Natural Product Synthesis

# The Enantioselective Synthesis of Phomopsin B**

Joshua S. Grimley, Andrew M. Sawayama, Hiroko Tanaka, Michelle M. Stohlmeyer, Thomas F. Woiwode, and Thomas J. Wandless

A variety of natural products and synthetic molecules cause mitotic arrest by interfering with microtubule function, and a few molecules of this mechanistic class are anticancer drugs.[1] The ustiloxin and phomopsin families of natural products (Scheme 1) are potent microtubule depolymerizers isolated from the fungus species *Ustilaginoidea virens* and *Phomopsis leptostromiformis*, respectively.[2] Members of both families possess a similar 13-membered macrolactam. The most striking differences between the ustiloxins and the phomopsins are that the latter contain an unsaturated side chain, display dehydration of the valine residue within the macrocycle, and possess the opposite configuration at the C10 position. Competitive tubulin binding assays demonstrated that one binding site for these compounds overlaps with the binding site for vinca alkaloids. A secondary tubulin binding

site for the phomopsins is as yet unidentified.[3] Because ustiloxin D (**1**) is only slightly less active than phomopsin A (**2**), it has been suggested that the macrocycle alone may be responsible for much of the biological activity.[4] Three total syntheses of ustiloxin D have been reported, one from this laboratory and two from the Joullié group.[5] We have used this knowledge, along with our previously reported progress, to synthesize the complex antimitotic phomopsin B (**3**).[6]

Phomopsin B is a hexapeptide although it contains none of the standard proteinogenic amino acids. Rather, there are four unsaturated residues, two oxidized residues, and an aryl alkyl ether side-chain connection. A logical initial disconnection for phomopsin B would rely on independent construction of both the macrocycle acid and side-chain amine, to be joined by peptide coupling. Unfortunately, such a strategy could not be realized,[5c] and our present synthesis necessitated incorporation of the side chain prior to macrocycle closure (Scheme 2). In spite of this accomodation, our established syntheses of both the tripeptide side-chain precursor **6**[6c] and ΔVal **7**[6b] could be used when these units were coupled in sequence with intermediate **5** (Scheme 2). We chose to incorporate a precursor to the last residue of the tripeptide side chain because *E*-ΔAsp isomerizes to *Z*-ΔAsp in alkaline solution.[7]

Although innovative approaches to prepare the vicinal C2,C3 stereocenters of aryl alkyl ether **5** had been reported,
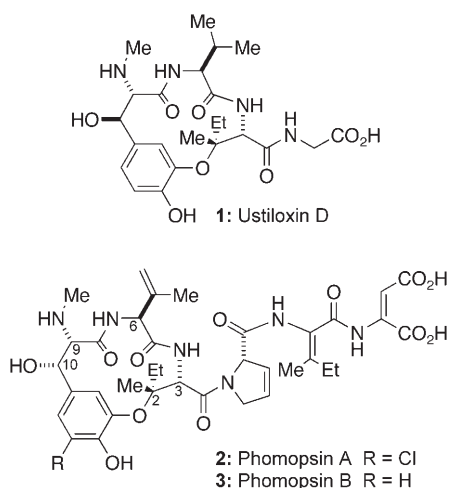
Scheme 1. Structures of ustiloxin D (**1**) and phomopsin A (**2**) and B (**3**).
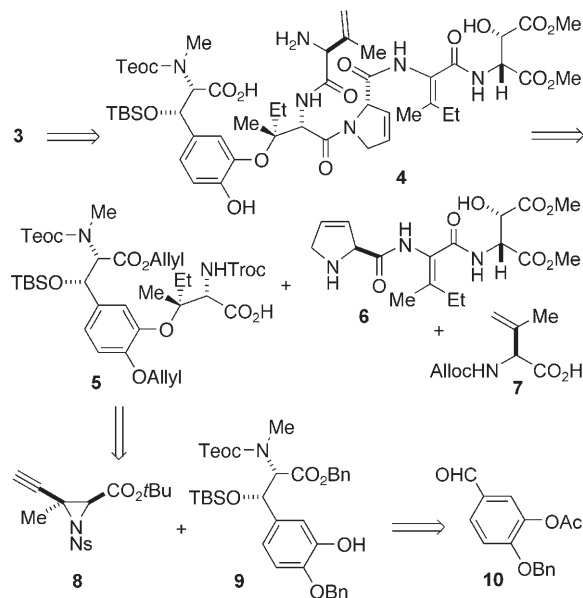
[*] Prof. T. J. Wandless
Department of Chemical and Systems Biology
Stanford University, Clark Center W350A
318 Campus Drive, Stanford, CA 94305 (USA)
Fax: (+1) 650-725-4665
E-mail: wandless@stanford.edu

Dr. J. S. Grimley,[+] Dr. A. M. Sawayama,[+] Dr. H. Tanaka,
Dr. M. M. Stohlmeyer, Dr. T. F. Woiwode
Department of Chemistry
Stanford University (USA)

[+] These authors contributed equally to this work.

Scheme 2. Retrosynthesis of phomopsin B (**3**). Ac = acetate, Alloc = allyloxycarbonyl, Bn = benzyl, Ns = 2-nitrobenzenesulfonyl, TBS = *tert*-butyldimethylsilyl, Teoc = 2-(trimethylsilyl)ethoxycarbonyl, Troc = 2,2,2-trichloroethoxycarbonyl.

these strategies were either lengthy[6a] or unselective.[5b] We envisioned that a recently described use of a phenolate to open a copper(I)-activated alkynyl aziridine[5d] could be used to construct this fragment. This convergent strategy would reduce the complexity to aziridine **8**, a derivative of a published intermediate,[5d] and phenol **9** (Scheme 2). Phenol **9** could be formed using an aldol reaction between 3,4-dihydroxybenzaldehyde derivative **10** and a glycine equivalent that we used in our synthesis of ustiloxin D.[5b]

Our synthesis began with the reaction of benzaldehyde derivative **10** with oxazole **11**[8] using Evans' chiral salen-Al catalyst[9] to produce the *cis*-oxazoline **13** (Scheme 3). As evidence of the versatility of this strategy, we had previously used the opposite enantiomer of the Al catalyst to construct a diastereomeric *trans*-oxazoline for the synthesis of ustiloxin D.[5b] In contrast to ustiloxin's robust *trans*-oxazoline, however, phomopsin's *cis*-oxazoline undergoes rapid isomerization under alkaline conditions, mandating its early unmasking to the underlying *N*-methyl amino alcohol. Fluoride-labile groups were installed as part of a global end-game deprotection strategy, followed by deacetylation under mild conditions to furnish phenol **9** in excellent yield over five steps. Gentle deacetylation conditions were required to prevent epimerization of the C9 Teoc-protected secondary amine.

Minor modifications to published procedures enabled facile construction of aziridine **8** (see the Supporting
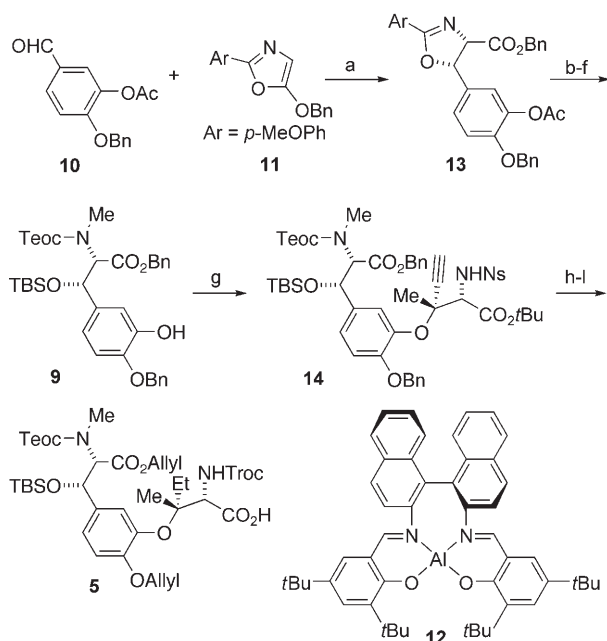
Information), setting the stage for the aryl alkyl etherification. Our initial attempts to form the Tyr-Ile ether followed the Joullié conditions of phenol/aziridine (mol ratio 2:1) with 1% copper(I) acetate.[5d] However, these conditions produced the desired product in only 18% yield. The majority of phenol **9** was recovered, but the aziridine was completely consumed. Optimized conditions required stoichiometric inversion using 2 equiv of aziridine **8** and 1% copper(I) acetate to provide the desired aryl alkyl ether **14** in 87% yield. The perfect regioselectivity of the aziridine opening sets the vicinal stereochemical centers at C2 and C3 in one step.
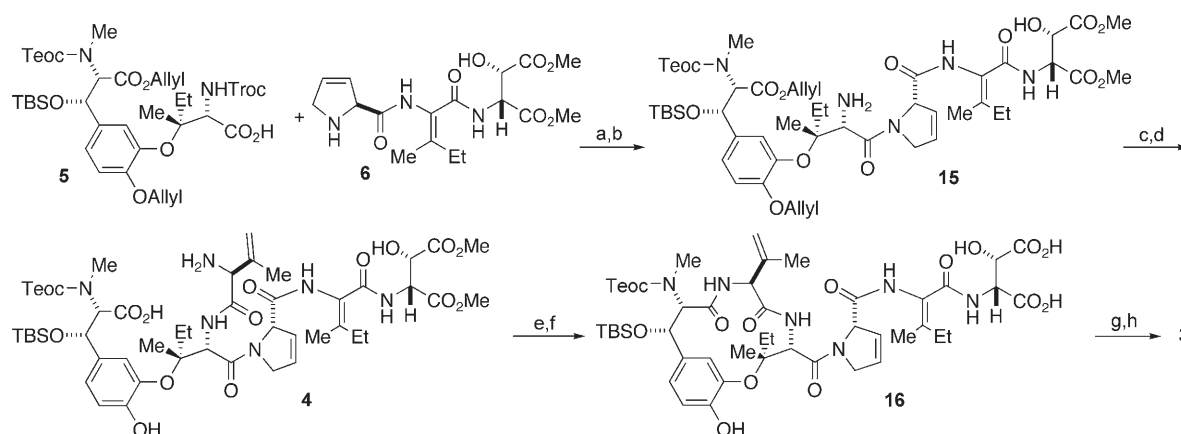
The next major hurdle in the synthesis involved appending the tripeptide side chain and ΔVal. However, before these unsaturated fragments could be incorporated, a redox adjustment of the alkynyl group was required. We therefore exchanged the C3-amine protecting group and exposed the fully protected Tyr-Ile dipeptide to hydrogen and Pearlman's catalyst to hydrogenate the alkyne and concomitantly remove the two benzyl groups in 99% yield. We next installed an orthogonal set of blocking agents to mask the newly liberated oxygens. We could now selectively reveal the C17 carboxylic acid by treatment with silica gel in toluene at 90°C [10] and produce intermediate **5**, which is poised for further elaboration with the dehydrated amino acids.

The preparation of tripeptide **6** has been outlined previously.[6c] Using our methodology, the (*E*)-ΔIle residue was synthesized by the *anti* elimination of cyclic sulfamidite diastereomers. Carboxylic acid **5** and tripeptide **6** were coupled in satisfactory yield to produce adduct **15** (Scheme 4). Following Troc deprotection the final amino acid, Alloc-ΔVal-OH **7**, was coupled to provide the complete carbon skeleton. We next employed palladium to deprotect the C8 allyl ester, the N7-Alloc-protected amine, and phenolic allyl ether to reveal the desired macrocycle precursor in amino acid **4**.[11] Macrocyclization was favored over intermolecular coupling by using high-dilution conditions (3 mM) to provide the desired macrocycle in moderate yield.

Saponification of the terminal methyl esters provided the β-hydroxy-Asp intermediate **16**. Model studies had shown that use of acetic anhydride and pyridine successfully dehydrated the β-hydroxyaspartic acid residue to produce a mixture of the desired (*E*)-ΔAsp and the doubly dehydrated cyclic anhydride. This mixture could be converged into the desired (*E*)-ΔAsp species following brief exposure to lithium hydroxide. Care was taken in the hydrolysis step because prolonged alkaline exposure results in isomerization to the undesired, thermodynamic (*Z*)-ΔAsp product. Finally, the TBS and Teoc groups were removed in one step using anhydrous fluoride conditions of TAS-F in DMF to yield phomopsin B (**3**) following reverse-phase HPLC purification.[12]

The identity of the synthetic phomopsin B was confirmed by MS, HPLC, and NMR spectroscopy. Interestingly, the ^{1}H, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC spectra of the HPLC-purified synthetic and natural compounds were similar but not identical.[13] Upon mixing equimolar amounts of the two samples the spectra display one set of resonances, demonstrating that both samples are phomopsin B (see the Supporting Information). HPLC analysis supports this con-

Scheme 3. Reagents and conditions: a) Na_2SO_4, **12**, $AgSbF_6$, 3-Å MS, PhCH$_3$, 25 h, 96%, 98% ee; b) MeOTf, CH$_2$Cl$_2$, 2 h then NaBH$_4$, NaHCO$_3$, H$_2$O, 0°C, 15 min, 72%; c) 0.1 N (COOH)$_2$, THF, 24 h, 99%; d) TBSOTf, 2,6-lutidine, CH$_2$Cl$_2$, 0°C, 30 min, 87%; e) Teoc-Cl, NaHCO$_3$, CH$_3$CN, 16 h, 98%; f) NH$_2$NH$_2$·H$_2$O, THF, 0°C, 3 h, 98%; g) **8**, CuOAc, DBU, PhCH$_3$, 0°C, 12 d, 87%; h) PhSH, Cs$_2$CO$_3$, DMF, 1 h, 92%; i) 2,2,2-trichloroethyl chloroformate, Na$_2$CO$_3$, THF, 1 h, 96%; j) H$_2$, 20% Pd(OH)$_2$/C, EtOAc, 4 d, 99%; k) allyl bromide, DBU, DMF, 5 h, 70%; l) SiO$_2$, PhCH$_3$, 90°C, 20 h. DBU = 1,8-diazabicyclo-[5.4.0]undec-7-ene, DMF = *N,N*-dimethylformamide, MS = molecular sieves, OTf = trifluoromethanesulfonate.

Scheme 4. Reagents and conditions: a) PyBOP, *i*Pr₂NEt, CH₃CN, 14 h, 28% (2 steps); b) Zn, HOAc, 12 h, 77%; c) **7**, PyBOP, *i*Pr₂NEt, CH₃CN, 1 h, 79%; d) [Pd(PPh₃)₄], *n*Bu₃SnH, AcOH, CH₂Cl₂, 1 h, 74%; e) PyAOP, *i*Pr₂NEt, DMF, 12 h, 48%; f) LiOH, THF, 0°C to RT, 75 min; g) Ac₂O, pyr, 0°C, 1.5 h then LiOH, THF, 15 min; h) TAS-F, DMF, 15 h, 17% (3 steps); PyAOP = (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, pyr = pyridine, TAS-F = tris(dimethylamino)-sulfonium difluorotrimethylsilicate.

clusion (see the Supporting Information). The HPLC analysis further revealed that phomopsin B exists as an equilibrium mixture of two species of significantly distinct polarities. We isolated each of the peaks by HPLC and observed that the two species interconvert, suggesting that one is not a decomposition product of the other (see the Supporting Information). Additionally, LC-MS analysis shows that both peaks report identical molecular ions (755 ($M+$H) and 753 ($M-$H) for $C_{36}H_{46}N_6O_{12}$). It is possible that the acidic HPLC conditions are responsible for the establishment of an equilibrium mixture, as there is no evidence of two distinct species by NMR analysis. Alternatively, it is possible that any spectroscopic differences between the two species are so slight as to prevent detection by NMR spectroscopy, especially given the limited quantities of sample and the existence of atropisomers (i.e., amide rotamers) within phomopsin B that complicate the spectra.

In summary, we accomplished the first total synthesis of phomopsin B in a longest linear sequence of 26 steps. Our convergent synthetic strategy will allow us to probe structure–activity relationships between members of this family of molecules and their microtubule targets. Of the key steps in our synthesis, the aldol reaction, the regioselective aziridine ring opening, and the modular coupling of a valine derivative can be exploited to introduce alternate configurations and altogether new connectivities within the macrocycle.

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