Carbohydrate Research 451 (2017) 59–71

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

The convergent synthesis and anticancer activity of broussonetinines related analogues

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ARTICLE INFO

Article history:
Received 5 September 2017
Received in revised form
14 September 2017
Accepted 14 September 2017
Available online 19 September 2017

Keywords:
Broussonetines
Broussonetinines
[3,3]-Sigmatropic rearrangements
Oxazolidinones pyrrolidine unit
Metathesis
Antiproliferative/cytotoxic activity

ABSTRACT

The convergent synthesis of broussonetinines related congeners **3** and **4** with the simple C_{13} alkyl side chain and differently configured pyrrolidine skeleton has been achieved. Our approach relied on the [3,3]-sigmatropic rearrangements of chiral allylic substrates derived from D-xylose. Cross metathesis of the common oxazolidinone intermediates **7** and **8** with tridec-1-ene followed by alkylative cyclization completed the construction of both *C*-alkyl iminosugars. The targeted compounds **3** and **4** were screened for antiproliferative/cytotoxic activities against multiple cancer cell lines by MTT assay. Compound **3** exhibited very good in vitro potency on Caco-2 and Jurkat cell lines with IC_{50} value of 5.1 μM and 5.8 μM, respectively.

1. Introduction

Broussonetines, along with diastereomeric broussonetinines, the representative structures of which are illustrated by broussonetine C (**1**) and broussonetinine A (**2**), were isolated by Kusano and co-workers [1] from the branches of the Asian deciduous tree *Broussonetia kazinoki* (Fig. 1). They represent a class of more than 30 well-identified and characterized polyhydroxylated pyrrolidine alkaloids, which possess variable side chains with the diverse types of functionalization [1]. Most of these *C*-alkyl iminosugars demonstrated significant glycosidase inhibitory activities with IC_{50} values in the micromolar to nanomolar range [1–3] and as such are expected to be promising anticancer [4], antidiabetic [5], antiviral [6] and anti-inflammatory agents [7], and pharmacological chaperones [8]. Due to these potent biological properties, several synthetic methods towards naturally occurring broussonetines [2,3,9] and their related analogues [2-3,8b,9d] have been reported.

In our continuing studies based on the feasibility of the [3,3]-sigmatropic rearrangements in the total synthesis of sphingoid base-like natural products and their analogues [10] we were interested in investigating the use of such transformation as the key reaction for the construction of broussonetinines related congeners, which possess the simple C_{13} hydrocarbon fragment. Herein, we would like to report the total synthesis and antiproliferative activity of two iminosugars **3** and **4**, starting from D-xylose. While this manuscript was in preparation, we published the approach towards other diastereomeric analogues of our final compounds **3** a **4** together with their cytotoxic profile [11]. Part of the reported synthetic strategy [11] is here effectively applied.

2. Results and discussion

2.1. Chemistry

As shown in Fig. 2, our retrosynthetic route to broussonetinines analogues **3** and **4** would involve pyrrolidine core construction through the intramolecular S_N2 cyclization of open chain scaffolds **5** and **6**, respectively. We planned to install the C_{13} side segment in

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https://doi.org/10.1016/j.carres.2017.09.009
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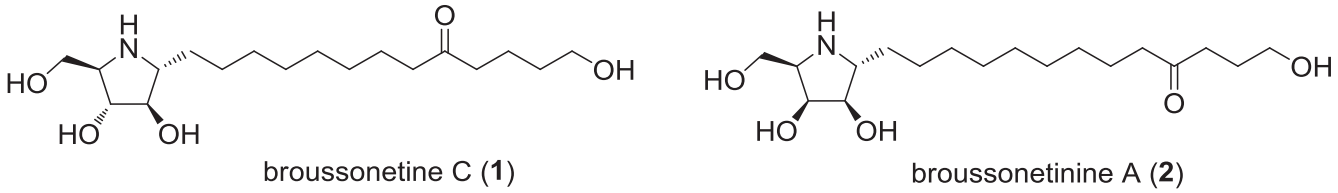


Fig. 1. Structures of broussonetine C (**1**) and broussonetinine A (**2**).

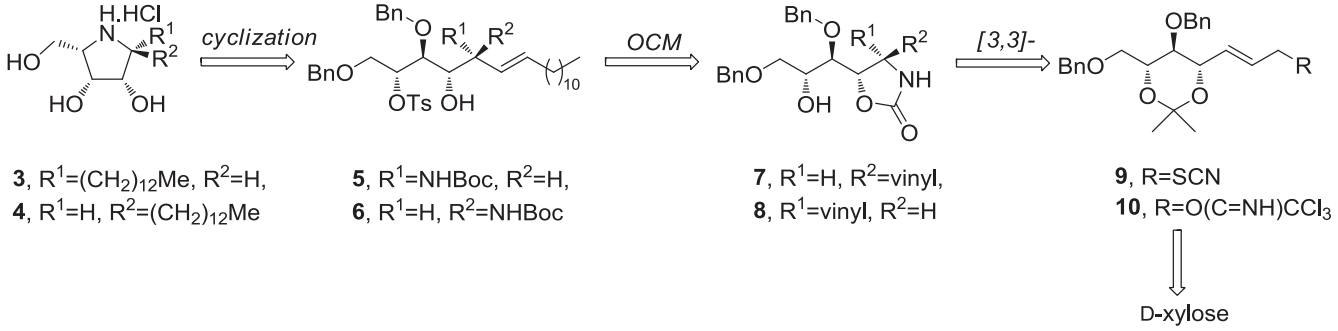
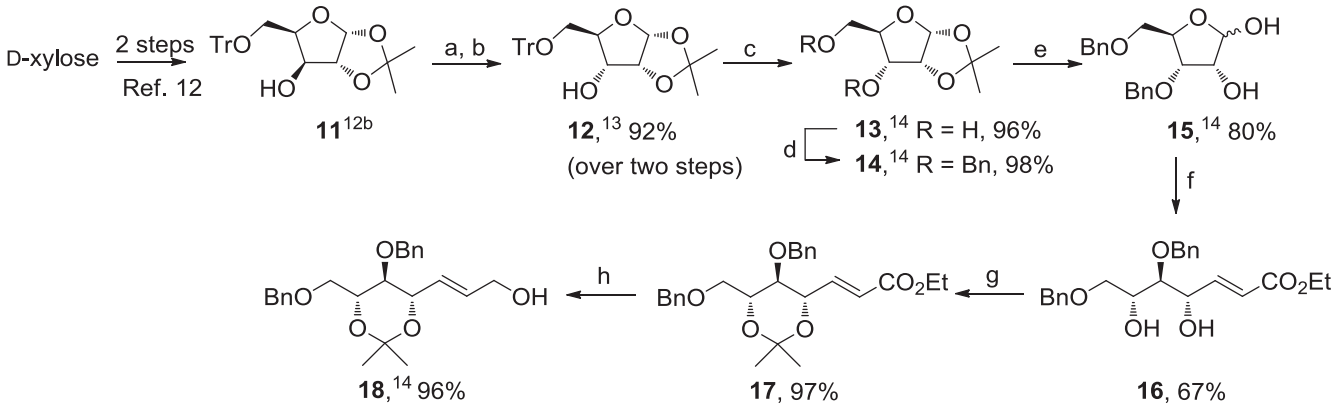


Fig. 2. Retrosynthetic route towards broussonetinines related analogues **3** and **4**.

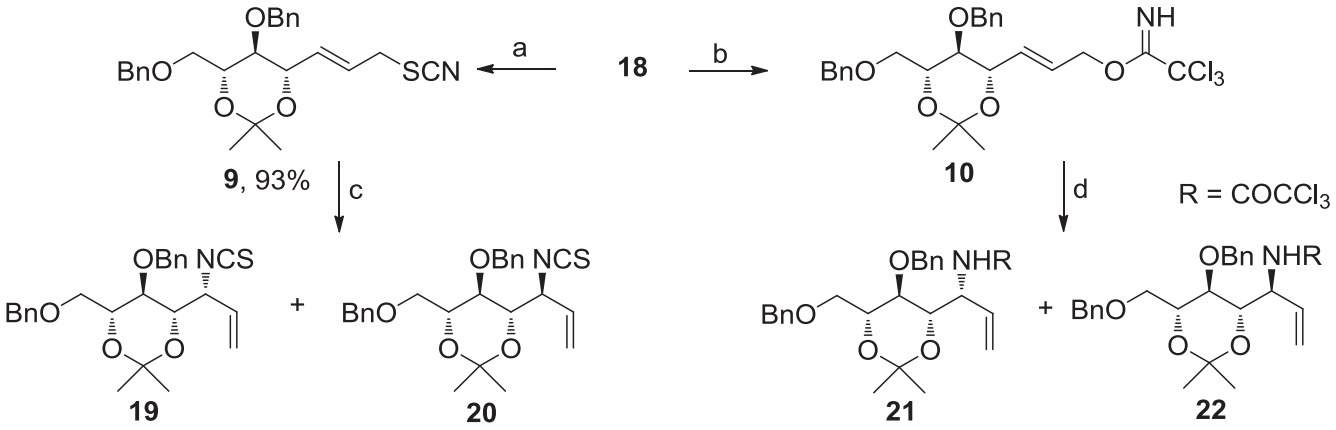
5 and **6** via cross metathesis chemistry of the common oxazolidinones **7** and **8**, which could be constructed by the [3,3]-sigmatropic rearrangements of the allylic substrates **9** and **10** derived from D-xylose.

The commercially available D-xylose was modified into the known 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose **11** [12b] according to the literature [12] (Scheme 1). The conversion of the C-3 stereochemistry in **11** was achieved through an oxidation-highly diastereoselective reduction protocol, affording the corresponding 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-ribofuranose **12** [13] in 92% yield over two steps via formation of the 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranos-3-ulose [13]. Removal of the *O*-trityl protecting group in **12** (CSA, MeOH/CH_2Cl_2) and subsequent treatment of the resulting diol **13** [14] (96%) with BnBr produced 3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose **14** [14] in 94% yield from **12**. Exposure of **14**—80% TFA resulted in cleavage of the isopropylideneketal fragment to provide lactol **15** [14] (80%) as a mixture of anomers. Wittig reaction of **15** with the stable ylide reagent ($Ph_3P=CHCO_2Et$) produced (*E*)-α,β-unsaturated ester **16** (67%, $J = 15.7$ Hz, this coupling constant value unambiguously supported an (*E*)-relationship between vinyl protons). It should be noted that no trace of the second geometric isomer was detected in the crude ^{1}H NMR spectrum. The lower yield is most likely due to generation of the minor unidentified side products. Further attempts to improve the efficiency of the olefination turned out to be ineffective and afforded **16** in the similar or lower amounts. After protection of the diol moiety in **16** with 2,2-DMP, the ester functionality in the created acetonide **17** (97%) was then reduced with DIBAl-H to give the corresponding alcohol **18** [14] (96%, 43% overall yield starting from **11**).

With the construction of the allylic scaffold **18** accomplished (Scheme 1), we were now in a position to examine the key [3,3]-sigmatropic rearrangements. For this purpose, compound **18** was converted into the appropriate substrates **9** and **10**, respectively. Thiocyanate **9** (93%) was produced via the known [10d] two-step approach involving the formation of a mesylate intermediate. On the other hand, the corresponding imidate **10** was prepared by treatment of **18** with Cl_3CCN and catalytic amounts of DBU and was used immediately in the Overman transformation [15] without purification (Scheme 2). The microwave-promoted thermal aza-



Scheme 1. *Reagents and conditions:* (a) IBX, MeCN, reflux, 93%; (b) $NaBH_4$, EtOH/CH_2Cl_2, 0 °C→rt, 99%; (c) CSA, MeOH/CH_2Cl_2, rt; (d) BnBr, NaH, DMF, TBAI, 0 °C→rt; (e) 80% TFA, rt; (f) $Ph_3P=CHCO_2Et$, CH_2Cl_2, benzoic acid, rt; (g) 2,2-DMP, *p*-TsOH, CH_2Cl_2, rt; (h) DIBAl-H, CH_2Cl_2, −30 °C.



Scheme 2. *Reagents and conditions:* (a) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C→rt; (ii) KSCN, MeCN, 0 °C→rt; (b) Cl_3CCN, DBU, CH_2Cl_2, 0 °C→rt; (c) *n*-heptane, 90 °C, Δ (60%) or MW (77%); (d) *o*-xylene, K_2CO_3, MW, 150 °C (85%) or 170 °C (94%).

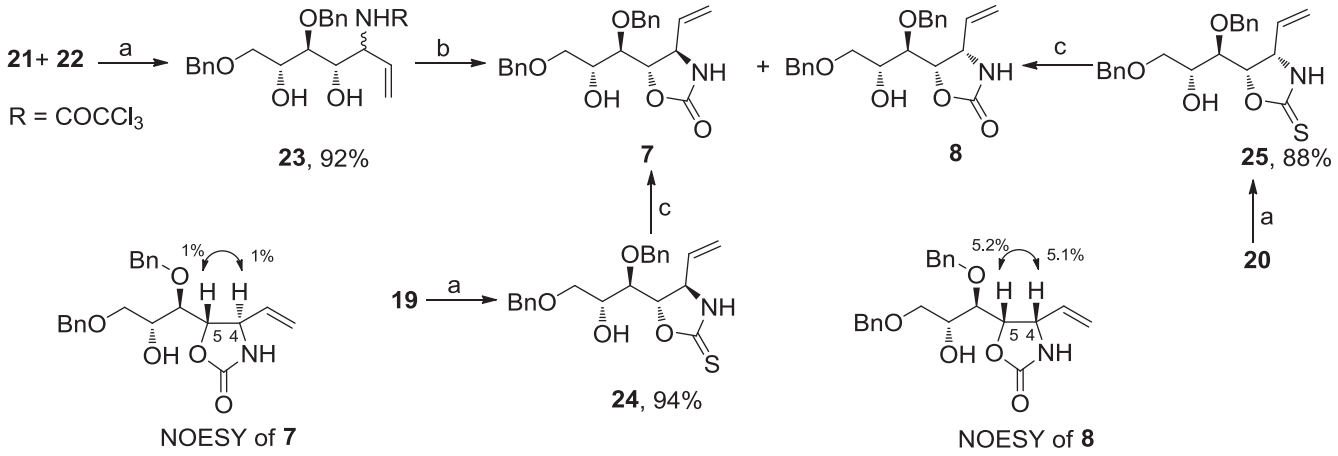
Claisen rearrangement of **9** was carried out in *n*-heptane at 90 °C to produce the rearranged products **19** and **20** in good combined yield (77%) after 1 h, as a separable mixture of diastereoisomers (**19**:**20** = 48:52, the ratio was determined by ^{1}H NMR spectroscopic analysis). The rearrangement reaction of **9** realized under the conventional heating (6 h) at the same temperature allowed the preparation of both isothiocyanates **19** and **20** with lower yield (60%) and similar selectivity (**19**:**20** = 44:56). In both cases we also recovered the starting thiocyanate **9** (11–13%). The thermal Overman rearrangement of **10** (Scheme 2) mediated by microwave irradiation was conducted in *o*-xylene in the presence of K_2CO_3 [16] either at 150 °C (3.5 h) or 170 °C (1 h) and provided an inseparable mixture of the corresponding trichloroacetamides **21** and **22** (**21**:**22** = 44:56 or 56:44 in both cases) in very good combined yields 85% and 94%, respectively, and with diastereoselectivities similar to those observed in the [3,3]-sigmatropic rearrangement of **9**. Although both used rearrangement reactions afforded an unsatisfactory *dr*, the relative short-step approach to the aforementioned intermediates **19**–**22** from **11** with respectable overall yields as well as and a possibility to demonstrate approach towards the new broussonetinines analogues led us to continue in our elaborated strategy.

The configuration of the newly generated stereocentre with nitrogen was confirmed by NOESY experiments which were conducted on the common more advanced oxazolidinones **7** and **8** derived from the rearranged products (vide infra).

To continue a synthesis towards **7** and **8**, treatment of Overman's products **21** and **22** with *p*-TsOH in MeOH removed the acetonide group and gave an inseparable mixture of diols **23** (92%, Scheme 3). Their subsequent reaction with DBU resulted in production of the desired cyclic carbamates **7** and **8** in 96% combined yield. Column chromatography then allowed the straightforward isolation of both diastereoisomers **7** and **8**. On the other hand, isothiocyanates **19** and **20** were initially converted into the cyclic thiocarbamates **24** (94%) and **25** (88%) by the same conditions as employed for the conversion of **21** and **22** to **23** (Scheme 3). The replacement of the sulfur atom to oxygen in **24** and **25** was accomplished with mesitylnitrile oxide in MeCN to afford scaffolds **7** and **8** in 89% and 92% yields, respectively.

At this stage, the relative C-4 stereochemistry of the common products **7** and **8** was assigned by 1D NOESY experiments (Scheme 3). In the case of cyclic carbamate **8**, the strong enhancements between H-4 and H-5 protons indicate that these protons are in the *cis*-relationship to each other. These findings demonstrate that the C-4 carbon in **8** is (*S*)-configured. On the other hand, for derivative **7**, the observed NOE interactions between H-4 and H-5 (1%) unambiguously establish their *trans* orientation revealing the opposite (4*R*)-configuration. Moreover, the configuration of the above mentioned newly constructed stereocentre in **24** was assigned to be (4*R*) through X-ray analysis (Fig. 3 and Table 1) of the suitable crystal which was provided by its recrystallization from a mixture of *n*-hexane and ethyl acetate, confirming that the rearranged product **19** must have the same stereochemistry.

Having successfully resolved the stereochemical assignment, we directed our efforts towards construction of the required pyrrolidine unit with the C-13 hydrophobic tail with the aim to obtain the final analogues **3** and **4**, and this was readily achieved by using the strategy depicted in Scheme 4. Formation of the *N*-Boc derivatives **26** (78%) and **27** (89%) from the corresponding carbamates **7** and **8** proceeded without problem [17]. It should be noted that during *tert*-butoxycarbonylation of **7** and **8** we also isolated the corresponding di-Boc derivative in 10% and 9% yields, respectively. After protection of the secondary hydroxy substituent in **26** and **27** as a tosyloxy group (TsCl, DMAP, Et_3N, Me_3N.HCl) [17], the subsequent hydrolysis of the oxazolidinone skeleton of **28** (95%) and **29** (87%) produced the amino alcohols **30** and **31** in 81% and 92% yields, respectively (Scheme 4). At this stage, the installation of the long alkyl chain through Grubbs' cross metathesis was performed and the aforementioned advanced vinyl scaffolds **30** and **31** were subjected to the coupling reaction with tridec-1-ene to afford the (*E*)-configured olefin **5** (79%, J = 15.6 Hz) and **6** (79%) as the sole product in both cases (Scheme 4). In the case of **6** it was not possible to determine correctly the value of the coupling constant of the vinyl protons. Therefore, the geometry of the double bond was unambiguously confirmed by ^{1}H NMR analysis of derivative **33** (vide infra). With the requisite alkenes **5** and **6** in hand, we then focused our attention on the preparation of the crucial pyrrolidine unit by adopting the recently reported Marco's protocol [9f]. Thus, removal of the temporary Boc protecting group of **5** and **6** followed by work up with aq NH_3 delivered the expected cyclic products **32** (96%) and **33** (86%, J = 15.2 Hz). Finally, the simultaneous saturation of the double bond and deprotection of the *O*-benzyl groups in **32** and **33** were achieved via catalytic hydrogenation to furnish the targeted broussonetinines congeners **3** and **4** in the nearly quantitative yields. The relative configuration of **3** was confirmed by ^{1}H NMR NOE analysis (Scheme 4). The observed larger enhancements between H-2 and H-3, between H-3 and H-4 and between H-4 and H-5 protons revealed that they all occupy the same face of the pyrrolidine skeleton. In the case of compound **4**, due to the overlap of the H-4 proton signal with the signals of the hydroxymethyl side



Scheme 3. *Reagents and conditions:* (a) *p*-TsOH, MeOH, rt, 92% for **23**; (b) DBU, CH_2Cl_2, rt, 96% (combined yield); (c) MNO, MeCN, rt, 89% for **7**, 92% for **8**.

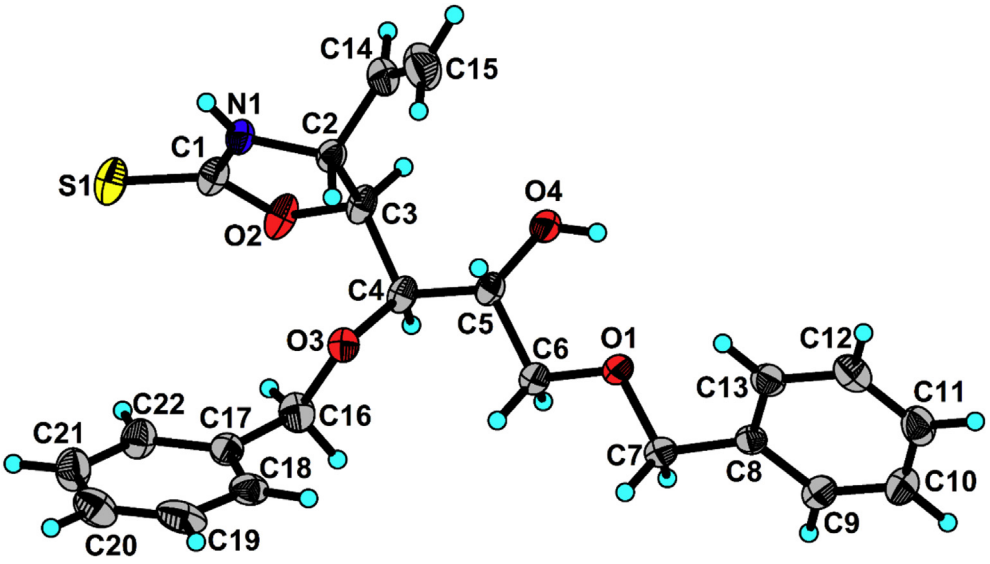


Fig. 3. ORTEP structure of **24** showing the crystallographic numbering.

chain in CD_3OD solution it was not possible to conduct the aforementioned measurement on this sample. To allow comparisons, the attached supplementary material includes ^1H and ^{13}C NMR data (see Tables 1 and 2) for the pyrrolidine core of our final compounds **3** and **4** and known analogues reported in the literature [18].

2.2. Antiproliferative/cytotoxic activity

The final synthesized compounds **3** and **4** were evaluated for in vitro antiproliferative/cytotoxic activities against seven human cancer cell lines A-549 (non-small cell lung cancer), MCF-7 (mammary gland adenocarcinoma), MDA-MB-231 (mammary gland adenocarcinoma), HTC-116 (human colon carcinoma), Caco-2 (human colon carcinoma), HeLa (cervical adenocarcinoma) and Jurkat (acute T-lymphoblastic leukaemia); and a non-malignant cell line NiH 3T3 (mouse fibroblasts) using the MTT assay [19]. The anticancer agent cisplatin was included as positive control, and all assays were carried out in triplicate. The obtained IC_{50} values (Table 2) showed that targeted compound **3** displays higher antiproliferative/cytotoxic potencies against Caco-2 and Jurkat cancer cell lines than cisplatin, whose IC_{50} values on these cells are at least 2.5–3 × higher. With the exception of HeLa cells, compound **4** was less active than its 5-*epi*-analogue **3** against all other evaluated cancer cell lines, indicating that the configuration of the pyrrolidine skeleton might be important for the biological activity. Iminosugar **4** was found to be more toxic on non-malignant mouse fibroblasts NiH 3T3 than on screened cancer cell lines.

3. Conclusions

In summary, we have accomplished the convergent synthesis of two *C*-alkyl iminosugars **3** and **4**. The key transformations of the developed approach are synthesis of the common oxazolidinone intermediates **7** and **8** using heterosigmatropic rearrangements to establish the new C-N bond and Grubbs' cross metathesis chemistry followed by intramolecular cyclization to create the carbon backbone and pyrrolidine function, respectively. The targeted compound **3** displayed very good antiproliferative/cytotoxic activity against Caco-2 and Jurkat cancer cell lines. Its 5-epimer **4** was found to be less potent on human cancer cell lines and more toxic against non-malignant cells (NiH 3T3).

Table 1
Crystal data and structure refinement parameters for compound **24**.

24	
Empirical formula	$C_{22}H_{25}NO_4S$
Formula weight	399.50
Temperature, T (K)	293 (2)
Wavelength, λ (Å)	1.54184
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	
a (Å)	7.07255 (13)
b (Å)	17.9943 (3) $\beta = 107.438$ (2)°
c (Å)	8.92418 (19)
V (Å^3)	1083.54 (4)
Formula per unit cell, Z	2
D_{calcd} (g/cm^3)	1.224
Absorption coefficient, μ (mm^{-1})	1.542
F (0 0 0)	424
Crystal size (mm)	0.86 × 0.58 × 0.12
θ Range for data collection (°)	5.195–75.716
Index ranges	$-8 \le h \le 8$ $-22 \le k \le 22$ $-10 \le l \le 11$
Independent reflections (Rint)	4456 (0.0388)
Completeness to $2\Theta = 74.98°$	98%
Reflections collected	56522
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4456/250/253
Goodness-of-fit on F^2	1.058
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0415$, $wR_2 = 0.1263$
R indices (all data)	$R_1 = 0.0461$, $wR_2 = 0.1334$
Flack x parameter	0.004 (8)
Largest diff. peak and hole (e/Å^{-3})	0.137 and −0.135

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. Thin layer chromatography was run on Merck silica gel 60 F_{254} analytical plates; detection was carried out with either ultraviolet light (254 nm), or spraying with a solution of phosphomolybdic acid, a basic potassium permanganate solution, or a solution of concentrated H_2SO_4, with subsequent heating. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD and C_6D_6 on a Varian Mercury Plus 400 FT NMR (400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C) or on a Varian Premium COMPACT 600 (599.87 MHz for ^{1}H and 150.84 MHz for ^{13}C) spectrometer using TMS as internal reference. For ^{1}H, δ are given in parts per million (ppm) relative to TMS ($\delta = 0.0$), CD_3OD ($\delta = 4.84$ or $\delta = 3.31$), C_6D_6 ($\delta = 7.15$) and for ^{13}C relative to $CDCl_3$ ($\delta = 77.00$), CD_3OD ($\delta = 49.05$) and C_6D_6 ($\delta = 128.02$). The multiplicity of the ^{13}C NMR signals concerning the ^{13}C-^{1}H coupling was determined by the HSQC method. Chemical shifts (in ppm) and coupling constants (in Hz) were obtained by first-order analysis; assignments were derived from COSY and H/C correlation spectra. Infrared (IR) spectra were measured with a Nicolet 6700 FT-IR spectrometer and expressed in ν values (cm^{-1}). Elemental analysis was performed on a Perkin-Elmer CHN 2400 elemental analyser. Optical rotations were measured on a P-2000 Jasco polarimeter and reported as follows: $[\alpha]_D$ (c in grams per 100 mL, solvent). 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 vessel were cooled rapidly using a stream of compressed air. Melting points were recorded on a Kofler hot block, and are uncorrected. Small quantities of reagents (μL) were measured with appropriate syringes (Hamilton). All reactions were performed under an atmosphere of nitrogen, unless otherwise noted.

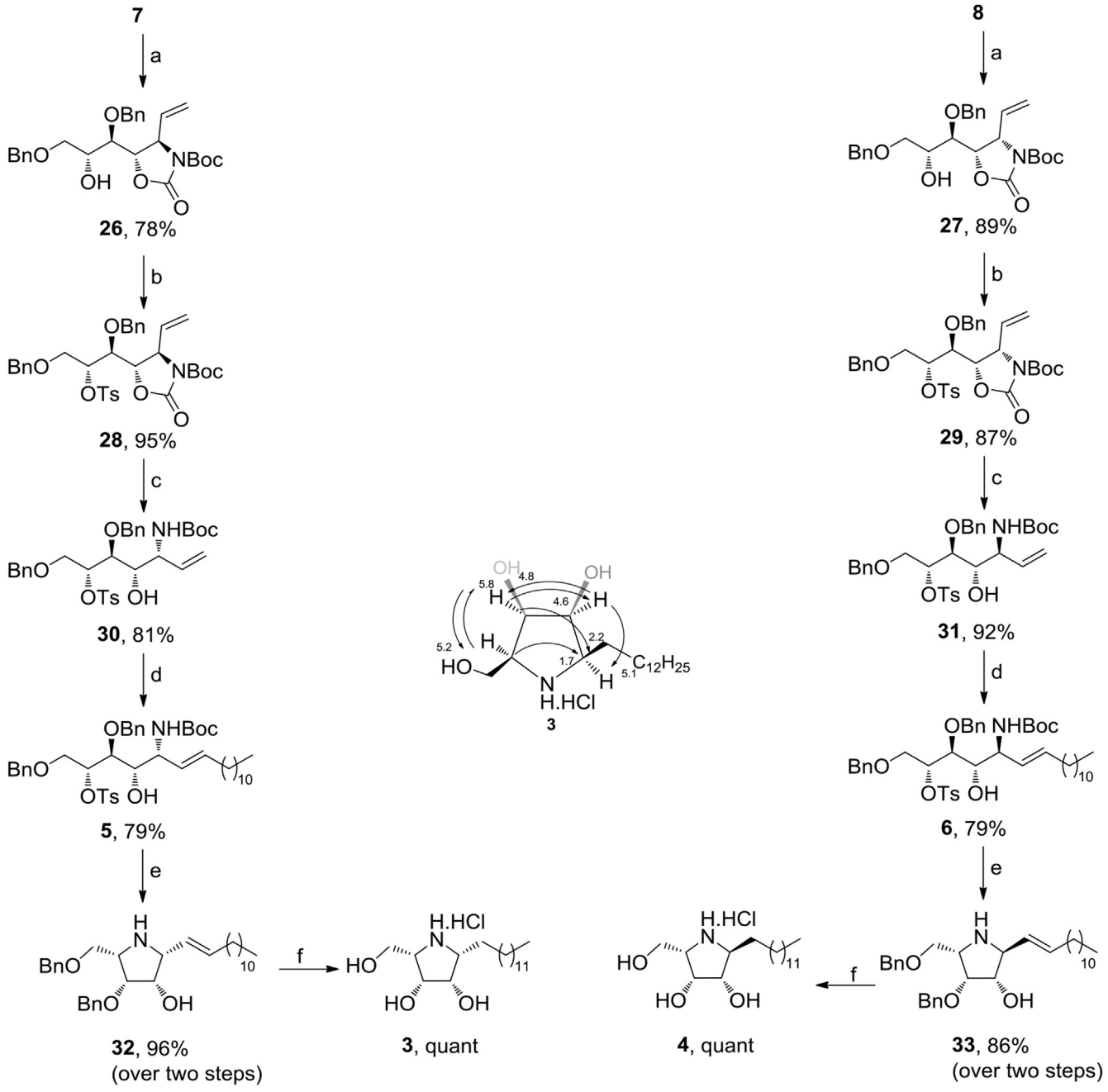
4.1.1. 1,2-O-Isopropylidene-5-O-trityl-α-D-ribofuranose (**12**) [13]

IBX was added to a solution of **11** [12b] (10.8 g, 0.025 mol) in MeCN (230 mL) and the resulting mixture was heated at reflux for 5 h. After cooling to room temperature, the solid parts were filtered off, and the filtrate was concentrated in vacuo. Chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 5:1) gave 10.0 g (93%) of 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranos-3-ulose [13] as a white amorphous solid; $[\alpha]_D^{26}$ +123.5 (*c* 0.20, $CHCl_3$), lit [13]. $[\alpha]_D$ +132 (*c* 4.7, $CHCl_3$, temperature at 21–22 °C). IR (neat) υ_{max} 1010, 1077, 1152, 1215, 1375, 1448, 1489, 1773 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.47 (s, 6H, 2 × CH_3), 3.32 (dd, 1H, $J = 2.6$ Hz, $J = 10.1$ Hz, H-5), 3.50 (dd, 1H, $J = 2.4$ Hz, $J = 10.1$ Hz, H-5), 4.41 (m, 1H, H-4), 4.54 (dd, 1H, $J = 1.0$ Hz, $J = 4.5$ Hz, H-2), 6.33 (d, 1H, $J = 4.5$ Hz, H-1), 7.23–7.25 (m, 3H, Ph), 7.28–7.31 (m, 6H, Ph), 7.34–7.36 (m, 6H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.2 (CH_3), 27.7 (CH_3), 64.4 (C-5), 76.9 (C-2), 80.0 (C-4), 87.4 (C_q), 103.6 (C-1), 114.2 (C_q), 127.2 (3 × CH_{Ph}), 127.9 (6 × CH_{Ph}), 128.6 (6 × CH_{Ph}), 143.2 (3 × C_i), 210.2 (C-3). Anal. Calcd for $C_{27}H_{26}O_5$: C, 75.33; H, 6.09. Found: C, 75.20, H, 6.18.

To a solution of the obtained ulose [13] (9.9 g, 0.023 mol) in a mixture of EtOH/CH_2Cl_2 (378 mL, 8:1) that had been pre-cooled to 0 °C was added $NaBH_4$ (1.74 g, 0.046 mol). After stirring at 0 °C for 10 min and another 20 min at room temperature, the solvents were evaporated, and the residue was partitioned between CH_2Cl_2 (290 mL) and brine (340 mL). The separated aqueous layer was then washed with CH_2Cl_2 (2 × 290 mL). The combined organic extracts were dried over Na_2SO_4, the solvent was evaporated, and the residue was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 5:1) to afford 9.85 g (99%) of compound **12** as a white foam; $[\alpha]_D^{26}$ +25.5 (*c* 0.20, $CHCl_3$); lit [13]. $[\alpha]_D$ +25.8 (*c* 4.5, $CHCl_3$, temperature at 21–22 °C). IR (neat) υ_{max} 1011, 1064, 1114, 1163, 1213, 1373, 1447, 1490 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 2.31 (d, 1H, $J = 9.6$ Hz, OH), 3.27 (dd, 1H, $J = 4.8$ Hz, $J = 10.4$ Hz, H-5), 3.41 (dd, 1H, $J = 3.0$ Hz, $J = 10.4$ Hz, H-5), 3.88–4.00 (m, 2H, H-3, H-4), 4.57–4.59 (m, 1H, H-2), 5.88 (d, 1H, $J = 3.8$ Hz, H-1), 7.21–7.26 (m, 3H, Ph), 7.27–7.32 (m, 6H, Ph), 7.44–7.48 (m, 6H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.5 (CH_3), 26.6 (CH_3), 63.0 (C-5), 72.3 (C-3), 78.5(C-2), 79.8 (C-4), 86.7 (C_q), 104.2 (C-1), 112.6 (C_q), 127.0 (3 × CH_{Ph}), 127.8 (6 × CH_{Ph}), 128.7 (6 × CH_{Ph}), 143.8 (3 × C_i). Anal. Calcd for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53. Found: C, 74.82; H, 6.64.

4.1.2. 1,2-O-Isopropylidene-α-D-ribofuranose (**13**) [14]

A solution of ribofuranose **12** (9.5 g, 0.022 mol) in CH_2Cl_2/MeOH (96 mL, 2:1) was treated with CSA (0.256 g, 1.10 mmol) at room temperature. After being stirred until no starting material was observed on TLC (3 h), the reaction was quenched with Et_3N (0.2 mL), and solvents were evaporated. Purification of the residue by flash chromatography on silica gel (*n*-hexane/ethyl acetate, 1:2) provided 4.0 g (96%) of compound **13** as colourless crystals; mp 86–87 °C; lit [14]. mp 85–86 °C; $[\alpha]_D$ [24] +43.2 (*c* 0.91, $CHCl_3$); lit [14]. $[\alpha]_D$ [21] +42.7 (*c* 1.32, $CHCl_3$). IR (neat) υ_{max} 1018, 1087, 1115, 1142, 1172, 1215, 1240, 1370, 1380, 2886, 2421, 2956, 3232 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.97 (br s, 1H, OH), 2.46 (br s, 1H, OH), 3.72–3.77 (m, 1H, H-5), 3.82–3.86 (m, 1H, H-5), 3.94–4.04 (m, 2H, H-3, H-4), 4.59 (t, 1H, $J = 4.4$ Hz, H-2), 5.83 (d, 1H, $J = 3.7$ Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.5



Scheme 4. *Reagents and conditions:* (a) Boc_2O, DMAP, Et_3N, CH_2Cl_2, 0 °C; (b) TsCl, Et_3N, $Me_3N.HCl$, CH_2Cl_2, rt; (c) Cs_2CO_3, EtOH, 0 °C; (d) tridec-1-ene, Grubbs II, CH_2Cl_2, reflux; (e) (i) TFA, CH_2Cl_2, 0 °C; (ii) aq NH_3, MeOH, rt; (f) H_2, 20% $Pd(OH)_2/C$, EtOAc/MeOH (1:3), 12 M HCl, rt.

Table 2
Antiproliferative activities of **3** and **4** on seven human cancer cell lines (A-549, MCF-7, MDA-MB-231, HCT-116, Caco-2, HeLa and Jurkat) and non-malignant mouse fibroblasts NiH 3T3.

Compd no.	Cell line, IC_{50}[a] ± SD (μmol × L^{-1})							
	A-549	MCF-7	HCT-116	Caco-2	HeLa	NiH 3T3	MDA-MB-231	Jurkat
3	ND	22.0 ± 4.11	9.3 ± 3.29	5.1 ± 0.87	22.4 ± 8.83	ND	24.6 ± 1.99	5.8 ± 1.41
4	23.8 ± 6.8	26.6 ± 3.22	30.9 ± 3.92	25.9 ± 0.56	18.4 ± 1.43	<10	28.4 ± 0.89	ND
cisplatin	9.5 ± 0.2	15.6 ± 0.3	15.3 ± 0.5	15.2 ± 0.3	13.1 ± 0.2	20.87 ± 0.3	17.5 ± 0.5	16.2 ± 0.6

ND-not detected.
[a] The potency of 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 amount of viable cells to 50% relative to untreated control cells, see *Experimental part*, section 4.2.2.).

(2 × CH_3), 60.8 (C-5), 70.8 (C-3), 78.7 (C-2), 80.7 (C-4), 104.0 (C-1), 112.8 (C_q). Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.39; H, 7.51.

4.1.3. 3,5-Di-O-benzyl-1,2-O-isopropylidene-α-D-ribofuranose (**14**) [14]

To an ice-cold solution of **13** (3.25 g, 17.1 mmol) in dry DMF (37 mL) were successively added NaH (1.97 g, 51.3 mmol, 60% dispersion in mineral oil), BnBr (4.5 mL, 37.6 mmol) and TBAI (0.189 g, 0.51 mmol). After stirring at 0 °C for 10 min and another 50 min at room temperature, the excess hydride was decomposed by addition of MeOH, and the resulting mixture was partitioned between Et_2O (200 mL) and cold water (370 mL). The separated aqueous layer was then washed with another portion of Et_2O (200 mL). The combined organic extracts were dried over Na_2SO_4, concentrated, and the residue was chromatographed on silica gel (*n*-hexane/ethyl acetate, 7:1) to give 6.2 g (98%) of compound **14** as a colourless oil; $[\alpha]_D$ [24] +90.3 (*c* 0.64, $CHCl_3$); lit [14]. $[\alpha]_D$ [21] +86.6 (*c* 2.66, $CHCl_3$). IR (neat) υ_{max} 1018, 1095, 1125, 1167, 1213, 1308, 1371, 1453, 1496, 2864, 2985 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.36 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 3.56 (dd, 1H, J = 3.9 Hz, J = 11.2 Hz, H-5), 3.76 (dd, 1H, J = 2.0 Hz, J = 11.2 Hz, H-5), 3.86 (dd, 1H, J = 4.4 Hz, J = 9.1 Hz, H-3), 4.16–4.19 (m, 1H, H-4), 4.48–4.58 (m, 4H, H-2, OCH_2Ph, *H*-OCH_2Ph), 4.73 (d, 1H, J = 12.0 Hz, OCH_2Ph), 5.75 (d, 1H, J = 3.7 Hz, H-1), 7.25–7.36 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.5 (CH_3), 26.8 (CH_3), 67.9 (C-5), 72.2 (OCH_2Ph), 73.5 (OCH_2Ph), 77.1 (C-3), 77.4 (C-2), 77.9 (C-4), 104.1 (C-1), 112.9 (C_q), 127.6 (CH_{Ph}), 127.7 (2 × CH_{Ph}), 128.0 (3 × CH_{Ph}), 128.3 (2 × CH_{Ph}), 128.4 (2 × CH_{Ph}), 137.6 (C_i), 138.0 (C_i). Anal. Calcd for $C_{22}H_{26}O_5$: C, 71.33; H, 7.07. Found: C, 71.25; H, 7.20.

4.1.4. Ethyl (4S,5S,6R,2E)-5,7-bis(benzyloxy)-4,6-dihydroxyhept-2-enoate (**16**)

Compound **14** (6.1 g, 16.5 mmol) was treated with a solution of 80% aq TFA (29 mL) at room temperature. After being stirred until no starting material was observed (judged by TLC, 2 h), the mixture was diluted with EtOAc (40 mL) and water (40 mL), and neutralized with the solid Na_2CO_3. The separated aqueous layer was washed with EtOAc (4 × 100 mL). The combined organic extracts were dried over Na_2SO_4, the filtrate was concentrated in vacuo, and the residue was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 1:1) to afford 4.35 g (80%) of compound **15** [14] as a white amorphous solid that was used immediately in the next reaction without spectral characterization.

Ylide, Ph_3P = $CHCO_2Et$, (2.85 g, 8.17 mmol) and benzoic acid (55.4 mg, 0.454 mmol) were successively added to a solution of **15** (1.5 g, 4.54 mmol) in dry CH_2Cl_2 (30 mL) at room temperature. After stirring for 8 h, the solvent was removed in vacuo, and the crude product was chromatographed through a short silica gel column (*n*-hexane/ethyl acetate, 2:1, then CH_2Cl_2/ethyl acetate, 10:1) to afford 1.22 g (67%) of compound **16** as a colourless oil; $[\alpha]_D$ [24] −11.8 (*c* 0.22, $CHCl_3$). IR (neat) υ_{max} 1071, 1267, 1655, 1701, 2867, 2901 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.29 (t, 3H, J = 7.1 Hz, CH_3), 2.71 (d, 1H, J = 5.9 Hz, OH), 3.22 (d, 1H, J = 4.8 Hz, OH), 3.53 (dd, 1H, J = 5.6 Hz, J = 7.3 Hz, H-5), 3.60 (dd, 1H, J = 5.3 Hz, J = 9.6 Hz, H-7), 3.67 (dd, 1H, J = 3.7 Hz, J = 9.6 Hz, H-7), 3.88–3.93 (m, 1H, H-6), 4.20 (q, 2H, J = 7.1 Hz, CH_2), 4.49–4.62 (m, 5H, H-4, 2 × OCH_2Ph), 6.14 (dd, 1H, J = 1.8 Hz, J = 15.7 Hz, H-2), 7.11 (dd, 1H, J = 4.7 Hz, J = 15.7 Hz, H-3), 7.24–7.39 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (CH_3), 60.4 (CH_2), 70.6 (C-7), 71.7 (C-6), 72.2 (C-4), 73.5 (OCH_2Ph), 74.1 (OCH_2Ph), 81.3 (C-5), 121.7 (C-2), 128.0 (3 × CH_{Ph}), 128.1 (CH_{Ph}), 128.2 (2 × CH_{Ph}), 128.5 (4 × CH_{Ph}), 137.5 (2 × C_i), 146.5 (C-3), 166.4 (C=O). Anal. Calcd for $C_{23}H_{28}O_6$: C, 69.98; H, 7.05. Found: C, 69.86; H, 6.96.

4.1.5. Ethyl (4S,5S,6R,2E)-5,7-bis(benzyloxy)-4,6-(isopropylidenedioxy)hept-2-enoate (**17**)

2,2-Dimethoxypropane (1.5 mL, 9.0 mmol) and *p*-TsOH.H_2O (57 mg, 0.3 mmol) were added to a solution of **16** (1.2 g, 3.0 mmol) in dry CH_2Cl_2 (17 mL). After stirring at room temperature for 30 min, the whole mixture was washed with a saturated $NaHCO_3$ solution (2 × 14 mL). The organic layer was dried over Na_2SO_4 and concentrated. Chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 11:1) furnished 1.28 g (97%) of compound **17** as a colourless oil; $[\alpha]_D^{25}$ −39.0 (*c* 0.42, $CHCl_3$). IR (neat) υ_{max} 1092, 1660, 1716, 2868, 2991, 3030 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.30 (t, 3H, J = 7.1 Hz, CH_3), 1.49 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 3.31 (t, 1H, J = 9.7 Hz, H-5), 3.63 (dd, 1H, J = 1.9 Hz, J = 10.9 Hz, H-7), 3.72 (dd, 1H, J = 4.2 Hz, J = 10.9 Hz, H-7), 3.91 (ddd, 1H, J = 1.9 Hz, J = 4.2 Hz, J = 9.6 Hz, H-6), 4.21 (q, 2H, J = 7.1 Hz, CH_2), 4.34–4.37 (m, 1H, H-4), 4.39 (d, 1H, J = 10.6 Hz, OCH_2Ph), 4.47 (d, 1H, J = 10.6 Hz, OCH_2Ph), 4.55 (d, 1H, J = 12.2 Hz, OCH_2Ph), 4.66 (d, 1H, J = 12.2 Hz, OCH_2Ph), 6.16 (dd, 1H, J = 1.5 Hz, J = 15.7 Hz, H-2), 7.06 (dd, 1H, J = 4.9 Hz, J = 15.7 Hz, H-3), 7.16–7.18 (m, 2H, Ph), 7.25–7.39 (m, 8H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2 (CH_3), 19.2 (CH_3), 29.3 (CH_3), 60.4 (CH_2), 69.1 (C-7), 72.2 (C-4), 73.1 (C-6), 73.5 (OCH_2Ph), 74.4 (C-5), 74.8 (OCH_2Ph), 98.9 (C_q), 122.2 (C-2), 127.7 (CH_{Ph}), 128.0 (3 × CH_{Ph}), 128.2 (2 × CH_{Ph}), 128.4 (2 × CH_{Ph}), 128.5 (2 × CH_{Ph}), 137.1 (C_i), 138.0 (C_i), 144.4 (C-3), 166.3 (C=O). Anal. Calcd for $C_{26}H_{32}O_6$: C, 70.89; H, 7.32. Found: C, 71.00; H, 7.41.

4.1.6. (4S,5S,6R,2E)-5,7-Bis(benzyloxy)-4,6-(isopropylidenedioxy) hept-2-en-1-ol (**18**) [14]

DIBAl-H (6.8 mL, 8.16 mmol, ~1.2 M solution in toluene) was added dropwise to a solution of ester **17** (1.2 g, 2.72 mmol) that had been pre-cooled to −30 °C. After being stirred at the same temperature until no starting material was observed on TLC (30 min), the reaction was quenched with MeOH (1.6 mL), and the whole mixture was treated with a 30% K/Na tartrate solution (43 mL) with vigorous stirring over 1 h at room temperature. The separated aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL). The combined organic extracts were dried over Na_2SO_4, concentrated, and the residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 2:1) to give 1.04 g (96%) of allylic alcohol **18** as white crystals; mp 89–90 °C; lit [14]. mp 92–93 °C; $[\alpha]_D$ [24] +5.2 (*c* 0.62, $CHCl_3$); lit [14]. $[\alpha]_D$ [24] +9.1 (*c* 1.22, $CHCl_3$). IR (neat) υ_{max} 1011, 1093, 2865, 2992, 3477 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.28 (br s, 1H, OH), 1.47 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 3.30 (t, 1H, J = 9.6 Hz, H-5), 3.65 (dd, 1H, J = 1.9 Hz, J = 10.9 Hz, H-7), 3.73 (dd, 1H, J = 4.3 Hz, J = 10.9 Hz, H-7), 3.90 (ddd, 1H, J = 1.9 Hz, J = 4.1 Hz, J = 9.6 Hz, H-6), 4.10–4.16 (m, 2H, 2 × H-1), 4.22 (dd, 1H, J = 7.2 Hz, J = 9.1 Hz, H-4), 4.39 (d, 1H, J = 10.8 Hz, OCH_2Ph), 4.48 (d, 1H, J = 10.8 Hz, OCH_2Ph), 4.56 (d, 1H, J = 12.3 Hz, OCH_2Ph), 4.68 (d, 1H, J = 12.3 Hz, OCH_2Ph), 5.72 (dd, 1H, J = 6.9 Hz, J = 15.5 Hz, H-3), 6.01 (td, 1H, J = 5.2 Hz, J = 15.5 Hz, H-2) 7.15–7.17 (m, 2H, Ph), 7.27–7.38 (m, 8H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.4 (CH_3), 29.5 (CH_3), 63.0 (C-1), 69.3 (C-7), 73.0 (C-6), 73.5 (OCH_2Ph), 73.7 (C-4), 74.4 (OCH_2Ph, C-5), 98.7 (C_q), 127.7 (CH_{Ph}), 127.9 (CH_{Ph}), 128.0 (2 × CH_{Ph}), 128.3 (2 × CH_{Ph}), 128.4 (4 × CH_{Ph}), 128.8 (C-3), 133.4 (C-2), 137.7 (C_i), 138.1 (C_i). Anal. Calcd for $C_{24}H_{30}O_5$: C, 72.34; H, 7.59. Found: C, 72.25; H, 7.67.

There are some differences in the coupling constants of the vinyl protons H-2 and H-3 between our compound **18** and the sample reported by Xie and co-workers [14]. Chemical shifts of these protons are in good agreement with those published in the literature [14].

4.1.7. (4R,5S,6S)-5-(Benzyloxy)-4-[(benzyloxy)methyl]-2,2-dimethyl-6-[(E)-3′-thiocyanatoprop-1′-en-1′-yl)-1,3-dioxane] (**9**)

To an ice-cold solution of alcohol **18** (0.667 g, 1.7 mmol) in dry

CH_2Cl_2 (14 mL) were successively added Et_3N (0.48 mL, 3.4 mmol) and MsCl (0.26 mL, 3.4 mmol). After being stirred at 0 °C for 10 min and another 20 min at room temperature, the mixture wad diluted with Et_2O, the solid part was filtered off, and the filtrate was concentrated in vacuo. The obtained residue was used immediately in the next reaction without purification.

KSCN (0.30 g, 3.07 mmol) was added to a solution of the crude mesylate (0.81 g, 1.70 mmol) in MeCN (14 mL) at 0 °C. After the mixture had stirred for 24 h at room temperature, the resulting yellow mixture was diluted with Et_2O. The insoluble material was removed by filtration and washed with Et_2O. After the solvent was evaporated, the residue was flash-chromatographed on silica gel (*n*-hexane/ethyl acetate, 9:1) to furnish 0.684 g (93%) of compound **9** as a colourless oil; $[\alpha]_D$ [23] −4.2 (*c* 0.38, $CHCl_3$). IR (neat) υ_{max} 970, 1093, 1201, 2153, 2865, 2992, 3029 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.48 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 3.31 (t, 1H, $J = 9.6$ Hz, H-5), 3.50−3.59 (m, 2H, 2 × H-3′), 3.63 (dd, 1H, $J = 2.0$ Hz, $J = 10.9$ Hz, H-1″), 3.71 (dd, 1H, $J = 4.3$ Hz, $J = 10.9$ Hz, H-1″), 3.90 (ddd, 1H, $J = 2.0$ Hz, $J = 4.3$ Hz, $J = 9.6$ Hz, H-4), 4.24 (dd, 1H, $J = 4.9$ Hz, $J = 9.5$ Hz, H-6), 4.43 (d, 1H, $J = 10.9$ Hz, OCH_2Ph), 4.56 (d, 1H, $J = 12.3$ Hz, OCH_2Ph), 4.58 (d, 1H, $J = 10.9$ Hz, OCH_2Ph), 4.66 (d, 1H, $J = 12.3$ Hz, OCH_2Ph), 5.90 (dd, 1H, $J = 4.9$ Hz, $J = 15.3$ Hz, H-1′), 5.97 (dd, 1H, $J = 6.6$ Hz, $J = 15.3$ Hz, H-2′), 7.17−7.38 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.3 (CH_3), 29.4 (CH_3), 35.9 (C-3′), 69.2 (C-1″), 72.7 (C-6), 73.1 (C-4), 73.5 (OCH_2Ph), 74.5 (OCH_2Ph), 74.6 (C-5), 98.8 (C_q), 111.9 (SCN), 124.9 (C-2′), 127.7 (CH_{Ph}), 127.9 (CH_{Ph}), 128.0 (2 × CH_{Ph}), 128.1 (2 × CH_{Ph}), 128.4 (4 × CH_{Ph}), 135.0 (C-1′), 137.6 (C_i), 138.1 (C_i). Anal. Calcd for $C_{25}H_{29}NO_4S$: C, 68.31; H, 6.65; N, 3.19. Found: C, 68.22; H, 6.73; N, 3.23.

*4.1.8. (4R,5S,6S)-5-(Benzyloxy)-4-[(benzyloxy)methyl]-6-[(R)-1′-isothiocyanatoallyl]-2,2-dimethyl-1,3-dioxane (19) and (4R,5S,6S)-5-(benzyloxy)-4-[(benzyloxy)methyl]-6-[(S)-1′-isothiocyanatoallyl]-2,2-dimethyl-1,3-dioxane (**20**)*

4.1.8.1. Conventional procedure. A solution of thiocyanate **9** (86 mg, 0.196 mmol) in dry *n*-heptane (1.7 mL) was heated at 90 °C for 6 h under a nitrogen atmosphere before cooling to room temperature. After the solvent was evaporated, the residue was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate, 17:1, then 9:1) to give a diastereomeric mixture of the corresponding isothiocyanates **19** and **20** (52 mg, 60%).

4.1.8.2. Microwave-assisted synthesis. Thiocyanate **9** (86 mg, 0.196 mmol) was weighed in a 10-mL glass pressure microwave tube equipped with a magnetic stirbar. *n*-Heptane (1.7 mL) was added, the tube was closed with a silicon septum, and the resulting mixture was subjected to microwave irradiation at 90 °C for 1 h. The mixture was allowed to cool to room temperature, the solvent was evaporated, and the residue was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate, 17:1, then 9:1) to afford a mixture of isothiocyanates **19** and **20** (66 mg, 77%).

Requiring a greater amount of the pure rearranged products **19** and **20**, they were obtained on a multi-gram scale by the microwave-assisted method in *n*-heptane at 90 °C using a big vessel. Column chromatography allowed the isolation of both stereoisomers in pure form.

Diastereoisomer **19**: colourless oil; $[\alpha]_D$ [21] −28.6 (*c* 0.28, $CHCl_3$). IR (neat) υ_{max} 1064, 1202, 1361, 2050, 2868, 2924 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 1.43 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 3.60 (t, 1H, $J = 9.5$ Hz, H-5), 3.67 (dd, 1H, $J = 2.1$ Hz, $J = 11.2$ Hz, H-1″), 3.71 (dd, 1H, $J = 2.0$ Hz, $J = 9.5$ Hz, H-6), 3.75 (dd, 1H, $J = 4.4$ Hz, $J = 11.2$ Hz, H-1″), 3.88 (ddd, 1H, $J = 2.1$ Hz, $J = 4.5$ Hz, $J = 9.5$ Hz, H-4), 4.30−4.32 (m, 1H, H-1′), 4.51 (d, 1H, $J = 11.0$ Hz, OCH_2Ph), 4.56 (d, 1H, $J = 11.0$ Hz, OCH_2Ph), 4.59 (d, 1H, $J = 12.2$ Hz, OCH_2Ph), 4.72 (d, 1H, $J = 12.2$ Hz, OCH_2Ph), 5.25−5.27 (m, 1H, H-3′), 5.32−5.35 (m, 1H, H-3′), 5.87 (ddd, 1H, $J = 6.3$ Hz, $J = 10.2$ Hz, $J = 16.8$ Hz, H-2′), 7.19−7.21 (m, 2H, Ph), 7.28−7.41 (m, 8H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$): δ 19.2 (CH_3), 29.2 (CH_3), 60.0 (C-1′), 69.4 (C-1″), 71.4 (C-5), 73.3 (C-4), 73.7 (OCH_2Ph), 74.7 (OCH_2Ph), 74.9 (C-6), 99.2 (C_q), 118.2 (C-3′), 127.7 (CH_{Ph}), 128.0 (2 × CH_{Ph}), 128.1 (2 × CH_{Ph}), 128.2 (CH_{Ph}), 128.4 (2 × CH_{Ph}), 128.6 (2 × CH_{Ph}), 133.0 (C-2′), 136.0 (NCS), 137.3 (C_i), 138.1 (C_i). Anal. Calcd for $C_{25}H_{29}NO_4S$: C, 68.31; H, 6.65; N, 3.19. Found: C, 68.25; H, 6.73; N, 3.26.

Diastereoisomer **20**: colourless oil; $[\alpha]_D^{26}$ −91.9 (*c* 0.58, $CHCl_3$). IR (neat) υ_{max} 1090, 1202, 1381, 2040, 2869, 2992 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 1.47 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 3.44 (t, 1H, $J = 9.6$ Hz, H-5), 3.64 (dd, 1H, $J = 2.2$ Hz, $J = 11.1$ Hz, H-1″), 3.74 (dd, 1H, $J = 4.0$ Hz, $J = 11.1$ Hz, H-1″), 3.84−3.87 (m, 1H, H-4), 3.99 (dd, 1H, $J = 2.4$ Hz, $J = 9.7$ Hz, H-6), 4.36−4.38 (m, 1H, H-1′), 4.42 (d, 1H, $J = 11.0$ Hz, OCH_2Ph), 4.44 (d, 1H, $J = 11.0$ Hz, OCH_2Ph), 4.56 (d, 1H, $J = 12.2$ Hz, OCH_2Ph), 4.69 (d, 1H, $J = 12.2$ Hz, OCH_2Ph), 5.23−5.26 (m, 1H, H-3′), 5.30 (d, 1H, $J = 10.2$ Hz, H-3′), 5.91 (ddd, 1H, $J = 7.1$ Hz, $J = 10.2$ Hz, $J = 17.2$ Hz, H-2′), 7.14−7.16 (m, 2H, Ph), 7.27−7.37 (m, 4H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$): δ 19.1 (CH_3), 29.1 (CH_3), 61.5 (C-1′), 69.2 (C-1″), 71.0 (C-5), 73.5 (C-4), 73.6 (OCH_2Ph), 73.8 (OCH_2Ph), 75.2 (C-6), 99.2 (C_q), 119.4 (C-3′), 127.7 (CH_{Ph}), 127.8 (2 × CH_{Ph}), 128.0 (3 × CH_{Ph}), 128.4 (2 × CH_{Ph}), 128.5 (2 × CH_{Ph}), 131.4 (C-2′), 136.3 (NCS), 137.4 (C_i), 138.0 (C_i). Anal. Calcd for $C_{25}H_{29}NO_4S$: C, 68.31; H, 6.65; N, 3.19. Found: C, 68.22; H, 6.71; N, 3.25.

*4.1.9. N-[(R)-1-{(4′S,5′S,6′R)-5′-(Benzyloxy]-6′-[(benzyloxy)methyl]-2′,2′-dimethyl-1′,3-dioxan-4′-yl}allyl]-2,2,2-trichloroacetamide (**21**) and N-[(S)-1-{(4′S,5′S,6′R)-5′-(benzyloxy]-6′-[(benzyloxy)methyl]-2′,2′-dimethyl-1′,3-dioxan-4′-yl}allyl]-2,2,2-trichloroacetamide (**22**)*

To an ice-cold solution of **18** (85 mg, 0.21 mmol) in dry CH_2Cl_2 (1.1 mL) was added DBU (3.2 μL, 0.021 mmol) followed by trichloroacetonitrile (42.7 μL 0.42 mmol). After being stirred at 0 °C for 10 min and then for a further 20 min at room temperature, the mixture was filtered through a small pad of silica gel. Removal of the solvent provided the crude imidate **10** that was used in the next reaction without purification.

Imidate **10** (0.115 g, 0.21 mmol) was weighed to a 10-mL glass pressure microwave tube equipped with a magnetic stirbar. *o*-Xylene (2.9 mL) followed by anhydrous K_2CO_3 (33.6 mg, 0.24 mmol) were added, the tube was closed with a silicone septum, and the reaction was subjected to microwave irradiation at 150 °C (3.5 h) or 170 °C (1 h). After cooling to room temperature, the insoluble parts were filtered off, and the filtrate was concentrated in vacuo. Purification of the residue on silica gel (*n*-hexane/ethyl acetate, 9:1) afforded an inseparable mixture of diastereoisomers **21** and **22** (98 mg, 85% for 150 °C; 0.108 g, 94% for 170 °C).

Requiring a greater amount of the rearranged products **21** and **22**, the procedure was repeated at 170 °C with 1 g of the starting imidate **10** using a big vessel.

*4.1.10. (4R,5S)-5-[(1′R,2′R)-1′,3′-Bis(benzyloxy)-2′-hydroxypropyl]-4-vinyloxazolidin-2-one (**7**) and (4S,5S)-5-[(1′R,2′R)-1′,3′-bis(benzyloxy)-2′-hydroxypropyl]-4-vinyloxazolidin-2-one (**8**)*

p-TsOH.H_2O (16.7 mg, 0.088 mmol) was added to a solution of a mixture of trichloroacetamides **21** and **22** (0.24 g, 0.44 mmol) in MeOH (8.7 mL). After stirring at room temperature for 22 h, the solvent was evaporated, and the residue was subjected to flash chromatography through a short silica gel column (*n*-hexane/ethyl acetate, 3:1) to yield 0.205 g (92%) of an inseparable mixture of diols **23**.

*4.1.10.1. From diols **23**.* DBU (6 μL, 38.0 μmol) was added to a solution of **23** (0.191 g, 0.38 mmol) in dry CH_2Cl_2 (4.4 mL) at room temperature. After stirring for 28 h, the solvent was evaporated,

and the residue was purified by flash chromatography (*n*-hexane/ethyl acetate, 1:1) to afford both cyclic carbamates **7** and **8** in combined yield of 96% (0.14 g). Repeated chromatography gave the pure oxazolidinones **7** and **8** as colourless oils.

*4.1.10.2. From thiocarbamates **24** and **25**, respectively. Modification of **24** into **7***: Mesitylnitrile oxide (48.4 mg, 0.30 mmol) was added to a solution of **24** (0.10 g, 0.25 mmol) in MeCN (2.4 mL) at room temperature. After stirring for 30 min, the solvent was removed in vacuo, and the residue was chromatographed on silica gel (*n*-hexane/ethyl acetate, 1:1) to give 85 mg (89%) of carbamate **7**.

*Modification of **25** into **8***: According to the same procedure described for the preparation of **7**, thiocarbamate **25** (80 mg, 0.20 mmol) and MNO (38.7 mg, 0.24 mmol) provided after flash chromatography on silica gel (*n*-hexane/ethyl acetate, 1:1) 71 mg (92%) of derivative **8**.

Diastereoisomer **7**: $[\alpha]_D$ [21] +72.8 (*c* 0.50, $CHCl_3$). IR (neat) υ_{max} 1088,1227, 1454, 1736, 2864, 3292 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.73 (d, 1H, J = 5.7 Hz, OH), 3.53 (dd, 1H, J = 5.5 Hz, J = 9.5 Hz, H-3′), 3.61 (dd, 1H, J = 3.1 Hz, J = 9.5 Hz, H-3′), 3.72–3.78 (m, 1H, H-2′), 3.85 (dd, 1H, J = 2.7 Hz, J = 8.2 Hz, H-1′), 4.39 (t, 1H, J = 6.6 Hz, H-4), 4.46–4.53 (m, 3H, OCH_2Ph, *H*-OCH_2Ph), 4.67 (dd, 1H, J = 2.7 Hz, J = 6.2 Hz, H-5), 4.75 (d, 1H, J = 11.0 Hz, OCH_2Ph), 5.18 (d, 1H, J = 10.2 Hz, H-2″), 5.25 (d, 1H, J = 17.1 Hz, H-2″), 5.82 (ddd, 1H, J = 7.0 Hz, J = 10.2 Hz, J = 17.1 Hz, H-1″), 5.96 (br s, 1H, NH), 7.22–7.36 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 54.9 (C-4), 69.1 (C-2′), 70.5 (C-3′), 73.4 (OCH_2Ph), 74.8 (OCH_2Ph), 78.2 (C-1′), 82.2 (C-5), 117.7 (C-2″) 127.9 (3 × CH_{Ph}), 128.0 (CH_{Ph}), 128.3 (2 × CH_{Ph}), 128.4 (2 × CH_{Ph}), 128.5 (2 × CH_{Ph}), 136.5 (C-1″), 137.4 (2 × C_i), 158.8 (C-2). Anal. Calcd for $C_{22}H_{25}NO_5$: C, 68.91; H, 6.57; N, 3.65. Found: C, 68.85; H, 6.49; N, 3.58.

Diastereoisomer **8**: $[\alpha]_D$ [21] −7.9 (*c* 0.48, $CHCl_3$). IR (neat) υ_{max} 1067, 1378, 1454, 1746, 2856, 2923, 3285 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.92 (d, 1H, J = 4.2 Hz, OH), 3.62–3.66 (m, 2H, 2 × H-3′), 3.85 (dd, 1H, J = 4.7 Hz, J = 7.2 Hz, H-1′), 4.12–4.18 (m, 1H, H-2′), 4.34 (t, 1H, J = 7.7 Hz, H-4), 4.48–4.55 (m, 2H, OCH_2Ph), 4.57 (d, 1H, J = 11.1 Hz, OCH_2Ph), 4.71 (d, 1H, J = 11.1 Hz, OCH_2Ph), 4.78 (t, 1H, J = 7.6 Hz, H-5), 5.23–5.28 (m, 2H, H-2″), 5.95 (ddd, 1H, J = 7.5 Hz, J = 10.2 Hz, J = 17.4 Hz, H-1″), 6.13 (br s, 1H, NH), 7.23–7.36 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 57.4 (C-4), 70.4 (C-2′, C-3′), 73.3 (OCH_2Ph), 73.6 (OCH_2Ph), 77.7 (C-1′), 78.1 (C-5), 119.0 (C-2″), 127.7 (3 × CH_{Ph}), 127.8 (3 × CH_{Ph}), 128.3 (2 × CH_{Ph}), 128.4 (2 × CH_{Ph}), 133.7 (C-1″), 137.6 (C_i), 137.7 (C_i), 158.8 (C-2). Anal. Calcd for $C_{22}H_{25}NO_5$: C, 68.91; H, 6.57; N, 3.65. Found: C, 68.82; H, 6.44; N, 3.56.

*4.1.11. (4R,5S)-5-[(1′R,2′R)-1′,3′-Bis(benzyloxy)-2′-hydroxypropyl]-4-vinyloxazolidine-2-thione (**24**)*

A solution of isothiocyanate **19** (0.15 g, 0.34 mmol) in MeOH (4.4 mL) was treated with *p*-TsOH (12.9 mg, 0.068 mmol). After being stirred at room temperature until no starting material was observed (judged by TLC, 24 h), the mixture was quenched with Et_3N, the solvent was evaporated, and the residue was flash-chromatographed on silica gel (*n*-hexane/ethyl acetate, 3:1) to furnish 0.128 g (94%) of compound **24** as colourless crystals; mp 107–109 °C; $[\alpha]_D$ [20] +87.0 (*c* 0.48, $CHCl_3$). IR (neat) υ_{max} 1091, 1249, 1374, 1497, 2861, 3249, 3589 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.41 (d, 1H, J = 6.2 Hz, OH), 3.55 (dd, 1H, J = 5.1 Hz, J = 9.4 Hz, H-3′), 3.61 (dd, 1H, J = 3.2 Hz, J = 9.4 Hz, H-3′), 3.65–3.71 (m, 1H, H-2′), 3.97 (dd, 1H, J = 2.1 Hz, J = 8.6 Hz, H-1′), 4.45–4.55 (m, 3H, OCH_2Ph, *H*-OCH_2Ph), 4.63 (t, 1H, J = 6.9 Hz, H-4), 4.80 (d, 1H, J = 10.6 Hz, OCH_2Ph), 5.03 (dd, 1H, J = 2.1 Hz, J = 6.6 Hz, H-5), 5.26–5.33 (m, 2H, 2 × H-2″), 5.86 (ddd, 1H, J = 7.5 Hz, J = 10.1 Hz, J = 17.4 Hz, H-1″), 6.74 (s, 1H, NH), 7.25–7.40 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 58.6 (C-4), 69.1 (C-2′), 70.2 (C-3′), 73.5 (OCH_2Ph), 74.9 (OCH_2Ph), 77.8 (C-1′), 88.5 (C-5), 119.3 (C-2″), 128.0 (2 × CH_{Ph}), 128.1 (2 × CH_{Ph}), 128.5 (2 × CH_{Ph}), 128.6 (4 × CH_{Ph}), 134.7 (C-1″), 137.1 (C_i), 137.2 (C_i), 188.9 (C-2). Anal. Calcd for $C_{22}H_{25}NO_4S$: C, 66.14; H, 6.31; N, 3.51. Found: C, 66.20; H, 6.27; N, 3.60.

*4.1.12. (4S,5S)-5-[(1′R,2′R)-1′,3′-Bis(benzyloxy)-2′-hydroxypropyl]-4-vinyloxazolidine-2-thione (**25**)*

By the same procedure as described for the preparation of thiocarbamate **24**, compound **20** (0.20 g, 0.455 mmol) and *p*-TsOH (17.3 mg, 0.091 mmol) afforded after flash chromatography on silica gel (*n*-hexane/ethyl acetate, 2:1) 0.16 g (88%) of compound **25** as white crystals; mp 114–116 °C; $[\alpha]_D$ [21] −17.4 (*c* 0.35, $CHCl_3$). IR (neat) υ_{max} 1082, 1156, 1455, 1511, 1734, 3275, 3567 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.81 (s, 1H, OH), 3.57–3.64 (m, 2H, 2 × H-3′), 3.95 (t, 1H, J = 5.9 Hz, H-1′), 4.05–4.11 (m, 1H, H-2′), 4.48 (d, 1H, J = 11.8 Hz, OCH_2Ph), 4.52–4.55 (m, 2H, *H*-OCH_2Ph, H-4), 4.61 (d, 1H, J = 11.0 Hz, OCH_2Ph), 4.67 (d, 1H, J = 11.0 Hz, OCH_2Ph), 5.10 (dd, 1H, J = 5.7 Hz, J = 8.8 Hz, H-5), 5.25 (d, 1H, J = 10.2 Hz, H-2″), 5.30 (d, 1H, J = 17.2 Hz, H-2″), 5.95–6.04 (m, 1H, H-1″), 7.23–7.37 (m, 10H, Ph), 7.83 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 61.2 (C-4), 70.0 (C-2′), 70.2 (C-3′), 73.4 (OCH_2Ph), 74.2 (OCH_2Ph), 77.8 (C-1′), 84.2 (C-5), 120.4 (C-2″), 127.8 (CH_{Ph}), 127.9 (5 × CH_{Ph}), 128.3 (2 × CH_{Ph}), 128.6 (2 × CH_{Ph}), 132.3 (C-1″), 137.4 (C_i), 137.5 (C_i), 188.6 (C-2). Anal. Calcd for $C_{22}H_{25}NO_4S$: C, 66.14; H, 6.31; N, 3.51. Found: C, 66.05; H, 6.36; N, 3.43.

*4.1.13. tert-Butyl (4R,5S)-5-[(1′R,2′R)-1′,3′-bis(benzyloxy)-2′-hydroxypropyl]-2-oxo-4-vinyloxazolidine-3-carboxylate (**26**)*

Boc_2O (0.116 g, 0.532 mmol), Et_3N (53.4 μL, 0.38 mmol) and DMAP (93 mg, 0.76 mmol) were successively added to a solution of oxazolidinone **7** (0.146 g, 0.38 mmol) in CH_2Cl_2 (10 mL) that had been pre-cooled to 0 °C. After stirring at the same temperature for 10 min, the mixture was poured into a saturated NH_4Cl solution (15 mL). The separated aqueous phase was then extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, the solvent was evaporated, and the residue was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate, 2:1) to give 0.143 g (78%) of compound **26** as a white solid; mp 91–92 °C; $[\alpha]_D$ [21] +67.1 (*c* 0.69, $CHCl_3$). IR (neat) υ_{max} 1088, 1146, 1311, 1732, 1759, 2923, 2976, 3443 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H, 3 × CH_3), 2.41 (d, 1H, J = 5.6 Hz, OH), 3.55 (dd, 1H, J = 4.7 Hz, J = 9.5 Hz, H-3′), 3.61 (dd, 1H, J = 2.9 Hz, J = 9.5 Hz, H-3′), 3.75–3.83 (m, 2H, H-2′, H-1′), 4.45 (d, 1H, J = 10.7 Hz, OCH_2Ph), 4.50 (d, 1H, J = 11.7 Hz, OCH_2Ph), 4.55 (d, 1H, J = 11.7 Hz, OCH_2Ph), 4.60 (dd, 1H, J = 2.1 Hz, J = 3.7 Hz, H-5), 4.69 (d, 1H, J = 10.7 Hz, OCH_2Ph), 4.75 (dd, 1H, J = 3.7 Hz, J = 7.1 Hz, H-4), 5.26–5.30 (m, 2H, 2 × H-2″), 5.87 (ddd, 1H, J = 7.1 Hz, J = 10.3 Hz, J = 17.3 Hz, H-1″), 7.18–7.40 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.8 (3 × CH_3), 57.1 (C-4), 69.1 (C-2′), 70.2 (C-3′), 73.5 (OCH_2Ph), 75.0 (OCH_2Ph), 78.6 (C-1′), 78.8 (C-5), 83.6 (C_q), 117.9 (C-2″), 128.0 (2 × CH_{Ph}), 128.1 (2 × CH_{Ph}), 128.4 (4 × CH_{Ph}), 128.6 (2 × CH_{Ph}), 135.2 (C-1″), 137.0 (C_i), 137.3 (C_i), 149.0 (C=O), 152.1 (C-2). Anal. Calcd for $C_{27}H_{33}NO_7$: C, 67.06; H, 6.88; N, 2.90. Found: C, 67.16; H, 6.79; N, 2.96.

During this reaction we also isolated 22 mg (10%) of *tert*-butyl (4*R*,5*S*)-5-{(1′*S*,2′*R*)-1′,3′-bis(benzyloxy)-2′-[(*tert*-butoxycarbonyl) oxy]propyl}-2-oxo-4-vinyloxazolidine-3-carboxylate as a colourless oil; $[\alpha]_D$ [21] +18.2 (*c* 0.78, $CHCl_3$). IR (neat) υ_{max} 1066, 1156, 1252, 1274, 1368, 1742, 1796, 1817, 2870, 2932, 2979 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, 3 × CH_3), 1.45 (s, 9H, 3 × CH_3), 3.65–3.72 (m, 2H, 2 × H-3′), 4.06 (dd, 1H, J = 2.6 Hz, J = 6.7 Hz, H-1′), 4.41–4.43 (m, 1H, H-5), 4.50 (d, 1H, J = 12.1 Hz, OCH_2Ph), 4.57 (d, 1H, J = 12.1 Hz, OCH_2Ph), 4.59 (d, 1H, J = 10.7 Hz, OCH_2Ph), 4.64 (d, 1H, J = 10.7 Hz, OCH_2Ph), 4.74 (dd, 1H, J = 3.4 Hz, J = 7.4 Hz, H-4), 4.94 (dt, 1H, J = 4.1 Hz, J = 6.7 Hz, H-2′), 5.23 (d, 1H, J = 10.3 Hz, H-

2″), 5.29 (d, 1H, J = 17.1 Hz, H-2″), 5.78–5.86 (m, 1H, H-1″), 7.20–7.36 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.6 (3 × CH_3), 27.8 (3 × CH_3), 57.4 (C-4), 67.7 (C-3′), 72.9 (C-2′), 73.3 (OCH_2Ph), 74.4 (OCH_2Ph), 76.7 (C-1′), 78.2 (C-5), 82.8 (C_q), 83.4 (C_q), 118.1 (C-2″), 127.7 (2 × CH_{Ph}), 127.8 (CH_{Ph}), 128.0 (CH_{Ph}), 128.2 (2 × CH_{Ph}), 128.4 (4 × CH_{Ph}), 134.8 (C-1″), 136.9 (C_i), 137.4 (C_i), 148.8 (C=O), 151.8 (C-2), 152.3 (C=O). Anal. Calcd for $C_{32}H_{41}NO_9$: C, 65.85; H, 7.08; N, 2.40. Found: C, 65.93; H, 6.99; N, 2.51.

4.1.14. *tert-Butyl (4R,5S)-5-[(1′S,2′R)-1′,3′-bis(benzyloxy)-2′-(tosyloxy)propyl]-2-oxo-4-vinyloxazolidine-3-carboxylate (**28**)*

Alcohol **26** (0.131 g, 0.27 mmol) was dissolved in dry CH_2Cl_2 (0.6 mL) and TsCl (0.129 g, 0.675 mmol), Et_3N (0.19 mL, 1.35 mmol) and Me_3N.HCl (13 mg, 0.135 mmol) were successively added at room temperature. The mixture was stirred until completion of the reaction (judged by TLC, 1 h), and was then diluted with CH_2Cl_2 (5 mL) before washing with a saturated NH_4Cl solution (5 mL). The organic layer was dried over Na_2SO_4, the solvent was evaporated in vacuo, and the residue was flash-chromatographed on silica gel (*n*-hexane/ethyl acetate, 5:1) to afford 0.164 g (95%) of compound **28** as a colourless oil; $[\alpha]_D$ [21] +12.7 (*c* 0.30, $CHCl_3$). IR (neat) υ_{max} 1061, 1175, 1366, 1725, 1813, 2929, 2979 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, 3 × CH_3), 2.37 (s, 3H, CH_3), 3.62 (dd, 1H, J = 5.0 Hz, J = 10.9 Hz, H-3′), 3.68 (dd, 1H, J = 4.6 Hz, J = 10.9 Hz, H-3′), 3.95 (t, 1H, J = 4.2 Hz, H-1′), 4.35–4.41 (m, 3H, H-5, OCH_2Ph), 4.52 (d, 1H, J = 11.0 Hz, OCH_2Ph), 4.57 (d, 1H, J = 11.0 Hz, OCH_2Ph), 4.63 (dd, 1H, J = 2.8 Hz, J = 6.8 Hz, H-4), 4.82–4.85 (m, 1H, H-2′), 5.23–5.27 (m, 2H, 2 × H-2″), 5.81 (ddd, 1H, J = 6.8 Hz, J = 10.2 Hz, J = 17.1 Hz, H-1″), 7.15–7.23 (m, 6H, Ph), 7.28–7.35 (m, 6H, Ph), 7.73–7.75 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.6 (CH_3), 27.8 (3 × CH_3), 57.8 (C-4), 67.6 (C-3′), 73.4 (OCH_2Ph), 74.2 (OCH_2Ph), 77.3 (C-5), 77.4 (C-1′), 78.2 (C-2′), 83.6 (C_q), 117.9 (C-2″), 127.7 (2 × CH_{Ph}), 127.8 (2 × CH_{Ph}), 127.9 (CH_{Ph}), 128.1 (CH_{Ph}), 128.2 (2 × CH_{Ph}), 128.4 (4 × CH_{Ph}), 129.8 (2 × CH_{Ph}), 133.4 (C_i), 134.3 (C-1″), 136.5 (C_i), 137.1 (C_i), 145.1 (C_i), 148.7 (C=O), 151.5 (C-2). Anal. Calcd for $C_{34}H_{39}NO_9S$: C, 64.03; H, 6.16; N, 2.20. Found: C, 64.10; H, 6.09; N, 2.28.

4.1.15. *(2R,3S,4S,5R)-1,3-Bis(benzyloxy)-5-[(tert-butoxycarbonyl)amino]-4-hydroxyhept-6-en-2-yl p-methylbenzenesulfonate (**30**)*

Cs_2CO_3 (81 mg, 0.25 mmol) was added to an ice-cold solution of **28** (0.153 g, 0.25 mmol) in EtOH (5.6 mL). After stirring for 2 h at 0 °C, the mixture was diluted wit H_2O (25 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, the filtrate was concentrated in vacuo, and the residue was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate, 5:1) to provide 0.124 g (81%) of compound **30** as a colourless oil; $[\alpha]_D$ [21] +3.1 (*c* 0.46, $CHCl_3$). IR (neat) υ_{max} 1095, 1174, 1364, 1496, 1689, 1713, 2850, 2920, 3420 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H, 3 × CH_3), 2.39 (s, 3H, CH_3), 2.95 (br s, 1H, OH), 3.63–3.70 (m, 2H, H-1, H-4), 3.80–3.84 (m, 2H, H-1, H-3), 4.34–4.40 (m, 3H, H-5, OCH_2Ph), 4.53 (d, 1H, J = 10.4 Hz, OCH_2Ph), 4.71 (d, 1H, J = 10.4 Hz, OCH_2Ph), 4.87 (d, 1H, J = 8.3 Hz, NH), 5.11–5.14 (m, 1H, H-2), 5.18–5.23 (m, 2H, 2 × H-7), 5.76–5.84 (m, 1H, H-6), 7.16–7.23 (m, 4H, Ph), 7.26–7.35 (m, 8H, Ph), 7.78–7.80 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.6 (CH_3), 28.3 (3 × CH_3), 53.6 (C-5), 67.8 (C-1), 72.2 (C-4), 73.3 (OCH_2Ph), 74.5 (OCH_2Ph), 79.2 (C-3), 79.7 (C_q), 81.7 (C-2), 116.1 (C-7), 127.6 (2 × CH_{Ph}), 127.8 (CH_{Ph}), 127.9 (CH_{Ph}), 128.0 (CH_{Ph}), 128.3 (2 × CH_{Ph})) 128.4 (2 × CH_{Ph}), 128.7 (CH_{Ph}), 129.6 (2 × CH_{Ph}), 133.6 (2 × CH_{Ph}), 136.2 (C-6), 137.3 (C_i), 137.6 (2 × C_i), 144.6 (C_i), 155.9 (C=O). Anal. Calcd for $C_{33}H_{41}NO_8S$: C, 64.79; H, 6.76; N, 2.29. Found: C, 64.86; H, 6.68; N, 2.35.

4.1.16. *(2R,3S,4S,5R,6E)-1,3-Bis(benzyloxy)-5-[(tert-butoxycarbonyl)amino]-4-hydroxyoctadec-6-en-2-yl p-methylbenzenesulfonate (**5**)*

Tridec-1-ene (0.214 mL, 0.90 mmol) and the second generation Grubbs catalyst (15.2 mg, 0.018 mmol) were added to a solution of **30** (0.11 g, 0.18 mmol) in dry CH_2Cl_2 (3.8 mL). After heating at reflux for 2 h, the mixture was allowed to cool to room temperature, and the solvent was evaporated. Chromatography of the residue on silica gel (*n*-hexane/ethyl acetate, 7:1) gave 0.109 g (79%) of alkene **5** as a colourless oil; $[\alpha]_D$ [21] +6.2 (*c* 0.29, $CHCl_3$). IR (neat) υ_{max} 1095, 1174, 1364, 1496, 1688, 1713, 2852, 2923, 3421 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.8 Hz, CH_3), 1.25–1.35 (m, 18H, 9 × CH_2), 1.45 (s, 9H, 3 × CH_3), 1.98–2.03 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 2.90 (br s, 1H, OH), 3.56–3.60 (m, 1H, H-4), 3.69 (dd, 1H, J = 6.3 Hz, J = 10.8 Hz, H-1), 3.79–3.84 (m, 2H, H-1, H-3), 4.28–4.40 (m, 3H, H-5, OCH_2Ph), 4.53 (d, 1H, J = 10.5 Hz, OCH_2Ph), 4.71 (d, 1H, J = 10.5 Hz, OCH_2Ph), 4.82 (d, 1H, J = 8.5 Hz, NH), 5.12–5.14 (m, 1H, H-2), 5.39 (dd, 1H, J = 4.5 Hz, J = 15.6 Hz, H-6), 5.57–5.65 (m, 1H, H-7), 7.15–7.22 (m, 4H, Ph), 7.27–7.35 (m, 8H, Ph), 7.78–7.80 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (CH_3), 21.6 (CH_3), 22.7 (CH_2), 28.3 (3 × CH_3), 29.2 (2 × CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (2 × CH_2), 29.7 (CH_2), 31.9 (CH_2), 32.3 (CH_2), 53.2 (C-5), 67.9 (C-1), 72.7 (C-4), 73.2 (OCH_2Ph), 74.4 (OCH_2Ph), 79.2 (C-3), 79.6 (C_q), 81.9 (C-2), 127.3 (C-6), 127.6 (2 × CH_{Ph}), 127.7 (CH_{Ph}), 127.9 (CH_{Ph}), 128.1 (2 × CH_{Ph}), 128.3 (2 × CH_{Ph}), 128.4 (3 × CH_{Ph}), 128.7 (CH_{Ph}), 129.6 (2 × CH_{Ph}), 133.0 (C-7), 133.7 (C_i), 137.4 (C_i), 137.6 (C_i), 144.5 (C_i), 155.9 (C=O). Anal. Calcd for $C_{44}H_{63}NO_8S$: C, 68.99; H, 8.29; N, 1.83. Found: C, 68.90; H, 8.36; N, 1.91.

4.1.17. *(2R,3S,4R,5S)-4-(Benzyloxy)-5-[(benzyloxy)methyl]-2-[(E)-tridec-1′-en-1′-yl]pyrrolidin-3-ol (**32**)*

To an ice-cold solution of **5** (0.10 g, 0.13 mmol) in CH_2Cl_2 (0.40 mL) was added TFA (0.40 mL). After stirring at 0 °C for 15 min, the mixture was concentrated in vacuo, the obtained residue was dissolved with MeOH (0.90 mL) and treated with aqueous NH_3 solution (~26%) until basic pH (three times). Evaporation of the solvents and chromatography of the crude product on silica gel (*n*-hexane/ethyl acetate, 1:2) afforded 61 mg (96%) of compound **32** as a pale yellow oil; $[\alpha]_D$ [21] −11.9 (*c* 0.35, $CHCl_3$). IR (neat) υ_{max} 969, 1027, 1095, 1454, 1670, 2851, 2921, 3404 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.8 Hz, CH_3), 1.25–1.38 (m, 18H, 9 × CH_2), 2.01–2.06 (m, 2H, CH_2), 3.10 (br s, 1H, OH), 3.39 (dd, 1H, J = 3.4 Hz, J = 6.9 Hz, H-2), 3.48–3.52 (m, 1H, H-5), 3.57 (dd, 1H, J = 3.9 Hz, J = 9.6 Hz, H-1″), 3.64 (dd, 1H, J = 3.8 Hz, J = 9.6 Hz, H-1″), 3.99–4.01 (m, 1H, H-3), 4.12 (dd, 1H, J = 4.5 Hz, J = 8.2 Hz, H-4), 4.50 (d, 1H, J = 11.6 Hz, OCH_2Ph), 4.53 (d, 1H, J = 11.9 Hz, OCH_2Ph), 4.58 (d, 1H, J = 11.9 Hz, OCH_2Ph), 4.71 (d, 1H, J = 11.6 Hz, OCH_2Ph), 5.58–5.72 (m, 2H, H-1′, H-2′), 7.24–7.37 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (CH_3), 22.7 (CH_2), 29.2 (2 × CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (2 × CH_2), 29.7 (CH_2), 31.9 (CH_2), 32.6 (CH_2), 58.3 (C-5), 62.7 (C-2), 68.3 (C-1″), 71.8 (C-3), 72.6 (OCH_2Ph), 73.6 (OCH_2Ph), 80.5 (C-4), 126.2 (C-1′), 127.7 (2 × CH_{Ph}), 127.8 (4 × CH_{Ph}), 128.4 (4 × CH_{Ph}), 134.9 (C-2′), 137.5 (C_i), 137.9 (C_i). Anal. Calcd for $C_{32}H_{47}NO_3$: C, 77.85; H, 9.60; N, 2.84. Found: C, 77.94; H, 9.53; N, 2.90.

4.1.18. *(2S,3R,4S,5R)-2-(Hydroxymethyl)-5-tridecylpyrrolidine-3,4-diol hydrochloride (**3**)*

To pyrrolidine derivative **32** (21 mg, 42.5 μmol) dissolved in a mixture of MeOH/EtOAc (3.5 mL, 3:1) was added 20% $Pd(OH)_2/C$ (23.5 mg) followed by 12 M aqueous HCl solution (35 μL) at room temperature. After being stirred an atmosphere of hydrogen until no starting material was observed (1 h, judged by TLC), the mixture was filtered through a small pad of Celite. After the solvent was removed, the residue was washed tree times with dry Et_2O and

dried under vacuum for 6 h to furnish compound **3** in the nearly quantitative yield as a white amorphous solid; $[\alpha]_D$ [22] −17.1 (*c* 0.28, MeOH). IR (neat) υ_{max} 1031, 1085, 1144, 1462, 1572, 1638, 2849, 2919, 3335 cm^{-1}; ^1H NMR (600 MHz, CD_3OD): δ 0.90 (t, 3H, J = 7.0 Hz, CH_3), 1.29–1.47 (m, 22H, 11 × CH_2), 1.68–1.74 (m, 1H, CH_2), 1.87–1.93 (m, 1H, CH_2), 3.37–3.40 (m, 1H, H-5), 3.63–3.67 (m, 1H, H-2), 3.84 (dd, 1H, J = 8.9 Hz, J = 12.0 Hz, H-1″), 3.89 (dd, 1H, J = 4.3 Hz, J = 12.0 Hz, H-1″), 4.19–4.20 (m, 1H, H-4), 4.41 (dd, 1H, J = 4.5 Hz, J = 7.5 Hz, H-3); ^{13}C NMR (150 MHz, CD_3OD): δ 14.5 (CH_3), 23.8 (CH_2), 27.4 (CH_2), 27.7 (CH_2), 30.5 (2 × CH_2), 30.6 (CH_2), 30.7 (CH_2), 30.8 (4 × CH_2), 33.1 (CH_2), 59.8 (C-1″), 62.4 (C-5), 63.2 (C-2), 71.6 (C-3), 71.8 (C-4). Anal. Calcd for $C_{18}H_{38}ClNO_3$: C, 61.43; H, 10.88; N, 3.98. Found: C, 61.35; H, 10.95; N, 3.92.

4.1.19. *tert*-Butyl (4*S*,5*S*)-5-[(1′*R*,2′*R*)-1′,3′-bis(benzyloxy)-2′-hydroxypropyl]-2-oxo-4-vinyloxazolidine-3-carboxylate (**27**)

By the same procedure as described for the preparation of **26**, compound **8** (0.10 g, 0.26 mmol) was converted into derivative **27** (0.112 g, 89%, *n*-hexane/ethyl acetate, 3:1, colourless oil); $[\alpha]_D$ [22] +8.0 (*c* 0.30, $CHCl_3$). IR (neat) υ_{max} 1062, 1156, 1315, 1726, 1805, 2923, 3486 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.49 (s, 9H, 3 × CH_3), 2.60 (d, 1H, J = 3.6 Hz, OH), 3.61–3.62 (m, 2H, 2 × H-3′), 3.88 (dd, 1H, J = 4.4 Hz, J = 7.5 Hz, H-1′), 4.14–4.19 (m, 1H, H-2′), 4.53 (m, 2H, OCH_2Ph), 4.57 (d, 1H, J = 11.1 Hz, OCH_2Ph), 4.66–4.72 (m, 2H, H-4, *H*-OCH_2Ph), 4.77 (t, 1H, J = 7.5 Hz, H-5), 5.30 (d, 1H, J = 17.1 Hz, H-2″), 5.35 (d, 1H, J = 10.3 Hz, H-2″), 5.87–5.95 (m, 1H, H-1″), 7.22–7.38 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.8 (3 × CH_3), 60.2 (C-4), 70.1 (C-3′), 70.2 (C-2′), 73.4 (OCH_2Ph), 73.6 (OCH_2Ph), 75.3 (C-5), 76.8 (C-1′), 83.7 (C_q), 119.9 (C-2″), 127.7 (2 × CH_{Ph}), 127.8 (3 × CH_{Ph}), 127.9 (CH_{Ph}), 128.4 (4 × CH_{Ph}), 131.5 (C-1″), 137.5 (2 × C_i), 148.6 (C=O), 151.4 (C-2). Anal. Calcd for $C_{27}H_{33}NO_7$: C, 67.06; H, 6.88; N, 2.90. Found: C, 66.96; H, 6.95; N, 2.96.

During this reaction we also isolated 14 mg (9%) of *tert*-butyl (4*S*,5*S*)-5-{(1′*S*,2′*R*)-1′,3′-bis(benzyloxy)-2′-[(*tert*-butoxycarbonyl) oxy]propyl}-2-oxo-4-vinyloxazolidine-3-carboxylate as a colourless oil; $[\alpha]_D$ [21] −7.6 (*c* 0.34, $CHCl_3$). IR (neat) υ_{max} 1058, 1155, 1254, 1368, 1741, 1820, 2853, 2923 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.48 (s, 18H, 6 × CH_3), 3.73 (dd, 1H, J = 5.2 Hz, J = 9.3 Hz, H-3′), 3.77 (dd, 1H, J = 4.3 Hz, J = 9.3 Hz, H-3′), 3.89 (dd, 1H, J = 2.7 Hz, J = 8.4 Hz, H-1′), 4.38 (d, 1H, J = 10.5 Hz, OCH_2Ph), 4.55 (m, 2H, OCH_2Ph), 4.68–4.79 (m, 3H, H-4, H-5, *H*-OCH_2Ph), 5.31 (d, 1H, J = 17.2 Hz, H-2″), 5.37–5.41 (m, 2H, H-2′, H-2″), 5.80–5.89 (m, 1H, H-1″), 7.25–7.38 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.7 (3 × CH_3), 27.9 (3 × CH_3), 60.0 (C-4), 67.9 (C-3′), 72.6 (OCH_2Ph), 73.2 (OCH_2Ph), 73.4 (C-2′), 74.5 (C-5), 75.6 (C-1′), 82.6 (C_q), 83.8 (C_q), 120.2 (C-2″), 127.6 (3 × CH_{Ph}), 128.0 (CH_{Ph}), 128.1 (2 × CH_{Ph}), 128.4 (4 × CH_{Ph}), 131.0 (C-1″), 137.1 (C_i), 137.9 (C_i), 148.7 (C=O), 151.1 (C-2), 152.8 (C=O). Anal. Calcd for $C_{32}H_{41}NO_9$: C, 65.85; H, 7.08; N, 2.40. Found: C, 65.92; H, 7.15; N, 2.34.

4.1.20. *tert*-Butyl (4*S*,5*S*)-5-[(1′*S*,2′*R*)-1′,3′-bis(benzyloxy)-2′-(tosyloxy)propyl]-2-oxo-4-vinyloxazolidine-3-carboxylate (**29**)

According to the same procedure described for the construction of **28** from **26**, compound **27** (0.102 g, 0.21 mmol) was transformed to derivative **29** (0.116 g, 87%, *n*-hexane/ethyl acetate, 5:1, white solid); mp 89–90 °C; $[\alpha]_D$ [20] −10.5 (*c* 0.42, $CHCl_3$). IR (neat) υ_{max} 1107, 1203, 1317, 1352, 1728, 1797, 2924 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.48 (s, 9H, 3 × CH_3), 2.39 (s, 3H, CH_3), 3.65 (dd, 1H, J = 6.5 Hz, J = 10.8 Hz, H-3′), 3.72 (dd, 1H, J = 5.5 Hz, J = 10.8 Hz, H-3′), 3.94 (dd, 1H, J = 2.1 Hz, J = 9.2 Hz, H-1′), 4.33–4.40 (m, 3H, OCH_2Ph, *H*-OCH_2Ph), 4.56 (dd, 1H, J = 7.2 Hz, J = 9.2 Hz, H-5), 4.70–4.73 (m, 1H, H-4), 4.82 (d, 1H, J = 10.8 Hz, OCH_2Ph), 5.02–5.06 (m, 1H, H-2′), 5.26 (d, 1H, J = 17.1 Hz, H-2″), 5.36 (d, 1H, J = 10.4 Hz, H-2″), 5.66–5.75 (m, 1H, H-1″), 7.16–7.36 (m, 12H, Ph), 7.77–7.80 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.7 (CH_3), 27.9 (3 × CH_3), 59.7 (C-4), 67.3 (C-3′), 73.2 (OCH_2Ph), 73.3 (OCH_2Ph), 74.2 (C-5), 76.1 (C-1′), 79.4 (C-2′), 84.0 (C_q), 120.6 (C-2″), 127.6 (2 × CH_{Ph}), 127.8 (3 × CH_{Ph}), 128.0 (CH_{Ph}), 128.1 (2 × CH_{Ph}), 128.4 (4 × CH_{Ph}), 129.7 (2 × CH_{Ph}), 130.6 (C-1″), 133.3 (C_i), 136.9 (C_i), 137.4 (C_i), 144.9 (C_i), 148.5 (C=O), 150.9 (C-2). Anal. Calcd for $C_{34}H_{39}NO_9S$: C, 64.03; H, 6.16; N, 2.20. Found: C, 64.11; H, 6.23; N, 2.14.

4.1.21. (2*R*,3*S*,4*S*,5*S*)-1,3-Bis(benzyloxy)-5-[(tert-butoxycarbonyl) amino]-4-hydroxyhept-6-en-2-yl p-methylbenzenesulfonate (**31**)

Using the same procedure as described for the preparation of **30**, compound **29** (96 mg, 0.15 mmol) and Cs_2CO_3 (49 mg, 0.15 mmol) afforded after column chromatography on silica gel (*n*-hexane/ethyl acetate, 4:1) 84 mg (92%) of derivative **31** as a colourless oil; $[\alpha]_D$ [20] +21.5 (*c* 0.53, $CHCl_3$). IR (neat) υ_{max} 1095, 1174, 1364, 1496, 1687, 1713, 2850, 2920, 3420 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H, 3 × CH_3), 2.36 (s, 3H, CH_3), 3.67–3.71 (m, 3H, H-1, H-3, H-4), 3.83 (dd, 1H, J = 4.9 Hz, J = 10.9 Hz, H-1), 4.27–4.30 (m, 1H, H-5), 4.35 (d, 1H, J = 11.8, OCH_2Ph), 4.39 (d, 1H, J = 11.8 Hz, OCH_2Ph), 4.45 (d, 1H, J = 11.2 Hz, OCH_2Ph), 4.75 (d, 1H, J = 11.2 Hz, OCH_2Ph), 5.09–5.16 (m, 4H, H-2, 2 × H-7, NH), 5.66 (ddd, 1H, J = 7.3 Hz, J = 9.9 Hz, J = 16.8 Hz, H-6), 7.15–7.20 (m, 4H, Ph), 7.24–7.34 (m, 8H, Ph), 7.77–7.79 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.5 (CH_3), 28.3 (3 × CH_3), 55.3 (C-5), 67.7 (C-1), 73.2 (OCH_2Ph, C-4), 73.4 (OCH_2Ph), 79.5 (C-3), 79.7 (C_q), 81.6 (C-2), 118.1 (C-7), 127.6 (2 × CH_{Ph}), 127.7 (CH_{Ph}), 127.8 (CH_{Ph}), 128.0 (3 × CH_{Ph}), 128.3 (5 × CH_{Ph}), 129.6 (2 × CH_{Ph}), 133.1 (C-6), 133.5 (C_i), 137.7 (C_i), 141.2 (C_i), 144.6 (C_i), 155.7 (C=O). Anal. Calcd for $C_{33}H_{41}NO_8S$: C, 64.79; H, 6.76; N, 2.29. Found: C, 64.69; H, 6.83; N, 2.35.

4.1.22. (2*R*,3*S*,4*S*,5*S*,6*E*)-1,3-Bis(benzyloxy)-5-[(tert-butoxycarbonyl)amino]-4-hydroxyoctadec-6-en-2-yl p-methylbenzenesulfonate (**6**)

By the same procedure as described for the preparation of **5** from **30**, compound **31** (73 mg, 0.12 mmol) was converted after stirring at reflux (2.5 h) to derivative **6** (73 mg, 79%, *n*-hexane/ethyl acetate, 5:1, colourless oil); $[\alpha]_D$ [21] −7.3 (*c* 0.40, $CHCl_3$). IR (neat) υ_{max} 1096, 1175, 1364, 1454, 1496, 1686, 2853, 2923, 3379 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.8 Hz, CH_3), 1.26–1.33 (m, 18H, 9 × CH_2), 1.43 (s, 9H, 3 × CH_3), 1.93–1.98 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 3.66–3.75 (m, 3H, H-1, H-3, H-4), 3.84 (dd, 1H, J = 4.9 Hz, J = 10.9 Hz, H-1), 4.22–4.25 (m, 1H, H-5), 4.34 (d, 1H, J = 11.7 Hz, OCH_2Ph), 4.39 (d, 1H, J = 11.7 Hz, OCH_2Ph), 4.45 (d, 1H, J = 11.2 Hz, OCH_2Ph), 4.75 (d, 1H, J = 11.2 Hz, OCH_2Ph), 4.94 (d, 1H, J = 8.1 Hz, NH), 5.12–5.14 (m, 1H, H-2), 5.24–5.30 (m, 1H, H-6), 5.48–5.58 (m, 1H, H-7), 7.15–7.21 (m, 4H, Ph), 7.26–7.35 (m, 8H, Ph), 7.77–7.79 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (CH_3), 21.6 (CH_3), 22.7 (CH_2), 28.3 (3 × CH_3), 29.1 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (2 × CH_2), 29.7 (CH_2), 31.9 (CH_2), 32.5 (CH_2), 55.1 (C-5), 67.9 (C-1), 73.2 (OCH_2Ph, C-4), 73.5 (OCH_2Ph), 79.7 (C-3), 79.8 (C_q), 81.9 (C-2), 124.4 (C-6), 127.6 (3 × CH_{Ph}), 127.7 (CH_{Ph}), 127.8 (CH_{Ph}), 128.1 (2 × CH_{Ph}), 128.3 (2 × CH_{Ph}), 128.4 (3 × CH_{Ph}), 129.6 (2 × CH_{Ph}), 133.6 (C_i), 135.5 (C-7), 137.4 (C_i), 137.5 (C_i), 144.5 (C_i), 155.7 (C=O). Anal. Calcd for $C_{44}H_{63}NO_8S$: C, 68.99; H, 8.29; N, 1.83. Found: C, 68.89; H, 8.37; N, 1.88.

4.1.23. (2*S*,3*S*,4*R*,5*S*)-4-(Benzyloxy)-5-[(benzyloxy)methyl]-2-[(*E*)-tridec-1′-en-1′-yl]pyrrolidin-3-ol (**33**)

According to the same procedure described for the preparation of **32** from **5**, compound **6** (61 mg, 0.08 mmol) was transformed to derivative **33** (pale yellow solid, 34 mg, 86%, *n*-hexane/ethyl acetate, 1:1); mp 46–47 °C; $[\alpha]_D$ [21] −20.9 (*c* 0.21, $CHCl_3$). IR (neat) υ_{max} 1093, 1122, 1204, 1454, 2849, 2920, 3025, 3327 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.9 Hz, CH_3), 1.25–1.38 (m, 18H, 9 × CH_2), 1.97–2.02 (m, 2H, CH_2), 2.52 (br s, 1H, OH), 3.52–3.55 (m, 1H, H-2), 3.56–3.62 (m, 3H, H-5, 2 × H-1″), 3.86 (t, 1H, J = 5.0 Hz, H-

3), 4.06–4.09 (m, 1H, H-4), 4.52 (d, 1H, J = 11.8 Hz, OCH_2Ph), 4.56 (d, 1H, J = 11.8 Hz, OCH_2Ph), 4.60 (d, 1H, J = 11.5 Hz, OCH_2Ph), 4.66 (d, 1H, J = 11.5 Hz, OCH_2Ph), 5.35 (ddt, 1H, J = 1.2 Hz, J = 7.0 Hz, J = 15.2 Hz, H-1′), 5.60–5.68 (m, 1H, H-2′), 7.24–7.37 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (CH_3), 22.7 (CH_2), 29.2 (2 × CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (3 × CH_2), 31.9 (CH_2), 32.3 (CH_2), 58.0 (C-5), 65.2 (C-2), 69.4 (C-1″), 73.5 (2 × OCH_2Ph), 76.0 (C-3), 79.7 (C-4), 127.7 (3 × CH_{Ph}), 127.8 (3 × CH_{Ph}), 128.4 (4 × CH_{Ph}), 129.7 (C-1′), 132.8 (C-2′), 137.8 (C_i), 138.0 (C_i). Anal. Calcd for $C_{32}H_{47}NO_3$: C, 77.85; H, 9.60; N, 2.84. Found: C, 77.77; H, 9.66; N, 2.76.

4.1.24. (2S,3R,4S,5S)-2-(Hydroxymethyl)-5-tridecylpyrrolidine-3,4-diol hydrochloride (**4**)

Using the same procedure as described for the preparation of **4** from **32**, compound **33** (25 mg, 50.6 μmol) was converted to pyrrolidine **4** (quantitative yield, white amorphous solid); $[\alpha]_D$ [20] −35.5 (*c* 0.22, MeOH). IR (neat) υ_{max} 1051, 1129, 1466, 1638, 2850, 2920, 3390 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 0.90 (t, 3H, J = 6.7 Hz, CH_3), 1.29–1.56 (m, 22H, 11 × CH_2), 1.69–1.78 (m, 1H, CH_2), 1.81–1.89 (m, 1H, CH_2), 3.43 (td, 1H, J = 5.4 Hz, J = 9.2 Hz, H-5), 3.67–3.72 (m, 1H, H-2), 3.86–3.99 (m, 3H, H-4, 2 × H-1″), 4.18 (t, 1H, J = 3.4 Hz, H-3); ^{13}C NMR (100 MHz, CD_3OD): δ 14.5 (CH_3), 23.8 (CH_2), 27.6 (CH_2), 30.4 (CH_2), 30.5 (2 × CH_2), 30.7 (CH_2), 30.8 (4 × CH_2), 32.0 (CH_2), 33.1 (CH_2), 59.4 (C-1″), 62.1 (C-5), 63.5 (C-2), 71.7 (C-3), 77.4 (C-4). Anal. Calcd for $C_{18}H_{38}ClNO_3$: C, 61.43; H, 10.88; N, 3.98. Found: C, 61.52; H, 10.95; N, 3.92.

4.1.25. X-ray techniques

Single crystal of **24** suitable for X-ray diffraction was obtained from a mixture of *n*-hexane/ethyl acetate by slow crystallization at 4 °C. X-ray diffraction data were collected at 293 K on an Oxford Diffraction Gemini R diffractometer equipped with a Ruby CCD detector and Mo Kα sealed-tube source. Selected crystallographic and other relevant data for compound **24** are listed in Table 1. Data collection and reduction were performed with Oxford Diffraction CrysAlis PRO version 171.38.43 software suite [20]. Crystal structures were solved by direct methods with charge-flipping algorithm Superflip [21] using OLEX2 [22] and refined by least-squares procedure on F^2 with SHELXL2013 [23]. All non-hydrogen atoms were refined with anisotropic thermal parameters. Positions of all H atoms were optimized under the constrain to ride on their parent atoms, with C–H (aromatic) bond length of 0.93 Å, C–H (aliphatic) bond length of 0.97 Å and bond length of 98 Å for hydrogen on asymmetric carbon, O–H group bond length of 0.82 Å and N–H group bond length of 0.86 Å. The DIAMOND programme was used for the molecular graphics [24].

4.1.26. Supplementary data

Complete crystallographic data for the structural analysis have been deposit with the Cambridge Crystallographic Data Centre, CCDC No. 1570020 for compound **24**. 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).

4.2. 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) and non-cancerous cell line NiH 3T3 (mouse fibroblasts). A-549, HCT-116, MCF-7, MDA-MB-231, Caco-2, Jurkat and HeLa cells were maintained in RPMI 1640 medium. NiH 3T3 cell line was maintained in growth medium consisting of high glucose Dulbecco's Modified Eagle Medium. Both of these media were supplemented with Glutamax, and with 10% (V/V) foetal calf serum, penicillin (100 IU × mL^{-1}), and streptomycin (100 mg × mL^{-1}) (all from Invitrogen, Carlsbad, CA USA), in the atmosphere of 5% CO_2 in humidified air at 37 °C. Cell viability, estimated by the trypan blue exclusion, was greater than 95% before each experiment.

4.2.2. Cytotoxicity assay

The cytotoxic effect of the tested compounds was studied using the colorimetric microculture assay with the MTT endpoint [19]. 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.

Acknowledgements

The present work was supported by the Scientific Grant Agency (No. 1/0168/15 and No. 1/0871/16) of the Ministry of Education, Slovak Republic. It was also supported by the Slovak Research and Development Agency (SRDA Grant No. APVV-14-0883 and No. APVV-15-0079), and by the project MediPark Košice: 26220220185 supported by Operational Programme Research and Development (OPVaV-2012/2.2/08-RO, contract No. OPVaV/12/2013). We wish to thank Dr. Mária Vilková (P.J. Šafárik University, Košice) for NOESY experiments.

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