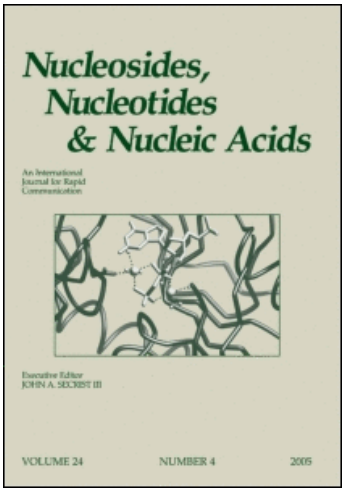

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Oligonucleotide Conjugates Derived from an Electrophilic Site: Conjugation to Baseless Carbohydrate Residue. Synthesis, Hybridization and Modeling Studies

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VIII. SPECIAL TOPICS

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1741-1744 (1997)

OLIGONUCLEOTIDE CONJUGATES DERIVED FROM AN ELECTROPHILIC SITE: CONJUGATION TO BASELESS CARBOHYDRATE RESIDUE. SYNTHESIS, HYBRIDIZATION AND MODELING STUDIES

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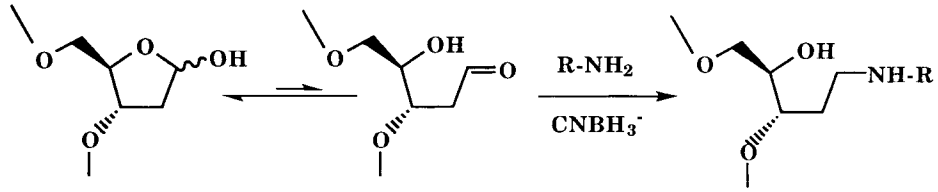
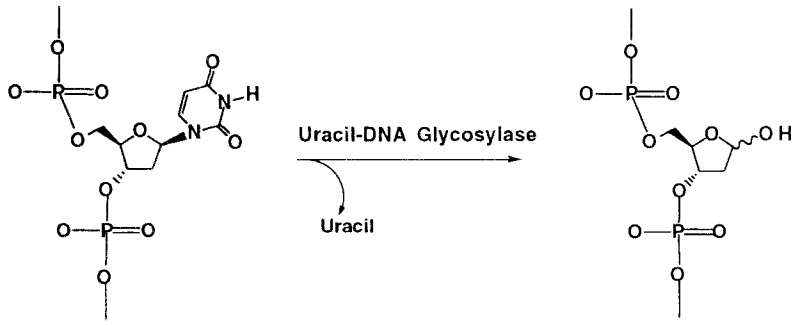
Conjugation of electrophiles to oligonucleotides via tethers carrying nucleophilic sites is well-known. However, for the reverse reaction, the available methods to generate electrophilic sites in oligonucleotides are not many: (e.g., periodate oxidation of terminal ribose sugar followed by reductive amination).

We have employed the enzyme uracil-DNA glycosylase to generate an aldehydo abasic site in the middle of an oligonucleotide; this site was further derivatized with several amino group containing ligands (via Schiff base formation and reduction). These ligands included several antisense property modifying agents having nucleophilic ends such as amines and hydrazides (Nucleic acid cleaving agents, intercalators and polyamines) and some antisense monitoring reporter groups. The conjugates were hybridized against both RNA and DNA targets and their hybridization properties were studied. In the second set of targets, the abasic conjugation site bulged out from the duplex since the target strands (RNA or DNA) had a missing nucleotide across the abasic site. Hybridization and molecular modeling studies were carried out to assess the relative stabilities of the various conjugates.



The abasic site in DNA has only the deoxyribose unit, and exists as an equilibrium mixture involving cyclic hemiacetals (nearly 1:1 α and β forms) and the aldehydo-open-chain form. The aldehyde form constitutes approximately 1% of the equilibrium population of the abasic site. The abasic (apurinic or apyrimidinic) sites arise from either spontaneous acid-

catalyzed depurination or as intermediates in DNA repair by N-glycosylase repair enzymes. During the repair process mediated by endonucleases, the aldehydo abasic site plays the vital role of facilitating a β-elimination of the 3'-phosphate. The enzymatic generation of the abasic sites and their use in further conjugation chemistry is not well studied.



Uracil Containing DNA **Aldehydic Abasic Site**

The 11-mer parent oligonucleotide sequence CGC AGU* CAG GC (ISIS 2746) has been synthesized. Thus, the abasic site (U*) lies in the position 6 of the oligonucleotide. Derivatization at the abasic site with the ligands shown below have been carried out. The corresponding 11-mer and 10-mer complementary DNA and RNA oligonucleotides have been synthesized as well and hybridization studies have been perfomed.

The stacking energy that had been lost in the abasic duplex, to some extent has been compensated by conjugation of selective ligands. The Tm studies indicate the following order of duplex stability:

11-mer DNA target

fluorescein < abasic < tripeptides < pyrene < ortho-phenanthroline

11-mer RNA target

fluorescein < abasic < pyrene < tripeptides < ortho-phenanthroline

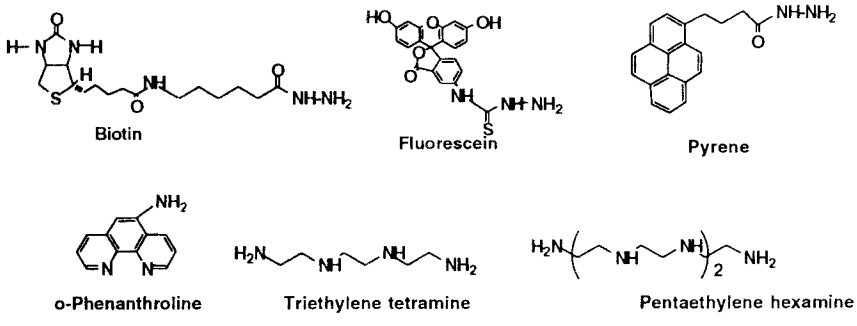
10-mer DNA target

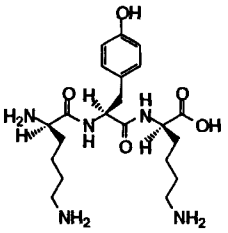
fluorescein < abasic < tripeptides < ortho-phenanthroline < pyrene

10-mer RNA target

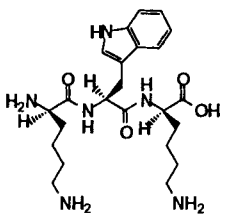
fluorescein < abasic < pyrene < ortho-phenanthroline < tripeptides

Ligands For Conjugation





LYS-TYR-LYS



LYS-TRP-LYS

The extraordinary thermodynamic stability of thye closed form over open form is borne out in the theretical computations of heats of formation. [*Ab initio* calculations have been performed using the 6-31G* basis set to optimize the geometries of both open aldehyde and closed furonoside forms of D-ribose]. Molecular modeling studies based energy minimization of the model built duplexes employing CFF93 forcefield as incorporated in the DISCOVER software [MSI, San Diego] corroborate the trends of the hybridization results.

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