DOI: 10.1002/ejoc.200900424

# Efficient Synthesis of β-Glycosphingolipids by Reaction of Stannylceramides with Glycosyl Iodides Promoted by TBAI/AW 300 Molecular Sieves

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**Keywords:** Glycosylation / Glycolipids / Sphingolipids / Tin / Ethers

TBAI and acid-washed molecular sieves efficiently promoted the glycosylation of stannylceramides with glycosyl iodides. This direct glycosylation reaction reduces the overall number of synthetic steps and provides rapid access to β-glycosphingolipids such as GalCer, β-lactosylceramide, and iGB3 in good yield and with complete chemo- and stereoselectivity.


## Introduction

Glycosphingolipids[1] (GSLs) constitute a heterogeneous group of biomolecules (Figure 1) displaying various structural biological functions in eukaryotic cells.[2] GSLs are endowed with unique chemical features that affect the structural dynamics and reactivity of the interface.[3] These lipids are composed of a sphingosine base and an amide-linked fatty acyl chain.[4,5] A variety of carbohydrates are present (mono- and oligosaccharides and sialic acid), which confer particular charge, hydrogen-bonding and hydration properties.[6] As building blocks of the plasma membrane, GSLs with their protruding oligosaccharide chains are involved in processes that have been extensively studied. For cell biologists, GSLs are chiefly involved in cellular trafficking and signaling functions. For pathologists, these compounds are preferential sites for host–pathogen/toxin interactions and for the generation of pathological/infectious forms of proteins associated with Alzheimer's and prion diseases[7] and HIV.[8]

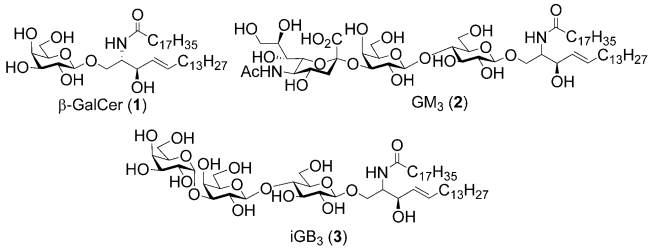


Figure 1. Naturally occurring glycosphingolipids.

As a result of the variety of their biological roles, it is little wonder that β-GalCer and its derivatives have become important synthetic targets. To provide homogeneous material for use in biochemical and pharmacological studies several synthetic routes have been developed.[4,9] Three key issues must be addressed for all GSLs synthesis. First, the sphingosine moiety must be attached to the sugar; second, N-acylation with a fatty acid must be performed; and third, the protecting groups must be eliminated. In this context, it is crucial to have good yield and high stereocontrol (α or β) in the glycoside bond-formation step. To accomplish this synthetic challenge, a variety of the glycosyl donors, trichloroacetimidates,[10] fluorides,[11] and sulfides,[12] have been commonly employed. The glycosylation approach for the synthesis of GSLs[13] can be classified into two categories: (i) based on direct glycosylation of ceramide **6** and (ii) based on the use of azidosphingosine **5** (Scheme 1). In this regard, the glycosylation of a ceramide unit is generally rather low yielding, whereas the use of azidosphingosine allows good yields to be obtained (Scheme 1). However, this last alternative requires further reduction of the azido group and acylation with an appropriate fatty acid. The difference in reactivity has been attributed to the low nucleophilicity of ceramides,[14] which are extremely ordered as a result of head-group hydrogen bonding. This driving force for molecular self-assembly in ceramides allows them to have hexagonal and orthorhombic phases with high stability.[15]

We recently reported that the use of stannyl ceramides in the synthesis of α-[16] and β-glycolipids[17] afforded excellent yield and complete chemo- and stereoselectivity in the glycosylation reactions. In these initial investigations, several types of glycosyl donors–promoters were tested, yielding

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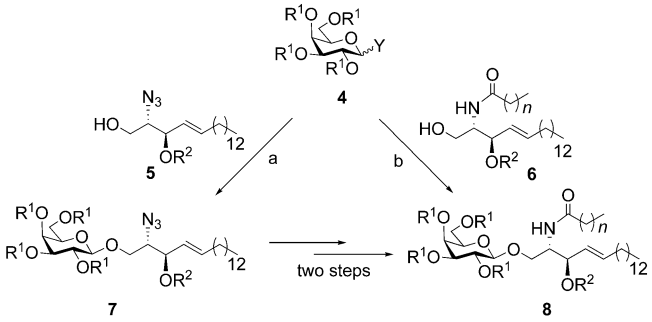
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200900424.



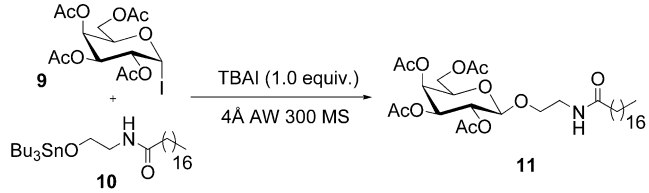

Scheme 1. Glycosylation strategies for GSL synthesis.

the orthoester when the reaction was carried out with a 2,3,4,6-tetra-*O*-acetylgalactose derivative or a mixture of the orthoester and the β-*O*-glycoside when a 2,3,4,6-tetra-*O*-pivaloylgalactose derivative was used. Thus, a second step, the migration of the orthoester under acidic conditions, was necessary to obtain the desired β-*O*-glycoside. Traditionally, this rearrangement is carried out by using strong and moisture-sensitive Lewis acids, including TMSOTf,[18,19] $BF_3{\cdot}Et_2O$,[18,20] TBDMSOTf,[18,21] and $Yb(OTf)_3$,[22] and more recently, $AuCl_3$[23] under strictly anhydrous conditions. In this context, it was recently observed that activated 4 Å acid-washed molecular sieves (4 Å AW MS, or AW 300), also catalyzed this rearrangement.[24] Other studies indicated that AW 300 can play both the role of drying agent and promoter[25] in the synthesis of glycosides and trisaccharides.[26] We describe here that β-*O*-galactosyl ceramides can be obtained in a one-pot fashion from 2,3,4,6-tetra-*O*-acetylgalactosyl iodide[27] and stannyl[28] ceramides in the presence of tetrabutylammonium iodide (TBAI) and AW 300.

## Results and Discussion

With the aim of obtaining the β-*O*-galactosyl ceramides in a one-pot manner from acetylated glycosyl iodides, we explored the compatibility of TBAI as promoter of glycosylation and several acids as rearrangement catalyst of the orthoester initially formed. In a model experiment, tetra-*O*-acetyl-α-galactosyl iodide (**9**) was treated with stannyl ceramide **10** in the presence of TBAI (1.0 equiv.) and various acids such as TfOH, TMSOTf, TBSOTf, $BF_3{\cdot}Et_2O$, and AW 300. No glycoside was observed when 0.2–1.0 equiv. of TfOH, TMSOTf, TBSOTf, was used. The use of $BF_3{\cdot}Et_2O$ was found successful, but two equiv. of $BF_3{\cdot}Et_2O$ were necessary to obtain 20% yield of **11**. However, initial experiments using 0.2 g of AW 300 per 0.366 mmol of **9** afforded a 22% yield of glycoside **11** (Scheme 2; Table 1, Entry 1).

We selected then AW 300 as a catalyst, and we focused on determining the optimum amount of AW 300. Thus, we followed the screening with a relation of 0.4, 0.6, 0.8, 1, and 1.2 g of AW 300/0.3 mmol of stannyl ceramide **10** in toluene at 80 °C (Table 1, Entries 2–6). All the experiments were carried out under the optimized conditions for glycosylation of ceramides by using glycosyl iodides as donors.




Scheme 2. Glycosylation of stannyl ether **11** derived from β-alcohol.

Table 1. Synthesis of compound **11** by glycosylation of stannyl amide **10** with iodide **9** in the presence of TBAI and 4 Å AW MS.[a]

| Entry | AW 300 [g] | Solvent | *T* [°C] | *t* [h] | Yield [%][b] |
|---|---|---|---|---|---|
| 1 | 0.2 | toluene | 80 | 18 | 22 |
| 2 | 0.4 | toluene | 80 | 18 | 35 |
| 3 | 0.6 | toluene | 80 | 18 | 37 |
| 4 | 0.8 | toluene | 80 | 18 | 42 |
| 5 | 1 | toluene | 80 | 18 | 48 |
| 6 | 1.2 | toluene | 80 | 18 | 64 |
| 7 | 1.2 | toluene | r.t. | 18 | 25 |
| 8 | 1.2 | $CH_2Cl_2$ | reflux | 18 | 50 |
| 9 | 1.2 | $CH_2Cl_2$ | r.t. | 18 | 29 |
| 10 | 1.2 | $Et_2O$ | reflux | 18 | 35 |
| 11 | 1.2 | $Et_2O$ | r.t. | 18 | 15 |

[a] Reactions conditions: **9** (0.366 mmol), **9**/**10**/TBAI, 1.2:1:1. [b] Yields of isolated product after chromatographic purification.

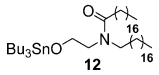
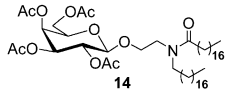
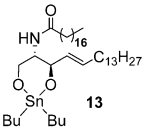
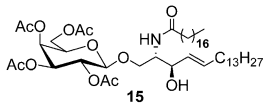
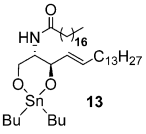
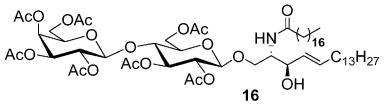
However, in the presence of 0.4–1 g of AW 300 yields were low (Table 1, Entries 2–5), but to our delight we found that corresponding β-glycoside **11** was obtained in 64% yield when 1.2 g of AW 300 was used (Table 1, Entry 6).

Encouraged by this result, we next examined the effects of temperature and solvents. The reaction conducted in refluxing $CH_2Cl_2$ gave moderate yield (50%; Table 1, Entry 8). When the reaction was performed in refluxing $Et_2O$, glycolipid **11** was obtained in 35% yield (Table 1, Entry 10). The yield dramatically decreased by lowering the reaction temperature under the same reaction conditions in $CH_2Cl_2$, toluene, and $Et_2O$ (Table 1, Entries 7, 9, and 11). In order to show the role of promoters and AW 300 in the process, **9** was treated with stannyl acceptor **10** in the presence of $Bu_4NI$, which provided exclusively the orthoester. Further treatment of the orthoester with AW 300 afforded β-glycoside **11**. This indicates that the rearrangement is exclusively produced by the action of AW 300.

With these results in hand, we tried to extend this methodology to other acceptors and donors (Table 2). Thus, ceramide **12** was glycosylated with donor **9** under the same reaction conditions used for **10**, giving glycosphingolipid **14** in good yield (65%; Table 2, Entry 1). The reaction conducted with ceramide **13** provided β-GalCer **15** in 67% yield (Table 2, Entry 2), whereas the reaction performed with hepta-*O*-acetyllactosyl iodide as donor afforded the corresponding glycosphingolipid **16** in similar yield with high stereoselectivity (63% yield; Table 2, Entry 3).

Table 2. Synthesis of glycosyl ceramides **14**–**16** by glycosylation of stannyloxy amides **12** and **13** with glycosyl iodides in the presence of TBAI and 4 Å AW MS.[a]

| Entry | Acceptor | Glycolipid | Yield [%][b] |
|---|---|---|---|
| 1 |  **12** |  **14** | 65 |
| 2 |  **13** |  **15** | 67 |
| 3[c] |  **13** |  **16** | 63 |

[a] Reaction conditions: **9** (0.211 mmol), **12**, or **13** (0.176 mmol), $Bu_4NI$ (0.176 mmol), 4 Å AW MS (700 mg), toluene, 80 °C, 18 h. [b] Yields of isolated product after chromatographic purification. [c] Hepta-*O*-acetyllactosyl iodide was used as donor under similar conditions to Entries 1 and 2.

The synthetic utility of the new approach was further demonstrated by rapid access to isoglobotrihexosylceramide (iGb3). Acceptor **19** and stannyl ceramide **20** would serve as the building blocks for glycosylation. Thus, isoglobotrihexose **17** was treated with acetic anhydride and pyridine in the presence of catalytic DMAP to provide peracetylated trisaccharide **18** in 98% (Scheme 3). Then, iodide donor **19** was generated in situ from **18** according to a procedure previously reported in the literature.[29] Finally, treatment of **19** with **20** in the presence of TBAI and AW 300, followed by the elimination of the acetate groups, furnished iGb3 **3** in 60% yield as a unique anomer. The $^1H$ and $^{13}C$ NMR spectroscopic data of **3** were consistent with the data reported for the synthetic product.[30]

## Conclusions

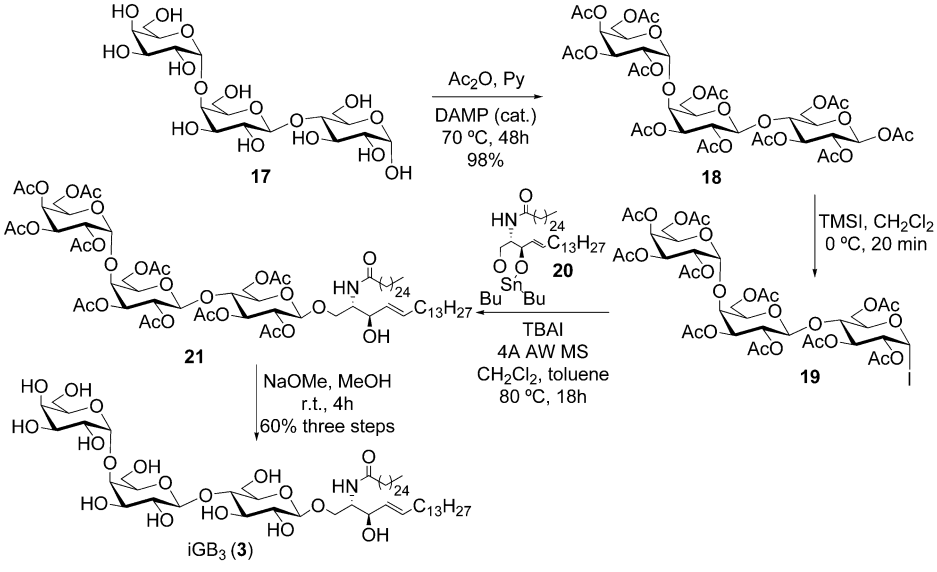
In summary, we have developed a process for the synthesis of glycosphingolipids based on a complete chemo- and stereoselective reaction of α-glycosyl iodides with stannyl ceramides in the presence of TBAI and AW 300. The synthetic scope was established by the use of a disaccharide and trisaccharide as donors. This is the first example of glycosylation of ceramides by using AW 300 as drying agent and catalyst of the 1,2-orthoester rearrangement. Moreover, it provides a solution for synthesizing glycolipids through the direct glycosylation of ceramides. We believe that this methodology will find use in the efficient assembly of glycosphingolipids, which should provide avenues for the synthesis of these therapeutically valuable compounds.

## Experimental Section

**General Procedure for Glycosylation. Synthesis of 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-octadecenoyl-2-aminoethanol (11):** The following protocol was followed prior to the glycosylation reaction: 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose and stannyl ether **10** were separately dried by co-distillation with toluene (3 × 5 mL) in dried flasks and then were placed under vacuum for 1 h.

TBAI was added to a dried flask with a magnetic stirring bar and was co-distilled with dry toluene (2 × 5 mL) in the dark. Activated 4 Å molecular sieves were added, and the mixture was co-distilled with toluene once more (5 mL) before being placed under vacuum for 1 h. Complete water exclusion is crucial to achieve good yields.

To a stirred solution of previously dried 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (142 mg, 0.366 mmol) in $CH_2Cl_2$ (3 mL)




Scheme 3. Synthesis of $iGB_3$.

cooled to 0 °C under an argon atmosphere in the dark was added TMSI (88 mg, 0.439 mmol). The reaction was stirred for 20 min at 0 °C. The reaction was stopped by adding dry toluene (3 mL) and co-distilling with dry toluene (3×) to obtain compound **9** as a slightly yellow oil, which was then dissolved in anhydrous toluene (5 mL) and kept under an atmosphere of argon.

To a stirred mixture of TBAI (111 mg, 0.305 mmol) and 4 Å acid washed molecular sieves (1.2 g) in anhydrous toluene (5 mL) under an atmosphere of argon at room temperature was added a solution of stannyl derivative **10** (188 mg, 0.305 mmol) in dry toluene (5 mL) and a solution of **9** (0.366 mmol) in dry toluene (5 mL) by syringe. The reaction mixture was stirred at 80 °C in the dark for 18 h and then diluted with AcOEt (15 mL) and cooled to 0 °C. The white precipitate was removed by filtration through a pad of Celite. The organic layer was concentrated in vacuo. The resulting residue was purified by flash column chromatography on silica gel (hexane/AcOEt/MeOH, 85:10:5) to give **11** (186 mg, 64%) as a unique anomer.

**Supporting Information** (see footnote on the first page of this article): $^{1}H$ and $^{13}C$ NMR spectra of compounds **11**, **14**, **15**, **16**, **18**, and **3**.

## Acknowledgments

Financial support from the Dirección General de Educación Superior e Investigación Científica (DGESIC) CTQ-2008-01569/BQU (Ministerio de Educación y Ciencia, Spain) is acknowledged. We are also grateful to the Servei de Recursos Cientifics (URV) for its technical assistance. Fellowship from DURSI (Generalitat de Catalunya) and Fons Social Euorpeu to J. A. M. S. is gratefully acknowledged.

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Received: April 17, 2009
Published Online: July 2, 2009

  