Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 7237

www.rsc.org/obc

PAPER

Syntheses of sulfur and selenium analogues of pachastrissamine *via* double displacements of cyclic sulfate†

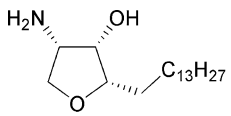
Hongjun Jeon,[a] Hoon Bae,[a] Dong Jae Baek,[a] Young-Shin Kwak,[b] Deukjoon Kim[a] and Sanghee Kim*[a]

Received 8th June 2011, Accepted 19th July 2011
DOI: 10.1039/c1ob05920c

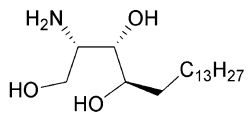
Bioisosteric analogues of pachastrissamine that contain sulfur and selenium atoms replacing the oxygen in the ring system, were efficiently prepared from a cyclic sulfate intermediate by sequential intermolecular and intramolecular S_N2 displacement reactions of the dianions. The analogues exhibited cytotoxicities comparable to that of pachastrissamine.

Introduction

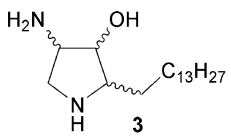
Pachastrissamine (also called jaspine B, **1**, Fig. 1)[1] is a marine natural product with a tetrahydrofuran ring possessing three contiguous stereogenic centers. It can be considered as an anhydrous derivative of the typical sphingoid base D-*ribo*-phytosphingosine (**2**). This natural product and its analogues have drawn much attention from synthetic and medicinal chemists[2,3] owing to both unusual structural features relative to the other sphingolipids and potent anticancer activity. In a recent report, the anticancer activity of pachastrissamine was ascribed to its ability to inhibit the activity of sphingomyelin synthase.[4] It was also postulated in a different report that an autophagy-related process might be responsible for the pachastrissamine's cytotoxic activity as the major mechanism of programmed cell death.[3k]



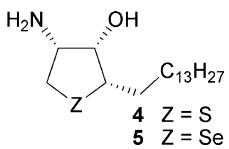
pachastrissamine (**1**)



D-*ribo*-phytosphingosine (**2**)



3



4 Z = S
5 Z = Se

Fig. 1 Chemical structures of compounds **1**–**5**.

Until now, studies on the structure–activity relationship (SAR) of pachastrissamine have mainly focused on the substituents on the tetrahydrofuran ring.[3b,3g–3i,3k] There is only one report concerning modification of the tetrahydrofuran core: Génisson *et al.* recently reported the synthesis and biological evaluation of aza analogues of pachastrissamine **3**.[3j] To elucidate the SAR of this promising natural product requires further exploration of its tetrahydrofuran core, for instance, by replacing it with a different ring system or size. With this in mind, the core ring-modified analogues of pachastrissamine **4** and **5** were designed.

The most common bioisosteric substitution is the replacement of C with N in an aromatic ring, and the second most common is the exchange of O and S.[5] Thus, the ring oxygen atom of pachastrissamine (**1**) was replaced with a sulfur atom to yield tetrahydrothiophene **4** (Fig. 1). The ring oxygen atom was also replaced by a selenium atom to create tetrahydroselenophene **5** because much attention has recently been given to selenium as a bioisostere of oxygen and sulfur.[6] Described herein is an efficient synthesis and a preliminary biological evaluation of the sulfur and selenium analogues of pachastrissamine, **4** and **5**.

Results and discussion

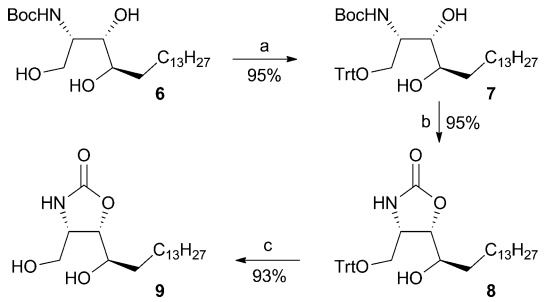
It was envisioned that the desired heterocyclic compounds **4** and **5** could be accessible from D-*ribo*-phytosphingosine **2** because three substituents on the heterocyclic rings of **4** and **5** are identical to those of **2**. The opposite absolute configuration at C-4 suggested that intermolecular substitution of the primary hydroxyl group of **2** by sulfur or selenium followed by subsequent intramolecular alkylation at C-4 with inversion of the stereochemistry would be a reasonable synthetic pathway towards **4** and **5**. For this synthetic strategy, the prior protection of the 2,3-amino alcohol functionality in **2** was required. To this end, the oxazolidinone ring was selected as a protecting group because the conformational restriction imposed by this cyclic group would facilitate intramolecular cyclization.

The starting material was the known *N*-Boc-protected D-*ribo*-phytosphingosine **6** (Scheme 1). The primary hydroxyl group of **6**

[a]*College of Pharmacy, Seoul National University, San 56-1, Shilim, Kwanak, Seoul 151-742, Korea. E-mail: pennkim@snu.ac.kr; Fax: +82-2-888-0649; Tel: +82-2-880-2487*

[b]*Korea Research Institute of Bioscience & Biotechnology (KRIBB), Chungbuk 363-883, Korea. E-mail: yskwak@kribb.re.kr; Fax: +82-43-240-6009; Tel: +82-43-240-6108*

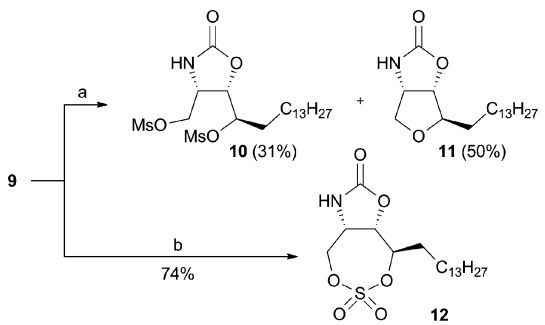
† Electronic supplementary information (ESI) available: Copies of ^{1}H NMR and ^{13}C NMR spectra of compounds **4**–**5**, **7**–**12** and **15**–**18**. See DOI: 10.1039/c1ob05920c



Scheme 1 (a) TrCl, DMAP, CH_2Cl_2–pyridine, 24 h; (b) NaH, DMF, 30 min; (c) $BF_3{\cdot}Et_2O$, toluene–MeOH, 24 h.

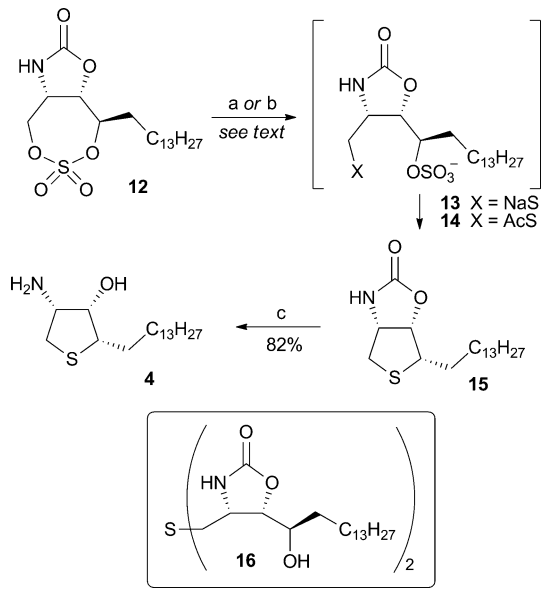
was selectively protected as its trityl ether to afford compound **7** in 95% yield. After several trials, it was found that treatment of **7** with sodium hydride in DMF gave the desired oxazolidinone **8** in 95% yield.[7] This reaction proceeded in an exclusively regioselective manner. No trace of 6-membered oxazinanone was detected in the crude ^{1}H NMR spectra.[8] Removal of the trityl group on **8** was achieved with boron trifluoride to afford diol **9** in 93% yield.

With the 2,3-amino alcohol-protected phytosphingosine **9** in hand, we examined the activation of both of its two hydroxyl groups as the mesylates (Scheme 2). Treatments of diol **9** under typical mesylation conditions furnished the desired bis-mesylate **10**, but in unacceptably low yield (31%). The major product was tetrahydrofuran **11** which resulted from the initial mesylation of the primary hydroxyl group of **9** and subsequent intramolecular cyclization involving the C-4 hydroxyl group. To circumvent this problem, a cyclic sulfate[9] was used as a diol-activating group instead. The formation of the 7-membered cyclic sulfate **12** proceeded smoothly when diol **9** was treated with thionyl chloride followed by oxidation with $RuCl_3$–$NaIO_4$ (74% overall yield).



Scheme 2 (a) MsCl, CH_2Cl_2–pyridine, 0 °C, 12 h; (b) (i) $SOCl_2$, $CHCl_3$, reflux, 1 h, (ii) $RuCl_3{\cdot}3H_2O$, $NaIO_4$, CCl_4–CH_3CN–H_2O (1 : 1 : 2), 1.5 h.

Although there are several examples of the formation of tetrahydrothiophenes from bis-sulfonates,[10] to the best of our knowledge no study has been reported of a 7-membered cyclic sulfate. Bis-sulfonates and cyclic sulfates could be regarded as synthetic equivalents. Thus, our initial synthetic attempts to prepare tetrahydrothiophene from cyclic sulfate **12** were made using the general reaction conditions employed for the transformation of bis-sulfonates to tetrahydrothiophenes. The reaction of **12** with a slight excess of Na_2S in DMF (0.1 M) at room temperature failed to give the desired tetrahydrothiophene **15** due to undesired dimerization (Scheme 3). The dimeric sulfide **16** was obtained in 91% yield after hydrolysis of the resulting reaction mixture.[11] The



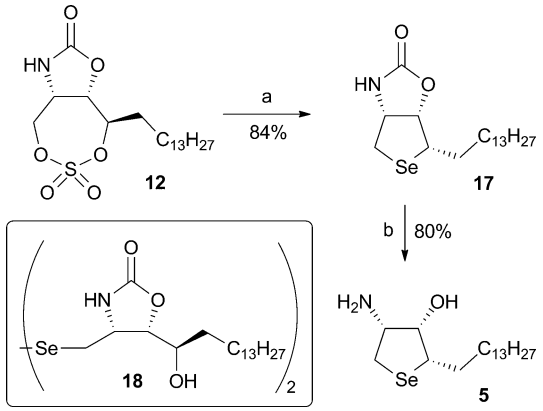
Scheme 3 (a) $Na_2S{\cdot}9H_2O$, DMF (see text); (b) (i) KSAc, DMF, 3 h, (ii) NaOMe, MeOH, 1 h; (c) LiOH, EtOH–THF–H_2O (3 : 2 : 1), 60 °C, 12 h.

formation of dimeric sulfide **16** could be attributed to the fact that the anionic sulfate group of intermediate **13** is a less reactive leaving group than a cyclic sulfate. To suppress the dimerization, the same reaction was performed under high dilution conditions (0.005 M), but still only dimeric sulfide **16** was obtained (71%). The desired tetrahydrothiophene **15** was obtained when the reaction was carried out with an excess of Na_2S (3.0 equiv) under high dilution conditions (DMF, 0.005 M), but with a low yield (31%) due to the formation of dimeric sulfide **16** (35%). Under the same conditions, the use of other solvent systems, such as MeOH, MeOH–DMF, THF, and MeOH–THF, did not improve the yield, but rather resulted in a lower yield of **15**. Finally, the competing dimerization could be minimized and the yield was improved to 79% when a diluted solution of cyclic sulfate **12** in DMF was slowly added *via* syringe pump over 0.5 h to a heated solution (60 °C) of Na_2S in DMF followed by additional stirring for 5 h.

As an alternative sulfide source, potassium thioacetate was employed.[12] Thioacetate can be regarded as a mono-protected form of S^{2-}. Thus, we anticipated that the use of this sulfide source would eliminate the possibility of dimerization and enable us to avoid high dilution conditions. The reaction of **12** with potassium thioacetate in DMF (0.1 M) gave the δ-acetylthio sulfate ester intermediate **14** by regiospecific attack at the less hindered primary position.[13] Treatment of the resulting intermediate **14** with NaOMe–MeOH gave tetrahydrothiophene **15** (69%), possibly *via* intramolecular substitution of sulfate ester group by thiolate.

After successfully achieving the transformation of the 7-membered cyclic sulfate to tetrahydrothiophene, the transformation of cyclic sulfate **12** into the tetrahydroselenophene was examined. As in the case of tetrahydrothiophenes, there is no precedent for such a transformation. With the information from the above studies with sulfide, reaction conditions were established for the synthesis of tetrahydroselenophene **17** from **12**. In this transformation, the selenium dianion provided the cyclized product with good yield. The desired tetrahydroselenophene **17** was obtained in 84% yield when a solution of cyclic sulfate **12** in THF

(0.005 M) was slowly added *via* syringe pump over 0.5 h to a heated solution (60 °C) of freshly prepared Na_2Se[14] (5.0 equiv) in EtOH followed by additional stirring for 3 h (Scheme 4). Under more concentrated conditions (0.1 M), the reaction gave diselenide **18** as the main product (73%).



Scheme 4 (a) Se, $NaBH_4$, EtOH–THF, 60 °C (see text); (b) LiOH, EtOH–THF–H_2O (3 : 2 : 1), 60 °C, 12 h.

The syntheses of the desired sulfur and selenium analogues of pachastrissamine, **4** and **5**, were completed by the cleavage of the oxazolidinone protecting groups of **15** and **17** with LiOH (Schemes 3 and 4).

Because pachastrissamine shows potent cytotoxicity against several cancer cell lines,[3i,3k] the cytotoxic activities of analogues **4** and **5** were examined as part of their preliminary evaluation (Table 1). Pachastrissamine **1** and D-*ribo*-phytosphingosine **2** were also tested to provide a direct comparison. As shown in Table 1, the sulfur and selenium analogues of pachastrissamine, **4** and **5**, were found to be much more effective in inducing cytotoxicity than the prototype sphingoid base **2**. They exhibited comparable potency to the natural product pachastrissamine against various cell lines. The sulfur analogue **4** was slightly more effective than the selenium analogue **5** at inhibiting cancer cell growth. The observed biological results indicate that the ring oxygen atom of pachastrissamine is amenable to bioisosteric replacement with sulfur and selenium atoms.

Conclusion

In summary, we report the design and synthesis of the sulfur and selenium analogues of pachastrissamine in which the tetrahydrofuran core of pachastrissamine was bioisosterically replaced with tetrahydrothiophene and tetrahydroselenophene. For the synthesis of the designed analogues, the 7-membered cyclic sulfate **12** was employed as a common intermediate. Double displacements of the cyclic sulfate **12** with the dianions of sulfur and selenium atoms allowed the efficient synthesis of the desired heterocycles. The natural product pachastrissamine and its sulfur and selenium analogues exhibited comparable cytotoxicity, yielding similar IC_{50} values. We believe that these analogues could be of value in the investigation of the biological functions of pachastrissamine and in the development of novel therapeutics as a lead scaffold because such bioisosteric replacement would systematically alter the physicochemical and biological properties of pachastrissamine.

Table 1 Cytotoxic activities for analogues of pachastrissamine

	IC_{50}[d] (μM)			
Cell lines	**1**	**2**	**4**	**5**
HCT 116[a]	0.22	6.64	0.14	0.69
A549[b]	0.18	5.09	0.14	0.28
PC-3[c]	0.17	5.29	0.06	0.10

[a] Human colon carcinoma. [b] Human lung carcinoma. [c] Human prostate adenocarcinoma. [d] 50% inhibition concentration.

Experimental section

A: Preparation of the compounds

General. All chemicals were of reagent grade and used as purchased. All reactions were performed under an inert atmosphere of dry argon or nitrogen using dry solvents. Reactions were monitored with TLC analysis using Merck silica gel 60 F-254 thin layer plates. Flash column chromatography was performed on silica gel (230–400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded in δ units relative to the deuterated solvent as internal reference at 300/400/500 and 75/100 MHz, respectively. IR spectra were measured on a Fourier Transform Infrared spectrometer. Mass spectra (MS) were recorded using fast atom bombardment (FAB). High resolution mass spectra (HRMS) were also recorded using FAB.

***tert*-Butyl (2*S*,3*S*,4*R*)-3,4-dihydroxy-1-(trityloxy)octadecan-2-ylcarbamate (7).** To a solution of **6** (2.4 g, 5.80 mmol) in CH_2Cl_2 (19.3 mL) and pyridine (11.6 mL) were added trityl chloride (1.94 g, 6.95 mmol) and 4-dimethylaminopyridine (142 mg, 1.16 mmol) at room temperature. After stirring for 24 h, the reaction mixture was poured into water and extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane–EtOAc 4 : 1) to give **7** (3.64 g, 95%) as a colorless oil. $[\alpha]_D^{24}$ +21.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, J = 6.8 Hz, 3H), 1.16–1.37 (m, 24H), 1.40 (s, 9H), 1.56–1.72 (m, 2H), 2.14 (d, J = 7.2 Hz, 1–OH), 2.84 (d, J = 7.8 Hz, 1–OH), 3.27–3.38 (m, 3H), 3.49–3.58 (m, 1H), 3.84–3.93 (m, 1H), 5.15 (d, J = 9.9 Hz, 1H), 7.15–7.28 (m, 9H), 7.34–7.39 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.7, 25.8, 28.4, 29.3, 29.5, 29.6, 29.7, 31.9, 33.0, 51.1, 63.2, 73.1, 75.8, 79.7, 87.5, 127.3, 128.0, 128.5, 143.3, 155.7; IR ($CHCl_3$) υ_{max} 3443, 2924, 2853, 1694, 1493, 1449, 1366, 1247, 1171, 1060 (cm^{-1}); HRMS (FAB) calcd for $C_{42}H_{61}NO_5Na$ 682.4447 ([M+Na]$^+$), found 682.4442.

(4*S*,5*S*)-5-((*R*)-1-Hydroxypentadecyl)-4-(trityloxymethyl)oxazolidin-2-one (8). To a solution of **7** (2.16 g, 3.27 mmol) in DMF (32.7 mL) was added sodium hydride (60% dispersion in mineral oil, 196 mg, 4.91 mmol) at room temperature. The reaction mixture was stirred for 30 min, quenched with a saturated aqueous NH_4Cl solution and extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane–EtOAc 3 : 1) to give **8** (3.64 g, 95%) as a colorless oil. $[\alpha]_D^{24}$ +11.5 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.5 Hz, 3H), 1.18–1.45 (m, 24H), 1.58–1.79

(m, 2H), 3.03 (d, J = 3.9 Hz, 1–OH), 3.21–3.32 (m, 2H), 3.40–3.51 (m, 1H), 3.98–4.09 (m, 1H), 4.32 (dd, J = 6.9, 9.0 Hz, 1H), 6.21 (br s, 1–NH), 7.18–7.32 (m, 9H), 7.35–7.40 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.6, 24.7, 29.3, 29.4, 29.57, 29.61, 29.64, 31.9, 33.6, 54.3, 61.7, 68.0, 80.9, 88.0, 127.5, 128.2, 142.6, 159.0; IR ($CHCl_3$) υ_{max} 3278, 2923, 2853, 1753, 1490, 1449, 1220, 1064 (cm^{-1}); HRMS (FAB) calcd for $C_{38}H_{52}NO_4$ 586.3896 ($[M+H]^+$), found 586.3910.

(4*S*,5*S*)-4-(Hydroxymethyl)-5-((*R*)-1-hydroxypentadecyl)oxazolidin-2-one (9). To a solution of **8** (1.61 g, 2.44 mmol) in toluene (6 mL) and methanol (6 mL) was added $BF_3{\cdot}OEt_2$ (905 µL, 7.33 mmol) at room temperature. After stirring for 24 h, reaction mixture was quenched with a saturated aqueous $NaHCO_3$ solution. The quenched reaction mixture was poured into water and extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane–EtOAc 1 : 1) to give **9** (779 mg, 93%) as a white solid. $[\alpha]_D^{24}$ −39.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$–MeOD 1 : 3, 400 MHz) δ 0.87 (t, J = 6.2 Hz, 3H), 1.26–1.48 (m, 24H), 1.49–1.62 (m, 1H), 1.65–1.78 (m, 1H), 3.66 (dd, J = 5.1, 11.4 Hz, 1H), 3.77 (dd, J = 5.6, 11.4 Hz, 1H), 3.83–3.92 (m, 2H), 4.32–4.40 (m, 1H); ^{13}C NMR ($CDCl_3$–MeOD 1 : 3, 75 MHz) δ 15.2, 24.3, 26.5, 31.0, 31.29, 31.32, 31.34, 33.6, 35.9, 58.0, 62.0, 69.9, 82.9, 162.1; IR ($CHCl_3$) υ_{max} 3340, 2917, 2850, 1717, 1691, 1054, 941, 705 (cm^{-1}); HRMS (FAB) calcd for $C_{19}H_{37}NO_4$ 344.2801 ($[M+H]^+$), found 344.2815.

Mesylation of compound 9. To a solution of diol **9** (55 mg, 0.16 mmol) in CH_2Cl_2 (1.6 mL) were added MsCl (125 µL, 1.60 mmol) and pyridine (130 µL, 1.60 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 12 h, poured into water and extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH 20 : 1) to provide bis-mesylate **10** and tetrahydrofuran **11**.

(*R*)-1-((4*S*,5*S*)-4-(Methylsulfonyloxymethyl)-2-oxooxazolidin-5-yl)pentadecyl methanesulfonate (10). As a colorless oil (25 mg, 31%). $[\alpha]_D^{24}$ −29.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, J = 6.3 Hz, 3H), 1.15–1.53 (m, 24H), 1.76–1.89 (m, 2H), 3.08 (s, 3H), 3.09 (s, 3H), 4.15–4.22 (m, 1H), 4.36 (dd, J = 6.9, 10.8 Hz, 1H), 4.49 (dd, J = 3.3, 10.9 Hz, 1H), 4.72 (m, 1H), 4.99 (dd, J = 5.7, 11.4 Hz, 1H), 6.54 (br s, 1–NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 24.3, 29.2, 29.31, 29.33, 29.5, 29.56, 29.60, 29.63, 29.64, 31.2, 31.9, 37.6, 39.1, 53.7, 67.5, 76.8, 77.2, 78.1, 157.6; IR ($CHCl_3$) υ_{max} 3364, 3028, 2924, 2853, 1769, 1467, 1352, 1262, 1223, 1175 (cm^{-1}); HRMS (FAB) calcd for $C_{21}H_{42}NO_8S_2$ 500.2352 ($[M+H]^+$), found 500.2342.

(3a*S*,6*R*,6a*S*)-6-Tetradecyltetrahydrofuro[3,4-*d*]oxazol-2(3*H*)-one (11). As a white solid (26 mg, 50%). $[\alpha]_D^{24}$ +8.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, J = 6.8 Hz, 3H), 1.19–1.55 (m, 26H), 3.75 (dd, J = 2.5, 10.2 Hz, 1H), 3.92 (dd, J = 4.9, 10.2 Hz, 1H), 4.01–4.06 (m, 1H), 4.30–4.34 (m, 1H), 4.69 (dd, J = 2.3, 8.1 Hz, 1H), 5.63 (br s, 1–NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.7, 25.4, 29.27, 29.35, 29.45, 29.51, 29.61, 29.64, 29.7, 30.6, 31.9, 56.3, 63.0, 72.6, 77.2, 84.0, 84.2, 158.7; IR ($CHCl_3$) υ_{max} 3249, 2954, 2920, 2850, 1758, 1728, 1703, 1469, 1408, 1250 (cm^{-1}); HRMS (FAB) calcd for $C_{19}H_{36}NO_3$ 326.2695 ($[M+H]^+$), found 326.2674.

(3a*S*,8*R*,8a*S*)-8-Tetradecyltetrahydro-[1,3,2]dioxathiepino[5,6-*d*]oxazol-2(3*H*)-one dioxide (12). To a solution of diol **9** (445 mg, 1.30 mmol) in $CHCl_3$ (25 mL) was added $SOCl_2$ (140 µL, 1.95 mmol) at room temperature. The reaction mixture was refluxed for 1 h, cooled to room temperature, poured into brine and extracted twice with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. After removal of solvent in the evaporator, the crude cyclic sulfite was dried *in vacuo* for 3 h using a vacuum pump, then dissolved in CCl_4–MeCN–H_2O (1 : 1 : 2, 25 mL) at room temperature. To the solution of crude cyclic sulfite, were added $RuCl_3{\cdot}3H_2O$ (15.0 mg, 0.06 mmol) and $NaIO_4$ (790 mg, 3.70 mmol). The reaction mixture was stirred at room temperature for 1.5 h, diluted with EtOAc and washed with a saturated aqueous $NaHSO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel (hexane–EtOAc 1.3 : 1) to give the cyclic sulfate **12** (390 mg, 74%) as a white solid. $[\alpha]_D^{24}$ +42.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, J = 6.2 Hz, 3H), 1.15–1.62 (m, 24H), 1.63–1.74 (m, 1H), 1.89–1.99 (m, 1H), 4.32–4.38 (m, 1H), 4.42 (d, J = 13.7 Hz, 1H), 4.52 (dd, J = 6.4, 13.8 Hz, 1H), 4.67 (t, J = 8.0 Hz, 1H), 4.79 (t, J = 9.3 Hz, 1H), 6.31 (br s, 1–NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.1, 22.7, 24.3, 29.0, 29.29, 29.34, 29.5, 29.57, 29.6, 29.7, 31.9, 32.2, 53.6, 66.3, 77.2, 80.5, 157.3; IR ($CHCl_3$) υ_{max} 3276, 2922, 2851, 1793, 1468, 1411, 1382, 1311, 1204, 1044 (cm^{-1}); HRMS (FAB) calcd for $C_{19}H_{36}NO_6S$ 406.2263 ($[M+H]^+$), found 406.2263.

(3a*R*,6*S*,6a*S*)-6-Tetradecyltetrahydrothieno[3,4-*d*]oxazol-2(3*H*)-one (15).

Method A. To an unclear solution of Na_2S (89 mg, 0.37 mmol) in DMF (10 mL) at 60 °C was added the solution of cyclic sulfate **12** (30 mg, 0.07 mmol) in DMF (5 mL) using a syringe pump over a 30 min period. After being stirred for an additional 5 h, the reaction mixture was cooled, poured into water and extracted twice with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH 20 : 1) to give **15** (20 mg, 79%) as a white solid.

Method B. To a solution of **12** (67 mg, 0.17 mmol) in DMF (1.6 mL) was added potassium thioacetate (38 mg, 0.33 mmol) at room temperature. After stirring for 3 h, the reaction mixture was concentrated *in vacuo*. The crude product was dissolved in methanol (1.6 mL). To the methanol solution was added a sodium methoxide solution (25 wt.% in methanol, 113 µL, 0.50 mmol). After stirring for 1 h, the reaction mixture was quenched with a saturated aqueous NH_4Cl solution. The quenched reaction mixture was poured into water and extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH 20 : 1) to give **15** (39 mg, 69%) as a white solid. $[\alpha]_D^{24}$ +57.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, J = 6.4 Hz, 3H), 1.15–1.48 (m, 24H), 1.69–1.90 (m, 2H), 2.75 (d, J = 13.1 Hz, 1H), 2.88 (dd, J = 4.7, 13.1 Hz, 1H), 3.17 (m, 1H), 4.49 (m, 1H), 4.99 (dd, J = 4.0, 6.8 Hz, 1H), 5.45 (br s, 1–NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.1, 22.7, 27.9, 28.8, 29.34, 29.4, 29.48, 29.55, 29.61, 29.64, 29.7, 31.9, 39.8, 55.5, 59.5, 83.4, 159.4; IR ($CHCl_3$) υ_{max}

3329, 2920, 2849, 1745, 1714, 1467, 1413, 1251, 1070, 1046 (cm^{-1}); HRMS (FAB) calcd for $C_{19}H_{36}NO_2S$, 342.2497 ([M+H]$^+$), found 342.2456.

(4*S*,5*R*)-5-((*S*)-1-Hydroxypentadecyl)-4-((((4*R*,5*S*)-5-((*R*)-1-hydroxypentadecyl)-2-oxooxazolidin-4-yl)methylthio)methyl)oxazolidin-2-one (16). To a solution of **12** (30 mg, 0.07 mmol) in DMF (0.74 mL, 0.1 M) was added $Na_2S{\cdot}9H_2O$ (21 mg, 0.09 mmol) at room temperature. After stirring for 30 min, the reaction mixture was concentrated *in vacuo* to remove DMF, and the concentrated residue was dissolved in THF (1 mL), H_2O (30 μL) and conc. H_2SO_4 (20 μL). The mixture was stirred for 1 h at room temperature, diluted with EtOAc and then washed with a saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to provide a crude mixture. Purification of the crude material by column chromatography on silica gel (CH_2Cl_2–MeOH 20 : 1) afforded only dimer **16** (23 mg, 91%) as a white solid. $[\alpha]_D^{24}$ −44.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$–MeOD 3 : 1, 400 MHz) δ 0.68 (t, J = 6.0 Hz, 6H), 1.10–1.29 (m, 48H), 1.29–1.40 (m, 2H), 1.51–1.62 (m, 2H), 2.39–2.48 (m, 2H), 2.82–2.90 (m, 2H), 3.52–3.60 (m, 2H), 3.70–3.78 (m, 2H), 4.10–4.17 (m, 2H); ^{13}C NMR ($CDCl_3$–MeOD 3 : 1, 100 MHz) δ 13.6, 22.3, 24.3, 29.0, 29.27, 29.29, 29.3, 31.6, 32.6, 34.3, 53.9, 67.7, 77.2, 80.6, 159.2; IR ($CHCl_3$) υ_{max} 3381, 2922, 2852, 1738, 1468, 1437, 1380, 1250, 1071 (cm^{-1}); HRMS (FAB) calcd for $C_{38}H_{73}N_2O_6S$ 685.5189 ([M+H]$^+$), found 685.5204.

(3a*R*,6*S*,6a*S*)-6-Tetradecyltetrahydroselenopheno[3,4-*d*]oxazol-2(3*H*)-one (17). To a suspension of selenium powder (47 mg, 0.60 mmol) in ethanol (500 μL) was added sodium borohydride (46 mg, 1.20 mmol) at room temperature. The mixture was heated to 60 °C and the cyclic sulfate **12** (48 mg, 0.12 mmol) in THF (24 mL, 0.005 M) was added to the mixture using a syringe pump over a 30 min period. After being stirred for an additional 3 h, the reaction mixture was cooled, poured into water and extracted twice with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH 20 : 1) to give **17** (39 mg, 84%) as a white solid. $[\alpha]_D^{24}$ +65.5 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, J = 6.2 Hz, 3H), 1.13–1.46 (m, 24H), 1.75–1.95 (m, 2H), 1.75–1.95 (m, 1H), 2.84 (d, J = 12.2 Hz, 1H), 3.00 (dd, J = 4.8, 12.4 Hz, 1H), 3.47 (m, 1H), 4.50–4.57 (m, 1H), 4.98–5.03 (m, 1H), 6.21 (br s, 1–NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.7, 29.0, 29.3, 29.4, 29.55, 29.61, 29.64, 29.7, 30.0, 31.9, 32.3, 51.3, 60.9, 77.2, 85.1, 159.6; IR ($CHCl_3$) υ_{max} 3285, 2920, 2850, 1737, 1709, 1469, 1412, 1252, 1047 (cm^{-1}); HRMS (FAB) calcd for $C_{19}H_{36}NO_2Se$ 390.1912 ([M+H]$^+$), found 390.1901.

(4*R*,5*R*)-4-((*R*)-1-Hydroxypentadecyl)-5-(((((4*R*,5*S*)-5-((*R*)-1-hydroxypentadecyl) - 2 - oxooxazolidin - 4 -yl)methyl)diselanyl)-methyl)oxazolidin-2-one (18). To a suspension of selenium powder (20 mg, 0.25 mmol) in ethanol (200 μL) was added sodium borohydride (19 mg, 0.50 mmol) at room temperature. The cyclic sulfate **12** (51 mg, 0.13 mmol) in THF (1.3 mL, 0.1 M) was added to the mixture. After stirring for 30 min, the reaction mixture was concentrated *in vacuo*, and the concentrated residue was dissolved in THF (2 mL), H_2O (60 μL) and conc. H_2SO_4 (40 μL). The mixture was stirred for 1 h at room temperature, diluted with EtOAc and then washed with a saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to provide a crude mixture. The crude mixture was purified by silica gel column chromatography (CH_2Cl_2–MeOH 20 : 1) to give only the dimer **18** (37 mg, 73%) as a white solid. $[\alpha]_D^{24}$ −75.8 (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, J = 6.1 Hz, 6H), 1.18–1.40 (m, 48H), 1.41–1.52 (m, 2H), 1.74–1.86 (m, 2H), 2.86 (t, J = 12.1 Hz, 2H), 2.94 (d, J = 4.5 Hz 2–OH), 3.67 (d, J = 12.5 Hz 2H), 3.75–3.87 (m, 2H), 3.95–4.08 (m, 2H), 4.38 (t, J = 8.3 Hz, 2H), 5.84 (br s, 2–NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.1, 22.7, 24.5, 29.36, 29.45, 29.56, 29.66, 29.69, 31.9, 32.7, 34.6, 55.0, 68.5, 77.2, 80.8, 158.6; IR ($CHCl_3$) υ_{max} 3301, 2922, 2852, 1744, 1468, 1379, 1241, 1066 (cm^{-1}); HRMS (FAB) calcd for $C_{38}H_{73}N_2O_6Se_2$ 813.3808 ([M+H]$^+$), found 813.3797.

General procedure for the cleavage of the oxazolidinone protecting groups of 15 and 17. To a solution of **15** or **17** (19 mg or 22 mg, 0.06 mmol) in EtOH–THF–H_2O (3 : 2 : 1, 3 mL) was added lithium hydroxide (14 mg, 0.60 mmol) at room temperature. The reaction mixture was stirred at 60 °C for 12 h, cooled to room temperature, poured into a saturated aqueous $NaHCO_3$ solution and extracted three times with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH–NH_4OH 100 : 10 : 0.5) to give **4** or **5**.

(2*S*,3*S*,4*R*)-4-Amino-2-tetradecyltetrahydrothiophen-3-ol (4). As a white solid (14 mg, 82%). $[\alpha]_D^{24}$ −10.3 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86 (t, J = 6.6 Hz, 3H), 1.18–1.36 (m, 24H), 1.52–1.62 (m, 1H), 1.76–1.85 (m, 1H), 2.66 (t, J = 10.1 Hz, 1H), 2.91 (dd, J = 7.4, 10.1 Hz, 1H), 3.39–3.46 (m, 1H), 3.46–3.54 (m, 1H), 3.89–3.93 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.7, 28.6, 29.3, 29.5, 29.57, 29.65, 29.7, 30.9, 31.9, 34.3, 51.2, 59.5, 76.4, 77.2; IR ($CHCl_3$) υ_{max} 2918, 2850, 1739, 1376, 1217 (cm^{-1}); HRMS (FAB) calcd for $C_{18}H_{38}NOS$ 316.2674 ([M+H]$^+$), found 316.2668.

(2*S*,3*S*,4*R*)-4-Amino-2-tetradecyltetrahydroselenophen-3-ol (5). As a white solid (16 mg, 80%). $[\alpha]_D^{24}$ −15.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, J = 6.3 Hz, 3H), 1.15–1.35 (m, 24H), 1.62–1.96 (m, 3H), 2.70 (t, J = 9.8 Hz, 1H), 3.0 (t, J = 8.6 Hz, 1H), 3.35–3.48 (m, 1H), 3.59–3.69 (m, 1H), 4.0 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.1, 22.7, 25.9, 29.3, 29.4, 29.49, 29.54, 29.61, 29.63, 29.7, 31.5, 31.9, 46.3, 61.0, 78.1; IR ($CHCl_3$) υ_{max} 2919, 2849, 1743, 1468, 1374, 1011 (cm^{-1}); HRMS (FAB) calcd for $C_{18}H_{38}NOSe$ 364.2119 ([M+H]$^+$), found 364.2112.

B: Bioassay

Measurements of cell viability. The cytotoxicity of individual compounds screened was determined by the SRB assay. Briefly, cells were plated in 96-well plates at a density of 5×10^4 cells $well^{-1}$ (HCT 116 and PC-3) or 2.5×10^4 cells $well^{-1}$ (A549) and incubated for 24 h. Test compounds (dissolved in pure DMSO) were diluted in the medium and the cells were treated for 48 h. The tested cells were then fixed with 10% trichloroacetic acid for 60 min at 4 °C. The fixed cells were stained with 0.4% sulforhodamine B (SRB) for 60 min at room temperature. The stained cells were then dissolved in 10 mM Tris (pH 10.0). The absorbance was measured at 515 nm. The cell survival (%) of each tested group was determined by comparison with solvent-treated control cells. The IC_{50} value, the

concentration of 50% cell survival, was estimated by non-linear regression analysis.

Acknowledgements

This work was supported by the SRC/ERC program (R-11-2007-107-02001-0) and the WCU program (R32-2008-000-10098-0) of the National Research Foundation of Korea (NRF) grant founded by Korea government (MEST).

Notes and references

1 (*a*) I. Kuroda, M. Musman, I. I. Ohtani, T. Ichiba, J. Tanaka, D. G. Gravalos and T. Higa, *J. Nat. Prod.*, 2002, **65**, 1505–1506; (*b*) V. Ledroit, C. Debitus, C. Lavaud and G. Massiot, *Tetrahedron Lett.*, 2003, **44**, 225–228.

2 For reviews, see: (*a*) E. Abraham, S. G. Davies, P. M. Roberts, A. J. Russell and J. E. Thomson, *Tetrahedron: Asymmetry*, 2008, **19**, 1027–1047; (*b*) S. Ballereau, M. Baltas and Y. Génisson, *Curr. Org. Chem.*, 2011, **15**, 953–986.

3 For recent total syntheses of **1** and studies of its derivatives published after the review ref. 2a, see: (*a*) M. Passiniemi and A. M. P. Koskinen, *Org. Biomol. Chem.*, 2011, **9**, 1774–1783; (*b*) J. Llaveria, Y. Díaz, M. I. Matheu and S. Castillón, *Eur. J. Org. Chem.*, 2011, 1514–1519; (*c*) H. Urano, M. Enomoto and S. Kuwahara, *Biosci., Biotechnol., Biochem.*, 2010, **74**, 152–157; (*d*) G. S. Rao, N. Sudhakar, B. V. Rao and S. J. Basha, *Tetrahedron: Asymmetry*, 2010, **21**, 1963–1970; (*e*) P. Vichare and A. Chattopadhyay, *Tetrahedron: Asymmetry*, 2010, **21**, 1983–1987; (*f*) S. Inuki, Y. Yoshimitsu, S. Oishi, N. Fujii and H. Ohno, *J. Org. Chem.*, 2010, **75**, 3831–3842; (*g*) Y. Yoshimitsu, S. Inuki, S. Oishi, N. Fujii and H. Ohno, *J. Org. Chem.*, 2010, **75**, 3843–3846; (*h*) Y. Salma, S. Ballereau, C. Maaliki, S. Ladeira, N. Andrieu-Abadie and Y. Génisson, *Org. Biomol. Chem.*, 2010, **8**, 3227–3243; (*i*) G. Jayachitra, N. Sudhakar, R. K. Anchoori, B. V. Rao, S. Roy and R. Banerjee, *Synthesis*, 2010, 115–119; (*j*) A. Rives, S. Ladeira, T. Levade, N. Andrieu-Abadie and Y. Génisson, *J. Org. Chem.*, 2010, **75**, 7920–7923; (*k*) D. Canals, D. Mormeneo, G. Fabrias, A. Llebaria, J. Casas and A. Delgado, *Bioorg. Med. Chem.*, 2009, **17**, 235–241; (*l*) G. Reddipalli, M. Venkataiah, M. K. Mishra and N. W. Fadnavis, *Tetrahedron: Asymmetry*, 2009, **20**, 1802–1805; (*m*) S. Inuki, Y. Yoshimitsu, S. Oishi, N. Fujii and H. Ohno, *Org. Lett.*, 2009, **11**, 4478–4481; (*n*) D. Enders, V. Terteryan and J. Paleček, *Synthesis*, 2008, 2278–2282.

4 Y. Salma, E. Lafont, N. Therville, S. Carpentier, M.-J. Bonnafé, T. Levade, Y. Génisson and N. Andrieu-Abadie, *Biochem. Pharmacol.*, 2009, **78**, 477–485.

5 R. P. Sheridan, *J. Chem. Inf. Comput. Sci.*, 2002, **42**, 103–108.

6 (*a*) J. K. Watts, B. D. Johnston, K. Jayakanthan, A. S. Wahba, B. M. Pinto and M. J. Damha, *J. Am. Chem. Soc.*, 2008, **130**, 8578–8579; (*b*) L. S. Jeong, D. K. Tosh, W. J. Choi, S. K. Lee, Y.-J. Kang, S. Choi, J. H. Lee, H. Lee, H. W. Lee and H. O. Kim, *J. Med. Chem.*, 2009, **52**, 5303–5306; (*c*) K. Jayakanthan, B. D. Johnston and B. M. Pinto, *Carbohydr. Res.*, 2008, **343**, 1790–1800; (*d*) Y. Ma, R. Liu, X. Gong, Z. Li, Q. Huang, H. Wang and G. Song, *J. Agric. Food Chem.*, 2006, **54**, 7724–7728; (*e*) J. Chin, J. Y. Hong, J. Lee, H. Hwang, H. Ko, H. Choi, D. Hahn, J. Ko, S. J. Nam, J. Tak, J. Ham and H. Kang, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7239–7242.

7 H.-J. Ha, D.-H. Yoon, L.-S. Kang, M. C. Hong and W. K. Lee, *Bull. Korean Chem. Soc.*, 2009, **30**, 535–536.

8 For a selective protection of 4–OH by oxazinanone formation, see ref. 3g.

9 For a review, see: (*a*) H.-S. Byun, L. He and R. Bittman, *Tetrahedron*, 2000, **56**, 7051–7091; (*b*) H. C. Kolb, M. S. VanNieuwenhze and K. B. Sharpless, *Chem. Rev.*, 1994, **94**, 2483–2547.

10 (*a*) H. W. Lee, H. O. Kim, W. J. Choi, S. Choi, J. H. Lee, S.-G. Park, L. Yoo, K. A. Jacobson and L. S. Jeong, *Bioorg. Med. Chem.*, 2010, **18**, 7015–7021; (*b*) S. Maity and S. Ghosh, *Tetrahedron*, 2009, **65**, 9202–9210; (*c*) R. Nasi, L. Sim, D. R. Rose and B. M. Pinto, *Carbohydr. Res.*, 2007, **342**, 1888–1894.

11 For the formation of dimeric sulfide from a 5-membered cyclic sulfate, see: F. G. Calvo-Flores, P. García-Mendoza, F. Hernández-Mateo, J. Isac-García and F. Santoyo-González, *J. Org. Chem.*, 1997, **62**, 3944–3961.

12 F. Santoyo-González, F. G. Calvo-Flores, P. García-Mendoza, F. Hernández-Mateo, J. Isac-García and M. D. Pérez-Alvarez, *J. Chem. Soc., Chem. Commun.*, 1995, 461–462.

13 The identity of **14** was confirmed by its conversion to the corresponding 4-hydroxy compound by hydrolysis with aqueous sulfuric acid.

14 (*a*) K. Schürrlea and W. J. Piepersberga, *Carbohydr. Chem.*, 1996, **15**, 435–447; (*b*) L. S. Jeong, D. K. Tosh, H. O. Kim, T. Wang, X. Hou, H. S. Yun, Y. Kwon, S. K. Lee, J. Choi and L. X. Zhao, *Org. Lett.*, 2008, **10**, 209–212.

