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Cycloaddition

Inverse-Electron-Demand Diels–Alder Reactions: Principles and Applications

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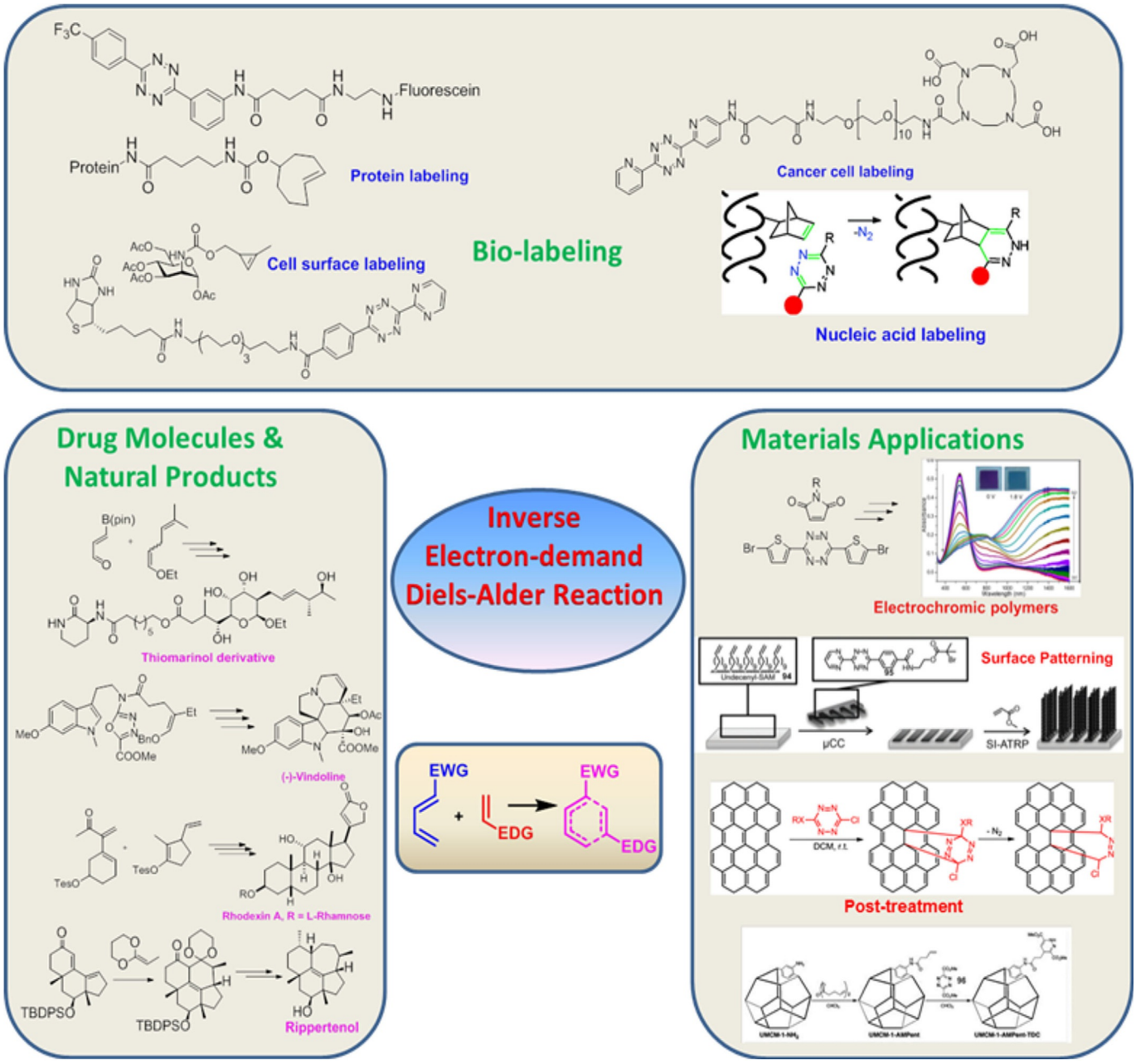

Abstract: Inverse-electron-demand Diels–Alder (iEDDA) reactions are an intriguing class of cycloaddition reactions that have attracted increasing attention for their application in bioorthogonal chemistry, the total synthesis of natural products, and materials science. In many cases, the application of the iEDDA reaction has been demonstrated as an innovative approach to achieve target structures. The theoretical aspects of this class of reactions are of particular interest for scientists as a means to understand the various factors, such as steric strain and electron density of the attached groups, that govern the reaction and thus to elucidate the reaction mechanism. This review aims to summarize both theoretical investigations and application-driven research work on the iEDDA reaction. First, the historical aspects and the theoretical basis of the reaction, especially recent advances in time-dependent density functional theory (TD-DFT) calculations, as well as catalysis strategies will be highlighted and discussed. Second, the applications of this novel reaction in the context of materials science, bioorthogonal chemistry, and total synthesis of natural products will be elaborated with selected recent examples. The challenges and opportunities of the iEDDA reaction will be highlighted to give more insight into its potential applications in many other research areas.

1. Introduction

The Diels–Alder reaction, discovered by Otto Diels and Kurt Alder in 1928,[1] is a key reaction in organic synthesis. Its popularity can be attributed to the reaction's simplicity, fast speed, extensive applicability, and mild reaction conditions. The efficacy of the Diels–Alder reaction can be increased by using electron-rich dienes and electron-poor dienophiles owing to matching of the diene's highest occupied molecular orbital (HOMO) with the dienophile's lowest unoccupied molecular orbital (LUMO). The inverse-electron-demand Diels–Alder (iEDDA) reaction was first discovered by Bachmann and Deno in 1949,[2] and it involves the reaction of an electron-rich dienophile with an electron-poor diene. In frontier molecular orbital (FMO) theory, this corresponds to interaction of the LUMO of the dienophile with the HOMO of the diene. It was independently recognized by both Hilderbrand[3] and Fox[4] in 2008 as a potential "click" chemistry method and has since been widely used in both the biochemical field and the materials field.

This review is divided into two main sections. First, the different mechanisms of the iEDDA reaction as well as the catalytic systems employed are discussed. Second, applications of the iEDDA reaction are reviewed, with examples from the synthesis of complex molecules, biolabeling, and materials science.

2. Mechanistic Studies of the iEDDA Reaction

Whereas the iEDDA reaction is usually recognized as a [4+2]-cycloaddition reaction,[5] its actual mechanism is sometimes disputed. Both concerted and stepwise mechanisms have been demonstrated in different studies. The exact mechanism may depend on the steric and electronic factors of the particular reaction partners.

2.1. Stepwise mechanism through a zwitterion

The De Rosa group has performed extensive research on the reaction of aminopyrroles with 1,3,5-triazines. As many as five intermediates have been identified by ^{1}H NMR, ^{13}C NMR, ^{15}N NMR, and ^{19}F NMR spectroscopy.[6,7] On this basis, a stepwise mechanism has been proposed (Figure 1). The first intermediate is zwitterion **3**. Zwitterions have also been isolated in other iEDDA experiments involving tetrazine.[8] In general, these zwitterions are detected if there are stabilizing electronic factors and steric factors inhibiting their cyclization.[7] A density functional theory (DFT) study performed on similar compounds[9] suggests that the 2-amino substituent helps to stabilize the zwitterionic intermediate by delocalizing the positive charge, which thus allows hydrogen bonding between the triazine ni-

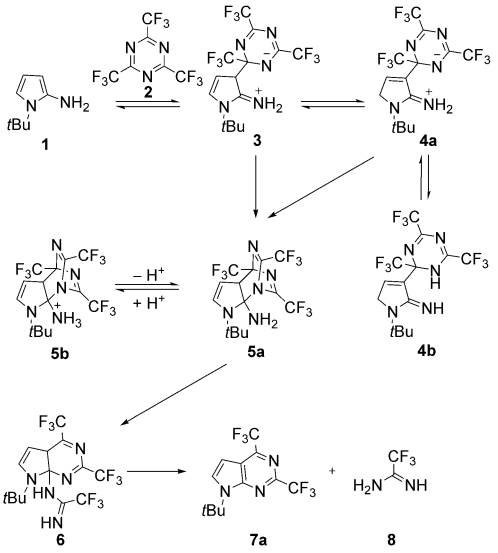

Figure 1. Proposed stepwise mechanism between 2-aminopyrrole **1** and 1,3,5-triazine **2**.

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trogen atom and the amino hydrogen atom. A certain amount of stabilization of the zwitterion has also been attributed to solvation energy. However, no direct evidence has been found for zwitterion **3**. Instead, its tautomer, zwitterion **4a**, has been detected. This is unsurprising given its greater stability caused by greater charge delocalization. The structure of **4a** has been confirmed by ^{1}H NMR and ^{15}N NMR spectroscopy. Another tautomer, imine **4b**, has also been detected. Upon cyclization, tricyclic adduct **5a** is formed, and it is in equilibrium with conjugate acid **5b**. Two possible paths follow from this adduct. The first is elimination of NH_3, which is followed by elimination of CF_3CN through a retro-Diels–Alder reaction. Conversely, retro-Diels–Alder elimination can take place before elimination of ammonia. A theoretical study suggests that the elimination of NH_3 should take place first, as the transition-state energy of this pathway is lower by as much as 50 kcal mol^{-1}.[10] However, there is no evidence for the generation of either NH_3 or CF_3CN. Instead, intermediate **6** is detected by ^{19}F NMR spectroscopy. Subsequent elimination generates the product and compound **8**. In a follow-up study, it was found that the addition of an acid to the mixture slightly increases the yield.[11] This is likely due to protonation of the triazine, which makes it more electrophilic.

2.2. Concerted mechanism

In the concerted mechanism, the product is obtained in a single step through a single transition state. At least two variations of the concerted mechanism may be distinguished. The mechanism is synchronous if both bonds are formed to the same extent during the transition state; conversely, the mechanism is asynchronous if one bond is formed to a greater extent than the other during the transition state.[12]

Gomez-Bengoa and co-workers have studied the iEDDA reaction between alkynylboronates and tetrazine by using both kinetic and DFT studies.[13] Compounds **9** and **10** are used as representatives of their respective functional groups, and the cycloaddition adduct is formed upon elimination of nitrogen (Figure 2). In principle, a bicyclic adduct is formed as an intermediate; in practice, this has never been observed, and computational studies indicate that the barrier to aromatization by elimination of nitrogen is virtually nonexistent.[14,15] The reaction can be followed by monitoring the disappearance of the absorption band of tetrazine at $\lambda = 533$ nm. The rate is second order, whereas an Eyring plot shows that the entropy of activation ($\Delta S^{\ddagger}$) is -41 cal mol^{-1} K^{-1}, which is consistent with the highly ordered transition state of a concerted mechanism. In

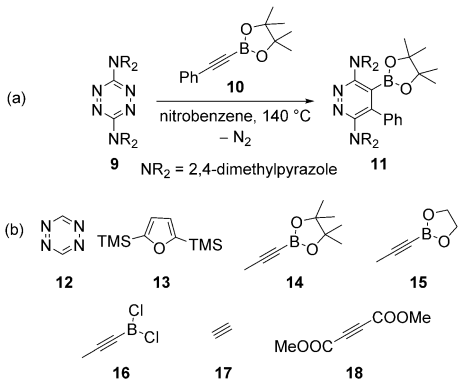

Figure 2. a) Reaction between alkynylboronate and tetrazine for kinetic studies. b) Tetrazine, furan **13**, and various alkynylboronates used in DFT calculations. TMS = trimethylsilyl.

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subsequent DFT calculations, the reactions between tetrazine **12** and alkynylboronates **14**–**18** were found to be highly synchronous with bond lengths during the transition state in a narrow range of 2.1 to 2.3 Å and bond orders between 0.28 to 0.37. This is in contrast with the same calculations for furan **13** and the same alkynylboronates, which are asynchronous with much greater differences in bond orders and bond lengths. For instance, the transition state of the reaction between compounds **13** and **16** has bond orders of 0.469 and 0.083, whereas the bond lengths are 1.97 and 2.88 Å, respectively.

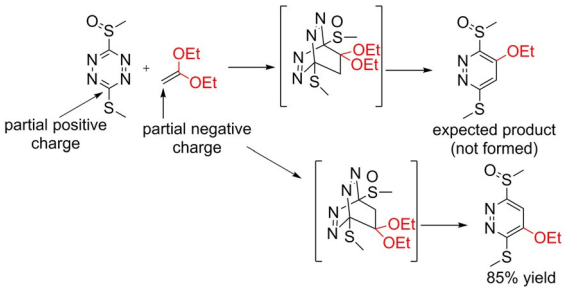

Figure 4. Unexpected regioselectivity of the iEDDA reaction.

2.3. Selectivity

2.3.1. Endo/exo selectivity

The selectivity of the iEDDA reaction generally follows that of the normal Diels–Alder reaction in that the *endo* adduct is usually the favored product.[16] A favorable secondary orbital interaction is the common explanation,[17] although this has been disputed by some researchers.[18] However, in some cases, the *endo* selectivity may be reversed.

For cases in which one of the cycloaddition partners is an ion, electrostatic repulsions may govern the *endo*/*exo* selectivity of the reaction. One such example is the reaction between the 2,3-dimethylisoquinolinium ion (**19**) and cyclopentadiene (**20**) (Figure 3a), for which the *exo* adduct is preferred over the *endo* adduct.[19] This has been validated computationally, as the *endo* transition state is approximately 2 kcal mol^{-1} higher in energy than the *exo* transition state owing to high electrostatic repulsive forces.[20] Another example of *exo* selectivity is the reaction of thione **21** with norbornene (**22**) (Figure 3b), and the selectivity is attributed to large distortions in norbornene and an eclipsed conformation of the transition state.[21]

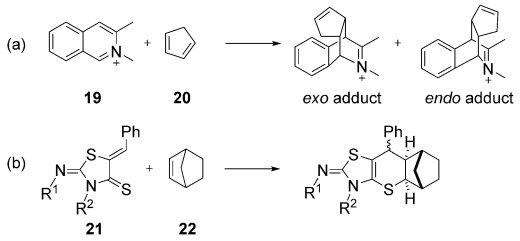

Figure 3. a) Reaction between the 2,3-dimethylisoquinolinium ion and cyclopentadiene. b) Reaction between norbornene and thione.

2.3.2. Regioselectivity

The regioselectivity of the iEDDA reaction can usually be predicted by zwitterionic models or FMO analysis.[22] The atom with the largest LUMO coefficient is expected to combine with the atom with the largest HOMO coefficient. Opposite formal charges are also expected to interact favorably. However, in 2006, Boger et al. found that sulfoxide-substituted tetrazines exhibit opposite regioselectivity if reacted with various electron-rich dienophiles.[23] One such example is shown in Figure 4. This remarkable regioselectivity was unexplainable by either FMO or zwitterionic models until 2009, at which time a DFT study performed by the Domingo group suggested that cyclization in the manner predicted by FMO is energetically unfavorable owing to a decrease in the electron density at the electronegative tetrazine core.[24] Although FMO theory is usually useful for predicting products, this study shows that it may give erroneous results for cases in which other factors are significant.

2.4. Catalytic systems for the iEDDA reaction

The iEDDA reaction is often a fast and simple reaction that takes place under mild conditions. However, catalysis is still required for some substrates owing to their lower reactivity. Some of these catalysis strategies include lowering the LUMO of the diene, raising the HOMO of the dienophile, or a combination of both to provide better HOMO–LUMO matching. This, according to FMO, results in more favorable interactions. Other strategies include transition-metal catalysis, the mechanism of which is not always clear. There is also interest in developing catalysis for asymmetric control of the reactions.

2.4.1. Lewis acidic lowering of the LUMO of dienes

1,2-Diazines are less often used in the iEDDA reaction owing to their relatively high-lying LUMOs, which makes them inert towards dienophiles. In 2010, Kessler and Wegner developed bidentate Lewis acid **25** to activate these diazines by complexing with the nitrogen atoms, thus lowering their LUMOs.[25] The proposed catalytic cycle is shown in Figure 5b. This catalyst has been tested by reaction with oxazolidine **24** (Figure 5a). In the absence of the catalyst, nearly no conversion is observed; the addition of just 5 mol% of the catalyst results in full conversion. Interestingly, the same reaction cannot be catalyzed by other common Lewis acids such as $BF_3{\cdot}OEt_2$, $MgCl_2$, $ZnBr_2$, and $TiCl_4$. DFT calculations predict lowering of the LUMO energy by 1.29 eV upon complexation with **25**, which is verified by an observable downward shift in the diazine signals in the ^{1}H NMR spectrum.[26] A wide range of dienophiles including enamines and dihydrofurans are also compatible with this mode of catalysis, despite the fact that water and free amines are released during the course of the reaction.[27] The application of this catalyst with oxyfurans results in a domino reaction involving first an iEDDA reaction and second a sigmatropic re-

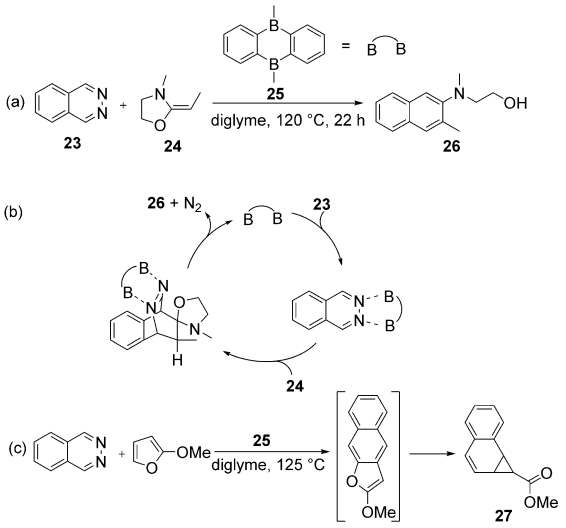

Figure 5. a) Catalytic iEDDA reaction of 1,2-diazine with oxazolidine. b) Proposed catalytic cycle of the above reaction. c) Domino reaction between 1,2-diazine and oxyfuran.

arrangement, which results in the formation of a benzonorcaradiene framework such as compound **27** (Figure 5c).[28] These are particularly useful building blocks for further transformation to increase the complexity in bioactive molecules.[29]

Another Lewis acid catalyst used in the iEDDA reaction was developed by Yamamoto to catalyze the reaction of tropone[30] (Figure 6). In the absence of the catalyst, the reaction with ethyl vinyl ether requires a high temperature of 120 °C and an ultrahigh pressure of 1.0 GPa.[31] In contrast, the presence of tris(pentafluoro)phenylborane enables the reaction to proceed at room temperature and atmospheric pressure. Other Lewis acid catalysts such as Me_3Al and Et_2Zn are ineffective and result in no conversion of tropone, decomposition of the ether, or formation of the [8+2] adduct.

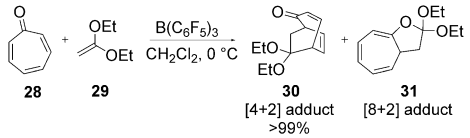

Figure 6. Reaction of tropone in the presence of tris(pentafluoro)phenylborone.

2.4.2. Raising the HOMO of the dienophile with impartation of chirality

Another strategy for catalysis is to raise the dienophile's HOMO. The Chen group reports the use of chiral amine **34** under acidic conditions to catalyze the reaction between crotonaldehyde (**32**) and diene **33** (Figure 7).[32] This strategy involves the use of the amine to convert crotonaldehyde into the corresponding dienamine, which thus raises its HOMO. Furthermore, the amine imparts chirality to the substrate, which results in an enantioselective reaction with *ee* values up to

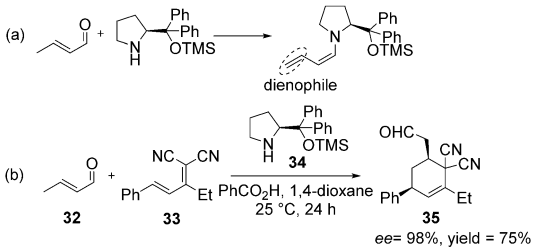

Figure 7. a) In situ conversion of an aldehyde into a chiral dienamine. b) Reaction between crotonaldehyde and a diene mediated by **34**.

98%. A similar strategy has been employed by the Wang group, who use L-proline as the amine catalyst.[33]

Whereas the method outlined by Chen makes use of steric shielding to increase regioselectivity (Figure 8a), Jørgensen makes use of a hydrogen-bond-directing approach[34] (Figure 8b,c). The hydrogen atom of the amino group of catalyst **38** forms a hydrogen bond with the ester oxygen atom of **37**, which directs the reaction. This method is more effective than the steric-shielding strategy in terms of chemical efficiency and stereoselectivity. For instance, in the reaction between compounds **36** and **37**, the use of steric-shielding catalyst **34** results in a conversion of 91% but only 25% *ee*. The same reaction performed with catalyst **38** gives the product with 78% *ee*.

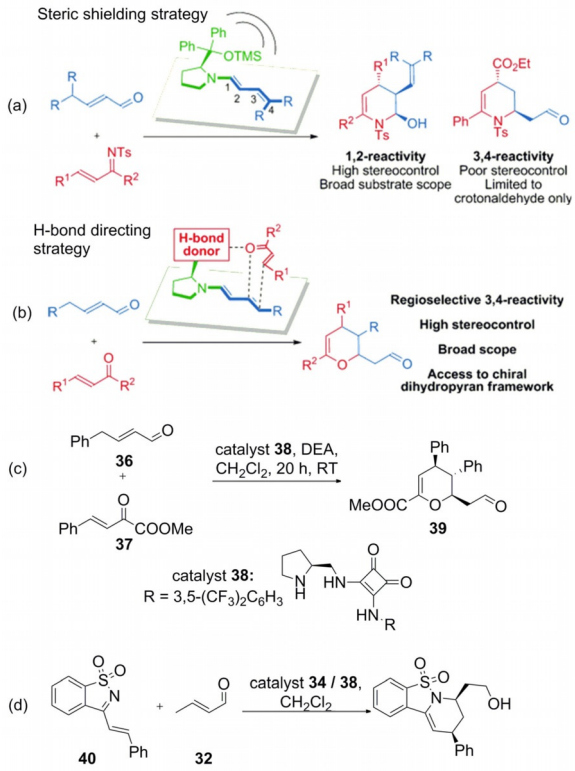

Figure 8. a) Chen's steric shielding strategy. b) Jørgensen's H-bond-directing strategy. c) Example of the effective use of the H-bond-directing strategy. d) Example of the effective use of the steric-shielding strategy. Ts = *para*-tolylsulfonyl, DEA = *N*,*N*-diethylacetamide. Panels a and b were reproduced with permission from Ref. [34]. Copyright 2012, John Wiley & Sons.

However, this method is less suitable for aza-Diels–Alder reactions, and Chen's method of steric shielding is able to catalyze those reactions.[35] In the reaction between **32** and **40** (Figure 8d), the use of hydrogen-bond-directing catalyst **38** results in the formation of the product in 45% yield with 55% *ee*. In contrast, making use of steric-shielding catalyst **34** results in the formation of the product in 80% yield with a much higher 88% *ee*.

2.4.3. Simultaneous raising of the HOMO and lowering of the LUMO

Raising the HOMO by using enamines is a feasible approach for aldehydes. However, this may not be sufficient to activate ketones, which are much less reactive. As such, there is motivation to develop a bifunctional catalyst to activate the reactants by both raising the HOMO of the dienophile and lowering the LUMO of the diene. The challenge lies in ensuring that the Lewis acid and Lewis base pair do not quench themselves.

Wang et al. have developed a catalytic system comprising primary or secondary amines bonded to a chelating agent with variable metal ions, as shown in Figure 9a.[36] Such a system has greater flexibility, as the Lewis acidic metal center may be changed at will to suit the reaction. Furthermore, the catalyst brings the pair of reactants together, which gives it intramolecular characteristics. This may also impart stereoselectivity to the reaction. As such, chelating catalyst **41** was developed. This catalyst has been tested against cyclohexanone and ester **42** (Figure 9b). Different metal salts have been screened, and $Cu(SbF_6)_2$, scandium(III) trifluoromethanesulfonate [$Sc(OTf)_3$], and europium(III) tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionate) [$Eu(fod)_3$] yield the aldol product, whereas $La(OTf)_3$, $Yb(OTf)_3$, and $Y(OTf)_3$ all give desired iEDDA product **44** with good chemoselectivity in yields of approximately 70%.

Whereas the catalytic system involving the use of **41** is useful in activating unreactive ketones, the requirement of metal ions may be inconvenient. Thus, in 2012 compound **45**, which is a single catalyst with bifunctional activation, was developed by Wang and co-workers.[37] This chiral catalyst contains an amine group to activate the dienophile through the in situ formation of an enolate and a thiourea group to activate the diene by hydrogen bonding (Figure 10). A similar thiourea–amine catalyst was previously reported in 2011 by Cai et al but was sidelined in favor of a cinchona alkaloid catalyst, which is also bifunctional.[38] Catalyst **45** has been tested in the reaction of unsaturated keto ester **46** with 2-nitrocycloketone **47**. Upon optimization of the reaction, the product is obtained in 76% yield with 93% *ee*. The catalyst can be further converted into a heterogeneous catalyst by covalent binding onto magnetic nanoparticles, which enables it to be recycled up to 10 times with little change in efficacy.[39] Magnetic nanoparticles are a good support owing to their ease of separation by magnets as well as their stability and low toxicity.[40] Thereafter, in 2013 and in 2014, Zhu et al.[41] and Li et al.[42] made further modifications to this thiourea-based catalyst to suit the requirements of their reactions.

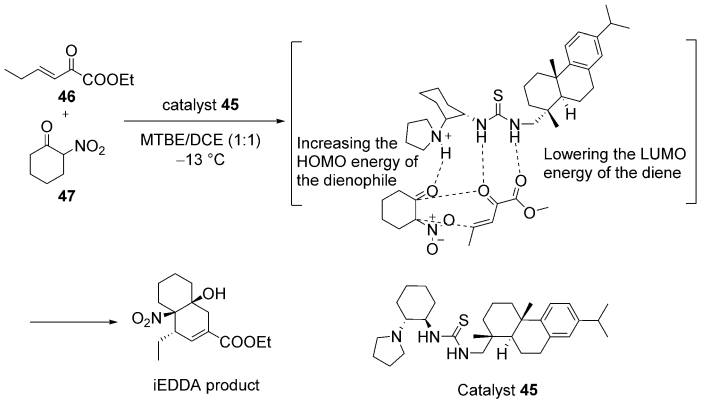

Figure 10. Proposed transition state for the bifunctional amine–thiourea catalyst. MTBE = methyl *tert*-butyl ether, DCE = 1,2-dichloroethane. Reproduced with permission from Ref. [37]. Copyright 2012, John Wiley & Sons.

2.4.4. Metal-catalyzed iEDDA reactions

In modern organic chemistry, metal catalysis is one of the most important areas of study with varying modes of activity.[43–46] It has also been used in some iEDDA reactions, although the mechanism of activation is not always clear.

We previously discussed the activation of 1,2-diazines with bidentate Lewis acids as well as their lack of activation by common Lewis acids. The only other known catalysts that are able to activate diazines are those developed by Rawal and coworkers. The first catalysts considered were those based on gold and silver owing to their high affinities for alkynes.[47] As expected, both gold and silver salts such as AuCl and AgOTf successfully give cycloaddition product **46**, although the yields are low. The low yields are partly attributed to bridging of the silver ions by the diazine, which results in a polymeric structure.[48] To surmount this, multidentate ligands such as ligands **47** and **48** have been used. A proposed mechanism is shown in Figure 11. There are two reasons to believe that the mode of activation is not merely coordination of the diazine to silver, which would result in lowering of the LUMO. First, other

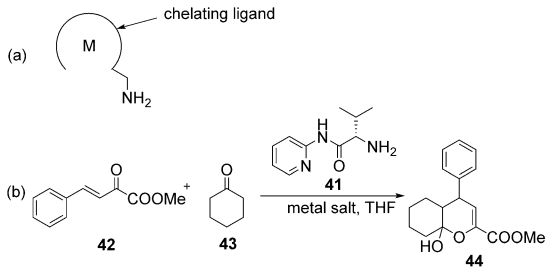

Figure 9. a) Primary amine attached to a chelating agent. b) Screening of the reaction between cyclohexanone and compound **42**. Reproduced with permission from Ref. [36]. Copyright 2011, John Wiley & Sons.

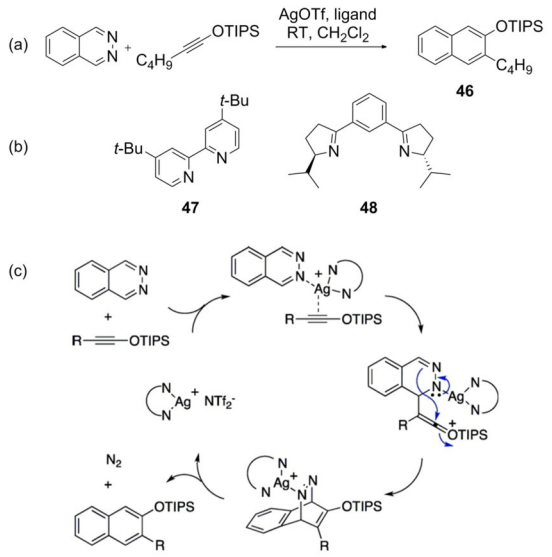

Figure 11. a) Silver-catalyzed reaction between a diazine and a siloxyalkyne. b) Ligands used for catalysis. c) Proposed mechanism for silver catalysis. TIPS = triisopropylsilyl. Reproduced with permission from Ref. [47]. Copyright 2012, American Chemical Society.

Lewis acids that are also able to coordinate to diazines are unable to catalyze the reaction. Second, no cycloaddition products are observed if ethoxyalkynes and -ynamides are used, although they are more electron-rich than siloxyalkynes. Furthermore, it is known that siloxyalkynes interact with silver bis(trifluoromethanesulfonyl)imide ($AgNTf_2$),[49] and other reports indicate that silver salts are able to catalyze iEDDA reactions between siloxyalkynes and pyridinium salts.[50] As such, a stepwise mechanism is proposed instead, with coordination of silver to both cycloaddition partners, followed by intramolecular addition and elimination of nitrogen. Other isoelectronic metals such as Ni^0 complexes and Cu^I salts have also been tested, and they are also effective in the same reaction, especially if large dissociated anions such as PF_6^- are used.[51]

Nickel has also been used to catalyze the iEDDA reaction between dienes and alkynes. It has previously been reported that nickel is able to co-oligomerize acrylates with alkynes to give dienes and that the reaction goes through a five-membered nickelacycle[52] (Figure 12a). Matsubara and co-workers report that a similar seven-membered nickelacycle results for the reaction between a diene and an alkyne and that subsequent reductive elimination results in a carbocycle[53] (Figure 12b). Various ligands have been screened, and PPh_3 has emerged as the most effective ligand. Highly functionalized arenes can be prepared by this method.

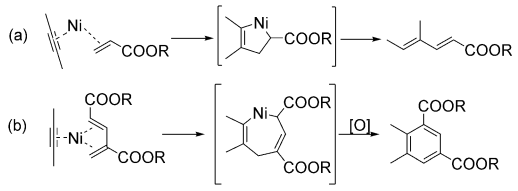

Figure 12. a) Nickel-catalyzed reaction of an alkyne with an acrylate. b) Proposed scheme for the nickel-catalyzed iEDDA reaction of a diene with an alkyne.

Similarly, asymmetric iEDDA reactions can be achieved by transition-metal catalysis. Evan and co-workers have developed enantioselective, chiral oxazoline–copper(II) catalysts for hetero-iEDDA reactions between acyl phosphonates and enol ethers[54] and for the preparation of dihydropyrans.[55] The Ishihara group has developed a chiral copper catalyst to enable enantioselective hetero-iEDDA reactions between keto esters and allylsilanes.[56] Feng et al. have also developed a copper catalyst by using chiral *N*,*N*′-dioxide–metal complex **51**[57] to control the competing hetero-iEDDA reaction and the Diels–Alder reaction between cyclopentadiene (**50**) and α,β-unsaturated carbonyl compounds such as **49** (Figure 13) with good control over selectivity. Diels–Alder product **52** is favored at room temperature, whereas hetero-iEDDA product **53** is favored at a low temperature of approximately −20 °C for a wide variety of substituents.

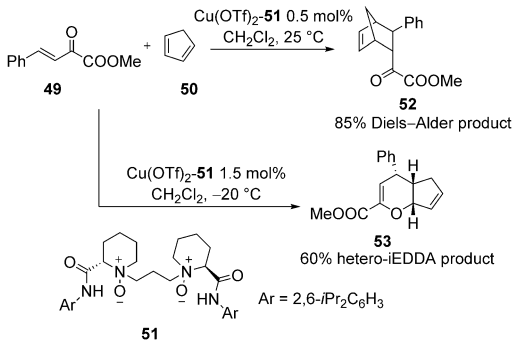

Figure 13. Copper-catalyzed iEDDA reaction with control of the chemoselectivity by temperature and control of the enantioselectivity by a chiral catalyst.

Recently, Lo et al. have developed a new class of luminogenic iridium pyridyltetrazine complexes.[58] The iridium center serves two purposes. First, coordination of the tetrazine to iridium enhances its reactivity towards strained alkynes. In addition, after the iEDDA reaction, the complex exhibits greatly enhanced emission spectra. This approach can potentially simplify the design of biorthogonal probes, as they would be inherently activated by the iEDDA reaction.

3. Applications of iEDDA Reactions

iEDDA reactions have been used in a wide variety of processes in both materials science and biochemical applications.

3.1. Biolabeling

Biolabeling is paramount in enabling the study of biological processes. One of the key technologies enabling this is the development of bioorthogonal chemistry. Bioorthogonal reactions are reactions that do not interfere with biological processes.[59] The ideal bioorthogonal reaction is stable under

physiological conditions yet is highly reactive towards the target group with a high rate of reaction.[60] In addition, the reagents must be bioavailable and nontoxic.[61] The ultimate aim for these reactions is for them to be applicable in live cells[62] or even living organisms.[63] Many bioorthogonal-ligation strategies have been developed, including the Staudinger ligation coupling reaction[64] and the strain-promoted azide–alkyne cycloaddition, which is performed in the absence of copper.[65] These methods and others have been extensively reviewed elsewhere.[66,67] The iEDDA reaction has been identified as an important bioorthogonal reaction,[68,69] and much work has been done to further its development. Its usefulness is greatly enhanced by its orthogonality to other click chemistries. For instance, the Slugovc group has demonstrated that the iEDDA reaction is orthogonal to both thiol-Michael addition and the copper-catalyzed azide–alkyne reaction.[70] Its selectivity is such that these three click reactions can be done sequentially in one pot with high yields and purity. Tetrazine, in particular, is the most widely used moiety, although other compounds, such as triazines, have also been reported.[71] In many cases, the synthesis and modification of tetrazines and their iEDDA partners are simpler than other bioorthogonal reactions. The following section reviews the recent exploitation of the iEDDA reaction in bioorthogonal biolabeling.

3.1.1. Dienophile for the iEDDA reaction with tetrazine

One of the first reports of the use of the iEDDA reaction as a bioorthogonal reaction was by Fox in 2008.[4] Tetrazine is a natural choice for bioorthogonal reactions because of its high reactivity, which allows for extremely rapid reactions or low concentrations. Its partner in this study is *trans*-cyclooctene, which is far more reactive than *cis*-cyclooctene. *trans*-Cyclooctene (**54**) reacts with tetrazine (**55**) with 100% conversion after 40 min at room temperature, even if the concentration is as low as 5×10^{-6} M (Figure 14a). The rate constant for the reaction is 2000 $M^{-1}s^{-1}$. This reaction has also been screened in the presence of EtSH and $BuNH_2$ and in the presence of cell media, all of which have no effect on the reaction. In fact, the reaction is faster in water than in methanol or THF. As such, thioredoxin can be functionalized with *trans*-cyclooctene (Figure 14b). The reaction is complete within 5 min of adding tetrazine (**55**), which shows the high rate of the reaction.

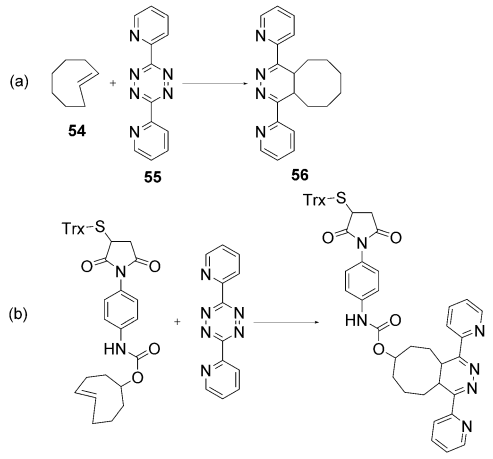

Figure 14. a) iEDDA reaction of *trans*-cyclooctene with a tetrazine at low micromolar concentrations. b) Testing the reaction with a functionalized thioredoxin. Trx = thioredoxin.

Whereas strained alkenes such as norbornene and *trans*-cyclooctene are well established, their hydrophobicity and steric bulk may have some effect on biomolecules. In view of that, Engelsma and co-workers have developed a more compact dienophile based on azetine[72] (Figure 15). The reasons for choosing azetine are threefold: First, strained alkenes such as azetine are activated for iEDDA reactions. Second, it has an electron-donating nitrogen atom next to the double bond, which also activates the alkene. Finally, it is the smallest possible core combining both effects, which thus reduces the hydrophobicity. This tag has been incorporated into epoxomicin and has been tested against a boron–dipyrromethene (BODIPY)-labeled tetrazine reporter reagent; it is just as effective as a norbornene reagent.

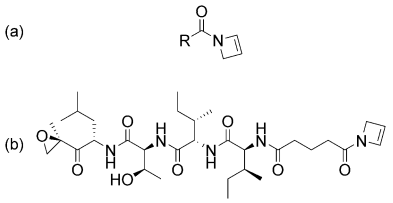

Figure 15. a) Azetine-based dienophile. b) Acylazetine-functionalized proteasome inhibitor epoxomicin.

3.1.2. Tetrazine in protein labeling

Chemical fluorophores are useful to image live cells. However, one of the challenges is to target them to a specific cellular protein. In 2011, Ting et al. made use of lipoic acid ligase (LplA) to introduce *trans*-cyclooctene derivative **57** into a protein[73] (Figure 16). Other variants of *trans*-cyclooctene have also been tested, but compound **57** is the most effectively ligated. Other research groups have made use of norbornene instead of *trans*-cyclooctene as the iEDDA partner.[74] LplA is able to recognize a LplA acceptor peptide (LAP) and site-selectively tag the protein of interest. The diene, tetrazine **58**, is conjugated to fluorescein. Upon reaction with the *trans*-cyclooctene-tagged protein, a 16.7-fold increase in the emission of fluorescein is observed, which allows for clear imaging of the tagged cells. Another site-selective tagging method involves expansion of the genetic code.[75] For instance, Mehl et al. have synthesized tetrazine-containing unnatural amino acid **59** and have incorporated it into a protein.[76] In its reaction with *trans*-cyclooctene **60**, the in cellulo rate constant is (72500 ± 1660) $M^{-1}s^{-1}$, which is fast enough to enable 95% labeling in less than 1 min at micromolar concentrations. This labeling method also does not require an additional washout step owing to the high rate of the reaction.

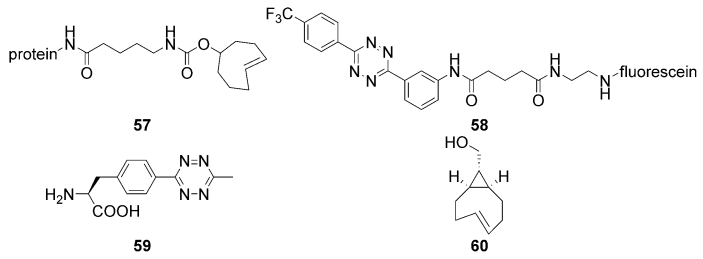

Figure 16. *trans*-Cyclooctene **57** tagged to a protein by enzymatic recognition, and tetrazine partner **58** tagged to fluorescein. Unnatural amino acid **59** with a tetrazine group in reaction with *trans*-cyclooctene **60**.

3.1.3. Tetrazine in cell-surface labeling

Other sites that can be labeled are the glycoconjugates on cell surfaces. These cell surfaces often change during disease, which makes their imaging-attractive targets. Wittmann and co-workers have prepared a mannosamine derivative with a cyclopropene group attached to it[77] (compound **61** in Figure 17). Incorporation of this compound into the growth medium of HEK 293T cells occurs over 2 days, and then the mannosamine derivative can be metabolically incorporated. Upon the addition of 25 μM tetrazine **62**, staining of the cell membrane is seen in 5 min, and in 15 min, staining is complete (Figure 17 b). In contrast, no staining of the cell membrane is seen if the labeled sugar is not added. Another strain-promoted azide–alkyne cycloaddition can also be performed simultaneously.

Cell-surface imaging can also be performed in a "double click" chemistry manner.[78] Initially, direct Staudinger ligation methods were used by treating azido-labeled sugars with a phosphine or cyclooctyne. This was unsuccessful, as the rate of the reaction was relatively slow, which required the use of high concentrations. As a result, there were significant background signals. A two-step labeling strategy, shown in Figure 18, was then developed. The first step is the conjugation of a bifunctional reagent to an azido-labeled sugar. The second step is the iEDDA reaction between the *trans*-cyclooctene group of the bifunctional reagent and a tetrazine conjugated to an imaging probe. The second step is four to five orders of magnitude faster than the first. Consequently, much lower concentrations of the tetrazine can be used, which lowers the background signal. This method allows imaging of surface tumor tissue. In principle, this is easily extendable to deeper tissues by using radio imaging.[79] This method can additionally reduce the radiation dose owing to the much lower concentration of the radiolabeled agent required.

3.1.4. Tetrazine in nucleic acid labeling

Traditionally, the copper-catalyzed azide–alkyne cycloaddition reaction has been the method of choice to modify nucleic acids,[80] especially after it was reported that the use of copper as a catalyst increases reaction rates.[81] However, the use of copper in an of itself has important drawbacks, including the oxidation of other products and its toxicity, which make its in vivo use difficult.[82] In contrast, the iEDDA reaction is naturally high yielding, catalyst free, and regioselective even under mild conditions, all of which make it a good choice for bioorthogonal reactions. As such, it has been employed to label DNA and RNA with fluorescent dyes. Jäschke et al. have post-synthetically modified DNA by introducing norbornene into oligonucleotides of 6 to 19 base pairs by solid-phase synthesis.[83] The norbornene-modified oligonucleotides are then conjugat-

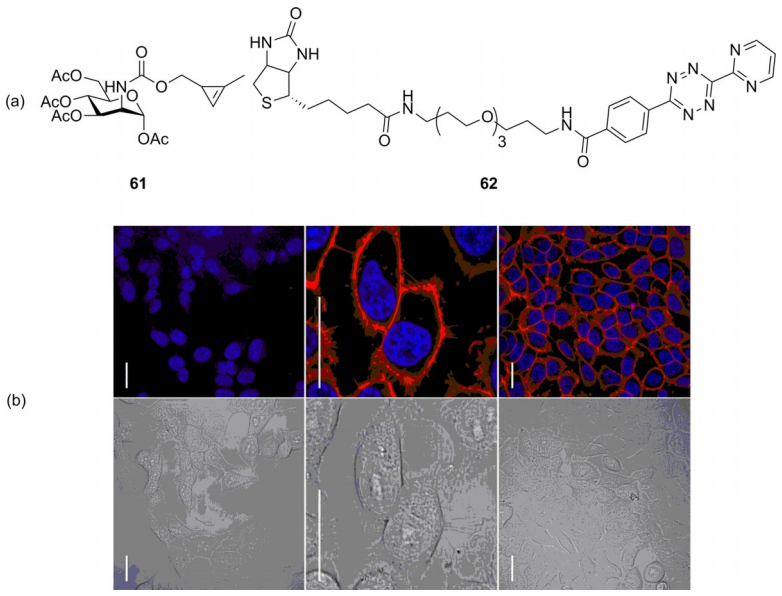

Figure 17. a) Cyclopropene **61** tagged to a cell membrane and activated by tetrazine **62**. b) Cells after staining with tetrazine **62** (25 μM, 15 min, 37 °C). Left) Cells grown without the addition of **61**. Middle and right) Cells grown with the addition of **61**. Scale bar: 30 μm. Nuclei stained with Hoechst33342. Reproduced with permission from Ref. [77]. Copyright 2014, American Chemical Society.

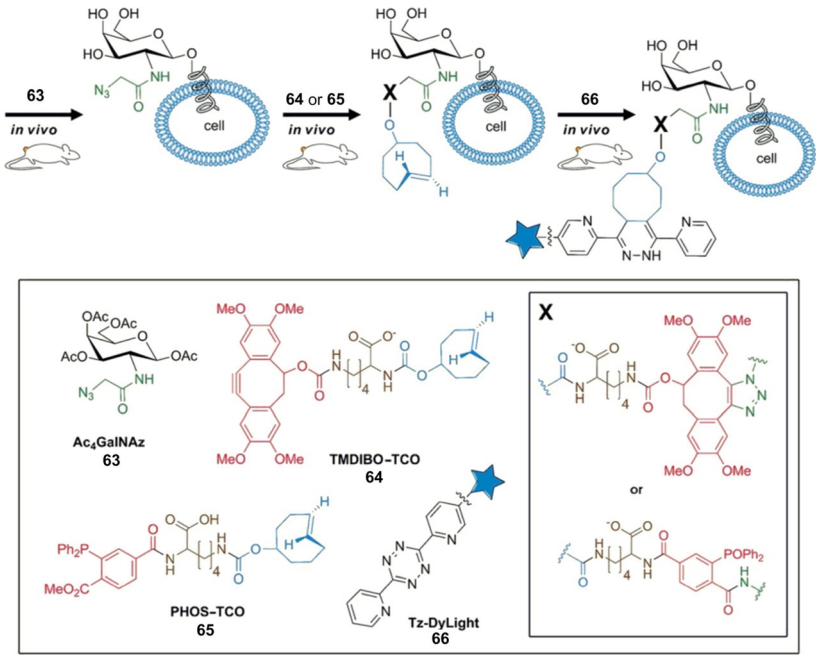

Figure 18. Mice were injected with **63** for 3 days, and it was incorporated into cell-surface glycans. The azido group reacted with the cyclooctyne moiety of the bifunctional group. The *trans*-cyclooctene group then reacted with the fluorophore-conjugated tetrazine. Reproduced with permission from Ref. [78]. Copyright 2013, American Chemical Society.

ed with tetrazine. Within 12 min, the reaction is approximately 50% complete, whereas up to 90% conversion is observed with a reaction time of 3 h. The oligonucleotides are then used as a primer to amplify a DNA with 109 base pairs by a polymerase chain reaction. This modified DNA is then treated with various derivatives of tetrazine (Figure 19a), and a product band is visible after 10 min. This shows that the iEDDA reaction can be used for the chemoselective tagging of both small chemically synthesized oligonucleotides as well as longer DNA strands. The same group has also made use of a similar method to label an RNA with up to 252 base pairs.[84] The use of *trans*-cyclooctene to tag RNA with a subsequent iEDDA reaction with tetrazine derivatives has also been reported.[85] The iEDDA reaction is also completely orthogonal to the copper-catalyzed azide–alkyne click reaction[86] (Figure 19b), which allows for dual tagging by both methods in a single step by mixing all the reagents together. This labeling has successfully been done in mammal cells,[87] although some difficulties can be encountered. For instance, the in vitro reactivity of tetrazine is different from its in-cell reactivity, and thus, optimization of individual reactions is required.

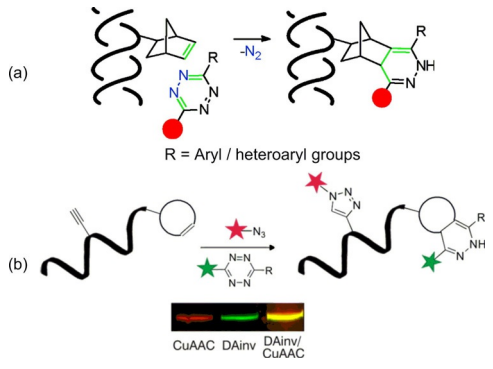

Figure 19. a) Tagging DNA oligonucleotides with norbornene, followed by labeling with fluorescent tetrazine dyes. b) Orthogonal labeling of DNA by both the copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction and the iEDDA reaction (labeled DA_{inv}). Reproduced with permission from Ref. [83]. Copyright 2011, Royal Society of Chemistry. Also reproduced with permission from Ref. [86]. Copyright 2012, American Chemical Society.

3.1.5. Tetrazine in cancer-cell labeling

The labeling of cancer cells has been performed by various research groups. The Weissleder group has targeted an anti epidermal growth factor receptor (EGFR) antibody, as EGFR is significant in cancer-cell signaling.[62] These antibodies are prelabeled with *trans*-cyclooctene as well as the Alexa Fluor 555 dye. The reaction is complete in 10 min after the addition of tetrazine conjugated with the Vivo-Tag 680 fluorophore. The resultant image shows excellent colocalization of both labels by the Alexa Fluor 555 dye and Vivo-Tag 680 (Figure 20), which indicates the high selectivity of the reaction of tetrazine.

Cancer-cell imaging has also been performed in live mice.[88] A tumor-targeting monoclonal antibody, CC49, is tagged with *trans*-cyclooctene and is then injected into live mice. One day later, tetrazine conjugated to radioactive [^{111}In] (Figure 21) is injected into the mice. After 3 h, single-photon emission shows accumulation of [^{111}In] at the site of the tumor. Some uptake is also observed in the blood and liver. Other probes that can be used in a similar manner include ^{68}Ga[89] and ^{18}F.[90] These radioactive tags are also often conjugated to tetrazine and are then reacted with a pretargeted group such as *trans*-cyclooctene. This pretargeting method is often superior to direct labeling

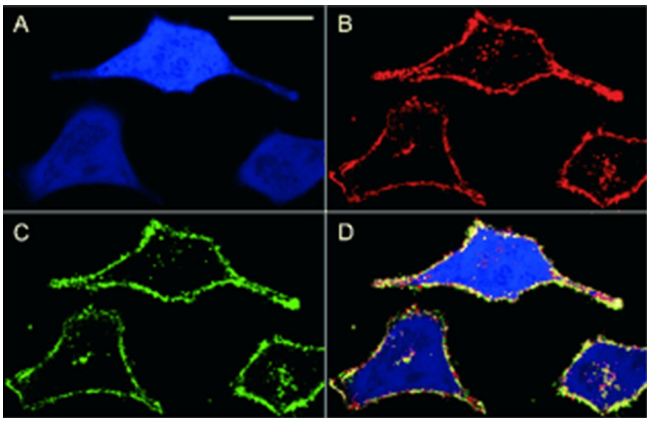

Figure 20. a) A549 Lung cancer cell. b) Red channel, indicating the labeling of Alexa Fluor 555. (c) Near-IR channel, indicating the labeling of tetrazine–Vivo-Tag 680 probe. d) Excellent colocalization of both red and near-IR channel shown. Reproduced with permission from Ref. [88]. Copyright 2009, John Wiley and Sons.

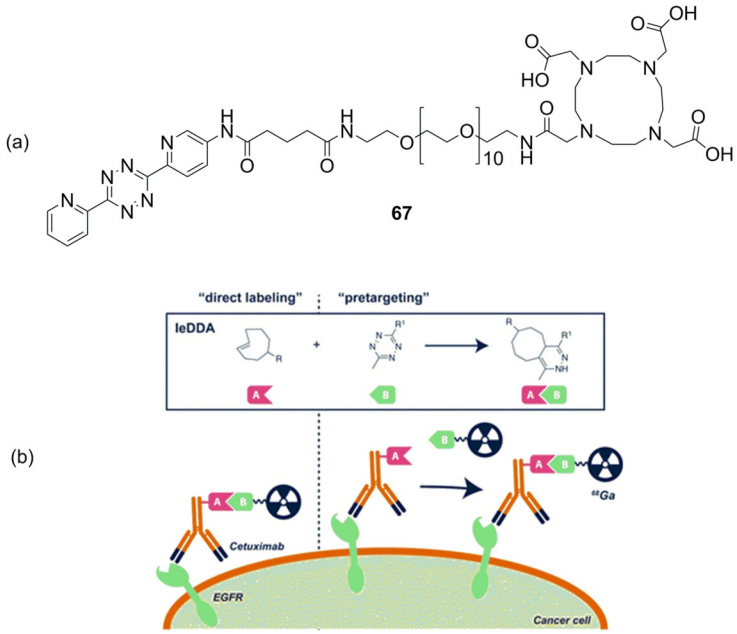

Figure 21. a) Tetrazine conjugated to a chelating agent for [^{111}In]. b) Schematic showing the difference between the pretargeting method and the direct-labeling method. Reproduced with permission from Ref. [89]. Copyright 2014, Royal Society of Chemistry.

methods, as the high rate of the iEDDA reaction enables the use of much lower concentrations of the radioactive probes, which reduces the radioactive dose to the patient.

3.1.6. 1,2,4-Triazines in bioorthogonal reactions

Tetrazines are the most popular substrates for iEDDA reactions owing to their high reactivity. However, this also results in lower stability in cells. 1,2,4-Triazines, on the other hand, are generally stable in physiological environments and can undergo iEDDA reactions with electron-rich dienophiles. Although they are less reactive than tetrazines, their overall higher stability still makes them useful for bioorthogonal reactions. Screening with *trans*-cyclooctene shows that the rate of the reaction is slower with 1,2,4-triazines than with most tetrazines but is similar to the rate of copper-free click reactions as well as some reactions with stabilized tetrazine.[71] Triazine can be incorporated into an amino acid and then incorporated into a protein by expansion of the genetic code. *trans*-Cyclooctene can then be successfully tagged to it, as shown in Figure 22. This shows that 1,2,4-triazines are also possible bioorthogonal reagents that may be appropriate if higher control of the rate of the reaction is required.

3.2. Synthesis of drug molecules and natural products

The iEDDA reaction is useful for the construction of many cyclic skeletons. Its mild and quick nature enables compatibility with many functional groups, whereas various catalysts also allow for control over stereochemistry. The following section gives an overview of some drug molecules and natural products synthesized with the iEDDA reaction as a key step.

3.2.1. Thiomarinol and its derivatives

Thiomarinol A is a natural product isolated from the bacterium *Alteromonas rava* sp. Nov. SANK 73390, which is found in the sea.[91] It is structurally similar to pseudomonic acid A, which has been used to treat skin infections. A derivative of thiomarinol (Figure 23) shows even greater potency than pseudomonic acid A. There is, thus, interest in the development of an efficient route for its synthesis. Gao and Hall report that the pyran core can be constructed by a stereoselective hetero-iEDDA reaction between **70** and enol ether **71**.[92] Substituted 2-enol ethers such as **71** are usually unreactive dienophiles. In this case, however, the activity of compound **70** helps to overcome this low reactivity, and the reaction is complete within 5 h at

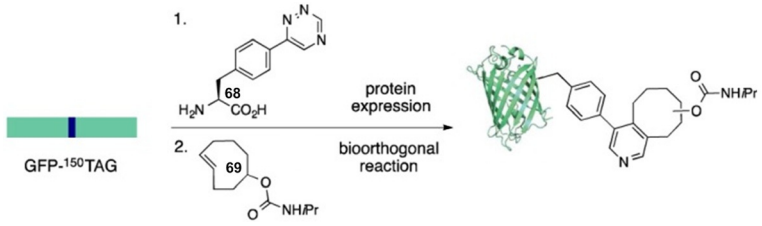

Figure 22. Protein with unnatural amino acid **68** tagged with triazine, and subsequent bioorthogonal reaction with *trans*-cyclooctene **69**. GFP-^{150}TAG = Green fluorescent protein tagged at amber codon 150. Reproduced with permission from Ref. [71]. Copyright 2015, American Chemical Society.

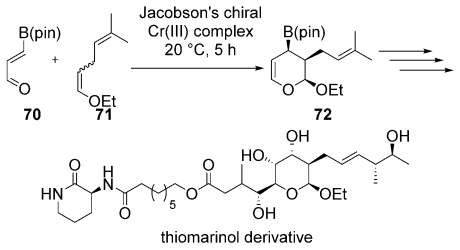

Figure 23. Asymmetric synthesis of thiomarinol derivative with a total yield of 22%. pin = pinacolyl.

room temperature. The use of Jacobson's chiral Cr^{III} complex has been previously reported[93] and allows for a highly enantioselective reaction. The rest of the synthesis is complete in an overall yield of 22%.

3.2.2. Vindoline

Vindoline is both a biosynthetic and a synthetic precursor of the natural products vinblastine and vincristine, which are used as antitumor drugs. Boger et al. describe a tandem intramolecular [4+2]/[3+2] reaction cascade enabling the creation of six stereocenters and substituents[94] (Figure 24). The key intermediate is compound **74**, which is prepared from compound **73** in three steps. The electron-rich enol ether first reacts with the electron-poor oxadiazole in an iEDDA reaction with loss of nitrogen to form carbonyl ylide **75**. Subsequently, the ylide undergoes a [3+2] cycloaddition with the indole group to form compound **76**, which can be carried forward to vindoline in four steps. This synthesis also allows for the convenient enantioselective synthesis of various related alkaloids, including minovine, *N*-methylaspidospermidine, and vindorosine.[95]

3.2.3. Pyrimidoblamic acid

Pyrimidoblamic acid is a key subunit of the anticancer drug bleomycin A_2. Its modification would enable an improvement in or the simplification of bleomycin analogues. Various total synthesis methods have been examined by Umezawa,[96] Hecht,[97] and Boger,[98] but there is often difficulty in controlling the stereochemistry of the tertiary carbon atom of the benzylic pyrimidine moiety Duerfeldt and Boger generate the pyrimidine core from an iEDDA reaction between a substituted electron-deficient triazine such as **78** (Figure 25) and an amidine such as **79**.[99] All the necessary stereochemistry is introduced before the cycloaddition. This method also enables the late-stage functionalization of pyrimidoblamic acid, which allows analogues to be easily synthesized. Recently, Glinkerman and Boger have adopted a similar strategy for the synthesis of methoxatin.[100]

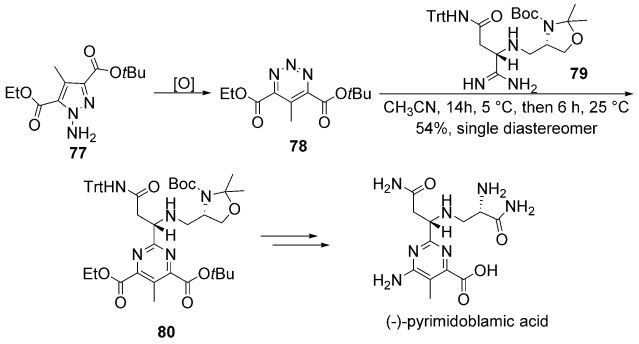

Figure 25. Synthesis of pyrimidoblamic acid with a late-stage cycloaddition. Trt = trityl, Boc = *tert*-butoxycarbonyl.

3.2.4. Rhodexin A

Rhodexin A is a natural product isolated from *Rhodea japonica*. It is active against human leukemia cells. Jung and Chu use an iEDDA reaction to prepare the four stereocenters of the tricyclic core in a single step[101] (Figure 26). This is done by treating diene **81** with dienophile **82** in the presence of Tf_2NH as the acid at −78 °C for 5 min. The reaction proceeds cleanly and gives the key intermediate, tricyclic compound **83**, in 86% yield. In later work, this key intermediate has been brought forward to rhodexin A[102] and three other analogues[103] by the same group, and in additional work, the authors make use of a more hindered diene, which necessitates the use of stronger Lewis acids and higher temperatures.[104]

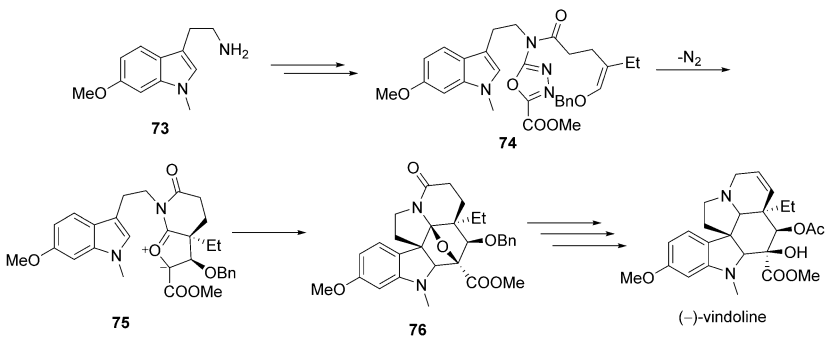

Figure 24. Synthesis of vindoline by a tandem intramolecular [4+2]/[3+2] cascade.

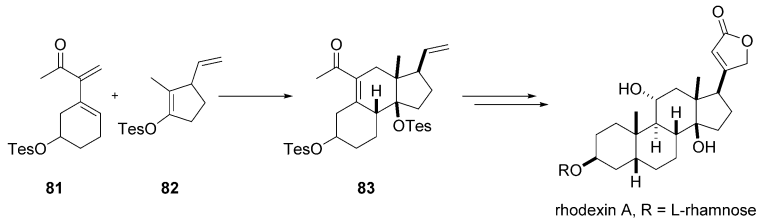

Figure 26. Synthesis of the key intermediate of rhodexin A by an iEDDA reaction. Tes = triethylsilyl.

3.2.5. Polemannone C

Polemannone C is a benzoxanthenone derivative from the tree *Polemannia montana*. Polemannon C is a homodimer of compound **84**. Lindsley et al. describe its total synthesis by using a novel catalytic system.[105] A previous biomimic synthesis of the related carpananone has been reported[106] with the use of $PdCl_2$ and NaOAc to catalyze the coupling and the iEDDA reaction. However, the same catalytic system is not successful for the more electron-rich polemannones. Instead, a new catalytic system comprising $CuCl_2$ and (−)-spartaine is used (Figure 27). The first step is an oxidative homocoupling reaction to form intermediate **85**. Intermediate **85** undergoes a spontaneous iEDDA reaction to give the desired compound, polemannone C, in an overall yield of 31.5% as a single diastereomer.

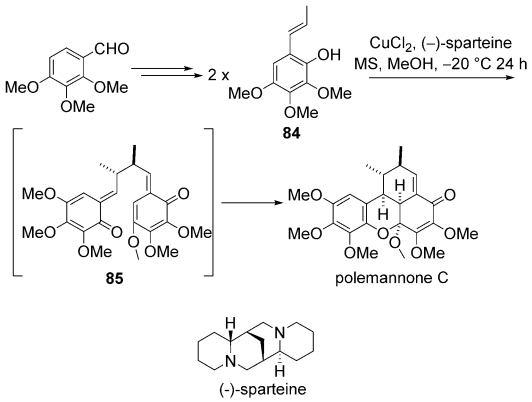

Figure 27. Homocoupling of compound **84**, followed by an intramolecular iEDDA reaction to form polemannone C. MS = molecular sieves.

3.2.6. Rippertenol

The total synthesis of rippertenol and its related compounds is typically difficult because of the lack of functionality on a stereochemically dense molecule with seven stereocenters. Snyder and co-workers have designed a total synthesis with early stereoselective installation of the quaternary methyl groups.[107] The seven-membered ring is the last to be formed, as Snyder notes that an iEDDA reaction would be able to control the diastereoselectivity. Subsequently, ring expansion would afford the seven-membered ring of rippertenol. Thus, the iEDDA reaction between **86** and **87** is complete in 10 min,

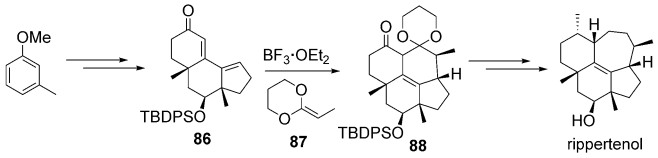

Figure 28. Total synthesis of rippertenol with late construction of the seven-membered ring. TBDPS = *tert*-butyldiphenylsilyl.

and the adduct is obtained in 68% yield with a diastereoselectivity of 2.6:1 (Figure 28). Interestingly, $BF_3{\cdot}OEt_2$ as a Lewis acid is required for the reaction to take place. As discussed previously, normal Lewis acids rarely directly catalyze iEDDA reactions. Subsequent ring expansion is then performed, and the target rippertenol is obtained in a stereocontrolled manner.

3.2.7. Halenaquinone

Halenaquinone is a natural product that is known to inhibit tumor cells.[108] Trauner et al. make use of an iEDDA reaction for the simultaneous construction of two rings[109] (Figure 29). This reaction proceeds slowly at room temperatures. Increasing the reaction temperature and adding a Lewis acid result in an increase in the rate of the reaction without an increase in the yield. Increasing the pressure to 1.0 GPa gives the product in 78% yield as a single isomer. DFT calculations reveal that the *exo* transition state is significantly lower in energy than the *endo* transition state, with synchronous bond formation. Subsequent aromatization yields (−)-halenaquinone.

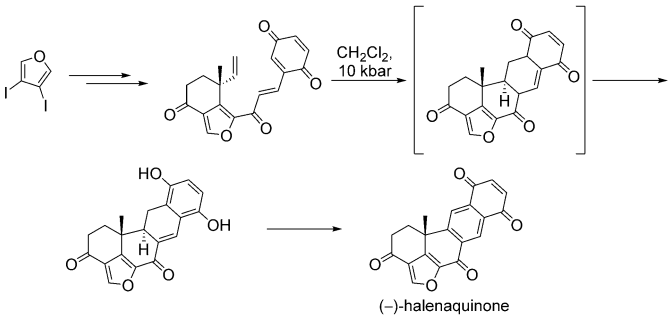

Figure 29. Total synthesis of halenaquinone by a high-pressure iEDDA reaction. 10 kbar = 1.0 GPa.

3.3. Materials applications

Apart from bio-labeling and total synthesis, the iEDDA reaction has also been used in a number of materials applications.

3.3.1. Electrochromic materials

Electrochromic materials give rise to a change in color with an applied electrical potential. One of the most effective materials is obtained through the donor–acceptor approach by alternating electron donors and acceptors, which results in low-band-gap polymers. Pyrrolo[3,4-*d*]pyridazine-5,7-dione **89**,[110] pyrrolophthalazine dione **90**,[111] and pyrroloacenaphthopyridazinedione **91**[112] are typical examples of electron acceptors prepared by our group (Figure 30). Similar monomers have also been applied to other fields such as organic solar cells.[113] These compounds can be synthesized by first preparing the perquisite tetrazine, which is followed by an iEDDA reaction with elimination of nitrogen to get the desired diazine electron acceptor. These iEDDA reactions are fairly challenging and require temperatures of 160 °C to proceed. This can be attributed to the electron deficiency of both the dienophile and the diene. However, no catalyst is required for the reaction. For compound **89**, DFT calculations show that the $HOMO_{dienophile}$–$LUMO_{diene}$ energy gap is 0.158 eV, whereas the $HOMO_{diene}$–$LUMO_{dienophile}$ energy gap is 0.127 eV.[110] Surprisingly, even though tetrazine is the diene, the $HOMO_{diene}$–$LUMO_{dienophile}$ gap is smaller, which indicates that a normal Diels–Alder pathway may be active for this reaction. This can be attributed to the highly electron-deficient character of the dienophile. The resultant materials have fairly good electrochromic properties with high optical contrast and good switching speeds.

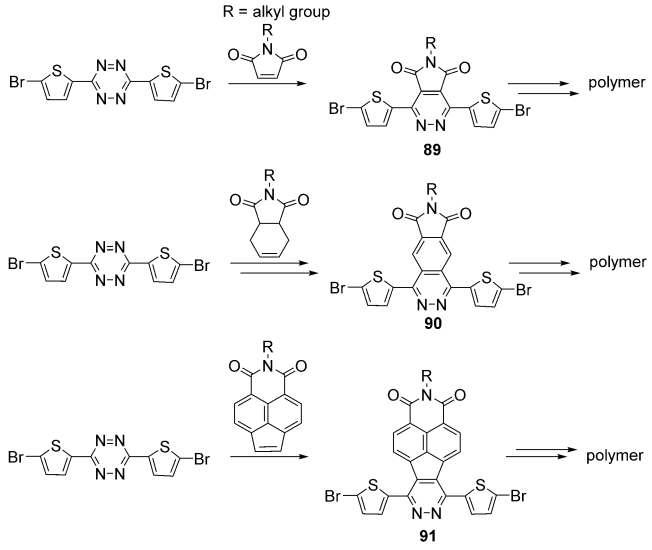

Figure 30. Pyrrolo[3,4-d]pyridazine-5,7-dione **89**, pyrrolophthalazine dione **90**, and pyrroloacenaphthopyridazinedione **91** as building blocks of donor–acceptor polymers.

3.3.2. Postpolymerization functionalization

Highly functionalized polymers are widely used in materials science with widespread applications. Efficient "click" chemistry may be used to prepare these polymers by a postpolymerization approach. Dove et al. have designed a polycarbonate scaffold with norbornene side chains that are able to undergo three different reactions under different conditions, namely, 1,3-cycloaddition with azides, the iEDDA reaction with tetrazines, and radical addition by thiols under UV radiation.[114] These three reactions are orthogonal to each other and may be performed in a sequential one-pot manner (Figure 31).

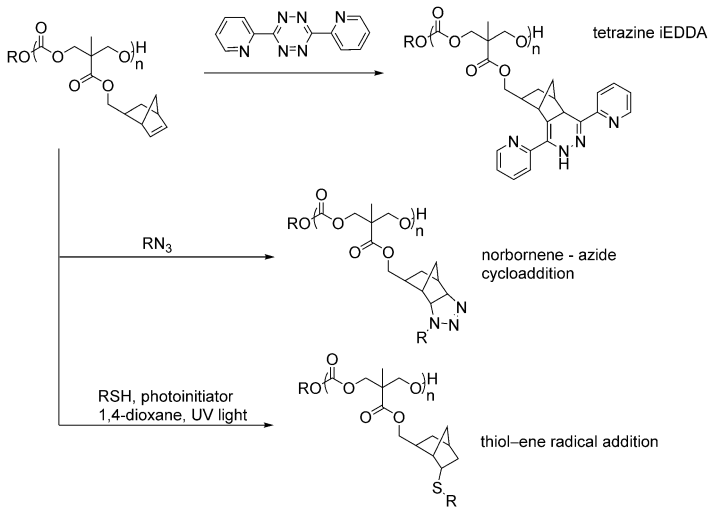

Figure 31. Orthogonal postprocessing of polymers in a sequential one-pot manner. Reproduced with permission from Ref. [114]. Copyright 2012, American Chemical Society.

This approach may be used to prepare a large number of functionalized polymers simply by varying the postprocessing conditions. A similar concept has also been applied for the postpolymerization modification of poly(ethylene glycol)s (PEGs).[115]

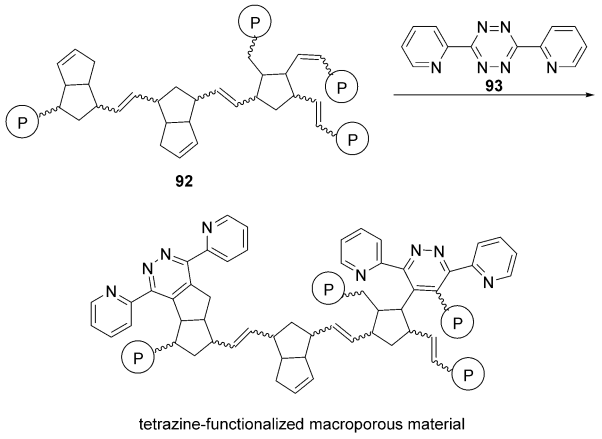

Figure 32. Postfunctionalization of macroporous materials with a tetrazine by the iEDDA reaction.

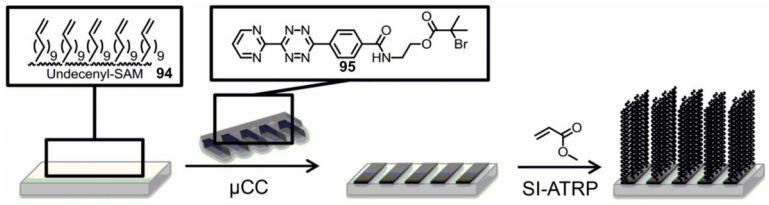

Figure 33. Surface functionalization by microcontact iEDDA chemistry. SAM = self-assembled monolayer, μCC = microcontact chemistry, SI-ATRP = surface-initiated atom-transfer radical polymerization. Reproduced with permission from Ref. [117]. Copyright 2014, Royal Society of Chemistry.

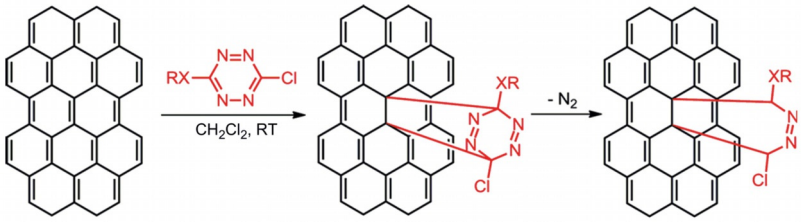

Figure 34. Functionalization of the graphite surface with gold nanoparticles by conjugation with tetrazine. RX = desired functionality. Reproduced with permission from Ref. [119]. Copyright 2014, American Chemical Society.

Another example of postpolymerization functionalization is the modification of macroporous foams. Macroporous foams are materials with highly interconnected pores at the microcellular level. Poly(dicyclopentadienes), such as **92**, are one such class of macroporous materials (Figure 32). Slugovc and coworkers report that the iEDDA reaction enables postfunctionalization of these materials in a single step.[116] Tetrazine **93** is added to the poly(dicyclopentadiene), and the mixture is heated for 2 days. Subsequent elemental analysis shows that every fourth double bond is functionalized. Surface analysis also shows that the open porous morphology of the polymer is maintained. This method can be further exploited by using other tetrazine derivatives for the postfunctionalization of materials.

3.3.3. Surface patterning

Surface patterning is an important aspect of materials science with applications in many areas. One method of surface patterning is microcontact chemistry, whereby reactive groups are brought into close physical contact with each other. Various click reactions including the azide–alkyne click reaction and the nitrile-oxide click reaction have been used on silicon and glass surfaces. The Diels–Alder and iEDDA reactions may also be used owing to their high reaction rates without the requirement for a catalyst. The Ravoo group has used alkene-functionalized self-assembled monolayer **94** as the support and have treated it with tetrazine **95**, which is conjugated to an atom-transfer radical-polymerization initiator[117] (Figure 33). Tetrazine undergoes an iEDDA reaction with the alkene, which results in functionalization of the surface with the radical initiator. Poly(methylacrylate) is then successfully grown on it, which shows the efficacy of the patterning. The reaction is efficiently complete within a few minutes at room temperature. Other applications include functionalization of surfaces with biosensors.

3.3.4. Carbon-surface functionalization

The functionalization of carbon nanotubes is of research interest owing to its potential applications in nanomaterials. For instance, nanoparticles bound to nanotubes may be used in drug delivery or catalysis. Covalent modification is generally

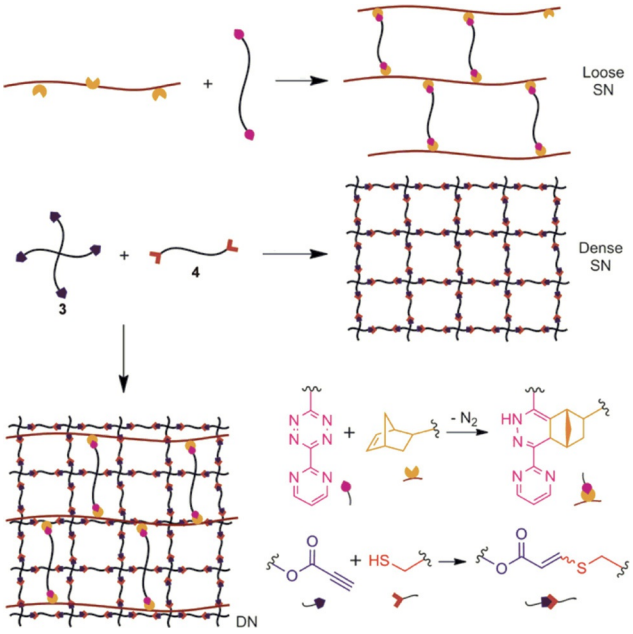

Figure 35. Formation of a double-network (DN) hydrogel by dual click chemistry. SN = swollen network. Reproduced with permission from Ref. [123]. Copyright 2015, American Chemical Society.

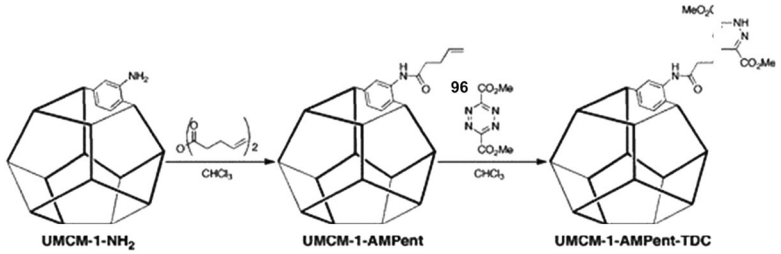

Figure 36. Postsynthetic functionalization of a MOF with a tetrazine. Reproduced with permission from Ref. [124]. Copyright 2011, American Chemical Society.

more useful than physical adsorption for functionalization owing to poor product stability. Schirrmacher and co-workers make use of the iEDDA reaction to functionalize carbon nanotubes by reaction with gold nanoparticles conjugated to tetrazine, and the sp^2 surface of the carbon nanotubes is the diene.[118] The reaction is performed at room temperature and at normal pressure. A similar method has also been used to functionalize a graphite surface with gold nanoparticles and fluorosilanes[119] (Figure 34). Fullerene may also be functionalized in the same way to achieve monoadducts.[120]

3.3.5. Hydrogels

Hydrogels are water-swollen network biomaterials with applications in cell encapsulation and drug delivery.[121,122] Double-network hydrogels are some of the strongest synthetic soft materials. However, they are, in general, difficult to synthesize, and free-radical polymerization and the use of toxic starting materials are often required. To simplify the synthesis of double-network hydrogels, Dove and co-workers make use of orthogonal click chemistry.[123] The two reactions are thiol–alkyne addition and the tetrazine–norbornene iEDDA reaction. These reactions take place under physiological conditions within 3 min of mixing the reactants (Figure 35). The synthesized materials have high mechanical strength and a water content of 90 wt%.

3.3.6. Postsynthetic modification

Metal–organic frameworks (MOFs) are crystalline solids with potential applications in gas adsorption and catalysis. Postsynthetic modification is a useful tool to introduce functionality into MOFs. The iEDDA reaction is an attractive reaction for this purpose. Cohen et al. have postsynthetically modified UMCM-1-NH_2 (University of Michigan Crystalline Material)[124] (Figure 36). UMCM-1-NH_2 is first modified with pentenoic anhydride to introduce an alkene group. Tetrazine **96** is then added to the modified MOF, and the reaction is complete at room temperature. A similar transformation has been done by the Rieger group on a zinc-based MOF.[125] These transformations help to circumvent the difficulties in the direct synthesis of prefunctionalized MOFs. Self-assembled supramolecular structures may also be modified in the same way, as reported by Nitschke.[126]

4. Conclusions

Like traditional Diels–Alder reactions, the inverse-electron-demand Diels–Alder (iEDDA) reaction has proven to be an invaluable addition to the chemist's toolbox. It joins two molecules together quickly and simply under mild conditions and with high yields, which allow it to be used in a wide variety of applications. It is also orthogonal to many other reactions, which makes it highly selective. All of these points give it the versatility and efficacy to take part in many types of applications. We have showcased various catalytic methods and understanding of the underlying theoretical model. Various applications in the synthesis of complicated molecules, biolabeling, and materials science have also been discussed. Whereas current applications are dominated by tetrazine-based dienes, other compounds such as triazines may also be further explored as potential iEDDA dienes. Further developments in this direction would allow for an even wider range of applications, and we expect the iEDDA reaction to be used more extensively in upcoming research.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biolabeling · cycloaddition · materials science · reaction mechanisms · synthetic methods

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