



Cite this: *Chem. Commun.*, 2019, 55, 11956

Received 9th August 2019,
Accepted 6th September 2019

DOI: 10.1039/c9cc06169j

rsc.li/chemcomm

Total synthesis of verucopeptin, an inhibitor of hypoxia-inducible factor 1 (HIF-1)[†]

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Verucopeptin is an inhibitor of hypoxia-inducible factor 1 (HIF-1), which is a promising target for cancer chemotherapy. Here, we report the first total synthesis of verucopeptin via condensation of the depsipeptide core and the polyketide side chain unit including three branched methyl groups after the synthesis of each segment.

Tumor cells are usually exposed to hypoxic conditions or a starvation state. For survival and cell growth of tumor cells in these severe environments, hypoxia-inducible factor 1 (HIF-1) plays an important role as a transcriptional factor that regulates the expression of a number of genes involved in angiogenesis, gluconeogenesis, and metastasis.^{1,2} Therefore, HIF-1 is a promising target for cancer chemotherapy, and studies on HIF-1 by using a chemical and biological approach have been carried out in our group.³

For the purpose of identifying new HIF-1 inhibitors, we screened natural resources using a hypoxia-responsive luciferase reporter gene assay and we re-discovered verucopeptin (**1**) as a potent inhibitor of HIF-1 from a culture broth of *Streptomyces* sp. KUSC_A08.^{3b,4} Verucopeptin (**1**) consists of a cyclic depsipeptide core and a polyketide side chain including three branched methyl groups. Unlike the other derivatives such as azinotricin^{5a} and dentigerumycin^{5b} **1** has a unique feature in that it exists in equilibrium between an open form and a closed form via its tetrahydropyran (THP) ring.

Recently, we determined the stereochemistry of **1** and revealed that **1** inhibits HIF-1 via the mTORC1 pathway.^{3b} For elucidation of the mode of action of **1** in more detail, investigation of various derivatives of **1** is necessary. Although the synthesis of the peptide core has been reported by the Hale group,⁶ the total synthesis of **1** has not been achieved.

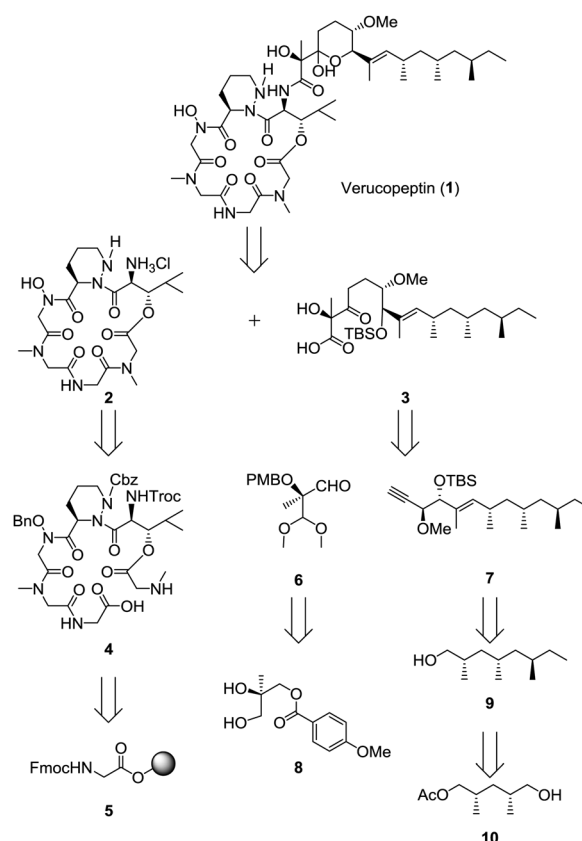
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[†] Electronic supplementary information (ESI) available: Supporting figures, procedures for the syntheses of verucopeptin (**1**) and copies of ¹H- and ¹³C-NMR spectra. See DOI: 10.1039/c9cc06169j

To investigate its chemical and biological properties, we carried out the total synthesis of verucopeptin (**1**) and report it herein.

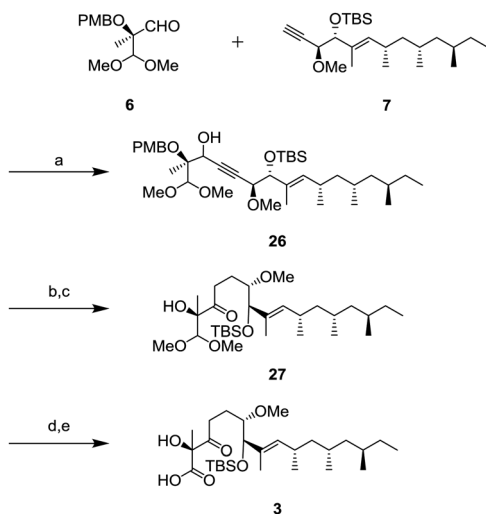
The summary of the total synthesis of **1** is shown in Scheme 1. Condensation of the depsipeptide core **2** and the side chain **3** followed by removal of the protective group for construction of the THP ring was carried out in the final stage. Carboxylic acid **3** was obtained by coupling alkyne **7** and aldehyde **6** following structural conversions. Aldehyde **6** was



Scheme 1 Retrosynthesis of verucopeptin (**1**).



Scheme 5 Synthesis of aldehyde **6**; (a) *p*-methoxybenzaldehyde dimethyl acetal, PPTS, CH₂Cl₂, r.t., 18 h, 98%; (b) NaOMe, MeOH, r.t., 18 h; (c) TBSCl, imidazole, CH₂Cl₂, r.t., 16 h, 92% (2 steps); (d) DIBAL-H, CH₂Cl₂, 0 °C, 4 h, 36%; (e) SO₃-pyridine, NEt₃, DMSO, r.t., 2 h, 82%; (f) PPTS, trimethyl orthoformate, CH₂Cl₂, r.t., 16 h; (g) TBAF, THF, r.t., 16 h, 91% (2 steps); and (h) SO₃-pyridine, NEt₃, DMSO, r.t., 4 h, 69%.



Scheme 6 Synthesis of carboxylic acid **3**; (a) LHMDS, -78 °C to 0 °C, 1 h, 95%; (b) DMP, CH₂Cl₂, r.t., 1 h, 96%; (c) Pd/C, AcOEt, r.t., 3 h; (d) TMSOTf, 2,4,6-collidine, CH₂Cl₂, 0 °C, 5 h; and (e) NaClO₂, NaH₂PO₄ monohydrate, 2-methyl-2-butene, H₂O, *t*BuOH, r.t., 3 h, 69% (3 steps).

We started the synthesis of the depsipeptide core as shown below. The building blocks for SPPS are described in Scheme 7. Although resin **5** and Fmoc sarcosine **28** were commercially available, *N*-hydroxy glycine **29**, piperazic acid **30** and dipeptide **31** needed to be synthesized. Among them, **30**¹⁷ and **31**⁶ were synthesized by previously reported methods. However, *N*-hydroxy glycine **29** was an unknown compound, and thus it needed to be synthesized. From the *tert*-butyl ester **32**, protection of the amino group with the Fmoc group and subsequent removal of the *tert*-butyl group under acidic conditions gave the amino acid **29**.

The SPPS was started from the Fmoc-glycine loaded Wang resin **5** (Scheme 8). Two cycles of removal of the Fmoc group using 20% piperidine/DMF and introduction of the amino acid (Fmoc-sarcosine **28** or hydroxy glycine **29**) in the presence of a condensing agent gave the tripeptide **33**. Then, synthesis of the

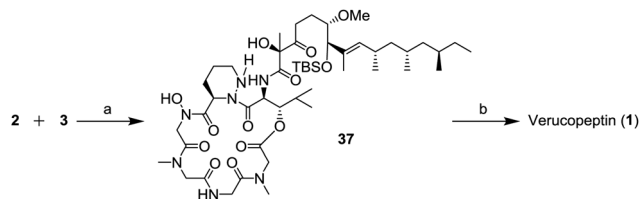


Scheme 7 Building blocks of the depsipeptide core **3**; (a) FmocCl, sat. NaHCO₃ aq., 1,4-dioxane, r.t., 1 h, 83% and (b) TFA, CH₂Cl₂, r.t., 1 h, 90%.



Scheme 8 Synthesis of cyclic depsipeptide **2**; (a) 20% piperidine/DMF, r.t., 1 h; (b) **28**, DIC, HOBT, DIEA, DMF, r.t., 2 h; (c) 20% piperidine/DMF, r.t., 1 h; (d) **29**, HATU, HOAt, DIEA, DMF, r.t., 1 h; (e) 20% piperidine/DMF, r.t., 1 h; (f) **30**, HATU, HOAt, DIEA, DMF, r.t., 12 h; (g) (COCl)₂ benzene, r.t., 2.5 h; (h) **35**, AgCN, toluene, 60 °C, 20 min; (i) 98% TFA, r.t., 1 h, 36% (overall yield of Fmoc-SPPS); (j) HATU, *N*-ethyl morpholine, CH₂Cl₂ (0.0004 M), 0 °C to r.t., 48 h, 54%; (k) Zn, AcOH/H₂O, r.t., 2 h; (l) CbzCl, 10% Na₂CO₃ aq., THF, r.t., 2 h, 79% (2 steps); and (m) Pd/C (degussa type), H₂, AcCl, MeOH, r.t., 24 h, 92%.

tetrapeptide **34** was examined. After deprotection of the Fmoc group, the resulting free amine was condensed with piperazic acid **30** using *O*-(7-aza-1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), and *N,N*-diisopropylethylamine (DIEA). Because the reactivity of the *N*α-amine in the piperazic acid of **34** was remarkably low,¹⁸ the protection of the *N*α-amine in **30** was not necessary under these conditions. In the final step of the SPPS, introduction of dipeptide **31** was investigated to generate the hexapeptide **36**. After conversion of **31** to the acid chloride **35**,^{6,18} a condensation reaction with **34** in toluene at 60 °C in the presence of AgCN gave **36**. Next, removal from the resin and deprotection of the *tert*-butoxycarbonyl (Boc) group was conducted. Treatment with 98% trifluoroacetic acid (TFA) and purification by HPLC gave a pure linear peptide **4** in 36% yield



Scheme 9 Completion of the total synthesis of verucopeptin (**1**); (a) PyBop, NEt₃, CH₂Cl₂, -78 °C to r.t., 3 h and (b) 1 N HCl aq., THF, r.t., 8 h, 23% (2 steps).

from resin **5**. Synthesis of the peptide core **2** from **4** was carried out by Hale's method.⁶ Macrolactamization, replacement of the 2,2,2-trichloroethoxycarbonyl (Troc) group to the benzyloxy-carbonyl (Cbz) group, and removal of a benzyl group and two Cbz groups gave **2**, whose spectral data were consistent with those in Hale's report.

The final stage of the synthesis is shown in Scheme 9. The depsipeptide core **2** and the side chain unit **3** were combined in the presence of 1*H*-benzotriazol-1-yloxy-tri(pyrrolidino)phosphonium hexafluorophosphate (PyBop) and trimethylamine (NEt₃) to afford compound **37**. In the last step, the TBS group of **37** was removed to generate verucopeptin (**1**) in 23% (2 steps). The spectral data of synthetic **1** were consistent with those of the natural compound.

In conclusion, verucopeptin (**1**) was successfully synthesized. First, the side chain unit **3** was created *via* construction of six chiral centers, whereas the Fmoc-SPPS unit was subjected to macrolactamization to obtain the depsipeptide core **2**. In the final stage, **2** and **3** were coupled to complete the first total synthesis of **1**. Taking advantage of the convergent properties in our present synthetic scheme, derivatization of the synthetic product by changing the partial structure in the units is underway for a structure–activity relationship (SAR) study and elucidation of its mode of action.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan (17H06401 and 19H02840) and a research grant (JP19fk0310112 and JP19am0101092) from the Japan Agency of Medical Research and Development (AMED), Japan.

Conflicts of interest

There are no conflicts to declare.

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