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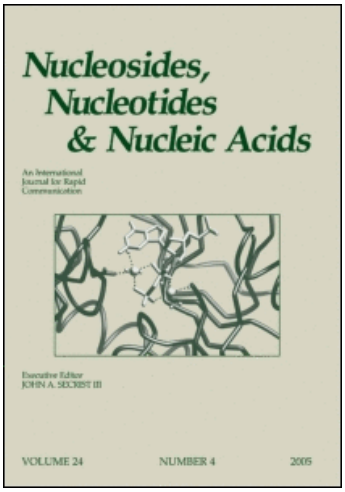

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INHIBITION OF 5-α-REDUCTASE (TYPE-II) EXPRESSION BY ANTISENSE 3'-DEOXY-(2'-5') OLIGONUCLEOTIDE CHIMERAS.

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ABSTRACT: 3'-Deoxy-(2'-5') oligonucleotides bind selectively to complementary RNA but not to DNA. 3'-Deoxy-(2'-5') phosphorothioate ODN chimeras embedded with a short stretch of 3'-5' phosphorothioate cassette are potent inhibitors of steroid 5-α-reductase expression with significantly less non-specific interactions in cell culture.

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The 2',5'-oligoadenylate system represents a classical example of naturally occurring constitutional RNA isomers that are suspected to be involved in the regulation of cell growth/differentiation and in the antiviral effect of interferon.[1] These unique oligoribonucleotides are reported to inhibit the activities of HIV-1 reverse transcriptase [2] and DNA topoisomerase-I in HIV-1 infected cells.[3] Furthermore, their selective hybridization to single stranded RNA[4] (ssRNA) over ssDNA with a preexisting ability to activate RNase-L,[5] has led to the possibility of their use in antisense applications.[6] One serious limitation restricting the potential utility of these oligonucleotides (ODN's), however, is in their rapid degradation by cellular nucleases[7] and towards this, there have been some recent efforts directed towards preparation of biologically stable analogs with 2',5'-internucleotide connections.[5a,7b,8] Earlier, 2',5'-linked 3'-deoxyoligonucleotides[8b,9] were found to retain their selective affinity for ssRNA[10] and had a markedly prolonged biological half life than comparable 3',5'-linked DNA.[10a]

3'-Deoxy guanosine was constructed by condensation of suitably protected base with the known 3'-deoxy sugar **1**,[9d] under Vorbruggen conditions[11] followed by purification and deprotection of the nucleoside with conc. ammonium hydroxide (Scheme 1). The nucleoside was then converted to the desired phosphoramidite by known methods. 3'-Deoxy pyrimidine and 3'-deoxy adenosine phosphoramidites were prepared by reported procedures.[9] 3'-Deoxy (2'-5') phosphorothioate oligonucleotides and their chimeras were

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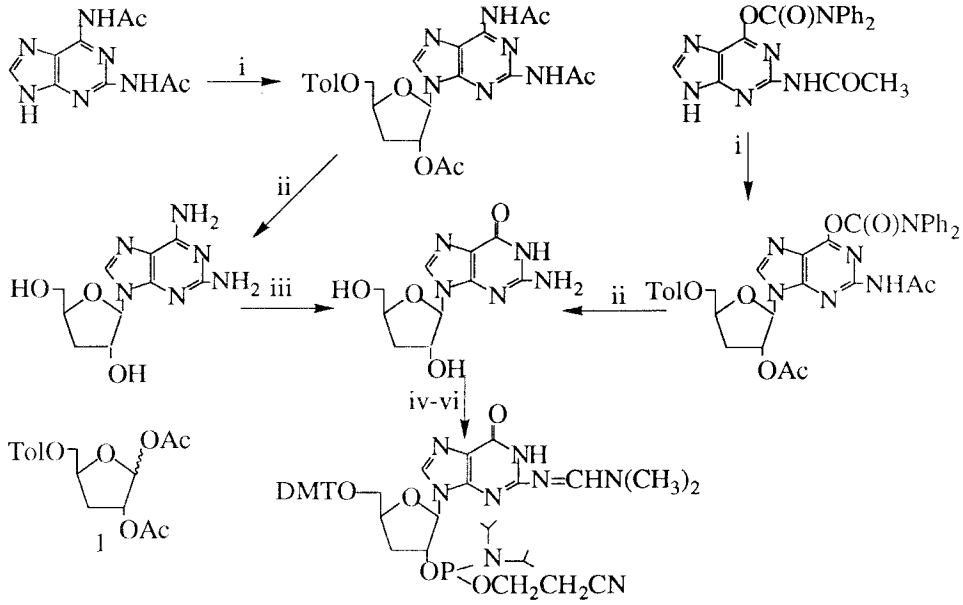

i: BSA/TMSOTf/DCE then 1 in Ph-CH_3, 66-68%; ii: NH_4OH/CH_3OH (1:1), 48h; 600C, 87%; iii: DMSO/ Adenosine deaminase/phosphate buffer pH 7.2, 100%; iv: $(CH_3)_2NCH(OCH_3)_2$/ CH_3OH; 92%; v: DMTCl/pyridine; 87% vi: $[(CH_3)_2CH]_2NP(Cl)OCH_2CH_2CN$/DIPEA, 82%.

SCHEME 1. Synthesis of 3'-deoxy Guanosine phosphoramidite.

TABLE 1. Thermal stability of 3'-deoxy (2'-5') phosphorothioate ODN's against RNA .

	Phosphorothioate-ODN's*	T_m (RNA)#
DP-1676	5'-CATCGCGCCGTGTTCCTCGCC-3'	60.5 ^{0}C
DP-5265	5'-CATGGCGCCGTCTTCCTCGCC-3'	42.0 ^{0}C
DP-5281	5'-CATCGCGCCGTGTTCCTCGCC-2'	56.0 ^{0}C
DP-5280	5'-CATGGCGCCGTCTTCCTCGCC-2'	35.5 ^{0}C
DP-5319	5'-CAT CGC Gcc gtg ttC CTC GCC-2'	58.0 ^{0}C
DP-5363	5'-CAT GGC Gcc gtc ttC CTC GCC-2'	38.0 ^{0}C
DP-5318	5'-CAT CGC Gcc gtg ttC CTC GCC-2' (PO)	60.0 ^{0}C
RNA Target:	GGCGAGGAACACGGCGCGAUGCAG	

Determined in 50 mM phosphate buffer (pH 7.2) containing 150 mM NaCl.
* Underlined bases are the mis-matches.

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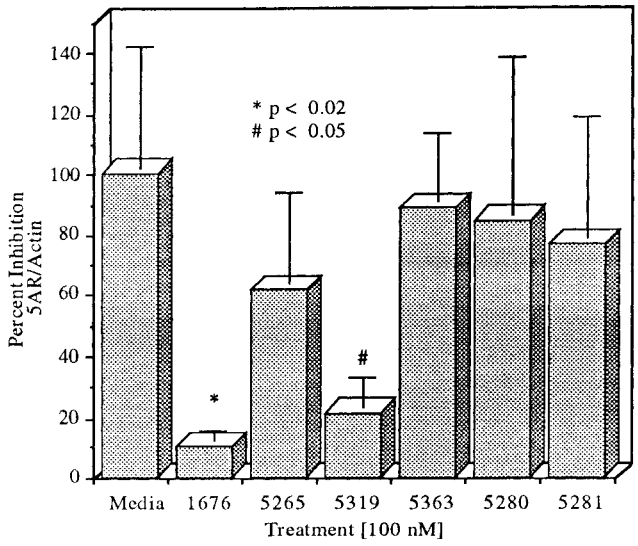

Figure 1. Inhibition of 5-α reductase expression by phosphorothioate ODN's.

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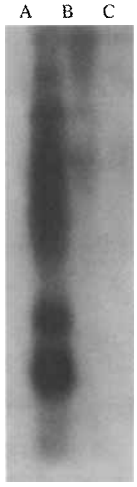

Lane A: 2'-Deoxy (3'-5') phosphorothioate ODN (DP-1676)
Lane B: 3'-Deoxy (2'-5') phosphorothioate ODN chimera (DP-5319)
Lane C: 3'-Deoxy (2'-5') phosphorothioate ODN (DP-5281)

FIGURE 2. Binding of ^{32}P-labeled phosphorothioate ODN's to cellular proteins

synthesized on a DNA synthesizer with established protocols using Beaucage reagent as the sulfur transfer reagent. The oligonucleotides were purified by ion-exchange chromatography and were judged as >95% pure by HPLC and PAGE analysis. The thermal stability of phosphorothioate ODN's against complementary RNA target is shown in Table 1. All the 3'-deoxy (2'-5') phosphorothioate ODN's form duplexes of comparable duplex stability against RNA when compared with their 3'-5' connected isomers.

The ability of 3'-deoxy (2'-5') phosphorothioate ODN's to inhibit 5 α-reductase expression was evaluated in Chinese hamster ovary cells transfected with the human 5 α-reductase type-II gene.[12]

While an antisense 2'-deoxy (3'-5') phosphorothioate ODN (DP-1676) was found to be a potent inhibitor of 5-α-reductase expression in 100 nM concentration, its 3'-deoxy (2'-5') analog (DP-5281) displayed no antisense activity (Figure 1). Incorporation of a cassette of seven 3'-5' linkage site (Table 1: lower case letters) in the 2'-5' ODN to restore putative RNase-H activity resulted in a chimeric ODN (DP-5319) that was a potent inhibitor of the 5-α-reductase expression.

Finally, 3'-deoxy (2'-5') phosphorothioate ODN's and their chimeras exhibit significantly less non-specific inhibitory effects on aortic smooth muscle cell proliferation (data not shown) and markedly less binding to cellular proteins than the corresponding 3'-5' phosphorothioate ODN's (Figure 2).

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12. Cells were treated with ODN's in the presence of a lipid carrier (Lipofectin) for three consecutive days after which the cells were lysed. All the cellular proteins were then separated by SDS-PAGE in a 12% acrylamide gel followed by transfer onto a PVDF membrane. Both 5-α-reductase-II and actin (as an internal control) were detected by electro-chemical luminance Western blotting techniques and results plotted as the ratio of the levels of 5 α-reductase (type-II) to actin.

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