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Note

Semi-synthesis of a 3-*O*-sulfated red seaweed galactan-derived disaccharide alditol

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Abstract—β-D-Gal*p*3-SO_3-(1→4)-3,6-anhydro-L-GalOH (agarobiitol 3^2-sulfate, **4**) was semi-synthetically prepared as follows: production of agarobiitol (**1**) from agarose by partial reductive hydrolysis, protection of the primary hydroxyl groups of **1** with trityl groups to produce the 1^1,6^2-di-*O*-tritylated derivative (**2**), regioselective dibutylstannylene-mediated sulfation of **2** to give the 3^2-*O*-sulfated-1^1,6^2-di-*O*-tritylated compound (**3**), and detritylation of compound **3** to give the final product (**4**). This semi-synthetic route allowed the preparation of a red seaweed galactan-derived disaccharide alditol with sulfate group located at C-3 of the galactopyranosidic ring. Because red seaweed galactans are glycosidically linked at C-3 of the β-D-Gal*p* unit, a sulfated derivative with this structure could not be obtained by partial reductive hydrolysis of sulfated red seaweed galactans.

Keywords: Sulfated oligosaccharide; Dibutylstannylene; Antivirals; Agarose; Reductive hydrolysis; Red seaweeds

Most of the galactans biosynthesized by red seaweeds (Rhodophyta) are found as anionic polymers with varying degrees of sulfation, presenting a wide variety of substitution patterns. However, these polysaccharides normally have a repetitive backbone; their basic structure contains the disaccharide-based repeating units of (1→3)-β-D-Gal*p*-(1→4)-α-Gal*p*.[1] In many cases, the latter residues appear as 3,6-anhydro-Gal*p* (3,6-An-Gal*p*) units. Depending on the enantiomeric configuration of the α-Gal*p* units, these galactans are classified as agarans (α-L-Gal*p*) and carrageenans (α-D-Gal*p*).[1] Sulfated oligosaccharides obtained from red seaweed galactans have been used in diverse areas, such as preparation of potential antiviral agents, model molecules in NMR spectroscopy/mass spectrometry studies,[2–7] and as standards in chromatographic and capillary electrophoresis separations.[7]

Agarans and carrageenans extracted from some selected red seaweed species present repetitive sulfation patterns and, therefore, can be attractive sources of oligosaccharides with specific sulfation positioning in relatively high yields.[2–7] For example, carrabiitol 4^2-sulfate and carratetraitol 4^2,4^4-disulfate can be readily prepared via partial reductive hydrolysis of *Kappaphycus alvarezii* (Solieriaceae) carrageenan.[7] This is possible because this seaweed produces mainly κ-carrageenan, a highly repetitive galactan containing (1→3)-β-D-Gal*p*4SO_3-(1→4)-α-D-3,6-An-Gal*p* units. The same approach has been applied using other repetitive galactans to produce a great variety of sulfated oligosaccharides (Scheme 1), for example, agarobiitol 6^2-sulfate from *Gracilaria domingensis* (Gracilariales) agaran (agarose 6-sulfate, 3-linked β-D-Gal*p* 6-sulfate and 4-linked 3,6-An-α-L-Gal*p*);[7] agarobiitol 2^2-sulfate from *Acanthophora spicifera* (Ceramiales) agaran (agarose 2-sulfate, 3-linked β-D-Gal*p* 2-sulfate and 4-linked 3,6-An-α-L-Gal*p*).[6,7]

As outlined in Scheme 1, it was possible to prepare disaccharide alditols with sulfate groups located at

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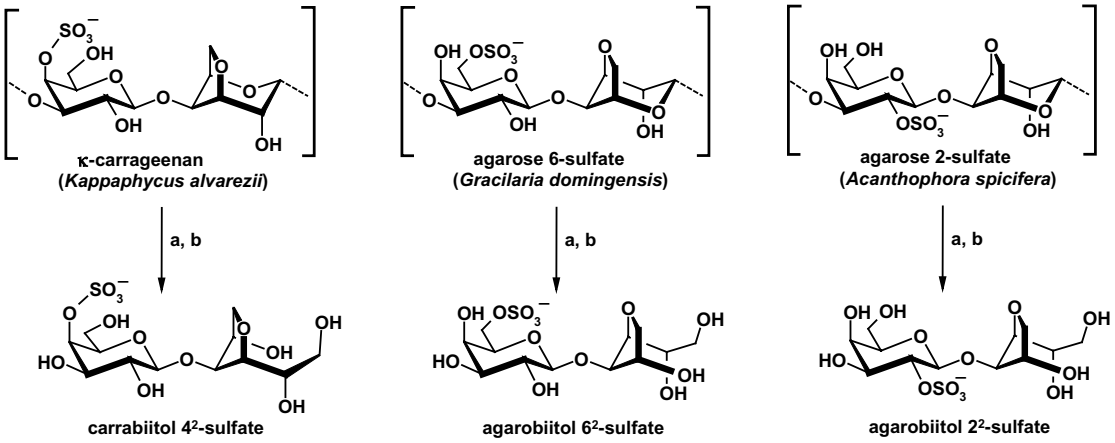


Scheme 1. Disaccharide alditols obtained from red seaweed galactans by partial reductive hydrolysis. The β-D-Gal*p* unit is sulfated at C-4, C-6, or C-2 for carrabiitol 4^2-sulfate, agarobiitol 6^2-sulfate, and agarobiitol 2^2-sulfate, respectively. **a**: TFA, 4-methylmorpholine borane, 8 h, 65 °C. **b**: anion exchange and gel filtration chromatography purification steps.[6,7]

C-4, C-6, or C-2 of the β-D-Gal*p* unit. However, the use of partial reductive hydrolysis for the production of oligosaccharides with the sulfate group located at C-3 of the β-D-Gal*p* unit is not possible because this position is involved in the glycosidic linkage. Considering that the 3-*O*-sulfated derivative would be important in our investigation of the antiviral properties of positional isomers, we semi-synthetically prepared a 3-*O*-sulfated oligosaccharide alditol, specifically, agarobiitol 3^2-sulfate.

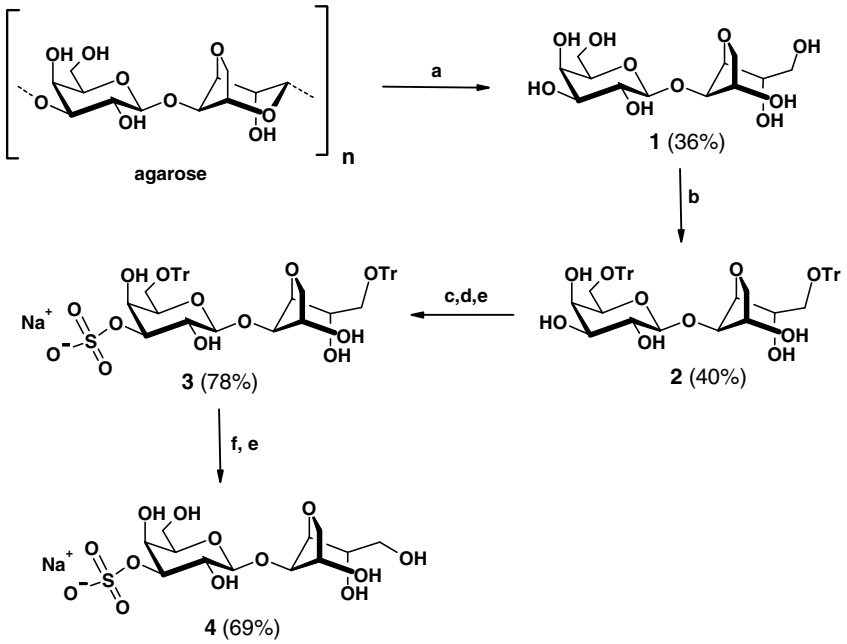
For this purpose, first we submitted neutral Type 1 agarose (3-linked β-D-Gal*p* and 4-linked 3,6-An-α-L-Gal*p*) to partial reductive hydrolysis.[2–7] TFA was immediately removed by co-distillation with water after hydrolysis and the residual polymeric material was precipitated with ethanol. The product was isolated by precipitation from methanol by the addition of excess ethyl acetate. The precipitate was purified by silica gel chromatography. The combined column fractions rendered pure neutral agarobiitol (**1**, 36%). Agarobiitol has been prepared by partial reductive hydrolysis from several seaweed galactans.[2,4,6] In all cases, the yields were very small due to the type of agaran utilized for the hydrolysis, which contained sulfate groups that caused a decrease in the neutral disaccharide alditol yield. Chromatographic steps of anion exchange followed by gel filtration chromatography are usually performed for the purification of oligosaccharide alditols. Now, we have utilized agarose, a completely neutral agaran, as a starting material and a combination of sequential precipitations with organic solvents and flash chromatography on a silica gel column for the purification of the products. The latter purification process was faster and allowed much higher yields than the aforementioned chromatographic steps.

For the sulfation of agarobiitol, we chose to activate *O*-3 of the β-D-Gal*p* unit through formation of its dibutylstannylene acetal. This strategy was defined based on the fact that in reactions of dibutylstannylene acetals, one of the two oxygen atoms reacts preferentially. In reactions of electrophiles with the dibutylstannylene acetal derived from an equatorial–axial pair of oxygen atoms of a *cis*-diol on a pyranose rings, the equatorial oxygen reacts preferentially; therefore, *O*-3 is the most reactive oxygen atom on the galactopyranosidic ring under these conditions.[8–12] However, another reactive vicinal-diol unit is present in the agarobiitol structure: the 1,2-diol containing a primary hydroxyl group in the 3,6-AnGalOH unit. To obtain regioselective sulfation at O-3, the primary hydroxyl groups of agarobiitol were protected with trityl groups as previously established.[13] The 1^1,6^2-di-*O*-trityl product (**2**, 40%) was unambiguously characterized with the help of an HMBC experiment, which showed correlations between the quaternary carbon of the trityl groups and the protons attached to the primary carbons.

The dibutylstannylene acetal of **2** was formed by heating with 1.08 equiv of dibutyltin oxide in dry methanol at reflux for 3 h, followed by removal of the methanol and any traces of water by azeotropic distillation with toluene for 2 h. The dibutylstannylene acetal was then reacted with the sulfur trioxide–trimethylamine complex in THF under argon.[13] The sulfated product was converted in its sodium salt by cation replacement using a cation exchange resin column. The deshielded position of the C-3 signal in the ^{13}C NMR spectrum indicated that the sulfation occurred as expected to give the 3^2-sulfate-1^1,6^2-di-*O*-trityl product (**3**, 78%).

Detritylation was performed treating compound **3** with 80% aqueous acetic acid for 2 h at 40 °C. After evaporation, the residue was purified by chromatography on a cationic resin followed by silica gel chromatography to give the final product, agarobiitol 3^2-sulfate (sodium salt, **4**, 69%) (Scheme 2).

The enzymatic machinery present in red seaweeds introduces sulfate groups at specific positions during the biosynthesis of their polysaccharides. We have



Scheme 2. Semi-synthetic route to agarobiitol 3-sulfate from agarose: Reagents and conditions: (a) borane 4-methylmorpholine complex, 0.5 M CF_3COOH in water, 65 °C, 8 h; (b) 2.20 equiv trityl chloride, pyridine, 5 °C, 24 h, then rt, 24 h; (c) 1.08 equiv dibutyltin oxide, methanol, reflux 3 h; (d) 1.92 equiv $Me_3N{\cdot}SO_3$, THF, rt, 15 h; (e) cation exchange resin, Na^+ form; (f) 80% aqueous CH_3COOH, 40 °C, 2 h.

extensively utilized this property as a facile method involving partial hydrolysis to prepare structures containing specifically sulfated galactopyranose units. Now, we have prepared agarobiitol 3^2-sulfate, a compound having its sulfate group attached at the position inaccessible to red seaweed enzymes. The synthesis of the 3-*O*-sulfated derivative and the preparation of the sulfated disaccharide alditols shown in Scheme 1, where the sulfates have been introduced by natural processes, make all four possible monosulfates on the galactopyranosidic ring available for investigation of their importance for antiviral activity.

1. Experimental

1.1. General methods

Melting points were determined using a Fisher–Johns melting point apparatus and were uncorrected. Optical rotations were determined with a Rudolph Instruments Digipol 781 automatic polarimeter. Thin-layer chromatography (TLC) was performed on silica gel coated aluminum sheets 60 F254 (Silicycle) using solvent mixtures measured on a v/v basis. After chromatography, products were visualized by spraying the plate with a solution of 0.5% orcinol in ethanol/concd H_2SO_4 (20:1) and heating on a hot plate until coloration was observed. Purification of compounds was carried out with flash column chromatography (1.5×25 cm, 5 mL/min) using TLC grade silica gel 60 (Silicycle). ^1H and ^{13}C NMR spectra were recorded in 5 mm NMR tubes on a Bruker Avance-500 NMR spectrometer operating at 500.13 and 125.77 MHz, respectively. Chemical shifts were reported relative to an internal acetone standard at 30.20 ppm for ^{13}C and 2.224 ppm for ^1H nuclei, for samples recorded in D_2O.[14] For samples recorded in acetone-d_6, chemical shifts were referenced to the central line of the deuterated solvent at 29.92 ppm for ^{13}C and 2.050 ppm for ^1H.[14] For samples in CD_3OD, central line was calibrated at 49.15 ppm (^{13}C) and 3.310 ppm (^1H).[14] All assignments were performed with the aid of COSY, HSQC and/or HMBC experiments. Assignments and magnitudes of coupling constants were obtained for ^1H NMR spectra by first-order analyses. The appearance of signals is indicated using the abbreviations b, s, d, t, q, p, and m for broad, singlet, doublet, triplet, quartet, pentet, and multiplet, respectively. Low-resolution electrospray mass spectra (LRMS) were recorded on a Finnegan LCQ. High-resolution electrospray mass spectra (HRMS) were recorded on a microTOF LC-Bruker Daltonics mass spectrometer with samples dissolved in methanol using the Tuning Mix from Agilent as reference. All solvents used in the experiments were dried and purified by standard methods and distilled before use.

1.2. β-D-Galactopyranosyl-(1→4)-3,6-anhydro-L-galactitol (1)

Commercial agarose Type 1 (Sigma–Aldrich) was submitted to partial reductive hydrolysis:[2] The polysaccharide (1.000 g) was dissolved in water (75 mL), the

solution was heated to 60 °C and borane 4-methylmorpholine complex (6.75 g) was added, followed by 25 mL of 2 M CF_3COOH aqueous solution (final concentration of 0.5 M CF_3COOH). The mixture was kept at 65 °C for 8 h and the acid was then evaporated with the aid of added water (150 mL, four times). The hydrolyzate was dissolved in water (50 mL), treated with three volumes of ethanol, and then filtered. The filtrate was concentrated and the residue was resuspended in methanol (50 mL) followed by addition of 10 volumes of ethyl acetate. The precipitate was collected by centrifugation and the pellet was chromatographed by flash chromatography on silica gel using ethyl acetate/methanol/water (8:2:1) as an eluent to give a colorless syrup (0.3617 g, 36%); $[\alpha]_D^{22}$ −8.3 (*c* 0.4, H_2O); R_f 0.29 (ethyl acetate/methanol/H_2O, 8:2:1); ^1H NMR (D_2O): δ 3.51 (t, 1H, H-2′), 3.65 (m, 3H, H-1a, H-1b, H-3′), 3.70 (m, 1H, H-5′), 3.76 (m, 2H, H-6a′, H-6b′), 3.87 (b, 1H, H-6a), 3.91 (b, 1H, H-3), 3.93 (b, 1H, H-2), 3.94 (b, 1H, H-4′), 3.97 (q, 1H, H-6b), 4.33 (b, 1H, H-4), 4.40 (b, 1H, H-5), 4.56 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1′); ^{13}C NMR (D_2O): δ 60.9 (1C, C-6′), 62.7 (1C, C-1), 68.5 (1C, C-4′), 70.6 (1C, C-2′), 70.9 (1C, C-2), 72.5 (1C, C-3′), 72.9 (1C, C-6), 75.2 (2C, C-5, C-5′), 83.5 (1C, C-3), 85.4 (1C, C-4), 102.2 (1C, C-1′); LRMS: *m*/*z* calcd for $C_{12}H_{22}O_{10}Na^+$: 349.11. Found: 349.1.

1.3. 6-*O*-Trityl-β-D-galactopyranosyl-(1→4)-3,6-anhydro-1-*O*-trityl-L-galactitol (2)

Compound **1** (0.435 g, 1.33 mmol) was dissolved in pyridine (2 mL), then cooled (carbon tetrachloride/liquid N_2 bath at ∼−20 °C), and a solution of trityl chloride (0.818 g, 2.94 mmol) in pyridine (2 mL) was then added dropwise to the vigorously stirred cooled solution. After the addition was complete, the reaction mixture temperature was raised to ∼5 °C for 24 h and, subsequently to rt for 24 h. The resulting mixture was concentrated under vacuum and the residue was purified by dry flash column chromatography using ethyl acetate as an eluent. Compound **2** was obtained as a colorless solid (0.420 g, 40%), that crystallized from hexanes/ethyl acetate (1:1) as colorless crystals, mp 130–133 °C; $[\alpha]_D^{22}$ −31.3 (*c* 0.3, MeOH); R_f 0.30 (ethyl acetate); ^1H NMR (acetone-d_6): δ 3.19 (m, 2H, H-1a, H-1b), 3.39 (q, 1H, H-6a′), 3.43 (m, 1H, H-6b′), 3.56 (b, 2H, H-2′, H-3′), 3.68 (b, 1H, H-5′), 3.72 (b, 1H, H-6b), 3.86 (q, 1H, H-6a), 3.93 (m, 1H, H-4′), 4.03 (b, 1H, H-2), 4.14 (b, 1H, H-3), 4.18 (b, 1H, H-5), 4.38 (b, 1H, H-4), 4.44 (d, 1H, $J_{1,2} = 7.3$ Hz, H-1′), 7.20–7.53 (complex m, 30H, Ar-H, Ar-H′); ^{13}C NMR (acetone-d_6): δ 64.0 (1C, C-6′), 66.0 (1C, C-1), 70.0 (1C, C-4′), 71.2 (1C, C-2), 72.2 (1C, C-2′), 74.7 (1C, C-3′), 75.0 (1C, C-5′), 75.2 (1C, C-6), 75.7 (1C, C-5), 85.3 (1C, C-3), 86.7 (1C, C-4), 87.5 (2C, O*C*Ph_3, O*C*Ph'_3), 103.8 (1C, C-1′), 127.9 (3ArCH, 3ArCH′), 128.7 (6ArCH, 6ArCH′), 129.7 (6ArCH, 6ArCH′), 145.3 (3ArqC, 3ArqC′); LRMS: *m*/*z* calcd for $C_{50}H_{50}O_{10}Na^+$: 833.33. Found: 833.3; for $C_{100}H_{100}O_{20}Na^+$: 1643.7. Found: 1643.9. HRMS: *m*/*z* calcd for $C_{50}H_{50}O_{10}Na^+$: 833.3296. Found: 833.3299.

1.4. Sodium 3-*O*-sulfonato-6-*O*-trityl-β-D-galactopyranosyl-(1→4)-3,6-anhydro-1-*O*-trityl-L-galactitol (3)

Compound **2** (0.151 g, 0.186 mmol) was reacted with dibutyltin oxide (0.0500 g, 0.201 mmol, 1.08 equiv) in dry methanol (8 mL) at reflux for 3 h, followed by removal of methanol and any traces of water by azeotropic distillation with toluene for 2 h. The dibutylstannylene acetal was then reacted with $Me_3N{\cdot}SO_3$ (0.0500 g, 0.358 mmol, 1.92 equiv) in THF (10 mL) under an argon atmosphere for 15 h at rt. The solvent was removed under vacuum and the residue was resuspended in methanol (5 mL) and loaded onto a cation exchange resin column (Dowex 50X2-100, Na+, 1.5 × 7 cm in methanol). The eluent was concentrated to a residue that was purified by flash chromatography on silica gel using ethyl acetate/methanol/H_2O (17:2:1) as an eluent to give compound **3** as a colorless film (0.134 g, 78%); $[\alpha]_D^{23}$ −14.1 (*c* 1.0, MeOH); R_f 0.36 (ethyl acetate/methanol/H_2O, 17:2:1); ^1H NMR (CD_3OD): δ 3.13 (d, 2H, H-1a, H-1b), 3.40 (m, 2H, H-6a, H-6b′), 3.54 (t, 1H, H-5′), 3.71 (q, 1H, $J_{2,3} = 9.7$ Hz, H-2′), 3.76 (b, 1H, H-6b), 3.90 (m, 1H, H-6a), 3.93 (dd, 1H, H-2), 4.08 (t, 1H, H-3), 4.22 (dd, 1H, $J_{3,4} = 3.2$ Hz H-3′), 4.25 (m, 1H, H-5), 4.28 (b, 1H, H-4), 4.34 (b, 1H, H-4′), 4.45 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1′), 7.16–7.46 (complex m, 30H, Ar-H, Ar-H′); ^{13}C NMR (CD_3OD): δ 63.7 (1C, C-6′), 66.2 (1C, C-1), 68.7 (1C, C-4′), 70.8 (1C, C-2′), 71.3 (1C, C-2), 75.1 (1C, C-5′); 75.2 (1C, C-6), 76.6 (1C, C-5), 82.2 (1C, C-3′), 85.7 (1C, C-3), 87.6 (1C, C-4), 88.1 (1C, O*C*Ph_3), 88.3 (1C, O*C*Ph'_3), 104.5 (1C, C-1′); 128.2 (3ArCH, 3ArCH′), 128.9–129.0 (6ArCH, 6ArCH′), 130.1 (6ArCH, 6ArCH′), 145.6 (3ArqC, 3ArqC′); HRMS: *m*/*z* calcd for $C_{50}H_{49}O_{13}S^-$: 889.2899. Found: 889.2883.

1.5. Sodium 3-*O*-sulfonato-β-D-galactopyranosyl-(1→4)-3,6-anhydro-L-galactitol (4)

Compound **3** (0.0212 g, 0.0231 mmol) was dissolved in 80% aqueous acetic acid (5 mL) and the solution was kept at 40 °C for 2 h, then concentrated. The residue was resuspended in methanol/water 3:1 and then purified by chromatography on a cationic resin column (Dowex 50 × 2-100, Na^+, 1.5 × 7 cm) using methanol/water 3:1 as eluent. Concentration of the appropriate fractions gave a residue that was again purified by chromatography on a silica gel column using ethyl acetate/methanol/water (6:3:1) as an eluent to give the title product (**4**) as a colorless syrup (0.0068 g, 69%); $[\alpha]_D^{23}$ −7.0 (*c* 0.2, H_2O); R_f 0.34 (ethyl acetate/methanol/H_2O, 6:3:1); ^1H NMR (D_2O): δ 3.66 (m, 1H, H-1a),

3.67 (m, 1H, H-2′), 3.72 (dd, 1H, H-1b), 3.78 (b, 1H, H-5′), 3.79 (b, 1H, H-6a′), 3.81 (b, 1H, H-6b′), 3.87 (b, 1H, H-6a), 3.93 (b, 2H, H-2, H-3), 4.00 (q, 1H, H-6b), 4.30 (b, 1H, H-4′), 4.34 (b, 1H, H-3′), 4.36 (b, 1H, H-4), 4.42 (b, 1H, H-5), 4.68 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1′); ^{13}C NMR (D_2O): δ 60.9 (1C, C-6′), 62.8 (1C, C-1), 66.8 (1C, C-4′), 68.7 (1C, C-2′), 70.9 (1C, C-2), 72.9 (1C, C-6), 75.1 (1C, C-5), 75.8 (1C, C-5′), 80.1 (1C, C-3′), 83.4 (1C, C-3), 85.4 (1C, C-4), 101.9 (1C, C-1′); HRMS: m/z calcd for $C_{12}H_{21}O_{13}S^-$: 405.0708. Found: 405.0705.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.02.002.

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