Accepted Manuscript

Total synthesis of cyclodepsipeptide spiruchostatin A on silyl-linked polymer-support

Masahito Yoshida, Ken-ichi Sasahara, Takayuki Doi

PII: S0040-4020(15)01141-2

DOI: 10.1016/j.tet.2015.07.064

Reference: TET 27009

To appear in: *Tetrahedron*

Received Date: 10 July 2015

Revised Date: 24 July 2015

Accepted Date: 25 July 2015

Please cite this article as: Yoshida M, Sasahara K-i, Doi T, Total synthesis of cyclodepsipeptide spiruchostatin A on silyl-linked polymer-support, *Tetrahedron* (2015), doi: 10.1016/j.tet.2015.07.064.

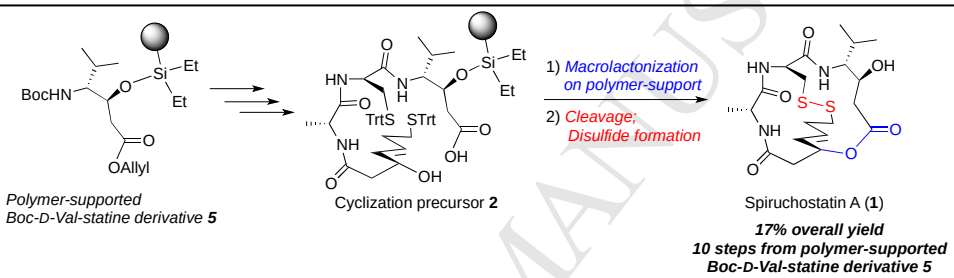
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Total synthesis of cyclodepsipeptide spiruchostatin A on silyl-linked polymer-support

Leave this area blank for abstract info.

Masahito Yoshida, Ken-ichi Sasahara and Takayuki Doi
Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3 Aza-Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan



Tetrahedron
journal homepage: www.elsevier.com

Total synthesis of cyclodepsipeptide spiruchostatin A on silyl-linked polymer-support

Masahito Yoshida[a], Ken-ichi Sasahara[a] and Takayuki Doi[a, *]

[a]*Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3 Aza-Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan*
** Corresponding author. E-mail: doi_taka@mail.pharm.tohoku.ac.jp*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Solid-phase synthesis
Silyl linker
Macrolactonization
Histone deacetylase
Cyclodepsipeptide

ABSTRACT

Solid-phase total synthesis of cyclodepsipeptide spiruchostatin A (**1**) has been achieved. Initially, immobilization of the Boc-D-Val-statine derivative **3** via the hindered β-hydroxy group was efficiently achieved using polymer-supported silyl triflate. Subsequently, the peptide chain was successfully extended on the polymer-support to yield the tetrapeptide cyclization precursor **2** with high purity. 2-Methyl-6-nitrobenzoic anhydride (MNBA)-mediated macrolactonization of **2** proceeded smoothly on the polymer-support to provide macrolactone **12**. Finally, release of the macrolactone from the polymer-support followed by in situ disulfide formation furnished spiruchostatin A (**1**) in 17% overall yield.

1. Introduction

Histone deacetylases (HDACs) are attractive targets for cancer therapy, because inhibition of HDACs can lead to apoptosis, cell cycle arrest, and inhibition of angiogenesis and metastasis.[1] Naturally occurring and synthetic HDAC inhibitors, such as romidepsin (FK228),[2] vorinostat (SAHA),[3] and belinostat (Beleodaq),[4] have recently been approved for clinical use as anticancer agents; several naturally occurring cyclodepsipeptides exhibiting HDAC inhibitory activity have also been evaluated in preclinical trials.[5] These advances indicate that inhibitors of HDAC activity can be viable candidates for the treatment of cancer. Spiruchostatin A (**1**), isolated from *Pseudomonas sp.* by Shin-ya et al. in 2001, is a 15-membered cyclodepsipeptide with an intramolecular disulfide bond and exhibits potent HDAC inhibitory activity (IC_{50} = 3.3 nM).[6] The structure of **1** bears similarities with that of the approved cyclodepsipeptide FK228; therefore, **1** is an attractive lead compound for the development of a drug for treating HDAC-dependent diseases. Because of its unique biological activity, spiruchostatin A (**1**) has been the target of total syntheses with efforts by our and other research group(s).[7, 8] We have synthesized **1** and its derivatives utilizing both solution- and solid-phase synthesis, in addition to the use of automated laboratory technologies.[8] Our previous solid-phase synthesis commenced with the immobilization of the *C*-terminus of the Fmoc-D-Val-statine derivative onto a polymer-support. The advanced intermediate, i.e., the precursor for the cyclization reaction, was prepared on the polymer-support using standard peptide synthesis protocols and then cleaved prior to in-solution cyclization. Therefore, the final macrolactonization and disulfide formation steps were performed in a stepwise fashion in the solution phase. Subsequently, a more concise synthesis of spiruchostatin analogs incorporating a radioactive probe performed entirely on a polymer-support,[9] allowing for the combinatorial synthesis of spiruchostatin analogs, was envisioned.[10] Here we describe the solid-phase total synthesis of spiruchostatin A (**1**), wherein 2-methyl-6-nitrobenzoic anhydride (MNBA)-mediated macrolactonization was performed on the polymer-supported intermediate and the final disulfide forming reaction was performed in situ after cleaving the macrocycle from the polymer-support.

2. Results and Discussion

The nature and position of the amino acid residue immobilized on the polymer support is a critical factor that ensures the successful total synthesis of **1** on the polymer-support. The retrosynthetic scheme for the polymer-supported synthesis of **1** is illustrated in Figure 1. We planned to immobilize the Boc-D-Val-statine derivative **3** via the β-hydroxy group onto the polymer-support using a trialkylsilyl linker,[11] because the silyl linker can be readily cleaved under mild acidic conditions without leading to decomposition of the cleaved product.[12, 13] Furthermore, such a scheme allows for the use of the Boc group for the protection of the amino group in **3**, thereby precluding the formation of the lactam under basic conditions. It is worth noting here that the peptide chain on the polymer-support can be elongated either from the *N*- or *C*-terminus of the D-Val-statine derivative **3**. Based on our prior experience,[8] we considered elongating the peptide at the *N*-terminus of the immobilized D-Val-statine derivative. We envisioned that elongation of the linear peptide on the polymer-support followed by macrolactonization of **2** and subsequent disulfide formation with concomitant cleavage from the polymer-support will afford the desired spiruchostatin A (**1**).

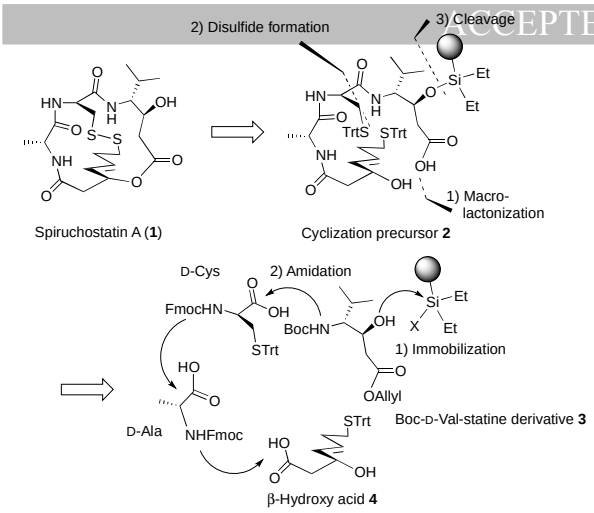
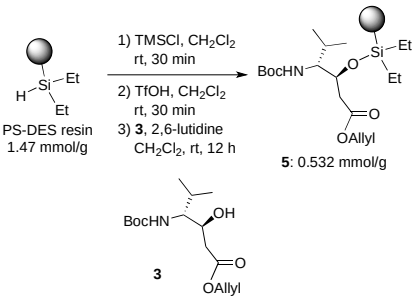


Figure 1. Retrosynthesis of spiruchostatin A (**1**)

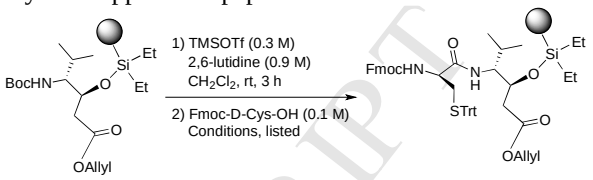
To immobilize the Boc-D-Val-statine derivative **3**[8] onto the polymer support (Scheme 1), commercially available PS-DES resin was first converted to PS-DES-OTf according to a reported protocol.[14] The modified resin was then treated with **3** under basic conditions to afford polymer-supported Boc-D-Val-statine **5**. The loading of resin-bound **5** was determined to be 0.532 mmol· g^{-1} by post-cleavage gravimetric analysis.



Scheme 1. Immobilization of the Boc-D-Val-statine derivative **3** onto the polymer-support

Next, we investigated the conditions for the coupling of **5** with Fmoc-D-Cys-OH to obtain polymer-supported dipeptide **6**. Selective removal of the Boc group in **5** in the presence of the acid-labile silyl ether linkage was carried out by treating **5** with TMSOTf/2,6-lutidine, followed by addition of MeOH for removal of the TMS group on the resulting amine.[15] It was necessary to use excess 2,6-lutidine (0.9 M) for the selective and efficient removal of the Boc group. The resulting free amine was immediately used in the next reaction to preclude any potential γ-lactamization. As Fmoc-D-Cys-OH is readily epimerized under the conditions typically used for condensation, we explored different reaction conditions to couple the polymer-supported amine with Fmoc-D-Cys-OH (Table 1). Our initial attempt based on our previous solid-supported synthesis [*N*, *N*'-diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt), Table 1, entry 1][8] yielded the desired dipeptide **6** in low yields. It is likely that acylation of the amino group is hindered by the bulkiness of both the isopropyl group at the γ-position and the trialkylsilyl group on the β-hydroxy group. The use of 1-hydroxy-7-azabenzotriazole (HOAt) instead of HOBt modestly increased the yield of **6** up to 57% (entry 2). Higher yield (82%) of the desired dipeptide 6 was successfully obtained with the use of *O*-(7-aza-1H-benzotriazol-1-yl)-*N*, *N*, *N*', *N*'-tetramethyluronium hexafluorophosphate (HATU)/*N*, *N*-diisopropylethylamine (DIEA). Epimerization at the α-position of the cysteine residue or the formation of a 5-membered lactam was not observed under these conditions (entry 4). Similar yields were observed when the reaction was performed utilizing 1*H*-benzotriazol-1-yloxy-tri(pyrrolidino)phosphonium hexafluorophosphate (PyBoP)[16]/DIEA (entry 3). Further investigations revealed that when utilizing HATU/DIEA, the reaction was completed within 3 h and the desired intermediate **6** was obtained in 85% yield (entry 5).[17]

Table 1. Optimizing the reaction conditions for the synthesis of polymer-supported dipeptide **6**

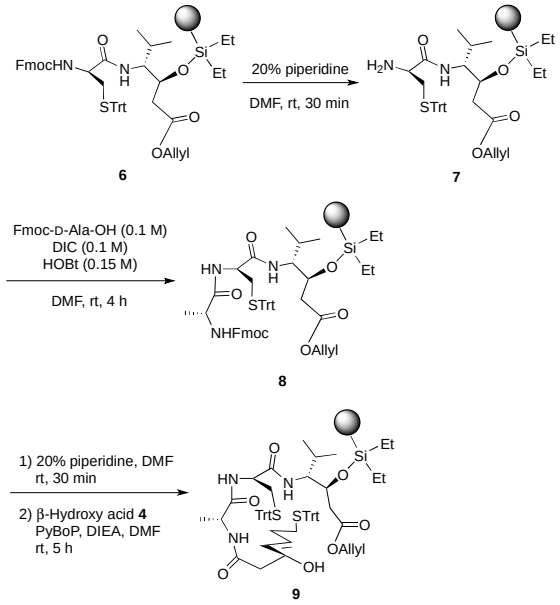


5 **6**

Entry	Reagent (M)	Additive (M)	Time (h)	Yield (%)[a]
1	DIC (0.1)	HOBt (0.15)	12	39
2	DIC (0.1)	HOAt (0.15)	12	57
3	PyBoP (0.1)	DIEA (0.15)	12	81
4	HATU (0.1)	DIEA (0.15)	12	82
5	HATU (0.1)	DIEA (0.15)	3	85

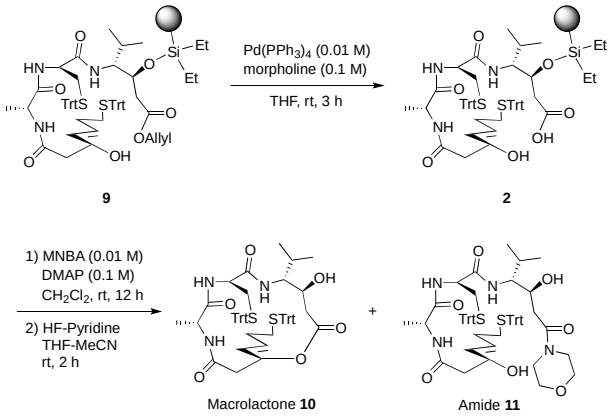
a) Isolated yield was determined after cleavage from the polymer-support (HF-Pyridine, THF-MeCN, rt, 2 h)

Further elongation of dipeptide **6** to afford polymer-supported tetrapeptide **9** was performed according to a protocol established by us previously. The details concerning the reaction conditions are displayed in Scheme 2. Removal of the Fmoc group in **6** under basic conditions (20% piperidine in DMF, rt, 30 min) followed by coupling of the resulting amine **7** with Fmoc-D-Ala-OH utilizing DIC/HOBt afforded polymer-supported tripeptide **8**. Removal of the Fmoc group in **8** and acylation with the β-hydroxy acid derivative **4**[8] in the presence of PyBoP/DIEA at room temperature provided **9** with high purity.



Scheme 2. Scheme for the synthesis of polymer-supported tetrapeptide **9**

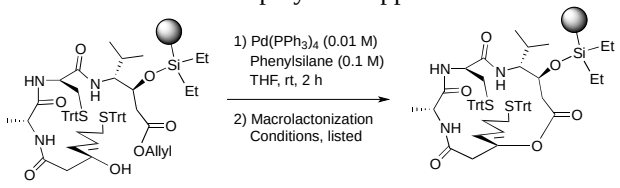
After obtaining the desired tetrapeptide **9**, we next explored the conditions for the removal of the allyl group in **9** and subsequent macrolactonization of the polymer-supported cyclization precursor **2** (Scheme 3). The allyl group was readily removed by treatment with morpholine[18] in the presence of $Pd(PPh_3)_4$. The polymer-supported cyclization precursor **2** was then subjected to macrolactonization in the presence of MNBA/4-(dimethylamino)pyridine (DMAP).[19] However, analysis of the products after cleavage of the reaction products from the polymer-support revealed an inseparable mixture of the desired macrolactone **10** and the unexpected amide **11** that was formed by condensation of the free carboxylic acid with the residual morpholine (left over from the allyl deprotection reaction). It is likely that the polymer-supported deallylated carboxylic acid retained morpholine through ionic interactions, which subsequently resulted in the amidation reaction. Therefore, we considered that use of amines was unsuitable for removal of the allyl group owing to the difficulty associated with the efficient removal of the amine on washing with organic solvents after completion of the reaction.



Scheme 3. Removal of the allyl group in **9** and macrolactonization on the polymer-support.

Owing to the presence of the unexpected amidation product, the conditions for the removal of the allyl group were re-evaluated. After extensive investigations, a combination of $Pd(PPh_3)_4$ and phenylsilane[20] was found to be effective in affording **2** in a pure form. Macrolactonization of the polymer-supported cyclization precursor **2** was subsequently performed utilizing MNBA/DMAP to afford macrolactone **12**. Macrolactonization of **2** was sluggish (a conversion of 60% in 3 h, Table 2, entry 1) when compared with that in the solution phase, which was completed within 1 h.[8] Extending the reaction time improved the conversion of the macrolactonization reaction (6 h, 77% conversion, entry 2). The reaction was complete in 12 h with >95% conversion, affording macrolactone **12** with 60% purity (Table 2, entry 3). It should be noted here that its dimer was not observed, therefore, performing the reaction at high dilutions is not necessary. Efforts directed at improving the purity of the macrolactonization product revealed that increasing the concentration of MNBA improved the purity of **12** (74%, entry 4 vs 60%, entry 3). Further improvement in the purity of the macrolactonization product was achieved when the polymer-support was washed with a solution of the carbamic acid sodium salt[21] (efficiently removes any residual Pd catalyst). With the wash step included in the protocol, the purity of released macrolactone from **12** increased to 86%. In summary, macrolactone **10** (17% overall yield, isolated) was synthesized in 9 steps starting from the polymer-supported Boc-D-Val-statine derivative **5** (entry 5).

Table 2. Optimization of the conditions for macrolactonization on the polymer-support

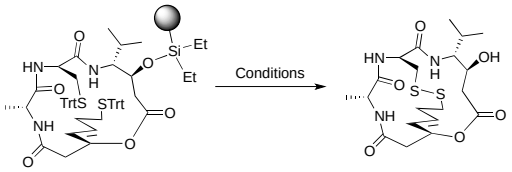


Entry	Conditions	Conv.(%)[a)]	Purity[a)]
1	MNBA (0.01 M) DMAP (0.1 M), rt, 3 h	60	-
2	MNBA (0.01 M) DMAP (0.1 M), rt, 6 h	77	-
3	MNBA (0.01 M) DMAP (0.1 M), rt, 12 h	>95	60
4	MNBA (0.04 M) DMAP (0.1 M), rt, 12 h	>95	74
5[b)]	MNBA (0.04 M) DMAP (0.1 M), rt, 12 h	>99	86 (17)[c)]

a) Determined by HPLC with UV peak area at 214 nm after cleavage from the polymer-support.
b) A solution of sodium diethyldithiocarbamate was used for washing out a trace amount of Pd catalyst after removal of the allyl group.
c) Isolated yield (9 steps from the polymer-supported **5**)

Finally, we explored the reaction conditions leading to the formation of the intramolecular disulfide bond on the polymer-support and resulting in polymer-supported total synthesis of spiruchostain A (**1**). Initial attempts to form the disulfide under oxidative conditions as reported previously (I_2 in CH_2Cl_2/MeOH, rt)[7, 8] did not yield **1** reproducibly on cleavage from the polymer-support (Table 3, entry 1). Despite extensive investigations, efficient conditions for solid-supported formation of the intramolecular disulfide bond were not identified. Therefore, we decided to pursue the intramolecular disulfide bond-forming reaction after first releasing the macrolactone from the polymer support. The silyl ether link between the polymer support and macrolactone in **12** was cleaved using a solution of HF-pyridine in THF, and the resulting solution, containing the released macrolactone **10**, was directly added to a solution of iodine in CH_2Cl_2/MeOH. Unexpectedly, a complex mixture, with no clear indication for the presence of **1**, was obtained (entry 2). It is likely that the pyridine retained in the solution from the prior reaction inhibits the disulfide-forming reaction.

Table 3. Optimization of the intramolecular disulfide formation



Entry	Conditions	Yield[a)]
1	I_2, CH_2Cl_2-MeOH, rt, 30 min	<2
2	HF-Pyridine (0.01 M), THF, rt, 2 h; filtered; I_2, CH_2Cl_2-MeOH, rt, 30 min	Complex mixture
3	5% aqueous HF, CH_2Cl_2-MeOH, rt, 2 h; addition of $CaCO_3$, then filtered; I_2, CH_2Cl_2-MeOH, rt, 30 min	17

a) Isolated yield (10 steps from the polymer-supported **5**)

Therefore, we utilized 5% aqueous HF/CH_2Cl_2/MeOH to release the macrolactone from the polymer support, and the resulting resin-free cleavage solution was neutralized by addition of $CaCO_3$.[22] Finally, the macrolactone-containing neutralized reaction mixture was filtered and the filtrate was directly added to a solution of iodine in CH_2Cl_2/MeOH to afford **1** in 17% overall yield. Because the precursor macrolatone **12** was obtained in 17% overall yield (see, Table 2), the yield of the disulfide formation was quantitative. Spectral data, including specific rotation, of the synthesized spiruchostatin A (**1**) were identical to those of the natural product.[6]

3. Conclusion

We have established a protocol for the solid-supported total synthesis of spiruchostatin A (**1**). The salient features of the synthesis include immobilization of the Boc-D-Val-statine derivative **3** via a silyl linker on the polymer support, sequential coupling of Fmoc-D-Cys-OH, Fmoc-D-Ala-OH, and β-hydroxy acid **4** at the *N*-terminus of **5** to provide polymer-supported tetrapeptide **9**, use of the $PhSiH_3$/Pd catalyst combination for the removal of the allyl group at the *C*-terminus in **9**, and MNBA-mediated macrolactonization of polymer-supported **2** to provide the advanced intermediate macrolactone **12**. It is noteworthy that use of a Pd scavenger for washing the polymer support after removal of the allyl group is a critical factor for improving the purity of the released macrolactone **10**. Finally, two sequential reactions, release of the macrolactone from the polymer support and subsequent disulfide formation, furnished the desired spiruchostatin A (**1**) in 17% overall yield. It should be noted here that this protocol, in addition to the synthesis of the linear peptide as demonstrated previously, allows for macrolactonization to take place on the polymer support; this approach can therefore be used for the synthesis of spiruchostatins, related natural products and their analogues. The synthesis and biological evaluation of spiruchostatin analogs are underway in our laboratory and the results will be reported in due course.

4. Experimental Section

4.1 General Techniques

All commercially available reagents were used as received. Dry THF and CH_2Cl_2 (Kanto Chemical Co.) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light, visualized by *p*-anisaldehyde solution, phosphomolybdic acid solution. Column chromatography and flash column chromatography were carried out with silica gel 60 N (Kanto Chemical Co. 100–210 μm) and silica gel 60 N (Kanto Chemical Co. 40–50 μm), respectively. Gel Permeation Chromatography was performed on LC-9201 (recycling preparative HPLC), with RI-50 refractive index detector and s-3740 ultra violet detector with polystyrene gel column (JAIGEL-1H, 20 mm x 600 mm), using chloroform as a solvent (3.5 mL/min). Preparative HPLC purification was performed on reversed-phase HPLC (Waters HPLC system), and the purity was determined with peak area at UV 214 nm. The column used was Waters X Bridge™ C18 5 μm, 10 x 150 mm. Analysis of the synthetic peptides was performed on reversed-phase HPLC (Waters LC/MS system ZQ-2000), and the purity was determined with peak area at UV 214 or 254 nm. The column used was Waters X Bridge™ C18 3.5 μm, 4.6 x 75 mm. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz and 150 MHz) were recorded on JEOL JNM-AL400 spectrometers and JEOL ECA-600 spectrometers, respectively, in the indicated solvent. Chemical shifts are reported in units parts per million (ppm) relative to tetramethylsilane (0.00 ppm for ^{1}H), chloroform (7.26 ppm for ^{1}H) and chloroform-*d* (77.0 ppm for ^{13}C) when internal standard is not indicated. Multiplicities are reported by the following abbreviations: s; singlet, d; doublet, t; triplet, q; quartet, dd; double doublet, dt; double triplet, ddt; double double triplet, m; multiplet, br; broad, *J*; coupling constants in Hertz. Mass spectra and high-resolusion mass spectra were measured on ThermoScientific™ Exactive™ Plus Orbitap Mass Spectrometer (for ESI). IR spectra were recorded on a JASCO FTIR-8400. Only the strongest and/or structurally important absorption are reported as the IR data afforded in cm^{-1}. Optical rotations were measured with a JASCO P-1010 polarimeter.

4.2 Immobilization of Boc-D-Val statine derivative 3 onto PS-DES resin

4.2.1 Immobilization of 3 onto PS-DES resin

To a PS-DES resin (500 mg) in a 20 mL syringe-shaped vessel (Varian Reservoir) was added CH_2Cl_2 (5.0 mL), and the mixture was shaken for 3 min and filtered. To this resin was added chlorotrimethylsilane (191 μL, 1.50 mmol, 0.3 M) in CH_2Cl_2 (5.0 mL) at room temperature. After the mixture was shaken for 30 min, the resin was filtered and washed three times with CH_2Cl_2. The resulting resin was treated with trifluoromethanesulfonic acid (132 μL, 1.5 mmol, 0.3 M) in CH_2Cl_2 (5.0 mL) at room temperature. After the mixture was shaken for 30 min, the resin was filtered and washed three times with CH_2Cl_2. To this resin was added a solution of **3** (452 mg, 1.50 mmol, 0.3 M) and 2,6-lutidine (230 μL, 2.0 mmol, 0.4 M) in CH_2Cl_2 (5.0 mL) at room temperature. After the mixture was shaken for 12 h, the resin was filtered and washed 10% triethylamine in CH_2Cl_2 x 3, THF/H_2O (1:1) x 3, MeOH x 3 and ether x 3. The resulting resin was dried under reduced pressure to give polymer-supported Boc-D-Val-statine-derivative **5**. IR (resin) 3451, 3372, 3026, 2925, 1738, 1722, 1494, 1454, 1235, 1176, 836, 768 cm^{-1}.

4.2.2 Cleavage of 5

To a polymer-supported **5** (30 mg) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added THF (1.5 mL), and the mixture was shaken for 3 min and filtered. To the suspension in THF□acetonitrile (300 μL, 2:1) was added HF-pyridine (100 μL) at room temperature. After the mixture was shaken for 2 h, the resin was filtered and washed with THF x 3. The filtrate was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, brine and dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (25% ethyl acetate in hexane) to give Boc-D-Val-statine derivative **3** (5.41 mg, 19.2 □mol) as a colorless oil, and the loading amount was determined to be 0.532 mmol•g^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ □5.92 (ddt, 1H, *J* = 16.8, 10.0, 6.0 Hz), 5.33 (dd, 1H, *J* = 16.8, 1.2 Hz), 5.26 (m, 1H), 4.62 (d, 2H, *J* = 6.0 Hz), 4.41 (d, 1H, *J* = 9.6 Hz, NH), 3.94 (m, 1H), 3.54 (m, 1H), 3.22 (brs, 1H, OH), 2.64 (dd, 1H, *J* = 16.4, 3.2 Hz), 2.51 (dd, 1H, *J* = 16.4, 11.2 Hz), 2.12 (m, 1H), 1.44 (s, 9H), 0.95 (d, 3H, *J* = 6.8 Hz), 0.87 (d, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 156.4, 131.8, 118.5, 79.5, 69.1, 65.4, 58.8, 38.3, 28.3, 27.5, 20.1, 16.2; IR ($CHCl_3$) 3444, 3372, 2964, 1717, 1695, 1521, 1367, 1171, 1068, 988 cm^{-1}; $[\alpha]_D^{21}$ □–8.42 (*c* 1.00, $CHCl_3$); HRMS(ESI) calcd for $C_{15}H_{27}NO_5Na$ $[M+Na]^+$ 324.1787, found 324.1776.

4.3 Synthesis of polymer-supported dipeptide 6

4.3.1 Amidation with Fmoc-D-Cys(Trt)-OH

To a polymer-supported Boc-D-Val-statine derivative **5** (50 mg) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added CH_2Cl_2 (1.0 mL) and the mixture was shaken for 3 min and filtered. To the mixture of resin and 2,6-lutidine (79 μL, 0.68 mmol) in CH_2Cl_2 (0.45 mL) was added a solution of trimethylsilyl trifluoromethanesulfonate (41 μL, 0.23 mmol) in CH_2Cl_2 (0.30 mL) at room temperature. After the mixture was shaken for 3 h, MeOH (0.15 mL) was added to the mixture at room temperature. After the mixture was shaken for 10 min, the resin was filtered and washed with CH_2Cl_2 x 3 and DMF x 3 to afforded Boc□deprotected resin. The resulting resin was immediately used for the next reaction.

To the above resin was added DMF (1.0 mL) and the mixture was shaken for 3 min and filtered. To a suspension of resin, Fmoc-D-Cys(Trt)-OH (44 mg, 0.075 mmol, 0.1 M) and DIEA (26 μL, 0.15 mmol, 0.2 M) in DMF (0.75 mL) was added HATU (29 mg, 0.075 mmol, 0.1 M) at room temperature. After the mixture was shaken for 3 h, the resin was filtered and washed with DMF x 3, THF x 3. The resulting resin was dried under reduced pressure to give polymer-supported dipeptide **6**. IR (resin) 3310, 3025, 2919, 1732, 1683, 1668, 1494, 1454, 1252, 1071, 1030, 839, 752 cm^{-1}.

*4.3.2 Cleavage of **6***

To a polymer-supported dipeptide **6** (50 mg) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added THF (1.0 mL) and the mixture was shaken for 3 min and filtered. To a suspension in THF□acetonitrile (300 μL, 2:1) was added HF□pyridine (100 μL) at room temperature. After the mixture was shaken for 2 h, the resin was filtered and washed three times with THF. The filtrate was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, brine and dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (50% ethyl acetate in hexane) to give dipeptide **S1** (17.4 mg, 22.6 □mol, 85%, 3 steps from **5**) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ□ □□□□□□ (t, 2H, *J* = 7.6 Hz), 7.54 (t, 2H, *J* = 8.0 Hz), 7.16□7.45 (m, 19H), 5.84 (m, 1H), 5.80 (d, 1H, *J* = 9.6 Hz, NH), 5.28 (d, 1H, *J* = 17.2 Hz), 5.20 (d, 1H, *J* = 10.4 Hz), 4.89 (d, 1H, *J* = 6.8 Hz, NH), 4.54 (m, 2H), 4.40 (m, 2H), 4.17 (t, 1H, *J* = 6.0 Hz), 3.87 (m, 1H), 3.78 (m, 1H), 3.69 (m, 1H), 3.23 (d, 1H, *J* = 4.4 Hz, OH), 2.66 (d, 2H, *J* = 7.6 Hz), 2.54 (d, 1H, *J* = 16.0 Hz), 2.40 (dd, 1H, *J* = 16.0, 10.0 Hz), 2.08 (m, 1H), 0.81 (d, 3H, *J* = 6.4 Hz), 0.80 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 170.7, 156.1, 144.3, 143.6, 143.6, 141.3, 131.7, 129.5, 128.1, 127.9, 127.8, 127.1, 126.9, 124.9, 124.9, 120.0, 118.6, 68.7, 67.5, 67.0, 65.5, 57.3, 54.2, 47.0, 38.1, 33.1, 27.3, 20.1, 16.2; IR ($CHCl_3$) 3311, 3026, 2959, 1703, 1699, 1661, 1528, 1446, 1033, 987, 741, 701 cm^{-1}; $[\alpha]_D^{26}$□ +□□□ (*c* 0.60, $CHCl_3$); HRMS(ESI) calcd for $C_{47}H_{48}N_2O_6SNa$ $[M+Na]^+$ 791.3131, found 791.3105.

4.4 Synthesis of polymer-supported tripeptide 8

4.4.1 Amidation with Fmoc-D-Ala-OH

To a polymer-supported dipeptide **6** (50 mg) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added DMF (1.0 mL) and the mixture was shaken for 3 min and filtered. The resin was treated with a solution of 20% piperidine in DMF (1.0 mL) at room temperature. After the mixture was shaken for 30 min, the resin was filtered and washed three times with DMF to afforded Fmoc-deprotected resin, and the resulting resin was immediately used for the next reaction.

To the resin was added DMF (1.0 mL) and the mixture was shaken for 3 min and filtered. To a mixture of resin, Fmoc-D-Ala-OH (15 mg, 0.045 mmol, 0.1 M), HOBt (9.2 mg, 0.068 mmol, 0.15 M) and DIEA (15 μL, 0.090 mmol, 0.2 M) in DMF (0.45 mL) was added DIC (7.0 μL, 0.045 mmol, 0.1 M) at room temperature. After the mixture was shaken for 4 h, the resin was filtered and washed three times with DMF and THF, respectively. The resulting resin was dried under reduced pressure to give polymer-supported tripeptide **8**. IR (resin) 3396, 3304, 3028, 2920, 1727, 1685, 1677, 1665, 1494, 1453, 1386, 1251, 1064, 1034, 844, 752 cm^{-1}.

*4.4.2 Cleavage of **8***

To a polymer-supported tripeptide **8** (50 mg) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added THF (1.0 mL) and the mixture was shaken for 3 min and filtered. To a suspension in THF□acetonitrile (300 μL, 2:1) was added HF□pyridine (100 μL) at room temperature. After the mixture was shaken for 2 h, the resin was filtered and washed three times with THF. The filtrate was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, brine and dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (50% ethyl acetate in hexane) to give tripeptide **S2** (19.5 mg, 23.3 □mol, 88%, 5 steps from **5**) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ□ □□□□□ (d, 2H, *J* = 7.2 Hz), 7.51 (t, 2H, *J* = 6.8 Hz), 7.10–7.46 (m, 19H), 6.24 (m, 2H, NH x 2), 5.92 (ddt, 1H, *J* = 17.2, 11.2, 5.0 Hz), 5.29 (d, 1H, *J* = 17.2 Hz), 5.21 (d, 1H, *J* = 11.2 Hz), 5.08 (d, 1H, *J* = 4.8 Hz, NH), 4.56 (d, 2H, *J* = 5.0 Hz), 4.36 (m, 2H), 3.95–4.14 (m, 4H), 3.83 (m, 1H), 3.23 (brs, 1H, OH), 2.94 (m, 1H), 2.59 (d, 1H, *J* = 16.0 Hz), 2.49 (m, 1H), 2.41 (dd, 1H, *J* = 16.0, 10.0 Hz), 2.13 (m, 1H), 1.30 (d, 3H, *J* = 6.8 Hz), 0.80 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 172.1, 170.2, 156.1, 144.3, 143.7, 143.4, 141.3, 141.3, 131.9, 129.4, 128.1, 127.8, 127.8, 127.1, 126.9, 125.0, 120.0, 118.3, 68.7, 67.2, 67.1, 65.3, 57.7, 52.6, 51.0, 47.0, 38.1, 32.7, 27.5, 20.2, 18.3, 16.6; IR ($CHCl_3$) 3289, 3245, 3063, 2961, 1718, 1706, 1701, 1647, 1540, 1401, 1245, 1077, 1034, 741, 701 cm^{-1}; $[\alpha]_D^{26}$□ □□□□ (*c* 0.85, $CHCl_3$); HRMS(ESI) calcd for $C_{50}H_{53}N_3O_7SNa$ $[M+Na]+$ 862.3502, found 862.3476.

4.5 Synthesis of polymer-supported tetrapeptide 9

*4.5.1 Amidation with β-hydroxy acid derivative **4***

To a polymer-supported tripeptide **8** (50 mg) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added DMF (1.0 mL) and the mixture was shaken for 3 min and filtered. The resin was treated with a solution of 20% piperidine in DMF (1.0 mL) at room temperature. After the mixture was shaken for 30 min, the resin was filtered and washed three times with DMF to afforded Fmoc-deprotected resin, and the resulting resin was immediately used for the next reaction.

To the above resin was added DMF (1.0 mL) and the mixture was shaken for 3 min and filtered. To a suspension of resin, β-hydroxy acid **4**[8] (31 mg, 0.075 mmol, 0.10 M) and DIEA (26 μL, 0.15 mmol, 0.20 M) in DMF (0.75 mL) was added PyBoP (59 mg, 0.11 mmol, 0.15 M) at room temperature. After the mixture was shaken for 12 h, the resin was filtered and washed three times with DMF and THF, respectively. The resulting resin was dried under reduced pressure to give polymer-supported tetrapeptide **9**. IR (resin) 3628, 3307, 3026, 2920, 1735, 1671, 1510, 1493, 1250, 1183, 1072, 1014, 843, 759, 698 cm^{-1}.

*4.5.2 Cleavage of **9***

To a polymer-supported tetrapeptide **9** (50 mg) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added THF (1.0 mL) and the mixture was shaken for 3 min and filtered. To the suspension in THF□acetonitrile (300 μL, 2:1) was added HF□pyridine (100 μL) at room temperature. After the mixture was shaken for 2 h, the resin was filtered and washed three times with THF. The filtrate was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, brine and dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on preparative TLC (10% methanol in chloroform) to give tetrapeptide **S3** (14.2 mg, 13.9 □mol, 52%, 7 steps from **5**) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ□7.15–7.47 (m, 30H), 6.97 (d, 1H, *J* = 7.6 Hz, NH), 6.18 (d, 1H, *J* = 9.6 Hz, NH), 6.09 (d, 1H, *J* = 6.4 Hz, NH), 5.87 (ddt, 1H, *J* = 17.6, 10.8, 5.6 Hz), 5.41 (m, 1H), 5.29 (m, 2H), 5.20 (d, 1H, *J* = 10.8 Hz), 4.57 (m, 2H), 4.32 (m, 1H), 4.26 (t, 1H, *J* = 6.8 Hz), 3.98–4.12 (m, 2H), 3.81 (m, 1H), 3.79 (d, 1H, *J* = 4.8 Hz, OH), 2.78 (brs, 1H, OH), 2.73 (dd, 1H, *J* = 13.2, 6.8 Hz), 2.59 (dd, 1H, *J* = 16.8, 2.4 Hz), 2.39–2.47 (m, 2H), 2.22 (dd, 1H, *J* = 14.0, 2.4 Hz), 2.16–2.24 (m, 3H), 2.02–2.12 (m, 2H), 1.34 (d, 3H, *J* = 7.2 Hz), 0.88 (d, 3H, *J* = 6.8 Hz), 0.88 (d, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.1, 171.8, 170.5, 144.8, 144.2, 132.3, 131.9, 130.4, 129.5, 129.4, 128.1, 127.9, 126.9, 126.6, 118.3, 69.8, 68.7, 66.9, 66.6, 65.3, 57.9, 52.7, 50.0, 43.9, 38.0, 33.0, 31.3, 31.2, 27.7, 20.1, 17.4, 16.9; IR ($CHCl_3$) 3275, 2959, 2929, 1735, 1623, 1529, 1443, 1260, 1032, 744, 699 cm^{-1}; $[\alpha]_D^{28}$ □□□□ (*c* 0.38, MeOH); HRMS(ESI) calcd for $C_{61}H_{67}N_3O_7S_2Na$ [M+Na]+ 1040.4318, found 1040.4296.

4.6 Synthesis of polymer-supported macrolactone 12

*4.6.1 Removal of the protecting groups in **9** and macrolactonization on polymer-support*

To a polymer-supported tetrapeptide **9** in a 6 mL syringe-shaped vessel (Varian Reservoir) was added THF (degassed, 1.0 mL) and the mixture was shaken for 3 min and filtered. To a suspension of resin and phenylsilane (9.2 μL, 0.075 mmol, 0.10 M) in THF (degassed, 0.75 mL) was added tetrakis(triphenylphosphine)palladium(0) (8.7 mg, 0.0075 mmol, 0.010 M) at room temperature. After the mixture was shaken for 2 h, the resin was filtered and washed three times with THF, 3% (w/v) sodium diethyldithiocarbamate in DMF, DMF and dichloromethane, respectively. The resulting resin was immediately used for the next reaction.

To the crude resin was added CH_2Cl_2 (1.0 mL) and the mixture was shaken for 3 min and filtered. To the suspension of resin and DMAP (9.2 mg, 0.075 mmol, 0.1 M) in CH_2Cl_2 (0.75 mL) was added MNBA (10 mg, 0.03 mmol, 0.04 M) at room temperature. After the mixture was shaken for 12 h, the resin was filtered and washed three times with CH_2Cl_2. The resulting resin was dried under reduced pressure to give polymer-supported macrolactone **12**. IR (resin) 3287, 3026, 2921, 1735, 1647, 1493, 1452, 1250, 1074, 1032, 842, 737, 694 cm^{-1}.

*4.6.2 Cleavage of **12***

To a polymer-supported macrolactone **12** (50 mg) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added THF (1.0 mL) and the mixture was shaken for 3 min and filtered. To a suspension in THF□acetonitrile (300 μL, 2:1) was added HF□pyridine (100 μL) at room temperature. After the mixture was shaken for 2 h, the resin was filtered and washed three times with THF. The filtrate was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, brine and dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on preparative TLC (9% methanol in chloroform) to give macrolactone **10** (4.2 mg, 4.4 □mol, 17%, 9 steps from **5**) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ□7.18–7.50 (m, 30H), 6.92 (d, 1H, *J* = 7.2 Hz, NH), 6.77 (m, 1H, NH), 5.91 (m, 1H, NH), 5.58–5.69 (m, 2H), 5.34 (dd, 1H, *J* = 15.6, 6.4 Hz), 4.39 (m, 1H), 4.23 (m, 1H), 3.78 (m, 1 H), 3.22–3.41 (m, 1H), 2.39–2.69 (m, 5H), 2.21 (t, 2H, *J* = 7.2 Hz), 2.03–2.12 (m, 3H), 1.36 (d, 3H, *J* = 7.2 Hz), 0.96 (d, 3H, *J* = 6.4 Hz), 0.88 (d, 3H, *J* = 6.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.8, 171.9, 170.4, 169.9, 144.4, 144.5, 132.8, 132.1, 132.0, 129.6, 129.5, 128.0, 127.9, 126.9, 126.7, 70.9, 68.2, 66.8, 66.6, 60.7, 50.0, 41,1, 37.5, 32.0, 31.3, 31.1, 29.7, 28.8, 20.4, 19.2, 17.8; IR ($CHCl_3$) 3301, 2926, 1734, 1669, 1540, 1444, 1260, 1182, 1034, 743, 700 cm^{-1}; $[\alpha]_D^{25}$ □–□6.0 (*c* 0.300, $CHCl_3$); HRMS(ESI) calcd for $C_{58}H_{61}N_3O_6S_2Na$ $[M+Na]^+$ 982.3899, found 982.3876.

4.7 Synthesis of spiruchostatin A (1)

To a polymer-supported macrolactone **12** (50 mg) in a 6 mL syringe-shaped vessel (Varian Reservoir) was added CH_2Cl_2 (1.0 mL) and the mixture was shaken for 3 min and filtered. The resin was treated 5% hydrogen fluoride solution (47% aqueous hydrogen fluoride was attenuated with CH_2Cl_2/MeOH (2:1)) at room temperature. After the mixture was shaken for 2 h, to the mixture was added $CaCO_3$ (150 mg) at room temperature. After the mixture was shaken for 20 min, the resin was filtered. The filtrate was subsequently added to a solution of iodine (48 mg, 0.38 mmol) in CH_2Cl_2 (10 mL) at room temperature. After being stirred for 30 min, the reaction mixture was quenched with 10% aqueous $Na_2S_2O_3$ and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and filtrated. The filtrate was concentrated in vacuo. The residue was purified by reverse-phase HPLC to give spiruchostatin A (**1**) (2.1 mg, 4.4 □mol, 17%, 10 steps from **5**) as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ□7.40 (d, 1H, *J* = 6.8 Hz, NH), 6.70 (d, 1H, *J* = 9.2 Hz, NH), 6.40 (m, 1H), 5.84 (m, 1H, NH), 5.65 (d, 1H, *J* = 15.2 Hz), 5.50 (m, 1H), 4.93 (dt, 1H, *J* = 8.8, 4.0 Hz), 4.60 (m, 1H), 4.28 (dq, 1H, *J* = 7.2, 4.0 Hz), 3.39 (m, 2H), 3.24 (m, 1H), 3.15 (m, 1H), 2.69–2.80 (m, 5H), 2.56 (d, 2H, *J* = 13.2 Hz), 2.36–2.51 (m, 2H), 1.52 (d, 3H, *J* = 7.2 Hz), 1.04 (d, 3H, *J* = 6.8 Hz), 0.93 (d, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 170.9, 170.6, 169.0, 133.6, 128.6, 70.5, 69.3, 63.8, 63.7, 52.3, 52.2, 40.8, 40.7, 39.6, 29.6, 20.6, 20.6, 19.6, 16.6; IR ($CHCl_3$) 3368, 3333, 2954, 2924, 1733, 1667, 1660, 1652, 1537, 1432, 1265, 1160, 752 cm^{-1}; $[\alpha]_D^{29}$□–60 (*c* 0.045, MeOH) [lit.[6] $[\alpha]_D$ –63.6 (*c* 0.14, MeOH)]; HRMS(ESI) calcd for $C_{20}H_{31}N_3O_6S_2Na$ $[M+Na]^+$ 496.1552, found 496.1534.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas “Chemical Biology of Natural Products” from The Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 2301). This work was partially supported by Platform for Drug Discovery, Informatics, and Structural Life Science from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References and Notes

1. a) Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat. Rev. Drug Discov.* **2006**, *5*, 769–784. b) Xu, W. S.; Parmigiani, R. B.; Marks, P. A. *Oncogene* **2007**, *26*, 5541–5552.
2. Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K.–H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. *Cancer Res.* **2002**, *62*, 4916–4921.
3. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188–193.
4. Plumb, J. A.; Finn, P. W.; Williams, R. J.; Bandara, M. J.; Romero, M. R.; Watkins, C. J.; La Thangue, N. B.; Brown, R. *Mol. Cancer Ther.* **2003**, *2*, 721–728.
5. Mottamal, M.; Zheng, S.; Huang, T. L.; Wang, G. *Molecules* **2015**, *20*, 3898–3941.
6. Masuoka, Y.; Nagai, A.; Shin-ya, K.; Furihata, K.; Nagai, K.; Suzuki, K.; Hayakawa, Y.; Seto, H. *Tetrahedron Lett.* **2001**, *42*, 41–44.
7. a) Yurek-George, A.; Habens, F.; Brimmell, M.; Packham, G.; Ganesan, A. *J. Am. Chem. Soc.* **2004**, *126*, 1030–1031. b) Takizawa, T.; Watanabe, K.; Narita, K.; Kudo, K.; Oguchi, T.; Abe, H.; Katoh, T. *Heterocycles* **2008**, *76*, 275–290. c) Calandra, N. A.; Cheng, Y. L.; Kocak, K. A.; Miller, J. S. *Org. Lett.* **2009**, *11*, 1971–1974. d) Narita, K.; Kikuchi, T.; Watanabe, K.; Takizawa, T.; Oguchi, T.; Kudo, K.; Matsuhara, K.; Abe, H.; Yamori, T.; Yoshida, M.; Katoh, T. *Chem. Eur. J.* **2009**, *15*, 11174–11186.
8. a) Doi, T.; Iijima, Y.; Shin-ya, K.; Ganesan, A.; Takahashi, T. *Tetrahedron Lett.* **2006**, 47, 1177–1180. b) Iijima, Y.; Munakata, A.; Shin-ya, K.; Ganesan, A.; Doi, T.; Takahashi, T. *Tetrahedron Lett.* **2009**, *50*, 2970–2972. c) Fuse, S.; Okada, K.; Iijima, Y.; Munakata, A.; Machida, K.; Takahashi, T.; Takagi, M.; Shin-ya, K.; Doi, T. *Org. Biomol. Chem.* **2011**, *9*, 3825–3833. d) Doi, T.; Otaka, H.; Umeda, K.; Yoshida, M. *Tetrahedron* **2015**, doi:10.1016/j.tet.2015.05.051.
9. Solid-phase synthesis is also useful tool for preparation of radiolabelled peptidic molecules because the labeling reagent would be readily recovered after the coupling reaction. a) Wiegand, H.; Wirz, B.; Schweitzer, A.; Gross, G.; Perez, M. I. R.; Andres, H. *Chem. Biodivers.* **2004**, *1*, 1812–1828. b) Marik, J.; Hausner, S. H.; Fix, L. A.; Gagnon, M. K. J.; Sutcliffe, J. L. *Bioconjugate Chem.* **2006**, *17*, 1017–1021.
10. a) Flanigan, E.; Marshall, G. R. *Tetrahedron Lett.* **1970**, *27*, 2403–2406. b) Isied, S. S.; Kuehn, C. G.; Lyon, J. M.; Merrifield. R. B. *J. Am. Chem. Soc.* **1982**, *104*, 2632–2634. c) Rovero, P.; Quartara, L.; Fabbri, G. *Tetrahedron Lett.* **1991**, *32*, 2639–2642. d) Lloyd-Williams, P.; Jou, G.; Albericio, F.; Giralt, E. *Tetrahedron Lett.* **1991**, *32*, 4207–4210. e) Tizeciak, A.; Bannwarth, W. *Tetrahedron Lett.* **1992**, *33*, 4557–4560. f) Kates, S. A.; Solé, N. A.; Johnson, C. R.; Hudson, D.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 1549–1552.
11. Hu, Y.; Porco, Jr. J. A.; Labadie, J. W.; Gooding, O. W.; Trost, B. M. *J. Org. Chem.* **1998**, *63*, 4518–4521.
12. a) Plunkett, M. J.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 6006–6007. b) Doi, T.; Hijikuro, I.; Takahshi, T. *J. Am. Chem. Soc.* **1999**, *121*, 6749–6750. c) Doi, T.; Sugiki, M.; Yamada, H.; Takahashi, T.; Porco, J. A. Jr. *Tetrahedron Lett.* **1999**, *40*, 2141–2144. d) Spring, D. R.; Krishnan, S.; Schreiber, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 5656–5657. e) Briehn, C. A.; Kirschbaum, T.; Baeuerle, P. *J. Org. Chem.* **2000**, *65*, 352–359. f) Ishii, A.; Hojo, H.; Kobayashi, A.; Nakamura, K.; Nakahara, Y.; Ito, Y.; Nakahara, Y. *Tetrahedron* **2000**, *42*, 1463–1466. g) Wang, B.; Chen, L.; Kim, K. *Tetrahedron Lett.* **2001**, *42*, 1463–1466. h) Matsuda, A.; Doi, T. Tanaka, H.; Takahashi, T. *Synlett* **2001**, 1101–1104. i) Hijikuro, I.; Doi, T.; Takahashi, T. *J. Am. Chem. Soc.* **2001**, *123*, 3716–3722. j) Paterson, I.; Temal-Laieb, T. *Org. Lett.* **2002**, *4*, 2473–2476. k) Gu, W.; Liu, S.; Silverman, R. B. *Org. Lett.* **2002**, *4*, 4174–4174. l) Kobori, A.; Miyata, K.; Ushioda, M.; Seio, K.; Sekine, M. *J. Org. Chem.* **2002**, *67*, 476–485. m) Kobori, A.; Miyata, K.; Ushioda, M.; Seio, K.; Sekine, M. *Chem. Lett.* **2002**, *31*, 16–17. n) Moura-letts, G.; DiBlasi, H. M.; Bauer, R. A.; Tan, D. S. *P. Natl. Acad. Sci. USA* **2011**, *108*, 6745–6750. o) Strack, M.; Metzler-Nolte, N.; Albaba, H. B. *Org. Lett.* **2013**, *15*, 3126–3129.
13. Silyl linker has also been utilized for the peptide synthesis, see: a) Lee, Y.; Silverman, R. B. *J. Am. Chem. Soc.* **1999**, *121*, 8407–8408. b) Lee, Y.; Silverman, R. B. *Org. Lett.* **2000**, *2*, 303–306. c) Gu, W.; Liu, S.; Silverman, R. B. *Org. Lett.* **2002**, *4*, 4171–4174. d) Gu, W.; Silverman, R. B. *J. Org. Chem.* **2003**, *68*, 8774–8779. e) Liu, S.; Gu, W.; Lo, D.; Ding, X.–Z.; Ujiki, M.; Adrian, T. E.; Soff, G. A.; Silverman, R. B. *J. Med. Chem.* **2005**, *48*, 3630–3638. f) Doi, T.; Hoshina, Y.; Mogi, H.; Yamada, Y.; Takahashi, T. *J. Comb. Chem.* **2006**, *8*, 571–582. g) Hoshina, Y.; Yamada, Y.; Tanaka, H.; Doi, T.; Takahashi, T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2904–2907.
14. a) Smith, E. M. *Tetrahedron Lett.* **1999**, *40*, 3285–3288. b) Hu, Y.; Porco, Jr. J. A. *Tetrahedron Lett.* **1999**, *40*, 3289–3292.
15. a) Sakaitani, M.; Ohfune, Y. *Tetrahedron Lett.* **1985**, *26*, 5543–5546. b) Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870–876. c) Zhang, A. J.; Russell, D. H.; Zhu, J.; Burgess, K. *Tetrahedron Lett.* **1998**, *39*, 7439–7442.
16. PyBoP: benzotriazolyloxy-tris(pyrrolidino)phosphonium hexafluorophosphate. Coste, J. Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205–208.
17. When the coupling of Fmoc-D-Cys-OH and Boc-D-Val-statine derivative was performed in solution-phase, we have observed that yield of dipeptide was gradually decreased in a time-dependent manner due to removal of the Fmoc group under basic conditions. Therefore, it is conceivable that yield of **6** in 12 h would be slightly lower than that in 3 h (entry 5 vs entry 4).
18. Lloyd–Williams, P.; Lou, G.; Albericio, F.; Giralt, E. *Tetrahedron Lett.* **1991**, *32*, 4207–4210.
19. a) Shiina, I.; Ibuka, R.; Kubota, M. *Chem. Lett.* **2002**, 286–287. b) Shiina, I. Kubota. M. Ibuka, R. *Tetrahedron Lett.* **2002**, *43*, 7535–7539. c) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822–1830. d) Shiina, I.; Hashizume, M.; Yamai, Y.; Oshiumi, H.; Shimazaki, T.; Takasuna, Y.; Ibuka, R. *Chem. Eur. J.* **2005**, *11*, 6601–6608. e) Shiina, I. *Bull. Chem. Soc. Jpn.* **2014**, *87*, 196–233.
20. Dessolin, M.; Guillerez, M.–G.; Thieriet, F.; Guibé, F.; Loffet, A. *Tetrahedron Lett.* **1995**, *36*, 5741–5744.
21. Kates, S. A.; de la Torre, B. G.; Eritja, R.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 1033–1034.
22. Kaburaki and Kishi reported that the use of calcium carbonate is effective to scavenge hydrogen fluoride (HF) and the resulting CaF_2 and H_2O are readily removed by filtration and evaporation, see; Kaburagi, Y.; Kishi, Y. *Org. Lett.* **2006**, *9*, 723–726.

Supplementary data

Copies of ^{1}H and ^{13}C NMR spectra for the synthetic spiruchostatin A (**1**) and the cleavage products. This material is available free of charge via the Internet.