Click Chemistry

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# Click Chemistry beyond Metal-Catalyzed Cycloaddition

*C. Remzi Becer, Richard Hoogenboom, and Ulrich S. Schubert**

click chemistry · catalyst-free method · cycloaddition · Diels–Alder reaction · thio-click reaction

*Dedicated to Professor Jean-Marie Lehn on the occasion of his 70th birthday*



*The overwhelming success of click chemistry encouraged researchers to develop alternative "spring-loaded" chemical reactions for use in different fields of chemistry. Initially, the copper(I)-catalyzed azide-alkyne cycloaddition was the only click reaction. In recent years, metal-free [3+2] cycloaddition reactions, Diels–Alder reactions, and thiol-alkene radical addition reactions have come to the fore as click reactions because of their simple synthetic procedures and high yields. Furthermore, these metal-free reactions have wide applicability and are physiologically compatible. These and other alternative click reactions expand the opportunities for synthesizing small organic compounds as well as tailor-made macromolecules and bioconjugates. This Minireview discusses the success and applicability of new, in particular metal-free, click reactions.*

## 1. Introduction

The click chemistry concept was introduced by Sharpless and co-workers in 2001.[1] Selected reactions were classified as click chemistry if they were modular, stereospecific, wide in scope, resulted in high yields, and generated only safe by-products. Furthermore, the reaction had to proceed under simple reaction conditions, with readily available starting materials, and without any solvent or in a benign solvent. The purification process of these reactions should be as easy as the synthesis process and, thus, nonchromatographic methods, that is, crystallization or distillation, are preferred for isolation of the products.

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A variety of click reactions exist, but the Huisgen 1,3-dipolar cycloaddition of azides and alkynes plays a particularly important role in organic synthesis, and had evolved into a common coupling procedure in all chemical disciplines within a few years.[2] The chemistry of azides has been neglected for a long time because of safety concerns. Despite their potentially explosive character, azide moieties have excellent properties, such as stability against dimerization or hydrolysis, compared to most other functional groups. The rate of the 1,3-dipolar cycloaddition of azides and alkynes is drastically increased in the presence of an appropriate catalyst such as transition-metal ions,[1] which at the same time provide stereospecificity, thus satisfying the requirements for classification of this cycloaddition as a click reaction.[1] This reaction is commonly performed in the presence of copper ions and a nitrogen-based ligand. However, concerns about the cytotoxicity of copper led to the investigation of other types of catalysts. In this regard, different ligands (PMDETA, bipyridine derivatives, terpyridine derivatives, and $Me_6$Tren) and other transition metals (Ru, Ni, Pd, Pt, and Fe) have been examined.[3]

In the last couple of years, there has been significant interest in developing click reactions that do not require any metal catalyst while exhibiting all the beneficial properties of the copper-catalyzed azide-alkyne click reaction (that is, fulfilling all the click requirements). In a recent highlight, Lutz provided an excellent overview of metal-free azide-alkyne cycloadditions.[4] However, click chemistry is not limited to cycloadditions and can be extended to other highly efficient reactions, such as nucleophilic substitution, radical additions, Michael additions, as well as Diels–Alder and retro-

Diels–Alder reactions. The advantage of these reactions is that they proceed in the absence of a metal catalyst.

This Minireview summarizes the tremendous recent progress in the development of metal-free click reactions. The alternative modular click reactions are discussed in detail, focusing on the potential of these reactions to fulfill the demanding requirements of a click reaction. Such an evaluation will serve as an indication of how broadly applicable the different approaches are and, thus, might predict their future potential. To aid the forthcoming discussion of the different metal-free click reactions, the requirements for click reactions as been defined by Sharpless and his co-workers are listed:

- modular and wide in scope;
- highly efficient and give high yields;
- no or inoffensive by-products;
- stereospecific;
- readily available starting materials and reagents;
- no solvent or a benign solvent;
- simple purification techniques.

## 2. Metal-Free Click Reactions

The potential toxicity of metal catalysts used in organic synthesis is a major issue when the products are designed to be used for biological applications.[5] Similarly, even though it is possible to synthesize a wide variety of compounds by the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC), traces of copper may still remain at ppm levels in the product after purification. Therefore, there has been significant interest in developing alternative click reactions that do not require any metal catalyst (Table 1).

### 2.1. Copper-Free [3+2] Cycloaddition Reactions with Azides

The preparation of a wide range of azides has been well-studied, and in recent years this readily available class of compounds has received increased attention because of their potential use in CuAAC click reactions. However, azides do not react easily with alkynes in the absence of a metal catalyst since they are generally poor 1,3-dipolar acceptors. Therefore, different approaches have been developed to increase the reactivity of the alkyne groups to allow metal-free azide-alkyne cycloadditions under mild conditions.

#### 2.1.1. Reaction of Azides with Substituted Cyclooctyne

One elegant approach, involving the reaction of azides with cyclooctyne derivatives, was reported by Bertozzi and co-workers (Scheme 1).[6] This strain-promoted azide-alkyne [3+2] cycloaddition (SPAAC) reaction was developed from the initial work of Wittig and Krebs.[7] However, the rate of SPAAC reactions with the first generation of cyclooctyne **1** are relatively slow compared to the corresponding CuAAC reactions. Therefore, monofluorinated (2nd generation, **2**) and difluorinated (3rd generation, **3**) derivatives of cyclooctyne were synthesized to decrease the lowest unoccupied molecular level (LUMO) level of the alkyne by introducing electron-withdrawing groups, which resulted in increased second order rate constants.[8] The corresponding second-order rate constants for cyclooctynes **1**, **2**, and **3** are 1.0, 1.8, and 31.8 $M^{-1}s^{-1}$, respectively.[6g]

Biomolecules have been labeled selectively by using difluorinated cyclooctyne (DIFO) derivatives in SPAAC reactions. Recently, Zou and Yin reported the use of biotin-conjugated DIFO derivatives for SPAAC with an azide-substituted substrate attached to a peptidyl carrier protein (PCP).[10] Boons and co-workers prepared an active cyclooctyne by introducing benzyl groups to increase the ring strain.[11] They used 4-dibenzocyclooctynol derivatives to label glycoconjugates of living cells metabolically for visualization.

The SPAAC reaction clearly fulfils many requirements of a click reaction. However, the demanding organic synthesis of cyclooctyne derivatives needs to be simplified before the SPAAC reaction can be used not only in chemical biology but also in other fields of chemistry. Alternatively, the commercial availability of **3** would significantly improve the scope and applicability of this reaction.

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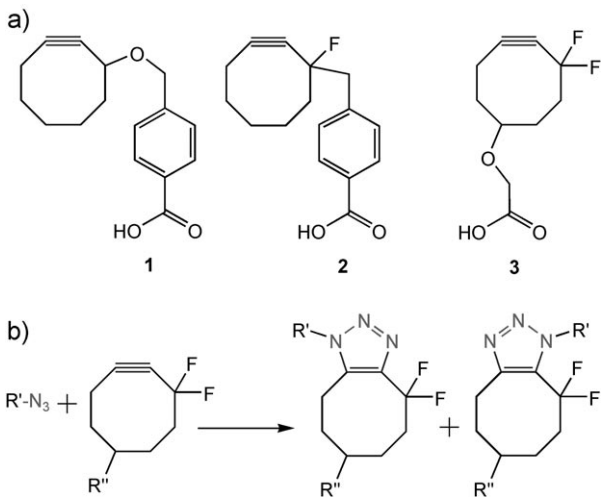
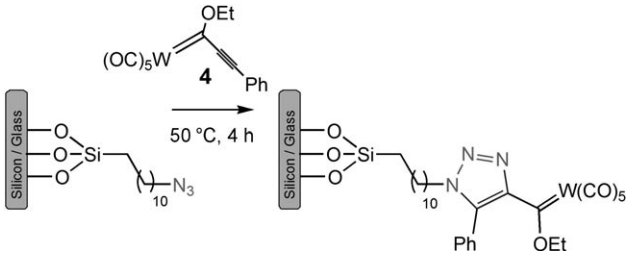
***Table 1:*** Click reactions that proceed in the absence of a metal-catalyst in comparison to the CuAAC (entry 0).

| | Reagent A | Reagent B | Mechanism | Notes on reaction[a] | Reference |
|---|---|---|---|---|---|
| 0 | azide | alkyne | Cu-catalyzed [3+2] azide-alkyne cycloaddition (CuAAC) | 2 h at 60 °C in $H_2O$ | [9] |
| 1 | azide | cyclooctyne | strain-promoted [3+2] azide-alkyne cycloaddition (SPAAC) | 1 h at RT | [6–8, 10, 11] |
| 2 | azide | activated alkyne | [3+2] Huisgen cycloaddition | 4 h at 50 °C | [12] |
| 3 | azide | electron-deficient alkyne | [3+2] cycloadditton | 12 h at RT in $H_2O$ | [13] |
| 4 | azide | aryne | [3+2] cycloaddition | 4 h at RT in THF with crown ether or 24 h at RT in $CH_3CN$ | [14, 15] |
| 5 | tetrazine | alkene | Diels–Alder retro-[4+2] cycloaddition | 40 min at 25 °C (100% yield) $N_2$ is the only by-product | [36–38] |
| 6 | tetrazole | alkene | 1,3-dipolar cycloaddition (photoclick) | few min UV irradiation and then overnight at 4 °C | [39, 40] |
| 7 | dithioester | diene | hetero-Diels–Alder cycloaddition | 10 min at RT | [43] |
| 8 | anthracene | maleimide | [4+2] Diels–Alder reaction | 2 days at reflux in toluene | [41] |
| 9 | thiol | alkene | radical addition (thio click) | 30 min UV (quantitative conv.) or 24 h UV irradiation (>96%) | [19–23] |
| 10 | thiol | enone | Michael addition | 24 h at RT in $CH_3CN$ | [27] |
| 11 | thiol | maleimide | Michael addition | 1 h at 40 °C in THF or 16 h at RT in dioxane | [24–26] |
| 12 | thiol | *para*-fluoro | nucleophilic substitution | overnight at RT in DMF or 60 min at 40 °C in DMF | [32] |
| 13 | amine | *para*-fluoro | nucleophilic substitution | 20 min MW at 95 °C in NMP as solvent | [30] |

[a] RT = room temperature, DMF = *N*,*N*-dimethylformamide, NMP = *N*-methylpyrolidone, THF = tetrahydrofuran, $CH_3CN$ = acetonitrile.




***Scheme 1.*** a) Cyclooctyne derivatives with different substituents. b) The SPAAC click reaction.




***Scheme 2.*** Copper-free click reaction between an azido SAM and an alkynyl Fischer carbene complex.

#### 2.1.2. Reaction of Azides with Activated Alkynes

Sarkar and co-workers reported an uncatalyzed click reaction of activated alkynes.[12] Glass and silicon surfaces modified with azido-functionalized self-assembled monolayers (SAMs) were treated with the Fischer carbene complex **4** under argon (Scheme 2). Detailed characterization of the surfaces was performed by ellipsometry, FT-IR, ATR-IR, AFM, and contact angle measurements. The Fischer carbene functionalized SAM formed in the click reaction was tested for nucleophilic substitution with a pyrene-based fluorescent probe.

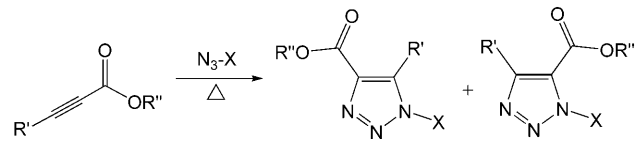
Although this reaction needs to be investigated in more detail with regard to, for example, yields and modularity, it represents a promising example of an uncatalyzed click reaction. However, activation of the alkyne with tungsten prevents this reaction from being classified as a metal-free click reaction. Even though the accessibility of the activated alkynes is easier than cyclooctyne derivatives, other activation methods have to be developed for it to be applicable in biological systems.

#### 2.1.3. Reaction of Azides with Electron-Deficient Alkynes

As shown in the previous examples, activated alkynes can undergo cycloaddition reactions with azides in the absence of a metal catalyst.[13] In 2004, Ju and co-workers reported a simple synthetic protocol for the 1,3-dipolar cycloaddition of azides with electron-deficient alkynes.[13c] 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 (Scheme 3). The yields obtained were in



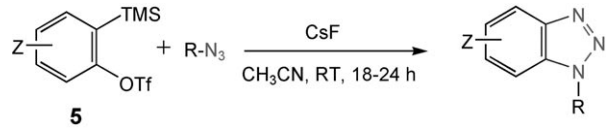
***Scheme 3.*** Click reaction between azides and electron-deficient alkynes (R′ = H, $CH_3$, or COOEt, R″ = Me or Et, and $N_3$-X = 5-azidovalerate or 5′-azido-DNA).

the range of 67 to 94%. 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

The promising results of Ju and co-workers might serve as a basis to extend this metal-free click reaction to other fields of chemistry. However, the yields of the described click reaction need to be improved, and the modularity and availability of the alkyne starting materials need to be evaluated.

#### 2.1.4. Reaction of Azides with Arynes

Larock and co-workers developed a facile, 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.[14] After optimization, such a "benzyne click" reaction between benzyl azide and *o*-(trimethylsilyl)phenyl triflate (**5**; Z = H) at room temperature for 18 h in acetonitrile and in the presence of CsF led to a yield of 76% (Scheme 4). The reaction scope was extended with various benzyne precursors and azides.



***Scheme 4.*** An example of benzyne click chemistry (R = benzyl). Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl.

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 a complementary crown ether and, furthermore, the reaction times could be reduced to less than 2 h.[15]

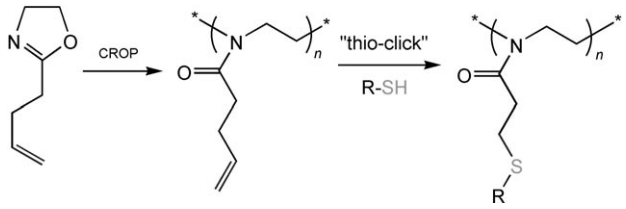
Both types of reactions meet the main requirements of a click reaction, and can be performed under optimized conditions in short reaction times, at room temperature, in air, and result in a single product in good yield. Unfortunately, azides bearing electron-withdrawing groups directly attached to the azide moiety do not react under those conditions. Furthermore, a fluoride source is necessary to conduct this reaction, and the availability of the required multifunctional aromatic starting materials might be problematic.

### 2.2. Thiol-Based Click Reactions

Thiols have been used in diverse chemical reactions for well over a century.[16] Initially, thiol-ene chemistry was used for the preparation of well-defined films or networks. However, there were some practical considerations regarding the thiols used, such as their odor, storage, and stability. Most of these challenges have now been solved by improved methods for the synthesis of the monomers and the development of various efficient stabilizers.[17] Extensive literature exists on the reaction pathways and kinetics of thiols, and a wide range of thiol compounds are relatively easy to prepare. These features result in thiols being good candidates for click reactions.[18]

#### 2.2.1. Radical Addition Reaction between Thiols and Alkenes

The radical addition of thiols to double bonds is a highly efficient method used for polymerizations, curing reactions, and for the modification of polymers.[19] Schlaad and co-workers demonstrated a post-polymerization modification of a well-defined poly[2-(3-butenyl)-2-oxazoline], which was polymerized by a living cationic ring-opening polymerization (CROP) process. Various fluorinated thiols, acetylated glucose thiols, and dihydroxy-functionalized thiols were used in model reactions (Scheme 5). These "thio-click" reactions



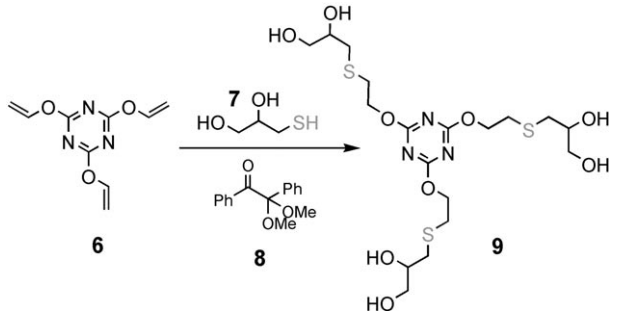
***Scheme 5.*** Synthesis and thio-click modification of poly[2-(3-butenyl)-2-oxazoline].

were performed under an inert atmosphere by exposure to UV light for 24 h,[20] but were also successful under irradiation with direct sunlight, since the thiol-ene photoaddition reaction can proceed at near-visible wavelengths ($\lambda$ = 365–405 nm).[21]

Hawker and co-workers reported a robust, efficient, and orthogonal synthesis of fourth generation dendrimers by using thiol-ene click reactions.[22] The solvent-free reaction between alkene **6** and thiol **7** was performed under ambient conditions by irradiation for 30 minutes with a hand-held UV lamp ($\lambda$ = 365 nm). Trace amounts of photoinitiator **8** were added to increase the radical concentration and, thus, increase the reaction rate. The first generation of dendrimer **9** is shown in Scheme 6. Higher generations were synthesized in the same manner, with purification by simple precipitation in diethyl ether.

Hoyle, Lowe, and co-workers demonstrated a convergent synthesis of three-arm star polymers by a combination of reversible addition/fragmentation chain transfer (RAFT) polymerization and a thiol-ene click reaction. Terminal thiol containing polymers were synthesized by RAFT polymeri-




***Scheme 6.*** Thiol-ene click chemistry for the synthesis of a G1 dendrimer.

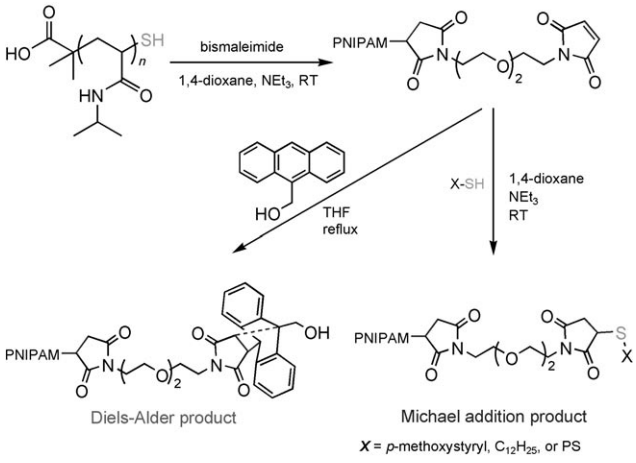
zation, and the chains were coupled to trimethylolpropane triacrylate to form star-shaped polymers.[23]

Thiol-ene chemistry has received renewed attention in the last two years, because of its simplicity, high reactivity, and the broad variety of available reagents. Furthermore, the reaction can be conducted under ambient conditions in relatively short reaction times. Thiol-ene click reactions indeed have a bright future for the synthesis not only of tailor-made macromolecules but also of small organic molecules and bioconjugates.

### 2.2.2. Michael Addition of Thiols

Thiol-terminated polymers can be easily prepared by RAFT polymerization, as mentioned in the previous section. This polymerization technique is well established, and various chain-transfer agents are available to yield well-defined functional polymers.[24]

Dove and co-workers reported metal-free thiol-maleimide click reactions for the functionalization of degradable polymers under mild conditions.[25] Moreover, Sumerlin and co-workers demonstrated the successful synthesis of block copolymers by Michael additions or Diels–Alder reactions on polymers prepared by the RAFT technique.[26] As illustrated in Scheme 7, the polymerization and following click reactions were all performed in the absence of any metal catalyst. The




***Scheme 7.*** End-group modification of poly(*N*-isopropyl acrylamide) (PNIPAM) with bismaleimide and subsequent Michael addition or Diels–Alder reaction.

Michael addition of maleimide-terminated poly(*N*-isopropylacrylamide) with sulfhydryl-terminated poly(styrene) (PS-SH) occurred under an inert atmosphere within 24 h at room temperature. The excess of PS-SH was removed from the reaction mixture by immobilization onto an insoluble iodoacetate-functionalized support, which represents an elegant method that avoids chromatographic purification steps. These model reactions confirm the potential of this methodology to combine RAFT-synthesized thiol-terminated polymers with a variety of other macromolecular thiols.

Another click reaction, reported by Nguyen and co-workers, was based on the base-catalyzed Michael addition of a thiosugar to a highly reactive enone.[27] $^1$H NMR spectroscopic analysis of the product obtained after 24 h at room temperature in acetonitrile revealed a completely stereoselective reaction and a yield of 94%.

Even though the reaction conditions employed in Michael additions do not (yet) meet the stringent criteria of click chemistry, these reactions provide a modular approach for the preparation of a variety of functional block copolymers synthesized by the RAFT technique. As such, Michael additions have the potential to become click reactions in the future.

### 2.2.3. Nucleophilic Substitution of Thiols with Amines

It is well-known in organic chemistry that the labile *para*-fluorine substituents of pentafluorophenyl ($C_6F_5$) groups can undergo nucleophilic substitution reactions with primary amino groups and thiols.[28] Mansuy and co-workers described a procedure for the preparation of functionalized polyhalogenated porphyrins in one step and high yields by selective substitution of the *para*-fluoro substituents of the $C_6F_5$ groups of meso-tetrakis(pentafluorophenyl)porphyrin by various nuchleophiles.[29]

Hoogenboom and Schubert demonstrated the application of this approach for functionalizing macromolecules to create well-defined multifunctional graft polymers (Scheme 8).[30]

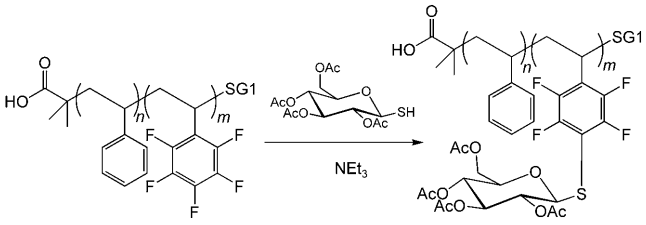

***Scheme 8.*** The *para*-fluoro-amine click reaction on a terpyridine (Tpy) functionalized well-defined copolymer of styrene and pentafluorostyrene. R-$NH_2$ represents 5-aminopentanol or α-amine-ω-hydroxypoly(ethyleneglycol). TIPNO = 2,2,5-trimethyl-4-phenyl-3-azahexane 3-nitroxide.

The reaction was performed in *N*-methylpyrrolidone under microwave irradiation using 5-aminopentanol or α-amino-ω-hydroxypoly(ethyleneglycol) as primary amines. This approach leads to the synthesis of graft polymers by the "grafting onto" method. Furthermore, side-chain functional-

ization of the polymer enables the synthesis of graft polymers by the "grafting from" method. For example, 5-aminopentanol can be attached to the polymer through a click reaction, and the hydroxy groups of the side chain initiated the ring-opening polymerization of L-lactide.

The synthesis of well-defined glycopolymers is often demanding since it requires mild reaction conditions to prevent degradation. "Grafting onto" a well-defined polymer can be preferred in the case when the monomeric units bear functional groups, such as alkyne or $C_6F_5$, on which sugar moieties can be anchored through a click reaction. However, the CuAAC reaction requires a copper catalyst, which makes the purification of the glycopolymers more challenging.[31] Therefore, metal-catalyst-free click reactions are valuable tools for the synthesis of glycopolymers. As illustrated in Scheme 9, well-defined copolymers of styrene and penta-




***Scheme 9.*** Synthesis of glycopolymers by the *para*-fluoro-thiol click reaction. SG1 = *N-tert*-butyl-*N*-(1-diethylphosphono-2,2-dimethylpropyl) nitroxide.

fluorostyrene (PFS) can be functionalized by a thioglucose derivative at room temperature in the presence of triethylamine as the base and *N*,*N*-dimethylformamide as the solvent. The kinetics of the substitution reaction were monitored by measuring $^{19}F$ NMR spectra at 40 °C and quantitative conversions were observed in less than one hour.[32]

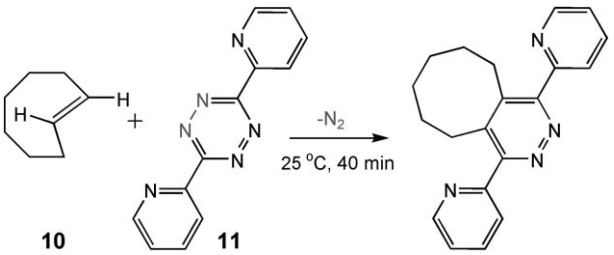
The versatility and efficiency of amine or thiol substitution at the *para* position of $C_6F_5$ satisfy most of the requirements of click chemistry. Furthermore, a wide range of primary amines and thiols are available in the databases, but the accessibility of $C_6F_5$ groups is rather limited, thus hindering the scope and modularity of the reaction.

### 2.3. Diels–Alder Reactions

Diels–Alder reactions were first documented in 1928[33] and they are amongst the most fascinating organic reactions, in terms of both their synthetic potential and reaction mechanism. Diels–Alder reactions involve the simultaneous formation and destruction of carbon–carbon bonds.[34] This reaction requires very little energy, and thus can be successful even below room temperature. There are several reports on Diels–Alder click reactions,[35] and here we highlight some recently published examples based on bioconjugates and macromolecules.

#### 2.3.1. Reaction of Tetrazines with Alkenes

Fox and co-workers reported a bioorthogonal reaction that proceeds with high reaction rates without the need for a catalyst.[36] The approach is based on the inverse electron demand Diels–Alder reaction of tetrazines with cyclooctynes. Subsequent retro-[4+2] cycloaddition produces $N_2$ as the only by-product. Scheme 10 shows the reaction of *trans*-cyclo-




***Scheme 10.*** [4+2] Retro-Diels–Alder reaction of *trans*-cyclooctene and tetrazine.

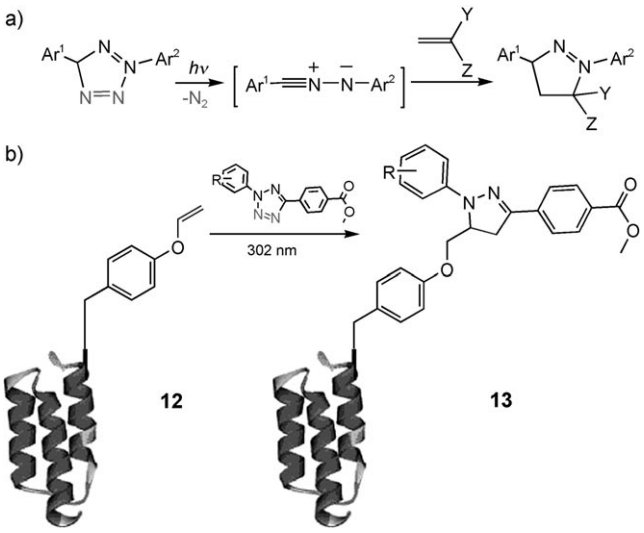
octene (**10**) and tetrazine **11**, which after 40 minutes at 25 °C gave a quantitative yield.[37] The reaction preserves its high reactivity in organic solvents, in water, and in cell media. In addition, functionalized *trans*-cyclooctene and dipyridyltetrazine were synthesized to broaden the scope of the retro-Diels–Alder click reaction.

Shortly after this report, Hilderbrand and co-workers demonstrated a tetrazine-based cycloaddition for the pretargeted imaging of living cells.[38] The reaction of norbornene and tetrazine led to multiple isomeric dihydropyridazines (as evidenced by LC/MS chromatography) since both norbornene and tetrazine are asymmetric compounds. The overall yield was reported to be greater than 93%. A similar approach using tetrazoles has been reported by Lin and co-workers.[39] As shown in Scheme 11 b, a genetically encoded alkene-containing protein in *Escherichia coli* could be selectively functionalized using a 1,3-dipolar cycloaddition with a photochemically generated nitrile imine. For this, BL21(DE3) cells expressing either wildtype Z or *O*-allyl-tyrosine containing Z-domain proteins **12** were suspended in the phosphate-buffered saline (PBS) buffer containing 5% glycerol and 100 μM of the tetrazole. Following incubation at 37 °C for 30 minutes, the cell suspensions were irradiated with UV light (302 nm) for 4 minutes. The bacterial cells were incubated at 4 °C overnight, which led to the cycloaddition reaction affording pyrazoline-Z (**13**) in quantitative yields. This photo-click reaction is illustrated in Scheme 11 a.[40]

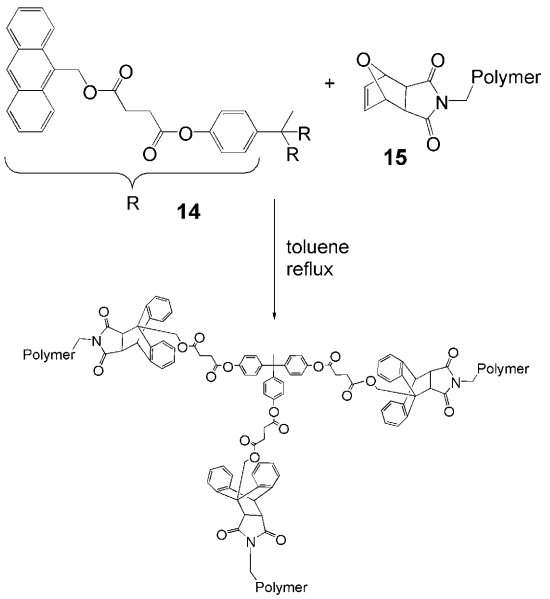
#### 2.3.2. Reaction of Anthracene with Maleimide

Tunca, Hizal, and co-workers reported the preparation of three-arm star polymers by Diels–Alder reactions.[41] In this approach they coupled furan-protected maleimide end-functionalized polymers **15** (poly(ethyleneglycol), poly(methyl methacrylate), and poly(*tert*-butyl acrylate)) with tri(anthracene) agent **14** (Scheme 12). Detailed size-exclusion chromatographic characterization showed the Diels–Alder click




***Scheme 11.*** a) Photoactivated 1,3-dipolar cycloaddition reaction between a 2,5-diaryl tetrazole and a substituted alkene dipolarophile. b) Selective functionalization of the Z-domain protein with an encoded *O*-allyltyrosine by a photoclick reaction.

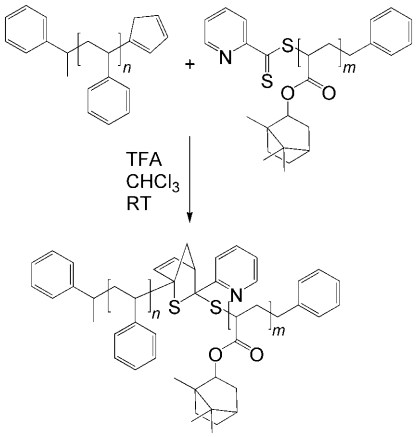



***Scheme 12.*** Schematic representation of the Diels–Alder click reaction for the preparation of star polymers.

reaction to be as successful as the CuAAC approach for the construction of star-shaped polymers. The authors demonstrated that a number of other copolymer architectures are accessible by Diels–Alder click reactions, sometimes in combination with CuAAC, thus exemplifying the wide scope and modularity.[35]

*2.3.3. Reaction of Dithioesters with Dienes*

Barner-Kowollik, Stenzel, and co-workers recently reported a convenient coupling method to access complex macromolecular systems.[42] In this approach, a RAFT polymerization of styrene was followed by a hetero-Diels–Alder cycloaddition reaction to yield a star-shaped polymer. By using an appropriate coupling agent and TFA as a catalyst, two-arm, three-arm, and four-arm star polymers were synthesized in yields of 91, 86, and 82%, respectively. Moreover, complete cleavage of the arms was achieved by heating the star polymers at 160°C for 24 h. This approach might be the inspiration for the development of reversible click reactions in the future. Very recently, the same authors reported ultrafast click conjugation of macromolecular building blocks at ambient temperature in just a few minutes in the absence of catalyst by using the more reactive cyclopentadiene (Scheme 13).[43] This click reaction is extremely efficient and




***Scheme 13.*** Formation of the poly(styrene-*b*-isobornyl acrylate) block copolymer by a fast hetero-Diels–Alder click reaction. TFA = trifluoroacetic acid.

allows the synthesis of block copolymers by macromolecular coupling just by shaking the reaction flask at room temperature.

There is no doubt that Diels–Alder reactions fulfill many of the requirements of click chemistry. However, long reaction times and high reaction temperatures, in particular for macromolecular systems, might be a limitation of this technique. It was noted by Tunca, Hizal, and co-workers that there could be some difficulties in accessing high molar mass star polymers because of steric hindrance caused by already formed arms, which is equally true for the copper(I)-catalyzed azide-alkyne cycloadditions.[41] Nevertheless, the use of electron-deficient dithioesters in combination with cyclopentadienes resulted in a dramatic improvement in the reaction rates in hetero-Diels–Alder reactions.[43] Therefore, this coupling procedure does qualify as a click reaction and should be seriously considered for the preparation of not only block copolymers but also bioconjugates.

## 3. Summary and Outlook

The use of the copper(I)-catalyzed azide-alkyne cycloaddition flourished in all fields of chemistry after the introduction of the click chemistry concept by Sharpless. Nonetheless, the need for metal-free click reactions, in

particular for biological applications, necessitated the development of alternative click reactions. In recent years, a wide variety of metal-free click reactions have been reported, such as copper(I)-free azide-alkyne cycloadditions with more reactive alkynes, radical addition between thiols and alkenes, Michael addition of thiols with maleimide, nucleophilic substitution of the *para*-fluoro substituent of pentafluorophenyl groups as well as regular and inverse electron demand Diels–Alder reactions. These alternative reactions comply with (most) of the requirements for click chemistry as formulated by Sharpless. However, additional investigations have to be performed before the modularity and scope of the different reactions can be determined.

The beauty and popularity of the azide-alkyne cycloaddition lies in the simple, readily available building blocks, whereas most metal-free click reactions involve rather large complicated reactive groups such as cyclooctyne, pentafluorophenyl, dipyridyltetrazine, and anthracene. Furthermore, the large size of the coupling units are disadvantageous for most applications. As such, it is probable that most of the hitherto-reported metal-free click reactions will remain beautiful academic examples rather than broadly applied methods.

The only simpler metal-free click reaction is the thiol-ene radical addition. The introduction of terminal alkene and thiol groups into a large variety of structures is straightforward, while the resulting thioether is even smaller than the 1,2,3-triazole that results from the azide-alkyne cycloaddition. Furthermore, the coupling procedure is even simpler than CuAAC since no catalysts are required other than UV light. As such, it is believed that thiol-ene click chemistry has the potential to become as broadly applied as CuAAC. In addition, the very recently discussed dithioester–cyclopentadiene conjugation seems to be a highly promising and simple method, although its wider applicability still has to be demonstrated.

The ongoing search for alternative metal-free click methods will definitely result in a range of other beautiful and/or broadly applicable coupling methods in the near future which might replace CuAAC in the long term. The development of these metal and catalyst-free click reactions will be of upmost importance for new applications in, for example, biological systems as well as lithography.

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