# Synthesis of [$^{211}$At]4-astato-L-phenylalanine by dihydroxyboryl-astatine substitution reaction in aqueous solution†

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Radiopharmaceutical therapy, including targeted alpha-particle therapy (TAT), has recently emerged as a novel therapeutic modality for the treatment of tumor. We previously demonstrated the utility of [$^{211}$At]4-astato-L-phenylalanine ([$^{211}$At]APA) for the treatment of glioma in tumor-bearing mice by means of TAT.[1] [$^{211}$At]APA specifically accumulates in tumor cells and are transported by the LAT1 transporters, which are predominantly expressed on the surface of various tumor cells including the glioma cells. In a mouse model xenografted with rat glioma cells, [$^{211}$At]APA significantly decreased the tumor volume after a single injection of the agent (dose range: 0.1–1.0 MBq/mouse) in the mouse.

Phenylalanine has been previously labeled with $^{211}$At using different methods.[2,3] However, most of the existing reactions for the synthesis of radiolabeled compounds either require the use of toxic and hazardous chemicals or are unable to yield a carrier-free final product.

We have developed a new and improved method for the preparation of [$^{211}$At]APA using 4-borono-L-phenylalanine (BPA) as the starting molecule. [$^{211}$At]APA was synthesized by the electrophilic substitution of $^{211}$At for a dihydroxyboryl (or borono) group on an aromatic ring of the corresponding precursor molecule BPA using N-bromosuccinimde (NBS; NBS method) as an oxidant or KI (KI method) as shown in Fig. 1. The radiochemical yield (RCY) and radiochemical purity (RCP) were better with the latter method. The reagents and compounds used for the synthesis of [$^{211}$At]APA were commercially approved drugs and physiologically relevant for clinical use. The entire synthesis could be accomplished in aqueous media; no organic solvents or toxic metals were required, suggesting that this method is relevant for practical applications. We also elucidated the differences between the chemical properties of iodine and astatine for the labeling reactions.

Based on our findings, the reaction scheme for the $^{211}$At labeling of arylboronic acids is depicted in Fig. 2. In the NBS method, $^{211}$At initially reacts with NBS to form the [$^{211}$At]astatosuccinimide intermediate (Fig. 2, top). Then, [$^{211}$At]astatosuccinimide reacts with the arylboronic acid to produce [$^{211}$At]arylastatide. In the KI method, $^{211}$At reacts with KI to form the [$^{211}$At]AtI and/or $AtI_2^-$ intermediate (Fig. 2, bottom). Several

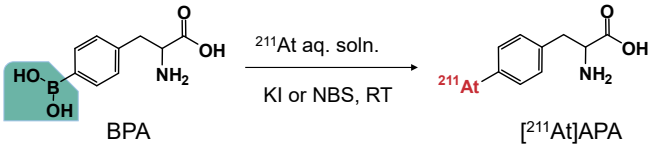


Fig. 1. Radioastatination reaction of boronophenylalanine.

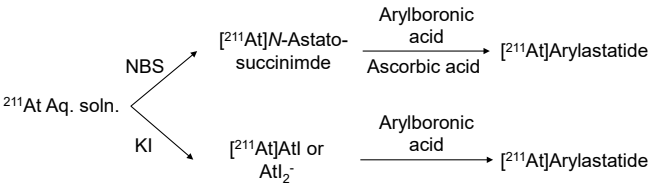


Fig. 2. Mechanism of astatination of arylboronic acids.

studies have suggested that astatine can react with iodide and produce inter-halogen compounds such as AtI and $AtI_2^-$.[4] The AtI and/or $AtI_2^-$ intermediate reacts with the arylboronic acid to give [$^{211}$At]arylastatide. Compared to NBS, KI was found to be more efficient for the substitution of the dihydroxyboryl group by astatine in the aqueous media. KI can probably reduce the hypervalent $^{211}$At species present in the aqueous solution (∼10%), forming the reactive [$^{211}$At]AtI and/or $AtI_2^-$ intermediate that allows higher RCYs compared to that obtained using the NBS method.

KI was originally intended to be used as the carrier atom of $^{211}$At since there are no natural stable isotopes of astatine. Unexpectedly, the radiolabeling yield of [$^{211}$At]APA using KI was much higher than 95%, due to the formation of [$^{211}$At]AtI and/or $AtI_2^-$ as a reaction intermediate in the aqueous solution.

It is well known that tyrosine can be labeled with radioactive iodine via an electrophilic substitution reaction in the presence of an oxidant, such as chloramine T, Iodogen, *N*-chlorosuccinimde, NBS, or $H_2O_2$. Astatination of tyrosine, however, gave a low yield of [$^{211}$At] astatotyrosine (<15%) with $H_2O_2$, and the product was unstable under neutral and basic conditions. Additionally, the labeling yield was low. These results were suggestive of the different chemical properties of astatine and iodine.

In summary, we developed a novel method for the synthesis and purification of [$^{211}$At]APA, which has potential clinical uses.

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