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Original article

Total synthesis of burkholdacs A and B and 5,6,20-tri-*epi*-burkholdac A: HDAC inhibition and antiproliferative activity

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ABSTRACT

The bicyclic depsipeptide histone deacetylase (HDAC) inhibitors burkholdacs A and B were efficiently synthesized in a highly convergent and unified manner. The synthesis features the amide coupling of a D-valine-D-cysteine- or D-*allo*-isoleucine-D-cysteine-containing segment with a D-methionine-containing segment to directly assemble the corresponding *seco*-acids, key precursors for macrolactonization. Using the same methodology, 5,6,20-tri-*epi*-burkholdac A was also synthesized. HDAC inhibitory assays and cell-growth inhibition analyses of the synthesized depsipeptides demonstrated the potency order of this class of bicyclic depsipeptides as compared to the clinically approved depsipeptide FK228 (romidepsin). Novel structure–activity relationships within this class of compounds were also revealed.

1. Introduction

In 2011, Brady et al. reported the isolation and structural elucidation of the two new bicyclic depsipeptide histone deacetylase (HDAC) inhibitors, burkholdacs A (**1**) and B (**2**) (Fig. 1) [1]. These compounds were identified through the systematic overexpression of transcription factors associated with natural product gene clusters encoded within *Burkholderia thilandensis* E264 [1]. The stereochemistry at the C5, C6 and C20 positions could not be unambiguously assigned at that time. The stereostructures of **1** and **2** were proposed as compounds **3** and **4** in Fig. 1, respectively, based on the biosynthesis gene cluster that contains only one epimerase domain [1]. Through two independent total syntheses by Ganesan et al. in 2011 [2] and by Ye et al. in 2012 [3], the stereochemistry of the two new HDAC inhibitors, which includes their absolute configurations, was determined, as shown in structures **1** and **2**.

In the course of our continuing research on the synthesis and biological evaluation of bicyclic depsipeptide HDAC inhibitors including FK228 (romidepsin) (**5**) [4] and the spiruchostatins A (**6**) [4,5], B (**7**) [4,6], C (**8**) [7] and D (**9**) [7], we became interested in the synthesis of **1** and **2** and their analogues with the aim of identifying novel mechanism-based anticancer agents [8]. Several total syntheses of **5**–**7** by other groups have been published in the literature [9]. In this study, we describe our total synthesis of **1** and **2** and the earlier proposed stereostructure **3** by applying a synthetic strategy developed previously in our laboratory [4–7]. The synthesized compounds **1**–**3** were subjected to HDAC inhibition assays and antiproliferative analyses to establish the potency order for this class of bicyclic depsipeptide HDAC inhibitors (**1**–**3** and **5**–**9**).

2. Results and discussion

2.1. Chemistry

2.1.1. Synthetic plan for burkholdacs A (*1*) and B (*2*)

Our synthetic plan for **1** and **2** is outlined in Scheme 1. We envisioned that target molecules **1** and **2** could be synthesized via the macrolactonization of the corresponding *seco*-acids **10** and **11**, followed by disulfide bond formation. The key macrolactonization

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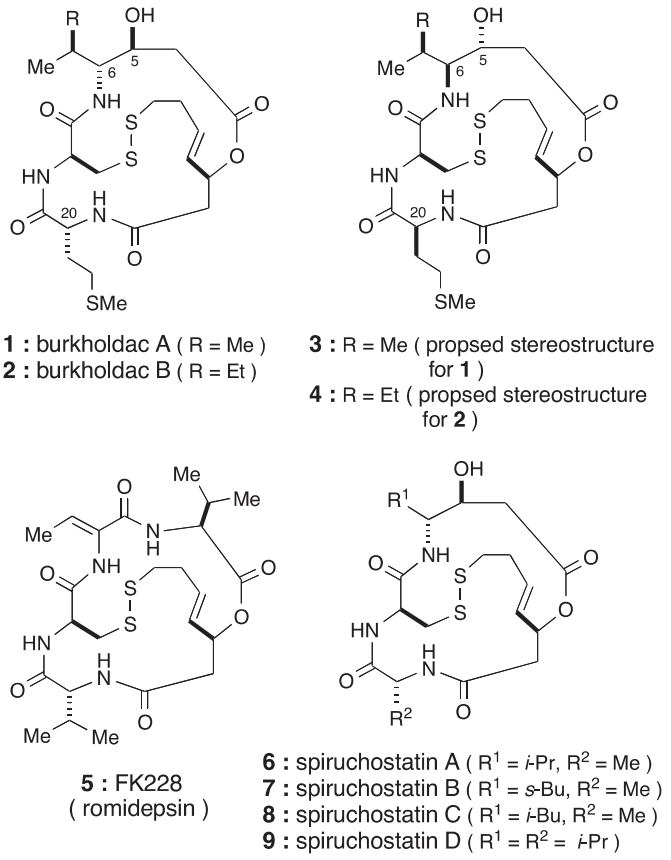


Fig. 1. Revised (**1**, **2**) and Brady's original (**3**, **4**) structures of burkholdacs A and B, FK228 (romidepsin) (**5**) and spiruchostatins A–D (**6**–**9**). *i*-Pr = isopropyl, *s*-Bu = *sec*-butyl, *i*-Bu = isobutyl.

precursors **10** and **11** could be prepared through the direct coupling of the D-valine-D-cysteine-containing segment **12** and D-*allo*-isoleucine-D-cysteine-containing segment **13**, respectively, with the D-methionine-containing segment **14**. The segment **14** was to be formed by condensation of commercially available D-methionine methyl ester (**15**) with the known carboxylic acid **16** (previously prepared from L-malic acid in our laboratory [4–6]). The two segments **12** and **13** were obtained as previously described in our total synthesis of **6** and **7** [4–6].

2.1.2. Synthesis of burkholdac A (*1*)

We initially pursued the synthesis of **1**. For the synthesis, the key segment **14** was efficiently prepared, as shown in Scheme 2. The condensation of **15** with **16** [4–6] afforded the desired coupling product **17** in excellent yield. Subsequent saponification of the methyl ester moiety in **17** furnished the requisite segment **14** in quantitative yield.

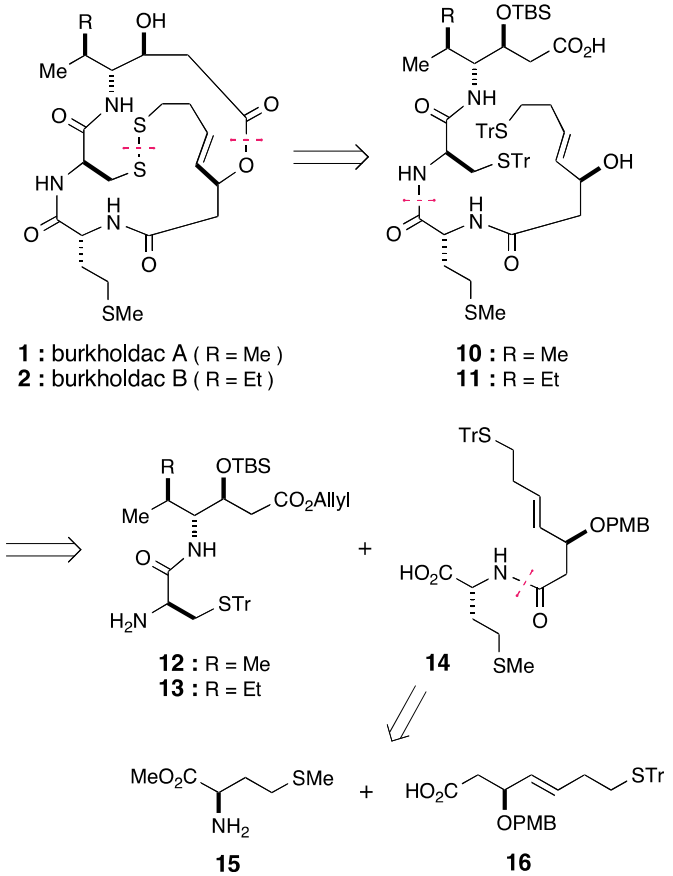
Subsequently, the synthesis of **1** was accomplished by assembling the two segments **12** [4,5] and **14**, as shown in Scheme 3. Thus, treatment of **12** and **14** with HATU (1.3 equiv) and HOAt (1.3 equiv) in the presence of *i*-Pr_2NEt (2.6 equiv) in CH_2Cl_2 at −30 °C for 3 h afforded the desired condensation product **18** in 83% yield without appreciable epimerization at the C20 stereogenic centre (burkholdacs numbering). The condensation product **18** was then converted to the requisite *seco*-acid **10** with an overall yield of 74% using alcohol **19** via the successive removal of both the PMB- and allyl-protecting groups. Notably, the sensitive methyl sulfide function present in the C20 side chain remained intact during the oxidative deprotection step (cf. DDQ, CH_2Cl_2/H_2O, rt, 3 h). The critical macrolactonization of **10** was successfully achieved by employing the Shiina method [10] [MNBA (1.3 equiv), DMAP (3.0 equiv), CH_2Cl_2 (10 mM), rt, 12 h], which afforded the expected cyclization product **20** in 79% yield. Subsequent disulfide bond formation accompanied by oxidative *S*-Tr deprotection of **20** was efficiently achieved by brief exposure to I_2 in a dilute CH_2Cl_2/MeOH solution (0.5 mM) at ambient temperature, which resulted in the production of the desired disulfide **21** in 81% yield. Finally, deprotection of the TBS group in **21** (89%) led to the completion of the total synthesis of **1**. The spectroscopic properties (IR, ^1H and ^{13}C NMR, and MS) of the synthesized sample **1** were identical to those reported for natural **1** [1].

2.1.3. Synthesis of burkholdac B (*2*)

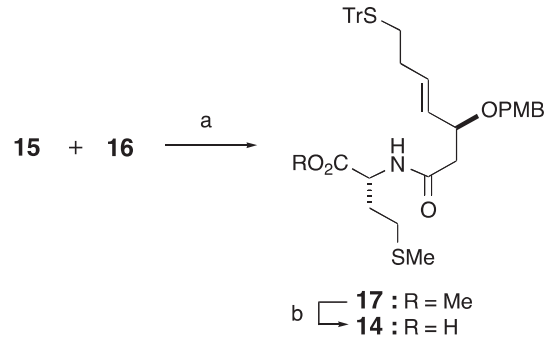
As shown in Scheme 4, using segments **13** [4,6] and **14** as the starting materials, **2** was synthesized in a manner similar to that described for the synthesis of **1** (cf. Scheme 3). The spectroscopic properties (IR, ^1H and ^{13}C NMR, and MS) of the synthesized sample **2** were identical with those reported for natural **2** [1].

2.1.4. Synthesis of 5,6,20-tri-epi-burkholdac A (*3*)

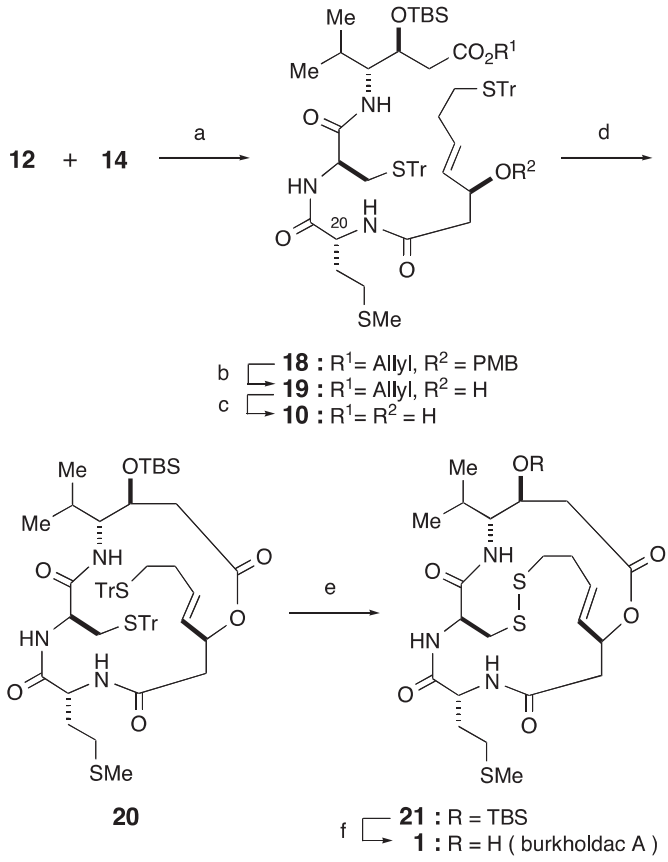
Moreover, we synthesized the Brady's proposed stereoisomer, 5,6,20-tri-*epi*-burkholdac A (**3**), to assess its biological activity. Using *N*-Boc-L-valinal (**26**) [11] as the starting material, the key segment **35** was synthesized according to a method developed previously in our laboratory [4,5] (Scheme 5). The other key segment **37** was readily prepared via the condensation of commercially available L-methionine methyl ester (*ent*-**15**) with



Scheme 1. Synthetic plan for burkholdacs A (**1**) and B (**2**). TBS = *tert*-butyldimethylsilyl, Tr (trityl) = triphenylmethyl, PMB = 4-methoxybenzyl.



Scheme 2. Synthesis of segment **14**. (a) PyBOP, *i*-Pr_2NEt, MeCN, rt, 99%; (b) LiOH, MeOH, rt, 100%. PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.



Scheme 3. Synthesis of burkholdac A (**1**). (a) HATU, HOAt, *i*-Pr_2NEt, CH_2Cl_2, −30 °C, 83%; (b) DDQ, CH_2Cl_2/H_2O, rt, 87%; c) $Pd(PPh_3)_4$, morpholine, THF, rt, 85%; (d) MNBA, DMAP, CH_2Cl_2, rt, 79%; (e) I_2, CH_2Cl_2/MeOH, rt, 81%; (f) HF·pyridine, pyridine, rt, 89%. 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.

carboxylic acid **16** (Scheme 6). The final target molecule **3** was synthesized, as shown in Scheme 7, through condensation of the two segments **35** and **37** in a manner similar to that described for the synthesis of **1** (cf. Schemes 2 and 3).

2.2. Biological evaluation

The synthesized compounds **1**–**3** were evaluated for their HDAC inhibitory activity and their cell-growth inhibitory activity to determine the order of their potency and reveal some novel aspects of structure–activity relationships (SARs) within this class of compounds.

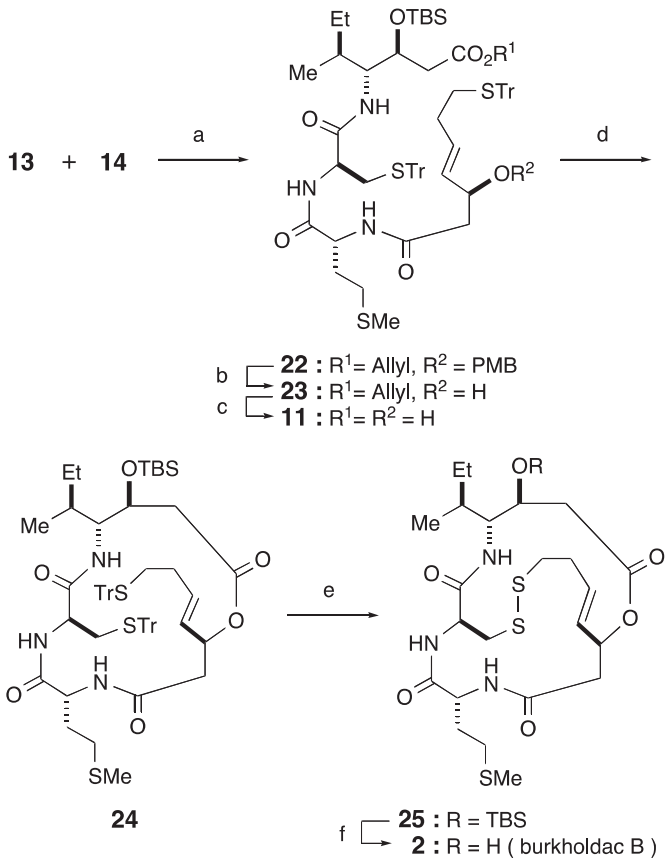
2.2.1. HDAC inhibition assay

HDAC enzymes are zinc metalloenzymes that catalyze the hydrolysis of acetylated lysine residues on proteins, particularly histones [12]. There are 18 human HDAC isoforms, which are grouped into the following four major classes: class I (HDACs 1, 2, 3 and 8), class II (class IIa: HDACs 4, 5, 7 and 9; 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 [13]. The inhibition of class I HDACs is considered to be a useful mechanism for anticancer agents, whereas the inhibition of class IIb HDACs may cause undesirable side effects such as serious cardiac hypertrophy [14]. In 2009, FK228 has been approved by the Food and Drug Administration in USA for treatment of cutaneous T-cell lymphoma [15]. However, this anticancer agent is associated with an unresolved cardiotoxicity that probably arises from insufficient class selectivity [15,16]. Therefore, the potent and selective inhibition of class I enzymes is highly desirable in next-generation cancer chemotherapy [17].

Compounds **1**–**3** were tested for their HDAC inhibitory activity against HDAC1 (class I) and HDAC6 (class IIb) enzymes to determine their degree of potency and isoform selectivity. In this assay, FK228 was used as a positive control. As summarized in Table 1, **1** and **2** exhibited extremely potent inhibitory activity against HDAC1 in the low nanomolar range (IC_{50} = 1.0–1.2 nM). Their potencies were observed to be approximately three-fold higher than that of FK228 (IC_{50} = 3.6 nM). In contrast, the unnatural stereoisomer **3** exhibited much lower inhibitory activity (IC_{50} = 336.6 nM) than the natural products (**1**: IC_{50} = 1.0 nM, **2**: IC_{50} = 1.2 nM), suggesting that the stereochemistry at the C5, C6 and C20 positions plays a key role in determining the pronounced HDAC1 inhibition efficacy. Combined with our previous data for spiruchostatins A (**6**), B (**7**), C (**8**) and D (**9**) [4,7], the potency order was estimated to be **9** (IC_{50} = 0.75 nM) ≥ **8** (IC_{50} = 0.93 nM) ≈ **1** (IC_{50} = 1.0 nM) ≥ **2** (IC_{50} = 1.2 nM) > **7** (IC_{50} = 2.2 nM) > **6** (IC_{50} = 3.3 nM) ≥ FK228 (**5**) (IC_{50} = 3.6 nM). As expected for HDAC6 inhibitory activity, both **1** and **2** were essentially inactive (**1**: IC_{50} = 1975 nM, **2**: IC_{50} = 992 nM). Isoform selectivity (class I/IIb) is expressed, for convenience, as a selective index (SI) value (HDAC6 IC_{50}/HDAC1 IC_{50}). Along with our previous data [4,7], the order of the selectivity was then determined to be **1** (SI = 1645) > **2** (SI = 992) > **7** (SI = 636) > **6** (SI = 485) > **8** (SI = 346) ≥ **9** (SI = 320) > FK228 (**5**) (SI = 108). Notably, the isoform selectivity of **1** and **2** is nine-to-fifteen-fold higher than that of FK228, which represents, to the best of our knowledge, the highest level of class I/II selectivity among the naturally occurring bicyclic depsipeptides known to date. In addition, a good correlation between enzymatic (HDAC1) activity and cellular HDAC inhibition efficacy [expressed as the EC_{10000} (effective concentration for 100 times increased induction) value measured via the p21 promoter assay] was determined for all of the compounds. From these results, it was revealed that structural alteration of the side chain at the C20 position is quite effective for improving the isoform selectivity without loss of the potent HDAC inhibitory activity.

2.2.2. Cell-growth inhibition assay

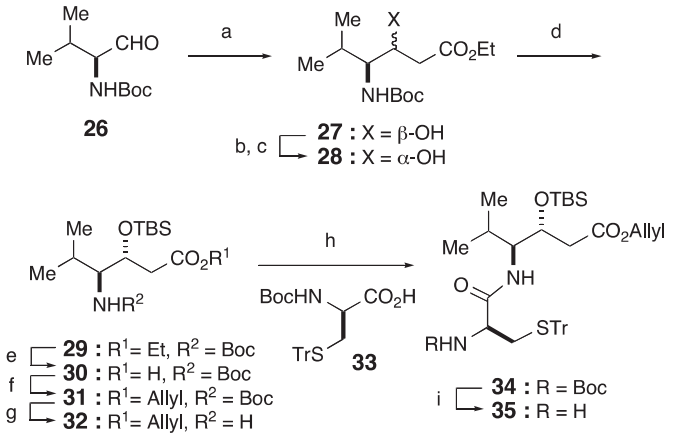
Next, the growth–inhibitory activity of compounds **1**–**3** was evaluated at the Japanese Foundation for Cancer Research simultaneously with FK228 as a reference compound using a panel of 39 human cancer cell lines [18]. The number of cell lines and their origin (organ) are as follows: 5 breast, 6 central nervous system (brain), 1 melanoma, 5 ovary, 2 kidney, 6 stomach and 2 prostate.



Scheme 4. Synthesis of burkholdac B (**2**). (a) HATU, HOAt, *i*-Pr_2NEt, CH_2Cl_2, −30 °C, 92%; (b) DDQ, CH_2Cl_2/H_2O, rt, 82%; (c) $Pd(PPh_3)_4$, morpholine, THF, rt, 94%; (d) MNBA, DMAP, CH_2Cl_2, rt, 81%; (e) I_2, CH_2Cl_2/MeOH, rt, 79%; (f) HF·pyridine, pyridine, rt, 97%.

Dose-response curves were measured at five different concentrations (from 10^{-10} to 10^{-6} M or from 10^{-8} to 10^{-4} M) for each compound, and the concentration causing 50% cell growth inhibition (GI_{50}) was compared with that of the control.

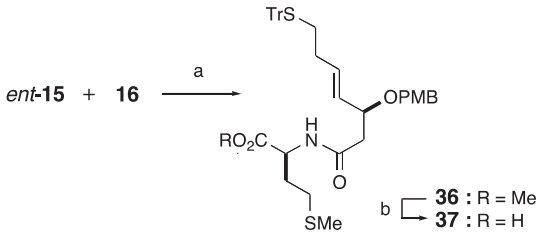
The GI_{50} values of the tested compounds **1**–**3** along with those for FK228 are shown in Table 2. Compounds **1** and **2** exhibited extremely potent growth–inhibitory activity against nearly all of the 39 cell lines in the sub-nanomolar to nanomolar range. Notably, the efficacy of **1** and **2** was superior to that of FK228 on brain cancer (SF-539, **1**: GI_{50} = 0.72 nM, **2**: GI_{50} = 0.70 nM), colon cancer (HCC2998, **1**: GI_{50} = 0.67 nM; KM-12, **1**: GI_{50} = 0.76 nM; HT-29, **1**: GI_{50} = 0.65 nM, **2**: GI_{50} = 0.74 nM; HCT-116, **1**: GI_{50} = 0.51 nM, **2**: GI_{50} = 0.51 nM), lung cancer (NCI-H23, **1**: GI_{50} = 0.64 nM; NCI-H226, **1**: GI_{50} = 0.47 nM; NCI-H522, **1**: GI_{50} = 0.20 nM, **2**: GI_{50} = 0.42 nM; NCI-H460, **1**: GI_{50} = 0.91 nM, **2**: GI_{50} = 0.53 nM; A549, **1**: GI_{50} = 0.57 nM, **2**: GI_{50} = 0.85 nM; DMS114, **1**: GI_{50} = 0.97 nM), melanoma cancer (LOX-IMVI, **1**: GI_{50} = 0.50 nM), ovary cancer (OVCAR-8, **1**: GI_{50} = 0.68 nM, **2**: GI_{50} = 0.72 nM; SK-OV-3, **1**: GI_{50} = 0.78 nM), stomach cancer (MKN1, **1**: GI_{50} = 0.67 nM) and prostate cancer (DU-145, **1**: GI_{50} = 0.82 nM) cells. In contrast, **3** exhibited fairly decreased activity against all of the 39 cell lines (GI_{50} = 230–15000 nM). The potency order of the bicyclic depsipeptides, including **5**–**9** (based on our previous data [4,7]), was estimated using the MG-MID value (mean value of GI_{50} over all of the cell lines tested) to be **1** (2.1 nM) ≥ **2** (3.2 nM) ≥ **9** (3.8 nM) > **7** (5.6 nM) ≈ FK228 (**5**) (6.2 nM) > **6** (15 nM) > **8** (28 nM). These results indicate that the antiproliferative activity of **1** and **2** is two-to-three-fold higher than that of FK228. Considering both the HDAC inhibitory and antiproliferative activities, **1** and **2** seem to be promising candidates for the development of novel anticancer agents with higher efficacy and lower toxicity.



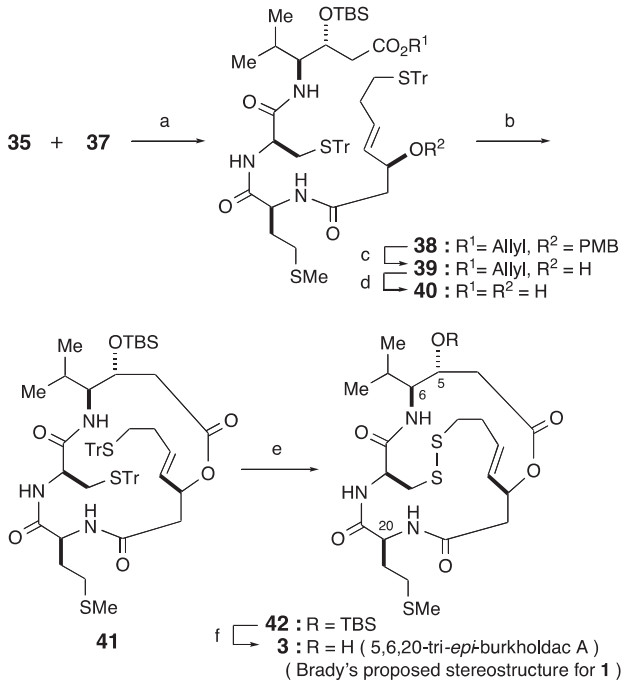
Scheme 5. Synthesis of segment **35**. (a) LDA, CH_3CO_2Et, THF, −78 °C; at −78 °C, add. **26**, 57% for **27**, 30% for **28** (**27**/**28** 2:1); (b) Jones reagent, acetone, 0 °C to rt, 86%; (c) KBH_4, MeOH, −40 °C, 79% for **28**, 5% for **27** (**28**/**27** 16:1); (d) TBSCl, imidazole, DMF, rt, 99%; (e) 1 M NaOH, EtOH, rt; (f) allyl bromide, K_2CO_3, DMF, rt, 81% (2 steps); (g) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt; MeOH, rt; (h) **33**, PyBOP, *i*-Pr_2NEt, MeCN, rt, 68% (2 steps); (i) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt; MeOH, rt; 98%. LDA = lithium diisopropylamide, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

3. Conclusion

The total synthesis of burkholdacs A (**1**) and B (**2**) and 5,6,20-tri-*epi*-burkholdac A (**3**) was achieved in a highly convergent and



Scheme 6. Synthesis of segment **37**. (a) PyBOP, *i*-Pr_2NEt, CH_2Cl_2, 0 °C to rt, 81%; (b) 1 M LiOH, MeOH, rt, 98%.



Scheme 7. Synthesis of 5,6,20-tri-*epi*-burkholdac A (**3**) (Brady's proposed stereostructure for **1**). (a) HATU, HOAt, *i*-Pr_2NEt, CH_2Cl_2, −30 °C, 81%; (b) DDQ, CH_2Cl_2/H_2O, rt, 83%; (c) $Pd(PPh_3)_4$, morpholine, THF, rt, 74%; (d) MNBA, DMAP, CH_2Cl_2, rt, 84%; (e) I_2, CH_2Cl_2/MeOH, rt, 85%; (f) HF·pyridine, pyridine, rt, 89%.

unified manner. Using the results of a preliminary biological evaluation of the synthesized compounds **1**–**3** and our previously obtained data for FK228 (**5**) and the spiruchostatins A–D (**6**–**9**), the order of the efficacy of the naturally occurring bicyclic depsipeptide HDAC inhibitors was established. Notably, the burkholdacs (**1** and **2**) were determined to be superior to **5** with respect to their HDAC1 inhibitory activity, isoform selectivity towards HDAC1 over HDAC6 and antiproliferative activity. These results should be useful for the design and development of anticancer agents with therapeutic potential that target the isoform-selective inhibition of HDACs. We are currently synthesizing additional analogues of burkholdacs with the aim of exploring their SARs. In addition, further investigation of the activity of the synthetic samples using animal models is in progress.

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

Table 1
HDAC inhibitory activity of burkholdacs A (**4**) and B (**5**) and 5,6,20-tri-*epi*-burkholdac A (**3**).

Compound	IC_{50}[a] (nM)			EC_{10000}[e] (nM)
	HDAC1 (class I)[b]	HDAC6 (class IIb)[b]	SI[d]	Cell HDAC
1	1.2	1975	1645	1.4
2	1.0	992	992	0.76
3	336.6	>10,000	–	>200
FK228[c]	3.6	390	108	3.8

[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 [4].
[d] Selectivity index (HDAC6 IC_{50}/HDAC1 IC_{50}) as the selectivity towards class I HDAC1 over class IIb HDAC6.
[e] The concentration that induces luciferase activity 100-fold higher than basal level.

Table 2
Growth inhibition of burkholdacs A (**1**) and B (**2**) and 5,6,20-tri-*epi*-burukholdac A (**3**) against a panel of 39 human cancer cell lines.

Origin of cancer	Cell line	GI_{50}[a] (nM)			FK228[b]
		1	**2**	**3**	
Breast	HBC-4	3.0	3.8	1300	6.9
	BSY-1	4.0	5.0	1600	8.5
	HBC-5	7.8	2.3	1900	13
	MCF-7	2.0	2.8	1800	4.2
	MDA-MB-231	1.8	2.6	1800	5.5
Central nervous system (Brain)					
	U-251	2.2	4.4	410	3.9
	SF-268	1.8	4.1	960	4.9
	SF-295	3.0	2.3	1600	4.0
	SF-539	0.72	0.70	1800	3.6
	SNB-75	3.6	1.4	1000	7.2
	SNB-78	460[c]	11	1700	9.6
Colon	HCC2998	0.67	2.6	1700	3.1
	KM-12	0.76	3.6	1600	3.4
	HT-29	0.65	0.74	1400	3.3
	HCT-15	300	100[c]	15,000[c]	450c
	HCT-116	0.51	0.51	540	3.1
Lung	NCI-H23	0.64	3.3	1600	4.6
	NCI-H226	0.47	2.7	1700	8.9
	NCI-H522	0.20[d]	0.42[d]	360	1.8d
	NCI-H460	0.91	0.53	1800	3.0
	A549	0.57	0.85	1400	2.6
	DMS273	3.0	2.7	2300	5.8
	DMS114	0.97	3.2	1300	3.6
Melanoma ovary	LOX-IMVI	0.50	2.5	230[d]	2.5
	OVCAR-3	2.2	2.8	1800	4.6
	OVCAR-4	5.8	5.0	1800	20
	OVCAR-5	1.0	1.6	1300	2.8
	OVCAR-8	0.68	0.72	1900	5.5
	SK-OV-3	0.78	1.9	1700	3.3
Kidney	RXF-631L	1.3	2.3	4000	6.6
	ACHN	3.4	6.6	4400	20
Stomach	St-4	8.1	1.1	3400	22
	MKN1	0.67	2.5	1400	3.2
	MKN7	4.1	10	2100	4.9
	MKN28	8.5	16	1900	17
	MKN45	7.6	14	3700	14
	MKN74	1.8	2.9	1500	3.0
Prostate	DU-145	0.82	3.1	1500	6.0
	PC-3	4.4	8.3	1600	18
MG-MID[e]		2.1	3.2	1600	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 [4].
[c] The least sensitive cell.
[d] The most sensitive cell.
[e] Mean value of GI_{50} over all cell lines tested.

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. (R)-Methyl 2-[(S,E)-3-(4-methoxybenzyloxy)-7-(tritylthio) hept-4-enamide]-4- (methylthio)butanoate (**17**)

i-Pr_2NEt (0.32 mL, 1.9 mmol) was added dropwise to a stirred solution of **16** (146 mg, 0.27 mmol) and D-methionine methyl ester (**15**) (108 mg, 0.54 mmol) in MeCN (15 mL) containing (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (281 mg, 0.54 mmol) at 0 °C under argon, and stirring was continued for 2 h at room temperature. The reaction mixture was diluted with EtOAc (120 mL), and the organic layer was washed successively with 10% aqueous HCl (2 × 40 mL), 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 2:1) to give **17** (183 mg, 99%) as a colorless oil. [α]25D −15.2 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.82–1.91 (1H, m), 1.99–2.17 (3H, m), 2.02 (3H, s), 2.21–2.25 (2H, m), 2.36–2.43 (3H, m), 2.51 (1H, dd, *J* = 8.8, 15.1 Hz), 3.73 (3H, s), 3.79 (3H, s), 4.10 (1H, dt, *J* = 2.9, 8.3 Hz), 4.31 (1H, d, *J* = 11.2 Hz), 4.51 (1H, d, *J* = 10.7 Hz), 4.65–4.70 (1H, m), 5.32 (1H, dd, *J* = 7.8, 15.6 Hz), 5.59 (1H, dt, *J* = 6.8, 15.1 Hz), 6.83 (2H, d, *J* = 8.3 Hz), 7.01 (1H, d, *J* = 8.3 Hz), 7.19–7.45 (17H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4, 29.9, 31.2, 31.4, 31.8, 42.7, 51.3, 52.3, 55.3, 66.6, 70.0, 76.7, 113.8 (3C), 126.6 (2C), 127.9 (6C), 129.6 (6C), 129.7 (2C), 129.9, 130.3, 133.0, 144.9 (3C), 159.3, 170.6, 172.4; IR (neat): 3438, 1734, 1647, 1514, 1443, 1363, 1300, 1248, 1174, 1077, 1032, 972 cm^{-1}; HRMS (EI): *m/z* calcd for $C_{40}H_{45}NO_5S_2$ (M^+) 683.2739, found 683.2741.

4.1.2. (R)-2-[(S,E)-3-(4-Methoxybenzyloxy)-7-(tritylthio)hept-4-enamide]-4-(methylthio)butanoic acid (**14**)

1 M LiOH (0.64 mL, 0.64 mmol) was added dropwise to a stirred solution of **17** (110 mg, 0.16 mmol) in MeOH (3.2 mL) at room temperature. After 3 h, 10% aqueous HCl was added to the mixture at 0 °C until the pH was 6. The resulting mixture was extracted with EtOAc (3 × 20 mL), and the combined extracts were washed with 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 ($CHCl_3$/MeOH 10:1) to give **14** (107 mg, 100%) as a white amorphous solid. [α]25D −3.2 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.84–1.91 (1H, m), 1.99 (3H, s), 2.10–2.24 (5H, m), 2.40–2.52 (4H, m), 3.76 (3H, s), 4.07–4.12 (1H, m), 4.26 (1H, d, *J* = 10.7 Hz), 4.41 (1H, d, *J* = 10.3 Hz), 4.57–4.61 (1H, m), 5.29 (1H, dd, *J* = 7.3, 15.4 Hz), 5.57 (1H, dt, *J* = 6.8, 15.1 Hz), 6.80–6.84 (3H, m), 7.15–7.4 (17H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.2, 29.8, 31.2, 31.3, 42.3, 51.8, 55.2, 66.5, 66.9, 70.0, 76.4, 113.8 (3C), 126.5 (2C), 127.8 (6C), 128.5, 129.6 (6C), 129.7 (2C), 130.0, 133.1, 144.8 (3C), 159.2, 171.7, 175.2; IR (neat): 3358, 3085, 2916, 1729, 1644, 1613, 1514, 1443, 1248, 1175, 1077, 1034, 970, 748, 701 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{39}H_{43}NO_5S_2Na$ ($M^+ + Na$) 692.2480, found 692.2454.

4.1.3. (3S,4R)-Allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-(4-methoxybenzyloxy)-7-(tritylthio)hept-4-enoylamino]-4-(methylthio)butanoylamino]-3-tritylthio(propionylamino)}-5-methylhexanoate (**18**)

i-Pr_2NEt (71 μL, 0.42 mmol) was added dropwise to a stirred solution of **12** (106 mg, 0.16 mmol) and **14** (107 mg, 0.16 mmol) in CH_2Cl_2 (3.2 mL) containing *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) (79.1 mg, 0.21 mmol) and 1-hydroxy-7-azabenzotriazole (HOAt) (28.3 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 **18** (175 mg, 83%) as a colorless viscous liquid. [α]25D +10.8 (*c* 1.02, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.01 (3H, s), 0.05 (3H, s), 0.79–0.84 (6H, m), 0.82 (9H, s), 1.66–1.73 (1H, m), 1.88–1.98 (2H, m), 1.97 (3H, s), 2.05–2.23 (4H, m), 2.30–2.45 (5H, m), 2.47–2.55 (2H, m), 2.73 (1H, dd, *J* = 8.3, 13.2 Hz), 3.74–3.80 (1H, m), 3.79 (3H, s), 3.87 (1H, dd, *J* = 5.4, 7.8 Hz), 4.01 (1H, dt, *J* = 3.4, 8.1 Hz), 4.09–4.17 (1H, m), 4.15 (1H, d, *J* = 10.2 Hz), 4.35–4.42 (1H, m), 4.37 (1H, d, *J* = 10.7 Hz), 4.45–4.55 (2H, m), 5.18–5.31 (3H, m), 5.45 (1H, dt, *J* = 6.8, 15.4 Hz), 5.82–5.92 (1H, m), 5.99 (1H, d, *J* = 10.2 Hz), 6.63 (1H, d, *J* = 7.3 Hz), 6.83 (2H, d, *J* = 8.8 Hz), 7.05 (1H, d, *J* = 7.3 Hz), 7.13–7.44 (32H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.6, 15.1, 16.8, 17.9, 20.4, 25.8 (3C), 27.9, 30.1, 30.6, 31.3, 31.4, 33.0, 39.6, 42.5, 52.1, 52.6, 55.3, 58.3, 65.3, 66.6, 67.1, 69.5, 70.1, 76.5, 114.0 (3C), 118.4 (3C), 126.6 (6C), 126.8 (2C), 127.9 (6C), 128.1 (6C), 128.7, 129.5 (6C), 129.7, 129.8 (2C), 130.0, 132.1, 133.1, 144.3 (3C), 144.8 (3C), 159.4, 169.7, 171.21, 171.22, 171.6; IR (neat): 3435, 2929, 2855, 1683, 1638, 1556, 1541, 1515, 1248, 1171, 1082, 1035, 828, 744, 701 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{77}H_{94}N_3O_8S_3Si$ ($M^+ + H$) 1312.5972, found 1312.5957.

4.1.4. (3S,4R)-Allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-hydroxy-7-(tritylthio)hept-4-enoylamino]-4-(methylthio) butanoylamino]-3-tritylthio(propionylamino)}-5-methylhexanoate (**19**)

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (64 mg, 0.23 mmol) was added in small portions to a stirred solution of **18** (146 mg, 0.11 mmol) in CH_2Cl_2/H_2O 9:1 (11 mL) at room temperature. After 3 h, the 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 **19** (114 mg, 87%) as a colorless viscous liquid. [α]25D +6.4 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.05 (3H, s), 0.79–0.84 (6H, m), 0.81 (9H, s), 1.86–1.97 (2H, m), 2.02–2.10 (3H, m), 2.06 (3H, s), 2.17–2.23 (3H, m), 2.30–2.33 (1H, m), 2.40 (1H, dd, *J* = 7.3, 15.6 Hz), 2.51–2.55 (3H, m), 2.63 (1H, dd, *J* = 8.3, 12.9 Hz), 3.30 (1H, br s), 3.89–3.97 (1H, m), 3.92–3.97 (1H, m), 4.14–4.18 (1H, m), 4.33–4.37 (1H, m), 4.42–4.54 (3H, m), 5.19–5.31 (2H, m), 5.36 (1H, dd, *J* = 6.3, 15.4 Hz), 5.49 (1H, dt, *J* = 6.8, 15.1 Hz), 5.81–5.91 (2H, m), 6.49 (1H, d, *J* = 7.8 Hz), 7.14–7.45 (32H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.7, 15.4, 16.9, 17.9, 20.2, 25.7 (3C), 28.0, 30.3, 30.4, 31.25, 31.33, 33.2, 39.4, 44.1, 52.6, 53.1, 58.5, 65.3, 66.6, 67.0, 69.3, 69.8, 118.4, 126.6 (3C), 126.8 (3C), 127.8 (6C), 128.0 (6C), 129.48 (6C), 129.53 (6C), 129.9, 132.0, 132.5, 144.3 (3C), 144.8 (3C), 170.0, 170.9,

171.5, 171.6; IR (neat): 3438, 2958, 2930, 2854, 1736, 1635, 1550, 1489, 1443, 1252, 1181, 1036, 828, 741, 701 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{69}H_{86}N_3O_7S_3Si$ (M^+ + H) 1192.5397, found 1192.5396.

4.1.5. (3S,4R)-3-(Tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-hydroxy-7-(tritylthio)hept-4-enoylamino]-4-(methylthio) butanoylamino]-3-tritylthio(propionylamino)}-5-methylhexanoic acid (**10**)

Morpholine (18.8 L, 0.22 mmol) was added dropwise to a stirred solution of **19** (117 mg, 97 μmol) in THF (10 mL) containing $Pd(PPh_3)_4$ (13.0 mg, 11 μmol) at room temperature under argon. After 30 min, the reaction mixture was diluted with EtOAc (30 mL). 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 **10** (98.9 mg, 85%) as a white amorphous solid. [α]25D +2.2 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.05 (6H, s), 0.80–0.87 (15H, m), 1.91–2.12 (5H, m), 2.04 (3H, s), 2.19–2.25 (3H, m), 2.33 (1H, dd, *J* = 2.9, 13.4 Hz), 2.40 (1H, dd, *J* = 4.4, 15.9 Hz), 2.49–2.53 (4H, m), 2.72 (1H, dd, *J* = 8.3, 12.4 Hz), 3.75–3.84 (1H, m), 4.07–4.13 (2H, m), 4.33–4.38 (1H, m), 4.49–4.54 (1H, m), 5.34 (1H, dd, *J* = 5.9, 15.6 Hz), 5.47 (1H, dt, *J* = 6.8, 15.1 Hz), 6.32 (1H, d, *J* = 10.3 Hz), 6.81 (1H, d, *J* = 7.3 Hz), 7.15–7.44 (31H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.5, 15.4, 16.4, 17.9, 20.3, 25.7 (3C), 27.7, 29.7, 30.2, 31.28, 31.32, 33.2, 39.7, 44.2, 53.30, 53.34, 59.3, 66.2, 67.0, 69.0, 69.8, 126.6 (3C), 126.9 (3C), 127.9 (6C), 128.1 (6C), 129.5 (6C), 129.6 (6C), 130.1, 132.3, 144.3 (3C), 144.8 (3C), 170.4, 171.5, 172.0, 173.9; IR (neat): 3435, 2955, 1646, 1557, 1541, 1507, 1437, 1396, 1255, 1184, 1088, 1011, 953, 742, 702 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{66}H_{82}N_3O_7S_3Si$ (M^+ + H) 1152.5084, found 1152.5062.

4.1.6. (2S,6R,9S,12R,13S)-13-(Tert-butyldimethylsilyloxy)-12-isopropyl-6-[2-(methylthio)ethyl]-2-[(E)-4-(tritylthio)but-1-enyl]-9-tritylthiomethyl-1-oxa-5,8,11-triazacyclopentadecane-4,7,10,15-tetraone (**20**)

A solution of **10** (119 mg, 0.10 mmol) in CH_2Cl_2 (10 mL) was added very slowly to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (46.3 mg, 0.14 mmol) in CH_2Cl_2 (100 mL, 1.0 mM concentration) containing 4-dimethylaminopyridine (DMAP) (38 mg, 0.31 mmol) at room temperature over 14 h. After 1 h, the mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was washed successively with saturated aqueous $NaHCO_3$ (2 × 40 mL), water (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, 1:1) to give **20** (89.0 mg, 79%) as a white amorphous solid. [α]25D −7.7 (*c* 1.01, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.08 (3H, s), 0.81–0.84 (6H, m), 0.87 (9H, s), 2.02–2.26 (7H, m), 2.06 (3H, s), 2.35–2.52 (4H, m), 2.58–2.70 (2H, m), 2.75 (1H, dd, *J* = 4.4, 12.7 Hz), 3.26–3.31 (1H, m), 3.40–3.46 (1H, m), 3.66–3.70 (1H, m), 4.08–4.12 (1H, m), 4.34–4.37 (1H, m), 5.40 (1H, dd, *J* = 6.8, 15.1 Hz), 5.56–5.60 (1H, m), 5.70 (1H, dt, *J* = 6.8, 15.1 Hz), 6.45 (1H, br s), 6.88 (1H, d, *J* = 5.9 Hz), 7.11 (1H, d, *J* = 10.3 Hz), 7.18–7.41 (30H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.1, 15.1, 15.5, 17.9, 20.5, 25.7 (3C), 27.3, 29.3, 30.3, 31.0, 31.3, 31.7, 42.0, 42.1, 52.9, 58.0, 58.9, 66.6, 66.9, 68.8, 71.5, 126.6 (3C), 126.8 (3C), 127.9 (6C), 128.0 (6C), 128.6, 129.49 (6C), 129.52 (6C), 133.3, 144.4 (3C), 144.8 (3C), 169.8, 170.16, 170.18, 171.8; IR (neat): 3430, 2955, 2928, 2857, 1733, 1715, 1648, 1575, 1540, 1507, 1490, 1445, 1260, 1082, 832, 771, 747, 699 cm^{-1}; HRMS (FAB): *m/z*: calcd for $C_{66}H_{80}N_3O_6S_3Si$ (M^+ + H) 1134.4979, found 1134.4996.

4.1.7. (1S,5S,6R,9S,20R,E)-5-(Tert-butyldimethylsilyloxy)-6-isopropyl-20-[2-(methylthio)ethyl]-2-oxa-11,12-dithia-7,19,22-triazabicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (**21**)

A solution of **20** (89 mg, 78 μmol) in CH_2Cl_2/MeOH 9:1 (20 mL) was added dropwise to a vigorously stirred solution of I_2 (198 mg, 0.78 mmol) in CH_2Cl_2/MeOH 9:1 (157 mL, 0.5 mM concentration) over 10 min at room temperature. After 10 min, the reaction was quenched with 0.2 M ascorbic acid/citric acid buffer (10 mL, adjusted to pH 4.0) at room temperature, and the resulting mixture was extracted with $CHCl_3$ (3 × 50 mL). The combined extracts were washed with brine (2 × 50 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 **21** (40.8 mg, 81%) as a white amorphous solid. [α]25D +27.4 (*c* 0.81, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD): δ −0.07 (3H, s), 0.00 (3H, s), 0.72 (3H, d, *J* = 6.8 Hz), 0.74 (9H, s), 0.79 (3H, d, *J* = 6.8 Hz), 1.87–1.95 (1H, m), 1.92 (3H, s), 2.00–2.06 (1H, m), 2.19–2.25 (1H, m), 2.37–2.61 (6H, m), 2.67–2.75 (2H, m), 2.79–2.83 (2H, m), 3.01–3.24 (3H, m), 4.06–4.09 (1H, m), 4.59–4.62 (2H, m), 5.46–5.47 (1H, m), 5.73–5.85 (2H, m), 6.84 (1H, d, *J* = 8.8 Hz), 7.47 (1H, d, *J* = 7.3 Hz), 8.38 (1H, br s); ^{13}C NMR (100 MHz, CD_3OD): δ −4.7, −4.0, 15.1, 18.1, 18.9, 21.6, 26.3 (3C), 30.5, 31.0, 31.3, 33.4, 41.4, 41.6, 42.1, 56.7, 57.3, 63.9, 69.1, 71.4, 79.5, 130.1, 132.4, 170.5, 170.9, 173.9, 174.3; IR (neat): 3342, 2957, 2928, 2856, 1744, 1662, 1542, 1432, 1257, 1158, 1137, 1080, 833, 776, 755 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{28}H_{50}N_3O_6S_3Si$ (M^+ + H) 648.2631, found 648.2629.

4.1.8. Burkholdac A (**1**)

HF·pyridine (2.5 mL) was added to a stirred solution of **21** (32.0 mg, 49 μmol) in pyridine (5 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 **1** (23.4 mg, 89%) as a white amorphous solid. [α]25D −57.3 (*c* 0.16, MeOH), {lit [3]. [α]20D −54 (*c* 0.23 in MeOH)}. ^1H NMR (400 MHz, CD_3CN): δ 0.78 (3H, d, *J* = 6.3 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 1.92–1.99 (1H, m), 2.02 (3H, s), 2.13–2.21 (1H, m), 2.36–2.43 (1H, m), 2.48–2.75 (9H, m), 2.88 (1H, dd, *J* = 7.3, 13.2 Hz), 3.02–3.05 (1H, m), 3.14–3.24 (3H, m), 4.04–4.09 (1H, m), 4.33–4.39 (1H, m), 4.62 (1H, dt, *J* = 3.9, 9.3 Hz), 5.43–5.45 (1H, m), 5.80 (1H, d, *J* = 12.2 Hz), 6.01–6.07 (1H, m), 6.78 (1H, d, *J* = 8.8 Hz), 7.17 (1H, br s), 7.29 (1H, d, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3CN): δ 15.1, 19.7, 21.1, 30.4, 30.6, 31.2, 33.3, 40.8, 40.9 (2C), 41.7, 56.3, 56.8, 63.2, 69.1, 71.6, 131.4, 132.4, 169.9, 171.7, 171.8, 172.8; IR (neat): 3361, 3335, 2960, 2921, 2849, 1733, 1717, 1683, 1654, 1541, 1523, 1508, 1263, 1162, 1020, 983, 754 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{22}H_{36}N_3O_6S_3$ (M^+ + H) 534.1766, found 534.1769. The ^1H and ^{13}C NMR, and MS spectrum are identical with those reported for natural burkholdac A [1].

4.1.9. (3S,4R,5R)-Allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-(4- methoxybenzyloxy)-7-(tritylthio)hept-4-enoylamino]-4-(methylthio)butanoylamino]-3- tritylthio(propionylamino)}-5-methylheptanoate (**22**)

i-Pr_2NEt (0.17 L, 1.0 mmol) was added dropwise to a stirred solution of **13** (260 mg, 0.39 mmol) and **14** (258 mg, 0.39 mmol) in CH_2Cl_2 (7.7 mL) containing HATU (190 mg, 0.50 mmol) and HOAt (68.1 mg, 0.50 mmol) at −30 °C under argon. After 3 h, the reaction mixture was diluted with $CHCl_3$ (40 mL). The organic layer was washed successively with 10% aqueous HCl (2 × 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 (hexane/EtOAc 1:1)

to give **22** (471 mg, 92%) as a colorless viscous liquid. [α]25D +12.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.06 (3H, s), 0.78–0.85 (6H, m), 0.83 (9H, s), 1.01–1.12 (1H, m), 1.15–1.24 (1H, m), 1.65–1.79 (2H, m), 1.88–1.97 (1H, m), 1.97 (3H, s), 2.04–2.15 (2H, m), 2.17–2.23 (2H, m), 2.30–2.47 (5H, m), 2.50–2.55 (2H, m), 2.73 (1H, dd, *J* = 8.3, 13.2 Hz), 3.78 (3H, s), 3.84–3.94 (2H, m), 4.00 (1H, dt, *J* = 3.4, 8.3 Hz), 4.10–4.17 (1H, m), 4.14 (1H, d, *J* = 10.7 Hz), 4.35–4.42 (1H, m), 4.37 (1H, d, *J* = 11.2 Hz), 4.43–4.54 (2H, m), 5.17–5.30 (3H, m), 5.44 (1H, dt, *J* = 6.8, 15.1 Hz), 5.81–5.91 (1H, m), 5.99 (1H, d, *J* = 10.2 Hz), 6.61 (1H, d, *J* = 7.8 Hz), 6.83 (2H, d, *J* = 8.3 Hz), 7.04 (1H, d, *J* = 7.3 Hz), 7.13–7.44 (32H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.5, 11.7, 13.6, 15.0, 17.9, 20.4, 25.7 (3C), 27.2, 30.0, 30.6, 31.2, 31.3, 32.9, 34.0, 40.0, 42.5, 51.9, 52.5, 55.2, 55.9, 65.2, 67.0, 69.4, 70.0, 76.4, 113.9 (2C), 118.3, 126.6 (3C), 126.7 (3C), 127.8 (6C), 128.0 (6C), 129.47 (6C), 129.49 (6C), 129.6, 129.8 (2C), 130.0, 132.0, 133.0, 144.2 (3C), 144.8 (3C), 159.3, 169.6, 171.1, 171.2, 171.5; IR (neat): 3287, 3059, 2957, 2928, 2856, 1733, 1639, 1539, 1514, 1443, 1248, 1172, 1084, 831, 744, 700 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{78}H_{96}N_3O_8S_3Si$ (M^+ + H) 1326.6129, found 1326.6110.

4.1.10. (3S,4R,5R)-Allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-hydroxy-7-(tritylthio)hept-4-enoylamino]-4-(methylthio)butanoylamino]-3-tritylthio(propionylamino)}-5-methylheptanoate (**23**)

DDQ (161 mg, 0.71 mmol) was added in small portions to a stirred solution of **22** (471 mg, 0.36 mmol) in CH_2Cl_2/H_2O 9:1 (18 mL) at room temperature. After 3 h, the 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 **23** (353 mg, 82%) as a colorless viscous liquid. [α]25D +12.3 (*c* 1.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.06 (3H, s), 0.78–0.86 (6H, m), 0.83 (9H, s), 1.05–1.12 (1H, m), 1.17–1.24 (1H, m), 1.72–1.78 (1H, m), 1.86–1.95 (1H, m), 2.01–2.09 (3H, m), 2.04 (3H, s), 2.17–2.23 (3H, m), 2.32 (1H, dd, *J* = 2.4, 13.7 Hz), 2.41 (1H, dd, *J* = 7.3, 16.1 Hz), 2.49–2.63 (5H, m), 3.40 (1H, d, *J* = 2.9 Hz), 3.87–3.97 (2H, m), 4.14 (1H, dt, *J* = 3.4, 7.2 Hz), 4.33–4.37 (1H, m), 4.39–4.52 (3H, m), 5.18–5.30 (2H, m), 5.39 (1H, dd, *J* = 6.3, 15.4 Hz), 5.49 (1H, dt, *J* = 6.8, 15.6 Hz), 5.79–5.88 (1H, m), 5.91 (1H, d, *J* = 10.3 Hz), 6.52 (1H, d, *J* = 7.8 Hz), 7.15–7.43 (31H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.5, 11.6, 13.7, 15.3, 17.9, 25.7 (3C), 27.0, 30.3, 30.4, 31, 2, 31.3, 33.1, 34.1, 39.9, 44.0, 52.6, 53.0, 56.1, 65.2, 66.6, 66.9, 69.3, 69.8, 118.3, 126.6 (3C), 126.8 (3C), 127.8 (6C), 128.0 (6C), 129.45 (6C), 129.52 (6C), 129.9, 132.0, 132.5, 144.2 (3C), 144.8 (3C), 170.0, 170.9, 171.5, 171.6; IR (neat): 3277, 3059, 2956, 2927, 2855, 1736, 1636, 1543, 1490, 1443, 1387, 1251, 1084, 836, 743, 700 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{70}H_{88}N_3O_7S_3Si$ (M^+ + H) 1206.5554, found 1206.5562.

4.1.11. (3S,4R,5R)-3-(Tert-butyldimethylsiloxy)-4-{(S)-2-[(R)-2-[(S,E)-3-hydroxy-7-(tritylthio)hept-4-enoylamino]-4-(methylthio)butanoylamino]-3-tritylthio(propionylamino)}-5-methylheptanoic acid (**11**)

Morpholine (51 μL, 0.59 mmol) was added dropwise to a stirred solution of **23** (353 mg, 0.29 mmol) in THF (15 mL) containing $Pd(PPh_3)_4$ (33.8 mg, 29 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 **11** (320 mg, 94%) as a white amorphous solid. [α]25D +4.8 (*c* 1.13, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (3H, s), 0.05 (3H, s), 0.77–0.85 (6H, m), 0.85 (9H, s), 1.04–1.11 (1H, m), 1.16–1.23 (1H, m), 1.75–1.81 (1H, m), 1.89–1.96 (1H, m), 2.01–2.10 (3H, m), 2.01 (3H, s), 2.18–2.34 (4H, m), 2.39 (1H, dd, *J* = 3.9, 16.1 Hz), 2.46–2.56 (4H, m), 2.70 (1H, dd, *J* = 8.3, 11.9 Hz), 3.89–3.94 (1H, m), 4.07–4.11 (2H, m), 4.32–4.37 (1H, m), 4.52 (1H, dd, *J* = 7.8, 12.7 Hz), 5.34 (1H, dd, *J* = 5.9, 15.6 Hz), 5.47 (1H, dt, *J* = 6.8, 15.1 Hz), 6.41 (1H, d, *J* = 10.2 Hz), 6.88 (1H, d, *J* = 7.3 Hz), 7.14–7.42 (31H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.4, 11.8, 13.5, 15.3, 17.8, 25.7 (3C), 27.1, 30.2, 30.4, 31.2, 31.3, 33.0, 34.1, 40.1, 44.0, 53.1, 53.4, 56.9, 66.5, 66.9, 68.8, 69.7, 126.6 (3C), 126.8 (3C), 127.8 (6C), 128.0 (6C), 129.4 (6C), 129.5 (6C), 129.9, 132.3, 144.2 (3C), 144.8 (3C), 170.3, 171.6, 171.9, 174.3; IR (neat): 3285, 3059, 3018, 2957, 2927, 2856, 1714, 1642, 1539, 1489, 1471, 1443, 1252, 1085, 836, 751, 700 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{67}H_{83}N_3O_7S_3SiNa$ (M^+ + Na) 1188.5060, found 1188.5057.

4.1.12. (2S,6R,9S,12R,13S)-12-[(S)-Isobutyl]-13-(tert-butyldimethylsilyloxy)-6-[2-(methylthio)ethyl]-2-[(E)-4-(tritylthio)but-1-en-1-yl]-9-tritylthiomethyl-1-oxa-5,8,11-triazacyclopentadecane-4,7,10,15-tetraone (**24**)

A solution of **11** (320 mg, 0.27 mmol) in CH_2Cl_2 (27 mL) was added very slowly to a stirred solution of MNBA (123 mg, 0.36 mmol) in CH_2Cl_2 (270 mL, 1.0 mM concentration) containing DMAP (101 mg, 0.82 mmol) at room temperature over 14 h. After 1 h, the mixture was diluted with CH_2Cl_2 (200 mL), and the organic layer was washed successively with saturated aqueous $NaHCO_3$ (2 × 80 mL), water (2 × 80 mL) and brine (2 × 80 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 **24** (256 mg, 81%) as a white amorphous solid. [α]25D −6.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.08 (3H, s), 0.83 (3H, d, *J* = 6.8 Hz), 0.89–0.93 (3H, m), 0.89 (9H, s), 1.04–1.12 (1H, m), 1.18–1.27 (1H, m), 1.87–1.92 (1H, m), 2.00–2.20 (4H, m), 2.05 (3H, s), 2.22–2.30 (2H, m), 2.36–2.66 (4H, m), 2.59–2.66 (2H, m), 2.72 (1H, dd, *J* = 10.2, 18.3 Hz), 3.34–3.41 (2H, m), 3.82 (1H, dt, *J* = 2.0, 9.8 Hz), 4.16 (1H, dt, *J* = 4.4, 9.8 Hz), 4.34 (1H, dd, *J* = 7.3, 14.6 Hz), 5.41 (1H, dd, *J* = 6.8, 15.6 Hz), 5.59–5.64 (1H, m), 5.71 (1H, dt, *J* = 6.8, 15.1 Hz), 6.48 (1H, d, *J* = 7.3 Hz), 7.00 (1H, d, *J* = 4.9 Hz), 7.16 (1H, d, *J* = 10.3 Hz), 7.25–7.49 (30H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.9, −4.0, 12.0, 12.9, 15.1, 17.8, 25.6 (3C), 27.3, 29.5, 30.2, 30.9, 31.3, 31.8, 34.0, 41.8, 42.2, 52.9, 57.0, 57.8, 66.6, 66.8, 68.6, 71.5, 126.6 (3C), 126.8 (3C), 127.8 (6C), 128.0 (6C), 128.1, 129.45 (6C), 129.48 (6C), 133.1, 144.4 (3C), 144.7 (3C), 169.8, 170.17, 170.24, 171.9; IR (neat): 3286, 3057, 2956, 2928, 2855, 1732, 1683, 1669, 1652, 1541, 1444, 1255, 1184, 1084, 834, 742, 699 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{67}H_{81}N_3O_6S_3SiNa$ (M^+ + Na) 1170.4954, found 1170.4971.

4.1.13. (1S,5S,6R,9S,20R,E)-6-[(S)-Isobutyl]-5-(tert-butyldimethylsilyloxy)-20-[2-(methylthio)ethyl]-2-oxa-11,12-dithia-7,19,22-triazabicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (**25**)

A solution of **24** (246 mg, 0.21 mmol) in CH_2Cl_2/MeOH 9:1 (50 mL) was added dropwise to a vigorously stirred solution of I_2 (544 mg, 2.1 mmol) in CH_2Cl_2/MeOH 9:1 (420 mL, 0.5 mM concentration) over 10 min at room temperature. After 10 min, the reaction was quenched with 0.2 M ascorbic acid/citric acid buffer (20 mL, adjusted to pH 4.0) at room temperature, and the resulting mixture was extracted with $CHCl_3$ (3 × 50 mL). The combined extracts were washed with brine (2 × 50 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 **25** (112 mg, 79%) as a white amorphous solid. [α]25D +13.1 (*c* 1.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.09 (3H, s), 0.16 (3H, s), 0.86–0.96 (6H, m), 0.93 (9H, s), 1.20–1.27 (1H, m), 1.36–1.43 (1H, m), 1.88 (1H, ddd, *J* = 2.4, 6.8, 14.0 Hz), 2.02–2.10 (1H, m), 2.19 (3H, s), 2.28–

2.36 (1H, m), 2.49–2.83 (8H, m), 2.96–3.14 (4H, m), 3.54–3.65 (1H, m), 4.41 (1H, dt, $J = 4.4$, 8.8 Hz), 4.86 (1H, dt, $J = 3.4$, 10.3 Hz), 4.98–5.02 (1H, m), 5.67–5.71 (2H, m), 6.33–6.41 (1H, m), 6.76 (1H, d, $J = 6.8$ Hz), 7.13 (1H, d, $J = 6.8$ Hz), 7.52 (1H, d, $J = 3.9$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.0, −4.3, 11.9, 14.5, 15.5, 17.9, 25.7 (3C), 25.8, 27.7, 28.0, 31.3, 34.5, 36.2, 40.0, 40.4, 41.3, 53.7, 57.3, 61.0, 67.0, 68.9, 129.3, 132.4, 168.9, 169.0, 170.1, 171.0; IR (neat): 3341, 2957, 2929, 2857, 1745, 1662, 1543, 1432, 1257, 1158, 1138, 1080, 949, 837, 756 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{29}H_{52}N_3O_6S_3Si$ ($M^+ + H$) 662.2788, found 662.2783.

4.1.14. Burkholdac B (**2**)

HF·pyridine (1.2 mL) was added to a stirred solution of **25** (77.3 mg, 0.12 mmol) in pyridine (2.3 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 **2** (62.0 mg, 97%) as a white amorphous solid. [α]25D −57.8 (*c* 1.00, MeOH), {lit. [2]. [α]32D −62.6 (*c* 0.34, MeOH)}. ^{1}H NMR (400 MHz, CD_2Cl_2): δ 0.88 (3H, d, $J = 6.8$ Hz), 0.91 (3H, t, $J = 7.3$ Hz), 1.16–1.24 (1H, m), 1.48–1.54 (1H, m), 1.99–2.09 (2H, m), 2.17 (3H, s), 2.20–2.29 (1H, m), 2.42–2.51 (1H, m), 2.61–2.68 (5H, m), 2.73–2.79 (2H, m), 2.88–2.93 (2H, m), 3.11 (1H, d, $J = 13.7$ Hz), 3.20–3.28 (2H, m), 3.32–3.36 (1H, m), 4.29 (1H, dt, $J = 8.3$, 4.4 Hz), 4.55–4.57 (1H, m), 4.83 (1H, dt, $J = 8.8$, 3.4 Hz), 5.49–5.51 (1H, m), 5.72 (1H, d, $J = 15.6$ Hz), 6.31 (1H, t, $J = 11.7$ Hz), 6.77 (1H, d, $J = 9.3$ Hz), 7.25 (1H, d, $J = 7.3$ Hz), 7.46 (1H, d, $J = 2.9$ Hz); ^{13}C NMR (100 MHz, CD_2Cl_2): δ 11.7, 15.56, 15.58, 27.5, 28.6, 31.5, 33.7, 36.6, 39.9, 41.1, 41.4, 41.8, 55.0, 57.6, 61.8, 68.5, 71.1, 129.5, 133.4, 169.4, 170.5, 171.4, 172.1; IR (neat): 3376, 2957, 2924, 2855, 1736, 1685, 1656, 1543, 1524, 1439, 1273, 1162, 980, 753 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{23}H_{38}N_3O_6S_3$ ($M^+ + H$) 548.1923, found 548.1924. The ^{1}H and ^{13}C NMR, and MS spectrum are identical with those reported for natural burkholdac B [1].

4.1.15. (3S,4S)-Ethyl 4-(tert-butoxycarbonylamino)-3-hydroxy-5-methylhexanoate (**27**) and its (3R,4S)-isomer (**28**)

A solution of EtOAc (6.0 mL, 62 mmol) in THF (8 mL) was added slowly to a stirred solution of lithium diisopropylamide (LDA) (62 mmol) [prepared from *n*-BuLi in hexane (1.6 M solution, 38.7 mL, 62 mmol) and *i*-Pr_2NH (9.3 mL, 65 mmol)] in THF (30 mL) at −78 °C. After 30 min, (2*S*)-2-(*tert*-butoxycarbonylamino)-3-methylbutylaldehyde (*N*-Boc-L-valinal) (**26**) [11] (3.66 g, 18 mmol) in THF (30 mL) was added to the above mixture at −78 °C. After 40 min, the reaction was quenched with 2 M HCl (20 mL) at −78 °C, and the resulting mixture was extracted with EtOAc (2 × 100 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and brine (2 × 50 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 **27** (2.98 g, 57%, less polar) and **28** (1.56 g, 30%, more polar).

Compound 27: colorless oil; [α]25D −39.8 (*c* 1.03, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, d, $J = 6.8$ Hz), 1.00 (3H, d, $J = 6.8$ Hz), 1.28 (3H, t, $J = 7.1$ Hz), 1.44 (9H, s), 1.83–1.91 (1H, m), 2.45 (1H, dd, $J = 2.7$, 16.7 Hz), 2.55 (1H, dd, $J = 9.8$, 16.7 Hz), 3.15 (1H, t, $J = 9.5$ Hz), 3.29 (1H, d, $J = 2.9$ Hz), 4.17 (2H, q, $J = 6.8$ Hz), 4.24–4.28 (1H, m), 4.85 (1H, d, $J = 9.3$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 19.5, 19.8, 28.4 (3C), 30.4, 39.1, 59.6, 60.8, 67.1, 79.1, 156.4, 173.7; IR (neat): 3393, 2977, 1716, 1507, 1392, 1367, 1309, 1279, 1247, 1174, 1042, 1023, 869, 777 cm^{-1}; HRMS (EI): *m/z* calcd for $C_{14}H_{27}NO_5$ (M^+) 289.1889, found 289.1882.

Compound 28: colorless oil; [α]25D +9.0 (*c* 1.02, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, d, $J = 7.3$ Hz), 0.95 (3H, d, $J = 6.8$ Hz), 1.28 (3H, t, $J = 7.3$ Hz), 1.44 (9H, s), 2.09–2.17 (1H, m), 2.46 (1H, dd, $J = 9.8$, 17.1 Hz), 2.59 (1H, dd, $J = 2.0$, 17.1 Hz), 3.30 (1H, d, $J = 4.9$ Hz), 3.50–3.56 (1H, m), 3.90–3.96 (1H, m), 4.18 (2H, dq, $J = 6.8$, 2.0 Hz), 4.43 (1H, br d, $J = 10.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 16.2, 20.1, 27.5, 28.3 (3C), 38.4, 58.7, 60.8, 69.2, 79.5, 156.4, 173.2; IR (neat): 3450, 3369, 2965, 1716, 1698, 1524, 1391, 1367, 1308, 1251, 1173, 1069, 984, 869, 775 cm^{-1}; HRMS (EI): *m/z* calcd for $C_{14}H_{27}NO_5$ (M^+) 289.1889, found 289.1891.

4.1.16. Conversion of compound **27** to compound **28**

2.6 M Jones reagent (5.2 mL, 14 mmol) was added dropwise to a stirred solution of **27** (2.68 g, 9.2 mmol) in acetone (80 mL) at 0 °C, and stirring was continued for 1 h at room temperature. The mixture was diluted with Et_2O (200 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and brine (2 × 50 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 (*S*)-ethyl 4-(*tert*-butoxycarbonylamino)-5-methyl-3-oxohexanoate (2.26 g, 86%) as a colorless oil.

KBH_4 (2.13 g, 40 mmol) was added in small portions to a stirred solution of the above product (2.26 g, 7.9 mmol) in MeOH (80 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 (30 mL) was added, and the resulting mixture was extracted with CH_2Cl_2 (4 × 30 mL). The combined extracts were washed with 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 **28** (1.80 g, 79%) along with **27** (112 mg, 5%). The IR, ^{1}H and ^{13}C NMR, mass spectra of these samples were identical with those recorded for **27** and **28**, respectively.

4.1.17. (3R,4S)-Ethyl 4-(tert-butoxycarbonylamino)-3-(tert-butyldimethylsiloxy)-5-methylhexanoate (**29**)

tert-Butyldimethylsilyl chloride (TBSCl) (1.26 g, 8.4 mmol) was added to stirred solution of **28** (807 mg, 2.8 mmol) in DMF (20 mL) containing imidazole (1.14 g, 17 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 **29** (1.11 g, 99%) as a colorless oil. [α]25D −5.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (3H, s), 0.09 (3H, s), 0.87–0.88 (12H, m), 0.92 (3H, d, $J = 6.8$ Hz), 1.27 (3H, t, $J = 7.3$ Hz), 1.43 (9H, s), 1.93–1.99 (1H, m), 2.43 (1H, dd, $J = 5.9$, 15.1 Hz), 2.53 (1H, dd, $J = 6.2$, 15.6 Hz), 3.47–3.53 (1H, m), 4.07–4.22 (3H, m), 4.46 (1H, br d, $J = 10.7$ Hz); ^{13}CNMR (100 MHz, $CDCl_3$): δ −4.9, −4.7, 14.1, 16.8, 18.0, 20.6, 25.7 (3C), 27.9, 28.4 (3C), 40.0, 59.5, 60.5, 70.1, 78.9, 155.9, 171.9; IR (neat): 3455, 3377, 2960, 2931, 1719, 1703, 1390, 1366, 1304, 1254, 1174, 1082, 837, 777 cm^{-1}; HRMS (EI): *m/z* calcd for $C_{20}H_{41}NO_5Si$ (M^+) 403.2754, found 403.2850.

4.1.18. (3R,4S)-Allyl 4-(tert-butoxycarbonylamino)-3-(tert-butyldimethylsiloxy)-5-methylhexanoate (**31**)

1 M NaOH (14 mL, 14 mmol) was added dropwise to a stirred solution of **29** (1.11 g, 2.8 mmol) in EtOH (30 mL) at room temperature. After 6 h, the mixture was diluted with 10% aqueous HCl (30 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*R*,4*S*)-4-(*tert*-butoxycarbonylamino)-3-(*tert*-butyldimethylsiloxy)-5-methylhexanoic acid (**30**) (1.03 g), which was used for the next reaction without further purification.

Allyl bromide (0.48 mL, 5.5 mmol) was added to a stirred solution of the crude carboxylic acid **30** (1.03 g, 2.8 mmol) in DMF (30 mL) containing K_2CO_3 (1.16 g, 8.3 mmol) at room temperature. After 6 h, the reaction was diluted with water (10 mL), 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 **31** (927 mg, 81%, 2 steps) as a pale yellow oil. $[\alpha]25_D$ −6.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04 (3H, s), 0.10 (3H, s), 0.87–0.88 (12H, m), 0.93 (3H, d, J = 6.8 Hz), 1.43 (9H, s), 1.91–2.00 (1H, m), 2.47 (1H, dd, J = 7.3, 15.6 Hz), 2.57 (1H, dd, J = 5.8, 15.6 Hz), 3.48–3.53 (1H, m), 4.21 (1H, dd, J = 6.8, 12.7 Hz), 4.43 (1H, br d, J = 10.7 Hz), 4.53–4.63 (2H, m), 5.23–5.35 (2H, m), 5.88–5.97 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.9, −4.7, 16.7, 17.9, 20.5, 25.7 (3C), 27.9, 28.4 (3C), 40.0, 59.5, 65.3, 70.1, 79.0, 118.5, 132.0, 155.9, 171.5; IR (neat): 3453, 3374, 2960, 2931, 2858, 1714, 1704, 1504, 1390, 1366, 1254, 1172, 1083, 990, 837, 777, 666 cm^{-1}; HRMS (EI): *m/z* calcd for $C_{21}H_{41}NO_5Si$ (M^+) 415.2754, found 415. 2752.

4.1.19. (3R,4S)-Allyl 4-[(S)-2-(tert-butoxycarbonylamino)-3-(tritylthio)propanamido]-3-(tert-butyldimethylsiloxy)-5-methylhexanoate (**34**)

Trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.69 mL, 3.8 mmol) was added to a stirred solution of **31** (200 mg, 0.48 mmol) in CH_2Cl_2 (10 mL) in the presence of 2,6-lutidine (0.56 mL, 4.8 mmol) at room temperature. After 30 min, MeOH (1.6 mL) was added to the reaction mixture at 0 °C, and stirring was continued for 3 h at room temperature. The reaction mixture was concentrated in vacuo to afford (3*R*,4*S*)-allyl 4-amino-3-(*tert*-butyldimethylsiloxy)-5-methylhexanoate (**32**) (151 mg) 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 (0.25 mL, 1.5 mmol) was added dropwise to a stirred solution of the crude amine **32** (151 mg, 0.48 mmol) and *N*-Boc-*S*-trityl-D-cysteine (**33**) (333 mg, 0.72 mmol) in MeCN (16 mL) containing PyBOP (300 mg, 0.58 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 to afford a residue, which was purified by column chromatography (hexane/EtOAc 1:1) to give **34** (247 mg, 68%, 2 steps) as a colorless oil. $[\alpha]25_D$ −3.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.05 (3H, s), 0.81 (3H, d, J = 6.3 Hz), 0.83 (9H, s), 0.86 (3H, d, J = 6.8 Hz), 1.42 (9H, s), 1.91–1.99 (1H, m), 2.44 (1H, dd, J = 6.3, 17.1 Hz), 2.50–2.58 (2H, m), 2.70–2.78 (1H, m), 3.80–3.86 (1H, m), 3.92 (1H, dt, J = 6.3, 5.4 Hz), 4.14 (1H, dt, J = 6.8, 4.4 Hz), 4.44–4.57 (2H, m), 4.70–4.76 (1H, m), 5.18–5.30 (2H, m), 5.80–5.90 (1H, m), 6.08 (1H, d, J = 10.7 Hz), 7.19–7.43 (15H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.6, 16.6, 17.9, 20.4, 25.7 (3C), 27.9, 28.2 (3C), 33.7, 39.7, 53.8, 57.9, 65.2, 67.2, 69.8, 80.2, 118.4, 126.8 (3C), 128.0 (6C), 129.5 (6C), 132.0, 144.4 (3C), 155.2, 170.3, 171.5; IR (neat): 3327, 2959, 2930, 2857, 1717, 1683, 1491, 1367, 1254, 1173, 1093, 937, 836, 751, 701 cm^{-1}; HRMS (EI): *m/z* calcd for $C_{43}H_{60}N_2O_6SSi$ (M^+) 760.3941, found 760.3934.

4.1.20. (3R,4S)-Allyl 4-[(S)-2-amino-3-(tritylthio)propanamido]-3-(tert-butyldimethylsiloxy)-5- methylhexanoate (**35**)

TMSOTf (0.29 mL, 1.6 mmol) was added dropwise to a stirred solution of **34** (241 mg, 0.32 mmol) in CH_2Cl_2 (4 mL) containing 2,6-lutidine (0.37 mL, 3.2 mmol) at room temperature. After 1 h, MeOH (0.7 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 5:1) to give **35** (206 mg, 98%) as a white amorphous solid. $[\alpha]25_D$ −4.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.03 (3H, s), 0.82–0.85 (15H, m), 1.30 (2H, br s), 1.93–2.01 (1H, m), 2.45 (1H, dd, J = 6.8, 15.6 Hz), 2.47–2.56 (2H, m), 2.75 (1H, dd, J = 3.9, 12.7 Hz), 3.04 (1H, dd, J = 3.9, 8.8 Hz), 3.74–3.79 (1H, m), 4.14 (1H, dd, J = 6.3, 11.2 Hz), 4.46 (1H, dd, J = 5.4, 13.2 Hz), 4.53 (1H, dd, J = 5.9, 13.2 Hz), 5.20 (1H, d, J = 10.7 Hz), 5.28 (1H, dd, J = 1.5, 17.1 Hz), 5.81–5.90 (1H, m), 7.08 (1H, d, J = 9.3 Hz), 7.18–7.44 (15H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.6, 16.9, 17.9, 20.6, 25.7 (3C), 27.8, 37.4, 40.0, 54.1, 57.7, 65.2, 66.9, 69.9, 118.4, 126.7 (3C), 127.9 (6C), 129.6 (6C), 132.0, 144.6 (3C), 171.5, 172.8; IR (neat): 3369, 3312, 2958, 2929, 2856, 1736, 1673, 1508, 1255, 1174, 1092, 936, 836, 777, 743, 701, 676 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{38}H_{53}N_2O_4SSi$ ($M^+ + H$) 661.3466, found 661.3476.

4.1.21. (S)-Methyl 2-[(S,E)-3-(4-methoxybenzyloxy)-7-(tritylthio) hept-4-enamide]-4-(methylthio)butanoate (**36**)

i-Pr_2NEt (0.15 L, 0.91 mmol) was added dropwise to a stirred solution of **16** (70.9 mg, 0.13 mmol) and L-methionine methyl ester (***ent*-15**) (51.9 mg, 0.26 mmol) in MeCN (6.5 mL) containing PyBOP (135 mg, 0.26 mmol) at 0 °C under argon, and stirring was continued for 2 h at room temperature. The reaction mixture was diluted with EtOAc (30 mL), and 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 2:1) to give **36** (87.0 mg, 98%) as a colorless oil. $[\alpha]25_D$ 1.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.84–1.91 (1H, m), 2.04 (3H, s), 2.05–2.16 (3H, m), 2.21–2.23 (2H, m), 2.36–2.49 (4H, m), 3.70 (3H, s), 3.78 (3H, s), 4.08–4.13 (1H, m), 4.25 (1H, d, J = 12.2 Hz), 4.52 (1H, d, J = 11.2 Hz), 4.68 (1H, dd, J = 7.3, 12.7 Hz), 5.35 (1H, dd, J = 8.3, 15.1 Hz), 5.59 (1H, dt, J = 6.8, 15.6 Hz), 6.83 (2H, d, J = 8.8 Hz), 6.87 (1H, d, J = 8.3 Hz), 7.19–7.45 (17H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 29.8, 31.2, 31.4, 31.8, 43.0, 51.3, 52.3, 55.2, 66.5, 69.8, 76.3, 113.8 (3C), 126.6 (2C), 127.8 (6C), 129.5 (6C), 129.6 (2C), 129.9, 130.2, 133.1, 144.8 (3C), 159.2, 170.3, 172.2; IR (neat): 3327, 3057, 2952, 2917, 1743, 1652, 1443, 1248, 1174, 1080, 1034, 822, 744, 701 cm^{-1}; HRMS (EI): *m/z* calcd for $C_{40}H_{45}NO_5S_2$ (M^+) 683.2739, found 683.2737.

4.1.22. (S)-2-[(S,E)-3-(4-methoxybenzyloxy)-7-(tritylthio)hept-4-enamide]-4-(methylthio)butanoic acid (**37**)

1 M LiOH (1.2 mL, 1.2 mmol) was added dropwise to a stirred solution of **36** (213 mg, 0.31 mmol) in MeOH (6.2 mL) at room temperature. After 3 h, 10% aqueous HCl was added to the mixture at 0 °C until the pH was 6. The resulting mixture was extracted with EtOAc (3 × 20 mL), and the combined extracts were washed with 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 ($CHCl_3$/MeOH 10:1) to give **37** (203 mg, 98%) as a white amorphous solid. $[\alpha]25_D$ −4.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.84–1.93 (1H, m), 2.00 (3H, s), 2.07–2.18 (3H, m), 2.21–2.25 (2H, m), 2.39–2.51 (4H, m), 3.77 (3H, s), 4.11 (1H, dt, J = 8.3, 3.4 Hz), 4.24 (1H, d, J = 10.7 Hz), 4.51 (1H, d, J = 11.7 Hz), 4.63–4.67 (1H, m), 5.32 (1H, dd, J = 8.3, 15.6 Hz), 5.59 (1H, dt, J = 6.8, 15.1 Hz), 6.82 (2H, d,

J = 8.3 Hz), 7.07 (1H, d, J = 8.3 Hz), 7.05–7.45 (17H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.2, 29.8, 31.2, 31.3, 31.4, 42.7, 51.6, 55.2, 66.5, 69.8, 76.2, 113.8 (3C), 126.6 (2C), 127.8 (6C), 129.5 (6C), 129.6, 129.7 (2C), 130.0, 133.4, 144.8 (3C), 159.2, 171.7, 175.1; IR (neat): 3336, 3015, 2918, 1733, 1614, 1541, 1514, 1489, 1444, 1248, 1177, 1034, 822, 747, 701 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{39}H_{44}NO_5S_2$ (M^+ + H) 670.2661, found 670.2684.

4.1.23. (3R,4S)-Allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(S)-2-[(S,E)-3-(4- methoxybenzyloxy)-7-(tritylthio)hept-4-enoylamino]-4-(methylthio)butanoylamino]-3-tritylthio(propionylamino)}-5-methylhexanoate (**38**)

i-Pr$_2$NEt (66 μL, 0.39 mmol) was added dropwise to a stirred solution of **35** (99.1 mg, 0.15 mmol) and **37** (100 mg, 0.15 mmol) in CH_2Cl_2 (3.0 mL) containing HATU (74.1 mg, 0.20 mmol) and HOAt (26.5 mg, 0.20 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 **38** (159 mg, 81%) as a colorless viscous liquid. [α]25D −1.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.04 (3H, s), 0.72 (3H, d, J = 6.3 Hz), 0.80 (9H, s), 0.85 (3H, d, J = 6.3 Hz), 1.72–1.81 (2H, m), 1.94–2.00 (1H, m), 1.94 (3H, s), 2.11–2.15 (2H, m), 2.19–2.44 (7H, m), 2.54 (1H, dd, J = 3.9, 15.6 Hz), 2.65 (2H, d, J = 6.8 Hz), 3.76–3.78 (1H, m), 3.78 (3H, s), 4.05 (1H, dt, J = 8.3, 3.4 Hz), 4.17–4.22 (4H, m), 4.44–4.56 (3H, m), 5.17–5.33 (3H, m), 5.56 (1H, dt, J = 6.3, 15.6 Hz), 5.80–5.90 (1H, m), 6.25–6.31 (2H, m), 6.80–6.90 (3H, m), 7.17–7.42 (32H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8, −4.7, 15.2, 17.5, 17.9, 20.3, 25.7 (3C), 28.4, 30.0, 30.6, 31.2, 31.4, 33.7, 39.4, 42.9, 52.6, 53.1, 55.3, 59.0, 65.3, 66.6, 67.2, 69.3, 69.9, 76.4, 113.9 (2C), 118.3, 126.6 (3C), 126.8 (3C), 127.9 (6C), 128.0 (6C), 129.52 (6C), 129.54 (6C), 129.6 (2C), 129.9, 130.2, 132.1, 133.2, 144.4 (3C), 144.8 (3C), 159.3, 169.3, 171.05, 171.09, 172.0 ppm; IR (neat): 3319, 2957, 2929, 2856, 1733, 1645, 1514, 1444, 1250, 1176, 1083, 1035, 836, 743, 701 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{77}H_{94}N_3O_8S_3Si$ (M^+ + H) 1312.5972, found 1312.5948.

4.1.24. (3R,4S)-Allyl 3-(tert-butyldimethylsiloxy)-4-{(S)-2-[(S)-2-[(S,E)-3-hydroxy-7-(tritylthio)hept-4-enoylamino]-4-(methylthio) butanoylamino]-3-tritylthio(propionylamino)}-5-methylhexanoate (**39**)

DDQ (93.1 mg, 0.34 mmol) was added in small portions to a stirred solution of **38** (212 mg, 0.16 mmol) in CH_2Cl_2/H_2O 9:1 (16 mL) at room temperature. After 3 h, the 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 **39** (158 mg, 83%) as a colorless viscous liquid. [α] 25D −7.7 (*c* 1.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.00 (3H, s), 0.04 (3H, s), 0.78 (3H, d, J = 6.3 Hz), 0.80 (9H, s), 0.87 (3H, d, J = 6.8 Hz), 1.81–1.88 (1H, m), 1.90–1.99 (1H, m), 2.02–2.14 (3H, m), 2.03 (3H, s), 2.18–2.22 (2H, m), 2.27 (1H, dd, J = 9.3, 15.1 Hz), 2.55–2.69 (5H, m), 2.59 (1H, dd, J = 5.4, 12.7 Hz), 2.66 (1H, dd, J = 7.8, 13.2 Hz), 3.25 (1H, d, J = 3.4 Hz), 3.80 (1H, dt, J = 9.8, 6.3 Hz), 4.14–4.18 (2H, m), 4.29 (1H, dd, J = 6.8, 13.2 Hz), 4.35–4.41 (1H, m), 4.40 (1H, dd, J = 4.9, 13.7 Hz), 4.54 (1H, dd, J = 5.9, 13.2 Hz), 5.18–5.30 (2H, m), 5.38 (1H, dd, J = 6.3, 15.6 Hz), 5.53 (1H, dt, J = 6.8, 15.1 Hz), 5.80–5.90 (1H, m), 6.17 (1H, d, J = 10.2 Hz), 6.51–6.56 (2H, m), 7.18–7.52 (30 H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.8 (2C), 15.2, 17.3, 17.9, 20.3, 25.7 (3C), 28.3, 30.15, 30.21, 31.3, 31.4, 33.6, 39.5, 42.9, 52.7, 52.9, 58.8, 65.4, 66.6, 67.1, 68.9, 69.2, 118.5, 126.6 (3C), 126.8 (3C), 127.8 (6C), 128.0 (6C), 129.47 (6C), 129.52 (6C), 130.0, 131.9, 132.3, 144.3 (3C), 144.8 (3C), 169.5, 170.8, 171.8, 172.0; IR (neat): 3340, 3318, 2958, 2928, 1732, 1634, 1539, 1444, 1255, 1179, 1094, 836, 743, 700 cm^{-1}; HRMS (FAB): *m/z*: calcd for $C_{69}H_{86}N_3O_7S_3Si$ (M^+ + H) 1192.5397, found 1192.5406.

4.1.25. (3R,4S)-3-(Tert-butyldimethylsiloxy)-4-{(S)-2-[(S)-2-[(S,E)-3-hydroxy-7-(tritylthio)hept-4-enoylamino]-4-(methylthio)butanoyl-amino]-3-tritylthio(propionylamino)}-5-methylhexanoic acid (**40**)

Morpholine (15.6 μL, 0.18 mmol) was added dropwise to a stirred solution of **39** (100 mg, 84 μmol) in THF (10 mL) containing $Pd(PPh_3)_4$ (11 mg, 9.5 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 **40** (71.6 mg, 74%) as a white amorphous solid. [α]25D −13.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 9:1): δ 0.03 (6H, s), 0.74 (3H, d, J = 7.3 Hz), 0.81 (3H, d, J = 7.3 Hz), 0.84 (9H, s), 1.83–1.92 (1H, m), 1.95–2.01 (1H, m), 2.01 (3H, s), 2.03–2.09 (3H, m), 2.17–2.20 (2H, m), 2.29 (1H, dd, J = 9.3, 14.1 Hz), 2.32–2.39 (3H, m), 2.48–2.52 (2H, m), 2.54–2.57 (2H, m), 3.71–3.76 (1H, m), 4.02–4.06 (2H, m), 4.33–4.38 (1H, m), 4.44 (1H, t, J = 7.1 Hz), 5.39 (1H, dd, J = 5.9, 15.1 Hz), 5.51 (1H, dt, J = 6.3, 15.6 Hz), 6.59 (1H, d, J = 9.8 Hz), 7.15–7.44 (32H, m); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ 9:1): δ −5.1, −4.7, 15.1, 16.1, 17.7, 20.3, 25.6 (3C), 27.6, 29.5, 29.9, 30.8, 31.2, 31.3, 33.4, 43.0, 52.5, 52.7, 59.3, 66.4, 67.0, 68.6, 69.5, 126.5 (3C), 126.7 (3C), 127.7 (6C), 127.9 (6C), 129.3 (6C), 129.4 (6C), 131.2, 132.4, 144.2 (3C), 144.7 (3C), 170.2, 171.9, 172.2, 172.5; IR (neat): 3420, 2956, 2927, 2855, 1646, 1577, 1489, 1444, 1253, 1184, 1083, 1034, 969, 836, 743, 700, 622 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{66}H_{81}N_3O_7S_3SiK$ (M^+ + K) 1190.4643, found 1190.4653.

4.1.26. (2S,6S,9S,12S,13R)-13-(Tert-butyldimethylsilyloxy)-12-isopropyl-6-[2-(methylthio)ethyl]-2-[(E)-4-(tritylthio)but-1-enyl]-9-tritylthiomethyl-1-oxa-5,8,11-triazacyclopentadecane-4,7,10,15-tetraone (**41**)

A solution of **40** (81.2 mg, 70 μmol) in CH_2Cl_2 (7 mL) was added very slowly to a stirred solution of MNBA (31.3 mg, 91 μmol) in CH_2Cl_2 (70 mL, 1.0 mM concentration) containing DMAP (25.7 mg, 0.21 mmol) at room temperature over 14 h. After 1 h, the mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was washed successively with saturated aqueous $NaHCO_3$ (2 × 40 mL), water (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, 1:1) to give **41** (66.3 mg, 84%) as a white amorphous solid. [α]25D −7.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 9:1): δ −0.03 (3H, s), 0.14 (3H, s), 0.90–0.98 (15H, m), 1.85–1.94 (1H, m), 1.97–2.04 (1H, m), 2.10–2.14 (1H, m), 2.10 (3H, s), 2.25–2.37 (6H, m), 2.46–2.55 (3H, m), 2.63–2.75 (3H, m), 3.65 (1H, dd, J = 7.8, 14.6 Hz), 3.78 (1H, dt, J = 10.7, 2.4 Hz), 4.07 (1H, dt, J = 10.2, 3.4 Hz), 4.45 (1H, dd, J = 7.3, 16.1 Hz), 5.45–5.54 (2H, m), 5.67 (1H, dt, J = 5.9, 14.6 Hz), 6.52 (1H, d, J = 10.2 Hz), 7.02 (1H, d, J = 8.3 Hz), 7.28–7.51 (30H, m), 7.93 (1H, d, J = 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ 9:1): δ −5.4, −4.6, 14.4, 15.2, 17.8, 20.2, 25.6 (3C), 26.4, 30.0, 31.0, 31.2, 31.3, 31.9, 40.5, 41.5, 51.7, 53.0, 57.9, 66.5, 67.1, 67.7, 70.8, 126.5 (3C), 126.7 (3C), 127.7 (6C), 127.9 (6C), 128.1, 129.4 (12C), 132.5, 144.3 (3C), 144.7 (3C), 169.1, 170.1, 171.4, 171.8; IR (neat): 3443, 2959, 2930, 1733, 1645, 1540, 1444, 1387, 1256, 1219, 1180, 1126, 1084, 1035, 834, 743, 700 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{66}H_{80}N_3O_6S_3Si$ (M^+ + H) 1134.4979, found 1134.4974.

*4.1.27. (1S,5R,6S,9S,20S,E)-5-(Tert-butyldimethylsilyloxy)-6-isopropyl-20-[2-(methylthio)ethyl]-2-oxa-11,12-dithia-7,19,22-triazabicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (**42**)*

A solution of **41** (56 mg, 49 μmol) in CH_2Cl_2/MeOH 9:1 (13 mL) was added dropwise to a vigorously stirred solution of I_2 (127 mg, 0.49 mmol) in CH_2Cl_2/MeOH 9:1 (98 mL, 0.5 mM concentration) over 10 min at room temperature. After 10 min, the reaction was quenched with 0.2 M ascorbic acid/citric acid buffer (10 mL, adjusted to pH 4.0) at room temperature, and the resulting mixture was extracted with $CHCl_3$ (3 × 50 mL). The combined extracts were washed with brine (2 × 50 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 **42** (27.4 mg, 85%) as a white amorphous solid. [α]25D −105.5 (*c* 1.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 9:1): δ 0.05 (3H, s), 0.15 (3H, s), 0.77 (3H, d, *J* = 5.4 Hz), 0.80–0.82 (12H, m), 2.02 (3H, s), 2.09–2.27 (3H, m), 2.31–2.47 (5H, m), 2.54–2.60 (3H, m), 2.80–2.87 (2H, m), 3.32–3.46 (2H, m), 3.75–3.83 (2H, m), 4.02–4.07 (1H, m), 4.78–4.83 (1H, m), 5.56 (1H, d, *J* = 4.4 Hz), 5.65–5.73 (2H, m), 7.04 (1H, d, *J* = 7.3 Hz), 7.13 (1H, d, *J* = 10.2 Hz), 7.96 (1H, d, *J* = 5.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD 9:1): δ −5.5, −4.4, 14.6, 14.7, 17.8, 20.5, 25.7 (3C), 26.3, 27.9, 29.6, 30.8, 31.4, 40.0, 40.3, 42.9, 53.9, 56.7, 60.0, 69.0, 69.6, 130.5 (2C), 170.0, 170.1, 170.3, 171.3; IR (neat): 3389, 3308, 2957, 2928, 2856, 1741, 1660, 1541, 1430, 1301, 1249, 1167, 1092, 947, 838, 778, 756 cm^{-1}; HRMS (FAB): *m/z*: calcd for $C_{28}H_{50}N_3O_6S_3Si$ (M^+ + H) 648.2631, found 648.2639.

*4.1.28. 5,6,20-Tri-epi-burkholdac A (**3**)*

HF·pyridine (2.0 mL) was added to a stirred solution of **42** (26 mg, 40 μmol) in pyridine (4 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 **3** (18.9 mg, 89%) as a white amorphous solid. [α]25D −114.3 (*c* 0.38, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3CN): δ 0.74 (3H, d, *J* = 6.8), 0.75 (3H, d, *J* = 6.8 Hz), 2.00 (3H, s), 2.00–2.09 (4H, m), 2.20 (1H, dd, *J* = 3.4, 13.7 Hz), 2.24–2.33 (2H, m), 2.36–2.44 (2H, m), 2.47–2.56 (3H, m), 2.61 (1H, dd, *J* = 6.8, 12.9 Hz), 3.13–3.35 (2H, m), 3.41 (1H, d, *J* = 7.3 Hz), 3.60 (1H, dt, *J* = 2.9, 10.2 Hz), 3.73–3.78 (1H, m), 3.84–3.90 (1H, m), 4.67 (1H, br s), 5.49–5.51 (1H, m), 5.69 (1H, d, *J* = 15.6 Hz), 5.84 (1H, br s), 6.88–6.90 (1H, m), 6.96 (1H, d, *J* = 9.8 Hz), 7.37 ppm (1H, d, *J* = 4.9 Hz); ^{13}C NMR (100 MHz, CD_3CN): δ 15.1, 15.6, 20.9, 28.3, 29.1, 30.3, 31.5, 41.07, 41.13, 41.30, 41.33, 43.8, 54.9, 60.0, 69.8, 69.9, 131.0, 131.4, 170.6, 171.0, 171.3, 172.1; IR (neat): 3383, 3301, 2961, 2923, 1733, 1717, 1653, 1541, 1507, 1429, 1286, 1245, 1166, 1021, 974, 754 cm^{-1}; HRMS (FAB): *m/z*: calcd for $C_{22}H_{36}N_3O_6S_3$ (M^+ + H) 534.1766, found 534.1764.

4.2. Biological evaluation

4.2.1. HDACs preparation and enzyme inhibition assay [19]

In a 100-mm dish, 293T 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 HDAC inhibition assay (p21 promoter assay) [19]

A luciferase reporter plasmid (pGW-FL) was constructed by cloning the 2.4 kb genomic fragment containing the transcription start site into HindIII and SmaI sites of the pGL3-Basic plasmid (Promega Co., Madison, WI). Mv1Lu (mink lung epithelial cell line) cells were transfected with the pGW-FL and a phagemid expressing neomycin/kanamycin resistance gene (pBK-CMV, Stratagene, La Jolla, CA) with the Lipofectamine reagent (Life Technology, Rockville, MD, USA). After the transfected cells had been selected by 400 μg mL^{-1} Geneticin (G418, Life Technology), colonies formed were isolated. One of the clones was selected and named MFLL-9. MFLL-9 expressed a low level of luciferase, whose activity was enhanced by TSA in a dose-dependent manner. MFLL-9 cells (1 × 10^5) cultured in a 96-well multi-well plate for 24 h were incubated for 20 h in the medium containing various concentrations of drugs. The luciferase activity of each cell lysate was measured with a LucLite luciferase Reporter Gene Assay Kit (Packard Instrument Co., Meriden, CT) and recorded with a Luminescencer-JNR luminometer (ATTO, Tokyo, Japan). Data were normalized to the protein concentration in cell lysates. Concentrations at which a drug induces the luciferase activity 100-fold higher than the basal level are presented as the 10,000% effective concentration 10,000% (EC_{10000}). The human wild-type p21 promoter luciferase fusion plasmid, WWP-Luc, was a kind gift from Dr. B. Vogelstein.

4.2.3. Cell-growth inhibition assay [18]

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.2014.02.044.

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