Synthesis of a Mannosyl-derived Glycolipid

CATALINA IONESCU1,*, VÉRONIQUE BARRAGAN-MONTERO2, JEAN-LOUIS MONTERO2

1 University of Craiova, Faculty of Chemistry, 107 I Calea București, 200144 Craiova, Romania
2 Equipe Glycochimie, Institut des Biomolécules Max Mousseron, UMR 5247 CNRS- UMI-UM2, ENSCM, CC 435, 8 rue de l’Ecole Normale, 34296 Montpellier Cedex 5, France

A synthetic route for the preparation of a mannosyl-derived glycolipid is described in a four-step reaction sequence starting from peracetylated mannose. The glycosylation step between 2,3,4,6-tetra-O-acetyl-α-D-mannopyranose trichloroacetimidate and 8-(Cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-ol was realized using Schmidt’s procedure, in the presence of BF₃.Et₂O as Lewis acid. The major glycosylation product was isolated and was identified as being the expected O-glycosylated product, which afforded the desired glycolipid after removal of acetyl protecting groups.

Keywords: glycolipid, mannose, TEG-Cholesteryl, Schmidt’s glycosylation

Liposomes are self-assembled vesicles with wide applications, e.g. in the active targeting of specific cell types and tissues or as drug carrier systems [1]. The special properties of liposomes in active targeting are due to their ability of being coated with various ligands that can be selectively recognized by cellular receptors. Although a number of papers described the covalent binding of ligands to liposomes, [2] most of the ligands are anchored in the lipid bilayer of liposomes through hydrophobic interactions. These ligands are surfactants with cholesterol [3] or alkyl chains (C₁₆ or C₁₈) as lipophilic part [4].

In previous papers, we have reported the preparation of "intelligent liposomes" coated with carbohydrate derivatives, able to recognize tissues or cell lines: breast cancer cells,[5] keratinocytes and skin sections [6]. The targeting of breast cancer cells (MCF-7 line) overexpressing the mannose 6-phosphate/insulin like growth factor-II receptor (M₆-P/IGF-II receptor) [7] was based on their interactions with liposomes containing mannose 6-phosphonate residues (M₆-Pₙ). The M₆-Pₙ residues linked to a cholesterylsuccinylanilinyl moiety formed synthetic amphiphilic molecules anchored in liposomes through hydrophobic interactions. Because degradation problems were encountered during the synthesis of this molecule, the preparation of a similar ligand was proposed, in which the succinylanilinyl spacer arm was replaced by a more stable triethyleneglycol moiety [8].

Our ongoing efforts in this direction focus on studying the influence of the carbohydrate moiety on the recognition process. Thus, we decided to synthesize an amphiphilic molecule with a non-functionalized mannose as hydrophilic head, for comparative recognition studies with the previously obtained M₆-Pₙ derivative. The target compound is Mann - TEG - Chol (Mannose - Triethylene-glycol – Cholesteryl) [9] (1) (fig. 1).

Results and discussions

Our strategy for the synthesis of Mann-TEG-Chol (1) consists in the coupling of mannose to the TEG-Chol moiety. The latter was obtained by refluxing cholesteryl toluenesulfonate and triethyleneglycol in dioxane (scheme 1) [10].

![Scheme 1. Synthesis of TEG-Chol (4). Reagents, conditions and yields: (i) tosyl chloride, pyridine, 12h at r. t., 90%; (ii) triethyleneglycol, dioxane, 2h at refluxing temperature, 72%](image)

Firstly, we tested the direct coupling of acetylated mannose with TEG-Chol, using boron trifluoride diethyletherate as Lewis acid (scheme 2). Although several experimental conditions were tested (Table 1), we failed to realize the glycosylation using this method. Firstly (entry 1), we used the conditions previously described for the glycosylation of acetylated mannose with a more stable compound, bromoethanol [11]. In our case, only decompositions in the reaction mixture were detected, even increasing the quantity of glycoside acceptor (entry 2). In order to use milder conditions, an attempt was also realised with only 2.5 equivalents of boron trifluoride diethyletherate (entry 3), but the same problems were encountered. We mention that the desired compound might have been present in a very little percent in the resulted mixture of products, but we were not interested...
in obtaining it in extremely little yields. Anyways, the fact that, although very short, this method is not a convenient route in the synthesis of this type of compounds is confirmed by the results reported in [12]. The researchers obtained 11-(Cholest-5-en-3b-yloxy)-3,6,9-trioxaundecanyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside using this method in only 3% yield.

Finally, we succeeded to realize the glycosylation reaction using Schmidt’s procedure [13], which employs only a catalytic amount of B,F$_3$.Et$_2$O, as the sugar is strongly activated as a trichloroacetimidate (scheme 3). In order to obtain the activated carbohydrate, we used peracetylated mannose as the starting material, which was selectively deprotected at its C1 position [14]. Afterwards, the trichloroacetimidate was prepared by addition of 2,3,4,6-tetra-O-acetyl mannopyranose to trichloroacetonitrile, in the presence of a catalytic amount of DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene) [15]. The trichloroacetimidate was subject to Schmidt’s glycosylation procedure. The major reaction product was isolated in 54% yield and it was identified as being the expected O-glycosylated compound. This represents one of the major advantages of this method, when compared to previously published results, [8] when the glycosylation reaction using Koenigs-Knorr’s procedure led to an orthoester in moderate yield. The latter was subject to isomerisation and afforded the desired O-glycoside, but the yields of this reaction were small when applied to this kind of compounds. Nevertheless, the target amphiphilic glycolipid (1) was obtained after deprotection of the acetyl protecting groups of (6) with sodium methoxide in methanol, with 37% overall yield stating from (5).

### Experimental part

Compounds were visualized on TLC plates using diluted 10% aqueous sulfuric acid solution or anisaldehyde solution, followed by heating. NMR spectra have been recorded on a Bruker DRX-400 spectrometer working at 400.13 MHz for $^1$H and 100.62 MHz for $^{13}$C. Chemical shifts are reported in ppm relative to residual CHCl$_3$ signals (δ = 7.26 ppm in $^1$H NMR spectrum and δ = 77.00 ppm in $^{13}$C NMR spectrum). Coupling constants (J) are measured in Hertz. Assignments given for the NMR spectra are based on COSY, HMQC pulse sequences and, in order to identify the type of different carbon atoms, C13DEPT135 sequences have been

<table>
<thead>
<tr>
<th>Entry</th>
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<td>1.2 eq.</td>
<td>2.5 eq.</td>
<td>O°C, ultrasounds</td>
<td>Decompositions</td>
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Table 1

EXPERIMENTAL CONDITIONS USED FOR THE GLYCOsyLATION TESTS OF PERACETYLATED MANNOSE (5) WITH TEG-CHOL (4)

Scheme 2. Attempt of direct coupling of acetylated mannose with TEG-Chol

Scheme 3. Synthesis of the target glycolipid 1. Reagents, conditions and yields: (i) Morpholine, CH$_2$Cl$_2$, 1h at r.t., 88%; (ii) Cl$_3$CCN, CH$_2$Cl$_2$, 1h at 0°C, then DBU, 30 min at 0°C, 82%; (iii) CH$_2$Cl$_2$, 30 min at 0°C, then BF$_3$.Et$_2$O, 30 min at 0°C and 1h at r.t., 54%; (iv) MeONa/MeOH, 30 min at r.t., 95%.
8-(Cholest-5-en-3-β-yloxy)-3,6-dioxaoctan-1-ol (4): Triethyleneglycol (50 mL, 369.79 mmol) was added to a solution of 3 (5g, 9.24 mmol) in dioxane (38 mL). The mixture was heated at refluxing temperature for 2 hours.

The reaction was monitored by TLC (petroleum ether/ethyl acetate 1/9). Afterwards, the reaction mixture was cooled at room temperature and poured into water. The aqueous phase was extracted three times with ethyl acetate. The organic phases put together were washed with NaHCO₃, water and brine and then dried (MgSO₄), filtered and concentrated. The compound was obtained as transparent oil and could be used without any further purification.

These atoms cannot be distinguished.

8-(Cholest-5-en-3-β-yloxy)-3,6-dioxaoctan-1-yl (5): A solution of 8 (5.21 g, 10.57 mmol) and 4 (5.49 g, 10.57 mmol) in anhydrous methylene chloride (85 mL) was stirred at -8°C for 30 min. Then, BF₃·Et₂O (0.50 mL, 4.08 mmol) was added and the mixture was stirred at -8°C for 30 min and at room temperature for other 30 min. The glycosylation reaction was monitored by TLC (petroleum ether/ethyl acetate 1/4). After complete consumption of the starting material, 20 mL of triethylamine were added and the reaction mixture was diluted with methylene chloride and washed with NaHCO₃ saturated solution. The
aqueous layer was extracted with methylene chloride and the organic phases put together were washed with brine, dried (Na$_2$SO$_4$), filtered and concentrated. The mixture was purified by silica gel column chromatography (petroleum ether/ethyl acetate 5/4) to afford the product as a transparent gum in 54% yield. R$_f$=0.26 (petroleum ether/ethyl acetate 6/4); SM : (ESI$^+$/MeOH) m/z : 871.5 [M+Na$^+$]$^+$; 887.4 [M+K$^+$]$^+$; (ESI$^-$/MeOH) m/z : 883.4 [M+Cl]$^-$; NMR$^H$ (400.13 MHz, CDCl$_3$) $\delta$ (ppm): 0.64 (s, 3H, H$_{18}$); 0.85 (d, 3H, H$_{25}$-$^{133}$O-); 0.88 (d, 3H, H$_{27}$-$^{133}$O-); 0.91 (d, 3H, 3JH$_{21}$H$_{20}$=6.4 Hz, H$_{21}$); 0.96 (s, 3H, H$_{19}$); 1.96, 2.01, 2.07, 2.12 (4s, 12H, -COH$_2$); 4.84 (d, 1H, 3JH$_{6}$H$_{5}$=5.1Hz, 2JH$_{6}$H$_{6}$=-12.4 Hz, H$_6$); 4.27 (dd, 1H, 3JH$_{6}$H$_{5}$=2.2Hz, 2JH$_{6}$H$_{6}$=-12.3 Hz, H$_6$); 4.06 (dd, 1H, 3JH$_{6}$H$_{5}$=2.2Hz, 2JH$_{6}$H$_{6}$=-12.3 Hz, H$_6$); 4.27 (dd, 1H, 3JH$_{6}$H$_{5}$=5.1Hz, 2JH$_{6}$H$_{6}$=-12.4 Hz, H$_6$); 4.84 (d, 1H, 3JH$_{6}$H$_{5}$=1.6 Hz, H$_5$); 5.24 (dd, 1H, 3JH$_{6}$H$_{5}$=1.8 Hz, 3JH$_{6}$H$_{6}$=3.4 Hz, H$_6$); 5.25-5.35 (m, 2H, H$_2$ and H$_3$); 5.29-5.33 (m, 1H, H$_4$); NMR $^{13}$C (100.62 MHz, CDCl$_3$) $\delta$ (ppm) : 11.8 (C$_{18}$); 18.7 (C$_{17}$); 19.3 (C$_{16}$); 20.6, 20.7 (2C) and 20.8 (-COH$_2$); 21.0 (C$_1$); 22.5 and 22.8 (C$_{26}$ and C$_{27}$); 23.8 (C$_{23}$); 24.3 (C$_{15}$); 28.0 (C$_7$ and C$_8$); 31.9 (C$_{20}$); 35.7 (C$_{22}$); 35.8 (C$_{20}$); 36.2 (C$_{22}$); 36.8 (C$_{10}$); 37.2 (C$_1$); 39.0 (C$_{4'}$); 39.5 (C$_{24'}$); 39.8 (C$_{12'}$); 42.2 (C$_{13'}$); 50.1 (C$_{9'}$); 56.2 (C$_{17'}$); 56.7 (C$_{16'}$); 60.6 (C$_5$); 66.0, 70.5, 71.1 and 72.3 (C$_{2'}$, C$_{3'}$, C$_{4'}$, C$_{21}$ and C$_{22}$); 66.7, 67.2, 70.1, 70.5 (2C) and 70.8 (-CH$_2$O-); 79.5 (C$_6$); 100.2 (C$_1$); 121.6 (C$_2$); 140.8 (C$_8$).

* These atoms cannot be distinguished.

Conclusions

In this paper, we describe the preparation of a glycolipid containing mannose as hydrophilic head. This surfactant was obtained using peracetylated mannose as the starting material (37% yield over four steps). The key-step of the synthesis was the glycosylation reaction realized using Schmidt’s procedure. An advantage of this method is represented by the fact that the major glycosylation product is the expected O-glycoside and not the orthoester, as proved by $^1$H and $^{13}$C NMR analysis. The obtained compound will be used in biological tests in order to study the recognition of liposomes coated with non-functionalized mannose by the M6-P/IGF-II receptor.

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References