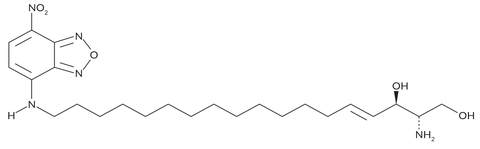
A New, Efficient Synthesis of ω-NBD Sphingosine

Samantha Hyder and Adam Uzieblo

Cayman Chemical, Ann Arbor, MI, United States

Abstract

ω-NBD sphingosine is a biologically active derivative of sphingosine that is tagged with a fluorescent nitrobenzoxadiazole (NBD) group. Sphingosine (d18:1) (Cayman Item No. 10007907) is an important precursor to sphingosine-1-phosphate. The two enzymes that are responsible for this process are the sphingosine kinases SPHK1 and SPHK2. The fluorescently tagged ω-NBD sphingosine (Cayman Item No. 25348) allows researchers to monitor the activity of these kinase enzymes in real time. ω-NBD sphingosine has been previously described in literature, and the former method of synthesis was thirteen steps long. We have developed a more efficient method of synthesis that is only six steps long.



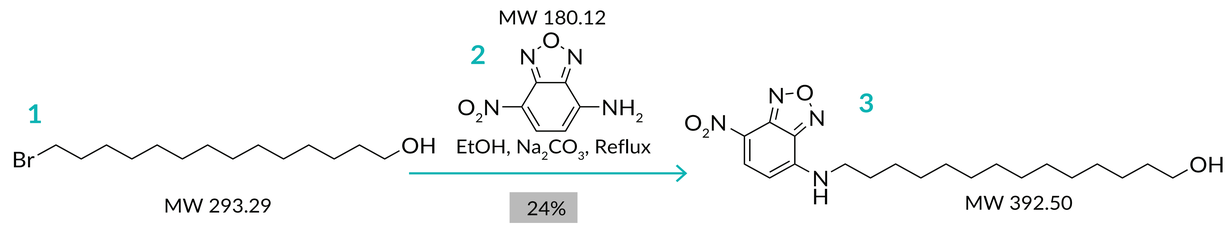
NBD Sphingosine

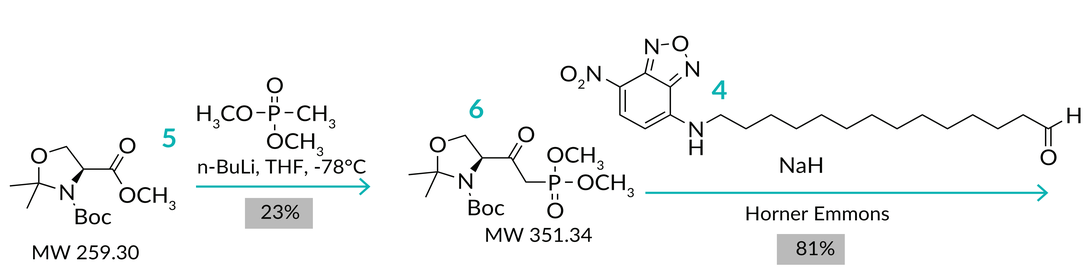
Introduction

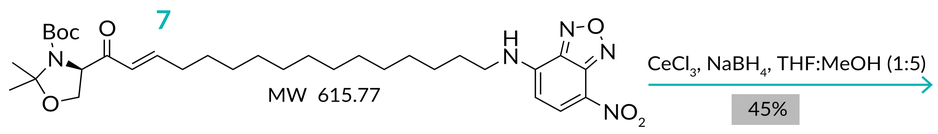
Sphingosine is a bioactive lipid that is formed from of the breakdown of ceramide and is the precursor to sphingosine-1-phosphate. Sphingosine has been known to induce apoptosis when added exogenously to cells.[1] Sphingosine also inhibits protein kinase C and phosphatidic acid phosphohydrolase, and activates diacylglycerol (DAG) kinase and phospholipase D.[2] When sphingosine is phosphorylated by the sphingosine kinase enzyme it becomes sphingosine-1-phosphate.[3] Sphingosine-1-phosphate has anti-apoptotic and proliferative activity. The enzyme sphingosine kinase has two isoforms, SPHK1 and SPHK2. SPHK1 and SPHK2 are expressed in all human tissues. The highest levels of SPHK1 are found in the lungs, spleen, and leukocytes. The highest levels of SPHK2 are found in the kidneys and liver.[4] SPHK1 has been shown in studies to increase cell survival and proliferation. Increased levels of SPHK1 have been found in many human cancers, and these increased levels seem to contribute to carcinogenesis. In many cancers, elevated levels of SPHK1 have been linked to poor patient prognosis.[4] SPHK1 has also been linked to promoting inflammation. SPHK2 has not been as well studied as SPHK1. Many studies suggest that SPHK2 may have some opposing function compared to SPHK1 and some overlap. Early studies looked at SPHK2 overexpression, which caused cell cycle arrest and apoptosis. However, other studies demonstrated that SPHK2 has a similar role to SPHK1 in promoting cancer. The role that SPHK2 plays in inflammation is controversial in that many studies suggest that SPHK2 may be anti-inflammatory.

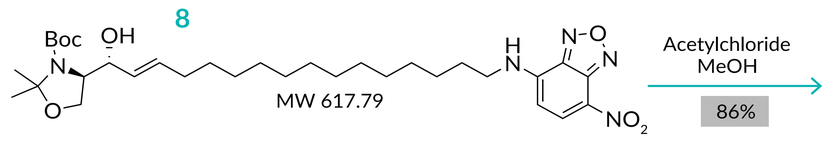
ω-NBD sphingosine has been used as a tool in several studies to examine the activity of SPHK1 and SPHK2 in the conversion of sphingosine to sphingosine-1-phosphate. Because of ω-NBD sphingosine's fluorescence, it is a useful tool in assays. It can be used to monitor SPHK1 and SPHK2 in real time without the need for organic partitioning of products, radioactive materials, or specialized equipment.[5] The originally published synthesis of ω-NBD sphingosine was thirteen steps long. The synthesis was started with 1,10-decanediol and ended with the attachment of the NBD (7-nitro-2,1,3-benzoxadiazol-4-amine) moiety.[6] In our work, we were able to create a more efficient synthesis that is comprised of six steps. This was possible because we were able to attach the NBD moiety at the beginning of the synthesis, and it did not interfere with the following steps of the synthesis.

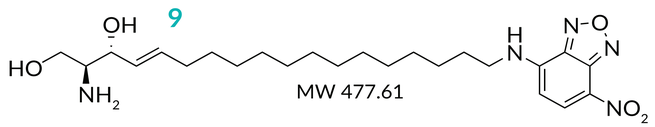
Synthesis of NBD Sphingosine











Methods

There were three critical steps to creating the shortened synthesis of ω-NBD sphingosine. The first step that was crucial for keeping the synthesis as short as possible was the direct transformation of the bromo-alcohol (1) with the NBD moiety (2) to give compound 3. The second crucial step in our approach was the application of protected L-serine (5) as a building block. The final crucial reaction was the diastereoselective reduction of the enone (7), which provided alcohol (8) without affecting the double bond.[7,8]

Results and Discussion

Overall, creating a shortened synthesis of ω-NBD sphingosine was successful. We have proven that the attachment of the NBD moiety did not interfere with the rest of the synthesis. Some of the key steps in this shortened synthesis include: a) the coupling of amino NBD to 14-bromo-1-tetradecanol, b) the conversion of 5 to 6, and c) the Luche reduction of the enone (7 to 8). There are still some improvements that need to be made to the synthesis. Further work will be done in the future to optimize the coupling of amino NBD to 14-bromo-1-tetradecanol to increase the yield of the reaction. The other reaction that we would like to improve the yield on is the transformation of 5 to 6.

Conclusion

We have demonstrated that the introduction of the NBD moiety at the beginning of the synthesis allowed us to construct a shorter path for the synthesis of ω-NBD sphingosine. We have proven that the attachment of NBD did not interfere with the remainder of the synthesis. The application of protected L-serine (5) also contributed to a significant simplification of the synthesis of the final product.

Reference

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