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MODIFIED OLIGONUCLEOTIDES : IV[1] SOLID PHASE SYNTHESIS AND PRELIMINARY EVALUATION OF PHOSPHOROTHIOATE RNA AS POTENTIAL ANTISENSE AGENTS.

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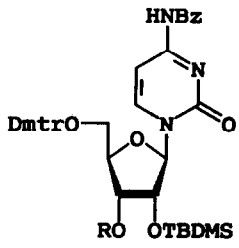
Abstract : For the first time a phosphorothioate oligoribonucleotide, namely PS-C_{14}, has been synthesized on solid support. This modified oligomer is more resistant to enzymatic degradation than the corresponding rC_{14} and binds to complementary RNA strands.

Various series of modified oligodeoxynucleotides have been described in the last few years and shown to be more resistant to nuclease degradation than the natural DNA and to still bind to complementary nucleic acid sequences.[2] Among these series, phosphorothioate analogues of oligodeoxyribonucleotides (PS-DNA) seem to be of great interest due to their potent anti-HIV activities.[3] Since modified RNAs[4] have been less extensively explored than modified DNAs, except for a recent preliminary note on α-RNA synthesis[5] and some publications on L-RNA[6] we decided to prepare phosphorothioate oligoribonucleotides (PS-RNA), in order to determine if they could eventually be used as antisense agents. In this communication we describe the first synthesis on a solid support of a PS-RNA, namely PS-rC_{14} and report on its nuclease resistance and its base-pairing properties.

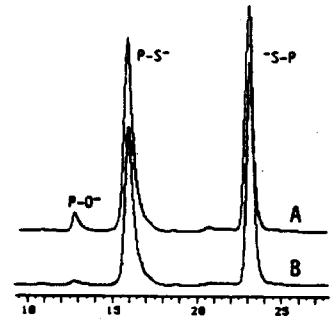
Automated syntheses of PS-DNAs have been previously performed by the H-phosphonate or phosphoramidite approach. In the latter case stepwise sulfurization with elemental sulfur[7] causes technical problems.[8] Very recently a new sulfurization reagent : 3H-1,2-benzodithiole-3-one-1,1-dioxide, was introduced by Beaucage,[9] and shown to lead, in only 30 sec,[10] to better than 99% stepwise yield of sulfurization during PS-DNA synthesis[11]. We therefore decided to evaluate the efficiency of this reagent in automated RNA synthesis on a simple model i.e. PS-r(CpC). The methyl phosphoramidite 1 and solid support derivative 2 (figure 1) were obtained and used following procedures reported in the literature.[12,13] After synthesis and usual deprotection,[13] it was possible to analyze the crude reaction products by HPLC as the natural dimer gave one peak at lower retention time than the ones corresponding to the diastereoisomeric phosphorothioates (figure 2)



1 : R= -P(CH$_3$O)-N(iPr)$_2$

2 : R= CPG-LCA succinyl

Figure 1



HPLC chromatogram of crude PS-r(CpC)

Figure 2

From various syntheses, we have observed that using standard conditions (15 min coupling time and 30 sec sulfurization time) the yield of sulfurization was only 95.5% (figure 2 A).[14] Increasing the sulfurization time (up to 60 sec) did not improve this yield, whereas decreasing the coupling time (3 min) gave rise to a yield better than 99% (figure 2 B) while quantitative couplings were still obtained[15]. Thus, Beaucage's reagent gives similar sulfurization pattern in both RNA and DNA series, at least when using the methyl phosphoramidite chemistry.

Under these conditions, PS-rC$_{14}$ was synthesized with an average coupling yield higher than 98%[16] as measured from the release of the dimethoxytrityl cation. After deprotection, the oligomer was purified by ion exchange chromatography and by reverse-phase HPLC and its homogeneity was checked by electrophoresis analysis,[17] ^{31}P-NMR spectroscopy[17] and HPLC.

	$t_{1/2}$ of	
	rC$_{14}$	PS-rC$_{14}$
Snake venom phosphodiesterase	14 min	170 min
Calf spleen posphodiesterase	34 min	260 min
Nuclease S1	400 min	4000 min
Ribonuclease A	*	22 min

Table I : Comparative half-life times of rC$_{14}$ and PS-rC$_{14}$, in presence of various nucleases. $t_{1/2}$ were determined by HPLC on the basis of disappearance of the signal corresponding to the studied 14-mer. * The natural oligomer was completely disappeared after 5 min reaction.

The substrate activity of PS-rC14 for four nucleases was studied and compared to that exhibited by rC14 The extent of enzymatic hydrolysis of those oligomers was monitored by HPLC and their half-life time was determined. As shown on Table I, the PS-rC14 exhibits, as expected,[18] a higher enzymatic stability than the natural oligomer.

Base pairing properties of PS-rC14 and rC14 with poly rI were determined by UV absorption spectroscopy. Under the same experimental conditions the PS-RNA exhibits a lower binding property (Tm 37°C) as compared to the corresponding RNA (Tm 46°C).[19] This result is not unexpected as it has been also observed in DNA series that phosphorothioate oligomers exhibit weaker binding capacities than the natural oligonucleotides.[20] This decrease in stability can be assigned among many factors to the presence of diastereoisomers.

In conclusion after having evaluated the capacity of Beaucage's reagent to form phosphorothioate bonds in the RNA series, we have synthesized a PS-RNA i.e. PS-rC14 Preliminary studies on the enzymatic stability and on the binding capacity of this modified RNA suggest that this series could be considered as potential antisense agents.

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