Abstract

Title of the master thesis: The development of reliable method for glucosylation and lactosylation of sphingolipids

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Ceramides are hydrophobic sphingolipids consisting of a sphingoid base and an acyl chain. They are found in the stratum corneum, the uppermost layer of the epidermis, where they are important part of the skin barrier, but they can also be found in brain tissue and inside human cells.

Glucosylceramides are the simplest glycosphingolipids, and they contain a single glucose molecule in their structure. They are found in all types of human cells, where they not only serve as precursors for the biosynthesis of other, more complex glycosphingolipids, but also have their own physiological functions. The most significant is their influence on cell growth, development, differentiation and apoptosis. Disruptions in their synthesis or degradation can lead to serious health problems and are also a risk factor for the future development of a wide range of diseases. Their total absence in the body is incompatible with life. A more thorough study of their roles and mechanisms of action in the human body may help to better understand important issues of modern medicine in the future, especially in the field of cancer and neurodegenerative diseases, and may also potentially provide new treatment options.

The aim of this thesis was to compare the current methods of glucosylceramide synthesis in the literature, to select the most suitable one for the environment of our laboratory and to validate and optimize the procedures. We were looking for a synthesis applicable to a wide range of different sugars and ceramides in the future, that would allow reliable synthesis of glucosylceramides for their further study.

In this work, we tested two methods of preparing glucosylceramides previously described in the literature and we attempted to optimize them. We successfully replicated and optimized the four-step preparation of the protected glucosyl donor several times in high yields exceeding those in the literature. This approach can be used to prepare a wider range of glycosyl donors in the future. The protected glucose was then used to react with the unprotected ceramide to form beta-glucosylceramide. The principle of the reaction involves the formation of electrophilic glucosyl mesylate, which subsequently reacts with ceramide in the presence of diphenylborinic anhydride. The latter forms a temporary complex with the C_1 and C_3 OH groups of the ceramide, amplifying the nucleophilicity of the free electron pair on the C_1 hydroxyl and allowing a stereoselective reaction. The overall yield of this six-step synthesis was 7 %.

The second procedure we tried in this work was the preparation of a glucosylceramide using a per-O-silylated glucosyl donor and its subsequent conversion to a glucosyl iodide, which should selectively provide alpha-glucosylceramides in the TBAI environment.