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SUGAR MODIFIED OLIGONUCLEOTIDES : II. SOLID PHASE SYNTHESIS OF NUCLEASE RESISTANT α-ANOMERIC URIDYLATES AS POTENTIAL ANTISENSE AGENTS

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Abstract : α-anomeric uridylates, α-rU_6 and α-rU_{12}, have been synthesized for the first time. These non natural α-oligoribonucleotides strongly resist to enzymatic degradation and bind to complementary RNA strands.

During the last few years, exogenous oligonucleotides binding specifically to complementary sequences of nucleic acids (RNA or DNA) through base pairing have been widely used *in vitro* as artificial regulators for gene expression[1-3] . Conceivable chemotherapeutic applications predicated on sequence specific hybridization require antisense oligonucleotides that are resistant to *in vivo* degradation by nucleases and strongly bind to their target mRNA. Thus a new class of nuclease resistant oligodeoxynucleotides consisting exclusively of α-anomeric nucleotide units has been recently developed in our laboratory[4]. Furthermore it is generally believed that the order of increasing stability of oligonucleotide.polynucleotide complexes is DNA.DNA < DNA.RNA < RNA. RNA[3] . So, we anticipated that hitherto unknown α-anomeric oligoribonucleotides would combine nuclease resistance and improved binding capacity to their complementary strand.

In this communication, we describe for the first time the synthesis on a solid support of α-hexauridylate (α-rU_6) and α-dodecauridylate (α-rU_{12}) and report our results related to their stability towards exo- and endonucleases and their binding properties.

α-uridine <u>1</u> was prepared according to an already published procedure[5]. The 5'- and 2'-hydroxy functions on the ribose moeity were respectively protected by the 4,4'-dimethoxytrityl group (DMTr)[6] and the *tert*-butyldimethylsilyl group (TBDMS)[7] (Figure 1). For the selective monosilylation of ribonucleosides, we have applied the procedure described by Ogilvie involving nitrate ion catalysed action of 1.1 molar equivalent of *tert*-butyldimethylsilyl chloride (TBDMS-Cl) in THF[8]. In contrast to the results observed in the β series, the TBDMS group was shown to be selectively introduced at the 3'position of the 5'-dimethoxytrityl-α-uridine <u>2</u>. When the reaction was carried out in pyridine in the presence of imidazole[9], a mixture of 2'-TBDMS

and 3'-TBDMS derivatives 3 and 4 was obtained in nearly equal amount. These two isomers were isolated by silica gel column chromatography and characterized by high field ^{1}H-NMR spectroscopic decoupling experiments[10].

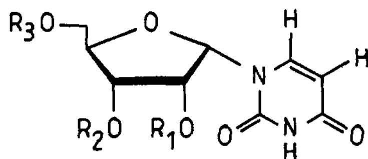

Figure 1

1 $R_1 = R_2 = R_3 = H$
2 $R_1 = R_2 = H$, $R_3 =$ DMTr
3 $R_1 =$ TBDMS, $R_2 = H$, $R_3 =$ DMTr
4 $R_1 = H$, $R_2 =$ TBDMS, $R_3 =$ DMTr
5 $R_1 =$ TBDMS, $R_2 = P(OCH_3)N(iPr)_2$, $R_3 =$ DMTr
6 $R_1 = P(OCH_3)N(iPr)_2$, $R_2 =$ TBDMS, $R_3 =$ DMTr
7 $R_1 = C(O)(CH_2)_2C(O)O(C_6Cl_5)$, $R_2 =$ TBDMS, $R_3 =$ DMTr
8 $R_1 =$ LCA-CPG succinyl, $R_2 =$ TBDMS, $R_3 =$ DMTr

Synthons 3 and 4 were then separately phosphitylated with methyl N,N-diisopropylchlorophosphoramidite[11] and the reaction products purified by chromatography on silica gel to give the corresponding phosphoramidites 5 and 6 respectively . The ^{31}P-NMR spectrum of 5 clearly showed only two signals at 152.49ppm and 151.31ppm different from those exhibited by 6 and corresponding to the expected diastereoisomeric mixture of ribonucleoside 3'-phosphoramidites . These data established that no detectable migration of the TBDMS group occured during the phosphitylation reaction (Figure 2).

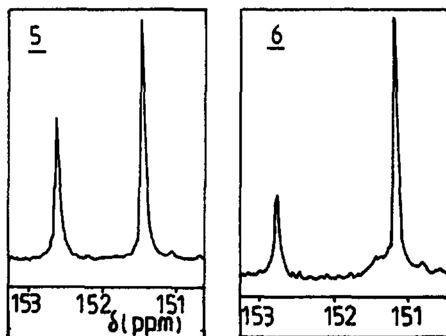

Figure 2. ^{31}P-NMR of α-uridine 2'- and 3'-phosphoramidites 6 and 5 obtained from separate preparations.

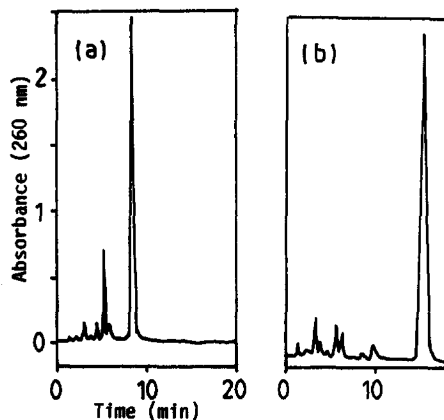

Figure 3. Reversed-phase HPLC analysis of the crude mixture containing U2'p5'U (a) and U3'p5'U (b) after deprotection using a C18 column.

The preparation of α-uridine derivatized support 8 was achieved via formation of a succinyl linkage between the 2'-hydroxy group of 3'-silylated α-uridine 4 and the amino group of the long chain alkylamine controlled pore glass (LCA-CPG). For this purpose the pentachlorophenyl succinate of the 3'-5'-protected α-uridine 7 was reacted with LCA-CPG according to the usual method[12].

The assembly of α-uridylates was achieved on an Applied Biosystems 381A synthesizer using 1 μmole protected nucleoside loaded support. As in the β ribo series[13], the condensation time required to obtain high coupling yields was 15 minutes using 0.5M tetrazole as activator and 0.15M phosphoramidite 5 in acetonitrile. Thus, the average coupling yield determined from the dimethoxytrityl cation release was 97% for the synthesis of α-rU_6 and α-rU_{12}. These two oligomers were deprotected[14] and purified by HPLC (spectrophotometric purity at 260nm was better than 97%).

In order to evaluate the possible occurence of phosphoryl migration under the deprotection conditions[14], we have synthesized the dimer α-(UpU). After cleavage of the protected dimer from the support and its deprotection, the products were analysed by reversed phase HPLC. Inspection of the chromatogram (Figure 3) showed one major peak corresponding to α-uridilyl-(3'→ 5')-α-uridine (R_T 15.11) and a minor peak (R_T 8.67) which was coeluted with α-uridilyl-(2'→ 5')-α-uridine (obtained from 6) and accounted for only 0.3% of the major peak. This very low extent of isomerisation compared well with previous results obtained in the β-series[15].

The substrate activity for four nucleases of rU_6 either in the all α- or all β-configuration was studied. The enzymatic hydrolysis were monitored by HPLC and the results are reported in Table 1. Under conditions where the β-hexamer was almost or completely degraded by either calf spleen phosphodiesterase, nuclease S1 or ribonuclease A, the corresponding α-hexamer remained intact. These results are consistent with the high nuclease resistance already observed with α- oligodeoxyribonucleotides[16].

Substrate / Nucleases	Time	α-rU_6*	β-rU_6*
Calf spleen phosphodiesterase	2 min	100%	89%
	60 min	100%	14%
Snake venom phosphodiesterase	2 min	94%	6.5%
	120 min	42%	0%
Nuclease S1	60 min	100%	68%
	300 min	100%	14%
Ribonuclease A	5 min	100%	0.3%
	35 min	100%	0%

Table 1. Time course of the cleavage of α- or β-rU_6 by four nucleases at 37°C. *Fraction of remaining hexamer after indicated time of incubation.

Base-pairing between α-rU_{12} and poly β-rA was followed by UV absorption spectroscopy[17]. Upon increasing the temperature of a 1:1 (A to U) mixture formed at 2°C a hyperchromicity was observed from which a melting temperature value (T_m) of 16°C was determined. This value is lower than the calculated melting temperature[18] of the duplex β-rU_{12}. β-rA_{12} (25°C). As this first data are only related to homopolymers, we are developing the synthesis of α-oligo-

ribonucleotides presenting mixed purine-pyrimidine sequences in order to fully evaluate their binding capacities.

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    For 4 : ^{1}H-NMR ($CDCl_3$) δ 8.73 (s, 1H, NH), 7.55 (d, 1H, $J_{5,6}$= 8.2 Hz, H-6), 7.45-6.82 (m, 13H, ArH), 6.34 (d, 1H, $J_{1',2'}$= 4.4 Hz, H-1'), 5.72 (d, 1H, H-5), 4.44 (m, 1H, H-2'), 4.35 (pseudo t, 1H, H-3'), 4.23 (m, 1H, H-4'), 3.80 (s, 6H, $O-CH_3$), 3.46-3.03 (m, 2H, H-5' and H-5"), 2.74 (d, 1H, OH-2'), 0.82 (s, 9H, $(CH_3)_3C$), 0.025 and -0.07 (2s, 6H, $(CH_3)_2Si$).
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