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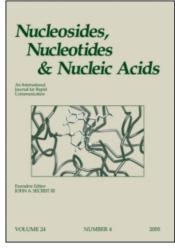
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# Protein and RNA of Human Telomerase as Targets for Modified Oligonucleotides

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## PROTEIN AND RNA OF HUMAN TELOMERASE AS TARGETS FOR MODIFIED OLIGONUCLEOTIDES

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**ABSTRACT.** Chimeric oligodeoxynucleotides containing phosphorothioate and N3´→P5`phosphoramidate linkages were synthesized. These oligomers show a high inhibitory activity against human telomerase.

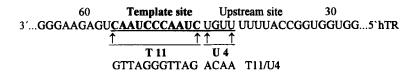
Telomerase was detected in most human tumors and is thought to be necessary for the sustained proliferation of cancer cells.<sup>1</sup> It is a polymerase carrying its own RNA template for the addition of multiples of TTAGGG telomeric repeats onto the ends of chromosomes.<sup>2</sup> Inhibition of telomerase may be considered as a potentially anticancer strategy changing the immortal state of cancer cells to a mortal one. One appropriate target might be the telomerase RNA.<sup>3</sup> Recently, we have found that phosphorothioate-modified oligodeoxynucleotides (PS-ODN) do not anneal sequence specifically to the RNA template but bind tightly in a sequence independent manner to a protein site of telomerase called primer binding site which produced a strong inhibition of telomerase.<sup>4</sup> Basing on these results we designed chimeric oligonucleotides (ODNs) combining strong protein and RNA binding properties thus retaining the high efficiency of PS-ODN but providing the required specificity by an antisense partner. Here we present the synthesis and first investigations of such chimeric oligomers.

A 15mer noncomplementary PS-ODN was extended by an antisense sequence covering T11/U4 of RNA (see below). This part of the oligomer was modified by N3'->P5'phosphoramidates as described.<sup>5</sup> We synthesized partially modified oligonucleotide analogs in which the 3'-oxygen of each thymidine residue was substituted with nitrogen. All oligomers were synthesized on an Applied Biosystems DNA 391

synthesizer, using 5'-cyanoethyl phosphoramidite methods. The monomer 5'-phosphoramidites and the solid supports were purchased from Glen Research.

Phosphorothioate linkages were generated by using tetraethylthiuram disulfide as the sulfurization reagent.<sup>6</sup> The 5'-(N,N-diisopropyl-amino-2-cyanoethyl)phosphoramidite-3'-(monomethoxytrityl)-amino-2',3'-dideoxythymidine monomer was prepared according a published method.<sup>5</sup> The oligonucleotides were estimated as inhibitors of telomerase in HL-60 cell extract using TRAP-eze<sup>TM</sup> detection kit (Oncor). <sup>7</sup> The quantitative evaluation of the radioactive telomerase products followed by the Bioimage Analyzer BAS 2000 (Fuji).

### Targeted RNA sequence of human telomerase



noncomplementary complementary PS sequence T11/U4/Pam/PO 5'-TCA GAT TAG GAC TGC-  $GT^{am}$  Tam AGG  $GT^{am}$  AG ACAA-3'  $IC_{50} = 1.1$  nM

5'-TCA GAT TAG GAC TGC- Tam CA GATam Tam AG GAC Tam GC-3' IC<sub>50</sub> = 4.9 nM noncomplementary noncomplementary
PS sequence Pam/PO sequence
Tam = 3'-Amino-2',3'-dideoxythymidine

The noncomplementary 15mer PS-ODN extended at the 3'-end with the T11/U4/Pam/PO sequence displayed an IC<sub>50</sub> of 1.1 nM. Replacement of the antisense sequence of the Pam-ODN part by a noncomplementary sequence gives an IC<sub>50</sub> value of 4.9 nM. These data indicate that the antisense part contributes really to the effect of the chimeric ODN. Further appropriate modifications adapted to both telomerase targets might be a way to develope effective and selective inhibitors of human telomerase at the cellular level.

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