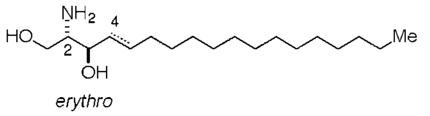
(−)-Rhizochalin is a Dimeric Enantiomorphic (2*R*)-Sphingolipid: Absolute Configuration of Pseudo-C_{2v}-Symmetric Bis-2-amino-3-alkanols by CD**

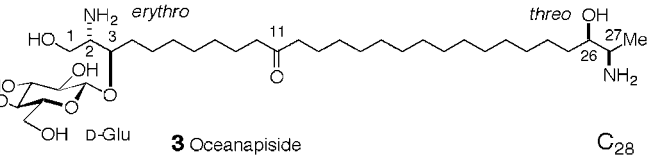
Tadeusz F. Molinski,* Tatyana N. Makarieva, and Valentin A. Stonik

Sphingolipids are well-known cellular components of all higher eukaryotes and have attracted renewed interest with the finding that *erythro*-D-sphingosine (**1a**) and its derivatives, such as ceramides (*N*-fatty acyl sphingosines) and sphinosine 1-*O*-phosphate, have important roles in higher mammals as "first messengers".[1] Highly modified sphingolipids, such as the sphingofungins,[2] occur in lower invertebrates and microbes and may also function as inhibitors of various pathways in mammalian sphingolipid biosynthesis. (−)-Rhizochalin (**2**), an antimicrobial galactopyranosyl pseudodimeric α,ω-bipolar sphingolipid, was isolated from the calcareous sponge *Rhizochalina incrustata*,[3] collected near Madagascar. Although the constitutional structure of **2** was established from spectroscopic and degradative analysis, the stereostructure remained undefined.

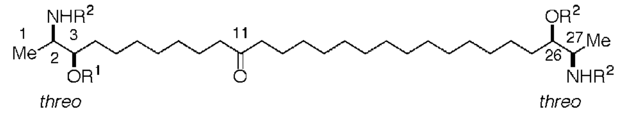
Configurational analysis of sphingosine diasteromers by circular dichroism (CD) has been reported.[4, 5] We have previously devised a CD method for α,ω-bis-sphingoid bases that exploits deconvolution of superposed exciton couplings in the CD spectrum of the corresponding tetrabenzoyl derivatives[6] and we applied this to the antifungal agent oceanapiside (**3**),[7] a related α,ω-sphingolipid isolated from a temperate-water marine sponge, *Oceanapia phillipensis* Dendy 1895 (Haplosclerida, Phloeodictyidae). The technique is noteworthy for simplicity, particularly with respect to dimeric amino alkanols, ample sensitivity (≈50 nmol), and complementarity with other CD methods for the configurational analysis of sphingolipids.[4, 5, 8]



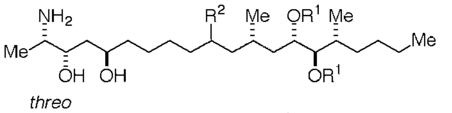
1a *erythro*-D-Sphingosine (Δ^4) C_{18}
1b 4,5-Dihydro-D-sphingosine (Sphinganine)



3 Oceanapiside C_{28}



2 Rhizochalin R^1 = β-D-galactopyranosyl, R^2 = H
4 $R^1 = R^2 = H$ C_{28}
5 $R^1 = R^2$ = Bz



8a Fumonisin B_1 R^1 = tricarballylic acid, R^2 = OH C_{20}
8b Fumonisin B_2 R^1 = tricarballylic acid, R^2 = H

Whereas the chain termini in **3** have dissimilar substitution, the pseudosymmetric structure of **2** presents the added challenge of correlating remote end groups in pseudo-C_{2v}-symmetric or *meso* dimeric stereoisomers of α,ω-difunctionalized long chains. Identification of *meso* compounds is trivial (lack of optical activity), but the former stereoisomers present a problem that is refractory to simple end-group analysis by NMR spectroscopy or measurement of optical rotation because the methods do not correlate the chain termini or they lack sensitivity. We show now, by the exciton-coupling CD superposition method, that the complete configuration of (−)-rhizochalin (**2**) is 2*R*,3*R*,26*R*,27*R* and we note a remarkable finding—both ends of **2** are *threo* and enantiomeric to those of known 2-amino-3-alkanol sphingolipids. This suggests that stepwise biosynthesis of bipolar sphingolipids in marine sponges is a highly ordered process that may self-regulate cognate precursor selection for chain assembly.

A suitable chromophoric derivative of **2** was prepared as follows (Scheme 1). The aglycone, **4**, obtained by methanolysis of **2** (MeOH, 1 M HCl, 75 °C, 24 h), was transformed into the corresponding tetrabenzoyl derivative **5** (*N*-benzoylimidazole, CH_3CN, catalytic DBU, 70 °C, 24 h),[9] which was subsequently purified by HPLC (≈50 nmol). Benzoylation of **4** with BzCl/pyridine (60 or 100 °C) was less suitable and resulted in extensive formation of the bis-oxazoline **6**.

The CD spectra of **5** in MeOH (Figure 1, dotted line) revealed Cotton effects (CEs) of moderate magnitude [$\Delta\varepsilon$ = +7.3 (221) and −5.0 (237 nm)] with the bisignate antiphase characteristics indicative of exciton coupling between vicinal benzoate–benzamide pairs. Ten of the sixteen stereoisomers of **4** are expected to show unique CD spectra and are depicted in Scheme 2. Several stereoisomers can be considered as almost pseudo-C_{2v}-symmetric or *meso* dimers, in which the

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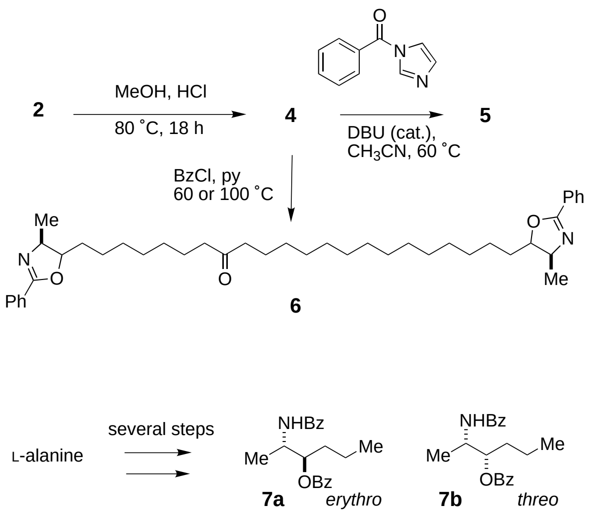
[*] Prof. T. F. Molinski
Department of Chemistry
University of California
Davis, CA 95616 (USA)
Fax: (+1) 530-752-8995
E-mail: tfmolinski@ucdavis.edu

T. N. Makarieva, V. A. Stonik
Pacific Institute of Bioorganic Chemistry
Far East Branch of the Russian Academy of Science
Vladivostok-22 (Russian Federation)

[**] We thank Jeff de Ropp and John MacMillan (University of California, Davis) for assistance with the 600 and 400 MHz ^{1}H NMR spectra, respectively; Gillian Nicholas (University of California, Davis) for measurement of the CD spectra of **7a,b**; Rich Kondrat (University of California, Riverside Mass Spectrometry Facility) for chemical ionization MS; and Carlito Lebrilla and Yongming Xie (University of California, Davis) for matrix-assisted laser desorption/ionization MS. This work was supported by the National Institutes of Health (NIH; grant no. AI39987). The NMR spectrometers were funded in part by the National Science Foundation (grant no. CHE-9808183; 400 MHz apparatus) and the NIH (grant no. RR11973; 600 MHz apparatus). CD = circular dichroism.

Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.



Scheme 1. Above: Synthesis of the tetrabenzoyl derivative **5** from (−)-rhizochalin (**2**). Below: Synthesis of stereodefined compounds **7a**, **b** from L-alanine.[6] DBU = 1,8-diazabicyclo[5.4.0]undecane, Bz = benzoyl, py = pyridine.

symmetry of the unbranched chain is broken only by the presence of a midchain keto group.[10] Inspection of the ten structures reveals that these include four "C_{2v}" isomers **4a**, **c**, **e**, **g** and two "*meso*" isomers **4i**, **j**. The isomers not depicted here correspond to structures obtained by interchange of configurations at the chain termini in **4b** and **4d** (and their enantiomers) along with enantiomers of the "*meso*" compounds **4i**, **j**. The former non-"*meso*" isomers arise when the end groups have different relative configurations—they can also be considered as the isomers resulting from placement of the midchain keto group at a position either proximal or distal to a given terminus.[11]

The observation of optical activity in the derivative **5** immediately eliminated the possibility of "*meso*" isomers, such as **4i**, **j**. Stereochemical correlation of the positive long-wavelength CE observed in **5** was not possible by inspection alone. Instead, the CD spectrum of **5** was compared to hybrid spectra of dimeric tetrabenzoyl "virtual molecules" constructed by linear combinations of spectra measured from stereodefined compounds **7a**, **b**, each synthesized from L-alanine, as

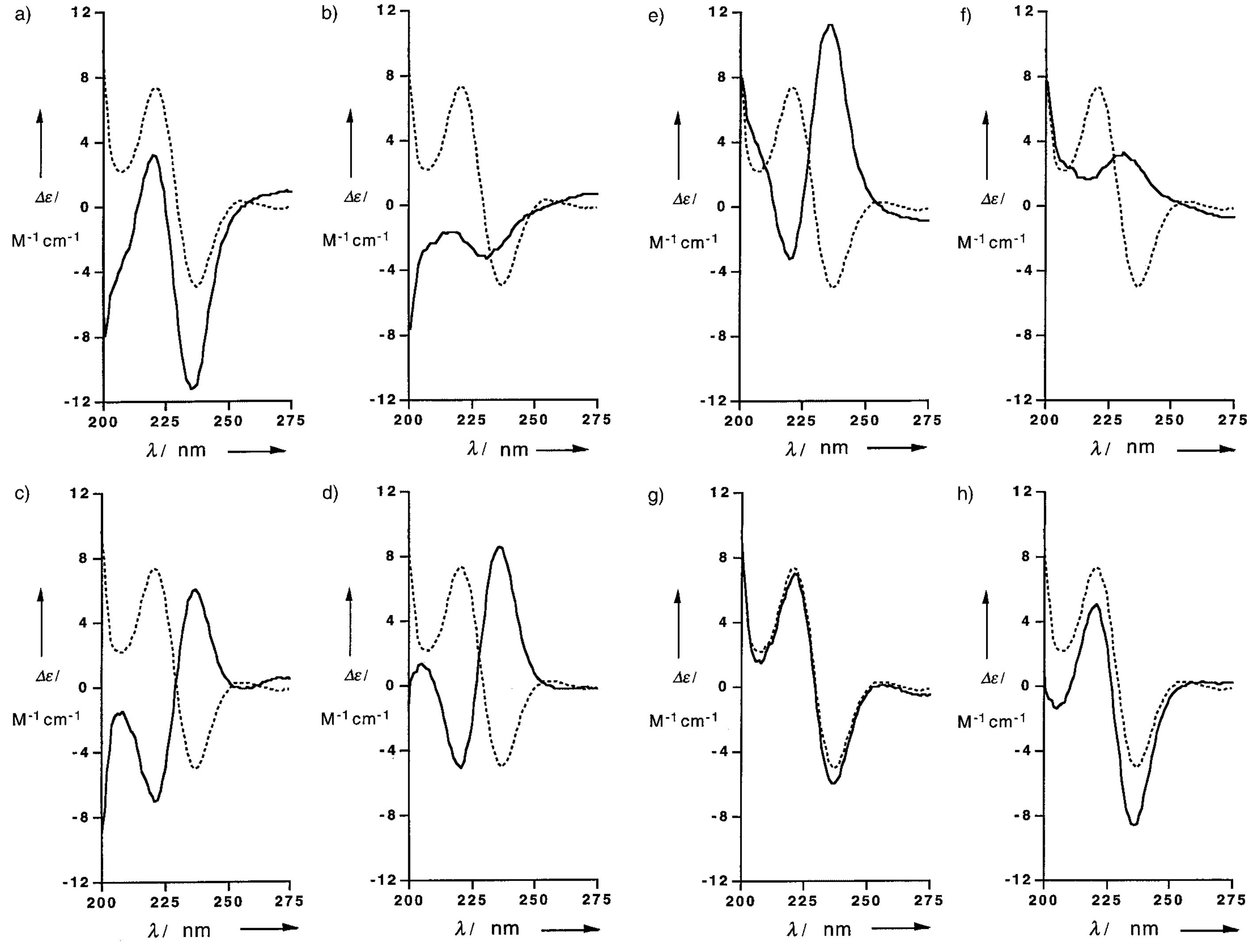
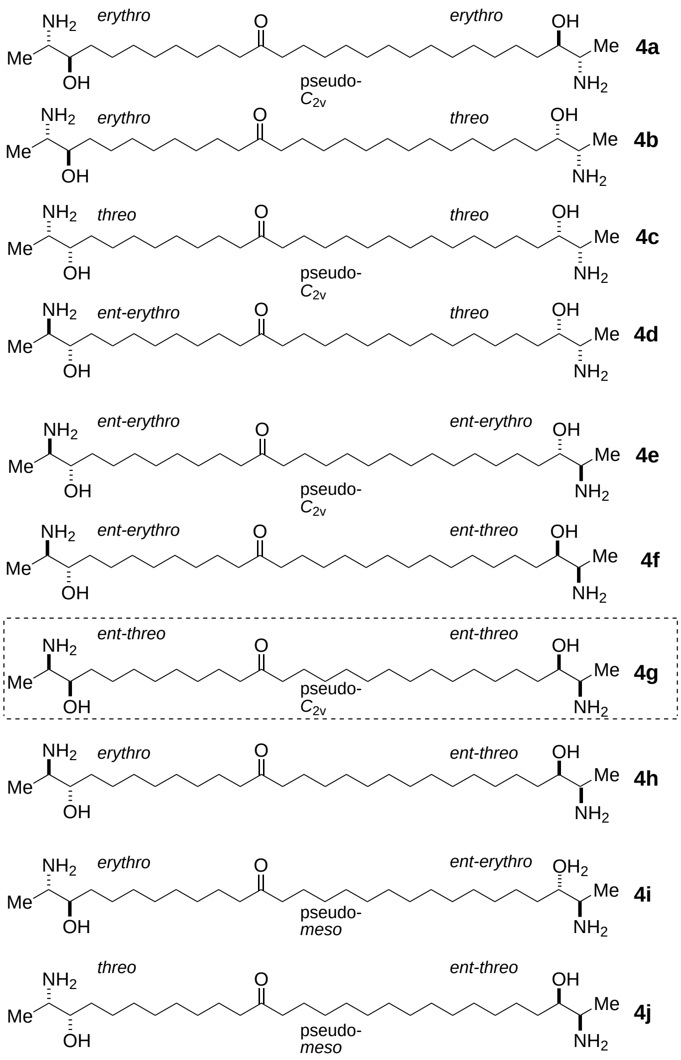


Figure 1. Hybrid CD spectra (solid lines) of eight diastereomers of "virtual molecules" related to **5a**–**h**, respectively (Scheme 2). These hybrid spectra are generated by linear combinations of the CD spectra of **7a** and **7b**. Spectra of pseudo-*meso* compounds are not shown. The overlaid CD spectrum (dashed line) is of the *N*,*N*′,*O*,*O*′-tetrabenzoyl derivative **5** ($c = 2.2 \times 10^{-4}$ M) of rhizochalin aglycone (**4**) derived from the natural (−)-rhizochalin (**2**). All spectra were recorded in MeOH at 25 °C.



Scheme 2. Structures of the unique stereoisomers of (−)-rhizochalin aglycone **4a–h** that could be distinguished by CD of the corresponding tetrabenzoyl derivatives **5a–h**. Another six stereoisomers (total = 16) are not shown—these correspond to interchange of the end-group configurations of **4b**, **d**, their enantiomers, and two additional pseudo-*meso* isomers which are enantiomers of **4i** and **4j**. The box indicates that **4g** is the compound derived from natural (−)-rhizochalin (**2**).

previously described.[6] The required spectra for *ent*-**7a**, **b** were not measured, but were obtained by simple inversion of the CD spectra of **7a**, **b**. Hybrid CD spectra corresponding to the combinations of end groups found in the tetrabenzoyl derivatives of the eight optically active stereoisomers are shown in Figure 1a–h (solid lines).

An excellent match was obtained for the CD spectrum of the tetrabenzoyl derivative **5** and the hybrid spectrum of the virtual molecule in Figure 1g. Both the relative and absolute configuration are provided by the CD spectrum, however, we confirmed the *threo,threo* configuration of **5** by ^{1}H NMR spectroscopic single-frequency decoupling of the tetrabenzoyl derivative. The pair of spin systems H-2/H-3 and H-26/H-27 were not resolved, even at 600 MHz, but COSY analysis and decoupling of the methyl signals (H-1/H-28) revealed a single, relatively large vicinal coupling constant ($J = 5.5$ Hz) for the H-2/H-3 and H-26/H-27 pairs that indicated a *threo* configuration (compared with *threo*-**7b**, $J = 5.2$ Hz and *erythro*-**7a**, $J = 2.6–2.7$ Hz).[6] The coupling-constant assignment was further corroborated by the characteristic upfield chemical shift ($\delta = 6.38$ ($2 \times$ br.d, 1H, $J = 9$ Hz)) of the signal assigned to NH for *N,O*-dibenzoyl derivatives of *threo*-2-amino-3-alkanols (the signal is shifted downfield in *erythro* compounds, $\Delta\delta \sim 0.6$).[6] Thus, the configurations of **2**, **4**, and **5** are 2*R*,3*R*,26*R*,27*R*.

The established configuration of (−)-rhizochalin (**2**) corresponds to a diastereomer with *threo,threo* termini, which is the opposite absolute configuration to known methyl-terminated sphingolipids, such as the fumonisins B1 and B2 (**8a**, **8b**)[12, 13] and C_{14} marine sphingolipids.[14] The configuration of other α,ω-bipolar sphingolipids are unknown. These include compound BRS1 from an unidentified sponge,[15] the coriacenins from the Mediterranean sponge *Clathrina coriacea*,[16] rhapsamine from the Antarctic sponge *Leucetta leptorhapsis*,[17] and leucettamols A and B,[18] although there is evidence that leucettamol A may possess *erythro*, *erythro* stereodiads.[18] Plants and higher organisms appear to select more conservatively for the 2*S* configuration at the committed step in the biosynthesis of sphinganine (**1b**)—the pyridoxal 5′-phosphate dependent condensation of palmitoyl CoA with (*S*)-serine or (*S*)-alanine, which proceeds with loss of CO_2 but retention of C-2 configuration.[19] The enzymes that catalyze the analogous biosynthesis of **1a** are a serine palmitoyl transferase[20] of the α-oxoamine synthase family,[21] followed by an NADPH-dependent ketoreductase. It is likely that the biosynthesis of **2**, like that of the fumonisins, is catalyzed by homologous enzymatic activity. We propose that at least two independent amino acid fatty acyl transferases, or homologous subunits in the same enzyme, are operative in the biosynthesis of dimeric sphingolipids in marine sponges—one incorporates alanine with 2*R*,3*R* stereoselectivity and a second incorporates serine with 2*S*,3*R* stereoselectivity. Comparing **2** with **3** suggests that commitment to a particular diastereomer is determined by selection of the amino acid and subsequent reduction.

Careful analysis of sponge extracts shows that only the single stereoisomers of **2** or **3** are likely to be present in each of the respective organisms. Indeed, **2** and **3** are the dominant polar lipids in extracts obtained from the sponges (0.42[7] and 0.11 %[3] dry weight, respectively)—no stereoisomers or regioisomers were detected.[7] Consequently, the enzymes controlling the bipolar biosyntheses of **2** and **3** must exhibit stringent control over the selection of cognate amino acids, the ordering of assembly at opposing chain termini by internal recognition, and the stereochemical outcome of each condensation reaction. It is clear that all-*threo* stereochemistry arises in **2** by enzyme selection for alanine with obligate *R* stereochemistry at C-2 and C-27 in the product, but it is not apparent whether D-alanine is employed as a precursor for **2**, with retention of configuration at the C-2 center, or L-alanine is used with an unorthodox inversion at C-2.

Both **2** and **3** have potent antifungal activity against the human pathogen *Candida glabrata* (minimum inhibition concentration (MIC) for **3** ≈ 3 μg mL^{-1}).[7] Recent findings show that an endogenous α,ω-diamine acts as a suppressor of sphinganine biosynthesis in J77A4.1-transformed murine cell lines; this has implications for the regulation of sphingolipid biosynthesis.[22] A detailed understanding of marine α,ω-bipolar sphingolipids may reveal mechanistic details that pertain to D-sphinganine biosynthesis in other organisms.

Experimental Section

All new compounds were purified by HPLC and gave satisfactory high-resolution mass spectra and ^{1}H NMR. See the Supporting Information for general procedures, CD measurements, and selected NMR spectra and MS results for compounds **5** and **6**.

Compound **4:** A solution of (−)-rhizochalin (**2**, 2 mg)[3] in 2% HCl/anhydrous MeOH (0.5 mL) was heated at 75 °C for 24 h in a sealed tube, after which the solution was cooled and concentrated under a stream of N_2. The residue was subjected to microcolumn chromatography (7 × 65 mm, silica) with elution in two stages: a) MeOH/$CHCl_3$ (1/4) to obtain 1-*O*-methylgalactopyranosides; b) CH_2Cl_2/MeOH/NH_4OH (9/4/1) which provided the ninhydrin-positive product **4** (1.3 mg). DCI-MS (NH_3): *m*/*z*: found, 471.4514 $[M+H]^+$; calcd for $C_{28}H_{59}N_2O_3$, 471.4526.

Compound **5:** A solution of **4** (100 µg, 0.11 µmol) in dry CH_3CN (0.30 mL) was treated with *N*-benzoylimidazole (2.1 µmol) and DBU (0.5 mg, see ref. [9]). The solution was heated at 70 °C for 24 h and allowed to cool to 25 °C over 24 h. Volatiles were removed under a stream of N_2 and the residue subjected to microcolumn chromatography (pipette, silica, EtOAc/*n*-hexane (2/7)) to obtain a nonpolar UV-active fraction (0.3 mg). Final purification was achieved by HPLC (Dynamax, 5 µ silica, 10 × 300 mm, EtOAc/*n*-hexane (3/7), 3.3 mL min^{-1}) to give pure **5** (retention time = 22.0 min). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 8.02$ (d, $^3J = 8.3$ Hz, 4H; *ortho*-PhCOO), 7.72 (d, $^3J = 8.3$ Hz, 4H; *ortho*-PhCONH), 7.58–7.38 (m, 12H; aryl H), 6.39, 6.38 (2 × br. d, $^3J = 8.5$ Hz, 2H; NH), 5.21 (2 × m, 2H; H-3/H-26), 4.525 (2 × qdd, $^3J = 8.5$, 7.2, 5.5 Hz, 2H; H-2/H-27), 2.34, 2.33 (2 × t, $^3J = 7.2$ Hz, 4H; H-10/H-12), 1.75 (m, 38H), 1.28 (2 × d, $^3J = 7.2$ Hz, 6H; H-1/H-28); matrix-assisted laser desorption/ionization Fourier transform MS: *m*/*z*: found, 909.5388 $[M+H]^+$; calcd for $C_{56}H_{74}N_2O_7Na$, 909.5394.

Attempted perbenzoylation of **4** (1 mg) using excess BzCl/pyridine (60 or 100 °C) resulted almost exclusively in formation of the bis-oxazoline **6** (≈0.5 mg after HPLC purification (silica, EtOAc/*n*-hexane (1/4), 3.3 mL min^{-1}, retention time = 22 min)). Selected data: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.04$ (2 × m, 2H; H-3/H-26), 5.22 (2 × m, 2H; H-2/H-27), 2.36, 2.35 (2 × t, $^3J = 7.4$ Hz, 4H; H-10/H-12), 1.591, 1.594 (2 × d, $^3J = 6.8$ Hz, 6H; H-1/H-28); DCI-MS (NH_3): *m*/*z* (%): found, 643 (4) $[M+H]^+$, 642.4780 (6) $[M^+]$, 105 (100); calcd for $C_{42}H_{62}N_2O_3$, 642.47604.

Received: June 9, 2000
Revised: August 2, 2000 [Z15254]

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[10] Strictly, compound **4** lacks an axis of rotation due to the presence of the ketone group in an even-numbered carbon chain. Mislow and co-workers comment on the natural tendency for chemists to ascribe a quantitative meaning to asymmetry; for example, molecules such as 1-stearoyl-2,3-dipalmitoylglycerin are described as having "very slight" asymmetry (A. B. Buda, T. Auf der Heyde, K. Mislow, *Angew. Chem.* **1992**, *104*, 1012–1031; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 989–1007). In the present context, one can think of **4** as possessing "very slight" C_{2v} symmetry because, for all intents, the molecule behaves spectroscopically and chemically as a C_{2v} entity, even though improper rotations are absent.

[11] Herein lies a limitation to the CD method as applied to dimeric sphingolipids with the same constitution at each chain terminus and midchain substitution. We can uniquely distinguish all isomers with the same local relative configuration at each end group (for example, all C_{2v} isomers), but cannot distinguish those stereoisomers with different local relative configurations at each end from their counterparts obtained by interchanging end-group configurations.

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Amphoteric Character of 2-Vinyloxiranes: Synthetic Equivalents of β,γ-Unsaturated Aldehydes and a Vinylogous Enolate**

Mark Lautens,* Stéphane G. Ouellet, and
Stéphane Raeppel

Oxiranes and 2-vinyloxiranes undergo a number of useful transformations including reactions with nucleophiles[1] and Lewis acid mediated rearrangements.[2] In contrast, the utility of vinyloxiranes as precursors to nucleophilic species has not been established. In this report we describe two reactions of

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[*] Prof. Dr. M. Lautens, S. G. Ouellet, Dr. S. Raeppel
Department of Chemistry
Lash Miller Chemical Laboratories
University of Toronto
80 St. George Street, Toronto, M5S 3H6, ON (Canada)
Fax: (+1) 416-978-6083
E-mail: mlautens@alchemy.chem.utoronto.ca

[**] We thank Professor Batey and his group for providing the allyl and crotyltrifluoroborates. This work was supported by the National Science and Engineering Research Council (NSERC) and the University of Toronto. S.G.O. thanks NSERC for a post-graduate scholarship.