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Bifunctional aryliodonium salts for highly efficient radioiodination and astatination of antibodies

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ABSTRACT

In this report we describe the development of an alternative approach to arylstannane chemistry for radiolabeling antibodies with radioiodine or astatine based on aryliodonium salts precursors. Bifunctional aryliodonium salts were designed and tested for the synthesis of ^{125}I and ^{211}At labeled prosthetic groups for bioconjugation. The nature of the electron rich aryl group was varied and its impact on the regioselectivity of radiohalogenation was evaluated. Unexpectedly, whereas the 2-thienyl group provided the best regioselectivity towards the radioiodination of the aryl moiety of interest (98:2), it was less selective for astatination (87:13); the anisyl group providing the best regioselectivity of astatination (94:6). Under optimized conditions, both radioiodination and astatination could be performed very efficiently in mild conditions (radiochemical yields > 85%). The ionic nature of the precursors was exploited to develop an efficient purification approach: the HPLC step that is usually necessary in conventionnal approaches to optimize removal of organotin toxic precursors and side products was replaced by a filtration through a silica cartridge with a significantly reduced loss of radiolabeled product. The purified radioiodinated and astatinated prosthetic groups were then conjugated efficiently to an anti-CD138 monoclonal antibody (75–80% conjugation yield). By using this novel and simple radiohalogenation procedure, higher overall radiochemical yields of astatination were obtained in comparison with the use of an arylstannane precursor and procedures of the litterature for labeling the same antibody. Overall, due to their simplicity of use and high robustness, these new precursors should simplify the labeling of proteins of interest with iodine and astatine radioisotopes for imaging and therapeutic applications.

1. Introduction

Radiolabeling antibodies and other proteins of biomedical interest with heavy halogens (radioiodine and astatine) has become a common step in a broad set of applications. Particularly, [^{125}I]radioiodination is useful in a number of non-invasive in vitro and in vivo detection methods for the study of biological properties of biomolecules, ^{123}I and ^{124}I are used for nuclear imaging, whereas ^{131}I allows production of radiopharmaceuticals for targeted β^--particle therapy.[1] On the other hand, ^{211}At is one of the most promising radionuclides for targeted α-particle therapy, and a growing number of [^{211}At]astatinated antibodies and derivatives are being investigated for cancer treatment.[2]

The simplest radioiodination procedure consists in a direct aromatic electrophilic substitution on tyrosine residues of proteins (Scheme 1a). Although very simple to execute, this approach exhibits suboptimal in vivo stability in several reported cases with uptake of free radioiodide in the thyroid which limits its use to in vitro applications.[3] This approach is however not applicable to astatination since highly unstable radiolabeling has been observed when attempted.[4] Consequently, radioiodination and astatination have evolved to two-step procedures via the development of bifunctional aromatic precursors comprised of a radiohalogenation site and a conjugation site to lysine or cysteine residues. Especially, radioiodinated *N*-succinimidyliodobenzoate (SIB) and *N*-succinimidylastatobenzoate (SAB) are the most frequently used prosthetic groups for conjugation to the amino group of lysines,[5] whereas maleimide-based derivatives for cysteine conjugation have been developed more recently.[6,7] Aryltrialkylstannane based precursors are by far the most used compounds for this application. They are easily prepared and are radiohalogenated in smooth conditions by electrophilic demetallation (Scheme 1b). However, several issues are associated with the use of organotin compounds.

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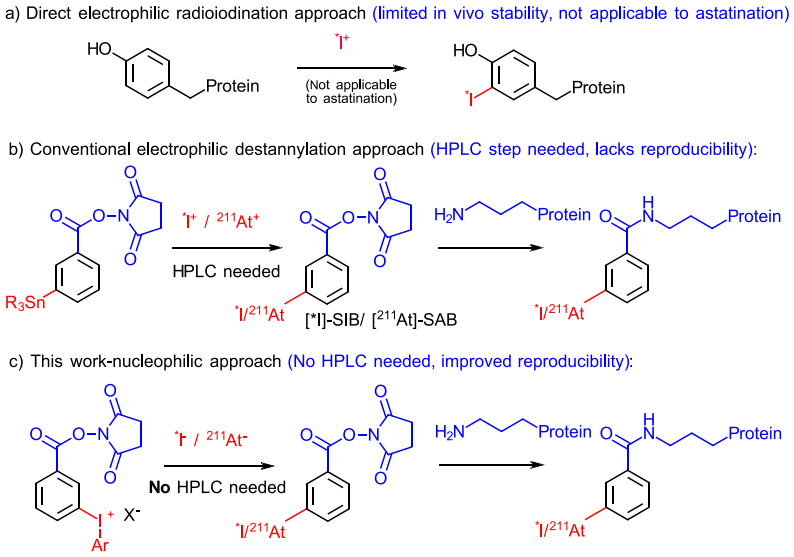


Scheme 1. Radioiodination and astatination of proteins.

First of all, trialkyltin precursors are toxic compounds (with a higher toxicity for the lighter alkyltin derivatives)[8,9] and thus require additional caution during their preparation, and efficient HPLC separation from labeled compounds to be injected in patients. However, a large part of the radiolabeled compound (up to 50%) can be lost by adsorption on HPLC components when working at the radiotracer level. Accordingly, improvements have recently been proposed to facilitate these purifications with the use of supported tin precursors that avoid the need of an HPLC step and which could facilitate the transfer to automated radiosynthesis.[10,11]

Another issue that is more apparent in the case of astatination, is the difficulty to control the +I oxidation state required for the electrophilic destannylation and which leads to low reproducibility of the radiolabeling from one ^{211}At batch to another.[12] This is a concern for the preparation of radiopharmaceuticals in clinical centers for patients treatment.

For these reasons we have recently initiated the investigation of alternative radioiodination and astatination approaches that would be more reliable and practical for use in clinics and that would preferably involve nucleophilic halogen species since I^- and At^- are much easier to stabilize than $I^+$and At^+that are readily overoxidized into several potentially nonreactive species in oxidizing media.[13] Most recent literature shows an interest in developing nucleophilic radioiodination approaches for these reasons, with for instance recent reports on the use of arylboron precursors.[14,15]

The approach introduced herein uses hypervalent iodine derivatives, a class of compounds that has recently replaced organometallic reagents in many chemical transformations without the concerns about toxicity.[16,17] In radiochemistry, aryliodonium salts have been increasingly used for radiofluorination reactions over the past ten years,[18] and we have recently shown that these precursors could also be radioiodinated and astatinated with an unexpected high efficiency on a series of model compounds.[19] We observed that radioiodination and especially astatination could be performed at much lower temperature than radiofluorination (which generally requires high temperatures in the absence of a catalyst), making these precursors potentially applicable to the radioiodination and astatination of more complex structures bearing heat sensitive functional groups.

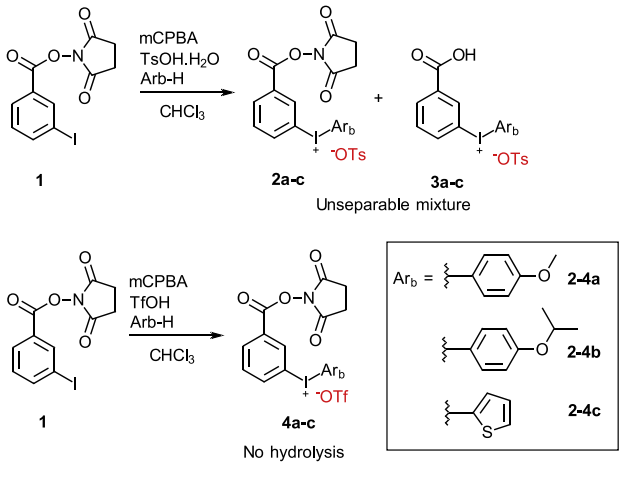
Motivated by developing radioiodination and astatination applications based on aryliodonium salts precursors, we herein report the development of bifunctional precursors for highly efficient protein radiolabeling as an alternative to the conventional arylstannane chemistry.

2. Results and discussion

2.1. Synthesis

Our approach was to design aryliodonium salts that would lead after aromatic nucleophilic substitution (S_NAr) with radioiodide or astatide to the widely used radiolabeled prosthetic groups *N*-succinimidyl-3-[^{125}I]iodobenzoate ([^{125}I]SIB) and *N*-succinimidyl-3-[^{211}At]astatobenzoate ([^{211}At]SAB) for antibody radioiodination and astatination (Scheme 1c).

To validate the feasibility of this approach, we designed unsymmetrical aryliodonium salts, with one of the aryl (Ar_a) bearing the *N*-hydroxysuccinimidyl ester group that would be converted into [^{125}I]SIB or [^{211}At]SAB after S_NAr, and the second (hetero)aryl (Ar_b) being chosen as an electron rich moiety to guide the substitution on the electron deficient Ar_a moiety (Scheme 2). Three aryl rich (hetero)arenes (Ar_b) were chosen for comparison of their



Scheme 2. Bifunctional aryliodonium salts preparation.

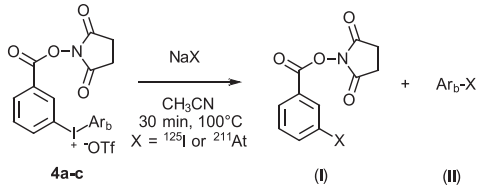
ability to induce this regioselectivity: (i) the 4-anisyl group previously reported as providing good to excellent selectivity of radioiodination and astatination,[19] (ii) the 4-isopropoxyphenyl group as an electron richer analogue of anisyl and (iii) the 2-thienyl group that has been reported as providing excellent regioselectivity of radiofluorination but which use has not yet been reported for radioiodination or astatination.[20]

The initial synthetic approach used a conventional one-pot-two-step procedure consisting in the oxidation of the *N*-succinimidyl-3-iodobenzoate to the corresponding aryliodane followed by ligand exchange with the desired (hetero)arene in the presence of tosylic acid to yield the iodonium tosylates.[19] However, partial hydrolysis of the activated ester was observed, resulting in an inseparable mixture of iodonium species (Scheme 2). This hydrolysis was attributed to the presence of water introduced in the reaction via the tosylic acid which is available in the monohydrated form, the electrophilicity of the carbonyl center of the activated ester being greatly enhanced by the electron withdrawing effect of the iodonium group. Accordingly, the procedure was adapted by replacing tosylic acid by anhydrous triflic acid. Although the counter ion associated to the iodonium salt can dramatically impact reactivity, this change was not expected to provide any marked difference since we observed previously that using either tosylate or triflate had no detectable impact on radioiodination and astatination results.[19]

2.2. Radiochemistry

Radiohalogenation efficiency in terms of radiochemical yield (RCY) and regioselectivity towards the formation of [^{125}I]SIB or [^{211}At]SAB were then assessed with $^{125}I^-$ and $^{211}At^-$ respectively. We have previously identified acetonitrile as an adequate solvent for radioiodination and astatination of aryliodonium salts. We have also previously observed that reaction kinetics were sensibly improved in methanol in the case of astatination (but not for radioiodination).[19] However our initial radiolabeling attempts with precursor **4a** indicated that the activated ester in the iodonium precursor was extensively transesterified by methanol only a few minutes after dissolution in this solvent, which was attributed to the strong electron withdrawing effect of the iodonium function on the aryl and the carbonyl center, making methanol incompatible for [^{211}At]SAB synthesis. Acetonitrile was thus kept for both radioiodination and astatination. Initial screening tests aimed at identifying the best orienting group (Ar_b) for providing the highest RCYs and regioselectivity using reverse phase HPLC analysis of crude labeling solutions. Striking differences were observed between radioiodination and astatination at 100 °C for 30 min (Table 1). As expected from our previous study, the astatination reaction was far more efficient than the radioiodination: nucleophilic substitution yields (conversion to products **I** + **II**) were >90% with all three precursors, whereas only the thienyl based precursor **4c** provided high RCYs (88%) of radioiodination, precursors **4a** and **4b** leading to only 36 and 50% RCY, respectively, the remaining activity corresponding to free radioiodide. Secondly, the regioselectivity of substitution for SIB/SAB formation differed sensibly: whereas it is excellent in the case of radioiodination (1–2% of side product **II** only), it is less marked for astatination, but still very good (6–12% of side product **II**). Despite its richer electronic density (Hammet constant $\sigma = -0.45$), the 4-isopropylphenyl group did not result in a significant change in astatination regioselectivity in comparison with the anisyl group ($\sigma = -0.268$), and surprisingly, the thienyl group was the less orienting group of astatination whereas it is the best for radioiodination. These observations add to many other reports highlighting the difficulty to translate astatine chemistry from known iodine and lighter halogens reactivity.[21] Nonetheless, it was possible to obtain high RCYs of [^{125}I]SIB and [^{211}At]SAB, but it must be noted that the best precursor for radioiodination (**4c**) was not the same as for astatination (**4a**) (see representative HPLC analyses in Fig. S1).

Table 1
Influence of the electron rich (hetero)aryl Ar_b on the radiochemical yield and regioselectivity of radiohalogenation of **4a–c** (n ≥ 3).



	^{125}I		^{211}At	
Iodonium	% (**I**)[a]	% (**II**)	% (**I**)[a]	% (**II**)
4a	36 ± 5	2 ± 1	90 ± 2	6 ± 1
4b	50 ± 3	1 ± 1	85 ± 3	6 ± 1
4c	87 ± 1	1 ± 1	79 ± 2	12 ± 1

[a] 1–3% of total activity of the reaction mixture contained also the carboxylic acid form of **I** due to hydrolysis of the *N*-hydroxysuccinimidyl ester.

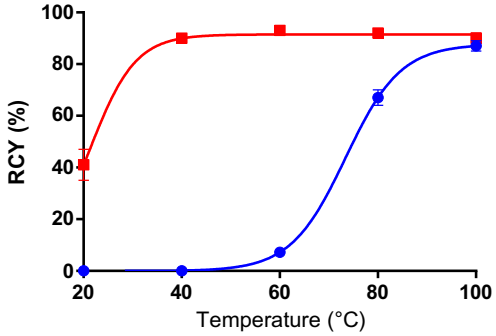


Fig. 1. Influence of the temperature on the RCY of formation of [^{125}I]SIB from **4c** (●) and [^{211}At]SAB from **4a** (■) after 30 min in CH_3CN determined by radio-HPLC analysis of the crude product (n ≥ 2).

Further optimization of reaction temperature and duration was then performed, keeping the best precursor for radioiodination and astatination, respectively. We found that the RCY of the radioiodination of **4c** decreased strongly below 100 °C (67% at 80 °C and only 7% at 60 °C) whereas astatination of **4a** remained highly efficient even at 40 °C (Fig. 1). Astatination RCY at 60 °C (93%) was even slightly higher than at 100 °C (90%) due to a lower amount of hydrolyzed side product ([^{211}At]astatobenzoic acid not detected at 60 °C vs. 1–2% at 100 °C). Kinetic studies indicated that the reaction was fast with optimal RCY reached after 10 min for radioiodination at 100 °C, and only 5 min for astatination at 60 °C (Fig. 2). Under optimized conditions, astatination yields exhibited high reproducibility (less than 2% variation from more than 10 tests performed with different astatine batches), which is consistent with our previous report on a larger number of iodonium precursors bearing various functional groups.[19] Such reproducibility for astatination is particularly attractive in comparison with electrophilic destannylation reactions which have often been reported to produce broader ranges of RCYs (ie RCYs ranging from 35 to 70% in 6 preparations of [^{211}At]MABG,[22] RCYs ranging from 33 to 66% in 16 clinical preparations of [^{211}At]SAB[23]).

Besides the robustness of this nucleophilic approach owing to the use of the radiohalogen in the stable halide form, another

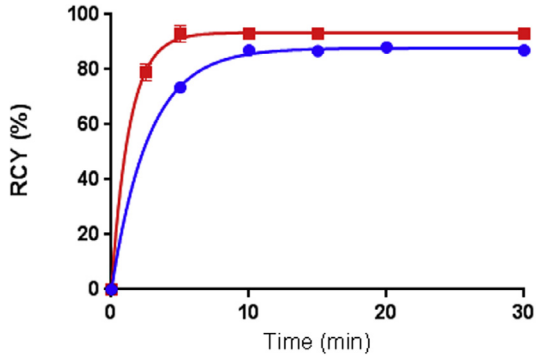


Fig. 2. Kinetics of formation of [^{125}I]-SIB from **4c** (●) at 100 °C and [^{211}At]-SAB from **4a** (■) at 60 °C in CH_3CN determined by radio-HPLC analysis of the crude product ($n \geq 2$).

advantage of aryliodonium salts precursors over conventional arylstannane is highlighted in the purification step. Aryliodonium salts are ionic species that are well retained on silica. This ionic character was thus advantageously used to isolate the radiohalogenated species by a simple filtration on a short silica cartridge instead of the HPLC procedure usually necessary for purification from arylstannane precursors. Three main advantages were found by this approach: i) it is time saving since elution takes less than a minute and the small recovery volume (≈300 μL) is faster to evaporate than the larger recovery volume from HPLC (typically 2–3 mL), ii) the loss of activity that is generally high on HPLC (typically up to 50% due to significant adsorption on materials at the radiotracer level) is largely decreased by using such cartridge purification (typically 10–15% activity was lost) and iii) it is ideally suited for transfer to automated synthesis. HPLC analyses indicated that >99.5% of initial aryliodonium salt was removed by this procedure, which corresponded to less than 2.5 nmol remaining precursor in our typical procedure that uses 450 nmol of precursor (Figs. S2 and S3 in ESI). However, the side products 2-[^{125}I]iodothiophene or [^{211}At]astatoanisole were not removed in this step. These volatile compounds were completely removed in the subsequent evaporation step together with the traces of non-reacted radioiodide and astatide, providing radiolabeled compounds of high radiochemical purities (>98.5%) for the bioconjugation step with a decay corrected RCY of 77–84% in a 80 ± 5 min procedure (n = 5).

2.3. Conjugation of prosthetic groups [^{125}I]SIB and [^{211}At]SAB to an anti-CD138 IgG

The [^{125}I]SIB and [^{211}At]SAB purified as described above were then conjugated to an IgG, our home-made murine anti-CD138 monoclonal antibody (9E7.4) that has proven successful in preclinical radioimmunotherapy with the α particle emitter ^{213}Bi.[24] Conjugation yields were high (75 ± 3% with [^{125}I]SIB and 77 ± 3% with [^{211}At]SAB) with good preservation of immunoreactivity (86 ± 2%).

The robustness of our approach for the production of astatinated mAb was compared with the two main labeling methods that use arylstannane precursors: method A, that includes a HPLC purification for excess arylstannane precursor removal prior to bioconjugation, and method B in which no purification is performed prior to bioconjugation. Results of the astatination of 9E7.4 mAb (Table 2) highlight the good reproducibility of the aryliodonium based radiosynthesis of [^{211}At]SAB (decay corrected RCY from 77 to 84%) in comparison with arylstannane based methods whose RCYs spread in a broader range of values (33–58% for method A and 75–93% for method B). Method A provided overall lower RCYs which was mainly due to lost activity during HPLC and evaporation of [^{211}At]SAB HPLC fractions. It is however important to note that RCYs obtained by us with method A were lower than the previously reported similar procedure used in 16 clinical preparation in which the author purified [^{211}At]SAB on normal phase silica cartridge instead of a HPLC with a RCY of 54 ± 10%.[23] However, it is unlikely that all the 2 mg arylstannane precursor was efficiently removed using such cartridge separation given the low difference of polarity between the precursor and the labeled product. The efficiency in removing residual tin content was not reported by the authors who commented only on the radiochemical purity of the purified product. On the other hand, method B provided lower conjugation yield which may be attributed to competitive reaction of [^{211}At]SAB with excess arylstannane precursor that was not removed at all (no purification performed) and that consequently leads to competition with the excess arylstannane precursor remaining in conjugation solution (24–29% overall RCY). Our results obtained with method B were somewhat inferior to previous literature reports (e.g. 37% ± 5% reported by Palm et al.[25]), which might be explained by the use of a different mAb as well as a different mAb concentration which is known to impact strongly such bioconjugation reaction, especially in our case where competitive arylstannane precursor is still present. Overall, our new radiolabeling procedure to produce the [^{211}At]astatinated 9E7.4 mAb led to significantly improved radiochemical yields in our comparison with a unique mAb used at a constant concentration (53–57% overall RCY, i.e. nearly twice than stannane based methods A and B) and with a higher consistency. The high SNAr reaction yields, the elimination of an HPLC purification step and the high purity of the astatinated synthon prior to the bioconjugation appear as essential factors that contributed to the improved overall RCY.

3. Conclusion

A novel radioiodination and astatination method was developed based on the S_NAr reaction of aryliodonium salts chemistry. Taking advantage of the easily controllable halogenide form compared to the X^+ species needed in the electrophilic approaches, high RCY and high reproducibility were obtained. The reaction conditions and precursors required to obtain optimal RCYs differed for radioiodination and astatination, highlighting the particular behavior of heavy halogenides with aryliodonium salts. Besides

Table 2
Comparison between arylstannane chemistry (method A & B, see details in ESI) from the literature and our aryliodonium salts approach for the astatination of the 9E7.4 antibody.

	[^{211}At]SAB RCY (%)[d]	Conjugation yield (%)[e]	Overall decay corrected RCY (%)	Procedure duration (min)
Method A[a]	33-58[f]	51–61	20–30	200 ± 10
Method B[b]	75–93	27–30	24–29	120 ± 10
This work[c]	77–84	54–60	53–57	140 ± 10

[a]SAB produced from the tin precursor with *N*-chlorosuccinimide as oxidizing agent and purified by HPLC before conjugation (n = 2). [b]SAB produced from the tin precursor with *N*-iodosuccinimide as oxidizing agent and conjugated crude without purification (n = 3). [c]SAB produced from aryliodonium **4a** and purified on a silica Sep-Pak Vac 3cc (500 mg) cartridge before conjugation (n = 5). [d]Decay corrected RCYs of purified [^{211}At]SAB. [e]Conjugation yields are given after purification by gel filtration. Such purification leads typically to 20–25% loss of radiolabeled antibody on the purification column. [f]Crude RCY was typically 70–90%; major loss due to HPLC purification.

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the excellent reactivity of these precursors, their separation from the radiolabeled prosthetic groups of interest was performed efficiently and rapidly using silica cartridges which greatly contributed to the overall radiosynthesis yield in comparison with the conventional arylstannane chemistry or the recently reported copper catalyzed arylboron chemistry[14,15] that require lengthy HPLC purification and thus leading to additional loss of radiolabeled product. Overall these precursors and the methods developed herein appear particularly attractive for the development of radioiodinated and astatinated proteins for preclinical applications and potentially for clinical applications, especially since toxicity concern are reduced and an HPLC step is not required, thus allowing easy transfer to fully automated synthesis. Such automated approaches are under development in our laboratory in preparation of future clinical trials with ^{211}At.

4. Experimental section

4.1. Syntheses

4.1.1. General

All reagents and solvents were obtained commercially and used without further purification unless otherwise noted. 3-iododobenzoate succinimidyl ester was prepared using a previously reported procedure.[26] ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker AC 400 instrument, and chemical shifts are reported in ppm on the δ scale relative to TMS. High-resolution mass spectrometry (HRMS) analyses were performed on a Synapt G2 HRMS Q-TOF mass spectrometer equipped with an electrospray ionization (ESI) interface operating in the positive mode (Waters Corporation, Milford, MA, USA)

4.1.2. 3-(Succinimidyloxycarbonyl)phenyl(4-methoxyphenyl) iodonium triflate (**4a**)

To 3-chloroperbenzoic acid dried in vacuo for 1 h prior to use (504 μmol) in dry dichloromethane (5 mL), was added 3-iododobenzoate succinimidyl ester (458 μmol) and the solution was stirred at room temperature for 15 min. Anisole (504 μmol) was then added, the reaction cooled to −20 °C and triflic acid (916 μmol) added. The solution turned dark. It was stirred for 15 min at −20°C and the volatiles were removed by rotary evaporation. To the dark residue was added Et_2O. The thick oily suspension was stirred for about 45 min upon which a deep blue precipitate formed. It was then purified by flash chromatography using a CH_2Cl_2/*t*BuOH gradient from 95:5 to 60:40. The solid obtained was then recrystallized from CH_3CN/Et_2O to afford white crystals (47 mg, 28%). ^{1}H NMR (CD_3CN, 400 MHz, ppm): δ 2.86 (s, 4H), 3.85 (s, 3H), 7.08 (d, 2H, J = 9.2 Hz), 7.73 (t, 1H, J = 8.2 Hz), 8.05 (d, 2H, J = 7.2 Hz), 8.32–8.38 (m, 2H), 8.75 (m, 1H). ^{13}C NMR (CD_3CN, 100 MHz, ppm): δ 26.5, 56.8, 102.2, 114.8, 119.3, 129.4, 134.0, 135.0, 137.0, 139.1, 141.7, 161.2, 164.6, 170.8. ^{19}F NMR (CD_3CN, 400 MHz, ppm): δ −79.35. HRMS: $C_{18}H_{15}INO_5^+$[M−OTf]$^+$ calc: 451.9995, found: 452.002

4.1.3. 3-(Succinimidyloxycarbonyl)phenyl(4-isopropoxyphenyl) iodonium triflate (**4b**)

The procedure was identical to the preparation of **4a**, with anisole replaced by isopropoxybenzene and afforded **4b** as colorless needles in 8% yield. ^{1}H NMR (CD_3CN, 400 MHz, ppm): δ 1.31 (d, 6H), 2.86 (s, 4H), 4.64–4.72 (m, 1H), 7.03 (d, 2H, J = 8.0 Hz), 7.72 (t, 1H, J = 8.0 Hz), 8.03 (d, 2H, J = 8.0 Hz), 8.35 (m, 2H), 8.74 (m, 1H). ^{13}C NMR (CD_3CN, 100 MHz, ppm): δ 21.9, 26.5, 72.0, 101.7, 115.1, 120.5, 123.6, 129.4, 134.0, 134.9, 137.0, 139.2, 141.7, 161.2, 163.1, 170.8. ^{19}F NMR (CD_3CN, 400 MHz, ppm): δ −79.70. HRMS: $C_{20}H_{19}INO_5^+$[M−OTf]$^+$ calc: 480.0308, found: 480.0315

4.1.4. 3-(Succinimidyloxycarbonyl)phenyl(2-thienyl)iodonium triflate (**4c**)

The procedure was identical to the preparation of **4a**, with anisole replaced by thiophene and afforded **4c** as a white solid in 17% yield. ^{1}H NMR (CD_3CN, 400 MHz, ppm): δ 2.86 (s, 4H), 7.22 (m, 1H), 7.74 (t, 1H, J = 8.0 Hz), 7.91 (m, 1H), 8.03 (m, 1H), 8.38 (m, 2H), 8.78 (m, 1H). ^{13}C NMR (CD_3CN, 100 MHz, ppm): δ 26,5, 95.6, 117.7, 129.4, 131.3, 134.0, 135.1, 136.7, 140.4, 141.4, 143.7, 157.6, 161.2, 170.8. ^{19}F NMR (CD_3CN, 400 MHz, ppm): δ −79.70. HRMS: $C_{15}H_{11}INO_4S^+$[M−OTf]$^+$ calc: 427.9453, found: 427.9467

4.2. Radiochemistry

4.2.1. General

[^{125}I]NaI was obtained commercially from Perkin Elmer in 10^{-5} M NaOH solution with a volumic activity of 50 μCi/μL (1.85 MBq/μL). ^{211}At was produced at the Arronax cyclotron facility using the ^{209}Bi(α,2n)^{211}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.[27] Before use, the ^{211}At solution was reduced to dryness under a gentle stream of nitrogen and dissolved in an appropriate volume of a 10 mg/mL sodium sulfite solution. Separation of radioiodinated or astatinated compounds from aryliodonium salt precursors were performed using disposable Sep-Pak Vac 3cc (500 mg) silica cartridges (Waters). HPLC analyses were performed on a Waters Alliance e2695 system equipped with a FlowStar LB 513 Radio Flow Detector (Berthold) and a C-18 column (Spherisorb ODS2 5 μ 4.6 mm × 25 cm, Waters) with the flow rate set at 1.50 mL/min with the following gradient: t = 0: 60% A, 40% B; t = 7 min: 30% A, 70% B; t = 11 min: 100% B with A = H_2O with 0.05% TFA and B = CH_3CN with 0.05% TFA. The non-radioactive iodinated compounds were analyzed using this HPLC system and their retention times (given in Table S1) were used as references for identification of their radioiodinated and astatinated analogues. Radioiodination and astatination of arylstannane precursors (methods A and B) are given in ESI

4.2.2. Radioiodination of aryliodonium salts **4a**, **4b** and **4c** at low activity for analytical purpose

A stock solution of [^{125}I]NaI was prepared by diluting the commercial solution twelve times in de-ionized water. For each experiment, 95 μL of a 2.5 mM aryliodonium salt solution in CH_3CN was placed in a 1.5 mL crimp top vial, and 5 μL (approx. 770 kBq) of the diluted [^{125}I]NaI solution was added. The vial was then sealed and placed in a heating block for an appropriate duration at the desired temperature. An aliquot was then diluted in water (0.05% TFA) and analyzed by reverse phase HPLC

4.2.3. Radioiodination of aryliodonium salt **4c** for protein labeling

The radioiodination was performed the same way using the thienyl version of aryliodonium salt **4c** without dilution of the [^{125}I]NaI commercial source, and keeping an aryliodonium/[^{125}I]NaI volume ratio of 95:5. Typically, radiolabeling procedures were performed with 190 μL aryliodonium salts solution incubated with 10 μL commercial [^{125}I]NaI solution for 60 min at 100°C. HPLC of an aliquot indicated the formation of [^{125}I]SIB with 88–90% RCY, 1–2% 3-[^{125}I]iodobenzoic acid and 1–2% 2-[^{125}I]iodothiophene. The crude reaction solution was then deposited directly on a dry disposable Sep-Pak Vac 3cc (500 mg) silica cartridge (Waters) and AcOEt was used as eluent. The [^{125}I]SIB activity eluted within the first 250 μL together with the 2-[^{125}I]iodothiophene. After evaporation under a stream of nitrogen, an HPLC analysis indicated that the final [^{125}I]SIB was recovered with a radiochemical purity >99% and less than 0.5% residual aryliodonium salt. HPLC analyses are given in Fig. S1

*4.2.4. Astatination of aryliodonium salts **4a**, **4b** and **4c** at low activity level for analytical purpose*

A stock solution of [^{211}At]NaAt was prepared as follow: the chloroform ^{211}At solution was evaporated to dryness under a gentle stream of nitrogen and redissolved in an appropriate volume of 10 mg/mL sodium sulfite solution to obtain a volumic activity of about 500 kBq/μL. For each experiment, 95 μL of a 2.5 mM aryliodonium salt solution in CH_3CN were placed in a 1.5 mL crimp top vial, and 5 μL of [^{211}At]NaAt solution was added. The vial was then sealed and placed in a heating block for an appropriate duration at the desired temperature. An aliquot was then immediately analyzed by reverse phase HPLC

*4.2.5. Astatination of aryliodonium salts **4a** for protein labeling*

Astatination was performed the same way using the anisyl version of aryliodonium salt **4a**, and keeping an aryliodonium/[^{211}At]NaAt volume ratio of 95:5. Typically, radiolabeling were performed with 190 μL aryliodonium salts solution incubated with 10 μL [^{211}At]NaAt solution for 30 min at 60°C. HPLC of an aliquot indicated the formation of [^{211}At]SAB with 92–94% RCY, and 5–6% [^{211}At]astatoanisole. The crude reaction solution was then deposited directly on a dry disposable Sep-Pak Vac 3cc (500 mg) silica cartridges (Waters) and AcOEt was used as eluent. The [^{211}At]SAB activity eluted within the first 250 μL together with the [^{211}At]astatoanisole and was collected in a 1.5 mL V-vial. After evaporation under a stream of nitrogen, an HPLC analysis indicated that the final [^{211}At]SAB was recovered with a radiochemical purity >98.5%, the remaining activity corresponding to 3-[^{211}At]astatobenzoic acid. Less than 0.5% residual aryliodonium salt was detected. HPLC analyses are given in Fig. S2

4.3. Protein radiolabeling

Conjugation of [^{125}I]SIB or [^{211}At]SAB were performed the same way: the dry [^{125}I]SIB or [^{211}At]SAB activity obtained in a 1.5 mL V-vial from aryliodonium salts as described above was dissolved in DMSO (10 μL), and the 9E7.4 IgG was added (60 μL of a 5 mg/mL solution in borate buffer 0.3 M, pH 8.6). After 30 min of incubation at 20 °C, the conjugation yield was assessed by elution of an aliquot deposited on a ITLC-SG plate (10% trichloroacetic acid as eluent), and integration of the plate using a Cyclone phosphorimager scanner (Perkin Elmer). Conjugation yields were in the 72–80% range with both radioisotopes. Purification was performed by gel filtration on a Sephadex G-25 resin loaded column (PD-10, GE healthcare) using PBS as eluent, affording the purified radiolabeled antibody with a >99.5% radiochemical purity as assessed by ITLC-SG. Immunoreactivity was preserved, with 86 ± 2% for both radioisotopes.

The whole radioiodination procedure was performed with an initial [^{125}I]NaI activity up to 25.9 MBq which led to 11.2 MBq radioiodinated 9E7.4 IgG with a specific activity of 50 MBq/mg corresponding to an overall RCY of 43%.

The whole astatination procedure was performed with an initial [^{211}At]NaAt activity up to 78 MBq which led to 35 MBq astatinated 9E7.4 IgG with a specific activity of 155 MBq/mg corresponding to a decay corrected overall RCY of 54% in a 2 h 30 min procedure.

4.4. Immunoreactivity assay

The immunoreactive fraction of [^{125}I]SIB-9E7.4 and [^{211}At]SAB-9E7.4 was determined using magnetic beads (Pierce, Thermo Scientific) labeled with a 40 amino acids peptide recognized by the 9E7.4 antibody according to the supplier's protocol. One picomole of radiolabeled 9E7.4 was incubated for 15 min at room temperature with 20 μL of coated magnetic beads (10 mg/mL). Using a magnetic rack, supernatants containing non-reactive antibodies and magnetic beads were collected separately and the radioactivity in each fraction was measured in a gamma counter.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2017.09.022.

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