Short Communication

# Simple method for high purity acylated steryl glucosides synthesis

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The present work covers an important gap in the field of analytic standards synthesis of the plants minor component, the acyl steryl glucosides (ASG). A simple and powerful procedure that allows the synthesis of different ASG has been introduced. The regioselective acylation of steryl glucosides on the C–6–OH of the glucose was produced using sym-collidine and acyl chloride. The reaction was optimized analyzing the conditions to maximize the isolated yield of the monoacylated product.

**Practical applications:** The identification and quantification of acyl steryl glucosides (ASG) and steryl glucosides (SG) is a difficult task and has become increasingly important in the food, oil and biodiesel industries. There is a lack of simple synthetic approaches to prepare ASG and commercial sources of those compounds as analytical standards are expensive and sometime do not have the required purity. In this paper we are introducing a simple, powerful and versatile procedure that allows the synthesis of different ASG in a rapid and efficient way, producing the products in high purity that will enable them to be use as standards.

**Keywords:** Acyl steryl glucosides / Phytosterols / Regioselective acylation / Steryl glucosides / Vegetable oil

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## 1 Introduction

Plant glycolipids is a group of compounds consisting mainly of steryl glucosides, sphingoglycolipids, and glyceroglycolipids [1]. Steryl glucosides (SG) can be found either as free molecules or esterified with fatty acids forming acylated steryl glucosides (ASG). SG are phytosterols conjugates where one glucose moiety binds at the C-3 position of the sterol residue and the anomeric carbon at the glucose. When the sugar moiety is acylated with a fatty acid in the C-6 hydroxyl group of the glucose, ASG are formed. Grille et al. [2] have recently reviewed the data on glycosylated plant sterols, and concluded that compared to other glycolipids or the other sterol conjugates, surprisingly little is known about the functions of glycosylated sterols in plants, as well as potential effects as minor food components. Until recently, the health benefits of glycosylated sterols have been questioned, as their metabolism in humans and other effects have been more or less unknown. Recent studies on glycosylated sterols of Lin et al. [3, 4] have demonstrated their potential health benefits as dietary components. That report has shown that SG decreased the absorption of cholesterol from the gut, being comparable to the decrease obtained with steryl esters. Lately, the same group focused on the effects of ASG in mice, demonstrating similar response in cholesterol absorption. Interestingly, the acyl group was cleaved from the ASG and the glycosidic bond remained intact in the digestive tract. Together, these studies introduced the potential of ASG as effective cholesterol lowering dietary component helping to decrease the risk of cardiovascular diseases [3, 4].

Quantification of these compounds is not an easy task. Although different GC and HPLC methods have been reported, most of them are highly time consuming and

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**Abbreviations: ASG,** acyl steryl glucosides; **PASG,** polyacylated SG; **SG,** steryl glucosides; **TLC,** thin layer chromatography; **TMS,** tetramethylsilane

 

required laborious sample preparation protocols by solid phase extraction [5, 6], preparative TLC [7], solvent extraction [8, 9], or combination of those [10]. One of the major drawbacks in ASG quantification is the lack of high purity standards in the market. According to the manufacturers, the commercially available standards of ASG are from natural sources, consisting of a mixture of esterified phytosteryl glucosides. Gomez Coca et al. [11] reported the analysis of commercial standard that were only 39% pure.

The synthesis of high purity ASG requires a very selective method to esterify the primary alcohol in the presence of the secondary alcohols from the glucose. In general terms, modern regioselective acylations can be achieved mainly through three different approaches. Generally those reactions provide mixtures of primary and secondary alcohols esterification products that are very difficult to isolate with high purity [12–14], One of the more conventional methods consists in using acyl chloride and a base. Pyridine [15] and collidine [16] are frequently used as non-sterically and sterically hindered bases, respectively. These reactions can be conducted using stoichiometric amount of the base and dichloromethane as solvent. Alternatively, bases can be used as solvent, being more adequate to dissolve high polar substrates. Temperature is another critical factor to achieve the expected selectivity. Another method to afford these products is the well-known Steglich esterification. This reaction has been used on the two step synthesis of the immunogenic bacterial glycolipid BbGL1 [12], a cholesteryl-(6′-O-palmitoyl)-β-D-galactopyranoside. In this report, the authors implement a very slow addition of the acylating reagent using a syringe pump and low temperature over the whole process, obtaining the product in moderate yield.

Finally, an enzymatic procedure reported by Paczkowski et al. [17] where immobilized lipase *Candida antarctica* was used to acylate SG with fatty acids to obtain ASG in variable yields depending on the nature of the acid.

As was mentioned, the preparation of steryl(6′-O-acylated)-β-D-glycosides is not an easy task, and we wanted to explore new and simple ways to prepare these important metabolites.

## 2 Materials and methods

### 2.1 General information

$^{1}H$ and $^{13}C$ NMR spectra were acquired on a Bruker Avance II 300 MHz (75.13 MHz) using $CDCl_3$ as solvent. Chemical shifts ($\delta$) were reported in ppm downfield from tetramethylsilane (TMS) at 0 ppm as internal standard and coupling constants ($\mathcal{J}$) are in hertz (Hz). Chemical shifts for carbon nuclear magnetic resonance ($^{13}C$ NMR) spectra are reported in parts per million relative to the center line of the $CDCl_3$ triplet at 76.9 ppm. The following abbreviations are used to indicate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, p = pentet, br = broad signal. IR spectra were obtained using an FT-IR Shimadzu spectrometer and only partial spectral data are listed. Melting points were measured on an Electrothermal 9100 apparatus and are uncorrected. Microwave heated reactions were conducted on a CEM Discover 300 W. GC-FID instrument description and methods are described in a separate section. Solvents were analytical grade or were purified by standard procedures prior to use. Yields were calculated for material judged homogeneous by thin layer chromatography (TLC) and nuclear magnetic resonance ($^{1}H$ NMR). All reactions were monitored by TLC performed on silica gel 60 $F_{254}$ precoated aluminum sheets, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 4-anisaldehyde. Column flash chromatography was performed using silica gel 60 (230–400 mesh). Collidine was anhydrated with sodium hydroxide lentils overnight followed by fractional distillation and stored under molecular sieves. All reagents were purchased from Sigma–Aldrich and used as received unless otherwise stated. The acyl chlorides were synthesized following the standard procedures [18].

### 2.2 Steryl glucosides

Reaction optimization was done with a mixture of steryl-β-D-glucosides (campesteryl-β-D-glucoside, stigmasteryl-β-D-glucoside, and β-sitosteryl-β-D-glucoside (1:1:2)) obtained from local plant biodiesel residues. These compounds were purified and isolated following the washing/centrifugation procedure reported by Bondioli [19]. Cholesteryl β-D-glucoside was purchased from Sigma–Aldrich.

### 2.3 General procedure for regioselective acylation under conventional heating conditions

0.17 mmol of SG were dissolved in 2.5 mL of *sym*-collidine. Then 0.26 mmol of acyl chloride were slowly added to the mixture at room temperature. After 20 min under continuous stirring at the desired temperature (80–160°C), the reaction was diluted with AcOEt (10 mL) and quenched with 10% HCl (10 mL). Centrifugation could improve the interface separation. The inorganic phase was extracted with AcOEt ($2 \times 10$ mL). The combined organic extracts were dried with $Na_2SO_{4(anh.)}$ and evaporated under vacuum.

### 2.4 General procedure for regioselective acylation under microwave heating conditions

0.17 mmol of SG were dissolved in 2.5 mL of *sym*-collidine. Then 0.38 mmol of acyl chloride were slowly added to the mixture at room temperature in a microwave vial, sealed, introduced in the reactor, and heated at constant temperature (160°C) for 3 min. Then the reaction mixture was diluted with AcOEt (10 mL) and quenched with 10% HCl

 

(10 mL). When was necessary centrifugation was used to improve the interface separation. The inorganic phase was extracted with AcOEt (2 × 10 mL). The combined organic extracts were dryed with $Na_2SO_{4(anh.)}$ and evaporated under vacuum.

#### 2.4.1 Purification

The organic reaction crude was purified by flash column chromatography with DCM:MeOH 1% gradient. (Purified yield: 35–45%).

#### 2.4.2 SG recovery from polyacylated fractions

The polyacylated steryl glucosides formed during the reaction were recovered from the early fractions of the flash column chromatography during ASG purification. After evaporation, the obtained solid was submitted to methanolysis to produce free steryl glucoside.

Hundred milligrams of polyacylated steryl glucosides were dissolved in anhydrous THF:MeOH (1:1) (5 mL) and 200 μL of sodium methoxide solution (5 mg Na in 500 μL of MeOH $_{(anh)}$) were added. After 3 h at RT, TLC analysis (hexane/ethyl acetate 70:30) shows the absence of the acetylated starting material. On the other hand, the presence of the product was observed by TLC in a highly polar mobile phase (DCM:MeOH 90:10). The reaction was then quenched with equivalent amount of AcOH. Solvent was evaporated and crude product directly purified by flash column chromatography with DCM:MeOH gradient.

### 2.5 GC-FID analysis

SG reaction mixtures were quenched with 1 mL of MeOH and a 10 μL aliquot dried under a nitrogen stream. Pyridine (200 μL) and MSTFA (200 μL) were added and the mixture incubated at 80°C for 2 h. Reaction was allowed to cool down, diluted with 1.6 mL of n-heptane and injected into the GC system (1 μL).

#### 2.5.1 GC-FID method

Gas chromatography analyses of the free and acylated steryl glucosides were carried out with a Varian GC450 Gas Chromatograph equipped with a flame ionization detector. Data was acquired with the Varian ChemStation for GC system program. The conditions for the GC assays were: DB-5HT column (5% diphenyl-95% dimethylpolysiloxane; 15 m × 0.32 mm ID × 0.10 μm film), 1.0 μL injection volume, hydrogen carrier gas at 30 mL/min, and cooled on-column injection. The oven temperature program was: 190°C (1 min), 15°C/min to 210°C (4 min), 15 °C/min to 230°C (4 min), and 30°C/min to 380°C (9 min). The detector temperature was 380°C.

### 2.6 ESI-HRMS analysis

High-resolution mass spectra were recorded on a MicroTOF II spectrometer (Bruker Daltonics, Bremen, Germany) with sample introduction performed by a syringe pump (KD Scientific, KDS 100) and an ESI source operating in a positive mode. General conditions were as follows: dyring gas temperature of 180°C, drying gas flow, 4 L/min capillary voltage of 4.5 kV, and nebulizing gas pressure, 0.4 bar. Mass spectra were acquired by scanning along the m/z 300–3000 range. The samples were diluted in acetonitrile to 50 μg/mL without any additives and loaded into a 500 μL volume Hamilton gastight 1700 series syringe operating at a flow rate of 3 μL/min. The accurate mass data of the molecular ions were processed through the software DataAnalysis 4.0 (Bruker Daltonics).

### 2.7 Products characterization

#### 2.7.1 Cholesteryl-(6′-O-palmitoyl)-β-D-glucopyranoside

*Creamy white solid*

IR 3990, 3545, 3446, 3421, 3408, 2954, 2933, 2885, 2868, 1674, 1473, 1332, 1282, 1253, 1166, 1070, 1031, 839, 680, 640 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 5.34 (br, 1H, C5 sterol), 4.48–4.21 (m, 3H, $CH_2$-6′, CH-1′), 3.63–3.22 (m, 4H, 3CH–OH glucose, CH C3 cholesterol), 2.3 (m, 4H, –C**H**$_2$–C=O–, –$CH_2$– C4 cholesterol), 2.09–1.73 (m, 5H, $CH_2$), 1.72–1.39 (m, 9H, $CH_2$, CH), 1.38–1.19 (m, 25H, $CH_2$), 0.99 (s, 3H, $CH_3$), 0.92 (s, 3H, $CH_3$), 0.89 (S, 3H, $CH_3$), 0.87 (br, $J = 7.2$ Hz, 3H, CH3), 0.85 (br, 3H, $CH_3$), 0.67 (s, 3H, $CH_3$).

$^{13}C$ NMR ($CDCl_3$) δ 174.2, 140.3, 122.1, 101.3, 79.8, 76.2, 72.2, 73.7, 73.4, 70.4, 63.6, 56.7, 56.3, 50.1, 42.3, 39.7, 39.8, 39.5, 38.9, 37.3, 36.7, 36.2, 35.8, 34.3, 31.9, 31.8, 29.8, 29.7, 29.5, 29.4, 28.2 28.0, 25.0, 24.3, 23.9, 22.8, 22.7, 22.6, 21.1, 19.3, 18.7, 14.1, 11.8. HRMS calc for $C_{49}H_{86}O_7Na^+$ $[M + Na]^+$ 809.6266, found 809.62516.

#### 2.7.2 Steryl-(6′-O-oleoyl)-β-D-glucopyranoside

(Mixture of campesteryl-(6′-O-oleoyl)-β-D-glucopyranoside, stigmasteryl-(6′-O-oleoyl)-β-D-glucopyranoside, and β-sitosteryl(6′-O-oleoyl)-β-D-glucopyranoside.)

*Yellowish oil*

IR 3990, 3446, 3421, 3408, 2954, 2933, 1670, 1473, 1253, 1031, 839, 680, 640 $cm^{-1}$. $^1H$ NMR ($CDCl_3$) δ 5.35 (m, 3H, C5 sterol, –CH=CH– oleoyl double bond), 5.02–4.97 (m, 2H stigmasterol exocyclic double bond), 4.44–4.24 (m, 3H, $CH_2$-6′, CH-1′), 3.63–3.22 (m, 4H, 3C**H**–OH glucose, CH C3 sterol), 2.3 (m, 4H, –C**H**$_2$–C=O–, –$CH_2$– C4 sterol),

 

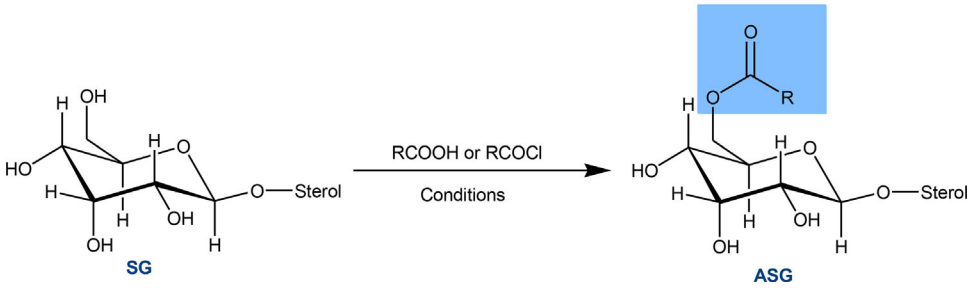


**Figure 1.** Synthetic strategy to obtain high purity ASG.

2.11–1.74 (m, 9H, $CH_2$), 1.73–1.39 (m, 11H, $CH_2$, CH), 1.38–1.21 (m, 22H, $CH_2$), 0.99 (m, 4H, $CH_3$),), 0.95–0.72 (m, 14H, $CH_3$), 0.67 (s, 3H, $CH_3$).

$^{13}$C NMR ($CDCl_3$) δ 174.2, 140.3, 138.1, 130.0, 129.7, 122.1, 101.3, 79.7, 76.2, 73.7, 73.3, 70.4, 63.6, 56.8, 56.2, 51.2, 50.2, 45.8, 42.3, 39.8, 38.9, 37.3, 36.7, 36.2, 34.3, 33.9, 31.9, 30.4, 29.8, 29.7, 29.5, 29.3, 29.2, 29.1, 28.2, 27.2, 26.2, 25.0, 24.3, 23.1, 22.7, 21.3, 21.1, 19.8, 19.4, 19.0, 18.83, 18.7, 14.1, 12.2, 12.0, 11.9, 11.8.

*HRMS*

Campesteryl-(6′-O-oleoyl)-β-D-glucopyranoside: calc for $C_{52}H_{90}NaO_7^+$ $[M+Na]^+$ 849.65788, found 849.65470.
Stigmasteryl-(6′-O-oleoyl)-β-D-glucopyranoside: See supporting information.
β-Sitosteryl-(6′-O-oleoyl)-β-D-glucopyranoside: calc for $C_{53}H_{92}NaO_7^+$ $[M+Na]^+$ 863.67353, found 863.66819.

### 2.7.3 Steryl(6′-O-palmitoyl)-β-D-glucopyranoside

(Mixture of campesteryl-(6′-O-palmitoyl)-β-D-glucopyranoside, stigmasteryl-(6′-O- palmitoyl)-β-D-glucopyranoside, and β-sitosteryl(6′-O-palmitoyl)-β-D-glucopyranoside.)

*Creamy white solid*

IR 3990, 3446, 3421, 3408, 2954, 2933, 1670, 1473, 1253, 1031, 839, 680, 640 $cm^{-1}$. $^1$H NMR ($CDCl_3$) δ 5.35 (br, 1H, C5 sterol), 5.02–4.97 (m, 2H stigmasterol exocyclic double bond), 4.48–4.21 (m, 3H, $CH_2$-6′, CH-1′), 3.63–3.22 (m, 4H, 3C**H**–OH glucose, CH C3 sterol), 2.3 (m, 4H, –C**H$_2$**–C=O–, –$CH_2$– C4 sterol), 2.09–1.73 (m, 5H, $CH_2$), 1.75–1.39 (m, 9H, $CH_2$, CH), 1.38–1.16 (m, 26H, $CH_2$), 1.16–0.75 (m, 28H, $CH_2$, $CH_3$), 0.67 (s, 3H, $CH_3$).

$^{13}$C NMR ($CDCl_3$) δ 174.2, 140.3, 138.1, 129.7, 122.1, 101.3, 79.7, 76.2, 73.7, 73.3, 70.4, 63.6, 56.8, 56.2, 51.2, 50.2, 45.8, 42.3, 39.8, 38.9, 37.3, 36.7, 36.2, 34.3, 33.9, 31.9, 30.4, 29.8, 29.7, 29.5, 29.4, 29.1, 28.2, 26.2, 25.0, 24.3, 23.1, 22.7, 21.1, 19.8, 19.4, 19.0, 18.8, 14.1, 12.2, 12.0, 11.9, 11.8.

*HRMS*

Campesteryl(6′-O-palmitoyl)-β-D-glucopyranoside: calc for $C_{50}H_{88}NaO_7^+$ $[M+Na]^+$ 823.64223 found 823.64125.
Stigmasteryl(6′-O-palmitoyl)-β-D-glucopyranoside: See supporting information.
β-sitosteryl(6′-O-palmitoyl)-β-D-glucopyranoside: calc $C_{51}H_{90}NaO_7^+$ $[M+Na]^+$ 837.65788 found 837.65600.

## 3 Results and discussion

Based on the reported reagents and conditions to prepare ASG, different approaches were explored, leaving behind the enzymatic reactions. To optimize the reaction, accessible natural SG was used. Residues from soybean oil biodiesel plants contain a mixture of steryl β-D-glucosides (campesteryl-β-D-glucosides, stigmasteryl-β-D-glucosides, and *β*-sitosteryl-β-D-glucosides (1:1:2)). Tank deposits from biodiesel local factories were purified, isolating pure SG following the washing/centrifugation procedure reported by Bondioli [19]. First it was washed with hexane and then methanol was used to remove all traces of polar compounds. The acylation optimization (Fig. 1) was then optimized following reported procedures.

Initially, the Steglich esterification reaction with DCC and DMAP was attempted using different anhydrous solvents and temperatures. Unfortunately, no product signals appeared in the GC-FID using DCM, THF, or AcCN going from low temperature to reflux. Having in mind that the SG was not soluble in those solvents, a reaction using anhydrous pyridine as a solvent was also attempted, but again no product was identified. As a conclusion of these initial experiments it was clear that SG solubility is a key limitation in this reaction. In fact, the solubility behavior of SG is very limited, being practically insoluble in the majority of the common organic solvents, with the exception of pyridine and mixture of organic solvents (THF:MeOH and DCM:MeOH). On the other hand, the reaction product (ASG) is significantly more soluble in the mentioned organic solvents.

To continue, it was necessary to explore reactions that are commonly conducted on the solvents where SG are soluble. One of those reaction is the regioselective acylation

 

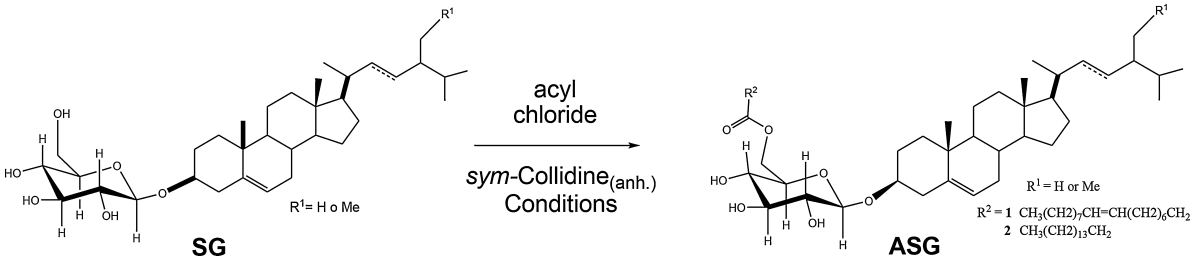


**Figure 2.** Synthetic strategy using collidine as sterically hindered base.

method [16], using a high polar, non-nucleophilic base, like *sym*-collidine as solvent, that is also sterically hindered (Fig. 2). Following reported procedure [16], the reaction on SG using palmitoyl chloride as acylating reagent was tested. The required selectivity is usually achieved at low temperature. Initial attempts were assayed between −40 to 0°C without producing a noticeable progress. When the reaction was conducted at room temperature promising results were obtained. Thus, reaction optimization was followed looking to improve the conversion, selectivity, yield, and to minimize the reaction time.

Conversion and products distribution on different reaction conditions were analyzed by GC-FID. The main variables of the reaction were optimized analyzing the reaction progress looking the product/starting material ratio (ASG/SG). The amount of palmitoyl chloride was increased from 1.5 to 5.0 equivalents, reaction time and temperature varied from 3 min to 72 h and room temperature to 160°C, respectively. The results are summarized in Table 1.

Entries 1 to 3 show the effect of the equivalent of palmitoyl chloride. A correlation was observed between the equivalents of acylating reagent and the isolated yield. When 1.5 or 2 equivalents of palmitoyl chloride were used (Entry 1 and 2) free SG was remaining. Increasing the acylating equivalents to 3, produced the total conversion of the SG without improving the isolated yield (Entry 3, Fig. S1). A new set of experiments was conducted to determine the time dependence of the conditions studied before. Entries 4–6 followed the same conditions than Entries 1–3 increasing the time to 72 h. When 1.5 equivalents were used (Entry 4) the isolated yield was 2.2 times higher, showing a progress on the reaction over the extended time. Increasing the equivalents of acylating reagent to 2 and 3 did not improve the isolated yield even when remaining SG was still present.

**Table 1.** Optimization of acylation conditions

| Entry | SG:Palm-Cl (Eq) | T (°C) | Time (h) | Isolated yield (%) |
|---|---|---|---|---|
| 1 | 1:1.5 | RT | 24 | 13 |
| 2 | 1:2 | RT | 24 | 28 |
| 3 | 1:3 | RT | 24 | 35 |
| 4 | 1:1.5 | RT | 72 | 29 |
| 5 | 1:2 | RT | 72 | 30 |
| 6 | 1:3 | RT | 72 | 31 |
| 7 | 1:1.5 | 80 | 40 min | 38 |
| 8 | 1:1.5 | 160 | 40 min | 45 |
| 9 | 1:3 | 160 | 40 min | PASG |
| 10 | 1:1.5 | 160 (MW) | 3 min | 38 |
| 11 | 1:1.8 | 160 (MW) | 3 min | 41 |
| 12 | 1:2.2 | 160 (MW) | 3 min | 42 |
| 13 | 1:3 | 160 (MW) | 3 min | PASG |
| 14 | 1:5 | 160 (MW) | 3 min | PASG |

The conclusion of the previous experiments demonstrated that after 24 h not all acylating agent was consumed and with more than 1.5 equivalents, the amount of polyacylated SG (PASG) rapidly increased without enhancing the ASG yield. In summary, the yield of isolated ASG is highly dependent on the stoichiometric amount of the acylating agent. At this point, we wanted to study the effect of the reaction temperature on the product distribution and isolated yield. The previous experiments demonstrated that the selectivity was not noticeable affected by increasing the temperature over 60°C range, what prompts us to test the reaction between room temperature and reflux. A time course of the reaction using 1.5 equivalents of the acylating reagent at 80°C was followed by GC-FID, concluding that 20 min is enough time to consume the entire acylating reagent leaving unreacted SG (Fig. S2). Increasing the temperature to reflux conditions produced the expected ASG in 45% yield in 40 min reaction (Entry 8). Then, the acylating equivalents were doubled producing a complete conversion of the SG, but only PASG were produced (Entry 9).

In summary, performing the reaction at higher temperature reduced the reaction time leading to better yields without affecting the selectivity.

Additionally, microwave irradiation was also examined looking to reduce the reaction time and to improve the yield. When 1.5 equivalents of acylating agent were used and the reaction was irradiated at 160°C for 3 min, unreacted SG were recovered (Entry 10). When the reaction time was extended to 10 min, similar results were obtained, showing again that starting material conversion is dependent on the acylating agent equivalents used. Then, a set of new reactions

 

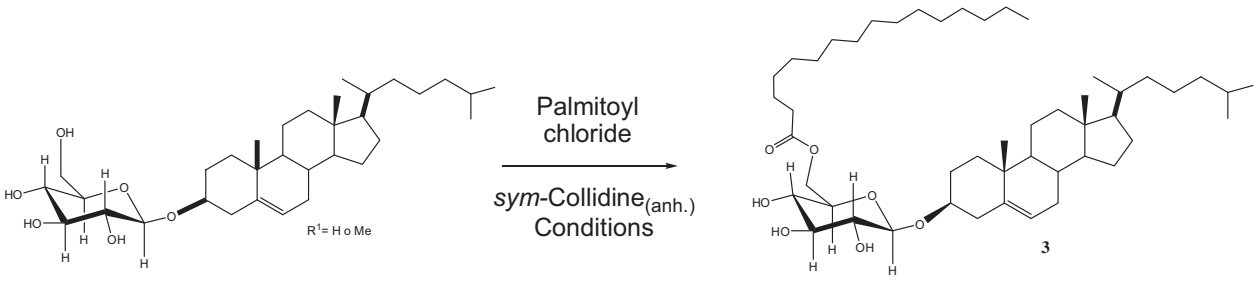


**Figure 3.** Synthesis of cholesteryl-(6′-O-palmitoyl)-β-D-glucopyranoside.

was analyzed increasing the equivalents of palmitoyl chloride from 1.5 to 5. The yield of the reactions using 1.8 and 2.2 equivalents were similar (around 41% yield, Entries 11 and 12). Finally, when more equivalents were used only PASG were obtained as was expected. (Entries 13 and 14**).**

The reaction byproduct, the PASG, can be easily isolated during the product purification by flash column chromatography. After separation, the SG can be recovered by methanolysis of the non-polar PASG.

In summary, there is a noticeable reaction time reduction when microwave heating was implemented being able to rich the same results than conventional heating in only 3 min.

Once the conditions were optimized, the substrate scope of the reaction was studied.

First, the nature of the sterol was analyzed using cholesteryl β-D-glucoside as substrate (Fig. 3). The optimized reaction conditions were implemented using palmitoyl chloride in *sym*-collidine, obtaining an isolated yield of 35% of the compound **3** that can be used as a new high purity standard (Fig. 4).

Also, different fatty acids have been analyzed in order to show the scope of the methodology introduced. Using SG again as starting material, steryl(6′-O-oleoyl)-β-D-glucopyranoside was prepared using oleoyl chloride as acylating agent. The product was obtained with an isolated yield of 36% demonstrating the reaction is independent of nature of the fatty acids.

$^{1}$H NMR data of all the synthesized final products can be unequivocally characterized by the shift of the C6′-H signal from 3.50 ppm on the steryl glucosides to 4.30 ppm due to the acylation of that position and an intense signal at 1.26 ppm due to the methylenes of the fatty acids. Additionally, they showed a broad signal at 5.34 ppm corresponding to the sterol proton endocyclic double bond, the acetalic C1′-H of the glucose at 4.30 ppm and the C3-H of the sterol at 3.55 ppm.

The mixture of steryl glucosides contains stigmasterol that is identified by a multiplet between 5.25 and 4.90 ppm corresponding to the exocyclic double bound. The oleoyl derivatives were identified by a multiplet at 5.35 ppm corresponding to Δ9 double bond.

Regioselectivity of the reaction was clearly demonstrated analyzing the products by HMBC experiments looking for the interaction of C6′-H of the glucose with the carbonyl carbon of the fatty acid (Fig. S3).

Additionally to the NMR experiments, all the products presented an intense band on IR in the region 1670 $cm^{-1}$ corresponding to the ester carbonyl group. Compound molecular formula were confirmed by HRMS ESI-TOF as the sodium adducts of the synthesized products.

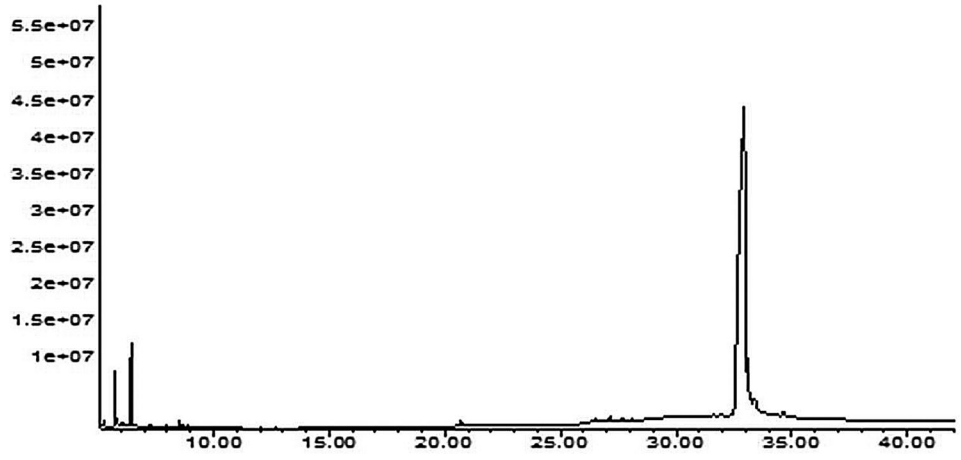


**Figure 4.** GC-FID chromatogram of the cholesteryl(6′-O-palmitoyl)-β-D-glucopyranoside.

 

## 4 Conclusions

A general methodology to synthesize any ASG requires a stereo-controlled glycosylation of the sterol and a regioselective acylation of SG. While there are different robust reactions that allowed selectively obtaining SG, their acylation have been barely studied. Starting from steryl glucosides mixtures, a simple method of ASG synthesis was developed. It was observed that the isolated yield is markedly dependant on the equivalents of acylating agent and microwave heating considerably reduce the reaction time. The scope of the reaction was demonstrated using different fatty acid chlorides and SG mixtures. Finally, the preparation of a pure single ASG was achieved preparing cholesteryl-(6′-O-palmitoyl)-β-D-glucopyranoside showing this method as a very useful tool for the regioselective synthesis of high purity ASG. The prepared compounds are clear examples of the versatility of the methodology introduced, allowing synthesizing any ASG if it is combined with known glycosylation reactions. The new procedure fulfilled an important gap on the synthesis of these plants minor components that can be used in the oleochemical and food industries as analytical standards or even as new cholesterol lowering agents.

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