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A solid-phase total synthesis of the cyclic depsipeptide HDAC inhibitor spiruchostatin A

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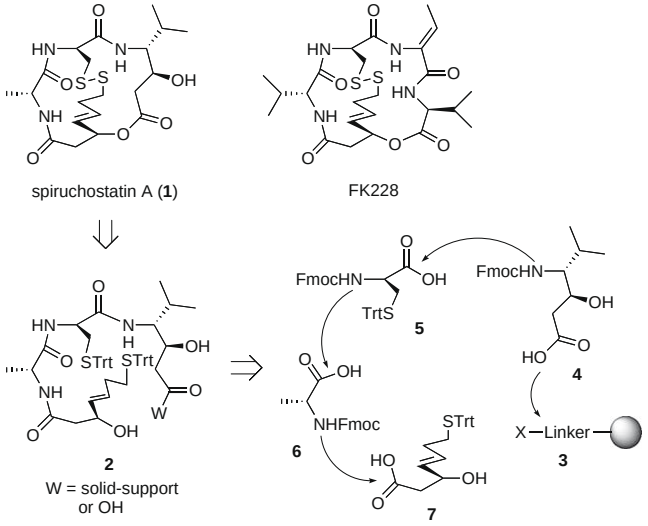
ABSTRACT

Spiruchostatin A, a potent histone deacetylase inhibitor, was efficiently synthesized from (3*S*,4*R*)-4-amino-3-hydroxy-5-methylhexanoic acid utilizing solid-phase peptide elongation with D-cysteine, D-alanine, and (*E*)-3-hydroxy-7-thio-4-heptenoic acid and solution-phase macrolactonization, followed by intramolecular disulfide formation.

Histone deacetylases (HDACs) play an important role in the regulatory post-translational modification of chromatin, and are considered to be promising targets for cancer therapy.[1] A variety of both natural products and synthetic compounds have provided access to potent HDAC inhibitors.[2] SAHA (Vorinostat) produced by Merck[3] is a synthetic example, and is the first approved HDAC inhibitor in the market as a therapeutic anticancer agent. Among natural products, the cyclic depsipeptide FK228[4] is the only one in clinical development, and is currently in Phase II trials.[5] Spiruchostatin A (**1**), isolated from *Pseudomonas* sp., is a more recent discovery with a structure similar to that of FK228 and with more potent activity.[6] Both compounds are bicyclic depsipeptides having an intramolecular disulfide bridge. The postulated mechanism of inhibition is that the disulfide bond of **1** is reduced in cells and the resulting thiol in the longer side chain binds to the catalytic zinc atom in the pocket of HDACs.[4b] We are interested in the potency of the parent skeleton of **1** with diversification of the side chains in the peptide components to discover subtype selective inhibitors.[7–9] As part of our studies on solid-phase combinatorial synthesis based on natural products, we report a solid-phase total synthesis of spiruchostatin A (**1**) suitable for the preparation of analogues.[10]

We previously reported a solution-phase total synthesis of **1**.[11,12] Based on the synthesis, solid-phase assembly of **4**–**7** would afford cyclization precursor **2**, which can undergo macrolactoniza-



Scheme 1. Strategy for the solid-phase-based total synthesis of spiruchostatin A (**1**).

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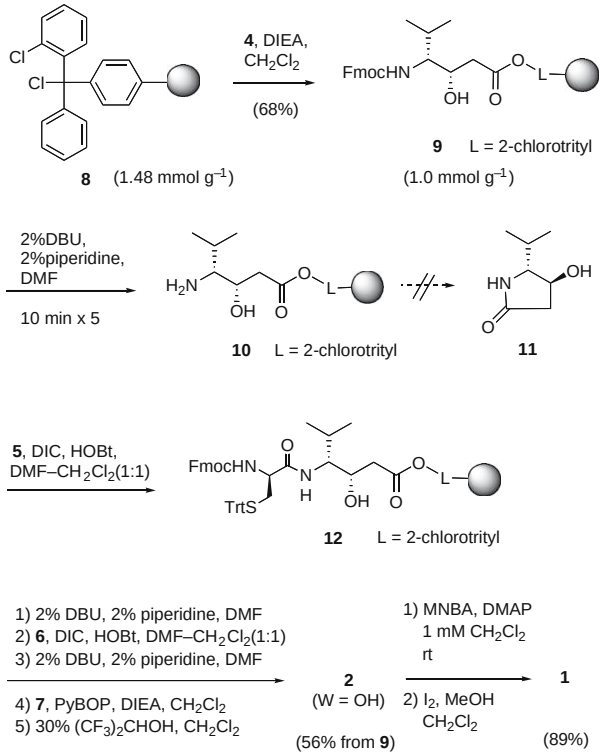
tion, followed by intramolecular disulfide bond formation either on the solid-phase or in the solution phase, leading to **1** (Scheme 1). Our initial attempts for solid-phase total synthesis utilizing safety-catch linkers, such as Kenner's sulfonamide linker[13] and a hydrazinobenzoyl linker[14] were unsuccessful.[15] Then, we chose the 2-chlorotrityl linker for the preparation of the cyclization precursor by an Fmoc strategy. The Fmoc derivative **4**, readily prepared from its Boc derivative,[11] was immobilized on the 2-chlorotrityl chloride resin (**8**) (Scheme 2).[16] The loading amount was determined to be 1.0 mmol g^{-1} by cleavage from the resin (1% CF_3COOH/CH_2Cl_2). Surprisingly, the Fmoc group in **9** could not be removed by conventional methods (20% piperidine/DMF). The complete deprotection, however, was performed by treatment of the well-swollen resins with 2% DBU and 2% piperidine in DMF five times.[17] The magic mixture (20% piperidine in 1% TritonX100 CH_2Cl_2/DMF/NMP = 1:1:1),[18] which is useful for deprotection of the N-terminus of aggregating peptide sequences, was also effective in our case. Fortunately, the 4-amino-3-hydroxy-5-methylhexanoate **10** was stable in such strongly basic conditions without forming lactam **11**, due to the steric hindrance of the trityl group.

The next step, involving condensation of **10** with Fmoc-D-Cys(Trt)-OH (**5**), was also initially problematic. When the resin **10** was treated with a mixture of **5**, diisopropylcarbodiimide (DIC), and 1-hydroxybenzotriazole (HOBt) in DMF–CH_2Cl_2 (4:1), the dipeptide **12** formed was a 77:23 mixture of diastereomers. The α position of the cysteine residue was partially racemized[19] prior to the acylation probably because the condensation of sterically hindered amine **10** would be too slow. We needed to overcome the problem by pre-incubation forming an activated ester in an appropriate mixed solvent. When a mixture of **5**, DIC, HOBt in DMF–CH_2Cl_2 (1:1) was incubated for 5 min in advance, the amidation was successfully performed without observation of severe epimerization (<5%).[20] The coupling with Fmoc–D-Ala–OH (**6**) (DIC/HOBt) and (*E*)-3-hydroxy-7-tritylthio-4-heptenoic acid (**7**)[11,21] (PyBOP/DIEA) was sequentially performed. The linear peptide **2** (W = OH) was cleaved from the polymer-support with 30% hexafluoroisopropyl alcohol in CH_2Cl_2.[22] After simple filtration through silica gel column, **2** (W = OH) was isolated in 56% overall yield.[23] According to our previous synthesis with modification of the reaction conditions, macrolactonization using Shiina's method (MNBA/DMAP/CH_2Cl_2/1 mM/rt),[24] followed by disulfide bond formation (I_2/MeOH) was performed in solution phase to furnish spiruchostatin A (**1**) in 89% overall yield. The spectral data of synthetic **1** were in good accordance with those of the natural product.[11]

In summary, we accomplished a solid-phase total synthesis of spiruchostatin A. The linear peptide was assembled by an Fmoc strategy using the 2-chlorotrityl linker on a polymer-support. After cleavage from the polymer-support, macrocyclization and disulfide formation in solution phase provided spiruchostatin A in a high overall yield. This solid-phase procedure was adapted to the combinatorial synthesis of a library of spiruchostatin analogues based on in silico design. The experimental details and their biological investigation will be reported in due course. Furthermore, it can be employed in the synthesis of other natural product HDAC inhibitors such as FK228 and the recently isolated largazole.[21,25,26]



Scheme 2. The solid-phase total synthesis of spiruchostatin A (**1**). DIEA = *N*,*N*-diisopropylethylamine, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DIC = diisopropylcarbodiimide, HOBt = 1-hydroxybenzotriazole, PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, MNBA = 2-methyl-6-nitrobenzoic anhydride, DMAP = 4-(dimethylamino)pyridine.

Acknowledgments

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15. In the case of a sulfonamide linker, immobilization of *N*-Boc (3*S*,4*R*)-3-amino-4-hydroxy-5-methylhexanoic acid using various condensation reagents

resulted in poor yields (<30%). On the other hand, with the hydrazine linker, immobilization and peptide elongation were successfully performed. However, activation of the linker for cyclization-cleavage from the polymer-support provided a complex mixture in the presence of trityl sulfide.

16. To a suspension of 2-chlorotrityl chloride resin (**8**) (250 mg, 1.48 mmol g^{-1}, 0.37 mmol) in CH_2Cl_2 (3 mL) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added acetyl chloride (0.3 mL) at room temperature. After being shaken for 3 h, the resin was filtered and washed with dry CH_2Cl_2 five times. To the resin were added acid **4** (567 mg, 1.48 mmol) and DIEA (517 μL, 2.96 mmol) in CH_2Cl_2 (3 mL) at room temperature and the mixture was shaken for 24 h. The resin was filtered and washed with CH_2Cl_2 three times, MeOH three times, and CH_2Cl_2 three times.
17. General procedure for removal of the Fmoc group. To the resin (50 mg, 0.050 mmol) in a 3 mL syringe-shaped vessel (Varian Reservoir) was added CH_2Cl_2 (1 mL) and the mixture was shaken for 1 h and filtered. To this resin were added 2% DBU and 2% piperidine in DMF (1 mL). After being shaken for 10 min, the resin was washed with CH_2Cl_2 five times. After this procedure was repeated five times, the resin was washed with CH_2Cl_2 five times and with DMF five times.
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20. General procedure for peptide formation using DIC–HOBt. To a solution of Fmoc-protected amino acid (0.20 mmol) and HOBt (27 mg, 0.20 mmol) in CH_2Cl_2 (0.5 mL) and DMF (0.5 mL) was added DIC (31 μL, 0.20 mmol) and the mixture was stirred for 5 min. This solution was added to the amine-free resin and the mixture was shaken for 2 h. The resin was filtered and washed with DMF three times and with CH_2Cl_2 three times.
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23. To the polymer-supported tripeptide was added a solution of **7** (84 mg, 0.20 mmol), DIEA (35 μL, 0.20 mmol), and PyBOP (104 mg, 0.20 mmol) in CH_2Cl_2 (1 mL) and the mixture was shaken for 24 h. The resin was filtered and washed with CH_2Cl_2 three times, MeOH three times, and CH_2Cl_2 three times. The resin was added 30% $(CF_3)_2CHOH$ in CH_2Cl_2 (1 mL) and the mixture was shaken for 30 min. The resin was filtered and washed with CH_2Cl_2 five times. The filtrate was concentrated in vacuo. The residue was purified by chromatography on silica gel (5% MeOH in $CHCl_3$) to afford desired **2** (27.2 mg, 0.0278 mmol, 56% from **9**).
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