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Dictazoles: Potential Vinyl Cyclobutane Biosynthetic Precursors to the Dictazolines

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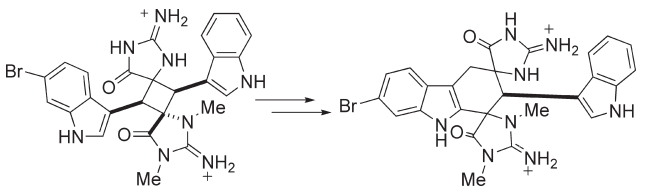
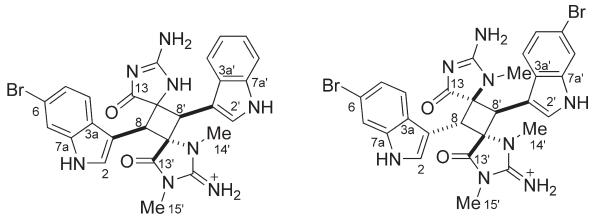


We report here the isolation of five new compounds, dictazoles A and B (**1** and **2**) and dictazolines C−E (**5**−**7**). Evidence is presented for the direct conversion of the cyclobutyl analogue **1** to its cyclohexyl constitutional isomer **5** via a vinyl cyclobutane rearrangement.

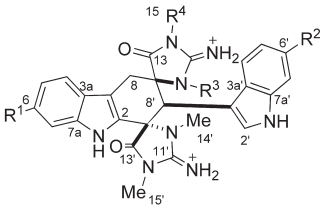
Marine sponges belonging to the family Thorectidae, and genus *Smenospongia* in particular, are well-known sources of indole alkaloids.[1] Consistent with these observations, we recently reported the isolation of two compounds, dictazolines A (**3**) and B (**4**),[2] from a Panamanian sponge identified as *S. cerebriformis* (Duchassaing & Michelotti, 1864) (order Dictyoceratida, family Thorectidae). Related alkaloids[3] are proposed to be Diels−Alder adducts of aplysinopsin (**8**),[4] but attempts to affect this transformation have been unsuccessful.[3b] Baran et al. have demonstrated the related alkaloid ageliferin (**11**), originally proposed to be formed via a Diels−Alder reaction of hymenidin,[5,6] can be efficiently synthesized via a vinyl cyclobutane rearrangement of sceptrin (**9**) (Scheme 1A).[7] This elegant synthesis supports an alternative unprecedented biosynthetic proposal for this dimeric compound.[7]

We report here the isolation of dictazoles A (**1**) and B (**2**) and dictazolines C−E (**5**−**7**) from the same extract which provided **3** and **4**. In addition, we present evidence for the direct conversion of **1** to the constitutional isomer **5**, presumably via a vinyl cyclobutane rearrangement (Scheme 1B). In this case, the rearrangement of the cyclobutyl ring system involves an indole rather than the imidazole ring found in **9**. These results suggest a more general role for this reaction in the biosynthesis of marine alkaloids and represent only the second example of a vinyl cyclobutane rearrangement featuring an indole ring.[8]

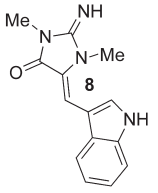
LC−MS analyses of the dictazoline-containing extract revealed the presence of several additional brominated metabolites. Extensive chromatographic separations eventually yielded **1**, which lacked the expected AB spin system for H_2-8 observed in the ^{1}H NMR spectra of **3** and **4**. Analyses of the DEPT and multiplicity-edited HSQC spectra confirmed this position in **1** was modified, as the compound contained only methine, methyl, and quaternary carbons.



Dictazole A (1) **Dictazole B (2)**



Dictazoline A (3) R^1 = Br R^2 = Br R^3 = Me R^4 = Me
Dictazoline B (4) R^1 = Br R^2 = Br R^3 = H R^4 = H
Dictazoline C (5) R^1 = Br R^2 = H R^3 = H R^4 = H
Dictazoline D (6) R^1 = Br R^2 = H R^3 = H R^4 = Me
Dictazoline E (7) R^1 = H R^2 = H R^3 = H R^4 = Me



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The structure of **1** was defined by analyses of the 2D NMR spectroscopic data (DMSO-d_6 and MeOH-d_4). Two indole rings substituted at C-3 were easily assembled based on a suite of HMBC and COSY correlations (Table 1). A spiro-2-iminoimidazolidin-4-one ring analogous to those

(1) Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2008**, *25*, 35–94.
(2) Dai, J.; Jiménez, J. I.; Kelly, M.; Barnes, S.; Lorenzo, P.; Williams, P. G. *J. Nat. Prod.* **2008**, *71*, 1287–1290.
(3) (a) Iwagawa, T.; Miyazaki, M.; Okamura, H.; Nakatani, M.; Doe, M.; Takemura, K. *Tetrahedron Lett.* **2003**, *44*, 2533–2535. (b) Mancini, I.; Guella, G.; Zibrowius, H.; Pietra, F. *Tetrahedron* **2003**, *59*, 8757–8762.
(4) Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J. *Tetrahedron Lett.* **1977**, *1*, 61–64.
(5) (a) Kobayashi, J.; Tsuda, M.; Murayama, T.; Nakamura, H.; Ohizumi, Y.; Ishibashi, M.; Iwamura, M.; Ohta, T.; Nozoe, S. *Tetrahedron* **1990**, *46*, 5579–5586. (b) Endo, T.; Tsuda, M.; Okada, T.; Mitsuhashi, S.; Shima, H.; Kikuchi, K.; Mikami, Y.; Fromont, J.; Kobayashi, J. *J. Nat. Prod.* **2004**, *67*, 1262–1267.
(6) For alternative proposals involving nonconcerted cyclizations, see: (a) Keifer, P. A.; Schwartz, R. E.; Koker, M. E. S.; Hughes, R. G. Jr.; Rittschof, D.; Rinehart, K. L. *J. Org. Chem.* **1991**, *56*, 2965–2975. (b) Mourabit, A. A.; Potier, P. *Eur. J. Org. Chem.* **2001**, *2*, 237–243.
(7) Baran, P. S.; O'Malley, D. P.; Zografos, A. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 2674–2677.
(8) Wenkert, E.; Moeller, P. D. R.; Piettre, S. R.; McPhail, A. T. *J. Org. Chem.* **1987**, *52*, 3404–3409.

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SCHEME 1. Vinyl Cyclobutane Rearrangements of Sceptrin (9) and Dictazole A (1)

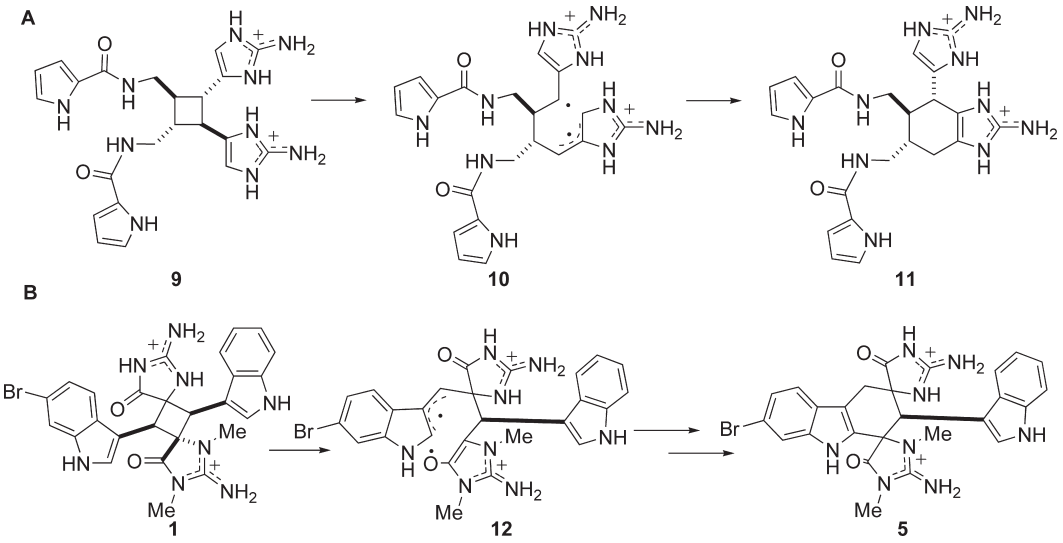


TABLE 1. NMR Spectroscopic Data for 1 in DMSO-d_6

C/H no.	δ_C	δ_H, mult (J in Hz)	HMBC	ROESY
2	124.6, CH	7.15, s		H-8, H-15′
3	106.5, C		H-2, H-4, H-8	
3a	126.4, C		H-2, H-4, H-5, H-7, H-8	
4	119.5, CH	7.25, d (8.3)		H-8, H-14′
5	121.9, CH	7.07, d (8.3)		
6	114.2, C		H-4, H-5, H-7	
7	114.3, CH	7.54, s		
7a	136.3, C		H-2, H-4	
8	43.4, CH	4.46, s	H-8′	H-4, H-2, H-14′
9	67.2, C		H-8′, H-8, H-10	
10		8.16, s		H-2′
11	170.9, C		H-10	
13	188.4, C		H-8, H-8′, H-10	
2′	123.6, CH	7.13, s		H-8, H-10
3′	105.9, C		H-2′, H-4′, H-8′	
3a′	127.4, C		H-2′, H-4′, H-5′, H-7′, H-8′	
4′	117.7, CH	7.31, d (8.0)		H-8, H-14′
5′	119.1, CH	6.95, t (8.0)		
6′	121.6, CH	7.05, t (8.0)		
7′	111.7, CH	7.32, d (8.0)		
7a′	135.3, C		H-2′, H-4′, H-6′	
8′	43.6, CH	4.49, s	H-8′	H-2′, H-14′
9′	72.7, C		H-8, H-8′, H-14′	
11′	153.5, C		H-14′, H-15′	
13′	172.5, C		H-8, H-8′, H-15′	
14′	25.8, CH_3	3.21, s		H-4′, H-8′, H-4, H-8
15′	25.1, CH_3	2.73, s		H-2

in **4** was deduced based on HMBC correlations from the *N*-methyls to the adjacent quaternary carbons (H-15′ to C-11′/13′ and H-14′ to C-9′/11′) and carbon chemical shift comparisons with **4** in MeOH-d_4. The two nonequivalent methine signals (H-8/H-8′) displayed HMBC correlations to C-9, C-9′, C-13, C-13′ and to either C-8 or C-8′. Together these data indicated **1** contained a cyclobutyl rather than a cyclohexenyl core. Analyses of the ROESY and 1D-DPFGSE NOE spectra established the configurations of C-8, C-8′, and C-9′ based on correlations between H-8′ and H-14′ and between H-8′ and H-8 (see Figures S13 and S14, Supporting Information). The relative configuration of C-9 remains undetermined though as attempts to crystallize our sample were unsuccessful due to decomposition.

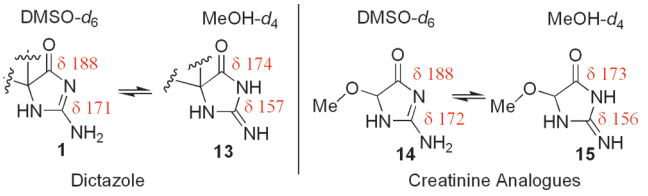


FIGURE 1. Solvent-dependent tautomerization.

The ^{13}C NMR spectrum of **1** was strongly dependent on the NMR solvent. Specifically, in MeOH-d_4 the "amide" C-13 and "guanidino" C-11 resonated as expected at 173.8 and 157.0 ppm, respectively, but in DMSO-d_6 these signals shifted downfield significantly to 188.4 (C-13) and 170.9 ppm (C-11). A solvent-dependent tautomerization between 2-aminoimidazolone (**1**) and 2-iminoimidazolidinone (**13**, Figure 1) explained these observations, as in the former tautomer (**1**) the lone pair on the "amide" nitrogen resided in a sp^2 orbital perpendicular to the π-system. These chemical shift assignments were consistent with spectroscopic data reported for the creatinine derivatives **14** and **15**.[9]

Several related analogues were also identified in the crude extract. In most cases, simple inspection of the ^1H NMR spectra in conjunction with HRMS data enabled the planar structures to be proposed (see the Supporting Information for tabulated NMR data). Briefly, compound **2** was bromo-10-*N*-methyl **1**, with a molecular formula of $C_{27}H_{24}Br_2N_8O_2$. The additional *N*-methyl group that was assigned as H-14, based on HMBC correlations, facilitated the assignment of the relative configuration of **2**. In 1D-DPFGSE NOE experiments, correlations were observed between H-8 and H-14 and between H-8′ and H-14′ (see Figures S32 and S33, Supporting Information). No correlation was present between H-8 and H-8′ for **2**, which contrast sharply with **1**,

(9) Krawczyk, H.; Pietras, A.; Kraska, A. *Spectrochim. Acta, Part A* **2007**, *66A*, 9−16 and references therein.

suggesting different relative configurations of the two compounds.[10] Additional circumstantial evidence in support of the epimeric nature of **1** and **2** was the notable chemical shift difference observed for these methines in DMSO-d_6 ($\Delta\delta^{1-2}{}_{C\text{-}8}$ −0.9; $\Delta\delta^{1-2}{}_{C\text{-}8'}$ −2.5; $\Delta\delta^{1-2}{}_{H\text{-}8}$ −0.51; $\Delta\delta^{1-2}{}_{H\text{-}8'}$ −0.63). As deduced by the ESI-MS data, compound **5** was a constitutional isomer of **1**. In contrast to **1** though, the ^{1}H NMR spectrum of **5** contained diagnostic signals for the H_2-8 AB system of the cyclohexenyl ring, which in conjunction with 2D NMR data, established the planar structure. Compound **6** was 12-*N*-methyl-**5**, based on the extra methylamide resonance in the ^{1}H NMR spectrum, the HMBC correlations to C-13 and C-11 from the new methyl resonance, and HR-ESI MS data. Finally, **7** was desbromo-**6** (see the Supporting Information). The relative configurations at C-8′ and 9′ of **5**−**7** were established after analyses of their 2D ROESY spectra, while the configuration of C-9 was deduced by comparison with ^{13}C NMR data for **3** and **4**.[2]

Dictazole A inhibited the aspartic protease BACE1 (memapsin 2). This protease is widely believed to have a central role in the pathology of Alzheimer's disease.[11] As such, pharmacological intervention that reduces BACE1 activity should be therapeutically beneficial. Dictazole A inhibited BACE1-mediated cleavage of amyloid precursor protein (APP) in a dose-dependent manner with an IC_{50} value of 50 μg/mL. Interestingly, the 2-iminoimidazolidinone moiety within the dictazoles is common in several BACE1 inhibitors and has led to the suggestion that this privileged subunit is responsible for the observed activity against BACE1.[12]

Compounds **1** and **2** are unusual. The closest related alkaloids containing cyclobutane rings are sceptrin (**9**) and orthidine E.[13] Baran et al. have proposed a biosynthesis of **11** involving a dicationic diradical vinyl cyclobutane rearrangement (Scheme 1) of **9**.[14] Evidence for this hypothesis includes computational data,[15] and the direct microwave conversion of **9** to **11**.[7] To date, no other potential examples of this biosynthetic rearrangement have been demonstrated.

Given these results, the isomers **1** and **5** are intriguing. The cyclobutyl alkaloid **1** could be a precursor to **5** via a related reaction (Scheme 1). Rearrangement of **1** via the intermediate **12** would result in ring expansion to the cyclohexenyl derivative **5** after double-bond isomerization. In this case, the rearrangement would involve an indole rather than a 2-aminoimidazole ring, and the electron-deficient intermediate **12** would be stabilized by the pendant 2-iminoimidazolidinone moiety as compared to a 2-aminoimidazole. Circumstantial evidence is the relative abundance of the two isolated compounds. As is the case with **9** and **11**, the cyclobutyl derivative **1** is isolated in higher yields than the cyclohexenyl analogue **5**.

To examine the feasibility of this transformation, two 100 μg aliquots were prepared from the same sample of **1**.

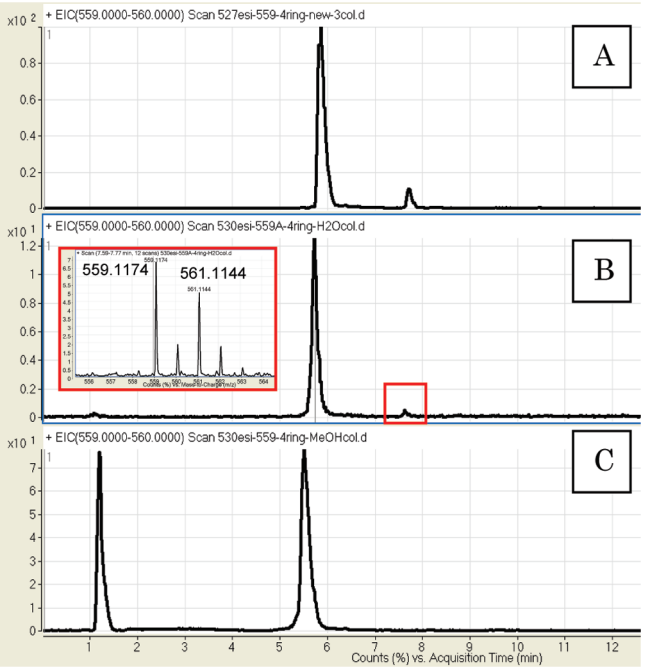
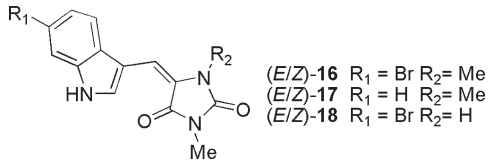


FIGURE 2. LC−MS extracted ion chromatograms (m/z 559−560): (A) standards **1** (major) and **5** (minor); (B) crude microwave reaction in H_2O of pure **1** after 1 min at 200 °C; (C) crude microwave reaction in MeOH of pure **1** after 1 min at 150 °C. The peak at 1 min is a result of deliberate overloading of the HPLC column to ensure **5** is not present in that reaction mixture.

One sample was dissolved in water, sealed, and heated in a microwave at 200 °C for 1 min, similar to the conditions reported for sceptrin.[16] The other sample was heated to 150 °C in methanol. While no product was observed in the methanol reaction mixture, remarkably, careful analysis of the aqueous reaction mixture by LC−MS (Figure 2B) revealed a new peak with the same retention time, m/z ratio, and M + 2 isotope pattern as **5**. HR-ESI mass spectrometry provided pseudomolecular ion peaks at 559.1174 and 561.1144 in approximately a 1:1 ratio that corresponded to the expected molecular formulas (errors of 5 and 7 ppm, respectively). The product was not observed in the starting material (see the Supporting Information Figure S54) or in the methanol control prepared from the same sample of **1**.[16] It should be noted that an identical solvent dependency was observed for the conversion of **9** to **11**. While the conversion proceeded smoothly in water, sceptrin decomposed when heated methanol.[17] Due to the limited amount of **1** isolated, the yield of this transformation has not been optimized, and the products have not been characterized by NMR. The tentative identification of **5** in the reaction mixture therefore rests on the standard practice of comparing the retention time and the ionization pattern of an unknown with a standard. It is possible though that another isomer, for example, derived from a single bond scission of the cyclobutane ring, may coelute with **5**. As such, final confirmation of this transformation

(10) C-9 epimers of compounds related to **3** have been previously reported by Mancini (see ref 3b).
(11) Hardy, J. *Curr. Alzheimer Res.* **2006**, *3*, 71–73.
(12) Hills, I. D.; Vacca, J. P. *Curr. Opin. Drug Disc.* **2007**, *10*, 383–391.
(13) Pearce, A. N.; Chia, E. W.; Berridge, M. V.; Maas, E. W.; Page, M. J.; Harper, J. L.; Webb, V. L.; Copp, B. R. *Tetrahedron* **2008**, *64*, 5748–5755.
(14) For a review on vinyl cyclobutane rearrangements, see: Baldwin, J. E.; Leber, P. A. *Org. Biomol. Chem.* **2008**, *6*, 36–47.
(15) Northrop, B. H.; O'Malley, D. P.; Zografos, A. L.; Baran, P. S.; Houk, K. N. *Angew. Chem., Int. Ed.* **2006**, *45*, 4126–4130.
(16) The injection amount in parts a and c of Figure 2 was approximately 10× the amount injected in Figure 2b.
(17) O'Malley, D. P.; Li, K.; Maue, M.; Zografos, A. L.; Baran, P. S. *J. Am. Chem. Soc.* **2007**, *129*, 4762–4775.

will likely require the synthesis of **1** to provide sufficient material to address these issues.



These results deserve comment. First, the reaction mixture containing the product **5** was composed mostly of starting material and fragmentation products. Specifically, pseudomolecular ions consistent with (*E*)- and (*Z*)-isomers of **16**−**18** were present.[18] Baran et al. have noted that the interconversion of **9** to **11** is strongly dependent on the counterion, with the highest yields obtained with formate or acetate salts.[15] It is possible the low yield of our reaction is attributable to a similar counterion dependency with the formate salt being less than ideal for this substrate.

These results suggest the possible involvement of a vinyl cyclobutane rearrangement in the biosynthesis of **3**−**7**, as opposed to the Diels−Alder reaction suggested by Mancini et al. for the cycloaplysinopsins.[19] Interestingly, during the isolation of this latter class of compounds, a constitutional isomer of cycloaplysinopsin A was identified by LC−MS that was attributed to a diastereomeric Diels−Alder adduct. Our results raise the possibility that this uncharacterized metabolite may instead be a cyclobutyl isomer.

On the basis of NMR experiments with chiral shift reagents in $CDCl_3$, the same group proposed that cycloaplysinopsin was a scalemic mixture. We attempted to duplicate these experiments with **1**. Unfortunately, **1** is not soluble in $CDCl_3$, and attempts to titrate this compound with $Eu(fod)_3$ in CD_3CN have been unsuccessful. This failure is due to the hygroscopic nature of the solvent required and the trace amounts of **1** remaining (200 μg).[20]

To the best of our knowledge, the conversion of **1** to **5** is only the second example of a vinyl cyclobutane rearrangement involving an indole ring and the first for a natural product. Our data suggests this rearrangement may play a larger role in the biosynthesis of alkaloids from marine invertebrates than previously appreciated and suggests a possible route toward the synthesis of this family of compounds.

(18) **16**: *m*/*z* 334.0200 (calcd for $C_{14}H_{13}{}^{79}BrN_3O_2{}^+$ 334.0191, +2.7 ppm), *m*/*z* 336.0160 (calcd for $C_{14}H_{13}{}^{81}BrN_3O_2{}^+$ 336.0171, −3.2 ppm). **17**: *m*/*z* 256.1086 (calcd for $C_{14}H_{14}N_3O_2{}^+$ 256.1086, 0.0 ppm), *m*/*z* 278.0902 (calcd for $C_{14}H_{13}N_3O_2Na^+$ 278.0902, −1.2 ppm). **18**: *m*/*z* 320.0033 (calcd for $C_{13}H_{11}{}^{79}BrN_3O_2{}^+$ 320.0035, −0.5 ppm), 321.9998 (calcd for $C_{13}H_{11}{}^{81}BrN_3O_2{}^+$ 322.0014, −5.0 ppm).

(19) The occurrence of these similar metabolites in two dissimilar sources suggests that the true producer may be microbial.

(20) The method development required for chiral HPLC analysis of **1** has not been undertaken.

Experimental Section

Extraction and Isolation of BMNH 2000.12.11.6. The freeze-dried sponge (114 g) was exhaustively extracted with 1:1 *i*-PrOH/CH_2Cl_2 (3 × 3 L) to afford 14.85 g of lipophilic extract. Partitioning using a modified Kupchan procedure yielded hexane (6.07 g), DCM (1.88 g), *n*-BuOH (2.94 g), and H_2O (5.78 g) fractions. The residue from the *n*-BuOH phase was separated on a Sephadex LH-20 column eluting with MeOH, and the resulting fractions were pooled based on TLC analyses into seven fractions. These fractions were subsequently separated by a combination of Si flash chromatography and RP-HPLC to yield **1**, **2**, and **5**−**7**.

Dictazole A (**1**, 4.5 mg, 3.0×10^{-2} % yield): colorless powder; $[\alpha]^{22}{}_D$ +8.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 223 (2.5) 284 (2.4) nm; IR (CaF_2) ν_{max} 3337, 1643, 1592, 1352 cm^{-1}; see Table S1 (DMSO-d_6) and Table S2 (MeOH-d_4) for tabulated spectral data; HRESI-TOFMS *m*/*z* 561.1206 $[M + H]^+$ (calcd for $C_{26}H_{24}{}^{81}BrN_8O_2{}^+$, 561.1185, +3.7 ppm).

Dictazole B (**2**, 0.8 mg, 5.0×10^{-3} % yield): colorless powder; $[\alpha]^{22}{}_D$ −42.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 228 (2.5) 288 (1.9) nm; IR (CaF_2) ν_{max} 3392, 1653, 1591, 1352 cm^{-1}; see Table S3 for tabulated spectral data; HRESI-TOFMS *m*/*z* $[M + H]^+$ 651.0490 (calcd for $C_{27}H_{25}{}^{79}Br_2N_8O_2{}^+$, 651.0467, +3.5 ppm).

Dictazoline C (**5**, 1.5 mg, 1.0×10^{-2} % yield): colorless powder; $[\alpha]^{22}{}_D$ −19.2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 225 (2.6) 289 (1.9) nm; IR (CaF_2) ν_{max} 3542, 1646 cm^{-1}; see Table S4 for tabulated spectral data; HRESI-TOFMS *m*/*z* 559.1221 $[M + H]^+$ (calcd for $C_{26}H_{24}{}^{79}BrN_8O_2{}^+$, 559.1206, +2.8 ppm).

Dictazoline D (**6**, 2.5 mg, 1.7×10^{-2} % yield): colorless powder; $[\alpha]^{22}{}_D$ −1.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 283 (9.14) nm; IR (CaF_2) ν_{max} 3422, 2930, 1656, 1586 cm^{-1}; See Table S5 for tabulated spectral data; HRESI-TOFMS *m*/*z* 573.1352 $[M + H]^+$ (calcd for $C_{27}H_{26}{}^{79}BrN_8O_2{}^+$, 573.1362, −1.7 ppm).

Dictazoline E (**7**, 0.5 mg, 3.4×10^{-3} % yield): colorless powder; $[\alpha]^{22}{}_D$ −22.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.6) 283 (3.8) nm; IR (CaF_2) ν_{max} 3542, 1646 cm^{-1}; See Table S5 for tabulated spectral data; HRESI-TOFMS *m*/*z* 495.2279 $[M + H]^+$ (calcd for $C_{27}H_{27}N_8O_2{}^+$, 495.2257, +4.4 ppm).

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Supporting Information Available: Experimental details, tabulated NMR data for **1** and **2**, copies of the relevant spectroscopic data, and LC−MS traces from the microwave conversion of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.