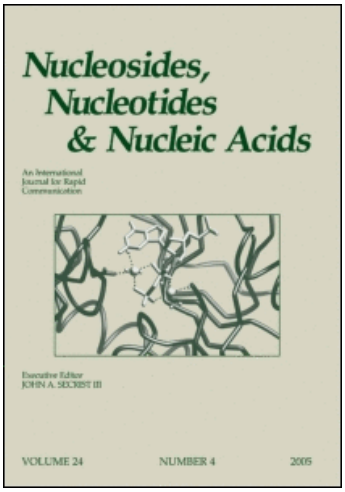

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COMPARATIVE STUDIES OF TRICYCLO-DNA- AND LNA-CONTAINING OLIGONUCLEOTIDES AS INHIBITORS OF HIV-1 GENE EXPRESSION

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□ Trans-*activation of HIV-1 transcription is triggered by the interaction of the protein Tat and host cellular factors with a 59-residue stem-loop RNA known as the* trans-*activation responsive element (TAR). Here we compare the* trans-*activation steric block inhibitory activity of 16-mer oligonucleotides targeted to TAR containing tricyclo-DNAs, and their mixmers with LNA or OMe residues, with LNA/OMe oligonucleotide. Despite generally weaker TAR RNA binding affinity, all tricyclo-DNA oligonucleotides showed similarly good activity levels to OMe/LNA oligonucleotide in a HeLa Tat-dependent* trans-*activation cell reporter assay with cationic lipid delivery, but mixmers of tricyclo-DNA were inactive. Tricyclo-DNA 16-mer showed sequence-specific inhibition of β-galactosidase expression in an anti-HIV HeLa cell reporter assay.*

Keywords HIV-1; *trans*-activation; steric block; tricyclo-DNA; LNA

INTRODUCTION

HIV-1 genome transcription is activated by the interaction of the viral protein Tat with the *trans*-activation responsive element (TAR) RNA at the 5′-leader sequence of the HIV-1 mRNA.[1] Synthetic oligonucleotides and their analogs complementary to the TAR RNA that are able to enter the cell nucleus and bind to the TAR RNA can prevent docking of Tat and thus inhibit Tat-mediated *trans*-activation.[2–4] We found previously that a 16-mer OMe/LNA mixmer oligonucleotide complementary to

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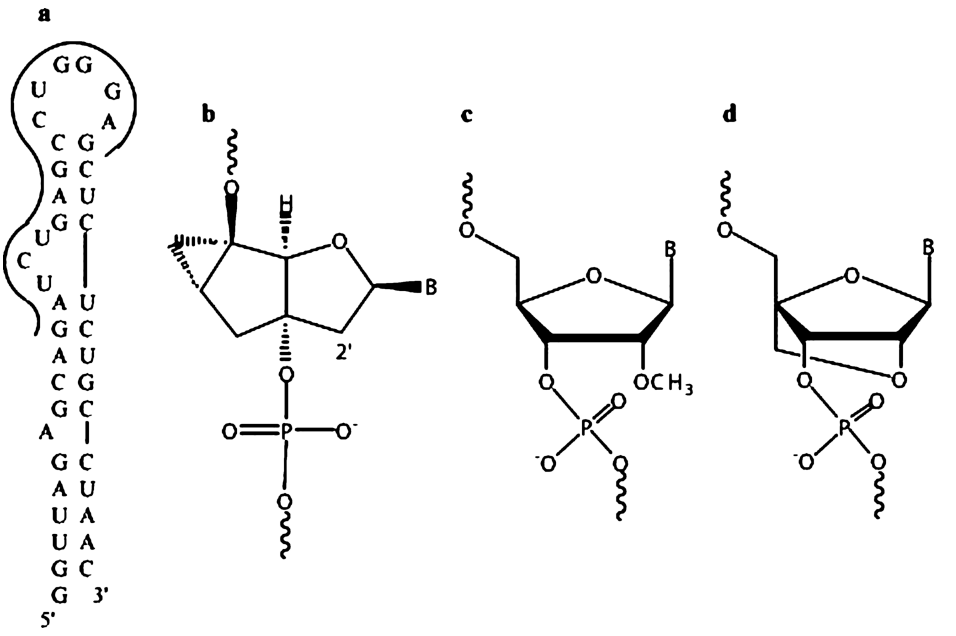

FIGURE 1 HIV-1 39-mer model TAR RNA (a) and the binding sites for oligonucleotides containing tricyclo-DNA (b), 2′-OMe (c) and LNA (d) monomers.

TAR when delivered by cationic lipids effectively inhibited Tat-dependent *trans*-activation in a HeLa cell reporter assay [5,6] and syncitia formation induced by HIV-1 infection in HeLa T4 LTR cells.[7] Here we compare the *trans*-activation inhibitory activity of the 16-mer OMe/LNA mixmer with oligonucleotide analogs containing another type of conformationally restricted monomers-tricyclo-DNA.[8–11]

RESULTS AND DISCUSSION

We synthesized a 16-mer with all tricyclo-DNA units **1** (Figure 1, Table 1), its mismatched sequence **2**, a 16-mer mixmer with six tricyclo-DNA and ten 2′-OMe units **3,** and a 15-mer with five tricyclo-DNA and ten LNA monomers **4**, complementary to the apical region of a 39-mer model TAR RNA (Figure 1a). We measured the ability of these oligonucleotides to bind to the model TAR RNA at 30°C in either of two buffers (Table 1). The results show that all of the exactly matched tricyclo-DNA containing oligonucleotides **1**, **3**, **4** have sufficiently strong binding (K_d 4.5 to 28.2 nM) to be considered for steric block agents though their binding is weaker than that of the 16-mer OMe/LNA mixmer **5**. 16-mer Tricyclo-DNA **1** showed stronger affinity to the target than corresponding mixmers **3–4** and its binding is sequence-specific,

TABLE 1 Oligonucleotide sequences and their binding to 39-mer TAR RNA

No.	Oligonucleotide analog (Sequence 5′-3′)	Binding Kd (nM) TK-80[b]	Binding Kd (nM) Transcription buffer[c]
1	16 TAR Tricyclo-DNA FAM (ctcccaggctcagatc-FAM)[a]	65.8 ± 7.5	5.5 ± 0.6
2	16 TAR Tricyclo-DNA-mism FAM (ctcccaccctcacatc-FAM)	n.b.[d]	n.b.[d]
3	16 TAR 10xOMe/6xTricyclo-DNA FAM (cUcCcAGGcUcAGAtC-FAM)	98.1 ± 4.9	4.5 ± 0.1
4	15 TAR 9xLNA/6xTricyclo-DNA (ccCcAGGcTcAGATc-FAM)	140.2 ± 17.5	28.2 ± 4.5
5	16 TAR 10xOMe/6xLNA FAM (CUCCCAGGCUCAGAUC-FAM)	9.3 ± 0.4[e]	3.3 ± 0.8[e]

[a]Tricyclo-DNA residues are shown in lower case. Capitals show 2′-*O*-Me nucleotides. Underlined monomers are LNA nucleotides. [b]TK-80: 50 mM Tris.HCl, pH 7.4; 80 mM KCl; [c]Transcription buffer: 20 mM HEPES, pH 7.9; 10 μM $ZnSO_4$; 2 mM DTT; 80 mM KCl; 3 mM $MgCl_2$; 10 mM creatine phosphate; [d]no binding up to 10 μm concentration; [e]data published before [6] and shown here for comparison.

since mismatched all tricyclo-DNA 16-mer **2** does not bind to the TAR RNA in concentrations up to 10 μm.

The nuclear inhibition activity of the oligonucleotides was tested in a double-luciferase HeLa cell reporter system described previously (Figure 2).[5,6] Dose-dependent knockdown of firefly luciferase expression was observed only for the 16 TAR all tricyclo-DNA **1** and the levels of inhibition were similar to those for the OMe/LNA control **5** (Figure 2a). At the same time the *Renilla* luciferase levels were not affected, which confirms the protein specificity of the cellular activity.

Furthermore, we tested the most promising steric block oligonucleotide candidate 16 TAR tricyclo-DNA **1** and the corresponding OMe/LNA mixmer **5** for inhibition of HIV-1 infectivity in a HeLa P4 cell line

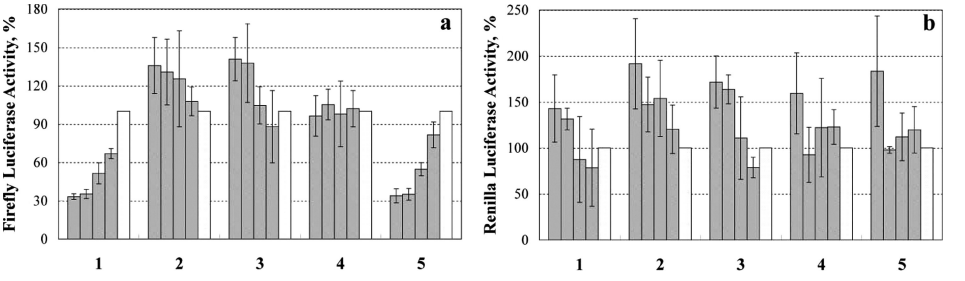

FIGURE 2 *Trans*-activation inhibitory activity of oligonucleotides **1–5** measured after oligonucleotide incubation with HeLa cells in the presence of Lipofectamine 2000 for 3 h, followed by cell growth for 18 h in media alone. (**a**) Firefly luciferase luminescence (normalized to cell viability count), shown as percentage of firefly luciferase luminescence of untreated cells (the last bar in each series). Bars left to right represent activity levels for oligonucleotide concentrations 500, 250, 125, 62.5, and 0 nM, respectively. (**b**) *Renilla* luciferase luminiscence.

expressing receptors CD4 and CXCR4 and carrying the stably integrated *lacZ* gene under the control of HIV-1 LTR.[12] The transfections were carried out with Lipofectamine 2000 assistance for 3 h; 18 h later HIV-1_{LAI} was added and the cells incubated for a further 24 h. Dose-dependent knockdown of HIV-1 induced β-galactosidase expression was observed for 16–mer tricyclo-DNA **1** (data not shown) for concentrations up to 10 μm (25% inhibition) whereas no inhibition was seen for the mismatched control **2**. A little higher inhibitory activity was measured for 16-mer OMe/LNA mixmer **5** (40% at 1 μM), which is very similar to that obtained in an analogous anti-HIV-1 syncitia reduction assay for the same oligonucleotide from another recent study.[7]

In conclusion, we have shown that a 16-mer all tricyclo-DNA oligonucleotide, targeted to the HIV-1 TAR region, similarly to 16 TAR OMe/LNA mixmer, blocks sequence-specific Tat-dependent *trans*-activation in Hela cells as well as HIV infectivity when delivered with cationic lipid. Thus, our study confirms that tricyclo-DNA oligonucleotides are promising antisense agents with potential for therapeutic applications.

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