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Biomimetic Synthesis of *ent*-(−)-Azonazine and Stereochemical Reassignment of Natural Product

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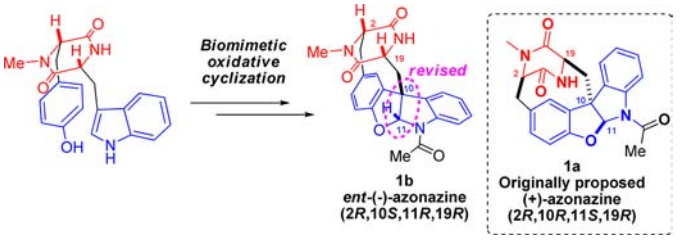
ABSTRACT



The first total synthesis of *ent*-(−)-azonazine has been accomplished using a hypervalent iodine-mediated biomimetic oxidative cyclization to construct the highly strained core. Based on the results from the completed synthesis, both the relative and absolute configurations of natural (+)-azonazine were revised.

The study of fungi within the genus *Aspergillus* provides an abundant source of bioactive small molecules possessing unparalleled structural diversity.[1] In 2010, Crews et al. reported a novel hexacyclic dipeptide, azonazine (**1a**, Figure 1), from a Hawaiian marine sediment-derived fungus, *Aspergillus insulicola*.[2] It was found to show anti-inflammatory activity by inhibiting NF-κB luciferase and nitrite production. Structurally, azonazine (**1a**) contains a benzofuranoindoline ring system bearing a quaternary center at the C10 position, which presents similarity to the core of diazonamide A[3] (**2**, Figure 1). However, a unique transannular 10-membered ring (F) connecting the tetracyclic core (A/B/C/D) and the diketopiperazine unit (E) makes the skeleton of azonazine much more rigid than that of diazonamide A. For its challenging structure and interesting biological activity, azonazine is now becoming a good platform for new methodology examination and also an attractive target for total synthesis.[4−6] Herein, we want to report the first total synthesis of (−)-azonazine, together with our structural revision and reassignment for the originally proposed structure of natural (+)-azonazine.

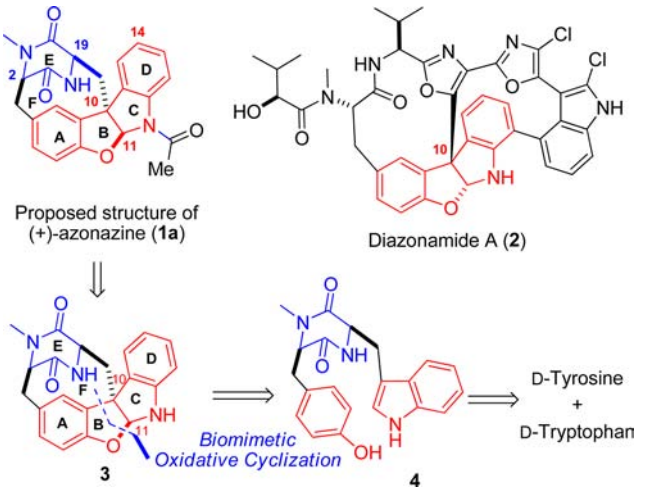


Figure 1. Retrosynthetic analysis of proposed azonazine (**1a**).

(1) Cole, R. J.; Jarvis, B. B.; Schweikert, M. A. *Handbook of Secondary Fungal Metabolites*; Academic Press: San Diego, 2003; Vols. I−III.
(2) Wu, Q. X.; Crews, M. S.; Draskovic, M.; Sohn, J.; Johnson, T. A.; Tenney, K.; Valeriote, F. A.; Yao, X. J.; Bjeldanes, L. F.; Crews, P. *Org. Lett.* **2010**, *12*, 4458.
(3) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 2303.

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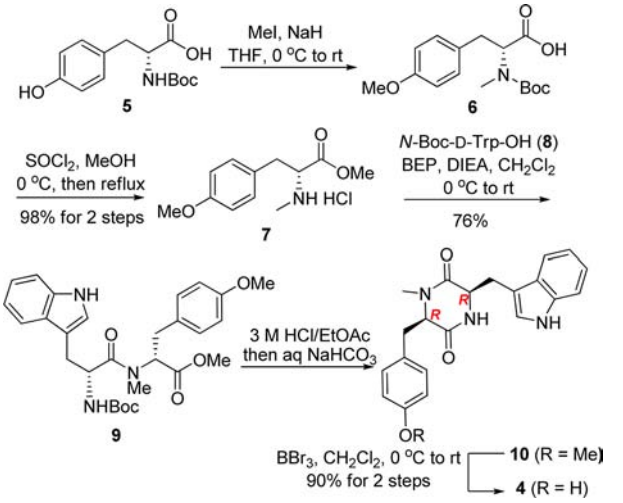
Scheme 1. Preparation of Diketopiperazine **4**[a]

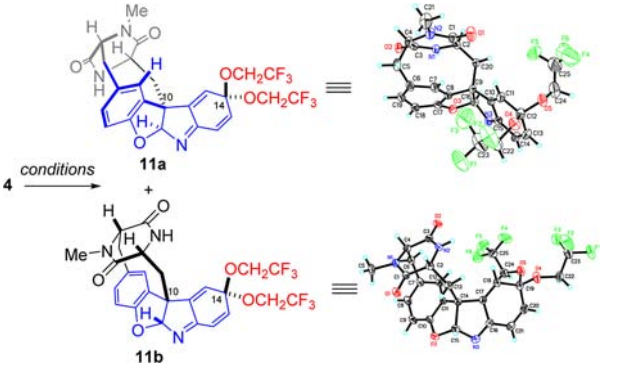


[a] BEP = 2-bromo-1-ethyl pyridinium tetrafluoroborate.

From a strategic point of view, construction of the benzofuranoindoline ring system (Figure 1, A/B/C/D rings of **1a**) containing a highly strained transannular 10-membered ring (F ring) is the most arduous task in the whole synthesis. To achieve high efficiency in constructing such a strained multiring system, proper oxidative free-radical cyclization mimicking the biogenesis pathway[2,5b] was taken into consideration. Azonazine (**1a**, the proposed structure) could be prepared from the corresponding indoline **3** by a selective *N*-acetylation. Formation of the benzofuranoindoline core of **3** could be achieved by a biomimetic oxidative cyclization of the diketopiperazine precursor **4**, which could be conveniently prepared from the commercially available D-Tyr and D-Trp derivatives.

D-Tyrosine derivative **5** and D-tryptopan derivative **8** were employed as the starting materials in our synthesis (Scheme 1). Double methylation of D-*N*-Boc-Tyr-OH (**5**) provided compound **6**,[7] which was transformed to D-*N*-Me-Tyr(OMe)-OMe hydrochloride salt (**7**) by treatment with thionyl chloride in methanol. BEP-mediated coupling[8] of **7** with D-*N*-Boc-Trp-OH (**8**) provided the dipeptide **9**. Cyclization of **9** to diketopiperazine **10** was carried out by deprotection of *N*-Boc functionality and subsequent base treatment. *O*-Demethylation of **10** was achieved by treatment with BBr_3 in dichloromethane, providing precursor **4** for the key oxidative cyclization.

Table 1. Optimization of Oxidative Cyclization of **4**[a]



	conditions	results or yield (%) 4	11a	11b
1	PIDA (1 equiv), LiOAc, TFE, −15 °C, 30 min	>30	<5	<5
2	PIFA (1 equiv), LiOAc, TFE, −15 °C, 30 min		complex	
3	HTIB (1 equiv), LiOAc, TFE, −15 °C, 30 min		complex	
4	PIDA (1 equiv), LiOAc, HFIP, −15 °C, 30 min		complex	
5	PIFA (1 equiv), LiOAc, HFIP, −15 °C, 30 min		complex	
6	HTIB (1 equiv), LiOAc, HFIP, −15 °C, 30 min		complex	
7	$Fe(acac)_3$ (2 equiv), *t*-BuOK, THF, −40 °C to rt		n.r.	
8	PIDA (2 equiv), LiOAc, TFE, −15 °C, 30 min	0	16	12
9	PIDA (2 equiv), LiOAc, TFE, −30 °C, 30 min	0	15	11

[a] PIDA: (diacetoxyiodo)benzene. PIFA: [bis(trifluoroacetoxy)-iodo]benzene. HTIB: [hydroxy(tosyloxy)iodo]benzene. TFE: 2,2,2-trifluoroethanol. HFIP: hexafluoroisopropanol.

With **4** in hand, we then turned our attention to the construction of the benzofuranoindoline ring systems. Due to the mild and highly selective oxidizing properties, a considerable number of hypervalent iodine reagents have been developed and used for various transformations of complex organic molecules in recent years.[5b,9] Several hypervalent iodine(III) reagents were examined in this study for the key oxidative cyclization, as well as $Fe(acac)_3$ (Table 1). When diketopiperazine **4** was added into a cold trifluoroethanol solution of $PhI(OAc)_2$, two unexpected diastereomeric prodcuts **11a** and **11b** bearing the hexacyclic core structure of azonazine were generated in very low isolated yields (entry 1, Table 1). Their structures were determined by NMR methods and finally confirmed by X-ray single crystal analyses. The results indicated that an overoxidation happened at the C14 position. Though the detailed mechanism is unclear as of yet, compound **15b** (a diastereomer of **3**, see Scheme 3 for its structure) is

(4) For a recent study on the synthesis of azonazine, see: Ghosh, S.; Kinthada, L. K.; Bhunia, S.; Bisai, A. *Chem. Commun.* **2012**, *48*, 10132.

(5) For total syntheses of diazonamide A, see: (a) Nicolaou, K. C.; Bella, M.; Chen, D. Y. K.; Huang, X.; Ling, T.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 3495. (b) Burgett, A. W. G.; Li, Q.; Wei, Q.; Harran, P. G. *Angew. Chem., Int. Ed.* **2003**, *42*, 4961. (c) Nicolaou, K. C.; Bheema Rao, P.; Hao, J.; Reddy, M. V.; Rassias, G.; Huang, X.; Chen, D. Y. K.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 1753. (d) Cheung, C.-M.; Goldberg, F. W.; Magnus, P.; Russell, C. J.; Turnbull, R.; Lynch, V. *J. Am. Chem. Soc.* **2007**, *129*, 12320. (e) Mai, C.-K.; Sammons, M. F.; Sammakia, T. *Angew. Chem., Int. Ed.* **2010**, *49*, 2397. (f) Knowles, R. R.; Carpenter, J.; Blakey, S. B.; Kayano, A.; Mangion, I. K.; Sinz, C. J.; MacMillan, D. W. C. *Chem. Sci.* **2011**, *2*, 308.

(6) A recent synthesis of benzofluoroindolines: Beaud, R.; Guillot, R.; Kouklovsky, C.; Vincent, G. *Angew. Chem., Int. Ed.* **2012**, *51*, 12546.

(7) Boger, D. L.; Yohannes, D. *J. Org. Chem.* **1988**, *53*, 487.

(8) (a) Li, P.; Xu, J. C. *Tetrahedron* **2000**, *56*, 8119. (b) Li, P.; Xu, J. C. *Chem. Lett.* **2000**, *29*, 204.

(9) For recent reviews on hypervalent iodines in organic synthesis, see: (a) Tohma, H.; Kita, Y. *Adv. Synth. Catal.* **2004**, *346*, 111. (b) Zhdankin, V. V.; Stang, P. J. *Chem. Rev.* **2008**, *108*, 5299. (c) Merritt, E. A.; Olofsson, B. *Angew. Chem., Int. Ed.* **2009**, *48*, 9052. (d) Dohi, T.; Ito, M.; Yamaoka, N.; Morimoto, K.; Fujioka, H.; Kita, Y. *Tetrahedron* **2009**, *65*, 10797. (e) Zhdankin, V. V. *J. Org. Chem.* **2011**, *76*, 1185. (f) Silva, L. F.; Olofsson, B. *Nat. Prod. Rep.* **2011**, *28*, 1722. (g) Duschek, A.; Kirsch, S. F. *Angew. Chem., Int. Ed.* **2011**, *50*, 1524.

speculated to be produced at the first stage of the oxidative cyclization (C, Figure 2). Unfortunately, the produced highly strained structure induces further $PhI(OAc)_2$-oxidation to easily happen on the indoline nitrogen (D). Subsequently, a TFE-addition takes place at the C14 position. As a final result, all of these $PhI(OAc)_2$-mediated oxidations enable ketals **11a** and **11b** to be formed at the C14 position.

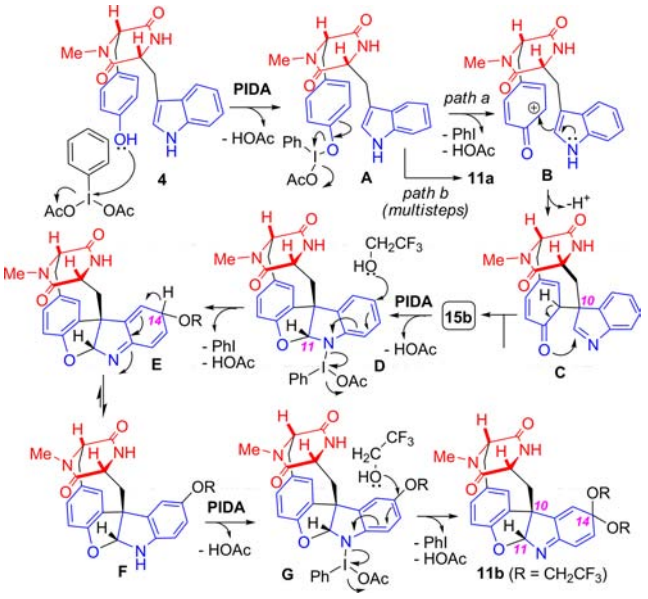


Figure 2. A proposed oxidative process for generation of **11b**.

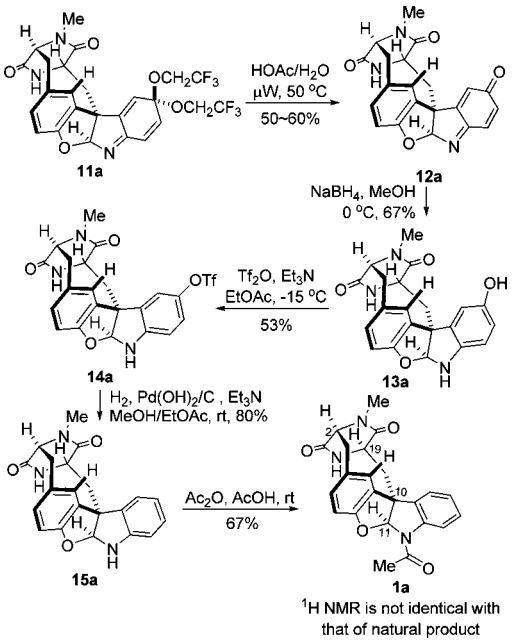
We attempted to improve the reaction by changing hypervalent iodine reagents (Table 1, entries 2−3) and the solvent (entries 4−6), but all failed. No reaction was observed when an Fe(III) oxidant was applied (entry 7). To avoid the overoxidation, modification of the electron density of the indole moiety of **4** was also attempted.[10] Disappointingly, no improvements were achieved. Finally, we decided to further optimize the conditions with $PhI(OAc)_2$. The reaction with 2 equiv of $PhI(OAc)_2$ gave the highest yields of the products at −15 °C (entry 8), and further lowering of the reaction temperature provided no improvement in the isolated yields (entry 9). Relatively low yields of the cyclized products **11a** and **11b** suggest that the high ring strain existing in the congested hexacyclic core makes the synthesis of such a ring system exceptionally difficult and inefficient. Crystallographic data of **11a** and **11b** (see Supporting Information for the details) also confirm that the products bore high strain,[11] which is consistent with previous computational calculation by Crews et al.[2]

(10) When the indole nitrogen was protected either by alkyl or Ts, the corresponding substrates displayed no reactivity in the following oxidations. When a bromine atom was introduced into the benzene ring of the indole moiety at different positions, the reaction was found to be very complicated.

(11) Bond angles and dihedral angles of **11a/11b** and **1b** were highly distorted according to the crystallographic data (see Table S1 of the Supporting Information for details).

Using the cyclized products **11a** and **11b**, we continued their transformations to the final products (Scheme 2). Deprotection of the unusal bis(trifluoroethoxy)acetal at the C14 of **11a** was found to be surprisingly problematic. After many experimental trials, it was successfully hydrolyzed with $HOAc/H_2O$ under microwave-assisted conditions, affording a moderate yield of quinone **12a**. This product was immediately reduced with $NaBH_4$ to afford stable phenol **13a**. Phenol **13a** was then converted into to the corresponding triflate **14a** under the optimized conditions (Tf_2O/Et_3N in EtOAc, −15 °C). Hydrogenative removal of the triflate functionality of **14a** (with a catalytic amount of $Pd(OH)_2$ on charcol) followed by *N*-acetylation under acidic conditions (Ac_2O, AcOH) finally afforded the originally proposed structure of (+)-azonazine (**1a**).

Scheme 2. Synthesis of the Proposed Structure of Azonazine

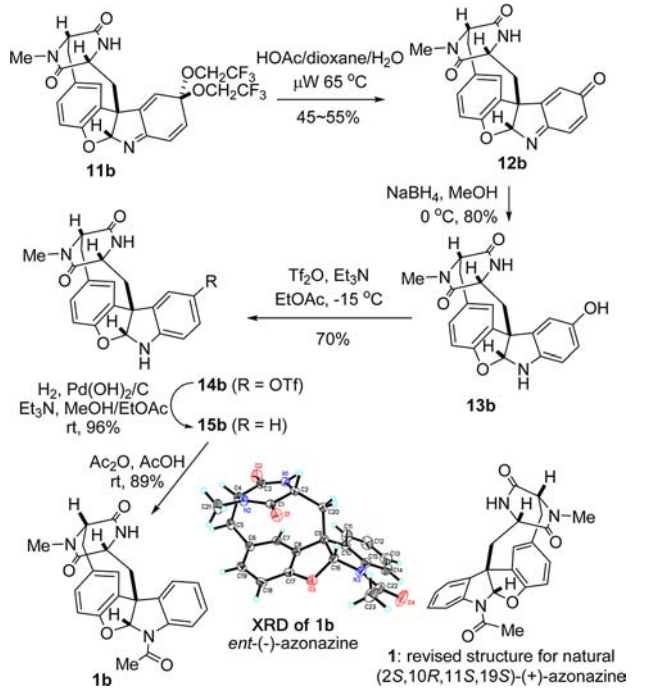


To our surprise, multiple apparent differences were found in the ^1H NMR spectrum of synthetic **1a** from those reported for the natural product. Careful analysis of the original data[2] found that the assignment of the relative configurations at C10 and C11 might be ambiguous.[12] By comparison with that of **11a**, we found the ^1H NMR of the previous intermediate **11b** was more similar to the natural product. We thus decided to synthesize the other C10/C11 diastereomer **1b** from **11b** for further analysis.

The C10/C11 diastereomeric target **1b** was synthesized with the same steps under similar conditions (Scheme 3). Hydrolysis of **11b** under microwave conditions followed by $NaBH_4$ reduction of quinone **12b** gave phenol **13b**. Deoxygenation of the C14 phenol group of **13b** was accomplished after *O*-triflation of **13b** and subsequent

(12) The authors (ref 2) favored to assign the absolute configuration of azonazine in parallel to that of diazonamide A (R10/S11), since the chemical shifts of dihydrobenzofuran of diazonamide A (ref 3) were virtually identical to those of (+)-azonazine.

Scheme 3. Completion of the Total Synthesis (−)-Azonazine (**1b**)

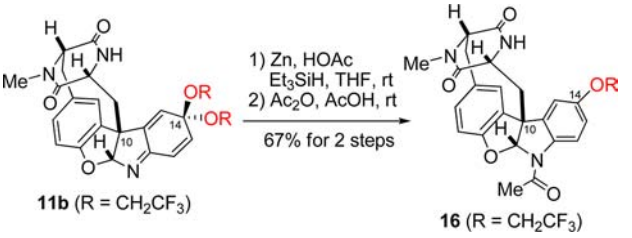


hydrogenation. Finally, a high-yield *N*-acetylation of **15b** provided the final product **1b**, whose structure was determined by NMR methods and confirmed by X-ray single crystal diffraction. With this solid evidence, the absolute chemistry of **1b**, a C10,C11-diastereomer of the originally proposed azonazine (**1a**), was unambiguously assigned as (2*R*,10*S*,11*R*,19*R*) (Scheme 3). With the exception of the opposite optical rotation {synthetic: $[\alpha]^{27}_{D}$ −299.3 (*c* 0.11, MeOH); natural: $[\alpha]^{23}_{D}$ +295.0 (*c* 0.1, MeOH)}, all the physical data of synthetic **1b** were in good agreement with those reported for the natural product.[2] Therefore, the absolute configuration of natural (+)-azonazine should be revised as (2*S*,10*R*,11*S*,19*S*)-**1**.

Selective introduction of fluorine atom(s) to bioactive natural and unnatural products is of extreme interest in pharmaceutical research.[13] The intermediates accidently generated in this synthesis provide a particular opportunity

(13) For a review on fluorine in medicinal chemistry, see: Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320.

Scheme 4. Synthesis of *ent*-Azonazine Analogue **16**



to explore the fluorinated analogues and derivatives of azonazine, which are usually difficult to achieve with traditional modification from the natural product. For instance, we could easily synthesize the C-14 trifluoroethoxy derivative **16** of *ent*-azonazine in two steps from ketal **11b** by reduction with zinc/HOAc under mild conditions[14] and subsequent *N*-acetylation (Scheme 4).

In conclusion, we have successfully accomplished the first total synthesis of (−)-azonazine, applying a biomimetic oxidative cyclization as the key step to construct the highly strained hexacyclic core. With the spectroscopic evidence and X-ray crystal data of the enantiopure diastereomeric products, the structure of natural (+)-azonazine was revised as (2*S*,10*R*,11*S*,19*S*). In addition, the intermediate produced in this synthesis could be converted into the fluorinated azonazine derivative in short convenient steps. The biological properties of these natural and unnatural products, as well as the fluorinated derivatives, are currently under investigation in our laboratory and will be reported in due course.

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Supporting Information Available. Experimental details and charaterizations of new compounds, NMR copies of new compounds (PDF), and X-ray data of compounds **1b**, **11a**, and **11b** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

(14) Ghaffarzadeh, M.; Joghan, S. S.; Faraji, F. *Tetrahedron Lett.* **2012**, *53*, 203.

The authors declare no competing financial interest.