Synthesis of Homoceramides, Novel Ceramide Analogues, and Their Lack of Effect on the Growth of Hippocampal Neurons

Steven De Jonghe,[†] Inge Lamote,[†] Krishnan Venkataraman,[§] Swetlana A. Boldin,[§] Ulrik Hillaert,[†] Jef Rozenski,[‡] Chris Hendrix,[‡] Roger Busson,[‡] Denis De Keukeleire,[†] Serge Van Calenbergh,*[,†] Anthony H. Futerman,[§] and Piet Herdewijn[†,‡]

Ghent University, Faculty of Pharmaceutical Sciences, Laboratory of Medicinal Chemistry, Harelbekestraat 72, 9000 Gent, Belgium; Catholic University of Leuven, REGA-Institute, Laboratory of Medicinal Chemistry, Minderbroedersstraat 10, 3000 Leuven, Belgium; and Weizmann Institute of Science, Department of Biological Chemistry, Rehovot 76100, Israel

serge.vancalenbergh@rug.ac.be

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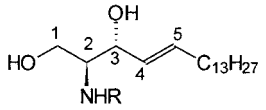
The synthesis of a new series of D-*erythro*-homoceramide analogues is described. Several synthetic approaches were investigated. Homoceramides can be successfully synthesized from L-homoserine as chiral building block and a protected Weinreb-amide as a key intermediate. The synthesis of short-chain analogues with a heptyl side chain, as well as with a phenyl residue in the sphingoid part (instead of the naturally occurring tridecyl side chain), was effected. The homoceramides **15**−**17** and **24** were investigated for their potential to reverse the inhibitory effect of fumonisin B_1 on axonal growth. Unfortunately, none of the tested compounds showed any biological activity due to their lack of metabolism to glucosylhomoceramide.

Introduction

Sphingolipids constitute one of the most, chemically and functionally, diverse classes of biomolecules. Although structurally diverse, sphingolipids share a hydrophobic component, generically referred to as ceramide. Ceramide is composed of a sphingoid base, sphingosine, with an amide-linked fatty acyl chain. Sphingosine is a 2-amino-1,3-diol with a 4,5-trans carbon−carbon double bond. The fatty acyl tail may vary in chain length (from 16 to 24 carbon atoms), saturation, and hydroxylation (Figure 1).[1] Ceramide acts as a signaling substance thereby affecting multiple aspects of cellular functioning, including cell proliferation, growth arrest, and apoptosis.[2]

Several synthetic analogues of natural ceramide have been prepared.[3] Chemically modified analogues of natural compounds serve as tools to understand the molecular specificity of enzyme−substrate interactions and the structural requirements needed to exert a specific function. Most of the structural modifications include stereochemical variations (D-*erythro*- and L-*threo*-epimers), alteration of the chain length of the fatty acid residue and the sphingoid base backbone, and the absence or presence of a 4,5-trans carbon−carbon double bond.



R = H: sphingosine
R = $C(O)(CH_2)_nCH_3$: ceramide (n = 14-22)

Figure 1. Structures of sphingosine and ceramide.

Recently, in our laboratory, we have developed strategies for the synthesis of short-chain aliphatic and aromatic ceramide analogues and have studied their effects on a number of biological parameters, including on the rate of growth of hippocampal neurons.[4] Inhibition of the biosynthesis of sphingolipids in hippocampal neurons by fumonisin B_1 (FB_1)[5,6] or D-*threo*-(1*R*,2*R*)-1-phenyl-2-decanoylamino-3-morpholino-1-propanol [D-*threo*-(1*R*,2*R*)-PDMP][5] reduced axonal growth between day 2 and day

* Corresponding author. Phone: +32 (0)9/264 81 24, fax: +32 (0)9/264.81.92.

[†] Ghent University.

[§] Weizmann Institute of Science.

[‡] Catholic University of Leuven.

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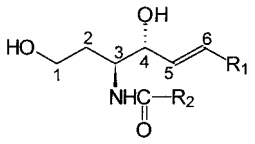

$R_1 = C_7H_{15}$ or C_6H_5
$R_2 = CH_3$, C_5H_{11} or C_6H_5

Figure 2. Structure of short-chain homoceramide analogues.

3 in culture. Short-acyl chain analogues of D-*erythro*-ceramide are able to reverse the disruptive effects of FB_1 on axonal growth.[4,7] Moreover, it has been demonstrated that ceramide must be metabolized to glucosylceramide to support axonal growth in hippocampal neurons.[7,8] In the current manuscript, we have studied the effects on axonal growth of a series of homoceramide analogues.

Homoceramides, containing an additional methylene group between the primary hydroxyl function and the *N*-acyl chain, are 3-amino-1,4-diols rather than the naturally occurring 2-amino-1,3-diols. As a consequence, homoceramides possess a 5,6-trans carbon−carbon double bond (Figure 2). These compounds have never been synthesized before. Because of the poor aqueous solubility of natural ceramide, it is necessary to present suitable derivatives with increased water solubility for in vitro and in vivo bioactivity studies. This aim was met by shortening the sphingoid backbone (a heptyl and a phenyl residue instead of the natural tridecyl side chain) and by introducing a short *N*-acyl chain instead of the naturally occurring C_{16}−C_{24}. Regarding stereochemical implications, we focused on D-*erythro*-homoceramides having the natural configuration at C(3) and C(4). The enantioselective synthesis of a D-*erythro*-ceramide with a 5,6-trans double bond has already been reported.[9] However, the secondary hydroxyl group of this compound is homoallylic with respect to the double bond. In contrast, homoceramides, which also possess a 5,6-trans double bond, retain the allylic position of the secondary hydroxyl group. This could be important to afford biologically active compounds.

In the current study, we report the synthesis of short-chain homoceramide analogues and demonstrate that, because of their lack of metabolism to homoglucosylceramide, these compounds are totally inactive in modulating rates of axon growth in hippocampal neurons.

Synthesis. In a first attempt to prepare homoceramides, we pursued a strategy similar to that described for the synthesis of ceramide. A straightforward synthesis of short-chain ceramide analogues uses L-serine as chiral substrate and the Garner-aldehyde as a key intermediate.[4,10]

Consequently, we opted for the use of L-homoserine as chiral building block for the synthesis of homoceramides, which should lead to the formation of a 4-formyl-2,2-dimethyl-tetrahydro-1,3-oxazine derivative (homo-Garner-aldehyde) (Figure 3). L-Homoserine (**1**) was converted to *N*-Boc-L-homoserine methyl ester (**2**) by using an established procedure.[11] Attempts to protect simultaneously the hydroxyl group and the NH−Boc group of **2** via formation of a 2,2-dimethyl-tetrahydro-1,3-oxazine ring failed, as only lactone **3** could be isolated (Scheme 1).

Simultaneous protection of the hydroxyl and the amino groups proved to be cumbersome; hence, a stepwise route was investigated. Phthalimide, which has already been used in the first serine-derived synthesis of D-*erythro*-sphingosine,[12] was selected as the *N*-protecting group. The reaction proceeds in aqueous solution at room temperature, which prevents lactonization of L-homoserine. *N*-Phthaloylation of L-homoserine (**1**) using *N*-(ethoxycarbonyl)phthalimide[13] afforded the phthalimide analogue **4** (Scheme 2). Since lactone formation was observed during purification of **4** on silica, the carboxylic acid function was in situ converted to the methyl ester **5** without prior purification. The hydroxyl group was transformed into a *tert*-butyldimethylsilyl (TBDMS) ether, yielding the fully protected **6**.

Unfortunately, reaction of **6** with DIBAL did not lead to the expected aldehyde. In contrast, cleavage of the TBDMS-group, followed by intramolecular cyclization and reduction of one of the amide carbonyl groups, led to the hemi-aminal **7**. Only one diastereomer was evident from the ^1H and ^{13}C NMR spectra, but the stereochemistry of both secondary alcohols was not determined. The structure of **7** was elucidated from the ^1H NMR, ^{13}C NMR, and 2D-NMR data (COSY, HMQC, and HMBC). A heteronuclear multiple quantum coherence (HMQC) spectrum was used to correlate the protons with the carbons to which they are directly attached. The absence of a TBDMS-group could easily be deduced from the ^1H NMR and ^{13}C NMR spectra. As homoserine derivatives are very prone to undergo intramolecular cyclizations, the potential formation of a hemi-acetal was considered. The presence of a tetrahydrofuran was confirmed by a 2D ^1H−^{13}C multiple bond correlation (HMBC) experiment. HMBC-interactions were observed between C(5) and the proton at C(2), and conversely between the proton at C(5) and C(2). Because HMBC-interactions only occur within a range of 2−3 bonds, the above evidence indicated that the cyclization of **6**, indeed, had afforded **7**. The hemi-acetal and hemi-aminal entities were confirmed by proton resonances at 6.02 ppm [H(2), doublet by coupling to H(3), as proven by a COSY-experiment] and at 6.14 ppm [H(9′), singlet]. Moreover, HMBC-correlations were detected between H(9′) and C(2′), C(3′), C(7′), and C(8′), as well as between C(9′) and H(3) and H(7′).

The final successful strategy that was applied for the synthesis of homoceramides, is outlined in Scheme 3. L-Homoserine (**1**) was converted to *N*-Cbz-L-homoserine lactone (**8**) by a known procedure.[11a] Reaction of **8** with trimethylaluminum[14] and *N*,*O*-dimethylhydroxylamine hydrochloride yielded the *N*-methoxy-*N*-methyl amide **9**, which is also known as a Weinreb-amide.[15] After protection of the hydroxyl group as a TBDMS-ether, Weinreb-

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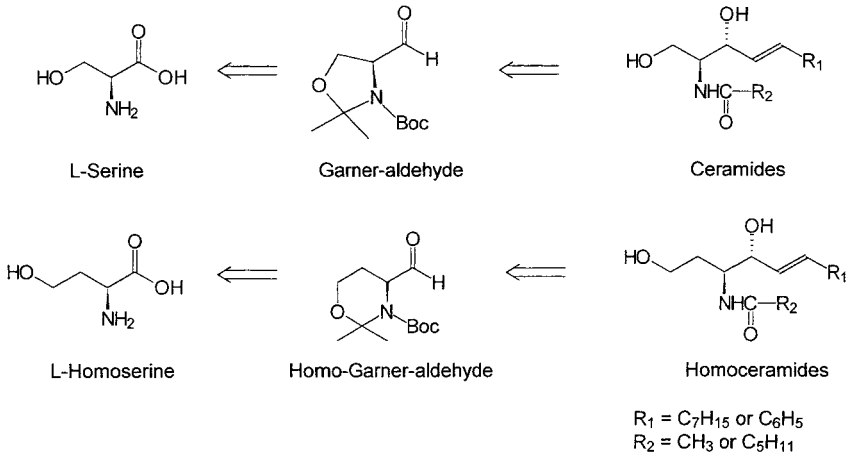
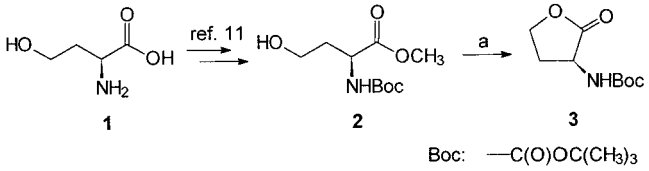


Figure 3. Retrosynthesis of short-chain ceramide and homoceramide analogues.

Scheme 1[a]



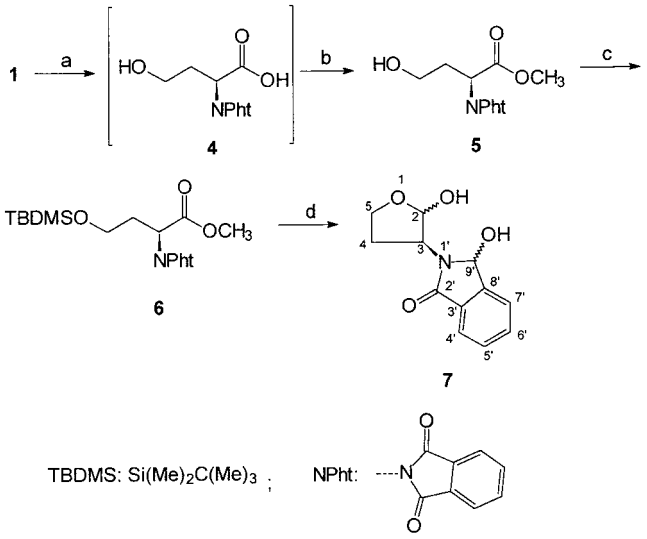
[a] (a) DMP, $BF_3{\cdot}OEt_2$, acetone, rt.

amide **10** was allowed to react with the lithium salt of non-1-yne, yielding the α′-amino-α,*β*-ynone **11**.[15] It is well-known that such compounds are very prone to undergo racemization on silica.[16] Therefore, **11** was treated in situ with $NaBH_4$ and $CeCl_3$ (Luche-conditions).[17] The resulting mixture of epimeric alkynols **12** could not be separated by HPLC on silica. The epimeric ratio was 8:3 as determined by ^{1}H NMR. Both epimers could be distinguished by the resonances of the C*H*N-proton (major epimer: $\delta = 3.98$ ppm; minor epimer: $\delta =$ 3.90 ppm). The relative configuration of the alkynols **12** was established by transformation of **12** to the corresponding cyclic urethanes on treatment with NaOMe (Scheme 4).[18] HPLC on silica provided the major isomer **18** in pure form. The minor isomer could not be obtained in pure form. The large vicinal coupling constant ($^3J_{4,5}$ = 7.9 Hz) of the major isomer indicates a *cis*-relationship of H-(4) and H-(5).[19]

The *cis*-stereochemistry was confirmed by the observation of a distinct NOE-effect (5.5%) between H(4) and H(5). These data are in agreement with the *anti*-diastereoselectivity of $NaBH_4$-reductions of α′-amino-α,*β*-ynones described in the literature.[16]

Acid deprotection of the TBDMS-group from **12** yielded the epimeric diols **13**, which could not be separated by HPLC on silica. Simultaneous removal of the Cbz-protecting group and reduction of the alkyne to a *trans*-alkene could be achieved with lithium in ethylamine

Scheme 2[a]



[a] (a) *N*-(Ethoxycarbonyl)phthalimide, Na_2CO_3, H_2O, rt; (b) (i) $(C_6H_{11})_2NH$, EtOH, rt; (ii) MeI, DMF, rt; (c) TBDMSCl, imidazole, DMF, rt; (d) DIBAL, toluene, −78 °C.

(Benkeser-conditions),[20] thereby affording the homo-sphingosine analogue. The *trans*-configuration of the double bond was evidenced by the large vicinal coupling constant of 15.4 Hz. At this stage of the synthesis, both epimers could be separated by flash chromatography on silica and the major *erythro*-epimer **14** was obtained in pure form. Subsequent *N*-acylation of **14** using varying acyl chlorides under Schotten−Baumann conditions[21] furnished the homoceramide analogues **15**−**17**. Later on, we found it more convenient to perform the acylation on the epimeric mixture of **14** and to isolate the desired epimers **15**−**17** from their C(4)-epimeric mixtures by flash chromatography.

Assignment of the C*H*OH- and C*H*N-protons in **16** was achieved from a COSY-experiment, which showed that the C*H*N-proton resonates at a higher field than the C*H*OH-proton. ^{13}C NMR resonances could readily be attributed to specific carbons by examination of the HMQC-spectrum. The ^{1}H NMR data of **15** and **17** were interpreted according to the analysis of **16**.

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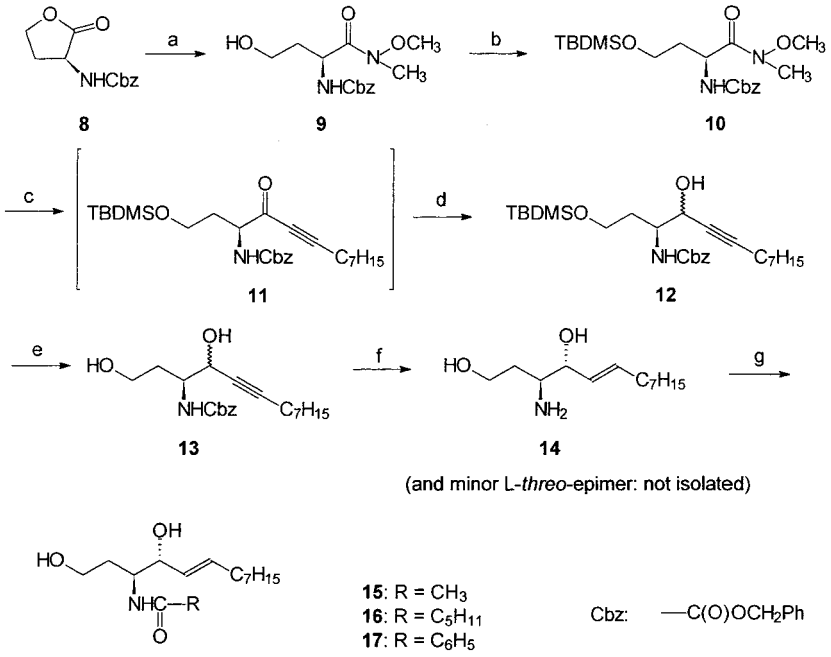
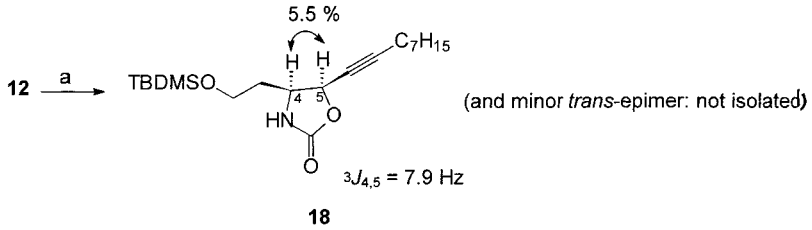
Scheme 3[a]



[a] (a) Me_3Al, $NH(CH_3)OCH_3$·HCl, pyridine, CH_2Cl_2, rt; (b) TBDMSCl, imidazole, DMF, rt; (c) *n*-BuLi, non-1-yne, THF, −78 °C to rt; (d) $NaBH_4$, $CeCl_3$·$7H_2O$, EtOH, 0 °C to rt; (e) HCl 37%, CH_3OH, rt; (f) Li, $EtNH_2$, −78 °C; (g) RC(O)Cl, THF, 50% aq NaOAc, rt.

Scheme 4[a]



[a] (a) NaOMe in MeOH, THF, rt.

It has been shown previously that D-*erythro*-ceramide analogues with a phenyl residue in the sphingoid moiety are still able to reverse the inhibitory effect of FB_1 on axonal growth.[4] In this context, we effected the synthesis of an aromatic homoceramide analogue. The synthetic approach followed a similar pathway as that described for the aliphatic analogues (Scheme 5).

Addition of lithium phenylacetylide to the Weinreb-amide **10** yielded α′-amino-α,β-ynone **19**, which was without purification subjected to $NaBH_4$-reduction, leading to an epimeric mixture of alkynols **20** in a ratio of 4:1, as determined by ^{1}H NMR. It was assumed that the major isomer was the *erythro*-epimer in accordance with the observation done for the aliphatic analogues. Again, both epimers could not be separated by HPLC on silica. Treatment of **20** with Red-Al[22] gave rise to reduction of the alkyne to a *trans*-alkene with concomitant intramolecular cyclization and cleavage of the carbobenzoxy group leading to the formation of an inseparable mixture of 1,3-oxazolidin-2-one derivatives **21**. The large vicinal coupling constant (^3J = 7.8 Hz) between H-(4) and H-(5) in compound **21** was consistent with a *cis*-configuration in the major epimer, which indicates that cyclization had occurred with retention of configuration at C(5).

The *cis*-relationship was confirmed by a significant NOE-effect of 11% between H(4) and H(5) (Figure 4). Removal of the TBDMS-group under acid conditions afforded an epimeric mixture of oxazolidinones **22**, from which the major (cis) epimer could be isolated. Base-catalyzed hydrolysis of the cyclic urethane **22**[23] afforded an aromatic homosphingosine analogue. At this stage, separation of D-*erythro*- and L-*threo*-homosphingosine proved to be feasible by flash chromatography on silica, yielding the pure D-*erythro*-homosphingosine analogue **23**. Acylation with hexanoyl chloride gave access to the aromatic homoceramide analogue **24**.

The integrity of the C(3) stereocenter through the series of reactions was determined by ^{1}H and ^{19}F NMR analysis of the corresponding Mosher ester of the primary alcohol group of **17**.

Biological Evaluation. It has been shown that, when cultured hippocampal neurons are incubated with basic fibroblast growth factor (bFGF), axonal growth rates increase between 2- and 4-fold.[8,24] The stimulatory effect of bFGF on neuronal growth is dependent on the synthesis of glucosylceramide from ceramide, and it can be

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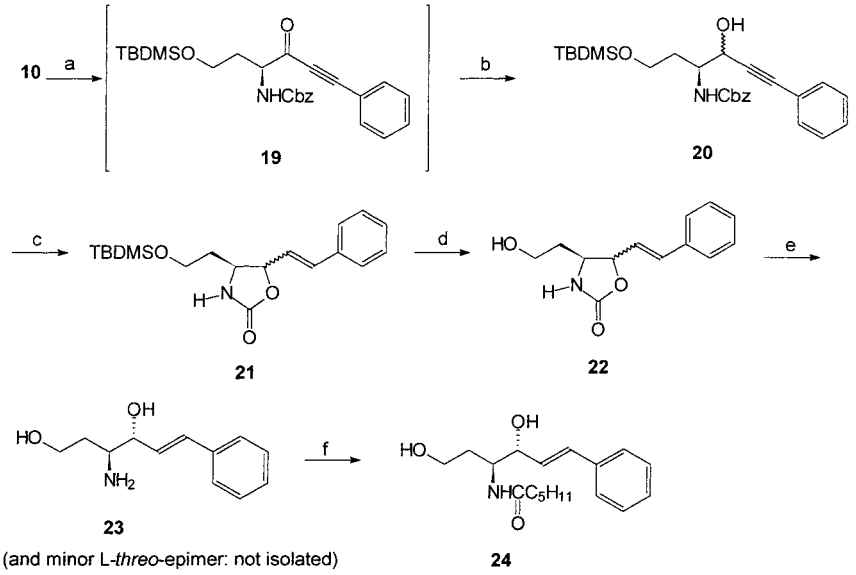
Scheme 5[a]



[a] (a) Lithium phenylacetylide, THF, −78 °C to rt; (b) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, EtOH, 0 °C; (c) Red-Al, Et_2O, −78 °C to rt; (d) CH_3COOH/H_2O 80:20, rt, (e) NaOH 2 N, EtOH, 80 °C; (f) $C_5H_{11}C(O)Cl$, THF, 50% aq NaOAc, rt.

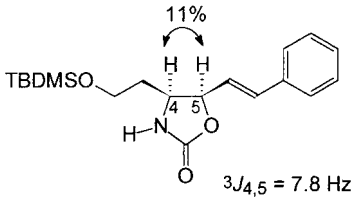


Figure 4. Relative stereochemistry of **21**.

completely abolished by coincubation with inhibitors of glucosylceramide synthesis (e.g., FB_1 or D-*threo*-(1*R*,2*R*)-PDMP). In our experiments, bFGF was added to hippocampal neurons after 48 h in culture, together with FB_1 and with C_6-D-*erythro*-ceramide or one of the homoceramide analogues **15**−**17** and **24**. C_6-D-*erythro*-ceramide was included as a positive control, since it is well-known that it reverses the inhibitory effect of FB_1.[7,8] After 3 h of incubation (51 h in culture), the coverslips were mounted for microscopic examination and the number of branch points counted per cell. The results are shown in Figure 5. None of the homoceramide analogues was able to reverse the inhibitory effect of FB_1 on accelerated axonal growth.

A possible reason for the biological inactivity could be due to the lack of metabolism to homoglucosylceramide. To test this hypothesis, a fluorescent homoceramide analogue **25** was synthesized from homosphingosine **14** and the *N*-hydroxysuccinimidyl (NHS) ester of ϵ-NBD-aminohexanoic acid (Figure 6).[25]

To determine whether homoceramides act as a substrate for glucosylceramide synthase, the metabolism of **25** in isolated rat liver Golgi fractions was analyzed in vitro (Figure 7). It is clear that C_6-NBD-D-*erythro*-homoceramide **25** is not metabolized to C_6-NBD-D-*erythro*-glucosylhomoceramide (lane E). In addition, conversion of C_6-NBD-D-*erythro*-homoceramide **25** to C_6-NBD-D-*erythro*-homosphingomyelin was not observed (lanes D and E). In contrast, C_6-NBD-D-*erythro*-ceramide was converted to C_6-NBD-D-*erythro*-glucosylceramide (lane C) and to C_6-NBD-D-*erythro*-sphingomyelin (lanes B and C). These data are consistent with the hypothesis[4,8,24] that ceramide and ceramide analogues must be glucosylated to exert their biological function in neurons.

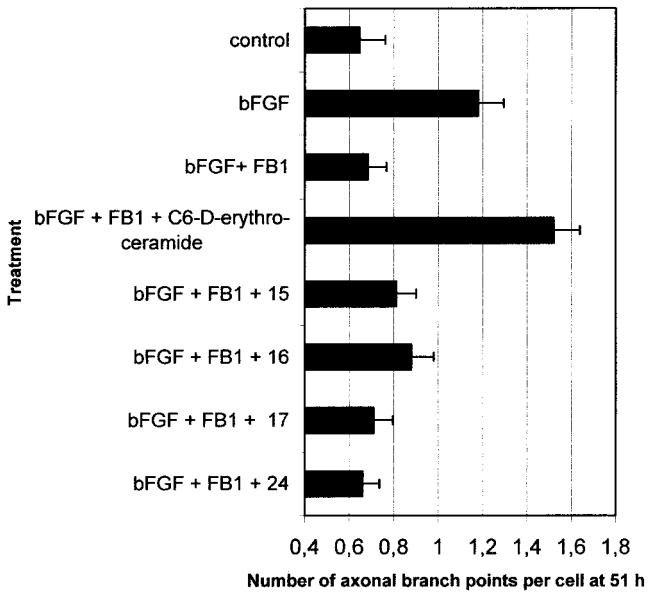


Figure 5. Average number of axonal branch points per neuron, determined after 3 h of incubation with bFGF, FB_1, and homoceramide analogues **15**−**17** and **24**. Values are means ± SEM. Per coverslip, 50 cells were counted for 2 individual coverslips per treatment.

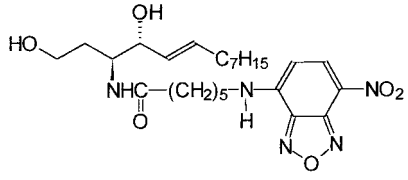


Figure 6. Structure of flourescent homoceramide analogue **25**.

(25) Schwarzmann, G.; Sandhoff, K. *Methods Enzymol.* **1987**, *138*, 319.

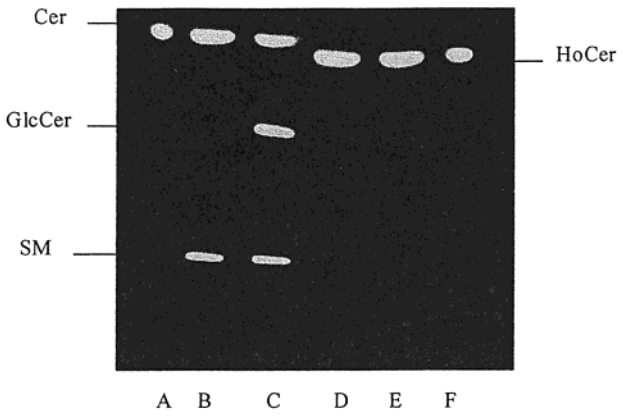


Figure 7. Metabolism of C_6-NBD-D-*erythro*-ceramide (lanes A−C) and C_6-NBD-D-*erythro*-homoceramide **25** (lanes D−F). Golgi fractions were incubated with 5 μM C_6-D-*erythro*-ceramide (lane B: absence of UDP-glucose; lane C: presence of UDP-glucose; lane A shows a C_6-NBD-D-*erythro*-ceramide marker) or 5 μM C_6-NBD-D-*erythro*-homoceramide **25** (lane D: absence of UDP-glucose; lane E: presence of UDP-glucose; lane F: C_6-NBD-homoceramide marker). Cer: ceramide; HoCer: homoceramide; GlcCer: glucosylceramide; SM: sphingomyelin.

Conclusion

In this paper, the synthesis of aromatic and aliphatic homoceramide analogues is described. L-homoserine was chosen as chiral building block and the reaction proceeds via a protected Weinreb-amide derivative. This methodology allows for the synthesis of a wide range of homoceramide analogues, differing in the chain length of the sphingoid base backbone and the *N*-acyl chain. Homoceramides **15**−**17** and **24** were not able to reverse the inhibitory effect of FB_1 on axonal growth of hippocampal neurons, which was due to the lack of conversion of homoceramide to glucosylhomoceramide. However, homoceramides are interesting compounds for study of the structure−activity relationship of other ceramide-mediated biological processes, such as apoptosis. Most metabolic enzymes in the biosynthesis and degradation of sphingolipid metabolism affect the functional groups at C(1), C(2), or C(4) in the sphingoid base backbone. Modifications in this part of the molecule are particularly interesting to see how they influence enzymatic activity. Homoceramides are therefore promising compounds to study as potential inhibitors and/or substrates of the different enzymes in sphingolipid metabolism.

Experimental Section

Hippocampal Cultures. Hippocampal neurons were cultured at low density as described previously.[4,26,27] Briefly, the dissected hippocampi of embryonic day 18 rats (Wistar), obtained from the Weizmann Institute Breeding Center (Rehovot, Israel), were dissociated by trypsinization (0.25% (w/v)) for 15 min at 37 °C. The tissue was washed in magnesium/calcium-free Hank's balanced salt solution 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 6000 cells/13-mm glass coverslip that had been precoated with poly-L-lysine (1 mg/mL). After 3−4 h, coverslips were transferred into 24-well multidishes containing a monolayer of astroglia. Neurons were placed with the cells facing downward and were separated from the glia by paraffin 'feet'. Cultures were maintained in MEM, which included N2-supplements, 0.1% (w/v) ovalbumin, and 0.1 mM pyruvate.

Addition of Compounds. 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. Homoceramide analogues were dissolved in EtOH and added to the culture medium, so that the final EtOH concentration did not exceed 1%. Control cultures were treated with 1% EtOH. bFGF (1 ng/mL) was added to the cultures after 48 h, together with FB_1 (10 μM) and homoceramides (5 μM), and the number of axonal branch points was measured after 51 h.

Analysis of Axonal Growth. After 51 h in culture, the coverslips 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 a Zeiss Axiovert 35 microscope (Achroplan 32x/n.a.0.4). An axon was considered to branch when the process that it gave rise to was more than 15 μm long. Thin filipodia, which were occasionally observed along the entire length of the axon, were not considered as branches.

Synthesis of C_6-NBD-D-*erythro*-homoceramide 25. Compound **25** was prepared from homosphingosine **14** and the NHS-ester of NBD-hexanoic acid, according to a procedure described in the literature.[25]

Substrate Specificity of Glucosylceramide Synthase and Sphingomyelin Synthase. Rat liver Golgi-fractions were prepared using modifications of the method of Bergeron et al.[28] Golgi-apparatus fractions were incubated with C_6-NBD-D-*erythro*-homoceramide **25** or with C_6-NBD-D-*erythro*-ceramide (5 μM) for 1 h at 37 °C in 0.25 M sucrose, 50 mM HEPES (pH 7.4), 100 mM KCl, 5 mM $MnCl_2$, in the presence or absence of 5 mM UDP-glucose. Lipids were extracted using the procedure of Bligh and Dyer[29] and separated by TLC using $CHCl_3$/MeOH/9.8 mM $CaCl_2$ (60:35:8 v/v) as the developing solvent. Fluorescent lipids were detected with a Biorad UV transilluminator.

Synthesis. General. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AN500 spectrometer (^{1}H NMR: 500 MHz; ^{13}C NMR: 125 MHz) or with a Varian Gemini-200 (^{1}H NMR: 200 MHz, ^{13}C NMR: 50 MHz) using tetramethylsilane as internal standard for ^{1}H NMR spectra and DMSO-d_6 (39.7 ppm) or $CDCl_3$ (76.9 ppm) for ^{13}C NMR spectra. Liquid secondary-ion mass spectra (LSIMS) were obtained using a Kratos concept ^{1}H mass spectrometer (Kratos, Manchester, UK). Glycerol, thioglycerol, NaOAc or 3-nitrobenzyl alcohol (NBA) were used as the matrix for the preparation of samples for LSIMS. Electron impact (EI) mass spectra were recorded on a Kratos MS50TC double focusing instrument with a Mach3 data system. Electrospray ionization mass spectra (ES) were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof 2, Micromass, Manchester, UK) equipped with a standard electrospray ionization interface. Samples were infused in a 2-propanol/water 1:1 mixture at 3 μL/min. Precoated Merck silica F254 plates were used for TLC and spots were examined with UV-light at 254 nm and/or a ninhydrin (0.5% in EtOH) solution or a phosphomolybdic acid (0.5% in EtOH) solution. Column chromatography was performed on SÜD-Chemie silica (0.2−0.05 mm). Semipreparative HPLC was performed on silica (10 μM; Biorad; Bio-Sil D90−10; 250 × 10 mm) with a RI-detector (Melz RI-detector LCD 312), and a Kontron-422 pump. Anhydrous solvents were obtained as follows: THF was distilled from sodium/benzophenone; pyridine was refluxed overnight over potassium hydroxide and distilled; dichloromethane and

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(27) Goslin, K.; Asmussen, H.; Banker, G. In *Culturing Nerve Cells*; Banker, G., Goslin, K., Eds.; MIT Press: Cambridge, MA, 1998; pp 339−370.
(28) Bergeron, J. J. M.; Rachubinski, R. A.; Sikstrom, R. A.; Posner, B. I.; Paiement, J. *J. Cell Biol.* **1982**, *92*, 139−146.
(29) Bligh, E. G.; Dyer, W. J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911−917.

toluene were stored over calcium hydride, refluxed, and distilled; DMF was stored over Linde 4 Å molecular sieves, followed by distillation under reduced pressure.

***tert*-Butyl (3*S*)-2-Oxotetrahydro-3-furanylcarbamate (3).** To a solution of **2** (122 mg, 0.52 mmol) and 2,2-dimethoxypropane (515 μl, 4.19 mmol) in acetone (2 mL) was added $BF_3{\cdot}OEt_2$ (7 μL of a 48% solution, 0.028 mmol). After stirring for 24 h at room temperature, the reaction mixture was concentrated in vacuo, and the residue was redissolved in CH_2Cl_2. The solution was washed with a mixture of a saturated $NaHCO_3$-solution and H_2O (1:1) and with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/pentane 1:9) yielded **3** as an oil (91 mg, 87%).

Spectral data are consistent with those reported in the literature.[4a]

(2*S*)-2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-4-hydroxybutanoic Acid (4). To a solution of l-homoserine **1** (1.01 g, 8.48 mmol) in water (6.3 mL) were added Na_2CO_3 (899 mg, 8.48 mmol) and *N*-(ethoxycarbonyl)phthalimide (1.86 g, 8.48 mmol). The reaction mixture was stirred at room temperature until complete dissolution of *N*-(ethoxycarbonyl)phthalimide (ca. 3 h). The reaction mixture was acidified (pH 2) with ice-cold 1 N HCl. The aqueous phase was extracted with EtOAc (3×). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo giving *N*-phthaloyl-L-homoserine **4**, which was used without further purification.

Methyl (2*S*)-2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-4-hydroxybutanoate (5). Crude *N*-phthaloyl-L-homoserine **4** (obtained from 1.01 g L-homoserine) was dissolved in EtOH (23 mL), and dicyclohexylamine (1.69 mL, 8.48 mmol) was added dropwise. The solvent was removed in vacuo affording the dicyclohexylammonium salt of **4** as a white solid. The solid was resuspended in Et_2O and collected by filtration (3.175 g, 90% yield from L-homoserine). To a stirred suspension of the dicyclohexylammonium salt (3.175 g, 7.63 mmol) in anhydrous DMF (53 mL) was added dropwise iodomethane (1.58 mL, 0.025 mol). The reaction mixture was stirred at room-temperature overnight, and the solvent was removed in vacuo. Water was added to the resulting residue and the aqueous phase was extracted with EtOAc (3×). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/pentane 3:7) yielded **5** as an oil (1.38 g, 62% from L-homoserine). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.30−2.40 (1 H, m), 2.50−2.58 (1 H, m), 3.52−3.60 (1 H, m), 3.73−3.80 (4 H, m), 5.10 (1 H, dd, J = 4.6 and 10.4 Hz) 7.75 (2 H, dd, J = 3.1 and 5.4 Hz), 7.87 (2 H, dd, J = 3.1 and 5.4 Hz) ppm; MS (CI): *m*/*z* (%) 264 ([M + H]$^+$, 46), 246 ([M + H − H_2O]$^+$, 100), 232 (100), 204 (19), 186 (9).

Methyl (2*S*)-4-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoate (6). To a solution of **5** (1.39 g, 5.28 mmol) in DMF (15 mL) were added imidazole (899 mg, 0.013 mol) and *tert*-butyldimethylsilyl chloride (955 mg, 6.34 mmol). The reaction mixture was stirred at room-temperature overnight. After 16 h, water was added, and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/hexane 35:65) furnished **6** as an oil (1.69 g, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.09 (6 H, s), 0.85 (9 H, s), 2.42−2.51 (2 H, m), 3.58−3.61 (1 H, m), 3.73−3.79 (4 H, m), 5.16 (1 H, dd, J = 5.4 and 9.6 Hz), 7.73 (2 H, dd, J = 3.1 and 5.4 Hz), 7.86 (2 H, dd, J = 3.1 and 5.4 Hz) ppm; MS (EI): *m*/*z* (%) 362 ([M-CH_3]$^+$, 4), 320 (100), 204 (8), 186 (12), 119 (20), 89 (32).

3-Hydroxy-2-[(3*S*)-2-hydroxytetrahydrofuran-3-yl]isoindolin-1-one (7). To a cooled (−78 °C) solution of **6** (76 mg, 0.20 mmol) in dry toluene (3 mL) was added dropwise a 1.5 M solution of DIBAL in toluene (242 μl, 0.36 mmol) via a syringe. The reaction mixture was stirred for 3 h at −78 °C. The reaction was quenched by slowly adding MeOH (caution: H_2-evolution). The resulting white emulsion was poured into ice-cold 1 N HCl and stirred for 15 min. The aqueous mixture was extracted with EtOAc (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/hexane 25:75) yielded **7** as an oil (19 mg, 41%). Further purification was achieved by HPLC (silica, EtOAc/hexane 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.25−2.30 (2 H, m, 2 H−C(4)), 4.07−4.12 (1 H, m, H_a-C(5)), 4.17−4.21 (1 H, m, H_b-C(5)), 4.82 (1 H, q, J = 4.5 Hz, H−C(3)), 6.02 (1 H, d, J = 4.2 Hz, H−C(2)), 6.14 (1 H, s, H−C(9′)), 7.55−7.59 (1 H, m, H−C(5′)), 7.62 (1 H, m, H−C(6′)), 7.64 (1 H, m, H−C(7′)), 7.81 (1 H, d, J = 7.3 Hz, H−C(4′)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 32.51 (C(4)), 58.65 (C(3)), 69.17 (C(5)), 91.74 (C(9′)), 110.85 (C(2)), 123.82 (C(6′)), 124.29 (C(4′)), 130.48 (C(5′)), 132.04 (C(3′)), 132.95 (C(7′)), 142.11 (C(8′)), 172.51 (C=O) ppm; MS (LSIMS): *m*/*z* (%) 218 ([M + H − H_2O]$^+$, 60), 133 (85), 69 (71), 55 (100).

Benzyl (1*S*)-3-Hydroxy-1-{[methoxy(methyl)amino]-carbonyl}propylcarbamate (9). To a solution of *N*,*O*-dimethylhydroxylamine·HCl (6.26 g, 0.064 mol) in CH_2Cl_2 (58 mL) were added pyridine (11.2 mL) and trimethylaluminum (32.1 mL of a 2 M solution in hexane, 0.064 mol) (Caution: CH_4-evolution). The mixture was stirred for 15 min at room temperature. Subsequently, a solution of **8** (5.43 g, 0.021 mol) in CH_2Cl_2 (20 mL) was added dropwise. The mixture was stirred at room-temperature for 3 days. The reaction was carefully quenched with 1 N HCl and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/pentane 7:3) yielded **9** as an oil (4.88 g, 77%). Further purification was achieved by HPLC (silica, EtOAc/hexane 75:25). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.99−2.18 (2 H, m), 3.21 (3 H, s), 3.68−3.72 (2 H, m), 3.77 (3 H, s), 4.80−4.95 (1 H, m), 5.06 (1 H, A of AB, J = 12.2 Hz), 5.12 (1 H, B of AB, J = 12.2 Hz), 5.84 (1 H, d, J = 7.7 Hz), 7.29−7.39 (5 H, m) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 32.08, 35.43, 48.43, 58.21, 61.54, 66.97, 128.05, 128.14, 128.38, 135.99, 156.76, 172.35 ppm; MS (LSIMS): *m*/*z* (%) 297 ([M + H]$^+$, 11), 164 (8), 91 ([benzyl]$^+$, 100).

(2*S*)-2-{[1-(Benzyloxy)vinyl]amino}-4-{[*tert*-butyl(dimethyl)silyl]oxy}-*N*-methoxy-*N*-methylbutanamide (10). To a solution of **9** (5.15 g, 0.017 mol) in DMF (27 mL) were added imidazole (2.958 g, 0.044 mol) and *tert*-butyldimethylsilyl chloride (3.14 g, 0.021 mol). The reaction mixture was stirred at room-temperature overnight and, after 16 h, diluted with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/hexane 2:8) yielded pure **10** as an oil (6.03 g, 84%). Further purification was achieved by HPLC (silica, EtOAc/hexane 25:75). $[\alpha]_D$ = −5.4 (*c* 0.47, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.04 (3 H, s), 0.06 (3 H, s), 0.89 (9 H, s), 1.74−1.80 (1 H, m), 1.96−2.04 (1 H, m), 3.21 (3 H, s), 3.67−3.75 (2 H, m), 3.79 (3 H, s), 4.82−4.88 (1 H, m), 5.05 (1 H, A of AB, J = 12.3 Hz), 5.15 (1 H, B of AB, J = 12.3 Hz), 5.78 (1 H, d, J = 8.0 Hz), 7.29−7.36 (5 H, m) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ −5.58, 18.11, 25.81, 32.17, 34.79, 49.23, 59.54, 61.44, 66.62, 127.83, 127.91, 128.37, 136.58, 156.14 ppm; MS (LSIMS): *m*/*z* (%) 411 ([M + H]$^+$, 8), 353 (9), 92 ([benzyl]$^+$, 100), 74 (30); Exact mass (LSIMS): calculated for $C_{20}H_{35}N_2O_5Si$ [M + H]$^+$ 411.2315, found 411.2353.

Benzyl (1*S*)-1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-2-oxo-undec-3-ynylcarbamate (11). *n*-BuLi (11.96 mL of a 1.6 M solution in hexane, 0.019 mol) was added dropwise to a solution of non-1-yne (3.35 mL, 0.020 mol) in dry THF (110 mL) at −20 °C. After stirring at −20 °C for 2 h, the mixture was cooled to −78 °C and a solution of **10** (2.618 g, 6.38 mmol) in dry THF (5 mL) was added dropwise. The mixture was allowed to warm to −40 °C and, after 3 h, the reaction was quenched by addition of a saturated NH_4Cl-solution (72 mL). The aqueous phase was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and evaporated in vacuo, yielding crude **11**. The crude product was used without further purification.

Benzyl (1*S*,2*RS*)-1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}-ethyl)-2-hydroxy-undec-3-ynyl-car-bamate (12). Crude **11** (obtained from 6.376 mmol **10**) was dissolved in EtOH (26 mL),

and the solution was cooled to 0 °C (ice-bath). Cerium(III) chloride ($CeCl_3 \cdot 7H_2O$, 713 mg, 1.91 mmol) was added until complete dissolution, followed by addition of $NaBH_4$ (265 mg, 7.01 mmol). The solution was stirred for 3 h at 0 °C, the solvent was evaporated, and the residue was suspended in 1 N HCl (69 mL). The aqueous phase was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/pentane 1:9) yielded an unseparable mixture of epimeric alkynols **12** as an oil (1.55 g, 51%). Further purification was achieved by HPLC (silica, EtOAc/hexane 2:8). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.06 (6 H, s), 0.86−0.90 (12 H, m), 1.24−1.38 (8 H, m), 1.45−1.51 (2 H, quintet, J = 7.3 Hz), 1.85−1.95 (2 H, m), 2.18 (2 H, td, J = 1.9 and 7.1 Hz), 3.67−3.77 (2 H, m), 3.88−3.92 (0.25 H, m, minor epimer), 3.95−4.01 (1 H, m, major epimer), 4.42−4.47 (1 H, m), 5.10 (2 H, s), 5.44 (1 H, d, J = 7.7 Hz), 7.34−7.36 (5 H, m) ppm; Exact mass (ES): calculated for $C_{27}H_{45}NO_4Si$ $[M + Na]^+$ 498.3015, found 498.3015.

Benzyl (1*S*,2*RS*)-2-Hydroxy-1-(2-hydroxylethyl)undec-3-ynylcarbamate (13). To a solution of **12** (924 mg, 1.94 mmol) in MeOH (10 mL) was added 1.0 mL concentrated HCl (37%). The reaction mixture was stirred at room-temperature for 1.5 h and then neutralized with 25% aqueous ammonia. Purification of the residue by flash chromatography (silica, EtOAc/pentane 1:1) afforded an inseparable mixture of alkynols **13** as a white solid (541 mg, 77%). Further purification was achieved by HPLC (silica, EtOAc/hexane 4:6). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3 H, t, J = 6.8 Hz), 1.24−1.36 (8 H, m), 1.44−1.52 (2 H, m), 1.59−1.68 (1 H, m), 1.90−1.98 (1 H, m), 2.19 (2 H, t, J = 7.1 Hz), 2.63 (2 H, br s), 3.64−3.75 (2 H, m), 3.95−3.99 (0.4 H, m, *threo*-epimer), 4.01−4.07 (1 H, m, *erythro*-epimer), 4.41−4.44 (0.4 H, m, *threo*-epimer), 4.46−4.50 (1 H, m, *erythro*-epimer), 5.09 (1 H, A of AB, J = 12.1 Hz), 5.15 (1 H, B of AB, J = 12.1 Hz), 5.25 (1 H, br s), 7.32−7.38 (5 H, m) ppm; MS (ES, *i*-Prop/H_2O): *m/z* (%) 384 ($[M + H + Na]^+$, 100), 362 ($[M + H]^+$, 27), 344 ($[M + H - H_2O]^+$, 26), 318 (26), 300 (28); Exact mass (ES): calculated for $C_{21}H_{32}NO_4$ $[M + H]^+$ 362.2331, found 362.2366.

(3*S*,4*R*,5*E*)-3-Aminotridec-5-ene-1,4-diol (14). A solution of Li (249 mg, 0.036 mmol) in $EtNH_2$ (10 mL) was stirred for 2 h at −78 °C, and a solution of **13** (866 mg, 2.40 mmol) in THF (10 mL) was added dropwise. The reaction mixture was stirred for 3 h at −78 °C, and the reaction was quenched by adding a saturated NH_4Cl-solution (8.5 mL). $EtNH_2$ and THF were removed under reduced pressure, and the residue was diluted with water and extracted several times with EtOAc. The organic layers were dried over $MgSO_4$ and evaporated in vacuo. The residue was subjected to flash chromatography (silica, CH_2Cl_2/MeOH/2 M NH_3 85:15:1). Both epimers could be separated, but only the major *erythro*-epimer **14** was obtained in pure form (313 mg, 57%). ^{1}H NMR (500 MHz, CD_3OD): δ 0.87 (3 H, t, J = 6.8 Hz), 1.20−1.38 (8 H, m), 1.40−1.48 (2 H, m), 1.68−1.77 (1 H, m), 1.80−1.88 (1 H, m), 2.10 (2 H, q, J = 7.0 Hz), 3.31 (1 H, quintet, J = 1.6 Hz), 3.68−3.76 (2 H, m) 4.21 (1 H, br t), 5.48 (1 H, dd, J = 6.7 and 15.4 Hz), 5.84 (1 H, dt, J = 6.8 and 15.0 Hz) ppm; MS (ES): *m/z* (%) 252 ($[M + Na]^+$, 16), 230 ($[M + H]^+$, 50), 212 ($[M + H - H_2O]^+$, 100); exact mass (ES): calculated for $C_{13}H_{28}NO_2$ $[M + H]^+$ 230.2120, found 230.2097.

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(2-hydroxyethyl)undec-3-enyl]acetamide (15).** To a solution of amine **14** (94 mg, 0.41 mmol) in THF (4 mL) were added a 50% aqueous NaOAc solution (4 mL) and acetyl chloride (26 μl, 0.37 mmol). After completion of the reaction (6 h), THF and brine were added. The organic phase was separated, washed with water, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, MeOH/CH_2Cl_2 7:93) yielded **15** as a white solid (82 mg, 74%). Further purification was achieved by HPLC (silica, MeOH/CH_2Cl_2 6:94). $[\alpha]_D = -7.9$ (*c* 0.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3 H, t, J = 6.9 Hz), 1.23−1.30 (10 H, m), 1.34−1.39 (1 H, m), 1.82−1.88 (1 H, m), 2.00−2.07 (5 H, m), 2.69 (2 H, br s), 3.57 (1 H, t, J = 9.5 Hz), 3.66 (1 H, dd, J = 4.1 and 11.6 Hz), 4.08−4.17 (1 H, m), 4.23−4.27 (1 H, m), 5.47 (1 H, dd, J = 5.8 and 15.5 Hz), 5.75 (1 H, dt, J = 6.6 and 14.8 Hz), 6.25 (1 H, d, J = 7.2 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.09, 22.65, 23.24, 29.11, 29.50, 29.69, 31.26, 31.79, 32.28, 50.94, 58.46, 74.42, 128.32, 134.19, 171.39; MS (ES): *m/z* (%) 294 ($[M + Na]^+$, 54), 272 ($[M + H]^+$, 40), 254 ($[M + H - H_2O]^+$, 100); exact mass (ES): calculated for $C_{15}H_{30}NO_3$ $[M + H]^+$ 272.2226, found 272.2211.

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(2-hydroxyethyl)undec-3-enyl]hexanamide (16).** Compound **16** was synthesized from amine **14** (124 mg, 0.54 mmol) and hexanoyl chloride (68 μl, 0.49 mmol) using the procedure described for the synthesis of compound **15**. Purification of the residue by flash chromatography (silica, MeOH/CH_2Cl_2 6:94) yielded **16** as a white solid (103 mg, 58%). Further purification was achieved by HPLC (silica, EtOAc/hexane 8:2). $[\alpha]_D = -10.7$ (*c* 0.43, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87−0.92 (6 H, m, 2 × CH_3), 1.26−1.38 (14 H, m, 7 × CH_2), 1.46−1.53 (1 H, m, C*H*$_2$CH$_2$OH), 1.62−1.68 (2 H, quintet, J = 7.3 Hz, NHC(O)CH$_2$C*H*$_2$), 1.83−1.89 (1 H, m, C*H*$_2$CH$_2$OH), 2.06 (2 H, q, J = 7.2 Hz, H−C(5)), 2.24 (2 H, t, J = 7.6 Hz, NHC(O)C*H*$_2$), 2.95 (2 H, br s, 2 × OH), 3.56 (1 H, td, J = 2.9 and 11.7 Hz, C*H*$_2$OH), 3.67 (1 H, td, J = 4.4 and 11.7 Hz, C*H*$_2$OH), 4.10−4.15 (1 H, tt, J = 2.9 and 8.8 Hz, H−C(1)), 4.24 (1 H, t, J = 4.6 Hz, H−C(2)), 5.48 (1 H, dd, J = 6.4 and 15.6 Hz, H−C(3)), 5.75 (1 H, dt, J = 6.8 and 15.1 Hz, H−C(4)), 6.12 (1 H, d, J = 8.3 Hz, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.92 (CH_3), 14.09 (CH_3), 22.37, 22.64 (2 × *C*H$_2$CH$_3$), 25.53 (NHC(O)CH$_2$*C*H$_2$), 29.09, 29.12, 29.16 (C(6), C(7) and C(8)), 31.35, 31.40 (C(9) and NHC(O)-CH$_2$CH$_2$*C*H$_2$), 31.80 (*C*H$_2$CH$_2$OH), 32.30 (C(5)), 36.70 (NHC-(O)*C*H$_2$), 50.80 (C(1)), 58.46 (CH$_2$OH), 74.46 (C(2)), 128.35 (C(4)), 134.12 (C(3)), 174.65 (C=O) ppm; MS (ES): *m/z* (%) 350 ($[M + Na]^+$, 39), 328 ($[M + H]^+$, 39), 310 ($[M + H - H_2O]^+$, 100); Exact mass (ES): calculated for $C_{19}H_{38}NO_3$ $[M + H]^+$ 328.2852, found 328.2866.

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(2-hydroxyethyl)undec-3-enyl]benzamide (17).** Compound **17** was prepared by the procedure described for the synthesis of **15** using **14** (110 mg, 0.48 mmol) and benzoyl chloride (50 μl, 0.43 mmol). Purification of the residue by flash chromatography (silica, MeOH/CH_2Cl_2 5:95) yielded **17** as a white solid (101 mg, 63%). For identification purposes and biological testing, a small sample was purified by HPLC (silica, EtOAc/hexane 7:3). $[\alpha]_D = -20.3$ (*c* 0.48, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3 H, t, J = 6.6 Hz), 1.23−1.31 (8 H, m), 1.35−1.40 (2 H, m), 1.60−1.67 (1 H, m), 1.95−2.01 (1 H, m), 2.07 (2 H, q, J = 7.1 Hz), 2.74 (2 H, br s), 3.63−3.68 (1 H, m), 3.71−3.75 (1 H, m), 4.32−4.39 (2 H, m), 5.55 (1 H, dd, J = 6.0 and 15.4 Hz), 5.80 (1 H, dt, J = 6.7 and 15.4 Hz), 6.87 (1 H, br s), 7.45 (2 H, t, J = 7.6 Hz), 7.53 (1 H, t, J = 7.3 Hz), 7.80 (2 H, d, J = 8.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.09, 22.65, 29.12, 29.16, 29.69, 31.52, 31.78, 32.33, 51.62, 58.72, 74.60, 127.03, 128.38, 128.66, 131.84, 133.80, 134.35, 168.51; MS (ES): *m/z* (%) 356 ($[M + Na]^+$, 37), 334 ($[M + H]^+$, 28), 316 ($[M + H - H_2O]^+$, 100), 298 (8); exact mass (ES): calculated for $C_{20}H_{32}NO_3$ $[M + H]^+$ 334.2382, found 334.2398.

(4*S*,5*R*)-4-(2-{[*tert*-Butyl)dimethyl)silyl]oxy}ethyl)-5-(non-1-ynyl)-1,3-oxazolidin-2-one (18). To a solution of **12** (106 mg, 0.22 mmol) in THF was added 100 μL (0.56 mmol) of a 30% NaOMe solution in MeOH. The reaction mixture was stirred at room-temperature for 4 h and diluted with Et_2O and water. The aqueous phase was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/hexane 15:85) gave the epimeric mixture of oxazolidinones as an oil (30 mg, 37%). The major isomer **18** could be obtained in pure form by HPLC (silica, EtOAc/hexane 2:8). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.09 (6 H, s, 2 × Si−CH_3), 0.88−0.92 (12 H, m, CH_3 and *tert*-Bu), 1.25−1.40 (8 H, m, 4 × CH_2), 1.53 (2 H, quintet, J = 7.3 Hz, C≡C−CH$_2$−C*H*$_2$), 1.88−1.93 (2 H, m, C*H*$_2$CH$_2$OSi), 2.26 (2 H, dt, J = 2.0 and 7.1 Hz, C≡C−C*H*$_2$), 3.73−3.78 (1 H, m, C*H*$_2$OSi), 3.85 (1 H, dt, J = 4.7 and 10.6 Hz, C*H*$_2$OSi), 3.98 (1 H, J = 4.8 and 8.1 Hz, H−C(4)), 5.29 (1 H, dt, J = 2.0 and 7.9 Hz, H−C(5)), 5.58 (1 H, br s, NH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ −5.52 (Si−CH_3), −3.60 (Si−CH_3), 14.02 (CH_3), 18.73

(C−Si), 22.57, 25.90, 28.25, 28.70, 31.68, 34.21 (CH_2CH_2OSi), 54.65 (C(4)), 61.27 (CH_2OSi), 70.51 (C(5)), 72.86 ($C{\equiv}C\text{-}CH_2$), 91.85 ($C{\equiv}C{-}CH_2$), 157.97 (C=O) ppm.

Benzyl (1*S*)-1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-2-oxo-4-phenyl-3-butynylcarbamate (19). To a cooled (−78 °C) solution of **10** (2.06 g, 5.02 mmol) in dry THF (10 mL) was added dropwise lithium phenylacetylide (10 mL of a 1 M solution in THF, 10.0 mmol). The reaction mixture was warmed to 0 °C over 5 h. The reaction was quenched with a 10% aqueous citric acid solution, and the aqueous phase was washed with EtOAc. The combined organic layers were washed with a 7% aqueous $NaHCO_3$-solution, giving crude **19** as an oil, which was further used without purification.

Benzyl (1*S*,2*RS*)-1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}-ethyl)-2-hydroxy-4-phenyl-3-butynylcarbamate (20). Crude **19** (obtained from 5.02 mmol **10**) was dissolved in ethanol (130 mL), and the solution was cooled to 0 °C. Cerium(III) chloride ($CeCl_3{\cdot}7H_2O$, 666 mg, 1.79 mmol) was added until complete dissolution and then $NaBH_4$ (248 mg, 6.55 mmol) was added. The solution was stirred for 2 h at 0 °C, the solvent was evaporated, and the residue was suspended in 1 N HCl (65 mL) and extracted with EtOAc. The combined organic layers were washed with 1 N HCl, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/pentane 15:85) yielded an unseparable mixture of epimers **20** as an oil (1.02 g, 45% from **10**). Further purification was achieved by HPLC (silica, EtOAc/hexane 2:8). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.07 (6 H, s), 0.09 (9 H, m), 1.95−2.09 (2 H, m), 3.72−3.81 (2 H, m), 4.03−4.08 (0.25 H, m, minor epimer), 4.10−4.16 (1 H, m, major epimer), 4.67−4.70 (1 H, m, major epimer), 4.71−4.73 (0.25 H, m, minor epimer), 5.12 (2 H, s), 5.53 (0.25 H, d, J = 7.8 Hz, minor epimer), 5.58 (1 H, d, J = 7.8 Hz, major isomer), 7.26−7.41 (10 H, m) ppm. MS (ES): m/z (%) 454 ($[M + H]^+$, 26), 436 ($[M + H - H_2O]^+$, 100), 392 (86), 260 (96). Exact mass (ES): calculated for $C_{26}H_{36}NO_4Si$ $[M + H]^+$ 454.2413, found 454.2455.

(4*S*,5*RS*)-4-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-5-[(*E*)-2-phenylethenyl]-1,3-oxazolidin-2-one (21). A solution of **20** (93 mg, 0.21 mmol) in dry Et_2O (5 mL) was added dropwise to a solution of Red-Al (616 μl of a 3.33 M solution in toluene, 2.1 mol) in Et_2O (5 mL). The solution was stirred at room temperature for 24 h. Excess Red-Al was destroyed with MeOH until H_2-evolution ceased. A saturated disodium tartrate solution (3 mL) was added, and the mixture was stirred for 3 h. The aqueous layer was separated and extracted with Et_2O. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/pentane 2:8) yielded an unseparable mixture of epimers **21** as an oil (29 mg, 41%). Further purification was achieved by HPLC (silica, EtOAc/hexane 3:7). ^{1}H NMR of cis isomer (200 MHz, $CDCl_3$): δ 0.08 (3 H, s, CH_3), 0.09 (3 H, s, CH_3), 0.95 (9 H, m, $C(CH_3)_3$), 1.64−1.76 (3 H, m, CH_2CH_2O), 3.62−3.83 (2.5 H, m, CH_2O), 4.02−4.19 (1.5 H, m, H−C(4)), 5.23 (1 H, td, J = 0.9 and 7.8 Hz, H−C(5)), 5.66 (1 H, br s, NH), 6.21 (1 H, dd, J = 7.7 and 15.9 Hz, $CH{=}CH{-}C_6H_5$), 6.73 (1 H, dd, J = 0.9 and 15.9 Hz, $CH{=}CH\text{-}C_6H_5$), 7.31−7.44 (5 H, m, arom H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): δ −5.58 (CH_3), 18.09 (C−Si), 25.83 ($C(CH_3)_3$), 33.14 (CH_2CH_2O, *cis*-epimer), 34.02 (CH_2CH_2O, *trans*-epimer), 55.91 (C(4), *cis*-epimer), 58.13 (C(4), *trans*-epimer), 61.14 (CH_2O, *trans*-epimer), 61.52 (CH_2O, *cis*-epimer), 80.22 (C(5), *cis*-epimer), 83.13 (C(5), *trans*-epimer), 122.10 ($CH{=}CH{-}C_6H_5$), 126.84 (arom C (*ortho*)), 128.57 (arom C (*para*)), 128.57 (arom C (*meta*)), 134.91 ($CH{=}CH{-}C_6H_5$), 135.64 (arom C (*ipso*)), 158.64 (C=O) ppm.

(4*S*,5*RS*)-4-(2-Hydroxyethyl)-5-[(*E*)-2-phenylethenyl]-1,3-oxazolidin-2-one (22). Epimers **21** (100 mg, 0.29 mmol) were dissolved in 7.5 mL of acetic acid and 1.9 mL of water. After stirring overnight at room-temperature, the mixture was neutralized with a 7% aqueous $NaHCO_3$-solution. The aqueous phase was extracted with EtOAc (3×). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash chromatography (silica, EtOAc/pentane 7:3) yielded a mixture of oxazolidinones **22** as an oil (55 mg, 82%). Repeated flash chromatography (silica, EtOAc/pentane 6:4→7:3) allowed isolation of the *cis*-oxazolidinone **22** as a semisolid. ^{1}H NMR (500 MHz, $CDCl_3$) (data for *cis*-**22**): δ 1.60−1.80 (2 H, m), 3.73−3.83 (1.5 H, m), 3.87−3.93 (1.5 H, m), 4.11−4.16 (1.5 H, m), 5.25 (1 H, t, J = 7.9 Hz), 6.19−6.28 (1 H, m), 6.72 (1 H, d, J = 15.9 Hz), 7.30−7.45 (5 H, m) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) (data for cis-**22**): δ 32.87, 55.90, 61.10, 80.37, 121.90, 126.79, 128.58, 128.74, 135.05, 135.50, 158.76.

(3*S*,4*R*,5*E*)-3-Amino-6-phenyl-5-hexene-1,4-diol (23). To a solution of **22** (58 mg, 0.25 mmol) in EtOH (2.0 mL) was added 2 N NaOH (2.0 mL). The mixture was stirred at 80 °C for 2.5 h. After cooling to room-temperature, water was added and the aqueous phase was extracted several times with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Both epimers could be separated by flash chromatography (silica, CH_2Cl_2/MeOH/NH_3 2 M 85:15:1), affording the pure major *erythro*-epimer **23** as a white solid (36 mg, 70%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.58−1.75 (1 H, m), 1.82−1.88 (1 H, m), 3.16 (1 H, quintet, J = 4.3 Hz), 3.73 (2 H, t, J = 6.2 Hz), 4.26 (1 H, t, J = 5.1 Hz), 6.29 (1 H, dd, J = 6.7 and 16.0 Hz), 6.69 (1 H, d, J = 15.9 Hz), 7.23 (1 H, t, J = 7.3 Hz), 7.30 (2 H, t, J = 7.6 Hz), 7.44 (2 H, d, J = 7.3 Hz) ppm. MS (LSIMS): m/z (%) 208 ($[M + H]^+$, 86), 190 ($[M + H - H_2O]^+$, 100), 155 (9), 145 (12), 91 (30), 55 (44); Exact mass (LSIMS): calculated for $C_{12}H_{18}NO_2$ $[M + H]^+$ 208.1337, found 208.1322.

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-(2-hydroxyethyl)-4-phenyl-3-butenyl]hexanamide (24).** Compound **24** was prepared by the procedure described for the synthesis of compound **15** using amine **23** (40 mg, 0.19 mmol) and hexanoyl chloride (27 μL, 0.19 mmol). Purification of the residue by flash chromatography (silica, MeOH/CH_2Cl_2 3:97) afforded compound **24** as a white solid (47 mg, 79%). For identification purposes and biological testing, a small sample was purified by HPLC (silica, EtOAc/hexane 85:15). $[\alpha]_D$ = −21.5 (*c* 0.35, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3 H, t, J = 6.7 Hz), 1.29−1.33 (4 H, m), 1.57−1.67 (3 H, m), 1.89−1.96 (1 H, m), 2.27 (2 H, t, J = 7.3 Hz), 2.40 (2 H, br s), 3.57−3.64 (1 H, m), 3.67−3.73 (1 H, m), 4.24−4.29 (1 H, m), 4.48−4.51 (1 H, m), 6.22 (1 H, dd, J = 5.5 and 15.7 Hz), 6.33 (1 H, br s), 6.68 (1 H, d, J = 15.7 Hz), 7.31−7.38 (5 H, m) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.90, 22.35, 25.52, 31.37, 31.53, 36.69, 51.20, 58.53, 74.42, 126.52, 127.94, 127.97, 128.63, 131.80, 136.14, 174.79; MS (LSIMS): m/z (%) 328 ($[M + Na]^+$, 100), 288 (6), 190 (28), 153 (7), 56 (13); Exact mass (LSIMS): calculated for $C_{18}H_{27}NO_3Na$ $[M + Na]^+$ 328.1889, found 328.1926.

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Supporting Information Available: ^{13}C spectra of compounds **10**, **15**, **16**, **17**, *cis*-**22**, and **24** as well as the ^{1}H and ^{19}F NMR spectra of the Mosher esters of **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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