Total Synthesis and Stereochemical Revision of Burkholdac A

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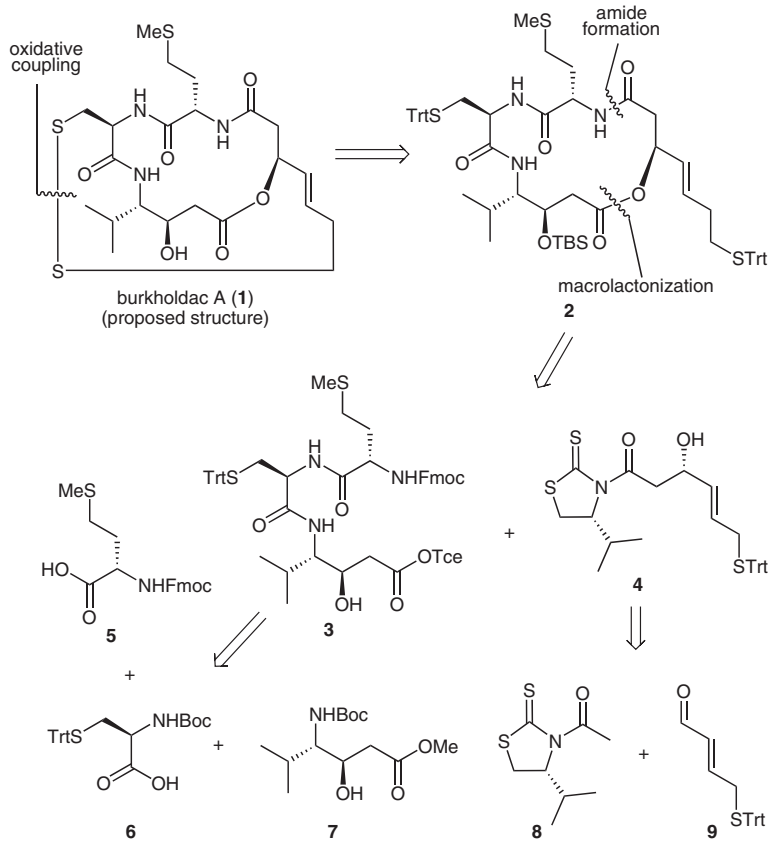
Abstract: A stereocontrolled total synthesis of burkholdac A was completed, leading to a revision of the reported stereochemistry.

Key words: burkholdac A, total synthesis, antitumor, stereochemical assignment

Natural products represent validated starting points for drug discovery. Their structural assignment and chemical synthesis potentially provide the foundation for research in the development of new therapeutic agents.[1] Advanced developments in isolation and analytical technologies have made structural elucidation of natural product a routine operation. However, numerous natural products were misassigned, including a substantial number of recently elucidated marine natural products. Total synthesis plays a critical role in natural product structure elucidation, which accounts for the overwhelming majority of natural product structural revisions.[2,3] We have been interested for some time in secondary metabolites and view their syntheses as a key route to structural confirmation, structural modification, and subsequent activity control.[4] While this manuscript was in preparation, Ganesan and



Scheme 1 Retrosynthetic analysis

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co-workers reported the first total synthesis of burkholdac B and corrected the originally misassigned structure.[5] The stereochemical revision of burkholdac B is identical with that described in the current manuscript. Here we wish to describe the total synthesis and revised stereochemical assignment of burkholdac A.

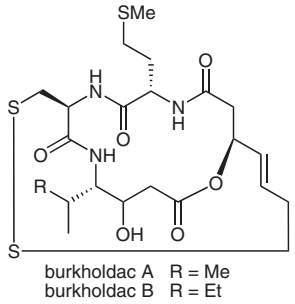


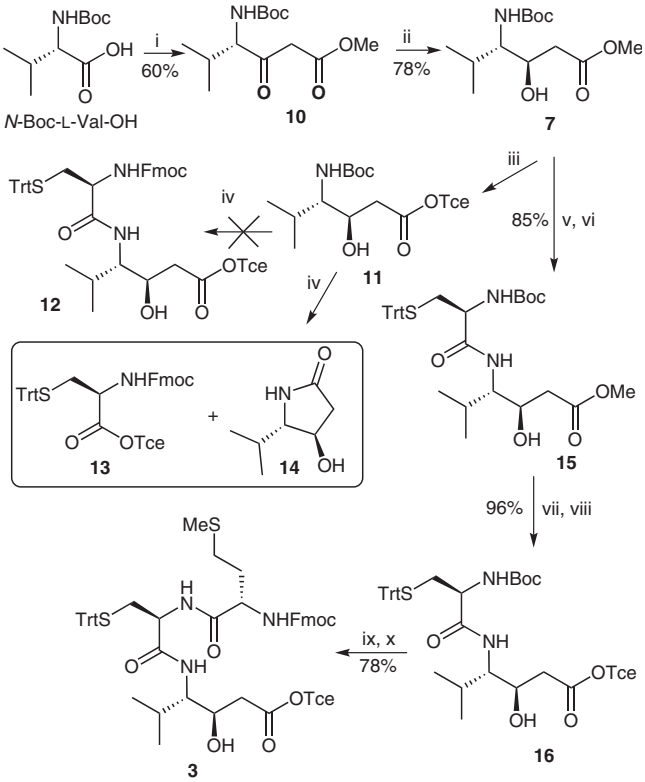
Figure 1 Structure of burkholdacs A and B

Through the systematic overexpression of transcription factors associated with natural product gene clusters encoded within *Burkholderia thailandensis* E264, Brady and co-workers[6] isolated burkholdacs A and B (Figure 1) as two new members of a small class of bicyclic depsipeptides that includes spiruchostatins, FK228, and FR901375.[7] The constitution of burkholdacs A and B was elucidated by extensive NMR spectroscopy studies, while their absolute stereochemistry, as illustrated in Figure 1, was proposed on the basis of the biosynthesis gene cluster contains only one epimerase domain.

As outlined in our retrosynthetic analysis, burkholdac A could be obtained from the advanced precursor **2** via disulfide formation. It was envisaged that the 15-membered macrolactone **2** could be constructed by macrolactonization of the corresponding precursor which was planned to be assembled from fragments **3** and **4**. Further disconnection of fragments **3** and **4** led to smaller subunits **5**–**9** (Scheme 1).

The synthesis of L-valine-derived statine methyl ester **7** commenced with the condensation of *N*-Boc-valine with methyl magnesium malonate,[8] to afford β-keto ester **10** in 60% yield (Scheme 2). Diastereoselective reduction of **10** with KBH_4 produced the desired alcohol **7** as the major isomer (dr >11:1) that was easily separated by column chromatography. Transesterification of a methyl ester to the trichloroethyl ester **11** was achieved according to the procedure of Ganesan.[9] However, in our hands, we were unable to reproduce dipeptide **12** by condensation of the amine derived from **11** and activated D-cysteine that Ganesan observed in his total synthetic of spiruchostatin A.[9] The major products isolated from our reaction were ester **13** and γ-lactam **14**, which could arise from an intramolecular cyclization of the amine derived from **11**, and nucleophilic attack of the activated D-cysteine by trichloroethanol. At this stage, we decided to slightly modify the synthetic sequence by formation of dipeptide **15** prior to a transesterification process. Thus, the Boc protective group of **7** was cleanly removed with trifluoroacetic acid, and the resulting free amine was immediately exposed to a PyBOP-mediated coupling process with *N*-Boc-D-cysteine to afford dipeptide **15** in 85% yield. Hydrolysis of ester **15** gave the corresponding acid, which was converted into **16** by Keck's modification[10] of Steglich's carbodiimide esterification. $BF_3{\cdot}OEt_2$-promoted removal of the Boc protective group of **16** followed by coupling of the resulting amine with *N*-Fmoc-L-methionine afforded **3** in 78% yield.

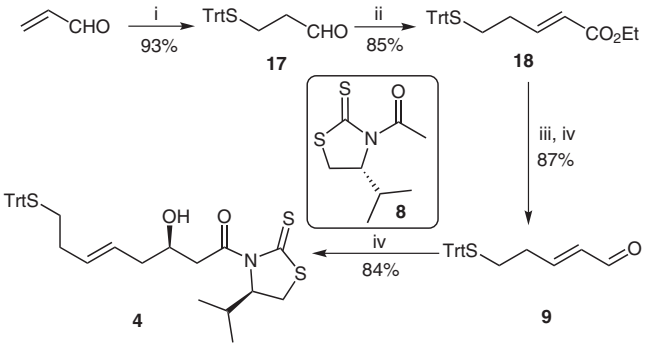
With fragment **3** in hand, we next turned our attention to the synthesis of the β-hydroxyl acid fragment **4** (Scheme 3). Thus, conjugate addition of triphenylmethanethiol to acrolein gave rise to aldehyde **17** in 93% yield. Subsequent Wittig olefination of **17** produced the *trans*-α,β-unsaturated ethyl ester **18** in 85% yield (*E*/*Z* >21:1). This conjugated ester was converted into the corresponding aldehyde **9** in 87% yield via a two-step sequence involving DIBAL reduction of ester followed by Dess–Martin oxidation of the resulting primary alcohol to give the corresponding aldehyde **9**. Treatment of aldehyde **9** with *N*-acetylthiazolidinethione (**8**) in the presence of $TiCl_4$ and Hünig's base in dichloromethane at –78 °C furnished the acetate aldol adduct in 84% yield (dr = ca. 9:1),



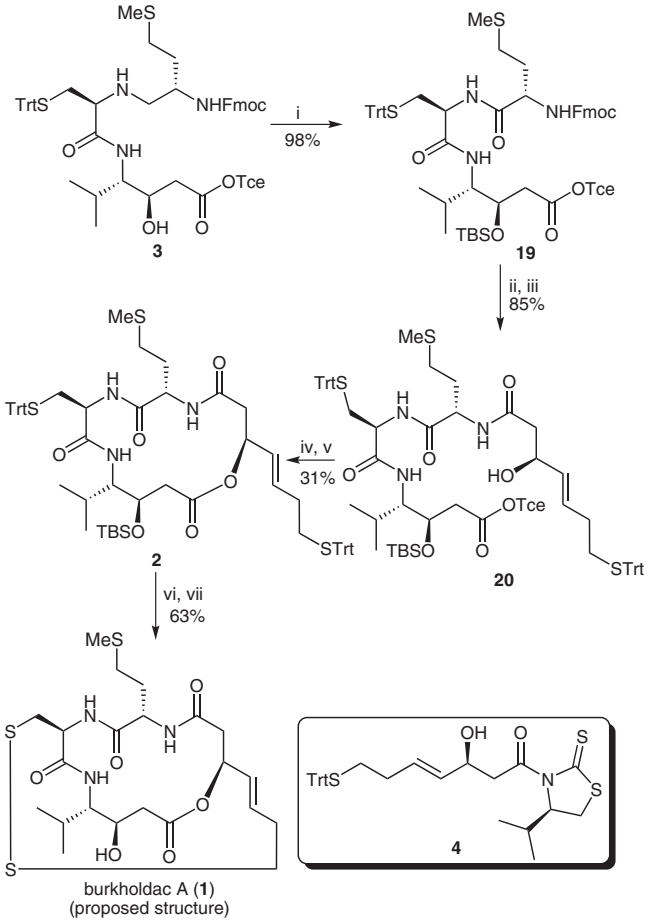
Scheme 2 Synthesis of fragment **3**. *Reagents and conditions*: (i) CDI, THF, then potassium methyl malonate, $MgCl_2$, 60%; (ii) KBH_4, MeOH, –78 °C to r.t., 78 °C; (iii) (a) LiOH, THF–H_2O (4:1), 0 °C; (b) TCEOH, DCC, DMAP, CH_2Cl_2; (iv) (a) TFA, CH_2Cl_2; (b) Fmoc-STrt-D-Cys, PyAOP, DIPEA, CH_2Cl_2, 74%; v, TFA, CH_2Cl_2; (vi) **6**, PyAOP, DIPEA, CH_2Cl_2, 85% (2 steps); (vii) NaOH, THF; (viii) TceOH, EDCI, DMAP, CH_2Cl_2, 96% (2 steps); (ix) $BF_3{\cdot}OEt_2$, CH_2Cl_2; (x) **5**, PyAOP, DIPEA, CH_2Cl_2, 78% (2 steps).

and the desired diastereomer **4** was readily separated by chromatography.[11]

At this juncture, the time had arrived to explore the assembly of two key fragments leading to linear peptide precursor **20** for the final macrocyclization reaction (Scheme 4).



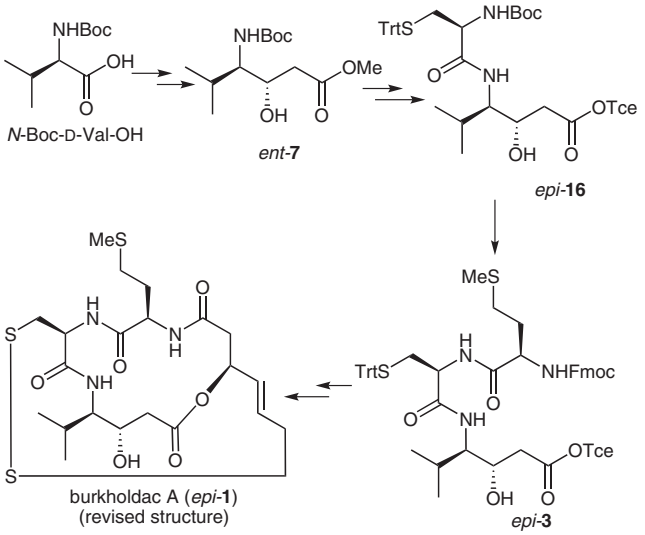
Scheme 3 Synthesis of fragment **4**. *Reagents and conditions*: (i) TrtSH, CH_2Cl_2, 93%; (ii) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2, 85%; (iii) DIBAL, CH_2Cl_2; (iv) Dess–Martin periodinane, CH_2Cl_2, $NaHCO_3$, 87% (2 steps); (v) **8**, $TiCl_4$, DIPEA, CH_2Cl_2, –78 °C, 84%.



Scheme 4 Synthesis of the proposed burkholdac A (**1**). *Reagents and conditions*: (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, –78 °C, 98%; (ii) diethyl amine, MeCN; (iii) **4**, DMAP, CH_2Cl_2, 0 °C to r.t., 85% (2 steps); (iv) Zn dust, NH_4OAc (aq), 40 °C; (v) MNBA, DMAP, CH_2Cl_2, 31% (2 steps); (vi) I_2, MeOH–CH_2Cl_2 (1:9); (vii) HF·py, 63% (2 steps).

Thus, the secondary alcohol in fragment **3** was protected as its TBS ether **18** in 98% yield. By taking advantage of the good leaving-group ability of the thiazolidine-2-thione moiety of fragment **4**, transamidification with suitable amine should be a spontaneous process. Thus, removal of the Fmoc group of **19** afforded the corresponding free amine, which reacted with fragment **4**, in the presence of DMAP, to produce **20** in 85% yield over the two steps. Reductive removal of the trichloroethyl ester by treating **20** with zinc and ammonium acetate afforded the corresponding *sec*-acid, which was subjected to Shiina's lactonization[12] protocol employing 2-methyl-6-nitrobenzoic anhydride (MNBA) and DMAP to provide macrolactone **2** in 31% yield over two steps. Oxidative deprotection of the bis(*S*-triphenylmethyl)lactone with iodine, followed by removal of the TBS protecting group with HF–pyridine complex to give rise to the proposed burkholdac A (**1**) in 63% yield.[13]

Unfortunately, on comparison of our spectra and the published data for natural burkholdac A, neither ^{1}H NMR nor ^{13}C NMR spectra for **1** were identical with those of the natural product. These data suggested that structure **1**, proposed by Brady and co-workers, must be incorrect for the true structure of burkholdac A. On the basis of the hypothesis that FK228, FR901228, FR901375, spiruchostatin, and burkholdacs are biosynthetically related, we hypothesized that all three amino acids of natural burkholdac are of D stereochemistry. We therefore elected to synthesize the diastereomer *epi*-**1** of the proposed structure **1**. As shown in Scheme 5, we prepared *ent*-**7** following the same synthesis as for **7**, but with *N*-Boc-D-valine as the starting material. Further elaboration of *ent*-**7** to the revised burkholdac A includes the incorporating of *N*-Fmoc-D-methionine into *epi*-**3** as the key intermediate. This was readily achieved, and *epi*-**1**[14] was obtained in 3.9% overall yield as previously performed. To our delight, the spectral data (^{1}H and ^{13}C NMR) of the synthetic burkholdac A (*epi*-**1**) are identical to those of the natural burkholdac A.



Scheme 5 Synthesis of the revised burkholdac A (*epi*-**1**)

The HDAC inhibitory evaluation of **1** and *epi*-**1** with the Biomol Fluor-de-Lys HDAC assay gave results of great interest (Figure 2). Both **1** and *epi*-**1** inhibited HDAC activity from a HeLa cell nuclear protein extract. Importantly, *epi*-**1** has a superior potency (IC_{50} = 31 pM) compared to **1** (IC_{50} = 720 nM). This indicated that the stereochemistry of burkholdac A and its diastereoisomer appears to be an important factor for its bioactivities. With an IC_{50} at picomolar level, burkholdac A (*epi*-**1**) is an exciting lead for further investigation.

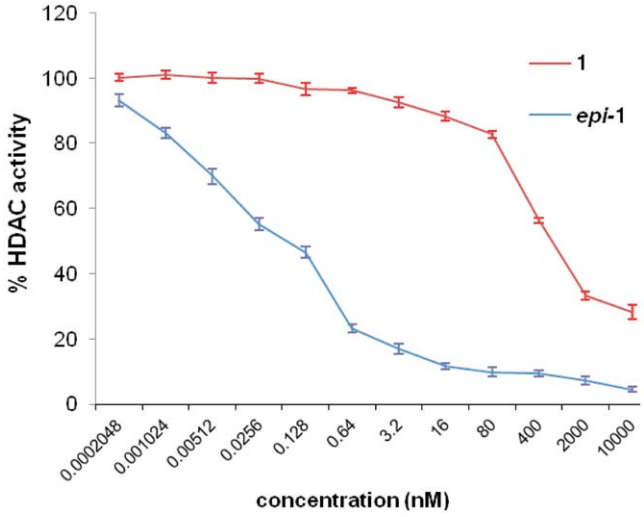


Figure 2 Effect of **1** and *epi*-**1** in HDAC activity assay

In summary, the first total synthesis of burkholdac A was completed, leading to a revision of the reported stereochemistry from structure **1** to *epi*-**1**.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

Acknowledgment

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(13) **Synthesis of the Proposed Burkholdac A (1)**
Compound **2** (46 mg, 0.04 mmol) was dissolved in MeOH–CH_2Cl_2 (50 mL, 1:9) and added to a vigorously stirred solution of I_2 (126 mg, 0.50 mmol) in MeOH–CH_2Cl_2 (200 mL, 1:9) at r.t. over 0.5 h. After 10 min, the reaction was quenched by addition of sat. aq solution of $Na_2S_2O_3$ (20 mL) and concentrated in vacuo. The residue was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with sat. aq solution of $Na_2S_2O_3$ (20 mL) and brine (30 mL), dried over anhyd Na_2SO_4 and concentrated in vacuo. The residue was dissolved in pyridine (1 mL), after HF·py (0.8 mL) was added at 0 °C, the reaction mixture was stirred at r.t. for 12 h. All volatiles were removed in vacuo; the residue was diluted with EtOAc (100 mL) and washed

with HCl (20 mL, 1.0 M in H_2O) and brine (20 mL), dried over anhyd Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (eluted with EtOAc–hexanes–MeOH = 3:1:0.3) to provide the desired compound **1** (13.4 mg, 63% yield over 2 steps) as a white amorphous solid: $[\alpha]_D^{20}$ –166.5 (*c* 0.35, MeOH). ^{1}H NMR (400 MHz, CD_3CN): δ = 7.71 (d, *J* = 5.4 Hz, 1 H), 7.09 (d, *J* = 9.8 Hz, 1 H), 7.02 (br s, 1 H), 5.90 (br s, 1 H), 5.77 (d, *J* = 15.5 Hz, 1 H), 5.58 (dd, *J* = 6.1, 2.3 Hz, 1 H), 4.74 (br s, 1 H), 3.97–3.92 (m, 1 H), 3.83 (ddd, *J* = 10.2, 6.5, 4.0 Hz, 1 H), 3.68 (td, *J* = 10.3, 3.0 Hz, 1 H), 3.40–3.22 (m, 2 H), 2.73–2.67 (m, 2 H), 2.64–2.56 (m, 3 H), 2.51–2.43 (m, 3 H), 2.40–2.26 (m, 3 H), 2.18–2.08 (m, 2 H), 2.06 (s, 3 H), 0.83 (d, *J* = 0.8 Hz, 3 H), 0.81 (d, *J* = 1.0 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, CD_3CN): δ = 172.4, 171.4, 171.2, 170.8, 131.9, 131.0, 70.0, 69.6, 60.1, 54.9, 54.8, 43.7, 41.2, 41.2, 41.1, 31.4, 29.1, 28.3, 20.8, 15.6, 15.0 ppm. ESI-HRMS *m/z* calcd for $C_{22}H_{36}N_3O_6S_3^+$ $[M + H]^+$: 534.1761; found: 534.1757.

(14) **The Analytical Data of the Revised Burkholdac A (*epi*-1)** $[\alpha]_D^{20}$ –54.0 (*c* 0.23, MeOH). ^{1}H NMR (500 MHz, CD_3CN): δ = 7.51 (s, 1 H), 7.41 (d, *J* = 6.9 Hz, 1 H), 6.90 (d, *J* = 8.9 Hz, 1 H), 6.12–6.06 (m, 1 H), 5.90 (d, *J* = 15.5 Hz, 1 H), 5.56–5.54 (m, 1 H), 4.70 (td, *J* = 9.2, 3.6 Hz, 1 H), 4.47–4.43 (m, 1 H), 4.18–4.13 (m, 1 H), 3.33–3.30 (m, 1 H), 3.21 (d, *J* = 10.5 Hz, 1 H), 3.13 (d, *J* = 14.0 Hz, 1 H), 2.96 (dd, *J* = 13.1, 7.2 Hz, 1 H), 2.86–2.57 (m, 8 H), 2.25 (dq, *J* = 13.6, 6.8 Hz, 1 H), 2.10 (s, 3 H), 2.08–2.01 (m, 2 H), 0.96 (d, *J* = 6.8 Hz, 3 H), 0.87 (d, *J* = 6.8 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, CD_3CN): δ = 173.0, 172.2, 171.7, 170.1, 132.4, 131.5, 71.7, 69.0, 63.2, 56.8, 56.4, 41.8, 41.1, 40.8, 33.3, 31.2, 30.6, 30.5, 21.1, 19.8, 15.2 ppm. ESI-HRMS: *m/z* calcd for $C_{22}H_{36}N_3O_6S_3^+$ $[M + H]^+$: 534.1761; found: 534.1760.