

SYNTHETIC APPROACH TOWARD THE MINIMAL ACTIVE STRUCTURE
OF PHOMOPSIN-USTILOXIN CLASS OF ANTIBIOTICS:
CONSTRUCTION OF 13-MEMBERED CYCLIC PEPTIDE SKELETON

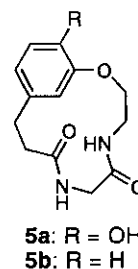
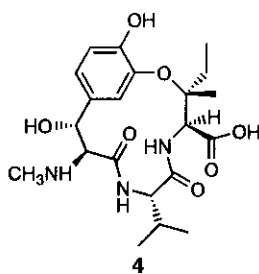
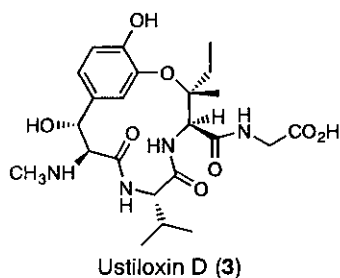
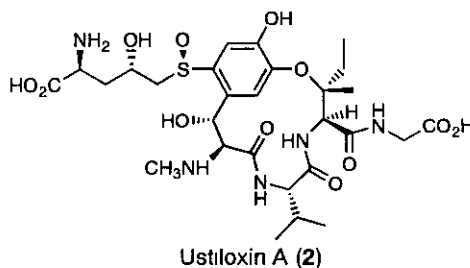
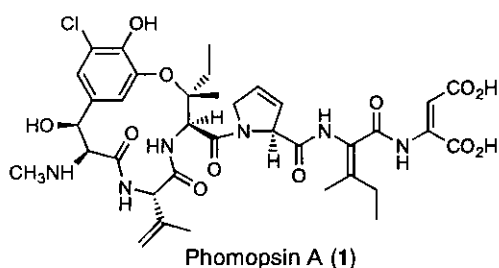
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Abstract The 13-membered cyclic peptides (**5a** and **5b**), the core structure of phomopsin-ustiloxin class of antibiotics, have been synthesized. The final macrolactamization was overcome by the intramolecular aminolysis of corresponding ω -amino pentafluorophenyl ester.

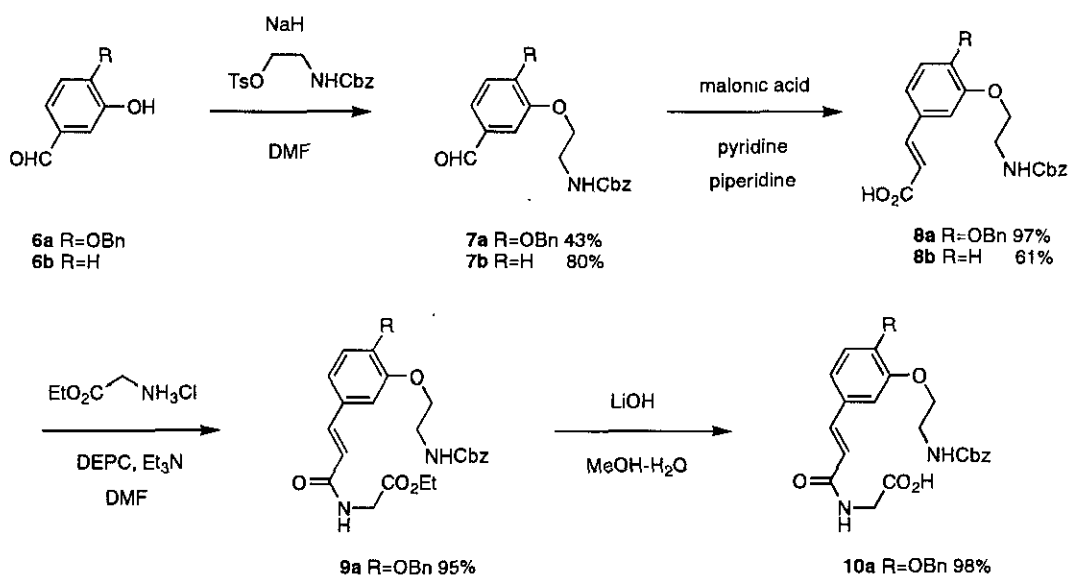
There are a number of antimittotic agents that interfere with the microtubule function by binding to tubulin. Maytansine,¹ rhizoxin,² dolastatin 10,³ phomopsin A (**1**)⁴ and ustiloxin A (**2**)⁵ are known to share the same binding site (rhizoxin site).^{6, 7} However, their structural diversity remains it difficult to find their common



structural elements to recognize the same binding site on tubulin. Among them, phomopsin A and ustiloxin A have a common 13-membered cyclic structure, and the newly isolated simplest ustiloxin congener, ustiloxin D (**3**),⁷ also exhibits potent *anti*-tubulin activity. Since we regarded ustiloxin D (**3**) as the promising candidate to elucidate the structural requirement for the rhizoxin site ligands, we planned to synthesize a series of 13-membered cyclic peptides such as **4** or the variants with other amino acid units to find the minimal structure responsible for *anti*-tubulin activity. Construction of 13-membered cyclic peptide skeleton, formed through a *meta*-phenylene substitution, is a challenging synthetic task. In this article, synthesis of *N*-protected seco- ω -amino acids (**10a**) and (**10b**), and their macrolactamization to simple 13-membered cyclic peptides (**5a**) and (**5b**) are described.

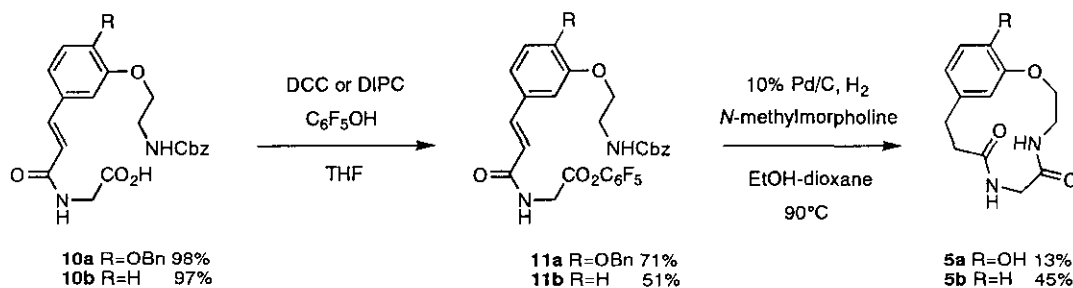
Synthesis of **10a** and **10b** are achieved as shown in Scheme 1. Aryl alkyl ethers (**7a**) and (**7b**) were synthesized by the alkylation of **6a**⁸ and **6b** with sodium hydride and *N*-carbobenzyloxyaminoethyl *p*-toluenesulfonate in 43% and 80% of respective yields. Knoevenagel condensation of **7a** and **7b** with malonic acid gave *m*-coumaric acid derivatives (**8a**) and (**8b**). Coupling of **8a** and **8b** with glycine ethyl ester (DEPC (diethylphosphoryl cyanide), Et₃N/DMF) followed by basic hydrolysis (LiOH/MeOH-H₂O) gave **10a** and **10b** in high yield.

Scheme 1



Macrolactamization of **10a** and **10b** was accomplished successfully according to the following procedure (Scheme 2). An activation of ω -*N*-protected seco amino acid with dialkylcarbodiimide (DCC (dicyclohexylcarbodiimide) for **10a**; DIPC (diisopropylcarbodiimide) for **10b**) and pentafluorophenol afforded pentafluorophenyl esters (**11a**) and (**11b**). Final cyclization reactions were achieved by hydrogenation condition (Pd/C, H₂) at elevated temperature (90°C) under high dilution condition.⁹ Under this reaction condition, hydrogenation of unsaturated carbon-carbon double bond, deprotection of *N*-Cbz group and macrolactamization took place in one pot to give **5a** and **5b**.¹⁰ On the other hand, macrolactamization of saturated ω -amino acid of **10b** with PyBroP (bromotrispyrrolidinophosphonium hexafluorophosphate) or DPPA (diphenylphosphoryl azide) failed. For macrolactamization to occur, the elevated temperature seems to be necessary to increase the conformational flexibility of the molecules.

Scheme 2



Our successful synthesis of **5a** and **5b** would provide a basis of future synthesis directed to the variously functionalized analogs of phomopsin-ustiloxin class of antibiotics. Currently, synthesis of cyclic peptides with additional functional groups are in progress in this laboratory for further approach to the minimal bioactive structure of phomopsin-ustiloxin class of antibiotics.¹¹

ACKNOWLEDGEMENT

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10. All new compounds gave satisfactory spectroscopic and analytical data. Representative ^1H -nmr data (500 MHz, CDCl_3) for selected compounds.
5a: δ 6.87 (d, $J = 8.1$ Hz, 1H), 6.85 (d, $J = 1.8$ Hz, 1H), 6.68 (dd, $J = 2.0$ Hz, $J' = 8.1$ Hz, 1H), 6.57 (br, 1H), 5.76 (br, 1H), 5.58 (br 1H), 4.41 (m, 2H), 3.74 (d, $J = 6.3$ Hz, 2H), 3.67 (m, 2H), 2.92 (m, 2H), 2.38 (m, 2H).
5b: δ 7.21 (dd, $J, J' = 7.8$ -8.0 Hz, 1H), 6.87 (br, 1H), 6.81 (dd, $J = 2.5$ Hz, $J' = 8.2$ Hz, 1H), 6.78 (d, $J = 7.3$ Hz, 1H), 6.62 (br, 1H), 5.87 (br, 1H), 4.32 (m, 2H), 3.69 (d, $J = 6.3$ Hz, 2H), 3.64 (m, 2H), 2.98 (m, 2H), 2.40 (m, 2H).
11. Although ustiloxin D exhibited strong *anti*-tubulin activity ($\text{IC}_{50} = 6.6\mu\text{M}$)⁷, both **5a** and **5b** did not inhibit the microtubule assembly ($\text{IC}_{50} > 100\mu\text{M}$). An inhibitory activity was determined as described previously. M. Takahashi, S. Iwasaki, H. Kobayashi, S. Okuda, T. Murai, Y. Sato, T. Haraguchi-Hiraoka, and H. Nagano, *J. Antibiot.*, 1987, **40**, 66.