DEVELOPMENT AND APPLICATIONS OF CLICK CHEMISTRY

Reported by Gregory C. Patton November 8, 2004

INTRODUCTION

Secondary metabolites produced in Nature contain diverse architectures with extensive carbon-carbon bond networks. These compounds often possess important biological activities that make them potential therapeutic agents.[1] However, drug discovery based on these natural products is generally slow, costly, and hindered by complex syntheses. The recent development of combinatorial chemistry and high-throughput screening has aided in the rapid generation of compounds in search of biological function but relies heavily on the success of the individual reactions to construct molecular frameworks.[2] Therefore, a set of criteria defining reliable reactions known as "click" chemistry was proposed by Sharpless and coworkers in order to accelerate the synthesis of drug-like molecules.[3]

Click chemistry enables a modular approach to generate novel pharmacophores utilizing a collection of reliable chemical reactions.[3] Those reactions thus give products stereoselectively in high yields, produce inoffensive byproducts, are insensitive to oxygen and water, utilize readily available starting materials, and have a thermodynamic driving force of at least 20 kcal mol^{-1}. Preferably, the reactions should be conducted in benign solvents and chromatography is not used for purification of reaction products.[3] Two types of click reactions that have influenced drug discovery are the nucleophilic opening of strained ring systems[4] and 1,3-dipolar cycloadditions.[5] Of particular interest is the Huisgen [3 + 2] cycloaddition between a terminal alkyne and an azide to generate substituted 1,2,3-triazoles, **1** (Figure 1).[6] This reaction has been termed the "cream of the crop" of click reactions[3] and has found application in various facets of drug discovery.[7] This review focuses on these two types of click reactions, as well as the use of click chemistry in generating natural product derivatives, target-guided synthesis, and activity-based protein profiling.

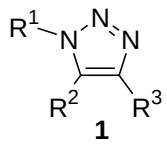

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Figure 1. Substituted 1,2,3-Triazole.

CLICK REACTIONS

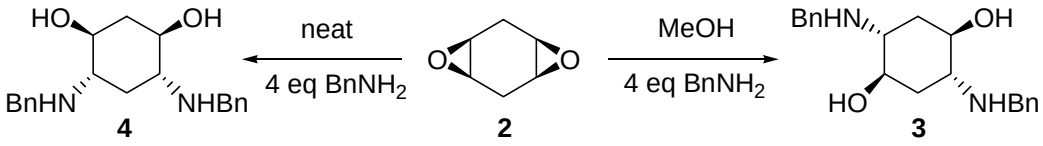
Nucleophilic ring opening and 1,3-dipolar cycloadditions are the most extensively studied click reactions to date. Preparation of potential drug candidates by these reactions utilizes building blocks such as acetylenes and olefins. These starting materials are readily available in Nature[8] or can be accessed through "steam cracking" of alkanes in the petrochemical industry[9] and can be functionalized by oxidative or addition reactions.

Nucleophilic Ring Opening

The ring opening of three membered heterocycles is generally facilitated by the release of strain energy[10] and therefore these compounds have been termed spring-loaded electrophiles.[3] Great diversity can be achieved from the nucleophilic opening of epoxides, aziridines, cyclic sulfates, episulfonium ions, and aziridinium ions. 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. The click reaction is frequently performed in alcohol/water mixtures or in the absence of solvents and products can be easily isolated in virtually quantitative yield.

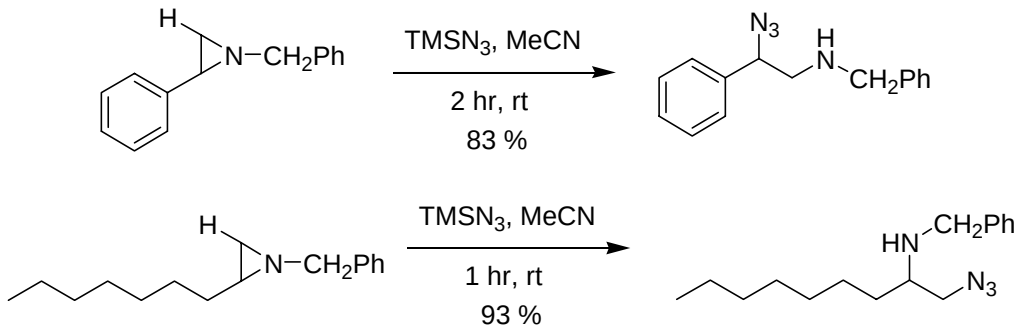
Regioselective oxirane opening to generate constitutional isomers can be regulated by the reaction conditions as demonstrated by the nucleophilic opening of diepoxide **2** with benzylamine (Scheme 1). In the presence of methanol, the 1,4-diol **3** is obtained in 90% yield where as in the absence of solvent, the 1,3-diol **4** is obtained obtained in 94% yield. Generation of the first hydroxyl group in **2** allows intramolecular activation of the remaining epoxide. This effect is less prevalent when the reaction is carried out in protic solvents which allows for nucleophilic attack from a more stable chair conformation, leading to the 1,4-diol-product.[3]

Scheme 1. Regioselectivity of Oxirane Opening.



Unlike the reactions of epoxides, regioselective opening of aziridines is primarily substrate controlled as shown by the opening of unsymmetrical aziridines using TMS azide (Scheme 2).[11] Benzyl substitution is more reactive than primary and secondary-carbon substitution, which obeys normal S_N2 behavior. In addition, greater product diversity can be achieved through variation of the nitrogen substitutent. Nucleophilic ring opening reactions of oxiranes and aziridines can be high yielding, stereospecific, and regioselective, fulfilling the requirements of click reactions.

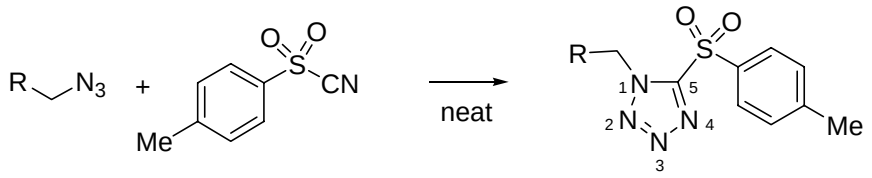
Scheme 2. Regioselective Opening of Aziridines.



1,3-Dipolar Cycloadditions

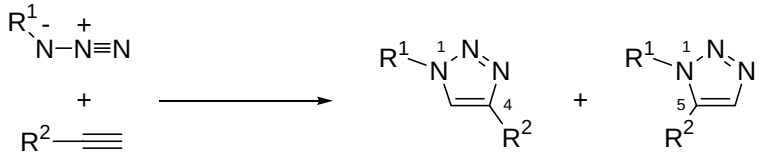
Reliable and quantitative triazole and tetrazole formation via 1,3-dipolar cycloadditions involving azides as one of the reaction partners are examples of ideal click chemical reactions.[12] Installation of the azido moiety within organic molecules by nucleophilic substitution of halides or ring opening of heterocycles with sodium azide is facile.[13] In addition, several cycloaddition partners can be utilized to generate a variety of heterocycles. The intermolecular [3 + 2] cycloaddition between nitriles and organic azides to synthesize tetrazoles is generally unsuccessful. However, the use of activated toluenesulfonyl cyanides in the presence of various unhindered azides produces the corresponding 1,5-disubstituted tetrazole in quantitative yield (Scheme 3).[12] The substituted tetrazole can be functionalized by the displacement of the sulfonyl group by a variety of nucleophiles. Expansion of this method to include the use of acyl cyanides, cyanoformates, and cyanoformamides with azide substrates generates acyltetrazoles.[14] For example, the reaction of benzoyl cyanide with various azides at 120 ^{o}C affords the [3 + 2] cycloaddition product regioselectively in high yield.

Scheme 3. Reaction of Unhindered Azides with Toluenesulfonyl Cyanide.



The Huisgen 1,3-dipolar cycloaddition between a terminal alkyne and an azide has rapidly become the most popular click reaction to date.[7] The formation of triazoles via the cycloaddition of azide and acetylene was first reported by Dimroth in the early 1900's but the generality, scope, and mechanism of these cycloadditions was not fully realized until the 1960's.[6] The reaction generates a mixture of 1,4- and 1,5-disubstituted triazoles (Scheme 4). Various attempts to control the regioselectivity have been reported without much success until the discovery of the copper(I)-catalyzed reaction in 2002, which exclusively yields the 1,4-disubstituted 1,2,3-triazole.[15,16] Several copper(I) salts such as CuI and CuOTf·C_6H_6, can be employed but the reactions generally must be run with acetonitrile as co-solvent, require a nitrogen base, and sometimes generate unwanted diacetylene and bis-triazole by-products.[15] The *in situ* reduction of copper(II) salts such as $CuSO_4 \cdot 5H_2O$ with sodium ascorbate in aqueous alcoholic solvents allows the formation of 1,4-triazoles at room temperature in high yield with less than 2 mol % catalyst loading. Primary, secondary, and tertiary substituted azides as

Scheme 4. 1,2,3-Triazole Formation via Huisgen 1,3-Dipolar Cycloaddition.



well as aromatic azides can be utilized. Numerous terminal acetylene components participate in the transformation and the reaction is compatible with various functional groups such as esters, acids, alkenes, alcohols, and amines. The copper-catalyzed reaction was later expanded by Yamamoto and coworkers using a bimetallic catalyst so that 1,4,5-substituted triazoles could be obtained from seemingly internal alkynes.[17]

The copper-catalyzed reaction is thought to proceed in a stepwise manner starting with the generation of copper(I) acetylide (**5**) (Figure 2). Density functional theory calculations show a preference for the stepwise addition (**5** → **6** → **7** → **8**) over the concerted cycloaddition (**5** → **8)** by approximately 12 to 15 kcal mol^{-1}, leading to the intriguing six-membered metallocycle **7**. Comparison of the thermal reaction between benzyl azide and phenyl propargyl ether with the copper-catalyzed reaction of the same substrates demonstrates the importance of copper catalysis (Scheme 5). The thermal reaction leads to the formation of two disubstituted triazole isomers while the copper(I)-catalyzed reaction selectively produces the 1,4-isomer in 91% yield after 8 hours.[15] This cycloaddition became even more powerful under microwave irradiation where a one-pot synthesis of various 1,2,3-triazoles was completed in less than 15 minutes.[18] Due to

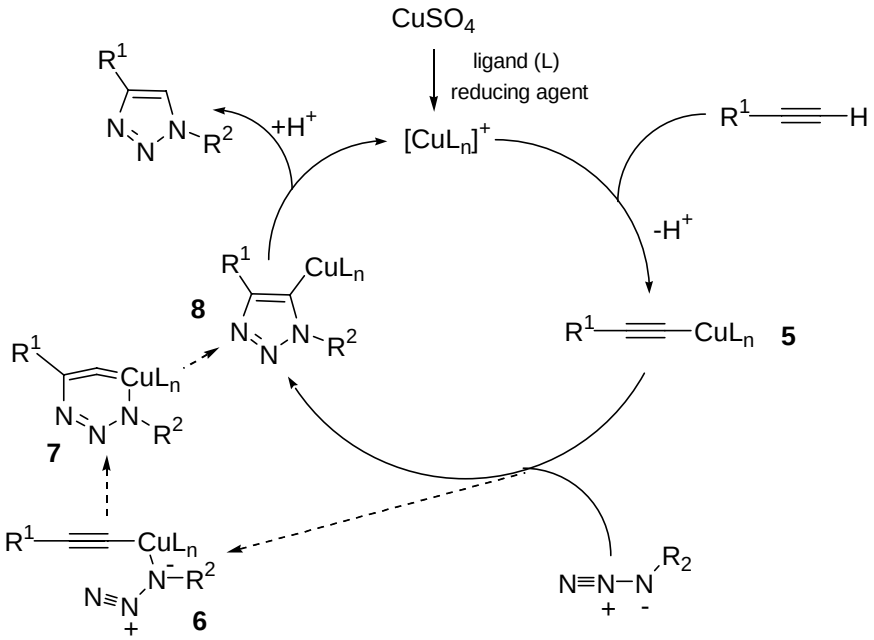
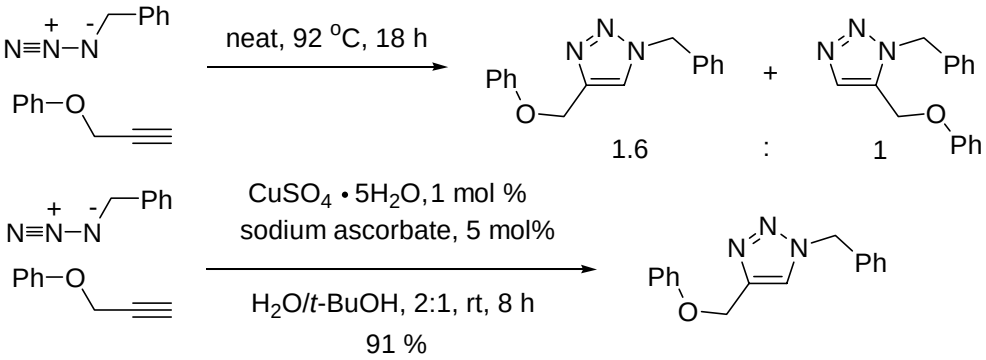


Figure 2. Postulated Catalytic Cycle for Azide-Alkyne Coupling.

Scheme 5. **Thermal and Cu(I)-Catalyzed 1,3-Dipolar Cycloadditions.**



the reliability and generality of the copper(I)-catalyzed azide-alkyne cycloaddition to generate N-heterocyclic pharmocophores, the reaction has been utilized for various aspects of drug discovery.

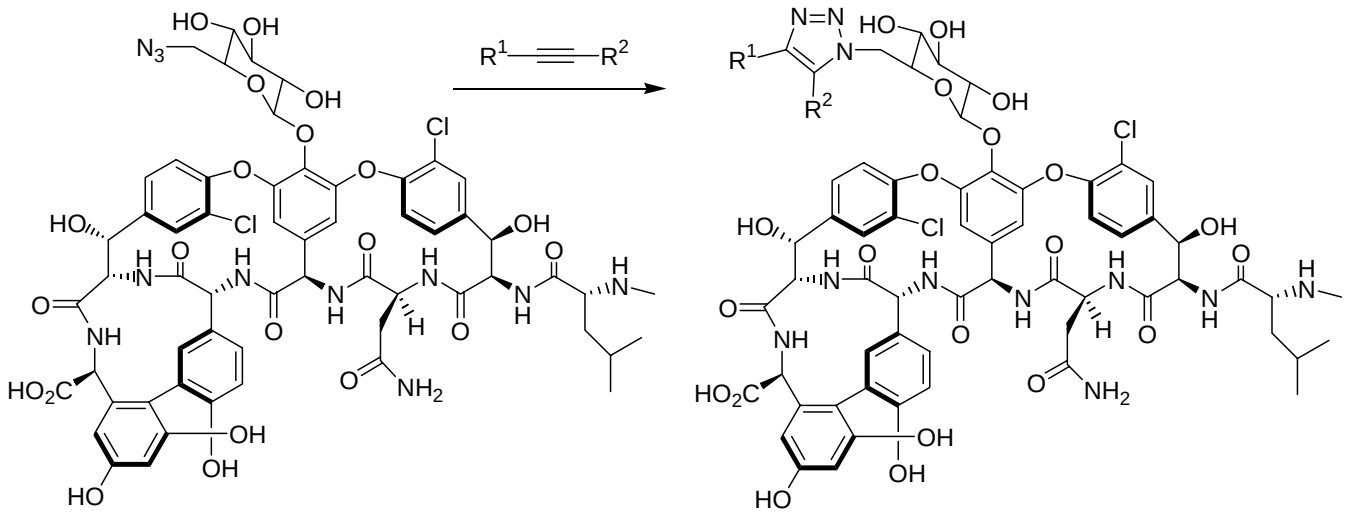
APPLICATIONS

The development of the copper(I)-catalyzed cycloaddition reaction between azides and terminal alkynes has led to many interesting applications of click reactions including the synthesis of natural product derivatives. Although azides and alkynes display high mutual reactivity, individually these functional groups are two of the least reactive in organic synthesis. They have been termed bioorthogonal because of their stability and inertness towards the functional groups typically found in biological molecules.[7] This bioorthogonality has allowed the use of the azide-alkyne [3 + 2] cycloaddition in various biological applications including target guided synthesis[19] and activity-based protein profiling.[20]

Generation of Natural Product Derivatives

Click reactions can be utilized to construct building blocks for the rapid synthesis of molecules with diverse structure and function. Thorson and coworkers recently utilized the Huisgen 1,3-dipolar cycloaddition of azide and acetylenes to generate fifty triazole analogs of the clinically available antibiotic vancomycin (Scheme 6).[21] Antibacterial screens of these compounds revealed several vancomycin derivatives that possessed similar biological activity to the natural product and could

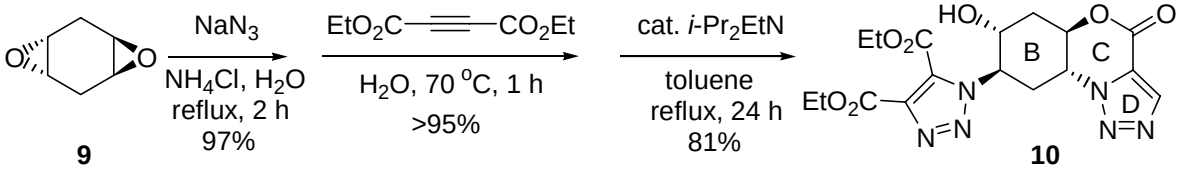
Scheme 6. Generation of Vancomycin Analogs.



possibly be utilized against vancomycin resistant bacterial strains as these analogs have a different target than the parent compound. Similarly, the copper-catalzyed azide-alkyne cycloaddition was utilized to create derivatives of the non-ribosomal decapeptide antibiotic tyrocidine.[22] Drug resistance to the

tyrocidine class of antibiotics is rare, making it an attractive therapeutic agent. However, this peptide also causes lysis of human red blood cells. In attempt to decrease tyrocidine's toxicity, sugar moieties were installed and generated several analogs that were six times less toxic than the natural decapeptide. Sharpless and coworkers demonstrated the power of nucleophilic ring opening and 1,3-dipolar cycloaddition click reactions in the construction of steroid-like skeletons from diepoxides (Scheme 7).[3] Tricyclic compound **10** can be prepared in a one-pot synthesis in three high yielding steps. The three

Scheme 7. **Formation of Steroid Mimics.**



rings formed in this sequence of reactions resemble the B, C, and D rings found in steroid natural products (Figure 3). Click chemistry methods demonstrate the promise of creating natural product derivatives quickly and efficiently and in addition, is now been employed in *in situ* synthesis in biomolecular active sites.

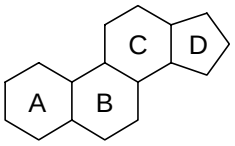
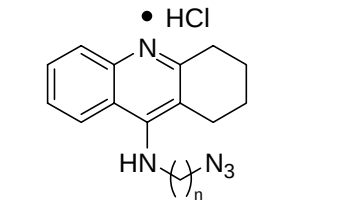


Figure 3. Ring Skeleton of Steroids.

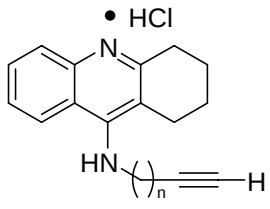
Target-Guided Synthesis

Target-guided synthesis (TGS) is the use of small molecule building blocks that can be assembled by specifically targeted enzymes to synthesize their own inhibitors. Only building blocks that interact with the active site of the protein will be in close proximity to react with one another and form potent inhibitors.[23] Installation of azides and alkynes within organic building blocks (Chart 1) has allowed the use of the Huisgen [3 + 2] cycloaddition to discover a femtomolar inhibitor of acetylcholinesterasae (AChE).[19] AChE hydrolyzes acetylcholine, a neurotransmitter, and plays a vital role in the central nervous system.[24] The active site is located in the base of a narrow 20 Å gorge lined with aromatic amino acids.[25]

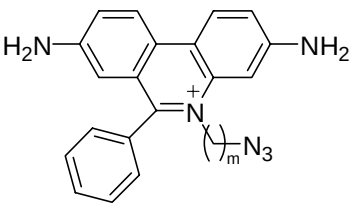
Chart 1. Building Blocks for Acetylcholinesterase TGS and Femtomolar AChE Inhibitor.



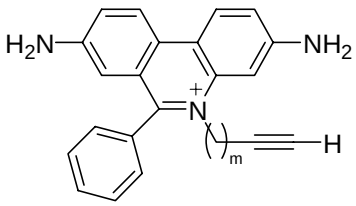
11a-e (n = 2-6)



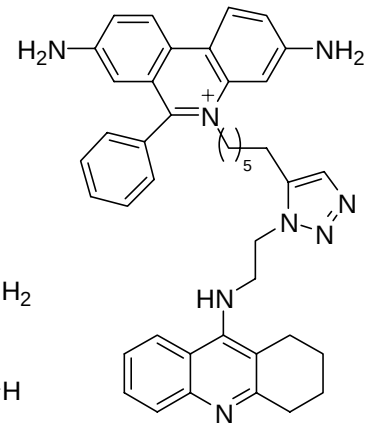
12a-c (n = 1-3)



13a-c (m = 6-8)



14a-e (m = 2-6)



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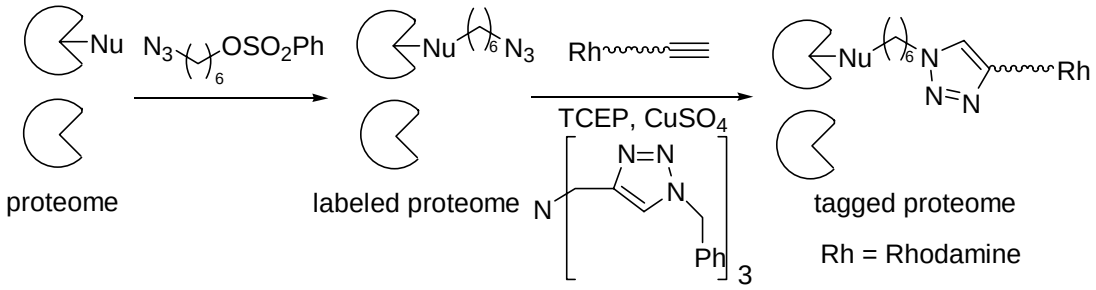
The design of the building blocks was based on tacrine and phenanthridinium, two known site specific ligands. Out of all possible azide-alkyne cycloaddition products, only the 1,5-triazole **15** was formed from the combination of **11a** and **14e** in the presence of AChE.[19] This inhibitor is currently the most potent inhibitor of AChE and has led to the discovery of three new inhibitors of AChE.[23]

Activity-Based Protein Profiling

Click chemistry as not only been utilized in the synthesis of potential therapeutic agents but also has assisted other areas of drug discovery such as target identification by activity-based protein profiling. Activity-based protein profiling (ABPP) utilizes active site-directed chemical probes with broad target selectivity to label active proteins within various enzyme classes and allows for the discovery of new drug targets.[26] The chemical probes contain a functional group that covalently reacts with specific classes of enzymes such as serine hydrolases.[27] Included in the probe is a chemical tag such as rhodamine that allows for the rapid detection and isolation of the enzymes covalently attached to the probe from a complex mixture of proteins. Until the development of the copper(I)-catalyzed azide-alkyne cycloaddition, ABPP experiments were conducted *in vitro* because the bulky chemical tags inhibited cellular uptake and caused the enzymes to be profiled outside their natural biological environments.[23] The discovery of the copper-catalyzed click reaction offered a solution to this problem by allowing enzymes to be profiled *in vivo*. The use of an azide containing a phenyl sulfonate ester reactive group allowed the *in vivo* profiling of glutathione *S*-transferases, aldehyde dehydrogenases, and enoyl CoA hydratases (Scheme 8).[20] The small azide reactive group is easily up taken by the cell and covalently labels active proteins. Addition of the alkyne cycloaddition reaction partner under copper-catalysis after lysing the cells tags the enzymes for detection and isolation. In fact, the use of click chemistry in ABPP resulted in the isolation of several enzymes in breast cancer cell lines that were never identified *in vitro* and can possibly serve as markers or novel targets for this disease.[28]

Scheme 8. **ABPP via Copper(I)-Catalyzed Click Reaction.**



CONCLUSIONS

"Click" chemistry was introduced as stringent criteria that define a set of dependable transformations that can be employed to construct novel pharmacophores in hopes of facilitating drug

discovery. 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 facets of drug discovery and will likely be utilized in many applications in the 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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