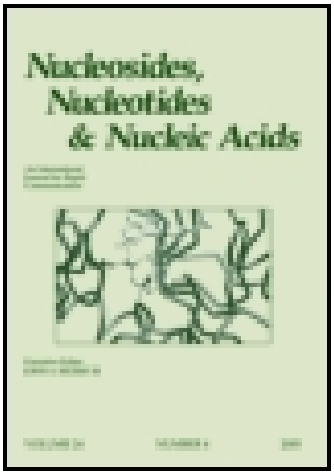


Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

Inhibition of 5-α-Reductase (TYPE-II) Expression by Antisense 3′-Deoxy-(2′-5′) Oligonucleotide Chimeras

Purshotam Bhan [a], Anila Bhan [a], Mikung Hong [a], John G. Hartwell [a], June M. Saunders [a] & Glenn D. Hoke [a]
[a] Dyad Pharmaceutical Corporation, 9110 Red Branch Road, Columbia, Maryland, 21045, USA
Published online: 16 Aug 2006.

To cite this article: Purshotam Bhan, Anila Bhan, Mikung Hong, John G. Hartwell, June M. Saunders & Glenn D. Hoke (1997) Inhibition of 5-α-Reductase (TYPE-II) Expression by Antisense 3′-Deoxy-(2′-5′) Oligonucleotide Chimeras, Nucleosides and Nucleotides, 16:7-9, 1195-1199, DOI: 10.1080/07328319708006156

To link to this article: http://dx.doi.org/10.1080/07328319708006156

NUCLEOSIDES & NUCLEOTIDES, 16(7–9), 1195–1199 (1997)

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

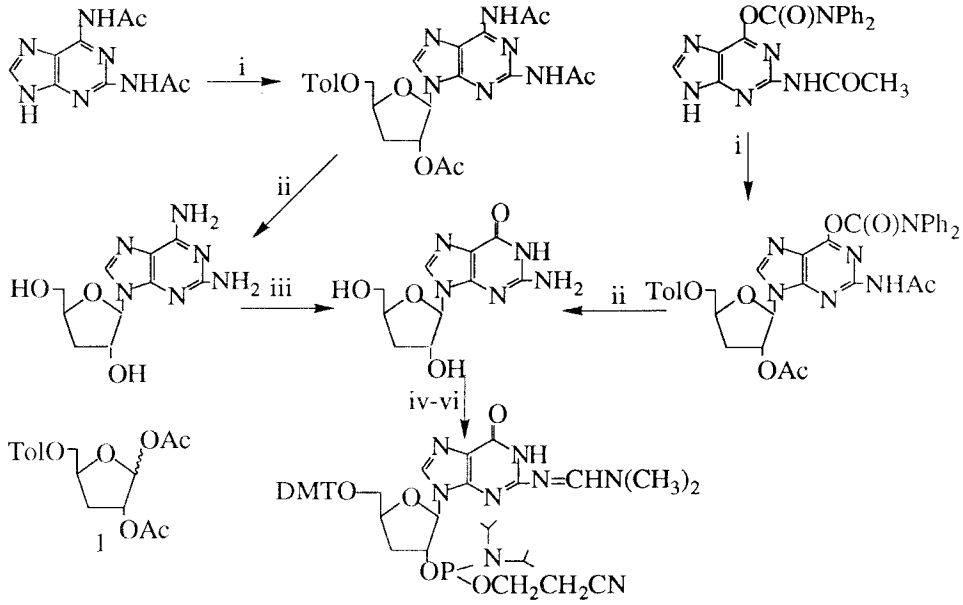
Purshotam Bhan*, Anila Bhan, Mikung Hong, John G. Hartwell, June M. Saunders and Glenn D. Hoke.

Dyad Pharmaceutical Corporation, 9110 Red Branch Road, Columbia, Maryland 21045. USA.

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.

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



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.

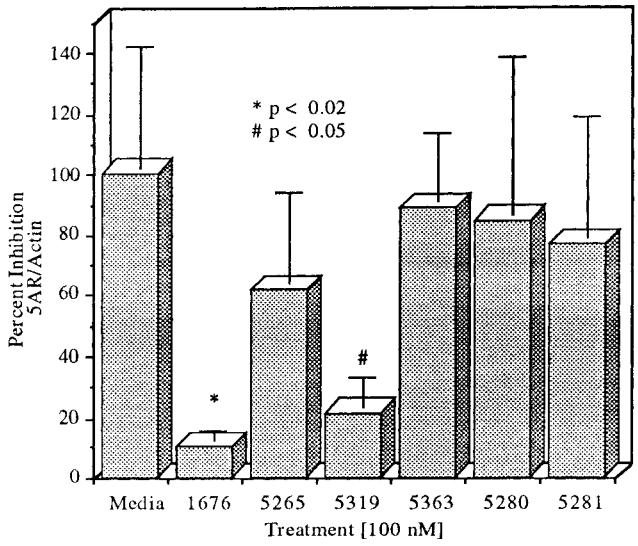
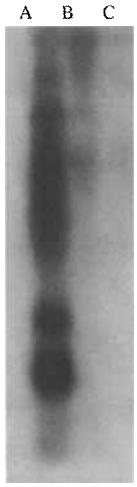


Figure 1. Inhibition of 5-α reductase expression by phosphorothioate ODN's.



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).

REFERENCES

1. Johnston, M. I.; Torrence, P. F., In *Interferon*, Friedman, M. Ed.; **1984**, vol *3*, pp. 189-298, Elsevier, New York.
2. Sobol, R. W.; Fisher, W. L.; Richenbach, N. L.; Kumar, A.; Beard, W. A.; Wilson, S. H.; Charubala, R.; Pfleiderer, W.; Suhadolnik, R. J. *Biochemistry*, **1993**, *32*, 12112.
3. Schroder, H. C.; Kelve, M.; Schacke, H.; Plfeiderer, W.; Charubala, R.; Suhadolnik, R. J.; Muller, W. E. G. *Chem. Biol. Interactions*, **1994**, *90*, 169.
4. Giannaris, P. A.; Damha. M. J. *Nucleic Acids Res.* **1993**, *21*, 4742.
5. (a) Kariko, K.; Sobol, R. W.; Suhadolnik, L.; Li, S. W.; Reichenbach, N. L.; Suhadolnik, R. J.; Charubala, R,; Pfleidereer, W. *Biochemistry*, **1987**, 26, 7127. (b) Kitade, Y.; Alster, D. K.; Pabuccuoglu, A.; Torrence, P. F. *Bioorg. Chem.*, **1991**, *19*, 283.
6. (a) P. F. Torrence, P. F.; Maitra, R. K.; Lesiak, K.; Khamnei, S.; Zhou, A.; Silverman, R. H. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 1300.
7. (a) Schmidt, A.; Chernajovsky, Y.; Shulman, L.; Federman, P.; Beressi, H.; Revel,

Proc. Natl. Acad. Sci. USA, **1979**, *76*, 4788. (b) Herdewijn, P.; Charubala, R.; De clercq, F.; Pfleiderer. W. *Helv. Chim Acta*, **1989**, *72*, 1739.

8. (a) Den Hartog, J. A. J.; Wijnands, Van Boom, J. H.; Crea, R. *J. Org. Chem.*. **1981**, *46*, 2242. (b) Wasner, M.; Henderson, E. E.; Suhadolnik, R. J.; Pfleiderer, W. *Helv. Chim. Acta*, **1994**, *77*, 1757 and references cited therein.
9. (a)Sheppard, T. L.; Rosenblatt, A. T.; Breslow, R. *J. Org. Chem.*, **1994**, *59*, 7243. (b) Rhie, S-Y.; Pfleiderer, W. *Nucleosides & Nucleotides*, **1994**, *13*, 1425.
10. (a) Alul, R.; Hoke, G. D. *Antisense Res & Develop.*, **1995**, *5*, 3. (b) Jin, R.; Chapman, W. H.; Srinivisan, A. R.; Olson, W. K.; Breslow, R.; Breslauer, K. J. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 10568. (c) Jung, K-E.; Switzer, *J. Am. Chem. Soc.*, **1994**, *116*, 1516.
11. Vorbruggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.*, **1981**, *114*, 1234.
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.