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Total Synthesis of Chloptosin, a Potent Apoptosis-Inducing Cyclopeptide

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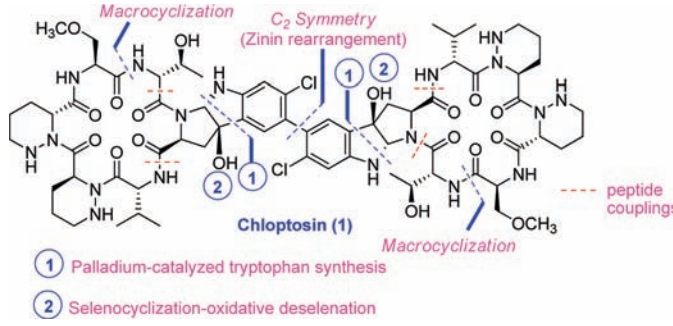
ABSTRACT



A bidirectional total synthesis of chloptosin has been achieved in 16 operations (32 individual reactions) and 3% overall yield from the readily available materials. Palladium-catalyzed tryptophan synthesis, diastereoselective selenocyclization and oxidative deselenation successfully served as key steps in construction of the dimeric core amino acid. 2-Bromo-1-ethyl pyridinium tetrafluoroborate was efficiently employed in the peptide couplings with spatial encumbrance in this synthesis.

Two types of cell death have been generally identified in metazoan animals. Among these, necrosis often triggers an inflammatory response when the body suffers from overwhelming cellular injuries. Apoptosis is the other distinct programmed cell death, which is often associated with characteristic morphological and biochemical changes. Apoptosis plays an indispensable role during their development, homeostasis, as well as in many diseases including cancers.[1] Many anticancer agents including adriamycin and paclitaxel have been known to induce apoptosis in a variety of cultured neoplastic cells.[2] However, human carcinoma cells are often resistant to apoptosis. This phenotype may partly explain the poor therapeutic effects of present cancer chemotherapies on most solid tumors.

Chloptosin (**1**, Figure 1), a natural cyclopeptide isolated as the third metabolite from the culture broth of the MK498−98F14 strain of *Streptomyces* by Umzawa and co-workers in 2000, was found to induce apoptotic activity in the apoptosis-resistant human pancreatic adenocarcinoma cell line AsPC-1 with an EC_{50} of 2.5 μg/mL after 24 h.[3] Chloptosin (**1**) also strongly inhibited the growth of Gram-positive bacteria, such as *Staphylococcus*, *Micrococcus* and *Bacillus* strains (MIC range 0.20−0.78 μg/mL), and its LD_{50} value was relatively high (50−100 mg/kg) to the 4-week-old ICR male mice.[3] Therefore, it shows considerable prospects as a promising lead for drug development and an excellent molecular tool for apoptosis studies.

Chloptosin (**1**) demonstrates a rare dumbbells-shaped C_2-symmetrical bis-cyclopeptide architecture, which contains a central core **3***a*-hydroxypyrrolo[2,3-*b*]indole di-

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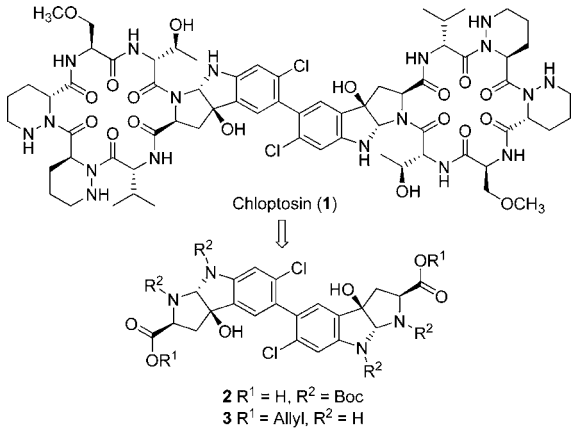


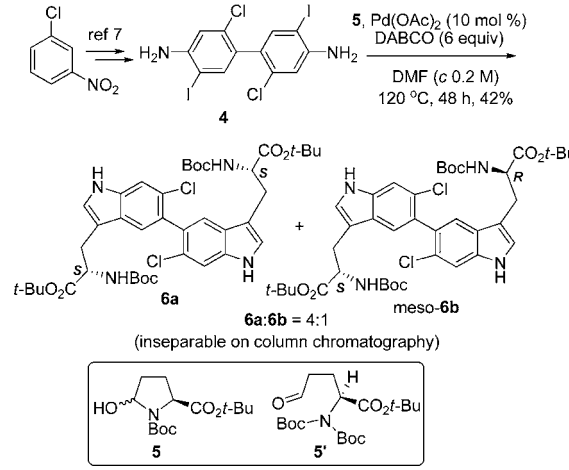
Figure 1. Structure of chloptosin (**1**) and its unique core amino acid (derivatives **2** and **3**).

meric amino acid and several other unusual amino acids. Structurally, it is similar to a previously reported natural product himastantin, whose structure was revised via a total synthesis by Danishefsky in 1998.[4] Besides some amino acid components, a major difference between these two natural compounds is the central core amino acid. Two additional chlorines are symmetrically embraced in the diphenyl moiety of chloptosin (**1**) in this case. Though a sound number of other natural products have been isolated having the pyrroloindole architecture,[5] the presence of a chlorine atom at the 6-position is apparently rare. In addition, existence of two vicinal piperazine-3-carboxylic acids in the cyclopeptide subunits further makes chloptosin more spatially crowded than himastatin. All these characteristics of the molecular architecture of chloptosin including crowded spatial environment would bring about some uncertain challenges during its total synthesis, especially in the peptide couplings and the macrocyclization.

For its unique structure and promising biological properties, chloptosin has attracted sound attentions in organic synthesis. Several methodology developments[6] and one synthesis of the central amino acid[7] have been reported in recent years. Due to the presence of 6-chlorine, a number of our initial attempts failed in the Csp^2−Csp^2 couplings to the diphenyl core under various metal-catalyzed conditions. Considering the stability of peptide substrates in those aryl−aryl couplings under relatively high temperatures in the further synthesis, an alternative bidirectional strategy[8] based on a Zinin rearrangement was thus developed in our successful synthesis of the dimeric core amino acid derivative **2** (Figure 1) in 2006.[7] In this communication, we report our achievement in the total synthesis of chloptosin using a bidirectional strategy.

Although the core amino acid derivative **3** (Figure 1) suitable for further synthesis could be prepared after several transformations from acid **2**,[7] relatively longer steps and lower overall yield made it disadvantageous in the material accumulation. Improvement of our previous synthesis of this core amino acid[7] was decided as the starting point of this work. Because both reactants are readily available, palladium-catalyzed heteroannulation[9] of 2,2′-dichloro-5,5′-diiodobiphenyl-4,4′-diamine **4**[7] and L-pyroglutamic acid derivative **5**[10] was then employed as a shortcut to the requested bis-tryptophan intermediate **6a**. After careful optimization of reaction conditions, a mixture of the needed dimeric tryptophan derivative **6a** and meso-product **6b** was afforded in 42% combined yield (Scheme 1, **6a**:**6b** > 4:1, measured by chiral HPLC). The corresponding reaction with aldehyde **5′** gave much lower yield. Diastereomers **6a** and **6b** are inseparable by silica gel chromatography. Because of the poor resolution under chiral HPLC conditions, the enantio purity of **6a** was finally dertemined after several further transformations (see below text).

Scheme 1. One-Step Synthesis of the Dimeric 6-Chlorotryptophan Derivatives **6a** and **6b**



After treatment of the mixture of **6a**/**6b** with Boc_2O, the resulting mixture of **7a**/**7b** was then subjected to the reaction with *N*-phenylselenophthalimide (*N*-PSP),[11] pro-

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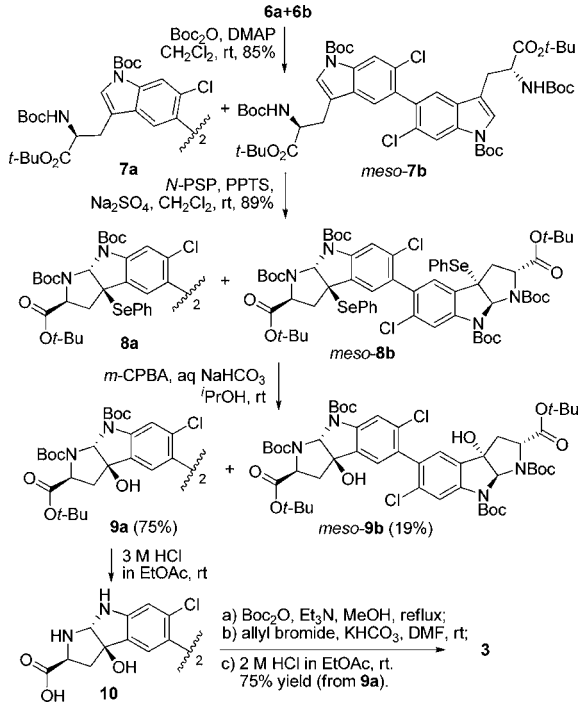
viding inseparable pyrroloindoles **8a**/**8b** in 89% yield (Scheme 2). Oxidative deselenation of **8a**/**8b** with excess

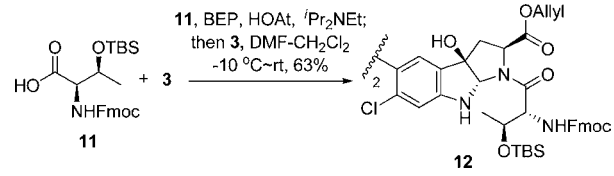
Scheme 2. Improved Synthesis of the Central Amino Acid **3**



of *m*-CPBA in the presence of aq $NaHCO_3$ in ^{i}PrOH afforded the C_2-symmetrical tertiary alcohol **9a** (95.9% ee, determined by chiral HPLC) in 75% yield and the other meso-compound **9b** in 19% yield after separation by routine silica gel column chromatorgraphy. The *N*-Boc protected compound **9a** was then converted to the required amine acid derivative **3** in 4 steps. Spectroscopic properties (^{1}HNMR,^{13}CNMR, IR, MS, and $[\alpha]_D$) of sample **3** obtained by this newly established route are in good agreement with the one obtained from our previous synthesis.[7] Compared to our first synthesis, this short synthesis greatly improves the overall yield of core amino acid and therefore facilitates the material accumulation in our total synthesis.

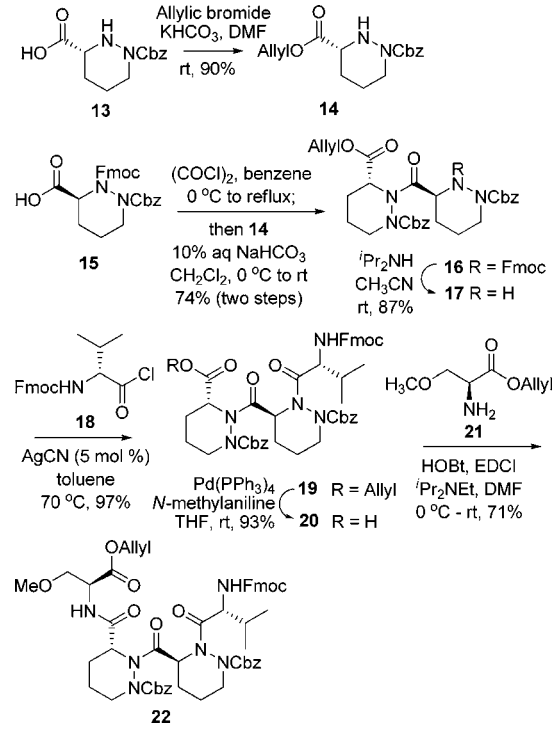
As our previous analysis, coupling of the core amino acid derivative **3** with the remaining pentapeptide was surprisingly difficult under various conditions. The initial attempts led to the decomposition of pentapeptide substrates. Shortening the pentapeptide to corresponding tetrapeptide, tripeptide, and dipeptide could not improve the results. Using the active ester or mixed anhydride of single amino acid threonine derivative **11** as the reactant, no desired product was given, either. When a combination of HATU-HOAt-^{i}Pr$_2$NEt was employed as the coupling reagents, the double-coupling product **12** was provided in 29% yield, along with the corresponding mono product in 28% yield. The yield of **12** was finally improved to 63% when 2-bromo-1-ethyl pyridinium tetrafluoroborate (BEP)[12] was used as the coupling reagent in the presence of HOAt and ^{i}Pr$_2$NEt (Scheme 3).

Scheme 3. Coupling of the Core Amino Acid **3** with the First Amino Acid Derivative **11**



Synthesis of the remaining tetrapeptide fragment **22** is outlined in Scheme 4. Protection of the carboxylic acid **13**

Scheme 4. Synthesis of the Tetrapeptide Fragment **22**



with allyl bromide gave ester **14**. Treatment of acid **15**[13] with oxalyl chloride (to give the corresponding acid chloride) followed by reaction with amine **14** in the presence of $NaHCO_3$ provided the dipeptide **16**.[14] Removal of the

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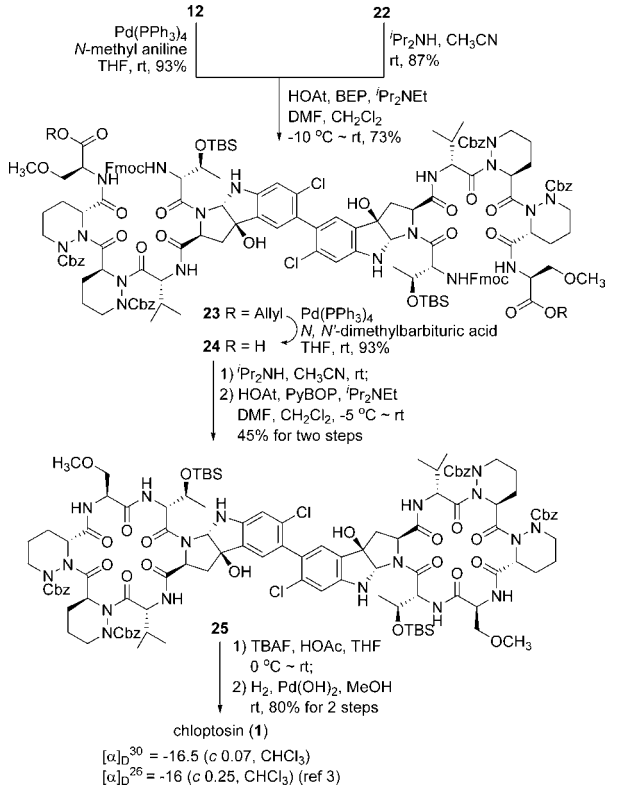
N-Fmoc protecting group of **16** with $^{i}Pr_2NH$ in acetonitrile gave the free amine **17**, which was immediately acylated with the valine acid chloride **18** in the presence of silver cyanide[15] to afford the tripeptide **19**. Deprotection of *O*-allyl group of **19** followed by coupling with amine **21** (for the preparation of **21**, see Supporting Information) afforded the tetrapeptide **22** in a satisfactory yield.

With both dimeric precursor **12** and tetrapeptide **22** in hand, further total synthesis of chloptosin was then performed (Scheme 5). Removal of the *O*-allyl protecting group of **12** under mild $Pd(PPh_3)_4$-catalyzed conditions in THF afforded the corresponding peptide acid, which was immediately coupled with the peptide amine freshly prepared in a parallel reaction from **22**. This coupling successfully provided the whole linear peptide **23** in 71% yield. Successive removal of the protecting groups at both the *C*-terminus and *N*-terminus of **23** led to the final linear precursor for the macrolactamization. Treatment of this linear head−tail "amino acid" with PyBOP-HOAt-$^{i}Pr_2NEt$ in CH_2Cl_2 and DMF (10:1) at rt for 4 days successfully afforded the dimeric cyclopeptide **25** in 45% yield.[16] Both two *O*-TBS protecting groups of cyclopeptide **25** were then removed with excess TBAF (20 equiv) and HOAc (22 equiv) in THF.[17] Further deprotection of all four *N*-Cbz groups of the resultant material failed either by hydrogenolysis in the presence of 10% Pd/C in MeOH, or by that using HOAc as an additive. Fortunately, this problem was resolved by hydrogenolysis (1 atm, rt) in the presence of 20% $Pd(OH)_2$ in MeOH, providing chloptosin (**1**) in 80% yield (2 steps). All characterization data of the synthetic chloptosin are in good agreement with those reported for natural sample,[3] incldiung the NMR spectra and optical rotation comparsions. To the best of our knowledge, few bidirectional total syntheses of cyclopeptides have been reported in the literature. Our achievement provides a new successful example to this category of total syntheses.

Scheme 5. Macrocyclization and Completion of the Total Synthesis



In summary, the first total synthesis of chloptosin, a potent apoptosis-inducing cyclopeptide, has been achieved in 16 operations (32 individual reactions) of the longest linear sequence and 3% overall yield from the readily available material **4**. A Pd-catalyzed tryptophan synthesis, a diastereoselective selenocyclization, and an oxidative deselenation successfully served as the key steps in the short bidirectional synthesis of core amino acid in this work. In addition, the coupling reagent 2-bromo-1-ethyl pyridinium tetraflouorborate shows great advantages in this synthesis through its successful application to the peptide couplings with spatial encumbrance.

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Supporting Information Available: Experimental details and charaterizations of new compounds, NMR copies of new compounds, and HPLC analysis reports. This material is available free of charge via the Internet at http://pubs.acs.org.

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