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Graphical Abstract

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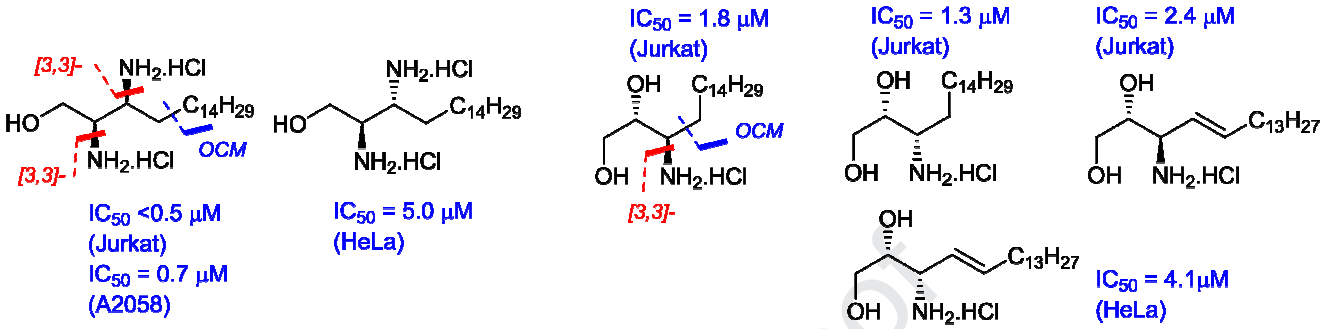

Synthesis and *in vitro* biological evaluation of 3-amino-3-deoxydihydrosphingosines and their analogues

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[†]This work is dedicated to the memory of Professor Jozef Gonda (1957-2019)

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ABSTRACT

The stereoselective synthesis of the 3-amino-3-deoxydihydrosphingosines and their isomeric analogues from dimethyl L-tartrate is described by means of [3,3]-sigmatropic rearrangements and the cross metathesis reaction as a cornerstone of the developed strategy. The configuration of two newly created stereocentres was unambiguously assigned via a single crystal X-ray analysis of the complex structure of di-*tert*-butyl [(2*R*,3*R*,4*S*)-1,2-

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dihydroxynonadecane-3,4-diyl]dicarbamate. The prepared unusual sphingoid bases were evaluated regarding their capacity to alter the viability of cancer cell lines.

1. Introduction

Sphingolipids (SLs) represent unique components of all eukaryotic cells. Besides their pivotal role in membrane construction, their metabolic pathways deliver compounds that participate in various significant cellular signalling processes, such as angiogenesis, cell proliferation, apoptosis, differentiation, inflammation and adhesion.[1] On the other hand, recent studies have revealed that defects in sphingolipid metabolism lead to several human diseases.[1b-f,2] This wide family of lipids shares common structural backbones, including D-*erythro*-sphingosine **1**,[3a,3d-e] D-*erythro*-dihydrosphingosine (sphinganine) **2**[3c] and D-*ribo*-phytosphingosine **3**[3b,3d-e] (Fig. 1). It should be noted that the mentioned sphingoid bases exhibit an interesting biological profile. Both sphingosine **1** and sphinganine **2** were found to inhibit protein kinase C (PKC).[4] Dihydrosphingosine **2** and its unnatural D/L-*threo*-diastereoisomers, the general structure of which is illustrated by safingol **4**[3c,5] (Fig. 1), are well-known sphingosine kinase inhibitors (SphK).[1f] Sphingosine kinases (SphKs)[1f,6] are enzymes that catalyze the conversion of **1** to sphingosine-1-phosphate (S1P). In addition to dihydrosphingosine **2** and its further *threo*-congeners, **3** also belongs to the family of SphK inhibitors.[6] D-*erythro*-Sphingosine **1** acts as a metabolic switch between ceramide (Cer) and S1P. These two structures are crucial regulators of cell functions. The former initiates an apoptotic process, while the latter is a recognized promoter of cell survival.[1c,7]

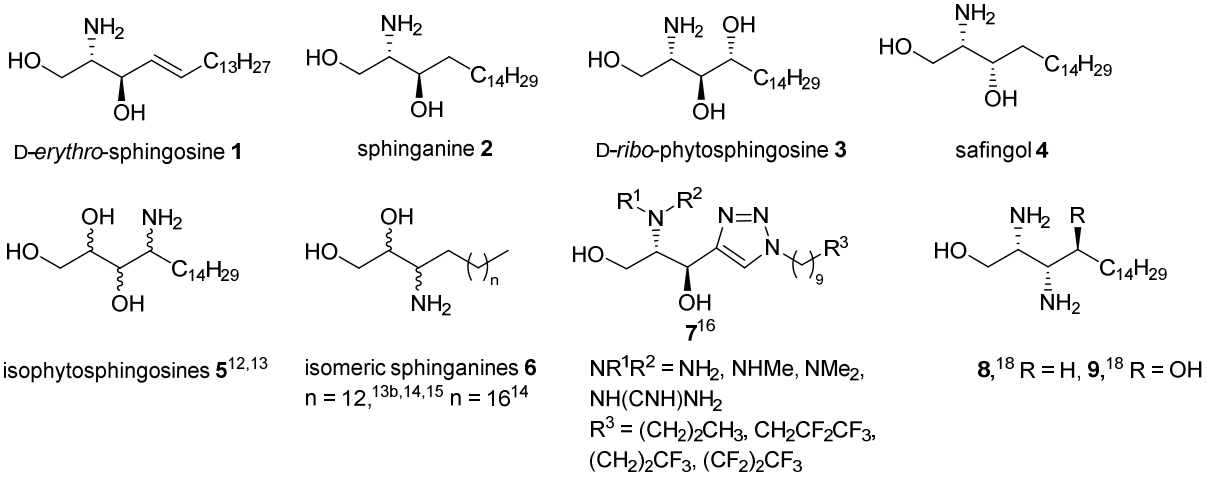


Figure 1. Representative natural sphingoid bases **1**-**3** and their synthetic analogues.

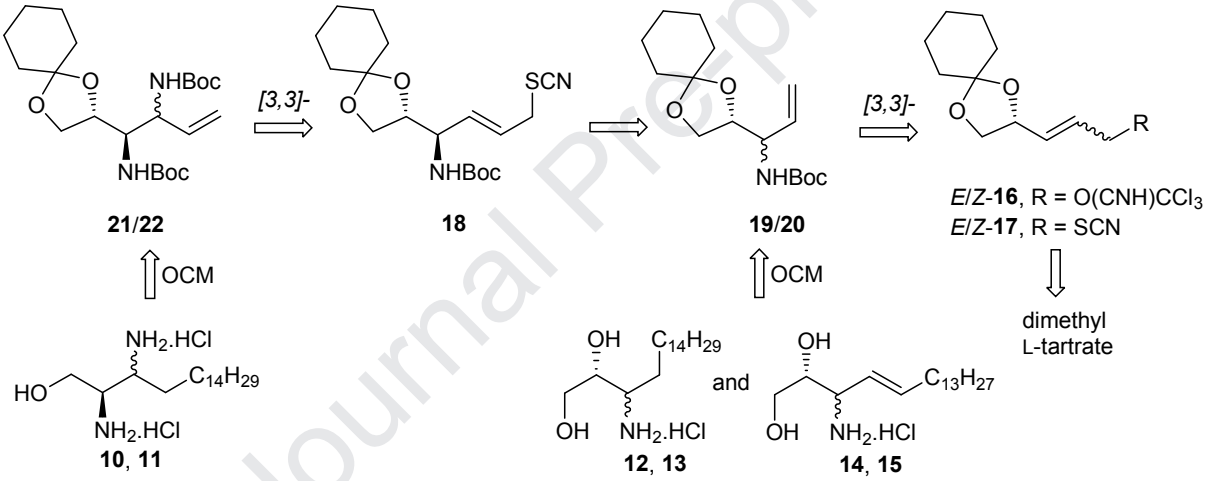
On the other hand, D-*ribo*-phytosphingosine **3** was identified as a heat-stress signalling molecule in yeast.[8] *N*-Acyl-linked derivatives of **3** (also called phytoceramides) have been found in large quantities in the extracellular spaces of the epidermis (*stratum corneum*) in humans.[9] Furthermore, phytosphingosine **3** exhibits a pro-apoptotic effect against different cancer cell lines.[10] Kim's[11] group revealed the cytotoxic activity of **3** on three different human malignant cells (PC-3, HCT-116 and A-549). Due to the potent biological properties of naturally occurring sphingoid bases, there has been extensive focus on the construction of various analogues of **1**-**3** in recent years with the aim of finding appropriate candidates or lead compounds for the design of novel sphingolipid metabolism modulators. Among the synthesized derivatives (for some examples, see Fig. 1), for example, the following stand out, isophytosphingosines **5**[12,13] and isomeric sphinganines **6**,[13b,14,15] which display remarkable cytotoxicity; fluorinated triazole-containing analogues[16] of **1** (up to 16 compounds depicted by the general structure **7**) with SphK's inhibitory activity; amide-linked derivatives[17] of **1** and **2** substituted with different types of nucleophiles at the C-1 position, with the ability to block glucosylceramide synthase and yeast inositol phosphorylceramide synthase; and last but not least, 3-amino-3-deoxy analogues[18] of **2** and **3**.

Cancer is the second leading cause of death worldwide. Modulation of sphingolipid biosynthesis and metabolism is expected to be one of the promising approaches for therapy of the aforementioned disease. Our group has been intensively dealing with the stereoselective construction of various types of sphingoid base-like compounds[13,15,19] having an anticancer profile, starting from simple and available chirons. As part of our continuing studies on the synthesis of biologically active sphingolipid related compounds, we herein report the preparation of the cytotoxic 3-amino-3-deoxydihydrosphingosines **10** and **11** along with their analogues **12**-**15** from dimethyl L-tartrate as the chiral pool material. It should be noted that we recently elaborated a small library of cytotoxic C_{17}-sphinganine analogues **6**[13b,15] (Fig. 1). In light of our recent synthetic development,[13b,15] we set out to extend the panel of targeted dihydrosphingosine and sphingosine analogues, including diamino congeners. We also bring improved diastereoselectivity of the aza-Claisen rearrangement of thiocyanates (*E*/*Z*)-**17** (*vide infra*) with an incorporated bulky cyclohexylidene ketal protecting group in comparison with the previous work, which used the corresponding acetonide.[15] Moreover, to the best of our knowledge, the present study will be the first report in the literature on the antiproliferative/cytotoxic activities of sphingoid bases with the vicinal diamino motif. Such production of sphingolipid analogue libraries can be useful for structure-activity studies in biological targets.

2. Results and discussion

2.1. Chemistry

The general synthetic strategy towards modified sphingoid bases **10**-**15** is outlined in Scheme 1. For all target compounds, the introduction of the aliphatic chain was planned to be realized at a later stage in the synthesis via an olefin cross-metathesis (OCM) reaction. It was anticipated that the [3,3]-sigmatropic rearrangements on the allylic substrates *E*/*Z*-**16**, *E*/*Z*-**17** and **18** would create the expected synthons **19**-**20** and **21**-**22**, respectively. Imidates *E*/*Z*-**16** and thiocyanates *E*/*Z*-**17** can be readily obtained from dimethyl L-tartrate. On the other hand, rhodanide **18** would be synthesized from the major template **19** through a short sequence involving an ozonolysis, Wittig olefination and reduction.



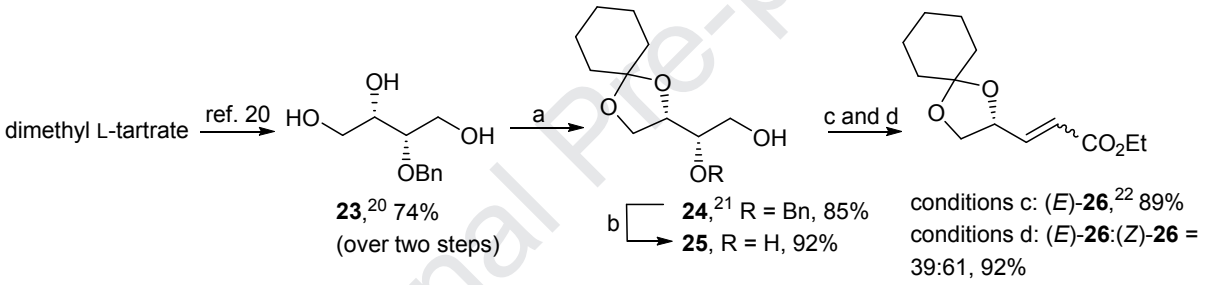
Scheme 1. Retrosynthetic analysis towards modified sphingoid bases **10**-**15**.

As seen in Scheme 2, the starting dimethyl L-tartrate was transformed to the known protected L-threitol **23** (74%, over two steps) according to the literature protocol.[20] Then the remaining hydroxyl groups of **23** were protected with cyclohexanone using catalytic amounts of *p*-TsOH to give **24**[21] in a yield of 85%. Compound **24** was converted into diol **25** (92%) by catalytic hydrogenolysis. The oxidative fragmentation of **25**, followed by Horner-Wadsworth-Emmons olefination with $(EtO)_2P(O)CH_2CO_2Et$ and NaH, delivered α,β-unsaturated ester (*E*)-**26**[22] exclusively in 89% yield. On the other hand, the Wittig reaction with a stable ylide reagent afforded a mixture of isomers **26** (*E*:*Z* = 39:61, combined yield of 92%) with a mild prevalence of (*Z*)-**26** (56%, >97:3 dr), $[\alpha]_D^{22}$ –77.0 (*c* 0.28, $CHCl_3$); for (*E*)-**26** (36%, >97:3

dr), $[\alpha]_D^{21}$ –31.9 (*c* 0.34, $CHCl_3$). Column chromatography on silica gel furnished each diastereoisomer in pure form.

It is known that Cinquini, Cozzi and co-workers[22] prepared the corresponding methyl (*R*,*Z*)-3-(1,4-dioxaspiro[4.5]decan-2-yl)acrylate, via the Still-Gennari protocol[23] as the sole product. It should be noted that this aforementioned procedure uses an exceedingly expensive reagent (methyl 2-[bis(2,2,2-trifluoroethoxy)phosphoryl]acetate). Therefore, we decided to apply the more available Wittig's conditions ($Ph_3P{=}CHCO_2Et$, benzene) towards (*Z*)-**26** (ethyl (*R*,*Z*)-3-(1,4-dioxaspiro[4.5]decan-2-yl)acrylate), although the selectivity of the olefination was moderate (Scheme 2).

The production of larger amounts of (*Z*)-**26** then allowed the preparation of four allylic substrates, (*E*)-**16**, (*Z*)-**16**, (*E*)-**17** and (*Z*)-**17** (*vide infra*), for the comparative study of subsequent [3,3]-sigmatropic rearrangements.

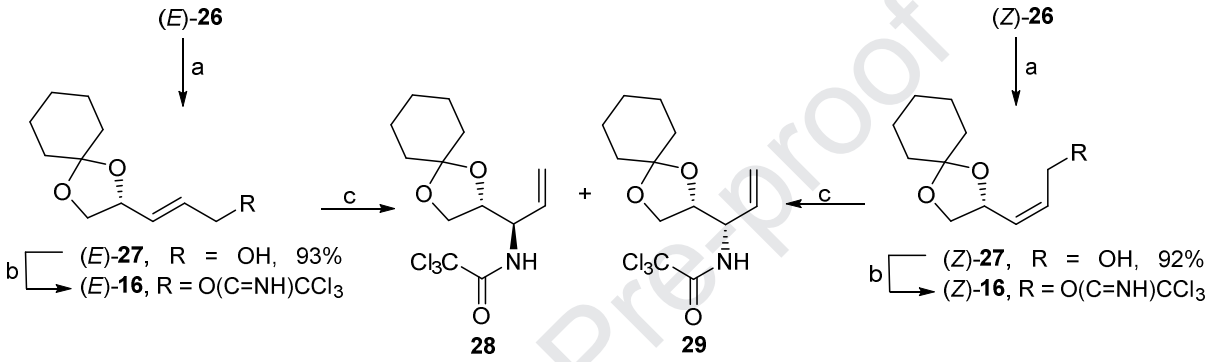


Scheme 2. *Reagents and conditions:* (a) cyclohexanone, *p*-TsOH, toluene, rt; (b) H_2, 10% Pd/C/20% $Pd(OH)_2$/C (1:1), EtOH, rt; (c) (i) $NaIO_4$, CH_2Cl_2/H_2O (2.5:1), rt; (ii) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, 0 °C; (d) (i) $NaIO_4$, CH_2Cl_2/H_2O (2.5:1), rt; (ii) $Ph_3P{=}CHCO_2Et$, benzene, reflux, (*E*)-**26** (36%), (*Z*)-**26** (56%).

Reduction of (*E*)-**26** and (*Z*)-**26** with DIBAl-H provided the corresponding alcohols (*E*)-**27** and (*Z*)-**27** in 93% and 92% yields, respectively (Scheme 3). To continue the synthesis, our attention focused on the Overman rearrangement of trichloroacetimidates (*E*)-**16** and (*Z*)-**16**, which were obtained from (*E*)-**27** and (*Z*)-**27** by the action of CCl_3CN and DBU. The microwave-promoted thermal rearrangement of both imidates (*E*)-**16**, (*Z*)-**16** took place in *o*-xylene in the presence of K_2CO_3.[24] In an effort to shorten the reaction time and improve the combined yield of rearranged products **28** and **29**, the microwave-promoted thermal Overman rearrangement of (*E*)-**16** and (*Z*)-**16** was tested at three temperatures: 150 °C, 170 °C and 190 °C. As seen in Table 1, the high temperature (190 °C, entries 3 and 6) was shown to be the most optimal for very good production of the corresponding trichloroacetamides **28** and **29**,

which, however, were isolated as an inseparable mixture of diastereoisomers due to their identical R_f values.

The observed diastereoselectivity in the thermal rearrangement of (*E*)-**16** showed that both faces of its allylic system are about equally preferred. In the case of (*Z*)-**16**, the geometry of the double bond in an allylic trichloroacetimidate system should be an important factor for the facial selectivity to prefer the production of the 1,2-*anti*-disatereoisomer **28** (Table 1, entries 4-6), and the energy differences between the transition states are key for explaining the observed diastereoselectivity.



Scheme 3. *Reagents and conditions:* (a) DIBAl-H, CH_2Cl_2, –50 °C; (b) CCl_3CN, DBU, CH_2Cl_2, 0 °C; (c) Table 1.

Table 1. Overman rearrangement of imidates (*E*)-**16** and (*Z*)-**16**

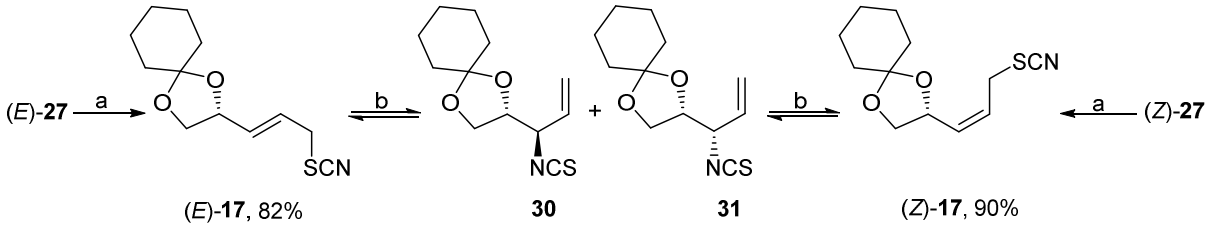
Entry	Imidate	Conditions[a,b]	Time (h)	Ratio[c] **28**:**29**	Yield[d] (%)
1	(*E*)-**16**	MW, 150 °C[a]	8	49:51	60
2	(*E*)-**16**	MW, 170 °C[a]	3.5	50:50	67
3	(*E*)-**16**	MW, 190 °C[a]	0.33	50:50	90
4	(*Z*)-**16**	MW, 150 °C[a]	19	80:20	60
5	(*Z*)-**16**	MW, 170 °C[a]	4	75:25	73
6	(*Z*)-**16**	MW, 190 °C[a]	1	74:26	85
7	(*E*)-**16**	Δ, 40 °C[b]	1	84:16	40
8	(*Z*)-**16**	Δ, 70 °C[b]	2	–	–[e]

[a] In *o*-xylene, in the presence of K_2CO_3.
[b] In toluene, in the presence of $PdCl_2(MeCN)_2$ and *p*-benzoquinone.
[c] Ratio in the crude reaction mixtures determined by ^{1}H NMR.
[d] Isolated combined yields.
[e] Decomposition.

Palladium(II)-catalyzed Overman rearrangement of (*E*)-**16** was carried out according to Sutherland's protocol[25] ($PdCl_2(MeCN)_2$, *p*-benzoquinone[26]) to afford a 84:16 diastereomeric ratio of the *anti*- and *syn*-amides **28** and **29**, respectively (entry 7, Table 1). The lower combined yield (40%) was due to the Lewis acidity of Pd(II), which induced the hydrolysis of the cyclohexylideneketal fragment[27] of (*E*)-**16**. Another competing pathway can be the

decomposition of the trichloroacetiminoyl group. The stereochemical outcome of the catalyzed rearrangement of (*E*)-**16** was in good agreement with the observations of Sutherland and Swift[27] and matched the *erythro* configuration of the vicinal aminoalcohol motif in the major product **28**. Upon applying Sutherland's conditions to (*Z*)-**16**, only unidentified decomposition products were generated in the reaction mixture (Table 1, entry 8).

Next, we explored the *aza*-Claisen rearrangement of thiocyanates (*E*)-**17** and (*Z*)-**17**. Incorporation of the –SCN group was accomplished by means of a nucleophilic substitution after mesylation of the primary alcohol functionality of (*E*)-**27** and (*Z*)-**27** to provide the corresponding products (*E*)-**17** and (*Z*)-**17** in 82% and 90% yields, respectively (Scheme 4). With the aim of finding the optimal reaction conditions, both the thermally driven and microwave-assisted [3,3]-sigmatropic rearrangements of (*E*)-**17** and (*Z*)-**17** were tested at three different temperatures (50 °C, 70 °C and 90 °C) in *n*-heptane to produce the required isothiocyanates **30** and **31** as readily separable diastereomeric mixtures. During these experiments the starting material, either in the pure form as (*E*)-**17** or (*Z*)-**17**, or as the corresponding mixture (*Z*/*E*)-**17**, was also recovered in amounts of 10-71% (Table 2). As seen in Table 2, high *erythro*-**30**/*threo*-**31** selectivity along with a satisfying yield were obtained in the case of (*Z*)-**17** at 50 °C and 70 °C (Table 2, entries 8, 9 and 11, respectively). The major isothiocyanate **30** is a kinetic reaction product, and the realization of the rearrangement at lower temperature (50 °C) enabled control of the selectivity (Table 2 entries 8 and 9) while still providing a satisfying yield (56% and 54%, respectively) of this transformation. The ratio of the rearrangement was also influenced by the bulky cyclohexylidene moiety in comparison to the isopropylidene group used in our previous study.[15] Prolonged heating and higher temperature were shown to be detrimental for the selectivity of the proceeding transformation (Table 2, entries 6, 7 and 12) and revealed that the equilibrium of the studied reaction was shifted to the thermodynamically more stable isothiocyanate **31** to decrease the 1,2-*anti*-diastereoselectivity. On the other hand, it furnished a greater amount of the 1,2-*syn*-product **31** as the synthon for the preparation of the sphingoid bases **13** and **15**. Analogously to our recent work,[15] the observed *anti*-vicinal stereochemistry in the [3,3]-sigmatropic rearrangement of thiocyanates (*E*)-**17** and (*Z*)-**17** would also be explained by the results of DFT calculations previously reported.[15]



Scheme 4. *Reagents and conditions:* (a) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C; (ii) KSCN, MeCN, rt; (b) Table 2.

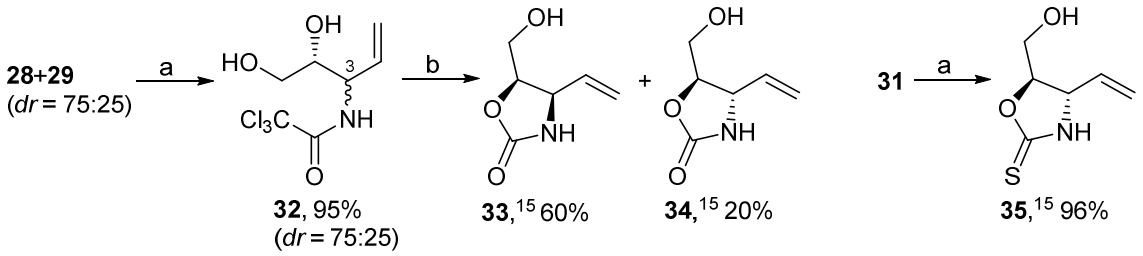
Table 2. [3,3]-Sigmatropic rearrangement of thiocyanates (*E*)-**17** and (*Z*)-**17**

Entry	Thiocyanate	Conditions[a]	Time (h)	Ratio[b] **30**:**31**	Yield[c] (%)	Isolated thiocyanates (%)
1	(*E*)-**17**	Δ, 50 °C	2.5	81:19	29	71[d]
2	(*E*)-**17**	Δ, 50 °C	12	75:25	59	37[d]
3	(*E*)-**17**	MW, 50 °C	4.5	81:19	58	39[d]
4	(*E*)-**17**	Δ, 70 °C	4.5	74:26	83	17[d]
5	(*E*)-**17**	MW, 70 °C	1.5	72:28	80	18[d]
6	(*E*)-**17**	Δ, 90 °C	0.75	72:28	78	17[d]
7	(*E*)-**17**	MW, 90 °C	0.33	72:28	81	14[d]
8	(*Z*)-**17**	Δ, 50 °C	12	94:6	56	42[e]
9	(*Z*)-**17**	MW, 50 °C	6	95:5	54	46[e]
10	(*Z*)-**17**	Δ, 70 °C	8	90:10	74	22[f] *E*/*Z* = 87:13
11	(*Z*)-**17**	MW, 70 °C	4	91:9	79	21[f] *E*/*Z* = 99:1
12	(*Z*)-**17**	Δ, 90 °C	1.5	75:25	90	10[f] E/Z = 75:25
13	(*Z*)-**17**	MW, 90 °C	0.75	85:15	80	16[f] *E*/*Z* = 67:33

[a] In *n*-heptane.
[b] Ratio in the crude reaction mixtures determined by ^{1}H NMR.
[c] Isolated combined yields.
[d] Isolated (*E*)-**17**.
[e] Isolated (*Z*)-**17**.
[f] Isolated a mixture of thiocyanates (*E*/*Z*)-**17**. Determined by ^{1}H NMR analysis.

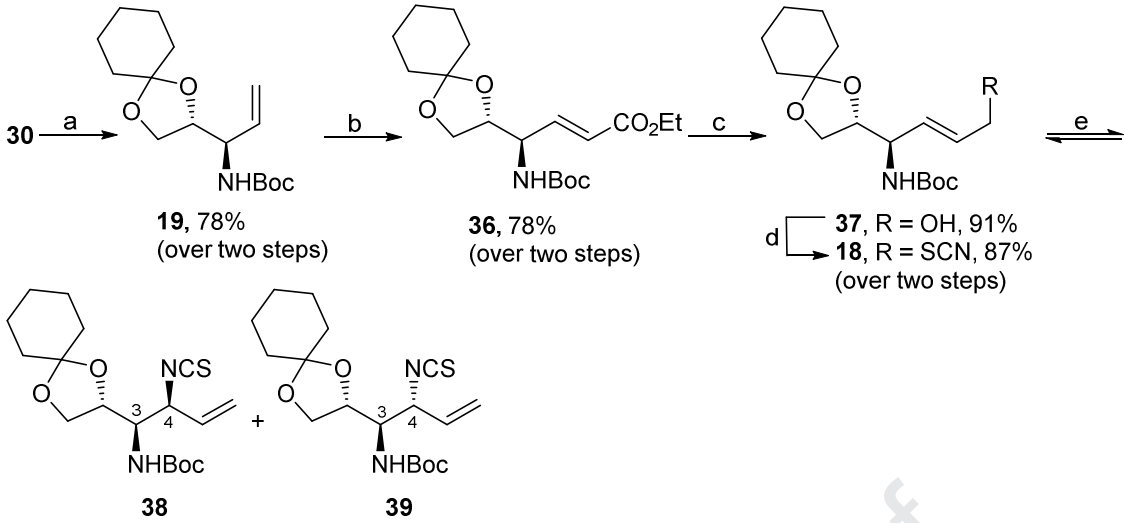
Then the C-3 stereochemistry in the rearranged products **28**-**31** had to be assigned (Scheme 5). For this purpose, the conversion of the mixture of amides **28** and **29** (**28**:**29** = 75:25, determined by ^{1}H NMR analysis) into known oxazolidin-2-ones[15] **33** (60%) and **34** (20%) was achieved via removal of the ketal moiety, delivering an inseparable mixture of diols **32** (95%, *dr* = 75:25) and a base induced ring-closure in **32**. Their NMR characteristics, optical rotation data and melting point were in accordance with those reported in the literature for the same compounds; for **33**: mp 102−104 °C; $[\alpha]_D^{22}$ –8.1 (*c* 0.40, MeOH); lit.[15] mp 102−105 °C; $[\alpha]_D^{26}$ –7.0 (*c* 0.37, MeOH); for **34**: $[\alpha]_D^{22}$ –85.1 (*c* 0.15, MeOH); lit.[15] $[\alpha]_D^{26}$ –87.5 (*c* 0.16, MeOH). On the other hand, treatment of the *threo*-isothiocyanate **31** with *p*-TsOH in MeOH mediated the spontaneous intramolecular addition of the liberated secondary alcohol to the –NCS group to produce the known cyclic thiocarbamate **35**[15] in a yield of 96% (Scheme 5). The isolated material had spectroscopic data, optical rotation and melting point in good agreement with those reported; mp 130−132 °C, $[\alpha]_D^{21}$ −158.3 (*c* 0.60, $CHCl_3$); lit.[15] mp 130−133 °C, $[\alpha]_D^{26}$

−144.6 (*c* 0.48, MeOH). These experiments revealed that both major products of the realized rearrangements (compounds **28** and **30**) are *erythro*-configured.



Scheme 5. *Reagents and conditions:* (a) *p*-TsOH, MeOH, 65 °C; (b) DBU, CH_2Cl_2, 0 °C→rt; (c) *p*-TsOH, MeOH, rt.

Following the retrosynthetic analysis depicted in Scheme 1, the stage was now set for the synthesis of the allylic substrate **18**, starting from the major isothiocyanate **30** as an appropriate synthon (Overman products **28** and **29** were not separable). Reaction of **30** with bis(*n*-tributyltin) oxide (TBTO) and subsequent *tert*-butoxycarbonylation of the amino group generated afforded *N*-Boc derivative **19** (78%, over two steps, Scheme 6). Ozonolysis of **19**, followed by Ph_3P treatment, furnished an aldehyde, which was then subjected to HWE olefination to afford (*E*)-α,β-unsaturated ester **36** (78%, over two steps) as the sole product. Reduction of the ester functionality delivered allylic alcohol **37** (91%), which was converted into the rhodanide **18** (87%, over two steps) using the same procedure as described for the transformation of (*E*)-**27** to (*E*)-**17**. The standard thermal reaction as well as the microwave irradiation of **18** resulted in the formation of 3,4-*syn*- and 3,4-*anti*-diastereoisomer **38** and **39**, respectively (for the reaction conditions, combined yields and ratios, see Table 3). Column chromatography then allowed the separation of both isothiocyanates **38** and **39**.



Scheme 6. *Reagents and conditions:* (a) (i) TBTO, toluene, 60 °C; (ii) Boc_2O, Et_3N, CH_2Cl_2, rt; (b) (i) O_3, EtOH, –78 °C, then Ph_3P, CH_2Cl_2, rt; (ii) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, 0 °C; (c) DIBAl-H, $BF_3.OEt_2$, CH_2Cl_2, –78 °C; (d) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C; (ii) KSCN, MeCN, rt; (e) Table 3.

Table 3. [3,3]-Sigmatropic rearrangement of thiocyanate **18**

Entry	Thiocyanate	Conditions[a]	Time (h)	Ratio[b] **38**:**39**	Yield[c] (%)	Isolated **18** (%)
1	**18**	Δ, 30 °C	14	84:16	51	43
2	**18**	MW, 30 °C	6.5	85:15	41	53
3	**18**	Δ, 50 °C	2.5	81:19	79	11
4	**18**	MW, 50 °C	1.25	83:17	71	25
5	**18**	Δ, 70 °C	1	78:22	81	11
6	**18**	MW, 70 °C	0.5	79:21	72	26
7	**18**	Δ, 90 °C	1	77:23	82	15
8	**18**	MW, 90 °C	0.33	79:21	81	9

[a] In *n*-heptane.
[b] Ratio in the crude reaction mixtures determined by ^1H NMR.
[c] Isolated combined yields.

The C-4 stereochemistry of **38** and **39** was assigned via a single crystal X-ray analysis (Fig. 2) of the more advanced intermediate **44** (*vide infra*) and revealed that the major rearranged product **38** has 3,4-*syn*-configuration.

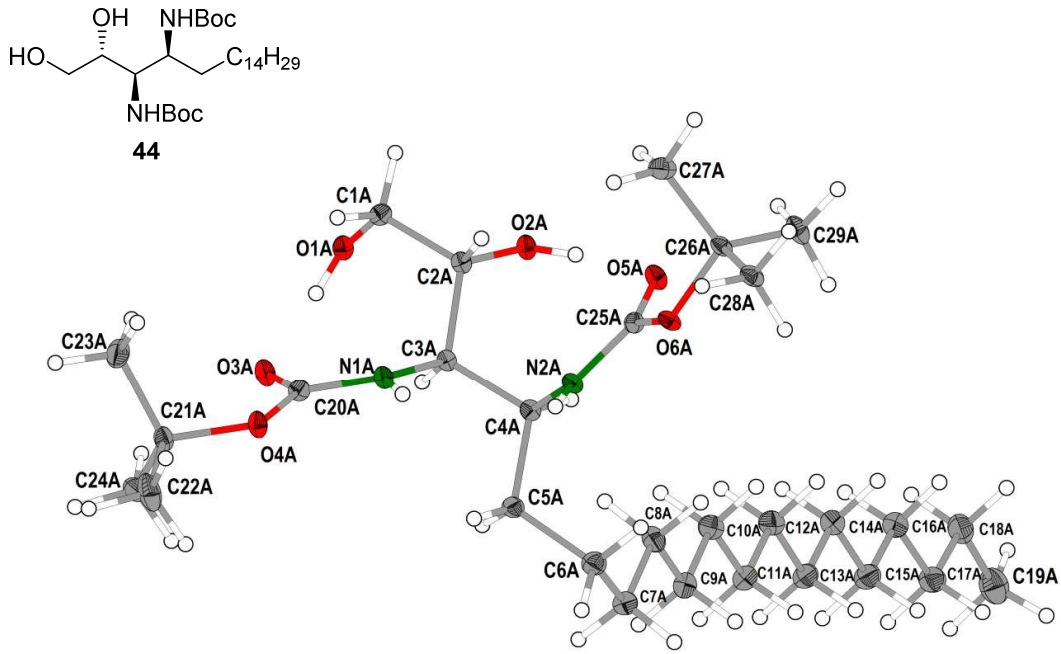
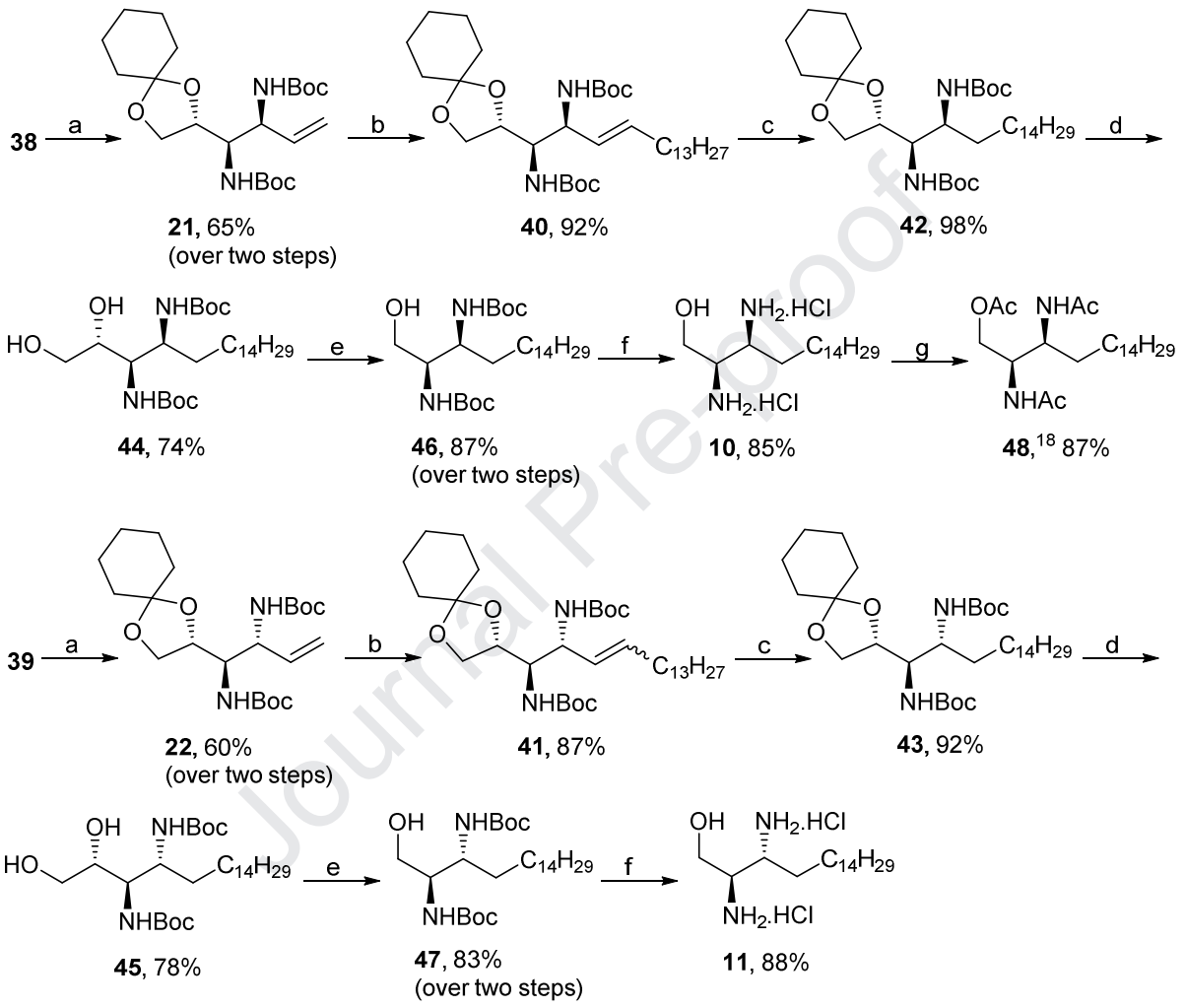


Figure 2. ORTEP structure of **44** showing the crystallographic numbering.

Having confirmed a structure of the key templates **30**, **31**, **38** and **39**, our focus was then shifted to the synthesis of the final compounds **10**-**15** (Schemes 7 and 8). To construct 3-amino-3-deoxydihydrosphingosines **10** and **11**, the corresponding isothiocyanates **38** and **39** were, after treatment with TBTO, submitted to the reaction with Boc_2O to furnish the protected derivatives **21** (65%) and **22** (60%, in both cases over two steps). The olefin cross metathesis reaction of **21** and **22** with pentadec-1-ene in the presence of second generation Grubbs catalyst (CH_2Cl_2, reflux for **21** or toluene, 60 °C for **22**) gave the (*E*)-configured coupling product **40** (92%) and a mixture of olefins **41** (87%), with a high prevalence of (*E*)-isomer. Subsequent reduction of the double bond under catalytic hydrogenation conditions (H_2, 5% Rh/Al_2O_3) led to the saturated products **42** (98%) and **43** (92%). Exposure of **42** and **43** to acid hydrolysis then resulted in cleavage of the cyclohexylidene ketal to provide diols **44** (74%) and **45** (78%). The modification of **44** and **45** into the diamino alcohols **46** (87%) and **47** (83%) was achieved via an oxidation/reduction process. Finally, exposure of **46** and **47** to 6 M HCl furnished the expected sphingoid bases **10** (85%) and **11** (88%), respectively

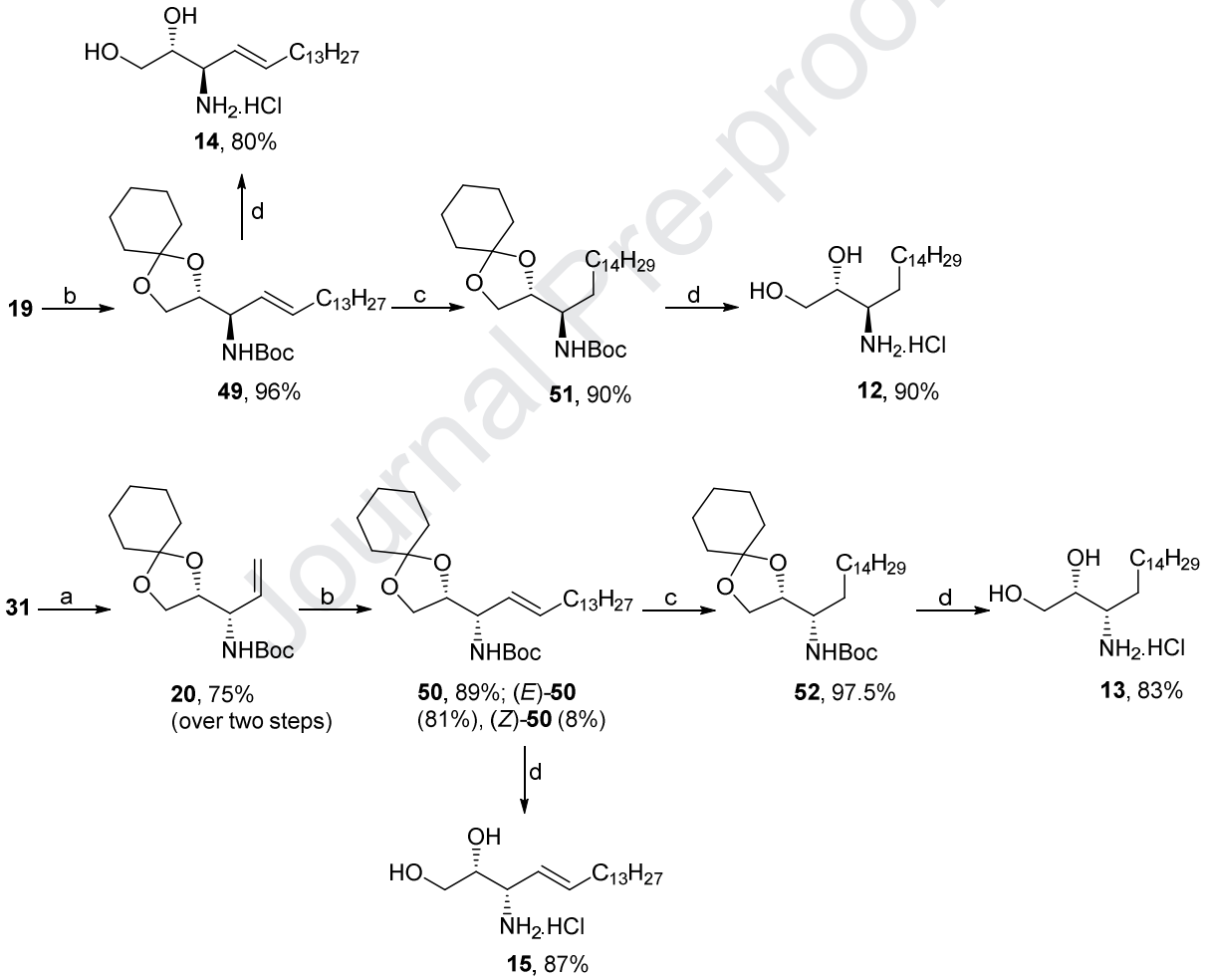
(Scheme 7). As an additional determination of structure, we converted **10** into the known acetylated derivative **48** (87%). The obtained material exhibited spectroscopic (^{1}H and ^{13}C NMR) and optical rotation data ($[\alpha]_D^{21}$ = –19.4, (*c* 0.3, $CHCl_3$); lit.[18] $[\alpha]_D^{22}$ = –16.1 (*c* 1, $CHCl_3$)) consistent with those reported in the literature for the same compound.[18] Melting point values were found to show small differences (mp 100–102 °C; lit.[18] mp 115–116 °C).



Scheme 7. *Reagents and conditions:* (a) (i) TBTO, toluene, 60 °C; (ii) Boc_2O, Et_3N, CH_2Cl_2, rt; (b) pentadec-1-ene, Grubbs II, CH_2Cl_2, reflux or toluene, 60 °C; (c) H_2, 5% Rh/Al_2O_3, EtOH or EtOH/THF (1:1), rt; (d) *p*-TsOH, $MeOH/H_2O$ (20:1), 55 °C; (e) (i) $NaIO_4$, $MeOH/H_2O$, rt; (ii) $NaBH_4$, EtOH, 0 °C; (f) 6 M aq HCl, 80 °C; (g) Ac_2O, pyridine, DMAP, rt.

A similar strategy as used in Scheme 7 was applied to the synthons **19** and **20** to obtain isomeric analogues of **1** and **2** (compounds **12**-**15**, Scheme 8). For this purpose, the minor isothiocyanate **31** was modified into *N*-Boc protected derivative **20** (75%, over two steps) according to the same reaction conditions employed for the conversion of **30** to **19** (see,

Scheme 6). The coupling of pentadec-1-ene with **19** and **20** was accomplished via cross-metathesis to give (*E*)-olefin **49** (96%), and a separable mixture of the geometric isomers **50** (89%) with the dominant production of (*E*)-isomer. Column chromatography on silica gel allowed the straightforward separation of both compounds to give pure (*E*)-**50** (81%, >98:2 dr) and (*Z*)-**50** (8%, >96:4 dr), respectively. Because of the overlap of proton signals in the ^{1}H NMR spectrum in the crude reaction mixture, we were unable to determine the *E*/*Z*-ratio. The catalytic hydrogenation of **49** and (*E*)-**50**, followed by global deprotection of **51** (90%) and **52** (97.5%), delivered the isodihydosphingosines **12** and **13** in 90% and 83% yields, respectively (Scheme 8). The corresponding isomeric sphingosines **14** (80%) and **15** (87%) were then prepared from the coupling products **49** and (*E*)-**50** after hydrolysis under acidic conditions.



Scheme 8. *Reagents and conditions:* (a) (i) TBTO, toluene, 60 °C; (ii) Boc_2O, Et_3N, CH_2Cl_2, rt; (b) pentadec-1-ene, Grubbs II, CH_2Cl_2, reflux; (c) H_2, 5% Rh/Al_2O_3, EtOH, rt; (d) 6 M aq HCl, 80 °C.

2.2. Antiproliferative/cytotoxic activity

A series of novel sphingoid bases **10-15** was evaluated for their *in vitro* cytotoxicity against several different human cancer cell lines, such as MDA-MB-231 (mammary gland adenocarcinoma), A-549 (non-small cell lung cancer), MCF-7 (mammary gland adenocarcinoma), Caco-2 (human colon carcinoma) and HCT-116 (human colon carcinoma), which were selected for compounds **12-15** (Table 4). A panel of four malignant cells – PaTu (human pancreatic adenocarcinoma), HeLa (human cervical adenocarcinoma), A2058 (human melanoma cells), Jurkat (human acute T-lymphoblastic leukaemia) – was used for all final derivatives **10-15** (Table 5). Two types of non-malignant cell lines, specifically NiH 3T3 (mouse fibroblasts) and MCF-10A (human mammary epithelial cells) were also included. The potency of the screened structures was determined by MTT assay with triplicate experiments.[28] Traditional anticancer substances cisplatin and etoposide (VP-16) were included as a positive control, and the corresponding results are shown in Tables 4 and 5.

The cytotoxicity results (Table 5) revealed that compounds **10-15** displayed remarkable antiproliferative activity against all the screened cell lines. Derivatives **12-15** were also significantly active on MDA-MB-231, A-549, MCF-7, HCT-116 and Caco-2 cells, with the IC_{50} values lower or comparable to that of cisplatin (Table 4).

Overall, all the tested derivatives were found to be more potent on leukaemia than on solid tumour cell lines (compare Tables 4 and 5). Moreover, 3-amino-3-deoxydihydrosphingosine **10** (IC_{50} < 0.5 μM for Jurkat, IC_{50} = 1.3 μM for HeLa) demonstrates higher potency against Jurkat and HeLa cells than commercially available etoposide (IC_{50} = 1.2 μM for Jurkat, IC_{50} = 3.9 μM for HeLa), respectively. Similarly, isomeric sphinganines **13** (IC_{50} = 1.3 μM) and **14** (IC_{50} = 1.8 μM) are at least 9-12 × more active against the Jurkat cell line than cisplatin (IC_{50} = 16.2 μM). Unfortunately, as seen in Table 5, all synthesized sphingoid bases **10-15** demonstrate very low values of SI (selectivity index) across all cell lines. This suggests that these compounds are equally or more toxic for non-malignant human mammary epithelial cells MCF-10A. Similarly, compounds **12-15** showed powerful cytotoxicity for mouse fibroblasts NiH 3T3 (Table 4). Therefore, the final sphingoid bases **10-15** could be further transformed to reduce their toxicity and to enhance selectivity.

Table 4. Antiproliferative/cytotoxic activities of compounds **12-15** on five human cancer cell lines (MDA-MB-231, A-549, MCF-7, HCT-116 and Caco-2) 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	NiH 3T3
12	6.9±0.4	7.0±0.7	8.2±1.6	3.3±0.6	6.3±2.1	<0.5

13	6.0±2.5	5.7±1.9	7.7±1.3	2.2±1.3	5.5±2.3	3.3±2.2
14	8.9±1.6	6.8±1.3	7.5±1.9	5.7±0.2	8.3±2.0	5.6±2.1
15	7.8±0.1	7.2±0.9	7.6±0.1	6.6±0.2	7.7±0.6	2.0±1.9
VP-16[29]	21.2±4.2	14.3±4.2	10.9±2.1	NT	NT	NT
cisplatin	17.5±0.5	9.5±0.2	15.6±0.3	15.3±0.5	15.2±0.3	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 the number of viable cells to 50% relative to untreated control cells, see Section 4.2).
NT-not tested.

Table 5. Antiproliferative/cytotoxic activities of compounds **10**-**15** on four human cancer cell lines (PaTu, HeLa, A2058, Jurkat) and non-malignant human mammary epithelial cells MCF-10A

Compd no.	Cell line, IC_{50}[a] ± SD (μmol × L^{-1})				
	PaTu	HeLa	A2058	Jurkat	MCF-10A
10	8.9±3.9	1.3±1.0	0.7±0.3	<0.5	0.8±0.2
11	23.1±12.7	5.0±5.9	21.6±5.8	7.0±1.4	16.5±10.5
12	7.8±2.7	6.1±1.4	6.4±1.9	2.4±1.0	6.9±0.1
13	6.2±2.3	5.9±2.2	4.3±2.8	1.3±0.9	7.3±4.6
14	5.3±1.8	6.7±1.2	7.0±1.4	1.8±2.0	6.2±2.2
15	7.8±1.7	4.1±2.5	6.5±1.8	4.2±2.2	9.4±0.9
cisplatin	NT	13.1±0.2	NT	16.2±0.6	NT
VP-16[29]	NT	3.9±2.3	NT	1.2±1.5	NT

[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 the number of viable cells to 50% relative to untreated control cells, see Section 4.2).
NT-not tested.

3. Conclusion

In summary, we successfully performed the diastereoselective construction of 3-amino-3-deoxydihydrosphingosines **10**-**11** and their analogues **12**-**15** by means of [3,3]-sigmatropic rearrangements of the allylic substrates (*E*/*Z*)-**16**, (*E*/*Z*)-**17** and **18**, and a late stage OCM reaction. The corresponding stereochemistries at C-2 and C-3 in **10**-**11** and at C-3 in **12**-**15** were unambiguously assigned through crystallographic analysis of the complex structure **44**. The target compounds **10**-**15** were evaluated *in vitro* for their capacity to alter the viability of the tested cancer cell lines. All prepared unusual sphingoid bases displayed cytotoxicity against screened malignant cell lines with a potency higher or comparable to those of the traditional anticancer agents cisplatin and etoposide. On the other hand, the observed strong toxicity towards non-malignant cell lines requires demands for further studies aiming at the construction of other analogues either with the alkyl chain modified by means of Grubbs coupling or with the installed ceramide functionality. An additional study to improve the activity profile of these compounds is already underway in our laboratory and progress will be reported in due course.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

Column chromatography was run on silica gel Kieselgel 60 (0.040–0.063 mm, 230–400 mesh, Merck) under pressure, with solvents that had been distilled prior to use. Analytical thin layer chromatography was performed on Merck silica gel 60 F_{254} plates and the visualization utilized either UV light (254 nm) or spraying with a solution of phosphomolybdic acid, with subsequent heating. NMR spectra were recorded on a Varian Mercury Plus 400 FT NMR (400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C) spectrometer or on a Varian Premium COMPACT 600 (599.87 MHz for ^{1}H and 150.84 MHz for ^{13}C). 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 $CDCl_3$ (δ = 7.26 ppm), CD_3OD (δ = 3.31 or δ = 4.87) and C_6D_6 (δ = 7.16 ppm) and for ^{13}C relative to $CDCl_3$ (δ = 77.16), CD_3OD (δ = 49.00) and C_6D_6 (δ = 128.06 ppm). 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 reported in wavenumber (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 determined using a P-2000 Jasco polarimeter. Melting points were recorded on a Kofler hot block and are uncorrected. Microwave reactions were carried out on a focused microwave system. The contents of the vessel were cooled rapidly using a stream of compressed air.

All commercial reagents were purchased from suppliers such as Sigma-Aldrich or Acros Organics. Solvents were dried and purified before use according to standard procedures.

4.1.2. (S)-2-(Benzyloxy)-2-{(S)-1',4'-dioxaspiro[4.5]decan-2'-yl}ethan-1-ol ***24***[21]

A solution of the known derivative **23**[20] (7.45 g, 35.1 mmol) in dry toluene (45 mL) was successively treated with cyclohexanone (36 mL, 0.35 mol) and *p*-TsOH (0.67 g, 3.51 mmol). After being stirred for 1 h at room temperature, the mixture was poured into a saturated NH_4Cl solution (45 mL) and extracted with EtOAc (2 × 70 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and the residue was subjected to flash chromatography

on silica gel (*n*-hexane/EtOAc, 3:1) to afford 8.70 g (85%) of compound **24** as a colourless oil; $[\alpha]_D^{21}$ –19.1 (*c* 1.16, $CHCl_3$). IR (neat) *υ* 3439, 2932, 2860, 1449, 1365, 1280, 1163, 1094, 1067, 1027 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.37−1.44 (m, 2H, CH_2), 1.55−1.69 (m, 8H, 4 × CH_2), 2.27 (t, *J* = 5.4 Hz, 1H, OH), 3.54–3.61 (m, 2H, H-1, H-2), 3.67−3.74 (m, 1H, H-1), 3.77−3.82 (m, 1H, H-3'), 4.00 (dd, *J* = 6.6, 8.2 Hz, 1H, H-3'), 4.26–4.32 (m, 1H, H-2'), 4.70 (d, *J* = 11.8 Hz, 1H, OCH_2Ph), 4.79 (d, *J* = 11.8 Hz, 1H, OCH_2Ph), 7.25−7.38 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 24.0 (CH_2), 24.2 (CH_2), 25.3 (CH_2), 34.9 (CH_2), 36.2 (CH_2), 61.9 (C-1), 65.4 (C-3'), 72.9 (OCH_2Ph), 76.5 (C-2'), 79.5 (C-2), 110.2 (C_q), 128.0 (3 × CH_{Ph}), 128.6 (2 × CH_{Ph}), 138.4 (C_i). ESI-HRMS: *m/z* calcd for $C_{17}H_{25}O_4$ $[M + H]^+$ 293.1747, found 293.1759.

*4.1.3. (S)-1-{(S)-1',4'-Dioxaspiro[4.5]decan-2'-yl}ethane-1,2-diol **25***

To a solution of **24** (8.60 g, 29.4 mmol) in dry EtOH (230 mL) were added a mixture of catalysts 10% Pd/C/20% $Pd(OH)_2$/C (1:1, 2.00 g). The resulting suspension was degassed three times and was stirred for 3 h at room temperature under an atmosphere of hydrogen. After this period, the mixture was filtered through a small pad of Celite, concentrated, and the crude product was chromatographed on silica gel (EtOAc) to provide 5.50 g (92%) of compound **25** as white crystals; mp 33−35 °C; $[\alpha]_D^{21}$ +7.3 (*c* 0.18, $CHCl_3$). IR (neat) *υ* 3408, 3363, 2934, 2852, 1445, 1366, 1285, 1092, 1066, 1028, 1012, 928 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.32–1.47 (m, 2H, CH_2), 1.52–1.72 (m, 8H, 4 × CH_2), 2.24−2.34 (m, 1H, OH), 2.57–2.64 (m, 1H, OH), 3.58–3.75 (m, 3H, H-1, 2 × H-2), 3.72–3.80 (m, 1H, H-3), 4.02–4.07 (m, 1H, H-3'), 4.15–4.21 (m, 1H, H-2'); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 23.9 (CH_2), 24.2 (CH_2), 25.2 (CH_2), 34.9 (CH_2), 36.3 (CH_2), 64.5 (C-2), 65.7 (C-3'), 71.5 (C-1), 76.5 (C-2'), 110.4 (C_q). ESI-HRMS: *m/z* calcd for $C_{10}H_{18}NaO_4$ $[M + Na]^+$ 225.1097, found 225.1101.

*4.1.4. Ethyl (R,E)-3-(1',4'-dioxaspiro[4.5]decan-2'-yl)acrylate (E)-**26**[22] and ethyl (R,Z)-3-(1',4'-dioxaspiro[4.5]decan-2'-yl)acrylate (Z)-**26***

Compound **25** (5.50 g, 27.2 mmol), which was dissolved in CH_2Cl_2 (150 mL), was treated with a solution of $NaIO_4$ (6.74 g, 31.5 mmol) in water (60 mL), and the resulting mixture was stirred for 40 min at room temperature. The layers were separated and the aqueous one was washed with further portions of CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated. The crude aldehyde[30] (4.63 g) was used in subsequent reaction without further purification.

Horner-Wadsworth-Emmons reaction: A suspension of NaH (1.40 g, 35.0 mmol, ~60% dispersion in mineral oil) in dry THF (45 mL) was treated with $(EtO)_2P(O)CH_2CO_2Et$ (3.20 mL, 16.0 mmol) at 0 °C. After being stirred for 25 min at 0 °C, a solution of the crude aldehyde (2.20 g, 12.90 mmol) in dry THF (12 mL) was added and stirring was continued for 20 min at the same temperature. The reaction was then quenched with a saturated NH_4Cl solution (30 mL) and the aqueous layer was washed with EtOAc (2 × 40 mL). The combined organic extracts were dried over Na_2SO_4, the solvent was evaporated, and the residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 15:1) to give 2.76 g (89%) of compound (*E*)-**26** as a colourless oil.

Wittig reaction: $Ph_3P{=}CHCO_2Et$ (6.40 g, 18.40 mmol) was added to a solution of the crude aldehyde (2.40 g, 14.10 mmol) in benzene (45 mL). After being stirred and refluxed for 25 min, the mixture was allowed to cool to room temperature, the solvent was evaporated, and the residue was chromatographed on silica gel (*n*-hexane/EtOAc, 15:1) to afford 1.90 g (56%, >97:3 dr) of (*Z*)-**26** and 1.22 g (36%, >97:3 dr) of (*E*)-**26** as colourless oils.

Ester (*E*)-**26**: R_f = 0.28 (*n*-hexane/EtOAc, 15:1); $[\alpha]_D^{21}$ –31.9 (*c* 0.34, $CHCl_3$); lit.[22] $[\alpha]_D^{22}$ +31.2 (*c* 1.2, $CHCl_3$); lit.[31] $[\alpha]_D^{30}$ +32.5 (*c* 0.7, $CHCl_3$) for *ent*-(*E*)-**26**. IR (neat) υ 2934, 2862, 1717, 1661, 1448, 1301, 1261, 1161 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (t, *J* = 7.1 Hz, 3H, CH_3), 1.38–1.45 (m, 2H, CH_2), 1.56–1.69 (m, 8H, 4 × CH_2), 3.67 (t, *J* = 7.6 Hz, 1H, H-3'), 4.15–4.24 (m, 3H, OCH_2, H-3'), 4.63–4.69 (m, 1H, H-2'), 6.11 (dd, *J* = 0.9, 15.6 Hz, 1H, H-2), 6.88 (dd, *J* = 5.6, 15.6 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4 (CH_3), 24.0 (CH_2), 24.1 (CH_2), 25.2 (CH_2), 35.4 (CH_2), 36.2 (CH_2), 60.7 (OCH_2), 68.6 (C-3'), 74.8 (C-2'), 111.0 (C_q), 122.5 (C-2), 145.1 (C-3), 166.2 (C=O). ESI-HRMS: *m/z* calcd for $C_{13}H_{20}NaO_4$ $[M + Na]^+$ 263.1254, found 263.1263.

Ester (*Z*)-**26**: R_f = 0.48 (*n*-hexane/EtOAc, 15:1); $[\alpha]_D^{22}$ –77.0 (*c* 0.28, $CHCl_3$); lit.[32] $[\alpha]_D^{27}$ +84.6 (*c* 1.05, $CHCl_3$) for *ent*-(*Z*)-**26**. IR (neat) υ 2981, 2934, 2862, 1715, 1643, 1448, 1367, 1278, 1161 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (t, *J* = 7.1 Hz, 3H, CH_3), 1.38–1.45 (m, 2H, CH_2), 1.56–1.69 (m, 8H, 4 × CH_2), 3.59–3.64 (m, 1H, H-3'), 4.17 (q, *J* = 7.1 Hz, 2H, OCH_2), 4.37 (t, *J* = 7.6 Hz, 1H, H-3'), 5.47–5.53 (m, 1H, H-2'), 5.83 (dd, *J* = 0.9, 11.7 Hz, 1H, H-2), 6.36 (dd, *J* = 6.7, 11.7 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3 (CH_3), 23.9 (CH_2), 24.0 (CH_2), 25.2 (CH_2), 35.0 (CH_2), 36.3 (CH_2), 60.5 (OCH_2), 69.1 (C-3'), 73.2 (C-2'), 110.5 (C_q), 120.7 (C-2), 149.7 (C-3), 165.7 (C=O). ESI-HRMS: *m/z* calcd for $C_{13}H_{20}NaO_4$ $[M + Na]^+$ 263.1254, found 263.1266.

*4.1.5. (R,E)-3-(1',4'-Dioxaspiro[4.5]decan-2'-yl)prop-2-en-1-ol (E)-**27***

DIBAl-H (40.5 mL, 48.6 mmol, ~1.2 M solution in toluene) was added dropwise to a solution of (*E*)-**26** (3.90 g, 16.2 mmol) in dry CH_2Cl_2 (100 mL) that had been pre-cooled to −50 °C. After being stirred for 20 min at −50 °C, the reaction was quenched by slow addition of MeOH (9.0 mL). The whole mixture was then poured into a 30% K/Na tartrate solution (200 mL) and vigorous stirring was continued for 2 h at room temperature. Then the layers were separated and the aqueous one was washed with CH_2Cl_2 (2 × 70 mL). The combined organic extracts were dried over Na_2SO_4, concentrated, and the residue was subjected to flash chromatography on silica gel (*n*-hexane/EtOAc, 2:1) to give 3.00 g (93%) of compound (*E*)-**27** as a colourless oil; $[\alpha]_D^{22}$ –11.0 (*c* 0.54, $CHCl_3$). IR (neat) *υ* 3467, 2933, 2860, 1716, 1664, 1448, 1364, 1279, 1162, 1095, 968 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.35–1.43 (m, 2H, CH_2), 1.53–1.67 (m, 8H, 4 × CH_2), 3.56–3.61 (m, 1H, H-3'), 4.08 (dd, *J* = 6.5, 7.8 Hz, 1H, H-3'), 4.13–4.18 (m, 2H, 2 × H-1), 4.49–4.56 (m, 1H, H-2'), 5.67–5.74 (m, 1H, H-3), 5.95 (dt, *J* = 5.2, 15.5 Hz, 1H, H-2); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 24.0 (CH_2), 24.1 (CH_2), 25.3 (CH_2), 35.6 (CH_2), 36.4 (CH_2), 62.8 (C-1), 69.2 (C-3'), 76.2 (C-2'), 110.1 (C_q), 128.6 (C-3), 133.4 (C-2). ESI-HRMS: *m/z* calcd for $C_{11}H_{18}NaO_3$ $[M + Na]^+$ 221.1148, found 221.1148.

*4.1.6. (R,Z)-3-(1',4'-Dioxaspiro[4.5]decan-2'-yl)prop-2-en-1-ol (Z)-**27***

Using the same procedure as described for the construction of (*E*)-**27**, ester (*Z*)-**26** (1.84 g, 7.66 mmol) was reduced with DIBAl-H (19.2 mL, 23.0 mmol, ~1.2 M solution in toluene) to afford after chromatography on silica gel (*n*-hexane/EtOAc, 2:1) 1.40 g (92%) of (*Z*)-**27** as a colourless oil; $[\alpha]_D^{22}$ +12.3 (*c* 0.44, $CHCl_3$); IR (neat) *υ* 3426, 2933, 2861, 1462, 1366, 1278, 1162, 1097, 1024, 847 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.36–1.44 (m, 2H, CH_2), 1.55–1.67 (m, 8H, 4 × CH_2), 2.07 (br s, 1H, OH), 3.54–3.59 (m, 1H, H-3'), 4.09 (dd, *J* = 6.7, 7.5 Hz, 1H, H-3'), 4.14–4.23 (m, 1H, H-1), 4.26–4.34 (m, 1H, H-1), 4.81–4.88 (m, 1H, H-2'), 5.53–5.60 (m, 1H, H-3), 5.79–5.86 (m, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 24.0 (CH_2), 24.1 (CH_2), 25.2 (CH_2), 35.6 (CH_2), 36.4 (CH_2), 58.8 (C-1), 69.3 (C-3'), 71.7 (C-2'), 110.3 (C_q), 129.9 (C-3), 133.1 (C-2). ESI-HRMS: *m/z* calcd for $C_{11}H_{18}NaO_3$ $[M + Na]^+$ 221.1148, found 221.1155.

*4.1.7. N-{(R)-1'-[(R)-1'',4''-Dioxaspiro[4.5]decan-2''-yl]allyl}-2,2,2-trichloroacetamide **28** and N-{(S)-1'-[(R)-1'',4''-dioxaspiro[4.5]decan-2''-yl]allyl}-2,2,2-trichloroacetamide **29***

*4.1.7.1. General procedure for the preparation of trichloroacetimidates (E)-**16** and (Z)-**16***

To a solution of (*E*)-**27** or (*Z*)-**27** (0.12 g, 0.61 mmol) in dry CH_2Cl_2 (3.0 mL) that had been pre-cooled to 0 °C were successively added DBU (9.0 μL, 0.06 mmol) and CCl_3CN (0.12 mL, 1.22 mmol). After stirring for 15 min at 0 °C, the mixture was filtered through a small pad of Celite, and the solvent was evaporated. This procedure yielded the corresponding crude imidates (*E*)-**16** or (*Z*)-**16**, which were then submitted to the next reaction without further purification.

4.1.7.2. Microwave-assisted thermal Overman rearrangement (general procedure)

The corresponding imidate (*E*)-**16** or (*Z*)-**16** (0.10 g, 0.29 mmol) was weighed in a 10-mL microwave tube equipped with a magnetic stirbar. *o*-Xylene (2.0 mL) and solid K_2CO_3 (46 mg, 0.33 mmol) were successively added, the tube was closed with septum, and the mixture was subjected to microwave irradiation (for the temperatures and reaction times, see Table 1). After completion, the insoluble parts were removed by filtration, the filtrate was concentrated, and the residue was chromatographed on silica gel (*n*-hexane/EtOAc, 9:1) to give trichloroacetamides **28** and **29** as an inseparable mixture of diastereoisomers (for the isolated combined yields, see Table 1).

4.1.7.3. Pd(II)-catalyzed Overman rearrangement (general procedure)

A solution of (*E*)-**16** or (*Z*)-**16** (0.17 g, 0.50 mmol) in dry toluene (2.0 mL) was successively treated with $PdCl_2(MeCN)_2$ (10 mg, 0.04 mmol) and *p*-benzoquinone (108 mg, 1.00 mmol). The resulting mixture was stirred and heated either for 40 °C or 70 °C (for the reaction times, see Table 1). After being cooled to room temperature, the mixture was filtered through a small pad of Celite, concentrated, and the residue was chromatographed on silica gel (*n*-hexane/EtOAc, 9:1) to provide the corresponding amides **28** and **29** in the combined yield of 40%, but only in the case of (*E*)-**16** (see Table 1).

Spectral data for both diastereoisomeric amides **28** and **29**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32−1.47 (m, 2.66H, CH_2-**28**, CH_2-**29**), 1.50−1.69 (m, 10.72H, 4 × CH_2-**28**, 4 × CH_2-**29**), 3.68 (dd, J = 6.5, 8.6 Hz, 1H, H-3"-**29**), 3.82 (dd, J = 5.2, 8.9 Hz, 1H, H-3"-**28**), 4.04−4.10 (m, 1.33H, H-3"-**28**, H-3"-**29**), 4.27−4.34 (m, 1.33H, H-2"-**28**, H-2"-**29**), 4.44−4.51 (m, 1.33H, H-1'-**28**, H-1'-**29**), 5.27−5.38 (m, 2.66H, H-3'$_{cis}$-**28**, H-3'$_{cis}$-**29**, H-3'$_{trans}$-**28**, H-3'$_{trans}$-**29**), 5.80−5.92 (m, 1.33H, H-2'-**28**, H-2'-**29**), 7.01−7.15 (m, 1.33H, NH-**28**, NH-**29**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.8 (CH_2-**28**, CH_2-**29**), 24.1 (CH_2-**28**, CH_2-**29**), 25.2 (CH_2-**28**, CH_2-**29**), 34.2 (CH_2-**28**), 34.5 (CH_2-**29**), 35.9 (CH_2-**28**), 36.2 (CH_2-**29**), 54.3 (C-1'-**29**), 56.0 (C-1'-**28**), 65.3 (C-3"-**28**), 66.2 (C-3"-**29**), 75.9 (C-2"-**28**), 76.3 (C-2"-**29**), 92.7 (CCl_3-**28**, CCl_3-**29**),

110.7 (C_q-**29**), 110.9 (C_q-**28**), 117.9 (C-3'-**29**), 119.3 (C-3'-**28**), 131.9 (C-2'-**28**), 134.1 (C-2'-**29**), 161.5 (C=O-**28**), 162.0 (C=O-**29**).

*4.1.8. (R,E)-2-(3'-Thiocyanatoprop-1'-en-1'-yl)-1,4-dioxaspiro[4.5]decane (E)-**17***

To a solution of (*E*)-**27** (2.70 g, 13.6 mmol) in dry CH_2Cl_2 (115 mL) were successively added Et_3N (3.82 mL, 27.2 mmol) and MsCl (2.10 mL, 27.2 mmol) at 0 °C. After being stirred for 20 min at the same temperature, the solvent was evaporated, and the residue was treated with Et_2O (40 mL). The insoluble parts were removed by filtration, the filtrate was concentrated, and the obtained mesylate was subjected to the subsequent reaction without further purification.

KSCN (2.00 g, 20.6 mmol) was added to a solution of the crude mesylate (3.76 g, 13.6 mmol) in dry acetonitrile (120 mL) and the resulting mixture was stirred for 2 h at room temperature. After completion of the reaction, the solvent was evaporated, and to the crude product was added Et_2O (40 mL). The generated solid material was filtered off, the solution was concentrated, and the residue was chromatographed on silica gel (*n*-hexane/EtOAc, 7:1) to furnish 2.67 g (82%) of thiocyanate (*E*)-**17** as a colourless oil; $[\alpha]_D^{21}$ –47.2 (*c* 2.94, $CHCl_3$). IR (neat) υ 2933, 2860, 2154, 1462, 1448, 1364, 1279, 1231, 1161, 1039 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.34–1.42 (m, 2H, CH_2), 1.52–1.65 (m, 8H, 4 × CH_2), 3.48–3.59 (m, 2H, 2 × H-3'), 3.59–3.64 (m, 1H, H-3), 4.12 (dd, *J* = 6.9, 7.7 Hz, 1H, H-3), 4.50–4.57 (m, 1H, H-2), 5.77–5.92 (m, 2H, H-1', H-2'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.9 (CH_2), 24.0 (CH_2), 25.2 (CH_2), 35.4 (CH_2), 35.5 (C-3'), 36.2 (CH_2), 69.2 (C-3), 75.3 (C-2), 110.5 (C_q), 111.6 (SCN), 125.2 (C-2'), 135.7 (C-1'). ESI-HRMS: *m/z* calcd for $C_{12}H_{17}NNaO_2S$ $[M + Na]^+$ 262.0872, found 262.0879.

*4.1.9. (R,Z)-2-(3'-Thiocyanatoprop-1'-en-1'-yl)-1,4-dioxaspiro[4.5]decane (Z)-**17***

By the same procedure as employed for the conversion of (*E*)-**27** into (*E*)-**17**, compound (*Z*)-**27** (1.00 g, 5.04 mmol) was transformed to thiocyanate (*Z*)-**17** (1.09 g, 90%, colourless oil, *n*-hexane/EtOAc, 7:1); $[\alpha]_D^{21}$ +132.7 (*c* 1.16, $CHCl_3$). IR (neat) υ 2933, 2859, 2154, 1462, 1448, 1367, 1278, 1231, 1161, 1039 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.35–1.44 (m, 2H, CH_2), 1.54–1.67 (m, 8H, 4 × CH_2), 3.54–3.65 (m, 2H, H-3, H-3'), 3.84–3.94 (m, 1H, H-3'), 4.15 (dd, *J* = 6.2, 8.3 Hz, 1H, H-3), 4.75–4.82 (m, 1H, H-2), 5.72–5.80 (m, 2H, H-1', H-2'); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.0 (CH_2), 24.1 (CH_2), 25.2 (CH_2), 31.0 (C-3'), 35.5 (CH_2), 36.4 (CH_2), 69.5 (C-3), 71.3 (C-2), 110.7 (C_q), 111.7 (SCN), 125.6 (C-2'), 134.3 (C-1'). ESI-HRMS: *m/z* calcd for $C_{12}H_{17}NNaO_2S$ $[M + Na]^+$ 262.0872, found 262.0880.

4.1.10. (R)-2-[(R)-1'-Isothiocyanatoallyl]-1,4-dioxaspiro[4.5]decane ***30*** *and (R)-2-[(S)-1'-isothiocyanatoallyl]-1,4-dioxaspiro[4.5]decane* ***31***

4.1.10.1. Conventional aza-Claisen rearrangement (general procedure)

A solution of the corresponding thiocyanate (*E*)-**17** or (*Z*)-**17** (0.10 g, 0.42 mmol) in *n*-heptane (2 mL) was stirred and heated under a nitrogen atmosphere (for the temperatures and reaction times, see Table 1). After completion of the reaction, the mixture was allowed to cool to room temperature, and the solvent was evaporated. Chromatography of the residue on silica gel (*n*-hexane/EtOAc, 19:1) delivered isothiocyanates **30** and **31** as a separable mixture of diastereoisomers (for the isolated combined yields, see Table 2).

4.1.10.2. Microwave-assisted synthesis (general procedure)

Thiocyanate (*E*)-**16** or (*Z*)-**16** (0.10 g, 0.42 mmol) was weighed in a 10-mL tube equipped with a magnetic stirbar. Then *n*-heptane (2 mL) was added, and the mixture was subjected to microwave irradiation (for the temperatures and reaction times, see Table 2). After completion of the reaction, the solvent was removed under reduced pressure, and the residue was chromatographed on silica gel (*n*-hexane/EtOAc, 19:1) to afford a mixture of **30** and **31** (for the combined yields, see Table 2).

Requiring greater amounts of the rearranged products **30** and **31**, they were prepared on a multigram scale by the conventional method at 70 °C starting from (*E*)-**17**.

Isothiocyanate **30**: colourless oil, R_f = 0.50 (*n*-hexane/EtOAc, 19:1); $[\alpha]_D^{21}$ +29.5 (*c* 0.42, $CHCl_3$). IR (neat) υ 2934, 2860, 2049, 2034, 1645, 1500, 1448, 1332, 1279, 1160 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.36–1.45 (m, 2H, CH_2), 1.52–1.72 (m, 8H, 4 × CH_2), 3.91 (dd, *J* = 5.2, 8.7 Hz, 1H, H-3), 4.05 (dd, *J* = 6.3, 8.7 Hz, 1H, H-3), 4.10–4.15 (m, 1H, H-2), 4.38–4.43 (m, 1H, H-1'), 5.33 (dd, *J* = 0.5, 10.3 Hz, 1H, H-3'$_{cis}$), 5.45 (dd, *J* = 0.5, 17.0 Hz, 1H, H-3'$_{trans}$), 5.81 (ddd, 1H, *J* = 5.0, 10.3, 17.0 Hz, 1H, H-2'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.8 (CH_2), 24.0 (CH_2), 25.1 (CH_2), 34.7 (CH_2), 36.3 (CH_2), 61.7 (C-1'), 65.6 (C-3), 76.9 (C-2), 111.3 (C_q), 118.4 (C-3'), 131.7 (C-2'), 135.1 (NCS). ESI-HRMS: *m/z* calcd for $C_{12}H_{17}NNaO_2S$ $[M + Na]^+$ 262.0872, found 262.0880.

Isothiocyanate **31**: colourless oil, R_f = 0.36 (*n*-hexane/EtOAc, 19:1); $[\alpha]_D^{21}$ –78.6 (*c* 0.46, $CHCl_3$). IR (neat) υ 2934, 2860, 2048, 2033, 1644, 1448, 1365, 1280, 1161 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.35–1.44 (m, 2H, CH_2), 1.51–1.72 (m, 8H, 4 × CH_2), 3.80 (dd, *J* = 5.3, 8.7 Hz, 1H, H-3), 4.04 (dd, *J* = 6.3, 8.7 Hz, 1H, H-3), 4.16–4.21 (m, 1H, H-2), 4.22–4.26 (m,

1H, H-1'), 5.35 (d, J = 10.3 Hz, 1H, H-3'$_{cis}$), 5.44 (d, J = 17.0 Hz, 1H, H-3'$_{trans}$), 5.73–5.87 (m, 1H, H-2'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.9 (CH_2), 24.1 (CH_2), 25.2 (CH_2), 34.7 (CH_2), 36.3 (CH_2), 62.3 (C-1'), 65.7 (C-3), 77.0 (C-2), 111.3 (C_q), 119.4 (C-3'), 131.6 (C-2'), 135.6 (NCS). ESI-HRMS: *m/z* calcd for $C_{12}H_{17}NNaO_2S$ $[M + Na]^+$ 262.0872, found 262.0878.

4.1.11. (4R,5R)-5-(Hydroxymethyl)-4-vinyloxazolidin-2-one ***33***[15] *and (4S,5R)-5-(hydroxymethyl)-4-vinyloxazolidin-2-one* ***34***[15]

To a solution of the mixture of amides **28** and **29** (0.16 g, 0.47 mmol, *dr* = 75:25) in MeOH (8 mL) was added *p*-TsOH (27 mg, 0.14 mmol) at room temperature. After being stirred and heated for 6 h at 65 °C, the mixture was quenched by neutralization with Et_3N, and the solvent was evaporated. Chromatography of the residue on silica gel (*n*-hexane/EtOAc, 1:2) afforded 0.12 g (95%) of diols **32** as an inseparable mixture of diastereoisomers.

The spectroscopic data for the corresponding mixture of diols **32**: ^{1}H NMR (400 MHz, CD_3OD) δ 3.53−3.56 (m, 0.66H, 2 × H-5'$_{min}$), 3.62–3.65 (m, 2H, 2 × H-5'$_{maj}$), 3.73–3.77 (m, 1H, H-4'$_{maj}$), 3.82 (td, 0.33H, J = 4.1, 6.1 Hz, 1H, H-4'$_{min}$), 4.46–4.50 (m, 1H, H-3'$_{maj}$), 4.50–4.51 (m, 0.33H, H-3'$_{min}$), 5.23–5.25 (m, 0.66H, 2 × H-1'$_{min}$), 5.25–5.32 (m, 2H, 2 × H-1'$_{maj}$), 5.90–6.01 (m, 1.33H, H-2'$_{maj}$, H-2'$_{min}$); ^{13}C NMR (100 MHz, CD_3OD) δ 57.0 (C-3'$_{min}$), 58.1 (C-3'$_{maj}$), 64.2 (C-5'$_{min}$), 64.3 (C-5'$_{maj}$), 73.2 (C-4'$_{maj}$), 73.4 (C-4'$_{min}$), 94.0 (CCl_{3maj}, CCl_{3min}), 117.4 (C-1'$_{min}$), 117.9 (C-1'$_{maj}$), 134.6 (C-2'$_{maj}$), 135.6 (C-2'$_{min}$), 163.4 (C=O$_{maj}$, C=O$_{min}$).

DBU (21 μL, 0.14 mmol) was added to a solution of stereoisomers **32** (0.12 g, 0.46 mmol) in dry CH_2Cl_2 (4.0 mL) at 0 °C and the resulting mixture was stirred for 21 h at room temperature. After completion of the reaction, the mixture was filtered through a small pad of Celite and concentrated. Chromatography of the residue on silica gel (EtOAc) gave 39 mg (60%) of compound **33** and 13 mg (20%) of **34**. The obtained oxazolidin-2-ones had spectroscopic data and optical rotation values in very good agreement with those reported in the literature for the same derivatives.

Diastereoisomer **33**: white crystals; mp 102−104 °C; $[\alpha]_D^{22}$ –8.1 (*c* 0.40, MeOH); lit.[15] mp 102−105 °C; $[\alpha]_D^{26}$ –7.0 (*c* 0.37, MeOH). IR (neat) υ 3438, 3239, 2978, 2953, 2931, 1742, 1690, 1408, 1314, 1246, 1117 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.65−3.68 (m, 2H, CH_2OH), 4.44–4.49 (m, 1H, H-4), 4.71 (ddd, J = 4.9, 6.0, 8.6 Hz, 1H, H-5), 5.29 (dt, J = 1.1, 10.3 Hz, 1H, H_{cis}-CH_2), 5.35 (dt, J = 1.1, 17.3 Hz, 1H, H_{trans}-CH_2), 5.94 (ddd, J = 7.1, 10.3, 17.3 Hz, 1H, CH_{vinyl}); ^{13}C NMR (100 MHz, CD_3OD) δ 58.1 (C-4), 61.7 (CH_2OH), 81.4 (C-5),

119.2 (CH_{2vinyl}), 134.6 (CH_{vinyl}), 161.5 (C=O). ESI-HRMS: *m/z* calcd for $C_6H_9NNaO_3$ [M + Na]$^+$ 166.0475, found 166.0483.

Diastereoisomer **34**: colourless oil; $[\alpha]_D^{22}$ –85.1 (*c* 0.15, MeOH); lit.[15] $[\alpha]_D^{26}$ –87.5 (*c* 0.16, MeOH). IR (neat) υ 3278, 2925, 1720, 1647, 1390, 1281, 1224, 1097 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.64 (dd, *J* = 4.1, 12.6 Hz, 1H, *H*-CH_2OH), 3.78 (dd, *J* = 3.2, 12.6 Hz, 1H, *H*-CH_2OH), 4.20–4.28 (m, 2H, H-4, H-5), 5.24 (dt, *J* = 0.9, 10.3 Hz, 1H, H_{cis}-CH_2), 5.33 (dt, *J* = 0.9, 17.1 Hz, 1H, H_{trans}-CH_2), 5.90 (ddd, *J* = 6.7, 10.3, 17.1 Hz, 1H, CH_{vinyl}); ^{13}C NMR (100 MHz, CD_3OD) δ 57.9 (C-4), 62.3 (CH_2OH), 84.0 (C-5), 118.2 (CH_{2vinyl}), 137.9 (CH_{vinyl}), 161.4 (C=O). ESI-HRMS: *m/z* calcd for $C_6H_9NNaO_3$ [M + Na]$^+$ 166.0475, found 166.0476.

*4.1.12. (4S,5R)-5-(Hydroxymethyl)-4-vinyloxazolidine-2-thione **35**[15]*

Compound **35** (32 mg, 96%) was prepared according to the same procedure as described in the literature[15] starting from the minor isothiocyanate **31** (50 mg, 0.21 mmol). Spectroscopic data, optical rotation and melting point of the obtained derivative **35** were in very good accord with those reported in the literature for the same compound; mp 130−132 °C; $[\alpha]_D^{21}$ −158.3 (*c* 0.60, $CHCl_3$); lit.[15] mp 130−133 °C; $[\alpha]_D^{26}$ −144.6 (*c* 0.48, MeOH). IR (neat) υ 3330, 3180, 2928, 1522, 1380, 1257, 1177 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.67 (dd, *J* = 4.1, 12.8 Hz, 1H, *H*-CH_2OH), 3.82 (dd, *J* = 4.1, 12.8 Hz, 1H, *H*-CH_2OH), 4.40 (t, *J* = 7.1 Hz, 1H, H-4), 4.50−4.55 (m, 1H, H-5), 5.29 (d, *J* = 10.2 Hz, 1H, H_{cis}-CH_2), 5.35 (d, *J* = 17.1 Hz, 1H, H_{trans}-CH_2), 5.89 (ddd, *J* = 7.1, 10.2, 17.1 Hz, 1H, CH_{vinyl}); ^{13}C NMR (100 MHz, CD_3OD) δ 61.6 (C-4), 62.0 (CH_2OH), 89.5 (C-5), 119.3 (CH_{2vinyl}), 136.4 (CH_{vinyl}), 190.6 (C=S). ESI-HRMS: *m/z* calcd for $C_6H_9NNaO_2S$ [M + Na]$^+$ 182.0246, found 182.0248.

*4.1.13. tert-Butyl {(R)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]allyl}carbamate **19***

TBTO (6.8 mL, 13.3 mmol) was added to a solution of **30** (1.60 g, 6.68 mmol) in dry toluene (37 mL) at room temperature. After being stirred and heated for 3 h at 60 °C, the mixture was allowed to cool to room temperature, and the solvent was evaporated. Chromatography of the residue on silica gel (EtOAc/MeOH/Et_3N, 80:20:1) afforded 1.25 g (95%) of the crude amine as a colourless oil, which was immediately submitted to the next reaction without spectral characterisation.

A solution of the obtained amine (1.25 g, 6.34 mmol) in dry CH_2Cl_2 (20 mL) was successively treated with Et_3N (1.0 mL, 7.12 mmol) and Boc_2O (2.10 g, 9.62 mmol). After stirring at room temperature overnight, the mixture was poured into a saturated NH_4Cl solution (20 mL) and was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and the residue was subjected to flash chromatography

on silica gel (*n*-hexane/EtOAc, 11:1) to give 1.55 g (82%) of **19** as white crystals; mp 66−68 °C; $[\alpha]_D^{21}$ +41.6 (*c* 0.44, $CHCl_3$). IR (neat) *υ* 3294, 2981, 2942, 2860, 1680, 1532, 1366, 1272, 1161 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.31–1.46 (m, 11H, 3 × CH_3, CH_2), 1.50–1.67 (m, 8H, 4 × CH_2), 3.77 (dd, *J* = 5.7, 8.5 Hz, 1H, H-3'), 4.01 (dd, *J* = 6.9, 8.5 Hz, 1H, H-3'), 4.10–4.23 (m, 2H, H-1, H-2'), 4.80 (br s, 1H, NH), 5.22 (d, *J* = 10.2 Hz, 1H, H-3_{cis}), 5.25 (d, *J* = 17.3 Hz, 1H, H-3_{trans}), 5.80−5.90 (m, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 23.9 (CH_2), 24.1 (CH_2), 25.3 (CH_2), 28.5 (3 × CH_3), 34.7 (CH_2), 36.1 (CH_2), 55.2 (C-1), 65.9 (C-3'), 77.2 (C-2'), 79.8 (C_q), 110.4 (C_q), 117.3 (C-3), 134.6 (C-2), 155.5 (C=O). ESI-HRMS: *m/z* calcd for $C_{16}H_{27}NNaO_4$ $[M + Na]^+$ 320.1832, found 320.1845.

4.1.14. Ethyl (R,E)-4-[(tert-butoxycarbonyl)amino]-4-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]but-2-enoate ***36***

Ozone was introduced to a solution of **19** (1.40 g, 4.71 mmol) in dry EtOH (40 mL) at –78 °C for 45 min. This resulted in the formation of a slightly blue solution. After excess ozone was purged with dry nitrogen, a solution of Ph_3P (1.24 g, 4.71 mmol) in dry CH_2Cl_2 (20 mL) was added at –78 °C, and the resulting mixture was stirred for another hour at room temperature. Then, the solvent was evaporated and the crude aldehyde was submitted to the subsequent reaction without further purification.

A suspension of NaH (0.50 g, 12.6 mmol, ~60% dispersion in mineral oil) in dry THF (9.5 mL) was treated with $(EtO)_2P(O)CH_2CO_2Et$ (1.18 mL, 5.89 mmol) at 0 °C. After stirring for 25 min at 0 °C, a solution of the obtained aldehyde (1.41 g, 4.71 mmol) in dry THF (6.5 mL) was added and stirring was continued for 30 min at the same temperature. The reaction was quenched with a saturated NH_4Cl solution (15 mL). The aqueous phase was separated and was washed with EtOAc (2 × 20 mL). The combined organic extracts were dried over Na_2SO_4, the solvent was evaporated, and the residue was chromatographed on silica gel (*n*-hexane/EtOAc, 7:1) to provide 1.36 g (78%) of compound **36** as a colourless oil; $[\alpha]_D^{21}$ +9.3 (*c* 0.44, $CHCl_3$). IR (neat) *υ* 3342, 2978, 2934, 2862, 1701, 1659, 1517, 1449, 1366, 1249, 1158 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.29 (t, *J* = 7.1 Hz, 3H, CH_3), 1.34–1.42 (m, 2H, CH_2), 1.45 (s, 9H, 3 × CH_3), 1.51–1.65 (m, 8H, 4 × CH_2), 3.79 (dd, *J* = 5.3, 8.7 Hz, 1H, H-3'), 4.04 (dd, *J* = 6.9, 8.7 Hz, 1H, H-3'), 4.13–4.24 (m, 3H, OCH_2, H-2'), 4.33−4.43 (m, 1H, H-4), 4.86 (d, *J* = 7.8 Hz, 1H, NH), 6.00 (d, *J* = 15.8 Hz, 1H, H-2), 6.93 (dd, *J* = 5.5, 15.8 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 14.3 (CH_3), 23.9 (CH_2), 24.1 (CH_2), 25.2 (CH_2), 28.5 (3 × CH_3), 34.6 (CH_2), 36.1 (CH_2), 54.1 (C-4), 60.7 (OCH_2), 65.9 (C-3'), 77.4 (C-2'), 80.3 (C_q),

110.9 (C_q), 123.1 (C-3), 144.2 (C-2), 155.2 (C=O), 166.1 (C=O). ESI-HRMS: *m/z* calcd for $C_{19}H_{31}NNaO_6$ $[M + Na]^+$ 392.2044, found 392.2046.

*4.1.15. tert-Butyl {(R,E)-4-hydroxy-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]but-2-en-1-yl}carbamate **37***

A solution of **36** (1.36 g, 3.68 mmol) in dry CH_2Cl_2 (17 mL) was treated with $BF_3.OEt_2$ (0.57 mL, 4.60 mmol) at −78 °C, and the resulting solution was stirred for 25 min at the same temperature. Then DIBAl-H (16.8 mL, 20.2 mmol, ~1.2 M solution in toluene) was added dropwise at –78 °C and stirring was continued for another 30 min. The reaction was quenched by slow addition of MeOH (4.2 mL), and the whole mixture was then poured into a 30% K/Na tartrate solution (85 mL). After stirring for 2 h at room temperature, the organic layer was separated and the aqueous one was washed with CH_2Cl_2 (2 × 35 mL). The combined organic extracts were dried over Na_2SO_4, stripped solvent, and the residue was chromatographed on silica gel (*n*-hexane/EtOAc, 1:1) to give 1.10 g (91%) of compound **37** as a colourless oil; $[\alpha]_D^{21}$ +12.3 (*c* 0.40, $CHCl_3$). IR (neat) *υ* 3435, 3336, 3005, 2976, 2933, 2862, 1690, 1517, 1365, 1249, 1160, 1041 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.29−1.47 (m, 11H, 3 × CH_3, CH_2), 1.49–1.66 (m, 8H, 4 × CH_2), 1.75 (br s, 1H, OH), 3.76 (dd, *J* = 5.6, 8.5 Hz, 1H, H-3'), 4.00 (dd, *J* = 6.6, 8.5 Hz, 1H, H-3'), 4.09–4.25 (m, 4H, H-1, H-2', 2 × H-4), 4.83 (br s, 1H, NH), 5.72 (dd, *J* = 5.9, 15.5 Hz, 1H, H-2), 5.85 (dt, *J* = 5.1, 15.5 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 23.9 (CH_2), 24.1 (CH_2), 25.3 (CH_2), 28.5 (3 × CH_3), 34.6 (CH_2), 36.1 (CH_2), 54.3 (C-1), 63.1 (C-4), 66.0 (C-3'), 77.3 (C-2'), 79.9 (C_q), 110.5 (C_q), 127.7 (C-3), 132.2 (C-2), 155.4 (C=O). ESI-HRMS: *m/z* calcd for $C_{17}H_{29}NNaO_5$ $[M + Na]^+$ 350.1938, found 350.1954.

*4.1.16. tert-Butyl {(R,E)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]-4-thiocyanatobut-2-en-1-yl}carbamate **18***

Using the same procedure as described for the transformation (*E*)-**27** into (*E*)-**17**, compound **37** (1.10 g, 3.36 mmol) was converted into thiocyanate **18** (colourless oil, 1.08 g, 87%, (*n*-hexane/EtOAc, 3:1); $[\alpha]_D^{21}$ −18.5 (*c* 1.12, $CHCl_3$). IR (neat) *υ* 3344, 2933, 2861, 2154, 1697, 1506, 1449, 1365, 1233, 1160 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.33–1.46 (m, 11H, 3 × CH_3, CH_2), 1.49–1.65 (m, 8H, 4 × CH_2), 3.51−3.62 (m, 2H, 2 × H-4), 3.75−3.80 (m, 1H, H-3'), 3.99–4.04 (m, 1H, H-3'), 4.10−4.17 (m, 1H, H-2'), 4.19−4.30 (m, 1H, H-1), 4.87 (br d, *J* = 6.9 Hz, 1H, NH), 5.75−5.88 (m, 2H, H-2, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 23.8 (CH_2), 24.1 (CH_2), 25.2 (CH_2), 28.4 (3 × CH_3), 34.5 (CH_2), 35.9 (C-4), 36.1 (CH_2), 54.1 (C-1), 65.8 (C-3'), 76.8 (C-2'), 80.1 (C_q), 110.6 (C_q), 111.7 (SCN), 125.1 (C-2 or C-3), 134.2 (C-2 or C-

3), 155.3 (C=O). ESI-HRMS: *m/z* calcd for $C_{18}H_{28}N_2NaO_4S$ $[M + Na]^+$ 391.1662, found 391.1672.

4.1.17. tert-Butyl {(1R,2S)-2-isothiocyanato-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]but-3-en-1-yl}carbamate ***38*** *and tert-butyl {(1R,2R)-2-isothiocyanato-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]but-3-en-1-yl}carbamate* ***39***

4.1.17.1. Thermal aza-Claisen rearrangement (general procedure)

By the same procedure employed for the thermally driven aza-Claisen rearrangement of thiocyanates (*E*/*Z*)-**17**, compound **18** (75 mg, 0.20 mmol) was converted into the corresponding isothiocyanates **38** and **39** (*n*-hexane/EtOAc, 9:1, for the temperatures, reaction times and combined yields, see Table 3).

4.17.2. Microwave-assisted synthesis (general procedure)

According to the same procedure described for the microwave-promoted [3,3]-sigmatropic rearrangement of thiocyanates (*E*/*Z*)-**17**, compound **18** (75 mg, 0.20 mmol) was transformed to rearranged products **38** and **39** (*n*-hexane/EtOAc, 9:1, for the temperatures, reaction times and combined yields, see Table 3).

Requiring greater amounts of the rearranged products **38** and **39**, they were prepared on a multi-gram scale by the microwave-assisted synthesis at 70 °C starting from **18**.

Isothiocyanate **38**: colourless oil; R_f = 0.32 (*n*-hexane/EtOAc, 9:1); $[\alpha]_D^{21}$ −101.6 (*c* 0.80, $CHCl_3$); IR (neat) *υ* 3338, 2927, 2853, 2054, 1705, 1499, 1449, 1248, 1158, 1096, 1041 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) *δ* 1.15−1.27 (m, 2H, CH_2), 1.35 (s, 9H, 3 × CH_3), 1.43–1.60 (m, 8H, 4 × CH_2), 3.60−3.67 (m, 1H, H-2'), 3.70−3.79 (m, 2H, 2 × H-3'), 3.83–3.90 (m, 1H, H-1), 4.18 (d, *J* = 10.0 Hz, 1H, NH), 4.61−4.65 (m, 1H, H-2), 4.89−4.94 (m, 1H, H-4_{cis}), 5.15−5.22 (m, 1H, H-4_{trans}), 5.48−5.58 (m, 1H, H-3); ^{13}C NMR (100 MHz, C_6D_6) *δ* 24.1 (CH_2), 24.4 (CH_2), 25.5 (CH_2), 28.3 (3 × CH_3), 35.3 (CH_2), 37.0 (CH_2), 56.5 (C-1), 61.7 (C-2), 67.3 (C-3'), 74.9 (C-2'), 79.8 (C_q), 110.9 (C_q), 117.2 (C-4), 133.2 (C-3), 137.3 (NCS), 155.4 (C=O). ESI-HRMS: *m/z* calcd for $C_{18}H_{28}N_2NaO_4S$ $[M + Na]^+$ 391.1662, found 391.1671.

Isothiocyanate **39**: colourless oil; R_f = 0.17 (*n*-hexane/EtOAc, 9:1); $[\alpha]_D^{21}$ −22.0 (*c* 0.46, $CHCl_3$). IR (neat) *υ* 3338, 2934, 2861, 2051, 1693, 1503, 1449, 1278, 1234, 1159, 1095, 1039 cm^{-1}; ^1H NMR (400 MHz, C_6D_6, 70 °C) *δ* 1.18−1.29 (m, 2H, CH_2), 1.44 (s, 9H, 3 × CH_3), 1.45–1.55 (m, 8H, 4 × CH_2), 3.48−3.55 (m, 1H, H-2'), 3.76 (dd, *J* = 6.1, 8.6 Hz, 1H, H-3'), 3.81 (dd, *J* = 6.0, 8.6 Hz, 1H, H-3'), 4.01–4.09 (m, 1H, H-1), 4.20 (d, *J* = 9.1 Hz, 1H, NH), 4.32−4.36 (m, 1H, H-2), 4.92 (dd, *J* = 0.7, 10.3 Hz, 1H, H-4_{cis}), 5.20 (dd, *J* = 0.7, 17.0 Hz,

1H, H-4$_{trans}$), 5.41−5.53 (m, 1H, H-3); ^{13}C NMR (100 MHz, C_6D_6) δ 24.2 (CH_2), 24.3 (CH_2), 25.4 (CH_2), 28.5 (3 × CH_3), 35.4 (CH_2), 36.7 (CH_2), 56.9 (C-1), 60.7 (C-2), 67.3 (C-3'), 75.0 (C-2'), 80.2 (C_q), 110.8 (C_q), 119.0 (C-4), 131.1 (C-3), 136.7 (NCS), 155.3 (C=O). ESI-HRMS: *m/z* calcd for $C_{18}H_{28}N_2NaO_4S$ $[M + Na]^+$ 391.1662, found 391.1670.

4.1.18. Di-tert-butyl {(1R,2S)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]but-3-ene-1,2-diyl}dicarbamate ***21***

To a solution of **38** (0.55 g, 1.49 mmol) in dry toluene (8.2 mL) was added TBTO (1.52 mL, 2.98 mmol) at room temperature. The resulting mixture was stirred and heated for 3 h at 60 °C. After completion of the reaction, the solvent was evaporated, and the residue was chromatographed on silica gel (EtOAc/0.5% Et_3N) to afford 0.36 g (73%) of the corresponding amine as a colourless oil, which was immediately subjected to the next reaction without spectral characterization.

A solution of the obtained amine (0.36 g, 1.10 mmol) in dry CH_2Cl_2 (3.6 mL) was successively treated with Et_3N (0.18 mL, 1.26 mmol) and Boc_2O (0.36 g, 1.65 mmol). After being stirred for 16 h at room temperature, the mixture was poured into a saturated NH_4Cl solution (4 mL) and was extracted with CH_2Cl_2 (2 × 10 mL). The combine organic extracts were dried over Na_2SO_4, stripped solvent, and the residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 5:1) to give 0.42 g (89%) of compound **21** as white crystals; mp 103−105 °C; $[\alpha]_D^{21}$ −44.6 (*c* 1.04, $CHCl_3$). IR (neat) υ 3368, 3024, 3005, 2970, 2932, 2862, 1737, 1725, 1692, 1518, 1449, 1230, 1160 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6, 70 °C) δ 1.23−1.30 (m, 2H, CH_2), 1.41 (s, 9H, 3 × CH_3), 1.44 (s, 9H, 3 × CH_3), 1.53–1.69 (m, 8H, 4 × CH_2), 3.85−4.02 (m, 4H, H-1, H-2', 2 × H-3'), 4.45−4.59 (m, 1H, H-2), 5.00 (d, *J* = 10.4 Hz, 1H, H-4$_{cis}$), 5.16 (d, *J* = 17.2 Hz, 1H, H-4$_{trans}$), 5.72−5.83 (m, 1H, H-3); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 24.3 (CH_2), 24.5 (CH_2), 25.7 (CH_2), 28.5 (3 × CH_3), 28.6 (3 × CH_3), 35.6 (CH_2), 36.9 (CH_2), 55.8 (C-1 or C-2), 57.0 (C-1 or C-2), 67.3 (C-3'), 76.0 (C-2'), 79.3 (C_q), 79.5 (C_q), 110.5 (C_q), 116.2 (C-4), 136.6 (C-3), 156.0 (C=O), 156.1 (C=O). ESI-HRMS: *m/z* calcd for $C_{22}H_{39}N_2O_6$ $[M + H]^+$ 427.2803, found 427.2816.

4.1.19. Di-tert-butyl {(1R,2R)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]but-3-ene-1,2-diyl}dicarbamate ***22***

By the same procedure as employed for the conversion of **38** to **21**, compound **39** (0.16 g, 0.43 mmol) was transformed over two steps to derivative **22** (white crystals, 0.11 g, 60%, *n*-hexane/EtOAc, 5:1); mp 196−197 °C; $[\alpha]_D^{21}$ +18.4 (*c* 0.58, $CHCl_3$). IR (neat) υ 3363, 3078,

2981, 2935, 2899, 2854, 1689, 1681, 1515, 1364, 1253, 1160 cm^{-1}; ^1H NMR (400 MHz, C_6D_6, 70 °C) δ 1.40 (s, 9H, 3 × CH_3), 1.42 (s, 9H, 3 × CH_3), 1.50–1.60 (m, 8H, 4 × CH_2), 1.63−1.68 (m, 2H, CH_2), 3.64−3.74 (m, 1H, H-2'), 3.83 (dd, J = 6.1, 8.4 Hz, 1H, H-3'), 3.88 (dd, J = 6.3, 8.4 Hz, 1H, H-3'), 4.00−4.08 (m, 1H, H-1), 4.43 (d, J = 7.8 Hz, 1H, NH), 4.67−4.74 (m, 1H, H-2), 4.95−5.07 (m, 2H, H-4_{cis}, NH), 5.10−5.17 (m, 1H, H-4_{trans}), 5.68 (ddd, J = 6.0, 10.5, 16.9 Hz, 1H, H-3); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 24.3 (CH_2), 24.4 (CH_2), 25.6 (CH_2), 28.5 (3 × CH_3), 28.6 (3 × CH_3), 35.6 (CH_2), 36.8 (CH_2), 55.1 (C-1), 57.5 (C-2), 67.6 (C-3'), 76.2 (C-2'), 79.2 (C_q), 79.7 (C_q), 110.7 (C_q), 116.7 (C-4), 135.5 (C-3), 155.5 (C=O), 156.0 (C=O). ESI-HRMS: *m/z* calcd for $C_{22}H_{39}N_2O_6$ $[M + H]^+$ 427.2803, found 427.2784.

4.1.20. Di-tert-butyl {(1R,2S,E)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]heptadec-3-ene-1,2-diyl}dicarbamate ***40***

To a solution of **21** (0.15 g, 0.35 mmol) in dry CH_2Cl_2 (5.9 mL) were successively added pentadec-1-ene (0.48 mL, 1.75 mmol) and second generation Grubbs catalyst (25.5 mg, 0.03 mmol). After stirring and heating under reflux for 7 h, the mixture was allowed to cool to room temperature. The solvent was evaporated, and the residue was subjected to flash chromatography on silica gel (*n*-hexane/EtOAc, 7:1) to furnish 0.20 g (92%) of compound **40** as a colourless oil; $[\alpha]_D^{21}$ −18.1 (*c* 0.90, $CHCl_3$). IR (neat) υ 3371, 2980, 2964, 2920, 2851, 1702, 1682, 1529, 1509, 1464, 1265, 1163 cm^{-1}; ^1H NMR (400 MHz, C_6D_6, 70 °C) δ 0.90 (t, J = 6.5 Hz, 3H, CH_3), 1.24−1.36 (m, 22H, 11 × CH_2), 1.44 (s, 9H, 3 × CH_3), 1.46 (s, 9H, 3 × CH_3), 1.55–1.73 (m, 10H, 5 × CH_2), 1.91−1.99 (m, 2H, CH_2), 3.86−4.11 (m, 4H, H-1, H-2', 2 × H-3'), 4.42−4.59 (m, 1H, H-2), 5.48 (dd, J = 7.0, 15.0 Hz, 1H, H-3), 5.61−5.73 (m, 1H, H-4), NH protons not seen; ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 14.2 (CH_3), 23.1 (CH_2), 24.3 (CH_2), 24.5 (CH_2), 25.7 (CH_2), 28.6 (3 × CH_3), 28.7 (3 × CH_3), 29.6 (CH_2), 29.7 (CH_2), 29.8 (CH_2), 30.0 (CH_2), 30.1 (2 × CH_2), 30.2 (3 × CH_2), 32.3 (CH_2), 32.7 (CH_2), 35.7 (CH_2), 37.0 (CH_2), 55.3 (C-1 or C-2), 57.3 (C-1 or C-2), 67.3 (C-3'), 76.2 (C-2'), 79.2 (C_q), 79.4 (C_q), 110.5 (C_q), 127.9 (C-3 or C-4), 133.3 (C-3 or C-4), 156.1 (C=O), 156.2 (C=O). ESI-HRMS: *m/z* calcd for $C_{35}H_{65}N_2O_6$ $[M + H]^+$ 609.4837, found 609.4847.

*4.1.21. Di-tert-butyl {(1R,2R,E)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]heptadec-3-ene-1,2-diyl}dicarbamate (E)-**41** and di-tert-butyl {(1R,2R,Z)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]heptadec-3-ene-1,2-diyl}dicarbamate (Z)-**41***

According to the same procedure described for the conversion of **21** to **40**, compound **22** (0.11 g, 0.26 mmol) was modified to a mixture of the inseparable olefins **41** isolated as a colourless oil (0.16 g, 87%, *n*-hexane/EtOAc, 9:1).

Some selected signals for the major isomer (*E*)-**41**: ^{1}H NMR (400 MHz, C_6D_6, 70 °C) δ 0.90 (t, J = 6.7 Hz, 3H, CH_3), 1.43 (s, 9H, 3 × CH_3), 1.43 (s, 9H, 3 × CH_3), 1.90−2.09 (m, 2H, CH_2), 4.60–4.71 (m, 1H, H-2), 4.99 (d, J = 7.8 Hz, 1H, NH), 5.30−5.50 (m, 2H, H-3, H-4); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 14.2 (CH_3), 23.1 (CH_2), 24.4 (CH_2), 24.5 (CH_2), 25.7 (CH_2), 28.6 (6 × CH_3), 29.6 (CH_2), 29.8 (CH_2), 30.1 (2 × CH_2), 30.2 (4 × CH_2), 32.3 (CH_2), 32.8 (CH_2), 35.7 (CH_2), 36.8 (CH_2), 54.3 (C-1 or C-2), 57.6 (C-1 or C-2), 67.5 (C-3'), 76.5, (C-2'), 79.1 (C_q), 79.2 (C_q), 110.5 (C_q), 126.6 (C-3 or C-4), 134.1 (C-3 or C-4), 155.9 (C=O), 156.1 (C=O).

4.1.22. Di-tert-butyl {(1R,2S)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]heptadecane-1,2-diyl}dicarbamate ***42***

5% Rh/Al_2O_3 (87 mg) was added to a solution of **40** (0.15 g, 0.25 mmol) in dry EtOH (3.0 mL). The resulting suspension was degassed three times and stirred for 4 h at room temperature under an atmosphere of hydrogen. After completion of the reaction, the mixture was filtered through a small pad of Celite and concentrated. Chromatography of the crude product on silica gel (*n*-hexane/EtOAc, 7:1) gave 0.15 g (98%) of compound **42** as a colourless oil; $[\alpha]_D^{21}$ −21.2 (*c* 1.00, $CHCl_3$). IR (neat) υ 3362, 2923, 2853, 1692, 1455, 1391, 1365, 1242, 1161 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6, 70 °C) δ 0.90 (t, J = 6.7 Hz, 3H, CH_3), 1.26−1.74 (m, 56H, 19 × CH_2, 6 × CH_3), 3.89−4.07 (m, 5H, H-1, H-2, H-2', 2 × H-3'), 4.72 (br s, 1H, NH), 5.19 (br s, 1H, NH); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 14.2 (CH_3), 23.1 (CH_2), 24.3 (CH_2), 24.5 (CH_2), 25.7 (CH_2), 26.6 (CH_2), 28.6 (3 × CH_3), 28.7 (3 × CH_3), 29.8 (CH_2), 29.9 (CH_2), 30.0 (CH_2), 30.1 (4 × CH_2), 30.2 (4 × CH_2), 32.3 (CH_2), 35.8 (CH_2), 37.1 (CH_2), 53.0 (C-1 or C-2), 57.3 (C-1 or C-2), 67.3 (C-3'), 76.6 (C-2'), 79.2 (2 × C_q), 110.2 (C_q), 156.6 (2 × C=O). ESI-HRMS: *m/z* calcd for $C_{35}H_{67}N_2O_6$ $[M + H]^+$ 611.4994, found 611.5009.

4.1.23. Di-tert-butyl {(1R,2R)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]heptadecane-1,2-diyl}dicarbamate ***43***

By the same procedure as employed for the preparation of **42**, a mixture of alkenes **41**(0.15 g, 0.25 mmol) was converted into the saturated compound **43** (white amorphous product, 0.14 g, 92%, *n*-hexane/EtOAc, 9:1); $[\alpha]_D^{21}$ +8.27 (*c* 0.56, $CHCl_3$). IR (neat) υ 3456, 3368, 2970, 2921, 2851, 1737, 1684, 1448, 1365, 1217, 1162 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6, 70 °C) δ

0.90 (t, J = 6.3 Hz, 3H, CH_3), 1.20−1.39 (m, 28H, 14 × CH_2), 1.43 (s, 9H, 3 × CH_3), 1.44 (s, 9H, 3 × CH_3), 1.51−1.74 (m, 10H, 5 × CH_2), 3.83−4.03 (m, 5H, H-1, H-2, H-2', 2 × H-3'), 4.51 (d, J = 8.0 Hz, 1H, NH), 4.93 (br s, 1H, NH); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 14.2 (CH_3), 23.1 (CH_2), 24.4 (CH_2), 24.5 (CH_2), 25.7 (CH_2), 26.7 (CH_2), 28.6 (6 × CH_3), 29.8 (CH_2), 29.9 (CH_2), 30.0 (CH_2), 30.1 (2 × CH_2), 30.2 (6 × CH_2), 32.3 (CH_2), 35.7 (CH_2), 36.8 (CH_2), 54.4 (C-1 or C-2), 57.5 (C-1 or C-2), 67.7 (C-3'), 76.5 (C-2'), 79.1 (C_q), 79.3 (C_q), 110.5 (C_q), 156.0 (C=O), 156.5 (C=O). ESI-HRMS: *m/z* calcd for $C_{35}H_{67}N_2O_6$ $[M + H]^+$ 611.4994, found 611.4995.

*4.1.24. Di-tert-butyl [(2R,3R,4S)-1,2-dihydroxynonadecane-3,4-diyl]dicarbamate **44***

A solution of **42** (0.14 g, 0.23 mmol) in a mixture of MeOH/H_2O (10.5 mL, 20:1) was treated with *p*-TsOH (13 mg, 0.07 mmol). After stirring and heating at 55 °C for 5 h, the mixture was allowed to cool to room temperature, then quenched by neutralization with Et_3N, and concentrated. Chromatography of the residue on silica gel (*n*-hexane/EtOAc, 2:1) yielded 90 mg (74%) of compound **44** as white crystals; mp 117−119 °C; $[\alpha]_D^{21}$ −32.8 (*c* 0.58, $CHCl_3$). IR (neat) υ 3423, 3274, 2918, 2850, 1674, 1511, 1457, 1393, 1366, 1251, 1159, 1082 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6, 70 °C) δ 0.91 (t, J = 6.5 Hz, 3H, CH_3), 1.18−1.36 (m, 28H, 14 × CH_2), 1.39 (s, 9H, 3 × CH_3), 1.42 (s, 9H, 3 × CH_3), 3.39−3.46 (m, 1H, H-1), 3.68−3.83 (m, 3H, H-1, H-2, H-3), 3.87−4.03 (m, 1H, H-4), 4.32 (br s, 1H, NH), 5.03 (br s, 1H, NH); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 14.2 (CH_3), 23.1 (CH_2), 26.7 (CH_2), 28.5 (3 × CH_3), 28.6 (3 × CH_3), 29.8 (CH_2), 30.0 (2 × CH_2), 30.1 (2 × CH_2), 30.2 (6 × CH_2), 32.3 (CH_2), 51.5 (C-3 or C-4), 56.0 (C-3 or C-4), 64.2 (C-1), 72.5 (C-2), 79.7 (C_q), 80.1 (C_q), 156.9 (C=O), 157.9 (C=O). ESI-HRMS: *m/z* calcd for $C_{29}H_{59}N_2O_6$ $[M + H]^+$ 531.4368, found 531.4378.

*4.1.25. Di-tert-butyl [(2R,3R,4R)-1,2-dihydroxynonadecane-3,4-diyl]dicarbamate **45***

Using the same procedure as employed for the preparation of **44**, compound **43** (0.14 g, 0.23 mmol) was converted into derivative **45** (white crystals, 95 mg, 78%, *n*-hexane/EtOAc, 2:1); mp 169−171 °C; $[\alpha]_D^{21}$ +12.1 (*c* 0.76, $CHCl_3$). IR (neat) υ 3370, 2979, 2920, 2852, 1678, 1518, 1457, 1389, 1364, 1305, 1247, 1163, 1042 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6, 70 °C) δ 0.90 (t, J = 6.5 Hz, 3H, CH_3), 1.20−1.37 (m, 28H, 14 × CH_2), 1.39 (s, 9H, 3 × CH_3), 1.41 (s, 9H, 3 × CH_3), 3.49 (dt, J = 3.4, 7.2 Hz, 1H, H-2), 3.61 (dd, J = 3.4, 12.0 1H, Hz, H-1), 3.71 (dd, J = 3.4, 12.0 Hz, 1H, H-1), 3.82−3.92 (m, 2H, H-3, H-4), 4.71 (d, J = 8.2 Hz, 1H, NH), 5.76 (br s, 1H, NH); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 14.2 (CH_3), 23.1 (CH_2), 27.0 (CH_2), 28.5 (6 × CH_3), 29.8 (2 × CH_2), 30.0 (CH_2), 30.1 (2 × CH_2), 30.2 (5 × CH_2), 32.3 (CH_2), 33.4

(CH_2), 54.7 (C-3 or C-4), 56.7 (C-3 or C-4), 64.1 (C-1), 72.9 (C-2), 79.5 (C_q), 79.8 (C_q), 157.2 (C=O), 157.5 (C=O). ESI-HRMS: *m/z* calcd for $C_{29}H_{59}N_2O_6$ $[M + H]^+$ 531.4368, found 531.4370.

4.1.26. Di-tert-butyl [(2R,3S)-1-hydroxyoctadecane-2,3-diyl]dicarbamate ***46***

A solution of **44** (90 mg, 0.17 mmol) in MeOH (2.1 mL) was treated with a solution of $NaIO_4$ (0.10 g, 0.48 mmol) in water (0.35 mL). After stirring for 1 h at room temperature, the mixture was diluted with CH_2Cl_2 and solid Na_2SO_4 was added. The insoluble material was removed by filtration, and the filtrate was concentrated. The crude product was used in the next reaction without further purification.

$NaBH_4$ (13 mg, 0.34 mmol) was added to a solution of the obtained aldehyde (85 mg, 0.17 mmol) in anhydrous EtOH (3.8 mL) at 0 °C. After stirring for 20 min at 0 °C, the reaction was quenched by neutralization with Amberlite IR 120 H^+ form. The solid parts were filtered and the filtrate was concentrated. The residue was chromatographed on silica gel (*n*-hexane/EtOAc, 4:1 to afford 74 mg (87%) of compound **46** as white crystals; mp 53−55 °C; $[\alpha]_D^{21}$ −39.1 (*c* 0.54, $CHCl_3$). IR (neat) υ 3496, 3375, 3347, 2964, 2916, 2848, 1668, 1464, 1389, 1281, 1249, 1169, 1060 cm^{-1}; ^1H NMR (400 MHz, C_6D_6, 70 °C) δ 0.91 (t, J = 6.6 Hz, 3H, CH_3), 1.17−1.37 (m, 28H, 14 × CH_2), 1.41 (s, 9H, 3 × CH_3), 1.45 (s, 9H, 3 × CH_3), 3.35−3.44 (m, 1H, H-1), 3.50−3.63 (m, 1H, H-2), 3.71−3.80 (m 1H, H-1), 3.80−3.91 (m, 1H, H-3), 4.36 (br s, 1H, NH), 4.76 (br s, 1H, NH); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 14.2 (CH_3), 23.1 (CH_2), 26.5 (CH_2), 28.5 (3 × CH_3), 28.6 (3 × CH_3), 29.8 (2 × CH_2), 30.0 (2 × CH_2), 30.1 (3 × CH_2), 30.2 (3 × CH_2), 32.3 (CH_2), 32.8 (CH_2), 51.5 (C-2 or C-3), 56.4 (C-2 or C-3), 63.2 (C-1), 79.2 (C_q), 79.7 (C_q), 156.4 (C=O), 157.4 (C=O). ESI-HRMS: *m/z* calcd for $C_{28}H_{57}N_2O_5$ $[M + H]^+$ 501.4262, found 501.4278.

4.1.27. Di-tert-butyl [(2R,3R)-1-hydroxyoctadecane-2,3-diyl]dicarbamate ***47***

Using the same procedure as described for the preparation of **46**, compound **45** (92 mg, 0.17 mmol) was converted into derivative **47** (colourless oil, 71 mg, 83%, *n*-hexane/EtOAc, 4:1); $[\alpha]_D^{21}$ +17.1 (*c* 0.54, $CHCl_3$). IR (neat) υ 3510, 3358, 2980, 2921, 2852, 1679, 1460, 1390, 1321, 1162, 1044 cm^{-1}; ^1H NMR (400 MHz, C_6D_6, 70 °C) δ 0.90 (t, J = 6.6 Hz, 3H, CH_3), 1.14−1.37 (m, 27H, 13 × CH_2, *H*-CH_2), 1.39 (s, 9H, 3 × CH_3), 1.45 (s, 9H, 3 × CH_3), 1.53−1.66 (m, 1H, *H*-CH_2), 3.18 (br s, 1H, OH), 3.42−3.68 (m, 4H, 2 × H-1, H-2, H-3), 4.36 (d, J = 6.9 Hz, 1H, NH), 5.21 (d, J = 8.4 Hz, 1H, NH); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 14.2 (CH_3), 23.1 (CH_2), 26.6 (CH_2), 28.5 (3 × CH_3), 28.6 (3 × CH_3), 29.8 (CH_2), 29.9 (CH_2),

30.0 (CH_2), 30.1 (2 × CH_2), 30.2 (5 × CH_2), 32.2 (CH_2), 32.3 (CH_2), 52.3 (C-2 or C-3), 56.4 (C-2 or C-3), 62.7 (C-1), 79.0 (C_q), 79.7 (C_q), 156.2 (C=O), 157.4 (C=O). ESI-HRMS: *m/z* calcd for pre $C_{28}H_{57}N_2O_5$ $[M + H]^+$ 501.4262, found 501.4265.

*4.1.28. (2R,3S)-2,3-Diaminooctadecan-1-ol dihydrochloride **10***

Compound **46** (70 mg, 0.14 mmol) was treated with 6 M HCl (6.6 mL) and the resulting solution was heated and stirred for 4 h at 80 °C. After being concentrated, the obtained product was washed three times with cold Et_2O (3 × 5 mL) and dried. This procedure yielded 44 mg (85%) of compound **10** as a white amorphous solid; $[\alpha]_D^{21}$ −8.1 (*c* 0.52, MeOH). IR (neat) *υ* 3239, 2953, 2917, 2849, 1586, 1510, 1471, 1375, 1122, 1079, 1020 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) *δ* 0.90 (t, *J* = 6.9 Hz, 3H, CH_3), 1.24–1.57 (m, 26H, 13 × CH_2), 1.68−1.89 (m, 2H, CH_2), 3.61–3.70 (m, 2H, H-2, H-3), 3.89 (dd, *J* = 4.8, 11.4 Hz, 1H, H-1), 3.93 (dd, *J* = 5.1, 11.4 Hz, 1H, H-1); ^{13}C NMR (100 MHz, CD_3OD) *δ* 14.5 (CH_3), 23.7 (CH_2), 26.7 (CH_2), 29.4 (CH_2), 30.4 (CH_2), 30.5 (2 × CH_2), 30.7 (CH_2), 30.8 (6 × CH_2), 33.1 (CH_2), 53.6 (C-2 or C-3), 53.7 (C-2 or C-3), 59.9 (C-1). ESI-HRMS: *m/z* calcd for $C_{18}H_{41}N_2O$ [M – HCl, −Cl]$^+$ 301.3213, found 301.3211.

*4.1.29. (2R,3R)-2,3-Diaminooctadecan-1-ol dihydrochloride **11***

By the same procedure as employed for the conversion of **46** to **10**, compound **47** (70 mg, 0.14 mmol) was modified into derivative **11** (white amorphous solid, 46 mg, 88%); $[\alpha]_D^{21}$ +21.2 (*c* 0.32, MeOH). IR (neat) *υ* 3346, 2952, 2917, 2849, 1597, 1509, 1467, 1053, 1030 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) *δ* 0.90 (t, *J* = 6.8 Hz, 3H, CH_3), 1.23–1.58 (m, 26H, 13 × CH_2), 1.68−1.88 (m, 2H, CH_2), 3.54–3.63 (m, 2H, H-2, H-3), 3.88−3.99 (m, 2H, 2 × H-1); ^{13}C NMR (100 MHz, CD_3OD) *δ* 14.4 (CH_3), 23.7 (CH_2), 26.7 (CH_2), 30.3 (CH_2), 30.4 (2 × CH_2), 30.6 (CH_2), 30.7 (6 × CH_2), 31.1 (CH_2), 33.0 (CH_2), 53.1 (C-2 or C-3), 54.0 (C-2 or C-3), 59.2 (C-1). ESI-HRMS: *m/z* calcd for $C_{18}H_{41}N_2O$ [M – HCl, −Cl]$^+$ 301.3213, found 301.3213.

*4.1.30. (2R,3S)-2,3-Diacetamidooctadecyl acetate **48**[18]*

A solution of **10** (20 mg, 53.6 μmol) in dry pyridine (0.75 mL) was successively treated with Ac_2O (0.75 mL, 7.93 mmol) and DMAP (0.65 mg, 5.36 μmol). After being stirred for 4 h at room temperature, the solvent was evaporated, and the residue was chromatographed on silica gel (CH_2Cl_2/MeOH, 95:5). This procedure yielded 20 mg (87%) of the protected derivative **48** as white crystals; mp 100−102 °C; lit.[18] mp 115–116 °C; $[\alpha]_D^{21}$ −19.4 (*c* 0.30, $CHCl_3$); lit[18] $[\alpha]_D^{22}$ = –16.1 (*c* 1, $CHCl_3$). IR (neat) *υ* 3279, 2951, 2849, 1733, 1645, 1548, 1464, 1371,

1255, 1039 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H, CH_3), 1.21−1.45 (m, 27H, 13 × CH_2, *H*-CH_2), 1.58−1.64 (m, 1H, *H*-CH_2), 1.97 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.94−4.00 (m, 1H, H-3), 4.05−4.10 (m, 1H, H-2), 4.14 (dd, J = 3.7, 11.7 Hz, 1H, H-1), 4.21 (dd, J = 5.0, 11.7 Hz, 1H, H-1), 5.75 (d, J = 9.1 Hz, 1H, NH), 6.34 (d, J = 8.8 Hz, 1H, NH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 20.8 (CH_3), 22.7 (CH_2), 23.3 (2 × CH_3), 25.6 (CH_2), 29.4 (2 × CH_2), 29.5 (CH_2), 29.6 (2 × CH_2), 29.7 (5 × CH_2), 31.5 (CH_2), 31.9 (CH_2), 50.5 (C-3), 52.7 (C-2), 63.7 (C-1), 170.8 (C=O), 171.0 (C=O), 171.1 (C=O). ESI-HRMS: *m/z* calcd for $C_{24}H_{47}N_2O_4$ $[M + H]^+$ 427.3530, found 427.3531.

*4.1.31. tert-Butyl {(S)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]allyl}carbamate **20***

According to the same procedure employed for the preparation of **19**, compound **31** (0.32 g, 1.34 mmol) was converted into product **20** (white crystals, 0.30 g, 75%, *n*-hexane/EtOAc, 9:1); mp 42−42.5 °C; $[\alpha]_D^{21}$ −30.8 (*c* 0.36, $CHCl_3$). IR (neat) υ 3348, 2978, 2931, 2861, 1686, 1524, 1365, 1248, 1161, 1120 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.29–1.48 (m, 11H, 3 × CH_3, CH_2), 1.49–1.68 (m, 8H, 4 × CH_2), 3.69−3.74 (m, 1H, H-3'), 3.99−4.04 (m, 1H, H-3'), 4.14–4.23 (m, 2H, H-1, H-2'), 4.81 (br s, 1H, NH), 5.19 (d, J = 10.4 Hz, 1H, H-3_{cis}), 5.26 (d, J = 17.2 Hz, 1H, H-3_{trans}), 5.77−5.90 (m, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.9 (CH_2), 24.1 (CH_2), 25.3 (CH_2), 28.5 (3 × CH_3), 34.6 (CH_2), 36.1 (CH_2), 53.8 (C-1), 66.1 (C-3'), 77.4 (C-2'), 79.8 (C_q), 110.2 (C_q), 116.3 (C-3), 136.3 (C-2), 155.9 (C=O). ESI-HRMS: *m/z* calcd for $C_{16}H_{27}NNaO_4$ $[M + Na]^+$ 320.1832, found 320.1845.

*4.1.32. tert-Butyl {(R,E)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]hexadec-2-en-1-yl}carbamate **49***

To a solution of **19** (0.15 g, 0.50 mmol) in dry CH_2Cl_2 (8.5 mL) were successively added pentadec-1-ene (0.68 mL, 2.50 mmol) and second generation Grubbs catalyst (42.4 mg, 0.05 mmol). After being stirred and refluxed for 5 h, the mixture was concentrated, and the residue was chromatographed on silica gel (*n*-hexane/EtOAc, 11:1) to furnish 0.23 g (96%) of alkene **49** as white crystals; mp 29−30 °C; $[\alpha]_D^{21}$ +2.9 (*c* 0.56, $CHCl_3$). IR (neat) υ 3342, 2922, 2852, 1702, 1496, 1449, 1365, 1280, 1098, 1041 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) δ 0.92 (t, J = 6.7 Hz, 3H, CH_3), 1.21–1.36 (m, 24H, 12 × CH_2), 1.45 (s, 9H, 3 × CH_3), 1.51–1.64 (m, 6H, 3 × CH_2), 1.66−1.74 (m, 2H, CH_2), 1.92−1.99 (m, 2H, CH_2), 3.64−3.74 (m, 2H, 2 × H-3'), 3.89−3.99 (m, 1H, H-2'), 4.30–4.43 (m, 1H, H-1), 4.70 (d, J = 8.0 Hz, 1H, NH), 5.48 (dd, J = 6.6, 15.5 Hz, 1H, H-2), 5.56−5.69 (m, 1H, H-3); ^{13}C NMR (100 MHz, C_6D_6) δ 14.4 (CH_3), 23.2 (CH_2), 24.2 (CH_2), 24.4 (CH_2), 25.6 (CH_2), 28.5 (3 × CH_3), 29.6 (CH_2), 29.9 (CH_2), 30.0

(CH_2), 30.1 (CH_2), 30.2 (5 × CH_2), 32.4 (CH_2), 32.8 (CH_2), 35.2 (CH_2), 36.5 (CH_2), 55.0 (C-1), 66.3 (C-3'), 78.0 (C-2'), 79.0 (C_q), 110.2 (C_q), 126.8 (C-2), 133.8 (C-3), 155.2 (C=O). ESI-HRMS: *m/z* calcd for $C_{29}H_{54}NO_4$ $[M + H]^+$ 480.4047, found 480.4056.

*4.1.33. tert-Butyl {(S,E)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]hexadec-2-en-1-yl}carbamate (E)-**50** and terc-butyl {(S,Z)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]hexadec-2-en-1-yl}carbamate(Z)-**50***

Using the same procedure as described for the preparation of **49**, compound **20** (0.10 g, 0.34 mmol) was converted into a separable mixture of olefins **50** (145 mg, 89%, *n*-hexane/EtOAc, 11:1).

Isomer (*E*)-**50**: 132 mg (81%, >98:2 dr); colourless oil; $[\alpha]_D^{21}$ −2.5 (*c* 1.48, $CHCl_3$). IR (neat) *υ* 3452, 2922, 2852, 1717, 1493, 1466, 1365, 1280, 1162, 1042 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) *δ* 0.92 (t, *J* = 6.7 Hz, 3H, CH_3), 1.23–1.35 (m, 24H, 12 × CH_2), 1.46 (s, 9H, 3 × CH_3), 1.48–1.73 (m, 8H, 4 × CH_2), 1.91−1.98 (m, 2H, CH_2), 3.69−3.76 (m, 2H, 2 × H-3'), 3.88−3.95 (m, 1H, H-2'), 4.28–4.41 (m, 1H, H-1), 4.85 (br d, *J* = 8.3 Hz, 1H, NH), 5.44 (dd, *J* = 6.5, 15.4 Hz, 1H, H-2), 5.62−5.74 (m, 1H, H-3); ^{13}C NMR (100 MHz, C_6D_6) *δ* 14.4 (CH_3), 23.2 (CH_2), 24.1 (CH_2), 24.4 (CH_2), 25.6 (CH_2), 28.5 (3 × CH_3), 29.5 (CH_2), 29.6 (CH_2), 29.9 (CH_2), 30.0 (CH_2), 30.1 (CH_2), 30.2 (4 × CH_2), 32.4 (CH_2), 32.7 (CH_2), 35.1 (CH_2), 36.5 (CH_2), 53.8 (C-1), 66.3 (C-3'), 77.8 (C-2'), 79.0 (C_q), 110.0 (C_q), 128.7 (C-2), 132.9 (C-3), 155.7 (C=O). ESI-HRMS: *m/z* calcd for $C_{29}H_{54}NO_4$ $[M + H]^+$ 480.4047, found 480.4044.

Isomer (*Z*)-**50**: 13 mg (8%, >96:4 dr); colourless oil; $[\alpha]_D^{21}$ −23.5 (*c* 0.16, $CHCl_3$). IR (neat) *υ* 3392, 2920, 2851, 1716, 1603, 1463, 1365, 1163, 1100, 1045 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) *δ* 0.92 (t, *J* = 6.8 Hz, 3H, CH_3), 1.19–1.41 (m, 24H, 12 × CH_2), 1.44 (s, 9H, 3 × CH_3), 1.48–1.75 (m, 8H, 4 × CH_2), 2.12−2.24 (m, 1H, H-4), 2.25−2.38 (m, 1H, H-4), 3.70−3.79 (m, 2H, 2 × H-3'), 3.90−3.96 (m, 1H, H-2'), 4.68–4.82 (m, 2H, H-1, NH), 5.40−5.54 (m, 2H, H-2, H-3); ^{13}C NMR (100 MHz, C_6D_6) *δ* 14.4 (CH_3), 23.2 (CH_2), 24.2 (CH_2), 24.4 (CH_2), 25.6 (CH_2), 28.3 (C-4), 28.5 (3 × CH_3), 29.8 (CH_2), 29.9 (CH_2), 30.1 (2 × CH_2), 30.2 (5 × CH_2), 32.4 (CH_2), 35.1 (CH_2), 36.6 (CH_2), 49.3 (C-1), 66.1 (C-3'), 78.1 (C-2'), 79.0 (C_q), 110.0 (C_q), 128.0 (C-2 or C-3), 133.6 (C-2 or C-3), 155.6 (C=O). ESI-HRMS: *m/z* calcd for $C_{29}H_{54}NO_4$ $[M + H]^+$ 480.4047, found 480.4051.

*4.1.34. tert-Butyl {(R)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]hexadecyl}carbamate **51***

By the same procedure as employed for the conversion of **40** to **42**, compound **49** (0.10 g, 0.21 mmol) was transformed to derivative **51** (white crystals, 90 mg, 90%, *n*-hexane/EtOAc, 11:1); mp 47−48 °C; $[\alpha]_D^{21}$ +14.5 (*c* 0.52, $CHCl_3$). IR (neat) *υ* 3374, 2914, 2847, 1689, 1524,

1464, 1447, 1365, 1247, 1166, 1105, 1061 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) δ 0.92 (t, J = 6.6 Hz, 3H, CH_3), 1.21–1.36 (m, 28H, 14 × CH_2), 1.45 (s, 9H, 3 × CH_3), 1.53–1.77 (m, 10H, 5 × CH_2), 3.71−3.89 (m, 4H, H-1, H-2', 2 × H-3'), 4.06 (d, J = 9.5 Hz, 1H, NH); ^{13}C NMR (100 MHz, C_6D_6) δ 14.4 (CH_3), 23.2 (CH_2), 24.3 (CH_2), 24.4 (CH_2), 25.6 (CH_2), 26.1 (CH_2), 28.5 (3 × CH_3), 29.9 (2 × CH_2), 30.1 (2 × CH_2), 30.2 (6 × CH_2), 31.7 (CH_2), 32.4 (CH_2), 35.5 (CH_2), 36.7 (CH_2), 53.4 (C-1), 67.1 (C-3'), 78.7 (C-2'), 78.8 (C_q), 110.1 (C_q), 155.8 (C=O). ESI-HRMS: *m/z* calcd for $C_{29}H_{56}NO_4$ $[M + H]^+$ 482.4204, found 482.4209.

*4.1.35. tert-Butyl {(S)-1-[(R)-1',4'-dioxaspiro[4.5]decan-2'-yl]hexadecyl}carbamate **52***

According to the same procedure described for the preparation of **51**, isomer (*E*)-**50** (55 mg, 0.11 mmol) was modified into derivative **52** (colourless oil, 54 mg, 97.5%, *n*-hexane/EtOAc, 11:1); $[\alpha]_D^{21}$ −26.1 (*c* 0.86, $CHCl_3$). IR (neat) υ 3452, 2922, 2852, 1717, 1497, 1464, 1364, 1280, 1101, 1041 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) δ 0.92 (t, J = 6.7 Hz, 3H, CH_3), 1.20–1.40 (m, 30H, 15 × CH_2), 1.46 (s, 9H, 3 × CH_3), 1.48–1.72 (m, 8H, 4 × CH_2), 3.71−3.79 (m, 3H, H-1, 2 × H-3'), 3.81−3.87 (m, 1H, H-2'), 4.64 (br d, J = 9.7 Hz, 1H, NH); ^{13}C NMR (100 MHz, C_6D_6) δ 14.4 (CH_3), 23.2 (CH_2), 24.2 (CH_2), 24.4 (CH_2), 25.6 (CH_2), 26.6 (CH_2), 28.5 (3 × CH_3), 29.9 (CH_2), 30.0 (CH_2), 30.1 (2 × CH_2), 30.2 (6 × CH_2), 32.4 (CH_2), 34.0 (CH_2), 35.1 (CH_2), 36.5 (CH_2), 51.0 (C-1), 66.3 (C-3'), 77.5 (C-2'), 78.8 (C_q), 109.5 (C_q), 156.3 (C=O). ESI-HRMS: *m/z* calcd for $C_{29}H_{56}NO_4$ $[M + H]^+$ 482.4204, found 482.4225.

*4.1.36. (2R,3R)-3-Aminooctadecane-1,2-diol hydrochloride **12***

Using the same procedure as employed for the preparation of **10**, derivative **51** (90 mg, 0.19 mmol) was converted into compound **12** (white amorphous solid, 57 mg, 90%); $[\alpha]_D^{21}$ +6.5 (*c* 0.66, MeOH). IR (neat) υ 3331, 2951, 2915, 2847, 1590, 1513, 1471, 1462, 1159, 1077, 1053, 1017 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.89 (t, J = 6.6 Hz, 3H, CH_3), 1.24–1.56 (m, 26H, 13 × CH_2), 1.58–1.76 (m, 2H, CH_2), 3.26−3.32 (m, 1H, H-3, CD_3OD), 3.63 (dd, J = 5.3, 11.4 Hz, 1H, H-1), 3.70 (dd, J = 4.8, 11.4 Hz, 1H, H-1), 3.78−3.83 (m, 1H, H-2); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4 (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 (2 × CH_2), 30.8 (5 × CH_2), 33.1 (CH_2), 56.0 (C-3), 63.7 (C-1), 71.1 (C-2). ESI-HRMS: *m/z* calcd for $C_{18}H_{40}NO_2$ $[M - Cl]^+$ 302.3054, found 302.3060.

*4.1.37. (2R,3S)-3-Aminooctadecane-1,2-diol hydrochloride **13***

According to the same procedure described for the preparation of **10**, compound **52** (50 mg, 0.10 mmol) was transformed to derivative **13** (white amorphous solid, 29 mg, 83%); $[\alpha]_D^{21}$

+3.1 (*c* 0.38, MeOH). IR (neat) *υ* 3454, 3390, 3004, 2949, 2917, 2848, 1740, 1435, 1366, 1229, 1217, 1205 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (t, J = 6.9 Hz, 3H, CH_3), 1.25–1.48 (m, 26H, 13 × CH_2), 1.56–1.66 (m, 1H, H-4), 1.71−1.80 (m, 1H, H-4), 3.27 (td, J = 3.1, 6.7 Hz, 1H, H-3), 3.65−3.72 (m, 3H, 2 × H-1, H-2); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4 (CH_3), 23.7 (CH_2), 26.4 (CH_2), 30.5 (3 × CH_2), 30.7 (CH_2), 30.8 (6 × 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 $C_{18}H_{40}NO_2$ $[M - Cl]^+$ 302.3054, found 302.3067.

*4.1.38. (2R,3R,E)-3-Aminooctadec-4-ene-1,2-diol hydrochloride **14***

By the same procedure as employed for the preparation of **10**, compound **49** (0.13 g, 0.27 mmol) was modified into derivative **14** (white amorphous solid, 73 mg, 80%); $[\alpha]_D^{21}$ +21.9 (*c* 0.26, MeOH). IR (neat) *υ* 3430, 3365, 3013, 2952, 2916, 2848, 1628, 1605, 1582, 1496, 1381, 1076 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (t, J = 6.6 Hz, 3H, CH_3), 1.24–1.38 (m, 20H, 10 × CH_2), 1.39−1.50 (m, 2H, CH_2), 2.08–2.18 (m, 2H, CH_2), 3.48 (dd, J = 6.0, 11.0 Hz, 1H, H-1), 3.56 (dd, J = 5.0, 11.0 Hz, 1H, H-1), 3.78−3.86 (m, 2H, H-2, H-3), 5.55 (dd, J = 8.8, 15.3 Hz, 1H, H-4), 5.89−6.02 (m, 1H, H-5); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4 (CH_3), 23.7 (CH_2), 29.9 (CH_2), 30.3 (CH_2), 30.5 (CH_2), 30.6 (CH_2), 30.7 (2 × CH_2), 30.8 (3 × CH_2), 33.1 (CH_2), 33.5 (CH_2), 57.2 (C-3), 64.0 (C-1), 72.1 (C-2), 122.2 (C-4), 141.0 (C-5). ESI-HRMS: *m/z* calcd for $C_{18}H_{38}NO_2$ $[M - Cl]^+$ 300.2897, found 300.2912.

*4.1.39. (2R,3S,E)-3-Aminooctadec-4-ene-1,2-diol hydrochloride **15***

According to the same procedure described for the construction of **10**, derivative (*E*)-**50** (70 mg, 0.15 mmol) was converted into compound **15** (white amorphous solid, 43 mg, 87%); $[\alpha]_D^{21}$ −8.3 (*c* 0.52, MeOH). IR (neat) *υ* 3390, 3273, 3123, 2953, 2917, 2849, 1676, 1604, 1473, 1377, 1104, 1049 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (t, J = 6.9 Hz, 3H, CH_3), 1.25–1.38 (m, 20H, 10 × CH_2), 1.39−1.49 (m, 2H, CH_2), 2.09–2.16 (m, 2H, CH_2), 3.56 (dd, J = 4.0, 10.8 Hz, 1H, H-1), 3.60−3.64 (m, 1H, H-2), 3.66 (dd, J = 3.3, 10.8 Hz, 1H, H-1), 3.70−3.76 (m, 1H, H-3), 5.49 (ddt, J = 1.4, 8.7, 15.4 Hz, 1H, H-4), 5.98 (dt, J = 6.8, 15.4 Hz, 1H, H-5); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4 (CH_3), 23.7 (CH_2), 29.8 (CH_2), 30.3 (CH_2), 30.5 (CH_2), 30.6 (CH_2), 30.7 (CH_2), 30.8 (4 × CH_2), 33.1 (CH_2), 33.5 (CH_2), 56.9 (C-3), 64.1 (C-1), 72.6 (C-2), 124.1 (C-4), 140.8 (C-5). ESI-HRMS: *m/z* calcd for $C_{18}H_{38}NO_2$ $[M - Cl]^+$ 300.2897, found 300.2907.

4.2. Antiproliferative/cytotoxic activity

4.2.1. 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) cells were maintained in RPMI 1640 medium, MCF-10A (human mammary epithelial cells) maintained in DMEM/F-12 supplemented with insulin (5 mg/mL) EGF (10 ng/mL) and cholera toxin (1 ng/mL), A2058 (human melanoma cells) maintained in DMEM + sodium pyruvate (1.5 g/L), and PaTu (human pancreatic adenocarcinoma) maintained in DMEM + sodium pyruvate (1.5 g/L) and Hepes (25 mM). NiH 3T3 cell line was maintained in growth medium consisting of high glucose Dulbecco's Modified Eagle Medium. 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.

4.2.2. Cytotoxicity assay

The cytotoxic effect of the tested compounds was studied using the colorimetric microculture assay with the MTT endpoint.[28] 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.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at [it will be filed by the Editorial office].

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Highlights

- Synthesis of unusual sphingoid bases was accomplished.
- Developed strategy relied on [3,3]-sigmatropic rearrangements.
- A single crystal X-ray analysis of the key substructure was performed.
- Grubbs coupling installed a hydrocarbon chain unit.
- The target compounds demonstrate significant antiproliferative/cytotoxic activity.

Synthesis and *in vitro* biological evaluation of 3-amino-3-deoxydihydrosphingosines and their analogues

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Conflicts of interest

The authors declare no conflicts of interest.

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