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Synthesis of the C-Terminal Macrocycle of Asperipin-2a

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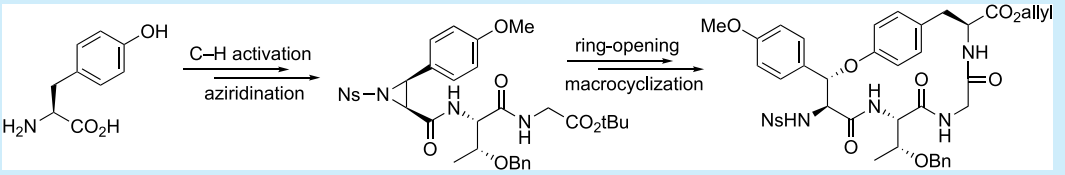
Supporting Information



ABSTRACT: A synthetic approach to the C-terminal macrocycle of asperipin-2a is presented. Two epimers were prepared, possessing *R*- and *S*-configurations at the *β*-position of Tyr[3]. Comparison of NMR data of the natural product with these isomers and X-ray crystallographic data for one macrocycle support assignment of the 2*S*,3*S*-configuration of Tyr[3]. Key steps in the synthesis include a stereoselective benzylic oxidation of the tyrosine residue and Lewis-acid-catalyzed ring opening of the subsequently generated aziridine.

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a class of peptide natural products in which the core structures are encoded directly in precursor proteins.[1−4] In the biosynthesis of RiPPs, a core peptide is fused to leader and/or follower peptides that facilitate installation of post-translational modifications (PTMs). Following modification of the core peptide, the leader and/or follower regions are removed to release the final product. PTMs dramatically increase the chemical and functional space of mature peptides. These modifications include hydroxylation, chlorination, and side-chain oxidation and cross-linking, leading to, for example, highly functionalized cyclic and bicyclic peptides such as the ustiloxins, amanitins, and asperipin-2a (Figure 1).

The ustiloxins were originally isolated from the pathogenic fungus *Ustilaginoidea virens*[5−8] and subsequently from *Aspergillus flavus*.[9,10] A biosynthetic gene cluster (*ust*) was identified in the genome of *A. flavus* as responsible for the biosynthesis of ustiloxin B **2**.[9−12] The ustiloxin gene cluster encodes the UstYa/UstYb proteins, which were identified as responsible for the hydroxylation of the benzylic position of the tyrosine residue and oxidative cyclization to generate the tertiary alkyl−aryl ether linkage present in the ustiloxins.[12,13]

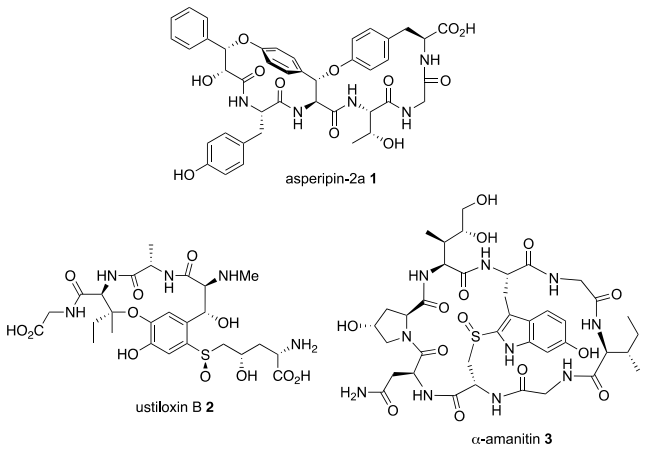


Figure 1. RiPP natural products.

Umemura and co-workers screened the genome sequences of *Aspergillus* for the presence of *ustYa*/*ustYb* gene homologues in a search for novel RiPP natural products.[9,11] From these studies, a RiPP metabolite was isolated and designated as asperipin-2a **1** (Figure 2).[9]

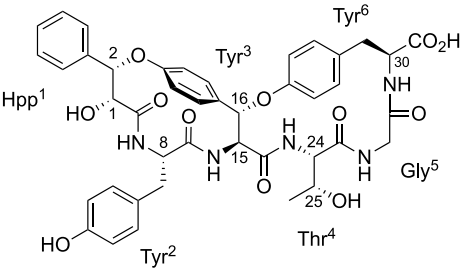


Figure 2. Structure and numbering of asperipin-2a.

Asperipin-2a **1** is a bicyclic peptide that contains three tyrosine residues, threonine and glycine residues, and a 2-hydroxy-3-phenylpropanoic acid (Hpp). The gene cluster encoding this bicyclic peptide includes an *ustYa/Yb* homologous gene, with the UstY-like protein identified as the sole oxidative enzyme.

Asperipin-2a was characterized by 2D NMR spectroscopy and mass spectrometry,[9] which indicated the presence of ether cross-

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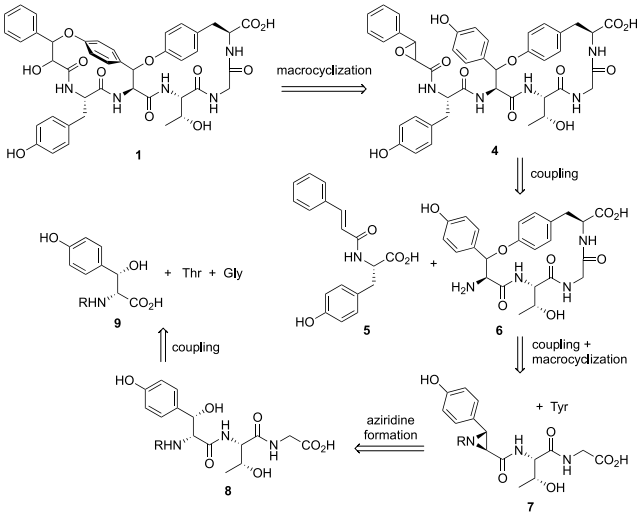
Org. Lett. XXXX, XXX, XXX−XXX

links between the phenolic oxygen of Tyr[6] and the β-position of Tyr[3] and between the phenolic oxygen of Tyr[3] and the β-position of Hpp[1] (Figure 2). More recently, improved production yield of **1** allowed determination of the absolute configuration by chemical degradation, chiral HPLC, and more detailed NMR analysis.[14]

The unusual structure of asperipin-2a, together with the lack of information from the initial report regarding the stereochemistry at positions C1, C2, and C16,[13] prompted us to investigate a route toward the total synthesis of asperipin-2a. Here, we report the first synthesis of the C-terminal macrocycle of asperipin-2a and confirmation of the stereochemical configuration of the central β-functionalized Tyr[3] residue.

The main challenge in the total synthesis of asperipin-2a is the formation of the benzyl−aryl ether linkages in the highly functionalized bicyclic system. A number of methods for the synthesis of benzyl−aryl ether linkages have been reported, including epoxide[15] and aziridine[16,17] ring openings and Mitsunobu reactions.[18,19] We postulated that an aziridine/epoxide ring opening approach would be suitable for the synthesis of both of the ether linkages in asperipin-2a. The devised retrosynthetic analysis is shown in Scheme 1.

Scheme 1. Retrosynthesis of Asperipin-2a 1

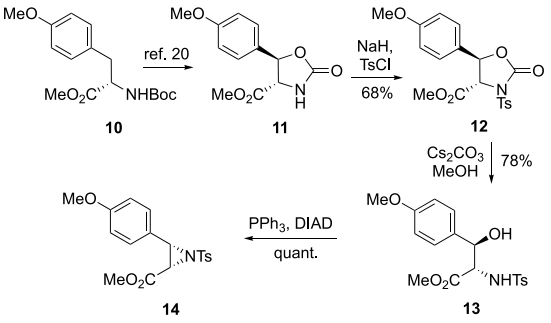


Generation of the N-terminal macrocycle to complete **1** could be approached through intramolecular substitution of a cinnamate-derived epoxide **4** with the phenolic group of Tyr[3]. The C-terminal macrocycle **6** could be approached through an analogous ring opening of aziridine **7** with the phenolic group of Tyr[6]. The required aziridine would be accessed via the corresponding β-hydroxytyrosine **9**.

The required aziridine component was synthesized through C−H activation of Boc-Tyr(Me)-OMe **10** as described by Shimamoto et al.[20] to give the oxazolidinone **11**. Oxazolidinone **11** was N-tosylated and subsequently hydrolyzed to give the β-hydroxytyrosine **13** in good yield. The β-hydroxytyrosine **13** was then converted to the aziridine **14** in quantitative yield through a Mitsunobu-type process (Scheme 2).

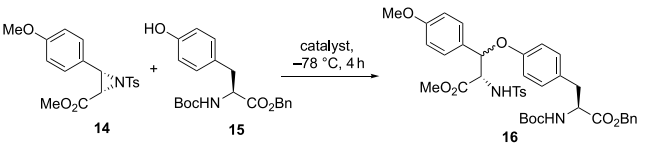
Aziridine **14** was employed in model studies with tyrosine derivative **15** to investigate ring opening reactions to furnish the required ether-linked adduct. The ring opening of aziridine-2-carboxylates with various nucleophiles has been employed in the synthesis of a range of β-substituted amino acid derivatives.[16,21−27] We and others have employed indole as the

Scheme 2. Synthesis of Aziridine 14



nucleophile in combination with metal triflate catalysts for the preparation of tryptophan derivatives.[23,28−30] Accordingly, combinations of aziridine **14** with tyrosine **15** in the presence of a range of Lewis acids were screened for their efficiency in generating ether adduct **16** (Table 1). Of the catalysts screened,

Table 1. Optimization of Aziridine Ring Opening



entry	catalyst	solvent	equiv	yield (%)
1	Cs_2CO_3	DMF	1.1	0
2	TBD	toluene	1.1	0
3	$Cu(OTf)_2$	toluene	1.1	10
4	$Sc(OTf)_3$	toluene	1.1	5
5	$Yb(OTf)_3$	toluene	1.1	0
6	$Bi(OTf)_3$	toluene	1.1	0
7	$BF_3{\cdot}OEt_2$	DCM	0.2	25
8	$BF_3{\cdot}OEt_2$	DCM	0.5	50
9	$BF_3{\cdot}OEt_2$	DCM	1.1	75

it was found that only $BF_3{\cdot}OEt_2$ was effective for activation of the aziridine. Under optimized conditions, treatment of aziridine **14** with **15** in the presence of 1.1 equiv of $BF_3{\cdot}OEt_2$ generated the ether adduct **16** in 75% yield and 65:35 dr. No evidence of C-alkylation was observed.[16] The poor diastereoselectivity presumably indicates the aziridine ring opening proceeds via an S_N1 process with the benzylic carbocation stabilized by the *p*-OMe group. Though the dr is poor, when these studies were initiated, no information regarding the stereochemistry at C16 of asperipin-2a was available, and as such, access to both diastereomers was deemed a favorable pathway.

With the conditions optimized for aziridine ring opening in the model study, application to a fully functionalized peptide was investigated. Accordingly, synthesis of aziridine-containing tripeptide **21** was undertaken. An *N*-nosyl-protected aziridine was chosen to facilitate subsequent sulfonamide deprotection. Accordingly, the oxazolidinone **11** was treated with nosyl chloride followed by hydrolysis of the oxazolidinone and methyl ester functionalities to give β-hydroxytyrosine **17** (Scheme 3). Boc-Thr(Bn)-OMe **18** was coupled with Gly-OtBu, followed by selective deprotection of the Boc group in the presence of the *tert*-butyl ester, to give dipeptide **19**. Coupling of the dipeptide **19** to β-hydroxytyrosine **17** in the presence of EDC gave tripeptide **20** in good yield. The β-hydroxytyrosine moiety in **20** was then converted to the aziridine **21** through a Mitsunobu-

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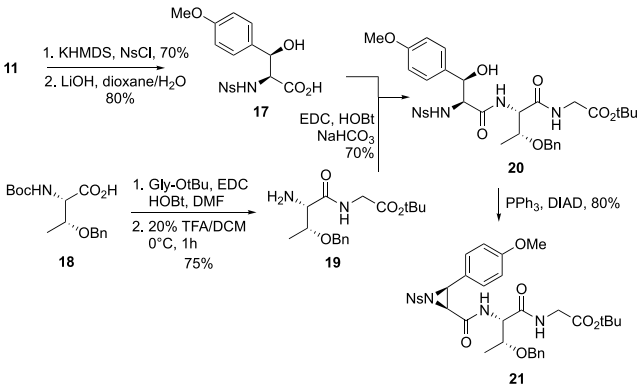
Org. Lett. XXXX, XXX, XXX−XXX

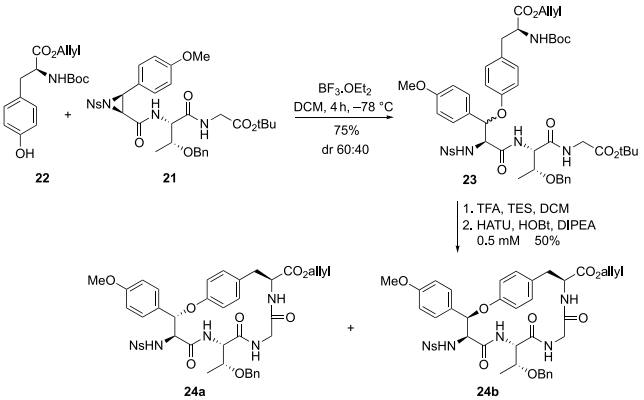
Scheme 3. Synthesis of Aziridine Tripeptide



type reaction (Scheme 3). The aziridine-containing tripeptide **21** was isolated as a single stereoisomer.

Treatment of the aziridine-containing tripeptide **21** with protected tyrosine **22** under the conditions developed in the optimized model study (1.1 equiv of $BF_3{\cdot}OEt_2$, −78 °C) yielded the ether adduct **23** in 75% yield with 60:40 dr (Scheme 4).

Scheme 4. Synthesis of the C-Terminal Macrocycle



Subsequent deprotection of the Boc and *t*Bu groups with TFA was followed by macrocyclization in the presence of HATU to provide the macrocycle **24** in 50% yield as a mixture of epimers at the tyrosine *β*-position.

The epimeric macrocycles **24a** and **24b** were separated by HPLC and analyzed by NMR spectroscopy, and their spectra were compared with that of asperipin-2a. A distinctive difference in the NMR spectra of isomers **24a** and **24b** was observed for the signal from the *β*-H of Tyr^3 (corresponding to H16 in asperipin-2a). For isomer **24a**, the signal for the Tyr *β*-H occurs as a doublet at *δ* 5.12 ppm with a coupling constant of 9.9 Hz, which is a reasonably close match to that for the equivalent *β*-H in asperipin-2a (*δ* 5.23 ppm, d, *J* = 8.4 Hz) (Figure 3A).[12,13] In contrast, the signal for the Tyr *β*-H of isomer **24b** occurs at *δ* 5.51 ppm with a much smaller coupling constant of 2.7 Hz. These data suggests that the configuration at position C16 of asperipin-2a matches that of isomer **24a**.

Further, isomer **24b** was successfully crystallized and analyzed by X-ray crystallography (Figure 3B), which showed this compound possesses the *R*-configuration at the *β*-position of Tyr^3. Thus, the combination of NMR and X-ray analysis suggest that the Tyr^3 in asperipin-2a is 2*S*,3*S*-configured, which corroborates the recent NMR analysis of Ye et al.[14]

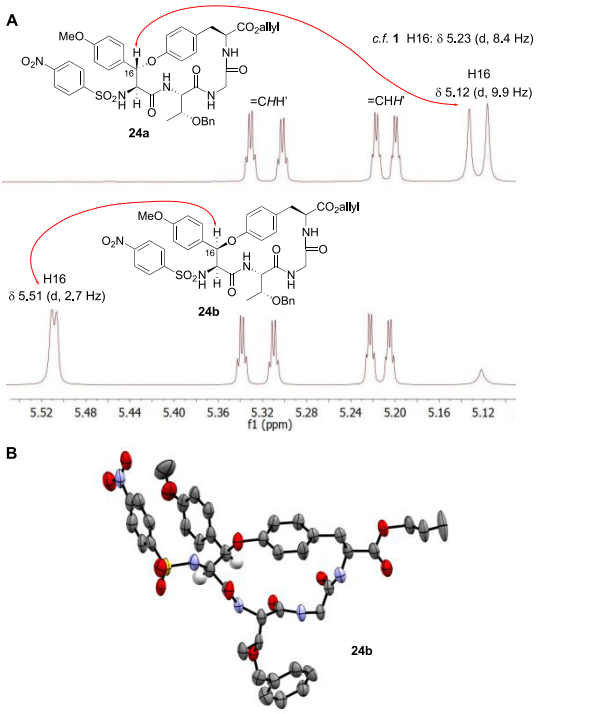


Figure 3. (A) ^1H NMR analysis of epimers of C-terminal macrocycle, **24a** and **24b**, showing signal from *β*-H of Tyr^3. (B) ORTEP diagram of macrocycle **24b**.

In conclusion, the C-terminal macrocycle of asperipin-2a has been synthesized employing an aziridine ring opening reaction to generate the Tyr^3−Tyr^6 alkyl−aryl ether linkage. We have confirmed that the configuration at the *β*-position of Tyr^3 in asperipin-2a is "*S*". Synthetic efforts toward the total synthesis of asperipin-2a are continuing.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b00488.

Experimental procedures, ^1H and ^{13}C NMR spectra of **10**−**14** and **16**−**24**, HPLC traces of **16** and **24** (PDF)

Accession Codes

CCDC 1886340 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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Org. Lett. XXXX, XXX, XXX−XXX

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