At-211 labeling of a somatostatin analog TATE via *closo*-decaborate for targeted alpha antitumor therapy

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Targeted alpha therapeutics are being researched and developed those conjugate alpha emitters with antibodies and peptides, as a new drug modality for cancer treatment. Among alpha emitters, At-211 is one of the safest and most promising nuclides for clinical use because of its simple, one-step decay process and not too long half-life (7.21 h). In At-211 labeling chemistry, various prosthetic groups have been proposed; by analogy with robust boron-iodine bonds, boron-astatine bonds in boron clusters have been shown to be particularly useful.[1] Most boron cluster-mediated At-211 labeling has been conducted for antibodies, and reports of labeling for peptidic ligands are very limited.

Tyr^3-octreotate (TATE), a somatostatin analog, is a peptidic ligand with high affinity for somatostatin receptor 2 (SSTR2). [^{68}Ga]Ga-DOTA-TATE has been approved by the U.S. Food and Drug Administration as a positron emission tomography probe for neuroendocrine tumors that highly express SSTR2,[2] and TATE is also expected to be used in targeted alpha antitumor therapy. In this report, we describe the At-211 labeling of TATE via *closo*-decaborate.

Azide-modified TATE and *closo*-decaborate were conjugated by DBCO-mediated strain-promoted azide-alkyne cycloaddition (SPAAC) to form a precursor (B10-TATE, Fig. 1). At-211 oxidized with chloramine T was reacted with the precursor to label At-211 at the B10 site. The labeling efficiency and side reactions were evaluated using a germanium semiconductor detector and a radio-high performance liquid chromatography (radio-HPLC). Comparative experiments were performed using TATE without B10 and bifunctional prosthetic group (B10-DBCO) as control compounds, and I-131 as a control radionuclide of the same halogen family. The following findings were obtained regarding the characteristics of the B10-mediated At-211 labeling reaction.

(1) Lower reactivity of At-211 to B10 and other groups than I-131: When B10-TATE was labeled with I-131 at a ratio of approximately 1 : 1500, the radiochemical yield was around 80% and many peaks were seen in radio-HPLC. TATE without B10 was also labeled when reacted with I-131. These results are in good agreement with the known binding of I-131 to the side chain of tyrosine.[3] In contrast, in the reaction with At-211, B10-TATE was labeled in approximately 40% radiochemical yield, but TATE was not. Finally, [^{211}At]At-B10-TATE was obtained with a molar radioactivity of around 10 MBq/nmol.

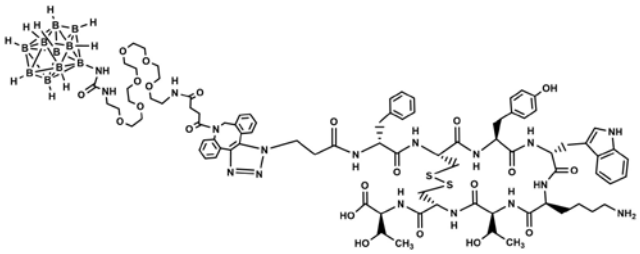

Fig. 1. Structure of *closo*-decaborate-conjugated Tyr^3-octreotate (B10-TATE).

(2) Isomer formation in the labeling to B10: In the reaction of B10-DBCO with I-131, three peaks with retention times assumed to be those of the labeled substances were observed in radio-HPLC. A similar result was obtained in the reaction with At-211, suggesting that isomers may be formed in the labeling of halogens to B10.

(3) Disapproved use of sodium metabisulfite to avoid ring-opening of macrocyclic peptides by disulfide reduction: The standard protocol for At-211 labeling of B10 with chloramine T includes stopping the reaction by adding a reducing agent, sodium metabisulfite ($Na_2S_2O_5$).[4] However, it was found that the disulfide of TATE was reduced in this process, causing the macrocyclic peptide to open its ring. Similar side reactions can occur with antibodies, and caution should be exercised because the higher-order structure of antibodies can be destroyed. Since chloramine T was removed in the purification by reversed-phase solid-phase extraction immediately afterwards, the solution was to skip this process. The addition of ascorbic acid for radiolysis prevention was not a problem.

(4) No SPAAC reactivity of previously At-211-labeled B10-DBCO with azide-modified TATE: The SPAAC reaction did not proceed when At-211 was added, while proceeded when not added, suggesting that the reaction was likely inhibited by the binding of At-211 to DBCO.

(5) Protection of [^{211}At]At-B10-TATE from radiolysis by cryopreservation: The addition of ascorbic acid alone did not sufficiently suppress radiolysis during overnight storage. We found that freezing the solution and storing at -80°C significantly suppressed radiolysis of [^{211}At]At-B10-TATE.

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References

1) M. Vanermen *et al.*, EJNMMI Radiopharm. Chem. **9**, 69 (2024).
2) A. Mullard, Nat. Rev. Drug Discov. **15**, 523 (2016).
3) E. B. de Araújo *et al.*, Appl. Radiat. Isot. **67**, 227 (2009).
4) D. S. Wilbur *et al.*, Bioconjug Chem. **22**, 1089 (2011).

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