Synthetic Methods

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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.*

It 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 3*H*-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 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*-NO_2PhSO_2) 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

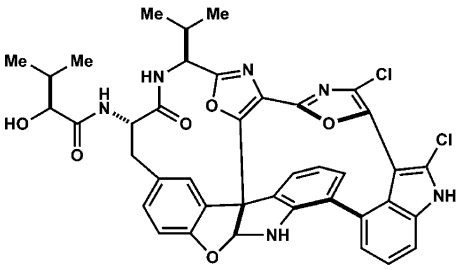

Figure 1. (−)-diazonamide A (**1**).

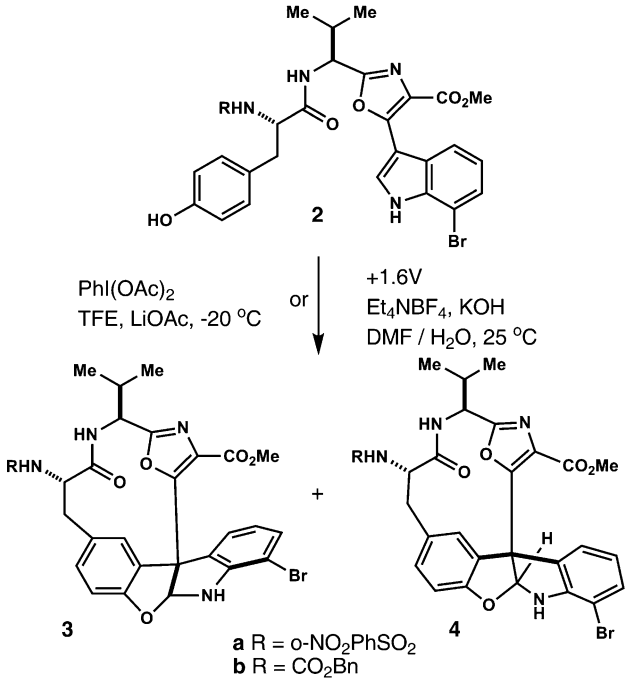

Figure 2. Two mechanistically distinct syntheses of diazonamide core structures.

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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.

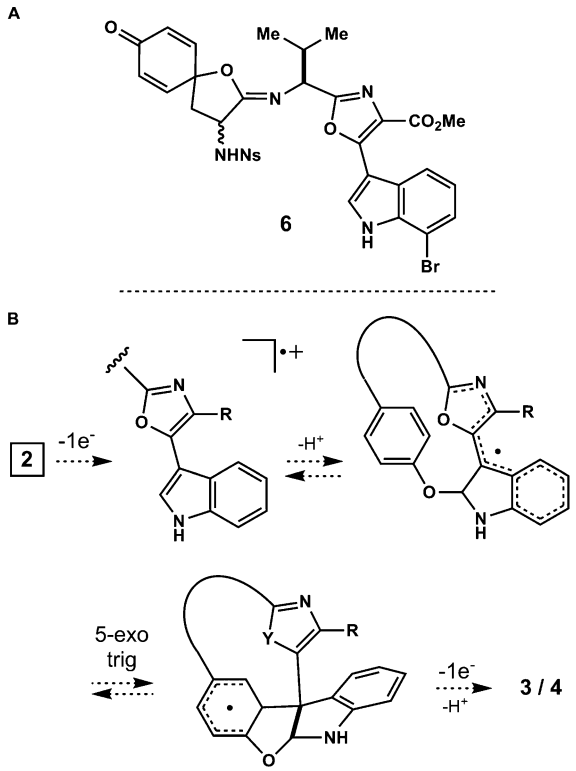


Figure 3. Working mechanistic hypothesis.

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 3 B,[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-

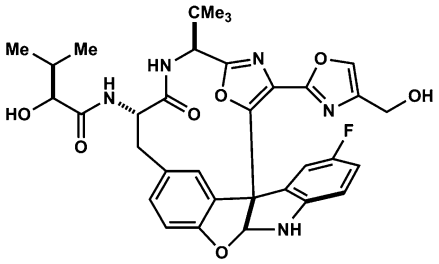
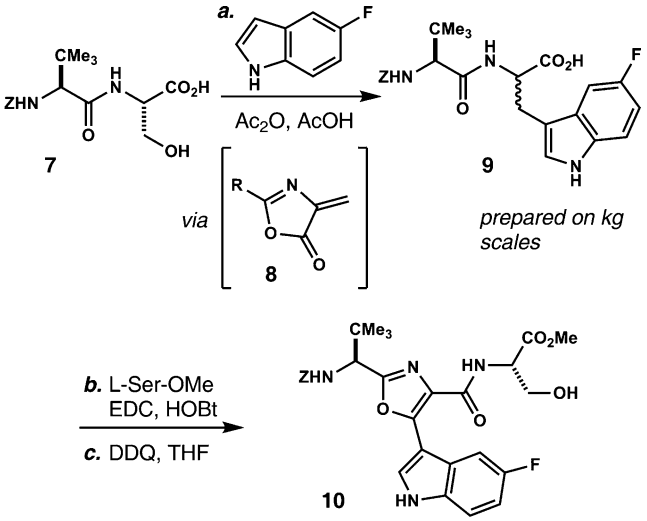


Figure 4. DZ-2384 (**5**).

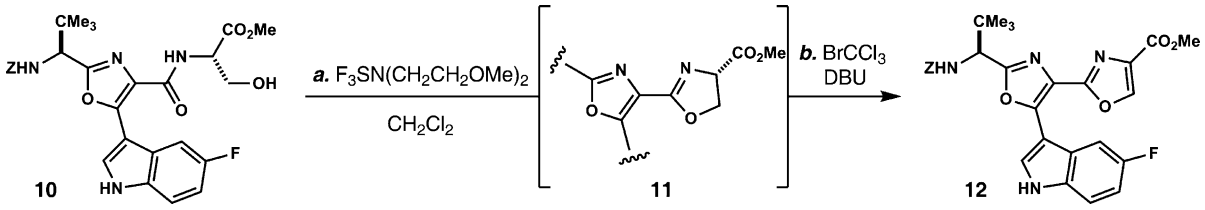
over, due to improved pharmacokinetics, compound **5** was 10- to 50-fold more efficacious than **1** as a cancer therapeutic in vivo (rodents).

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, iPr_2NEt, 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.



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 $BrCCl_3$, (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_2CO_3[b]	5	64

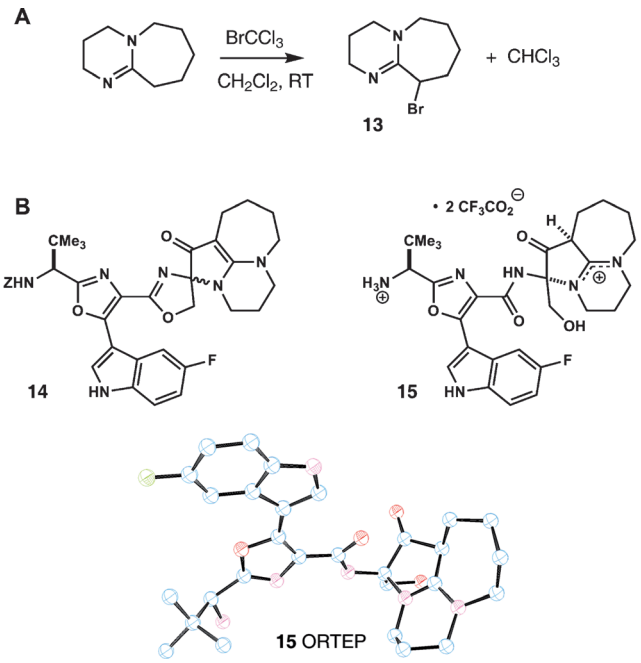
[a] Stepwise yield (90 g scale). [b] 2.2 equiv (2.5 equiv DBU used).

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

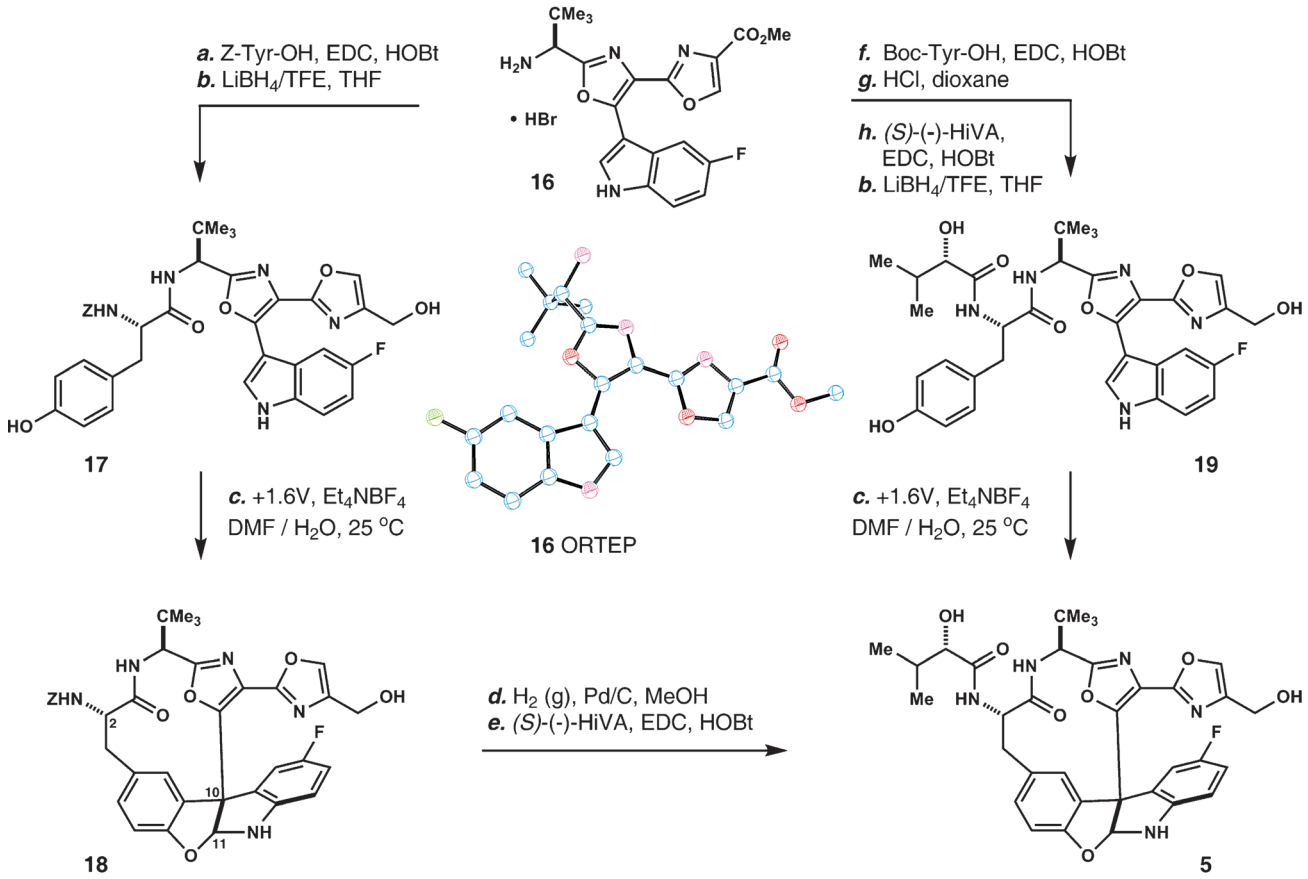
Our intent was to assemble DZ-2384 outwardly from a central dipeptide, namely tert-Leu-5-F-Trp-OH (**9**, Scheme 1). Peptide **9** can be prepared in three steps from 5-fluorotryptophan.[12] However, a four-step route proved more cost effective. In that case, L-*tert*-leucine was acylated with $ClCO_2Bn$ 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 Ac_2O 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 ^{1}H 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 $BrCCl_3$ and DBU.[18] This process proved interesting. The dehydration step was uneventful. Oxazoline **11** could be cleanly isolated. However, subsequent treatment with $BrCCl_3$/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 decomposing 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 $BrCCl_3$ 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 $BrCCl_3$ 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 3 B and the Supporting Information). Mechanistic details notwithstanding,[23] **14** was thought to derive from DBU being oxidized with $BrCCl_3$, 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 Na_2CO_3. 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.



Scheme 4. Two concise syntheses of DZ-2384 employing electrolytic macrocyclization. Reagents and Conditions: a) Z-Tyr-OH, EDC, HOBt, iPr$_2$NEt, DMF, 0 °C to RT, 3 h. b) LiBH$_4$ (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, Et$_4$NBF$_4$, (NH$_4$)$_2$CO$_3$, 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, Et$_4$NBF$_4$, (NH$_4$)$_2$CO$_3$, 2% aq. DMF, 56 h (35%, 48% based on recovered **19**, d.r. = 2.3:1; see the Supporting Information). d) H$_2$ (1 atm), Pd/C (10 mol%), tBuNH$_2$ (1.5 equiv), MeOH, 4 h. e) (*S*)-(−)-hydroxyisovaleric acid, EDC, HOBT, iPr$_2$NEt, 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 iPr$_2$O 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 LiBH$_4$ 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 LiBH$_4$) 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/H$_2$O/Et$_4$NBF$_4$), 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 (NH$_4$)$_2$CO$_3$ 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 4 N HCl. The resultant C2 amine was coupled to (*S*)-2-hydroxyisovaleric acid and treated with TFE modified LiBH$_4$ to afford phenolic diol **19** (63% from **16**, 87% purity, no chromatography). Anodic

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.

Keywords: anodic oxidation · electrosynthesis · macrocycles · natural products · oxazoles

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[22] The +30 Da by-product (30–35%) was largely absent in $BrCCl_3$-mediated oxidations of isolated **11**, implicating residual Deoxo-Fluor or a product thereof acting in concert with a reduction product of $BrCCl_3$ in the one-pot procedure as its source. See the Supporting Information.
[23] Spiroaminals **14** were not detected when **11**, AIBN and **13** were refluxed in $(ClCH_2)_2$. The same was true when **12**, DBU and **13** were stirred at room temperature in CH_2Cl_2.
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