SYNTHETIC STUDY OF USTILOXIN ANALOGS: BENZYLIC OXIDATION OF 13-MEMBERED CYCLIC PEPTIDE BY LEAD TETRAACETATE[1]

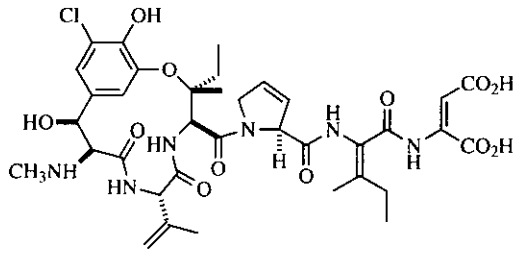
Masato Takahashi, Ryuichi Shirai, Yukiko Koiso, and Shigeo Iwasaki*

Institute of Molecular and Cellular Biosciences (IMCB)
The University of Tokyo
1-1-1, Yayoi, Bunkyo-ku, Tokyo 113, Japan

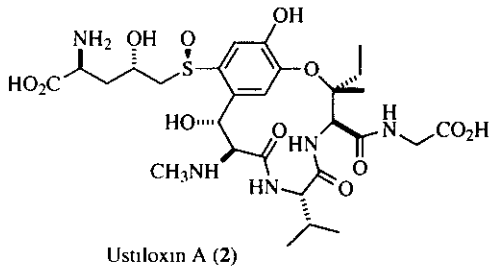
Abstract- The 13-membered cyclic peptides (**5**, **6**), analogs of phomopsin-ustiloxin class of antibiotics, have been synthesized. Benzylic hydroxyl group was introduced in desired stereochemistry by lead tetraacetate oxidation followed by methanolysis.

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

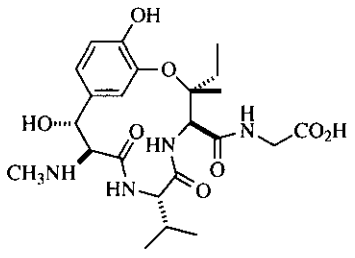
Figure 1



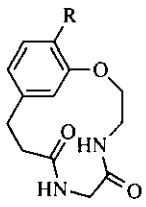
Phomopsin A (**1**)



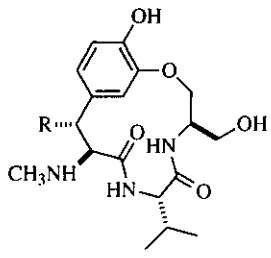
Ustiloxin A (**2**)



Ustiloxin D (**3**)



4a: R = OH
4b: R = H



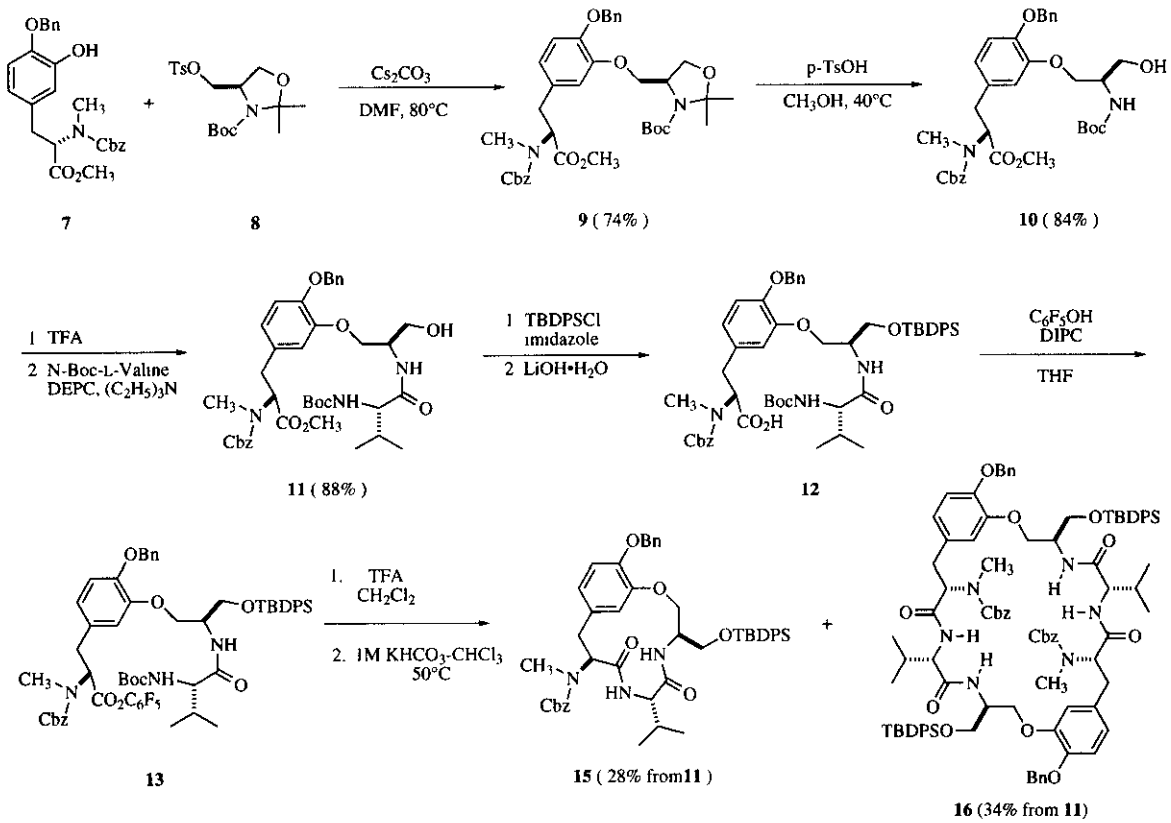
5: R = H
6: R = OH

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} >100μ 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

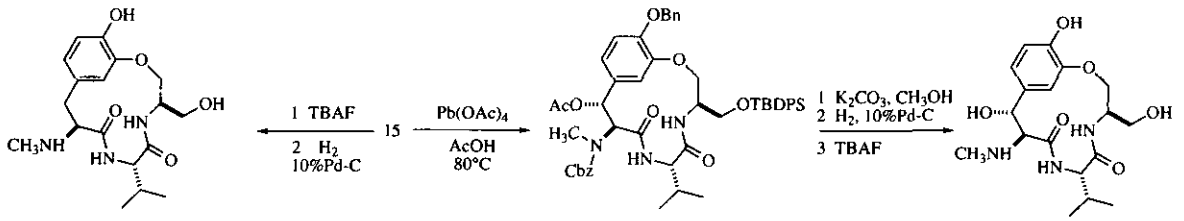


7 **8** **9** (74%) **10** (84%)

11 (88%) **12**

13 **15** (28% from **11**) **16** (34% from **11**)

Scheme 2

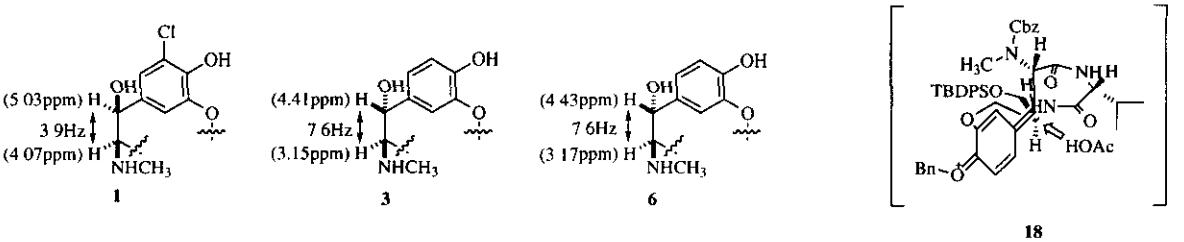


5 (78%, 2 steps) 17 (30%) 6 (29%, 3steps)

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 K_2CO_3/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}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



1 **3** **6** **18**

Our successful synthesis of **5** and **6** would provided 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]

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