Effect of Aromatic Short-Chain Analogues of Ceramide on Axonal Growth in Hippocampal Neurons

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Received March 1, 1999

A series of D-*erythro*- and L-*threo*-ceramide analogues was synthesized and investigated for their ability to reverse the inhibitory effects of fumonisin B_1 (FB_1) on axonal growth in hippocampal neurons. The analogues contained either a C_7 side chain or a phenyl group substituted for the C_{13} residue present in naturally occurring ceramides, while the *N*-acyl chain length was reduced from C_{16}−C_{24} to C_2−C_8. D-*erythro*-Ceramide **18a** with a C_7 side chain and an *N*-acetyl residue and D-*erythro*-ceramide **20c** with an aromatic side chain and an *N*-hexanoyl residue were most active in reversing the inhibitory effects of FB_1 on axonal growth, although the mechanism remains unclear.

Introduction

Sphingolipids in the cell membrane are important in regulating cell proliferation, cell differentiation, and neuronal adhesion, while they interfere in signal transduction and programmed cell death and act as receptors for cellular communication.[1,2,3] The biosynthesis of sphingolipids occurs in the endoplasmic reticulum and the Golgi apparatus. D-*erythro*-Sphinganine is acylated in the endoplasmic reticulum,[4] and subsequent introduction of the 4,5-double bond by dihydroceramide desaturase[5,6] leads to formation of ceramide, which is further metabolized in the Golgi apparatus to sphingomyelin and glycosphingolipids.[7] The metabolism of ceramide can be inhibited by a number of compounds (Scheme 1). The best known inhibitor is fumonisin B_1 (FB_1),[8] which inhibits sphingosine *N*-acyltransferase (IC_{50} = 0.1 μM).[9] D-*threo*-1-Phenyl-2-(decanoylamino)-3-morpholino-1-propanol (PDMP) is an inhibitor of glucosylceramide (GlcCer) synthesis,[10] and conduritol B-epoxide (CBE) inhibits lysosomal glucocerebrosidase, the enzyme that degrades GlcCer.[11]

Neuronal growth is a vital process affected by metabolism of ceramide. Studies using cultured hippocampal neurons have shown that normal growth[12−14] and growth accelerated by growth factors[15] can be blocked by inhibition of sphingolipid synthesis. Interestingly, the effects of FB_1 can be reversed by co-incubation with short-acyl-chain analogues of ceramide (C_6-D-*erythro*-ceramide and C_6-NBD-D-*erythro*-ceramide (*N*-[7-(4-nitrobenz-2-oxa-1,3-diazolyl)]-ϵ-aminocaproylsphingosine)).[12,15] It has been proposed that continuous synthesis of GlcCer, the simplest glycosphingolipid, is required for axonal growth in hippocampal neurons.[14,15] The prerequisite that ceramide must be metabolized to GlcCer to sustain growth was supported by contrasting observations on using stereoisomeric ceramides. Whereas C_6-D-*erythro*-ceramide (or C_6-NBD-D-*erythro*-ceramide), as a result of metabolism to GlcCer,[16,17] is able to antagonize the inhibitory effect of FB_1 on axonal growth, the L-*threo*-analogue resists metabolism;[18,19] hence, it is not effective in reversing the effects of FB_1. Moreover, the inhibitory effect of PDMP on the axonal growth cannot be reversed by C_6-NBD-D-*erythro*-ceramide.[14,15]

The use of primary cultures of hippocampal neurons is well-suited to investigate interferences with nerve growth as they develop by a well-characterized and regular sequence of events giving rise to fully differentiated axons and dendrites.[20] Study of new compounds that may activate nerve growth could lead to the development of drugs for counteracting nerve degeneration or insufficient nerve development. In this context, we synthesized new series of D-*erythro*- and L-*threo*-ceramide analogues and investigated their potential to reverse the inhibitory effects of FB_1 on axonal growth in hippocampal neurons. Features of interest are shortening of the "sphingoid" part with respect to the natural sphingosine, introducing a phenyl group in the sphingoid moiety, and varying the length of the *N*-acyl groups. Natural sphingolipids having a C_{18} sphingosine chain, and C_{16}−C_{24} *N*-acyl groups are difficult to study due to the high lipophilic character. Thus, natural ceramides are poor candidates for in vitro and in vivo studies in view of the poor biodistribution. Bioactivity studies would, therefore, profit from ceramides modified with shorter hydrocarbon chains to enhance aqueous solubility and cell permeability. We achieved this prerequisite by introducing heptyl and phenyl residues, respectively, in the sphingoid part and short (up to C_8) acyl chains in the amide moiety.

Chemistry

The known 'Garner aldehyde' (**1**) (Scheme 2) served as chiral building block for the synthesis of ceramide analogues with a different sphingoid backbone. This protected L-serine-derived configurationally stable al-

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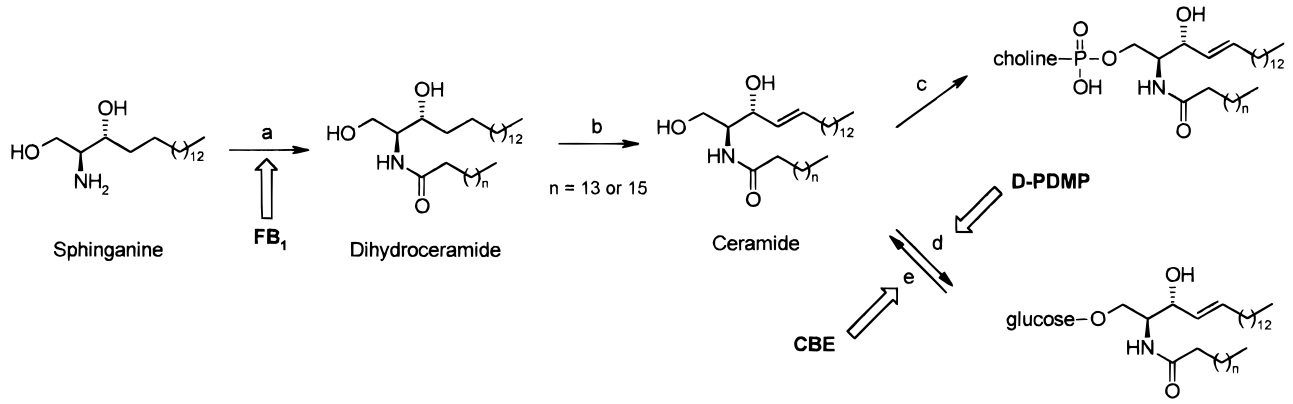
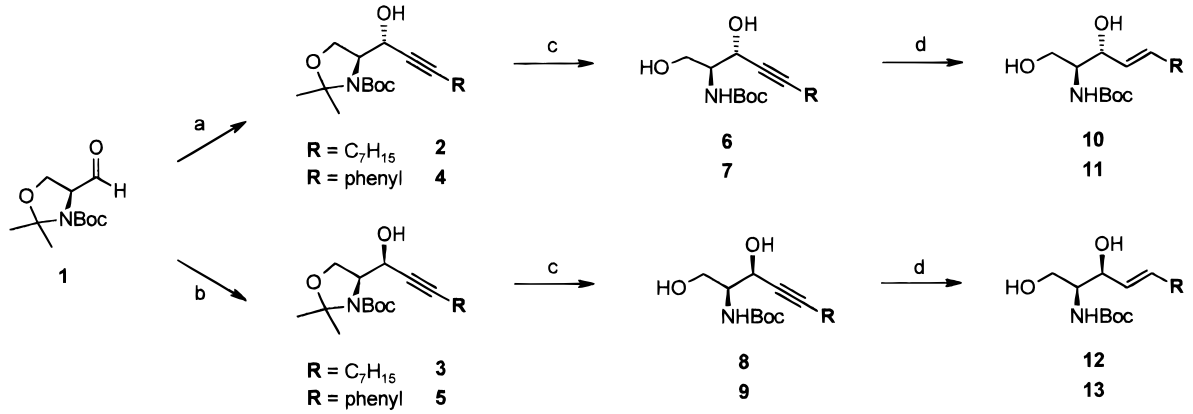
10.1021/jm990091e CCC: $18.00 © 1999 American Chemical Society
Published on Web 06/25/1999

Scheme 1. Inhibitors of Ceramide Metabolism[a]



[a] (a) Sphinganine *N*-acyltransferase; (b) dihydroceramide desaturase; (c) sphingomyelin synthase; (d) glucosylceramide synthase; (e) glucocerebrosidase. FB_1, fumonisin B_1; D-PDMP, D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; CBE, conduritol B-epoxide.

Scheme 2[a]



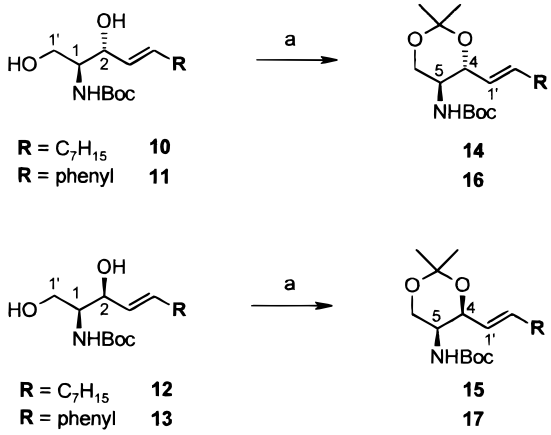
[a] (a) 1-Nonyne, *n*-BuLi/lithium phenylacetylide, THF, HMPA; (b) 1-nonyne/phenylacetylene, *n*-BuLi, Et_2O, $ZnBr_2$; (c) Amberlyst 15 or TsOH, MeOH; (d) Red-Al, Et_2O, 0 °C to rt.

dehyde can be obtained in two steps from commercially available *N*-Boc-L-serine methyl ester.[21] We adopted slightly the Herold approach to the synthesis of D-*erythro*- and L-*threo*-*N*-Boc-protected sphingosines.[22]

Nucleophilic addition of an appropriate lithium acetylide to **1** results in either the *erythro*- or the *threo*-alkynol, depending on specific reaction conditions. When hexamethylphosphoramide (HMPA) is used as a cation-complexing agent, the *erythro*-alkynol is predominantly formed, resulting from stereoselective *re*-attack.[23] *threo*-Selectivity is effected by zinc dibromide-induced chelation between the *N*-Boc group and the aldehyde thereby favoring addition from the *si*-face.[24,25]

Thus, addition of the lithium salt of 1-nonyne to **1** at −78 °C in THF in the presence of 2 equiv of HMPA gave alkynol **2**. Using anhydrous zinc dibromide in diethyl ether, the epimeric *threo*-**3** was formed in preference to the *erythro*-compound (*threo*/*erythro* 9:1). Both isomers were separated by flash chromatography and distinguished by the ^1H NMR resonance of the C(1′) protons, whereby H−C(1′) of the 1′*R* (*erythro*) epimer was distinctively observed at higher field with respect to the 1′*S* (*threo*) analogue.[22] Similar results were obtained on using lithium phenylacetylide leading to the alkynols **4** and **5**. Deprotection involving cleavage of the oxazolidine ring was carried out with Amberlyst 15 or TsOH in CH_3OH, which afforded the *erythro*-propargylic alcohols **6** and **7** and their *threo*-epimers **8** and **9**, respectively. Partial hydrogenation to the corresponding *trans*-alkenes was achieved using Red-Al (bis(2-methoxyethoxy)-aluminum hydride) in diethyl ether at 0 °C. This short synthetic sequence afforded the *N*-Boc-protected D-*erythro* (**10**, **11**) and L-*threo* (**12**, **13**) sphingosine analogues.

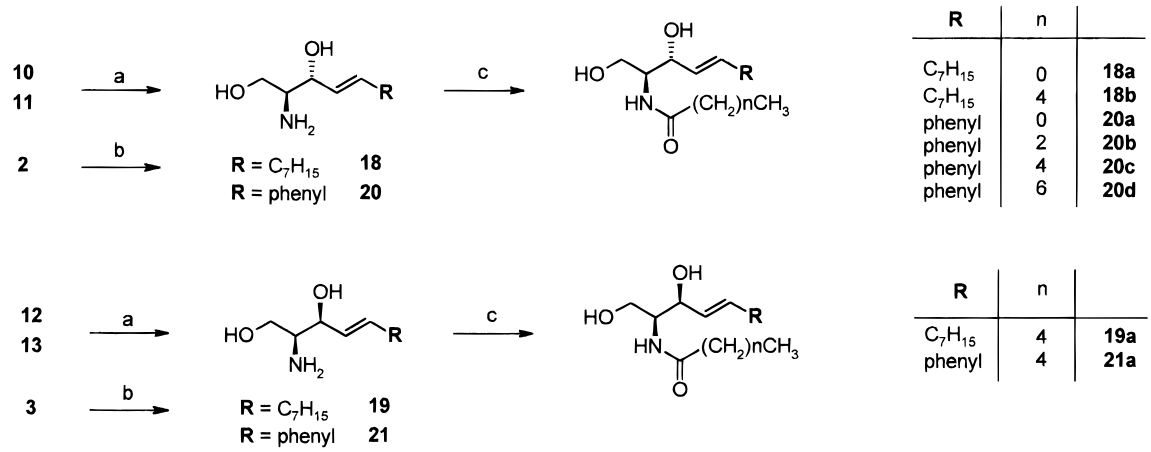
Scheme 3[a]



[a] (a) DMP, PPTS, CH_2Cl_2.

The relative configuration at C(1) and C(2) was unambiguously established via conversion of **10** and **12** to the corresponding acetonides **14** and **15** on acetalization with 2,2-dimethoxypropane in the presence of pyridinium *p*-toluenesulfonate (PPTS) in CH_2Cl_2 (Scheme 3).[22] Although the H−C(4) and H−C(5) protons were not

Scheme 4[a]



[a] (a) 1 N HCl, dioxane, 100 °C; (b) Li, $EtNH_2$, THF, −78 °C; (c) $CH_3(CH_2)_nCOOH$, DIC, HOBT, CH_2Cl_2 or $CH_3(CH_2)_nCOCl$, 50% aq NaOAc, THF or $[CH_3(CH_2)_nCO]_2O$, Et_3N, THF.

Table 1. Ability of Short-Chain Ceramide Analogues To Reverse the Inhibitory Effects of FB_1 on Axonal Growth[a]

treatment	no. of axonal branch points/cell at 51 h	statistical differences (p-values) with bFGF-treated cells	statistical differences (p-values) with (bFGF + FB_1)-treated cells
none	1.11 ± 0.03	$p < 0.001$	
bFGF	1.52 ± 0.09		$p < 0.001$
bFGF + FB_1	1.12 ± 0.06	$p < 0.001$	
bFGF + FB_1 + C_6-D-*erythro*-Cer	1.47 ± 0.10		$p < 0.005$
bFGF + FB_1 + C_6-L-*threo*-Cer	1.13 ± 0.05	$p < 0.005$	
bFGF + FB_1 + **18a** (C_2-D-*erythro*)	1.50 ± 0.07		$p < 0.001$
bFGF + FB_1 + **18b** (C_6-D-*erythro*)	1.30 ± 0.10		$p < 0.050$
bFGF + FB_1 + **19a** (C_6-L-*threo*)	1.18 ± 0.05	$p < 0.010$	
bFGF + FB_1 + **20a** (C_2-D-*erythro*)	1.30 ± 0.09	$p < 0.050$	$p < 0.050$

[a] bFGF (1 ng/mL) was added to hippocampal neurons at 48 h in culture together with FB_1 (10 μM) and with or without short-acyl-chain ceramide analogues (5 μM). Values are means ± SEM of measurements from six individual cultures in which 50 cells were counted per coverslip, for two individual coverslips per treatment. The number of axonal branch points is proportional to the length of the axon plexus.[15] Statistical differences, calculated using the Student's t-test, are shown as p-values. When no p-value is given, no statistical differences ($p > 0.05$) were obtained.

resolved in the ^{1}H NMR spectrum of **14**, couplings of 5.4 and 1 Hz for H−C(4) in the *syn*-compound **15** referred to H−C(1′) and H−C(5), respectively. These values are in agreement with reported $J_{(4,5)}$ values of ca. 10 and 1 Hz for *anti*- and *syn*-acetonides, respectively.[22,26] Acetonides **16** and **17** provided supportive data.

The *N*-Boc-protected sphingoids are suitable precursors for the synthesis of ceramide analogues with short acyl chains. Hydrolysis with 1 N HCl in dioxane resulted in formation of the free amino diols (Scheme 4). These compounds were purified for identification, but due to their relative instability, it was preferable to proceed with the acylation reaction without prior purification. It is known, indeed, that sphingosine is very sensitive to air oxidation.[22,26] The D-*erythro*-sphingosine analogues **18** and **20** and their L-*threo*-epimers **19** and **21** are distinguishable on TLC (R_f *erythro* > R_f *threo*), but the respective ^{1}H NMR spectra reveal only minor differences, as was reported for D-*erythro*- and L-*threo*-sphingosine.[27] Ceramide analogues **18a**,**b**, **19a**, **20a**−**d**, and **21a** were obtained by amide formation of their corresponding sphingosine analogues (**18**−**21**). Acylation was accomplished by using suitable carboxylic acids, in the presence of 1,3-diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole hydrate (HOBT) as coupling reagents, or acid chlorides in a mixture of THF and 50% aqueous sodium acetate.

A short approach to sphingoids **18** and **19** involves treatment of the alkynols with lithium in ethylamine (Birch reduction).[27] Reduction to the *trans*-double bond proceeds concomitantly with deprotection of both the oxazolidine and the carbamate functions. Thus, compound **3** was converted to **19** in a single manipulation. Unfortunately, this efficient sequence was not applicable to the synthesis of **20** and **21** as Birch reduction of alkynols **4** and **5** resulted in the formation of cyclohexenes.

Biological Evaluation

Previous studies reported that incubation of hippocampal neurons with FB_1 disrupts axonal growth between days 2 and 3 in culture.[12] On the other hand, the growth of axons of these neurons can be stimulated by basic fibroblast growth factor (bFGF).[28] For the first 3 h after addition of bFGF to the culture medium, the number of axonal branch points per cell increases at a rate 4-fold greater than in untreated cells.

We subjected our synthetic ceramide analogues to a bioassay in which incubation with FB_1 led to a particular number of axonal branch points per cell. The results are summarized in Tables 1 and 2 and Figure 1. As reported previously, bFGF stimulates axonal growth, but the combination with FB_1 blocked the stimulation.[15] Interestingly, C_6-D-*erythro*-ceramide reverses the effect of FB_1 in contrast to C_6-L-*threo*-ceramide. Compounds

Table 2. Influence of the *N*-Acyl Chain Length on Axonal Growth[a]

treatment	no. of axonal branch points/cell at 51 h	statistical differences (p-values) with bFGF-treated cells	statistical differences (p-values) with (bFGF + FB_1)-treated cells
bFGF + FB_1 + **20a** (C_2-D-*erythro*)	1.30 ± 0.09		$p < 0.050$
bFGF + FB_1 + **20b** (C_4-D-*erythro*)	1.38 ± 0.09		$p < 0.010$
bFGF + FB_1 + **20c** (C_6-D-*erythro*)	1.45 ± 0.09		$p < 0.005$
bFGF + FB_1 + **20d** (C_8-D-*erythro*)	1.40 ± 0.10		$p < 0.010$
bFGF + FB_1 + **21a** (C_6-L-*threo*)	1.22 ± 0.08	$p < 0.010$	

[a] Short-chain ceramide analogues were added as indicated in Table 1. Values are means ± SEM of measurements from three to six individual cultures, in which 50 cells were counted per coverslip, for two individual coverslips per treatment. Corresponding values for control cells, bFGF-treated cells, and (bFGF + FB_1)-treated cells are shown in Table 1.

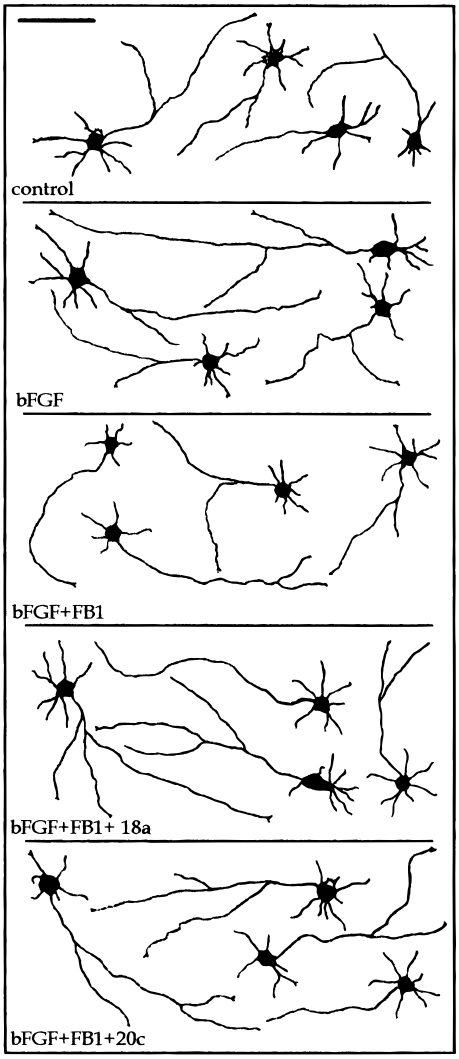


Figure 1. Ability of short-chain ceramide analogues to reverse the inhibitory effects of FB_1 on accelerated axonal growth. Camera lucid drawings of representative cells are shown. bFGF (1 ng/mL) was added to hippocampal neurons at 48 h in culture together with FB_1 (10 μM) and with or without **18a** (D-*erythro* C_2) or **20c** (D-*erythro* C_6). The bar corresponds to 50 μm.

18a,**b**, i.e., D-*erythro*-ceramides with a sphingosine moiety of 12 carbon atoms, cause partial or full reversal of the FB_1 effect (Table 1), although no direct influence on axonal growth in the absence of bFGF and FB_1 was apparent (not shown). Compound **18b** with a C_6 acyl group is less effective than compound **18a** with a C_2 acyl chain. The L-*threo*-congener **19a** did not show any activity. Ceramide **20a** with a C_2 acyl chain and a phenyl group in the sphingosine moiety partially reverses the effect of FB_1. As this short-chain aromatic ceramide still exhibited biological activity, we screened for an optimal length of the *N*-acyl side chain (Table 2). Ceramides with increasing chain lengths (**20a**-C_2; **20b**-C_4; **20c**-C_6; **20d**-C_8) were synthesized together with the L-*threo*-analogue **21a**. Compound **20c** with a C_6 acyl chain retains full activity when compared with the D-*erythro*-ceramide **18a**. L-*threo*-Ceramide **21a** with a phenyl group and a C_6-acyl chain has a small but statistically insignificant effect.

The mechanism by which the examined ceramide analogues stimulate axonal growth could be conversion to GlcCer analogues, as shown for C_6-ceramide stereoisomers (with a C_{18} sphingoid backbone).[15] During the period of axonal growth, GlcCer synthesis might be necessary to supply the growing axon of newly synthesized GlcCer or to deliver a protein, essential for axon growth, to the axonal membrane.[14,15] In this regard, we synthesized the radioactive ^{14}C analogue of compound **20a** (labeled at the acetyl group), but its glucosylation could not be demonstrated in vitro nor in vivo (in hippocampal neurons), in the experimental conditions[15,29] where glucosylation is observed for [^{14}C]-C_6-D-*erythro*-ceramide (not shown).

Study of the substrate specificity of rat liver GCS with fluorescent ceramide analogues showed that the C_6- and C_8-NBD-ceramides were the most active substrates,[19] while less activity for shorter (C_3/C_5) or longer (C_{14}) ceramide analogues was found. Moreover, the yield of glucosylation of [^{14}C]-C_6-ceramide was only 25% relative to that of C_6-NBD-ceramide. These preliminary results suggest that the analogues with the styryl group are not recognized by GCS, which would indicate that they stimulate axonal growth by a different, as yet unknown, mechanism. Further studies on the metabolism of **18a** and **20c** are needed to clarify the mode of action.

Conclusion

We investigated the bioactivity of a series of new ceramide analogues with modified sphingosine and *N*-acyl chains. Their influence on the axonal growth of hippocampal neurons was compared with that of C_6 *N*-acyl analogues of natural ceramides. Only the D-*erythro*-analogues reversed the inhibitory effect of fumonisin B_1 on the axonal growth of hippocampal neurons. Interestingly, substitution of the alkenyl chain of ceramide by a styryl group did not result in a decrease of activity. The finding of a new active ceramide analogue with an aromatic chain allows the study of further structure−activity relationships.

Experimental Section

Hippocampal Cultures. Hippocampal neurons were cultured at low density as previously described,[30] however, with some modifications.[12,13] The dissected hippocampi of embryonic

day 18 rats (Wistar), obtained from the Weizmann Institute Breeding Center, were dissociated by trypsinization (0.25% w/v, for 15 min at 37 °C). The tissue was washed in Mg^{2+}/Ca^{2+}-free Hank's balanced salt solution (HBSS) (Gibco) and dissociated by repeated passage through a constricted Pasteur pipet. Cells were plated in minimal essential medium (MEM) with 10% horse serum, at a density of 6×10^3 cells/13-mm glass cover slip (Assistent, Germany) that had been precoated with poly-L-lysine (1 mg/mL). After 3−4 h, cover slips were transferred into 24-well multidishes (Nunc) containing a monolayer of astroglia. Cover slips were placed with the neurons facing downward and separated from the glia by paraffin 'feet'. Cultures were maintained in serum-free medium (MEM), which included N_2 supplements,[30] ovalbumin (0.1% w/v), and pyruvate (0.1 mM). In some experiments, neurons were removed from multidishes containing glia and placed in new dishes that contained fresh medium but not a glial monolayer. Neurons cultured at high density (23×10^4 cells/24-mm glass coverslip in 100-mm Petri dishes) were used for biochemical analysis.[13,31]

Analysis of Axon Growth. Stock solutions of FB_1 were dissolved in Hepes buffer (20 mM, pH 7.4) and added to cultures to give final concentrations of 10 μM. Sphingolipid analogues were dissolved in ethanol and added to the culture medium whereby the final ethanol concentration did not exceed 1%; control cultures were treated with 1% ethanol. After appropriate times, cover slips were removed from the 24-well multidishes; neurons were fixed in 1% (v/v) glutaraldehyde in PBS for 20 min at 37 °C and mounted for microscopic examination in 50% glycerol in PBS. Neurons were examined by phase contrast microscopy using an Achroplan 32×/0.4 n.a. phase 2 objective of a Zeiss Axiovert 35 microscope. An axon was considered to branch when the process that it gave rise to was more than 15 μm long.[15] Thin filipodia, which were occasionally observed along the entire length of the axon, were not considered as branches.

Addition of Basic Fibroblast Growth Factor (bFGF). bFGF (1 ng/mL) was added to cultures after 48 h together with FB_1 (10 μM); sphingolipid analogues (5 μM) were added after 48 h as indicated, and the number of axonal branch points was measured after 51 h. Statistical analysis was performed using the Student's *t*-test.

Synthesis. General. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20 °C. UV spectra were recorded in CH_3OH with a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were recorded with a Perkin-Elmer 1600 Series FTIR spectrometer. ^1H and ^{13}C NMR spectra were obtained with a Brucker WH 360 spectrometer (^1H NMR, 360 MHz; ^{13}C NMR, 90 MHz), unless otherwise indicated, using tetramethylsilane as internal standard. Liquid secondary-ion mass spectra (LSIMS) were obtained using a Kratos concept ^1H mass spectrometer (Kratos, Manchester, U.K.). Elemental analyses were performed at the University of Konstanz, Germany, and are within ±0.4% of theoretical values unless otherwise specified.

Precoated Merck silica gel F_{254} plates were used for TLC, and spots were examined with UV light at 254 nm and/or ninhydrin (0.3% in EtOH) solution, phosphomolybdic acid (0.5% in EtOH) solution and sulfuric acid−anisaldehyde spray. Column chromatography was performed on SÜD-Chemie silica gel (0.2−0.05 mm). THF and Et_2O were distilled with sodium and benzophenone. CH_2Cl_2 was obtained by distillation after reflux overnight with CaH_2.

***tert*-Butyl (4*S*)-4-[(1*R*)-1-Hydroxy-2-decynyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (2).** To a stirred and cooled (−25 °C) solution of 1-nonyne (3.5 mL, 21 mmol) in dry THF (100 mL) was added dropwise a 1.6 M solution of *n*-BuLi in hexane (12 mL, 19 mmol). After 1.5 h, the solution was cooled to −78 °C and HMPA (5 mL, 29 mmol) was added, followed by addition of a cooled (−78 °C) solution of **1**[21] (3.27 g, 14 mmol) in dry THF (8 mL). After 4 h at −78 °C, the reaction mixture was warmed to −25 °C and quenched by the addition of a saturated NH_4Cl solution (110 mL). The aqueous layer was extracted with Et_2O (three times), and the combined organic layers were washed with 0.5 N HCl and brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (pentane/EtOAc, 9:1) yielded 4.15 g (84%) of **2** as an oil: $[\alpha]_D = -42$ (*c* 1.4, $CHCl_3$); IR (cm^{-1}, KBr) 3439, 2932, 1700; ^1H NMR (DMSO-d_6) δ 0.85 (3 H, t, CH_3CH$_2$), 1.15−1.6 (25 H, m, *t*-Bu, 2 CH$_3$, (CH$_2$)$_5$), 2.12−2.2 (2 H, m, CH_2(CH$_2$)$_5$CH$_3$), 3.75−4.08 (3 H, m, 2H−C(5), H−C(4)), 4.45 (1 H, br s, *H*COH), 5.42 (1 H, d, OH); HRMS (LSIMS) calcd for $C_{20}H_{36}NO_4$ $(M + H)^+$ 354.2644, found 354.2643.

***tert*-Butyl (4*S*)-4-[(1*S*)-1-Hydroxy-2-decynyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (3).** To a stirred and cooled (−20 °C) solution of 1-nonyne (0.36 mL, 2.21 mmol) in dry Et_2O (7 mL) was added dropwise a 1.6 M solution of *n*-BuLi in hexane (1.38 mL, 2.21 mmol). After 1.5 h, anhydrous $ZnBr_2$ (0.54 g, 2.38 mmol) was added at 0 °C and the reaction was stirred for 2 h. Then, a cooled (−78 °C) solution of **1**[21] (0.43 g, 1.88 mmol) in dry Et_2O (2.5 mL) was added dropwise at −78 °C. The mixture was allowed to warm to room temperature overnight and was then quenched by adding a saturated NH_4Cl solution (7 mL). The aqueous layer was extracted with Et_2O (three times), and the combined organic layers were washed with 0.5 N HCl and brine, dried over $MgSO_4$, and concentrated under reduced pressure. After flash chromatography (hexane/EtOAc, 4:1) 0.43 g (64%) of addition product was obtained. The reaction mixture contained a considerable amount of the *erythro*-epimer (*threo*/*erythro* ≈ 9:1) and chromatographic separation yielded 20% (0.13 g) of the *threo*-isomer **3**, 1% (7 mg) of the *erythro*-isomer **2**, and 43% (0.29 g) of a mixed fraction: $[\alpha]_D = -36.1$ (*c* 1.12, $CHCl_3$); IR (cm^{-1}, KBr) 3431, 2931, 1700; ^1H NMR (DMSO-d_6) δ 0.85 (3 H, t, CH_3CH$_2$), 1.2−1.55 (25 H, m, *t*-Bu, 2 CH$_3$, (CH$_2$)$_5$), 2.12−2.2 (2 H, m, CH_2(CH$_2$)$_5$CH$_3$), 3.75−3.85 (1 H, m, H−C(4)), 3.9−4.1 (2 H, m, 2H−C(5)), 4.6−4.7 (1 H, m, *H*COH), 5.5−5.55 (1 H, d, OH); HRMS (LSIMS) calcd for $C_{20}H_{36}NO_4$ $(M + H)^+$ 354.2644, found 354.2633.

***tert*-Butyl (4*S*)-4-[(1*R*)-1-Hydroxy-3-phenyl-2-propynyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (4).** To a cooled (−78 °C) solution of lithium phenylacetylide (1.0 M solution in THF, 90 mL, 90.1 mmol) in dry THF (500 mL) was added HMPA (24.3 mL, 138.6 mmol). A solution of **1**[21] (15.89 g, 69.3 mmol) in dry THF (40 mL) was added dropwise over a period of 45 min. After 5 h at −78 °C, the reaction mixture was warmed to −25 °C and quenched by the addition of a saturated NH_4Cl solution (785 mL). The aqueous layer was extracted with Et_2O (three times), and the combined organic layers were washed with 0.5 N HCl and brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (pentane/EtOAc, 9:1) yielded 14.94 g (65%) of **4** as an oil. Additionally, a mixed fraction (1.02 g, 4.5%) containing **4** and the *threo*-epimer was obtained: $[\alpha]_D = -71.65$ (*c* 1.85, $CHCl_3$); UV (CH_3OH) λ_{max} 242 nm (log ϵ 4.18); IR (cm^{-1}, KBr) 3435, 1692; ^1H NMR (DMSO-d_6) δ 1.3−1.6 (15 H, m, *t*-Bu, 2 CH$_3$), 3.9−4.15 (3 H, m, 2H−C(5), H−C(4)), 4.65 (1 H, br s, *H*COH), 5.82 (1 H, br s, OH), 7.3−7.45 (5 H, m, arom.); HRMS (LSIMS) calcd for $C_{19}H_{26}NO_4$ $(M + H)^+$ 332.1862, found 332.1868.

***tert*-Butyl (4*S*)-4-[(1*S*)-1-Hydroxy-3-phenyl-2-propynyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (5).** To a stirred and cooled (−20 °C) solution of phenylacetylene (4.86 mL, 44.28 mmol) in dry Et_2O (180 mL) was added dropwise *n*-BuLi (2.5 M in hexane, 18 mL, 44.28 mmol). After 1 h, anhydrous $ZnBr_2$ (10 g, 44.28 mmol) was added at 0 °C and the reaction was stirred for 2 h. Then, a cooled (−78 °C) solution of **1**[21] (6.77 g, 29.52 mmol) in dry Et_2O (40 mL) was added dropwise at −78 °C. The mixture was allowed to warm to room temperature overnight and was then quenched by the addition of a saturated NH_4Cl solution (150 mL). The aqueous layer was extracted with Et_2O (three times), and the combined organic layers were washed with 0.5 N HCl and brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (pentane/EtOAc, 85:15) yielded 2.95 g of *threo*-**5** (30%) and a mixed fraction containing both epimers (4.9 g, 50%) in a ratio *erythro*/*threo* ≈ 1:9: $[\alpha]_D = -104.6$ (*c* 1.01, $CHCl_3$); UV (CH_3OH) λ_{max} 242 nm (log ϵ 4.24); IR (cm^{-1}, KBr) 3426, 1692; ^1H NMR (DMSO-d_6) δ 1.3−1.6 (15 H, m, *t*-Bu,

2 CH_3), 3.9−4.08 (2 H, m, H−C(4), H−C(5)), 4.12−4.18 (1 H, m, H−C(5)), 4.82−4.92 (1 H, m, *H*COH), 5.85−5.95 (1 H, m, OH), 7.35−7.45 (5 H, m, arom.); HRMS (LSIMS) calcd for $C_{19}H_{26}NO_4$ $(M + H)^+$ 332.1862, found 332.1864.

***tert*-Butyl (1*S*,2*R*)-2-Hydroxy-1-(hydroxymethyl)-3-undecynylcarbamate (6).** To a solution of **2** (2.52 g, 7,13 mmol) in CH_3OH (70 mL) was added Amberlyst 15 (4 g), and the heterogeneous mixture was stirred at room temperature for 48 h. The mixture was filtered over Celite, and the solvent was evaporated. Flash chromatography of the residue (pentane/EtOAc, 6:4) afforded 1.77 g of **6** (79%) as an oil: $[\alpha]_D = -11.5$ (*c* 1.2, $CHCl_3$); IR (cm^{-1}, KBr) 3416, 1689; ^1H NMR (DMSO-d_6) δ 0.85 (3 H, t, CH_3CH$_2$), 1.2−1.45 (19 H, m, *t*-Bu, $(CH_2)_5$), 2.15 (2 H, t, 2H−C(5)), 3.38−3.55 (3 H, m, HOCH_2, H−C(1)), 4.2 (1 H, br s, H−C(2)), 4.5 (1 H, br s, OH), 5.31 (1 H, d, OH), 6.18 (1 H, d, NH); HRMS (LSIMS) calcd for $C_{17}H_{32}NO_4$ $(M + H)^+$ 314.2331, found 314.2338.

***tert*-Butyl (1*S*,2*R*)-2-Hydroxy-1-(hydroxymethyl)-4-phenyl-3-butynylcarbamate (7).** This compound was prepared as described for **6**, using 0.45 g (1.36 mmol) of **4** and 0.55 g of Amberlyst 15. Column chromatographic purification (pentane/EtOAc, 6:4) yielded 0.23 g of **7** (58%) as an oil: $[\alpha]_D = -9.72$ (*c* 1.06, $CHCl_3$); UV (CH_3OH) λ_{max} 242 nm (log ϵ 4.18); IR (cm^{-1}, KBr) 3391, 1694; ^1H NMR (DMSO-d_6) δ 1.3−1.45 (9 H, m, *t*-Bu), 3.25−3.68 (3 H, m, HOCH_2, H−C(1)), 4.42 (1 H, t, J = 6.6 Hz, H−C(2)), 4.58 (1 H, t, J = 5.5 Hz, OH), 5.64 (1 H, d, J = 6.4 Hz, OH), 6.48 (1 H, d, J = 8.9 Hz, NH), 7.35−7.45 (5 H, m, arom.); HRMS (LSIMS) calcd for $C_{16}H_{22}NO_4$ $(M + H)^+$ 292.1549, found 292.1528.

***tert*-Butyl (1*S*,2*S*)-2-Hydroxy-1-(hydroxymethyl)-3-undecynylcarbamate (8).** This compound was prepared as described for **6**, using 0.28 g (0.79 mmol) of **3** and 0.41 g of Amberlyst 15. Column chromatographic purification (pentane/EtOAc, 6:4) yielded 0.23 g of **8** (93%) as an oil: $[\alpha]_D = -15.45$ (*c* 1.76, $CHCl_3$); IR (cm^{-1}, KBr) 3446, 1738; ^1H NMR (DMSO-d_6) δ 0.85 (3 H, t, CH_3CH$_2$), 1.2−1.48 (19 H, m, *t*-Bu, $(CH_2)_5$), 2.15 (2 H, t, 2H−C(5)), 3.25−3.5 (3 H, m, HOCH_2, H−C(1)), 4.29−4.34 (1 H, m, H−C(2)), 4.59 (1 H, t, J = 5.3 Hz, OH), 5.18 (1 H, d, J = 6.7 Hz, OH), 6.15 (1 H, d, J = 7.8 Hz, NH); HRMS (LSIMS) calcd for $C_{17}H_{32}NO_4$ $(M + H)^+$ 314.2331, found 314.2344.

***tert*-Butyl (1*S*,2*S*)-2-Hydroxy-1-(hydroxymethyl)-4-phenyl-3-butynylcarbamate (9).** This compound was prepared as described for **6**, using 0.6 g (1.82 mmol) of **5** and 0.94 g of Amberlyst 15. Column chromatographic purification (pentane/EtOAc, 6:4) yielded 0.28 g of **9** (53%) as an oil: $[\alpha]_D = -19.36$ (*c* 1.1, $CHCl_3$); UV (CH_3OH) λ_{max} 242 nm (log ϵ 4.22); IR (cm^{-1}, KBr) 3415, 1692; ^1H NMR (DMSO-d_6) δ 1.35−1.42 (9 H, m, *t*-Bu), 3.4−3.69 (3 H, m, HOCH_2, H−C(1)), 4.55−4.59 (1 H, m, H−C(2)), 4.68 (1 H, t, J = 5.5 Hz, OH), 5.51 (1 H, d, J = 6.6 Hz, OH), 6.41 (1 H, d, J = 8.3 Hz, NH), 7.35−7.45 (5 H, m, arom.); HRMS (LSIMS) calcd for $C_{16}H_{22}NO_4$ $(M + H)^+$ 292.1549, found 292.1590.

***tert*-Butyl (1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-3-undecenylcarbamate (10).** A solution of **6** (1.67 g, 5.33 mmol) in dry Et_2O (10 mL) was added dropwise to Red-Al (3.33 M in toluene, 16 mL, 53.28 mmol) and dry Et_2O (20 mL) at 0 °C. The mixture was stirred at room temperature for 24 h and then quenched by adding CH_3OH (8 mL) at 0 °C. After dilution with Et_2O (80 mL) and saturated disodium tartrate (80 mL), the mixture was stirred for 2 h. The aqueous layer was extracted with Et_2O (3 × 80 mL), and the organic layers were washed with saturated disodium tartrate (2 × 50 mL) and brine and dried over $MgSO_4$. Flash chromatography (pentane/EtOAc, 6:4) yielded 1.14 g (68%) of **10**: $[\alpha]_D = -1.57$ (*c* 1.02, $CHCl_3$); IR (cm^{-1}, KBr) 3386, 1688; ^1H NMR (DMSO-d_6) δ 0.85 (3 H, t, CH_3CH$_2$), 1.17−1.45 (19 H, m, *t*-Bu, $(CH_2)_5$), 1.9−2.02 (2 H, m, 2H−C(5)), 3.24−3.35 (1 H, m, H−C(1)), 3.37−3.51 (2 H, m, HOCH_2), 3.85 (1 H, td, J = 6.5 and 5.8 Hz, H−C(2)), 4.39 (1 H, t, J = 5.5 Hz, OH), 4.76 (1 H, d, J = 5.1 Hz, OH), 5.4 (1 H, dd, J = 15.5 and 6.7 Hz, H−C(3)), 5.53 (1 H, dt, J = 15.4 and 6.4 Hz, H−C(4)), 6.18 (1 H, d, J = 9 Hz, NH); HRMS (LSIMS) calcd for $C_{17}H_{34}NO_4$ $(M + H)^+$ 316.2489, found 316.2491.

***tert*-Butyl (1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-phenyl-3-butenylcarbamate (11).** The same procedure as described for the synthesis of **10** was followed using 1.18 g (4.04 mmol) of **7** and 48.28 mmol of Red-Al. After flash chromatography (pentane/EtOAc, 1:1) 0.83 g of **11** was obtained (70%): $[\alpha]_D = 5.12$ (*c* 1.23, $CHCl_3$); UV (CH_3OH) λ_{max} 252 nm (log ϵ 4.22); IR (cm^{-1}, KBr) 3426, 1682; ^1H NMR (DMSO-d_6) δ 1.25−1.45 (9 H, m, *t*-Bu), 3.4−3.6 (3 H, m, HOCH_2, H−C(1)), 4.05−4.13 (1 H, m, H−C(2)), 4.5 (1 H, br s, OH), 5.05 (1 H, d, J = 5.7 Hz, OH), 6.26 (1 H, dd, J = 16 and 6.7 Hz, H−C(3)), 6.35 (1 H, d, J = 8.6 Hz, NH), 6.5 (1 H, d, J = 15.9 Hz, H−C(4)), 7.2−7.45 (5 H, m, arom.); HRMS (LSIMS) calcd for $C_{16}H_{24}NO_4$ $(M + H)^+$ 294.1705, found 294.1713.

***tert*-Butyl (1*S*,2*S*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-3-undecenylcarbamate (12).** The same procedure as described for the synthesis of **10** was followed using 0.18 g (0.57 mmol) of **8** and 5.7 mmol of Red-Al. After flash chromatography (pentane/EtOAc, 1:1), 0.11 g (59%) of **12** was obtained: $[\alpha]_D = -4.02$ (*c* 0.97, $CHCl_3$); IR (cm^{-1}, KBr) 3403, 1691; ^1H NMR (DMSO-d_6) δ 0.85 (3 H, t, J = 6.7 Hz, CH_3CH$_2$), 1.2−1.45 (19 H, m, *t*-Bu, $(CH_2)_5$), 1.91−2 (2 H, m, 2H−C(5)), 3.25−3.45 (3 H, m, HOCH_2, H−C(1)), 4.1 (1 H, br s, H−C(2)), 4.54 (1 H, t, J = 5.7 Hz, OH), 4.62 (1 H, d, J = 6.1 Hz, OH), 5.4 (1 H, dd, J = 15.3 and 5.4 Hz, H−C(3)), 5.55 (1 H, dt, J = 15.5 and 6.5 Hz, H−C(4)), 5.95 (1 H, d, J = 8.3 Hz, NH); HRMS (LSIMS) calcd for $C_{17}H_{34}NO_4$ $(M + H)^+$ 316.2489, found 316.2472.

***tert*-Butyl (1*S*,2*S*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-phenyl-3-butenylcarbamate (13).** The same procedure as described for the synthesis of **10** was followed using 0.32 g (1.1 mmol) of **9** and 13 mmol of Red-Al. After flash chromatography (pentane/EtOAc, 1:1), 0.24 g (75%) of **13** was obtained: $[\alpha]_D = 8.61$ (*c* 1.37, $CHCl_3$); UV (CH_3OH) λ_{max} 252 nm (log ϵ 4.08); IR (cm^{-1}, KBr) 3405, 1687; ^1H NMR (DMSO-d_6) δ 1.3−1.4 (9 H, m, *t*-Bu), 3.32−3.6 (3 H, m, HOCH_2, H−C(1)), 4.31−4.37 (1 H, m, H−C(2)), 4.61 (1 H, t, J = 5.4 Hz, OH), 4.96 (1 H, d, J = 5.7 Hz, OH), 6.15 (1H, d, J = 8.7 Hz, NH), 6.28 (1 H, dd, J = 15.9 and 5 Hz, H−C(3)), 6.54 (1 H, d, J = 16.2 Hz, H−C(4)), 7.22−7.45 (5 H, m, arom.); HRMS (LSIMS) calcd for $C_{16}H_{24}NO_4$ $(M + H)^+$ 294.1705, found 294.1676.

***tert*-Butyl (4*R*,5*S*)-2,2-Dimethyl-4-[(*E*)-1-nonenyl]-1,3-dioxan-5-ylcarbamate (14).** Pyridinium *p*-toluenesulfonate (13 mg, 0.05 mmol) was added to a solution of **10** (16 mg, 0.05 mmol) and 2,2-dimethoxypropane (0.125 mL, 1.01 mmol) in dry CH_2Cl_2 (1 mL). The reaction was stirred at room temperature overnight, concentrated under reduced pressure, and purified by flash chromatography (pentane/EtOAc, 9:1) yielding 12 mg of **14** (66%): ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3 H, t, J = 6.4 Hz, CH_3CH$_2$), 1.2−1.5 (25 H, m, *t*-Bu, 2 CH_3, $(CH_2)_5$), 2−2.1 (2 H, m, HC=CHCH_2), 3.55−3.65 (1 H, m, H−C(5)), 3.71 (1 H, dd, J = 11.4 and 3.7 Hz, H−C(6)), 3.94 (1 H, dd, J = 11.4 and 3.7 Hz, H−C(6)), 4.28−4.35 (1 H, m, H−C(4)), 5.24−5.35 (1 H, m, NH), 5.52 (1 H, dd, J = 15.5 and 6.4 Hz, *H*C=CHCH$_2$), 5.78 (1 H, dt, J = 15.4 and 6.6 Hz, HC=C*H*CH$_2$); HRMS (LSIMS) calcd for $C_{20}H_{38}NO_4$ $(M + H)^+$ 356.2801, found 356.2796.

***tert*-Butyl (4*S*,5*S*)-2,2-Dimethyl-4-[(*E*)-1-nonenyl]-1,3-dioxan-5-ylcarbamate (15).** Following the procedure described for **14**, **12** (16 mg, 0.05 mmol) was converted to **15** (11 mg, 61%): ^1H NMR ($CDCl_3$) δ 0.87 (3 H, t, CH_3CH$_2$), 1.18−1.6 (25 H, m, *t*-Bu, 2 CH_3, $(CH_2)_5$), 1.98−2.06 (2 H, m, HC=CHCH_2), 3.55 (1 H, dq, J = 9.8 and 1.9 Hz, H−C(5)), 3.75 (1 H, dd, J = 11.9 and 1.8 Hz, H−C(6)), 4.4 (1 H, dd, J = 11.9 and 1.9 Hz, H−C(6)), 4.46 (1 H, dd, J = 5.5 and 1 Hz, H−C(4)), 5.31 (1 H, d, J = 9.8 Hz, NH), 5.4 (1 H, dd, J = 15.6 and 5.8 Hz, *H*C=CHCH$_2$), 5.75 (1 H, dt, J = 14.3 and 6.5 Hz, HC=C*H*CH$_2$); HRMS (LSIMS) calcd for $C_{20}H_{38}NO_4$ $(M + H)^+$ 356.2801, found 356.2778.

***tert*-Butyl (4*R*,5*S*)-2,2-Dimethyl-4-[(*E*)-2-phenylethenyl]-1,3-dioxan-5-ylcarbamate (16).** This compound was prepared as described for **14**, using 20 mg (0.068 mmol) of **11**, 17 mg of PPTS, and 0.168 mL (1.36 mmol) of DMP: ^1H NMR ($CDCl_3$) δ 1.25−1.7 (15 H, m, *t*-Bu, 2 CH_3), 3.67−3.81 (2 H, m, H−C(5), H−C(6)), 4.01 (1 H, dd, J = 11.1 and 3.6 Hz, H−C(6)), 4.54−4.61 (1 H, m, H−C(4)), 6.28 (1H, dd, J = 16 and 6 Hz,

*H*C═CHPh), 6.71 (1 H, d, J = 16 Hz, HC═C*H*Ph), 7.3−7.45 (5 H, m, arom.).

***tert*-Butyl (4*S*,5*S*)-2,2-Dimethyl-4-[(*E*)-2-phenylethenyl]-1,3-dioxan-5-ylcarbamate (17).** The same procedure as described for **16** was followed, using 27 mg (0.09 mmol) of **13**, 23 mg of PPTS, and 0.226 mL (1.83 mmol) of DMP: ^{1}H NMR ($CDCl_3$) δ 1.32−1.6 (15 H, m, *t*-Bu, 2 CH_3), 3.68 (1 H, d, J = 9.8 Hz, H−C(5)), 3.82 (1H, d, J = 12 Hz, H−C(6)), 4.17 (1 H, d, J = 12 Hz, H−C(6)), 4.69−4.74 (1 H, m, H−C(4)), 5.36 (1 H, d, J = 10 Hz, NH), 6.13 (1H, dd, J = 16 and 4.8 Hz, *H*C═CHPh), 6.65 (1 H, d, J = 16 Hz, HC═C*H*Ph), 7.28−7.42 (5 H, m, arom.).

(2*S*,3*R*,4*E*)-2-Amino-4-dodecene-1,3-diol (18). To a solution of **10** (0.28 g, 0.87 mmol) in dioxane (9 mL) was added 1 N HCl (4.35 mL). The solution was stirred for 30 min at 100 °C and, after cooling to room temperature, neutralized with 1 N NaOH (4.35 mL). The mixture was extracted with Et_2O (3×), and the combined organic layers were washed with brine and dried over $MgSO_4$. For identification purposes, a small amount of the obtained (0.18 g) crude amine was purified by flash chromatography (CH_2Cl_2/CH_3OH/2 M NH_3, 80:20:2): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (3 H, t, J = 7 Hz, CH_3−C(11)), 1.24−1.43 (10 H, m, $(CH_2)_5$), 1.82 (4 H, br s, NH_2, 2 OH), 2.08 (2 H, q, J = 7.1 Hz, 2H−C(6)), 2.95 (1 H, br s, H−C(2)), 3.58−3.78 (2 H, m, 2H−C(1)), 4.1 (1 H, br s, H−C(3)), 5.49 (1 H, dd, J = 14.9 and 6.1 Hz, H−C(4)), 5.77 (1 H, dt, J = 15.3 and 6.8 Hz, H−C(5)); HRMS (LSIMS) calcd for $C_{12}H_{26}NO_2$ $(M + H)^+$ 216.1964, found 216.1966. Anal. ($C_{12}H_{25}NO_2$) N; C: calcd, 66.93; found, 66. H: calcd, 11.7; found, 11.02.

(2*S*,3*R*,4*E*)-2-Acetamido-4-dodecene-1,3-diol (18a). The crude amine **18** (0.18 g, 0.82 mmol) was dissolved in dry CH_2Cl_2 (12 mL), to which HOBT (0.12 g, 0.87 mmol), acetic acid (40 μL, 0.7 mmol), and DIC (136 μL, 0.87 mmol) were added. The mixture was kept overnight at room temperature and then concentrated under reduced pressure. Flash chromatography (CH_2Cl_2/CH_3OH, 95:5) afforded 0.15 g of **18a**. The mixture of esters formed during the reaction was treated with a 0.15 M solution of K_2CO_3 in CH_3OH. After hydrolysis to **18a**, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic layers were dried over $MgSO_4$, and after flash chromatography (CH_2Cl_2/CH_3OH, 95:5), the total yield of **18a** was 0.16 g (71%): $[\alpha]_D$ = −6.94 (*c* 0.72, $CHCl_3$); IR (cm^{-1}, KBr) 3334, 2927, 1651; ^{1}H NMR ($CDCl_3$) δ 0.87 (3 H, t, J = 6.8 Hz, CH_3−C(11)), 1.1−1.43 (12 H, m, $(CH_2)_6$), 2.01−2.09 (3 H, m, CH_3CO), 3.69 (1 H, dd, J = 11.3 and 3.2 Hz, H−C(1)), 3.86−3.91 (1 H, m, H−C(2)), 3.94 (1 H, dd, J = 11.2 and 3.8 Hz, H−C(1)), 4.28−4.33 (1 H, m, H−C(3)), 5.52 (1 H, dd, J = 15.4 and 6.3 Hz, H−C(4)), 5.78 (1 H, dt, J = 15.4 and 6.8 Hz, H−C(5)), 6.43 (1 H, d, J = 7.4 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 13.9, 22.5, 23.3, 29.0, 31.7, 32.1, 54.5 (C-2), 62.2 (C-1), 74.5 (C-3), 128.7 (C-5), 134.1 (C-4), 170.1 (C═O); HRMS (LSIMS) calcd for $C_{14}H_{28}NO_3$ $(M + H)^+$ 258.2069, found 258.2067.

(2*S*,3*R*,4*E*)-2-(Hexanoylamino)-4-dodecene-1,3-diol (18b). To a solution of the crude amine **18** (0.68 g) (prepared from **10** (0.8 g, 2.54 mmol)) in dry CH_2Cl_2 (25 mL) were added HOBT (0.34 g, 2.54 mmol), hexanoic acid (0.22 mL, 1.78 mmol), and DIC (0.4 mL, 2.54 mmol). The mixture was stirred overnight at room temperature and then concentrated under reduced pressure. Column chromatographic purification (CH_2Cl_2/CH_3OH, 95:5) afforded 0.64 g (81%) of **18b**: $[\alpha]_D$ = −5.91 (*c* 1.02, $CHCl_3$); IR (cm^{-1}, KBr) 3333, 2923, 1610; ^{1}H NMR ($CDCl_3$) δ 0.82−0.95 (6 H, m, 2 C*H*$_3$CH$_2$), 1.15−1.42 (14 H, m, 7 CH_2), 1.58−1.70 (2 H, m, C*H*$_2$CH$_2$CO)), 2.05 (2 H, q, J = 7 Hz, 2H−C(6)), 2.23 (2 H, t, J = 7.7 Hz, C*H*$_2$CO), 3.18−3.3 (2 H, m, 2 OH, exchangeable with D_2O), 3.63−3.73 (1 H, m, H−C(1)), 3.86−3.98 (2 H, m, H−C(1), H−C(2)), 4.25−4.32 (1 H, m, H−C(3)), 5.5 (1 H, dd, J = 15.4 and 6.4 Hz, H−C(4)), 5.76 (1 H, dt, J = 15.4 and 6.7 Hz, H−C(5)), 6.37 (1 H, d, J = 7.3 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 13.8, 14.0, 22.3, 22.6, 25.3, 29.0, 31.3, 31.7, 32.2, 36.7, 54.5 (C-2), 62.4 (C-1), 74.4 (C-3), 128.8 (C-5), 134.1 (C-4), 173.9 (C═O); HRMS (LSIMS) calcd for $C_{18}H_{36}NO_3$ $(M + H)^+$ 314.2695, found 314.2716. Anal. ($C_{18}H_{35}NO_3$) H, N; C: calcd, 68.97; found, 70.01.

(2*S*,3*S*,4*E*)-2-Amino-4-dodecene-1,3-diol (19). The same procedure as described for **18** was followed, starting from compound **12**. Alternatively, **19** can be obtained by treatment of **3** with lithium and $EtNH_2$. Thus, $EtNH_2$ (6 mL) was added to lithium (0.15 g, 0.02 mol) at −78 °C under N_2. After 2 h, a solution of **3** (0.5 g, 1.41 mmol) in dry THF (8 mL) was added dropwise to the mixture. The reaction was quenched after 4.5 h by adding a saturated NH_4Cl solution (5 mL). $EtNH_2$ and THF were removed under reduced pressure, and the residue was diluted with H_2O (20 mL) and extracted with Et_2O (4 × 25 mL). The organic layers were washed with brine and dried over Na_2SO_4, which afforded 0.4 g of crude **19**. For identification purposes, a small amount was purified by flash chromatography (CH_2Cl_2/CH_3OH/2 M NH_3, 80:20:2): $[\alpha]_D$ = −16.79 (*c* 0.53, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.88 (3 H, t, J = 6.8 Hz, CH_3−C(11)), 1.2−1.42 (10 H, m, $(CH_2)_5$), 2.05 (2 H, q, J = 6.9 Hz, 2H−C(6)), 2.9 (1 H, br s, H−C(2)), 3.05 (4 H, br s, exchangeable with D_2O, NH_2 and 2 OH), 3.53−3.6 (1 H, m, H−C(1)), 3.68−3.75 (1 H, m, H−C(1)), 4.05 (1 H, br s, H−C(3)), 5.44 (1 H, dd, J = 15.4 and 6.7 Hz, H−C(4)), 5.75 (1 H, dt, J = 15.4 and 6.6 Hz, H−C(5)); HRMS (LSIMS) calcd for $C_{12}H_{26}NO_2$ $(M + H)^+$ 216.1964, found 216.1978.

(2*S*,3*S*,4*E*)-2-(Hexanoylamino)-4-dodecene-1,3-diol (19a). To a solution of crude amine **19** (0.4 g) in THF (9 mL) and a 50% aqueous NaOAc solution (9 mL) was added hexanoyl chloride (0.16 mL, 1.16 mmol). After 2.5 h, the reaction mixture was diluted with THF (20 mL) and brine (20 mL). The organic layer was washed with brine and dried over $MgSO_4$. Flash chromatography (CH_2Cl_2/CH_3OH, 97:3) afforded 0.3 g (62% overall from alkynol **3**) of ceramide **19a**: $[\alpha]_D$ = −7.82 (*c* 1.01, $CHCl_3$); IR (cm^{-1}, KBr) 3333, 2923, 1646; ^{1}H NMR ($CDCl_3$) δ 0.85−0.95 (6 H, m, 2 C*H*$_3$CH$_2$), 1.2−1.4 (14 H, m, 7 CH_2), 1.59−1.72 (2 H, m, C*H*$_2$CH$_2$CO), 2.03 (2 H, q, J = 6.9 Hz, 2H−C(6)), 2.22 (2 H, t, J = 7.6 Hz, C*H*$_2$CO), 2.72 (1 H, br s, OH), 2.88 (1 H, br s, OH), 3.8−3.85 (2 H, m, 2H−C(1)), 3.88−3.96 (1 H, m, H−C(2)), 4.37−4.43 (1 H, m, H−C(3)), 5.48 (1 H, dd, J = 15.4 and 6.4 Hz, H−C(4)), 5.75 (1 H, dt, J = 15.3 and 6.8 Hz, H−C(5)), 6.13 (1 H, d, J = 7.5 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 13.8, 14.0, 22.3, 22.6, 25.4, 29.0, 31.3, 31.7, 32.2, 36.7, 54.6 (C-2), 64.4 (C-1), 73.1 (C-3), 128.9 (C-5), 133.9 (C-4); HRMS (LSIMS) calcd for $C_{18}H_{34}NO_3$ $(M - H)^+$ 312.2539, found 312.2519. Anal. ($C_{18}H_{35}NO_3$) H, N; C: calcd, 68.97; found, 68.29.

(2*S*,3*R*,4*E*)-2-Amino-5-phenyl-4-pentene-1,3-diol (20). To a solution of **11** (0.9 g, 3 mmol) in dioxane (30 mL) was added 1 N HCl (15 mL). The solution was stirred for 30 min at 100 °C and, after cooling to room temperature, neutralized with 1 N NaOH (15 mL). The mixture was extracted with Et_2O (3×) and EtOAc (3×), and the combined organic layers were washed with brine and dried over $MgSO_4$ to give 0.45 g (2.31 mmol) of the crude amine **20**. For identification purposes, a small amount was purified by column chromatography (CH_2Cl_2/CH_3OH/2 M NH_3, 90:10:1): $[\alpha]_D$ = 30.8 (*c* 0.38, CH_3OH); ^{1}H NMR (CD_3OD) δ 2.94 (1 H, td, J = 5.9 and 5.3 Hz, H−C(2)), 3.56 (1 H, dd, J = 10.9 and 7 Hz, H−C(1)), 3.73 (1 H, dd, J = 11 and 4.5 Hz, H−C(1)), 4.24 (1 H, t, J = 6.4 Hz, H−C(3)), 6.3 (1 H, dd, J = 15.9 and 6.9 Hz, H−C(4)), 6.66 (1 H, d, J = 16 Hz, H−C(5)), 7.2−7.45 (5 H, m, arom.); HRMS (LSIMS) calcd for $C_{11}H_{16}NO_2$ $(M + H)^+$ 194.1181, found 194.1188.

(2*S*,3*R*,4*E*)-2-Acetamido-5-phenylpent-4-ene-1,3-diol (20a). To a solution of **20** (0.23 g, 1.18 mmol) in dry CH_2Cl_2 (10 mL) were added HOBT (0.16 g, 1.18 mmol), acetic acid (47 μL, 0.83 mmol), and DIC (185 μL, 1.18 mmol), at 0 °C. The reaction was kept overnight and then concentrated under reduced pressure. Flash chromatography (CH_2Cl_2/CH_3OH, 93:7) afforded 0.24 g of **20a** (86%): $[\alpha]_D$ = −6.5 (*c* 0.43, CH_3OH); UV (CH_3OH) λ_{max} 252 nm ($\log \epsilon$ 4.13); ^{1}H NMR (DMSO-d_6) δ 1.8 (3 H, s, CH_3CO), 3.45−3.6 (2 H, m, 2H−C(1)), 3.75−3.85 (1 H, m, H−C(2)), 4.15−4.21 (1 H, m, H−C(3)), 4.56 (1 H, t, J = 5.5 Hz, OH), 5.1 (1 H, d, J = 5.3 Hz, OH), 6.27 (1 H, dd, J = 16 and 6.3 Hz, H−C(4)), 6.52 (1 H, d, J = 16 Hz, H−C(5)), 7.15−7.41 (5 H, m, arom.), 7.62 (1 H, d, J = 8.7 Hz, NH); ^{13}C NMR (DMSO-d_6) δ 22.9 (C-2′), 55.8 (C-2), 60.4 (C-1), 71.3 (C-3), 126.3 (arom.), 127.3 (arom.), 128.6 (arom.), 129.5 (C-5),

131.6 (C-4), 137.0 (C_{ipso}), 169.4 (C═O); HRMS (LSIMS) calcd for $C_{13}H_{18}NO_3$ $(M + H)^+$ 236.1287, found 236.1281. Anal. ($C_{13}H_{17}NO_3$) H, N; C: calcd, 66.36; found, 65.9.

(2*S*,3*R*,4*E*)-2-(Butanoylamino)-5-phenylpent-4-ene-1,3-diol (20b). To a solution of the crude amine **20** (0.19 g, 0.98 mmol) in a mixture of THF (10 mL) and 50% aqueous NaOAc solution (10 mL) was added butanoyl chloride (71 μL, 0.69 mmol). After 2 h at room temperature, an additional amount of butanoyl chloride (20 μL, 0.2 mmol) was added. After 40 min, the reaction mixture was diluted with THF (50 mL), washed with brine, and dried over $MgSO_4$. Flash chromatography ($CHCl_3$/CH_3OH, 95:5) yielded 0.17 g (67%) of **20b**: UV (CH_3OH) λ_{max} 252 nm (log ϵ 4.39); ^{1}H NMR (DMSO-d_6) δ 0.8 (3 H, t, J = 7.4 Hz, CH_3CH$_2$), 1.4−1.53 (2 H, m, CH$_3$CH_2), 2.04 (2 H, t, J = 6.5 Hz, CH_2CO), 3.45−3.6 (2 H, m, 2H−C(1)), 3.75−3.85 (1 H, m, H−C(2)), 4.15−4.21 (1 H, m, H−C(3)), 4.56 (1 H, t, J = 4.3 Hz, OH), 5.11 (1 H, d, J = 4 Hz, OH), 6.26 (1 H, dd, J = 15.8 and 6.3 Hz, H−C(4)), 6.51 (1 H, d, J = 15.9 Hz, H−C(5)), 7.15−7.4 (5 H, m, arom.), 7.54 (1 H, d, J = 8.6 Hz, NH); ^{13}C NMR (DMSO-d_6) δ 13.6, 18.6, 37.5, 55.6 (C-2), 60.5 (C-1), 71.4 (C-3), 126.2 (arom.), 127.3 (arom.), 128.6 (arom.), 129.6 (C-5), 131.6 (C-4), 137.1 (C_{ipso}), 172.2 (C═O); HRMS (LSIMS) calcd for $C_{15}H_{22}NO_3$ $(M + H)^+$ 264.1600, found 264.1603. Anal. ($C_{15}H_{21}NO_3$) H; C: calcd, 68.42; found, 67.82. N: calcd, 5.32; found, 4.81.

(2*S*,3*R*,4*E*)-2-(Hexanoylamino)-5-phenylpent-4-ene-1,3-diol (20c). The crude amine **20** (0.35 g), which was prepared from **11** (0.47 g, 1.62 mmol), was dissolved in dry THF (30 mL) and Et_3N (1 mL). A solution of hexanoic anhydride (0.25 mL, 1.08 mmol) in THF (13 mL) was added dropwise, and the mixture was stirred overnight at room temperature. After concentration, the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, 95:5). The byproducts (esters) were treated with a 0.15 M K_2CO_3 solution in CH_3OH. The reaction was worked up by dilution with H_2O and extraction with CH_2Cl_2 as described for **18a**. After chromatography (CH_2Cl_2/CH_3OH, 95:5), the total yield of isolated **20c** was 0.34 g (72%). Using an alternative method, amine **20** (0.22 g, 1.13 mmol) was dissolved in dry CH_2Cl_2 (10 mL), and HOBT (0.15 g, 1.13 mmol), hexanoic acid (99 μL, 0.79 mmol), and DIC (177 μL, 1.13 mmol) were added at 0 °C to the solution. The reaction mixture was kept overnight and then concentrated under reduced pressure. Flash chromatography (CH_2Cl_2/CH_3OH, 95:5) afforded 0.32 g of **20c** (96% overall from the crude amine): $[\alpha]_D$ = −3.02 (*c* 0.63, $CHCl_3$); UV (CH_3OH) λ_{max} 252 nm (log ϵ 4.32); IR (cm^{-1}, KBr) 3442, 1639; ^{1}H NMR (DMSO-d_6) δ 0.75 (3 H, t, J = 6.9 Hz, CH_3CH$_2$), 1.10−1.23 (4 H, m, (CH$_2$)$_2$), 1.36−1.48 (2 H, m, CH_2CH$_2$CO), 2−2.1 (2 H, m, CH_2CO), 3.46−3.57 (2 H, m, 2H−C(1)), 3.74−3.82 (1 H, m, H−C(2)), 4.09−4.17 (1 H, m, H−C(3)), 4.56 (1 H, t, J = 5.5 Hz, OH), 5.11 (1 H, d, J = 5.3 Hz, OH), 6.24 (1 H, dd, J = 15.9 and 6.5 Hz, H−C(4)), 6.49 (1 H, d, J = 15.9 Hz, H−C(5)), 7.13−7.38 (5 H, m, arom.), 7.54 (1 H, d, J = 8.8 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 13.8, 22.3, 25.4, 31.3, 36.7, 54.5 (C-2), 62.3 (C-1), 74.4 (C-3), 126.5 (arom.), 127.9 (arom.), 128.3 (C-5), 128.6 (arom.), 131.8 (C-4), 136.2 (C_{ipso}), 174.1 (C═O); HRMS (LSIMS) calcd for $C_{17}H_{26}NO_3$ $(M + H)^+$ 292.1913, found 292.1909. Anal. ($C_{17}H_{25}NO_3$) C, H, N.

(2*S*,3*R*,4*E*)-2-(Octanoylamino)-5-phenylpent-4-ene-1,3-diol (20d). This compound was prepared as described for **20b**, using 0.14 g (0.7 mmol) of crude amine **20** and 108 μL (0.63 mmol) of octanoyl chloride. Chromatographic purification ($CHCl_3$/CH_3OH, 97:3) yielded 0.14 g (62%) of **20d**: UV (CH_3OH) λ_{max} 252 nm (log ϵ 4.26); ^{1}H NMR (DMSO-d_6) δ 0.82 (3 H, t, J = 7.1 Hz, CH_3CH$_2$), 1.08−1.28 (8 H, m, (CH$_2$)$_4$), 1.35−1.48 (2H, m, CH_2CH$_2$CO), 2.04 (2 H, t, J = 7.1 Hz, CH_2CO), 3.47−3.6 (2 H, m, 2H−C(1)), 3.74−3.83 (1 H, m, H−C(2)), 4.11−4.18 (1 H, m, H− C(3)), 4.55 (1 H, t, J = 5.4 Hz, OH), 5.11 (1 H, d, J = 5.4 Hz, OH), 6.24 (1 H, dd, J = 15.9 and 6.5 Hz, H−C(4)), 6.5 (1 H, d, J = 15.9 Hz, H−C(5)), 7.15−7.4 (5 H, m, arom.), 7.52 (1 H, d, J = 8.8 Hz, NH); ^{13}C NMR (DMSO-d_6) δ 14.0, 22.1, 25.5, 28.6, 31.1, 35.7, 55.5 (C-2), 60.6 (C-1), 71.4 (C-3), 126.2 (arom.), 127.3 (arom.), 128.6 (arom.), 129.6 (C-5), 131.6 (C-4), 137.0 (C_{ipso}), 172.4 (C═O); HRMS (LSIMS) calcd for $C_{19}H_{30}NO_3$ $(M + H)^+$ 320.2226, found 320.2225. Anal. ($C_{19}H_{29}NO_3$) C, H; N: calcd, 4.38; found, 3.84.

(2*S*,3*S*,4*E*)-2-Amino-5-phenyl-4-pentene-1,3-diol (21). The same procedure as described for the synthesis of **20** was followed, using 0.34 g (1.16 mmol) of **13** and 6 mL of 1 N HCl, which gave 0.23 g of crude **21**. For identification purposes, a small amount was purified by column chromatography (CH_2Cl_2/CH_3OH/2 M NH_3, 90:10:1): $[\alpha]_D$ = −28.8 (*c* 0.34, CH_3OH); ^{1}H NMR (CD_3OD) δ 2.86 (1 H, td, J = 6.2 and 4.7 Hz, H−C(2)), 3.53 (1 H, dd, J = 11 and 6.4 Hz, H−C(1)), 3.68 (1 H, dd, J = 11.1 and 4.6 Hz, H−C(1)), 4.22 (1 H, t, J = 6.2 Hz, H−C(3)), 6.28 (1 H, dd, J = 15.9 and 7 Hz, H−C(4)), 6.66 (1 H, d, J = 15.9 Hz, H−C(5)), 7.18−7.45 (5 H, m, arom.); HRMS (LSIMS) calcd for $C_{11}H_{16}NO_2$ $(M + H)^+$ 194.1181, found 194.1214.

(2*S*,3*S*,4*E*)-2-(Hexanoylamino)-5-phenylpent-4-ene-1,3-diol (21a). Crude amine **21** (0.122 g, 0.63 mmol) was dissolved in a mixture of THF (6 mL) and a 50% aqueous NaOAc solution (6 mL), to which hexanoyl chloride (71 μL, 0.51 mmol) was added dropwise. After 2 h, the reaction mixture was diluted with THF and brine. The organic layer was washed with brine and dried over $MgSO_4$. Flash chromatography on silica gel (CH_2Cl_2/CH_3OH, 95:5) afforded 0.1 g (55%) of **21a**: $[\alpha]_D$ = −13.7 (*c* 0.57, $CHCl_3$); UV (CH_3OH) λ_{max} 252 nm (log ϵ 4.31); IR (cm^{-1}, KBr) 3415, 1641; ^{1}H NMR (DMSO-d_6) δ 0.77 (3 H, t, J = 6.8 Hz, CH_3CH$_2$), 1.12−1.25 (4 H, m, (CH$_2$)$_2$), 1.36−1.5 (2 H, m, CH_2CH$_2$CO), 2−2.15 (2 H, m, CH_2CO), 3.35−3.45 (1 H, m, H−C(1)), 3.48−3.56 (1 H, m, H−C(1)), 3.82−3.91 (1 H, m, H−C(2)), 4.39−4.45 (1 H, m, H−C(3)), 4.65 (1 H, t, J = 5.3 Hz, OH), 5.12 (1 H, d, J = 4.9 Hz, OH), 6.24 (1 H, dd, J = 15.9 and 5.4 Hz, H−C(4)), 6.54 (1 H, d, J = 15.9 Hz, H−C(5)), 7.15−7.42 (6 H, m, arom., NH); ^{13}C NMR ($CDCl_3$) δ 13.7, 22.3, 25.4, 31.3, 36.8, 54.5 (C-2), 64.1 (C-1), 73 (C-3), 126.5 (arom.), 127.8 (arom.), 128.5 (arom., C-5), 131.6 (C-4), 136.2 (C_{ipso}), 174.3 (C═O); HRMS (LSIMS) calcd for $C_{17}H_{26}NO_3$ $(M + H)^+$ 292.1913, found 292.1956. Anal. ($C_{17}H_{25}NO_3$) H; C: calcd, 70.07; found, 68.89. N: calcd, 4.81; found, 3.72.

Acknowledgment. We are grateful to Prof. J. Rozenski and Prof. A. De Bruyn (Rega Institute, K.U. Leuven) for recording the mass and NMR spectra, respectively. This work was financially supported by the Flemish Institute for the Promotion of Scientific-Technological Research in Industry, IWT, Brussels, Belgium (doctoral fellowship to I.V.O.), the Special Research Fund of the University of Gent (BOZF Project No. 01101497), the Israel Science Foundation, and the Buddy Taub Foundation (research grant to A.H.F.).

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JM990091E