Synthesis of Piperazic Acid-Containing Cyclodepsipeptide Core of Verucopeptin

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Abstract

Chemically, N1 nitrogen of piperazic acid is more nucleophilic than N2 nitrogen, but amide bonds predominantly formed at N2 nitrogen are prevalent in piperazic acid-containing natural products, with only one exception of sanglifehrin. Thus two orthogonal protecting groups of nitrogen are often employed to realize selective coupling of N2 nitrogen, resulting in increased synthetic steps and low synthetic efficiency. However, we developed selective deprotection of N2-Cbz from the N1,N2-diCbz piperazic acid-containing peptide to form the N2 amide exclusively, avoiding the tedious orthogonal protection strategy commonly applied to the easily-accessible N1,N2-diCbz piperazic acid as the building block. We employ this method to achieve an efficient synthesis of piperazic acid-containing cyclodepsipeptide core of verucopeptin with an overall yield of 21%. The key steps include late stage coupling of piperazic acid with 3-hydroxyleucine derivatives, and HATU-mediated macrolactamization of 19-membered macrocycle at N9 and C10. The selective deprotection of N2-Cbz from the N1,N2-diCbz-piperazic acid at late-stage would greatly facilitate the total syntheses of piperazic acid-containing cyclodepsipeptides of biological interest.

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Synthesis of Piperazic Acid-Containing Cyclodepsipeptide Core of Verucopeptin

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Keywords

Cyclodepsipeptide | Piperazic Acid | Verucopeptin | Total Synthesis

Comprehensive Summary

Chemically, N1 nitrogen of piperazic acid is more nucleophilic than N2 nitrogen, but amide bonds predominantly formed at

Background and Originality Content

Piperazic acid (Piz), a non-proteinogenic amino acid bearing a unique cyclic hydrazine side chain, has been found in more than 30 families of natural products (over 140 compounds), many of which possess potent biological activities.[1]Like conformationally rigid proline, the Piz residue may also act as a turn-inducing motif in the structure of these peptides, furthermore another nucleophilic nitrogen of Piz potentially confers

some significant structural and biological effects.[2]Chemically, N1 nitrogen is more nucleophilic than N2 nitrogen in the Piz structure, but biological outcomes are often surprising as N2 nitrogen is usually selected to form the amide bond in the backbone of most Piz-containing natural products only except for sanglifehrin[3]. As free Piz has been proved to be directly adenylated and incorporated into the nonribosomal peptides,[4] enzymes may overcome this barrier via active site orientation, acid-base reactions, and/or coupled enzymatic reactions. However, chemists tend to solve this synthetic problem by adopting two various protective groups of nitrogens, leading to a prolonged synthetic route and lowered synthetic efficiency.[5,6] Free and *N* -protected Piz can be obtained from diverse asymmetric synthetic strategies: chiral substrate[7], chiral auxiliary[8-10], or chiral catalyst[11], as such. Among them, Chen *et al* developed one of the most concise synthetic method of N1,N2-diCbz-Piz via proline-catalyzed asymmetric ∝-hydrazination, oxidation, and cyclization (3 steps from commercially available reagents).[11] Thus N1,N2-diCbz-Piz is preferred as the building block to construct Piz-containing natural products and analogs. However, the tedious handlings of protecting groups were also needed to realize selective amide condensation of N2 nitrogen (Figure 1A). Since selective removal of N2-Cbz at N1,N2-diCbz Piz was mentioned available with the aid of adjacent carboxy group,[11] we speculated that selective deprotection of N2-protecting group (Cbz) at late-stage, posterior to the direct coupling of the carboxylic acid within the N1,N2-diCbz Piz residue would be superior for the syntheses of Piz-containing natural products (Figure 1B), to the usually adopted orthogonal protecting strategy with deprotecting N1,N2-diCbz into free Piz at initial stage (Figure 1A)

Figure 1 Synthetic strategies to assemble piperazic acid-containing peptides.

Therefore, we commenced to try late-stage selective deprotection strategy to synthesize the Piz-containing cyclodepsipeptide core of Verucopeptin (VE), an antibiotic isolated from Philippino soil microorganism *Actinomadura verrucospora* in 1993. Structurally, VE is composed of a cyclodepsipeptide core, a hemiketal formed tetrahydropyran ring, and a tetramethyl-substituted carbon chain with 3 chiral centers. [12-15] A pyranylated dipeptide composed of Piz and (2*S* ,3*S*)-3-hydroxyleucine, and an N-hydroxylated amide in C-terminal of Piz (red segments in Figure 2) are common features in the class of cyclic hexadepsipeptides including azinothricin, diperamycin, aurantimycin A and VE (Figure 2). Among them, VE exhibits potent antiproliferative activities against a wide range of cancer cell lines and was recently identified as a potent chemotherapeutic agent against multidrug-resistant (MDR) cancers, by targeting vacuolar H^+-ATPase.[16]

Figure 2 Representative structures of Verucopeptin family with the Piz-containing cyclodepsipeptide core.

Hale's group reported a [2 + 2 + 2]-fragment condensation strategy to construct the cyclodepsipeptide core of VE in total 18 steps from known compound.[17] Until 2019, Kakeya et al accomplished the first total synthesis of VE, in which the cyclodepsipeptide core **2** was synthesized via the combination of solid phase peptide synthesis (SPPS), and macrolactamization as well as dispositions of protecting groups in solution phase (Scheme 2).[18]However, more efficient synthesis is still in demand for the further mechanism study and drug-like derivatization of VE members.

Herein, we report an efficient chemical synthesis toward cyclodepsipeptide core**2** of VE (Scheme 2). We envision that the amide bond between N9 and C10 can serve as the final lactamization site for the cyclic peptide core, and the resulting cyclization precursor **3** can be constructed via challenging amide condensation between acid chloride**4** and dipeptide **5** , which allows reduced synthetic steps of substrates bearing the expensive non-proteinogenic amino acids. Although AgCN has been proved to promote the coupling of N2 nitrogen in Piz with 3-hydroxyleucine,[19] the applicability of this strategy to more complex acid derivatives **4** also needs verifying. Piz-containing dipeptide **5** would be prepared via selective deprotection of N2-Cbz in **8** , posterior to the coupling of **9** and **10** . Esterification between readily available tripeptide **6** and alcohol **7** , followed by deprotection and chlorination of carboxylic acid, would furnish**4** in 3 steps.

Scheme 1 Synthetic strategies to cyclodepsipeptide fragment of VE

Results and Discussion

We started the synthesis of the cyclodepsipeptide **2** from the building blocks described in Figure 3.

Among them, N-protected sarcosine **11a** and**11b** , dipeptide **12** are commercially available. Protected 3-hydroxyleucine **7** , [20,21]*tert* -butyl (benzyloxy)glycinate**9** ,[22] N1-Cbz,N2-Cbz Piz**10a** ,[11] and N1-Cbz Piz**10b** [11] were synthesized via previously reported methods.

Figure 3 Building blocks to construct the cyclodepsipeptide core.

With these building blocks in hand, we firstly explored the direct coupling of **9**with N1-Cbz Piz **10b** in which N2 nitrogen was not protected, in the presence of HATU and DIEA. However, the yield of coupling was only 19% because of nucleophilic competition between N2 nitrogen of **10b** and the hydroxylamine of **9** . To avoid this side reaction, Fmoc group was introduced to protect N2 nitrogen before the coupling of **9** and **13** . But Fmoc deprotection of the dipeptide **14** under the treatment of piperidine also gave rise to a low yield, probably because of the undesired cleavage of N-O bond in hydroxylamine motif. These experimental results indicated that tedious handlings of protecting groups or direct coupling in the presence of free N2 nitrogen could impair the synthetic efficiency, especially for the condensation of Piz and hydroxylamine derivatives. Then we tried to realize the selective hydrogenation of N2-Cbz over the N1-Cbz and the benzyl group in hydroxylamide, after smooth coupling of **10a** and **9** . Fortunately, we can control the reaction time to maximize the amount of**5** under the condition of 10% Pd/C and H_2. Five grams of **5** could be obtained in one batch in 58% yield when hydrogenation time was 2 h and the solvent was THF. Further prolonging the reaction time or replacing THF with MeOH as the solvent could result in excessive hydrogenation, while insufficient reaction time would lead to low conversion rate. (Scheme 2)

Scheme 2 Synthetic routes to Piz-containing dipeptide**5** [a]

[a] Reagents and conditions: (a) TMSCl, FmocCl, DIEA, THF, rt, 5 h, 76%; (b) oxalyl chloride, toluene, 60°C, 1 h; (c) **9** , Et_3N, toluene, 60 °C, 1 h; (d) piperidine, THF, rt, 4 h, 52%; (e) **9** , HATU, DIEA, THF, rt, 4 h; (f) H_2, 10% Pd/C, solvents, rt.

Then amidation of **12** and **11a** under EDCI and HOBt conditions proceeded smoothly to deliver tripeptide **13** in 86% yield. After hydrolysis of methyl ester **13** , the resulting acid**6a** was subjected to esterification reaction with **7a**(the simplified derivative of **7**), which was synthesized from commercially available reagents, to yield isopeptide **14** . The treatment of **14** with $Pd(PPh_3)_4$and N-methylaniline removed the allyl group to give acid **15** . We have attempted to carry out the chlorination of **15** with excess oxalyl chloride and amide condensation under AgCN-assisted conditions[19] between acid chloride and dipeptide**5** . However, no desired product **16** was observed probably due to the lability of Boc group in excess oxalyl chloride. (Scheme 3)

Scheme 3 First attempt to generate the cyclodepsipeptide core[a]

[a] Reagents and conditions: (a) **11a** , EDCI, HOBt, CH_2Cl_2, rt, 4 h, 86%; (b) LiOH, THF/H_2O=3/1, rt, 2 h, 89%; (c) **7a** , DIC, DMAP, CH_2Cl_2, rt, 4 h, 76%; (d) $Pd(Ph_3)_4$, N-methylaniline, rt, 3 h; (e) oxalyl chloride, CH_2Cl_2, rt, 1 h; (f)**5** , AgCN, toluene, 60 °C, 30 min.

Correspondingly, acid-sensitive Boc group in **6a** was replaced with Fmoc group. The acid **6b** was obtained in 2 steps from**12** , and then transformed to isopeptide **17** in a yield of 85%. Then, the allyl group of **17** was removed and the resulting acid **18** was subjected to the chlorination under excess oxalyl chloride condition. However, only trace of desired product **3** was obtained after amidation with dipeptide **5** under AgCN-assisted conditions. Oxalylation at N6 site was deemed to be the major side reaction, which was detected by liquid chromatography-mass spectrometry after quenching of chlorination reaction with H_2O. The undesired oxalylation reaction could be circumvented by using thionyl chloride instead of oxalyl chloride condition. The chlorination of **18** under thionyl chloride and amidation with dipeptide **5** afforded **3** . Terminal Fmoc group and tertiary butyl group in cyclization precursor**3** were removed successively under diethylamine and TFA conditions to produce trifluoroacetate **19** . Notably, the Fmoc deprotection should be monitored carefully to avoid much prolonging the reaction, and the use of more reactive piperidine instead of diethylamine was not recommended, as N-OBn moiety was not stable under these treatments. Macrolactamization was conducted using Carpino's HATU reagent in high dilution to afford **20** in 76% yield over 3 steps. After detaching

the Troc group with Zn powder in aqueous acid, we directly removed benzyl and Cbz protecting groups by hydrogenolysis under Hale's condition.[17]Cyclodepsipeptide core **2**was obtained as a hydrochloride, and the spectral data were consistent with that previously reported. [18] (Scheme 4)

Scheme 4 Construction of the cyclodepsipeptide core[a]

[a] Reagents and conditions: (a) **11b** , EDCI, HOBt, CH_2Cl_2, rt, 4 h; (b) LiOH, THF/H_2O=3/1, rt, 2 h, 82% over 2 steps; (c) **7** , EDCI, DMAP, CH_2Cl_2, rt, 4 h, 85%; (d) $Pd(Ph_3)_4$, N-methylaniline, THF, rt, 2 h, 91%; (e) oxalyl chloride, CH_2Cl_2, rt, 1 h; (f) **5** , AgCN, toluene, 60 °C, 30 min, 85% over 2 steps; (g) 2%(v/v) diethylamine in MeCN, rt, 4 h; (h) TFA/ CH_2Cl_2=3/1, rt; (i) HATU, DIEA, THF, rt, 24 h 65% over 3 steps; (j) Zn powder, AcOH/H_2O=9/1, rt, 2 h; (k) H_2, 10% Pd/C, 0.01 M HCl in MeOH, 24 h, 60% over 2 steps.

Conclusions

Piz-containing natural products display potent biological activities and Piz residue potentially exerts significant structural and biological effects. However, the assembly of Piz-containing peptides suffered from tedious handlings of protecting groups on the Piz residue. We found that selective deprotection of N2-protecting group at late-stage posterior to the direct coupling of carboxylic acid within N1,N2-diCbz Piz would be efficient method, facilitating the syntheses of Piz-containing natural products. As such, we developed an efficient synthetic method to construct cyclodepsipeptide core **2** of VE in total 13 steps with an overall yield of 21%. Our strategy also features late stage coupling of Piz and 3-hydroxyleucine derivatives, which could reduce the synthetic steps to handle substrates bearing the infrequent unnatural amino acids. Macrolactamization at N9 and C10 realized the cyclization of 19-membered macrocycle in high yield.

Experimental

Experimental procedures are available in the Supporting information.

Supporting Information

The supporting information for this article is available on the WWW under https://doi.org/10.1002/cjoc.2023xxxxx.

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Herein we reported an innovative strategy which enables the selective deprotection of N2-Cbz from N1,N2-diCbz piperazic a