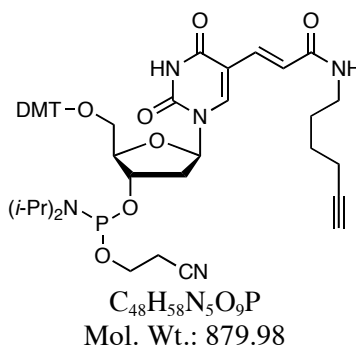


Alkynyl-Modifier-C6-dT CEP

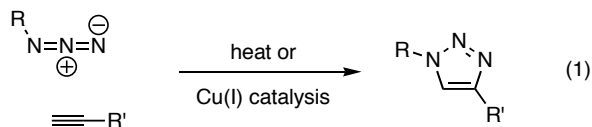
Product No. BA 0316

Product Information



May be used to install an alkyne into an oligonucleotide internally or at the 5'-terminus. Useful for click conjugation with azide-bearing labeling agents.

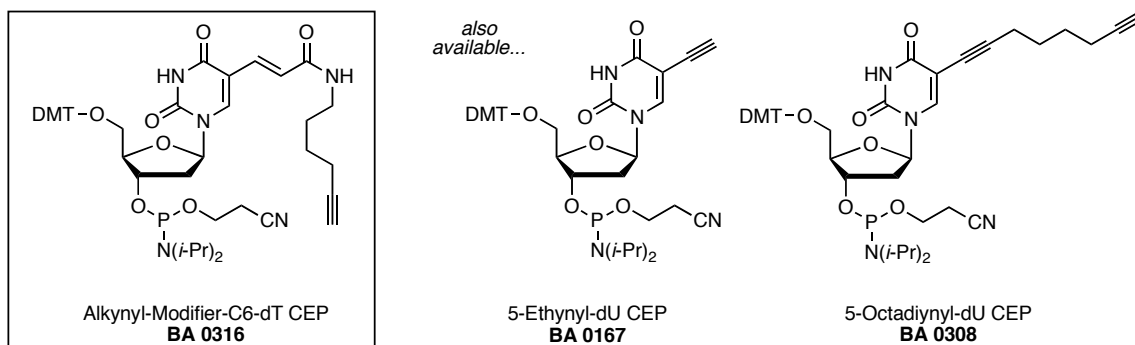
Introduction: Huisgen¹ pioneered the field of 1,3-dipolar cycloaddition chemistry, wherein five-membered heterocycles are formed by the combination of two molecules that bear certain unsaturated groups. A powerful example is the 1,3-dipolar cycloaddition of alkynes with azides (RN_3) to form triazoles, a reaction that occurs thermally with no additional reagents (eq. 1).² A major advance was made independently by the groups of Sharpless³ and Meldal,⁴ who discovered that Cu(I) catalysis allows the cycloaddition to be performed at room temperature. Sharpless and co-workers coined the term "click reaction" to refer to simple reactions that require only benign conditions, occur in high yield, and are easy to perform. The copper-catalyzed azide/alkyne cycloaddition reaction⁵ is the most popular example of a click reaction and is now essentially implied when one encounters the term "click".



In the oligonucleotide field, the click reaction of azides with alkynes is of increasing importance, and provides an alternative to nucleophile-electrophile chemistry for the conjugation of various species to nucleic acid. The click reaction of azides with alkynes is bioorthogonal to conjugation techniques such as the acylation of amines or the alkylation or conjugate addition reactions of thiols.

Alkyne-bearing nucleoside phosphoramidites enable click chemistry. An attractive strategy for nucleic acid conjugation involves the click reaction of alkyne-bearing oligonucleotides with azide-bearing species to join them via a triazole linkage.⁶ For the installation of an alkyne-bearing nucleoside into an oligonucleotide, we offer several options: (1) Ethynyl-dU CEP (BA 0167)⁸ 5-Octadiynyl-dU CEP (BA 0308),⁷⁻¹⁰ and the

new compound described herein, Alkynyl-Modifier-C6-dT CEP (BA 0316). Ethynyl-dU CEP allows the synthesis of modified oligonucleotides capable of undergoing click reactions with azides bearing a variety of groups (sugar, coumarin, fluorescein). However, the short, rigid nature of the alkyne group in 5-ethynyl-dU limits the utility of the click reaction if multiple incorporations are desired.⁸ The longer, more flexible tether in 5-Octadiynyl-dU CEP allows efficient click chemistry, even with multiple incorporations.⁷⁻¹¹ The same should be true for Alkynyl-Modifier-C6-dT CEP, which relies on well-known linker chemistry between the nucleobase and the alkyne functional group.



Use of Alkynyl-Modifier-C6-dT CEP: Employ acetonitrile diluent at the concentration recommended by the synthesizer manufacturer. Use standard coupling protocols; extended coupling times are not required. Cleavage from the solid support may be carried out by standard procedures. Standard nucleobase deprotection conditions may be employed.

Click conjugation: Copper(I) salts can cause strand breaks, perhaps by hydroxyl radical production.⁸ To avoid this problem, modified conditions have been studied that protect the integrity of biomolecules.¹² Instead of using air-sensitive Cu(I) salts, copper sulfate pentahydrate is reduced in situ to the Cu(I) oxidation state with water-soluble reducing agents such as sodium ascorbate or tris(carboxyethyl)phosphine hydrochloride (TCEP, Aldrich). Further, the Cu(I)-stabilizing ligand tris(benzyltriazolylmethyl)amine (TBTA, Aldrich) is added, which accelerates the rate of the reaction and protects the Cu(I) center from oxidation under aerobic conditions.^{12,13}

For a representative example of a relevant click conjugation, see Seela and co-workers,⁹ who reported that a trityl-off alkynyl oligonucleotide derived from the similar alkyne-bearing phosphoramidite 5-Octadiynyl-dU CEP (BA 0308), the desired azide (in 50% aqueous dioxane), 1:1 CuSO₄ : TBTA (in 1:9 *t*-BuOH:H₂O), and TCEP (in water) were combined in 10% aqueous *t*-BuOH for 12 h at room temperature to afford the conjugate, which was purified by RP-HPLC. Carell and co-workers reported⁸ a similar procedure.

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