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Evolution of the Total Syntheses of Ustiloxin Natural Products and Their Analogues

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Abstract: Ustiloxins A−F are antimitotic heterodetic cyclopeptides containing a 13-membered cyclic core structure with a synthetically challenging chiral tertiary alkyl-aryl ether linkage. The first total synthesis of ustiloxin D was achieved in 31 linear steps using an S_NAr reaction. An NOE study of this synthetic product showed that ustiloxin D existed as a single atropisomer. Subsequently, highly concise and convergent syntheses of ustiloxins D and F were developed by utilizing a newly discovered ethynyl aziridine ring-opening reaction in a longest linear sequence of 15 steps. The approach was further optimized to achieve a better macrolactamization strategy. Ustiloxins D, F, and eight analogues (14-MeO-ustiloxin D, four analogues with different amino acid residues at the C-6 position, and three (9*R*,10*S*)-*epi*-ustiloxin analogues) were prepared via the second-generation route. Evaluation of these compounds as inhibitors of tubulin polymerization demonstrated that variation at the C-6 position is tolerated to a certain extent. In contrast, the *S* configuration of the C-9 methylamino group and a free phenolic hydroxyl group are essential for inhibition of tubulin polymerization.

Introduction

Antimitotic Agents. Antimitotic agents have long been of interest to scientists and physicians, even before their precise mechanism of action could be articulated. Most of these compounds interfere with microtubule assembly and functions, thus resulting in mitotic arrest of eukaryotic cells. There are a number of natural and synthetic compounds that interfere with tubulin function to inhibit the formation of microtubules.[1] Such antimitotic agents exhibit a broad range of biological activities and can be used for medicinal and agrochemical purposes, acting as anticancer, antifungal, or anthelmintic agents. Moreover, tubulin-binding compounds are valuable for understanding basic mechanisms involved in the dynamics of the microtubule network.[2] Mitotic arrest is of importance in cancer chemotherapy, since tubulin is an established chemotherapeutic target. In addition to the widely used vinca alkaloids and taxoids, many other antimitotic agents are currently in clinical or preclinical development.[3] Newer potential targets that may cause mitotic arrest are in development as well. Although cancer chemotherapy is a very important goal being extensively investigated, the mechanism of action of the naturally occurring peptides that inhibit tubulin polymerization remains a challenging problem. Attempts to establish a relationship among the tubulin binding peptides have had only limited success.[4−8] As a result, the mechanism of action of peptidic antimitotic agents is not completely elucidated and deserves further investigation.

Ustiloxins. The first peptide shown to interact with tubulin was phomopsin A (Figure 1).[9−13] Since then, a number of peptides and depsipeptides were found to disrupt cellular microtubules. These compounds universally inhibit the assembly of the α,β-tubulin dimer into its polymer and, in the cases investigated, strongly suppress microtubule dynamics at low

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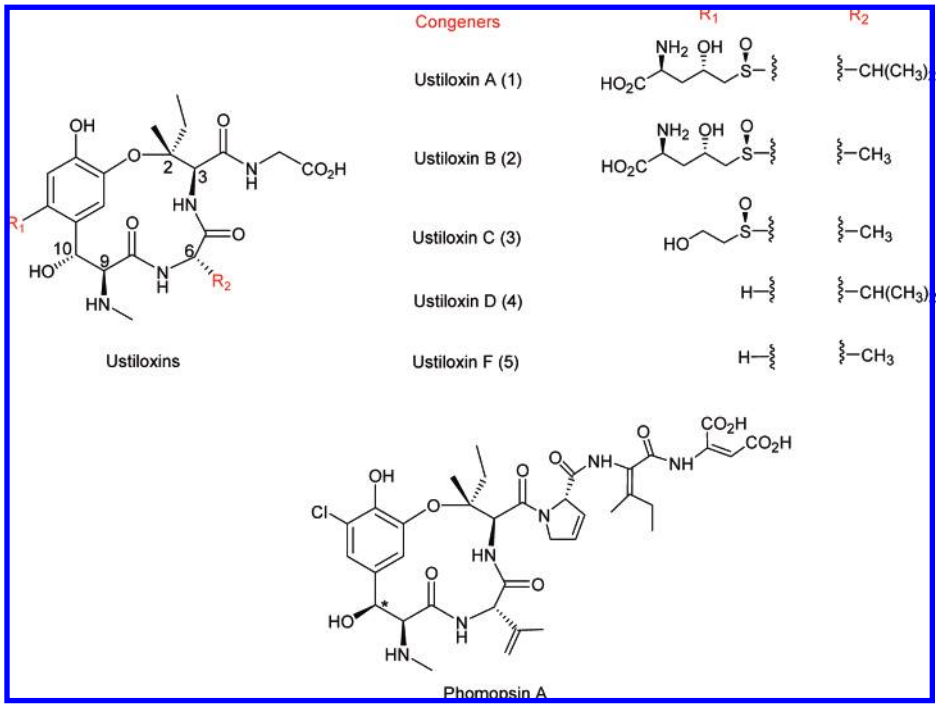

Figure 1. Structures of ustiloxins and phomopsin A.

concentrations. Among these compounds are ustiloxins A−F (**1**−**5**) (Figure 1), which were isolated from the water extracts of false smut balls, caused by the parasitic fungus *Ustilaginoidea virens*, found on the panicles of the rice plant.[14−16]

Ustiloxins are structurally similar to phomopsin A, and their structures were elucidated by amino acid analyses, X-ray analysis of ustiloxin A,[16] and other spectroscopic data. Ustiloxins cause mycotoxicosis and inhibit the polymerization of brain tubulin at micromolar concentrations but have no growth inhibitory effect on bacteria or fungi.[17] Interestingly, the ustiloxins and phomopsin A are relatively weak cytotoxins compared with other antimitotic agents, which are lethal to cells at subnanomolar concentrations.[5]

The structures of the ustiloxin congeners contain a 13-membered heterodetic peptide macrocycle that includes a rare chiral tertiary alkyl-aryl ether linkage. The ustiloxin congeners (**1**−**5**) are composed of permutations of two separate sites: the side chain attached to the macrocyclic aromatic ring para to the ether linkage (see R^1 in Figure 1) and the presence of either alanine or valine in the macrocycle (see R^2 in Figure 1). Both ustiloxins A (**1**) and B (**2**) have a sulfinyl norvaline-based amino acid attached to the aromatic ring at the R^1 position. Ustiloxins A and B differ only in the side chain of a single macrocyclic amino acid. Ustiloxin C (**3**) contains a hydroxyethanesulfinyl group at the R^1 position and the alanine variation of the macrocycle. Ustiloxins D (**4**) and F (**5**) have no sulfinyl appendage on the aromatic ring; they are differentiated from each other only by the presence of valine or alanine in the macrocycle. A major difference between the phomopsins[12] and the ustiloxins[16] is that, although both have a hydroxyl substituent at C-10, they are of opposite configurations.

It is important to determine the mechanism of action of the naturally occurring peptides that inhibit tubulin polymerization so that they may be more efficiently applied in cancer chemotherapy. In the literature, descriptions of potential binding sites of antimitotic peptides and depsipeptides to tubulin are somewhat contradictory.[6,18−20] The ustiloxins appeared to provide fertile ground for studying antimitotic peptide natural products and their inhibitory effects on tubulin polymerization. Synthesis of ustiloxins could provide access to a variety of analogues of the natural product for SAR studies, which would elucidate the interaction between tubulin and the ustiloxin ligands. Ultimately, these analogues would provide information that could lead to a better understanding of the underlying mechanism of microtubule assembly inhibition, as well as the dynamics and impact of modulating these processes on cancer chemotherapy. Given our longstanding interest in the synthesis of cyclodepsipeptides, we present two generations of total syntheses of ustiloxins D and F and a full account of recent progress in the total synthesis of several analogues.

Synthetic Strategy and Retrosynthetic Analysis

Ustiloxin D was chosen as the lead compound because it was one of the simplest congeners of the ustiloxin family and it possessed high biological activity. The total synthesis of ustiloxin D provided a scaffold for the syntheses of other congeners and analogues. From a synthetic perspective, ustiloxin D is composed of four amino acid residues (Figure 2). Two of them are naturally occurring glycine and valine. The other two

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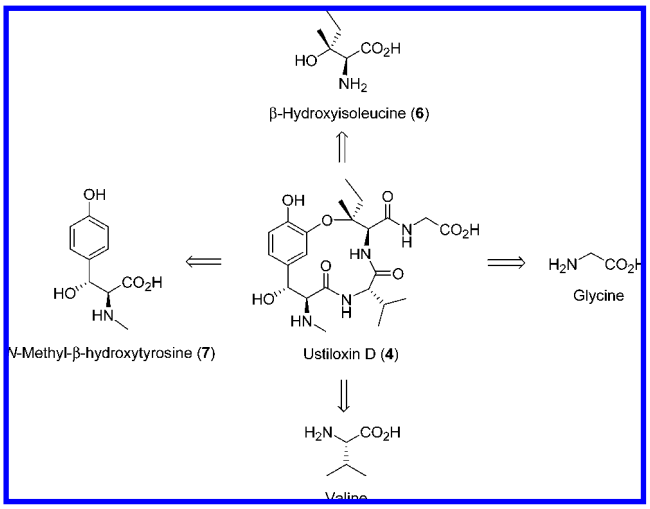


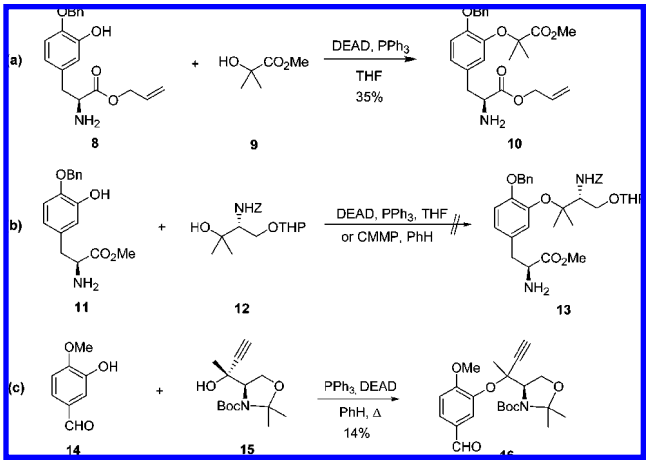
Figure 2. Amino acid components of ustiloxin D.

are non-proteinogenic amino acid residues, namely β-hydroxyisoleucine (**6**) and *N*-methyl-β-hydroxytyrosine derivative **7**. A chiral tertiary alkyl-aryl ether linkage exists between compounds **6** and **7**. The main challenges of the total synthesis are the construction of **6** and **7** by short and stereoselective approaches and the formation of a chiral tertiary alkyl-aryl ether between these two amino acid residues without affecting other functional groups and the existing stereochemistry. The latter specification was especially challenging, given the very limited methods that were available for the transformation and the highly functionalized structure of the ustiloxins.

The Challenges

An alkyl-aryl ether may be retrosynthetically disconnected in two ways, one providing an alkyl alcohol and an aromatic compound with a leaving group. The C−O bond may be formed using a nucleophilic aromatic substitution (S_NAr) reaction or a transition metal-catalyzed coupling reaction between the same reagents. The other disconnection would provide a phenol derivative and an aliphatic compound with a leaving group. In this approach, the most common methods are nucleophilic substitution of an alkyl halide by a phenol or a Mitsunobu reaction between a phenol derivative and an alkyl alcohol. Although these methods are available, the formation of a tertiary alkyl-aryl ether remains a challenging transformation. Stereoselective formation of this functionality is even more difficult. In the first disconnection, the alcohol substrate needs to be a tertiary alcohol. Tertiary alkoxides are more often employed as non-nucleophilic bases, but rarely used as nucleophiles because of steric bulkiness and low nucleophilicity. The second disconnection is not a good alternative since tertiary alkyl species with a leaving group tend to form tertiary alkyl cations readily, which would destroy the stereochemistry of the tertiary carbon center. The competing elimination reaction would inhibit the effectiveness of the nucleophilic substitution reaction. As a result, very few reactions have been reported to form tertiary alkyl-aryl ethers stereoselectively. To the best of our knowledge, only four types of reactions have been reported to construct such an ether motif, namely, S_NAr reactions,[21−23] Pd-catalyzed intermolecular cross-coupling of aryl halides with alcohols,[24−26] Mitsunobu-type reactions[27−29] and Pd-catalyzed asymmetric allylic *O*-alkylation (AAA) reactions.[30−33]

Scheme 1. Ether Formation Study Using the Mitsunobu Reaction



Since the project began in our laboratory, extensive investigations were carried out to identify a method to form the challenging tertiary alkyl-aryl ether bond. It was envisioned that a mild reaction tolerant of a broad range of functionalities would lead to an expedient total synthesis. The Mitsunobu reaction was the first choice. We had successfully synthesized a number of cyclopeptide alkaloids bearing alkyl-aryl ether linkages using the Mitsunobu approach.[34,35] Model studies used phenol derivative **8**, which was synthesized in six steps from tyrosine. Among all the Mitsunobu reaction conditions tested, the best result was obtained by treatment of **8** with methyl-2-hydroxyisobutyrate (**9**) with PPh_3 and DEAD in THF, which provided the desired ether **10** in 35% yield (Scheme 1a).

However, when using a more complex tertiary alcohol **12**, the desired ether **13** was not produced under Mitsunobu conditions (PPh_3, DEAD). A newly developed Mitsunobu reagent, cyanomethylenetrimethylphosphorane (CMMP),[36] was also used but failed to give the desired ether (Scheme 1b). When propargyl tertiary alcohol **15** was treated with isovanillin (**14**) under a Mitsunobu protocol, tertiary alkyl-aryl ether **16** was obtained in a very low yield (Scheme 1c). Although the propargylic alcohol **15** is a more reactive Mitsunobu substrate, no further improvement to this reaction was achieved. Our unsuccessful model studies indicated that the scope of substrates in the Mitsunobu reaction for the tertiary ether formation was limited.[37,38] As a result, we investigated the S_NAr reaction as an alternative approach toward stereoselective alkyl-aryl synthesis.

The advantage of the S_NAr reaction for generation of alkyl-aryl ethers is retention of configuration of the chiral alcohol

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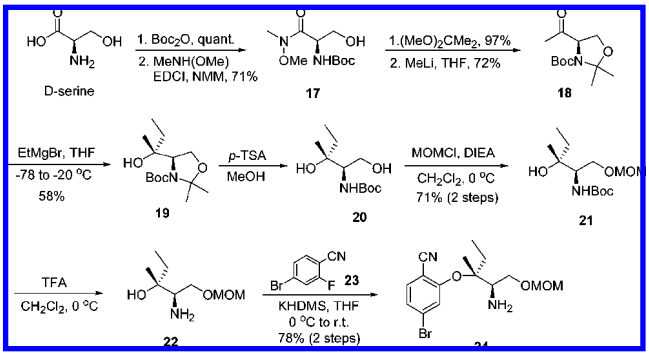
Scheme 2. Synthesis of Chiral Tertiary Alkyl−Aryl Ether **24** via S_NAr Reaction



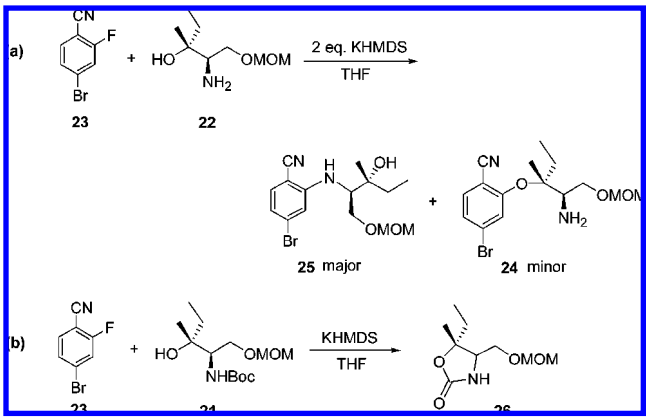
substrates, as no new stereogenic centers are formed in the transformation. For many years, our group has focused on building the ether linkage in cyclopeptide alkaloids using S_NAr reactions. These efforts resulted in the total syntheses of sanjoinine A[39,40] and sanjoinine G1.[41] We used 4-fluorobenzonitrile as an effective reagent for the arylation of alcohols,[42] and several S_NAr reactions on activated aryl halides have been reported.[23,43,44] Wandless and co-workers developed an efficient method for the preparation of hindered alkyl-aryl ethers using potassium bis(trimethylsilyl)amide (KHMDS) to generate the alkoxide.[23] The enantiopure tertiary alcohols were prepared prior to this challenging bond formation, and no selectivity issues were noted during the reaction. However, functional group compatibility remained an issue as the reaction uses strong basic conditions. Nonetheless, Zhu and co-workers applied the intramolecular S_NAr reaction to synthesize a cyclic depsipeptide using tetrabutylammonium fluoride (TBAF) as the base.[44]

The First Total Synthesis of Ustiloxin D

On the basis of the reported success of S_NAr reactions, this approach was used in our first generation total synthesis of ustiloxin D. The key aspects of our synthetic strategy were the installation of the ether linkage at an early stage to circumvent potential problems at the later stages and the stereoselective generation of the β-hydroxytyrosine moiety in a single step. The first total synthesis of ustiloxin D began with the preparation of the protected amino alcohol **19** (Scheme 2). This alcohol was synthesized from D-serine in five steps according to a literature procedure.[45] Treatment of D-serine with di-*tert*-butyl dicarbonate and $NaHCO_3$ protected the amino group as its Boc carbamate, and the carboxylic acid was then coupled with *N*,*O*-dimethylhydroxylamine to afford Weinreb amide **17** using 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl) as the coupling reagent. The amino and hydroxyl groups of **17** were protected as the *N*-*tert*-butoxycarbonyl-*N*,*O*-isopropylidene derivative. Treatment with methyllithium converted the Weinreb amide to methyl ketone **18**. Addition of ethylmagnesium bromide to the ketone proceeded with very good diastereoselectivity, giving rise to the *anti*-Felkin diastereomer of tertiary alcohol **19** as the major product. The absolute configuration of the newly generated stereocenter was confirmed by X-ray crystallography.

Treatment of **19** with *p*-toluenesulfonic acid in methanol gave diol **20**, and the primary hydroxyl group was selectively protected as its MOM ether **21**. Subsequent removal of the Boc group with trifluoroacetic acid (TFA) afforded amino alcohol **22**. Treatment of **22** with aryl fluoride **23** and 1.1 equiv of KHMDS in THF[23] successfully constructed the ether linkage in **24** in 78% yield. A number of problems were encountered during this S_NAr reaction. Two of these are shown in Scheme 3: (a) when **22** and **23** were treated with 2.0 equiv of KHMDS, two products were observed with the undesired aryl amine **25** as the major product due to a faster reaction between the in situ generated amide and aryl fluoride **23**; (b) when the amino group was protected as its *N*-carbamate **21**, an undesired oxazolidinone **26** was produced via intramolecular cyclization. The S_NAr reaction is highly sensitive to the functional groups on the aromatic ring as well. When the cyano group was replaced by a nitro group, no reaction occurred under the same reaction conditions.[38]

Scheme 3. Problems Observed during the S_NAr Reaction Optimization



With ether **24** in hand, the amino group was protected as its Boc carbamate **27** (Scheme 4). Later we found that the three-step sequence from compound **21** to **27** could be performed without purification of crude intermediates to provide better overall yield (78% for three steps). Raney nickel reduction with NaH_2PO_2 in aqueous buffer solution converted nitrile **27** to aldehyde **28**. Treatment of **28** with 30% H_2O_2 and catalytic di-(*o*-nitrophenyl)diselenide via Dakin oxidation afforded the corresponding formate, and subsequent hydrolysis with KOH in methanol gave phenol **29**, which was protected as a benzyl ether **30**. Treatment of **30** with ethyl acrylate, $Pd(OAc)_2$, and $(o\text{-tolyl})_3P$ under Heck conditions afforded cinnamate **31** in 96% yield.

With cinnamate **31** in hand, the regioreversed Sharpless asymmetric aminohydroxylation (AA) protocol[46] was successfully applied to install two stereocenters in a single step, giving the desired (2*S*,3*R*)-β-hydroxy-α-amino ester **32** in 58% yield (Figure 3). To determine the regioselectivity and diastereoselectivity of this reaction, the diastereomer **33** and the regioisomer

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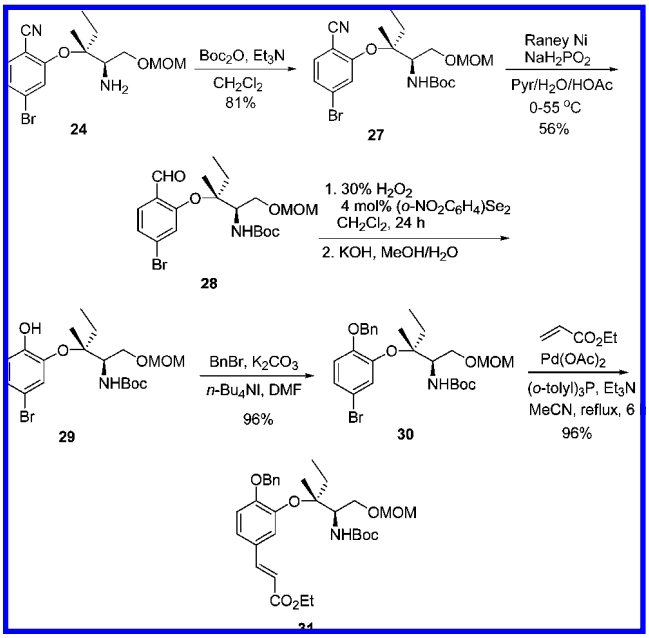
Scheme 4. Synthesis of Intermediate **31** for Use in the Sharpless Aminohydroxylation



34 of compound **32** were synthesized using different chiral ligands. With $(DHQD)_2AQN$ or $(DHQ)_2AQN$ as the ligand, isomer **32** or the diastereomeric (2*R*,3*S*)-*β*-hydroxy-α-amino ester **33** were the major products, respectively. When $(DHQD)_2PHAL$

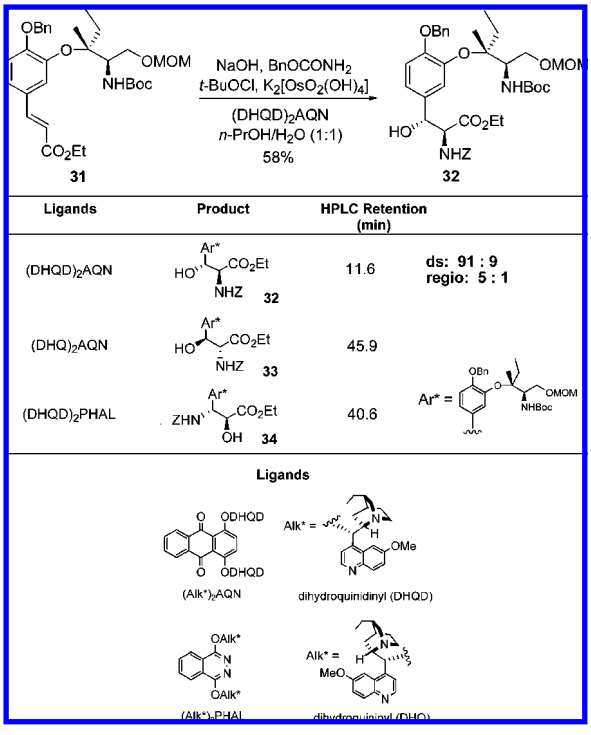
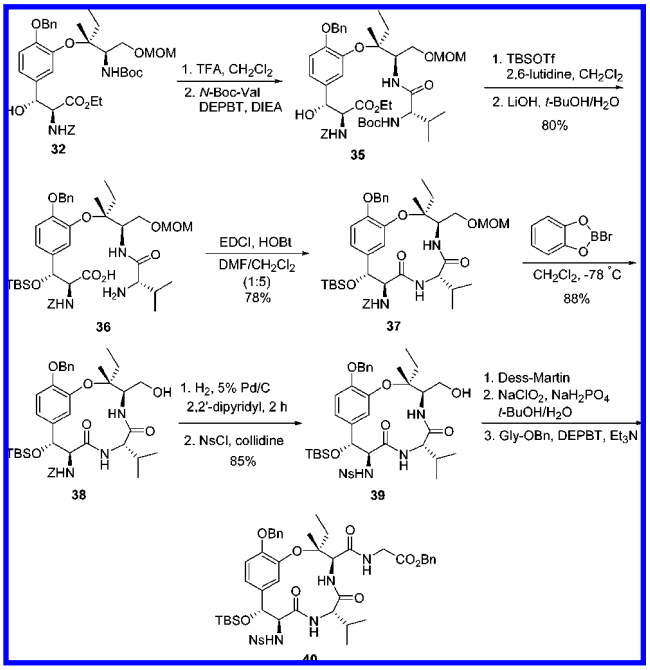


Figure 3. Synthesis of *β*-hydroxy-α-amino ester via regioreversed Sharpless AA reaction.

was used as the ligand, the regioisomeric (2*S*,3*R*)-α-hydroxy-*β*-amino ester **34** was the major product. Using isomers **33** and **34** as internal references, regioselectivity (5:1) and diastereoselectivity (91:9) were determined. The desired compound **32** was separated from other isomers by flash column chromatography on aluminum oxide.

With the amino ester **32** in hand, the Boc group was removed by TFA to afford an amine, which was then coupled with *N*-Boc-L-valine using the DEPBT reagent to give **35** (Scheme 5). Treatment of **35** with TBSOTf protected the secondary hydroxyl group as its silyl ether, as well as transformed the *tert*-butyl carbamate to the corresponding *tert*-butyldimethylsilyl carbamate. Basic hydrolysis with LiOH in *tert*-BuOH−H_2O afforded amino acid **36**, which was then cyclized via macrolactamization to afford macrocycle **37** in 78% yield using EDCI−HOBt in a mixed solvent DMF−CH_2Cl_2 (1:5) as the optimized conditions for macrocyclization. The MOM group was removed using *B*-bromocatecholborane at −78 °C to afford alcohol **38** in 88% yield (Scheme 5).

Scheme 5. Synthesis of Protected Macrocycle **40**



In order to differentiate the nitrogen of the tyrosine α-amino group from the two backbone nitrogens in the macrocycle and to achieve methylation later in the synthesis, conversion of the *N*-carbamate to *N*-sulfonamide was necessary to decrease the pK_a of the nitrogen. The benzyl carbamate was selectively removed by catalytic hydrogenolysis in the presence of the benzyl ether, using 2,2′-dipyridyl as a catalyst poison (Scheme 5). The intermediate amine was subsequently protected as the 2-nitrobenzenesulfonamide **39** in 85% yield for two steps. Sequential oxidation of the primary hydroxyl group in **39** with the Dess−Martin reagent and then with $NaClO_2$ afforded a carboxylic acid, which was coupled with glycine benzyl ester to give **40**. Selective deprotonation of sulfonamide **40** was achieved with the base MTBD (1,3,4,6,7,8-hexahydro-1-methyl-2*H*-pyrimido[1,2-*a*]-pyrimidine),[47] and subsequent methylation with methyl 4-nitrobenzenesulfonate afforded **41** (Scheme 6). Treatment of compound **41** with *n*-Bu_4NF−HOAc deprotected the silyl ether to give alcohol **42**. Removal of the 2-nitrobenzenesulfonyl group with *β*-mercaptoethanol and DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) gave secondary amine **43**.[48,49]

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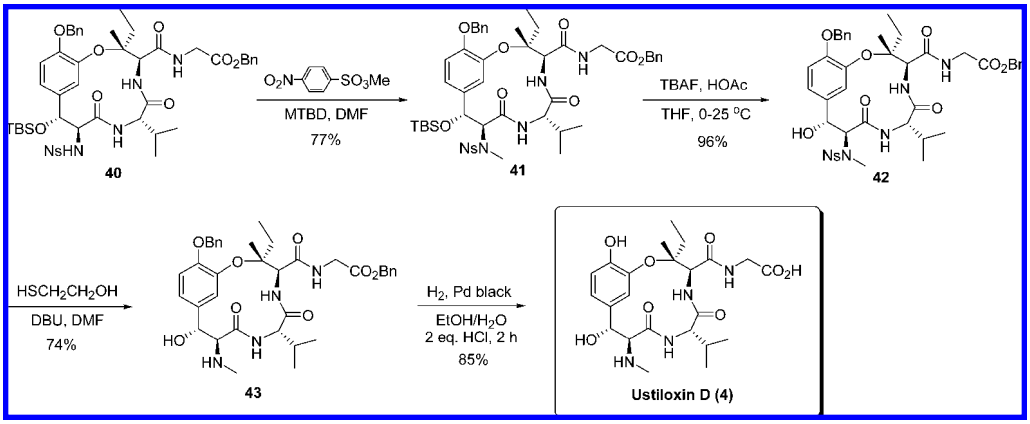
Scheme 6. Completion of the Synthesis of Ustiloxin D



The final step involved hydrogenolysis to remove both the benzyl ester and the benzyl ether. Direct hydrogenolysis with 10% Pd/C, as well as transfer hydrogenolysis with 10% Pd/C and 1,4-cyclohexadiene, removed the benzyl ester but not the benzyl ether. A more active catalyst, palladium black, was needed to remove the protecting groups and afford ustiloxin D (**4**) in 85% yield. The ^{1}H NMR, ^{13}C NMR, HRMS, and optical rotation of synthetic ustiloxin D were identical to those of the natural product.

Atropisomerism

Like many other macrocyclic natural products, such as vancomycins, ustiloxin D may potentially exist as two atropisomers. To clarify the atropisomerism issue, a two-dimensional ^{1}H−^{1}H NOESY experiment was carried out. Ustiloxin D exhibited diagnostic NOE cross-peaks between protons on C-16 and C-10, C-12 and C-9, and C-16 and C-21 (Figure 4). No NOE cross-peaks were observed between protons on C-12 and C-10, C-16 and C-9, or C-12 and C-21. This observation suggested that ustiloxin D was present as a single atropisomer instead of as a mixture of two atropisomers. The three-dimensional structure in Figure 4 was generated based on the information from NOE signals.

The first total synthesis of ustiloxin D (**4**) was accomplished from readily available D-serine and aryl fluoride starting materials. The longest linear sequence of the synthetic route was 31 steps, and the average yield was 82% for each step.[50] We next initiated efforts to improve the efficiency of the synthesis. The strategy was based on an intramolecular S_NAr reaction at a late stage to make the synthesis more convergent. Although the linear precursor was synthesized very efficiently, many attempts using different S_NAr reaction conditions failed to afford the desired macrocycle.[51]

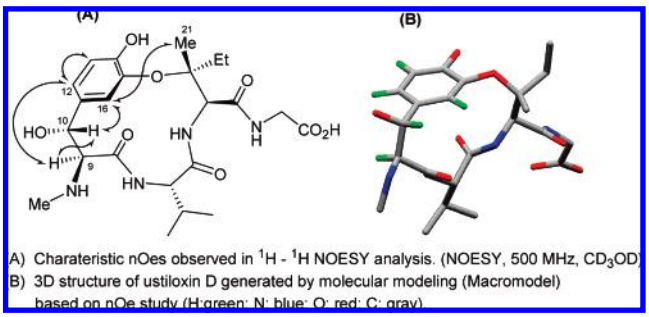


Figure 4. Assignment of atropstereochemistry of ustiloxin D by ^{1}H−^{1}H NOE study.

It was clear that a new method to construct the chiral tertiary alkyl-aryl ether linkage was necessary for a more efficient and convergent synthesis. The new reaction should ideally be performed after β-hydroxyisoleucine **6** and *N*-methyl-β-hydroxytyrosine derivative **7** were built. The conditions for ether formation had to be mild to tolerate all the functionalities of these substrates and not affect any existing chiral centers. Furthermore, as the main difference between the macrocyclic core structures of ustiloxins and phomopsins[12,16] is the stereochemistry of the C-10 hydroxyl group of the β-hydroxytyrosine moiety, adoption of a single method to access all the diastereomers of *N*-methyl-β-hydroxytyrosine was preferred as it would be easily modifiable for the synthesis of phomopsin natural products.

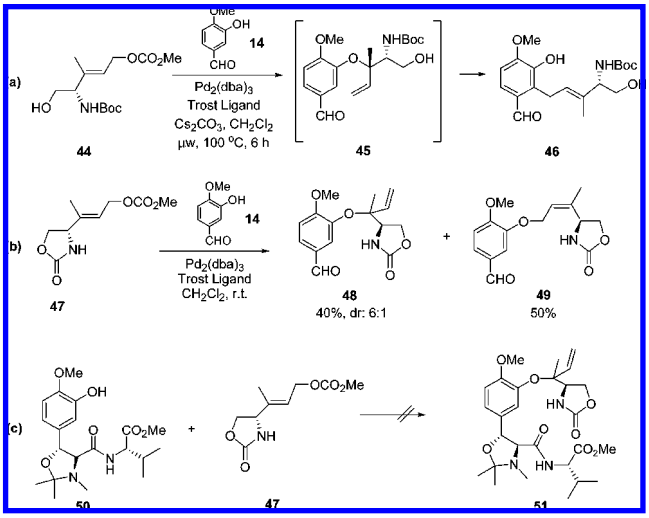
Investigation of AAA Reaction

The Pd-catalyzed AAA reaction has been widely used to construct chiral alkyl-aryl ethers in many natural product syntheses.[31,32] However, applications of the AAA reaction to form a tertiary alkyl-aryl ether stereoselectively are rare because of steric effects.[52] Nonetheless, the mild reaction conditions and broad functional group tolerance would be beneficial for the late-stage synthesis of highly functionalized natural products such as ustiloxins with a proper allylic methyl carbonate. To investigate this approach, allylic carbonate **44**, bearing a nitrogen-containing chiral carbon center and an adjacent hydroxyl group, was subjected to standard AAA reaction conditions with isovanillin (**14**, Scheme 7a).

No reaction was observed at room temperature, but a small amount of product **46** was isolated when the reaction was heated to 100 °C in a microwave reactor. The initial Pd-catalyzed allylic *O*-alkylation presumably formed the desired tertiary alkyl-aryl ether **45**, but it was subsequently converted to the isolated product via a Claisen rearrangement because of the high temperature. To increase the reactivity of the allylic carbonate and allow for lower reaction temperatures, allylic carbonate **47** was synthesized[38] and reacted with isovanillin to provide products **48** and **49** in a 4:5 ratio. Product **48** was shown by ^{1}H NMR to be a diastereomeric mixture in a 6:1 ratio. Although introduction of this cyclic constraint improved the reactivity with isovanillin, carbonate **47** failed to participate in the AAA

(50) Cao, B.; Park, H.; Joullie, M. M. *J. Am. Chem. Soc.* **2002**, *124*, 520.
(51) Li, P.; Evans, C. D.; Forbeck, E. M.; Park, H.; Bai, R.; Hamel, E.; Joullié, M. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4804.
(52) Trost, B. M.; Toste, F. D. *J. Am. Chem. Soc.* **1998**, *120*, 9074.

Scheme 7. Studies of the AAA Reaction



reaction with dipeptide **50**. Attempts to favor the AAA pathway by either adding a base to increase the concentration of phenoxide nucleophile or using the naphthoyl-based ligand did not provide any desired product (**51**). A low conversion was observed in toluene and THF, but the major product was the undesired regioisomer. Using an iridium phosphoramidite catalyst[53] also did not yield any product.

On the basis of these results, we concluded that the more substituted allylic carbon center was too sterically hindered or that the nucleophile was too bulky. At that time, Wandless and co-workers reported a total synthesis of ustiloxin D utilizing an AAA reaction.[54,55] However, a 2:1 ratio of diastereoselectivity was obtained with a relatively simple allylic carbonate, and this inseparable mixture was carried through all subsequent manipulations to the end of the total synthesis.[54,55] On the basis of these results and our model studies, we decided to develop a new stereoselective method to construct tertiary alkyl-aryl ethers.

Discovery of an Aziridine Ring-Opening Reaction by Phenol Derivatives

We next investigated methods used for the formation of tertiary alkyl-aryl ethers with the aim to extend these to a stereoselective method. The aziridine ring-opening reaction by phenol derivatives was very attractive for this purpose.[56−60] Having learned from the results of our investigations of the Mitsunobu reaction that the propargylic carbon center was more reactive toward nucleophilic attack than a normal tertiary carbon center, ethynyl aziridine **52** was used for the study of tertiary alkyl-aryl ether formation by aziridine ring-opening. Investigation of a trisubstituted aziridine ring-opening by phenol derivatives with Lewis acids[51] or phosphines[57] did not provide **53** in acceptable yield (Figure 5). Our results suggested that aziridine ring-opening at the tertiary carbon center was very difficult

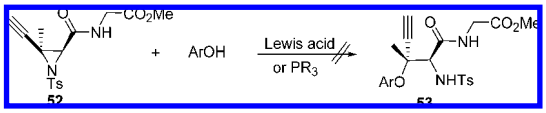


Figure 5. Attempted Lewis acid-promoted ring-opening of aziridine by phenol derivatives.

because of steric effects. The size and nucleophilicity of the phenol were also significant factors.

The formation of aryl 1,1-dimethylpropargyl ethers mediated by copper catalysts appeared to be worthy of investigation.[61,62] The reaction conditions were mild, and the yields were excellent. Although the method had not been used to form stereogenic centers, we felt that a stereochemical induction from a vicinal stereocenter might provide useful levels of selectivity. The chiral amino acid carbon center next to the C-2 center could potentially induce diastereoselectivity. Subjecting propargylic carbonate **54** and isovanillin (**14**) to the conditions of the copper-catalyzed aryl 1,1-dimethylpropargyl etherification afforded the desired tertiary alkyl-aryl ether **55** in 37% yield (Figure 6a). The more highly functionalized β-hydroxytyrosine derivative **56** also exhibited similar reactivity to isovanillin, albeit in poor yield (Figure 6b).

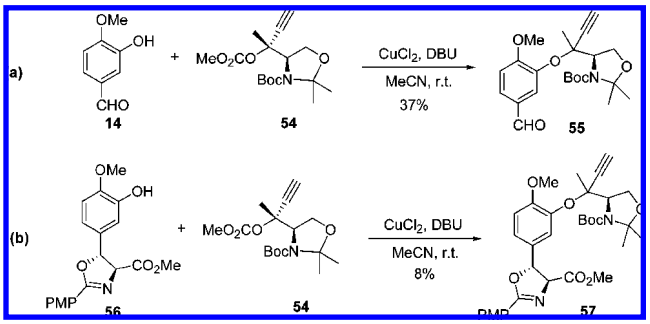


Figure 6. Diastereoselective copper-catalyzed tertiary alkyl-aryl ether formation.

Although these results were very promising, reaction optimization and determination of the stereochemistry of the coupled product were problematic. The reactive propargylic carbonate **54** participated in various nonproductive reaction pathways, and a less reactive leaving group was desired.

Aziridine-2-carboxylate **52**, prepared in the previous study of the Lewis acid promoted aziridine ring-opening, had shown low reactivity where the strained N−C bond behaved as a leaving group. Therefore, we investigated the reaction between isovanillin (**14**) and **52** and found that it afforded a tertiary alkyl-aryl ether **58** as a single product in modest yield (Figure 7a). An advantage of this aziridine ring-opening reaction was that

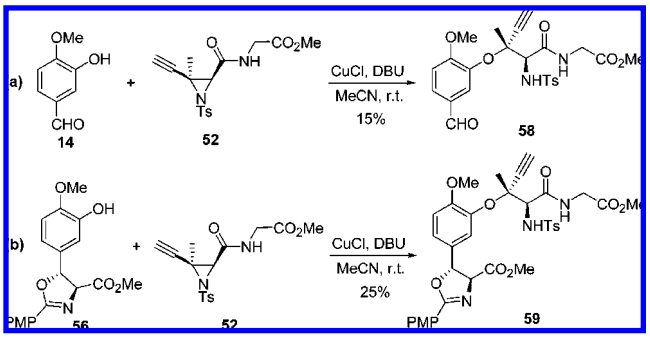


Figure 7. Ethynyl aziridine ring-opening reactions.

(53) Lopez, F.; Ohmura, T.; Hartwig, J. F. *J. Am. Chem. Soc.* **2003**, *125*, 3426.
(54) Sawayama, A. M.; Tanaka, H.; Wandless, T. J. *J. Org. Chem.* **2004**, *69*, 8810.
(55) Tanaka, H.; Sawayama, A. M.; Wandless, T. J. *J. Am. Chem. Soc.* **2003**, *125*, 6864.
(56) Fan, R.-H.; Hou, X.-L. *J. Org. Chem.* **2003**, *68*, 726.
(57) Hou, X.-L.; Fan, R.-H.; Dai, L.-X. *J. Org. Chem.* **2002**, *67*, 5295.
(58) Palais, L.; Chemla, F.; Ferreira, F. *Synlett* **2006**, 1039.
(59) Pineschi, M.; Bertolini, F.; Haak, R. M.; Crotti, P.; Macchia, F. *Chem. Commun.* **2005**, 1426.
(60) Prasad, B. A. B.; Sanghi, R.; Singh, V. K. *Tetrahedron* **2002**, *58*, 7355.

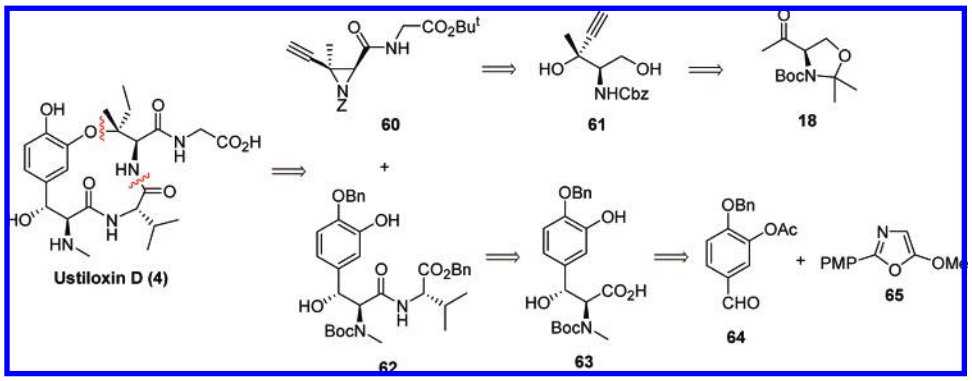
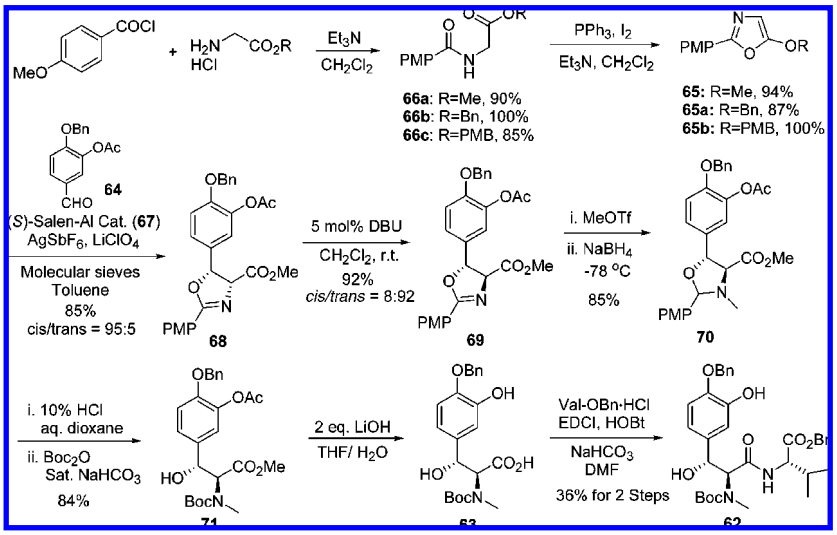


Figure 8. Convergent approach to ustiloxin D.

Scheme 8. Synthesis of β-Hydroxytyrosine Fragment **62**



the product stereochemistry was easily controlled since it was stereoselective and yielded a single diastereomer. Should the C-2 configuration prove to be opposite of what we proposed, the desired product could be obtained from a diastereomer of aziridine **52**. The reaction between **52** and β-hydroxytyrosine derivative **56** afforded the tertiary alkyl-aryl ether **59** as well (Figure 7b).

A number of conditions, including different copper species, bases, solvents, and temperatures were screened to optimize the reaction shown in Figure 7a. Toluene was the solvent of choice. During the screening of different copper species, CuOAc was found to provide the best reaction when combined with DBU as the base. Using these optimized conditions, the desired tertiary alkyl-aryl ether product **58** was isolated in 60% yield after 24 h at room temperature. Moreover, X-ray structure analysis showed that the stereochemical configuration of the product matched that of ustiloxin D.[63]

Second-Generation Total Synthesis of Ustiloxins D and F

Given the mild reaction conditions and broad functional group tolerance, the new regio- and stereoselective ethynyl aziridine ring-opening reaction provided an ideal solution for a convergent ustiloxin total synthesis. Two key intermediates (**60** and **62**) were necessary for the construction of the tertiary alkyl-aryl ether via ethynyl aziridine ring-opening reaction (Figure 8).

First Convergent Approach toward Ustiloxin. The synthesis of fragment **62** started from 5-methoxy-2-(4-methoxyphenyl)-oxazole) **65** (Scheme 8). The oxazole **65** was originally obtained by dehydration of (4-methoxybenzoylamino)acetic acid methyl ester using P_2O_5.[64] However, the reaction was low yielding and not easy to handle, especially under humid conditions. Furthermore, replacing the 5-methoxy group with other alkoxy groups, such as a benzyloxy group, failed to give the desired oxazoles. The problem was solved by using the cyclodehydration conditions developed by Wipf,[65] oxazole **65** was obtained in good yield along with **65a**−**b**. The condensation of *p*-anisoyl chloride with glycine derivatives smoothly afforded **66a**−**c** in excellent yield. Treatment of **66a**−**c** with triphenylphosphine, iodine, and triethylamine presumably formed a carbene that underwent a formal cyclodehydration to yield the oxazoles **65** and **65a**−**b**.

A salen−aluminum complex (**67**)-catalyzed aldol-type reaction between oxazole **65** and orthogonally protected 3,4-dihydroxybenzaldehyde **64** afforded the desired oxazoline-2-

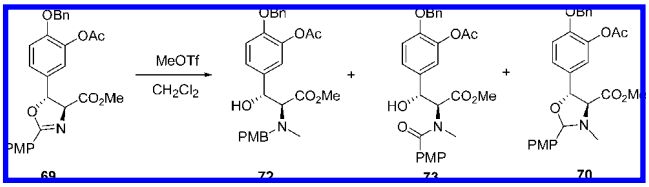
(61) Bell, D.; Davies, M. R.; Geen, G. R.; Mann, I. S. *Synthesis* **1995**, 707.
(62) Godfrey, J. D., Jr.; Mueller, R. H.; Sedergran, T. C.; Soundararajan, N.; Colandrea, V. J. *Tetrahedron Lett.* **1994**, *35*, 6405.
(63) Li, P.; Forbeck, E. M.; Evans, C. D.; Joullié, M. M. *Org. Lett.* **2006**, *8*, 5105.
(64) Karrer, P.; Miyamichi, E.; Storm, H. C.; Widmer, R. *Helv. Chim. Acta* **1925**, *8*, 205.
(65) Wipf, P.; Miller, C. P. *J. Org. Chem.* **1993**, *58*, 3604.

carboxylate **68**.[66] Electron-rich aldehydes undergo slower turnover, so catalyst loading was increased to 10% to ensure full conversion of aldehyde **64** to **68**. Other investigators reported nearly identical results in this aldol-type reaction.[54] The diimine ligand was easily recovered by flash column chromatography, and the catalyst could be regenerated without loss of activity and stereoselectivity. The enantiopure *cis*-oxazoline **68** was obtained in 85% yield after a single recrystallization. However, when oxazole **65a** was subjected to the aluminum−salen-catalyzed aldol-type reaction, only a small amount of the cis product was formed even with high catalyst loadings. No cis product was observed when oxazole **65b** was used in the aldol-type reaction. It appeared that the bulky aluminum−salen catalyst was sensitive to the increased size of the benzyl or *p*-methoxybenzyl substituents, and it could not effectively promote the aldol-type reactions of oxazoles **65a** and **65b**. The electronic properties of the aromatic ring are also critical. No reaction occurred when the acetyl group was replaced by a more electron-donating silyl ether.

DBU-mediated isomerization of **68** afforded the thermodynamically more stable *trans*-oxazoline **69**,[66] with the correct absolute stereochemistry for the construction of ustiloxins. At this stage, it was decided to install the requisite *N*-methyl group by formation of an oxazolinium ion with methyl triflate, followed by in situ reduction.[67] This protocol would replace the four-step selective *N*-methylation sequence employed in our first total synthesis.

During the in situ reduction of the oxazolinium ion, we encountered the same over-reduction problem that Wandless and co-workers had observed.[54] While applying the commonly used conditions of $NaBH_4$ in a 1:4 mixture of methanol and THF at 0 °C to the methoxy phenol analogue of **69**, the desired *N*,*O*-acetal was obtained without any difficulty. However, when **69** was subjected to the same protocol, the C−O bond was cleaved to afford **72** as the major product (Table 1). The relative stereochemical configuration of the adjacent two carbon centers was confirmed by an X-ray crystallographic analysis of **72**.

Table 1. Investigation of Iminium Ion Reduction



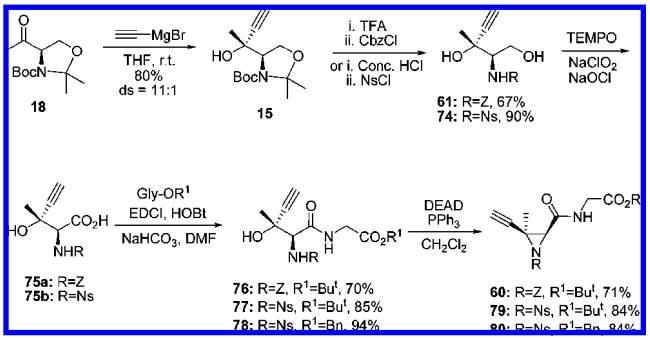
reductant	solvents	temp (°C)	product[a]	yield[b] (%)
$NaBH_4$	MeOH/THF (1:4)	0	**72**	64
L-Selectride	CH_2Cl_2	0	**73**	67
$NaBH_4$	H_2O/THF (1:4)	0	**73**	63
$NaBH_4$	EtOH	−78	**70**	85

[a] Major product. [b] Yield of major product

L-Selectride or $NaBH_4$ in a mixture of water and THF as the reductants provided compound **73** as the major product. Wandless and co-workers developed a biphasic system which successfully prevented over-reduction from occurring,[54] but we found that using ethanol as the solvent and lowering the reaction temperature to −78 °C provided the desired oxazolidine **70** in higher yield. Since diastereomers were formed upon reduction, the *p*-methoxybenzylidene **70** was cleaved by 10% aqueous HCl, and the resulting secondary amine was protected as Boc-carbamate **71** in one pot. Simultaneous hydrolysis of the acetate and methyl ester of compound **71** was next performed using lithium hydroxide, followed by coupling with valine benzyl ester to afford dipeptide **62** in a sequence of eight steps (Scheme 8).

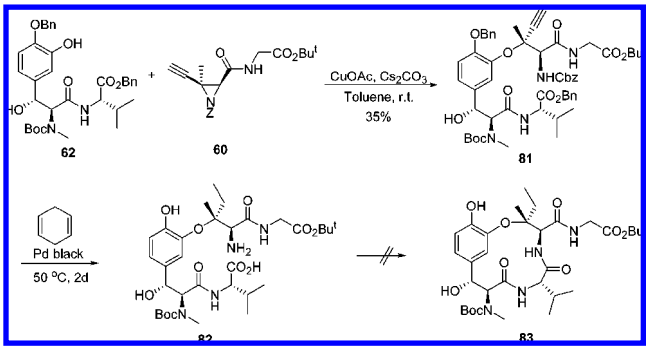
The ethynyl aziridines were synthesized utilizing the methyl ketone intermediate **18** of the first total synthesis of ustiloxin D. The synthesis of ketone **18** was improved[68] via a four-step sequence without column chromatography with an overall 60% yield. Grignard addition of ethynylmagnesium bromide to **18** afforded tertiary alcohol **15** in 80% yield with an 11:1 diastereomeric ratio. The amino alcohol was liberated by acidic full deprotection of intermediate **15**, followed by protection of the primary amino group as its Cbz carbamate (**61**) or nosyl sulfonamide (**74**) in one pot. A one-step TEMPO-catalyzed oxidation protocol directly oxidized the primary hydroxyl group of diols **61** and **74** to carboxylic acids **75a**−**b**, which were coupled with glycine esters to provide the dipeptides without affecting the tertiary hydroxyl group. The Mitsunobu reaction converted the amino alcohols **76**−**78** to the ethynyl aziridines **60**, **79**, and **80** (Scheme 9).[63]

Scheme 9. Trisubstituted Ethynyl Aziridine Synthesis



The aziridine ring-opening reaction between **60** and **62** successfully generated the desired tertiary alkyl-aryl ether **81**. A Pd-catalyzed transfer hydrogenolysis simultaneously removed the Cbz carbamate, benzyl ester, and benzyl ether and reduced the ethynyl group to an ethyl group to provide the linear precursor **82** (Scheme 10).

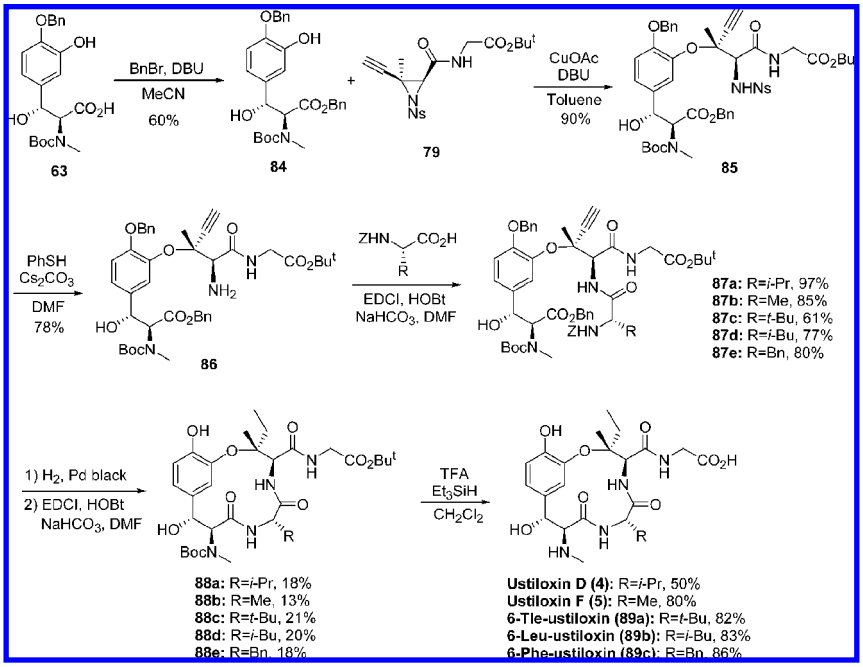
Scheme 10. First Attempt to Ustiloxin D via Aziridine Ring-Opening



To our disappointment, macrolactamization of **82** was unsuccessful with various coupling reagents, bases, and solvents. Wandless and co-workers also experienced difficulties in

(66) Evans, D. A.; Janey, J. M.; Magomedov, N.; Tedrow, J. S. *Angew. Chem., Int. Ed.* **2001**, *40*, 1884.

Scheme 11. Synthesis of Ustiloxins D and F and C-6 Analogues



macrolactamization with a linear ustiloxin precursor at this position. Although only macrolactamization and simultaneous deprotection of the *tert*-butyl ester and the Boc carbamate were needed to finish the total synthesis of ustiloxin D in 14 steps, no further progress was achieved.

Total Synthesis of Ustiloxins D and F, and Three C-6 Analogues. On the basis of these results, it was decided that the macrolactamization between the *N*-terminus of *β*-hydroxyisoleucine and the *C*-terminus of valine was not viable. Another choice was to attempt macrocyclization between the *N*-terminus of valine and the *C*-terminus of *β*-hydroxytyrosine, as both previous total syntheses of ustiloxin D closed the macrocycle at this position.

To improve the protecting group strategy, acid **63** was selectively protected as its benzyl ester **84** (Scheme 11). The ethynyl aziridine ring-opening reaction of **84** with **79** successfully provided the desired chiral tertiary alkyl-aryl ether **85** in 90% yield. The *o*-nosyl group was removed to afford the primary amine **86** in 78% yield.[69] The amine was coupled with *N*-Cbz-valine and *N*-Cbz-alanine using EDCI and HOBt to install all the required amino acid components for ustiloxin D and F respectively. Palladium black catalyzed hydrogenolysis of **87a**−**b** simultaneously removed the benzyl carbamate, the benzyl ester, and the benzyl ether and reduced the ethynyl group to the requisite ethyl group to afford the linear precursors, which underwent macrolactamization to provide macrocycles **88a**−**b** in moderate yield in two steps. Cleavage of the Boc carbamate and *tert*-butyl ester by TFA in the presence of triethylsilane[70] completed the total syntheses of ustiloxin D (**4**)[71] and ustiloxin F (**5**).[51] The longest linear sequence of this second-generation approach was 15 steps. Since the amino acid residue at the C-6 position is one of the two variable positions in the ustiloxin family, it was valuable to investigate the structural requirements at this position. As the new approach introduces the C-6 amino acid residue at the late stage, three analogues (6-Tle-ustiloxin, 6-Leu-ustiloxin, and 6-Phe-ustiloxin, **89a**−**c**) were synthesized using the same strategy.

An Improved Total Synthesis Strategy. Although the new total syntheses of ustiloxins D and F were convergent, concise, and versatile, the approach suffered from a low-yielding macrocyclization step. To facilitate the synthesis of other analogues, the macrolactamization needed to be improved. The yields of macrocyclization were consistently in the range of 10−20%, and coupling reagents seemed to have little effect on the yield. However, in a nonpolar solvent, such as methylene chloride, no product was observed, whereas in a polar solvent, such as DMF, macrolactamization occurred with various coupling reagents. This observation suggested that the solution conformation of the linear precursor, likely due to intramolecular H-bonding, could be preventing macrocyclization. A comparison of the linear precursors of the new route with those of the previous two routes[50,54,55] showed a major difference between the precursors: the phenolic hydroxyl group ortho to the tertiary alkyl-aryl ether linkage was not protected in the new linear precursor, while the other two were both protected as their benzyl ethers in the macrocyclization reaction. A study of the unusual effects remote substituents may have on macrocyclization reactions was reported by Boger and Yohannes.[72] The formation of 17-membered cyclic tripeptides bearing a diaryl ether was thoroughly examined using a full range of substrates. The study showed that remote substituents have substantial, conformationally derived effects on the rate of macrocyclization reactions. Interestingly, in contrast with our results, substrates bearing a free phenol were found to undergo macrocyclization more productively.[73]

The observation led us to hypothesize that intramolecular H-bonding between the free phenolic hydroxyl group and other

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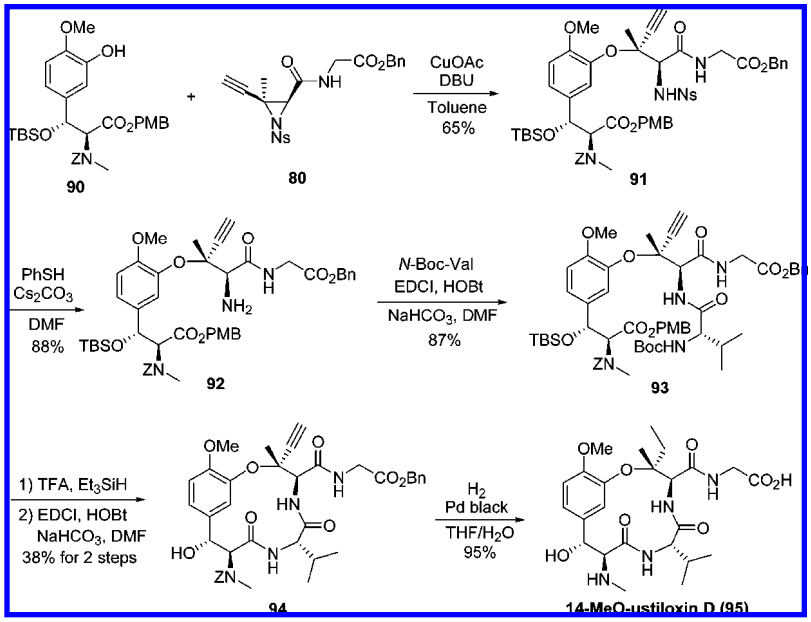
(68) Campbell, A. D.; Raynham, T. M.; Taylor, R. J. K. *Synthesis* **1998**, 1707.
(69) Nihei, K.; Kato, M. J.; Yamane, T.; Palma, M. S.; Konno, K. *Synlett* **2001**, 1167.
(70) Mehta, A.; Jaouhari, R.; Benson, T. J.; Douglas, K. T. *Tetrahedron Lett.* **1992**, *33*, 5441.
(71) Li, P.; Evans, C. D.; Joullié, M. M. *Org. Lett.* **2005**, *7*, 5325.
(72) Boger, L. D.; Yohannes, D. *Tetrahedron Lett.* **1989**, *30*, 5061.
(73) Boger, L. D.; Yohannes, D. *J. Org. Chem.* **1990**, *55*, 6000.

Scheme 12. Synthesis of 14-MeO-ustiloxin D



oxygen or nitrogen atoms in the linear precursor was the problem for the macrocyclization reaction. Computational results supported the hypothesis, as some of the lowest-energy conformations of the linear precursor calculated by MacroModel indicated hydrogen bonding between the phenolic hydroxyl group and other oxygen and nitrogen atoms was causing the distance between the *N*-terminus and *C*-terminus to be too large for amide bond formation.

Model Study: Synthesis of 14-MeO-Ustiloxin. To achieve a better macrocyclization reaction, a linear precursor with a protected phenolic hydroxyl group was needed. At that time, aryl methyl ether **90** was available. This intermediate not only was ideal for the model study but also could provide an analogue to test the importance of the free phenolic functionality for biological activity.

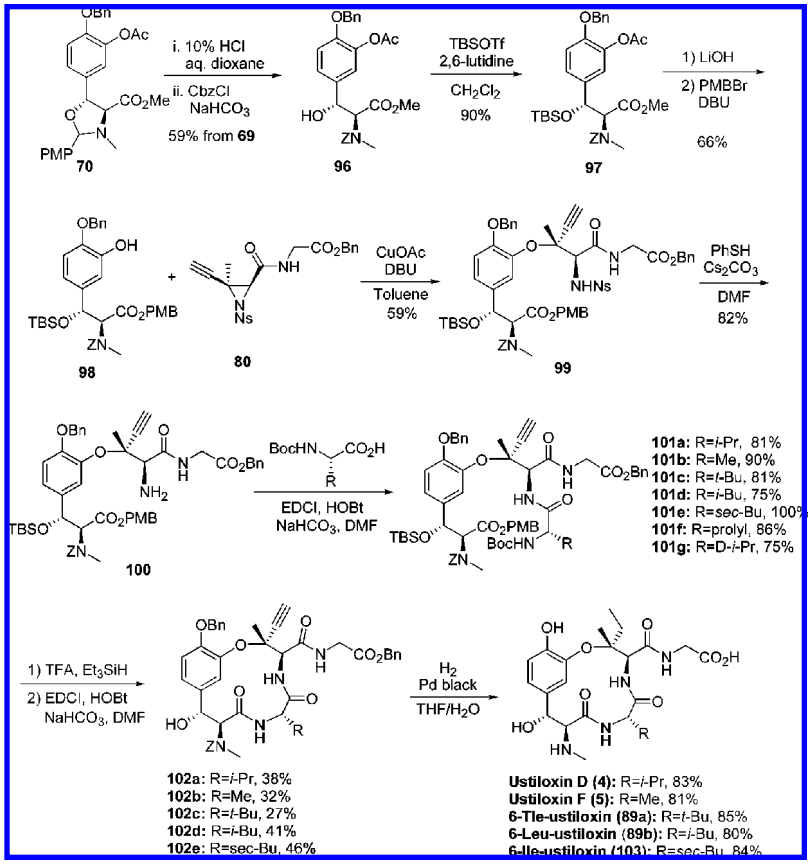
It was realized that if the model study went as proposed and the benzyl ether was present for the macrocyclization, this approach would require avoiding hydrogenolysis in the linear precursor formation as in the total synthesis route. The protecting strategy was changed accordingly to employ the acid labile *p*-methoxybenzyl (PMB) ester and Boc carbamate to protect the termini of the linear precursor. Thus, the linear precursor would be generated by treatment with acid without affecting the phenolic benzyl ether. After macrolactamization, protecting group removal by hydrogenolytic cleavage would provide the natural product analogue.

The ethynyl aziridine ring-opening reaction of **80** with *β*-hydroxytyrosine derivative **90** successfully formed the tertiary alkyl-aryl ether **91**. Deprotection of the *o*-nosyl group and coupling of the resulting free amine **92** and *N*-Boc-valine provided compound **93**. Treatment of **93** with TFA liberated the carboxylic acid and free amino group and deprotected the TBS ether simultaneously. The linear precursor was cyclized to give **94** in 38% yield for the two steps, which was twice the yield that was obtained when the linear precursor had a free phenolic hydroxyl group. This result confirmed the hypothesis that the free phenol made macrocyclization more difficult. The final hydrogenolysis step deprotected the Cbz carbamate and benzyl ester and reduced the ethynyl group to the ethyl group to provide the 14-MeO-ustiloxin D analogue **95** (Scheme 12).[51]

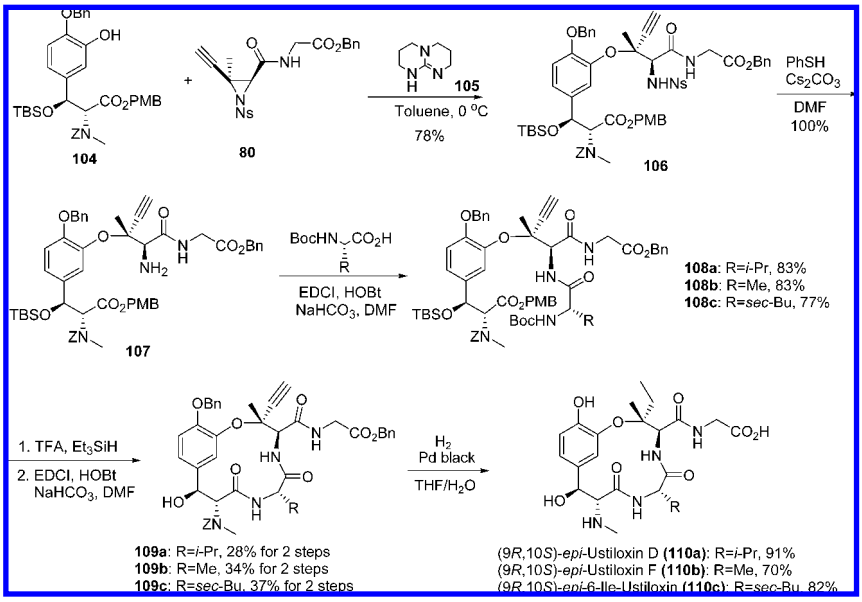
Final Optimization of the New Total Synthesis Route. With the results from the model study, a slight modification of the second-generation synthesis was pursued. In this new plan, *β*-hydroxytyrosine **98** was reacted with the ethynyl aziridine **80**. (Scheme 13). The synthesis of *β*-hydroxytyrosine derivative **98** started from *trans*-oxazolidine **70**. Acidic deprotection of the *N*,*O*-acetal, followed by Cbz carbamate formation of the free amino group, afforded product **96** that, unlike the Boc carbamate **71**, easily formed an oxazolidinone through an intramolecular cyclization under basic conditions. The C-9 benzylic hydroxyl was masked as its TBS ether **97**. Following hydrolysis of acetate and methyl ester, the free acid was selectively protected as the PMB ester to give compound **98**.

The ethynyl aziridine ring-opening reaction between the *β*-hydroxytyrosine derivative **98** and aziridine **80** successfully constructed the desired tertiary alkyl-aryl ether **99**. The free amino group of **100**, liberated from the thiol-assisted nosyl sulfonamide cleavage, was coupled with various Boc-protected amino acids. The resulting products **101a**−**g** were then subjected to TFA with triethylsilane as a cation scavenger. These acidic conditions simultaneously cleaved the Boc carbamate, the PMB ester, and the TBS ether to afford the linear precursors. Macrolactamization successfully provided the macrocycles **102a**−**e**. The two-step yields (**102a**−**d**) were significantly better than those obtained in Scheme 11. Unfortunately, proline- and D-valine-based linear precursors **101f**−**g** did not undergo successful macrolactamization to provide ustiloxin analogues. The (6*R*)-ustiloxin D would have provided data on the role of configuration at C-6 for biological activity. Simultaneous reduction of the ethynyl group to the ethyl group and deprotection of Cbz carbamate, benzyl ester, and benzyl ether by hydrogenolysis afforded ustiloxin D (**4**), ustiloxin F (**5**), 6-Tle-ustiloxin (**89a**), 6-Leu-ustiloxin (**89b**), and 6-Ile-ustiloxin (**103**). A mixture of THF−H_2O instead of ethanol was the solvent of choice for the hydrogenolysis, because it was observed that the carboxylic acid of the glycine side chain was easily esterified

Scheme 13. Improved Synthesis of Ustiloxins



Scheme 14. Synthesis of Three (9*R*,10*S*)-*epi*-Ustiloxin Analogues



under acidic conditions, during both the first total synthesis of ustiloxin D and the synthesis of ustiloxin F.

Syntheses of (9*R*,10*S*)-*epi*-Ustiloxin Analogues. Another goal of the research program was to prepare (9*R*,10*S*)-*epi*-ustiloxin analogues to evaluate their ability to inhibit tubulin polymerization. Comparison of the ustiloxin and phomopsin[12,16] core structures shows that the configuration of the C-10 hydroxyl group is not critical for tubulin binding, because the two natural products have opposite configurations at C-10. This observation suggested that the C-10 hydroxyl group may not play a role in the interaction of these peptides with tubulin. Since it is known that tubulin polymerization activity is sensitive to dimethylation of the β-hydroxytyrosine nitrogen,[74] we felt that investigation of the stereochemical configuration of the 9-amino group was important. As we had developed a robust route to all diastereomers of the β-hydroxytyrosine moiety using Evans'

(74) Morisaki, N.; Mitsui, Y.; Yamashita, Y.; Koiso, Y.; Shirai, R.; Hashimoto, Y.; Iwasaki, S. *J. Antibiot.* **1998**, *51*, 423.

aldol-type chemistry, we chose to prepare (9*R*,10*S*)-*epi*-ustiloxin analogues, and their synthesis is shown in Scheme 14.

β-Hydroxytyrosine **104** was successfully prepared by the same approach used for its enantiomer (**98**) using *R*-salen−aluminum catalyst. At this stage, our investigations showed that the use of TBD (**105**) as a base gave better results than DBU and CuOAc combined and also minimized side reactions in the ethynyl aziridine ring-opening reaction.[75] Indeed, the reaction between **104** and **80** in the presence of TBD gave superior results and afforded the chiral tertiary alkyl-aryl ether **106** in 78% yield. An additional advantage was that a 1:1 ratio of **104** and **80** was enabled by the use of TBD versus a 1.5:1 ratio when DBU was employed.[76] This result greatly improved the material throughput for the synthesis of analogues, and this efficient joining of the complex β-hydroxytyrosine and aziridine components highlights the convergence of our synthetic route. The *N*-nosyl group was cleaved by treatment with benzenethiol to provide the free amine **107** in quantitative yield. The amine was used as a branching point to incorporate three amino acid residues, specifically valine, alanine, and isoleucine. The amino acids valine and alanine were chosen because these residues are found in ustiloxin D (**4**) and F (**5**), respectively. In previous investigations, an active 6-Ile-ustiloxin analogue had been prepared, so we chose to introduce isoleucine to provide a comparable (9*R*,10*S*)-*epi*-6-Ile-ustiloxin analogue.

The coupling of amine **107** with valine, alanine, or isoleucine provided the desired protected linear precursors **108a**−**c**, respectively, in excellent yields. Using the improved macrolactamization and same end-game strategy, (9*R*,10*S*)-*epi*-ustiloxin analogues **110a**−**c** were prepared efficiently. These three (9*R*,10*S*)-*epi*-ustiloxin analogues are the first analogues synthesized to probe the structural basis for the interaction of the β-hydroxytyrosine region of the ustiloxin family with tubulin.

Bioactivity

Disturbance of the dynamic equilibrium of microtubules has critical consequences for cell survival. Since disruption of the mitotic pathway represents a challenging and significant target for oncology, the investigation of ligands that interact with tubulin is essential to better understand their mechanisms of action and biological activity. The course of our investigations led to the preparation of suitable analogues to initiate structure activity relationship studies in the ustiloxin−phomopsin class of antimitotic peptides. The analogues were designed to probe the importance of different structural regions of these peptides for biological activity. Thus far, modifications at the valine residue (C-6), the β-hydroxytyrosine residue (C-9 and C-10), and the phenolic hydroxyl have been investigated.

The biological property we examined was the effect of these compounds on the polymerization of purified tubulin. Tubulin showed a tolerance for structural variation at the C-6 macrocyclic position of the ustiloxins (Figure 9). The most active analogues had the highest degree of branching at the C-6 position, with the synthetic 6-Tle-ustiloxin (**89a**) and ustiloxin D (**4**) displaying comparable activities (IC_{50} = 1.2 and 1.5 μM, respectively). When an isoleucine residue was incorporated at

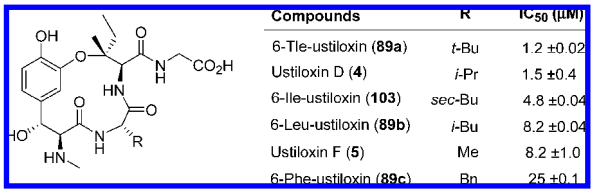


Figure 9. Evaluation of C-6 substitution on inhibition of tubulin polymerization.

C-6 (6-Ile-ustiloxin **103**), a slight decrease in activity was observed. The isoleucine residue possesses the same degree of branching as the valine residue in **4** but is extended by an additional methylene unit, which may cause less effective binding of **103** relative to **4**. When the branching element at the C-6 position was moved further from the macrocycle or removed, as in 6-Leu-ustiloxin **(89b)** or ustiloxin F (**5**), respectively, inhibition of tubulin polymerization was further decreased (IC_{50} = 8.2 μM). An aromatic residue, such as phenylalanine (6-Phe-ustiloxin **89c**), resulted in a still less active compound (IC_{50} = 25 μM). These results suggest that a relatively compact, but highly branched aliphatic residue at the C-6 position is optimal for inhibition of tubulin polymerization.

Biological evaluation of 14-MeO-ustiloxin D (**95**) showed no inhibition of tubulin polymerization (IC_{50} > 40 μM), demonstrating that the phenol moiety of the ustiloxin family is essential for binding to the α,β-tubulin dimer. Iwasaki also reported loss of activity upon methylation of the phenol in a semisynthetic ustiloxin analogue.[74] Similarly, (9*R*,10*S*)-*epi*-ustiloxin D (**110a**), (9*R*,10*S*)-*epi*-ustiloxin F (**110b**), and (9*R*,-10*S*)-*epi*-6-Ile ustiloxin (**110c**) yielded IC_{50} values >40 μM (Figure 10). Since the phomopsin peptides[13] have an *S* configuration at C-10, these results imply that the naturally occurring

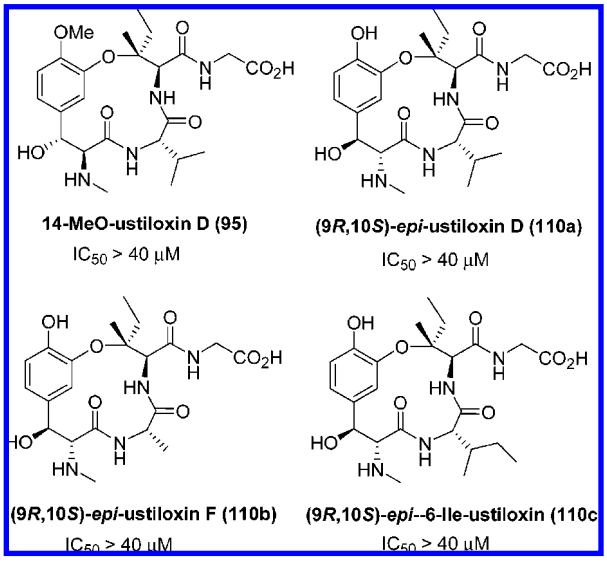


Figure 10. Inactivity of 14-MeO-ustiloxin D and (9*R*,10*S*)-*epi*-ustiloxin analogues.

9*S*-methylamino group is critical for binding to the α,β-tubulin heterodimer. Structural changes that alter the macrocyclic conformation, such as stereochemical modifications, likely place the 9*R*-methylamino group at a distance too great to make contact with the tubulin protein and results in the observed loss

(75) Forbeck, E. M.; Evans, C. D.; Gilleran, J. A.; Li, P.; Joullié, M. M. *J. Am. Chem. Soc* **2007**, *129*, 14463.
(76) Evans, C. D. Ph.D. Thesis. University of Pennsylvania, Philadelphia, PA, 2007.

of activity. Future studies to investigate other regions of the ustiloxin/phomopsin structure should reveal other critical points for binding to tubulin and advance our understanding of the interaction of tubulin with these heterodetic peptides.

Conclusion

Two total syntheses of ustiloxin natural products were achieved. The first total synthesis of ustiloxin D featured application of an S_NAr reaction to construct the tertiary alkyl-aryl ether and regioreversed Sharpless aminohydroxylation to install the two chiral centers of the β-hydroxytyrosine moiety in one step. The synthesis provided a scaffold for further development. The second-generation synthesis took advantage of a newly developed trisubstituted aziridine ring-opening by phenol derivatives and resulted in a concise and convergent synthesis. Ustiloxin D, ustiloxin F, and eight ustiloxin analogues were prepared using this highly versatile strategy. Studies on the inhibition of tubulin polymerization revealed that limited variation at the C-6 position is tolerated and that the free phenolic hydroxyl group and the *S* configuration of the C-9 methylamino group are essential for bioactivity. Investigation of other analogues of the ustiloxin family is ongoing.

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Supporting Information Available: Experimental detail and characterization for all new compounds in the synthesis of ustiloxin D, ustiloxin F, and eight analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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