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-workers¹ 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 (3S,4R)-statine (blue-colored part), D-cysteine (orange-colored part), D-alanine (green-colored part), (3R,4E)-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 coworkers,^{4a}[‡] and two total syntheses of spiruchostatin A (1) have been reported by Ganesan and co-workers⁵ 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 olefination⁹ 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-isolucinal $(7)^7$ 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 KBH₄ (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 fourstep operation involving the formation of S-tetrazole



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

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

product¹² (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 ¹H NMR) in 66% yield. The mixture **19** was subjected to regioselective acetal opening with DIBAL¹⁴ 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 conditions¹⁵ (e.g., PyBOP, EDCI/HOBT, or HATU, rt) failed; the condensation product was produced in good yield $(\sim 80\%)$ 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*-Pr₂NEt (2.5 equiv.) in CH₂Cl₂ 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) KBH₄, 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, K₂CO₃, DMF, rt, 98%; (g) TMSOTf, 2,6-lutidine, CH₂Cl₂, rt; MeOH, rt, 92%; (h) 9, PyBOP, *i*-Pr₂NEt, MeCN, rt, 86%; (i) TMSOTf, 2,6lutidine, CH₂Cl₂, 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-6nitrobenzoic 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, PPh₃, 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, PPh₃, 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, NaHCO₃, CH_2Cl_2 , rt, 88%; (h) NaClO₂, NaH₂PO₄, DMSO-H₂O, rt, 75%; (i) D-alanine methyl ester (12), PyBOP, *i*-Pr₂NEt, CH₂Cl₂, 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*-Pr₂NEt, CH₂Cl₂, -30 °C, 94%; (b) DDQ, CH₂Cl₂/H₂O, rt, 85%; (c) Pd(Ph₃)₄, morpholine, THF, rt, 99%; (d) MNBA, DMAP, CH₂Cl₂, rt, 89%; (e) I₂, MeOH–CH₂Cl₂, rt, 94%; (f) HF·pyridine, pyridine, rt, 93%. HATU = *O*-(7-azabenzotriazol-1yl)-*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 [α]_D –58.6 (c = 0.11, MeOH)}, in 87% yield in two steps. The spectroscopic properties (IR, ¹H and ¹³C NMR, MS) of the synthetic sample **2** were identical with those reported¹ 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

 \ddagger 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 NaBH₄, LiBH₄ and KBH₄ were examined; the best result was obtained by the use of KBH₄.

¶ In the previous two total syntheses of spiruchostatin A (1), Ganesan and co-workers⁵ successfully achieved the crucial macrolactonization using the Yamaguchi method (2,4,6-Cl₃C₆H₂COCl, Et₃N, 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 Shina method (MNBA, DMAP, CH₂Cl₂, 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 synthesized 5"*-epi*-spiruchostatin B (id not match those of natural spiruchostatin B (see ESI[†]).

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