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Straightforward Access to Spisulosine and 4,5-Dehydrospisulosine Stereoisomers: Probes for Profiling Ceramide Synthase Activities in Intact Cells

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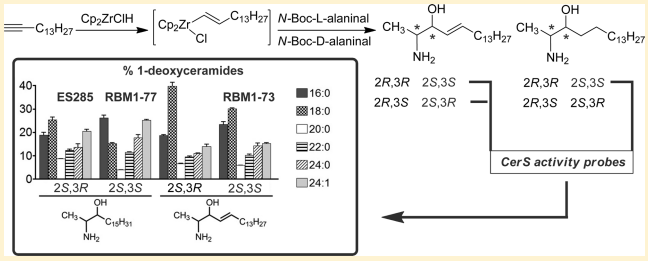
Supporting Information

ABSTRACT: A stereoselective synthesis of spisulosine (**ES285**) and 4,5-dehydrospisulosine stereoisomers is described. Hydrozirconation of 1-pentadecyne with Schwartz reagent, followed by diastereocontrolled addition to L- or D-alaninal afforded the required 2-amino-1,3-diol framework. The resulting sphingoid bases revealed as excellent probes for the profiling of ceramide synthase activity in intact cells. Among the sphingoid bases described in this work, spisulosine (**ES285**), **RBM1-77**, and **RBM1-73** were the most suitable ones because of their highest acylation rates. These molecules should prove useful to study the role of the different ceramide synthases and the resulting *N*-acyl (dihydro)ceramides in cell fate.



INTRODUCTION

Sphingolipids (SLs) constitute a group of natural products characterized by the presence of a long chain 2-amino-1,3-diol scaffold or sphingoid base. Structurally, this central scaffold can accommodate a variety of functionalities that account for the different families of SLs found in nature. In particular, dihydrosphingosine shows a C18 2-amino-1,3-diol core with a defined 2*S*,3*R* stereochemistry that is common to the most representative group of mammal SLs (Figure 1). Acylation of the amino group of sphingosine with acyl chains of different lengths and unsaturations, followed by metabolic oxidation to install an *E* unsaturation at C4 position of the sphingoid base, affords ceramides. These are essential constituents of SL metabolism in mammals, with crucial roles in cell fate and homeostasis. Likewise, polar head groups, such as phosphate derivatives or sugars, can be appended at the C1-hydroxy position of ceramides to afford sphingomyelins and glycosphingolipids, respectively (Scheme 1). Besides playing a structural role and regulating the physical properties of cell membranes, these SL metabolites also participate in cell signaling and in the control of numerous cellular functions in mammals.[1]

Several 1-deoxysphingolipids, derived from a central core lacking the C1-OH group present in dihydrosphingosine, are also found in nature. This is the case of spisulosines, a group of antiproliferative compounds of marine origin isolated from the clam *Spisula polynyma* (syn. *Mactromeris polynima*).[2] Although several spisulosine analogs of different chain lengths have been reported,[3,4] **ES285** (Figure 1) resembles the natural SL in the C18 sphingoid backbone and in the (2*S*,3*R*) configuration of the amino and hydroxyl groups, respectively.

Interestingly, prior to its isolation and characterization as a marine natural product, the structure of spisulosine had already been described as a synthetic simplified analog of the ceramide synthase (CerS) inhibitor Fumonisin B1 (FB1), together with enigmol and other related 2-amino-3,5-octadecanediols with different stereochemistries at the sphingoid backbone (Figure 1).[5] Although spisulosine was initially developed as a promising anticancer agent due to its ability to inhibit proliferation in the prostate tumor PC-3 and LNCaP cell lines,[6] it was discontinued from phase I in 2008.[7,8] Nevertheless, its close structural relationship with other related 1-deoxysphingoids with remarkable cytotoxic properties, such as obscuraminols,[9] clavaminols,[10,11] crucigasterins,[12] and xestoaminols,[13] (Figure 1) makes this type of compound an attractive lead for anticancer-oriented drug discovery programs. In addition, recent evidence of the presence of 1-deoxysphingolipids as natural metabolites in mammalian cells have been postulated from studies of a mutation in the *SPTLC1* gene that is found in human sensory neuropathy type 1 (HSN1).[14] Interestingly, in a recent study, the presence of several 1-deoxysphingolipids in plasma has also been proposed as a novel class of biomarkers for the metabolic syndrome.[15]

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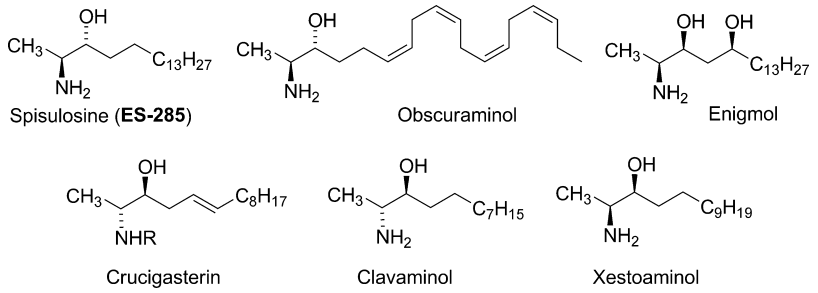
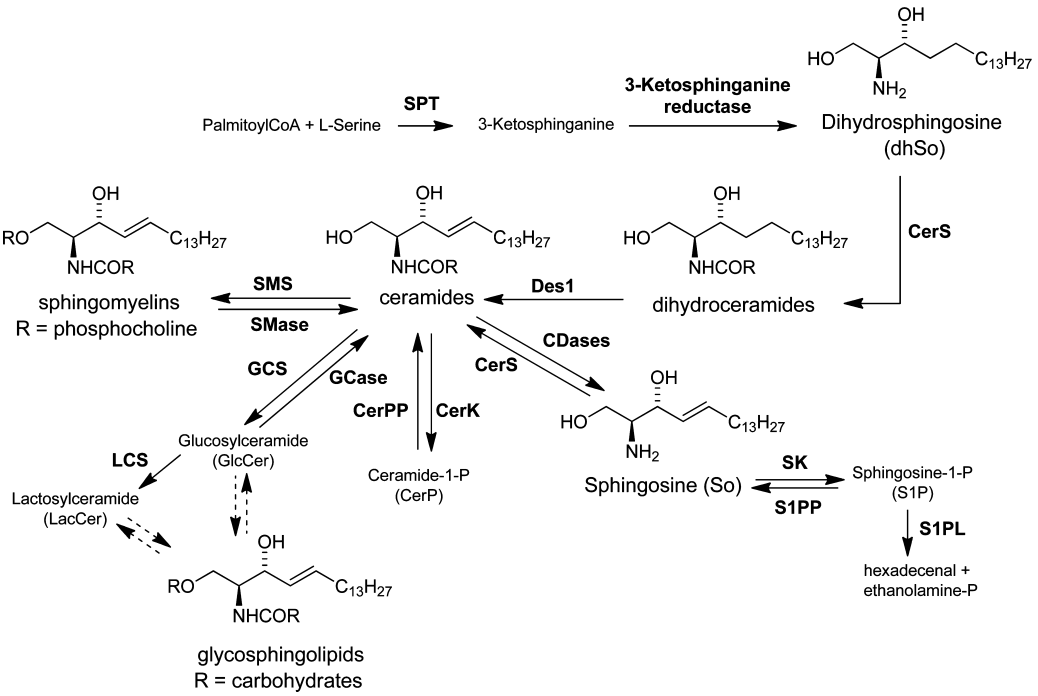


Figure 1. Spisulosine (**ES285**) and related 1-deoxysphingolipids.

Scheme 1. Main Metabolic Pathways in Sphingolipids[a]



[a]SPT, serine palmotyl transferase; CerS, ceramide synthase; Des1, dihydroceramide desaturase; SMS, sphingomyelin synthase; SMase, sphingomyelinase; GCS, glucosylceramide synthase; GCase, glucocerebrosidase; CerPP, CerP phosphatase; CerK, ceramide kinase; CerS, ceramide synthase; CDases, ceramide hydrolases; LCS, lactosyl ceramide synthase; SK, sphingosine kinase; S1PP, S1P phosphatase.

In light of the above considerations, we have recently undertaken a work program aimed at the study of the cellular properties of spisulosine and related analogs as SL modulators. In this paper, we report on the design of efficient synthetic protocols for this type of 1-desoxysphingolipids, with special attention to the stereochemical control at C2 and C3 positions and the presence of a C4−C5 unsaturation as part of the sphingoid backbone. The usefulness of some of the compounds as probes for ceramide synthase activity profiling in intact cells is also reported.

RESULTS AND DISCUSSION

Aside from its interesting cytotoxic properties, spisulosine has also been used as a target to illustrate the applicability of new synthetic methodologies. In this context, several multistep syntheses of enantiopure spisulosine, or that of advanced precursors thereof, have been reported in the literature. Examples are found in the reduction of enantiomerically pure 2-acylaziridines,[16] the Cu-catalyzed Grignard addition to a suitable *N*-Boc aminoepoxide,[17] the organometallic addition to *N*-*tert*-butylsulfinyl imines,[18] the diastereoselective reduction of ketimines,[19] the regioselective azidolysis of epoxyalcohols,[20] the reaction of carbon nucleophiles with cyclic sulfamidates,[21] or, in its racemic form, the functional group transformations from a Morita-Baylis-Hillman adduct.[22] In addition, spisulosine has also been obtained by multistep synthesis from Garner's aldehyde.[23] In view of the above precedents, we reasoned that a direct approach, based on the use of D-or L-alanine as starting materials, would be more suitable for the straightforward synthesis of spisulosine analogs with different chiralities and degrees of unsaturation in the sphingoid backbone. Surprisingly, despite its apparent simplicity, the use of D- or L-alanine derivatives as starting materials for the synthesis of spisulosine analogs has been scarcely reported in the literature. Thus, the addition of Grignard reagents to *N*,*N*-dibenzylalaninal[24,25] or to the Weinreb amide of *N*-Boc alanine[26] are the only reported examples along this line.

In this work, we report on an alternative, straightforward approach to spisulosines based on the diastereocontrolled addition of a suitable alkenylzirconocene to *N*-Boc L- or D-alaninal according to the general approach described in Scheme 2.[27]

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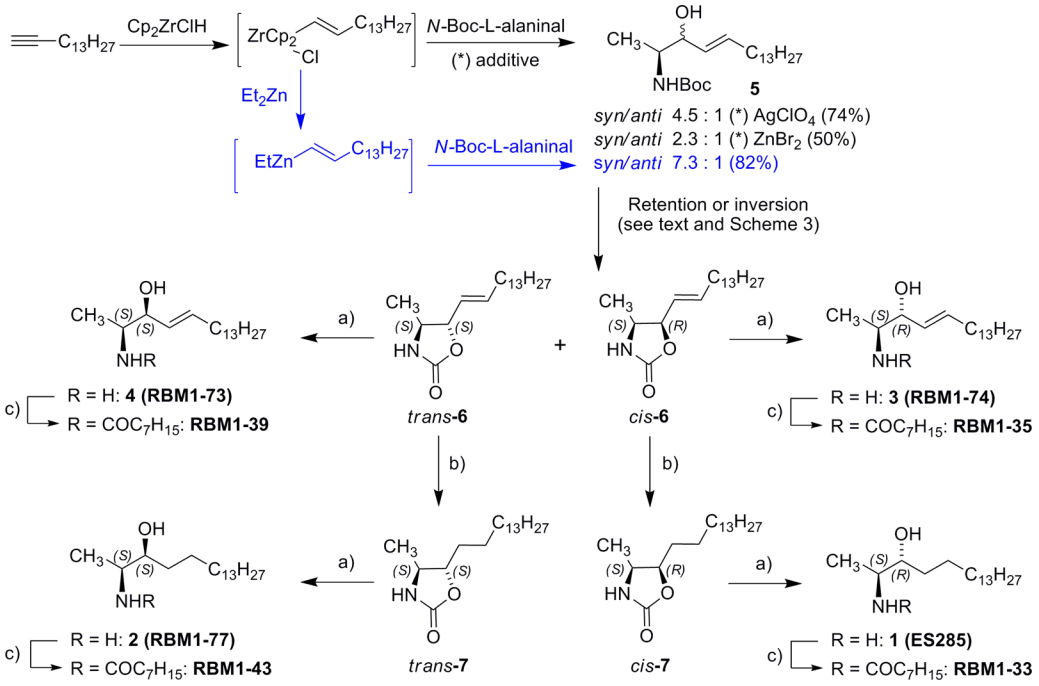
Scheme 2. General Approach to Spisulosine 1 (ES285) and the Analogs Described in This Work from *N*-Boc-L-alaninal[a]



[a]Conditions: (a) EtOH, aqueous 2 M NaOH, rfx (90-95%); (b) H_2, 5% Rh in Al_2O_3 (quant); (c) EDC, HOBt, octanoic acid, CH_2Cl_2 (80−85%).

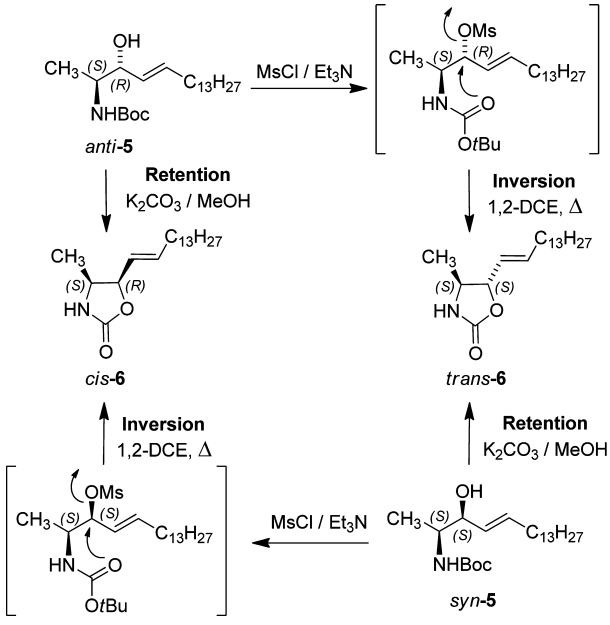
Hydrozirconation of 1-pentadecyne with Schwartz reagent,[28,29] followed by reaction with *N*-Boc-L-alaninal, afforded the required sphingoid base **5** as a mixture of diastereomers, the *syn* adduct being predominant in all cases (see below). The highest, albeit modest (4.5/1), diastereoselection in the alkenylzirconocene addition was achieved in the presence of $AgClO_4$ (10 mol %) in CH_2Cl_2,[30] while a modest 2.3/1 *syn*/*anti* selectivity was observed in the presence of $ZnBr_2$ as additive (50 mol %, THF as solvent).[31,32]

Interestingly, transmetalation with Et_2Zn of the initially formed alkenylzirconocene to the corresponding organozinc reagent,[33,34] followed by addition to *N*-Boc alaninal in CH_2Cl_2, increased the *syn*/*anti* ratio up to 7.3/1, in agreement with reported results from Garner aldehyde.[28,29] The major formation of the *syn* adduct in all the conditions tested can be explained either by operation of an apparent "chelation controlled" transition state, following a classical Cram's model,[35] or by a coordinated delivery from an alternative nonchelated transition state.[36,37]

The resulting mixture of diastereomers *syn*/*anti* **5** was difficult to separate at this stage. For preparative purposes, they were converted into the corresponding oxazolidinones **6** (Scheme 2), which could be cleanly separated by flash chromatography. Formation of **6** was initially carried out by base-promoted (K_2CO_3 in MeOH) intramolecular cyclization of the syn/anti mixture **5** (Scheme 3). Since this reaction implied the formation of a transient alkoxide and the nucleophilic intramolecular attack upon the *N*-Boc group,[38] the ratio *trans*-**6**/*cis*-**6** of the ensuing oxazolidinones matched the *syn*-**5**/*anti*-**5** ratio of the starting mixture, via an overall "retention" process (Scheme 3).

In light of the well established intramolecular reactivity of the *N*-Boc group toward electrophiles,[38,39] we reasoned that conversion of the hydroxyl group of **5** into a suitable leaving group would favor the formation of a mixture of oxazolidinones

Scheme 3. Formation of Oxazolidinones 6 by Retention or Inversion Processes[a]



[a]Yields for *cis*-**6**: 70% (inversion); 10% (retention); *trans*-**6**: 12% (inversion); 70% (retention).

6 through an "inversion" process via *N*-Boc promoted intramolecular S_N2 displacement (Scheme 3). As expected, treatment of the diastereomeric mixture **5** (*syn*/*anti* 7.3/1) with MsCl and Et_3N followed by *in situ* intramolecular cyclization, led to a mixture of oxazolidinones **6** in a roughly 7/1 *cis*/*trans* ratio.[40] Interestingly, this inversion process has been judiciously used in our case to gain access to the corresponding *anti*

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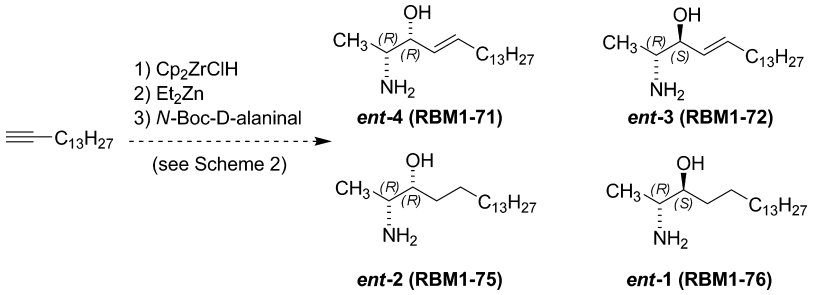
Scheme 4. General Approach to Enantiomeric Spisulosines and Dehydrospisulosines *ent*-1 to *ent*-4



spisulosines after hydrolysis of the oxazolidinone system (see below and Scheme 2).

Double bond reduction of oxazolidinones **6** afforded, uneventfully, the saturated systems **7** present in natural spisulosine. Finally, access to natural spisulosine **1** (**ES285**), the unsaturated analog **3** (**RBM1-74**), and the diastereomeric spisulosine analogs **2** (**RBM1-77**) and **4** (**RBM1-73**) required the alkaline hydrolysis of oxazolidinones **6** or **7**, a process that took place in good overall yields. It is worthy of mention that attempts to obtain spisulosines by direct addition of pentadecylzirconocene to *N*-Boc-L-alaninal were of no synthetic utility, both in terms of diastereoselectivities and overall yields. Using *N*-Boc D-alaninal as starting material, the above process (Scheme 2) allowed the access to the enantiomeric series *ent*-**1** to *ent*-**4**, as summarized in Scheme 4.

Stereochemical Assignments. Initial attempts to assign the stereochemistry of oxazolidinones **6** and **7** by NOE experiments were not conclusive, since only a weak NOE effects (around 2−3%) were observed for C(5)H in *cis*-**6** *cis*-**7** on irradiation at C(4)H (Figure 2).

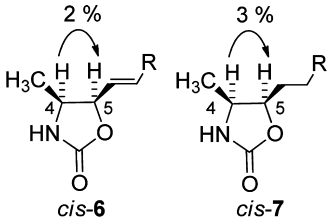
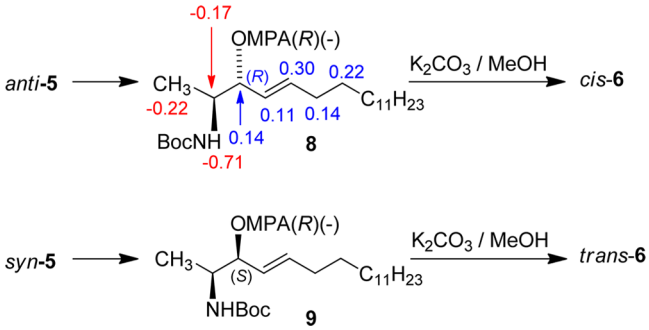


Figure 2. NOE effects from oxazolidionones *cis*-**6** and *cis*-7.

Alternatively, we considered an indirect approach to determine of the absolute configuration of the newly formed stereogenic centers in *syn*/*anti*-**5**. Derivatization by means of the α-methoxy-α-phenylacetic (MPA) esters is one of the most frequently used auxiliary reagents for the assignment of the absolute configuration of secondary alcohols by ^{1}HNMR.[41] In our case, derivatization of the mixture *syn*/*anti*-**5** with (*R*)-(−)-MPA, followed by chromatographic separation of the resulting diastereomeric (*R*)-(−)-OMPA esters **8** and **9**, allowed the configurational assignment of ester **8** as the 3*R* isomer (sphingoid base numbering). Thus, positive $\Delta\delta$ for H4 to H7 and negative $\Delta\delta$ for Me and H2 are indicative of the assigned configuration for ester **8**. This was nicely corroborated by its conversion into oxazolidinone *cis*-**6** by "one-pot" ester hydrolysis with *in situ* cyclization by treatment with methanolic K_2CO_3 under the above "retention conditions". Similar reactivity was observed from the diastereomeric ester **9**, which was cleanly converted into *trans*-**6** under identical conditions (Scheme 5). The unambiguous configurational assignment of **8** was crucial for that of the remaining spisulosine analogs described in this work.

Scheme 5. Derivatization of *syn* and *anti*-5 as (*R*)-(−)-MPA Esters and Assignment of the Absolute Configuration at C3 (Sphingoid Numbering) based on $\Delta\delta^{RS}$ between the C_3(*R*)- and C_3(*S*)-(*R*)-(−)-OMPA Esters



Based on the above results, we can conclude that hydrozirconation of 1-pentadecyne followed by diastereoselective addition of the intermediate zirconocene to D- or L-alaninal represents a straightforward approach to stereochemically defined 1-deoxysphingolipid analogs. Intramolecular, stereocontolled oxazolidinone formation from the mixture of the initially formed *syn*/*anti* adducts **5** is crucial for the overall efficiency of this synthetic approach. Interestingly, this sequence can be easily extended to other related 1-deoxysphingolipid analogs by a judicious choice of the starting alkyne in the hydrozirconation step.

Enzymatic *N*-Acylation of Spisulosines. One of the steps of *de novo* ceramide synthesis is the acylation of dihydrosphingosine to form dihydroceramide. This reaction is catalyzed by ceramide synthases (CerS), genetically encoded by longevity assurance homologue of yeast *lag1* (Lass) genes. Six mammalian ceramide synthases (CerS1-CerS6) have been identified. Each isoform has the ability to produce ceramides with characteristic acyl-chain distributions, which may be, at least in part, associated to specific enzyme compartmentalization in a context-dependent manner. By participating in the control of SL's acyl-chain structure, CerS regulates multiple aspects of sphingolipid-mediated cell biology.[42−44] Recent studies suggest that *de novo*-generated ceramides may have different and opposing activities in tumor promotion/suppression. In these different roles, the subcellular location of the generated amide and its *N*-acyl moiety are probably of importance by activating different downstream targets. For example, CerS6/C16Cer is associated with increased tumor growth in head and neck cancer cells, whereas CerS1/C18Cer

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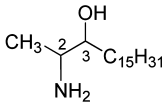
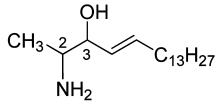
Table 1. Effect of Compounds (CC_{50}, μM) on Viability of MDA MB 468 Cells[a]

									
2S,3R	*2S,3S*	*2R,3S*	*2R,3R*	*2S,3R*	*2S,3R*	*2S,3S*	*2R,3S*	*2R,3R*	*2S,3R*
ES285	**RBM1-77**	**RBM1-76**	**RBM1-75**	**dhSo**	**RBM1-74**	**RBM1-73**	**RBM1-72**	**RBM1-71**	**So**
8.3±1.9	10.6±0.6	13.7±3.4	13.5±1.5	14.2±2.8	12.7±2.2	10.1±2.6	12.1±2.8	9.9±1.3	16.8±3.4

[a]Cell viability was determined by MTT reduction. The CC_{50} values were obtained from dose−response curves. The % of viability numbers were adjusted with the four parameter logistic equation using GraphPad Prism Software with top and bottom constraints at 100 and 0%, respectively. The data were obtained from 2 or 3 experiments with triplicates. dhSo: dihydrosphingosine; So: sphingosine

has been shown to suppress tumor growth in several cancer models.[43] On the other hand, CerS1/C18Cer mediates lethal mitophagy in human cancer cells,[45] while CerS5/C14Cer are required for cardiomyocyte autophagy in relation to lipotoxic cardiomyopathy and hypertrophy.[46]

The increasing interest in CerS activity, compartmentalization and function underscores the need for appropriate CerS probes. Characterization of (dihydro)ceramidomes is not a suitable means to profile CerS activities in intact cells, since (dihydro)ceramide populations are the result of the overall activities of ceramide metabolism enzymes. The compounds described here were envisaged as useful tools in CerS activity profiling, as they are inert toward enzymes of 1-*O*-functionalization.

As a proof of concept, the metabolic *N*-acylation of spisulosine **1** (**ES285**), the stereoisomeric **2** (**RBM1-77**), the dehydroanalogs **3** (**RBM1-74**) and **4** (**RBM1-73**) and the corresponding enantiomeric counterparts (***ent*-1**: **RBM1-76**, ***ent*-2**: **RBM1-75**, ***ent*-3**: **RBM1-72**, and ***ent*-4**: **RBM1-71**, see Schemes 2 and 4) was investigated in the MDA MB 468 breast cancer cell line. The effect of compounds on cell viability was similar, as concluded from the CC_{50} values of the dose response curves, which ranged from 8.3 to 13.2 μM in 24 h treatments (one way ANOVA, $P > 0.05$, see Table 1).

Moreover, the natural bases sphingosine and dihydrosphingosine (Scheme 1) had comparable CC_{50} values in the same experimental conditions. Furthermore, similar viabilities were observed for MCF-7 and MDA MB 231 cell lines in the presence of the compounds (data not shown). The cytotoxicities found here are lower than those reported for **ES285**, **RBM1-76** and **RBM1-77** in other cell lines.[24] Unfortunately, the lack of detailed experimental information, including incubation time, precludes any comparison.

On the other hand, the eight 1-deoxyamines were *N*-acylated in intact MDA MB 468 cells, although the total amount of amides varied according to the probe stereochemistry (Figure 3A). Not surprisingly, the 4 stereoisomers having the natural configuration at the CNH_2 center (**RBM1-73**, **RBM1-74**, **RBM1-77** and **ES285**) gave significantly higher quantities of *N*-acylated derivatives than the corresponding analogs with opposite CNH_2 configuration (Figure 3A). Furthermore, spisulosine (**ES285**) afforded the highest amounts of amide metabolites, which were significantly higher than those produced from the 4,5-dehydro analog (**RBM1-74**). These results indicate that CerS are not only selective for the fatty acyl donor, but also discriminate between acceptor bases, even between the saturated and the unsaturated ones.

Analysis of the different *N*-acylated species showed as the most striking feature that, for compounds with the natural

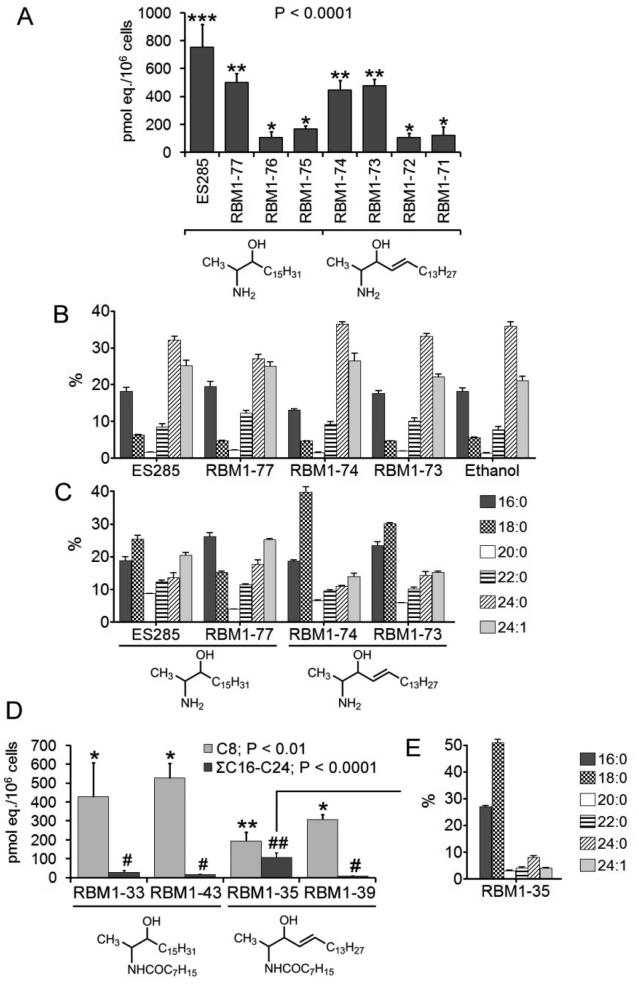


Figure 3. (A) Amounts (pmol) relative to C12Cer of *N*-acylated derivatives of **ES285** and **RBM1-71** to **RBM1-77** as measured by UPLC/TOF analyses of lipid extracts from cells incubated with the compounds (5 μM, 3 h). (B) Percentage of endogenous ceramides and (C) 1-deoxyceramides found in extracts from cells treated with **ES285**, **RBM1-77**, **RBM1-74**, **RBM1-73**, and vehicle. (D) Total amounts of recovered octanamides (C8) and their *N*-transacylated derivatives (ΣC16−C24), as measured by UPLC/TOF analyses of lipid extracts from cells incubated with the compounds (5 μM, 3 h). (E) Percentage of 1-deoxyceramides formed in extracts from cells treated with **RBM1-35**. Data, presented as mean ± SD, were obtained from 2 separate experiments with duplicates. In panels A and D, means are statistically different at the indicated *P* values (one way ANOVA). Symbols on top of the SD bar indicate results from Bonferroni's Multiple Comparison post-test; ($p < 0.05$). See Table 1 and Scheme 2 for compounds stereochemistry.

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stereochemistry at $CHNH_2$, which undergo the highest acylation levels, the percentages of the *N*-C18 1-deoxyamides (Figure 3C) were remarkably higher (30.2%, 39.7%, 15.2% and 25.3% for **RBM1-73**, **RBM1-74**, **RBM1-77**, **ES285**, respectively) than those of the natural *N*-C18 ceramide (4.6%, 4.6%, 4.7% and 6.2% for **RBM1-73**, **RBM1-74**, **RBM1-77** and **ES285**, respectively) (compare Figure 3B and C). In contrast, the percentages of the *N*-C24 species, mainly C24:0 were significantly lower (Figure 3B and C), while other *N*-acyl species remained essentially unchanged. Importantly, the ceramides profile is not affected by the treatments, as similar percentages of the different species are found in vehicle treated controls (ethanol, Figure 3B). These data suggest that the levels of endogenous CerS-generated-C18-ceramide are reduced by metabolic interconversions that result in increased amounts of the C24 species. In the case of the 1-deoxyamides, the amide function is the only possible metabolic site, amenable to transacylation by the sequential action of ceramidases and CerS. To assess whether *N*-transacylation occurs, the formation of different *N*-acyl species from the *N*-octanoyl derivatives of amines **RBM1-73**, **RBM1-74**, **RBM1-77** and **ES285**, namely **RBM1-39**, **RBM1-35**, **RBM1-43** and **RBM1-33**, respectively (Scheme 2), was investigated using non toxic concentrations of amides at 3 h incubation time.

As shown in Figure 3D, the compound undergoing the highest transacylation (55.5%) was **RBM1-35**, (*N*-octanoyl-1-deoxyceramide). The metabolization of **RBM1-35** was paralleled with a decrease in the amounts of the given probe (Figure 3D). In contrast, the saturated counterpart, **RBM1-33**, was only modestly transacylated (6.1%), while, the amounts of the different amides formed from the other octanamides were negligible (**RBM1-39**, 2.2%; **RBM1-43**, 3.0%). Regarding the different *N*-transacylated species formed from **RBM1-35**, the C16 and C18 species were the most abundant ones, with twice as much C18 than C16 (Figure 3E). This profile resembles the obtained from the parent amine, **RBM1-74** (Figure 3C). Likewise, not remarkably different *N*-acyldeoxy(dh)ceramide profiles are produced from the other amine/octanamide pairs (Figure S1, Supporting Information). These results suggest that the different transacylation rates of the octanamides result from the substrate specificity of ceramidase(s), which hydrolyze **RBM1-35** preferentially. Which of the different ceramidases are involved in the hydrolysis will be investigated in the near future. In summary, **ES285**, **RBM1-77** and **RBM1-73** are suitable probes for CerS profiling in intact cells, as they are metabolically stable at both C1 and the amide linkage and thus the resulting amide composition reflects the overall CerS activities in a given experimental condition. Among the three compounds, **ES285** gives the highest acylation rates, thus being the compound of choice. These molecules should prove useful to study the role of the different CerS and *N*-acyl (dihydro)-ceramides in cell fate.

EXPERIMENTAL SECTION

General. Solvents were distilled prior to use and, if required, dried by standard methods. ^{1}H and ^{13}CNMR spectra were obtained in $CDCl_3$ solutions at 400 MHz (for ^{1}H) and 100 MHz (for ^{13}C), respectively, unless otherwise indicated. Chemical shifts (δ) are reported in ppm relative to the solvent ($CDCl_3$) signal. All reactions were monitored by thin layer chromatography (TLC) on aluminum foil precoated silica gel plates. Flash column chromatography was performed with the indicated solvents using silica gel 60 (particle size 0.035−0.070). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Optical rotations were measured at the sodium D line (589 nm) and are reported as $[\alpha]_D$ (c, g/100 mL, solvent). UPLC-TOF analyses were carried out as previously reported.[47] Electrospray ionization was used for HRMS analysis.

(2*S*,3*RS*,*E*)-*tert*-Butyl 3-hydroxyoctadec-4-en-2-yl)carbamate (*syn*- and *anti*-5). *a. Via Schwartz Reagent in the Presence of $ZnBr_2$.* To an ice-cooled stirred suspension of $Cp_2Zr(H)Cl$ (520 mg, 2.0 mmol) in THF (1 mL) under Ar was added neat 1-pentadecyne (530 μL, 2.0 mmol). After stirring at rt for 1h, the reaction mixture was cooled to 0 °C and a solution of *N*-Boc L-alaninal[48] (173 mg, 1.0 mmol) in THF (1.5 mL) was added dropwise, followed by $ZnBr_2$ (112 mg, 0.5 mmol), previously dried by evaporation from an anh THF solution. The resulting mixture was stirred for 24 h at rt, diluted with EtOAc (10 mL) and aq. potassium sodium tartrate (10 mL) and stirred for additional 10 min. The resulting suspension was filtered off and washed with EtOAc (10 mL). The combined filtrate and washings were washed with brine, dried, and evaporated. The residue was flash chromatographed using a stepwise gradient from 0 to 20% hexanes−EtOAc to afford a 2.3:1 mixture of *syn*/*anti*-**5** in 50% yield on elution with a 88:12 hexanes−EtOAc mixture.

b. Via Schwartz Reagent in the Presence of $AgClO_4$. Neat 1-pentadecyne (135 μL, 0.5 mmol) was added dropwise to $Cp_2Zr(H)Cl$ (130 mg, 0.5 mmol) in CH_2Cl_2 (4 mL) at 0 °C under Ar. The reaction mixture was allowed to warm to rt (around 20 min) to give a clear pale yellow solution. A solution of *N*-Boc L-alaninal (87 mg, 0.5 mmol) in CH_2Cl_2 (1 mL) was next added followed by $AgClO_4$ (10 mg, 0.05 mmol). After 30 min, the dark brown reaction mixture was diluted with Et_2O (5 mL) and the organic phase was washed with sat $NaHCO_3$ aqueous solution and then filtered through a pad of Celite. The organic extracts were washed with aqueous sat $NaHCO_3$ and brine. Usual workup afforded a 4.5:1 mixture of *syn*/*anti*-**5** in 74% yield.

c. Via Transmetalation with Et_2Zn.[28] To an ice-cooled stirred suspension of $Cp_2Zr(H)Cl$ (260 mg, 1.0 mmol) in CH_2Cl_2 (4 mL) under Ar was added neat 1-pentadecyne (265 μL, 1 mmol). After stirring at rt for 1h, the reaction mixture was cooled to −40 °C and next treated with a 1.0 M solution of Et_2Zn in hexane (1 mL, 1.0 mmol), followed by *N*-Boc L-alaninal (173 mg, 1.0 mmol) in CH_2Cl_2 (1.5 mL). The resulting mixture was allowed to warm gradually and stirred at rt for 1 h. The mixture was next diluted with EtOAc (10 mL) and aq. potassium sodium tartrate (10 mL). A similar workup as described in a) afforded a 7.3:1 mixture of *syn*/*anti*-**5** in 82% yield.

^{1}HNMR ($CDCl_3$, 400 MHz); data for the major syn isomer: 5.71−5.59 (dt, J = 13.4, 6.7, C(5)H), 5.46−5.35 (m, 1H, C(4)H), 3.93 (dd, J = J' = 6.2, 1H, C3H), 3.63 (m, 1H, C(2)H), 2.03 (m, 2H), 1.43 (s, 9H), 1.35 (broad, 2H), 1.25 (broad, 20H), 1.13 (d, J = 6.8, 3H, C(1)H3), 0.87 (t, J = 6.9, 3H, C(18)H3). ^{13}CNMR ($CDCl_3$, 101 MHz): 134.2 (C5), 129.4 (C4), 79.6 ($C(CH_3)_3$), 76.1 (C3), 51.2 (C2), 32.5 (C6), 32.1 (C16), 29.8−29.2, 28.3 ($C(CH_3)_3$), 17.8 (C1), 14.3 (C18). HRMS (mixture of *syn*/*anti*-**5**): Calculated for $C_{23}H_{46}NO_3$ $(M + 1)^+$: 384.3472; Found 348.3478. Application of this protocol using *N*-Boc D-alaninal afforded *ent*-*syn*/*anti*-**5** in comparable yields.

(4*S*,5*RS*)-4-Methyl-5-[(*E*)-pentadec-1-en-1-yl]oxazolidin-2-one (*cis*-6 and *trans*-6). *a. Retention Conditions.* A solution of **5** (*syn*/*anti* 7.3/1 mixture, 380 mg, 1 mmol) in MeOH (10 mL) is treated with K_2CO_3 (600 mg, 4.3 mmol) and warmed gently in a water bath at 50 °C until consumption of the starting material (TLC). The reaction mixture is next evaporated to dryness, taken up in H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). Usual workup afforded a crude that was flash chromatographed on a stepwise gradient of hexanes/EtOAc from 1 to 40%. Carbamate *trans*-**6** was isolated on elution with a 72:28 gradient mixture in 70% yield and *cis*-**6** was obtained in 10% yield on elution with a 66:34 gradient mixture.

b. Inversion Conditions. A solution of mesyl chloride (170 mg, 1.5 mmol) in CH_2Cl_2 (4 mL) was added dropwise to a solution of aminoalcohols **5** or *ent*-**5** (syn/anti 7.3/1 mixture, 380 mg, 1 mmol) in CH_2Cl_2 (6 mL) containing Et_3N (415 μL,303 mg, 3.0 mmol). After stirring for 1h at rt, the reaction mixture was quenched with H_2O (5 mL) and the organic phase was separated, dried over Na_2SO_4 and

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evaporated to dryness. The resulting crude was taken up in 1,2-DCE (10 mL) and Et_3N (690 μL, 505 mg, 5.0 mmol) was next added dropwise. The resulting solution was refluxed until TLC indicated the total formation carbamates **6** (typically around 4h). Evaporation to dryness afforded a crude mixture that was flash chromatographed under the above conditions to give *trans*-**6** in 12% yield and *cis*-**6** in 70% yield.

trans-**6**: ^{1}HNMR ($CDCl_3$, 400 MHz, sphingoid base numbering): 5.84 (dt, J = 15.3, 6.8, 6.8, 1H, C(5)H), 5.49 (dd, J = 15.3, 7.9, 1H, C(4)H), 4.42 (dd, J = J' = 7.9, 1H, C(3)H), 3.62 (m, 1H, C(2)H), 2.08 (m, 2H, C(6)H_2), 1.38 (m, 2H), 1.26 (m, broad, 23H), 0.87 (t, 3H). ^{13}CNMR ($CDCl_3$, 101 MHz, sphingoid base numbering): 159.3, 137.9, 125.5, 85.3, 54.5, 32.3, 32.1, 29.9−29.8 (3C), 29.7, 29.6, 29.5, 29.2, 28.8, 22.8, 19.4, 14.2. $[\alpha]_D$ −33.0 (c 1, $CHCl_3$). HRMS: Calculated for $C_{19}H_{36}NO_2$ $(M + 1)^+$: 310.2741; Found 310.2747.

cis-**6**:[24] ^{1}HNMR ($CDCl_3$, 400 MHz, sphingoid base numbering): 5.86 (dd, J = 14.6, 7.6, 1H, C(5)H), 5.51 (dd, J = 14.6, 7.9, 1H, C(4)H), 4.99 (dd, J = J' = 7.9, 1H, C(3)H), 3.95 (m, 1H, C(2)H), 2.12 (m, 2H), 1.38 (m, 2H), 1.25 (m, broad, 20H), 1.14 (d, J = 6.5, 3H, C(1)H3), 0.88 (t, 3H). ^{13}CNMR ($CDCl_3$, 101 MHz, sphingoid base numbering): 138.4, 122.6, 81.7, 51.8, 32.4, 32.0, 29.8−28.9, 22.8, 17.2, 14.3. $[\alpha]_D$ +1.0 (c 1, $CHCl_3$). HRMS: Calculated for $C_{19}H_{36}NO_2$ $(M + 1)^+$: 310.2741; Found 310.2736.

Starting from *ent*-**5** (syn/anti mixture) carbamates *ent-cis*-**6** and *ent-trans*-**6** were obtained in comparable yields; *ent-trans*-**6**: $[\alpha]_D$ +29.6 (c 1, $CHCl_3$). HRMS: Calculated for $C_{19}H_{36}NO_2$ $(M + 1)^+$: 310.2741; Found 310.2750. *ent-cis*-**6**: $[\alpha]_D$ −1.0 (c 1, $CHCl_3$). HRMS: Calculated for $C_{19}H_{36}NO_2$ $(M + 1)^+$: 310.2741; Found 310.2752. Both compounds showed ^{1}H and ^{13}C spectra identical to those of *trans*-**6** and *cis*-**6**, respectively.

(4*S*,5*R*)-4-Methyl-5-pentadecyloxazolidin-2-one (*cis*-7) and (4*S*,5*S*)-4-Methyl-5-pentadecyloxazolidin-2-one (*trans*-7). A solution of the starting carbamate *cis*-**6** or *trans*-**6** (309 mg, 1.0 mmol) in EtOAc (20 mL) was hydrogenated at 1 atm and rt in the presence of 5% (w/w) Rh on alumina. After stirring for 12 h, the catalyst was removed by filtration over Celite and the resulting clear solution was evaporated to dryness to afford the required carbamates **7** in quantitative yield.

trans-**7**: ^{1}HNMR ($CDCl_3$, 400 MHz, sphingoid base numbering): 4.07 (m, 1H, C(3)H), 3.56 (m, 1H, C(2)H), 1.65 (m, 2H,C(4)H_2), 1.25 (broad, 29H), 0.87 (t, 3H). ^{13}CNMR ($CDCl_3$, 101 MHz): 159.6, 84.4, 53.8, 34.3, 32.1, 29.8−29.4, 25.0, 22.8, 20.8, 14.2. $[\alpha]_D$ −34.6 (c, 1.0 $CHCl_3$). HRMS: Calculated for $C_{19}H_{38}NO_2$ $(M + 1)^+$: 312.2897; Found 312.2892.

cis-**7**[24]: ^{1}HNMR ($CDCl_3$, 400 MHz, sphingoid base numbering): 4.55 (m, 1H, C(3)H), 3.89 (m, 1H, C(2)H), 1.72 (m, 2H), 1.52 (m, 2H), 1.35−1.20 (broad, 24H), 1.15 (d, J = 6.4, 3H, C(1)H_3), 0.87 (t, 3H, C(18)H_3). ^{13}CNMR ($CDCl_3$, 101 MHz, sphingoid base numbering): 159.7, 80.4 (C3), 51.3 (C2), 32.1, 29.8−29.3, 26.0, 22.83, 16.1 (C1), 14.3 (C18). $[\alpha]_D$ +11.5 (c, 1.0 $CHCl_3$). HRMS: Calculated for $C_{19}H_{38}NO_2$ $(M + 1)^+$: 312.2897; Found 312.2903.

The enantiomeric series was obtained similarly from carbamates *ent*-**6**: *ent-cis*-**7**: $[\alpha]_D$ −15.2 (c, 1.0 $CHCl_3$). HRMS: Calculated for $C_{19}H_{38}NO_2$ $(M + 1)^+$: 312.2897; Found 312.2906. *ent-trans*-**7**: $[\alpha]_D$ +33.6 (c, 1.0 $CHCl_3$). HRMS: Calculated for $C_{19}H_{38}NO_2$ $(M + 1)^+$: 312.2897; Found 312.2901. Both compounds showed ^{1}H and ^{13}C spectra identical to those of *trans*-**7** and *cis*-**7**, respectively.

Spisulosines and Dehydrospisulosines by Hydrolysis of Carbamates 6 and 7. A solution of the starting carbamate **6**, *ent*-**6**, **7** or *ent*-**7** (0.5 mmol) in a 1:1 EtOH−aqueous 2*N* NaOH (20 mL) was refluxed for 2 h or until disappearance of the starting carbamate. The reaction mixture was next concentrated, diluted with H_2O (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). Usual workup afforded the required spisulosines and dehydrospisulosines in optimal purities and yields.

***1* (2*S*,3*R*, Spisulosine, ES285).**[24] Yield: 90% from carbamate *cis*-**7**. ^{1}HNMR (CD_3OD, 400 MHz, sphingoid base numbering): 3.40 (m, 1H), 2.78 (m, 1H), 1.51 (m, 2H), 1.39−1.23 (m, 26H), 1.04 (d, J = 6.4, 3H), 0.90 (t, 3H). ^{13}CNMR (CD_3OD, 101 MHz): 76.5, 52.1, 33.4, 33.1, 30.8−30.7, 30.5, 27.3, 23.7, 17.2, 14.5. HRMS: Calculated for $C_{18}H_{40}NO$ $(M + 1)^+$: 286.3104; Found 286.3107. Mp: 76−77 °C; lit[23] 65−67 °C; lit[17] 64.5−66 °C. Base: $[\alpha]_D$ +8.2 (*c* 1, $CHCl_3$); lit[3] +24.9 (c 1, $CHCl_3$); lit[18] +5.32 (c 0.36, MeOH); lit[20] +21.4 (c 0.5 $CHCl_3$); lit[17] +24.0 (*c* 1, $CHCl_3$); lit[23] +25.3 (*c* 0.95, $CHCl_3$); lit[25] +24.2 (*c* 1, $CHCl_3$). Hydrochloride: lit[17] +3.2 (*c* 1, MeOH, HCl); lit[21] +7.15 (*c* 0.42, MeOH).

***2* (2*S*,3*S*, RBM1-77).**[24] Yield: 92% from carbamate *trans*-**7**. ^{1}HNMR (CD_3OD, 400 MHz, sphingoid base numbering): 3.21 (m, 1H, C(3)H), 2.69 (m, 1H, C(2)H), 1.50 (m, 2H), 1.29 (m, 26H), 1.04 (d, J = 9.2, 3H, C(1)H_3), 0.90 (t, 3H, C(18)H_3). ^{13}C NMR (CD_3OD, 101 MHz): 77.2, 52.4, 34.6, 33.1, 30.9−30.8, 30.5, 26.9, 23.7, 19.4, 14.5. HRMS: Calculated for $C_{18}H_{40}NO$ $(M + 1)^+$: 286.3104; Found 286.3100. Mp: 87−88 °C. Base: $[\alpha]_D$ −7.6 (c 1, $CHCl_3$); lit[20] +3.4 (c 1.8, MeOH); no alfaD.[24]

***3* (2*S*,3*R*, RBM1-74).**[18,26] Yield: 91% from carbamate *cis*-**6**. ^{1}HNMR (CD_3OD, 400 MHz, sphingoid base numbering): 5.71 (dt, 1H, J = 15.6, J' = J'' = 6.4, C(5)H), 3.82 (m, 1H, C(3)H), 2.80 (m, 1H, C(2)H), 2.07 (m, 2H, C(6)H_2), 1.40 (m, 2H), 1.30 (m, 22H), 1.04 (d, J = 6.6, 3H, C(1)H_3),), 0.90 (t, 3H, C(18)H_3). ^{13}CNMR (CD_3OD, 101 MHz, sphingoid base numbering): 135.1, 130.5, 78.1, 52.2, 33.5, 33.1, 30.8−30.7, 30.6, 30.5, 30.4, 30.3, 23.7, 18.2, 14.5. HRMS: Calculated for $C_{18}H_{38}NO$ $(M + 1)^+$: 284.2948; Found 284.2949. Mp: 70−71 °C; Base: $[\alpha]_D$ +7.5 (*c* 1, $CHCl_3$).

***4* (2*S*,3*S*, RBM1-73).**[49] Yield: 94% from carbamate *trans*-**6**. ^{1}HNMR (CD_3OD, 400 MHz, sphingoid base numbering): 5.69 (dt, 1H, J = 15.4, J' = 6.6, C(5)H), 5.43 (dd, 1H, J = 15.4, J' = 7.44, C(4)H), 3.65 (t, 1H, C(3)H), 2.71 (m, 1H, C(2)H), 2.07 (m, 2H, C6(H)$_2$), 1.39 (m, 2H), 1.30 (broad, 20H), 1.01 (d, J = 6.8, 3H, C(1)H_3), 0.91 (t, 3H, C(18)H_3). ^{13}CNMR (CD_3OD, 101 MHz): 134.7 (C5), 131.6 (C4), 79.0 (C3), 52.4 (C2), 33.4 (C6), 33.1, 30.8−30.7, 30.6, 30.5, 30.4, 30.3, 23.8, 18.9 (C1), 14.5 (C18). HRMS: Calculated for $C_{18}H_{38}NO$ $(M + 1)^+$: 284.2948; Found 284.2946. Mp: 71−72 °C; Base: $[\alpha]_D$ +6.5 (*c* 1, $CHCl_3$).

The enantiomeric spisulosines (*ent*-**1** and *ent*-**2**) and dehydrospisulosines (*ent*-**3** and *ent*-**4**) were obtained similarly from the corresponding carbamates *ent*-**6** or *ent*-**7**

ent-**1** (2*R*,3*S*, **RBM1-76**):[24] Yield: 93% from carbamate *ent-cis*-**7**. ^{1}H and ^{13}C spectra identical to those of **1** (**ES285**), see above. Mp: 74−75 °C; Base: $[\alpha]_D$ −8.5 (*c* 1, $CHCl_3$). HRMS: Calculated for $C_{18}H_{40}NO$ $(M + 1)^+$: 286.3104; Found 286.3103.

ent-**2** (2*R*,3*R*, **RBM1-75**): Yield: 95% from carbamate *ent-trans*-**7**. ^{1}H and ^{13}C spectra identical to those of **2** (**RBM1-77**), see above. Mp: 84−85 °C; Base: $[\alpha]_D$ +6.5 (*c* 1, $CHCl_3$). HRMS: Calculated for $C_{18}H_{40}NO$ $(M + 1)^+$: 286.3104; Found 286.3115.

ent-**3** (2*R*,3*S*, **RBM1-72**): Yield: 94% from carbamate *ent-cis*-**6**. ^{1}H and ^{13}C spectra identical to those of **3** (**RBM1-74**), see above. Mp: 67−68 °C; Base: $[\alpha]_D$ −6.8 (*c* 1, $CHCl_3$). HRMS: Calculated for $C_{18}H_{38}NO$ $(M + 1)^+$: 284.2948; Found 284.2963.

ent-**4** (2*R*,3*R*, **RBM1-71**): Yield: 92% from carbamate *ent-trans*-**6**. ^{1}H and ^{13}C spectra identical to those of **4** (**RBM1-73**), see above. Mp: 69−70 °C; Base: $[\alpha]_D$ −7.0 (*c* 1, $CHCl_3$). HRMS: Calculated for $C_{18}H_{38}NO$ $(M + 1)^+$: 284.2948; Found 284.2945.

Synthesis of MPA Esters 8 and 9. EDC (120 mg, 0.65 mmol) was added portionwise to a solution of 155 mg (0.4 mmol) of a mixture *syn*/*anti*-**5** in CH_2Cl_2 (10 mL) containing (*R*)(−)MPA (100 mg, 0.6 mmol), DMAP (73 mg, 0.6 mmol) and Et_3N (111 μL, 0.8 mmol). After stirring for 2h at rt, the reaction mixture was washed with aq. sat $NaHCO_3$, the organic phase was separated, dried, evaporated to dryness and the resulting residue was flash chromatographed (hexanes/EtOAc from 0 to 10% gradient).

Compound **8** was obtained on elution with hexanes/EtOAc 8%, (R_f 0.66, hexanes/EtOAc 7/3) in 82% isolated yield. ^{1}HNMR ($CDCl_3$, 400 MHz, sphingoid base numbering): 7.45 (m, 2H), 7.38 (m, 3H), 5.72 (m, 1H, C(5)H), 5.34 (m, 1H, C(3)H), 5.32 (m, 1H, C(4)H), 4.77 (s, 1H), 3.78 (broad, 1H, NHBoc), 3.66 (m, 1H, C(2)H), 3.42 (s, 3H), 2.00 (m, 2H, C(6)H_2), 1.62 (broad, 2H), 1.39 (s, 9H), 1.25 (broad, 20H), 0.88 (t, 3H, C(18)H_3), 0.81 (d, J = 6.8, 1H, C(1)H_3). ^{13}CNMR ($CDCl_3$, 101 MHz, sphingoid base numbering): 169.9 (CO), 154.9 (CO), 136.9, 136.3 (C5), 129.0, 128.9, 127.4, 124.5 (C4), 82.6,

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79.4, 77.2 (C3), 57.4, 49.3 (C2), 32.4 (C6), 32.1, 29.8–29.5, 29.2, 28.9, 28.5, 22.8, 14.7 (C1), 14.2 (C18).

Compound **9** was obtained on elution with hexanes/EtOAc 10%, (R_f 0.60, hexanes/EtOAc 7/3) in 86% isolated yield. ^{1}HNMR ($CDCl_3$, 400 MHz, sphingoid base numbering):): 7.43 (m, 2H), 7.33 (m, 3H), 5.42 (m, 1H, C(5)H), 5.21 (m, 1H, C(3)H), 5.20 (m, 1H, C(4)H), 4.76 (s, 1H), 4.48 (broad, 1H, NHBoc), 3.83 (m, 1H, C(2)H), 3.40 (s, 3H), 1.86 (m, 2H, C(6)H_2), 1.42 (s, 9H), 1.29–1.15 (broad, 22H), 1.03 (d, J = 6.8, 1H, C(1)H_3), 0.87 (t, 3H, C(18)H_3). ^{13}CNMR ($CDCl_3$, 101 MHz, sphingoid base numbering): 169.9 (CO), 155.3 (CO), 136.7 (C5), 136.2, 128.8, 128.6, 127.3, 124.1 (C4), 82.7, 79.4, 77.4 (C3), 57.4, 49.1 (C2), 32.2 (C6), 32.1, 29.8–29.7, 29.6, 29.5, 29.4, 29.1, 28.8, 28.5, 22.8, 17.8 (C1), 14.2 (C18).

One-pot Hydrolysis and Cyclization of MPA Esters 8 and 9. A solution of the MPA ester **8** (50 mg, 94 μmol) in MeOH (1 mL) is treated with K_2CO_3 (60 mg, 0.43 mmol) at rt until consumption of the starting material (TLC). The reaction mixture is next evaporated to dryness, taken up in H_2O (1 mL) and extracted with CH_2Cl_2 (3 × 1.5 mL). Usual workup afforded 26 mg (89% yield) of oxazolidinone *cis*-**6** (TLC, hexanes/EtOAc 70:30), whose spectroscopical data were identical to those reported above. Following the same procedure, MPA ester **9** afforded 24 mg (83% yield) of oxazolidinone *trans*-**6**.

General Method for the Synthesis of *N*-octanoyl Amines. To a solution of EDC (1.62 mmol) and HOBt (1.19 mmol) in anhydrous CH_2Cl_2 (5 mL) was added the corresponding carboxylic acid (1.08 mmol) under argon atmosphere. The resulting mixture was vigorously stirred at rt for 10 min, and next added dropwise to a solution of the corresponding amine (1.00 mmol) and Et_3N (3.90 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 1 h under argon atmosphere. The mixture was next diluted by addition of CH_2Cl_2 (10 mL), and washed successively with 1 M $NaHCO_3$ solution and brine (5 mL each). The organic layer was dried over $MgSO_4$, and filtered. Concentration under reduced pressure afforded crude compounds, which were purified by flash chromatography (hexanes/EtOAc, 7:3) to give the corresponding amides in 80–85% yield.

(2*S*,3*R*, **RBM1-33**): ^{1}HNMR ($CDCl_3$, 400 MHz): 5.81 (broad, NH), 4.00 (m, 1H), 3.62 (m, 1H), 2.16 (t, 2H), 1.62 (m, 2H), 1.25 (broad, 36H), 1.09 (d, J = 8 Hz, 3H), 0.87 (2*t*, 6H). ^{13}C NMR ($CDCl_3$, 101 MHz): 173.3, 74.5, 49.6, 37.0, 33.7, 32.0, 31.8, 29.8–29.7, 29.5, 29.4, 29.1, 26.6, 25.9, 22.8, 22.7, 14.3, 14.2. HRMS: Calculated for $C_{26}H_{54}NO_2$ $(M + 1)^+$: 412.4149; Found 412.4153.

(2*S*,3*R*, **RBM1-35**): ^{1}H NMR ($CDCl_3$, 400 MHz): 5.69 (m, 2H, C(5)H + NH), 5.41 (dd, 1H, J = 16 Hz, J'= 8 Hz, C(4)H), 4.10 (m, 2H, C(2)H, C(3)H), 2.17 (t, 2H, C(2′)H_2), 2.04 (m, 2H, C(6)H_2), 1.62 (m, 2H), 1.28 (m, 30H), 1.09 (d, J = 8 Hz, 3H, C1(H)$_3$), 0.87 (2*t*, 6H, C8′(H)$_3$ + C18(H)$_3$). ^{13}C NMR ($CDCl_3$, 101 MHz): 173.9, 134.2 (C5), 128.3 (C4), 75.8 (C3), 50.2 (C2), 37.0 (C2′), 32.5 (C6), 32.1, 32.8, 29.8–29.7, 29.6, 29.5, 29.4, 29.2, 26.0, 22.8, 22.7, 15.4 (C1), 14.3, 14.2. HRMS: Calculated for $C_{26}H_{52}NO_2$ $(M + 1)^+$: 410.3993; Found 410.4011.

(2*S*,3*S*, **RBM1-39**): ^{1}H NMR ($CDCl_3$, 400 MHz): 5.67 (m, 2H, C(5)H + NH), 5.44 (dd, 1H, J = 16 Hz, J'= 8 Hz, C(4)H), 3.97 (m, 2H, C(2)H, C(3)H), 2.16 (t, 2H, C(2′)H_2), 2.02 (m, 2H, C(6)H_2), 1.61 (m, 2H), 1.28 (m, 30H), 1.16 (d, J = 8 Hz, 3H, C1(H)$_3$), 0.88 (2*t*, 6H, C8′(H)$_3$ + C18(H)$_3$). ^{13}C NMR ($CDCl_3$, 101 MHz): 173.7, 134.2 (C5), 129.7 (C4), 76.2 (C3), 49.5 (C2), 37.1 (C2′), 32.4 (C6), 32.1, 31.8, 29.8–29.2, 26.0, 22.8, 17.7 (C1), 14.3, 14.2. HRMS: Calculated for $C_{26}H_{52}NO_2$ $(M + 1)^+$: 410.3993; Found 410.3991.

(2*S*,3*S*, **RBM1-43**): ^{1}H NMR ($CDCl_3$, 400 MHz): 5.78 (broad, NH), 3.96 (m, 1H, C(2)H), 3.52 (m, 1H, C(3)H), 2.17 (t, 2H), 1.60 (t, 2H), 1.40 (m, 2H), 1.28 (broad, 36H), 1.18 (d, J = 8 Hz, 3H), 0.88 (2*t*, 6H). ^{13}C NMR ($CDCl_3$, 101 MHz): 173.5, 74.9 (C3), 48.9 (C2), 37.2 (C2′), 34.6, 32.1, 31.9, 29.8–29.7, 29.5, 29.4, 29.2, 26.0, 25.8, 22.8, 22.7, 18.5 (C1), 14.3, 14.2. HRMS: Calculated for $C_{26}H_{54}NO_2$ $(M + 1)^+$: 412.4149; Found 412.4147

Cell Culture. MDA MB 468 cells were cultured at 37 °C 5% CO_2 in DMEM medium with high glucose supplemented with 10% FBS and 1% penicillin-streptomycin.

Cell Viability. Cells were seeded in 96-well plates at a density of 50,000 cells/mL (0.1 mL/well). Twenty four hours after seeding, the medium was replaced with fresh complete medium containing vehicle (0.3% ethanol, 100% viability) or the test compounds at concentrations of 30, 20, 13.3, 8.9, 5.9, 4.0, 2.6, and 1.8 μM (2/3 serial dilution). The 30 μM solution was made by dilution of 0.9 μL of a 10 mM solution in ethanol in 300 μL of complete medium. After 24 h of treatment, the medium was removed and cell viability was determined by the MTT test.

Metabolization of Compounds in Intact Cells. Cells were seeded in 6-well plates at a density of 500 000 cells/mL (2 mL/well). Twenty four hours after seeding, the medium was removed and 1 mL of complete fresh medium containing the test compounds at 5 μM (0.5 μL of a 10 mM solution in ethanol) was added. After 3 h of incubation at 37 °C, 5% CO_2 cells were collected by trypsinization, pelleted by centrifugation and lipids were extracted and analyzed as reported.[47]

■ ASSOCIATED CONTENT

Supporting Information

Copies of NMR spectra for the compounds described in this work, UPLC-TOF analyses of the percentage of 1-deoxyceramides formed in extracts from cells treated with **RBM1-33**, **RBM1-43**, and **RBM1-39**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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