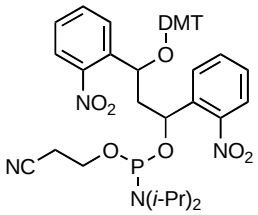
Caged Strand-Breaker CEP

Product No. BA 0315

Product Information



$C_{45}H_{49}N_4O_9P$

Mol. Wt.: 820.87

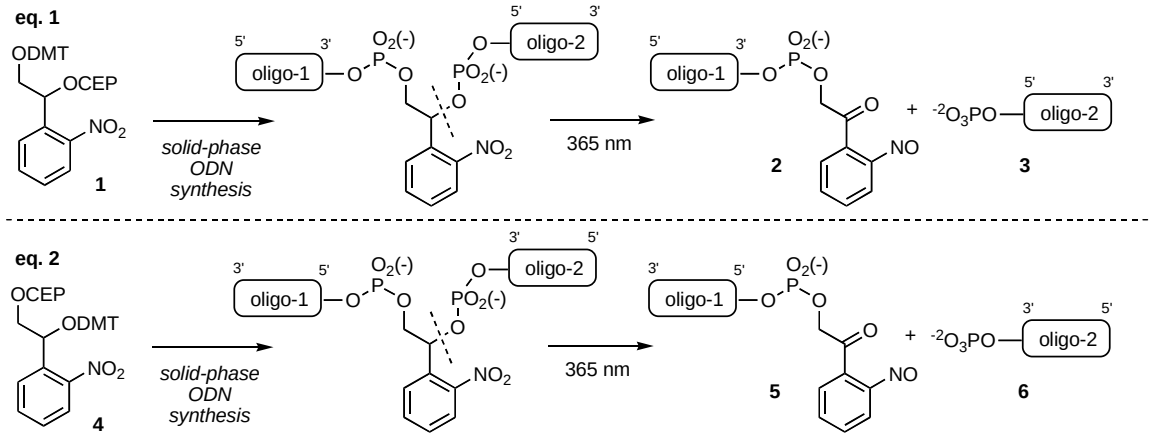
Once installed into an oligonucleotide, irradiation at 365 nm causes strand cleavage, leaving phosphate groups on both daughter strands.

Introduction. Placing a photochemically labile group on or within a molecule of interest opens many possibilities for altering its properties by simple illumination.[1] For example, one popular strategy is to install a photolabile protecting group that interferes with an important property, rendering the molecule inactive, or "caged". Illumination of the sample removes the protecting group, uncaging the molecule and turning on its biological function. An example in the oligonucleotide field is the management of gene expression by blocking hydrogen bonding with photolabile protecting groups.

A very different process that is aided by photochemistry is the introduction of nicks in DNA using photolabile nucleotides or nucleotide surrogates. [1a] These so-called *"caged strand-breaks"* can be affected through the use of the Caged Strand-Breaker CEP (BA 0315).

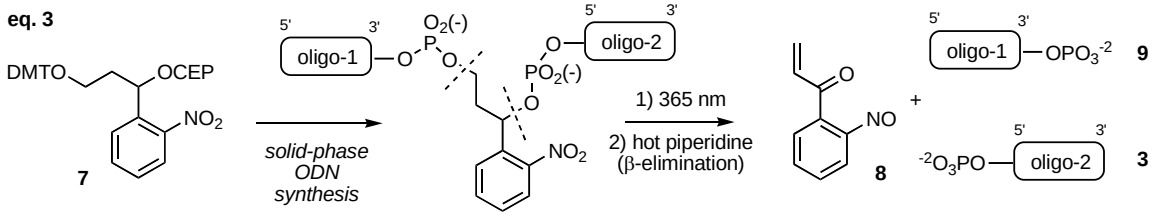
Overview of known non-nucleosidic caged strand-breaking monomers. Several monomers have been developed that can produce caged strand-breaks. Only those that are non-nucleosidic are considered here. Nucleosidic caged strand-breaking monomers are also known from the groups of Giese, Marx, Sheppard, Kotera, and Pirrung; see the review by Mayer and Heckel.[1a]

Urdea and Horn (US 5,258,506, 1993)[2] reported the non-nucleosidic monomers **1** and **4** (equations 1 and 2 below), which produce oligonucleotides that can be cleaved with UV light to give a single daughter strand bearing either a 5'-phosphate (**3** in eq. 1) or a 3'-phosphate (**6** in eq. 2), respectively. The other strands remain attached to the protecting group by-product (see **2** and **5**).

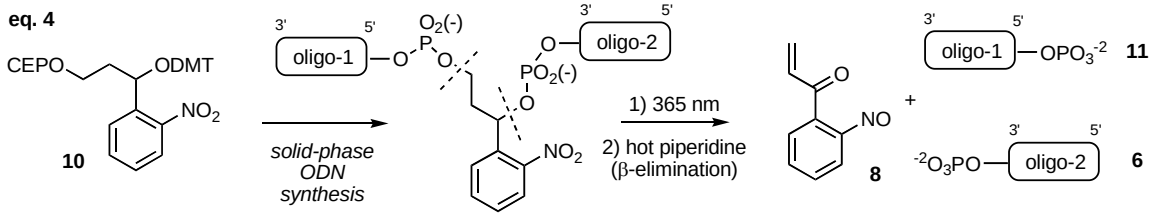


Taylor, *et al*.[3-6] and Raynor, *et al*.[7] have reported several non-nucleosidic monomers that cleave to liberate *both* daughter strands (eq. 3-6).

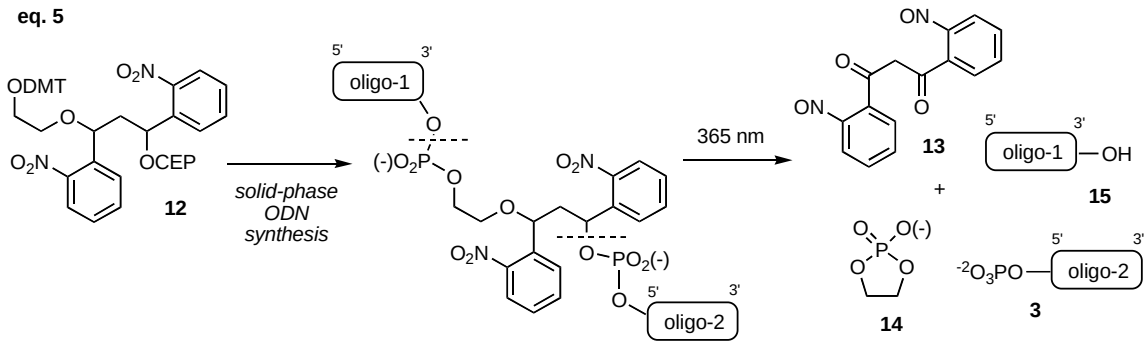
Monomer **7** produces an oligonucleotide that may be subjected to illumination, producing the phosphorylated daughter strands **3** and **9** plus the by-product **8** (eq. 3).[3,5] Unfortunately, only the 5'-phosphorylated daughter strand was produced at neutral pH; production of the 3'-phosphorylated strand required hot piperidine treatment to go to completion via a β-elimination reaction.[3]



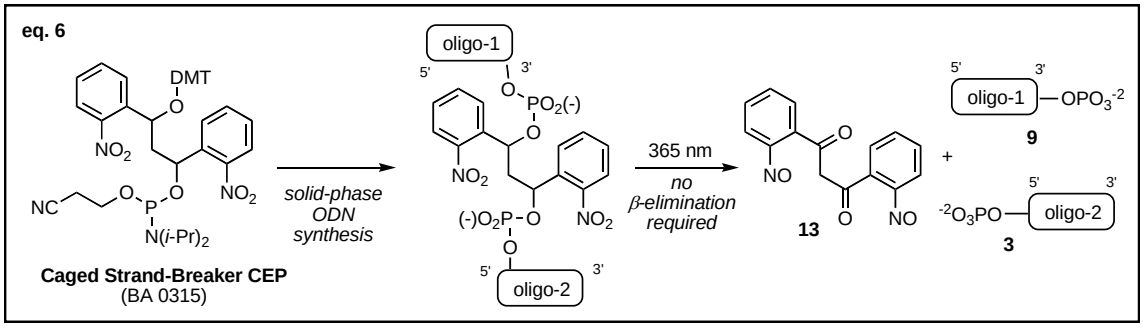
The complementary monomer **10** produces the phosphorylated daughter strands **6** and **11** with the phosphate ends switched, again requiring a separate β-elimination step (eq. 4).[5,7] Monomers **7** and **10** could be used in tandem to solve the β-elimination problem,[5] but the requirement for two specialty monomers decreases the attractiveness of this approach.



Zhang and Taylor have developed a complementary monomer that leaves one of the daughter strands with a 3'-OH group (eq. 5).[4] Thus, the monomer **12** ultimately leads to the daughter strands **3** and **15** and the by-products **13** and **14**.



In response to the challenges encountered in their earlier work described above, Zhang and Taylor[6] developed a novel monomer that we now offer as **Caged Strand-Breaker CEP (BA 0315)**. Caged Strand-Breaker-containing oligonucleotides produce the phosphorylated daughter strands **3** and **9** and the by-product **13** upon irradiation (eq. 6). *This monomer is the most advanced iteration of the Urdea/Taylor/Raynor strategy, and allows complete removal of the photolabile protecting group from both daughter strands (compare to **1** and **4**), the phosphorylation of both daughter strands (compare to **12**), and the avoidance of a second β-elimination step (compare to **7** and **10**).* For applications requiring strand-cleavage resulting in two phosphorylated daughter strands, BA 0315 offers the simplest approach.



Using Caged Strand-Breaker CEP (BA 0315). *Where possible, the phosphoramidite and the resultant oligonucleotides should be protected from light until strand cleavage is desired.*

Zhang and Taylor[6] report that this monomer can be incorporated into oligonucleotides under standard conditions except for the use of a 30 min coupling time, leading to a coupling efficiency of about 80%. In our hands, the use of standard protocols on an Expedite 8909 instrument led to >85% coupling in 15 min. Longer coupling did not lead to an improvement in the yield. Cleavage from the solid support and nucleobase deprotection was accomplished using concentrated ammonium hydroxide under standard conditions.

Zhang and Taylor[6] reported that photochemical decaging and strand cleavage occurred quantitatively at pH 7.0 (20 mM Tris-HCl, 1.5 mL Eppendorf tube) in 32 min after subjecting the sample to 365 nm light from a hand-held UV lamp (Ultra-Violet Products, UVGL-25, 720 μW/cm^2) at a distance of 7.6 cm, consistent with results in our labs.

If your research required strand breaks that result in daughter strands with a phosphate group at the 5'-terminus and a hydroxyl residue on the 3'-terminus, please consider our Caged Strand-Breaker II CEP (BA 0420).

Literature:

1. Reviews: (a) Mayer, G.; Heckel, A. *Angew. Chem. Int. Ed*. **2006**, *45*, 4900-4921. (b) Young, D. D.; Deiters, A. *Org. Biomol. Chem*. **2007**, *5*, 999-1005. (c) Tang, X. Dmochowski, I. J. *Mol. BioSyst*. **2007**, *3*, 100-110.

2. Urdea, M. S.; Horn, T. US Patent 5,258,506, Nov. 2, 1993.

3. Ordoukhanian, P.; Taylor, J.-S. *J. Am. Chem. Soc*. **1995**, *117*, 9570-9571.

4. Zhang, K.; Taylor, J.-S. *J. Am. Chem. Soc*. **1999**, *121*, 11579-11580.

5. Ordoukhanian, P.; Taylor, J.-S. *Bioconj. Chem*. **2000**, *11*, 94-103.

6. Zhang, K.; Taylor, J.-S. *Biochemistry* **2001**, *40*, 153-159.

7. Dell'Aquila, C.; Imbach, J.-L.; Rayner, B. *Tetrahedron Lett*. **1997**, *38*, 5289-5292.