (**6b**) and 26% (**6c**). The phosphoramidites synthesized were easily soluble in anhydrous acetonitrile and employed in a 0.15 M solution in the following DNA synthesis.

2-Cyanoethyl phosphoramidite (Expedite™ purchased from Millipore) was used for DNA synthesis. The modified oligonucleotide synthesis was carried out employing the standard synthetic cycle with an extended coupling time (5 min.) during the phosphoramidite coupling. The coupling efficiency of **6a-c** caluculated by dimethoxytrityl cation assay was more than 96%. Cleavage from the solid support and deprotection was accomplished with conc. ammonia for 2 hours at room temperature yielding 5'-dimethoxytritylated oligonucleotide. The crude product was purified by the reversed-phase HPLC. Finally, the dimethoxytrityl substrate was removed with 80% acetic acid for 15 min. and then again purified by the reversed-phase HPLC. It is noteworthy that for the phosporamidites containing 9-amino-6-chloro-3-methoxyacridine aq. NaOH solution was employed instead of conc. ammonia for the purpose of release from the solid support to avoid cleavage of the 9-aminoacridine bond.[12,13] Actually, treatment with conc. ammonia (55 °C, 8 hours) gave completely decomposed products. On the other hand, when Expedite phosporamidite was used under the deprotection time of less than 2 hours at room temperature, this decomposition could be suppressed to less than 10% and the impurities could be easily removed by HPLC. The following three types of acridine modified DNA were synthesized, where [Acr**n**] means acridine tethered to the $(CH_2)_n$ linker. At the same time, we used the oligonucleotides **II** and **III** as the "normal" complementary and the "bulged-adenine" complementary sequence, respectively (see Figure 3).

Ia : 5'-ATAATAA[Acr**3**]AATTAAT
Ib : 5'-ATAATAA[Acr**4**]AATTAAT
Ic : 5'-ATAATAA[Acr**5**]AATTAAT
II : 5'-ATTAATTTTATTAT
III : 5'-ATTAATTATTATTAT

Interactions of **Ia-c** with the complementary oligonucleotides **II** and **III** were investigated by use of UV absorption spectra and melting curves of the duplexes.

The absorption spectrum of the acridine moiety of **Ic** is shown in Figure 1A. When **Ic** bound to complementary sequence **II**, the hypochromic effect, that suggests the intercalation of the acridine ring into the DNA helix[7,14], was observed. As the concentration of the

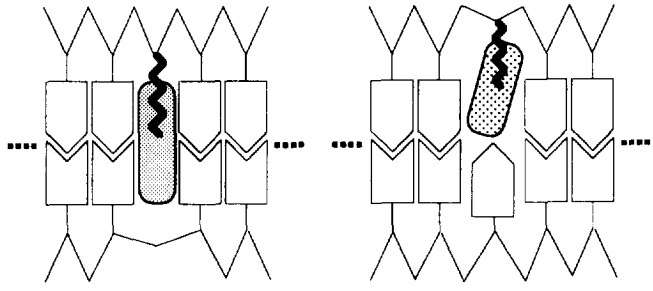


Figure 3. Schematic representation of intercalative geometries of acridine in double stranded DNA. The orientation in 'normal' DNA (left) and in 'bulged' DNA (right).

complementary sequence increased, the absorbance at 428 nm decreased (Figure 1B). The decrease in the absorbance definitely ceased at a specific concentration of **II**. This observation proves a strong interaction between the acridine and the bases which can be most plausibly explained in terms of the intercalation.[15] On the contrary, **Ia** and **Ib** having a shorter linker chain did not show any decrease at 428 nm (Figure 1B). Moreover, when **Ic** was mixed with **III** in which an adenine base exists at the opposite side of acridine, increase of absorbance was observed. These results suggest that the acridine ring was selectively intercalated into the neighboring abasic site and that existence of the large purine base prevents this intercalation[16] (Figure 3).

All of the oligonucleotides showed a clear sigmoid and the melting temperatures of **Ia**, **Ib**, and **Ic** were 34.7, 34.1 and 33.1 °C, respectively (Figure 2A). On the other hand, **Ic**-**III** duplex showed a lower value of 27.5 °C supporting the above result that the adenine base interferes with the intercalation of the acridine ring (Figure 3). Figure 2B shows melting curves at 428 nm characteristic of the acridine ring. Although there was some noise due to the low absorbance of the acridine ring, the thermodynamic behavior of **Ic** at 428 nm and 260 nm was nearly the same. In **Ia** and **Ib**, however, this behavior was rather different at the two wavelengths. These observations suggest that all of the modified oligonucleotides can form double-stranded DNA with complementary oligonucleotides, but only the longer chain and the defined sequence more favorably permit the intercalation of the acridine ring like intermolecular intercalation.[17]

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