Highly efficient and stereoselective synthesis of β-glycolipids†

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β-Galactosylceramide and glycolipid analogues were prepared in high yield and with complete chemo and stereoselectivity by reaction of α-iodo glycosides with stannyl ceramides, formed *in situ*. TBAI was used to activate both the iodogalactose and the stannyl ether.

In recent years there has been a rapid increase in the knowledge of cellular processes in which lipids are involved. In particular, glycosphingolipids (GSLs) are widely distributed in the membrane of eukaryotic cells[1] and play a critical role in many stages of the cell cycle including growth, proliferation, differentiation, adhesion, senescence and apoptosis. GSLs serve a variety of functions through interaction with many biofactors[2] by inhibiting or interfering with the physiological effects of these factors or cells.[3] GSLs can also interact in the cell surface with toxins, viruses and bacteria.[4] In addition, some intermediates in the GSLs metabolism, such as ceramide, sphingosine and phosphorylated derivatives, have been identified as signals.[5]

GSLs (Fig. 1) and related compounds have mainly been investigated in reference to storage diseases,[6] which are a group of genetic diseases where, most commonly, GSLs accumulate due to specific defects in lysosomal hydrolases. However, in recent years, these compounds have been studied as a strategy for pharmacological prevention of microbial infections,[7] cancer chemotherapy,[8] modifying the activity of receptors for insulin,[9] epidermal growth factor[10] and nerve growth factor[11] which may have potential effects in Alzheimer's[12] and Parkinson's[13] diseases.

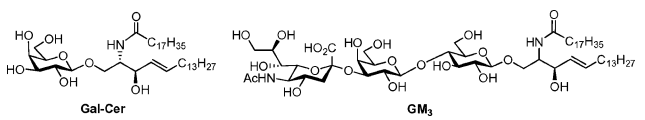
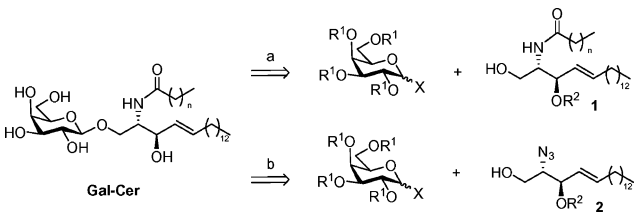


Fig. 1 Naturally occurring β-glycosphingolipids.

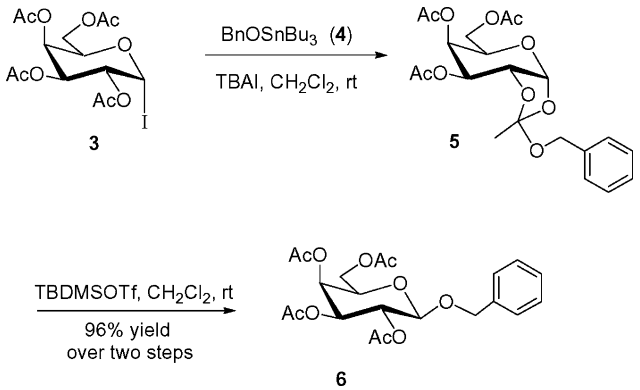
With this stimulating biological background and the complicated availability of GSLs from natural sources, many organic chemists have focused on developing methods for synthesizing GSLs. Crucial steps in these protocols involve the formation of the glycosidic bond between a properly protected carbohydrate and sphingosine or azidosphingosine.[14] The most classical and efficient glycosylation procedures[15] that have been used in the GSLs' synthesis include glycosyl trichloroacetimidates[16] and fluorides.[17] In these cases the stereochemistry of the reaction is determined by the participation of the neighbouring groups. Recently, it has been shown that glycosyl iodides[18] are excellent glycosyl donors for glycosylation reactions and they have been successfully used in the synthesis of α-glycosphingolipids.[19]

One of the drawbacks in the synthesis of GSLs is that the yields in the direct glycosylation of ceramides are low. This has been attributed to the low nucleophilicity of sphingosines and ceramides, and has been circumvented by using azidosphingosine **2** (Scheme 1b), a precursor of ceramide, instead of the ceramide **1** (Scheme 1a). This strategy allows obtaining high yields in the glycosylation step but increases the number of steps. Consequently, the development of simple and direct synthesis of glycolipids is still a challenge. In this study, we report a simple and very efficient procedure for the glycosylation of β-amidoalcohols and ceramides by using glycosyl iodides as glycosyl donors.



Scheme 1 Retrosynthetic analysis of β-galactosylceramide.

Our approach uses stannyl ethers,[20] derived from ceramides with the purpose of increasing the nucleophilicity of oxygen without significantly modifying the basicity (Scheme 2). We selected glycosyl iodides as the glycosyl donors because they can be activated by tetrabutylammonium iodide (TBAI), which can also activate the stannyl ether.



Scheme 2 Glycosylation–isomerization reactions of $BnOSnBu_3$ **4** with the iodogalactose **3** in the presence of TBAI and TBDMSOTf to give **6**.

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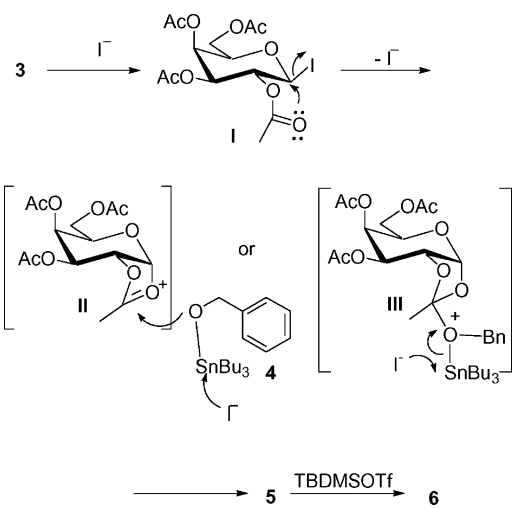
Initially we studied the reaction of tetra-*O*-acetyl-α-iodogalactose **3**, prepared by reaction of penta-*O*-acetylgalactose with TMSI, with the stannyl ether **4**. We tried the reaction in toluene at 80 °C using TBAI as the catalyst, and we obtained the orthoester **5** (Scheme 2), which is the product usually obtained when the reaction is driven in neutral conditions. Treatment of **5** with *tert*-butyldimethylsilyl triflate (TBDMSOTf)[21] afforded the β-*O*-glycoside **6** in 90% overall yield. In the absence of TBAI, and in similar conditions, the reaction doesn't evolve. When the reaction was carried out at room temperature, only the starting material was recovered and at 150 °C, decomposition products were observed in the crude reaction mixture. The reaction was then tried in CH_2Cl_2 at reflux in the presence of catalytic amounts of TBAI obtaining the orthoester **5**, which after treatment with TBDMSOTf gave **6** in 95% yield. Interestingly, the reaction also evolved at room temperature providing similar yields although in a longer reaction time. In both cases, the presence of TBAI was necessary for the reaction to take place.

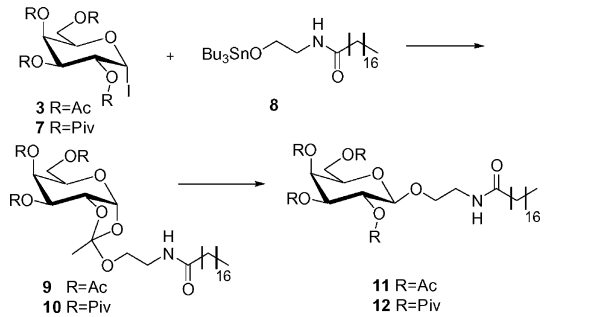
Finally, we tried the reaction with the benzyl alcohol as the glycosyl acceptor in boiling dichloromethane and toluene, and neither the glycosylated product or the orthoester were observed. It can be concluded that the use of TBAI and stannyl ether derivatives allow the efficient activation of disarmed glycosyl iodides affording the corresponding orthoester in excellent yields. This orthoester can be isomerized to the β-glycoside in excellent yields by using TBDMSOTf or $BF_3{\cdot}OEt_2$.

Scheme 3 shows the proposed mechanism of the reaction, which must start by the attack of an iodide anion on **3** to form the β-iodo-intermediate **I**.[22] In principle, it would be expected, according to the well known mechanism for orthoester formation, that intermediate **II** would be formed by intramolecular attack of the acetate group. But the fact that benzyl alcohol doesn't react in these conditions seems to indicate that the attack of iodide is faster, thereby making the reaction reversible. Then, it is reasonable to think that iodide, removed from **II** or from TBAI, will attack the tin in **II** or **III** to form the thermodynamically stable stannyl iodide, driving the reaction to the orthoester **5** (Scheme 3). Consequently, TBAI should have two functions, to form the reactive β-iodo-glycoside and make the reaction irreversible.[23,24]



Scheme 3 Proposed reaction mechanism ion.

Table 1 Synthesis of compound **11** by glycosylation of stannyl amide **8** with iodides **3** and **7** in the presence of TBAI followed by isomerization[a]



Entry	Solvent	Temp/°C	Yield (%)[b]	Ratio (α–β)
1	CH_2Cl_2	Rt	62	0 : 1
2	CH_2Cl_2	Reflux	88	0 : 1
3	Toluene	80	93	0 : 1
4[c]	Toluene	80	—	—

[a] Reaction conditions: **3** (1.2 mmol), **8** (1 mmol), Bu_4NI (0.10 mmol), toluene, 80 °C, 18 h and then $BF_3{\cdot}Et_2O$ (3 mmol), CH_2Cl_2 (20 mL), 0 °C, 30 min. [b] Yields of isolated product after chromatographic purification over two steps. [c] In absence of Bu_4NI.

Next we explored the reaction of amide **8**, a simplified model of ceramides, with glycosyl donor **3** (Table 1). The reaction was carried out in dichloromethane and toluene at different temperatures and in the presence or absence of TBAI. When the reaction was performed at room temperature in dichloromethane, the orthoester **9** was obtained, and further treatment with TBDMSOTf or $BF_3{\cdot}OEt_2$ afforded **11** in 62% yield over two steps (entry 1, Table 1). The yield increased to 88% (entry 2) when the reaction was heated to reflux. The best result, 93% yield, was obtained performing the reaction in toluene at 80 °C (entry 3). In this case the presence of TBAI was also necessary for the reaction to evolve (entry 4). These results contrast with those obtained in the glycosylation of ceramides using other common leaving groups such as trichloroacetimidate, iodide or bromide, where yields were lower than 50%.[15]

It has been reported that the use of pivaloyl protecting groups avoids the formation of the orthoester.[25] However, in our hands, when starting from pivaloyl protected donor **7**, this led to the formation of a mixture of orthoester **10** and β-glycoside **12** similar to that reported for related glycosyl donors.[26]

In order to examine the scope of the reaction we performed different experiments with stannyl ethers **13–15** (Table 2). The reaction of the stannyl derivative of ceramide analogue **13** with donor **3** was carried out under the optimized reaction conditions to initially give the corresponding orthoester which was treated with $BF_3{\cdot}Et_2O$ to afford glycolipid **16** in excellent yield (entry 1, Table 2). The azido-sphingosine and ceramide have two hydroxyl groups and we considered the possibility of protecting both as a stannyl acetal. Thus, compounds **14** and **15** were prepared by reaction of azido-sphingosine and ceramide with dibutyltinoxide with water exclusion. The glycosylation of **14** and **15** with α-iodogalactose **3** under the optimized conditions provided, after treatment with $BF_3{\cdot}OEt_2$, excellent yields of glycolipid **17** (entry 2) and galactosylceramide **18** (entry 3). Glycosylation of **15** with

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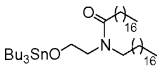
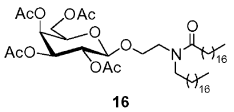
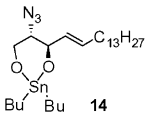
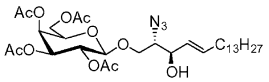
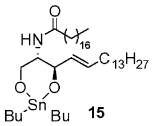
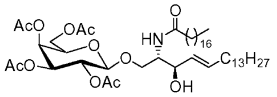
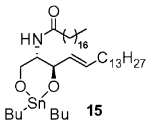
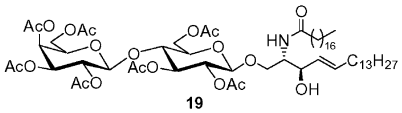
Table 2 Glycosylation of ceramide and ceramide analogues[a]

Entry	Acceptor	Glycolipid	Yield (%)[b]
1[c]	 **13**	 **16**	93
2[c]	 **14**	 **17**	94
3[c]	 **15**	 **18**	90
4[d]	 **15**	 **19**	90

[a] Reaction conditions: **3** (1.2 mmol), **13**, **14** or **15** (1 mmol), Bu_4NI (0.10 mmol), toluene, 80 °C, 18 h and then $BF_3{\cdot}Et_2O$ (3 mmol), CH_2Cl_2 (20 mL), 0 °C, 30 min. [b] Yields of isolated product after chromatographic purification over two steps. [c] **3** was used as glycosyl donor. [d] **20** was used as glycosyl donor.

hepta-*O*-acetyllactosyl iodide (**20**) also afforded corresponding glycolipid **19** in excellent yield. The overall process takes place with complete chemo and stereoselectivity. The yields obtained are close to those obtained by enzymatic procedures by using glycosyl fluorides as glycosyl donors.[25] In addition, when the reaction crudes were analyzed by ^{1}H NMR no α anomer or the undesired elimination product (glycal) were observed.

In conclusion, we have developed a new and highly efficient protocol for the glycosylation of ceramides consisting in the reaction of stannyl ethers with α-iodogalactose derivatives in the presence of TBAI as an activator. The procedure provides very efficient access to β-galactosyl-ceramide and derivatives, and is fully chemo and stereoselective. Because the ceramide acceptor does not need to be protected, and no special protecting groups in the donor are required, this protocol can be readily utilized for the simple and efficient preparation of glycolipids with important biological properties. Furthermore, this direct glycosylation protocol reduces the overall number of steps and provides a rapid access to complex target molecules.

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