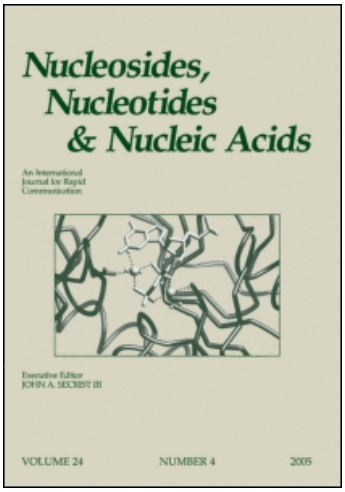

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BIOLOGICALLY ACTIVE OLIGODEOXYRIBONUCLEOTIDES-VII[1]: ANTI-HIV-1 ACTIVITY OF HEXADEOXYRIBONUCLEOTIDES BEARING 3'- AND 5'-END-MODIFICATIONS

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Abstract: It has been determined that hexadeoxyribonucleotides (5'TGGGAG3'), which have modified aromatic groups such as the trityl group at the 5'-end, exhibit anti-HIV-1 activity *in vitro*. The 6-mer (S-1443) bearing a 3, 4-dibenzyloxybenzyl (3, 4-DBB) group at the 5'-end and a 2-hydroxyethylphosphate group at the 3'-end exhibited the most potent activity and the least cytotoxicity. Moreover, it was found that the S-1443 was the most stable, when the 6-mer analogues were incubated with mouse, rat, or human plasma.

INTRODUCTION

We have found that the pentadecadeoxyribonucleotide bearing the 4, 4'-dimethoxytrityl (DMTr) group at the 5'-end exhibits high anti-HIV activity.[2] Furthermore, the chain length of the oligodeoxyribonucleotide (ODN) was deleted, and the acid-lable DMTr was substituted with a variety of aromatic groups, resulting in hexadeoxyribonucleotide, TGGGAG, bearing a 3, 4-DBB group at the 5'-end, which exhibited high anti-HIV-1 activity and the least cytotoxicity.[3] The mechanism for the activity of these ODNs was the inhibition of the adsorption of HIV-1 to the $CD4^+$ cell.[2]

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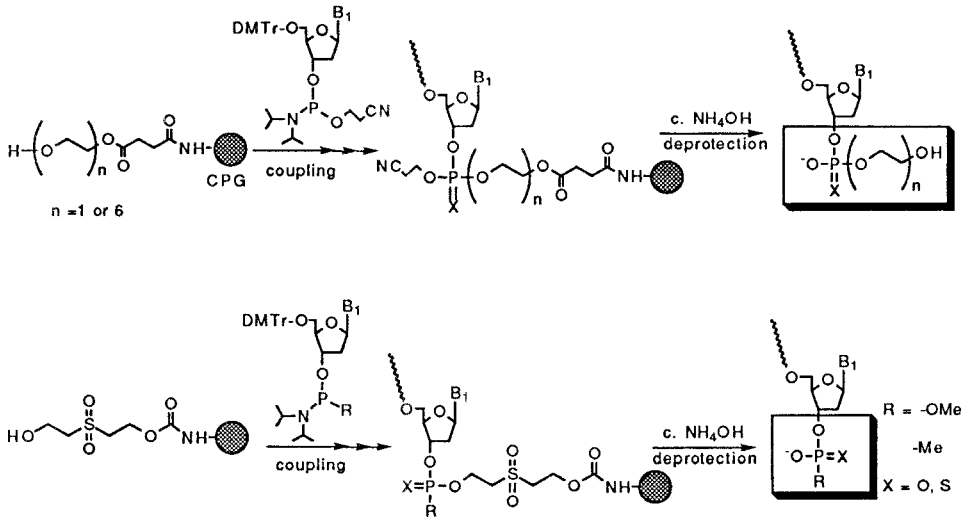


FIG. 1 Synthesis of 3', 5'-modified ODN.

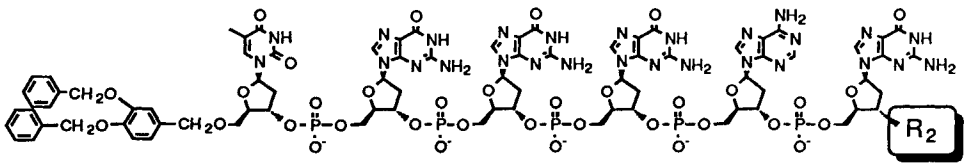
In this study, various modified phosphate groups were introduced into the 6-mer at the 3'-end to get resistance to 3'-exonuclease. These compounds exhibited higher anti-HIV-1 activity and more resistance in plasma than the 6-mer without the 3'-modification.

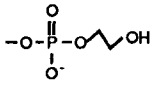
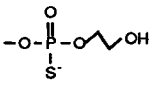
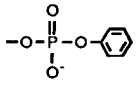
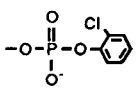
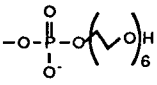
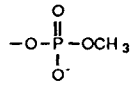
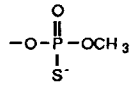
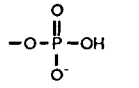
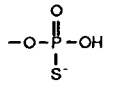
RESULTS AND DISCUSSION

Chemistry

Two types of controlled pore glass (CPG) were prepared to introduce 3'-modified phosphate groups into the 6-mers (FIG. 1). One type bound a diol such as ethylene glycol or hexa(ethylene glycol) via a succinate linker. The another one had a hydroxyethylsulfonyl group that could be cleaved from the support in alkaline conditions. For example, when a nucleoside 3'-*O*-methylphosphoramidite was coupled with this CPG, a methylphosphate could be introduced into the ODN at the 3'-end. The chain-elongation of 3'- and 5'-end-modified ODNs was performed using these CPGs by the phosphoramidite method or the phosphotriester method. The 5'-*O*-(3, 4-DBB) thymidine 3'-*O*-β-cyanoethyl-phosphoramidite was finally coupled to the ODN-linked CPG for the 5'-end modification.[4] The CPG was treated with 28% aqueous ammonia solution and the purification of ODNs was performed by C18 reverse phase column chromatography. The modifications and the sequences of ODNs were determined by negative ion LSI mass spectroscopy.

TABLE 1. Anti-HIV-1 activity of 3', 5'-modified ODNs.



R_2	IC_{50} (μg/ml)	CC_{50} (μg/ml)
-OH	0.3	>100
	0.19	>100
	0.15	>100
	0.29	>100
	0.50	>100
	0.35	>100
	0.15	>100
	0.15	>100
	0.15	>100
	0.25	>100

Anti-HIV-1 activity of 3', 5'-modified ODNs

The 50% inhibitory concentration (IC_{50}) for the cytopathic effect induced by HIV-1_{IIIB} and the 50% cytotoxic concentration (CC_{50}) of 3', 5'-end-modified ODNs were determined by MTT assay *in vitro* according to the procedure reported previously.[4] We reported that the 6-mer bearing a 3, 4-DBB group at the 5'-end without 3'-modification was excellent for less cytotoxicity up to 100 μg/ml and IC_{50} = 0.3 μg/ml.[3] The anti-HIV-1 activity of ODNs having a hexa(ethylene glyceryl)phosphate, a 2-chlorophenylphosphate or a phenylphosphate group at the 3'-end, was not improved (TABLE 1). However, the ODNs having the other modification as shown in TABLE 1, exhibited about 2-fold higher anti-HIV-1 activity than that of the ODN without the 3'-modification. On the basis of these results, it was found that the relatively small substituted groups (a 2-hydroxyethylphosphate, a 2-hydroxyethylthiophosphate, a methylphosphate, a methylthiophosphate, a phosphate, and a thiophosphate) were the highly active modifications. However, since the methylphosphate and the methylthiophosphate groups of the ODN were partially demethylated in alkaline conditions for the deprotection (data not shown), these ODNs might not be suitable for large scale synthesis. On the other hand, the phosphate group and the thiophosphate group of the ODN may be easily dephosphorylated

by phosphatases *in vivo*. Therefore, we selected the two modified groups, the 2-hydroxyethylphosphate and the 2-hydroxyethylthiophosphate, for investigation of the stability in plasma.

3', 5'-Modified ODNs stability in plasma

These ODNs bearing the 2-hydroxyethylphosphate or the 2-hydroxyethylthiophosphate at the 3'-end with high anti-HIV-1 activity were tested for stability in mouse, rat, and human plasma. These 3', 5'-modified ODNs were incubated with the plasma for 4 hr, and then these reaction mixtures were analyzed by reverse-phase HPLC to quantify the remaining amounts of ODNs. The 5'-modified ODN without the 3'-modification was used as a control. The 3', 5'-modified ODNs were stable in the plasma of all species in comparison to the ODN without the 3'-modification. Further, we found that the ODN bearing the 2-hydroxyethylphosphate was more stable than the ODN bearing the 2-hydroxyethylthiophosphate. This 6-mer bearing the 3, 4-DBB group at the 5'-end and the 2-hydroxyethylphosphate group at the 3'-end (S-1443) was chosen as the best candidate among the 6-mers for its anti-HIV-1 activity.

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