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Review of the Doctoral Thesis of M.S. Patrycja Mała

Author: **Patrycja Mała**

Title: **"Self-promoted Glycosylations with Trichloroacetimidate Glycosyl Donors: Synthesis of N-glycosides"**

Thesis Supervisor: Prof. Henryk Koroniak

Thesis Supervisor: Assoc. Prof. Christian Marcus Pedersen

By the resolution of the Scientific Council of the Chemical Disciplines of the Adam Mickiewicz University in Poznań on June 23, 2023, I was appointed a reviewer of the doctoral dissertation entitled "Self-promoted glycosylations with trichloroacetimidate glycosyl donors: synthesis of N-glycosides", whose author is M.S. Patrycja Mała. This dissertation was carried out in cooperation between two research centres Adam Mickiewicz University in Poznan and the University of Copenhagen under the supervision of Professor Henryk Koroniak (Adam Mickiewicz University in Poznan) and Assoc. Prof. Christian Marcus Pedersen (University of Copenhagen) within the international and interdisciplinary program ChemInter co-financed by the European Union.

The subject of the reviewed work focuses on the development of a novel synthetic approach toward N-glycosides containing amide, sulfonamide, and carbamate functions. The designed route involves the N-glycosylation step, which was the central subject of this study and the orthogonal protection strategy. In the literature, many methods of obtaining glycoconjugates are described and developed. In my opinion, the development of a protocol for the synthesis of N-glycosides has so far been a challenge for scientists primarily due to the reaction yields, as well as chemoselectivity and stereoselectivity. Unfortunately, the literature describes only synthetic methods for obtaining glycoconjugates, which use, for example, as catalysts, promoters, or scavengers, and in the case of some synthetic procedures. It is also necessary to

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use drastic reaction conditions. It is contrary to the principles of green chemistry which is one of the forms of action aimed at reducing environmental threats and is an important element of sustainable development. Therefore, one of the most important goals of the long-term chemistry strategy for sustainable development is to achieve zero environmental pollution - free of toxic substances - announced in the European Green Deal. On this basis, I believe that there is a need for research aimed at developing practical methods for the synthesis of N-glycoconjugates by the principles of green chemistry.

The doctoral dissertation of M.S. Patrycja Mała is written in English. It contains 249 pages and follows the classic structure of a doctoral dissertation in exact sciences. The work includes abstract, acknowledgements, Author information, table of contents, abbreviations, chapter 1 (pages 1-29), goals of research (page 30), chapter 2 (pages 31-118), chapter 3 (pages 119- 217) and references.

When evaluating the presented work, I will first refer to the objectives of the work. The main aim of the work was to develop an efficient and stereoselective approach involving self-promoted glycosylation and the orthogonal protection strategy for the preparation of selectively functionalized N-glycosides.

The main objective of the doctoral dissertation has been extended with specific objectives, which I have listed below:

Objective 1: Investigate whether the N-sulfonyl carbamates and N-sulfonyl amides can activate the acid-labile TCA glycosyl donors in the presence of no additives.

Objective 2: Establish the optimal glycosylation procedure and study its generality and versatility by testing various TCA glycosyl donors and electron-poor acceptors. At this point, the author tried to assess how different structural features of the reactants, such as size, stereochemistry and particular functional groups influence the reaction rate, yield, and anomeric ratio.

Objective 3: Chemoselective removal of the sulfonyl and carbamate functions (N-glycosides)

Objective 4: Determine the structures and conformations of the obtained products using different spectroscopic methods.

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After a thorough reading of the doctoral dissertation by M. S. Patrycja Mała, I can say that the assumed main goal together with specific goals have been clearly articulated and fully achieved.

In Chapter 1, the PhD student introduces us to the world of glycosylation reactions and N-glycosides. Glycosylation is an irreversible enzymatic reaction involving the addition of sugar residues to proteins and lipids. Thanks to the glycosylation process, for example, proteins have additional properties that enable them to perform their functions. N-glycosides, on the other hand, are one of the main classes of carbohydrate derivatives that are widely distributed in nature in the form of N-glycoconjugates. N-glycoconjugates are the most structurally diverse group of compounds in living organisms and play a very important role in many biological processes, e.g., viral infections). In Chapter 1, the PhD candidate also described trichloroacetimidate glycosyl donors including commonly utilized thioglycosides, glycals, glycosyl halides, esters, orthoesters, phosphates and imidates. In this part of the doctoral dissertation, I paid particular attention to the description of N-glycosides as important targets in organic synthesis. I read this chapter with great interest.

In the second chapter entitled: "Self-promoted glycosylation: synthesis of N-glycosyl sulfonamides and carbamates" and in the third chapter entitled "Self-promoted glycosylation: synthesis of N-glycosyl sulfonyl amides" the PhD student describes the results of her research. The obtained results of the research show that the PhD student has developed a protocol for the synthesis of N-glycosides with an amide, sulfonamide, and carbamate group. Based on the experimental research on the synthesis of N-glycosyl sulfonamides and carbamates, the PhD student concluded that N-glycosides are formed in two key steps: the first - the self-promoted N-glycosylation with trichloroacetimidate (TCA) glycosyl donors and the second - the orthogonal deprotection. Particularly noteworthy is the fact that during the N-glycosidic bond formation stage, the PhD student showed that electron-poor acceptors can efficiently activate acid-labile TCA glycosyl donors giving rise to N-glycosyl sulfonyl carbamates. The advantage of this method is the lack of the use of additional factors, e.g., catalysts or promoters. In the literature, such reactions are known, which mainly occur with the use of catalysts or promoters. I consider it a great research success for the PhD student. Other advantages of this synthesis method can also be mentioned: e.g., the process takes place at room temperature in a solvent -

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dichloromethane, take place complete β-stereospecificity when using the α-glucosyl donor and the stereochemistry of the starting material is preserved during the Alloc and tosyl cleavage. However, each experimental method has advantages and disadvantages. The following can be considered disadvantages: synthesis of the acceptors involves toxic and unstable isocyanates, and the glycosylation and deprotection reactions require dry conditions and poor yield of the glycosylation.

In Chapter 3, the PhD student describes the synthesis of N-glycosyl sulfonyl amides. In glycosylation, the PhD student used TCA glycosyl donors and sulfonyl amides. It is worth noting that this reaction does not require use of catalysts, because the electron-poor acceptors can act as activators of TCA donors. Other advantages of using this method of synthesis that should be emphasized are primarily the fact that the glycosylation process takes place in various conditions and high β-selectivity can also be observed when using the α-donors. On the other hand, this process takes place at high temperatures and dry conditions, which is a major limitation of this method. In summary, N-linked glycosylation is an example of a self-promoted reaction, i.e., without the need for additives. The results of the obtained studies indicate that the preparation of selected N-glycosides takes place with very good yield and high stereoselectivity. In my opinion, this is a great success for the PhD student. In the research work, the use of several research methods by the PhD student deserves special attention {^{1}H and ^{13}C-NMR, Infrared spectroscopy (IR), Total Correlation Spectroscopy (TOCSY), 1D and 2D gradient-selected NOESY} which are a valuable source of information in the design of the proposed methods synthesis of N-glycosides.

It is also worth paying attention to the development of research results by a PhD student. In the work, it is supported by data presented in the form of tables and charts, which makes the work more visual and less monotonous for the reader. The analysis and description of the issues contained in the work indicate that its Author uses the nomenclature in the field of chemistry well.

In her doctoral thesis, the PhD student supported herself with 236 publications, the vast majority of which are publications from the Philadelphia list. In my opinion, this proves the great ability of the author of the doctoral dissertation to use the rich literature on the subject.

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The latest publications published in the last few years constitute a significant part of the entire literature, which is a fully satisfactory indicator of the current high dynamics of the circulation of knowledge in science. In terms of quality, the literature is selected correctly and is also varied and rich.

It is also worth drawing attention to the fact that the author can conduct scientific work independently and interpret the obtained results. However, while reading the doctoral dissertation, a few questions and comments came to my mind, which are as follows:

1) Page 64 - What methods was the product phenyl 1-thio-α-D-mannopyranoside identified? In the same point, the author writes "*The filtration and evaporation of solvent afforded the crude product 2.10α as a white solid which was used in the next step without further purification.*" Why was the compound not purified and how will this fact affect the reaction yield?

2) Page 138 - On the scheme the Author wrote "solvent (0.05 M)". How was the molar concentration of the solvent determined?

3) Page 140 - The PhD student writes: "*Nevertheless, it was found that higher reaction temperatures generally favored the β-linked O- and N-glycosides.*" Why high temperature of the reaction favors formation of the β-form rather than the α-form? Perhaps for this purpose, it would be worthwhile to perform additional research, e.g., theoretical calculations? I am very interested in PhD students' opinions on this topic.

4) Page 140 - How were the reactant equivalents determined? Have optimization studies been carried out?

5) Page 145 - (Figure 3.11A). What is the peak at about 5.25 ppm?

6) Are the proposed synthesis methods of N-glycosyl sulfonamides and carbamates and N-glycosyl sulfonyl amides more environmentally friendly/green chemistry compared to existing methods well described in the literature? I am very interested in PhD students' opinions on this topic.

7) Has there been a tendency to increase the yield of the reaction depending on the viscosity of the solvents used? In the case of glycosylation reactions, the rate of diffusion of substrates (donor and acceptor monosaccharides) to the active site of the enzyme is important. If the solvent is highly viscous, it makes the diffusion of molecules in the solution much more

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difficult. This, in turn, may lead to limited access of substrates to the enzyme and lower efficiency of the glycosylation reaction. If the solvent is highly viscous, it can make mixing difficult and lead to the inhomogeneous distribution of the reactants, which can negatively affect the efficiency of the glycosylation reaction.

I am asking a PhD student to provide answers to the above questions.

To sum up, I believe that the doctoral thesis by M.S. Patrycja Mała is a work of significant cognitive and application value. Gathering such rich research material required a lot of work on the part of the PhD student. I can say with all conviction that the research carried out and presented in the work expands the interdisciplinary knowledge in the field of research on the broadly understood synthesis of N-glycosides. I would also like to emphasize the high scientific importance of the research carried out by the PhD student and I rate the doctoral dissertation very highly. It contains a lot of valuable and original research results, which significantly expand the current state of knowledge in the researched area.

Finally, I would like to emphasize that M.S. Patrycja Mała is a co-author of 4 scientific articles which were published in specialist international journals (referred to in the ISI Web of Knowledge database) with high influence factors, for example, Journal of Photochemistry and Photobiology A: Chemistry (IF = 3.8), Chemical Science (IF = 8.6), European Journal of Organic Chemistry (IF = 2.5), Journal of Medicinal Chemistry (IF = 7.3). During her scientific career, she has presented her work at several international and domestic conferences.

In my opinion, the doctoral dissertation by M.S. Patrycja Mała is an original solution to a scientific problem and proves the PhD student's general theoretical knowledge in chemistry, as well as the ability to independently conduct scientific work, as well as the ability to formulate and solve scientific problems. Therefore, I strongly recommend without hesitation M.S. Patrycja Mała to receive the doctorate degree.

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