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## Expanding the bioorthogonal chemistry toolbox: innovative synthetic strategies for cyclooctynes

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Cyclooctyne derivatives represent an important class of compounds that play a key role in bioorthogonal chemistry. The presence of an endocyclic triple bond endows these molecules with the necessary reactivity for strain-promoted azide–alkyne cycloaddition (SPAAC); however, stability issues may hamper their use in biological systems. Many research groups, with the aid of computational studies, are devoting their efforts to finding ideal cyclooctyne candidates that strike a delicate balance between reactivity and stability. In this context, providing reliable and general synthetic procedures for accessing such chemical scaffolds is of critical importance. This review covers the recent synthetic strategies found in the literature to achieve this goal. Specifically, six main methodologies are discussed, highlighting the synthetic pathways, the key precursors for each, the applicability to a wide range of cyclooctyne derivatives and the challenges encountered in fulfilling this target.

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## 1. Introduction

Since their first appearance in a paper by Blomquist<sup>1</sup> in 1953, cyclooctynes have long been considered curiosity-driven molecules, with synthetic methodologies for their formation seen merely as exercises to test chemists' ability to synthesize highly reactive and strained molecular structures. However, at the beginning of the new century, interest in the unique reactivity profile of this class of compounds surged as they were recognized as valuable moieties in different fields of chemistry for a variety of specialized applications. Their most remarkable use is found in the field of bioorthogonal chemistry,<sup>2–4</sup> where they participate in the strain-promoted azide–alkyne cycloaddition (SPAAC) reaction.<sup>5</sup> This transformation avoids the use of metal catalysts and efficiently occurs under physiological conditions, making cyclooctynes exceptionally suitable for *in vivo* and *in situ* applications. Specifically, cyclooctynes enable the precise labelling of biomolecules with fluorophores or other spectroscopic probes, facilitating dynamic studies of biological processes without perturbing cellular environments.<sup>6,7</sup> The success of cyclooctynes is strictly related to their reactivity, which directly depends on the ideal ring size that provides the necessary balance between stability and reactivity. Smaller cycloalkynes usually exhibit high ring strain due to a strong deviation of the triple bond angle from linearity (180°),

making them challenging to synthesize and store. In contrast, larger ones, in which the triple bond angle resembles that of the linear alkynes, lack sufficient reactivity under these conditions.<sup>8,9</sup> Thus, cyclooctynes, featuring a triple bond angle of 155°,<sup>10</sup> just slightly deviating from linearity (180°), stand out as the optimal choice for these important chemical transformations.

Early methodologies for the synthesis of cycloalkynes date back to the 1970s, including the classical protocol for introducing a triple bond, *i.e.* the two-step procedure involving bromination of an alkene followed by elimination, and also the electrocyclic ring opening of 1,1-dibromo derivatives followed by the capture of the corresponding *trans*-allylic cation to yield the vinyl bromide intermediate, which is finally converted to the desired cyclooctyne under basic conditions.

Such methodologies were applied to the synthesis of several cyclooctynes;<sup>11–16</sup> in particular, the bromination/elimination strategy proved to be very efficient for the synthesis of dibenzoazacyclooctyne (DBCO) on a gram scale, without the aid of column chromatography for the purification steps.<sup>17</sup> Even though this methodology allowed access to the cyclooctyne moiety, it appeared to be inadequate for exploiting a wider variety of such strained molecules. Computational studies in fact, along with the first data on kinetic studies, called for the synthesis of new and improved cyclooctynes with the right lipophilicity profile (as these molecules are primarily intended for *in vivo* use) and the introduction of different heteroatoms, which could be useful either for electronic effects or for further functionalization. It was clear that the synthetic aspect could not dictate a limitation for the construction of novel and

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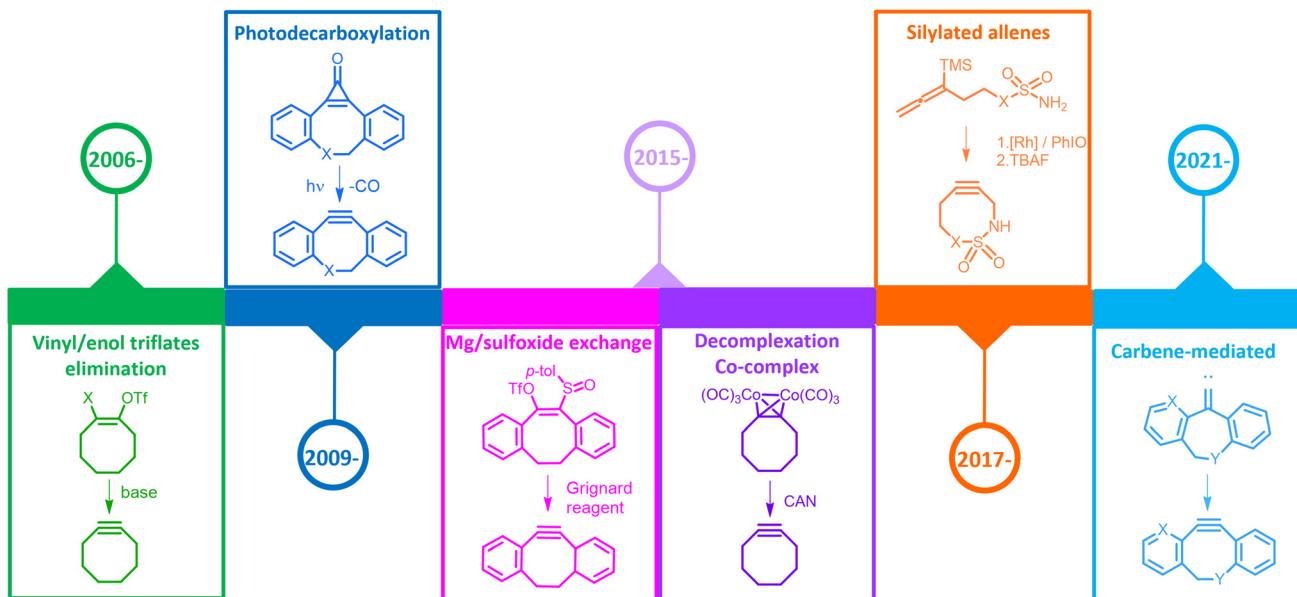


Fig. 1 Timeline of synthetic methodologies applied to cyclooctyne synthesis.

promising cyclooctynes. Therefore, a series of new methodologies have been developed over time with the aim of effectively tackling the most diverse classes of cyclooctynes. This review will illustrate such synthetic procedures, highlighting the scope, the key steps and the strengths and weaknesses. Bromination/elimination reactions and ring opening reactions of 1,1-dibromo derivatives will not be discussed here, as these methodologies were already known before the advent of SPAAC in chemical biology. The main procedures discussed in this review for the synthesis of cyclooctyne derivatives are reported in Fig. 1 in the order of chronological appearance of the first publication.

## 2. Synthetic methodologies

### 2.1. Vinyl/enol triflate elimination

Over the years, Bertozzi and co-workers have made a fundamental contribution to the development of bioorthogonal chemistry. For the synthesis of the cycloalkyne moiety, they initially employed the 1,1-dibromo derivative strategy.<sup>15,16</sup> However, this approach was not adequate for the synthesis of peculiar cyclooctynes, such as dibenzo-substituted substrates or cyclooctynes bearing an electron-withdrawing moiety at the propargylic position. The urgent need for such molecules arises from their outstanding reactivities toward bioorthogonal reactions in various biological applications.

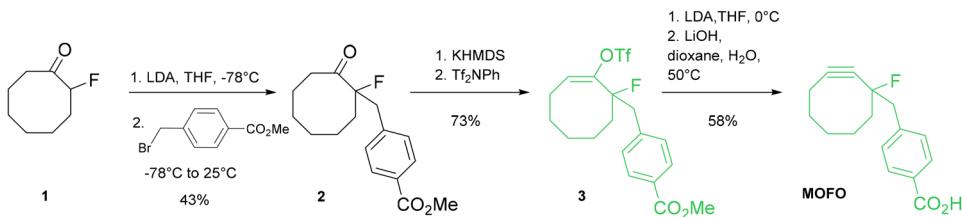
Therefore, the introduction of a synthetic methodology that was feasible, scalable and modular was urgent. In order to access fluorine-substituted cyclooctynes with promising electronic and reactivity features, the authors explored the chemistry of vinyl triflates. Stang and co-workers were the first to synthesize 3,3-dimethyl-1-buten-2-yl triflate to yield, in the

presence of pyridine, the corresponding *tert*-butylacetylene.<sup>18</sup> Later, other examples on the use of vinyl triflates appeared in the literature.<sup>19–21</sup> This methodology was then applied to the synthesis of cyclooctynes by Bertozzi. Specifically, the key steps in this protocol involved the formation of vinyl triflates from the corresponding substituted cyclooctanones in the presence of potassium bis(trimethylsilyl)amide (KHMDS) and a triflate source, and the final step was a base-mediated elimination reaction (Scheme 1).

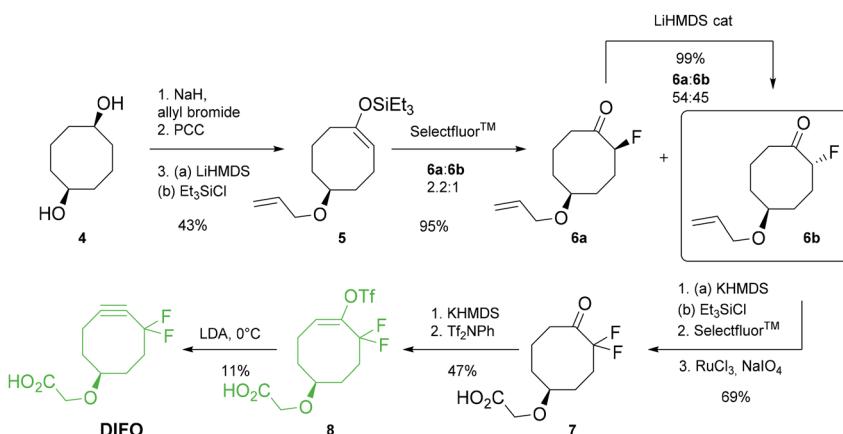
The first cyclooctyne synthesized using this strategy was a monofluorinated cyclooctyne (**MOFO**),<sup>16</sup> in which a fluorine atom was introduced at the propargylic position. This substitution was performed with the aim of lowering the LUMO level and enhancing the interaction with the HOMO of the azide partner,<sup>22</sup> as recently elucidated by density functional theory calculations.<sup>23</sup> Deprotonation at  $-78\text{ }^\circ\text{C}$  with lithium diisopropylamide (LDA) at the 2-position of 2-fluorocyclooctan-1-one<sup>24</sup> (1), followed by alkylation with methyl 4-(bromomethyl) benzoate, resulted in an overall yield of 43% and yielded the intermediate fluoroketone 2. The corresponding vinyl triflate 3 was obtained (73% yield) after deprotonation mediated by KHMDS and triflation with *N*-phenyl-bis(trifluoromethanesulfonimide) ( $\text{Tf}_2\text{NPh}$ ). The final step was performed using LDA as a base at  $0\text{ }^\circ\text{C}$  in THF, followed by hydrolysis with LiOH in dioxane/water at  $50\text{ }^\circ\text{C}$  to yield the desired cyclooctyne **MOFO** in 58% yield.

A structural evolution of **MOFO** is the difluorinated cyclooctyne named **DIFO**,<sup>25</sup> in which an electron-withdrawing moiety, *i.e.*, the difluoride unit, is introduced at the propargylic position (Scheme 2). This moiety, as similarly described for **MOFO**, increases the reactivity toward the azide in SPAAC reactions; furthermore, it is easy to install and has the advantage of being inert toward biological systems. The synthesis employed





Scheme 1 Synthesis of MOFO using the vinyl triflate elimination strategy.



Scheme 2 Synthesis of DIFO employing the vinyl triflate elimination strategy.

*cis*-1,5-cyclooctanediol (**4**) as the starting material, which was first *O*-monoalkylated with allyl bromide, and then the remaining hydroxyl group was oxidized with pyridinium chlorochromate (PCC). Subsequent treatment with lithium bis(trimethylsilyl)amide (LiHMDS) and chlorotriethylsilane ( $\text{Et}_3\text{SiCl}$ ) afforded the key intermediate  $\text{Et}_3\text{Si}$ -enolate **5**. Selectfluor™ was used to introduce the first fluorine unit to afford the corresponding diastereoisomeric mixture of fluoroketones **6a** and **6b** in a 2.2 : 1 ratio. Epimerization in the presence of catalytic LiHMDS increased the amount of the desired stereoisomer **6b**, which was transformed into the corresponding  $\alpha$ -difluoroketone intermediate after treatment with KHMDS and  $\text{Et}_3\text{SiCl}$ , followed by Selectfluor™. The terminal alkene was then converted into a carboxylic moiety with  $\text{RuCl}_3$  and  $\text{NaIO}_4$  to yield the desired intermediate **7**. Treatment of **7** with KHMDS and  $\text{Tf}_2\text{NPh}$  yielded the desired vinyl triflate **8** in 47% yield. Finally, the cyclooctyne **DIFO** was obtained by LDA-mediated elimination of intermediate **8**.

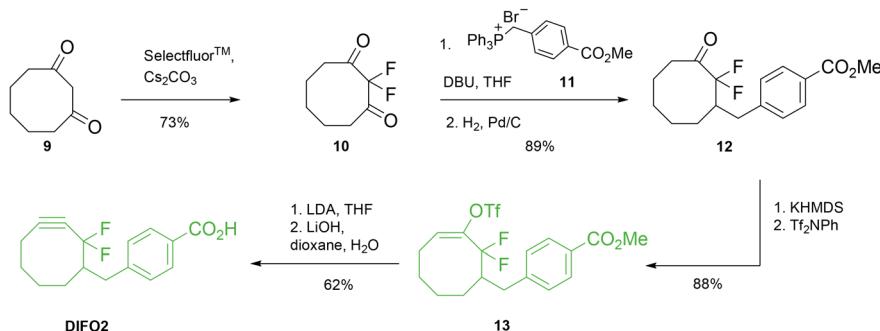
**DIFO** cyclooctyne displays outstanding reactivity, labelling azides linked to biomolecules within minutes through the Cu-free [3 + 2] dipolar cycloaddition; however, the synthetic process to obtain this molecule is quite lengthy, with a very low overall yield of 1.4% from commercially available **4**.

In search of alternative **DIFO**-like cyclooctynes with comparable reactivity but with a simpler synthetic pathway, Bertozzi and co-workers proposed a second generation of **DIFO**, namely **DIFO2**,<sup>26</sup> which retains the same difluorinated moiety but features a benzyl chain instead of an alkoxy handle. This struc-

tural modification allowed for a more straightforward synthesis (Scheme 3).

1,3-Cyclooctanedione (**9**) was fluorinated with Selectfluor™ and cesium carbonate ( $\text{Cs}_2\text{CO}_3$ ) to give the intermediate 2,2-difluoro-1,3-cyclooctanedione **10** in 73% yield. To install the handle for further functionalization, a Wittig reaction between **10** and phosphonium salt **11** in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was performed, followed by double bond reduction with  $\text{H}_2$  over  $\text{Pd/C}$  to yield the corresponding intermediate **12**. The usual step for vinyl triflate formation (*i.e.* treatment with KHMDS and  $\text{Tf}_2\text{NPh}$ ) was performed to obtain the desired compound **13** in 88% yield. The step to afford the triple bond was run using LDA as a base, and final hydrolysis of the ester moiety yielded the terminal carboxylic acid. The overall yield of **DIFO2** was 36%, approximately 25 times higher than that obtained for **DIFO**, even though the starting material for this synthesis, the 1,3-cyclooctanedione, was not commercially available but obtained in 67% yield in two steps from diethyl pimelate.<sup>27</sup> Furthermore, a similar synthetic procedure that employed intermediate **10** was also used to synthesize an analogue of **DIFO2** without a phenyl moiety in the handle, in order to decrease the hydrophobicity that could lead to undesired nonspecific interactions.<sup>26</sup>

A successful alternative to the use of vinyl triflates was a strategy inspired by the synthesis of cyclohexyne reported by Guitan and co-workers, which envisioned the formation of the parent enol triflate compounds.<sup>28</sup> Such intermediates were

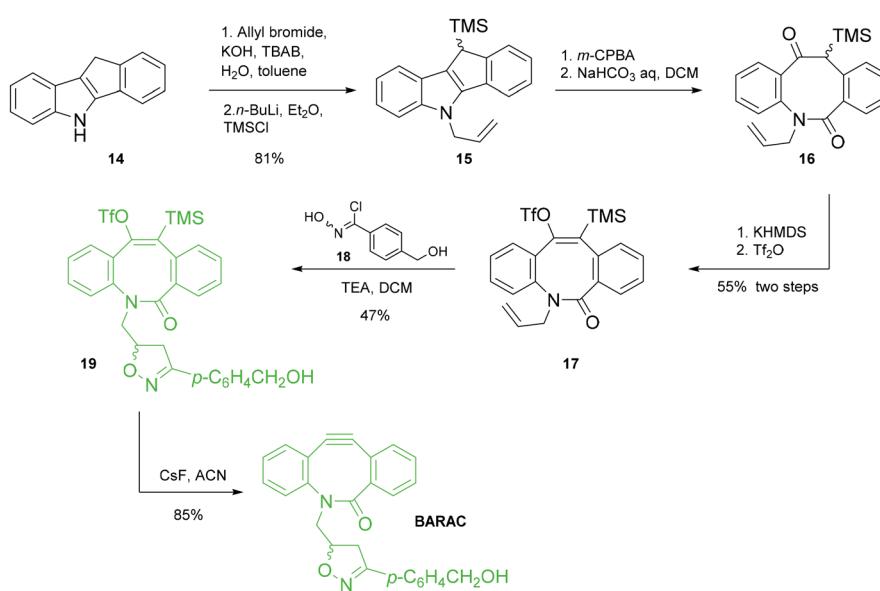


Scheme 3 Synthesis of DIFO2.

obtained from the corresponding  $\alpha$ -silyl ketones, which were in turn converted to cyclooctynes by treatment with cesium fluoride (CsF).

Bertozzi and co-workers employed the latter strategy for their research, as they were interested in establishing a modular protocol for the synthesis of a biarylazacyclooctynone (**BARAC**)<sup>29</sup> (Scheme 4). The synthesis of this new cyclooctyne was inspired by the 4-dibenzocyclooctynol (**DIBO**)<sup>30</sup> introduced by Boons, which exhibits enhanced reactivity due to the increased strain energy of the ring. In the synthesis of **BARAC**, the authors aimed to enhance the reactivity of the cyclooctyne skeleton by introducing an additional degree of unsaturation through the amide moiety, which could also serve as a handle for further functionalization. The synthetic strategies introduced so far were not feasible for such a substrate as they were too long and low-yielding,<sup>31,32</sup> so the authors developed a new procedure that involved the formation of an  $\alpha$ -silyl ketone as a key intermediate, which was converted into the corresponding enol triflate and eventually transformed into the desired strained cyclooctyne.<sup>28</sup>

Commercially available indole **14** was used as the starting material for this synthesis. This precursor is ideal, as a large variety of derivatives can also be synthesized using the Fisher indole synthesis, eventually leading to different **BARAC** substrates. *N*-Alkylation with allyl bromide was performed under phase transfer conditions using tributylammonium bromide (TBAB). Then the trimethylsilyl group was installed using trimethylsilylchloride (TMSCl) after deprotonation with *n*-BuLi to yield intermediate **15**, with an overall yield of 81% from **14**. Oxidation of indole **15** in the presence of *meta*-chloroperbenzoic acid (*m*-CPBA) led to ring opening and the formation of keto-amide **16**. Enolate formation using KHMDS followed by  $Tf_2O$  yielded the key intermediate enol triflate **17**. Notably, the early formation of the carbon–silicon bond is critical for a positive outcome of this reaction, as the formation of  $\alpha$ -silyl ketones under enolate forming conditions is very difficult due to the high affinity of the silicon atom for oxygen.<sup>33</sup> The final functionalization of the terminal double bond was achieved using a nitrile oxide, which was prepared *in situ* from chlorooxime **18** upon treatment with triethylamine (TEA), affording



Scheme 4 BARAC synthesis using the enol triflate elimination strategy.



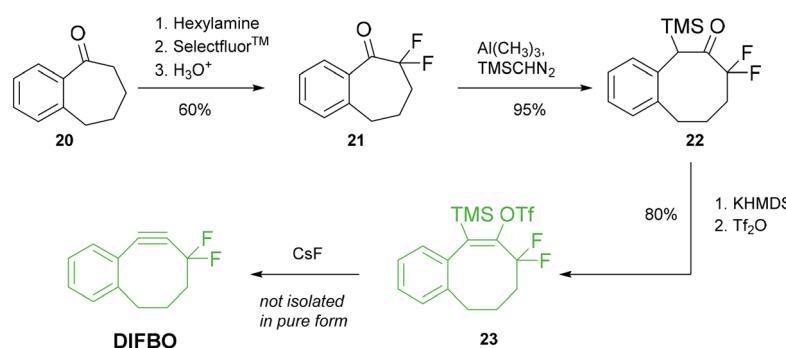
intermediate **19** decorated with the desired handle for further conjugation reaction. The formation of the triple bond to yield **BARAC** (85% yield) was successfully achieved in only 30 min at room temperature using CsF. This synthetic pathway affords an overall yield of 36%, and **BARAC** is also stable during chromatographic purification as well as on a benchtop. Such features make **BARAC** an ideal candidate for bioconjugation reactions.

A similar strategy was employed by the same authors for the synthesis of dibenzofluorocyclooctyne (**DIFBO**),<sup>34</sup> aiming to combine the beneficial effects of the electron-withdrawing moiety of the difluoro group (**DIFO**) on reactivity with the presence of the aromatic units (**DIBO**). Studies by Goddard's group, in fact, highlighted that, unlike **DIBO**, the presence of just one aryl moiety could enhance cycloaddition reactivity with benzyl azide, favoring the formation of at least one regioisomer.<sup>35</sup> The synthetic pathway employed, as a starting material, the commercially available 1-benzosuberone **20**, which, following a procedure described by Stavber and colleagues,<sup>36</sup> was allowed to react with hexylamine and then fluorinated using Selectfluor™ to afford  $\alpha$ -difluoroketone **21** in 60% yield. A Lewis acid-mediated homologation reaction using (trimethylsilyl)diazomethane ( $\text{TMSCN}_2$ ) was used for the first time in this work on fluoroketones and successfully yielded the desired intermediate **22** in 95% yield. Treatment with KHMDS followed by  $\text{Tf}_2\text{O}$  afforded enol triflate **23**, which, according to the previous synthesis, easily yields the desired cyclooctyne **DIFBO** (Scheme 5). Unfortunately, this product was highly unstable and difficult to isolate, as it underwent spontaneous homotrimerization. However, its structure was confirmed through *in situ* trapping with benzyl azide, and stabilization was also achieved by complexation with  $\beta$ -cyclodextrins.

As previously mentioned, **DIFBO** is a promising substrate for bioorthogonal reactions, but unfortunately stability issues hindered its use. Bertozzi and co-workers suggested that a promising structural modification to release part of the strain in the cyclooctyne moiety while maintaining high reactivity could be the insertion of a bulkier heteroatom into the 8-membered skeleton. Specifically, they investigated the installation of a sulfur atom in the cyclooctyne ring. The replacement of a

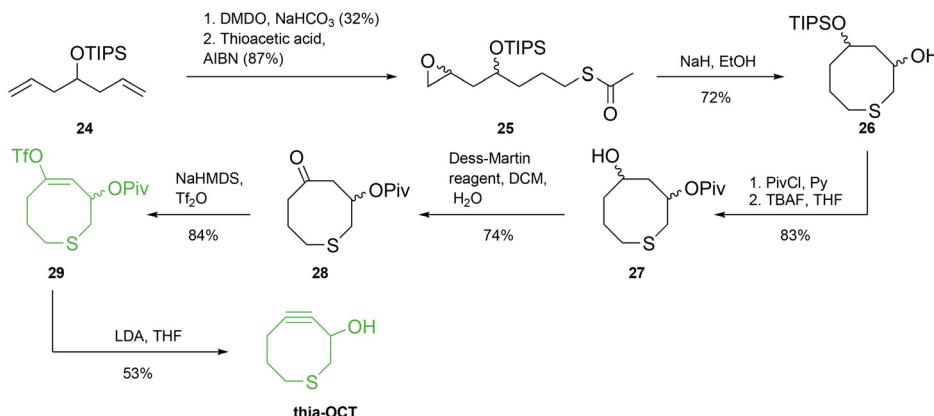
carbon atom with sulfur resulted in a longer atom-to-atom length (C-C length is equal to 1.54 Å, whilst that of C-S is 1.84 Å), leading to a small but significant ring expansion. In order to test the effect of the sulfur atom, the authors synthesized two different molecules, namely thia-cyclooctyne (**thia-OCT**) and thia-dibenzofluorocyclooctyne (**thia-DIFBO**).<sup>37</sup> The synthetic strategy that involved the formation of a vinyl triflate intermediate was also applied in this work (Scheme 6). Specifically, for the synthesis of **thia-OCT**, 1,6-dienyl-4-oxysilane **24** was monoepoxidized with dimethyldioxirane (DMDO), and the unreacted double bond underwent a thiol-ene reaction with thioacetic acid to yield intermediate **25**. Intermolecular cyclization was induced through deprotection with NaH to give intermediate **26** in 72% yield. A two-step procedure involving the protection of the free hydroxyl group with pivaloyl chloride (PivCl) and the removal of the triisopropylsilyl ether group (TIPS) with tetrabutylammonium fluoride (TBAF) yielded the corresponding alcohol **27**, which was oxidized using Dess–Martin periodinane to afford ketone **28**. Finally, intermediate **28** yielded vinyl triflate **29** after treatment with sodium bis(trimethylsilyl)amide (NaHMDS) and  $\text{Tf}_2\text{O}$ . LDA-mediated *syn*-elimination afforded the desired cyclooctyne **thia-OCT** in 53% yield.

The second-order rate constant of the reaction between **thia-OCT** and benzyl azide was found to be one order of magnitude lower than that of **OCT**, indicating that the sulfur atom had indeed rendered the cycloalkyne more stable to be isolated, but at the same time it had also considerably reduced the reactivity of the new cyclooctyne. Next, the authors sought the right structural balance by synthesizing the sulfur analogue of **DIFBO**, *i.e.* **thia-DIFBO**, that featured a propargylic *gem*-difluoro group and a fused aryl ring, but in this case also, the rate constant for the cycloaddition reaction with benzyl azide was not satisfactory, resulting in a value twenty times lower than that obtained using **DIFBO**. Notably, the authors were able to find a balance between stability and reactivity only by employing a sulfur-containing seven-membered ring, namely 3,3,6,6-tetramethylthiacycloheptyne (**TMTH**), previously synthesized by Krebs and co-workers.<sup>38</sup> When **TMTH** was allowed to react with benzyl azide in  $\text{CD}_3\text{CN}$ , a second-order rate constant of  $4 \text{ M}^{-1} \text{ s}^{-1}$  was observed.



Scheme 5 DIFBO synthesis.





Scheme 6 Thia-OCT synthesis employing the vinyl triflate elimination strategy.

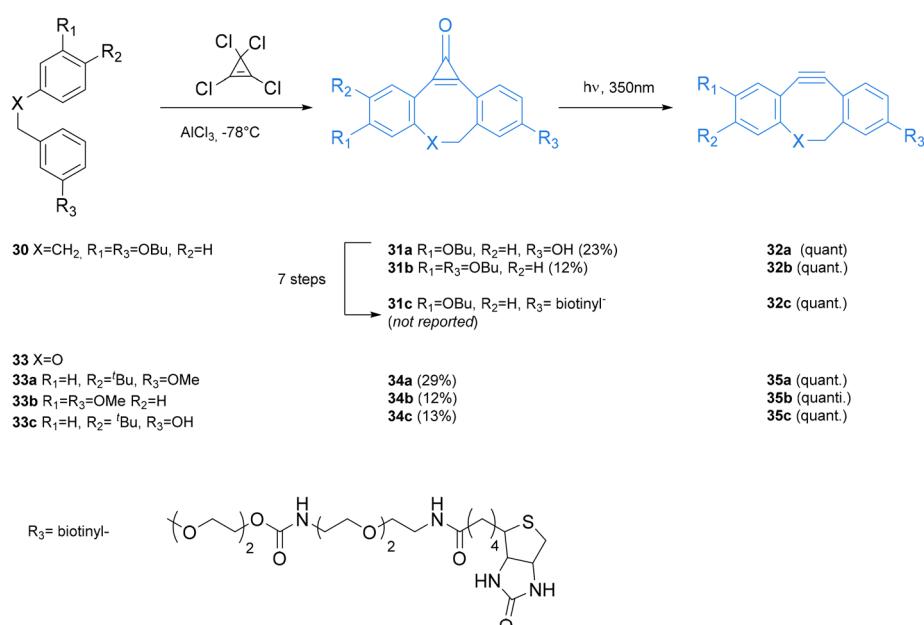
In conclusion, the vinyl enol/triflate strategy has been applied to the synthesis of a wide variety of cyclooctynes, ranging from dibenzosubstituted scaffolds like **BARAC**, mono- and difluorinated derivatives like **MOFO** and **DIFO** and heterocycle-containing targets such as **thia-DIFBO**. Some procedures rely on a lengthy synthetic pathway, and their optimization should be considered.

## 2.2. Photodecarboxylation of cyclopropenones

Popik and co-workers<sup>39</sup> proposed an alternative strategy to synthesize dibenzocyclooctyne derivatives named **DIBOs**.<sup>30</sup> This strategy involves their photochemical generation from the corresponding cyclopropenone derivatives. Previous studies by the same authors<sup>40,41</sup> had already demonstrated that the photodecarboxylation of diaryl-substituted cyclopropenones was a very efficient process that yielded the corresponding

alkynes quantitatively. Inspired by these results, they extended this strategy to the synthesis of strained cyclooctynes. In this context, photoactivation was particularly convenient as the formation of the triple bond could be rapidly achieved and therefore could trigger for instance a SPAAC reaction in the desired biological environment.

As reported in Scheme 7, **DIBO** dibenzylcyclopropenone intermediates **31a** and **31b** were obtained *via* a Friedel-Crafts reaction between **3,3'-bisbutoxybibenzyl** **30** and tetrachlorocyclopropene in the presence of  $\text{AlCl}_3$ , followed by acidic hydrolysis, with a yield of 23% and 12%, respectively. Cyclopropenone **31a** was further functionalized to yield, after 7 steps, the biotinylated intermediate **31c** to be used for labelling living cells through photo-triggered click reactions. The final dibenzocyclooctynes **32a-c** were obtained after de-carboxylation induced by efficient irradiation with 350 nm



Scheme 7 Synthesis of DIBOs and ODIBOs using the photodecarboxylation strategy.



light ( $\Phi_{355} = 0.33$ ); the transformation was monitored by UV-vis spectroscopy.

A few years later, Popik<sup>42</sup> and co-workers applied the same synthetic strategy to the synthesis of the oxygenated analogues of **DIBO**, *i.e.* the oxadibenzocyclooctynes, referred to as **ODIBOs**. Cyclopropenone derivatives **34a–c** were obtained in moderate yields (29% to 12%) starting from the substituted phenyl benzyl ethers **33a–c**, which allowed the quantitative synthesis of the corresponding **ODIBOs** **35a–c** (Scheme 7).

Later, such cyclopropenone intermediates were employed through multiphoton activation in an AAC reaction for *in situ* labeling.<sup>43</sup> Furthermore the same synthetic strategy was also applied to the synthesis of a 9-membered sulfate-linked dibenzocycloalkyne, the bis-methoxydiphenylacetylene diol cyclic sulfate (**DPAD-CS**).<sup>44</sup>

The photodecarboxylation strategy proved to be a valuable methodology for yielding dibenzocyclooctyne derivatives in a rapid and efficient way. Cyclopropenone intermediates have a long shelf life if protected from light. However this methodology is so far restricted to the synthesis of only dibenzocycloalkynes, and key cyclopropenone intermediates are often synthesized with long and complex synthetic procedures.

### 2.3. Sulfoxide–magnesium exchange

Hosoya<sup>45</sup> and co-workers described a new procedure for the synthesis of cycloheptynes and cyclooctynes employing a sulfoxide–magnesium exchange reaction that involved 2-sulfinylcycloalkenyl triflates. Inspired by their own studies on the chemistry of arynes,<sup>46</sup> the authors established an efficient three-step procedure, *i.e.*  $\alpha$ -thiolation, triflylation and oxidation, to obtain the desired 2-sulfinyl derivatives from the corresponding cyclic ketones. Reaction with a Grignard reagent triggered a sulfur–magnesium substitution and subsequent  $\beta$ -elimination of the triflyloxy group afforded the desired cycloalkyne (Scheme 8). Phenylmagnesium bromide (PhMgBr) was identified as the optimal Grignard reagent after thorough

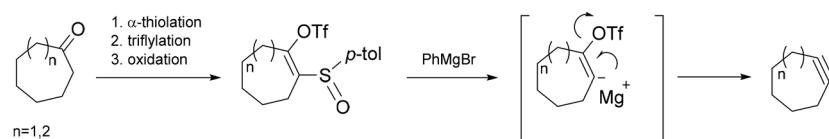
screening using a cycloheptyne precursor and benzyl azide as the ynone.

The described general procedure was suitable for the synthesis of unsubstituted cyclooctyne, which was not isolated but readily reacted with benzyl azide. **DIBO**, instead, was synthesized as described and isolated with 18% overall yield (Scheme 9). 11,12-Dihydrodibenzo[*a,e*]cycloocten-5(6*H*)-one **36**, obtained as reported in the paper from the catalytic hydrogenation of commercially available dibenzo[*a,e*]cycloocten-5(6*H*)-one, was deprotonated employing LDA and thiolated at the  $\alpha$ -position with *S-p*-tolyl *p*-toluenethiosulfonate at  $-78\text{ }^\circ\text{C}$  to afford intermediate **37** in 34% yield. Addition of KHMDS and  $\text{Tf}_2\text{NPh}$  at  $-78\text{ }^\circ\text{C}$ , followed by final oxidation of the sulfur atom with *m*-CPBA, yielded enol triflate **38** in 58% overall yield. Finally, sulfoxide–magnesium exchange in the presence of PhMgBr afforded the desired **DIBO** in 92% yield. The described procedure for the synthesis of **38** could also be applied to the formation of different 2-sulfinylcycloalkenyl triflates with six- or seven-membered rings, but the isolation of the corresponding cyclohexyne and cycloheptyne derivatives was not possible, and an alternative procedure involving *in situ* formation of the alkyne species followed by reaction with benzyl azide was proposed.

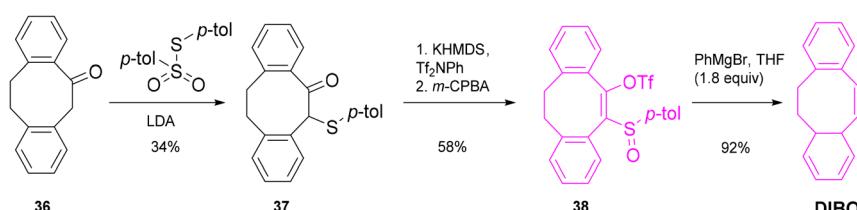
This synthetic strategy proposed by Hosoya is a short and simple procedure for the synthesis of a variety of cycloheptynes and two cyclooctynes. However, there might be some challenges in applying this strategy to a broader set of triflate precursors, as the exchange reaction with the Grignard reagent may not be suitable for base-sensitive substrates.

### 2.4. Decomplexation of Co-complexes

Tomooka<sup>47</sup> and co-workers reported the synthesis of heteroatom-embedded cycloalkynes with medium-sized rings. These compounds are amenable to further functionalization through the insertion of lateral chains attached to the heteroatom. The formation of the triple bond relies on the use of alkyne-dicobalt hexacarbonyl complexes. These intermediates, known



**Scheme 8** General procedure for sulfoxide–magnesium exchange.



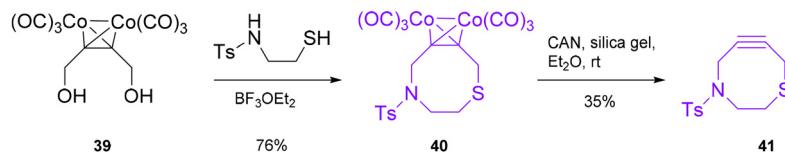
**Scheme 9** DIBO synthesis applying the sulfur–magnesium exchange strategy.



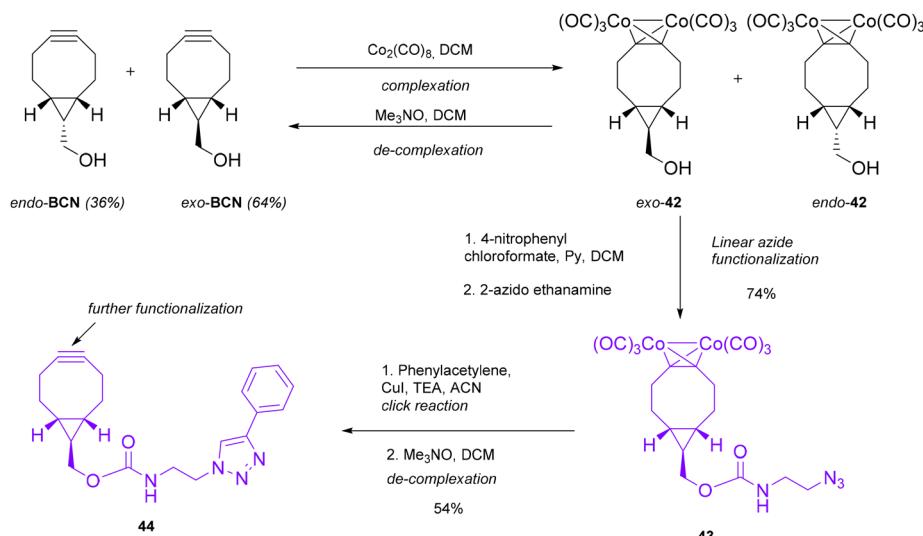
from the pioneering work of Nicholas,<sup>48</sup> give, in the presence of a Lewis acid, a dicobalt hexacarbonyl-stabilized propargylic cation. Subsequent addition of a nucleophile and application of mild oxidative conditions yield the desired alkyne. The authors applied this approach to a variety of cycloalkynes with rings containing 8 to 11 atoms and embedded with N, O, or S atoms. Regarding cyclooctyne synthesis, they reported a protocol for the formation of a N,S-containing cyclooctyne. In the first step, the reaction of a cobalt complex **39**, decorated with two hydroxyl moieties as leaving groups,<sup>49</sup> and *N*-(2-mercaptoprotoethyl)-4-methylbenzenesulfonamide yielded, through a double Nicholas reaction promoted by  $\text{BF}_3\text{Et}_2\text{O}$ , the corresponding complex **40** (76% isolated yield). The next step was performed using the optimized conditions for cobalt removal, *i.e.* the use of ammonium cerium(IV) nitrate (CAN) with silica gel, which afforded the desired alkyne **41** (Scheme 10). Unfortunately, the yield of the decomplexation step was only modest (35%), especially when compared to those of larger cycloalkyne rings (from 64% to 99%), due to the instability of the cyclooctyne ring under the oxidative reaction conditions. The positive outcome of the Nicholas reaction was ascribed to the use of a sulfonamide group on the heteroatom, which acted as the nucleophile in the first step of the transformation. Kinetic studies on the influence of the heteroatom in the cycloalkyne rings highlighted an increased reactivity in the cycloaddition reaction with benzyl azide. Specifically, cyclooctyne **41** showed higher reactivity than its carbocyclic analogue **OCT**.

Workentin<sup>50</sup> and co-workers demonstrated for the first time that a strained alkyne, specifically the *endo/exo* bicyclo[6.1.0]nonyne-methanol (**BCN**), can be efficiently complexed and decomplexed with dicobalt octacarbonyl ( $\text{Co}_2(\text{CO})_8$ ), (Scheme 11). This strategy allowed the construction of more complex cyclooctynes, particularly in cases where the highly reactive triple bond of the cyclooctyne moiety needed to be protected to introduce different functional groups in the structure. A similar approach was also studied by Yoshida<sup>51</sup> and co-workers, who temporarily protected the triple bond moiety of a strained alkyne with a Cu-complex to further functionalize the molecule with a linear azide to perform the SPAAC reaction and finally deprotected the product using an excess of  $\text{NH}_3$ . Co-complexes of alkynes might be considered preferable due to their high tolerance to a variety of reaction conditions, allowing the introduction of different functional groups to perform bioorthogonal chemistry reactions. The authors synthesized the **BCN** alkyne as an *endo/exo* mixture following a literature procedure.<sup>52</sup> This mixture was quantitatively converted to the corresponding Co-complex *exo*-**42** and *endo*-**42** by reaction with  $\text{Co}_2(\text{CO})_8$  after 60 min at r.t. The de-complexation reaction could be performed under mild reaction conditions employing trimethyl *N*-oxide ( $\text{Me}_3\text{NO}$ ) to afford the original strained alkyne *exo*-**BNC** and *endo*-**BCN** in 85% yield. As proof of concept, a chemical modification was performed on the protected alkyne, which would have been incompatible with the unprotected **BCN**.

Specifically, the *endo* and *exo* isomers of **42** were separated, and the most abundant *exo* component was further functionalized.



Scheme 10 Cyclooctyne **41** synthesis using the Co-complex decomplexation strategy.



Scheme 11 Use of Co-complex decomplexation for **BCN** functionalization.

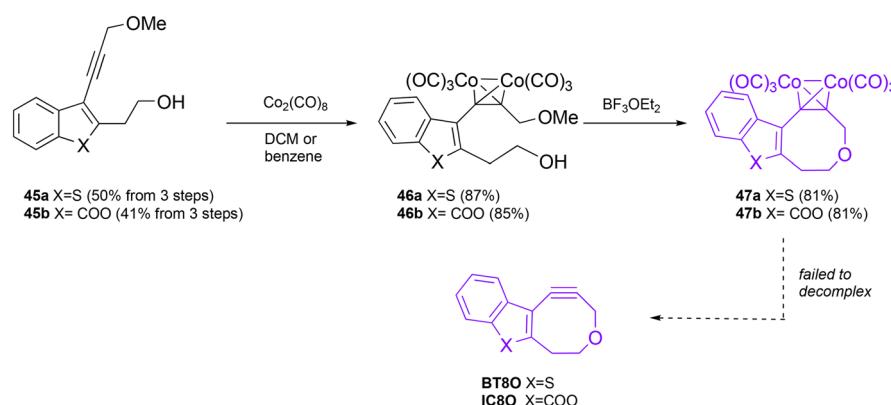


lized through the reaction of its alcoholic moiety with 4-nitrophenyl chloroformate to yield the corresponding carbonate, which was further functionalized with 2-azidoethanamine to yield the linear azide derivative **43** in 74% yield. The synthesis of this compound would not have been possible without the Co-complexation of the highly reactive triple bond. After the introduction of this new functional group, the highly functionalized intermediate **43** reacted smoothly with phenylacetylene to yield the corresponding triazole in a click reaction catalyzed by CuI. The most critical step, *i.e.* decomplexation, which can often be very difficult, was successfully accomplished, releasing the desired cyclooctyne **44**.

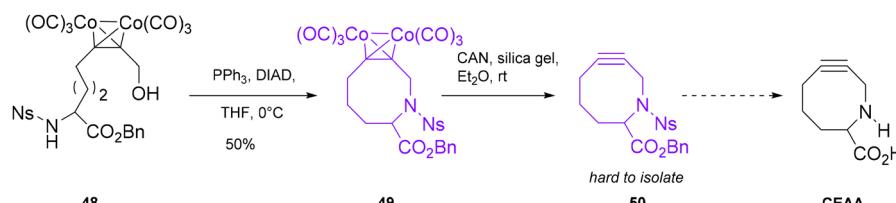
Balova<sup>53</sup> and co-workers attempted the synthesis of a new class of cycloalkynes, where a heterocyclic core is fused with a heterocycloalkyne moiety, such as benzothiophene and isocoumarin, with the goal of achieving a balance between stability and reactivity, thereby enabling their use in SPAAC reactions (Scheme 12). In order to synthesize the 8-membered benzothiophene (**BT8O**) and isocoumarin (**IC8O**) cyclooctynes, the alkyne intermediates **45a** and **45b**, obtained from a Sonogashira reaction described in the paper, were allowed to react with  $\text{Co}_2(\text{CO})_8$  to form the Co-complexes **46a** and **46b**, which were then employed in the following Nicholas reaction mediated by  $\text{BF}_3\text{Et}_2\text{O}$  to yield cyclooctyne precursors **47a** and **47b**. Unfortunately, all the attempts to decomplex intermediates **47a** and **47b** with common reagents (*e.g.*, CAN, NMO, TBAF, EDA) failed. The authors pointed out that the structure of this new oxygen-containing analogue of monobenzocyclooctyne (**MOBO**) was not stable enough. On the other hand, the

corresponding cyclononynes were successfully synthesized using the described strategy.

Rothman described an attempt to synthesize a cyclooctyne endocyclic amino acid (CEAA).<sup>54</sup> The general aim of this work was to create a novel class of cycloalkynes that resemble a proline analogue and, therefore, more similar to natural amino acids, to be employed in bioorthogonal reactions and likely presenting fewer issues of perturbation of the native structural configuration (Scheme 13). Moreover, the inclusion of endocyclic heteroatoms in the ring increased the reactivity of the triple bond compared to that of a carbocyclic ring, because of the hyperconjugation effect as a sigma-acceptor in an antiperiplanar position. The synthetic strategy proposed by the author envisioned a mild Mitsunobu reaction using triphenylphosphine ( $\text{PPh}_3$ ) and diisopropyl azodicarboxylate (DIAD) as the key step for the cyclization of a dicobalt adduct **48** (its synthesis is reported in the paper), yielding the intermediate **49**, which is then converted to the free cycloalkyne **50** through a Nicholas-related procedure. Unfortunately, the decomplexation process of cyclooctyne **49** proved very challenging, and unlike 9–11-membered cycloalkynes, its isolation was difficult, and the complete synthetic strategy could not be accomplished. Hosoya<sup>55</sup> and co-workers applied a synthetic strategy that involved the use of a cobalt complex triple bond intermediate for the synthesis of dibenzoazacyclooctyne (**DIBAC**, also referred to as **DBCO**<sup>17</sup>) derivatives (Scheme 14). These derivatives take advantage of the ring strain of an  $\text{sp}^2$  center, such as the aromatic ring, fused to the cyclooctyne to increase the reactivity. Furthermore, the endocyclic nitrogen

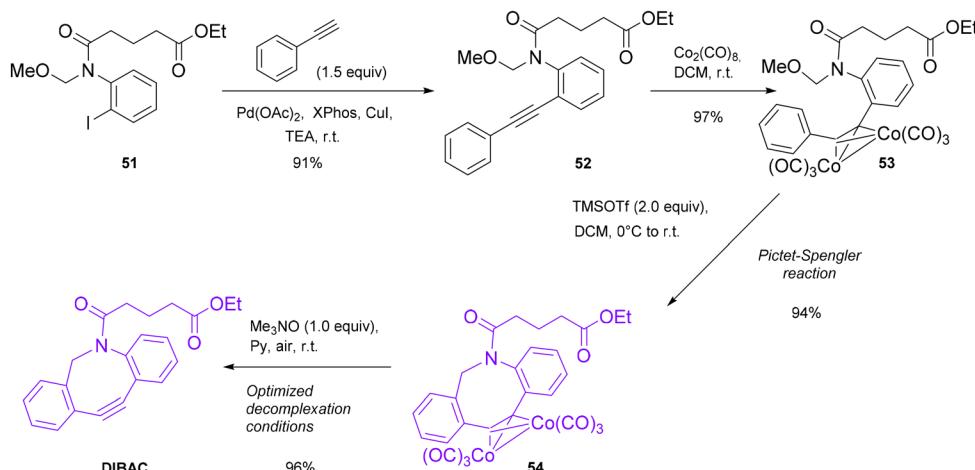


Scheme 12 Synthesis attempts of **BT8O** and **IC8O**.



Scheme 13 Synthesis attempt of CEAA.





Scheme 14 DIBAC synthesis using the Co-complex decomplexation methodology.

increases hydrophilicity, and it can be used as a functionalization handle. Compared with the classic strategy reported for this moiety, *i.e.* bromination of the double bond/elimination,<sup>13</sup> this procedure was more group-tolerant and enabled the formation of a series of functionalized **DIBACs**. Specifically, amide **51**, obtained in 2 steps in 91% yield from *o*-iodoaniline, was allowed to react through a Sonogashira coupling with phenylacetylene to form the alkyne precursor **52**, which was subsequently complexed with  $\text{Co}_2(\text{CO})_8$  to afford intermediate **53** in 97% yield. The key step in performing the cyclization of the azacyclooctyne moiety was accomplished using the Pictet-Spengler reaction,<sup>50</sup> where trimethylsilyl triflate (TMSOTf) activated the Co-complex, with the formation of an iminium salt that finally afforded the desired complexed-dibenzocyclooctyne **54**. For the final decomplexation step, classical literature procedures, *e.g.*  $\text{Me}_3\text{NO}$  in  $\text{CH}_2\text{Cl}_2$  at r.t. or CAN in silica gel/ $\text{Et}_2\text{O}$  at 0 °C, gave only poor yields; therefore, a screening for this step was performed using the Co-complex of dibenzocyclooctenol as a test substrate. Optimal reaction conditions were achieved, in terms of yield, reaction time and amount of additive, when 1 equivalent of  $\text{Me}_3\text{NO}$  was employed in pyridine without any protective atmosphere at r.t. for 3 hours. Such conditions were successfully applied to Co-complex **54**, yielding the desired **DIBAC** in 96% yield. This strategy was effectively applied to the other **DIBAC** derivatives functionalized with the Halotag ligand, coumarin and biotin. The **bisDIBAC** synthesized through this procedure is a bis-reactive molecule with important applications in azido-installed protein modification through a strain-promoted double click reaction (SPDC).<sup>56</sup>

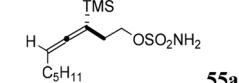
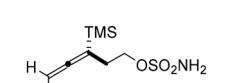
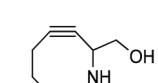
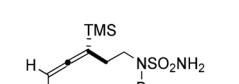
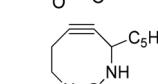
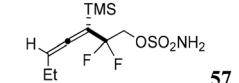
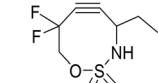
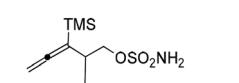
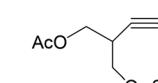
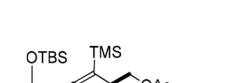
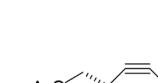
The introduction of a Co-masked triple bond using the Nicholas reaction for the synthesis of cyclooctynes showed good efficacy for producing dibenzocyclooctyne derivatives but presented some limitations due to the difficulties in the decomplexation step for other substrates. On the other hand, this methodology is a valid strategy for temporarily protecting a strained triple bond to insert other functionalities on the alkyne moiety.

## 2.5. Silylated allene rearrangement

Schomaker<sup>57–59</sup> and co-workers extensively studied a class of sulfamate/amide-based cyclooctynes bearing heteroatoms at the homopropargylic position, namely sulfur, nitrogen, and oxygen-containing cyclooctynes (**SNO-OCTs**). The idea behind the synthesis of molecules with this structure, confirmed by computational studies, lies in the beneficial effect of the heteroatoms on stability and reactivity. In addition, they display multiple sites that are amenable to functionalization. The general synthetic strategy involved a two-step one-pot procedure using  $\alpha$ -silyl allene sulfamate/sulfamide, which underwent Rh-catalyzed aziridination,<sup>60</sup> followed by ring expansion triggered by TBAF, promoting TMS elimination and ring-opening of the aziridine moiety (scheme of Table 1). The presence of the trimethylsilyl group was of critical importance, as it regiochemically directed the formation of the aziridine to the distal allene carbon for steric reasons, as well as for stabilization of the incipient positive charge through hyperconjugation.<sup>61</sup> The procedure had the advantage of being mild and rapid as the aziridine intermediate was not isolated.

The first generation of **SNO-OCTs**<sup>57</sup> (**56a–c**), featuring alkyl functionalization at the C1 position (Table 1, part A), was obtained in excellent to good yields (89% to 66%) from homoallenic sulfamates **55a** and **55b** and sulfamide **55c** under the reaction conditions described in Scheme 15. The carbon skeleton of precursors **55a–c** was derived from the  $\beta$ -allenic esters **61a** and **61b**, which were synthesized *via* a propargylic Claisen rearrangement of a TMS-propargylic alcohol with an orthoester.<sup>62</sup> These intermediates were subjected to reduction of the ester moiety with  $\text{LiAlH}_4$  to yield homoallenic alcohols **62a** and **62b** in 81% and 99% yields, respectively. Final functionalization of **62a** and **62b** employing chlorosulfonyl isocyanate (CSI) and formic acid yielded sulfamates **55a** and TBS-**55b**. The latter was completely deprotected during the ring expansion stage using an excess of TBAF. On the other hand, **62a** was converted into precursor **55c** using *tert*-butyl aminosulfonylcarba-

Table 1 Synthesis of three generations of SNO-OCTs

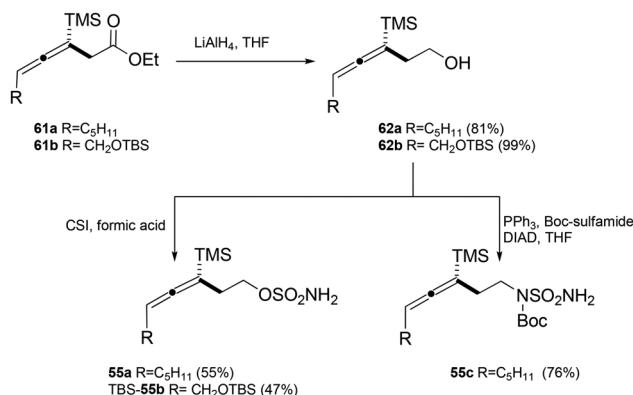
SNO-OCT generation	Synthesis of homoallenic precursor	Homoallenic reactant	SNO-OCT (yield%)
<b>Part A</b>			
	Acid-catalyzed Claisen rearrangement	 <b>55a</b>	 <b>56a (89%)</b>
<i>First generation: alkyl group at C1-carbon</i>			
		 <b>55b</b>	 <b>56b (66%)</b>
		 <b>55c</b>	 <b>56c (65%)</b>
<b>Part B</b>			
	Zn-mediated Reformatsky–Claisen rearrangement	 <b>57</b>	 <b>58 (72%)</b>
<b>Part C</b>			
	Au(i)-catalyzed Claisen rearrangement	 <b>59a</b>	 <b>60 (61%)</b>
<i>Third generation: functional handle at C4 or C1–C4</i>			
		 <b>59b</b>	 <b>60b (81%)</b>
		 <b>59c</b>	 <b>60c (41%)</b>

mate (Boc-sulfamide) in the presence of diisopropyl dicarboxylate (DIAD) with 76% yield.

Cyclooctyne **56a** proved to be very stable under both basic and acidic conditions. Furthermore, the 1,3-dipolar cycloaddition with benzyl azide delivered very high rate constants, greater than those obtained with fluorinated cyclooctynes like **DIFO**. Bioconjugation studies were performed to assess the viability of using **SNO-OCTs** in a biological context and for the functionalization of polymers.<sup>63</sup>

The second generation of **SNO-OCT** (**58**)<sup>58</sup> (Table 1, part B) consisted of a difluorinated derivative that displayed improved rate constants in the cycloaddition reaction with diazoacetamides, greater than  $5.13 \text{ M}^{-1} \text{ s}^{-1}$ , and in general with Type I-II dipoles, whilst showing a diminished preference for engaging in inverse electron demand Diels–Alder (IEDDA) reactions. Additionally, such a substrate could be further manipulated to afford a triple ligation system for bioorthogonal reactions. The synthetic strategy envisioned the use of a chlorodifluoro ester





**Scheme 15** Synthesis of homoallenic sulfamates **55a** and **55b** and sulfamide **55c**.

precursor **63**, synthesized according to the literature,<sup>58</sup> for the introduction of a *gem*-difluoro moiety at C4 and an ethyl group at C1 (Scheme 16). This precursor underwent a Zn-mediated Reformatsky–Claisen rearrangement<sup>64</sup> to yield the corresponding allenic difluoroacid, which was promptly methylated with trimethylsilyldiazomethane (TMSCHN<sub>2</sub>), giving the desired homoallenic methyl ester intermediate **64** in 78% overall yield. Finally, the homoallenic sulfamate **57** was obtained after reduction of the ester moiety with sodium borohydride (NaBH<sub>4</sub>), followed by the introduction of the sulfamate group using CSI and formic acid.

The third generation of SNO-OCTs<sup>59</sup> (**60a–c**) is represented by highly functionalized cyclooctynes at C4 or C4–C1, designed to be employed as labelling reagents (Table 1, part C). The synthesis of the first SNO-OCT **60a** with only a C4 handle allowed the authors to optimize a new strategy for this class of compounds (Scheme 17). Specifically, the first step involved an Au(i)-catalyzed Claisen rearrangement<sup>65</sup> of intermediate **65**, which was synthesized as described in the paper from a TMS-protected propargylic alcohol. Careful screening of the catalytic

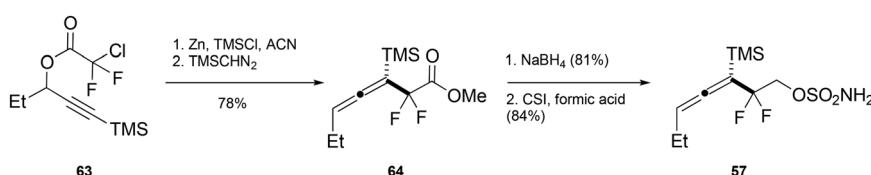
conditions for the first step revealed that gold(i) chloride (AuCl, 5 mol%) in the presence of sodium tetrafluoroborate (NaBF<sub>4</sub>, 10 mol%) as an additive provided the best experimental conditions to be employed. Compound **65**, which contained both a propargyl unit and an allylic acetate, yielded, after Claisen rearrangement followed by reduction with NaBH<sub>4</sub>, the corresponding alcohol intermediate **66** with a tertiary allenic center. Intermediate **66** was then converted into homoallenic precursor **59a** with CSI and formic acid with a 68% yield.

Under these optimized conditions, intermediate **67** (obtained from the reaction of a propargylic alcohol previously synthesized by the authors<sup>57</sup> and methyl propiolate) was subjected to Au(i)-catalyzed rearrangement, yielding the products **68a** and **68b** as a diastereoisomeric mixture, which was separated by column chromatography (Scheme 18). Finally, treatment with hexafluoroisopropyl sulfamate (HFIPS) yielded the desired homoallenic sulfamides **59b** (66% yield) and **59c** (72% yield), which were employed for the synthesis of third-generation SNO-OCTs (**60b** and **60c**).

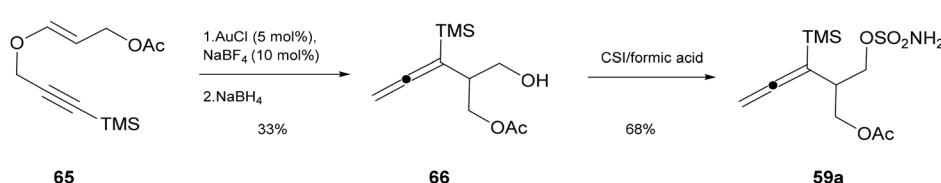
The strategy proposed by Schomaker and co-workers, *i.e.* the synthesis of diverse SNO-OCTs *via* a rearrangement of silylated allenes, proved to be very versatile, as the cyclooctyne moiety can be decorated with different handles at different positions, allowing the formation of highly dense functionalized molecules. However, the drawback of this procedure is that it is limited to the synthesis of heteroatom-containing cyclooctynes.

## 2.6. Carbene-mediated strategy

Raines and co-workers<sup>66</sup> sought the synthesis and application of a new kind of heterocycle-containing cyclooctyne moiety, namely 2-azadibenzocyclooctyne (**ABC**). The structure of this molecular scaffold was proposed based on computational studies, which identified the **ABC** moiety as offering an optimal combination of increased strain and electronic tuning to achieve better rate constants in 1,3-dipolar cycloaddition

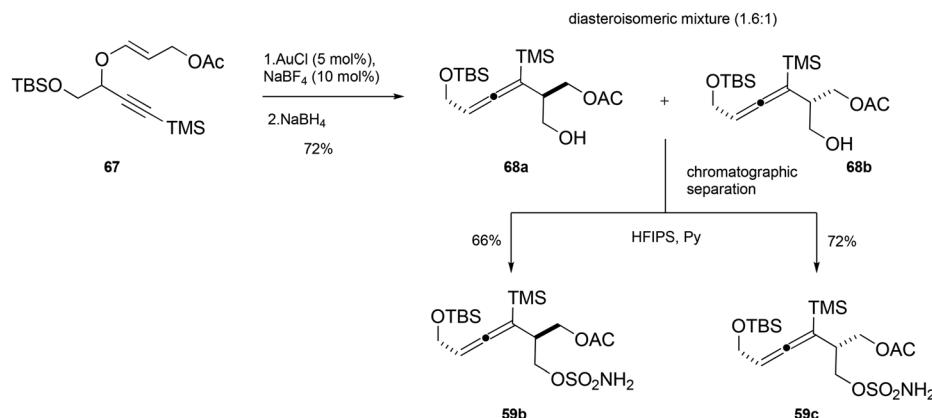


**Scheme 16** Synthesis of homoallenic sulfamate **57**.



**Scheme 17** Synthesis of homoallenic precursor **59a**.





Scheme 18 Synthesis of homoalleenic sulfamides 59b and 59c.

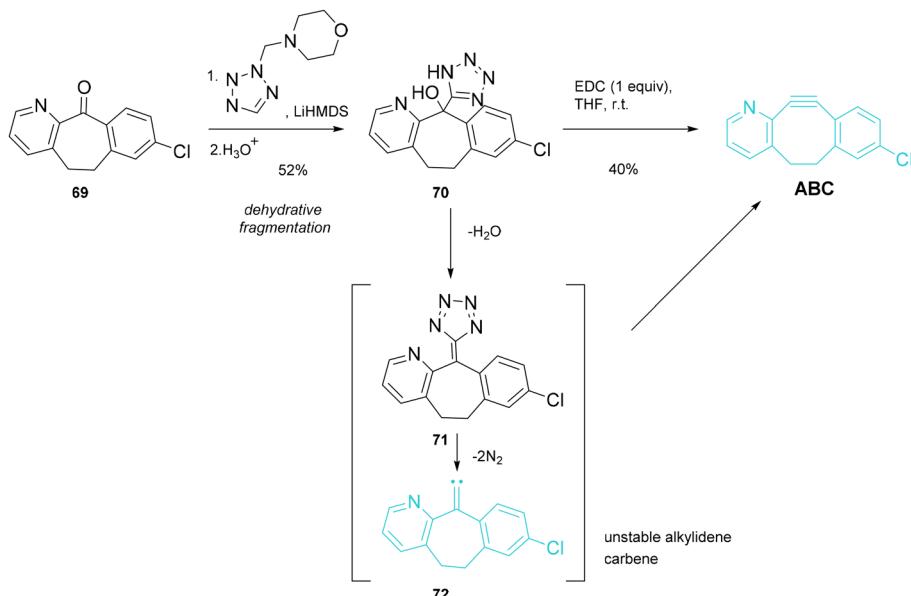
reactions. The synthetic strategy toward **ABC** involved, as a key reaction step, a ring-expansion through a 1,2 rearrangement of an alkylidene carbene. These intermediates have a very short lifetime, as they immediately undergo a 1,2 migration of a  $\beta$ -substituent upon formation, generating an alkyne.<sup>67–69</sup>

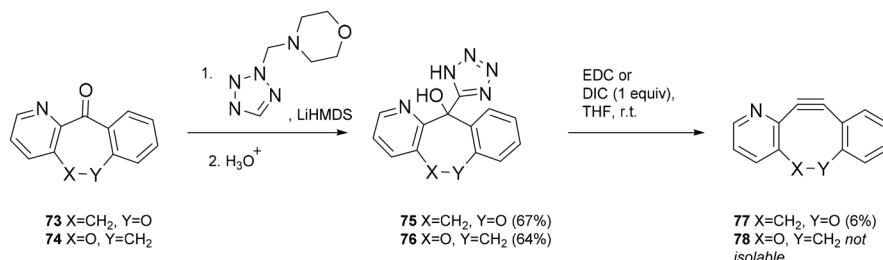
The starting material of the synthetic pathway was the commercially available ketone **69**, which reacted with *N*-morpholinomethyl-5-lithiotetrazole (formed *in situ* from 4-((*N*-tetrazolyl)methyl)morpholine and a 1 M solution of LiHMDS) to yield the corresponding 5-hydroxalkyl-1*H*-tetrazole **70** in 52% yield after acidic hydrolysis.<sup>70</sup> Tetrazole **70** was treated with 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDC) to afford, through a dehydrative elimination, the intermediate tetraazafulvene **71**, which rapidly expelled two nitrogen molecules to yield the unstable alkylidene carbene **72** that, in turn, after a [1,2] rearrangement, afforded the final cyclooctyne product **ABC** in 40% yield (Scheme 19).<sup>69,71</sup>

The same synthetic strategy has been applied in this paper to the synthesis of the known **DIBO**<sup>30</sup> employing the commercially available dibenzosuberone as the starting material, which yielded the corresponding cyclooctyne in 51% yield.

Later, Gold<sup>72</sup> and co-workers attempted to employ this approach for the synthesis of the oxygen-containing analogues oxa-azabenzocyclooctynes (**O-ABCs**), as reported in Scheme 20. The starting materials employed were ketones **73** and **74** synthesized using a literature procedure that envisioned the use of a two-step substitution–Parham-type cyclization.<sup>73</sup>

The cyclooctyne **O-ABC-77**, derived from **73**, showed stability issues upon solvent concentration after flash chromatography or when exposed to air; therefore, it was isolated only in 6% yield. However, it was possible to prove its formation by trapping with benzyl azide. **O-ABC-78**, derived from ketone **74**, was too unstable to be isolated, but the *in situ* reaction with

Scheme 19 Carbene-mediated synthesis of **ABC**.



Scheme 20 Carbene-mediated synthesis of O-ABCs.

benzyl azide afforded the corresponding cycloadduct in 53% yield from the tetrazole intermediate.

In conclusion, Raines and Gold proposed a convenient strategy for the synthesis of ABC cyclooctyne employing the commercially available starting material **69**. However, extending this strategy to the corresponding oxygenated derivatives **O-ABCs** presented issues in terms of low yield and stability. Additionally, in the latter case, starting compounds need to be synthesized, as they are not readily available.

### 3. Conclusions

Cyclooctyne derivatives are scaffolds of strategic importance in bioorthogonal chemistry. Researchers in this field face a stimulating challenge in synthesizing these derivatives, as success relies on the essential balance between reactivity and stability. In addition, the high selectivity these substrates must exhibit in biological environments adds an additional layer of complexity. Many efforts have been made in recent years to develop efficient synthetic protocols applicable to a wide range of substrates. While much progress has been attained over the years, there is still room for improvement. All the procedures reported so far, despite their differences, share a common feature, *i.e.* the formation or release of the endocyclic triple bond at a late stage in the synthetic protocol, due to its inherent reactivity or instability. The vinyl enol/triflate strategy has been successfully applied to the synthesis of a wide range of cyclooctynes such as **MOFO**, **DIFOs** and **BARAC**. This method can be considered one of the most flexible strategies, even though it does present challenges in achieving good yields in certain cases. On the other hand, the Co-complex decomplexation strategy has proven effective for the synthesis of various cyclooctyne derivatives, with some ongoing studies still needed to complete the final step of decomplexation in some cases. Other procedures, although more specific, still contribute to expanding and diversifying the pool of available cyclooctynes. As an example, the silylated allene rearrangement strategy for the synthesis of **SNO-OCTs**, developed by the research group of Schomaker, effectively yielded a wide variety of heteroatom-containing cyclooctynes. Future research in this field will hopefully lead to the development of new synthetic protocols that combine wide applicability for the target molecules with a reasonable synthetic pathway and an adequate

overall yield. These advancements will undoubtedly provide a winning combination for the effective use of cyclooctyne derivatives in bioorthogonal reactions within biological environments.

### Data availability

No primary research results, software or code have been included. Multistep and overall yield data were calculated from the individual yields reported in the literature sources cited in this review.

### Conflicts of interest

There are no conflicts to declare.

### Acknowledgements

A. D. gratefully acknowledges the Ministero dell'Università e della Ricerca (MUR) for the PON PhD fellowship DOT1423134-3 and E. P. and D. L. acknowledge MUR for PRIN project no. 20207CNBE4 "FAITH".

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