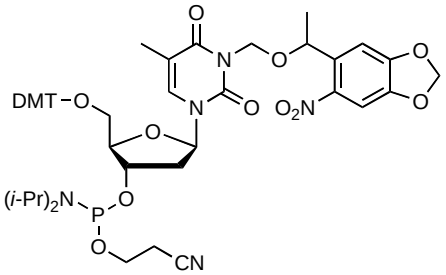
NPOM-Caged-dT CEP

Product No. BA 0317

Product Information



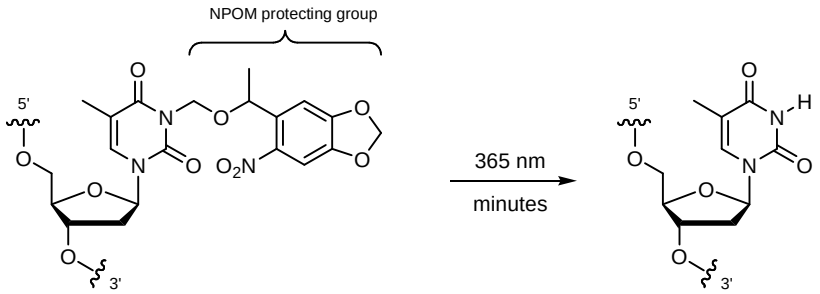
$C_{50}H_{58}N_5O_{13}P$

Mol. Wt.: 968.00

Installs a thymidine residue bearing a bulky NPOM protecting group at N1, thus efficiently disrupting Watson-Crick base-pairing. Upon brief exposure to UV light of 365 nm, the "caged" oligonucleotide is uncaged, restoring base-pairing ability.

Introduction to caging: Light-induced activation of biological processes is most commonly achieved through the initial deactivation of a particular molecule via installation of a photo-protecting group at a critical position, e.g., disrupting a hydrogen bond. This renders the molecule inactive, a practice known as "caging".[1] The photo-protecting group (caging group) is removed upon irradiation with UV light, thus restoring the biological activity, i.e., "uncaging". This process enables precise regulation of biological processes, since light irradiation can be easily controlled in a spatial and a temporal fashion. Moreover, light irradiation is a non-invasive technique that results in minimal secondary perturbations of cellular processes. An example of caging/decaging in the oligonucleotide field is the management of gene expression.

Nucleobase caging with NPOM-Caged-dT CEP: Many strategies have surfaced for the caging of oligonucleotides using photochemically labile groups. The caging group may be placed on internucleotide phosphates, various positions on the sugar, or on the nucleobase. Caging the nucleobase is particularly attractive.[1,2] One of the most promising strategies for doing so involves the use of the 6-nitropiperonyloxymethyl (NPOM) group, developed by Deiters and co-workers at North Carolina State



University.[3-5] This new group is useful for the caging of nitrogen heterocycles and does not involve a carbamate linkage as is typical of many photolabile nitrogen protecting groups. Deiters, *et al.*, have installed the NPOM group at N1 of the thymidine phosphoramidite NPOM-Caged-dT CEP (BA 0317) described herein and incorporated it into oligonucleotides using standard automated DNA synthesis protocols, resins, and reagents.[3,4] *They found that the bulky NPOM group effectively blocks hydrogen-bond formation in duplexes. The caged thymidine is stable to a wide range of chemical and physiological conditions, but the NPOM caging group is removed within minutes by irradiation with 365 nm UV light.* This can be achieved using a standard fluorescent microscope, UV LED fiberoptic instruments, or even with a simple hand-held 25 watt UVA lamp. This wavelength is long enough to avoid damage to the oligonucleotides or cells.

In their initial work,[3] Deiters and co-workers prepared DNAzymes with one to three thymidine residues replaced by NPOM-Caged-dT residues. A single caged thymidine placed at a crucial position was sufficient to inhibit DNAzyme activity. Three substitutions at non-critical positions were found to be useful for the inhibition of DNA/RNA hybridization. Uncaging with UVA light restored RNA cleaving ability in all cases.

In later work,[4] Deiters and co-workers were able to control DNA amplification via PCR using the same caging strategy. The placement of three NPOM-Caged-dT residues throughout several 19-mer PCR primers was sufficient to prevent annealing to the cognate DNA template, thus blocking PCR amplification. Uncaging the primers with light effectively activated PCR. It was also found that PCR could be stopped using the caging strategy. Thus, a primer that bears a self-complementary region and a propensity to exist as a hairpin was employed. Placing NPOM-Caged-dT residues in the complementary region prevented hairpin formation and allowed the primer to operate. Illumination caused uncaging, thus allowing hairpin formation and disrupting the annealing of the primer to the cognate DNA strand, stopping the amplification process. The power of these techniques was further illustrated using the "start" and "stop" type of primers simultaneously to stop the production of one PCR product while triggering the start of the amplification of a different PCR product. Another potential application is heating a start-type caged primer with its target at the annealing temperature followed by irradiation, constituting a hot-start PCR protocol.

Use of NPOM-Caged-dT CEP: Employ acetonitrile diluent at the concentration recommended by the synthesizer manufacturer. Use standard coupling protocols; in our hands, extended coupling times were not required. Cleavage from the solid support may be carried out by standard procedures. Standard nucleobase deprotection conditions may be employed; the NPOM protecting group is quite stable to ammonium hydroxide and AMA.

References:

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5. Lusic, H.; Deiters, A., *Synthesis* **2006**, 2147-2150.