J|A|C|S
ARTICLES
Published on Web 07/27/2004

Studies toward Diazonamide A: Development of a Hetero-Pinacol Macrocyclization Cascade for the Construction of the Bis-Macrocyclic Framework of the Originally Proposed Structure

K. C. Nicolaou,* Scott A. Snyder, Nicolas Giuseppone, Xianhai Huang, Marco Bella, Mali V. Reddy, Paraselli Bheema Rao, Alexandros E. Koumbis, Paraskevi Giannakakou,† and Aurora O'Brate†

Contribution from the Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, and Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093

Received March 29, 2004; E-mail: kcn@scripps.edu

Abstract: In this article, we describe further studies toward the originally proposed structure of diazonamide A (**1**). After confronting a number of failures in synthesizing the heterocyclic core of that structure, success was finally realized through the development of a novel hetero-pinacol-based macrocyclization cascade sequence. Subsequent elaboration led to an advanced compound bearing both of the 12-membered rings of the target molecule. In addition, preliminary biological studies with intermediates and simplified analogues obtained via the developed sequences are also described.

Introduction

In the preceding article in this issue,[1] we delineated our first-generation approach to the originally proposed structure of diazonamide A (**1**, Scheme 1), one which proved capable of delivering the entire heteroaromatic core of the molecule in model systems but untenable in its late stages when applied to fully functionalized intermediates. Fortunately, such challenges often serve to extend the boundaries of established chemistry by forcing the practitioner to develop more creative solutions to unprecedented problems. As this article will detail, one such answer did eventually become apparent in the form of a designed and efficient cascade sequence using a hetero-pinacol-based macrocyclization to forge the final C−C bond needed to close the heterocyclic domain of **1** (see Scheme 1). This new approach not only accelerated our studies to access the remainder of the originally proposed structure of diazonamide A, work which culminated in the construction of both macrocyclic units of **1**, but it also led to the invention of several new synthetic methods. Equally important, it fueled initial chemical biology studies that revealed a number of unexpected structure−activity relationships within the diazonamide class.

Results and Discussion

1. Studies toward Alternate Macrocyclization. Anytime that a strategy toward a complex molecule requires revision, one faces the difficult decision of either scrapping the original approach entirely, or retreating within the developed sequence to a point where some of the established chemistry can still be employed. In assessing which of these options to pursue, we felt that the first was far too extreme and instead anticipated that the viability of our original strategy to diazonamide A (**1**) could be resurrected by altering the manner in which we forged the C29−C30 bond to close the heteroaromatic core. Indeed, since the problem we had encountered with our first-generation approach was not the ring closure itself but obtaining subsequent functionalization, maybe that issue could be circumvented if the needed functional groups were installed directly during the cyclization step. For example, if an aldol-type ring closure of compounds of type **3** could be accomplished, with X representing a masked form of an amine such as a phthalimide, cyanide, nitro group, or azide, then hopefully it would be straightforward to form **2**. From there, the A-ring oxazole could be built either through an oxidation followed by Robinson−Gabriel cyclodehydration, or oxazoline formation followed by aromatization.

Apart from its appeal as an idea that would be easy to test (since we would only need to construct different versions of **4**), our failure to accomplish macrocyclic ring closures at other sites during our model studies enhanced our desire to devote time to its pursuit. For example, our attempts to form the 12-membered heteroaromatic ring through the C16−C18 biaryl axis both by intramolecular Ullmann-type[2] and radical-based reactions of compound **9** (see Scheme 2) met with resistance, even though the test intermediate possessed an open F-ring to allow for maximal flexibility.

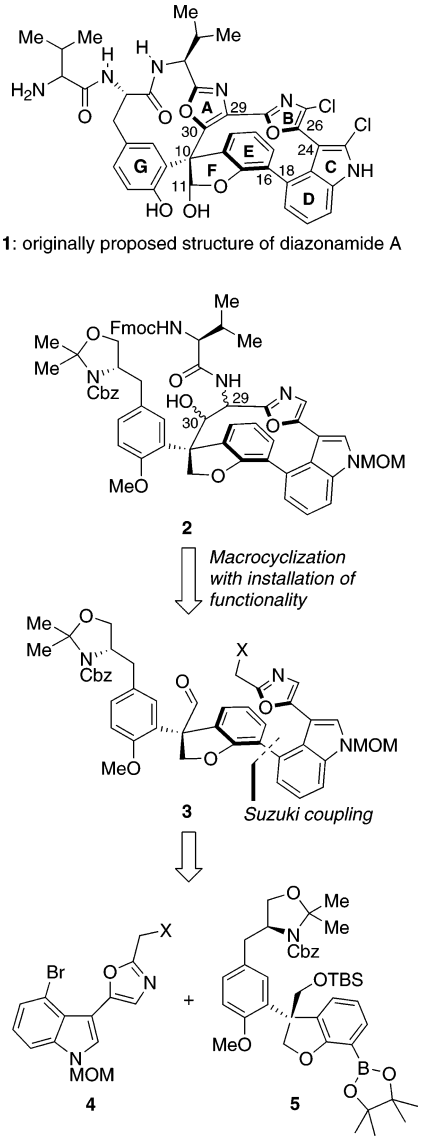
† Emory University School of Medicine, Winship Cancer Institute.

(1) Nicolaou, K. C.; Snyder, S. A.; Huang, X.; Simonsen, K. B.; Koumbis, A. E.; Bigot, A. *J. Am. Chem. Soc.* **2004**, *126*, 10162−10173.

(2) Both classical, as well as milder, variants of this reaction were attempted. For leading references, see: Hennings, D. D.; Iwama, T.; Rawal, V. H. *Org. Lett.* **1999**, *1*, 1205−1208 and references therein.

10.1021/ja040091q CCC: $27.50 **© 2004 American Chemical Society**

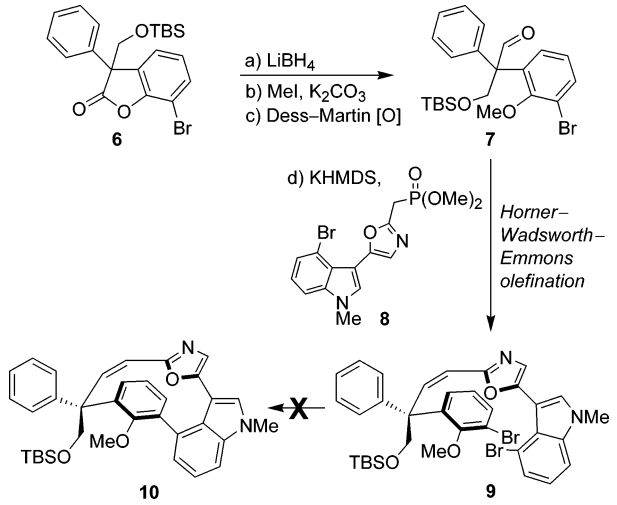
Scheme 1. Revised Retrosynthetic Analysis of the Originally Proposed Structure of Diazonamide A (**1**) Based on Alternate Methods of Ring Closure



Accordingly, our renewed efforts to accomplish C29−C30 macrocyclization via the plan delineated in Scheme 1 began by converting the alcohol within indole-oxazole **11** (Scheme 3) to a series of masked amines. Thus, treatment of **11** with phthalimide under Mitsunobu conditions[3] led to the efficient formation of **12** in 87% yield after just 3 h of reaction time at 0 °C. Similar smoothness was observed in the formation of azide **13** upon reaction of **11** with the *p*NO_2 derivative[4] of diphenylphosphoryl azide. Finally, cyanide **14** was obtained over two steps in 57% yield overall via initial halogen exchange followed by nucleophilic displacement with cyanide from KCN as promoted by 18-crown-6.

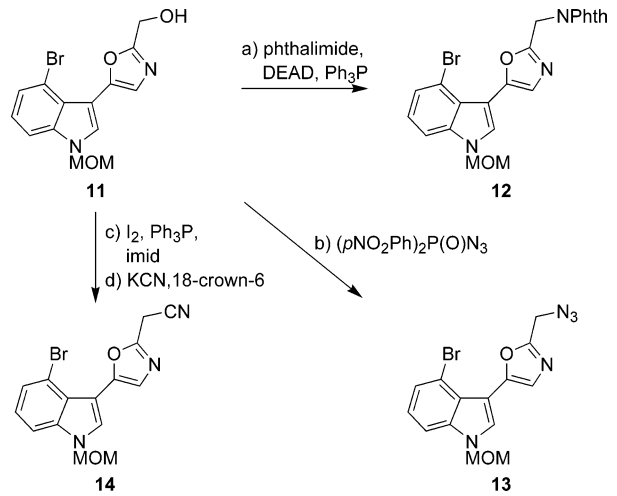
These three building blocks were then serially coupled to the previously synthesized EFG boronate fragment **5**[1] (see Scheme 4) using Suzuki coupling conditions[5] described earlier [Pd(dppf)-Cl_2·CH_2Cl_2, K_2CO_3, DME, 85 °C, 12 h]. Subsequent elaboration of these intermediates (i.e., **15**, **16**, and **17**) to the desired test substrates (i.e., **18**, **19**, and **20**) was then accomplished via HF-mediated silyl ether cleavage, reformation of the acetonide that broke apart in the previous reaction, and then oxidation (using Swern conditions to access **18** and **19** and Dess−Martin periodinane to generate **20**). Unfortunately, despite the ease by which these three substrates were prepared with the established chemistry, effecting their macrocyclization proved impossible. Exposure of each to a variety of bases (NaH, KHMDS, LiHMDS,

Scheme 2. Alternate Approaches To Close the Heterocyclic Macrocycle of Diazonamide A (**1**) Based on Different Sites of Ring Closure Fail[a]



[a] Reagents and conditions: (a) $LiBH_4$ (2.0 M in THF, 2.0 equiv), Et_2O, 0 °C, 2 h, 90%; (b) MeI (1.5 equiv), K_2CO_3 (3.0 equiv), DMF, 25 °C, 2 h, 94%; (c) Dess−Martin periodinane (2.0 equiv), $NaHCO_3$ (10 equiv), CH_2Cl_2, 0 °C, 2 h, 87%; (d) **8** (1.0 equiv), KHMDS (0.5 M in toluene, 1.0 equiv), THF, −78 °C, 5 min; **7** (1.0 equiv), −78 → 0 °C, 3 h, 54% (18% **9** + 36% *trans* isomer). TBS = *tert*-butyldimethylsilyl; DMF = *N*,*N*-dimethylformamide; KHMDS = potassium bis(trimethylsilyl)amide.

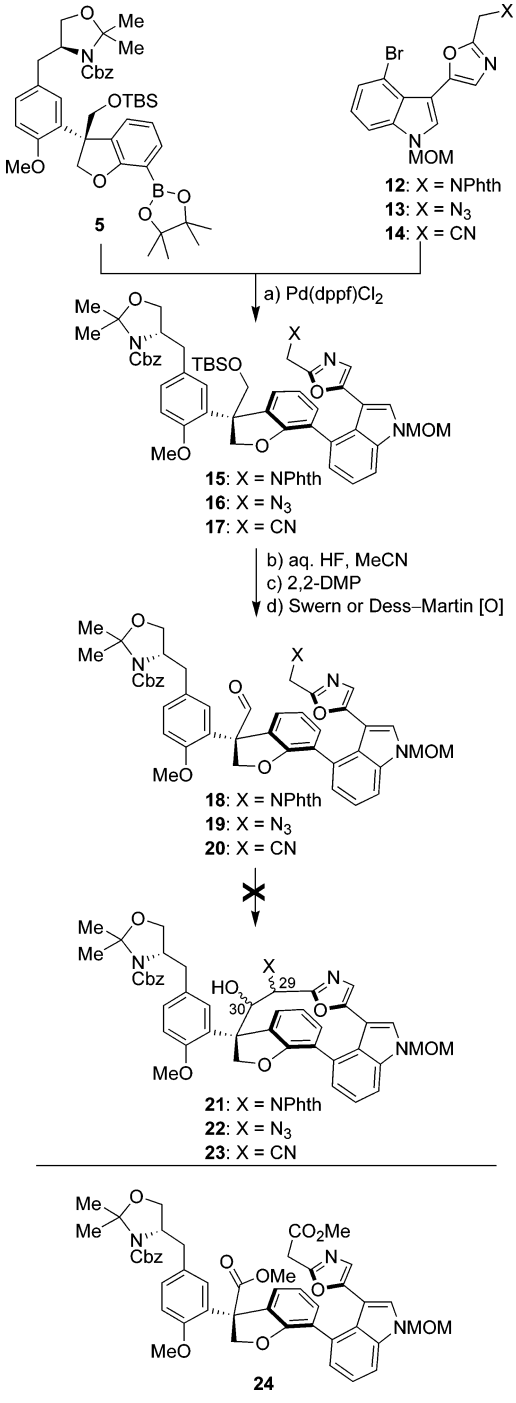
Scheme 3. Conversion of Indole **11** into Alternate Building Blocks **12**, **13**, and **14**[a]



[a] Reagents and conditions: (a) phthalimide (1.2 equiv), Ph_3P (1.2 equiv), DEAD (1.1 equiv), THF, 0 °C, 3 h, 87%; (b) ($p$$NO_2$Ph)$_2$P(O)$N_3$ (1.2 equiv), DBU (1.2 equiv), toluene, 25 °C, 2 h, 83%; (c) Ph_3P (1.7 equiv), imidazole (2.0 equiv), I_2 (1.5 equiv), CH_2Cl_2, 0 °C, 20 min, 94%; (d) KCN (2.0 equiv), 18-crown-6 (0.5 equiv), MeCN, 25 °C, 12 h, 61%. DEAD = diethyl azodicarboxylate; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; imid = imidazole.

(3) Gilbert, A. M.; Antane, M. M.; Argentieri, T. M.; Butera, J. A.; Francisco, G. D.; Freeden, C.; Gundersen, E. G.; Graceffa, R. F.; Herbst, D.; Hirth, B. H.; Lennox, J. R.; McFarlane, G.; Norton, N. W.; Quagliato, D.; Sheldon, J. H.; Warga, D.; Wojdan, A.; Woods, M. *J. Med. Chem.* **2000**, *43*, 1203−1214.
(4) Mizuno, M.; Shiori, T. *Chem. Commun.* **1997**, 2165−2166.
(5) Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508−7510.

Scheme 4. Attempts to Accomplish Anion-Based Macrocyclizations[a]



[a] Reagents and conditions: (a) **5** (1.1 equiv), **12**, **13**, or **14** (1.0 equiv), $Pd(dppf)Cl_2$ (0.2 equiv), K_2CO_3 (5.0 equiv), DME, 85 °C, 12 h; (b) aq HF (48%, excess), MeCN, 0 °C, 45 min; (c) 2,2-DMP, acetone, 25 °C, 5 min; (d) DMSO (10 equiv), $(COCl)_2$ (5.0 equiv), CH_2Cl_2, −78 °C, 45 min; Et_3N (20 equiv), CH_2Cl_2, −78 °C, 15 min or Dess−Martin periodinane (3.0 equiv), $NaHCO_3$ (10 equiv), CH_2Cl_2, 25 °C, 1 h, 51% overall for **18**, 10% overall for **19**, 40% overall for **20**. dppf = diphenylphosphinoferrocene; DME = ethylene glycol dimethyl ether; 2,2-DMP = 2,2-dimethoxypropane.

LDA, or KO*t*-Bu) universally failed to deliver anything resembling the desired product. Instead, we typically observed decomposition and, in some cases, recovered starting material.

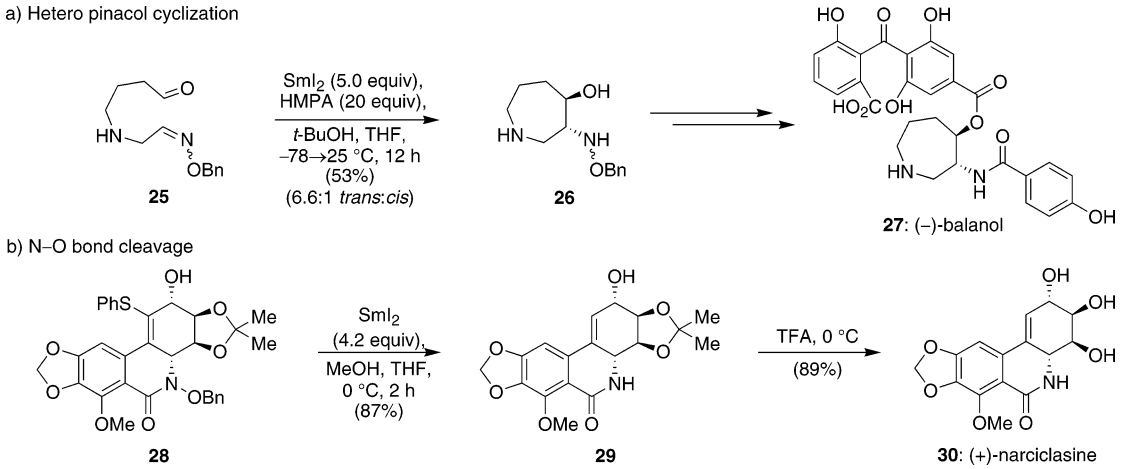
Recognizing that the failures in these couplings could reflect the high proclivity for retro-aldol reactions, since nothing existed to prevent such an outcome in the same way that the loss of a phosphonate irreversibly drove our previously successful Horner−Wadsworth−Emmons (HWE) macrocyclizations,[1,6] we then turned to the synthesis of compound **24**. Our expectation was that Dieckmann condensation would overcome this problem,[7] especially since the Vedejs group had reported[8] success in a related approach in their model studies toward **1**. Unfortunately, this advanced intermediate similarly proved recalcitrant to macrocyclization under any conditions probed (including NaH, LiHDMS, KO*t*-Bu, and NaOMe).

2. Revised Retrosynthetic Analysis and Execution of the New Strategy. Although this litany of failures was certainly frustrating, it pushed us to think even more deeply about solving the problem of C29−C30 functionalization. Mindful of our previous use of the McMurry reaction (a pinacol coupling) to form a highly strained eight-membered ring in our total synthesis of Taxol,[9] we hypothesized that perhaps we could enlist its hetero variant to fashion a fully functionalized C29−C30 bond for diazonamide A from a precursor aldehyde-oxime. This idea seemed encouraging since intramolecular hetero-pinacol couplings[10] have been used on numerous occasions to fashion a diverse range of rings ever since the late 1970s when the Corey, Hart, and Bartlett groups independently demonstrated[11] that oximes could serve as competent radical acceptors. For example, as shown in Part A of Scheme 5, the Naito group recently employed a hetero-pinacol cyclization initiated by SmI_2[12] to efficiently convert **25** into a seven-membered ring (**26**) appropriately functionalized to complete a total synthesis of balanol (**27**).[13]

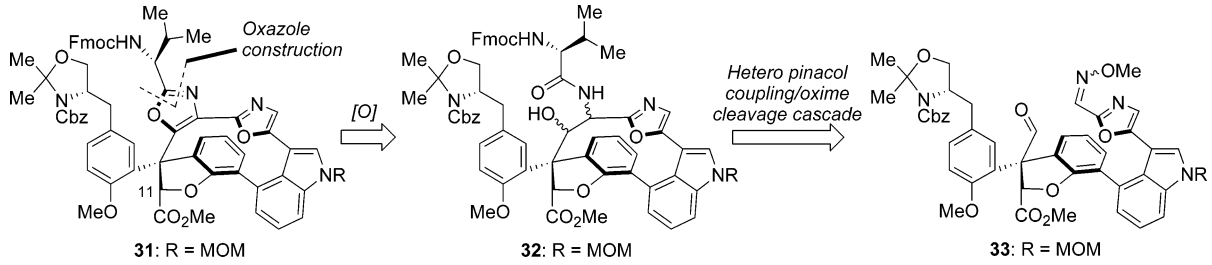
Despite this wealth of precedent, however, up to the end of the year 2000, no variant of the hetero-pinacol reaction had been successfully applied in a macrocyclization event to generate a ring size greater than seven, despite precedent for medium-size ring formation in related systems employing dialdehydes. In fact, a number of studies seeking to form such rings met only with failure.[10a] Nevertheless, we thought that the diazonamide

(6) Nicolaou, K. C.; Snyder, S. A.; Simonsen, K. B.; Koumbis, A. E. *Angew. Chem., Int. Ed.* **2000**, *39*, 3473−3478.
(7) Numerous applications of this strategy in the synthesis of complex molecules have appeared. For a representative survey, see: Davis, B. R.; Garratt, P. J. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 2, pp 806−829.
(8) Vedejs, E.; Zajac, M. A. *Org. Lett.* **2001**, *3*, 2451−2454. For the successful application of the same approach to the revised structure of diazonamide A, see: Vedejs, E.; Zajac, M. A. *Org. Lett.* **2004**, *6*, 237−240.
(9) Nicolaou, K. C.; Yang, Z.; Liu, J. J.; Ueno, H.; Nantermet, P. G.; Guy, R. K.; Claiborne, C. F.; Renaud, J.; Couladouros, E. A.; Paulvannan, K.; Sorensen, E. J. *Nature* **1994**, *367*, 630−633.
(10) (a) Ritte, D.; Hazell, R.; Skrydstrup, T. *J. Org. Chem.* **2000**, *65*, 5382−5390. (b) Keck, G. E.; Wager, T. T.; Rodriquez, J. F. D. *J. Am. Chem. Soc.* **1999**, *121*, 5176−5190. (c) Keck, G. E.; McHardy, S. F.; Murry, J. A. *J. Org. Chem.* **1999**, *64*, 4465−4476. (d) Tormo, J.; Hays, D. S.; Fu, G. C. *J. Org. Chem.* **1998**, *63*, 201−202. (e) Keck, G. E.; Wager, T. T. *J. Org. Chem.* **1996**, *61*, 8366−8367. (f) Kiguchi, T.; Tajiri, K.; Ninomiya, I.; Naito, T.; Hiramatsu, H. *Tetrahedron Lett.* **1995**, *36*, 253−256. (g) Camps, P.; Font-Bardia, M.; Munoz-Torrero, D.; Solans, X. *Liebigs Ann.* **1995**, 523−535. (h) Shono, T.; Kise, N.; Fujimoto, T.; Yamanami, A.; Nomura, R. *J. Org. Chem.* **1994**, *59*, 1730−1740. (i) Naito, T.; Tajiri, K.; Harimoto, T.; Ninomiya, I.; Kiguchi, T. *Tetrahedron Lett.* **1994**, *35*, 2205−2206. (j) Marco-Contelles, J.; Martinez, L.; Martinez-Grau, A.; Pozuelo, C.; Jimeno, M. L. *Tetrahedron Lett.* **1991**, *32*, 6437−6440.
(11) (a) Corey, E. J.; Pyne, S. G. *Tetrahedron Lett.* **1983**, *24*, 2821−2824. (b) Hart, D. J.; Seely, F. L. *J. Am. Chem. Soc.* **1988**, *110*, 1631−1633. (c) Bartlett, P. A.; McLaren, K. L.; Ting, P. C. *J. Am. Chem. Soc.* **1988**, *110*, 1633−1634.
(12) (a) Namy, J. L.; Girard, P.; Kagan, H. B. *Nouv. J. Chim.* **1977**, *1*, 5−7. (b) Girard, P.; Namy, J. L.; Kagan, H. B. *J. Am. Chem. Soc.* **1980**, *102*, 2693−2698. For recent review articles on the application of SmI_2 in organic synthesis, see: (c) Kagan, H. B. *Tetrahedron* **2003**, *59*, 10351−10372. (d) Krief, A.; Laval, A.-M. *Chem. Rev.* **1999**, *99*, 745−778. (e) Molander, G. A.; Harris, C. R. *Tetrahedron* **1998**, *54*, 3321−3354. (f) Skrydstrup, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 345−347. (g) Molander, G. A.; Harris, C. R. *Chem. Rev.* **1996**, *96*, 307−338.
(13) Miyabe, H.; Torieda, M.; Inoue, K.; Tajiri, K.; Kiguchi, T.; Naito, T. *J. Org. Chem.* **1998**, *63*, 4397−4407.

Scheme 5. Selected Uses of SmI_2 in Organic Synthesis as Pertinent Precedents to the Diazonamide Problem



Scheme 6. Revised Retrosynthetic Analysis of the Originally Proposed Structure of Diazonamide A (**1**) Based on a Heteropinacol Coupling Sequence



framework might provide a particularly unique case of a 12-membered ring, as $\pi-\pi$ stacking between the B- and E-rings could bring the aldehyde and oxime motifs quite close and significantly reduce their rotational freedom. Thus, the reaction might have at least some chance for success, although conventional wisdom seemed to point to a challenging proposition.

Since we would ultimately need an amide product, and not an N−O bond as that observed in the formation of compound **26** (see Scheme 5), following cyclization, we would have to break that linkage apart. As shown in Part B of Scheme 5, we were pleased to discover recent reports from the Keck group detailing that SmI_2 is particularly effective in this task.[14] Thus, it appeared to us that it should be possible to accomplish both hetero-pinacol coupling and N−O cleavage in the same pot. Although a seemingly safe assumption, an extensive search of the literature revealed its success on only one occasion through a protocol which involved treating a substrate with 6.0 equiv of SmI_2 and then stirring for a prolonged period in deoxygenated H_2O.[15,16] Our analysis of this unique result suggested that its success reflected the amount of SmI_2 employed, since if 4−5 equiv of SmI_2 were required to accomplish just one of the reactions in Scheme 5, then at least 8−10 equiv would probably be required to effect both. Accordingly, our new plan for the synthesis of diazonamide A was to treat an intermediate such as **33** (see Scheme 6) with a gross excess of SmI_2, hoping that both cyclization and N−O cleavage could be accomplished concomitantly to afford a 1,2-amino alcohol product. Rather than isolate that likely polar intermediate, we would then try and couple that product directly with a protected form of L-valine to obtain **32**. Thus, if this sequence could be realized, not only would it constitute the first example of a hetero-pinacol reaction leading to a medium- or large-sized ring, but it also would productively combine this transformation into a new reaction cascade that could potentially have wider applications.

Our explorations of this strategy began with the synthesis of a new EFG building block, as shown in Scheme 7, altered only from the fragment employed earlier (i.e., **5**) by the use of a methyl ester as the methylene activating group in the 5-*exo*-tet cyclization leading to **36**. Although the yield for this key conversion was lower (38%) than that achieved previously[1] with cyanide due to a number of side-reactions initiated by the ester functionality, the presence of that motif provided a reactive handle easily convertible to a C-11 lactol and incapable of benzofuran fragmentation. As an added benefit, its incorporation also shortened the overall sequence from L-tyrosine methyl ester to the final boronate ester (**37**) by three steps.

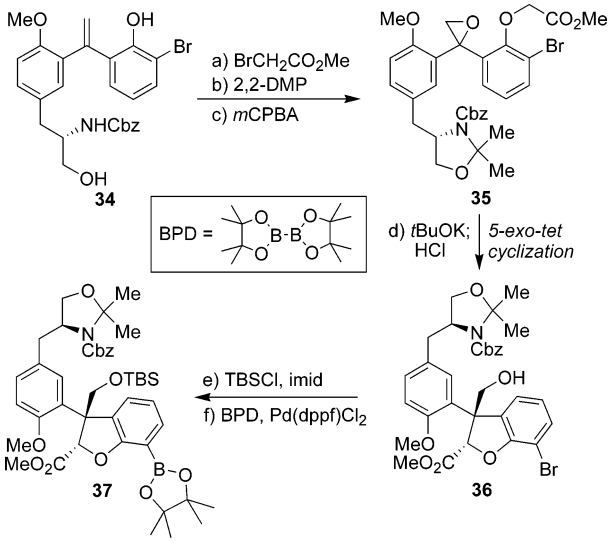
Once this fragment was completed, it was coupled with the previously obtained indole-oxazole **38**[1] through the standard Miyaura conditions[5] for Suzuki coupling of boronate esters, leading to the assembly of **39** (see Scheme 8) in 83% yield. This new intermediate was then cleanly converted into aldehyde-oxime **33** through a tandem deprotection/oxidation sequence, followed by selective oxime capture of the more-activated

(14) (a) Keck, G. E.; Wager, T. T.; McHardy, S. F. *Tetrahedron* **1999**, *55*, 11755−11772. (b) Chiara, J. L.; Destabel, C.; Gallego, P.; Marco-Contelles, J. *J. Org. Chem.* **1996**, *61*, 359−360. (c) Keck, G. E.; McHardy, S. F.; Wager, T. T. *Tetrahedron Lett.* **1995**, *36*, 7419−7422.

(15) (a) Marco-Contelles, J.; Gallego, P.; Rodríguez-Fernández, M.; Khiar, N.; Destabel, C.; Bernabé, M.; Martínez-Grau, A.; Chiara, J. L. *J. Org. Chem.* **1997**, *62*, 7397−7412. For a preliminary disclosure of the same reductive coupling/N−O cleavage and an example with altered protecting groups, see (b) Chiara, J. L.; Marco-Contelles, J.; Khiar, N.; Gallego, P.; Destabel, C.; Bernabé, M. *J. Org. Chem.* **1995**, *60*, 6010−6011. (c) Bobo, S.; Storch de Gracia, I.; Chiara, J. L. *Synlett* **1999**, 1551−1554.

(16) The role of H_2O is either as a proton source, or, more likely, as a donor ligand which increases the reducing power of SmI_2. For leading references, see: (a) Hanessian, S.; Girard, C. *Synlett* **1994**, 861−862. (b) Hasegawa, E.; Curran, D. P. *J. Org. Chem.* **1993**, *58*, 5008−5010.

Scheme 7. Synthesis of Advanced EFG Fragment **37**[a]



[a] Reagents and conditions: (a) $BrCH_2CO_2Me$ (1.5 equiv), K_2CO_3 (1.5 equiv), acetone, 56 °C, 3 h, 95%; (b) 2,2-dimethoxypropane (10 equiv), *p*TsOH (0.1 equiv), acetone, 25 °C, 30 min, 87%; (c) *m*CPBA (77%, 2.0 equiv), $NaHCO_3$ (3.0 equiv), CH_2Cl_2, 0 °C, 8 h, 84%; (d) *t*BuOK (1.0 M in THF, 1.5 equiv), THF/DMF (1:1), −78 °C, 5 min, then 1 M aq HCl, −78 °C, 5 min, 38%; (e) TBSCl (3.0 equiv), imidazole (6.0 equiv), DMF, 25 °C, 6 h, 88%; (f) bis(pinacolato)diboron (1.1 equiv), $Pd(dppf)Cl_2$ (0.2 equiv), KOAc (3.0 equiv), DMSO, 85 °C, 6 h, 81%. *p*TsOH = *p*-toluenesulfonic acid; *m*CPBA = *m*-chloroperoxybenzoic acid; BPD = bis(pinacolato)diboron.

aldehyde after just 10 min of reaction with $MeONH_2 \cdot HCl$ in DMSO at 25 °C. Use of neat DMSO as solvent was critical for the success of this final operation leading to **33**, as the same reaction in any alcoholic media was attended by a significant degree of acetonide cleavage (likely due to trace HCl derived from the oxime source). In any case, with an efficient synthesis of **33** achieved in 70% yield from **39**, explorations into the key reaction of the proposed sequence could begin. Most gratifyingly, little reaction scouting was required. Following treatment of aldehyde-oxime **33** with a premixed complex of 9 equiv of freshly prepared SmI_2 and 36 equiv of HMPA in THF at 25 °C for 1 h, followed by an aqueous NH_4Cl reaction quench, extraction, solvent removal, and subsequent peptide coupling using a DMF solution of Fmoc-protected L-valine (**44**), EDC, and HOBt, we obtained compound **32** as a mixture of stereoisomers in an isolated yield of 45%.

Mechanistically, we believe that the initial exposure of **33** to SmI_2/HMPA led to the generation of diradical intermediate **40**, which then cyclized to provide **41**. The presence of excess SmI_2 complexed with HMPA then effected N−O cleavage, leading to intermediate **42**, which, upon workup, provided the desired amino alcohol that was later trapped through peptide formation. Consequently, each step in the sequence proceeded in an average yield of 75%. Although this picture differs from the more typical representation[17] of this reaction where a ketyl radical attacks an intact oxime to account for the generation of **41**, the isolation of noncyclized material with both the aldehyde and oxime reduced suggests that the existence of diradical **40** cannot be excluded. We also believe that our alternative takes into account the unique framework of diazonamide A (**1**), as our experience with compounds such as **33** suggests that the steric hindrance around that aldehyde would make it quite difficult to form a ketyl radical without touching the highly accessible oxime. As an alternative to either of these pictures, one could also invoke a samarium-bridged diradical such as **45** to account for the eventual formation of the bridged bond in **41**.

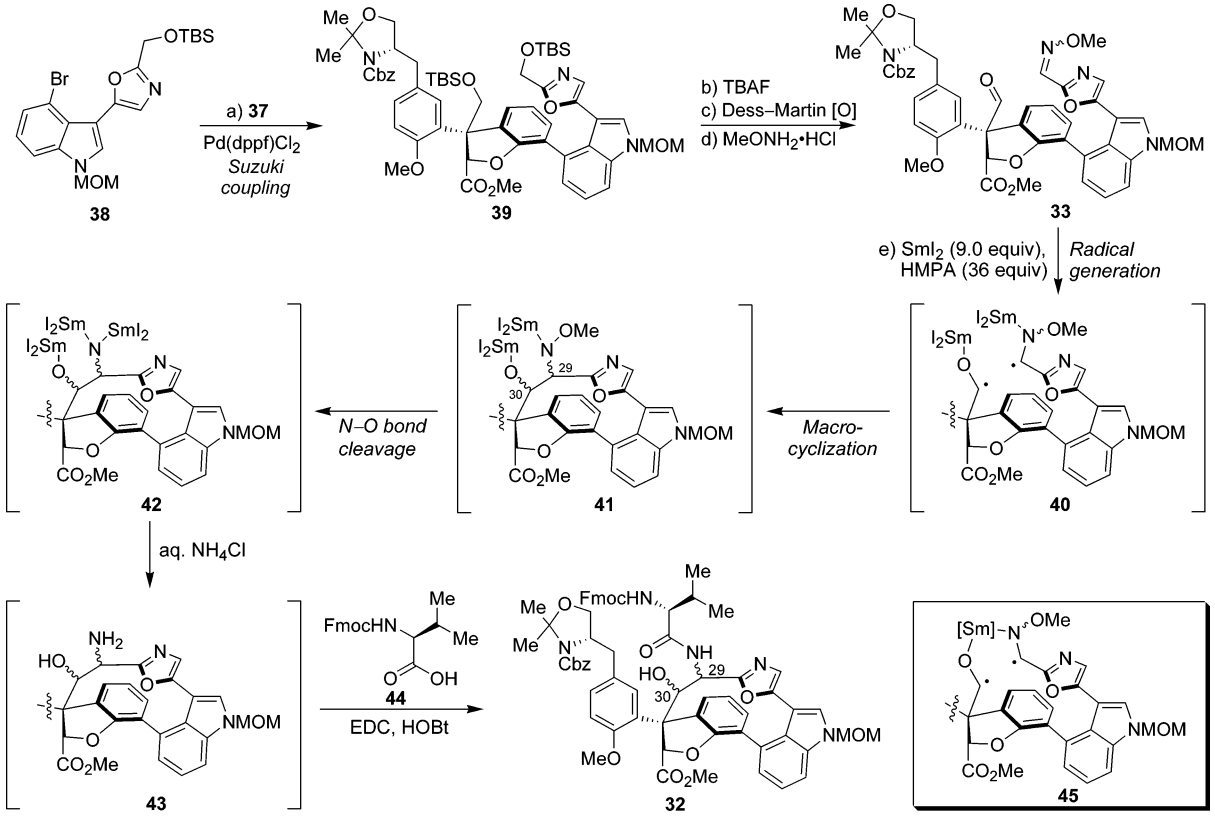
Apart from these mechanistic considerations, it is worth noting that, in accordance with earlier reports exploring the hetero-pinacol coupling of aldehyde-oximes,[10] the macrocyclization of **33** did not proceed in the absence of HMPA. Moreover, if the ratio of HMPA/SmI_2 was reduced from 4:1 to 2:1 (still using 9 equiv of SmI_2), **32** was observed, along with significant amounts of cyclized product, with the N−O linkage firmly intact, indicating that the presence of a suitable donor ligand in conjunction with excess SmI_2 is the combination required for reliable oxime cleavage following reductive cyclization. These results suggest that in cases where one would desire to effect only N−O cleavage, the addition of HMPA might greatly facilitate the transformation in circumstances that prove difficult or low-yielding with SmI_2 alone.

Having finally established a functionalized C29−C30 bridge after numerous failures, we could now investigate means by which to form the A-ring oxazole and complete the entire heteroaromatic core of **1**. As mentioned earlier, two direct pathways were available to accomplish this goal from advanced intermediate **32**: oxidation followed by Robinson−Gabriel dehydration, or oxazoline formation and subsequent aromatization. While both might appear equally feasible on paper, our model studies suggested that the first was unlikely to succeed with **32** since only *p*TsOH in refluxing toluene proved effective in initiating the needed cyclodehydration on a substrate that lacked its numerous acid-labile functionalities.[18] Accordingly, we elected to probe oxazoline formation and found that the desired ring system (i.e., **46**, Scheme 9) could indeed be formed in 24% yield over the course of 12 h using (diethylamino)sulfur trifluoride (DAST) in THF at −10 °C.[19] The remaining material balance from this event was the enamide that resulted from simple dehydration of the C-30 alcohol. Although the yield for this operation was low, that outcome partially reflects the fact that only one of the two possible oxazoline products (which we have tentatively assigned as drawn in Scheme 9) was formed in the event.[20] Consequently, only the portion of starting material that possessed the proper C-29 and C-30 stereochemistry required to form this product could have participated productively in the cyclization. In truth, the efficiency of this reaction (or lack thereof) was ultimately of no consequence since we could never accomplish the subsequent oxidation leading to **31**, despite nearly a dozen attempts.[21]

With this approach reaching a roadblock, we turned to the second alternative for oxazole formation, and in line with our previous expectations, Robinson−Gabriel cyclodehydration proved impossible to achieve following the formation of **47** either with Dess−Martin periodinane on small scale or TPAP/

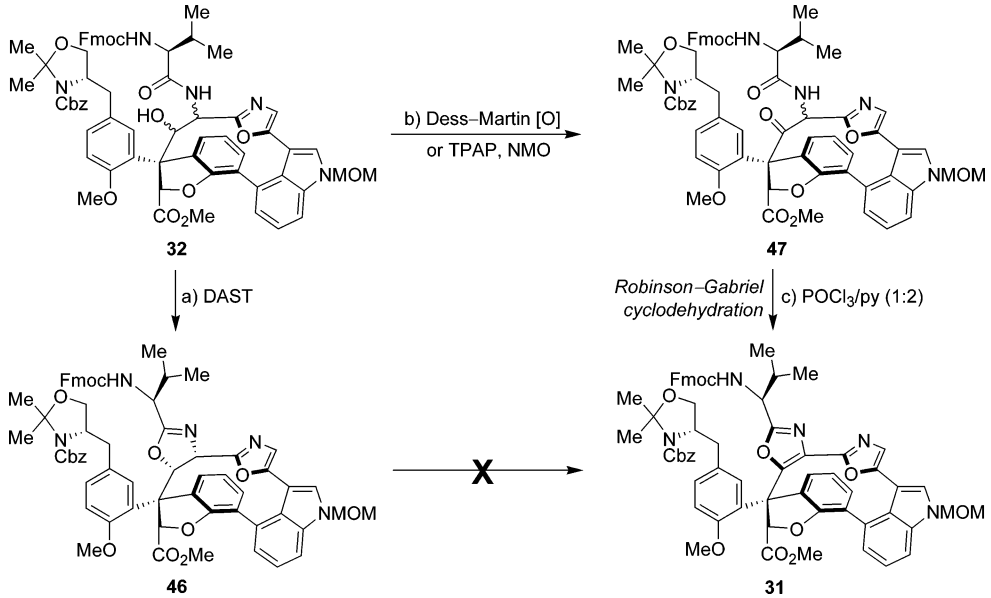
(17) For example, see: Robertson, G. M. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 3, pp 563−611.
(18) Similar findings were presented in another diazonamide study: Wipf, P.; Methot, J.-L. *Org. Lett.* **2001**, *3*, 1261−1264.
(19) Lafargue, P.; Guenot, P.; Lellouche, J.-P. *Heterocycles* **1995**, *41*, 947−958.
(20) This assignment is based, in part, on the fact that the alternate oxazoline would suffer from severe steric interactions with the protons from the C-11 position in ring F. Such strain also renders the approach of DAST or any other reagent from that side of the molecule quite unlikely.
(21) Efforts to accomplish this conversion included several known oxidants for the process, including: MnO_2 in refluxing benzene or CH_2Cl_2, DDQ in benzene at 25 °C, Pd/C in benzene at 25 °C, $BrCCl_3$ and DBU in MeCN at 25 °C, and NCS in CCl_4 at 25 °C and reflux.

Scheme 8. Successful Generation of the Fully Functionalized Heterocyclic Core (**33**) of the Originally Proposed Structure of Diazonamide A (1) Using Suzuki and Hetero-Pinacol Couplings[a]



[a] Reagents and conditions: (a) **37** (1.1 equiv), **38** (1.0 equiv), $Pd(dppf)Cl_2$ (0.2 equiv), K_2CO_3 (5.0 equiv), DME, 85 °C, 12 h, 83%; (b) TBAF (4.0 equiv), THF, 25 °C, 20 min, 93%; (c) Dess−Martin periodinane (3.0 equiv), $NaHCO_3$ (10 equiv), CH_2Cl_2, 25 °C, 1 h, 87%; (d) $MeONH_2$·HCl (5.0 equiv), DMSO, 25 °C, 10 min, 87%; (e) SmI_2 (0.1 M in THF, 9.0 equiv), HMPA (36 equiv), THF, 25 °C, 1 h; then saturated aq $NaHCO_3$, 25 °C, 1 h; solvent removal; then **44** (3.0 equiv), EDC (3.0 equiv), HOBt (3.0 equiv), DMF, 25 °C, 10 h, 40−45% overall, ca. 75% per synthetic operation in the designed reaction sequence. TBAF = tetra-*n*-butylammonium fluoride; HMPA = hexamethylphosphoramide; EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide; HOBt = 1-hydroxy-1*H*-benzotriazole.

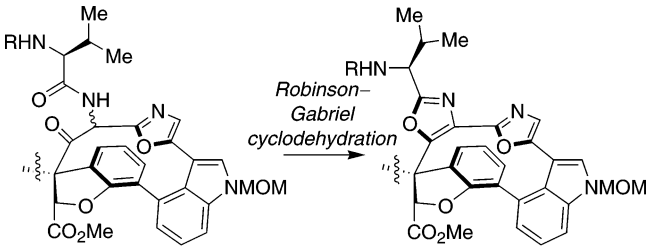
Scheme 9. Formation of the A-ring Oxazole Subunit of the Proposed Structure of Diazonamide A (**1**) from Advanced Intermediate **32**[a]



[a] Reagents and conditions: (a) DAST (5.0 equiv), THF, −10 °C, 12 h, 24%; (b) Dess−Martin periodinane (3.0 equiv), $NaHCO_3$ (10 equiv), CH_2Cl_2, 25 °C, 1 h, 83% or TPAP (1.0 equiv), NMO (5.0 equiv), CH_2Cl_2, 25 °C, 2 h, 68%; (c) $POCl_3$/pyridine (1:2), 70 °C, 6 h, 45%, 68% based on recovered starting material. DAST = (diethylamino)sulfur trifluoride; TPAP = tetra-*n*-propylammonium perruthenate; NMO = 4-methylmorpholine *N*-oxide; py = pyridine.

NMO (TPAP = tetra-*n*-propylammonium perruthenate, NMO = 4-methylmorpholine *N*-oxide) on larger scale. As shown in Table 1, no published oxazole formation protocol[22] proved successful in converting **47** into **31**, with some providing

Table 1. Screening of Conditions To Accomplish the Formation of the A-ring Oxazole and Complete the Heterocyclic Core of Diazonamide A



47: R = Fmoc
48: R = Boc

31: R = Fmoc
49: R = Boc

entry	R	conditions	yield
1	Fmoc	Burgess reagent, THF, Δ, 10 h	no reaction
2	Fmoc	Ph_3P, I_2, Et_3N, CH_2Cl_2, 25 °C, 10 h	no reaction
3	Fmoc	Ph_3P, Cl_3CCCl_3, Et_3N, 25 °C, 10 h	no reaction
4	Fmoc	TFA[a], TFAA[b], 60 °C, 2 h	decomposition
5	Fmoc	$POCl_3$, 70 °C, 2 h	decomposition
6	Fmoc	P_2O_5, $CHCl_3$, 25 °C, 5 h	decomposition
7	Fmoc	$POCl_3$/pyridine (1:2), 70 °C, 6 h	45% **31** (33% rsm)
8	Boc	$POCl_3$/pyridine (1:2), 70 °C, 6 h	34% **49** (20% rsm)

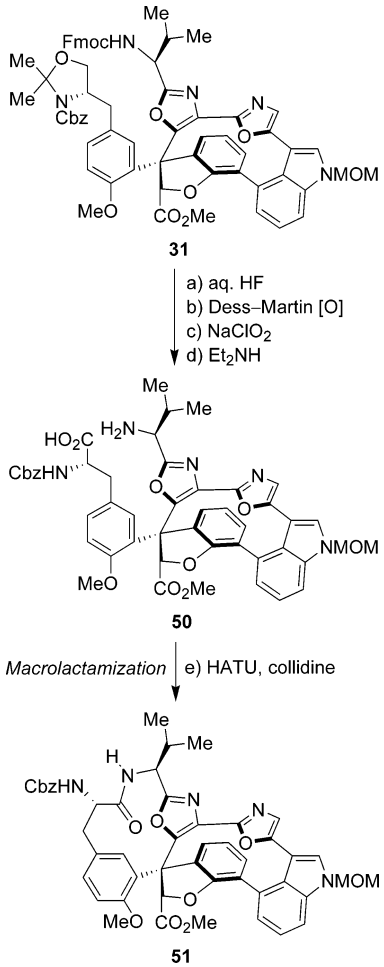
[a] TFA = trifluoroacetic acid. [b] TFAA = trifluoroacetic anahydride.

recovered starting material (entries 1−3) and others leading to complete decomposition (entries 4−6). Use of the same protocols with microwave activation[23] instead of direct heat or efforts to minimize the steric bulk of the valine residue through alternate nitrogen protection (such as Boc or Alloc instead of Fmoc, as well as the use of N_3 for the entire amine) provided no improvement either. Even probing the formation of a far simpler oxazole by using an acetate in lieu of the L-valine attached to the amine in **47** failed to afford any desired product. Our approach was seemingly at another dead end.

After examining the collected results more carefully, we wondered if a new opportunity for success might arise simply by modifying an existing procedure to accomplish Robinson−Gabriel cyclodehydration. More specifically, although $POCl_3$ has proven to be quite effective at oxazole synthesis in a number of contexts,[22a] perhaps when we attempted that reaction with **47**, trace amounts of HCl from the reagent led its functionality to break apart before oxazole formation could occur. Thus, if we buffered that dehydrating reagent with a base such as pyridine, then maybe this problem could be circumvented. As an added benefit, it seemed reasonable to expect that rendering the reaction media basic could also accelerate the overall rate of the reaction by promoting the amide enolization required to displace the $POCl_3$-activated alcohol. When this protocol was put to the test by adding a 2:1 mixture of pyridine/$POCl_3$ to ketone **47** and heating the resultant solution at 70 °C for 6 h, we were pleased to discover that oxazole **31** could be obtained in 45% yield with virtually all of the remaining material balance (33%) constituting recovered starting material (Table 1, entry 7). Nearly equal levels of success were observed with related substrates bearing alternate L-valine protection (entry 8).[24]

Having finally overcome this major synthetic hurdle, we felt that the targeted structure (**1**) would soon be within reach

Scheme 10. Completion of the Second Macrocyclic Subunit of the Originally Proposed Structure of Diazonamide A (**1**)[a]



[a] Reagents and conditions: (a) aq HF (48%, excess), MeCN, 0 °C, 45 min, 96%; (b) Dess−Martin periodinane (3.0 equiv), $NaHCO_3$ (10 equiv), CH_2Cl_2, 25 °C, 1 h; (c) $NaClO_2$ (5.0 equiv), NaH_2PO_4 (5.0 equiv), resorcinol (5.0 equiv), DMSO/H_2O (10:1), 25 °C, 3 h, 65% over two steps; (d) Et_2NH (excess), THF, 25 °C, 4 h, 97%; (e) HATU (2.0 equiv), collidine (6.0 equiv), DMF/CH_2Cl_2 (9:1, 1.0×10^{-4} M), 25 °C, 12 h, 5−10%. HATU = 2-(1*H*-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

following formation of the second macrocyclic subunit through lactamization, a transformation for which we anticipated few difficulties on the basis of the wealth of literature precedent to effect such ring closures. Indeed, our initial forays along these lines were encouraging, as **31** was converted into **50** (see Scheme 10) without incident in 61% overall yield through HF-mediated cleavage of the acetonide, generation of a free acid from the resultant alcohol through a two-stage oxidation protocol, and finally, Et_2NH-induced lysis[25] of the Fmoc group guarding the amine appended to the A-ring oxazole. Unfortunately, as revealed in Table 2, initial explorations to effect the formation of **51** from this advanced amino acid (**50**) met with significant resistance (entries 1−4),[26] leading in all cases either to decomposition or dimerization (even when the reaction was

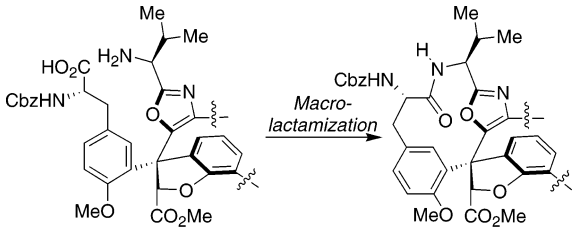
(22) Examples of protocols employed include: (a) Dow, R. L. *J. Org. Chem.* **1990**, *55*, 386−388. (b) Wasserman, H. H.; Vinick, F. J. *J. Org. Chem.* **1973**, *38*, 2407−2408. (c) Wipf, P.; Miller, C. P. *J. Org. Chem.* **1993**, *58*, 3604−3606. (d) Parsons, R. L.; Heathcock, C. H. *J. Org. Chem.* **1994**, *59*, 4733−4734. (e) Fukuyama, T.; Xu, L. *J. Am. Chem. Soc.* **1993**, *115*, 8449−8450.

(23) Brain, C. T.; Paul, J. M. *Synlett* **1999**, 1642−1644.

(24) The scope of this new protocol is explored more fully in the final article in this series and has proven applicable to the formation of diverse oxazoles from ketoamides, thiazolines from thioamide-alcohols, thiazoles from ketothioamides, and furans from 1,4-dicarbonyls.

(25) The same operation could also be accomplished in slightly lower yield using TBAF. For an application of this alternative protocol in total synthesis, see: Jiang, W.; Wanner, J.; Lee, R. J.; Bounard, P.-Y.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 5288−5290.

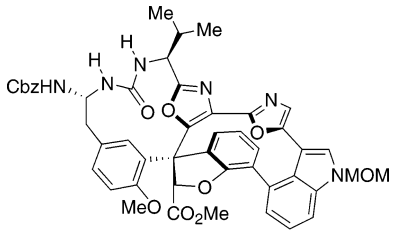
Table 2. Screening of Conditions To Accomplish Peptide Formation and Thereby Complete Both Macrocycles of Diazonamide A



50 **51**

entry	conditions	yield (%)
1	PyBroP, $NaHCO_3$, DMF/CH_2Cl_2 (1:1)[a]	dimer/trimer
2	FDPP, $NaHCO_3$, DMF/CH_2Cl_2 (1:1)[a]	decomposition
3	EDC, HOBt, DMF/CH_2Cl_2 (1:1)[a]	dimer/trimer
4	DEPBT, *i*-Pr_2NEt, DMF/CH_2Cl_2 (1:1)[a]	decomposition
5	DPPA, $NaHCO_3$, DMF/CH_2Cl_2 (1:1)[a]	~60 of **52**
6	HATU, collidine, DMF/CH_2Cl_2 (1:1)[a]	trace
7	HATU, collidine, DMF/CH_2Cl_2 (9:1)[b]	5−10[c]

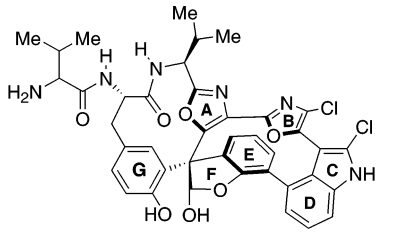
[a] Concentration of **50** = 5.0×10^{-4} M. [b] Concentration of **50** = 1.0×10^{-4} M. [c] Range indicates maximum and minimum values obtained for several runs. PyBroP = bromotripyrrolidinophosphonium hexafluorophosphate; FDPP = pentafluorophenyl diphenylphosphinate; DEPBT = 3-diethyloxyphophoryloxy)-1,2,3-benzotriazin-4(3*H*)-one; DPPA = diphenylphosphoryl azide.



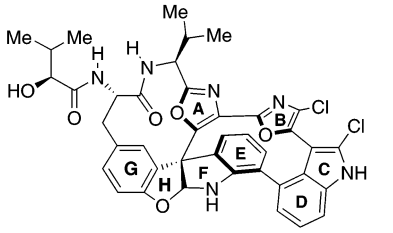
52

run at exceedingly low concentration). Although molecular models suggested that the reactive units should be quite close, the steric hindrance within the system and the entropic penalties associated with the formation of the highly strained 12-membered ring must have been the culprits that led to such difficulties. Indeed, the only smooth reaction which we ever observed in our first attempts occurred when **50** was exposed to diphenylphosphoryl azide (DPPA)[27] in $NaHCO_3$-buffered CH_2Cl_2, conditions which led to the formation of a product which we have assigned as the more flexible 13-membered cyclic urea **52** (the product of a room-temperature Curtius rearrangement).[28]

Thankfully, after more experimentation, we were able to form the desired macrolactam (**51**) in yields of 5−10% if **50** was added dropwise over several hours to a mixture of 2-(1*H*-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and 2,4,6-collidine dissolved in DMF/CH_2Cl_2 (9:1) at a final concentration of 1.0×10^{-4} M.[29] The remainder of the material from this experiment was dimeric in nature, and could not be separated from the desired product



1: originally proposed structure of diazonamide A



53: revised structure of diazonamide A

Figure 1. Structural reassignment of diazonamide A (**1**) to **53** based on the work of Harran and co-workers.[30]

(**51**) via column chromatography. Despite the disappointing outcome for this key reaction in terms of yield, the fact that it could be achieved marked a milestone for our overall plan to synthesize **1**, especially since we felt that its outcome could be improved through optimization. Sadly, though, we would never get that opportunity since it was at this juncture that the original structure of diazonamide A (**1**) was revised to **53** (see Figure 1) by the Harran group,[30] leading us to abandon any further synthetic efforts toward the "oxygen analogue" of the real diazonamide A.

3. Analogue Synthesis and Chemical Biology Explorations. While we would not complete the synthesis of the originally proposed structure of diazonamide A (**1**), the developed sequences did allow us to make a few preliminary forays into the chemical biology of the diazonamide class. Such explorations were certainly of value, even though a key part of our intermediates did not exactly match the structure now proposed for the natural product, since the Harran team had disclosed that advanced synthetic materials bearing the central oxygen atom of **1** could induce the same phenotype as natural diazonamide A in several tumor cell lines.[30b] Accordingly, we not only screened every synthetic compound that we had in hand as part of our program to synthesize **1**, but we also constructed and tested a series of simplified analogues. The preparation of these substances is shown in Schemes 11 and 12.[31]

As revealed in Figure 2, with selected data for a few of the compounds examined, these studies unveiled a number of interesting structure−activity relationships. For instance, although analogues **60**, **62**, **63**, and **64** are all several-hundred-

(26) 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.

(27) Qian, L.; Sun, Z.; Deffo, T.; Mertes, K. B. *Tetrahedron Lett.* **1990**, *31*, 6469−6472.

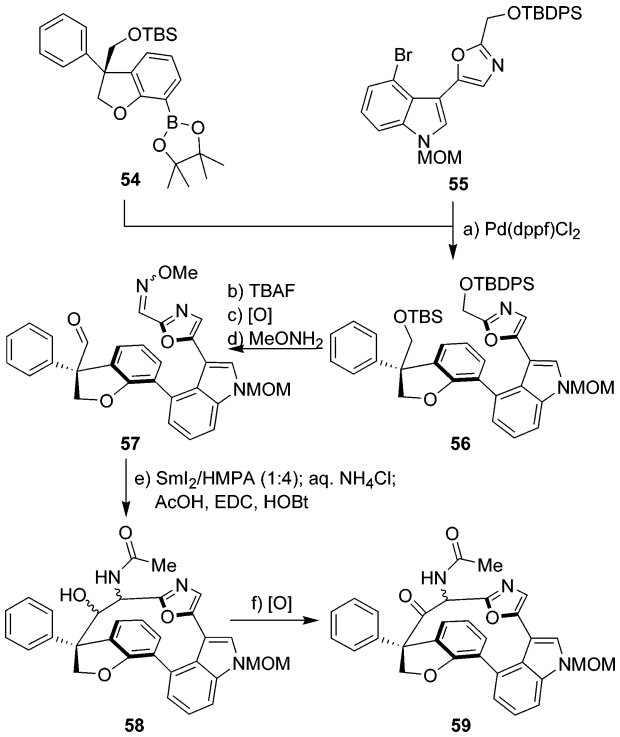
(28) To achieve such a smooth rearrangement at a low temperature typically requires the presence of a protic or Lewis acid; in this case, the particular spatial arrangement of the reactive groups in **17** must facilitate this conversion as no such activators were needed.

(29) For other 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.

(30) (a) Li, J.; Jeong, S.; Esser, L.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4765−4769. (b) Li, J.; Burgett, A. W. G.; Esser, L.; Amezcua, C.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4770−4773.

(31) For a detailed discussion of this sequence, see: 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. For the sake of comparison, however, it is interesting to note here the decreased yield observed for the key SmI_2/HMPA-based hetero-pinacol sequence in this model system versus the fully functionalized intermediates presented in Scheme 8.

Scheme 11. Execution of the Developed Pinacol Coupling Cascade Sequence to Efficiently Prepare Ketoamide **59**[a]



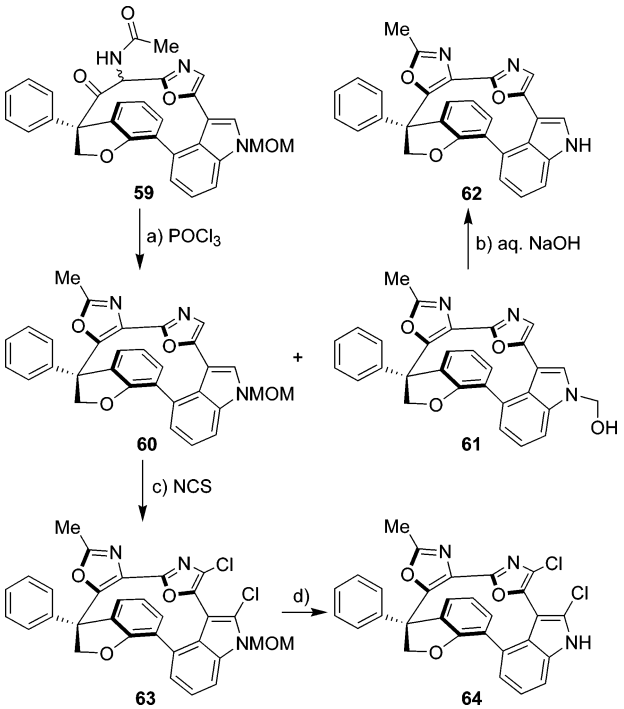
[a] Reagents and conditions: (a) **54** (1.0 equiv), **55** (1.0 equiv), $Pd(dppf)Cl_2$ (0.2 equiv), K_2CO_3 (5.0 equiv), DME, 110 °C, 8 h, 66%; (b) TBAF (3.0 equiv), THF, 25 °C, 10 min, 93%; (c) Dess−Martin periodinane (3.0 equiv), $NaHCO_3$ (10.0 equiv), CH_2Cl_2, 25 °C, 1 h, 88%; (d) $MeONH_2 \cdot HCl$ (10.0 equiv), DMSO, 25 °C, 10 min, 91%; (e) SmI_2 (0.1 M in THF, 9.0 equiv), HMPA (36 equiv), THF, 25 °C, 1 h; then saturated aq NH_4Cl, 25 °C,1 h; solvent removal; then AcOH (3.0 equiv), EDC (3.0 equiv), HOBt (3.0 equiv), DMF, 25 °C, 1 h, 25% overall, 71% per synthetic operation in the cascade sequence; (f) Dess−Martin periodinane (3.0 equiv), $NaHCO_3$ (10.0 equiv), CH_2Cl_2, 25 °C, 1.5 h, 94%.

fold less cytotoxic than diazonamide A (**53**) against the 1A9 human ovarian carcinoma cell line, these four simple structural congeners indicate the importance of the two aryl chlorine residues (**60** vs **63** and **62** vs **64**) in conferring biological efficacy and that substitution of the indole nucleus (**63** vs **64**) can potentially lead to increased potency. Apart from these preliminary findings, however, we were unable to identify any intermediate or analogue possessing potency remotely commensurate to diazonamide A, even among compounds bearing both of the macrocyclic subunits of **1**.

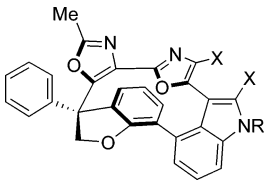
Conclusion

Despite the interruption of the sequence developed to access the originally proposed structure for diazonamide A (**1**), the campaign waged to reach advanced intermediate **51** was far from fruitless. Indeed, the adopted strategy inspired a novel extension of the hetero-pinacol reaction as part of a unique cascade sequence to construct complex macrocyclic systems. It also led to the discovery of a series of valuable synthetic methodologies, the most important in this article being the identification of a powerful method to accomplish Robinson−Gabriel cyclodehydration in hindered settings using $POCl_3$/pyridine. In addition, biological screening of analogues and intermediates synthesized as part of this program revealed several significant findings, particularly in terms of structure−activity relationships for the diazonamide class. Most important, as the remaining two articles

Scheme 12. Generation of a Series of Advanced Model System Analogs Using the Developed Chemistry[a]



[a] Reagents and conditions: (a) $POCl_3$, 70 °C, 2 h, **60**/**61** (2.4:1), 65%; (b) aq NaOH (15%, excess), THF, 25 °C, 10 min, 74%; (c) NCS (3.0 equiv), THF/CCl_4 (1:1), 55 °C, 8 h, 73%; (d) BBr_3 (1.0 M in CH_2Cl_2, 2.0 equiv), CH_2Cl_2, −78 °C, 20 min; then aq NaOH (15%, excess), THF, 25 °C, 10 min, 61%. NCS = *N*-chlorosuccinimide.



	X	R	IC_{50} (μM)
60	H	MOM	>30
62	H	H	>30
63	Cl	MOM	3.6
64	Cl	H	9.4

Figure 2. Preliminary biological screening of simple heterocyclic core analogues (**60**, **62**, **63**, and **64**) against 1A9 human ovarian carcinoma cells.

in this series will detail,[32] the developed chemistry proved directly translatable into a successful total synthesis of the revised structure of diazonamide A (**53**).

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 (USA), predoctoral fellowships from the National Science Foundation, Pfizer, and Bristol-Myers Squibb (all to S.A.S.), postdoctoral fellowships from the Association pour la Recherche sur le Cancer (N.G.) and The Skaggs Institute for Chemical Biology (X.H.), and a grant from American Biosciences.

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

JA040091Q

(32) (a) Nicolaou, K. C.; Chen, D. Y.-K.; Huang, X.; Ling, T.; Bella, M.; Snyder, S. A. *J. Am. Chem. Soc.* **2004**, *126*, in press. (b) 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*, in press.