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Chemistry and Biology of Diazonamide A: First Total Synthesis and Confirmation of the True Structure

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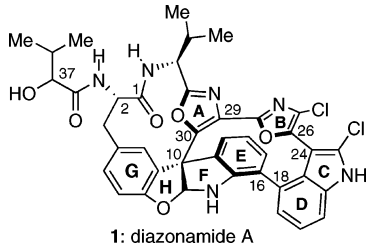
Abstract: With the addition of a tenth ring, the exchange of an oxygen atom for a nitrogen in the heart of the molecule, and a different terminal residue, the revised architecture for diazonamide A (**1**) provided an even more challenging molecular puzzle for chemical synthesis than its predecessor. In this article, we detail the first successful total synthesis of diazonamide A, an endeavor which not only verified its proper connectivities and established the stereochemistry of its previously unassignable C-37 chiral center, but which also was attended by the development of several new synthetic strategies and tactics.

Introduction

Although structural revisions of natural products are quite common, few of these reassignments occur with as many profound changes as the ones applied to diazonamide A (**1**, Figure 1). Indeed, in addition to the complete alteration of the terminal amino acid attached to the C-2 amine and the incorporation of a tenth ring in the form of an aminal (ring H), what was previously thought to be an oxygen atom at its epicenter was exchanged for a nitrogen. As such, any synthetic plan that had been developed for diazonamide A's originally proposed structure (see previous articles)[1] would need to be significantly retooled to gain access to building blocks appropriately functionalized for the new target. Once these fragments were built, however, the total synthesis of **1** was not necessarily near or guaranteed. For example, as experience garnered during campaigns toward the original structure had consistently demonstrated, while it was relatively easy to construct fragments bearing the disparate elements of the diazonamide structure, it was quite difficult to merge them into structures containing its highly strained and rigid 12-membered macrocyclic systems.[2]

Faced with this conundrum, we elected to confront the structural complexity posed by the "new" diazonamide A (**1**) from not one, but two, completely different directions to enhance our chances for success. Our first approach was patterned after the chemistry which had been developed to access the originally proposed structure of diazonamide A as described in the previous two articles in this series.[1] According to this plan, we would attempt to form the heterocyclic core first through a heteropinacol macrocyclization, and then build the second macrocycle of **1** through macrolactamization. The second plan of attack, which is the subject of this article, sought to reach diazonamide A (**1**) via the alternate order of macrocycle formation. Our hope was that at least one of these approaches would prove capable of delivering diazonamide A (**1**) to verify its structure and, if both plans worked in part to produce one of the 12-membered rings, then we would be able to generate simplified analogues of both domains for the purposes of biological investigations.



1: diazonamide A

Figure 1. The revised structural assignment of diazonamide A (**1**). Note: the C-37 stereochemistry is unassigned.

Results and Discussion

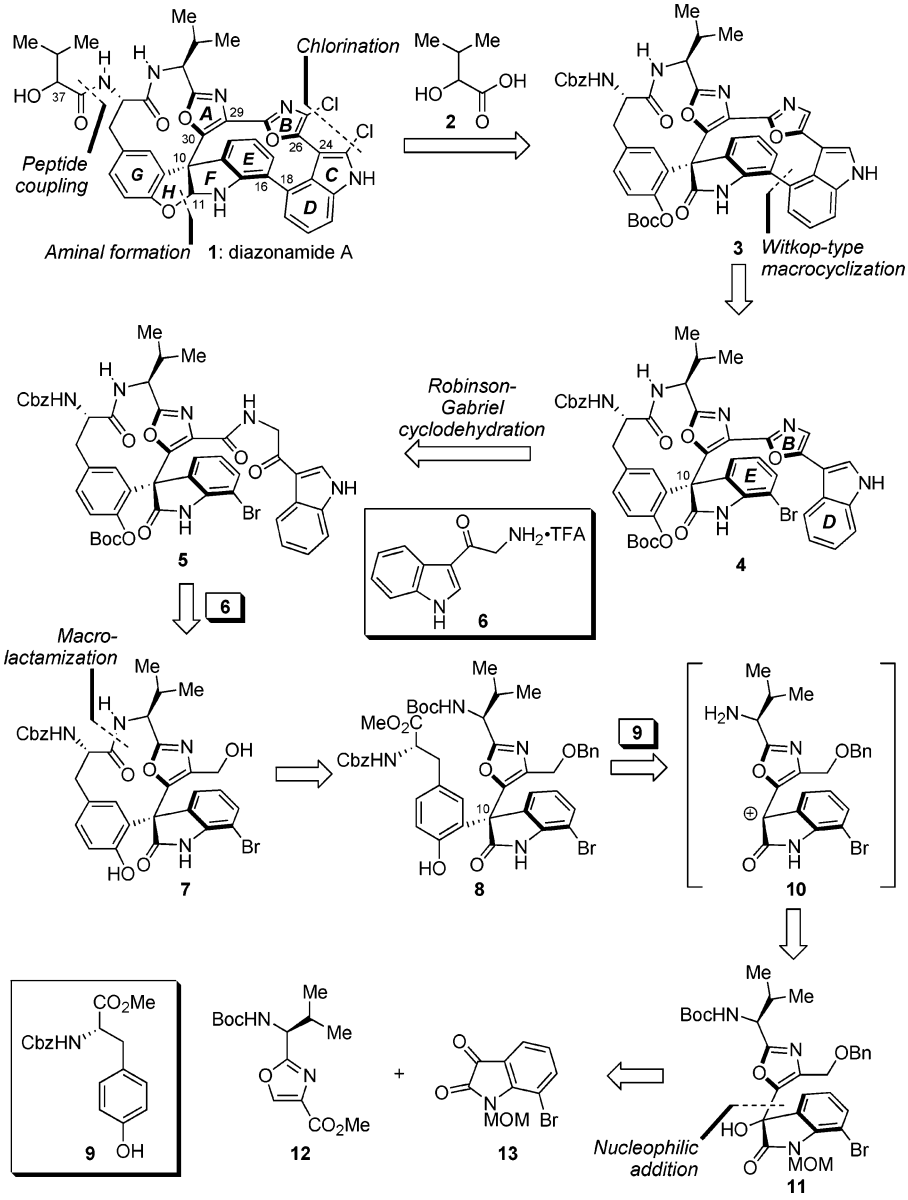
Retrosynthetic Analysis. Our new retrosynthetic plan for diazonamide A (**1**) is shown in Scheme 1, starting with essentially the same modifications that we had applied in our analysis of the originally proposed structure to prepare the ground for retrons seeking to open its macrocyclic systems. These were the removal of the C-2 hydroxyisovaleric acid side

(1) (a) Nicolaou, K. C.; Snyder, S. A.; Huang, X.; Simonsen, K. B.; Koumbis, A. E.; Bigot, A. *J. Am. Chem. Soc.* **2004**, *126*, 10162−10173. (b) Nicolaou, K. C.; Snyder, S. A.; Giuseppone, N.; Huang, X.; Bella, M.; Reddy, M. V.; Bheema Rao, P.; Koumbis, A. E.; Giannakakou, P.; O'Brate, A. *J. Am. Chem. Soc.* **2004**, *126*, 10174−10182.

(2) In truth, only a few of the creative strategies developed toward the original structure managed to identify a sequence that could generate either of these 12-membered systems in any measurable yield. See, for example: (a) Nicolaou, K. C.; Snyder, S. A.; Simonsen, K. B.; Koumbis, A. E. *Angew. Chem., Int. Ed.* **2000**, *39*, 3473−3478. (b) Nicolaou, K. C.; Huang, X.; Giuseppone, N.; Bheema Rao, P.; Bella, M.; Reddy, M. V.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 4705−4709. (c) Vedejs, E.; Zajac, M. A. *Org. Lett.* **2001**, *3*, 2451−2454. (d) Reference 3 in this article.

10.1021/ja040092i CCC: $27.50

Scheme 1. General Retrosynthetic Analysis of Diazonamide A (**1**) on the Basis of Constructing the Heterocycle-Based Macrocycle Last



chain (**2**), since its lone chiral center (C-37) could not be assigned,[3] and the excision of the two aryl chlorines and the FH aminal ring on the basis of considerations of their stability to diverse reaction conditions. In the forward direction, we expected that these latter two motifs could be generated in either order, with the chloro groups arising through a site-selective electrophilic aromatic substitution reaction and the aminal ring resulting from the addition of the C-7 phenol onto an iminium species derived from the F-ring lactam in **3**.

Having reached an appropriate subgoal structure to tackle the question of macrocycle formation, we then unfurled the heterocycle-based ring system at its C16−C18 biaryl linkage through a Witkop-type photocyclization transform.[4] This same disconnection had been used to great success by the Harran group in their synthesis of the originally proposed structure.[3,5] Accordingly, the chances that **4** could be converted into **3** during the actual synthesis were relatively high, although the presence of new functionality within the E-ring of **4** meant that some modification of the reaction conditions would likely be needed to promote the single-electron-transfer step in this case. Equally important, from the standpoint of design, the macrocyclization should occur atropselectively as governed by the chirality of the C-10 quaternary center and the likely strong driving force to orient the B- and E-rings in a stacked arrangement through $\pi-\pi$ interactions (an orientation that only the correct atrop-isomer can accomplish).

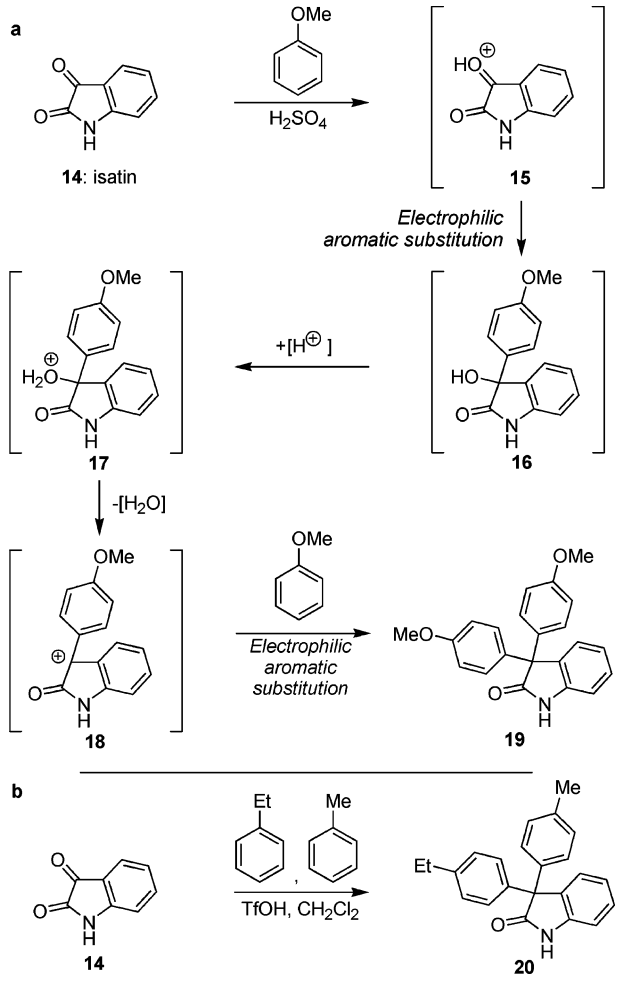
Accepting these key alterations, the heterocyclic chain of this new target (**4**) was then simplified to **7** by unraveling its B-ring oxazole to a keto amide precursor (**5**) and breaking apart the

(3) (a) Li, J.; Jeong, S.; Esser, L.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4765−4770. (b) Li, J.; Burgett, A. W. G.; Esser, L.; Amezcua, C.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4770−4773.

(4) (a) Yonemitsu, O.; Cerutti, P.; Witkop, B. *J. Am. Chem. Soc.* **1966**, *88*, 3941−3945. (b) Theuns, H. G.; Lenting, H. B. M.; Salemink, C. A.; Tanaka, H.; Shibata, M. S.; Ito, K.; Lousberg, R. J. J. *Heterocycles* **1984**, *22*, 2007−2011. (c) Mascal, M.; Moody, C. J. *J. Chem. Soc., Chem. Commun.* **1988**, 589−590.

(5) For the third successful total synthesis of diazonamide A, one which also utilized a Witkop reaction to great success, see Burgett, A. W. G.; Li, Q.; Wei, Q.; Harran, P. G. *Angew. Chem., Int. Ed.* **2003**, *42*, 4961−4966.

Scheme 2. Precedent for the Reaction of Aromatics with Isatin (**14**) under Acidic Conditions



newly unveiled amide linkage to excise the CD-indole portion as amine **6**. With the formidable heterocycle-based macrocycle of **1** effectively stripped away, we now needed to dissect the remaining 12-membered ring of diazonamide A (**1**). As indicated, we unlocked this ring at its seemingly most logical site, its central amide, to provide an amino acid precursor (**8**) modified by the introduction of a few protecting groups. However, while this choice was a relatively obvious one, the operations that could, in turn, convert this new target (**8**) into simple building blocks were far from self-evident since that task entailed the detachment of three disparate aromatic systems from a quaternary carbon. Indeed, literature precedent for how to form such systems was relatively sparse.

For example, at the end of the 19th century, Baeyer and Lazarus were able to attach two identical aromatic systems to a molecule of isatin (**14**, Scheme 2) through iterative electrophilic aromatic substitution reactions proceeding by way of intermediates **15**−**18**.[6] Over one hundred years later, a report from Olah and co-workers described how the same process could be employed to attach two different aromatics to isatin (**14**), albeit in random fashion, by using two aromatic reactants simultaneously to obtain a statistical mixture of all three possible products favoring **20**.[7] While both were important advances in heterocyclic chemistry, the successful translation of these studies to the diazonamide context was not clear, especially in light of the fact that an oxazole ring (ring A) and a phenol (ring G) have far different reactivities.

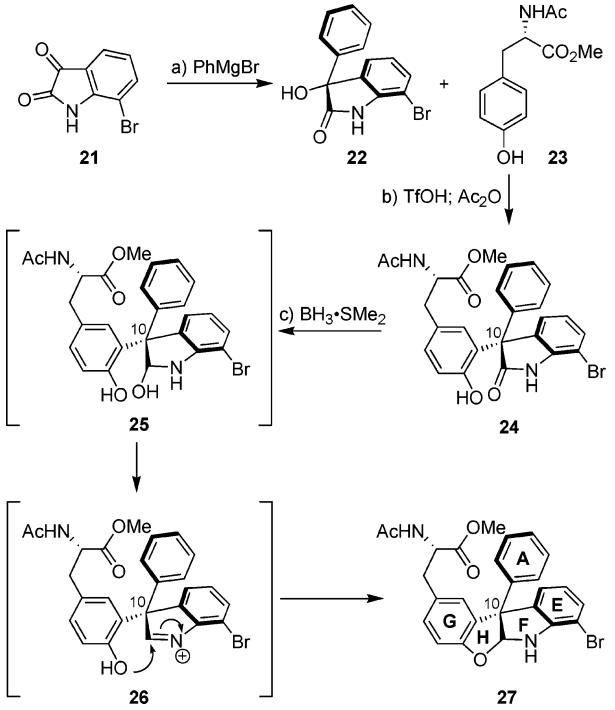
Upon further consideration, we wondered if success could be achieved by forming a compound such as **16** through a different reaction, and then introducing the second aromatic moiety through the acid-catalyzed electrophilic aromatic substitution process that Baeyer and Olah had used so productively in the past. As shown in Scheme 1, this idea was expressed through the initial nucleophilic addition of a dianion derived from oxazole **12** onto the more reactive C-3 carbonyl group of isatin **13**, a process that would lead to the requisite test compound (**11**) bearing a tertiary hydroxyl group. Subsequent exposure of this product (**11**) to acid in the presence of the electron-rich L-tyrosine-derived building block **9** was then expected to give rise to **8** by way of **10**. Of course, while this strategy would afford an expedient entry into the diazonamide skeleton if it worked, it could never lead to the stereocontrolled synthesis of the C-10 center in the absence of additional tricks. However, because we found it challenging to envision a more expeditious synthesis of such a fragment, the loss of a portion of material because of shortcomings imposed by its conciseness was anticipated to provide a level of material throughput that could match, or even surpass, a longer route featuring stereocontrol.[8] Thus, the formidable architecture of diazonamide A (**1**) had been reduced to five simple building blocks (**2**, **6**, **9**, **12**, and **13**) in what amounted to a convergent synthetic plan.[9]

Preliminary Model Studies. We began our evaluation of this plan's feasibility with several model studies directed toward the construction of the C-10 quaternary center of diazonamide A with all of its peripheral structural motifs. Pleasingly, as indicated in Scheme 3, such travail would ultimately bear fruit in the form of a successful construction of **27**.

The first key operation was merging Cbz-protected L-tyrosine methyl ester[10] and isatin derivative **22** [the product of chemoselective Grignard addition of phenylmagnesium bromide onto 7-bromoisatin (**21**)[11]] through electrophilic aromatic substitution. At first, forming this key carbon−carbon bond proved elusive, as treatment of 1.00 equivalents of **22** and 1.05 equivalents of **23** with a variety of acids in catalytic quantities led exclusively to decomposition (Table 1, entries 1−7). Thankfully, though, when the amount of acid was increased to stoichiometric (entry

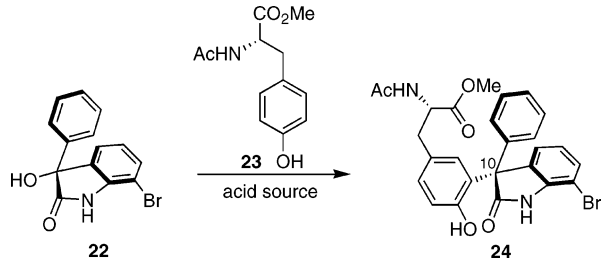
(6) Baeyer, A.; Lazarus, M. J. *Chem. Ber.* **1885**, *18*, 2637−2643.
(7) Klumpp, D. A.; Yeung, K. Y.; Prakash, G. K. S.; Olah, G. A. *J. Org. Chem.* **1998**, 4481−4484.
(8) Several such outcomes are known. For instance, in their elegant studies toward the natural product *meso*-chimonanthine, the Overman group developed a 13-step asymmetric synthesis of this compound which proceeded in an overall yield of ∼30%: (a) Overman, L. E.; Larrow, J. F.; Stearns, B. A.; Vance, J. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 213−215. (b) Overman, L. E.; Paone, D. V.; Stearns, B. A. *J. Am. Chem. Soc.* **1999**, *121*, 7702−7703. Later, another group developed a biomimetic, three-step approach to the same target. Although it lacked any stereocontrol, it afforded the same product in 30% yield following separation from the undesired stereoisomers: Ishikawa, H.; Takayama, H.; Aimi, N. *Tetrahedron Lett.* **2002**, *43*, 5637−5639.
(9) For the preliminary communication of this total synthesis, see Nicolaou, K. C.; Bella, M.; Chen, D. Y.-K.; Huang, X.; Ling, T.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 3495−3499.
(10) The synthesis of this compound was achieved by Cbz-protection of L-tyrosine methyl ester hydrochloride using benzyl chloroformate and Na_2CO_3 as described in ref 2a.
(11) 7-Bromoisatin was prepared via the standard Sandmeyer procedure, namely, reacting 2-bromoaniline with chloral hydrate and $HONH_2$·HCl, and then cyclizing the resultant product with H_2SO_4: (a) Lisowski, V.; Robba, M.; Rault, S. *J. Org. Chem.* **2000**, *65*, 4193−4194. (b) Ma, D.; Wu, Q. *Tetrahedron Lett.* **2000**, *41*, 9089−9093. (c) Tokunaga, T.; Hume, W. E.; Umezome, T.; Okazaki, K.; Ueki, Y.; Kumagai, K.; Hourai, S.; Nagamine, J.; Seki, H.; Taiji, M.; Noguchi, H.; Nagata, R. *J. Med. Chem.* **2001**, *44*, 4641−4649.

Scheme 3. Model Studies Exploring the Generation of the C-10 Quaternary Center of the Revised Structure of Diazonamide A (**1**)[a]



[a] Reagents and conditions: (a) PhMgBr (2.5 equiv), THF, 0 °C, 96%; (b) **23** (1.05 equiv), TfOH (5.0 equiv), CH_2Cl_2, 0 °C, 20 min; then Ac_2O (10 equiv), CH_2Cl_2, 25 °C, 20 min, 84%; (c) BH_3•SMe_2 (4.0 equiv), THF, 25 °C, 2 h, 40%. TfOH = trifluoromethanesulfonic acid. Compounds **24**−**27** are mixtures of C_{10} epimers (ca. 1:1).

Table 1. Attempts to Form the Triaryl-Substituted Quaternary Center of a Diazonamide A (**1**) Model System through Electrophilic Aromatic Substitution[a]

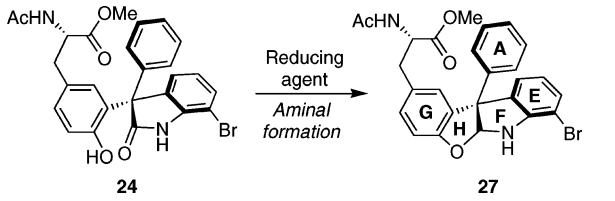


entry	conditions	yield (%)
1	$Yb(OTf)_3$ (cat.), CH_2Cl_2, 25 °C	decomposition
2	$Sc(OTf)_3$ (cat.), CH_2Cl_2, 25 °C	decomposition
3	*p*TsOH (1.0 equiv), CH_2Cl_2, 25 °C	decomposition
4	*p*TsOH (1.0 equiv), toluene, 25 °C	decomposition
5	CSA[b] (1.0 equiv), CH_2Cl_2, 25 °C	decomposition
6	PPTS[c] (1.0 equiv), CH_2Cl_2, 25 °C	decomposition
7	TfOH (0.2 equiv), CH_2Cl_2, 25 °C	decomposition
8	$MeSO_3H/CH_2Cl_2$ (1:5), 0 °C	47
9	TfOH (2 equiv), CH_2Cl_2, 25 °C	63
10	$TfOH/CH_2Cl_2$ (1:5), 25 °C	84

[a] Compound **24** is a mixture of C_{10} epimers (ca. 1:1). [b] CSA = 10-camphorsulfonic acid. [c] PPTS = pyridinium *p*-toluenesulfonate.

8) or gross excess levels (entries 9 and 10), triaryl intermediate **24** could be obtained in yields as high as 84% following acetylation of the free amine liberated during the event. We hypothesize that the amount of acid required reflects a need to fully ionize the indole-based starting material in a relatively short period of time to get it to participate in the desired pathway. With this critical stage reached, subsequent exposure

Table 2. Attempts to Form the FH Aminal Ring System of a Diazonamide A Model System through Reductive Closure[a]



entry	conditions	yield (%)
1	DIBAL-H,[b] THF, 25 °C	23
2	Red-Al, THF, 25 °C	31
3	$LiBEt_3H$, THF, 25 °C	32
4	BH_3•SMe_2, THF, 25 °C	40
5	$NaBH_4$, THF, 25 °C	37
6	BH_3•THF, THF, 25 °C	26
7	TMSOTf (1.0 equiv), Et_3SiH (20 equiv), THF, 0→25 °C	18

[a] Compounds **24** and **27** are mixtures of C_{10} epimers (ca. 1:1). [b] DIBAL-H = diisobutylaluminum hydride.

of **24** to virtually any of the common reducing agents (see Table 2) led to the formation of the bicyclic aminal system **27**, presumably by way of intermediates **25** and **26** [Compounds **24**−**27** are mixtures of C-10 epimers (ca. 1:1)].[12] Although the best yield that we obtained in this final step was only 40% (unoptimized), we were hopeful that when it came time to close the same ring system with fully elaborated intermediates, the restricted rotational freedom of the G-ring phenol as part of a completed left-hand macrocyclic unit would drive the ring closure with higher efficiency than that observed here. In any case, we knew that we could form the C-10 quaternary center of diazonamide A (**1**) through this approach, and we had concurrently identified a method that could be applied in the late stages of the synthesis to form its aminal ring.

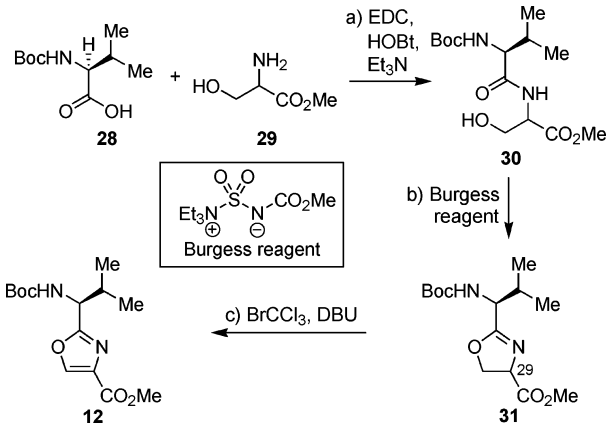
Synthesis of Building Blocks. With these studies complete, we sought to apply our designed sequence to building blocks appropriately decorated to access diazonamide A (**1**), a task which required supplies of 2-hydroxyisovaleric acid (**2**), keto amide **6**, oxazole **12**, and MOM-protected 7-bromoisatin (**13**, see Scheme 1). Of these, only the last three demanded any route development since the first was commercially available.

The construction of oxazole fragment **12** is shown in Scheme 4, starting with the merger of Boc-protected L-valine (**28**) and DL-serine methyl ester (**29**) into **30** in 92% yield through a standard peptide-coupling reaction as conducted by EDC and HOBt. Although this reaction was a routine one, it served to provide a product (**30**) bearing all the structural motifs required to cast the target's oxazole ring. Indeed, following cyclization to oxazoline **31** through the action of Burgess reagent[13] in

(12) For examples of related iminium closures, see (a) Shishido, K.; Shitara, E.; Komatsu, H.; Hiroya, K.; Fukumoto, K.; Kametani, T. *J. Org. Chem.* **1986**, *51*, 3007−3011. (b) Shishido, K.; Hiroya, K.; Komatsu, H.; Fukumoto, K.; Kametani, T. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2491−2495. (c) Ezquerra, J.; Pedregal, C.; Yruretagoyena, B.; Rubio, A.; Carreño, M. C.; Escribano, A.; Ruano, J. L. G. *J. Org. Chem.* **1995**, *60*, 2925−2930. (d) Collado, I.; Ezquerra, J.; Mateo, A. I.; Rubio, A. *J. Org. Chem.* **1998**, *63*, 1995−2001. (e) Downham, R.; Ng, F. W.; Overman, L. E. *J. Org. Chem.* **1998**, *63*, 8096−8097.

(13) For reviews on the chemistry of the Burgess reagent, see (a) Taibe, P.; Mobashery, S. In *Encyclopedia of Reagents for Organic Synthesis, Vol. 5;* Paquette, L. A., Ed.; John Wiley & Sons: Chichester, U.K., 1995; pp 3345−3347. (b) Burckhardt, S. *Synlett* **2000**, 559. For the initial report of this reagent, see (c) Atkins, G. M.; Burgess, E. M. *J. Am. Chem. Soc.* **1968**, *90*, 4744−4745. (d) Atkins, G. M.; Burgess, E. M. *J. Am. Chem. Soc.* **1972**, *94*, 6135−6141. (e) Burgess, E. M.; Penton, H. R.; Taylor, E. A. *J. Org. Chem.* **1973**, *38*, 26−31.

Scheme 4. Synthesis of Oxazole Building Block **12**[a]



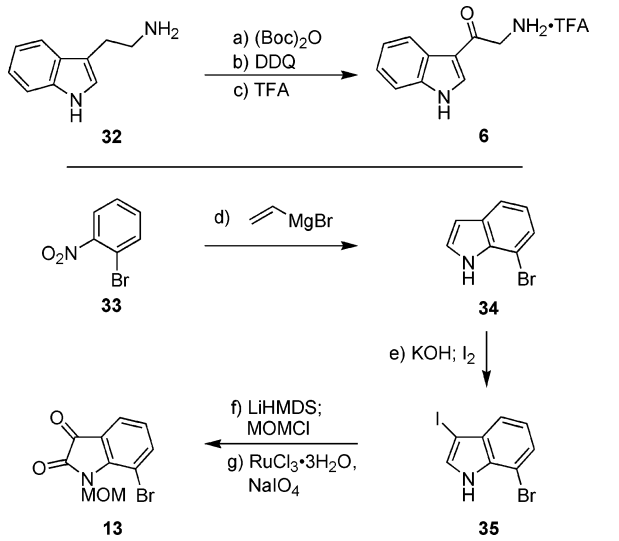
[a] Reagents and conditions: (a) **28** (1.0 equiv), **29** (1.0 equiv), EDC (1.05 equiv), HOBt (1.05 equiv), THF, 25 °C, 5 min; then Et_3N (2.0 equiv), 25 °C, 2 h, 92%; (b) Burgess reagent (1.5 equiv), THF, 65 °C, 4 h, 80%; (c) $BrCCl_3$ (1.1 equiv), DBU (1.1 equiv), CH_2Cl_2, 0→25 °C, 3 h, 90%. EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, HOBt = 1-hydroxybenzotriazole, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene. Compound **31** is a mixture of diastereomers (ca. 1:1).

refluxing THF over the course of 4 h, subsequent aromatization as effected by $BrCCl_3$ and DBU in CH_2Cl_2 at 25 °C delivered the requisite fragment (**12**) in 72% overall yield.[14] The targeted fragment could also be obtained in the same number of steps through Robinson−Gabriel cyclodehydration of the aldehyde congener of **30**, but in significantly reduced yields (~30% over two steps).[15]

With one fragment in hand, we then sought to prepare the remaining two. As shown in Scheme 5, we were able to access indole-fragment **6** by selectively protecting the free primary amine of tryptamine (**32**), installing a ketone carbonyl through the action of DDQ in aqueous THF,[16] and then exposing the resultant product to TFA to concomitantly cleave the now-extraneous Boc protecting group and deliver the product as its stable TFA salt. MOM-protected 7-bromoisatin (**13**) required almost as few steps to prepare as **6**, ultimately arising after four operations from 1-bromo-2-nitrobenzene, by way of 7-bromoindole (**34**), in 43% yield. Although this fragment (**13**) could also be prepared in two steps through MOM-protection of 7-bromoisatin (**21**, Scheme 3) formed by the more classical Sandmeyer procedure,[11] an inability to consistently secure large amounts of commercial chloral hydrate on the basis of its recent classification as a regulated substance led to the development of the alternate approach shown here.

4. Synthesis of the First Macrocyclic Unit. Having accomplished this preparative work, attempts to merge the synthesized pieces into the diazonamide skeleton began in earnest, starting with the operations needed to effect the generation of the C-10 center. At first, the initial task required to achieve this goal, the addition of oxazole **12** to isatin **13**, met with failure because of an inability to generate a dianion from the oxazole fragment without touching its ester. However, when that motif was converted into a protected primary alcohol ($LiBH_4$, THF; then LiHMDS, TBAI, BnBr, THF),[17] these

Scheme 5. Synthesis of Building Blocks **6** and **13**[a]

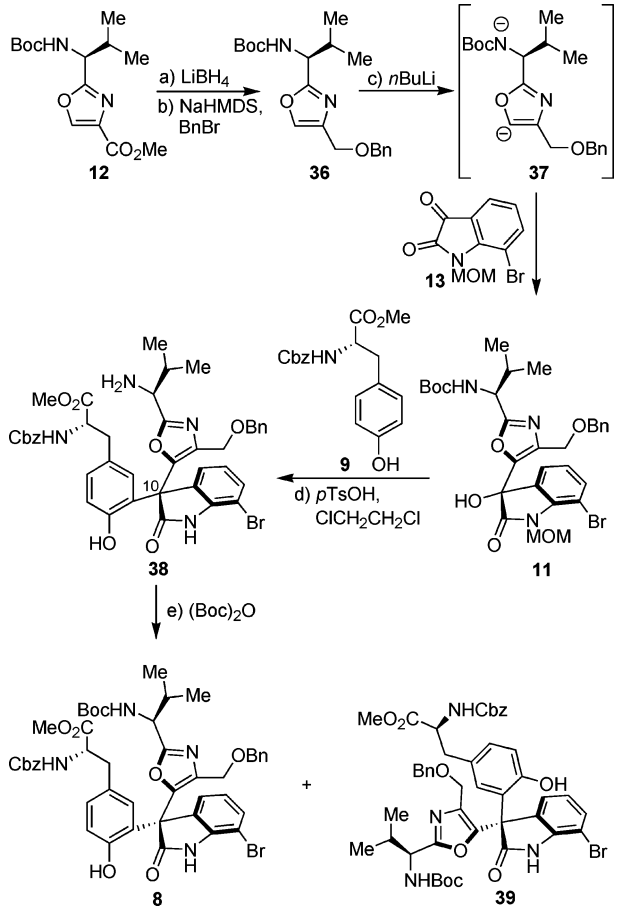


[a] Reagents and conditions: (a) $(Boc)_2O$ (1.1 equiv), aq $NaHCO_3$/dioxane (1:2), 25 °C, 2 h; (b) DDQ (2.0 equiv), THF/H_2O (9:1), 0 °C, 2 h; (c) TFA, 25 °C, 10 min, 79% over three steps; (d) vinylmagnesium bromide (3.0 equiv), THF, −45 °C, 45 min, 63%; (e) KOH (2.5 equiv), DMF, 0 °C, 5 min; I_2 (1.05 equiv), DMF, 0 °C, 15 min; (f) LiHMDS (1.0 M in THF, 1.2 equiv), THF, −78 °C, 15 min; MOMCl (1.4 equiv), −78→25 °C, 2 h; (g) $RuCl_3 \cdot 3H_2O$ (0.1 equiv), $NaIO_4$ (3.0 equiv), CH_3CN/THF/H_2O (8.3:1.0:1.7), 0 °C, 30 min, 68% over three steps. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, TFA = trifluoroacetic acid, DMF = *N*,*N*-dimethylformamide, LiHMDS = lithium bis(trimethylsilyl)amide, MOM = methoxy methyl.

fragments were smoothly converted into **11** (Scheme 6) in 73% yield by treating a THF solution of that new oxazole (**36**) with 2.0 equiv of *n*-BuLi at −78 °C for 20 min, and then adding **13** to the reaction flask. With this operation unveiling a tertiary hydroxyl group at the carbon corresponding to the C-10 position of the target molecule, this new intermediate (**11**) was perfectly outfitted to attempt the incorporation of the final aromatic ring of the AG system in the next step. After extensive optimization of the conditions originally identified for this task in our model studies (vide supra), this goal was accomplished in 47% yield by refluxing a solution containing **9** (4.0 equiv), **11** (1.0 equiv), and *p*-TsOH (4.0 equiv) in 1,2-dichloroethane for 25 min. Although the material return for this reaction was modest, this shortcoming was not a measure of the efficiency of the reaction itself but rather reflected the difficulty in isolating the final product because of the presence of the free amine (**38**) which had been unveiled unintentionally. Unable to improve this outcome any further, we pressed forward and reprotected this site in the next operation, now as a *tert*-butyl carbamate, to provide **8** and **39** as a mixture of chromatographically separable diastereomers in a combined yield of 76%. However, despite our ability to obtain both C-10 epimers of this advanced intermediate in stereochemically pure form following this operation, we could not definitively assign their C-10 configurations through any form of noncrystallographic analysis. Consequently, both **8** and **39** were processed separately through the ensuing steps of the sequence, hoping that at some stage their physical data, or a crystal structure, would indicate which one

(14) Williams, D. R.; Lowder, P. D.; Gu, Y.-G.; Brooks, D. A. *Tetrahedron Lett.* **1997**, *38*, 331−334.
(15) For the synthesis of a related fragment, see Downing, S. V.; Aguilar, E.; Meyers, A. I. *J. Org. Chem.* **1999**, *64*, 826−831.
(16) Oikawa, Y.; Toshioka, T.; Mohri, K.; Yonemitsu, O. *Heterocycles* **1979**, *12*, 1457−1462.
(17) The use of LiHDMS was crucial in obtaining the desired benzylated product (**36**) in high yield. Other bases such as NaH or LDA led to significant amounts of bis-benzylated material by enabling engagement of the Boc-protected amine. The reason for this strange difference in behavior, especially as relates to the two lithium bases, is currently unknown.

Scheme 6. Synthesis of Advanced Intermediate **8**[a]

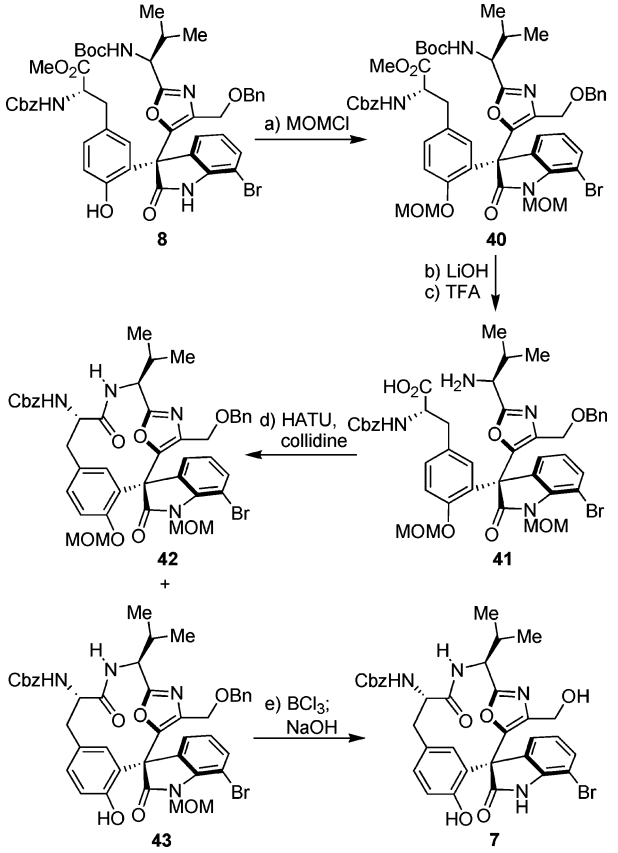


[a] Reagents and conditions: (a) $LiBH_4$ (2.0 M in THF, 3.0 equiv), EtOH (10 equiv), Et_2O/THF (2:1), 0→25 °C, 3 h, 83%; (b) NaHMDS (1.0 M in THF, 2.0 equiv), THF, −78 °C, 10 min, then TBAI (0.1 equiv), BnBr (10 equiv), −78→25 °C, 24 h, 72%; (c) *n*BuLi (2.0 equiv), THF, −78 °C, 20 min, then **13** (1.05 equiv), 5 min, 73%; (d) **9** (4.0 equiv), *p*TsOH (4.0 equiv), $ClCH_2CH_2Cl$, 83 °C, 25 min, 47%; (e) $(Boc)_2O$ (1.1 equiv), aq $NaHCO_3$/dioxane (1:2), 25 °C, 2 h, 65%. TBAI = tetra-*n*-butylammonium iodide, Bn = benzyl, *p*TsOH = *p*-toluenesulfonic acid, Boc = *t*-butoxycarbonyl.

possessed stereochemistry corresponding to that of (−)-diazonamide A (**1**).

The next objective of the sequence was to construct the only major structural element still missing from the AG system of **1**, namely, the amide bond that would complete its 12-membered ring. Thus, to set the stage for the macrolactamization reaction that would hopefully accomplish that goal, both **8** and **39** were converted into amino acid **41** (see Scheme 7, shown as a single sequence from **8**) in near quantitative yield via initial protection of their oxindole nitrogen and G-ring phenol as MOM ethers (MOMCl, K_2CO_3, acetone), followed by ester hydrolysis and Boc removal. Probes to form the final linkage of the AG macrocycle then began. Despite the difficulty encountered in forming this ring system in our studies to synthesize the originally proposed structure for diazonamide A,[1a] no such troubles were encountered here in the absence of a formed heterocyclic core. Indeed, several conditions rose to the occasion, with the use of HATU and 2,4,6-collidine in a 1:2 mixture of DMF and CH_2Cl_2 at a final concentration of 3.0×10^{-4} M constituting the best protocol by delivering **42** along with a small amount of phenol **43** in 36% combined yield.[18] In the absence of high dilution, we observed significant amounts of dimeric and higher oligomeric materials. Although this outcome was

Scheme 7. Completion of the Peptide-Based Macrocycle **7**[a]



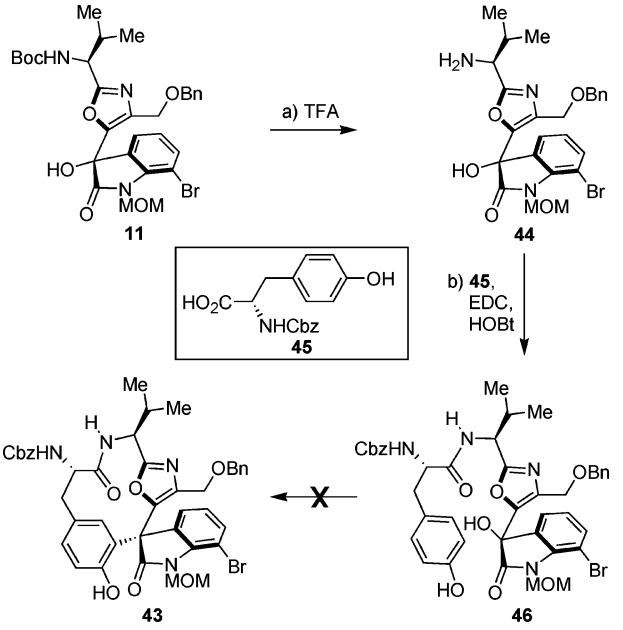
[a] Reagents and conditions: (a) MOMCl (20 equiv), K_2CO_3 (40 equiv), acetone, 0→25 °C, 1 h, 85%; (b) LiOH (20 equiv), MeOH/THF/H_2O (2:10:1), reflux, 20 min, 96%; (c) TFA (neat), 10 min, 98%; (d) HATU (2.2 equiv), collidine (6.6 equiv), DMF/CH_2Cl_2 (1:2, 3.0×10^{-4} M), 25 °C, 12 h, 36%; (e) BCl_3 (1.0 M in hexanes, 20 equiv), CH_2Cl_2, −78 °C, 2 h; then THF, saturated aq $NaHCO_3$, 10% aq NaOH, 25 °C, 1 h, 65%. HATU = 2-(1*H*-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

not especially surprising, the result that did confound us was the observation that only one of the two C-10 epimers of **41** underwent macrocyclization successfully, with the other leading primarily to dimerized material irrespective of reaction concentration.[19] Thus, the troubling question raised by this finding was whether it was the correct diastereomer that had cyclized as drawn in Scheme 7, because if the wrong C-10 epimer of **42** had been formed, then we would never be able to access **1** through this strategy. Some positive evidence that the correct epimer had indeed cyclized was provided once the MOM and benzyl ethers attached to our mixture of products (**42** and **43**)

(18) For other total syntheses which benefited from this reagent combination, see (a) Nicolaou, K. C.; Koumbis, A. E.; Takayanagi, M.; Natarajan, S.; Jain, N. F.; Bando, T.; Li, H.; Hughes, R. *Chem. Eur. J.* **1999**, *5*, 2622−2647. (b) Hu, T.; Panek, J. S. *J. Org. Chem.* **1999**, *64*, 3000−3001. (c) Evans, D. A.; Wood, M. R.; Trotter, B. W.; Richardson, T. I.; Barrow, J. C.; Katz, J. L. *Angew. Chem., Int. Ed.* **1998**, *37*, 2700−2704.

(19) Other examples of such selective cyclizations as dictated by the stereochemistry of the intervening chain exist. See, for example, one of the classic total syntheses of erythronolide: Woodward, R. B.; Logusch, E.; Nambiar, K. P.; Sakan, K.; Ward, D. E.; Au-Yueng, B.-W.; Balaram, P.; Browne, L. J.; Card, P. J.; Chen, C. H.; Chênevert, R. B.; Fliri, A.; Frobel, K.; Gais, H.-J.; Garratt, D. G.; Hayakawa, K.; Heggi, W.; Hesson, D. P.; Hoppe, D.; Hoppe, I.; Hyatt, J. A.; Ikeda, D.; Jacobi, P. A.; Kim, K. S.; Kobuke, Y.; Kojima, K.; Krowicki, K.; Lee, V. J.; Leutert, T.; Malchenko, S.; Martens, J.; Matthews, R. S.; Ong, B. S.; Press: J. B.; Rajan Babu, T. V.; Rosseau, G.; Sauter, H. M.; Suzuki, M.; Tatsuta, K.; Tolbert, L. M.; Truesdale, E. A.; Uchida, I.; Ueda, Y.; Uychara, T.; Vasella, A. T.; Vlauchick, W. C.; Wade, P. A.; Williams, R. M.; Wong, N.-C. *J. Am. Chem. Soc.* **1981**, *103*, 3213−3215.

Scheme 8. An Alternate Approach to Form the C-10 Quaternary Center and the Peptide-Based Macrocycle Concomitantly Met with Failure[a]



[a] Reagents and conditions: (a) TFA, 25 °C, 10 min; (b) **45** (1.5 equiv), EDC (1.5 equiv), HOBt (1.5 equiv), 25 °C; then Et_3N, 0 °C; then 25 °C, 12 h, 63% over two steps.

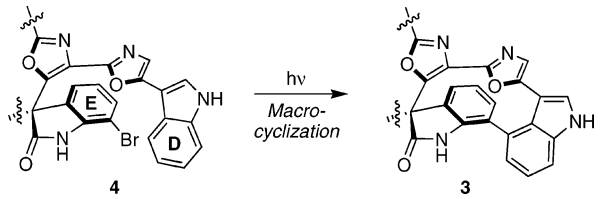
were removed through the action of BCl_3 in CH_2Cl_2 at −78 °C, as the ^1H NMR data of the resultant compound (**7**) was remarkably similar to the natural product. While not definitive proof, it was enough to induce us to continue our studies and attempt to construct the second macrocyclic unit.

Before describing the next endeavors, it is important to recognize that though the presence of the MOM groups throughout the sequence shown in Scheme 7 may appear unnecessary, they served to improve the yield of the ring closure leading to **42** by facilitating this product's isolation. In their absence, we could never obtain more than a 15% yield of the macrolactamization product. In addition, we also attempted to execute several other strategies for macrocycle formation using elements of this same sequence from intermediate **11**, such as ring closure through an acid-catalyzed intramolecular electrophilic aromatic substitution of compound **46** (see Scheme 8). Unfortunately, these approaches did not lead to the formation of macrocycle **43** despite screening numerous activating candidates of both Brønsted and Lewis acidic types.[20]

5. Completion of the Synthesis. With a route to **7** and at least some faith in its proper stereochemistry, work was now directed toward the construction of the heterocyclic core. As drawn in Scheme 9, these efforts began by selectively protecting its H-ring phenol as a Boc carbonate using $(Boc)_2O$ in a solvent mixture of aqueous $NaHCO_3$ and 1,4-dioxane (1:2) over the course of 24 h. With this operation leading to **47** in 91% yield on the basis of the recovery of a small amount of starting material (**7**), the remaining free hydroxyl group was then converted into a carboxylic acid through two iterative oxidations (IBX; $NaClO_2$) to afford **48** in 63% overall yield from **47**. This newly unveiled motif was then enlisted to complete the remaining structural elements of the target, namely, the B-, C-, and D-rings, through an initial peptide coupling with **6** to afford keto amide **27**, followed by a Robinson−Gabriel cyclodehydration using pyridine-buffered $POCl_3$ at 25 °C. Interestingly, other oxazole forming protocols, such as Cl_3CCCl_3, Ph_3P, and Et_3N[21] or the Burgess reagent,[13] also accomplished the formation of **4** from **5**, but none afforded a yield commensurate to that obtained with the above conditions which were developed as part of our campaign toward the original structure of diazonamide A.[1a,2]

(20) While we would be unable to accomplish this closure through cationic chemistry, the anionic alternative does work (see ref 5).

Table 3. Optimization of the Photochemical Conditions for Witkop Closure



entry	conditions[a]	yield (%)
1	quartz filter, Pyrex vessel, 200 W lamp, 30 min	5−10
2	quartz filter, Pyrex vessel, 200 W lamp, 12 h	dec
3	borosilicate filter, Pyrex vessel, 200 W lamp, 30 min	trace
4	sodium filter, Pyrex vessel, 200 W lamp, 30 min	5
5	quartz filter, Pyrex vessel, 450 W lamp, 30 min	15
6	quartz filter, Pyrex vessel, 450 W lamp, 12 h	dec
7	sodium filter, Pyrex vessel, 450 W lamp, 10 min	20
8	sodium filter, quartz vessel, 450 W lamp, 12 min	33

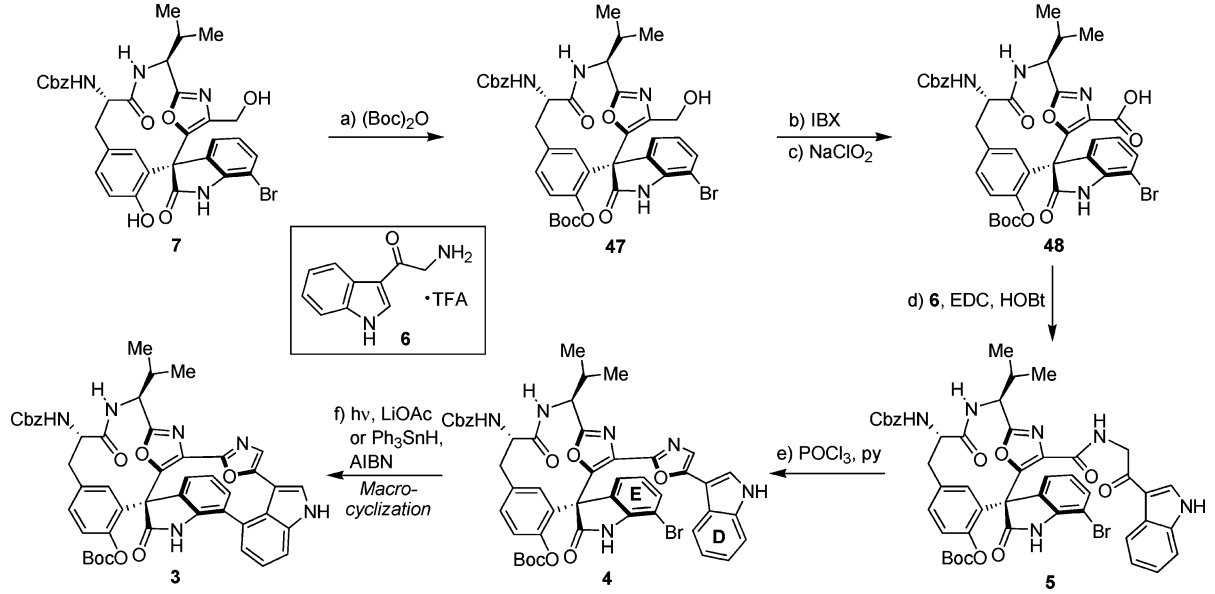
[a] All reactions were carried out in a 3:1 mixture of $MeCN/H_2O$ at 25 °C in the presence of 3.0 equiv of epichlorohydrin and 2.0 equiv of LiOAc.

With the synthesis of compound **4** achieved in 34% overall yield from **48**, we now had in hand a substrate which could be subjected to Witkop-type photocyclization conditions. Pleasingly, little alteration of the protocol developed by the Harran group[3] to accomplish this ring closure in their synthesis of the originally proposed structure of diazonamide A was required here, with excitation at 200 nm in the presence of excess epichlorohydrin (an acid scavenger) and LiOAc leading to the assembly of **3** in a reproducible yield of 33%. Key to obtaining this yield was the use of a quartz reaction vessel, a 450 W lamp, and a short reaction time (15 min), as various alternatives (see Table 3) either led to no product whatsoever or to a decreased yield of **3**. Importantly, though, when the reaction proceeded it did so with complete atropselectivity for both the C16−C18 and C24−C26 biaryl axes. Equally significant, most of the remaining material balance constituted recovered starting material that could be recycled to enhance the overall throughput of this process.[22] As an additional note, we were also able to convert **4** into **3** under strict radical conditions by treating **4** with a slight excess of Ph_3SnH (2 equiv) and catalytic amounts of AIBN in refluxing benzene. This alternate macrocyclization proceeded in 10% yield.[23]

At this juncture, the completion of the target molecule (**1**) required only a few finishing touches: the installation of the

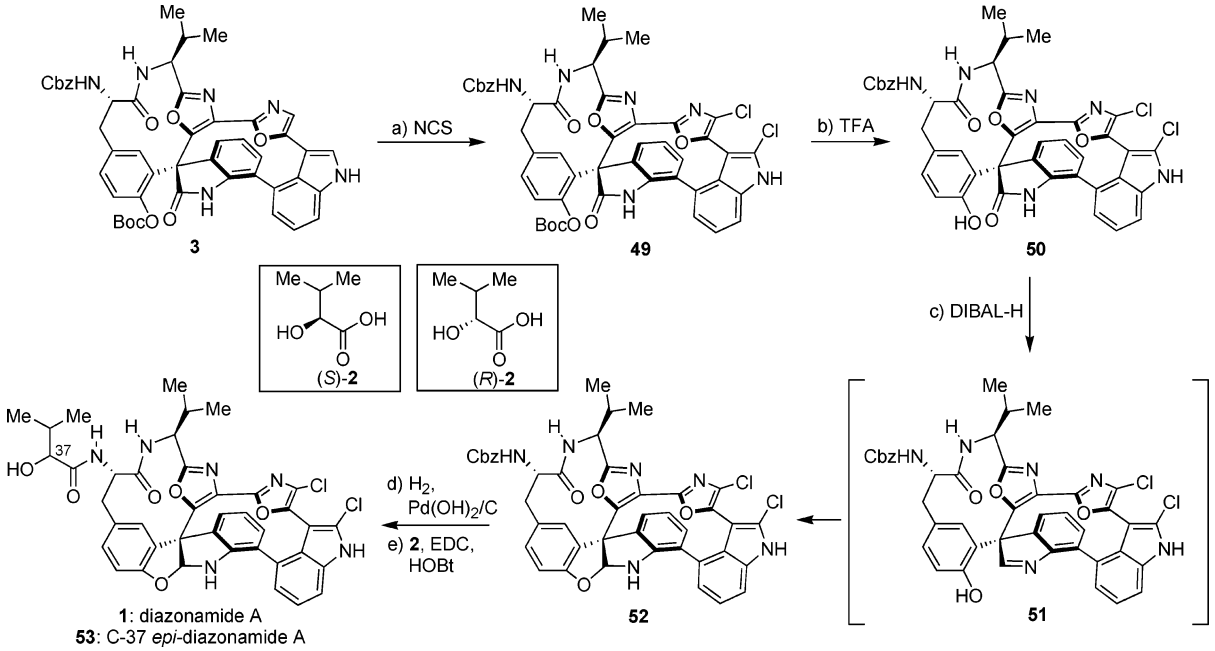
(21) Wipf, P.; Miller, C. P. *J. Org. Chem.* **1993**, *58*, 3604−3606.
(22) A typical yield based on recovered starting material for this process was around 50%.
(23) Because the majority of the substrate was reductively debrominated in the Ph_3SnH-induced reaction while the bulk of the remaining material from the Witkop conditions remained unchanged from starting material, this observation lends further support that this particular macrocyclization reaction proceeds through a mechanism based on intramolecular photoinduced electron transfer from the D-ring indole chromophore to the adjacent benzenoid E-ring.

Scheme 9. Elaboration of Advanced Intermediate **7** to **3**, a Compound Bearing Both Macrocyclic Domains of the Revised Structure of Diazonamide A (**1**)[a]



[a] Reagents and conditions: (a) $(Boc)_2O$ (30 equiv), aq $NaHCO_3$/dioxane (1:2), 25 °C, 24 h, 91% based on recovered starting material; (b) IBX (3.0 equiv), DMSO, 25 °C, 2 h; (c) $NaClO_2$ (5.0 equiv), NaH_2PO_4 (5.0 equiv), resorcinol (5.0 equiv), DMSO/H_2O (10:1), 0→25 °C, 2 h, 66% over two steps; (d) **6** (3.0 equiv), EDC (3.0 equiv), HOBt (3.0 equiv), $NaHCO_3$ (15 equiv), DMF, 25 °C, 12 h, 65%; (e) $POCl_3$/pyridine (1:4), 25 °C, 2 h, 52%; (f) *hν* (200 nm), epichlorohydrin (3.0 equiv), LiOAc (2.0 equiv), MeCN/H_2O (3:1), 25 °C, 15 min, 33% or Ph_3SnH (2.0 equiv), AIBN (0.1 equiv), C_6H_6, 80 °C, 3 h, 10%. IBX = 1-hydroxy-1,2-benziodoxol-3(1*H*)-one, py = pyridine, AIBN = 2,2′-azobisisobutyronitrile.

Scheme 10. Final Stages and Completion of the First Total Synthesis of Diazonamide A (**1**)[a]



[a] Reagents and conditions: (a) NCS (4.0 equiv), CCl_4/THF (1:1), 60 °C, 2 h, 53%; (b) TFA, 25 °C, 10 min, 98%; (c) DIBAL-H (1.0 M in toluene, 10 equiv, added portionwise), THF, −78→25 °C, 3 h, 56%; (d) H_2 (2.0 atm), $Pd(OH)_2$/C (20 wt %, catalytic), EtOH, 25 °C, 2 h; (e) **2** (5.0 equiv), EDC (5.0 equiv), HOBt (5.0 equiv), $NaHCO_3$ (15 equiv), DMF, 25 °C, 12 h, 82% over two steps. NCS = *N*-chlorosuccinimide.

two aryl chlorines, the formation of diazonamide A's FH aminal system, and the attachment of the final terminal residue (2-hydroxyisovaleric acid). As shown in Scheme 10, the first of these tasks was accomplished by treating **3** with NCS at 60 °C for 2 h in a 1:1 mixture of THF and CCl_4, conditions that led to the site- and atropselective incorporation of the two requisite chlorines. Although our earlier chlorination forays[1a] using just the BCD indole-oxazole subunit suggested that indole protection was required for this event, we did not observe any of the *N*-chlorination that plagued those studies in our reactions with **3**.

Seeking now to form the FH aminal system along the lines employed previously (cf. Scheme 3), we deprotected the Boc group from the G-ring phenol in **49** (TFA, 25 °C, 10 min) to obtain **50**, and then we exposed this new compound to a variety of hydride sources. Amazingly, this cyclization proved quite

challenging despite the ease by which a plethora of reducing agents had managed to convert **24** into **27** earlier (see Table 2). Following extensive scouting, we discovered that this task could be accomplished in 56% yield through the portionwise addition of 100 equiv of DIBAL-H (5 × 20 equiv) to a solution of **50** in THF over 3 h, using a cooling and warming cycle between −78 °C and 25 °C after each addition.[24] Although a seemingly esoteric protocol, adding the hydride source in batches was critical for the success of this cascade sequence, as otherwise the reaction stalled and far reduced yields of **52** were consistently obtained with starting material recovered instead. No other reducing agent screened provided any product (**52**).

At this stage, the spectral data for synthetic **52** and that published[25] for the natural product were quite similar, suggesting that **1** was perhaps the correct structure for diazonamide A. However, the moment of truth would not occur until the final 2-hydroxyisovaleric acid residue was attached to the molecule. Thus, we chemoselectively excised the Cbz group guarding the C-2 amine through a standard hydrogenation protocol using Pearlman's catalyst [20% $Pd(OH)_2/C$] in EtOH and then separately installed both (*S*)-**2** and (*R*)-**2** onto the resultant product through a peptide coupling reaction as orchestrated by EDC and HOBt. Of the two resultant materials, both formed in 82% overall yield from **52**, one [the product obtained from the use of (*S*)-**2** in the coupling reaction] matched the data reported for natural diazonamide A exactly (on the basis of ^{1}H NMR, optical rotation, HPLC, and TLC comparisons in a variety of solvent systems).[26] As such, the first total synthesis of **1** was complete through a route that required 21 steps in its longest linear sequence, and all questions regarding the structural identity of diazonamide A were finally laid to rest.[27]

Conclusion

Unique and challenging molecular motifs within secondary metabolites have long presented synthetic chemists with golden opportunities for discovery, whether as a source of inspiration leading to creative strategies or as a testing ground revealing weaknesses in the power of available methodology to effectively fashion such complexity worthy of repair. Diazonamide A (**1**) certainly is a natural product endowed with several such domains, as the completion of the total synthesis described in this article could not have been accomplished without the development of several unique synthetic strategies and complexity-building reaction cascades. Most noteworthy among these are (1) the expeditious construction of the C-11 quaternary center and its adjoining aromatic systems, (2) the successful use of the Witkop-photocyclization reaction to forge the final bond needed to close the highly hindered 12-membered heterocyclic core, and (3) DIBAL-H initiated aminal formation.

Importantly, though, the story of the developed chemistry did not stop once we reached this pinnacle. As the following article will describe,[28] not only did we use this sequence to prepare a series of simpler analogues for biological evaluation, but we also devised and executed an entirely different approach to the target molecule that proved equally capable of handling its most nefarious structural motifs.

Acknowledgment. We thank Drs. D. H. Huang and G. Siuzdak for NMR spectroscopic and mass spectrometric assistance, respectively. Financial support for this work was provided by The Skaggs Institute for Chemical Biology, the National Institutes of Health, predoctoral fellowships from the National Science Foundation, Pfizer, and Bristol-Myers Squibb (all to S.A.S.), a postdoctoral fellowship from the Skaggs Institute for Chemical Biology (X.H.), and a grant from American BioScience.

Supporting Information Available: Experimental procedures and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) Neither of the conditions developed for our key reactions in our model studies proved directly applicable to fully functionalized intermediates. Although such outcomes often occur, its consistency throughout the diazonamide campaign is indicative of the severely compact nature of the target in which the alteration of a single group or the addition of a few atoms can instigate several new problems with established chemistry.

(25) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 2303−2304.

(26) While most of the signals for both **1** and **53** were identical, a few were diagnostic. Especially revealing was the C-37 proton signal (400 MHz, MeOH-d_4) which, in **1**, coincided precisely with the reported value for the natural product (3.88 ppm), whereas in **53** this proton appeared at 3.92 ppm.

(27) In line with expectations, synthetic diazonamide A (**1**) exhibited potent cytotoxic activity at single digit nM concentrations against a variety of human cancer cell lines of distinct origin, including ovarian carcinoma 1A9, lung carcinoma A549, prostate carcinoma PC-3, breast carcinoma MCS-7, and the Taxol-resistant 1A9/PTX10 cell line. We thank Dr. Paraskevi Giannakakou and Aurora O'Brate, Emory University Winship Cancer Center, for performing these studies. More detailed discussion and additional biological studies are described in the following article.[28]

(28) Nicolaou, K. C.; Hao, J.; Reddy, M. V.; Bheema Rao, P.; Rassias, G.; Snyder, S. A.; Huang, X.; Chen, D. Y.-K.; Brenzovich, W. E.; Giuseppone, N.; Giannakakou, P.; O'Brate, A. *J. Am. Chem. Soc.* **2004**, *126*, XXXX−XXXX.