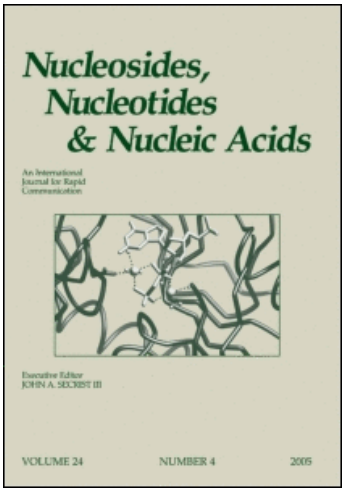

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CLIP-PHEN CONJUGATES FOR THE SPECIFIC CLEAVAGE OF NUCLEIC ACIDS

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□ *For the first time Clip-Phen (**1**) was conjugated to oligonucleotides to provide very efficient tools for the cleavage of nucleic acids at specific positions. The synthesis of the conjugates as well as the cleavage experiments are reported.*

Keywords Clip-Phen; cleavage; DNA targets

INTRODUCTION

The synthetic nuclease, Clip-Phen copper complex (**1**), promotes a very efficient oxidative cleavage of nucleic acids in the presence of a reductant. This molecule is a promising tool for nucleic acids based diagnostics where there is a need to fragment the long DNA, or RNA targets, prior to their amplification or hybridization on DNA arrays. However, even if Clip-Phen mediated oxidation occurs selectively at the desoxy or ribose moiety, the resulting cleavage is random. In order to control such a cleavage and make this complex sequence selective, Clip-Phen was synthesized and properly derivatized for conjugation with 5'amino oligonucleotides. We have demonstrated that these conjugates can easily discriminate between specific and non specific DNA targets. The cleavage of the target occurs at specific position in less than 1 hour.

RESULTS AND DISCUSSION

3-Clip-Phen and its acidic derivatives were synthesized following published procedures[1,2] and further derivatized under its NHS form for

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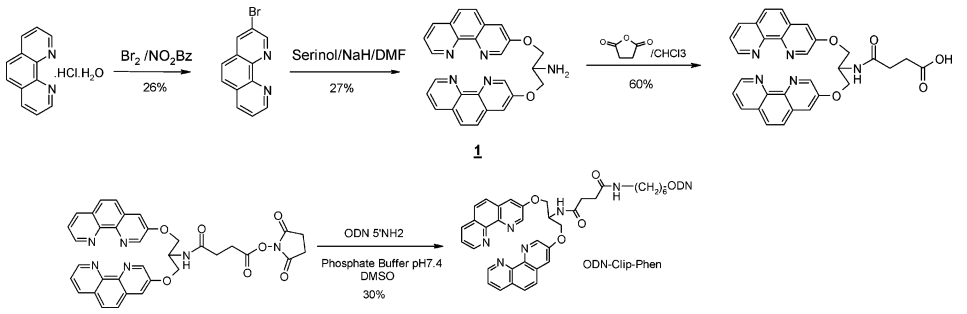
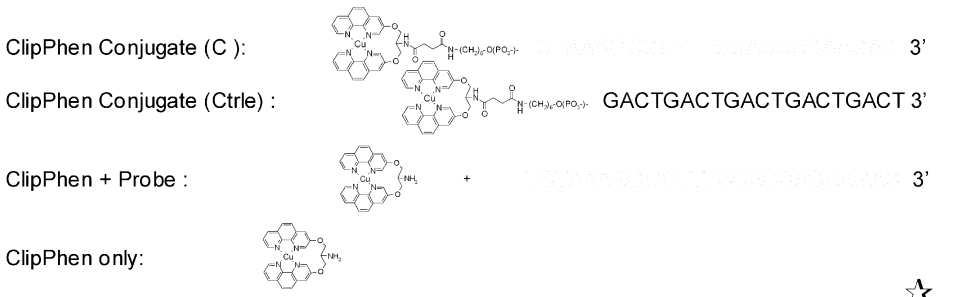


FIGURE 1 Synthesis of Clip-Phen conjugates.

conjugation to oligonucleotides (Figure 1). All of these compounds were purified by HPLC and characterized by NMR and mass spectroscopy. The conjugates as well as free Clip-Phen were metallated by CuCl2 and incubated with their 5′ P32 labelled targets before ascorbate addition. After 1 hour incubation the cleaved targets were analyzed by PAGE electrophoresis (Figure 2). Free Clip-Phen, at equimolar concentration, hydrolyses very quickly the target (C) or (NC) without any sequence selectivity (data not showed). We demonstrated that after dilution in a large amount of non specific salmon sperm DNA, the same free Clip-Phen is unable to hydrolyze a specific P^{32} labelled DNA sequence (column 4, Figure 3). On the contrary, conjugate (C) at high or low concentration, is always able to find its target even in the absence or in the presence of nonspecific DNA (column 1 and 3, Figure 3). The target is cleaved at specific positions around the junction single/double stand at more that 50% in 30 minutes. We also demonstrated



- Metallated Conjugates (**1µM-100 nM**) + ^{P32} Labelled target (**1 µM-10 nM**+/- exces salmon sperm DNA) in phosphate buffer 40mM pH 7.2, 50mM NaCl, 10mM MgCl2.
- Incubation 37°C 1h and ascorbate addition (**100 µM**)
- Incubation for 1h 37°C. Precipitation and analysis by PAGE + phospho imager

FIGURE 2 Sequences of the Clip-Phen conjugates and the controls.

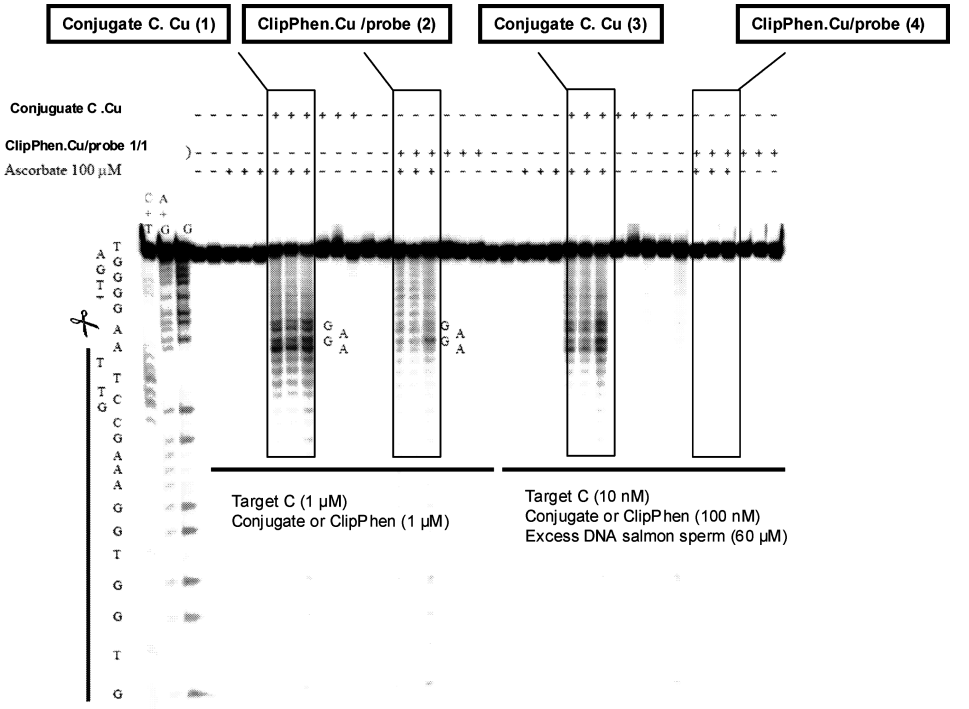


FIGURE 3 Cleavage of a specific target in solution by Clip-Phen conjugate. A comparison between the activity of Conjugate, Clip-Phen.Cu Probe, and free Clip-Phen.Cu.

that a nonconjugated probe incubated with Clip-Phen in the presence of the target induces also cleavage specificity (column 2, Figure 3) at the same junction. This effect totally disappears after dilution with non specific DNA (column 4, Figure 3). Following these experiments, the demonstration was done that Clip-Phen conjugate enhances the specificity and the efficiency of the cleavage. The quantity of cleavage products is reported in Table 1.

TABLE 1 Percentage of cleaved target estimated by autoradiography

		Pourcentage of cleaved target	
	Hybridation	Without ascorbate	With ascorbate
Target **C** 1 mM/ClipPhen Conjugate C1 mM	37°C 1 h	~10	60
Target **C** 1 mM/Probe **C** 1 mM/3-Clip-Phen·Cu 1 mM	37°C 1 h	~0	43
Target **C** 10 nM/ClipPhen Conjuguate C 100 nM ADN random	37°C 1 h	0	51
Target **C** 10 nM/Probe **C** 100 nM/ 3-Clip-Phen-Cu 100 nM ADN random	37°C 1 h	~0	0

Ascorbate 100 mM/1 hour at 37°C.
PAGE + Phospho Imager quantification.

CONCLUSION

For the first time Clip-Phen molecule was conjugated to oligonucleotides to obtain a very efficient and sequence-specific nucleic acid fragmentation tool. Clip-Phen conjugate showed a very good selectivity toward its specific target where hydrolysis occurs at the junction single/double strand. Moreover, Clip-Phen conjugate was more efficient (50% target cleavage) than free Clip-Phen (0%) in the presence of a large amount of nonspecific DNA. These new cleaving tools will be applied to control DNA fragmentation before hybridization on DNA Chips.

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