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Click and bioorthogonal reactions with mesoionic compounds.

Karine Porte⁺, Margaux Riomet⁺, Carlotta Figliola, Davide Audisio and Frédéric Taran*

Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), SCBM, 91191 Gif-sur-Yvette, France.

+ These authors contributed equally to this review.

ABSTRACT: Click and bioorthogonal reactions are dominated by cycloaddition reactions in general and 1,3-dipolar cycloadditions in particular. Among the dipoles routinely used for click chemistry, azides, nitrones, isonitriles and nitrile oxides are the most popular. This review is focused on the emerging click chemistry that uses mesoionic compounds as dipoles partners. Mesoionics are a very old family of molecules but their use as reactants for click and bioorthogonal chemistry is quite recent. The facility to derivatize these dipoles and to tune their reactivity towards cycloaddition reactions makes mesoionics an attractive opportunity for future click chemistry development. In addition, some compounds from this family are able to undergo click and release reactions, finding interesting applications in cells, as well as in animals. This review covers the synthetic access to main mesoionics, their reaction with dipolarophiles and recent applications in chemical biology and heterocycle synthesis.

CONTENTS

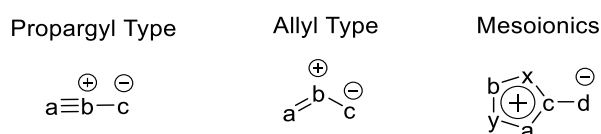
1. Introduction
2. General Information on Mesoionics
 - 2.1. Structure and properties of mesoionics
 - 2.2. Synthetic access to main families of mesoionics
 - 2.3. Reactivity of mesoionics in thermal cycloaddition reactions
3. Metal-catalyzed click reactions with mesoionics
 - 3.1. Copper(I)-catalyzed sydnone-alkyne cycloaddition
 - 3.2. Copper(II) promoted Sydnone cycloaddition with alkynes
 - 3.3. Copper(I)-catalyzed azaminosydnone-alkyne cycloaddition
4. Photo-induced click reactions with mesoionics
 - 4.1. "Photo-click" cycloaddition of sydnones with alkenes and alkynes
 - 4.2. Photo-catalytic reactions with sydnones

5. Strained promoted bioorthogonal reactions with mesoionics
 - 5.1. Sydnone-Cycloalkyne Cycloaddition
 - 5.2. Dithiolium- Cycloalkyne Cycloaddition
 - 5.3. Iminosydnone- Cycloalkyne Cycloaddition
6. Conclusions and Outlook

1. INTRODUCTION

The general principle of 1,3-dipolar cycloaddition introduced by Huisgen in 1961⁽¹⁾ for the synthesis of five-membered heterocycles, has turned out to be not only a very powerful tool for organic synthesis but also participated, 40 years later, to the foundation of click chemistry. This chemical transformation occupies indeed a prominent place in the 2001 seminal paper of K. B. Sharpless, introducing for the first time the concept of click chemistry⁽²⁾ and was the first example of click reaction, *i.e* the Cu-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC), published one year later concomitantly by K. B. Sharpless⁽³⁾ and M. Meldal.⁽⁴⁾ CuAAC is still one of the most popular click reaction because of its

incredible efficiency, chemoselectivity and compatibility with functional groups allowing ligation of very complex molecules. The Cu-free version of the reaction, developed by the group of Bertozzi using strained cycloalkynes, proved to be more biocompatible and bioorthogonal allowing the cycloaddition to proceed even inside living organisms.^{(5),(6),(7)} Since then, numerous other 1,3-dipolar cycloaddition reactions have been successfully developed for click and bioorthogonal ligations using either propargyl (such as isonitriles or nitrile oxides)^{(8),(9)} or allyl (such as nitrones)⁽¹⁰⁾ type dipoles.



Scheme 1: Types of dipoles used in click cycloaddition reactions.

Besides propargyl and allyl type dipoles, the mesoionics (Scheme 1) represent a vast family of dipoles which are very little explored and used for click reactions. Huisgen himself identified several mesoionic compounds able to react with dipolarophiles to form a diverse array of heterocycles,^{(11),(12)} but these transformations usually required harsh conditions and therefore did not fulfil the definition of click chemistry. The first example of click reaction using such dipole was published in 2013 by our group. Since then, a significant number of publications were reported in this area. Because of their cyclic structure, these dipoles have the capacity to form bicyclic intermediates following the [3+2] cycloaddition reaction with dipolarophiles, which can undergo subsequent reactions such as retro-Diels Alder reactions. This property makes mesoionics of particular interest to develop not only ligation but also cleavage reactions. In this review, we summarized the efforts accomplished during the last years to develop such click transformations.

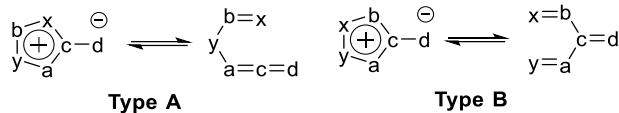
2. GENERAL INFORMATION ON MESOIONICS

The structure of mesoionic compounds is quite disconcerting at first glance. Significant investigations into mesoionic compounds began in the early 1930s with the work of Earl and Mackney who prepared *N*-phenylsydnone.⁽¹³⁾ It continued in the 1949 with Baker, Ollis and Poole who coined the term mesoionic (initially meso-ionic) as a combination of mesomeric and ionic to define this peculiar family of heterocycles.^{(14),(15)} Accordingly, belong to this group the heterocycles which cannot be represented satisfactorily by any Lewis structure not involving charge separation, and possessing a sextet of electrons in association with the atoms comprising the ring.^{(15),(16)} This definition was later restricted by C. Ramsden to five-membered rings.⁽¹⁷⁾ This early history of mesoionics was quite controversial. In 1955, Katritzky publically asked the scientific community to stop using this term and only qualify them as mesomeric betaines.⁽¹⁸⁾ The classification of dipolar compounds was still quite confusing until 1985, when Ollis, Stanforth and Ramsden reported a new classification according to the different types of conjugation of the heterocycles.⁽¹⁹⁾ Yet, we can still find papers erroneously associating the term mesoionic with zwitterions, ylides and six-membered betaines.

2.1. Structure and properties of mesoionics

Early on, mesoionics were grouped in two types **A** and **B**, according to the arrangement of their heteroatoms as depicted in Scheme 2.⁽²⁰⁾ Those two types show a clear resemblance, being only position isomers. Indeed, for type **B** mesoionics, the atoms contributing with two electrons to the π -system, **x** and **y**, are adjacent whereas they are separated by one of the atom with one-electron contribution **a**, **b**, **c**, **d** for type **A**. One interesting parameter depicting efficiently those two types is the hypothetical open-chain associated tautomer (Scheme 2). In the case of type **A**, this species would be linear whereas it is branched in the case of type **B** mesoionics. This tautomerism

has indeed been observed for some mesoionics and can explain their reactivity.



Scheme 2: Type **A** and Type **B** mesoionics. **a**, **b**, **c** and **d** represent atoms with one-electron contribution to the π -system. **x** and **y** represent atoms with two-electrons contribution to the π -system.

This categorization was supported by Ramsden's classification of heterocyclic mesomeric betaines, built *via* connectivity-matrix analysis.^{(19),(21),(22)} These studies, applied to a larger family than only mesoionics, recognized new classes and subclasses within heterocyclic mesomeric betaines. Type **A** mesoionics should be considered as conjugated betaines, while type **B** belongs to semi-conjugated heterocyclic mesomeric betaines. As suggested by their intrinsic definition, the representation of mesoionics is debatable.⁽²³⁾ For many years, the representation used in Scheme 2 was recommended, suggesting aromaticity of the heterocycle. Even if the conjugated system follows Hückel's rule, counting 6 π -electrons, the aromaticity of those rings was controversial and has been investigated by several groups. The mesoionic's reactivity towards electrophiles, similar to SnAr , and the strong mesomeric resonance were first used to justify the aromatic character of mesoionics.⁽²⁴⁾ On the contrary, the capacity of mesoionics to react as 1,3-dipoles is typically against aromatic behavior. Several calculations showed that substituents have large effects on the mesoionic properties and shouldn't be neglected.⁽²⁵⁾ The general atoms layout was studied by Bird, who discovered for instance that an endocyclic oxygen would reduce aromaticity.⁽²⁶⁾ In 2012, through four different calculated parameters in 54 mesoionics, Morzherin concluded that 1,2,3-triazoles and 1,2,3,4-tetrazoles are more aromatic than other mesoionic compounds, which can be considered more like masked dipoles.⁽²⁷⁾ The most recent

calculations were carried out by Champagne and Houk.⁽²⁸⁾ In accordance with other recent works, they concluded that bonding, charge and magnetic distributions confirm the absence of aromaticity. With these results, they highlighted that the most used representation of mesoionics is not the most accurate, with the exocyclic **c-d** bond showing more a double bond character according calculations, X-ray and IR. The recommended representations for both types **A** and **B** are depicted in Scheme 3 and will be the ones we choose throughout this review.

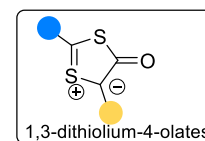
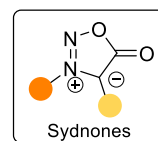
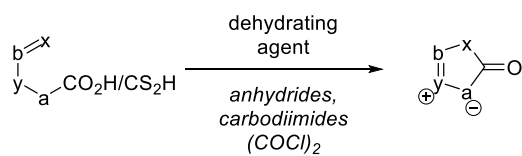


Scheme 3: Recommended representations of mesoionics.

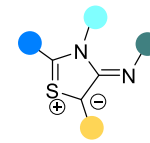
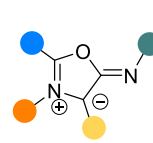
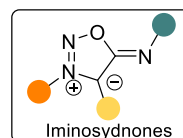
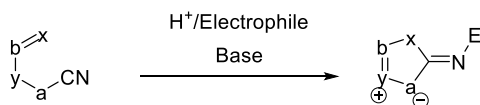
2.2. Synthetic access to main families of mesoionics

The synthesis of mesoionics has been the topic of numerous reviews.^{(20),(29)(30),(31)} Here, we will develop only the most representative mesoionics that have found applications in click and bioorthogonal reactions. Historically, four main strategies were used to construct the scaffolds of mesoionics. For most mesoionics having an exocyclic oxygen atom, the cyclodehydration of the corresponding carboxylic acid was utilized (Scheme 4a). This method was notably employed to prepare münchnones, sydnones and 1,3-dithiolium-4-olates. For their counterparts bearing an exocyclic nitrogen atom, the corresponding nitrile can be cyclized in presence of an electrophile and/or under acidic conditions (Scheme 4b). This method is typically used to obtain iminosydnones. A third strategy relies on mesoionic formation *via* the reaction of the linear precursor with phosgene and its derivatives (Scheme 4c). Finally, münchnones undergo 1,3-dipolar cycloaddition with heterocumulenes to form other mesoionics (Scheme 4d).

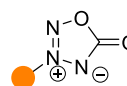
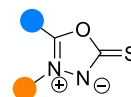
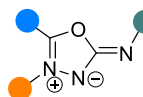
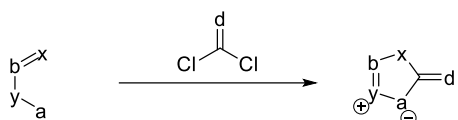
a) Cyclodehydration (C=O-mesoionics)



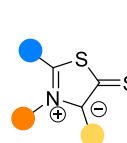
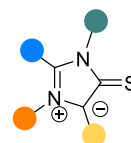
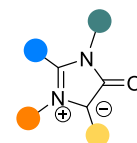
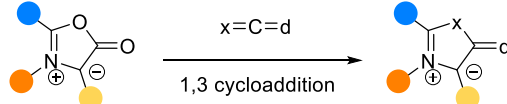
b) Nitrile activation (C=N-mesoionics)



c) Addition to dichlorocarbonyls



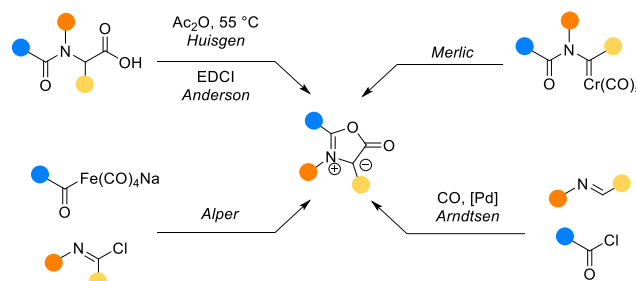
d) 1,3 Cycloaddition with Münchnones



Scheme 4: General synthetic strategies for the preparation of mesoionics.

Münchnones are one of the most relevant families of mesoionics. Despite their poor stability,⁽³²⁾ they are widely used for cycloaddition reactions, but are often generated *in situ* and only rare articles describe their isolation.⁽³³⁾ They are traditionally obtained *via* cyclodehydration of *N*-acylamino acids. Different activating agents were investigated to promote this step, including acetic anhydride,⁽³⁴⁾ carbodiimides or silicon tetrachloride (Scheme 5).⁽³⁵⁾ Activation of the amino acids with trifluoroacetic anhydride provided the 4-trifluoroacetylated münchnones, which were stable enough to be isolated.⁽³⁶⁾ A first alternative synthetic approach was provided in 1979 by Alper *et al.* using the reaction between acyl tetracarbonyl ferrates and imidoyl chlorides (Scheme 5).⁽³⁷⁾ A few other approaches were also described for the *in situ* generation of münchnones, including the alkylation of azlactones⁽³⁸⁾ or deprotection of siloxy

oxazoles.⁽³⁹⁾ They could also be efficiently assembled *via* CO-insertion in a chromium carbene complex (Scheme 5).⁽⁴⁰⁾ Finally, in 2002 the group of Arndtsen developed a new palladium-catalyzed synthesis of münchnones (Scheme 5).^{(41),(42)}



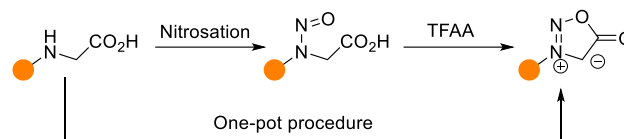
Scheme 5: Selected methods for the synthesis of münchnones.

This strategy was recently applied to the construction of poly-münchnone derivatives; their further derivatization enabled the authors

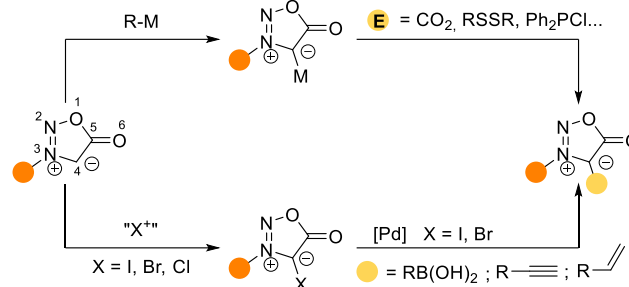
to obtain a vast series of polymers exploiting the whole array of münchnone reactivities.⁽⁴³⁾

Since their first preparation in 1935 by Earl and Mackney, the synthetic approach to generate sydrones did not evolved much (Scheme 6a).^{(13),(44)} This still sole synthetic route consists in the cyclodehydration of *N*-nitroso-glycine derivatives. Some improvements and modifications have been made, either concerning the nitrosation of glycines or the cyclization step. First achieved with acetic anhydride, the cyclization has proved to be faster using trifluoroacetic anhydride (TFAA).^{(15)a} It was also shown that other reagents, such as 2-halogeno-1,3-dimethylimidazolium salts, could promote the sydnone formation.⁽⁴⁵⁾ The nitrosilation step was also improved by Turnbull by using isoamyl nitrite, a milder reagent which requires only evaporation as work-up.⁽⁴⁶⁾ Finally, one-pot strategies were developed, avoiding the isolation of the toxic nitroso-amine intermediate.^{(47),(48)} In 2019, an alternative method using mechano-chemistry was developed by the group of Lamaty.⁽⁴⁹⁾ Interestingly, the sydnone core offers various post-functionalization possibilities, which have been the topic of several works, including reviews (Scheme 6b).^{(31),(50),(51),(52)} Indeed, the proton in position C₄ possesses an acidic character⁽⁵³⁾ and can be abstracted in presence of different organometallic species. The intermediate sydnone anion can further react with a large variety of electrophiles or participate in metal-catalyzed cross-coupling reaction *via* transmetalation.^{(31),(54),(55),(56),(57)} Among the possible functionalizations, halogenations (typically achieved in presence of *N*-halosuccinimides) were of particular interest as 4-halo-sydrones are good substrates for cross-coupling reactions.^{(58),(59),(60)} More recently, Moran discovered that direct arylation, alkenylation and alkynylation could be performed with 4-*H*-sydrones.^{(61),(62)} Following this work, Suzuki couplings and oxidative Heck reactions were developed.^{(63),(64)}

a) Synthesis of the sydnone core



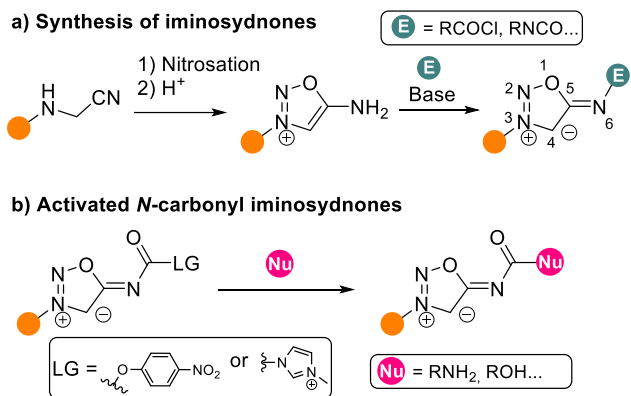
b) Derivatization of sydrones



Scheme 6: Synthesis and functionalization of sydrones.

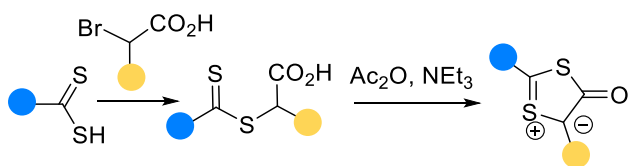
Less popular than sydrones, iminosydrones (or sydnonimines) share common structural features and properties with them.^{(52),(65)} They are obtained *via* a unique synthetic route used since their discovery (Scheme 7a).^{(66),(67)} This strategy relies on the acidic cyclisation of *N*-nitroso derivatives of α -aminoacetonitriles, thus generating the resulting iminosydnone scaffolds as salts. These iminosydnone salts, which do not bear any substituent on the exocyclic nitrogen atom (position N₆), are not stable under basic conditions and undergo ring opening into the corresponding nitrile precursors.⁽⁶⁸⁾ Iminosydnone salts can however react with diverse electrophiles resulting in stable products (Scheme 7a).^{(69),(70),(71),(72),(73)} Nonetheless, depending on the nature of substituents on the mesoionic and the electrophile, this operation might be less trivial than expected. In order to facilitate the exocyclic *N*-functionalization, two alternative strategies using activating groups on the N₆ position have been developed: one using a *p*-nitrobenzyl carbamate and the other with an imidazolium urea (Scheme 7b). Those key intermediates can be further functionalized in presence of a large variety of nucleophiles.^{(74),(75)} The other possibilities to further derivatize iminosydrones are relatively similar to those used for sydrones, since the C₄ position is

nucleophilic and acidic, as well. However, lithiated iminosydones are less stable and less nucleophilic than their sydnone counterparts.^{(31),(76)} Halogenation of the C₄ position is also possible, but might be capricious.⁽⁷⁷⁾



Scheme 7: Synthesis of iminosydones.

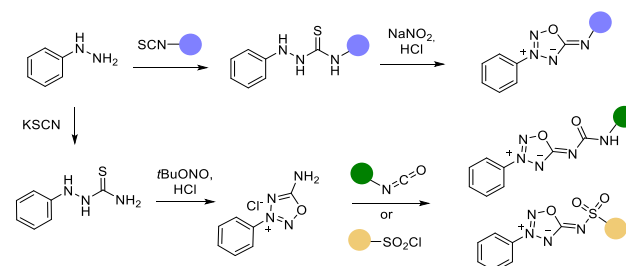
1,3-Dithiolium-4-olates (DTOs) are quite reactive mesoionics (*vide infra*), although their chemistry was poorly explored.⁽⁷⁸⁾ First reported in 1964,⁽⁷⁹⁾ the synthetic access to DTOs generally follows a three-step approach. A Grignard addition onto carbon disulphide (CS₂), followed by nucleophilic substitution of α -bromophenyl acetic acids by the dithioic acids, and final cyclodehydration, with an acetic anhydride/triethylamine system, will provide the desired mesoionic (Scheme 8).^{(80),(81),(82)}



Scheme 8: Common route to 1,3-dithiolium-4-olates (DTOs).

Azaminosydones (AIS) are a family of mesoionics which recently drew a new attention. Compared to the other mesoionics, they were first reported quite recently by Jaźwiński *et al.*⁽⁸³⁾ They are obtained in a two-step sequence starting from the corresponding hydrazine and isothiocyanate, which react to generate the

substituted thiosemicarbazide intermediate and, after nitrosylation, cyclizes to the corresponding mesoionic. While this strategy is suitable to introduce aryl and alkyl substituents on the exocyclic nitrogen, a different procedure is necessary to install a carbonyl or sulfonyl group (Scheme 9). This requires the use of potassium thiocyanate to access the unsubstituted thiosemicarbazide, which is next cyclized to the corresponding acid chloride salt and finally substituted in presence of a suitable electrophile.

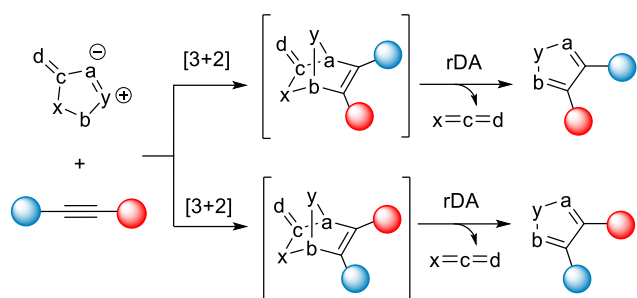


Scheme 9: Synthesis of aza-iminosydones (AIS).

2.3. Reactivity of mesoionics in thermal cycloaddition reactions

The ability to undergo thermal cycloadditions with dipolarophiles is a common property shared by many mesoionics.^{(30),(84)} The overall transformation, depicted in Scheme 10, is a two-step process, involving an initial [3+2] cycloaddition between the mesoionic dipole and the dipolarophile, followed by a subsequent retro-Diels Alder (rDA) reaction. The process generates the heterocyclic product, as well as the rDA byproduct with general structure $x=c=d$ (Scheme 10). The reaction with the activated alkyne dimethyl acetylene dicarboxylate (DMAD) is a benchmark transformation, that allows to assess the reactivity of a mesoionic. Despite its activated nature, the cycloaddition with DMAD requires high temperatures. In addition, when non-symmetrical alkynes are utilized, the low regioselectivity of the cycloaddition is often a practical limitation of this synthetic strategy. Therefore, efforts have been made to obtain reactivity under milder conditions and to control the regioselectivity of the cycloaddition. This can

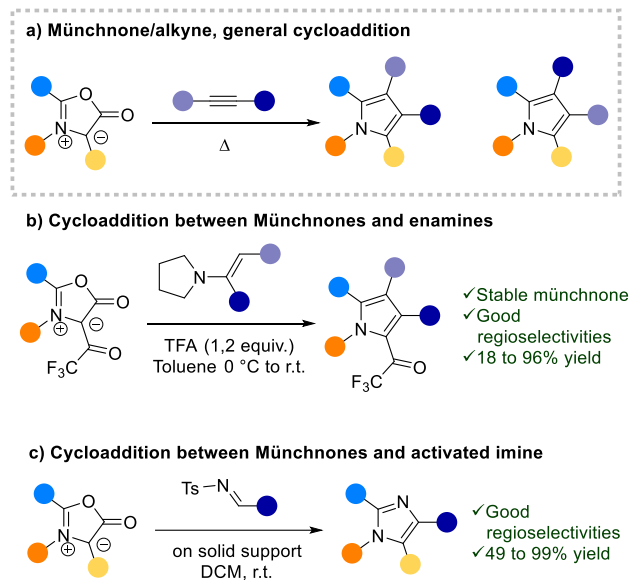
be achieved either by engineering the substrates or by using additives.



Scheme 10: General representation of the thermal cycloaddition between mesoionics and alkynes.

One of the most studied mesoionics in cycloaddition chemistry are münchnones.⁽³³⁾ Their reactivity with alkynes, discovered by Huisgen in 1964, leads to the formation of pyrroles (Scheme 11a).⁽³⁴⁾ The reaction was expanded to diverse alkynes and its regioselective outcome was extensively studied.^{(85),(86),(87)} Many insaturated compounds were used as dipolarophiles with münchnones under thermal conditions. Among them can be found maleimides and other electron-deficient olefins.^{(88),(89),(90),(91),(92),(93),(94),(95)} In 2019, enamines could be activated under acidic conditions to react at 0 °C with 4-trifluoroacetylated münchnones and generate substituted pyrroles with a high degree of selectivity (Scheme 11b).⁽⁹⁶⁾ Münchnones could also react with carbon-heteroatom insaturations. Typically, they exhibited excellent reactivity with activated tosylimines providing imidazoles with good to excellent yields and as one single isomer (Scheme 11c).⁽⁹⁷⁾ In the case of strained imines such as 2-phenylbenzazete, the cycloadduct product undergoes a ring expansion and extrusion of carbon dioxide to obtain benzodiazepines.⁽⁹⁸⁾ Finally, münchnones were able to react smoothly with some nitrogen-based dipolarophiles such as diazoniums and nitroso-benzene.^{(99),(100)} Münchnones derivatives bearing a phosphorus atom instead of the nitrogen atom, called phospha-münchnones or montréalones were very reactive with alkynes, electron poor olefins and imines.^{(101),(102),(103),(104)} Although

those cycloadditions could take place at room temperature, montréalones, like münchnones, have to be generated *in situ*.⁽¹⁰⁴⁾ This drawback has thus far precluded their utilization in the field of click and bioorthogonal chemistry.

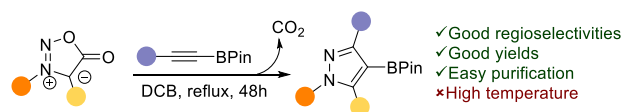


Scheme 11: Reactivity of münchnones.

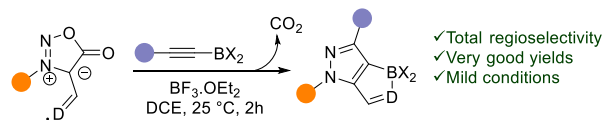
Sydnone is another major class of mesoionics whose reactivity was extensively investigated. Generally less reactive than münchnones, they are stable compounds that can be isolated and stored overtime, without particular precautions.⁽¹⁰⁵⁾ Their thermal cycloaddition with alkynes, first reported by Huisgen in 1962, leads to the formation of pyrazoles.^{(11)a} Despite the moderate degree of selectivity observed in presence of unsymmetrical alkynes, the high relevance of this reaction, originally performed at high temperatures, encouraged chemists to improve its chemo- and regio-selectivity.^{(106),(107)} One of the first efficient way to overcome regioselectivity issues in thermal sydnone-alkyne cycloadditions was the utilization alkynyl boronates, which was introduced by Harrity (Scheme 12a).^{(108),(109)} The substituted pyrazole boronic esters were further functionalized by means of a series of Suzuki cross-coupling reactions.⁽¹¹⁰⁾ The use of alkynyl silanes also demonstrated excellent regio-selectivity in the cycloaddition.^{(111),(112)} To overcome thermal

activation, Harrity developed sydnone bearing a Lewis-basic substituent at position C₄ with alkynyl trifluoroborate (Scheme 12b).⁽¹¹³⁾ Besides controlling the regioselectivity, the Lewis acid/base pair accelerates the cycloaddition, which proceeds at room temperature. In addition, the pyrazole products can be easily post-functionalized *via* well-known boron chemistry.

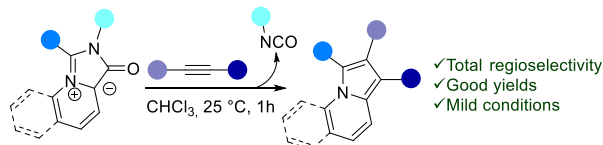
a) Sydnone/alkynylboronates, Harrity 2007



b) 4-PyridylSydnone/alkynyltrifluoroborate, Harrity 2019



c) Imidazo[1,5-a]pyridin-2-ium-1-olate/alkynes, Arndtsen 2017



Scheme 12: Mesoionic cycloadditions fulfilling most of the click criteria. D: Lewis-basic substituent

Arynes also proved excellent reactivity with sydnone. Pioneered by Gotthard, Huisgen and Knorr,⁽¹¹⁴⁾ this reaction was more recently exploited and studied in details by Larock and Shi.^{(115),(116)} This reactivity was also applied to the synthesis of novel hetero-helicenes, with good to excellent regioselectivities.⁽¹¹⁷⁾ Upon heating, sydnone can also react with a variety of olefins, which is obviously favored when the reaction is intramolecular.⁽¹¹⁸⁾ However, the favored olefinic partner for sydnone thermal cycloadditions remains maleimides.^{(88)b,(119)} Similarly to münchnones, the primary formed cycloadduct is more reactive than the sydnone itself and undergoes a second cycloaddition to form a 2:1 adduct.^{(119),(120)} This double cycloaddition was also observed with cyclooctadiene yielding structurally interesting diaza-tetracycles.^{(121),(122)}

New mesoionic scaffolds allowing mild conditions have been discovered in the recent years. In 2017, Arndtsen developed a new class of pyridine-based mesoionics, which reacted fast and regioselectively with alkynes at room temperature to provide polycyclic pyrazoles in a reaction that may be qualified as click (Scheme 12c).⁽¹²³⁾

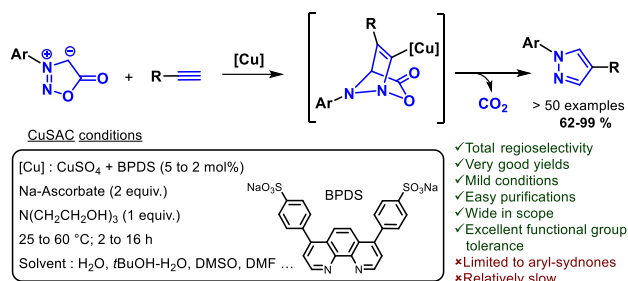
According to the definition formulated by Sharpless,⁽²⁾ a click reaction should involve readily accessible starting materials, simple purification and no toxic solvents. Those requirements exclude reactions, which need an *in situ* generation of the mesoionic partner such as münchnones that are generated under harsh conditions. On the other hand, the latest developments regarding the regioselectivity and reaction conditions are notable improvements of these cycloaddition reactions. We can easily imagine that some of the above described mesoionic-based cycloadditions can be further developed to fulfill all the criteria of click chemistry. The recent calculations described by Houk regarding the reactivity of 24 mesoionic cores in cycloaddition reactions⁽²⁸⁾ might be a valuable guide for designing new cycloadditions with mesoionics and unveil new click reactions.

3. METAL-CATALYZED CLICK REACTIONS WITH MESOIONICS

3.1. Copper(I)-catalyzed sydnone-alkyne cycloaddition

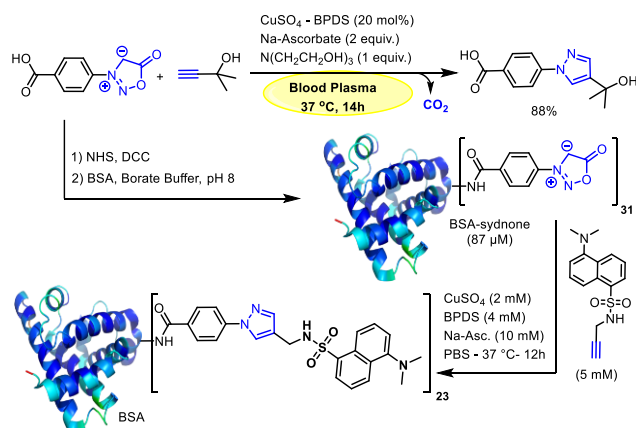
As indicated in the previous section, classical Huisgen cycloadditions with sydnone and terminal alkynes require elevated temperatures, long reaction times and may generate mixtures of pyrazole regioisomers. Using a screening approach, our group discovered that Cu(I) salts are able to catalyze this reaction quite efficiently to form only 1,4-disubstituted pyrazoles at room temperature or upon gentle heating (Scheme 13).⁽¹²⁴⁾ By analogy with the well-known Cu-catalyzed Azide-Alkyne cycloaddition (CuAAC), this reaction was called

CuSAC for Cu-catalyzed Sydnone-Alkyne Cycloaddition.



Scheme 13: Cu-catalyzed sydnone-alkyne cycloaddition (CuSAC). BPDS: bathophenanthroline disulfonate.

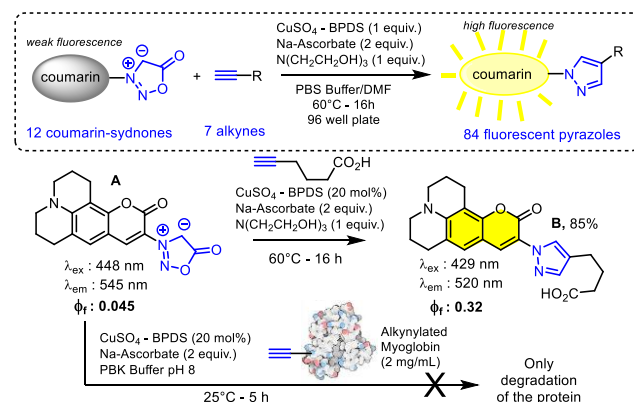
The optimal reaction conditions are very close to those of CuAAC with however one major difference: the presence of a phenanthroline-based ligand is mandatory for the CuSAC reaction. Contrary to CuAAC, other Cu-ligands such as tris-triazole or benzimidazole ligands are much less effective and absence of ligand prevents any reaction from taking place. The CuSAC reaction is also slower than CuAAC and usually requires higher amounts of copper catalyst, typically 20 % mol. However, apart from these limitations, the reaction has many advantages and meets most of the criteria of click chemistry: yields are generally high, regioselectivity is total, conditions are mild and the tolerance towards chemical functional groups is excellent. The reaction was also found chemoselective towards biological functions: the CuSAC reaction works very well in biological media, such as blood plasma, and with proteins (Scheme 14).



Scheme 14: Compatibility of CuSAC with biological media and biomolecules.

According to MALDI-TOF analysis, 75% of sydnes linked to bovine serum albumin (BSA) were successfully converted into pyrazoles upon reaction with a dansylated alkyne under standard CuSAC conditions, demonstrating that CuSAC can be successfully used for bioconjugation.

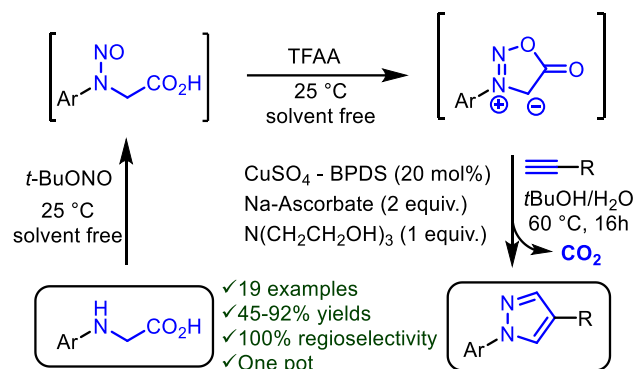
The CuSAC reaction was recently exploited to construct a library of 84 coumarin-pyrazoles fluorescent products by combining 12 sydnone-coumarin substrates with 7 terminal alkynes (Scheme 15).⁽¹²⁵⁾



Scheme 15: CuSAC with coumarin-sydnes.

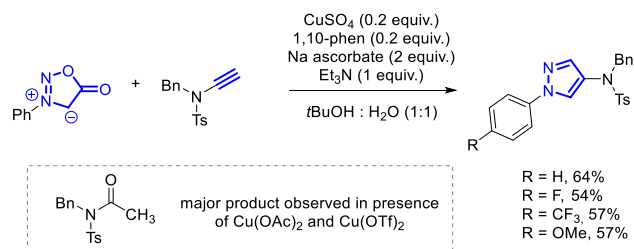
Most of the reactions were completed after overnight heating at 60 °C offering a pyrazole library with globally more than 80% conversion. The sydnone-coumarin compounds were found to be interesting turn-on probes allowing strong fluorescence enhancement upon CuSAC reaction. Coumarin A for example allowed an increase of the fluorescence intensity of more than 2 orders of magnitude after CuSAC reaction with terminal alkynes. However, this probe failed to react with alkynylated proteins provoking essentially degradation probably due to the excess of copper required for the reaction. The need of higher amounts of copper, when compared to CuAAC conditions, is probably the reason why CuSAC is not much used for bioconjugation applications, but rather in field of pyrazole synthesis.

A one pot reaction from aryl-glycine derivatives was first developed to allow facile preparation of a large panel of 1,4-disubstitued pyrazole products without the need to isolate neither the sydnone nor the *N*-nitroso intermediate (Scheme 16).⁽¹²⁶⁾



Scheme 16: One-pot synthesis of pyrazoles from glycine derivatives.

Recently, the group of Harrity explored the reactivity of sydnone with *N*-substituted alkynes in order to access to new 4-amino-pyrazoles.⁽¹²⁷⁾ As their reactivity with ynamides was unreported, they decided to start their optimization with copper(II) salts as catalyst. Unfortunately, when $\text{Cu}(\text{OAc})_2$ and $\text{Cu}(\text{OTf})_2$ were utilized only traces amounts of cycloadduct product were detected, while the main reaction product was the parasite formation of the sulfonyl amide, from the hydrolysis of sulfonyl ynamide. The formation of the desired pyrazoles was possible only when the CuSAC conditions were utilized (Scheme 17). Importantly, the authors reported that only C_4 -unsubstituted sydnone are competent in the transformation.

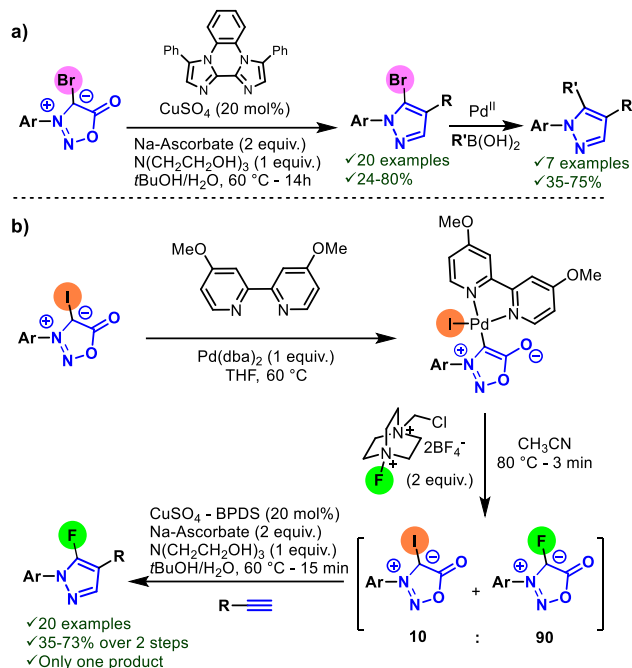


Scheme 17: CuSAC with ynamide substrates.

Although the CuSAC reaction provides a nice access to 1,4-disubstitued pyrazoles, its use for more substituted pyrazoles preparation is more

complicated. The reaction is limited to terminal alkynes and substituents in position C_4 of the sydnone core strongly inhibits the reaction, with the exception of halogens, which are tolerated. Unlike 4-iodosydnone, which are not good substrates for the CuSAC reaction, 4-chloro and 4-bromosydnone are fully converted under standard CuSAC conditions, but regioselectivity was altered giving a mixture of 1,3,5- and 1,4,5-trisubstitued pyrazoles. Optimization studies identified di-imidazoquinoxalines as better ligands than BPDS for this transformation enabling the formation of the desired 1,4,5-trisubstitued pyrazoles with high yields and complete regioselectivity (Scheme 18a).⁽¹²⁸⁾ The presence of bromide was further exploited by adding a third substitution on the pyrazole using Suzuki coupling.

4-Fluorosydnone are excellent substrates for CuSAC but their preparation is difficult using traditional electrophilic fluorination protocols. A specific procedure based on electrophilic fluorination of sydnone-Pd(II) complexes with Selectfluor was thus developed to prepare 4-fluorosydnone in a fast and clean reaction. This strategy involves a selective reductive elimination key step from high-valent Pd(IV) intermediates favoring the formation of a C–F bond over a C–I bond. As 4-iodosydnone are not substrates for CuSAC, the subsequent CuSAC reaction afforded 5-fluoropyrazoles as pure products (Scheme 18b).⁽¹²⁹⁾

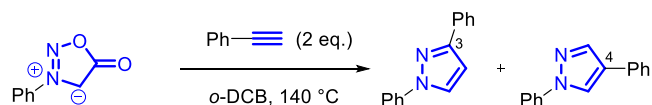


Scheme 18: CuSAC with 4-halogenated sydnes.

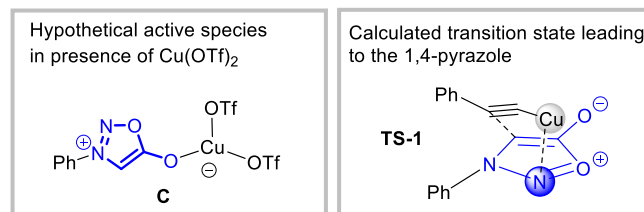
3.2. Copper(II) promoted Sydnone cycloaddition with alkynes

Following their interest in sydnone/alkyne cycloaddition chemistry, the group of Harrity reported in 2015 their investigations on the effect of Lewis acid activation on the reaction with terminal alkynes.⁽¹³⁰⁾ Based on their previous work,⁽¹⁰⁹⁾ they speculated that a Lewis acid activation might positively affect the transformation. The authors screened a variety of Lewis acids and evaluated their influence over the reaction. Under thermal conditions (at 140 °C in 1,2-dichlorobenzene), it was found that only copper(II) salts displayed an enhancement of the speed and reduced reaction times generally from > 12h to < 2h. The best results were obtained with Cu(OTf)₂ and Cu(OAc)₂ leading respectively to the formation of the opposite isomers: the 1,3- and the 1,4-disubstituted pyrazoles (Scheme 19). The selectivity observed towards the 1,3-disubstituted pyrazole isomer obtained in presence of 1 equiv. of Cu(OTf)₂ was the same as the background reaction, in the absence of Lewis acid. In this case, the reaction between *N*-phenyl sydnone and phenylacetylene takes place by interaction of the alkyne/HOMO and the sydnone/LUMO orbitals. Here, the role of copper is to enhance the reactivity of the dipole by

coordination to the sydnone, thus decreasing the energy of the sydnone/LUMO and increasing its electrophilicity. Experimentally, it was observed by IR analysis that the 1:1 adduct with Cu(OTf)₂ had a significant impact on the C₅-O₆ bond stretching frequency: from 1756 cm⁻¹ (in the absence of copper) to 1698 cm⁻¹ (with Cu(OTf)₂). Finally, computational studies suggest that the active species in the reaction is the complex **C** (Scheme 19). On the other hand, the presence of Cu(OAc)₂ induced a selectivity towards the 1,4-pyrazole, which is highly reminding of the CuSAC reported by our group. The authors suggested that *in situ* reduction of copper(II) to copper(I) is responsible of the observed reactivity. Evidence on the Cu(OAc)₂ reduction during the reaction was provided by the isolation of the Glaser phenylacetylene homo-coupling product.



Additive	Time	1,3 : 1,4	Yield
No	24 h	10 : 1	62%
Cu(OTf) ₂ (1 eq)	1 h	> 90 : 10	69%
Cu(OAc) ₂ (1 eq)	5 h	> 05 : 95	53%

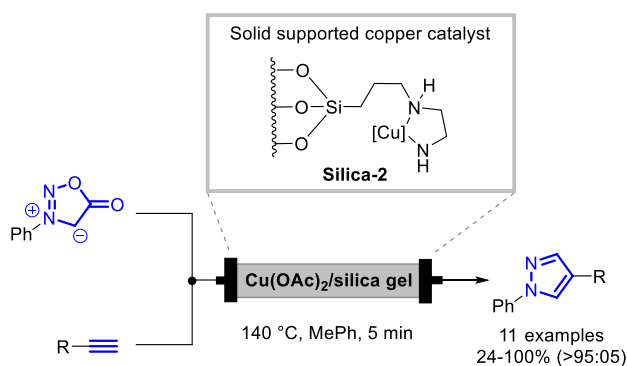


Scheme 19. Selective copper(II) promoted sydnone-alkyne cycloaddition.

The authors performed DFT experiments to investigate the mechanism of the CuSAC in presence of sydnone and phenyl-alkynyl-Cu(I) species. They found that the transition state (**TS-1**) with a strong copper-nitrogen interaction seems to be responsible with a lower activation barrier. Interestingly, **TS-1** corresponds to a concerted, but asynchronous, formation of the pyrazole: since during the transition state the C-C bond is forming to some extent, while the Cu-N

interaction delays the formation of the C-N bond to a later stage of the cycloaddition.

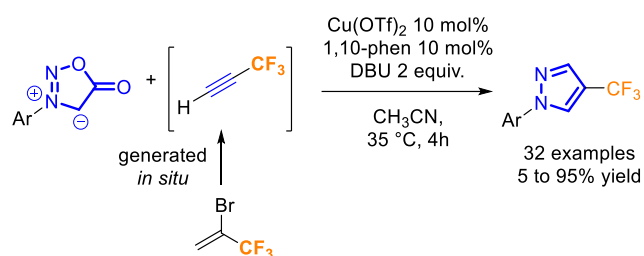
In 2016, the groups of Harrity and Rutjes reported the use of solid supported copper catalyst and flow chemistry to implement a continuous flow synthesis of pyrazoles.⁽¹³¹⁾ Their aim was to optimize the performance of the Cu(OAc)₂ promoted cycloaddition and reduce the amount of copper, which was utilized as a stoichiometric reagent in their previous report. At first, a series of solid supported catalysts were prepared. The most effective turned out to be a silica gel modified with additional amine groups to assist the copper coordination (**Silica-2**, Scheme 20). Next, the authors investigated the use of the catalyst in batch reactions. They found that the use of catalytic amounts of **Silica-2** (25/30 mol%) were sufficient to achieve high conversion. They observed that using one equivalent of the alkyne was enough for the transformation to reach full conversion, thus suggesting an alternative mechanism for the *in situ* reduction of Cu(II) to Cu(I) to the Glaser homo-coupling, such as Cu(II) disproportionation at temperature above 120 °C, as previously reported by Mao *et al.*⁽¹³²⁾ The procedure was next implemented in a flow system and used for the fast synthesis of a series of pyrazoles (Scheme 20).



Scheme 20. Cu-promoted pyrazoles synthesis in continuous flow.

Trifluoromethylated pyrazoles are present in several drugs such as Celecoxib, Razaxaban and Penthiopyrad. Very recently, Lin, Weng and co-authors explored Cu-catalysis to selectively

obtain a library of 4-trifluoromethyl pyrazoles from sydnones.⁽¹³³⁾ The authors used 2-bromo-3,3,3-trifluoropropene as a readily available source of 3,3,3-trifluoropropyne, which was generated *in situ* under basic conditions.⁽¹³⁴⁾ In presence of catalytic amounts of Cu(OTf)₂ and 1,10-phenanthroline the desired product was isolated with full control over the selectivity and under mild reaction conditions (35 °C, 4h). The regioselectivity of this reaction suggests that Cu(II) salts might have been reduced *in situ* to form Cu(I) which would act as the catalyst. The scope turned out to be large: *N*-arylsydnones bearing electron-donating substituents performed well, while the presence of electron-poor groups furnished the desired products with lower yields, but still maintaining a high selectivity. Finally, it was shown that a gram scale reaction could be performed on the model substrate *N*-phenyl sydnone with 80% yield.

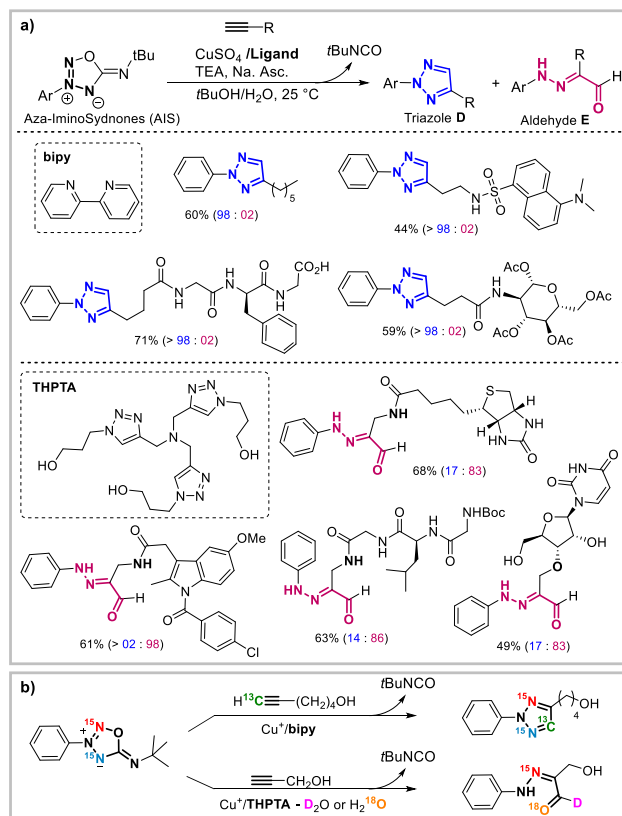


Scheme 21. Synthesis of trifluoromethylated pyrazoles.

3.3. Copper(I)-catalyzed azaiminosydnone-alkyne cycloaddition

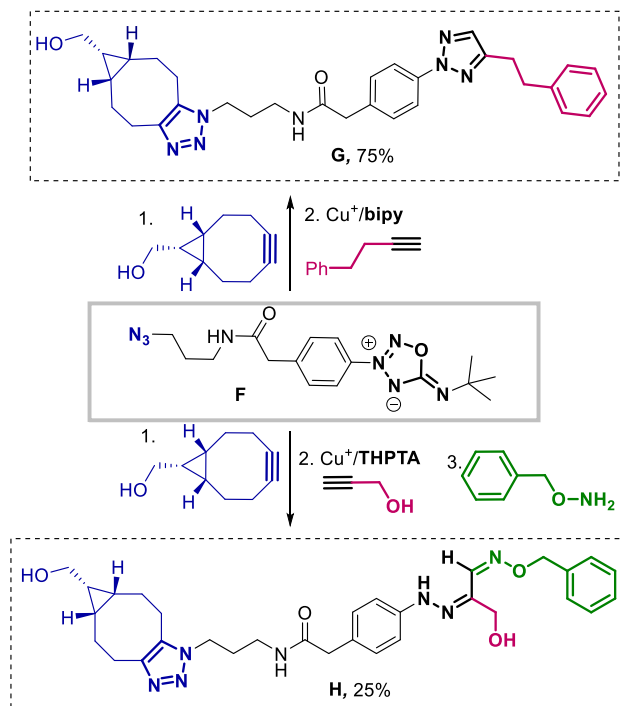
With the aim to explore the reactivity of a library of mesoionics in presence of metal catalysts, our group has developed in 2018 an ESI-MS screening platform using an MS-tag labeled alkyne to accelerate the read-out of detection.⁽¹³⁵⁾ Twenty four mesoionics with large structural diversity were mixed with tris(2,4,6-trimethoxyphenyl)-phosphonium (TMPP)-tagged terminal alkyne, in presence of copper and nine selected ligands. The screening highlighted the peculiar reactivity of aza-iminosydnones (AIS), a family of mesoionic derivatives, which has been only sporadically reported, and whose reactivity is virtually unexplored.^{(136),(137),(138),(139)} The reactivity of AIS

under copper catalysis was surprisingly unexpected. It was found that the nature of the ancillary ligand and the structures of both the mesoionic and alkyne were capable to affect the selectivity of the reaction in favor either of the triazole **D** or the aldehyde **E** products. In presence of 2,2'-bipyridine and a bulky *tert*-butyl substituent on the hexocyclic nitrogen, the triazole was found as major product with generally high selectivity (up to 98:02). On the contrary, when tris(3-hydroxy-propyl-triazolylmethyl)amine (THPTA) was employed as ligand and alkynes with a nitrogen or oxygen atom in propargylic position, the 2-(arylhydrazineylidene)propanal was generated in good selectivity (up to 02:98). The scope of the reaction was investigated utilizing mild conditions at 30 °C and in organoaqueous solvent; representative examples are illustrated in Scheme 22a. A series of stable isotope labeling experiments with carbon-13, nitrogen-15, oxygen-18 and deuterium allowed understanding the precise evolution of the reaction from the starting AIS mesoionic to the final products (Scheme 22b). While the mechanistic pathway leading to the triazole follows very closely the same mechanism as the CuAAC and the CuSAC, the formation of the aldehyde product has yet to be fully understood and remains as a pure speculation.



Scheme 22. Cu-catalyzed AIS-alkyne cycloaddition. a) Scope of the reaction. % are isolated yields of the most major product, (**D**:**E** ratios). b) Mechanistic studies with stable isotope labeling.

Surprisingly, it was found that AIS were unreactive towards strained cyclooctynes, such as **BCN**, and activated alkynes such as dimethylacetylene dicarboxylate (DMAD). Driven by this unexpected selectivity, which is in contrast to what is known with sydrones, a bifunctional AIS-azide **F** was synthesized to explore the orthogonality of the transformation. The SPAAC proceeded smoothly and with complete selectivity towards the azide. Afterwards, the AIS was converted to the corresponding bis-triazole **G** in presence of bipyridine ligand in 75% yield. Alternatively, when THPTA was used the corresponding aldehyde could be synthesized and immediately derivatized by means of the oxime ligation to provide product **H** resulting of the triple ligation (Scheme 23).

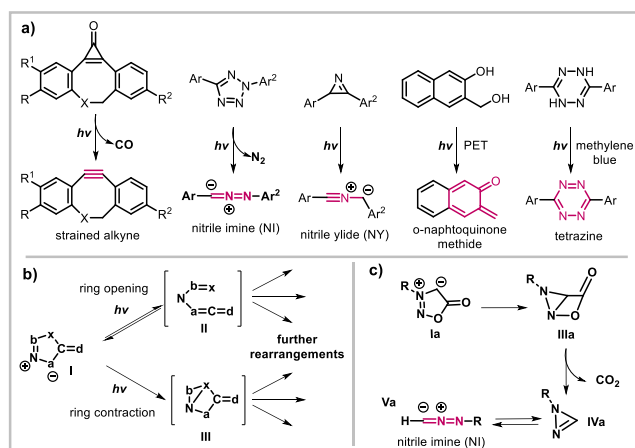


Scheme 23. Application of the Cu-catalyzed azamidosynone-alkyne reaction to double and triple click reactions.

4. PHOTO-INDUCED CLICK REACTIONS WITH MESOIONICS

The ability to control chemical reactions with high spatiotemporal resolution is highly suitable in biological, medicinal and material sciences.⁽¹⁴⁰⁾ Light allows to guide a transformation in space and in time by irradiating a precise area at a specific wavelength for an exact time. In the last two decades photo-activated bioorthogonal reactions (“photo-click” reactions), possessing the advantages of both photochemical and bioorthogonal transformations, became a new chemical tool not only for a large number of biological applications, but also for polymer synthesis and surface patterning.^{(140),(141),(142),(143)} Particular attention has been given to various cycloadditions, including azide-alkyne *via* the photo-decarbonylation of cyclopropenone,^{(144),(145),(146),(147)} tetrazole-alkene *via* the nitrile imine (NI) intermediate,^{(148),(149),(150),(151),(152),(153),(154)} azirine-alkene *via* the nitrile ylide (NY) intermediate,⁽¹⁵⁵⁾ phenanthrenequinone (PQ)- and *o*-

naphthoquinone methide-alkene *via* photo-induced electron transfer (PET).^{(156),(157),(158)} Furthermore, the photo-catalytic oxidation of dihydrotetrazine for IEDDA⁽¹⁵⁹⁾ and the photo-activated thiol-based conjugations have been also developed.⁽¹⁶⁰⁾ The role of the light is to trigger the generation of the reactive species *via* extrusion of CO (e.g. cyclopropenones)^{(161),(162),(163)} or N₂ (e.g. tetrazoles),^{(164),(165),(166),(167)} ring-opening (e.g. azirines),⁽¹⁶⁸⁾ photo-induced electron transfer (PET) (e.g. PQ, *o*-naphthoquinone methide and tetrazine)^{(156),(157),(158),(169),(170),(171)} and formation of radicals with the aid of a photo-initiator (e.g. thiols) (Scheme 24a).⁽¹⁷²⁾



Scheme 24. a) Some examples of the *in situ* light-triggered generation of reactive species for 1,3-dipolar cycloaddition reactions. b) Light-triggered decomposition pathways of mesoionic compounds (**x** = C, N, O, S; **b** = C, N, S; **a** = C, N, O, S; **d** = N, O, S). c) Light-triggered decomposition pathway of sydnone to the reactive species NI.

Mesoionic compounds **I** have also been reported to respond to light and undergo light-triggered ring-opening or contraction to the heterocumulene intermediate **II**^{(173),(174)} or the bicyclic lactone **III**,^{(175),(176)} respectively (Scheme 24b). According to the nature of **I**, as well as the temperature and the medium of the photolysis, these intermediates and their subsequent rearrangements have been identified and characterized by UV/IR spectroscopy^{(177),(178),(179),(180),(181),(182),(183),(184)} or trapped as cycloaddition products.^{(185),(186)}

Among these structures, early studies on sydrones **1a**^{(187),(188)} have reported their rearrangements to **IIIa**, which historically was the first speculated structure of sydrones made by Earl and Mackney, in their pioneering work in 1935.⁽¹³⁾ Intermediate **IIIa** further rearranges to the nitrile imine (NI) species **Va** via successive ring-opening and CO₂-extrusion from the diazarene **IVa** (Scheme 24c). Based on its 1,3-dipole nature, the intermediate **Va** has been trapped either intramolecularly⁽¹⁸⁹⁾ or intermolecularly⁽¹⁹⁰⁾ to form pyrazoline or pyrazole cycloadducts.

4.1. “Photo-click” cycloaddition of sydrones with alkenes and alkynes

Cycloaddition of sydrones with alkenes and alkynes under thermal activation has been reported to require high temperatures and long reaction times to reach completion.^{(51),(106),(107)} Furthermore, while alkynes afford consistently the pyrazole products, the cycloaddition with alkenes gives various cyclic products with moderate reaction yields, substrate scope and reproducibility.^{(88b),(119),(191),(192),(193)} Following early attempts,^{(194),(195),(196)} the first light-induced cycloadditions of sydrones compatible with proteins was reported in 2018 by Yu and co-workers with diarylsydrones (**DASyds**, Figure 1a).⁽¹⁹⁷⁾ Aryl groups on the N₃- and C₄-positions have been used to facilitate the formation of the reactive species **NI** by disrupting the planarity of the sydnone core. The formation of 1,3-pyrazoline over the 1,5-regioisomer was favored due to steric factors orienting the addition of dipolarophiles on the π-orbital of the mesoionic core.⁽¹⁸¹⁾ In order to increase the reaction rate and the selectivity of the cycloaddition, the authors synthesized a series of **DASyds** and found that electron-rich and electron-poor aryl groups on the N₃ and C₄ respectively, were the most promising substrates. The tested dipolarophiles, electron-deficient alkenes **MMA** and **DMF**, as well as with norborene (**NOR**), *trans*-cyclooct-4-

en-1-ol (**TCO**) and 5-vinyl-2'-deoxyuridine (**VdU**) gave the expected cycloadducts (Figure 1a).

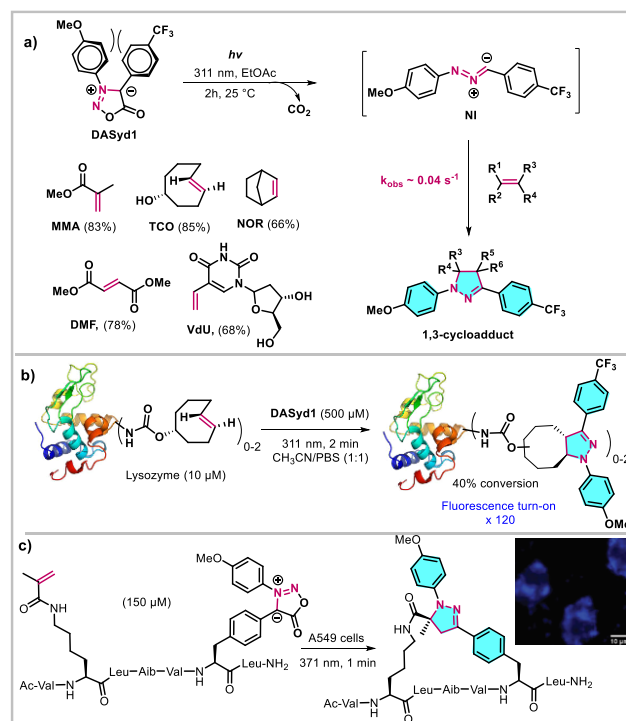


Figure 1. a) Fluorogenic photoclick reaction [3+2] cycloaddition involving **DASyds** and alkenes. b) Application to bioconjugation. c) Peptide photocyclisation inside A549 cells.

The fluorescent properties of the final pyrazolines enabled determining the apparent first-order rate constant (k_{obs}) by time-dependent fluorescence measurements. It resulted that k_{obs} is independent from both the nature of alkene and the concentration, but dependent on the **DASyds** itself, suggesting that the photo-decarboxylation is the rate-determining step and that the [3 + 2] cycloaddition occurs fast.

Subsequently, the reaction was successfully applied to fluorescent labeling of proteins. Chicken lysozyme and the suprafolder green fluorescent mutant protein (sGFP) were first derivatized with **TCO** and then reacted with **DASyds** under irradiation at 311 nm for 2 min (Figure 1b). Fluorescence imaging and MS analysis confirmed the formation of the expected bioconjugates in moderate conversions (40% for Lysozyme, 14% for sGFP). In a following study,

the authors applied this photo-triggered chemistry to form and localize a cyclopeptide in living cells.⁽¹⁹⁸⁾ The **DASyd** and the olefin **MMA** moieties were branched to a phenylalanine and lysine residues respectively incorporated into a short peptide, which was incubated with EGFR-positive cancer cells (A549). Irradiation with a 371 nm LED array for 1 min allowed formation of the cyclopeptide via intramolecular 1,3-dipolar cycloaddition inside the cells according to fluorescence imaging (Figure 1c).

In situ photo-isomerization of stable *cis*-cycloalkenes to the corresponding highly-strained and reactive *trans*-isomer has been shown as an interesting possibility to control cycloadditions by light.⁽¹⁹⁹⁾ In the search for such photo-triggered reactions, Yu and co-workers tested the *in situ* photo-isomerization of ring-strained azobenzenes as potential dipolarophiles.⁽²⁰⁰⁾ A series of dibenzo[*c,f*][1,2]diazepine analogs was reacted with **DASyds** under irradiation of 311 nm lamp and/or 405 nm laser in ACN/H₂O and only **DBTD** gave the stable macrocycle cycloproducts **M1** and **M2** (Figure 2a).

The *trans*-**DBTD** reacted faster than the *cis*-isomer with a second-order reaction rate (k_2) of $1.6 \pm 0.16 \times 10^5$ and $2.4 \pm 0.24 \times 10^4$ M⁻¹s⁻¹, respectively. Sydnone **DASyd2** was found interesting for further applications due to the effective formation of the **NI** intermediate upon irradiation at 405 nm, the same wavelength used for isomerization of **DBTD**. The authors synthesized the cyanine derivative **DASyd3** and studied the selectivity of the cycloaddition towards **DBTD**-tagged Lysozyme (Figure 2b) and BSA in a cell lysate. The resultant mixtures were analyzed *via* in-gel fluorescence demonstrating the efficiency of the labeling for both proteins. Subsequently, the authors demonstrated the bioorthogonality of this photo-induced ligation by performing labeling experiments at the surface of living cells. A549 cells were incubated with the antibody Cetuximab previously derivatized with fluorescein-5-fluoroisocyanate (**FITC**) and **DBTD**. After washing steps, the photo-click reaction was performed with **DASyd3**, the appearance of the **Cy3** signal was observed only

from the cell treated with **DBTD**-tagged antibodies, colocalisation of **FITC** and **Cy3** *via* fluorescence microscopy imaging confirmed that the photoclick occurred at the right place (Figure 2c).

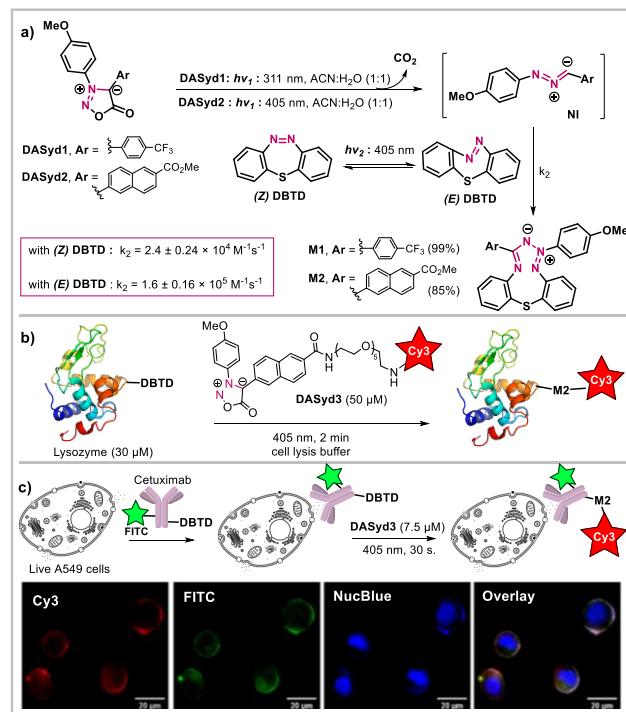
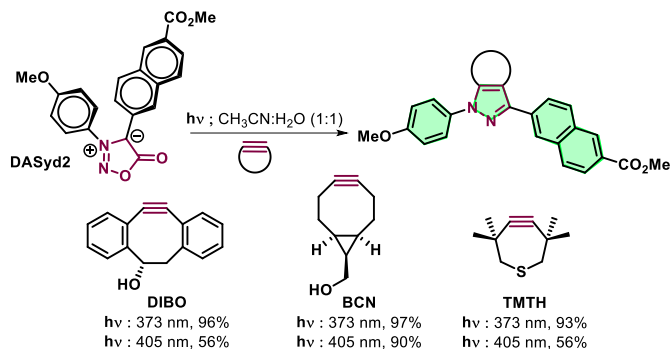


Figure 2. a) Photo-isomerization assisted reaction of **DASyds** with the strained azobenzene **DBTD**. b) Application to bioconjugation. c) Schematic illustration of the photo-click cycloaddition of **DASyd3** with **DBTD**-tagged antibodies on A549 cells.

The photo-click [3+2] cycloaddition reaction of **DASyds** was also investigated with strained alkynes (Scheme 25).⁽²⁰¹⁾ Several combinations of **DASyds** and cycloalkynes were screened and the best results were obtained with **DASyd2** and **BCN**. With these substrates irradiated at 373 nm, the apparent kinetic constant was determined to be as $k_{obs} = 0.65$ s⁻¹ and the kinetic constant for the [3+2] cycloaddition step was $k_2 = 3.3 \times 10^4$ M⁻¹ s⁻¹. The kinetics were found independent of the illumination intensity and of the concentration of **BCN** confirming that the rate-limiting step is the photo-induced decarboxylation.⁽¹⁹⁷⁾ The photo-click cycloaddition was successfully used for protein labeling and cell imaging in similar applications as those illustrated in Figure 2.

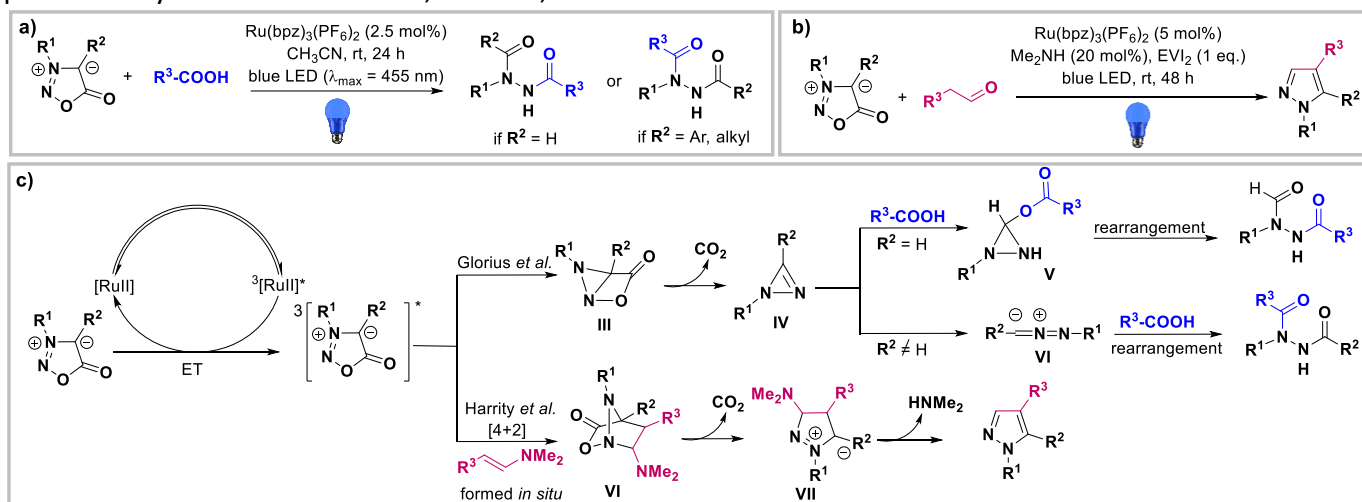


Scheme 25. Photo-click [3+2] cycloaddition of **DASyd2** with cyclic alkenes.

4.2. Photo-catalytic reactions with sydnones

Previous studies on the photo-decomposition of sydnones reported a fast decay of the singlet excited state to its long-lived triplet state,^{(182),(202)} but whether it is involved in photochemical reactions or in the formation of the NI intermediate (**Va**, Scheme 24) has not been clarified in the literature.⁽¹⁸¹⁾ In the search of new photo-catalytic transformations, Glorius, Taran

and co-workers showed that photo-catalysts ([Ir] or [Ru]) under blue LED light ($\lambda_{\text{max}} = 455 \text{ nm}$) catalyze a mild photosensitized decarboxylative hydrazide synthesis from sydnones and carboxylic acids (Scheme 26a).⁽²⁰³⁾ The process proved to have high applicability to a wide range of carboxylic acids and sydnones, yields from moderate to high (36-85%), excellent chemical robustness and good scale-up performances. The proposed mechanism involves visible light excitation of [RuII] to its triplet excited state $^3[\text{RuII}]^*$ (ET = 48.4 kcal/mol), which undergoes triplet-triplet energy transfer to sydnone (ET = 48.5 kcal/mol)^{(181),(182),(202)} returning to its ground state. The sensitization of the sydnone substrate promotes the formation of bicyclic lactone **III** which decarboxylate to diazirene **IV** to form the reactive intermediates **V** or **VI** depending of the structure of the sydnones.



Scheme 26. Photocatalytic reactions with sydnones. a) Photo-catalytic decarboxylative hydrazide synthesis. b) [4+2] Cycloaddition of sydnones to enamines. c) Proposed mechanisms of the two photo-catalytic transformations.

For C_4 -unsubstituted sydnones, the hydrazide is formed after the addition of the carboxylic acid to **IV** and consecutive rearrangement. For aryl- or alkyl- C_4 sydnones, the carboxylic acid adds to the intermediate **VI** and the product is formed after rearrangement (Scheme 26).

Harrity and co-workers also investigated the sensitization of sydnones to promote cycloadditions with enamines.⁽²⁰⁴⁾ Sydnones and aldehydes gave the disubstituted 1,4-pyrazoles in

46% to 85% yields as single regioisomer in the presence of $\text{Ru}(\text{bpy})_3(\text{PF}_6)_2$, sub-stoichiometric quantities of dimethylamine as organocatalyst and ethyl viologen diiodide (EVI_2) as “electron sink” under blue LED light irradiation (Scheme 26b). In contrast with the previous report,⁽²⁰³⁾ the excited sydnones undergo a direct [4+2] cycloaddition with the *in situ*-formed enamine giving the bridged intermediate **VI**, which rapidly loses CO_2 and amine leading to the final product

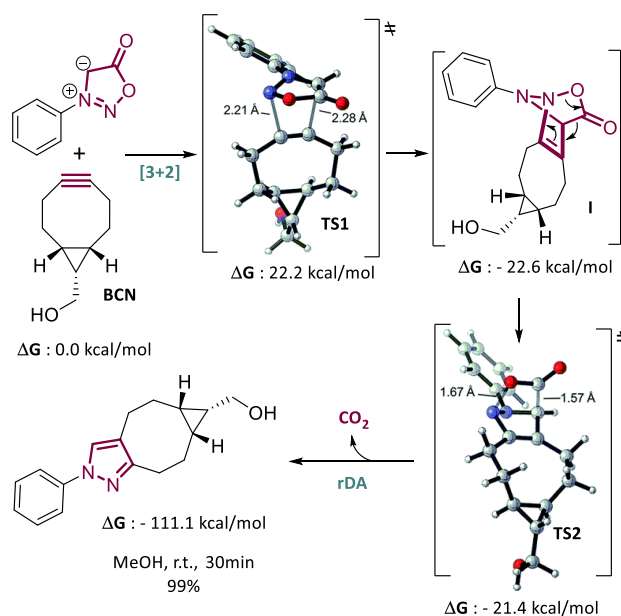
(Scheme 26c). In accordance with the previous report,⁽²⁰³⁾ the Stern–Volmer analysis of the photo-catalyst luminescence quenching confirmed the sydnone as a prominent quencher as well as the expected enamine ($K_{SV} = 2.01 \times 10^2 \text{ M}^{-1}$). Cyclic voltammetry analysis of sydnones demonstrated that PET would be unlikely and that the luminescence quenching of the photo-catalyst operates *via* a triplet-triplet energy transfer.

5. STRAINED PROMOTED BIOORTHOGONAL REACTIONS WITH MESOIONICS

As observed by Wittig and Krebs in the early 1960s, cyclooctynes are the smallest stable cycles containing a triple bond and react efficiently with phenyl azide.⁽²⁰⁵⁾ This particular reactivity is due to the tension resulting from the distortion of the angles of the alkyne (158° instead of 180°). Attracted by their particular reactivity, the use of cyclooctynes has been largely explored in click and bioorthogonal chemistry, and this has been the case with mesoionics, too.

5.1. Sydnone-Cycloalkyne Cycloaddition

In 2014, Chin's group published the first example of Strain-Promoted Sydnone-Alkyne Cycloaddition (SPSAC) by reacting *N*-phenyl sydnone with the cyclooctyne **BCN** in a clean and complete reaction at room temperature (Scheme 27).⁽²⁰⁶⁾ This reaction occurs *via* a two steps process: a [3+2] cycloaddition and a retro Diels-Alder, with concomitant emission of CO_2 and formation of a pyrazole cycloadduct. DFT calculation performed by Houk and Murphy⁽²⁰⁷⁾ indicated that the [3+2] cycloaddition occurring *via* transition state **TS1** requires an activation free energy of $22.2 \text{ kcal}\cdot\text{mol}^{-1}$ in water and the formation of the bicyclic intermediate **I** is exergonic by $22.6 \text{ kcal}\cdot\text{mol}^{-1}$. The conversion of **I** to the pyrazole product *via* transition state **TS2** has almost no barrier. This calculation indicates that the reaction rate should be entirely controlled by the [3 + 2] cycloaddition step.



Scheme 27. Strain-Promoted Sydnone-Alkyne Cycloaddition (SPSAC) between phenylsydnone and **BCN**. ΔG values were calculated in water.

The reaction of *N*-Phenyl-sydnone with **BCN** was found 5 times slower than with azides (SPAAC reaction), therefore limiting potential biological applications and stimulating efforts to improve its speed. In parallel to this report, our group published the same year a kinetic study with various substituted sydnones, with the aim to understand the substitution patterns enabling the acceleration of the SPSAC reaction.⁽²⁰⁸⁾ A strong influence of the nature of the substitution on the N_3 and C_4 positions was observed (Figure 3). *N*-alkyl sydnones did not react with **BCN** under standard reaction conditions. In contrast, *N*-aryl sydnones have a good reactivity, especially when carrying an electron-withdrawing group at the *para* position, which allows to increase the reaction speed by one order of magnitude. At the C_4 position, the insertion of a C-C bond does not have important impact on the kinetic constant while an electron-withdrawing group leads to a decrease of the speed and an electron-donating group has a positive effect. The most remarkable effect on the rate constant was observed when halogens are present at position C_4 . Interestingly, the beneficial effect was correlated to the electronegativity of the halogen atom

culminating with fluorine, whose presence allows an increase of the SPSAC kinetics by several orders of magnitude (Figure 3). This halogen effect was explained recently by Houk and Liang by computational studies.⁽²⁰⁹⁾ Using the distortion/interaction model to analyze computed activation free energies, they demonstrated that the presence of a fluorine substituent in C₄ lowers the LUMO energy and makes the sydnone much easier to distort, decreasing dramatically the activation barrier of the [3+2] cycloaddition step.

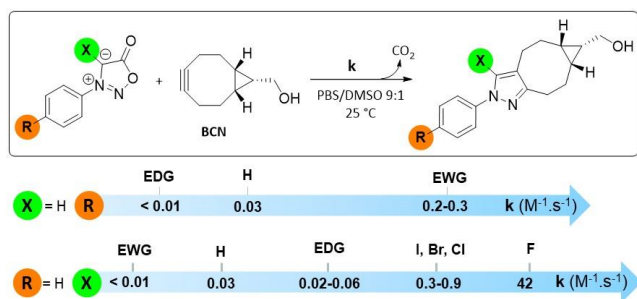
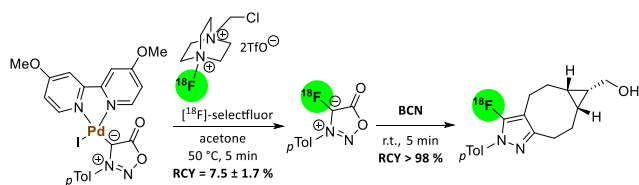


Figure 3. Influence of the substituents in positions N₃ and C₄ of the sydnone core on the SPSAC with **BCN**.

With a kinetic constant higher than 40 M⁻¹.s⁻¹, the reaction of 4-fluorosydnone with **BCN** offers the possibility to manage complex reactions at very low concentrations. This property can be exploited for the preparation of PET (positron emission tomography) tracers. As a proof of concept, the radiosynthesis of *N-pTolyl-4-[¹⁸F]-fluorosydnone* was successfully achieved by the groups of Gouverneur and Solin through electrophilic fluorination of the corresponding pallado(II)-sydnone precursor with [¹⁸F]-selectfluor bis(triflate).⁽¹²⁹⁾ The [¹⁸F]-fluorosydnone then reacted quantitatively with **BCN**, to afford the resulting [¹⁸F]-pyrazole cycloadduct in only 5 minutes at room temperature (Scheme 28).



Scheme 28. Preparation of a [¹⁸F]-fluoropyrazole upon reaction of a [¹⁸F]-fluorosydnone with **BCN**.

Kinetic experiments carried out by our group⁽¹²⁹⁾ and recent DFT calculations from A. Varnek and co-workers⁽²¹⁰⁾ confirmed the two-step mechanism of the SPSAC with the [3+2] cycloaddition step being the rate-limiting step of this reaction. Thus, an efficient strategy to increase the speed of the [3+2] step and improve the kinetics of SPSAC would involve the use of cycloalkynes with a higher degree of strain. In this spirit, the groups of Houk and Murphy performed a series of DFT calculations to study the potential reactivity of *N*-phenyl sydnone with various cycloalkynes and cycloalkenes.⁽²⁰⁷⁾ **DBC** and **BARAC**, two dibenzocyclooctynes derivatives were found theoretically and experimentally highly reactive when compared to **BCN** (Figure 4).

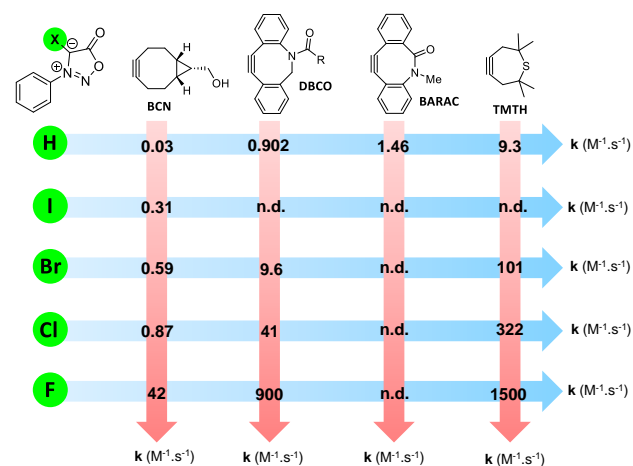


Figure 4. Reactivity of *N*-phenyl-sydnone with cyclooctynes. *n.d.* : not determined.

Furthermore, these dibenzocyclooctynes are more pre-distorted, due to smaller bond angle, and less energy is needed to reach the transition state of the reaction. These properties have later been confirmed by other calculations studies on sydnone and other mesoionics compounds, performed by Garcia de la Concepcion and Cintas.⁽²¹¹⁾ The combination of highly strained cyclooctynes and 4-halogeno-sydnone was found particularly efficient to develop ultra-fast ligation reactions. This observation was also established by the measurement of really short life time (< 80 μs) of the bicyclic intermediate formed by the [3+2] step.⁽²¹²⁾ The reaction of 4-

fluorosydnone and **DBCO** displayed particularly interesting kinetic values as determined using stopped-flow techniques: $k_{[3+2]} = 900 \pm 300 \text{ M}^{-1} \cdot \text{s}^{-1}$, $k_{[\text{rDA}]} = 0.06 \pm 0.01 \text{ s}^{-1}$.⁽¹²⁹⁾ However, fluorosydnone suffer from stability issues in biological media and the best compromise between reactivity and stability has been obtained with the 4-chlorosydnone, bearing electron withdrawing groups in position N_3 . These mesoionics are stable, easy to prepare and their reactivity with **DBCO** derivatives is high enough ($k \sim 300 \text{ M}^{-1} \cdot \text{s}^{-1}$ in blood plasma) to allow interesting biological applications. Chlorosydnone were first used *in vitro* for bioconjugation applications. After covalent attachment of 28 chlorosydnone moieties to the BSA protein, almost all of them were converted into pyrazoles by SPSAC reaction with a **BCN**-rhodamine (TAMRA) conjugate.⁽²⁰⁸⁾ This experience allowed an efficient labeling of the model protein in only 5 minutes at low concentrations (Figure 5a). More challengingly, our group demonstrated recently the possibility to click chlorosydnone and **DBCO** derivatives inside living mice (Figure 5b).⁽²¹³⁾ To this purpose, we injected (*i.v.*) a Cetuximab-chlorosydnone conjugate (**CTX-ClSyd**) to tumor-bearing mice and three days later, the time to reach maximal uptake of the antibody in the tumor, injected (*i.v.*) a [^{18}F]-**DBCO** cyclooctyne. PET imaging revealed specific tumoral radiolabeling when compared to control experiments carried out without injection of the antibody. This study was the first and is still the only example of the use of SPSAC in living animals.

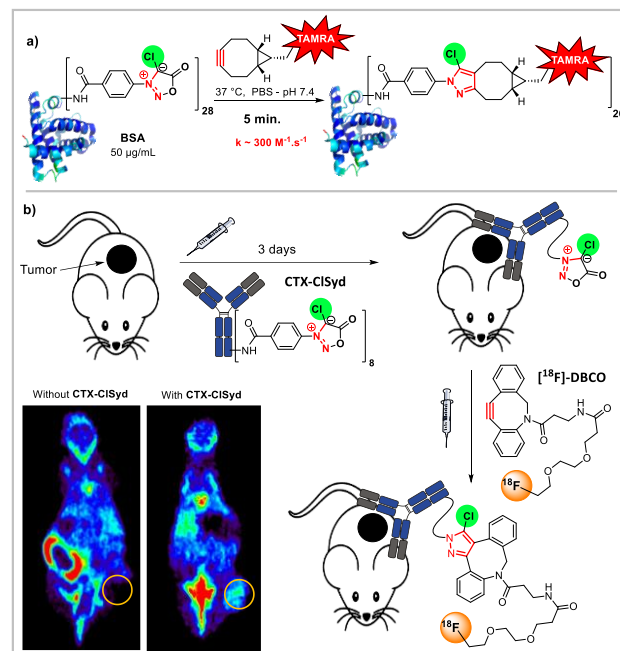


Figure 5. Use of chlorosydnone for a) protein labeling and b) pretargeting PET imaging with [^{18}F]-**DBCO**. CTX = Cetuximab.

More examples of use of the SPSAC reaction for cell biology are described in the literature. In 2019, Friscourt and co-workers demonstrated the use of chlorosydnone as bioorthogonal reporters for metabolic oligosaccharide engineering of living cells.⁽²¹⁴⁾ The authors introduced a chlorosydnone moiety at the 9-position of a neuraminic acid (**Neu9SydCl**, Figure 6). The reactivity of this unnatural neuraminic acid with a **BCN**-biotin conjugate followed by the addition of Streptavidin-AlexaFluor 568 led to robust cell surface labeling. Interestingly, **Neu9SydCl** was found to be preferentially accepted by the specific sialyltransferases leading to the favored incorporation of the reporter into linkage-specific α 2,6-N-linked sialoproteins affording the possibility to investigate the roles of specific sialosides.

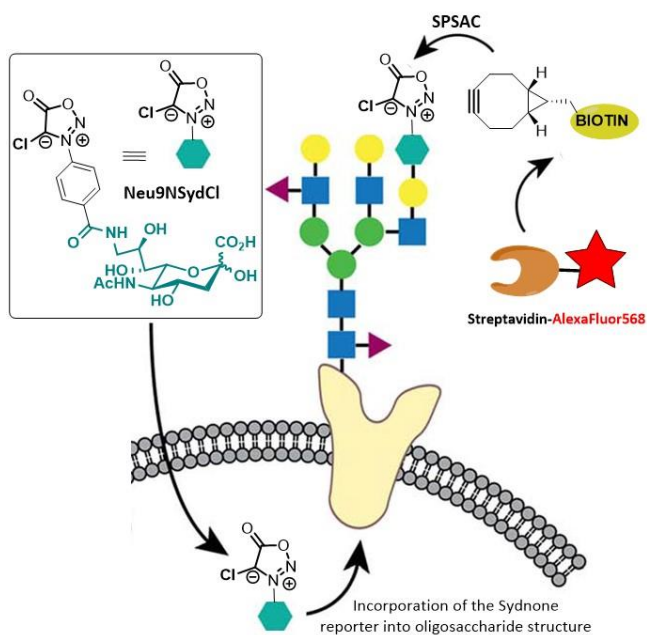
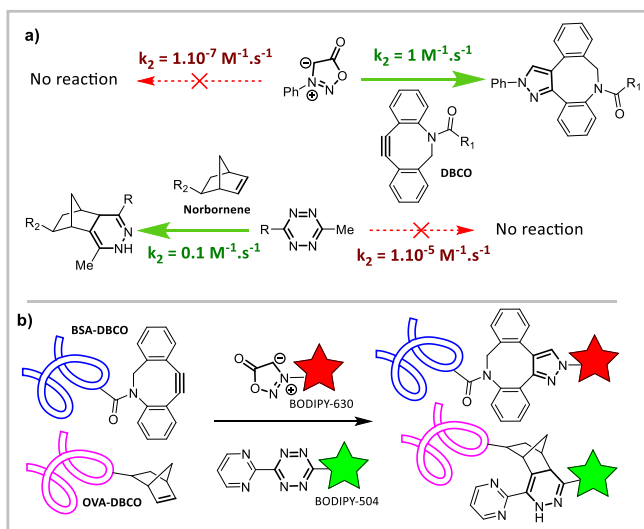


Figure 6. Use of chlorosydnone for metabolic engineering of sugars.

Interestingly, the SPSAC reaction has proved to be orthogonal to other click reactions, such as tetrazine ligation (Scheme 29a).^{(207), (215)} The orthogonality between these two transformations was successfully applied to the simultaneous fluorescent labeling of two model proteins (BSA and OVA), previously functionalized with DBCO or norbornene (Scheme 29b).



Scheme 29. a) Orthogonality of the SPSAC vs. Tetrazine/Norbornene ligation. b) Application to one pot double labeling of proteins.

In the last two years, sydnone have started to be studied as potential fluorogenic partners for the development of novel pro-fluorophores, whose optical properties can be activated and enhanced by bioorthogonal reactions. In 2018, our group⁽¹²⁵⁾ and others^{(216), (217)} first described the use of sydnone-modified coumarins as a tool for protein labeling. It was shown that sydnone have the capability to quench the fluorescence emission of the coumarin core, when connected at position N₃. The bioorthogonal reaction with cyclooctynes, such as BCN, induces an important turn-on of the fluorescence of the coumarin. In order to improve kinetic properties of the probes and to develop clickable sydnone with variable detection wavelengths, other classes of fluorogenic sydnone have been developed (Figure 7a).

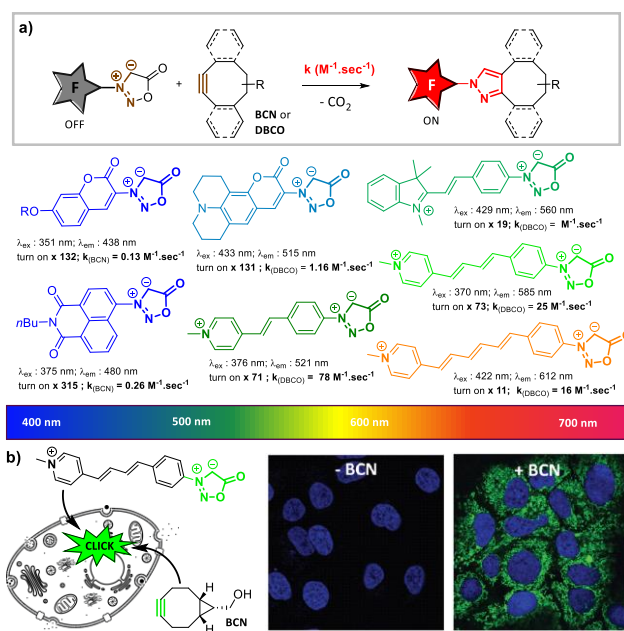
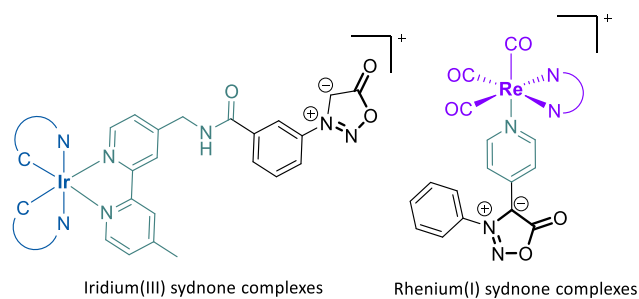


Figure 7. Turn-on sydnone probes for biological applications. a) Structures of main turn-on probes, photophysical properties of corresponding pyrazoles and kinetic constants of the SPSAC reactions. b) Example of turn-on experiments in Huh-7 cells.

Naphthalimide based probes were developed to detect the pyrazole click products in the blue region of the visible.⁽²¹⁸⁾ This probe was successfully used to detect cyclooctynes present at the surface of cells. Our group prepared a

series of aromatic-styryl-sydnones allowing the detection of the click products at higher wavelengths.⁽²¹⁹⁾ Among them, probes constructed with a pyridinium moiety separated from the sydnone core by one or two double bonds were found particularly interesting. These probes react fast with **DBCO** and the large Stokes shift and high fluorescence enhancements observed upon SPSAC reaction make them very suitable for wash-free protein labeling and cell imaging. Further experiments demonstrated that one of these probes was able to click with **BCN** inside living hepatocyte carcinoma cells Huh-7 (Figure 7b).

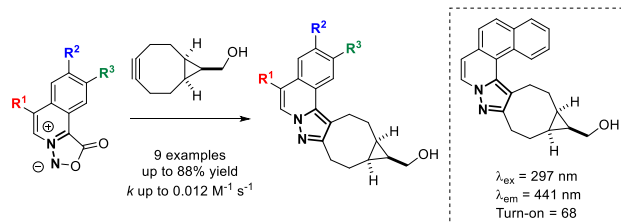
In 2018, the group of K.W. Lo described luminescent iridium (III) complexes bearing a clickable sydnone and displaying significant emission enhancement and lifetime elongation upon SPSAC reaction.^{(220),(221)} These complexes were utilized to label cyclooctyne-modified proteins and ceramide molecules in living cells. In order to develop clickable luminophores with improved properties, Lo and co-workers described in 2020 the photophysical properties of rhenium(I) poly-pyridine complexes containing a sydnone moiety.⁽²²²⁾ In that case, the Re complexes are introduced at the C₄ position of the sydnone moiety (Scheme 30). These complexes showed good turn-on capabilities after click reaction with **BCN** and were successfully used for lysosomal imaging.



Scheme 30. Iridium(III) and Rhenium(I) sydnone complexes.

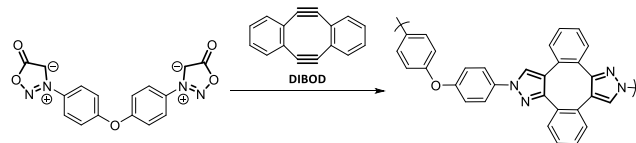
In 2020, our group reported a series of poly-aromatic sydnone derivatives and their behavior as turn-on SPSAC probes (Scheme 31).⁽²²³⁾ While fluorogenic sydnones have been previously

designed on (hetero)aromatic units conjugated to each other by single or double bonds that inherently allow a high flexibility, we decided to explore the presence of rigid poly-aromatic motifs and their effect on the optical properties of the probes. Nine poly-aromatic sydnones were synthesized, reacted with **BCN** and their optical properties and kinetics measured. The turn-on fluorescent values were generally good, and the emission wavelength of the pyrazoles ranging between 370 and 441 nm. While promising, the hydrophobic nature of the scaffold and low kinetics will challenge their applications in biological media.



Scheme 31. Polyaromatic sydnones for fluorogenic SPSAC.

The SPSAC has not only been successfully applied to various biological applications, but has also been found useful for material science. The reaction has been recently used for the preparation of polymers through 1,3-dipolar cycloaddition polymerization.⁽²²⁴⁾ Reaction with **DIBOD**, allowed the formation of varied macromolecular structures under ambient temperature, catalyst-free conditions in less than 1h (Scheme 32).

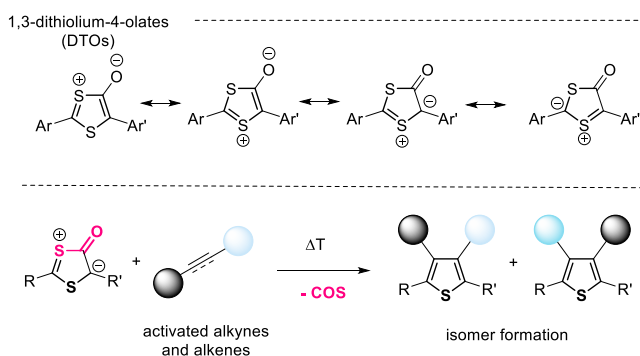


Scheme 32. Formation of macromolecular structures by polymerization of a di-sydnone with **DIBOD**.

5.2. Dithiolium-CycloAlcyne Cycloaddition

While nitrogen-containing mesoionics, such as for example münchnones and sydnones, are

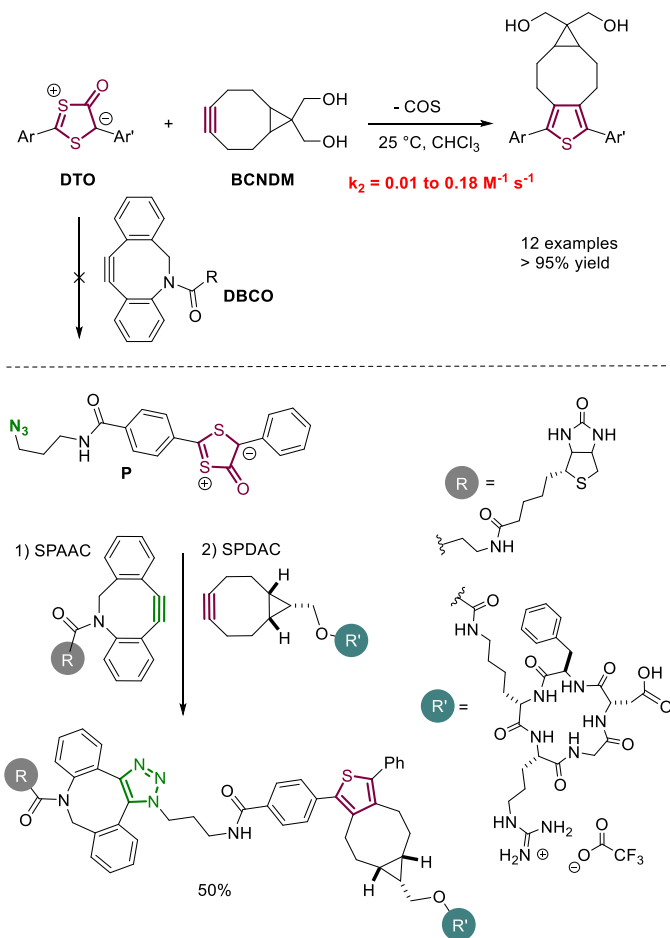
known to be competent dipole partners for strain-promoted cycloadditions and have been extensively investigated, this is certainly not the case for sulfur-based mesoionics. In particular, the family of 1,3-dithiolium-4-olates (**DTOs**) is considered as a mere academic curiosity and its reactivity lies largely unexplored. A general characteristic of **DTOs** are the dark purple/blue color appearance with a UV absorption in the range of 500-600 nm. Their synthesis and thermal reactivity were first reported over the 60s by the groups of Gotthardt^{(80),(81),(225),(226),(227),(228)} and Potts.⁽²²⁹⁾ After 1,3-dipolar cycloaddition and subsequent *retro*-Diels Alder, substituted thiophenes can be accessible with simultaneous release of carbonyl sulfide (COS). Despite its potential interest, this transformation was mainly limited to activated alkynes^{(225)c, (230)} and olefins such as fulvene,⁽²³¹⁾ maleimides⁽²³²⁾ and azirines.⁽²³³⁾ The rather poor number of literature available certainly portrays a lack of interest probably due to limitations of the transformation, which displays no control of regioselectivity and required somehow harsh conditions (Scheme 33).



Scheme 33. Top, mesomeric structures of **DTOs**. Bottom, thermal reactivity with activated alkynes and alkenes.

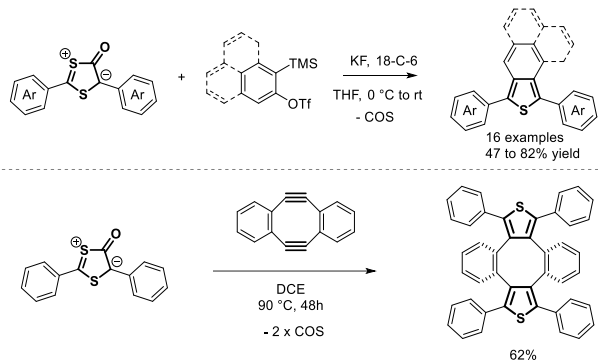
Intrigued by such mesoionics, our group has recently investigated the reactivity of **DTOs** with strained alkynes. In presence of the easily available cyclooctyne **BCNDM**,⁽²³⁴⁾ several **DTOs** reacted to deliver the corresponding thiophenes in a quantitative manner. Measured kinetic values of the Strain-Promoted 1,3-Dithiolium-4-olates/Alkyne Cycloaddition (SPDAC) reaction,

ranging from 0.02 to 0.18 M⁻¹ sec⁻¹, are in line with strain-promoted azide-alkyne cycloaddition (SPAAC) click reaction. Surprisingly, the electronic nature of substituents on the aromatic rings did not affect much the rate of the reaction. Unexpectedly, when **DTOs** were mixed with **DBCO**, no reaction occurred at room temperature, despite the higher strain of this cyclooctyne. This lack of reactivity might be a consequence of the steric congestion of the substituted **DTO** and the bulky cyclooctyne. Capitalizing on this selectivity, it was possible to prepare the dual probe **P**, bearing a **DTO** and an azide moiety (Scheme 34). In a proof-of-concept experiment, **P** reacted with a **DBCO**-biotine conjugate first and sequentially with a **BCN**-RGD conjugate providing the double clicked adduct in 50% yield (isolated by HPLC).



Scheme 34: Orthogonality between SPDAC and SPAAC cycloaddition reactions (only one regioisomer of triazole product is represented).

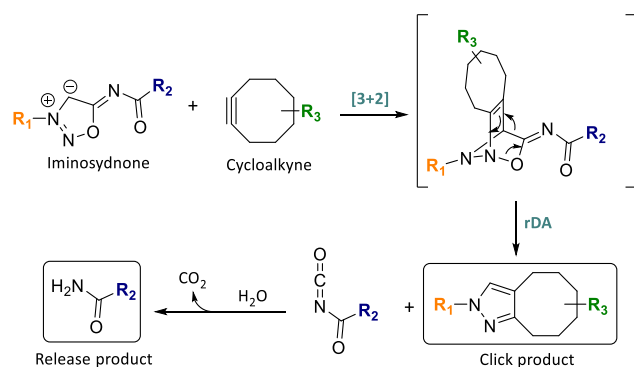
Finally, the reactivity of **DTOs** with arynes and with Sondheimer-Wong diyne was explored, providing a novel access to poly-aromatic thiophene derivatives that would be otherwise difficult to obtain by classical approaches (Scheme 35).



Scheme 35. Reactivity of **DTOs** with benzyne derivatives and Sondheimer-Wong diyne.

5.3. Iminosydnone-Cycloalkyne Cycloaddition

In 2017, our group reported the discovery of a new bioorthogonal click-and-release reaction between iminosydnone and cycloalkynes, a transformation called Strain-Promoted Iminosydnone-Cycloalkyne Cycloaddition (SPICC).⁽²³⁵⁾ Similarly to SPSAC, this reaction occurs *via* a two steps process, but in this case two compounds of interest are concomitantly formed: a “click” product and a “release” product (Scheme 36).



Scheme 36. Click-and-release reaction between iminosydnone and cycloalkynes (SPICC).

The iminosydnone reacts first with the cycloalkyne through a suprafacial [3+2]

cycloaddition to form a bicyclic intermediate which undergoes *retro*-Diels-Alder (rDA) to afford a pyrazole (click product) and an acyl isocyanate that is instantly converted to the corresponding terminal amide (release product), through hydrolysis and decarboxylation. Our group conducted a structure/reactivity relationship study on the iminosydnone core to explore substituent effects on the reaction kinetics.⁽²³⁶⁾ Over the course of this study, it was found that the presence of an urea or a sulfonamide moiety at the N₆ position has a positive effect on the reaction rate, while the presence of an amide or a carbamate moiety decreased the reactivity of the iminosydnone (Figure 8a).

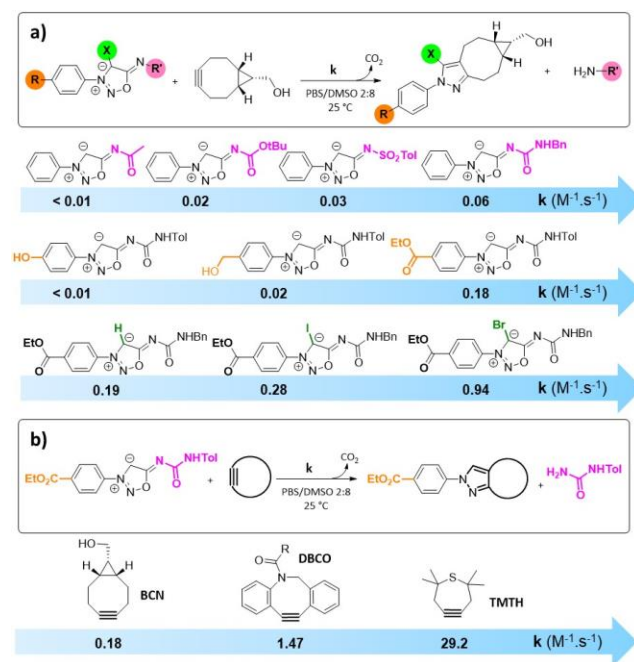
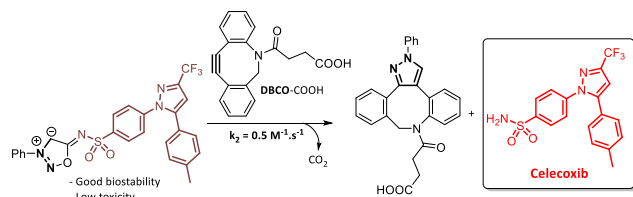


Figure 8. Structure reactivity relationship of the reaction rate of iminosydnone with cycloalkynes. Influence of a) iminosydnone substituents and b) cycloalkyne structures on the SPICC kinetics.

A positive effect on the kinetic of the reaction was also observed when electron-withdrawing groups (EWG) are present at the aryl moiety in position N₃ (R substituent, Figure 8). In analogy to what was previously observed with sydnone, the presence of halogens, and the use of more strained cycloalkynes such as **DBCO** or **TMTH** (Figure 8b), drastically increase the reaction rate.

Interestingly, all these positive effects of the different substitutions of the iminosydnone scaffold on the reaction rate were found to be cumulative. When an iminosydnone bearing an urea on position N₆, an electron-withdrawing ester group on position N₃ and a bromide on position C₄, reacted with **BCN**, a kinetic of $k = 3.7 \text{ M}^{-1} \cdot \text{s}^{-1}$ was observed. This value can be increased to $5.7 \text{ M}^{-1} \cdot \text{s}^{-1}$ with the use of a more strained alkyne, such as **DBCO**. Such kinetic values point to possible biological applications.

Shortly after, Liang and co-workers developed a N₆-sulfonyl iminosydnone for the bioorthogonal release of drugs.⁽²³⁷⁾ This strategy allowed the authors to release Celecoxib, a non-steroidal anti-inflammatory drug, through the SPICC click and release reaction with **DBCO-COOH** (Scheme 37).



Scheme 37. Click and release SPICC reaction between N₆-sulfonyl iminosydnone and **DBCO-COOH** for the release of Celecoxib.

The SPICC reaction has been found to be orthogonal to tetrazine cycloadditions⁽²³⁷⁾ and to CuAAC.⁽²³⁶⁾ This property has notably been used for applications in target fishing (Figure 9). In this strategy, compound **P**, bearing a biotin and an azide function separated by an iminosydnone moiety, was added to a liver extract containing alkynylated Myoglobin (**MyoAlk**) used as model protein. After CuAAC reaction, the corresponding biotinylated Myoglobin (**MyoBiot**) was captured by Streptavidin beads. **MyoBiot** was then smoothly released from Streptavidin beads through addition of a **DBCO-TAMRA** (Figure 9). The results indicated quantitative capture and conversion of **MyoBiot** into fluorescent labeled Myoglobin **MyoTAMRA**.

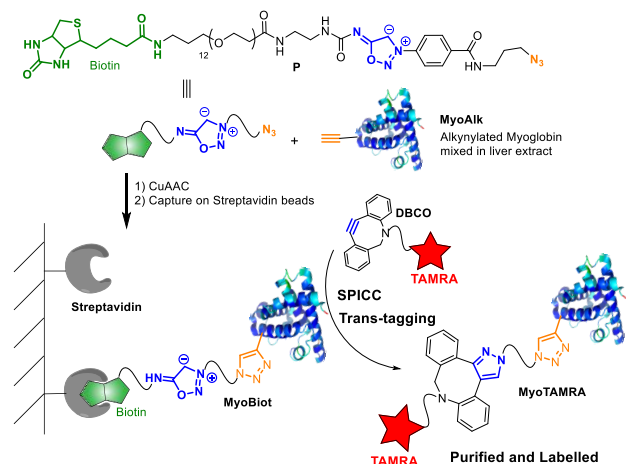


Figure 9. Iminosydnone as cleavable linker for capture and release of alkynylated-myoglobin from a liver extract.

This result confirmed the high selectivity and bioorthogonality of the iminosydnone motif and opened new opportunities to use iminosydnone as a bioorthogonal cleavable linker for trans-tagging of proteins, under physiological conditions. This property has been recently used to develop a technology to produce monodisperse antibody-drug conjugates (ADC) and to control the nature and the number of drugs attached to the antibody (Figure 10).⁽²³⁸⁾ First, an iminosydnone-biotin is conjugated to an antibody, which is present in large excess in the mixture. In this manner, only a minimal percentage of the protein is biotinylated and only with one single biotin. The mixture is next loaded into an affinity column with streptavidin. The excess of non-reacted antibody is eliminated, and recycled for another experiment, while the antibody-iminosydnone-biotin is immobilized onto the column. Subsequently, a cyclooctyne-drug derivative is loaded into the column, to trigger the SPICC provoking both the attachment of the drug and the liberation of a mono-modified ADC. This innovative strategy is a general method for the preparation of monodisperse protein conjugates having precise stoichiometry.

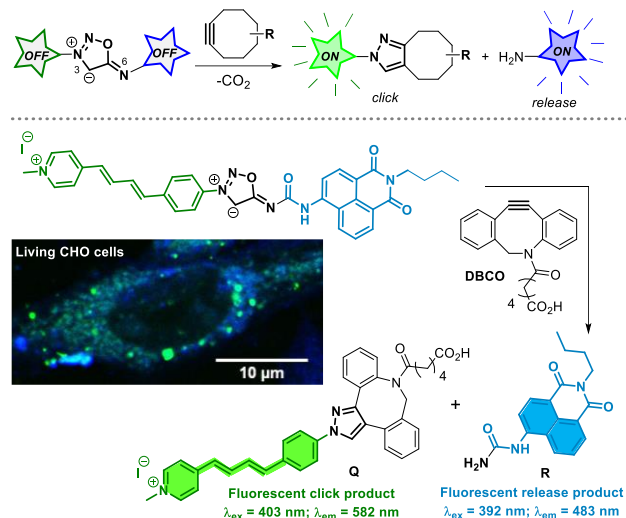
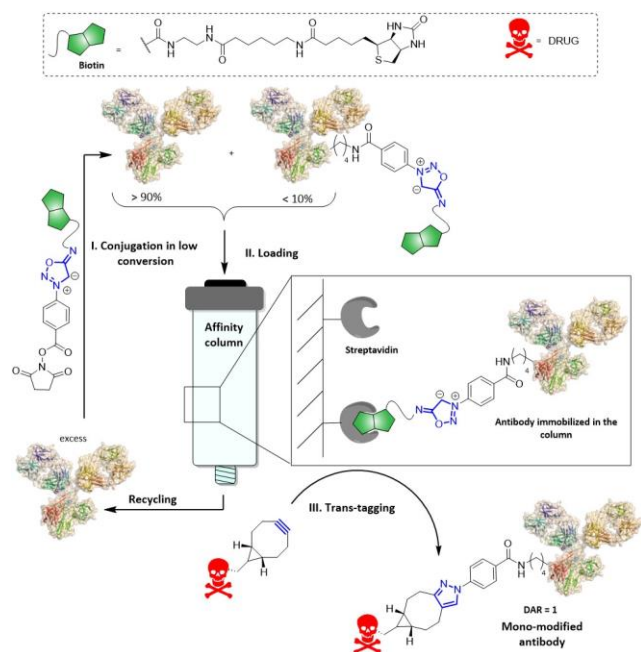


Figure 10. Use of the SPICC reaction for the preparation of DAR-controlled antibody drug conjugate. DAR: Drug-Antibody Ratio.

Like sydnone, iminosydnone were found to be able to quench several fluorophores and therefore can be used to develop efficient turn-on probes. In 2020, we reported the first utilization of iminosydnone as fluorogenic probes in SPICC (Scheme 38).⁽²³⁹⁾ While much efforts have been given by the scientific community to develop fluorogenic probes, the attention has been almost exclusively focused on the generation of only one single fluorescent molecule upon click reaction. Capitalizing on the previous works on parent sydnone, we decided to look whether it was possible to generate, at the same time, two distinct fluorescent motifs after SPICC reaction. This challenging concept has so far only a couple of precedents in the literature.^{(240),(241)} After much optimization, a series of fluorogenic probes were synthesized. The most promising displays a pyridinium derivative connected to the N₃-position of the mesoionic and a naphthalimide motif on the exocyclic N₆ position (Scheme 38).

Scheme 38: Fluorogenic iminosydnone for double turn-on click-and-release SPICC reactions.

Following SPICC, the simultaneous release of the fluorescent naphthalimide-urea **R** and pyrazolopyridinium **Q** was studied *in vitro*, using the more reactive cycloalkyne **DBCO**. The probe has shown promising kinetic constants (between 0.18 and 0.82 M⁻¹.s⁻¹, depending on the medium) and was tested in fixed and living CHO cells. Confocal microscopy imaging confirmed that the double turn-on click and release reaction was efficient inside the cells, highlighting the biocompatibility of the reaction.

Recently, our group demonstrated that SPICC can not only be useful for chemistry in cells, but also inside living animals. By designing the amphiphilic compound **S** containing an iminosydnone as cleavable linker between the lipophilic and the hydrophilic moieties, our group succeeded in obtaining the first micelles that can be cleaved by a bioorthogonal reaction.⁽²⁴²⁾ Compound **S** is able to form 10 nm sized micelles in water by self-assembly. Addition of cyclooctynes to these micelles induces depegylation of **S** destroying the self-assembly. The reaction is complete in few seconds when hydrophobic cyclooctynes are used (Figure 11).

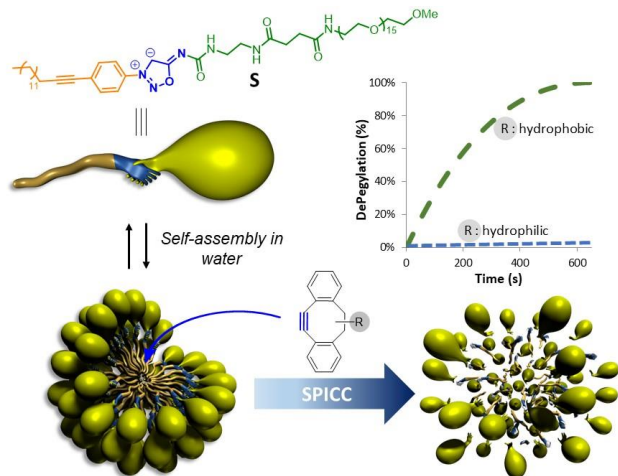


Figure 11. Reactivity of iminosynnone-based micelles towards cyclooctynes.

Taking advantage of this property, our group developed a strategy of sequential enzymatic and bioorthogonal reaction for the release of the micelle content under physiological conditions. We developed an enzyme-sensitive cycloalkyne **DBCO-Glu** (Figure 12a), which hydrophilicity avoids its entry inside the micelles. On the contrary, in the presence of the tumor-associated enzyme β -glucuronidase, **DBCO-Glu** is transformed into the more hydrophobic cycloalkyne **DBCO-NH₂** able to enter inside the micelles and destroy their nanostructures. As a proof of concept, micelles **M-DiR** containing the hydrophobic cyanine **DiR** were used; upon SPICC reaction **M-DiR** are destroyed and liberate the cyanine from their core, a phenomenon that can be monitored by following the subsequent fluorescence enhancement due to **DiR** release. This strategy was first validated in cells: the release of **DiR** from micelles **M-DiR** was possible only in the presence of the **DBCO-Glu** and β -glucuronidase (Figure 12b). Finally, mice bearing subcutaneous KB mouth epidermal carcinoma xenografts were treated with **M-DiR**. The Enhanced Permeability Retention effect (EPR effect) allowed accumulation of the micelles at the tumor site 24h post-administration as observed by fluorescence monitoring. The mice were then treated with **DBCO-Glu** and euthanized after 45 min. The fluorescence signal intensity at the tumor was significantly higher in

mice that receive both reactants than the ones that receive only the micelles. Furthermore, the pyrazole obtained by sequential enzymatic and bioorthogonal SPICC reaction was exclusively observed in mice that receive both reactants confirming the disassembly of the nanomaterial and the liberation of the dye at the tumor (Figure 12c).

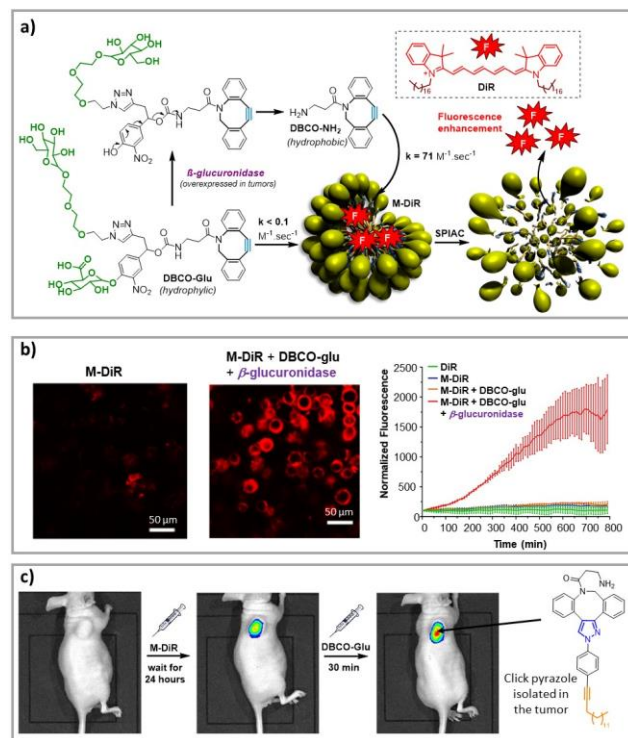


Figure 12. Bioorthogonal release of **DiR** from micelles. a) Enzymatic release of **DBCO-NH₂** from **DBCO-Glu** and its reaction with micelles **M-DiR**. b) Release of **DiR** from **M-DiR** upon enzymatic and SPICC reactions in KB cells. [**DBCO-Glu**] = 150 μ M, [**M-DiR**] = 200 μ M. c) *In vivo* release of **DiR** at the tumoral site after intravenous injection of **M-DiR** (1 mg) and **DBCO-Glu** (200 mg/kg) 24 hours later in KB tumor xenografted mice.

This study demonstrated the possibility to use SPICC in animals and opened new avenues for *in vivo* click chemistry.

5. CONCLUSIONS AND OUTLOOK

Over the last few years, mesoionic compounds have raised novel attention in the literature.

Besides their fascinating chemistry and reactivity in 1,3-dipolar cycloadditions, which led to fundamental milestones over the last century, a new facet of their reactivity has caught increasing attention in the field of click and bioorthogonal chemistry. In this review, we tried to assemble and critically review the many endeavors accomplished worldwide to explore the potential of this underrated family of dipoles. In the large chemical space of all possible mesoionics, only five families have found real applications, so far. While münchnones have been well-known for their reactivity, their limited stability, which requires the *in situ* assembly of the dipolar motif, has thus far prevented any application in the field. On the other hand, sydnones play by far the undiscussed leading role (Scheme 39). Their versatility has been explored both in copper catalyzed (CuSAC) and in strain promoted (SPSAC) ligation strategies, with many successful

in vitro and *in vivo* applications. Besides sydnones, iminosydnones and aza-iminosydnones have started to be recognized as new tools for bioconjugation. The structural advantages of these two families is that they undergo a sequential ligation/fragmentation process, which opens alternative avenues for bioorthogonal applications. Finally, sulfur-based mesoionic **DTOs** have also recently been reported as promising tools for click chemistry.

Mesoionics represent a large, mostly unexplored, chemical space that should be a source of inspiration for new reaction discoveries. Given the variety of possible mesoionic structures, future developments in this area are expected, the major challenge will focus on finding a right balance between reactivity, stability and synthetic accessibility.

	Cu-catalyzed	Strain-promoted	Photochemical
Münchnones	<ul style="list-style-type: none"> ✗ Unexplored ✗ require <i>in situ</i> formation of the mesoionic 	<ul style="list-style-type: none"> ✓ with arynes ✗ Unexplored with cyclooctynes ✗ fragility of this family has hampered its utilization 	<ul style="list-style-type: none"> ✗ Unexplored
Sydnones	<ul style="list-style-type: none"> ✓ Click ✓ High regioselectivities ✓ High yields ✗ Slower than CuAAC ✓ Labeling of proteins ✓ Suitable for turn-on probes 	<ul style="list-style-type: none"> ✓ Click ✓ Protein labeling ✓ Cell imaging ✓ In vivo pretargeting ✓ Suitable for turn-on probes ✓ ¹⁸F-radiolabeling 	<ul style="list-style-type: none"> ✓ Click ✓ with alkynes ✓ with alkenes ✓ Protein labeling ✓ Cell imaging
Aza-iminosydnones	<ul style="list-style-type: none"> ✓ Click and Release ✓ Ligand dependent behavior ✗ Substrate dependent behavior ✓ Labeling of small molecules ✓ Orthogonal to SPAAC 	<ul style="list-style-type: none"> ✗ Not reactive 	<ul style="list-style-type: none"> ✗ Unexplored
Iminosydnones	<ul style="list-style-type: none"> ✗ Unreactive 	<ul style="list-style-type: none"> ✓ Click and Release ✓ Protein labeling ✓ Drug release ✓ In vivo chemistry ✓ Suitable for turn-on probes ✗ Moderate kinetics 	<ul style="list-style-type: none"> ✗ Unexplored
1,3-Dithiolium-4-olates (DTO)	<ul style="list-style-type: none"> ✗ Poorly reactive 	<ul style="list-style-type: none"> ✓ Click ✓ Small molecule labeling ✓ Suitable for turn-on probes ✓ Orthogonal to SPAAC with DBCO-like cyclooctynes ✗ Moderate kinetics 	<ul style="list-style-type: none"> ✗ Unexplored

Scheme 39. Overview of click and bioorthogonal reactions with mesoionics.

AUTHOR INFORMATION

Corresponding Author

*F.T.: Phone, +33-169082685; Email, frederic.taran@cea.fr

ORCID

Carlotta Figliota: 0000-0001-7299-1083

Davide Audisio: 0000-0002-6234-3610

Frédéric Taran: 0000-0001-5461-329X

Notes

The authors declare no competing financial interest.

Biographies

Karine Porte joined the group of Dr. Taran for her PhD degree in chemistry at the Atomic Energy and

alternative Energy Commission (CEA) in 2016. Her PhD research was focus on the use of bioorthogonal chemistry for drug delivery. After obtaining her PhD in September 2019, she joined the group of Prof. Glorius at the University of Münster for a postdoctoral fellow.

Margaux Riomet studied chemistry at Ecole Nationale Supérieure de Chimie de Paris where she completed her Engineer Degree and Msc degree in 2015 in partnership with Université Pierre et Marie Curie. In 2018, she obtained her PhD degree under the supervision of Dr. Frédéric Taran at CEA Saclay. Her work was dedicated to the chemistry of iminosydnones and their application in biology. After graduating she joined the group of Pr. Nuno Maulide at the University of Vienna.

Carlotta Figliola obtained her Ph. D in chemistry at the University of Birmingham (UK) in 2014 with Dr. Richard S. Grainger working on Fe-catalysis for the hydrogen production. After a first postdoctoral experience in medicinal chemistry and bioimaging at Dalhousie University (Nova Scotia, Canada) with Dr. Alison Thompson, she joined the University of Aix-Marseille (France) focusing her research on the diastereoselective synthesis of borane catalysts with Dr Jean-Luc Parrain, Dr. Olivier Chuzel and Dr. Gaelle Chouraqui. She then worked on photodynamic therapy at the Ecole Normale Supérieure in Lyon (France) with Dr. Frédéric Chaput and then joined the research group of Dr. Frédéric Taran and Dr. Davide Audisio at CEA-Saclay (France).

Davide Audisio holds a PhD degree in medicinal chemistry from the University Paris Sud (France, Dr. M. Alami and Dr. S. Messaoudi). After a postdoctoral experience working on asymmetric catalysis at the Max-Planck-Institut für Kohlenforschung (Germany, Prof. N. Maulide), in 2012 he joined the chemistry department of Eli Lilly & Co (UK). In 2014, he joined the group of Dr. Frédéric Taran at CEA, the Alternative Energies and Atomic Energy Commission (France) and since 2016, he is the head of the Laboratory of Carbon-14 Labeling. His research interests span diverse areas within organic chemistry, including carbon radiolabeling methodologies, the development of new tools for bio-orthogonal chemistry and the investigation of alternatives synthetic access to poly-aromatic compounds. In 2019, he was the recipient of an ERC Consolidator Grant devoted to the development of new methods for the late-stage radiocarbon labeling of organic molecules.

Frédéric Taran secured a PhD in chemistry at the Paris XI University under the supervision of Dr. Charles Mioskowski and then moved to a post-doctoral position with Prof. Sir Derek Barton at Texas A&M University. Since 1998, he works for the French Alternative Energies and Atomic Energy Commission (CEA) located at Saclay, close to Paris and is now in charge of the department of bioorganic chemistry.

His research aims at developing new reagents for bioorthogonal chemistry to address important problems in the fields of bioconjugation, labeling, imaging and drug delivery.

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