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Total synthesis of bicyclic depsipeptides spiruchostatins C and D and investigation of their histone deacetylase inhibitory and antiproliferative activities

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ABSTRACT

The bicyclic depsipeptide histone deacetylase (HDAC) inhibitors spiruchostatins C and D were synthesized for the first time in a highly convergent and unified manner. The method features the amide coupling of a D-leucine–D-cysteine- or D-valine–D-cysteine-containing segment with a D-alanine- or D-valine-containing segment to directly assemble the corresponding *seco*-acids, key precursors of macrolactonization. The HDAC inhibitory assay and cell-growth inhibition analysis of the synthesized depsipeptides determined the order of potency of spiruchostatins A–D in comparison with the clinically approved depsipeptide FK228 (romidepsin). Novel aspects of structure–activity relationships (SAR) were revealed.

1. Introduction

Histone deacetylase (HDAC) inhibitors have attracted significant attention because of their potentials as novel mechanism-based anticancer agents [1]. It has been reported that HDAC inhibitors exhibit prominent antitumour activity in several human tumour xenograft models by inducing transcriptional events involved in growth arrest, differentiation and apoptotic cell death [1,2]. HDAC enzymes remove an acetyl group from the lysine residues of the core histones, leading to a changed chromatin structure that impacts transcriptional activity [3]. There are 18 human HDAC isoforms, which are grouped into four major classes: class I (HDACs 1, 2, 3 and 8), class II (class IIa: HDACs 4, 5, 7 and 10; class IIb: HDACs 6 and 10), and class IV (HDAC 11) are Zn^{2+}-dependent metallohydrolases, while class III HDACs (7 members) are NAD^{+}-dependent sirtuins [4]. The inhibition of class I HDACs is considered to be a useful mechanism for anticancer agents, whereas the inhibition of class II HDACs may cause undesirable side effects such as serious cardiac hypertrophy [5]. Therefore, the potent and selective inhibition of class I enzymes is highly desirable in cancer chemotherapy.

In 2009, the Food and Drug Administration (FDA) in the USA approved the bicyclic depsipeptide HDAC inhibitor FK228 (romidepsin, **1**, Fig. 1) for the treatment of cutaneous T cell lymphoma (CTCL) [6]. However, this anticancer agent is associated with an unresolved cardiotoxicity issue [6b,7], which highlights the need for further identification and development of new HDAC inhibitors with high efficacy and low toxicity [1n,4a]. FK228 was originally isolated from a culture broth of *Chromobacterium violaceum* (No. 968) by Fujisawa Pharmaceutical Co. Ltd. (now Astellas Pharma Inc.) in 1994 [8]. The structural features of this natural product include a 16-membered bicyclic depsipeptide consisting of (*Z*)-2-amino-2-butenoic acid, L-valine, D-cysteine, D-valine, (3*R*,4*E*)-3-hydroxy-7-mercapto-4-heptenoic acid and a characteristic disulfide bond linkage [8b]. Subsequent to the discovery of FK228, the structurally similar 15-membered bicyclic depsipeptide HDAC inhibitors — spiruchostatins A (**2**) [9], B (**3**) [9], C (**4**) [10,11] and D (**5**) [12] — were successively isolated from a culture broth of the *Pseudomonas* sp. by Shin-ya and Yamanouchi Pharmaceutical Co. Ltd. (now Astellas Pharma Inc.).

The attractive biological properties and unique structural features of **1**–**5** have made them intriguing and timely targets for

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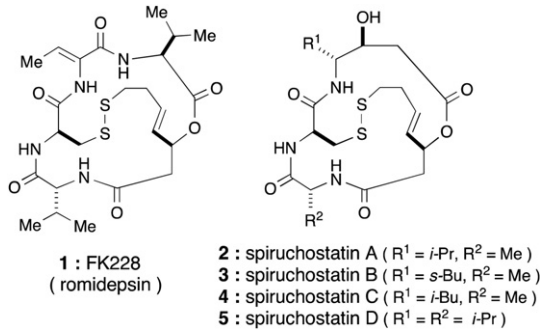


Fig. 1. Structures of FK228 (romidepsin) (**1**) and spiruchostatins A (**1**), B (**2**), C (**3**) and D (**4**). *i*-Pr = isopropyl, *s*-Bu = *sec*-butyl, *i*-Bu = isobutyl.

total synthesis. Several total syntheses of FK228 (**1**) [13], spiruchostatins A (**2**) [13a,14] and B (**3**) [13a,15] have been published. However, to the best of our knowledge, the total synthesis of spiruchostatins C (**4**) and D (**5**) has not been reported, and their biological properties have not been described in the patent literatures [10,12]. We have previously presented the total synthesis of **1**–**3** using a highly convergent and unified scheme [13a,14c,15b], and demonstrated that **1**–**3** exhibit a potent class I HDAC inhibitory activity (IC_{50} = 2.2–3.6 nM) [13a]. During our efforts on the synthesis and biological evaluation of the bicyclic depsipeptide HDAC inhibitors, we became interested in the biological potencies of **4** and **5** as compared with those of **1**–**3**. In this paper, we describe the total synthesis of **4** and **5** for the first time applying the synthetic strategy developed in our laboratory [13a]. HDAC inhibition assay and antiproliferative analysis of the synthesized **4** and **5** were also performed to establish the potency order of the bicyclic depsipeptides family (**1**–**5**).

2. Results and discussion

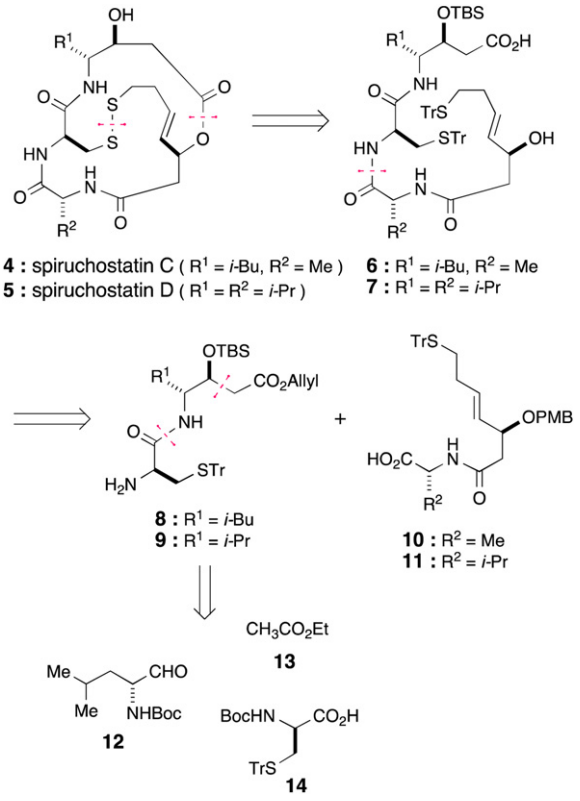
2.1. Chemistry

2.1.1. Synthetic plan for spiruchostatins C (*4*) and D (*5*)

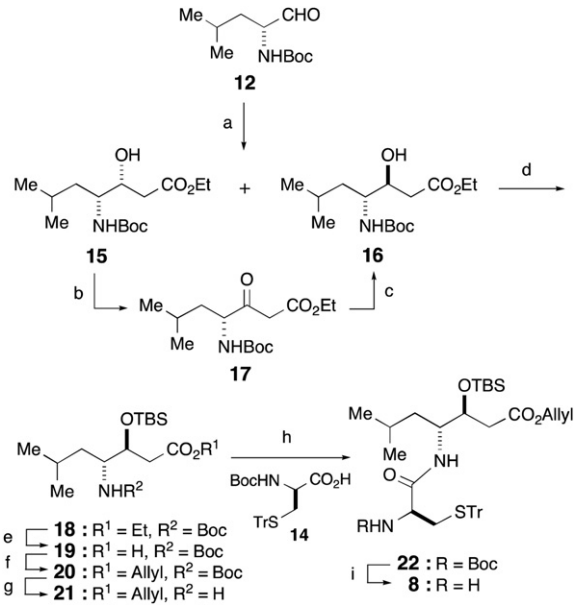
Our synthetic plan for spiruchostatins C (**4**) and D (**5**) is outlined in Scheme 1. We envisioned that the targeted molecules **4** and **5** could be synthesized by the macrolactonization of the corresponding *seco*-acids **6** and **7**, followed by disulfide bond formation. The key element of this scheme is the highly convergent assembly of **6** and **7** by the direct coupling of a D-leucine–D-cysteine-containing segment **8** and a D-alanine-containing segment **10** and a D-valine–D-cysteine-containing segment **9** and a D-valine-containing segment **11**, respectively. The segment **8** was to be prepared by the aldol coupling of *N*-Boc-D-leucinal (**12**) with ethyl acetate (**13**) and subsequent condensation with the D-cysteine derivative **14**. The remaining three segments **9**, **10** and **11** were obtained by our previously described total synthesis of **1**–**3** [13a].

2.1.2. Synthesis of segment *8*

The synthesis of **8**, starting from the known *N*-Boc-D-leucinal (**12**) [16], is shown in Scheme 2; the route is based on our previous study [13a]. Thus, the aldol coupling of **12** with the lithium enolate of ethyl acetate (**13**) gave the desired coupling product **16** (33%) and the undesired stereoisomer **15** (54%). The conversion of **15** to **16** was achieved by Jones oxidation (80%) and the stereoselective reduction of the resulting ketone **17** (90%). The ethyl ester **16** was then transformed to the allyl ester **21** via a four-step operation involving the *tert*-butyldimethylsilyl (TBS) protection of the hydroxy group in **16** (99%), the saponification of the ester moiety in the resulting TBS ether **18**, the formation of an allyl ester from



Scheme 1. Synthetic plan for spiruchostatins C (**4**) and D (**5**). TBS = *tert*-butyldimethylsilyl, Tr (trityl) = triphenylmethyl, Boc = *tert*-butoxycarbonyl, PMB = 4-methoxybenzyl.

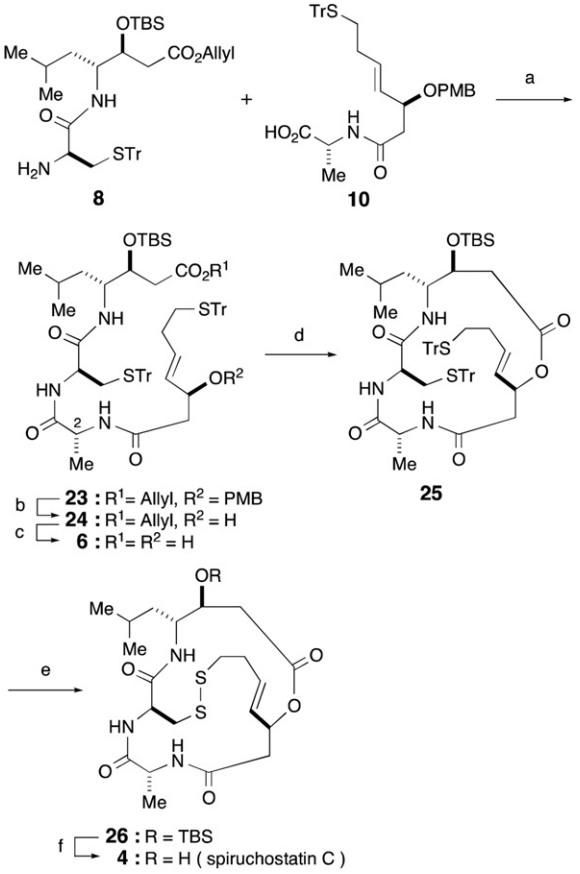


Scheme 2. Synthesis of segment **8**. (a) LDA, CH_3CO_2Et (**13**), THF, −78 °C; at −78 °C, add. **12**, 54% for **15**, 33% for **16** (**15**/**16** *ca.* 3:2); (b) Jones reagent, acetone, 0 °C to rt, 80%; (c) KBH_4, MeOH, −40 °C, 90%; (d) TBSCl, imidazole, DMF, rt, 99%; (e) 1 M NaOH, EtOH, rt; (f) allyl bromide, K_2CO_3, DMF, rt. 77% (2 steps); (g) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt; MeOH, rt; (h) **14**, PyBOP, *i*-Pr_2NEt, MeCN, rt, 69% (2 steps); (i) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt, 88%. LDA = lithium diisopropylamide, TMSOTf = trimethylsilyl trifluoromethanesulfonate, PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.

the liberated carboxylic acid **19** (77% in two steps) and the deprotection of the *N*-Boc group in **20**. The condensation of the amine **21** with *N*-Boc-*S*-trityl-L-cysteine (**14**) furnished the desired coupling product **22** with a 69% yield from **20**. Finally, the deprotection of the *N*-Boc group in **22** afforded the requisite segment **8** in 88% yield.

2.1.3. Synthesis of spiruchostatin C (**4**)

After obtaining the key segment **8**, we performed the synthesis of the first target spiruchostatin C (**4**), as shown in Scheme 3. The condensation of **8** with segment **10** proceeded well under the mild conditions [HATU (1.3 equiv), HOAt (1.3 equiv), *i*-Pr_2NEt (2.6 equiv), −30 °C, 6 h] explored in our previous studies [13a]. The desired coupling product **23** was obtained in 84% yield without appreciable epimerization at the C2 stereogenic centre (D-alanine part in **23**). The coupling product **23** was converted to the requisite *seco*-acid **6** with an overall yield of 98% using the alcohol **24** by the successive removal of both the PMB and allyl protecting groups. The subsequent macrolactonization of **6** was achieved by employing the Shiina method [17] [MNBA (1.3 equiv), DMAP (3.0 equiv), CH_2Cl_2 (10 mM), rt, 12 h], which resulted in the desired cyclization product **25** in 75% yield. Finally, simultaneous *S*-Tr deprotection and disulfide bond formation of **25** (95%) followed by the deprotection of the TBS group of the resulting disulfide **26** (91%) furnished spiruchostatin C (**4**), $[\alpha]_D^{25} = -59.4°$ (c = 0.20 in $CHCl_3$) {lit. [10] $[\alpha]_D^{25} = -60.0°$ (c = 0.10 in $CHCl_3$)}. The spectroscopic properties (IR, ^1H and ^{13}C NMR, and MS) of the synthetic sample **4** were identical with those reported for natural **4** [10].



Scheme 3. Synthesis of spiruchostatin C (**4**). (a) HATU, HOAt, *i*-Pr_2NEt, CH_2Cl_2, −30 °C, 84%; (b) DDQ, CH_2Cl_2/H_2O, rt, 99%; (c) $Pd(PPh_3)_4$, morpholine, THF, rt, 99%; (d) MNBA, DMAP, CH_2Cl_2, rt, 75%; (e) I_2, MeOH/CH_2Cl_2, rt, 95%; (f) HF·pyridine, pyridine, rt, 91%. HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAt = 1-hydroxy-7-azabenzotriazole, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, MNBA = 2-methyl-6-nitrobenzoic anhydride, DMAP = 4-dimethylaminopyridine.

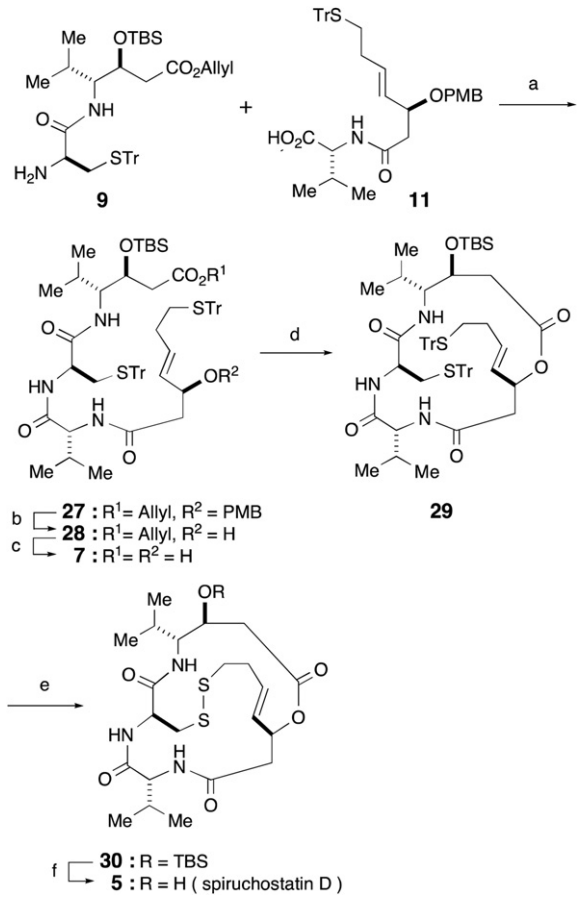
2.1.4. Synthesis of spiruchostatin D (**5**)

As shown in Scheme 4, using segments **9** [13a] and **11** [13a] as the starting materials, the second target spiruchostatin D (**5**) was synthesized in a manner similar to that described for **4** (cf. Scheme 3). The spectroscopic properties (IR, ^1H and ^{13}C NMR, and MS) of the synthesized sample **5** were identical with those reported for natural **5** [12]. The optical rotation of synthesized **5** {$[\alpha]_D^{25} = -59.2°$ (c = 0.29 in MeOH)} corresponded to that of natural **5** {$[\alpha]_D^{25} = -65.3°$ (c = 0.20 in MeOH)} [12].

2.2. Biological evaluation

2.2.1. HDAC inhibition assay

The synthesized spiruchostatins C (**4**) and D (**5**) were tested for their HDAC inhibitory activity against HDAC1 (class I) and HDAC6 (class IIb) to determine the degree of potency and isoform selectivity. In this assay, FK228 was used as a positive control. As summarized in Table 1, the two compounds **4** and **5** that were tested exhibited extremely potent inhibitory activity against HDAC1 in the sub-nanomolar range (IC_{50} = 0.75–0.93 nM). The potencies of **4** and **5** were observed to be 3-to-4-fold higher than that of FK228. Combined with our previous data for spiruchostatins A (**2**) and B (**3**) [13a], the order of potency was estimated to be **5** (IC_{50} = 0.75 nM) ≥ **4** (IC_{50} = 0.93 nM) > **3** (IC_{50} = 2.2 nM) ≥ **2** (IC_{50} = 3.3 nM) ≈FK228 (**1**) (IC_{50} = 3.6 nM). Spiruchostatin D (**5**)



Scheme 4. Synthesis of spiruchostatin D (**5**). (a) HATU, HOAt, *i*-Pr_2NEt, CH_2Cl_2, −30 °C, 94%; (b) DDQ, CH_2Cl_2/H_2O, rt, 79%; (c) $Pd(PPh_3)_4$, morpholine, THF, rt, 90%; (d) MNBA, DMAP, CH_2Cl_2, rt, 85%; (e) I_2, MeOH/CH_2Cl_2, rt, 88%; (f) HF·pyridine, pyridine, rt, 93%.

Table 1
HDAC inhibitory activity of spiruchostatins C (**4**) and D (**5**).

Compound	IC_{50}[a] (nM)		SI[d]
	HDAC1 (class I)[b]	HDAC6 (class IIb)[b]	
4	0.93	320	346
5	0.75	240	320
FK228[c]	3.6	390	108

[a] Concentration that induces 50% inhibition against HDACs.
[b] Enzyme assay was performed in the presence of 100 μM dithiothreitol (DTT).
[c] Positive control used in this study was synthesized in our laboratory.
[d] Selectivity index (HDAC6 IC_{50}/HDAC1 IC_{50}) as the selectivity toward class I HDAC1 over class IIb HDAC6.

exhibited the most potent HDAC1 inhibitory activity among the spiruchostatins family (**2**–**5**), suggesting that sterically bulky terminal-branched alkyl groups are preferable for the R^1 and R^2 side chains in the spiruchostatins (cf. Fig. 1). As expected for HDAC6 inhibitory activity, both **4** and **5** were essentially inactive (**4**: IC_{50} = 320 nM, **5**: IC_{50} = 240 nM), which is similar to the behavior exhibited by FK228 (IC_{50} = 390 nM). Isoform selectivity (class I/IIb) is expressed, for convenience, as a selective index (SI) value (HDAC6 IC_{50}/HDAC1 IC_{50}). The isoform selectivity of **4** (SI = 346) and **5** (SI = 320) is approximately 3-fold higher than that of FK228 (SI = 108). These results suggested that the minor structural alteration of the R^1 and R^2 side chains in spiruchostatins improves HDAC inhibitory activity and isoform selectivity. This is in agreement with a previous SAR study on synthetic FK228 analogues reported by Ganesan et al. [18].

2.2.2. Cell-growth inhibition assay

The growth-inhibitory activity of compounds **4** and **5** was evaluated simultaneously with FK228, a reference compound, using a panel of 39 human cancer cell lines at the Japanese Foundation for Cancer Research [19]. The number of cell lines and their origin (organ) are as follows: 5 breast, 6 central nervous system (brain), 1 melanoma, 5 ovary, 2 kidney, 6 stomach and 2 prostate. Dose–response curves were measured at five different concentrations (10^{-10}–10^{-6} or 10^{-8}–10^{-4} M) for each compound, and the concentration causing 50% cell-growth inhibition (GI_{50}) was compared with that of the control. The GI_{50} values of the tested compounds **4** and **5** along with FK228 are shown in Table 2. Compound **5** exhibited extremely potent growth-inhibitory activity against almost all 39 cell lines in the sub-nanomolar to nanomolar range and its efficacy was superior to that of FK228. In contrast, compound **4** exhibited decreased activity compared to FK228. The order of potency, combined with our previous data for **2** and **3** [13a], was estimated by the MG-MID value (mean value of GI_{50} over all cell lines tested) to be **5** (3.8 nM) ≥ **3** (5.6 nM) ≈ FK228 (**1**) (6.2 nM) > **2** (15 nM) > **4** (28 nM).

Considering the HDAC inhibitory and antiproliferative activities, spiruchostatin D (**5**) seems to be a promising candidate for the development of novel anticancer agents with high efficacy and low toxicity. Interestingly, the order of potency in antiproliferative activity is not necessarily in agreement with that in HDAC inhibitory activity. This is probably influenced by bioavailability and/or cell permeability. Another possible explanation for this discrepancy is that this class of HDAC inhibitors has an additional mechanism of action, albeit the molecular target is unknown at present. This hypothetical issue is now under investigation in our laboratories.

3. Conclusion

We have achieved the first total synthesis of spiruchostatins C (**4**) and D (**5**) in a highly convergent and unified manner. The present synthesis demonstrated that our explored synthetic strategy has the potential for producing spiruchostatin analogues that possess various alkyl side chains. The preliminary biological evaluation of the synthesized **4** and **5** together with our previous data for **2** and **3** determined the order of the efficacy of the spiruchostatins family (**2**–**5**). These results would be useful for the design and development of anticancer agents with therapeutic potential that target the isoform-selective inhibition of HDACs. Further investigations concerning the synthesis of unnatural spiruchostatin analogues and SAR study are currently underway and will be reported in due course.

Table 2
Growth inhibition of spiruchostatins C (**4**) and D (**5**) against a panel of 39 human cancer cell lines.

Origin of cancer	Cell line	GI_{50}[a] (nM)		
		4	**5**	FK228[b]
Breast	HBC-4	20	4.1	6.9
	BSY-1	25	5.2	8.5
	HBC-5	150	18	13
	MCF-7	47	2.9	4.2
	MDA-MB-231	14	1.7	5.5
Central nervous system (brain)	U-251	52	2.7	3.9
	SF-268	25	3.5	4.9
	SF-295	32	5.1	4.0
	SF-539	10[c]	0.58	3.6
	SNB-75	10[c]	1.9	7.2
	SNB-78	78	41	9.6
Colon	HCC2998	11	1.5	3.1
	KM-12	10[c]	2.6	3.4
	HT-29	10[c]	2.2	3.3
	HCT-15	3800[d]	210[d]	450[d]
	HCT-116	10[c]	1.3	3.1
Lung	NCI-H23	10[c]	2.5	4.6
	NCI-H226	53	4.0	8.9
	NCI-H522	10[c]	0.39[c]	1.8[c]
	NCI-H460	16	2.8	3.0
	A549	10[c]	0.97	2.6
	DMS273	32	5.1	5.8
	DMS114	10[c]	3.6	3.6
Melanoma	LOX-IMVI	10[c]	1.8	2.5
Ovary	OVCAR-3	16	2.2	4.6
	OVCAR-4	58	4.7	20
	OVCAR-5	10[c]	1.2	2.8
	OVCAR-8	10[c]	2.7	5.5
	SK-OV-3	15	2.3	3.3
Kidney	RXF-631L	35	5.9	6.6
	ACHN	130	21	20
Stomach	St-4	190	16	22
	MKN1	16	2.0	3.2
	MKN7	150	5.8	4.9
	MKN28	130	9.5	17
	MKN45	100	9.6	14
	MKN74	15	2.9	3.0
Prostate	DU-145	10[c]	4.0	6.0
	PC-3	23	8.7	18
MG-MID[e]		28	3.8	6.2

[a] Concentration that induces 50% inhibition of cell growth compared to the control.
[b] Positive control used in this study was synthesized in our laboratory.
[c] The most sensitive cell.
[d] The least sensitive cell.
[e] Mean value of GI_{50} over all cell lines tested.

4. Experimental

4.1. Chemistry

All reactions involving air- and moisture-sensitive reagents were carried out using oven dried glassware and standard syringe-septum cap techniques. Routine monitorings of reaction were carried out using glass-supported Merck silica gel 60 F_{254} TLC

plates. Flash column chromatography was performed on Kanto Chemical Silica Gel 60N (spherical, neutral 40–50 nm) with the solvents indicated.

All solvents and reagents were used as supplied with following exceptions. Tetrahydrofuran (THF) was freshly distilled from Na metal/benzophenone under argon. *N*,*N*-dimethylformamide (DMF), CH_2Cl_2, MeCN, pyridine, *i*-Pr_2NH, and *i*-Pr_2NEt were distilled from calcium hydride under argon.

Measurements of optical rotations were performed with a JASCO DIP-370 automatic digital polarimeter. ^1H and ^{13}C NMR spectra were measured with a JEOL AL-400 (400 MHz) spectrometer. Chemical shifts were expressed in ppm using Me_4Si ($\delta = 0$) as an internal standard. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Infrared (IR) spectral measurements were carried out with a JASCO FT/IR-4100 spectrometer. Low- and high-resolution mass (HRMS) spectra were measured on a JEOL JMS-DX 303/JMA-DA 5000 SYSTEM high-resolution mass spectrometer.

4.1.1. (3R,4R)-ethyl 4-(tert-butoxycarbonylamino)-3-hydroxy-6-methylheptanoate (**15**) and its (3S,4R)-isomer (**16**)

A solution of EtOAc (3.05 mL, 31 mmol) in THF (3 mL) was added slowly to a stirred solution of lithium diisopropylamide (LDA) (31 mmol) [prepared from *n*-BuLi in hexane (1.6 M solution, 20.6 mL, 33 mmol) and *i*-Pr_2NH (4.4 mL, 31 mmol)] in THF (15 mL) at −78 °C. After 30 min, (2*R*)-2-(*tert*-butoxycarbonylamino)-4-methylpentylaldehyde (*N*-Boc-D-leucinal) (**12**) [16] (1.98 g, 9.2 mmol) in THF (9 mL) was added to the above mixture at −78 °C. After 40 min, the reaction was quenched with 2 M HCl (10 mL) at −78 °C, and the resulting mixture was extracted with EtOAc (2 × 50 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (2 × 30 mL) and brine (2 × 30 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 5:1 → 4:1) to give **15** (1.51 g, 54%, less polar) and **16** (0.93 g, 33%, more polar).

Compound **15**: colorless oil; $[\alpha]_D^{25}$ +33.8 (*c* 1.00, $CHCl_3$); IR (neat): 3441, 3381, 2957, 2933, 2870, 1714, 1695, 1513, 1506, 1470, 1455, 1392, 1367, 1327, 1251, 1173, 1114, 1098, 1046, 1027 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.93 (6H, d, $J = 6.8$ Hz), 1.25–1.28 (1H, m), 1.27 (3H, t, $J = 7.3$ Hz), 1.44–1.56 (1H, m), 1.44 (9H, s), 1.63–1.68 (1H, m), 2.47–2.58 (2H, m), 3.41 (1H, d, $J = 2.9$ Hz), 3.62 (1H, d, $J = 5.4$ Hz), 4.02 (1H, s), 4.17 (2H, dd, $J = 6.8$, 6.8 Hz), 4.79 (1H, d, $J = 9.8$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.2, 22.9, 24.7, 28.3 (3C), 38.8, 41.6, 51.9, 60.7, 69.7, 79.0, 156.0, 173.2; HRMS (EI): *m*/*z* calcd for $C_{15}H_{29}NO_5$ (M^+) 303.2046, found 303.2039.

Compound **16**: colorless oil; $[\alpha]_D^{25}$ +20.2 (*c* 1.00, $CHCl_3$); IR (neat): 3444, 3371, 2977, 2957, 2935, 2871, 1714, 1695, 1522, 1391, 1367, 1252, 1174, 1043, 1026 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, d, $J = 6.3$ Hz), 0.94 (3H, d, $J = 6.8$ Hz), 1.27 (3H, t, $J = 7.3$ Hz), 1.32–1.35 (2H, m) 1.44 (9H, s), 1.64–1.71 (1H, m), 2.46–2.48 (2H, m), 3.54 (1H, d, $J = 2.9$ Hz), 3.67 (1H, br s), 4.01 (1H, br s), 4.17 (2H, dd, $J = 6.8$, 7.3 Hz), 4.68 (1H, d, $J = 8.8$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 21.5, 23.6, 24.7, 28.3 (3C), 38.1, 38.8, 52.7, 60.7, 71.3, 79.4, 156.1, 172.7; HRMS (EI): *m*/*z* calcd for $C_{15}H_{29}NO_5$ (M^+) 303.2046, found 303.2039.

4.1.2. (R)-ethyl 4-(tert-butoxycarbonylamino)-6-methyl-3-oxoheptanoate (**17**)

2.6 M Jones reagent (2.21 mL, 5.8 mmol) was added dropwise to a stirred solution of **15** (1.16 g, 3.8 mmol) in acetone (40 mL) at 0 °C, and stirring was continued for 1 h at room temperature. The mixture was diluted with Et_2O (150 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 40 mL) and brine (2 × 40 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 8:1 → 4:1) to give **17** (0.98 g, 86%) as a colorless oil. $[\alpha]_D^{25}$ +18.4 (*c* 1.03, $CHCl_3$); IR (neat): 3358, 2961, 2935, 2872, 1748, 1714, 1513, 1391, 1367, 1317, 1252, 1167, 1044, 1030 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, d, $J = 3.1$ Hz), 0.96 (3H, d, $J = 3.4$ Hz), 1.28 (3H, t, $J = 7.3$ Hz), 1.36–1.42 (1H, m), 1.45 (9H, s), 1.56–1.77 (1H, m), 3.55 (2H, dd, $J = 15.6$, 15.6 Hz), 4.20 (2H, dd, $J = 7.3$, 7.3 Hz), 4.34–4.39 (1H, m), 4.93 (1H, d, $J = 7.3$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 21.6, 23.2, 24.8, 28.3 (3C), 39.9, 46.3, 58.2, 61.4, 80.1, 155.5, 167.0, 172.7; HRMS (EI): *m*/*z* calcd for $C_{15}H_{27}NO_5$ (M^+) 301.1889, found 301.1883.

4.1.3. Conversion of compound **17** to compound **16** by stereoselective reduction

KBH_4 (1.30 g, 4.3 mmol) was added in small portions to a stirred solution of **17** (1.30 g, 4.3 mmol) in MeOH (40 mL) at −40 °C. After 5 h, the reaction was quenched with 10% aqueous citric acid at 0 °C (adjusted to pH 3). After concentration of the reaction mixture in vacuo, water (15 mL) was added, and the resulting mixture was extracted with CH_2Cl_2 (4 × 15 mL). The combined extracts were washed with brine (2 × 15 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 5:1 → 4:1) to give **16** (1.04 g, 90%). The IR, ^1H and ^{13}C NMR, mass spectra of this sample were identical with those recorded for compound **16**.

4.1.4. (3S,4R)-ethyl 4-(tert-butoxycarbonylamino)-3-(tert-butyldimethylsiloxy)-6-methylheptanoate (**18**)

tert-butyldimethylsilyl chloride (TBSCl) (1.15 g, 7.7 mmol) was added to a stirred solution of **16** (0.78 g, 2.3 mmol) in DMF (30 mL) containing imidazole (1.04 g, 15 mmol) at room temperature. After 24 h, the reaction mixture was diluted with Et_2O (120 mL), and the organic layer was washed successively with 3% aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 30 mL) and brine (2 × 30 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 10:1 → 5:1) to give **18** (1.08 g, 99%) as a colorless oil. $[\alpha]_D^{25}$ +15.5 (*c* 1.01, $CHCl_3$); IR (neat): 3371, 3273, 2957, 2931, 2901, 2857, 1703, 1504, 1472, 1390, 1366, 1284, 1253, 1174, 1094, 1051, 1028, 1007, 836, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.01 (3H, s), 0,07 (3H, s), 0.84 (9H, br s), 0.84–0.90 (6H, m), 1.21–1.25 (5H, m), 1.40 (9H, s), 1.57–1.65 (1H, m), 2.40 (1H, d, $J = 6.4$ Hz), 3.61 (1H, s), 4.06–4.16 (3H, m), 4.39 (1H, d, $J = 8.3$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.9, −4.8, 14.1, 18.0, 21.5, 23.7, 24.6, 25.7 (3C), 28.3 (3C), 38.5, 39.8, 53.0, 60.4, 71.7, 78.8, 155.3, 171.2; HRMS (EI): *m*/*z* calcd for $C_{21}H_{43}NO_5Si$ (M^+) 417.2911, found 417.2893.

4.1.5. (3S,4R)-allyl 4-(tert-butoxycarbonylamino)-3-(tert-butyldimethylsiloxy)-6-methylheptanoate (**20**)

1 M NaOH (16 mL, 16 mmol) was added dropwise to a stirred solution of **18** (1.30 g, 3.1 mmol) in EtOH (30 mL) at room temperature. After 6 h, the mixture was diluted with 10% aqueous HCl (20 mL) at 0 °C, and the resulting mixture was extracted with EtOAc (3 × 30 mL). The combined extracts were washed with brine (2 × 20 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded (3*S*,4*R*)-4-(*tert*-butoxycarbonylamino)-3-(*tert*-butyldimethylsiloxy)-6-methylheptanoic acid (**19**) as a white amorphous solid, which was used for the next reaction without further purification.

Allyl bromide (0.54 mL, 6.2 mmol) was added to a stirred solution of **19** (1.21 g, 3.1 mmol) in DMF (30 mL) containing K_2CO_3 (1.31 g, 9.4 mmol) at room temperature. After 6 h, the reaction was diluted with water (15 mL) at room temperature, and the resulting mixture was extracted with Et_2O (4 × 30 mL). The combined

extracts were washed successively with 3% aqueous HCl (2 × 20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL) and brine (2 × 20 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 5:1) to give **20** [1.03 g, 77% (2 steps from **18**)] as a pale yellow oil. $[\alpha]_D^{25}$ +16.1 (*c* 1.03, $CHCl_3$); IR (neat): 2956, 2930, 2898, 2857, 1739, 1717, 1703, 1389, 1366, 1253, 1171, 1095, 836, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ −0.02 (3H, s), −0.05 (3H, s), 0.82 (9H, s), 0.82–0.88 (6H, m), 1.19–1.24 (2H, m), 1.38 (9H, s), 1.55–1.63 (1H, m), 2.42 (2H, d, *J* = 6.4 Hz), 3.59 (1H, br s), 4.16 (1H, br s), 4.35 (1H, d, *J* = 8.3 Hz), 4.48–4.57 (2H, m), 5.17–5.29 (2H, m), 5.82–5.92 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.7, 18.0, 21.6, 23.7, 24.6, 25.8 (3C), 28.3 (3C), 38.5, 39.7, 53.1, 65.3, 70.5, 71.7, 118.4, 132.0, 155.3, 171.0; HRMS (EI): *m*/*z* calcd for $C_{22}H_{43}NO_5Si$ (M^+) 429.2911, found 429. 2927.

4.1.6. (3S,4R)-allyl 4-[(S)-2-(tert-butoxycarbonylamino)-3-(tritylthio)propanamido]-3-(tert-butyldimethylsiloxy)-6-methylheptanoate (22)

Trimethylsilyl trifluoromethanesulfonate (TMSOTf) (2.13 mL, 12 mmol) was added to a stirred solution of **20** (632 mg, 1.5 mmol) in CH_2Cl_2 (30 mL) in the presence of 2,6-lutidine (1.70 mL, 15 mmol) at room temperature. After 1 h, MeOH (5.0 mL) was added to the reaction mixture at 0 °C, and stirring was continued for 1 h at room temperature. The reaction mixture was concentrated in vacuo to afford (3*S*,4*R*)-allyl 4-amino-3-(*tert*-butyldimethylsiloxy)-6-methylheptanoate (**21**) as a colorless oil, which was immediately used for the next reaction due to its instability (prone to form a γ-lactam ring).

i-Pr_2NEt (0.37 mL, 2.21 mmol) was added dropwise to a stirred solution of the crude amine **21** (480 mg, 15 mmol) and *N*-Boc-*S*-trityl-D-cysteine (**14**) [13a] (1.02 g, 2.21 mmol) in MeCN (50 mL) containing (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (918 mg, 1.8 mmol) at room temperature under argon. After 3 h, the mixture was diluted with Et_2O (80 mL), and the organic layer was washed successively with 3% aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 30 mL) and brine (2 × 30 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 1:1) to give **22** (781 mg, 69%, 2 steps from **20**) as a colorless oil. $[\alpha]_D^{25}$ +5.0 (*c* 1.00, $CHCl_3$); IR (neat): 2956, 2930,1736, 1692, 1512, 1492, 1366, 1252, 1169, 833, 777, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ −0.01 (3H, s), 0.05 (3H, s), 0.83–0.88 (6H, m), 0.85 (9H, s), 1.25–1.31 (2H, m), 1.40 (9H, s), 1.52–1.58 (1H, m), 2.41–2.51 (3H, m), 2.76 (1H, dd, *J* = 6.8, 6.8 Hz), 3.88–3.98 (2H, m), 4.10–4.14 (1H, m), 4.53–4.56 (2H, m), 4.72 (1H, d, *J* = 6.8 Hz), 5.21–5.33 (2H, m), 5.85–5.94 (1H, m), 6.08 (1H, d, *J* = 8.3 Hz), 7.19–7.42 (15H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.6, 18.0, 21.5, 23.9, 24.5, 25.8 (3C), 28.2 (3C), 33.4, 38.7, 39.8, 51.8, 53.5, 65.3, 67.1, 71.7, 80.3, 118.5, 126.8 (3C), 128.0 (6C), 129.6 (6C), 132.0, 144.5 (3C), 149.8, 170.0, 170.9 ppm; HRMS (FAB): *m*/*z* calcd for $C_{44}H_{62}N_2O_6SSi$ (M^+) 774.4098, found 774.4091.

4.1.7. (3S,4R)-allyl 4-[(S)-2-amino-3-(tritylthio)propanamido]-3-(tert-butyldimethylsiloxy)-6-methylheptanoate (8)

TMSOTf (0.90 mL, 5.0 mmol) was added dropwise to a stirred solution of **22** (781 mg, 1.0 mmol) in CH_2Cl_2 (11 mL) containing 2,6-lutidine (0.91 mL, 5.0 mmol) at room temperature. After 1 h, MeOH (2.2 mL) was added to the reaction mixture at 0 °C, and stirring was continued for 1 h at room temperature. The mixture was concentrated in vacuo to afford a residue, which was purified by column chromatography (hexane/EtOAc 2:1) to give **8** (593 mg, 88%) as a white amorphous solid. $[\alpha]_D^{25}$ +3.8 (*c* 1.00, $CHCl_3$); IR (neat): 2954, 2929, 2895, 2856, 1736, 1671, 1508, 1471, 1445, 1254, 1172, 1084, 835, 777, 743, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.82–0.89 (6H, m), 0.86 (9H, s), 1.25–1.37 (2H, m), 1.49–1.51 (1H, m), 2.42–2.52 (2H, m), 2.58 (1H, dd, *J* = 8.3, 8.3 Hz), 2.69 (1H, dd, *J* = 3.4, 3.9 Hz), 3.10 (1H, dd, *J* = 3.4, 3.9 Hz), 3.89–3.94 (1H, m), 4.11–4.15 (1H, m) 4.55 (2H, d, *J* = 5.9 Hz), 5.22–5.33 (2H, m), 5.85–5.94 (1H, m), 7.14 (1H, d, *J* = 9.3 Hz), 7.21 (2H, d, *J* = 7.3 Hz), 7.26–7.44 (15H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.6, 17.9, 21.6, 23.8, 24.6, 25.8 (3C), 37.3, 38.3, 40.1, 51.5, 53.7, 65.3, 66.8, 71.8, 118.5, 126.7 (3C), 127.9 (6C), 129.6 (6C), 132.0, 144.6, 171.0, 172.2; HRMS (FAB): *m*/*z* calcd for $C_{39}H_{55}N_2O_4SSi$ (M^+ + H) 674.3574, found 675.3662.

4.1.8. (3S,4R)-allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-(4-methoxybenzyloxy)-7-(tritylthio)hept-4-enoylamino]propionylamino]-3-tritylthio(propionylamino)}-6-methylheptanoate (23)

i-Pr_2NEt (73 μL, 0.43 mmol) was added dropwise to a stirred solution of **8** (108 mg, 0.16 mmol) and (*R*)-2-[(*S*,*E*)-3-(4-methoxybenzyloxy)-7-(tritylthio)hept-4-enamido]propanoic acid (**10**) [13a] (100 mg, 0.16 mmol) in CH_2Cl_2 (3.2 mL) containing *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) (81.2 mg, 0.21 mmol) and 1-hydroxy-7-azabenzotriazole (HOAt) (28.6 mg, 0.21 mmol) at −30 °C under argon. After 3 h, the reaction mixture was diluted with $CHCl_3$ (30 mL). The organic layer was washed successively with 10% aqueous HCl (2 × 10 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL) and brine (2 × 10 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 1:1) to give **23** (169 mg, 84%) as a colorless viscous liquid. $[\alpha]_D^{25}$ +12.0 (*c* 1.00, $CHCl_3$); IR (neat): 282, 2956, 2930, 1735, 1626, 1541, 1512, 1444, 1247, 1172, 1083, 835, 743, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ −0.01 (3H, s), 0.05 (3H, s), 0.81–0.86 (15H, m), 1.16 (3H, d, *J* = 7.3), 1.32 (2H, m), 1.45–1.48 (1H, m), 2.08–2.12 (2H, m), 2.19–2.23 (2H, m), 2.31 (1H, dd, *J* = 3.4, 2.9 Hz), 2.40–2.52 (4H, m), 2.83 (1H, dd, *J* = 7.3, 7.8 Hz), 3.79 (3H, s), 3.89–4.02 (2H, m), 4.06–4.14 (3H, m), 4.19–4.27 (1H, m), 4.36 (1H, d, *J* = 10.7 Hz), 4.53–4.55 (2H, m), 5.19–5.45 (4H, m), 5.85–5.94 (1H, m), 6.19 (1H, d, *J* = 9.3 Hz), 6.53 (1H, d, *J* = 8.3 Hz), 6.82–6.84 (2H, m), 6.96 (1H, d, *J* = 6.3 Hz), 7.14–7.29 (20H, d), 7.36–7.43 (12H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.6, 17.0, 18.0, 21.5, 23.8, 24.7, 25.8 (3C), 29.6, 31.2, 33.1, 39.1, 39.6, 42.4, 49.0, 52.1, 52.2, 55.3, 65.2, 66.6, 66.9, 70.0, 71.7, 76.4, 113.9 (2C), 118.3, 126.6 (3C), 126.8 (3C), 127.8 (6C), 128.0 (6C), 129.48 (6C), 129.52 (8C), 129.6, 129.8 (2C), 132.1, 144.3 (3C), 144.8 (3C), 159.4, 169.3, 171.2, 171.23, 172.0; HRMS (FAB): *m*/*z* calcd for $C_{76}H_{92}N_3O_8S_2Si$ (M^+ + H) 1266.7665, found 1266.6104.

4.1.9. (3S,4R)-allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-hydroxy-7-(tritylthio)hept-4-enoylamino]propionylamino]-3-tritylthio(propionylamino)}-6-methylheptanate (24)

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (73.5 mg, 0.27 mmol) was added in small portions to a stirred solution of **23** (160 mg, 0.13 mmol) in CH_2Cl_2/H_2O 9:1 (13 mL) at room temperature. After 3 h, the reaction mixture was diluted with $CHCl_3$ (60 mL), and the organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and brine (2 × 20 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 3:2) to give **24** (147 mg, 99%) as a colorless viscous liquid. $[\alpha]_D^{25}$ +10.4 (*c* 1.00, $CHCl_3$); IR (neat): 3283, 2955, 2929, 1734, 1626, 1525, 1489, 1444, 1253, 1169, 1090, 833, 742, 699, 675 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.01 (3H, s), 0.06 (3H, s), 0.83–0.88 (15H, m), 1.26–1.34 (5H, m), 1.47–1.54 (1H, m), 2.04–2.09 (2H, m), 2.17–2.26 (2H, m), 2.31 (1H, dd, *J* = 2.4, 2.4 Hz), 2.41–2.54 (4H, m), 2.71 (1H, dd, *J* = 7.3, 7.3 Hz), 3.08 (1H, d, *J* = 1.9 Hz), 3.91–3.96 (1H, m), 4.09–4.22 (2H, m), 4.30–4.35 (2H, m), 4.48–4.57 (2H,m), 5.20–5.48 (4H, m), 5.84–5.94 (1H, m), 6.06 (1H, d,

J = 7.3 Hz), 6.14 (1H, d, J = 9.3 Hz), 6.89 (1H, d, J = 8.3 Hz), 7.17–7.31 (18H, m), 7.37–7.43 (12H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.6, 17.5, 18.0, 21.5, 23.8, 24.7, 25.8 (3C), 31.28, 31.34, 33.3, 39.3, 39.6, 43.9, 49.6, 52.3 (2C), 65.3, 66.6, 66.9, 69.7, 71.7, 118.3, 126.6 (3C), 126.9 (3C), 127.9 (6C), 128.1 (6C), 129.5 (6C), 129.6 (6C), 130.0, 132.1, 132.5, 144.3 (3C), 144.9 (3C), 169.5, 171.3, 171.6, 171.8; HRMS (FAB): m/z calcd for $C_{68}H_{84}N_3O_7S_2Si$ (M^+ + H) 1146.6180, found 1146.5529.

4.1.10. (7S,11R,14S,17R,18S,E)-18-(tert-butyldimethylsilyloxy)-7-hydroxy-17-isobutyl-11-methyl-9,12,15-trioxo-1,1,1,-triphenyl-14-tritylthiomethyl-2-thia-10,13,16-triazaicos-5-en-20-oic acid (**6**)

Morpholine (7.3 μL, 84 μmol) was added dropwise to a stirred solution of **24** (47.8 mg, 42 μmol) in THF (4.2 mL) containing $Pd(PPh_3)_4$ (4.8 mg, 4.2 μmol) at room temperature under argon. After 30 min, the reaction mixture was diluted with EtOAc (30 mL), and the organic layer was washed with 10% aqueous HCl (2 × 10 mL) and brine (2 × 10 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography ($CHCl_3$/MeOH 20:1) to give **6** (45.7 mg, 99%) as a white amorphous solid. $[\alpha]_D^{25}$ −11.2 (c 1.00, $CHCl_3$); IR (neat): 3295, 3081, 3058, 3018, 2955, 2928, 2856, 1714, 1659, 1651, 1644, 1635, 1538, 1488, 1444, 1256, 1094, 835, 752, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.07 (6H, d, J = 4.9 Hz), 0.79–0.91 (15H, m), 1.35 (3H, d, J = 7.3 Hz), 1.38–1.49 (2H, m), 1.52–1.57 (1H, m), 2.02–2.07 (2H, m), 2.17–2.26 (3H, m), 2.31–2.37 (2H, m), 2.44 (1H, dd, J = 3.4, 3.9 Hz), 2.53 (1H, dd, J = 6.3, 6.3 Hz), 2.82 (1H, dd, J = 6.8, 6.8 Hz), 3.86–3.96 (2H, m), 4.25–4.35 (3H, m), 5.28–5.44 (2H, m), 6.38 (1H, d, J = 5.9 Hz), 6.54 (1H, d, J = 8.3 Hz), 7.06 (1H, d, J = 8.3 Hz), 7.15–7.30 (18H, m), 7.34–7.43 (12H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.5, 17.2, 17.9, 21.4, 24.0, 24.8, 25.8 (3C), 29.7, 31.3, 33.1, 39.9, 39.96, 44.03, 50.3, 52.6, 53.1, 66.7, 66.8, 69.7, 71.7, 126.7 (3C), 127.0 (3C), 127.9 (6C), 128.1 (6C), 129.4 (6C), 129.6 (6C), 132.0, 132.1, 144.1 (3C), 144.8, 170.4, 172.25, 172.30, 172.4; HRMS (FAB): m/z calcd for $C_{65}H_{80}N_3O_7S_2Si$ (M^+ + H) 1106.5542, found 1106.5216.

4.1.11. (2S,6R,9S,12R,13S)-13-(tert-butyldimethylsiloxy)-12-isobutyl-6-methyl-2-[(E)-4-(tritylthio)but-1-enyl]-9-tritylthiomethyl-1-oxa-5,8,11-triazacyclopentadecane-4,7,10,15-tetraone (**25**)

A solution of **6** (45.7 mg, 41 μmol) in CH_2Cl_2 (4.1 mL) was added very slowly to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (18.5 mg, 54 μmol) in CH_2Cl_2 (41 mL, 1.0 mM concentration) containing 4-dimethylaminopyridine (DMAP) (18.5 mg, 54 μmol) at room temperature over 14 h. After 1 h, the mixture was diluted with CH_2Cl_2 (50 mL), and the organic layer was washed successively with saturated aqueous $NaHCO_3$ (2 × 20 mL), water (2 × 20 mL) and brine (2 × 20 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 1:1) to give **25** (33.5 mg, 75%) as a white amorphous solid. $[\alpha]_D^{25}$ −9.3 (c 1.01, $CHCl_3$); IR (neat): 3468, 3323, 2954, 2927, 1735, 1651, 1537, 1489, 1444, 1259, 1094, 832, 743, 700, 668 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.01 (3H, s), 0.05 (3H, s), 0.78–0.93 (15H, m), 1.22–1.40 (5H, m), 1.72–1.83 (1H, m), 1.94–2.08 (2H, m), 2.16–2.20 (2H, m), 2.33–2.41 (2H, m), 2.47–2.53 (2H, m), 2.71 (1H, d, J = 13.2 Hz), 3.28–3.35 (2H, m), 3.70 (1H, br s), 4.18 (1H, t, J = 6.3, 6.8 Hz), 4.30–4.33 (1H, m), 5.29–5.35 (1H, m), 6.23 (1H, br s), 6.60 (1H, br s), 6.94 (1H, d, J = 7.3 Hz), 7.18–7.40 (30H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.3, 17.20, 17.9, 21.3, 23.8, 25.0, 25.8 (3C), 31.1, 31.3, 32.5, 38.8, 39.4, 41.5, 50.4, 53.1, 54.4, 66.7, 69.8, 70.2, 77.2, 126.7 (3C), 126.8 (3C), 127.9 (6C), 128.0 (6C), 128.5, 129.50 (6C), 129.54 (6C), 131.9, 144.5 (3C), 144.8 (3C), 169.4, 169.9, 170.5, 171.5; HRMS (FAB): m/z calcd for $C_{65}H_{78}N_3O_6S_2Si$ (M^+ + H) 1088.5389, found 1088.5118.

4.1.12. (1S,5S,6R,9S,20R,E)-5-(tert-butyldimethylsilyloxy)-6-isobutyl-20-methyl-2-oxa-11,12-dithia-7,19,22-triazabicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (**26**)

A solution of **25** (33.5 mg, 31 μmol) in CH_2Cl_2/MeOH 9:1 (7.5 mL) was added dropwise to a vigorously stirred solution of I_2 (78.4 mg, 0.31 mmol) in CH_2Cl_2/MeOH 9:1 (62 mL, 0.5 mM concentration) over 10 min at room temperature. After 10 min, the reaction was quenched with 10% aqueous $Na_2S_2O_3$ (30 mL) at room temperature. The resulting mixture was diluted with CH_2Cl_2 (90 mL), and the organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 30 mL) and brine (2 × 30 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography ($CHCl_3$/MeOH 30:1) to give **26** (17.6 mg, 95%) as a white amorphous solid. $[\alpha]_D^{25}$ −3.4 (c 1.00, $CHCl_3$); IR (neat): 3426, 2953, 2857, 1747, 1653, 1541, 1259, 1146, 1127, 833 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.09 (3H, s), 0.13 (3H, s), 0.89–0.93 (15H, m), 1.50 (3H, d, J = 7.3 Hz), 1.54–1.66 (2H, m), 1.96–2.05 (1H, m), 2.44–2.57 (3H, m), 2.64–2.70 (3H, m), 3.03–3.11 (2H, m), 3.21 (2H, dd, J = 7.3, 7.3 Hz), 3.41 (1H, br s), 4.24–4.31 (1H, m), 4.50–4.54 (1H, m), 4.91 (1H, br s), 5.64–5.68 (2H, m), 5.88 (1H, s), 6.08 (1H, br s) 6.72 (1H, d, J = 9.8 Hz), 7.47 (1H, d, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.3, 16.7, 17.9, 21.1, 23.7, 25.3, 25.7 (3C), 33.3, 38.8, 38.9, 39.9, 40.6, 40.9, 52.2, 54.5, 55.9, 69.3, 70.8, 129.6, 132.5, 168.5, 168.8, 171.2, 171.3; HRMS (FAB): m/z calcd for $C_{27}H_{48}N_3O_6S_2Si$ (M^+ + H) 601.8941, found 602.2757.

4.1.13. Spiruchostatin C (**4**)

HF pyridine (0.55 mL) was added to a stirred solution of **26** (35.4 mg, 58 μmol) in pyridine (1.1 mL) at room temperature. After 14 h, the reaction mixture was diluted with EtOAc (60 mL), and the organic layer was washed successively with 3% aqueous HCl (3 × 15 mL), saturated aqueous $NaHCO_3$ (2 × 15 mL) and brine (2 × 15 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography ($CHCl_3$/MeOH 10:1) to give **4** (spiruchostatin C) (26.0 mg, 93%) as a white amorphous solid. $[\alpha]_D^{25}$ −59.4 (c 0.20, $CHCl_3$) {lit. [10] $[\alpha]_D^{25}$ −60.0 (c 0.10, $CHCl_3$)}; IR (neat): 3455, 3369, 3311, 2998, 2953, 2868, 1728, 1659, 1546, 1530, 1275, 1161 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (6H, d, J = 6.3), 1.50–1.56 (4H, m), 1.59–1.68 (1H, m), 2.05–2.13 (1H, m), 2.42 (1H, dd, J = 11.7, 11.7 Hz), 2.57 (1H, d, J = 13.2 Hz), 2.64–2.76 (5H, m), 2.99–3.06 (1H, m), 3.17–3.20 (2H, m), 3.30 (1H, br s), 3.41 (1H, dd, J = 7.3, 7.3 Hz), 4.23–4.29 (1H, m), 4.34–4.41 (1H, m), 4.84–4.89 (1H, m) 5.46 (1H, br s), 5.67 (1H, d, J = 15.1 Hz), 5.93 (1H, d, J = 3.4 Hz), 6.36 (1H, t, J = 12.2, 13.7 Hz), 6.72 (1H, d, J = 9.3 Hz), 7.49 (1H, d, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.6, 21.2, 23.5, 25.2, 29.7, 32.9, 38.7, 38.8, 40.7, 41.1, 52.3, 54.8, 56.3, 70.6, 70.9, 128.7, 133.5, 168.8, 170.8, 171.0, 171.6; HRMS (FAB): m/z calcd for $C_{21}H_{34}N_3O_6S_2$ (M^+ + H) 487.6332, found 488.1890. The IR, ^{1}H and ^{13}C NMR, and HRMS spectrum are essentially identical with those reported for natural spiruchostatin C [10].

4.1.14. (3S,4R)-allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-(4-methoxybenzyloxy)-7-(tritylthio)hept-4-enoylamino]isovaleroylamino]-3-tritylthio(propionylamino)}-5-methylhexanoate (**27**)

i-Pr_2NEt (0.17 mL, 1.0 mmol) was added dropwise to a stirred solution of 4-[(*S*)-2-amino-3-(tritylthio)propanamido]-3-(*tert*-butyldimethylsiloxy)-5-methylhexanoate (**9**) [13a] (258 mg, 0.39 mmol) and (*R*)-2-[(*S*,*E*)-3-(4-methoxybenzyloxy)-7-(tritylthio) hept-4-enamido]-3-methylbutanoic acid (**11**) [13a] (247 mg, 0.39 mmol) in CH_2Cl_2 (8.0 mL) containing HATU (193 mg, 0.51 mmol) and HOAt (69.2 mg, 0.51 mmol) at −30 °C under argon. After 3 h, the reaction mixture was diluted with $CHCl_3$ (50 mL). The organic layer was washed successively with 10% aqueous HCl (2 × 20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL) and brine

(2 × 20 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 2:1) to give **27** (469 mg, 94%) as a colorless viscous liquid. $[\alpha]_D^{25}$ +5.6 (*c* 1.00, $CHCl_3$); IR (neat): 3290, 3058, 2958, 2930, 2857, 1734, 1637, 1540, 1514, 1444, 1389, 1248, 1173, 1092, 1036, 830, 743, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.02 (3H, s), 0.06 (3H, s), 0.68 (3H, d, *J* = 6.8 Hz), 0.76–0.86 (9H, m), 0.82 (9H, s), 1.86–2.13 (4H, m), 2.17–2.23 (2H, m), 2.34–2.57 (6H, m), 2.76 (1H, dd, *J* = 7.8, 12.9 Hz), 3.75–3.82 (1H, m), 3.78 (3H, s), 3.93–4.02 (2H, m), 4.09 (1H, dd, *J* = 5.9, 7.3 Hz), 4.15–4.22 (2H, m), 4.39 (1H, d, *J* = 10.7 Hz), 4.46 (1H, dd, *J* = 5.9, 13.2 Hz), 4.53 (1H, dd, *J* = 5.9, 12.7 Hz), 5.18–5.31 (3H, m), 5.49 (1H, dt, *J* = 6.8, 15.5 Hz), 5.81–5.91 (1H, m), 6.01 (1H, d, *J* = 10.2 Hz), 6.30 (1H, d, *J* = 7.8 Hz), 6.80 (2H, d, *J* = 8.3 Hz), 7.03 (1H, d, *J* = 7.8 Hz), 7.16–7.45 (32H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.6, 16.9, 17.6, 17.9, 19.5, 20.3, 25.7 (3C), 28.0, 29.9, 31.2, 31.3, 33.2, 39.4, 42.5, 52.4, 55.3, 58.4, 58.5, 65.2, 66.5, 67.1, 69.4, 70.0, 76.5, 113.8 (2C), 118.3, 126.6 (3C), 126.8 (3C), 127.9 (6C), 128.1 (6C), 129.5 (12C), 129.7, 129.9 (2C), 129.9, 132.1, 133.2, 144.3 (3C), 144.8 (3C), 159.3, 169.8, 171.1, 171.2, 171.6; HRMS (FAB): *m*/*z* calcd for $C_{77}H_{93}N_3O_8S_2SiNa$ (M^+ + Na) 1318.5810, found 1318.5787.

4.1.15. *(3S,4R)-allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-hydroxy-7-(tritylthio)hept-4-enoylamino]isovaleroylamino]-3-tritylthio(propionylamino)}-5-methylhexanate (**28**)*

DDQ (166 mg, 0.73 mmol) was added in small portions to a stirred solution of **27** (469 mg, 0.37 mmol) in CH_2Cl_2/H_2O 9:1 (18 mL) at room temperature. After 3 h, the reaction mixture was diluted with $CHCl_3$ (60 mL), and the organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and brine (2 × 20 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 1:1) to give **28** (336 mg, 79%) as a colorless viscous liquid. $[\alpha]_D^{25}$ +11.2 (*c* 1.00, $CHCl_3$); IR (neat): 3270, 3058, 2958, 2928, 2856, 1736, 1673, 1633, 1549, 1490, 1444, 1390, 1254, 1174, 1093, 837, 743, 700, 674 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.05 (3H, s), 0.79–0.85 (9H, m), 0.81 (9H, s), 0.92 (3H, d, *J* = 6.8 Hz), 1.87–1.92 (1H, m), 2.05 (1H, d, *J* = 7.3 Hz), 2.17–2.26 (4H, m), 2.33 (1H, dd, *J* = 2.7, 13.7 Hz), 2.41 (1H, dd, *J* = 7.3, 15.6 Hz), 2.49–2.61 (4H, m), 3.43 (1H, br s), 3.73–3.78 (1H, m), 3.99 (1H, dd, *J* = 8.3, 14.9 Hz), 4.17 (1H, dt, *J* = 3.4, 7.2 Hz), 4.23 (1H, dd, *J* = 4.9, 8.3 Hz), 4.33–4.37 (1H, m), 4.49 (1H, dd, *J* = 5.9, 13.3 Hz), 4.52 (1H, dd, *J* = 5.9, 13.2 Hz), 5.19–5.31 (2H, m), 5.36 (1H, dd, *J* = 5.9, 15.4 Hz), 5.51 (1H, dt, *J* = 6.3, 15.5 Hz), 5.81–5.93 (2H, m), 6.04 (1H, d, *J* = 8.3 Hz), 6.93 (1H, d, *J* = 8.3 Hz), 7.17–7.43 (30H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.7, 16.9, 17.4, 17.9, 19.5, 20.2, 25.7 (3C), 28.1, 29.7, 31.30, 31.34, 33.3, 39.34, 44.0, 52.6, 58.5, 58.6, 65.3, 66.6, 67.0, 69.3, 69.8, 118.4, 126.6 (3C), 126.8 (3C), 127.9 (6C), 128.0 (6C), 129.48 (6C), 129.54 (6C), 129.9, 132.0, 132.5, 144.3 (3C), 144.8 (3C), 170.2, 170.9, 171.6, 171.7 ppm; HRMS (FAB): *m*/*z* calcd for $C_{69}H_{86}N_3O_7S_2Si$ (M^+ + H) 1159.5598, found 1160.5654.

4.1.16. *(7S,11R,14S,17R,18S,E)-18-(tert-butyldimethylsilyloxy)-7-hydroxy-11,17-diisopropyl-9,12,15-trioxo-1,1,1-triphenyl-14-tritylthiomethyl-2-thia-10,13,16-triazaicos-5-en-20-oic acid (**7**)*

Morpholine (46 μL, 0.53 mmol) was added dropwise to a stirred solution of **28** (308 mg, 0.27 mmol) in THF (13 mL) containing $Pd(PPh_3)_4$ (30.7 mg, 27 μmol) at room temperature under argon. After 30 min, the reaction mixture was diluted with EtOAc (30 mL), and the organic layer was washed with 10% aqueous HCl (2 × 10 mL) and brine (2 × 10 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography ($CHCl_3$/MeOH 20:1) to give **7** (270 mg, 90%) as a white amorphous solid. $[\alpha]_D^{25}$ −0.8 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.00 (6H, s), 0.74–0.79 (9H, m), 0.79 (9H, s), 0.87 (3H, d, *J* = 6.8 Hz), 1.94–2.02 (3H, m), 2.12–2.36 (6H, m), 2.41–2.50 (2H, m), 2.63–2.68 (1H, m), 3.69–3.75 (1H, m), 4.02–4.07 (2H, m), 4.18–4.21 (1H, m), 4.27–4.32 (1H, m), 5.32 (1H, dd, *J* = 5.9, 15.4 Hz), 5.41 (1H, dt, *J* = 6.4, 15.1 Hz), 6.22 (1H, d, *J* = 9.8 Hz), 6.33 (1H, br s), 7.02 (1H, br s), 7.10–7.42 (30H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.5, 16.7, 17.4, 17.9, 19.5, 20.3, 25.7 (3C), 27.9, 29.6, 31.3, 33.2, 39.7, 43.9, 53.2, 58.8, 59.0, 66.5, 66.9, 69.1, 69.6, 126.6 (3C), 126.7 (3C), 128.5 (6C), 128.6 (6C), 129.45 (6C), 129.51 (6C), 131.5, 132.5, 144.3 (3C), 144.8 (3C), 170.4, 171.5, 172.2, 174.0; IR (neat): 282, 3058, 2958, 1717, 1637, 1544, 1489, 1438, 1388, 1255, 1181, 1120, 1093, 1034, 836, 744, 723, 699 cm^{-1}; HRMS (FAB): *m*/*z* calcd for $C_{66}H_{81}N_3O_7S_2SiK$ (M^+ + K) 1158.4922, found 1158.4901.

4.1.17. *(2S,6R,9S,12R,13S)-13-(tert-butyldimethylsiloxy)-6,12-diisopropyl-2-[(E)-4-(tritylthio)but-1-enyl]-9-tritylthiomethyl-1-oxa-5,8,11-triazacyclopentadecane-4,7,10,15-tetraone (**29**)*

A solution of **7** (34.8 mg, 31 μmol) in CH_2Cl_2 (3.0 mL) was added very slowly to a stirred solution of MNBA (13.9 mg, 31 μmol) in CH_2Cl_2 (30 mL, 1.0 mM concentration) containing DMAP (11.4 mg, 93 μmol) at room temperature over 14 h. After 1 h, the mixture was diluted with CH_2Cl_2 (50 mL), and the organic layer was washed successively with saturated aqueous $NaHCO_3$ (2 × 20 mL), water (2 × 20 mL) and brine (2 × 20 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography (hexane/EtOAc 1:1) to give **29** (28.9 mg, 85%) as a white amorphous solid. $[\alpha]_D^{25}$ −6.3 (*c* 0.64, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ −0.10 (3H, s), 0.00 (3H, s), 0.76–0.80 (6H, m), 0.80 (9H, s), 0.95 (3H, d, *J* = 6.8 Hz), 1.00 (3H, d, J = 6.8 Hz), 1.93–2.09 (4H, m), 2.16–2.20 (2H, m), 2.31 (1H, dd, *J* = 6.3, 16.1 Hz), 2.38 (1H, dd, *J* = 2.9, 16.6 Hz), 2.45–2.52 (2H, m), 2.61 (1H, d, *J* = 8.3 Hz), 3.24–3.29 (2H, m), 3.54–3.60 (1H, m), 3.94–3.99 (2H, m), 5.37 (1H, dd, *J* = 6.8, 15.4 Hz), 5.44–5.49 (1H, m), 5.67 (1H, dt, *J* = 6.3, 15.6 Hz), 6.11 (1H, d, *J* = 7.8 Hz), 6.60 (1H, d, *J* = 4.9 Hz), 7.20–7.43 (31H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.9, −3.9, 15.6, 17.9, 18.6, 19.4, 20.5, 25.8 (3C), 27.2, 29.7, 30.4, 30.9, 31.4, 32.0, 41.2, 42.1, 58.3, 59.6, 66.6, 66.9, 68.9, 72.2, 126.6 (3C), 126.8 (3C), 127.9 (6C), 128.0 (6C), 128.2, 129.47 (6C), 129.52 (6C), 133.4, 144.4 (3C), 144.8 (3C), 169.6, 170.1, 170.6, 173.1 ppm; IR (neat): 3468, 3323, 2954, 2927, 1735, 1651, 1537, 1489, 1444, 1259, 1094, 832, 743, 700, 668 cm^{-1}; HRMS (FAB): *m*/*z* calcd for $C_{66}H_{79}N_3O_6S_2SiNa$ (M^+ + Na) 1124.5077, found 1124.5040.

4.1.18. *(1S,5S,6R,9S,20R,E)-5-(tert-butyldimethylsilyloxy)-6,20-diisopropyl-2-oxa-11,12-dithia-7,19,22-triazabicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (**30**)*

A solution of **29** (45.0 mg, 42 μmol) in CH_2Cl_2/MeOH 9:1 (17 mL) was added dropwise to a vigorously stirred solution of I_2 (170 mg, 0.67 mmol) in CH_2Cl_2/MeOH 9:1 (84 mL, 0.5 mM concentration) over 10 min at room temperature. After 10 min, the reaction was quenched with 10% aqueous $Na_2S_2O_3$ (30 mL) at room temperature. The resulting mixture was diluted with CH_2Cl_2 (90 mL), and the organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 30 mL) and brine (2 × 30 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography ($CHCl_3$/MeOH 30:1) to give **30** (36.1 mg, 88%) as a white amorphous solid. $[\alpha]_D^{25}$ +13.6 (*c* 0.28, $CHCl_3$); IR (neat): 3335, 2956, 1747, 1670, 1541, 1258, 1158, 948, 831, 781 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.10 (3H, s), 0.16 (3H, s), 0.79 (3H, d, *J* = 6.8 Hz), 0.83 (9H, s), 0.87 (3H, d, *J* = 6.8 Hz), 0.95 (3H, d, *J* = 6.8 Hz), 0.99 (3H, d, *J* = 6.8 Hz), 2.06–2.14 (1H, m), 2.18–2.26 (1H, m), 2.47–2.53 (3H, m), 2.58–2.91 (6H, m), 3.04 (1H, dd, *J* = 7.3, 13.2 Hz), 3.70–3.72 (1H, m), 4.07 (1H, t, *J* = 5.2 Hz), 4.75 (1H, dt, *J* = 3.4, 9.8 Hz), 4.95–4.99 (1H, m), 5.54 (1H, d, *J* = 15.1 Hz), 5.61–5.62 (1H, m), 5.71 (1H, d, *J* = 4.4 Hz), 6.50 (1H, t, *J* = 13.4 Hz), 6.62

(1H, d, $J = 9.8$ Hz), 7.00 (1H, $J = 6.8$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.2, 17.0, 18.0, 19.1, 19.8, 21.0, 25.7 (3C), 29.0, 29.2, 35.2, 39.2, 40.6, 41.3, 44.2, 53.6, 61.8, 62.8, 67.4, 68.7, 128.3, 133.4, 168.8, 169.1, 170.0, 171.2 ppm; HRMS (FAB): m/z calcd for $C_{28}H_{49}N_3O_6S_2Si$ ($M^+ + H$) 615.2832, found 615.2914.

4.1.19. Spiruchostatin D (**5**)

HF·pyridine (0.55 mL) was added to a stirred solution of **30** (35.4 mg, 58 μmol) in pyridine (1.1 mL) at room temperature. After 14 h, the reaction mixture was diluted with EtOAc (60 mL), and the organic layer was washed successively with 3% aqueous HCl (3 × 15 mL), saturated aqueous $NaHCO_3$ (2 × 15 mL) and brine (2 × 15 mL), then dried over Na_2SO_4. Concentration of the solvent in vacuo afforded a residue, which was purified by column chromatography ($CHCl_3$/MeOH 10:1) to give **5** (spiruchostatin D) (26.8 mg, 93%) as a white amorphous solid. $[\alpha]_D^{25}$ −59.2 (c 0.29, MeOH) {lit. [12] $[\alpha]_D^{25}$ −65.3 (c 0.20, MeOH)}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, d, $J = 6.8$ Hz), 1.04 (3H, d, $J = 6.8$ Hz), 1.07 (3H, d, $J = 6.8$ Hz), 1.09 (3H, d, $J = 7.3$ Hz), 2.25–2.34 (1H, m), 2.36–2.44 (1H, m), 2.46–2.54 (1H, m), 2.62 (1H, d, $J = 13.2$ Hz), 2.66–2.79 (5H, m), 2.98–3.15 (3H, m), 3.38 (1H, d, $J = 6.8$, 13.2 Hz), 3.51–3.57 (1H, m), 4.10 (1H, t, $J = 5.1$ Hz), 4.56–4.58 (1H, m), 4.93–4.98 (1H, m), 5.50–5.52 (1H, m), 5.68 (1H, d, $J = 15.1$ Hz), 5.92–5.95 (1H, m), 6.54 (1H, t, $J = 12.7$ Hz), 6.74 (1H, d, $J = 9.3$ Hz), 7.39 (1H, d, $J = 7.3$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.3, 19.6, 19.7, 20.6, 29.1, 29.6, 34.0, 39.7, 40.2, 40.8, 42.6, 54.3, 62.1, 63.4, 69.4, 70.4, 128.1, 133.9, 168.9, 170.0, 171.0, 171.8 ppm; IR (neat): 3375, 3342, 2964, 2932, 2875, 1731, 1660, 1534, 1519, 1266, 1162, 979, 911, 755, 733 cm^{-1}; HRMS (FAB): m/z calcd for $C_{20}H_{32}N_3O_6S_2$ ($M^+ + H$) 501.196, found 501.2057. The IR, ^{1}H and ^{13}C NMR, and HRMS spectrum are essentially identical with those reported for natural spiruchostatin D [12].

4.2. Biological evaluation

4.2.1. HDACs preparation and enzyme inhibition assay [20]

In a 100-mm dish, 293 T cells (1–2 × 10^7) were grown for 24 h and transiently transfected with 10 μg each of the vector pcDNA3-HDAC1 for human HDAC1 or pcDNA3-mHDA2/HDAC6 for mouse HDAC6, using the LipofectAMINE2000 reagent (Invitrogen). After successive cultivation in DMEM for 24 h, the cells were washed with PBS and lysed by sonication in lysis buffer containing 50 mM Tris–HCl (pH 7.5), 120 mM NaCl, 5 mM EDTA, and 0.5% NP40. The soluble fraction collected by microcentrifugation was precleared by incubation with protein A/G agarose beads (Roche). After the cleared supernatant had been incubated for 1 h at 4 °C with 4 μg of an anti-FLAG M2 antibody (Sigma–Aldrich Inc.) for HDAC1 and HDAC6, the agarose beads were washed three times with lysis buffer and once with histone deacetylase buffer consisting of 20 mM Tris–HCl (pH 8.0), 150 mM NaCl, and 10% glycerol. The bound proteins were released from the immune complex by incubation for 1 h at 4 °C with 40 μg of the FLAG peptide (Sigma–Aldrich Inc.) in histone deacetylase buffer (200 μL). The supernatant was collected by centrifugation. For the enzyme assay, 10 μL of the enzyme fraction was added to 1 μL of fluorescent substrate (2 mM Ac-KGLGK(Ac)-MCA) and 9 μL of histone deacetylase buffer, and the mixture was incubated at 37 °C for 30 min. The reaction was stopped by the addition of 30 μL of trypsin (20 mg/mL) and incubated at 37 °C for 15 min. The released aminomethylcoumarin (AMC) was measured using a fluorescence plate reader. The 50% inhibitory concentrations (IC_{50}) were determined as the means with SD calculated from at least three independent dose–response curves.

4.2.2. Cell-growth inhibition assay [19]

This experiment was carried out at the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research. The screening panel consisted of the following 39 human cancer cell lines (HCC panel): breast cancer HBC-4, BSY-1, HBC-5, MCF-7, and MDA-MB-231; brain cancer U-251, SF-268, SF-295, SF-539, SNB-75, and SNB-78; colon cancer HCC2998, KM-12, HT-29, HCT-15, and HCT-116; lung cancer NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273, and DMS114; melanoma LOX-IMVI; ovarian cancer OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; renal cancer RXF-631L and ACHN; stomach cancer St-4, MKN1, MKN7, MKN28, MKN45, and MKN74; prostate cancer DU-145 and PC-3. The GI_{50} (50% cell-growth inhibition) value for these cell lines was determined by using the sulforhodamine B colorimetric method.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.12.023.

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