Electrolytic Macrocyclizations: Scalable Synthesis of a Diazonamide-Based Drug Development Candidate**

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Abstract: An electrochemical method to synthesize the core macrolactam of diazonamides is described. Large ring-forming dehydrogenation is initiated by anodic oxidation at a graphite surface. The reaction requires no tailoring of the substrate and occurs at ambient temperature in aqueous DMF in an undivided cell open to air. This unique chemistry has enabled a concise, scalable preparation of DZ-2384; a refined analog of diazonamide A slated for clinical development as a cancer therapeutic.

t is not uncommon to observe internal cross-links between electron-rich aromatic side chains in peptide-derived natural products. These bonds rigidify structure and often improve pharmacological properties. Products of net dehydrogenation are frequently encountered, wherein C–C bonding occurs at the expense of two aryl C–H bonds. Examples include oxygenated biphenyls^[1] derived from tyrosine oxidations and bis-indoles resultant from tryptophan oxidations.^[2] Some time ago we began studying a structure that contains a linkage hybrid of the above two; namely diazonamide A (1, Figure 1),^[3] wherein a tyrosine residue has internally bonded to an adjacent tryptophan. The 3-linked *o*-phenolic 3H-indole in that case exists as its aminal tautomer; giving a core motif that was unknown at the time it was first described in diazonamides.^[4]

Structure 1 drew intense interest from the synthetic community and methods were forged to prepare the natural

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Figure 1. (-)-diazonamide A (1).

product.^[5-7] Assembling the core of the molecule was a particular challenge.^[7,8] Among solutions to the problem, ours was an attempt to parallel biosynthesis. We found that PhI(OAc)₂ would mediate the conversion of peptidyl derivative **2** (Figure 2, R = o-NO₂PhSO₂) to isomeric aminals **3a** and **4a**.^[5c] The directness of the construction was valuable, particularly as our interest in the pharmacology of diazonamides increased.^[9] Numerous congeners of the natural product were prepared and those molecules fueled early in vivo experiments.^[10] As rodent models made clear that



Figure 2. Two mechanistically distinct syntheses of diazonamide core structures.

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diazonamides may have utility as cancer therapy, additional analogs with refined properties were sought, and material consumption grew. In this regard, the original preparation of **3a** became a bottleneck. Alongside **3a** and **4a**, $PhI(OAc)_2$ oxidation of **2** invariably gave comparable amounts of spirocyclohexadienones **6** (Figure 3 A).^[5c] These conventional Kita oxidation^[11] products limited the yield of **3a** and complicated its purification.



Figure 3. Working mechanistic hypothesis.

An alternate method to prepare 3 was sought, wherein oxidation of 2 could initiate at the indole rather than the phenol. Presumably this would circumvent by-product formations inherent to the Kita protocol. To date, only one technique has achieved outcomes consistent with selective internal oxidation. When substrate 2 $(R = CO_2Bn)$ was electrolyzed at a controlled potential of +1.6 V, anodic oxidation occurred to give **3b** and **4b** in a 3:1 ratio.^[12] Relative to the PhI(OAc)₂ procedure, the process was a major practical advance. The reaction was run in wet DMF at 25°C in an undivided cell open to air. Substrate was stirred from the center of a circular array of graphite rods. These served alternately as cathode and anode. In line with the working mechanistic hypothesis shown in Figure 3B,^[13] products 3/4b were obtained free of spirocyclohexadienone contaminants. This simplified isolations of **3b** and accelerated the medicinal chemistry program. Hundreds of diazonamide analogs were subsequently prepared-varying both in core structure and peripheral substitutions. These efforts led to the identification of DZ-2384 (5, Figure 4) as a simplified structure that fully retained diazonamide anti-mitotic characteristics.^[14] More-



Figure 4. DZ-2384 (5).

over, due to improved pharmacokinetics, compound **5** was 10to 50-fold more efficacious than **1** as a cancer therapeutic in vivo (rodents).

DZ-2384 was selected as a development candidate in 2012.^[15] Attention turned to establishing a practical synthesis of the molecule. The medicinal chemistry route was an adaptation of our original synthesis of **1**. Because **5** lacked the right hand macrocycle (as drawn in Figure 1) of the natural product, the main issue to be addressed was building the core triarylacetaldehyde in proper diarylaminal form. Magnus,^[6b] Macmillan,^[5a] and Sammakia^[6a] had each described diastereoselective chemistry for this purpose. However, when evaluated in context of overarching goals for the project, the electrochemical method above seemed especially promising. It operated on substrates derived from common amino acids and formed the target motif at the requisite oxidation state directly from a linear precursor.

The electrochemical step had been positioned in the middle of the synthetic sequence. To further differentiate oxidation potential of the indolic chromophore, we sought to shift this reaction to a later stage. Here we describe a refined



Scheme 1. Assembling an oxidized tripeptide. Reagents and conditions: a) 5-F-indole (2 equiv), Ac_2O (2.2 equiv), AcOH, 60-65 °C. b) L-Ser-OMe, EDC, HOBt, *i*Pr₂NEt, DMF, RT. c) DDQ (2 equiv), THF, 85 °C, 1.5 h. 73% from **9**. EDC = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, HOBt = 1-hydroxybenzotriazole, DMF = dimethylformamide, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, THF = tetrahydrofuran.

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Scheme 2. One-pot cyclodehydration/oxidation sequence. Reagents and conditions: a) Deoxo-Fluor (1.1 equiv), CH_2Cl_2 (0.37 M), -20°C, 10 min. b) add BrCCl₃, (2.5 equiv), DBU (3.2 equiv) and additives (see Table 1) at RT, 3–4 h. Deoxo-Fluor = bis(2-methoxyethyl)aminosulfur trifluoride, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

Table 1: Effects of protocol and additives on the synthesis of 12 from 10 (Scheme 2).

Entry	Additive	Galvinoxyl (mol%)	Yield of 12 [%]
1	none	0	31 ^[a]
2	none	0	38
3	none	5	50
4	MgO ^[b]	5	38
5	Na ₂ CO ₃ ^[b]	5	64

[a] Stepwise yield (90 g scale). [b] 2.2 equiv (2.5 equiv DBU used).

route to DZ-2384 wherein electrolytic macrocyclization can, in fact, be the final step in the process.

Our intent was to assemble DZ-2384 outwardly from a central dipeptide, namely tert-Leu-5-F-Trp-OH (9, Scheme 1). Peptide 9 can be prepared in three steps from 5fluorotryptophan.^[12] However, a four-step route proved more cost effective. In that case, L-*tert*-leucine was acylated with CICO₂Bn and the product was coupled to L-serine methyl ester. After saponification with aq. LiOH, 7 was condensed with 5-fluoroindole in the presence of Ac₂O to afford dipeptide 9. The overall yield of 9 from L-*tert*-leucine was 62% on kilogram scales. Compound 9 was isolated as an inconsequential mixture of diastereomers; consistent with in situ ¹H NMR analyses by Yokoyama et al.^[16] These authors identified methylidene oxazolones (i.e. 8) as intermediates in closely related reactions.

Coupling of 9 to L-serine methyl ester provided a tripeptide that was then incrementally oxidized, first with DDQ to afford a single isomer of 3-(5-oxazolyl)indole 10.^[17] Following dessication of crude 10 with Deoxo-Fluor, the resultant oxazoline (11) was further oxidized using a combination of BrCCl₃ and DBU.^[18] This process proved interesting. The dehydration step was uneventful. Oxazoline 11 could be cleanly isolated. However, subsequent treatment with BrCCl₃/DBU gave bis-oxazole 12 in poor yield (25–35 % from 10, see Scheme 2 and Table 1, entry 1).

In an attempt to minimize handling, we implemented Wipf and Williams' streamlined protocol.^[19] Unmodified, this one-pot procedure did little to improve yield (Table 1, entry 2). Full conversion at a useful rate in the oxidation step required 3 equiv of DBU. Significant by-products accompanied formation of **12** under those conditions. One of these was a compound 30 Da greater in mass than **12** that degraded to **12** upon attempted isolation. It was as if **12** had reacted with a formaldehyde synthon in situ, leading to a hydroxymethylated adduct upon workup that was decom-

posing via retro-addition. It was unclear how this might occur, although there was precedent for DBU reducing CCl_{4} via single-electron transfers.^[20] Along those lines, mixing equimolar amounts of BrCCl₃ and DBU produced brominated bicycle 13 and chloroform (1:1, 52% conversion after 1 h at RT, see Scheme 3 A). We discovered that Galvin Coppinger's radical^[21] inhibited this reaction. In turn, 5 mol% of Galvinoxyl added together with BrCCl₃ and DBU to crude solutions of 11 improved the synthesis of 12 from 10 by eliminating the + 30 Da contaminant.^[22] A second by-product accompanying 12 was a composite of 11 and DBU. Its structure was assigned as spiroaminals 14 following X-ray crystallographic analysis of congener 15 (see Scheme 3B and the Supporting Information). Mechanistic details notwithstanding,^[23] 14 was thought to derive from DBU being oxidized with BrCCl₃, not unlike the +30 Da contaminant discussed above. Galvinoxyl suppressed formation of 14 and this benefit increased when a portion of the DBU in the oxidation mixture was replaced with Na₂CO₃. Combined, these two modifications more than doubled the yield of 12 (Table 1, entry 5). For the synthesis of



Scheme 3. Insights into the one-pot cyclodehydration/oxidation sequence depicted in Scheme 2. Thermal ellipsoids drawn at 50% probability.

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Scheme 4. Two concise syntheses of DZ-2384 employing electrolytic macrocyclization. Reagents and Conditions: a) Z-Tyr-OH, EDC, HOBt, *i*Pr₂NEt, DMF, 0°C to RT, 3 h. b) LiBH₄ (5 equiv), TFE (5 equiv), THF, RT; for **17**: t=12 h, 85% from **16**; for **19**: t=3 h, 63% from **16**. c) **18**: +1.6 V, Et₄NBF₄, (NH₄)₂CO₃, 1.8% aq. DMF, 5 d (35%, 43% based on recovered **17**, d.r. = 2.7:1; see the Supporting Information); **5**: +1.6 V, Et₄NBF₄, (NH₄)₂CO₃, 2% aq. DMF, 56 h (35%, 48% based on recovered **19**, d.r. = 2.3:1; see the Supporting Information). d) H₂ (1 atm), Pd/C (10 mol%), tBuNH₂ (1.5 equiv), MeOH, 4 h. e) (S)-(-)-hydroxyisovaleric acid, EDC, HOBT, *i*Pr₂NEt, DMF, 4 h, 91% from 18. f) Boc-Tyr-OH, EDC, HOBt, *N*-methylmorpholine, DMF, 14 h. g) 4 M HCl in dioxane, 20 min. h) (S)-(-)-hydroxyisovaleric acid, EDC, HOBt, N-methylmorpholine, DMF, 20 h. TFE = 2,2,2-trifluoroethanol. Thermal ellipsoids drawn at 50% probability.

DZ-2384, it was best to subject crude **10** directly to the optimized, one-pot procedure. Following work-up, crude **12** (ca. 60% purity) was treated with HBr in AcOH to afford amine salt **16** (Scheme 4) in 52% overall yield (95% purity, > 98% ee) following precipitation with *i*Pr₂O and trituration with cold EtOH.

With **16** in hand, two pathways to DZ-2384 were developed. In the first, **16** was coupled to Z-Tyr-OH and the product reduced with LiBH₄ to afford **17**. The reduction step was complicated by limited solubility of the substrate in ether solvents. It was found that adding trifluoroethanol (1 equiv relative to LiBH₄) formed a strongly reducing system^[24] as well as a homogenous solution. This allowed **17** to be isolated in 85 % yield (two steps from **16**) on 70 gram scale.

Compound **17** was an electrolysis substrate. Cyclic voltammetry indicated the molecule oxidized irreversibly at +1.15 V (graphite anode vs. Ag/AgCl, DMF/H₂O/Et₄NBF₄), while oxidation of Z-Tyr-OMe under identical conditions required a higher potential (+1.39 V). These data suggested the mechanism invoked to explain anodic oxidation of **2** (Figure 3B) would remain plausible for **17**. On preparative scale, **17** was most efficiently converted to **18** by electrolysis at a potential of +1.6 V while adding solid (NH₄)₂CO₃ portionwise.^[25] Oxidizing 60 grams of **17** in this manner gave 21.0 g of **18** (d.r. 2.7:1) and 11.0 g of unreacted **17** after chromatography on silica gel. Product yield peaked at about 80% conversion. While HPLC analyses did not detect isolable byproducts, further electrolysis was detrimental. Based on recovered **17**, the yield of the macrocyclization was 43%.

Following hydrogenolysis of **18**, the two isomeric amines were separated (see the Supporting Information) and the major isomer was acylated with (*S*)-2-hydroxyisovaleric acid to give DZ-2384 (**5**). Twenty grams of **5** were prepared in this manner and characterized as a free-flowing, white powder (>99% purity).^[26]

A final series of experiments tested if electrolysis could generate **5** directly. Because the batch reaction was run to partial conversion and produced isomeric products, chromatography was required. It was desirable to delay purification until the last step. Hydrobromide **16** was condensed with Boc-Tyr-OH and the product treated with 4N HCl. The resultant C2 amine was coupled to (*S*)-2-hydroxyisovaleric acid and treated with TFE modified LiBH₄ to afford phenolic diol **19** (63% from **16**, 87% purity, no chromatography). Anodic

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oxidation of crude **19**, under conditions identical to those used for **17**, afforded DZ-2384, its *epi*-C10,C11 diastereoisomer **S7** (35%, >98% purity, d.r. 2.3:1) and unreacted **19** (16%)following chromatography on silica gel. Based on the purity of starting material and the amount of **19** recovered in the experiment, the yield of the macrocyclization was 48%.

The formation of a macrolactam-containing heptacyclic diol directly from unprotected **19** was a milestone for the project. It established a synthesis of DZ-2384 (**5**) that proceeded in 13 total operations and 5.7% overall yield from *L-tert*-leucine—wherein each kilogram of *L-tert*-leucine would translate into 280 grams of DZ-2384.^[27] The route utilizes easily sourced raw materials and was completely free of heavy metals. It also highlights the special potential of electrochemical methods to elicit reactivity that is otherwise elusive.^[28] Further research along these lines is ongoing, as are implementations of this chemistry according to good manufacturing practice.

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Communications

Synthetic Methods

H. Ding, P. L. DeRoy, C. Perreault, A. Larivée, A. Siddiqui, C. G. Caldwell, S. Harran, P. G. Harran* _____ IIII-IIII

Electrolytic Macrocyclizations: Scalable Synthesis of a Diazonamide-Based Drug Development Candidate



Electrifying chemistry! The core macrolactam of diazonamides can be synthesized electrochemically. This large ring forming dehydrogenation has enabled a concise preparation of DZ-2384, a refined analog of diazonamide A slated for clinical development as a cancer therapeutic.

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