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Synthesis of the Sulfonate Analogue of Seminolipid via Horner−Wadsworth−Emmons Olefination

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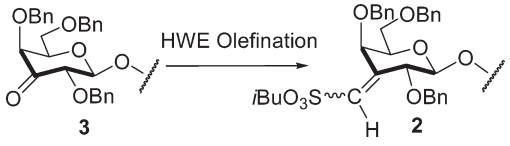
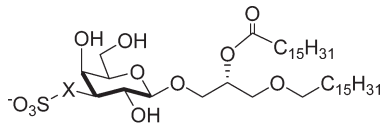


The first synthesis of the sulfonate analogue of seminolipid, the main sulfoglycolipid in mammalian sperm, is reported. Installation of the sulfonate unit was accomplished by a quite unexplored strategy based on Horner−Wadsworth−Emmons olefination on a 3′-keto-galactoside, followed by stereoselective double bond reduction.

Seminolipid SGG **1a** (Figure 1) is a sulfated glycolipid found in mammalian spermatozoa and testes in substantial amount.[1a] SGG from mammalian spermatozoa is mainly a single molecular species: 1-*O*-alkyl-2-*O*-acyl-3-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-*sn*-glycerol, distinguished by an almost homogeneous composition in acyl (hexadecanoyl) and alkyl (hexadecyl) chains.[1b]

Seminolipid **1a** was first synthesized by Gigg[1c] and its properties confirmed the structure of the natural material. Also, the synthesis of deuterium-labeled SGG isotopomers for the quantification of SGG in biological samples has been reported.[1d]



1a X = O **1b** X = CH_2

FIGURE 1. Structure of SGG **1a** and SGG sulfonate **1b**.

SGG is an integral component of sperm lipid rafts[2] and participates on recognition events taking place during sperm−egg interaction. According to a postulated model[2] SGG and its binding protein arylsulfatase-A (AS-A) form a complex that engages in multivalent binding with the glycan moiety of the zona pellucida (ZP), a family of sulfated egg glycoproteins with sperm-binding ability.[3] Whether the interaction between SGG and AS-A is crucial for fertilization is actually a matter of investigation,[4] although analogous involvement of gangliosides in cell-adhesion events is acknowledged.[5]

Seminolipid could also be significant in the frame of sexually transmitted diseases. HIV-1 viral entry into a host cell involves binding of the envelope-glycoprotein gp-120 to CD4 receptor, chemokine coreceptors, and several galactose-containing cell surface glycolipids[6] such as galactosylceramide,[7] GM3 ganglioside,[7] sulfatide,[8] and globotriaosylceramide.[9] SGG exhibits the same receptor functions showing high affinity for gp-120[10] and interaction with viruses and other pathogen microbials.[11] In this context the possibility to inhibit the interaction between gp-120 and glycolipid receptors has driven the development of HIV-1 entry inhibitors with a simplified glycolipid structure.[12]

Inhibitors and probes based on SGG structure would be very useful as tools for studying how SGG acts in combination with AS-A as an adhesion molecule and to discover new SGG analogues with HIV-1 entry inhibitor activity. To this aim we developed the first synthesis of SGG sulfonate **1b**, a mimetic of SGG resistant to hydrolysis, in which the ester oxygen of the sulfate moiety is replaced with a CH_2 unit. The replacement of the sulfate ester with a C-sulfonate results in a

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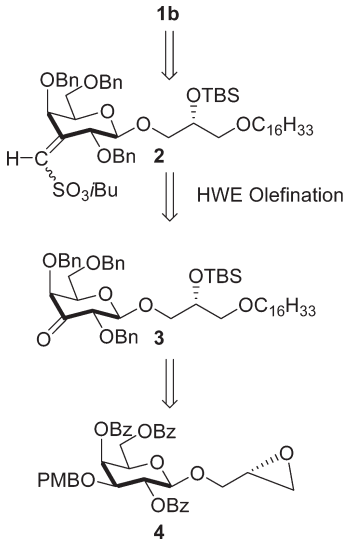
SCHEME 1. Retrosynthesis of 1b



stable, isosteric mimic of SGG that preserves the negative charge of the sulfate.

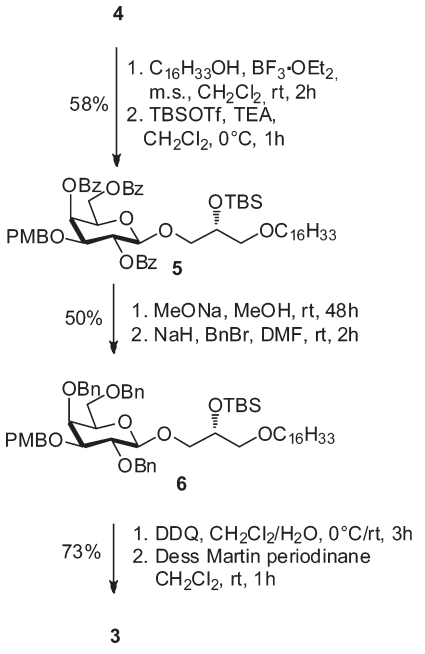
Examples concerning the synthesis of sulfonate analogues of bioactive carbohydrates are reported in literature, including the sialyl Lewis X and sialyl Lewis A tetrasaccharides,[13a,b] glucose-6-sulfate,[13c] *N*-acetylneuraminic acid,[13d] heparin,[13e] nucleosides,[13f] and mannose-6-phosphate.[13g]

Most of these approaches rely on the "mesylate anion chemistry"[13f] (addition of the methanesulfonate ester carbanion to a carbonyl function), while strategies based on the Horner−Wadsworth−Emmons (HWE) olefination are still quite unexplored.[14a,b]

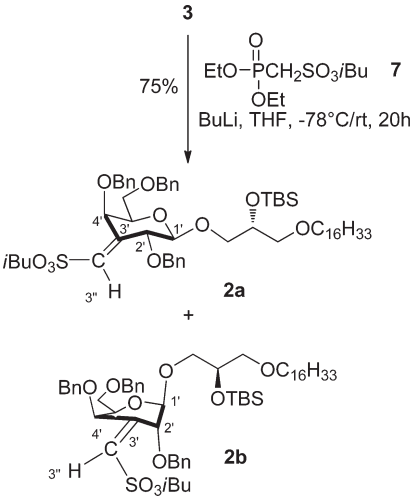
Retrosynthetic analysis of the target sulfonate **1b** is shown in Scheme 1. The synthesis involves the suitably protected 3′-keto-β-galactosylglycerolipid **3** as key intermediate, originated from the β-glycidylgalactoside **4**; a HWE olefination to the α,β-unsaturated sulfonate **2**, followed by the stereoselective double bond reduction should give access to **1b**.

Ketone **3**, the key building block for the HWE olefination, was obtained starting from β-glycidyl galactoside **4**[15−17] (Scheme 2). A regioselective epoxide opening by hexadecyl alcohol promoted by boron trifluoride diethylether complex, followed by conventional silylation, gave the 1-*O*-hexadecyl-ether **5** in 58% overall yield. At this stage benzoyl groups were exchanged for benzyls to avoid any problems during the planned HWE olefination of ketone **3**, and carefully controlled Zemplèn transesterification of compound **5**, followed by benzylation of the crude triol afforded the desired product **6**.[18]

SCHEME 2. Synthesis of 3′-Keto-β-galactosylglycerolipid 3



SCHEME 3. HWE Olefination: Synthesis of Sulfonates 2a,b



Compound **6** was next deprotected by selective removal of the PMB group, and Dess−Martin oxidation of the 3-OH of galactose finally furnished ketone **3**.

Horner−Wadsworth−Emmons olefination is an extremely versatile reaction providing access to a variety of alkene compounds bearing different functional groups.[19] Previous investigations[14b,20] demonstrated that different sulfonate-stabilized

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(15) β-Galactoside **4** was prepared by benzoylation of phenyl 3-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (see ref 16) followed by β-glycosylation with *S*-glycidol under DMTST-activation according to Konradsson's procedure (see ref 17). The compound was produced exclusively as the β-anomer; the anomeric configuration was readily inferred from the coupling costant: $J_{1',2'} = 8.0$ Hz.

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(18) Zemplèn transesterification, performed with 1 molar eq. of sodium methoxide and stopped after 48 h, avoided TBS cleavage; in these conditions, 15% of the byproduct benzylated at position 4 and 6 but retaining the benzoate at position 2 was recovered after flash chromatography.

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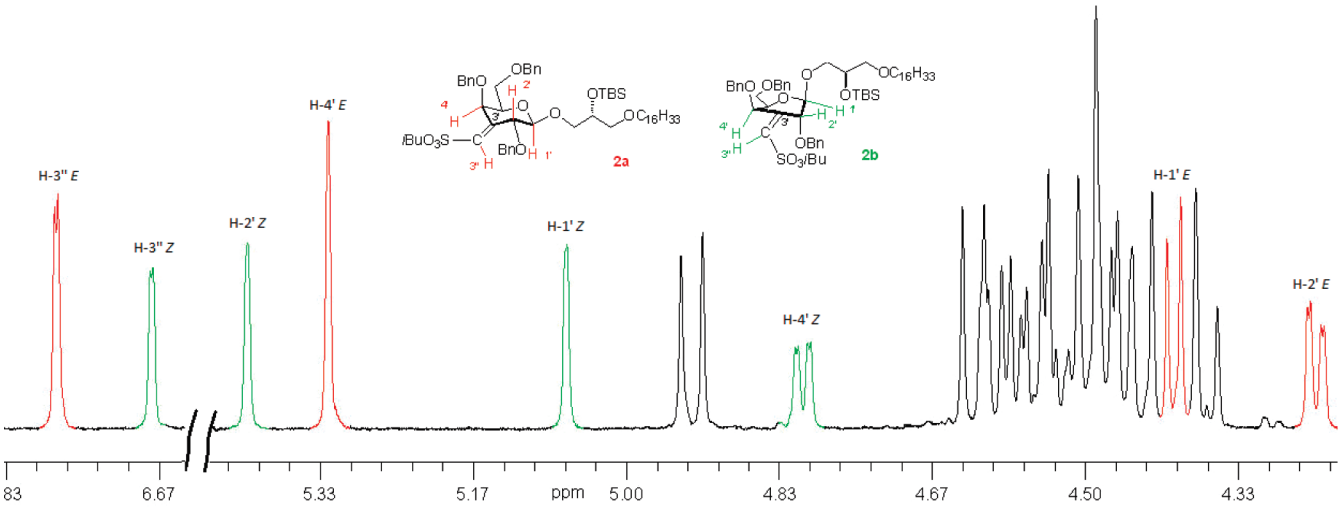


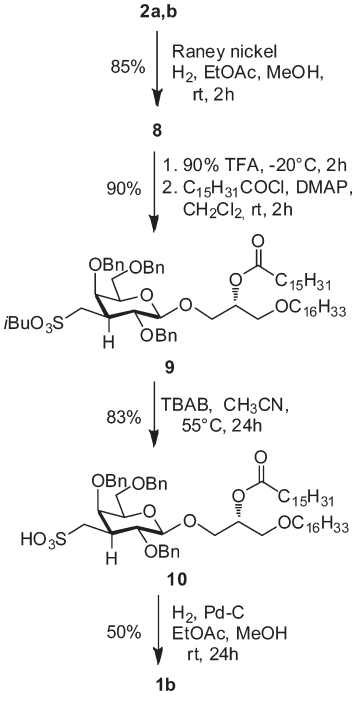
FIGURE 2. Selected ^{1}H NMR signals of sulfonates **2a** and **2b** ($CDCl_3$).

phosphonates are efficient olefinating agents allowing the preparation of α,β-unsaturated sulfonates from both aldehydes and ketones. Toward our target sulfonate **1**, the reactivity of isobutyl sulfonylphosphonate **7** was examined (Scheme 3). Deprotonation of **7** was effected with BuLi at −78 °C. The produced anion reacted smoothly with ketone **3**[21] and α,β-unsaturated sulfonates **2a**,**b** were isolated as a mixture in 75% yield after chromatographic purification.[22]

^{1}H NMR analysis of the **2a,b** mixture ascertained the presence of two geometric sulfonate isomers that were found in different conformations. However, the interpretation of the spectroscopic data was not trivial, as the ^{1}H NMR spectrum (Figure 2) showed some not easily assignable couples of signals having a similar shape but different integrations and correlations in COSY spectrum. Careful examination of the HSQC spectrum allowed the correct assignment of the signals. NMR data, together with a very preliminary conformation analysis of compounds **2a** and **2b** suggested that whereas the pyranose ring of *E*-isomer **2a** adopts a chairlike conformation, the *Z*-isomer **2b** prefers a twist boatlike conformation (see Scheme 3), attributable to an allylic strain between sulfonate and 2′-OBn, with a significant change of both H-1′ chemical shift and $J_{1',2'}$ value.[23] Diagnostic NMR signals and *J* values corroborating this assignment for **2a** and **2b** are reported in Table 1 (see Supporting Information).

Hence attention was focused on the double bond reduction, which had to afford stereoselectively from both conformers **2a** and **2b** the 3′-equatorially arranged isomer with the correct *galacto*-configuration. The best choice was catalytic hydrogenation, and the best results were obtained using

SCHEME 4. Double Bond Reduction, Acylation, and Deprotections: Synthesis of 1b



Raney nickel, which gave exclusively galactoside **8** in satisfactory 85% yield[24] (Scheme 4). The conformations of both isomers account for the observed stereoselectivity of the double bond reduction as a 4′ substituent for **2a** and both 1′ and 4′ substituents for **2b** hamper the attack from the top side.

The configuration at C-3′ was deduced from the vicinal coupling constants ($J_{2',3'}$ = 10.5 Hz and $J_{3',4'}$ = 3.0 Hz), and NOE contacts for compound **8**, corroborating this assignment, are shown in Figure 3.

Completion of the synthesis was then accomplished by means of selective desilylation of the glycerol 2-OH, followed by acylation with palmitoyl chloride, which produced compound **9** in high yield (90%). Nucleophilic displacement on isobutyl sulfonate ester **9** by tetrabutylammonium bromide

(21) Preliminary attempts on tetradecanal or cyclohexanone models afforded the corresponding α,β-unsaturated sulfonates in 60−70% yield.

(22) For our purposes the very difficult separation of sulfonates **2a** and **2b** was not required as both isomers had to stereoselectively converge to a single reduction product.

(23) *E*/*Z* ratio was 2:1 according to integration of the olefinic proton (δ 6.78 ppm for *E*-isomer and δ 6.68 ppm for *Z*-isomer).

(24) Attempts to reduce compounds **2a,b** by catalytic hydrogenation in the presence of Pt/C resulted in an incomplete reaction, and sodium borohydride showed stereoselectivity in favour of the *gulo*-isomer. Moreover, a difference of reactivity between **2a** and **2b** was observed in the hydrogenation reaction where TLC analysis showed a faster reduction of compound **2a** with respect to compound **2b**.

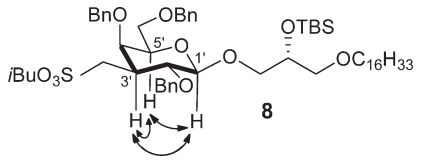


FIGURE 3. Diagnostic NOE contacts for galactoside **8**.

gave compound **10**, which was purified by flash column chromatography and then subjected to hydrogenolysis in the presence of palladium over charcoal to remove the benzyl groups, affording the target sulfonate **1b** (Scheme 4).

In summary, the first synthesis of the seminolipid sulfonate analogue **1b** was developed using a HWE olefination to install a vinylsulfonate, followed by a highly stereoselective reduction of unsaturated sulfonates **2a,b**.

Variations on the lipid moiety of **10** will allow to generate soluble or labeled or fluorescent sulfonate analogues of SGG; moreover, the flexibility of this strategy could also be exploited to obtain sulfonate analogues of other naturally occurring sulfated glycolipids.

Experimental Section

3-*O*-[2,4,6-Tri-*O*-benzyl-3-deoxy-3-(*E*)-isobutylsulfonomethylene-*β*-D-galactopyranosyl]-2-*O*-*tert*-butyldimethylsilyl-1-*O*-hexadecyl-*sn*-glycerol (2a) and 3-*O*-[2,4,6-Tri-*O*-benzyl-3-deoxy-3-(*Z*)-isobutylsulfonomethylene-*β*-D-galactopyranosyl]-2-*O*-*tert*-butyldimethylsilyl-1-*O*-hexadecyl-*sn*-glycerol (2b). To a solution of isobutylsulfonylphosphonate **7** (see Supporting Information) (0.13 g, 0.45 mmol) in dry THF (1.2 mL), kept under argon and cooled at −78 °C, was added dropwise *n*-BuLi (0.17 mL of a 2.5 M solution in hexanes, 0.43 mmol). After 20 min a solution of ketone **3** (0.30 g, 0.35 mmol) in THF (3.0 mL) was slowly added. The mixture was stirred at −78 °C for 1 h and then allowed to warm to room temperature. Stirring was continued at room temperature for 20 h; the pale yellow solution was diluted with EtOAc (40 mL) and washed with water (20 mL), dried over Na_2SO_4, and evaporated under reduced pressure. Purification of the crude material by flash chromatography (*n*-hexane/EtOAc 8:2) afforded sulfonates **2a,b** (0.26 g, 75%) as a colorless oil. Their *E* and *Z* configurations and the *E*/*Z* ratio (2:1) were established by NMR analysis on the mixture of compounds **2a** and **2b**. ESI-MS m/z = 1017.5 $[M + Na]^+$; Anal. Calcd. for $C_{57}H_{90}O_{10}SSi$: C 68.77, H 9.11; found C 68.51, H 9.33. In Table 1 are reported significant ^1H and ^{13}C chemical shifts and coupling constants for **2a,b**. (see Supporting Information).

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Supporting Information Available: Experimental procedures, full characterization of new compounds and proton, carbon NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.