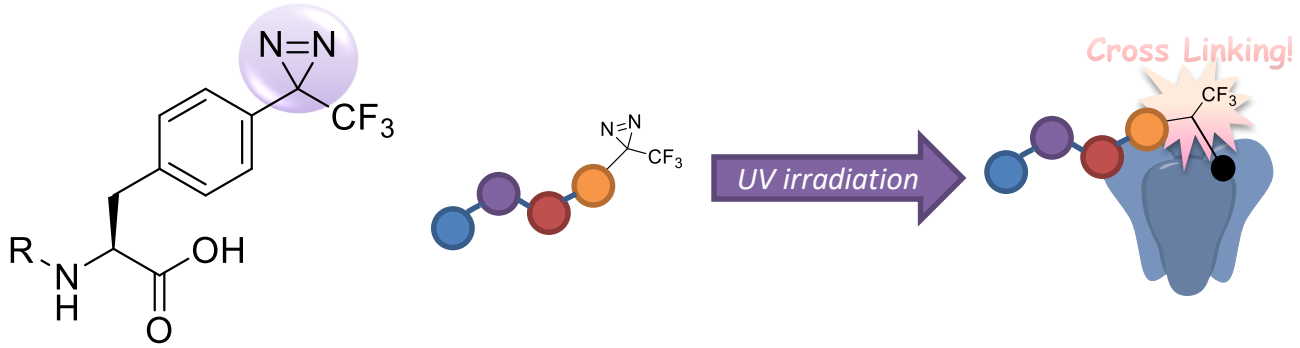
Watanabe Chemical News

WN-190930-662

Amino acids containing Diazirine for Photoaffinity Labeling



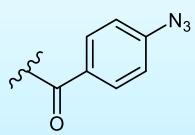
Functional proteins such as receptors and enzymes in vivo recognize and bind with specific ligands and exert biological function via various transmission mechanisms. However, how those molecular mechanisms are composed is not known in most cases. Yet it is known that drugs and bioactive molecules have pharmacological and physiological activities or cause side effects. Finding out how molecules recognize proteins and where to bind within the protein helps research of biochemical function in vivo molecules and to identify new drug discovery targets.

Photoaffinity Labeling

Interaction of ligands and functional proteins which have physiological activities is reversible by non-covalent bond such as electrostatic interaction and often provides strong physiological activities despite its low affinity.

It is difficult to identify such weak interaction directly and specifically where there are many non-target proteins and H_2O molecules.

Photoaffinity labeling is one of the chemical modification methods which generates high reactivity chemical species and non-reversible covalent bonds, and label. The only proteins which interact with ligands in miscellaneous mixes containing various proteins can be identified and detected, and their binding site at the amino acid level can be identified in principal. Photoaffinity labeling has been extensively applied as a necessary tool for chemical biology research to identify drug receptors and their binding site in protein molecules.



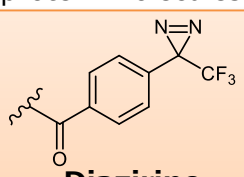
Phenyl azide

Nitrene is generated by UV irradiation

○ structure is small and easy to be combined

× low length of UV light which could cause side reaction is required

× reducible by thiol



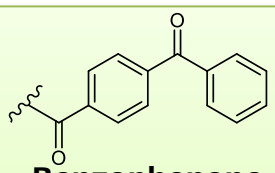
Diazirine

Carbene is generated by UV irradiation

○ relatively long length of UV light can be used

○ has high activity and can label in short time
→prevent side reaction

× take time to synthesis



Benzophenone

Biradical is generated by UV irradiation

○ excitation is reversible and it is considered that the labeling efficiency is good

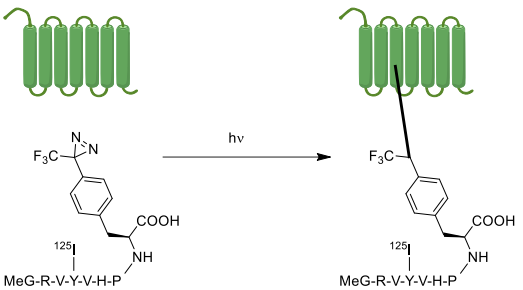
× structure is large and could have an impact after labeling

Photoreactive group Diazirine

Diazirine absorbs ultraviolet light around 350nm and generates carbene. This carbene is inserted to the close C-H, N-H bond and generates stable covalent bond. This covalent bond is generated only at a close site and a non-specific covalent bond is not generated.

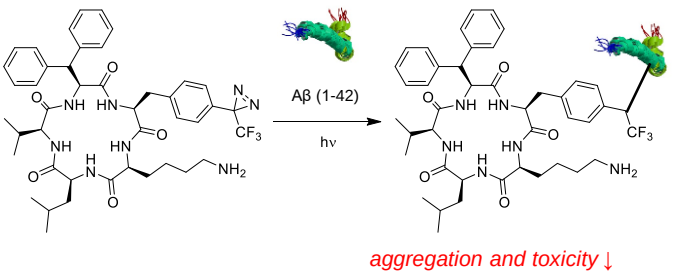
Diazirine containing amino acid Tdf can be bonded with other molecules such as peptides and low molecule compounds through the carboxyl group or amino group. As the structure of diazirine is chemically stable, Fmoc-Tdf-OH (M03645) for instance can be used for solid-phase peptide synthesis using an automatic synthesizer.

Prepare Tdf which is combined with ligand such as peptide and low molecule compound and it is irradiated by ultra-violet light around 350nm when it consists with target proteins, the ligand and the protein are interacted to generate covalent bond when they are close, and they form one molecule. Ligand-target protein conjugate can be detected by mass analysis or SDS-PAGE. The interaction between ligand and target protein becomes stronger, labeling efficiency becomes higher accordingly however weak interaction can also be detected. And the carbene generated from Tdf is also useful for biomolecule research because it doesn't need to use short length of ultra-violet light which can cause degradation of protein.



The angiotensin II, which is incorporated with Tdf into the C-terminal by Fmoc-Tdf-OH (M03465), formed covalent bond with G-protein coupled angiotensin II receptor, by UV irradiation. It is useful for precise determination of ligand binding site in peptide receptor.

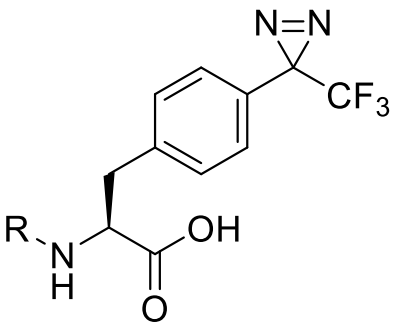
D. Fillion *et. al., J. Med. Chem., 49* (7), 2200–2209 (2006)



The cyclic peptide incorporated with Tdf which has affinity to Aβ, causative substance of Alzheimer's disease, formed covalent bond with Aβ. It reduced aggregation and toxicity of Aβ. It is confirmed that Tyr10 of Aβ is the covalent bond site with the Tdf-containing peptide.

R. Kino *et. al., Bioorg. Med. Chem. Lett., 25* (15), 2972-2975 (2015)

Products



R=	Code	Symbol Name	CAS RN®	Price in USD	
Fmoc	M03465	**Fmoc-Tdf-OH**	[133342-64-0]	100 mg	$364
				250 mg	$728
				500 mg	$1,091
				1 g	$1,819
H	J00971	**H-Tdf-OH**	[92367-16-3]	50 mg	$455
				100 mg	$728
Boc	M02824	**Boc-Tdf-OH**	[92367-17-4]	100 mg	$455
				250 mg	$910
				500 mg	$1,364
				1 g	$2,273

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