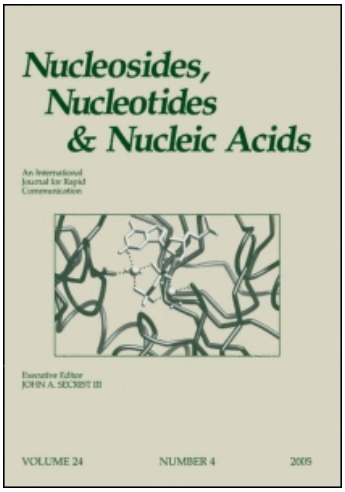

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Enzymatic Hydrolysis and Biological Activity of Oligonucleotides Containing 5-Substituted Pyrrmidine Bases

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To cite this Article Ötvös, L. , Sági, J. , Sági, Gy. , Szemzõ, A. , Tóth, F. D. and Jeney, A.(1999) 'Enzymatic Hydrolysis and Biological Activity of Oligonucleotides Containing 5-Substituted Pyrrmidine Bases', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1665 — 1666
To link to this Article: DOI: 10.1080/07328319908044816
URL: http://dx.doi.org/10.1080/07328319908044816

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1665–1666 (1999)

ENZYMATIC HYDROLYSIS AND BIOLOGICAL ACTIVITY OF OLIGONUCLEOTIDES CONTAINING 5-SUBSTITUTED PYRIMIDINE BASES.

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ABSTRACT. Two series of alternating ODNs containing 5-n.alkyl-, alkenyl- and alkynyl-dU and -dC units have been prepared in order to study the kinetics of their hydrolysis by SV PDE and human serum, respectively. Both in $(r^5dUpdA)_{10}$ and $(r^5dCpdG)_6$ series the rate of hydrolysis decreased with increasing length of side-chain. Replacement of thymidines by 5-hexynyl-dU in different antisense oligomers resulted in considerably higher biological activity relative to that of the thymidine-containing counterparts.

Oligonucleotides composed of natural building blocks are good substrates of extra- and intracellular nucleases, which is the major obstacle in their use as potential chemotherapeutic agents [1]. Although the necessary nuclease resistance is usually attained by phosphate modifications , the most commonly used phosphorothioate antisense oligos sometimes show non-sequence-specific effects, as well [2,3]. However, certain base-modified oligonucleotides were also found to be stable against endo- and exonuclease degradation [4].

In this study we investigated the enzymatic stability of two series of modified, self-complementary oligonucleotides containing 5-n.alkyl-, (E)-5-(1-alken-1-yl)-, and 5-(1-alkyn-1-yl)-dU and -dC units, respectively. The synthesized $(r^5dUpdA)_{10}$ and $(r^5dCpdG)_6$ analogues were hydrolysed by snake venom phosphodiesterase (SV PDE) and human serum, respectively. In both cases the initial rate of degradation was

determined by HPLC. We found that in the case of SV PDE the hydrolysis rate of alkyl- and alkenyl-substituted oligomers continuously decreased with increasing length of carbon side-chain up to hexyl and hexenyl analogues, which were quite resistant within the investigated periods. It may be explained by supposing that the bulky 5-substituents sterically protect the phosphate moieties against nuclease attack. Beside steric effect the thermal stability of oligonucleotides also seems to play important role in resistance to nucleases. It was confirmed by the enhanced stability of alkynyl oligomers that correlates well with their higher duplex stability [5] in addition, the shorter but still much more thermostable dCpdG analogues were found to be more resistant than the corresponding dUpdA derivatives. Total nuclease activity of human serum was lower than that of SV PDE, especially towards (r^5dCpdG)$_6$ series, where even the analogues of short side-chain (ethyl, vinyl, ethynyl) proved nearly or quite resistant.

Due to the advantageous biochemical properties mentioned above, the 5-hexynyl-dU was selected and incorporated into two series of antisense oligonucleotides in place of thymidines to study its effect on biological activity. Thus, four 18-mers, complementary to a mRNA region of 92 kDa type human collagenase IV, and four 20-mers, targeted to splice-donor region of HIV-1 mRNA, were synthesized. Both series consisted of unmodified, base-modified, thio-modified, and base+thio-modified analogues. In the case of human collagenase inhibition —that may have great importance in cancer chemotherapy—the oligomers containing 5-hexynyl-dU have significantly lower IC_{50} values than their thymidine-containing counterparts. Similar results were obtained with the four anti-HIV agents, as well. Twenty days after HIV-1 infection the unmodified analogue proved practically ineffective, whereas the base- and thio-modified variants reduced the HIV-1 replication by 62 and 78%, respectively. The base+thio-modified derivative produced complete supression of virus multiplication not only after 20 days but through 4 weeks, so it can be regarded as a most promising anti-HIV agent.

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