# Absolute Configuration of the α,ω-Bifunctionalized Sphingolipid Leucettamol A from *Leucetta microrhaphis* by Deconvoluted Exciton Coupled CD[⊥]

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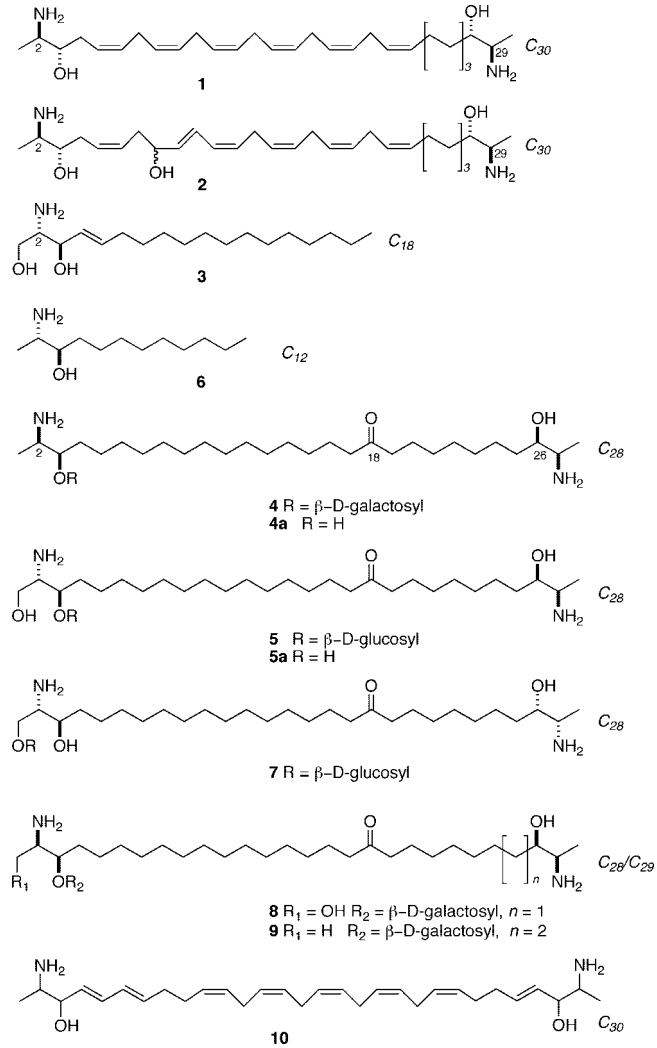
The configuration of leucettamol A (**1**), a known long-chain "two-headed" sphingolipid (dimeric sphingolipid) from the marine sponge *Leucetta microrhaphis*, was determined by conversion to an *N*,*N*′,*O*,*O*′-tetrabenzoyl derivative, measurement of the exciton coupled circular dichroism spectrum (ECCD), and quantitative analysis by deconvolution of superposed exciton couplets. Contrary to the earlier assignment that claimed leucettamol A (**1**) was racemic, the CD approach unambiguously reveals the natural product is chiral and optically active and displays pseudo-$C_2$ symmetry. The configuration of each end of the chain has *erythro* stereochemistry with an absolute configuration of 2*R*,3*S*,28*S*,29*R*. We show that deconvolution ECCD reliably predicts *erythro* versus *threo* vicinal amino alcohols in all cases with greater sensitivity (<5 nmol) compared to $^1$H NMR *J*-based methods and provides verification of optical purity and unequivocal elucidation of absolute configuration in this difficult class of natural products.

Leucettamols A (**1**) and B (**2**) are long-chain bifunctionalized sphingolipids reported by Kong and Faulkner from *Leucetta microrhaphis* from Palau.[1] Dimeric sphingolipids (DSs) are comprised of long-chain $C_{28}$−$C_{30}$ vicinal amino alcohols or 2-amino-1,3-alkanediols that are related to sphingosine (**3**). Analogues of D-sphingosine and phytosphingosine, including their ceramides, are commonly encountered in marine invertebrates, but bifunctionalized sphingolipids (dimeric bis-α,ω-amino alcohols) are rare and, so far, have been reported only from marine sponges. In addition to **1** and **2**, the known members include rhizochalin (**4**) from *Rhizochalina incrustata* from Madagascar,[2] oceanapiside (**5**)[3] and oceanalin A[4] from two different species of *Oceanapia* from Australia, calyxoside (**7**) from *Calyx* sp. from Sulawesi,[5] rhizochalins C (**8**) and D (**9**),[6] and a compound named BSR1 (**10**) from an unidentified sponge[7a] and *L. microrhaphis*,[7b] both from the Great Barrier Reef. DSs display a range of biological activity: **4** exhibits weak antibacterial activity against *Staphylococcus aureus* and cytotoxic activity against mouse Ehrlich carcinoma cells. The glucoside (**7**) induces DNA damage in cells and inhibits protein kinase C.[3] Significantly, the aglycone **5a** of oceanapiside shows approximately 10-fold greater antifungal activity against the pathogenic fluconazole-resistant yeast *Candida glabrata*[2a] than monomeric vicinal amino alcohols such as **3**, implying amplification of activity through a multivalent effect.[8] Leucettamol A (**1**) has recently been shown to inhibit the Ubc13−Uev1A ubiquitin conjugating enzyme complex.[9]

Analysis of the stereostructure of DSs presents interesting challenges. The biosynthesis of DSs displays stereochemical heterogeneity between sponges; each terminus may be elaborated with the same or different relative and absolute configurations. Since there are four stereogenic centers in each DS, the maximum number of possible stereoisomers is 16. If the biosynthesis of DSs follows the well-established pathways for sphingosine and phytosphingosine, the first step is predicted to be condensation of alanine or serine with an activated fatty acyl precursor, with concomitant decarboxylation followed by reduction. The products are 2-aminoalkanols or 2-amino-1,3-alkanediols, respectively, with either




*threo* and *erythro* relative configuration and *S* or *R* absolute configuration (with respect to the α-amino acid). Nevertheless, the biosynthesis is not stereorandom. Each species of sponge appears to produce a limited number of DS analogues but always with *only*

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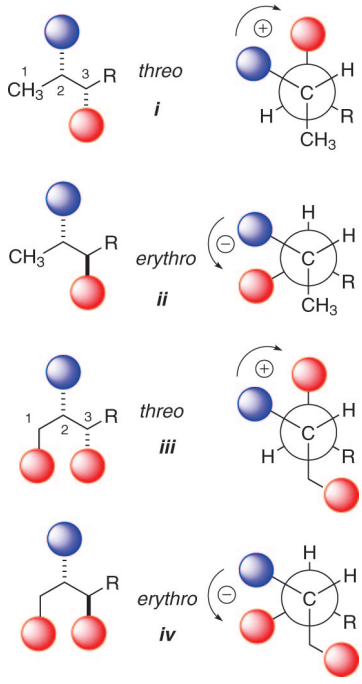


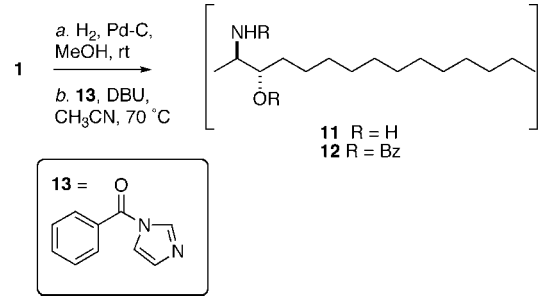

**Figure 1.** Representations of major conformers contributing to exciton coupling CD[11a] in *threo* and *erythro* isomers of *N*,*O*-arenecarboxy derivatives of 2-amino-3-alkanols (***i*** and ***ii***) and 2-amino-1,3-alkanediols (***iii*** and ***iv***). Blue-shaded spheres represent *N*-arenecarboxamide chromophores, and red spheres are *O*-arenecarboxylate chromophores (e.g., arenecarboxy = Ph(CO)O, benzoate, $\lambda_{max} \approx 228$ nm). A positive exciton coupling (+ sign) is defined by a split Cotton effect with a maximum CD signal at longer wavelengths and minimum at shorter wavelengths. Contributions to ECCD from the primary C-1 chromophore (e.g., benzoate) in compounds ***iii*** and ***iv*** modulate the fine structure and intensity of the split Cotton effect, but the signs remain the same as their respective counterparts ***i*** and ***ii***. The enantiomers of ***i***−***iv*** would show mirror image CD spectra.

one stereostructure, but this configuration may differ in compounds from different sponges.

Two claimed exceptions to this motif are **1** and **2**, which were each described as "an optically inactive...oil". On the basis of analysis of the relative configuration of **1** and a specific rotation of $[\alpha]_D = 0$, the authors conclude, "it must be assumed that leucettamol A...is racemic".[10] Since we were aware that low specific rotations were characteristic of natural long-chain dimeric amino alcohols, and enzyme-catalyzed C−C bond formation in sphingosine is highly enantiospecific, this anomaly may be explained if the leucettamols were actually chiral and optically pure but of very low rotatory power. Fortuitous procurement of a sample of **1** by reisolation from *Leucetta* aff. *microrhaphis* (Haekel, 1872), collected in Indonesia, allowed us to make a complete configurational assignment of **1** and **2**, which is the subject of this report.

Exciton coupled circular dichroism (ECCD) between vicinal dibenzoates is a powerful nonempirical method for assignment of absolute configuration of glycols[11] and also cyclic[11b] and acyclic amino alcohols[11c] including "picomole" scale stereochemical analyses of sphingosine and phytosphingosines.[11d] On the basis of this work, we devised a method for quantitative ECCD to assign all four stereocenters in DSs *simultaneously*[3b] that takes advantage of pairwise exciton couplings between vicinal pairs of benzamide−benzoate chromophores (Figure 1). Stereochemical assignment requires a single CD measurement of the corresponding perbenzoyl derivative, only requiring samples as low as 5 nmol, but effectively deconvolutes the superposed exciton couplings between as many as six vicinal *N*,*O*-dibenzoyl pairwise interactions with nanomolar sensitivity. The method readily converges upon a unique stereochemical assignment and was used by one of us (T.F.M.) to assign **5**,[3b] **4**,[2c] **6**,[12] **8**, and **9**[5] and by Kingston and co-workers to assign **7**.[5] Applying this method, we now report that **1** is not racemic as first suggested, but an *optically active* compound with pseudo-$C_2$ symmetry.

**Scheme 1**




## Results and Discussion

Leucettamol A (**1**) was isolated from MeOH extracts of *L. microrhaphis* by solvent partition and reversed-phase HPLC.[13] The compound showed spectroscopic values identical with literature values,[1] was highly prone to decomposition by autoxidation, and was kept frozen (−20 °C) until needed. The structures of **1** and **2** are each unsymmetrical due to the offset placement of skipped polyolefins in the $C_{30}$ linear carbon long chain; however if one considers only end groups for stereochemical analysis, the problem can be reduced in complexity after removal of the double bonds. In order to achieve this aim, a sample of **1** (ca. 0.5 mg) was hydrogenated (Scheme 1) to give the constitutionally symmetrical dimeric $C_{30}$ bis-2,29-diamino-3,28-diol **11** (*m*/*z* 485.36 $[M + H]^+$), which was immediately converted without purification to the corresponding *N*,*N*′,*O*,*O*′-tetrabenzoyl derivative **12** (freshly prepared benzoyl *N*-imidazolide **13**, DBU, dry $CH_3CN$, 60−70 °C).[14] The final product was purified by HPLC (6:94 *i*-PrOH−*n*-hexane) and characterized by MS (*m*/*z* 923.43, $[M + Na^+]$) and $^1$H NMR ($CDCl_3$, 600 MHz, 1.7 mm microcryoprobe), which showed half the number of H signals compared to the formula $C_{58}H_{80}N_2O_6$, consistent with a structure of **12** with either $C_2$ or *meso* symmetry. In particular, downfield methine signals were observed due to H2/H29 CH-NHBz groups ($\delta$ 4.46, m, 2H), H3/H28 CH-OBz groups ($\delta$ 5.22, m, 2H), and the benzamide NH ($\delta$ 6.99, d, $J$ = 7.8 Hz, 2H).

Elucidation of the stereostructure assignment of **1** can be reduced to defining which of the 10 unique stereoisomers **12a**−**j** (Figure 2) corresponds to **12** formed by hydrogenation of **1**. It should be noted that symmetry considerations reduce the maximum number of possible stereoisomers from 16 for **1** to only 10 for **12**; these consist of two enantiomeric pairs of $C_2$ isomers, **12a** and **12d**, two enantiomeric pairs of $C_1$ isomers, **12b** and **12c**, belonging to the group that lacks symmetry, and two achiral compounds, the *meso* dimers **12i** and **12j**.[15]

Assignment of relative configuration and absolute configuration followed from ECCD of the tetrabenzoyl derivative **12** (see below); however, independent verification of the *erythro*/*erythro* relative configuration of **1** reported by Kong and Faulkner's assignment[1] was evident in the $^1$H NMR spectrum of **12**. We had earlier shown[2b] that the vicinal coupling constant $^3J_{H2-H3}$ in *N*,*O*-dibenzoyl-2,3-aminoalkanols can be used to assign *threo* ($^3J$ = 5.2 Hz) versus *erythro* ($^3J$ = 2.6−2.7 Hz) relative configurations; however, the differences between these values diminish in the corresponding 2-amino-1,3-alkanediol derivatives ($^3J$ = 3.8 Hz *threo*; $^3J$ = 3.9−4.1 Hz, *erythro*). The $J$ values also become difficult to measure accurately in the latter compound due to the strong mutual coupling of the C-1 oxymethylene protons and magnetically inequivalent vicinal coupling to H-2. Both factors may lead to erroneous

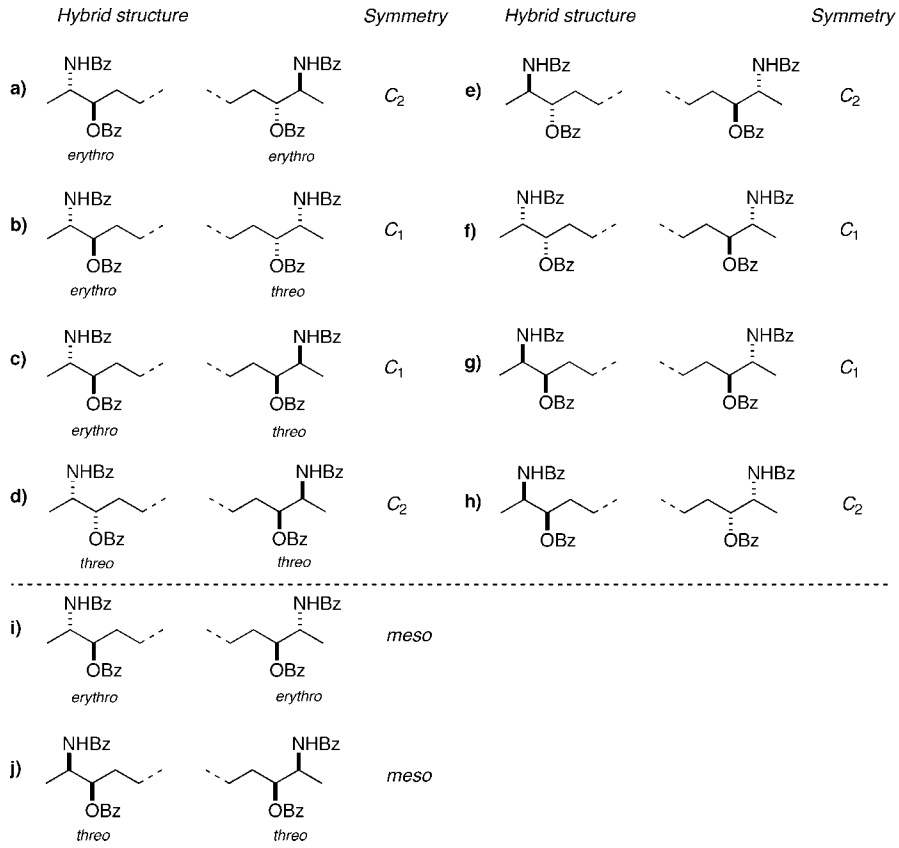
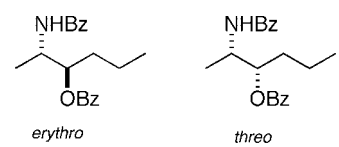



**Figure 2.** Representation of all possible stereoisomers of dimeric α,ω-dimeric amino alcohols **12a**−**j** (only end groups depicted) corresponding to the hydrogenation product of leucettamol A (**1**). The first four rows depict chiral stereoisomers in the left column and their corresponding enantiomers in the right column.

assignments of configuration in amino alcohols. Fortunately, a highly reliable diagnostic indicator for relative configuration of the vicinal amino alcohols is the chemical shift of the amide NH in perbenzoyl derivatives in *both* 2-amino-3-alkanols and 2-amino-1,3-alkanediols. Differences in the degree of intramolecular hydrogen bonding lead to an NH signal in the *threo* stereoisomer that occurs at an unusually high field ($\delta \approx 6.38-6.63$, d, $J = 8.9-9.2$ Hz) compared to that of the *erythro* isomer ($\delta \approx 6.97-7.10$, d, $J = 7.5-8.7$ Hz).[2b] The leucettamol A derivative **12** showed a downfield NH signal ($\delta$ 6.99, 2H, $J = 7.8$ Hz) strongly suggestive of the *erythro* configuration at each end of the chain.[16] This is entirely consistent with the relative configuration that Kong and Faulkner assigned to **1** and **2**, as depicted in the reported structures.[17]

The specific rotation of **12**, with attendant errors of a low rotatory compound, is not expected to be informative of the configuration at all four stereocenters. In contrast, CD readily revealed **12** was chiral and nonracemic even with only 90 $\mu$g of sample. Tetrabenzoyl derivative **12** gave a positive bisignate CD spectrum (split Cotton effect, MeOH, *c* 20 $\mu$M) (Figure 3) of moderate intensity ($\lambda$ 222 nm, $\Delta\epsilon$ −2.80; $\lambda$ 238 nm, $\Delta\epsilon$ +10.29) due to ECCD between vicinal *N*-benzamide-*O*-benzoate pairs. Two *erythro*/*erythro* configurations can be formulated for **12**; one in which the absolute configuration at each corresponding stereogenic center is the same (overall $C_2$ symmetry, **12a**) or one in which the absolute stereostructures of the opposing termini are inverted with respect to one another by a mirror plane that bisects C-15−C-16 (overall *meso* symmetry, **12i**). Clearly, the *meso* isomers—and for that matter, racemates of $C_1$ and $C_2$ stereoisomers—are optically inactive and can be eliminated here by observation of a nonzero CD spectrum for **12** (Figure 3). The final assignment of **1** was readily made by quantitative comparison of the CD spectrum of its derivative **12** with "hybrid CD spectra" generated by all possible linear combinations of CD spectra of two well-characterized model compounds **14** and **15** prepared from (2*S*)-alanine[2b] and their enantiomeric "virtual CD spectra" obtained by inversion of the first two. Figure 3 shows overlays of the measured CD spectrum of **12** with "hybrid CD spectra" (note: only nonzero combinations **12a**−**h** are shown here). An excellent match is seen between the hybrid CD spectrum of the *ent-erythro*/*ent-erythro* pair **12e** and the measured CD spectrum of **12**, showing the two share the same absolute configuration. The same match was found when the spectra were recorded in $CH_3CN$, even though the nonprotic solvent produces slightly different amplitudes of maxima and minima for all CD spectra, due to slightly different blue shifts in $\lambda_{max}$ benzamide and benzoate chromophores and other minor changes in fine structure at ~200 nm. These CD results provide a second independent verification of the *erythro*/*erythro* relative configuration assigned first by Kong and Faulkner[1] and corroborated here by NMR (see above). Therefore, leucettamol A (**1**) is not racemic but a $C_2$ diastereomer of the configuration (2*R*,3*S*,28*S*,29*R*). Since leucettamol B (**2**) co-occurs with **1** in *L. microrhaphis*,[1] it is almost certain both compounds have the same configuration at C-2, C-3, C-28, and C-29.[18]




**14** **15**

Leucettamol A (**1**) has *erythro* end groups, like D-sphingosine (**3**), but is of the opposite absolute configuration. The minor anomaly of reported "zero rotation" can now be explained. Leucettamol A (**1**) is clearly chiral and its optical rotation cannot be zero, except by coincidence. In our hands, the specific rotation of **1** was

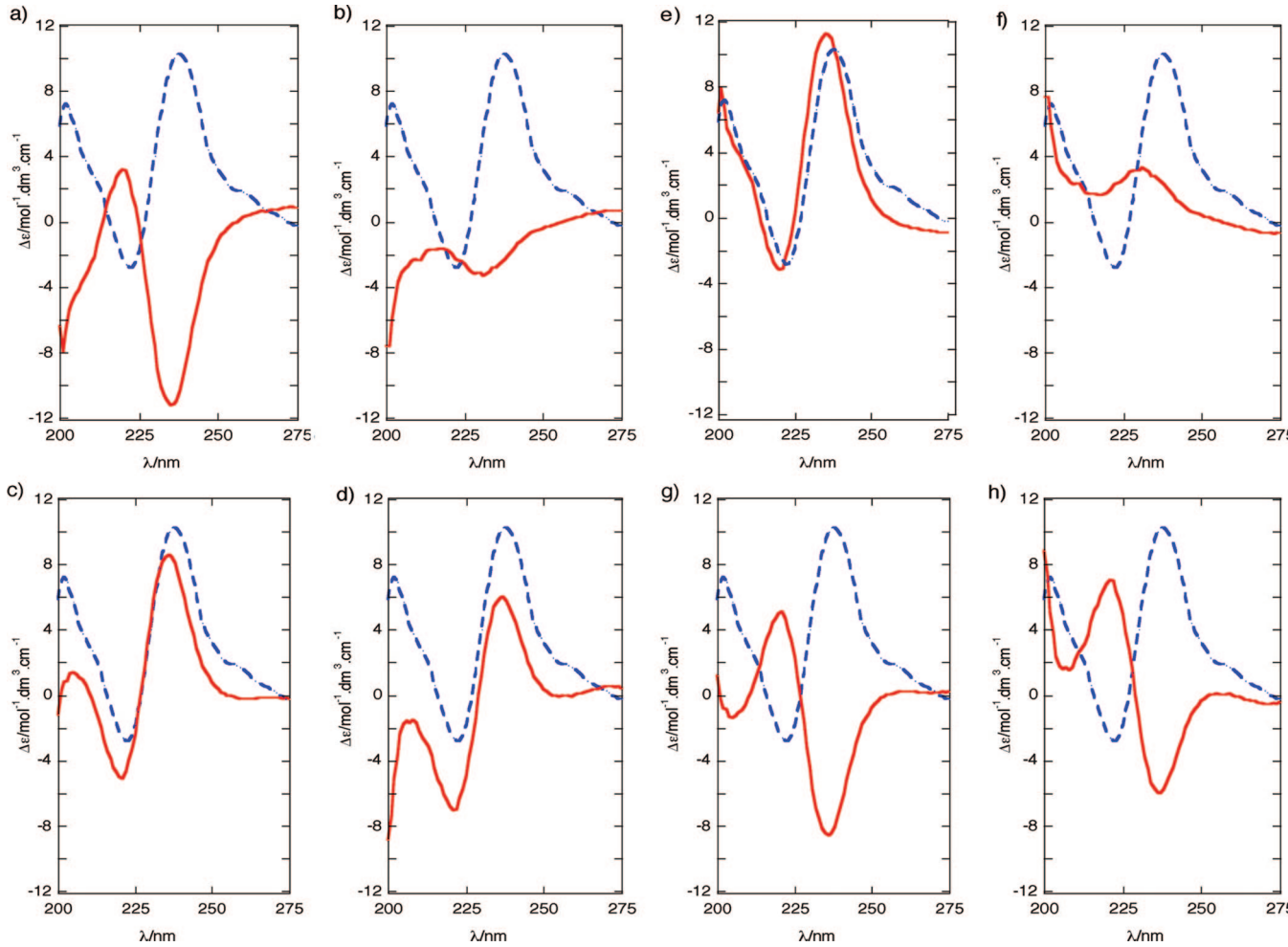



**Figure 3.** Circular dichroism (CD) spectra of leucettamol A derivative **12** ($c = 2.5 \times 10^{-5}$ M, MeOH, 23 °C) (dashed line) overlaid with "hybrid CD" spectra (solid) generated by linear combinations of the measured CD spectra of *erythro*-**14** and *threo*-**15** and their enantiomers (*ent*-). ($c \approx 2 \times 10^{-4}$ M, MeOH, 23 °C, see ref 3b) as follows: (a) *erythro*-**14** + *erythro*-**14**, (b) *erythro*-**14** + *threo*-**15**, (c) *ent-erythro*-**14** + *threo*-**15**, (d) *threo*-**15** + *threo*-**15**, (e) *ent-erythro*-**14** + *ent-erythro*-**14**, (f) *ent-erythro*-**14** + *ent-threo*-**15**, (g) *erythro*-**14** + *ent-threo*-**15**, (h) *ent-threo*-**15** + *ent*-threo **15**.

established as $[\alpha]_D$ −3.8 ± 0.1 (*c* 4.4, MeOH) (averaged measurements $N = 10$). Therefore, the $[\alpha]_D = 0$ reported for **1** by Kong and Faulkner[1] might have been close to the limits of detection of α under the conditions of measurement.[19] Specific rotations $[\alpha]_D$'s of optically pure natural product amines and amino alcohols are characteristically low and often masked by very low measured optical rotations, especially with small samples, contamination with highly rotatory substances, and differences between free base and protonated salt forms. Derivatization of the $NH_2$ and OH groups can lead to more reliable measurements. For example, the magnitudes of $[\alpha]_D$ increase by 2- to 3-fold upon acetylation or benzoylation of sphingosine.[20] Discrimination of *threo* from *erythro* isomers of sphingosine by $^1$H NMR has been demonstrated with the corresponding Mosher's acid derivatives, which are also useful for estimating optical purities of sphingosine samples containing as little as 1% of the minor enantiomer.[21] Nevertheless, errors of assignment in configuration of even simple monomeric marine amino alcohols have been made, and sole reliance upon some methods may be risky. For example, the xestaminols[22] first reported by Gulavita and Scheuer as (2*S*,3*R*) compounds were later corrected to (2*R*,3*S*) after total synthesis and $[\alpha]_D$ comparisons of their diacetyl derivatives.[23]

*N*,*O*-Dibenzoyl derivatives of sphingolipids show characteristic CD spectra useful for characterization of very small amounts of compound. Munesada and co-workers first applied CD to perbenzoyl sphingosine obtained from frog brain cerebrosides for stereochemical assignment by correlation of configuration with the split Cotton effects that arise from exciton coupled benzoyl chromophores.[24] Nakanishi and co-workers showed greatly improved sensitivity of ECCDs using *N,O,O*-trinaphthoyl derivatives of sphinganine (dihydrosphingosine) and phytosphingosine,[14,25] and even more distinct CD fingerprints have been obtained by differential acylation of primary and secondary $NH_2$ and OH groups.[25]

When the dihedral angle in staggered conformations of vicinal benzoate−benzamide pairs rotates in the positive direction (positive helicity, see Figure 4), a positive split Cotton effect is observed; a negative sign is observed for a negative helicity.[11a] The magnitude of the ECCD is related to the dihedral angle and is maximum when the angle subtended by the electronic transition dipole moments $\theta = 70°$ but zero when $\theta = 0°$ or 180°.[11a] The motion-averaged direction of the electronic transition dipole moments lies approximately along the C−N and C−O single bond, respectively. Unlike vicinal benzamide−benzoate pairs in rigid cyclic systems, where the dihedral angles are fixed, the net magnitude and sign of the split Cotton effect in acyclic systems are subject to conformational dependences. The observed CD spectrum of *threo*-***i*** or *erythro*-***ii*** is expected to be the sum of ECCD contributions for all the possible conformers *of each diastereomer*, and the net effect is largely determined by the Boltzmann-weighted staggered conformations ***a***−***c*** shown in Figure 4.

Investigations of dibenzoyl and derivatives of acyclic vicinal 2-amino-1-alkanols[11b] show that prediction of the sign of the split

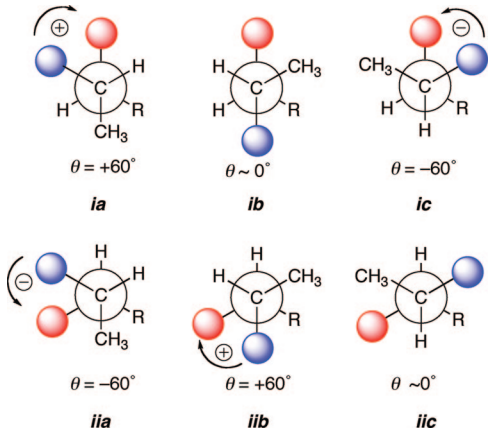



**Figure 4.** Staggered conformers contributing to exciton coupling CD in *threo* (***ia***−***ic***) and *erythro* (***iia***−***iic***) isomers of acyclic dibenzoyl 2-amino-3-alkanols. Blue- and red-shaded spheres represent *N*-Bz and *O*-Bz chromophore groups, respectively ($\lambda_{max} \approx$ 228 nm). The net signs of the observed split Cotton effects are consistent with predominant contributions from conformers ***ia*** and ***iia***, respectively. Conformers ***ib*** and ***iic*** have antiparallel transition dipole moments and give zero contributions to the ECCDs. See also Figure 1.

Cotton effect is upheld by both CD measurements and calculations of conformer populations. Our own empirical CD and NMR investigations of 3-amino-2-alkanols[3b] also support this model; for example, the larger H2−H3 vicinal coupling constant in the *threo* diastereomer (Figure 4, R = *n*-Pr, $J$ = 5.2 Hz) shows the dominant conformer is ***ia*** with the antiperiplanar arrangement of the H2−C2−C3−H3 bonds, but in the *erythro* isomer ($J$ = 2.6 Hz) it is *gauche* ***iia***. It is of interest to note that *erythro* and *threo* diastereomers of vicinal benzamide−benzoates are opposite in sign, with magnitudes that are similar but not equal (Figure 1). This is in contrast to vicinal dichromophoric derivatives of 1,2-glycols in which the magnitude of the ECCD in the *threo isomer* is almost zero due to a dihedral angle $\theta$ = 180° between the C−O bonds in the predominant *anti*-periplanar conformer.[26] The reason for the difference is likely related to hydrogen bonding of the NH in the amide group to the vicinal benzoate oxygen in both ***ia*** and ***iia*** that stabilize these *gauche* conformations with consequently larger contributions to the net ECCDs.

We have exploited *N*,*O*-benzoyl derivatives by extension to *dimeric* sphingolipids using a simple one-step perbenzoylation and interpretation of the resultant unique CD pattern. The results are easily read; simple superposition of the exciton coupled Cotton effects from expected linear combinations of *threo* and *erythro* isomeric end groups in 2-amino-3-alkanols can be deconvoluted by permutations of the constituent short-chain perbenzoyl derivatives.[3b]

The unambiguous assignment of **1** now allows some summary comparative notes on this interesting class of natural products and outcomes from application of the deconvolution of ECCD. The $\Delta\epsilon$ values measured in the ECCD of benzoate−benzamide pairs in these studies are recorded using highly dilute solutions ($10^{-4}$−$10^{-5}$ M) under conditions where, like UV−vis spectroscopy, the Beer−Lambert law is obeyed.[27] The method is applicable even to natural products that are obtained in submilligram amounts, since accurate concentrations of perbenzoyl derivatives are reliably measured from UV absorbance values together with the precisely recorded molar absorbtivities, $\epsilon$, that we reported in the original study.[3b] In turn, this allows confidence in estimating stereochemical purity and possible inhomogeneity or "contamination" of natural product long-chain DSs. Homochiral perbenzoyl derivatives of homologues and some analogues of DSs exhibit CD spectra that are matched *both* by sign and by magnitude of the Cotton effects. Cross-contamination by even small amounts of another diastereomer (e.g., an *ent-erythro*/*erythro*-diasteromeric contaminant in an *ent-erythro*/*ent-erythro* sample) would amplify mismatches between hybrid and measured CD spectra and be quickly revealed. This is not the case with measurements of optical activity, which are notoriously unreliable for natural products with weak $[\alpha]_D$'s or cross-contamination by highly rotatory congeners.

In the five dimeric sphingolipids we have examined so far, the natural product has been isolated as a homochiral entity; that is, compounds from a single sponge specimen appear as only one diastereomeric modification of one enantiomeric form (>95% ee). Nature appears to show high fidelity in enantiospecific biosynthesis of each DS, and this may reflect a tightly coupled enzymic process that unifies construction of the amino alcohol end group with long-chain dimerization, although the details of the latter process are still unclear.

The mis-assignment of **1** and **2** as "racemic"[1] is understandable from one point of view. Low molar rotations are often observed for acyclic and alicyclic aminoalkanes and amino alcohols and can be further lowered when functional groups contribute molar rotations of opposing signs. Nevertheless, the biosynthesis of sphingolipids can be expected to occur under stringent enzyme control with high enantioselectivity, although the stereospecificity may vary between different species of sponge and produce stereodiads at termini with different configurations. Although racemic and partially racemic chiral long-chain lipids[28] and terpenoids[29] have been observed in Nature, these seem to be exceptions. It seems unlikely that leucettamols would be biosynthesized with complete racemization. Even if they were, it seems even less likely that only one diastereomer of **1** instead of two or more would be present in the same sponge.

Finally, it is of interest to note that *L. microrhaphis*, the sponge that produces **1** and **2**, belongs to the less common group of calcareous sponges; all other DSs come from Demospongia of the family Oceanapiidae. The outstanding compound in the series, the polyene dimeric sphingolipid BRS1 (**10**), is clearly a leucettamol analogue and has been isolated from two sponges from the Great Barrier Reef: another specimen of *L. microrhaphis*[7b,30] and the earlier finding[7a] from a sponge of uncertain identity.

In conclusion, we have completed the stereochemical assignment of leucettamol A (**1**) by CD and report the complete configuration. The deconvolution ECCD method for assignment of dimeric sphingolipids,[2c,3] as well as simple monomeric 2-amino-3-alkanols (e.g., **6**),[12] is well suited to distinguish enantiomers of both *threo* and *erythro* stereoisomers of amino alcohols at nanomole sensitivity. This is particularly useful for those natural products with $[\alpha]_D$'s that, through happenstance, have magnitudes near zero and deceptively present themselves as racemic or *meso*.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured using a Jasco P1020 polarimeter. $^1$H NMR spectra were recorded on a Bruker DRX-600 (600 MHz, $^1$H, 1.7 mm CPTCI microcryoprobe) and Varian Mercury 400 (400 MHz) spectrometers in $CDCl_3$ using residual $CHCl_3$ ($\delta$ 7.26 ppm) as an internal standard. CD spectra were measured on a Jasco J810 spectropolarimeter using spectroscopic grade solvents (Fisher EM) and a 2 mm quartz cell with 50 nm/min scan rate and 1 nm slit. FTIR was measured using a Jasco FT-4100 spectrometer equipped with a Pike MIRacle ZnSe ATR plate. LR ESI mass spectra were obtained on a ThermoElectron MSQ single quad mass spectrometer coupled to an Accela UPLC. HRESI mass spectra were provided by the University of California, Riverside, mass spectrometry facility. Semipreparative HPLC was carried out using a Rainin binary HPLC system (Dynamax $C_{18}$ column, 10 × 250 mm, 5 $\mu$m, 3 mL/min or Dynamax silica 4.6 × 250 mm, 3 $\mu$m in specified solvent systems) coupled to a refractive index detection (Waters R401) and Isco UV-6 detector equipped with a filter for 250 nm. Flash column chromatography was carried out on silica (43−60 $\mu$m, EM 360) using a low-pressure solvent delivery system, and TLC was performed on silica

gel coated 0.2 mm aluminum backed plates with visualization by vanillin−$H_2SO_4$−EtOH or ceric ammonium nitrate(aq).

**Animal Material.** The sponge *Leucetta* aff. *microrhaphis* (Haekel, 1872) was collected by hand using scuba at a depth of 10 m in North Sulawesi, Indonesia (September 2006), and immediately soaked in EtOH. The sponge was identified by Rob van Soest, and a voucher sample (ZMAPOR20126) was deposited in the Institute for Systematics and Ecology, University of Amsterdam, The Netherlands.

**Extraction and Isolation.** The EtOH extract of the sponge was evaporated, and the aqueous residue extracted with EtOAc followed by *n*-BuOH. The *n*-BuOH fraction (0.61 g) was subjected to reversed-phase column chromatography (2:3 $CH_3CN$−$H_2O$) to afford leucettamol A (**1**, 202 mg, 0.25% w/w) with spectroscopic properties (MS, $^1$H NMR, $^{13}$C NMR) identical with literature values.[1] $[\alpha]_D$ −3.8 ± 0.1 (*c* 4.4, MeOH, 10 measurements), lit.[1] $[\alpha]_D$ 0 (*c* 1.26, MeOH).

No leucettamol B (**2**) was detected in this sample of *L.* aff. *microrhaphis*.

**Hydrogenation of Leucettamol A (1). Hexahydroleucettamol A (11).** A mixture of **1** (0.5 mg) in MeOH (2.0 mL) and 10% Pd−C (0.2 mg) was stirred under an atmosphere of hydrogen for 18 h. The mixture was filtered through Celite and concentrated to give crude **11** (ca. 0.1 mg). LR ESIMS *m/z* 485.4 $[M + H]^+$; calcd 485.5 for $C_{30}H_{65}N_2O_2$. This material was used immediately in the next step.

***N*-Benzoylimidazolide (13).**[31] A suspension of imidazole (4.54 g, 0.0668 mol) in dry benzene (200 mL) was treated with a solution of benzoyl chloride (4.69 g, 0.0333 mol) in benzene (20 mL) at 8 °C in a flask fitted with a drying tube. The mixture was allowed to warm to room temperature and stirred for 16 h. The precipitated imidazole hydrochloride was removed by filtration through a fritted funnel, and the clear filtrate concentrated under reduced pressure to give *N*-benzoyl imidazolide (**13**) as a viscous, hygroscopic oil (5.78 g, quant.), which was stored at 0 °C in a tightly stoppered vessel. $^1$H NMR ($CDCl_3$) 8.05 (s, 1H), 7.78 (bd, 2H, $J$ = 8.2 Hz), 7.67 (tt, 2H, $J$ = 8.2, 1.5 Hz), 7.58 (bt, 1H, $J$ = 7.4 Hz), 7.53 (bs, 1H), 7.16 (bs, 1H).

***N*,*N*′,*O*,*O*′-Tetrabenzoylhexahydroleucettamol A (12).** A solution of **11** (0.1 mg, 0.2 μmol) in dry $CH_3CN$ (0.5 mL) was treated with a solution of freshly prepared *N*-benzoylimidazole **13** (0.36 mg, 2.0 μmol) in dry $CH_3CN$ and DBU (0.28 mg, 1.8 μmol) at room temperature, then heated (70 °C) with stirring under an atmosphere of nitrogen for 18 h. The volatiles were removed under a stream of $N_2$, and the residue was dissolved in 0.3 mL of $CHCl_3$, loaded in a pipet column (silica), and eluted with 3:7 EtOAc−hexanes. After elution of nonpolar UV-active byproducts, the more polar product **12** was eluted and further purified by HPLC (6:94 *i*-PrOH−*n*-hexane, Alltech 5 μm silica, 4.6 × 250 mm, $t_R$ = 7.25 min, 1.5 mL/min). UV (MeOH) λ 227 nm (ε 39 900). CD (MeOH) λ 222 nm (Δε −2.80), 229 (Δε 0), 238 (Δε +10.30). CD ($CH_3CN$) λ 221 nm (Δε −4.59), 229 (Δε 0), 237 (Δε +10.03). See Figure 3 for CD spectra in MeOH and Supporting Information for CD spectra in $CH_3CN$. FTIR (ATR) ν 3600bs, 2264, 2362, 1623 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 8.09 (d, 4H, $J$ = 8.4 Hz; *ortho*-PhCOO), 7.76 (d, 4H, $J$ = 8.4 Hz; *ortho*-PhCONH), 7.41−7.60 (m, 12H; aryl H), 6.99 (d, 2 × 1H, $J$ = 7.8 Hz, C2/C29-NH), 5.22 (m, 2 × 1H, H3/H28), 4.46 (m, 2 × 1H, H2/H29), 1.20−1.30 (m, 48H), 1.29 (d, 2 × 3H, $J$ = 6.6 Hz, H1/H30); LR ESIMS *m/z* 923.5908 $[M + Na]^+$, calcd for $C_{58}H_{80}N_2NaO_6$ 923.5914.

**CD Measurements and Generation of "Hybrid CD" Spectra.**[3] CD spectra were measured on a Jasco J-810 spectropolarimeter using solutions prepared in HPLC grade MeOH or $CH_3CN$. All measurements were carried out with $N$ = 20 scans in dilute solutions (20−200 μM) in cells of CD grade quartz (2 mm path); scan speed 50 nm/min, slit width 1 nm. No smoothing or noise reduction was applied to CD data. Digitized spectral files (1 nm/data point) were exported as ASCII files and parsed into Excel spreadsheets (MS Office 2004) prior to column additions−subtractions to yield "hybrid CD" spectra. Tabulated CD data of **14** and **15** were obtained under similar conditions as reported earlier[3b] and plotted against "hybrid spectra" using Kaleidagraph 4.0 (Synergy Software) running on an iMac computer (Apple, Inc.).

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**Supporting Information Available:** Measured CD spectra of **12** and "hybrid spectra" in $CH_3CN$, MS and $^1$H NMR spectra of **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

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(10) Also implicit in the logic of their deduction is that leucettamols must be chiral—and racemic—because both ends of each molecule have the same configuration and that the positions of the skipped olefins in the long chains make them constitutionally unsymmetrical, but the molecules would still be chiral even if they were *constitutionally symmetrical* (with the exception of the *meso* form, in which each end has the same relative configuration but with mirror image absolute configurations; see text and Figure 1 for further discussion). Comparison of the rotation of $C_{28}$ rhizochalin aglycone (**4a**, $[\alpha]_D$ +11, ref 2a) with the zero specific rotation observed for **1** and **2** is also invoked to further support the conclusion that "*both leucettamols. .are racemic*"; however as we have shown elsewhere (ref 2c), the former molecule represents a pseudo-$C_2$ molecule that is fully expected to be chiral, regardless of the presence of the near mid-chain keto group.
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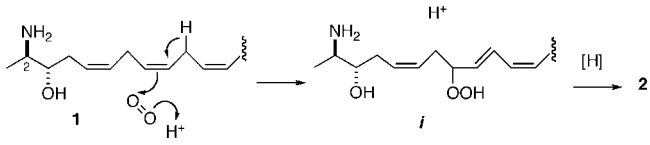
(15) Although the hydrogenation product **11** of leucettamol A (**1**) was revealed to be a constitutional $C_2$ dimer, the assignment of configuration in **1** would have been made more complicated if it were $C_1$ because of the need to place each inequivalent end group at correct ends of the unsymmetrical skipped polyene chain. Herein lies a limitation to the CD method. However the correct assignment could still be made by 2D NMR spin correlation (e.g., TOCSY, HMBC) of the compound prepared by per-benzoylation of **1** without hydrogenation and using the empirical NH chemical shift method described in the text to identify *threo* and *erythro* end groups.
(16) In our opinion, the (CO)NH $^1$H NMR chemical shift *in $CDCl_3$ only* is more reliable for assignment of relative configuration, in most cases, than measurements of vicinal couplings or NOEs in the corresponding cyclic oxazolidinone[1] or thiazolidinone derivatives.
(17) In the text of the paper (ref 1), the authors state, apparently in contradiction to their depicted structure, that **1** has, "*threo* stereochemistry at both ends of the molecule". This appears to be a typographical error since the $^1$H NMR data support the all-*erythro* configuration for leucettamol A as shown.

(18) Leucettamol B (**2**) is also an expected product of oxidation of **1** by a singlet oxygen ene-reaction followed by disproportionation of the resultant hydroperoxide **i** and may possibly be an artifact.




(19) The different $[\alpha]_D$ we observed may partly be due to instrumental limitations. In our work, a standard 1 cm "microcell" (volume = 200 $\mu$L) and solution of 44 mg/mL of **1** (MeOH) was used for rotation measurements (Jasco P2000 digital polarimeter), giving a measured rotation of −0.017 millidegrees for the solution of **1**. Back-calculating the measured $\alpha$ from the specific rotation reported by Kong and Faulkner, $[\alpha]_D$ 0 (*c* 1.26, MeOH), gives $\alpha = -0.0478$ in a 10 cm cell or $\alpha = -0.00478$ in a 1 cm cell. The latter value is close to the "limit of detection" (twice the S/N) of older model polarimeters.

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