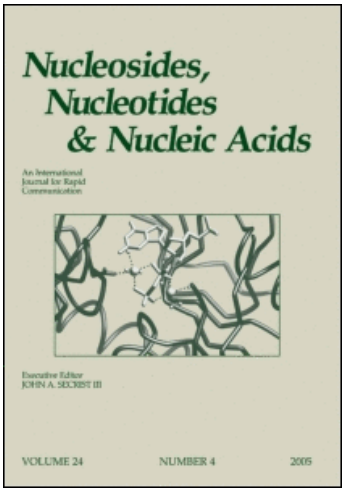

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Hybridization Properties of 4′-Branched Oligonucleotides

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Key Words: 4′-Branched oligonucleotides; Hybridization; Triplexes; Sugar conformation; Nuclease stability.

The positive results of our previous work encouraged the continuation of preparation, and evaluation of the properties of specific DNA/RNA analogs.[1] The findings that 4′-methoxy substituent (unlike 4′-methyl) in modified oligonucleotides enhances hybridization with RNA and in lesser extent with DNA[2,3] prompted us to investigate this phenomenon in detail. The 4′-substituents of oligonucleotide strand in a duplex are oriented into the minor groove, and could interfere with a hydration and/or with groups of a complementary strand. The detailed view of the image obtained from the molecular mechanics calculation shows the 4′-substituents as the bridging across the minor groove. In addition, the 4′-substitution itself influences significantly the ratio of individual sugar ring conformers so that it could lead to thermodynamically more stable duplexes eventually.

4′-Modified monomers (4′-N_3, 4′-CH_3O, 4′-$CH_3OCH_2CH_2O$, ...) were prepared, in spite of chemical difficulties, and significant decomposition of the compound during the key reaction steps. Olefin, 4′,5′-didehydro-2′,5′-dideoxythymidine, was prepared from 5′-iodo-2′,5′-dideoxythymidine using sodium methoxide,

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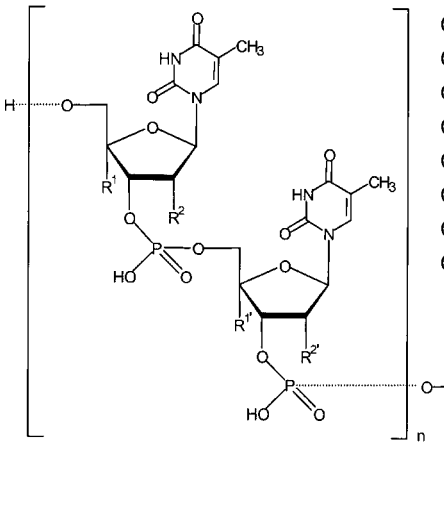
potassium *tert*-butoxide, or DBU. The oxidative addition of appropriate alcohol with *m*-chloroperoxybenzoic acid yielded 4′-alkoxy branched 2′-deoxynucleotide, which was separated from unwanted diastereoisomer and consequently dimethoxytritylated. The esterification of its 3′ position with novel reagent, bis(4-methoxy-1-oxido-2-picolyl) phosphate and subsequent partial deesterification gave desired monomer for the synthesis of oligonucleotide by the triester approach. The 4′-azido-5′-iodo-2′,5′-dideoxythymidine, the intermediate for the oligonucleotide ON-7, was prepared by modified method.[4]

The set of unique 4′-branched dT_{15} analogues (ON-1, ON-3 to ON-8) containing phosphodiester internucleotide linkage was synthesized, isolated, and characterized by MALDI-TOF MS. Some of them were prepared from the appropriate fully protected nucleoside 3′-phosphoramidites.



	R′	R²	R¹′	R²′	n
ON-1	CH_3O	H	CH_3O	H	7
ON-2	H	CH_3O	H	CH_3O	7
ON-3	CH_3O	H	H	CH_3O	7
ON-4	$CH_3OCH_2CH_2O$	H	$CH_3OCH_2CH_2O$	H	7
ON-5	$CH_3OCH_2CH_2O$	H	H	CH_3O	7
ON-6	$CH_3OCH_2CH_2O$	H	CH_3O	H	7
ON-7	N_3	H	N_3	H	6
ON-8	CH_3	H	CH_3	H	7

The 2′-*O*-methyl oligoribothymidylate ON-2 was prepared for the hybridization comparative study. Pentofuranose rings of both 4′-methoxy- and 2′-*O*-methyl-oligothymidylates adopt 3′-*endo* conformation (RNA type). The equation[5] $\%S = [(J_{(1',2')} + J_{(1',2'')} - 9.8)/5.9] \times 100$ was used for the calculation of pentofuranose ring conformation from NMR interaction constants.

The enzymatic resistance of the oligonucleotides was checked; oligonucleotide ON-1 is nuclease P1 resistant, however it has got no resistance towards snake venom exonuclease.

Our findings, as well as the results of molecular dynamics simulation (AMBER 5.0), convince us that the hybridization properties, namely, the deoxyribo vs. ribo selectivity, could be controlled by the modification at the 4′-position which do not affect, despite principal difference in pentofuranoside ring conformation, the duplex forming ability; therefore, such motif could be used in the construction of specific sequences.

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The T_m [°C] values of the 2:1 complexes of the modified dT_{15} with dA_{15} (rA_{15}) are compared with those of dT_{15}*dA_{15}(dT_{15}*rA_{15}).

	ON-1	ON-2	ON-3	ON-6	ON-5	ON-4	dT_{15}
$dA_{15}(Mg^{2+})$	55	53	44	49	49	53	45
$dA_{15}(Na^{+})$	40	34	25	19	23	16	36
$rA_{15}(Mg^{2+})$	55	55	52	52	57	58	36
$rA_{15}(Na^{+})$	50	48	41	41	48	48	34

The obtained NMR data sets revealed an interesting relationship between the character of 4′-substituent (e.g., alkyl vs. alkoxy) and T_m values.

GENERAL METHODS

Nuclear Magnetic resonance spectra were measured on Varian UNITY-500 spectrometer (^1H at 500 MHz; ^{13}C at 125.7 MHz frequency). Proton 2D-COSY spectra were used for the structural assignment of coupled protons and 2D-ROESY spectra for detection of the NOE constants.

Mass spectra (m/z) were recorded on ZAB-EQ (VG Analytical) instrument, using FAB+ and/or FAB- technique (Xe, 8 kV) with glycerol and thioglycerol as matrices.

MALDI-TOF mass spectra were recorded on Bruker Reflex4 spectrometer. N_2 laser 337 nm UV; 3-hydroxypicolinic and picolinic acid (9:1) in acetonitrile and mQ-water (1:1, v/v).

Melting temperature curves were recorded on Cary 100 Bio spectrophotometer (THERMAL program).

Final oligonucleotides were purified by preparative HPLC on reverse phase column (Phenomenex, JUPITER 5μ C18 300A).

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