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Total synthesis and the anticancer activity of (+)-spisulosine

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ABSTRACT

The total synthesis of the anticancer agent (+)-spisulosine has been accomplished. The strategy involved a substrate-controlled aza-Claisen rearrangement to establish the *erythro*-configured amino-alcohol motif followed by deoxygenation to create a methyl side-chain. Subsequent Wittig olefination then permitted the construction of the carbon backbone of the target molecule. To investigate the antiproliferative effect of **1**, its biological profile was examined on a panel of 6 human malignant cell lines and demonstrated the significant anticancer activity of **1** on at least five of the evaluated lines with IC_{50} < 1 μM (MCF-7, HTC-116, Caco-2, Jurkat and HeLa).

1. Introduction

Simple saturated 1-deoxysphingoid bases of marine origin, represented by spisulosine **1** [1], xestoaminol C (**2**) [2], its 3-epimer **3** [3], clavaminols [4], the general structure of which is illustrated by clavaminol A (**4**) [4a] (Fig. 1), and related analogues have become an attractive and timely target for total synthesis due to their impressive biological activities as well as their simple, but unique structures possessing an *erythro*- or *threo*-configured amino alcohol moiety.

Spisulosine **1** was first isolated from the clam *Spisula polynyma* (syn. *Mactrometris polynyma*) [1] and displayed very strong cytotoxic activity on both leukaemia and solid cancer cells with IC_{50} values down to the nanomolar range [1,5]. Padrón and co-workers further revealed that **1** behaved as a selective CK1ε inhibitor and demonstrated its remarkable antiproliferative potency on several cancer cells (HBL-100, HeLa, SW1573, T-47D and WiDr) [6]. Bittman and co-workers have found that spisulosine selectively blocks sphingosine kinase (SphK1) to induce its ubiquitin-proteasomal degradation in human pulmonary arterial smooth muscle cells (PASMC) with an IC_{50} of 7.1 ± 0.75 μM [7]. In addition, **1** significantly inhibits DNA synthesis in PASMC [7]. Spisulosine has also been found to reduce prostate tumor cell growth via de novo synthesis of ceramide and atypical protein kinase C (PKCζ) activation [8]. Its truncated analogue xestoaminol C (**2**) was discovered independently by two research groups. The first of these isolated **2** from the Fijian sponge *Xestospongia* sp [2a] while the second group obtained it from the tunicate *Pseudodistoma obscurum* [2b]. Xestoaminol C (**2**) exhibits activity against reverse transcriptase [2] as well as remarkable cytotoxic/antiproliferative potency in several human cancer cells A-549 [5], HT-29 [5], MeL-28 [5], DU-145 [5] and SHG-44 [9]. It should be noted that **1** was found to possess higher in vitro anticancer activity than xestoaminol C. In 2014, Keyzers and co-workers [3] reported the discovery of 3-*epi*-xestoaminol C (**3**), an effective antimicrobial compound present in the New Zealand brown alga *Xiphophora chondrophylla*. The aforementioned bases, especially spisulosine have stimulated a number of synthetic efforts and several elegant and efficient approaches towards **1** have been developed. The chemistry of **1** dates back to 1957 when this molecule was synthesized for the first time by Prostenik and Alaupović [10] before its isolation from the natural source [1]. To date, several total syntheses of **1** in the form of its free base and/or its HCl salt [1,5,7,9,11] and *N*-Boc-derivative [11a,11c,12] have been reported, together with one construction of racemic (±)-spisulosine [13]. Elaborated strategies have garnered both the Chiron approach [1,5,7,9,11a-c,11i-j,12] as well as asymmetric methods [11d-11h]. Thus, Reinhart and co-workers [1b], Huang's group [9], Delgado and co-workers [11i], Padrón's group [11j], and Ghosal and Shaw

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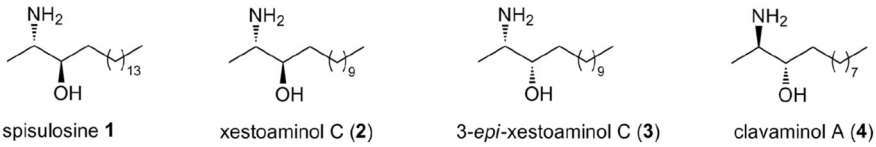


Fig. 1. Structures of the natural 1-deoxysphingoid bases.

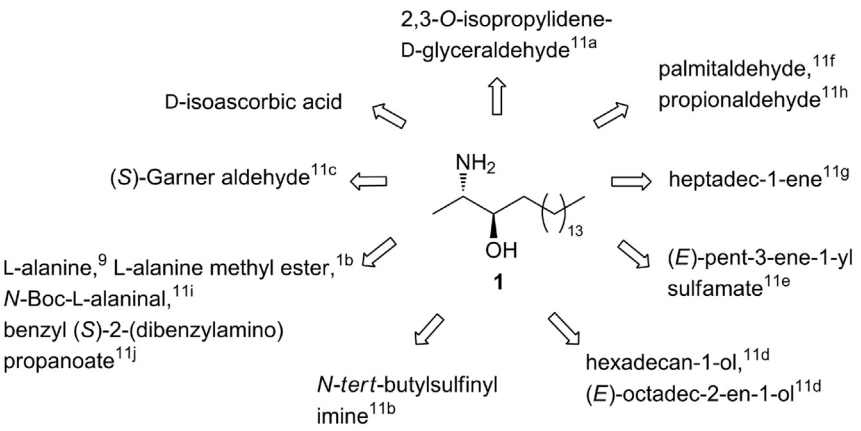
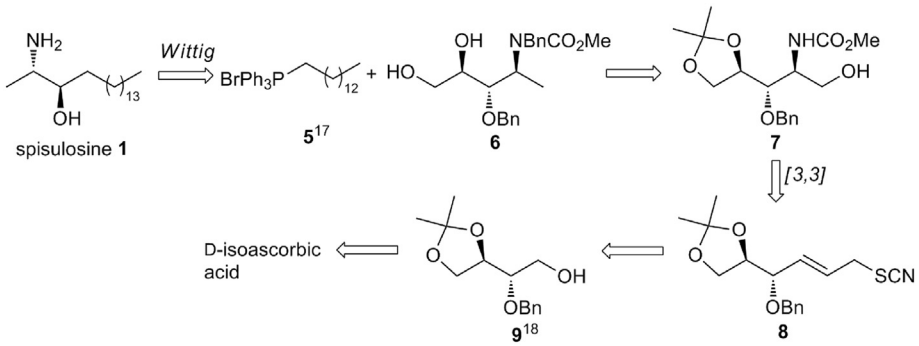


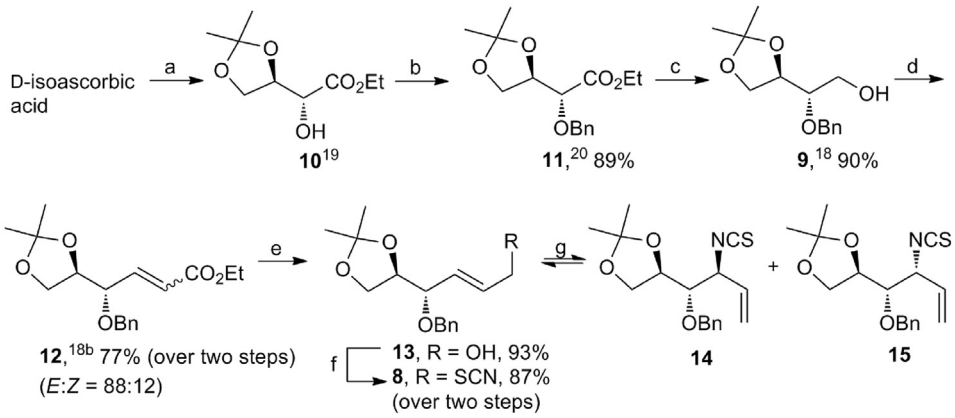
Fig. 2. Reported approaches to spisulosine **1**.

[11c] have commenced their syntheses of spisulosine **1** from the alanine and serine scaffolds, namely L-alanine methyl ester hydrochloride, L-alanine, *N*-Boc-L-alaninal, benzyl (*S*)-2-(dibenzylamino)propanoate, (*S*)-Garner's aldehyde, respectively, in which the addition of diverse organometallic species was employed as the key step. As well, approaches of Gálvez's [11a] and Chemla's groups [11b] have relied on the diastereoselective addition of these aforementioned reagents to the *N*-benzylimine derived from 2,3-*O*-isopropylidene-D-glyceraldehyde and *N*-*tert*-butylsulfinyl imine, respectively. Sutherland and co-workers [11g] have developed a MOM ether-directed palladium(II)-catalyzed Overman rearrangement of an allylic trichloroacetimidate, which was prepared from heptadec-1-ene, to create an *erythro*-configured amino alcohol foldamer. Wang's group [11f] has reported a synthesis of **1** from propionaldehyde, which utilized a highly *anti*-selective asymmetric Henry reaction to build up the carbon backbone with both stereogenic centres. Panda's group [11d] construction of spisulosine from hexadecan-1-ol has employed the Sharpless asymmetric dihydroxylation and S_N2 displacement with a nitrogen nucleophile (azide) as the crucial transformations. Their alternative route from (*E*)-octadec-2-en-1-ol has involved the asymmetric epoxidation and Miyashita's boron-directed C-2 regioselective azidolysis. Dauban and co-workers [11e] have applied a copper-catalyzed intramolecular alkene aziridination of sulfamates and S_N2 ring-opening of the generated bicyclic product to provide the advanced intermediate bearing two required stereocentre. Kumar's group [11h] strategy has used a L-proline-catalyzed α-amination of propionaldehyde and subsequent indium-mediated allylation, assembling the truncated carbon fragment possessing *anti*-amino alcohol unit enatio- and diastereoselectively. Our work (vide infra) is based on a [3,3]-heterosigmatropic rearrangement to install the required C–N bond while a Wittig reaction allowed incorporation of the long chain (Fig. 2).

On the other hand, spisulosine truncated analogue xestoaminol C [5,9,14] as well as its 3-epimer **3** [14a,14e,15] have received less attention. It should be noted that *ent*-**1** [11i] and (2*S*,3*S*)-stereoisomer [11d,11i], together with its (2*R*,3*R*)-antipode [11i] have been synthesized. Delgado's group [11i] evaluated **1** and all of its stereoisomers for in vitro cytotoxicity against the cancer MDA-MB-468 cell line with CC_{50} levels in the micromolar range. In 2011, Dauban and co-workers [11e] communicated their preparation of the 3-fluoro analogue of spisulosine, (2*S*,3*R*)-3-fluorooctadecan-2-



Scheme 1. Retrosynthetic analysis of spisulosine **1**.



Scheme 2. *Reagents and conditions*: (a) Ref. [19]; (b) BnBr, Ag_2O, CH_2Cl_2, rt; (c) $LiAlH_4$, THF, rt; (d) (i) IBX, MeCN, reflux; (ii) $Ph_3P=CHCO_2Et$, CH_2Cl_2, rt; (e) DIBAl-H, CH_2Cl_2, −50 °C; (f) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C→rt; (ii) KSCN, MeCN, 0 °C→rt; (g) Table 1.

Table 1
[3,3]-Sigmatropic rearrangement of thiocyanate **8**.

Entry	Thiocyanate	Conditions[a]	Time (h)	Ratio[b] **14:15**	Yield[c] (%)	Yield[d] (%)
1	**8**	MW, 70 °C	1	78:22	64	34
2	**8**	Δ, 70 °C	21	70:30	67	32
3	**8**	MW, 90 °C	1	56:44	73	25
4	**8**	MW, 90 °C	4	56:44	67	32
5	**8**	Δ, 90 °C	1	83:17	65	33
6	**8**	Δ, 90 °C	2	80:20	68	30
7	**8**	Δ, 90 °C	17	58:42	65	25

[a] In *n*-heptane.
[b] Ratio in the crude reaction mixtures. Determined by ^1H NMR spectroscopy.
[c] Isolated combined yields.
[d] Isolated yields of thiocyanate **8**.

amine, and screened this molecule along with **1** for anticancer activity on KB, HCT-116 and HL-60 cell lines. Cytotoxic activity of spisulosine was confirmed in all cell lines, with IC_{50} values in the nanomolar range. The aforementioned fluoro derivative proved completely inactive even at micromolar concentrations [11e].

Recently, we reported a novel approach to the total synthesis of heterogeneously configured deoxysphingoid bases employing a diastereoselective substrate-controlled aza-Claisen rearrangement [16]. In this paper, we would like to show an extension of this methodology for the construction of the known cytotoxic marine product **1** starting from D-isoascorbic acid as the chiral template.

2. Results and discussion

2.1. Chemistry

The retrosynthetic plan is outlined in Scheme 1. For spisulosine **1**, disconnection of the carbon framework led to the known phosphonium salt **5** [17] and the polar scaffold **6** with the desired D-*erythro*-configured amino alcohol unit. The deoxygenation at C-1 would be required, and it could be accomplished en route to **6** from the pivotal compound **7**. The aza-Claisen rearrangement of the allylic substrate **8** was expected to create a new C—N bond. The thiocyanate **8** was built up from protected D-erythritol **9** [18] derived from D-isoascorbic acid.

As shown in Scheme 2, our investigation commenced with the known ester **10** which was prepared on a multi-gram scale from D-(−)-isoascorbic acid adopting the known protocol.

elaborated by Abushanab's group [19]. The remaining hydroxyl group in **10** was protected as the corresponding benzyl ether **11** [20] (95%) employing BnBr and Ag_2O in dry CH_2Cl_2. The subsequent reduction of **11** ($LiAlH_4$, THF) [18b] resulted in the formation of **9** [18] in 89% yield. To continue the synthesis, a one-pot IBX oxidation/Wittig olefination of **9** furnished a mixture of α,β-unsaturated

Table 2
Crystal data and structure refinement parameters for compound **14**.

14	
Empirical formula	$C_{17}H_{21}NO_3S$
Formula weight	319.41
Temperature, T (K)	173(2)
Wavelength, λ (Å)	0.71073
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	
a (Å)	8.4431(3)
b (Å)	11.1105(5)
c (Å)	17.9408(8)
V (Å^3)	1682.97(12)
Formula per unit cell, Z	4
D_{calcd} (g/cm^3)	1.261
Absorption coefficient, μ (mm^{-1})	0.204
F(0 0 0)	680
Crystal size (mm)	0.46 × 0.37 × 0.27
θ Range for data collection (°)	2.919–26.487
Index ranges	$-10 \le h \le 10$ $-13 \le k \le 11$ $-22 \le l \le 22$
Independent reflections (*Rint*)	3474(0.0288)
Absorption correction	Analytical
Max. and min. transmission	0.955 and 0.906
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3474/0/201
Goodness-of-fit on F^2	1.058
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0343$, $wR_2 = 0.0809$
R indices (all data) Flack x parameter	$R_1 = 0.0430$, $wR_2 = 0.0858$ 0.06(4)
Largest diff. peak and hole (e/Å^{-3})	0.205 and −0.179

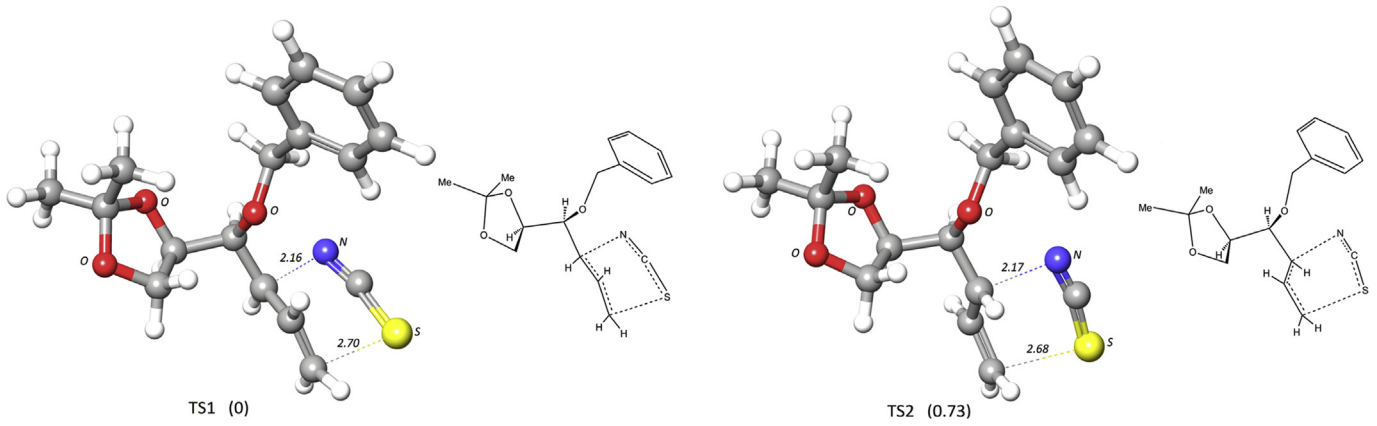


Fig. 3. Transition state structures for the rearrangement **8**→[TS1]/[TS2]→**14** + **15**. Relative energies of transition states (in kcal/mol) and bond distances (in Å) are shown.

esters **12** in 77% yield over two steps (*E*:*Z* = 88:12 as determined by ^{1}H NMR spectroscopy). After their chromatographic separation, the major ester (*E*)-**12** [18b] (*J* = 15.8 Hz) was then reduced with DIBAl-H providing the required allylic alcohol **13** (93%, Scheme 2). As such, thiocyanate **8** (87%) was cleanly produced from **13** through the nucleophilic displacement of the corresponding in situ-generated mesylate by KSCN. Subsequent aza-Claisen rearrangement of **8** was achieved under conventional heating as well as under microwave-promoted thermal conditions at 70 °C or 90 °C in *n*-heptane and afforded a separable mixture of the corresponding isothiocyanates **14** and **15** in good combined yields (Table 1) and with moderate selectivity (Table 1, entry 5). Prolonged heating in an effort to increase the yield of the rearranged products (Table 1, entry 7) was shown to be detrimental for the diastereoselective outcome of the proceeding reaction. Table 1 (entry 7) shows that realization of the rearrangement under thermal conditions (90 °C, 17 h) exhibited an equilibrium shifted to the thermodynamically more stable isothiocyante **15** to decrease the desired 1,2-*anti*-selectivity. On the other hand, these conditions could be eventually used to gain access to a greater amount of the 1,2-*syn*-diastereomer **15** as a precursor of the corresponding 2-*epi*-congener of spisulosine **1**.

In order to rationalise the observed stereoselectivity in the [3,3]-sigmatropic rearrangement of thiocyanate **8**, high-level density functional theory (DFT) calculations, including electron correlation effects, were carried out. The solvent effect was taken into account via a single-point calculation in a dielectric continuum representing *n*-heptane as the solvent.

The rearrangement of **8** occurs via transition states TS1 and TS2 with relative free energies 0 and 0.727 kcal/mol (Fig. 3). The process is concerted but asynchronous. From the calculations, for the pathway **8**→TS1→**14**, the activation energy was found to be 0.727 kcal/mol lower than for the pathway **8**→TS2→**15**. Thus, the predicted diastereomeric ratio of **14**:**15** at 90 °C was 73.3:26.7. These results are in very good agreement with the experimental data (Table 1) with the correct prediction of isothiocyanate **14** as being the predominant diastereoisomer.

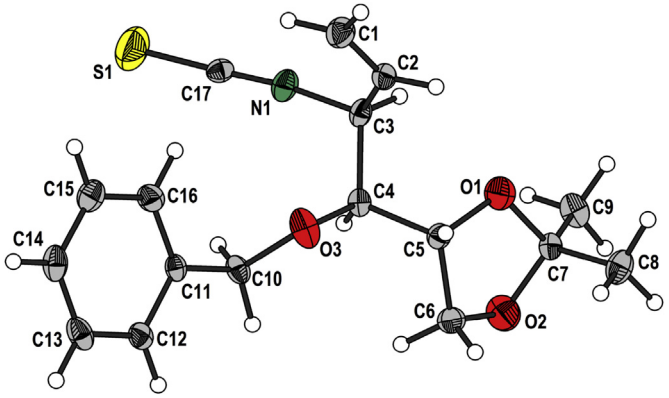
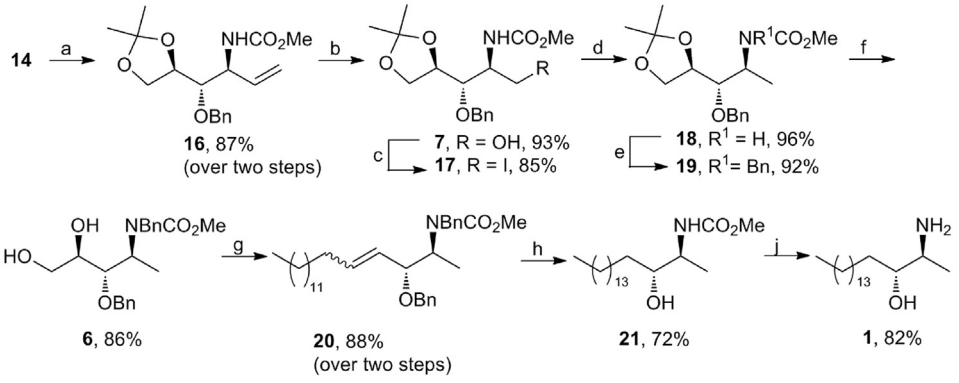
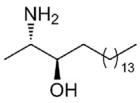
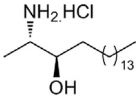


Fig. 4. ORTEP structure of isothiocyanate **14** showing the crystallographic numbering.



Scheme 3. *Reagents and conditions*: (a) (i) MeONa, MeOH, 0 °C→rt, 93%; (ii) mesitylnitrile oxide (MNO), MeCN, rt; (b) (i) O_3, MeOH/CH_2Cl_2, –78 °C; (ii) $NaBH_4$, –78 °C→0 °C; (c) Ph_3P, I_2, imidazole, Et_2O/MeCN, rt; (d) H_2, 10% Pd/C, MeOH, Et_3N, rt; (e) BnBr, NaH, TBAI, DMF, 0 °C→rt; (f) *p*-TsOH, MeOH, rt; (g) (i) $NaIO_4$, MeOH/H_2O (1:1), rt; (ii) LHMDS, THF, **5**, rt; (h) H_2, 10% Pd/C/20% $Pd(OH)_2$/C (1:1), MeOH, 60 °C; (j) 2 M aq NaOH, EtOH, reflux.

Table 3
Optical rotation values for spisulosine **1** and its corresponding HCl salt **1.HCl**.

Compound	Literature	Optical rotation
 spisulosine **1**	Rinehart et al. (Ref. [1b])	$[\alpha]_D^{26} = +24.9$ (*c* 1.0, $CHCl_3$)
	Chemla et al. (Ref. [11b])	$[\alpha]_D^{20} = +5.2$ (*c* 0.36, MeOH)
	Gálvez et al. (Ref. [11a])	$[\alpha]_D^{25} = +24.0$ (*c* 1.0, $CHCl_3$)
	Huang et al. (Ref. [9])	$[\alpha]_D^{20} = +24.2$ (*c* 1.0, $CHCl_3$)
	Delgado et al. (Ref. [11i])	$[\alpha]_D = +8.2$ (*c* 1.0, $CHCl_3$)[a]
	Padrón et al. (Ref. [11j])	$[\alpha]_D^{25} = +25.3$ (*c* 0.94, $CHCl_3$)
	Kumar et al. (Ref. [11h])	$[\alpha]_D^{25} = +19.3$ (*c* 0.23, $CHCl_3$)
	Wang et al. (Ref. [11f])	$[\alpha]_D^{20} = +8.2$ (*c* 0.80, MeOH)
	Panda et al. (Ref. [11d])	$[\alpha]_D^{25} = +21.4$ (*c* 0.50, $CHCl_3$)
	Ghosal and Shaw (Ref. [11c])	$[\alpha]_D^{28} = +25.3$ (*c* 0.95, $CHCl_3$)
	Martinková et al.	$[\alpha]_D^{26} = +16.5$ (*c* 0.20, $CHCl_3$)
 **1.HCl**	Sutherland et al. (Ref. [11g])	$[\alpha]_D^{23} = +7.5$ (*c* 0.30, MeOH)
	Dauban et al. (Ref. [11e])	$[\alpha]_D^{24} = +7.15$ (*c* 0.42, MeOH)
	Gálvez et al. (Ref. [11a])	$[\alpha]_D^{25} = +3.2$ (*c* 1.0, MeOH)

[a] Temperature was not reported.

It should be noted that during this reaction we also recovered the starting thiocyanate **8** (from 25% to 34%, Table 1) which was repeatedly utilized in the rearrangement transformation. Purification by column chromatography allowed the quantitative separation of both compounds **14** and **15**. The major isothiocyanate **14** crystallized well from Et_2O to afford suitable crystals for X-ray diffraction analysis which unambiguously assigned the *S* configuration of the newly formed stereocentre possessing the masked amino functionality (Fig. 4).

As we had prepared the skeletal framework with an *anti*-vicinal amino-alcohol motif, our next task was to develop a suitable route toward the final molecule **1**. For this purpose, isothiocyanate **14** was converted into carbamate **16** (87%, over two transformations) via a two-stage process involving its reaction with MeONa followed by mesitylnitrile oxide (MNO) treatment (Scheme 3). Subsequent ozonolysis of **16**, coupled with a reductive work up ($NaBH_4$), provided **7** in 93% yield. To achieve the required methyl side-chain, replacement of the free hydroxyl function in **7** with a hydrogen atom would create the key intermediate for the synthesis of the aforementioned 1-deoxysphingoid bases. This deoxygenation was accomplished in a two-step approach. First, the alcohol **7** was transformed to iodide **17** in 85% yield according to the protocol reported by Garegg's group [22]. Having obtained compound **17**, we next searched for appropriate conditions for the reductive dehalogenation. After several trials, it was found that catalytic hydrogenation (10% Pd/C) in the presence of Et_3N was the most effective and led to the desired derivative **18** in excellent yield of 96%.

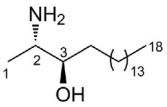
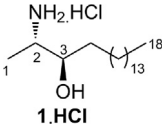
With **18** in hand, we focused on installing of the long alkyl side-chain by the Wittig olefination. To overcome the problems with low yields of this transformation, protection of the nitrogen atom of the carbamate in **18** with a benzyl group was carried out to afford **19** in 92% yield (Scheme 3). Its subsequent *p*-toluenesulfonic acid-mediated acetonide hydrolysis resulted in the formation of diol **6** (86%). This functionalized intermediate was then subjected to the oxidative fragmentation with $NaIO_4$ to produce an aldehyde, which was then treated with a non-stabilized ylide produced from the corresponding salt $CH_3(CH_2)_{13}PPh_3Br$ **5** [17], to give a barely separable mixture of olefins **20**.

(88%, *Z*:*E* = 94:6). Repeated chromatography of small amounts of the obtained mixture of **20** provided only pure (*Z*)-**20** as an analytical sample. To complete the total synthesis, saturation of the double bond and removal of both benzyl ether protecting groups was achieved under catalytic hydrogenation (H_2, 10% Pd/C/20% $Pd(OH)_2$/C, 60 °C). With this procedure, compounds **20** were successively converted into **21** (72%). Final base hydrolysis (2 M aq NaOH, EtOH) furnished the corresponding base **1** in 82% yield (Scheme 3). The spectroscopic data, melting point and optical rotation value for the prepared spisulosine **1** showed good concordance with those, previously reported (for the comparison of values of optical rotations and NMR data, see Tables 3–5, respectively).

2.2. Antiproliferative/cytotoxic activity

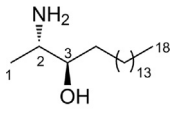
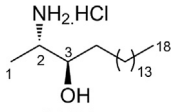
Four newly prepared compounds along with the commercially available anticancer substance cisplatin were evaluated for their in vitro antiproliferative activity on a panel of six human cancer cell lines MDA-MB-231 (mammary gland adenocarcinoma), MCF-7 (mammary gland adenocarcinoma), HTC-116 (colon carcinoma), Caco-2 (colon carcinoma), Jurkat (acute T-lymphoblastic leukaemia), HeLa (cervical adenocarcinoma), and a non-malignant cell line NiH 3T3 (mouse fibroblasts) using the MTT assay. The results are summarized in Table 6 as IC_{50} values. It should be noted that cytotoxicities shown here are lower than those published for **1** on the other cancer cells (P-388, A-549, HT-29, MeL-28, DU-145) by Acena and co-workers [5b]. However, the lack of more detailed

Table 4
^{1}H NMR data for spisulosine **1** and its corresponding HCl salt **1.HCl**.

Compound	Literature	Solvent	H-1 (CH_3)	H-2	H-3	H-18 (CH_3)
 spisulosine **1**	Chemla et al. (Ref. [11b])	CD_3OD	1.24 d	3.29 dq	3.72 m	0.93 t
	Gálvez et al. (Ref. [11a])	CD_3OD	1.03.d	2.77 qd	3.40 ddd	0.89 t
	Huang et al. (Ref. [9])	CD_3OD	1.05 d	2.75–2.87 m	3.44 m	0.91 t
	Delgado et al. (Ref. [11i])	CD_3OD	1.04 d	2.78 m	3.40 m	0.90 t
	Padrón et al. (Ref. [11j])	$CDCl_3$	1.05 d	3.02 br s	3.52 br s	0.88 t
	Kumar et al.(Ref. [11h])	CD_3OD	1.17 d	3.21–3.24 m	3.65–3.71 m	0.83–0.87 m
	Wang et al. (Ref. [11f])	$CDCl_3$	1.00 d	2.97 m	3.44 m	0.88 t
	Panda et al.(Ref. [11d])	CD_3OD	1.20 d	3.22–3.34 m	3.68 m	0.89 t
	Ghosal and Shaw (Ref. [11c])	$CDCl_3$	0.99 d	2.94 br m	3.42 br m	0.84–0.88 m
	Coelho et al. (Ref. [13])[a]	$CDCl_3$	1.01 d	2.88–3.06 m	3.39–3.47 m	0.88 t
	Reinhart et al.(Ref. [1b])	CD_3OD	1.05 d	2.81 qd	3.42 dt	0.89 t
	Martinková et al.	CD_3OD	1.03 d	2.76–2.82 m	3.38–3.42 m	0.87 t
 **1.HCl**	Sutherland et al. (Ref. [11g])	CD_3OD	1.12 d	3.14–3.20 m	3.57–3.62 m	0.80 t
	Dauban et al. (Ref. [11e])	CD_3OD	1.21 d	3.27 qd	3.69 td	0.90 t
	Gálvez et al. (Ref. [11a])	CD_3OD	1.17 d	3.23 qd	3.62–3.70 m	0.86 t

[a] Data for (±)-spisulosine.

Table 5
^{13}C NMR data for spisulosine **1** and its corresponding HCl salt **1.HCl**.

Compound	Literature	Solvent	C-1	C-2	C-3	C-18
 spisulosine **1**	Chemla et al. (Ref. [11b])	CD_3OD	15.3	53.5	72.6	13.1
	Gálvez et al. (Ref. [11a])	CD_3OD	17.3	52.2	76.6	14.5
	Huang et al. (Ref. [9])	CD_3OD	17.0	52.3	76.3	14.5
	Delgado et al. (Ref. [11i])	CD_3OD	17.2	52.1	76.5	14.5
	Padrón et al. (Ref. [11j])	$CDCl_3$	16.0	50.6	74.0	14.1
	Kumar et al. (Ref. [11h])	$CDCl_3$	17.0	51.6	70.6	13.4
	Wang et al. (Ref. [11f])	$CDCl_3$	17.0	NR[a]	NR[a]	14.3
	Panda et al. (Ref. [11d])	$CDCl_3$	17.1	50.5	74.9	14.3
	Ghosal and Shaw (Ref. [11c])	$CDCl_3$	17.3	50.8	75.1	14.5
	Coelho et al. (Ref. [13])[b]	$CDCl_3$	16.8	50.3	74.7	14.1
	Reinhart et al. (Ref. [1b])	CD_3OD	16.8	52.3	76.2	14.6
	Martinková et al.	CD_3OD	16.8	52.2	76.1	14.5
 **1.HCl**	Sutherland et al. (Ref. [11g])	CD_3OD	14.5	52.6	71.7	12.1
	Dauban et al. (Ref. [11e])	CD_3OD	14.4	52.6	71.7	12.1
	Gálvez et al. (Ref. [11a])	CD_3OD	14.5	52.7	71.7	12.1

[a] Not reported.
[b] Data for (±)-spisulosine.

Table 6
Antiproliferative activities on six human cancer cell lines (MDA-MB-231, MCF-7, HCT-116, Caco-2, Jurkat, HeLa) and non-malignant mouse fibroblasts NiH 3T3.

Compd no.	Cell line, IC_{50}[a] ± SD (μmol × L^{-1})						
	MDA-MB-231	MCF-7	HCT-116	Caco-2	Jurkat	NiH 3T3	HeLa
1	3.45 ± 1.08	≤1	<1	<1	≤1	3.25 ± 0.68	≤1
14	31.4 ± 1.7	33.03 ± 7.13	<10	30.4 ± 13.44	23 ± 5	29.1 ± 0.14	<10
17	>100	>100	>100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100	>100	>100
cisplatin	17.5	15.6	20.3	17.2	16.2	20.87	14.1

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

experimental data, including incubation time, given by the aforementioned research group has concluded further comparison.

The antiproliferative activity results (Table 6) revealed that spisulosine exhibited significant potency, mainly against MCF-7, HeLa, CaCo-2, HCT-116 and Jurkat cells. On the other hand, the protected form of spisulosine, carbamate **21**, was completely inactive in all tested cells suggesting a key role of the free vicinal amino alcohol moiety. The major isothiocyanate **14** demonstrated comparable or higher in vitro activity than the commercially available anticancer drug cisplatin on HCT-116 and HeLa cell lines, respectively. These finding observed from the comparison of the cytotoxic activity of compounds **14** and **21** could be explained either via the different mode of action of **14** due to presumably by the presence of the NCS group or by its diverse targets in the tested cancer lines.

3. Conclusions

In summary, we have reported the synthesis of spisulosine **1**. The described approach relied on the substrate-controlled aza-Claisen rearrangement which established the desired amino-alcohol unit and a subsequent deoxygenation step which installed the methyl side framework. A Wittig olefination step permitted coupling of the polar fragment **6** with the hydrophobic chain **5** to create the final carbon backbone of **1**. Spisulosine **1** was assessed for its antiproliferative activity against six cancer cell lines (Jurkat, HeLa, MDA-MB-231, MCF-7, HTC-116 and Caco-2) and displayed significant potency with IC_{50} values down to sub-micromolar range. In addition, substance **1** demonstrates quite promising selectivity in cytotoxicity discriminating between the tested cancer cell lines (MCF-7, CaCo-2, HCT-116, Jurkat and HeLa) and non-malignant mouse fibroblasts (NiH 3T3). These findings support our further screening of **1** against HUVEC (human umbilical vein endothelial cells) and progress will be reported in due course.

4. Experimental

4.1. Chemistry

All commercial reagents were used in the highest available purity from Aldrich, Merck or Acros Organics without further purification. Solvents were dried and purified before use according to standard procedures. For flash column chromatography on silica gel, Kieselgel 60 (0.040–0.063 mm, 230–400 mesh, Merck) was used. Solvents for flash chromatography (hexane, ethyl acetate, methanol, dichloromethane) were distilled before use: hexane with $CaCl_2$, ethyl acetate with K_2CO_3, methanol with BaO and CH_2Cl_2 with CaH_2. Thin layer chromatography was run on Merck silica gel 60 F_{254} analytical plates; detection was carried out with either ultraviolet light (254 nm), or spraying with a solution of phosphomolybdic acid, a basic potassium permanganate solution, or a solution of concentrated H_2SO_4, with subsequent heating. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD and C_6D_6 on a Varian Mercury Plus 400 FT NMR (400.13 MHz for ^1H and 100.61 MHz for ^{13}C) spectrometer using TMS as the internal reference. For ^1H, δ are given in parts per million (ppm) relative to TMS ($\delta = 0.0$), CD_3OD ($\delta = 4.84$), C_6D_6 ($\delta = 7.15$) and for ^{13}C relative to $CDCl_3$ ($\delta = 77.00$), CD_3OD ($\delta = 49.05$), C_6D_6 ($\delta = 128.02$). For the assignment of resonances, homonuclear COSY and heteronuclear 2D correlated (HSQC, HMBC) techniques were used. Infrared (IR) spectra were measured with a Nicolet 6700 FT-IR spectrometer and

expressed in *v* values (cm^{-1}). Optical rotations were measured on a P-2000 Jasco polarimeter and reported as follows: $[\alpha]_D$ (*c* in grams per 100 mL, solvent). Melting points were recorded on a Kofler hot block, and are uncorrected. Microwave reactions were carried out on the focused microwave system (CEM Discover). The temperature content of the vessel was monitored using a calibrated infrared sensor mounted under the vessel. At the end of all reactions the contents of the vessel were cooled rapidly using a stream of compressed air. Small quantities of reagents (μL) were measured with appropriate syringes (Hamilton). All reactions were performed under an atmosphere of nitrogen, unless otherwise noted.

4.1.1. Ethyl (R)-2-[(4′R)-2′,2′-dimethyl-1,3-dioxolan-4′-yl]-2-hydroxyacetate (10) [19]

For the preparation of **10** we used the known procedure reported in Ref. [20]. Here we would like to present the full spectral data of **10** along with its value of the optical rotation; $[\alpha]_D^{25}$ − 24.3 (*c* 0.72, MeOH); lit [19]. $[\alpha]_D$ − 29.14 (*c* 1.565, MeOH, temperature not reported). IR υ_{max} 3447, 2985, 2937, 2904, 1733, 1371, 1250, 1206, 1065 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (t, 3H, $J = 7.1$ Hz, CH_3), 1.36 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 2.89 (d, 1H, $J = 6.3$ Hz, OH), 4.00–4.06 (m, 2H, 2 × H-5′), 4.24–4.35 (m, 4H, H-2, CH_2, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (CH_3), 25.1 (CH_3), 26.3 (CH_3), 61.9 (CH_2), 65.0 (CH_2, C-5′), 71.1 (CH, C-2), 77.0 (CH, C-4′), 109.9 (C_q), 172.0 (C=O). Anal. Calcd for $C_9H_{16}O_5$: C, 52.93; H, 7.90. Found: C, 52.85; H, 7.98.

4.1.2. Ethyl (R)-2-(benzyloxy)-2-[(4′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]acetate (11) [20]

Applying the same reaction conditions described in Ref. [20a], compound **10** [19] (0.50 g, 2.40 mmol), benzyl bromide (0.30 mL, 2.60 mmol) and Ag_2O (0.85 g, 3.60 mmol) in dry CH_2Cl_2 (5 mL) afforded after stirring (1.5 h), treatment and chromatography on silica gel (*n*-hexane/ethyl acetate, 9:1) 0.68 g (95%) of compound **11** as a colourless viscous oil; $[\alpha]_D^{25}$ + 43.5 (*c* 0.41, $CHCl_3$); lit [20a]. $[\alpha]_D^{20}$ + 26.5 (*c* 2.0, MeOH); lit [21]. $[\alpha]_D^{20}$ − 46.0 (*c* 2.0, $CHCl_3$) for *ent*-**11**. IR υ_{max} 2985, 2936, 2875, 1741, 1455, 1371, 1074 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.29 (t, 3H, $J = 7.1$ Hz, CH_3), 1.33 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 3.95 (d, 1H, $J = 6.4$ Hz, H-2), 3.98–4.05 (m, 2H, 2 × H-5′), 4.18–4.28 (m, 2H, CH_2), 4.31–4.36 (m, 1H, H-4′), 4.50 (d, 1H, $J = 11.6$ Hz, OCH_2Ph), 4.68 (d, 1H, $J = 11.6$ Hz, OCH_2Ph), 7.28–7.37 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2 (CH_3), 25.3 (CH_3), 26.6 (CH_3), 61.1 (CH_2), 66.2 (CH_2, C-5′), 72.8 (OCH_2Ph), 76.0 (CH, C-4′), 79.1 (CH, C-2), 109.8 (C_q), 128.0 (CH_{Ph}), 128.1 (2 × CH_{Ph}), 128.4 (2 × CH_{Ph}), 137.0 (C_i), 170.4 (C=O). Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.23; H, 7.61.

4.1.3. (2S)-2-(Benzyloxy)-2-[(4′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]ethanol (9) [18]

Using the same reaction conditions as described in Ref. [18b], compound **11** (0.68 g, 2.30 mmol) and $LiAlH_4$ (0.175 g, 4.61 mmol) in THF (9 mL) afforded after stirring at ambient temperature (1 h), treatment and chromatography through a short column of silica gel (*n*-hexane/ethyl acetate, 3:1) 0.52 g (89%) of **9** as a colourless oil; $[\alpha]_D^{26}$ + 22.1 (*c* 0.67, $CHCl_3$); lit [18a]. $[\alpha]_D^{27}$ + 21.7 (*c* 1.0, $CHCl_3$); lit [18b]. $[\alpha]_D^{25}$ + 23.3 (*c* 1.1, $CHCl_3$). IR υ_{max} 3446, 2985, 2919, 1454, 1370, 1210, 1068 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 2.18 (br s, 1H, OH), 3.52 (app. td, 1H, $J = 6.7$ Hz, $J = 4.2$ Hz, H-2), 3.65–3.74 (m, 1H, H-1), 3.83 (dd, 1H, $J = 11.9$ Hz, $J = 3.9$ Hz, H-1), 3.87 (dd, 1H, $J = 8.4$ Hz, $J = 5.9$ Hz, H-5′), 4.08 (dd, 1H, $J = 8.4$ Hz, $J = 6.4$ Hz, H-5′), 4.15–4.22 (m, 1H, H-4′), 4.63 (d, 1H, $J = 11.6$ Hz, OCH_2Ph), 4.67 (d, 1H, $J = 11.6$ Hz, OCH_2Ph) 7.25–7.40 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.2 (CH_3), 26.6 (CH_3), 61.7 (CH_2, C-1), 66.9 (CH_2, C-5′), 72.6 (OCH_2Ph), 75.8 (CH, C-4′), 79.6 (CH, C-2), 109.2 (C_q), 127.9 (2 × CH_{Ph}), 128.0 (CH_{Ph}), 128.5 (2 × CH_{Ph}), 137.9 (C_i). Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.65; H, 7.99. Found: C, 66.72; H, 7.93.

4.1.4. Ethyl (4S,2E)-4-(benzyloxy)-4-[(4′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]but-2-enoate (E-12) and ethyl (4S,2Z)-4-(benzyloxy)-4-[(4′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]but-2-enoate (Z-12)

To a solution of **9** (2.82 g, 11.2 mmol) in MeCN (85 mL) was added IBX (6.27 g, 22.4 mmol) and the resulting mixture was stirred at reflux for 1 h. After cooling to room temperature, the insoluble parts were filtered off, the solvent was removed in vacuo, and the residue was subjected to the next reaction without further purification.

A solution of the crude aldehyde (2.80 g, 11.2 mmol) in dry CH_2Cl_2 (95 mL) was treated with the stabilized ylide ($Ph_3P = CHCO_2Et$, 4.67 g, 13.4 mmol) and stirring was continued for 1 h at room temperature. After completion of the reaction, the solvent was evaporated, and the residue was chromatographed on silica gel (*n*-hexane/ethyl acetate, 11:1) to afford 2.76 g (77%) of a mixture of esters **12** [18b]. Only (*E*)-**12** was isolated in the pure form as a colourless oil; $[\alpha]_D^{27}$ + 33.5 (*c* 0.53, $CHCl_3$); lit [18b]. $[\alpha]_D^{25}$ + 11.0 (*c* 1.3, $CHCl_3$). IR υ_{max} 2984, 2936, 2874, 1717, 1658, 1455, 1369, 1264, 1210, 1174, 1070 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.31 (t, 3H, $J = 7.2$ Hz, CH_3), 1.33 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 3.88 (dd, 1H, $J = 8.4$ Hz, $J = 5.2$ Hz, H-5′), 3.96 (dt, 1H, $J = 6.4$ Hz, $J = 6.4$ Hz, $J = 1.0$ Hz, H-4), 4.05 (dd, 1H, $J = 8.4$ Hz, $J = 6.4$ Hz, H-5′), 4.09–4.13 (m, 1H, H-4′), 4.22 (q, 2H, $J = 7.1$ Hz, CH_2), 4.43 (d, 1H, $J = 11.7$ Hz, OCH_2Ph), 4.65 (d, 1H, $J = 11.7$ Hz, OCH_2Ph), 6.10 (dd, 1H, $J = 15.8$ Hz, $J = 0.9$ Hz, H-2), 6.92 (dd, 1H, $J = 15.8$ Hz, $J = 6.2$ Hz, H-3), 7.21–7.44 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2 (CH_3), 25.2 (CH_3), 26.6 (CH_3), 60.6 (CH_2), 66.6 (CH_2, C-5′), 71.7 (OCH_2Ph), 77.2 (CH, C-4′), 78.8 (CH, C-4), 109.8 (C_q), 124.0 (CH, C-2), 127.8 (2 × CH_{Ph}), 127.9 (CH_{Ph}), 128.4 (2 × CH_{Ph}), 137.4 (C_i), 144.6 (CH, C-3), 165.8 (C=O). Anal. Calcd for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55. Found: C, 67.54; H, 7.49.

4.1.5. 4.1.5. (4S,2E)-4-(Benzyloxy)-4-[(4′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]but-2-en-1-ol (13)

To a solution of (*E*)-**12** (2.43 g, 7.58 mmol) in dry CH_2Cl_2 (34 mL) that had been pre-cooled to −50 °C was added dropwise DIBAl-H (18.9 mL, 22.7 mmol, ~1.2 M solution in toluene) for 15 min, and the resulting solution was stirred at −50 °C for another 30 min. The excess hydride was decomposed by the cautious addition of MeOH (4.4 mL). Then, a 30% solution of K/Na tartrate (114 mL) was added and stirring was continued at room temperature for 2 h. After separation of the organic layer, the aqueous phase was extracted with CH_2Cl_2 (2 × 150 mL). The combined organic extracts were dried over Na_2SO_4, the solvent was removed, and the residue was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 2:1). This procedure yielded 1.96 g (93%) of compound **13** as a colourless oil; $[\alpha]_D^{27}$ + 53.3 (*c* 0.78, $CHCl_3$). IR υ_{max} 3417, 2985, 2869, 1454, 1371, 1253, 1209, 1155, 1046 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 2.02 (br s, 1H, OH), 3.78 (app. t, 1H, $J = 6.9$ Hz, H-4), 3.87 (dd, 1H, $J = 8.1$ Hz, $J = 5.3$ Hz, H-5′), 4.03–4.13 (m, 2H, H-4′, H-5′), 4.18–4.22 (m, 2H, 2 × H-1), 4.41 (d, 1H, $J = 11.7$ Hz, OCH_2Ph), 4.63 (d, 1H, $J = 11.7$ Hz, OCH_2Ph), 5.69 (dd, 1H, $J = 15.7$ Hz, $J = 7.7$ Hz, H-3), 5.92 (app. td, 1H, $J = 15.6$ Hz, $J = 5.2$ Hz, H-2), 7.25–7.36 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.2 (CH_3), 26.5 (CH_3), 62.7 (CH_2, C-1), 66.8 (CH_2, C-5′), 70.6 (OCH_2Ph), 77.6 (CH, C-4′), 80.0 (CH, C-4), 109.5 (C_q), 127.6 (CH_{Ph}), 127.8 (2 × CH_{Ph}), 128.0 (CH, C-3), 128.3 (2 × CH_{Ph}), 134.5 (CH, C-2), 138.1 (C_i). Anal. Calcd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97. Found: C, 69.11; H, 7.89.

*4.1.6. 4.1.6. (4R)-4-[(1′S,2′E)-1′-(Benzyloxy)-4′-thiocyanatobut-2′-en-1′-yl]-2,2-dimethyl-1,3-dioxolane **(8)***

To a solution of **13** (1.91 g, 6.86 mmol) in dry CH_2Cl_2 (59 mL) that had been pre-cooled to 0 °C were successively added Et_3N (1.5 mL, 10.3 mmol) and MsCl (0.80 mL, 10.3 mmol), and stirring was continued for 30 min at 0 °C. Then, the solvent was evaporated and the residue was diluted with Et_2O (65 mL) to produce the insoluble salt, which was removed by filtration. The filtrate was concentrated in vacuo and the obtained product was subjected to the next reaction without further purification.

A solution of the crude mesylate (2.44 g, 6.86 mmol) in dry MeCN (49 mL) was treated with KSCN (1.27 g, 13.0 mmol) at 0 °C. After stirring for 15 min at 0 °C and then at room temperature for another 20 h, the solvent was evaporated, the residue was diluted with Et_2O (45 mL), and the solid part was filtered off. Evaporation of the solvent and chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 7:1) gave 1.91 g (87%) of compound **8** as a light yellow oil; $[\alpha]_D^{27}$ + 35.9 (*c* 0.72, $CHCl_3$). IR υ_{max} 2985, 2934, 2873, 2154, 1496, 1454, 1370, 1209, 1070 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 3.59 (dd, 1H, J = 13.0 Hz, J = 5.7 Hz, H-4′), 3.64 (dd, 1H, J = 13.0 Hz, J = 6.6 Hz, H-4′), 3.80–3.85 (m, 1H, H-1′), 3.85–3.92 (m, 1H, H-5), 4.05–4.12 (m, 2H, H-4, H-5), 4.47 (d, 1H, J = 11.7 Hz, OCH_2Ph), 4.70 (d, 1H, J = 11.7 Hz, OCH_2Ph), 5.80–5.92 (m, 2H, H-2′, H-3′), 7.24–7.38 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.2 (CH_3), 26.6 (CH_3), 35.7 (CH_2, C-4′), 66.8 (CH_2, C-5), 71.0 (OCH_2Ph), 77.5 (CH, C-4), 79.2 (CH, C-1′), 109.6 (C_q), 111.6 (C, SCN), 126.9 (CH, C-3′), 127.8 (CH_{Ph}), 128.0 (2 × CH_{Ph}), 128.4 (2 × CH_{Ph}), 134.6 (CH, C-2′), 137.7 (C_i). Anal. Calcd for $C_{17}H_{21}NO_3S$: C, 63.92; H, 6.63; N, 4.39. Found: C, 63.99; H, 6.57; N, 4.44.

*4.1.7. 4.1.7. (4R)-4-[(1′S,2′S)-1′-(Benzyloxy)-2′-isothiocyanatobut-3′-en-1′-yl]-2,2-dimethyl-1,3-dioxolane (14) and (4R)-4-[(1′S,2′R)-1′-(benzyloxy)-2′-isothiocyanatobut-3′-en-1′-yl]-2,2-dimethyl-1,3-dioxolane **(15)***

4.1.7.1. Conventional method. A solution of **8** (0.10 g, 0.31 mmol) in *n*-heptane (2.6 mL) was stirred and heated under a nitrogen atmosphere (for the temperatures and reaction times, see Table 1). After completion of the reaction (judged by TLC), the mixture was cooled to room temperature and concentrated. The residue was purified by flash chromatography through a short column of silica gel (*n*-hexane/ethyl acetate, 11:1) to afford the corresponding isothiocyanates **14** and **15** (for the combined yields, see Table 1).

4.1.7.2. Microwave-assisted synthesis. A starting thiocyanate **8** (0.1 g, 0.31 mmol) was weight into a 10-mL glass pressure microwave tube equipped with a magnetic stirbar and dissolved in *n*-heptane (2.6 mL). The tube was closed with a silicon septum and the mixture was subjected to microwave irradiation (for the temperatures and reaction times, see Table 1). After cooling to room temperature, the solvent was evaporated, and the residue was chromatographed on silica gel (*n*-hexane/ethyl acetate, 11:1) to give isothiocyanates **14** and **15** (for the combined yields, see Table 1).

To require a greater amount of the rearranged products, they were prepared at 90 °C under the conventional heating.

Diastereomer **14**: white crystals; mp 53–54 °C (recrystallized from Et_2O); $[\alpha]_D^{26}$ − 37.8 (*c* 0.64, $CHCl_3$). IR υ_{max} 2978, 2927, 2885, 2183, 2132, 1648, 1454, 1412, 1380, 1370, 1354, 1262, 1207, 1154, 1103, 1065, 1044, 1009 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 3.69 (dd, 1H, J = 6.9 Hz, J = 2.8 Hz, H-1′), 3.81–3.89 (m, 1H, H-5), 3.99–4.05 (m, 2H, H-4, H-5), 4.52 (tdd, 1H, J = 6.6 Hz, J = 2.6 Hz, J = 1.2 Hz, J = 1.2 Hz, H-2′), 4.66 (d, 1H, J = 11.2 Hz, OCH_2Ph), 4.83 (d, 1H, J = 11.2 Hz, OCH_2Ph), 5.35 (dd, 1H, J = 10.2 Hz, J = 0.7 Hz, H-4′), 5.42 (dd, 1H, J = 16.9 Hz, J = 0.7 Hz, H-4′), 5.92 (ddd, 1H, J = 16.9 Hz, J = 10.2 Hz, J = 6.6 Hz, H-3′), 7.22–7.40 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.2 (CH_3), 26.7 (CH_3), 61.5 (CH, C-2′), 66.6 (CH_2, C-5), 74.4 (OCH_2Ph), 74.8 (CH, C-4), 81.6 (CH, C-1′), 109.4 (C_q), 119.3 (CH_2, C-4′), 128.0 (2 × CH_{Ph}), 128.1 (CH_{Ph}), 128.5 (2 × CH_{Ph}), 131.2 (CH, C-3′), 134.8 (C, NCS), 137.3 (C_i). Anal. Calcd for $C_{17}H_{21}NO_3S$: C, 63.92; H, 6.63; N, 4.39. Found: C, 63.99; H, 6.58; N, 4.34.

Diastereomer **15**: colourless viscous oil; $[\alpha]_D^{25}$ + 125.2 (*c* 0.54, $CHCl_3$). IR υ_{max} 3031, 2986, 2930, 2884, 2079, 2052, 1645, 1454, 1371, 1254, 1210, 1070 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 3.57 (dd, 1H, J = 7.2 Hz, J = 2.5 Hz, H-1′), 3.82 (dd, 1H, J = 8.5 Hz, J = 5.4 Hz, H-5), 4.04 (dd, 1H, J = 8.5 Hz, J = 6.3 Hz, H-5), 4.13–4.19 (m, 1H, H-4), 4.48–4.51 (m, 1H, H-2′), 4.60 (d, 1H, J = 11.4 Hz, OCH_2Ph), 4.74 (d, 1H, J = 11.4 Hz, OCH_2Ph), 5.32 (dd, 1H, J = 10.3 Hz, J = 1.1 Hz, H-4′), 5.48 (dd, 1H, J = 16.8 Hz, J = 1.3 Hz, H-4′), 5.93 (ddd, 1H, J = 16.8 Hz, J = 10.3 Hz, J = 5.5 Hz, H-3′), 7.24–7.39 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.1 (CH_3), 26.7 (CH_3), 61.2 (CH, C-2′), 66.5 (CH_2, C-5), 74.7 (OCH_2Ph), 75.1 (CH, C-4), 81.5 (CH, C-1′), 109.4 (C_q), 117.8 (CH_2, C-4′), 127.7 (2 × CH_{Ph}), 128.0 (CH_{Ph}), 128.4 (2 × CH_{Ph}), 133.2 (CH, C-3′), 135.3 (C, NCS), 137.3 (C_i). Anal. Calcd for $C_{17}H_{21}NO_3S$: C, 63.92; H, 6.63; N, 4.39. Found: C, 63.86; H, 6.68; N, 4.44.

*4.1.8. 4.1.8. Methyl {(1S,2S)-1-(benzyloxy)-1-[(4′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]but-3-en-2-yl}carbamate **(16)***

Sodium methoxide (98 mg, 1.82 mmol) was added to a solution of **14** (0.45 g, 1.4 mmol) in dry MeOH (14 mL) that had been pre-cooled to 0 °C. After stirring for 30 min at 0 °C and then at room temperature for another 7 h, the solvent was evaporated, and the residue was partitioned between CH_2Cl_2 (30 mL) and water (15 mL). The organic layer was separated and the aqueous one was washed with another portion of CH_2Cl_2 (30 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 9:1) to give 0.46 g (93%) of thiocarbamate as a colourless oil; $[\alpha]_D^{24}$ − 39.7 (*c* 0.37, $CHCl_3$), which was used in the next transformation without spectral characterization due to the presence of rotamers.

A solution of the obtained thiocarbamate (0.45 g, 1.28 mmol) in dry MeCN (12.6 mL) was treated with MNO (0.27 g, 1.66 mmol) and the resulting mixture was stirred at room temperature for 1 h. Evaporation of the solvent and chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 5:1) gave 0.40 g (93%) of compound **16** as a colourless oil; $[\alpha]_D^{23}$ − 18.5 (*c* 0.86, $CHCl_3$). IR υ_{max} 3330, 2985, 2935, 1702, 1643, 1510, 1454, 1370, 1218, 1067 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.33 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 3.65 (s, 3H, OCH_3), 3.70–3.74 (m, 1H, H-1), 3.88 (dd, 1H, J = 8.2 Hz, J = 6.3 Hz, H-5′), 4.01 (dd, 1H, J = 8.2 Hz, J = 6.3 Hz, H-5′), 4.10 (m, 1H, H-4′), 4.39–4.47 (m, 1H, H-2), 4.62 (d, 1H, J = 11.5 Hz, OCH_2Ph), 4.73 (d, 1H, J = 11.5 Hz, OCH_2Ph), 4.96 (br d, 1H, J = 6.2 Hz, NH), 5.20 (d, 1H, J = 10.4 Hz, H-4), 5.27 (d, 1H, J = 17.4 Hz, H-4), 5.84 (ddd, 1H, J = 17.4 Hz, J = 10.4 Hz, J = 7.2 Hz, H-3), 7.24–7.40 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.1 (CH_3), 26.6 (CH_3), 52.1 (OCH_3), 55.2 (CH, C-2), 66.3 (CH_2, C-5'), 74.3 (OCH_2Ph), 76.2 (CH, C-4′), 81.5 (CH, C-1), 108.8 (C_q), 117.6 (CH_2, C-4), 127.8 (CH_{Ph}), 127.9 (CH_{Ph}), 128.5 (3 × CH_{Ph}), 134.1 (CH, C-3), 137.9 (C_i), 156.2 (C=O). Anal. Calcd for $C_{18}H_{25}NO_5$: C, 64.46; H, 7.51; N, 4.18. Found: C, 64.39; H, 7.56; N, 4.23.

*4.1.9. 4.1.9. Methyl {(1S,2S)-1-(benzyloxy)-1-[(4′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]-3-hydroxypropan-2-yl}carbamate **(7)***

Ozone was introduced to a solution of **16** (0.39 g, 1.2 mmol) in EtOH (12.6 mL) that had been pre-cooled to −78 °C for 10 min. After the complete consumption of the starting material, nitrogen was passed through the cold solution for 5 min in order to remove the excess ozone. The resulting mixture was treated with $NaBH_4$

(0.20 g, 5.4 mmol), and stirring was continued for 30 min at −78 °C and then at 0 °C for further 30 min. The reaction was quenched by neutralization with a 1 M HCl solution and the solvent was evaporated. The residue was diluted with CH_2Cl_2 (15 mL) and gradually washed with H_2O (10 mL) and a saturated NaCl solution (10 mL). The organic layer was dried over Na_2SO_4, the solvent was evaporated, and the residue was flash-chromatographed on silica gel (*n*-hexane/ethyl acetate, 1:1). This procedure yielded 0.37 g (93%) of alcohol **7** as a colourless oil; $[\alpha]_D^{24}$ − 1.47 (*c* 0.62, $CHCl_3$). IR υ_{max} 3329, 2985, 2936, 1698, 1521, 1455, 1371, 1212, 1060 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 3.63–3.68 (m, 4H, OCH_3, H-3), 3.70 (dd, 1H, J = 6.1 Hz, J = 4.6 Hz, H-1), 3.87–3.93 (m, 2H, H-3, H-5′), 3.93–3.96 (m, 1H, H-2), 4.08–4.14 (m, 1H, H-5′), 4.16–4.22 (m, 1H, H-4′), 4.65 (d, 1H, J = 11.3 Hz, OCH_2Ph), 4.70 (d, 1H, J = 11.3 Hz, OCH_2Ph), 5.41 (d, 1H, J = 8.3 Hz NH), 7.26–7.39 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.2 (CH_3), 26.4 (CH_3), 52.2 (OCH_3), 52.3 (CH, C-2), 62.1 (CH_2, C-3), 66.8 (CH_2, C-5′), 74.0 (OCH_2Ph), 75.3 (CH, C-4′), 80.5 (CH, C-1), 109.5 (C_q), 128.1 (3 × CH_{Ph}), 128.6 (2 × CH_{Ph}), 137.4 (C_i), 156.9 (C═O). Anal. Calcd for $C_{17}H_{25}NO_6$: C, 60.16; H, 7.42; N, 4.13. Found: C, 60.23; H, 7.36; N, 4.17.

*4.1.10. 4.1.10. Methyl {(1S,2R)-1-(benzyloxy)-1-[(4′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]-3-iodopropan-2-yl}carbamate **(17)***

To a solution of **7** (0.24 g, 0.71 mmol) in a mixture of 3:1 Et_2O/MeCN (7.2 mL) were successively added Ph_3P (0.28 g, 1.08 mmol) and imidazole (71.3 mg, 1.07 mmol), and the resulting solution was stirred at room temperature for 15 min before addition of I_2 (0.22 g, 1.78 mmol). After stirring for another 1 h, the mixture was washed with a saturated $Na_2S_2O_3$ solution (35 mL) and then extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate, 5:1) to afford 0.27 g (85%) of compound **17** as white crystals; mp 65–66 °C (recrystallized from *n*-hexane/ethyl acetate); $[\alpha]_D^{27}$ − 7.22 (*c* 0.62, $CHCl_3$). IR υ_{max} 3365, 2984, 2912, 1702, 1515, 1454, 1370, 1328, 1226, 1157, 1093, 1070, 1022 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 3.43 (dd, 1H, J = 10.0 Hz, J = 3.3 Hz, H-3), 3.51 (dd, 1H, J = 10.0 Hz, J = 3.9 Hz, H-3), 3.66–3.75 (m, 5H, OCH_3, H-1, H-2), 3.93 (app. t, 1H, J = 7.3 Hz, H-5′), 4.08 (app. t, 1H, J = 7.3 Hz, H-5′), 4.18–4.25 (m, 1H, H-4′), 4.69 (d, 1H, J = 11.0 Hz, OCH_2Ph), 4.81 (d, 1H, J = 11.0 Hz, OCH_2Ph), 4.95 (d, 1H, J = 5.5 Hz, NH), 7.25–7.40 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.2 (CH_2, C-3), 25.1 (CH_3), 26.5 (CH_3), 52.5 (OCH_3 or CH, C-2), 52.6 (OCH_3 or CH, C-2), 66.0 (CH_2, C-5'), 74.5 (OCH_2Ph), 76.0 (CH, C-4′), 79.5 (CH, C-1), 109.4 (C_q), 128.0 (2 × CH_{Ph}), 128.5 (3 × CH_{Ph}), 137.7 (C_i), 156.2 (C═O). Anal. Calcd for $C_{17}H_{24}INO_5$: C, 45.45; H, 5.38; N, 3.12. Found: C, 45.52; H, 5.34; N, 3.16.

*4.1.11. 4.1.11. Methyl {(1S,2S)-1-(benzyloxy)-1-[(1′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]propan-2-yl}carbamate **(18)***

To a solution of **17** (0.25 g, 0.56 mmol) in MeOH (45 mL) was added 10% Pd/C (0.42 g) and the resulting suspension was then treated with Et_3N (0.41 mL, 2.94 mmol). The mixture was stirred for 1 h at room temperature under an atmosphere of hydrogen. After completion of the reaction, the catalyst was removed by filtration through a small pad of Celite, and the filtrate was concentrated. Chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 4:1) gave 0.17 g (96%) of compound **18** as a colourless oil; $[\alpha]_D^{27}$ − 28.4 (*c* 0.45, $CHCl_3$). IR υ_{max} 3331, 2984, 2935, 1701, 1525, 1454, 1370, 1222, 1068 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.16 (d, 3H, J = 6.9 Hz, CH_3), 1.34 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.59–3.69 (m, 4H, OCH_3, H-1), 3.86–3.92 (m, 1H, H-5′), 3.96–4.12 (m, 3H, H-2, H-5′, H-4′), 4.61 (d, 1H, J = 11.5 Hz, OCH_2Ph), 4.65–4.76 (m, 2H, OCH_2Ph, NH), 7.25–7.39 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.6 (CH_3), 25.2 (CH_3), 26.6 (CH_3), 48.1 (CH, C-2), 52.0 (OCH_3), 66.8 (CH_2, C-5′), 74.4 (OCH_2Ph), 76.1 (CH, C-4′), 81.6 (CH, C-1), 109.0 (C_q), 127.8 (CH_{Ph}), 127.9 (CH_{Ph}), 128.5 (3 × CH_{Ph}), 138.1 (C_i), 156.2 (C═O). Anal. Calcd for $C_{17}H_{25}NO_5$: C, 63.14; H, 7.79; N, 4.33. Found: C, 63.20; H, 7.74; N, 4.37.

*4.1.12. 4.1.12. Methyl benzyl{(1S,2S)-1-(benzyloxy)-1-[(4′R)-2′,2′-dimethyl-1′,3′-dioxolan-4′-yl]propan-2-yl}carbamate **(19)***

To a solution of **18** (0.16 g, 0.49 mmol) in dry DMF (1.8 mL) that had been pre-cooled to 0 °C were successively added NaH (23.5 mg, 0.98 mmol, ~60% suspension in mineral oil), BnBr (0.12 mL, 0.98 mmol) and TBAI (3.7 mg, 9.8 μmol). After stirring for 15 min at 0 °C and then at room temperature for further 45 min, MeOH (0.1 mL) was added to decompose the excess hydride followed by addition of the cold water (18 mL). The mixture was extracted with Et_2O (2 × 20 mL), the combined organic extracts were dried over Na_2SO_4, and the solvent was evaporated in vacuo. Chromatography of the residue through a short column of silica gel (*n*-hexane/ethyl acetate, 5:1) gave 0.19 g (92%) of compound **19** as a colourless oil; $[\alpha]_D^{27}$ − 22.0 (*c* 0.20, $CHCl_3$). IR υ_{max} 2985, 2935, 1696, 1451, 1370, 1209, 1059 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 60 °C): δ 1.17 (d, 3H, J = 6.9 Hz, CH_3), 1.30 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 3.76–3.83 (m, 1H, H-5′), 3.84–3.92 (m, 2H, H-1, H-5′), 3.93–4.04 (m, 1H, H-2), 4.06–4.13 (m, 1H, H-4′), 4.38 (d, 1H, J = 16.1 Hz, NCH_2Ph), 4.48–4.59 (m, 2H, *1H*–OCH_2Ph, *1H*–NCH_2Ph), 4.76 (d, 1H, d, J = 11.3 Hz, OCH_2Ph), 7.14–7.36 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 60 °C): δ 14.6 (CH_3), 25.1 (CH_3), 26.5 (CH_3), 49.0 (NCH_2Ph), 52.6 (OCH_3), 54.3 (CH, C-2), 65.5 (CH_2, C-5′), 74.9 (OCH_2Ph), 76.9 (CH, C-4′), 80.8 (CH, C-1), 108.8 (C_q), 127.0 (CH_{Ph}), 127.7 (CH_{Ph}), 127.8 (2 × CH_{Ph}), 128.3 (3 × CH_{Ph}), 128.4 (3 × CH_{Ph}), 138.5 (C_i), 139.2 (C_i), 157.0 (C═O). Anal. Calcd for $C_{24}H_{31}NO_5$: C, 69.71; H, 7.56; N, 3.39. Found: C, 69.78; H, 7.50; N, 3.44.

*4.1.13. 4.1.13. Methyl benzyl[(2S,3S,4R)-3-(benzyloxy)-4,5-dihydroxypentan-2-yl]carbamate **(6)***

p-TsOH (16.6 mg, 0.08 mmol) was added to a solution of **19** (0.18 g, 0.44 mmol) in MeOH (6.8 mL) and the mixture was stirred at room temperature for 6 h. After neutralization with Et_3N, the solvent was removed in vacuo, and the residue was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 1:2) to afford 0.14 g (86%) of compound **6** as a colourless oil; $[\alpha]_D^{26}$ − 25.4 (*c* 0.37, $CHCl_3$). IR υ_{max} 3419, 3029, 2952, 1671, 1451, 1403, 1240, 1213, 1046, 1027 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 60 °C): δ 1.23 (d, 3H, J = 7.0 Hz, CH_3), 3.63–3.75 (m, 6H, OCH_3, 2 × H-5, H-4), 3.76–3.81 (m, 1H, H-3), 4.05–4.15 (m, 1H, H-2), 4.45–4.48 (m, 2H, NCH_2Ph), 4.54 (d, 1H, J = 11.3 Hz, OCH_2Ph), 4.67 (d, 1H, J = 11.3 Hz, OCH_2Ph), 7.14–7.35 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 60 °C): δ 14.6 (CH_3), 49.3 (NCH_2Ph), 52.7 (OCH_3), 54.1 (CH, C-2), 63.4 (CH_2, C-5), 72.3 (CH, C-4), 74.4 (OCH_2Ph), 83.2 (CH, C-3), 127.1 (2 × CH_{Ph}), 127.9 (5 × CH_{Ph}), 128.5 (3 × CH_{Ph}), 138.1 (C_i), 139.0 (C_i), 157.4 (C═O). Anal. Calcd for $C_{21}H_{27}NO_5$: C, 67.54; H, 7.29; N, 3.75. Found: C, 67.48; H, 7.34; N, 3.69.

4.1.14. Methyl benzyl[(2S,3R,Z)-3-(benzyloxy)octadec-4-en-2-yl) carbamate (Z)-20 and methyl benzyl[(2S,3R,E)-3-(benzyloxy) octadec-4-en-2-yl)carbamate (E)-20

A solution of **6** (0.104 g, 0.28 mmol) in MeOH (0.6 mL) was treated with a solution of $NaIO_4$ (72 mg, 0.34 mmol) in H_2O (0.3 mL) and stirring was continued for 30 min at room temperature. After completion of the reaction, the insoluble parts were filtered off, and the filtrate was concentrated in vacuo. The residue was washed with CH_2Cl_2 (2 × 5 mL), the combined organic layers were dried over Na_2SO_4, and the solvent was evaporated. The obtained crude product was used immediately in the next reaction without further purification.

To a solution of 1,1,1,3,3,3-hexamethyldisilazane (190 μL, 0.9 mmol) in dry THF (0.8 mL) was added *n*-BuLi (0.56 mL, 0.9 mmol, ~1.6 M solution in *n*-hexane) at room temperature. The solution of LHMD such generated was treated with the known salt **5** [17] (0.5 g, 0.9 mmol) and the resulting dark mixture was stirred for 5 min at the same temperature. Then, a solution of the crude aldehyde (95 mg, 0.28 mmol) in dry THF (0.8 mL) was added and stirring was continued for 30 min. After completion of the reaction (judged by TLC), the mixture was washed with a saturated NH_4Cl solution (5 mL). The organic layer was separated, and the aqueous phase was extracted with further portions of EtOAc (2 × 10 mL). The combined organic extracts were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was chromatographed on silica gel (*n*-hexane/ethyl acetate, 25:1) to give 0.129 g (88%) of a mixture of alkenes **20**. Repeated chromatography on silica gel (*n*-hexane/ethyl acetate, 25:1) afforded only (*Z*)-**20** in pure form as an analytical sample.

Isomer (*Z*)-**20**: colourless oil; $[\alpha]_D^{26}$ − 19.6 (*c* 0.49, $CHCl_3$). IR υ_{max} 2922, 2852, 1698, 1496, 1452, 1213, 1062 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 60 °C): δ 0.88 (t, 3H, J = 6.8 Hz, CH_3), 1.22 (d, 3H, J = 6.9 Hz, CH_3), 1.25–1.37 (m, 22H, 11 × CH_2), 1.99–2.14 (m, 2H, CH_2), 3.66 (s, 3H, OCH_3), 3.82–3.91 (m, 1H, H-2), 4.24 (d, 1H, J = 11.7 Hz, OCH_2Ph), 4.37–4.56 (m, 4H, OCH_2Ph, *2H*–NCH_2Ph, H-3), 5.21–5.26 (m, 1H, H-4), 5.65 (app. td, 1H, 11.0 Hz, J = 7.4 Hz, H-5), 7.16–7.32 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 60 °C): δ 14.0 (2 × CH_3, C-1, C-18), 22.7 (CH_2), 27.9 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.8 (CH_2), 31.9 (CH_2), 50.1 (NCH_2Ph), 52.3 (OCH_3), 57.1 (CH, C-2), 70.4 (OCH_2Ph), 76.8 (CH, C-3), 126.8 (CH_{Ph}), 127.4 (CH_{Ph}), 127.6 (CH, C-4), 128.2 (4 × CH_{Ph}), 128.3 (4 × CH_{Ph}), 135.5 (CH, C-5), 138.9 (C_i), 139.5 (C_i), 156.6 (C=O). Anal. Calcd for $C_{34}H_{51}NO_3$: C, 78.26; H, 9.85; N, 2.68. Found: C, 78.32; H, 9.80; N, 2.64.

4.1.15. 4.1.15. Methyl [(2S,3R)-3-hydroxyoctadecan-2-yl]carbamate (21)

To a solution of **20** (0.11 g, 0.22 mmol) in dry MeOH (6.2 mL) was added successively 10% Pd/C (59 mg) and 20% $Pd(OH)_2$/C (59 mg) and the resulting suspension was stirred at 60 °C for 48 h under an atmosphere of hydrogen. After cooling to room temperature, the catalyst was filtered through a small pad of Celite, and the solvent was evaporated. Chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 5:1) furnished 52 mg (72%) of compound **21** as white crystals; mp 101–103 °C (recrystallized from *n*-hexane/ethyl acetate); $[\alpha]_D^{26}$ − 4.6 (*c* 0.26, $CHCl_3$). IR υ_{max} 3322, 2914, 2847, 1696, 1541, 1464, 1285, 1243, 1094, 1047 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.8 Hz, CH_3-18), 1.09 (d, 3H, J = 6.8 Hz, CH_3-1), 1.17–1.35 (m, 26H, 13 × CH_2), 1.33–1.44 (m, 2H, CH_2), 3.62–3.79 (m, 5H, H-2, H-3, OCH_3), 4.99 (d, 1H, J = 5.6 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (CH_3, C-1, CH_3, C-18), 22.7 (CH_2), 26.0 (CH_2), 29.3 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2), 33.5 (CH_2), 50.9 (CH, C-2), 52.1 (OCH_3), 74.2 (CH, C-3), 156.8 (C=O). Anal. Calcd for $C_{20}H_{41}NO_3$: C, 69.92; H, 12.03; N, 4.08. Found: C, 69.97; H, 11.98; N, 4.12.

4.1.16. 4.1.16. (2S,3R)-2-Aminooctadecan-3-ol (1)

A solution of **21** (52 mg, 0.15 mmol) in EtOH (3 mL) was treated with 2 M NaOH (3 mL) and the resulting mixture was stirred and heated under reflux for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure, and the obtained residue was washed with CH_2Cl_2 (2 × 5 mL). The combined organic layers were dried over Na_2SO_4, the solvent was evaporated, and the crude product was purified by flash chromatography through a short column of silica gel (MeOH/CH_2Cl_2, 1:1). This procedure yielded 35 mg (82%) of the crystalline compound **1**; mp 68–70 °C; lit [9]. mp 65–66 °C; lit [11a]. mp 64.5–66 °C; lit [11b]. mp 115–116 °C; lit [11c]. 65–67 °C; lit [11d]. mp 67–68 °C; lit [11f]. mp not reported; lit [11h]. mp 65–67 °C; lit [11i]. mp 76–77 °C; lit [11j]. mp 65–67 °C; $[\alpha]_D^{26}$ + 16.5 (*c* 0.20, $CHCl_3$); lit [1b,9,11a-d,11f,11h-j]. (see Table 3). IR υ_{max} 2956, 2913, 2847, 1573, 1465, 1374, 1043 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 0.87 (t, 3H, J = 6.6 Hz, CH_3-18), 1.03 (d, 3H, J = 6.6 Hz, CH_3-1), 1.18–1.39 (m, 28H, 14 × CH_2), 2.76–2.82 (m, 1H, H-2), 3.38–3.42 (m, 1H, H-3); ^{13}C NMR (100 MHz, CD_3OD): δ 14.5 (CH_3, C-18), 16.8 (CH_3, C-1), 23.8 (CH_2), 27.3 (CH_2), 30.5 (CH_2), 30.8 (CH_2), 30.9 (CH_2), 33.1 (CH_2), 34.0 (CH_2), 52.2 (CH, C-2), 76.1 (CH, C-3). Anal. Calcd for $C_{18}H_{39}NO$: C, 75.72; H, 13.77; N, 4.91. Found: C, 75.77; H, 13.81; N, 4.87.

4.1.17. X-ray techniques

Single crystals of **14** suitable for X-ray diffraction were obtained from Et_2O by slow evaporation at room temperature. The intensities were collected at 173 K on an Oxford Diffraction XCalibur2 CCD diffractometer using Mo Kα radiation (λ = 0.71073 Å). Selected crystallographic and other relevant data for compound **14** are listed in Table 2. The structure was solved by direct methods [23]. All non-hydrogen atoms were refined anisotropically by full-matrix least squares calculations based on *F* [2,23] All hydrogen atoms were included in calculated positions as riding atoms, with SHELXL97 [23] defaults. The PLATON [24] programme was used for structure analysis and molecular and crystal structure drawings.

4.1.18. Supplementary data

Complete crystallographic data for the structural analysis have been deposit with the Cambridge Crystallographic Data Centre, CCDC No. 1456896 for compound **14**. These data can be obtained free of charge from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or via: www.ccdc.cam.ac.uk).

4.1.18.1. DFT calculations. A thorough conformational search was performed on all transition states, although only the lowest energy conformers are discussed herein. The potential energy surface scans were used to explore the conformational space of transition states TS1 and TS2 with the constrained transition bond lengths C–N and C–S. The systematic conformational search was performed using semiempirical PM3 methodology. The conformers within a 3 kcal/mol energy range were then optimized using B3LYP/6-31G(d) for a more accurate description of the conformer distribution. The low energy conformers of the transition states were later fully optimized using B3LYP/6-31G(d,p) with the Jaguar 7.7 program [27]. The potential energy surface scans were used to explore the conformational space of the reactant and products (thiocyanate **8** and isothiocyanates **14** and **15**) with the later full optimization using the same level of theory; however we only focused on the energy differences between TSs as being crucial for the explanation of the observed diastereoselectivity. The nature of the vacuum B3LYP transition states was verified with frequency calculations, yielding only one large imaginary frequency. Harmonic zero-point energy corrections at B3LYP/6-31G(d,p) obtained from the frequency calculations of the vacuum transition states were applied to the transition-state energies. Single-point energies were computed by the M06-2X density functional method and the cc-pvTZ basis set. The solvent effect was taken into account via a single-point calculation in a dielectric continuum representing *n*-heptane as the solvent. A standard PBF solvation model was applied as implemented in Jaguar 7.7 program [27].

4.2. Antiproliferative/cytotoxic activity

4.2.1. Cell culture

The following human cancer cell lines were used for this study:

MCF-7(breast cancer cells MDA-MB-231 (breast cancer cells), Jurkat (human acute T-lymphoblastic leukaemia), HCT-116 (human colon carcinoma), Caco-2 (human colon carcinoma) and HeLa (cervical adenocarcinoma), and non-cancerous cell line NiH 3T3 (mouse fibroblasts). MCF-7, HCT-116, Caco-2, HeLa and Jurkat cells were maintained in RPMI 1640 medium. MDA-MB-231 and NiH 3T3 cell lines were maintained in growth medium consisting of high glucose Dulbecco's Modified Eagle's Medium. Both of these media were supplemented with Glutamax, and with 10% (V/V) foetal calf serum, penicillin (100 IU $\times$ mL^{-1}), and streptomycin (100 mg $\times$ 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 tryptan 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 [25,26]. 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 $\times$ L^{-1}. After 72 h incubation, 10 μL of MTT (5 mg $\times$ 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 was added into each well and another 12 h were allowed for the formazan to be dissolved. The absorbance was measured at 540 nm using the automated uQuant™ Universal Microplate Spectrophotometer (Biotek Instruments Inc., Winooski, VT USA). The blank corrected absorbance of the control wells was taken as 100% and the results were expressed as a percentage of the control.

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Appendix A. Supplementary data

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