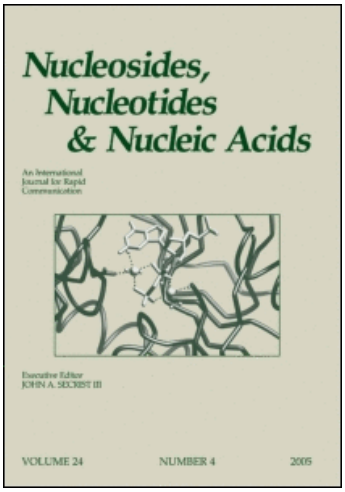

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Abasic Oligodeoxyribonucleoside Phosphorothioates as Inhibitors of the Human Immunodeficiency Virus-1 (HIV-1) Phosphorothioate Inhibition of HIV-1 Reverse Transcriptase and Interactions with Syrian Hampster Fibroblast (V79) Cells

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ABASIC OLIGODEOXYRIBONUCLEOSIDE PHOSPHOROTHIOATES AS INHIBITORS OF THE HUMAN IMMUNODEFICIENCY VIRUS-1 (HIV-1): PHOSPHOROTHIOATE INHIBITION OF HIV-1 REVERSE TRANSCRIPTASE AND INTERACTIONS WITH SYRIAN HAMPSTER FIBROBLAST (V79) CELLS.§

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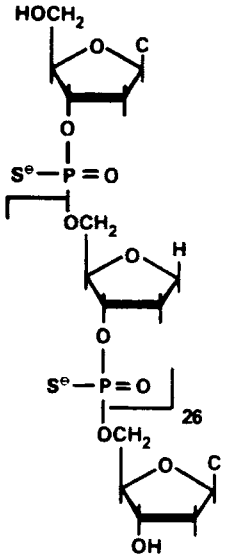
Abstract. An abasic oligonucleoside phosphosphorothioate has been synthesized and evaluated as an inhibitor of HIV-1 reverse transcriptase. The cellular binding to and mutagenicity of the abasic phosphorothioate with Syrian hampster fibroblasts have also been evaluated.

Oligodeoxyribonucleotide phosphorothioates (S-ODNs) have been shown to block the *de novo* infection of H9 cells by the human immunodeficiency virus, HIV-1[1]. This inhibitory effect is dependent on both the length and the composition of the S-ODN, but not on its specific sequence[1]. In a *de novo* infection assay, the α and β forms of phosphoric acid diester linked oligonucleotides show either markedly reduced or no inhibitory effect relative to the corresponding phosphorothioates[2,3]. These observations suggest that it is the $P(O)S^-$ functionality that is fundamentally responsible for the potent anti-HIV-1 activity of the S-ODNs, such as S-dC_{28} ($IC_{50} \approx 0.1\ \mu M$)[1]. To test this hypothesis and to provide insight into the molecular basis for the anti-HIV-1 activity of phosphorothioates, such as the role of reverse transcriptase (RT) inhibition in phosphorothioate blocking of HIV-1 infection, we have begun studies with abasic phosphorothioate sequences. The synthesis and anti-HIV-1 activity of S-d[$C(E)_{26}C$], 1, wherein "E" derives from 1,2-dideoxy-D-ribofuranose, have recently been reported by us[4]; comparative studies of 1 and S-dC_{28} as inhibitors of HIV-1 RT are herein communicated. The relative mutagenic potential and the ability to bind and be taken up by V79 cells is additionally reported.



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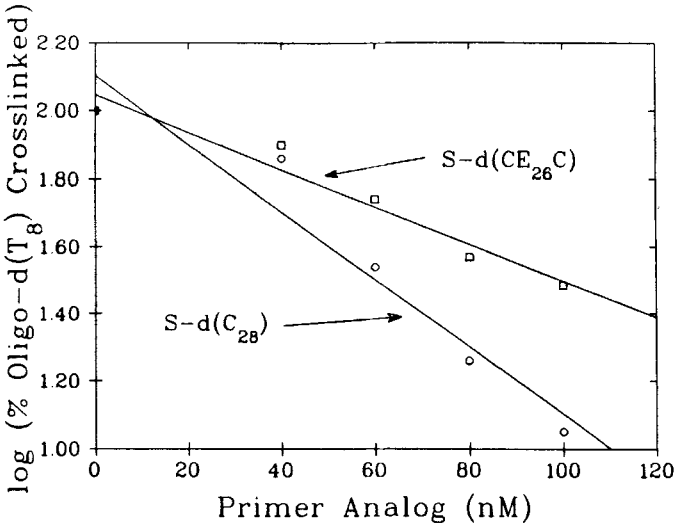

FIG. 1. Plot of competition between 1 and S-dC_{28} for the ^{32}P-labelled dT_8binding site of HIV-1 reverse transcriptase; the [dT_8] was *ca.* 1.2 μM.

RESULTS

Inhibition of HIV- Reverse Transcriptase.

The phosphorothioate, 1, was synthesized and characterized as described[4]. The ability of 1 to inhibit HIV-1 RT was evaluated in a standard assay[5] using a poly(rA) template, oligo(dT) primer, and ^{3}H-dTTP nucleotide substrate. This study (data not shown) demonstrated that 1 is approximately as potent an inhibitor of RT as is S-dC_{28}.

The ability of 1 to inhibit HIV-1 RT was also monitored in an assay wherein 1 competes with dT_8 for the RT primer binding site. A recent study[6], based on results with other DNA-binding proteins[7], has shown that dT_8 is efficiently photo-crosslinked to RT. Using ^{32}P-dT_8 as a primer and measuring the radioactivty incorporated into RT following UV-irradiation, the extent to which various primer analogs compete with ^{32}P-dT_8 for the primer binding site can be measured. The results of this assay with S-dC_{28} and 1 are displayed in Figure 1. As seen from Figure 1, 1 competes nearly as well as S-dC_{28} for the primer binding site; the K_i values for S-dC_{28} and 1 were found to be 1.2 $\times$ 10^{-8}M and 2.0 $\times$ 10^{-8}M, respectively. Under similar conditions, the K_i value for the phosphoric acid diester linked homo-oligomer, dC_{28}, is *ca.* 1.0 $\times$ 10^{-7}M.

Binding to V79 Cells, Mutagenicity, Hydroxy Radical Production.

Syrian hampster fibroblast (V79) cells were separately incubated for 60 min at room temperature with *ca.* 10 μM of internally ^{35}S labelled 1, S-dT_{20} and S-dC_{28}. Following incubation, the cells were washed, removed from the plate, and the cell-

associated radioactivity determined. The cells treated with 1 showed an approximately 100-fold greater radioactivity than those treated with either S-dC_{28} or S-dT_{20}, with the latter two oligonucleotides yielding approximately equal counts.

An autoradiographic study of V79 cells that were incubated for various periods of time with 30 μM 1 at 37 ^{o}C showed that very few of the cells internalized the phosphorothioate. This is in contrast to the behavior of other phosphorothioates, such as the anti-*rev* sequence of HIV-1[8], wherein significant internalization is observed, with localization initially in the cytoplasm and then in the cell nucleus and perinuclear organelles. Disruption of the V79 cells and cell component fractionation by ultracentrifugation, following incubation with the three S-ODNs noted above, furnished similar results.

The phosphorothioates were tested for mutagenicity in V79 cells[9]. Relative to the natural mutation frequency, the α-*rev* sequence (as P(O)S^-), resulted in six-fold increase in mutation frequency (*ca.* 26 mutations/10^6 cells); by contrast, the α-*rev* sequence (as P(O)O^-) resulted in only a doubling of the mutation frequency. No mutations were observed with 1; that is, the rate of mutations was below control. Such behavior is indicative of a very high mutational frequency, that is, frequently occurring double mutations, and is observed, for example, with N-methylnitroso urea at higher concentrations (used as a control).

S-ODNs are inducers of hydroxy radicals in V79 cells, with 1 being the strongest producer, among the S-ODNs, of hydroxy radicals found to date. On a relative scale, with S-dC_4 being 1.0, α-*rev* is 1.6, S-dC_{28} is 1.4, and 1 is 7.6. Hydroxy radical production is significantly reduced by pre-treatment or concomitant treatment of the cells with indomethacin.

DISCUSSION

The present study has shown that 1 and S-dC_{28} are nearly equivalent in ability to inhibit HIV-1 RT and that they inhibit RT through competition at the primer binding site. Although it is not unexpected that S-dC_{28} competes at the primer binding site, it is, on the surface, surprising that 1 also acts on this site. However, this finding is in accord with the previous notion[6] that primer binding to RT is through the backbone and that the binding is primarily dependent on non-electrostatic forces.

As an inhibitor of HIV-1 replication in a *de novo* infection assay, S-dC_{28} is approximately 50-fold more potent than 1[4]. Since 1 is virtually as good an inhibitor of RT as S-dC_{28}, differences in the ability of these two compounds to block HIV-1 replication in the *de novo* infection assay, cannot be due to differences in ability of the two compounds to inhibit RT within the H9 cells, assuming that the two materials would

be similarly free to react within the H9 cellular milieu. Either **1** is unable to be internalized within the H9 cell to the same extent as S-dC_{28} and then block RT or the two compounds differ in their ability to block viral attachment and penetration of the cells, or both. We are currently investigating the effect of **1** and S-dC_{28} on HIV-1 attachment and entry into cells.

Finally, it has been shown that S-ODNs are strong mutagens and that **1** appears to be a more potent mutagen than other tested S-ODNs, although studies at lower concentrations of **1** must be first completed to support this point. The mutagenicity of S-ODNs is possibly related to the ability of these materials to stimulate the production of hydroxyl radicals, as both effects are blocked by indomethacin pre-treatment.

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