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## COMMUNICATION

## Selective Chlorination of Iminosydnone for Fast Release of Amide, Sulfonamide and Urea-Containing Drugs

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Accepted 00th January 20xxMinghao Feng,<sup>a†</sup> Léa Madegard,<sup>a†</sup> Margaux Riomet,<sup>a</sup> Manon Louis,<sup>a</sup> Pier Alexandre Champagne,<sup>b</sup> Grégory Pieters,<sup>a</sup> Davide Audisio<sup>a</sup> and Frédéric Taran\*<sup>a</sup>

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Herein, we describe a methodology for iminosydnone chlorination and we demonstrate the high beneficial effect of this modification on the reactivity of these mesoionic dipoles in strain-promoted cycloaddition reactions. Exploiting their reaction with cyclooctynes, we used these new iminosydnone for bioorthogonal release of amide, urea and sulfonamide containing drugs. Notably, drugs containing a terminal amide function were released for the first time with good kinetic constants.

The use of prodrugs - caged bioactive substances whose activities can be restored in vivo - is a well-established strategy that has led to the marketing of several pharmaceutical products.<sup>1</sup> Prodrugs not only improve the solubility,<sup>2</sup> permeability<sup>3</sup> and biostability<sup>4</sup> of drugs, but also allow their release in specific tissues. Conventional prodrug activation strategies typically rely on physiological characteristics of pathological cells, such as pH and redox variations or the presence of an over-expressed enzyme. Upon activation, the active parent drug is released and can then exert the desired pharmacological effect.<sup>5</sup> However, healthy and pathological tissues are often poorly differentiable, resulting in a non-selective activation of prodrugs, therefore decreasing their efficiency at reducing undesired side-effects.<sup>6</sup> An emerging alternative strategy is the use of selective, bioorthogonal reactions, which can be triggered on demand where the disease is taking place.<sup>7</sup> Bioorthogonal click-and-release reactions appear as particularly suitable for such prodrug strategies.<sup>8</sup> In this approach, a bioorthogonal function is attached to a key part of the drug in order to mask its original activity. Addition of the complementary bioorthogonal trigger then allows the decaging of the drug, restoring its biological activity. The most representative examples include Staudinger-type reductive

cleavage reactions,<sup>9</sup> transition-metal-catalysed deallylation<sup>10</sup> or depropargylation<sup>11</sup> and the inverse electron demand Diels-Alder reaction (iEDDA, Fig. 1a) between tetrazines (Tz) and trans-cyclooctenes (TCO).<sup>12</sup> The latter is considered as the most effective among all reported decaging reactions both in terms of kinetics and biocompatibility.

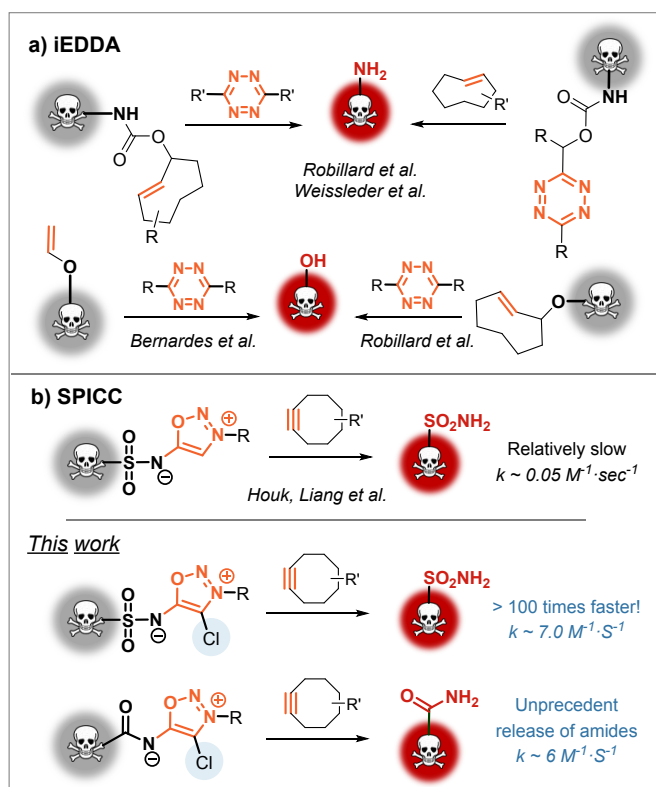


Fig. 1. Drug release after bioorthogonal click and release reactions.

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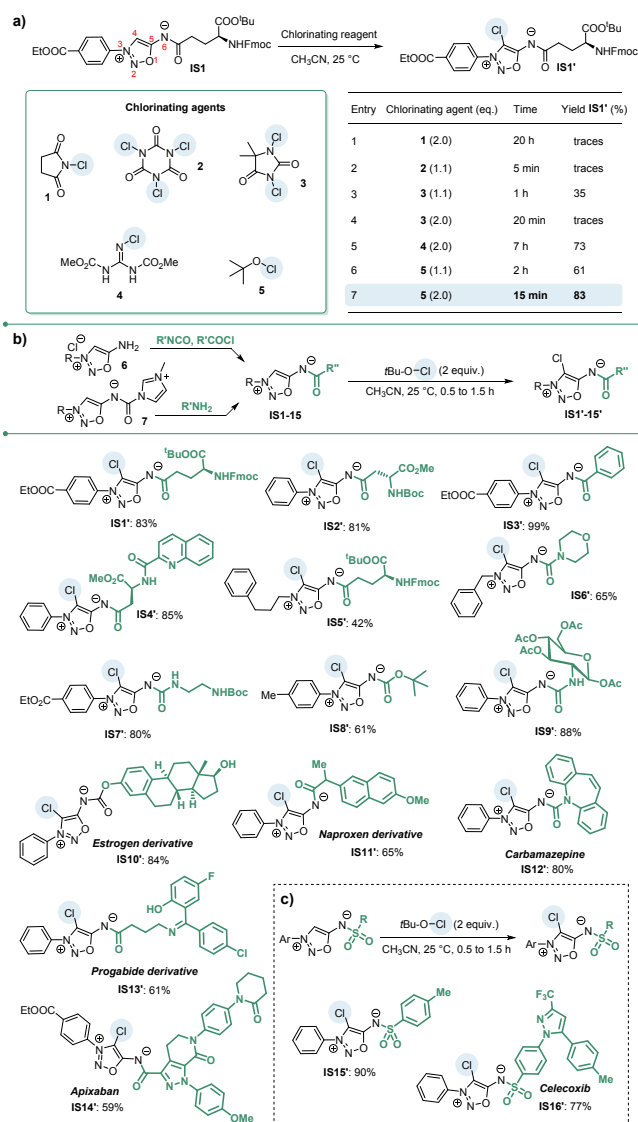
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The use of this reaction for prodrug development has been pioneered in 2013 by Robillard and co-workers who reported the bioorthogonal release of the drug Doxorubicin upon reaction of a Tz with a TCO bearing a carbamate in allylic position.<sup>13</sup> Since then, several key developments have been reached notably by Weissleder<sup>14</sup> and Robillard<sup>15</sup> to improve the efficiency of the iEDDA reaction for drug release. These efforts

recently led to the development of a TCO-doxorubicin prodrug, currently under clinical trials for solid tumor therapy in human patients.<sup>16</sup> First used to release NH<sub>2</sub>-containing drugs, iEDDA was extended in 2016 to the release of alcohols by the group of Bernardes using a vinyl ether/Tz pair<sup>17</sup> and to phenols release from TCO-ethers as described in 2018 by Robillard<sup>18</sup> (Fig. 1a). As of today, decaging technologies were mostly focused on the release of alcohols, phenols and amines, either directly or with the intermediate of a self-immolative linker. In 2017, our group reported a new bioorthogonal click and release reaction involving iminosydones and cyclooctynes (SPICC: Strain-Promoted Iminosydnone-Cyclooctyne Cycloaddition, Fig. 1b), which allows the release of ureas, carbamates and sulfonamides.<sup>19</sup> This reaction was later used to release Celecoxib, a sulfonamide-containing anti-inflammatory drug.<sup>20</sup> While the SPICC reaction has the major advantage of being quantitative, its moderate kinetics represent a limitation for its applicability. Herein, we demonstrate that the selective chlorination at position C-4 of iminosydones significantly boosts their reactivity towards cyclooctynes and allows the rapid release of bioactive substances containing a terminal amide function for which no bioorthogonal prodrug strategy was possible until now (Fig. 1b).

Our group previously showed that halogenation at position C-4 of sydones, another family of mesoionics, dramatically enhanced the kinetics of their cycloaddition with cyclooctynes.<sup>21</sup> Computational studies carried out by the group of Houk revealed that the origin of this halogen effect is due to lowering the distortion energy of sydones.<sup>22</sup> We later confirmed this halogen effect on iminosydones, where their bromination improved the kinetics of the SPICC reaction by one order of magnitude.<sup>23</sup> Motivated by this remarkable effect, we turned our attention to the incorporation of more electronegative halogen atoms. Unfortunately, all attempts to fluorinate iminosydones in position 4 were unsuccessful, probably due to the apparent instability of the expected fluoro-iminosydones. We then tested various chlorinating conditions on iminosydnone **IS1**, used as the model substrate (Scheme 1a). We first began our investigation with *N*-chlorosuccinimide **1**, and trichlorocyanuric acid **2** but unfortunately with no success. The desired chlorinated product could finally be observed when 1,3-dichloro-5,5-dimethylhydantoin **3** was used, albeit in low yield and along with some of remaining unreacted iminosydnone. Unfortunately, rapid degradation happened when a higher stoichiometry of **3** was used (entry 2, Scheme 1a). Palau'chlor<sup>24</sup> **4** gave an encouraging 73% yield after 7 hours of reaction at room temperature (entry 3, Scheme 1a), however, separation of the desired chlorinated iminosydnone from the polar by-products proved challenging. Finally, *t*BuOCl **5** was found to be the most efficient and practical reagent affording 83% of **IS1'** in only 15 min at room temperature (entry 7, Scheme 1a). With these optimised conditions in hands, we further explored the tolerance and selectivity of our chlorination method. To this end, an array of iminosydones **IS2-15** were prepared using established protocols.<sup>25</sup> Carbamoyl and urea derivatives of iminosydones were prepared in one step by *N*-exocyclic functionalisation involving the addition of

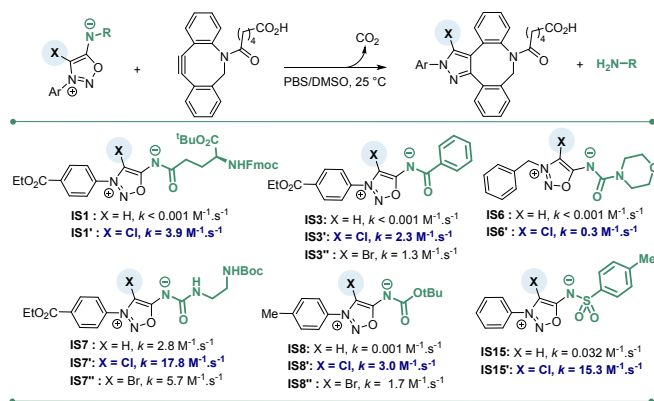
strong electrophiles (activated esters, chloroformates or isocyanates) on **6** under slightly basic conditions or the addition of alcohols and amines on **7** (see ESI for details). The chlorination protocol was found quite general and afforded a variety of 4-chloro-iminosydones in good yields (Scheme 1b).



**Scheme 1.** a) Optimisation of iminosydnone chlorination. b) and c) scope of the chlorination reaction. All yields are isolated yields.

The presence of amino-acids, alcohols, phenols, carbohydrate and heterocyclic moieties did not affect the reaction. No additional chlorination was observed even in the case of substrates bearing electron-rich aromatic sites (**IS10**, **11**, **13** and **15**), showing that position C-4 of the iminosydnone core displays a particular reactivity towards chlorination. The chemoselectivity of this reaction allowed us to successfully perform the chlorination of iminosydones carrying biologically relevant compounds such as Estradiol (hormone, **IS10**), Naproxen (nonsteroidal anti-inflammatory drug, **IS11**), Progabide (GABA analogue, **IS13**), Carbamazepine (antiepileptic drug, **IS14**) and Apixaban (anticoagulant drug, **IS15**). The chlorination reaction was also effective on sulfonamide

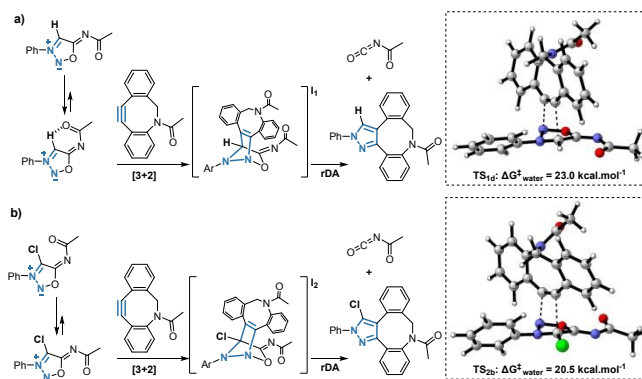
compounds as shown by the preparation of prodrug **IS17'** of the COX-2 inhibitor Celecoxib (Scheme 1c). Kinetic studies were then carried out to evaluate the aptitude of chlorinated iminosydones to react with cyclooctynes. We selected a series of iminosydones bearing amide, urea, carbamate and sulfonamide functions at position N-6 and compared the rate constants of the non-chlorinated versus chlorinated compounds with dibenzocyclooctyne (DBCO). As shown in Scheme 2, the effect of the chlorination on the reaction rate is quite significant, as it induced an increase of several orders of magnitude regardless of the iminosydnone substitution pattern.



**Scheme 2.** Halogen effect on the SPICC reaction. Rate constants  $k$  were determined by monitoring the reaction between **IS** and DBCO in 0.01M PBS/DMSO (80/20) at 25 °C; PBS: Phosphate buffered-saline.

The most spectacular effect was observed for iminosydones bearing an exocyclic amide function (**IS1** and **IS3**). While the parent compounds ( $X = H$ ) are almost unreactive, the corresponding chloro-iminosydones (**IS1'** and **IS3'**) react smoothly with rate constants  $k > 1 \text{ M}^{-1}\cdot\text{sec}^{-1}$  at room temperature. These rate constants are in the same range as the well-established strain promoted azide-alkyne cycloaddition (SPAAC) reaction and support the use of chloro-iminosydones as masking agents for bioactive compounds containing terminal amide functions. The beneficial influence of the chlorine atom was also observed for iminosydones bearing carbamate and sulfonamide moieties (Scheme 2), inducing a rate increase of the SPICC reaction by more than 100 (**IS8** vs **IS8'**, **IS16** vs **IS16'**). The chlorine effect was also significant on the urea derivative **IS7**, but to a lesser extent. Notably, 3-*N*-alkyl iminosydnone **IS6'** was also effective in the reaction with DBCO whereas its non-chlorinated analogue **IS6** was completely unreactive. We also confirmed the beneficial impact of bromination at position C-4 of iminosydones although it is slightly lower than chlorination (compare **IS3'**, **7'** and **8'** with **IS3''**, **7''** and **8''** in scheme 2). To better understand the origin of the chlorine atom effect on the kinetics, we performed a theoretical study using DFT calculations at the M06-2X/6-31+G(d,p)/CPCM(H<sub>2</sub>O) level of theory (Fig. 2, see ESI for computational details). Using two model iminosydones **mis1** and **mis2** (Fig. 2a), the transition structures (TS) and activation free energies ( $\Delta G_{\text{water}}^{\ddagger}$ ) for their reactions with a DBCO model were computed. As the cycloaddition step is rate-determining

and irreversible in similar systems, we compared the corresponding [3+2] TSs for the two systems. The  $\Delta G_{\text{water}}^{\ddagger}$  for the chlorinated iminosydnone **mis2** (20.5 kcal mol<sup>-1</sup>) was found to be significantly lower than the one for **mis1** (23.0 kcal mol<sup>-1</sup>). This free energy difference of 2.5 kcal mol<sup>-1</sup> results into an almost 100-fold increase in rate at room temperature due to chlorination, which is in line with the experimentally-observed rate enhancement.

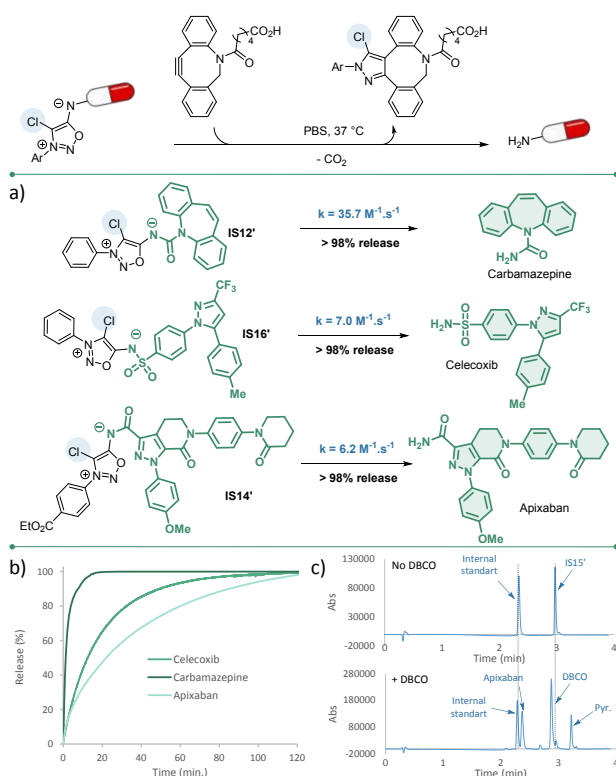


**Fig. 2.** Theoretical study on the SPICC reaction. a) Mechanism of the reaction and transition structure involving **mis1** (TS<sub>1d</sub>), b) Mechanism of the reaction and transition structure involving **mis2** (TS<sub>2b</sub>), with a DBCO model (M06-2X/6-31+G(d,p)/CPCM(H<sub>2</sub>O) level of theory). Only one pyrazole regioisomer was considered for clarity reasons.

As already demonstrated by Houk and co-workers in the case of sydones,<sup>22</sup> the origin of the beneficial halogen effects on cycloaddition kinetics could be due to the lower distortion energy of the 4-chloro-iminosydnone compared with the non-halogenated structure. In addition, we also found that the presence of the halogen atom on C-4 prevents the iminosydnone from adopting a low-energy conformation with an intramolecular C-H...O interaction (Fig. 2a), and as such chlorinated iminosydones might be less stabilized. Overall, these theoretical and experimental results demonstrated that the chlorination of iminosydones accelerates the SPICC reaction to a degree that allows its use for drug decaging applications. To this end, we investigated the bioorthogonal release of the drugs Carbamazepine, Apixaban and Celecoxib from their corresponding prodrugs **IS14'**, **IS15'** and **IS17'** (Fig. 3). We first checked the stability of these prodrugs and found  $t_{1/2} > 7$  days in PBS (see ESI). Decaging was then carefully monitored at 37 °C in PBS buffer by following both the disappearance of the starting 4-chloro-iminosydones and the appearance of the drugs released through the retro-Diels-Alder (rDA) step of the reaction. As expected, all drugs were rapidly released upon addition of DBCO. Importantly, according to HPLC analysis, the SPICC reactions were quantitative (> 98% of drug release) and perfectly selective, as no byproduct was observed. The beneficial influence of the chlorine atom can be highlighted by the rate constant obtained with compound **IS17'** which allows a 100 times faster release of Celecoxib compared to published data.<sup>20</sup> These results validate the approach of using chloro-iminosydones as masking agents for drugs containing terminal amide, urea or sulfonamide drugs.



In summary, we have developed a general method for iminosydones chlorination and have demonstrated the beneficial impact of this modification on the SPICC reaction. 4-chloro-iminosydones were thus found particularly reactive leading to a fast click and release reaction with cyclooctynes. These mesoionic compounds can be employed to temporarily mask terminal amide, urea or sulfonamide functional groups in bioactive substances. This approach can be used for the development of new prodrugs but also in the design of new drug delivery technologies such as antibody-drug conjugates (ADCs), offering additional tools for chemical biology.



**Fig. 3.** Release of drugs upon SPICC reactions. a) reactions and rate constants values, b) drug release as function of time after addition of DBCO, c) chromatogram after 1 h reaction of **IS15'** with DBCO. Reaction conditions: [IS] = 30 μM, [DBCO] = 45 μM, 37 °C in 0.01M PBS containing 20% DMSO. Internal standard = 3-fluoro-4-nitrophenol.

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