

**SYNTHETIC STUDY OF USTILOXIN ANALOGS:
BENZYLIC OXIDATION OF 13-MEMBERED CYCLIC PEPTIDE BY
LEAD TETRAACETATE¹**

Masato Takahashi, Ryuichi Shirai, Yukiko Koiso, and Shigeo Iwasaki*

Institute of Molecular and Cellular Biosciences (IMCB)

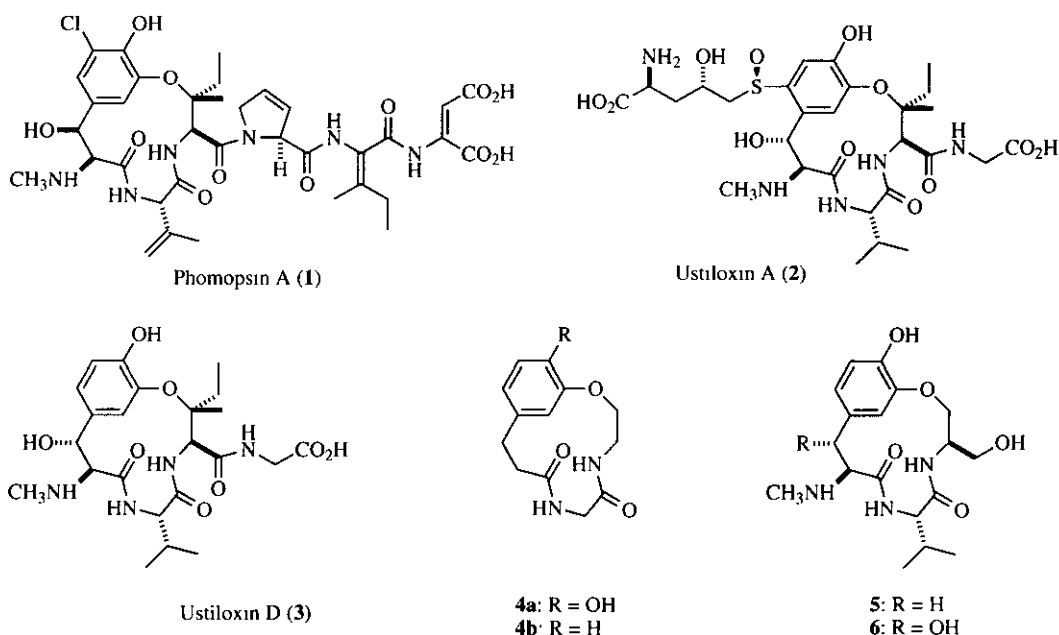
The University of Tokyo

1-1-1, Yayoi, Bunkyo-ku, Tokyo 113, Japan

Abstract- The 13-membered cyclic peptides (**5**, **6**), analogs of phomopsin-ustiloxin class of antibiotics, have been synthesized. Benzylic hydroxyl group was introduced in desired stereochemistry by lead tetraacetate oxidation followed by methanolysis.

There are a number of natural and synthetic compounds that interfere with microtubule function by binding to tubulin. Maytansine,² rhizoxin,³ dolastatin 10,⁴ phomopsin A(**1**)⁵ and ustiloxin A(**2**)⁶ are known to share the same binding site (RZX-MAYsite)^{7,8} on tubulin. However, their structural diversity remains it difficult to find their common structural elements to recognize the same binding site. Ustiloxin D(**3**),⁸ isolated as the minor component of ustiloxin family from the water extract of false smut balls, also exhibits potent anti-tubulin activity. The common structure found in **1**, **2** and **3** demonstrates that 13-membered core structure is

Figure 1

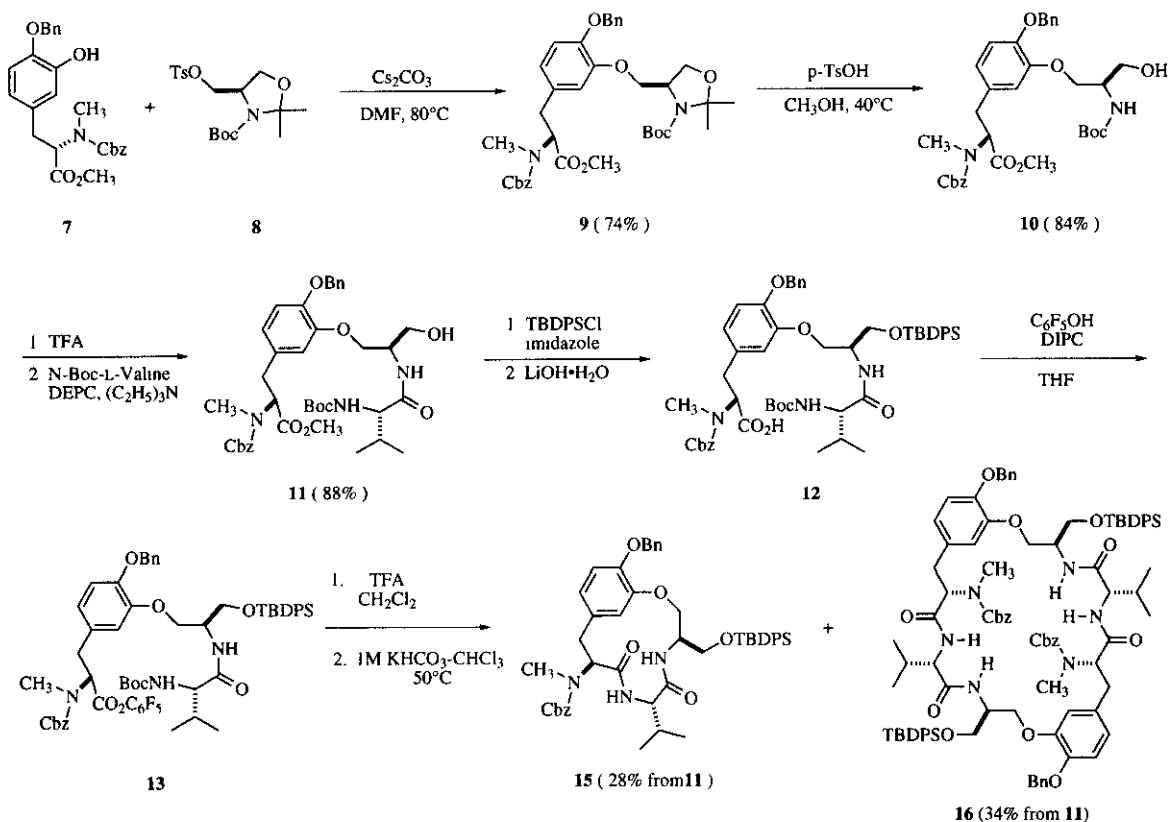


responsible for anti-tubulin activity. Therefore, ustiloxin D was regarded as the promising candidate to elucidate the structural requirement for the RZX-MAYsite ligands.

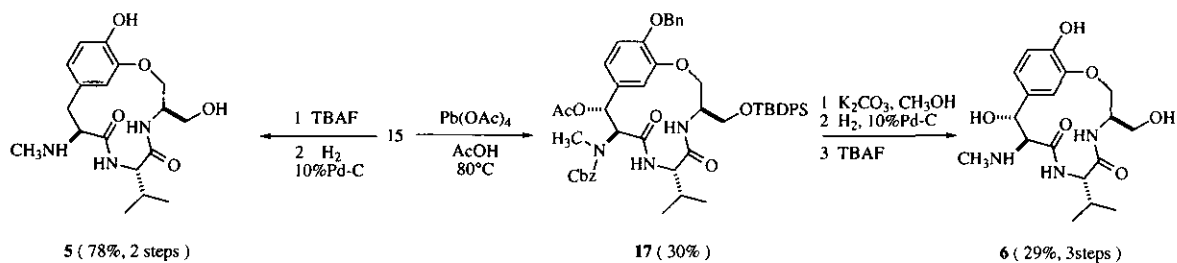
We have been working in the synthesis of ustiloxin analogs to find the minimal structure responsible for anti-tubulin activity. In the previous report, synthesis of non-substituted 13-membered cyclic peptides (**4a**) and (**4b**) was described.⁹ However, both compounds did not inhibit the microtubule assembly even at higher concentration ($IC_{50} > 100 \mu M$). Therefore, as the next challenge, we planned to synthesize more functionalized 13-membered cyclic peptides (**5**) and (**6**) to elucidate the minimal active structure as effective RZX-MAYsite ligand. Our synthetic strategy described below should make it possible not only to introduce a variety of functional groups into the 13-membered ring but also to modify the side chain easily.

Synthesis of **5** and **6** are achieved as shown in **Scheme 1** and **Scheme 2**. Starting L-DOPA derivative (**7**) was prepared from L-tyrosine by Boger's procedure.¹⁰ Williamson alkylation of **7** with tosylate (**8**) derived from D-serine, with Cs_2CO_3 as base, gave the corresponding aryl alkyl ether (**9**). Deprotection of *N*-Boc group of **10** with TFA gave the ammonium salt, which was condensed with *N*-Boc-L-valine to give the amide (**11**). Protection of primary hydroxy group as TBDPS (*tert*-butyldiphenylsilyl) ether followed by basic saponification afforded carboxylic acid (**12**). Macrolactamization of **12** was accomplished successfully according to the protocol developed by Schmidt.¹¹ An activation of **12** with DIPC (diisopropylcarbodiimide) and pentafluorophenol provided pentafluorophenyl ester (**13**), which was used without purification for the next

Scheme 1



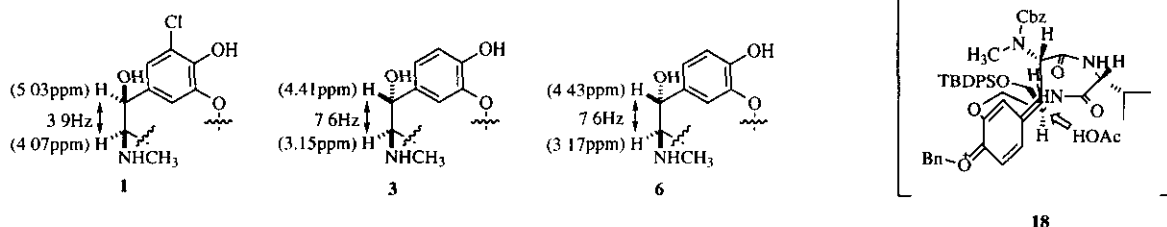
Scheme 2



cyclization. Removal of *N*-Boc-protective group with TFA and successive macrolactamization in two phases system (CHCl_3 -1M KHCO_3) under high dilution at 50°C gave 13-membered cyclic peptide (**15**) in 28% from **11** along with 34% of dimeric cyclic peptide (**16**). Removal of TBDPS group by tetra-*n*-butylammonium fluoride (TBAF), *N*-Cbz group and Bn group by hydrogenolysis gave **5**. In order to introduce the oxygen function at benzylic carbon of **15**, lead tetraacetate oxidation in AcOH was performed at 80°C to give acetate (**17**) as a single diastereomer in 30%.¹²⁻¹⁴ Removal of acetyl, TBDPS, *N*-Boc and Bn groups by $\text{K}_2\text{CO}_3/\text{MeOH}$, TBAF and hydrogenolysis gave **6** with hydroxyl group at benzylic center.

The absolute configuration of benzylic center of **6** was determined to be *R*, the same configuration as **3**, by the comparison of its $^1\text{H-NMR}$ chemical shift and coupling constant with these of **1** and **3** (**Figure 2**).¹⁵ The benzylic and phenethylic protons of **6** appear at 4.43 and 3.17 ppm whereas these protons of **3** appear at 4.41 and 3.15 ppm, respectively. On the other hand, these protons of **1** appear at 5.03 and 4.07 ppm, respectively. Similarly, coupling constant between above protons are 3.9Hz for **1** and 7.6Hz for **3** and **6**. The course of stereoselectivity is reasonably understood that the conjugate addition of acetic acid took place from the less hindered face of quinone methide transition state **18**.

Figure 2



Our successful synthesis of **5** and **6** would provide a basis of future synthesis of phomopsin-ustiloxin class of antibiotics. Further studies on the structure-activity relationship of ustiloxin by synthetic approach and chemical degradation of ustiloxin A are in progress.¹⁶

ACKNOWLEDGMENT

This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. We thank Dr. Naoko Morisaki for FABMS and HRFABMS measure-

ments, Mrs. Hiroko Hino for elemental analyses.

REFERENCES AND NOTES

1. Dedicated to Professor Koji Nakanishi, Columbia University, on the occasion of his 75th birthday.
2. S. M. Kupchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltwanger, and R. F. Bryan, *J. Am. Chem. Soc.*, 1972, **94**, 1354.
3. S. Iwasaki, H. Kobayashi, J. Furukawa, M. Namikoshi, S. Okuda, Z. Sato, I. Matsuda, and T. Noda, *J. Antibiot.*, 1984, **37**, 354.
4. G. R. Pettit, Y. Kamano, C. L. Herald, A. A. Tuinman, F. E. Boetter, H. Kizu, J. M. Schmidt, L. Baczynskyj, K. B. Tomer, and R. J. Bontems, *J. Am. Chem. Soc.*, 1987, **109**, 6883.
5. M. F. Mackay, A. V. Donkelaar, and C. C. J. Culvenor, *J. Chem. Soc., Chem. Commun.*, 1986, 1219.
6. Y. Koiso, M. Natori, S. Iwasaki, S. Sato, R. Sonoda, Y. Fujita, H. Yaegashi, and Z. Sato, *Tetrahedron Lett.*, 1992, **33**, 4157.
7. S. Iwasaki, *Med. Res. Rev.*, 1993, **13**, 183.
8. Y. Koiso, Y. Li, S. Iwasaki, K. Hanaoka, T. Kobayashi, R. Sonoda, Y. Fujita, H. Yaegashi, and Z. Sato, *J. Antibiot.*, 1994, **47**, 765.
9. R. Mutoh, R. Shirai, Y. Koiso, and S. Iwasaki, *Heterocycles*, 1995, **41**, 9.
10. D. L. Boger and D. Yohannes, *J. Org. Chem.*, 1987, **52**, 5283.
11. U. Schmidt, H. Griesser, A. Lieberknecht, and J. Talbiersky, *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 280. U. Schmidt, H. Griesser, A. Lieberknecht, and J. Talbiersky, *J. Org. Chem.*, 1982, **47**, 3261.
12. O. Dimroth and R. Schweizer, *Ber.*, 1923, **56**, 1375.
13. W. S. Johnson, J. M. Anderson, and W. E. Shelberg, *J. Am. Chem. Soc.*, 1944, **66**, 218.
14. G. W. K. Cavill and D. H. Solomon, *J. Chem. Soc.*, 1954, 3943.
15. ¹H-NMR spectra of **6**, phomopsin A and ustiloxin D were measured in d₆-DMSO at rt.
16. An inhibitory activity of microtubule assembly 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. Although ustiloxin D exhibited strong anti-tubulin activity (IC₅₀ = 3.6 μM), both **5** and **6** did not inhibit the microtubule assembly (IC₅₀ > 100 μM).

Received, 12th May, 1997