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Florida International University
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Doctoral Dissertation Defense

Abstract

Strain Promoted Click Chemistry of 8-Azidopurine and 5-Azidopyrimidine Nucleosides and Nucleotides with Cyclooctynes and Applications to Living Cell Imaging.

by

Jessica Zayas

The strain promoted azide alkyne cycloaddition (SPAAC) of azido nucleobase modified nucleosides and nucleotides with cyclooctynes to give fluorescent triazoles has been relatively unexplored. Thus, SPAAC between azido-nucleobases and various cyclooctynes in aqueous solution at ambient temperature resulted in the efficient formation (3 min - 2 h) of triazole products with inherent fluorescent properties. The 2- and 8-azidoadenine nucleosides reacted with fused cyclopropyl cyclooctyne, dibenzylcyclooctyne or monofluorocyclooctyne to produce click products functionalized with hydroxyl, amino, *N*-hydroxysuccinimide, or biotin moieties. The previously unexplored 5-azidouridine and labile 5-azido-2'-deoxyuridine were similarly converted to the analogous triazole products in quantitative yields in less than 5 minutes. The 8-azido-ATP quantitatively afforded the triazole product with fused cyclopropyl cyclooctyne (3 h). Addition of a triazole ring at the 2 or 8 position of adenine or 5-position of uracil induces fluorescent properties which were used for direct imaging with fluorescent microscopy in MCF-7 cancer cells without the need for traditional fluorogenic reporters. Fluorescent lifetime imaging microscopy of the click adducts in live cells were used to determine the lifetime of each fluorophore in the cellular nuclei demonstrating the potential utility of the synthesized triazole adducts for dynamic measuring and tracking of events inside single living cancer cells.

The SPAAC methodology developed has also been applied to study the cellular targets in protozoal parasite, *Trichomonas Vaginalis* and bacteria, *Pseudomonas aeruginosa*. The 9-(2-deoxy-2-fluoro-β,D-arabino-furanosyl)adenine (*arabino*-F-Ado) was modified with an azido moiety at the C8 position for use in click chemistry. Tagging and subcellular localization studies using azido modified *arabino*-F-Ado could provide insight into the mechanism of action of *arabino*-F-Ado.

An activated analogue of S-adenosyl-L-methionine (SAM) with an EnYn group on the sulfur instead of a methyl group was prepared to study the transfer of the methyl group from SAM. We found that the EnYn group was transferred from SAM to a guanosine on tRNA by methytransferase Trm1. Thus, AdoEnYn is a competitive inhibitor of SAM and can be incorporated into tRNA in place of SAM.

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Department: Chemistry and Biochemistry
Major Professor: Dr. Stanislaw Wnuk