

Large Scale Synthesis of Analogs of Azidosphingosine and Preparation of the Corresponding GM₂ Analogs

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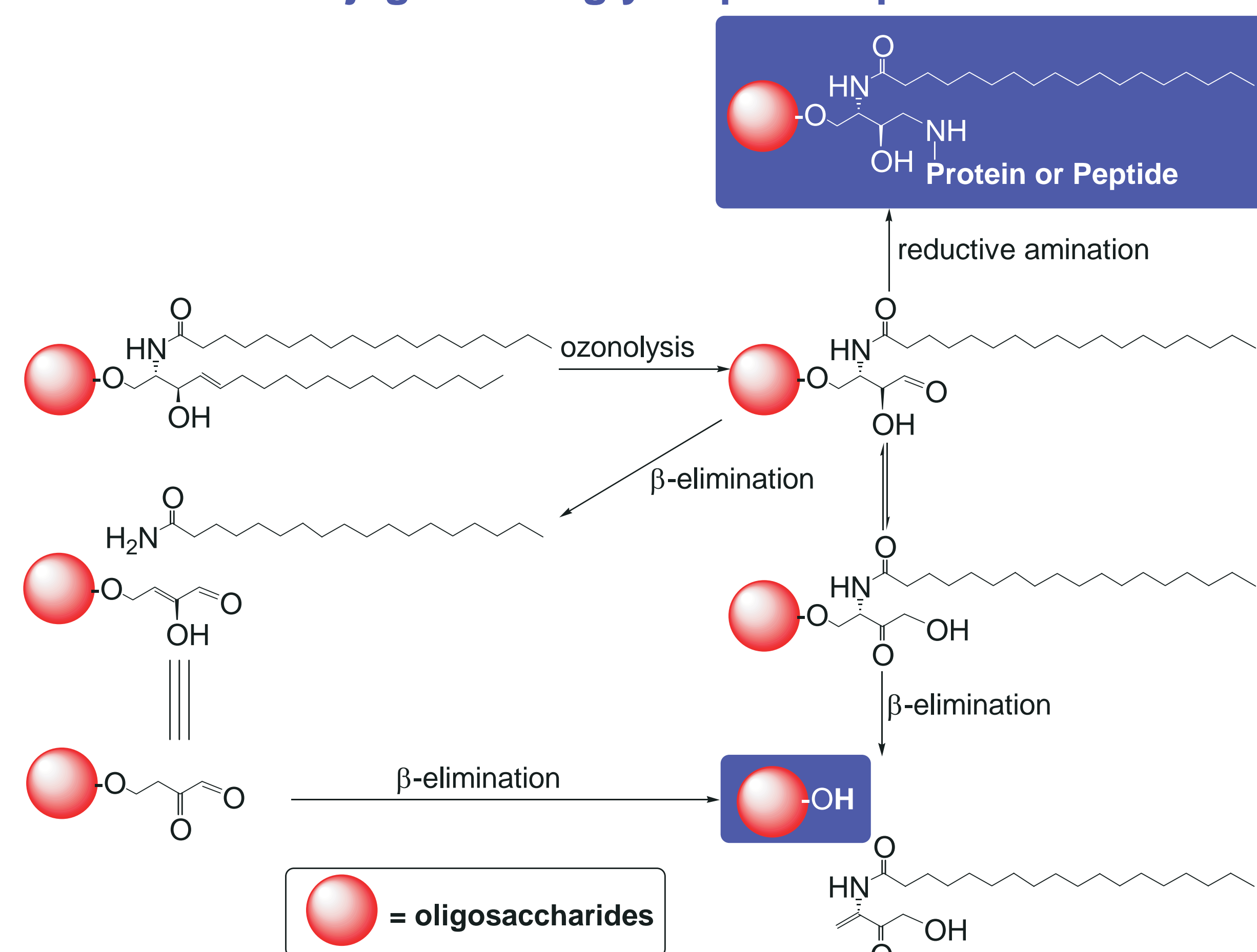
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Introduction

Gangliosides, glycosphingolipids containing sialic acid, as well as neutral glycosphingolipids such as Lewis Y are used in several cancer vaccines.¹ To render these glycolipids immunogenic the lipid portion of the molecule, the ceramide, is cleaved at its carbon-carbon double bond by an ozonolysis procedure and the resulting aldehyde group is used to couple to the primary amine groups on the surface of protein through a reductive amination procedure.² Usually during the coupling step, the intermediate aldehyde undergoes serious side reactions leading to partial or even total elimination of the lipid moiety. The side reactions occur via two potential pathways involving tautomerization and β -elimination (Scheme 1). Consequently this process is highly inefficient and wasteful of extremely costly synthetic or natural product.

Scheme 1. Conjugation of glycolipids to protein



Objective

We have designed a synthetic route to a truncated analog (**2**) that corresponds to the core sphingosine structure of natural ceramide (Scheme 2). Because of its smaller size, the use of analog **2** should allow improvement in the chemical synthesis of gangliosides and in the conjugation chemistry.

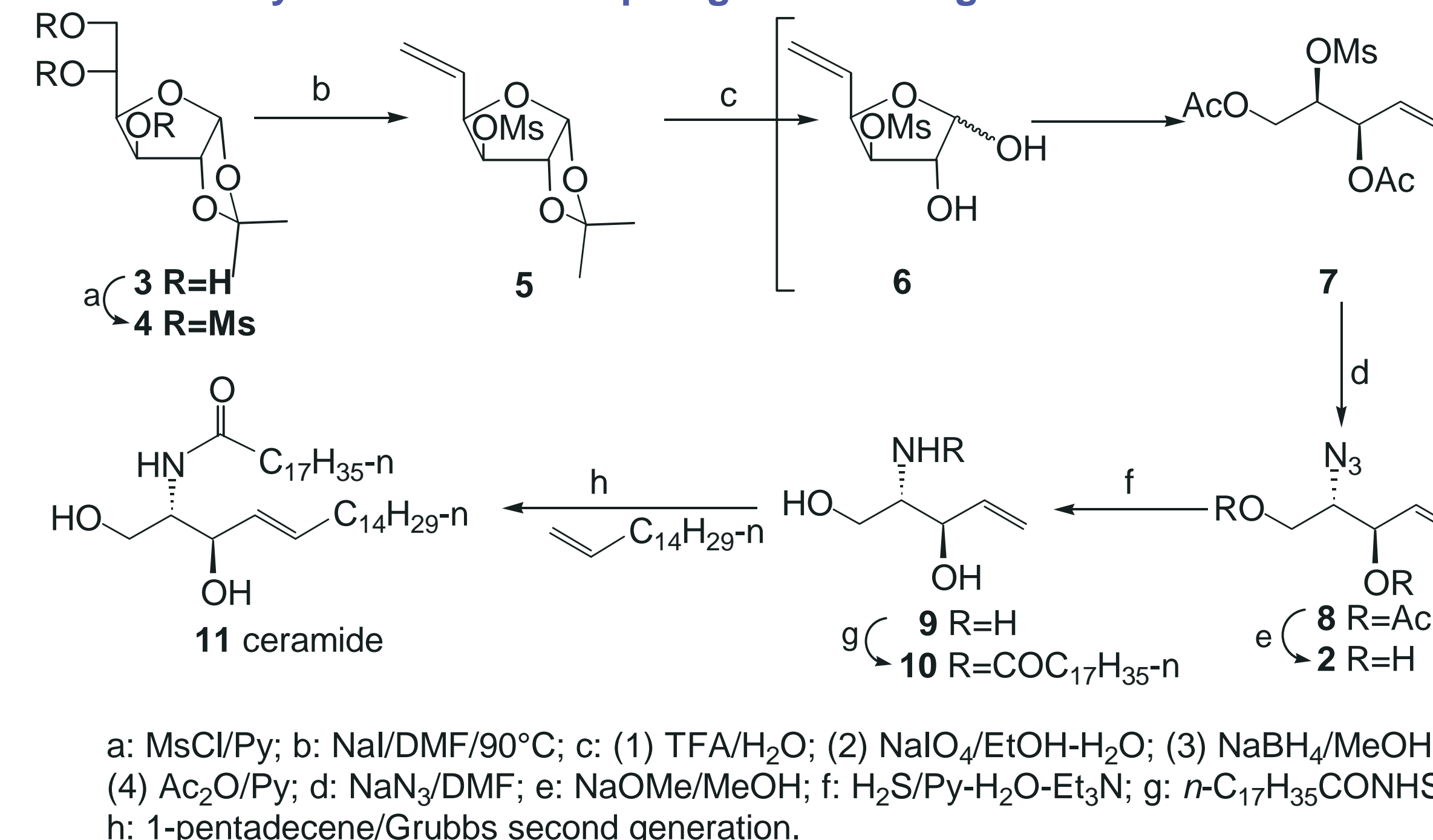
Scheme 2



Synthesis of azidosphingosine and ceramide

The truncated azido sphingosine **2** was prepared from monoacetone glucose **3** according to scheme 3. The synthesis has been scaled up to a hundred gram scale. The whole process involves only two chromatography steps with overall yields of 41%. The azido sphingosine **2** could be transformed to the truncated ceramide **10** as shown in scheme 3. Compound **10** could be converted to the natural ceramide (in ~30% efficiency) by cross-methathesis with 1-pentadecene using 2nd generation Grubbs' catalyst.

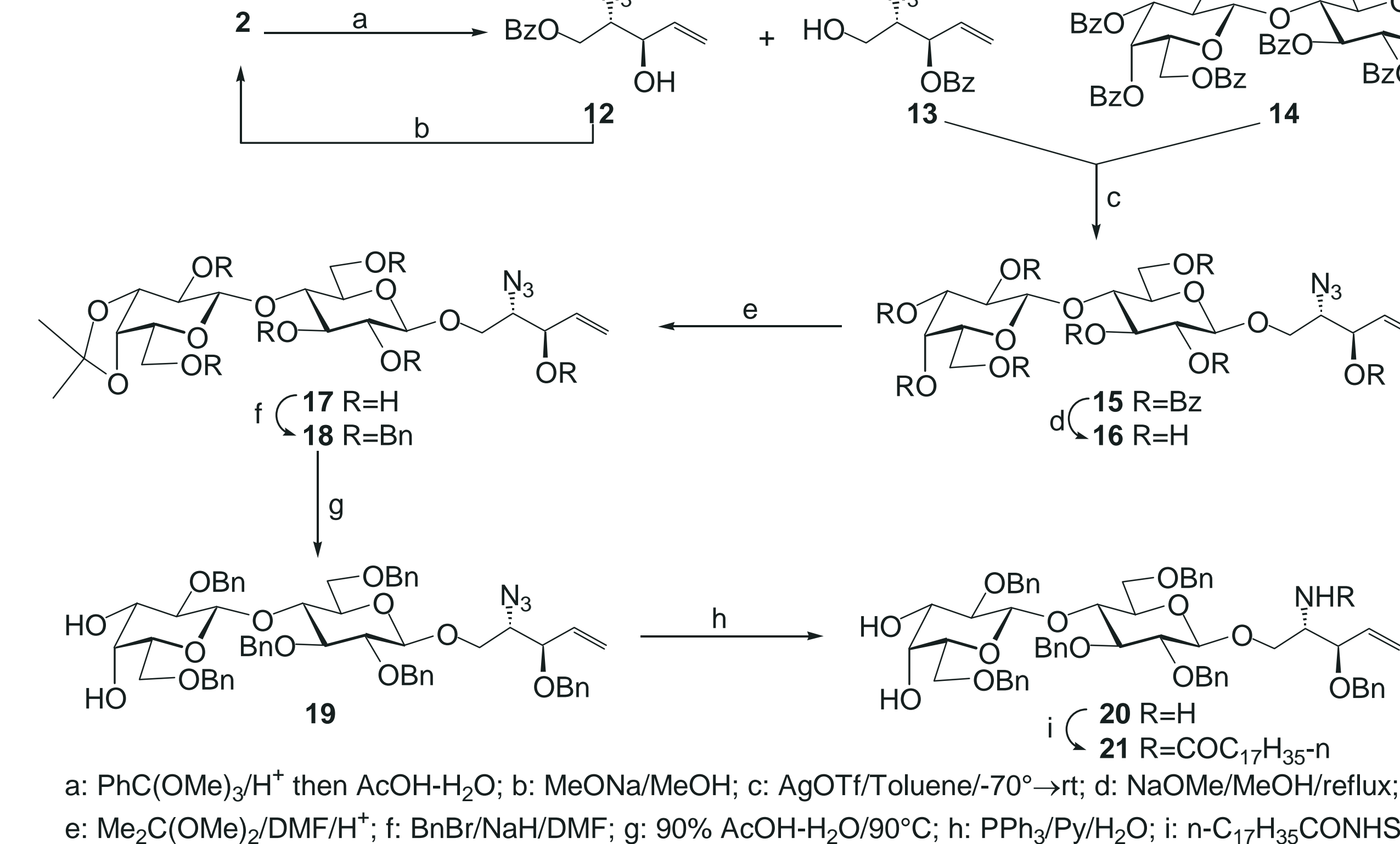
Scheme 3. Synthesis of azidosphingosine analog 2 and ceramide



Synthesis of lactosyl azidosphingosine

In contrast to the chemistry of **1**, the glycosylation of **13** with lactosyl donor **14** proceeded quantitatively to afford **16** directly after a debenzoylation. The desired lactosyl acceptor **19** as well as **21** were obtained following conventional transformation steps. Acceptor **21** was found to be unsuitable for sialylation.

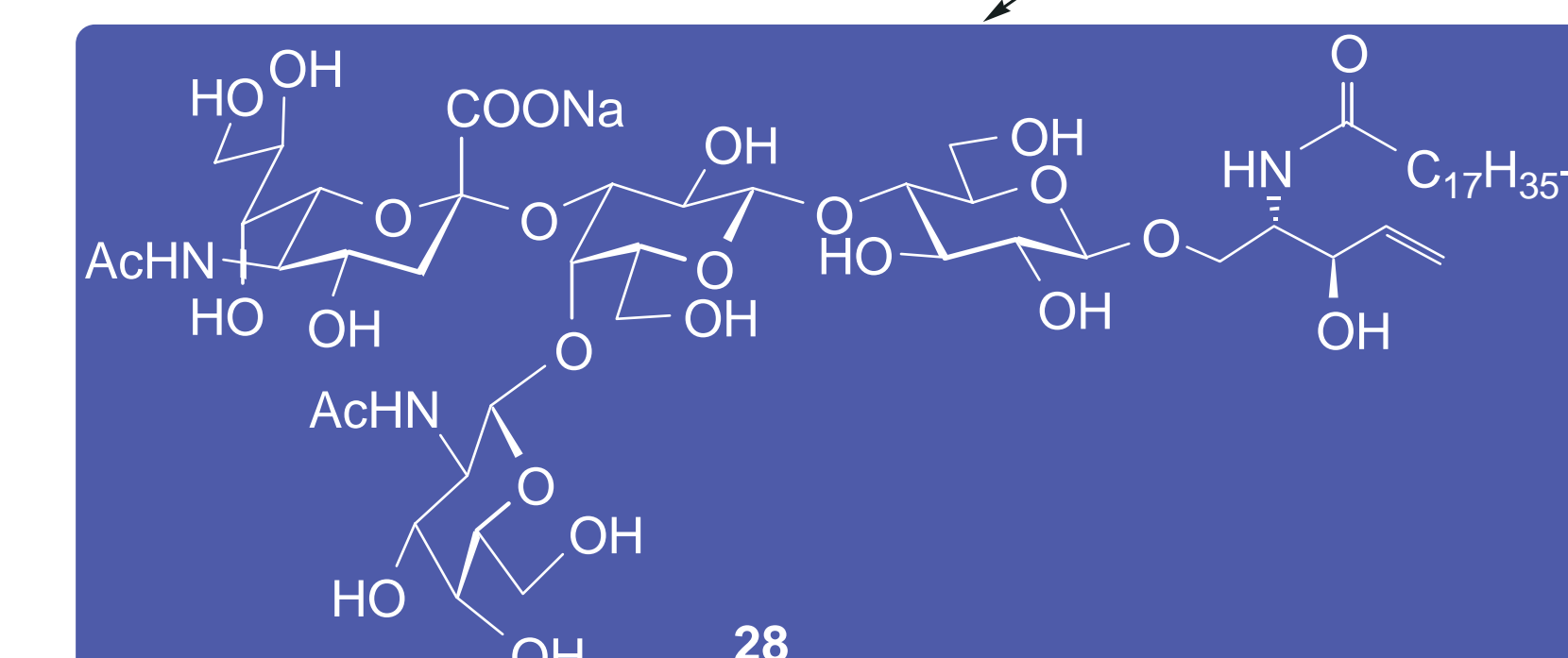
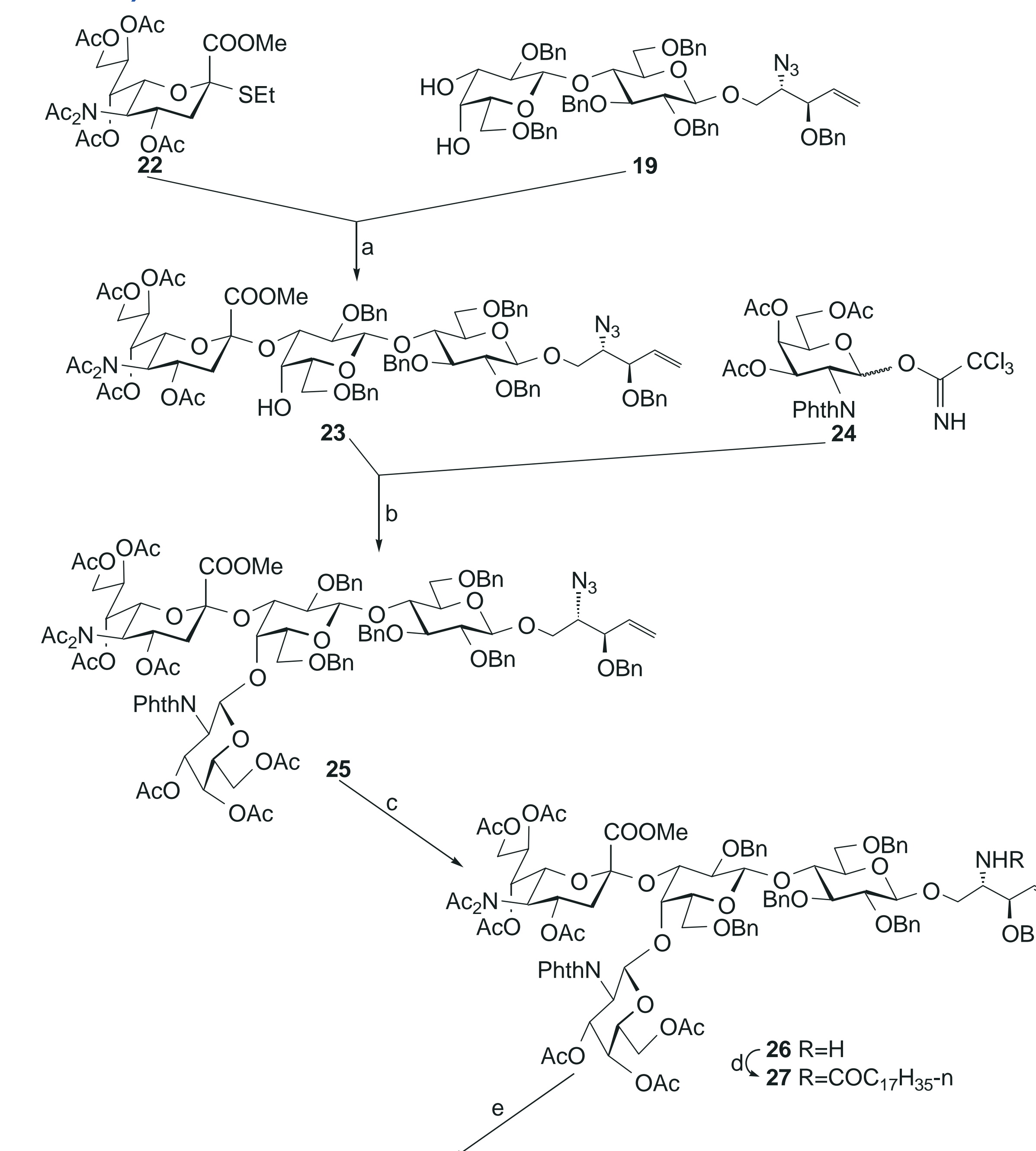
Scheme 4



Synthesis of GM₂ analog

Acceptor **19** was sialylated with donor **22** in ~ 60% yield (α/β 4:1). The GM₃ trisacchride **23** was glycosylated with **24** in 54% yield to afford **25**. The GM₂ analog was finally obtained after 6 additional transformations.

(Scheme 5)



a: NIS/TfOH/CH₃CN-CH₃CH₂CN/-60° C; b: TMSOTf/CH₃CN; c: H₂S/Py/H₂O/NEt₃/0° C; d: n-C₁₇H₃₅CONHS/Py; e: (1) NaOMe/MeOH, then H₂O; (2) NH₂CH₂CH₂NH₂/n-BuOH/110° C; (3) Ac₂O/MeOH; (4) Na/NH₃/-78° C.

Reference

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- Helling, F., Shang, A., Calves, M., Zhang, S., Ren, S., Yu, R. K., Oettgen, H. F., and Livingston, P. O. *Cancer Res.* **24**, 197-203 (1994).

Acknowledgement

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