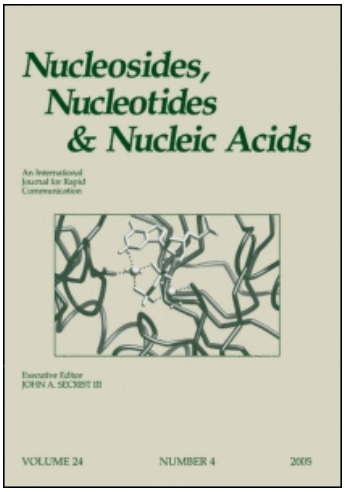


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Imidazolethyl-Phosphoramidate α-Oligonucleotides

T. Michel[a]; L. Mouls[a]; F. Debart[a]; J. -J. Vasseur[ab]
[a] Laboratoire de Chimie Organique Biomoléculaire de Synthèse, Université de Montpellier II, Montpellier, France [b] Université de Montpellier II, Montpellier Cedex 5, France

Online publication date: 09 August 2003

To cite this Article Michel, T. , Mouls, L. , Debart, F. and Vasseur, J. -J.(2003) 'Imidazolethyl-Phosphoramidate α-Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1263 — 1265
To link to this Article: DOI: 10.1081/NCN-120022942
URL: http://dx.doi.org/10.1081/NCN-120022942

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1263–1265, 2003

Imidazolethyl-Phosphoramidate α-Oligonucleotides

T. Michel, L. Mouls, F. Debart, and J.-J Vasseur*

Laboratoire de Chimie Organique Biomoléculaire de Synthèse, Université de Montpellier II, UMR 5625 CNRS-UMII, Montpellier, France

ABSTRACT

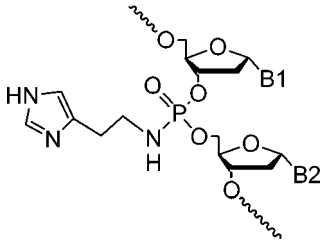
α-ODNs conjugated to imidazole groups via phosphoramidate internucleosidic linkages were synthesized. The presence of the imidazolethyl-phosphoramidate linkage improved the affinity of α-ODNs for their nucleic acid targets.

Key Words: Imidazole; Phosphoramidate; Alpha oligonucleotides.

Sequence specific RNA cleaving molecules have been obtained by conjugating imidazole rings to the 5′- or 3′-ends of ODNs.[1] However, the efficiency of the RNA cleavage with these compounds bearing a low number of imidazoles is limited. Enhancing the number of imidazoles through conjugation to the phosphate backbone of the ODN is a potential approach to increase the efficacy of such conjugates. However, phosphate modifications have a detrimental impact on the stability of duplexes formed with nucleic acid targets.[2] Fortunately, inversion of the anomeric configuration (from β to α) of the sugar moieties in phosphate-modified ODNs, such N-alkyl phosphoramidates, increases the affinity for their targets.[3]

*Correspondence: J.-J Vasseur, Université de Montpellier II, CC008, Place E. Bataillon, F-34095 Montpellier Cedex 5, France; Fax: +33 4 6704 2029; E-mail: vasseur@univ-montp2.fr.

DOI: 10.1081/NCN-120022942
1525-7770 (Print); 1532-2335 (Online)



Here, we present first results concerning the synthesis and the properties of α-ODNs conjugated to imidazole groups via phosphoramidate internucleosidic linkages.

ODNs containing phosphodiester (PO) and phosphoramidate (PN) internucleosidic links could be obtained using H-phosphonate chemistry by treatment of H-phosphonate diesters with an I_2/H_2O/pyridine solution to give PO bonds and with a 10% solution of the desired amine in CCl_4/pyridine[4] to give PN bonds. In this work, the efficiency of the oxidative amidation with histamine, a β-dT_{10} ODN bearing 3 imidazolethyl-phosphoramidate links (PNHImE) at its 5′-end and 6 PO linkages was synthesized. However, HPLC and MALDI-TOF analyses revealed a mixture of 3 main compounds corresponding to β-dT_{10} ODN bearing 3, 2 and 1 imidazolethyl-phosphoramidates. This fact could be explained by the possible reaction of one imidazole nitrogen atom of the histamine during the oxidative amidation giving rise to an unstable phosphoroimidazolidate further hydrolyzed in PO.

To avoid this reaction, the oxidative amidation was performed using histamines where the imidazole ring was protected with a monomethoxytrityl or a dinitrophenyl group. However, here again, the oxidative amidation was unsuccessful giving a mixture of ODNs containing 0, 1, 2 and 3 imidazole moieties.

The effect of the imidazolethyl-phosphoramidate linkage (PNHImE) on the hybridization with DNA and RNA targets, was studied with an α-ODN heterododecamer containing 3 PN linkages at its 5′-end and 8 PO at its 3′-end α-$^{5'}$d(T*C*T*TAACCCACA)$^{3'}$ (α-PNHImE) prepared using unprotected histamine.

The Tm values of the hybrids of the α-PNHImE ODN were compared to those previously obtained[3] with β-PO and α-PO ODNs.

Tm studies showed significant increases for the α-PNHImE ODN with the DNA target ($\approx +3.3$°C and 2.4°C/mod with respect to the α- and β-ODNs) and similar Tm with the RNA target ($+0.9$ and ≈ -0°C/mod with respect to the α- and β-ODNs).

To evaluate the ability of the modified ODNs to act as Rnase mimics, the hybridization medium of the α-PNHImE ODN and its complementary RNA was allowed to stand at room temperature with or without $Zn(OAc)_2$[5] for one week without any observable degradation of the RNA.

ACKNOWLEDGEMENT

This work was supported by grant from ''Association pour le Recherche contre le Cancer'' (ARC).

REFERENCES

1. a) Hovinen, J.; Guzaev, A.; Azhayeva, E.; Azhayev, A.; Lonnberg, H. Imidazole tethered oligodeoxyribonucleotides: synthesis and RNA cleaving activity. J. Org. Chem. **1995**, *60*, 2205–2209; b) Vlassov, V.; Abramova, T.; Godovikova, T.; Giege, R.; Silnikov, V. Sequence-specific cleavage of yeast $tRNA^{phe}$ with oligonucléotides conjugated to a diimidazole construct. Antisense Nucleic Acid Drug Develop. **1997**, *7*, 39–42.
2. Lebedev, A.V.; Wickstrom, E. The chirality problem in P-substituted oligonucleotides. Perspect. Drug Discov. Des. **1996**, *4*, 17–40.
3. Laurent, A.; Naval, M.; Debart, F.; Vasseur, J.-J.; Rayner, B. Chiral and steric effects in the efficient binding of α-anomeric deoxyoligonucleoside *N*-alkylphosphoramidates to ssDNA and RNA. Nucleic Acids Res. **1999**, *27*, 4151–4159.
4. Froehler, B.C.; Matteucci, M.D. Phosphoramidate analogues of DNA: synthesis and thermal stability of hetroduplexes. Nucleic Acids Res. **1988**, *16*, 7831–4839.
5. Santoro, S.W.; Joyce, G.F.; Sakthivel, K.; Gramatikova, S.; Barbas, C.F. RNA cleavage by a DNA enzyme with exrended chemical functionality. J. Am. Chem. Soc. **2000**, *122*, 2433–2439.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016