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Note

Thermal glycosylations: Reactions of β-cellobiosyl fluoride heptaacetate with tigogenin and its trimethylsilyl ether

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Abstract

Thermal reaction of β-cellobiosyl fluoride heptaacetate with ticogenin in toluene or *o*-xylene at 112–142 °C gave ticogenyl β-cellobioside heptaacetate in 69–75% yields with good stereoselectivity. The procedure avoids the use of Lewis acid or traditional glycosidation promotors. The deacetylated product is a hypocholesterolemic and anti-atherosclerosis agent. © 1997 Published by Elsevier Science Ltd.

Keywords: Stereoselective thermal glycosylations; β-Cellobiosyl fluoride heptaacetate; Tigogenin; Me_3Si-O-tigogenin; Tigogenyl β-cellobioside heptaacetate; Tigogenyl β-cellobioside; Cholesterol-lowering agent

Tigogenyl β-cellobioside, a synthetic spirostane glycoside, represents a novel hypocholesterolemic and anti-atherosclerosis agent which acts by inhibiting the adsorption of dietary and biliary cholesterol, thereby reducing plasma LDL-cholesterol levels [1–4]. Tigogenyl β-cellobioside is an exciting and potentially very safe therapeutic agent since it reduces plasma cholesterol without significant absorption of the drug. To support the clinical development of tigogenyl β-cellobioside, new synthetic methods which do not use any Lewis acids nor Koenigs–Knorr promoters for the construction of the β-glycosidic linkage were investigated. Literature methods to prepare tigogenyl β-cellobioside heptaacetate used $Hg(CN)_2$ [5–7], $SnCl_4$ [8] or Ag_2CO_3 [9] [1] to activate glycosidic couplings. The use of mercuric, stannic, or silver salts for glycoside syntheses on a large commercial scale would present major toxicity, handling, disposal and environmental problems. Schmidt's trichloracetimidate method was also evaluated by Urban [10] who found that BF_3 etherate or $ZnBr_2$ activation of α-cellobiosyl trichloracetimidate heptaacetate afforded glycoside **4** in only 35–60% yields. Classical glycosyl fluoride activators such as $SnCl_2$–$AgClO_4$ [11], Cp_2TiCl_2–$AgClO_4$ [12], Me_3SiOTf [13], SiF_4 [13], or Me_2GaCl [14] were not evaluated, again to eliminate the use of inorganic promoters and their potential deleterious reaction byproducts.

Thermal glycosylations of β-cellobiosyl fluoride heptaccetate (**1**) with tigogenin (**2**) or *O*-Me_3Si-tigogenin (**3**) were investigated for the stereoselective formation of tigogenyl β-cellobioside heptaacetate (**4**) as shown in Scheme 1. Thermal glycosidic couplings are potentially very practical for large-scale syntheses, since no activators are required and these

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[1] These authors studied the reactions of tigogenin with tetra-O-acetyl-α-D-glucopyranosyl bromide under the influence of a variety of silver salts.

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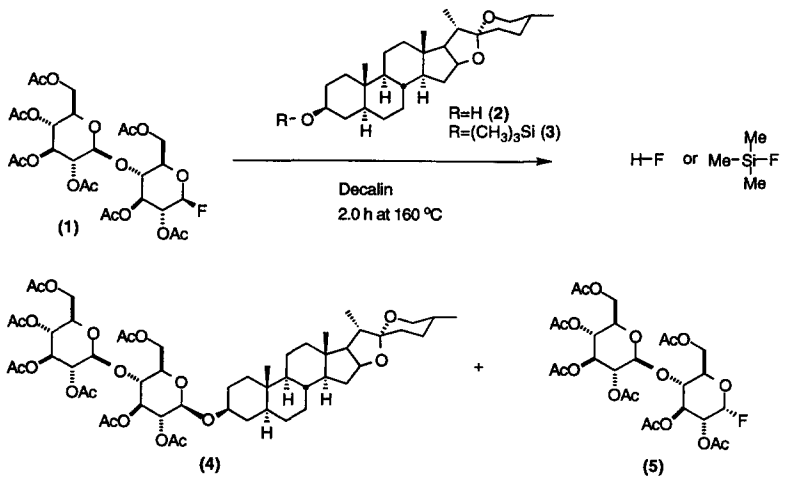

Scheme 1.

glycosidic coupling reactions would liberate HF or Me_3SiF as volatile byproducts, which could easily be removed from the reaction and trapped in an aqueous, alkali base solution. Thermal glycosylations using 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride [15], 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl chloride [16,17] or 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl chloride [16,17] with sterols have been reported, but these literature methods required the addition of acid scavengers and generally afforded poor stereoselectivity.

Thermal reactions of β-cellobiosyl fluoride heptaacetate (**1**) with tigogenin (**2**) occurred in toluene or *o*-xylene at 112–142 °C to afford tigogenyl β-cellobioside heptaacetate (**4**) in 69–75% yields with good stereoselectivity, as shown in Table 1. The highest yield of β-glycoside was obtained when the reaction was conducted in toluene (run 1) at lower reaction temperatures. At higher temperatures in *o*-xylene (runs 2–4), the glycoside yields remained high, but anomeric stereoselectivity was lower and variable, perhaps because of HF-catalyzed anomerization of the β-glycoside. The thermal glycosylations of **1** and **2** in refluxing toluene or *o*-xylene showed variable induction periods which appeared to depend upon whether the reflux condenser was air- (25 °C) or water-cooled (15 °C) and also whether volatile byproduct — HF (bp 19.5 °C) was removed from the

Table 1
Thermal glycosylations of β-cellobiosyl fluoride (**1**)

Run	Aglycone (equiv)	Solvent	Reflux Condenser	Reaction		Yield		Hplc
			Cooling (°C)	Time (h)	Temp (°C)	Glycosides	αF(5)	Stereoselectivity (β:α)
1	2 (1.0)	toluene	water (15 °C)	6.5	112	75%	< 1%	30:1
2	2 (1.0)	*o*-xylene	water (15 °C)	1.0	142	69%	< 1%	7:1
3	2 (1.0)	*o*-xylene	air (25 °C)	1.0	142	70%	< 1%	6:1
4	2 (1.0)	*o*-xylene	air (25 °C)	1.0	142	69%	< 1%	1:1
5	2 (1.0)	*o*-xylene	air (25 °C) N_2 sweep	11	142	no reaction		
6	3 (1.0)	neat	water (15 °C)	0.25	190	36%	33%	13:1
7	3 (1.0)	decalin	water (15 °C)	2.0	165	28%	33%	30:1
8	3 (1.0)	*o*-xylene	water (15 °C)	2.0	142	41%	31%	85:1
9	3 (2.0)	*o*-xylene	water (15 °C)	3.0	142	39%	25%	90:1
10	3 (0.5)	*o*-xylene	water (15 °C)	4.0	142	48%	5%	1:1

reaction by a stream of N_2 bubbled through the reaction solution. When HF was removed from the reaction solution with a nitrogen sparge (run 5), no reaction occurred. In addition, when quinoline (1.0 equiv) was added to the thermal glycosylation in *o*-xylene, longer reactions times (8 h) were required and glycoside **4** was formed in only 25% yield, without any α-glycoside formation. Although thin-layer chromatography did not detect any reaction intermediates, additional mechanistic experiments are needed to determine whether the thermal glycosylation of **1** with **2** may be catalyzed by the liberated HF.

Thermal reactions of β-cellobiosyl fluoride heptaacetate (**1**) with *O*-Me_3Si-tigogenin (**3**) in the absence of any Lewis acids or Koenigs–Knorr promoters occurred only at higher reaction temperatures (140–190 °C) under neat conditions (run 6) or in high boiling, non-polar solvents such as decalin (run 7) or *o*-xylene (runs 8–10) to afford tigogenyl β-cellobioside heptaacetate (**4**) in 27–41% yields. Thermal glycosylation reactions with *O*-Me_3Si-tigogenin did not show any induction periods and consistently afforded high β-stereoselectivity, except when **1** was used in excess (run 10). However, α-cellobiosyl fluoride heptaacetate (**5**) was always produced as a major, unreactive byproduct. In a control experiment, β-cellobiosyl fluoride heptaacetate was heated in *o*-xylene for 6 h at 145 °C without any α-glycosyl fluoride (**5**) formation. Attempts to decrease the formation of **5** by changing reaction stoichiometry (runs 9–10), reaction solvents (CH_2Cl_2, diglyme, pyridine, Me_2SO), or trying to catalyze (HF, *p*-toluenesulfonic acid, SiO_2, or F^-) the thermal glycosylations at lower reaction temperatures were unsuccessful. When *O*-Me_3Si-tigogenin reaction was conducted in decalin (run 6), thin-layer chromatography (SiO_2, 1:1 hexanes–ethyl acetate) showed that the β-cellobiosyl fluoride (**1**) was completely consumed within 2.0 h at 165 °C while tigogenyl β-cellobioside heptaacetate (27%), α-cellobiosyl fluoride heptaacetate (33%), and tigogenyl α-cellobioside heptaacetate (1%) were formed. Volatile trimethylsilyl fluoride (bp 16 °C) was also isolated from the decalin reaction using a cold trap (−78 °C), and identified by ^{1}H NMR. Small amounts of tigogenin and some unknown polar by-products were also formed. Tigogenyl β-cellobioside heptaacetate (**4**), tigogenyl α-cellobioside heptaacetate, and α-cellobiosyl fluoride heptaacetate (**5**) were isolated by column chromatography and their spectral and physical properties were consistent with literature values [6]. Finally, tigogenyl β-cellobioside heptaacetate was suspended in methanol and deprotected using the Zemplén method [18] to give tigogenyl β-cellobioside in 85% yield as a white crystalline solid.

In summary, thermal glycosylations of β-cellobiosyl fluoride heptaacetate (**1**) with tigogenin (**2**) afforded 69–75% yields of tigogenyl β-cellobioside heptaacetate (**4**), but these thermal glycosylations with tigogenin showed variable induction periods and variable stereoselectivity. Thermal glycosylations of β-cellobiosyl fluoride heptaacetate (**1**) with *O*-Me_3Si-tigogenin (**3**) at higher temperatures (142–190 °C) in hydrocarbon solvents were more reproducible and afforded high β-stereoselectivity, but tigogenyl β-cellobioside heptaacetate (**4**) was produced in only 27–41% yields. Although no Koenigs–Knorr activators, Lewis acids, nor acid scavengers were used in these thermal glycosylations, initial attempts to simplify the large-scale, commercial synthesis of tigogenyl β-cellobioside heptaacetate were only partially successful and further studies will be required to understand the induction period and variable stereoselectivity observed in the tigogenin reactions.

1. Experimental

General methods.—Melting points were determined on a Thomas Hoover capillary melting-point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 Polarimeter. ^{1}H NMR (400 Hz) and ^{13}C NMR (100 Hz) spectra were measured in $CDCl_3$ or Me_2SO-d_6 with a Varian Unity Plus spectrometer; NMR spectra are reported in ppm (δ) and referenced to the deuterium lock signal of the solvent. Thin-layer chromatography was conducted on Merck Kieselgel 60 F_{254} plates (5 × 10 cm) using 1:1 hexanes–EtOAc eluant. The TLC plates were visualized by spraying the dried plate with 10% aqueous H_2SO_4 and then heating the plate to visualize the spots: *O*-Me_3Si-tigogenin (R_f = 0.90), tigogenin (R_f = 0.55), tigogenyl β-cellobioside heptaacetate (R_f = 0.36), and β-cellobiosyl fluoride heptaacetate (R_f = 0.26). Column chromatography was conducted using 32–63 mm Silica Gel 60 using 2:3 hexanes–EtOAc eluant. High-pressure liquid chromatography was performed on LDC Analytical ConstaMetric 3200 HPLC pump using a Water's Novapak silica column — 4 μm (3.9 × 150 mm) and 2:3 hexanes–EtOAc mobile phase, flow rate = 1.0 mL/min, injection volume = 50 μL, LDC Refracto Monitor IV refractive index detector, retention times:

tigogenyl β-cellobioside heptaacetate (3.9 min), tigogenyl α-cellobioside heptaacetate (4.7 min), and β-cellobiosyl fluoride heptaacetate (6.8 min). Tigogenin was purchased from Sigma Chemical Company of St. Louis, Missouri 63178. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory of Woodside, New York 11377.

O-Trimethylsilyl-tigogenin (**2**).—Tigogenin (50.0 g, 0.12 mol) and MeCN (0.5 L) were added to a 1-L, 3-neck round-bottom flask which was equipped with a mechanical stirrer, thermometer, and a distillation head. The flask was purged with N_2 and then maintained under an inert N_2 atmosphere while the slurry was heated to reflux (83 °C), and 100 mL of distillate was removed. The slurry was cooled to 45 °C and then sampled for a Karl Fischer determination (H_2O = 0.08%). 1,1,1,3,3,3-hexamethyldisilazane (12.1 g, 0.075 mol) was added and the mixture was reheated to 70–75 °C. After heating for 6 h at reflux, TLC using 1:1 hexanes–EtOAc eluant showed that only a trace of tigogenin remained and that the reaction was complete. The slurry was cooled to 25 °C and stirred overnight. The slurry was filtered and the filter cake was washed with MeCN (40 mL). The off-white solid was dried in vacuo for 16 h at 40 °C to give 53.6 g (91% yield) of white needles: mp 196–198 °C; ^{13}C NMR ($CDCl_3$): δ 109.18, 80.84, 71.83, 66.81, 62.24, 56.24, 54.44, 45.02, 41.59, 40.57, 40.09, 38.55, 37.15, 35.58, 35.11, 32.30, 31.82, 31.77, 31.38, 30.30, 28.80, 28.66, 21.03, 17.13, 16.48, 14.51, and 12.35. Anal. Calcd for $C_{30}H_{52}SiO_3$: C, 73.7; H, 10.70. Found: C, 73.70; H, 11.12.

General procedure for thermal glycosylations.—*Solution glycosylation with tigogenin.* β-Cellobiosyl fluoride heptaacetate (500 mg, 0.78 mmol), tigogenin (326 mg, 0.78 mmol), and 27 mL of toluene were added to a 50 mL round bottom flask equipped with a thermometer topped with a N_2 inlet, water-cooled condenser, and a magnetic stirring bar. The flask was flushed with N_2 and then maintained under a static N_2 atmosphere while the gelatinous mixture was heated to reflux (112 °C). After the clear solution had been heated for 3 h at reflux, TLC showed only the presence of starting materials. The reaction was heated at reflux for an additional hour when TLC showed the complete consumption of the reactants and the formation of **4** as the predominant product. No transient spots (intermediates) were detected on the TLC plate. The solution was cooled to room temperature, diluted to 50 mL with CH_2Cl_2, and analyzed by HPLC. Tigogenyl β-cellobioside heptaacetate was formed in 74.4% yield with a stereoselectivity > 30:1 as measured against reference standards. The solution was concentrated under diminished pressure to a solid which was triturated with 10 mL of EtOH. The product (**4**) was filtered, washed with 2 mL of EtOH, and then dried in vacuo at 40 °C to give 557 mg (71.6% yield) of a white crystalline solid: mp 229–232 °C; ^{13}C NMR ($CDCl_3$): δ 170.38, 170.21, 170.09, 169.78, 169.43, 169.22, 168.97, 109.10, 100.65, 99.33, 80.72, 79.62, 76.56, 72.86, 72.57, 72.46, 71.81, 71.68, 71.55, 67.76, 66.71, 62.15, 62.01, 61.50, 56.19, 54.24, 44.63, 41.51, 40.48, 39.94, 36.83, 35.58, 34.99, 34.52, 32.14, 31.67, 31.30, 30.20, 29.17, 28.73, 28.60, 20.95, 20.79, 20.64, 20.55, 20.45, 17.09, 16.41,14.45, and 12.22.

Solution glycosylation with O-trimethylsilyl-tigogenin. β-Cellobiosyl fluoride heptaacetate (10.45 g, 15.1 mmol), *O*-trimethylsilyl-tigogenin (8.00 g, 16.4 mmol), and 56 mL of *o*-xylene were added to a 100 mL round-bottom flask which was equipped with a mechanical stirrer and thermometer. After the flask had been purged with N_2, the white slurry was heated to give a clear solution which was then held for 2 h at 140–143 °C, when TLC showed that the reaction was complete and that only a trace of β-cellobiosyl fluoride (**1**) remained. The solution was cooled to room temperature, and the crude mixture was analyzed by HPLC. HPLC and TLC analyses showed that tigogenyl β-cellobioside heptaacetate, tigogenyl α-cellobioside heptaacetate, and α-cellobiosyl fluoride heptaacetate were formed as the main reaction products in 40, 0.4 and 31% yields respectively. The crude reaction solution was concentrated under diminished pressure to afford a waxy solid. The solid was dissolved in CH_2Cl_2 (20 mL) affording a hazy yellow solution. The yellowish solution (10 mL) was chromatographed on a silica gel column using an 2:3 hexanes–EtOAc eluant to give pure samples of tigogenyl β-cellobioside heptaacetate (4.29 ppm, d, J_{H1-2} = 7.8 Hz), tigogenyl α-cellobioside heptaacetate (5.10 ppm, d, J_{H1-2} = 3.4 Hz), and α-cellobiosyl fluoride heptaacetate. The spectral, physical, and chromatographic properties of these glycosides matched literature values [6] and were identical to authentic samples.

Neat glycosylation with O-trimethylsilyl-tigogenin. β-Cellobiosyl fluoride heptaacetate (4.00 g, 6.26 mmol), O-trimethylsilyl-tigogenin (3.06 g, 6.25 mmol), and CH_2Cl_2 (10 mL) were added to a 15 mL, 3-neck round-bottom flask which was equipped with a magnetic stirring bar, thermometer, and a short-path distillation head. The colourless solution was heated to reflux under N_2 and CH_2Cl_2 was removed by an

atmospheric distillation to give a white solid. The flask containing the solid was immersed in a hot oil-bath (200–210 °C) affording a yellow melt. After heating for 15 min at 190–195 °C, TLC showed that the β-cellobiosyl fluoride (**1**) was completely consumed and that the reaction was complete. The melt was cooled to 25 °C and CH_2Cl_2 (10 mL) was added. The solution was transferred to a 50-mL volumetric flask, diluted with CH_2Cl_2 to volume, and then an aliquot was analyzed by HPLC. The solution from the neat reaction contained β-tigogenyl cellobioside heptaacetate (35%), α-tiogenyl cellobioside heptaacetate (1.2%), and α-cellobiosyl fluoride heptaacetate (33%).

Tigogenyl β-cellobioside. Tigogenyl β-cellobioside heptaacetate (3.9 g, 3.76 mmol), MeOH (36 mL), and NaOMe (10 mg) were combined under N_2 to give a white slurry which was heated to reflux (66 °C). After 1.5 h at reflux, TLC (4:1 CH_2Cl_2–MeOH) showed that the reaction was complete. The slurry was cooled to 25 °C and stirred overnight. The slurry was vacuum filtered, the filter cake washed with fresh MeOH (7.5 mL), and then dried in vacuo for 32 h at 40 °C to give tigogenyl β-cellobioside (2.4 g, 85% yield) as a white crystalline solid: mp 258–260 °C dec.; $[a]_D^{25}$ −56.6 °C (*c*, 1.1, N,N-dimethylacetamide); ^{13}C NMR (Me_2SO-d_6): δ 108.82, 103.67, 100.84, 81.15, 80.63, 77.24, 76.93, 75.48, 75.11, 73.74, 73.60, 70.49, 66.38, 62.41, 61.48, 60.96, 56.11, 54.17, 44.48, 41.56, 36.97, 35.74, 35.11, 34.49, 32.29, 31.85, 31.38, 30.27, 29.48, 28.94, 28.74, 21.05, 17.59, 16.70, 15.08, and 12.55. Anal. Calcd for $C_{39}H_{64}O_{13}$ dihydrate: C, 60.29; H, 8.82. Found: C, 60.48; H, 8.84.

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