Total synthesis of spiruchostatin B, a potent histone deacetylase inhibitor, from a microorganism†

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The first total synthesis of spiruchostatin B, a potent histone deacetylase inhibitor, was achieved in a convergent manner; the synthesis established stereochemistry at the C5″ position.

Spiruchostatins A (**1**) and B (**2**) (Fig. 1), isolated from a culture broth of *Psuedomonas* sp. by Shin-ya and co-workers[1] in 2001, exhibit potent histone deacetylase (HDAC) inhibitory activity.[2] HDAC inhibitors have been reported to exhibit prominent antitumor activity against various types of mammalian solid tumors;[3] therefore, these natural products are expected to be promising candidates for novel molecular-targeted anticancer agents. Structurally, **1** and **2** are bicyclic depsipeptides consisting of (3*S*,4*R*)-statine (blue-colored part), D-cysteine (orange-colored part), D-alanine (green-colored part), (3*R*,4*E*)-3-hydroxy-7-mercapto-4-heptenoic acid (red-colored part), and disulfide bond linkage. These structures are similar to that of FK228 (FR901228) (**3**), a powerful HDAC inhibitor isolated from the fermentation broth of *Chromobacterium violaceum* by Fujisawa Pharmaceutical Co. Ltd. (now Astellas Pharm Inc.).[2]

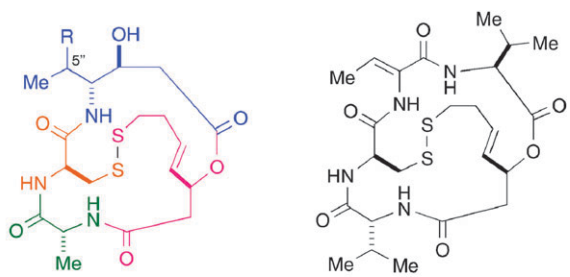
The remarkable biological properties and attractive structural features prompted us to undertake a project directed toward the total synthesis of **1–3**. Till date, only one total synthesis of FK228 (**3**) has been reported by Simon and co-workers,[4a]‡ and two total syntheses of spiruchostatin A (**1**) have been reported by Ganesan and co-workers[5] followed by Doi, Takahashi *et al.*[6] However, total synthesis of **2** has not yet been mentioned in the literature, and the stereochemistry at C5″ (spiruchostatin numbering) of **2** has not been clearly assigned.

In this communication, we describe the first total synthesis of **2**, which established the stereochemistry at C5″ as described in Scheme 1.

Our synthetic plan is outlined in Scheme 1. Spiruchostatin B (**2**) should be synthesized by macrolactonization of *seco*-acid **4** followed by a disulfide bond formation according to the protocols described by previous literature.[5,6] The major challenge of this scheme is a convergent assembly of **4** by amide coupling of segment **5** with segment **6** while avoiding epimerization at the C2 position (D-alanine part) in **6**. Segment **5** would be produced through an aldol reaction of D-allo-isoleucine derivative **7**[7] with ethyl acetate (**8**) and subsequent condensation with D-cysteine derivative **9**.[8] However, segment **6** would be prepared through Julia–Kocienski olefination[9] of sulfone **10** accessible from 1,3-propanediol with aldehyde **11** available from L-malic acid,[10] and subsequent condensation with D-alanine methyl ester (**12**).

We initially pursued the synthesis of segment **5** as shown in Scheme 2. Aldol reaction of the lithium enolate of ethyl acetate (**8**) with the known *N*-Boc-D-allo-isoluecinal (**7**)[7] produced the desired product **14** (31%) and the undesired stereoisomer **13** (62%). Conversion of **13** to **14** was successfully performed by inversion of the hydroxy group (77% yield in two steps); the sequence involved Jones oxidation and stereoselective reduction with KBH_4 (**14** : **13** = 15 : 1).§ Ethyl ester **14** was then transformed to allyl ester **15** *via* a four-step sequence involving TBS protection of the secondary hydroxy group (96%), saponification of the ester moiety (80%), formation of an allyl ester from the liberated carboxylic acid (98%), and deprotection of the *N*-Boc group (92%). Subsequent condensation of amine **15** with *N*-Boc-*S*-trityl-D-cysteine (**9**)[8] furnished the desired product **16** in 86% yield. Ultimately, deprotection of the *N*-Boc group in **16** afforded the requisite segment **5** in a quantitative yield.

We then performed the synthesis of segment **6**, as described in Scheme 3. Sulfone **10**, a crucial substrate for the Julia–Kocienski olefination,[9] was efficiently prepared from the known 3-(4-methoxybenzyloxy)propan-1-ol (**17**)[11] *via* a four-step operation involving the formation of *S*-tetrazole



1 : spiruchostatin A (R = Me)
2 : spiruchostatin B (R = Et)
3 : FK228 (FR901228)

Fig. 1 Structures of spiruchostatins A (**1**), B (**2**) and FK228 (FR901228) (**3**).

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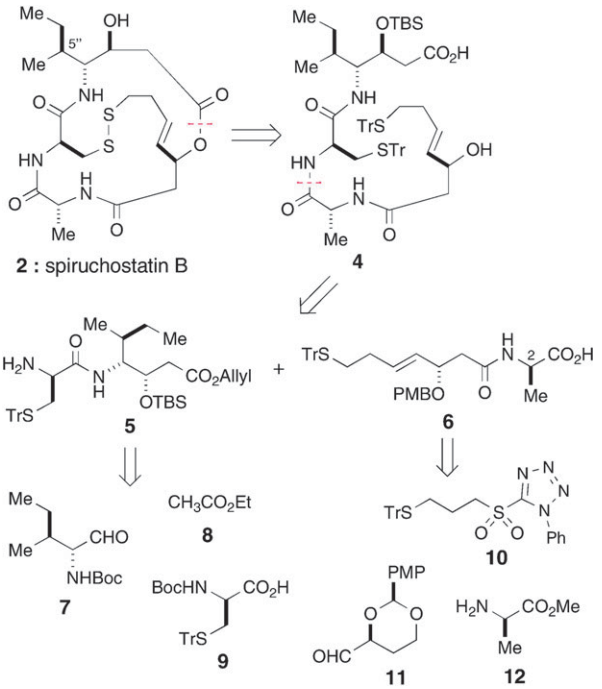
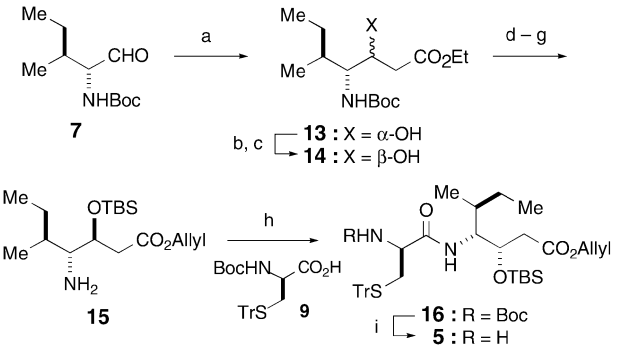
† Electronic supplementary information (ESI) available: Experimental procedures and characterization data for all new compounds along with copies of ^1H and ^{13}C NMR spectra. See DOI: 10.1039/b718310k



Scheme 1 Synthetic plan for spiruchostatin B (**2**). TBS = *tert*-butyldimethylsilyl, Tr (trityl) = triphenylmethyl, PMB = 4-methoxybenzyl, Boc = *tert*-butoxycarbonyl, PMP = 4-methoxyphenyl.

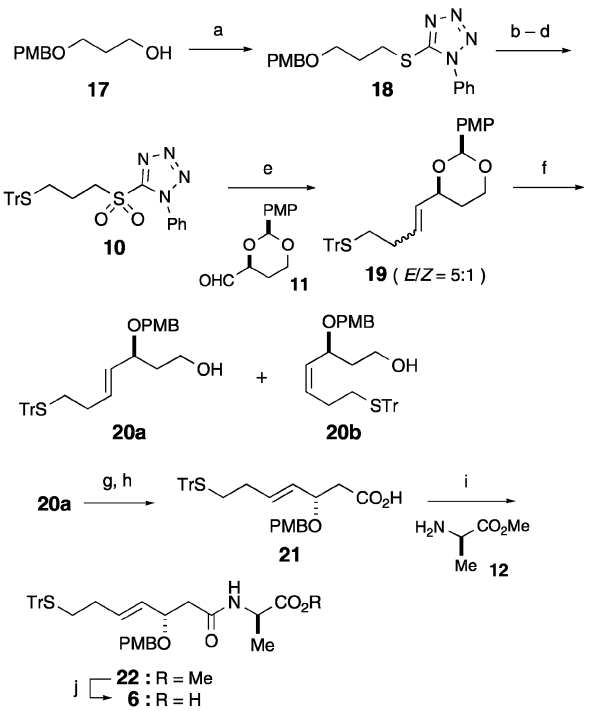
product[12] (95%), Molybdenum-mediated oxidation,[9b,13] deprotection of the PMB group (94% in two steps), and formation of the *S*-trityl product (96%). The crucial Julia–Kocienski olefination of **10** with the known aldehyde **11**,[10] readily prepared from L-malic acid, produced the desired product **19** as an inseparable mixture of *E*/*Z*-stereoisomers (*E* : *Z* = 5 : 1 by 400 MHz ^{1}H NMR) in 66% yield. The mixture **19** was subjected to regioselective acetal opening with DIBAL[14] at 0 °C; the desired *E*-olefinic alcohol **20a** was produced as a major product (60%) along with the undesired *Z*-olefinic isomer **20b** (12%). Twofold oxidation of **20a** afforded carboxylic acid **21** (66% in two steps), which was finally converted to the requisite segment **6** (88% in two steps) by condensation with D-alanine methyl ester (**12**) and saponification of the ester function.

With the key segments **5** and **6** synthesized, we next investigated the synthesis of spiruchostatin B (**2**) by assembling the two segments as shown in Scheme 4. Initial attempts to achieve the pivotal condensation of **5** with **6** under conventional conditions[15] (*e.g.*, PyBOP, EDCI/HOBT, or HATU, rt) failed; the condensation product was produced in good yield (~80%) but with considerable epimerization at the C2 stereogenic center (D-alanine part). After screening several reaction conditions, we solved this problem using a combination of HATU and HOAt at low temperature. Treatment of **5** and **6** with HATU (1.3 equiv.) and HOAt (1.3 equiv.) in the presence of *i*-Pr_2NEt (2.5 equiv.) in CH_2Cl_2 at −30 °C for 2 h produced the desired condensation product (94%) without appreciable epimerization at C2. The resulting product was then converted to *seco*-acid **4** (84% in two steps), a crucial substrate for macrolactonization, by deprotection of the PMB and allyl groups. The critical macrolactonization of **4** was best achieved by employing the Shiina protocol.[16]¶ Thus, treatment of a

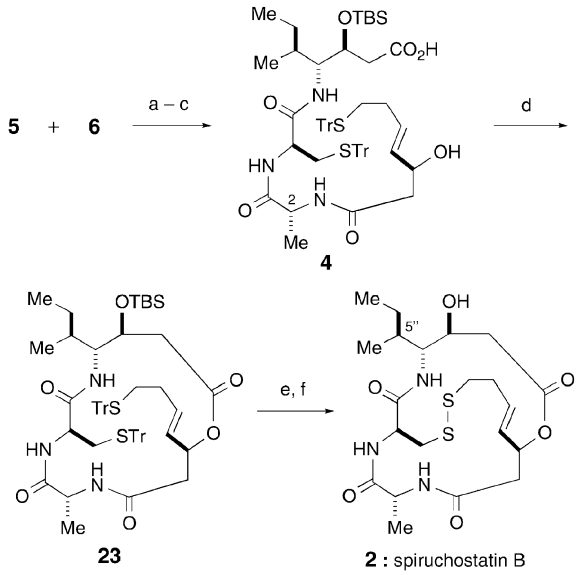


Scheme 2 Synthesis of segment **5**. *Reagents and conditions*: (a) LDA, EtOAc (**8**), THF, −78 °C; add. **7**, 62% for **13**, 31% for **14** (**13** : **14** = 2 : 1); (b) Jones' reagent, acetone, rt, 86%; (c) KBH_4, MeOH, −40 °C, 90% for **14**, 6% for **13** (**14** : **13** = 15 : 1); (d) TBSCl, imidazole, DMF, rt, 96%; (e) 1M NaOH, EtOH, rt, 80%; (f) allyl bromide, K_2CO_3, DMF, rt, 98%; (g) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt; MeOH, rt, 92%; (h) **9**, PyBOP, *i*-Pr_2NEt, MeCN, rt, 86%; (i) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt, 99%. TMSOTf = trimethylsilyl trifluoromethanesulfonate, PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.

dilute solution of **4** in CH_2Cl_2 (0.001 M) with 2-methyl-6-nitrobenzoic anhydride (MNBA) (1.3 equiv.) and DMAP (3.0 equiv.) at room temperature for 15 h produced the desired macrocycle **23** in high yield (89%). Finally, disulfide bond



Scheme 3 Synthesis of segment **6**. *Reagents and conditions*: (a) 1-phenyl-1*H*-tetrazole-5-thiol, DEAD, PPh_3, THF, rt, 95%; (b) $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$, 30% H_2O_2, EtOH, rt; (c) DDQ, CH_2Cl_2/H_2O, rt, 94% (2 steps); (d) TrSH, DEAD, PPh_3, CH_2Cl_2, reflux, 96%; (e) $LiN(SiMe_3)_2$, DMF, −60 °C; at −60 °C, add. **11**, −60 to 0 °C, 66% (*E* : *Z* = 5 : 1); (f) DIBAL, toluene, 0 °C, 60% for **20a**, 12% for **20b**; (g) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt, 88%; (h) $NaClO_2$, NaH_2PO_4, DMSO–H_2O, rt, 75%; (i) D-alanine methyl ester (**12**), PyBOP, *i*-Pr_2NEt, CH_2Cl_2, 0 °C to rt, 90%; (j) 1 M LiOH, MeOH, rt, 98%. DEAD = diethyl azodicarboxylate, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DIBAL = diisobutylaluminium hydride.



Scheme 4 Synthesis of spiruchostatin B (**2**). *Reagents and conditions*: (a) HATU, HOAt, *i*-Pr_2NEt, CH_2Cl_2, −30 °C, 94%; (b) DDQ, CH_2Cl_2/H_2O, rt, 85%; (c) $Pd(PPh_3)_4$, morpholine, THF, rt, 99%; (d) MNBA, DMAP, CH_2Cl_2, rt, 89%; (e) I_2, MeOH–CH_2Cl_2, rt, 94%; (f) HF·pyridine, pyridine, rt, 93%. HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAt = 1-hydroxy-7-azabenzotriazole, MNBA = 2-methyl-6-nitrobenzoic anhydride, DMAP = 4-dimethylaminopyridine.

formation of **23** by exposure to iodine in dilute MeOH solution[4–6,17] followed by deprotection of the TBS group completed the total synthesis of **2**, $[\alpha]_D^{20}$ −59.8 (*c* = 1.02, MeOH) {lit.[1] $[\alpha]_D$ −58.6 (*c* = 0.11, MeOH)}, in 87% yield in two steps. The spectroscopic properties (IR, ^1H and ^{13}C NMR, MS) of the synthetic sample **2** were identical with those reported[1] for natural spiruchostatin B, which resulted in the establishment of the C5″ stereochemistry in **2** to be (*S*)-configuration as depicted in Scheme 4.‖

In conclusion, we have accomplished a total synthesis of spiruchostatin B (**2**) in a convergent manner starting from D-allo-isoleucinal **7**, aldehyde **11** derived from L-malic acid, and 1,3-propanediol derivative **17**. The pivotal steps of the synthesis involve (i) Julia–Kocienski olefination of sulfone **10** and aldehyde **11** to install the requisite (*E*)-olefin unit present in the critical segment **6**, (ii) condensation of segments **5** and **6** to directly assemble the crucial *seco*-acid **4**, and (iii) macrolactonization of **4** using the Shiina reagent to efficiently construct the desired macrocycle **23**. The C5″ stereochemistry of **2** was determined by the present synthesis.

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Notes and references

‡ A total synthesis of FR901375, a structurally closely related bicyclic depsipeptide isolated from a microorganism along with FK228 (**3**), has been reported.[4b]

Note added at proof: After submission of this manuscript, we learned that the second, improved total synthesis of **3** has been reported by Williams and co-workers.[18]

§ In this stereoselective reduction, several reducing agents such as $NaBH_4$, $LiBH_4$ and KBH_4 were examined; the best result was obtained by the use of KBH_4.

¶ In the previous two total syntheses of spiruchostatin A (**1**), Ganesan and co-workers[5] successfully achieved the crucial macrolactonization using the Yamaguchi method (2,4,6-$Cl_3C_6H_2COCl$, Et_3N, MeCN–THF, 0 to 20 °C; DMAP, toluene, 50 °C, 53%); on the other hand, Doi, Takahashi *et al.*[6] efficiently performed the macrolactonization event with the Shiina method (MNBA, DMAP, CH_2Cl_2, rt, 67%).

‖ By employing (2*R*,3*R*)-D-isoleucine derivative instead of (2*R*,3*S*)-D-allo-isoleucine derivative **7**, we have also synthesized 5″-*epi*-spiruchostatin B, $[\alpha]_D^{20}$ −49.3 (*c* = 0.58, MeOH), in the same manner as described for the synthesis of spiruchostatin B (**2**). The ^1H and ^{13}C NMR spectra of the synthesized 5″-*epi*-spiruchostatin B did not match those of natural spiruchostatin B (see ESI†).

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