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Growing Advances and Applications of Click Reactions

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ABSTRACT

Examination of nature's favorite molecules revealed that nucleic acids, proteins and polysaccharides are condensation polymers of small subunits stitched together by carbon ± heteroatom bonds. Taking clue from the natures approach, a set of powerful, highly reliable and selective reactions were developed for the rapid synthesis of useful new compounds, an approach called as "click reactions". Thus click chemistry is a modular synthetic approach that utilizes the most practical and reliable chemical transformations. Its applications are increasingly found in all aspects of drug discovery, ranging from lead finding through combinatorial chemistry and target-template in situ chemistry, to proteomics and DNA research, using bioconjugation reactions. One of the reactions of click chemistry i.e. copper (I)-catalyzed 1, 2, 3-triazole forming reaction has become the gold standard of click chemistry due to its reliability, specificity and biocompatibility of the reactants. The triazole products are more than just passive linkers; they readily associate with biological targets, through hydrogen bonding and dipole interactions. This review gives a brief overview about some of the advances and applications of these click reactions.

Keywords: Azides, Alkynes, Click chemistry, Triazole

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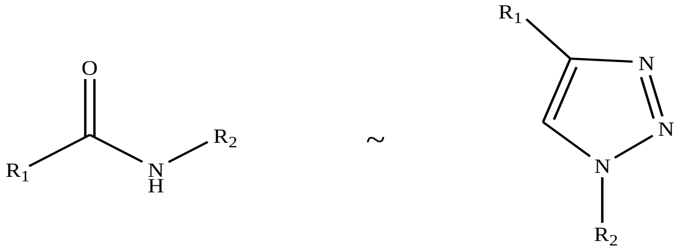
INTRODUCTION

Click chemistry is a newer approach which utilizes a few practical and reliable reactions to generate substances quickly and reliably by joining small unit's together [1]. The traditional process of drug discovery is generally slow, costly and hindered by complex synthesis [2]. Therefore, a set of criteria defining reliable reactions known as "click" reactions was proposed by Sharpless and coworkers of "The Scripps Research Institute" in 2001 in order to accelerate the synthesis of drug-like molecules [1, 3]. Click chemistry does not replace existing methods for drug discovery, but rather, it complements and extends them. It also makes each reaction in a multistep synthesis fast, efficient and predictable [1]. This concept was inspired by the fact that nature also generates substances by joining small modular units. Click chemistry is not a specific reaction rather it is a concept that mimics nature [3, 4]. For example, the triazole formed in click reactions share useful topological and electronic features with the ubiquitous amide moiety found in nature (**Figure1**) [1].



Amide linkage

Triazole

Figure 1: Comparison of structural and electronic features of amide and triazole moiety.

Characteristics of Click Reaction

The reactions in click chemistry must be modular, selective, wide in scope, stereo-specific and physiologically stable. The reactions should also be easy to perform, insensitive to oxygen and water, quick, quantitative, pH-insensitive and bio-compatible. The reactions should give very high chemical yields, should exhibit a large thermodynamic driving force >84 kJ/mol to favor a reaction with a single reaction product or if not then generate only inoffensive byproducts [1, 4 -6].

Conditions Required for Click Reaction

The process should have simple reaction conditions, should use readily available starting materials and should use simple product isolation techniques such as crystallization or distillation. The reactions should use no solvent or use a solvent that is benign or easily removed, preferably water. It was found that when water was omitted, the reactions were much slower, less selective and on a larger scale became explosive, because click reactions are highly exothermic. Therefore it was found that the presence of water in these reactions is beneficial because water is the best heat-sink for handling this enormous heat output [1, 3, 4, 7].

CLASSIFICATION OF CLICK REACTIONS [4, 8]

Till date, five major classes of click reactions have been identified:

1. Cycloaddition
    - 1.1. Huisgen 1, 3 dipolar cycloaddition
        - 1.1. A. Thermal catalyzed- Huisgen reaction
        - 1.1. B. Copper catalyzed- Huisgen reaction
        - 1.1. C. Ruthenium catalyzed- Huisgen reaction
    - 1.2. Diels Alder reaction
2. Nucleophilic ring-openings
    - 2.1. Oxirane opening
    - 2.2. Aziridine opening
3. Carbonyl chemistry
4. Staudinger reaction
5. Michael addition reaction

1. <u>Cycloaddition</u>

In this reaction, azides reacts with alkyne to produce a cyclic product i.e. triazole. This reaction is further divided into two types: Huisgen 1, 3 dipolar cycloaddition and Diels Alder reaction (**Figure2)**.

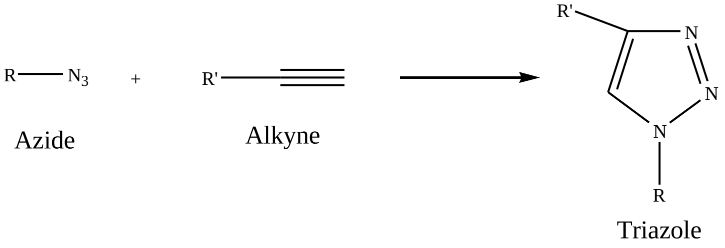


Figure 2: Cycloaddition reaction [8].

1.1. Huisgen 1, 3 dipolar cycloaddition

In the click universe, the "perfect" example of cycloaddition is the Huisgen 1,3-dipolar cycloaddition of a terminal or internal alkynes to azides to form mixture of 1,2,3-triazoles. American chemist K. Barry Sharpless has referred to this cycloaddition as "the cream of the crop" of click chemistry [9, 10]. This reaction is further classified into three types:

1.1. A. Thermal catalyzed- Huisgen reaction

1.1. B. Copper catalyzed- Huisgen reaction

1.1. C. Ruthenium catalyzed- Huisgen reaction

1.1. A. Thermal catalyzed- Huisgen reaction

In this reaction, alkyne and azide react to produce a mixture of triazole in presence of heat (**Figure 3**).

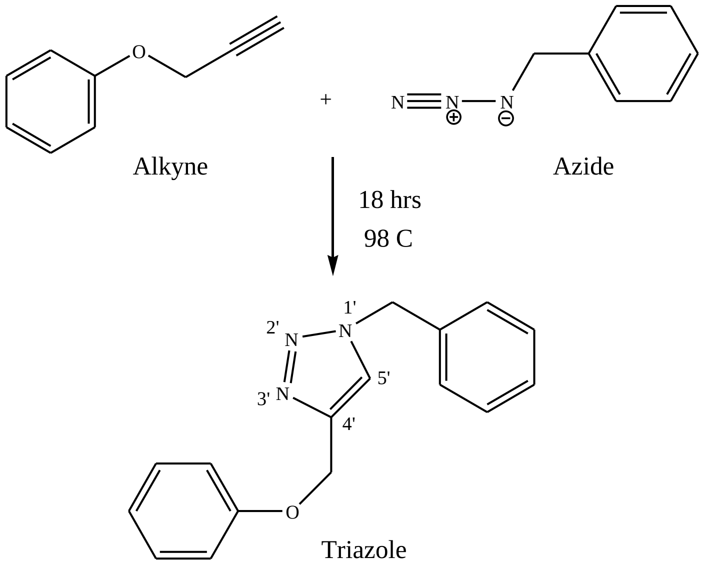


Figure 3: Thermal catalyzed- Huisgen reaction [10].

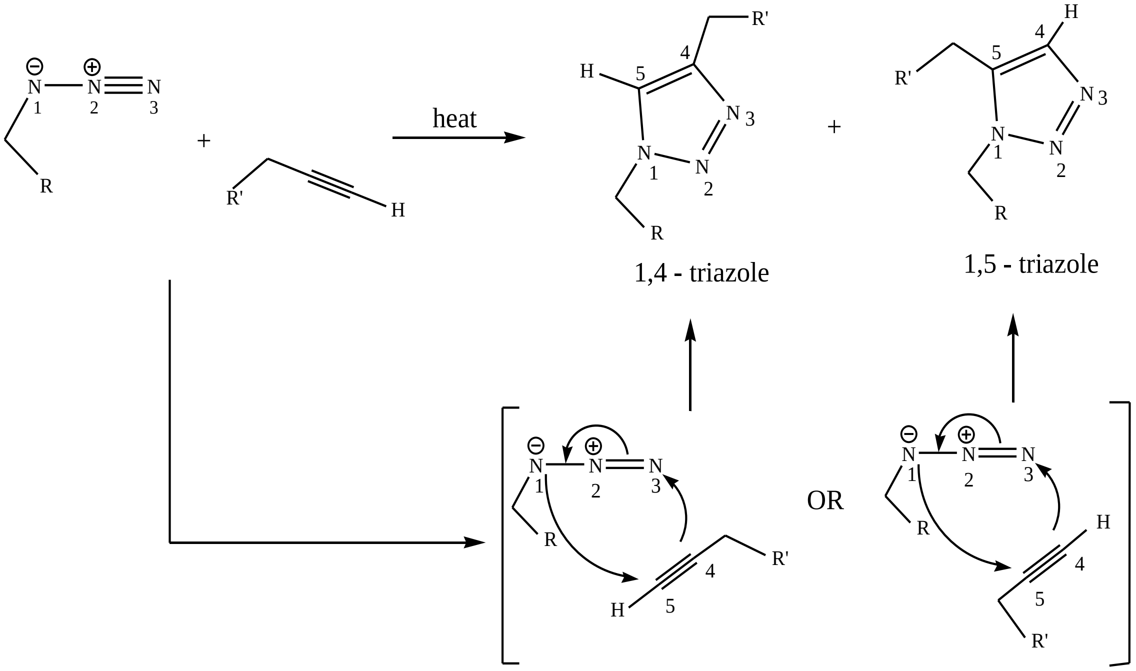


Figure 4: Mechanism of Thermal catalyzed- Huisgen reaction [11-13].

1.1. B. Copper catalyzed- Huisgen reaction

In this reaction, organic azides and terminal alkynes are united to form 1,4-regioisomers of 1,2,3-triazoles as sole product in presence of Cu(I) and hence this reaction is termed as Copper

(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) (**Figure 5**). Some of the advantages of CuAAC over thermal catalyzed- Huisgen reactions are the reaction is mild, very efficient, requires no protecting groups and results in the ~10^6 fold increase in reaction rate and hence the traditional thermal catalyzed- Huisgen reaction is replaced by CuAAC [4, 14].

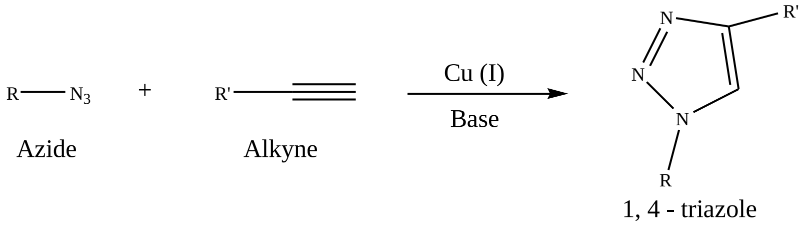


Figure 5: Copper catalyzed- Huisgen reaction [4].

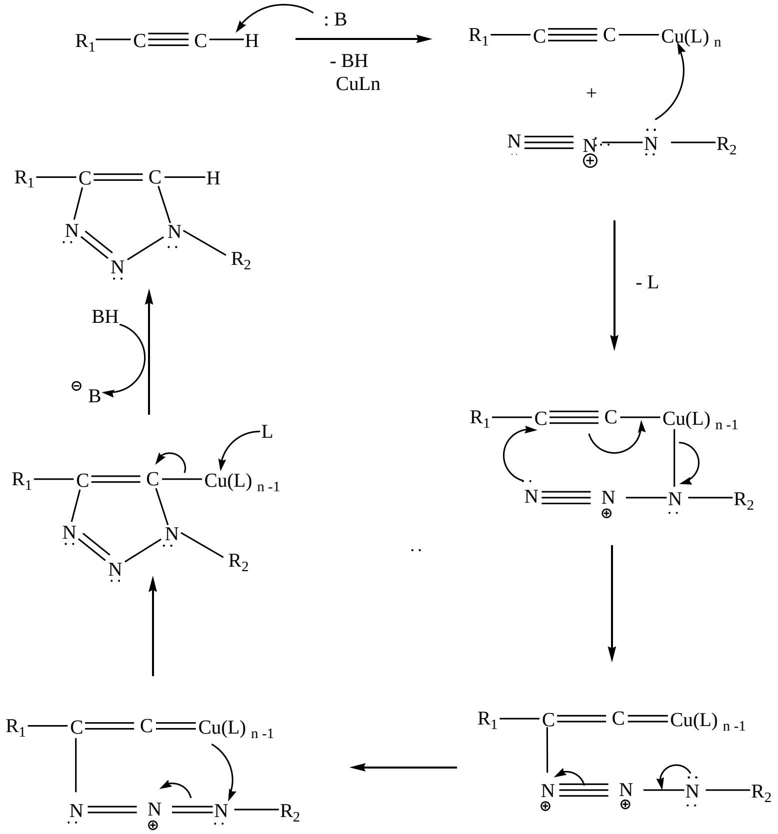


Figure 6: Mechanism of Copper catalyzed- Huisgen reaction[16]

Mechanism [4, 8, 15]

In presence of a base, the terminal hydrogen, being the most acidic is deprotonated first to give a Cu acetylide intermediate. It has been found from the literature survey that the transition state involves two Cu atoms. One Cu atom is bonded to the acetylide while the other Cu atom serves to activate the azide. The metal center coordinates with the electrons on the nitrogen atom. The ligands employed are labile and are weakly coordinating. The azide displaces one ligand to generate a copper-azide-acetylide complex. At this point cyclisation takes place. This is followed by protonation and the source of proton being the hydrogen which is pulled off from the terminal acetylene by the base. The product is formed by dissociation and the catalyst ligand complex is regenerated for further reaction cycles (**Figure 6**).

Following are the elements required in CuAAC reactions:

i. Cu (I) species:

The Cu (I) species may either be introduced in CuAAC reactions as preformed complexes or may be generated in the reaction in situ by one of the following ways:

a) A Cu compound (in which copper is present in the +2 oxidation state) is added to the reaction in presence of a reducing agent (e.g. sodium ascorbate) which reduces the Cu from the (+2) to the (+1) oxidation state. The advantage of generating the Cu (I) species in this manner is it eliminates the need of a base in the reaction. Also the presence of reducing agent reduces oxygen which may be present in the system; otherwise oxygen present will oxidize the Cu (I) to Cu (II) that will impede the reaction and will result in low yields. One of the commonly used Cu compounds is $CuSO_4$ (**Figure 7**).

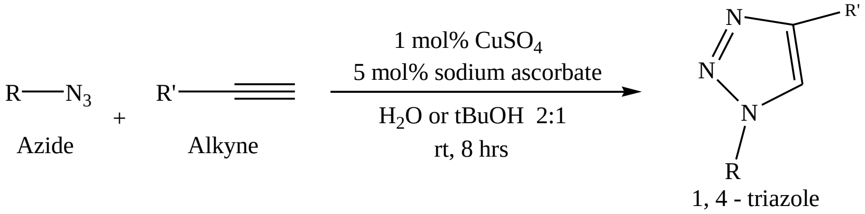


Figure 7: In situ formation of Cu (I) in CuAAC reaction.

b) Halides of copper such as the iodide and bromide Cu salts may be used. But here solubility is a problem. However, this can be overcome either by carrying reaction in the presence of amines or at higher temperatures [17, 18].

ii. Ligands:

Ligands are effective for improving the reaction outcome as it stabilizes the unstable Cu (I) catalyst in aqueous solvents, e.g. tris-(benzyltriazolylmethyl)amine (TBTA) (Figure 8).

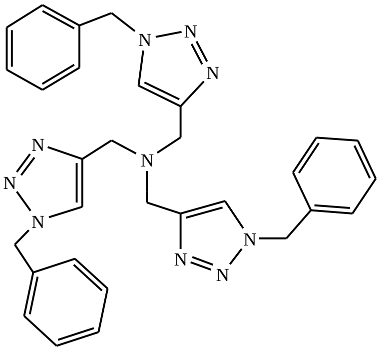


Figure 8: tris-(benzyltriazolylmethyl)amine.

Finn and co-workers showed that tris-(2-benzimidazolylmethyl) amines (**Figure 9**) are the most promising family of accelerating ligands for the Cu catalyzed azide-alkyne cycloaddition reaction. These tripodal benzimidazole derivatives give substantial improvements in rate and yields.

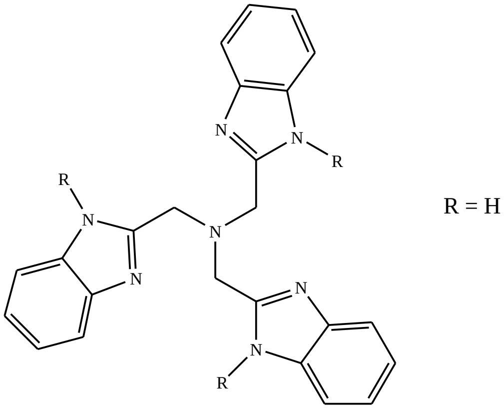


Figure 9: tris-(2-benzimidazolylmethyl)amine.

The presence of a ligand has following advantages:

a) It protects the Cu ion from interactions leading to degradation and formation of side products.

b) It prevents the oxidation of the Cu (I) species to the Cu (II).

c) It functions as a proton acceptor [19, 20].

iii. Solvents:

- Polar aprotic solvents such as THF, DMSO, Acetonitrile, DMF
- Non-polar aprotic solvents such as toluene
- Neat solvent (i.e. water) or a mixture of solvents
- Variety of partially miscible organic solvents including alcohols, DMSO, DMF, *t*BuOH and acetone

iiii. Bases: DIPEA (N, N-Diisopropylethylamine) and Et_3N (triethylamine) [8, 14]

1.1. C. Ruthenium catalyzed- Huisgen reaction [6]

The ruthenium-catalyzed 1, 3-dipolar azide-alkyne cycloaddition (RuAAC) gives the sole product i.e. the 1, 5-triazole. Unlike CuAAC in which only terminal alkynes react, in RuAAC both, terminal and internal alkynes can participate. Several ruthenium complexes were employed, but the pentamethylcyclopentadienyl (Cp*) analogues gave the best results e.g. Cp*RuCl $(PPh_3)_2$ (**Figure 10**).

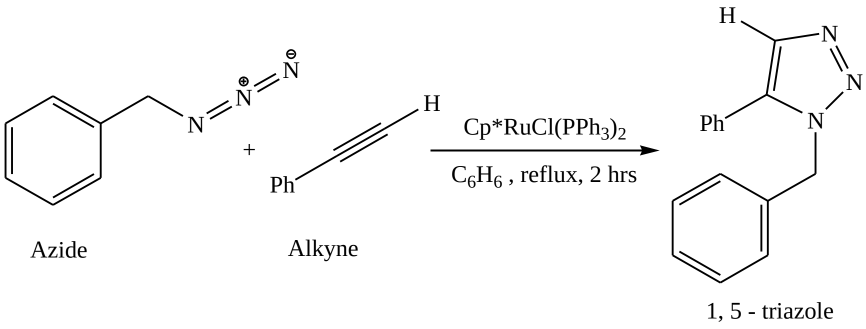


Figure 10: Ruthenium catalyzed- Huisgen reaction.

1.2. Diels-Alder Reaction [21]

The Diels-Alder reaction is a conjugate addition reaction of a conjugated diene to an alkene (the dienophile) to produce a cyclohexene. The simplest example is the reaction of 1, 3-butadiene with ethene to form cyclohexene (**Figure 11**).

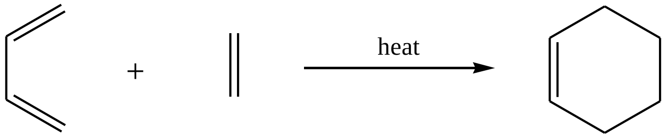


Figure 11: Reaction between 1, 3-butadiene and ethane.

The analogous reaction of 1, 3-butadiene with ethyne to form 1, 4-cyclohexadiene is also known (**Figure 12**).

Figure 12: Reaction between 1, 3-butadiene and ethyne.

Since the reaction forms a cyclic product, via a cyclic transition state, it is described as a “cycloaddition”. Due to the high degree of regio and stereo selectivity, the Diels-Alder reaction is a very powerful reaction and is widely used in synthetic organic chemistry (**Figure 13**).

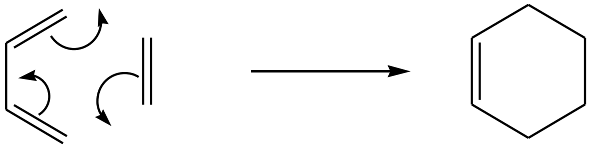
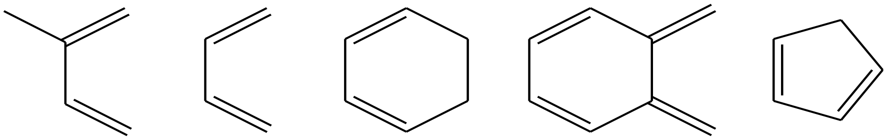


Figure 13: Mechanism of Diels Alder reaction.

This reaction is thermodynamically favorable due to the conversion of 2 π bonds into 2 new stronger sigma bonds. Diels-Alder reaction is favored by electron withdrawing groups on the electrophilic dienophile and by electron donating groups on the nucleophilic diene (**Figure 14**). Some common examples are shown below.

Dienes :



Dienophiles :

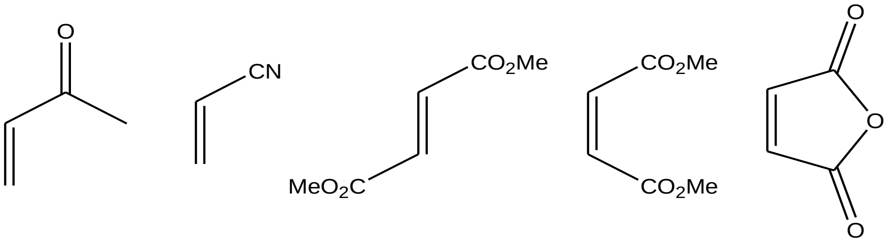


Figure 14: Examples of diene and dienophile.

2. Nucleophilic ring opening [22]

This reaction refers to the opening of strained heterocyclic electrophiles such as aziridines, epoxides, cyclic sulfates, aziridinium ions, episulfonium ions, etc. Of these heterocycles, epoxides and aziridines are the most common substrates for click reactions and their regioselective ring opening is highly useful for the formation diverse compounds (**Figure 15**). The ring opening of three membered heterocycles is generally facilitated by the release of strain energy and therefore these compounds have been termed as spring-loaded electrophiles. Nucleophilic ring opening reactions of oxirane and aziridine are high yielding, stereospecific and regioselective thus fulfilling the requirements of click reactions.

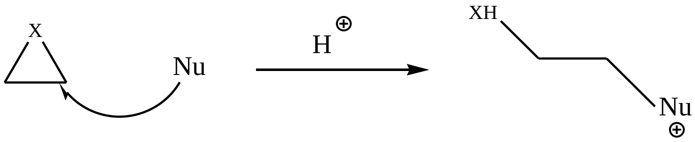


Figure 15: Nucleophilic ring opening reaction.

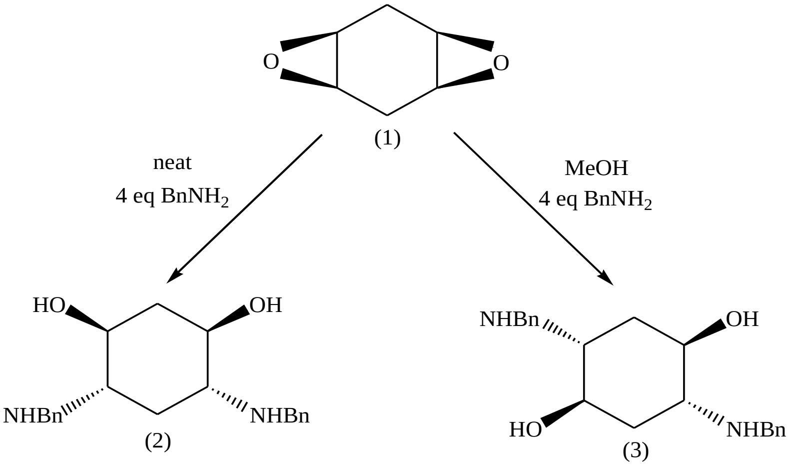


Figure 16: Oxirane opening.

2.1. Oxirane opening [5, 22]

Regioselective oxirane opening to generate constitutional isomers can be carried out by the nucleophilic opening of diepoxide (1) with benzyl amine. In the presence of methanol, the 1, 4-diol (3) is obtained in 90% yield where as in the absence of solvent, the 1, 3-diol (2) is obtained in 94% yield (**Figure 16**).

2.2. Aziridine opening [23]

Regioselective opening of aziridines is primarily substrate controlled as shown by the opening of unsymmetrical aziridines using Trimethylsilyl azide (TMS azide) (**Figure 17**).

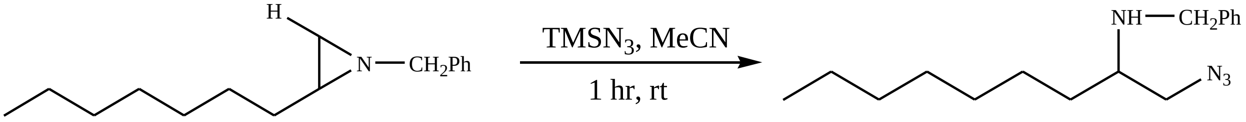


Figure 17: Aziridine opening.

3. Carbonyl chemistry [8,24]

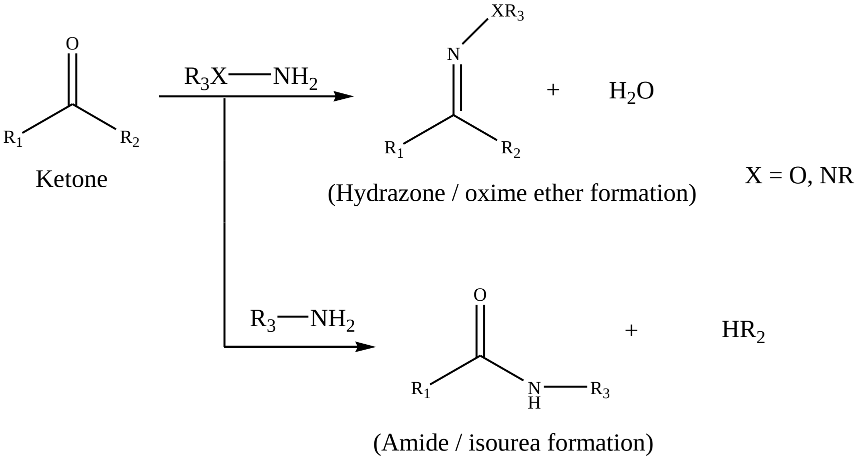


Figure 18: Reactions involving carbonyl groups.

Carbonyl chemistry of the non-aldol type includes examples of the formations of ureas, thioureas, hydrazones, oximes, ethers, amides, aromatic heterocycles, etc (**Figure 18**). These reactions are easy to perform and have high thermodynamic driving forces, hence are classified as click reactions. While carbonyl reactions of the aldol type generally have low thermodynamic driving forces; hence they have longer reaction times and give side products and therefore cannot be considered click reactions.

4. Staudinger reaction [25-27]

Azides may be converted to amines by hydrogenation (Figure 19). Staudinger reaction is another possibility, which is a very mild azide reduction. As there are a variety of methods for preparing azides readily, the Staudinger reaction makes it possible to use $^{-}N_3$ as $^{-}NH_2$ synthon.

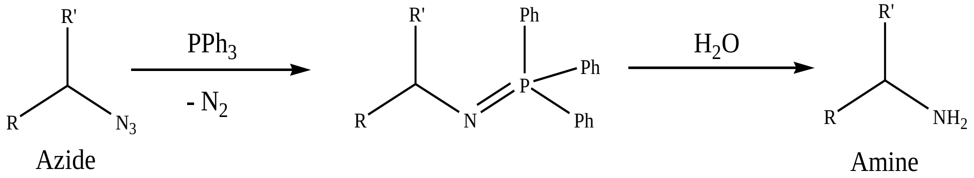


Figure 19: Staudinger reaction.

Mechanism

Triphenylphosphine reacts with the azide to generate a phosphazide, which loses N_2 to form an iminophosphorane. Aqueous work up leads to the amine and the very stable phosphine oxide (Figure 20).

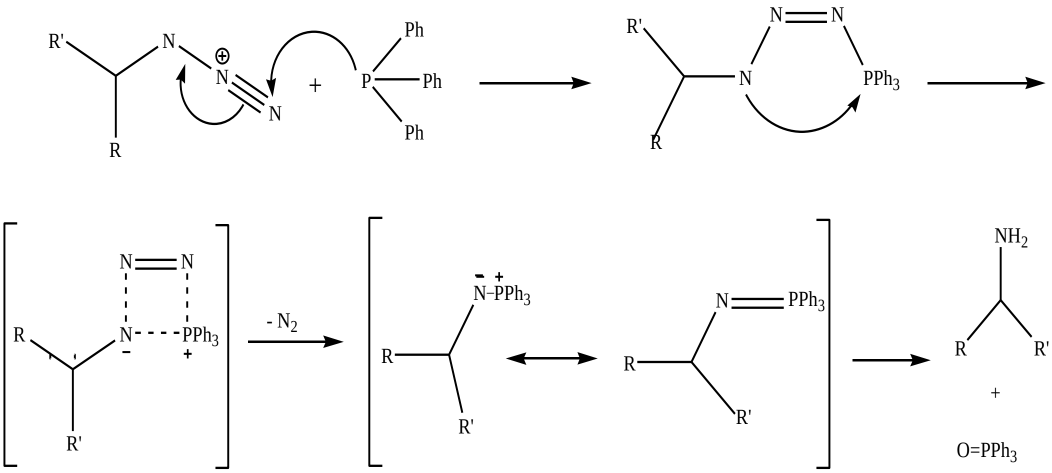
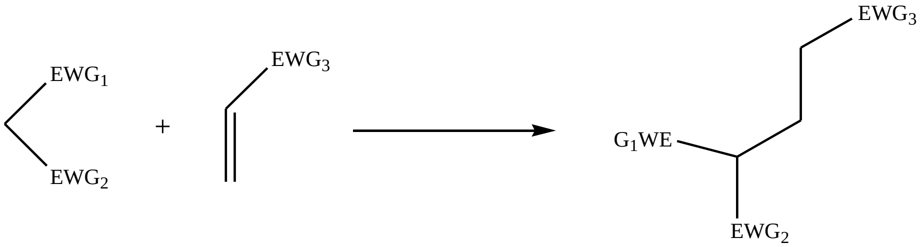


Figure 20: Mechanism of Staudinger reaction.

5. <u>Michael addition reaction</u> [28,29]

Michael addition is the <u>nucleophilic addition</u> of a <u>carbanion</u> or another <u>nucleophile</u> to an <u>α, β-unsaturated carbonyl compound</u> (**Figure 21**). It belongs to the larger class of <u>conjugate additions</u>. This is one of the most useful methods for the mild formation of C-C bonds.



EWG = electron withdrawing group

Figure 21: Michael Addition.

ADVANCES: COPPER- FREE CLICK REACTIONS

1. Reaction of Azides with Substituted Cyclooctyne [1]

A new reagent developed by Carolyn R. Bertozzi and co-workers eliminated the toxicity to living cells that is usually associated with the copper catalyzed Huisgen 1, 3-dipolar cycloaddition. By using a Cyclooctyne instead of the usual terminal alkyne a rapid cycloaddition reaction takes place even without a catalyst and in 1hr at room temperature (**Figure 22**) [30].

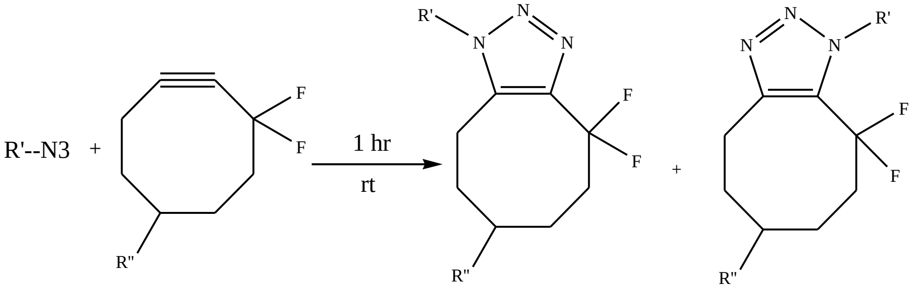


Figure 22: Reaction of Azides with substituted Cyclooctyne.

The rate of these reactions with the 1st generation of Cyclooctyne (a) was relatively slow compared to the corresponding CuAAC reactions. Therefore, monofluorinated (2nd generation, b) and difluorinated (3rd generation, c) derivatives of Cyclooctyne were synthesized (**Figure 23**). In this, the ring strain and the electron-withdrawing difluoro group activated the alkyne group and hence were termed as strain-promoted azide-alkyne [3+2] cycloaddition (SPAAC) reaction [31, 32].

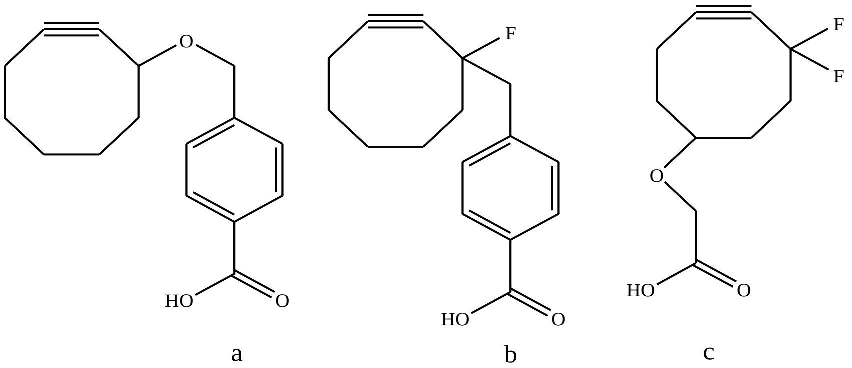


Figure 23: 1st, 2nd and 3rd generation cyclooctynes.

This method was used to attach fluorescent label to cells with azide containing sialic acid in their surface glycans. This is an impressive example were copper-free click chemistry is used as a biologically friendly method to label and track biomolecules in living cells (**Figure 24**) [33].

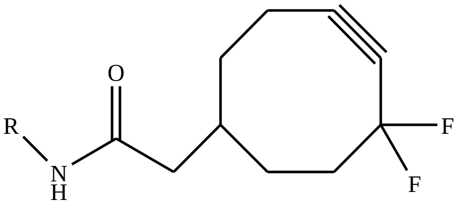


Figure 24: Fluorescent dye or biotin.

2. Reaction of Azides with Electron-Deficient Alkynes [1, 34]

In 2004, Ju and co-workers reported a simple synthetic protocol for the 1, 3-dipolar cycloaddition of azides with electron-deficient alkynes. A series of alkynes with at least one electron-withdrawing group were investigated in the click reaction with 5-azidovalerate at room temperature in water. The yields obtained were in the range of 67 to 94% (**Figure 25**). This click reaction has been extended to the coupling of an azido-DNA molecule to demonstrate the potential for introducing functional groups to DNA under physiological conditions.

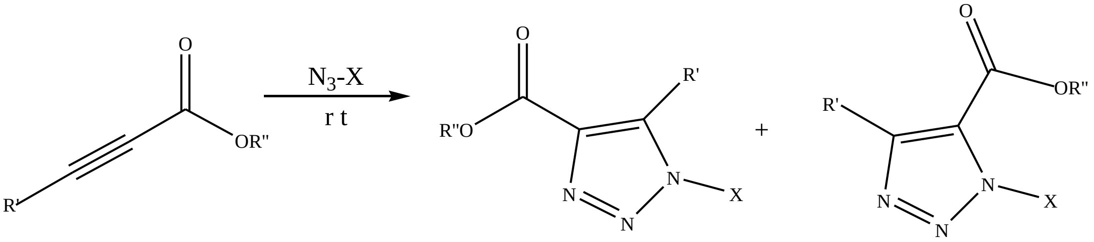


Figure 25: Click reaction between azides and electron-deficient alkynes (R' = H, CH_3 or COOEt; R" = Me or Et; N_3-X = 5-azidovalerate or 5'-azido-DNA).

3. Reaction of Azides with Arynes

Larock and co-workers developed an efficient and general method for the synthesis of functionalized benzotriazoles by the 1, 3-dipolar cycloaddition of benzynes with azides under very mild reaction conditions and called this reaction as "benzyne click" reaction (**Figure 26**).

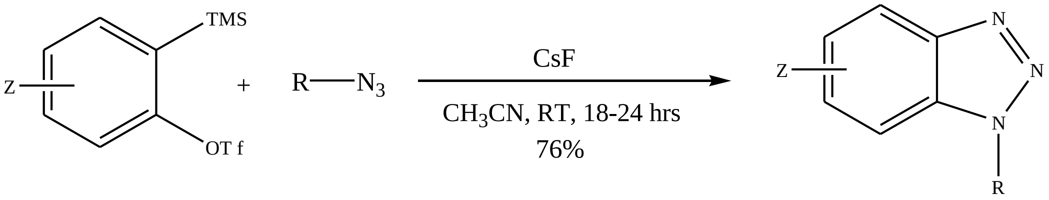


Figure 26: Reaction of azides with arynes.

A similar study was reported by Feringa and co-workers, who found that the yields of this reaction could be improved by the use of complementary crown ether and also the reaction times, could be reduced to less than 2 hrs [34].

APPLICATIONS

A. Click in peptide chemistry [20,35]

This process allows the combination of two unprotected peptide segments by the reaction of α-thioester with a cysteine-peptide (**Figure 27**). The result of this reaction is a native amide bond at the ligation site, rendering this method highly attractive for the synthesis of large peptides. Usually, α-alkylthioesters are used because of their ease of preparation. Since they are unreactive, the ligation reaction is catalyzed by *insitu* trans-thioesterification with thiol additives.

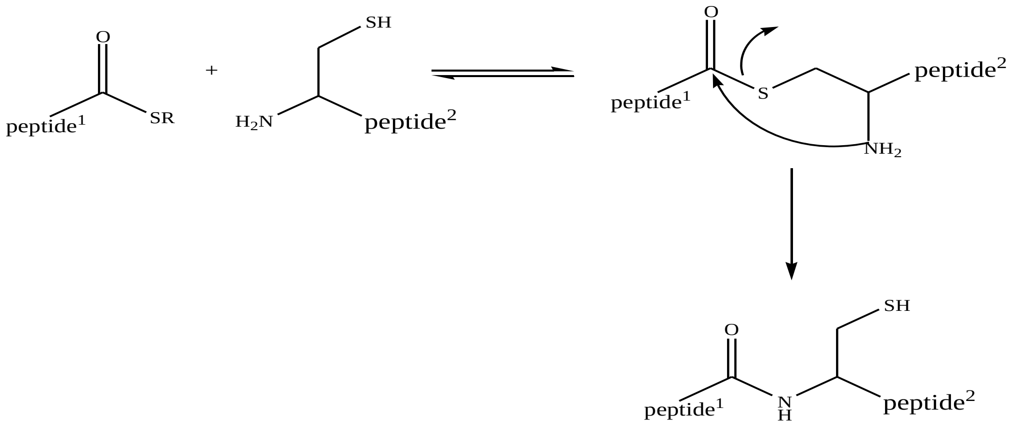


Figure 27: Click in peptide chemistry.

The most common thiol catalyst used is either a mixture of thiophenyl/benzyl mercaptan, or 2-mercaptoethanesulfonate (MESNa). In a recent study, it was shown that MESNa is a poor catalyst, requiring reaction times of 24–48 hrs and was replaced by certain aryl thiol such as 4-mercaptophenylacetic acid (MPAA) wherein proteins were synthesized much more rapidly i.e. within an hour and with high yields (**Figure 28**).

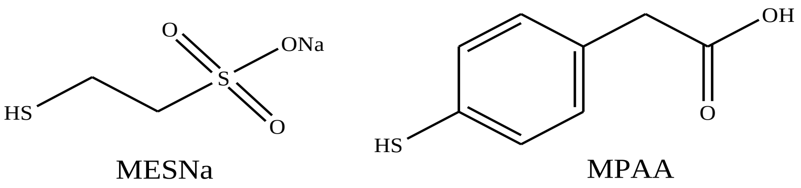


Figure 28: Structures of thiol catalysts.

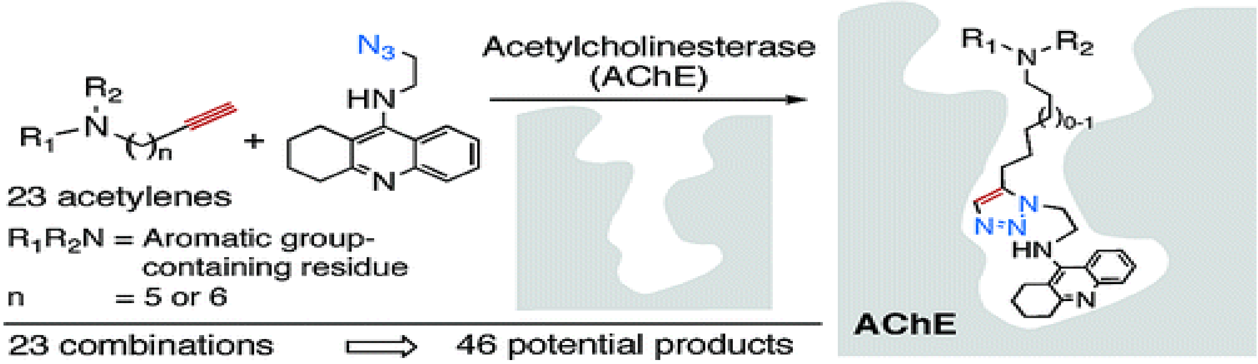


Figure 29: Target-guided optimization of acetyl cholinesterase inhibitors [36].

B. Target-guided *in situ* Click Chemistry [8, 36]

The target-guided *in situ* click chemistry approach to lead discovery has been successfully employed for discovering acetyl cholinesterase (AChE) inhibitors by incubating a selected enzyme/tacrine azide combination with a variety of acetylene reagents that were not previously known to interact with the enzyme's peripheral binding site. The triazole products formed by the enzyme were identified by HPLC-mass spectrometry analysis of the crude reaction mixtures.

From 23 acetylene reagents, the enzyme selected two phenyltetrahydroisoquinoline (PIQ) building blocks that combined with the tacrine azide within the active center formed multivalent inhibitors that simultaneously associate with the active and peripheral binding sites. These new inhibitors are up to three times as potent as previous discovered phenylphenanthridinium-derived compounds. They are the most potent non-covalent AChE inhibitors known (**Figure29**).

C. Click in Radiochemistry [8, 37, 38]

The use of click chemistry is receiving more interest in the field of radio pharmacy. Radio labeling of biologically active molecules has become an important tool to assess novel drug candidates. Nonmetallic positron-emitting isotopes, such as ^{18}F and ^{11}C, possessing short half-lives ($t_{1/2}$ = 109.8 minutes and $t_{1/2}$ = 20.5 minutes respectively) and have to be produced on-site. Another conflict for labeling biomolecules of higher molecular weight with high specificity is the requirement of harsh reaction conditions. To overcome these, a bifunctional approach is usually applied, wherein the ^{18}F or ^{11}C is linked to or incorporated into a small molecule that is subsequently capable of being attached to biomolecules such as proteins and peptides, under mild conditions.

In the synthesis of ^{18}F-labeled peptides through click chemistry, ^{18}F-fluoroalkynes were conjugated to various azide-functionalized peptides and found good radiochemical yields being achieved within 10 minutes. This synthetic method to prepare functionalized peptides attached to ^{18}F-fluoroalkynes (e.g. butyne, pentyne and hexyne) achieved high radiochemical purities (81% – 99%). The ^{18}F-fluoroalkynes were prepared from the reaction of the corresponding tosylalkynes with [^{18}F] KF/K222 complex and purified by co-distillation with acetonitrile in 10 minutes and the labeling was achieved by reacting peptides bearing N-(3-azidopropionyl)-groups with ω-[^{18}F] fluoroalkynes (**Figure 30**).

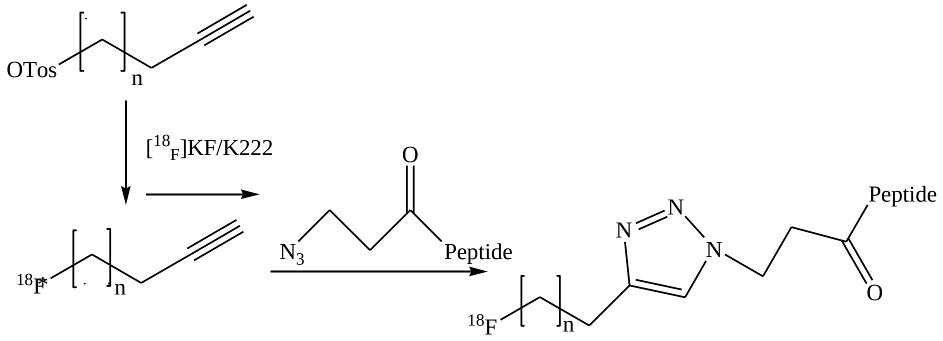


Figure 30: Click in radiochemistry.

D. Labeling of biomolecules [38]

Click chemistry describes a class of chemical reactions that use bio-orthogonal or biologically unique moieties to label and detect a molecule of interest. The azide and alkyne moieties can be used interchangeably; that is either one can be used to tag the molecule of interest while other is used for subsequent detection (**Figure 31**). Click chemistry can be used when methods such as direct labeling or the use of antibodies are not applicable or efficient. Click chemistry label is small enough that tags molecules (e.g. nucleotides, sugars and amino acids) which are acceptable substrates for the enzymes that assemble these building blocks into biopolymers.

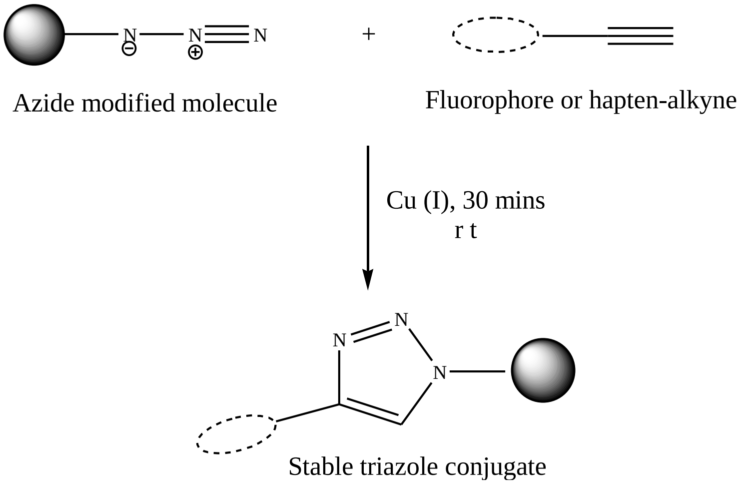


Figure 31: Click in labeling of biomolecules.

E. Activity-based protein profiling (ABPP) [39]

ABPP is a chemical method that employs active site-directed probes to tag proteins and monitor their expression levels and function in complex proteomes. However, the bulky reporter tags that are currently used require cell homogenization before analysis, thereby, preventing measurements in living organisms. Cravatt have solved this problem with small, cell-permeable reagents that carry an azide group for later dye attachment via the bio-orthogonal, copper-(I) catalyzed reaction with acetylenes. He detected glutathione S-transferases (GSTO 1–1), aldehyde dehydrogenases (ALDH-1) and enoyl CoA hydratases (ECH-1) at endogenously expressed levels in viable cells, by incubation with 6-azidohexyl benzenesulfonate (PS-N_3), followed by homogenization and tagging with Rhodamine-acetylene (Rh-acetylene). Quantification of GSTO 1–1 levels in breast cancer cell lines yielded results that were comparable with traditional methods (**Figure 32**). The Cravatt approach works even in live animals, allowing ECH-1 to be observed in the heart muscle of mice one hour after injection with PS-N_3 and ‘staining’ of the unique protein from the crude heart-homogenate, via copper-catalyzed conjugation with Rh-acetylene. By enabling the determination of protein expression levels in living organisms, this new in vivo ABPP method provides more unbiased results, because tagging occurs before cell death.

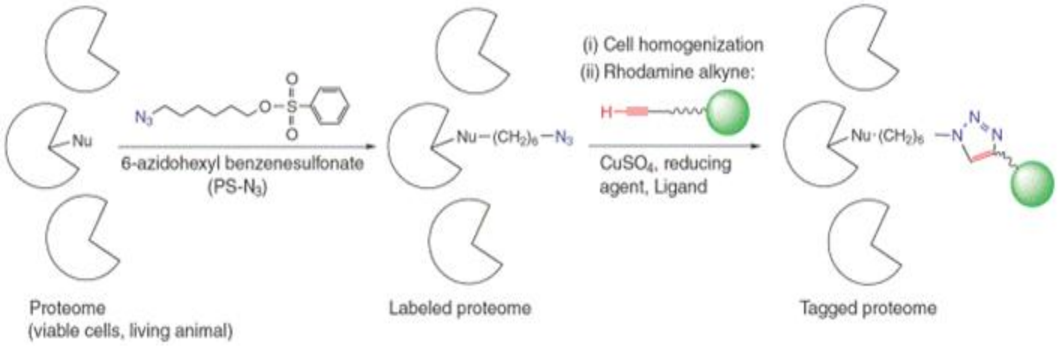


Figure 32: Activity-based protein profiling [39].

ADVANTAGES OF CLICK CHEMISTRY [1, 2, 5, 8]

- Aqueous conditions are usually required, hence no byproducts are formed
- Shorter reaction time
- High yield
- High purity
- Regio-specificity
- Cost effective method
- As there are no protecting groups in click reaction, structural uncertainties do not exist, rendering purification unnecessary

DISADVANTAGES OF CLICK CHEMISTRY [1, 8]

1) One of the major disadvantages is alkyne homocoupling. Alkynes can react with another alkyne instead of the azide.
2) For the click reaction to occur efficiently, both the alkyne and the azide should be at the terminal position of an alkyl chain.
3) Tornoe et al. found that a sterically hindered azide failed to react even at elevated temperatures and with extended reaction times.
4) Some click chemistry reactions require metal such as copper, as a catalyst, which can be incompatible in vivo. Excess copper is known to cause physiologic side-effects such as hepatitis, neurologic and renal diseases.
5) Another concern is the stability of some azide derivatives. Some heavy metal azides or methyl azide are known to be explosive, but this property certainly should not be a major issue at the small-scale pharmaceutical research level.
6) One of the drawbacks for the Staudinger ligation reaction is air oxidation of phosphine reagents that can be problematic and affect reaction kinetics.

CONCLUSION

Click chemistry is a stringent criteria that defines a set of dependable transformations that can be employed to construct novel pharmacophores to facilitate drug discovery. Of the various click reactions that are available to us, the union of azides and acetylenes to give triazoles deserves special recognition. Azides and acetylenes are stable across a broad range of organic reaction conditions and in biological environments, yet they are highly energetic functional groups and thus widely used. Currently, the term click chemistry has become synonymous with the Huisgen 1, 3-dipolar cycloaddition because it is an ideal reaction. This reaction has been exploited in various fields of drug discovery and will likely be utilized in many applications in the near future. Ultimately, other reactions that fit the click criteria should be examined for their use towards the synthesis of biological active molecules in order to access greater structural diversity.

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