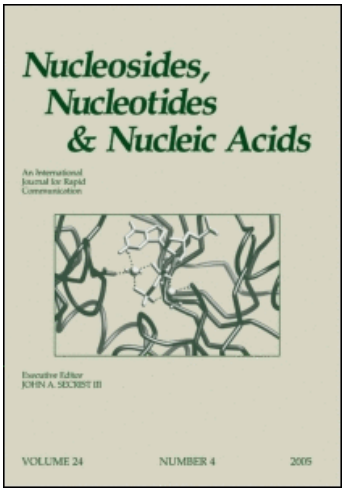

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Synthesis of Oligonucleotides Bearing Polyamine Groups for Recognition of DNA Sequences

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SYNTHESIS OF OLIGONUCLEOTIDES BEARING POLYAMINE GROUPS FOR RECOGNITION OF DNA SEQUENCES

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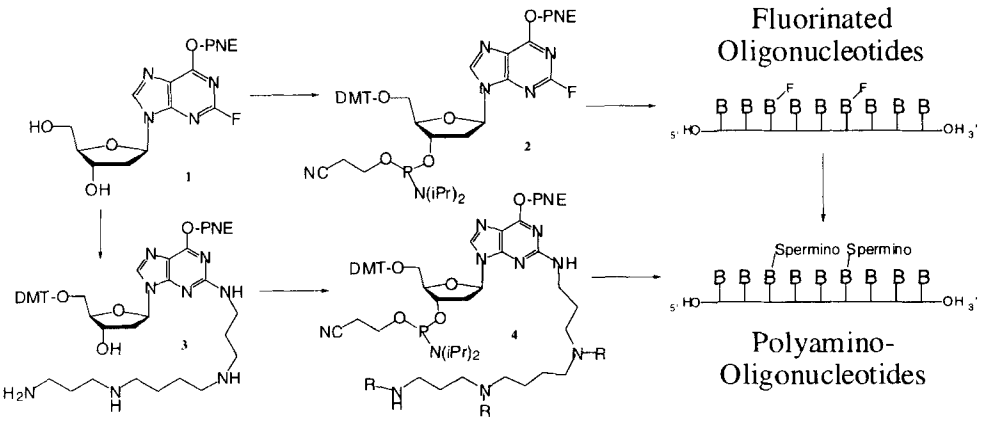
ABSTRACT: A 2-sperminoguanosine nucleotide has been synthesized and incorporated into oligonucleotides which showed increased duplex melting termperature.

Regulation of gene expression by the specific recognition of double-stranded DNA is a challenging therapeutic goal that antigene stategy may achieve. The field has been extensively explored and results using triple helix[1], peptide nucleic acids (PNA)[2] or pyrrole/imidazole in hairpin structures[3] are promising. As so far no strategy offers a general answer in a cellular context, alternative solutions are worth considering.

Tethering polyamines to oligonucleotides (ODN) enables the stabilization of duplex DNA structures. When attached to the C(2) position of a modified guanosine, a polyamine such as spermine is able to form a network of hydrogen bonds with the O(2) atoms of pyrimidines and N(3) of purines[4]. These modifications increase both association kinetics and duplex stability and thus are expected to help invading the double helix and hybridizing with the target sequence.



R= CF3CO- ; DMT= 4-4'-Dimethoxytrityl- ; PNE= 2-(4-Nitrophenyl)ethyl- ; Spermino= H_2N-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-

SCHEME: The post - and pre -oligomerization strategies

In a first synthetic pathway, the convertible[5] nucleotide **2** was introduced into ODN's which were then functionalized with spermine (SCHEME); however this *post-oligomerization* strategy was not suitable for high levels of substitution[6]. We therefore turned ourselves to the *pre-oligomerization* introduction of spermine. Starting from fluoroinosine **1**[6] we synthesized nucleoside **3** which was converted to **4** after protection and activation for solid support synthesis of DNA. Three ODN's having up to six modifications were synthesized with satisfactory trytyl cation titration profiles.

ODN's were isolated by reverse phase HPLC and characterized by MALDI-TOF mass spectrometry (TABLE 1). For **22dG1** and **22dG3** mass spectra showed the exact mass peaks together with their sodium salts. **22dG6** exhibited a peculiar behaviour during purification which may be related to its zwitterionic nature. Furthermore **22dG6** gave a large broad molecular peak of mass higher than expected.

TABLE 1: Oligonucleotide characterization

Name	Sequence	M/z (Da)	Mw (Da)
22dG0	5'-ATGAG ATGTG ACGAA CGTGT AC-3'	-	-
22dG1	5'-ATGAG ATGTG ACGAA CGT**G**T AC-3'	7008	7009
22dG3	5'-ATGA**G** ATGTG AC**G**AA C**G**TGT AC-3'	7376	7379
22dG6	5'-ATGA**G** AT**G**T**G** AC**G**AA C**G**T**G**T AC-3'	~8150	7935

TABLE 2: Melting temperature versus NaCl concetration

	50 nM			100mM			150mM		
Name	Tm	ΔTm	ΔTm/mod	Tm	ΔTm	ΔTm/mod	Tm	ΔTm	ΔTm/mod
22dG0	62.0	-	-	66.4	-	-	68.5	-	-
22dG1	65.5	+3.5	+3.5	69.4	+3.0	+3.0	71.0	+2.5	+2.5
22dG3	73.4	+11.4	+3.8	76.1	+9.7	+3.2	77.6	+9.1	+3.0

Duplex melting temperatures (Tm) were measured for **22dG0-3** in various salt concentrations and are listed in TABLE 2. Depending on the conditions, the tethered polyamines stabilize the duplex structure by an average ΔTm= +3°C per modification. Extrapolation of the Tm increase for **22dG6** leads to an estimated Tm~86°C at 150mM in NaCl, similar to DNA itself in the same conditions (data not shown).

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