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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.

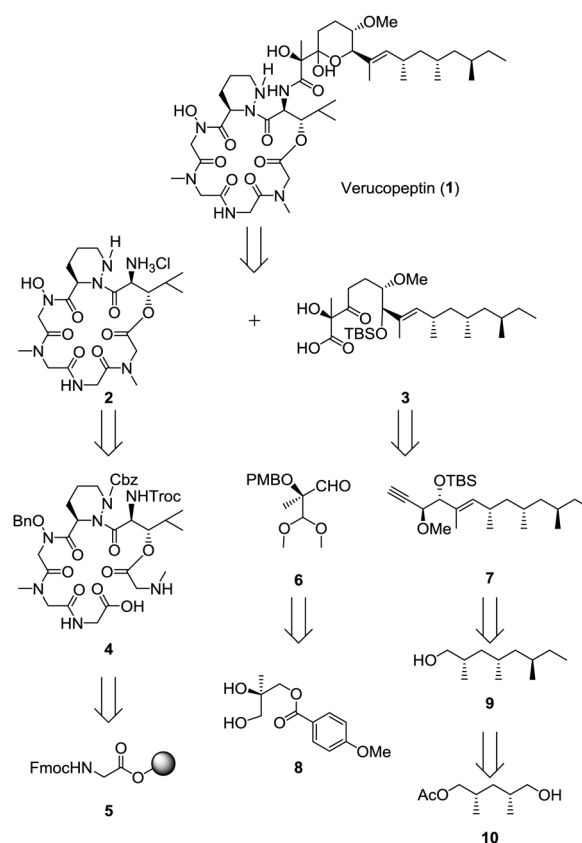
Tumor cells are usually exposed to hypoxic conditions or a starvation state. For survival and cell growth of tumor cells in these severe environments, hypoxia-inducible factor 1 (HIF-1) plays an important role as a transcriptional factor that regulates the expression of a number of genes involved in 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.

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**).

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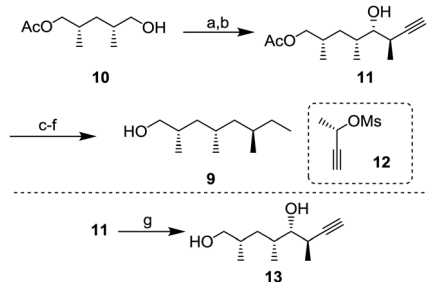
[†] Electronic supplementary information (ESI) available: Supporting figures, procedures for the syntheses of verucopeptin (**1**) and copies of ¹H- and ¹³C-NMR spectra. See DOI: 10.1039/c9cc06169j

transformed from known alcohol **8**. Alkyne **7** was obtained from alcohol **9**, which was prepared from known monoacetate **10**.⁷ Depsipeptide **2** was transformed from the linear peptide **4** by the same method as reported in Hale's work.⁶ Compound **4** was prepared by Fmoc-based solid phase peptide synthesis (SPPS) from Fmoc glycine loaded resin **5**.

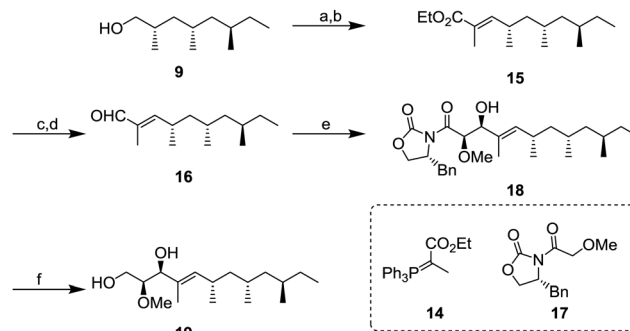
Alcohol **9** with three branched methyl groups was prepared from known monoacetate **10**⁷ in 6 steps (Scheme 2). After oxidation of **10** with 2-iodoxybenzoic acid (IBX), the resulting aldehyde was converted to propargyl alcohol **11** by Pd-catalyzed propargylation⁸ using chiral (*S*)-mesylate **12**.⁹ To determine the absolute configuration, alcohol **11** was transformed to diol **13** whose enantiomer is a known compound¹⁰ and the spectral data, including the sign of optical rotation, were compared. After hydrogenation of the alkyne, the unnecessary hydroxy group was removed by radical deoxygenation in two steps. Next, hydrolysis of the acetyl group was conducted to afford alcohol **9** in 96% yield.

Next, we focused on the construction of the two adjacent chiral centers of the methoxy group and allylic hydroxy group (Scheme 3). After oxidation of **9** with 85% yield, the resulting aldehyde was transformed to α,β -unsaturated ester **15** by the Wittig reaction using **14**. Conversion of **15** into α,β -unsaturated aldehyde **16** was conducted by diisobutylaluminium hydride (DIBAL-H) reduction and followed by oxidation with 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) and PhI(OAc)₂ with 75% yield (2 steps). Next, the aldehyde **16** was subject to the Evans aldol reaction with oxazolidinone **17**. Under the typical conditions with boron enolate, the diastereomerically pure aldol product **18** was obtained in 96% yield. Next, reductive removal of the chiral auxiliary of **18** gave diol **19**¹¹ in 55% yield.

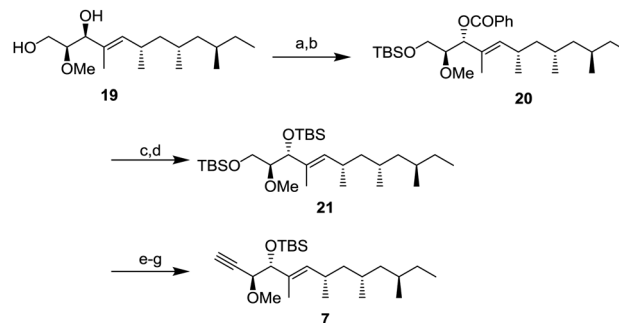
Next, the diol **19** was transformed to alkyne **7** for a coupling reaction with aldehyde **6** (Scheme 4). After protection of the primary alcohol with a *tert*-butyldimethylsilyl (TBS) group to obtain the intermediate in 81% yield,¹² the Mitsunobu reaction was conducted to obtain benzoate **20** whose stereochemistry was the same as that of verucopeptin (**1**). The benzoate group was removed by methanolysis with K₂CO₃ and the resulting alcohol was protected by a TBS group to obtain a di-silylated compound **21**. The compound **21** was converted to alkyne **7** by



Scheme 2 Synthesis of alcohol **9**; (a) IBX, DMSO, r.t., 3 h, 85%; (b) **12**, Pd(OAc)₂, PPh₃, ZnEt₂, THF, −78 °C to −20 °C, 6 h, 70%; (c) Pd/C, H₂, MeOH, r.t., 18 h, 85%; (d) phenyl chlorothionoformate, DMAP, pyridine, CH₂Cl₂, r.t., 3.5 h, 74%; (e) *n*Bu₃SnH, AIBN, toluene, 100 °C, 16 h; (f) K₂CO₃, MeOH, r.t., 2 h, 96% (2 steps); and (g) K₂CO₃, MeOH, r.t., 3 h, 85%.



Scheme 3 Synthesis of diol **6**; (a) TEMPO, PhI(OAc)₂, CH₂Cl₂, r.t., 3 h, 88%; (b) **14**, CH₃CN, 80 °C, 12 h, 75%; (c) DIBAL-H, toluene, −78 °C, 4 h; (d) DMP, CH₂Cl₂, r.t., 2 h, 75% (2 steps); and (e) **17**, *n*Bu₂BOTf, NEt₃, CH₂Cl₂, −78 °C to 0 °C, 1 h, 96%; (f) LiCl, NaBH₄, EtOH, THF, r.t., 2 h, 55%.

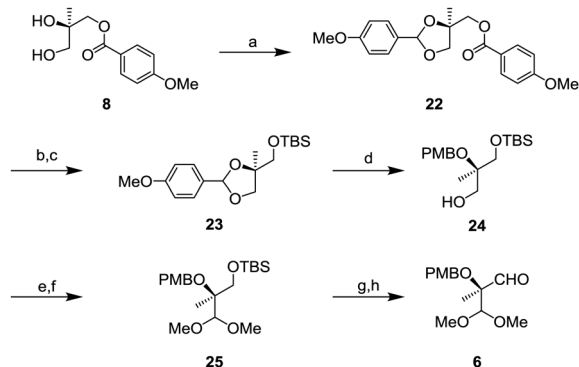


Scheme 4 Synthesis of alkyne **7**; (a) TBSCl, imidazole, CH₂Cl₂, r.t., 2 h, 81%; (b) PhCOOH, DEAD, PPh₃, THF, r.t., 15 h, 95%; (c) K₂CO₃, MeOH, r.t., 12 h; (d) TBSOTf, 2,6-lutidine, CH₂Cl₂, −78 °C, 2 h, 98% (2 steps); (e) PPTS, EtOH, r.t., 16 h, 80%; (f) DMP, CH₂Cl₂, r.t., 2 h; and (g) dimethyl(1-diazo-2-oxopropyl)phosphonate, K₂CO₃, MeOH, r.t., 18 h, 58% (2 steps).

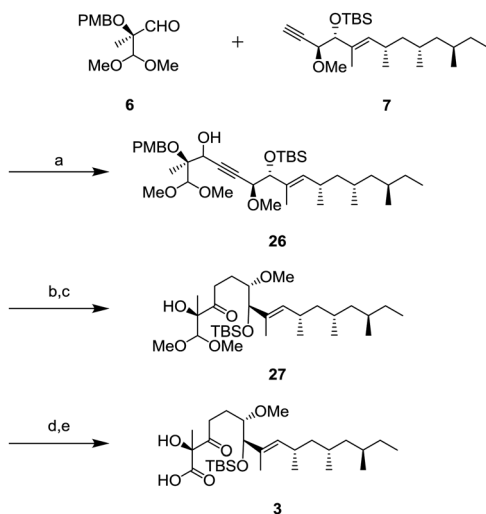
selective deprotection of the primary alcohol, Dess–Martin periodinane (DMP) oxidation, and alkyne formation using the Ohira–Bestmann reagent.¹³

Aldehyde **6** was prepared from known diol **8**¹⁴ as shown in Scheme 5. Protection of the diol by *p*-methoxybenzylidene acetal gave compound **22** in quantitative yields.¹⁵ After removal of the *p*-methoxybenzyl (PMB) ester group, TBS protection of the hydroxy group gave *O*-silylated compound **23**. Next, reduction of the *p*-methoxybenzylidene acetal with DIBAL-H was conducted to afford **24** whose tertiary alcohol was protected by the PMB group. After oxidation of **24**, the resulting aldehyde was transformed to a dimethyl acetal to generate **25**. Finally, removal of the TBS group followed by oxidation gave the aldehyde **6**.

Completion of the side chain unit is described in Scheme 6. The addition of alkyne **7** to aldehyde **6** using lithium hexamethyldisilazide (LHMDS) gave **26** in 95% yield as a mixture of diastereomers. Oxidation of the resulting secondary alcohol with DMP and the subsequent hydrogenation of the triple bond were performed to obtain ketone **27**. After deprotection of the dimethyl acetal in the presence of trimethylsilyl triflate (TMSOTf) and 2,4,6-collidine,¹⁶ the resulting aldehyde was oxidized to carboxylic acid **3** by the Pinnick oxidation.



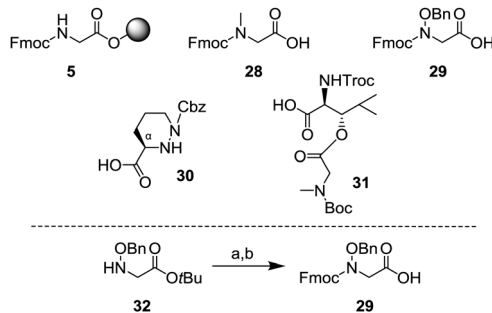
Scheme 5 Synthesis of aldehyde **6**; (a) *p*-methoxybenzaldehyde dimethyl acetal, PPTS, CH₂Cl₂, r.t., 18 h, 98%; (b) NaOMe, MeOH, r.t., 18 h; (c) TBSCl, imidazole, CH₂Cl₂, r.t., 16 h, 92% (2 steps); (d) DIBAL-H, CH₂Cl₂, 0 °C, 4 h, 36%; (e) SO₃-pyridine, NEt₃, DMSO, r.t., 2 h, 82%; (f) PPTS, trimethyl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



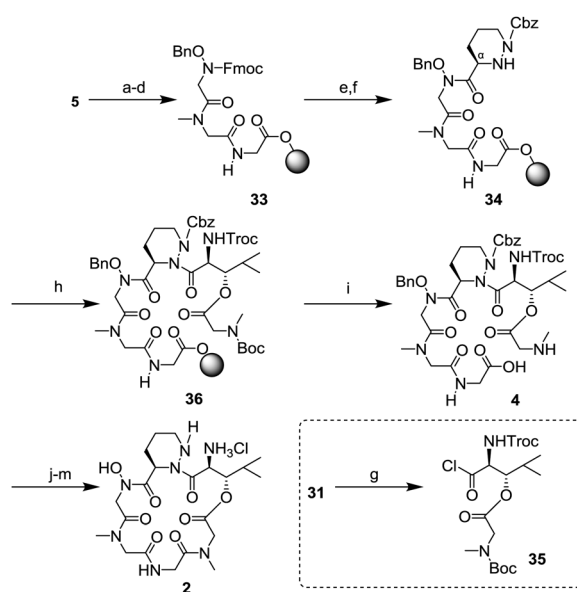
Scheme 6 Synthesis of carboxylic acid **3**; (a) LHMDS, −78 °C to 0 °C, 1 h, 95%; (b) DMP, CH₂Cl₂, r.t., 1 h, 96%; (c) Pd/C, AcOEt, r.t., 3 h; (d) TMSOTf, 2,4,6-collidine, CH₂Cl₂, 0 °C, 5 h; and (e) NaClO₂, NaH₂PO₄ monohydrate, 2-methyl-2-butene, H₂O, *t*BuOH, r.t., 3 h, 69% (3 steps).

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

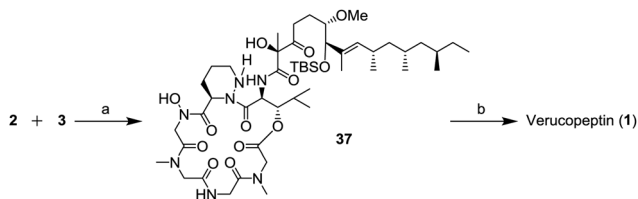


Scheme 7 Building blocks of the depsipeptide core **3**; (a) FmocCl, sat. NaHCO₃ aq., 1,4-dioxane, r.t., 1 h, 83% and (b) TFA, CH₂Cl₂, r.t., 1 h, 90%.



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, NEt_3 , CH_2Cl_2 , -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 benzyloxycarbonyl (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 (NEt_3) 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.

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Conflicts of interest

There are no conflicts to declare.

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- To confirm whether we obtained the *syn* aldol product, diol **19** was converted to an acetonide-protected compound and an NOE experiment was conducted (for details see the ESI[†]).
- The absolute configuration of the allylic position generated by the Evans aldol reaction was determined by a modified Mosher method (for details see the ESI[†]).
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