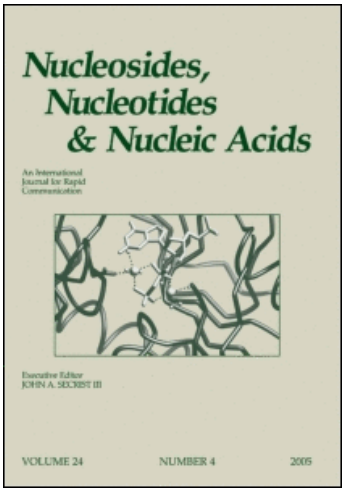


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# Modified Oligonucleotides with Triple-Helix Stabilization Properties

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# MODIFIED OLIGONUCLEOTIDES WITH TRIPLE-HELIX STABILIZATION PROPERTIES.

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**ABSTRACT:** Triple helix binding properties of several purine and pyrimidine derivatives are described. Introduction of an amino group at position 8 of adenine and guanine stabilize triple helix.



Several years ago, it was shown that oligonucleotides could bind to homopurine-homopyrimidine sequences of double stranded DNA by forming triple helices. The formation of nucleic acid triple helices offers the possibility of designing sequence specific DNA binding molecules with therapeutic and diagnostic uses (1).

Using the structure of the d(T.A.T) triple helix obtained by molecular dynamics simulations (2), a defined pattern of hydration was found in the narrow groove between A and the O2 of the Hoogsteen strand thymine (mM groove). We predicted that the introduction of a polar group near C8 will produce a notable increase in the stability of the triplex by displacement of the hydration spine as well as by the formation of an extra H-bond.

For these reasons we have prepared oligodeoxynucleotides carrying 8-aminoadenine (8-AA) and 8-aminoguanine (8-AG). The triple helix binding properties of these modified oligonucleotides were studied together with other modified oligonucleotides carrying 5-substituted pyrimidines (5-MeC: 5-methyl-C, 5-PrC: 5-propynyl-C, 5-BrU: 5-bromo-U, 5-IC: 5-iodo-C, 5-FU: 5-fluoro-U, 5-PrU:5-propynyl-U). Some of the 5-substituted pyrimidines are known to stabilize triplex (1). Previous to our work triplex stabilization properties for 8-aminoadenine (3) and 8-aminoguanine (4) were predicted but no

**TABLE 1**: Melting temperatures (°C) in 1M NaCl, 100 mM sodium phosphate/ citric acid buffer) for the triplex $h_{26}$:$s_{11}$. The duplex Tm values of $h_{26}$ occurred between 82 °C and 75 °C. n.d. not determined

$h_{26}$: $^{5'}$GAZGWZWGZWATTTTTCTCCTCCTTC$^{3'}$

$s_{11}$: $^{5'}$XYYXXYXXYXT$^{3'}$

| X,Y | Z,W | pH 5.5 | pH 6.0 | pH 6.5 | pH 7.0 |
|---|---|---|---|---|---|
| C,U | A, G | 42 | 28 | -- | -- |
| C,T | A, G | 40 | 20 | -- | -- |
| 5-MeC, T | A, G | 56 | 34 | 27 | -- |
| 5-PrC, T | A, G | 31 | -- | -- | -- |
| C, 5-BrU | A, G | 53 | 33 | 26 | -- |
| C, 5-IU | A, G | 40 | 27 | -- | -- |
| C, 5-FU | A, G | 40 | 23 | -- | -- |
| C, 5-PrU | A, G | 50 | 38 | n.d. | n.d. |
| C,T | 8-AA, G | 65 | 49 | 40 | 27 |
| C,T | A, 8-AG | 59 | 47 | 40 | 32 |



experimental data were reported. During the preparation of this manuscript, the triplex stabilization properties of 8-aminoadenine were described (5).

Melting curves have been measured using a triple helix model formed by a self-complementary hairpin of 26 bases and an all pyrimidine single stranded oligonucleotide (6). Results are shown in **TABLE 1**. As described previously (1), the presence of 5-bromouracil, 5-propynyluracil and 5-methylcytidine stabilize triple helix (2-3°C per substitution at pH 6.0 compared with T) . Triplex containing 5-iodouracil, 5-fluorouracil, thymine, uracil have similar stabilities. Substitution of C for 5-propynylcytosine gave a less stable triplex.

Substitution of A and G for 8-AA and 8-AG produced a large increase on the melting temperature of the triplex (4-5 °C per substitution at pH 6.0), and therefore, the observation of triple helices at neutral pH becomes possible. The high degree of stabilization obtained by this simple substitution will be of interest in the development of new applications based on triple helix formation such as DNA-based diagnostic kits and antigene therapy.

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