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Total synthesis and biological evaluation of ustiloxin natural products and two analogs

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Abstract—Synthetic investigations of ustiloxin natural products are described. The first total synthesis of ustiloxin F was completed in 15 steps via ethynyl aziridine ring-opening by a phenol derivative. The results of biological tests of synthetic ustiloxins D and F, and two analogs, *O*-Me-ustiloxin D and 6-Ile-ustiloxin, demonstrated that the free hydroxyl group *ortho* to the ether linkage is critical for activity and variations at the Val/Ala site produce changes in the biological activity suggesting the need for further perturbations at this site to more extensively study the tubulin binding.

Ustiloxins and phomopsins (Fig. 1) are antimitotic heterodetic peptides isolated from distinctly different sources. Ustiloxins A–F (**1**–**5**) were isolated from the water extracts of false smut balls on rice plant panicles caused by the fungus *Ustilaginoidia virens*.[1] Phomopsins A (**6**) and B (**7**) were isolated from cultures of *Phomopsis leptostromiformis*.[2] Phomopsin A (**6**) is the natural product responsible for lupinosis, a potentially fatal liver disease occurring in livestock in Australia and other countries.[3]

The biological properties of ustiloxins and phomopsins are similar in that they both cause mycotoxicosis and inhibit polymerization of tubulin but have no growth inhibitory effect on bacteria and fungi.[4] Both ustiloxin A (**1**) and phomopsin A (**6**) are believed to interact with tubulin in the Vinca domain, a region which also binds to vinca alkaloids, rhizoxin, dolastatin 10, spongistatin, and several other antimitotic agents.[5,6] However, the mechanism of action of peptide-based antimitotic agents, including dolastatin 10 and cryptophycin 1 as well as ustiloxin A (**1**) and phomopsin A (**6**), is poorly understood in terms of the structural aspects of their interaction with tubulin. Interpretation of the actions and toxicities of these

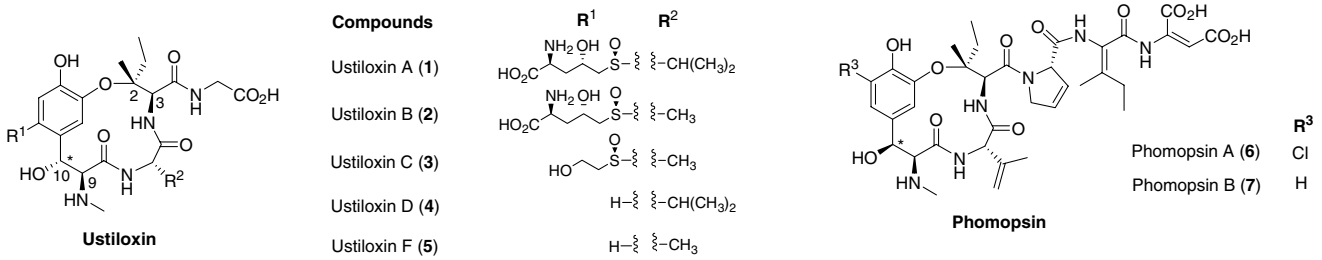

Figure 1. Structures of ustiloxins and phomopsins.

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natural products suffers from a lack of receptor mapping and structural detail.

One of the most striking aspects of the biology of ustiloxin A (**1**) and phomopsin A (**6**) is that these compounds are only modestly cytotoxic (IC_{50} = 2–17 μM) to virtually all tumor cells in vitro, even though they are potent inhibitors of tubulin polymerization.[6] Dolastatin 10, for example, is much more toxic to cells (IC_{50} = 0.5 nM) than **1** or **6**, although they are all comparable inhibitors of tubulin polymerization and phomopsin A (**6**) is a competitive inhibitor of dolastatin 10 binding to tubulin.[5–7] The reasons for the variation in toxicity of tubulin-binding natural products are not well understood. A detailed SAR study would help to determine whether the difference derives from the overall structure of the molecules or from specific substituents.

Further complicating matters is the finding that phomopsin A (**6**) is quite toxic to animals (LD_{50} = 20 mg/kg in sheep) in spite of its moderate toxicity against cell lines. This activity was later attributed to the action of **6** on liver cells,[8] suggesting a possible application in the treatment of liver cancer. The reason for the discrepancy between cellular and animal toxicity remains unexplained. One possibility is that a metabolite of **6** is more toxic than the natural product. Alternatively, the differential toxicity within this family of natural products might be due to widely differing sensitivity of various cell types.

Therefore, ustiloxins and phomopsins provide an attractive entry to the study of antimitotic natural products and their biochemical effects at the molecular and cellular levels. Chemical synthesis would be a suitable approach to access a diverse class of compounds carefully designed to critically examine the biological functions of these natural products. In our efforts to achieve the total synthesis of ustiloxin and phomopsin natural products, ustiloxin D (**4**) was chosen as the lead compound because it is one of the simplest congeners of the ustiloxin and phomopsin natural product families, and it retains fairly high biological activity. The total synthesis of ustiloxin D would provide a scaffold for the other congeners and analogs.

One of the main challenges of the total synthesis of ustiloxin and phomopsin natural products is the stereoselective formation of the chiral tertiary alkyl–aryl ether. Extensive investigations to identify a method to form the challenging tertiary alkyl–aryl ether were carried out since the project began in our laboratory in 1999.[9] The first total synthesis of ustiloxin D was completed in our laboratory in 2002.[9,10] The possibility of atropisomerism was investigated by NOE experiments, which showed that ustiloxin D existed in only one isomeric form at room temperature.[9]

After the completion of the first total synthesis of ustiloxin D, efforts to make the route more convergent were undertaken to facilitate access to other ustiloxin and phomopsin congeners and their analogs. The strategy of the approach was based on an intramolecular S_NAr reaction to form the 13-membered macrocycle (Fig. 2).

Disconnection of the amide bond of **8** gave two key fragments, β-hydroxyphenylalanine derivative **9** and a tripeptide **10** containing a noncoded β-hydroxyisoleucine residue. Regioreversed asymmetric aminohydroxylation could be used to install the requisite stereochemistry of the vicinal amino alcohol functionalities in **9**.[11] Disconnection of amide bonds of compound **10** provides diol **11**, which was an intermediate of the first total synthesis of ustiloxin D.[10]

The synthesis began with sequential treatment of the primary hydroxyl group in **11** with Dess–Martin periodinane and then with $NaClO_2$ to afford the corresponding carboxylic acid, which was coupled with glycine benzyl ester to give **12**. After acid deprotection of the *N*-Boc carbamate, the free amine was coupled with *N*-Boc-valine, followed by a second acid cleavage of the Boc protecting group to afford the tripeptide **10** with a free amino group necessary for the next coupling reaction.

The amino group of the β-hydroxytyrosine derivative **13**[11] was methylated by oxazolidine formation. Sequential reduction[12] was followed by protection of the amino group as its carbobenzyloxy (Z) carbamate. The methyl ester of compound **14** was converted to the corresponding carboxylic acid **9** by $AlBr_3$ and tetrahydrothiophene[13] because basic hydrolysis induced the formation of an oxazolidinone between the benzylic hydroxyl group and the Z carbamate. The coupling of **9** and **10** using EDCI–HOBt provided the macrocyclization precursor **8** in high yield (Scheme 1). The longest linear sequence of the synthesis of **8** was 13 steps.

At this point, if the intramolecular reaction had provided the desired macrocycle **15**, manipulation of the nitro group to the requisite hydroxyl group *ortho* to the ether linkage and global hydrogenation would complete the total synthesis. However, attempts using basic conditions (TBAF, molecular sieves in DMF) as reported by Zhu or under microwave conditions did not afford

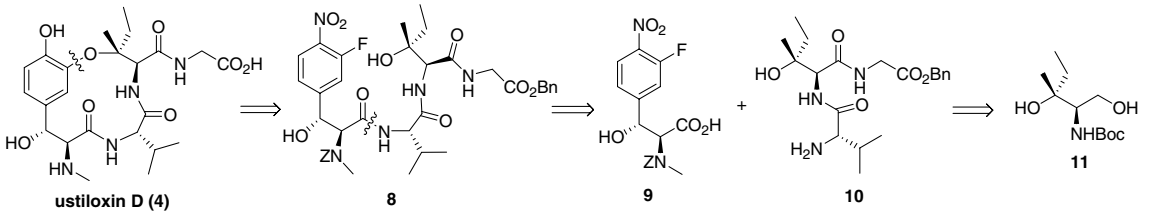
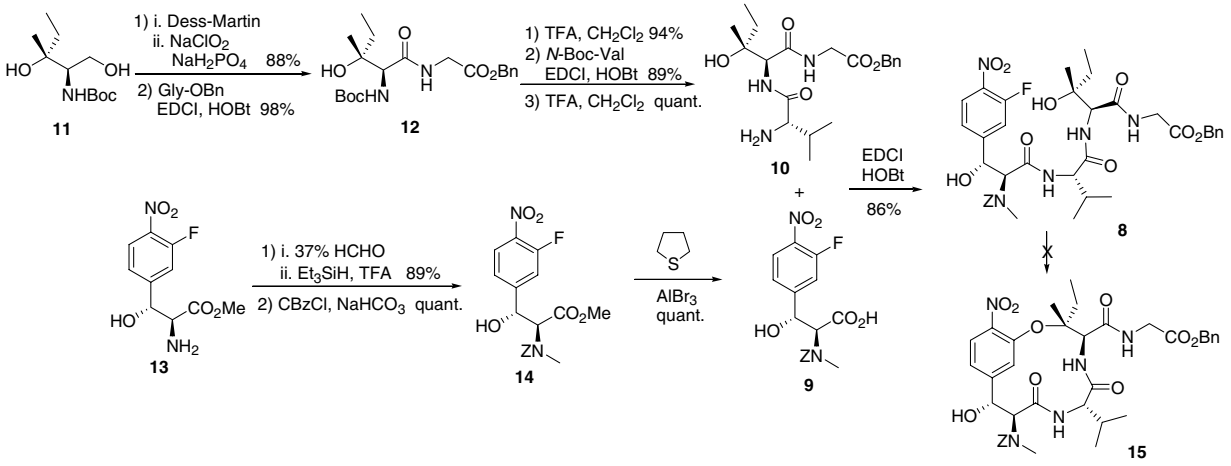


Figure 2. Retrosynthetic analysis of ustiloxin D (**4**).



Scheme 1. Synthesis of linear precursor for S_NAr ring closure.

the macrocycle although a similar strategy was able to provide simplified ustiloxin analogs.[14] These results suggested that the intramolecular S_NAr reaction is not suitable for the synthesis of tertiary alkyl–aryl ethers and prompted us to evaluate alternative approaches to synthesize this motif.

While we were investigating a new method to build the unusual chiral tertiary alkyl–aryl ether motif under mild conditions, Wandless and co-workers reported a second total synthesis of ustiloxin D using the Pd-catalyzed asymmetric allylic *O*-alkylation (AAA) reaction to form the challenging ether linkage. Although the synthesis significantly reduced the linear sequence to 20 steps, it suffered from a diastereoselectivity issue brought by the AAA reaction.[15] In the meantime, an unprecedented copper-catalyzed ethynyl aziridine ring-opening reaction by phenol derivatives was discovered and optimized in our group. This reaction was the key to a convergent approach to the ustiloxin and phomopsin natural products because of its high stereo- and regioselectivity and broad functional group tolerance. Based on the new ethynyl aziridine ring-opening reaction, a convergent total synthesis of ustiloxin D was completed in 15 steps.[16]

As shown in Figure 1, the C-6 amino acid residue is one of the two variations among the ustiloxin natural product family. To take advantage of the new approach in which the C-6 amino acid residue was installed at a late stage, ustiloxin F (**5**) was synthesized via a slight modification (Scheme 2). Amine **19** was synthesized from the ring-opening of ethynyl aziridine **17** by β-hydroxytyrosine derivative **16**, followed by *o*-nosyl deprotection. The coupling of **19** and *N*-Z-alanine provided the precursor **21** for ustiloxin F. Following the simultaneous removal of the benzyl carbamate, benzyl ester, benzyl ether, and reduction of the ethynyl group to the requisite ethyl group by hydrogenation, the resulting linear precursor was cyclized to provide macrocycle **23** in 13% for two steps. We believe that difficulties in the macrolactamization step are responsible for the moderate yield as hydrogenation completely converted **23** to the desired linear precursor. Global acidic deprotection of the Boc

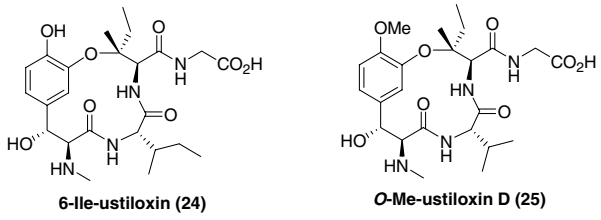
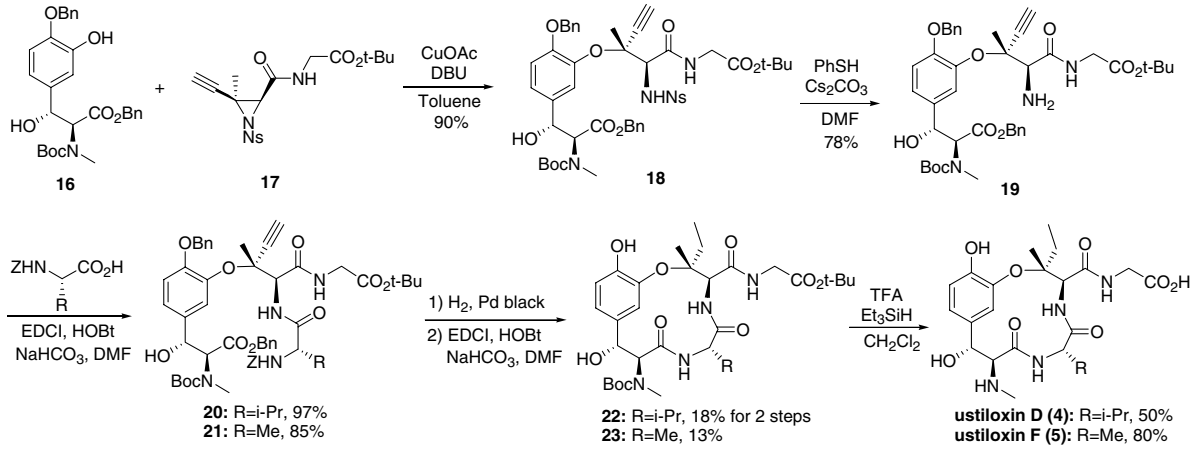


Figure 3. Structures of *O*-Me-ustiloxin and 6-Ile-ustiloxin analogs.



Scheme 2. Synthesis of ustiloxin D (**4**) and ustiloxin F (**5**).

Table 1. Inhibitory effects of ustiloxins on purified tubulin polymerization

Compound	Ustiloxin A (**1**)	Ustiloxin D (**4**) (synthetic)	Ustiloxin F (**5**) (synthetic)	6-Ile-ustiloxin (**24**)	*O*-Me-ustiloxin D (**25**)
IC_{50} (μM)	1.1 ± 0.2	1.5 ± 0.06	8.2 ± 1	4.8 ± 0.4	>40

carbamate and *tert*-butyl ester completed the synthesis of ustiloxin F (**5**) in 80% yield. The first total synthesis of ustiloxin F was completed in 15 steps with a 1.8% overall yield.

Recently, two ustiloxin analogs, 6-Ile-ustiloxin (**24**) and *O*-Me-ustiloxin D (**25**), were synthesized in our laboratory by slight modification of the newly developed route (Fig. 3).

Together with ustiloxin A (**1**) (provided by Professor Iwasaki), synthetic ustiloxin D (**4**), ustiloxin F (**5**), 6-Ile-ustiloxin (**24**), and *O*-Me-ustiloxin D (**25**) were evaluated for their inhibitory effects on the polymerization of purified tubulin.[17] The biological evaluation provided information on the importance of the Ala/Val variant site and the free hydroxyl group *ortho* to the ether linkage to the biological activity of the ustiloxin natural product family.

The IC_{50} values of the inhibition of several ustiloxins on purified tubulin polymerization were obtained (Table 1). The activities of our synthetic ustiloxins D and F, relative to the natural ustiloxin A, are similar to those previously reported for the natural products, although we find greater relative activity in ustiloxin D (**4**) as compared with ustiloxin A (**1**).[2–4] The greater than 3-fold and 5-fold reduced activity of **24** and **5**, respectively, relative to **4** indicates a limited tolerance for size changes, either smaller or larger, for the alkyl substituent at C-6. Even more important for tubulin inhibition is the unsubstituted hydroxyl group on the phenyl ring, since methylation at this position resulted in an almost inactive compound (**25**), although slight inhibitory activity was observed when compound **25** was present in the reaction mixture at 40 μM.

In addition, we performed a cytotoxicity evaluation of these five compounds with the human Burkitt lymphoma cell line CA46. The highest drug concentration examined was 2.5 μM, and only ustiloxin A (**1**) showed any activity, with an IC_{50} value of 2.5 μM. For comparison, the potent antimitotic peptide dolastatin 10[18] was examined simultaneously and yielded an IC_{50} value of 30 nM. The limited cytotoxicity observed here with the ustiloxins is also in agreement with previous results.[2]

In conclusion, synthetic studies of a convergent approach to ustiloxin D were investigated. The first total synthesis of ustiloxin F was completed in 15 steps. Tubulin inhibition studies of natural ustiloxin A, synthetic ustiloxins D and F, 6-Ile-ustiloxin and *O*-Me-ustiloxin D showed that tolerance of substitution at the C-6 position is limited and that the free hydroxyl group *ortho* to the ether linkage is essential for biological activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.06.071.

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