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A Convergent Synthesis of the Proposed Structure of Antitumor Depsipeptide Stereocalpin A

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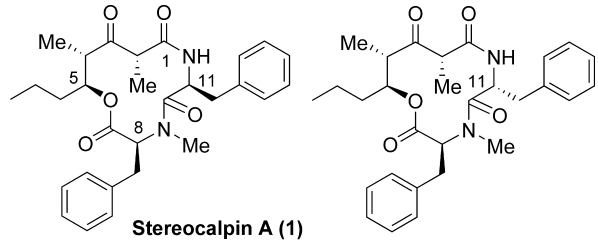
ABSTRACT



Stereocalpin A (1) (proposed structure)

11-*epi*-Stereocalpin A

The total synthesis of the proposed structure of anticancer agent stereocalpin A is described. The synthesis features a diastereoselective synthesis of a 5-hydroxy-2,4-dimethyl-3-oxooctanoic acid unit with asymmetric *anti*- and *syn*-aldol reactions as the key steps. Initial cycloamidation led to complete epimerization at the C-11 stereocenter due to unique steric constraints in the 12-membered depsipeptide ring. A late-stage methylation strategy led to the synthesis of the proposed structure of stereocalpin A.

A variety of cyanobacterial metabolites have shown diverse biological properties including antitumor, antibiotic, antiviral, antimycobacterial, analgesic, and antipyretic properties.[1] Stereocalpin A (**1**), a new cyclic depsipeptide, was isolated from the dry lichen *Ramalina terebrata* of Antarctica in 2008, by Oh et al.[2] Initial testing for cytotoxicity against solid tumor cell lines has shown good activity against human colon carcinoma cell lines (HT-29, IC_{50} = 6.5 μM), human skin carcinoma cell lines (B16F10, IC_{50} = 11.9 μM), and human liver carcinoma cell lines (HepG2, IC_{50} = 13.4 μM). In addition, stereocalpin A displayed a protein tyrosine phosphatase 1B (PTP1B) inhibitory activity in a dose-dependent manner with an IC_{50} value of 40 μM. Further biological investigations could not be carried out because of lack of material.

The structure of stereocalpin A (**1**, Figure 1) was elucidated by extensive use of NMR and HPLC analyses of derivatives. Acid degradation (6 N HCl, 120 °C, 24 h) of **1** followed by derivatization with Marfey's reagent,[3] and subsequent HPLC analysis revealed that stereocalpin A contains a L-Phe, a L-*N*-Me-Phe, and a 5-hydroxy-2,4-dimethyl-3-oxooctanoic acid unit, which has not been previously reported as a component of any natural product. The absolute configuration of the octanoate derivative was resolved by a thorough analysis of NOESY data. The unique structure, interesting biological activity, and our interest in cyclic depsipeptides as antitumor agents[4] led us to explore the chemistry and biology of stereocalpin A. Herein, we report our preliminary investigation leading to the stereoselective synthesis of the proposed structure of stereocalpin A and 11-*epi*-stereocalpin A. The present work suggested an incorrect assignment of the reported structure for stereocalpin A.[2] Furthermore, we

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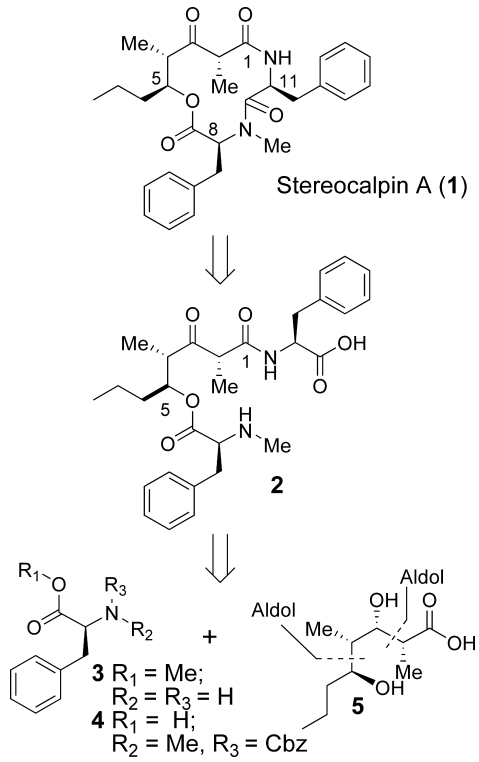


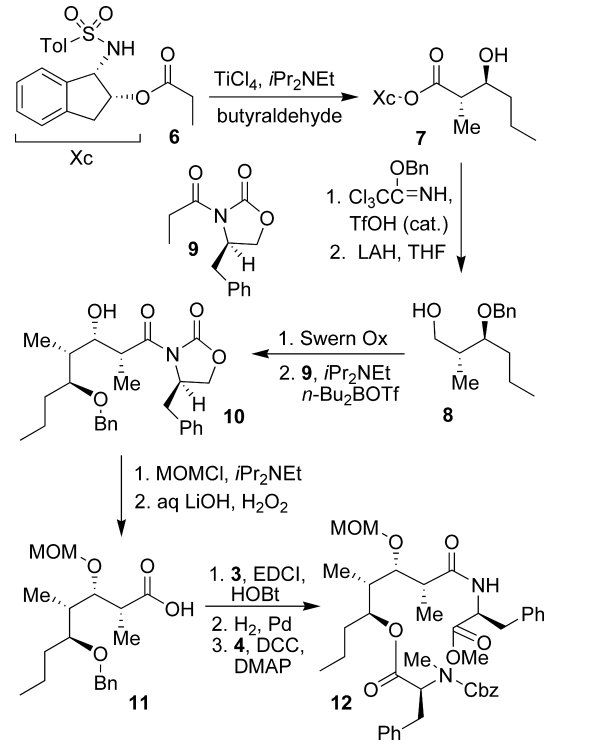
Figure 1. Retrosynthesis of stereocalpin A.

observed that the proposed structure has an unusual propensity to epimerize at the L-phenylalanine stereocenter.

As illustrated in Figure 1, our initial retrosynthetic analysis of stereocalpin A led us to disconnect the peptide bond between L-Phe and *N*-Me-Phe residues to provide amino acid derivative **2**. The C2 chiral center is located between the C1 amide and C3 ketone, making this chiral center prone to epimerization. Therefore, we planned to keep the C3 ketone as a protected alcohol that will be oxidized into a ketone at the end of the synthesis. The acid **2** can be further disconnected to provide the octanoic acid subunit **5**, Cbz-*N*-Me-Phe **4**, and Phe-OMe **3**. We planned to synthesize subunit **5** using Ghosh's $TiCl_4$-promoted *anti*-aldol reaction and Evans' *syn*-aldol reaction as the key steps.

As depicted in Scheme 1, we utilized an ester-derived titanium enolate-based highly diastereoselective *anti*-aldol reaction to install the C4 and C5 stereocenters of stereocalpin A.[5] In a slightly modified protocol, tosylaminoindanol ester **6** was treated with $TiCl_4$ (1.1 equiv) and diisopropylethylamine (3.8 equiv) in CH_2Cl_2 at 23 °C for 2 h. Addition of the resulting titanium enolate to the premixed *n*-butyraldehyde (2 equiv), $TiCl_4$ (2 equiv), and MeCN (2 equiv) at −78 °C afforded the *anti*-aldol product **7** in 65% yield. The ^1H

Scheme 1. Synthesis of Cyclization Precursor **12**



NMR and ^{13}C NMR analyses determined the *anti*-aldol diastereoselectivity to be 37:1 for aldol adduct **7**. Protection of the resulting alcohol as a benzyl ether followed by reductive cleavage of the chiral auxiliary with LAH afforded alcohol **8** in 85% yield over two steps.[6] We then employed Evans' *syn*-aldol reaction to install the C2 and C3-stereocenters of **5**. Thus, Swern oxidation of **8** followed by *syn*-aldol[7] reaction with chiral imide **9** generated aldol product **10** in 89% yield (7:1 *dr*). Protection of alcohol **10** as its MOM ether using MOMCl, DIPEA, and DMAP afforded the corresponding MOM ether in 90% yield. The removal of chiral imide with LiOOH at 0−23 °C for 12 h provided the acid **11** in 65% yield. It was coupled with L-phenylalanine methyl ester to provide the corresponding amide in 90% yield.[8] The benzyl protecting group was removed by catalytic hydrogenation over Pearlman's catalyst in a mixture (1:1) of ethyl acetate and methanol. The resulting alcohol was esterified with Cbz-*N*-Me-phenylalanine **4** using DCC and DMAP to afford the key amino acid derivative **12** in 85% yield.

As shown in Scheme 2, saponification of amine ester **12** with lithium hydroxide in *tert*-butyl alcohol/H_2O at 0 °C for 2 h followed by exposure of the resulting acid to catalytic hydrogenation over $Pd(OH)_2$ afforded the corresponding amino acid precursor for cycloamidation. Treatment of the resulting amino acid with HATU (1.2 equiv) and HOAt (2 equiv) in a mixture (5:1) of CH_2Cl_2 and DMF at 23 °C for 24 h resulted in the cycloamide **13** as a single product in 95% yield.[9] To complete the synthesis, the MOM group in **13** was deprotected using dimethylboron bromide.[10] Oxidation of the resulting alcohol with Dess−Martin periodinane[11]

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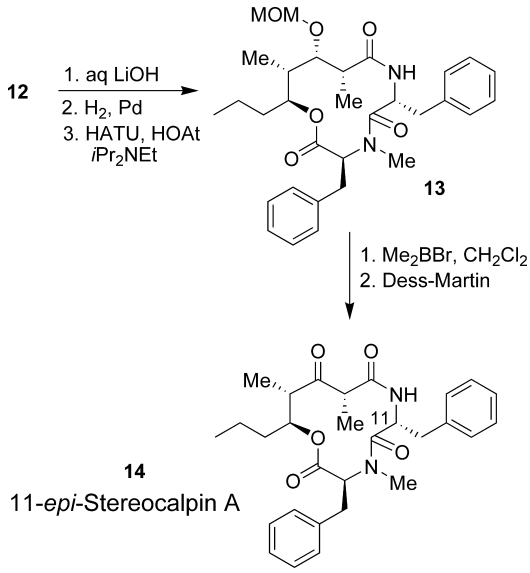
Scheme 2. Synthesis of 11-*epi*-Stereocalpin A



afforded **14**, the presumed structure of stereocalpin A. However, the ^{1}H NMR and ^{13}C NMR of **14** did not match the reported data for the natural stereocalpin A.[2] To our surprise, the X-ray crystal structure of **14** (Figure 2)[12]

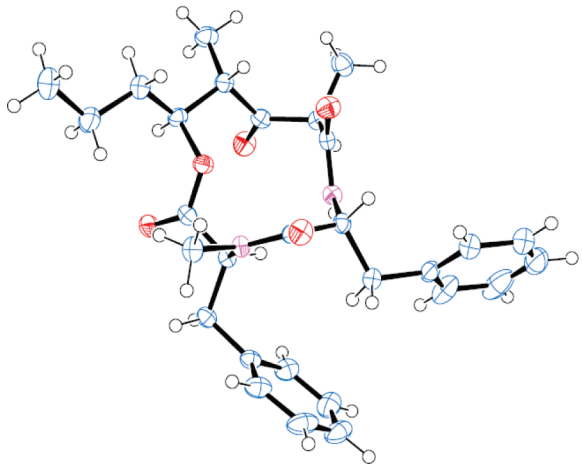
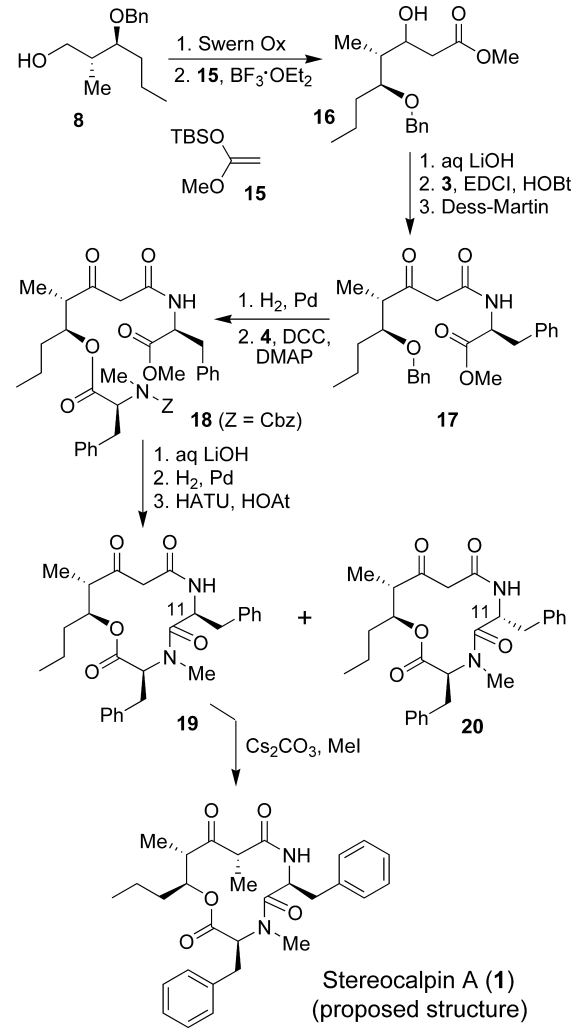


Figure 2. ORTEP drawing of compound **14**.

revealed that the C11-stereocenter of L-phenylalanine had completely epimerized to D-phenylalanine, as shown in Scheme 2. For further confirmation of the structure, cycloamide **14** was hydrolyzed with 6 N HCl at 110 °C for 24 h. The resulting amino acids were exposed to Marfey's reagent,[3] and the resulting products were analyzed by HPLC which confirmed the presence of D-phenylalanine and *N*-Me-L-phenylalanine. To avoid this epimerization, other coupling reagents, such as TBTU and BOP reagents, have been examined. However, in each case, the same epimerization product was obtained. Although partial epimerization of amino acids during coupling reactions has been reported,[13] a complete epimerization during a macrolactamization reaction is unprecendented. The observed facile epimerization is presumably due to developing steric compression between the C2 methyl, C3 MOM group, and the C11 benzyl group in the 12-membered cycloamide.

Assuming that the C2 methyl and C3 MOM groups may be involved in facilitating the epimerization of L-phenylalanine during cyclization, we decided to assemble the methyl group at the C2 position at a later stage after the formation of the 12-membered ring. As outlined in Scheme 3, Swern

Scheme 3. Synthesis of Stereocalpin A (Proposed Structure)



oxidation of alcohol **8** followed by Mukaiyama aldol reaction of the resulting aldehyde with ketene acetal **15** afforded the corresponding aldol product,[14] the β-hydroxy ester **16** in 72% yield (5:1 *dr*). Saponification of methyl ester **16** with aqueous LiOH and 30% H_2O_2 afforded the corresponding acid which was coupled with L-phenylalanine methyl ester to provide the β-hydroxy amide derivative. Dess−Martin oxidation[11] of the resulting β-hydroxy amide furnished amide **17** in 71% yield over two steps. Removal of the benzyl protecting group,

followed by esterification of the resulting alcohol with *N*-Cbz-*N*-Me-phenylalanine, afforded amino acid derivative **18** in 89% yield. Saponification of ester **18** with LiOH in aqueous *tert*-butyl alcohol, and removal of the Cbz group by catalytic hydrogenation over $Pd(OH)_2$ in a mixture (1:1) of EtOAc and MeOH, afforded the corresponding amino acid. The resulting amino acid was exposed to macrolactamization conditions with HATU and HOAt in a mixture of CH_2Cl_2 and DMF (5:1) at 23 °C for 36 h as described above. The desired cycloamide **19** was formed in 47% yield, along with 10% of the C11-epimer **20**. Methylation of **19** was carried out stereoselectively in the presence of cesium carbonate and methyl iodide in DMF for 24 h to afford the proposed stereocalpin A (**1**) in 50% yield (90% based on recovered starting material) as a single isomer. It appears that the alkylation presumably proceeded from the less hindered face, away from both benzyl and propyl substituents on the 12-membered ring. Interestingly, however, the spectral data of synthetic stereocalpin A (**1**) did not match with the data reported for the natural stereocalpin A.[2] Our detailed structural analysis using 2D-NMR and NOESY of synthetic **1** fully supported our assignment of **1** as the proposed structure of stereocalpin A. Therefore, our stereocontrolled synthesis of the stereocalpin structure (**1**) now suggested that the structure of natural stereocalpin A had been assigned incorrectly. The comparison of NMR data is shown in the supporting information.

In conclusion, we have developed a short synthesis of the proposed structure of stereocalpin A. Our synthetic studies now ascertain that the original assignment of the stereocalpin A structure is incorrect. Interestingly, a combination of substituents, their stereochemistry, and the developing steric strain during the formation of a 12-membered depsipeptide ring led to an unprecedented complete epimerization at the C-11 amino acid center. Further investigation leading to the assignment of stereocalpin A's structure and structure−activity studies are in progress.

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Supporting Information Available: Experimental procedures and ^1H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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