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Review Article

A REVIEW ON 1,3-DIPOLAR CYCLOADDITION REACTIONS IN BIOCONJUGATION AND IT'S IMPORTANCE IN PHARMACEUTICAL CHEMISTRY

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ABSTRACT

1,3-dipolar Cycloaddition (DC) reactions have emerged as one of the important type of cycloaddition reactions from the view point of chemical and biological industry. Particularly the Azide-Alkyne Cycloaddition, also known as "Click Chemistry" has opened up new challenges in front of medicinal and pharmaceutical chemists. Bioconjugation is a technique of coupling two different biomolecules by covalent linkage, employed for synthesizing protein conjugates, immobilized enzymes and antibodies and also in purification of proteins. In the current work, a discussion is made with the mechanisms of Cu catalyzed Azide-Alkyne Cycloaddition (CuAAC) and Ru catalyzed Azide-Alkyne Cycloaddition (RuAAC) followed by their applicability in bioconjugation techniques. The CuAAC is already gained importance in bioconjugation while the RuAAC shows huge promise for the future.

Keywords: Cycloaddition, Bioconjugation, Azide-Alkyne Reaction, Copper, Ruthenium.

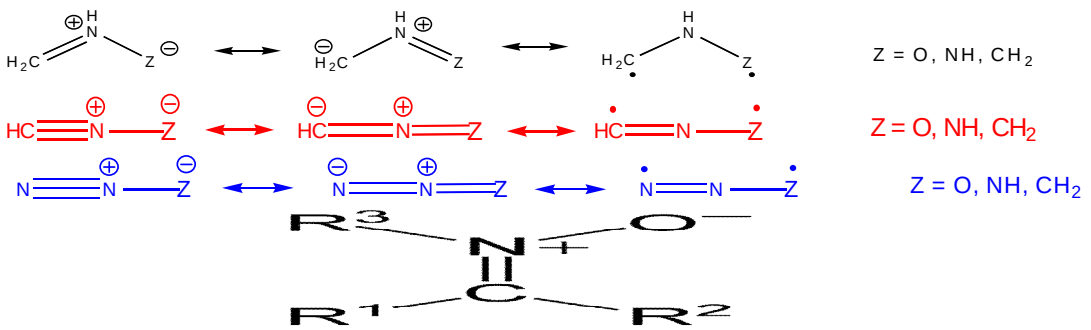
INTRODUCTION

The vibrant dynamics of the pharmaceutical industry has lead to many novel technologies and innovative ideas for development of new chemical entities. Over the past two decades the pericyclic reactions like electrocyclic addition, cycloaddition and sigmatropic rearrangement has gained prime importance among the scientists in relevant field. The most noteworthy among these remains to be Cycloaddition reactions[1].

There are several types of Cycloaddition reactions like (2+2), (4+2), (1+3) etc. An example for the (4+2) cycloaddition reaction is the Diels Alder reaction where a dienophile reacts with a diene at around 25^0C to yield cycloaddition adduct which includes anthraquinones, a pharmaceutically important principle present in various plants like Senna, Rhubarb, Cascara, Aloe and responsible for laxative action[2-3]. In the current work, these authors would like to highlight some of the non-familiar or current cycloaddition reactions and it's importance to the pharmacy fraternity.

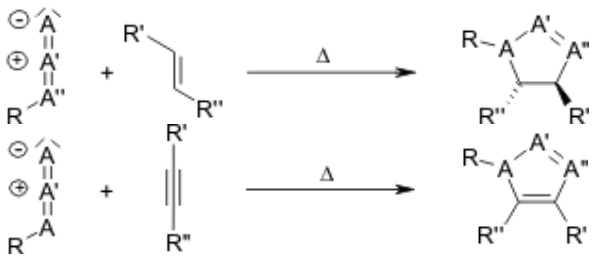
Huisgen Cycloaddition: (1,3-Dipolar Cycloaddition)

The 1,3-dipolar species includes azomethines, nitrilium, diazoniums and nitrones (given below):

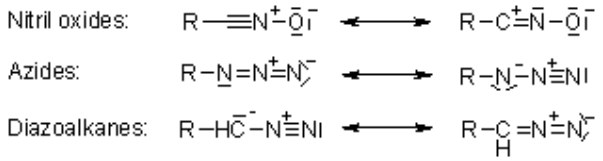


Among these nitrones are the most important species with a general formula $R^1R^2C=N^+R^3O^-$. Nitrones are N-oxide of an imine and a functional group. Though their chemistry is hugely varied, yet they are dominated by their use as 1,3-dipoles in cycloaddition reactions (DC reactions). The most important 1,3-DC of nitrones is the formation of oxazolidines, a five membered heterocycle including three carbon, one oxygen and one nitrogen atom. These can utilized in the synthesis of antibacterial agents like Streptomycin, Nystatin etc. [4]

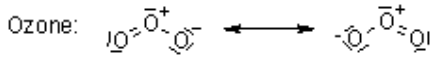
The Huisgen Cycloaddition is basically the reaction of a dipolarophile with a 1,3-dipolar compound that leads to 5-membered heterocycles. Examples of dipolarophiles are alkenes and alkynes and molecules that possess related heteroatom functional groups (such as carbonyls and nitriles). 1,3-Dipolar compounds contain one or more heteroatoms and can be described as having at least one mesomeric structure that represents a charged dipole.



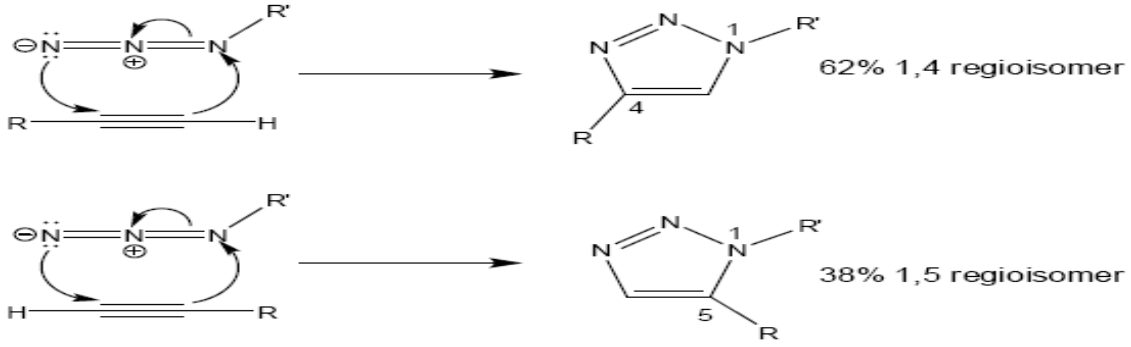
Examples of linear, propargyl-allenyl-type dipoles



An example of an allyl-type dipole.



Mechanism of the Huisgen 1,3-Dipolar Cycloaddition

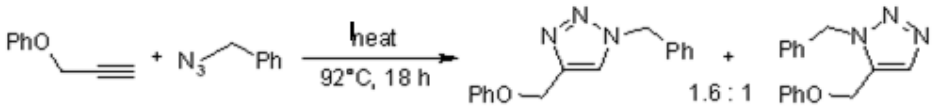


2 π-electrons of the dipolarophile and 4 electrons of the dipolar compound participate in a concerted, pericyclic shift. The addition is stereoconservative (suprafacial), and the reaction is therefore a $[2_s+4_s]$ cycloaddition similar to the Diels Alder Reaction[5].

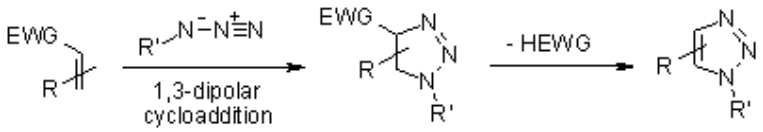
A condition for such a reaction to take place is a certain similarity of the interacting HOMO and LUMO orbitals, depending on the relative orbital energies of both the dipolarophile and the dipole. Electron-withdrawing groups on the dipolarophile normally favour an interaction of the LUMO of the dipolarophile with the HOMO of the dipole that leads to the formation of the new bonds, whereas electron donating groups on the dipolarophile normally favour the inverse of this interaction.

Diazomethane as an electron-rich dipolar compound therefore rapidly reacts with electron-poor alkenes, such as acrylates[5].

The regioselectivity of the reaction depends on electronic and steric effects and is somewhat predictable. For example, the addition of alkynes to azides, which is an interesting reaction for the generation of 1,2,3-triazole libraries by the simple reaction of two molecules leads to regioisomers.[6]



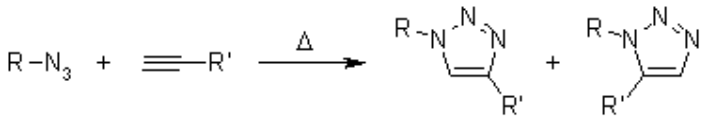
The reaction has been modified to a more regioselective, copper-catalyzed stepwise process by the Sharpless group, which is no longer a classic Huisgen Cycloaddition. Another approach prefers the use of a directing electron withdrawing group, which is removable later.[7]



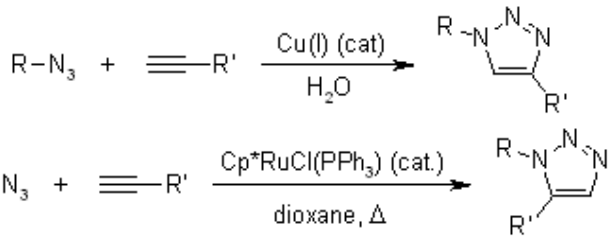
Thus, the 1,3-dipolar cycloaddition allows the production of various 5-membered heterocycles. Many reactions can be performed with high regioselectivity and even with enantioselective transformations of prochiral substrates.

Azide-Alkyne Cycloaddition (Click Chemistry)

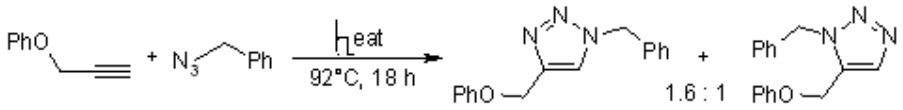
"Click Chemistry" is a term that was introduced by Nobel Laurette Prof. K. B. Sharpless in 2001. Click Chemistry describe reactions that are high yielding, wide in scope, create only byproducts that can be removed without chromatography, stereospecific, simple to perform, and can be conducted in easily removable or benign solvents. This concept was developed in parallel with the interest within the pharmaceutical, materials, and other industries in capabilities for generating large libraries of compounds for screening in discovery research. Several types of reaction have been identified that fulfill these criteria, thermodynamically-favored reactions that lead specifically to one product, such as nucleophilic ring opening reactions of epoxides and aziridines, non-aldol type carbonyl reactions, such as formation of hydrazones and heterocycles, additions to carbon-carbon multiple bonds, such oxidative formation of epoxides and Michael Additions, and cycloaddition reactions. An examination of the azide-alkyne cycloaddition shows that it fulfills many of the prerequisites. Many of the starting monosubstituted alkynes and organic azides are available commercially, many others can easily be synthesized with a wide range of functional groups, and their cycloaddition reaction selectively gives 1,2,3-triazoles.



The thermal Huisgen 1,3-dipolar cycloaddition of alkynes to azides requires elevated temperatures and often produces mixtures of the two regioisomers when using asymmetric alkynes. In this respect, the classic 1,3-dipolar cycloaddition fails as a true click reaction. A copper-catalyzed variant that follows a different mechanism can be conducted under aqueous conditions, even at room temperature. Additionally, whereas the classic Huisgen 1,3-dipolar cycloaddition often gives mixtures of regioisomers, the copper-catalyzed reaction allows the synthesis of the 1,4-disubstituted regioisomers specifically. By contrast, a later developed ruthenium-catalyzed reaction gives the opposite regioselectivity with the formation of 1,5-disubstituted triazoles. Thus, these catalyzed reactions comply fully with the definition of click chemistry and have put a focus on azide-alkyne cycloaddition as a prototype click reaction.

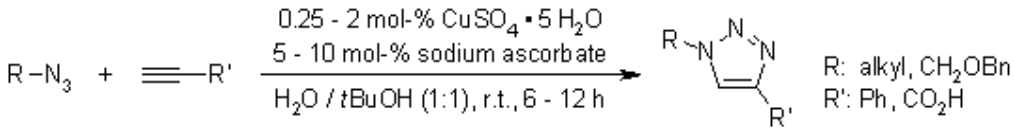


This reaction is highly exothermic, but the high activation barrier is responsible for a very low reaction rate, even at elevated temperature. Another drawback is the formation of regioisomers, as the two possible HOMO-LUMO interactions of the substrates are closely related in terms of energy. The thermal reaction therefore often gives approximately 1:1 mixtures of both the 1,4-substituted and the 1,5-substituted regioisomers.[6]



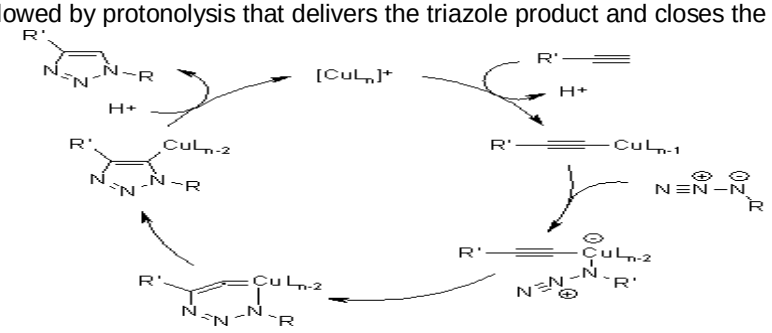
Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC)

As one of the best click reactions to date, the copper-catalyzed azide-alkyne cycloaddition features an enormous rate acceleration of 10^7 to 10^8 compared to the uncatalyzed 1,3-dipolar cycloaddition. It succeeds over a broad temperature range, is insensitive to aqueous conditions and a pH range over 4 to 12, and tolerates a broad range of functional groups. Pure products can be isolated by simple filtration or extraction without the need for chromatography or recrystallization.[8]



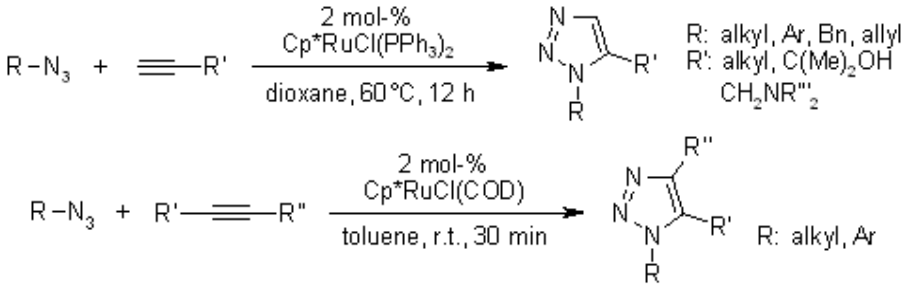
The active Cu(I) catalyst can be generated from Cu(I) salts or Cu(II) salts using sodium ascorbate as the reducing agent. Addition of a slight excess of sodium ascorbate prevents the formation of oxidative homocoupling products. Disproportionation of a Cu(II) salt in presence of a Cu wire can also be used to form active Cu(I).

DFT calculations have shown that coordination of Cu(I) to the alkyne is slightly endothermic in MeCN, but exothermic in water, which is in agreement with an observed rate acceleration in water. However, coordination of Cu to the acetylene does not accelerate a 1,3-dipolar cycloaddition. Such a process has been calculated to be even less favorable than the uncatalyzed 1,3-dipolar cycloaddition. Instead, a copper acetylide forms, after which the azide displaces another ligand and binds to the copper. Then, an unusual six-membered copper(III) metallacycle is formed. The barrier for this process has been calculated to be considerably lower than the one for the uncatalyzed reaction. The calculated rate at room temperature is 1 s^{-1}, which is quite reasonable. Ring contraction to a triazolyl-copper derivative is followed by protonolysis that delivers the triazole product and closes the catalytic cycle.

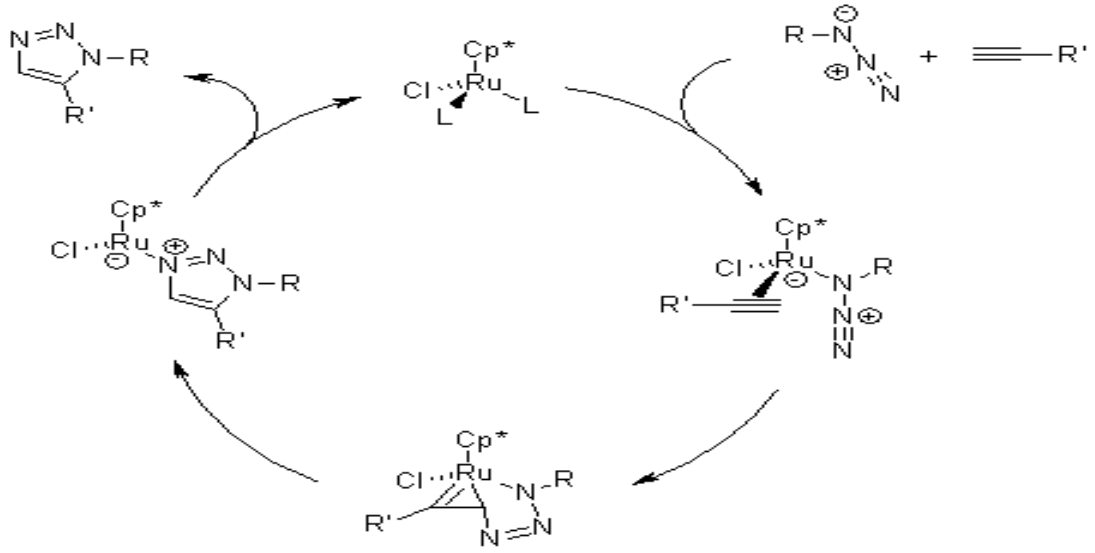


Ruthenium-Catalyzed Azide-Alkyne Cycloaddition (RuAAC)

A search for catalysts revealed that pentamethylcyclopentadienyl ruthenium chloride [Cp*RuCl] complexes are able to catalyze the cycloaddition of azides to terminal alkynes regioselectively leading to 1,5-disubstituted 1,2,3-triazoles. In addition, RuAAC can also be used with internal alkynes, providing fully substituted 1,2,3-triazoles, which contrasts with CuAAC.[9]



The ruthenium-catalyzed azide-alkyne cycloaddition (RuAAC) appears to proceed via oxidative coupling of the azide and the alkyne to give a six-membered ruthenacycle, in which the first new carbon-nitrogen bond is formed between the more electronegative carbon of the alkyne and the terminal, electrophilic nitrogen of the azide. This step is followed by reductive elimination, which forms the triazole product. DFT calculations support this mechanistic proposal and indicate that the reductive elimination step is rate-determining.



Bioconjugation: An Introduction

Bioconjugation, simply the conjugation of two biomolecules through a covalent linkage is currently a burgeoning field of research. Novel methods for the mild and site-specific derivatization of proteins, DNA, RNA, and carbohydrates have been developed for applications such as ligand discovery, disease diagnosis, and high-throughput screening. These powerful methods owe their existence to the discovery of chemoselective reactions that enable bioconjugation under physiological conditions—a tremendous achievement of modern organic chemistry.[10]

Bioconjugation by Cu-catalyzed azide alkyl reaction

The copper-catalyzed cycloaddition reaction between azides and alkynes functions efficiently in aqueous solution in the presence of a tris(triazolyl)amine ligand. The process has been employed to make rapid and reliable covalent connections to micromolar concentrations of protein decorated with either of the reactive moieties. The chelating ligand plays a crucial role in stabilizing the Cu(I) oxidation state and protecting the protein from Cu(triazole)-induced denaturation. Because the azide and alkyne groups themselves are unreactive with protein residues or other biomolecules, their ligation is of potential utility as a general bioconjugation method.

Activity based protein profiling by Cu catalyzed cycloadditions

Toward the goal of assigning function to the tens of thousands of protein products encoded by eukaryotic and prokaryotic genomes, the field of proteomics requires new technologies that can functionally characterize proteins within the dynamic environment of the cell, where these biomolecules are subject to myriad posttranslational modifications and the actions of endogenous activators and inhibitors. Several enzymes can be labeled in an activity-based manner both in vitro and in vivo by an azido-sulfonate ester probe and these labeling events can be detected in whole proteomes by copper-catalyzed ligation with a rhodamine-alkyne reagent. This click chemistry-based strategy for ABPP represents a unique and versatile method for functional proteome analysis.

Ruthenium catalyzed azide-alkyne cycloaddition in bioconjugation

In 2005, ruthenium cyclopentadienyl complexes were found to catalyze the formation of the complementary 1,5-disubstituted triazole from azides and terminal alkynes, and also to engage internal alkynes in the cycloaddition (already discussed). As one would imagine from these differences, this sister process, designated RuAAC (*ru*thenium-catalyzed *a*zide–*a*lkyne *c*ycloaddition), is mechanistically quite distinct from its cuprous cousin, although the underlying activation of the alkyne component appears to be fundamentally similar: the nucleophilicity of its π-system is increased by the back donation from the ruthenium center. While the scope and functional group compatibility of RuAAC are excellent, the reaction is more sensitive to the solvents and the steric demands of the azide substituents than CuAAC. Applications of RuAAC are only beginning to appear.[11]

DISCUSSION

Bioconjugation provides novel methods for the mild and site-specific derivatization of proteins, DNA, RNA, and carbohydrates. This stream has been developed for applications in ligand discovery, disease diagnosis, and high-throughput screening. These powerful methods owe their existence to the discovery of chemoselective reactions that enable bioconjugation under physiological conditions—a tremendous achievement of modern organic chemistry. Bioconjugation also helps in producing immobilized enzymes and certain antibodies. On the other hand, cycloaddition provides a major breakthrough in synthetic organic chemistry where the requirement of a novel product with a very simplified way of reaction is fulfilled. This study was intended to provide a widespread knowledge to the pharmaceutical chemists for synthesizing newer drugs with ease and advantage of less time consumption. CuAAC has a major disadvantage as Cu has variable oxidation states. On the contrary no other metal like Pd, Hg, Ag shown any success in such type of DC (Dipolar Cycloaddition) reactions. After the success with Ru, there has been a major search for the thermodynamically stable oxidation state of the metal that ranges from “0” to “+8” and also “-2” . Hence, a more detailed study can be conducted in the section of Ruthenium catalyzed Azide-Alkyne Cycloaddition (RuAAC) for conducting the catalysis in a much convenient manner than Cu. The success with RuAAC can lead to new vistas to synthesizing novel and commercially viable protein conjugates which may be helpful in diagnostics, medical and pharmaceutical field.

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