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Site-Specific RNA Cleavage Using Terpyridine-Cu(II)-Linked 2'-O-Methyloligonucleotides

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SITE-SPECIFIC RNA CLEAVAGE USING TERPYRIDINE•Cu(II)-LINKED 2'-O-METHYLOLIGONUCLEOTIDES

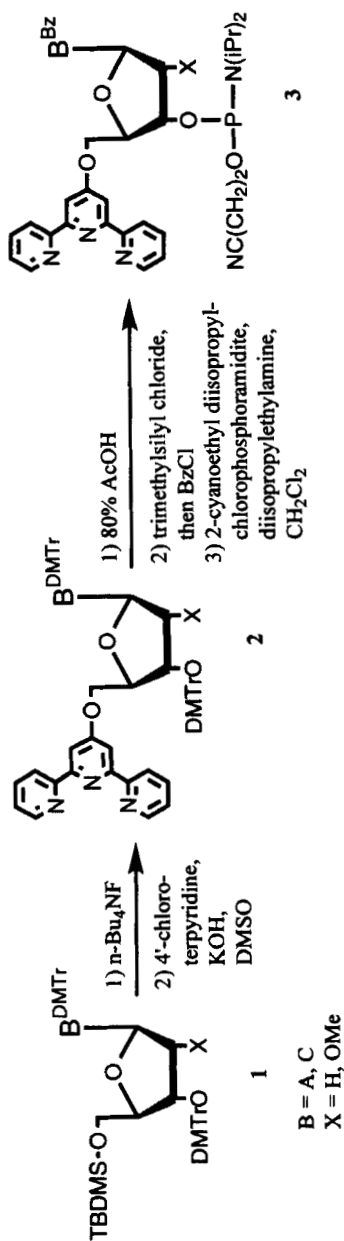
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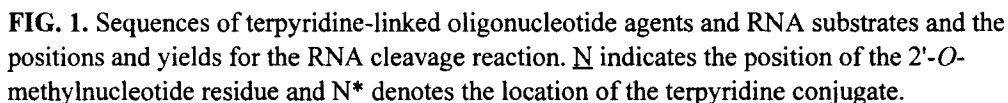
ABSTRACT: 2'-Deoxy- and 2'-O-methyl-5'-O-terpyridyl derivatives of adenosine and cytidine were synthesized and used to construct 5'-end-modified oligonucleotides. These antisense agents complexed with Cu(II) exclusively cleaved a complementary RNA oligomer at the site opposite the terpyridine-nucleoside residue. We also found that the terpyridine•Cu(II) moiety stabilizes 2'-O-methyl RNA duplex. These suggest that after RNA hybridization, the terpyridine moiety is close to the RNA strand, presumably in an end capping manner.

Oligonucleotides that combine sequence-specific recognition with cleavage within the recognition region may be useful for studies on structure-function relationships of native RNAs. They may be also good candidates for antisense chemotherapy because cleavage of a specific mRNA will lead to loss of its function. Studies of site-specific RNA cleavage using terpyridine¹- or derivatives² of this ligand-linked agents have been recently reported. In these and related studies, flexible linker arms have been used to attach reactive groups to DNA oligomers for sequence-specific recognition.¹ Here, we describe an alternative arrangement consisting of a 2'-O-methyl RNA oligomer as an efficient RNA hybridization probe³ and a terpyridine•Cu(II) complex which was directly attached to the nucleoside-sugar 5'-oxygen atom.

A terpyridine-containing building unit **3** for oligonucleotide synthesis was constructed using standard methods for the most steps (SCHEME 1). Compound **1** was prepared by tert-butyldimethylsilyl (TBDMS) protection of 2'-deoxyadenosine, 2'-O-methyladenosine, 2'-deoxycytidine or 2'-O-methylcytidine. This was followed by di-



SCHEME 1. Synthesis of terpyridine-linked nucleoside 3'-phosphoramidite derivatives.



Oligonucleotides synthesized for RNA cleavage reaction are shown in FIG. 1. The reaction of the agent (complexed with Cu(II), 10 molar equivalent) with a target RNA (5'-end-labeled with ^{32}P) was carried out at pH 7.5 and 45 °C for 20 hr. Analysis of the products by denaturing polyacrylamide gel electrophoresis indicated site-specific cleavage of the RNA as shown in FIG. 1. The cleavage position was observed at the junction of the hybrid and single-stranded RNA regions. Mispairing at the terpyridine site reduced the site-specificity (data not shown). In order to explore the spatial orientation of the terpyridine moiety in the oligonucleotide hybrid, we prepared a terpyridine-linked duplex with a self-complementary sequence (dA*CmAmGmCmUmGmUm) and examined its thermal stability. It was found that the T_m value (72°C) was very high, compared with that (52°C) for the control duplex without terpyridine moieties. These results may mean that the terpyridine moiety interacts with the end of the hybrid in an end capping manner. Thus, this study shows that our terpyridine agent can cleave RNA with high specificity at the predetermined site.

REFERENCES

- 1) Trawick, B. N.; Daniher, A. T.; Bashkin, J. K. *Chem. Rev.*, **1998**, *98*, 939-960.
- 2) Hall, J.; Hüsken, D.; Häner, R. *Nucleic Acids Res.*, **1996**, *24*, 3522-3526.
- 3) Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.*, **1987**, *15*, 6131-6148.