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Bioactive lipids from the sponge *Spirastrella abata*

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ABSTRACT

Three sphingosine 4-sulfates (**1**–**3**) and a lysophosphatidylglycerol (**4**) were isolated from the Korean sponge *Spirastrella abata*. The structures of these compounds were determined based on the combined results of spectroscopic analyses. Based on the results of combined synthesis and comparison of specific rotation and circular dichroism, the absolute configurations of **1**–**3** were found to be enantiomeric to the previously isolated metabolites. The configurations of **4** were also partially determined by similar chemical and spectroscopic methods. The compounds exhibited significant cytotoxicity and weak antimicrobial activity (**1**), as well as weak-to-moderate inhibitory activity against isocitrate lyase and Na^+/K^+-ATPase. A structure–activity relationship was found for the sphingosine 4-sulfates.

Sphingolipids and lysophospholipids are important membrane components that function in cell recognition and cell regulation.[1] Breakdown metabolites of sphingolipids, such as ceramide, sphingosine, and sphingosine-1-phosphate, have been implicated in cell regulation and exhibit a wide variety of activities related to signal transduction as agonists or second messengers.[1,2] These and related metabolites are widely distributed among marine organisms, such as algae, coelenterates, echinoderms, soft corals, tunicates, and sponges,[3,4] including those of the genus *Spirastrella*.[5a–c]

During the course of our search for bioactive metabolites from Korean water organisms, we encountered the violet-colored sponge *Spirastrella abata* (Order Hadromerida, Family Spirastrellidae), the organic extract of which exhibited significant lethality (LC_{50} = 51 ppm) towards brine-shrimp larvae. Bioassay-guided separation of the crude extract using various chromatographic techniques yielded several lipids. Here, we report the isolation and structural determination of four new metabolites: three sulfated sphingosines (**1**–**3**) and a lysophosphatidylglycerol (**4**) (Fig. 1). These compounds exhibited significant cytotoxicity and weak antimicrobial activity, as well as weak-to-moderate inhibitory activity against isocitrate lyase and Na^+/K^+-ATPase.

The molecular formula of compound **1** was deduced to be $C_{18}H_{37}NO_6S$ by HRFABMS analysis. The linear nature of this compound was evident from the presence of several signals in the regions of δ_C 30.8∼30.4 and δ_H 1.36∼1.28 in the ^{13}C and ^1H NMR data, respectively. This linearity, in conjunction with the presence of nitrogen in the mass data, suggested that **1** was a sphingosine, consistent with the presence of signals for a linear double bond and heteroatom-bearing methines in the NMR data (Experimental Section). Additionally, the presence of a sulfate group was deduced from the strong absorption band at 1250 cm^{-1} in the IR spectrum,

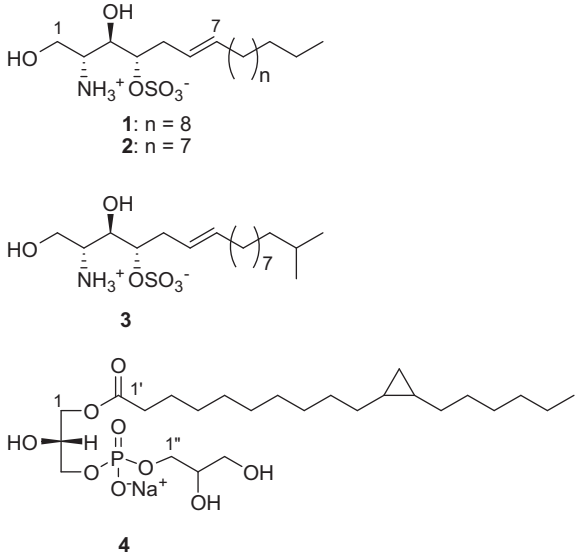

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Figure 1. Structures of compounds **1**–**4**.

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combined with the presence of a sulfur and several oxygens in the mass data.

Given this information, the planar structure of **1** was determined by a combination of ^{1}H COSY, *g*HSQC, and *g*HMBC analyses. These data readily defined a linear array of downfield NMR signals to form a partial structure: $-CH_2-CH-CH-CH-CH_2-CH=CH-CH_2-$. The placement of heteroatoms in this framework was accomplished by observing the chemical shifts and comparing the spectroscopic data with those in the literature. That is, two hydroxy and an amine group were placed at C-1, C-3, and C-2, respectively, by carbon chemical shifts: δ_C 59.3 (C-1), 56.7 (C-2), and 70.6 (C-3). The downfield shift of the C-4 methine (δ_H 4.30, δ_C 79.1) assigned the sulfate group to this location. The remaining portion of the molecule, consisting of one methyl and nine methylene groups, was linearly attached at C-8 on the basis of proton and carbon chemical shifts, thus forming a sulfated sphingosine, 2-amino-1,3-dihydroxyoctadec-6-ene-4-sulfate, for **1**. The *E* configuration of the C-6 double bond was assigned by the downfield shifts of allylic methylene carbons at δ_C 34.9 (C-5) and 33.8 (C-8), as well as cross peaks of H-5 and H-8 with olefinic ones in the NOESY data.

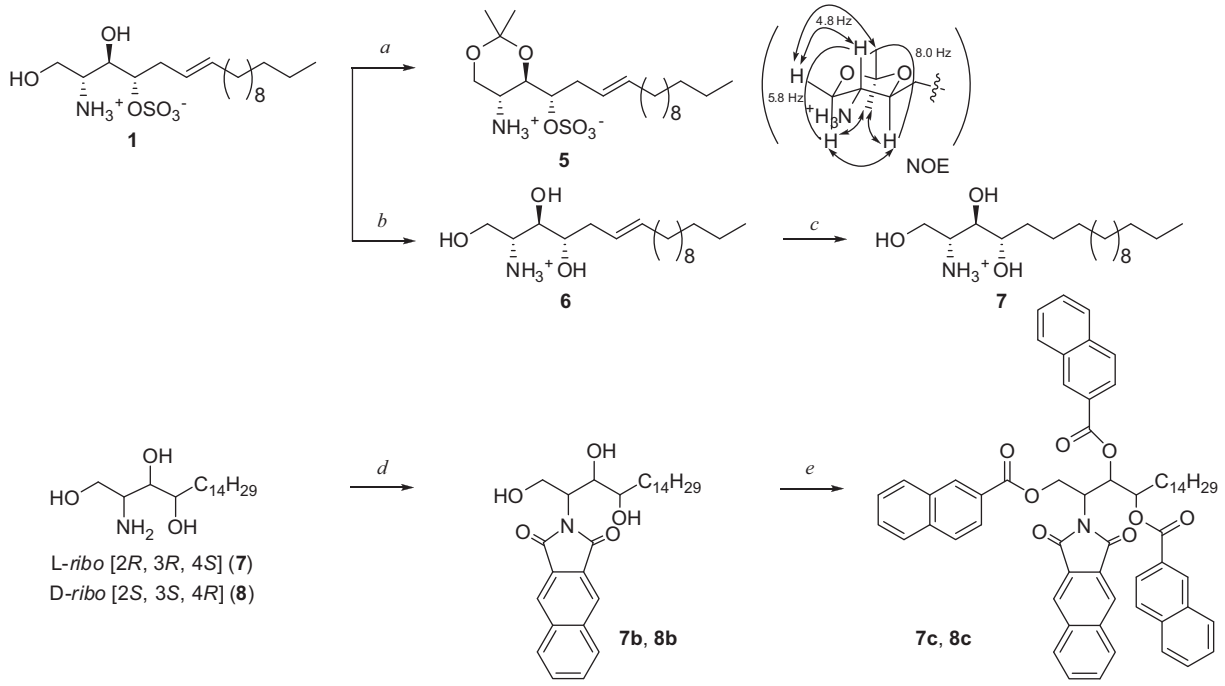
A literature survey revealed that the structure of **1** was identical to that of a sulfated sphingosine previously isolated from the same *S. abata*. However, the specific rotations measured for **1** and its per-acetylated derivative **1a** ($[\alpha]_D^{25}$ −16.8 and −20.4 for **1** and **1a**, respectively in $CHCl_3$) were found to be the opposite of that reported previously ($[\alpha]_D^{25}$ +26 in $CHCl_3$ for the per-acetylated derivative).[6a–c] This prompted us to extensively investigate the stereochemistry of this compound by diverse chemical derivatizations followed by measurements of specific rotation and CD.[5c,7,8a,b]

First, as shown in Scheme 1, the relative configurations at C-2 to C-4 were approached by a ketal formation.[4] Treatment of **1** with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate (PPTS) in acetone yielded the corresponding 1,3-cyclic ketal, **5**. The vicinal coupling constants ($J_{1ax,2}$ = 5.8 Hz, $J_{1eq,2}$ = 4.8 Hz, $J_{2,3}$ = 8.0 Hz) analysis, aided by NOESY cross peaks at H-1$_{ax}$ (δ_H 3.65)/H-3, H-1$_{ax}$/ketal-Me (δ_H 1.41), H-3/ketal-Me, and H-1$_{eq}$ (δ_H 3.99)/ketal-Me (δ_H 1.36), positioned H-2 and H-3 in the *anti* orientation, and thus indicated the 2*R**, 3*R** relative configurations. Due to the free rotation of the side chain, however, the relative configuration at the sulfate-bearing C-4 remained unassigned at this stage.

Given this information, **1** was further derivatized to compare spectroscopic data with known compounds. That is, treatment with H_2SO_4/H_2O/THF converted **1** to 2-amino-1,3,4-trihydroxyoctadec-6-enoate (**6**). The ^{1}H NMR spectrum and LC–ESIMS data (*m/z* 317 $[M+H]^+$) of the product confirmed the sulfate hydrolysis. Hydrogenation of **6** with H_2 with Pd/C furnished the phytosphingosine (**7**). Comparison of the ^{1}H NMR spectra of **7** with reported data for commercial phytosphingosine (D-*ribo*) **8** and its synthetic diastereomers (D-*arabino*, D-*lyxo*, D-*xylo*) showed identity between **7** and a phytosphingosine (D-*ribo*) **8**.[7] However, the specific rotations of **7** and its per-acetyl derivative **7a**, prepared for the enhancement of specific rotation, were the opposite of both phytosphingosine **8** and its per-acetyl derivative **8a** ($[\alpha]_D^{25}$ −10.3, −4.0, +11.6, and +26.0 in $CHCl_3$ for **7**, **7a**, **8**, and **8a**, respectively). Despite the remarkable difference in the scalar values of optical rotations, these results assigned the 2*R*, 3*R*, and 4*S* configurations for **1**.

The stereochemistry of **1** was also approached by another chemical derivatization followed by CD measurements.[8a,b] As shown in Scheme 1, in the first step, the natural product-derived phytosphingosine (**7**) and commercial phytosphingosine (**8**) were converted into *N*-naphthimide derivatives (**7b** and **8b**), then esterified to yield the pernaphthoate derivatives (**7c** and **8c**, respectively). Although the NMR data of these compounds were the same, the CD spectra were opposite to each other [**7c**, extrema at 240 nm ($\Delta\varepsilon$ −5.0), 248 nm (0.0), 263 nm (+2.2); **8c**, 240 nm (+5.1), 248 nm (0.0), 263 nm (−2.2)] (Fig. 2). Thus, the structure of compound **1** was determined to be (*E*)-(2*R*,3*R*,4*S*)-2-amino-1,3-dihydroxyoctadec-6-ene-4-sulfate by application of the Cotton effect.[8b] It is noteworthy that sphingosines from the same sponges were enantiomeric to each other.[5c]

Spectroscopic analyses readily determined that the structure of compound **2**, having the molecular formula $C_{17}H_{35}NO_6S$, was analogous to its congener **1**. Combined 2-D NMR experiments revealed this compound to be a desmethylene derivative of **1**.



Scheme 1. Reagents and conditions: (a) N_2, 2,2-dimethoxypropane, PPTS, acetone, rt, 2 h; (b) H_2SO_4/H_2O/THF, rt, 1 h; (c) H_2, Pd/C, MeOH, rt, 10 h; (d) 2,3-naphthalenedicarboxylic acid anhydride, pyridine, reflux, 15 h; (e) 2-naphthoylimidazole, DBU, MeCN, rt, 3 h.

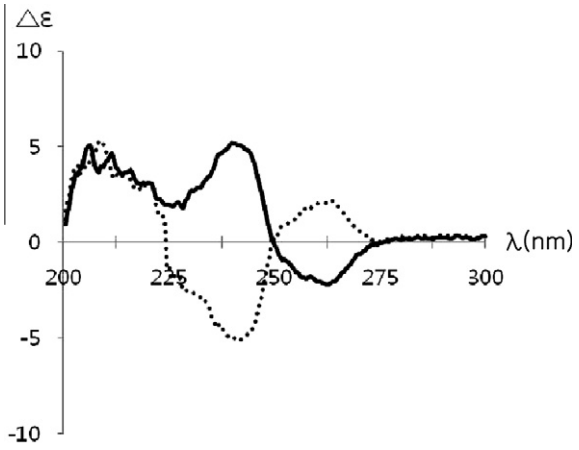


Figure 2. CD spectra of *N*-naphthimide-*O*-trinaphthoate derivatives (**7c**, dotted line; **8c**, solid line) in MeOH.

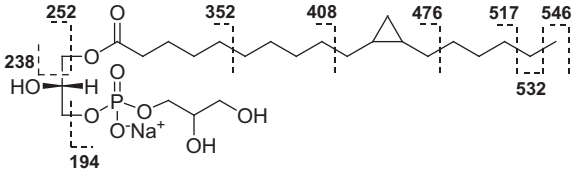


Figure 3. FAB–CID–MS/MS fragmentations of compound **4**.

Configurations identical to those of **1** were assigned based on chemical shifts and coupling constants of key protons. And the specific rotation of **2** was the opposite of previously reported sphingosine from *S.abata*. ($[\alpha]_D^{25}$ −20.4 and +11.6 for **2** and reference, respectively in $CHCl_3$).[5c] Thus, the structure of **2** was (*E*)-(2*R*,3*R*,4*S*)-2-amino-1,3-dihydroxyheptadec-6-ene-4-sulfate, another enantiomer of the previously reported sphingosine from *S. abata*.

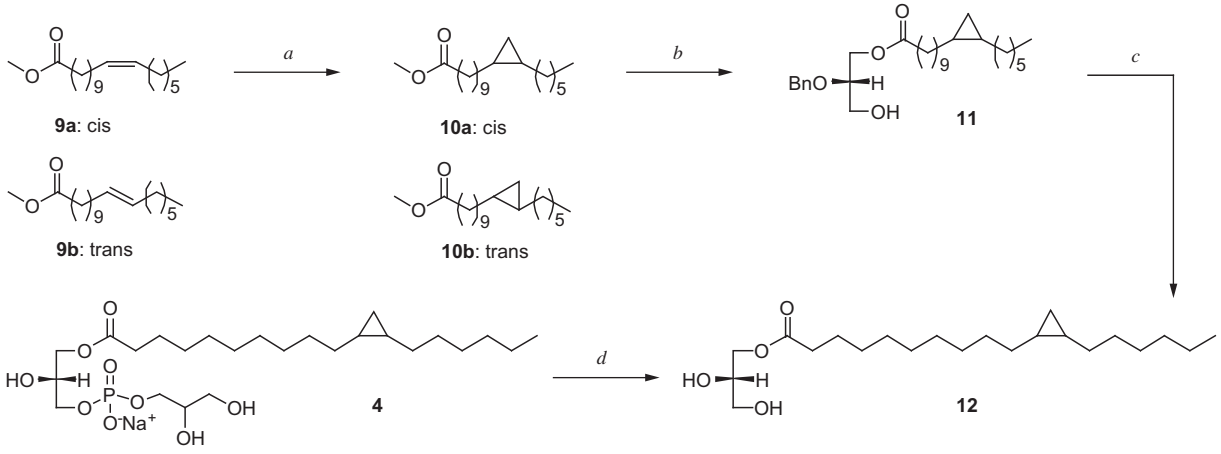
The molecular formula of **3** was found to be $C_{18}H_{37}NO_6S$ by HR-FABMS. Spectroscopic data of this compound were very similar to those of **1** and **2**, with the appearance of a signal for a dimethyl group (δ_H 0.87, 6 H, d, J = 6.6 Hz; δ_C 23.0) as the most significant change in the NMR spectra. Mutual long-range correlations with the C-16 methine (δ_H 1.51, δ_C 29.2) in the *g*HMBC data located the dimethyl group at this position, determining the structure of **3** to be (*E*)-(2*R*,3*R*,4*S*)-2-amino-1,3-dihydroxyisooctadec-6-ene-4-sulfate.

In addition to the sphingosine sulfates, compound **4** was isolated as a colorless gum with the molecular formula $C_{25}H_{48}O_9PNa$, deduced from HRFABMS. The observation of a carbonyl carbon at δ_C 175.5 in the ^{13}C NMR spectra and an absorption band at 1760 cm^{-1} in the IR spectrum were indicative of an ester linkage. The NMR data displayed signals of six oxygenated carbons at δ_C 72.7∼63.9 and their attached protons at δ_H 4.2∼3.5. ^1H COSY, *g*HSQC, and *g*HMBC correlations among these signals revealed the presence of two glycerol-type moieties. Several carbons and protons showed additional splittings that differed from the anticipated findings based on their glycerol moieties; in conjunction with the molecular formula, these were attributed to long-range couplings with the phosphorous atom ($^2J_{CP}$, $^3J_{CP}$, $^3J_{HP}$), indicating the insertion of a phosphate group between the glycerols.

The remaining portion of the molecule, containing the ester and all of the upfield carbons and protons, was found to form a long chain by ^1H COSY analysis and was connected to the C-1 of glycerol, based on long-range correlations between the ester carbon and H-1 methylene protons in the *g*HMBC data. Thus, the gross structure of compound **4** was defined as a *sn1*-lysophosphatidylglycerol.

The fatty acid chain of **4** possessed a cyclopropane (δ_C 16.8, 16.8, 11.6; δ_H 0.66, 0.66, 0.57, −0.33) that was placed at C-11′ by conspicuous ion clusters, including those at *m/z* 476 and 408, attributed to the characteristic γ-cleavage in FAB collision-induced dissociation (FAB–CID) MS/MS data (Fig. 3). Significant differentiations of the H-19 methylene protons (δ 0.57 and −0.33) and large vicinal coupling constants ($J_{11,19}$ = $J_{12,19}$ = 8.2 and 5.2 Hz) suggested a *cis*-orientation for the cyclopropane.

Although a literature survey revealed that the structurally related lysophospholipid was isolated from *S. abata*, the stereochemistry at the asymmetric centers including the cyclopropane moiety was not determined.[5a] The structure and stereochemistry of compound **4** were further pursued by synthetic methods (Scheme 2). Treatment with CH_2I_2 and Et_2Zn converted the commercially available methyl *cis*-(**9a**) and *trans*-11-octadecenoate (**9b**) to the corresponding *cis*-(**10a**) and *trans*-cyclopropane-containing ester (**10b**), respectively. Significant differences in proton chemical shifts in the cyclopropane portion (δ_H 0.64, 0.64, 0.57, −0.34 for **10a**; δ_H 0.33, 0.33, 0.10, 0.10 for **10b**) showed that **4** contained a *cis*-cyclopropane with 11*R**, 12*S** configurations at its fatty acid chain. However, the absolute configurations remained undetermined because the optical rotations of both *cis*-cyclopropane esters from the natural and commercial products were zero. This could have been because the natural and synthetic compounds were mixtures with opposite absolute configurations (11*R*, 12*S* and 11*S*, 12*R*). Alternatively, the contribution to the optical rotation



Scheme 2. Reagents and conditions: (a) CH_2I_2, Et_2Zn, benzene, 70 °C, 6 h; (b) 2-*O*-benzylglycerol, lipase, $CHCl_3$,30 °C, 6 days; (c) H_2, Pd/C, MeOH, rt, 24 h; (d) phospholipase C, Tris–HCl buffer, $CaCl_2$, rt, 22 h.

Table 1
Results of bioactivity test

Compound	MIC(μg/mL)										K562	ICL	Na^+/K^+−APTase
	Gram(+) bacterium			Gram(−) bacterium			Fungus				LC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
	A	B	C	D	E	F	G	H	I	J			
1	50	6	25	100	25	>100	100	>100	50	50	8	2	25
2	>100	12	100	>100	25	>100	>100	>100	>100	>100	7	20	21
3	>100	100	100	100	100	>100	>100	>100	>100	>100	8	24	21
4	>100	NT	NT	NT	>100	NT	>100	NT	NT	13	4	87	4
Ampicillin	1	2	2	2	1	6							
Amphotericin B							1	2	2	1			
Doxorubicin											7		
3-NP[a]												1	
Ouabain													4

A: *Staphylococcus aureus* (ATCC 6538p), B: *Bacillus subtilis* (ATCC 6633), C: *Micrococcus luteus* (IFO 12708), D: *Salmonella typhimurium* (ATCC 14028), E: *Proteus vulgaris* (ATCC 3851), F: *Escherichia coli* (ATCC 35270), G: *Aspergillus fumigatus* (HIC 6094), H: *Trichophyton rubrum* (IFO 9185), I: *Trichophyton mentagrophytes* (IFO 40996), J: *Candida albicans* (ATCC 10231).

[a] 3-Nitropropionic acid. NT: Due to the limited amounts of isolated materials, test was not performed.

of the cyclopropane in the middle part of the fatty acid chain could be minimal.

The configuration at the C-2 of the glycerol moiety was also approached by chemical reactions. The lipase from *Pseudomonas cepacia* (lipase PS)-catalyzed transesterification of **10a** with 2-*O*-benzylglycerol in $CHCl_3$ yielded the monoester (**11**). Based on the stereoselectivity of this enzyme-catalyzed reaction, the configuration at C-2 was assigned as *S*.[9] Then, palladium-catalyzed deprotection of **11** under a H_2 atmosphere gave the desired compound **12a**. Also, **4** was hydrolyzed to **12b** by phospholipase *C* and $CaCl_2$.[10] The ^1H NMR spectrum and specific rotation of the synthetic **12a** were very similar to those of natural product-derived **12b** ($[\alpha]_D^{25}$ −5.2 and −6.1 in $CHCl_3$ for **12a** and **12b**, respectively). Thus, an absolute configuration of *S* (*R* for **4**) was assigned at C-2 of **12b**. The configuration at the remote C-2″ remained unassigned since several attempts to synthesize **4** from **12a** or **12b** with asymmetric glycerol derivatives were unsuccessful. Overall, the structure of compound **4** was determined to be 1-*O*-(*cis*-11,12-methyleneoctadecanoyl)-*syn*-glycero-3-phosphoglycerol.

Sponge-derived phytosphingosine sulfates and lysophospholipids have been reported to exhibit diverse bioactivities.[5a–c] In our measurements, all of the compounds described here displayed significant cytotoxicity against the K562 cell line that was comparable to that of doxorubicin (Table 1). However, their antimicrobial activities were much weaker and only **1** exhibited marked inhibition against diverse bacterial and fungal strains. Additionally, these compounds exhibited weak-to-moderate inhibition against isocitrate lyase (ICL) and Na^+/K^+-ATPase. It is noteworthy that **1** displayed much more potent antimicrobial activity and ICL inhibition than the similar sphingosine sulfates **2** and **3**, indicating that the bioactivity of these sphingosine sulfates depended significantly on chain length and terminal substituents. On the other hand, the lysophosphatidylglycerol **4** showed far more significant cytotoxicity and Na^+/K^+-ATPase inhibition than the sphingosine sulfates.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.105.

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