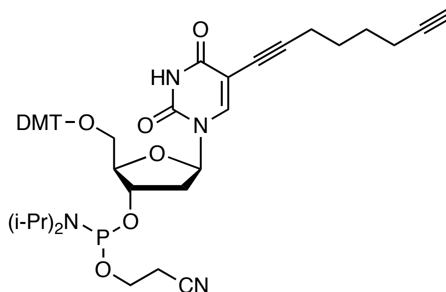
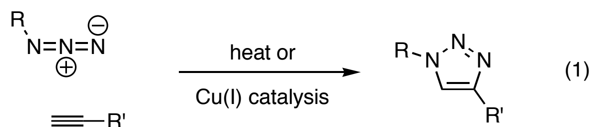
5-Octadiynyl-dU CEP
Product No. BA 0308
Product Information



$C_{47}H_{55}N_4O_8P$
Mol. Wt.: 834.94

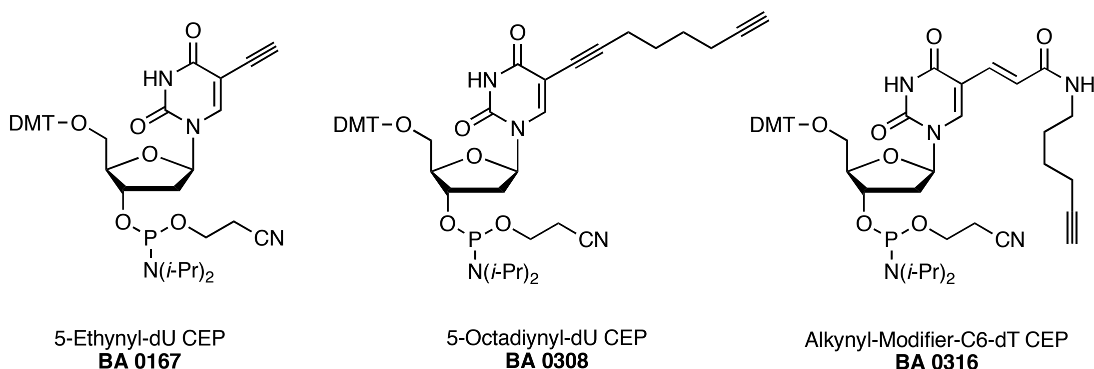
Introduction: Huisgen[1] pioneered the field of 1,3-dipolar cycloaddition chemistry, wherein five-membered heterocycles are formed by the combination of two molecules that bear certain unsaturated groups. A powerful example is the 1,3-dipolar cycloaddition of alkynes with azides (RN_3) to form triazoles, a reaction that occurs thermally with no additional reagents (eq. 1).[2] A major advance was made independently by the groups of Sharpless[3] 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 reaction[5] 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, Ethynyl-dU CEP (BA 0167)[8] and 5-Octadiynyl-dU CEP (BA 0308) have been shown to be useful.[7-10] Most recently, we now offer Alkynyl-Modifier-C6-dT CEP (BA 0316), which relies on a

venerable amide linker between the nucleobase and the tethered alkyne. 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] Click reactions with the above azides[8] as well as AZT,[7,9] a pinacyanol dye,[10] and azide-terminated slides[11] were successful. Further, the 5-octadienyl-dU modification is similar to the well-known propynyl-dU modification, resulting in a slight stabilization of DNA duplexes.[9]



Coupling, deprotection, and purification: Seela and co-workers employed normal protocols for the use of 5-Octadiynyl-dU CEP.[7,9] The trityl-on oligonucleotide was cleaved from the support with 25% aqueous ammonia and deprotection was carried out in the same solution by heating 14-16 h at 60 °C. RP-HPLC was used to purify this material, which was then detritylated with 2.5% dichloroacetic acid in dichloromethane for 5 min at 0 °C. The resultant oligonucleotide was purified by RP-HPLC. Carell and co-workers[8] recommend double coupling, extended coupling times, elongated de-blocking, and benzylthiotetrazole (BTT) as activator. This may be due to their emphasis on multiple incorporations of 5-Octadiynyl-dU CEP. Cleavage from the support and nucleobase deprotection was carried out with concentrated aqueous ammonia : ethanol (3:1) for 3 h at 40 °C. RP-HPLC purification was performed on either the trityl-off or trityl-on oligonucleotide.

In our hands, coupling of 5-Octadiynyl-dU CEP (single incorporation) proceeded with >95% efficiency using standard protocols as recommended by the instrument manufacturer. Cleavage and nucleobase deprotection was achieved with concentrated ammonium hydroxide using standard techniques.

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]

The conjugation of 5-octadiynyl-dU-modified oligonucleotides may be performed in solution or while still bound to the CPG solid support.[7-10]

Conjugation in solution: Seela and co-workers[9] reported that the trityl-off alkynyl oligonucleotide, the desired azide (in 50% aqueous dioxane), 1:1 $CuSO_4$: TBTA (in 1:9 *t*-BuOH:H_2O), 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 reported[8] a similar procedure for such solution-phase conjugations.

Conjugation on solid support: Seela and co-workers[9] reported that the oligonucleotide may be retained on the CPG in DMT-on and protected form (*t*-BPA-protected dA and dC, iBu-protected dG) while the cycloaddition is performed over 12 h at room temperature using a mixture of reagents and solvents similar to that described above. The crude CPG was then shaken with 50% aqueous methanol to wash away the reagents, then the oligonucleotide was cleaved and deprotected with ammonium hydroxide for 14 h at 60 °C. The DMT-on oligonucleotide was purified by RP-HPLC, detritylated, then purified again with RP-HPLC.

References:

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