Enantioselective Synthesis of a Novel Trans Double Bond Ceramide Analogue via Catalytic Asymmetric Dihydroxylation of an Enyne. The Role of the Trans Double Bond of Ceramide in the Fusion of Semliki Forest Virus with Target Membranes

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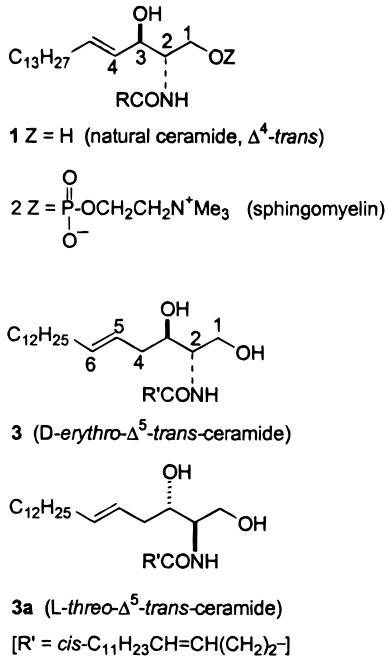
Abstract: Intensive interest is currently focused on the role of ceramide (**1**), a key lipid molecule that functions as a second messenger. The first asymmetric synthesis of a D-*erythro*-ceramide analogue that contains a C(5)−C(6) trans double bond (**3**), together with its biological evaluation in a viral−liposome fusion system, is described. Sharpless asymmetric dihydroxylation of 4′-methoxyphenyl *trans*-5-octadecyn-2-enyl ether (enyne **8a**), prepared by the coupling reaction of 1-[(*E*)-(4′-bromo-2′-butenyl)oxy]-4-methoxybenzene (**7**) with lithium tetradecyne, generated yne-diol **9** in 96% yield with the desired stereochemistry at the C(2) and C(3) positions and high enantiomeric purity (95% enantiomeric excess). Birch reduction (Li/$EtNH_2$) of yne-diol **9** furnished (2*R*,3*R*,5*E*)-octadecene-1,2,3-triol (**10**) stereospecifically. The latter was converted to 2-azido derivative **13** in three steps (via a 2-*O*-triflate-1,3-*O*-benzylidene intermediate) and 55% overall yield. Reduction of azide **13** and in situ *N*-acylation with *p*-nitrophenyl *cis*-hexadec-4-enoate provided D-*erythro*-Δ^5-*trans*-ceramide (**3**) in 91% yield. The role of the trans double bond of ceramide in mediating fusion of an alphavirus (Semliki Forest virus) was assessed in a liposomal model system, using target phospholipid/cholesterol vesicles containing either D-*erythro*-ceramide **1** or **3**. The kinetics of virus fusion, as monitored by a change in pyrene excimer fluorescence over a period of 60 s, showed that Δ^5-*trans*-ceramide **3** was completely inactive, indicating that there is an absolute requirement for the trans double bond to be located between C(4) and C(5). These data indicate that the molecular determinants on the viral envelope glycoprotein are highly specific for recognition of the unsaturated site in the ceramide molecule.

Introduction

Sphingolipid messengers have been implicated in the regulation of a myriad of physiological events. Recent interest has focused on ceramide (*N*-acylsphingosine, **1**, Chart 1), a long-chain aliphatic 2-amido-1,3-diol with a C(4)−C(5) trans double bond; the long-chain sphingoid base predominant in mammalian cells has 18 carbons.[2] Rapid increases in the intracellular availability of ceramide are elicited by diverse stimuli, ranging from the activation of cytotoxic receptor systems (such as those induced by the binding of inflammatory cytokines) or the exposure to environmental stresses (such as ionizing radiation, heat shock, and oxidative trauma) to the response to antineoplastic agents. At least two distinct mechanisms for ceramide generation have been characterized: (a) by hydrolysis of sphingomyelin (*N*-acylsphingosine-1-phosphocholine, SM, **2**, Chart 1) catalyzed by sphingomyelinase and (b) by de novo biosynthesis from sphingoid bases catalyzed by ceramide synthase. Ceramide arising from either pathway participates in intracellular signaling, mediating critical aspects of cell survival such as the initiation of differentiation or apoptosis (programmed cell death) and suppression of cell growth.[3] These regulatory processes appear to be mediated through the actions of several ceramide-sensitive protein kinases and protein phosphatases.[4]

Chart 1



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Ceramide (in the form of at least six structurally heterogeneous types of *N*-acylated sphingosines) is also the principal lipid component of human skin, comprising about 35% of the total lipids in human stratum corneum (the apical skin layer).[5] Ceramides play an essential role in normal skin tissue function, maintaining the permeability barrier and water-binding properties of the outer layer.[6] Ceramide is also present as a building block of SM and glycosphingolipids in the membranes of mammalian cells.

Recently, it has been found that sphingolipids act as a target membrane cofactor in the fusion of Semliki Forest virus (SFV), ceramide representing the sphingolipid minimally required.[7] The molecular basis for SFV's specificity for sphingolipids has not yet been established. It is clear, however, that a stereospecific recognition is involved between the sphingolipid and protein(s) present in the viral envelope, since only D-*erythro*-ceramide (2*S*,3*R*, **1**) (the stereoisomer that occurs naturally in mammalian systems) supported fusion of SFV, whereas the three unnatural stereoisomers of ceramide were inactive.[8] Since the four diastereomeric ceramides are expected to possess similar physical properties in membranes, and since only ~1.5 mol % of **1** or **2** is needed to obtain half-maximal fusion,[7] it was concluded that 2*S*,3*R*,4*E*-sphingolipids have a cofactor-type role in mediating the viral fusion process. Although a specific binding site for ceramide on the viral envelope glycoprotein has not yet been identified, studies with synthetic analogues of ceramide have shown that the following molecular features are critical in the headgroup portion of the ceramide molecule for retention of SFV fusion activity: carbon 1 may bear a hydroxy group or a phosphocholine or carbohydrate moiety;[7a] the length of the amide chain at carbon 2 may be varied from 8 to 18 carbons without significant change in activity, but deletion of the carboxamide moiety abolishes fusion activity entirely;[7a] carbon 3 must bear a hydroxy group,[7b,9] since 3-deoxyceramide and 3-methoxyceramide do not induce fusion; and carbons 4 and 5 must be part of a π bond, since fusion activity is lost when the double bond is hydrogenated[9] but is retained when the trans double bond of **1** is converted into a triple bond.[10]

Reports that 4,5-dihydroceramide generally has a significantly lower activity than **1** with respect to several biological processes (such as apoptosis[11] and metabolic conversion into glycosphingolipids and intracellular transport in several cell lines[12]) suggested that the C(4)−C(5) trans double bond in the sphingoid base of **1** may be essential for ceramide's capacity to modulate various fundamental biological functions. However, the biological inactivity of dihydroceramide may not reflect a specific structural feature, since the 4,5-dihydro analogue of **1** is expected to have a higher molecular flexibility and a perturbed geometry relative to that of an analogue in which the trans double bond is maintained. Moreover, we are intrigued to find that several receptor and enzyme systems that bind to lipids have been found to be dependent on the presence of a specific double bond in the ligand or substrate molecule. For example, a 9-cis double bond is required in synthetic analogues of the sleep-inducing lipid oleamide for binding to the 5-HT_{2A} serotonin receptor;[13] dihydro analogues of *all-trans*-retinoic acid display modified binding to retinoid receptors;[14] modification of the unsaturation of leukotriene B4 reduces binding to the LTB4 receptor;[15] and double-bond regioisomers of 15,32-substituted lanost-8-en-3-ol display varying degrees of inhibition of the enzyme lanosterol 14α-methyl demethylase.[16] Therefore, we planned to prepare a series of ceramide analogues in which the trans double bond is moved along the sphingolipid backbone rather than removed altogether. A goal of the present work was to determine whether the position of the trans double bond of Δ^4-**1** is important for the ceramide-mediated fusion of SFV with cholesterol-containing phospholipid vesicles. Our first synthetic target is D-*erythro*-Δ^5-*trans*-ceramide (**3**, Chart 1), a positional isomer of **1** in which the 3-hydroxy group is homoallylic with respect to the double bond (rather than allylic, as in the natural compound **1**) and the natural stereochemistry is retained.

Results and Discussion

Outline of the Synthetic Plan. Here we report the first enantioselective total synthesis of a regioisomer of **1**, D-*erythro*-(5*E*)-ceramide (**3**), starting from commercially available and inexpensive methyl 4-bromocrotonate (**4**).[17] Crotonate **4** was readily converted to 1-[(*E*)-(4′-bromo-2′-butenyl)oxy]-4-methoxybenzene (**7**) in three steps (Scheme 1). Coupling of allylic bromide **7** with lithium tetradecyne gave *E*-enyne **8a** (Scheme 2). Asymmetric dihydroxylation of the trans double bond in the presence of $(DHDQ)_2$-PHAL (AD-mix-β) provided yne-diol **9** with the necessary stereochemistry at the C(2) and C(3) positions

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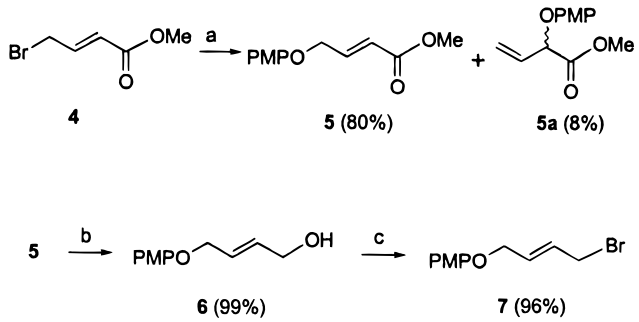
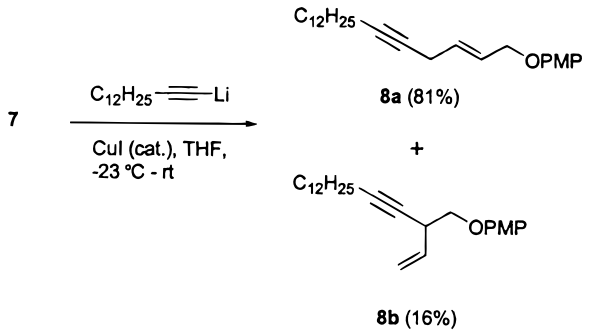
Scheme 1. Synthesis of 1-[(*E*)-(4′-Bromo-2′-butenyl)oxy]-4-methoxybenzene (**7**)[a]



[a] Reagents: (a) *p*-$MeOC_6H_4OH$, K_2CO_3, $(CH_3)_2CO$, (catalytic) 18-crown-6, room temperature. (b) DIBAL-H, THF, −78 °C. (c) Ph_3P, NBS, CH_2Cl_2, −23 °C to room temperature.

Scheme 2. Coupling of Allylic Bromide **7** with Lithium Tetradecyne

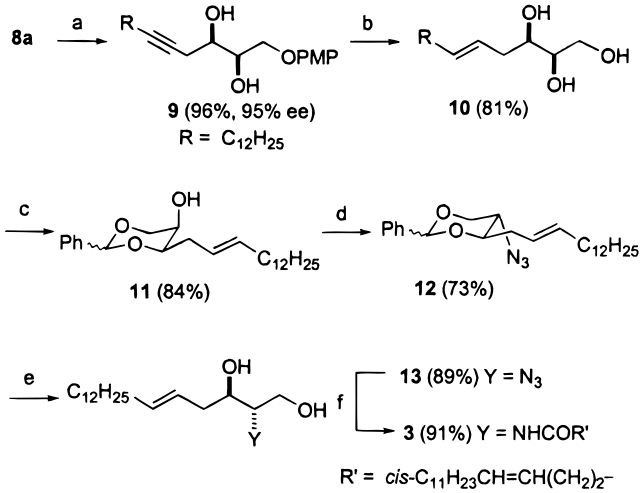


(Scheme 3). ^1H NMR analysis (Figure 1) of the bis-Mosher ester derived from diol **9** indicated that the dihydroxylation reaction gave **9** in high enantiomeric purity (95% ee). Birch reduction of yne-diol **9** with Li in $EtNH_2$ afforded (2*R*,3*R*)-*trans*-5-ene-triol **10**. Selective protection of the 1- and 3-hydroxy groups, followed by activation of the 2-hydroxy group as a triflate, inversion by azide substitution, then deprotection, azide reduction, and in situ *N*-acylation, afforded ceramide analogue **3**. The overall yield for the 11 steps from bromocrotonate **4** to product **3** was 24%. This synthetic route also permits access to L-*erythro*-(5*E*)-ceramide (**3a**, Chart 1) via asymmetric dihydroxylation of **8a** with $(DHQ)_2$-PHAL (AD-mix-α).

Ceramide analogue **3** was designed to have a minimal positional change of the double bond with respect to naturally occurring **1**. However, it is noteworthy that the asymmetric dihydroxylation approach described here is well suited for the preparation of other analogues having the trans double bond at different positions along the sphingolipid backbone.

Preparation of Allylic Bromide 7. As shown in Scheme 1, 1-[(*E*)-(4′-bromo-2′-butenyl)oxy]-4-methoxybenzene (**7**) was prepared from methyl 4-bromocrotonate (**4**) by the following three steps. Bromide displacement of crotonate **4** with *p*-methoxyphenol in the presence of K_2CO_3 and a catalytic amount of phase-transfer catalyst, 18-crown-6, in dry Me_2CO at room temperature provided methyl 4-(4′-methoxyphenyloxy)crotonate (**5**)[18] in 80% yield, together with the S_N2' regioisomer **5a** in 8% yield. After methyl ester **5** was isolated either by silica gel chromatography or by recrystallization in hexane/EtOAc (15:1), DIBAL-H reduction in THF for 1 h at −78 °C furnished the corresponding allylic alcohol **6** in almost quantitative yield.

Scheme 3. Synthesis of D-*erythro*-Δ^5-Ceramide (**3**)[a]



[a] Reagents: (a) (i) AD-mix-*β*, $CH_3SO_2NH_2$, *t*-BuOH/H_2O 1:1, 0 °C; (ii) Na_2SO_3, room temperature; (b) Li/$EtNH_2$, THF, −78 °C; (c) PhCHO, *p*-TsOH·H_2O, CH_2Cl_2, room temperature; (d) (i) $(CF_3SO_2)_2O$, py, CH_2Cl_2, −78 °C, 2 h; (ii) NaN_3, DMF, −78 °C to room temperature; (f) *p*-TsOH·H_2O, MeOH, ethylene glycol, room temperature; (g) Ph_3P, *p*-nitrophenyl *cis*-4-hexadecenoate, THF/H_2O (9:1), room temperature.

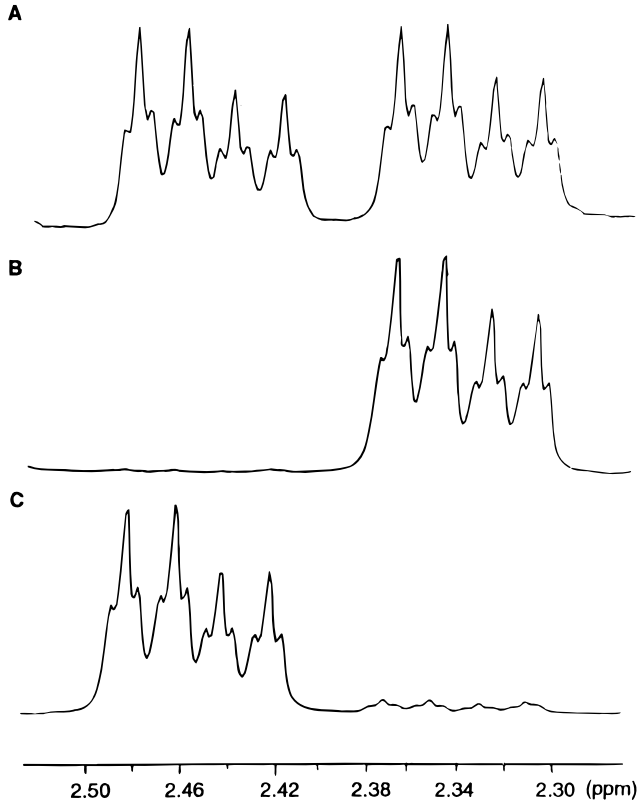


Figure 1. Partial ^1H NMR spectra of bis-(*R*)-(+)-MTPA ester of **9**: (A) bis-Mosher esters derived from 1:1 mixture of enantiomers of **9**; (B) bis-Mosher ester of **9** prepared by AD-mix-*β*; (C) bis-Mosher ester of the enantiomer of **9** prepared by AD-mix-α.

(17) To the best of our knowledge, no syntheses of regioisomers of ceramide (**1**) have yet been reported. For a review of chiral syntheses of sphingosine and its stereoisomers from chiral starting materials (such as L-serine derivatives, carbohydrates, tartaric acid, and cyclohexadiene-*cis*-1,2-diol) or via enantioselective reactions (such as aldol condensation and Sharpless epoxidation), see: (a) Byun, H.-S.; Bittman, R. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, NY, 1993; pp 97−140. (b) Nugent, T. C.; Hudlicky, T. *J. Org. Chem.* **1998**, *63*, 510−520. (c) Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998**, 1075−1091. (d) Jung, K.-H.; Schmidt, R. R. In *Lipid Synthesis and Manufacture*; Gunstone, F. D., Ed.; Sheffield Academic Press: Sheffield, U.K.; CRC Press: Boca Raton, FL, 1999; pp 208−249.

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Treatment of alcohol **6** with Ph_3P-NBS in CH_2Cl_2 afforded allylic bromide **7** in 96% yield.

Coupling of 1-Tetradecyne with Allylic Bromide 7. Our original efforts focused on the direct coupling of 1-tetradecyne with methyl 4-bromocrotonate (**4**) via an organocuprate intermediate. Although coupling reactions of allylic halides with terminal alkynes are well documented via Cu(I) catalysis,[19] it seemed that the reactivities of allylic halides conjugated with an ester (e.g., **4**) were so poor that the coupling reaction did not proceed,[20] even in the presence of cocatalysts such as palladium, zinc, or manganese and in different solvents such as THF, HMPA, or DMF. However, coupling of lithium tetradecyne with allylic bromide **7** in THF in the presence of a catalytic amount of CuI proceeded smoothly and rapidly at room temperature (the same coupling reaction could be completed within 1 h, even at −23 °C) to give enyne **8a** in 81% yield and the regioisomer **8b** in 16% yield (Scheme 2). A cosolvent such as HMPA was not required. Lowering the reaction temperature retarded the reaction rate, but the ratio of product to S_N2' isomer was not improved. Excess lithium tetradecyne (2.5 equiv, based on allylic bromide **7**) was required for the completion of the coupling reaction.

Asymmetric Dihydroxylation of Enyne 8a. Asymmetric dihydroxylation of enyne **8a** on a 1-mmol scale by typical Sharpless procedures[21] provided the desired diol **9** in 96% yield after 24 h of reaction. However, on a larger scale (e.g., 15 mmol), the reaction proceeded very sluggishly, even when much larger volumes of solvents were used, probably because of the poor solubility of the starting material in aqueous *tert*-butyl alcohol at 0 °C. We found that, by dissolving the starting material in Et_2O and then transferring it to the reaction pot, followed by evaporation of the ether, **8a** was converted into a fine powder, which accelerated the reaction.

Evaluation of Chiral Purity of Diol 9. To determine the chiral purity of diol **9**, both enantiomers of diol **9** were prepared by asymmetric dihydroxylation of **8a** by using either AD-mix-β or AD-mix-α according to the procedure described above; then the enantiomers were converted to the corresponding bis-Mosher esters, and the resulting diastereomeric ratio was analyzed by 400-MHz ^{1}H NMR. Figure 1A shows the partial ^{1}H NMR spectrum (δ 2.27−2.53) of the bis-Mosher esters derived from a 1:1 mixture of the enantiomers of diol **9**. Figure 1B,C illustrates the ^{1}H NMR spectra of the bis-Mosher esters of diols prepared by AD-mix-β and -α, respectively. The double double triplet (ddt) between δ 2.30−2.39 is assigned to one of the C(4) protons in the bis-Mosher ester of diol **9** (Figure 1B), whereas the ddt between δ 2.40−2.50 arises from one of the C(4) protons in the bis-Mosher ester derived from the enantiomer of **9** (Figure 1C). A clear baseline separation for those two groups of signals was obtained (Figure 1B,C). The integration of each ddt in Figure 1B on an expanded scale indicated an enantiomeric purity of 95% ee for diol **9**. On the other hand, the enantiomer of **9** (produced by use of AD-mix-α) had 89% ee (Figure 1C).[22]

Synthesis of D-*erythro*-(5*E*)-Ceramide (3). As shown in Scheme 3, the synthesis of ceramide analogue **3** from yne-diol **9** was completed in a stereospecific fashion by dissolving-metal reduction, followed by selective introduction of an amide functionality to the C(2) position. The reduction by Li in $EtNH_2$ at −78 °C not only generated the necessary trans geometry of the double bond but also removed the PMP protecting group.[23] Low temperature was required for reduction, since at 16 °C (bp of $EtNH_2$) the generated double bond is susceptible to reduction by Li/$EtNH_2$.[24]

To introduce an amide group to the C(2) position of (2*R*,3*R*)-*trans*-5-octadecene-1,2,3-triol (**10**), the hydroxy groups at positions C(1) and C(3) must be masked first. Thus, triol **10** was reacted with benzaldehyde in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O). However, the formation of the desired product, 1,3-acetal **11**, faced competing side reactions, such as formation of the 1,2-*O*-benzylidene and 2,3-*O*-benzylidene derivatives. The problem was overcome by isolating product **11** and allowing the two byproducts to reequilibrate with the 1,3-*O*-benzylidene derivative in the presence of *p*-TsOH·H_2O and benzaldehyde. A yield of 84% of the desired 1,3-acetal **11** in two runs was obtained.

Ohashi et al.[25] reported that (2*R*,3*R*)-1,3-*O*-benzylidene-(4*E*)-octadecene-1,2,3-triol, the Δ^4-regioisomer of **11**, could be converted to its 2-azido derivative via a mesylate intermediate in 64% yield. We prepared the mesylate of 1,3-acetal **11** in almost quantitative yield but found that the subsequent azide displacement reaction (e.g., NaN_3, DMF, 93 °C) was troublesome, because azido acetal **12** was unstable at this temperature. Lowering the reaction temperature or using $(PhO)_2P(O)N_3$, $Zn(N_3)_2$, or LiN_3 in the presence of a phase-transfer catalyst was also unsuccessful. Finally, we found that activation of the 2-hydroxy group of **11** as the 2-*O*-triflate followed by in situ substitution with NaN_3 (DMF, −78 °C to room temperature) provided azido acetal **12** in 73% yield.

The benzylidene protecting group was removed by using a catalytic amount of *p*-TsOH·H_2O in MeOH in the presence of ethylene glycol at room temperature. Staudinger reduction of 2-azido-1,3-diol **13** with Ph_3P in aqueous THF followed by in situ *N*-acylation with *p*-nitrophenyl *cis*-4-hexadecenoate[26] proceeded smoothly at room temperature and delivered the target ceramide analogue **3** in 91% yield.

Kinetics of SFV Fusion with Liposomes Prepared with 1 or 3. SFV is an enveloped positive-strand RNA virus, belonging to the genus *Alphavirus* of the family of the Togaviridae. The virus enters cells through receptor-mediated endocytosis, which directs the virions to the endosomal compartment of the cell.[27] Fusion of the viral envelope with the endosomal membrane requires the mildly acidic pH that is present in the lumen of the endosome. Through fusion, the viral genome gains access to the cell cytosol. A molecular model of SFV fusion with target membranes is lacking; nevertheless, a considerable amount of information is available concerning the key properties required for the fusion process. It is mediated by the E1/E2 heterodimeric

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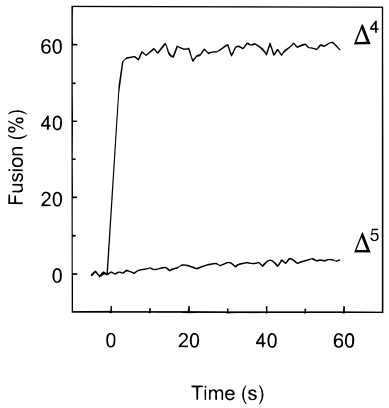

Figure 2. Effects of ceramide **1** and **3** on the fusion of pyrene-labeled SFV with PC/PE/Chol/Cer liposomes. On-line fusion measurements were performed at 37 °C and pH 5.5, as described in the Experimental Section. Fusion was recorded as a *decrease* of the pyrene excimer fluorescence. Liposomes consisted of PC/PE/Chol (molar ratio, 1.0: 0.65:1.0), supplemented with 10 mol % of **1** (Δ^4-ceramide) or **3** (Δ^5-ceramide).

envelope glycoprotein of the virus.[28] At low pH, this protein undergoes a series of conformational changes,[29] resulting in the formation of a homotrimeric structure of E1, which presumably represents the fusion competent conformation of the viral spike.[30] The low-pH-induced fusion of SFV in a liposomal model system requires the presence of both cholesterol[30,31] and sphingolipid[7] in the target liposomes. Cholesterol is essential for the low-pH-dependent irreversible binding of the virus to the liposomes. On the other hand, the sphingolipid appears to be directly involved in the subsequent fusion reaction,[7a] probably as a cofactor inducing a conformational change in the viral envelope glycoprotein essential for fusion.

To evaluate the capacity of ceramides **1** and **3** to support fusion of SFV with target membranes, liposomes were prepared consisting of phosphatidylcholine (PC), phosphatidylethanolamine (PE), cholesterol (Chol), and ceramide **1** or **3** (molar ratio, 1.0:0.65:0.35:1.0). These liposomes were used as target membrane vesicles for fusion of pyrene-labeled SFV, essentially as described before.[7a,30] As shown in Figure 2, the natural Δ^4-ceramide **1** supported efficient fusion of the virus with the liposomes at pH 5.5, whereas Δ^5-ceramide **3** was completely inactive. This demonstrates conclusively that the location of the trans double bond in the sphingosine backbone is critical for the fusion-supporting capacity of ceramide.

Conclusions

We present an efficient and enantioselective synthesis of **3**, a new derivative of the biologically important ceramide **1**, in 24% overall yield. The synthesis proceeds by asymmetric dihydroxylation of enyne **8a**, followed by dissolving-metal reduction of PMP yne-diol **9**, and introduction of the azido group at C(2) via the triflate of **11**. Placement of the trans double bond at the adjacent position in the sphingoid base gives a novel analogue for investigating the role of the Δ^4-trans double bond in a variety of sphingolipid-regulated processes. We provide herein evidence that the C(4)−C(5)-trans double bond plays a *critical* role in the ceramide-mediated fusion of SFV with target membranes.

Experimental Section

General Information. See ref 23 for general experimental protocols. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz on a Bruker spectrometer, respectively, and were referenced to the residual $CHCl_3$ at δ 7.24 (^{1}H) and δ 77.00 ppm (^{13}C). Deuterated chloroform ($CDCl_3$) was used as the only solvent for all of the NMR analyses. (*R*)-(−)-α-Methoxy-α-trifluoromethylphenyl acetic acid chloride was purchased from Sigma-Aldrich. Melting points are reported without correction.

Preparation of SFV and Liposomes. Pyrene-labeled SFV was purified from the medium of infected baby hamster kidney (BHK-21) cells, cultured beforehand in the presence of the fluorescent fatty acid 16-(1-pyrenyl)hexadecanoic acid (Molecular Probes, Eugene, OR), essentially as described before.[30,32] The fatty acid is biosynthetically incorporated into the cellular membrane lipids. Thus, virus particles budding from these cells contain pyrene-labeled phospholipids in their envelopes.[7a,9,30,32] The concentration of viral phospholipid was determined, after extraction of membrane lipids,[33] by phosphate analysis.[34] Liposomes (large unilamellar vesicles) were prepared by a freeze−thaw/extrusion procedure, as described before.[7a,9,30b] Briefly, lipid mixtures, dried from $CHCl_3$/MeOH, were hydrated in HNE buffer (5 mM HEPES, 150 mM NaCl, 0.1 mM EDTA, pH 7.4) and subjected to five cycles of freeze−thawing.[35] The vesicles were sized by extrusion[36] through two stacked Unipore polycarbonate filters with a pore size of 0.2 μm (Nucleopore, Inc., Pleasanton, CA) in a LiposoFast syringe extruder (Avestin, Ottawa, Canada). The liposomes consisted of a mixture of egg yolk PC, PE (prepared by transphosphatidylation of egg PC), and Chol (all from Avanti Polar Lipids, Alabaster, AL), supplemented with 10 mol % of ceramide **1** or **3**, as described in the caption to Figure 2.

Fusion Assay. The kinetics of fusion of pyrene-labeled SFV with liposomes was monitored as a *decrease* of the pyrene excimer fluorescence due to dilution of the pyrene-labeled lipids from the viral into the liposomal membrane.[7,30] On-line measurements were carried out in an Aminco Bowman series 2 fluorometer (SLM/Aminco, Urbana, IL) at excitation and emission wavelengths of 343 and 480 nm, respectively. Virus (final concentration, 0.5 μM phospholipid) and liposomes (final concentration, 0.2 mM phospholipid) were mixed in the cuvette of the fluorometer in a final volume of 0.7 mL of HNE buffer, pH 7.4. The contents of the cuvette were stirred and maintained at a temperature of 37 °C. Fusion was initiated by injection of a small, pretitrated volume of 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES), 0.1 M acetic acid, pH 4.9, to achieve a final pH of 5.5. The fusion scale was calibrated such that 0% fusion corresponded to the initial excimer fluorescence level and 100% fusion to the fluorescence intensity after addition of 10 mM (final concentration) of the detergent octa(ethylene glycol) *n*-dodecyl monoether (Fluka, Buchs, Switzerland), representing infinite dilution of the fluorophore.

1-[(*E*)-(4′-Bromo-2′-butenyl)oxy]-4-methoxybenzene (7). Allylic bromide **7** was prepared from methyl 4-bromocrotonate via the following three steps, as shown in Scheme 1. Step a: To a mixture of 13.8 g (100.0 mmol) of ground anhydrous K_2CO_3, 7.5 g (60.0 mmol) of *p*-methoxyphenol, and 0.66 g (2.5 mmol) of 18-crown-6 in 300 mL of dry acetone was added 8.0 mL (~58.0 mmol, technical grade) of methyl 4-bromocrotonate. After the mixture was stirred for 17 h at room temperature under N_2, the salts were removed by filtration in a sintered glass funnel. The filtrate was concentrated under reduced

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pressure to give a brown solid residue. Purification either by silica gel column chromatography (elution with hexane/EtOAc 8:1) or by recrystallization from hexane/EtOAc (15:1) gave 10.3 g (80%) of methyl 4-(4′-methoxyphenyloxy)crotonate (**5**) as white crystals and 1.0 g (8%) of the S_N2' regioisomer **5a** as a light yellow oil: R_f of **5** and **5a**, 0.40 and 0.47, respectively (hexane/EtOAc 6:1). **5**: mp 53.6−54.3 °C (lit.[18] mp 43.0−45.0 °C); IR (NaCl) 1720, 1508, 1443, 1226, 1032, 826 cm^{-1}; ^1H NMR δ 3.74 (s, 3H), 3.75 (s, 3H), 4.63 (dd, 2H, J = 4.0, 2.0 Hz), 6.18 (dt, 1H, J = 15.8, 2.0 Hz), 6.82 (s, 4H), 7.06 (dt, 1H, J = 15.8, 4.1 Hz); ^{13}C NMR δ 51.67, 55.69, 67.16, 114.69, 115.64, 121.43, 143.10, 152.16, 154.22, 166.55. Step b: To a solution of 4.3 g (19.4 mmol) of crotonate **5** in 140 mL of freshly distilled THF was injected 33.5 mL (50.0 mmol, 1.5 M solution in toluene) of DIBAL-H at −78 °C. The reaction mixture was stirred at −78 °C until starting material **5** was consumed (∼1 h) and then was warmed to room temperature. After MeOH (4 mL) was added dropwise to destroy the excess DIBAL-H, the reaction mixture was poured into 400 mL of 5% aqueous potassium sodium tartrate solution and extracted first with 400 mL of hexane/EtOAc (1:1), then with EtOAc (2 × 200 mL). The combined extracts were dried (Na_2SO_4), and the solvents were removed, giving 3.7 g (99%) of pure 4-(4′-methoxyphenyloxy)-(2*E*)-buten-1-ol (**6**) as a white solid: mp 65.3−66.2 °C; IR (NaCl) 3307, 1514, 1237, 1032, 826 cm^{-1}; ^1H NMR δ 1.57 (t, 1H, J = 7.1 Hz), 3.75 (s, 3H), 4.20 (m, 2H), 4.49 (dd, 2H, J = 5.0, 0.8 Hz), 5.98 (m, 2H), 6.81 (m, 4H); ^{13}C NMR δ 55.72, 62.91, 68.51, 114.63, 115.65, 126.50, 132.68, 152.64, 153.92. Step c: To a solution of 3.7 g (19.1 mmol) of alcohol **6** and 5.5 g (21.0 mmol) of Ph_3P in 150 mL of dry CH_2Cl_2 was added 4.1 g (22.9 mmol) of NBS at −23 °C. The reaction mixture was stirred under N_2 at this temperature for 1 h and then warmed to room temperature for another hour. Hexane (200 mL) was added to precipitate Ph_3PO, and the reaction mixture was passed through a pad of silica gel in a sintered glass funnel, which was rinsed with 400 mL of hexane/EtOAc (8:1). Concentration gave 4.8 g (97%) of pure allylic bromide **7** as a white solid: mp 60.0−60.5 °C; IR 1501, 1225, 1037, 820 cm^{-1}; ^1H NMR δ 3.75 (s, 3H), 3.98 (d, 2H, J = 7.4 Hz), 4.49 (d, 2H, J = 4.7 Hz), 6.05 (m, 2H), 6.89 (s, 4H); ^{13}C NMR δ 31.73, 55.71, 67.94, 114.63, 115.68, 129.07, 130.40, 152.48, 154.03; HRMS [FAB, M^+] calcd for $C_{11}H_{13}BrO_2$ *m*/*z* 256.0099, 258.0079, found 256.0096, 258.0081.

4-Methoxyphenyl 5-Octadecyn-(2*E*)-enyl Ether (8a). To a solution of 5.0 g (26.0 mmol) of 1-tetradecyne in 300 mL of THF was injected 16.3 mL (26.0 mmol, 1.6 M solution in hexane; in some reactions, we used 22.5 mmol instead of 26.0 mmol of *n*-butyllithium at −23 °C. The mixture was stirred at this temperature under N_2 for 2.5 h and then warmed to room temperature. A catalytic amount of CuI (300 mg, 1.6 mmol) was added, and the milklike solution was stirred at room temperature for 15 min, followed by injection of 2.3 g (9.0 mmol) of allylic bromide **7** in 20 mL of THF. After 1 h, 10% aqueous $NaHSO_4$ solution (100 mL) was added to quench the reaction, and the product was extracted with hexane/EtOAc (50:1) (3 × 100 mL). After the combined extracts were dried (Na_2SO_4), the solvents were removed under vacuum. The yellow solid residue was purified by column chromatography (elution with pentane/Et_2O 100:1) to give 2.68 g (81%) of the desired enyne **8a** as a white solid; in addition to **8a**, 0.52 g (16%) of the S_N2' isomeric byproduct **8b** was also isolated as a yellow oil. **8a**: mp 53.0−53.8 °C; R_f 0.51 (hexane/EtOAc 20:1); IR (NaCl) 1508, 1454, 1237, 1032, 826 cm^{-1}; ^1H NMR δ 0.86 (t, 3H, J = 7.0 Hz), 1.24 (m, 16H), 1.37 (m, 2H), 1.50 (m, 2H), 2.15 (tt, 2H, J = 7.0, 2.3 Hz), 2.95 (m, 2H), 3.75 (s, 3H), 4.45 (dd, 2H, J = 5.7, 1.3 Hz), 5.78−5.84 (m, 1H), 5.93−5.99 (m, 1H), 6.81 (m, 4H); ^{13}C NMR δ 14.11, 55.69, 68.80, 76.31, 83.05, 114.57, 115.63, 126.40, 129.33, 152.79, 153.82; HRMS [FAB, M^+] calcd for *m*/*z* $C_{25}H_{38}O_2$ 370.2872, found 370.2874. **8b**: R_f 0.59 (hexane/EtOAc 20:1); IR (NaCl) 1502, 1467, 1232, 1044, 826 cm^{-1}; ^1H NMR δ 0.86 (t, 3H, J = 7.0 Hz), 1.24 (m, 16H), 1.36 (m, 2H), 1.49 (dt, 2H, J = 14.9, 7.1 Hz), 2.20 (dt, 2H, J = 7.0, 2.1 Hz), 3.49 (m, 1H), 3.75 (s, 3H), 3.85 (t, 1H, J = 8.4 Hz), 4.00 (dd, 1H, J = 8.9, 6.1 Hz), 5.20 (dt, 1H, J = 10.1, 1.4 Hz), 5.45 (dt, 1H, J = 17.0, 1.5 Hz), 5.92 (ddd, 1H, J = 17.0, 10.2, 5.8 Hz), 6.82 (m, 4H); ^{13}C NMR δ 14.12, 35.99, 55.70, 71.84, 77.16, 85.03, 114.57, 115.96, 116.71, 135.08, 152.77, 154.01; HRMS [FAB, M^+] calcd for $C_{25}H_{38}O_2$ *m*/*z* 370.2872, found 370.2881.

(2*R*,3*R*)-1-(4 -Methoxyphenyloxy)-5-octadecyne-2,3-diol [(+)-9]. After a solution of 7.9 g of AD-mix-β in 100 mL of *tert*-butyl alcohol and 100 mL of water was stirred for 45 min at room temperature, 538 mg (5.7 mmol) of $MeSO_2NH_2$ was added, and the reaction mixture was chilled to 0 °C. At this moment, a solution of 2.1 g (5.7 mmol) of enyne **8a** in 12 mL of Et_2O was added. A gentle stream of air was used for about 1 h to evaporate the Et_2O. The heterogeneous solution was stirred for 48 h at 0 °C, and then 8.5 g (68 mmol) of Na_2SO_3 was added. The reaction mixture was allowed to warm to room temperature and stirred for another 30 min. The product was extracted first with 100 mL of EtOAc, and then with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4. After the solvents were removed under vacuum, the yellow solid residue was dissolved in a minimum volume of $CHCl_3$ and filtered through a pad of silica gel in a sintered glass funnel to remove the chiral ligand. The pad was washed with hexane/EtOAc 1:1 (400 mL). The filtrate was concentrated and lyophilized from benzene to give 2.25 g (96%) of pure diol **9** as a white solid: mp 91.0−92.3 °C; $[\alpha]^{25}_D$ +9.6° (*c* 1.27, $CHCl_3$); IR (NaCl) 3272, 2073, 1508, 1461, 1232, 1055, 814 cm^{-1}; ^1H NMR δ 0.86 (t, 3H, J = 7.0 Hz), 1.25 (m, 16H), 1.37 (m, 2H), 1.45 (m, 2H), 2.13 (tt, 2H, J = 7.0, 2.2 Hz), 2.51 (m, 3H), 2.60 (d, 1H, J = 4.9 Hz), 3.80 (s, 3H), 3.87 (ddt, 1H, J = 6.0, 5.8, 2.1 Hz), 4.03 (m, 3H), 6.83 (m, 4H); ^{13}C NMR δ 14.11, 55.69, 70.27, 70.43, 70.08, 75.43, 83.47, 114.65, 115.54, 152.48, 154.18; HRMS [FAB, M^+] calcd for $C_{25}H_{40}O_4$ *m*/*z* 404.2926, found 404.2915. The enantiomeric purity was determined to be 95% ee by ^1H NMR analysis of the bis-Mosher ester of diol **9** (see Figure 1).

(2*R*,3*R*,5*E*)-Octadecene-1,2,3-triol [(+)-10]. In a 500-mL two-neck round-bottom flask was collected 160 mL of $EtNH_2$ at −78 °C; then, 1.4 g (200 mmol) of lithium metal (prewashed with hexane, then with Et_2O, and finally with MeOH until it was shiny) was added. After the blue solution was stirred for 30 min, a solution of 4.5 g (11.1 mmol) of yne-diol **9** in 50 mL of THF was injected slowly. The reaction mixture was stirred at this temperature for 10 h under N_2 and then was quenched with 40 mL of MeOH and 160 mL of water. The reaction mixture was warmed to room temperature, and the product was extracted with $CHCl_3$ (4 × 150 mL). The extracts were dried over Na_2SO_4 and concentrated. After the brown semisolid residue was dissolved in 200 mL of $CHCl_3$ and washed with 200 mL of 3 N HCl, the aqueous layer was further extracted with $CHCl_3$ (2 × 100 mL). The combined extract was dried (Na_2SO_4) and concentrated to give a brown solid that was purified by column chromatography (elution with $CHCl_3$/MeOH 15:1). The white solid obtained was dissolved in $CHCl_3$ and filtered through a Cameo filter (Fisher Scientific) to remove the dissolved silica gel. Concentration gave 2.7 g (81%) of the desired triol **10** as a white solid: mp 66.5−67.5 °C; $[\alpha]^{25}_D$ +4.3° (*c* 1.5, $CHCl_3$/MeOH 10:1); IR (NaCl) 3342, 1461 cm^{-1}; ^1H NMR δ 0.85 (t, 3H, J = 7.0 Hz), 1.25 (m, 20H), 2.03 (dt, 2H, J = 6.9, 6.8 Hz), 2.27 (m, 4H), 2.60 (d, 1H, J = 5.5 Hz), 3.55 (dt, 1H, J = 5.0, 3.7 Hz), 3.64 (m, 1H), 3.67 (dd, 1H, J = 11.5, 5.2 Hz), 3.73 (dd, 1H, J = 11.5, 3.6 Hz), 5.40 (dt, 1H, J = 15.2, 7.5 Hz), 5.57 (dt, 1H, J = 15.2, 6.5 Hz); ^{13}C NMR δ 14.11, 64.96, 72.03, 73.03, 124.84, 135.13; HRMS [FAB, MH^+] calcd for $C_{18}H_{37}O_3$ *m*/*z* 301.2742, found 301.2735.

(2*R*,3*R*)-1,3-*O*-Benzylidene-(5*E*)-octadecene-1,2,3-triol [(+)-11]. To a solution of 245 mg (0.8 mmol) of ene-triol **10** and 252 mg (2.4 mmol) of benzaldehyde in 40 mL of CH_2Cl_2 was added 20 mg (0.1 mmol) of *p*-TsOH·H_2O. The reaction mixture was stirred at room temperature for 4 h under N_2, quenched by addition of 30 mg (0.22 mmol) of K_2CO_3, and stirred for another 10 min. After the solvent was removed, the slurry was purified by column chromatography (elution with hexane/EtOAc 6:1). The top spot (R_f 0.40, hexane/EtOAc 6:1), acetal **11**, was isolated as a waxy solid, and the 1,2- and 2,3-*O*-benzylidene isomers were combined and allowed to equilibrate again with the 1,3-*O*-benzylidene derivative by the addition of 5 mg (0.025 mmol) of *p*-TsOH·H_2O, 100 mg (0.9 mmol) of benzaldehyde, and 8 mL of CH_2Cl_2. The combined yield of the desired acetal **11** in the two runs was 276 mg (84%): $[\alpha]^{25}_D$ +16.9° (*c* 4.13, $CHCl_3$); IR (NaCl) 3436, 1085, 1026, 744, 691 cm^{-1}; ^1H NMR δ 0.85 (t, 3H, J = 7.0 Hz), 1.24 (m, 20H), 2.00 (dt, 2H, J = 6.9, 6.6 Hz), 2.39 (t, 2H, J = 7.1 Hz), 2.57 (d, 1H, J = 11.6 Hz), 3.49 (d, 1H, J = 11.6 Hz), 3.84 (t, 1H, J = 7.1 Hz), 4.03 (dd, 1H, J = 11.8, 0.9 Hz), 4.22 (dd, 1H, J = 11.8, 1.7 Hz), 5.41 (dt, 1H, J = 15.2, 7.0 Hz), 5.55 (s, 1H), 5.59 (dt,

1H, J = 15.3, 6.6 Hz), 7.33−7.50 (m, 5H); ^{13}C NMR δ 14.11, 64.59, 72.85, 80.16, 101.42, 124.12, 125.88, 128.26, 128.95, 134.46, 137.90; HRMS [FAB, MH$^+$] calcd for $C_{25}H_{41}O_3$ m/z 389.3056, found 389.3058.

(2*S*,3*R*)-2-Azido-(1,3-*O*-benzylidene)hexadec-(5*E*)-ene-1,3-diol [(+)-12]. Method A: Via a Triflate Intermediate (Not Isolated). To a stirred solution of 200 mg (0.51 mmol) of acetal **11** in 12 mL of dry CH_2Cl_2 was injected 300 μL (3.7 mmol) of pyridine under nitrogen. The mixture was then chilled to −78 °C, followed by the slow injection of 140 μL (0.85 mmol) of $(CF_3SO_2)_2O$. After the reaction mixture was stirred at this temperature for 1 h, dry DMF (30 mL) was injected slowly, followed by rapid addition of 650 mg (10 mmol) of NaN_3 (dried on a vacuum pump for 2 h at 0.5 Torr) using a Pasteur pipet. The cold bath was removed to allow the reaction mixture to warm to room temperature with vigorous stirring under N_2. When complete consumption of the starting alcohol was noted (∼2 h) by TLC analysis (hexane/EtOAc 20:1), H_2O (80 mL) was added, and the product was extracted with Et_2O (3 × 60 mL). The combined ether extract was washed with brine (2 × 20 mL), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (elution first with 400 mL of hexane, followed by hexane/EtOAc 80:1) to provide 155 mg (73%) of azido acetal **12** as a light brown oil: $[\alpha]^{25}_D$ +7.63° (*c* 2.7, $CHCl_3$); IR (NaCl) 2260, 1162, 735, 692 cm^{-1}; ^{1}H NMR δ 0.86 (t, 3H, J = 7.0 Hz), 1.24 (m, 20H), 2.02 (dt, 2H, J = 7.0, 6.8 Hz), 2.39 (dt, 1H, J = 14.4, 6.7 Hz), 2.55 (dt, 1H, J = 14.9, 4.6 Hz), 3.50 (m, 1H), 3.59 (m, 1H), 3.60 (dd, 1H, J = 10.4, 5.0 Hz), 4.34 (dd, 1H, J = 10.8, 5.0 Hz), 5.44 (s, 1H), 5.56 (m, 2H), 7.33−7.45 (m, 5H); ^{13}C NMR δ 14.11, 56.35, 68.99, 80.51, 101.03, 123.93, 126.00, 128.26, 129.01, 134.66, 137.50; HRMS [FAB, MH$^+$] calcd for $C_{25}H_{40}N_3O_2$ m/z 414.3120, found 414.3119 (40%); [FAB, MH$^+$ − N_2] calcd for $C_{25}H_{40}NO_2$ m/z 386.3059, found 386.3055 (100%).

Method B: Via the Mesylate Intermediate, (2*R*,3*R*)-2-(*O*)-Methanesulfonyl-1,3-*O*-benzylidene-(5*E*)-octadecene-1,3-diol. To a solution of 2.3 g (5.9 mmol) of acetal **11** and 2.4 g (23.7 mmol) of Et_3N in 60 mL of dry CH_2Cl_2 was added slowly a solution of 2.0 g (71.0 mmol) of $MeSO_2Cl$ in 10 mL of CH_2Cl_2 through an addition funnel at 0 °C. The reaction mixture was stirred for 2 h at this temperature and then quenched by the addition of 120 mL of saturated aqueous $NaHCO_3$ solution. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). After the combined organic layers were dried (Na_2SO_4), the solvent was removed under vacuum to yield 2.70 g (98%) of pure mesylate as a white solid: mp 96.5−97.3 °C; $[\alpha]^{25}_D$ −7.6° (*c* 3.1, $CHCl_3$); IR (NaCl) 1173, 738, 697 cm^{-1}; ^{1}H NMR δ 0.86 (t, 3H, J = 7.0 Hz), 1.18 (m, 20H), 1.94 (dt, 2H, J = 7.0, 7.0 Hz), 2.30−2.45 (m, 2H), 3.13 (s, 3H), 3.95 (dt, 1H, J = 6.9, 1.1 Hz), 4.02 (dd, 1H, J = 13.2, 0.8 Hz), 4.48 (dd, 1H, J = 13.3, 1.40 Hz), 4.56 (d, 1H, J = 1.2 Hz), 5.34 (m, 1H), 5.52 (s, 1H), 5.56 (m, 1H), 7.26−7.45 (m, 5H); ^{13}C NMR δ 14.12, 39.26, 69.65, 72.35, 78.31, 101.27, 123.22, 126.04, 128.28, 129.09, 135.35, 137.49; HRMS [FAB, MH$^+$] calcd for m/z $C_{26}H_{43}O_5S$ 467.2831, found 467.2814. A suspension of 4.0 g (8.8 mmol) of the mesylate and 5.2 g (80.0 mmol) of dry NaN_3 in 60 mL of dry DMF was stirred at 93 °C for 12−14 h under N_2. Prolonged heating at this temperature caused severe decomposition of the product; after 72 h, almost all of the product decomposed. The reaction mixture was poured into 500 mL of 2% brine, and the product was extracted with Et_2O (4 × 200 mL). The combined ether extracts were dried (Na_2SO_4) and concentrated to give a light brown residue, which was purified by column chromatography (elution first with hexane/EtOAc 9:1 to collect azide **12**, and then with hexane/EtOAc 1:1 to recover the starting mesylate). The above procedure was repeated using the recovered mesylate. Azide **12** was obtained in 62% yield (based on the consumption of 0.8 g of mesylate) as a light brown oil.

(2*S*,3*R*)-2-Azido-(5*E*)-octadecene-1,3-diol [(+)-13]. A solution of 400 mg (1.0 mmol) of azido acetal **12**, 580 mg (9.3 mmol) of ethylene glycol, and 32 mg (0.2 mmol) of *p*-TsOH·H_2O in 50 mL of MeOH was stirred under N_2 at room temperature for 12 h. The reaction was quenched by addition of 66 mg (0.50 mmol) of K_2CO_3. After the heterogeneous solution was stirred for 10 min, the solvent was removed, leaving a yellow residue that was purified by column chromatography (elution with hexane/EtOAc 2:1) to give 281 mg (89%) of azido diol **13** as a white solid: mp 55.2−56.3 °C; $[\alpha]^{25}_D$ +12.52° (*c* 2.0, $CHCl_3$); IR (NaCl) 3420, 2263, 2120 cm^{-1}; ^{1}H NMR δ 0.86 (t, 3H, J = 7.0 Hz), 1.24 (m, 18H), 1.34 (m, 2H), 2.03 (dt, 2H, J = 6.9, 6.9 Hz), 2.10 (d, 1H, J = 3.1 Hz), 2.18 (m, 2H), 2.37 (dt, 1H, J = 14.0, 4.9 Hz), 3.42 (dt, 1H, J = 5.8, 5.7 Hz), 3.70 (m, 1H), 3.86 (m, 2H), 5.37 (m, 1H), 5.60 (m, 1H); ^{13}C NMR δ 14.11, 62.85, 66.07, 71.39, 124.06, 136.52; HRMS [FAB, MH$^+$] calcd for $C_{18}H_{36}N_3O_2$ m/z 326.2808, found 326.2813.

(2*S*,3*R*)-2-*N*-(*cis*-4-Hexadecenoyl)-(5*E*)-octadecene-1,3-diol [(+)-3]. To a solution of 40 mg (0.12 mmol) of azido diol **13** and 93 mg (0.24 mmol) of *p*-nitrophenyl *cis*-4-hexadecenoate in 10 mL of 9:1 THF/H_2O was added 65 mg (0.25 mmol) of Ph_3P. The reaction mixture was stirred under nitrogen at room temperature for 24 h. Removal of the solvents (2-PrOH was added to remove water) gave a yellow residue that was purified by column chromatography (elution with $CHCl_3$/MeOH 50:1). The resulting white solid was dissolved in $CHCl_3$, and the solution was passed through a Cameo filter to remove dissolved silica gel. There was obtained 60 mg (91%) of Δ^5-ceramide **3** as a white solid: mp 91.5−93.0 °C; $[\alpha]^{25}_D$ +5.3° (*c* 3.65, $CHCl_3$); IR (NaCl) 3356, 1656 cm^{-1}; ^{1}H NMR δ 0.86 (t, 6H, J = 7.0 Hz), 1.23 (m, 38H), 2.00 (m, 4H), 2.23 (m, 4H), 2.34 (dt, 2H, J = 7.3, 7.2 Hz), 2.50 (br s, 1H), 2.82 (br s, 1H), 3.68 (dd, 1H, J = 11.4, 3.0 Hz), 3.77 (dt, 1H, J = 8.6, 4.3 Hz), 3.85 (dt, 1H, J = 7.7, 3.8 Hz), 4.01 (dd, 1H, J = 11.4, 3.2 Hz), 5.28−5.45 (m, 3H), 5.56 (dt, 1H, J = 15.2, 7.5 Hz), 6.23 (d, 1H, J = 7.8 Hz); ^{13}C NMR δ 14.12, 36.67, 37.84, 53.28, 62.26, 72.98, 124.79, 127.41, 131.80, 135.77, 172.78; HRMS [FAB, MH$^+$] calcd for $C_{34}H_{66}NO_3$ m/z 536.5042, found 536.5042.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **3** and **5**−**13**, including **8b** and the mesylate derivative of **11** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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