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Stereoselective synthesis and antiproliferative activity of the isomeric sphinganine analogues

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ABSTRACT

A flexible synthetic approach to biologically active sphingoid base-like compounds with a 3-amino-1,2-diol framework was achieved through a [3,3]-sigmatropic rearrangement and late stage olefin cross-metathesis as the key transformations. The stereochemistry of the newly created stereogenic centre was assigned via a single crystal X-ray analysis of the (4*S*,5*R*)-5-(hydroxymethyl)-4-vinyloxazolidine-2-thione. In order to rationalise the observed stereoselectivity of the aza-Claisen rearrangement, DFT calculations were carried out. The targeted isomeric sphingoid bases were screened *in vitro* for anticancer activity on a panel of seven human malignant cell lines. Cell viability experiments revealed that C_{17}-homologues are more active than their C_{12} congeners.

1. Introduction

D-*erythro*-Dihydrosphingosine **1** [1] (also known as sphinganine, Fig. 1) represents an earlier intermediate in the *de novo* biosynthesis [1b,2] of the more complex mammalian groups of sphingolipids, such as sphingomyelins and glycosphingolipids, as well as being identified as the structural backbone of the aforementioned classes in at least small amounts. Sphinganine **1** (Fig. 1), together with the unsaturated widespread D-*erythro*-sphingosine, inhibits protein kinase C (PKC) [3]. It was found that **1** and its unnatural D/L-*threo*-isomers are well-known sphingosine kinase inhibitors [4]. Furthermore, L-*threo*-dihydrosphingosine **2** (safingol) exhibits antineoplastic and antipsoriatic properties [5]. It is known that safingol possesses remarkable *in vitro* anticancer activity and acts synergistically with various chemotherapeutic agents [6]. Cammue at al [7a]. revealed the fungicidal activity of the truncated C_{15}— and C_{17}-homologues of **1** against *Candida albicans* and *Candida glabrata*, where both synthetic molecules were found to be more potent than native dihydrosphingosine **1**. Devi's group [7b] also showed promising antifungal and antimicrobial activities of D-*erythro*-congener of the natural sphinganine **1** with a C_{12} carbon backbone and its several 1,2,3-triazole derivatives. A naturally occurring L-*erythro*-C_{12} analogue of **1**, referred to as clavaminol H **3**, was isolated from the Mediterranean ascidian *Clavelina phlegraea* by Menna's group [8] in 2009, and its deacetylated form **4** demonstrates *in vitro* cytotoxic [8,9] and antibacterial properties [9]. Surprisingly, **3** exhibited none of the aforementioned activities [8,9]. These results indicate that the liberated vicinal amino-alcohol motif is indeed important for the biological profile of such compounds. Due to the promising activities associated with the relatively simple structural features, numerous synthetic methods towards **1** [10,11], **2** [10,11a,11f,11j,11n,12], **3** [9,11h,13-14] and **4** [9,11h,13a,14-16] have been reported. The elaborated methodologies have utilized both the Chiron Approach, as well as asymmetric construction. Our ongoing interest in the total synthesis of bioactive sphingolipid structures and their analogues [17] has stimulated us to develop a convenient strategy leading to the construction of the isomeric congeners of **1** and/or **4** (see compounds **5–8** in Scheme 1) in order to obtain new derivatives for evaluation of their anticancer profile, with the aim of searching for chemical entities with potential therapeutic use. Besides the study of Génisson's group [18] oriented towards “isophytosphingosines” and the work of Kokotos and coworkers [19a], which has reported the remarkable *in vitro* cytotoxicity of **6** [19a] (Scheme 1) and its congeners on a panel of six different cancer cell lines (A-2780, H-322, WiDr, UMSCC-22B, LL and C26-10), little is known about the structural-activity relationships in this novel group of the isomeric sphingoid bases, the general structure of which is depicted in Fig. 1. With these factors in mind, we recently undertook the synthesis and biological evaluation of **6** and **8** (Scheme 1) along with their antipodes from D-isoascorbic acid [19b]. The remarkable

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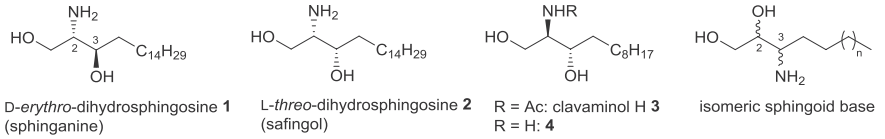
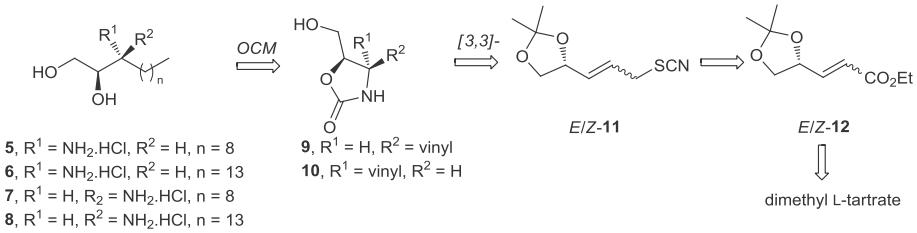


Fig. 1. Dihydrosphingosine **1**, its analogues **2–4** and the general structure of isomeric sphinganines.



Scheme 1. Retrosynthetic analysis of isomeric sphingoid bases **5–8**.

antiproliferative/cytotoxic potency of these aforementioned compounds has prompted us to develop a more economic approach towards them and their truncated analogues **5** and **7**, which are expected to have promising antifungal[7a,b] and/or antimicrobial activity [7b].

2. Results and discussion

2.1. Chemistry

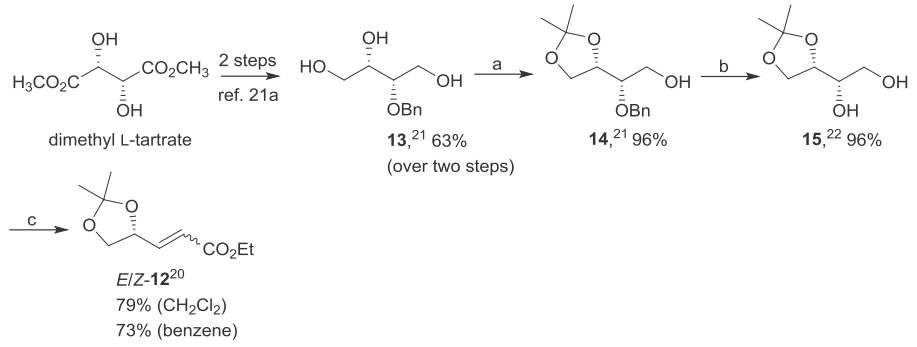
The retrosynthetic strategy towards **5–8** is delineated in Scheme 1. We planned to prepare our targeted molecules **5–6** and **7–8** from the common oxazolidinones **9** and **10**, respectively. The required long chain segments could be installed in **9** and **10** via Grubbs' cross metathesis chemistry. It was anticipated that cyclic carbamates **9** and **10** can be obtained by subjecting the allylic substrate *E/Z*-**11** to the [3,3]-sigmatropic rearrangement, which would be expected to stereoselectively create the new C—N bond. Thiocyanates *E/Z*-**11** can be prepared from the corresponding esters *E/Z*-**12**, which were envisioned as arising from dimethyl L-tartrate. It should be noted that both derivatives (*E*)-**12** and (*Z*)-**12** are commercially available compounds. Seeing that they are too costly, we decided to prepare these isomers. Our initial attempts to prepare **12** according to Hubschwerlen's original protocol [20], which uses L-ascorbic acid as the starting material, turned out to be ineffective. The corresponding esters **12** were isolated in a maximum overall yield of about 10%. L-Tartrate was then investigated as an alternative and proved to be a viable starting material.

As seen in Scheme 2, our synthesis commenced with a gram-scale preparation of the known protected L-threitol **13** [21] (63%, over two steps). The obtained product **13** had NMR data, optical rotation and a melting point in agreement with those reported in the literature [21b]. Exposure of **13** to acetone in the presence of *p*-TsOH and 4 Å molecular sieves resulted in the formation of the acetonide **14** [21] in a 96% yield. After deprotection of the *O*-benzyl group in **14**, the obtained diol **15** [22] (96%) was subjected to oxidative fragmentation ($NaIO_4$, CH_2Cl_2) followed by the Wittig reaction using a stable ylide reagent to afford a separable mixture of the α,β-unsaturated esters (*E/Z*)-**12** [20].

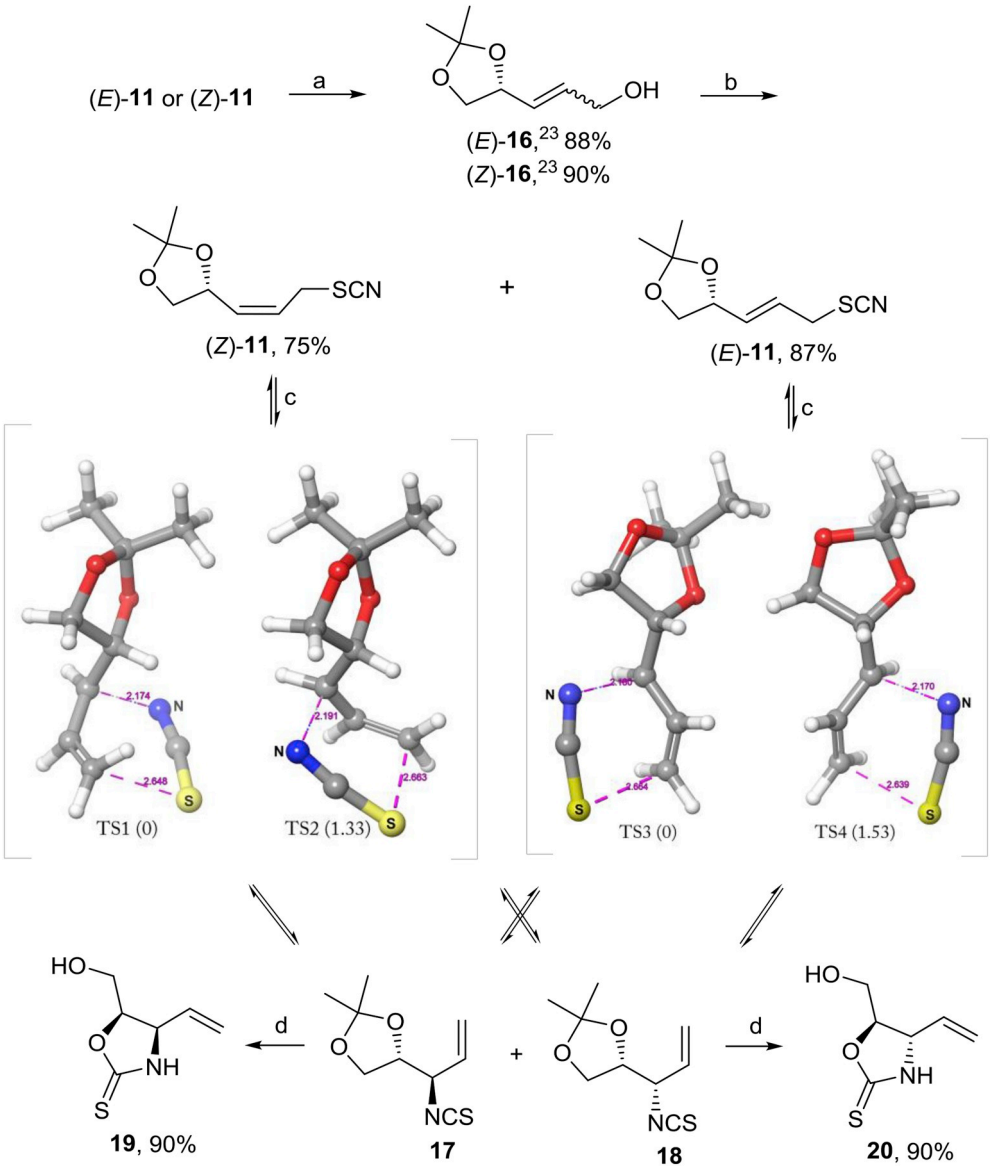
The aforementioned olefination was carried out either in CH_2Cl_2 at room temperature (*E*:*Z* = 40:60, combined yield of 79% over two steps) or in benzene at reflux (*E*:*Z* = 72:28, 73%); their structures including configuration of the double bonds, were determined by NMR analysis (J_{trans} = 15.6 Hz, J_{cis} = 11.6 Hz). After chromatographic separation, the subsequent DIBAL-H reduction of (*E*)-**12** and (*Z*)-**12** gave the corresponding alcohols (*E*)-**16** [23] and (*Z*)-**16** [23] in 88% and 90% isolated yields, respectively (Scheme 3). To continue the synthesis, a two-step sequence involving activation of the hydroxyl group of (*E*)-**16** and (*Z*)-**16** with MsCl and followed by KSCN treatment, furnished the desired thiocyanates (*E*)-**11** (87%) and (*Z*)-**11** (75%).

With the substrates (*E*)-**11** and (*Z*)-**11** in hand, our focus then shifted to the [3,3]-sigmatropic rearrangement to create the vicinal amino alcohol unit. The key aza-Claisen rearrangement of both thiocyanates **11** took place either at 70 °C or at 90 °C in *n*-heptane using the conventional thermal conditions as well as microwave heating and provided the corresponding isothiocyanates **17** and **18** as a separable mixture of diastereoisomers in good yields (Table 1). During these experiments we also recovered the starting material, either (*E*)-**11** or a mixture of isomers (*E*:*Z* ~ 64:32) in approximately 7–32% yields. As shown in Table 1, the best *anti*-**17**/*syn*-**18** ratio was achieved using the thermally driven reaction at 70 °C in the case of (*Z*)-**11** (entries 9 and 10).

In order to rationalise the observed stereoselectivity in the [3,3]-sigmatropic rearrangement of thiocyanates (*E*)-**11** and (Z)-**11**, high-level density functional theory (DFT) calculations [24], including electron correlation effects, were carried out. A thorough conformational search was performed on all transition states, though only the lowest energy conformers are discussed here. The rearrangements of (*Z*)-**11** and (*E*)-**11** occur via transition states TS1/TS2 and TS3/TS4, respectively (Scheme 3). The calculated diastereoisomeric ratio of **17**:**18** was 85:15 for the rearrangement of (*Z*)-**11** and 90:10 for (*E*)-**11** (both at 90 °C). These results are in relatively good agreement with the experimental data (see Table 1), with the correct prediction of isothiocyanate **17** as the major diastereoisomer.



Scheme 2. *Reagents and conditions:* (a) acetone, *p*-TsOH, 4 Å MS, rt; (b) H_2, 10% Pd/C/20% $Pd(OH)_2$/C (2:1), EtOH, rt; (c) (i) $NaIO_4$, CH_2Cl_2, rt; (ii) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2, rt or $Ph_3P{=}CHCO_2Et$, benzene, reflux.



Scheme 3. Relative energies of transition states (in kcal/mol) and bond distances (in Å) are shown.
Reagents and conditions: (a) DIBAL-H, CH_2Cl_2, –50 °C; (b) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C; (ii) KSCN, MeCN, rt; (c) Table 1; (d) *p*-TsOH, MeOH, rt.

Table 1
[3,3]-Sigmatropic rearrangement of thiocyanates (*E*)-**11** and (*Z*)-**11**.

Entry	Thiocyanate	Conditions[a]	Time (h)	Ratio[b] 17:18	Yield[c] (%)	Isolated thiocyanates (%)
1	(*E*)-**11**	Δ, 90 °C	0.5	70:30	81	12[d]
2	(*E*)-**11**	Δ, 90 °C	2	66:34	80	7[d]
3	(*E*)-**11**	MW, 90 °C	0.4	65:35	81	9[d]
4	(*E*)-**11**	Δ, 70 °C	6	75:25	88	8[d]
5	(*E*)-**11**	Δ, 70 °C	3	72:28	84	14[d]
6	(*E*)-**11**	MW, 70 °C	1	70:30	70	19[d]
7	(*Z*)-**11**	Δ, 90 °C	2	83:17	79	14[e]
8	(*Z*)-**11**	MW, 90 °C	1	82:18	81	12[e]
9	(*Z*)-**11**	Δ, 70 °C	6	89:11	71	22[e]
10	(*Z*)-**11**	Δ, 70 °C	4	89:11	64	32[e]
11	(*Z*)-**11**	MW, 70 °C	4	80:20	60	27[e]

[a] In *n*-heptane.
[b] Ratio in the crude reaction mixtures determined by ^{1}H NMR.
[c] Isolated combined yields.
[d] Isolated (*E*)-**11**.
[e] Isolated a mixture of thiocyanates (*E*/*Z*)-**11** (*E*:*Z* ~ 64:32). Determined by ^{1}H NMR analysis.

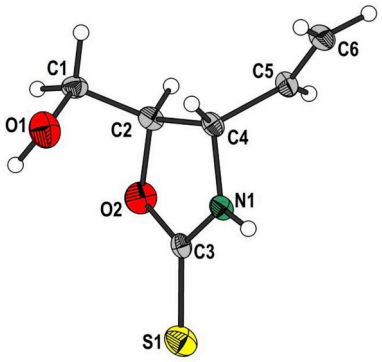


Fig. 2. ORTEP structure of **20** showing the crystallographic numbering.

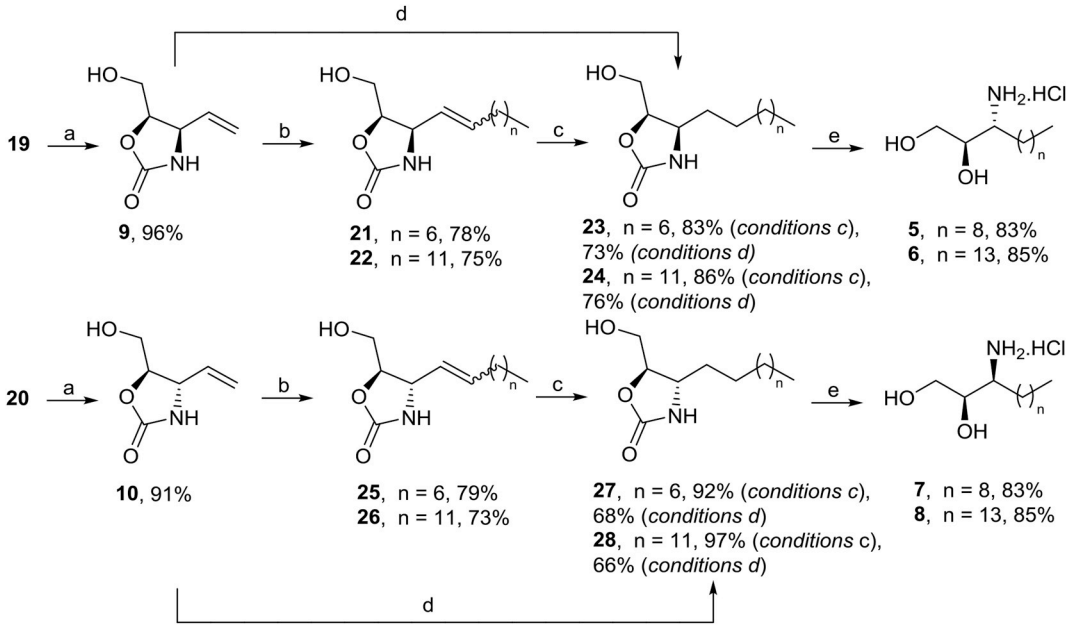
The configuration of the newly generated stereocentre in both aza-Claisen products **17** and **18** was determined by their transformation to oxazolidine-2-thiones **19** (90%) and **20** (90%), respectively. This modification involved the acid hydrolysis of the protecting isopropylidene group, followed by the spontaneous intramolecular cyclization providing derivatives **19** and **20** as crystalline compounds (Scheme 3). The crystallographic analysis of thiocarbamate **20** confirmed its (4*S*,5*R*)-stereochemistry (Fig. 2), revealing that the minor *syn*-diastereoisomer **18** possesses the same configuration.

The stage was now set for the completion of construction of the targeted isomeric sphinganine analogues **5**–**8**. After treatment of **19** and **20** with mesitylnitrile oxide (MNO) in acetonitrile, the incorporation of the long alkyl chains into the carbamates **9** and **10** was achieved via cross-metathesis using Grubbs' second generation catalyst. The coupling reaction of **9** with the commercial non-1-ene and tetradec-1-ene in refluxing CH_2Cl_2 resulted in the production of the corresponding mixtures of alkenes **21** and **22** in 78% and 75% combined yields, respectively (Scheme 4). Because of overlap of the proton signals in the crude aforementioned mixtures in both the $CDCl_3$ and C_6D_6 solutions, it was not possible to determine the corresponding ratio of the geometric isomers. On the other hand, their repeated column chromatography allowed the pure major isomers (*E*)-**21** and (*E*)-**22** to be isolated. The subsequent catalytic hydrogenation of **21** and **22** was performed in the presence of 5% Rh/Al_2O_3 [25] to furnish the corresponding saturated derivatives **23** (83%) and **24** (86%). With the aim of being practical, we tried to obtain **23** and **24** through a more economic sequence using the tandem OCM/transfer hydrogenation [26]. When oxazolidinone **9** was subjected to Grubbs' catalyst-mediated cross metathesis with the corresponding alkenes (non-1-ene or tetradec-1-ene), followed by $NaBH_4$ addition, products **23** and **24** were isolated in 73% and 76% yields, respectively. On the other hand, the two-stage process mentioned above led to their lower yields (to both 65% over two steps). In the case of **27** and **28** (Scheme 4), they were obtained in lower, but satisfying amounts when compared with a two-step sequence (73% for **27** and 71% for **28** over two steps). Finally, the treatment of **23** and **24** with 6 M HCl resulted in the production of the isomeric sphinganines **5** and **6** as HCl salts in 83% and 85% isolated yields, respectively.

In a parallel fashion, the minor oxazolidinone **10** was elaborated into the corresponding analogues **7** and **8** (Scheme 4). The spectroscopic and optical rotation data of **6** and **8** were in very good agreement with the values reported in the literature for the same compounds [19b].

2.2. Antiproliferative/cytotoxic activity

The final compounds **5** and **7** and also several intermediates of our synthetic route (derivatives **17**, **18**, (*E*)-**21** and (*E*)-**22**) were screened for their antiproliferative/cytotoxic activities against seven different human cancer cell lines – MDA-MB-231 (mammary gland adenocarcinoma), A-549 (non-small cell lung cancer), MCF-7 (mammary gland adenocarcinoma), Caco-2 (human colon carcinoma), HCT-116 (human colon carcinoma), HeLa (cervical adenocarcinoma), Jurkat (human acute T-lymphoblastic leukaemia), and a non-malignant cell line NiH 3T3 (mouse fibroblasts), using MTT assay with triplicate experiments [27]. The commercially available anticancer substance cisplatin was included as a positive control, and the obtained results are summarised in Table 2. Isothiocyanate **17** exhibited selective antiproliferative potency against the MDA-MB-231 cell line with an IC_{50} value comparable to those of cisplatin. To allow comparison, Table 2 also includes IC_{50} values for **6** and **8**, which were prepared in our laboratory in a parallel fashion [19b]. Whereas for compounds **5** and **7** cytotoxicity was significantly reduced, isomeric sphinganines **6** and **8** displayed remarkable activity for all tested cancer cell lines, revealing that antiproliferative/cytotoxic profile was highly dependent on the chain length. In addition,



Scheme 4. *Reagents and conditions:* (a) MNO, MeCN, rt; (b) Grubbs II, non-1-ene or tetradec-1-ene, CH_2Cl_2, reflux; (c) H_2, 5% Rh/Al_2O_3, EtOAc or MeOH, rt; (d) Grubbs II, non-1-ene or tetradec-1-ene, CH_2Cl_2, reflux, then $NaBH_4$, MeOH, rt; (e) 6 M aq HCl, EtOH, 100 °C.

Table 2
Antiproliferative activities of compounds **5–8**, **17**, **18**, (*E*)-**21**, (*E*)-**22** on seven human cancer cell lines (MDA-MB-231, A-549, MCF-7, HCT-116, Caco-2, HeLa and Jurkat) and non-malignant mouse fibroblasts NiH 3T3.

Compd no.	Cell line, IC_{50}[a] ± SD (μmol × L^{-1})							
	MDA-MB-231	A-549	MCF-7	HCT-116	Caco-2	HeLa	Jurkat	NiH 3T3
17	<10	68.3 ± 16.9	> 100	51.3 ± 11.7	15 ± 6.8	71.8 ± 5.9	26 ± 5.4	53.5 ± 16.9
18	> 100	72.1 ± 14.6	> 100	65.8 ± 10.5	73.3 ± 16	≥100	36 ± 0.5	> 100
(*E*)-**21**	81.8 ± 9.8	73.7 ± 5.8	75.4 ± 12.4	73.6 ± 0.3	70.5 ± 11.2	79.5 ± 13.2	67.3 ± 10.9	71.1 ± 9.9
(*E*)-**22**	> 100	44.2 ± 0.9	56 ± 10.3	36.5 ± 4.9	31.1 ± 7.1	40.8 ± 1.8	> 100	81.2 ± 14.1
5	75.8 ± 4.6	43.6 ± 15.3	78.3 ± 5.2	56.5 ± 18.2	32.5 ± 4.1	85.2 ± 14.9	53.8 ± 1.1	36.5 ± 11.4
6 [19b]	7.1 ± 0.9	8.4 ± 0.5	7.7 ± 0.1	7.3 ± 0.6	3.3 ± 2.3	7.2 ± 1.5	7 ± 1.3	7.6 ± 0.1
7	81.8 ± 7.1	33.2 ± 20	79.6 ± 5.1	72.8 ± 23.3	56.5 ± 2.2	> 100	59.5 ± 3.1	23.2 ± 1.9
8 [19b]	6.3 ± 1.6	3.7 ± 2.1	7.3 ± 0.1	1.4 ± 1.3	1.7 ± 1.2	4.9 ± 0.2	0.7 ± 0.1	0.7 ± 0.1
cisplatin	17.5 ± 0.5	9.5 ± 0.2	15.6 ± 0.3	15.3 ± 0.5	15.2 ± 0.3	13.1 ± 0.2	16.2 ± 0.6	20.87 ± 0.3

[a] The potency of compounds was determined using MTT assay after 72 h incubation of cells and given as IC_{50} (concentration of a tested compound that decreased number of viable cells to 50% relative to untreated control cells, see Section 4.2).

isodihydrosphingosine analogue **8** is significantly more potent on the HCT-116 and Jurkat cell lines than derivative **6**, whose IC_{50} values on these cells are at least 5 and 10 × higher, respectively (Table 2). The low potency of alkene (*E*)-**22** relative to compound **6** or **8** emphasizes the importance of the liberated vicinal amino-alcohol unit. Unsaturation of the carbon backbone can also be detrimental for the cytotoxicity in such series of compounds [8,28].

3. Conclusion

In summary, we have accomplished the synthesis of isomeric sphinganine analogues **5–8** starting from dimethyl L-tartrate. The key transformation of our approach was the implementation of an amino-bearing asymmetric centre through the [3,3]-sigmatropic rearrangement of thiocyanates **11**. In order to rationalise the stereochemical outcome of the realised rearrangements, DFT calculations were carried out. The alkyl chain of the common oxazolidinones **9** and **10** was extended via olefin cross-metathesis, thus completing the construction of the targeted molecules. The several newly prepared compounds including final isomeric sphingoid bases **5** and **7**, were screened *in vitro* for antiproliferative/cytotoxic activities against seven human cancer cell lines. Cell viability experiments revealed that C_{17}-homologues are more active than their C_{12} congeners. On the other hand, the short-chain analogues **5** and **7** were chosen as promising candidates for evaluation of their antifungal and/or antimicrobial activity. Biological screening is currently underway, and results will be reported in due course.

4. Experimental section

4.1. General methods

All commercial reagents were used in the highest available purity from Aldrich or Acros Organics without further purification. Solvents were dried and purified before use according to standard procedures. For flash column chromatography on silica gel, Kieselgel 60 (0.040–0.063 mm, 230–400 mesh, Merck) was used. Solvents for flash chromatography (hexane, ethyl acetate, methanol, dichloromethane) were distilled before use. Thin layer chromatography was run on Merck silica gel 60 F_{254} analytical plates; detection was carried out with either ultraviolet light (254 nm), or spraying with a solution of phosphomolybdic acid, a basic potassium permanganate solution, or a solution of concentrated H_2SO_4, with subsequent heating. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and CD_3OD on a Varian Mercury Plus 400 FT NMR (400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C) spectrometer. For ^{1}H, δ are given in parts per million (ppm) either relative to TMS (δ = 0.0) as the internal standard or to the solvent signals CD_3OD (δ = 4.84 or δ = 3.31) and for ^{13}C relative to $CDCl_3$ (δ = 77.00) and CD_3OD (δ = 49.05). The multiplicity of the ^{13}C NMR signals concerning the ^{13}C—^{1}H coupling was determined by the HSQC method. Chemical shifts (in ppm) and coupling constants (in Hz) were obtained by first-order analysis; assignments were derived from COSY and H/C correlation spectra. Infrared (IR) spectra were measured with a Nicolet 6700 FT-IR spectrometer and expressed in ν values (cm^{-1}). High-resolution mass spectra (HRMS) were recorded on a micrOTOF-Q II quadrupole-time of flight hybrid mass spectrometer (Bruker Daltonics). Optical rotations were measured on a P-2000 Jasco polarimeter and reported as follows: $[\alpha]_D$ (*c* in grams per 100 mL, solvent). Melting points were recorded on a Kofler hot block, and are uncorrected. Microwave reactions were carried out on a focused microwave system (CEM Discover). The temperature content of the vessel was monitored using a calibrated infrared sensor mounted under the vessel. At the end of all reactions the contents of the vessel were cooled rapidly using a stream of compressed air. Small quantities of reagents (μL) were measured with appropriate syringes (Hamilton). All reactions were performed under an atmosphere of nitrogen, unless otherwise noted.

4.2. (S)-2-(Benzyloxy)-2-[(S)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]ethan-1-ol (14) [21]

To a stirring solution of **13** [21] (2.0 g, 9.42 mmol) in dry acetone (518 mL) were successively added 4 Å molecular sieves (2 g) and *p*-TsOH (0.717 g, 3.77 mmol) at room temperature. After 12 h, the solid K_2CO_3 (1.7 g) was added, and the mixture was stirred for another 2 h. Filtration through a small pad of Celite, followed by evaporation of the solvent left a residue that was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate, 3:1). This procedure yielded 2.27 g (96%) of compound **14** as a colourless oil; $[\alpha]_D^{27}$ −21.9 (*c* 1.6, $CHCl_3$); lit [21a]. $[\alpha]_D$ −17.0 (*c* 0.2, $CHCl_3$, temperature 21–24 °C); lit [21b]. $[\alpha]_D^{20}$ −17.5 (*c* 1.08, $CHCl_3$); IR (neat cm^{-1}) 3447, 2985, 2933, 2880, 1454, 1370, 1253, 1210, 1156, 1048, 1027 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.37 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 2.26 (br s, 1H, OH), 3.53–3.63 (m, 2H, H-1, H-2), 3.68–3.76 (m, 1H, H-1), 3.81 (dd, 1H, *J* = 7.0, 8.4 Hz, H-5′), 4.01 (dd, 1H, *J* = 6.6, 8.4 Hz, H-5′), 4.25–4.34 (m, 1H, H-4′), 4.69 (d, 1H, *J* = 11.8 Hz, OCH_2Ph), 4.77 (d, 1H, *J* = 11.8 Hz, OCH_2Ph), 7.26–7.38 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.3 (CH_3), 26.4 (CH_3), 61.7 (C-1), 65.4 (C-5′), 72.7 (OCH_2Ph), 76.6 (C-4′), 79.2 (C-2), 109.4 (C_q), 127.8 (3 × CH_{Ph}), 128.4 (2 × CH_{Ph}), 138.2 (C_i). ESI-HRMS: *m/z* calcd for $C_{14}H_{21}O_4$ $[M + H]^+$ 253.143, found 253.141.

4.3. (S)-1-[(4′S)-2′,2′-Dimethyl-1′,3′-dioxolan-4′-yl]ethane-1,2-diol (15) [22]

To a solution of **14** [21] (5.70 g, 22.6 mmol) in dry EtOH (180 mL) was added a mixture of catalysts (10% Pd/C/20% $Pd(OH)_2$/C, 2.4 g, 2:1). The resulting suspension was stirred at room temperature for 2 h

under an atmosphere of hydrogen, then filtered through a small pad of Celite and concentrated. Chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 1:2) furnished 3.51 g (96%) of compound **15** as a colourless oil; $[\alpha]_D^{25}$ +2.7 (*c* 0.78, $CHCl_3$); lit [22]. $[\alpha]_D^{20}$ +2.1 (*c* 1.0, $CHCl_3$). IR (neat cm^{-1}) 3404, 2985, 2934, 2886, 1371, 1252, 1211, 1156, 1049; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 1.37 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.62–3.67 (m, 2H, H-1, H-2), 3.70 (dd, 1H, *J* = 6.0, 12.9 Hz, H-2), 3.86 (dd, 1H, *J* = 6.7, 8.3 Hz, H-5′), 4.06 (dd, 1H, *J* = 6.6, 8.3 Hz, H-5′), 4.15–4.21 (m, 1H, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 25.2 (CH_3), 26.4 (CH_3), 64.1 (C-2), 65.8 (C-5′), 71.7 (C-1), 76.6 (C-4′), 109.6 (C_q). ESI-HRMS: *m/z* calcd for $C_7H_{15}O_4$ $[M + H]^+$ 163.096, found 163.092.

4.4. Ethyl (R,E)-3-(2′,2′-dimethyl-1′,3′-dioxolan-4′-yl)acrylate (*E*−**12**) [20] and ethyl (R,Z)-3-(2′,2′-dimethyl-1′,3′-dioxolan-4′-yl)acrylate (*Z*-**12**) [20]

A solution of **15** (0.25 g, 1.54 mmol) in CH_2Cl_2 (8.3 mL) was treated with a solution of $NaIO_4$ (0.38 g, 1.78 mmol) in water (1.7 mL) at room temperature. After being stirred for 30 min, the insoluble parts were filtered off, and the filtrate was diluted with CH_2Cl_2. The separated aqueous phase was then extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated. The obtained crude aldehyde [20] was used immediately in the next reaction without purification.

*Wittig reaction in CH_2Cl_2*To a solution of the crude aldehyde (0.2 g, 1.54 mmol) in CH_2Cl_2 (10 mL) was added the ylide reagent, $Ph_3P{=}CHCO_2Et$, (1.2 g, 3.46 mmol). After stirring at room temperature for 30 min, the solvent was evaporated in vacuo, and the residue was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 25:1) to afford 0.243 g (79%) of a mixture of α,β-unsaturated esters (*E/Z*-**12**).

Wittig reaction in benzene: To a solution of $Ph_3P{=}CHCO_2Et$ (1.2 g, 3.46 mmol) in dry benzene (5 mL) was added a solution of the crude aldehyde (0.2 g, 1.54 mmol) in the same solvent (5 mL), and the mixture was heated at reflux for 1 h. After the solvent was removed, the residue was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate, 25:1) to give 0.225 g (73%) of a mixture of the corresponding esters (*E/Z*-**12**).

The repeated chromatography allowed the separation of both geometric isomers, which were isolated as colourless oils.

Ester (*E*)-**12**: $[\alpha]_D^{27}$ −47.7 (*c* 0.44, $CHCl_3$); lit [20]. $[\alpha]_D^{20}$ –46.0 (*c* 1.0, $CHCl_3$); IR (neat cm^{-1}); 2985, 2937, 2875, 1717, 1661, 1370, 1301, 1257, 1213, 1174, 1154, 1058, 1032, 976; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 1.30 (t, 3H, *J* = 7.1 Hz, CH_3), 1.41 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.68 (dd, 1H, *J* = 7.1, 8.3 Hz, H-5′), 4.16–4.25 (m, 3H, CH_2, H-5′), 4.64–4.70 (m, 1H, H-4′), 6.10 (dd, 1H, *J* = 1.4, 15.6 Hz, H-2), 6.88 (dd, 1H, *J* = 5.7, 15.6 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 14.2 (CH_3), 25.7 (CH_3), 26.4 (CH_3), 60.5 (CH_2), 68.8 (C-5′), 74.9 (C-4′), 110.2 (C_q), 122.4 (C-2), 144.6 (C-3), 166.0 (C═O). ESI-HRMS: *m/z* calcd for $C_{10}H_{16}NaO_4$ $[M + Na]^+$ 223.094, found 223.096.

Ester (*Z*)-**12**: $[\alpha]_D^{27}$ −126.4 (*c* 0.5, $CHCl_3$); lit [20]. $[\alpha]_D^{20}$ –126.0 (*c* 5.2, $CHCl_3$); IR (neat cm^{-1}) 2986, 2937, 1717, 1412, 1381, 1372, 1188, 1155, 1059, 1030; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 1.29 (t, 3H, *J* = 7.1 Hz, CH_3), 1.39 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.63 (dd, 1H, *J* = 6.7, 8.3 Hz, H-5′), 4.18 (q, 2H, *J* = 7.1 Hz, CH_2), 4.38 (dd, 1H, *J* = 6.9, 8.3 Hz, H-5′), 5.46–5.54 (m, 1H, H-4′), 5.85 (dd, 1H, *J* = 1.7, 11.6 Hz, H-2), 6.36 (dd, 1H, *J* = 6.6, 11.6 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 14.1 (CH_3), 25.4 (CH_3), 26.5 (CH_3), 60.4 (CH_2), 69.4 (C-5′), 73.5 (C-4′), 109.7 (C_q), 120.8 (C-2), 149.2 (C-3), 165.6 (C═O). ESI-HRMS: *m/z* calcd for $C_{10}H_{16}NaO_4$ $[M + Na]^+$ 223.094, found 223.096.

4.5. (R,E)-3-(2′,2′-dimethyl-1′,3′-dioxolan-4′-yl)prop-2-en-1-ol (*E*−**16**) [23]

DIBAL-H (9.5 mL, 34.2 mmol, ~1.2 M solution in toluene) was added dropwise to a solution of ester (*E*)-**12** (2.28 g, 11.4 mmol) in dry CH_2Cl_2 (52 mL) that had been pre-cooled to −50 °C. The resulting mixture was stirred at the same temperature for 30 min before quenching with MeOH (6.4 mL) to remove the excess hydride. The whole mixture was then treated with a 30% K/Na tartrate solution (170 mL) with vigorous stirring over 2 h at room temperature. The separated aqueous phase was extracted with EtOAc (3 × 150 mL). The combined organic layers were dried over Na_2SO_4, the solvents were evaporated, and the residue was chromatographed on silica gel (*n*-hexane/ethyl acetate, 1:1) to afford 1.59 g (88%) of compound (*E*)-**16** as a colourless oil; $[\alpha]_D^{26}$ −35.1 (*c* 0.70, $CHCl_3$); lit [23]. $[\alpha]_D$ −27.6 (*c* 0.29, $CHCl_3$, temperature is not reported); lit [29]. $[\alpha]_D^{25}$ +33.7 (*c* 1.0, $CHCl_3$) for *ent*-(*E*)-**16**; IR (neat cm^{-1}) 3404, 2985, 2934, 2870, 1370, 1213, 1154, 1052, 1008; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 1.39 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 2.06 (br s, 1H, OH), 3.58–3.64 (m, 1H, H-5′), 4.10 (dd, 1H, *J* = 6.2, 8.2 Hz, H-5′), 4.13–4.19 (m, 2H, 2 × H-1), 4.50–4.58 (m, 1H, H-4′), 5.68–5.76 (m, 1H, H-3), 5.92–6.00 (m, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 25.8 (CH_3), 26.6 (CH_3), 62.5 (C-1), 69.3 (C-5′), 76.4 (C-4′), 109.3 (C_q), 128.3 (C-3), 133.5 (C-2). ESI-HRMS: *m/z* calcd for $C_8H_{14}O_3$ $[M + Na]^+$ 181.084, found 181.085.

4.6. (R,Z)-3-(2′,2′-dimethyl-1′,3′-dioxolan-4′-yl)prop-2-en-1-ol (*Z*-**16**) [23]

By the same procedure as described for the preparation of (*E*)-**16** from (*E*)-**12**, compound (*Z*)-**12** (1.77 g, 8.8 mmol) was transformed to alcohol (*Z*)-**16** (colourless oil, 1.25 g, 90%, *n*-hexane/ethyl acetate, 1:1); $[\alpha]_D^{25}$ −15.2 (*c* 0.56, $CHCl_3$); lit [23]. $[\alpha]_D$ −16.5 (*c* 0.22, $CHCl_3$, temperature is not reported). IR (neat cm^{-1}) 3405, 2985, 2935, 2871, 1371, 1212, 1154, 1053; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 1.40 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 2.22 (br s, 1H, OH), 3.54–3.60 (m, 1H, H-5′), 4.10 (dd, 1H, *J* = 6.1, 8.2 Hz, H-5′), 4.18 (dd, 1H, *J* = 6.0, 13.3 Hz, H-1), 4.30 (dd, 1H, *J* = 7.0, 13.3 Hz, H-1), 4.81–4.91 (m, 1H, H-4′), 5.53–5.60 (m, 1H, H-3), 5.84 (ddd, 1H, *J* = 6.0, 7.0, 11.2 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 25.9 (CH_3), 26.7 (CH_3), 58.5 (C-1), 69.5 (C-5′), 71.8 (C-4′), 109.4 (C_q), 129.4 (C-3), 133.1 (C-2). ESI-HRMS: *m/z* calcd for $C_8H_{14}NaO_3$ $[M + Na]^+$ 181.084, found 181.085.

4.7. (R,E)-2,2-dimethyl-4-(3′-thiocyanatoprop-1′-en-1′-yl)-1,3-dioxolane (*E*−**11**)

To a solution of (*E*)-**16** (1.59 g, 10.05 mmol) in dry CH_2Cl_2 (86 mL), that had been pre-cooled to 0 °C, was added Et_3N (2.08 mL, 15.07 mmol), followed by addition of MsCl (1.15 mL, 15.07 mmol). After being stirred for 30 min at 0 °C, the solvent was evaporated and the residue was treated with Et_2O (86 mL). The solid parts were filtered off, washed with Et_2O, and the filtrate was concentrated. The obtained crude mesylate (2.37 g, 10.05 mmol) was dissolved in acetonitrile (80 mL). To this solution, anhydrous KSCN (1.68 g, 17.08 mmol) was added at room temperature, and the resulting mixture was stirred for 6 h before evaporating of the solvent. Chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 7:1) gave 1.74 g (87%) of compound (*E*)-**11** as a colourless oil; $[\alpha]_D^{27}$ −56.1 (*c* 0.66, $CHCl_3$); IR (neat cm^{-1}) 2985, 2935, 2874, 2153, 1370, 1212, 1153, 1056, 966; ^1H NMR (400 MHz, $CDCl_3$): *δ* 1.40 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 3.52–3.68 (m, 3H, 2 × H-3′, H-5), 4.16 (dd, 1H, *J* = 6.3, 8.3 Hz, H-5), 4.55–4.60 (m, 1H, H-4), 5.81–5.95 (m, 2H, H-1′, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 25.7 (CH_3), 26.5 (CH_3), 35.3 (C-3′), 69.4 (C-5), 75.6 (C-4), 109.7 (C_q), 111.4 (SCN), 125.3 (C-2′), 135.2 (C-1′). ESI-HRMS: *m/z* calcd for $C_9H_{13}NNaO_2S$ $[M + Na]^+$ 222.056, found 222.058.

4.8. (R,Z)-2,2-dimethyl-4-(3′-thiocyanatoprop-1′-en-1′-yl)-1,3-dioxolane (*Z*-**11**)

Using the same reaction sequence as employed for the conversion of (*E*)-**16** to (*E*)-**11**, compound (*Z*)-**16** (1.25 g, 7.9 mmol) was converted to

thiocyanate (*Z*)-**11** (colourless oil, 1.18 g (75%), *n*-hexane/ethyl acetate, 7:1); $[\alpha]_D^{26}$ +126.1 (*c* 0.82, $CHCl_3$); IR (neat cm^{-1}) 2986, 2935, 2872, 2154, 1380, 1371, 1212, 1153, 1055; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 1.40 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 3.55–3.60 (m, 1H, H-3′), 3.65 (dd, 1H, *J* = 7.6, 8.4 Hz, H-5), 3.87–3.92 (m, 1H, H-3′), 4.18 (dd, 1H, *J* = 6.2, 8.4 Hz, H-5), 4.78–4.83 (m, 1H, H-4), 5.74–5.83 (m, 2H, H-1′, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 25.8 (CH_3), 26.6 (CH_3), 30.7 (C-3′), 69.7 (C-5), 71.3 (C-4), 109.8 (C_q), 111.5 (SCN), 125.6 (C-2′), 133.9 (C-1′). ESI-HRMS: *m/z* calcd for $C_9H_{13}NNaO_2S$ $[M + Na]^+$ 222.056, found 222.058.

4.9. (R)-4-[(R)-1′-Isothiocyanatoallyl]-2,2-dimethyl-1,3-dioxolane (17) and (R)-4-[(S)-1′-isothiocyanatoallyl]-2,2-dimethyl-1,3-dioxolane (18)

4.9.1. Conventional method (general procedure)

The corresponding thiocyanate (*E*)-**11** or (*Z*)-**11** (0.10 g, 0.50 mmol) was dissolved in *n*-heptane (3.5 mL), and the resulting solutions were stirred and heated under a nitrogen atmosphere (for the temperatures and reaction times, see Table 1). After cooling to room temperature, the solvent was evaporated, and the residue was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 30:1) to provide the rearranged products **17** and **18** (for the combined yields, see Table 1).

4.9.2. Microwave-assisted synthesis (general procedure)

Thiocyanate (*E*)-**11** or (*Z*)-**11** (0.10 g, 0.5 mmol) was weighed in a 10-mL glass pressure microwave tube equipped with a magnetic stirbar. *n*-Heptane (3.5 mL) was added, the tube was closed with a silicon septum, and the resulting mixture was subjected to microwave irradiation (for the temperatures and reaction times, see Table 1). After being cooled to room temperature, the solvent was taken down, and the residue was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate, 30:1) to afford isothiocyanates **17** and **18** (for the combined yields, see Table 1).

Requiring greater amounts of the pure products **17** and **18**, they were prepared on a multigram scale by the conventional method in *n*-heptane at 70 °C starting from (*E*)-**11**.

Diastereoisomer **17**: colourless oil; $[\alpha]_D^{26}$ +42.6 (*c* 0.68, $CHCl_3$); IR (neat cm^{-1}) 2986, 2935, 2885, 2048, 1372, 1209, 1151, 1069; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 1.36 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 3.93 (dd, 1H, *J* = 5.1, 8.8 Hz, H-5), 4.08 (dd, 1H, *J* = 6.4, 8.8 Hz, H-5), 4.13–4.17 (m, 1H, H-4), 4.40–4.43 (m, 1H, H-1′), 5.35 (dd, 1H, *J* = 1.0, 10.3 Hz, H-3′$_{cis}$), 5.47 (dd, 1H, *J* = 1.0, 16.9 Hz, H-3′$_{trans}$), 5.81 (ddd, 1H, *J* = 5.1, 10.3, 16.9 Hz, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 25.0 (CH_3), 26.5 (CH_3), 61.5 (C-1′), 65.8 (C-5), 77.1 (C-4), 110.6 (C_q), 118.5 (C-3′), 131.4 (C-2′), 135.0 (NCS). ESI-HRMS: *m/z* calcd for $C_9H_{13}NNaO_2S$ $[M + Na]^+$ 222.056, found 222.057.

Diastereoisomer **18**: colourless oil, $[\alpha]_D^{26}$ −98.2 (*c* 0.78, $CHCl_3$); IR (neat cm^{-1}) 2986, 2935, 2885, 2044, 1371, 1211, 1152, 1065; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 1.36 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 3.83 (dd, 1H, *J* = 5.2, 8.8 Hz, H-5), 4.06 (dd, 1H, *J* = 6.3, 8.8 Hz, H-5), 4.19–4.26 (m, 2H, H-1′, H-4), 5.37 (d, 1H, *J* = 10.3 Hz, H-3′), 5.46 (d, 1H, *J* = 16.9 Hz, H-3′), 5.78–5.86 (m, 1H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 25.0 (CH_3), 26.4 (CH_3), 61.9 (C-1′), 65.8 (C-5), 77.1 (C-4), 110.6 (C_q), 119.3 (C-3′), 131.4 (C-2′), 135.3 (NCS). ESI-HRMS: *m/z* calcd for $C_9H_{13}NNaO_2S$ $[M + Na]^+$ 222.056, found 222.057.

4.10. (4R,5R)-5-(hydroxymethyl)-4-vinyloxazolidine-2-thione (19)

Isothiocyanate **17** (2.08 g, 10.4 mmol) was dissolved in dry MeOH (190 mL) at room temperature and *p*-TsOH (0.20 g, 1.05 mmol) was added. After being stirred at the same temperature for 17 h, the solvent was evaporated in vacuo, and the residue was chromatographed through a short column of silica gel (*n*-hexane/ethyl acetate, 1:1) to furnish 1.49 g (90%) of compound **19** as white crystals; mp 98–100 °C; $[\alpha]_D^{26}$ +42.7 (*c* 0.26, MeOH); IR (neat cm^{-1}) 3410, 3221, 2924, 1505, 1386, 1265, 1176, 1142, 1053, 1003; ^1H NMR (400 MHz, CD_3OD) *δ*: 3.67–3.75 (m, 2H, 2 × H-6), 4.62–4.66 (m, 1H, H-4), 4.93 (ddd, 1H, *J* = 4.5, 6.1, 9.3 Hz, H-5), 5.32–5.38 (m, 2H, 2 × H-8), 5.92 (ddd, 1H, *J* = 7.3, 10.3, 17.4 Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 61.3 (C-6), 61.7 (C-4), 86.8 (C-5), 120.3 (C-8), 133.1 (C-7), 190.7 (C═S). ESI-HRMS: *m/z* calcd for $C_6H_{10}NO_2S$ $[M + H]^+$ 160.043, found 160.044.

4.11. (4S,5R)-5-(hydroxymethyl)-4-vinyloxazolidine-2-thione (20)

By the same procedure as described for the conversion of **17** to **19**, diastereomeric isothiocyanate **18** (0.70 g, 3.50 mmol) was transformed to oxazolidine-2-thione **20** (white crystals, 0.50 g, 90%, *n*-hexane/ethyl acetate, 1:1); mp 130–133 °C; $[\alpha]_D^{26}$ −144.6 (*c* 0.48, MeOH); IR (neat cm^{-1}) 3315, 3185, 2927, 1522, 1379, 1257, 1176, 1093, 1000; ^1H NMR (400 MHz, CD_3OD) *δ*: 3.67 (dd, 1H, *J* = 4.2, 12.8 Hz, H-6), 3.82 (dd, 1H, *J* = 3.4, 12.8 Hz, H-6), 4.39–4.42 (m, 1H, H-4), 4.51–4.55 (m, 1H, H-5), 5.28–5.30 (m, 1H, H-8), 5.33–5.38 (m, 1H, H-8), 5.89 (ddd, 1H, *J* = 7.2, 10.2, 17.2 Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 61.6 (C-4), 62.1 (C-6), 89.5 (C-5), 119.3 (C-8), 136.4 (C-7), 190.6 (C═S). ESI-HRMS: *m/z* calcd for $C_6H_{10}NO_2S$ $[M + H]^+$ 160.043, found 160.042.

4.12. (4R,5R)-5-(hydroxymethyl)-4-vinyloxazolidin-2-one (9)

Mesitylnitrile oxide (1.80 g, 11.22 mmol) was added to a solution of **19** (1.49 g, 9.35 mmol) in dry MeCN (91 mL), and the resulting mixture was stirred for 0.5 h at room temperature. After the solvent was evaporated, the residue was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 1:3) to give 1.28 g (96%) of compound **9** in the form of white crystals; mp 102–105 °C; $[\alpha]_D^{26}$ −7.0 (*c* 0.37, MeOH); IR (neat cm^{-1}) 3438, 3239, 2932, 1754, 1408, 1319, 1245, 1087; ^1H NMR (400 MHz, CD_3OD) *δ*: 3.63–3.71 (m, 2H, 2 × H-6), 4.45–4.49 (m, 1H, H-4), 4.69–4.73 (m, 1H, H-5), 5.29 (d, 1H, *J* = 10.3 Hz, H-8$_{cis}$), 5.35 (d, 1H, *J* = 17.2 Hz, H-8$_{trans}$), 5.94 (ddd, 1H, *J* = 7.2, 10.3, 17.2 Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 58.2 (C-4), 61.8 (C-6), 81.4 (C-5), 119.3 (C-8), 134.6 (C-7), 161.6 (C═O). ESI-HRMS: *m/z* calcd for $C_6H_{10}NO_3$ $[M + H]^+$ 144.066, found 144.067.

4.13. (4S,5R)-5-(hydroxymethyl)-4-vinyloxazolidin-2-one (10)

By the same procedure as described for the preparation of **9**, compound **20** (0.49 g, 3.07 mmol) was converted into derivative **10** (colourless oil, 0.40 g, 91%, *n*-hexane/ethyl acetate, 1:3); $[\alpha]_D^{26}$ −87.5 (*c* 0.16, MeOH); IR (neat cm^{-1}) 3402, 3275, 2925, 1720, 1390, 1223, 1096, 1026; ^1H NMR (400 MHz, CD_3OD) *δ*: 3.64 (dd, 1H, *J* = 4.1, 12.6 Hz, H-6), 3.78 (dd, 1H, *J* = 3.2, 12.6 Hz, H-6), 4.20–4.28 (m, 2H, H-4, H-5), 5.24 (d, 1H, *J* = 10.2 Hz, H-8$_{cis}$), 5.33 (d, 1H, *J* = 17.0 Hz, H-8$_{trans}$), 5.90 (ddd, 1H, *J* = 6.7, 10.2, 17.0 Hz, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 56.4 (C-4), 61.5 (C-6), 82.5 (C-5), 118.7 (C-8), 135.8 (C-7), 159.4 (C═O). ESI-HRMS: *m/z* calcd for $C_6H_{10}NO_3$ $[M + H]^+$ 144.066, found 144.067.

4.14. (4R,5R)-5-(Hydroxymethyl)-4-[(E)-non-1′-en-1′-yl]oxazolidin-2-one (E−21) and (4R,5R)-5-(hydroxymethyl)-4-[(Z)-non-1′-en-1′-yl] oxazolidin-2-one (Z-21)

Non-1-ene (0.60 mL, 3.50 mmol) and Grubbs catalyst II (59 mg, 0.07 mmol) were successively added to a solution of oxazolidinone **9** (0.10 g, 0.70 mmol) in dry CH_2Cl_2 (15 mL). After being stirred at reflux for 1 h, the solvent was evaporated, and the residue was chromatographed on silica gel (*n*-hexane/ethyl acetate, 1:1) to furnish 0.13 g (78%) of a mixture of olefins **21** as a white solid. Repeated chromatography allowed the separation of (*E*)-**21** as the major product in the form of white crystals.

(*E*)-isomer **21**: mp 109–112 °C; $[\alpha]_D^{21}$ −12.6 (*c* 0.35, $CHCl_3$); IR (neat cm^{-1}) 3417, 3253, 2924, 2851, 1755, 1412, 1247, 1080, 1037, 963; ^1H NMR (400 MHz, CD_3OD) *δ*: 0.90 (t, 3H, *J* = 6.9 Hz, CH_3),

1.31–1.44 (m, 10H, 5 × CH_2), 2.06–2.11 (m, 2H, CH_2), 3.65–3.67 (m, 2H, 2 × H-6), 4.40–4.44 (m, 1H, H-4), 4.67 (td, 1H, J = 5.4, 8.6 Hz, H-5), 5.51 (tdd, 1H, J = 1.3, 7.9, 15.3 Hz, H-1′), 5.72–5.80 (m, 1H, H-2′); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.5 (CH_3), 23.8 (CH_2), 30.2 (CH_2), 30.3 (2 × CH_2), 33.1 (CH_2), 33.2 (CH_2), 57.9 (C-4), 62.0 (C-6), 81.9 (C-5), 126.1 (C-1′), 137.1 (C-2′), 161.7 (C═O). ESI-HRMS: m/z calcd for $C_{13}H_{24}NO_3$ $[M + H]^+$ 242.175, found 242.177.

4.15. (4R,5R)-5-(hydroxymethyl)-4-[(E)-tetradec-1′-en-1′-yl]oxazolidin-2-one (E−22) and (4R,5R)-5-(hydroxymethyl)-4-[(Z)-tetradec-1′-en-1′-yl]oxazolidin-2-one (Z-22)

Using the same reaction conditions as in the previous case, compound **9** (0.14 g, 0.978 mmol) was converted into a mixture of olefins **22** (0.23 g, 75%, *n*-hexane/ethyl acetate, 1:1). Repeated chromatography allowed the separation of (*E*)-**22** as the major product as white crystals.

(*E*)-isomer **22**: mp 112–115 °C; $[\alpha]_D^{21}$ −14.2 (*c* 0.24, $CHCl_3$); IR (neat cm^{-1}) 3271, 2916, 2848, 2532, 1756, 1478, 1033; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.88 (t, 3H, J = 6.8 Hz, CH_3), 1.26–1.41 (m, 20H, 10 × CH_2), 1.92–1.95 (m, 1H, OH); 2.04–2.09 (m, 2H, CH_2), 3.70–3.84 (m, 2H, 2 × H-6), 4.40–4.44 (m, 1H, H-4), 4.69–4.74 (m, 1H, H-5), 5.03 (br s, 1H, NH), 5.50 (dd, 1H, J = 8.5, 15.5 Hz, H-1′), 5.74–5.81 (m, 1H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$, 50 °C) δ: 14.0 (CH_3), 22.7 (CH_2), 28.9 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.6 (2 × CH_2), 29.7 (2 × CH_2), 31.9 (CH_2), 32.1 (CH_2), 56.7 (C-4), 61.6 (C-6), 79.8 (C-5), 124.1 (C-1′), 137.5 (C-2′), 158.1 (C═O). ESI-HRMS: m/z calcd for $C_{18}H_{34}NO_3$ $[M + H]^+$ 312.253, found 312.252.

4.16. (4R,5R)-5-(hydroxymethyl)-4-nonyloxazolidin-2-one (23)

4.16.1. Modification of 21 into 23

5% Rh/Al_2O_3 (27 mg) was added to a solution of a mixture of alkenes **21** (0.13 g, 0.54 mmol) in dry EtOAc (11 mL), and the resulting suspension was stirred at room temperature under an atmosphere of hydrogen. After completion of the reaction (2 h, judged by TLC), the mixture was filtered through a small pad of Celite and concentrated. Flash chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 1:1) furnished 0.109 g (83%) of compound **23** as white crystals.

4.16.2. Modification of 9 into 23

A solution of **9** (50 mg, 0.35 mmol) in dry CH_2Cl_2 (7.5 mL) was successively treated with non-1-ene (0.3 mL, 1.75 mmol) and Grubbs II (15 mg, 0.018 mmol). After being stirred at reflux until no starting material was observed (judged by TLC, 2 h), the mixture was allowed to cool to room temperature, and then, MeOH (1.5 mL) and $NaBH_4$ (0.132 g, 3.5 mmol) were added. After 3 h with stirring, the reaction mixture was partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The separated aqueous phase was washed with the further portions of CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 1:1) to give 62 mg (73%) of **23**; mp 115–116 °C; $[\alpha]_D^{21}$ −2.3 (*c* 0.26, $CHCl_3$); IR (neat cm^{-1}) 3413, 3282, 2920, 2849, 1754, 1420, 1250, 1097, 1034; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.88 (t, 3H, J = 6.6 Hz, CH_3), 1.26–1.43 (m, 14H, 7 × CH_2), 1.51–1.60 (m, 2H, CH_2), 2.55–2.58 (m, 1H, OH), 3.79–3.95 (m, 3H, 2 × H-6, H-4), 4.66–4.71 (m, 1H, H-5), 6.16 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (CH_3), 22.6 (CH_2), 26.6 (CH_2), 29.2 (CH_2), 29.4 (2 × CH_2), 29.5 (CH_2), 29.6 (CH_2), 31.8 (CH_2), 54.8 (C-4), 60.7 (C-6), 79.7 (C-5), 159.2 (C═O). ESI-HRMS: m/z calcd for $C_{13}H_{26}NO_3$ $[M + H]^+$ 244.191, found 244.194.

4.17. (4R,5R)-5-(hydroxymethyl)-4-tetradecyloxazolidin-2-one (24)

4.17.1. Modification of 22 to 24

Compound **24** (white crystals, 65 mg, 86%, *n*-hexane/ethyl acetate, 1:1) was prepared by the catalytic hydrogenation in MeOH (4.8 mL) from a mixture of olefins **22** (75 mg, 0.24 mmol) according to the procedure used to convert **21** to **23**.

4.17.2. Modification of 9 to 24

To a solution of **9** (0.15 mg, 1.05 mmol) in dry CH_2Cl_2 (22 mL) were successively added tetradec-1-ene (1.3 mL, 5.25 mmol) and Grubbs II (45 mg, 0.053 mmol). After being stirred at reflux until no starting material was observed (judged by TLC, 1 h), the mixture was allowed to cool to room temperature. Then, MeOH (4.5 mL) and $NaBH_4$ (0.396 g, 10.5 mmol) were added, and the resulting mixture was stirred for 2 h. After washing with water and extraction with CH_2Cl_2 (3 × 20 mL), the combined organic layers were dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (*n*-hexane/ethyl acetate, 1:1) to give 0.25 g (76%) of compound **24**; mp 111–113 °C; $[\alpha]_D^{21}$ −10.0 (*c* 0.15, $CHCl_3$); IR (neat cm^{-1}) 3410, 3281, 2914, 2848, 1758, 1469, 1047; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.88 (t, 3H, J = 6.7 Hz, CH_3), 1.26–1.37 (m, 24H, 12 × CH_2), 1.52–1.58 (m, 2H, CH_2), 2.05–2.08 (m, 1H, OH); 3.78–3.94 (m, 3H, H-4, 2 × H-6); H-6), 4.66–4.71 (m, 1H, H-5), 5.52 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (CH_3), 22.7 (CH_2), 26.7 (CH_2), 29.4 (3 × CH_2), 29.5 (CH_2), 29.6 (4 × CH_2), 29.7 (2 × CH_2), 31.9 (CH_2), 54.7 (C-4), 60.8 (C-6), 79.6 (C-5), 158.6 (C═O). ESI-HRMS: m/z calcd for $C_{18}H_{36}NO_5$ $[M + H]^+$ 314.269, found 314.271.

4.18. (2R,3R)-3-Aminododecane-1,2-diol hydrochloride (5)

A solution of **23** (30 mg, 0.136 mmol) in EtOH (0.6 mL) was treated with 6 M aq HCl solution (6.4 mL). The resulting mixture was stirred and heated at 100 °C for 21 h. After evaporating of the solvents, the solid residue was washed several times with dry Et_2O and dried under high vacuum for 6 h. This procedure yielded 28 mg (90%) of compound **5** as a white amorphous solid; $[\alpha]_D^{21}$ +6.4 (*c* 0.44, MeOH); IR (neat cm^{-1}) 3331, 3025, 2915, 2848, 1508, 1463, 1050; ^{1}H NMR (400 MHz, CD_3OD) δ: 0.91 (t, 3H, J = 7.0 Hz, CH_3), 1.32–1.56 (m, 14H, 7 × CH_2), 1.62–1.78 (m, 2H, CH_2), 3.30–3.36 (m, 1H, H-3 + CD_3OD), 3.65 (dd, 1H, J = 5.3, 11.4 Hz, H-1), 3.72 (dd, 1H, J = 4.9, 11.4 Hz, H-1), 3.81–3.85 (m, 1H, H-2); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.5 (CH_3), 23.7 (CH_2), 26.8 (CH_2), 29.1 (CH_2), 30.4 (CH_2), 30.5 (CH_2), 30.6 (CH_2), 30.7 (CH_2), 33.1 (CH_2), 56.1 (C-3), 63.7 (C-1), 71.1 (C-2). ESI-HRMS: m/z calcd for $C_{12}H_{27}NNaO_2$ $[M + Na]^+$ 240.193, found 240.195.

4.19. (2R,3R)-3-Aminoheptadecane-1,2-diol hydrochloride (6) [19b]

Using the same procedure as described for the preparation of **5**, compound **24** (23 mg, 0.073 mmol) was converted to derivative **6** (white amorphous solids, 20 mg, 87%); $[\alpha]_D^{21}$ +12.9 (*c* 0.28, MeOH); lit [19b]. $[\alpha]_D^{20}$ +12.3 (*c* 0.26, MeOH); IR (neat cm^{-1}) 3339, 3039, 2914, 2847, 1462; ^{1}H NMR (400 MHz, CD_3OD) δ: 0.89 (t, 3H, J = 6.7 Hz, CH_3), 1.29–1.54 (m, 24H, 12 × CH_2), 1.60–1.76 (m, 2H, CH_2), 3.28–3.35 (m, 1H, H-3 + CD_3OD), 3.63 (dd, 1H, J = 5.3, 11.4 Hz, H-1), 3.71 (dd, 1H, J = 4.9, 11.4 Hz, H-1), 3.80–3.84 (m, 1H, H-2); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.5 (CH_3), 23.7 (CH_2), 26.8 (CH_2), 29.1 (CH_2), 30.5 (2 × CH_2), 30.6 (CH_2), 30.7 (CH_2), 30.8 (5 × CH_2), 33.1 (CH_2), 56.1 (C-3), 63.7 (C-1), 71.1 (C-2). Anal. Calcd for $C_{17}H_{38}ClNO_2$: C, 63.03; H, 11.82; N, 4.32. Found: C, 63.16; H, 11.91; N, 4.21. ESI-HRMS: m/z calcd for $C_{17}H_{38}NO_2$ $[M + H]^+$ 288.290, found 288.292.

4.20. (4S,5R)-5-(Hydroxymethyl)-4-[(E)-non-1′-en-1′-yl]oxazolidin-2-one (E−25) and (4S,5R)-5-(hydroxymethyl)-4-[(Z)-non-1′-en-1′-yl] oxazolidin-2-one (Z-25)

Using the same procedure as described for the preparation of **21**, compound **10** (55 mg, 0.38 mmol) was converted into a mixture of olefins **25** (72 mg, 79%, *n*-hexane/ethyl acetate, 1:1). Repeated

chromatography allowed the separation of (*E*)-**25** as the major product as white crystals; mp 54–56 °C; $[\alpha]_D^{21}$ − 70.3 (*c* 0.58, $CHCl_3$); IR (neat cm^{-1}) 3432, 3311, 2952, 2919, 2854, 1760, 1698, 1663, 1467, 1418, 1341, 1217, 1098, 1032; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 0.88 (t, 3H, *J* = 6.9 Hz, CH_3), 1.27–1.40 (m, 10H, 5 × CH_2), 2.01–2.07 (m, 2H, CH_2), 2.63 (br s, 1H, OH), 3.61–3.70 (m, 1H, H-6), 3.89–3.93 (m, 1H, H-6), 4.22–4.28 (m, 2H, H-4, H-5), 5.39–5.44 (m, 1H, H-1′), 5.49 (br s, 1H, NH), 5.75 (td, 1H, *J* = 6.8, 15.1 Hz, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 14.1 (CH_3), 22.6 (CH_2), 28.8 (CH_2), 29.1 (2 × CH_2), 31.8 (CH_2), 32.0 (CH_2), 56.1 (C-4), 61.5 (C-6), 82.8 (C-5), 127.1 (C-1′), 136.7 (C-2′), 158.6 (C═O). ESI-HRMS: *m/z* calcd for $C_{13}H_{24}NO_3$ $[M + H]^+$ 242.175, found 242.173.

4.21. (4S,5R)-5-(hydroxymethyl)-4-[(E)-tetradec-1′-en-1′-yl]oxazolidin-2-one (E−26) and (4S,5R)-5-(hydroxymethyl)-4-[(Z)-tetradec-1′-en-1′-yl]oxazolidin-2-one (Z-26)

According to the same procedure as employed for the conversion of **9** to **22**, compound **10** (0.10 g, 0.70 mmol) was transformed to a mixture of alkenes **26** (0.16 g, 73%, *n*-hexane/ethyl acetate, 1:1). Repeated chromatography allowed the separation of (*E*)-**26** as the major product in the form of white crystals; mp 72–74 °C; $[\alpha]_D^{21}$ − 53.6 (*c* 0.56, $CHCl_3$); IR (neat cm^{-1}) 3433, 3308, 2952, 2919, 2845, 1770, 1687, 1668, 1467, 1420, 1214, 1099, 1032; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 0.88 (t, 3H, *J* = 6.7 Hz, CH_3), 1.26–1.37 (m, 20H, 10 × CH_2), 2.01–2.06 (m, 2H, CH_2), 3.13 (br s, 1H, OH), 3.63–3.66 (m, 1H, H-6), 3.89–3.92 (m, 1H, H-6), 4.20–4.30 (m, 2H, H-4, H-5), 5.38–5.44 (m, 1H, H-1′), 5.74 (td, 1H, *J* = 6.7, 15.3 Hz, H-2′), 5.82 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 14.1 (CH_3), 22.7 (CH_2), 28.8 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.6 (3 × CH_2), 29.7 (CH_2), 31.9 (CH_2), 32.0 (CH_2), 56.1 (C-4), 61.5 (C-6), 82.8 (C-5), 127.1 (C-1′), 136.7 (C-2′), 158.6 (C═O). ESI-HRMS: *m/z* calcd for $C_{18}H_{34}NO_3$ $[M + H]^+$ 312.253, found 312.251.

4.22. (4S,5R)-5-(hydroxymethyl)-4-nonyloxazolidin-2-one (27)

4.22.1. Modification of 25 to 27

According to the same procedure as employed for the conversion of **21** to **23**, compound **25** (52 mg, 0.215 mmol) was transformed to derivative **27** (white crystals, 48 mg, 92%, *n*-hexane/ethyl acetate, 1:1).

4.22.2. Modification of 10 to 27

Using the same procedure as described for the conversion of **9** to **23**, compound **10** (40 mg, 0.28 mmol) was transformed to derivative **27** (46 mg, 68%, *n*-hexane/ethyl acetate, 1:1); mp 74–75 °C; $[\alpha]_D^{21}$ − 55.8 (*c* 0.98, $CHCl_3$); IR (neat cm^{-1}) 3288, 3199, 2919, 2851, 1721, 1406, 1378, 1258, 1086; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 0.88 (t, 3H, *J* = 6.8 Hz, CH_3), 1.26–1.31 (m, 14 H, 7 × CH_2), 1.56–1.60 (m, 2H, CH_2), 2.42 (br s, 1H, OH), 3.65–3.73 (m, 2H, H-4, H-6), 3.84–3.88 (m, 1H, H-6), 4.27 (ddd, 1H, *J* = 3.2, 4.7, 5.7 Hz, H-5), 5.63 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 14.1 (CH_3), 22.7 (CH_2), 25.2 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.4 (2 × CH_2), 31.8 (CH_2), 35.4 (CH_2), 53.7 (C-4), 63.0 (C-6) 82.5 (C-5), 158.8 (C═O). ESI-HRMS: *m/z* calcd for $C_{13}H_{26}NO_3$ $[M + H]^+$ 244.191, found 244.193.

4.23. (4S,5R)-5-(hydroxymethyl)-4-tetradecyloxazolidin-2-one (28)

4.23.1. Modification of 26 to 28

Using the same procedure as described for the preparation of **24**, compound **26** (0.10 g, 0.32 mmol) was transformed to derivative **28** (white crystals, 98 mg, 97%, *n*-hexane/ethyl acetate, 1:1).

4.23.2. Modification of 10 to 28

According to the same procedure employed for the transformation of **9** to **24**, compound **10** (50 mg, 0.35 mmol) was converted into derivative **28** (72 mg, 66%, *n*-hexane/ethyl acetate, 1:1); mp 80–84 °C; $[\alpha]_D^{21}$ − 38.9 (*c* 0.70, $CHCl_3$); IR (neat cm^{-1}) 3286, 3199, 2914, 2847, 1720, 1467, 1406, 1251, 1094; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 0.88 (t, 3H, *J* = 6.8 Hz, CH_3), 1.26–1.33 (m, 24H, 12 × CH_2), 1.52–1.62 (m, 2H, CH_2), 2.81 (t, 1H, *J* = 6.4 Hz, OH), 3.62–3.73 (m, 2H, H-4, H-6), 3.83–3.88 (m, 1H, H-6), 4.26 (ddd, 1H, *J* = 3.2, 4.6, 5.6 Hz, H-5), 5.99 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 14.1 (CH_3), 22.7 (CH_2), 25.2 (CH_2), 29.3 (2 × CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (3 × CH_2), 29.7 (2 × CH_2), 31.9 (CH_2), 35.4 (CH_2), 53.7 (C-4), 62.9 (C-6), 82.6 (C-5), 159.1 (C═O). ESI-HRMS: *m/z* calcd for $C_{18}H_{36}NO_3$ $[M + H]^+$ 314.269, found 314.275.

4.24. (2R,3S)-3-Aminododecane-1,2-diol hydrochloride (7)

Compound **27** (40 mg, 0.164 mmol) was dissolved in a minimum volume of EtOH (0.7 mL) and then treated with a 6 M aq solution of HCl (7.7 mL). The resulting mixture was stirred and heated at 100 °C for 30 h. After the solvent was removed, the residue was further washed three times with Et_2O and dried under high vacuum for 10 h. This procedure yielded 35 mg (83%) of compound **7** as white amorphous solids; $[\alpha]_D^{21}$ +1.9 (*c* 0.46, MeOH); IR (neat cm^{-1}) 3388, 3120, 2998, 2950, 2918, 2849, 1484, 1374, 1105, 1072; ^1H NMR (400 MHz, CD_3OD) *δ*: 0.90 (t, 3H, *J* = 6.8 Hz, CH_3), 1.30–1.48 (m, 14H, 7 × CH_2), 1.58–1.67 (m, 1H, CH_2), 1.71–1.80 (m, 1H, CH_2), 3.26–3.29 (m, 1H, H-3), 3.66–3.71 (m, 3H, H-2, 2 × H-1); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 14.5 (CH_3), 23.7 (CH_2), 26.4 (CH_2), 30.4 (CH_2), 30.5 (CH_2), 30.6 (CH_2), 30.7 (CH_2), 31.3 (CH_2), 33.1 (CH_2), 55.0 (C-3), 65.0 (C-1), 70.5 (C-2). ESI-HRMS: *m/z* calcd for $C_{12}H_{27}NNaO_2$ $[M + Na]^+$ 240.193, found 240.193.

4.25. (2R,3S)-3-Aminoheptadecane-1,2-diol hydrochloride (8) [19b]

According to the same procedure as employed for the conversion of **27** to **7**, compound **28** (40 mg, 0.128 mmol) was modified into derivative **8** (white amorphous solid, 33 mg, 88%); $[\alpha]_D^{21}$ +2.2 (*c* 0.18, MeOH); lit [19b]. $[\alpha]_D^{21}$ +1.4 (*c* 0.28, MeOH); IR (neat cm^{-1}) 3383, 3116, 2917, 2848, 1486, 1085; ^1H NMR (400 MHz, CD_3OD) *δ*: 0.90 (t, 3H, *J* = 6.8 Hz, CH_3), 1.29–1.48 (m, 24H, 12 × CH_2), 1.57–1.66 (m, 1H, CH_2), 1.71–1.80 (m, 1H, CH_2), 3.24–3.29 (m, 1H, H-3), 3.66–3.71 (m, 3H, H-2, 2 × H-1); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 14.5 (CH_3), 23.8 (CH_2), 26.5 (CH_2), 30.5 (CH_2), 30.6 (2 × CH_2), 30.7 (CH_2), 30.8 (3 × CH_2), 30.9 (2 × CH_2), 31.4 (CH_2), 33.1 (CH_2), 55.0 (C-3), 65.1 (C-1), 70.5 (C-2). ESI-HRMS: *m/z* calcd for $C_{17}H_{37}NNaO_2$ $[M + Na]^+$ 310.272, found 310.273.

4.26. Antiproliferative/cytotoxic activity

4.26.1. Cell culture

The following human cancer cell lines were used for this study: A-549 (non-small cell lung cancer), HeLa (cervical adenocarcinoma), MCF-7 (mammary gland adenocarcinoma), MDA-MB-231 (mammary gland adenocarcinoma), HCT-116 (human colon carcinoma), Caco-2 (human colon carcinoma), Jurkat (acute T-lymphoblastic leukaemia) and non-cancerous cell line NiH 3T3 (mouse fibroblasts). A-549, HCT-116, MCF-7, MDA-MB-231, Caco-2, Jurkat and HeLa cells were maintained in RPMI 1640 medium. NiH 3T3 cell line was maintained in growth medium consisting of high glucose Dulbecco's Modified Eagle Medium. Both of these media were supplemented with Glutamax, and with 10% (V/V) foetal calf serum, penicillin (100 IU × mL^{-1}), and streptomycin (100 mg × mL^{-1}) (all from Invitrogen, Carlsbad, CA USA), in the atmosphere of 5% CO_2 in humidified air at 37 °C. Cell viability, estimated by the trypan blue exclusion, was greater than 95% before each experiment.

4.26.2. Cytotoxicity assay

The cytotoxic effect of the tested compounds was studied using the colorimetric microculture assay with the MTT endpoint [27]. The

amount of MTT reduced to formazan was proportional to the number of viable cells. Briefly, 5×10^3 cells were plated per well in 96-well polystyrene microplates (Sarstedt, Germany) in the culture medium containing tested chemicals at final concentrations 10^{-4}–10^{-6} mol × L^{-1}. After 72 h incubation, 10 μL of MTT (5 mg × mL^{-1}) were added into each well. After an additional 4 h, during which insoluble formazan was formed, 100 μL of 10% (m/m) sodium dodecylsulfate were added into each well and another 12 h were allowed for the formazan to be dissolved. The absorbance was measured at 540 nm using the automated uQuant™ Universal Microplate Spectrophotometer (Biotek Instruments Inc., Winooski, VT USA). The blank corrected absorbance of the control wells was taken as 100% and the results were expressed as a percentage of the control.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2018.09.008.

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