Pergamon

Tetrahedron 55 (1999) 935–942

TETRAHEDRON

Synthesis of Didemnolines A-D, N9-Substituted β-Carboline Alkaloids from the Marine Ascidian *Didemnum* sp.

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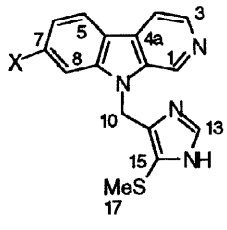
Received 21 October 1998; revised 12 November 1998; accepted 20 November 1998

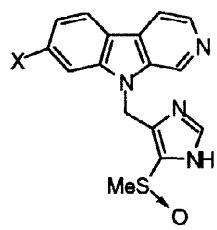
Abstract: Didemnolines A-D (**1-4**) were synthesized and their structures were confirmed through comparisons of data for the synthetic material with those obtained for natural **1-4**. The synthesis of didemnolines A (**1**) and C (**3**) involved the coupling of 1-[(benzyloxy)methyl]-4-chloromethyl-5-(thiomethyl)imidazole (**6**) with 7-bromo-β-carboline (**5**), while didemnolines B (**2**) and D (**4**) were formed through the analogous coupling of **6** with norharmon (**7**). Intermediate **6** was efficiently prepared from 1-[(benzyloxy)methyl]-2,4,5-tribromoimidazole using a sequential one-pot halogen-metal exchange reaction and 7-Br-β-carboline was synthesized using a new approach.

Keywords: Natural Products, Alkaloids, Imidazoles,

Marine ascidians have gained recognition in recent years as excellent sources of a wide variety of novel amino acid-derived natural products,[1] many of which have been the focus of further biological studies as well as targets for synthetic chemists. In addition to cyclic peptides, ascidians have yielded many heteroaromatic alkaloids, including pyridoacridines[2] and β-carbolines.[1] Although ascidians of the genus *Eudistoma* (family Polycitoridae) have been the most common source of biologically active β-carboline derivatives,[3,4,5] several tetrahydro-β-carboline alkaloids, e.g., lissoclin C[6] and bengacarboline,[7] have been isolated from ascidians belonging to the genera *Lissoclinum* and *Didemnum*, respectively, both members of the family Didemnidae.



1 X = Br
2 X = H



3 X = Br
4 X = H

In 1995, we reported the structures of didemnolines A-D (**1-4**), new β-carboline alkaloids isolated from an ascidian of the genus *Didemnum*, collected near the island of Rota, Northern Mariana Islands.[8] The didemnolines are unusual in that their β-carboline rings are substituted at the N-9 position, rather than at the more common C-1 point of attachment. In addition, didemnolines A (**1**) and C (**3**) were biologically active, with **3** being roughly an order of magnitude more cytotoxic to KB cells (IC_{50} values of 0.28 μg/mL for **3** *versus* 6.1 μg/mL for **1**) and **1** being slightly more antimicrobial toward *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. In order to confirm our proposed structures and allow us to ascertain the structures of several additional didemnolines, we undertook the synthesis of didemnolines A-D. We now report the results of these synthetic efforts.

Because we previously demonstrated that didemnoline C (**3**) could be generated from didemnoline A (**1**) by treatment with $NaIO_4$,[8] our retrosynthetic analysis (see Scheme 1) involved the straightforward disconnection of the N-9/C-10 bond. Therefore, compounds **1** and **3** could be formed through the coupling of 7-bromo-β-carboline (**5**, = eudistomin O), previously prepared by Rinehart and co-workers from 4-methyl-3-nitroaniline,[3c]

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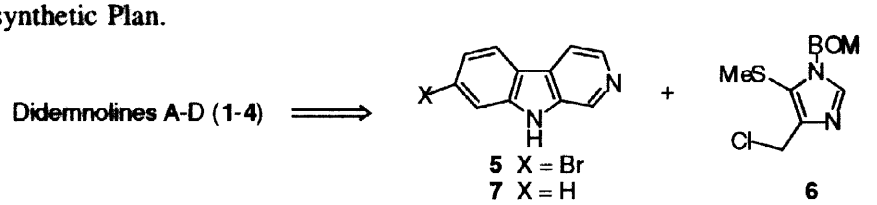
0040-4020/99/$ - see front matter
PII: S0040-4020(98)01100-4

with an appropriately substituted imidazole moiety (**6**) that may be formed directly from imidazole. Similarly, didemnolines **B** (**2**) and **C** (**4**) could be formed from imidazole **6** and commercially available norharmon (**7**, = β-carboline).

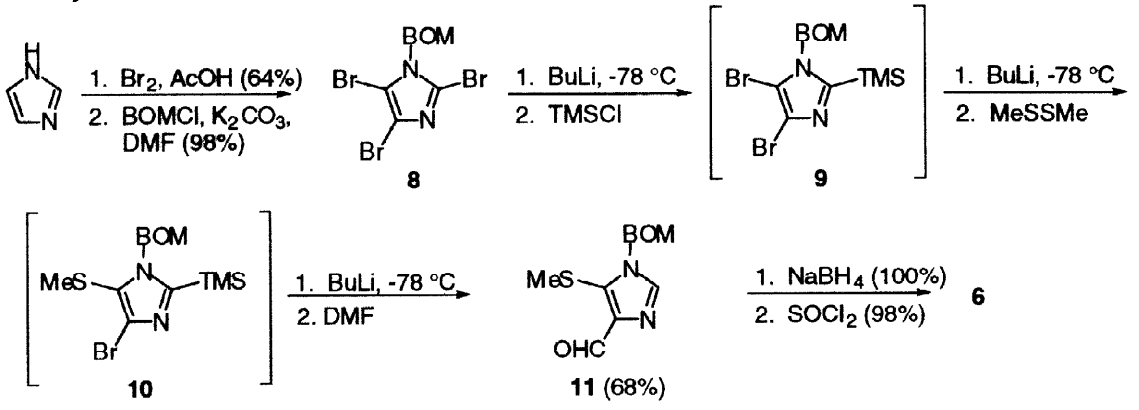
Scheme 1. Retrosynthetic Plan.



Synthesis of Imidazole 6.

The imidazole ring system has been studied more than any of the other azole ring systems because many imidazole derivatives, e.g., histamine and nucleosides, possess biological activity. The development and refinement of synthetic methods for the functionalization of imidazole rings continues to be a central concern in the pursuit of biologically active synthetic analogs of naturally occurring imidazole-based compounds. Our approach for the formation of compound **6** involved the use of the sequential halogen-metal exchange method.[9] Although it has been known for some time that the rate of halogen-metal exchange in 2,4,5-trihalogenated imidazoles occurs in the order C-2 > C-5 >> C-4, early reports describing this regioselectivity were filled with contradictions as to whether 4,5-dihalogenated imidazoles could be functionalized directly at the C-4 and C-5 positions, without first protecting the C-2 position. In 1991, Groziak and co-workers clearly showed that upon treatment of 2-unprotected-4,5-diiodoimidazoles with *n*-BuLi, the initially formed 5-yllithium species equilibrates to form a 2-yllithium species, which can then react with an electrophile,[9d] demonstrating the need for C-2 substitution when the C-5 position is to be functionalized. Based on this finding, the authors developed a one-pot procedure for preparing 2H-4,5-disubstituted imidazoles using a transient protecting group at C-2.[9f] This approach provides a method for the preparation of 2-unsubstituted-4,5-unsymmetrically disubstituted imidazoles such as **6**.

Scheme 2. Synthetic Route to Imidazole **6**.



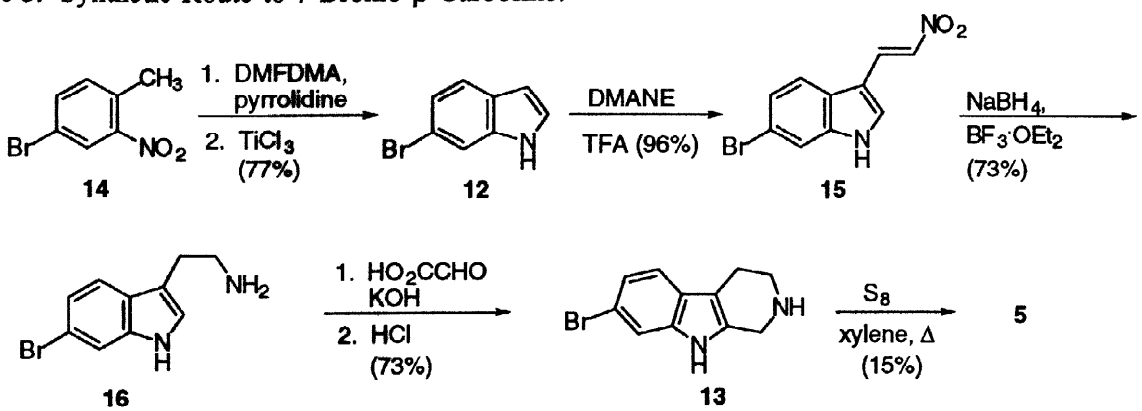
The requisite *N*-protected, polyhalogenated imidazole was prepared in two steps by treatment of imidazole with Br_2 in dry acetic acid to give the 2,4,5-tribromoimidazole product, which was then protected at N-1 by treatment with BOMCl and K_2CO_3 in DMF, yielding compound **8** in 63% yield from imidazole. As discussed earlier, in order to functionalize the C-4 and C-5 positions, the C-2 position must be protected. Therefore, treatment of **8** with *n*-BuLi was followed by addition of TMSCl to afford 2-trimethylsilyl protected intermediate **9**, which was not isolated but instead treated immediately with another equivalent of *n*-BuLi. Addition of MeSSMe yielded the intermediate **10**, substituted at the C-5 position with a thiomethyl group, which was once more treated with *n*-BuLi. The resulting 4-yllithium species was quenched with DMF. Upon aqueous work-up, the silyl

group was hydrolyzed and chromatographic purification then gave compound **11** in 68% yield. Reduction of the aldehyde with $NaBH_4$, followed by treatment of the resulting alcohol with $SOCl_2$ provided compound **6** in 98% from **11** and in 43% overall yield from imidazole.

Synthesis of 7-Bromo-β-Carboline (5).

7-Bromo-β-carboline (**5**, eudistomin O) was previously synthesized by Rinehart and co-workers in 8 steps with a 7% yield.[3c] However, because their route included several troublesome steps, we chose a modified reaction sequence, which like the Rinehart synthesis targets 6-bromoindole (**12**) and 1,2,3,4-tetrahydro-β-carboline (**13**) as intermediates (Scheme 3). Treatment of 4-bromo-2-nitrotoluene (**14**) with dimethylformamide dimethylacetal (DMFDMA) and pyrrolidine gave an intermediate β-pyrrolidinostyrene, which was immediately reduced with $TiCl_3$, yielding 6-bromoindole (**12**) in 77% yield.[10] Compound **12** was treated with 1-dimethylamino-2-nitroethylene (DMANE)[11] in dry TFA giving compound **15** (96%), which was reduced with $NaBH_4/BF_3{\cdot}OEt_2$[12] to yield 6-bromotryptamine (**16**) in 75%. A Pictet-Spengler reaction using glyoxylic acid monohydrate[13] then resulted in the formation of tetrahydro-β-carboline **13** in 73% yield.

Scheme 3. Synthetic Route to 7-Bromo-β-Carboline.



Oxidation of **13** to give the β-carboline ring system proved very difficult. Attempts to use diphenylselenium bis-(trifluoroacetate), as had Rinehart,[3c] were unsuccessful. Furthermore, MnO_2, Pd/C, $LiAlH_4$, chloranil, and DDQ all failed to give the desired product, although each of these reagents had been reported to work in high yield when the C-1 position is substituted.[14] Ultimately, dehydrogenation to give **5** was achieved using S_8 in refluxing xylene,[15] albeit in only 15% yield. In terms of ease, availability of reagents, and yield, this route provides a significant improvement for the synthesis of 7-bromo-β-carboline over that previously reported by Rinehart.[3c]

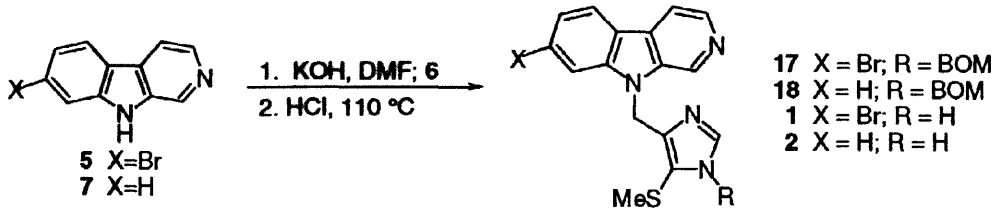
Formation of Didemnolines A (1) and B (2).

The final coupling of β-carbolines **5** and **7** with imidazole **6** to form didemnolines A (**1**) and B (**2**) was accomplished as follows (Scheme 4). Addition of **6** to a solution of **5** and powdered KOH in DMF afforded BOM-didemnoline A (**17**) in 68% yield. Removal of the BOM group with 6*N* HCl at 110 °C then provided didemnoline A (**1**) in 91% yield. A similar protocol using **7** instead of **5** gave didemnoline B in 65% yield over two steps, via **18**. The spectral data for synthetic **1** and **2** matched closely those of natural didemnolines A and B, respectively, unambiguously confirming the proposed structures.[8]

Conversion of Didemnolines A and B to Didemnolines C and D.

As previously shown, didemnolines A and B could be used to form didemnolines C and D, respectively.[8] Synthetic **1** was treated with $NaIO_4$ in $MeOH/H_2O$ for 24 h at RT yielding a product, which, after chromatographic purification was indistinguishable from natural didemnoline C (**3**). Likewise, treatment of synthetic **2** with $NaIO_4$ provided a product yielding NMR data that matched the signals assigned to didemnoline D (**4**) in the original spectrum containing 3 and 4,[8] thus confirming the proposed structure.

Scheme 4. Final Steps Towards Didemnolines A and C.



Summary.

Didemnolines A-D (**1-4**) have been synthesized. The synthesis of didemnolines A (**1**) and C (**3**) involved the coupling of 7-bromo-β-carboline (**5**) with 1-[(benzyloxy)methyl]-4-chloromethyl-5-(thiomethyl)imidazole (**6**), which was prepared in a single step from 1-[(benzyloxy)methyl]-2,4,5-tribromoimidazole using an approach involving sequential halogen-metal exchange. Although 7-bromo-β-carboline (**5**), also known as the natural product eudistomin O,[3c] had been previously synthesized, an improved route was developed. Didemnolines B (**2**) and D (**4**) were similarly prepared by coupling imidazole **6** with norharmon (**7**). These results have allowed the proposed structures to be confirmed, provided sufficient material for further biological testing, and have furnished a method by which the structures of four additional, and as yet unpublished, didemnolines can be confirmed. Their structures will be reported in the near future.

EXPERIMENTAL SECTION

General. Unless otherwise noted, materials and solvents were obtained from commercial suppliers and used without further purification. THF and pyrrolidine were distilled from Na/benzophenone immediately prior to use. Anhydrous solvents were added using syringes. Chromatography was carried out using silica gel (Merck 60, 60 Å, 230-400 mesh) according to the procedure described by Still.[16] Reactions and chromatography fractions were monitored by TLC, using 2 × 5 cm aluminum-backed plates covered with a 0.20 mm layer of silica gel 60 F_{254}, Art. 5554 (E. Merck, Darmstadt). UV light and iodine were used to visualize TLC spots. All NMR experiments were performed on either a General Electric QE-300 or GE Omega 500 spectrometer. NMR spectra were recorded in either $CDCl_3$ [^{1}H, 7.26 ppm (residual $CHCl_3$); ^{13}C, 77.0 ppm] or DMSO-d_6 [^{1}H, 2.49 ppm (residual DMSO-d_5); ^{13}C, 39.0 ppm). EI mass spectral data were obtained on a VG-70SE spectrometer and UV spectra were obtained on a Milton Roy spectronic 3000 diode array spectrophotometer. Melting points were determined on a Laboratory Devices Mel-Temp II apparatus and are uncorrected.

2,4,5-Tribromoimidazole: To a stirred solution of imidazole (1.36 g, 0.02 mmol) and NaOAc (20 g) in glacial HOAc (180 mL), was added a solution of Br_2 (9.6 g, 0.6 mmol) in HOAc (20 mL) over 30 min. When about one third of the bromine had been consumed, more NaOAc (5 g) was added. Stirring was continued for 2.5 h, during which time the tribromoimidazole began to precipitate. The acetic acid was evaporated and water (600 mL) was added. The white precipitate was collected, washed with water, and dried under vacuum, yielding pure 2,4,5-tribromoimidazole (3.8 g, 64%): mp 218-221 °C (lit.[17] 221-222 °C); ^{1}H NMR ($CDCl_3$) δ 10.20 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 116.30, 108.61; EIMS *m/z* (rel int) 308 (28), 306 (93), 304 (100), 302 (36), 279 (52), 277 (49), 225 (44), 198 (22), 93 (26); HREIMS 301.7712 ($C_3H^{79}Br_3N_2$; Δ -2.2 mmu).

1-[(Benzyloxy)methyl]-2,4,5-tribromoimidazole (8): A solution of 2,4,5-tribromoimidazole (8 g 26 mmol) in anhydrous DMF (100 mL) was treated with excess powdered K_2CO_3 (35 g). To the vigorously stirred suspension was added dropwise benzyloxymethyl chloride (4.8 g, 31 mmol) and the reaction was stirred overnight. The reaction mixture was filtered, after which the resulting oily residue slowly crystallized yielding 10.8 g (98%) of large translucent rhomboids: IR (film) ν 3249, 3017, 2932, 2857, 1494, 1456, 1419, 1360, 1317, 1253, 1200, 1098, 965, 740, 692, 654, 601 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.35-7.33 (m), 5.41 (s), 4.59 (s); ^{13}C NMR ($CDCl_3$) δ 135.7, 128.2, 127.9, 127.5, 127.3, 118.8, 117.3, 105.5, 75.4, 70.8; HRFABMS (3-NBA/Gly/TFA) 422.8330 ($C_{11}H_{10}Br_3N_2O$, MH$^+$; Δ -3.1 mmu).

1-[(Benzyloxy)methyl]-5-thiomethylimidazole-4-carboxaldehyde (11): To a solution of **8** (1.5 g, 3.56 mmol) in dry THF (20 mL) was added *n*-BuLi (3.56 mmol, 1.4 mL of 2.5 M) at -78 °C. After stirring for 20 min, TMSCl (452 μL, 3.56 mmol) was added dropwise via syringe and the solution was allowed to warm to RT for 2 h. The reaction was cooled to -78 °C and another aliquot of *n*-BuLi (3.56 mmol, 1.4 mL of 2.5 M) was added. After stirring for another 20 min at –78 °C, MeSSMe (321 μL, 3.56 mmol) was added and the solution was allowed to warm to room temperature for 2 h with stirring. The solution was again cooled to -78 °C, stirred for 30 min, and DMF (300 μL) was added. The reaction was allowed to warm to RT and after stirring for 2 h, it was poured into a separatory funnel containing saturated aqueous NH_4Cl. The aqueous solution was extracted with EtOAc, dried ($MgSO_4$), and concentrated, resulting in a crude yellow oil that was chromatographed over silica gel (97:3 CH_2Cl_2:MeOH) yielding pure **11** (631 mg) in 68 % as a colorless oil: UV (EtOH) λ_{max} 252 (16,500), 209 (7,100) nm; IR (film) ν 3423, 3091, 2920, 1681, 1488, 1450, 1333, 1247, 1216, 1082, 842, 789, 746, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.94 (1H, s), 7.70 (1H, s), 7.25-7.16 (5H, m), 5.41 (2H, s), 4.43 (2H, s), 2.34 (3H, s); ^{13}C NMR ($CDCl_3$) δ 184.6, 142.5, 140.1, 135.5, 133.1, 128.2, 127.9, 127.4, 73.0, 70.5, 19.8; EIMS *m/z* (rel int) 262 (27), 247 (49), 232 (17), 203 (17), 113 (16), 92 (57), 91 (100), 65 (55).

1-[(Benzyloxy)methyl]-4-hydroxymethyl-5-(thiomethyl)imidazole: To a solution of **11** (200 mg, 0.76 mmol) in MeOH (5 mL) was added $NaBH_4$ (58 mg, 1.52 mmol) at RT and the solution was stirred for 15 min until the aldehyde was consumed, as determined by TLC. The solution was poured into water and extracted with EtOAc. The extract was dried ($MgSO_4$) and evaporated under reduced pressure to give 201 mg (100 %) of alcohol: UV (EtOH) λ_{max} 252 (28,000), 214 (4,100) nm; IR (film) ν 3380, 2910, 2857, 1643, 1488, 1451, 1376, 1210, 1082, 1002, 788, 746 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.79 (1H, s), 7.34-7.26 (5H, m), 5.41 (2H, s), 4.71 (2H, s), 4.59 (1H, br s), 4.48 (2H, s), 2.34 (3H, s); ^{13}C NMR ($CDCl_3$) δ 147.4, 139.3, 136.3, 128.7, 128.3, 127.9, 121.6, 73.5, 70.5, 57.0, 21.2; EIMS *m/z* (rel int) 264 (70), 234 (34), 143 (44), 91 (100), 65 (76); HREIMS 264.0910 ($C_{13}H_{16}N_2O_2S$; Δ +2.3 mmu).

1-[(Benzyloxy)methyl]-5-thiomethyl-4-(chloromethyl)imidazole (6): Freshly distilled $SOCl_2$ (119 mg, 1.0 mmol) was added to 1-[(benzyloxy)methyl]-5-thiomethyl-4-(hydroxymethyl)imidazole (132 mg, 0.5 mmol) and the neat reaction was warmed on a steam bath with stirring for 15 min. The resulting HCl salt was washed with satd $NaHCO_3$, dried ($MgSO_4$), and concentrated, giving 138 mg (98%) of **6**: UV (EtOH) λ_{max} 253 (15,600), 218 (12,500), 213 (2,800) nm; IR (film) ν 3391, 1643, 1547, 1494, 1456, 1381, 1253, 1211, 1093, 1023, 746, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68 (1H, s), 7.31-7.22 (5H, m), 5.36 (2H, s), 4.66 (2H, s), 4.46 (2H, s), 2.34 (3H, s); ^{13}C NMR ($CDCl_3$) δ 138.2, 135.5, 135.5, 128.4, 128.2, 127.8, 126.9, 76.6, 72.3, 33.0, 20.2; EIMS *m/z* (rel int) 284 (4), 282 (5), 126 (78), 108 (42), 106 (47), 92 (67), 91 (100), 79 (67), 77 (91), 65 (83); HREIMS 282.0535 ($C_{13}H_{15}{}^{35}ClN_2OS$; Δ +5.0 mmu).

6-Bromoindole (12): A solution of 4-bromo-2-nitrotoluene (1.0 g, 4.65 mmol), dimethylformamide dimethyl acetal (1.6 mL, 12 mmol), and pyrrolidine (1 mL, 12 mmol) was heated for 4 h at 100-110 °C under argon with stirring. The dark red solution of the intermediate β-pyrrolidinostyrene was allowed to cool to RT and a solution of 4 M NH_4OAc (20 mL) was added. To the stirred solution was added dropwise 20% w/v aqueous $TiCl_3$ (17 mL, 22.0 mmol) and the resulting gray solution was stirred for 20 min and poured into a separatory funnel containing H_2O (150 mL). It was made basic (pH 10) with 1 M NaOH and the basic solution was extracted with ether (3 × 50 mL). The combined organic phase was dried ($MgSO_4$) and concentrated to give a red oil which was chromatographed over silica gel using CH_2Cl_2 to give **12** as a light tan solid (0.71 g, 77%): mp 91-94 °C (lit.[3c] 94 °C); ^1H NMR ($CDCl_3$) δ 8.02 (1H, br *s*), 7.55 (1H, d, *J* = 8.3 Hz), 7.50 (1H, d, *J* = 0.1 Hz), 7.28 (1H, dd, *J* = 8.3, 0.1 Hz), 7.15 (1H, dd, *J* = 3.2 Hz), 6.60 (1H, m); ^{13}C NMR ($CDCl_3$) δ 136.4, 126.6, 124.8, 123.0, 121.8, 115.3, 113.9, 102.6; EIMS *m/z* (rel int) 197 (98), 195 (100), 116 (93), 89 (38); HREIMS 194.9696 ($C_8H_6{}^{79}BrN$; Δ -1.2 mmu).

3-[(E)-2-Nitroethenyl]-6-bromoindole (15): To a mixture of 6-bromoindole (200 mg, 1.03 mmol) 1-dimethylamino-2-nitroethylene (102 mg, 0.88 mmol),[11] was added anhydrous TFA (1 mL) and the reaction was

stirred under N_2 for 30 minutes. The reaction mixture was then poured into saturated aqueous $NaHCO_3$ and the solution was extracted with EtOAc (2×). The combined organic phase was dried ($MgSO_4$), concentrated, and chromatographed over silica gel (CH_2Cl_2) to give **15** (261 mg, 96%) as yellow crystals: UV (EtOH) λ_{max} 385 (15,900), 287 (4,900), 229 (10,100) nm; ^{1}H NMR (DMSO-d_6) δ 12.28 (1H, s), 8.38 (1H, d, J = 13.5 Hz), 8.25 (1H, s), 8.02 (1H, d, J = 13.5 Hz), 7.93 (1H, d, J = 8.4 Hz), 7.71 (1H, d, J = 0.1 Hz), 7.33 (1H, dd, J = 8.4, 0.1 Hz). ^{13}C NMR (DMSO-d_6) δ 138.4, 136.5, 133.9, 131.8, 124.4, 123.6, 122.1, 115.8, 115.3, 108.1; EIMS *m/z* (rel int) 268 (11), 266 (10), 234 (36), 232 (40), 208 (26), 207 (100), 126 (27); HREIMS 265.9699 ($C_{10}H_7{}^{79}BrN_2O_2$; Δ -0.8 mmu).

6-Bromotryptamine (16): A dry 100 mL flask equipped with a septum inlet, magnetic stirring bar, and a reflux condenser was cooled to 0 °C and then charged with $NaBH_4$ (135 mg, 4.0 mmol) followed by sequential additions of THF (10 mL) and $BF_3{\cdot}OEt_2$ (564 μL, 4.4 mmol) at 0 °C. The ice bath was removed and the contents were stirred at RT for 15 min. A solution of **15** (200 mg, 0.75 mmol) in THF (2 mL) was then added dropwise into the reaction flask via syringe and the reaction mixture was heated at reflux for 2 h. After cooling to room temperature, the reaction was quenched by careful addition of ice. The mixture was acidified (1*N* HCl), and then heated at 80-85 °C for another 2 h. After cooling to RT, the acidic solution was washed with ether (2 × 15 mL), after which it was made basic with aqueous NaOH to liberate the amine. Solid NaCl was added and the product was extracted into ether (3 × 25 mL). The solution was dried ($MgSO_4$) and concentrated under reduced pressure to yield 6-bromotryptamine (131 mg, 73%): ^{1}H NMR ($CDCl_3$) δ 9.24 (1H, s), 7.43 (1H, s), 7.41 (1H, d, J = 8.6 Hz), 7.18 (1H, dd, J = 8.6, 0.1 Hz), 6.90 (1H, s), 2.98 (2H, t, J = 6.4 Hz), 2.84 (2H, t, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 137.4, 126.4, 123.1, 122.4, 120.1, 115.4, 114.3, 42.2, 29.1; EIMS *m/z* (rel int) 238 (16), 210 (98), 208 (100), 129 (61), 128 (33), 102 (24); HREIMS 238.0092 ($C_{10}H_{11}{}^{79}BrN_2$; Δ +1.4 mmu).

7-Bromo-1,2,3,4-tetrahydro-β-carboline (13): A solution of glyoxylic acid monohydrate (165 mg, 1.8 mmol) in H_2O (32 mL) was added dropwise with shaking to a solution of **16** (460 mg, 1.8 mmol) in H_2O (3 mL). A cooled solution of KOH (90 mg) in water (1.6 mL) was slowly added and the mixture was stirred for 1 h, during which time a precipitate formed. The solution was filtered and the damp yellow filter cake was collected and resuspended in H_2O (16 mL). Concentrated HCl (635 mL) was added and the solution was boiled for 30 min. Another 635 mL conc HCl was added and the solution stirred for 15 min as a dark precipitate formed. Basification to pH 10 with aqueous NaOH yielded pure **13** (307 mg, 73 %) as a tan solid: mp 180-183 °C; ^{1}H NMR (10% CD_3OD in $CDCl_3$),) δ 7.36 (1H, d, J = 0.1 Hz), 7.24 (1H, d, J = 8.6 Hz), 7.10 (1H, dd, J = 8.6, 0.1 Hz), 3.81 (2H, m), 3.08 (2H, m), 2.67 (2H, m); ^{13}C NMR (10% CD_3OD in $CDCl_3$) δ 136.5, 132.5, 126.1, 122.1, 118.8, 114.4, 113.6, 107.8, 43.3, 42.4, 21.7; HRFABMS (3-NBA/Gly/TFA) 251.0186 ($C_{11}H_{13}BrN_2$, MH^+; Δ +0.8 mmu)

7-Bromo-β-Carboline (5): To a solution of **13** (25-100 mg) in freshly distilled xylene (1-5 mL) was added S_8 (4 equiv.) and the resulting suspension was stirred at 128 °C for 4 h. The reaction was diluted with H_2O and extracted with EtOAc. The organic phase was concentrated and the crude material was chromatographed over silica gel (95:5, $CHCl_3$:MeOH) giving **5** in 5-15% yield: mp 203-204 °C; ^{1}H NMR ($CDCl_3$) δ 8.71 (1H, s), 8.25 (1H, d, J = 5.4 Hz), 7.91 (1H, d, J = 8.4 Hz), 7.88 (1H, d, J = 5.4 Hz), 7.63 (1H, d, J = 1.5 Hz), 7.30 (1H, dd, J = 8.4, 1.5 Hz); ^{1}H NMR (DMSO-d_6) δ 8.92 (1H, s), 8.35 (1H, d, J = 5.3 Hz), 8.20 (1H, d, J = 8.8 Hz), 8.12 (1H, d, J = 5.3 Hz), 7.77 (1H, d, J = 1.8 Hz), 7.38 (1H, dd, J = 7.7, 1.8 Hz); ^{13}C NMR (DMSO-d_6) δ 242.3, 238.6, 136.1, 134.4, 126.9, 123.6, 122.2, 120.8, 119.8, 114.7, 114.57; EIMS *m/z* (rel int) 248 (96), 246 (100), 223 (40), 221 (42), 167 (50), 140 (52); HREIMS 245.9796 ($C_{11}H_7{}^{79}BrN_2$; Δ -0.3 mmu).

14-[(Benzyloxy)methyl]didemnoline A (17): To a solution of powdered KOH (17 mg, 0.3 mmol) in dry DMSO (5 mL), which had stirred for 0.5 h at RT, was added **5** (12 mg, 0.05 mmol) and the mixture was stirred for another 2 h at RT. A solution of imidazole **7** (226 mg, 0.8 mmol) in DMSO (1 mL) was added dropwise and the reaction was stirred overnight. The solution was poured into H_2O and the resulting aqueous mixture was extracted with EtOAc (3×). The combined organic phase was then dried ($MgSO_4$) and concentrated

to **17** (16 mg) in 65% yield: UV (EtOH) λ_{max} 357 (2,900), 343 (2,800), 297 (9,900), 289 (7,300), 244 (24,600) nm; IR (film) ν 3420, 1723, 1654, 1617, 1554, 1477, 1434, 1327, 1254, 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.21 (1H, s),9.04 (1H, s), 8.38 (1H, br d, J = 4.8 Hz), 8.21 (1H, d, J = 8.4 Hz), 8.13 (2H, m), 8.00 (1H, s), 7.41 (1H, dd, J = 8.4, 1.9 Hz), 7.31-7.22 (5H, m), 5.63 (2H, s), 5.44 (2H, s), 4.44 (2H, s), 2.11 (3H, s); ^{13}C NMR (DMSO-d_6) δ 142.9, 141.6, 140.8, 139.0, 137.1, 136.2, 133.7, 128.3, 127.7, 127.6, 126.9, 123.6, 122.5, 121.1, 120.8, 119.7, 114.6, 113.8, 73.7, 69.8, 48.6, 20.4; HRFABMS (3-NBA/Gly/TFA) 493.0694 ($C_{24}H_{22}{}^{79}BrN_4OS$, MH^+; Δ -0.7 mmu))

14-[(Benzyloxy)methyl]didemnoline B (18): To a solution of powdered KOH (170 mg, 3.0 mmol) in dry DMSO (5 mL), which had stirred for 0.5 h, was added **6** (84 mg, 0.5 mmol) and the mixture was stirred for another 2 h at RT. A solution of imidazole **6** (226 mg, 0.8 mmol) in DMSO (1 mL) was added dropwise and the reaction was stirred overnight. The solution was poured into H_2O and the resulting aqueous mixture was extracted with EtOAc (3×). The combined organic phase was then dried ($MgSO_4$) and concentrated to **18** (125 mg) in 60% yield: UV (EtOH) λ_{max} 356 (3,000), 343 (25,000), 288 (7,500), 296 (10,000) nm; IR (film) ν 3054, 2923, 2862, 1727, 1673, 1620, 1557, 1489, 1366, 1323, 1258, 1208, 1077 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.30 (1H, s), 8.45 (1H, d, J = 5.4 Hz), 8.13 (1H, d, J = 7.8 Hz), 7.99 (1H, d, J = 5.4 Hz), 7.93 (1H, d, J = 8.3 Hz), 7.64 (1H, t, J = 7.1 Hz), 7.64 (1H, s), 7.31 (1H, t, J = 7.6 Hz), 7.29-7.20 (5H, m), 5.59 (2H, s), 5.37 (2H, s), 4.45 (2H, s), 2,03 (3H, s); ^{13}C NMR ($CDCl_3$) δ 143.4, 139.7, 136.3, 136.1, 131.1, 129.9, 129.3, 128.7, 128.3, 127.9, 122.2, 122.0, 120.9, 120.4, 114.9, 110.9, 73.6, 70.6, 40.9, 21.0; HRFABMS (3-NBA/Gly/TFA) 415.1597 ($C_{24}H_{24}N_4OS$, MH^+; Δ +1.0 mmu).

Didemnoline A (1): To a solution of **17** (25 mg, 0.05 mmol) in EtOH (5 mL) was added 6*N* HCl (1 mL) and the solution was stirred at 110 °C for 0.5 h. The reaction was poured into saturated $NaHCO_3$ and extracted with EtOAc. The organic phase was then concentrated to give pure **1** (17 mg) in 91% yield: UV (EtOH) λ_{max} 240 (38,700), 290 (13,500), 344 (sh), 358 (5,000) nm; IR (film) ν 3443, 1683, 1493, 1441, 1327, 1200, 1138, 1053, 963, 840, 802, 721 cm^{-1}; ^1H NMR (5% TFA-*d* in DMSO-d_6) δ 9.58 (1H, s), 9.06 (1H, s), 8.84 (1H, d, J = 6.2 Hz), 8.70 (1H, d, J = 6.2 Hz), 8.49 (1H, d, J = 8.4 Hz), 8.14 (1H, d, J = 1.6 Hz), 7.65 (1H, dd, J = 8.4, 1.6 Hz), 6.01 (2H, s), 2.30 (3H, s); ^{13}C NMR ($CDCl_3$) δ 141.6, 139.0, 137.6, 136.3, 133.6, 127.0, 123.7, 122.6, 121.2, 119.7, 114.7, 113.8, 39.0, 19.6; HRFABMS (3-NBA/Gly/TFA) 373.0119 ($C_{16}H_{14}{}^{79}BrN_4S$, MH^+; Δ -0.9 mmu).

Didemnoline B (2): To a solution of **18** (82.8 mg, 0.2 mmol) in EtOH (7 mL) was added 6*N* HCl (1 mL) and the solution was stirred at 110 °C for 0.5 h. The reaction was poured into saturated $NaHCO_3$ and extracted with EtOAc. The organic phase was then concentrated to give pure **2** (52 mg) in 87% yield: UV (EtOH) λ_{max} 358 (4,900), 243 (sh), 290 (14,100), 283 (sh), 238 (39,800), 216 (3,300) nm; IR (film) ν 3049, 1622, 1493, 1451, 1328, 1264, 1211, 1208, 1028, 815 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.14 (1H, s), 8.23 (1H, d, J = 5.4 Hz), 8.03 (1H, d, J = 7.8 Hz), 7.88 (1H, d, J = 5.4 Hz), 7.79 (1H, d, J = 8.3 Hz), 7.57 (1H, t, J = 8.1 Hz), 7.24 (1H, t, J = 7.3 Hz), 5.83 (2H, s), 2.76 (3H, s); ^{13}C NMR ($CDCl_3$) δ 141.8, 137.2, 136.8, 136.3, 131.4, 129.5, 129.1, 127.3, 123.1, 121.9, 120.8, 120.3, 114.9, 110.6, 39.5, 20.2; HRFABMS (3-NBA/Gly/TFA) 295.1021 ($C_{16}H_{15}N_4S$, MH^+; Δ +1.2 mmu).

Didemnoline C (3): To a solution of **1** (2 mg) in MeOH:H_2O (9:1, 2 mL) was added $NaIO_4$ (1.6 mg) at 0 °C. After stirring for 24 h at 25 °C, the solid particulate was removed by filtration and the filtrate was concentrated to give a residue that was chromatographed over and amino-bonded column ($CHCl_3$:MeOH, 95:5) to give **3** (1.5 mg) in 75% yield: UV (EtOH) λ_{max} 352 (4,000), 342 (sh), 288 (1,400), 243 (sh), 227 (34,000) nm; ^1H NMR (5% TFA-*d* in DMSO-d_6) δ 9.59 (1H, s), 8.86 (1H, d, J = 6.1 Hz), 8.70 (1H, d, J = 6.1 Hz), 8.51 (1H, d, J = 8.4 Hz), 8.27 (1H, d, J = 1.5 Hz), 8.23 (1H, s), 7.65 (1H, dd, J = 8.4, 1.5 Hz), 6.09 (2H, s), 2.88 (3H, s); ^{13}C NMR ($CDCl_3$) δ 142.9, 141.7, 138.8, 136.9, 135.6, 135.4, 133.9, 126.8, 123.4, 122.3, 121.3, 119.6, 114.4, 114.1, 39.0, 38.5; HRFABMS (3-NBA/Gly/TFA) 389.0061 ($C_{16}H_{14}BrN_4OS$, MH^+; Δ -2.7 mmu).

Didemnoline D (4): See procedure for synthetic didemnoline C above: UV (EtOH) λ_{max} 355 (3,400), 338 (3,400), 289 (10,200), 281 (sh), 236 (27,000) nm; IR (film) ν 3423, 3046, 2923, 1731, 1673, 1619, 1557, 1593, 1446, 1327, 1266, 1208 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.08 (1H, s), 8.36 (1H, d, J = 5.1 Hz), 8.24 (1H, d, J = 7.6 Hz), 8.11 (1H, d, J = 5.1 Hz), 7.77 (1H, s), 7.73 (1H, d, J = 8.3 Hz), 7.56 (1H, t, J = 7.6 Hz), 7.26 (1H, t, J = 7.6 Hz), 6.10 (2H, s), 2.88 (3H, s); ^{13}C NMR ($CDCl_3$) δ 140.5, 138.6, 138.0, 137.8, 136.0, 133.0, 132.9, 128.1, 127.4, 121.6, 120.6, 119.6, 114.3, 110.4, 39.2, 37.4; EIMS *m/z* (rel int) 310 (4), 293 (11), 207 (30), 169 (13), 168 (100), 141 (14), 140 (18),127 (16), 125 (25),91 (11); HREIMS 310.0919 ($C_{16}H_{14}N_4OS$, Δ -3.1 mmu)

ACKNOWLEDGEMENTS

This work was supported in part by the American Cancer Society, in the form of a Junior Faculty Research Award (JFRA-528) to B.S.D. Mass spectrometry was provided by the Washington University Mass Spectrometry Resource with support from the NIH National Center for Research Resources (Grant No. P41RR0954).

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