User project report

User project identifier: 1729007705_cw4c6

User project title: Novel prosthetic groups for the ^{211}At-astatination of biomolecules via disulphide rebridging

Main contact of the user group: Dr A. Krzyczmonik

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1. Authors

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2. Context of the project (800 characters max. including spaces)

We have designed novel prosthetic groups for radioiodine and astatine-211 which can be used for the modification of biomolecules *via* disulphide rebridging. Disulphide rebridging is a new strategy for prosthetic group conjugation[1,2]. Most medically relevant biomolecules contain at least one disulphide bridge available on the solvent accessible surface. The disulphide bridges are formed between two cysteine moieties and play a crucial role in the stability and biological activity of biomolecules. As a preparation for the project a series of prosthetic group precursors was prepared. During this project we tested several labelling strategies. The most traditional approach being electrophilic radioastatination with stannylated precursor, or direct radioastatination.

3. Results and discussion (1000 characters max. including spaces)

During the project were provided with At-211 for the labelling of the prepared prosthetic groups for disulphide rebridging. Boronic esters precursors were tested in presence of reducing agent (0.1M NaOH in either MeOH or water solution), $Cu(py)_4(OTf)_2$ and 1,10-phenantroline n MeOH/MeCN solution. Reactions were carried out for 30 min in room temp and later analysed with radioTLC and radioHPLC. All boronic esters gave a high RCY of 71-87%.

For electrophilic reactions with stannylated precursors 3 different oxidizing agents were used: NCS, NIS and Iodogen with either AcOH or TFA. Reactions were carried out for 30 min in room temp. All reactions were analysed with radioTLC and the most promising one were also analysed with radioHPLC. From the performed reaction the best results were obtained with NIS in MeOH with addition of AcOH and resulted in RCY of over 90 % for both precursors.

Direct astatination of the compound 7 showed only slight conversion with hydrogen peroxide and in Cu catalyzed halogen exchange at high temperature. This indicates the substrate is unsuitable for direct astatination, and activation *via* a boronic ester or stannylated intermediate is needed to enhance reactivity.

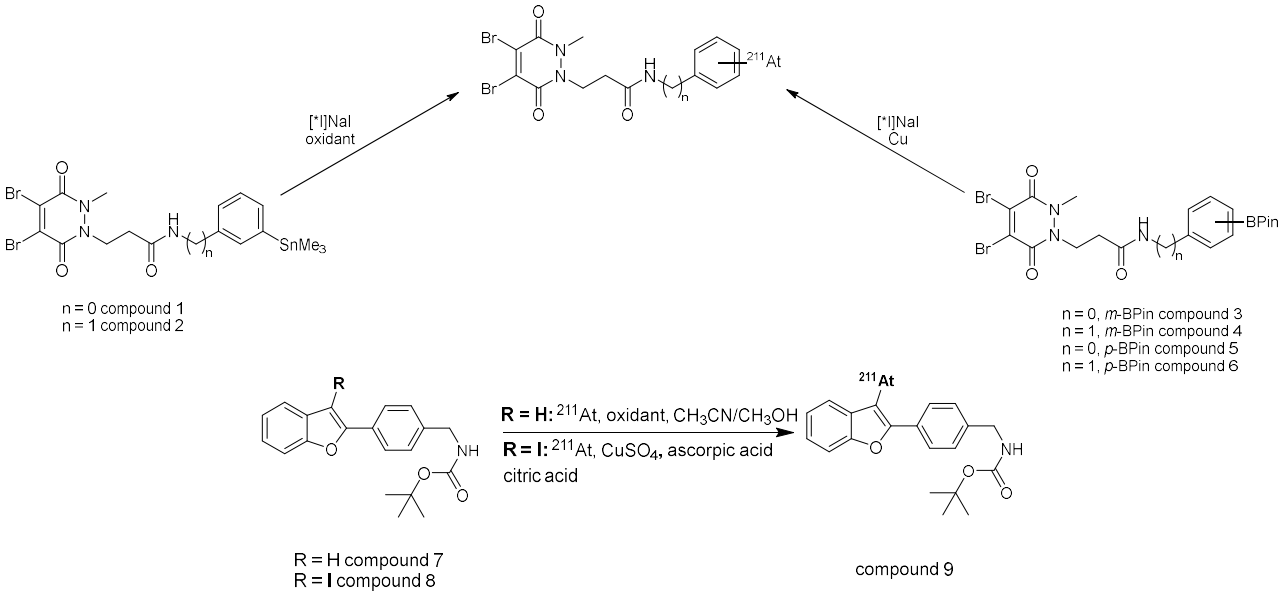

Figure 1. Radioastatination reactions tested during PRISMAP project

4. Conclusions (800 characters max. including spaces)

Radioastatination of most of the proposed prosthetic groups was very successful. Both, boronic ester and stannylated precursors provided high radiochemical yield with a very simple synthesis strategy. The direct approach did not provide a desired product with various reaction conditions. For further development a corresponding boronic ester will be prepared for radioastatodeboronation according to the procedure applied for other precursors. Developed prosthetic groups will be further used for the labelling of octreotide via disulphide rebridging.

Table 1. Labelling results for compounds 1-6

Compound	TLC RCY (%)	HPLC RCY (%)
1	94	92
2	95	90
3	71	75
4	87	82
5	82	85
6	78	73

5. Involvement of the PRISMAP services (600 characters max. including spaces)

Through the PRISMAP initiative, we were granted access to astatine-211. Due to the relatively short half-life of ^{211}At (7.21 hours), transporting the isotope from France to Poland was impractical. Consequently, the experiments planned within the PRISMAP project were carried out directly at the site of astatine production at ARRONAX in France. This arrangement not only provided us with the opportunity to work with a new and valuable radionuclide, but also allowed us to greatly benefit from the expertise and support of the ARRONAX staff.

6. Feedback to PRISMAP (600 characters max. including spaces)

Our experience from start to finish was very positive. Contact with our partner institute ARRONAX was excellent, and the people who supported us were very helpful and understanding. As far as access to the isotope and the facility is concerned, the whole process went extremely smoothly, and the institute's staff were exceptionally helpful and supportive. We believe that one of the benefits of the project will be further collaboration between our institutes.

7. Publications and other dissemination activities (conferences etc.)

Authors intend to use the results obtained in the project for conference presentation, as well as, for the publications however, they have not yet been prepared .

Appendix 1. Dissemination guidelines for user projects as agreed in the signed User Agreement

Dissemination rules

Only user groups that are allowed to disseminate the results which they have generated under the project may benefit from the access, unless they are working for SMEs.

For each user group project, a publishable project summary and a publishable summary of the results will be published on the European Union Horizon 2020 PRISMAP project website www.prismap.eu. The publication of results in journals or at conferences is strongly encouraged.

To ensure the long-term sustainability of the PRISMAP initiative, proper recognition of the contributing facilities, their services and the involved persons is necessary. All participating PRISMAP facilities shall be acknowledged in the publication. Acknowledgement and co-authorship of PRISMAP staff members who participated in the experiment shall be considered according to the research field best practices and verified with the PRISMAP Technical Manager before any publication.

The user group shall contact the PRISMAP Technical Manager 30 days prior to submission of publications or other communications of results that were obtained by making use of services provided by PRISMAP (radionuclides delivered or medical services provided). The Technical Manager will communicate to the user group the list of PRISMAP facilities and persons that have contributed to each specific project and the way this contribution must be acknowledged in the publication/communication or where co-authorship is required to reflect specific scientific contributions.

Users must comply with Horizon 2020 dissemination rules (i.e. acknowledge that their work was financially supported by the European Union's Horizon 2020 Research and Innovation Programme by including the following acknowledgement: "This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008571 (PRISMAP)"), and grant open access to resulting publications and related data.

Dissemination shall take place only once legitimate interests regarding intellectual property have been safeguarded. A maximum publication delay of 90 days may be granted for this purpose.

Acknowledgements

The list of name(s) to be mentioned in the acknowledgment section is sent to the technical manager by the main contact of the involved facilities.

A general sentence will be added by the corresponding author of the article (user side):

"The authors would like to thank the members of the PRISMAP consortium and of the PRISMAP user selection panel, coordination and management team for their advice and support."

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