was assumed that there were 39.5 water molecules present in all, which filled the space available for solvent. For both **1** and **2** it was not possible to locate all cations because of the large amount of disordered solvent present. Charge balance requires a total of ten cations for **1** and sixteen for **2**; the relative proportions of missing $Na^+$ and $NMe_4^+$ have been assigned on the basis of microanalysis results. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-155973 (**1**) and -155972 (**2**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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# Synthesis of a Cyclopropene Analogue of Ceramide, a Potent Inhibitor of Dihydroceramide Desaturase**

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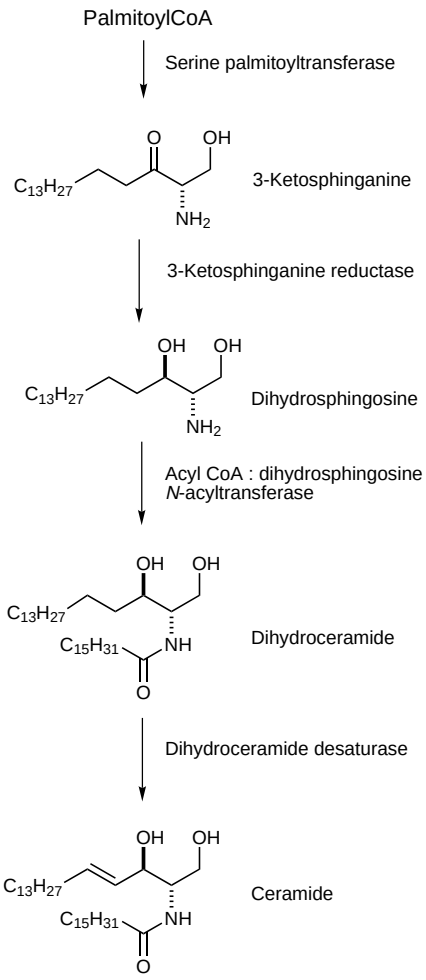
*Dedicated to Professor Francisco Camps*

Ceramide is a biologically relevant sphingolipid, which is intracellularly generated by either the breakdown of glycosphingolipids, the hydrolysis of sphingomyelin (sphingomyelin pathway), or the de novo biosynthesis (Scheme 1).[1] Despite the general acceptance of ceramide as a key second messenger, some aspects of ceramide-mediated signal transduction are controversial. The selective inhibition of the different enzymes involved in the biosynthesis and metabolism of ceramide can help solve such discrepancies and to better understand the precise role of ceramide in cell biology. This fact has led to the discovery of several inhibitors of these enzymes.[1–3]

The de novo biosynthetic pathway of ceramide (Scheme 1) is initiated with the condensation of palmitoyl CoA with serine to give 3-ketosphinganine, which is transformed into dihydroceramide upon reduction and acylation. The last step involves the introduction of the C4–C5 *trans* double bond into dihydroceramide to give ceramide. This reaction is catalyzed by dihydroceramide desaturase, for which no inhibitor has been reported previously.[4]




Scheme 1. De novo biosynthesis of ceramide.

Although little information is available about dihydroceramide desaturase,[5–10] it appears to be similar to the better known fatty acyl desaturases. After early reports on the activity of sterculic acid (9,10-methyleneoctadec-9-enoic acid) as a potent inhibitor of the $\Delta^9$ stearoyl-CoA desaturase,[11] the inhibitory effect of cyclopropene fatty acids on different acyl-CoA desaturases has been the subject of several publications.[12] Although the mechanism of inhibition is still controversial,[12] it is well established that a) sterculic acid inhibits the $\Delta^9$ desaturation of different aliphatic acids, regardless of the chain length;[13] b) among several synthetic cyclopropene fatty acids, only those compounds with the ring at C9 and/or C10 are effective inhibitors of the $\Delta^9$ desaturase;[14] and c) a structural analogue of sterculic acid with an exocyclic double bond does not inhibit the desaturation of stearic to oleic acid.[15]

In light of these reports, the cyclopropene-containing ceramide *erythro*-**1** was designed as a putative inhibitor of dihydroceramide desaturation. We describe herein the synthesis of cyclopropene ceramide analogue *erythro*-**1**, as well as inhibition experiments that show that *erythro*-**1** is a potent inhibitor of dihydroceramide desaturase.

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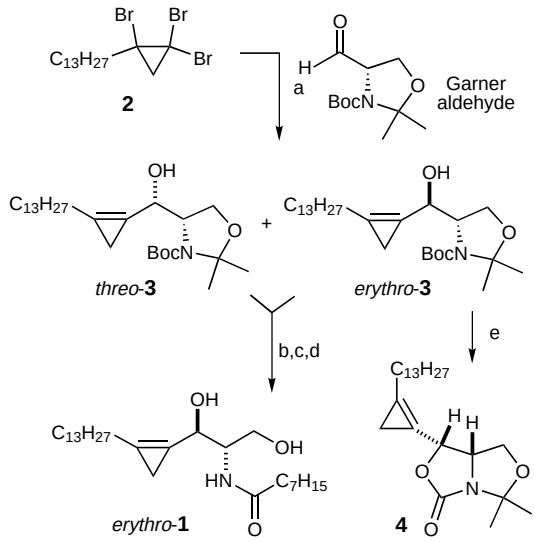
[**] This work was supported by the Dirección General de Enseñanza Superior e Investigación Científica (grant PB97-1171) and the Departament d'Universitats, Recerca i Societat de la Informació, Generalitat de Catalunya (grant 1999-SGR 00187 and a Predoctoral fellowship to G.T.). We thank Dr. J. Casas, Dr. A. Delgado, and Dr. J. Joglar for their help in different aspects of this work.

  0044-8249/01/11310-2014 $ 17.50+.50/0 

The synthesis of *erythro*-**1** was carried out as depicted in Scheme 2. 1-Lithio-2-tridecylcyclopropene, which was generated from 1,1,2-tribromo-2-tridecylcyclopropane **2** according to a reported procedure,[16] was treated with Garner aldehyde to afford a 3:1 diastereomeric mixture of *erythro* and *threo* alcohols **3** in 75% yield. The diastereoselectivity of the




Scheme 2. Synthesis of the ceramide analogues **1**. Reagents and conditions: a) 2.2 equiv *n*BuLi, THF, $-78$ to 25 °C, 45 min, then Garner aldehyde, THF, $-23$ °C, 2 h; b) flash chromatography; c) 2.2 equiv trimethylsilyl triflate, 2,6-lutidine, THF; d) octanoyl chloride, pyridine; e) NaH, THF.

reaction was improved by using hexamethyl phosphoramide (HMPA) as cosolvent.[17] In this case, *erythro* and *threo* alcohols **3** were obtained in a 10:1 ratio. Pure *erythro*-**3** was isolated after flash chromatography. The stereochemistry of the major diastereomer formed in the addition reaction was confirmed by NOE studies on a bicyclic oxazolidinone derivative **4**.[18] The protecting groups were removed by using trimethylsilyl triflate.[19] Other common procedures used to cleave the Boc group resulted in the complete decomposition of alcohol **3**, probably because of the instability of the cyclopropene ring under acidic conditions. Final acylation with octanoyl chloride[20] afforded *erythro*-**1** in 14% yield from 1-pentadecyne over 5 steps.

The inhibitory effect of *erythro*-**1** on dihydroceramide desaturase was investigated in rat liver microsomes,[5] by using D-*erythro*-*N*-octanoyldihydrosphingosine as the substrate. The formation of the unsaturated product was monitored by gas chromatography coupled to mass spectrometry of the trimethylsilyl derivatives.[21] Compound *erythro*-**1** inhibited dihydroceramide desaturase in a dose-dependent manner, with an $IC_{50}$ of 20 μM without preincubation and a substrate concentration of 50 μM (Figure 1). Biochemical studies of *threo*-**1** showed no inhibition at a concentration of 50 μM (equimolar with substrate),[22] thus demonstrating that the inhibitory effect of ceramide analogues **1** is stereoselective, and that the relative stereochemistry on the aminodiol head of the substrate is a prerequisite for inhibition.

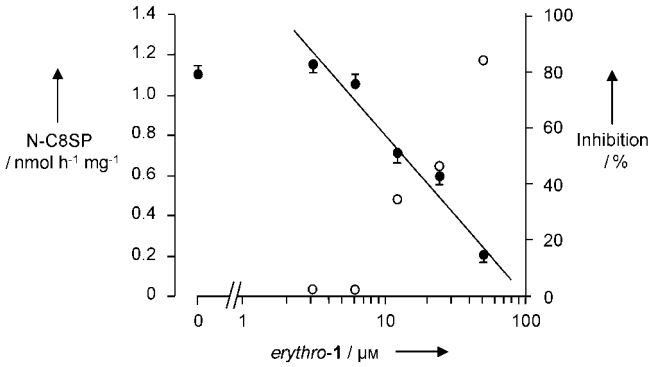



Figure 1. Dose dependence of the inhibition of dihydroceramide desaturase by ceramide analogues *erythro*-**1**. Experiments were conducted at a concentration of 50 μM (15 nmol) of substrate without preincubation. Data correspond to the average ± standard deviation of three replicates. Left ordinate axis (closed circles): amounts of desaturated product (linear fittings yield: $y = 1.59 - 0.77 \log x$ ($R^2 = 0.96$)). Right ordinate axis (open circles): percentage of inhibition.

A comprehensive investigation of the inhibition mechanism is in progress in our laboratories. Cyclopropenes react with thiols and, although it has never been demonstrated, experimental evidence suggests that the inhibition of fatty acyl CoA desaturases by cyclopropene fatty acids is irreversible and occurs by the reaction of the cyclopropene ring with a cysteine residue of the active site of the enzyme.[12] This might also occur in the inhibition of dihydroceramide desaturase by *erythro*-**1**. Michel et al.[5] have also demonstrated that the activity of dihydroceramide desaturase was inhibited by dithiothreitol and suggested that dihydroceramide desaturase might require one or more disulfide bonds for protein stability and/or catalytic activity. However, it has not been determined whether the active site of the enzyme contains reduced cysteine residues that are susceptible to react with the cyclopropene moiety of *erythro*-**1**.

In summary, the introduction of a cyclopropene unit to replace the double bond in the ceramide structure has led to the first potent inhibitor of dihydroceramide desaturase. This compound will be a valuable tool for the elucidation of the role of this enzyme in ceramide-mediated biological processes. Since dihydroceramide lacks the biological effects of ceramide, compound *erythro*-**1** could lead to opportunities for the therapeutic intervention in different diseases related to ceramide accumulation (caused by increased de novo biosynthesis[23]) by interfering with the last step of this pathway, namely the desaturation reaction.

## *Experimental Section*

**1**: 1-Pentadecyne was converted into 1,1,2-tribromo-2-tridecylcyclopropane (**2**) in two steps (addition of HBr to give 2-bromo-1-pentadecene, followed by the addition of dibromocarbene).[16] A solution of *n*BuLi in hexane (1.55 M, 6.7 mL, 10.45 mmol) was added to a solution of **2** (2.17 g, 4.75 mmol) in THF (40 mL) at $-78$ °C under argon. The reaction mixture was warmed to room temperature over 45 min and cooled again to $-23$ °C. A solution of *tert*-butyl-(*S*)-(−)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate (0.90 g, 3.9 mmol) in THF (14 mL) was added at $-23$ °C and stirred for 2 h at this temperature. The reaction was quenched with aqueous saturated $NH_4Cl$ and extracted with diethyl ether. Evaporation of the solvent and flash chromatography (hexane/AcOEt 14:1) of the crude residue (which contained a 3:1 mixture of *erythro*-**3** and *threo*-**3**) allowed

the partial separation of the pure diastereomers, which were obtained in a combined yield of 75%. Removal of the protecting groups with trimethylsilyl triflate,[19] *N*-acylation with octanoyl chloride[20] and final chromatography afforded *erythro*-**1** in 14% overall yield from pentadecyne.

*erythro*-**1**: $[\alpha]_D = +3.0$ ($c = 0.8$ in $CHCl_3$); IR (NaCl): $\tilde{\nu} = 1550, 1644, 3010, 3287\ cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta = 0.87$ (t, 6H, $J = 6.2$ Hz), 1.01 (s, 2H), 1.25 (s, 28H), 1.60 (m, 4H), 2.24 (t, 2H, $J = 7.2$ Hz), 2.45 (dt, 2H, $J = 7.6$, $J' = 1.2$ Hz), 3.02 (br s, 1H), 3.37 (br s, 1H), 3.71 (dd, 1H, $J = 11.4$, $J' = 3.4$ Hz), 3.87 (dd, 1H, $J = 11.2$, $J' = 4$ Hz), 4.18 (m, 1H), 4.85 (s, 1H), 6.37 (br d, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta = 8.36, 14.04, 14.10, 22.58, 22.66, 25.70, 25.82, 27.27, 28.99, 29.19, 29.33, 29.37, 29.44, 29.57, 29.63, 29.64, 29.64, 29.67, 31.65, 31.89, 36.77, 53.74, 63.12, 70.52, 107.52, 115.40, 174.00$. HR-MS calcd for $C_{27}H_{51}NO_3$: 437.386895; found: 437.387000.

The dihydroceramide desaturase inhibition assay was performed by using rat liver microsomes, which were prepared as reported.[5] The activity of dihydroceramide desaturase was determined in phosphate buffer (0.1 M, pH 7.4), with D-*erythro*-*N*-octanoylsphingosine as substrate. The inhibitor (at the indicated concentrations) and the substrate (15 nmol) were solubilized (15 nmol of BSA in phosphate buffer/ethanol 9:1 v/v, 100 μL), combined with the microsome suspension (1 mg of protein) and NADH (30 μL, 1 μM in phosphate buffer), and made up to a final volume of 300 μL with phosphate buffer. The suspension was incubated for 30 min at 37 °C, and the reactions were terminated by the addition of $CHCl_3$ (0.5 mL) containing D-*erythro*-*N*-hexanoylsphingosine (1 nmol) as an internal standard for quantification. The lipids were extracted with $CHCl_3$ ($2 \times 250$ μL), the combined organic layers were evaporated under a stream of nitrogen, and the residue was derivatized with bistrimethylsilyltrifluoroacetamide (50 μL, 25 °C, 60 min). After derivatization, $CHCl_3$ (50 μL) was added and the samples were stored at −80 °C. Instrumental analyses were carried out by gas chromatography coupled to electron impact (70 eV) mass spectrometry using a Fisons gas chromatograph (8000 series) coupled to a Fisons MD-800 mass-selective detector. The system was equipped with a nonpolar Hewlett–Packard HP-1 capillary column (30 m × 0.20 mm i.d.), which was programmed from 100 °C to 340 °C at 7 °C min$^{-1}$. Analyses were carried out in the selected ion monitoring mode. Selected ions were $m/z =$ 311, 313, 230, and 258. Dwell (time for which a given mass is monitored) was set at 0.02 s and the mass span at 0.5.

Received: January 31, 2001
Revised: March 8, 2001 [Z16535]

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## Synthesis of Cored Dendrimers with Internal Cross-Links**

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The physical properties of a dendrimer may be manipulated by post-synthetic modification of its periphery, core, or interior. Such alterations can tune the bulk properties of the dendritic system and frequently convert simple dendrimers into functional macromolecules. For example, incorporation of molecular recognition elements on the periphery of a dendrimer produces multivalent receptors capable of ligand binding or supramolecular assembly.[1] Organometallic functional groups can also be added to the periphery, thus creating macromolecular catalysts.[1–3] In contrast to the many reports of end-group functionalization, there are only a few examples of covalent modification of the interior of the dendrimer.[4] We recently reported[5] the "coring" of dendrimer **1** by ring-closing metathesis (RCM) of the peripheral homoallyl ether groups[6] using ruthenium benzylidene catalyst **2**[7] followed by hydrolytic removal of the core. In some respects, "cored" dendrimers resemble hollow, polymeric nanospheres or core-shell nanoparticles.[8] Consequently, the potential of "cored" dendrimers to encapsulate or complex substances makes them outstanding candidates for delivery agents or molecular sensors.

Our reported synthesis of cored dendrimers[5] is limited in its scalability by the requirement of high dilution (about

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[**] This work was supported by the National Institute of Health (GM 39782). L.G.S. acknowledges the Department of Chemistry, University of Illinois, and Pharmacia & Upjohn for fellowship support.

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