Nonsymmetrical *β*-Carboline Dimers from an Ascidian, *Didemnum* sp.

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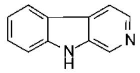
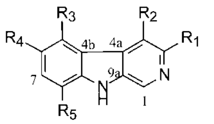
In addition to the previously reported symmetrical *β*-carboline dimer **1**, an ascidian *Didemnum* sp. yielded trace amounts of *β*-carboline and the nonsymmetrical *β*-carboline dimers **2**, **3**, and **5**. The structures of the nonsymmetrical dimers were confirmed by nonselective synthesis of **2** and **3** from *β*-carboline, which also yielded additional dimers **4** and **6**.

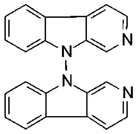
Ascidians have yielded a wide variety of secondary metabolites, many presumably derived from tryptophan, including gelliusines A and B,[1] woodinine,[2] and tiruchanduramine,[3] the last two incorporating the *β*-carboline (9*H*-pyrido[3,4-*b*]indole) structural unit. In an earlier publication,[4] we reported that the symmetrical *β*-carboline dimer **1** was the major product isolated from a *Didemnum* species of ascidian. We also noted in that report that there were trace amounts of several other UV fluorescing metabolites present in the extract, most likely nonsymmetrical *β*-carboline dimers, but not in sufficient quantities to enable full characterization.



β-carboline

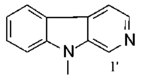


2 R_1 = Cby, R_2 = R_3 = R_4 = R_5 = H
3 R_1 = R_2 = R_3 = H, R_4 = Cby, R_5 = H
4 R_1 = R_2 = R_3 = R_4 = H, R_5 = Cby
5 R_1 = R_2 = H, R_3 = Cby, R_4 = R_5 = H
6 R_1 = H, R_2 = Cby, R_3 = R_4 = R_5 = H



1

Cby =



As further specimens of the ascidian were not found during subsequent collection trips to various areas of the Great Barrier Reef, we turned our attention to possible nonselective synthesis of the nonsymmetrical dimers, to allow full characterization of the natural metabolites. Although UV irradiation of *β*-carboline was reported to yield only the symmetrical dimer **1**,[5] irradiation of *β*-carbolines with a substituent at C-1 did yield N′,C-3 nonsymmetrical dimers,[6] in addition to the symmetrical dimer. We therefore reasoned that UV irradiation of a mixture of *β*-carboline and a suitable *N*-9 derivative of *β*-carboline might yield one or more unsymmetrical dimers.

Synthetic *β*-carboline was prepared from tryptophan,[7] and the product proved identical to the least polar of the *Didemnum* minor metabolites by TLC and ^{1}H NMR spectroscopy. *β*-Carboline has been previously reported from the ascidian *Ritterella sigillinoides* and other organisms.[8] As we did not observe **1** to undergo reduction, we believe *β*-carboline is a *Didemnum* metabolite, and not an artifact of the isolation.

Although we initially envisaged using one of the silyl protecting groups to block dimerization at N-9, it transpired that the N-9 lithium salt, formed by reacting *β*-carboline with BuLi, itself provided a derivative that allowed formation of small amounts of three nonsymmetrical dimers on photolysis. UV irradiation of a 1:2 mixture of *β*-carboline and its N-lithiated derivative in CH_2Cl_2 for 40 h yielded **1** as the major product, along with three minor products, shown below to be the nonsymmetrical *β*-carboline dimers **2**, **3**, and **4**, plus recovered *β*-carboline. All three synthetic dimers gave HRMS molecular ions in agreement with the formula $C_{22}H_{14}N_4$.

The ^{1}H NMR spectrum of *β*-carboline contains two isolated spin systems for the nonexchangeable protons: a broad singlet (H-1) and two doublets with a coupling constant of about 4 Hz (H-3, H-4) from the pyridine-like ring; two 8 Hz "doublets" (H-5, H-8) and two 8 Hz "triplets" (H-6, H-7) (with additional meta coupling and second-order effects) from the benzene-like ring, hereafter referred to as spin systems 1 and 2, respectively. The least polar of the *β*-carboline dimer products, **2**, showed ^{1}H NMR (Table 1) and COSY spectra with one exchangeable proton and four spin systems, one of type 1, two of type 2, and a fourth derived from type 1 with two singlet signals, most consistent with the N′,C-3, nonsymmetrical dimer **2**. All protons and carbons for **2** were fully assigned (Table 1) using HMBC and HMQC spectra. Substitution at C-3 was confirmed by NOESY correlations. One of the above singlet signals (H-4) of **2** gave correlations to H-5 in the same *β*-carboline unit, plus H-1′ and H-8′ in the second *β*-carboline unit; the other singlet (H-1) gave a correlation to the exchangeable proton. Dimer **2** was identical (TLC, HPLC retention time, ^{1}H NMR, UV fluorescence color) to the intermediate polarity minor *Didemnum* metabolite. Slight chemical shift differences between ^{1}H NMR spectra of **2** and the natural dimer were due to higher solute concentration in the synthetic dimer solution.

The second nonsymmetrical dimer **3** gave a ^{1}H NMR spectrum (Table 1) that showed two type 1 and one type 2 spin systems (as defined above), plus a spin system derived from type 2, consistent with N′-substitution at either C-6 or C-7. After full assignment of the proton and carbon signals (Table 1) by 2D NMR, the isomer **3** was confirmed by NOESY correlations. The doublet signal with meta coupling (H-5) gave correlations to H-4 in the same *β*-carboline unit and to both H-1′ and H-8′ in the other *β*-carboline unit. Dimer **3** was identical to the most polar minor *Didemnum* metabolite.

The third, most polar synthetic nonsymmetrical dimer **4** isolated from the product mixture was not observed in the *Didemnum* extracts. Its ^{1}H NMR spectrum (Table 1) also showed a spin system derived from type 2, but consistent with substitution at either C-5 or C-8. After assignment of all proton and carbon signals by 2D NMR (Table 1), C-8 substitution for **4** was confirmed from the NOESY spectrum, which showed correlations from the H-7 8 Hz "doublet" to H-1′ and H-8′ in the other *β*-carboline unit and a correlation from the H-5 "doublet" to H-4 in the same *β*-carboline unit.

One further nonsymmetrical dimer of *β*-carboline was isolated in very low yield from the *Didemnum* extracts. It could not be fully characterized; however ^{1}H NMR and COSY spectra from **5** showed a spin system derived from type 2, also consistent with substitution

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Table 1. NMR Spectroscopic Data for Dimers **2**, **3**, **4**, and **6** in DMSO-d_6

	2		**3**		**4**		**6**	
no.	δ_C	δ_H, mult.	δ_C	δ_H, mult.	δ_C	δ_H, mult.	δ_C	δ_H, mult.
1	133.2	9.12 s	134.5	9.10 br s	132.4	8.95 br s	131.3	8.86 br s
3	140.0		138.4	8.48 br d^{b}	136.3	8.61 br d^{b}	112.0	9.05 br s
4	111.1	8.68 s	115.2	8.30 d^{b}	115.8	8.56 d^{b}	130.9^{a}	
4b	130.4		127.4^{a}		129.5		134.9	
4c	120.7		122.1		123.1^{a}		120.5	
5	122.5	8.49 d^{c}	120.7	8.70 d^{d}	123.4	8.72 d^{c}	123.8	8.78 d^{c}
6	119.6	7.39 t^{c}	127.7^{a}		120.8	7.69 t^{c}	122.7	7.65 t^{c}
7	128.9	7.71 t^{c}	127.1	7.88 dd^{c}	128.5	8.02 d^{c}	130.5	7.77 t^{c}
8	112.2	7.78 d^{c}	113.6	7.99 d^{c}	120.1^{a}		109.7	7.25 d^{c}
8b	141.7		139.9		138.0		141.7	
9b	135.2		137.0		135.9		143.2^{a}	
1′	133.6	9.18 br s	132.7	8.83 br s	131.1	8.70 br s	133.3	9.33 br s
3′	139.4	8.60 br d^{b}	139.4	8.58 br d^{b}	137.7	8.67 br d^{b}	139.1	8.64 br d^{b}
4′	114.8	8.38 d^{b}	114.8	8.37 d^{b}	115.6	8.61 d^{b}	116.1	8.46 d^{b}
4b’	128.4		127.9		129.9		129.2	
4c’	121.0		120.7		121.1		121.4	
5′	122.0	8.50 d^{c}	122.1	8.49 d^{c}	122.7	8.64 d^{c}	122.3	8.54 d^{c}
6′	120.8	7.51 t^{c}	120.5	7.46 t^{c}	121.2	7.57 t^{c}	121.2	7.56 t^{c}
7′	128.9	7.74 t^{c}	128.9	7.70 t^{c}	129.7	7.70 t^{c}	129.4	7.80 t^{c}
8′	111.5	7.89 d^{c}	110.4	7.54 d^{c}	110.7	7.33 d^{c}	111.8	8.03 d^{c}
8b’	140.7		141.9		142.1		140.7	
9b’	136.1		137.2		136.6		135.8	
*N*9		12.0 br s		12.1 br s		11.9 br s		12.0 br s

[a] Values in the same column may be interchanged. [b] $J = 4$ Hz. [c] $J = 8$ Hz, with additional ($J = 1.5$ Hz) meta coupling. [d] $J = 1.5$ Hz.

at either C-5 or C-8. As the N′,C-8 dimer **4** has already been described, **5** must be the N′,C-5 isomer.

The oxidative coupling of β-carboline by $KMnO_4$ in acetone[9] was also briefly investigated to determine whether any of the *Didemnum* dimers might be synthesized by this route. However, apart from **1**, a very complex mixture of UV-absorbing minor products was formed, from which only one nonsymmetrical dimer was isolated in pure form. This isomer, like **2**, showed a ^{1}H NMR spectrum (Table 1) with two type 2 and one type 1 spin systems, plus a modified type 1 spin system with two broadened singlets. It must therefore be the N′,C-4 dimer **6**. All further spectra for **6** were consistent with this structure. Re-examination of the *Didemnum* extracts did not provide any evidence that **6** was a natural product from the ascidian.

Experimental Section

General Experimental Procedures. Ultraviolet spectra were recorded on a Varian Cary 4E spectrophotometer. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR as KBr disks. All NMR spectral data were recorded on an Avance spectrometer at 400 and 100 MHz, respectively, using standard parameter sets, and calibrated using the respective solvent peaks. DMSO-d_6: δ 2.60 and 39.5 ppm; $CDCl_3$: δ 7.26 and 77.0 ppm. A small quantity of Et_3N was added to very dilute NMR solutions, to reduce signal broadening due to trace acid impurities in the solvents. EIMS and HREIMS spectra were recorded on a Micromass Autospec mass spectrometer. UV photolysis experiments were performed using a Hanovia 450 W medium-pressure Hg lamp, without filter. Normal-phase TLC was performed on Merck DC-Alufolien Kieselgel 60 F_{254} sheets. The TLC plates were visualized at both short (254 nm) and long (366 nm) wavelengths. Crude extracts and other impure mixtures were subjected to vacuum-assisted liquid chromatography[10] on Merck Kieselgel 60PF (TLC) SiO_2. HPLC separations were performed using a NovaPak Si gel analytical column (60 Å, 4 μm, 3.9 × 150 mm) and compounds detected using a GBC LC1200 UV−visible detector.

Animal Material. As per previous description.[4]

Extraction and Isolation. The freeze-dried ascidian (42 g) was successively extracted using hexanes, EtOAc, and $(CH_3)_2CO$, as previously described.[4] After removal of **1**, the residual components were purified by column chromatography on SiO_2 [EtOAc−HOAc (99:1)] to afford β-carboline (1 mg, 0.002% dry weight) and **2** (2 mg, 0.004% dry weight). The more polar dimers were rechromatographed on SiO_2 using EtOAc−$(CH_3)_2CO$−Et_3N (80:20:1) as the solvent and afforded two fractions rich in **3** and **5**. Dimer **3** was further purified by HPLC [EtOAc−$(CH_3)_2CO$−Et_3N (79.5:20:0.5)] (0.5 mg, 0.0007% dry weight). Dimer **5** was purified by HPLC [EtOAc−Et_3N (99.5:0.5)] (<0.5 mg). HPLC retention times, 1 mL/min, EtOAc−$(CH_3)_2CO$−Et_3N (79.5:20:0.5): **1**, 3.6; β-carboline, 5.5; **2** and **5**, 7.8; **3**, 22 min.

β-Carboline: pale yellow film; ^{1}H NMR ($CDCl_3$) 7.29 (t, $J = 8$ Hz), 7.54 (m), 7.58 (m), 7.98 (br d, $J = 4$ Hz), 8.16 (d, $J = 8$ Hz), 8.32 (br s), 8.48 (br d, $J = 4$ Hz), 8.94 (br s); EIMS *m/z* 168 (5), 167 (34), 140 (14), 35 (5), 32 (100).

N′,C-3 Nonsymmetrical dimer (2): pale yellow film; ^{1}H NMR (DMSO-d_6) 7.30 (t, $J = 8$ Hz), 7.41 (t, $J = 8$ Hz), 7.62 (t, $J = 8$ Hz), 7.65 (t, $J = 8$ Hz), 7.70 (t, $J = 8$ Hz), 7.80 (d, $J = 8$ Hz), 8.28 (br d, $J = 4$ Hz), 8.40 (d, $J = 8$ Hz), 8.40 (d, $J = 8$ Hz), 8.50 (d, $J = 4$ Hz), 8.59 (s), 9.02 (s), 9.06 (br s).

N′,C-6 Nonsymmetrical dimer (3): pale yellow film; ^{1}H NMR (DMSO-d_6) 7.40 (t, $J = 8$ Hz), 7.45 (d, $J = 8$ Hz), 7.61 (t, $J = 8$ Hz), 7.80 (dd, $J = 8$, 1.5 Hz), 7.89 (d, $J = 8$ Hz), 8.20 (br d, $J = 4$ Hz), 8.28 (br d, $J = 4$ Hz), 8.39 (br d, $J = 8$ Hz), 8.40 (d, $J = 8$ Hz), 8.49 (br d, $J = 4$ Hz), 8.62 (d, $J = 1.5$ Hz), 8.75 (br s), 9.02 (br s).

5,9′-Bi-9*H*-pyrido[3,4-*b*]indole (N′,C-5 nonsymmetrical dimer) (5): pale yellow film; ^{1}H NMR ($CDCl_3$) 7.26 (d, $J = 8$ Hz), 7.40 (t, $J = 8$ Hz), 7.45 (t, $J = 8$ Hz), 7.54 (d, $J = 8$ Hz), 7.79 (t, $J = 8$ Hz), 8.05 (d, $J = 4$ Hz), 8.08 (d, $J = 4$ Hz), 8.30 (d, $J = 8$ Hz), 8.35 (d, $J = 8$ Hz), 8.56 (d, $J = 4$ Hz), 8.58 (d, $J = 4$ Hz), 8.62 (s), 8.80 (s); EIMS *m/z* 334 (100), 167 (25).

Synthesis of β-Carboline Dimers. BuLi (2.45 mL, 1.23 M in *n*-hexane) was added slowly, via a syringe, to a solution of β-carboline (386 mg, 2.3 mmol) in dry THF (8 mL) at −78 °C under an atmosphere of dry Ar. The mixture was stirred for 10 min at this temperature, then raised to room temperature, and the THF was evaporated. CH_2Cl_2 (70 mL) and β-carboline (202 mg, 1.2 mmol) were then added, and the mixture was irradiated in Pyrex for 40 h. The mixtures obtained were chromatographed on SiO_2 using gradient chromatography with CH_2Cl_2−Et_3N (99:1) to $(CH_3)_2CO$−Et_3N (99:1), via EtOAc−Et_3N (99:1), as the solvents. The fractions from each column were combined on the basis of TLC analysis to give fractions rich in **1** (38 mg), β-carboline (430 mg), **2** (5 mg), **3** (6 mg), and **5** (5 mg). The fractions rich in **2** and **3** were purified by column chromatography on SiO_2 [EtOAc−Et_3N (99:1)] and [EtOAc−$(CH_3)_2CO$−Et_3N (79:20:1)], respectively, to afford pure **2** (2.7 mg, 0.008 mmol, 0.67% yield) and **3** (3.7 mg, 0.011 mmol, 0.92% yield). The fractions that were rich in **5** were purified by SiO_2 HPLC [EtOAc−Et_3N (99.8:0.2)] to afford pure **5** (3 mg, 0.009 mmol, 0.75% yield).

3,9′-Bi-9*H*-pyrido[3,4-*b*]indole (2): pale yellow, amorphous solid; UV (CH_3OH) λ_{max} (log ϵ) 235 (5.07), 261 (4.83), 277 (4.38), 287 (4.76), 353 (4.26) nm; IR (KBr) ν_{max} 3446, 3042, 2932, 1640, 1540, 1500, 1380, 1240, 1155, 1040, 833, 745 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 335 (23), 334 (100), 333 (44), 307 (5), 167 (20), 166 (5), 140 (7); HREIMS *m/z* 334.1220 (calcd for $C_{22}H_{14}N_4$, 334.1218).

6,9′-Bi-9*H*-pyrido[3,4-*b*]indole (3): pale yellow, amorphous solid; UV (EtOH) λ_{max} (log ϵ) 231 (4.72), 288 (4.22), 353 (3.76) nm; IR (KBr) ν_{max} 3419, 3050, 2950, 1624, 1560, 1499, 1438, 1384, 1330, 1278, 1239, 1150, 1037, 821, 747 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 335 (23), 334 (100), 333 (15), 168 (13), 167 (13), 140 (5); HREIMS *m/z* 334.1220 (calcd for $C_{22}H_{14}N_4$, 334.1218).

8,9′-Bi-9*H*-pyrido[3,4-*b*]indole (4): pale yellow, amorphous solid; UV (EtOH) λ_{max} 235 (4.27), 287 (3.82), 353 (3.43) nm; IR (KBr) ν_{max} 3445, 3078, 2960, 1633, 1535, 1500, 1372, 1236, 1150, 1039, 962, 831, 745 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 335 (17), 334 (100), 333 (36), 332 (12), 212 (5), 167 (10), 149 (10); HREIMS *m/z* 334.1209 (calcd for $C_{22}H_{14}N_4$, 334.1218).

$KMnO_4$ Coupling of *β*-Carboline. $KMnO_4$ (3 g, 19 mmol) was added to a $(CH_3)_2CO$ solution of β-carboline (534 mg, 3.2 mmol, 40 mL) and heated under refluxing conditions for 17.5 h. Column chromatography of the reaction mixture on SiO_2 afforded two fractions: A (237 mg) and B (192 mg). The first fraction was purified by column chromatography on SiO_2 using CH_2Cl_2−EtOAc gradients to afford **1** (151 mg, 0.45 mmol, 28% yield) and recovered β-carboline (86 mg, 0.51 mmol). Rechromatography of fraction B on SiO_2 using $CHCl_3$−EtOAc mixtures as the solvents afforded **6** (4 mg, 0.012 mmol, 0.75% yield). The other components of this fraction were inseparable.

4,9′-Bi-9*H*-pyrido[3,4-*b*]indole (6): pale yellow, amorphous solid; UV (EtOH) λ_{max} (log ϵ) 202 (4.35), 231 (4.21), 278 (3.91), 351 (3.28) nm; IR (KBr) ν_{max} 3392, 3057, 2953, 1627, 1541, 139 1497, 1374, 1241, 1145, 1080, 1036, 962, 832, 745 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 334 (100), 333 (45), 281 (20), 243 (15), 231 (25), 205 (10), 181 (27), 167 (10), 153 (7); HREIMS *m/z* 334.1205 (calcd for $C_{22}H_{14}N_4$, 334.1218).

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