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Automated synthesis of N-succinimidyl 3-[^{211}At]astatobenzoate (SAB) for antibodies radiolabelling.

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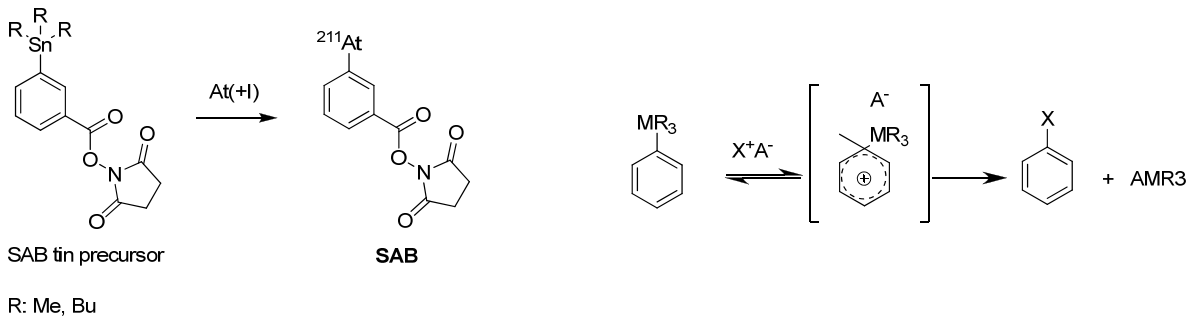
Introduction

Astatine-211 is one of the most promising alpha emitters for micrometastatic diseases treatment considering its decay and physical properties[1,2]. As micrometastatic disease often present specific or overexpressed receptors on abnormal cells, monoclonal antibodies can be selected as vectors for astatine[3]. As mAb direct halogenation led to rapid deastatination *in vitro* and *in vivo*[4], radiolabelling with astatine-211 can be achieved using a prosthetic group (radiolabelling and conjugation to the vector). Succinimidyl astatobenzoate (SAB) is one of the most commonly used prosthetic group[5,6] (**Scheme 1**). This compound is synthesized from a stannyl precursor bearing an activated ester using the electrophilic At(+I) species for an halodemetallation reaction.



Scheme 1: SAB synthesis and halodemetallation mecanism.

As the SAB prosthetic group is conventionnaly used in our group for antibodies radiolabelling, we propose to develop an automated synthesis of this prosthetic group using the Synthera automated system (IBA).

Materiel and methods

(1) Zalutsky MR, McLendon RE, Garg PK, Archer GE, Schuster JM, Bigner DD [1994] Cancer Res. 54: 4719-4725.
(2) Lucignani G [2008] Eur. J. Nucl. Med. Mol. Imaging 35: 1729-1733.
(3) Zalutsky MR, Reardon DA, Pozzi OR, Vaidyanathan G, Bigner DD [2007] Nucl. Med. Biol. 34:779-785.
(4) Chérel M, Davodeau F, Kraeber-Bodéré F, Chatal JF [2006] Q. J. Nucl. Med. Mol. Imaging 50: 322-329.
(5) Zalutsky MR, Narula AS [1988] Appl. Radiat. Isot. 39: 227-232.
(6) Bourgeois M, Guerard F, Alliot C, Mougin-Degraef M, Rajerison H, Remaud-Le Saëc P, Gestin JF, Davodeau F, Chérel M, Barbet J, Faivre-Chauvet A, [2008] J. Label. Compd. Radiopharm. 51: 379-383.

The non-carrier added astatine has been distillated from a bismuth-209 target and trapped in a methanol solution. 70 to 90% of the radioactivity was isolated in methanol. Minimal volumes (reagents vials and reactor) for proper use of the Synthera system have been firstly determinated. Then SAB reaction conditions have been optimized from non-automated synthesis and then adapted for automatisation. Optimal reaction conditions have been determinated to be 45.7 µl of N-chlorosuccinimide (2.7 mg/ml in methanol/acetic acid (95:5)) as oxidant for 500 µl astatine (4-20 MBq) and 70.3 µl of stannic precursor (1 mg/ml in methanol/acetic acid (95:5)). The separation of SAB from tin precursor is conventionnaly obtained using HPLC purification (Silica column, Heptane/AcOEt (80:20)). As no HPLC system is integrated on the Synthera automatizer, the SAB is obtained as a ready-to-inject solution (for HPLC) at the end of the sequence using a separated HPLC system. This sequence has been optimized and divided into 6 steps: astatine sample and transfer, reagents adding, reaction, solvent (methanol/acetic acid) evaporation, solubilization in dichloromethane, tranfer to HPLC vial and finally partial evaporation of dichloromethane. Radiochemical yield has been controlled after the reaction step (TLC on Silica gel, $CHCl_3$/AcOEt (9:1)) and radiochemical purity was measured by HPLC.

Results

Transfer loss during reagents adding has been estimated to 50 µl. This result led us to use a pre-mixed solution of tin precursor and N-chlorosuccinimide in the same vial. The minimal volume of astatine has been estimated to 500 µl, as this volume is needed to avoid neat reactions. Using optimal reaction conditions, SAB was synthesized in acceptable radiochemical yield (65-70% vs. 65-85% for non-automated synthesis) in 30 min. at 60°C. Similar global yields, for the sequence from the astatine sample to the ready-for-coupling SAB, have been obtained for non-automated and automated synthesis (respectively 37.0 ± 11.7 % (n = 9) and 35.0 ± 2.5 % (n = 3)).

Discussion/Conclusion

This study highlights the efficiency of automated SAB synthesis. Reaction conditions for automation are close to the “conventional” conditions. Similar global yields are obtained for automated and non-automated synthesis. However the automated synthesis is more reproducible. Next investigations will concern the development of a fully-automated system with an integrated HPLC-based purification system.