Communication

Total Synthesis of Herquline B and C

Joshua B. Cox, Aoi Kimishima, and John L. Wood

J. Am. Chem. Soc., **Just Accepted Manuscript** • Publication Date (Web): 18 Dec 2018

Total Synthesis of Herquline B and C

Joshua B. Cox[#], Aoi Kimishima[#], and John L. Wood*

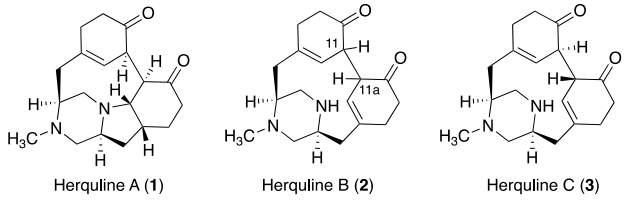
Department of Chemistry and Biochemistry, Baylor University, One Bear Place 97348, Waco, Texas 76798, U.S.A.

Supporting Information Placeholder

ABSTRACT: The total syntheses of (-)-herquline B (**2**) and a heretofore-unrecognized congener, (+)-herquline C (**3**) are described. The syntheses require 14 and 13 steps, respectively, and feature a key oxazoline reduction that sets the stage for piperazine construction.

In 1979 and 1996 Ōmura and co-workers reported the isolation of herqulines A and B (**1** and **2**, Figure 1), two secondary metabolites produced by a fungal strain originally isolated from a soil sample collected in the Saitama Prefecture of Japan, *Penicillium herquei* Fg-372.[1] Preliminary evidence from these studies suggested tyrosine as a biosynthetic precursor to both congeners and screens for biological activity revealed **1** and **2** to be inhibitors of platelet aggregation. In a subsequent study, **1** was demonstrated to be an effective inhibitor of influenza virus replication.[2] The structure of **1** was confirmed via single crystal X-ray analysis,[3] which allowed complete assignment of the illustrated relative stereochemistry. In contrast, the structure of **2** was deduced solely from spectral data and the stereochemistry at C(11) and C(11a) was left unassigned.

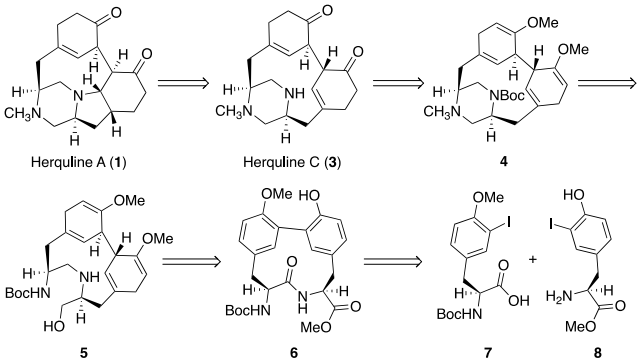
Figure 1. The Herqulines



In the nearly three decades that have passed since the initial isolation efforts, several total syntheses of the herqulines have been attempted but none have been completed.[4,5] Intrigued by the synthetic challenge these strained alkaloids were presenting, we set out, in early 2016, to develop a synthesis of the more complex congener, herquline A (**1**). Thus, we took great interest later that year when Tang and co-workers reported an extensive study into the biosynthetic origins of the herqulines, demonstrating that a six-gene NRPS-containing cluster, that also includes P450, dehydrogenase and methyltransferase enzymes, produces "herquline B", which after extensive NMR analysis was assigned the structure illustrated as **3**. Thus, it appeared this latter study had addressed the stereochemical question that was left unanswered in Ōmura's structural elucidation of **2**. Additionally, and of particular interest to us, it was reported that **3** undergoes conversion to herquline A (**1**) via a nonenzymatic process (e.g., exposure to pH 8.0 buffer).[6] Based on this latter observation, the most efficient synthetic strategy would be one designed to initially deliver **3** and thereby formally **1**. Accordingly, based on these observations we modified our synthetic approach and began targeting **3**. Herein, we report the results of recent investigations which have culminated in completed syntheses of **2** and **3** and the discovery that the "herquline B" isolated by Tang (i.e., **3**), is in fact a new herquline congener (herquline C) and diastereomeric to herquline B isolated by Ōmura (cf. **2** and **3** in Scheme 4).[7] Additionally, our efforts have revealed that **3** isomerizes to **2** upon exposure to pH 8.0 buffer, and neither **2** nor **3** furnish **1** under these conditions.

As illustrated retrosynthetically in Scheme 1, in accord with the biosynthetic studies mentioned above, our synthetic strategy evolved into an approach wherein **1** was expected to derive from **3** which, in turn, was seen as arising via deprotection of piperazine **4**. Based on a review of previous synthetic studies directed toward the herqulines,[4,5] we chose to delay installation of the piperazine until after biaryl coupling and viewed **5** as a viable precursor. The latter was seen as deriving from reduction of the ester, amide, and aryl moieties in **6**,[8] an aryl-linked dityrosine derivative that would arise from known precursors **7**[9] and **8**.[10]

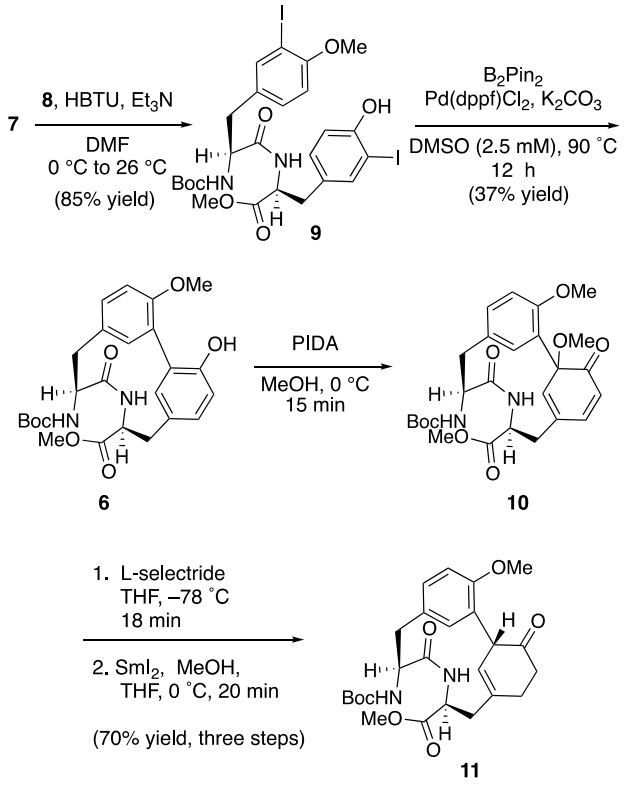
Scheme 1. Retrosynthetic Analysis



In the forward sense (Scheme 2), **7** and **8** were found to readily undergo conversion to dipeptide (+)-**9** under standard amidation conditions. To our delight, subsequent application of a Miyaura-Suzuki cross-coupling reaction, employing conditions developed by Hutton et al. in their mycocyclosin synthesis,[11] proved

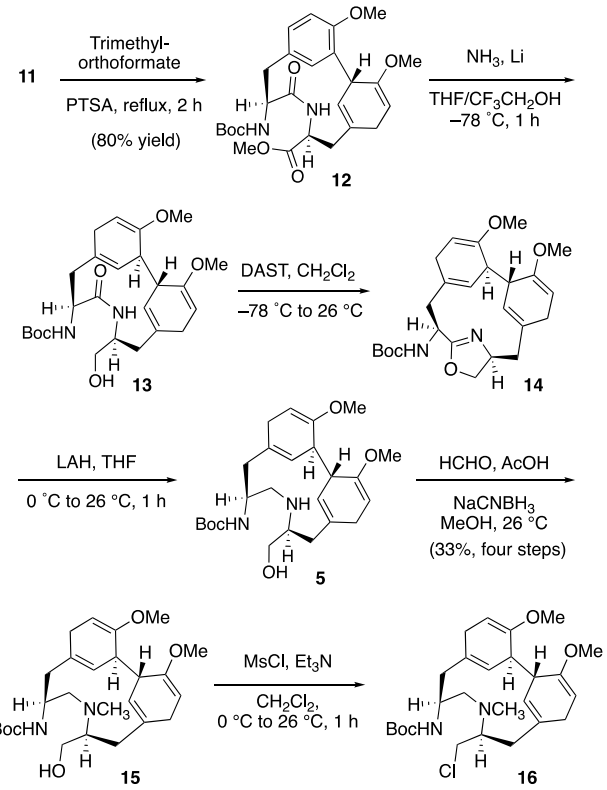
satisfactory for converting (+)-**9** to (+)-**6**. As mentioned above, our early work was directed toward herquline A (**1**); thus, in these initial efforts the goal was to construct the 6,5-fused bicycle imbedded in **1** via conjugate addition of a pendant amine. We began setting the stage for this ring closure by exposing (+)-**6** to phenyliodide diacetate in MeOH, which resulted in conversion to (+)-**10** in excellent yield. Subsequent reduction of (+)-**10** by treatment with L-selectride followed by SmI_2 proved extremely efficient in producing (+)-**11**.[12] It was while attempting to advance (+)-**11** toward herquline A (**1**) that we became aware of Tang's observations (*vide supra*) and decided to redirect our approach away from **1** and toward **3**.[13]

Scheme 2. Biaryl Coupling and Phenol Reduction



Transforming (+)-**11** into **3** requires reduction of the aryl, amide, and ester moieties as well as piperazine formation and *N*-methylation. In developing this end-game strategy, we took inspiration from the work of Yang and Simpkins who, in their efforts toward the herqulines, had noted difficulty in performing Birch Reductions on advanced biaryl intermediates containing either diketopiperazines or piperazines.[4d] Thus, we opted to attempt aryl reduction prior to closing the piperazine ring. To maintain integrity of (+)-**11**'s ketone moiety during the subsequent chemistry, it was masked as its corresponding methyl enol ether ((+)-**12**, Scheme 3). As illustrated, reduction of (+)-**12** employing Li/NH_3 results in regio- and stereoselective reduction of the aromatic ring and conversion of the methyl ester to the corresponding primary alcohol to produce **13**.[14]

Scheme 3. Completing the Requisite Reductions



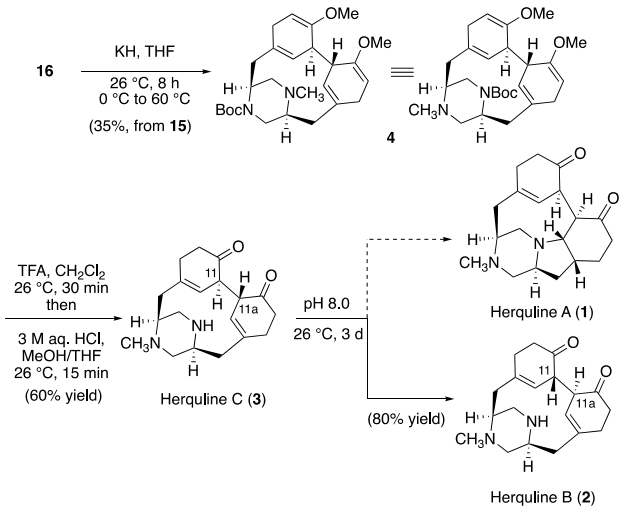
Having accessed the cyclohexyl moieties in their proper oxidation states, we turned toward the remaining issues of piperazine formation, *N*-methylation, and enol ether hydrolysis. At this stage, it was not clear what would be the best order in which to effect these changes; however, in practice, **13** as well as other similar amide substrates proved recalcitrant toward ring closure to the corresponding keto-piperazines. Attributing this to deleterious conformational effects imparted by the amide we next explored initiating this sequence via reduction. Surprisingly this too proved challenging with success being found only after first converting **13** to the corresponding oxazoline (**14**, Scheme 3) via treatment with DAST.[15] Importantly, the macrocyclically embedded oxazoline displays enhanced reactivity, undergoing smooth reduction to amino alcohol **5** upon treatment with LAH.[16] Methylation of **5** via reductive amination with formaldehyde furnished (+)-**15** which,[17] upon treatment with mesyl-chloride, provided **16** and set the stage for piperazine formation.

Once again, considerable experimentation was required but eventually KH was found to be effective in advancing **16** to the cyclized product (+)-**4** (Scheme 4). Exposure of

(+)-**4** to TFA followed by HCl removed the Boc and enol ether protecting groups, respectively, and produced (+)-**3**, which was found to be spectroscopically identical to material isolated by Tang. Turning to the preparation of herquline A (**1**), we followed Tang's procedure and exposed (+)-**3** to pH 8.0 buffer solution. Indeed, after three days at room temperature a new compound was observed to form in 80% yield. However, to our surprise the ^{1}H NMR spectrum of this product still showed two vinyl protons, thus the reaction had not produced **1** but, more likely, a diastereomer of (+)-**3**. In efforts to assign a structure to this unexpected product, we closely reviewed our spectral data along with that reported by Ōmura and Tang. During this review, we discovered that although our data for synthetic (+)-**3** matched Tang's, neither matched that reported by Ōmura for herquline B. However, the herquline B data did match that obtained by us for the product produced upon exposure of synthetic (+)-**3** to pH 8.0 conditions.[18] At his point it was clear that (+)-**3** was a new herquline congener (herquline C) capable of undergoing isomerization to (-)-**2**. Further NMR analysis of these diastereomers established them to be epimeric at C(11) and C(11a) Scheme 4.[19]

In an effort to understand why Tang and Ōmura had isolated different herquline congeners we reviewed the isolation procedures and noted that Ōmura reports bringing the fermentation broth to pH 10 by the addition of aqueous ammonia prior to isolation, whereas Tang reports adjusting the broth to pH 6.0 and makes no mention of adjusting to a higher pH. Prior to our observations the importance of these differences was not appreciated and it now seems probable that both groups had produced (+)-**3**. The isolation of (-)-**2** by Ōmura is likely the result of epimerization promoted by the high pH employed in their isolation procedure.

Scheme 4. Completion of Herqulines B and C



Having established that (+)-**3** undergoes epimerization to (-)-**2** after prolonged exposure to pH 8.0 conditions, we reviewed Tang's results in hopes of discerning what had led them to conclude that these conditions produce **1**. We noted that although in initial large-scale fermentation studies Tang had successfully isolated both **3** and **1**,[20] the reported conversion of **3** to **1** was based on observations made during small-scale *in vitro* efforts to reconstitute the biosynthetic pathway. In these latter studies the various metabolites were detected using HPLC/MS in selected-ion monitoring mode. Thus, the reported conversion of **3** to **1** was determined solely by comparing HPLC/MS retention times to those previously obtained for authentic samples of **1** and **3**. Since **1** and **2** have the same mass, if by chance they also have the same HPLC retention times, these two products would be indistinguishable by this analytical method. To explore this possibility, we obtained an authentic sample of herquline A from the Ōmura group and compared the HPLC/MS retention times of **1**, **2**, and **3** using a column and conditions identical to that reported by Tang. Indeed, this study revealed that **1** and **2** possess virtually identical retention times.[21]

In conclusion, efforts to develop a synthesis capable of delivering herqulines A and B have been successful in providing access to (-)-herquline B (**2**) and a heretofore unrecognized congener (+)-herquline C (**3**). These studies have also revealed that, in contrast to earlier reports, **3** does not undergo conversion to herquline A (**1**) upon exposure to pH 8.0 buffer; thus, the biosynthetic origins of **1** have yet to be fully delineated and it remains an intriguing target for synthesis.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures, analytical data, spectra (^{1}H NMR, ^{13}C NMR and IR) and crystallographic data (CIF)

AUTHOR INFORMATION

Corresponding Author

*** John_L_Wood@baylor.edu**

Author Contributions

[#]JBC and AK contributed equally to the completion of these studies

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

The authors thank Dr. Sam Yruegas for her assistance in obtaining and analyzing X-ray crystallographic data and Professors Satoshi Ōmura and Toshiaki Sunazuka for providing an authentic sample of herquline A. We also acknowledge the gracious assistance of Professor Yi Tang (UCLA) in providing authentic samples, biosynthetic intermediates, and insight regarding their research efforts. Dr. Jacob Timmerman is acknowledged for helpful discussions. The authors would also like to thank Professor Phil Baran for the collegial exchange of information (ref. 18b) that occurred after our having received reviews and submitted a revision of this manuscript. Financial support was provided from Baylor University, the Welch Foundation (Chair, AA-006), the Cancer Prevention and Research Institute of Texas (CPRIT, R1309), and the NSF (CHE-1764240). Finally, the authors would like to dedicate this manuscript to Professor E. J. Corey on the occasion of his 90th birthday.

REFERENCES

(1) (a) Ōmura, S.; Hirano, A.; Iwai, Y.; Masuma, R. Herquline, A New Alkaloid Produced by *Penicillium Herquei* Fermentation, Isolation and Properties *J. Antibiot*. **1979**, *32*, 786. (b) Enomoto, Y.; Shiomi, K.; Hayashi, M.; Masuma, R.; Kawakubo, T.; Tomosawa, K.; Iwai, Y.; Ōmura, S. Herquline B, a New Platelet Aggregation Inhibitor Produced by *Penicillium herquei* Fg-372 *J. Antibiot.* **1996**, *49*, 50.

(2) Chiba, T.; Asami, Y.; Suga, T.; Watanabe, Y.; Nagai, T.; Momose, F.; Nonaka, K.; Iwatsuki, M.; Yamada, H.; Ōmura, S.; Shiomi, K. Herquline A, Produced by *Penicillium herquei* FKI-7215, Exhibits Anti-Influenza Virus Properties *Bioscience, Biotechnology, and Biochemistry* **2017**, *81*, 59.

(3) Furusaki, A.; Matsumoto, T.; Ogura, H.; Takayanagi, H.; Hirano, A.; Ōmura, S. *J.* X-Ray Crystal Structure of Herquline, a New Biologically Active Piperazine from *Penicillium herquei* Fg-372 *C. S. Chem. Comm.* **1980**, 698.

(4)For Ph.D. theses describing efforts directed toward the herqulines, see: (a) Kim, G. T. Ph.D. Thesis, Korea Advanced Institute of Science and Technology, November 1997. (b) Hart, J. M. Ph.D. Thesis, University of Leeds, June 2004. (c) Stawski, P. S. Ph.D. Thesis, Ludwig-Maximilians-Universität München, December 2012. (d) Yang, H. Ph.D Thesis, University of Birmingham, August 2015.

(5) For conference proceedings describing efforts toward the herqulines, see: Kawai, N.; Atsumi, T.; Arai, N.; Kuwajima, I. *Nippon Kagakkai, Koen Yokushu* **2003**, *83*, 777.

(6) Yu, X.; Liu, F.; Zou, Y.; Tang, M.-C.; Hang, L.; Houk, K. N.; Tang, Y. Biosynthesis of Strained Piperazine Alkaloids-Uncovering the Concise Pathway of Herquline A *J. Am. Chem. Soc.* **2016**, *138*, 13529.

(7) Tabulated and physical comparisons of the spectral data reported for herquline B by Ōmura and Tang are included as supporting information.

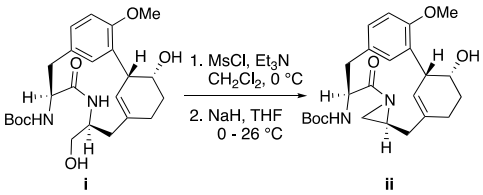
(8) Differentiation of the aryl rings in **6** as a phenol and anisole is a strategic remnant of early investigations targeting **1**. This differentiation may not be necessary to access **2** and avoiding it could lead to a more efficient strategy; however, to date, investigations along these lines have met with limited success.

(9)David, N.; Pasceri, R.; Kitson, R. R. A.; Pradal, A.; Moody, C. J. Formal Total Synthesis of Diazonamide A by Indole Oxidative Rearrangement *Chem. Eur. J.* **2016**, *22*, 10867.

(10)Krenitsky, P. J.; Boger, D. L. Synthesis of the (S,S,S)-Diastereomer of the 15-Membered Biaryl Ring System of RP 66453 *Tetrahedron Lett.* **2003**, *44*, 4019.

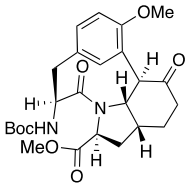
(11) Cochrane, J. R.; White, J. M.; Wille, U.; Hutton, C. A. Total Synthesis of Mycocyclosin *Org. Lett.* **2012**, *14*, 2402. For a recent modification of these coupling conditions, see: Zhu, X.; McAtee, C. C.; Schindler, C. S. Scalable Synthesis of Mycocyclosin *Org. Lett.* **2018**, *20*, 2862.

(12) The stereochemistry illustrated for **11** is based upon the single crystal X-ray analysis of aziridine **ii**, a product formed while attempting an alternative route that involved advancing **i**. These data are included as supporting information.



i ii

(13) Attempts were made to advance **11** to compounds possessing the 6,5-azabicycle found in herquline A (e.g., **iii**). However, these limited efforts proved unsuccessful.



iii

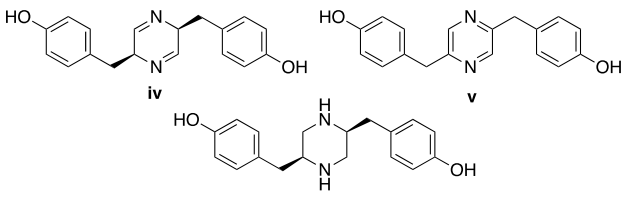
(14) The stereochemistry illustrated in **13** was assigned retrospectively and is based upon an X-ray analysis of **15**. This stereochemical outcome and that observed in the preparation of **11** were not predicted and both are best described as serendipitous.

(15) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno M. J.; Williams, D. R. Synthesis of Functionalized Oxazolines and Oxazoles with DAST and Deoxo-Fluor *Org. Lett.* **2000**, *2*, 1165.

(16) Meyers, A. I.; Himmelsbach, R. J.; Reuman, M. Reductive Cleavage of Aryl Oxazolines to Benzaldehydes and Substituted Toluenes *J. Org. Chem.* **1983**, *48*, 4053.

(17) The structure of compound **15** was confirmed by single crystal X-ray analysis. See supporting information for details.

(18) (a) Optical rotation data for naturally derived **3** was not reported by Tang[6] but determined by us to be +160°. In an initial study, the rotation of **2** was also found to be dextrorotatory thus opposite in sign to that reported by Ōmura.[1b] This observation led to concern that the presumed L-tyrosine-derived stereochemistry had been inverted during the biosynthesis. Specifically, the stereochemistry of **vi** was questioned given the epimerizability of it's proposed precursor **iv** and/or the potential intermediacy of achiral pyrazine **v**. To address this possibility, enantiopure (+)- and (-)-**vi** were prepared from D- and L-tyrosine. Comparison of this synthetic material to that isolated by Tang during their biosynthetic studies revealed that the stereochemistry of L-tyrosine was maintained in **vi**. In view of this we repeated the synthesis and more carefully purified synthetic **2** and found its chiroptic properties in accord with those reported by Omura. This has been further substantiated by Baran, who has recently completed an elegant synthesis of herqulines B and C.[18b] The preparation of **vi** and HPLC comparison data are included in the supporting information. (b) He, C.; Stratton, T. P.; Baran, P. S. Concise Total Synthesis of Herqulines B and C. s*ubmitted*.



iv v vi

(19) The stereochemistry indicated for compound (+)-**3** was assigned here based upon X-ray analysis of intermediate **15**. This analysis confirms the structure of naturally derived **3**, which was assigned by Tang on the basis of NOE data, and establishes the veracity of applying the latter technique in these systems.[6] The stereochemistry illustrated for (-)-**2** is based upon a similar NOE analysis and is supported by computational data reported by Tang and Houk which predicts this diastereomer to be ca. 8 kcal/mol lower in energy than both of the two remaining cis-C(11/11a) diastereomers and 3 kcal/mole more stable than (+)-**3**.[6] The NOE data for (-)-**2** are included as supporting information.

(20) In contrast to **2** and **3**, ^{1}H and ^{13}C NMR data reported by Tang and Ōmura for **1** were found to be identical.

(21) This data is included as supporting information.

TOC Graphic