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## Sydnone-based prosthetic groups for radioiodination

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### ABSTRACT

The potential of Strained-Promoted Sydnone-Alkyne Cycloaddition (SPSAC) for radioiodination was evaluated with model cyclooctyne-conjugated peptides. Starting with a series of sydnones with varying N<sub>3</sub> and C<sub>4</sub> substitution, a preliminary kinetic study with non-radioactive iodinated compounds highlighted the superiority of an arylsydnone substituted by a chlorine atom in C<sub>4</sub> position. Interestingly, reaction rate up to 11 times higher than using an azide was achieved with the best system. Access to <sup>125</sup>I-labelled sydnones was granted with high efficiency from arylboronic acid precursors by copper catalyzed nucleophilic substitution. Application of SPSAC on the model peptide in radiotracer conditions showed the same trend than in non-radioactive kinetic study and complete reactions could be achieved within less than an hour for the best systems. These results are favorable for use in the production of radiopharmaceuticals with heavy halogens and increase the diversity of available bioorthogonal reaction for nuclear imaging and therapy.

### 1. Introduction

Interest in heavy radiohalogens in nuclear medicine is now well established whether for imaging or therapeutic applications. Several radioisotopes of iodine such as <sup>123</sup>I (γ, 13.2 h), <sup>124</sup>I (β<sup>+</sup>, 4.1 d), <sup>125</sup>I (γ, Auger e<sup>-</sup>, 59.4 d) and <sup>131</sup>I (β<sup>-</sup>, γ, 8.0 d) have demonstrated a significant relevance for Single Photon Emission Computed Tomography (SPECT) or Positron Emission Tomography (PET) imaging but also for targeted radiotherapy.<sup>1</sup> Historically, radioiodination of biological vectors such as peptides or proteins was performed by direct electrophilic substitution of tyrosine residues at *ortho* position of the phenol ring using oxidizing agents, in particular Iodogen®, to generate electrophilic iodine species.<sup>2</sup> Although apparently simple and fast, this strategy is limited by the need for accessible tyrosine residues on the biomolecule, which is not always the case, especially for small proteins. In addition, alteration of the protein properties can be observed in case of sensitivity to the oxidizing conditions required. Finally, for *in vivo* use, this labelling approach shows a lack of stability due to dehalogenation induced by deiodinases known to metabolize the iodotyrosine motif found in thyroid hormones and leading to uptake of free radioiodine by stomach and thyroid.<sup>3,4</sup>

Consequently, use of a more stable prosthetic group is commonly preferred. For instance, the preparation of *N*-succinimidyl-3-[<sup>\*</sup>I]iodobenzoate ([<sup>\*</sup>I]SIB), that is then grafted to natural amino acids, mainly lysine residues, is often reported.<sup>5</sup> However, in addition to the lack of specificity of the bioconjugation step, competitive hydrolysis of the activated ester occurs at pH required for the bioconjugation (pH = 8–9) limiting the conjugation yields achievable. This reactivity issue can be minimized using a high concentration of protein (4–5 mg/mL or higher), but at the expense of a lower specific activity of the final radioconjugate, or a risk of aggregation or precipitation of the protein.

Over the last decade, bioorthogonal click chemistry has emerged as an interesting tool for optimization of conjugation strategies in the field of radiopharmaceutical sciences.<sup>6</sup> This approach demonstrated advantages compared to conventional ones with fast kinetics, mild aqueous reaction conditions and chemo- and regio-selectivity of the resulting product. Based on this approach, our group<sup>7</sup> and others<sup>8–10</sup> have previously reported the development of prosthetic groups for radioiodination and/or astatination of peptides and proteins (Scheme 1).

In particular, we compared five bioorthogonal systems, with the kinetic evaluation of Cu(I)-catalysed Azide-Alkyne Cycloaddition

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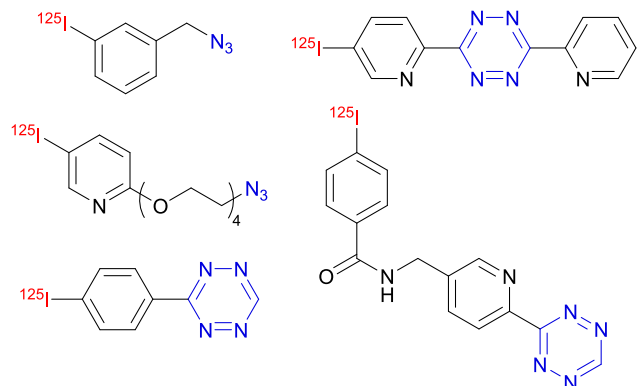
E-mail addresses: [jean-francois.gestin@univ-nantes.fr](mailto:jean-francois.gestin@univ-nantes.fr) (J.-F. Gestin), [romain.eychenne@univ-nantes.fr](mailto:romain.eychenne@univ-nantes.fr) (R. Eychenne), [francois.guerard@univ-nantes.fr](mailto:francois.guerard@univ-nantes.fr) (F. Guérard).

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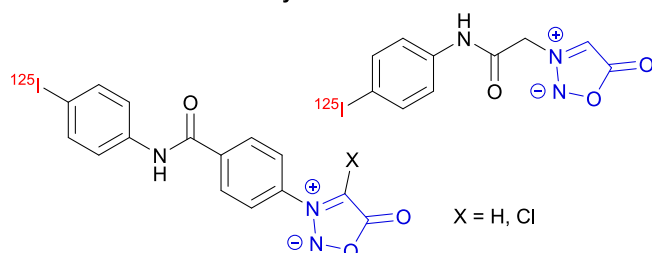
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Previously reported radioiodinated clickable prosthetic groups <sup>7-10</sup>

## This work : radioiodinated sydnones



Scheme 1. Compounds discussed in this report.

(CuAAC), Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC) with dibenzozacyclooctyne/azide (DIBAC/ $N_3$ ) or bicyclononyne/azide (BCN/ $N_3$ ) and Inverse Electron-Demand Diels–Alder reaction (IEDDA) with *trans*-cyclooctene/tetrazine (TCO/Tz) or BCN/Tz. Initially evaluated with a model peptide, further experiments demonstrated the possibility to transfer this strategy for the radiolabelling of an anti-CD138 monoclonal antibody with high radiochemical yield (RCY) and without impact on the immunoreactivity of the vector.<sup>7</sup> However, despite promising advances, systems studied also show some limitations: (i) low stability of TCO function due to isomerisation to *cis*-cyclooctene,<sup>11</sup> limiting the long term storage of the pre-modified mAb; (ii) use of copper catalyst for CuAAC, not always compatible with biomolecules<sup>12,13</sup>, or (iii) use of bulky and lipophilic moieties like DIBAC that can impair vectors pharmacokinetics.<sup>14</sup> Consequently, the development of novel bioconjugation strategies to optimize the approach based on clickable prosthetic groups remains necessary.

This work aims to extend the range of clickable systems for radiohalogenation of biomolecules and for this purpose our interest focused on Strain-Promoted Sydnone-Alkyne Cycloaddition (SPSAC).<sup>15,16</sup> Sydnones are described as easily accessible *via* three-step synthesis from anilines and to have a high chemical stability, making them the most studied mesoionic compounds.<sup>17</sup> In addition to have an effect on reactivity, substitution on  $N_3$  or  $C_4$  position also allows functionalization leading to various applications.<sup>16,18,19</sup> Finally, among all the systems reported in the literature, SPSAC has previously shown a high reactivity that in some cases can compete with the IEDDA system.<sup>20</sup>

The high reaction rate of SPSAC promotes a use with low reactant concentration, that is particularly suitable for the development of radiotracers. Recent reports in literature illustrate the scope of SPSAC for development of radiofluorinated PET tracers especially based on *N*-arylsydnones. The proof of concept of radiofluorination using sydnone-based prosthetic group was described with the preparation of 4-<sup>18</sup>F fluorosydnones and their reaction with BCN<sup>20</sup> or to label peptides using a [<sup>18</sup>F]fluorophenylsydnone prosthetic group.<sup>21</sup> Radiolabeling with SPSAC reaction resulted in higher RCYs than using a conventional oxime ligation (12 % vs 3 %). Even if PET/CT imaging revealed a high liver uptake due to the highly lipophilic DIBAC moiety conjugated to the

peptides, this could be in part balanced by the addition of a histidine/glutamic acid linker. More recently, SPSAC system was also reported as a potential pretargeting strategy.<sup>22</sup>

Herein we report the development of a series of sydnones for radioiodination of biomolecules by click chemistry and their comparison with the previously studied SPAAC system that uses instead the azide clickable handle. It is known that sydnone substituents on  $N_3$  or  $C_4$  positions can have a drastic impact on SPSAC reaction rate. Consequently, we chose to modulate the nature of  $C_4$  substituent (H or Cl) since it was previously reported a 30 times increase in reaction rate with Cl.<sup>16</sup> On the other hand, we compared the reaction with  $N_3$  substituent being either an alkyl or an aryl, the latter having also shown more favorable reaction rates. The sydnones were prepared as non-radioactive iodinated references as well as precursors for <sup>125</sup>I-labelling. For this, we chose boronic acid as a leaving group that was previously reported as an efficient strategy for radioiodination.<sup>23-25</sup> A model peptide bearing a DIBAC or a BCN group was then used for evaluation of reaction rates with non-radioactive iodinated sydnones and their <sup>125</sup>I-labelled homologues.

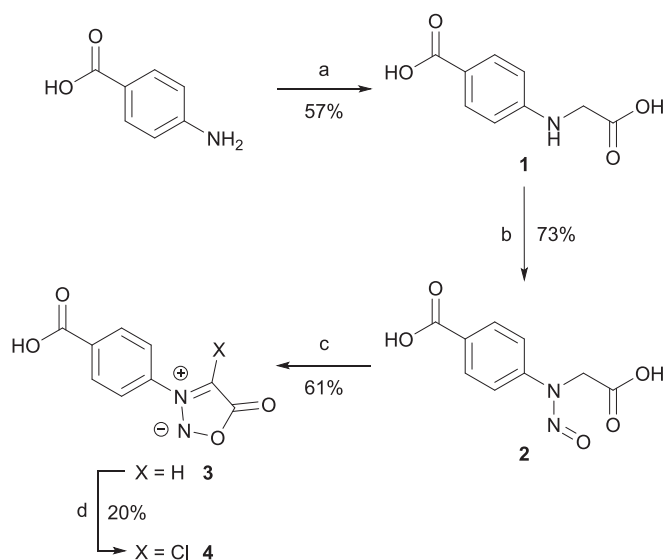
## 2. Results and discussion

## 2.1. Synthesis

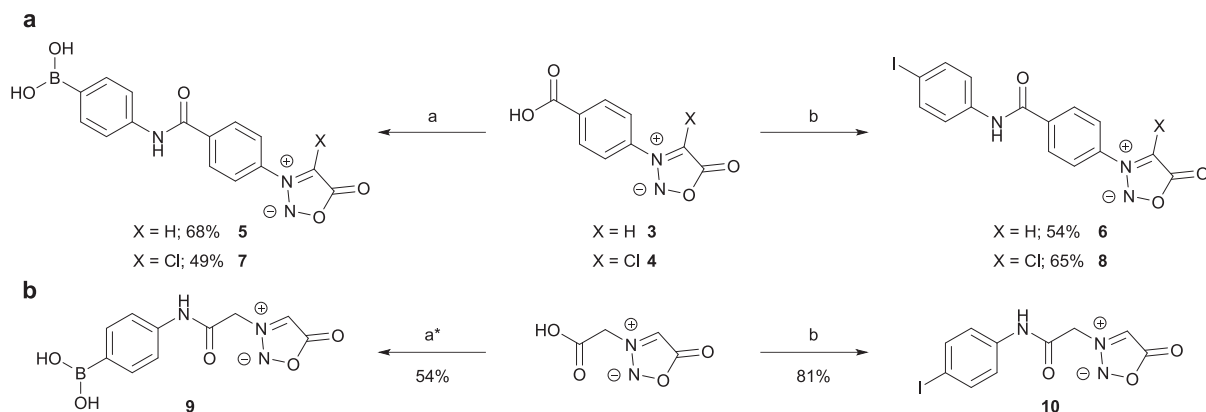
The preparation of the bifunctional precursors started with the synthesis of *N*-arylsydnones moiety **3** and **4**, bearing a carboxylic acid function in *para* position, from 4-aminobenzoic acid (Scheme 2).

Boronic acid precursor **5** and iodinated reference **6** were then obtained by formation of an amide bond with 4-aminophenylboronic acid hydrochloride and *p*-iodoaniline with yields of 68 % and 54 %, respectively (Scheme 3a). Two additional precursors with different substitution on sydnone  $N_3$  and/or  $C_4$  position were also synthesized with the same procedure in order to investigate the influence on reaction rate. *N*-arylchlorosydnones **7** and **8** were prepared with the same procedure than detailed above starting from chlorosydnone **4**, with yields of 49 % and 65 %. *N*-alkylsydnones **9** and **10** were obtained in one step after reaction of 3-carboxymethylsydnone with the corresponding aniline, leading to 54 % and 81 % yields, respectively (Scheme 3b).

For comparison with previously studied 3-iodobenzyl azide, we developed a new precursor also based on a boronic acid leaving group



Scheme 2. Preparation of intermediate sydnones **3** and **4** from 4-aminobenzoic acid. Reagents and conditions: (a) Bromoacetic acid, H<sub>2</sub>O, reflux, 16 h; (b) NaNO<sub>2</sub>, H<sub>2</sub>O, conc. HCl, 0 °C to rt, 5 min; (c) Ac<sub>2</sub>O, 100 °C, 3 h; (d) NaOCl, dioxane/HCl 1 M (2:1), rt, 3 h.



**Scheme 3.** Synthesis of bifunctional sydrones and iodinated references. Reagents and conditions: (a) 4-aminophenylboronic acid hydrochloride, EDC.HCl, HOBT, NMM, DMF, rt, 18 h to **5** d; (b) *p*-iodoaniline, EDC.HCl, HOBT, DMF, rt, 16 to 22 h. \*Reaction made without NMM.

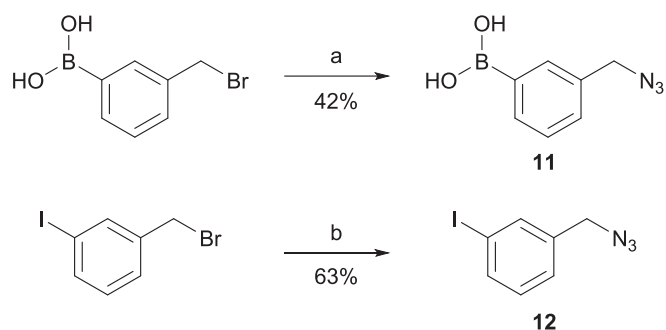
that was expected to provide improved RCY and purity of radioiodinated compound compared to the previously reported arylidonium salt strategy.<sup>7</sup> Precursor **11** was obtained by nucleophilic substitution between 3-(bromomethyl)phenylboronic acid and sodium azide, with a moderate yield of 42%. The non-radioactive iodinated reference **12**, on the other hand, was prepared from 3-iodobenzyl bromide, with a yield of 63% (Scheme 4).

For the kinetic study, the previously used tripeptide Gly-Lys-Gly bearing DIBAC or BCN clickable handle was selected as a simple comparative model.<sup>7</sup> In order to identify the formation of SPSAC cycloadducts during kinetic studies in non-radioactive and radioactive experiments, iodinated clicked peptides serving as chromatographic references were prepared by clicking peptides **13** and **14** with the iodinated sydrones (Scheme 5). These cycloadditions led to two isomers that were clearly visible in HPLC analyses only for the reaction between DIBAC and arylsydrones or 3-iodobenzyl azide (35:65 (**15**); 18:82 (**16**); 43:57 (**21**)).

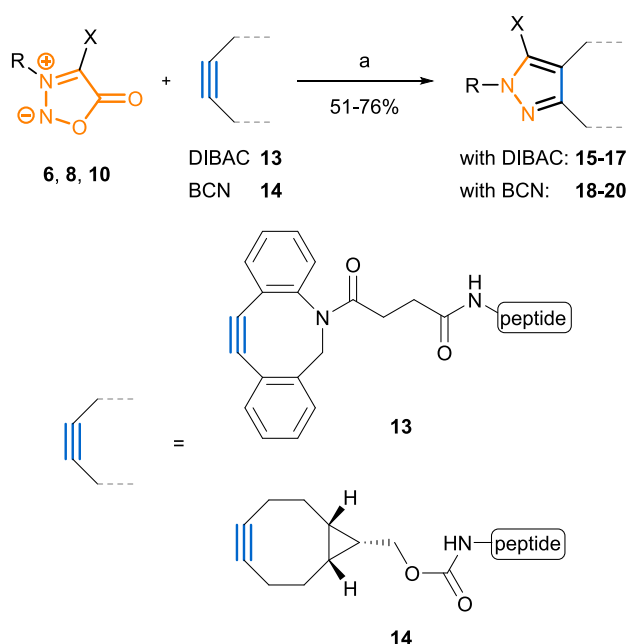
## 2.2. Kinetic study

In order to evaluate the impact of N<sub>3</sub> and C<sub>4</sub> substituents on sydrones reactivity, a preliminary kinetic study was performed with non-radioactive iodinated sydrones and model peptides bearing DIBAC (**13**) or BCN (**14**) to measure reaction rate constants. Reactions were performed at room temperature in water but in the presence of 50% DMSO that was necessary to avoid sydnone precipitation, and kinetics were monitored by HPLC for 2 h with measurements every 30 min (Table S1, ESI<sup>†</sup>).

Cycloaddition reactions of sydrones were first studied with the DIBAC peptide, and as expected, *N*-alkylsydnone **10** showed slowest reaction kinetic among all derivatives tested (Table 1, entry 1). Concerning *N*-arylsydrones, substitution of hydrogen atom in C<sub>4</sub> position



**Scheme 4.** Preparation of benzyl azide compounds. (a) NaN<sub>3</sub>, DMF, rt, 20 h; (b) NaN<sub>3</sub>, DMSO, 70 °C, 16 h.



**Scheme 5.** SPSAC reactions between sydrones **6**, **8** or **10** and cyclooctynes DIBAC **13** and BCN **14**. Reagents and conditions: (a) DMF, rt, 4 to 48 h.

**Table 1**  
Influence of the sydnone structure on the SPSAC reaction.<sup>a</sup>

Entry	Sydnone	Peptide	Product	$k_{\text{syd}}$ (M <sup>-1</sup> .s <sup>-1</sup> )	$k_{\text{syd}}/k_{\text{N3}}$ <sup>b</sup>
1	<b>10</b>	<b>13</b>	<b>17</b>	0.104 ± 0.010	0.03
2	<b>6</b>	<b>13</b>	<b>15</b>	5.675 ± 0.424	1.62
3	<b>8</b>	<b>13</b>	<b>16</b>	5.693 ± 0.419	1.62
4	<b>10</b>	<b>14</b>	<b>20</b>	0.042 ± 0.007	0.13
5	<b>6</b>	<b>14</b>	<b>18</b>	0.320 ± 0.022	0.60
6	<b>8</b>	<b>14</b>	<b>19</b>	6.017 ± 0.299	10.96

<sup>a</sup> Reactions were conducted with 100 μM of sydnone and 150 μM of peptide in 10 mM (pH 7.4) PBS containing 50% DMSO.

<sup>b</sup> Rate constant ratio calculated from the value obtained with 3-iodobenzyl azide ( $k_{\text{DIBAC}} = 3.506 \pm 0.328 \text{ M}^{-1}.\text{s}^{-1}$ ;  $k_{\text{BCN}} = 0.549 \pm 0.053 \text{ M}^{-1}.\text{s}^{-1}$ ).

with chlorine atom appeared to have no significant impact on reactivity (Table 1, entries 2 and 3). SPSAC with BCN model peptide also highlighted a small rate constant with *N*-alkylsydnone **10** (Table 1, entry 4). However, unlike with DIBAC peptide, results with *N*-arylchlorosydnone **8** were much higher compared to the C<sub>4</sub> hydrogenated analogue (Table 1, entries 5 and 6). *N*-Arylchlorosydrones are reported in the

literature to be more reactive than *N*-arylsydnone, which is confirmed in our experiments with BCN peptide but not with DIBAC peptide. Recent work reported by Wagenknecht et al. suggests that steric hindrance of DIBAC counterbalance the reactivity brought by chlorine atom, explaining the difference in reactivity.<sup>26</sup> Comparison with the SPAAC system using 3-iodobenzyl azide showed a slight superiority of sydnone cycloaddition rate with DIBAC. More strikingly, reaction rate of cycloaddition with BCN proved nearly 11 times higher with the chlorosydnone compared to azide.

### 2.3. Radiochemistry

Radioiodinated sydnones (**6a**, **8a** and **10a**) and benzyl azide **12a** were obtained by iododeboronation reaction catalysed by copper (II) using previously reported conditions.<sup>23</sup> Radiolabelling of benzyl azide was performed in a mixture of MeOH/H<sub>2</sub>O (9:1) whereas 5 % DMSO were necessary to help solubilization of sydnone precursors (Scheme 6). Even if reactions appeared to be completed after 5 to 10 min, the labelling mixtures were stirred for 30 min at room temperature and HPLC analyses of crude product showed excellent RCY (>97 %) for all compounds. Radioiodinated compounds were then purified by SPE using C<sub>18</sub> Sep-Pak cartridges providing sydnones **6a**, **8a**, **10a** and 3-iodobenzyl azide **12a** with high radiochemical purity (>95 %) and good to excellent RCY (76–91 %). Moreover, we observed that isolated radioiodinated sydnones could be stored without degradation over at least one month at 4 °C in acetonitrile.

Radioiodinated prosthetic groups were then evaluated in the cycloaddition reaction with model peptides. This study showed a similar trend than the evaluation without radioactivity, first illustrated with a very low reactivity of *N*-alkylsydnone **10a** (RCY of 0 % with BCN or 8 % with DIBAC after 24 h). Then, *N*-arylsydnone **6a** demonstrated an interesting reaction rate with DIBAC-peptide reaching RCY >95 % after

60 min while values obtained with BCN-peptide were much lower (17 % after 60 min). Finally, *N*-arylchlorosydnone **8a** appeared to be the most reactive with RCYs >95 % after 60 min whatever the peptide used (Fig. 1). Finally, the superiority of arylsydnones over azide, and in particular the arylchlorosydnone was again observed in these radiotracer conditions, especially when cycloaddition is performed with BCN.

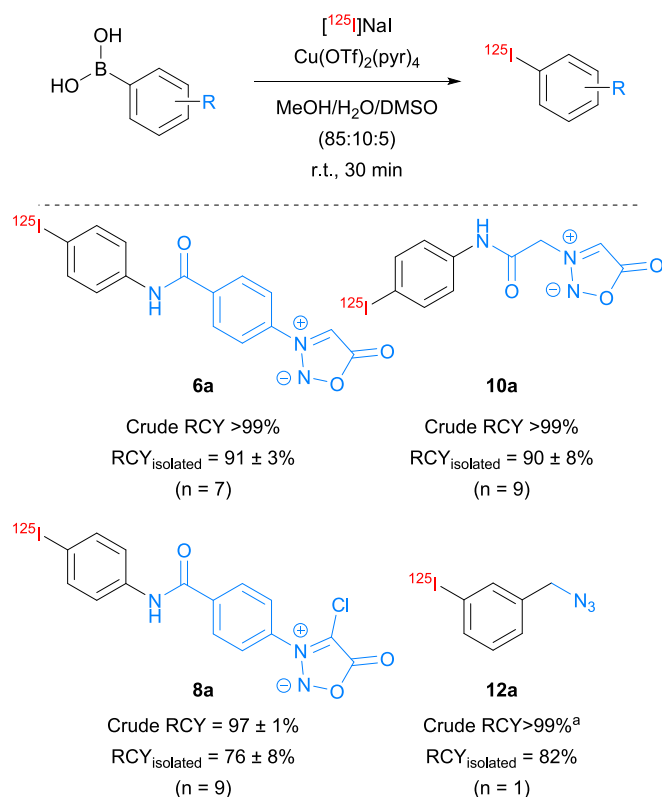
### 3. Conclusion

The beneficial impact of click chemistry in the design of radiopharmaceutical is now clearly established. New radiolabelling procedures providing high RCY with short reaction times and low reagent concentration have been reported for a wide range of molecules, from small compounds to peptides and proteins. However, most bioorthogonal couples considered up to now are Tz/TCO in IEDDA, and azide/alkyne in CuAAC and SPAAC, which may in some case exhibit limitations. These systems were reported in recent years for labelling peptides and proteins with radioiodine, and expanding the scope of system available is of interest. The modularity of sydnones for the SPSAC reaction appeared to us interesting in order to tune reactivity and chemical functionality for optimal bioconjugation. Accordingly, we have designed 3 iodinated sydnones, differing by the substituent at N<sub>3</sub> and C<sub>4</sub> position and we studied the impact of the cyclooctyne on the cycloaddition rate. While the presence of a phenyl instead of an alkyl group at N<sub>3</sub> is essential to reach high reaction rates, the advantage of placing a chlorine atom in C<sub>4</sub> was only visible in the SPSAC reaction with BCN. This optimized system outperformed previously reported SPAAC reaction between a benzyl azide and DIBAC or BCN on a model peptide. This places BCN as an excellent complementary handle for the click reaction unlike in the SPAAC reaction with azides where its reactivity is known as lower than the bulkier DIBAC moiety. Interestingly the same trend was observed at the radiotracer scale using <sup>125</sup>I-labelled analogues and the same peptides. This excellent correlation in the comparison of the prosthetic group tested highlight that the non-radioactive kinetics assay is a robust predictor of the radioactive conditions. This system appears thus valid to perform preselection of motifs of interest and could avoid the hassle of preparing precursors and radiolabelled compounds that would show low efficiency in the search for optimal prosthetic groups. In addition, as astatine exhibits a similar reactivity than iodine as shown in our previous work,<sup>7</sup> methods reported here are of interest for labelling with <sup>211</sup>At, a promising alpha emitter for targeted alpha therapy of cancers.<sup>27</sup> In particular, the reaction rate appears appropriate regarding its relatively short half-life (7.2 h) to carry out bioconjugation in a reasonable time in the perspective of the preparation of radiopharmaceuticals for patient treatment. Applications to relevant carrier molecules for imaging and therapy of cancers based on these results are now in progress and will be reported in due time.

### 4. Experimental section

#### 4.1. Kinetic studies with <sup>127</sup>I-compounds (non-radioactive)

All procedures for the preparation of substrates is provided in ESI. Reactions of sydnones (**6**, **8**, **10**) and azide (**12**) with peptide **13** or **14** were carried out in PBS/DMSO (1:1) mixtures at 100 μM concentration of sydnone/azide and 150 μM concentration of peptide using the following procedure: To 232.5 μL of DMSO was added 5 μL of benzamide solution (internal standard, 100 mM in DMSO) and 5 μL of azide or sydnone solution (10 mM in DMSO). After 10 s of stirring, 250 μL of phosphate buffered saline (PBS, 10 mM) and 7.5 μL of peptide solution (10 mM in DMSO) were added. After 1 min of stirring at room temperature, a 50 μL aliquot of the resulting mixture was injected in HPLC and the next injections were carried out every 30 min until 2 h. The conversion was followed by measuring the normalized azide/sydnone peak area. HPLC analyses were carried out on a Waters Alliance e2695 system using a C<sub>18</sub> column (Spherisorb ODS2 5 μm, 4.6 mm × 25 cm,



**Scheme 6.** <sup>125</sup>I-radiolabelling of boronic acid precursors. Standard conditions: precursor (100 μM) in 100 μL of MeOH/H<sub>2</sub>O/DMSO (85:10:5), Cu(OTf)<sub>2</sub>(pyr)<sub>4</sub> (10 mM) and [<sup>125</sup>I]NaI (0.5–12 MBq), for 30 min at rt. <sup>a</sup> Reaction performed with 250 μM of precursor in 100 μL of MeOH/H<sub>2</sub>O (9:1).

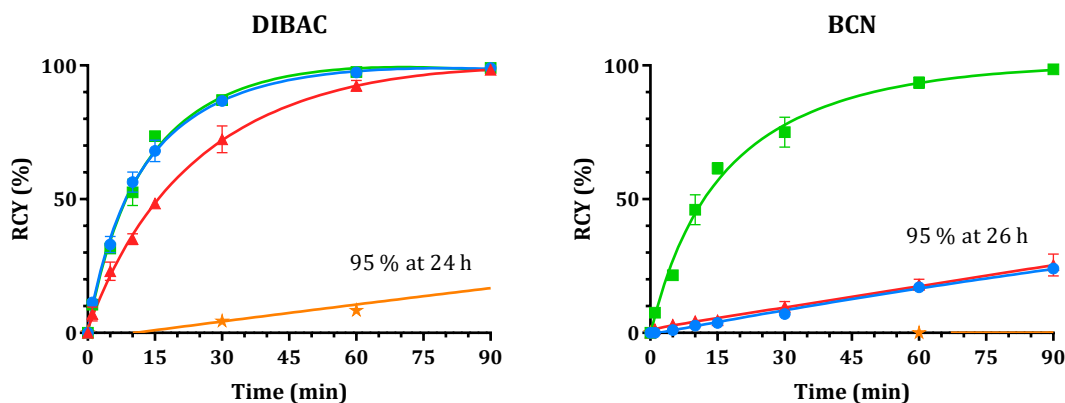


Fig. 1. Kinetics of bioorthogonal conjugation between radioiodinated prosthetic groups and clickable peptides. Sydnone 6a (●); sydnone 8a (■); sydnone 10a (★); azide 12a (▲). RCYs based on the HPLC analysis of the crude product (n = 3).

Waters) with the flow rate set at 1.50 mL/min with the following gradient: t = 0: 60 % A, 40 % B; t = 15 min: 100 % B; 17 min 100 % B. A = H<sub>2</sub>O with 0.05 % TFA and B = CH<sub>3</sub>CN with 0.05 % TFA. UV-Vis detection was achieved with a Waters 2489 UV/Vis PDA detector set at 254 nm.

Second order reaction rate k was determined by plotting  $\ln([A]/[B])/([A]_0 - [B]_0)$  versus time with [A] and [B] corresponding respectively to azide or sydnone and peptide concentration at measurement time and [A]<sub>0</sub> or [B]<sub>0</sub> their initial concentration. Analysis by linear regression on GraphPad Prism software (version 8.0.0 for Windows, GraphPad Software, San Diego, California USA) provided k as the slope of this linear regression.

#### 4.2. Radiochemistry

[<sup>125</sup>I]NaI was obtained commercially from Revity in 10<sup>-5</sup> M NaOH solution with a volumic activity of 100 μCi/μL (3.7 MBq/μL). Radio-HPLC analyses were performed on the same system as the non-radioactive iodinated references. After UV detection cell, the eluent was redirected to a Flow Star LB 513 radioactivity detector (BERTHOLD Technologies) equipped with a BGO cell.

##### 4.2.1. General labeling method

A stock solution of [<sup>125</sup>I]NaI was prepared by diluting the commercial solution an appropriate volume of deionized water. To 70 μL of MeOH was added 10 μL of a solution of sydnone (1 mM in MeOH/DMSO 1:1) or azide (2.5 mM in MeOH) boronic acid precursor and 10 μL of a solution of Cu(OTf)<sub>2</sub>(pyr)<sub>4</sub> (100 mM in MeOH). Then, 10 μL of a stock solution of [<sup>125</sup>I]NaI (0.5–12 MBq) was added and the reaction mixture was stirred at room temperature for 30 min before analysis. After 30 min reaction, samples for reverse-phase HPLC analyses were prepared by diluting an aliquot of the reaction solution in a 3:2 H<sub>2</sub>O/MeCN solution before injection on the system. All reactions led to crude RCY >97 %. The reaction mixture was diluted in 1 mL of H<sub>2</sub>O, dropped off on a pre-washed Sep-Pak C<sub>18</sub> Plus Long cartridge (Waters), washed with 45 mL of H<sub>2</sub>O/MeCN solution (85:15 (10a), 80:20 (6a, 12a) or 75:25 (8a)) to remove precursor, catalyst and free radioiodide, and <sup>125</sup>I-product was recovered by passing 2 mL of MeCN. This solution was then diluted in 10 mL of H<sub>2</sub>O, dropped off on a pre-washed Sep-Pak C<sub>18</sub> Plus Light cartridge (Waters) and washed with 600 μL of MeCN to recover the expected product. Samples for reverse-phase HPLC analyses were prepared by diluting an aliquot of the reaction solution in a 3:2 H<sub>2</sub>O/MeCN solution before injection. All compounds were obtained with RCP >95 %.

##### 4.2.2. Kinetic study of the cycloaddition with <sup>125</sup>I-compounds (radioactive)

To a solution of radioiodinated sydnones or azide (0.5 MBq, 10 μL) in DMSO was added a solution of clickable peptide 13 or 14 (90 μL, 200 μM) in a PBS/DMSO (55:45) mixture. The solution was stirred at room temperature and at selected times, a 10 μL aliquot (50 kBq) of the resulting mixture was added to 40 μL of H<sub>2</sub>O/MeCN (3:2). The resulting solution was analyzed by reverse-phase HPLC and the conversion was followed by measuring the compound peak area.

#### CRediT authorship contribution statement

**Ludovic Le Saux:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. **Ferid Haddad:** Writing – review & editing, Funding acquisition. **Jean-François Gestin:** Writing – review & editing, Supervision, Resources. **Romain Eychenne:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **François Guérard:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmc.2024.117904>.

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