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Hybridization Properties and Polymerase Acceptance of Nitropiperonyl Deoxyriboside. Photochemical DNA Backbone Cleavage

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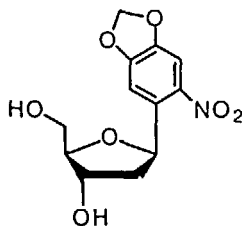
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A novel C-nucleoside has been developed that can substitute for three of the four heterocyclic bases in double-stranded nucleic acids. It has the further property that it can be activated by light, leading to backbone cleavage and thereby dissociation of longer, double-stranded nucleic acids into shorter, single-stranded nucleic acids.

Keywords: nucleotides; fragmentation; photochemistry

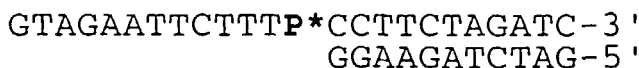
In a number of modern techniques for analysis of nucleic acids,^[1] small (<50nts) fragments of analyte are desirable. Several strategies have been developed for the cleavage of larger nucleic acid fragments, such as PCR products, to smaller ones, including limited restriction digests, transcription to RNA and limited hydrolysis, or low-level incorporation of uracil, generation of abasic sites with uracil-N-glycosylase, and backbone cleavage. All have drawbacks related to sequence bias, efficiency, and reagents that interfere with subsequent processing.^[2] A universal, photochemically cleavable base analog that could be doped into PCR reactions and randomly incorporated at low levels into amplification products would therefore be desirable. The

evaluation of a novel C-nucleoside, nitropiperonyl deoxyriboside (NPdR), for this purpose has been undertaken.



nitropiperonyl deoxyriboside
(NPdR, P*)

NPdR can be converted to its 5'-DMTr-3'-CE-phosphoramidite and incorporated into pentacosanucleotides by conventional techniques. The effect on hybrid formation when this P* base opposes native bases was evaluated, as was its ability to promote incorporation of native dNTPs when in the template strand of a primer-template complex. As the triphosphate, its incorporation into the replicated strand was also shown. Brief irradiation ($\lambda > 360$ nm) of duplexes containing the P* base and piperidine treatment cause single-strand cleavage events giving 3'-phosphates. Thus, two significant recent interests, universal/non-hydrogen bonding base analogs and photochemical backbone cleavage, have been combined in a single molecule that serves as a light-based DNA scissors.



Acknowledgments

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