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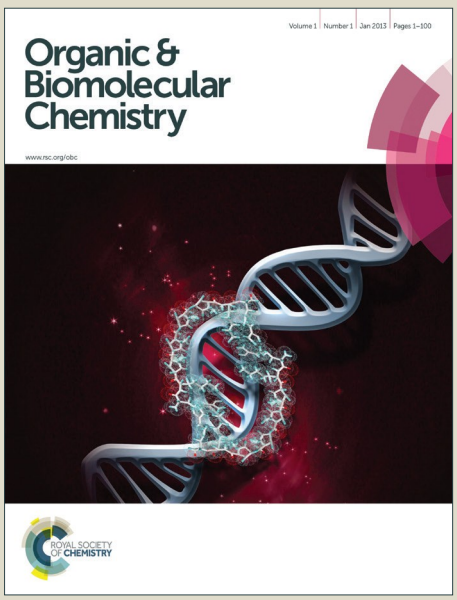

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PAPER

Ionic liquid supported organotin reagents to prepare molecular imaging and therapy agents

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Efficiency of ionic liquid supported organotin reagents in dehalogenation reaction has been investigated. High radiochemical yields of astatinated and iodinated compounds have been obtained using simple work-up procedure. This methodology represents a straightforward approach for the preparation of molecular imaging and therapy agents in nuclear medicine.

Introduction

The use of radiopharmaceuticals in nuclear medicine is opening significant perspectives for diagnostic and functional imaging of tumors. Indeed, the characterization (phenotype, proliferation, response to treatment) and the better comprehension of tumor environment (vascularization, hypoxia, inflammation, and immune response) can be crucial for personalized therapy, another valuable application for radiopharmaceuticals. Various radionuclides may be used, especially radioactive isotopes of halogens (i.e. radiohalogens).[1] Radiohalogens are widely represented in nuclear medicine as Fluorine-18 is the most common used radioisotope for PET imaging.[2]^{18}F-FDG is considered now as a standard with applications not only for oncology but also for neurology or cardiology. Another heavy radiohalogen, astatine, is currently gaining attention. Indeed, astatine with its isotope 211 is considered as one of the most promising radionuclides for the development of targeted alpha-radionuclide therapy of disseminated cancerous cells considering its physical properties ($T_{1/2}$: 7.2 hours; $E_{ave.}$: 6.79 MeV (100%); LET: 99 keV.m^{-1}, mean range : 65 µm).[3] Its chemistry is similar to the one of iodine which radioisotopes are applicable for both targeted β-therapy (I-131) more convenient for larger tumors, SPECT (I-125, I-123,) and PET (I-124).[4] Theranostic (diagnostic and therapy with the same or similar molecule(s) radiolabeled with a pair of radionuclide) for personalized medicine can then be considered with the astatine/iodine pair.[5]

Except for [I-131/I-123]NaI for thyroid diseases imaging, radiopharmaceuticals labeled with radiohalogens are generally constituted by two entities: the vector and the radionuclide. Vectors may be antibodies and derivatives, peptides or small organic molecules targeting tumors. The labeling of the vector may be performed either directly or requires the use of a small radiolabeled organic compound (prosthetic group) bearing a reactive function able to link the vector.

Among the direct radiolabeling strategies, electrophilic substitution of organometallic precursors with heavy radiohalogens represents one of the most convenient methods. Based on the high reactivity of carbon-metal bond, organometallic derivatives allow fast and regioselective reactions on a wide variety of substrates with high yields under mild conditions. In addition, these reactions display a good tolerance towards functionalities present in the substrate (e.g. esters, activated esters). Halodemetalation reaction for the preparation of radiohalogenated compounds have been studied with organometallics[6] containing mercury,[7] boron,[8] tin,[9] silicon,[10] germanium,[11] thallium[12] and lead.[13]Triorganotin derivatives display thus great potential for demetalation reaction. Unfortunately, drawbacks such as contamination of halogenated products by tin residues limit the scope of these reagents.

Efforts have been made to overcome these problems, leading, for example, to the development of solid-phase synthesis methods,[14] and fluorous phases.[15] As a part of our ongoing research program on the discovery of potentialities of TSILs (Task Specific Ionic Liquids),[16] we decided to investigate organotin reagents supported on ionic liquids in demetalation reactions to produce radiohalogenated compounds. The use of organotin reagents immobilized on ionic liquids would have the combined advantages of safe handling (non-volatile properties) and minimizing the product contamination by tin.

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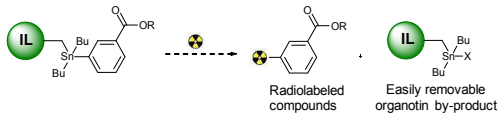


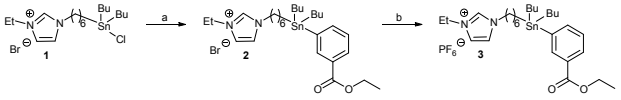
Scheme 1 General strategy for a "clean" synthesis of radiolabeled compounds and making the purification easier, by filtration (Scheme 1).

Results and discussion

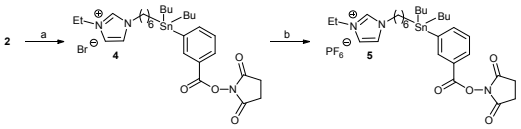
The first part of this study relies in the preparation of organotin reagents immobilized on ionic liquids bearing an ester function or an activated ester group. In this context, starting from organotin chloride derivative **1**,[16] new organotin reagent supported on ionic liquid **2** has been isolated in 70% yield after reaction of 3-(ethoxycarbonyl)phenyl zinc reagent. To avoid any side reactions involving the bromide counter anion instead of the radiohalogen, anionic metathesis reaction between bromide and hexafluorophosphate anions has been achieved to lead **3** in 94% yield (Scheme 2).

Succinimidyl ester **4** was obtained in 55% yield by saponification of precursor **2** using NaOH aqueous solution in EtOH followed by esterification with *N*-hydroxysuccinimide. Bromide anion was exchanged by PF_6^- to afford **5** in a good yield. All new compounds **2**-**5** were fully characterized with conventional methods (Scheme 3).

The reactivity of ionic liquid supported organotin reagents in halodemetalation reactions was first evaluated with the organotin derivative **3** and carrier-added iodine-125. In that case, the halodemetalation reaction occurred quantitatively in only five minutes to form the ethyl [I-125/127]iodobenzoate **6.**



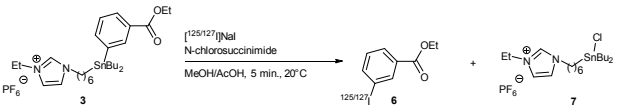
Scheme 2 Synthesis of Ethyl 3-(dibutylstannyl)benzoate supported on ionic liquid. Reagents and conditions: (a) 3-(ethoxycarbonyl)phenylzinc, THF, room temperature, overnight; (b) $NaPF_6$, acetone, 24h.



Scheme 3 Preparation of 3-(Dibutylstannyl)succinimidyl ester supported on ionic liquid. Reagents and conditions: (a) i) aq NaOH (15% w/w), EtOH, 78°C, 2h; ii) *N*-hydroxysuccinimide, DCC, THF, overnight ; 55 %(b) $NaPF_6$, acetone, 24h.

using an equimolar ratio between the oxidant (*N*-Chlorosuccinimide) and the ionic liquid supported tin precursor **3** (Scheme 4).Pure product was then easily obtained with simple filtration on silica cartridge which was validated with HPLC control of the purified product **6** (Fig. 1). Contrarily to conventional radiolabeling techniques,[17] it is noteworthy that purification was facilitated here since the by-product **7** can be easily separated from **6** limiting also the tin contamination.

We were then interested in the radiolabeling with astatine-211 for the synthesis of the SuccinimidylAstatoBenzoate ([At-211]SAB) which is the most frequently described prosthetic group for astatine radiolabeled vectors (Scheme 5).[18]



Scheme 4 Radiolabeling of Ethyl 3-(dibutylstannyl)benzoate supported on ionic liquid **3**. Reagents and conditions: [I-125/127]NaI, N-chlorosuccinimide, MeOH/AcOH, 5 min., 20°C.

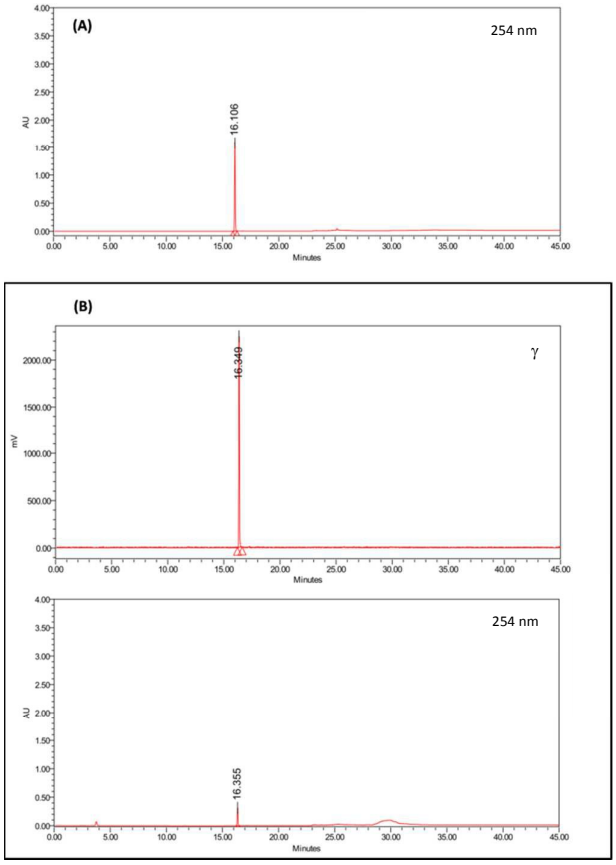
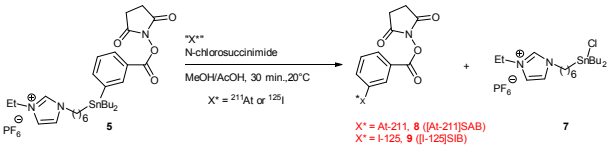


Fig. 1 HPLC chromatogram of cold ethyl iodobenzoate (A), and purified [I-125/127]**6** (B).



Scheme 5 Radiolabeling of 3-(Dibutylstannyl)succinimidyl ester supported on ionic liquid **5**. Reagents and conditions: *N*-chlorosuccinimide, MeOH/AcOH, 30 min.,20°C.

Table 1 Radiochemical yields (RCY) and concentration in oxidant for production of [At-211]SAB from organotin supported ionic liquid **5**.

Entry	NCS (mg.mL^{-1})	RCY (%)
1	0.25	27
2	0.50	39
3	1.00	49
4	1.35	53
5	2.70	62
6	4.05	63
7	5.40	66

Table 2 Radiochemical yields and concentration in ionic liquid supported organotin **5** for production of [At-211]SAB

Entry	**5** (mg.mL^{-1})	RCY (%)
1	0.6	48
2	1.3	53
3	2.5	56
4	5.0	64
5	10.0	63
6	20.0	64

The demetalation reaction involving organotin derivative **5** and no-carrier-added astatine-211 was completed in 30 minutes in MeOH / AcOH (95/5) mixture at 20 °C. Unsurprisingly, previous reaction time with carrier-added radioiodine is shorter as the carrier generally enhanced reaction kinetics. However, it should be noted that astato-demetalation with a conventional tin derivative and our astatine source generally required heating at 60°C.[19] This decrease of reaction temperature is likely due to the fact that ionic liquid can act as a catalyst or can enhance the reactivity of radiohalogen.[20]

We then studied the influence of NCS (*N*-Chlorosuccinimide) concentration on the astato-demetalation reaction. Results are summarized in Table 1. This study shows that optimal concentration of NCS is 2.70 mg.mL^{-1} and leads to [At-211]SAB in 62 % radiochemical yield (Table 1, entry 5). Below this value the yield is lower (Table 1, entries 1-4) and higher concentration does not afford better performance (Table 1, entries 6-7).

To further optimize reaction conditions, the influence of concentration of ionic liquid supported organotin reagent **5** was investigated (Table 2). Using an organotin **5** concentration of 5.0 mg.mL^{-1} with a NCS concentration of 2.70 mg.mL^{-1}, the reaction proceeded with 64% optimized radiochemical yield (Table 2, entry 4).

Interestingly, no time-consuming purification protocols such as HPLC technique was required to isolate the reaction product from tin precursors and unreacted astatine species. The pure [At-211]SAB was conveniently isolated by filtration through silica gel cartridge from the crude mixture. This single purification led to a time saving estimated about 2 hours compared with conventional methodologies. RP-HPLC analysis of the purified product shows that hydrophilic species such as free astatine was also eliminated during the fast filtration (Fig. 2). According to the analytical HPLC, the [At-211]SAB **8** was obtained up to 91% radiochemical purity (Fig.2).Purified [At-211]SAB allowed successfully the astatine radiolabeling of the 9E7 mAb in 76% yields with good immunoreactivity[21].Similarly, halodemetalation optimized conditions were directly applied to reaction with Iodine-125 and **5** (Scheme 5). [I-125]SIB **9** was thus obtained with 67% RCY and coupled to bovine serum albumin modified with about 50 Di-HSGL heteropeptide[22] residues per protein (BSA-Di-HSGL) after purification (Fig. 3). The coupling yield was interestingly good (54%) despite the low protein concentration (1.5 mg.ml^{-1}, 20 μM). Moreover, the immunoreactivity test has demonstrated that recognition of HSG residues on the radiolabeled BSA-Di-HSGL is maintained for mAb-679 (anti-HSG)[23].

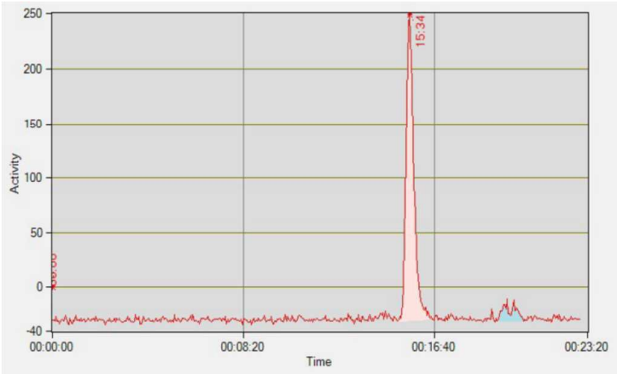


Fig. 2 HPLC chromatogram of [At-211]SAB **8** (t_R: 15.34 min.) after purification.[17]

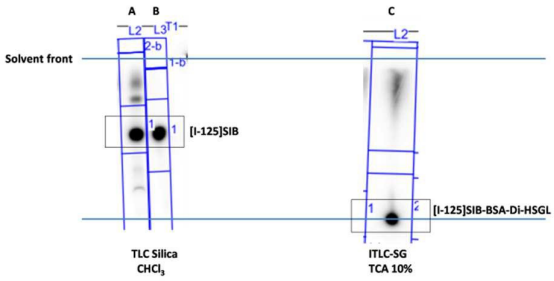


Fig. 3 TLC of [I-125]SIB **9** before (A) and after (B) purification, (C) ITLC-SG of radiolabeled BSA-Di-HSGL.

Conclusion

In summary, we have developed a new ionic liquid–solution phase radiolabeling method avoiding time consuming purification methods, leading to astatinated and radioiodinated radiopharmaceuticals compounds in good yields and purities. Interestingly, halodemetalation radiochemical yields are similar to those obtained with conventional tin precursors (trimethylstannyl derivatives). It is noteworthy that this new original methodology led to final products with higher global yields since no product was lost neither by decay (thanks to this time-saving process) nor by undesirable retention on chromatographic systems. An innovative automated system based on this methodology is currently underway to routine production.

Experimental section

General Information

Commercially available reagents and solvents were purified and dried, when necessary, by standard methods prior to use. ^{1}H (300 MHz), ^{13}C (75 MHz) NMR spectra were recorded on a Bruker Avance 300 spectrometer. The compounds studied were measured in $CDCl_3$ and ^{1}H and ^{13}C chemical shifts, reported in ppm, were referred to the central signal of the solvent. ^{13}C NMR spectra were recorded with complete proton decoupling. High resolution mass spectra measurements were recorded on Waters-Micromass GCT Premier spectrometers. Analytical thin layer chromatography was performed on pre-coated silica gel 60-F254 plates. For cold references, *m*-iodoethylbenzoate is commercially available and *m*-succinimidyl iodobenzoate has been previously described.[17]

Radioactive materials. All radioactive materials were handled according to the approved protocols at the Centre de Recherche en Cancérologie Nantes/Angers. Astatine-211 was produced at the CEMTHI, Orléans, France using the ^{209}Bi(α, 2n)^{211}At nuclear reaction (28 MeV). Astatine-211 was recovered from targets by sublimation and subsequent trapping into methanol.[3a] Total activity was determined in an ACAD 2000 ionization chamber (LemerPax, Carquefou, France). Volumic activities were close to 100 MBq.mL^{-1}. Iodine-125 was purchased as a solution of [I-125]NaI in 0.048 M NaOH at 3.7 GBq.mL^{-1} (Perkin Elmer, Courtaboeuf, France). Methanol, acetic acid and sodium metabisulfite were purchased in trace select grade and diethyl ether in analytical grade. All organic solvents were HPLC grade and water (18.2 MΩ.cm) was obtained from a Milli-Q® Gradient system (Millipore).

Radio-chromatography. Radio-TLC were eluted on precoated silica gel 60 F254 TLC plastic sheets (Merck, Darmstadt, Germany) and examined using a Cyclone Plus Phosphor Imager (Perkin Elmer). Data were analyzed with the OptiQuant software (Perkin Elmer). Sep-Pak Silica Vac Cartridges prepacked with 200 mg of silica (Waters) were used for fast purifications of small radioactive compounds. Radiolabelings of proteins were controlled using Instant Thin Layer Chromatography-Silica Gel (ITLC-SG paper, Agilent) eluted with trichloroacetic acid 10%. Radiolabeled proteins were purified on NAP-5 column (GE Healthcare) eluted with PBS/BSA 0.1%.

Normal phase HPLC analysis were conducting using an Waters Alliance system equipped with a dual wavelength UV-Vis detector (λ = 280 nm) and a BertoldFlowstar LB513 detector shielded module. Radioactive species were separated on a Spherisorb S5W silica column (5 µm, 250 × 4.6 mm) with a gradient elution of Heptane (A) and AcOEt (B) (0-5 min.: 100% A, 5-15 min.: 100% A - 99% A, 15-20 min.: 99% A, 20-35 min.: 99% A - 100% B, 35-45 min.: 100% B) at 2 ml.min^{-1}. Data were collected and analyzed using the Empower 3 software (Waters). RP-HPLC analysis were conducting using an Eckert&Ziegler HPLC system consisting of Knauer pumps K120, Knauer HPLC Degasser, Knauersmartline UV 2520 (λ = 254 nm), and a Eckert&Ziegler detector shielded module. Radioactive species were separated on a ACE C18 column (3 µm, 150 × 3 mm) with a gradient elution of TFA 0.05% (A) and ACN/TFA 0.05% (B) (0-3 min.: 1% B, 3-20,3 min.: 1% B - 100% B) at 0.5 ml.min^{-1}. Data were collected and analyzed using the Modular-Lab software (Eckert&Ziegler).

1-(6-(dibutyl(3-(ethoxycarbonyl)phenyl)stannyl)hexyl)-3-ethyl-1H-imidazol-3-ium bromide 2. Commercially available (3-(ethoxycarbonyl)phenyl)zinc bromide (0.5M in THF, 12.8 mL, 6.4 mmol, 5.6 eq) was introduced dropwise to the ionic liquid **1** (529 mg, 1.15 mmol, 1 eq) in solution in anhydrous THF (6 mL). After 18 h of stirring at room temperature, the resulting mixture was filtered through a short pad of silica gel then extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 90:10) to afford compound **2** as a viscous yellow oil (450 mg, 70 %).^{1}H NMR ($CDCl_3$, ppm) δ 10.17 (s, 1H), 8.00 (bs, 1H), 7.86-7.81 (m, J = 7.8 Hz, 1H), 7.58 (bs, 1H), 7.55-7.50 (m, J = 7.5 Hz, 1H), 7.37 (bs, 1H), 7.32-7.25 (m, 1H), 4.38-4.17 (m, 6H), 1.81-1.70 (m, 2H), 1.55-1.38 (m, 9H), 1.35-1.18 (m, 11H), 1.02-0.90 (m, 6H), 0.77 (t, J = 7.2 Hz, 6H). ^{13}C NMR ($CDCl_3$, ppm)δ 167.1, 142.2, 140.9, 137.1, 136.2, 129.6, 129.0, 127.7, 122.2, 122.1, 60.8, 50.0, 45.3, 33.6, 30.2, 28.9, 27.2, 26.5, 25.7, 15.7, 14.3, 13.6, 9.6, 9.5. HRMS (FAB) calcd. for $C_{28}H_{47}N_2O_2Sn$ 563.2654 $[M\text{-}Br]^+$; found 563.2675.

1-(6-(dibutyl(3-(ethoxycarbonyl)phenyl)stannyl)hexyl)-3-ethyl-1H-imidazol-3-ium hexafluorophosphate 3. Compound **2** (150 mg, 0.233 mmol, 1eq) was dissolved in acetone (4 mL) and stirred with $NaPF_6$ (78 mg, 0.464 mmol, 2 eq) at room temperature for 24 h to exchange the anion. The reaction mixture was filtered and the acetone was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 90:10) to afford compound **3** as a viscous yellow oil (156 mg, 94%).^{1}H NMR ($CDCl_3$, ppm)δ 8.55 (bs, 1H), 8.10 (s, 1H), 7.95-7.91 (m, , 1H), 7.69-7.55 (m, , 1H), 7.42-7.26 (m, 3H), 4.35 (q, J = 7.3 Hz, 2H), 4.21 (q, J = 7.3 Hz, 2H), 4.10 (t, 2H, J = 7.2 Hz), 1.89-1.75 (m, 2H), 1.61-1.49 (m, 9H), 1.41-1.24 (m, 12H), 1.11-0.99 (m, 5H), 0.87 (t, J = 7.2 Hz, 6H). ^{13}C NMR ($CDCl_3$, ppm)δ 167.5, 142.5, 141.2, 137.4, 135.1, 129.9, 129.3, 128.1, 122.4, 122.3, 61.2, 50.3, 45.5, 33.7, 30.1, 29.2, 27.5, 26.8, 25.9, 15.3, 14.6, 13.9,

9.8. HRMS (FAB) calcd. for $C_{28}H_{47}N_2O_2Sn$ 563.2654 $[M\text{-}PF_6]^+$; found 563.2655.

1-(6-(dibutyl(3-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)phenyl)stannyl)hexyl)-3-ethyl-1H-imidazol-3-ium bromide 4. To a solution of 600 mg of compound **2** (0.934 mmol, 1 eq) in ethanol (5 mL) were added 0.97 mL of an aqueous solution of NaOH (15% w/w). The resulting mixture was stirred for 20 min at room temperature, then refluxed 2 h and the ethanol was removed under reduced pressure. The residue was acidified with 3 mL of HCl 1M, and then extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude acid was used in next step without further purification (510 mg, HRMS (FAB) calcd. for $C_{26}H_{43}N_2O_2Sn$ 535.2341 $[M\text{-}Br]^+$; found 535.2336). A mixture of crude acid (510 mg, 0.83 mmol, 1 eq), *N*-hydroxysuccinimide (105 mg, 0.913 mol, 1.1 eq.) and DCC (188 mg, 0.913 mol, 1.1 eq) in anhydrous THF (10 mL) was stirred for 12h at room temperature under argon. The reaction mixture was filtered and solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 98:02 to 90:10) to afford compound **4** as a yellow oil (371 mg, 55% over 2 steps). ^{1}H NMR ($CDCl_3$, ppm)δ 10.23 (s, 1H), 8.12 (bs, 1H), 8.00-7.95 (m, , 1H), 7.78-7.64 (m, 1H), 7.51 (bs, 1H), 7.45-7.38 (m, 1H), 7.30 (bs, 1H), 4.36 (q, J = 7.3 Hz, 2H), 4.21 (t, J = 7.3 Hz, 2H), 2.90 (s, 4H), 1.82-1.70 (m, 2H), 1.58-1.40 (m, 9H), 1.32-1.18 (m, 9H), 1.10-0.95 (m, 5H), 0.83 (t, J = 7.3 Hz, 6H). ^{13}C NMR ($CDCl_3$, ppm)δ169.7, 162.5, 143.6, 143.2, 138.1, 136.7, 130.1, 128.4, 124.5, 122.0, 50.1, 45.3, 33.5, 30.2, 29.0, 27.3, 26.5, 25.9, 25.8, 15.7, 13.7, 9.8. HRMS (FAB) calcd. for $C_{30}H_{46}N_3O_4Sn$ 632.2505 $[M\text{-}Br]^+$; found 632.2522.

1-(6-(dibutyl(3-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)phenyl)stannyl)hexyl)-3-ethyl-1H-imidazol-3-ium hexafluorophosphate 5. Compound **4** (110 mg, 0.154 mmol, 1eq) was dissolved in acetone (4 ml) and stirred with $NaPF_6$ (52 mg, 0.308 mmol, 2 eq) at room temperature for 24 h to exchange the anion. The reaction mixture was filtered then solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 90:10) to afford compound **5** as viscous yellow oil (81 mg, 67%). ^{1}H NMR ($CDCl_3$, ppm) δ 8.50 (s, 1H), 8.16 (bs, 1H), 8.01 (bd, J = 7.2 Hz, 1H), 7.75 (bd, J = 7.2 Hz, 1H), 7.49-7.42 (m, 1H), 7.32 (bs, 1H), 7.23 (bs, 1H), 4.18 (q, J = 7.3 Hz, 2H), 4.06 (t, J = 7.3 Hz, 2H), 2.90 (s, 4H), 1.79-1.67 (m, 2H), 1.58-1.45 (m, 9H), 1.38-1.21 (m, 9H), 1.13-1.00 (m, 5H), 0.87 (t, J = 7.3 Hz, 6H). ^{13}C NMR ($CDCl_3$, ppm)δ170.0, 162.7, 143.8, 143.3, 138.2, 135.0, 130.2, 128.5, 124.6, 122.4, 122.3, 50.2, 45.4, 33.4, 30.0, 29.2, 27.5, 26.6, 25.9, 25.8, 15.2, 13.9, 9.9. HRMS (FAB) calcd. for $C_{30}H_{46}N_3O_4Sn$ 632.2505 $[M\text{-}PF_6]^+$; found 632.2484.

Ethyl 3-[I-125/127]iodobenzoate [I-125/127]-6. To NaI (1 µl, 26 nmol. including 1.2 pmol (100 kBq) of [I-125]NaI) in NaOH 0.048 M was added NCS (8.7 µl, 130 nmol.) in MeOH/AcOH (95/5). The solution was stirred 30 s at 20°C. **3** (20 µl, 130 nmol) in MeOH/AcOH (95/5) was then added. After 5 minutes stirring, the radioiodinated ethyl iodobenzoate [I-125/127]-**6** was obtained in 98% RCY. The solution was evaporated to dryness and the crude product was recovered in 400 µl of diethyl ether. After filtration using a silica gel cartridge and diethyl ether as eluent, the product [125/127I]-**6** was obtained with a good radiochemical purity (RCP: 100%). Rf ($CHCl_3$) : 0.75, t_R: 15.3 min.

Succinimidyl 3-[At-211]astatobenzoate [At-211]-8. To astatine (50 µl, 4.2 MBq) in methanol was added to NCS (4µl, 81 nmol.) in MeOH/AcOH (95/5). The solution was stirred 30 s at 20°C. **5** (20 µl, 130nmol) in MeOH/AcOH (95/5) was then added. After 30 minutes stirring, 2 µl of an aqueous solution of sodium metabisulfite (20 mg/ml) were added. The succinimidyl m-astatobenzoate [At-211]-**8** was obtained (64% RCY). The solution was evaporated to dryness and recovered in 400 µl of diethyl ether. After filtration using a silica gel cartridge and diethyl ether as eluent, the product [At-211]-**8** was obtained with a good RCP (90%). Volatiles were evaporated under argon and the purified [At-211]SAB was obtained as a dry residue ready for the coupling to the vector. Rf ($CHCl_3$ /AcOEt9 :1) : 0.4, t_R: 15.3 min.

Succinimidyl 3-[I-125]iodobenzoate [I-125]-9. To NaI (1 µl, 46.2 pmol (3.7 MBq) of [I-125]NaI) in NaOH 0.048 M was added NCS (4 µl, 81 nmol.) in MeOH/AcOH (95/5). The solution was stirred 30 s at 20°C. **5** (20 µl, 130 nmol) in MeOH/AcOH (95/5) was then added. After 30 minutes stirring, the radioiodinated succinimidyliodobenzoate was obtained (67 % RCY). The solution was evaporated to dryness and the crude product was recovered in 400 µl of diethyl ether. After filtration using a silica gel cartridge and diethyl ether as eluent, the product was obtained with a good RCP (100%). Volatiles were evaporated under argon and the purified [I-125]-**9** was obtained as a dry residue ready for the coupling to the vector. Rf ($CHCl_3$ /AcOEt 8:2) : 0.5.

[At-211]9E7-SAB. The mAb 9E7 (50 µl, 22 µM in 300 mM borate buffer pH 8.6) was added to the dry [At-211]-**8**. The solution was stirred 30 min at 20°C. The coupling yield was determined by ITLC-SG (76%). The radiolabeled BSA was then purified on NAP-5 cartridge eluted with PBS/BSA 0.5% (RCP (ITLC-SG) > 90%).

Radioimmunoassay for the radiolabeled mAb-9E7[21]. **R**adioimmunoassay was assessed using magnetic beads grafted with the synthetic mousse CD138 peptide (SNTETAFTSVLPAGEKPEEGEPVLHVEAEPGFTARDKEKE-Ahx-C-NH_2). 2 µL of astatinated 9E7 (mouse anti-CD138) were diluted with 1 mL of PBS/BSA 0.5%. Series of 20 µL (200 µg) aqueous suspension of grafted magnetic beads were aliquoted in an Eppendorf vial, washed 4 times with PBS/BSA 0.5% and then suspended in 120 µl of PBS/BSA. 50 µL of the diluted solution of astatinated 9E7 (0.45 pmol) were then added and stirred for 1 h. at 20°C (A). Supernatants were separated and magnetic beads are washed twice with 200 µL of PBS/BSA 0.5%. Finally, 600 µL of PBS/BSA 0.5% were added to the washed magnetic beads (B). Activities contained in (A) and (B) were determined using a γ-counter (Wallac 1480-Wizard®3, Perkin-Elmer, Paris) and immunoreactivity was calculated as the ratio between (B) and (A). The [At-211]SAB-9E7 exhibits a good immunoreactivity (78%).

[I-125]Di-HSGL-BSA-SIB. Di-HSGL-BSA[22] (40 µl, 20 µM in Borate buffer pH 8.6 300 mM) was added to the dry [I-125]-**9**. The solution was stirred 30 min at 20°C. The coupling yield was determined by ITLC-SG (54%). The radiolabeled BSA was then purified by size-exclusion chromatography using a NAP5 column (Sephadex G25, Bio-Rad) eluted with PBS/BSA 0.5% (RPC (ITLC-SG) > 90%).

Radioimmunoassay for the radiolabeled Di-HSGL-BSA. Two concentrations of [I-125]Di-HSGL-BSA-SIB were tested and each test was duplicated. Radioimmunoassay was assessed using polypropylene tubes (MAXISORP, VWR) coated with 10 µg (66 pmol.) of mAb-679 (anti-HSG).[23] After washing propylene tubes 3 times with 500 µL of PBS/BSA 0.5%, the radiolabeled protein (40 or 80 fmol.) diluted in 500 µL of PBS/BSA 0.5% was added (A). The solution was incubated at 37°C for 1 h. Supernatants were separated and coated PP tubes are washed twice with 500 µL of PBS/BSA 0.5%. Finally 500 µL of PBS/BSA 0.5% were added. Activities contained in (A) and radioactivity fixed in the coated polypropylene tube (B) were determined using a γ-counter (Wallac 1480-Wizard®3, Perkin-Elmer, Paris). Immunoreactivity was calculated as the ratio between (B) and (A). The radiolabeled BSA-Di-HSGL exhibits a good immunoreactivity (70%).

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