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Halisphingosines A and B, Modified Sphingoid Bases from *Haliclona tubifera*. Assignment of Configuration by Circular Dichroism and van't Hoff's Principle of Optical Superposition†

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Abstract

Halisphingosines A (**1**) and B (**2**), modified long-chain sphingoid bases, from the marine sponge *Haliclona tubifera* collected in Brazil, were characterized after conversion to their *N*-Boc derivatives. The 2*R*,3*R*,6*R* configuration of halisphingosine A, a compound first reported from *Haliclona* sp. South Korea, was confirmed using a novel CD approach: deconvolution of exciton coupling from mono- and tri-naphthoyl derivatives obtained by derivatization of the natural product. The sensitive CD deconvolution method, applicable to sub-milligram samples, simultaneously predicted the relative and absolute configuration of three stereocenters in halisphingosine A with precision and accuracy. Halisphingosine B was assigned by correlation to halisphingosine A.

Diverse variants of the C₁₈ long-chain bases sphingosine and phytosphingosine and the corresponding ceramides occur throughout the five Kingdoms¹ including marine phyla² such as sponges, tunicates and macroalgae. Modifications include variable chain lengths, chain branching by methyl groups, hydroxylation, and polar head-group modifications. Linear and branched aminoalkanols from sponges and tunicates exhibit substantial antifungal activity. ‘Two-headed’ sphingolipids, constituting C₂₈ or C₃₀ chains functionalized at both termini as aminoalkanols or aminoalkane-diols with four stereocenters,³ show activity against the fungal pathogens *Candida albicans*, *C. glabrata* and other species. Heterocyclic analogs derived from linear sphingoid bases have also been characterized from sponges and tunicates; these include azirines⁴ and piperidines (e.g. pseudodistomins A and B, from *Pseudodistoma* sp.⁵). Other heterocyclic sphingoids reported from marine invertebrates are the so-called ‘anhydrophytosphingosines’ including

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Supporting Information Available. Additional experimental details and ¹H NMR data for compounds **1b**, **2b**, **6a**, **7a**, **12**, **13** are available free of charge via Internet at <http://pubs.acs.org>.

azetidines (penaresidines A and B from *Penares* sp.⁶) and tetrahydrofurans (pachastrissamine from *Pachastrissa* sp.⁷ – synonymous with jaspine B⁸ – and penasins A-E from *Penares* sp.⁹).

Although mammalian sphingosines are almost always of the 2*S*,3*R* configuration (D-*erythro*), invertebrate-derived sphingoid bases exhibit broad stereochemical heterogeneity. Examples of α,ω -bifunctionalized sphingolipids are known with almost all possible permutations of the four stereocenters ($N = 16$).³ While the modified Mosher's method has been the applied method for assignment of absolute configuration of sphingoid bases, the method suffers from the usual limitations; requirement of sufficient sample for preparation of *S*- and *R*-MTPA derivatives and NMR analysis, and equivocal interpretations for molecules with multiple contiguous –NH₂ and –OH groups. Other approaches based on degradation have similar drawbacks and erroneous assignments have been made in the past.¹⁰ Here, we report the *N*-Boc derivative **1a** of halisphingosine A (**1**), a C₁₈ sphingoid base first isolated from a *Haliclona* sp. collected off Ulleung Island, South Korea,¹¹ and reisolated in this work from a Brazilian marine sponge, *Haliclona tubifera* collected off Ilha do Arvoredo, Santa Catarina.¹² A new derivative, the *N*-Boc analog **2a** of halisphingosine B (**2**), is also described and characterized by NMR and MS. Unlike common marine-derived sphingolipids related to D-*ribo*-phytosphingosine (**i**) the new compounds are L-*threo*. The configuration of **1a** was verified at the nanomole scale as 2*R*,3*R*,6*R* [L-*threo* at the head group], by interpretation of the CD spectra of mono- and tetra-naphthoyl derivatives of **1**; specifically, deconvolution of the Cotton effect (CE) arising from different molecular exciton couplings through application of van't Hoff's principle of optical superposition.¹³

Results and Discussion

Ethyl acetate soluble extracts of the sponge *Haliclona tubifera* were fractionated by flash chromatography (NH₃-saturated MeOH: CH₂Cl₂) to obtain a polar ninhydrin-positive fraction containing a mixture of long-chain bases. In order to simplify the purification, the long-chain base mixture was converted to the corresponding *N*-Boc derivatives (di-*tert*-butylcarbonate, NaHCO₃, aq. THF), and then separated by multiple rounds of reversed-phase HPLC (C₁₈-bonded silica), leading to pure *N*-Boc derivatives **1a** and **2a**. Characterization of **1a** by MS and NMR (Table 1) demonstrated that it was the *N*-Boc derivative of **1** (2-aminooctadec-7-ene-1,3,6-triol), a compound first reported by Mansoor and coworkers in 2007.¹¹ Analysis of the COSY spectrum (CD₃OD, 500 MHz) assigned downfield signals to a secondary allylic alcohol (H-6, δ 4.37, dt, $J = 9.0, 5.7$; H-7, δ 5.31, dddd $J = 9.3, 7.8, 1.5, 1.5$; H-8, δ 5.45, dt, $J = 9.3, 6.4$) and a contiguous spin system associated with the terminal head group: CH₂OH (H-1a, δ 3.55, m; H1b, δ 3.60, m) adjacent to C-2 CH-NH-(*t*-Boc) (H-2, δ 3.54, m) which in turn was coupled to a hydroxymethine signal H-3 (δ 3.75, m). The C-1–C-3 segment was separated from the C-6 allylic CH-OH by two CH₂ groups and the remainder of the molecule was a linear *n*-alkyl chain. Taken all together, the constitution of **1a** was identical to that published,¹¹ except for the presence of the *N*-Boc group.

The challenges of assignment of multiple stereocenters in acyclic natural products can be formidable.¹⁴ The original configurational analysis of **1** was carried out by comparison of ¹H NMR spectra of the tetra-Mosher's ester derivative of **1**¹¹ with those of the corresponding four synthetic stereoisomers of sphinganine.¹⁵ Although, strictly not an accurate comparison (**1** contains an additional allylic OH at C-6), the conclusion of the analysis suggested a likely match for the (2*R*,3*R*,6*R*) isomer. In order to verify the configuration of **1** and refine methods for sub-milligram stereoanalysis of complex sphingolipids, we adapted a variant of Nakanishi and Berova's method¹⁶ for phytosphingosines based on CD of naphthimide-naphthoate derivatives obtained by two-step

sequential derivatization. Nakanishi's method introduces a C-2 naphthimide group selectively by conversion of the primary NH_2 in the first step by condensation with naphthalene-2,3-dicarboxylic acid, followed by per-*O*-naphthoylation of the remaining OH groups. Degeneracy is avoided in the superposition of pairwise exciton couplings because of the different UV λ_{max} of naphthimide-naphthoate chromophore pairs. This choice provides 'fingerprint' CE that sufficiently discriminate all possible diastereomers and their corresponding enantiomers. We favored, instead, a single-step exhaustive naphthoylation of **1a** that we expected would provide the same discrimination of *erythro* and *threo* diastereomers by pairwise-interactions of naphthamide-naphthoate pairs, but overlaid with an additional CE from the distal allylic 2-naphthoate at C-6. Application of the latter CE for assignment of allylic alcohols – arising from helicity of the exciton coupled 1L_b transition of the 2-naphthoate chromophore with the ene $\pi-\pi^*$ transition (Figure 2) – was first introduced by Nakanishi and coworkers¹⁷ for secondary alcohols from their allylic benzoates, and successfully validated by others¹⁸ to allylic 2-naphthoate esters. Because the exciton coupled chromophores at the head-group C-1–C-3 and the C-6–C-8 are separated by two CH_2 groups in an acyclic chain backbone, little interaction is expected between the two stereoclusters. In other words, the ensemble should be interpretable as the superposition of each set of *independent* exciton coupled systems according to van't Hoff's principle of optical superposition.¹³ This was shown to be the case and the configuration of **1** was verified as follows.

The *N*-Boc derivative **1a** (ca. 1 mg) was converted to the acetonide **3** (Scheme 1) under standard conditions. Analysis of the ^1H NMR (500 MHz, CDCl_3) spectrum of **3**, in particular the vicinal coupling constants, verified a chair conformation of the 1,3-dioxane ring, possessing an axial C-2 *N*-Boc group and an equatorial C-3 substituent. It follows from the small vicinal coupling observed between H-2 and H-3 ($J = 1.9$ Hz) that **3** has the *threo* relative configuration at C-2–C-3 using arguments similar to those applied by Braekman to the acetonide of a related *threo* sphingolipid from *Haliclona vansoesti*.¹⁹ Attempted naphthoylation of **3** was sluggish and incomplete under standard conditions (2-naphthoyl chloride, pyridine, 100 °C, 24 h), however, smooth conversion to mononaphthoate **4** was achieved with the alternative acylating reagent, *N*-(2'-naphthoyl)imidazole (DBU, CH_3CN , 80 °C, ~4 h).²⁰ The CD spectrum of **4** (CH_3CN , Figure 4b) revealed the long-wavelength half of the expected split CE as a simple broad negative band [λ 236 ($\Delta\epsilon -21.9$) rising to a weak, positive CE ($\Delta\epsilon \sim 8$, $\lambda = 190$ nm, truncated). Thus the C-6 configuration of **4** and **1b** is verified as *R*.

Naphthoylation of a fraction enriched in **1** gave, after HPLC purification, the tetra-(2'-naphthoyl) derivative **5**. In contrast to **4**, the CD spectrum (CH_3CN) of **5** (Figure 4c) exhibited expectedly more complex CEs [λ 243 ($\Delta\epsilon -20.1$), 235 (+17.4), 224 (–12.5)] generated by superposed exciton coupling contributions of the stereoclusters at C-1–C-3 and C-6–C-7–C-8.

For the purposes of CD stereoanalysis of the head group in **5**, model compounds of known configuration were required. The *erythro* and *threo* tri-(2'-naphthoyl) diastereomers **6b** and **7b** were prepared (Scheme 2) from L-serine using a variation of the method reported earlier.^{3c} The CD spectrum of *threo*-**7b** (Figure 3) showed a strong negative split CE [λ 237 ($\Delta\epsilon -19.3$), 221 (+7.2), peak to trough, $A = 26.5$] while the CD of *erythro*-**6b** (Figure 3) was distinctly different: although the sign of the split CE was still negative the magnitude was far larger [λ 226 ($\Delta\epsilon +56$), 242 (–68.6), $A = 124$]. Both *erythro*-**6b** and *threo*-**7b** displayed biphasic CEs that were higher in magnitude than their corresponding tri-benzoyl derivatives^{3c} and, as with the latter compounds, easily discriminated the four possible stereoisomers with the added advantage of approximately five- to ten-fold higher sensitivity.

The CD spectrum of **5** was compared with 'hybrid CD' spectra generated by simple linear combinations of the CD spectra of **7b**, **4** (Figure 4) and their mirror images (not shown; see Supporting Information for hybrid CD of **4** and *erythro-6b*). The only combination (Figure 3c) that matched the observed CEs of **5** corresponds with the 2*R*,3*R*,6*R* configuration, thereby confirming the assignment for **1** proposed by Mansoor and coworkers.¹¹

Halisphingosine B (**2a**) was isolated in low yield (~0.4 mg) from an earlier-eluting fraction that yielded **1a**. The formula C₂₃H₄₇NO₅Na established from ESI HRMS suggested one double bond equivalent less than **1a**. Inspection of the ¹H NMR spectrum of **2a** (Table 1) showed absence of the signals of the disubstituted olefin in **1a** but preservation of the spin system corresponding to H-1–H-6, suggesting the new compound was 7,8-dihydro-halisphingosine A. Halisphingosine derivatives **1a** and **2a** were correlated as follows (Scheme 3). Compound **1a** was hydrogenated (H₂, Pd-C, MeOH) and the product converted to the per-acetyl derivative **8** by removal of the Boc group (TFA, CH₂Cl₂) and acetylation (Ac₂O, pyridine, rt) followed by purification by HPLC. Compound **8** was also obtained by two step conversion of **2a** (~0.1 mg, TFA, CH₂Cl₂; Ac₂O, pyridine). Both samples of **8** were identical by HPLC (rt = 34.5 min, RP C₁₈, 7:3 CH₃CN-H₂O; one peak by coinjection), HRMS and ¹H NMR (CDCl₃, 500 MHz, See Supporting Information), in particular, by comparison of the chemical shifts of the four narrow-line CH₃(C=O) signals and H-1–H-3 (Table 1). Thus, the configurations of **1a** and **2a** are the same and given that the two natural products co-occur in *Haliclona tubifera*, it's highly likely the absolute configurations are also identical.

The application of CD combined with deconvolution of superposed CEs is exceedingly sensitive; in the case of **5** we estimate the limits of detection (LOD) to be approximately 30 pmol. The method should be applicable to the structures of other sphingoid base natural products containing similar remotely separated stereoelements.

The pathogenic yeast *Candida albicans* is modestly inhibited by sphingosine or phytosphingosine, however, many modified sphingolipids possess significantly more potent antifungal activity.²¹ Unfortunately, homogeneous samples of **1** for testing could not be purified from the limited amounts of sponge material available, or by deprotection of pure **1a**,²² and insufficient amounts of **2a** were available for assay. Efforts in our laboratory to procure additional samples of **1** by total synthesis are underway.

In conclusion, a new sphingolipid halisphingosine B was characterized as its per-acetyl derivative **8** along with **1a**, the *N*-Boc derivative of halisphingosine A (**1**). A variant of Nakanishi's CD protocol¹⁶ for assignment of sphingolipids, combined with van't Hoff's principle of optical superposition, was applied to verify the absolute configuration of the known compound **1**. These tools should find general application for configurational analysis of sphingoid base natural products.

Experimental Section

General Experimental Procedures

General experimental procedures are described elsewhere.²³ CD spectra were recorded on a Jasco J810 spectropolarimeter in 1 mm pathlength under the following parameters: slit 2 nm, scan speed 100 nm/min, digital resolution 1 nm, 8–16 accumulations. ¹H NMR spectra were recorded on a dual channel Jeol ECA 500 MHz spectrometer (¹H NMR, 500.1599 MHz), an Agilent 500 MHz spectrometer equipped with an XSENS ¹³C{¹H} cryoprobe, or a dual channel Bruker 600 MHz spectrometer (¹H NMR, 599.5560 MHz) equipped with an Avance III dual channel console and a 1.7 mm ¹H{¹³C,¹⁵N} microcryoprobe. Spectra are referenced to residual solvent signals for CD₃OD or CDCl₃ (¹H, δ 3.31 ppm or δ 7.26 ppm; ¹³C, δ 49.0

or 77.0 ppm, respectively). Accurate mass measurements were carried out on an Agilent 6230 ESI-TOF mass spectrometer, equipped with an Agilent 1200 capillary HPLC system. Solvents for sub-milligram preparative HPLC were distilled from commercial 'HPLC-grade' stock.

Animal Material

Specimens of *Haliclona tubifera* were collected by hand with scuba (−15 to −20 m) in 2010 at Ilha do Arvoredo, Santa Catarina, Brazil. The samples were freeze-dried and stored at −18 °C for 4 months before use. The sponge was identified by João Luis de Fraga Carraro, and specimens are deposited in the Museu de Ciências Naturais–Porifera (MCNPOR) collection of the Fundação Zoobotânica do Rio Grande do Sul, Brazil.

Purification of Halisphingosines A and B: *N*-Boc derivatives, 1a, 2a

A sample of *Haliclona tubifera* (56.1g wet wt.) was extracted with MeOH (3 × 500 mL). The resulting extract was adjusted to 9:1 MeOH/H₂O and partitioned with hexanes (3 × 300 mL). The aqueous MeOH layer was removed and the H₂O layer was extracted with EtOAc (3 × 300 mL). The EtOAc soluble fraction was partially (500 mg) purified by flash chromatography on a silica column using a gradient of MeOH (NH₃ satd.):CH₂Cl₂ (5:95–10:90, v/v). The ninhydrin positive bands were combined resulting in seven fractions. The sixth fraction (46.8 mg) was subjected to a reversed-phase (C₁₈) cartridge with a solvent gradient of MeOH:H₂O (50:50–100:0, v/v). The fractions eluted with 80% and 100% of MeOH (29.3 mg and 10 mg, respectively) were treated with excess (Boc)₂O and K₂CO₃ in THF:H₂O (4:1) and the mixture stirred for 2 h. After filtration of the solids and concentration, the entire filtrate, was subjected to RP-HPLC (Luna C₁₈, 10 × 250 mm, 5 μm; gradient elution, 65:45 to 100:0 CH₃CN:H₂O over 35 min, ELSD detection) to give *N*-Boc derivative **1a** (8.0 mg). The entire fourth *N*-Boc fraction was subjected to RP-HPLC (Luna C₁₈, 10 × 250 mm, 5 μm; 7:3 CH₃CN:H₂O, 2.5 mL min^{−1}) to give *N*-Boc-halisphingosine B (**2a**, 0.4 mg).

***N*-Boc-halisphingosine A (1a)**—Colorless oil. [α]_D +12.7 (c, 0.20, CHCl₃); ¹H NMR (CD₃OD, 500 MHz), see Table 1; ¹³C NMR (CDCl₃) 156.4 (NH-(C=O)), 132.5* (C-7), 132.3* (C-8), 79.6 (O-C(CH₃)₃), 73.2 (C-3), 67.7 (C-6), 65.6 (C-1), 54.2 (C-2), 37.4 (C-4[§]), 34.2 (C-5[§]), 31.7 (C-16), 29.7 (C-9), 29.41 (C-15[§]), 29.38 (C-10[§]), 28.94 (C-11[§]), 28.35 (3xC, OC(CH₃)₃[§]), 27.7 (C-12[§]), 25.41 (C-13[§]), 25.23 (C-14[§]), 22.6 (C-17), 14.1 (C-18). (*, § interchangeable); HRMS *m/z* 438.3192 [M+Na]⁺ (calcd for C₂₃H₄₅NO₅Na, 438.3195).

***N*-Boc-halisphingosine B (2a)**—Colorless oil.; ¹H NMR (CD₃OD, 600 MHz) δ 3.75 (1H, t, *J*=5.6), 3.60 (1H, m), 3.50–3.54 (3H, m), 1.45 (9H, s), 1.27–1.38 (30H, m), 0.90 (3H, t, *J*=6.7); HRMS *m/z* 440.3343 [M+Na]⁺ (calcd for C₂₃H₄₇NO₅Na, 440.3352).

Preparation of Acetonide 3

A solution of *N*-Boc halisphingosine A (**1a**) (2.0 mg) in acetone and 2,2-dimethoxypropane (12 μL), CH₂Cl₂ (0.2 mL) and pyridinium *p*-toluenesulfonate (1.2 mg, 4.8 μmol) was stirred at room temperature overnight. The mixture was treated with solid K₂CO₃, filtered and concentrated under a stream of N₂. The residue was resuspended and the soluble-portion separated by column chromatography (oversized pipet, silica, 4:1 hexanes:EtOAc) to obtain **3** as a colorless oil pale yellow oil (1.3, 60%). ¹H NMR (CDCl₃, 500 MHz) δ 5.48 (1H, dt, *J* = 9.3, 6.4 Hz), 5.36 (1H, dddd, *J* = 9.3, 7.8, 1.5, 1.5 Hz), 5.29 (1H, d, *J* = 10.0 Hz), 4.41 (1H, dt, *J* = 7.6, 6.0 Hz), 4.05 (1H, dd, *J* = 10.2, 1.7 Hz), 3.89 (1H, m), 3.75 (1H, dd, *J* = 10.2, 1.7 Hz), 3.5 (1H, dddd, *J* = 10.0, 1.9, 1.7, 1.7 Hz), 2.08 (2H, m), 1.45 (6H, s,

$C(CH_3)_2$, 1.40 (2H, *t*-BuO, s), 1.35–1.24 (21H, m), 0.88 (3H, t, $J = 7.0$ Hz); ESI HRMS m/z 478.3504 $[M+Na]^+$ (calcd for $C_{26}H_{49}NO_5Na$, 478.3503).

Preparation of Mononaphthoate Ester 4

A mixture of acetonide **3** (0.6 mg), DBU 3.0 μ L, 20 μ mol and *N*-(2'-naphthoyl)imidazole²⁰ (1.4 mg, 6.5 μ mol) were dissolved in CH_3CN (0.2 mL), sealed in a vial and heated at 60 °C overnight. After cooling, the volatiles were removed under a stream of N_2 , and the residue purified by HPLC (Luna, 3 μ m silica, gradient 65:35 hexanes:EtOAc to 100:0 over 60 min, 0.5 mL.min⁻¹) to give the mononaphthoate ester **4** (0.6 mg, 76%) as a colorless oil. UV (CH_3CN) λ_{max} (log ϵ) 290 (3.67), 280 (3.83), 270 (3.76), 237 (4.76) nm; CD: See Figure 4 and Supporting Information; ¹H NMR ($CDCl_3$) 8.58 (s, H-1'), 8.05 (d, $J = 8.6$ Hz, H-3'), 7.95 (1H, d, $J = 8.1$ Hz, H-8'*), 7.880 (1H, d, $J = 8.6$ Hz, H-4'), 7.875 (1H, d, $J = 8.1$ Hz, H-5'*), 5.83 (m, H-6), 5.61 (m, H-8), 5.47 (1H, bd, $J = 9.9$ Hz, H-7), 5.28 (1H, d, J , 10.0 Hz, NH), 4.02 (1H, d, $J = 12$ Hz, H1a) 3.87 (1H, bt, $J = 6.7$ Hz, H-3), 3.73 (1H, d, $J = 12$ Hz, H-1b), 3.47 (1H, bd, $J = 10.4$ Hz, H-2), 2.23 (H-9, m), 1.85 (m), 1.65 (m), 1.43 (s, *t*-BuO), 1.42 (s, CCH_3), 1.38 (s, CCH_3), 1.45–1.23 (m), 0.87 (3H, t, $J = 6.9$ Hz, H-18) *interchangeable. HRMS m/z 632.3919 $[M+Na]^+$ (calcd for $C_{37}H_{55}NO_6Na$, 632.3922).

Preparation of *N,O',O″,O'''*-Tetranaphthoyl Derivative 5

A partially purified fraction (0.5 mg, ~1.2 μ mol) containing largely **1** in a solution of DBU (5.4 μ L, 36 μ mol) and excess *N*-(2'-naphthoyl)imidazole (2.7 mg, 12.0 μ mol) in CH_3CN (0.2 mL) and heated in a sealed vial at 70 °C overnight. After cooling, the volatiles were removed under a stream of N_2 and the residue purified by analytical HPLC (silica, Luna 3 μ m, hexanes to EtOAc over 60 min, 0.5 mL.min⁻¹) to give the tetranaphthoyl derivative **5** (0.3 mg, 27%). UV (CH_3CN) λ_{max} (log ϵ) 290 (4.10), 280 (4.26), 270 (4.20), 233 (5.14) nm. CD: See Figure 4 and Supporting Information; ¹H NMR ($CDCl_3$) 8.56 (1H, s, H-1'), 8.56 (H-1', s), 8.51 (1H, s, H-1'), 8.27 (H-1', s), 8.2 – 7.2 (m), 6.90 (1H, d, $J = 9.5$ Hz, NH), 5.78 (1H, m), 5.67 (1H, m), 5.55 (1H, m), 5.42 (1H, m), 5.03 (1H, m), 4.71 (1H, s, m, H-1a), 4.63 (1H, s, m, H-1b), 2.18 (2H, m), 1.95 (2H, m), 1.81 (2H, m), 1.25–1.65 (m), 0.83 (3H, t, $J = 6.7$ Hz, H-18). HRMS m/z 932.4517 $[M+H]^+$ (calcd 932.4521 for $C_{62}H_{62}NO_7$, 932.4521).

Preparation of L-erythro- and L-threo-Trinaphthoyl Model Compounds 6a and 7a

The starting aminodiols L-erythro-**6a** and L-threo-**7a** were prepared from **8** derived from L-serine as described previously^{3c} and used without further purification. A solution of crude **6a** (5.0 mg, 0.0257 mmol), DBU (117 mg, 0.771 mmol) and *N*-(2'-naphthoyl)imidazole (57 mg, 0.257 mmol) in CH_3CN (1.0 mL) was sealed in a vial and heated at 70 °C overnight. After removal of the volatiles, the residue was first purified by TLC (4:1 hexanes:EtOAc) and then semipreparative HPLC (RP C_{18} Luna column, gradient elution with 60:40 to 100:0 $CH_3CN:H_2O$ over 15min, 4.0 mL min⁻¹) to give erythro-**6b** as a white powder (0.7 mg, 5%). Conversion of **7a** into threo-**7b** was achieved in a similar fashion (2.9 mg, 14%).

L-erythro-6a—UV (CH_3CN) λ_{max} (log ϵ) 233 (5.14), 280 (4.52) nm; $[\alpha]_D^{23} -67.0$ (c 0.060, $CHCl_3$); CD: see Figure 3 and Supporting Information; ¹H NMR (600 MHz, $CDCl_3$) δ 8.58 (1H, s), 8.49 (1H, s), 8.35 (1H, s), 8.03 (1H, dd, $J=8.5, 1.7$ Hz), 7.94 (1H, dd, $J=8.6, 1.7$ Hz), 7.92 – 7.85 (4H, m), 7.85 – 7.76 (5H, m), 7.73 (1H, d, $J=8.6$ Hz), 7.59 – 7.42 (5H, m), 7.39 (1H, d, $J=8.6$ Hz), 5.55 (1H, dt, $J=8.6, 4.3$ Hz), 5.01 (1H, ddt, $J=8.6, 6.0, 4.3$ Hz), 4.84 (1H, dd, $J=11.8, 6.0$ Hz), 4.76 (1H, dd, $J=11.8, 4.3$ Hz), 2.15 – 2.05 (1H, m), 2.00 – 1.90 (1H, m), 1.69 – 1.60 (2H, m), 1.02 (3H, t, $J=7.4$ Hz). ¹³C NMR (126 MHz, $CDCl_3$) δ 167.39, 167.35, 167.1, 135.8, 135.7, 135.0, 132.8, 132.51, 132.47, 131.6, 131.51, 131.50, 129.51, 129.50, 129.2, 128.7, 128.6, 128.5, 128.3, 127.93, 127.87, 127.85, 127.84, 127.80,

126.92, 126.87, 126.84, 126.7, 125.3, 125.2, 123.7, 76.0, 63.3, 52.2, 34.6, 19.2, 14.0. HRMS m/z 618.2250 $[M+Na]^+$ (calcd for $C_{39}H_{33}NO_5Na$, 618.2251).

L-threo-7b—UV (CH_3CN) λ_{max} (log ϵ) 231 (5.22), 280 (4.61) nm; $[\alpha]_D^{23} +29.6$ (c 0.071, $CHCl_3$); CD: see Figure 3 and Supporting Information; 1H NMR (500 MHz, $CDCl_3$) δ 8.58 (1H, s), 8.53 (1H, s), 8.27 (1H, s), 8.02 (1H, dd, $J=8.8$, 1.6 Hz), 7.98 (1H, dd, $J=8.5$, 1.6 Hz), 7.92–7.73 (10H, m), 7.62–7.49 (5H, m), 7.46 (1H, dd, $J=8.4$, 6.7 Hz), 6.92 (N-H, 1H, d, $J=9.2$ Hz), 5.71 (1H, dt, $J=8.9$, 4.8 Hz), 5.03 (1H, ddt, $J=8.9$, 5.8, 4.8 Hz), 4.72 (1H, dd, $J=11.6$, 5.8 Hz), 4.65 (1H, dd, $J=11.6$, 4.8 Hz), 2.05–1.95 (1H, m), 1.95–1.86 (1H, m), 1.64–1.49 (2H, m), 1.00 (3H, t, $J=7.3$ Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.8, 166.9, 166.8, 135.8, 135.7, 134.9, 132.7, 132.55, 132.49, 131.54, 131.50, 131.47, 129.52, 129.50, 129.1, 128.8, 128.6, 128.51, 128.49, 128.36, 127.90, 127.89, 127.87, 127.82, 127.78, 127.02, 126.95, 126.92, 126.86, 126.7, 125.21, 125.16, 123.5, 73.8, 64.9, 51.9, 34.1, 18.9, 14.0. HRMS m/z 618.2254 $[M+Na]^+$ (calcd for $C_{39}H_{33}NO_5Na$, 618.2251).

Conversion of 1a and 2a to Per-acetyl Derivative 8

A sample of **1a** (0.5 mg) and Pd/C (1.0 mg) was taken up in MeOH (0.4 mL) and purged with H_2 for 2 min. The heterogeneous mixture was allowed to stir at rt under H_2 (1 atm) for 4 h. Complete hydrogenation was verified by LCMS. The mixture was then filtered (0.45 μm syringe filter), and then concentrated under a stream of N_2 . The resultant oil (0.5 mg) was then reconstituted in CH_2Cl_2 :TFA (3:1, 0.2 mL) and stirred at rt for 30 min. The solvent was then removed under a stream of N_2 , and the oil was reconstituted in pyridine (0.1 mL) and acetic anhydride (0.1 mL). The reaction was allowed to stir at 35 °C under N_2 for 3 hr. The volatile reagents were removed, and the crude oil was purified by RP-HPLC (Luna C_{18} , 4.6×250 mm, 5 μm ; 7:3 $CH_3CN:H_2O$, 0.85 mL min⁻¹) to give 7,8-dihydro-peracetate-halisphingosine A (**8**, 0.4 mg). Conversion of **2a** using the conditions of the last two of the three preceding reactions followed by purification gave **8** identical (HRMS, 1H NMR, LC) with the product obtained from **1a**. 1H NMR ($CDCl_3$, 600 MHz) see Table 1. 1H NMR (C_6D_6 , 600 MHz) δ 5.16 (1H, m), 5.08 (2H, m), 4.61 (1H, dddd $J=13$, 9.7, 6.6, 3.1 Hz), 4.05 (1H, ddd, $J=11$, 6.5, 1.7 Hz), 3.94 (1H, ddd, $J=11$, 6.5, 1.7 Hz), 1.79 (3H, s), 1.70 (3H, s), 1.69 (3H, s), 1.55 (2H, m), 1.51 (3H, s); 1.15–1.47 (24H, m), 0.91 (3H, t, $J=6.7$ Hz); HRMS, m/z 508.3244 $[M+Na]^+$ (calcd for $C_{26}H_{47}NO_7Na$, 508.3245). Separate and co-injections of **8** derived from **1a** or **2a** gave a single peak (LCMS, rt 13.38 min, linear gradient from 60%–100% CH_3CN aq: 0.1 % formic acid, over 15 min; Kinetex C_{18} , 4.6×150 mm, 2.7 μm).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Pruett ST, Bushnev A, Hagedorn K, Adiga M, Haynes CA, Sullards MC, Liotta DC, Merrill AH Jr. *J Lipid Res.* 2008; 49:1621–1639.

2. Carter GT, Rinehart KL. *J Am Chem Soc.* 1978; 100:7441–2. Cuadros R, Montejo de Garcini E, Wandosell F, Faircloth G, Fernández-Sousa JM, Avila J. *Cancer Lett.* 2000; 152:23–29. For a review of bioactive aminoalcohols from marine organisms, see Molinski T. *Curr Med Chem: Anti-Infect Agents.* 2004; 3:197–220.
3. (a) Makarieva TN, Denisenko VA, Stonik VA. *Tetrahedron Lett.* 1989; 30:6581–6584. (b) Molinski TF, Makarieva TN, Stonik VA. *Angew Chem Intl Ed.* 2000; 39:4076–4079. (c) Nicholas GM, Molinski TF. *J Am Chem Soc.* 2000; 122:4011–4019. (d) Makarieva TN, Zakharenko AM, Dmitrenok PS, Guzii AG, Denisenko VA, Savina AS, Dalisay DS, Molinski TF, Stonik VA. *Lipids.* 2009; 44:1155–1162. (e) Makarieva TN, Guzii A, Denisenko VA, Dmitrenok PS, Santalova EA, Pokanevich EV, Molinski TF, Stonik VA. *J Nat Prod.* 2005; 68:255–257. (f) Makarieva TN, Dmitrenok PS, Zakarenko AM, Denisenko VA, Guzzi AG, Li R, Skepper CK, Molinski TF, Stonik VA. *J Nat Prod.* 2007; 70:1991–1998. (g) Zhou BN, Mattern MP, Johnson RK, Kingston DGI. *Tetrahedron.* 2001; 57:9549–9554.
4. (a) Molinski TF, Ireland CM. *J Org Chem.* 1988; 53:2103–5. (b) Salomon CE, Williams DH, Faulkner DJ. *J Nat Prod.* 1995; 58:1463–1466. (c) Skepper CK, Molinski TF. *J Org Chem.* 2008; 73:2592–2597.
5. (a) Kobayashi J, Naitoh K, Doi Y, Deki K, Ishibashi M. *J Org Chem.* 1995; 60:6941–6945. (b) Kiguchi T, Yuimoto Y, Ninomiya I, Naito T. *Chem Pharm Bull.* 1997; 45:1212–1215. and key references within.
6. Kobayashi, Ji; Cheng, J-F.; Ishibashi, M.; Walchli, MR.; Yamamura, S.; Ohizumi, Y. *J Chem Soc Perkin Trans.* 1991; 1:1135–1137.
7. Kuroda I, Musman M, Ohtani II, Ichiba T, Tanaka J, Gravalos DG, Higa T. *J Nat Prod.* 2002; 65:1505–1506.
8. Ledroit V, Debitus C, Lavaud C, Massiot G. *Tetrahedron Lett.* 2003; 44:225–258.
9. Ando H, Ueoka R, Okada S, Fujita T, Iwashita T, Imai T, Yokoyama T, Matsumoto Y, van Soest RWM, Matsunaga S. *J Nat Prod.* 2010; 73:1947–1950.
10. The absolute configurations of diastereomeric erythro and threo 2-aminotetradec-5,7-dien-3-ols from a Papua New Guinea sponge, *Haliclona* sp. were first misassigned as (2S,3R) and (2S,3S), respectively, Gulavita NK, Scheuer PJ. *J Org Chem.* 1989; 54:366–369. and revised upon total synthesis. Mori K, Matsuda H. *Liebigs Ann Chem.* 1992; 2:131–137.
11. Mansoor TA, Park T, Luo X, Hong J, Lee CO, Jung JH. *Nat Prod Sci.* 2007; 13:247–250.
12. Compound **1** (Ref. 11) ((2R,3R,6R,Z)-2-aminooctadec-7-ene-1,3,6-triol) had not been assigned a trivial name. Here, the name ‘halisphingosine A’ is coined for **1** for convenience of referencing and relational properties to the co-occurring new compound, halisphingosine B (**2**).
13. van't Hoff, JH. *Die Lagerung der Atome im Raume.* Vol. chp 8. Vieweg: Braunschweig; 1908. p. 95–97.
14. For a contemporary review of integrated approaches to assignment of configuration in marine natural products, see Morinaka BI, Molinski TF. *Tetrahedron.* 2012; 68:9307–9343.
15. Li S, Wilson WK, Schroepfer GJ Jr. *J Lipid Res.* 1999; 40:117–1125.
16. (a) Shirota O, Nakanishi K, Berova N. *Tetrahedron.* 1999; 55:13643–13658. (b) Kawamura A, Berova N, Dirsch V, Mangoni A, Nakanishi K, Schwartz G, Bielawska A, Hannun Y, Kitagawa I. *Bioorg Med Chem Lett.* 1996; 4:1035–1043.
17. Gonnella NC, Nakanishi K, Martin VS, Sharpless KB. *J Am Chem Soc.* 1982; 104:3775–1376.
18. (a) Schneider C, Schreier P, Humpf HU. *Chirality.* 1997; 9:563–567. (b) Molinski TF, Brzezinski LJ, Leahy JW. *Tetrahedron-Asymmetry.* 2002; 13:1013–1016.
19. Devijver C, Salmoun M, Daloze D, Braekman JC, De Weerd WH, De Kluijver MJ, Gomez R. *J Nat Prod.* 2000; 63:978–980.
20. Ikemoto N, Lo LC, Nakanishi K. *Angew Chem Int Ed.* 1992; 31:890–891.
21. Nicholas GN, Li R, MacMillan JB, Molinski TF. *Bioorg Med Chem Lett.* 2002; 12:2159–2162.
22. Attempted removal of the N-Boc group in **1a** using the standard protocol (50% CF₃COOH in CH₂Cl₂, rt) or milder conditions (10 equiv, –20 °C) resulted in decomposition, most likely through S_N1 solvolysis of the allylic OH. More benign conditions for deprotection of N-Boc-alkanamines (H₂O, 100 °C, overnight, reported by Wang J, Liang YL, Qu J. *J C S Chem Commun.* 2009:5144–5146. when applied to **1a**, returned only unreacted starting material.

23. Dalisay DS, Rogers EW, Edison A, Molinski TF. *J Nat Prod.* 2009; 72:732–738.

Figure 1.
Sphingoid bases from *Haliclona tubifera* and erythro-D-ribosphingosine.

Figure 2.

Newman diagrams of major contributing conformers and predicted signs of exciton coupled CD spectra in (a), (b) *S* and *R* secondary allylic 2-naphthoate esters,^{16,18} (c), (d) and *N,O,O*-tris-naphthoyl derivatives of 2-aminoalkane-1,3-diols, (*R,R*)-*i*, and (*R,S*)-*ii*, and (e), (f), (*S,S*)-*i*, and (*S,R*)-*ii* (contributions from 1-naphthoate-2-naphthamide pairs are not shown, but their Cotton effects have the same signs with amplified magnitude *A*). Np = 2-naphthoyl. Blue bar = 2-naphthoate, red bar = 2-naphthamide.

Figure 3.

Measured circular dichroism spectra (CH_3CN , 24 °C) of per-naphthoyl derivatives prepared from L-serine (see Scheme 2). (a) *erythro*-**6b** ((2*S*,3*R*), dashed line, and (b) *threo*-**7b** (2*S*, 3*S*), solid line.

Figure 4.

Measured circular dichroism spectra (CD, CH_3CN , 24 °C) and hybrid CD spectra. (a) *N,O,O',O''*-tetranaphthoyl compound (**5**) prepared from natural product **1**. (b) Mononaphthoate, **4**, prepared from **3** (see Scheme 1) and *N,O,O'*-trinaphthoyl compound *threo*-**7b** (see Scheme 2). (c) CD of **5** (solid line) overlayed with hybrid CD spectrum (dashed) = [CD(**4**) – CD(**7b**), dashed]. (d) CD of **2** (solid line) overlayed with hybrid CD spectrum (dashed) = [CD(**4**) + CD(**7b**)]. See Supporting Information for comparisons of the CD of **5** with hybrid spectra derived from **4** and *erythro*-**6b**.

Scheme 1.
Synthesis of acetone **3** and mono- and tri-naphthoyl derivatives **4** and **5** from **1**.

Scheme 2. Synthesis of model compounds *erythro*-**6b** and *threo*-**7b**.

Scheme 3.
Conversion of **1a** and **2a** to peracetyl-compound **8**.

Table 1

¹H and ¹³C NMR Data of Halisphingosine A (**1a**, CD₃OD, 500 and 125 MHz) and Per-acetyl Halisphingosine B (**8**, CDCl₃, 600 MHz).

Position	1a		8	
	δ_C , type ^b	δ_H , (J in Hz) ^a	δ_C , type ^b	δ_H , (J in Hz) ^a
1	62.8, CH ₂	3.55 m, 3.60 m	63.1, CH ₂	4.03 ddd (15.9, 11.2, 6.2)
2	56.5, CH	3.54 m	49.8, CH	4.41 m
2-NH	–	–	–	5.63 br t (5.8)
3	70.7, CH	3.75 m	71.9, CH	5.06 m
4	34.7, CH ₂	1.45 m	30.5, CH ₂	1.54 m
5	38.5, CH ₂	1.38 m, 1.56 m	34.0, CH ₂	1.50 m
6	67.9, CH	4.37 dt (9.0, 5.7)	73.8, CH	4.84 p (12.5, 6.0)
7	133.7, CH	5.31 dddd (9.3, 7.8, 1.5, 1.5)	30.5, CH ₂	1.54 m
8	131.8, CH	5.45 dt (9.3, 6.4)		
9	28.4, CH ₂	2.09 m	22.6–31.5, CH ₂	1.20–1.32 m
10–17	23.5–32.5, CH ₂	1.29–1.41 m		
18	14.1, CH ₃	0.91 t (6.7)	14.2, CH ₃	0.88 t (6.7)
N-Boc CH ₃	28.5, CH ₃	1.45 s	–	–
Ac ^c	–	–	23.0, CH ₃	2.08 s
Ac ^c	–	–	21.0, CH ₃	2.06 s
Ac ^c	–	–	21.0, CH ₃	2.04 s
Ac ^c	–	–	23.0, CH ₃	2.03 s

^abr=broad; s=singlet; d=doublet; dt=doublet of triplets; t=triplet; m=multiplet; p=pentet.

^bMultiplicities of ¹³C NMR signals established by edited HSQC and DEPT.

^cAcetyl ¹H and ¹³C signals cross correlated by HSQC but not assigned.