Published in final edited form as:
J Nat Prod. 2009 September ; 72(9): 1651–1656. doi:10.1021/np900336f.

Carteriosulfonic Acids A-C, GSK-3β Inhibitors from a *Carteriospongia* sp

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Abstract

Modulators of Wnt signaling have therapeutic potential in a number of human diseases. A fractionated library from marine invertebrates was screened in a luciferase assay designed to identify modulators of Wnt signaling. A fraction from a *Carteriospongia* sp. sponge activated Wnt signaling and was subsequently shown to inhibit GSK-3β, which inhibits Wnt signaling through phosphorylation of β-catenin. Three novel natural products, carteriosulfonic acids A (**1**), B (**2**) and C (**3**), were identified as active constituents. The carteriosulfonic acids contain unprecedented 4,6,7,9-tetrahydroxylated decanoic acid subunits. Their structures were elucidated through analysis of NMR data and a detailed analysis of pseudo MS^3 spectra.

The Wnt signaling pathway plays major roles in controlling cell proliferation and differentiation; therefore, misregulation of the Wnt pathway has been implicated in a number of human diseases including cancer and neurodegenerative diseases.[1] The kinase GSK-3β negatively regulates mammalian Wnt signaling *via* phosphorylation of β-catenin in the destruction complex. Upon phosphorylation of β-catenin by GSK-3β, β-catenin is targeted and ubiquitinated by b-TrCP and subsequently degraded by the proteasome. Activation of Wnt signaling leads to disheveled-mediated inhibition of GSK-3β allowing β-catenin to activate transcription of Wnt/β-catenin responsive genes.

We recently outlined a screen to identify modulators of Wnt signaling from a fractionated marine natural products library. Recently, we reported studies on bromotyrosine derivatives that activated the Wnt signaling reporter in a non-specific manner through HDAC inhibition.[2] Herein, we report biological and chemical studies on another hit from the screen. A fraction derived from a *Carteriospongia* sp. was a Wnt signaling activator and yielded three new low μM inhibitors of GSK-3β, carteriosulfonic acids A (**1**), B (**2**) and C (**3**). These natural products contain an unprecedented 4,6,7,9-tetrahydroxylated decanoic acid subunit that is derivatized as an amide with taurine and further esterified at *O*-9 with long-chain allylic-alcohol-containing fatty acid groups. The differences between **1**, **2** and **3** lie in the long-chain fatty acid components.

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Supporting Information **Available:** The following supporting information is available: ^{1}H NMR spectra of **1-3**, **7**, **11** and **12**; gHMBC spectra of **1-3**, and **11**; gHSQC spectra of **11** and **12**; a ^{13}C NMR spectrum of **7**; and dose response curves for carteriosulfonic acids A, B and C against GSK-3β. This material is available free of charge via the internet at http://pubs.acs.org.

Structurally, the carteriosulfonic acids are most closely related to taurospongin A and irciniasulfonic acid B. All of these compounds contain taurine functionalized decanoic acid subunits; however, they differ in the substitution patterns on the decanoic subunits and in the make up of the long chain fatty acid components. Taurospongin A was obtained from a *Hippospongia* sp. and exhibited activity against DNA polymerase β and HIV reverse transcriptase.[3] Irciniasulfonic acid B, obtained from an *Ircinia* sp., was a mixture of two related compounds that reversed multi-drug resistance in KB/VJ300 cells.[4]

Results and Discussion

Following the previously reported library screen,[2] we hypothesized that some of the Wnt signaling activators might work through inhibition of GSK-3β. Thus, when an activator of Wnt signaling from a *Carteriospongia* sp. in the library (library code: 6CB8) was found to inhibit GSK-3β the extract was selected for further chemical analysis.

The initial approach to analyzing 6CB8 utilized the previously described automated LCMS fractionation protocol.[5,6] A one milligram archived sample of 6CB8 was chromatographed on a monolithic C-18 column to generate twenty fractions in a 96-well plate. Screening of fractions indicated concentration of activity in well seven, which eluted between nine and ten min. The (+)ESI-MS of the active fraction revealed several sodium-containing clusters between *m/z* 700 and *m/z* 810. An analysis to reconcile the accurate mass data with the source taxonomy yielded no known natural product candidates.

NMR analysis of the active well (~50 μg) revealed a mixture of related oxygenated fatty acid derivatives. At this stage, major sub-structural elements of the carteriosulfonic acids (**1**, **2** and **3**) were elucidated by gCOSY, gHSQC and gHMBC experiments.

In order to purify and fully characterize the GSK-3β inhibitors observed in the active LCMS fractions, a scaled up extraction of the *Carteriospongia* sp. was conducted. A MeOH extract of the sponge was chromatographed on HP20SS resin using a gradient of 100% H_2O to 75% isopropyl alcohol (IPA), followed by 100% MeOH. The 50% IPA fraction contained the same compounds observed in LCMS fraction seven. Carteriosulfonic acids A (**1**), B (**2**) and C (**3**) were purified on LH20 followed by RP-HPLC. The structural elucidation of **1**, **2** and **3** relied on extensive 2D-NMR and MSMS analyses.

Carteriosulfonic acid A (**1**) was isolated as an optically active, amorphous white solid (0.9 mg). FT-MS analysis of **1** supported a molecular formula of $C_{36}H_{67}NO_{11}S$ (*m/z* 722.4511 $[M+H]^+$), which was consistent with NMR data. The IR spectrum showed stretches indicative of hydroxy (3200-3700 cm^{-1}), ester (1724 cm^{-1}) and amide (1676 cm^{-1}) functional groups, while the weak UV chromophore implied limited conjugation.[7]

The NMR data of **1** (Table 1) showed three separate spin systems and was suggestive of a functionalized fatty acid derivative with six exchangeable protons. The majority of the structure of **1** was elucidated from gHSQC, gHMBC, gCOSY and TOCSY data (Figure 1) that revealed two carbonyls, two terminal methyl groups, six oxygenated methines and a number of overlapped methylene resonances.

The simplest spin system in **1**, an AM_2X_2 system, consisted of an exchangeable amide proton (δ_H 7.66) adjacent to a $-CH_2CH_2-$ moiety (δ_H 3.27, δ_C 35.2; & δ_H 2.51, δ_C 50.2). These chemical shifts were consistent with a taurine amide substructure,[7,8] and this was further supported by the IR spectrum that suggested a sulfonate (1205, 1047 cm^{-1}).[7–9]

The taurine containing substructure was connected by gHMBC correlations to a tetra-oxygenated decanoic acid that formed the second separate spin system. The assignment of the

4,6,7,9-oxygenation substitution pattern on the decanoic acid was assisted by selective 1D TOCSY experiments in addition to 2D NMR experiments. The oxygenated methine protons at C-4, C-6 and C-7 were *J*-coupled with exchangeable protons that were assigned to OH groups. The remaining oxygenated methine at C-9 was an ester based on the HMBC spectrum, to a fatty acid unit ($C_{24}H_{43}O_4$ based on the molecular formula) that formed the final spin system. This long-chain fatty acid subunit contained two allylic alcohols that possessed *E* double bond geometry (~15 Hz coupling constant by gDQCOSY). It was not possible to assign the location of the allylic systems on the C_{24} chain by NMR due to overlap of the methylene resonances. Therefore, pseudo-MS^3 experiments were utilized to complete the structural elucidation.

The (-)ESI-MSMS spectrum of **1** fully supported the structural assignments elucidated from the NMR data (Figure 2). Since allylic alcohols undergo (-)ESI-MSMS fragmentations between the oxygenated allylic carbon and the adjacent sp^2 carbon[10] the allylic subunits in the C_{24} fatty acid moiety were located using pseudo-MS^3 (see Figure 2). In source CID of **1** was induced using a 70 V cone voltage, and then the ion (*m/z* 395) corresponding to the C_{24} fatty acid chain was selected for MSMS fragmentation (see Figure 2). From these experiments, the gross structure of carteriosulfonic acid A (**1**) was completed.

Carteriosulfonic acid B (**2**) was isolated as an optically active, amorphous solid. FT-MS analysis of **2** supported a molecular formula of $C_{38}H_{69}NO_{12}S$ (*m/z* 764.46151 $[M+H]^+$) and suggested that **2** was an acetylated analog of **1**. Additionally, the (-)ESI-MSMS spectrum of **2** showed a significant M-AcOH peak (loss of 60.0211) with the remaining fragmentations being nearly identical to those observed in the (-)ESI-MSMS spectrum of **1**. Furthermore, the NMR data for **2** was largely identical to **1** (Table 1), except that the allylic systems in **2** were no longer degenerate. These data implied that **2** contained an allylic acetate, and this was supported by gCOSY and gHMBC correlations (see Table 1). The configuration of each double bond in **2** was assigned as *E* on the basis alkene coupling constants of ~15 Hz in each system.

While the combined spectroscopic evidence supported the structure of carteriosulfonic acid B (**2**) as an allylic acetate derivative of **1**, additional MS studies were required to identify which allylic oxygen was acetylated in **2**. In the (-)ESI-MSMS spectrum of **2** a weak ion at *m/z* 437.3, corresponding to the C_{24} chain, was assigned as either **4** or **5** depending on the location of the acetate in the parent compound (Figure 3). A daughter ion, at *m/z* 377.3, resulting from a loss of acetic acid, was selected for further fragmentation in a pseudo-MS^3 experiment, which supported the structure of the fragment as **6**. Thus, the data supported the structure of the C_{24} fragment as **5** and therefore carteriosulfonic acid B as **2**.

Carteriosulfonic acid C (**3**) was isolated as an optically active, amorphous solid and had nearly identical IR and UV spectra as **1**. (-)ESI-MS analysis of **3** supported a molecular formula of $C_{34}H_{65}NO_{10}S$ (*m/z* 678.4247 $[M-H]^-$). The (-)ESI-MSMS spectrum of **3** showed similar cleavage patterns to **1** and **2** that suggested **3** was yet a third member of a the same series with a different long-chain fatty acid component. This proposition was confirmed by the NMR data for **3** (Table 1), which was nearly identical to **1**; however, **3** exhibited only one allylic alcohol spin system. Thus, **3** was assigned as a C_{22} fatty acid analog of **1** that possessed one allylic alcohol. The configuration of the double bond in the allylic system was assigned as *E* on the basis of a 16 Hz vicinal coupling constant.

The location of the allylic alcohol in **3** was identified by a pseudo-MS^3 experiment. In source CID of **3** (cone voltage = 70V) yielded an ion corresponding to the C_{22} fatty acid chain that was selected for MSMS fragmentation. Ions resulting from fragmentation between the oxygenated allylic carbon and the sp^2 alkene carbon were observed that placed the former at

C-15 and the latter at C-16 on the C_{22} chain (Figure 4). Thus, the gross structure of carteriosulfonic acid C (**3**) was completed.

In a ^{32}P labeling assay **1**, **2** and **3** inhibited GSK-3β with IC_{50} values of 12.5, 6.8 and 6.8 μM respectively (Supporting Information, Figure S14). Desacyl-carteriosulfonic acid (**7**) was prepared to investigate whether the long-chain fatty acid portion of the carteriosulfonic acids was necessary for the GSK-3β inhibitory activity. A sample containing crude carteriosulfonic acids was hydrolyzed with LiOH to give a mixture of **7** and the long-chain allylic-alcohol-containing fatty acid fragments (Scheme 1). Interestingly, **7** showed no GSK-3β inhibitory activity at concentrations up to 50 μM.

In order to investigate the configuration of each oxygenated allylic methine in the carteriosulfonic acids, we chose Riguera's variable temperature NMR method given this requires the preparation of only a single MPA derivative.[11] The crude long-chain-fatty-acid-containing SPE fraction obtained from the above hydrolysis was methylated with diazomethane to give a mixture containing **8** and **9** that was then coupled without further purification with (*S*)-(+)-MPA (Scheme 1) to give the MPA esters **10** and **11**. The ^{1}H NMR spectrum of **11** showed a doubling of several signals, most notably those corresponding to the olefinic, MPA-methoxy and oxygenated methine protons. This implied that **11** was a mixture of diastereomers and that C-15 was a racemic center.

The ^{1}H NMR data observed for **10** was similar to that observed for **11**; however, the signals corresponding to the methyl ether in **10** occurred as a multiplet of overlapping peaks. Given that **10** contained two (*S*)-MPA groups, this suggested that both asymmetric centers in **10** were also racemic.

It is possible that the allylic alcohols racemized during our work up or reaction sequences; however, there are several literature precedents that suggest this is unlikely.[12,13] The most likely points where racemization might have occurred were at the pH extremes, that is, the LiOH hydrolysis or the neutralization with AcOH. Chiral allylic alcohols have been subjected to more basic conditions (1N NaOH)[12] and more acidic conditions (HCl, pH = 1)[13] without reported racemization. Additionally, a precedent in which a racemic allylic alcohol natural product was isolated exists in the obscurolides.[14]

In order to investigate the relative configuration of the 4,6,7-triol system in the decanoic acid subunit of the carteriosulfonic acids, the respective five (**12**) and six-membered (**13**) acetonides were prepared (Scheme 2). After purification by SPE and gel permeation chromatography, the acetonides **12** and **13** were obtained (~7:3 ratio by NMR). While these compounds were not separated, due to the paucity of material and lack of chromophore, two clear sets of acetonide signals were observed by ^{1}H, gHSQC and gHMBC NMR experiments; their structures were also consistent with the reaction sequence and the spectroscopic data. In accordance with literature precedents for the assignment of the relative configuration of 1,2 diols,[12,15-18] the major five-membered acetonide, **12**, was assigned the *syn* configuration: with a relatively small coupling constant ($J_{6\text{-}7}$ = 5.8 Hz, by DQCOSY) and relatively dissimilar methyl ^{1}H chemical shifts (Δδ 0.9 ppm). Furthermore, in accordance with Rychnovsky's NMR protocol for the assignment of 1,3 diols,[19] the minor six-membered acetonide, **13**, was assigned a *syn* configuration based on the ^{1}H and ^{13}C shifts of the acetonide methyls, and the presence of a ROESY correlation between one of the acetonide methyls and both of the acetonide methines (Scheme 2). The combination of both of these analyses suggests the carteriosulfonic acids possess a 4*R**, 6*R**, 7*S** relative configuration in the decanoic acid subunit.

The carteriosulfonic acids (**1**, **2** and **3**) are three new natural products isolated from a *Carteriospongia* sp. These compounds contain unprecedented 4,6,7,9-tetrahydroxylated decanoic acid substructures that are esterified at *O*-9 with long-chain fatty acids. The carteriosulfonic acids are low μM inhibitors of GSK-3β, and by a preparation of a hydrolysis product we found that the long-chain fatty acid component is necessary for this activity. Additional biological studies on the carteriosulfonic acids will be reported in a separate publication.

Experimental Section

General Experimental Procedures

Optical rotations were measured on a Perkin Elmer Model 343 Digital Polarimeter. UV spectra were acquired on a Hewlett-Packard 8452A spectrophotometer. Infrared spectra were recorded on NaCl plates, using a JASCO FTIR-420 spectrophotometer. NMR spectra were obtained on Varian INOVA spectrometers operating at 500/600 and 125/150 MHz for ^{1}H and ^{13}C, respectively. Chemical shifts are reported in ppm and were referenced to residual solvent signals: CD_3OD (δ_H 3.30; δ_C 49.15), DMSO-d_6 (δ_H 2.50; δ_C 39.5), $CDCl_3$ (δ_H 7.26; δ_C 77.0). ESI-MS and MS^n spectra were obtained using a Micromass Q-Tof micro mass spectrometer. FT-MS spectra were obtained using a ThermoFinnigan LTQ-FT mass spectrometer.

GSK-3β Inhibitory Assays

The GSM peptide RRRPASVPPSPSLS RHS(pS)HQRR was purchased (Upstate), dissolved to 1.87 mM in H_2O for use as a GSK-3β substrate, and stored at -20 °C. Purified rabbit GSK-3β and reaction buffer were purchased from New England Biolabs. Reactions were performed in reaction buffer with addition of fresh DTT to 1 mM. Two to six units of GSK-3β were used per reaction. Substrate peptide concentration was 47 μM. ATP was added to 100 μM. P^{32} ATP was added to 2 mCi/reaction. Reactions were incubated at 37 °C for 1 h and stopped by addition of phosphoric acid to 25 mM final concentration. Stopped reactions were spotted onto P81 filter papers and allowed to dry. After drying, filter papers were washed 6 times in 650 mL of 75 mM phosphoric acid. Filters were then put into scintillation vials containing 2 mL of H_2O and counted. GSK-3β inhibition assays on 6CB8, the purified carteriosulfonic acids **1**, **2** and **3**, and desacyl-carteriosulfonic acid **7** were performed by addition of a range of concentrations of each from stocks dissolved in DMSO. Final DMSO concentration was 1%. Data were analyzed in Excel and Prism 4.

Biological Material

The *Carteriospongia* sp. sponge (PSO-04-3-79) was collected by SCUBA from San Miguel Island, Sorsogon, Philippines (Latitude: 12° 71.842′, Longitude: 123° 59.238′, Depth: 10 m). The sponge was identified by Mary Kay Harper from the University of Utah, where a voucher specimen is retained.

Isolation

The sponge was extracted with MeOH (3 × 100 mL), and the combined extracts were concentrated *in vacuo* to give an extract (2.4 g), which was adsorbed onto HP20SS (3 g) in a minimum volume of MeOH. The resulting slurry was then rigorously dried *in vacuo* and then loaded on top of a packed HP20SS column (8 g, 10 × 2 cm) pre-equilibrated with H_2O. The column was eluted with a gradient of H_2O/IPA (four steps 100%, 75%:25%, 50%:50%, 25%:75%, 100%) followed by 100% MeOH. The 50% IPA fraction (250 mg) was selected for further purification. A portion of this material (115 mg) was dissolved in MeOH/H_2O (1.5 mL, 2:1) and chromatographed on a Sephadex LH20 column (30 × 1.25 cm, 1:1 MeOH/H_2O) to yield 11 fractions. The fifth LH20 fraction (10 mg) was purified by RP-HPLC (Phenomenex, C-18, 5 μm, 250 × 10 mm, 3.9 mL/min) using a gradient of MeCN and aqueous NaCl (0.2 M) [55:45 for 2 minutes, then to 80:20 from 2-22 mins] to give **1** (5.9 min), **2** (9.9 min) and **3** (12.7 min).

The samples were desalted using C-18 cartridges (500 mg) which were eluted with H_2O (15 mL) followed by MeOH. Concentration of the respective MeOH fractions gave pure **1**, (0.9 mg), **2** (1.5 mg) and **3** (1.5 mg) as amorphous white solids.

Carteriosulfonic acid A (1)—amorphous, white solid; $[\alpha]^{25}_{D}$ -20 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 206 (3.88), 284 (2.87) nm; IR ν_{max} 3300, 2924, 2854, 1724, 1676, 1585, 1205, 1047 $cm^{-1;}$ ^1H and ^{13}C NMR data, see Table 1; (+) FT-MS *m/z* 722.45111 $[M+H]^+$ (calcd for $C_{36}H_{68}NO_{11}S$, 722.45131); (-)ESI-MS *m/z* 720.4349 $[M-H]^-$ (calcd for $C_{36}H_{66}NO_{11}S$, 720.4357).

Carteriosulfonic acid B (2)—amorphous, white solid; $[\alpha]^{25}_{D}$ -13 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 206 (3.82), 280 (2.70) nm; IR ν_{max} 3300, 2922, 2854, 1724, 1709, 1664, 1580, 1207, 1057 $cm^{-1;}$ ^1H and ^{13}C NMR data, see Table 1; (+) FT-MS *m/z* 764.46151 $[M+H]^+$ (calcd for $C_{38}H_{70}NO_{12}S$, 764.46187); (-)ESI-MS *m/z* 762.4462 $[M-H]^-$, (calcd for $C_{38}H_{68}NO_{12}S$, 762.4462).

Carteriosulfonic acid C (3)—amorphous, white solid; $[\alpha]^{25}_{D}$ -43 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 206 (3.49), 266 (2.64, shoulder) nm; IR ν_{max} 3300, 2922, 2852, 1724, 1662, 1587, 1207, 1059 $cm^{-1;}$ ^1H and ^{13}C NMR data, see Table 1; (-)ESI-MS *m/z* 678.4247 $[M-H]^-$ (calcd for $C_{34}H_{64}NO_{10}S$, 678.4251).

Preparation of desacyl-carteriosulfonic acid (7)

The remaining portion of the 50% IPA fraction off the HP20SS (135 mg) was dissolved in 1:1 MeOH/H_2O (0.3 mL) and chromatographed on a Sephadex LH20 column (30 × 1.25 cm, 1:1 MeOH/H_2O) to yield 15 fractions. Fraction 10 contained crude **1**, **2** and **3** (10.2 mg) and this material was hydrolyzed overnight with LiOH (0.5 M, 1:1 MeOH/H_2O, 4 mL), and the reaction was then neutralized (AcOH) and concentrated *in vacuo*. The products were separated on a C-18 SPE cartridge (500 mg, H_2O/MeOH gradient). The polar desacyl-carteriosulfonic acid (**7**) eluted with 100% H_2O and the long-chain fatty acid containing fragments eluted with 75-100% MeOH. Desacyl-carteriosulfonic acid (**7**) was further purified from the Li salts on an LH20 column (30 × 1.25 cm, 1:1 MeOH/H_2O) and then subjected to a final round of RP-HPLC purification (Phenomenex, phenylhexyl, 5 μm, 250 × 4.6 mm, 0.9 mL/min) using a gradient of MeCN in aqueous AcOH (0.1%) [3.0% for 3 minutes, then to 30.0% over 3-15 mins] to give **7** (eluted across 6 to 14 min) as an amorphous solid (0.9 mg).

Desacyl-carteriosulfonic acid (7)—amorphous, white solid; $[\alpha]^{25}_{D}$ -5 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 206 (3.20) nm; IR ν_{max} 3300, 2927, 1648, 1572, 1211, 1064 $cm^{-1;}$ ^1H NMR (CD_3OD, 500 MHz) δ 3.99 (1H, m, H-9), 3.82 (1H, m, H-4), 3.68 (1H, m, H-7), 3.63 (1H, m, H-6), 3.59 (2H, t, *J* = 6.5 Hz, H-2′), 2.97 (2H, t, *J* = 6.5 Hz, H-1′), 2.32 (2H, m, H-2), 1.84 & 1.67 (2H, m, H-3), 1.71 & 1.59 (2H, m, H-5), 1.61 & 1.48 (2H, m, H-8), 1.19 (3H, t, *J* = 6.0 Hz, H-10); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.2 (C-1), 75.1 (C-6), 72.9 (C-7), 70.9 (C-4), 65.6 (C-9), 51.5 (C-1′), 42.5 (C-8), 40.3 (C-5), 36.8 (C-2′), 34.1 (C-3), 33.4 (C-2), 24.6 (C-10); (-)ESI-MS *m/z* 342.1234 $[M-H]^-$ (calcd for $C_{12}H_{24}NO_8S$, 342.1223).

Preparation of MPA derivative 11

The S-MPA ester **11** was prepared by DCC facilitated coupling according to the literature method.[20] The product was purified by LH20 chromatography.

(S)-MPA derivative 11 (racemic mixture of 2-diastereomers)—amorphous, white solid; UV (MeOH) λ_{max} (log ε): 218 (3.89) nm; IR ν_{max} 2926, 2854, 1749, 1743, 1462, 1175 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 500 MHz) δ 7.43 (2H, m, ArH), 7.34 (3H, m) ArH), 5.67 (0.5H, m, olefinic), 5.44 (0.5H, m, olefinic), 5.37 (1H, m, olefinic), 5.22 (1H, m, H-15), 4.74 (0.5H, s, H-23), 4.73 (0.5H, s, H-23), 3.67 (3H, s, CO_2CH_3), 3.42 (1.5H, s, H-24), 3.41 (1.5H, s, H-24), 2.31 (2H, t, *J* = 7.5 Hz, H-2), 2.01 & 1.87 (2H, m, H-20), 1.62 (2H, m, H-3), 1.51-1.41 (2H, m, H-14), 1.29-1.25 (broad overlapped methylene peak), 0.87 (3H, m, H-22); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.6 (C-1), 170.2 (MPA-ester carbonyl), 136.7 (aromatic), 135.2 (olefinic), 134.8 (olefinic), 130.2 (olefinic), 128.8 (aromatic), 128.5 (aromatic), 128.2 (olefinic), 127.3 (aromatic), 83.1(C-23), 83.0 (C-23), 76.1 (C-15), 57.8 (C-24), 51.7 (methyl ester CH_3), 34.7 (C-14), 34.5 (C-14), 34.3 (C-2), 32.4 (C-18), 32.1 (C-18), 29.7 (multiple methylenes, 25.4, 22.6, 14.0 (C-22); (+)ESI-MS *m/z* 539.3933 $[M+Na]^+$ (calcd for $C_{32}H_{62}O_5Na$, 539.3712).

Preparation of acetonide 12

A sample of crude carteriosulfonic acids (7 mg), 2,2-dimethoxypropane (0.5 mL, excess) and TsOH (< 1 mg) was dissolved in DCM (3 mL) and stirred, under N_2, for 24 h. The reaction was treated with NEt_3 (100 μL), concentrated *in vacuo* and then re-dissolved in a 0.3 M solution of LiOH in MeOH/H_2O (5:2, 7 mL). After hydrolyzing overnight the sample was concentrated and then partially purified on a C-18 SPE plug (500 mg) that was eluted with a gradient of MeOH/H_2O/NEt_3 (1:99:0.1 to 100:0:0.1). The 30% MeOH SPE fraction was then further purified by LH20 chromatography (30 × 1.25 cm, MeOH/H_2O/NEt_3 [1:1:0.001]), to give the 5-membered acetonide **12*** as an amorphous solid (2 mg).

5-Membered acetonide 12*—amorphous, white solid; UV (MeOH) λ_{max} (log ε): 210 (3.71) nm; IR ν_{max} 3292, 2933, 1684, 1587, 1437, 1219, 1054 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 4.33 (1H, m, H-7), 4.28 (1H, m, H-6), 3.92 (1H, m, H-9), 3.74 (1H, m, H-4), 3.58 (2H, m, H-2′), 2.96 (2H, m, H-1′), 2.35 & 2.28 (2H, m, H-2), 1.85 & 1.69 (2H, m, H-3), 1.66 & 1.59 (2H, m, H-5), 1.55 & 1.46 (H-8), 1.40 (3H, s, acetonide CH_3), 1.33 (3H, s, acetonide CH_3), 1.20 (3H, d, 6.4 Hz, H-10); ^{13}C NMR (CD_3OD, 125 MHz) δ 175.7 (C-1), 108.8 (acetal), 76.7 (C-6), 76.0 (C-7), 70.1 (C-4), 65.4 (C-9), 51.3 (C-1′), 40.1 (C-8), 37.9 (C-5), 36.5 (C-2′), 33.4 (C-3), 33.3 (C-2), 28.9 (acetonide CH_3), 26.2 (acetonide CH_3), 24.4 (C-10); (-)ESI-MS *m/z* 382.1548 $[M-H]^-$ (calcd for $C_{15}H_{28}NO_8S$, 382.1536). *Note: NMR data is for the major 5-membered component, the sample contained about 30% of the isomeric 6-membered acetonide **13**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dr. C. Nelson for the FTMS data, J. Reppart for help with ESI-MS, and Dr. J. Skalicky for help with NMR experiments. The following NIH and NSF grants funded NMR instrumentation, RR14768, RR06262, RR13030, DBI-0002806. This research was also supported by The Huntsman Cancer Institute, a University of Utah Seed Grant: Pharmacologic regulation of Wnt signaling (2006-2007), and by NIH grant CA 36622.

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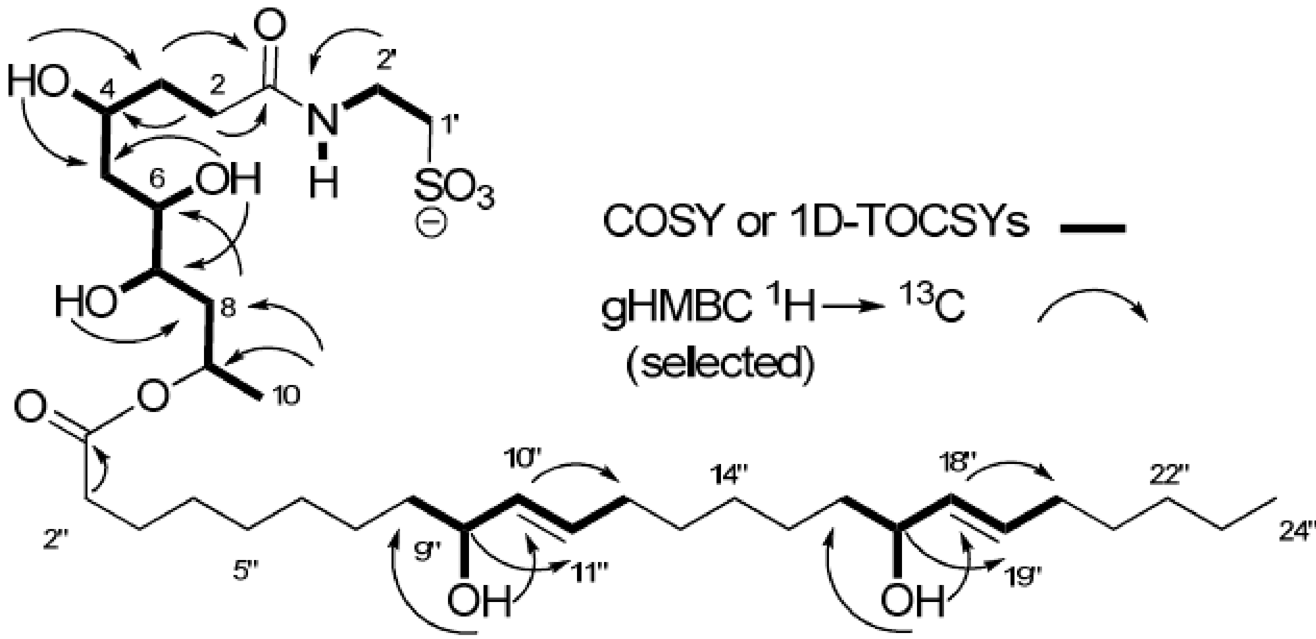


Figure 1.
Key NMR correlations in the structural elucidation of carteriosulfonic acid A (**1**).

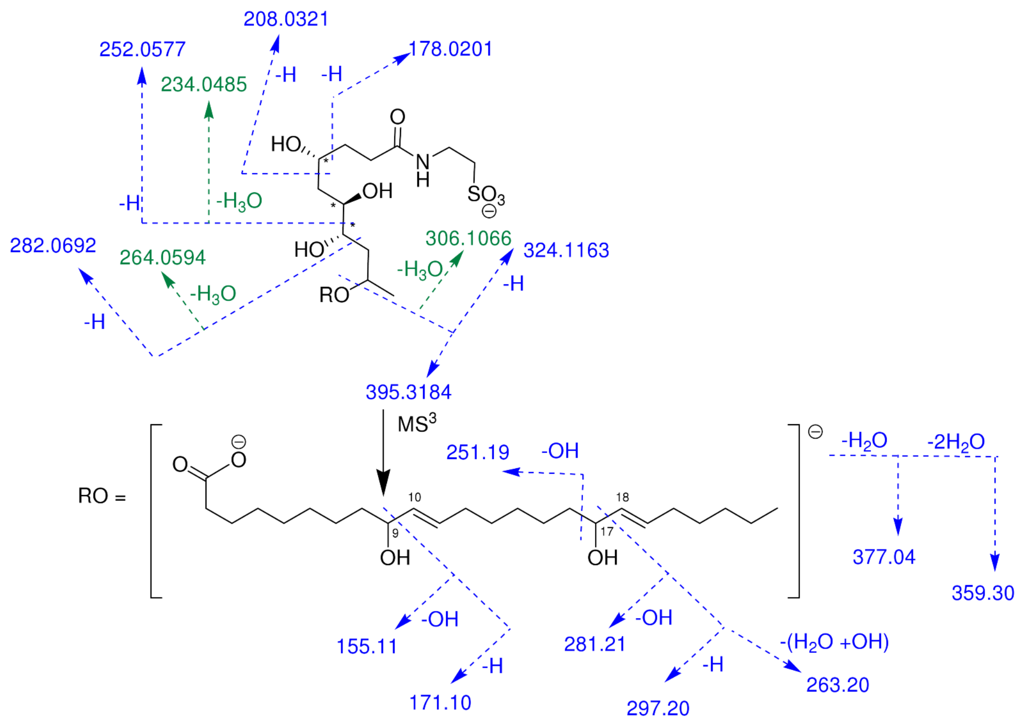


Figure 2.

(-)ESI-MSMS and pseudo-MS^3 analysis of carteriosulfonic acid A (**1**).

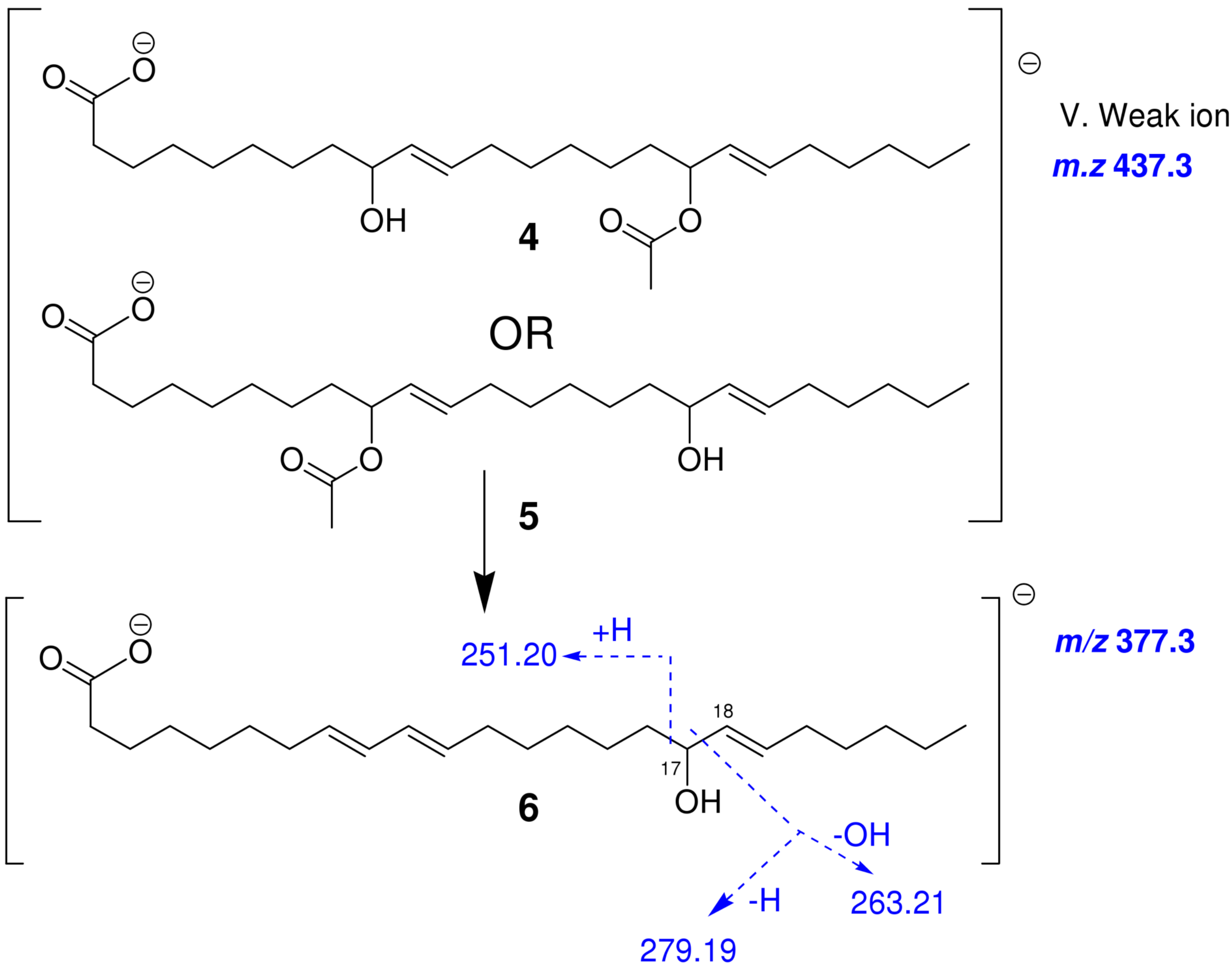


Figure 3.
(-)ESI-MSMS of the two possible C_{24} chain fragments in carteriosulfonic acid B (**2**) showed cleavage consistent with **6.**

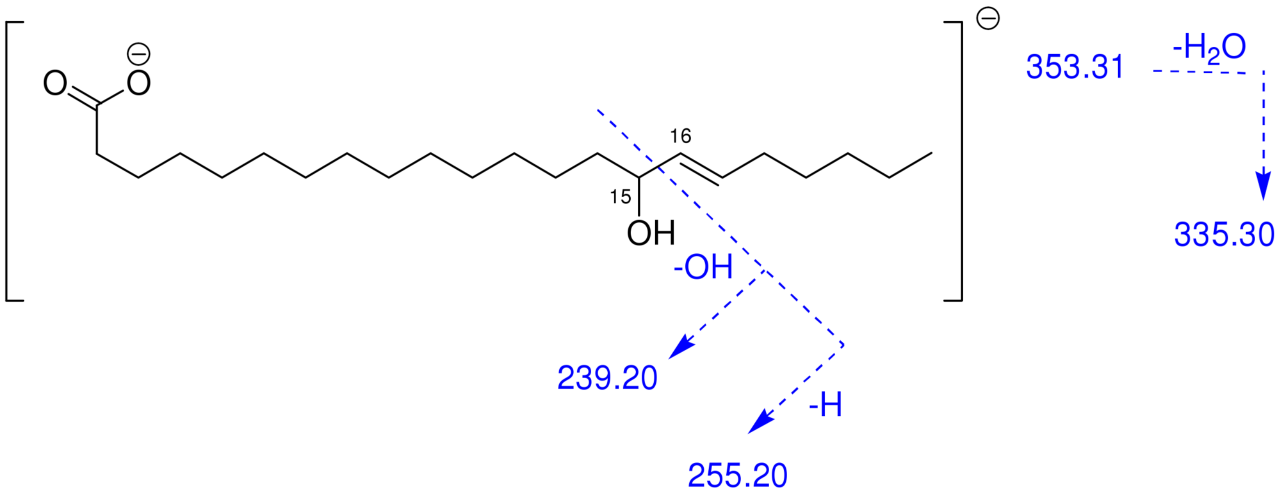
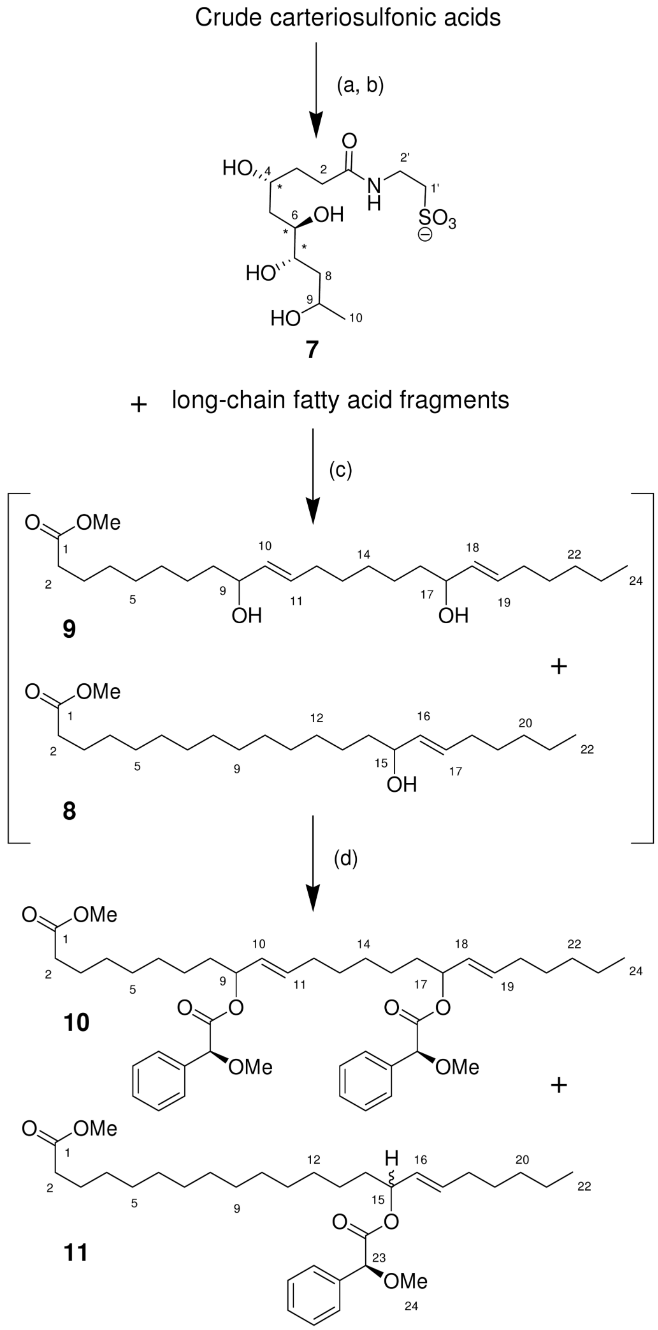
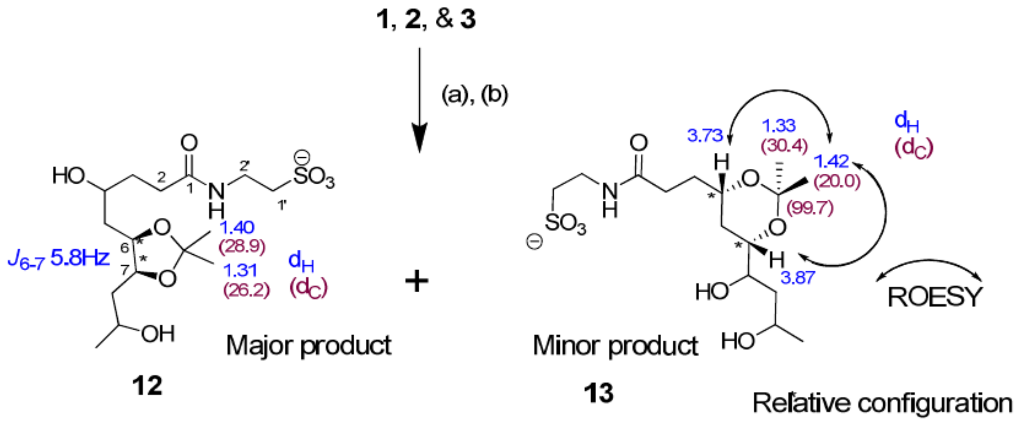


Figure 4.
(-) Pseudo-MS^3 analysis of C_{22} chain of carteriosulfonic acid C (**3**)



Scheme 1.
Hydrolysis of the carteriosulfonic acids and synthesis of (*S*)-MPA derivatives[a].
[a](a) LiOH, MeOH/H_2O, 7 hr. (b) neutralization (AcOH), SPE C-18 $H_2O \rightarrow$ MeOH (c) Fatty acid containing fragment: CH_2N_2, DCM, overnight. (d) (*S*)-(+)-MPA, DCC, DMAP, DCM.



Scheme 2.

Synthesis and relative configuration of acetonide derivatives[a].

[a](a) 2,2-Dimethoxy propane, TsOH, DCM, 24 hr. (b) LiOH MeOH/H_2O, 20 hr.

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Table 1

NMR Data for Compounds **1**, **2** and **3**

Position[a]	1[b]		2[b]		3[b]	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
Decanoic subunit						
1	171.8	-	171.7	-	171.7	-
2 a	31.6	2.15, m	31.7	2.14, m	31.6	2.15, m
b	-	2.06, m	-	2.06, m	-	2.05, m
3 a	32.5	1.65, m	32.3	1.65, m	32.5	1.65, m
b	-	1.46, m	-	1.45, m	-	1.45, m
4	68.1	3.62, m	68.2	3.62, m	68.1	3.62, m
4-OH	-	4.69, d (4.2)	-	4.69, d (4.5)	-	4.70, m
5 a	39.4	1.58, m	39.4	1.57, m	39.4	1.57, m
b	-	1.35, m	-	1.35, m	-	1.36, m
6	73.1	3.35, m	73.1	3.35, m	73.3	3.34, m
6-OH	-	4.61, d (4.4)	-	4.61, d (4.5)	-	4.62, m
7	70.0	3.22, m	70.0	3.22, m	70.0	3.22, m
7-OH	-	4.44, d (6.4)	-	4.45, d (6.1)	-	4.45, m
8 a	38.5	1.78, dd (11.0, 14.0)	38.6	1.78, m	38.5	1.78, m
b	-	1.34, m	-	1.33, m	-	1.33, m
9	67.4	4.97, m	67.5	4.97, m	67.4	4.97, m
10	20.5	1.16, d (6.2)	20.5	1.16, d (6.5)	20.5	1.15, d (6.2)
Taurine subunit						
1′	50.2	2.51, m	50.2	2.52, m	50.1	2.52, m
2′	35.2	3.27, m	35.2	3.28, m	35.2	3.28, m
3′-NH	-	7.66, t (5.5)	-	7.67, t (5.5)	-	7.66, t (5.0)
Long-chain fatty subunit						
1″/1″	172.5	-	172.5	-	172.5	-
2″/2″	33.0	2.22, t (7.3)	33.5	2.22, t (7.3)	33.6	2.22 t (7.4)
3″/3″	24.2	1.49, m	24.2	1.50, m	24.2	1.50, m
8″/- a	37.1	1.37, m	33.6	1.53, m	-	-
b	-	1.30, m	-	1.49, m	-	-

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Position[a]	1[b]		2[b]		3[b]	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
9″/-	70.5	3.83, m	73.7	5.08, m	-	-
9″/- OH	-	4.49, m	-	-	-	-
10″/-	134.3	5.35, m	128.2	5.38, m	-	-
11″/-	128.6	5.47, m	132.9	5.61, m	-	-
12″/- a	31.2	1.99, m	26.2	1.99, m	-	-
b	-	1.96, m	-	1.98, m	-	-
16″/14″ a	37.1	1.37, m	37.1	1.36, m	37.1	1.37, m
b	-	1.30, m	-	1.30, m	-	1.30, m
17″/15″	70.5	3.83, m	70.5	3.82, m	70.5	3.82, m
17″/15″-OH	-	4.49, m	-	4.49, m	-	4.49, m
18″/16″	134.3	5.35, m	134.3	5.35, m	134.2	5.34, dd (6.3, 16.0)
19″/17″	128.6	5.47, m	128.7	5.47, m	128.7	5.47, m
20″/18″ a	31.2	1.99, m	31.2	1.96, m	31.2	1.96, m
b	-	1.96, m	-	1.96, m	-	1.96, m
24″/22″	13.6	0.85, t (6.5)	13.5	0.85, t (6.5)	13.6	0.85, t (7.2)
25″/-	-	-	169.5	-	-	-
26″/-	-	-	20.7	1.98, s	-	-
Overlapping methylenes						
4″-7″/4″-13″, 13″-15″ /19″-21″, 21″-23″/-	28.4, 26.2, 24.6, 24.2, 22.8, 21.7	1.23-1.31, br	30.9, 28.5, 28.2, 28.0, 24.4. 21.8	1.23-1.31, br	24.7, 28.6, 28.3 31.0, 21.7,	1.23-1.31 br

[a]For the long chain fatty acid substructure: numbering for **1** and **2** / numbering for **3**.

[b]DMSO-d6, 600 MHz (^{13}C: 150 MHz).