Synthesis and Biological Evaluation of Ceramide Analogues with Substituted Aromatic Rings or an Allylic Fluoride in the Sphingoid Moiety

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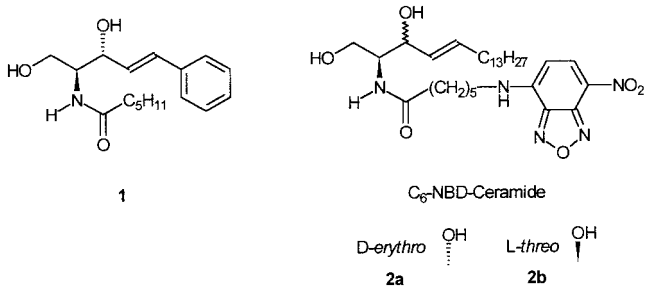
The biological activity of synthetic ceramide analogues, having modified sphingoid and *N*-acyl chains, as well as fluorine substituents in the allylic position, was investigated in hippocampal neurons. Their influence on axonal growth was compared to that of C_6-*N*-acyl analogues of natural ceramides. D-*erythro*-Ceramides with a phenyl group in the sphingoid moiety and a short *N*-acyl chain were able to reverse the inhibitory effect of fumonisin B_1 (FB_1), but not of D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), on accelerated axonal growth in hippocampal neurons. Moreover, we demonstrated that a ceramide analogue with an aromatic ring in the sphingoid moiety is recognized as a substrate by glucosylceramide synthase, which suggests that the observed biological effects are mediated by activation of the ceramide analogue via glucosylation. Introduction of a methyl, pentyl, fluoro, or methoxy substituent in the *para* position of the phenyl ring in the sphingoid moiety yielded partly active compounds. Likewise, substitution of the benzene ring for a thienyl group did not abolish the ability to reverse the inhibition of accelerated axonal growth by FB_1. Both D-*erythro*- and L-*threo*-ceramide analogues, having an allylic fluorine substituent, partly reversed the FB_1 inhibition.

Introduction

Ceramide, an intermediate in sphingolipid biosynthesis and degradation, influences a variety of cellular processes, e.g. cell differentiation and apoptosis.[1,2] Although the majority of studies on the biological activity of ceramide make use of nonneuronal cell systems, sphingolipids, and especially gangliosides, are at least as important in the nervous system, where their abundance is high.[3]

Inhibition of sphingolipid synthesis causes dramatic effects on neuronal development. In a murine neuroblastoma cell line, neurite growth was inhibited by D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), an inhibitor of glucosylceramide synthesis.[4,5] Studies with fumonisin B_1 (FB_1), an inhibitor of ceramide synthesis, demonstrated that ongoing synthesis of ceramide is necessary to sustain the axonal growth of hippocampal neurons.[6] Likewise, in cerebellar Purkinje cells, the dendrite growth was inhibited by FB_1.[7] Later it was established that glucosylceramide synthesis is required to maintain the axonal growth in hippocampal neurons.[8,9]

We previously described the synthesis of a new class of short-chain ceramides, which possess a styryl group instead of the alkenyl chain of naturally occurring sphingolipids. D-*erythro*-Ceramide analogue **1** was able to fully reverse the inhibition of FB_1 on accelerated axonal growth, when tested in hippocampal neurons.[10] However we were unable to resolve the mechanism of action of these modified ceramides. It is described that D-*erythro*-C_6-ceramide and D-*erythro*-C_6-NBD-ceramide (**2a**) must be metabolized to glucosylceramide (GlcCer) to be able to reverse the inhibition of axonal growth caused by FB_1.[8,9] This was demonstrated using different stereoisomers of C_6-ceramides. L-*threo*-C_6-NBD-ceramide (**2b**) is not a substrate for GlcCer synthase and does not reverse the inhibition by FB_1. Furthermore, neither D-*erythro*- nor L-*threo*-C_6-ceramide (or C_6-NBD-ceramide) can reverse the inhibitory effect of PDMP on accelerated axonal growth.[9]



We extended our studies by synthesizing new aromatic ceramide analogues to try to get insight in the structure−activity relationship (SAR) of the lead compound **1**. These ceramide analogues possess either a *para*-substituted benzene ring (**8a**−**d**, Scheme 1) or a thienyl group (**8e**, Scheme 1) in the sphingoid part. Regarding stereochemical implications, we focused only on the D-*erythro*-ceramides, since the L-*threo*-epimer of **1** was previously shown to be unable to reverse the inhibition of accelerated axonal development by FB_1.[10] In most of the new compounds a hexanoyl chain represented the fatty acid residue, since this was the optimum chain length for the lead compound **1**. From one of the congeners (**8b**) we also synthesized the analogues with a shorter acid residue (**8f**,**g**), because

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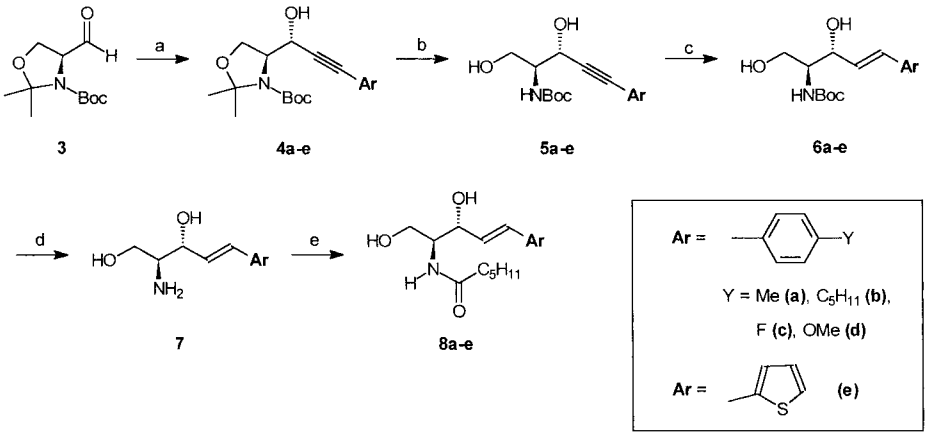
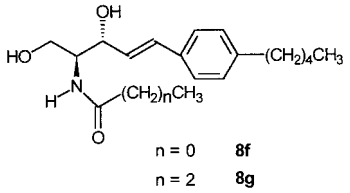
Scheme 1



(a) Ar-C≡CH, *n*-BuLi, THF, HMPA, -78 °C (23-92%); (b) *p*-TsOH, MeOH (40-82%); (c) Red-Al, Et_2O, 0 °C to rt (63-90%); (d) 1 N HCl, dioxane, 100 °C; (e) $CH_3(CH_2)_4COCl$, 33% aq NaOAc, THF (30-66%).

the introduction of a pentyl substituent on the aromatic ring increases the hydrophobicity of the molecule considerably. We, likewise, analyzed the mechanism of action of these aromatic short-chain ceramides.

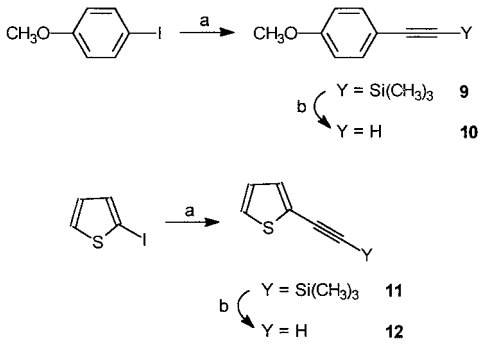


Additionally, we investigated the biological activity of fluorinated short-chain ceramides. Unlike the role of the primary hydroxyl group of sphingolipids, little is known about the function of the allylic alcohol group. Whereas the primary hydroxyl group serves as a glycosyl or phosphate acceptor site resulting in the formation of glycosphingolipids and phosphosphingolipids (sphingomyelin, ceramide phosphate), no biochemical modification of the secondary hydroxyl group is known. To further explore the importance of the allylic hydroxyl group of sphingolipids on axonal growth, we synthesized fluorinated D-*erythro*- and L-*threo*-ceramide analogues (**18** and **19**, Scheme 3) and investigated their activity in hippocampal neurons.

Results

Chemistry. A. Ceramides with an Aromatic Sphingoid Moiety. These compounds were synthesized according to the procedure described previously for the synthesis of ceramide analogues with different sphingoid chains.[10] Addition of the lithium salts of *para*-substituted phenylacetylenes to the *N*-*t*-Boc-protected oxazolidine aldehyde **3**[11] in the presence of HMPA gave *erythro* alkynols **4a**−**e** (Scheme 1). Exclusive *erythro* selectivity is explained by the Felkin−Ahn model for additions to chiral aldehydes[12] and confirmed by conversion of **4a**−**e** to the corresponding acetonides (data not shown). As reported previously,[10] determination of the $J_{4,5}$ allowed unambiguous assignment of the *erythro* configuration. Opening of the oxazolidine ring of **4a**−**e** by treatment with *p*-TsOH in CH_3OH (**5a**−**e**), followed by reduction of the triple bond to a *trans* alkene with

Scheme 2



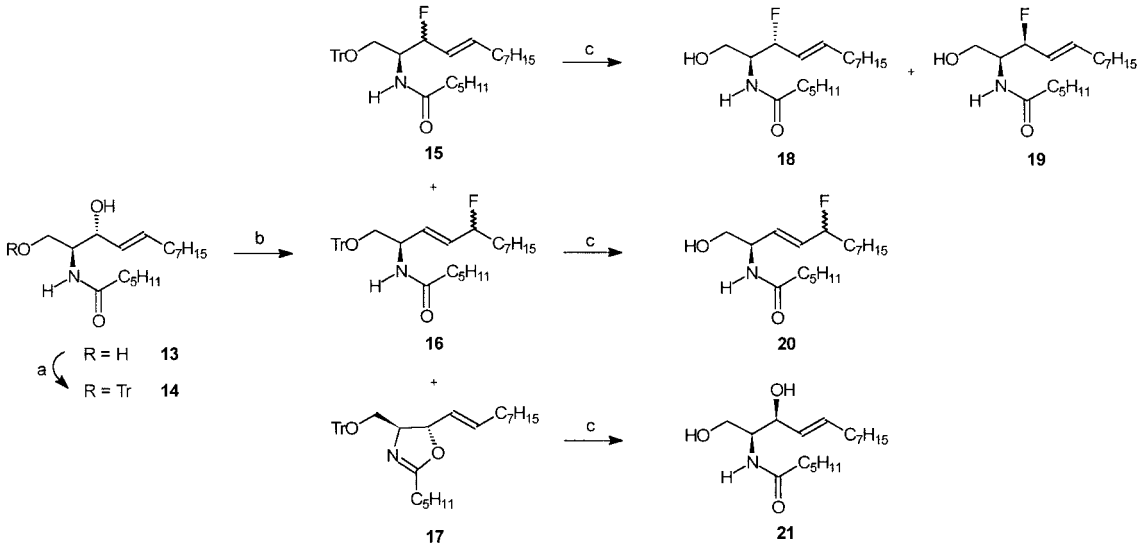
(a) $Pd(II)Cl_2(PPh_3)_2$, CuI, $(CH_3)_3SiC{\equiv}H$, Et_3N (quant.); (b) TBAF, THF.

Red-Al in Et_2O, gave the *N*-*t*-Boc-protected sphingosine analogues **6a**−**e**. Hydrolysis of the carbamates by treatment with 1 N HCl and subsequent *N*-acylation with hexanoyl chloride under Schotten−Baumann conditions[13] afforded the ceramide analogues **8a**−**e**. Additionally, amine **7b** was *N*-acylated using acetyl chloride and butanoyl chloride to afford ceramide analogues **8f**,**g**, respectively. Compounds **8a**−**c**, having a methyl, pentyl, and fluoro substituent in the *para* position of the phenyl ring, were readily obtained, as the required phenylacetylenes are commercially available.

The alkynes 4-methoxyphenylacetylene (**10**) and 2-ethynylthiophene (**12**) (Scheme 2), required for the synthesis of ceramides **8d,e**, respectively, were prepared by reaction of 4-iodoanisole and 2-iodothiophene, respectively, with trimethylsilylacetylene in the presence of bis(triphenylphosphine)palladium(II) chloride and cuprous(I) iodide and triethylamine.[14] Desilylation of **9** and **11** was carried out by treatment with TBAF in THF which afforded acetylenes **10** and **12** as volatile oils.[15] The corresponding ceramides having a *p*-chloro or *p*-bromo substituent could not be obtained in the same way as the *p*-fluoro compound as reduction with Red-Al resulted in dehalogenation of the phenyl ring.

B. Fluorinated Short-Chain Ceramides. The substitution of the secondary hydroxyl group of ceramide analogue **13**[10] (which has a short sphingoid and fatty acid chain) by a fluorine atom was investigated using diethylaminosulfur trifluoride (DAST) as fluorinating reagens.[16,17] Thus, the primary hydroxyl group of **13** was

Scheme 3



(a) TrCl, pyridine, 100 °C (90%); (b) DAST, CH_2Cl_2, -78 °C (**15**: 28%, **16**: 57%, **17**: 14%); (c) Amberlyst 15, CH_3OH (**18** and **19**: 50%, **20**: 65%, **21**: 20%).

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

treatment	no. of axonal branch points/cell at 51 h (normalized)[b]	statistical differences (p values) with bFGF-treated cells	statistical differences (p values) with (bFGF + FB_1)-treated cells
none	1.03 ± 0.06	<0.005	
bFGF	1.63 ± 0.10		<0.005
bFGF + FB_1	0.89 ± 0.08	<0.005	
bFGF + FB_1 + D-*erythro*-C_6-NBD-Cer	1.66 ± 0.08		<0.01
bFGF + FB_1 + L-*threo*-C_6-NBD-Cer	1.01 ± 0.09	<0.005	
bFGF + FB_1 + **1**	2.09 ± 0.14		<0.005
bFGF + FB_1 + **8a**	1.46 ± 0.12		<0.05
bFGF + FB_1 + **8b**	1.54 ± 0.10		<0.05
bFGF + FB_1 + **8c**	1.50 ± 0.10		<0.05
bFGF + FB_1 + **8d**	1.35 ± 0.06		<0.01
bFGF + FB_1 + **8e**	1.38 ± 0.09		<0.05
bFGF + FB_1 + **8f**	1.67 ± 0.09		<0.001
bFGF + FB_1 + **8g**	1.83 ± 0.12		<0.005

[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 three individual cultures in which 50 cells were counted per cover slip, for two individual cover slips per treatment. The number of axonal branch points is proportional to the length of the axon plexus.[9] Statistical differences, calculated using the Students' *t*-test, are shown as p values. When no p value is given, no statistical differences ($p > 0.05$) were obtained. [b] The numbers are normalized to 1 at 48 h for the particular set of data in the table.

protected as trityl ether **14** (Scheme 3). Reaction of **14** with 1.5 equiv of DAST in CH_2Cl_2 at −78 °C gave compounds **15**−**17** in a ratio of 2:4:1. ^{1}H NMR analysis proved that **15** appeared as an epimeric mixture. Based on the observation that D-*erythro* and L-*threo* epimers can be distinguished by the upfield resonance of *H*-C-OH in the ^{1}H NMR spectrum of the *erythro* compound,[10,18] it was deduced from the δ values for *H*-C-F that the L-*threo* fluoride of **15** was predominantly formed (L-*threo*/D-*erythro* ≈ 2.5:1). The main compound **16** is formed by a S_N2' type substitution of the hydroxyl group in **14**.[19]

After deprotection of **15** with Amberlyst 15 in CH_3-OH, both epimers **18** (D-*erythro*) and **19** (L-*threo*) could be separated by preparative HPLC and were obtained in pure form. In contrast, the mixture of epimers **20**, obtained from **16**, could not be separated. From the ^{13}C NMR spectrum of **20** the ratio of epimers was estimated to be ≈ 1:1. Prolonged exposure of the oxazoline derivative **17** to acid furnished the L-*threo*-ceramide **21**,[10] indicating that inversion of the configuration at the allylic position had occurred during cyclization from **14**. It also provided us with a reference compound (**21**) to investigate the biological activity of the fluorinated ceramide **19**.

Biological Evaluation of New Compounds. The ability of compounds **8a**−**g** to reverse the inhibitory effect of FB_1 on accelerated axonal growth (by basic fibroblast growth factor (bFGF)) of hippocampal neurons was evaluated as previously described.[10] D-*erythro*- and L-*threo*-C_6-NBD-ceramide (**2a**,**b**) were used as reference compounds as well as the lead compound **1**. All new compounds were able to partially or fully reverse the FB_1 inhibition (Table 1). The nature of the *p*-phenyl substituent has only a minor influence on the activity (**8a**−**d**). Substitution of the benzene ring for a thienyl group also yielded an active compound (**8e**). However, the activity of the new compounds, having a methyl (**8a**), pentyl (**8b**), fluoro (**8c**), or methoxy (**8d**) substituent, is decreased compared to that of the unsubstituted ceramide analogue **1**. When evaluating the activity of analogues **8b**,**f**,**g**, the *N*-acyl chain length was found to have a more pronounced effect on the activity. The aromatic ceramides having a *p*-pentyl substituent and

Table 2. Comparison of the Influence of Short-Chain Fluorinated and Nonfluorinated Ceramide Analogues on Axonal Growth[a]

treatment	no. of axonal branch points/cell at 51 h (normalized)[b]	statistical differences (p values) with bFGF-treated cells	statistical differences (p values) with (bFGF + FB_1)-treated cells
none	1.08 ± 0.05	<0.001	
bFGF	1.56 ± 0.07		<0.001
bFGF + FB_1	1.07 ± 0.05	<0.001	
bFGF + FB_1 + D-*erythro*-C_6-NBD-Cer	1.46 ± 0.06		<0.005
bFGF + FB_1 + L-*threo*-C_6-NBD-Cer	1.06 ± 0.05	<0.001	
bFGF + FB_1 + **18**	1.39 ± 0.07		<0.05
bFGF + FB_1 + **19**	1.29 ± 0.07	<0.05	<0.05
bFGF + FB_1 + **13**	1.43 ± 0.06		<0.01
bFGF + FB_1 + **21**	1.11 ± 0.05	<0.005	

[a] Short-chain ceramide analogues were added as indicated in Table 1. Values are means ± SEM of measurements from three individual cultures in which 50 cells were counted per cover slip, for two individual cover slips per treatment. [b] The numbers are normalized to 1 at 48 h for the particular set of data in the table.

Table 3. Inability of Ceramide Analogues To Reverse the Inhibitory Effects of PDMP on Accelerated Axonal Growth[a]

treatment	no. of axonal branch points/cell at 51 h (normalized)[b]	statistical differences (p values) with bFGF-treated cells	statistical differences (p values) with (bFGF + PDMP)-treated cells
none	1.03 ± 0.06	<0.005	
bFGF	1.63 ± 0.10		<0.005
bFGF + PDMP	1.06 ± 0.10	<0.005	
bFGF + PDMP+ D-*erythro*-C_6-NBD-Cer	0.86 ± 0.13	<0.005	
bFGF + PDMP + L-*threo*-C_6-NBD-Cer	0.94 ± 0.06	<0.005	
bFGF + PDMP + **1**	0.92 ± 0.10	<0.005	

[a] bFGF (1 ng/mL) was added to hippocampal neurons at 48 h in culture together with PDMP (50 μM) and with or without short-acyl-chain ceramide analogues (5 μM). Values are means ± SEM of measurements from three individual cultures in which 50 cells were counted per cover slip, for two individual cover slips per treatment. [b] The numbers are normalized to 1 at 48 h for the particular set of data in the table.

Table 4. Inability of Ceramide Analogues To Reverse the Inhibitory Effects of PDMP on Accelerated Axonal Growth[a]

treatment	no. of axonal branch points/cell at 51 h (normalized)[b]	statistical differences (p values) with bFGF-treated cells	statistical differences (p values) with (bFGF + PDMP)-treated cells
none	1.08 ± 0.05	<0.001	
bFGF	1.56 ± 0.07		<0.001
bFGF + PDMP	1.02 ± 0.04	<0.001	
bFGF + PDMP+ D-*erythro*-C_6-NBD-Cer	1.08 ± 0.06	<0.005	
bFGF + PDMP + L-*threo*-C_6-NBD-Cer	1.06 ± 0.06	<0.005	
bFGF + PDMP + **18**	1.12 ± 0.06	<0.005	
bFGF + PDMP + **19**	1.16 ± 0.07	<0.05	
bFGF + PDMP + **8e**	1.04 ± 0.06	<0.005	

[a] Short-chain ceramide analogues were added as indicated in Table 3. Values are means ± SEM of measurements from three individual cultures in which 50 cells were counted per cover slip, for two individual cover slips per treatment. [b] The numbers are normalized to 1 at 48 h for the particular set of data in the Table.

a C_2- or C_4-acyl chain length are the most active new compounds of the series and are able to fully reverse the FB_1 inhibition.

Likewise, the biological activity of the fluorinated compounds **18** and **19** was investigated and compared to that of the hydroxylated counterparts **13** and **21**, respectively (Table 2). D-*erythro*- and L-*threo*-C_6-NBD-ceramide served as reference compounds. Both fluorinated epimers (**18** and **19**) partly reversed the FB_1 inhibition of axonal branching in a similar manner, whereas among nonfluorinated compounds, only D-*erythro* epimers (i.e. **13**) were active.

Glucosylation as a Bioactivity Mechanism. The ability of short *N*-acyl chain ceramides (D-*erythro*-C_6-NBD-ceramide and D-*erythro*-C_6-ceramide) to affect axonal branching of hippocampal neurons has been suggested to involve the formation of GlcCer.[9] Two observations have led to the conclusion that glucosylation is important as activation step, leading to the biological activity of aromatic ceramide analogues.

First, we investigated the ability of **1**, the most active of the ceramides having an aromatic residue, to reverse the inhibition of the axonal growth by PDMP. It has been shown before that accelerated axonal growth by treatment with bFGF is inhibited by co-incubation with PDMP.[9] Neither D-*erythro*-C_6-NBD-ceramide nor L-*threo*-C_6-NBD-ceramide can reverse the PDMP effect (Tables 3 and 4). If co-incubation of PDMP and **1** would lead to a reversal of the PDMP inhibition, this should imply that the activity of **1** is independent of its metabolism to GlcCer. However, if **1** does not counteract the PDMP effect, then glucosylation may be involved in its mode of action, supposing that the compound is a substrate for GlcCer synthase. Thus, hippocampal neurons were incubated at 48 h in culture with bFGF, PDMP, and **1** and the number of axonal branch points was determined at 51 h. It was clearly demonstrated that the inhibitory effect of PDMP is not counteracted by co-incubation with ceramide analogue **1** (Table 3).

In a second step, we investigated whether glucosylation of **1** by GlcCer synthase may occur. Therefore, we examined the metabolism of its ^{14}C-labeled analogue (label in the carbonyl group) in a rat liver Golgi membrane fraction, which is a rich source of GlcCer synthase.[20,21] [^{14}C]-D-*erythro*-C_6-ceramide was used as the reference compound (Figure 1). The in vitro reactions

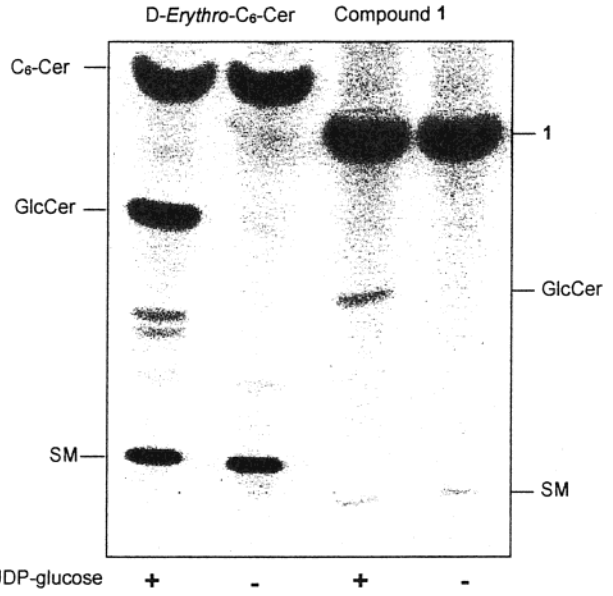


Figure 1. Metabolism of [^{14}C]-D-*erythro*-C_6-ceramide and [^{14}C]-**1** in a rat liver Golgi membrane fraction (GlcCer = glucosylceramide, SM = sphingomyelin). The in vitro reaction mixtures contained rat liver Golgi membranes (127 μg of protein/mL), UDP-glucose (5 mM), [^{14}C]-D-*erythro*-C_6-ceramide or [^{14}C]-**1** (2 nmol/mL), $MnCl_2$ (5 mM), and protease inhibitors in a total volume of 1 mL of buffer (50 mM TRIS-HCl, 25 mM KCl, pH 7.4), in the presence or absence of UDP-glucose (5 mM). The reactions were terminated after 2 h at 37 °C. Lipids were extracted[28] and separated on TLC using $CHCl_3/CH_3OH$/9.8 mM $CaCl_2$ (60:35:8, v/v/v) as the developing solvent.

of [^{14}C]-D-*erythro*-C_6-ceramide and [^{14}C]-**1** with GlcCer synthase were performed during 2 h at 37 °C, in either the presence or absence of UDP-glucose. TLC examination of the extracted lipids revealed formation of a metabolite of [^{14}C]-**1**, which, based on its R_f value, is suggested to be the glucosylated form (Figure 1, lane 3: incubation with UDP-glucose). This compound is not formed in the absence of UDP-glucose (Figure 1, lane 4). Since [^{14}C]-**1** serves as a substrate of GlcCer synthase, the presence of an alkyl chain in the sphingoid moiety is not necessary to be recognized by this enzyme. On the basis of the observations that ceramide analogue **1** reverses the inhibitory effect of FB_1 on accelerated axonal growth, but not the PDMP inhibition, and that it may act as a substrate for GlcCer synthase, we suggest that its glucosylated form is involved in the mediation of the biological effects.

In analogy with the proposed mechanism of action for **1**, it is tempting to speculate that glucosylation of the ceramide analogues, having a substituted aromatic ring in the sphingoid part (**8a**−**g**), is a prerequisite for the observed biological activity in hippocampal neurons. The inability of compound **8e** to reverse the inhibition by PDMP on accelerated axonal growth further supports this hypothesis (Table 4). The lower activity of ceramide analogues **8a**−**g** with respect to **1** (Table 1) may be related to the altered cellular uptake or recognition by GlcCer synthase.

Due to the similar activity of both epimers of the fluorinated ceramides (**18** and **19**, Table 2), their mode of action was studied in more detail. First, the potential ability to reverse the inhibitory actions of PDMP on axonal growth was assayed. Co-incubation of bFGF with PDMP and with or without fluorinated ceramide analogues was performed as described for **1**. D-*erythro*- and L-*threo*-C_6-NBD-ceramide were used in the same neuronal cultures as reference compounds (Table 4). Neither **18** nor **19** caused reversal of the PDMP-inhibition.

It is known that GlcCer synthase does not recognize L-*threo*-ceramides as substrates.[20,22] In this regard, it seemed interesting to investigate the metabolism of D-*erythro* **18** and L-*threo* **19**. To this end, the corresponding ^{14}C-labeled analogues were synthesized (label at the carbonyl group). [^{14}C]-**18** and [^{14}C]-**19** were separately incubated for 2 h at 37 °C with a rat liver Golgi membrane fraction and UDP-glucose. After reaction, the lipids were extracted and examined by TLC. The observation of a number of unknown bands on the TLCs (not shown) indicated that these ^{14}C-labeled fluorinated ceramides are unstable compounds. We also noticed that they progressively degrade upon storage in solution, even at −20 °C. However, for D-*erythro* compound **18**, comparison of incubations with and without UDP-glucose clearly indicated that **18** might be glucosylated (not shown). Thus, the allylic hydroxyl group of D-*erythro*-ceramide does not seem to be critical for recognition by GlcCer synthase. The complexity of the thin layer chromatogram, obtained for L-*threo* compound **19**, prevented us to conclusively establish whether this compound may be glucosylated or not by GlcCer synthase. Therefore, the biological activities of the fluorinated epimers **18** and **19** on axonal development (Table 2) must be interpreted with caution, since some of the effects could be due to degradation products.

Conclusion

New ceramide analogues, having either a *para*-substituted phenyl ring in the sphingoid moiety or an allylic fluoride, were synthesized, and their ability to reverse the inhibited axonal growth of hippocampal neurons, by FB_1, was investigated. Introduction of a *para* substituent on the phenyl ring of aromatic D-*erythro* analogues **8a**−**d** gave compounds of somewhat lower activity than the unsubstituted lead compound **1**. However, variation of the *N*-acyl chain length more clearly influenced the activity of *para*-substituted aromatic ceramide analogues **8f**,**g**. Substitution of the benzene ring for a thiophene group gave a partially active compound (**8e**). The activity of a D-*erythro*-ceramide analogue having an allylic fluorine substituent (**18**) was comparable to that of its hydroxylated counterpart.

We provided indications for the involvement of a glucosylation step in the mechanism of action of D-*erythro* aromatic ceramide analogues. For the fluorinated ceramide analogues the mechanism of action was not completely elucidated as the compounds were found to be unstable on prolonged incubation. These findings increase our understanding of the role of the sphingoid alkyl chain and of the allylic hydroxyl group of ceramides in the axonal growth in hippocampal neurons.

Experimental Section

Hippocampal Cultures. Hippocampal neurons were cultured at low density as previously described,[23] however, with some modifications.[6,24] The dissected hippocampi of embryonic day 18 rats (Wistar) 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 12×10^3 cells/13-mm glass cover slip (Assistent, Germany) that had been precoated with poly-L-lysine (1 mg/mL). After allowing 3−4 h for the cells to adhere to the substrate, cover slips were transferred into 24-well multidishes (Nunc) containing a monolayer of glial cells. Cover slips were placed with the neurons facing downward and separated from the glial cells by paraffin 'feet'. Cultures were maintained in serum-free medium (MEM), which included N_2 supplements,[23] ovalbumin (0.1%, w/v), and pyruvate (0.1 mM).

Addition of Compounds. Stock solutions of FB_1 and PDMP were dissolved in Hepes buffer (20 mM, pH 7.4) and added to cultures to give final concentrations of 10 and 50 μM, respectively. 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. bFGF (1 ng/mL) was added to cultures after 48 h, together with FB_1 (10 μM) or PDMP (50 μM), and sphingolipid analogues (5 μM), as indicated, and the number of axonal branch points was measured after 51 h.

Analysis of Axonal Growth. After appropriate periods of time, cover slips were removed from the 24-well multidishes; neurons were fixed in 1% (v/v) glutaraldehyde in phosphate buffered saline for 20 min at 37 °C, and mounted for microscopic examination in 50% glycerol in phosphate buffered saline. 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.[9] Thin filipodia, which were occasionally observed along the entire length of the axon, were not considered as branches. Statistical analysis was performed using the Students' *t*-test.

Synthesis of ^{14}C-Labeled Ceramides. The ^{14}C-labeled analogue of ceramide **1** was synthesized by *N*-acylation of (2*S*,3*R*,4*E*)-2-amino-5-phenyl-4-pentene-1,3-diol[10] with [^{14}C]-hexanoic acid (55 mCi/mmol) (Biotrend, Köln, Germany), in the presence of diethylphosphoryl cyanide and Et_3N.[25] Purification was performed by HPLC and UV detection using a 250 × 10 mm column (Biorad, Bio-Sil D90-10) filled with 10 μm silica, a Waters pump (model 510), a Philips Pye Unicam PU 4025 UV-detector, and a Shimadzu C-R6A Chromatopac recorder. The ^{14}C-labeled analogues of ceramides **18** and **19** were synthesized by *N*-acylation of (2*S*,3*R*,4*E*)-2-amino-3-fluoro-4-dodecen-1-ol and (2*S*,3*S*,4*E*)-2-amino-3-fluoro-4-dodecen-1-ol,[17] respectively, using the *N*-hydroxysuccinimide ester of 1-[^{14}C]hexanoic acid (55 mCi/mmol) (Biotrend, Köln, Germany).[26] Attempts to purify the compounds by preparative TLC failed due to decomposition. Therefore, the ceramides were used without further purification after isolation (Et_2O extraction) from the reaction mixture.

Glucosylceramide Synthase Assay in Rat Liver Golgi Membranes with ^{14}C-Labeled Ceramides. A Golgi membrane-enriched fraction was isolated from rat liver by the method of Dominguez et al.,[27] as follows. Rat liver was homogenized in ice-cold 0.25 M sucrose, 50 mM Tris-HCl, pH 7.4, 25 mM KCl, 5 mM $MgCl_2$, 4.5 mM $CaCl_2$ (STKCM buffer) using a motorized Potter-Elvehjem homogenizer. After centrifugation at 400*g* for 10 min, supernatants were adjusted to 0.2 M sucrose in STKCM buffer and underlayed beneath a discontinuous gradient of 0.90 and 0.4 M sucrose in STKCM. After centrifugation at 25 000 rpm for 3 h in a SW 28 rotor at 4 °C, Golgi fractions were collected at the 0.4/0.9 M sucrose interface.

The in vitro reaction mixtures contained a rat liver Golgi membrane fraction[20,21] (127 μg of protein/mL), UDP-glucose (5 mM), [^{14}C]-D-*erythro*-C_6-ceramide or [^{14}C]-**1** (2 nmol/mL), $MnCl_2$ (5 mM), and protease inhibitors (Complete, EDTA-free tablets, Boehringer Mannheim) in a total volume of 1 mL of buffer (50 mM TRIS-HCl, 25 mM KCl, pH 7.4). Control experiments were performed in the absence of UDP-glucose. The reactions were terminated after 2 h at 37 °C by addition of 3 mL of $CHCl_3/CH_3OH$ (1:2 v/v). Lipids were extracted[28] and separated on TLC using $CHCl_3/CH_3OH$/9.8 mM $CaCl_2$ (60:35:8, v/v/v) as the developing solvent.

For ^{14}C-labeled **18** and **19**, the same procedure was followed using a Golgi membrane fraction (100 μg of protein/mL), UDP-glucose (5 mM), [^{14}C]-D-*erythro*-C_6-ceramide or ^{14}C-labeled **18** or **19** (5 nmol/mL), $MnCl_2$ (5 mM), and protease inhibitors (Complete, EDTA-free) in a total volume of 1 mL of buffer (pH 7.4). Lipids were, after extraction, separated on TLC using $CHCl_3/CH_3OH/NH_4OH/H_2O$ (160:40:1:3, v/v/v/v) as the developing solvent.

Synthesis. General. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20 °C. IR spectra were recorded with a Perkin-Elmer 1600 Series FTIR spectrometer. ^{1}H NMR spectra were recorded at 500 MHz (Brücker AM-500 or Varian Unity 500) or at 360 MHz (Brücker WH-360). ^{13}C NMR spectra were recorded at 50 MHz (Varian Gemini-200), 90 MHz (Brücker WH-360) or 125 MHz (Brücker AM-500 or Varian Unity 500), as indicated. Proton and carbon chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$ (^{1}H NMR, 7.23 ppm; ^{13}C NMR, 77.00 ppm), CD_3OD (^{1}H NMR, 3.35 ppm) or DMSO-d_6 (^{1}H NMR, 2.50 ppm; ^{13}C NMR, 39.70 ppm). Liquid secondary-ion mass spectra (LSIMS) were obtained using a Kratos concept ^{1}H 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). Some compounds were purified by HPLC using a RI-detector (Metz RI-detector LCD 312), a 250 × 10 mm column (Biorad, Bio-Sil D90-10) filled with 10 μm silica, and a Kontron 422 pump. 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-3-(4-methylphenyl)-2-propynyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (4a).** To a stirred and cooled (−20 °C) solution of 4-ethynyltoluene (1.37 mL, 10.8 mmol) in dry THF (50 mL) was added dropwise *n*-BuLi (1.6 M in hexane, 5 mL, 8.1 mmol). After standing for 2 h, the solution was further cooled to −78 °C and HMPA (1.89 mL, 10.8 mmol) was added, followed by a cooled (−78 °C) solution of **3**[11](1.46 g, 6.38 mmol) in dry THF (4 mL). After standing for 3 h at −78 °C, the reaction mixture was warmed to −20 °C and quenched by addition of a saturated NH_4Cl solution (75 mL). The aqueous layer was extracted with Et_2O (3×) 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 1.31 g (70%) of **4a** as a colorless oil: IR (cm^{-1}, KBr) 3432, 2975, 2250, 1696; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35−1.50 (15 H, m, *t*-Bu, 2 CH_3), 2.30 (3 H, s, CH_3−Ph), 3.90−4.12 (3 H, m, 2H−C(5), H−C(4)), 4.64 (1 H, t, J = 5.3 Hz, *H*COH), 5.74−5.79 (1 H, m, OH), 7.15−7.20 (2 H, m, arom), 7.30 (2 H, d, J = 7.9 Hz, arom); HRMS (LSIMS) calcd for $C_{20}H_{28}NO_4$ $(M + H)^+$ 346.2018, found 346.2001.

***tert*-Butyl (1*S*,2*R*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-methylphenyl)-3-butynylcarbamate (5a).** To a solution of **4a** (1.06 g, 3.06 mmol) in CH_3OH (20 mL) was added *p*-TsOH (0.07 g, 0.37 mmol); the reaction mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with saturated $NaHCO_3$ and H_2O, and dried over $MgSO_4$. Flash chromatography (pentane/EtOAc, 6:4) afforded 0.6 g of **5a** (64%) as a colorless oil: IR (cm^{-1}, KBr) 3412, 2975, 2250, 1690; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30−1.50 (9 H, m, *t*-Bu), 2.30 (3 H, s, CH_3−Ph), 3.45−3.65 (3 H, m, HOCH_2, H−C(1)), 4.40 (1 H, t, J = 6.5 Hz, H−C(2)), 4.58 (1 H, m, OH), 5.61 (1 H, d, J = 6.3 Hz, OH), 6.48 (1 H, d, J = 8.8 Hz, NH), 7.18 (2 H, d, J = 7.7 Hz, arom), 7.30 (2 H, d, J = 7.9 Hz, arom); HRMS (LSIMS) calcd for $C_{17}H_{24}NO_4$ $(M + H)^+$ 306.1705, found 306.1729.

***tert*-Butyl (1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-methylphenyl)-3-butenylcarbamate (6a).** A solution of **5a** (0.44 g, 1.44 mmol) in dry Et_2O (2 mL) was added dropwise to Red-Al (3.33 M in toluene, 5.2 mL, 17 mmol) and dry Et_2O (5 mL) at 0 °C. The mixture was stirred overnight at room temperature and then quenched by adding CH_3OH (2.5 mL) at 0 °C. After dilution with Et_2O (35 mL) and saturated disodium tartrate (35 mL), the mixture was stirred for 2 h. The aqueous layer was extracted with Et_2O (3×) and the organic layers were washed with saturated disodium tartrate (2×) and brine and dried over $MgSO_4$. Flash chromatography (pentane/EtOAc, 6:4) yielded 0.35 g (79%) of **6a**: IR (cm^{-1}, KBr) 3432, 1685; ^1H NMR (500 MHz, DMSO-d_6) δ 1.32 (9 H, s, *t*-Bu), 2.28 (3 H, s, CH_3−Ph), 3.40−3.56 (3 H, m, HOCH_2, H−C(1)), 4.04−4.09 (1 H, m, H−C(2)), 4.48 (1 H, t, J = 5.4 Hz, OH), 5.03 (1 H, d, J = 5.0 Hz, OH), 6.19 (1 H, dd, J = 15.9 and 6.8 Hz, H−C(3)), 6.36 (1 H, d, J = 8.6 Hz, NH), 6.44 (1 H, d, J = 15.9 Hz, H−C(4)), 7.11 (2 H, d, J = 7.8 Hz, arom), 7.27 (2 H, d, J = 7.9 Hz, arom); HRMS (LSIMS) calcd for $C_{17}H_{25}NO_4Na$ $(M + Na)^+$ 330.1681, found 330.1659.

(2*S*,3*R*,4*E*)-2-Amino-5-(4-methylphenyl)-4-pentene-1,3-diol (7a). To a solution of **6a** (0.198 g, 0.64 mmol) in dioxane (6.5 mL) was added 1 N HCl (3.25 mL). The solution was stirred for 30 min at 100 °C and, after cooling to room temperature, neutralized with 1 N NaOH (3.25 mL). The aqueous layer was extracted several times with EtOAc and the combined organic layers were concentrated under reduced pressure. Flash chromatography (CH_2Cl_2/CH_3OH/2 M NH_3, 80:20:2) gave 0.097 g (73%) of **7a** (*erythro*/*threo* as estimated from TLC: 95:5): ^1H NMR (500 MHz, CD_3OD) δ 2.30 (3 H, s, CH_3−Ph), 3.03−3.08 (1 H, m, H−C(2)), 3.61 (1 H, dd, J = 11.3 and 7.5 Hz, H−C(1)), 3.76 (1 H, dd, J = 11.2 and 4.4 Hz, H−C(1)), 4.30 (1 H, t, J = 6.2 Hz, H−C(3)), 6.22 (1 H, dd, J = 15.9 and 7.0 Hz, H−C(4)), 6.65 (1 H, d, J = 15.9 Hz, H−C(5)), 7.13 (2 H, d, J = 8.0 Hz, arom), 7.32 (2 H, d, J = 8.1 Hz, arom).

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-methylphenyl)-3-butenyl]hexanamide (8a).** To a solution of crude **7a** (0.062 g, 0.3 mmol) in a mixture of THF (2.5 mL) and a 33% aqueous NaOAc (2.5 mL) was added hexanoyl chloride (34 μL, 0.24 mmol). The mixture was stirred at room temperature overnight and then diluted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Flash chromatography ($CHCl_3$/CH_3OH, 97:3) and HPLC ($CHCl_3$/CH_3OH, 98:2) afforded 0.06 g (66%) of **8a**: IR (cm^{-1}, KBr) 3426, 1641; ^1H NMR (500 MHz, DMSO-d_6) δ 0.76 (3 H, t, J = 7.0 Hz, CH_3CH$_2$), 1.10−1.20 (4 H, m, $(CH_2)_2$), 1.36−1.44 (2 H, m, CH_2), 2.04 (2 H, t, J = 7.5 Hz, COCH_2), 2.28 (3 H, s, CH_3−Ph), 3.47−3.56 (2 H, m, HOCH_2), 3.74−3.80 (1 H, m, H−C(1)), 4.10−4.15 (1 H, dd, J = 6.3 and 5.8 Hz, H−C(2)), 4.56 (1 H, t, J = 5.3 Hz, OH), 5.08 (1 H, d, J = 5.3 Hz, OH), 6.18 (1 H, dd, J = 15.9 and 6.6 Hz, H−C(3)), 6.45 (1 H, d, J = 15.9 Hz, H−C(4)), 7.12 (2 H, d, J = 8.0 Hz, arom), 7.25 (2 H, d, J = 8.1 Hz, arom), 7.53 (1 H, d, J = 8.8 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.8, 20.8, 22.0, 25.2, 31.0, 35.6, 55.5 (C-1), 60.5 (HOCH_2), 71.4 (C-2), 126.2 (arom), 129.2 (arom), 129.5 (C-3), 130.5 (C-4), 134.2 (C$_{ipso}$), 136.5 (C$_{ipso}$), 172.3 (C=O); HRMS (LSIMS) calcd for $C_{18}H_{27}NO_3Na$ $(M + Na)^+$ 328.1889, found 328.1862.

***tert*-Butyl (4*S*)-4-[(1*R*)-1-Hydroxy-3-(4-pentylphenyl)-2-propynyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (4b).** The same procedure, as described for the synthesis of **4a**, was followed using 1-ethynyl-4-pentylbenzene. Thus, 1.5 g (6.54 mmol) of **3**,[11] 2 g (12 mmol) of 1-ethynyl-4-pentylbenzene, 5.4 mL *n*-BuLi (1.6 M in hexane, 8.7 mmol), and 2 mL (12 mmol) HMPA gave, after flash chromatography (pentane/EtOAc, 9:1), 2 g (76%) of **4b**: IR (cm^{-1}, KBr) 3415, 3056, 2985, 2933, 2239, 1692, 1663; ^1H NMR (360 MHz, DMSO-d_6) δ 0.84 (3 H, t, J = 7 Hz, CH_3CH$_2$), 1.20−1.33 (4 H, m, $(CH_2)_2$), 1.37−1.60 (17 H, m, *t*-Bu, 2 CH_3, CH_2), 2.56 (2 H, t, J = 7.6 Hz, CH_2−Ph), 3.90−4.11 (3 H, m, 2H−C(5), H−C(4)), 4.63−4.68 (1 H, m, *H*COH), 5.79 (1 H, m, OH), 7.15−7.22 (2 H, m, arom), 7.32 (2 H, d, J = 8.1 Hz, arom); HRMS (LSIMS) calcd for $C_{24}H_{36}NO_4$ $(M + H)^+$ 402.2644, found 402.2616.

***tert*-Butyl (1*S*,2*R*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-pentylphenyl)-3-butynylcarbamate (5b).** The same procedure, as described for the synthesis of **5a**, was followed using 2 g (5 mmol) of **4b** and 0.19 g (1 mmol) of *p*-TsOH. Thus, 1.48 g (82%) of **5b** was obtained: IR (cm^{-1}, KBr) 3405, 2934, 2227, 1692; ^1H NMR (500 MHz, DMSO-d_6) δ 0.84 (3 H, t, J = 7.1 Hz, CH_3CH$_2$), 1.20−1.33 (4 H, m, $(CH_2)_2$), 1.37 (9 H, s, *t*-Bu), 1.54 (2 H, quintet, J = 7.5 Hz, CH_2), 2.56 (2 H, t, J = 7.6 Hz, CH_2−Ph), 3.45−3.51 (1 H, m, HOC*H*H), 3.55−3.65 (2 H, m, HOCH*H*, H−C(1)), 4.40 (1 H, m, H−C(2)), 4.58 (1 H, t, J = 5.3 Hz, OH, exchangeable with D_2O), 5.61 (1 H, d, J = 5.0 Hz, OH, exchangeable with D_2O), 6.45 (1 H, d, J = 8.9 Hz, NH, exchangeable with D_2O), 7.17 (2 H, d, J = 8.0 Hz, arom), 7.30 (2 H, d, J = 8.0 Hz, arom); HRMS (LSIMS) calcd for $C_{21}H_{32}NO_4$ $(M + H)^+$ 362.2331, found 362.2384.

***tert*-Butyl (1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-pentylphenyl)-3-butenylcarbamate (6b).** The same procedure, as described for the synthesis of **6a**, was followed using 1.48 g (4.09 mmol) of **5b** and 13.3 mL Red-Al (3.33 M in toluene, 0.044 mol). After standing for 24 h, the reaction was worked up in the usual way. Flash chromatography (pentane/EtOAc, 6:4) yielded 1.21 g (90%) of **6b**: IR (cm^{-1}, KBr) 3426, 2933, 1687; ^1H NMR (500 MHz, DMSO-d_6) δ 0.85 (3 H, t, J = 7.1 Hz, CH_3CH$_2$), 1.20−1.40 (13 H, m, *t*-Bu, $(CH_2)_2$), 1.54 (2 H, t, J = 6.7 Hz, CH_2), 2.53 (2 H, t, J = 7.0 Hz, CH_2−Ph), 3.40−3.56 (3 H, m, HOCH_2, H−C(1)), 4.08 (1 H, br s, H−C(2)), 4.47 (1 H, br s, OH), 5.00 (1 H, br s, OH), 6.20 (1 H, dd, J = 15.3 and 6.4 Hz, H−C(3)), 6.34 (1 H, d, J = 8.0 Hz, NH), 6.45 (1 H, d, J = 15.9 Hz, H−C(4)), 7.12 (2 H, d, J = 7.0 Hz, arom), 7.27 (2 H, d, J = 6.9 Hz, arom); HRMS (LSIMS) calcd for $C_{21}H_{34}NO_4$ $(M + H)^+$ 364.2488, found 364.2419.

(2*S*,3*R*,4*E*)-2-Amino-5-(4-pentylphenyl)-4-pentene-1,3-diol (7b). The same procedure, as described for the synthesis of **7a**, was followed using 0.76 g (2.09 mmol) of **6b**, 10.5 mL 1 N HCl and 21 mL dioxane. An analytical sample was obtained by purification of the crude amine (0.95 g obtained) using flash chromatography ($CHCl_3$/CH_3OH/2 M NH_3, 90:10:1): ^1H NMR (500 MHz, CD_3OD) δ 0.89 (3 H, t, J = 7.1 Hz, CH_3CH$_2$), 1.26−1.38 (4 H, m, $(CH_2)_2$), 1.60 (2 H, quintet, J = 7.5 Hz, CH_2), 2.57 (2 H, t, J = 7.7 Hz, CH_2−Ph), 2.87−2.92 (1 H, m, H−C(2)), 3.54 (1 H, dd, J = 10.9 and 7.0 Hz, H−C(1)), 3.72 (1 H, dd, J = 11.0 and 4.6 Hz, H−C(1)), 4.20 (1 H, dt, J = 6.5 and 0.9 Hz, H−C(3)), 6.25 (1 H, dd, J = 15.9 and 7.2 Hz, H−C(4)), 6.62 (1 H, d, J = 15.9 Hz, H−C(5)), 7.12 (2 H, d, J = 8.1 Hz, arom), 7.34 (2 H, d, J = 8.1 Hz, arom); HRMS (LSIMS) calcd for $C_{16}H_{25}NO_2Na$ $(M + Na)^+$ 286.1783, found 286.1817.

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-pentylphenyl)-3-butenyl]hexanamide (8b).** To a solution of crude amine **7b** (0.137 g, 0.52 mmol) in a mixture of THF (4 mL) and 33% aqueous NaOAc (4 mL) was added hexanoyl chloride (72 μL, 0.51 mmol). The reaction mixture was stirred at room temperature overnight and then diluted with Et_2O. The organic layer was washed with brine and separated. The aqueous layer was extracted with Et_2O (2×). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. HPLC purification (CH_2Cl_2/CH_3OH, 97:3) yielded 0.092 g (49%) of **8b**: IR (cm^{-1}, KBr) 3426, 1641; ^1H NMR (500 MHz, DMSO-d_6) δ 0.80−0.88 (6 H, m, 2 CH_3CH$_2$), 1.10−1.15 (4 H, m, $(CH_2)_2$), 1.22−1.30 (4 H, m, $(CH_2)_2$), 1.35−1.43 (2 H, m, COCH$_2$CH_2), 1.50−1.58 (2 H, m, CH_2), 2.02−2.08 (2 H, m, COCH_2), 2.55−2.57 (2 H, m, CH_2−Ph), 3.46−3.55 (2 H, m, HOCH_2), 3.75−3.80 (1 H, m, H−C(1)), 4.12 (1 H, t, J = 6.5 Hz, H−C(2)), 4.55 (1 H, br s, OH), 5.10 (1 H, br s, OH), 6.17 (1 H, dd, J = 15.9 and 6.6 Hz, H−C(3)), 6.44 (1 H, d, J = 15.9 Hz, H−C(4)), 7.12 (2 H, d, J = 8.0 Hz, arom), 7.26 (2 H, d, J = 8.0 Hz, arom), 7.52 (1 H, d, J = 8.8 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.9, 22.0, 25.2, 30.9, 34.9, 35.6, 55.5 (C-1), 60.5 (HOCH_2), 71.5 (C-2), 126.2 (arom), 128.5 (arom), 129.6 (C-3), 130.6 (C-4), 134.5 (C$_{ipso}$), 141.5 (C$_{ipso}$), 172.3 (C=O); HRMS (LSIMS) calcd for $C_{22}H_{35}NO_3Na$ $(M + Na)^+$ 384.2515, found 384.2513. Anal. ($C_{22}H_{35}NO_3 \cdot {}^1/_6CH_3OH$) C, N; H: calcd, 9.76; found, 9.26.

***tert*-Butyl (4*S*)-4-[(1*R*)-3-(4-Fluorophenyl)-1-hydroxy-2-propynyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate**

(4c). The same procedure, as described for the synthesis of **4a**, was followed using 1-ethynyl-4-fluorobenzene. Thus, 0.95 g (4.2 mmol) of **3**,[11] 1 g (8.3 mmol) of 1-ethynyl-4-fluorobenzene, 4.55 mL *n*-BuLi (1.6 M in hexane, 7.3 mmol), and 1.46 mL (8.3 mmol) HMPA gave 1.3 g (89%) of **4c**. An analytical sample was obtained by HPLC purification (hexane/EtOAc, 82.5:17.5): IR (cm^{-1}, KBr) 3354, 2242, 1692, 1662; ^1H NMR (500 MHz, DMSO-d_6) δ 1.35−1.53 (15 H, m, *t*-Bu, 2 CH_3), 3.90−4.10 (3 H, m, 2H−C(5), H−C(4)), 4.60−4.67 (1 H, m, *H*COH), 5.84 (1 H, t, J = 7.1 Hz, OH), 7.19−7.25 (2 H, m, arom), 7.45 (2 H, t, J = 6.3 Hz, arom); HRMS (LSIMS) calcd for $C_{19}H_{24}NO_4FNa$ $(M + Na)^+$ 372.1587, found 372.1583.

***tert*-Butyl (1*S*,2*R*)-4-(4-Fluorophenyl)-2-hydroxy-1-(hydroxymethyl)-3-butynylcarbamate (5c).** The same procedure, as described for the synthesis of **5a**, was followed using 1.3 g (3.74 mmol) of **4c** and 0.142 g (0.75 mmol) of *p*-TsOH. Thus, 0.83 g (72%) of **5c** was obtained. A small amount was purified by HPLC (hexane/EtOAc, 1:1): IR (cm^{-1}, KBr) 3413, 3056, 2985, 2262, 1703; ^1H NMR (500 MHz, DMSO-d_6) δ 1.35 (9 H, s, *t*-Bu), 3.44−3.51 (1 H, m, HOC*H*H), 3.53−3.66 (2 H, m, HOC*H*H, H−C(1)), 4.40 (1 H, t, J = 6.7 Hz, H−C(2)), 4.60 (1 H, t, J = 5.6 Hz, OH), 5.65 (1 H, d, J = 6.4 Hz, OH), 6.50 (1 H, d, J = 9.0 Hz, NH), 7.22 (2 H, t, J = 8.8 Hz, arom), 7.45 (2 H, dd, J = 8.5 Hz en 5.7 Hz, arom); HRMS (LSIMS) calcd for $C_{16}H_{20}NO_4FNa$ $(M + Na)^+$ 332.1274, found 332.1298.

***tert*-Butyl (1*S*,2*R*,3*E*)-4-(4-Fluorophenyl)-2-hydroxy-1-(hydroxymethyl)-3-butenylcarbamate (6c).** The same procedure, as described for the synthesis of **6a**, was followed using 0.7 g (2.26 mmol) of **5c** and 3.4 mL Red-Al (3.33 M in toluene, 11.3 mmol). After standing for 24 h, the reaction was worked up in the usual way. Flash chromatography (hexane/EtOAc, 45:55) yielded 0.44 g (63%) of **6c**: IR (cm^{-1}, KBr) 3436, 1641; ^1H NMR (500 MHz, DMSO-d_6) δ 1.30 (9 H, s, *t*-Bu), 3.40−3.57 (3 H, m, HOCH_2, H−C(1)), 4.00−4.10 (1 H, m, H−C(2)), 4.50 (1 H, br s, OH), 5.08 (1 H, br s, OH), 6.20 (1 H, dd, J = 15.7 and 6.6 Hz, H−C(3)), 6.39 (1 H, d, J = 8.0 Hz, NH), 6.47 (1 H, d, J = 15.9 Hz, H−C(4)), 7.14 (2 H, t, J = 8.4 Hz, arom), 7.38−7.48 (2 H, m, arom); HRMS (LSIMS) calcd for $C_{16}H_{22}NO_4FNa$ $(M + Na)^+$ 334.1431, found 334.1431.

***N*-[(1*S*,2*R*,3*E*)-4-(4-Fluorophenyl)-2-hydroxy-1-(hydroxymethyl)-3-butenyl]hexanamide (8c).** To a solution of **6c** (0.215 g, 0.69 mmol) in dioxane (7 mL) was added 1 N HCl (3.45 mL). The solution was stirred for 30 min at 100 °C and, after cooling to room temperature, neutralized with 1 N NaOH (3.45 mL). The aqueous layer was extracted with EtOAc (3×) and the combined organic layers were concentrated under reduced pressure. The crude amine (**7c**) was dissolved in a mixture of THF (6 mL) and 33% aqueous NaOAc (6 mL), to which hexanoyl chloride (78 μL, 0.56 mmol) was added. After standing for 2 h, the reaction mixture was diluted with Et_2O and brine. The aqueous layer was extracted with Et_2O (2×) and the organic layers dried over $MgSO_4$. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 95:5) and HPLC (CH_2Cl_2/CH_3OH, 97:3) yielded 0.074 g (35%) of **8c**: IR (cm^{-1}, KBr) 3426, 1641; ^1H NMR (500 MHz, DMSO-d_6) δ 0.74 (3 H, t, J = 6.8 Hz, CH_3CH$_2$), 1.08−1.20 (4 H, m, $(CH_2)_2$), 1.33−1.45 (2 H, m, COCH$_2$CH_2), 2.03 (2 H, dt, J = 7.9 and 1.7 Hz, COCH_2), 3.46−3.58 (2 H, m, HOCH_2), 3.75−3.83 (1 H, m, H−C(1)), 4.10−4.15 (1 H, m, H−C(2)), 4.58 (1 H, br s, OH), 5.11 (1 H, d, J = 4.7 Hz, OH), 6.18 (1 H, dd, J = 15.9 and 6.6 Hz, H−C(3)), 6.48 (1 H, d, J = 15.9 Hz, H−C(4)), 7.14 (2 H, t, J = 8.8 Hz, arom), 7.40 (2 H, dd, J = 8.3 and 5.8 Hz, arom), 7.52 (1 H, d, J = 8.8 Hz, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 13.7, 21.9, 25.1, 30.8, 35.6, 55.4 (C-1), 60.5 (HOCH_2), 71.4 (C-2), 115.2 (arom), 115.7 (arom), 128.0 (arom), 128.2 (arom), 128.5 (C-3), 131.7 (C-4), 133.7 (C_{ipso}), 172.5 (C=O); HRMS (LSIMS) calcd for $C_{17}H_{24}NO_3FNa$ $(M + Na)^+$ 332.1638, found 332.1668. Anal. ($C_{17}H_{24}NO_3F\cdot{}^1/_6CH_3OH$) C; H: calcd, 7.82; found, 7.64; N: calcd, 4.53; found, 3.71.

***tert*-Butyl (4*S*)-4-[(1*R*)-1-Hydroxy-3-(4-methoxyphenyl)-2-propynyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (4d).** The same procedure, as described for the synthesis of **4a**, was followed using 4-methoxyphenylacetylene. Thus, 0.91 g (3.97 mmol) of **3**,[11] 1.05 g (7.94 mmol) of 4-methoxyphenylacetylene (**10**), 17.4 mL *n*-BuLi (1.6 M in hexane, 2.8 mmol), and 1.4 mL (7.94 mmol) HMPA gave, after flash chromatography (hexane/EtOAc, 82.5:17.5), 0.33 g (23%) of **4d**: IR (cm^{-1}, KBr) 3436, 2235, 1656; ^1H NMR (500 MHz, DMSO-d_6) δ 1.40−1.48 (15 H, m, *t*-Bu, 2 CH_3), 3.75 (3 H, s, CH_3O), 3.95−4.10 (3 H, m, 2H−C(5), H−C(4)), 4.63 (1 H, br s, *H*COH), 5.75 (1 H, br s, OH), 6.92 (2 H, br s, Ph-3-H, Ph-5-H), 7.34 (2 H, d, J = 8.4 Hz, Ph-2-H, Ph-6-H); HRMS (LSIMS) calcd for $C_{20}H_{27}NO_5Na$ $(M + Na)^+$ 384.1787, found 384.1795.

***tert*-Butyl (1*S*,2*R*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-methoxyphenyl)-3-butynylcarbamate (5d).** The same procedure, as described for the synthesis of **5a**, was followed using 0.52 g (1.44 mmol) of **4d** and 0.055 g (0.29 mmol) of *p*-TsOH. After standing for 3 h, the reaction was worked up in the usual way. Flash chromatography (hexane/EtOAc, 6:4) gave 0.185 g (40%) of **5d**. A small amount was purified by HPLC (hexane/EtOAc, 1:1): IR (cm^{-1}, KBr) 3427, 2965, 2233, 1693; ^1H NMR (500 MHz, DMSO-d_6) δ 1.36 (9 H, s, *t*-Bu), 3.45−3.65 (3 H, m, HOCH_2, H−C(1)), 3.75 (3 H, s, CH_3O), 4.35−4.42 (1 H, m, H−C(2)), 4.59 (1 H, br s, OH), 5.59 (1 H, d, J = 6.0 Hz, OH), 6.46 (1 H, d, J = 8.7 Hz, NH), 6.92 (2 H, d, J = 8.6 Hz, Ph-3-H, Ph-5-H), 7.33 (2 H, d, J = 8.6 Hz, Ph-2-H, Ph-6-H); HRMS (LSIMS) calcd for $C_{17}H_{23}NO_5Na$ $(M + Na)^+$ 344.1474, found 344.1451.

***tert*-Butyl (1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-methoxyphenyl)-3-butenylcarbamate (6d).** The same procedure, as described for the synthesis of **6a**, was followed using 0.15 g (0.47 mmol) of **5d** and 0.7 mL Red-Al (3.33 M in toluene, 2.34 mmol). After standing for 6 h, the reaction was worked up in the usual way to yield 0.125 g (83%) of **6d**. An analytical sample was obtained by HPLC purification (hexane/EtOAc, 45:55): ^1H NMR (500 MHz, DMSO-d_6) δ 1.28 (9 H, s, *t*-Bu), 3.40−3.56 (3 H, m, HOCH_2, H−C(1)), 3.75 (3 H, s, CH_3O), 4.00−4.08 (1 H, m, H−C(2)), 4.49 (1 H, br s, OH), 5.00 (1 H, d, J = 4.7 Hz, OH), 6.09 (1 H, dd, J = 15.8 and 6.8 Hz, H−C(3)), 6.35 (1 H, d, J = 8.4 Hz, NH), 6.41 (1 H, d, J = 15.9 Hz, H−C(4)), 6.87 (2 H, d, J = 8.3 Hz, Ph-3-H, Ph-5-H), 7.31 (2 H, d, J = 8.4 Hz, Ph-2-H, Ph-6-H); HRMS (LSIMS) calcd for $C_{17}H_{25}NO_5Na$ $(M + Na)^+$ 346.1631, found 346.1626.

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-methoxyphenyl)-3-butenyl]hexanamide (8d).** A solution of **6d** (0.12 g, 0.37 mmol) in dioxane (4 mL) was treated with 1 N HCl (1.9 mL) for 20 min at 100 °C. After cooling to room temperature, the mixture was neutralized with 1 N NaOH (1.9 mL) and the aqueous layer was extracted with Et_2O (5×) and with EtOAc (6×). The combined organic layers were concentrated to yield the crude amine (**7d**). The residue was dissolved in a mixture of THF (4 mL), 33% aqueous NaOAc (4 mL), and hexanoyl chloride (0.03 mL, 0.21 mmol). After standing for 2 h, the reaction mixture was diluted with Et_2O and brine. The aqueous layer was extracted with Et_2O (2×) and the organic layers dried over $MgSO_4$. HPLC purification (CH_2Cl_2/CH_3OH, 97:3) gave 0.046 g of **8d** (39% from **6d**): ^1H NMR (500 MHz, DMSO-d_6) δ 0.83 (3 H, t, J = 7.0 Hz), 1.12−1.29 (4 H, m, $(CH_2)_2$), 1.33−1.53 (2 H, m, CH_2), 2.00−2.12 (2 H, m, CH_2), 3.43−3.49 (2 H, m, HOCH_2), 3.56−3.62 (1 H, m, H−C(1)), 3.72 (3 H, s, CH_3O), 4.32−4.36 (1 H, m, H−C(2)), 4.61 (1 H, t, J = 5.5 Hz, OH), 5.04 (1 H, d, J = 5.0 Hz, OH), 6.05 (1 H, dd, J = 15.9 and 5.9 Hz, H−C(3)), 6.44 (1 H, d, J = 16.1 Hz, H−C(4)), 6.86 (2 H, d, J = 8.5 Hz, Ph-3-H, Ph-5-H), 7.28 (1 H, d, J = 8.6 Hz, NH), 7.44 (2 H, d, J = 8.7 Hz, Ph-2-H, Ph-6-H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 13.9, 21.9, 25.1,30.9, 35.5, 55.1, 60.9, 70.1, 114.1, 127.5, 129.3, 172.2; HRMS (LSIMS) calcd for $C_{18}H_{27}NO_4Na$ $(M + Na)^+$ 344.1838, found 344.1814.

***tert*-Butyl (4*S*)-4-[(1*R*)-1-Hydroxy-3-(2-thienyl)-2-propynyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (4e).** The same procedure, as described for the synthesis of **4a**, was followed using 2-ethynylthiophene. Thus, 0.42 g (1.85 mmol) of **3**,[11] 0.4 g (3.7 mmol) of 2-ethynylthiophene (**12**), 1.7 mL *n*-BuLi (1.6 M in hexane, 2.78 mmol), and 0.65 mL (3.7 mmol) HMPA gave 0.57 g (91%) of **4e**. An analytical sample was obtained by HPLC purification (hexane/EtOAc, 85:15): ^1H NMR (500 MHz, DMSO-d_6) δ 1.30−1.50 (15 H, m, *t*-Bu, 2 CH_3), 3.87−4.08 (3 H, m, 2H−C(5), H−C(4)), 4.55−4.65 (1 H, m,

H-C−OH), 5.84−5.93 (1 H, m, OH), 7.08 (1 H, br s, H−C(4′)), 7.21−7.30 (1 H, m, H−C(3′)), 7.57 (1 H, br s, H−C(5′)); HRMS (LSIMS) calcd for $C_{17}H_{23}NO_4SNa$ (M + Na)$^+$ 360.1246, found 360.1236.

***tert*-Butyl (1*S*,2*R*)-2-Hydroxy-1-(hydroxymethyl)-4-(2-thienyl)-3-butynylcarbamate (5e).** The same procedure, as described for the synthesis of **5a**, was followed using 0.55 g (1.63 mmol) of **4e** and 0.062 g (0.33 mmol) of *p*-TsOH. Thus, 0.32 g (66%) of **5e** was obtained. A small amount was purified by HPLC (hexane/EtOAc, 6:4): IR (cm^{-1}, KBr) 3427, 2233, 1698; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.37 (9 H, s, *t*-Bu), 3.43−3.64 (3 H, m, HOCH_2, H−C(1)), 4.42 (1 H, t, J = 6.5 Hz, H−C(2)), 4.60 (1 H, br s, OH), 5.73 (1 H, d, OH), 6.53 (1 H, d, J = 9.0 Hz, NH), 7.05 (1 H, t, J = 4.4 Hz, H−C(4′)), 7.23 (1 H, d, J = 3.3 Hz, H−C(3′)), 7.56 (1 H, d, J = 5.1 Hz, H−C(5′)); HRMS (LSIMS) calcd for $C_{14}H_{19}NO_4SNa$ (M + Na)$^+$ 320.0933, found 320.0931.

***tert*-Butyl (1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(2-thienyl)-3-butenylcarbamate (6e).** The same procedure, as described for the synthesis of **6a**, was followed using 0.27 g (0.9 mmol) of **5e** and 2.75 mL Red-Al (3.33 M in toluene, 9 mmol). After standing for 4 h, the reaction was worked up in the usual way. Thus, 0.218 g (81%) of **6e** was obtained. A small amount was purified by HPLC (hexane/EtOAc, 4:1): IR (cm^{-1}, KBr) 3415, 1687; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.33 (9 H, s, *t*-Bu), 3.36−3.42 (1 H, m, H−C(1)), 3.43−3.49 (1 H, m, HOC*H*H), 3.50−3.56 (1 H, m, HOC*H*H), 3.99−4.05 (1 H, m, H−C(2)), 4.49 (1 H, t, J = 5.5 Hz, OH), 5.10 (1 H, d, J = 5.3 Hz, OH), 6.03 (1 H, dd, J = 15.7 and 6.4 Hz, H−C(3)), 6.40 (1 H, d, J = 9.0 Hz, NH), 6.65 (1 H, d, J = 15.7 Hz, H−C(4)), 6.98 (1 H, dd, J = 4.8 and 3.6 Hz, H−C(4′)), 7.01 (1 H, d, J = 2.6 Hz, H−C(3′)), 7.36 (1 H, d, J = 4.9 Hz, H−C(5′)); HRMS (LSIMS) calcd for $C_{14}H_{21}NO_4SNa$ (M + Na)$^+$ 322.1089, found 322.1071.

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(2-thienyl)-3-butenyl]hexanamide (8e).** A solution of **6e** (64 mg, 0.21 mmol) in dioxane (2 mL) was treated with 1 N HCl (1 mL) for 30 min at 100 °C. After cooling to room temperature, the mixture was neutralized with 1 N NaOH (1 mL) and extracted with EtOAc. The combined organic layers were concentrated and the crude amine (**7e**) was dissolved in a mixture of THF (1 mL) and 33% aqueous NaOAc (1 mL). Hexanoyl chloride (22 μL, 0.16 mmol) was added and the mixture was stirred overnight at room temperature and then diluted with Et_2O and brine. The aqueous layer was extracted with Et_2O (3×) and the organic layers were dried over $MgSO_4$. Purification by HPLC (CH_2Cl_2/CH_3OH, 98:2) gave 0.03 g (48%) of **8e**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85−0.93 (3 H, m, CH_3-CH_2), 1.25−1.40 (4 H, m, $(CH_2)_2$), 1.62−1.70 (2 H, m, $COCH_2CH_2$), 2.24 (2 H, t, J = 7.5 Hz, COCH_2), 3.73−3.79 (1 H, m, HOC*H*H), 3.98−4.40 (2 H, m, HOCH*H*, H−C(1)), 4.55 (1 H, br s, H−C(2)), 6.10 (1 H, dd, J = 15.7 and 5.7 Hz, H−C(3)), 6.40 (1 H, br s, NH), 6.84 (1 H, d, J = 15.8 Hz, H−C(4)), 6.95−7.00 (2 H, m, H−C(3′), H−C(4′)), 7.17 (1 H, d, J = 4.4 Hz, H−C(5′)); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.8, 22.3, 25.3, 31.3, 36.6, 54.4 (C-1), 62.3 (HOCH_2), 69.5 (C-2), 124.9, 126.2, 126.7, 127.3, 130.7; HRMS (LSIMS) calcd for $C_{15}H_{23}NO_3SNa$ (M + Na)$^+$ 320.1297, found 320.1277.

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-pentylphenyl)-3-butenyl]acetamide (8f).** The same procedure, as described for the synthesis of **8b**, was followed using 0.22 g (0.83 mmol) of crude amine **7b** and 47 μL (0.67 mmol) acetyl chloride. Flash chromatography ($CHCl_3$/CH_3OH, 95:5) and HPLC (CH_2Cl_2/CH_3OH, 95:5) yielded 0.154 g (61%) of **8f**: IR (cm^{-1}, KBr) 3415, 1646; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (3 H, t, J = 7.0 Hz, CH_3CH$_2$), 1.20−1.33 (4 H, m, $(CH_2)_2$), 1.48−1.58 (2 H, m, CH_2), 1.74−1.84 (3 H, m, CH_3CO), 2.53 (2 H, t, J = 7.6 Hz, CH_2−Ph), 3.43−3.53 (2 H, m, HOCH_2), 3.72−3.85 (1 H, m, H−C(1)), 4.12−4.18 (1 H, m, H−C(2)), 4.58 (1 H, br s, OH), 5.08 (1 H, d, J = 5.2 Hz, OH), 6.21 (1 H, dd, J = 15.9 and 6.4 Hz, H−C(3)), 6.47 (1 H, d, J = 15.7 Hz, H−C(4)), 7.13 (2 H, d, J = 8.0 Hz, arom), 7.28 (2 H, d, J = 8.0 Hz, arom), 7.63 (1 H, d, J = 8.7 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.0, 22.0, 22.9, 30.7, 30.9, 34.9, 55.8 (C-1), 60.3 (HOCH_2), 71.3 (C-2), 126.2 (arom), 128.6 (arom), 129.5 (C-3), 130.5 (C-4), 134.5 (C_{ipso}), 141.6 (C_{ipso}), 169.4 (C=O); HRMS (LSIMS) calcd for $C_{18}H_{27}NO_3Na$ (M + Na)$^+$ 328.1889, found 328.1907. Anal. ($C_{18}H_{27}NO_3 \cdot {}^1/_{12}CH_3OH$) C, N; H: calcd, 8.91; found, 8.76.

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(hydroxymethyl)-4-(4-pentylphenyl)-3-butenyl]butanamide (8g).** The same procedure, as described for the synthesis of **8b**, was followed, using 0.22 g (0.83 mmol) of crude amine **7b** and 70 μL (0.68 mmol) butanoyl chloride. Chromatographic purification ($CHCl_3$/CH_3OH, 95:5) and HPLC (CH_2Cl_2/CH_3OH, 98:2) yielded 0.083 g (30%) of **8g**: IR (cm^{-1}, KBr) 3415, 1636; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.77 (3 H, t, J = 7.4 Hz, CH_3CH$_2$), 0.85 (3 H, t, J = 7.0 Hz, CH_3CH$_2$), 1.15−1.30 (4 H, m, $(CH_2)_2$), 1.38−1.48 (2 H, m, CH_2), 1.49−1.58 (2 H, m, CH_2), 2.03 (2 H, t, J = 7.3 Hz, COCH_2), 2.53 (2 H, t, J = 7.6 Hz, CH_2−Ph), 3.43−3.56 (2 H, m, HOCH_2), 3.72−3.82 (1 H, m, H−C(1)), 4.10−4.17 (1 H, m, H−C(2)), 4.56 (1 H, t, J = 5.3 Hz, OH), 5.08 (1 H, d, J = 5.2 Hz, OH), 6.19 (1 H, dd, J = 15.8 and 6.6 Hz, H−C(3)), 6.46 (1 H, d, J = 15.7 Hz, H−C(4)), 7.12 (2 H, d, J = 7.8 Hz, arom), 7.26 (2 H, d, J = 7.9 Hz, arom), 7.53 (1 H, d, J = 8.7 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.6, 14.0, 18.8, 22.0, 30.6, 30.9, 34.8, 37.5, 55.6 (C-1), 60.5 (HOCH_2), 71.4 (C-2), 126.2 (arom), 128.5 (arom), 129.5 (C-3), 130.6 (C-4), 134.5 (C_{ipso}), 141.5 (C_{ipso}), 172.2 (C=O); HRMS (LSIMS) calcd for $C_{20}H_{31}NO_3Na$ (M + Na)$^+$ 356.2202, found 356.2201. Anal. ($C_{20}H_{31}NO_3 \cdot {}^1/_8CH_3OH$) C, N; H: calcd, 9.37; found, 9.22.

Trimethyl[2-(4-methoxyphenyl)ethynyl]silane (9). To a solution of 4-iodoanisole (4.97 g, 0.021 mol) in dry Et_3N (55 mL) were added $Pd^{II}Cl_2(PPh_3)_2$ (0.147 g, 0.21 mmol), CuI (0.04 g, 0.21 mmol), and trimethylsilylacetylene (6 mL, 0.042 mol). After standing for 1 h at room temperature, the volatiles were removed in vacuum and the residue was dissolved in CH_2Cl_2 (100 mL). The organic layer was washed with 5% aqueous disodium EDTA and H_2O and dried over Na_2SO_4. Flash chromatography (pentane/EtOAc, 99:1) yielded ≈4.3 g (100%) of **9** as a colorless oil: IR (cm^{-1}, KBr) 2955, 2155; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.25 (9 H, s, Si−Me_3), 3.80 (3 H, s, CH_3O), 6.82 (2 H, d, J = 7.9 Hz, Ph-3-H, Ph-5-H), 7.40 (2 H, d, J = 7.9 Hz, Ph-2-H, Ph-6-H).

4-Methoxyphenylacetylene (10). To a solution of **9** (5.1 g, 0.025 mol) in THF (300 mL) was added TBAF (1 M in THF, 27.5 mL, 0.0275 mol). After standing for 3 h at room temperature, the mixture was diluted with Et_2O and saturated aqueous $NaHCO_3$. The organic layer was removed and the aqueous layer was extracted with Et_2O (2×). The combined organic layers were dried over $MgSO_4$. Flash chromatography (pentane/Et_2O, 95:5) yielded 2.4 g (72%) of **10** as a colorless oil: IR (cm^{-1}, KBr) 3303, 2122; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.00 (1 H, s, ethynyl H), 3.75 (3 H, t, J = 6.6 Hz, CH_3O), 6.84 (2 H, d, J = 8.8 Hz, Ph-3-H, Ph-5-H), 7.47 (2 H, d, J = 8.8 Hz, Ph-2-H, Ph-6-H).

Trimethyl[2-(2-thienyl)ethynyl]silane (11). To a solution of 2-iodothiophene (2.8 mL, 0.025 mol) in dry Et_3N (63 mL) were added $Pd^{II}Cl_2(PPh_3)_2$ (0.175 g, 0.25 mmol), CuI (0.048 g, 0.25 mmol), and trimethylsilylacetylene (5 g, 0.051 mol). After standing for 2 h at room temperature, the volatiles were removed in vacuum and the residue was dissolved in CH_2Cl_2 (150 mL). The organic layer was washed with 5% aqueous disodium EDTA and H_2O and dried over Na_2SO_4. Flash chromatography (pentane) yielded 4.67 g (100%) of **11** as a colorless oil: IR (cm^{-1}, KBr) 2964, 2144; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.25 (9 H, s, Si−Me_3), 6.95 (1 H, dd, J = 4.9 and 3.9 Hz, H−C(4′)), 7.24 (2 H, d, J = 4.3 Hz, H−C(3′), H−C(5′)).

2-Ethynylthiophene (12). To a solution of **11** (4.67 g, 0.023 mol) in THF (295 mL) was added TBAF (1 M in THF, 28 mL, 0.028 mol). After standing for 2 h at room temperature, the reaction mixture was diluted with Et_2O (100 mL) and saturated aqueous $NaHCO_3$ (100 mL). The aqueous layer was removed, and the organic layer was washed with H_2O (2×) and brine and dried over $MgSO_4$. After concentration in vacuum, the resulting oil (2.54 g) was dissolved in pentane and filtered over silica to give **12** as a volatile oil: IR (cm^{-1}, KBr) 3304, 2111; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.35 (1 H, s,

ethynyl H), 6.98 (1 H, dd, J = 5.1 and 3.6 Hz, H−C(4)), 7.20 (2 H, m, H−C(3), H−C(5)).

***N*-{(1*S*,2*R*,3*E*)-2-Hydroxy-1-[(trityloxy)methyl]-3-undecenyl}hexanamide (14).** A mixture of **13**[9] (0.3 g, 0.96 mmol) and triphenylmethyl chloride (0.47 g, 1.67 mmol) in dry pyridine (27 mL) was heated at 100 °C for 3 h. After cooling to room temperature, it was poured into ice-cold 1 N HCl (370 mL) and stirred for 15 min. The aqueous layer was extracted with Et_2O (3×), and the combined organic layers were washed with 2.5% $NaHCO_3$ and H_2O and dried over $MgSO_4$. Flash chromatography (hexane/EtOAc, 75:25) yielded 0.48 g of **14** (90%) as a colorless oil: $[\alpha]_D^{20} = -1.98$ (c 1.36, $CHCl_3$); UV (CH_3OH) λ_{max} 210 nm (log ϵ 4.32); IR (cm^{-1}, KBr) 3327, 2920, 1651; ^1H NMR (360 MHz, $CDCl_3$) δ 0.86−0.96 (6 H, m, 2 CH_3), 1.20−1.40 (14 H, m, 7 CH_2), 1.62−1.70 (2 H, m, $COCH_2CH_2$), 1.87−1.96 (2 H, m, 2H−C(5)), 2.20 (2 H, t, J = 7.7 Hz, $COCH_2$), 3.31 (1 H, dd, J = 9.7 and 4.0 Hz, TrOC*H*H), 3.37−3.42 (2 H, m, TrOCH*H*, OH), 4.03−4.10 (1 H, m, H−C(1)), 4.16−4.21 (1 H, m, H−C(2)), 5.26 (1 H, dd, J = 15.4 and 6.2 Hz, H−C(3)), 5.64 (1 H, dt, J = 15.4 and 6.6 Hz, H−C(4)), 6.08 (1 H, d, J = 7.9 Hz, NH), 7.20−7.45 (15 H, m, arom); HRMS (LSIMS) calcd for $C_{37}H_{49}NO_3Na$ $(M + Na)^+$ 578.3610, found 578.3602.

***N*-{[1*S*,2(*RS*),3*E*]-2-Fluoro-1-[(trityloxy)methyl]-3-undecenyl}hexanamide (15).** To a cooled (−78 °C) solution of DAST (0.19 mL, 1.43 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise a solution of **14** (0.53 g, 0.95 mmol) in dry CH_2Cl_2 (10 mL). After standing for 45 min at −78 °C, examination by TLC (hexane/EtOAc, 75:25) indicated completion of the reaction. The reaction mixture was washed with 5% aqueous $NaHCO_3$ (3×) and the organic layer was dried over $MgSO_4$. Flash chromatography (hexane/EtOAc, 94:6) gave 0.07 g (14%) of **17**. Further elution (hexane/EtOAc, 9:1) afforded 0.15 g (28%) of **15** and 0.3 g (57%) of **16**. The pure *threo* compound was obtained after HPLC purification of **15** (hexane/EtOAc, 9:1): ^1H NMR (500 MHz, $CDCl_3$) (data for *threo*) δ 0.85 (6 H, m, 2 CH_3), 1.20−1.40 (14 H, m, 7 CH_2), 1.55−1.60 (2 H, m, CH_2), 1.98−2.08 (2 H, m, 2H−C(5)), 2.13 (2 H, t, J = 7.6 Hz, $COCH_2$), 3.18−3.25 (2 H, m, TrOCH_2), 4.26−4.37 (1 H, m, H−C(1), $^3J_{H,F}$ = 22.7 Hz), 5.14−5.18 (0.5 H, m, H−C(2)), 5.23−5.27 (0.5 H, m, H−C(2), $^2J_{H,F}$ = 47.6 Hz), 5.44−5.53 (1 H, m, H−C(3)), 5.57 (1 H, d, J = 9.4 Hz, NH), 5.79−5.88 (1 H, m, H−C(4)), 7.20−7.45 (15 H, m, arom); HRMS (LSIMS) calcd for $C_{37}H_{48}NO_2$ $(M - HF + H)^+$ 538.3685, found 538.3669.

***N*-{[1*R*,2*E*,4(*RS*)]-4-Fluoro-1-[(trityloxy)methyl]-2-undecenyl}hexanamide (16).** Procedure: see **15**; ^1H NMR (360 MHz, $CDCl_3$) δ 0.85−0.95 (6 H, m, 2 CH_3), 1.20−1.50 (14 H, m, 7 CH_2), 1.50−1.80 (4 H, m, 2 CH_2), 2.15 (2 H, t, J = 7.5 Hz, $COCH_2$), 3.19−3.29 (2 H, m, TrOCH_2), 4.67−4.74 (1 H, m, H−C(1)), 4.79−4.86 (0.5 H, m, H−C(4)), 4.93−4.99 (0.5 H, m, H−C(4), $^2J_{H,F}$ = 48.2 Hz), 5.61−5.87 (3 H, m, NH, H−C(2), H−C(3)), 7.20−7.45 (15 H, m, arom); HRMS (LSIMS) calcd for $C_{37}H_{48}NO_2FNa$ $(M + Na)^+$ 580.3567, found 580.3586.

(4*S*,5*S*)-5-[(*E*)-1-Nonenyl]-2-pentyl-4-[(trityloxy)methyl]-4,5-dihydro-1,3-oxazole (17). Procedure: see **15**; ^1H NMR (500 MHz, $CDCl_3$) δ 0.85−0.92 (6 H, m, 2 CH_3), 1.20−1.42 (16 H, m, 8 CH_2), 1.66−1.75 (2 H, m, CH_2), 2.02−2.09 (2 H, m, HC=CHCH_2), 3.25−3.30 (2 H, m, TrOCH_2), 3.94−3.98 (1 H, m, H−C(4)), 4.80−4.85 (1 H, m, H−C(5)), 5.47 (1 H, dd, J = 15.3 and 8.1 Hz, *H*C=CHCH$_2$), 5.72 (1 H, dt, J = 15.3 and 6.7 Hz, HC=C*H*CH$_2$), 7.20−7.45 (15 H, m, arom); HRMS (LSIMS) calcd for $C_{37}H_{48}NO_2$ $(M + H)^+$ 538.3685, found 538.3711.

***N*-[(1*S*,2*R*,3*E*)-2-Fluoro-1-(hydroxymethyl)-3-undecenyl]-hexanamide (18).** A solution of **15** (0.147 g, 0.26 mmol) in CH_3OH (5 mL) was treated with Amberlyst 15 (0.256 g) at room temperature for 24 h. The heterogeneous mixture was then filtered over Celite. Before concentration, the methanolic solution was diluted with H_2O and extracted with Et_2O. The Et_2O layers were washed with saturated aqueous $NaHCO_3$ and dried ($MgSO_4$). Flash chromatography (CH_2Cl_2/CH_3OH, 98:2) yielded 0.04 g (50%) of a mixture of **18** and **19** which was separated by HPLC (CH_2Cl_2/CH_3OH, 99:1): ^1H NMR (500 MHz, $CDCl_3$) δ 0.86−0.93 (6 H, m, 2 CH_3), 1.20−1.43 (14 H, m, 7 CH_2), 1.60−1.67 (2 H, m, $COCH_2CH_2$), 2.04−2.11 (2 H, m, 2H−C(5)), 2.21 (2 H, dt, J = 7.7 and 2.0 Hz, $COCH_2$), 3.73 (1 H, dd, J = 11.5 and 3.5 Hz, HOC*H*H), 3.92 (1 H, dd, J = 11.2 and 4.1 Hz, HOCH*H*), 4.07−4.17 (1 H, m, H−C(1), $^3J_{H,F}$ = 21.0 Hz), 4.97 (0.5 H, t, J = 5.9 Hz, H−C(2)), 5.06 (0.5 H, t, J = 6.0 Hz, H−C(2), $^2J_{H,F}$ = 48.2 Hz), 5.51−5.60 (1 H, m, H−C(3)), 5.84−5.92 (1 H, m, H−C(4)), 6.00 (1 H, d, J = 8.0 Hz, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.8, 14.0, 22.3, 22.6, 25.3, 28.7, 29.0, 31.3, 31.7, 32.2, 36.7, 53.6 (C-1, $^2J_{C,F}$ = 22.9 Hz), 61.3 (HOCH_2), 93.6 (C-2, $^1J_{C,F}$ = 170.9 Hz), 124.5 (C-3, $^2J_{C,F}$ = 18.3 Hz), 137.8 (C-4, $^3J_{C,F}$ = 12.2 Hz), 173.7 (C=O); HRMS (LSIMS) calcd for $C_{18}H_{34}NO_2FNa$ $(M + Na)^+$ 338.2471, found 338.2431.

***N*-[(1*S*,2*S*,3*E*)-2-Fluoro-1-(hydroxymethyl)-3-undecenyl]-hexanamide (19).** Procedure: see **18**; ^1H NMR (500 MHz, $CDCl_3$) δ 0.87−0.97 (6 H, m, 2 CH_3), 1.20−1.45 (14 H, m, 7 CH_2), 1.60−1.67 (2 H, m, $COCH_2CH_2$), 2.02−2.11 (2 H, m, 2H−C(5)), 2.23 (2 H, t, J = 7.5 Hz, $COCH_2$), 3.70−3.80 (2 H, m, HOCH_2), 4.04−4.09 (0.5 H, m, H−C(1)), 4.10−4.15 (0.5 H, m, H−C(1), $^3J_{H,F}$ = 23.8 Hz), 5.07 (0.5 H, dd, J = 6.5 and 3.5 Hz, H−C(2)), 5.16 (0.5 H, dd, J = 6.5 and 3.5 Hz, H−C(2), $^2J_{H,F}$ = 47.7 Hz), 5.46−5.55 (1 H, m, H−C(3)), 5.80−5.95 (2 H, m, NH en H−C(4)); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.8, 14.0, 22.3, 22.6, 25.3, 28.7, 29.0, 31.3, 31.7, 32.2, 36.7, 54.3 (C-1, $^2J_{C,F}$ = 19.8 Hz), 62.6 (HOCH_2), 92.2 (C-2, $^1J_{C,F}$ = 169.4 Hz), 124.5 (C-3, $^2J_{C,F}$ = 18.7 Hz), 137.6 (C-4, $^3J_{C,F}$ = 10.7 Hz), 174.2 (C=O); HRMS (LSIMS) calcd for $C_{18}H_{35}NO_2F$ $(M + H)^+$ 316.2652, found 316.2696.

***N*-{[1*R*,2*E*,4(*RS*)]-4-Fluoro-1-(hydroxymethyl)-2-undecenyl}hexanamide (20).** A solution of **16** (0.174 g, 0.31 mmol) in CH_3OH (3 mL) was treated with Amberlyst 15 (0.203 g) for 48 h at room temperature. The mixture was filtered over Celite and concentrated. Flash chromatography (CH_2Cl_2/CH_3OH, 98:2) yielded 0.06 g of **20** (61%): ^1H NMR (360 MHz, $CDCl_3$) δ 0.83−0.97 (6 H, m, 2 CH_3), 1.20−1.48 (14 H, m, 7 CH_2), 1.60−1.82 (4 H, m, 2 CH_2), 2.18−2.28 (2 H, m, $COCH_2$), 3.64−3.77 (2 H, m, HOCH_2), 4.51−4.63 (1 H, m, H−C(1)), 4.78−4.85 (0.5 H, m, H−C(4)), 4.92−4.98 (0.5 H, m, H−C(4), $J_{H,F}$ = 48.5 Hz), 5.66−5.80 (2 H, m, H−C(2), H−C(3)), 5.92−6.00 (1 H, m, NH); ^{13}C NMR (90 MHz, $CDCl_3$) δ 13.8, 14.0, 22.3, 22.5, 24.6, 25.3, 29.0, 29.2, 31.3, 31.7, 35.2, 35.4, 36.7, 52.3 (C-1), 52.4 (C-1), 65.2 (CH_2OH), 92.6 (C-4, $^1J_{C,F}$ = 167.0 Hz), 92.8 (C-4, $^1J_{C,F}$ = 166.1 Hz), 129.3 (C-2, $^3J_{C,F}$ = 11.1 Hz), 129.7 (C-2, $^3J_{C,F}$ = 11.0 Hz), 131.1 (C-3, $^2J_{C,F}$ = 18.9 Hz), 131.3 (C-3, $^2J_{C,F}$ = 18.9 Hz), 173.4 (C=O); HRMS (LSIMS) calcd for $C_{18}H_{35}NO_2F$ $(M + H)^+$ 316.2652, found 316.2746. Anal. ($C_{18}H_{34}NO_2F \cdot {}^1/_{10}CH_3OH$) C, N; H: calcd, 10.86; found, 10.49.

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Supporting Information Available: Analytical data for compounds **8a**−**g** and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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