

Chemistry and Biology of Diazonamide A: Second Total Synthesis and Biological Investigations

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Abstract: As an especially unique target for chemical synthesis, diazonamide A has the potential to be constructed through a plethora of synthetic routes, each attended by different challenges and opportunities for discovery. In this article, we detail our second total synthesis of diazonamide A through a sequence entirely distinct from that employed in our first campaign, one whose success required the development of several special strategies and tactics. We also disclose our complete studies regarding the chemical biology of diazonamide A and its structural congeners, and more fully delineate the scope of our protocol for Robinson–Gabriel cyclodehydration using pyridine-buffered POCl₃.

Introduction

Although we had accomplished one total synthesis of diazonamide A as discussed in the previous article,¹ an endeavor which finally proved the correct molecular connectivities of this intriguing structure, we were still curious to know if the alternate order of macrocycle construction could also deliver the target molecule. It was our hope that the pursuit of this second line of investigation would not only lead to the discovery of new chemistry, but would also confer the ability to prepare simplified structural analogues of the other half of diazonamide A to achieve a complete structure–activity relationship picture. As this article will detail, both of these goals would be met.

Results and Discussion

Retrosynthetic Analysis. As indicated in Scheme 1, our second approach for the total synthesis of diazonamide A (**1**) was patterned after our previous work toward the originally proposed structure.² Thus, following the excision of the terminal 2-hydroxyisovaleric acid subunit (**2**), opening of the aminal ring,

and removal of the two aryl chlorines, the AG macrocycle was unlocked at its central amide bond to reveal **3**. The oxazole ring within this target molecule was then unfurled via a Robinson–Gabriel cyclodehydration transform to provide **4**, a new subgoal structure whose 12-membered macrocyclic ring could potentially arise via our heteropinacol coupling/oxime cleavage sequence from compound **5**. Finally, if that compound's C16–C18 biaryl linkage was severed through a palladium-mediated coupling reaction transform, such as the venerable Suzuki reaction, then this intermediate could be dissected into two fragments of commensurate size, indole-oxazole **6** and EFG building block **7**. We expected that the first of these (**6**) could be prepared in a single step from material already in hand, while the second (**7**) could be traced to an initial reaction between L-tyrosine methyl ester (**8**) and 7-bromoisatin (**9**) through a carefully controlled acid-catalyzed merger of the Friedel–Crafts type on the basis of precedent showing the ability of phenols to add to ketones.³ All in all, this analysis led to a convergent plan with a fair amount of experimental support for the likely success of its key steps. As matters would transpire, its execution would prove to be relatively smooth with the issue of C-11 functionalization serving as one of the most important elements in its successful reduction to practice.⁴

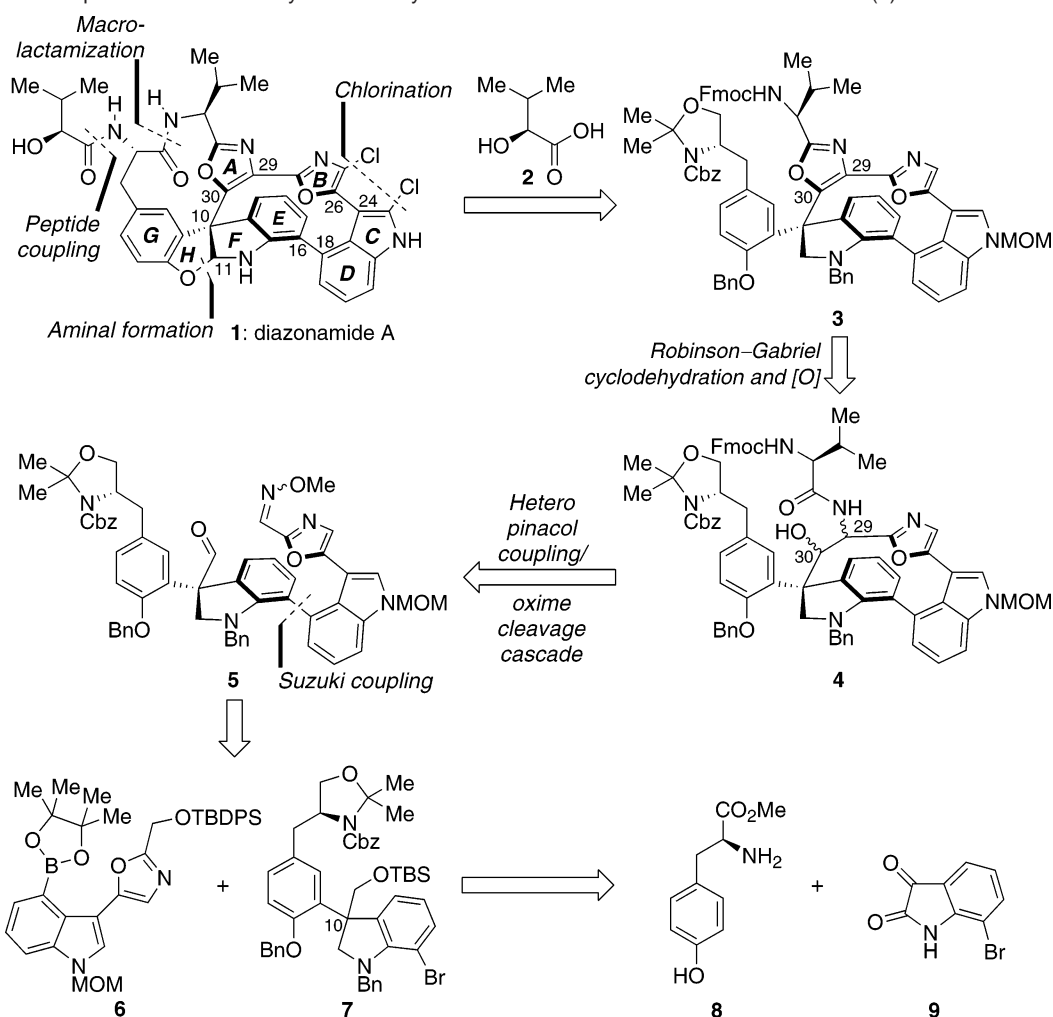
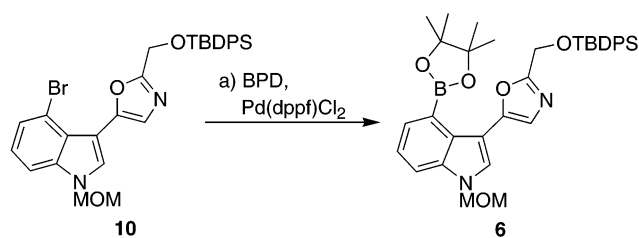
Synthesis of Building Blocks. Our efforts began with the construction of the desired building blocks, starting with the projected BCD indole-oxazole fragment (**6**). As indicated in

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- (1) Nicolaou, K. C.; Chen, D. Y.-K.; Huang, X.; Ling, T.; Bella, M.; Snyder, S. A. *J. Am. Chem. Soc.* **2004**, *126*, XXXX – XXXX. 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.
- (2) (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. (c) Nicolaou, K. C.; Snyder, S. A.; Simonsen, K. B.; Koumbis, A. E. *Angew. Chem., Int. Ed.* **2000**, *39*, 3473–3478. (d) 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.

(3) Citterio, A.; Gandolfi, M.; Piccolo, O.; Filippini, L.; Tinucci, L.; Valoti, E. *Synthesis* **1984**, 760–763.

(4) For the preliminary communication of this work, see 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*, 1753–1758.

Scheme 1. The Heteropinacol-Based Retrosynthetic Analysis of the Revised Structure of Diazonamide A (1)**Scheme 2.** Synthesis of Indole-Oxazole Building Block 6^a

^a Reagents and conditions: (a) BPD (2.5 equiv), Pd(dppf)Cl₂·CH₂Cl₂ (0.1 equiv), KOAc (3.0 equiv), 1,4-dioxane, 95 °C, 6 h, 85%. MOM = methoxy methyl, TBDPS = *tert*-butyldiphenylsilyl, BPD = bis(pinacolato)diboron, dppf = diphenylphosphinoferrocene.

Scheme 2, only a single step was required from the known fragment 10^{2a} (prepared in seven steps from 4-bromoindole⁵) by exchanging its aryl bromide for a boronic ester in 85% yield through the action of Pd(dppf)Cl₂·CH₂Cl₂, bis(pinacolato)diboron, and KOAc.⁶ While these general conditions are roughly the same as seen on several occasions throughout the diazonamide program, key to obtaining a high yield of the desired product in this case was the use of 2.5 equiv of the boron source and 1,4-dioxane, instead of DME, as solvent. If either of these

two parameters were not adhered to fully, then significant amounts of homocoupled product, as well as desbromo 10, were obtained in addition to 6.

With this fragment in hand, its eventual coupling partner, EFG fragment 7, was prepared as delineated in Scheme 3, starting with the protection of commercially available L-tyrosine methyl ester (8) as its corresponding Cbz derivative, a step that proceeded in 94% yield. While a conventional beginning, this operation served to set the stage for a subsequent acid-catalyzed Friedel-Crafts merger with 7-bromoindole (9). Following extensive screening of acidic activators, we were eventually able to execute this strategy and obtain the desired product (12) bearing the majority of the targeted fragment's architecture in 58% yield (70% based on recovered starting material) through the action of 1.2 equiv of TiCl₄ in CH₂Cl₂ at 25 °C for 6 h.^{7,8}

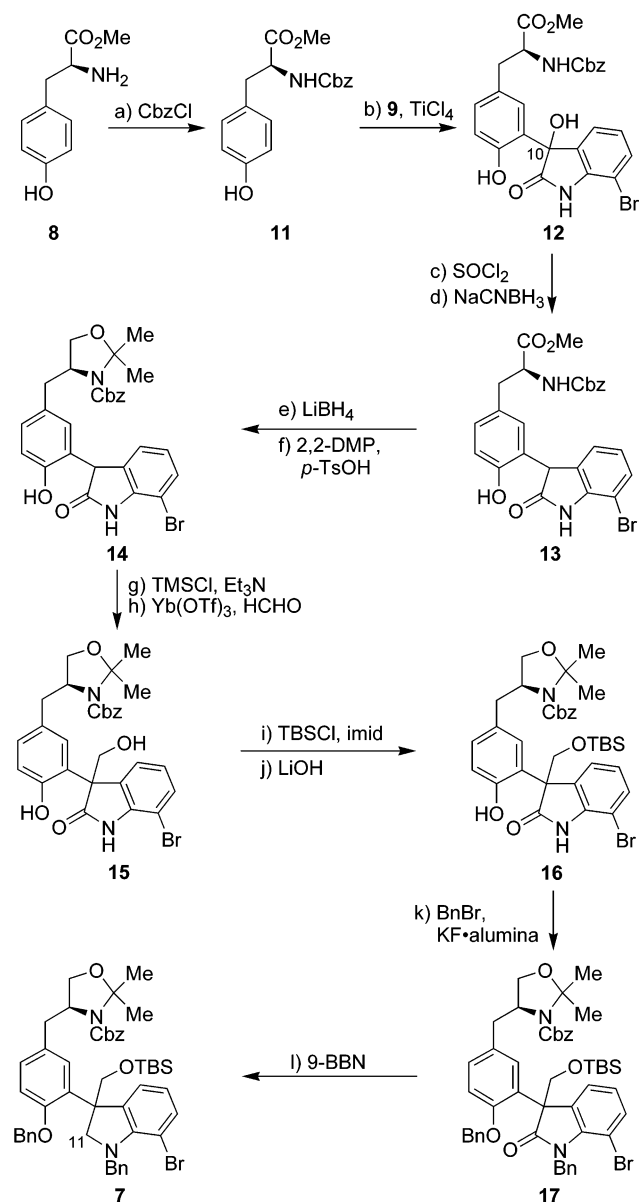
Having achieved success in this key operation, the only major structural element separating 12 from 7 was appropriate functionalization of its C-10 center. In preparation for the sequence

(5) Harrington, P. J.; Hegedus, L. S. *J. Org. Chem.* **1984**, *49*, 2657–2662.

(6) Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508–7510. For a later preparation of aryl boronic esters, see Murata, M.; Watanabe, S.; Masuda, Y. *J. Org. Chem.* **1997**, *62*, 6458–6459.

(7) Other protocols attempted include the use of TiF₄, TiBr₄, TiI₄, ZrF₄, VCl₅, BF₃, as well as ZrCl₂ and HfCl₂ with or without added silver salts. None of these alternatives worked. A greatly reduced yield of 12 could be obtained through the use of SnCl₄, whereas a slightly reduced yield of 12 occurred when ZrCl₄ was the acid catalyst.

(8) In attempts to extend the scope of this reaction, we have found that *meta*- and *para*-substituted phenols can react with isatin in the presence of TiCl₄ to deliver products bearing the isatin ring *ortho* to the phenol. However, no *ortho*-substituted starting phenol has ever served as a willing participant in this process.

Scheme 3. Synthesis of EFG Fragment 7^a

^a Reagents and conditions: (a) CbzCl (1.0 equiv), aq Na₂CO₃ (excess), CHCl₃, 25 °C, 3 h, 94%; (b) **9** (1.1 equiv), TiCl₄ (1.0 M in CH₂Cl₂, 1.2 equiv), CH₂Cl₂, 25 °C, 6 h, 58% (70% based on recovered **11**); (c) SOCl₂, 25 °C, 10 h, 90%; (d) NaCNBH₃ (3.0 equiv), MeCN, 0–25 °C, 5 h, 84%; (e) LiBH₄ (4.0 equiv), 0–25 °C, THF, 2 h, 97%; (f) 2,2-dimethoxypropane (10 equiv), *p*-TsOH (0.1 equiv), acetone, 25 °C, 2 h, 98%; (g) TMSCl (4.0 equiv), Et₃N (6.0 equiv), CH₂Cl₂, 0 °C, 2 h; (h) HCHO (37% in H₂O, 10 equiv), Yb(OTf)₃ (0.25 equiv), THF, 25 °C, 24 h, 70% over two steps; (i) TBSCl (4.0 equiv), imidazole (8.0 equiv), CH₂Cl₂, 25 °C, 6 h, 84%; (j) LiOH (3.0 equiv), DMF, 25 °C, 5 h; (k) BnBr (4.0 equiv), KF·alumina (5.0 equiv), DME, 25 °C, 12 h, 85% over two steps; (l) 9-BBN (0.5 M in THF, 5.0 equiv), THF, 65 °C, 36 h, 72%. 2,2-DMP = 2,2-dimethoxypropane, *p*-TsOH = *p*-toluenesulfonic acid, TMS = trimethylsilyl, TBS = *t*-butyldimethylsilyl, imid = imidazole, 9-BBN = 9-borabicyclo[3.3.1]nonane. Note: compounds **12**–**17** and **7** are mixtures of C₁₀ diastereomers.

that would eventually rectify this deficiency, the newly formed tertiary alcohol in **12** was exchanged for a hydrogen (**13**) in 76% yield over two steps by way of an intermediate chloride formed upon several hours of exposure to SOCl₂ at 25 °C followed by its reduction with NaCNBH₃. Next, the upper tyrosine domain was transformed into an acetonide (**14**) in near quantitative yield via methyl ester reduction using LiBH₄ in THF followed by a *p*TsOH-catalyzed reaction with 2,2-

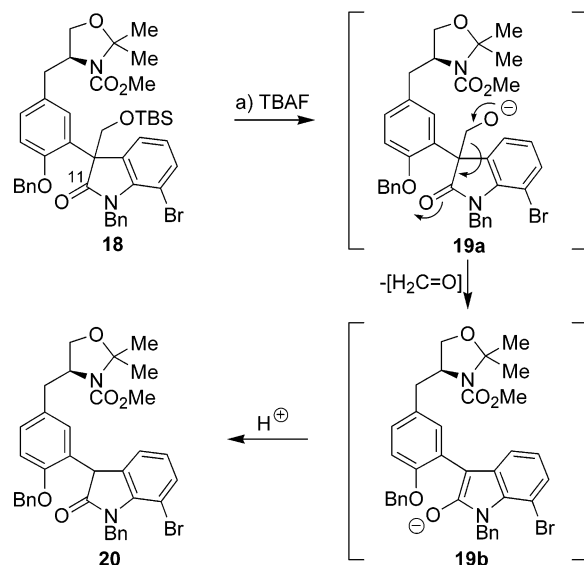
dimethoxypropane. As such, we now had a substrate onto which the needed C-10 hydroxymethyl function could be attached. This task was accomplished by enlisting a two-step procedure developed by the Padwa group for this purpose with lactones,⁹ in which the initial formation of a silyl enol ether (TMSCl, Et₃N) served to create a latent nucleophile that was subsequently unleashed upon reaction with Yb(OTf)₃ to engage the excess formaldehyde present in solution. These operations provided intermediate **15** in 70% overall yield as a mixture of both C-10 stereoisomers. Despite being diastereomers, though, these compounds could not be separated at this stage either through flash column chromatography or selective crystallization. As a result, they were carried forward together in the hopes that the incorporation of an appropriate functional group would eventually confer sufficient differences in their physical properties to allow their separation.

Pressing forward, with the complete architectural framework of the EFG fragment now established as expressed in **15**, only a few protecting and functional group manipulations remained before the substrate would be suitably outfitted to attempt Suzuki coupling with indole-oxazole **6**. These adjustments began with silylation of the free primary hydroxyl group within **15** through the action of TBSCl and imidazole in CH₂Cl₂, conditions that also led to protection of the free G-ring phenol. Since the latter event was unintended, a subsequent reaction with LiOH served to excise this superfluous group. With the phenol now free, the desired protecting group array was then completed by engaging both the phenol as well as the lactam nitrogen with benzyl groups through reaction with BnBr and KF·alumina in DME,¹⁰ leading to the formation of **17** in 80% overall yield for these three operations.

While **7** was our target for specific EFG functionalization, the intermediate that we had just synthesized (**17**) possessed, in principle, all of the motifs that the projected strategy required to move forward. Of particular importance was its E-ring carbonyl, as our first total synthesis of diazonamide A had productively employed this functional group to construct its FH aminal system.¹ However, on the basis of our experiences toward the originally proposed structure of diazonamide A, where fragmentation reactions leading to benzofuran products were a rampant problem with such C-11 functionalization,^{2ab} we expected that the carbonyl's presence would likely cause similar side reactions here. Indeed, experimentation seeking to explore this possibility on a related compound (**18**, Scheme 4) confirmed these fears, as exposure of **18** to TBAF led to **20**, presumably through the indicated sequence invoking the intermediacy of a fully aromatic enolate (**19b**). Thus, in light of these problems with **18** and related compounds such as **17**, we completed our desired EFG fragment (**7**) by fully reducing the oxindole system of **17** to an indolene (see Scheme 3) through the action of excess 9-BBN in refluxing THF. Of course, while this reduction ensured that we would not encounter any benzofuran fragmentation during the steps that would hopefully lead to the synthesis of the heteroaromatic core of **1**, it also meant that the completion of diazonamide A (**1**) would be predicated upon eventually finding an oxidative method for its reintroduction.

- (9) Padwa, A.; Dehm, D.; Oine, T.; Lee, G. A. *J. Am. Chem. Soc.* **1975**, *97*, 1837–1845.
 (10) For a related protection using this reagent combination, see (a) Ando, T.; Yamawaki, J.; Kawate, T.; Sumi, S.; Hanafusa, T. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2504–2507. (b) Yamawaki, J.; Ando, T.; Hanafusa, T. *Chem. Lett.* **1981**, 1143–1146.

Scheme 4. Problems with the C-11 Carbonyl: Deformylation through a Cascade Sequence Observed during Attempts to Create the Originally Proposed Structure of Diazonamide A^a



^a Reagents and conditions: (a) TBAF (1.3 equiv), THF, 0 °C, 1 h; then aq NH₄Cl, 95%. TBAF = tetra-*n*-butylammonium fluoride. Note: all compounds are mixtures of C₁₀ diastereomers.

Completion of the Second Total Synthesis of Diazonamide

A. With the preparative work required to access these two fragments behind us, we could now explore the key steps of the strategy, starting with the operations to synthesize the heterocyclic core. First, as shown in Scheme 5, the previously divergent nature of our sequence converged through the successful merger of **6** and **7** into **21** via a Suzuki reaction employing catalytic Pd(dppf)Cl₂·CH₂Cl₂ and K₂CO₃ in refluxing DME, an event which proceeded in 78% yield after 12 h of reaction. Next, this new adduct (**21**) was converted into a dialdehyde through a tandem deprotection/oxidation sequence employing TBAF at a slightly elevated temperature in THF (45 °C) followed by treatment with SO₃py. The projected aldehyde-oxime substrate (**5**) was then completed, as before,^{2b} through selective oxime capture of this intermediate's sterically more accessible and reactive aldehyde as accomplished with excess MeONH₂·HCl in DMSO at 25 °C.

The opportunity to harvest some benefits from this labor through a successful heteropinacol cyclization cascade sequence was now at hand. Most gratifyingly, this reaction was executed smoothly along the same lines as before using a protocol altered only by the choice of activating ligand for SmI₂ [i.e., *N,N*-dimethylacetamide (DMA)¹¹ instead of HMPA], giving rise to **4** (as a mixture of several diastereomers) in reproducible yields of 45–50%.¹² On the basis of the three distinct operations of macrocyclization, N–O bond cleavage, and peptide coupling, this outcome correlates to an average yield of 79% per step. With this operation providing functionalized material separated

(11) Nishiura, M.; Katagiri, K.; Imamoto, T. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 1417–1424.

(12) Exchanging the activating ligand from that employed during our earlier studies (HMPA) toward the originally proposed structure of diazonamide A does not reflect its failure to accomplish the same sequence leading to **4**. Indeed, HMPA also facilitated this sequence, but in inferior yield (25–30%). In truth, given that DMA possesses less toxicity than that associated with HMPA, we were pleased to be able to make this exchange from an experimental perspective. We also believe that on the basis of its success as an activating ligand for SmI₂ in this sequence, DMA could serve as a competent replacement for HMPA in several other SmI₂-related processes.

Table 1. Screening of Conditions to Accomplish the Formation of the A-ring Oxazole and Complete the Heterocyclic Macrocycle of **1**

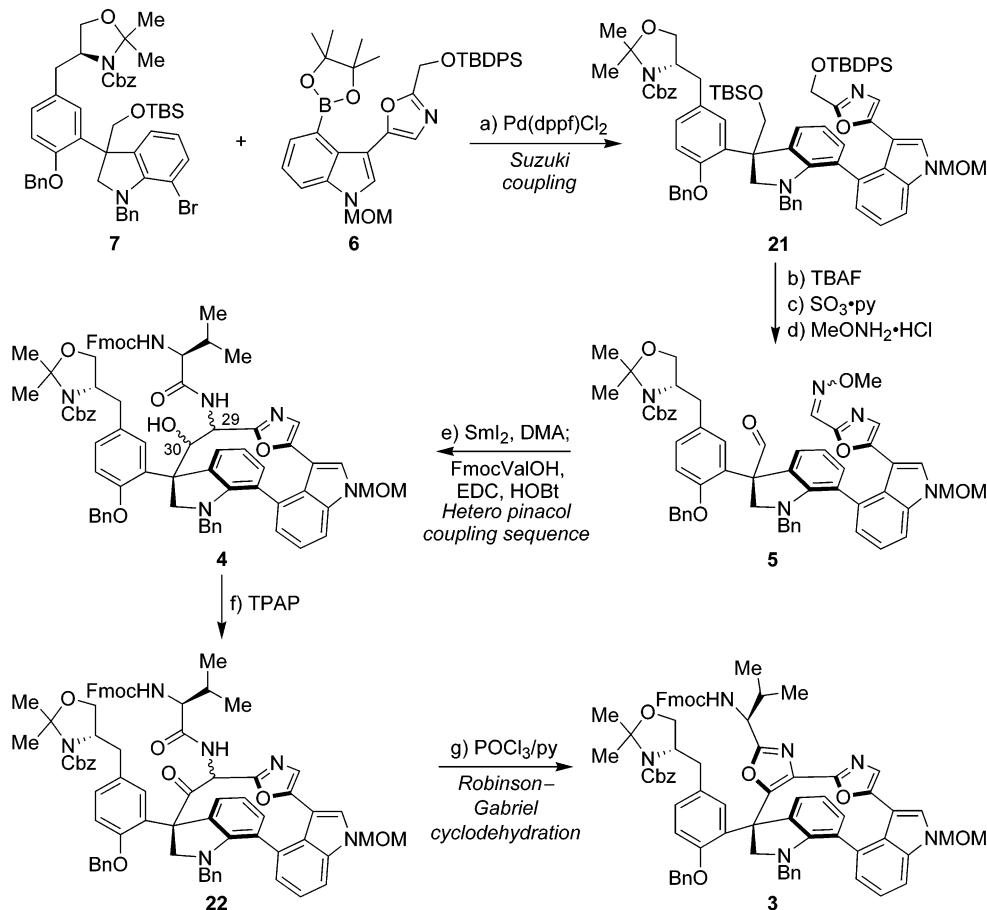
entry	conditions	yield (%)
1	PyBroP, NaHCO ₃ , DMF/CH ₂ Cl ₂ (1:1) ^a	dimer/trimer
2	FDPP, NaHCO ₃ , DMF/CH ₂ Cl ₂ (1:1) ^a	dimer/trimer
3	DEPBT, <i>i</i> -Pr ₂ NEt, DMF/CH ₂ Cl ₂ (1:1) ^a	decomposition
4	HATU, 2,4,6-collidine, DMF/CH ₂ Cl ₂ (1:1) ^a	5–10 ^b
5	HATU, 2,4,6-collidine, DMF/CH ₂ Cl ₂ (9:1) ^a	0–5 ^b
6	HATU, 2,4,6-collidine, DMF/CH ₂ Cl ₂ (1:2) ^a	10–15 ^b

^a Concentration of **23** = 1.0 × 10⁻⁴ M. ^b Range indicates maximum and minimum values obtained for several runs. PyBroP = bromotripyrrolidynophosphonium hexafluorophosphate, FDPP = pentafluorophenyl diphenylphosphinate, DEPBT = 3-diethoxyphosphoryloxy-1,2,3-benzotriazin-4(3*H*)-one, DPPA = diphenylphosphoryl azide.

from the complete heteroaromatic macrocycle by only two more steps, namely, oxidation of its C-30 hydroxyl group followed by a Robinson–Gabriel reaction to form the A-ring oxazole, we immediately set out to finish this portion of the molecule. As indicated by the last two operations in Scheme 5, these tasks were ultimately accomplished in 33% combined yield through an initial TPAP oxidation to provide **22**,¹³ followed by the needed cyclodehydration as effected by a 1:2 mixture of POCl₃ and pyridine at 70 °C.² While the material throughput for this sequence leading to **3** was lower than we would have liked, it accurately reflects the severely strained and highly hindered nature of the diazonamide heteroaromatic core since both operations were quite difficult to accomplish. In fact, in line with our experiences elsewhere in the diazonamide program, only pyridine-buffered POCl₃ proved capable of forming the A-ring oxazole of **3** from **22** in any yield in the latter of these two seemingly simple steps.

Having reached this key juncture, we expected that the completion of diazonamide A (**1**) was not that far off, with the remaining primary task at this point being the formation of the second macrocyclic subunit through macrolactamization. Thus, to prepare the needed amino acid substrate projected for this event (i.e., **23**, Scheme 6), **3** was first converted into a carboxylic acid through HF-mediated acetonide cleavage, followed by a two-stage oxidation protocol (IBX; NaClO₂). With these three events proceeding in 85% overall yield, the Fmoc group guarding the needed amine appended to the A-ring oxazole was then removed by the action of Et₂NH in THF at 25 °C over the course of 4 h.¹⁴ We were now in the same position where our campaign toward the originally proposed structure of diazonamide A met with significant resistance.^{2a,b} In concert with our experiences in that context, attempts to accomplish macrolactam formation with **23** also met with difficulty.¹⁵ Fortunately, as indicated in Table 1, when we employed HATU and 2,4,6-collidine, the only reagent combination that had provided some

(13) For an excellent review on this oxidant, see Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, 639–666. For its initial report, see Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625–1627.

Scheme 5. Construction of the Fully Functionalized Heterocyclic Core (**3**) of Diazonomide A via Suzuki and Heteropinacol Couplings^a

^a Reagents and conditions: (a) **6** (1.05 equiv), **7** (1.0 equiv), Pd(dppf)Cl₂·CH₂Cl₂ (0.2 equiv), K₂CO₃ (5.0 equiv), DME, 85 °C, 12 h, 78%; (b) TBAF (6.0 equiv), THF, 45 °C, 2.5 h, 94%; (c) Et₃N (10 equiv), SO₃py (8.3 equiv), DMSO/CH₂Cl₂ (1:1), 0 °C, 2 h, 91%; (d) MeONH₂·HCl (5.0 equiv), DMSO, 25 °C, 15 min, 91%; (e) Sml₂ (0.1 M in THF, 9.0 equiv), DMA (36 equiv), THF, 25 °C, 1 h; then saturated aq NH₄Cl, 25 °C, 1 h; solvent removal, then FmocValOH (3.0 equiv), EDC (3.0 equiv), HOBT (3.0 equiv), DMF, 25 °C, 10 h, 45–50% overall, ca. 79% per synthetic operation in the cascade sequence; (f) TPAP (1.0 equiv), NMO (5.0 equiv), 4 Å molecular sieves, CH₂Cl₂, 25 °C, 2 h, 65%; (g) POCl₃/pyridine (1:2), 70 °C, 6 h, 35% (53% based on recovered starting material); DME = ethylene glycol dimethyl ether, DMA = *N,N*-dimethylacetamide, EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide, HOBT = 1-hydroxy-1*H*-benzotriazole, TPAP = tetra-*n*-propylammonium perruthenate, NMO = 4-methylmorpholine *N*-oxide, py = pyridine. Note: all compounds are mixtures of C₁₀ diastereomers and **4** and **22** are mixtures of several diastereomers.

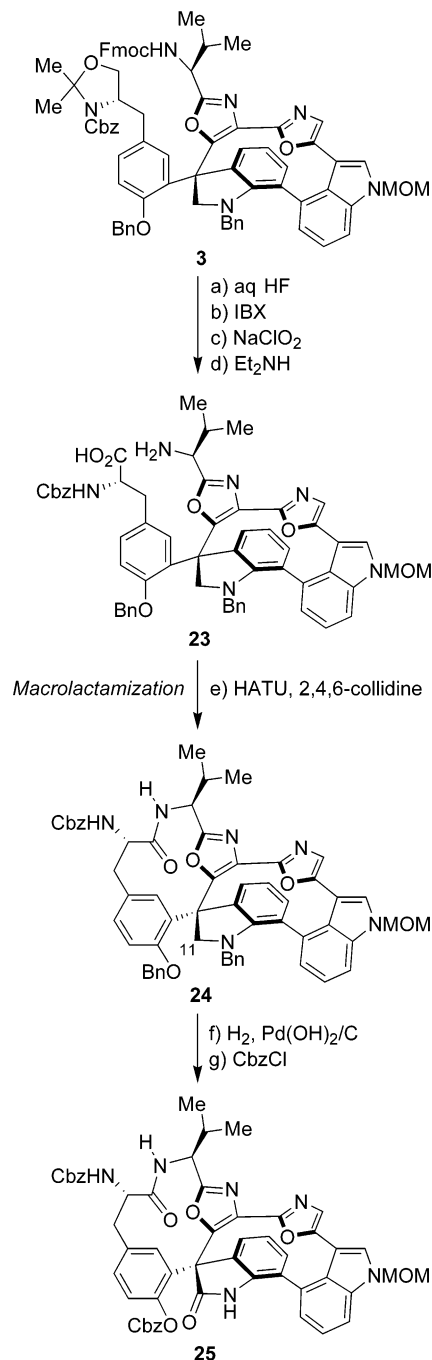
glimmer of hope from those previous investigations, we were able to form compound **24** in modest yield (entries 4 and 5).¹⁶ Following several attempts at optimization, we identified a protocol with these reagents that could reliably deliver the desired product (**24**) in 10–15% yield: adding HATU and 2,4,6-collidine in a single portion to a solution of **23** in DMF/CH₂Cl₂ (1:2, final concentration of **23** = 1.0 × 10⁻⁴ M) at 25 °C and stirring for 7 days. Importantly, apart from completing the second highly strained macrocyclic domain, this step also served to resolve the previously formed mixture of C-10 epimers, as only material with the stereochemical disposition drawn for **24**

resulted (an assignment based on its NMR homology to the natural product). Thus, just as in the first total synthesis of diazonomide A,¹ the success or failure of this macrolactamization was intricately linked to the stereochemistry of its intervening chain. Taking this fact into consideration, the yield for the macrolactamization step could really be viewed as 20–30% on the basis of the one and only C-10 diastereomer of **23** willing to cyclize.

Having constructed both macrocycles of the target molecule, we now had to confront the issue of oxidizing the E-ring indoline of **24** to an oxindole (the C-11 position) in advance of introducing the aminal ring system. Expecting that the deprotection of its E-ring benzyl ether would facilitate this task, we attempted to remove both it as well as the one attached to the G-ring phenol in **24** through a standard hydrogenation reaction facilitated by Pd(OH)₂/C (Pearlman's catalyst). Amazingly, when we used an excess amount of this catalyst and quenched the reaction product with benzyl chloroformate to capture the free C-2 amine liberated during the event, the spectral data of the resultant product indicated that not only had the debenzylations occurred as intended, but the desired indoline to oxindole conversion had also taken place! As such, the conditions

- (14) For another example of this set of conditions to remove an Fmoc group, see the total synthesis of calicheamicin γ_1 : Nicolaou, K. C.; Hummel, C. W.; Nakada, M.; Shibayama, K.; Pitsinos, E. N.; Saimoto, H.; Mizuno, Y.; Baldenius, K.-U.; Smith, A. L. *J. Am. Chem. Soc.* **1993**, *115*, 7625–7635.
- (15) Lead references for alternate peptide couplings employed include DEPBT: Li, H.; Jiang, X.; Ye, Y.-h.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91–93. FDPP: Chen, S.; Xu, J. *Tetrahedron Lett.* **1991**, *32*, 6711–6714. PyBrop: Coste, J.; Frerot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437–2446.
- (16) 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.

Scheme 6. Elaboration of Advanced Intermediate **3** to **25**, an Intermediate Bearing Both Macrocyclic Domains of Diazonamide A (**1**)^a

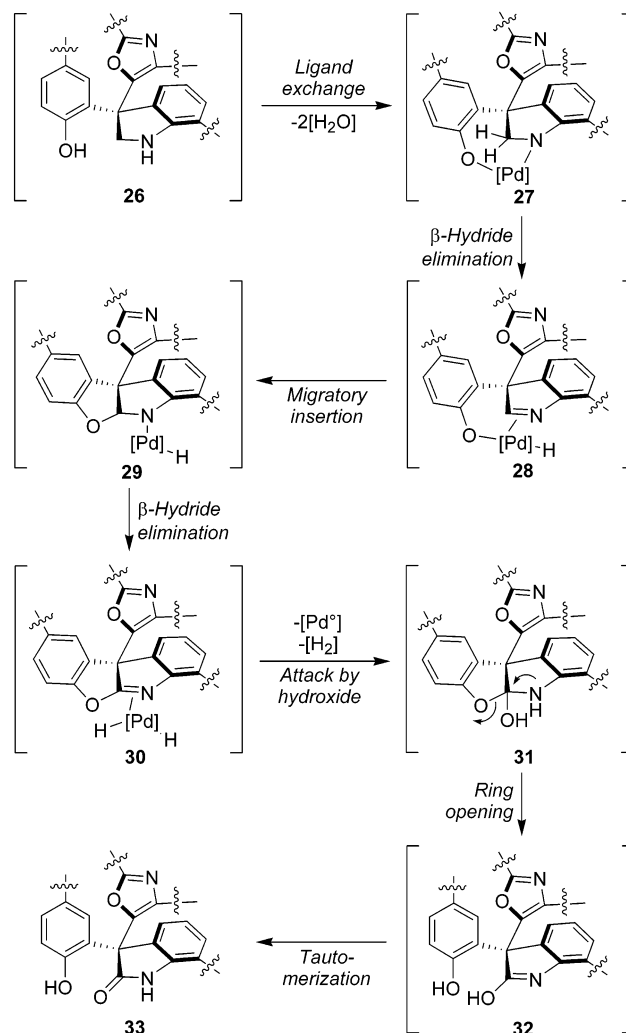


^a Reagents and conditions: (a) aq HF (48%, 3.0 equiv), MeCN, 0 °C, 2 h, 95%; (b) IBX (5.0 equiv), DMSO, 25 °C, 4 h; (c) NaClO₂ (5.0 equiv), NaH₂PO₄ (5.0 equiv), resorcinol (5.0 equiv), DMSO/H₂O (10:1), 25 °C, 1 h, 89% over two steps; (d) Et₂NH/THF (1:5), 25 °C, 4 h, 97%; (e) HATU (2.0 equiv), 2,4,6-collidine (6.0 equiv), DMF/CH₂Cl₂ (1:2, 1.0 × 10⁻⁴ M), 25 °C, 7 d, 10–15%; (f) H₂ (1.0 atm), Pd(OH)₂/C (20 wt %, excess), EtOH, 25 °C, 12 h; (g) CbzCl (5.0 equiv), aq NaHCO₃/1,4-dioxane (1:2), 25 °C, 12 h, 35% over two steps. IBX = 1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide, HATU = 2-(1H-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

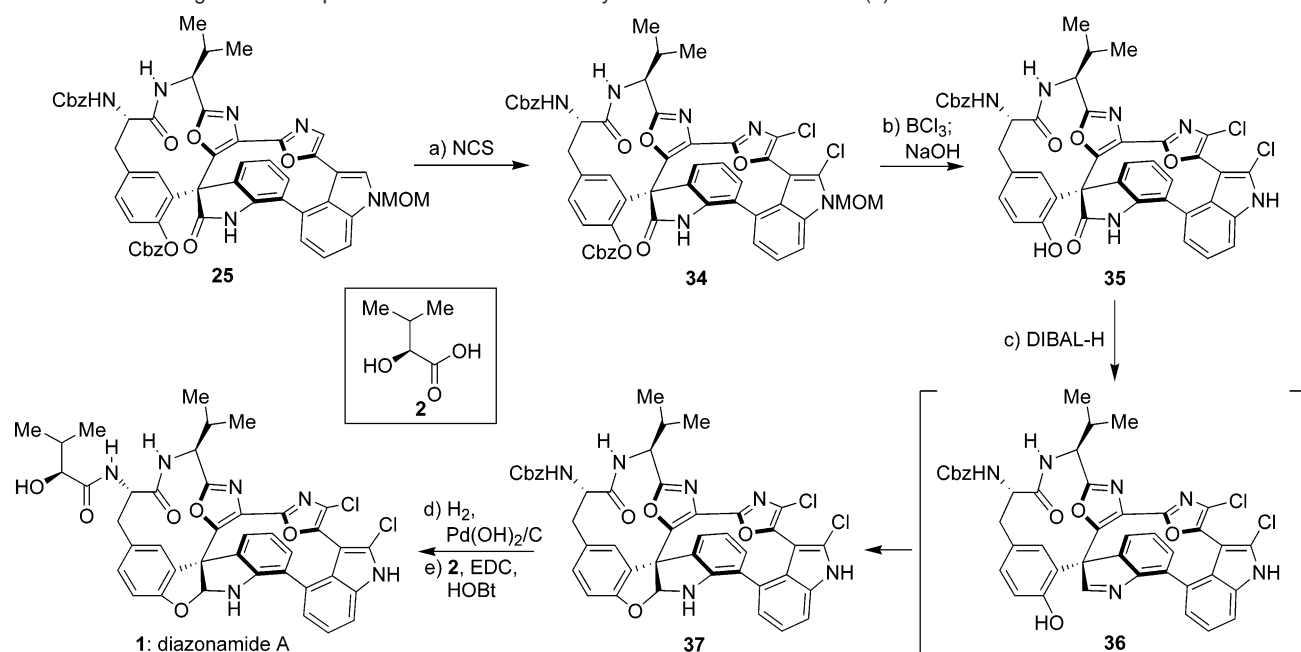
employed in this hydrogenation reaction leading to **25** had formally served to both reduce and oxidize the substrate.

A mechanistic proposal to account for this unusual transformation is shown in Scheme 7. Thus, following the removal of the two benzyl groups to afford **26**, one could envision the

Scheme 7. Proposed Mechanism to Account for Concomitant Reduction/Oxidation of Intermediate **26** to Lactam **33** in the Presence of Excess Pd(OH)₂/C



reduced Pd⁰ formed by its initial reaction with hydrogen to insert into this intermediate's phenolic and amino groups to afford a species of type **27**. While the insertion of a palladium species into an amine would not normally lead to a subsequent β-hydride elimination, in this case it might be reasonable to presume that coordination with the adjacent phenol altered its typical geometry so that this event could occur, thereby leading to **28**. Intramolecular attack of the phenol onto the C-11 position would then afford **29**, in which the palladium species is now bound exclusively to the nitrogen. A second β-hydride elimination to afford an imine whose capture by hydroxide (or water) could account for the formation of **31**, an intermediate that then could collapse to the observed product through the indicated ring-opening and tautomerization sequence. Although we have been unable to obtain direct evidence for this chain of events, this conjecture appears reasonable because of its close similarity to the mechanistic underpinnings of the Wacker oxidation.¹⁷ It also is in accordance with other experimental findings such as an inability to accomplish this reaction without an excess of Pd(OH)₂/C and the fact that it is substrate specific; for example, application of the same conditions to **7** (cf. Scheme 5) served only to excise this compound's benzyl protecting groups. The latter of these observations is reflected by the delineated

Scheme 8. Final Stages and Completion of the Second Total Synthesis of Diazonamide A (**1**)^a

^a Reagents and conditions: (a) NCS (4.0 equiv), CCl₄/THF (1:1), 60 °C, 4 h; (b) BCl₃ (1.0 M in CH₂Cl₂, 5.0 equiv), CH₂Cl₂, -78 °C, 10 min, then aq NaOH (10%, excess), THF, 25 °C, 10 min, 75% over two steps; (c) DIBAL-H (1.0 M in toluene, 10 equiv, added portionwise), THF, -78→25 °C, 3 h, 56%; (d) H₂ (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), NaHCO₃ (15 equiv), DMF, 25 °C, 12 h, 82% over two steps. NCS = *N*-chlorosuccinimide, DIBAL-H = diisobutylaluminum hydride.

mechanism in which the rigidity of the substrate is the crucial feature leading to the initial formation of **27**.

In any case, with this fortuitous transformation accomplished, the completion of diazonamide A (**1**) required only a few finishing touches. These efforts began with the installation of the two requisite chlorines onto **25** (see Scheme 8), a task accomplished through an electrophilic aromatic substitution reaction using NCS at 60 °C in a 1:1 mixture of THF and CCl₄. Just as in the first total synthesis of diazonamide A, this reaction leading to **34** proceeded with complete atropselectivity because of the constraints of the macrocyclic system. Next, the long-resilient MOM protecting group was cleaved from the C-ring indole through a one-pot, two-step protocol involving initial reaction with BCl₃ to selectively cleave its methyl group, followed by exposure of the resultant intermediate to NaOH to expel the residual hydroxymethyl chain as formaldehyde.¹⁸ These conditions also served to remove the Cbz group residing on the phenol, thereby generating **35** in 75% overall yield from **25**. Since this intermediate was the same as the one prepared during the final stages of our first total synthesis of diazonamide A, we could compare its spectral data to those obtained earlier to verify the integrity of the developed sequence. To our delight, all their data matched perfectly. As such, we could then apply the same three terminating steps used in the first total synthesis to complete the second total synthesis of diazonamide A (**1**). Overall, this total synthesis was the longer of the two (31 steps

in its longest linear sequence), but afforded a completely unique solution to its many intricate problems.

3. Chemical Biology Explorations

With a second total synthesis of diazonamide A (**1**) complete, we now sought to explore its structure from the standpoint of chemical biology. Given that the natural product possesses nanomolar cytotoxicity against several tumor cell lines with a mechanism of action involving tubulin stabilization at a binding site seemingly unique from similar agents such as vinblastine or dolastatin,^{10,19} we felt that such work was of the utmost importance. We began by assessing the activity of our synthetic diazonamide A (**1**) as well as its C-37 epimer (compound **53** in previous article) in growth inhibition assays with six human cancer cell lines of distinct origin. Four of these were relatively standard: 1A9 human ovarian carcinoma, PC-3 human prostate carcinoma, MCF-7 human breast carcinoma, and A549 human lung carcinoma cell lines. Two were drug-resistant cell lines derived from the 1A9 parental cells: A2780/AD10 cells with a multidrug resistant phenotype due to expression of high levels of P-glycoprotein and 1A9/PTX10 cells, which are Taxol-resistant because of an acquired β -tubulin mutation at this agent's binding site on tubulin.

Our findings with just these two compounds were quite interesting.²⁰ Synthetic diazonamide (**1**) possessed IC₅₀ values in the low nanomolar range (2–5 nM) against every cell line tested except for one, the A2780/AD10 line. Such selective lack of activity suggests that diazonamide A (**1**) is a good substrate

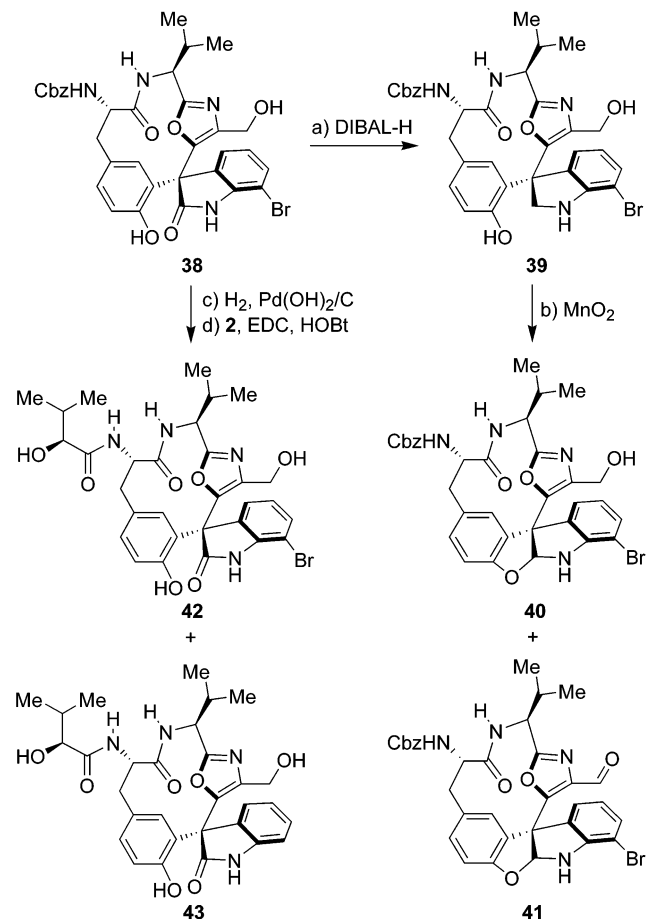
(17) For a review, see Takacs, J. M.; Jiang, X. *Curr. Org. Chem.* **2003**, *7*, 369–396.

(18) This protocol represents a new method for MOM-cleavage on indoles, particularly for acid sensitive substrates since typical deprotection procedures utilize HCl at elevated temperatures. Since previous reports have already established the acid sensitivity of the aryl chlorines on the diazonamide skeleton bearing a free indole (see Li, J.; Jeong, S.; Esser, L.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4765–4770), the ability to initially cleave the methyl ether only, followed by basic hydrolysis, is crucial for the survival of the chlorines.

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(20) Cells were plated in 96-well plates and incubated with either the test substrate or Taxol (as a positive control) for 72 h. The cells were then fixed and processed for growth inhibition using the sulforhodamine-B assay.

Scheme 9. Use of Advanced Intermediate **38** from the First Total Synthesis of Diazonamide A as a Platform to Generate Structural Analogs **39–43** for Biological Testing^a



^a Reagents and conditions: (a) DIBAL-H (1.0 M in toluene, 4.0 equiv, added portionwise), CH_2Cl_2 , 25 °C, 12 h, 73%; (b) MnO_2 (10 equiv), benzene, 80 °C, 1 h, 48% for **40**, 23% for **41**; (c) H_2 (2.0 atm), $\text{Pd}(\text{OH})_2/\text{C}$ (20 wt %, catalytic), EtOH , 25 °C, 1.5 h; (d) **2** (1.1 equiv), EDC (1.5 equiv), HOBt (1.5 equiv), NaHCO_3 (7.5 equiv), DMF, 25 °C, 12 h, 47% over two steps for **42**, 21% over two steps for **43**.

for the drug-efflux pump P-glycoprotein. Similar findings were observed with *epi*-C-37 diazonamide A, except that its activity levels were approximately 3- to 5-fold less potent (10–15 nM) in the five cell lines that **1** was active against. Perhaps the most important finding, however, was the ability of both of these compounds to combat Taxol-resistant 1A9/PTX10 cells, suggesting that the β -tubulin mutation these cells harbor has no effect on their activity. As such, this outcome lends further support for diazonamide A possessing a tubulin binding site distinct from that of other chemotherapeutic agents with a similar mechanism of action.

Spurred by these findings, as well as the preliminary structure–activity relationships that we had established during our studies toward the originally reported structure of diazonamide A,^{2b} we sought to expand the scope of our tests by screening intermediates obtained from both of our successful total syntheses of **1**. Moreover, since our developed sequences approached the framework of diazonamide A from two completely different directions, we also prepared a series of analogues of each of the macrocyclic domains of **1**. For example, as shown in Scheme 9, we were able to convert intermediate **38** into analogues **40–43** through a simple series of steps,

providing materials separately possessing the exocyclic 2-hydroxyisovaleryl residue or an aminal function in addition to the 12-membered AG macrocycle. Surprisingly, however, when we screened these and other analogues, as well as protected and fully deprotected compounds from the established sequences toward **1**, we were unable to discern any additional structure–activity features from what we had already established. The only real finding was the discovery that in the absence of the complete diazonamide architecture, no compound possessed cytotoxicity that broke the low micromolar barrier. Indeed, it was only **1** and its C-37 epimer that ever possessed levels of activity in the low nanomolar range. While a slightly disappointing outcome from the standpoint of improving diazonamide A's activity or finding a simplified analogue that could become a clinical agent, this result is in concert with studies toward other bis-oxazole containing natural products where only minor structural changes are tolerated before activity drops off precipitously.

Methodology Development

Gratifyingly, our efforts to create diazonamide A's architecture were rewarded with a wealth of new synthetic methodologies. Some that we have already described, such as a means to create 3-arylbenzofurans²¹ and the deoxygenation of sulfoxides using titanocene methylidenes,²² were the direct result of insights into chemical reactivity afforded by the unique diazonamide skeleton. Others, such as the heteropinacol macrocyclization sequence used in the successful total synthesis described in this article, were the result of challenges imposed by the difficulty in accessing its most challenging domains.²³ As a final entry into this collection of methods, we wish to expand in this section on the generality of our unique conditions to accomplish Robinson–Gabriel cyclodehydration on hindered substrates, a protocol that succeeded where all others failed on three different occasions in our diazonamide campaign, including both total syntheses.

As indicated in Table 2, several keto amides (entries 1–6) were smoothly converted into their oxazole counterparts through the action of $\text{POCl}_3/\text{pyridine}$ (1:5) at 25 °C over the course of 3 h. The success of the dehydration in entry 3 serves as a useful diagnostic measure for the power of this reaction on relatively simple substrates, since this same dehydration was accomplished in similar yield with a variety of other protocols during our efforts to prepare BCD fragments for both the original and revised structures of diazonamide A. The most important example, however, may be the one in entry 7 that comes from the elegant studies of Vedejs and Zajac²⁴ toward the synthesis of the 12-membered heterocyclic core of **1**. On a compound bearing a highly unstable aminal, an acid-labile Boc group, and a benzyl ether, $\text{POCl}_3/\text{pyridine}$ proved to be the only set of

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(23) Challenges encountered during A-ring oxazole formation also inspired a series of new synthetic methodologies related to the chemistry of the Burgess reagent. (a) Nicolaou, K. C.; Huang, X.; Snyder, S. A.; Bheema Rao, P.; Bella, M.; Reddy, M. V. *Angew. Chem., Int. Ed.* **2002**, *41*, 834–838. (b) Nicolaou, K. C.; Longbottom, D. A.; Snyder, S. A.; Nalbandian, A. Z.; Huang, X. *Angew. Chem., Int. Ed.* **2002**, *41*, 3866–3870. (c) Nicolaou, K. C.; Snyder, S. A.; Nalbandian, A. Z.; Longbottom, D. A. *J. Am. Chem. Soc.* **2004**, *126*, 6234–6235. (d) Nicolaou, K. C.; Snyder, S. A.; Longbottom, D. A.; Nalbandian, A. Z.; Huang, X. *Chem. Eur. J.* **2004**, *13*, in press.

(24) Vedejs, E.; Zajac, M. A. *Org. Lett.* **2004**, *6*, 237–240.

Table 2. Exploration of the Scope of Oxazole Formation Using the Novel Robinson–Gabriel Cyclodehydration Conditions Pioneered as Part of the Diazonamide A Synthetic Program

Entry	Starting Material	Product	Yield [%]
1			77
2			75
3			77
4			83
5			90
6			81
7			80

56: R = Boc 57: R = Boc

conditions capable of forming the A-ring oxazole of their model compound (**57**) from precursor **56**. Taking this result in concert

Table 3. Formation of Various Heterocycles with POCl₃/Pyridine

Entry	Starting Material	Product	Yield [%]
1			77
2			93
3			79
4			26
5			41

with those presented earlier during our syntheses of **1**, we believe that it is safe to state that POCl₃/pyridine is the condition of choice in accomplishing Robinson–Gabriel oxazole synthesis with highly hindered keto amides.

Equally important, these conditions are also effective at accomplishing cyclodehydrations leading to other heterocycles such as thiazoles (Table 3, entry 1), thiazolines (entries 2 and 3), and, in much less efficiency, furans (entries 4 and 5).²⁵ For some reason, however, all attempts at forming oxazolines failed to proceed with the smoothness observed in oxazole formation, leading to a multiplicity of products in all cases probed. Nevertheless, these conditions do appear to have a broad applicability in heterocycle synthesis, especially on highly elaborate substrates bearing acid-labile functionality.

Conclusion

From the time a structure for diazonamide A was disclosed in 1991,^{19a} synthetic chemists have been both captivated and experimentally frustrated by its stunning molecular architecture. The same remains true today as several groups around the world continue to employ **1** as a platform for discovery in both synthesis and chemical biology.^{24,26} What important findings these studies will reveal only time can tell, though it is certain that these efforts will not go without some reward. Indeed, our

(25) One potential reason for the decreased yield of furan products could be a high enol content within the starting materials employed.

prospecting with this target molecule for a period of five years has provided considerable wealth in the form of a series of new methodologies, reaction cascades, and synthetic strategies that stand both as a tribute to its innate complexity and a triumph of modern organic synthesis over it. The true dividend for this research program, however, resides in its demonstration that the total synthesis of complex natural products is a powerful catalyst for the invention, discovery, and development of enabling technologies for chemical synthesis, biology, and medicine.²⁷

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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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