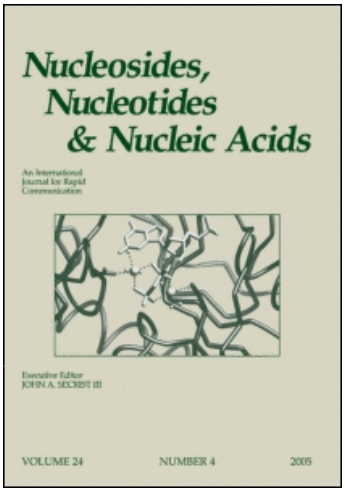

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Anti-HIV Activity of the Antisense Oligonucleotides Bearing Lipophilic and Alkylating Groups at the 5'-Terminus

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To cite this Article Abramova, T. V. , Blinov, V. M. , Vlassov, V. V. , Gorn, V. V. , Zarytova, V. F. , Ivanova, E. M. , Konevets, D. A. , Plyasunova, O. A. , Pokrovsky, A. G. , Sandahchiev, L. S. , Svinarchuk, F. P. , Starostin, V. P. and Chapligina, S. R.(1991) 'Anti-HIV Activity of the Antisense Oligonucleotides Bearing Lipophilic and Alkylating Groups at the 5'-Terminus', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 419 — 422
To link to this Article: DOI: 10.1080/07328319108046493
URL: http://dx.doi.org/10.1080/07328319108046493

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 419-422 (1991)

ANTI-HIV ACTIVITY OF THE ANTISENSE OLIGONUCLEOTIDES BEARING LIPOPHILIC AND ALKYLATING GROUPS AT THE 5'-TERMINUS.

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Abstract: Experiments with the HIV-1 infected MT-4 cells evidence that coupling of lipophilic and alkylating groups to the antisense oligonucleotides improve their anti-HIV efficiency.

Antisense deoxyribooligonucleotides complementary to the HIV-1 RNA inhibit proliferation of the virus in cell culture[1]. It was shown that more efficient inhibitors may be developed by chemical modifications of the oligonucleotides which facilitate cellular uptake of the compounds and protect them from the nucleases degradation. We investigated the effect of coupling of lipophilic and reactive groups to the oligonucleotides terminus on the anti-HIV activity of the compounds.

Oligonucleotides complementary to different regions of HIV-1 RNA (table 1) were synthesized as described. Lipophile groups X,Y,Z,W were coupled according to the published method[2] (fig.1).

The groups were expected to anchor the oligonucleotides to the cells membrane and to increase the efficiency of their uptake. Indeed, in experiments with the radiolabeled oligonucleotide derivatives, it was found that the oligonucleotides bearing groups X,Y,Z, bind to the MT-4 cells 100,

TABLE 1

N N	oligonucleotides	target sequence in the HIV RNA	nucleo-tides
1	pTGGCGTACTCACCAGTCGCCGC	donor-splice site	278-290
2	pTCCGCTTCTTCCTGCCATA	acceptor-splice site, genes env, art	5548-5566
3	pTTTTTTTTTTTTTTT	poly-A sequence	
4	pTGACCCTCTTCCCATT	control,may form 10 bp	7388-7403
5	pGAGACCGAGA	control,may form 8 bp	-3 --7, 9113-9122
6	pTTGAAACCCGAGAACATCAT	control (anti-fos)	

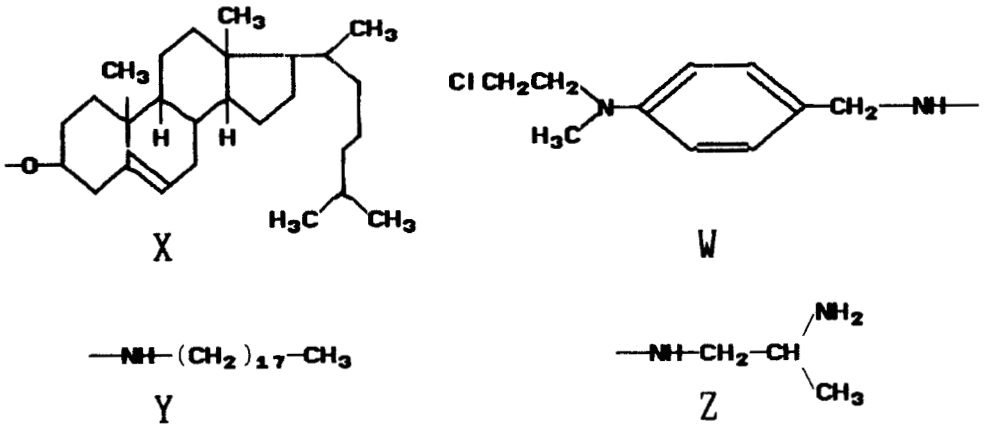

FIG.1. Chemical structures of the coupled groups.

10, 3 times better as compared to the parent nonmodified oligonucleotides. Alkylating groups were expected to bind the oligonucleotides to the target RNA covalently.

The modifications used protect oligonucleotides against nucleases. After 24h incubation with MT-4 cells, elect- rophoresis analysis has revealed that 70% of the derivatives remained intact as compared to 20% for the parent oligonucleotide. The antiviral activity of the oligonucleotide derivatives was assayed in experiments with MT-4 cells. The cells ($5 \cdot 10^5$ ml^{-1}) were infected with HIV-1 (10-100 ID per cell). One hour post infection, the oligonucleotides were introduced in the medium. The virus yield was determined 96

TABLE 2

inhibitor	reverse transcriptase			HIV-antigene		
	oligonucleotide concentration (μM)					
	1 A	1 B	10 A	1 A	1 B	10 A
(1)	12	44	30	19	47	22
(1)-Z	6	64	45	15	13	70
(1)-Y	32	75	72	54	62	79
(2)-Y	5	74	64	10	50	68
(3)	6	73	63	8	70	83
(3)-Z	2	41	73	0	35	73
(3)-Y	1	63	66	36	73	88
(1)-Z + (3)-Z	56	92	86	46	88	86
(4)-Y	2	-	24	54	-	65
(5)-Z	-	-	19	-	-	0
(1)-W	13	95	3	-	70	-
(1)-X	7	92	37	-	89	-
(6)-Z	24	8	50	-	13	-
AZT (35 μM)	64	97	-	88	96	-

(A) and 144 (B) hours post infection by immunoassay and by the reverse transcriptase assay. Results of the experiments shown in table 2 confirm that the modifications introduced dramatically increase the inhibitory potential of the oligonucleotides. Simultaneous introduction of oligonucleotides (1) and (3) results in the synergistic action on the virus proliferation. It is seen that control oligonucleotides also cause some inhibition of the virus proliferation, in accordance with the previous communications[3], probably due to a partial complementarity to the viral RNA. The results of the experiments suggest that significant improvement of the antiviral properties can be achieved by coupling of the lipophilic and alkylating groups to the oligonucleotides.

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