Stereoselective preparation of quaternary 2-vinyl sphingosines and ceramides and their effect on basal sphingolipid metabolism

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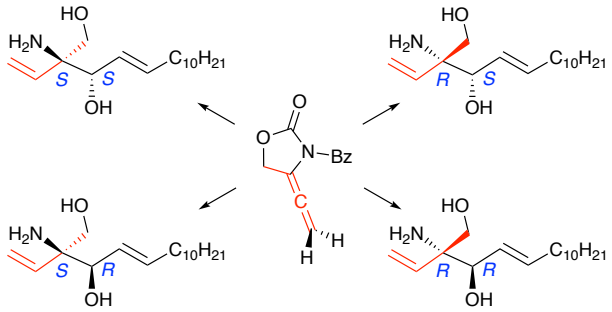
GRAPHICAL ABSTRACT:



ABSTRACT: The dicyclohexylborane–mediated addition of allene **1** to (E)-2-tridecenal affords a quaternary protected 2-amino-2-vinyl-1,3-diol in good yield as a single diastereomer. This compound is readily transformed into the four stereoisomers of the quaternary (*E*)-2-vinyl analogs of sphingosine. The metabolic fate and the effect of these compounds on the basal sphingolipid metabolism in human A549 lung adenocarcinoma cells has been studied, together with the ceramide analog of the most relevant vinylsphingosine derivative.

Introduction

Sphingolipids are a family of natural products that play essential roles as structural cell membrane components and also in cell signaling through a complex metabolic network involving specific enzymes. From a structural point of view, most mammalian sphingolipids share a common 2-amine-1,3-diol moiety arising from (*E*)-2-amino-4-octadecen-1,3-diol (sphingosine). Interestingly, some structural analogs of sphingosine and/or ceramides (*N*-acyl sphingosines) can act as selective inhibitors of sphingolipid metabolism enzymes and they exhibit interesting pharmacological properties.[1–3] In particular, Boumendjel and Miller reported that compound **2** (Figure 1, mixture of isomers), a quaternary vinyl analog of dihydrosphingosine 1-phosphate, exhibits potent inhibition of sphingosine 1-phosphate lyase.[4]

Based on the above precedents, it is conceivable that the introduction of the 2-amino-2-vinyl-1,3-diol core as part of the sphingolipid framework represents an attractive modification for the design of new modulators of sphingolipid metabolism. In this work, we present an adaptation of our previously reported protocol[5] to the enantioselective synthesis of quaternary 2-vinyl sphingosines **9** (Figure 1). In addition, studies on their cellular metabolism, cellular toxicity and effects on basal sphingolipid metabolism in human A549 lung adenocarcinoma cells are discussed.

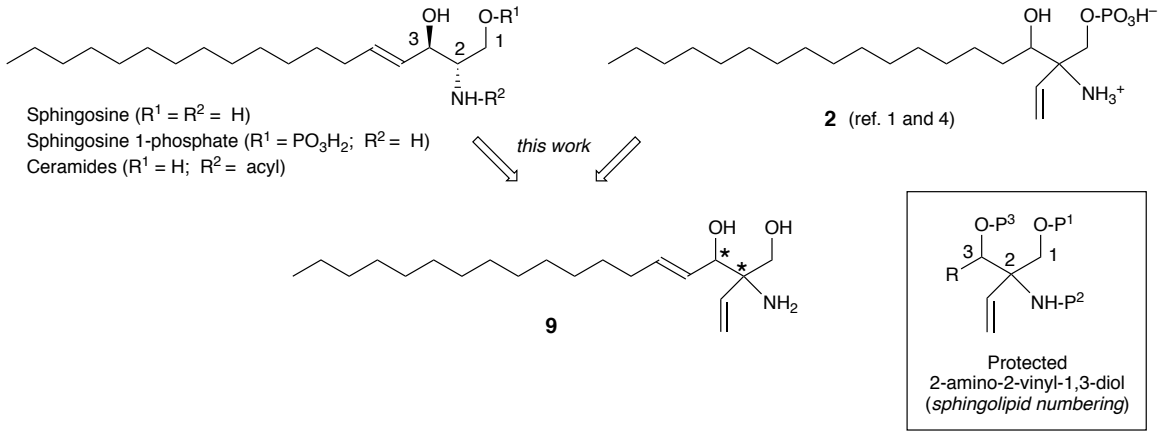
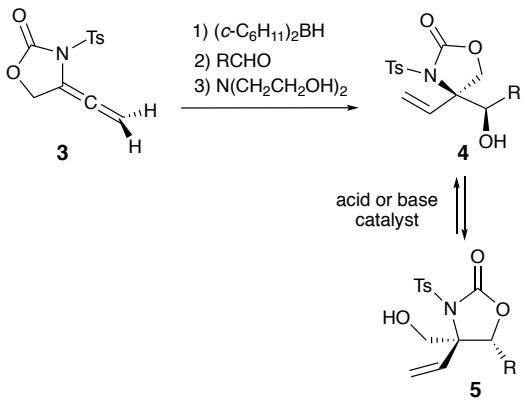


Fig. 1 Sphingolipids, 2-vinyl dihydrosphingosine 1-phosphate (**2**), and 2-vinylsphingosines (**9)**

Results and discussion

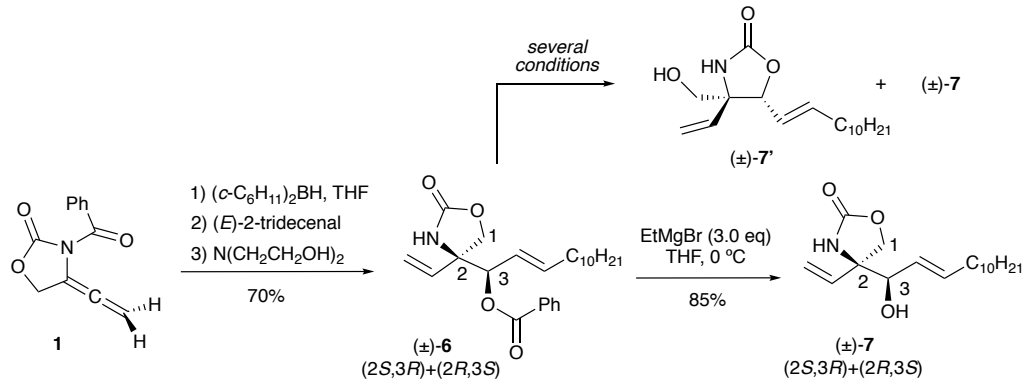
Chemistry

We recently reported a highly stereoselective addition of *N*-tosyl allene **3** to aldehydes leading to protected tosylcarbamates **(±)-4**.[5] This one-pot process is based on the hydroboration of the allene with $(c\text{-}C_6H_{11})_2BH$ at the less hindered face of the terminal double bond to generate an allylborane which could be added *in situ* to an aldehyde (Scheme 1). However, in practice, the use of tosyl as *N*-protecting group suffers from some drawbacks. First, the robust tosyl group may be difficult to remove in the final steps of a multi-step synthetic sequence. Secondly, the *N*-tosyl group favors an facile acid or base-catalyzed partial isomerization to the corresponding inner *N*-tosylcarbamates **5** during work-up and/or chromatographic purification of compounds **4**, leading to mixtures that are difficult to separate.



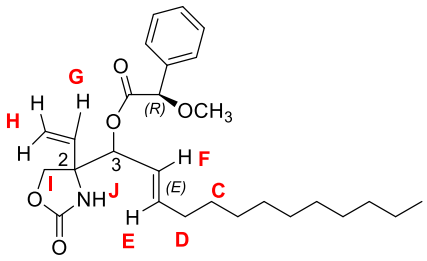
Scheme 1 Preparation of *N*-tosylcarbamates (±)-**4** and their isomerization to (±)-**5**

These disadvantages can be avoided almost completely by using allene **1** (Scheme 2), obtained from but-2-yn-1,4-diol in 61% yield,[6] in which the more easily removable *N*-benzoyl group is used as protecting group.[7] We envisaged that the addition of allene **1** to (*E*)-2-tridecenal would provide access to 2-vinyl sphingoid analogs **9** (Figure 1), structurally related to sphingosine. Considering the incorporation of the vinyl unit in **9**, the use of (*E*)-2-tridecenal would afford a C17 long chain base, which is expected to be endowed with appropriate properties for cell permeabilization. As expected, hydroboration of **1** with (*c*-$C_6H_{11})_2BH$, in CH_2Cl_2 at 0 ºC followed by addition of (*E*)-2-tridecenal, afforded carbamate (±)-**6** in very high diastereochemical purity (>95:5 by ^1H NMR) after a hydrolytic work-up with triethanolamine and column chromatography. It should be remarked that complete migration of the benzoyl protecting group from the nitrogen atom to the newly formed secondary alcohol was observed, in this way avoiding the isomerization of **6** to the inner carbamate (±)-**7'** (Scheme 2), as was observed in the isomerization of **4** into **5** (Scheme 1). Thus, compound (±)-**6** was obtained in an acceptable 75% yield, after chromatographic purification, as a single diastereomer (as racemic mixture of both enantiomers).



Scheme 2 Synthesis of sphingoid precursor (±)-**7** from allene **1** and attempts of selective hydrolysis of benzoate (±)-**6**

Hydrolysis of the benzoate group in (±)-**6** to the desired alcohol (±)-**7** avoiding isomerization to the inner carbamate was not a trivial task. A number of hydrolytic treatments with different basic (K_2CO_3, LiOH) or acidic (H_2SO_4, HCl) aqueous or methanolic media, as well as reductive treatment of (±)-**6** with $LiBH_4$ in THF, were performed leading to mixtures of both carbamates arising from the primary or the secondary alcohol ((±)-**7** and (±)-**7'**). Gratifyingly, the use of EtMgBr in THF at 0 ºC afforded the required alcohol (±)-**7** in good yield with negligible isomerization. Since we were interested in the influence of the different stereoisomers of 2-vinyl sphingosines in sphingolipid metabolism, we undertook the resolution of (±)-**7**. This was accomplished by transformation of (±)-**7** into a mixture of the corresponding diastereomeric esters **8** derived from (*R*)-methoxyphenylacetic acid [(*R*)-MPA][8] using EDC as coupling reagent in the presence of a catalytic amount of DMAP. Esters **8** were then easily isolated by column chromatography and their configuration at C3 was inferred from Δδ between selected pair of protons, following the empirical method of Riguera *et al*,[9] as indicated in Table 1. Since the relative configuration between C2 and C3 had already been established based on mechanistic grounds,[5] the absolute configuration at both stereogenic centers in esters **8** was thus assigned.

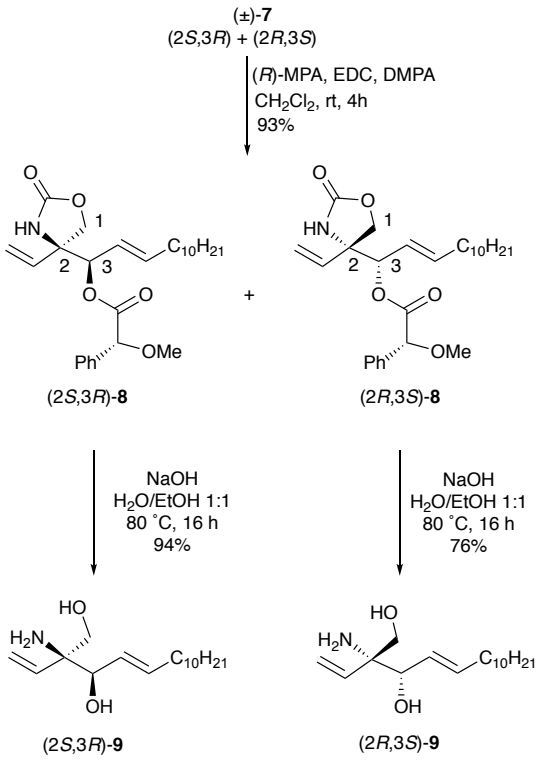


3*S* configuration assigned from Δδ [(2*R*,3*S*) or (2*S*,3*R*)] (*R*)-MPA esters								
	δH_C(*)	δH_D(*)	δH_E	δH_F	δH_G(*)	δH_H	δH_I(*)	δH_J
2*R*3*S*	1.16	1.88	5.59	5.18	5.83	5.28	4.11	5.90
2*S*3*R*	1.29	2.02	5.91	5.30	5.62	5.06	3.85	5.72
$\Delta\delta^{SR}$	-0.13	-0.14	-0.32	-0.12	+0.21	+0.22	+0.26	+0.18
3*R* configuration assigned from Δδ [(2*R*,3*R*) or (2*S*,3*S*)] (*R*)-MPA esters								
	δH_C(*)	δH_D(*)	δH_E	δH_F	δH_G(*)	δH_H	δH_I(*)	δH_J
2*R*3*R*	1.32	2.01	5.82	5.29	5.62	5.16	3.90	5.42
2*S*3*S*	1.01	1.80	5.24	5.09	5.81	5.34	4.17	5.78
$\Delta\delta^{RS}$	+0.31	+0.21	+0.58	+0.20	-0.19	-0.18	-0.27	-0.36

(*) δ from the center of the system

Table 1: Configurational assignment at C3 position of (*R*)-MPA esters **8**

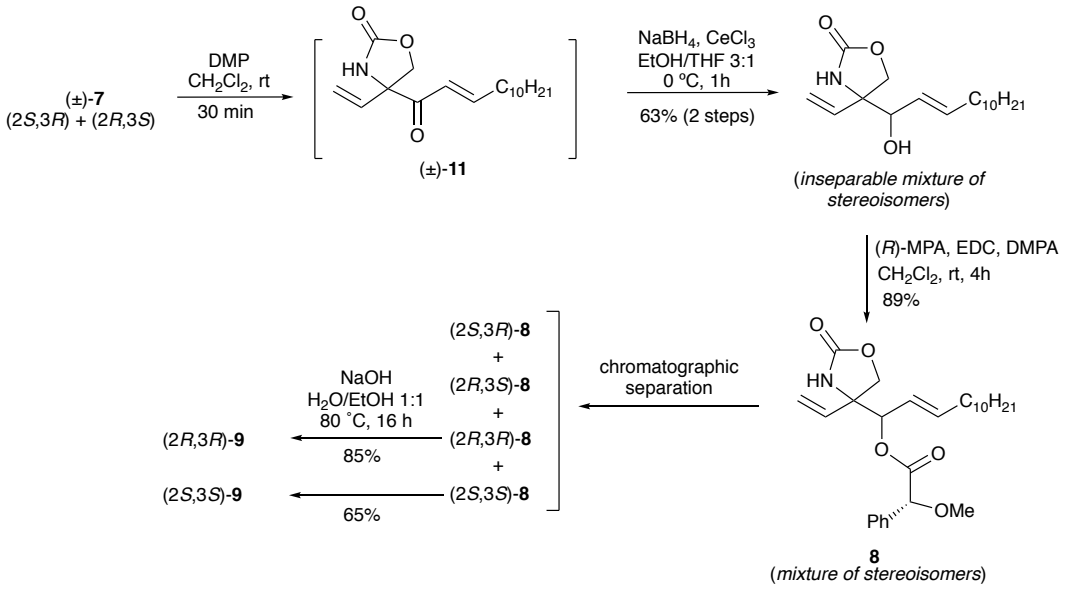
Enantiopure (2*S*,3*R*)-**8** and (2*R*,3*S*)-**8** we independently treated in basic aqueous-alcoholic media to obtain free aminodiols (2*S*,3*R*)-**9** and (2*R*,3*S*)-**9**, respectively, in good yields (Scheme 3).



Scheme 3 Resolution of **(±)-7** and conversion of enantiopure esters **8** into (2*S*,3*R*)-**9** and (2*R*,3*S*)-**9**

Regarding the preparation of the enantiomeric series, (2*S*,3*S*) and (2*R*,3*R*), we first attempted a direct inversion of the secondary alcohol in (±)-**7** by Mitsunobu reaction, using benzoate as nucleophile, without success. We then turned our attention to a redox two-step process based on the oxidation of (±)-**7** to ketone (±)-**11**, followed by reduction. In practice, the Dess-Martin oxidation of (±)-**7** gave ketone (±)-**11**, which was used without further purification. The reduction of (±)-**11** under Luche conditions (Ce(III)/$NaBH_4$)[10] turned out to be only minimally stereoselective, since a roughly 1:1 mixture of the corresponding diastereomeric alcohols in a satisfactory 63% yield (over two steps) was obtained (Scheme 4). Unfortunately, we were not able to efficiently isolate both diastereomeric racemates by column chromatography. Thus, we attempted the resolution of the mixture of alcohols through the formation of the corresponding diastereomeric esters **8** derived from (*R*)-MPA (Scheme 4). To our satisfaction, the four stereoisomeric esters exhibited sufficient separation by TLC.

After column chromatographic isolation of (2*R*,3*R*)-**8** and (2*S*,3*S*)-**8**, the amino diols (2*R*,3*R*)-**9** and (2*S*,3*S*)-**9** were obtained in 85% and 65% yield, respectively, by basic hydrolysis. Again, the configuration at C3 of the diasteromeric esters (2*R*,3*R*)-**8** and (2*S*,3*S*)-**8** was inferred by the method of Riguera *et al*, based on the Δδ values indicated in Table 1.



Scheme 4 Resolution of (±)-**11** and preparation of (2*S*,3*S*) and (2*R*,3*R*) **8** and **9**.

Metabolism of amino diols 9.

Treatment of A549 cells with sub-toxic concentrations (Fig. S1) of amino diols **9** revealed that only the 2*S*,3*R* stereomer was *N*-acylated (Fig. 2A,B). Interestingly, only the C22, C24 and C24:1 acyl derivatives were formed (Fig. 2A). Since A549 cells also produce ceramides with other *N*-acyl chains, with C16 being also abundant[11,12] (See Figure 3), this result suggests that (2*S*,3*R*)-**9** is a substrate of ceramide synthase 2 (CerS2), which produces long chain ceramides, but it is not a good substrate of CerS5 and CerS6, which catalyze the formation of C16 ceramide.[13] This is the first example of a sphingoid base selectively used by a specific CerS. On the other hand, only (2*S*,3*R*)-**9** and, to a significantly lower extent, (2*S*,3*S*)-**9** are phosphorylated at C1OH (Figure 2B), which supports the idea that, despite the

presence of the vinyl group, sphingosine kinase activity takes place preferentially on the 2*S* stereoisomer. Importantly, the above long chain *N*-acyl derivatives of (2*S*,3*R*)-**9** were metabolically stable, as neither the sphingomyelin analogs nor the glucosylceramide analogs were detected in the extracts.

To investigate the metabolic stability of amides of (2*S*,3*R*)-**9** against ceramidases, *N*-octanoylamide **10**, obtained by acylation of (2*S*,3*R*)-**9** with octanoic acid (EDC/DMAP), was incubated with A549 cells at sub-toxic concentrations (Figure S1). As shown in Figure 2C, the free base (2*S*,3*R*)-**9** was detected in the extracts. Furthermore, the C22, C24 and C24:1 acyl derivatives of (2*S*,3*R*)-**9** were also formed (Figure 2D), indicating that amide **10** is hydrolyzed by ceramidases and further reacylated with other acyl moieties. However, which of the 5 different ceramidases[13] is responsible for the hydrolysis of (2*S*,3*R*)-**10** is, so far, unknown.

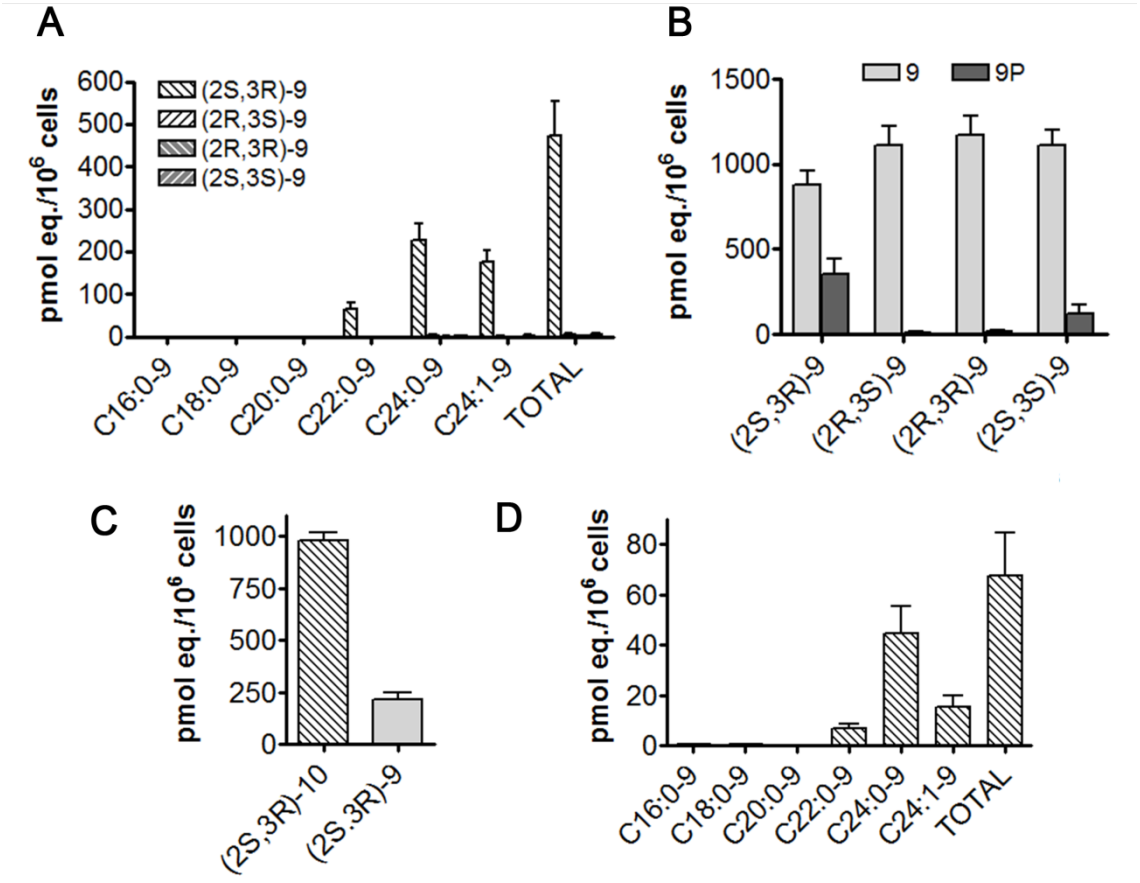


Fig. 2 Metabolism of compounds **9** and (2*S*,3*R*)-**10** . Cells were incubated with: A-B, the four stereoisomers of **9** (15 μM) and C-D, amide (2*S*,3*R*)-**10** (30 μM) for 24 h. Lipid analysis was carried out by UPLC/TOF MS in ESI + (free bases and amides) or ESI – (phosphates). A-B, amounts of amides (A) and phosphates (B) present in cells treated with the different stereoisomers of **9**; (C): amounts of incorporated amide (2*S*,3*R*)-**10** and of its hydrolysis product (2*S*,3*R*)-**9**; (D): reacyclation products present in cells treated with amide (2*S*,3*R*)-**10**. Data were obtained from two independent experiments with triplicates.

Effect on natural sphingolipids

Cells treated with amino diols **9** did not give rise to any remarkable change in the natural sphingolipids content (data not shown). In contrast, cells exposed to amide (2*S*,3*R*)-**10** (30 μM/24 h) contained significantly lower total ceramide levels than vehicle treated controls, all the different *N*-acyl species being similarly reduced (Figure 3A). This reduction is translated into significantly lower levels of glucosylceramide (GlcCer) (Fig. 3B) and sphingomyelin (SM) (Fig. 3C).

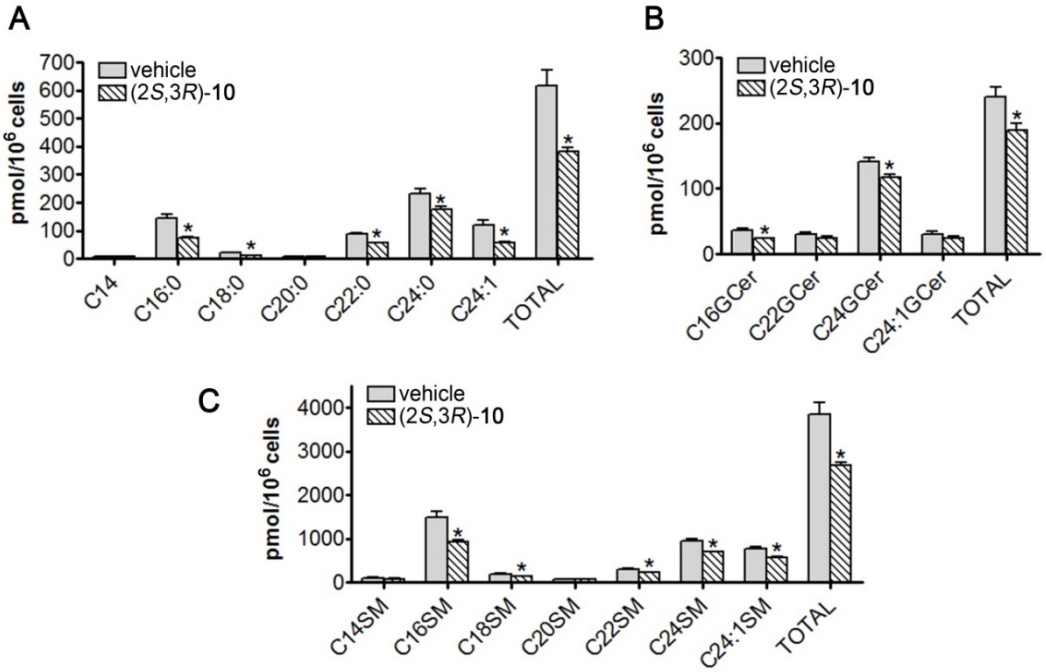


Fig. 3 Effect of (2*S*,3*R*)-**10** on natural sphingolipids. Cells were incubated with (2*S*,3*R*)-**10** (30 μM/24 h). Lipids were extracted and analyzed by UPLC/TOF MS. (A): amounts of

natural ceramides (Cer); (B): amounts of natural glucosyl ceramides (GlcCer) and (C): amounts of natural sphingomyelins (SM). Data were obtained from 2 independent experiments with triplicates. Asterisks indicate statistical difference with vehicle (control) ($P<0.05$. Unpaired, two-tail *t* test).

These results are consistent with inhibition of ceramide synthesis *de novo*. Since no increase in dihydroceramides was observed upon treatments (data not shown), dihydroceramide desaturase is not a likely candidate for inhibition. On the other hand, a decrease in CerS activity results in an increase in long chain bases and their phosphates.[14,15] However, either sphinganine, sphingosine or their corresponding phosphates were not detected in extracts after cell treatment with amide (2*S*,3*R*)-**10**, arguing against inhibition of CerS. Furthermore, no accumulation of 3-ketosphinganine was found in extracts from cells incubated with (2*S*,3*R*)-**10**, which is against inhibition of 3-ketosphinganine reductase. Therefore, we suggest that serine palmitoyl transferase (SPT), the rate-limiting enzyme in ceramide synthesis *de novo*, is the likely target of (2*S*,3*R*)-**10**. Although this amide is *N*-deacylated to (2*S*,3*R*)-**9**, this amino alcohol has no effect on Cer, GlcCer and SM levels, which argues against its involvement in the observed inhibition. Collectively, our data support that (2*S*,3*R*)-**10** could be responsible for the putative SPT inhibition.

Conclusions

The first stereoselective preparation of the four stereoisomers of quaternary 2-vinyl analogs of sphingosine has been achieved in a small number of steps and the effect of these compounds on basal sphingolipid metabolism in human A549 lung adenocarcinoma cells has been studied. A stereoselective borane-mediated addition of allene **1** to (*E*)-2-tridecenal very recently developed in our research group was used, giving only a single diastereomer [(*R*,*S*)-**7**

and (*S*,*R*)-**7**]. Resolution of enantiomers was readily accomplished by chromatography of the corresponding mixture of (*R*)-MPA diastereomeric esters followed by basic hydrolysis. Among the 2-vinyl sphingosines, only the 2*S*,3*R* isomer was *N*-acylated to produce long chain ceramides, presumably by CerS2. The metabolic stability of these amides was studied with the corresponding (2*S*,3*R*)-*N*-octanoyl amide **10**, which confirmed the operation of deacylation and reacylation metabolic pathways. Interestingly, the effects of amide **10** on natural sphingolipidome are in agreement with the inhibition of SPT, the first enzyme of the *de novo* biosynthesis of sphingolipids.

Experimental section

Chemistry

All reactions involving moisture- or air-sensitive reagents were performed in oven-dried glassware under N_2. Chemical shifts (δ) are quoted in parts per million and referenced for ^{1}H NMR to internal TMS (for $CDCl_3$) or residual solvent peak d 2.50 ppm (for DMSO-d_6). ^{13}C NMR are referenced to $CDCl_3$ (d 77.0 ppm) or DMSO-d_6 (d 39.5 ppm). Column chromatography was performed on silica gel (Merck 230-400 mesh). HRMS analyses were recorded on a LC/MSD-TOF mass spectrometer.

3-Benzoyl-4-vinylideneoxazolidin-2-one (1). A solution of benzoyl isocyanate (4.20 g, 25.60 mmol) in anhydrous CH_2Cl_2 (20 mL) was added to 2-butyn-1,4-diol (1.00 g, 11.60 mmol) at 0 ºC under N_2 atmosphere. The mixture was stirred for 5 hours at rt and the solvent was removed. A solution of $Pd_2(dba)_3 \cdot CHCl_3$ (0.055 g, 0.05 mmol) in anhydrous THF (40 mL) and triethylamine (0.087 mL, 0.64 mmol) were added under N_2 atmosphere. The mixture was stirred for 16 h at rt and filtered through a pad of Celite. The solid was washed with AcOEt.

The solvent was removed and the crude residue was purified by column chromatography (hexanes/AcOEt 4:1) to afford 0.595 g (61%) of allene **1**.

Compound 1, yellow solid; mp 102-103 ºC (lit.[6] 101.2-103 ºC); $\mathbf{R_f}$ (hexanes/AcOEt 2:1) = 0.35; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.68 (m, 2H, Ar*H*), 7.57 (m, 1H, Ar*H*), 7.45 (m, 2H, Ar*H*), 5.57 (t, 2H, *J* = 4.7 Hz, =CH_2), 5.04 (t, 2H, *J* = 4.7 Hz, CH_2O); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 193.6, 167.3, 151.9, 132.8, 129.2, 128.1, 128.0, 103.9, 90.4, 63.9; **IR** (film, cm^{-1}): 1792, 1689, 1331, 1308, 1157, 1068; **HRMS** (ESI+) calculated for $C_{12}H_{10}NO_3$ $[M+H]^+$ = 216.0655, found = 216.0651.

(*RS*,*E*)-1-[(2-oxo-4-vinyloxazolidin-4-yl]tridec-2-en-1-yl benzoate [**(±)-6**].

A solution of the allene **1** (1.24 g, 5.76 mmol, 1.00 eq) in anhydrous CH_2Cl_2 (4 mL) was added to a suspension of dicyclohexylborane[16] (1.23 g, 6.91 mmol, 1.2 eq) in CH_2Cl_2 (6 mL) at 0 ºC and under nitrogen atmosphere. The resulting mixture was stirred for 10 min at 0 ºC and for 1 h at rt. The resulting solution was then cooled to – 78 ºC, and (*E*)- 2-tridecenal (1.58 g, 8.06 mmol, 1.4 eq) was added. The reaction was stirred for 4 h at rt, and was then quenched by addition of triethanolamine (2.2 g, 2.5 eq). The resulting mixture was stirred for 1 h at rt. Evaporation of the solvent under vacuum gave a crude that was purified by *flash* column cromatography (hexanes/AcOEt 7:3) to afford 1.78 g (4.30 mmol, 75%) of adduct (±)-**6**.

Compound (±)-6, yellow oil; $\mathbf{R_f}$ (hexanes/AcOEt 3:2) = 0.60; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.06-8.00 (m, 2H, Ar*H*), 7.58-7.53 (m, 1H, Ar*H*), 7.46-7.40 (m, 2H, Ar*H*), 6.65 (bs, 1H, N*H*), 6.05-5.96 (m, 2H, =C*H*, C*H*OBz), 5.54-5.41 (m, 3H, =C*H*H, C*H*=C*H*), 5.34 (d, 1H, *J* = 10.8 Hz, =CH*H*), 4.46 (d, 1H, *J* = 8.7 Hz, OC*H*H), 4.14 (d, 1H, *J* = 8.7 Hz, OCH*H*), 2.05 (td, 2H, *J* = 7.9, 1.1 Hz, =CHCH_2), 1.38-1.19 (m, 16H, C_8H_{16}), 0.87 (t, 3H, *J* = 6.9 Hz, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.4, 159.3, 140.6, 136.2, 133.5, 129.8, 129.7, 128.6, 121.8, 117.3, 77.8, 72.2, 64.4, 32.5, 32.0, 29.7, 29.7, 29.5, 29.4, 29.3, 28.8, 22.8, 14.2; **IR** (ATR, cm^-

^{1}): 3240, 2923, 1755, 1707, 1263, 709; **HRMS** (ESI+) calculated for $C_{25}H_{35}NNaO_4$ $[M+Na]^+$ = 436.2458, found = 436.2466.

(*RS,E*)-4-(1-hydroxytridec-2-en-1-yl)-4-vinyloxazolidin-2-one [**(±)-7**].

A 3M solution of EtMgBr in Et_2O (1.3 mL, 3 eq) was dropwise added to a solution of compound (±)-**6** (537 mg, 1.30 mmol, 1 eq) in dry THF (8 mL) at 0 ºC. The reaction was followed by TLC (hexanes/AcOEt 3:2). After 2 h at 0 ºC, the reaction was partitioned by addition of *p*H 7 buffer and CH_2Cl_2. The organic layer was washed with additional *p*H 7 buffer solution, dried over $MgSO_4$ and filtered. The evaporation of the solvent under vacuum gave a crude which was purified by *flash* column cromatography (hexanes/AcOEt 3:2) to afford compound (±)-**7 (**341 mg, 1.11 mmol, 85%):

Compound (±)-7, mp = 70-2 ºC; $\mathbf{R_f}$ (hexanes/AcOEt 3:2) = 0.34; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.70 (bs, 1H, N*H*), 5.92 (dd, 1H, *J* = 17.3, 10.7 Hz, =C*H*), 5.80 (dt, 1H, *J* = 15.4, 6.7 Hz, =C*H*CH_2), 5.39-5.25 (m, 3H, C*H*=CH, =CH_2), 4.44 (d, 1H, *J* = 8.5 Hz, OC*H*H), 4.05 (d, 1H, *J* = 7.1 Hz, C*H*OH), 4.01 (d, 1H, *J* = 8.5 Hz, OCH*H*), 3.68 (bs, 1H, O*H*), 2.01 (q, 2H, *J* = 6.9 Hz, =CHCH_2), 1.37-1.19 (m, 16H, C_8H_{16}), 0.85 (t, 3H, *J* = 6.9 Hz, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 160.2, 137.1, 136.9, 125.6, 116.4, 75.9, 71.7, 65.5, 32.5, 32.0, 29.7, 29.5, 29.4, 29.3, 29.1, 22.7, 14.2; **IR** (ATR, cm^{-1}): 3287, 2922, 2851, 1742, 1042, 936; **HRMS** (ESI+) calculated for $C_{18}H_{32}NO_3$ $[M+H]^+$ = 310.2377, found = 310.2384.

(4*SR*,5*RS,E*)-5-(dodec-1-en-1-yl)-4-(hydroxymethyl)-4-vinyloxazolidin-2-one [(±)-**7'].**

A solution of NaOH (0.04 g, 1.0 mmol) in 9:1 MeOH/H_2O (1 mL) was added to a stirred solution of compound (±)-**6** (300 mg, 0.73 mmol, 1 eq) in 9:1 MeOH/H_2O (10 mL) at r.t. The advance of the reaction was followed by TLC (hexanes/AcOEt 3:2). After 6 h, the mixture was heated at 50 ºC for additional 3 h and no more changes was observed (TLC). The reaction was partitioned by addition of a saturated aqueous solution of NH_4Cl (20 mL) and CH_2Cl_2 (20

mL). The phases were decanted and the aqueous phase was washed with more CH_2Cl_2 (2 x 10 mL). The combined organic portions were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography using silica gel (hexanes/AcOEt 7:3) to afford (±)-**7'** (0.112 g, 50%) and (±)-**7** (0.072 g, 32%). **Compound (±)-7'**: colourless oil; $\mathbf{R_f}$ (hexanes/AcOEt 3:2) = 0.18; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.70 (broad band, 1H, N*H*), 5.86 (dt, 1H, *J* = 15.2, 6.7 Hz, =C*H*CH_2), 5.74 (dd, 1H, *J* = 17.4, 10.8 Hz, =C*H*), 5.72-5.66 (m, 1H, C*H*=CH), 5.37 (dd, 1H, *J* = 17.4, 0.5 Hz, =C*H*H), 5.27 (dd, 1H, *J* = 10.8, 0.5 Hz, =CH*H*), 4.65 (d, 1H, *J* = 8.4 Hz, OC*H*), 3.71 (d, 1H, *J* = 12.0 Hz, C*H*HOH), 3.56 (d, 1H, *J* = 12.0 Hz, CH*H*OH), 2.12-2.06 (m, 2H, =CHCH_2), 1.29-1.23 (m, 16H, C_8H_{16}), 0.87 (t, 3H, *J* = 6.5 Hz, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 160.3, 140.0, 135.9, 121.7, 117.2, 86.6, 66.6, 64.6, 32.4, 32.0, 29.7, 29.7, 29.6, 29.5, 29.3, 28.9, 22.8, 14.2; **IR** (ATR, cm^{-1}): 3277, 2922, 2853, 1736, 1350, 976; **HRMS** (ESI+) calculated for $C_{18}H_{32}NO_3$ $[M+H]^+$ = 310.2377, found = 310.2384.

Isomerization of (±)-**7**

A solution of Dess-Martin periodinane (DMP) (1.930 g, 4.414 mmol, 1.1 eq) in dry CH_2Cl_2 (10 mL) was dropwise added to a stirred solution of (±)-**7** (1.240 g, 4.007 mmol, 1 eq) in dry CH_2Cl_2 (10 mL) at r.t. The course of the reaction was followed by TLC. After 30 min, the reaction was quenched by adding sat. $NaHCO_3$ aqueous solution (10 mL) and solid $Na_2S_2O_3$ (0.30 g). The mixture was extracted with CH_2Cl_2 (5 x 10 mL) and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give 1.180 g of crude ketone (±)-**11** [$\mathbf{R_f}$ (hexanes/AcOEt 3:2) = 0.58]. A mixture of $NaBH_4$ (0.443 g, 11.710 mmol, 3 eq) and $CeCl_3$:$7H_2O$ (2.90 g, 7.807 mmol, 2 eq) was added to a solution of the crude ketone (1.180 g) in THF (20 mL) and EtOH (60 mL) at 0 ºC. After 6 h, the reaction was quenched by addition of a sat. solution of NH_4Cl (40 mL) and water (60 mL). The mixture was extracted with CH_2Cl_2 (5 x 20 mL) and the combined organic layers

were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The ^{1}H NMR of the residue showed a roughly 1:1 mixture of isomers (±)-**7** and its racemic diastereomer (0.781 g, 2.44 mmol, 63% two-steps yield).

Typical procedure for the preparation of the (*R*)-MPA esters 8.

A solution of (*R*)-methoxyphenylacetic acid [(*R*)-MPA, 0.135 g, 0.81 mmol, 1.4 eq] and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 0.167 g, 0.87 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 ºC for 30 min. Then, a solution of (±)-**7** (0.180 g, 0.58 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP, 0.010 g) in CH_2Cl_2 (10 mL) was added and the solution was stirred for 2 h at r.t. The reaction was quenched by addition of 2M HCl (10 mL) and the organic phase was washed with more 2M HCl (10 mL), sat. $NaHCO_3$ (10 mL) and brine (10 mL). The combined organic portions were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography in silica gel (4:1 hexanes/ EtOAc) to afford 0.117 g (0.27 mmol, 46%) of (2*S*,3*R*)-**8** and 0.120 g (0.28 mmol, 47%) of (2*R*,3*S*)-**8**.

(2*S*,3*R*)-**8**: colourless oil; $\mathbf{R_f}$ (hexanes/AcOEt 3:2) = 0.82; $\mathbf{[\alpha]_D^{25}}$ = +6.7 (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.42-7.30 (m, 5H, Ar*H*), 5.91 (dt, 1H, *J* = 14.7, 6.8 Hz, =C*H*CH_2), 5.72 (bs, 1H, N*H*), 5.62 (dd, 1H, *J* = 17.3, 10.7 Hz, =C*H*), 5.36-5.23 (m, 2H, OC*H*C*H*=), 5.06 (d, 1H, *J* = 17.2 Hz, =C*H*H), 5.06 (d, 1H, *J* = 10.7 Hz, =CH*H*), 4.73 (s, 1H, C*H*Ph), 3.96 (d, 1H, *J* = 8.8 Hz, OC*H*H), 3.75 (d, 1H, *J* = 8.8 Hz, OCH*H*), 3.38 (s, 3H, OCH_3), 2.07-1.98 (m, 2H, =CHCH_2), 1.38-1.19 (m, 16H, C_8H_{16}), 0.87 (t, 3H, *J* = 6.9 Hz, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 169.4, 158.4, 141.3, 136.2, 135.5, 129.1, 128.9, 127.3, 121.3, 116.9, 82.5, 77.8, 72.0, 63.9, 57.5, 32.5, 32.0, 29.7, 29.7, 29.5, 29.5, 29.3, 28.7, 22.8, 14.2; **IR** (ATR, cm^{-1}): 2923, 2853, 1748, 1169, 1108, 752; **HRMS** (ESI+) calculated for $C_{27}H_{40}NO_5$ $[M+H]^+$ = 458.2901, found = 458.2913.

(2*R*,3*S*)-**8**: colourless oil; $\mathbf{R_f}$ (hexanes/AcOEt 3:2) = 0.69; $\mathbf{[\alpha]_D^{25}}$ = –71.0 (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.42-7.32 (m, 5H, Ar*H*), 5.90 (bs, 1H, N*H*), 5.83 (dd, 1H, *J* = 17.2, 10.7 Hz, =C*H*), 5.59 (dt, 1H, *J* = 14.5, 6.8 Hz, =C*H*CH_2), 5.33-5.26 (m, 3H, OC*H*CH=, =CH_2), 5.22-5.13 (m, 1H, C*H*=CH), 4.76 (s, 1H, OC*H*Ph), 4.23 (d, 1H, *J* = 8.8 Hz, OC*H*H), 4.00 (d, 1H, *J* = 8.8 Hz, OCH*H*), 3.39 (s, 3H, OCH_3), 1.94-1.87 (m, 2H, =CHCH_2), 1.34-1.14 (m, 16H, C_8H_{16}), 0.88 (t, 3H, *J* = 6.9 Hz, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 169.6, 158.4, 140.6, 136.0, 135.9, 129.1, 128.8, 127.3, 120.8, 117.3, 82.6, 77.4, 71.8, 63.9, 57.5, 32.4, 32.1, 29.8, 29.7, 29.5, 29.5, 29.2, 28.7, 22.8, 14.3; **IR** (ATR, cm^{-1}): 2923, 2858, 1748, 1169, 1108, 752; **HRMS** (ESI+) calculated for $C_{27}H_{40}NO_5$ $[M+H]^+$ = 458.2901, found = 458.2908.

(2*S*,3*S*)-**8**: colourless oil; $\mathbf{R_f}$ (hexanes/AcOEt 3:2) = 0.40; $\mathbf{[\alpha]_D^{25}}$ = +6.5 (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.50-7.31 (m, 5H, Ar*H*), 5.81 (dd, 1H, *J* = 17.2, 10.7 Hz, CH_2=C*H*), 5.78 (bs, 1H, N*H*), 5.39-5.30 (m, 3H, =CH_2, C*H*OCO), 5.24 (dt, 1H, *J* = 15.6, 6.7 Hz, =C*H*CH_2), 5.09 (dd, 1H, *J* = 15.5, 6.0 Hz, CHC*H*=), 4.84 (s, 1H, C*H*OMe), 4.27 (d, 1H, *J* = 8.8 Hz, OC*H*H), 4.07 (d, 1H, *J* = 8.8 Hz, OCH*H*), 3.41 (s, 3H, OCH_3), 1.80 (q, 2H, *J* = 6.0 Hz, =CHCH_2), 1.33-1.07 (m, 16H, C_8H_{16}), 0.88 (t, 3H, *J* = 6.8 Hz, CH_3) **^{13}C NMR** (101 MHz, $CDCl_3$): δ 169.6, 158.9, 138.3, 136.0, 135.5, 129.0, 128.8, 127.5, 120.7, 117.4, 82.3, 77.1, 64.0, 57.4, 32.3, 32.0, 29.8, 29.7, 29.5, 29.5, 29.1, 28.7, 22.8, 14.3; **IR** (ATR, cm^{-1}): 2923, 2853, 1748, 1169, 1108, 752; **HRMS** (ESI+) calculated for $C_{27}H_{40}NO_5$ $[M+H]^+$ = 458.2901, found = 458.2895.

(2*R*,3*R*)-**8**: colourless oil; $\mathbf{R_f}$ (hexanes/AcOEt 3:2) = 0.62; $\mathbf{[\alpha]_D^{25}}$ = –98.7 (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.49-7.32 (m, 5H, Ar*H*), 5.82 (dt, 1H, *J* = 15.2, 6.8 Hz, =C*H*CH_2), 5.62 (dd, 1H, *J* = 17.2, 10.7 Hz, CH_2=C*H*), 5.42 (bs, 1H, N*H*), 5.29 (dd, 1H, *J* = 15.3, 7.2 Hz, CHC*H*=), 5.25-5.11 (m, 3H, =CH_2, C*H*OCO), 4.78 (s, 1H, C*H*OMe), 3.93 (d, 1H, *J* = 8.8 Hz, C*H*HO), 3.86 (d, 1H, *J* = 8.8 Hz, CH*H*O), 3.39 (s, 3H, OCH_3), 2.01 (q, 2H, *J* = 7.1 Hz, =CHCH_2), 1.35-1.17 (m, 16H, C_8H_{16}), 0.87 (t, 3H, *J* = 6.8 Hz, CH_3); **^{13}C NMR**

(101 MHz, $CDCl_3$): δ 169.6, 158.4, 139.6, 136.2, 135.5, 129.3, 129.0, 127.3, 121.3, 117.0, 82.4, 77.8, 71.7, 63.7, 57.4, 32.5, 32.0, 29.7, 29.7, 29.5, 29.4, 29.2, 28.8, 22.8, 14.2; **IR** (ATR, cm^{-1}): 2923, 2853, 1748, 1169, 1108, 752; **HRMS** (ESI+) calculated for $C_{27}H_{40}NO_5$ $[M+H]^+$ = 458.2901, found = 458.2891.

Typical procedure for hydrolysis of the (*R*)-MPA esters 8 to amino diols 9.

A solution of ester (2*S*,3*R*)-**8** (0.170 g, 0.372 mmol) in a 1:1 EtOH/2M aq. NaOH mixture (6 mL) was heated to reflux. The progress of the reaction was followed by TLC (hexanes/AcOEt 3:2). After 20 h, the EtOH was evaporated under vacuum and the aqueous residue was extracted with CH_2Cl_2 (4 x 10 mL). The combined organic portions were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography in silica gel (9:1 CH_2Cl_2/MeOH) to afford 0.099 g (0.35 mmol, 94%) of (2*S*,3*R*)-**9**.

(2*S*,3*R*)-**9**: colourless oil; $[\alpha]_D^{25}$ = +14.4 (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.97 (dd, 1H, *J* = 17.7, 10.7 Hz, C*H*=CH_2), 5.74 (dt, 1H, *J* = 15.4, 6.7 Hz, =C*H*CH_2), 5.45 (dd, 1H, *J* = 15.4, 7.6 Hz, CHC*H*=), 5.33-5.24 (m, 2H, =CH_2), 3.96 (d, 1H, *J* = 7.6 Hz, C*H*OH), 3.71 (d, 1H, *J* = 11.0 Hz, OC*H*H), 3.43 (d, 1H, *J* = 10.9 Hz, OCH*H*), 2.21 (bs, 4H, NH_2, 2xO*H*), 2.04 (q, 2H, *J* = 7.2 Hz, =CHCH_2), 1.44-1.14 (m, 16H, C_8H_{16}), 0.87 (t, 3H, *J* = 6.8 Hz, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 140.0, 136.0, 127.7, 115.8, 77.0, 66.7, 60.7, 32.5, 32.1, 29.8, 29.6, 29.5, 29.4, 29.4, 29.3, 22.8, 14.3; **HRMS** (ESI+) calculated $C_{17}H_{34}NO_2$ $[M+H]^+$ = 284.2584, found = 284.2581.

(2*R*,3*S*)-**9**: $[\alpha]_D^{25}$ = –15.1 (*c* = 1.0, $CHCl_3$); **HRMS** (ESI+) calculated $C_{17}H_{34}NO_2$ $[M+H]^+$ = 284.2584, found = 284.2584.

(2*R*,3*R*)-**9**: mp 63-5 ºC; $[\alpha]_D^{25}$ = –7.0 (*c* = 1.1, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.82 (dd, 1H, *J* = 17.7, 10.8 Hz, CH_2=C*H*), 5.73 (dt, 1H, *J* = 15.2, 7.1 Hz, =C*H*CH_2), 5.41 (dd, 1H, *J* = 15.4, 6.6 Hz, CHC*H*=), 5.31-5.25 (m, 2H, =CH_2), 4.10 (d, 1H, *J* = 6.3 Hz, C*H*OH), 3.65

(d, 1H, *J* = 11.0 Hz, OC*H*H), 3.48 (d, 1H, *J* = 11.0 Hz, OCH*H*), 2.03 (q, 2H, *J* = 7.1 Hz, =CHCH_2), 1.38-1.20 (m, 16H, C_8H_{16}), 0.88 (t, 3H, *J* = 6.9 Hz, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 138.6, 134.7, 127.5, 116.0, 75.6, 67.3, 61.3, 32.4, 32.1, 29.6, 29.5, 29.3, 29.3, 29.2, 29.1, 22.7, 14.1; **HRMS** (ESI+) calculated $C_{17}H_{34}NO_2$ $[M+H]^+$ = 284.2584, found = 284.2581.

For (2*S*,3*S*)-**9**: **$[\alpha]_D^{25}$** = +7.6 (*c* = 1.0, $CHCl_3$); **HRMS** (ESI+) calculated $C_{17}H_{34}NO_2$ $[M+H]^+$ = 284.2584, found = 284.2583.

(3'*S*,4'*R*,*E*)-N-(4-hydroxy-3-(hydroxymethyl)hexadeca-1,5-dien-3-yl)octanamide (11)

A solution of octanoic acid (7.2 mg, 0.05 mmol) and EDC (10 mg, 0.05 mmol) in anhydrous CH_2Cl_2 (1 mL) was stirred for 30 min. To this mixture, a solution of the starting alcohol (2*S*,3*R*)-**9** (10 mg, 0.035 mmol) and DMAP (5 mg, 0.04 mmol) in CH_2Cl_2 (1 mL) was added dropwise. After stirring 15h at rt, the mixture was diluted with CH_2Cl_2 (5 mL) and washed successively with HCl 1N, water, $NaHCO_3$ and water (3 mL each). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude was purified by flash chromatography (Hexanes-EtOAc 8:2), to yield amide **10** (10.7 mg, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.04 (br s, 1H, N*H*), 5.93 (dd, *J* = 17.3, 10.7 Hz, 1H, =C*H*), 5.79 – 5.66 (m, 1H, =C*H*$C_{10}H_{21}$), 5.38 (ddd, *J* = 7.6, 7.0, 1.7 Hz, 1H, =C*H*CHOH), 5.24 (dd, *J* = 82.8, 14.0 Hz, 2H, =CH_2), 4.45 (br s, 1H, CH_2O*H*), 4.07 (d, *J* = 7.4 Hz, 1H, C*H*OH), 3.74 (s, 1H, CHO*H*), 3.60 (ddd, *J* = 15.4, 12.0, 4.3 Hz, 2H, CH_2OH), 2.31 – 2.24 (m, 2H, CH_2C(O)), 2.04 (dd, *J* = 14.5, 7.0 Hz, 2H, =CHCH_2), 1.65 (dt, *J* = 14.9, 7.6 Hz, 2H, CH_2 CH_2C(O)), 1.45 – 1.17 (br m, 24H, $C_{12}H_{24}$), 0.88 (td, *J* = 6.8, 2.6 Hz, 6H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.9 (C), 136.56 (CH), 136.3 (CH), 126.6 (CH), 116.17 (CH_2), 74.7 (CH), 66.9 (CH_2), 66.0 (CH_2), 37.3 (CH_2), 32.5 (CH_2), 32.0 (CH_2), 31.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 26.09

(CH_2), 22.8 (CH_2), 22.7 (CH_2), 14.3 (CH_3), 14.2 (CH_3). **HRMS** (ESI+) calculated $C_{25}H_{47}NO_3$ $[M+H]^+$ = 410,3634, found 410,3595.

Biology

Cell culture

Human A549 lung adenocarcinoma cells were obtained from the American Type Culture Collection (ATCC) and grown in Ham's F-12 medium supplemented with 10% fetal bovine serum (FBS), 1% Penicillin and Streptavidin and 2mM Glutamine at 37 ^{0}C in 5% CO_2/95% air.

Cell viability of amide 11

Cells were seeded in complete medium at 10.000 cells per well in 96-well plates. Twenty-four hours after seeding, media were replaced with fresh medium and compounds were added to give final concentrations of 0.02–400 μM. After 24 h, the number of viable cells was quantified by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. Vehicle (0.25 % methanol) was used in controls. The viability for amide **11** (expressed as IC_{50}) ranged between 33 to 58 μM.

Lipid analysis

A549 cells were seeded in 1 mL of medium with 10% FBS-penicillin, streptavidin, glutamine in a 6-well plates (250.000 cells/well). Twenty-four hours later, media were replaced with fresh medium containing the compounds at the specified concentrations. After 24 h, the medium was removed and cells were washed in PBS, collected by brief trypsinization, transferred to Eppendorf vials and resuspended in 0.1 mL of PBS. An aliquot (0.01 mL) was

taken for cell counting. The remaining suspension was transferred to glass vials and 0.75 mL of chloroform/methanol (2:1) containing the internal standards (C17-sphinganine, *N*-dodecanoylsphingosine, N-dodecanoylglucosylsphingosine and *N*-dodecanoylsphingosylphosphorylcholine, 0.2 nmol each), were added. Samples were heated at 48 ºC overnight and 0.075 mL of 1 M KOH in methanol was added, followed by 2 h incubation at 37 ºC. Finally, the mixtures were neutralized with 0.075 mL of 1 M acetic acid, dried under nitrogen and the residue was dissolved in 0.150 mL of methanol. The liquid chromatography–mass spectrometer consisted of a Waters Aquity UPLC system connected to a Waters LCT Premier orthogonal accelerated time of flight mass spectrometer (Waters, Millford, MA), operated in positive or negative electrospray ionisation mode. Full scan spectra from 50 to 1500 Da were acquired and individual spectra were summed to produce data points each 0.2 s. Mass accuracy and reproducibility were maintained by using an independent reference spray via the LockSpray interference. The analytical column was a 100 mm x 2.1 mm id, 1.7 µm C8 Acquity UPLC BEH (Waters). The two mobile phases were phase A: MeOH/H_2O/HCOOH (74:25:1 v/v/v); phase B: MeOH/HCOOH (99/1 v/v), both also contained 5 mM ammonium formate. A gradient was programmed—0.0 min, 80% B; 3 min, 90% B; 6 min, 90% B; 15 min, 99% B;18 min, 99% B; 20 min, 80% B. The flow rate was 0.3 $mL \cdot min^{-1}$. The column was held at 30 ºC. Quantification was carried out using the extracted ion chromatogram of each compound, using 50 mDa windows. The linear dynamic range was determined by injecting standard mixtures. Positive identification of compounds was based on the accurate mass measurement with an error <5 ppm and its LC retention time, compared to that of standards (±2%)

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