Isolation, Structure Elucidation, Total Synthesis, and Evaluation of New Natural and Synthetic Ceramides on Human SK-MEL-1 Melanoma Cells

Francisco León,[†,‡] Ignacio Brouard,[†] Augusto Rivera,[⊥] Fernando Torres,[§] Sara Rubio,[§] José Quintana,[§] Francisco Estévez,[§] and Jaime Bermejo*,[†]

Instituto de Productos Naturales y Agrobiología, C.S.I.C., Instituto Universitario de Bio-Orgánica "Antonio González", Avda. Astrofísico F. Sánchez 3, 38206 La Laguna, Tenerife, Spain, Instituto Canario de Investigación del Cáncer, Avda. Astrofísico F. Sánchez 2, 38206 La Laguna, Tenerife, Spain, Departamento de Bioquímica y Biología Molecular, Centro de Ciencias de la Salud, Universidad de Las Palmas de Gran Canaria, Avenida S. Cristóbal, 35016 Las Palmas de Gran Canaria, Spain, and Departamento de Química, Facultad de Ciencias, Universidad Nacional de Colombia, Apartado Aéreo 14490, Bogotá, D.C., Colombia

Received May 8, 2006

Two new long-chain ceramides, trametenamides A (**1**) and B (**2**), were isolated from the methanolic extract of the fruiting body of the fungus *Trametes menziesii*. The structures were elucidated by spectroscopic analyses and chemical transformations, and the absolute stereochemistry of trametenamide B (**2**) was determined by stereoselective total synthesis of four possible diastereomers. The acetyl derivative of the natural ceramide (**1a**) and synthetic ceramides (**24**−**27**) showed cytotoxicity on the human melanoma cell line SK-MEL-1, which was caused by induction of apoptosis as determined by DNA fragmentation, poly-(ADP-ribose) polymerase cleavage, and procaspase-9 and -8 processing.

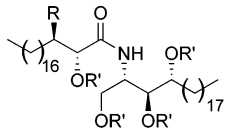
Introduction

Programmed cell death or apoptosis is triggered by a variety of stimuli, including cell surface receptors such as FAS, the mitochondrial response to stress, and factors released from cytotoxic T cells. It is well established that mitochondria play a central role in the process of apoptosis. Numerous signals converge on this intracellular organelle across the activation of diverse proapoptotic Bcl-2 family members that include Bak, Bad, Bid, and Bax. In response to apoptotic stimuli these proteins are redistributed from the cytosol to the mitochondria and result in the permeabilization of the outer mitochondrial membrane.

Cytochrome *c* is an essential component of the mitochondrial respiratory chain, and it is localized in the intermembrane space. Release of cytochrome *c* from the mitochondria provides the signal for the initiation of the assembly of the apoptosome, a large multisubunit complex formed from constitutive proteins. This complex recruits and activates procaspase-9, which then activates downstream effector caspases.[1] Caspase-3 is one of the key executioners of apoptosis, being responsible either partially or totally for the proteolytic cleavage of many key proteins, such as the nuclear enzyme poly(ADP-ribose) polymerase.[2] Other mitochondrial proteins such as the protein Smac and its murine homologue DIABLO have been described that promote caspase activation by eliminating IAP (inhibitor of apoptosis proteins) inhibition of caspases. IAPs inhibit apoptosis primarily by direct inhibition of distinct caspases.

Ceramides have been proposed as a second messenger for events as diverse as differentiation, senescence, proliferation, and cell cycle arrest, although most research has focused on their role in apoptosis.[3] In addition, ceramides are involved in the processes of neuronal death during the development of neurodegenerative diseases.[4] In some cells, apoptosis induction requires ceramide generation at the plasma membrane,[5] and ceramide generation probably drives the clustering of sphingolipid rafts into platforms.[6] Recently, a mitochondrial sphingomyelinase activity has been described that generates enough ceramide in this organelle to activate the apoptotic pathway.[7] Ceramides also seem to be involved in the mitochondrial membrane permeabilization allowing the exit of proapoptotic factors such as cytochrome *c*, AIF, Smac/DIABLO, and the endonuclease Endo G.[8] Recent evidence suggests that ceramides can also induce apoptosis by a caspase-independent mechanism.[9]

In the course of our research on biologically active metabolites from higher fungi, we recently described a series of new triterpene compounds isolated from *Ganoderma lucidum*,[10] *G. concinna*,[11] *G. australe*,[12] and *Laetiporus sulphureus*[13] as well as their biological activities. The present work deals with the chemical study of the fungus *Trametes menziesii*, which is mainly distributed in tropical and subtropical regions (American Samoa region) and has not hitherto been chemically investigated. Two new ceramides were isolated and characterized as their peracetylated derivatives, trametenamide A (**1**) and trametenamide B (**2**), the latter being synthesized along with three of its diastereomers and analyzed as bioactive compounds.



1. R = H; R' = H **1a**. R = H; R' = Ac
2. R = OH; R' = H **2a**. R = OAc; R' = Ac

This fungus belongs to a group of fungi (Polyporaceae) that break down woody plants into their basic elements and are a critical part of the tropical ecosystem including Colombia.[14] Although few species of Polyporaceae fungi have been evaluated for biological activity, most of the tested polypore fungi have shown significant antimicrobial activity as well as antiviral, cytotoxic, and/or antineoplastic activities properties. Therefore, these fungi may constitute a good source for the development of new drugs.[15] To assess whether acetylated ceramides (**1a** and

* To whom correspondence should be addressed. Phone: (34) 922-318583. Fax: (34) 922-318571. E-mail: jbermejo@ull.es.
† Instituto de Productos Naturales y Agrobiología, C.S.I.C.
‡ Instituto Canario de Investigación del Cáncer.
⊥ Universidad Nacional de Colombia.
§ Universidad de Las Palmas de Gran Canaria.

10.1021/jm0605334 CCC: $33.50
Published on Web 08/22/2006

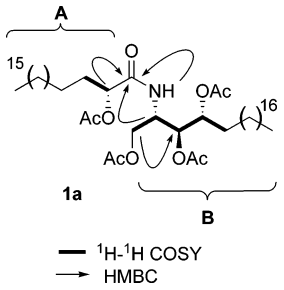


Figure 1. ^{1}H−^{1}H COSY and HMBC correlations for **1a**.

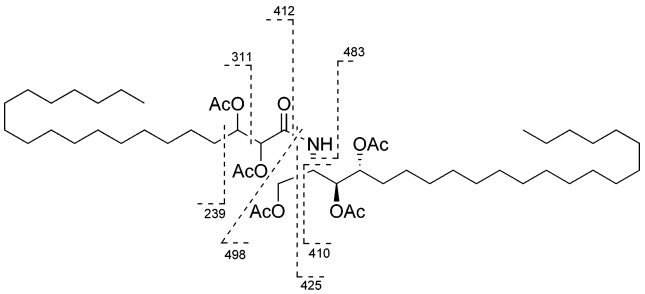


Figure 2. EIMS fragmentation of **1a**.

24−**27**) display cytotoxic activities, we studied their effects on the viability of the human melanoma cell line SK-MEL-1.

Results and Discussion

Isolation and Structural Elucidation. The fungus was collected in the Valencia region (Córdoba, Colombia). The ethanolic extract of the fresh fruiting bodies of the fungus was separated by extensive column chromatography over silica gel to yield five known products, linoleic acid, ergosta-7,22-diene-3-one,[11] ergosta-7,22-diene-3β-ol,[11] ergosterol peroxide,[12] and cerevisterol,[13] as well as an inseparable mixture of two compounds. Acetylation of this mixture and purification of the products by gradient flash silica gel chromatography gave pure compounds **1a** and **2a**. The structures of the known compounds were determined by a combination of spectroscopic analysis and comparison with reported data.

Compound **1a** gave a $[M]^+$ ion at *m*/*z* 851.6778 in HR-FABMS appropriate for a molecular formula of $C_{50}H_{93}NO_9$. The typical IR absorptions at 1667 and 1530 cm^{-1} suggested that compound **1a** contains a secondary amide, which was supported by the presence of a nitrogen-attached carbon signal at δ 46.9 and a carbonyl signal at δ 169.1 in the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR spectra of **1a** showed the presence of two terminal methyls, aliphatic methylenes, three oxygenated methines, one oxygenated methylene, a nitrogenous methine, four acetates, and a carbon and a proton of an amide function. The ^{1}H−^{1}H COSY, HSQC, and HBMC spectra of **1a** afforded two partial regions **A** and **B** (Figure 1). ^{1}H−^{1}H COSY experiment correlations from the nitrogeneous methine to the oxygenated methylene and to one oxygenated methine together with correlations from this methine to another one gave the partial structure of sphingosine **B**. The correlations in the ^{1}H−^{1}H COSY experiment from an oxygenated methine to an aliphatic methylene gave the partial structure of acyl chain **A**. The HMBC correlations of the amide carbonyl with the oxygenated methine proton of C-2′ and with the nitrogenous methine proton of C-2 gave the connectivity between partial structures **A** and **B**.

Amide **1a** was subjected to basic methanolysis in order to determine the nature of the alkyl chains of the ceramide (Scheme 1). Two products were separated chromatographically affording an acid and a sphingosine. The fatty acid was methylated with CH_2N_2 to give the methyl ester **3**, which was directly analyzed by GC−MS and identified as the methyl ester of unbranched 2-hydroxy fatty acid comprising 20 carbon atoms. The presence of this methyl ester **3** was supported by a HR-FABMS ion peak at *m*/*z* 366.2540. The second product from the methanolysis was identified as the aminotriol **4** having 22 carbon atoms based on an ion peak at *m*/*z* 396.3371 as well as major peak fragments at 377, 307, and 289. The stereochemistry of the molecule was established by comparison of ^{1}H NMR and optical rotation data from a similar analogue isolated by Lourenço et al.[16] On the basis of the above data, compound **1** was identified as (2*S*,3*S*,4*R*,2′R)-2-(2′-hydroxyeicosanoylamino)docosane-1,3,4-triol and named as trametenamide A (**1**).

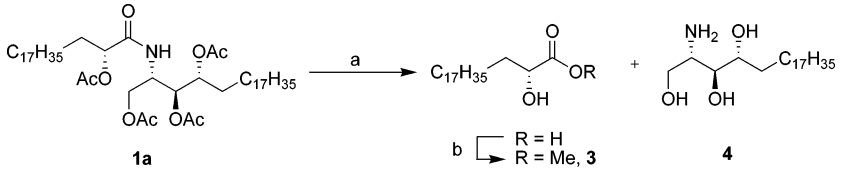
Compound **2**, isolated as the peracetylated derivative **2a**, showed an ion peak at *m*/*z* 932.8060 in the HR-FABMS spectrum. This mass spectrometric datum is in accordance with the molecular formula $C_{52}H_{95}NO_{11}$, suggesting that **2a** should possess an extra acetoxy group compared to peracetate ceramide **1a**. This hypothesis was corroborated by the observation of an additional acetyl methyl singlet in the ^{1}H NMR spectrum of **2a**, as well as an additional oxymethine signal in its ^{13}C NMR spectrum. In the EIMS data of **2a**, characteristic fragment peaks were observed at *m*/*z* 498, 483, 425, 412, 410, 311, and 239, which were rationalized according to the pattern shown in Figure 2 indicating a C_{22} long chain for the sphingosine and a C_{20} long chain for the acid including two acetoxy groups.

Furthermore, the chemical shifts and coupling constants of the sphinganine protons are almost identical to those of the corresponding protons in peracetate ceramide **1a**, thus indicating the same relative stereochemistry in **2a**. As a consequence of those data and on the basis of the results of ^{1}H−^{1}H COSY and HMBC experiments, the additional oxygenated function could only be located at the C-3 position of the acyl chain. However, we could not determine the stereochemistry at C-2′ and C-3′ in the fatty acid chain because a minute amount of compound **2a** hampered further chemical transformation and 2D NMR studies. To unambiguously fully characterize compound **2a**, we carried out the total synthesis of the four possible diastereomers of 2,3-dihydroxyeicosanoic acid. Subsequent coupling of these acids with synthetic common sphingosine **4** will lead us to four peracetylated ceramides and will resolve the remaining absolute stereochemistry of **2a** and, ultimately, provide access to additional material for biological evaluation. Compound **2** is one of the few examples of natural ceramides that contain that particular hydroxylation profile in the fatty acid region.[17] To the best of our knowledge, there is no example of a complete stereochemical characterization of such compounds.

Chemical Synthesis. The first step was the preparation of common sphingosine **4**. The construction of this optically pure amine was achieved by application of the strategy developed by Asai et al.[18] starting from β-D-galactose pentaacetate (Scheme 2) employing a 17-carbon alkylphosphonium salt in the synthesis of compound **8**. Subsequent derivatization of alkene **8** afforded sphingosine **4**.

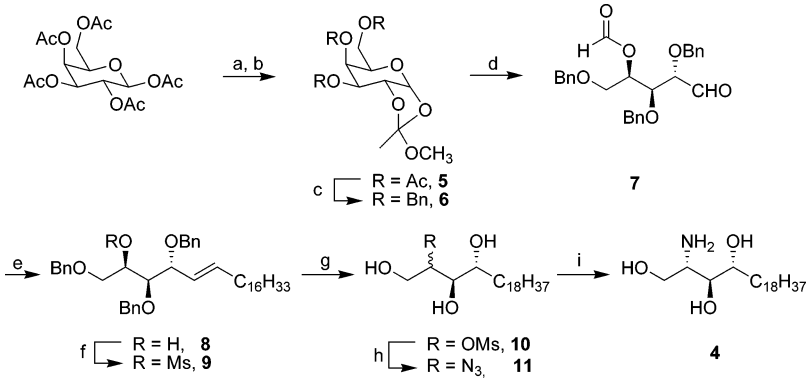
The four acid partners were synthesized following two different strategies depending on the threo or erythro series. For the threo series we took advantage of the Sharpless asymmetric dihydroxylation process performed using both AD-mix-α and AD-mix-β separately. As starting material we used α,β-unsaturated ester **14** obtained from the Horner−Wadsworth−Emmons reaction (HWE) over octadecanal **13** (Scheme 3). Treatment of ester **14** with AD-mix-α and AD-mix-β gave access to diols **15a** and **15b**, respectively, each with a relatively

Scheme 1[a]



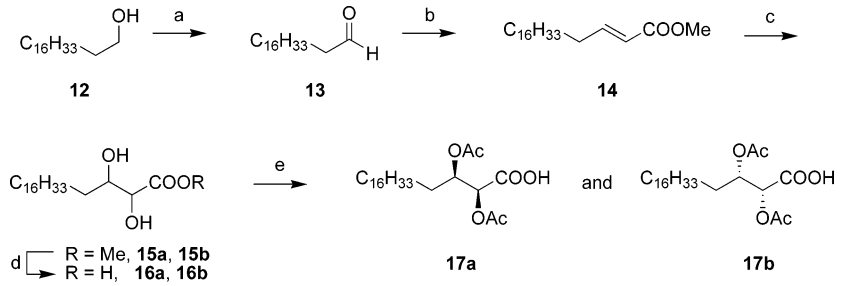
[a] Reagents and conditions: (a) KOH, MeOH, room temp; (b) CH_2N_2, Et_2O, room temp.

Scheme 2[a]



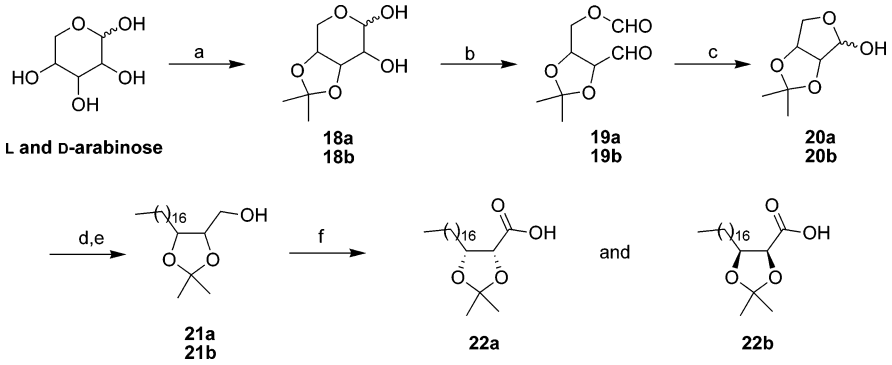
[a] Reagents and conditions: (a) HBr/AcOH (30%); (b) MeOH, Et_3N, $ClCH_2CH_2Cl$; (c) KOH, BnBr, toluene; (d) $NaIO_4$, $EtOH/H_2O$; (e) phosphonium salt, *n*-BuLi, THF; (f) MsCl, Py; (g) H_2, 10% Pd/C, EtOH; (h) NaN_3, DMF; (i) H_2, 10% Pd/C, EtOH.

Scheme 3[a]



[a] Reagents and conditions: (a) PCC, CH_2Cl_2, room temp; (b) methyl phosphonoacetate, NaH, benzene; (c) AD-mix-β or *t*-BuOH−H_2O, methanesulfonamide; (d) NaOH, H_2O, THF; (e) Ac_2O, Py, room temp.

Scheme 4[a]



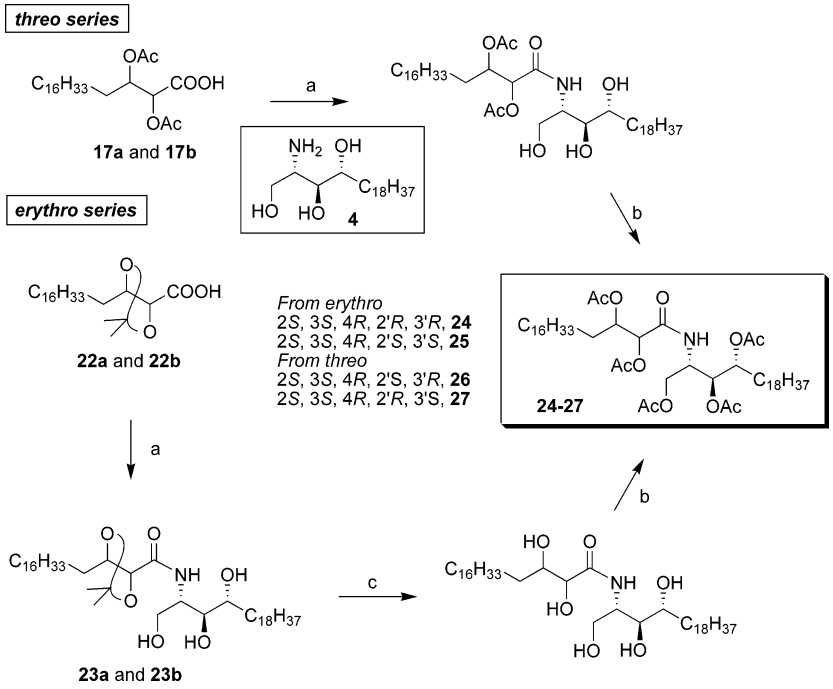
[a] Reagents and conditions: (a) dimethoxypropane, DMF, room temp; (b) $NaIO_4$, CH_2Cl_2−EtOH−H_2O, room temp; (c) K_2CO_3, MeOH, room temp; (d) phosphonium salt, *n*-BuLi, THF, −20 °C; (e) H_2, Pd/C (10%), room temp; (f) $RuCl_3$, $NaIO_4$, $CHCl_3$, room temp.

poor yield, presumably due to solubility problems. Subsequent basic hydrolysis followed by alcohol protection as acetates gave efficient access to the pair of threo enantiomeric acids **17a** and **17b** in a very straightforward manner.

We next explored the synthesis of the erythro acid series via Sharpless asymmetric epoxidation of the allylic alcohol derived from reduction of the above-mentioned ester **14**. Unfortunately, attempts to achieve regioselective epoxide opening induced by Ti(*i*-PrO)$_4$ in the presence of AcOH and subsequent selective protection led us to an unseparable mixture of transesterification byproducts. In our reasoning, the low solubility of the long-chain intermediates could be partly responsible for the inefficiency of this process.

As an alternative, we examined a new strategy using arabinose as starting material (Scheme 4), which contains the appropriate stereochemistry for both chiral centers in the acid chain. Treatment of commercially available D- and L-arabinose, separately, with 2,2-dimethoxypropane afforded diols **18a** and

Scheme 5[a]



[a] Reagents and conditions: (a) HOBt, EDCI or *N*-hydroxysuccinimide, sphingosine **4**; (b) Ac_2O, Py, room temp; (c) HCl (3 N), MeOH, room temp.

Table 1. Effects of Natural and Synthetic Ceramides on the Growth of Human Melanoma Cell Line SK-MEL-1[a]

ceramide	IC_{50} (μM)
1a	7.7 ± 0.8
24	5.6 ± 0.5
25	10.2 ± 3.2
26	3.9 ± 0.9
27	7.1 ± 0.5

[a] The data shown represent the mean ± SEM of two independent experiments with three determinations in each. The IC_{50} values were calculated from experiments such as those shown in Figure 3 using the methodology described in the Experimental Section.

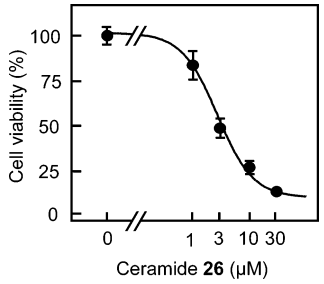


Figure 3. Effect of ceramide **26** on human SK-MEL-1 cell viability. Cells were cultured in the presence of the indicated concentrations (doses) of ceramide **26** for 72 h, and thereafter, cell viability was determined by the MTT assay as described in the Experimental Section. The results of a representative experiment are shown. Each point represents the average of triplicate determinations.

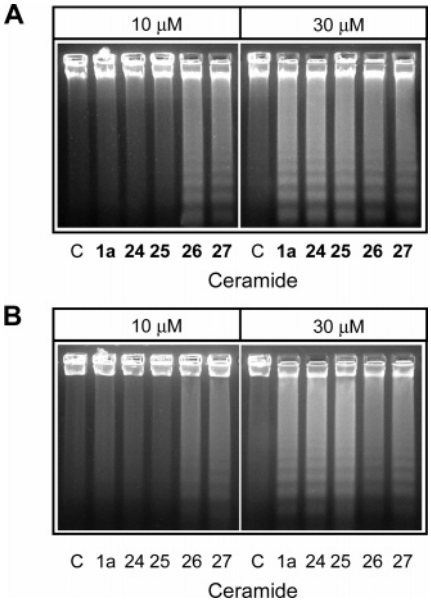


Figure 4. DNA fragmentation. Qualitative assessment of apoptotic DNA damage. SK-MEL-1 human melanoma cells were incubated in absence (C, control) or presence of 10−30 μM of the indicated ceramides for 6 h (A) or 12 h (B). Laddered electrophoretic patterns of oligonucleosomal DNA fragments were resolved by conventional agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light.

18b[19] as their respective anomeric mixtures. Oxidative fragmentation employing $NaIO_4$ followed by basic hydrolysis furnished dimethyl ketal protected erythroses **20a** and **20b**. Both compounds were submitted separately to the Wittig coupling reaction with the phosphonium bromide salt generated from 1-hexadecanol to provide the complete carbon chain. Subsequent hydrogenation of the double bond led us to alcohols **21a** and **21b**, which were oxidated to acids **22a** and **22b** in moderate yields using $RuCl_3$−$NaIO_4$.[20]

With these four fatty acids **17a**, **17b**, **22a**, and **22b** in hand, we turned our attention to the amide generation by coupling amine **4**, separately, with each acid. Toward this end we first applied the reaction conditions used by Schultz et al.[21] in the synthesis of a wide combinatorial ceramide library. This methodology consists of the activation of the acid as a resin-bound ester derived from nitrophenol. To our dismay, attempts to couple activated acid **17a** with the amine partner **4** proved to be unrewarding. When the same coupling was performed using stearic acid, the reaction occurred easily. We therefore

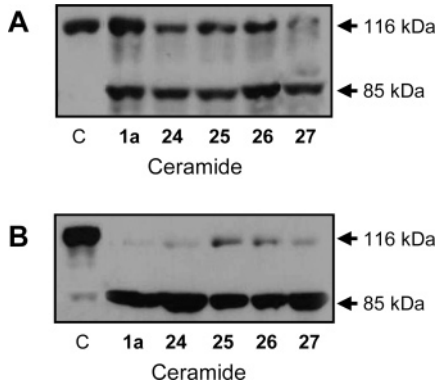


Figure 5. Western blot analysis for the cleavage of poly(ADP-ribose) polymerase (PARP). Control lanes refer to untreated cells. In the other lanes, SK-MEL-1 cells were treated with 10 μM (upper panel, A) or 30 μM (lower panel, B) of the indicated ceramides for 12 h. Cell lysates were subjected to SDS−PAGE followed by blotting with an anti-poly-(ADP-ribose) polymerase that also recognizes the 85 kDa fragment.

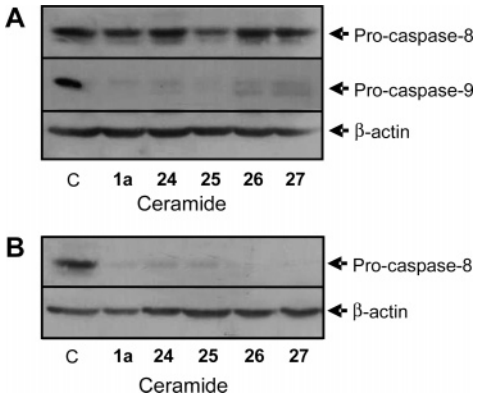


Figure 6. Western blot analysis for procaspase-8 and -9. SK-MEL-1 cells were cultured in the presence of 10 μM (A) or 30 μM (B) ceramides and harvested at 12 h. Total cell lysates were then analyzed by immunoblotting with anti-procaspase-8 or anti-procaspase-9 antibodies. β-Actin was used as loading control.

assumed that the presence of the acetoxy groups close to the reaction center creates a negative effect during the coupling process.

In light of the above, we turned to the acid activation employing either *N*-hydroxysuccinimide or HOBT/EDCI (Scheme 5). In all four cases acetylated ceramides **24**−**27** were obtained in similar and moderate yields. Comparison of the ^{1}H and ^{13}C NMR spectra and physical data of synthetic compound **24** and the peracetylated isolated ceramide **2a** confirmed the absolute configuration of trametenamide B **2** as (2*S*,3*S*,4*R*,2′*R*,3′*R*)-2-(2′,3′-dihydroxyeicosanoylamino)docosane-1,3,4-triol.

Induction of Apoptosis by Ceramides. We also report here on the cytotoxic activity of the acetyl derivative of natural ceramide (**1a**) and synthetic ceramides (**24**−**27**) against human melanoma SK-MEL-1 cells (Table 1, Figure 3), using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) dye-reduction assay. These ceramides show similar IC_{50} values (3−10 μM), indicating that the configurations at carbons C-2′ and C-3′ of the ceramide skeleton do not play a significant role in cytotoxic activity (Table 1, Figure 3). Similar results were obtained in the human myeloid leukaemia cell line HL-60 (results not shown).

To determine whether the decrease in human SK-MEL-1 cell viability (Table 1) observed after treatment with ceramides **1a** and **24**−**27** occurs by apoptosis, we performed DNA fragmentation and poly(ADP-ribose) polymerase cleavage experiments. DNA fragmentation is considered the end point of the apoptotic pathway, and poly(ADP-ribose) polymerase cleavage is a hallmark of apoptosis that indicates activation of caspase.

We observed the appearance of internucleosomal DNA fragmentation in SK-MEL-1 cells treated with ceramides **1a** and **24**−**27**. As illustrated in Figure 4, SK-MEL-1 cells underwent apoptosis in a dose-dependent fashion after treatment with ceramides. These ceramides also induced poly(ADP-ribose) polymerase cleavage; the typical 85 kDa band was observed after treatment with ceramides **1a** and **24**−**27** (Figure 5). By use of Western blot analysis, the cleavage of procaspase-9 (46−50 kDa) and procaspase-8 (55−57 kDa) was observed after exposure to ceramides. Procaspase-9 processing exhibited a higher level than procaspase-8 processing at 10 μM ceramides (Figure 6).

Conclusions

The polyporaceae fungus *Trametes menziesii* has been chemically studied for the first time, and two new long-chain ceramides have been identified along with five known compounds. One of these ceramides contains an unusual dihydroxylation profile for sphingolipids. The scarcity of novel isolated ceramide **2** encouraged us to develope the synthesis of such a compound, together with three of its diastereomers, to fully determine the stereochemistry of the chiral centers in the fatty acid chain and to provide enough material to perform biological assays. For the first time in the literature an example of this kind of ceramides is unambiguously described.

The exposure of SK-MEL-1 cells to ceramides **1a** and **24**−**27** described in this work elicits a strong antiproliferative effect and induces apoptosis. These ceramides induced internucleosomal DNA fragmentation and poly(ADP-ribose) polymerase cleavage characteristic of apoptotic cell death. It was found that the induction of apoptosis by ceramides **1a** and **24**−**27** in SK-MEL-1 melanoma cells correlated with enhanced poly(ADP-ribose) polymerase cleavage and procaspase-9 and -8 processing. The results of the biological experiments allow us to conclude that the stereochemistry of the hydroxyl groups in fatty acids has no significant influence on the antiproliferative activity of the ceramides.

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi B-540 apparatus and are uncorrected. Optical rotations were recorded in a Perkin-Elmer model 343 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker models AMX-400, Avance 400, and Avance 300 spectrometers with standard pulse sequences operating at 400 and 300 MHz in ^{1}H NMR and at 100 and 75 MHz in ^{13}C NMR. $CDCl_3$ and C_5D_5N were used as solvents. EIMS, HR-EIMS, FABS, and HR-FABS data were taken on a Micromass model Autospec (70 eV) spectrometer. Column chromatography was carried out on silica gel 60 (Merck 230−400 mesh) and preparative TLC on silica gel 60 $PF_{254+366}$ plates (20 cm × 20 cm, 1 mm thickness) and Sephadex LH-20 (Aldrich).

Plant Material. The fungus *Trametes menziesii* (Berk) Ryv. was collected in the region of Valencia, Córdoba Department, Colombia, in May 2000. The fungus was identified by Professor Luis G. Henao of the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, where a voucher specimen is deposited (Col. 344005).

Extraction and Isolation. The body fungi (380 g) were ground and steeped in EtOH (96%) for a week. The ethanolic extract (3.62 g) was chromatographed on silica gel (400 g), the fractions were eluted with *n*-hexane, *n*-hexanes−EtOAc mixtures (9:1; 4:1; 7:3; 1:1), and EtOAc (each 200 mL), yielding six fractions. Fraction 1 (150 mg) was rechromatographed over silica gel, eluting with

n-hexanes−EtOAc (9:1) to yield linoleic acid (50 mg). Fraction 2 (200 mg) and fraction 3 (500 mg) were further chromatographed with a Sephadex LH-20 column, eluting with *n*-hexane-$CHCl_3$-MeOH (3:3:1), and preparative TLC was carried out with toluene−acetone (9:1) and three elutions, affording ergosta 7,22-dien-3-one (90 mg) and ergosta 7,22-dien-3β-ol (130 mg). Fraction 4 (200 mg) was rechromatographed on preparative TLC with *n*-hexanes−EtOAc (8:2) and four elutions, yielding ergosterol peroxide (70 mg). Fraction 5 (150 mg) was chromatographed by column silica gel, eluting with $CHCl_3$−MeOH (50:1), and preparative TLC was carried out with *n*-hexanes−EtOAc (7:3) and three elutions, affording cerevisterol (2 mg). Fraction 6 (300 mg), after acetylation with Ac_2O/pyridine and subsequent purification by preparative TLC chromatography with *n*-hexanes−EtOAc (4:1), eluting twice, afforded tetraacetyltrametenamide A (**1**) (10 mg) and pentaacetyltrametenamide B (**2**) (3 mg).

(2*S*,3*S*,4*R*,2′*R*)-2-[2′-Acetoxyeicosanoylamino]-1,3,4-triacetoxydocosane (1a). Amorphous solid; $[\alpha]^{25}_D$ +25.0 (*c* 0.08, $CHCl_3$); IR ν_{max} (film, NaCl) 3341, 2920, 2850, 1737, 1667, 1530, 1470,1374, 1229, 1040 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.87 (6H, t, *J* = 7.1 Hz, Me-22 and Me-20′), 1.27−1.41 (64H, br s, 32-CH_2), 1.60 (4H, m, CH_2-5 and CH_2-3′), 2.02 (3H, s, OAc), 2.05 (3H, s, OAc), 2.08 (3H, s, OAc), 2.18 (3H, s, OAc), 4.00 (1H, dd, *J* = 3.2, 11.8 Hz, H_a-1), 4.33 (1H, dd, *J* = 6.4, 11.2 Hz, H_b-1), 4.44 (1H, ddd, *J* = 3.2, 7.3, 9.7 Hz, H-2), 4.94 (1H, dd, *J* = 3.3, 10.0 Hz, H-2′), 5.09 (1H, m, H-3), 5.09 (1H, m, H-4), 6.60 (1H, d, *J* = 9.1 Hz, N−H); ^{13}C NMR (δ, $CDCl_3$) 13.2 (C-22 and C-20′), 19.8 (OAc), 19.9 (OAc), 20.0 (OAc), 20.1 (OAc), 21.8−31.0 [C-(5−21) and C-(3′-19′)], 46.9 (C-2), 61.5 (C-1), 71.4 (C-3), 71.8 (C-2′), 73.1 (C-4), 169.1 (C-1′), 169.1 (2 × C═O), 170.0 (C═O), 170.4 (C═O); FABMS *m*/*z* (relative intensity) 875 $[M + Na]^+$ (9), 852 $[M + H]^+$ (30), 851 $[M]^+$ (1), 820 (20), 806 (25), 792 (55), 790 (11), 264 (13), 154 (70), 137 (70), 107 (22), 69 (60), 54 (100); HR-FABMS 851.6778 ($C_{50}H_{93}NO_9$, calcd 851.6850).

Basic Hydrolysis of 1a. A solution of 1 N KOH in MeOH−H_2O (9:1) (5 mL) was added to acetylated trametenamide A (4 mg), and the mixture was maintained at 80 °C under argon with constant stirring. After 24 h, the mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was removed in vacuo, and the residue was subjected to preparative TLC and eluted with $CHCl_3$−methanol (5:1) to give phytosphingosine **4** (1.0 mg) as an amorphous solid. $[\alpha]^{25}_D$ +12.3 (*c* 0.008, pyridine); ^1H NMR (δ, pyridine-d_5) 0.85 (3H, t, *J* = 5.5 Hz, CH_3-22), 1.1−1.3 (30H, br s, 15-CH_2 (7−21)), 1.61 (1H, m, H_b-6), 1.82 (2H, m, H_a-6 and H_b-5), 2.20 (1H, m, H_a-5), 4.03 (1H, d, *J* = 4.7 Hz, H-2), 4.18 (1H, t, *J* = 8.0 Hz, H-4), 4.33 (1H, dd, *J* = 5.4, 7.6 Hz, H-3), 4.49 (2H, m, 2 × H-1); FABMS *m*/*z* (relative intensity) 396 $[M + Na]^+$ (11), 377 $[M + Na - H_2O]^+$ (9), 307 (28), 289 (13), 176 (8), 154 (100), 137 (66), 107 (17); HR-FABMS 396.3371 ($C_{22}H_{47}NO_3Na$ calcd 396.3453). The aqueous phase was then acidified with 5% H_2SO_4, extracted with CH_2Cl_2, and concentrated. The residue was treated with CH_2N_2 in diethyl ether, and the mixture was kept at room temperature for 12 h. Concentration of the reaction mixture under vacuo furnished methyl (2*R*)-2-hydroxyeicosanoate (0.6 mg) as an oil. $[\alpha]^{25}_D$ −2.3 (*c* 0.048, $CHCl_3$); ^1H NMR (δ, $CDCl_3$) 0.96 (3H, t, *J* = 7.4 Hz), 1.25 (30H, s br, 15-CH_2), 1.42 (2H, m), 1.71 (2H, m), 3.66 (3H, s, OMe), 4.10 (1H, dd, *J* = 6.8, 10.1 Hz); HR-FABMS *m*/*z* (relative intensity) 366.2540 ($C_{21}H_{43}O_3Na$ calcd 366.2489) $[M + Na + H]^+$ (11), 327 (5), 313 (18), 311 (17), 299 (7), 284 (5).

(2*S*,3*S*,4*R*,2′*R*,3′*R*)-2-[2′,3′-Diacetoxyeicosanoylamino]-1,3,4-triacetoxydocosane (2a). Amorphous solid; IR ν_{max} (film, NaCl) 2923, 2853, 1747, 1697, 1660, 1546, 1371, 1225, 1047 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.87 (6H, t, *J* = 6.8 Hz, Me-22 and Me-20′), 1.20−1.50 (62H, br s, 31-CH_2), 1.60 (4H, m, CH_2-5 and CH_2-4′), 2.02 (3H, s, OAc), 2.05 (3H, s, OAc), 2.06 (3H, s, OAc), 2.08 (3H, s, OAc), 2.09 (3H, s, OAc), 4.02 (1H, dd, *J* = 3.4, 11.8 Hz, H_a-1), 4.28 (1H, dd, *J* = 6.4, 11.8 Hz, H_b-1), 4.82 (1H, ddd, *J* = 3.2, 6.8, 9.6 Hz, H-2), 4.98 (1H, dt, *J* = 3.2, 6.6 Hz, H-4), 5.11 (1H, dd, *J* = 4.0, 7.4 Hz, H-3), 5.25 (1H, dt, *J* = 4.0, 7.4 Hz, H-3′), 5.36 (1H, d, *J* = 3.2 Hz, H-2′), 6.78 (1H, d, *J* = 9.2 Hz, N−H); ^{13}C NMR (δ, $CDCl_3$) 14.1 (C-22 and C-20′), 20.5 (OAc), 20.6 (OAc), 20.7 (OAc), 20.9 (OAc), 21.0 (OAc), 22.6−31.9 [C-(5−21) and C-(4′-19′)], 47.9 (C-2), 62.5 (C-1), 72.1 (C-3), 72.8 (C-3′), 72.9 (C-4), 73.7 (C-2′), 166.6 (C-1′), 169.3 (C═O), 170.0 (C═O), 170.5 (C═O), 170.8 (C═O), 171.2 (C═O); EIMS (70 eV) *m*/*z* (relative intensity 498 (4), 483 (7), 439 (24), 425 (35), 424 (35), 412 (7), 410 (26), 369 (23), 341 (8), 325 (6), 311 (18), 239 (4); HR-FABMS *m*/*z* (relative intensity) 932.8060 $[M + Na]^+$ (5), 911 $[M + H]^+$ (3), 910 $[M]^+$ (7), 864 (3 850 (7), 790 (4), 308 (3), 264 (8), 154 (10), 137 (22), 107 (22), 69 (60), 54 (100).

Synthesis of Phytosphingosine 4. 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose (5). To penta-*O*-acetyl-β-D-galactopyranose (16.0 g, 41.0 mmol) was added 30% HBr/AcOH (30 mL), and the mixture was stirred for 3 h at room temperature and then evaporated with toluene under reduced pressure to afford the bromide derivative (∼90%). ^1H NMR (δ, $CDCl_3$) 1.97 (3H, s, OAc), 2.01 (3H, s, OAc), 2.07 (3H, s, OAc), 2.11 (3H, s, OAc), 4.07 (1H, dd, *J* = 6.8, 11.4 Hz, H_a-6), 4.15 (1H, dd, *J* = 6.3, 11.4 Hz, H_b-6), 4.45 (1H, t, *J* = 6.6 Hz, H-5), 5.00 (1H, dd, *J* = 3.9, 10.6 Hz, H-2), 5.36 (1H, dd, *J* = 3.2, 10.6 Hz, H-3), 5.48 (1H, dd, *J* = 1.1, 3.2 Hz, H-4), 6.66 (1H, d, *J* = 3.9 Hz, H-1); ^{13}CNMR (δ, $CDCl_3$) 20.4−20.6 (4 × OAc), 60.7 (C-6), 66.9 (C-4), 67.6 (C-2), 67.9 (C-3), 70.9 (C-5), 88.0 (C-1), 169.7−170.3 (4 × OAc). This compound, without purification, was dissolved in 1,2-dichloroethane (150 mL), and triethylamine (12 mL, 86.0 mmol), methanol (1.82 mL, 45.0 mmol), and tetrabutylammonium bromide (6.8 g, 21.2 mmol) were added. The mixture was stirred for 20 h at 45 °C, and the precipitated salt was removed by filtration. The filtrate was washed with brine and concentrated in vacuo. The residue was chromatographed over silica gel and eluted with *n*-hexanes−EtOAc (7:3) to give **5** (12 g, 33.0 mmol, 80%). The physical and spectroscopic data were identical to those reported in the literature.[18]

3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose (6). To a stirred solution of **5** (11.0 g, 30.3 mmol) in toluene (250 mL) were added KOH (17.5 g, 310.0 mmol) and benzyl bromide (25 mL, 35.9 g, 210.2 mmol). The mixture was gradually heated and refluxed for 4 h. It was then diluted with toluene and washed with water and brine. The organic phase was concentrated in vacuo, chromatographed over silica gel, and eluted with *n*-hexanes−EtOAc (4:1) to afford compound **6** (9.96 g, 19.69 mmol, 65%). The physical and spectroscopic data were identical to those reported in the literature.[18]

(2*S*,3*S*,4*R*)-2,3,5-Tribenzyloxy-4-formyloxypentanal (7). To a stirred solution of **6** (9.0 g, 17.77 mmol) in 1,4-dioxane was added 1 M H_2SO_4 (40 mL), and the mixture was refluxed for 3 h. The reaction mixture was cooled to room temperature, and then solid $NaHCO_3$ was carefully added until the mixture was neutralized. The precipitated salts were removed by filtration, and the filtrate was evaporated to dryness. The resulting residue was dissolved in $CHCl_3$ and washed with brine and concentrated. This residue was dissolved in EtOH−H_2O (4:1, 80 mL). Sodium metaperiodate (7.5 g, 35.34 mmol) was added, and the mixture was stirred for 24 h at room temperature. Then the solution was diluted with ether, washed with brine, and dried over $MgSO_4$ and the solvent was evaporated to give aldehyde **7** (6.52 g, 14.57 mmol, 82%) as an oil. $[\alpha]^{25}_D$ −3.2 (*c* 0.06, $CHCl_3$); IR ν_{max} (film, NaCl) 3429, 3063, 2927, 1790, 1734, 1698, 1501, 1496, 1454, 1250, 738, 698 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 3.70 (2H, m, H_2-4), 4.02 (1H, dd, *J* = 1.7, 4.5 Hz, H-1), 4.17 (1H, dd, *J* = 4.6, 4.7 Hz, H-2), 4.47−4.76 (6H, m, 3-CH_2-benz), 5.44 (1H, dd, *J* = 5.0, 10.1 Hz, H-3), 7.28−7.41 (15H, m, 15CH-aromatic), 8.03 (1H, s, OCHO), 9.64 (1H, s, CHO); ^{13}C NMR (δ, $CDCl_3$) 67.8 (C-4), 71.8 (C-3), 73.0 (CH_2-benz), 73.2 (CH_2-benz), 74.2 (CH_2-benz), 77.5 (C-2), 82.6 (C-1), 127.0−128.6 (15C-aromatic), 136.8, 137.2, 137.5 (3C-aromatic), 160.3 (OCHO), 200.9 (CHO); HR-EIMS (70 eV) *m*/*z* (relative intensity) $[M]^+$ (absent), 327.1215 $[M - C_8H_{10}O]^+$ ($C_{19}H_{19}O_5$, calcd 327.1232) (3), 253 (8), 181 (7), 122 (5), 107 (7), 105 (15), 91 (100), 77 (9).

(2*R*,3*S*,4*R*)-1,3,4-Tribenzyloxy-5-docosen-2-ol (8). A suspension of heptadecyltriphenylphosphonium bromide (23.35 g, 40.2 mmol, prepared by the method of Duclos[22]) in THF (160 mL) was

stirred at 0 °C. *n*-BuLi (2.0 M in cyclohexane, 14 mL) was added dropwise. The resulting solution was stirred for 30 min, and then a solution of **7** (6.0 g, 13.4 mmol) in THF (10 mL) was added dropwise. The mixture was warmed to room temperature and stirred for 1 h. The reaction was quenched by adding water and extracted with ether, and the organic phase was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed over silica gel and eluted with *n*-hexanes−EtOAc (4:1), giving olefin **8** (4.39 g, 6.93 mmol, 51%) as an oil. $[\alpha]^{25}_D$ −13.3 (*c* 0.08, $CHCl_3$); IR ν_{max} (film, NaCl) 3446, 3031, 2922, 2853, 1723, 1604, 1496, 1454, 1270, 1091, 1027, 734, 697 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.94 (3H, t, J = 6.3 Hz, CH_3-22), 1.2−1.4 (28H, br s, $14CH_2$ (8−21)), 2.00 (2H, m, H_2-7), 3.58 (2H, d, J = 5.8 Hz, 2 × H-1), 3.63 (1H, dd, J = 2.8, 5.5 Hz, H-3), 4.17 (1H, m, H-2), 4.40 (1H, d, J = 11.7 Hz, 1H-benz), 4.51 (4H, m, 3H-benz and H-4), 4.69 (1H, d, J = 11.3 Hz, 1H-benz), 4.77 (1H, d, J = 10.6 Hz, 1H-benz), 5.52 (1H, dd, J = 9.4, 10.5 Hz, H-5), 5.80 (1H, m, H-6), 7.26−7.36 (15H, m, 15CH-aromatic); ^{13}C NMR, (δ, $CDCl_3$) 14.1 (C-22), 22.7, 28.0, 29.3, 29.6, 29.7, 32.0 (C-7-C-21), 69.9 (C-2), 70.2 (CH_2-benz), 71.1 (C-1), 73.3 (CH_2-benz), 73.8 (CH_2-benz), 74.6 (C-4), 80.2 (C-3), 127.0 (C-5), 127.6−128.4 (15C-aromatic), 136.3 (C-6), 136.9, 138.2, 138.3 (3C-aromatic); HR-EIMS (70 eV) *m*/*z* (relative intensity) $[M]^+$ (absent), 491.3937 $[M - C_9H_{11}O_2]^+$ ($C_{34}H_{51}O_2$, calcd 491.3889) (1), 443 (1), 384 (2), 371 (3), 293 (2), 279 (3), 181 (7), 108 (4), 91 (100), 79 (6).

(5*E*)-(2*R*,3*R*,4*R*)-1,3,4-Tribenzyloxy-2-methanesulfonyloxy-5-docosene (9). A solution of **8** (4.2 g, 6.53 mmol) in pyridine (10 mL) was ice-cooled at 0 °C, and then mesyl chloride (1.05 mL, 13.57 mmol) was added. The mixture was stirred for 8 h at room temperature, then toluene was added, and the solution was concentrated in vacuo. The resulting residue was dissolved in ether, and the solution was washed successively with water and brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness, and the residue was chromatographed over silica gel and eluted with *n*-hexanes−EtOAc (9:1) to afford sulfonate **9** (4.6 g, 6.38 mmol, 97%) as an oil. $[\alpha]^{25}_D$ +3.3 (*c* 0.008, $CHCl_3$); IR ν_{max} (film, NaCl) 2924, 2853, 1454, 1359, 1174, 1092, 971, 920, 803, 736, 698, 526 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.88 (3H, t, J = 6.4 Hz, CH_3-22), 1.2−1.4 (30H, br s, $15CH_2$ (8−21)), 2.00 (2H, m, H_2-7), 2.94 (3H, s, SO_3CH_3), 3.51 (1H, dd, J = 3.6, 10.9 Hz, H_a-1), 3.67 (1H, dd, J = 4.0, 7.0 Hz, H_b-1), 3.78 (1H, dd, J = 4.1, 6.4 Hz, H-3), 4.17 (3H, m, 2 × benz and H-4), 4.51 (3H, m, benz), 4.77 (1H, d, J = 11.2 Hz, benz), 5.06 (1H, ddd, J = 3.8, 7.0, 7.4 Hz, H-2), 5.50 (1H, dd, J = 9.6, 10.9 Hz, H-5), 5.81 (1H, ddd, J = 7.2, 10.8, 11.2 Hz, H-6), 7.26−7.36 (15H, m, 15CH-aromatic); ^{13}C NMR (δ, $CDCl_3$) 14.1 (C-22), 22.7, 28.0, 29.3, 29.6, 29.7, 31.9 (C-7-C-21), 38.6 ($C-SO_3CH_3$), 69.5 (C-1), 70.0 (CH_2-benz), 73.4 (C-4), 73.4 (CH_2-benz), 74.8 (CH_2-benz), 79.3 (C-3), 80.8 (C-2), 126.3 (C-5), 127.5−128.4 (15C-aromatic), 137.4 (C-aromatic), 137.8 (C-aromatic), 138.3 (C-aromatic), 155.2 (C-6); EIMS (70 eV) *m*/*z* (relative intensity) 720 $[M]^+$ (absent), 371 (3), 279 (3), 253 (36), 181 (6), 91 (100).

(2*R*,3*R*,4*R*)-2-Methanesulfonyloxy-1,3,4-docosanetriol (10). To a solution of **9** (4.34 g, 6.02 mmol) in EtOH (40 mL) was added 10% Pd/C (0.5 g). The mixture was stirred for 24 h under a hydrogen atmosphere at room temperature and filtered through a pad of Celite, and the filtrate was concentrated to give **10** (2.6 g, 5.7 mmol, 95%) as an amorphous solid. $[\alpha]^{25}_D$ +16.8 (*c* 0.006, pyridine); IR ν_{max} (film, NaCl) 3367, 3209, 2915, 2849, 1166, 1020, 950, 920, 483 cm^{-1}; ^1H NMR (δ, pyridine-d_5) 0.74 (3H, t, J = 6.4 Hz, CH_3-22), 1.1−1.3 (32H, br s, $16CH_2$ (6−21)), 1.82 (2H, m, CH_2-5), 3.32 (3H, s, SO_3CH_3), 4.11 (1H, ddd, J = 2.1, 8.2, 10.5 Hz, H-4), 4.18 (1H, dd, J = 2.0, 8.5 Hz, H-3), 4.40 (2H, m, CH_2-1), 5.62 (1H, dt, J = 1.9, 5.9 Hz, H-2); ^{13}C NMR (δ, pyridine-d_5) 15.2 (C-22), 23.8, 26.9, 30.5, 30.9, 31.1, 33.0, 35.5 (C-(7−21)), 39.4 ($C-SO_3CH_3$), 63.2 (C-1), 71.7 (C-4), 74.9 (C-3), 85.7 (C-2); HR-EIMS (70 eV) *m*/*z* (relative intensity) $[M]^+$ (absent), 356.3282 $[M - OMs]^+$ ($C_{22}H_{44}O_3$, calcd 356.3290) (3), 338 (21), 325 (13), 320 (47), 281 (100), 264 (15), 151 (16), 137 (12), 123 (28), 113 (13).

(2*S*,3*S*,4*R*)-2-Azido-1,3,4-docosanetriol (11). Sodium azide (81.9 g, 29.38 mmol) was added to a solution of **10** (2.49 g, 5.51 mmol) in DMF (65 mL) at room temperature. The mixture was slowly heated to 100 °C, stirred for 3 h, then diluted with EtOAc and washed with water and brine. The organic phase was dried on Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed over silica gel with $CHCl_3$−MeOH (9:1) to give triol **11** (1.45 g, 3.65 mmol, 66%) as an amorphous solid. $[\alpha]^{25}_D$ +26.6 (*c* 0.01, pyridine); IR ν_{max} (film, NaCl) 3339, 2915, 2848, 2113, 1463, 1350, 1200 cm^{-1}; ^1H NMR (δ, pyridine-d_5) 0.71 (3H, t, J = 6.5 Hz, CH_3-22), 1.1−1.3 (30H, m, $15CH_2$ (7−21)), 1.51 (1H, m, H_b-6), 1.75 (2H, m, H_a-6, H_b-5), 2.02 (1H, m, H_a-5), 4.08 (1H, br d, J = 6.0 Hz, H-4), 4.16 (1H, br d, J = 5.4 Hz, H-3), 4.26 (1H, br t, J = 2.5 Hz, H-2), 4.40 (1H, m, H_b-1), 4.52 (1H, m, H_a-1); ^{13}C NMR (δ, pyridine-d_5) 15.5 (C-22), 24.2, 27.6, 30.8, 31.3, 31.5, 33.3 (C-(6−21)), 35.4 (C-5), 63.2 (C-1), 67.9 (C-2), 73.6 (C-4), 77.3 (C-3); HR-EIMS (70 eV) *m*/*z* (relative intensity) $[M]^+$ (absent), 340.3352 $[M - OH - N_3]^+$ ($C_{22}H_{44}O_2^+$, calcd 340.3341) (11), 322 (10), 313 (18), 283 (15), 281 (88), 264, 137 (8), 123 (14), 109 (26). Anal. ($C_{22}H_{45}N_3O_3$) C, H, N.

(2*S*,3*S*,4*R*)-2-Amino-1,3,4-docosanetriol (4). To a solution of azide **11** (1.3 g, 3.25 mmol) in EtOH (100 mL) was added 10% Pd/C (0.3 g). The mixture was stirred for 24 h under a hydrogen atmosphere at room temperature. The Pd/C was then removed by filtration through a pad of Celite, and the filtrate was concentrated in vacuo. The resulting residue was chromatographed over silica gel with $CHCl_3$−MeOH−H_2O−25% NH_4OH (8:2:0.15:0.05) to give amine **4** (0.95 g, 2.54 mmol, 78%) as an amorphous solid. $[\alpha]^{25}_D$ +10.6 (*c* 0.01, pyridine); IR ν_{max} (film, NaCl) 3250, 3019, 2916, 2846, 1216, 757, 658 cm^{-1}; ^1H NMR (δ, pyidine-d_5) 0.82 (3H, t, J = 5.5 Hz, CH_3-22), 1.1−1.3 (30H, br s, $15CH_2$ (7−21)), 1.61 (1H, m, H_b-6), 1.80 (2H, m, H_a-6 and H_b-5), 2.23 (1H, m, H_a-5), 4.01 (1H, d, J = 4.5 Hz, H-2), 4.16 (1H, t, J = 8.0 Hz, H-4), 4.31 (1H, dd, J = 5.3, 7.6 Hz, H-3), 4.47 (2H, m, CH_2-1); ^{13}C NMR (δ, pyridine-d_5) 15.5 (C-22), 24.2, 27.3, 30.8, 31.2, 31.5, 33.4 (C-6−C-21), 36.1 (C-5), 58.7 (C-2), 63.6 (C-1), 75.2 (C-4), 76.0 (C-3); HR-EIMS (70 eV) *m*/*z* (relative intensity) $[M]^+$ (absent), 342.3383 $[M - CH_2OH]^+$ ($C_{21}H_{44}NO_2^+$, calcd 342.3372) (6), 324 (8), 308 (8), 90 (10), 60 (100). Anal. ($C_{22}H_{47}NO_3$) C, H, N.

Synthesis of Threo Series of Acids. Methyl (2*E*)-Eicosenoate (14). To a suspension of pyridinium chlorochromate (PCC) (17 g, 83.24 mmol) in anhydrous CH_2Cl_2 was added 1-octadecanol (15 g, 55.49 mmol). The mixture was stirred for 40 min. Then ether was added and the supernatant decanted from the black gum. The insoluble residue was washed with ether four times whereupon it became a black granular solid. The combined organic solutions were passed through a pad of Celite, and the filtrate was concentrated yielding the crude aldehyde **13**, which was used without further purification in the following reaction. To a nitrogen-flushed solution of NaH (1.33 g, 110.98 mmol) in 250 mL of freshly distilled benzene at 0 °C, trimethyl phosphonoacetate (110.98 mmol, 17.95 mL) was injected dropwise. After the solution was stirred for 30 min, crude aldehyde (∼15 g, 55.5 mmol) in benzene (10 mL) was added. The reaction mixture was stirred vigorously at room temperature until full consumption of the aldehyde (3 h) was observed (TLC). The mixture was diluted with EtOAc and washed with water and brine. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed over silica gel with *n*-hexanes−EtOAc (19:1) to give **14** (10.48 g, 32.33 mmol, 58%) as an amorphous solid. IR ν_{max} (film, NaCl) 2916, 2850, 1722, 1656, 1471, 1436, 1266, 975, 716 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.84 (3H, t, J = 6.5 Hz, CH_3-20), 1.2−1.4 (30H, br s, $15CH_2$ (5−19)), 2.17 (2H, m, CH_2-4), 3.68 (3H, s, OMe), 5.78 (1H, d, J = 15.6 Hz, H-2), 6.93 (1H, ddd, J = 6.9, 14.4, 15.6 Hz, H-3); ^{13}C NMR (δ, $CDCl_3$) 14.1 (C-20), 22.6, 29.1, 29.3, 29.5, 29.7, 31.9, 32.2 (C-(4−19)), 51.2 (OMe), 120.8 (C-2), 149.7 (C-3), 167.0 (C-1); HR-EIMS (70 eV) *m*/*z* (relative intensity) 324.3011 $[M]^+$ ($C_{21}H_{40}O_2$, calcd 324.3028) (20), 311 (21), 292 (100), 250 (21), 227 (28), 113 (18), 97 (14), 87 (32).

General Experimental Procedure for Asymmetric Dihydroxylation of α,β-Unsaturated Ester (14). A solution of 0.88 g of AD-mix-β (or AD-mix-α) in *t*-BuOH−H_2O (1:1, 50 mL) was stirred vigorously at room temperature for 30 min. Then methane-

sulfonamide (73.4 mg, 0.77 mmol) was added, and the stirring was continued for 10 min. After the reaction mixture was cooled to 0 °C, ester **14** (215 mg, 0.66 mmol) was added and the reaction mixture was stirred vigorously at this temperature until no change was observed by TLC (4 days). Then a solution of sodium sulfite was added to quench the reaction and stirring was continued for 60 min while the reaction mixture was allowed to warm to room temperature. The mixture was diluted with EtOAc and washed with water and brine. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed over silica gel with *n*-hexanes−EtOAc (3:1) to give diol **15a** (92.4 mg, 0.25 mmol, 37%).

Methyl (2*S*,3*R*)-2,3-Dihydroxyeicosanoate (15a). Amorphous solid; $[\alpha]^{25}_D$ +6.0 (*c* 0.004, $CHCl_3$); IR ν_{max} (film, NaCl) 3382, 2916, 2848, 1731, 1454, 1278, 1116, 1071, 721, 495, 479 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.90 (3H, t, J = 5.5 Hz, CH_3-20), 1.10−1.30 (28H, br s, 14CH_2 (5−18)), 1.63 (3H, m, CH_2-19 and H_a-4), 1.91 (1H, d, J = 8.3 Hz, H_b-4), 3.85 (3H, s, OMe), 3.89 (1H, m, H-3), 4.12 (1H, dd, J = 1.9, 5.2 Hz, H-2); ^{13}C NMR (δ, $CDCl_3$) 14.1 (C-20), 22.7, 25.7, 29.3, 29.4, 29.5, 29.7, 31.9, 33.7 (C-(4−19)), 52.8 (OMe), 72.4 (C-3), 73.0 (C-2), 174.1 (C-1); HR-EIMS (70 eV) *m*/*z* (relative intensity) 358.3054 $[M]^+$ ($C_{21}H_{42}O_4$, calcd 358.3083) (0.1), 340 (6), 299 (6), 281 (11), 269 (3), 125 (2), 119 (2), 111 (4), 97 (8), 90 (100), 83 (9), 71 (5), 69 (8).

Methyl (2*R*,3*S*)-2,3-Dihydroxyeicosanoate (15b). Amorphous solid; $[\alpha]^{25}_D$ −4.4 (*c* 0.006, $CHCl_3$). The IR, MS, and ^1H and ^{13}C NMR data were identical to those of **15a**.

General Experimental Procedure for the Preparation of Diacetoxy Acids. To a solution of **15a** (90 mg, 0.25 mmol) in THF was added a solution of 1 M NaOH, and the mixture was stirred at 50 °C for 24 h. Then the mixture was acidified with 5% HCl, extracted with ether, washed with water, dried over $MgSO_4$, and concentrated affording acid **16a**, which was treated with acetic anhydride in pyridine. The solution was stirred for 16 h at room temperature. The product was dried under vacuo to furnish **17a**.

(2*S*,3*R*)-2,3-Diacetoxyeicosanoic Acid (17a). Amorphous solid (95 mg, 0.22 mmol, 88%); $[\alpha]^{25}_D$ +16.0 (*c* 0.002, $CHCl_3$); IR ν_{max} (film, NaCl) 3417, 3063, 2919, 2872, 1722, 1651, 1496, 1459, 1362, 1275, 1207, 1071, 1026, 737, 698 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.86 (3H, t, J = 6.8 Hz, CH_3-20), 1.10−1.30 (30H, br s, 15CH_2 (5−19)), 1.64 (1H, m, H_a-4), 1.70 (1H, m, H_b-4), 2.09 (OAc), 2.21 (OAc), 5.19 (1H, s, H-2), 5.39 (1H, br s, H-3); ^{13}C NMR (δ, $CDCl_3$) 14.1 (C-20), 20.4 (OAc), 20.8 (OAc), 22.7, 25.2, 29.2, 29.3, 29.4, 29.5, 29.7, 30.3, 31.9 (C-4 and C-19), 72.0 (C-3), 72.1 (C-2), 170.3 (OAc), 170.6 (OAc), 171.7 (C-1); HR-EIMS (70 eV) *m*/*z* (relative intensity) 428.3144 $[M]^+$ ($C_{24}H_{44}O_6$, calcd 428.3138) (0.2), 383 (10), 369 (4), 354 (3), 350 (7), 341 (11), 326 (52), 325 (4), 311 (8), 308 (72), 295 (9), 290 (10), 281 (36), 264 (8), 135 (9), 125-(11), 118 (17), 100 (100).

(2*R*,3*S*)-2,3-Diacetoxyeicosanoic Acid (17b). Amorphous solid; $[\alpha]^{25}_D$ −12.0 (*c* 0.003, $CHCl_3$). The IR, MS, and ^1H and ^{13}C NMR data were identical to those of **17a**.

Synthesis of Erythro Series of Acids. General Experimental Procedure for the Preparation of Alcohols 21a and 21b. Hexadecylphosphonium bromide (850 mg, 1.5 mmol) and anhydrous tetrahydrofuran (10 mL) were mixed in a round-bottom flask at −20 °C under argon atmosphere. *n*-Butyllithium (1.7 M in *n*-hexane, 0.93 mL, 1.5 mmol) was added dropwise to the solution. After 30 min at 0 °C, the mixture was cooled to −20 °C, followed by the addition of **20a** (80 mg, 0.5 mmol) in THF (5 mL). After 1 h at −20 °C, the mixture was allowed to warm to room temperature. The mixture was extracted with CH_2C1_2 (10 mL) and then washed with water (10 mL). The organic layers were dried over $MgSO_4$. Removal of the solvent afforded the crude olefin, which was further purified by silica gel column chromatography to yield a cis−trans mixture of the olefin (145 mg, 78%). A suspension of this alcohol (120 mg, 0.326 mmol) and Pd/C (10% w/w, 5 mg) in MeOH (10 mL) was stirred for 12 h under an atmosphere of hydrogen. Then the catalyst was filtered off and carefully rinsed with CH_2Cl_2. The combined filtrates were evaporated and the crude product was purified by flash chromatography to give compound **21a** (120 mg, 99%).

(2*S*,3*R*)-2,3-*O*-Isopropylideneicosanol (21a). Amorphous solid; $[\alpha]^{25}_D$ −6.0 (*c* 0.015, $CHCl_3$); IR ν_{max} (film, NaCl) 3443, 2919, 2852, 1464, 1375, 1246, 1217, 1044 cm^{-1}; ^1H NMR (δ, $CDCl_3$, 400 MHz) 0.84 (3H, br s), 1.22−1.30 (32H, m), 1.36 (3H, s), 1.44 (3H, s), 3.57 (1H, br s), 4.11 (1H, br s); ^{13}C NMR (δ, $CDCl_3$) 13.8, 22.4, 25.2, 26.4, 28.0, 28.6, 29.1, 29.2, 29.3, 29.4, 31.6, 61.5, 76.8, 77.8, 107.7; HR-EIMS (70 eV) *m*/*z* (relative intensity) 369.3375 $[M - H]^{+\bullet}$ ($C_{23}H_{45}O_3$, calcd 369.3369) (3), 355.3204 $[M - CH_3]^+$ ($C_{22}H_{43}O_3$, calcd 355.3212) (100), 340.2971 $[M - 2CH_3]^+$ ($C_{21}H_{40}O_3$, calcd 340.2977) (14), 339.3251 $[M - CH_2OH]^+$ ($C_{22}H_{43}O_2$, calcd 339.3263) (58).

(2*R*,3*S*)-2,3-*O*-Isopropylideneicosanol (21b). Amorphous solid; $[\alpha]^{25}_D$ +7.7 (*c* 0.012, $CHCl_3$). The IR, MS, and ^1H and ^{13}C NMR data were identical to those of **21a**.

General Experimental Procedure for the Preparation of Isopropylidene Acids. Alcohol **21a** (100 mg, 0.27 mmol) was dissolved in a mixture of $CH_3CN-CCl_4-H_2O$ (2:2:3) (0.54 mL: 0.54 mL:0.81 mL) and treated with sodium metaperiodate (202 mg, 0.945 mmol). To this biphasic solution, an amount of 4.3 mg (2.2 mol %, 5.9×10^{-3} mmol) of ruthenium trichloride hydrate was added, and the entire mixture was stirred vigorously for 2 h at room temperature. Then CH_2C1_2 (10 mL) was added, and the phases were separated. The upper aqueous phase was extracted three times with CH_2C1_2. The combined organic extracts were dried ($MgSO_4$) and concentrated. The resulting residue was diluted with ether (20 mL), filtered through a Celite pad, and concentrated. The crude product was purified by column chromatography, affording 40 mg (38%) of acid **22a**.

(2*R*,3*R*)-2,3-*O*-Isopropylideneicosanoic Acid (22a). Amorphous solid; $[\alpha]^{25}_D$ −15 (*c* 0.01, $CHCl_3$); IR ν_{max} (film, NaCl) 3421, 2916, 2849, 1700, 1462, 1410, 1297, 942, 721 cm^{-1}; ^1H NMR (δ, $CDCl_3$, 400 MHz) 0.85 (3H, m), 1.17−1.33 (28H, m), 1.33 (3H, s), 1.50 (3H, s), 4.26 (1H, br s), 4.52 (1H, br s); ^{13}C NMR (δ, $CDCl_3$) 13.8, 22.4, 29.1, 29.4, 29.5, 31.6, 77.0, 78.5, 108.7, 187.5; HR-FABMS (relative intensity) 385.3315 $[M + H]^+$ ($C_{23}H_{45}O_4^+$, calcd 385.3318), 384.3250 $[M]^+$ ($C_{23}H_{44}O_4^+$, calcd 384.3240), 383.3152 $[M - H]^+$ ($C_{23}H_{43}O_4^+$, calcd 383.3161), 369.2995 $[M - CH_3]^+$ ($C_{23}H_{41}O_4^+$, calcd 369.3005) (100), 339.3251 $[M - COOH]^+$ ($C_{22}H_{43}O_2^+$, calcd 339.3263) (23), 326 (6), 309.2804 $[M - COOH - 2CH_3]^+$ ($C_{20}H_{37}O_2^+$, calcd 309.2794) (8), 281 (8), 264 (6), 149 (11), 137 (11).

(2*S*,3*S*)-2,3-*O*-Isopropylideneicosanoic Acid (22b). Amorphous solid; $[\alpha]^{25}_D$ +18 (*c* 0.02, $CHCl_3$). The IR, MS, and ^1H and ^{13}C NMR data were identical to those of **22a**.

General Experimental Procedure for the Preparation of Amides 23a and 23b. Activation Using HOBt/EDCI. To a mixture of acid **22a** (22 mg 0.057 mmol), amine **4** (21.4 mg, 0.0128 mmol), and 1-hydroxybenzotriazole (HOBt) (23.2 mg, 0.17 mmol) in dry CH_2Cl_2 (2 mL) was added EDCI (16.4 mg, 0.086 mmol). The reaction mixture was stirred for 24 h, and then it was extracted with AcOEt. The organic layers were washed using a saturated solution of NaCl and dried over $MgSO_4$. Removal of the solvent afforded the crude product **23a**, which was further purified by silica gel column chromatography to yield 14 mg (33%) of the coupling product.

Acid Activation Using Hydroxysuccinimide. To a solution of acid **22a** (25 mg, 0.065 mmol) and *N*-hydroxysuccinimide (9 mg, 0.078 mmol) in dry CH_2Cl_2 (0.8 mL) at 0 °C was added EDCI (13 mg, 0.070 mmol) in dry CH_2Cl_2 (1.0 mL). The solution was stirred for 12 h at room temperature and then filtered, and the solvent was removed in vacuo. The remaining solid was dissolved in CH_2-Cl_2 and filtered and the solvent was removed in vacuo to give a crude product that was used after purification. Sphingosine **4** (21 mg, 0.056 mmol) was dissolved in THF (1 mL), followed by addition of Et_3N (27 μL, 2 equiv) and the solution of *N*-succinimidyl derivative (3.72 mmol) in THF. After the mixture was stirred for 15 h, the precipitate was removed by filtration. The organic layer was then concentrated under reduced pressure, and the crude product

was purified by column chromatography to give 22 mg of a colorless solid (53%).

(2*S*,3*S*,4*R*,2′*R*,3′*R*)-2-(2′,3′-*O*-Isopropylideneicosanoylamino)-1,3,4-docosanetriol (23a). Amorphous solid; $[\alpha]^{25}_D$ −11.8 (*c* 0.022, $CHCl_3$); IR ν_{max} (film, NaCl) 2917, 2850, 1658, 1529, 1467, 1216, 1081 cm^{-1}; ^1H NMR (δ, $CDCl_3$, 400 MHz) 0.87 (6H, m), 1.21−1.35 (62H, m), 1.37 (3H, s), 1.35−1.60 (2H, m), 1.60−1.80 (2H, m), 1.56 (3H, s), 3.55−3.70 (2H, m), 3.75 (1H, dd, *J* = 5.7, 11.5 Hz), 3.88 (1H, dd, *J* = 2.4, 11.5 Hz), 4.12 (1H, m), 4.36 (1H, m), 4.46 (1H, d, *J* = 7.2 Hz), 7.33 (1H, d, *J* = 8.0 Hz); ^{13}C NMR (δ, $CDCl_3$) 14.1, 22.7, 24.7, 25.7, 26.6, 27.4, 29.4, 29.7, 30.4, 31.9, 33.2, 52.6, 61.4, 72.4, 76.5, 77.6, 77.7, 109.4, 170.7; HR-FABMS (relative intensity) 740.6797 $[M + H]^+$ ($C_{49}H_{90}NO_6^+$, calcd 740.6768) (22), 682 (16), 664 (17), 356 (10), 338 (16).

(2*S*,3*S*,4*R*,2′*S*,3′*S*)-2-(2′,3′-*O*-Isopropylideneicosanoylamino)-1,3,4-docosanetriol (23b). Amorphous solid; $[\alpha]^{25}_D$ −5 (*c* 0.018, $CHCl_3$); IR ν_{max} (film, NaCl) 2917, 2849, 1660, 1530, 1468, 1371, 1214, 1066 cm^{-1}; ^1H NMR (δ, $CDCl_3$, 400 MHz) 0.88 (6H, m), 1.15−1.37 (62H, m), 1.38 (3H, s), 1.40−1.80 (4H, m), 1.55 (3H, s), 3.63 (3H, m), 3.75 (1H, dd, *J* = 5.8, 11.1 Hz), 3.93 (1H, b dd, *J* = 2.5, 11.4 Hz), 4.12 (1H, m), 4.33−4.39 (1H, m), 4.47 (1H, d, *J* = 7.4 Hz), 7.35 (1H, d, *J* = 7.7 Hz); ^{13}C NMR (δ, $CDCl_3$) 13.8, 22.4, 24.4, 25.4, 26.3, 27.1, 29.1, 29.2, 29.3, 29.42, 29.46, 30.0, 31.6, 33.0, 52.4, 61.6, 72.5, 76.0, 77.4, 77.5, 109.2, 170.4; HR-FABMS (relative intensity) 740.6732 $[M + H]^+$ ($C_{49}H_{90}NO_6^+$, calcd 740.6768) (100), 682 (32), 664 (52), 398 (45), 356 (34), 338 (26).

General Experimental Procedure for the Preparation of Pentaacetate Ceramides 24 and 25. The acetonide **23a** (13 mg, 0.017 mmol) was treated with 3% HCl−MeOH (3 mL) and stirred at room temperature for 12 h. Then the mixture was neutralized with Et_3N. After removal of the MeOH in vacuo, the obtained white solid was dissolved in pyridine (2 mL) and an excess of Ac_2O was added. After 8 h, the reaction product was extracted with CH_2Cl_2 (3 × 10 mL) and the organic layer was washed with brine, dried ($MgSO_4$), and filtered. The solvent was removed in vacuo to give a white solid, which was purified by column chromatography to yield pure **24** (10 mg, 65%).

(2*S*,3*S*,4*R*,2′*R*,3′*R*)-2-(2′,3′-Diacetoxyeicosanoylamino)-1,3,4-triacetoxydocosane (24). Amorphous solid; $[\alpha]_D$ −7.5 (*c* 0.02, $CHCl_3$). Anal. ($C_{52}H_{95}NO_{11}$) C, H. N: calcd, 10.52; found, 10.07. The IR, MS, and ^1H and ^{13}C NMR data were identical to those of **2a**.

(2*S*,3*S*,4*R*,2′*S*,3′*S*)-2-(2′,3′-Diacetoxyeicosanoylamino)-1,3,4-triacetoxydocosane (25). Amorphous solid; $[\alpha]^{25}_D$ −2.0 (*c* 0.035, $CHCl_3$); IR ν_{max} (film, NaCl) 2918, 2850, 1746, 1698, 1523, 1467, 1371, 1225, 1047 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.87 (6H, m), 1.15−1.35 (62H, m), 1.55−1.75 (4H, m), 2.04 (9H, br s), 2.08 (3H, s), 2.22 (3H, s), 4.07 (1H, dd, *J* = 3.2, 11.7 Hz), 4.20 (1H, dd, *J* = 5.6, 11.6 Hz), 4.44−4.50 (1H, m), 4.81−4.85 (1H, m), 5.04 (1H, dd, *J* = 3.6, 7.5 Hz), 5.19−5.26 (1H, m), 5.33 (1H, d *J* = 3.0 Hz), 6.76 (1H, d *J* = 9.2 Hz); ^{13}C NMR (δ, $CDCl_3$) 13.9, 20.5, 20.6, 20.8, 22.4, 25.2, 25.2, 28.4, 29.1, 29.2, 29.3, 2935, 31.7, 47.3, 62.3, 72.1, 72.5, 72.8, 73.8, 166.4, 169.2, 170.0, 170.1, 170.5, 170.8; HR-FABMS *m*/*z* (relative intensity) 932 (6), 910.6927 $[M + H]^+$ ($C_{52}H_{96}NO_{11}^+$, calcd 910.6983) (12), 850 (20), 790 (22), 320 (22). Anal. ($C_{52}H_{95}NO_{11}$) C, H. N: calcd, 10.52; found, 9.97.

General Experimental Procedure for the Preparation of Pentaacetate Ceramides 26−27. To a mixture of acid **17a** (8 mg, 0.0128 mmol), amine **4** (5.5 mg, 0.0128 mmol), and 1-hydroxybenzotriazole (HOBt) (5 mg, 0.037 mmol) in dry CH_2Cl_2 (2 mL) was added EDCI (3.68 mg, 0.0192 mmol). The reaction mixture was stirred for 48 h, and then it was extracted with AcOEt. The organic layers were washed using a saturated solution of NaCl and dried over $MgSO_4$. This residue was dissolved in pyridine. Acetic anhydride was added, and the solution was stirred for 24 h at room temperature. The product was dried in vacuo to furnish **26**.

(2*S*,3*S*,4*R*,2′*S*,3′*R*)-2-(2′,3′-Diacetoxyeicosanoylamino)-1,3,4-triacetoxydocosane (26). Amorphous solid (6.8 mg, 0.0074 mmol, 58%); $[\alpha]^{25}_D$ +8.5 (*c* 0.02, $CHCl_3$); IR ν_{max} (film, NaCl) 3285, 2918, 2850, 1746, 1698, 1469, 1372, 1220, 1044 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.87 (6H, t, *J* = 6.5 Hz, Me-22 and Me-20′), 1.20−1.40 (62H, br s, 31-CH_2), 1.60 (4H, m, CH_2-5 and CH_2-4′), 2.03 (3H, s, OAc), 2.04 (6H, s, OAc), 2.08 (3H, s, OAc), 2.27 (3H, s, OAc), 4.04 (1H, dd, *J* = 3.1, 11.6 Hz, H_a-1), 4.13 (1H, dd, *J* = 5.4, 11.7 Hz, H_b-1), 4.45 (1H, m, H-2), 4.80 (1H, dd, *J* = 6.7, 9.7 Hz, H-4), 5.05 (1H, dd, *J* = 3.4, 7.9 Hz, H-3), 5.23 (1H, d, *J* = 3.1 Hz, H-3′), 5.31 (1H, m, H-2′), 6.62 (1H, d, *J* = 9.4 Hz, N−H); ^{13}C NMR (δ, $CDCl_3$) 14.1 (C-22 and C-20′), 20.6 (2 × OAc), 20.7 (OAc), 20.9 (2 × OAc), 25.1−31.9 [C−(5−21) and C−(4′-19′)], 47.6 (C-2), 62.7 (C-1), 72.0 (C-3), 73.3 (C-3′), 72.8 (C-4), 74.0 (C-2′), 167.4 (C-1′), 169.3 (C═O), 169.8 (C═O), 170.3 (C═O), 170.7 (C═O), 171.0 (C═O); HR-EIMS (70 eV) *m*/*z* (relative intensity) $[M]^+$ (absent), 790.6172 $[M - C_5H_{11}O_3]^+$ ($C_{47}H_{84}NO_8^+$, calcd 790.6197) (83), 776 (42), 747 (36), 730 (26), 687 (13), 669 (26); 656 (27), 627 (18), 599 (11), 512.3546 ($C_{28}H_{50}NO_7^+$, calcd 512.3587) (86), 484.3729 ($C_{28}H_{52}O_6^+$, calcd 484.3764) (75), 452 (44), 428 (24), 424 (19), 411.3085 ($C_{24}H_{43}O_5^+$, calcd 411.3110) (46), 392 (100), 383 (33), 369 (42), 364 (12), 341 (39), 320 (20). Anal. ($C_{52}H_{95}NO_{11}$) C, H. N: calcd, 10.52; found, 11.0.

(2*S*,3*S*,4*R*,2′*R*,3′*S*)-2-(2′,3′-Diacetoxyeicosanoylamino)-1,3,4-triacetoxydocosane (27). Amorphous solid (7.1 mg, 0.0074 mmol, 44%); $[\alpha]^{25}_D$ +15.3 (*c* 0.004, $CHCl_3$); IR ν_{max} (film, NaCl) 3286, 2917, 2852, 1744, 1699, 1470, 1370, 1220, 1044 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.86 (6H, t, *J* = 6.5 Hz, Me-22 and Me-20′), 1.20−1.40 (62H, br s, 31-CH_2), 1.59 (4H, m, CH_2-5 and CH_2-4′), 2.00 (3H, s, OAc), 2.06 (3H, s, OAc), 2.07 (3H, s, OAc), 2.08 (3H, s, OAc), 2.27 (3H, s, OAc), 4.01 (1H, dd, *J* = 3.6, 11.7 Hz, H_a-1), 4.33 (1H, dd, *J* = 7.1, 11.7 Hz, H_b-1), 4.46 (1H, m, H-2), 4.95 (1H, td, *J* = 3.4, 7.5 Hz, H-4), 5.01 (1H, dd, *J* = 4.3, 6.0 Hz, H-3), 5.24 (1H, d, *J* = 3.6 Hz, H-3′), 5.33 (1H, m, H-2′), 6.68 (1H, d, *J* = 9.1 Hz, N−H); ^{13}C NMR (δ, $CDCl_3$) 14.1 (C-22 and C-20′), 20.6 (2OAc), 20.7 (OAc), 20.8 (OAc), 21.0 (OAc), 25.1−31.9 [C-(5−21) and C-(4′−19′)], 48.4 (C-2), 62.1 (C-1), 72.3 (C-3), 72.6 (C-3′), 72.9 (C-4), 73.7 (C-2′), 167.4 (C-1′), 169.3 (C═O), 169.7 (C═O), 170.3 (C═O), 170.6 (C═O), 171.1 (C═O); EIMS (70 eV) *m*/*z* (relative intensity) $[M]^+$ (absent), 792 (14), 790 (84), 777 (22), 776 (41), 748 (21), 747 (36), 730 (26), 729 (23), 716 (7), 687 (12), 669 (23); 657 (12), 599 (12), 424 (19), 411 (46), 392 (100), 383 (33), 369 (43). Anal. ($C_{52}H_{95}NO_{11}$) C, H, N.

Cell Culture. Human SK-MEL-1 melanoma cells (DSMZ No. ACC 303, DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were grown in RPMI 1640 (Sigma) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Sigma) and 100 units/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. The cell numbers were counted by a hematocytometer, and the viability was always greater than 95% in all experiments as assayed by the 0.025% trypan blue exclusion method. Stock solutions of 10 mM ceramides were made in ethanol/dodecane (49:1), and aliquots were frozen at −20 °C. Further dilutions were made in culture media just before use. In all experiments, the final concentration of ethanol/dodecane (49:1) did not exceed 0.5% (v/v), a concentration that is nontoxic to the cells. Cells were resuspended in fresh medium 24 h before each treatment.

Assay for Growth Inhibition and Cell Viability. The cytotoxicity of ceramides was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay.[23] Briefly, 1 × 10^4 exponentially growing cells were seeded in 96-well microculture plates with various ceramide concentrations in a volume of 100 μL for 72 h at 37 °C. Controls were always treated with the same amount of ethanol−dodecane (49:1) as used in the corresponding experiments. Surviving cells were detected on the basis of their ability to metabolize 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) into formazan crystals. Optical density at 570 nm was used as a measure of cell viability. Cell survival was calculated as the fraction of cells alive relative to control for each point:

$$\text{cell survival (\%)} = \frac{\text{mean absorbance in treated cells}}{\text{mean absorbance in control wells}} \times 100$$

Concentrations inducing a 50% inhibition of cell growth (IC_{50}) were determined graphically for each experiment using the curve-fitting

routine of the computer software Prism 2.0 (GraphPad) and the equation derived by DeLean et al. (1978).[24]

Western Blot for PARP. Cells were treated with ceramides **1a** and **24**−**27**. After different times cells were pelleted by centrifugation and washed with ice-cold phosphate-buffered saline. Western blot analysis for PARP was performed as described (Rivero et al., 2003).[25]

Analysis of DNA Fragmentation. The presence of apoptosis was evaluated by agarose gel electrophoresis of DNA extracted from SK-MEL-1 melanoma cells. Briefly, cells (4×10^5) were washed with phosphate-buffered saline and incubated in 20 μL of 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.5% SDS, and 1 μg/μL RNase A (Sigma) at 37 °C for 1 h. Then 10 μg/μL proteinase K (Sigma) (2 μL) was added, and the mixture was incubated at 50 °C for 2 h more. DNA was extracted with 100 μL of phenol−chloroform−isoamyl alcohol and mixed with 5 μL of loading buffer. Samples were separated by electrophoresis at 40 V for 4 h through a 2% agarose gel in TAE buffer (40 mM Tris-acetate and 1.0 mM EDTA, pH 8.3). DNA bands were visualized under UV light after staining with ethidium bromide (0.5 μg/mL), and the images were captured by a digital camera (Kodak).

Acknowledgment. This work was supported in part by the Instituto Canario de Investigación del Cáncer (Grant G-05-09 to J.B. and F.L.), Programa de Iniciativa Comunitaria INTERREG IIIB Açores-Madeira-Canarias (Grant 04/MAC/3.5/C5), the Ministerio de Educación y Ciencia of Spain, and FEDER (Grant SAF 2004-07928 to F.E.), and the Dirección General de Universidades e Investigación of the Canary Islands Government (Grants GRUP-2004/44 and INFRA 2004:59 to F.E. and F.T. for a research studenship).

Supporting Information Available: ^{1}H NMR spectra of compounds **1a**, **2a**, and **24**−**27** and enlargement of the ^{1}H NMR spectra of compounds **1a** and **24**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bratton, S. B.; Walker, G.; Srinivasula, S. M.; Sun, X. M.; Butterworth, M.; Alnemri, E. S.; Cohen, G. M. Recruitment, activation and retention of caspases-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes. *EMBO J.* **2001**, *20*, 998−1009.

(2) (a) Cohen, G. M. Caspases: the executioners of apoptosis. *Biochem. J.* **1997**, *326*, 1−16. (b) Earnshaw, W. C.; Martins, L. M.; Kaufmann, S. H. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu. Rev. Biochem.* **1999**, *68*, 383−424.

(3) (a) Perry, D. K.; Hannun, Y. A. The role of ceramide in cell signalling. *Biochim. Biophys. Acta* **1998**, *1436*, 233−243. (b) Andrieu-Abadie, N.; Gouazé, V.; Salvayre, R.; Levade, T. Ceramide in apoptosis signaling: relationship with oxidative stress. *Free Radical Biol. Med.* **2001**, *31*, 717−728. (c) Mimeault, M. New advances on structural and biological functions of ceramide in apoptotic/necrotic cell death and cancer. *FEBS Lett.* **2002**, *530*, 9−16. (d) Reynolds, C. P.; Maurer, B. J.; Kolesnick, R. N. Ceramide synthesis and metabolism as a target for cancer therapy. *Cancer Lett.* **2004**, *206*, 169−180.

(4) Luberto, C.; Kraveka, J. M.; Hannun, Y. A. Ceramide regulation of apoptosis versus differentiation: A walk on a fine line. Lessons from neurobiology. *Neurochem. Res.* **2002**, *27*, 609−617.

(5) Kolesnick, R.; Fuks, Z. Radiation and ceramide-induced apoptosis. *Oncogene* **2003**, *22*, 5897−5906.

(6) Gulbins, E.; Kolesnick, R. Raft ceramide in molecular medicine. *Oncogene* **2003**, *22*, 7070−7077.

(7) Birbes, H.; Luberto, C.; Hsu, Y. T.; El Bawab, S.; Hannun, Y. A.; Obeid, L. M. A mitochondrial pool of sphingomyelin is involved in TNFα-induced Bax translocation to mitochondria. *Biochem. J.* **2005**, *386*, 445−451.

(8) Anishkin, A.; Sukharev, S.; Colombini, M. Searching for the molecular arrangement of transmembrane ceramide channels. *Biophys. J.* **2006**, *90*, 2414−2426.

(9) (a) Thon, L.; Mohlig, H.; Mathieu, S.; Lange, A.; Bulanova, E.; Winoto-Morbach, S.; Schutze, S.; Bulfone-Paus, S.; Adam, D. Ceramide mediates caspase-independent programmed cell death. *FASEB J.* **2005**, *19*, 1945−1956. (b) Rotolo, J. A.; Zhang, J.; Donepudi, M.; Lee, H.; Fuks, Z.; Kolesnick, R. Caspase-dependent and -independent activation of acid sphingomyelinase signaling. *J. Biol. Chem.* **2005**, *280*, 26425−34.

(10) González, A. G.; León, F.; Rivera, A.; Muñoz, C. M.; Bermejo, J. Lanostanoids triterpenes from *Ganoderma lucidum. J. Nat. Prod.* **1999**, *62*, 1700−1701.

(11) González, A. G.; León, F.; Rivera, A.; Padrón, J. I.; González-Plata, J.; Zuluaga, J.; Quintana, J.; Estévez, F.; Bermejo, J. New lanostanoids from *Ganoderma concinna. J. Nat. Prod.* **2002**, *65*, 417−421.

(12) León, F.; Valencia, M.; Rivera, A.; Nieto, I.; Quintana, J.; Estévez, F.; Bermejo, J. Novel cytostatic lanostanoid triterpenes from *Ganoderma australe. Helv. Chim. Acta* **2003**, *86*, 3088−3095.

(13) León, F.; Quintana, J.; Rivera, A.; Estévez, F.; Bermejo, J. Isolation of lanostane triterpenes from *Laetiporus sulphureus* that induce apoptosis in human myeloid leukemia cells. *J. Nat. Prod.* **2004**, *67*, 2008−2011.

(14) Setliff, E. C.; Ryvarden, L. Los hongos de Colombia VII: Some aphyllophoraceous wood-inhabiting fungi. *Mycotaxon* **1983**, *28*, 509−525.

(15) Zjawiony, J. K. Biologically active compounds from aphyllophorales (polypore) fungi. *J. Nat. Prod.* **2004**, *67*, 300−310.

(16) Lourenço, A.; Lobo, A. M.; Rodríguez, B.; Jimeno, M. L. Ceramides from the fungus *Phellinus pini. Phytochemistry* **1996**, *43*, 617−620.

(17) (a) Zhan, Z. J.; Yue, J. M. New glycosphingolipids from the fungus *Catathelasma ventricosa. J. Nat. Prod.* **2003**, *66*, 1013−1016. (b) Gao, J. M.; Zhang, A. L.; Zhang, C. L.; Liu, J. K. Paxillamide: A novel phytosphingosine derivative from the fruiting of *Paxillus panuoides. Helv. Chim. Acta* **2004**, *87*, 1483−1487.

(18) Asai, N.; Fusetani, N.; Matsunaga, S. Sex pheromones of the hair crab *Erimacrus isenbeckii.* II. Synthesis of ceramides. *J. Nat. Prod.* **2001**, *64*, 1210−1215.

(19) Gelas, J.; Horton, D. Acetonation of D-ribose and D-arabinose with alkyl isopropenyl ethers. *Carbohydr. Res.* **1975**, *45*, 181−195.

(20) Fokina, N. A.; Kornilov, A. M.; Kulik, I. B.; Kukhar, V. P. Towards optically pure mono- and difluorinated amino acids: common methodology based on (*R*)-2,3-*O*-isopropylideneglyceraldehyde. *Synthesis* **2002**, *17*, 2589−2596.

(21) Chang, Y. T.; Choi, J.; Ding, S.; Prieschl, E. E.; Baumruker, T.; Lee, J. M.; Chung, S. K.; Schultz, P. G. The synthesis and biological characterization of a ceramide library. *J. Am. Chem. Soc.* **2002**, *124*, 1856−1857.

(22) Duclos, R. I., Jr. The total syntheses of D-*erythro*-sphingosine, *N*-palmitoylshingosine (ceramide), and glucosylceramide (cerebroside) via an azidosphingosine analog. *Chem. Phys. Lipids* **2001**, *111*, 111−138.

(23) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.

(24) DeLean, A.; Munson, P. J.; Rodbard, D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves. *Am. J. Physiol.* **1978**, *235*, E97−E102.

(25) Rivero, A.; Quintana, J.; Eiroa, J. L.; López, M.; Triana, J.; Bemejo, J.; Estévez, F. Potent induction of apoptosis by germacranolide sesquiterpene lactones on human myeloid leukemia cells. *Eur. J. Pharmacol.* **2003**, *482*, 77−84.

JM0605334