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# <sup>211</sup>At and <sup>125</sup>I-labeling of (hetero)aryliodonium ylides: astatine wins again.

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**Abstract:** Despite the growing interest in radioiodine and <sup>211</sup>At-labeled radiopharmaceuticals, the search for radiolabeling reactions has been somewhat neglected, resulting in a limited number of available radiosynthetic strategies. Herein we report a comparative study of nucleophilic <sup>125</sup>I and <sup>211</sup>At-labeling of aryliodonium ylides. Whereas radioiodination efficiency was low, <sup>211</sup>At-labeling performed efficiently on a broad scope of precursors. The most activated aryliodonium ylides led rapidly to quantitative reactions at room temperature in acetonitrile. For deactivated precursors, heating up to 90°C in glyme and addition of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) as radical scavenger appeared essential to avoid precursor degradation and to achieve high radiochemical yields and molar activity. The approach was applied successfully to the preparation of 4-[<sup>211</sup>At]astatophenylalanine (4-APA), an amino acid derivative increasingly studied as radiotherapeutic drug for cancers. This validated aryliodonium ylides as a valuable tool for nucleophilic <sup>211</sup>At-labeling and will complement the short but now growing list of available astatination reactions.

## Introduction

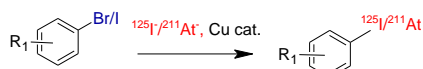
Iodine and astatine, the heaviest elements in the halogen series, exhibit radioisotopes of interest for medical applications. <sup>123</sup>I and <sup>124</sup>I have favorable decay characteristics for nuclear imaging whereas <sup>131</sup>I has long been used for β<sup>-</sup> therapy of cancers.<sup>[1]</sup> On the other hand, <sup>211</sup>At is at the center of a growing attention due to its nearly ideal decay characteristics for targeted α therapy (t<sub>1/2</sub> = 7.21 h, 100% α-emitter, E<sub>α</sub> = 5.9 and 7.5 MeV, decays to stable <sup>207</sup>Pb), a promising modality for the treatment of disseminated micrometastases, blood cancers and residual disease.<sup>[2-5]</sup> Despite several decades of medical research with these radioisotopes, the available radiolabeling chemistry has long been restricted to a few approaches that consist mainly in the nucleophilic halogen (or isotope) exchange reaction (Scheme 1a)<sup>[6]</sup> and, by far, the electrophilic halodestannylation reaction, to form (hetero)aryl radiohalogenides (Scheme 1b).<sup>[7]</sup> Now that <sup>211</sup>At is becoming increasingly available, a growing body of knowledge on the chemistry of this element is being built. A better understanding of the chemical behavior of astatine allows the

development of more rational approaches to design new radiolabeling reactions and ultimately benefits to the progress in <sup>211</sup>At-labelled radiopharmaceuticals.<sup>[8]</sup> Based on the observation that the astatide species (At<sup>-</sup>) can be stable in a much broader pH/Eh domain than the At<sup>+</sup> species required for electrophilic reactions,<sup>[9]</sup> we recently initiated the investigation of nucleophilic <sup>211</sup>At-labeling approaches of aryl compounds with the intention to propose more robust radiolabeling procedures in the perspective of future clinical trials. The interest in the use of nucleophilic astatine is also found in most recent reports such as the <sup>211</sup>At-labeling of the promising neopentyl glycol scaffold.<sup>[10]</sup> We first reported the aromatic nucleophilic substitution of aryliodonium salts, which consisted in the first novel reaction in nearly 30 years to form astatine-carbon bonds, also applicable to radioiodination (Scheme 1c).<sup>[11]</sup> Then, still using nucleophilic astatine or radioiodine, we studied, among other groups, the potential of copper catalyzed halodeboronation of arylboronic acids and esters (Scheme 1d).<sup>[12-14]</sup> Despite this recent interest in new radioiodination and astatination reactions, the choice remains rather restricted and new methods have to emerge to face the growing need in small radiohalogenated compounds. For instance, aryliodonium salts can be very efficient to produce [<sup>211</sup>At]SAB or [<sup>125</sup>I]SIB,<sup>[15]</sup> the main prosthetic groups used to radiolabel proteins that we recently applied in a preclinical therapy study.<sup>[16]</sup> However the use of aryliodonium salt chemistry appears restricted to electron deficient compounds, radiochemical yields (RCYs) being drastically reduced for electron rich compounds. In this unfavorable case, selectivity of halogenation at the aryl position of interest decreases versus the aryl auxiliary. On the other hand, a similar class of trivalent organoiodine compounds, aryliodonium ylides, have emerged in recent years as efficient reagents for radiofluorination.<sup>[17-19]</sup> With these precursors, the auxiliary group is not reactive towards nucleophilic substitution, which eliminates chemoselectivity issues encountered in diaryliodonium salts and which motivated us to investigate their reactivity with nucleophilic radioiodine and astatine. Herein we report methods to produce (hetero)aryl compounds labeled with nucleophilic <sup>125</sup>I and <sup>211</sup>At from aryliodonium ylides (Scheme 1e). On the basis of the significantly superior reactivity observed with astatide over radioiodide, the method was applied

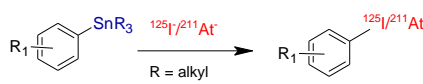
successfully to the preparation of [ $^{211}\text{At}$ ]4-astato-*L*-phenylalanine, a promising agent for LAT1 expressing tumors.

#### Previous works

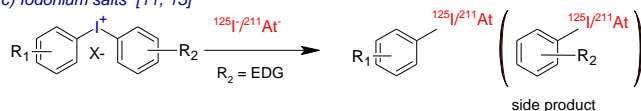
##### a) Iodine and Bromine derivatives [6]



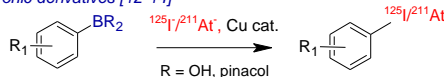
##### b) Stannyl derivatives [7]



##### c) Iodonium salts [11, 15]

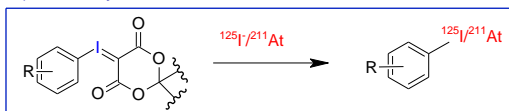


##### d) Boronic derivatives [12-14]



#### This work

##### e) Iodonium ylides



**Scheme 1.** Overview of radioiodination and astatination reactions of aryl compounds.

## Results and Discussion

### Influence of auxiliary and solvent on radiolabeling.

As a preliminary study, we investigated the influence of spirocyclic auxiliary on the  $^{211}\text{At}$ -labeling of arylidonium ylides. Six arylidonium ylide precursors (**2a-c** and **2a'-c'**) were prepared as model, containing either a cyclopentyl or an adamantyl auxiliary (respectively **1** and **1'**) previously reported as some of the best choices in the case of radiofluorination.<sup>[20,21]</sup> The aryl moiety was substituted by a methyl, a chlorine or a cyano group in *para* position. These three substituents were chosen in order to cover the scale of electronic contribution from deactivating to strongly activating (Hammett constant  $\sigma_{p-\text{Me}} < \sigma_{p-\text{Cl}} < \sigma_{p-\text{CN}}$ ). Dithiothreitol (DTT) was used as reducing agent to form the reactive species  $\text{At}^-$  which was then dried by azeotropic evaporation in the presence of  $\text{K}_2\text{CO}_3$  and Kryptofix® 222 ( $\text{K}_{222}$ ) before addition of the precursor, similarly to protocols generally used in radiofluorination reactions. This step appeared essential to provide high RCYs as illustrated by the astatination of **2a-c** and **2a'-c'** (Table 1). First assays were performed at room temperature over 30 minutes in acetonitrile (procedure denoted as Method A), a solvent that we previously determined to be excellent for radioiodination and astatination of arylidonium salts.<sup>[11]</sup> The most activated iodonium ylides **2b** and **2c** associated to spirocyclopentyl auxiliary **1** showed excellent RCYs ( $\geq 90\%$ ) whereas the methyl substituted precursor **2a** provided significantly lower RCY. On the contrary, adamantyl analogues **2a'-c'** led to no conversion or only traces of expected product, which was most likely due to their limited solubility in the solvent used. All RCYs were determined by TLC and HPLC and provided consistent results as illustrated in Table 1. Consequently, only HPLC results are shown thereafter (radio-HPLC and radio-TLC can be found in Supporting Information).

**Table 1.** Investigation of  $^{211}\text{At}$ -labeling of arylidonium ylides **2a-c** and **2a'-c'**. RCYs determined by radio-HPLC and radio-TLC analysis of the crude product<sup>a</sup>

Auxiliary	R	$^{211}\text{At}$ -product	RCY (%)	
			Radio-HPLC	Radio-TLC
1	Me ( <b>2a</b> )	<b>3a</b>	15±1(0 <sup>[b]</sup> )	18±2(2 <sup>[b]</sup> )
	Cl ( <b>2b</b> )	<b>3b</b>	99±3 (0 <sup>[b]</sup> )	>99 (0 <sup>[b]</sup> )
	CN ( <b>2c</b> )	<b>3c</b>	90±4(42 <sup>[b]</sup> )	93±2(52 <sup>[b]</sup> )
1'	Me ( <b>2a'</b> )	<b>3a</b>	0	0
	Cl ( <b>2b'</b> )	<b>3b</b>	1	0
	CN ( <b>2c'</b> )	<b>3c</b>	0	0

<sup>a</sup> Standard condition: DTT (7.7 mmol), precursor (3.0  $\mu\text{mol}$ ) in 100  $\mu\text{L}$  MeCN,  $\text{K}_{222}$  (2.8  $\mu\text{mol}$ ),  $\text{K}_2\text{CO}_3$  (1.4  $\mu\text{mol}$ ) and  $^{211}\text{At}$  (0.5–2 MBq), at r.t for 30 min. Average of  $n = 3$  runs. <sup>b</sup> RCY obtained in the absence of  $\text{K}_2\text{CO}_3$  /  $\text{K}_{222}$  and azeotropic drying of astatide before reaction.

With arylidonium ylides containing the adamantyl auxiliary **1'** that exhibited solubility issues in MeCN, better results were obtained in glyme or in toluene (Table 2). Ylide **2b'** and **2c'** provided nearly quantitative RCYs in toluene after 30 min at r.t. whereas RCY with the less reactive compound **2a'** was improved with glyme and especially toluene to up to 71 ± 10%.

**Table 2.** Influence of solvent on  $^{211}\text{At}$ -labeling of iodonium ylides **2a'-c'** with adamantyl auxiliary **1'**. RCYs determined by radio-HPLC analysis of the crude product<sup>a</sup>

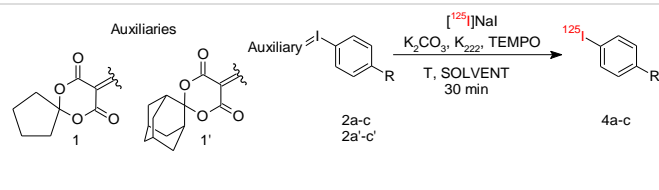
R	$^{211}\text{At}$ -product	Solvent	RCYs (%)
Me ( <b>2a'</b> )	3a	Glyme	50±9
		Toluene	71±10
Cl ( <b>2b'</b> )	3b	Glyme	63±8
		Toluene	95±3
CN ( <b>2c'</b> )	3c	Glyme	>99%
		Toluene	95±3

<sup>a</sup> Standard conditions: DTT (0.33  $\mu\text{mol}$ ), precursor (3.0  $\mu\text{mol}$ ) in 100  $\mu\text{L}$  solvent,  $\text{K}_{222}$  (2.7  $\mu\text{mol}$ ),  $\text{K}_2\text{CO}_3$  (1.4  $\mu\text{mol}$ ) and  $^{211}\text{At}$  (0.5–2 MBq), at r.t for 30 min ( $n = 3$ ).

Given previously observed similarities between astatine and iodine reactivity, the  $^{125}\text{I}$ -labeling of these 6 arylidonium ylides (**2a-c** and **2a'-c'**) was also investigated. Unlike  $^{211}\text{At}$ , no reducing agent was necessary to form the reactive  $\text{I}^-$  species since  $^{125}\text{I}$  is available commercially as a sodium iodide solution. Rather, we noticed that the addition of a reducing agent to mimic the conditions we set up for  $^{211}\text{At}$  labeling was detrimental to the reaction. A difference in reactivity compared to astatine was observed, similarly to the halogenation of arylidonium salts we reported earlier.<sup>[11]</sup> It was indeed necessary to heat the medium up to 90°C in glyme or 110°C in toluene and to add 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) as radical scavenger to limit the degradation of the heat sensitive precursor. Under these conditions, we observed the formation of expected  $^{125}\text{I}$ -labeled products in moderate RCYs except with compounds **2b** and **2b'** (R = Cl) that provided significantly higher RCYs (Table 3). Unlike

$^{211}\text{At}$ -labeling, the superiority of the cyclopentyl auxiliary was not observed and appeared to be substituent dependent. Regardless of auxiliary, no formation of  $^{125}\text{I}$ -labeled product **4c** was detected.

**Table 3.** Investigation of  $^{125}\text{I}$ -labeling of arylidonium ylides **2a-c** and **2a'-c'**. RCYs determined by radio-HPLC analysis of the crude product<sup>a</sup>



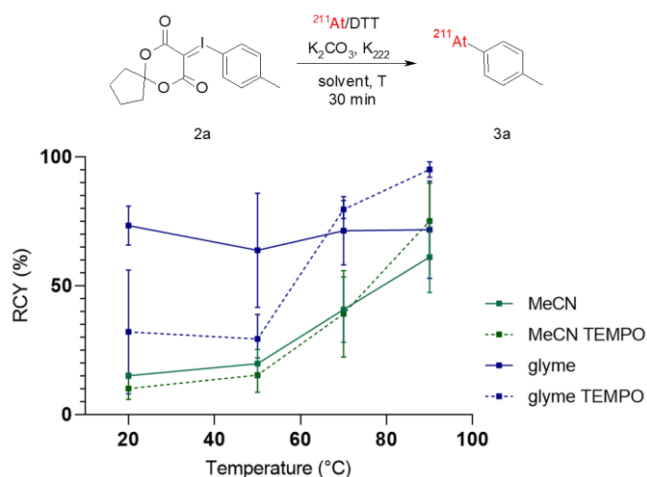
Auxiliary	R	$^{125}\text{I}$ -product	Conditions	RCY (%)
1	Me (2a)	4a	Glyme, 70°C Toluene, 110°C	21±10 (2 <sup>b</sup> ) 48±2
	Cl (2b)	4b	Glyme, 70°C Toluene, 110°C	84±4 (12 <sup>b</sup> ) 60±10
	CN (2c)	4c	Glyme, 70°C Toluene, 110°C	0 0
	Me (2a')	4a	Glyme, 70°C Toluene, 110°C	11±1 36±6
1'	Cl (2b')	4b	Glyme, 70°C Toluene, 110°C	86±7 81±10
	CN (2c')	4c	Glyme, 70°C Toluene, 110°C	0 0

<sup>a</sup> Standard conditions: precursor (3.0 μmol) in 100 μL solvent,  $\text{K}_{222}$  (2.7 μmol),  $\text{K}_2\text{CO}_3$  (1.4 μmol) TEMPO (57.7 μmol) and  $^{125}\text{I}$ NaI (0.2–1 MBq), for 30 min. <sup>b</sup> Reaction performed without TEMPO. (n = 2).

Overall, this preliminary study highlighted the superior reactivity of the cyclopentyl auxiliary thanks to the resulting better solubility of precursors over the adamantyl auxiliary, leading to higher RCYs in most cases. The cyclopentyl auxiliary was thus selected for the rest of the study. Radiolabeling reaction performed with radioiodine were quite disappointing and do not compete with other reported radioiodination approaches. Conversely, the promising results observed with astatine led us to focus our attention on the use of  $^{211}\text{At}$  as reported in the following sections.

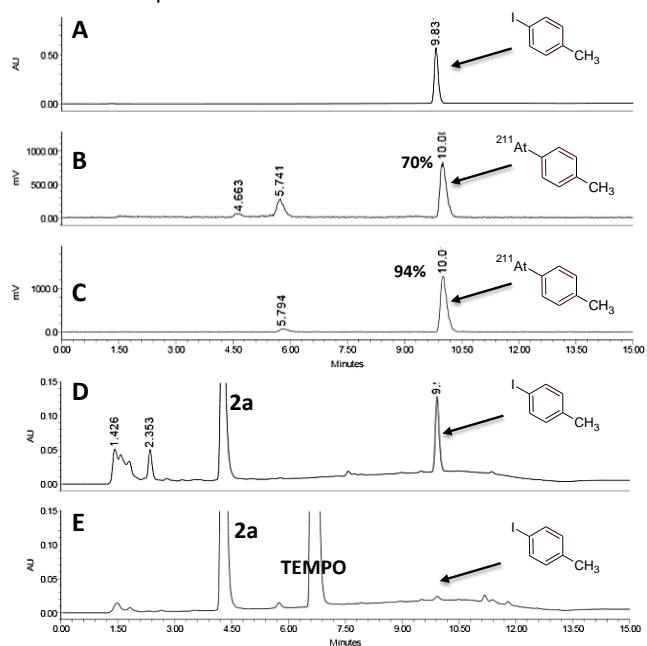
### Optimization of $^{211}\text{At}$ -labeling conditions and substituent scope.

Regardless of solvent used, deactivated compound **2a** provided significantly lower RCYs at room temperature whereas quantitative labeling was observed in the case of precursors with electron deficient aryls. Consequently, an optimization of reaction conditions was performed to expand the scope of application to electron rich arylidonium ylides. The conditions set up for radioiodination served as a base for this optimization and thus, the use of glyme as solvent, heating up to 90°C and addition of TEMPO was investigated (Figure 1). The temperature did not impact RCY in glyme (65–75% from 20 to 90°C) whereas this RCY value was reached only at 90°C in acetonitrile. However, the addition of TEMPO led to an excellent RCY of 96 ± 2 % in glyme at 90°C, a result again superior to acetonitrile (71%). Glymes were previously reported in reactions involving arylidonium ylides.<sup>[22]</sup> They are known as excellent for solvation of metal cations similarly to crown ethers.<sup>[23]</sup> In our case improved solvation of potassium cation leading to an enhanced nucleophilicity of astatide may explain the improved RCY.



**Figure 1.** Optimization of reaction conditions of  $^{211}\text{At}$ -labeling of arylidonium ylide **2a**. RCY determined by HPLC of crude product (n = 3). Standard conditions: DTT (7.7 mmol), precursor **2a** (3.0 μmol) in 100 μL of MeCN or glyme,  $\text{K}_{222}$  (2.7 μmol),  $\text{K}_2\text{CO}_3$  (1.4 μmol), TEMPO (19.2 μmol) for 30 min (n = 3).

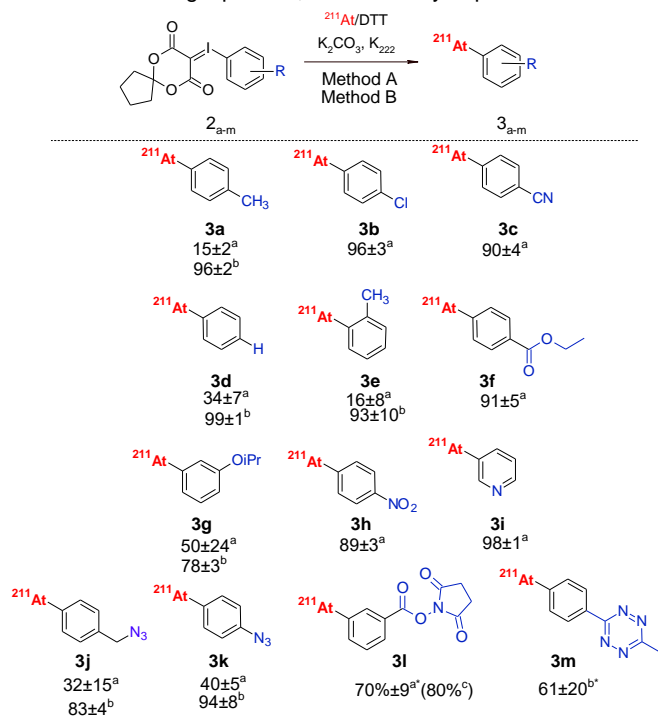
We also looked at the degradation of the precursor during the reaction. This point is crucial since one of the possible degradation products is the non-radioactive arylidide analogue of the expected  $^{211}\text{At}$ -labeled product. Formation of this  $^{127}\text{I}$ -side product has to be limited since it cannot be separated from the astatinated derivative due to nearly identical polarity, impacting negatively the resulting molar activity ( $A_m$ ). The UV traces of HPLC analyses for the reaction performed at 90°C with and without TEMPO were compared and showed a significant decrease in arylidide formation when the radical scavenger was used (Figure 2). Consequently, not only to improve RCYs but also to optimize  $A_m$ , TEMPO appeared essential for the  $^{211}\text{At}$ -labeling of electron rich precursors.



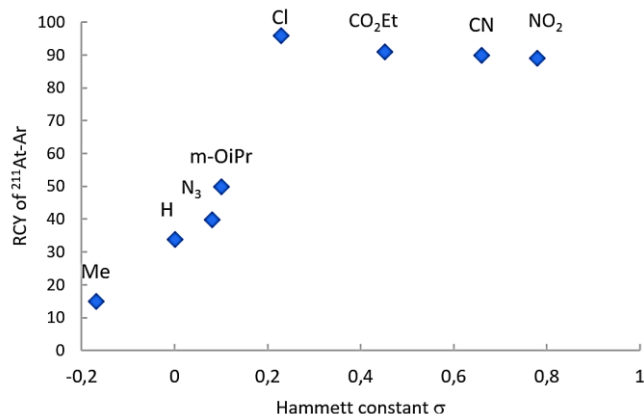
**Figure 2.** Comparisons of HPLC analyses of the  $^{211}\text{At}$ -labeling of **2a** obtained with or without TEMPO. A) UV trace of  $^{127}\text{I}$ -analogue **3a** as reference; B)  $\gamma$ -trace of the crude product without TEMPO; C)  $\gamma$ -trace of the crude product with TEMPO (most of unreacted  $^{211}\text{At}$  is visible in the column wash run chromatogram, see Supporting Information for details); D) UV-trace of the crude product of the  $^{211}\text{At}$ -labeling of **2a** with TEMPO; E) UV-trace of the crude product of the  $^{211}\text{At}$ -labeling of **2a** without TEMPO. Standard conditions: DTT (7.7

mmol), precursor **2a** (3.0  $\mu$ mol) in 100  $\mu$ L of glyme,  $K_{222}$  (2.7  $\mu$ mol),  $K_2CO_3$  (1.4  $\mu$ mol), TEMPO (0 or 19.2  $\mu$ mol) for 30 min ( $n = 3$ ).

The scope of the reaction was then studied using a set of arylodonium ylides from strongly deactivated (compound **2g**,  $\sigma_{m-OiPr} = -0.1$ ) to strongly activated (compound **2h**,  $\sigma_{p-NO_2} = 0.78$ ) with initial Method A applied (room temperature,  $CH_3CN$ ) (Scheme 2). Electron-deficient compounds provided higher RCYs than electron-rich ones, confirming the  $SN_{Ar}$  mechanism expected for this reaction in good correlation with their Hammett constants, (Figure 3) and suggesting a similar reaction pathway than reported previously with fluoride by Roststein *et al.*<sup>[20]</sup> The reaction can proceed very rapidly with, for instance, precursor **3b** reaching a RCY > 90% within less than 5 min at room temperature (figure S4). Interestingly, no *ortho* effect was observed, as illustrated by the comparison between **2a** (*p*-Me) and **2e** (*o*-Me) which provided nearly identical RCYs. This result is the opposite of observations made with the very similar arylodonium salts that exhibited significantly higher RCY when substituted in *ortho* position.<sup>[11]</sup> Method B (90°C, glyme, TEMPO), determined by optimized  $^{211}At$ -labeling of compound **2a** as detailed above, was then applied to compounds that had provided suboptimal RCYs under Method A, leading in this case to good or excellent RCYs. Precursors functionalized with conjugation moiety (activated ester **3l**) or click chemistry handles (tetrazine **3m** or azide groups **3j** and **3k**) were also labeled efficiently as well as the heteroaryl precursor **3i**. It was however necessary to avoid DTT in the astatine reduction step prior to labeling reaction in the case of activated ester and tetrazine precursors **3l** and **3m** to prevent from degradation of these more sensitive functional groups. In these cases, sodium metabisulfite was preferred. Overall, this study confirmed the high potential of this class of precursors to produce astatinated small molecules. For most compounds, RCYs obtained were superior than using the arylodonium salts chemistry thanks to the formation of a single product, as we initially hoped for.



**Scheme 2.** Substituent scope. <sup>a</sup> Method A: precursor (3.0  $\mu$ mol) in 100  $\mu$ L  $CH_3CN$ ,  $K_{222}$  (2.7  $\mu$ mol),  $K_2CO_3$  (1.4 mmol) and  $^{211}At$  in DTT (0.5–2 MBq), at r.t for 30 min. <sup>b</sup> Method B: precursor (3.0  $\mu$ mol) in 100  $\mu$ L glyme,  $K_{222}$  (2.7  $\mu$ mol),  $K_2CO_3$  (1.4 mmol), TEMPO (19.2  $\mu$ mol),  $^{211}At$  in DTT (0.5–2 MBq), 90°C for 30 min. <sup>c</sup> Adamantyl was used as auxiliary instead of cyclopentyl ( $n = 2$ ). <sup>d</sup> DTT was replaced by sodium metabisulfite as reducing agent. RCYs were determined by radio-HPLC analysis of the crude product ( $n = 3$ ).

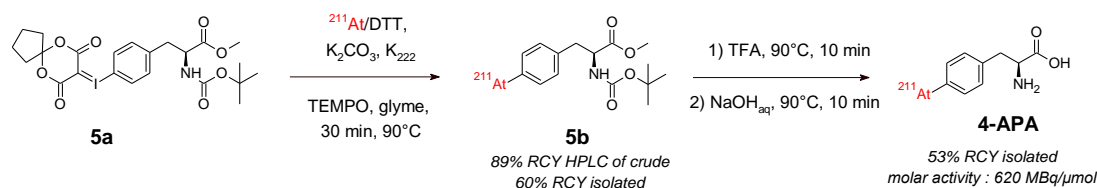


**Figure 3.** RCY of  $^{211}At$ -labeling of arylodonium ylides using method A for 30 min in function of their Hammett constant.

#### Preparation of 4-[ $^{211}At$ ]astatophenylalanine and in vitro evaluation.

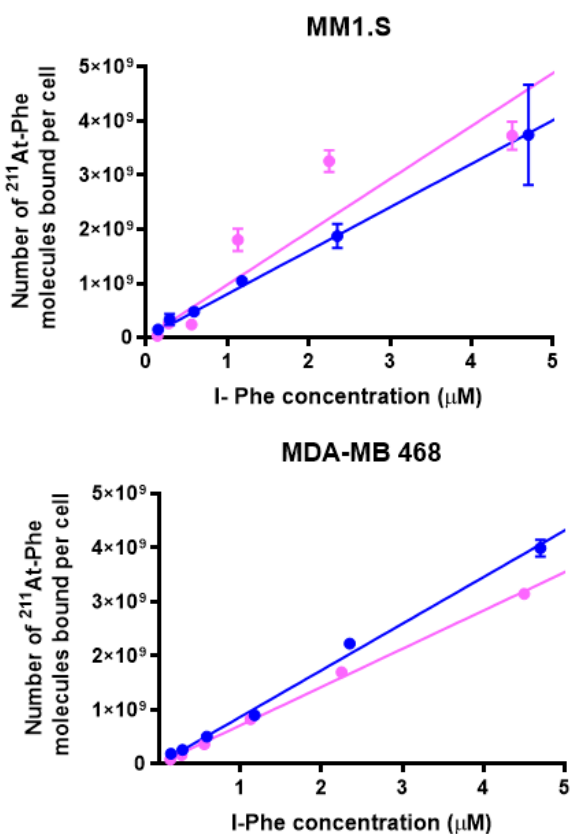
4-[ $^{211}At$ ]astatophenylalanine (4-APA) is an amino acid derivative whose efficiency has already been demonstrated to target cancer cells via LAT1, a transporter overexpressed by tumor cells.<sup>[24]</sup> This includes glioblastoma cells<sup>[25]</sup> or multiple myeloma for which 4-APA received the Orphan Drug Designation by FDA in 2020.<sup>[26]</sup> As a result, efforts have been directed towards improving access to this compound. Originally reported using a halogen exchange reaction from the iodine analogue,<sup>[27]</sup> novel approaches have recently been reported such as the electrophilic desilylation<sup>[28]</sup> and deboronation reactions.<sup>[29]</sup> 4-APA appears thus as a relevant example to apply our labeling strategy.

Protected arylodonium ylide precursor **5a** was prepared as previously reported<sup>[17]</sup> providing access to 4-APA in a two-step procedure (Scheme 3). The first step, performed according to Method B, led to a good RCY of 89% (HPLC analysis of crude product) which after purification on a C18 cartridge led to the isolation of protected 4-APA **5b** with a 60% RCY. Successive deprotections with TFA and NaOH led to 4-APA with 53% RCY and 98% RCP. Starting with a  $^{211}At$  activity of 70 MBq, the activity yield was 27 MBq and the  $A_m$  was 620 MBq/ $\mu$ mol. In comparison, the previous procedure reported by Meyer *et al.*<sup>[27]</sup> using copper(I) assisted halogen exchange was also applied. It led to significantly less favorable results than our approach starting with a similar activity of 77 MBq and leading to 4-APA with RCY of 95% (crude product), an activity yield of 27 MBq and more importantly, an  $A_m$  of only 9.56 MBq/ $\mu$ mol.



**Scheme 3.** Preparation of 4- $^{211}\text{At}$ astatophenylalanine from precursor **5a**

Cell uptake of 4-APA obtained by both methods was then compared in two tumor cell lines MM1.S (multiple myeloma cells ATCC, CRL-2974) and MDA-MB-468 (breast cancer cells ATCC, HTB-132). Results showed nearly identical results of both labeling strategies in both cell lines with an increasing uptake when 4-iodophenylalanine concentration increased (Figure 4). This experiment validated the viability of our novel labeling approach, with the advantage of an improved starting  $A_m$  that is expected to be more favorable to adjust required  $A_m$  in the context of future in vivo use.



**Figure 4.** Uptake of 4-APA in MM1.S and MDA-MB 468 cell lines. (●) 4-APA prepared via halogen exchange reaction; (●) 4-APA prepared via aryliodonium ylide precursor **5a**.

## Conclusion

Herein we demonstrated the high efficiency of aryliodonium ylides for the introduction of nucleophilic astatine at an aryl position. Rapid reaction at room temperature was achieved for the most reactive precursors whereas optimized conditions with heating and use of TEMPO for electron rich compounds were needed to reach high RCYs and preservation of  $A_m$ . The reaction was readily applied to the preparation of prosthetic groups and also to the clinically relevant 4-APA. The role of TEMPO appears essential

and to our knowledge, this is the first example of its use in a radioiodination or astatination reaction. The use of this radical scavenger distinguishes our work from a related study published during the preparation of this manuscript in which the drastic conditions used may lead to suboptimal  $A_m$ , and compromise applications with resulting radiopharmaceuticals.<sup>[30]</sup> Additionally, we compared astatide and radioiodide reactivity, revealing the significantly lower reactivity of  $^{125}\text{I}^-$ , similarly to aryliodonium salts chemistry. This result adds to the growing list of examples that distinguishes astatine behavior from trends within lighter halogens.

Our method appears as a valuable addition to the rather restricted list of available reactions for nucleophilic astatination of organic compounds. In comparison with the halogen exchange reaction, the efficiency and  $A_m$  appears largely improved, whereas high RCYs are achievable in a broader scope of compounds compared to aryliodonium salts. As for arylboron chemistry, a comparable efficacy appears achievable with the advantage that no metal catalyst is needed with aryliodonium ylides. Overall, we believe this work will contribute to the development of novel  $^{211}\text{At}$ -labeled compounds for applications in the growing field of  $\alpha$ -particle targeted therapy of cancers.

## Experimental Section

**Radiochemistry.** [ $^{125}\text{I}$ ]NaI was obtained commercially from Perkin Elmer in  $10^{-5}$  M NaOH solution with a volumic activity of 50  $\mu\text{Ci}/\mu\text{L}$  (1.85 MBq/mL) and was diluted 10 times in MeCN before use.  $^{211}\text{At}$  was produced at the Arronax cyclotron facility using the  $^{209}\text{Bi}(\alpha, 2n)^{211}\text{At}$  reaction and recovered from the irradiated target in chloroform using a dry distillation protocol adapted from the procedure previously reported by Lindegren et al.<sup>[31]</sup> [ $^{211}\text{At}$ ]At $^-$  was then obtained by reducing to dryness the chloroformic astatine solution under a gentle stream of nitrogen to obtain dry astatine, followed by dissolution with a 5 mg/mL DTT solution in MeCN, or with a 10 mg/mL aqueous sodium metabisulfite solution for iodonium ylides **3l** and **3m**. Aryliodonium ylides were synthesized as described in Supporting Information

**Reaction of aryliodonium ylides with  $^{125}\text{I}^-$  and  $^{211}\text{At}^-$ .**  $\text{K}_{222}$  (100  $\mu\text{L}$ , 10 mg/mL in MeCN) and  $\text{K}_2\text{CO}_3$  (2.7  $\mu\text{L}$ , 0.5 M in  $\text{H}_2\text{O}$ ) were added to a solution of [ $^{125}\text{I}$ ]NaI or [ $^{211}\text{At}$ ]At/DTT solution (10  $\mu\text{L}$ , typically 0.5-1.5 MBq), prepared as described above. Azeotropic evaporation were performed by reducing to dryness astatine or radioiodine/ $\text{K}_{222}/\text{K}_2\text{CO}_3$  solution under a gentle stream of nitrogen, 250  $\mu\text{L}$  of MeCN were added, and the evaporation step was repeated twice. Then, Method A or B was applied.

**Method A:** Freshly prepared solution of iodonium ylide (3.0  $\mu\text{mol}$ ) in 100  $\mu\text{L}$  of MeCN was added to fresh At/DTT/ $\text{K}_{222}/\text{K}_2\text{CO}_3$  complex. The reaction mixture was vortexed for 30 min at room temperature.

**Method B:** Freshly prepared solution of iodonium ylide (3.0  $\mu\text{mol}$ ) in 100  $\mu\text{L}$  of glyme and TEMPO (3 mg in 30  $\mu\text{L}$  MeCN) were added to fresh At/DTT/ $\text{K}_{222}/\text{K}_2\text{CO}_3$  complex. The reaction mixture was heated for 30 min at 90°C.

After 30 min reaction, aliquots were withdrawn and deposited on a silica gel TLC plate and eluted with hexane/ethyl acetate (1:1). In parallel, samples for reverse-phase HPLC analyses were prepared by diluting an aliquot of the reaction solution in a 1:1 water/MeCN solution before analysis using system I detailed in Supporting Information. Retention indices and elution systems used for all compounds of this study are given in Supporting Information. Aromatic <sup>125</sup>I and <sup>211</sup>At species were identified by comparison of the retention indices of the non-radioactive iodinated analogue.

**Preparation of 4-[<sup>211</sup>At]astatophenylalanine (4-APA) from arylodonium ylide 5a.** 4-[<sup>211</sup>At]astatophenylalanine was prepared from iodonium ylide **5a** according to Method B. The reaction mixture was diluted with water (1 mL) and deposited on a Sep-Pak C<sub>18</sub> Plus Long cartridge (Waters). The <sup>211</sup>At-product **5b** was then eluted with 2 mL MeCN before being evaporated under a gentle stream of nitrogen. Then TFA (20 µL) was added and the solution heated at 90°C for 10 min. After cooling and evaporation of TFA under a stream of nitrogen, 2 M NaOH (100 µL) was added to the residue, and the solution was heated at 90°C for 10 min. After evaporation under a stream of nitrogen with heating (50°C), the dry labeled compound was dissolved in about 1 mL of PBS for use in biological experiments. An aliquot of the solution was withdrawn for TLC and HPLC analyses (System II, details provided in Supporting Information).

**Preparation of (4-APA) by halogen exchange.** The preparation of 4-APA was performed by previous procedure reported by Meyer et al.<sup>[27]</sup> with some modifications. Briefly, 0.5 mg of SnSO<sub>4</sub>, 28 µL of a CuSO<sub>4</sub> solution (1.3x10<sup>-2</sup> µmol/µL), 2.5 mg of citric acid, 1.25 mg of gentisic acid and 1 mg of 4-iodophenylalanine were added to the dry astatine-211. After 60 min at 120°C, the labeling efficiency was determined by TLC (1-butanol/H<sub>2</sub>O/CH<sub>3</sub>COOH – 4:1:1) and HPLC (System II). These two methods indicated the formation of 4-APA with 95% radiochemical yield (RCY) and 5% of unreacted <sup>211</sup>At. The reaction mixture was then dropped off on a Sep-Pak C<sub>18</sub> Plus Short cartridge (Waters), washed with 20 mL of H<sub>2</sub>O and 2 mL of EtOH to recover the expected product. After evaporation under a stream of nitrogen with heating (50°C), the dry labeled compound was dissolved in about 1 mL of PBS for use in biological experiments.

**Cellular uptake study of 4-[<sup>211</sup>At]astatophenylalanine prepared from arylodonium ylide precursor and by halogen exchange.** MM.1S multiple myeloma cells (ATCC, CRL-2974) and MDA-MB-468 breast cancer cells (ATCC, HTB-132) were plated at 0.2x10<sup>6</sup> cells per well in 24 well-plates and incubated with 4-APA, obtained either by halogen exchange or from arylodonium ylide precursor, in presence of increasing concentrations of 4-iodophenylalanine (amount added according to initial A<sub>m</sub>) ranging from 0.14 to 4.7 µM. After 1h incubation at 4°C, supernatants and cells were collected and counted in a γ counter (WALLAC/Perkin Elmer). Each condition was assessed in triplicate. Amounts of 4-[<sup>211</sup>At]astatophenylalanine bound per cell were calculated based on counted activity in the cell fractions.

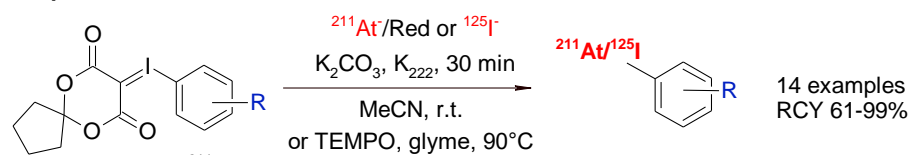
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**Keywords:** astatine-211 • iodine-125 • iodonium ylides • radiolabeling • halogenation

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### Graphical abstract



The nucleophilic  $^{211}\text{At}$ -labeling of arylidonium ylides was investigated and proved significantly more efficient than radioiodination. Reaction is rapid at room temperature with electron-poor activated substrates whereas heating and addition of the radical scavenger TEMPO was essential in the case of deactivated precursors to reach high RCY and molar activity.