Review Article

CLICK CHEMISTRY: A NEW APPROACH FOR DRUG DISCOVERY

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ABSTRACT

Click chemistry is a chemical philosophy introduced by K. Barry Sharpless of The Scripps Research Institute, in 2001 and describes chemistry tailored to generate substances quickly and reliably by joining small units together. This is inspired by the fact that nature also generates substances by joining small modular units. Click chemistry is not a specific reaction; it is a concept that mimics nature. Many of the Click chemistry criteria are subjective; and even if measurable and objective criteria could be agreed upon, it's unlikely that any reaction will be perfect for every situation and application. Click chemistry also has been used for selectively labeling biomolecules within biological system. A click reaction that is to be performed in a living system needs to meet an even more rigorous set of criteria; it must be bioorthogonal, meaning the reagents used may not interact with the biological system in any way, nor may they be toxic. The reaction must also be carried out at neutral pH and at or around body temperature. Most click reactions have a high energy content which make the reactions irreversible and involve carbon-hetero atom bonding processes. The aim of present article is to provide in depth knowledge about Click chemistry, its wide spread applications as well as various concept of click chemistry.

Keywords: Click Chemistry, stereospecific, 1, 3-dipolar cycloaddition reaction, azides.

INTRODUCTION

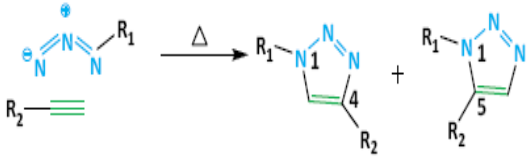
The "click" concept, proposed by Sharpless in 2001, is undeniably one of the most noticeable synthetic trends in the research area of chemistry and material science of this new century[1]. The catchy term "click" refers to energetically favored, specific and versatile chemical transformations, which lead to a single reaction product. Click chemistry is therefore not a new type of chemistry, but rather a term used for a class of reactions that can create complex molecules in a very efficient manner. This exciting concept seems to perfectly answer the needs of modern scientists working in research areas as diverse as molecular biology, drug design, biotechnology, macromolecular chemistry or materials science[2]. It is important to make focus that since last few years, complicated reactions requiring either complex apparatus or harsh experimental conditions, have been less frequently studied than in the last century and gradually replaced by simpler tools. Click chemistry explains chemistry tailored to generate substances quickly and reliably by joining small units together as nature does. It is defined as a fast, modular, process-driven approach to irreversible connections of the substrates involved in click reactions. Click chemistry uses only the most reliable reactions to build complex molecules from olefins, electrophiles, and heteroatom linkers. The criteria for being classified as click chemistry contain a yield close to 100% as well as a preferential and rapidly occurring irreversible, highly selective and orthogonal reaction. The reaction conditions should be mild, insensitive to oxygen and water and use either no solvents or benign solvents like water. Click reactions in organic solvents have also a high significance in polymer and material science. The bonds generated in the product should be chemically stable under a range of physiological conditions. Additionally, for click reactions involved in polymerizations, the counter functionalities of the reagents should be unreactive under free radical polymerization conditions or be easily protected during the polymerization stage and functionalized afterwards.

BASIC PRINCIPLES OF CLICK CHEMISTRY

1. Huisgen 1, 3–dipolar cycloaddition of azides and alkynes

Out of all identified click reactions, the heteroatom cycloaddition class of reactions is the most reliable and versatile. Within this class, the Huisgen 1, 3-dipolar cycloaddition of azides and alkynes is known for being closest to an "ideal" click reaction. Cu (I)-catalyzed Huisgen 1, 3-dipolar cycloaddition of azides and alkynes yields 1, 2, 3-triazole products.

Traditionally, uncatalyzed cycloadditions of azides and alkynes require long reaction times, high temperatures and result in the formation of two products, 1, 4- and 1, 5-regioisomers.



The synthesis of 1, 2, 3-triazoles by 1, 3-dipolar cycloaddition was discovered by Michael at the end of the 19th century and significantly advanced by Huisgen in the 1960s. The groups of Sharpless and Meldal discovered the Cu (I)-catalyzed variation of this reaction, which allows very fast and efficient formation of exclusively 1, 4-triazoles at mild reaction conditions. This breakthrough led to a renaissance of Huisgen cycloadditions in synthetic chemistry. Hence, research in this direction has led to its widespread application in all fields of polymer chemistry and biochemistry over the last few years. Moreover, since azide and alkyne functions are widely absent in the biological world, azide–alkyne chemistry constitutes a very interesting chemo selective platform for the functionalization or ligation of biomaterials, such as stationary phases for bioseparation, site-specific modified proteins or viruses, drug- or gene-delivery carriers, protein or oligonucleotide microarrays, and functionalized cell surfaces.[21-25] The high kinetic stability of azide and alkyne groups that was disadvantageous in uncatalyzed cycloadditions is an advantage in the Cu(I)-catalyzed process, meaning the two functional groups are inert under a wide range of conditions and do not interact with water, oxygen, biological molecules or other functionalities present in the reaction. Both the azide and alkyne groups can also be added easily to different molecules, requiring minimal initial functionalization stages or protective chemistry[3] (Fig.1).

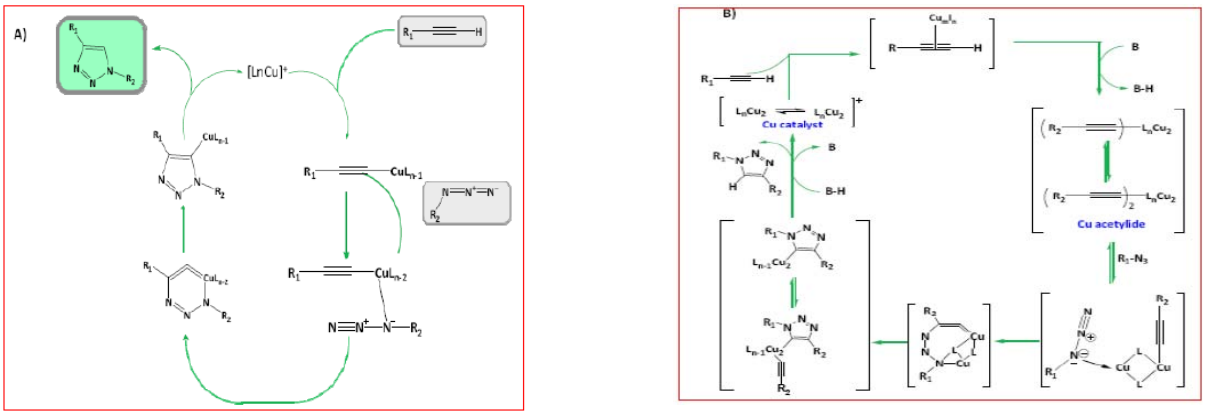


Figure 1: Proposed catalytic cycle of stepwise Cu (I)-catalyzed Azide- Alkyne Cycloaddition; A) First order with respect to copper; and B) second order with respect to copper.

In both mechanisms the first copper group initiates the formation of copper acetylide. In the first order mechanism the acetylide formed is thought to be capable of immediately forming an acetylide-azide complex, while in the second order mechanism a second copper component in the acetylide group is required to activate the azide molecule and form a copper acetylide-azide complex. The copper acetylide-azide complex then undergoes cyclization and formation of a metallocycle due to the nucleophilic attack of an acetylide carbon by the azide group. Finally, ring contraction occurs and the catalyst dissociates and is regenerated via protonation of the triazole-copper molecule.

2. Metal-Free Click Reaction

In some cases it is found that click reactions, which involve transition metal as catalysts, are hindered. Some examples of in vitro copper-induced degradation of viruses or oligonucleotide strands have been reported[4]. Additionally, the use of copper (I)-catalyzed azide–alkyne cycloaddition (CuAAC) for *in vivo* applications is limited because copper ions are potentially toxic for living organisms. The development of this class of reaction is relevant. In recent years, metal-free [3+2] cycloaddition reactions, Diels–Alder reactions, and thiol-alkene radical addition reactions have come in the picture as click reactions because of their simple synthetic procedures and high yields[5] (Fig. 2).

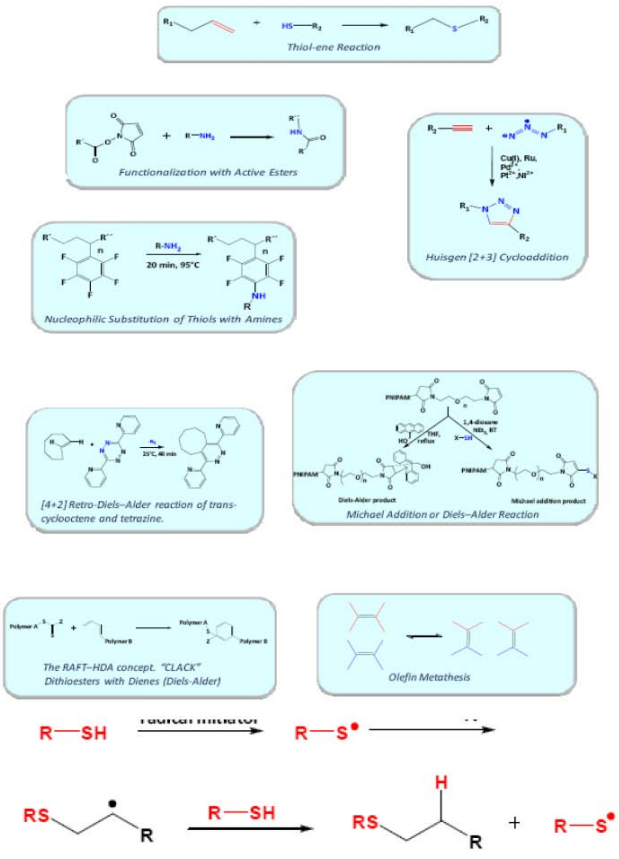


Figure 2: The thiol-ene radical reaction[29, 30]

Schlaad and coworkers demonstrated a post-polymerization modification of a well-defined poly [2-(3-butenyl)-2-oxazoline]. The reactions were performed by exposure to UV light, as well as under irradiation with direct sunlight. Earlier they demonstrated the free-radical addition of ω-functional mercaptans onto 1, 2-polybutadienes[6]. The great potential of thiol-ene chemistry was exploited by Hawker and coworkers in the synthesis of poly (thio-ether) dendrimers.

2. Polymer Architectures

Due to the various architectures and functionalities of nanostructures, soft materials like polymers have been playing extremely important roles in the templated synthesis, surface protection and surface fictionalizations. Some excellent works on this was done by Schlaad et al[7].

(A) Cyclic Polymers

Ring-shaped polymers have gained increasing attention in polymer science not only because they are found in the natural products such as circular DNA molecules, cyclic peptides, and cyclic polysaccharides[8], but they can also be used in polymer recycling based on chain-ring equilibria. Cyclization reactions represent an inherent, unavoidable component of step-growth polymerization and may be dominant factor for limitation of chain growth. The concurrent formation of linear and cyclic molecules is a general characteristic of polymer systems containing reactive functions. Cyclic polymers formed in linear-ring step and chain polymerizations are often undesirable side-products. The absence of chain ends and consequently the topological restriction imposed by the cyclic architecture result in a variety of molecular characteristics and physical properties that significantly distinguish them from their linear counterparts.

The most appropriate methods for the synthesis of cyclic polymers of controlled size and narrow poly dispersity are based on the end-to-end chain coupling of α, ω-difunctional linear chains in highly dilute reaction conditions. The use of living polymerization techniques (e.g. anionic or RAFT polymerization) for the preparation of the linear precursors allows control over the molar mass and a narrow molar mass distribution. Different approaches exist for the end-to-end closure: Cassasa[9] proposed the direct coupling of α, ω- polymer dianions, where the polymer has two identical end-functionalized groups and the ring-closure requires the use of a bifunctional coupling agent. The unimolecular ring closure corresponds to the reaction between the α-and ω-polymer ends. The high dilution, required to favour the cyclization versus chain extension, is unfavorable to the quantitative formation of the hetero-difunctional polymer intermediate.

To overcome this difficulty another approach involves the direct synthesis of and α, ω- hetero difunctional linear precursor. The cyclization is then performed in a separate step under high dilution. The concept of unimolecular end-to-end closure was used in the click- approach for the synthesis of cyclic polystyrene. Pre-organization of macromolecular precursors bearing specific ionic end-functions via electrostatic non-covalent interactions is an interesting and original strategy that was recently applied to the preparation of various types of chain architectures. The concept of cyclic polymers was extended to the synthesis of ring-shaped polymer brushes. Macrocyclic polymer brushes can be considered as a special case of cylindrical polymer brushes, in which the two ends of the brushes meet each other by a coupling reaction. Although macrocyclic polymers were first obtained 40 years ago[10], the preparation of large macrocyclic (co)polymer brushes is limited by the difficulty to get pure, difunctional high molar mass precursors, the drastic decrease of the end-to-end ring closing efficiency when increasing the distance between the chain ends and the separation from linear contaminants of comparable molar mass.

3. Reversible Addition Fragmentation Chain Transfer Polymerization (RAFT)

Currently, there are three main types of "living"/controlled radical polymerization (CRP): (1) atom transfer radical polymerization (ATRP), (2) stable free radical polymerization (SFRP) including nitroxide mediated polymerization (NMP), and (3) reversible addition fragmentation chain transfer (RAFT) polymerization.

(1) ATRP involves a reversible chain termination using the exchange of an organic halide via a reversible redox reaction in the presence of a transition metal catalyst.

(2) SFRP also uses reversible chain termination by means of exchange of a stable radical group. In the case of NMP, the stable radical is a nitroxide group. NMP benefits from the absence of copper and sulfur compounds, yet it is limited by low reaction rates, limited monomer compatibility and requires high reaction temperatures. With the discovery of CRP techniques, a great variety of complex macromolecular architectures became available under non-demanding reaction conditions. Polymers with well-defined structure and different functionality can be created[11, 12]. RAFT polymerization has proven to be a versatile tool, as RAFT reactions are less oxygen sensitive, proceed at lower temperatures than ATRP and NMP and are compatible with a wider range of monomers, including acrylate, methacrylate and styrenic monomers.

(3) RAFT polymerization a thiocarbonylthio group containing compound, with a general structure of Z-C (=S) S-R, is added to an otherwise conventional free radical polymerization in order to obtain a controlled radical polymerization. These controlling agents are reversible chain transfer agents (CTAs) or RAFT agents. First, a radical initiator decomposes, creating radicals that initiate the polymerization. A propagating radical then adds to the thiocarbonyl group of the CTA molecule and forms an intermediate radical (also called "dormant" species). Eventually the intermediate radical undergoes a β-scission reaction, either re-forming the original radical or creating a new propagating radical from the leaving group

(R group). Additionally, the CTA molecule is recovered in this reaction step. After the initial phase equilibrium is established between the propagating radicals and the intermediate radical species. Only when a certain chain length is exceeded, the rate coefficients become independent of the chain length. Therefore, one has to distinguish between a so-called "pre-equilibrium", where low molecular weight CTAs are still present, and the "main-equilibrium" with polymeric RAFT agents. With the polymerization being of a radical nature, side reactions like transfer, recombination and disproportion cannot fully be suppressed. Still, RAFT polymerizations show a linear growth of the molecular weight with respect to conversion and yield polymers with narrow molecular weight distributions when the equilibrium reactions are fast compared to propagation[2].

4. Click Chemistry in Combination with RAFT Polymerizations

Living free radical polymerization and click pericyclic reactions are independently known for having many similar advantages, including reaction under mild conditions and tolerance of a range of functionalities. Recently, research groups have begun combining these click reactions with different polymerization techniques to synthesize new polymeric materials previously inaccessible via traditional polymerization methods. For example block copolymers, which are currently difficult to synthesize because of different polymerization mechanisms, have been successfully prepared via RAFT polymerization of homopolymer chains with the requisite azide and alkyne end functionalities and subsequent post-polymerization click additions[13]. Combining RAFT polymerization and click pericyclic reactions is a relatively novel concept, which provides many useful opportunities and benefits. The ability to synthesize well- defined amphiphilic block copolymers and other complex polymer architectures from highly reactive monomers, will allow for the potential development of many new materials with wide industrial and biomedical applications[14, 15].

Nature of reactions involved in click chemistry[16]

A concerted research effort in laboratories and industries has yielded a set of extremely reliable processes for the synthesis of building blocks and compound libraries:
• Cycloaddition reactions, especially from the 1, 3-dipolar family but also hetero-Diels-Alder reactions.

• Nucleophilic ring-opening reactions, especially of strained heterocyclic electrophiles, such as epoxides, aziridines, cyclic sulfates, cyclic sulfamidates, aziridinium ions and episulfonium ions.

• Carbonyl chemistry of the non-aldol type (e.g. the formation of oxime ethers, hydrazones and aromatic heterocycles).
• Addition to carbon–carbon multiple bonds; particularly oxidation reactions, such as epoxidation, dihydroxylation, aziridination, and nitrosyl and sulfenyl halide additions, but also certain Michael addition reactions.

It observed that the very best click reaction classes proceed most rapidly and in highest yield, not in water or water–co-solvent mixtures, but floating on water.

Drug discovery with respect to click chemistry

In biomedical research, many efforts have been done by researchers in last few years. This is expended from new lead formation and optimization, to tagging of biological systems like proteins, whole organism, PCR assays, PCR primers and labeling of large fragments, DNA and RNA labeling, FISH probes and FISH experiments, PEGylation, Flow cytometry, cell feeding, cell tracking and cell-based assays, Nanoparticles, Bioconjugation, Micro arrays.

The strength of this approach is highlighted here by reviewing some applications.

Synthesis of Lead Discovery Libraries

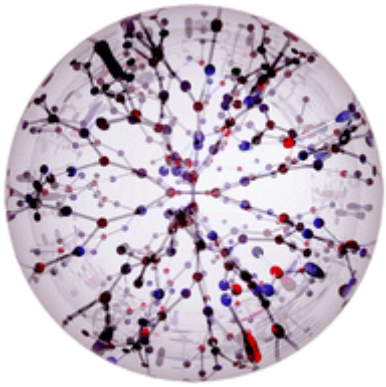
After working long time, click chemistry laboratories have used solution-phase chemistry to produce a variety of screening libraries, containing a total of 200 000 individual com-pounds, each more than 85% pure. According to the click chemistry concept, each library compound was produced in only one or two synthetic steps, from key building block reagents, using automated liquid handling platforms.

Although in click chemistry synthesis for the compounds short synthetic sequences used yet much compound diversity and novelty was achieved by starting with non-commercial building block reagents, prepared in-house on multi-gram or, even, kilogram scales. Examples include 'spring-loaded' epoxides and aziridines for the formation of 1,2-difunc-tionalized compounds by nucleophilic opening, imidoesters for the generation of five-membered aromatic heterocycles, azides for the synthesis of 1,2,3-triazole-derived libraries via 1,3-dipolar cycloaddition with β - ketoesters , and 3-aminoazetidines for the preparation of non-aromatic heterocyclic libraries. Also, targeted libraries were made, one of which led to the discovery of potent Peroxisome Proliferator-Activated Receptor γ (PPAR-γ) agonists. The copper-(I)-catalyzed formation of 1, 2, 3-triazoles has recently been used to prepare functionalized resins for the solid phase synthesis of a library of dopaminergic arylcarbamides. In another resin-based approach, Yli-Kauhaluoma et al. prepared 1,2,3-triazoles via thermal 1,3-dipolar cycloaddition of polymer-bound azides to alkynes, followed by cleavage from the solid support with TFA[17].

How leads are synthesized by click chemistry

Dendritic polymers are highly branched polymer structures, with complex, secondary architectures and well-defined spatial location of functional groups. Due to their unique physical and chemical features, applications in areas such as targeted drug-delivery, macromolecular carriers, catalysis, sensors, light harvesting, surface engineering and biomimetic materials have been proposed. However, only a few dendritic materials have

been exploited commercially due to time consuming syntheses and the generation of significant waste/presence of unreacted starting materials. The traditional synthesis of dendritic materials as well as recent advances in synthetic strategies, for example the use of Click chemistry, as a tool to efficiently obtain complex, functional dendritic structures.



The inhibition of NAD synthesis or salvage pathways has been proposed as a novel target for antitumoral drugs. Two molecules with this mechanism of action are at present undergoing clinical trials. In searching for similar novel molecules, we exploited copper-catalyzed [3 + 2] cycloaddition between azides and alkynes (click chemistry) to synthesize 185 novel analogues. The most promising compound displays an IC_{50} for cytotoxicity in vitro of 3.8 ± 0.3 nM and an IC_{50} for NAD depletion of 3.0 ± 0.4 nM. Herein, we strengthen previous data suggesting that this class of compounds induces autophagic cell death. In addition to characterizing this compound and providing a rationale via molecular docking, we reinforce the excellent potential of click chemistry for rapidly generating structure activity relationships and for drug screening.

In situ click chemistry[18]

Using various combinatorial chemistry and structure based approach; a large number of molecules can be synthesized from a small set of starting materials. Then all the synthesized molecules need to be tested for pharmacological activity and toxicity to find the lead, its optimization and finally to offer the potent candidate. Thus these are very time consuming processes. The idea of *in vitro* combinatorial chemistry represents a variety of technologies where the selection and synthesis of novel molecules is performed in one single process. In situ click chemistry, pioneered by Sharpless, is a class of *in vitro* combinatorial chemistry. Here biological target is itself used to select a compound from a large pool of potential combinatorial libraries means biological structures are used as a reaction vessel. Sharpless and coworkers used this approach to find new inhibitors of acetylcholinesterase (AChE), a pivotal central nervous system neurotransmitter. The Huisgen cycloaddition reaction between azides and alkynes was selected for this study. Although azides and alkynes are spring loaded reactants but still they are very slow reacting under physiological conditions unless and until they are activated; either by any catalyst or by using a biological target which acts as template (reaction vessel) to bring the reactants close enough to react.

Click chemistry related with bioconjugation

(I) In Vivo Tumor Cell Targeting with "Click" Nanoparticles:

The *in vivo* fate of nonmaterials strongly determines their biomedical efficacy. Accordingly, much effort has been invested into the development of library screening methods to select targeting ligands for a diversity of sites *in vivo*.

Still, broad application of chemical and biological screens to the *in vivo* targeting of nanomaterials requires ligand attachment chemistries that are generalizable, efficient, covalent, orthogonal to diverse biochemical libraries, applicable under aqueous conditions, and stable *in vivo* environments. To date, the copper (I)-catalyzed Huisgen 1, 3-dipolar cycloaddition or "click" reaction has shown considerable promise as a method for developing targeted nanomaterials *in Vitro*. Here, we investigate the utility of "click" chemistry for the *in vivo* targeting of inorganic nanoparticles to tumors. We find that "click" chemistry allows cyclic LyP-1 targeting peptides to be specifically linked to azido-nanoparticles and to direct their binding to p32-expressing tumor cells *in Vitro*. Moreover, "click" nanoparticles are able to stably circulate for hours *in vivo* following intravenous administration (>5 h circulation time), extravasate into tumors, and penetrate the tumor interstitium to specifically bind p32-expressing cells in tumors. In the future, *in vivo* use of "click" nanomaterials should expedite the progression from ligand discovery to *in vivo* evaluation and diversify approaches toward multifunctional nanoparticle development (Fig.3).

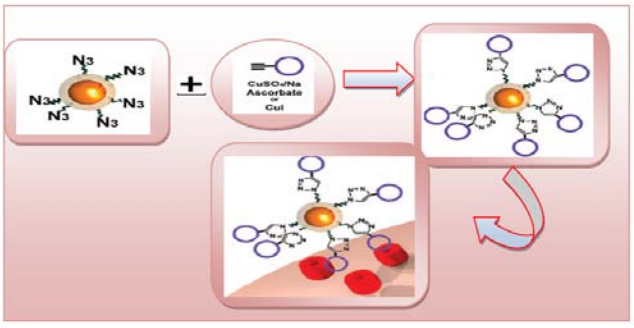


Figure 3: Design of a "Click" Nanoparticle That Targets Tumor Cells *in vitro* and *in vivo*

FUTURE OF CLICK CHEMISTRY, APPLICATIONS AND CONCLUSIONS

Polyurethanes are versatile plastics which occupy an important position in the world market of high performance synthetic polymers, with a global consumption around 8 million tons in 2000. They cover almost all the daily life aspects, such as building thermo insulation, wood substitutes or coatings. Polyurethanes

are traditionally prepared by reacting an oligomeric polyol and a disocyanate.

Whereas the isocyanate component is always derived from petrochemical feed stocks, the polyol component could come from bio-based resources. Vegetable oil-based polyols are synthesized from varied oils which, except castor oil, have to be chemically modified to meet the polyurethane production requirements. Transesterification and epoxydation are already industrially used for the production of polyols from oleo chemicals. The thiol-ene reaction represents another interesting toolbox for the functionalization of unsaturated vegetable oils. Indeed, this reaction of "click chemistry" allows photochemical or thermal initiation, undemanding synthesis conditions thanks to the insensitivity to oxygen inhibition, and leads to high yields with basic purification procedures. Some of click chemistry's proponents dream that the philosophy could one day help people in the developing world to make pharmaceuticals on the spot. Click chemistry is a chemical philosophy introduced by K. Barry Sharpless of The Scripps Research Institute, in 2001[19, 20] and describes chemistry tailored to generate substances quickly and reliably by joining small units together. This is inspired by the fact that nature also generates substances by joining small modular units. Click chemistry has widespread applications, some of them are:

- Preparative organic synthesis of 1, 4-substituted triazoles
- modification of peptide function with triazoles
- modification of natural products and pharmaceuticals
- drug discovery
- macrocyclizations using Cu(I) catalyzed triazole couplings
- modification of DNA and nucleotides by triazole ligation
- supramolecular chemistry: calixarenes, rotaxanes, and catenanes
- dendrimer design
- carbohydrate clusters and carbohydrate conjugation by Cu(1) catalyzed triazole ligation reactions
- polymers
- material science
- nanotechnology [21], and
- Bioconjugation, for example, azidocoumarin.

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