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Click chemistry: a fascinating method of connecting organic groups

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Abstract: Click chemistry, a modular synthetic strategy for synthesizing the assembly of novel molecular entities, has made a tremendous impact in the field of science since its debut. This powerful strategy relies mainly upon the construction of carbon–heteroatom bonds using spring-loaded reactants. Its growing number of applications are found in nearly all areas of modern chemistry ranging from drug discovery to materials science. This manuscript includes important aspects of the copper-catalyzed Huisgen cycloaddition reaction, which is considered a gold standard of click chemistry due to its biocompatibility and reliability, along with its applications in bioconjugation, drug delivery and polymer chemistry. A bird′s eye view of recent progress in developing the copper-free click chemistry protocols such as catalyst-free strain-promoted alkyne–azide cycloaddition (SPAAC) click chemistry has also been provided.

Keywords: Click chemistry; Huisgen 1,3-dipolar cycloaddition; drug delivery; bioconjugation.

1. Introduction

The term "click chemistry" was first introduced by Dr. Barry Sharpless group in 1999 at the 217th American Chemical Society annual meeting,[1] later in 2001, Kolb, Finn and Sharpless, in their land mark review, described it as concept for conducting organic reactions, which was based upon the premise that organic synthesis should focus attention on highly selective, simple orthogonal reactions that give heteroatom-linked molecular systems with high efficiency under mild reaction conditions.[2] Several efficient reactions, which are capable of producing a wide catalogue of functional synthetic molecules and organic materials have been grouped accordingly under the term click reactions. The rule of thumb for this approach was that "…all searches must be restricted to molecules that are easy to make".

Click chemistry concerns with a generation of the substances by joining small, selective and modular building blocks together with heteroatom links (C-X-C). These building blocks contain a high built-in energy content that drives a spontaneous and irreversible linkage reaction with complementary sites in other blocks and work reliably in both small- and large-scale applications. Click Chemistry dictates a "guiding principle"- A principle born to meet the demands of modern day chemistry.

In order to reach up to a status of click reaction, a process must fulfil certain conditions**.** Reaction must be

a) modular
b) with wider scope
c) high yielding

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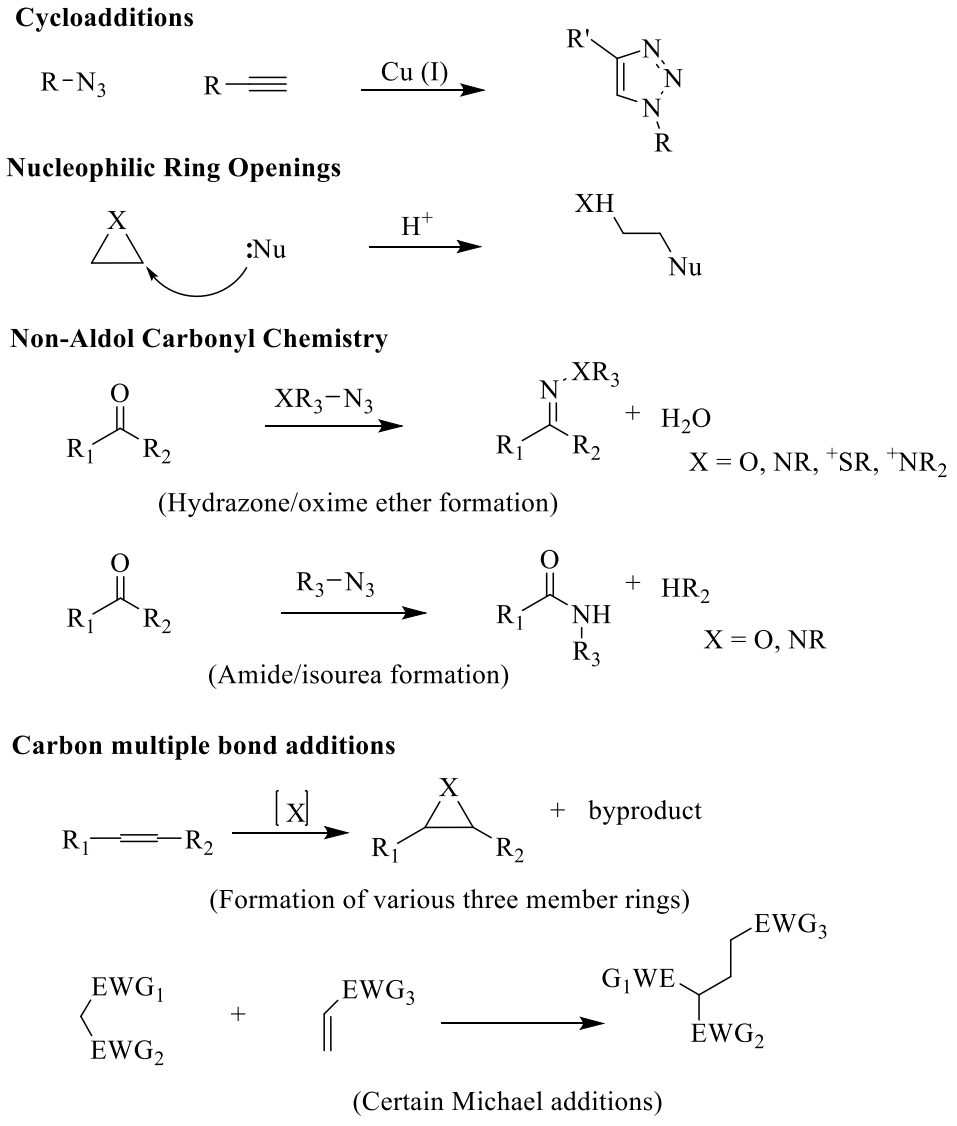
d) generating only harmless byproducts which could be removed easily by employing nonchromatographic methodologies
d) stereospecific (but not necessarily enantioselective)
e) under milder reaction conditions (ideally insensitive to air and water)
f) conducted with readily available starting materials and reagents
g) under either no or some benign solvent
h) generating stable product under physiological conditions
e) orthogonality with other common organic synthesis reactions

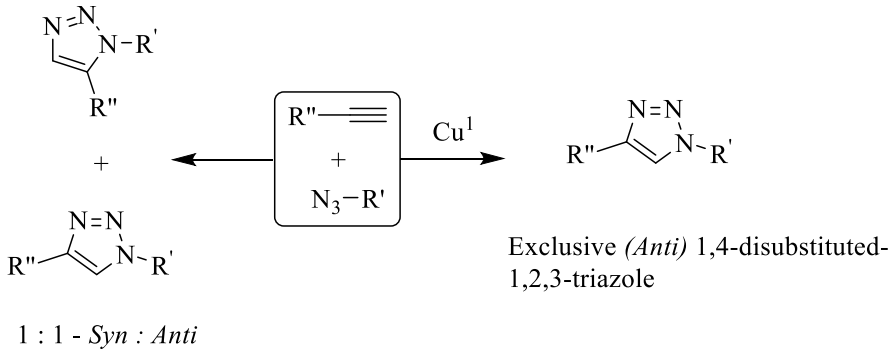
Carbon-heteroatom bond forming reactions comprise the most common examples (Scheme 1), including the following classes of chemical transformations:

a) nucleophilic ring opening reactions: epoxides, aziridines, aziridinium ions etc.[2]
b) non-aldol carbonyl chemistry: formation of ureas, oximes and hydrazones etc.
c) additions to carbon–carbon multiple bonds: especially oxidative reactions such as aziridation,[3] hydroxylation,[4] epoxidation,[5] nitrosyl and sulfenyl halide additions,[6] and Michael additions of Nu–H reactants.
d) Cycloaddition reactions: especially 1,3-dipolar cycloaddition reactions as well as hetero- Diels-Alder reactions.[7-9]



Scheme 1. Summary of the most popular click reactions

Amongst all the reactions which achieve "click status", the Huisgen 1,3-dipolar cycloaddition of alkynes and azides to yield 1,2,3-triazoles is undoubtedly the premier example of a click reaction.[10-14] The most primitive azide-alkyne cycloaddition was explored by Huisgen and coworkers during 1950-70s. Although the reaction was highly exothermic with low reaction rate and yield, it had a great impact on further research. The requirement of high temperature and pressures for this reaction were the biggest constraint for its application in living systems. It was not that popular until the use of copper as a catalyst, which solved this issue. The discovery of the Cu(I) catalyzed azide–alkyne cycloaddition in 2002 (Scheme 2) transformed CC from a working concept to an accepted reality. This reaction is usually conducted under mild conditions using diverse available substrates, highly yielding 1,4-regioisomers of 1,2,3- triazoles as sole products instead of regiorandom triazole adducts which complies fully with the definition of the conceptual click chemistry. This "near perfect" reaction has become synonymous with CC, and is often referred to as "The Click Reaction". This water-tolerant reaction is thermodynamically favorable by approximately 30-35 kcalmol^{-1}.



Scheme 2. The Cu(I) catalyzed Huisgen 'click reaction' results in exclusive formation of 1,4-triazole, whilst the thermally induced Huisgen cycloaddition usually results in an approximately 1 : 1 mixture of 1,4- and 1,5-triazole stereoisomers

Over the past five years, it has been observed that the very best click reaction classes proceed most rapidly and in highest yield, not in water or water–co-solvent mixtures[15] but floating on water.[1] For instance, 1,3-dipolar cycloadditions between diethyl acetylenedicarboxylate and diazido-cyclohexanediols proceed best in pure water.[1] When water is omitted so that the above reactants are mixed neat, the reactions are much slower and less selective, and, on a larger scale, become dangerous, as click chemistry reactions are highly exothermic. The presence of water in these reactions is beneficial, not just for reactivity but also because water is the best heat-sink for handling the enormous heat output when click reactions are performed on larger scales. Another advantage of employing water as a reaction solvent is that its presence prevents interference from simple protic functional groups, like amides and alcohols, which are enormously present in biologically active organic molecules.[16]

The advent of click chemistry has had a profound influence on almost every branch of chemical science and it has become a ubiquitous chemical tool with applications in nearly all areas of modern chemistry from chemical to material science. An overview of the recent advancements of click chemistry in different fields have been given below.

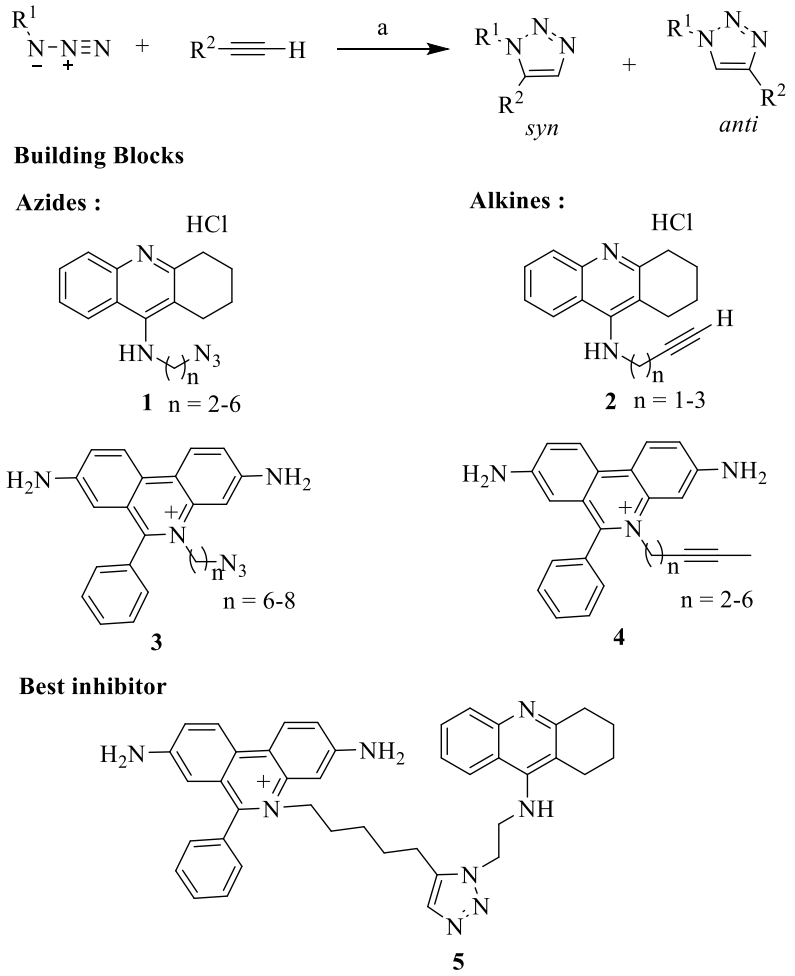
2. Bioconjugation

Bioconjugation encompasses a broad arena of science at the interface between chemistry and molecular biology, involving the formation of covalent links between synthetic labels and biomolecular frameworks. It involves biocompatible reactions joining substrates to biomolecules in a selective, fast and high-yielding manner.[17] Covalently linking two molecular entities is a challenge for molecular biologists and chemists while studying the biological systems, for instance, attaching a small molecular probe (fluorescent dye, radical probe, affinity tag, etc.) onto a biopolymer or linking a complex carbohydrate with a peptide. Because of the diverse functional reactivity and structural complexity, one has to find out selective ligation reactions, allowing the coupling of two mutually and uniquely reactive functional

groups, usually in an aqueous environment under physiological conditions. These functional groups should be selective for each other as well as capable to tolerate other functionalities, hence circumventing the need to use protecting groups and ideally allowing applications of the molecules in the complex environment of a living cell.

Reactions employing azide moiety as a functional group are mostly employed in bioconjugation and they are known to offer following advantages;[18-21] (i) the azide moiety is absent in almost all natural existing compounds and (ii)despite a high intrinsic reactivity, azides allow selective ligation with a very limited set of reaction partners.

The Huisgen 1,3-dipolar cycloaddition of azides and acetylenes to give 1,2,3-triazoles was identified as an interesting candidate after Sharpless et al. Realization of achieving higher functional group compatibility by employing electrocyclic reactions instead of encountering limitations imposed by electrophile-nucleophile reactions. The groups of Sharpless and Finn employed the azide-alkyne coupling in the parallel synthesis of a highly active inhibitor of the enzyme acetylcholinesterase (AchE) **5**.[21] Sharpless and Finn chose a strategy that involved a biological target that should literally guide the inhibitor synthesis by serving as a template for the assembly of building blocks instead of using a conventional approach in which a diverse set of chemical compounds is first synthesized and subsequently screened against the biological target in question.

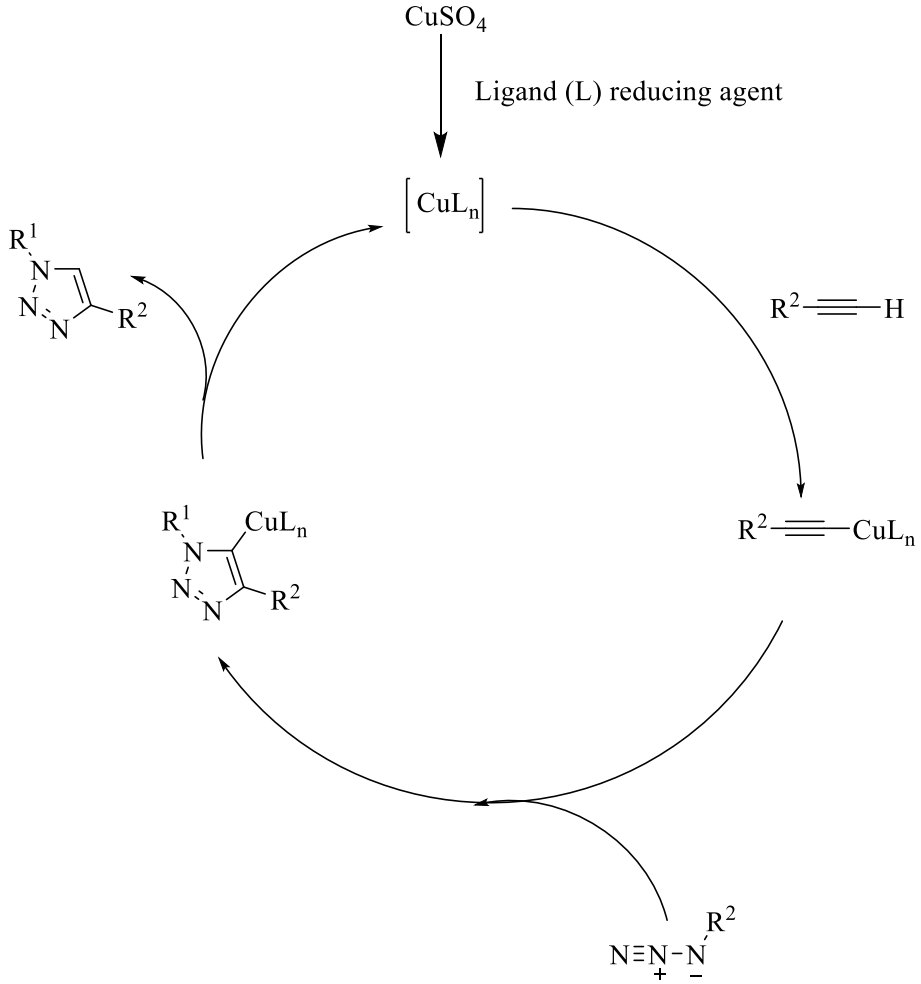


Scheme 3. Template-guided synthesis of the highly active AChE inhibitor 1 by azide-alkyne coupling. a) AchE (0.03 equiv), pH 7.4 buffer, RT, 49 parallel reactions

Complementary pairs of a selection of site-specific inhibitors based on tacrine and phenanthridinium motifs containing alkyl acetylenes **1** and **3** and alkyl azides **2** and **4** of different chain

lengths were incubated in the presence of electrophorus AChE at room temperature (Scheme 3). In the absence of enzymesl rate of reaction under these conditions was negligible, hence, detectable amounts of triazole products formed only when the azide and alkyne were brought together by the enzyme, enforcing propinquity and proper alignment of the reactants. Amongst 49 reactions, only one combination yielded a detectable amount of coupled product. This substance **1** turned out to be the most potent noncovalent AChE inhibitor known, with Kd values ranging from 77 fM (AChE from Torpedo californica) to 410 fM. The fact that azide and alkyne tags may easily be introduced into small molecules and proteins through chemical synthesis and common *in vitro* protein modification procedures, have revolutionized bioconjugate chemistry, particularly when Cu(I)-chelating ligands are employed in the coupling reaction.

However, the low intrinsic reaction rate of the azide-alkyne coupling-key for the experiment described above, was supposed to be dramatically increased in order to make this reaction attractive as a general bioconjugation strategy.[22] A catalyst was needed to be identified for accelerating the reaction as increasing the temperature was not an option due to the sensitivity of the biological materials towards heat. Catalytic amounts of Cu(I) salts was found to accelerate the rate of reaction as well as improve the regioselectivity to deliver exclusively the 1,4-disubstituted product (Scheme 4). Cu(I)-catalyzed azide-alkyne coupling proved to be compatible with the numerous functional groups[23-25] such as thioethers, carboxylic acids, esters, amides, ethers, thiols, alcohols, phenols, amines, guanidines and carbamates.



Scheme 4. Catalytic cycle of the Cu(I)-catalyzed azide-alkyne coupling

Bioconjugation based on crosslinking primary amines to carboxylic acid groups has found broad applications in protein modification, nanomaterial functionalization and drug development. However, proteins tend to give nonselective bioconjugation when primary amine-based crosslinking is employed.

Thus, selective bioconjugation is required for controlling the protein orientation and activity after conjugation.

Yuan Liu and co-workers reported an efficient and cysteine-selective thiol-ene click reaction-based bioconjugation protocol employing colloidal nanoparticles.[26] The resulting thiol-ene based enzyme nanoconjugates and aptamer displayed excellent enzymatic activity and target binding ability, respectively. Colloidal nanoparticle-based bioconjugates containing manganese oxide, iron oxide and UCNP were designed and crosslinkers were tested by HS-aptamer labeling, HS-PEG Pegylation and enzyme immobilization. Thiol–ene crosslinker was found to be stable and robust by Gel electrophoresis. Furthermore, the binding ability of aptamer to its target cells or the catalytic activity of HRP enzyme was not affected by the stable and robust thiol–ene linkage between dopamine acrylamide functionalized UCNPs and aptamer or HRP. Bioconjugates based on reactions with nanomaterials have enormous potential in such fields as material sciences and biology. In particular, the stability and superior selectivity of the thiol–ene make it a potential candidate to be used in multifunctional nanomaterial bioconjugates, making this a powerful tool with broad spectrum of applications in bioimaging, bioanalysis, biosensing, drug delivery and theranostics.

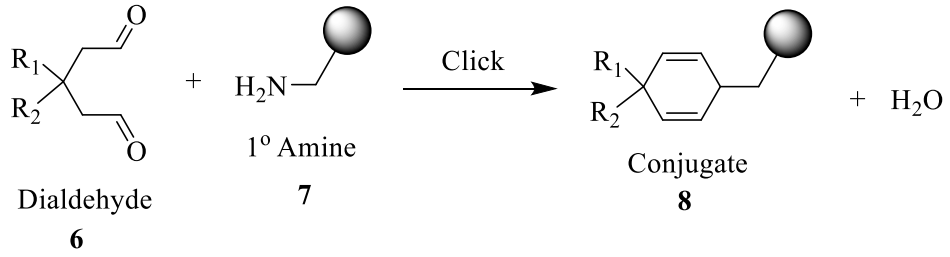
Bioorthogonal reactions have revolutionized the way low-molecular-weight compounds are linked to biomolecules. Low-molecular-weight compounds are not able to evoke a secondary, adaptive immune response on their own. In order to trigger the cascade of events leading to the proliferation of plasma and memory B cells and to the production of high-affinity IgG antibodies, for these so-called haptens, the compound have to be linked to an entity carrying epitopes for helper T-cells.[27-34] Proteins are the most commonly used carriers such as globulins, albumins and hemocyanins, viruslike particles, or toxoids. Formation of stable amide bonds between carboxylate groups of the hapten and amine groups of the protein has been the most extended protocol for the bioconjugation of small compounds. *N*-Hydroxysuccinimidyl esters synthesized *via* chemistry-mediated carbodiimides were able to efficiently react, with solvent-accessible lysine residues, which were ubiquitous in most carrier proteins, under mild reaction conditions.[35,36]

Daniel López-Puertollano and co-workers reported for the first time that CuAAC chemistry is a convenient approach for the preparation of protein-hapten bioconjugates intended for the generation of suitable antibodies for immunodiagnostics of small organic chemicals, using mycotoxin ochratoxin A as a model compound.[37] These results revealed that the participation of the triazole moiety in antibody binding is not as critical as previously thought, hence, its presence in the spacer arm does not preclude the production of antibodies with apparent affinity constants for the analyte in the subnanomolar range. These finding opened the door for achieving highly valuable biotechnological immunoreagents for other relevant compounds, like biotoxins, antibiotics and drugs, through unexplored chemical strategies involving innovative positions for linker attachment and hapten functionalization. Further studies should certainly contribute to clarifying the generalizability of the strategy reported herein.

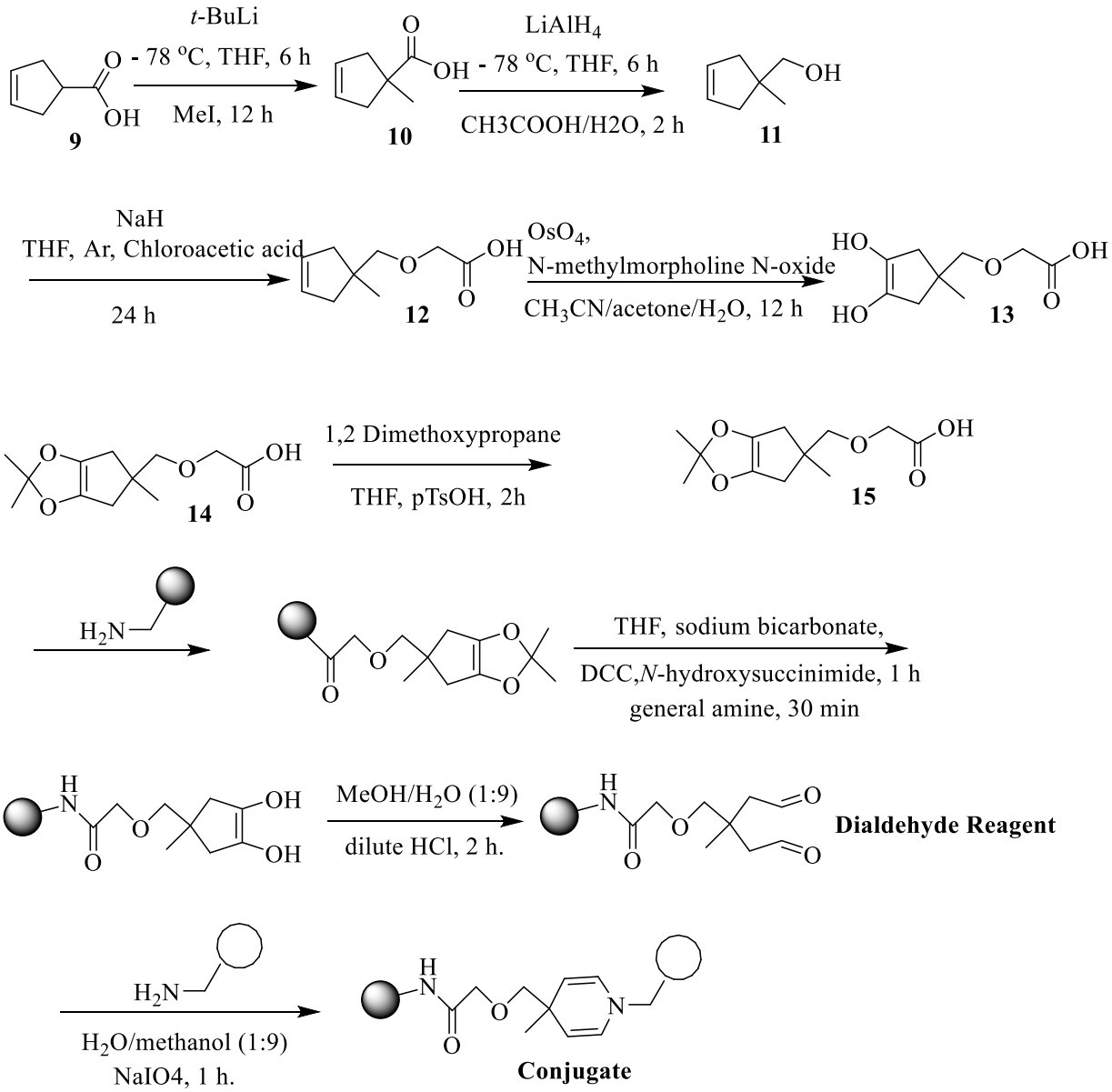
The primary amine is a key functional group and one of the most important nucleophiles and bases used in all of synthetic chemistry.[38-41] The development of methods for conjugating a range of molecules to primary amine functional groups has revolutionized the fields of chemistry, biology, and material science. Tremendous efforts have been dedicated for the synthesis of molecules containing primary amines and methodologies to devise chemical reactions to react with primary amines. In particular, primary amines are an abundant functional group found in biological systems as free amino acids, in metabolites and signaling molecules and are also present in many classes of natural products. The primary amine is the most convenient functional group handle in molecules for ligation to other molecules for a broad spectrum of applications due to its ubiquity.[42-46]

Sina Elahipanah and co-workers developed a novel traceless, high-yielding, robust click-chemistry protocol based on the fast and efficient trapping of amine groups through a functionalized dialdehyde group.[47] The general reaction has been shown in Scheme 5, for the conjugation of a disubstituted 1,5-pentanedial **6** with a primary-amine-containing molecule **7** to form a disubstituted 1,4-dihydropyridine **8** conjugate. This click reaction was conducted under mild reaction conditions, in aqueous medium or in the presence of organic solvent and proceeded in high yield with stable starting dialdehyde reagent and resulting dialdehyde click conjugates. No dialdehyde-activating group or catalyst was needed for this reaction and only water is formed as a byproduct. Reaction proceeded with high atom economy and the

starting dialdehyde and the resulting conjugate were both easy to characterize. A scheme was designed to synthesize a suite of dialdehyde reagents inorder to demonstrate the broad scope of this new click-conjugation protocol. The dialdehyde molecules were used for tailoring surfaces for material science applications and in cell-surface engineering. The broad utility of the general dialdehyde click chemistry to primary amines is anticipated in all areas of chemical research, ranging from bioconjugation and polymers to nanotechnology and material science.



Scheme 5. Schematic of a general click-reaction strategy to ligate a primary amine group **7** with a dialdehyde group **6**



Scheme 6. Synthetic route to generating a suite of dialdehyde reagents

The dialdehyde reagents were synthesized with minimal steps and in high yields. Acid **9** was converted to **10** in the presence of *n*-BuLi and excess of iodomethane. Alcohol **11** was obtained by treating **10** with $LiAlH_4$. 2-((1-Methylcyclopent-3-enyl)methoxy) acetic acid **12** was afforded by the addition of NaH and 2-chloroacetic acid to **11**. Catalytic amount of OsO_4 and excess *N*-methylmorpholine *N*-oxide 50% in H_2O at pH 8, were used to obtain compound **13**, which was subsequently converted to the key intermediate **14** employing catalytic amount of *p*-TsOH and then large excess of 2,2-dimethoxypropane. **15** allowed for many dialdehyde-containing reagents to be synthesized. Once the intermediate **10** is obtained, a range of probes, monomers, ligands, and molecules could be connected to generate a broad suite of dialdehyde reagents (Scheme 6).

3. Polymer Chemistry and Macromolecular Engineering

Click reactions, being versatile and simple, can greatly facilitate the synthesis and modification of polymeric materials. There is a wide range of polymers that have been synthesized by employing various click reactions including (multi)block copolymers[48-51] and micelles,[52-56] terminal- and pendant-functional polymers,[57-61] gels and networks,[62-65] complex architectures such as graft,[66-77] star,[78-84] brush,[57,85-87] hyperbranched polymers[88-92] and polymers conjugated to nanomaterials.[93-103] Many of the polymers that are used for further modifications were originally synthesized through controlled polymerization techniques. Polymers with pre-determined molecular weight, chain end functionality, narrow molecular weight distribution and controlled architecture are usually prepared by using controlled radical polymerization (CRP).[104-106] The most commonly employed CRP methodologies include reversible addition-fragmentation transfer (RAFT) polymerization,[107-110] atom transfer radical polymerization (ATRP)[111-115] and stable free radical polymerization (such as nitroxide-mediated polymerization, NMP).[116,117] In these strategies, composition, molecular weight and topology is controlled by a fast dynamic equilibrium between a dormant and propagating state which sustains a low concentration of radicals, hence, suppressing chain termination reactions.[118-130] Click chemistry combines particularly well with these reactions because the species used to initiate polymerization or mediate the dynamic equilibrium can provide functional groups either inherently present or intentionally added that are easily converted to "clickable" functionality.

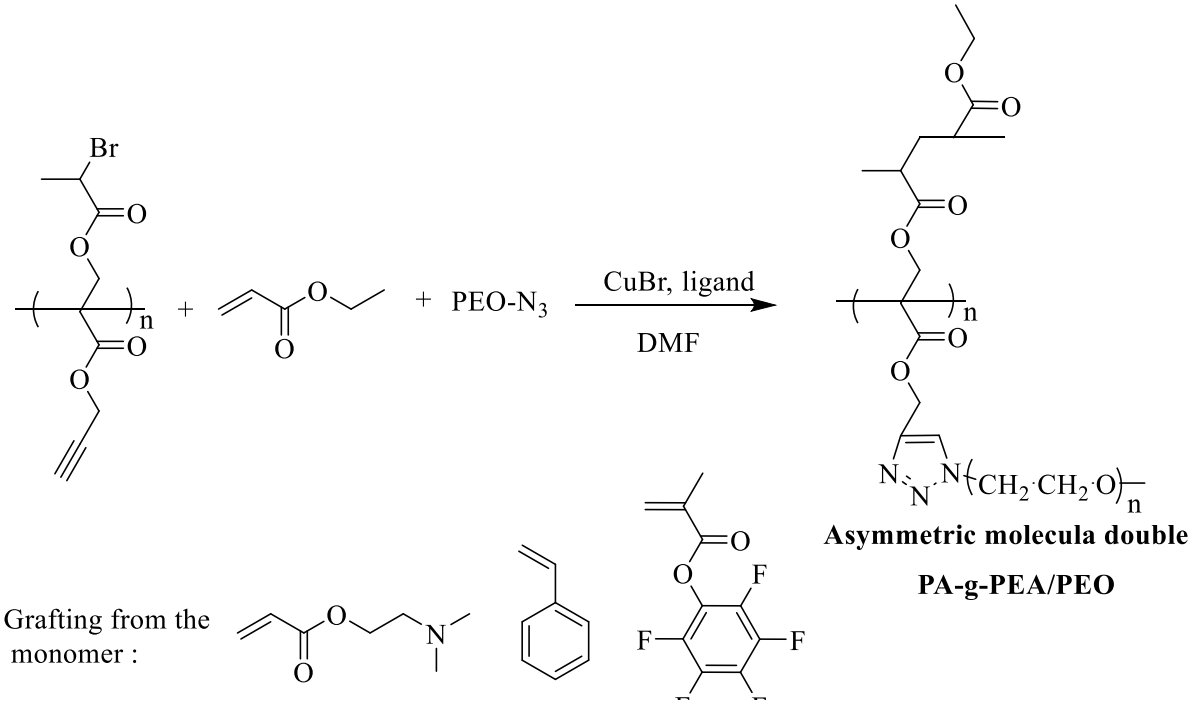
Modern polymeric material design often involves precise macromolecular synthesis in order to get macroscopic material properties of choice. This concept is referred as macromolecular engineering which includes the rational design of the macromolecular structure, accurate synthesis, assembly, and processing to nanodevices or upramolecular objects, thorough characterization, and theoretical modeling for aiding and optimizing the macromolecular design procedure.[131] Precise tailoring of the molecular and supramolecular structures required for achieving desirable features such as molecular size, topology, uniformity, functionality and composition, is considered core of the macromolecular engineering.[132] Click chemistry is one of the available tools at researchers' disposal to tailor the molecular architectures. ATRP and CuAAC are often combined together in macromolecular architecture design[133] due to the following reasons:

a) Cu(I) is used as catalyst in both CuAAC and ATRP.
b) Both azide and alkyne groups are stable during the course of ATRP as long as they do not coexist in the same reaction.
c) ATRP furnishes polymers containing halogens at their chain ends which can easily be converted to the azide groups.

Concurrent ATRP/CuAAC proved to be a very efficient tool to synthesize an array of diverse polymeric materials including brush polymers,[134-136] block copolymers,[137] networks[138] and polymers with functional side groups.[139,140]

Binbin Xu and coworkers reported the synthesis of well-defined asymmetric molecular double-brushes consisting of two different side chains linked to the same repeat unit along the backbone by one-pot concurrent ATRP and Cu-catalyzed azide/alkyne cycloaddition (CuAAC) reaction.[141] The double-brushes were based on a poly(Br-acrylate-alkyne) homopolymer possessing an alkynyl for CuAAC

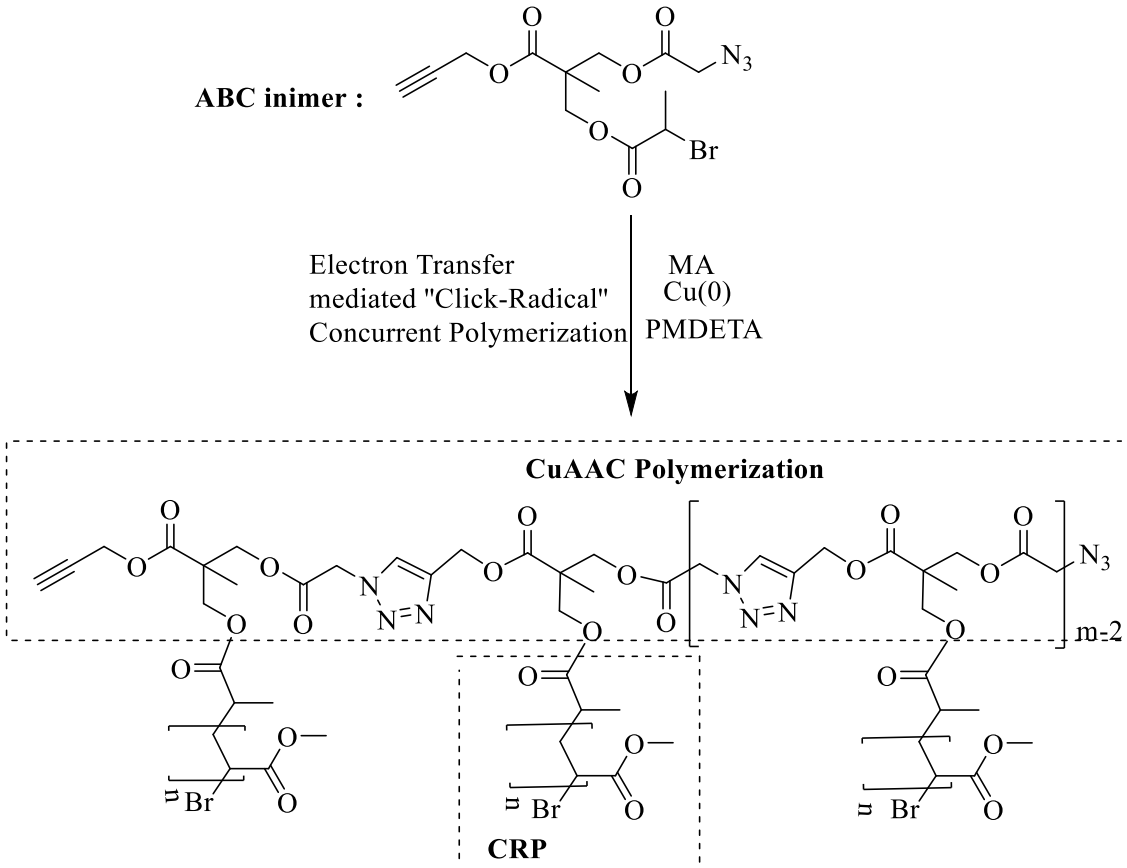
reaction and a 2-bromopropionate initiating group for ATRP in each repeat unit. Employing an acrylate type monomer containing both an ATRP initiationsite, *i.e.*, tert-butyl 2-((2-bromopropanoyloxy)methyl)acrylate (tBBPMA) and an alkyne group, was the key to the successful synthesis of Janus armed bottlebrush copolymer. This monomer tBBPMA was polymerized *via* RAFT to afford a polymer with two types of functional side groups on each monomeric unit. Finally, the Janus armed bottlebrush copolymer was synthesized in one-pot by concurrent ATRP of vinyl monomers and CuAAC coupling of poly(ethylene oxide) with an azide end group (PEO-N_3) to afford well-defined copolymers with and narrow molecular weight distribution and controlled architecture (Scheme 7).



Scheme 7. Synthesis of Janus armed bottlebrush copolymer by concurrent ATRP and CuAAC click chemistry

In recent years, various researchers have reported number of excellent examples which involved CuAAC reactions to design functional materials with improved properties. However, the majority of previous investigations utilized CuAAC reactions alone or multistep reactions; the controlled chain-growth CuAAC polymerization has rarely been reported because it was hard to be well-controlled and furnished polymers with broad molecular weight distribution. Recent developments in the electron transfer mediated controlled/"living" radical polymerization (CRP) facilitated the further development of the CuAAC reactions because the mechanisms of CuAAC reactions and CRP may have little in common using the same copper(I)-catalyst for both protocols.

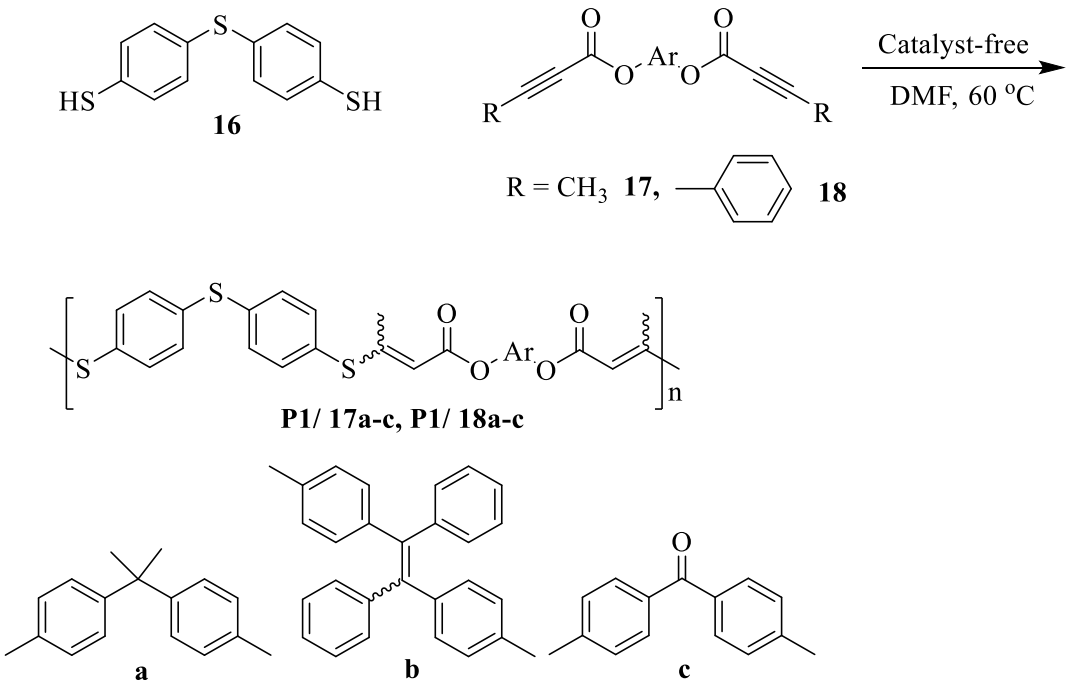
Wentao Xue and co-workers successfully employed the electron transfer mediated "click- radical" concurrent polymerization utilizing Cu(0)/PMDETA as catalyst for the generation of well-defined copolymers, where controlled CuAAC polymerization of clickable ester monomer was performed in the main chain acting as the polymer backbone, the CRP of the acrylic monomer was conducted in the side chain.[142] Furthermore, strong collaborative effect and compatibility between CRP and CuAAC polymerization was observed for improving the controllability (Scheme 8).



Scheme 8. One-step synthesis of brush polymer from small molecules by concurrent ATRP and copper-catalyzed azide-alkyne cycloaddition (CuAAC) click polymerization

Click reaction has also been explored for the synthesis of π-conjugated polymers for organic electronics including CuAAC and without CuAAC, featuring essential ‘’CLICK’’ attributes. The alkyne-based click polymerizations, derived from click reactions, have attracted much attention because of their advantageous characteristics such as good functional group tolerance, mild reaction conditions, atom economy, high regio- and stereo-selectivity and high yields.[143-148] Recently, thiol-yne click polymerizations have become significant synthetic tools for the preparation of linear as well as hyperbranched polysulfides with diverse structure and properties. The alkyne monomers used in majority of thiol-yne polymerizations are mainly terminal alkynes while internal alkynes are rarely employed because of their relatively low reactivity.

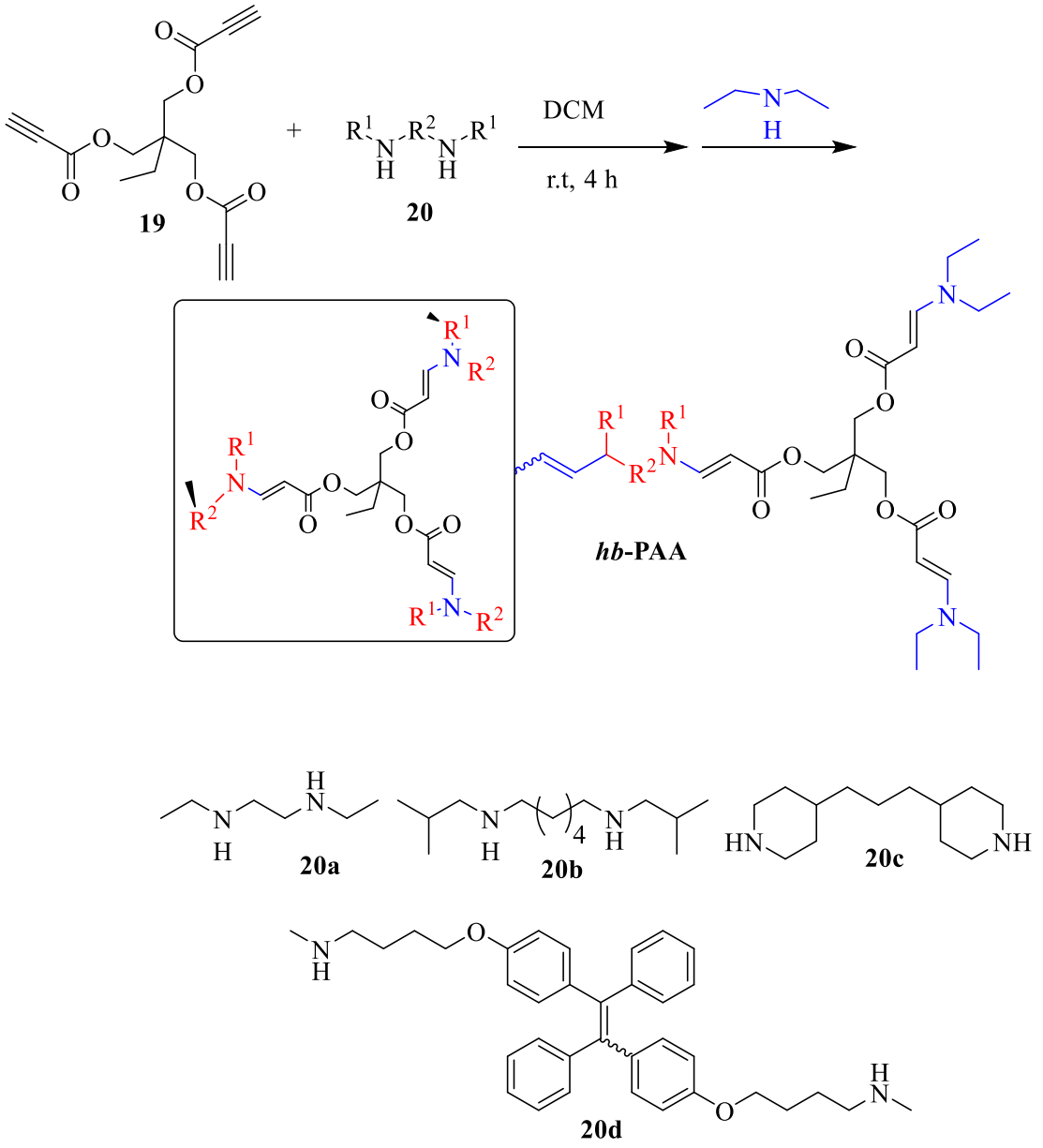
Jun Du and co-workers developed a catalyst-free click polymerization of activated internal alkynes and thiol.[149] The polymerization reactions between thiol **16** and electron deficient internal alkynes (**17** and **18**) proceeded smoothly in DMF to furnish soluble poly(β-thioacrylate)s (PTAs) with high molecular weights and Z-stereoregularities in high yields.



Scheme 9. Synthesis of **P1/17a-c** and **P1/18a-c** by catalyst free click polymerization of thiol **16** with internal diynes **17** and **18**

Hyperbranched polymers is an emerging class of functional polymeric materials, which has gained increasing attention and has found widespread use in the areas of nanomaterials, adhesives, coatings, additives, biomaterials, drug delivery carriers, etc. because of their fascinating attributes, such as excellent solubility, low intrinsic viscosities, interior cavities and abundant surface functional groups.[150-157] Various polymerization methods such as condensation polymerization, ring-opening polymerization, self-condensing vinyl polymerization, proton transfer polymerization and addition polymerization have been used for the construction of multifunctional hyperbranched polymers.[158-165] However, most of these polymerizations require harsh conditions, which complicate the experimental operation and greatly limit their applications. Click polymerization has become a powerful tool for the preparation of functional polymers due to their distinct features of high efficiency, high selectivity, atom economy and mild reaction conditions. At present, functional hyperbranched polymers have been prepared *via* thiol-ene click polymerization, Cu(I)- and Ru(II)-catalyzed azide-alkyne click polymerizations, metal-free azide-alkyne click polymerizations and thiol-yne click polymerizations. Recently, another powerful click polymerization, *i.e.*, amino-yne click polymerization, has been developed.[166-168] This click polymerization protocol belongs to the hydroamination reaction, which is a direct and efficient way for the synthesis of nitrogen containing functional materials. This polymerization can take place at room temperature without the need of any external catalyst, and poly(β-aminoacrylate)s (PAAs) with high molecular weights could be obtained in high yields. More importantly, this click polymerization can proceed in a regio- and stereospecific fashion, and 100% E-isomers are afforded in an anti-Markovnikov addition manner.

Benzhao He and co-workers synthesized a series of multifunctional hyperbranched poly(β-aminoacrylate)s (hb-PAAs) *via* spontaneous amino-yne click polymerization by employing ester activated triyne **19** and diamines **20a-d** as monomers.[169] Various soluble and thermally stable hb-PAAs with high weight-average molecular weights (M_w, up to 18,290) were afforded in high yields (up to 99%) under mild reaction conditions. Furthermore, by introducing the aggregation-induced emission (AIE)-active tetraphenylethene (TPE) moiety into the backbones, the resultant polymers also displayed a unique AIE feature, and their nanoaggregates could be used for the detection of explosives. The special three-dimensional topologies of hyperbranched polymers could effectively improve their detection sensitivity (Scheme 10).



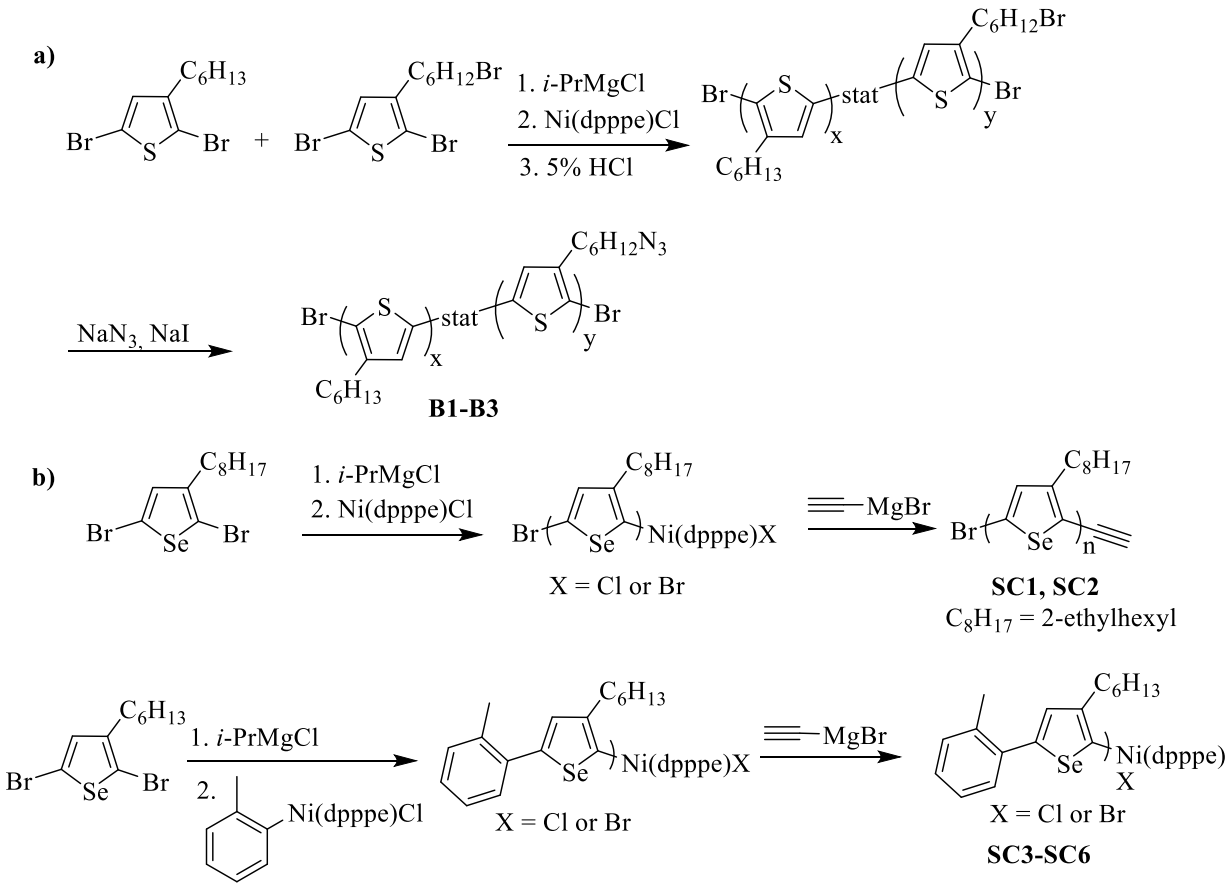
Scheme 10. Syntheses of hyperbranched poly(β-aminoacrylate)s by spontaneous amino-yne click polymerization

Graft polymers contain complex architecture with multiple polymeric side chains. The synthesis of graft copolymers has an advantage of incorporating properties of two or more polymers that are functionally distinct from their linear copolymer relatives. Conjugated organic polymers are both interesting and feasible to study from a complex architecture viewpoint. Studying complex all-conjugated polymers is advantageous for observing the effect of introducing multidimensional architectures on their optoelectronic properties. Moreover, the overall increased rigidity of conjugated polymers as compared to the nonconjugated polymers is hypothesized to affect the material properties of the resulting graft copolymers.

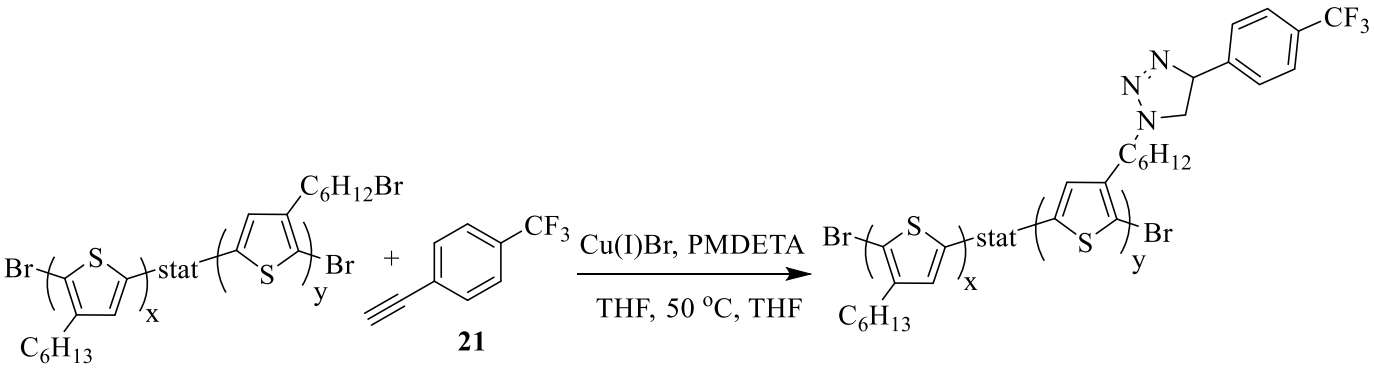
Nimrat K. Obhi and co-workers used the copper-catalyzed azide-alkyne click (CuAAC) reaction as the grafting-to reaction.[170] A library of well-defined polythiophene backbones and polyselenophene side chains with click-active azide and acetylene functional groups was synthesized using Kumada catalyst transfer polymerization (KCTP). Control test reactions displayed excellent azide group tolerance to CuAAC click conditions, and successful synthesis of a 100% graft copolymer using a polythiophene

backbone and small molecule 4-ethynyl-α,α,α-trifluorotoluene (ETFT) was also performed under these conditions.

Click chemistry reaction conditions were assessed by performing two test reactions. In the first test reaction, it was observed that the CuAAC click conditions did not affect the integrity of the azide groups on the polythiophene backbone, hence, eliminating the possibility of azide crosslinking during the comb reactions. Specifically, a 14.5 kDa polythiophene backbone with 53% N_3 was treated with the CuAAC click conditions in the absence of any alkyne-functionalized molecules. CuAAC conditions were used which are most commonly employed for polymer-polymer click reactions, involving the use of Cu(I)Br in conjunction with amine ligand PMDETA with the application of heat (Scheme 11). In the second reaction, efficiency of the grafting-to click procedure was assessed by using small molecule alkyne 4-ethynyl-α,α,α-trifluorotoluene (ETFT) **21**. 100% grafting of ETFT to the 53% N_3 backbone was obtained using the same CuAAC conditions described for the first reaction. The backbone (B1) was treated with a stoichiometric amount of the small molecule and 1.2 equivalents of Cu(I)Br/PMDETA relative to the percentage of azide groups. Excellent efficiency of the grafting-to procedure using the Cu(I)Br/PMDETA click system with a small molecule suggested that this grafting-to procedure could be appropriate for comb copolymer synthesis (Scheme 12).



Scheme 11. Synthesis of **(a)** Polythiophene backbone copolymers and **(b)** Polyselenophene side chains using Kumada catalyst-transfer polycondensation



Scheme 12. General scheme for control click reactions using small molecule 4-ethynyl-α,α,α-trifluorotoluene **21**

4. Drug Delivery

CC is of tremendous help to meet the demand of modern-day chemistry research, mainly drug discovery. It uses pairs of functional groups that rapidly and selectively react (click reaction) with each other in ecofriendly, mild, aqueous conditions as well as in organic solvents. The selection of each click reaction is based on its selectivity, reactivity, biocompatibility, and stability. Click chemistry has greatly facilitated the overall drug discovery process by providing easy access to the synthesis of building blocks for new molecular entities (NMEs). Although it has by no means replaced existing methods for drug discovery, it has complemented and extended them by aiding lead discovery and optimization.[171]

Nanosystems that release cancer drugs in response to an external stimulus offers unique advantages over commonly used conventional carriers that release their payload in a passive fashion. Reduced graphene oxide (rGO) nanosheets with restored sp^2 network have been shown to be ideally suited for drug loading and release based applications.[172-180] Since effective functionalization of the rGO through covalent transformations is limited, the non-covalent π- π stacking based functionalization route of rGO scaffold has been extensively explored.[181-186] On the other hand, this strategy was found to be effective for the molecules which are able to form efficient non-covalent interactions. Conjugation of molecules unable to undergo such associations with rGO is quite challenging. For the functionalization of rGO, aromatic anchoring groups such as tetrathiafulvalene (TTF),[183,185] pyrene[181,187] and dopamine derivatives[184,186] are of great significance. Dopamine containing clickable azide and alkyne groups have also been used for the functionalization of rGO.[184,186] The azide containing rGO could be further modified with alkynyl-terminated molecules such as ferrocene using Cu(I) catalyzed azide-alkyne Huisgen-type click reaction. The azide-alkyne based functionalization of rGO was effective but using copper as a catalyst could be of concern for biomedical applications as the cytotoxicity of nanostructures could be enhanced by any trace of copper. This necessitates to develop click reactions that do not require metal catalysts such as nucleophilic or radical thiol-ene reactions and strain-promoted azide-alkyne cycloaddition (SPAAC).[188] Nucleophilic thiol-ene based reactions using the thiolmaleimide functional group dyad has been extensively utilized in bio-conjugations. The maleimide-thiol addition reaction is an efficient protocol that could be used under mild reaction conditions without using any catalyst.

Yavuz Oz and co-workers investigated the maleimide-containing catechol (dopa-MAL) ligand as a versatile surface anchor onto reduced graphene oxide (rGO) nanosheets.[189] Thiol-maleimide chemistry allowed facile attachment of thiol-containing molecules under ambient metal-free conditions. While the attachment of glutathione and 6-(ferrocenyl)hexanethiol was used as models, the attachment of a cancer cell targeting cyclic peptide, c(RGDfC), opened the possibility of using the dopa-MAL modified rGO as a targeted drug delivery system for doxorubicin (DOX). This approach was shown to be effective not only for model molecules such as glutathione and ferrocene containing a thiol group, but it was also adaptable to the covalent linking of thiol containing tripeptides, such as the cancer cell targeting peptide c(RGDfC). The facile fabrication and functionalization to readily obtain a functional material in a modular fashion make this clickable-rGO construct an attractive candidate for various useful applications.

A metal-free "click" conjugation protocol using strain-promoted click chemistry was explored as a facile and fast tool for the functionalization of microbubbles with drug encapsulated nanoparticles, siRNA encapsulated micelles and protein molecules.[190] This microbubble–therapeutic "click" conjugation established in the current study did not require any catalyst or initiator, did not involve toxic agents, had ultra-fast reaction speed, and was versatile for the ligation of different anticancer or therapeutic agents to the microbubbles. These advantages made it a favorable protocol for use in various biomedical research and clinical applications such as therapeutic delivery for inflammation, tumor treatment, thrombosis and angiogenesis in different organs and real-time imaging.

The combination of copper-free click chemistry with metabolic labeling have opened new avenues in drug delivery. Nicolas Alcaraz and co-workers conducted a study to determine whether cubosomes functionalized with azide or dibenzocyclooctyne (DBCO) groups were able to undergo copper-free click chemistry with a strained cyclooctyne or azide, respectively.[191] Phytantriol-based cubosomes were functionalized by phospholipids containing an azide or DBCO group. The efficiency of "clickability" was estimated by the reaction of cubosomes with a complementary dye and determining bound and unbound dye through size exclusion chromatography. The clickable cubosomes reacted specifically with a click-Cy5 dye with minor changes to the shape, size and structure of the cubosomes. This showed that cubosomes could retain their unique internal structure while participating in copper-free click chemistry. This proof of concept study could pave the way for the utilization of copper-free click chemistry and metabolic labeling with cubosomes for targeted drug delivery and imaging.

In the past few years, Smart hydrogels have become an attractive candidate in biomaterials fields for promising applications in drug delivery and tissue engineering.[192-197] Hydrogels can change their permeability, volume and phase state in response to the external stimuli such as pH,[198] temperature,[199] UV-light,[200] enzymes,[201] electric or magnetic fields[202] and/or oxidizing or reducing agents.[203] For biological applications, pH/temperature responsive hydrogels are considered more useful because temperature and pH are the most affected environmental stimuli under in vitro and in vivo conditions.[204] Thiol-ene chick chemistry offers many advantages for hydrogel formation, such as insensitivity to oxygen and rapid stoichiometrically controlled polymerization *via* photoinitiation.[205,206] Stimuli-responsive chitosan (CS) hydrogels displayed a great potential for drug delivery and tissue engineering; however, the structure of these stimuliresponsive CS hydrogels, such as dual pH- and thermo-responsive hydrogels, is difficult to control or needs additional crosslinking agents.

Haichang Ding and co-workers reported a novel dual pH- and thermo-responsive hydrogel system by combining pH-responsive C_6-OH allyl-modified CS (OAL-CS) with thermo-responsive poly(N-isopropylacrylamide) (PNIPAM).[207] The thiol groups in PNIPAM and the allyl groups in OAL-CS could rapidly form crosslinking hydrogel network by "thiol-ene" click chemistry under UV irradiation. The swelling ratio of the OAL-CS/PNIPAM hydrogel could be controlled by changing temperature and pH. Furthermore, the hydrogel displayed non-cytotoxic nature toward human bone marrow mesenchymal stem cells, and the histological analyses revealed the subcutaneous tissue with no signs of inflammation after five days of injection in vivo. The results showed that the new OAL-CS/PNIPAM hydrogel had potential to serve as a smart injectable platform to be utilized in drug delivery and tissue engineering.

Clara García-Astrain and co-workers reported a series of chemically cross-linked alginate hydrogels, synthesized through click chemistry via Diels-Alder reaction by reacting furan-modified alginate and bifunctional cross-linkers.[208] Furfurylamine was used for the functionalization of alignate. Then, 3D architectures were synthesized with water-soluble bismaleimides. Different substitution degrees were obtained for investigating the effect of alginate modification and the cross-linking extent over the behavior of the hydrogels. The ensuing hydrogels were analyzed in terms of swelling, microstructure, rheological behavior and structure modification. The materials response to external stimuli such as pH was also studied, revealing a pulsatile behavior in a large pH range (1–13) and a clear pH-dependent swelling. Finally, vanillin release studies were conducted in order to display the potential of these biobased materials for drug delivery applications.

4. Conclusion

Click chemistry has become one of the most powerful strategies in materials and biomedical sciences due to the efficiency, selectivity and tolerance of this class of reactions to a variety of solvents and functional groups. By far the most widely utilized of these efficient transformation reactions is the CuI-catalyzed azide–alkyne cycloaddition. This reaction has been creatively employed to facilitate the preparation of versatile molecules to be employed in different areas of chemistry including bioconjugation, drug delivery and polymer chemistry. Click chemistry has enabled the researchers to explore complex materials while simplifying their preparation methods. Additional reactions that can provide the benefits of click chemistry have been increasingly investigated, which are helpful in expanding the range of available functional groups that can participate in highly efficient chemical transformations. Overall, the results from research to date suggest that click chemistry has emerged as a valuable tool in biomedical fields as well as in material chemistry.

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