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PAPER

Asymmetric synthesis and cytotoxic activity of isomeric phytosphingosine derivatives†

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New phytosphingosine analogues have been conceived, synthesised and their cytotoxicity in B16 murine melanoma cells tested. These compounds embed an isomeric substitution pattern resulting from a formal permutation of the C-2 and C-4 substituents along the aliphatic skeleton of the original sphingoid base. Five different stereoisomers have been accessed through regio- and stereocontrolled opening of the oxirane of long chain epoxyamine precursors. The corresponding *N*-hexyl and *N*-octanoyl derivatives have also been prepared. In cell viability experiments all the primary amines were found to be more active than the natural phytosphingosine with IC_{50} in the low μM range for the most potent compounds.

Introduction

Sphingosine represents the archetypal structural backbone common to all sphingolipids. Two main C_{18} derivatives are typically encountered in living organisms: D-*erythro*-sphingosine or D-*ribo*-phytosphingosine (Fig. 1).[1] Due to the strong contribution of sphingolipids as signalling molecules in cell growth and differentiation regulation,[2] the sphingosine framework has inspired intense synthetic studies.[3]

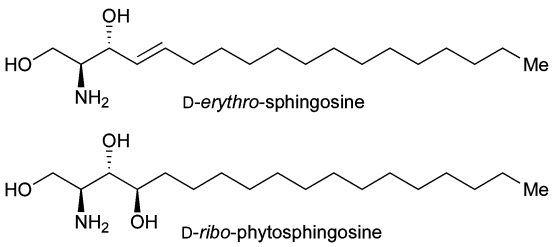

Fig. 1 Structure of the two most widespread sphingoid bases.

D-*erythro*-Sphingosine, the most common form in mammals, is ubiquitous in eukaryotic cells. It has been described to induce cell cycle arrest and apoptosis,[4] through modulation of protein kinases.[5] Additionally, this sphingoid base constitutes a metabolic switch between two major players of cell fate regulation, *i.e.* the proapoptotic ceramide and the proliferative and angiogenic sphingosine-1-phosphate.[6] Close structural analogues of sphingosine of high anti-cancer pharmacological relevance have thus been developed, such as the naturally occurring spisulosine[7] or the nature-inspired synthetic safingol,[8] and enigmol (Fig. 2).[9]

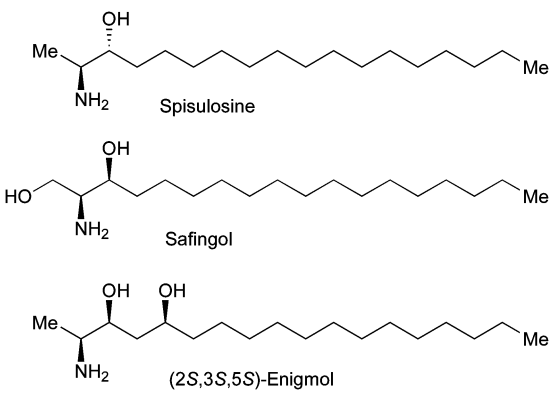

Fig. 2 Structure of pharmacologically relevant sphingosine analogues.

D-*ribo*-Phytosphingosine is more frequent in plants and fungi.[10] In yeast it has been identified as a signalling molecule involved in the cell cycle arrest in response to heat stress.[11] It is also encountered in mammals, such as in intestinal[12] and skin cells.[13] Phytosphingosine has been shown to have a pro-apoptotic effect on different cancer cell lines, either alone,[14] or in combination with other treatments.[15] Related phytoceramides, obtained upon *N*-acylation, were described to be more cytotoxic than the corresponding sphingosine-derived ceramides.[16] Other important biologically active phytosphingosine-related compounds have also been reported,[17] including cyclised analogues.[18]

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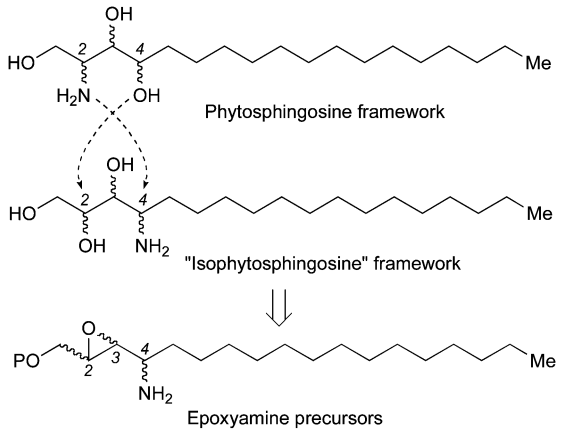
† Electronic supplementary information (ESI) available: copies of the ^{1}H NMR spectra for **2**,**4**, **6–18** and ^{13}C NMR spectra for **6–18**. CCDC reference numbers 836021 and 836022. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob06195j

In the course of our recent work on the preparation of aza-analogues of the anhydrophytosphingosine jaspine B, we developed a straightforward access to enantioenriched long-chain sphingosine-related epoxyamines (Scheme 1).[19] We envisioned that these intermediates could give rise to phytosphingosine-like aminotriol derivatives upon formal epoxide hydrolysis. In particular, the location of the amino group would lead to an unprecedented isomeric substitution pattern resulting from a formal permutation of the C-2 and C-4 substituents along the aliphatic skeleton of the original sphingoid base. We will refer to these entities as "isophytosphingosines" in the rest of the article.



Scheme 1 General approach.

We thus report herein the enantioselective preparation of long chain 4-amino-1,2,3-triols as well as several *N*-substituted derivatives thereof. The present study includes the first biological evaluation of these novel isophytosphingosine derivatives regarding their effect on melanoma cell viability.

Results and discussion

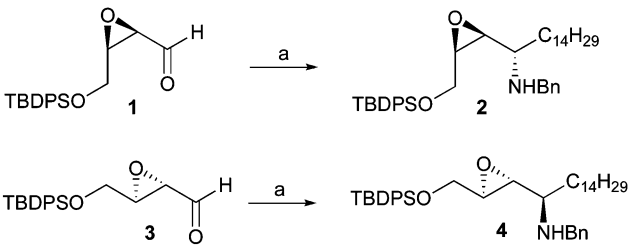
Synthetic plan

Taking advantage of the flexibility of our synthetic route, we targeted a panel of stereoisomeric forms. Indeed, there is typically a strong influence of the configuration of the sphingosine analogues on their bioactivity. For example the ceramide synthase inhibitory activity of a series of 1-deoxy sphingosine analogues, including enigmols, was shown to strongly depend on their stereochemical pattern.[20] Our plan was to rely on a stereochemical manifold based on the following key elements: 1) the configuration of the starting epoxide; 2) the 3,4-*anti* epoxyamine relationship and 3) the regio- and stereocontrolled C-2 or C-3 epoxide opening.[21]

Preparation of the starting epoxyamines

The representative starting epoxyamines were obtained *via* the *anti*-selective addition of the tetradecyl Grignard reagent onto an intermediate epoxyimine (Scheme 2).[19]

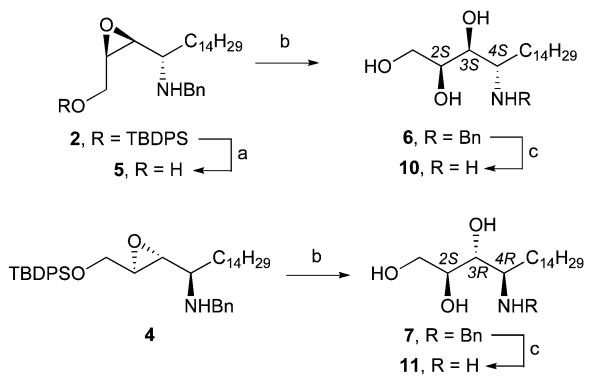
The *cis* and *trans* derivatives **2** and **4** were obtained in 70% (dr > 95 : 5) and 53% (dr 80 : 20) yields respectively from the starting epoxyaldehyde. The *cis* epoxyamine *ent*-**2** was prepared in a similar fashion from the enantiomeric aldehyde precursor.



Scheme 2 Preparation of the epoxyamines. *Reagents and conditions*: a) i) $BnNH_2$, 4 Å MS, Et_2O, rt, 18 h, 70%; ii) Et_2OBF_3, THF, −78 °C to −40 °C then $C_{14}H_{29}MgCl$, −78 °C, 2 h, 53%.

Access to 3,4-*anti* aminotriols

Starting from 3,4-*anti* epoxyamines, a regio- and stereocontrolled C-2 epoxide hydrolysis was expected to yield 2,3-*syn*/3,4-*anti* or 2,3-*anti*/3,4-*anti* from a *cis* or a *trans* epoxide respectively. The *cis* epoxyamine **2** was first desilylated and the resulting primary alcohol **5** was treated with diluted H_2SO_4 in dioxane (Scheme 3). To our satisfaction, these conditions, previously developed for vinyl derivatives, proved equally efficient in the aliphatic series.[22] The expected aminotriol **6** was isolated in 66% yield. Only traces (*ca.* 5%) of another isomer were detected in HPLC/MS analysis of the crude mixture (*m*/*z* 408). The same procedure was directly applied to the *O*-silylated *trans* epoxyalcohol **4** to selectively deliver the expected 2,3-*anti*/3,4-*anti* isomer **7** in 65% yield.



Scheme 3 Hydrolysis of the epoxyamines toward 3,4-*anti* aminotriols. *Reagents and conditions*: a) TBAF/SiO_2, THF, rt, 16 h, 81%. b) 3 M H_2SO_4, 1,4-dioxane, reflux, 5 h, 66–65%. c) 12 bar H_2, $Pd(OH)_2$, MeOH, 72 h, 77–95%.

Gratifyingly, a crystal sample of the latter proved suitable for X-ray diffraction analysis. The resulting structure allowed unambiguous confirmation of the (*2S**,*3R**,*4R**) configuration (Fig. 3).

A final hydrogenolysis afforded the targeted isophytosphingosines **10** and **11** in high yields (Scheme 3). *Ent*-**10** was prepared from *ent*-**2** using a reaction sequence identical to that employed for **10**.

Access to 3,4-*syn* aminotriols

The preparation of 3,4-*syn* aminotriols from 3,4-*anti* epoxyamines required an inversion of configuration at C-3. This was planned through the implementation of our carbonation/intramolecular cyclisation sequence ensuring neat C-3 epoxide opening.[24]

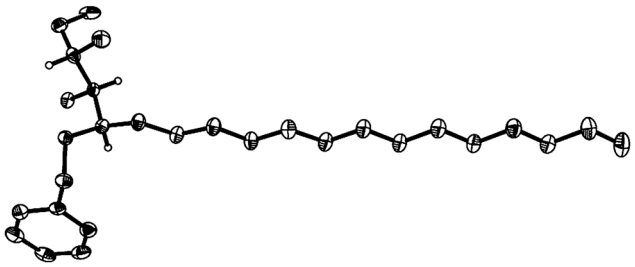
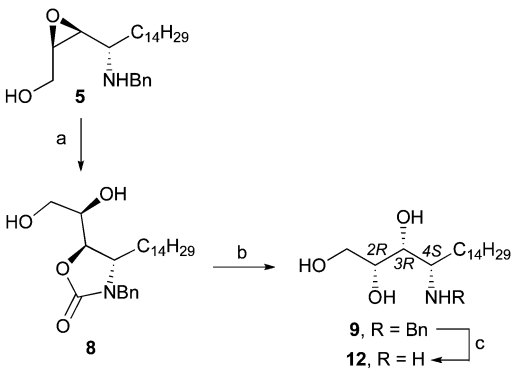


Fig. 3 Molecular view of the (2*S*,3*R*,4*R*)-aminotriol **7** in the solid state (thermal ellipsoids at 50% probability); hydrogens are omitted for clarity except on asymmetric carbons.[23]

Treatment of the deprotected *cis* epoxyamine **5** with ammonium carbonate in THF/H_2O led to the efficient and selective formation of the desired oxazolidinone **8** (Scheme 4).



Scheme 4 The oxazolidinone route towards 3,4-*syn* aminotriols. *Reagents and conditions*: a) $(NH_4)_2CO_3$, THF/H_2O, rt, 8 h, 95%. b) NaOH, $EtOH/H_2O$, reflux, 6 h, 83%. c) 12 bar H_2, $Pd(OH)_2$, MeOH, 72 h, 62%.

The latter was saponified to give the 2,3-*syn*/3,4-*syn* aminotriol **9** in 83% yield. Again, a crystal structure of this *N*-benzyl intermediate was obtained by X-ray diffraction analysis confirming the (*2R**,*3R**,*4S**) stereochemical assignment (Fig. 4).

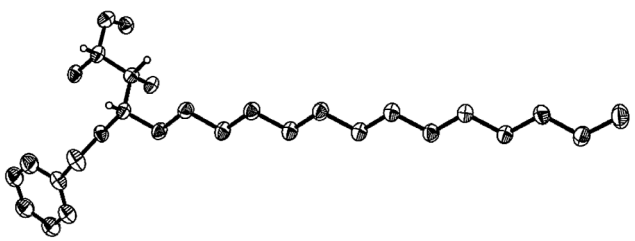


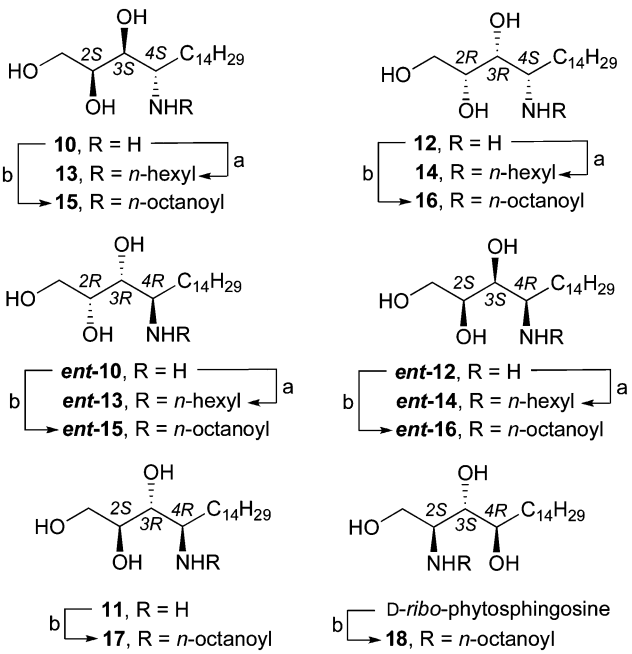
Fig. 4 Molecular view of the (2*R*,3*R*,4*S*)-aminotriol **9** in the solid state (thermal ellipsoids at 50% probability); hydrogens are omitted for clarity except on asymmetric carbons.[25]

Hydrogenolytic *N*-benzyl deprotection afforded the expected isophytosphingosine **12**. *Ent*-**12** was similarly obtained starting from the epoxyamine *ent*-**5**.

N-Functionalisation of the aminotriols

In modulating the steric and electronic environment of the nitrogen atom, *N*-functionalisation of sphingoid bases deeply impacts their biological profile. For example, a direct relationship between the degree of *N*-methylation of the sphingosine and its signalling activity has been recently evidenced.[26] Depending on the nature of the *N*-substituent, additional physicochemical factors such as the hydrophobicity of double-chained derivatives may also be involved. In the context of the present study, two representative transformations were selected, *i.e.* the *N*-alkylation and the *N*-acylation. An aliphatic residue of moderate chain length (C_6 to C_8) was selected in both cases so as to mimic ceramide while retaining reasonable hydrophilicity.

Applying a general procedure of reductive amination with *n*-hexanal, the four *N*-hexyl amines **13**, **14**, *ent*-**13**, *ent*-**14** were obtained in reasonable yields from the corresponding isophytosphingosines (Scheme 5). Alternatively, *N*-acylation was conducted with *p*-nitrophenyl octanoate to give the expected isophytoceramides **15–17**, *ent*-**15** and *ent*-**16** with good efficiency. For the sake of comparison, the natural phytoceramide **18** was prepared similarly from commercially available D-*ribo*-phytosphingosine.



Scheme 5 Preparation of *N*-functionalised (iso)phytosphingosines. *Reagents and conditions*: a) *n*-hexanal, $NaBH_3CN$, HCl conc., MeOH, rt, 16 h, 50–65%. b) *p*-nitrophenyl octanoate, THF, rt, 48 h, 60–63%.

Biological evaluation

Compounds were evaluated for their capacity to alter murine melanoma B16 cells viability. Melanoma is indeed considered as a radiation-, immunotherapy-, and chemotherapy-refractory neoplasm.

Regarding the amide derivatives, none of the five compounds **15–17**, *ent*-**15** and *ent*-**16** tested displayed a significant cytotoxicity at concentrations up to 50 μM. Surprisingly, the same trend was observed for the natural D-*ribo*-phytosphingosine-derived ceramide **18** (up to 25 μM).

Among the four *N*-alkyl derivatives evaluated, the 4*R* compounds *ent*-**13** and *ent*-**14** proved slightly more active (40–50% cytotoxicity at 10 μM) than the 4*S* stereoisomers **13** and **14** (30–35% cytotoxicity at 10 μM).

The more significant activities were recorded in the unsubstituted isophytosphingosine series. Apart from the 4*S* derivative **10** (50% cytotoxicity at 10 μM), all the primary amines showed fair cytotoxicity (70–79% at 10 μM). The two more potent compounds *ent*-**10** and **11** were evaluated at different concentrations. The all-*R* *ent*-**10** displayed a dose-dependent effect with an IC_{50} of 3 μM. The (2*S*,3*R*,4*R*) isomer **11**, possessing a stereochemical pattern analogous to that of the natural D-*ribo*-phytosphingosine, displayed a similar behaviour, whereas the natural phytosphingosine itself gave an IC_{50} of 15 μM in B16 cells.

Conclusions

The extension of our regio- and stereocontrolled epoxide opening procedures to long chain *cis* or *trans anti*-epoxyamines led to novel isomeric phytosphingosine analogues. The 3,4-*anti* series was accessed *via* acidic hydrolysis while the use of oxazolidine intermediates allowed preparation of the 3,4-*syn* analogues. Five different stereoisomers were thus prepared and *N*-functionalisation was also explored through the preparation of four *N*-hexyl and five *N*-octanoyl derivatives. Cell viability experiments showed that the amide series was inactive and that the secondary amines displayed only modest cytotoxicity. On the other hand, all the primary amines were revealed to be more active than the natural phytosphingosine with the most potent derivatives being the all-*R* and the (2*S*,3*R*,4*R*) isomers *ent*-**10** and **11**. The present work opens up prospects for further studies aiming at synthesizing other stereoisomers and elucidating the mode of action of these novel phytosphingosine-type derivatives.

Experimental section

General considerations

The following solvents and reagents were dried prior to use: CH_2Cl_2, MeOH, DMF (from calcium hydride), 1,2-dimethoxyethane, Et_2O, petroleum ether, THF, toluene (freshly distilled from sodium/benzophenone), Et_3N (from calcium hydride, stored over KOH pellets). Analytical thin layer chromatography (TLC) was performed using Merck silica gel $60F_{254}$ precoated plates. Chromatograms were observed under UV light and/or were visualised by heating plates that were dipped in 10% phosphomolybdic acid in EtOH or Dragendorff reagent. Column chromatography was carried out with SDS 35–70 mm flash silica gel. NMR spectroscopic data were obtained with Bruker Avance 300. Chemical shifts are quoted in parts per million (ppm) relative to residual solvent peak. *J* values are given in Hz. Infrared (IR) spectra were recorded on a Perkin–Elmer FT-IR 1725X spectrometer. Mass spectrometry (MS) data were obtained on a ThermoQuest TSQ 7000 spectrometer. High-resolution mass spectra (HRMS) were performed on a ThermoFinnigan MAT 95 XL spectrometer. LC–MS analyses and preparative HPLC purification were performed using a Waters Autopurif apparatus. Optical rotations were measured on a Perkin–Elmer model 241 spectrometer. Crystallographic data were collected at low temperature (193 K) on a Bruker SMART Apex II diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) by using phi- and omega-scans. Single crystals were mounted in inert oil and transferred to the cold gas stream of the diffractometer. The data were integrated with SAINT, and an empirical absorption correction with SADABS was applied.[27,28] The structures were solved by direct methods, using SHELXS-97[29] and refined using the least–squares method on F^2.[30] All non-H atoms were treated anisotropically. 3-(4,5-Dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide (MTT) was supplied from Euromedex. D-*ribo*-Phytosphingosine was purchased from Aventi Polar Lipids. Murine B16 melanoma cell line was purchased from American Type Culture Collection.

General procedures

General procedure for the epoxide hydrolysis. A 3 M aqueous H_2SO_4 solution (8.00 eq) was added to a solution of product (1.00 eq) in 1,4-dioxane (0.2 M). The mixture was refluxed for 4 h and allowed to cool down before neutralisation with 3 M aqueous NaOH solution (16.5 eq) followed by solid $NaHCO_3$. 1,4-Dioxane was then evaporated off under reduced pressure and the resulting aqueous phase extracted with EtOAc. The combined organic phases were successively washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure.

General procedure for the hydrogenolysis. 10% $Pd(OH)_2/C$ (20–30% w/w) and 12 N HCl aqueous solution (1–2 drops) were successively added to a solution of *N*-benzylamine (1.00 eq) in MeOH (0.1 M). The flask was purged with N_2 and then loaded with H_2 (10–12 bars). The mixture was stirred at room temperature until disappearance of the starting material (24–90 h). The catalyst was then removed by filtration through Celite® and the filtrate evaporated to dryness. The residue was taken up in 2 : 1 MeOH/water (25 mL $mmol^{-1}$) and Dowex 50WX8-200 ion-exchange resin (12.0 g $mmol^{-1}$) was added. After being stirred for 1 h, the resin was successively filtered and washed with water and MeOH. A 3 M ammonium hydroxide solution was then added (50 mL $mmol^{-1}$) and the suspension was stirred for 1 h before being filtered and rinsed with a 3 M ammonium hydroxide solution (500 mL $mmol^{-1}$). The resulting solution was evaporated to dryness under reduced pressure.

General procedure for the reductive amination. To aminotriol hydrochloride (prepared by treating the free base with a methanolic solution of HCl or obtained as the crude product of hydrogenolysis) (1.00 eq) in MeOH (0.07 M) at room temperature and under nitrogen atmosphere was added $NaBH_3CN$ (1.60 eq) and hexanal (1.00 eq). The mixture was stirred for 16 h after which it was diluted with water. The mixture was extracted three times with EtOAc and the combined extracts were washed with brine, dried over Na_2SO_4 and the solvent was evaporated *in vacuo*.

General procedure for the *N*-acylation. To a solution of aminotriol (1.00 eq) in anhydrous THF (0.03 M) at room temperature and under nitrogen atmosphere was added 4-nitrophenyl caprylate (2.00 eq). The mixture was stirred for 2 days after which the solvent was removed *in vacuo*.

Specific procedures and characterisation data

((2*R*,3*S*)-3-((*S*)-1-(Benzylamino)pentadecyl)oxiran-2-yl)methanol (5). TBAF on silica gel (6.97 g at *ca.* 1.25 mole of fluoride/g, *ca.* 8.71 mmol, *ca.* 2 eq) was added to a solution of **2** (2.69 g, 4.38 mmol) in anhydrous THF (44 mL). The reaction mixture

was vigorously stirred until TLC analysis showed no remaining starting material (*ca.* 16h) and then filtrated. Silica gel was rinsed several times with EtOAc (3 × 50 mL) and the combined filtrates concentrated *in vacuo*. The resulting crude product was purified by flash column chromatography on silica gel eluting with CH_2Cl_2/MeOH (98 : 2) to afford primary alcohol **5** (1.34 g, 81%) as a colourless oil. R_f = 0.26 (CH_2Cl_2/MeOH 95 : 5). $[\alpha]_D^{25}$ −13.4 (*c* 0.9, $CHCl_3$). **IR (neat)** 3435 (O–H), 1042 (C–O) cm^{-1}. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 0.85 (t, ^3J = 6.6 Hz, 3H), 1.10–1.48 (m, 24H), 1.52–1.84 (m, 2H), 2.37–2.44 (m, 1H), 2.91 (dd, ^3J = 8.6 Hz, ^3J = 4.1 Hz, 1H), 3.18–3.24 (m, 1H), 3.52 (dd, $^2J_{gem}$ = 11.0 Hz, ^3J = 6.9 Hz, 1H, H1) 3.69–3.73 (AB system, $^2J_{gem}$ = 12.4 Hz, $\delta a - \delta b$ = 17.7 Hz, 2H), 3.86 (dd, $^2J_{gem}$ = 11.0 Hz, ^3J = 5.2 Hz, 1H), 7.24–7.40 (m, 5H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 14.1, 22.6, 25.1, 29.3–29.7 (9C), 31.9, 32.5, 50.5, 55.4, 59.5, 60.9, 127.4, 128.3, 128.6, 139.0. **MS (ES)**: *m*/*z* = 390 (100%) [M + H$^+$]. **HRMS (ESI+)**: $C_{25}H_{44}NO_2$ calc. 390.3405, found 390.3372.

((2*S*,3*R*)-3-((*R*)-1-(Benzylamino)pentadecyl)oxiran-2-yl)methanol (*ent*-5). Compound *ent*-**5** was prepared in the same way as its enantiomer. It displayed identical analytical data expect for its optical rotation. $[\alpha]_D^{25}$ +12.6 (*c* 1.1, $CHCl_3$).

(2*S*,3*S*,4*S*)-4-(Benzylamino)octadecane-1,2,3-triol (6). Epoxyamine **5** (300 mg, 0.79 mmol) was reacted according to the general procedure for epoxide hydrolysis. The crude product was purified by flash column chromatography on silica gel (EtOAc/Ether/MeOH 85 : 10 : 5, 1.2% NH_4OH) to give **6** (210 mg, 66%). R_f = 0.10 (EtOAc/Ether/MeOH 85 : 10 : 5, 1.2% NH_4OH). $[\alpha]_D^{25}$ −6.3 (*c* 0.7, $CHCl_3$). **IR (neat)** 3400 (O–H), 1056 (C–O) cm^{-1}. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 0.85 (t, ^3J = 6.7 Hz, 3H), 1.05–1.29 (m, 24H), 1.35–1.54 (m, 2H), 2.86–2.92 (m, 1H), 3.74–3.89 (m, 6H) 7.28–7.33 (m, 5H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 14.1, 22.6, 26.2, 29.3, 29.4, 29.5, 29.6–29.7 (6C), 30.7, 31.9, 52.9, 60.8, 65.5, 70.3, 73.6, 127.2, 128.5, 128.6, 139.5. **MS (ES)**: *m*/*z* 408 (100) [M + H$^+$]. **HRMS (ESI+)**: $C_{25}H_{46}NO_3$ calc. 408.3478, found 408.3487

(2*R*,3*R*,4*R*)-4-(Benzylamino)octadecane-1,2,3-triol (*ent*-6). Compound *ent*-**6** was prepared in the same way as its enantiomer. It displayed identical analytical data expect for its optical rotation. $[\alpha]_D^{25}$ +6.1 (*c* 1.1, $CHCl_3$).

(2*S*,3*R*,4*R*)-4-(Benzylamino)octadecane-1,2,3-triol (7). *trans*-Epoxyamine **4** (507 mg, 0.81 mmol) was reacted according to the general procedure for epoxide hydrolysis. The crude product was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 95 : 5) to give **7** (180 mg, 65%). R_f = 0.18 (CH_2Cl_2/MeOH 95 : 5). $[\alpha]_D^{25}$ −14.2 (*c* 1.0, $CHCl_3$). **IR (neat)** 3376 (O–H), 1051 (C–O) cm^{-1}. **^{1}H NMR (300 MHz, MeOD + traces of $CDCl_3$)** δ 0.85 (t, ^3J = 6.7 Hz, 3H), 1.05–1.29 (m, 24H), 1.35–1.54 (m, 2H), 2.79–2.82 (m, 1H), 3.53–3.84 (m, 6H) 7.15–7.34 (m, 5H). **^{13}C NMR (75 MHz, MeOD + traces of $CDCl_3$)** δ 14.4, 23.1, 25.7, 29.7, 29.9, 30.2–30.5 (8C), 32.5, 51.8, 61.0, 63.7, 71.7, 74.5, 127.9, 128.9, 129.1, 139.7. **MS (ES)**: *m*/*z* 408 (100%) [M + H$^+$]. **HRMS (ESI+)**: $C_{25}H_{46}NO_3$ calc. 408.3478, found 408.3471. **Selected crystal data for 7**: $C_{25}H_{45}NO_3$, *M* = 407.62, Orthorhombic, space group $P2_12_12_1$, *a* = 4.9152(2) Å, *b* = 9.6665(4) Å, *c* = 50.7258(18) Å, *V* = 2410.12(16) Å^3, *Z* = 4, crystal size 0.42 × 0.10 × 0.04 mm^3, 25 744 reflections collected (4399 independent, R_{int} = 0.1026), 269 parameters, R_1 [$I > 2\sigma(I)$] = 0.0509, wR_2 [all data] = 0.1049, largest diff. peak and hole: 0.164 and –0.153 *e* Å^{-3}.

(4*R*,5*R*)-3-Benzyl-5-((*R*)-1,2-dihydroxyethyl)-4-tetradecyloxazolidin-2-one (8). To a solution of epoxyamine **5** (200 mg, 0.53 mmol) in a 4 : 1 THF/water mixture (2 mL) was added $(NH_4)_2CO_3$ (407 mg, 4.24 mmol, 8.00 eq) and the heterogeneous mixture was vigorously stirred at room temperature until TLC analysis showed disappearance of the starting material. The THF was then evaporated off under reduced pressure and the resulting aqueous phase extracted with Et_2O. The combined organic phases were successively washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 95 : 5) to give **8** (205 mg, 95%). R_f = 0.28 (CH_2Cl_2/MeOH 95 : 5). $[\alpha]_D^{25}$ +58.1 (*c* 1.1, $CHCl_3$). **IR (neat)** 3365 (O–H), 1728 (C═O) cm^{-1}. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 0.86 (t, ^3J = 6.9 Hz, 3H), 1.05–1.29 (m, 24H), 1.39–1.55 (m, 2H), 3.55–3.75 (m, 5H) 4.09 (d, ^2J = 15.5 Hz, 1H), 4.19–4.21 (m, 1H), 4.67 (d, ^2J = 15.5 Hz), 7.20–7.31 (m, 5H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 14.0, 22.6, 23.4, 29.3–29.6 (9C), 31.4, 31.9, 46.1, 56.1, 63.0, 72.5, 78.7, 127.7, 127.8, 128.6, 139.5, 158.3. **MS (ES)**: *m*/*z* 456 (100%) [M + Na$^+$]. **HRMS (ESI+)**: $C_{26}H_{43}NO_4Na$ calc. 456.3090, found 456.3083.

(4*R*,5*S*)-3-Benzyl-5-((*S*)-1,2-dihydroxyethyl)-4-tetradecyloxazolidin-2-one (*ent*-8). Compound *ent*-**8** was prepared in the same way as its enantiomer. It displayed identical analytical data expect for its optical rotation. $[\alpha]_D^{25}$ −57.6 (*c* 1.4, $CHCl_3$).

(2*R*,3*R*,4*S*)-4-(Benzylamino)octadecane-1,2,3-triol (9). NaOH (92.0 mg, 2.30 mmol, 10.0 eq) was added to a solution of oxazolidinone **8** (98.0 mg, 0.23 mmol) in a 8 : 2 EtOH/H_2O mixture (4.6 mL). The reaction mixture was heated at 85 °C for 6 h before being cooled to room temperature. The mixture was extracted three times with EtOAc and the combined extracts were concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 95 : 5) to give **9** (76 mg, 82%). R_f = 0.17 (CH_2Cl_2/MeOH 95 : 5). $[\alpha]_D^{25}$ −51.3 (*c* 0.8; $CHCl_3$). **IR (neat)** 3420 (O–H), 2892 (C–H) cm^{-1}. **^{1}H NMR (300 MHz, CD_3OD + traces of $CDCl_3$)** δ 0.87 (t, ^3J = 6.9 Hz, 3H), 1.19–1.45 (m, 24H), 1.59–1.74 (m, 2H), 2.83–2.89 (m, 1H), 3.59–3.62 (m, 2H), 3.64–3.72 (m, 2H), 3.82 (d, ^2J = 12.7 Hz), 4.03 (d, ^2J = 12.7 Hz, 1H), 7.24–7.38 (m, 5H). **^{13}C NMR (75 MHz, CD_3OD + traces of $CDCl_3$)** δ 14.4, 23.5, 27.0, 30.1, 30.2, 30.4–30.6 (7C), 32.8, 51.2, 61.6, 63.9, 69.6, 75.3, 128.8, 129.5, 129.6, 138.0. **MS (ES)**: *m*/*z* 408 (100%) [M + H$^+$]. **HRMS (ESI+)**: $C_{25}H_{46}NO_3$ calc. 408.3478, found 408.3439. **Selected crystal data for 9**: $C_{25}H_{45}NO_3$, *M* = 407.62, Monoclinic, space group $P2_1$, *a* = 10.2997(5) Å, *b* = 4.9870(3) Å, *c* = 24.6551(13) Å, β = 92.668(4)°, *V* = 1265.03(12) Å^3, *Z* = 2, crystal size 0.60 × 0.40 × 0.10 mm^3, 17 243 reflections collected (5095 independent, R_{int} = 0.0388), 321 parameters, R_1 [$I > 2\sigma(I)$] = 0.0545, wR_2 [all data] = 0.1557, largest diff. peak and hole: 0.200 and –0.170 *e* Å^{-3}.

(2*S*,3*S*,4*R*)-4-(Benzylamino)octadecane-1,2,3-triol (*ent*-9). Compound *ent*-**9** was prepared in the same way as its enantiomer. It displayed identical analytical data expect for its optical rotation. $[\alpha]_D^{25}$ +50.2 (*c* 0.8, $CHCl_3$).

(2*S*,3*S*,4*S*)-4-Aminooctadecane-1,2,3-triol (10). *N*-Benzyl amine **6** (34.0 mg, 0.09 mmol) was reacted according to the general procedure for hydrogenolysis. The crude product was purified by flash column chromatography on silica gel

(MeOH/EtOH/NH_4OH/CH_2Cl_2 3 : 12 : 6 : 79) to give **10** (24 mg, 95%). R_f = 0.22 (MeOH/EtOH/NH_4OH/CH_2Cl_2 12 : 12 : 6 : 70). $[\alpha]_D^{25}$ +2.3 (*c* 1.1, MeOH). **IR (neat)** 3426 (O–H), 2917 (C–H) cm^{-1}. **^{1}H NMR (300 MHz, CD_3OD + traces of $CDCl_3$)** δ 0.88 (t, ^3J = 6.3 Hz, 3H), 1.31–1.42 (m, 24H), 1.43–1.73 (m, 2H), 2.82–2.92 (m, 1H), 3.38 (dd, ^3J = 6.3 Hz, ^3J = 2.4 Hz 1H), 3.54–3.64 (m, 2H), 3.75–3.79 (m, 1H). **^{13}C NMR (75 MHz, CD_3OD)** δ 14.5, 23.8, 27.2, 30.5, 30.8–30.9 (7C), 31.1, 33.1, 34.1, 54.9, 64.9, 72.8, 74.5. **MS (ES)**: *m/z* 318 (100%) [M + H^+]. **HRMS (ESI+)**: $C_{18}H_{40}NO_3$ calc. 318.3008, found 318.3042.

(2*R*,3*R*,4*R*)-4-Aminooctadecane-1,2,3-triol (*ent*-10). Compound *ent*-**10** was prepared in the same way as its enantiomer. It displayed identical analytical data expect for its optical rotation. $[\alpha]_D^{25}$ −2.1 (*c* 1.0, MeOH).

(2*S*,3*R*,4*R*)-4-Aminooctadecane-1,2,3-triol (11). *N*-Benzyl amine **7** (76.5 mg, 0.09 mmol) was reacted according to the general procedure for hydrogenolysis. The crude product was purified by flash column chromatography on silica gel (MeOH/EtOH/NH_4OH/CH_2Cl_2 3 : 12 : 6 : 79) to give **11** (24.0 mg, 77%). R_f = 0.22 (MeOH/EtOH/NH_4OH/CH_2Cl_2 12 : 12 : 6 : 70). $[\alpha]_D^{25}$ +2.3 (c 1.1, MeOH). **IR (neat)** 3426 (O–H), 2917 (C–H) cm^{-1}. **^{1}H NMR** (300 MHz, CD_3OD) δ 0.88 (t, ^3J = 6.3 Hz, 3H), 1.31–1.42 (m, 24H), 1.43–1.73 (m, 2H), 2.88–2.94 (m, 1H), 3.42–3.46 (m, 1H), 3.54–3.63 (m, 2H), 3.68–3.76 (m, 1H). **^{13}C NMR** (75 MHz, CD_3OD) δ 14.4, 23.2, 26.6, 30.0–30.4 (9C), 33.1, 34.1, 54.5, 63.8, 73.8, 74.9. **MS (ES)**: *m/z* 318 (100%) [M + H^+]. **HRMS (ESI+)**: $C_{18}H_{40}NO_3$ calc. 318.3008, found 318.3024.

(2*R*,3*R*,4*S*)-4-Aminooctadecane-1,2,3-triol (12). *N*-Benzyl amine **9** (31.0 mg, 0.08 mmol) was reacted according to the general procedure for hydrogenolysis. The crude product was purified by flash column chromatography on silica gel (MeOH/EtOH/NH_4OH/CH_2Cl_2 3 : 12 : 6 : 79) to give **12** (15 mg, 62%). R_f = 0.27 (MeOH/EtOH/NH_4OH/CH_2Cl_2 12 : 12 : 6 : 70). $[\alpha]_D^{25}$ +4.2 (*c* 1.1, MeOH). **IR (neat)** 3425 (O–H), 2905 (C–H) cm^{-1}. **^{1}H NMR (300 MHz, CD_3OD)** δ 0.90 (t, ^3J = 6.4 Hz, 3H), 1.24–1.47 (m, 24H), 1.48–1.65 (m, 2H), 2.80–2.90 (m, 1H), 3.45 (dd, ^3J = 4.4 Hz, ^3J = 2.6 Hz, 1H), 3.57–3.63 (m, 2H), 3.65–3.72 (m, 1H). **^{13}C NMR (75 MHz, CD_3OD)** δ 14.5, 23.8, 27.2, 30.5, 30.8–30.9 (7C), 31.1, 33.1, 35.4, 54.6, 64.4, 73.5, 74.5. **MS (ES)**: *m/z* 318 (100%) [M + H^+]. **HRMS (ESI+)**: $C_{18}H_{40}NO_3$ calc. 318.3008, found 318.2997.

(2*S*,3*S*,4*R*)-4-Aminooctadecane-1,2,3-triol (*ent*-12). Compound *ent*-**12** was prepared in the same way as its enantiomer. It displayed identical analytical data expect for its optical rotation. $[\alpha]_D^{25}$ −4.1 (*c* 1.1, MeOH).

(2*S*,3*S*,4*S*)-4-(Hexylamino)octadecane-1,2,3-triol (13). Aminotriol hydrochloride **10** (51.0 mg, 0.14 mmol) was reacted according to the general procedure for reductive amination. The crude material was purified by column chromatography on silica gel eluted with EtOAc/MeOH 98 : 2 to give **13** (28.1 mg, 50%). R_f = 0.32 (EtOAc/MeOH 95/5). $[\alpha]_D^{25}$ −11.0 (*c* 1.5, MeOH). **IR (neat)** 3407 (O–H), 2925, 2853 (C–H) cm^{-1}. **^{1}H NMR (300 MHz, CD_3OD)** δ 0.88–1.00 (m, 6H), 1.28–1.44 (m, 36H) 2.62–2.73 (m, 2H), 2.74–2.82 (m, 1H), 3.56–3.72 (m, 3H), 3.77–3.84 (m, 1H).**^{13}C NMR (75 MHz, CD_3OD)** δ 13.1, 13.0, 22.4, 22.3, 25.7, 26.7, 29.1, 29.3, 29.4, 29.5, 29.6, 30.4, 31.6, 31.7, 47.9, 60.9, 63.2, 71.0, 71.7. **MS (ES)**: *m/z* = 402 (100%) [M + H^+]. **HRMS (ESI+)**: $C_{24}H_{52}NO_4$ calc. 402.3947, found 402.3987.

(2*R*,3*R*,4*R*)-4-(Hexylamino)octadecane-1,2,3-triol (*ent*-13). Compound *ent*-**13** was prepared in the same way as its enantiomer. It displayed identical analytical data expect for its optical rotation. $[\alpha]_D^{25}$ +12.3 (*c* 0.8, MeOH).

(2*R*,3*R*,4*S*)-4-(Hexylamino)octadecane-1,2,3-triol (14). Aminotriol hydrochloride **12** (35.6 mg, 0.10 mmol) was reacted according to the general procedure for procedure for reductive amination. The crude material was purified by column chromatography on silica gel eluted with EtOAc/MeOH 98 : 2 to give **14** (27.9 mg, 65%) as a white amorphous solid. R_f = 0.3 (EtOAc/MeOH 95 : 5). $[\alpha]_D^{25}$ −31.8 (*c* 1.2, MeOH). **IR (neat)** 3408 (O–H), 2924 (C–H) cm^{-1}. **^{1}H NMR (300 MHz, CD_3OD)** δ 0.84–1.04 (m, 6H), 1.26–1.70 (m, 36H) 2.44–2.62 (m, 1H), 2.66–2.76 (m, 1H), 2.76–2.94 (m, 1H), 3.58–3.68 (m, 3H), 3.70–3.82 (m, 1H). **^{13}C NMR (75 MHz, CD_3OD)** δ 14.4, 14.5, 23.7, 23.8, 27.4, 28.1, 30.5, 30.7, 30.8 (2 peaks), 31.0, 31.1, 31.3, 33.0, 33.1, 48.0, 62.0, 64.3, 70.7, 75.6. **MS (ES)**: *m/z* 402 (100%) [M + H^+]. **HRMS (ESI+)**: $C_{24}H_{52}NO_4$ calc. 402.3947, found 402.3965.

(2*S*,3*S*,4*R*)-4-(Hexylamino)octadecane-1,2,3-triol (*ent*-14). Compound ***ent*-14** was prepared in the same way as its enantiomer. It displayed identical analytical data expect for its optical rotation. $[\alpha]_D^{25}$ +29.4 (*c* 1.2, MeOH)

***N*-((2*S*,3*S*,4*S*)-1,2,3-Trihydroxyoctadecan-4-yl)octanamide (15).** Aminotriol **10** (31.0 mg, 0.10 mmol) was reacted according to the general procedure for *N*-acylation. The crude material was purified by column chromatography on silica gel eluted with EtOAc/MeOH 98 : 2 to give **15** (26.2 mg, 63%). R_f = 0.3 (EtOAc/MeOH 95/5). $[\alpha]_D^{25}$ +1.9 (*c* 1.0, MeOH). **IR (neat)** 3405 (O–H), 2851 (C–H), 1631 (C═O) cm^{-1}. **^{1}H NMR (300 MHz, CD_3OD)** δ 0.88–0.98 (m, 6H), 1.24–1.54 (m, 34H) 1.58–1.74 (m, 2H), 2.26 (t, ^3J = 7.2 Hz, 2H), 3.34–3.36 (m, 1H), 3.60–3.65 (m, 3H), 3.86–3.93 (m, 1H). **^{13}C NMR (75 MHz, CD_3OD)** δ 14.5, 23.8 (2 peaks), 27.3 (2 peaks), 30.3, 30.4, 30.5, 30.6, 30.8 (3 peaks), 31.7, 33.0, 33.1, 37.2, 52.6, 64.4, 72.2, 74.2, 177.2. **MS (ES)**: *m/z* 466 (100%) [M + Na^+]. **HRMS (ESI+)**: $C_{26}H_{53}NO_4Na$ calc. 466.3872, found 466.3896.

***N*-((2*R*,3*R*,4*R*)-1,2,3-Trihydroxyoctadecan-4-yl)octanamide (*ent*-15).** Compound *ent*-**15** was prepared in the same way as its enantiomer. It displayed identical analytical data expect for its optical rotation. $[\alpha]_D^{25}$ −1.6 (*c* 1.2, MeOH).

***N*-((2*R*,3*R*,4*S*)-1,2,3-Trihydroxyoctadecan-4-yl)octanamide (16).** Aminotriol **12** (33.0 mg, 0.10 mmol) was reacted according to the general procedure for *N*-acylation. The crude material was purified by column chromatography on silica gel eluted with EtOAc/MeOH 98 : 2 to give **16** (28.4 mg, 62%) as a white amorphous solid. R_f = 0.3 (EtOAc/MeOH 95 : 5). $[\alpha]_D^{25}$ +7.9 (*c* 1.1, MeOH). **IR (neat)** 3405 (O–H), 2919 (C–H), 1631 (C═O) cm^{-1}. **^{1}H NMR (300 MHz, CD_3OD)** δ 0.84–0.94 (m, 6H), 1.24–1.42 (m, 34H), 1.50–1.70 (m, 2H), 2.21 (t, ^3J = 7.2 Hz, 2H), 3.46–3.58 (m, 2H), 3.59–3.68 (m, 2H), 3.94–4.03 (m, 1H). **^{13}C NMR (75 MHz, CD_3OD)** δ 14.5, 23.8 (2 peaks), 27.3 (2 peaks), 30.3, 30.4, 30.5, 30.6, 30.8 (3 peaks), 33.0, 33.1, 33.2, 37.3, 52.1, 64.4, 73.7, 74.0, 176.3. **MS (ES)**: *m/z* 466 (100%) [M + Na^+]. **HRMS (ESI+)**: $C_{26}H_{53}NO_4Na$ calc. 466.3872, found 466.3896.

***N*-((2*S*,3*S*,4*R*)-1,2,3-Trihydroxyoctadecan-4-yl)octanamide (*ent*-16).** Compound *ent*-**16** was prepared in the same way as its enantiomer. It displayed identical analytical data expected for its optical rotation. $[\alpha]_D^{25}$ −6.5 (*c* 1.3, MeOH).

***N*-((2*S*,3*R*,4*R*)-1,2,3-Trihydroxyoctadecan-4-yl)octanamide (17).** Aminotriol **11** (18.7 mg, 0.10 mmol) was reacted according to the general procedure for *N*-acylation. The crude material was purified by column chromatography on silica gel eluted with EtOAc/MeOH 98 : 2 to give **17** (16.1 mg, 60%). R_f = 0.3 (EtOAC/MeOH 95 : 5). $[\alpha]_D^{25}$ +10.3 (*c* 0.6, MeOH). **IR (neat)** 3405 (O–H), 2851 (C–H), 1631 (C═O) cm^{-1}. **^{1}H NMR (300 MHz, CD_3OD)** δ 0.88–0.99 (m, 6H), 1.24–1.52 (m, 34H) 1.58–1.74 (m, 2H), 2.32 (t, ^3J = 7.2 Hz, 2H), 3.32–3.38 (m, 1H), 3.60–3.65 (m, 3H), 3.86–3.93 (m, 1H). **^{13}C NMR (75 MHz, CD_3OD)** δ 14.6, 23.8 (2 peaks), 27.3 (2 peaks), 30.3, 30.4, 30.5, 30.6, 30.8 (3 peaks), 31.7, 33.0, 33.1, 37.2, 52.6, 64.4, 72.2, 74.2, 177.2. **MS (ES)**: *m*/*z* = 444 (100%) [M + H^+]. **HRMS (ESI+)**: $C_{26}H_{54}NO_4$ calc. 444.4053, found 444.4060.

***N* - ((2*S*,3*S*,4*R*) - 1,3,4 - Trihydroxyoctadecan - 2 - yl)octanamide (18).** D-*ribo*-Phytosphingosine (18.7 mg, 0.10 mmol) was reacted according to the general procedure for *N*-acylation. The crude material was purified by column chromatography on silica gel eluted with EtOAc/MeOH 98 : 2 to give **18** (16.1 mg, 60%). R_f = 0.3 (EtOAc/MeOH 95 : 5). $[\alpha]_D^{25}$ +8.9 (*c* 1.1, MeOH). **^{1}H NMR (300 MHz, CD_3OD)** δ 0.88–0.99 (m, 6H), 1.24–1.52 (m, 34H) 1.58–1.74 (m, 2H), 2.32 (t, ^3J = 7.2 Hz, 2H), 3.32–3.38 (m, 1H), 3.60–3.65 (m, 3H), 3.86–3.93 (m, 1H). **^{13}C NMR (75 MHz, CD_3OD)** δ = 14.5, 23.8 (2 peaks), 27.1, 27.2, 30.3, 30.4, 30.5, 30.6, 30.8, 30.9 (2 peaks), 33.0, 33.1 (2 peaks), 37.3, 53.6, 62.2, 73.4, 76.0, 176.1. **MS (ES)**: *m*/*z* 444 (100%) [M + H^+]. **HRMS (ESI+)**: $C_{26}H_{54}NO_4$ calc. 444.4053, found 444.4051.

Biological evaluations

Cell viability experiments. Murine B16 melanoma cells were grown in a humidified 5% CO2 atmosphere at 37 °C in DMEM medium containing Glutamax (2 mM), and heat-inactivated FCS (10%). All compounds were added to the cells as ethanolic solution. Control cells were treated with the same concentration of solvent (which did not exceed 0.5%). After treatment with the compounds for 24 h in the absence of FCS, viability of murine B16 melanoma cells was evaluated by using the MTT assay based on the cleavage of the tetrazolium salt MTT to formazan crystals by metabolically active cells.[31] The formazan crystals formed were solubilised by adding dimethylsulfoxide for 1 h at 37 °C and quantified spectrophotometrically using an ELISA reader (the absorbance was measured at λ = 560 nm).

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