Synthesis & biological applications of glycosylated iminosugars

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Synthesis of α- and β-Cholesteryl Glucosides

6.1 Introduction

Steryl glycosides are abundant in nature.[1] For example the gram-negative bacterium *Helicobacter pylori*, a common human pathogens which is known to cause ulcers contains several steryl glycosides, such as cholesteryl-6-*O*-acyl-α-D-glucopyranoside, cholesteryl-α-D-glucopyranoside and cholesteryl-6-*O*-phosphatidyl-α-D-glucopyranoside.[2] Additionally various steryl-β-glucosides are synthesized by plants, including sitosteryl-β-glucoside which can serve as primer in the biosynthesis of cellulose.[3] Studies reporting on the occurrence of endogenous cholesterol-β-glucoside in mammals are surprisingly scarce. Only Murakami-Murofushi and co-workers have reported on the formation of cholesterol-β-glucoside in cultured fibroblasts as a rapid response to heat stress.[4] Very recently they presented evidence that glucosylceramide (GC) and not UDP-glucose acts as a sugar donor in the biosynthesis of cholesteryl glucoside.[5] The enzyme responsible for the synthesis of cholesterol-β-glucoside in man has not yet been identified.

The limited knowledge on endogenous cholesterol-β-glucosides in mammals is surprising since there are numerous speculations that steryl glucosides may act as (neuro)toxins. An example thereof is the neurological disorder amyotrophic lateral sclerosis-parkinsomism dementia complex (ALS-PDC) in which features of parkinsomism are presented and which is linked to the consumption of flour made from cycad fruits (*Cycas micronesica*) that is known to contain a high concentration of steryl glycosides.[6–9]

Parkinsonism and glucosylceramide metabolism also appear to be linked given, the high incidence of neurodegenerative conditions in Gaucher disease patients.[10] Gaucher disease is a rare lysosomal storage disorder, which is caused by the inefficient catabolism of GC by mutant glucocerebrosidase (GBA1).[11] This causes accumulation of GC-laden macrophages which leads to the enlargement of organs (spleen and liver) and inflammation. It may be speculated that GC acts as a donor in the biosynthesis of the potentially neurotoxic steryl-β-glucosides, implying that cholesteryl-β-glucoside is a missing link between parkinsonism and Gaucher.

To further investigate this hypothesis pure samples of α- and β-glucosylated cholesterol are needed. This chapter describes the synthesis of α-cholesteryl glucoside **204** and β-cholesteryl glucoside **207** (Scheme 6.1).

6.2 Results and Discussion

In carbohydrate chemistry the glycosylation of naturally occurring terpenes and steroids present a special challenge. Besides controlling the stereoselectivity, the reactivity of the functional groups in the steroids is an important issue. The secondary 3-OH function in cholesterol is moderately nucleophilic and the alkene function is sensitive to hydrogenation.[12]

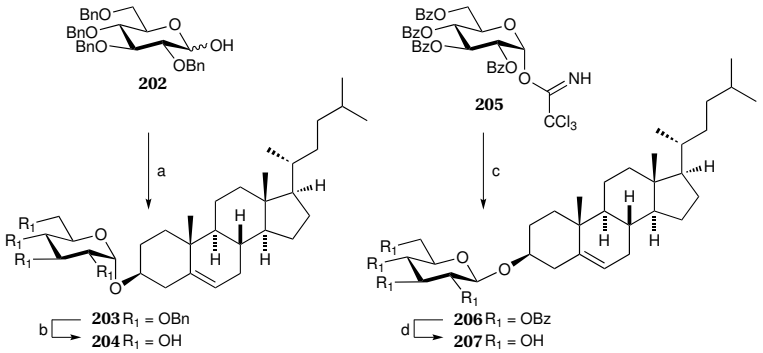
The synthesis of α-cholesteryl glucoside **204** and β-cholesteryl glucoside **207** is shown in Scheme 6.1. For the synthesis of α-cholesteryl glucoside **204**,[13] donor 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranose **202**[14] was used bearing benzyl groups which were cleaved by transfer hydrogenation leaving the alkene in cholesterol intact. *In situ* tosylation of the anomeric alcohol in **202** and coupling with an excess of cholesterol, to prevent self condensation gave steryl glycoside **203**. In almost complete anomeric selectivity (β<5%) and 90% overall yield.[15] Careful deprotection of the benzyl groups using Pearlman's catalyst in EtOH:cyclohexene to prevent the reduction of the endocyclic unsaturated bond in cholesterol, gave **204** which was purified by HPLC.[16]

β-Cholesteryl glycoside **207** was synthesized according to literature.[17] Activation of imidate **205** using TMSOTf followed by addition of cholesterol gave **206** (Scheme 6.1). Saponification of **206** under Zemplén conditions and purification by HPLC gave target compound **207** as a white solid.

6.3 Conclusion

The syntheses of α-cholesteryl glycoside **204** and β-cholesteryl glycoside **207** were successfully executed. The use of Pearlman's catalyst and cyclohexene, as hydrogen source, for the hydrogenation of the benzyl ethers in **203** prevented saturation of the double bond in cholestrol. Both **204** and **207** are currently used as internal

Scheme 6.1: Synthesis of α- and β-cholesteryl glucoside **204** and **207**.



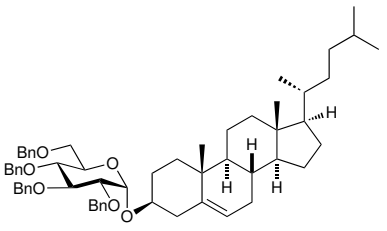
Reagents and conditions: a) TosCl, TEBA, DCM, $NaOH_{aq}$ 3M, 90%; b) $Pd(OH)_2$, H_2, EtOH:cyclohexene, 30%; c) TMSOTf, DCM, -40°C, 83%; d) NaOMe, MeOH ,89%.

standards for the investigation of cholesterol glucosides as common denominator for parkinsonism and Gaucher disease.

6.4 Experimental section

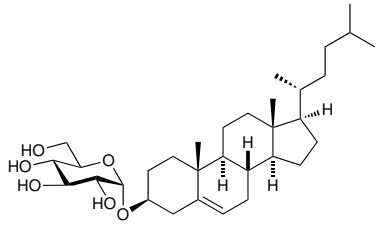
All reagent were of commercial grade and used as received (Acros, Fluka, Merck, Schleicher & Schuell) unless stated otherwise. Diethyl ether (Et_2O), light petroleum ether (PE 40-60), en toluene (Tol) were purchased from Riedel-de Haën. Dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), methanol (MeOH), pyridine (pyr) and tetrahydrofuran (THF) were obtained from Biosolve. THF was distilled over $LiAlH_4$ before use. Dichloromethane was boiled under reflux over P_2O_5 for 2 h and distilled prior to use. Molecular sieves 3Å were flame dried under vacuum before use. All reactions sensitive to moisture or oxygen were performed under an inert atmosphere of argon unless stated otherwise. Solvents used for flash chromatography were of pro analysis quality. Flash chromatography was performed on Screening Devices silica gel 60 (0.004 - 0.063 mm). TLC-analysis was conducted on DC-alufolien (Merck, Kieselgel60, F245) with detection by UV-absorption (254 nm) for UV-active compounds and by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4\,H_2O$ 25 g/L, $(NH_4)_4Ce(SO_4)_4\cdot 2\,H_2O$ 10 g/L, 10% H_2SO_4 in H_2O followed by charring at ~150 °C. ^1H and ^{13}C NMR spectra were recorded on a Bruker DMX-400 (400/100 MHz), a Bruker AV 400 (400/100 MHz), a Bruker AV 500 (500/125 MHz) or a Bruker DMX-600 (600/150 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to the chloroform residual solvent peak or tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. High resolution mass spectra were recorded on a LTQ-Orbitrap (Thermo Finnigan) Mass spectrometer. LC/MS analysis was performed on a Jasco HPLC-system (detection simultaneous at 214 nm and 245 nm) equipped with an analytical Alltima C_{18} column (Alltech, 4.6 mmD x 50 mmL, 3μ particle size) in combination with buffers A: H_2O, B: MeCN and C: 0.5% aq. TFA and coupled to a Perkin Almer Sciex API 165 mass spectrometer. Optical rotations were measured on a Propol automatic polarimeter. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}.

Cholesteryl 2,3,4,6-*tetra*-*O*-benzyl-α-D-glucopyranoside(203):



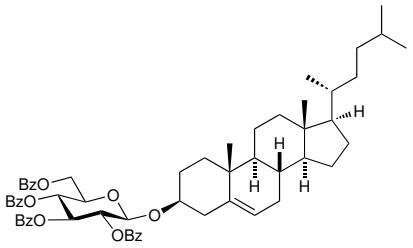
A solutions of known **202**[14] (0.54 g, 1 mmol), TosCl (0.21 g, 1.1 mmol, 1.1 quiv.), benzyltriethyl ammonium chloride (TEBA) (70 mg, 0.3 mmol, 0.3 equiv) and cholesterol (1.54 g, 4 mmol, 4 equiv) in dry DCM (10 mL) was stirred with 40% aqueous NaOH (5 mL) at room temperature. After 10 minutes an addition 5 mL of DCM was added to dilute the mixture. The reaction mixture was stirred overnight after which TLC analysis showed full conversion of the starting material. The mixture was diluted with DCM and H_2O, followed by seperation of the layers. The organic layer was washed thrice with H_2O and dried using $MgSO_4$. After filtration the mixture was concentrated *in vacuo* and purified using a short silica column (EtOAc/Tol 2.5%) gave **203** in 90% yield as a colourless oil (0.28 g, 0.31 mmol).The recorded data agree with those of Vankayalapati *et al.*[18] However, ^{1}H and ^{13}C NMR are given. TLC: 10% EtOAc/Tol; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.33 - 7.11 (m, 20H), 5.38 - 5.35 (m, 1H), 5.03 - 4.41 (m, 9H), 4.00 - 3.44 (m, 7H), 2.37 - 2.10 (m, 2H), 1.99 - 1.68 (m, 5H), 1.55 - 0.85 (m, 33H), 0.68 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 140.8, 138.9, 138.2, 128.3 - 121.7, 102.2, 94.6, 93.4, 84.7, 82.0, 79.9, 79.7, 77.9, 76.6, 75.6, 75.1, 73.4, 72.9, 70.0, 69.1, 68.6, 56.7, 56.1, 42.3, 39.7, 39.4, 36.7, 36.2, 35.7, 31.8, 28.2, 27.9, 24.3, 23.8, 22.8, 22.5, 21.0, 19.3, 18.7, 11.8.

Cholesteryl α-D-glucopyranoside(204):



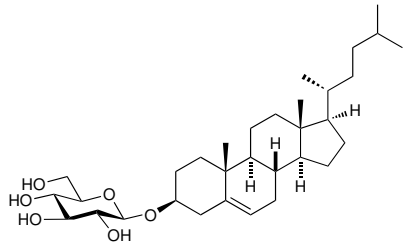
Compound **203** was dissolved in a mixture of ethanol (8 mL) and cyclohexene (4 mL). The reaction mixture was purged thrice with argon followed by addition of a catalytic amount of $Pd(OH)_2$ (20% on carbon). The suspension was stirred under reflux on till TLC analysis showed complete deprotection of the benzyl groups. The mixture was filtered over Whatman® filter paper and concentrated *in vacuo*. Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA) followed by evaporation of MeOH and lyophilizing H_2O yielded **207** (0.131 mg, 0.24 mmol, 30%) as white fluffy solid. The recorded data agree with those of Nagarajan *et al.*[13] However, ^{1}H and ^{13}C NMR are given. ^{1}H NMR (400 MHz, (D_6) DMSO): δ = 5.36 - 5.26 (d, J = 4.5 Hz, 1H), 4.90 - 4.82 (d, J = 5.3 Hz, 1H), 4.82 - 4.76 (d, J = 3.7 Hz, 1H), 4.75 - 4.66 (d, J = 4.7 Hz, 1H), 4.52 - 4.40 (m, 2H), 3.66 - 3.55 (d, J = 9.4 Hz, 1H), 3.50 - 3.41 (m, 3H), 3.19 - 3.11 (m, 1H), 3.09 - 3.00 (m, 1H), 2.41 - 2.18 (m, 2H), 2.02 - 1.77 (m, 5H), 1.61 - 0.80 (m, 34H), 0.69 - 0.64 (s, 3H); ^{13}C NMR (100 MHz, (D_6) DMSO): δ = 140.7, 121.1, 96.9, 76.4, 73.2, 72.8, 71.8, 70.4, 61.0, 56.2, 55.6, 49.5, 41.8, 40.2, 39.9, 39.8, 36.6, 36.2, 35.6, 35.2, 31.4, 31.3, 27.7, 27.4, 27.4, 23.9, 23.2, 22.6, 22.4, 20.6, 19.1, 18.5, 11.7.

Cholesteryl 2,3,4,6-*tetra*-*O*-benzoyl-β-D-glucopyranoside(206):



Known imidate **205**[17] (0.74 g, 1 mmol) and cholesterol (0.32 g, 0.83 mmol, 0.83 equiv) were coevapporated thrice with toluene and dissolved in dry DCM (5 mL). To this mixture 3Å molsieves were added and the mixture was cooled to -40°C. After 10 minutes the mixture was activated by addition of TMSOTf (9 μL, 0.05 mmol) and stirring was continued for 1 hour at -40°C. After complete consumption of the donor the mixture was quenched using 2 mL Et_3N, diluted with DCM and washed twice with H_2O and once with brine. The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by column chromatography (EtOAc/Tol 2%) gave **206** in 83% yield (0.67 g, 0.69 mmol). The recorded data agree with those of Deng *et al.*[17] However, ^{1}H and ^{13}C NMR are given. TLC: 10% EtOAc/Tol;^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 - 7.80 (m, 9H), 7.58 - 7.17 (m, 11H), 6.01 - 5.87 (t, J = 9.6 Hz, 1H), 5.72 - 5.61 (t, J = 9.7 Hz, 1H), 5.61 - 5.47 (dd, J = 9.8, 7.9 Hz, 1H), 5.28 - 5.18 (m, 1H), 5.02 - 4.93 (d, J = 7.9 Hz, 1H), 4.68 - 4.46 (m, 2H), 4.22 - 4.13 (m, 1H), 3.60 - 3.52 (m, 1H), 2.26 - 2.12 (m, 2H), 2.07 - 0.80 (m, 38H), 0.73 - 0.60 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.1 - 165.1, 140.3, 133.5 - 122.0, 100.2, 80.5, 73.2, 72.2, 72.1, 70.2, 63.4, 56.8, 56.2, 50.2, 42.4, 39.8, 39.6, 38.9, 37.2, 36.7, 36.3, 35.8, 31.9, 31.8, 29.7, 28.3, 28.1, 24.4, 23.9, 22.9, 22.6, 21.1, 19.3, 18.8

Cholesteryl β-D-glucopyranoside(207):



Compound **206** (0.31 g, 0.33 mmol) was dissolved in a mixture of MeOH:dioxane (10:1 mL). To this mixture a catalytic amount of NaOMe (30% in MeOH) was added and the mixture was stirred for 1.5 hours. After TLC analysis showed complete deprotection of all acetyl groups, the mixture was neutralized using Amberlite® H^+ till $\sim$ pH 7, filtered and concentrated *in vacuo*. Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA) followed by evaporation of MeOH and lyophilizing H_2O yielded **207** (145 mg, 0.26 mmol, 81%) as white fluffy solid. The recorded data agree with those of Nagarajan *et al.*[13] However, ^{1}H and ^{13}C NMR are given. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44 - 5.25 (d, J = 4.2 Hz, 1H), 4.99 - 4.77 (m, 3H), 4.47 - 4.38 (t, J = 5.7 Hz, 1H), 4.30 - 4.20 (d, J = 7.3 Hz, 1H), 3.73 - 3.60 (m, 1H), 3.53 - 3.40 (m, 2H), 3.19 - 2.98 (m, 3H), 3.00 - 2.86 (m, 1H), 2.46 - 2.31 (m, 1H), 2.24 - 2.05 (t, J = 12.1 Hz, 1H), 2.06 - 1.89 (m, 2H), 1.87 - 1.73 (m, 3H), 1.56 - 0.83 (m, 34H), 0.80 - 0.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 140.4, 129.2, 128.5, 121.2, 100.8, 76.9, 76.8, 73.5, 70.1, 61.1, 56.2, 55.6, 49.6, 41.8, 38.3, 36.8, 36.2, 35.6, 35.2, 31.4, 31.4, 29.2, 27.5, 27.4, 23.9, 23.2, 22.6, 22.4, 20.6, 19.1, 18.5, 11.7

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