Total Synthesis of Floridoside

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Abstract: The native floridoside [2′-*O*-(α-D-galactopyranosyl)-glycerol], isolated from the red marine alga *Rhodymenia palmata*, has demonstrated a potent immunostimulating activity. In order to carry out further biological investigations, the synthesis of this compound appeared necessary and is reported here, for the first time.

Key words: floridoside, 2′-*O*-(α-D-galactopyranosyl)glycerol, glycosylation, bioactive glycosides, antitumor agent

The native floridoside [2′-*O*-(α-D-galactopyranosyl)-glycerol, Figure 1] was first isolated in 1930 by Colin[1] and Gueguen from the red marine alga *Rhodymenia palmata*. Its molecular structure was first established by Putman and Hassid[2] and the complete ^{1}H NMR and ^{13}C NMR spectra were unambiguously assigned by Simon–Colin[3] et al. Floridoside is considered to be the main photosynthetic carbon-reserve product in most red algae,[4–7] where it is also believed to behave as an intracellular osmotic regulator.

Some hemolytic tests were carried out using human serum deficient in C2, C4, or C1q proteins in order to check the activity of the natural floridoside towards the complement system. These tests have shown that floridoside was able to activate the classical pathway of the complement system.

The floridoside has an α-Gal structure, and Galili[8] has already demonstrated that an α-Gal epitope was highly immunogenic.

The results obtained with the floridoside have promoted an increasing interest and to go further in biological investigations, especially to make the coupling of floridoside to a therapeutic antibody, larger amounts of pure **1** are therefore needed. We report here on a facile route for the synthesis of floridoside (**1**), which could be easily scaled up to several grams.

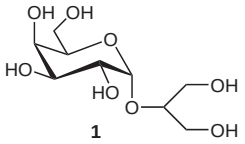
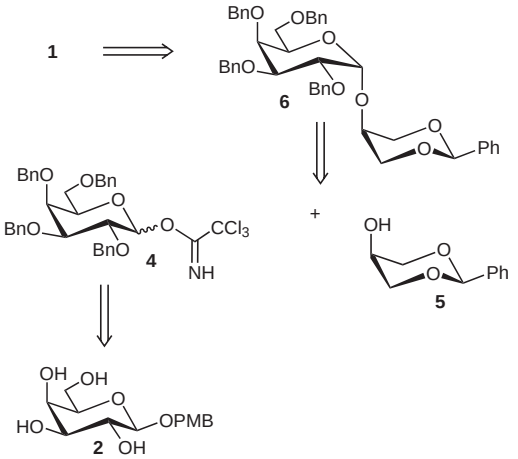


Figure 1 Floridoside (**1**)

The retrosynthetic analysis of compound **1** is depicted in Scheme 1. The α-galactoside synthon **6** was obtained starting from a suitable protected galactosyl donor **4** and, as alcohol acceptor, commercially available **5**. The trichloroacetimidate **6** can be readily prepared from the 4-methoxyphenyl β-D-galactopyranoside[9] (**2**).



Scheme 1 Retrosynthetic analysis of floridoside

The β-galactoside **2** (Scheme 2) was 2,3,4,6-tetra-O-benzylated by treatment with benzyl bromide and sodium hydride in DMF. It was purified by flash chromatography (toluene–ethyl acetate), then crystallized from hexane–ethyl acetate and finally obtained in 85% yield.

Oxidative removal of the 4-methoxyphenyl group in **3** with ceric ammonium nitrate followed by imidoylation[10] with trichloroacetonitrile and 1,8-diazabicyclo[5,4,0]undec-7-ene afforded a mixture of α- and β-imidate **4** in 58% overall yield. The analytical data of compound **4** were in accordance with those published by Schmidt[11] et al.

The galactosylation of *cis*-1,3-*O*-benzylideneglycerol (**5**, 1.5 equiv) with imidate **4** (1 equiv) in anhydrous dichloromethane in the presence of trimethylsilyl trifluoromethanesulfonate gave, after separation by flash chromatography (hexane–ethyl acetate), **6** in 65% yield and **7** in 20% yield (α/β = 3:1).[12]

The ^{1}H NMR spectrum of **6** clearly shows a doublet at δ = 5.08 ppm with a coupling constant of 2.8 Hz corresponding to the anomeric proton and demonstrates an α-stereochemistry of the glycosidic bond. The ^{1}H NMR spectrum of **7** shows a doublet at δ = 4.84 ppm with a coupling constant of 8.0 Hz that corresponds to the anomeric proton β.[13]

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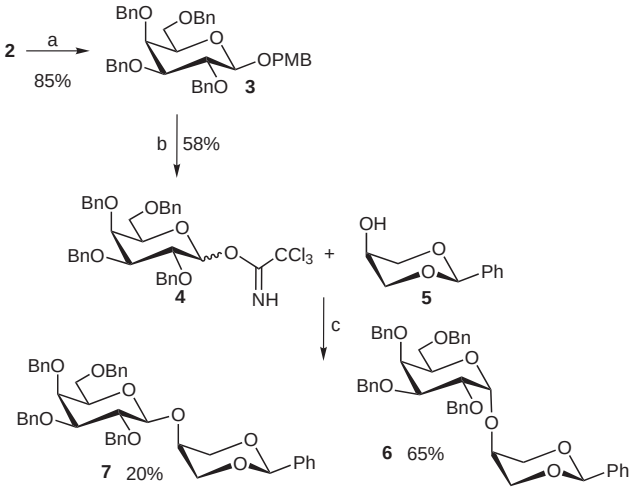
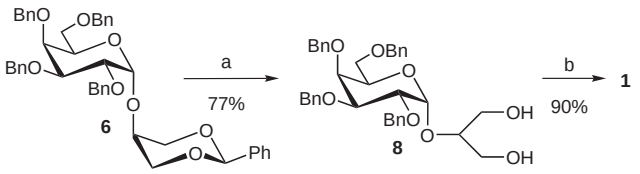


Scheme 2 *Reagents and conditions*: a) $PhCH_2Br$, NaH, DMF, r.t., 2 h; b) CAN, toluene–MeCN–H_2O, r.t., 20 min; then CCl_3CN, DBU, CH_2Cl_2, r.t., 30 min; c) **5** (1.5 equiv), TMSOTf (1 M), MS 4 Å, CH_2Cl_2, r.t., 30 min.

Alternative condensation conditions were also tested in toluene at –10 °C using a trimethylsilyl trifluoromethanesulfonate catalysis, but the yield and the ratio α/β was not modified.

The 1′,3′-*O*-benzylidene group was removed by acidic hydrolysis with aqueous acetic acid (60%) at 100 °C for 30 minutes. Compound **8**[14] was obtained in 77% yield (Scheme 3), after purification by flash chromatography (toluene–ethyl acetate then dichloromethane–methanol).



Scheme 3 *Reagents and conditions*: a) AcOH (60%), 100 °C, 30 min; b) H_2, 10% Pd/C, EtOAc–MeOH–H_2O, r.t., 18 h.

Final hydrogenation of **8** in aqueous methanol afforded the target molecule **1** in a 90% yield.[15]

The ^1H NMR and ^{13}C NMR data of the synthetic floridoside were in accordance with those published for the natural and purified compound by Simon–Colin[3] et al.

In conclusion, the total synthesis of compound **1** was successfully achieved starting from 4-methoxyphenyl β-D-galactopyranoside (**2**) in five steps. This sequence easily allows the scaling up of this synthesis in order to obtain bulk quantities of **1** for further biological studies.

References and Notes

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(12) Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations are measured at 20–25 °C with Perkin-Elmer 341 polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded at 25 °C with a Bruker DPX400 spectrometer operating at 400 and 100 MHz, respectively. Assignments were based on homo- and heteronuclear correlations using supplier's software. Flash column chromatography was performed on silica gel (E. Merck, 40–63 μm). Microanalyses were performed by the department of microanalysis of CNRS (ICSN, Gif sur Yvette, France).
(13) **General Procedure for the Glycosylation**
A mixture of trichloroacetimidate (600 mg, 0.75 mmol), *cis*-1,3-*O*-benzylideneglycerol (315 mg, 1.75 mmol) and activated powdered MS 4 Å in anhyd CH_2Cl_2 (10 mL) was stirred for 1 h at r.t. under dry N_2, then cooled to 0 °C. A solution of TMSOTf in toluene (1 M, 131 μL, 15%) was added, and the mixture was stirred for 30 min at r.t., then was quenched with Et_3N, filtered and concentrated. Flash silica gel chromatography (hexane–EtOAc = 3:1, containing 0.1% of Et_3N) gave **7** (125 mg, 20%) and **6** (400 mg, 65%).
***cis*-1′,3′-*O*-Benzylidene-2′-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)glycerol (6)**
$[\alpha]_D$ +7 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.40–7.10 (m, 25 H, arom. H), 5.57 (s, 1 H, PhCH), 5.10 (d, 1 H, $J_{1,2}$ = 2.8 Hz, H-1), 5.00–4.53 (m, 6 H, $PhCH_2$), 4.48–4,38 (m, 5 H, $PhCH_2$, OCH_2, OCH), 4.20–4.13 (m, 1 H, H-5), 4.10–3.90 (m, 5 H, H-2, H-3, H-4, OCH_2), 3.58–3.49 (m, 2 H, H-6a, H-6b). ^{13}C NMR ($CDCl_3$): δ = 138.9–126.3 (arom. C), 101.4 (PhCH), 96.7 (C-1), 78.8 (C-4), 76.7 (C-2), 75.1 (C-3), 74.8–73.0 ($PhCH_2$), 70.2 (OCH_2), 69.6 (OCH), 69.5 (C-5), 69.1 (C-6), 68.5 (OCH_2). Anal. Calcd for $C_{44}H_{46}O_8$: C, 74.19; H, 6.60. Found: C, 74.09; H, 6.71.
(14) **2′-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)glycerol (8)**
^1H NMR ($CDCl_3$): δ = 7.40–7.10 (m, 25 H, arom. H), 4.95 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.94–4.35 (m, 8 H, $PhCH_2$), 4.13–4.07 (m, 2 H, H-2, H-5), 3.98 (dd, 1 H, $J_{3,2}$ = 10.1 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 3.87 (d, 1 H, H-4), 3.79–3.70 (m, 1 H, OCH), 3.60–3.50 (m, 5 H, 2 OCH_2, H-6a), 3.28 (dd, 1 H, $J_{6a,6b}$ = 4.1 Hz, $J_{6b,5}$ = 9.6 Hz, H-6b). ^{13}C NMR ($CDCl_3$): δ = 138.2–127.5 (arom. C), 99.4 (C-1), 85.4 (OCH), 79.4 (C-3), 76.4 (C-2), 74.6 (C-4), 74.5-73.6 ($PhCH_2$), 70.7 (C-5), 69.7 (C-6), 62.9 (OCH_2), 62.3 (OCH_2). Anal. Calcd for $C_{37}H_{42}O_8$: C, 72.99; H, 6.89. Found: C, 71.13; H, 7.42.

(15) **General Procedure for the Catalytic Hydrogenation**
A mixture of compound **8** (300 mg, 0.488 mmol) and Pd/C 10% in EtOAc–MeOH–H_2O (4:4:2 mL) was stirred under a H_2 atmosphere at r.t. for 18 h. The mixture was filtered, concentrated, and co-evaporated with toluene. The compound was eluted from a column (3.0 × 30 cm) of Sephadex LH-20 with H_2O to give **1** (112 mg, 90%) as a white powder.

2′-*O*-(α-D-Galactopyranosyl)glycerol (1)
$[\alpha]_D$ +164 (*c* 1.0, H_2O), lit. 2: $[\alpha]_D$ +165. ^{1}H NMR (D_2O): δ = 5.13 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1), 4.12–4.09 (m, 1 H, H-5), 3.95 (d, 1 H, $J_{4,3}$ = 2.8 Hz, H-4), 3.87 (dd, 1 H, $J_{3,2}$ = 10.1 Hz, H-3), 3.82–3.78 (m, 1 H, H-2), 3.75–3.70 (m, 7 H, 2 × OCH_2, OCH, H-6a, H-6b). ^{13}C NMR ($CDCl_3$): δ = 100.8 (C-1), 81.4 (OCH), 74.0 (C-5), 72.2 (C-3), 72.1 (C-4), 71.3 (C-2), 64.2 (OCH_2), 63.9 (OCH_2), 63.2 (C-6); cf. lit. 3. Anal. Calcd for $C_9H_{18}O_8$: C, 42.52; H, 7.14. Found: C, 42.36; H, 7.25.