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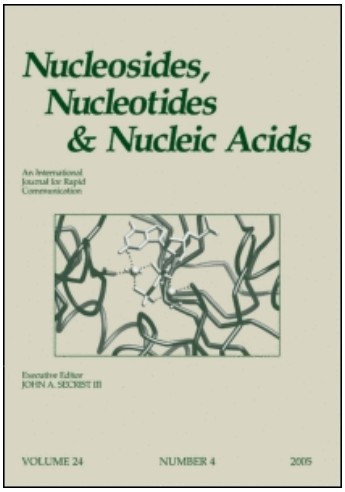

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SYNTHESIS AND ANTI-HIV PROPERTY OF NOVEL OLIGO-DNA PHOSPHOROTHIOATE ANALOGS BEARING AN INTERCALATIVE MOIETY AND/OR POLYAMINE RESIDUES

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ABSTRACT: Novel oligoDNA phosphorothioate analogs (S-ODN-1 to -3) bearing an intercalative moiety at the 5'-terminus and/or polyamine at the C-5 position of certain uridine residues substituting for normal thymidine residues were synthesized and their physicochemical properties as well as the anti-HIV activities were studied. The analogs have identical base sequence which is complementary to *art/tris* region of human immunodeficiency virus type 1 (HIV-1). The polyamine moiety on the analogs is found to be effective not only to enhance the hybridization ability but also reduce the non-specific cytotoxicity and strengthen the anti-HIV activity of the analogs.

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Introduction: Antisense technique utilizing single-stranded DNA or RNA has been attracting a great interest as a potential new therapeutic method since the discovery of its possible application as an anti-HIV agent.[1,2] Several synthetic analogs of DNA have been developed and studied as an antisense agent.[3] Among them, phosphorothioate analog of DNA (S-ODN) is the most widely used synthetic DNA as a suitable antisense reagent. However, S-DNA has several shortcomings such as low hybridization ability compared to the corresponding natural DNA and relatively high non-specific cytotoxicity.[4,5] We have conducted chemical modification of S-ODN to overcome these shortcomings. Here, we wish to report the synthesis of novel phosphorothioate DNA analogs bearing an intercalative moiety at the 5'-terminus and/or polyamine moiety at the C-5 position of certain uridine residues (S-ODN-1 to -3, **SCHEME 1**) and their physicochemical as well as anti-HIV properties.

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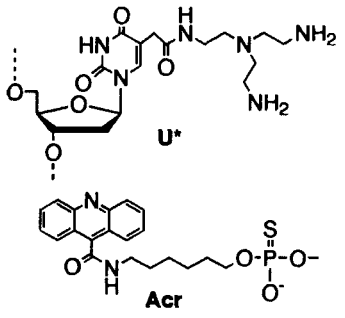
S-ODN-N 5' - TCG TCG CTG TCT CCG - 3'

S-ODN-1 5'- Acr - TCG TCG CTG TCT CCG - 3'

S-ODN-2 5' - TCG U*CG CTG TCU* CCG - 3'

S-ODN-3 5' -Acr- TCG U*CG CTG TCU* CCG - 3'



SCHEME 1. The structure of S-ODNs bearing an intercalative (acridine) moiety at 5'-terminus and/or polyamine moiety at C-5 position of certain uridine residues.

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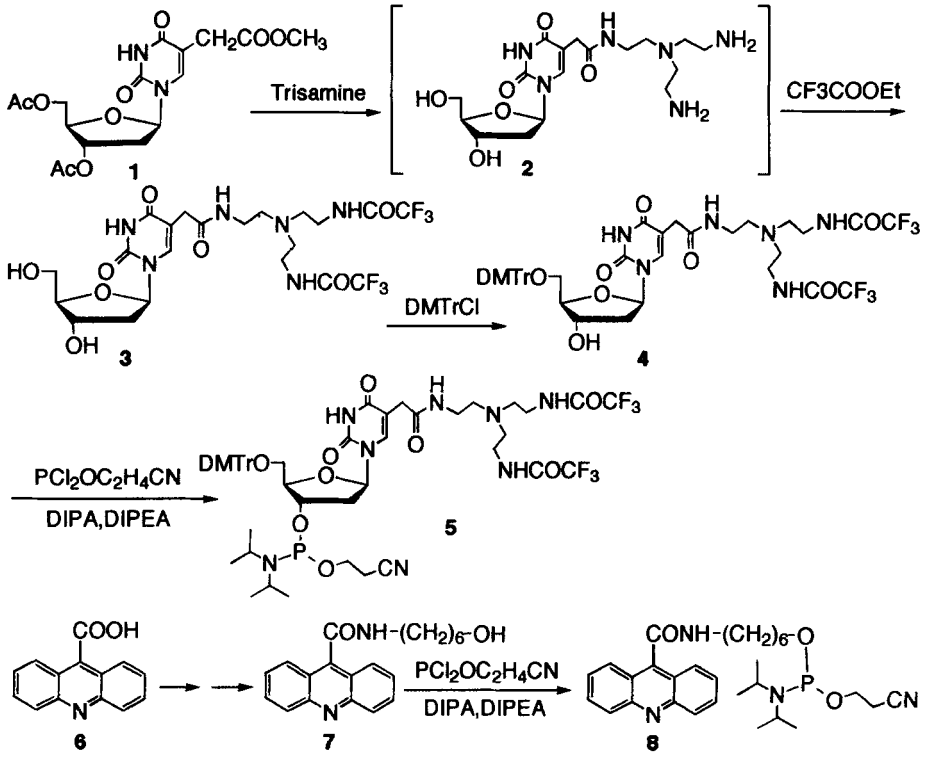
Synthesis of the S-ODNs bearing an intercalative moiety at the 5'-terminus and/or polyamine moiety at the C-5 position of certain uridine residues (S-ODN-1 to -3).

Incorporation of the polyamine-bearing uridine residue into phosphorothioate oligonucleotides was accomplished in a similar manner as described previously.[6] Thus, 5-[*N*-[2-[*N*,*N*-bis(2-trifulioroacetylaminoethyl)-amino]ethyl]carbonylmethyl-2'-deoxyuridine (**3**) was protected at its 5'-position with a dimethoxytrityl group (DMTr) and converted to the corresponding 3'-phosphoramidite derivative **5** by the standard phosphitylation procedure.[7] The compound **5** was incorporated into S-DNA-1 and -3 using an automated DNA synthesizer (ABI 381-A) in 1 μmol scale synthesis. The incorporation of an intercalative moiety (acridine moiety) at the 5'-terminus of S-ODN was also accomplished by use of the phosphoramidite derivative (**8**) derived from acridine carboxylic acid (**6**). These are summarized in **SCHEME 2**.

Hybridization ability of the S-ODNs

The duplex forming ability of the S-ODNs was examined by their Tm values determined by the UV-melting curves. An oligoDNA having normal phosphodiester linkage was used as the complement. Under near physiological condition (50 mM sodium phosphate, 100 mM NaCl, pH 7.2), the modified S-ODNs gave higher Tm values than that of the unmodified S-ODN (S-ODN-N, 41.6 °C). Thus, the incorporation of polyamine-bearing deoxyuridine substituting normal thymidine residdues and/or acridine into the S-ODN molecule is proved to be effective to increase the duplex forming ability. S-ODN-3 possessing both the polyamine-bearing deoxyuridine residues and the acridine moiety exhibited the highest Tm value (54.3 °C)



SCHEME 2. Preparation of phosphoramidite derivatives of C-5 substituted 2'-deoxyuridine and acridine carboxylic acid.

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among all. S-ODN-2 possessing only the polyamine-bearing deoxyuridine residues exhibited the second highest Tm value (51.5 °C).

Anti HIV activity of the S-ODNs.

Anti-HIV activity of the S-ODNs were assayed by monitoring p-24 gag protein expression using chronically HIV infected cells. Also, ^{3}H-thymidine uptake was measured to estimate the cytotoxicity of the oligomers. The chain elongated version (27-mer) of the S-ODNs (S-ODN-1L - 3L and S-ODN-NL) was used as the antisense agent.

All modified S-ODNs exhibited enhanced anti-HIV activity compared to the unmodified S-ODN-NL. S-ODN-1L, bearing an intercalator, and S-ODN-2L, bearing C-5 polyamine-substituted uridine residue, exhibited comparable anti-HIV activity while S-ODN-3L, bearing both an intercalator and C-5 polyamine-substituted uridine residue, showed the highest anti-HIV activity. For example, the expression of p-24 protein was

reduced to less than 10 % by S-ODN-3L while 20 % by S-ODN-NL at 10 μM concentration, compared to the control. The results of ^{3}H-thymidine uptake experiments also indicate that the introduction of polyamine moiety to an antisense agent is effective to reduce its cytotoxicity while the introduction of an intercalator slightly enhances the cytotoxicity.

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