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Protected Sphingosine from Phytosphingosine as an Efficient Acceptor in Glycosylation Reaction

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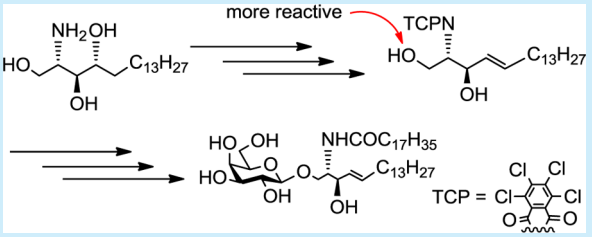
Supporting Information

ABSTRACT: A convenient, simple, and high-yielding five-step synthesis of a sphingosine acceptor from phytosphingosine is reported, and its behavior in glycosylation reactions is described. Different synthetic paths to sphingosine acceptors using tetrachlorophthalimide as a protecting group for the sphingosine amino function and different glycosylation methods have been explored. Among the acceptors tested, the easiest accessible acceptor, unprotected on the two hydroxyl groups in positions 1 and 3, was regioselectively glycosylated on the primary position, the regioselectivity depending on the donor used.



It is well-known that glycosphingolipids (GSLs), components of the plasma membrane where their hydrophilic portions are exposed toward the cell surface and the hydrophobic moieties are inserted into the membrane layer, play essential roles in cellular trafficking, signaling functions,[1] and interactions of the cells with various agents,[2] proliferation, differentiation, apoptosis, and cellular embryogenesis.[3] Most GSLs are composed of a hydrophobic moiety, ceramide, and a hydrophilic group of core monosaccharides.

Ceramide, in turn, is composed of sphingosine, a long-chain 2-amino-1,3-diol in the D-erythro configuration containing a C4−C5 trans double bond, linked to a fatty acid, usually with a long chain, which is sometimes hydroxylated or unsaturated.

The biological properties of GLS and the need for pure, structurally well-defined compounds prompted the development of various synthetic ways for their preparation.

The two most relevant aspects in GLS synthesis are the availability of sphingosine and the glycosylation of either sphingosine or ceramide precursors.[4] A number of sphingosine syntheses are described in the literature.[5] Among these, efficient preparations of sphingosine from much cheaper phytosphingosine have been recently described.[6] We therefore decided to exploit this approach, not with the major aim of developing an efficient route to sphingosine but to obtain a properly protected sphingosine acceptor for effective glycosylation by an accurate choice of the protecting groups, minimizing the protection−deprotection steps and therefore shortening the synthesis.

We here describe the results obtained from application of different protection strategies to phytosphingosine, two different conversions of phytosphingosine to sphingosine and the glycosylation properties of the so-obtained protected sphingosines.

We started by selecting the protecting group to be introduced on the amino function. It is now commonly accepted that the presence of a hydrogen bond between the electron pair on the C-1 oxygen and the amide hydrogen withdraws the electron density from this oxygen, making the primary hydroxyl group less nucleophilic.[4]

Therefore, we decided to choose a protecting group that should allow us to avoid this drawback and, possibly, to have a positive effect, and we selected tetrachlorophthalimide[7] (TCP) (Figure 1) for this purpose.

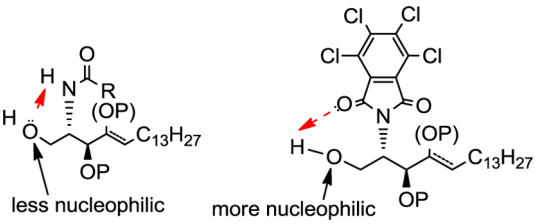


Figure 1. Hydrogen bonding in sphingosines.

TCP is safer in comparison to the most commonly used azide, does not require the use of harmful triflic azide, and was also expected to be easy to remove at the end of the synthesis.

Our first approach to the synthesis of sphingosine from phytosphingosine is shown in Scheme 1.

Treatment of phytosphingosine **1** with tetrachlorophthalic anhydride in DMF at 150 °C, after pouring the mixture in water and filtration of the precipitate, gave almost pure **2** in a very satisfactory yield.[8]

Silylation of the primary hydroxyl group with TBDPSCl furnished **3**, again in excellent yield. Compound **3** was efficiently converted in two steps into the corresponding cyclic sulfate **4** by reacting the remaining 3,4-hydroxyl groups with $SOCl_2$ and oxidation of the intermediate cyclic sulfite with $RuCl_3/NaIO_4$.[6]

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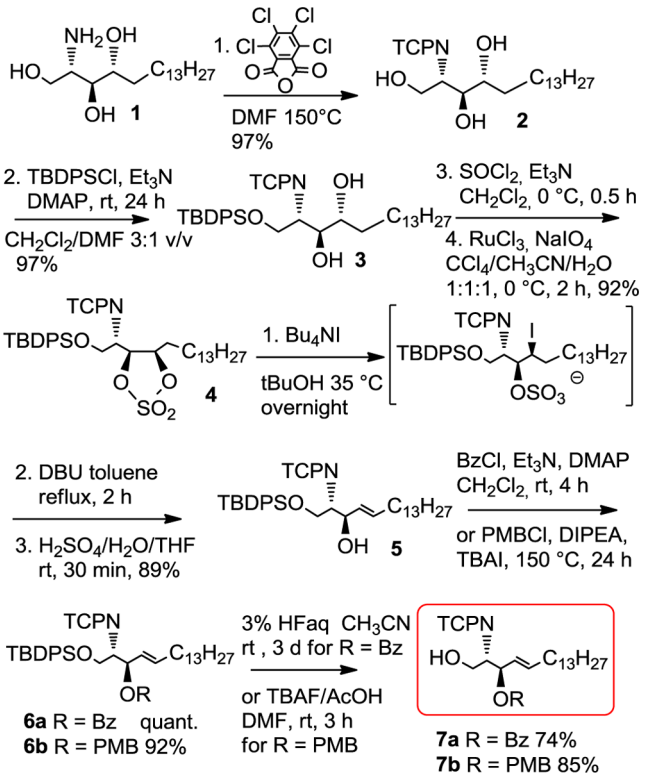
Scheme 1. First Synthesis of Sphingosine Acceptors



Opening of the cyclic sulfate **4** with tetrabutylammonium iodide, DBU-promoted elimination,[9] and sulfate hydrolysis afforded sphingosine **5** in excellent yield. It should be noted that when the reaction was attempted as described,[6] namely using toluene for the sulfate opening, for unknown reasons the overall yield was around 35−40%. We obtained good results only by using the solvents indicated in Scheme 1.

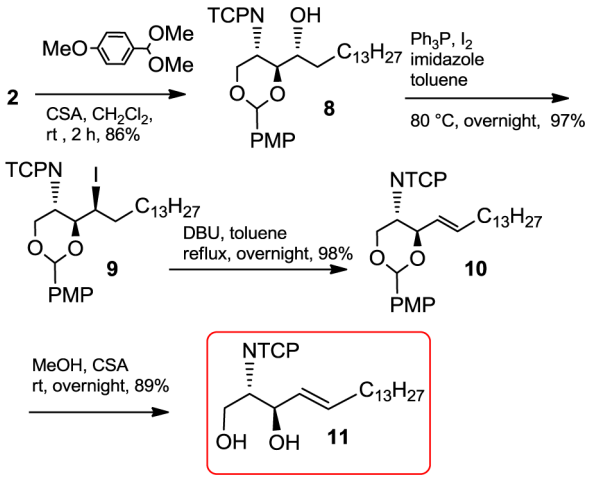
Protection of the 3-OH group either as benzoate or as a *p*-methoxybenzyl ether[10] followed by desilylation of the 1-OH afforded two different acceptors, **7a** and **7b**.

However, we were looking for a shorter synthetic scheme to the sphingosine acceptor. Toward this goal, we protected the 1- and 3-OH groups as *p*-methoxybenzylidene acetal. Then we efficiently introduced the 4,5 double bond through 4-OH substitution with an iodine atom, brought about by treatment with I_2, PPh_3, and imidazole[11] followed by DBU-promoted elimination, which provided the protected sphingosine **10** (Scheme 2).

In order to obtain the acceptor **7b**, we attempted to perform a regioselective reductive ring-opening of the *p*-methoxybenzylidene acetal using $NaCNBH_3$ and different acidic promoters (Me_3SiCl, *t*-$BuMe_2SiCl$, *t*-$BuMe_2SiOTf$),[12] but we invariably obtained a mixture of 1- and 3-PMB derivatives in a ratio of 2:1. On the other hand, Castillon has described the efficient regioselective glycosylation of a 1,3-diol at the C-1 position exploiting a 1,3-stannylene derivative.[13] Therefore, we decided to explore the possibility of exploiting a similar approach with diol **11**, either as such or as the stannylene derivative. Diol **11** was easily obtained by conventional removal of the *p*-methoxybenzylidene acetal from compound **10**.

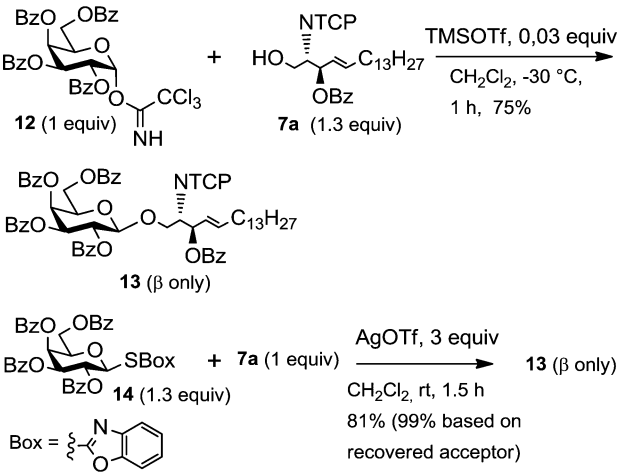
With the acceptors **7a**, **7b**, and **11** in hand, their properties as glycosyl acceptors were then explored. As donors we chose the perbenzoylated galactopyranosyl trichloroacetimidate **12**[14] or SBox galactopyranoside **14**.[15]

Scheme 2. Second Synthesis of a Sphingosine Acceptor



Both glycosylation reactions on **7a** gave satisfactory results without relevant differences between the two galactosyl donors affording product **13** in good yield (Scheme 3).

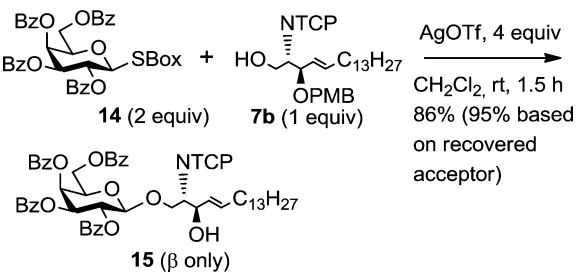
Scheme 3. Glycosylations of Acceptor 7a



When acceptor **7b** was submitted to glycosylation using donor **14**, product **15** was obtained in 86% yield (Scheme 4), and the PMB group was simultaneously removed under the reaction conditions, avoiding a separate deprotection step.

The most interesting results, however, were obtained with acceptor **11** (Scheme 5). The use of imidate **12** as donor (1.3 equiv) gave, besides the expected product **15**, some unwanted diglycosylated derivative **16**. When glycosylation was performed with donor **14** in the same conditions used for acceptors **7a** and

Scheme 4. Glycosylation of Acceptor 7b



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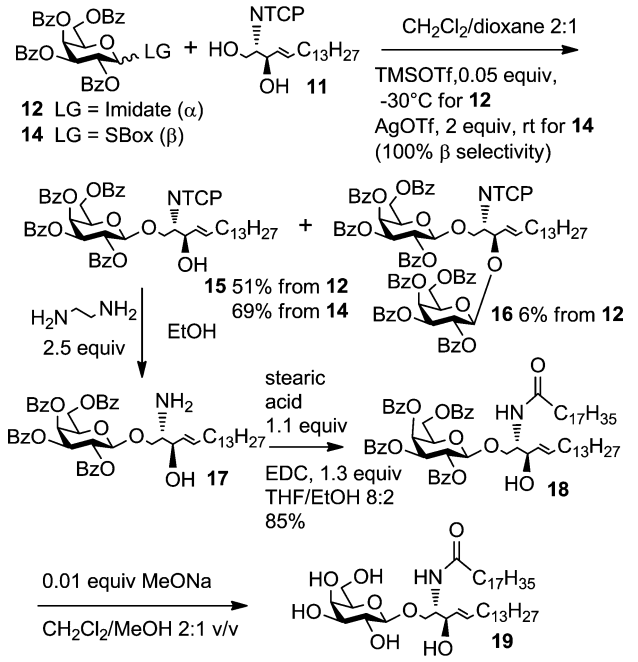
Scheme 5. Glycosylation of Acceptor 11 and Synthesis of Galactocerebroside



7b, we were delighted to obtain the expected glycosylation product **15** on the primary hydroxyl group almost exclusively, *without the use of any tin derivative*. When a small excess of donor (1.1 equiv) was used the yield was 62% (85% based on recovered acceptor). Investigation of the reaction mixture revealed, as expected, that a tiny amount of diglycosylated product **16** was also formed (together with traces of other unidentified byproducts). Therefore the reaction was repeated using an excess of acceptor (2 equiv) in order to avoid the formation of **16**. Under these conditions, compound **15** was obtained in a satisfactory 69% yield (90% based on recovered acceptor).

To demonstrate the feasibility and efficiency of the whole scheme, we performed the TCP deprotection, the acylation, and the final debenzoylation steps. When TCP deprotection was attempted on compound **13**, under the usual conditions[16] (1,2-ethylendiamine, EtOH, 60−80 °C), we observed a significant migration of the benzoyl group from the 3 to the 2 position to give the corresponding benzamide as main product (data not shown). Similar behavior has already been described in literature,[17] and the use of milder conditions for TCP deprotection did not give satisfactory results. On the other hand, TPC deprotection on compound **15** using slightly modified literature conditions,[16] in particular working at room temperature rather than at 60 °C, smoothly gave amine **17**, which was used directly for the next step. Acylation of **17**[18] and debenzoylation[19] of compound **18** were very satisfactory, affording galacto cerebroside **19**[20] in good yield.

In conclusion, we have described the use of tetrachlorophthalimide as an effective protecting group for the amino function of phytosphingosine, allowing its efficient conversion to sphingosine acceptors for glycolipid synthesis. The overall yield competes well with other sphingosine synthesis: compound **11**, from which sphingosine can be obtained in one step, has been obtained in 72% overall yield. A recent, efficient sphingosine synthesis[6b] reports a 67% overall yield, while a more classical synthesis starting from Garner's aldehyde[5c] proceeded in 42% yield. The overall yield from **1** to **19** was 34%. Our results show the importance of a proper tuning of the donor reactivity. In the present case, SBox donor **14** was better than donor **12** not only as it had the correct reactivity for good selectivity but also because it is easy to prepare and shelf-stable and the reaction conditions do not require low temperature.

We are currently extending the use of TCP to phytosphingosine glycosylation en route to αGalCer derivatives.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and hard copies of NMR spectra of new and known but not fully characterized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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