ES-242 Derivatives and Cycloheptapeptides from *Cordyceps* sp. Strains BCC 16173 and BCC 16176

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Five new ES-242 analogues (**1**–**5**) were isolated together with nine known compounds (**6**–**14**) from the insect pathogenic fungus *Cordyceps* sp. BCC 16173. A closely related strain, BCC 16176, provided cordyheptapeptide A (**15**) and small amount of its new analogue, cordyheptapeptide B (**16**), along with known ES-242s. Structures of the new bioxanthracenes, **1**–**5**, were determined to be 6′-*O*-desmethyl analogues of **6** (ES-242-4), **8**, **9** (ES-242-2), **12**, and **13**, respectively, primarily by spectroscopic analyses. Cordyheptapeptide B (**16**) has an *N*-methyl-L-phenylalanine residue instead of the *N*-methyl-L-tyrosine in **15**.

ES-242s are bioxanthracenes isolated from a fungus, *Verticillium* species.[1] These compounds are known to exhibit potent activity as *N*-methyl-D-aspartate (NMDA) receptor antagonists.[2] Tatsuta and co-workers have achieved the synthesis of key ES-242s and their analogues, which also established the relative and absolute stereochemistry of this class of compounds.[3] We previously reported the isolation, structure elucidation, and antimalarial activity of ES-242s and their analogues from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620 (on a Lepidoptera larva).[4,5] Recently, a cycloheptapeptide, cordyheptapeptide A, and some known ES-242s were isolated from an unidentified *Cordyceps* species, strain BCC 1788 (on an elaterid larva).[6] In a continuing search for bioactive compounds from insect pathogenic fungi,[7] we noticed that culture extracts of six *Cordyceps* sp. strains, collected in the same location (on Coleoptera larvae, at Doi Innthanon National Park, Chiang Mai Province, Thailand) during a collection trip in 2004, showed similar ^{1}H NMR spectra. This suggested the presence of bioxanthracenes (ES-242s) and cordyheptapeptide A as major constituents. Two of these strains, BCC 16173 (relatively rich in bioxanthracenes) and BCC 16176 (relatively rich in cordyheptapeptide A), have been subjected to mass fermentation (15 L) and chemical investigation. As a result, five new ES-242 analogues, **1**–**5**, along with nine known compounds, **6**–**14**,[5] and cordyheptapeptide A (**15**)[6] were isolated from *Cordyceps* sp. BCC 16173. A new analogue of **15**, named cordyheptapeptide B (**16**), was isolated, together with **15** and known ES-242s, from the fermentation of strain BCC 16176. We report here the isolation, structure elucidation, and biological activities of the new compounds **1**–**5** and **16**.

Results and Discussion

Cordyceps sp. BCC 16173 was fermented in yeast extract sucrose medium (60 × 250 mL; 15 L). The organic extract from mycelia was subjected to silica gel column chromatography and preparative HPLC (ODS column) to furnish 14 bioxanthracenes (**1**–**14**) and cordyheptapeptide A (**15**). Major constituents were ES-242-4 (**6**, 330 mg), ES-242-2 (**9**, 523 mg), and **15** (252 mg).

The ^{1}H and ^{13}C NMR spectra of compound **1** were similar to those of ES-242-4 (**6**),[1b] but it was nonsymmetrical and lacked one methoxyl resonance. The molecular formula of **1** ($C_{31}H_{32}O_{10}$), determined by HRMS (ESI-TOF), suggested that this compound was an *O*-desmethyl analogue of ES-242-4 (**6**). The corresponding phenolic proton resonance was not observed in the ^{1}H NMR ($CDCl_3$) spectrum. The chemical shift of the three OCH_3 groups, δ_H 4.00 and 4.02 (δ_C 56.2 and 56.3) and δ_H 3.46 (δ_C 55.2), indicated that the missing OCH_3 group was the upfield one; hence, the free OH in **1** was assignable to C-6/C-6′. Detailed analyses of NMR data (^{1}H, ^{13}C, DEPT, COSY, HMQC, and HMBC) confirmed that two tricyclic units joined at the two quaternary carbons C-10 and C-10′ (Figure 1). The relative configuration at C-3/C-4 and at C-3′/C-4′ was addressed by analysis of the vicinal *J* values and NOESY correlations. The NOESY cross-peak of H-3 (δ_H 3.64, m) and one of the diastereotopic H-1 methylene (δ_H 4.72, d, J = 15.7 Hz) indicated pseudoaxial orientation of these protons. H-4 resonated as a broad and narrow singlet, showing a small *J* value between H-3 and H-4. Therefore, H-4 was placed in the pseudoequatorial position. Similarly, H-3′ and H-4′ should occupy pseudoaxial and pseudoequatorial positions, respectively (Figure 1). These relative configurations are the same as in ES-242-4 (**6**) and all other ES-242s. Finally, the structures of **1** and **6** were correlated by O-methylation. Compound **1** was treated with excess MeI/K_2CO_3 in 2-butanone (rt, 12 h) to afford a major reaction product identical to **6** (MS, ^{1}H NMR). O-Methylation of chelated hydroxyl groups, 9-OH and 9′-OH, did not occur under the reaction conditions.

Compound **2** was obtained in very low quantity (1.7 mg). The molecular formula ($C_{31}H_{32}O_{10}$), determined by HRMS (ESI-TOF), was the same as that of **1**. The ^{1}H and ^{13}C NMR chemical shifts for **2** were similar to those of compound **8**, the atropisomer of ES-242-4 (**6**),[3a,5] and the spectra also indicated the presence of three OCH_3 groups. The chemical shifts of the OCH_3 protons, δ_H 4.09, 4.07, and 3.47, were consistent with the 6′-*O*-desmethyl structure. Detailed analyses of 2D-NMR data revealed that this compound was a C-10–C-10′ dimer of two tricyclic units and that the relative configurations at C-3/C-4 and C-3′/C-4′ are the same as those of other ES-242s. On the basis of the comparison of the ^{1}H and ^{13}C NMR data with those of **1**, **6**, and **8**, compound **2** was proposed to be the atropisomer of **1**, hence, the 6′-*O*-desmethyl derivative of **8**.

Compound **3** ($C_{35}H_{36}O_{12}$, HRESIMS) possessed two acetoxy groups, as in the known co-metabolite ES-242-2 (**9**).b In particular, the characteristic upfield acetyl protons resonated at δ_H 1.14 and 1.16, due to shielding by an aromatic ring from another side, and were similar to those of the symmetric molecule **9** (δ_H 1.13, 6H). Since compound **3** lacked one of the relatively upfield OCH_3 groups, it was assigned as the 6′-*O*-desmethyl derivative of **8**. Methylation (MeI/K_2CO_3, in 2-butanone) of **3** gave **9** as the major product.

The molecular formula of compound **4** was determined by HRESIMS as $C_{31}H_{32}O_{10}$, the same as **1** and **2**. The ^{1}H NMR and ^{13}C NMR spectra resembled those of the known C-10–C-5′ dimer **12**.[5] Detailed analysis of the NMR data revealed that C-10 and C-5′ were quaternary aromatic carbons, while C-5 and C-10′ were methines (Figure 2). Compound **4** also possessed three OCH_3 groups (δ_H 4.14, 4.08, and 3.45). The HMBC and NOESY correlation data enabled assignments of OCH_3 groups at C-6 (δ_H 3.45) and C-8 (δ_H 4.08); therefore, the remaining position, C-6′ or C-8′, was substituted with an OH group. The downfield shift of the remaining

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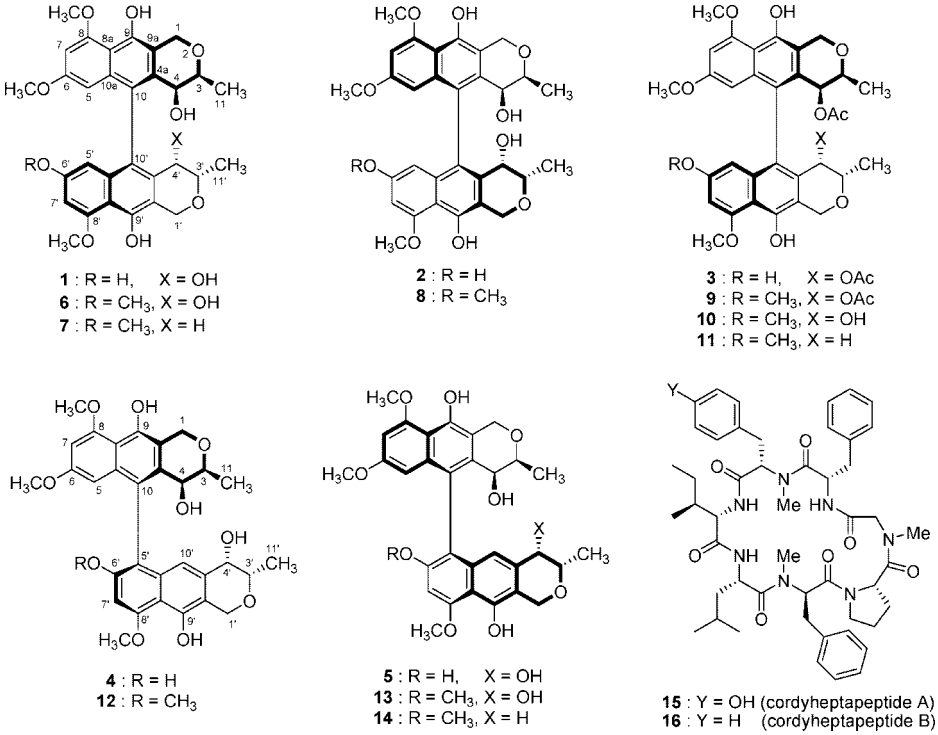
Chart 1



OCH_3 signal (δ_H 4.14) was in good agreement with its attachment to C-8 but not to C-6.[5] On the basis of the NOESY analysis and *J* values, as described for **1**, the relative configurations at C-3/C-4 and C-3′/C-4′ were the same as those of other ES-242s. The intense NOESY cross-peak for H-4 and H-10′ indicated the configuration at the C-10–C-5′ junction to be as depicted in structure **4** (Figure 2). Again, methylation of **4** gave the major product whose MS and ^{1}H NMR ($CDCl_3$) spectra were identical to those of the co-metabolite **12**.

Interpretation of the spectroscopic data for compound **5** ($C_{31}H_{32}O_{10}$, HRESIMS) led to the conclusion that it possessed the same planar structure as **4**. Again, the relative configuration at C-3/C-4 and C-3′/C-4′ was identical to that of other ES-242s. The ^{1}H and ^{13}C NMR chemical shifts for **5** were more similar to those of **13** than **12**.[5] Compound **5** is, therefore, the atropisomer of **4**, which is also designated as the 6′-*O*-desmethyl analogue of **13**.

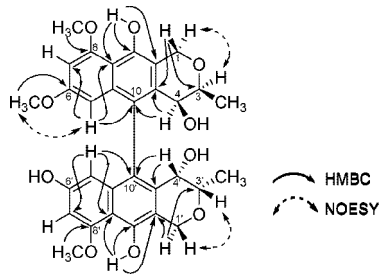


Figure 1. Selected HMBC and NOESY correlations for **1**.

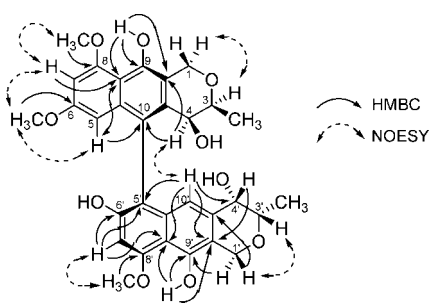


Figure 2. Selected HMBC and NOESY correlations for **4**.

The mycelia extract of *Cordyceps* sp. BCC 16176 contained a large amount of cordyheptapeptide A (**15**, 603 mg)[6] and a new minor analogue, cordyheptapeptide B (**16**, 6.3 mg). The molecular formula of **16** ($C_{49}H_{65}N_7O_7$) was determined by HRESIMS, which was one oxygen atom less than **15**. The ^{1}H and ^{13}C NMR spectra of **16** were similar to those of **15**. The only difference was the absence of the *p*-hydroxyphenyl group (two doublets, 2 × 2H) proton resonances of *N*-methyl-tyrosine (*N*-Me-Tyr) in **16** and the presence of three phenyl groups. This difference suggested the replacement of *N*-Me-Tyr with *N*-methyl-phenylalanine (*N*-Me-Phe). The structures of the seven amino acid residues were elucidated by analysis of 2D NMR data: two *N*-Me-Phe, a phenylalanine (Phe), a leucine (Leu), an isoleucine (Ile), a proline (Pro), and a sarcosine (Sar). The absolute configuration of cordyheptapeptide A (**15**) was previously determined by combination of X-ray crystallography (relative configuration) and chiral column HPLC analysis of the acid hydrolyzate. Only the *N*-Me-Phe residue possessed a D-(2*R*)-configuration.[6] The amino acid configurations of **16** were also determined by HPLC analysis of the acid hydrolyzate using a chiral column and by comparison with **15**. The difference of the HPLC chromatograms was the absence of the *N*-Me-L-Tyr peak (t_R 15.0 min) in the hydrolyzate of **16** and the appearance of an additional peak at 42.2 min. Other peaks in the hydrolyzate of **15** and **16** were identical, as confirmed by co-injection. The new peak in the hydrolyzate of **15** was that of *N*-Me-L-Phe, identical to that of the standard amino acid.

New compounds **1**, **3**, **5**, and **16**, and respective closely related compounds **6**, **9**, **13**, and **15**, were tested for activity against the malarial parasite *Plasmodium falciparum* K1 and cytotoxicity to KB cells (oral human epidermoid carcinoma), BC cells (human breast cancer), NCI-H187 cells (human small cell lung cancer), and noncancerous Vero cells (African green monkey kidney fibroblasts). Because of the sample shortage, compounds **2** and **4** were not tested. Among the ES-242s, compound **5** exhibited moderate antimalarial activity (IC_{50} 3.3 μM), whereas other analogues showed less than 50% inhibition at 10 μg/mL (15−18 μM). However, compounds **6** (ES-242-4), **9** (ES-242-2), and **13** showed respective IC_{50} values of 8.1, 3.3, and 12 μM in our previous assay.[4] Cordyheptapeptide A (**15**) exhibited antimalarial activity with an IC_{50} value of 3.8

μM, while cordyheptapeptide B (**16**) was inactive at 10 μg/mL (12 μM). In contrast, both cyclic peptides showed moderate cytotoxicity. IC_{50} values of **15** against KB, BC, NCI-H187, and Vero cells were 0.78, 0.20, 0.18, and 14 μM, respectively. Corresponding IC_{50} values of **16** were 2.0, 0.66, 3.1, and 1.6 μM.

Experimental Section

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a Varian CARY 1E UV–visible spectrophotometer. FT-IR spectra were recorded on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on a Bruker AV500D spectrometer. ESI-TOF mass spectra were measured with a Micromass LCT mass spectrometer.

Fungal Material. *Cordyceps* species used in this study were isolated on Coleoptera larvae collected in Doi Innthanon National Park, Chiang Mai Province, Thailand, by Ms. Kanoksri Tasanatai of the BIOTEC. These fungi were deposited in the BIOTEC Culture Collection (BCC) as BCC 16173 and BCC 16176 on November 12, 2004.

Fermentation of BCC 16173, Extraction, and Isolation. *Cordyceps* sp. BCC 16173 was maintained on potato dextrose agar at 25 °C. The agar was cut into pieces (1 × 1 cm) and inoculated into 6 × 250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB; potato starch 4.0 g, dextrose 20.0 g, per liter). After incubation at 25 °C for 7 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated at 25 °C for 7 days on a rotary shaker (200 rpm). Each 25 mL portion of the secondary cultures (in 6 flasks) was transferred into 60 × 1 L Erlenmeyer flasks each containing 250 mL of yeast extract sucrose medium (YES; yeast extract 20 g, sucrose 150 g, per liter), and final fermentation was carried out at 25 °C for 24 days under static conditions. The cultures were filtered to separate mycelia and filtrate. Mycelia were macerated in MeOH (2.5 L, rt, 2 days), then filtered. To the filtrate were added H_2O (200 mL) and hexane (600 mL). The aqueous MeOH layer was concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with H_2O, and concentrated under reduced pressure to leave a brown solid (4.60 g). This crude extract was subjected to column chromatography (CC) on silica gel (5.5 × 20 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 15:85) to obtain seven fractions (1–7): 1 (1780 mg), 2 (77 m g), 3 (23 mg), 4 (20 mg), 5 (19 mg), 6 (81 mg), and 7 (352 mg). Fraction 7 contained mostly **15**, which was further purified by silica gel CC (elution with MeOH/CH_2Cl_2, 252 mg). Each of the other fractions was repeatedly fractionated by CC on silica gel (MeOH/CH_2Cl_2) and preparative HPLC using a reversed-phase column (Nova-Pak HR C_{18}, 2.5 × 10 cm, 6 μm; mobile phase MeCN/H_2O, 45:55 or 40:60; flow rate 8 mL/min) to furnish 14 compounds: **1** (54.9 mg, from fraction 1), **2** (1.7 mg, from fraction 6), **3** (37.2 mg, from fraction 1), **4** (13.7 mg, from fractions 1 and 2), **5** (36.2 mg, from fractions 2 and 6), **6** (ES-242-4; 330.2 mg, from fractions 1 and 2), **7** (ES-242-5; 48.9 mg, from fraction 1), **8** (19.5 mg, from fractions 2, 3, and 4), **9** (ES-242-2; 523 mg, from fractions 1 and 2), **10** (ES-242-3; 28.3 mg), **11** (ES-242-1; 31.5 mg) and **12** (24.9 mg) from fraction 1, **13** (5.0 mg, from fraction 6), and **14** (ES-242-8; 4.9 mg, from fraction 5).

Fermentation of BCC 16176, Extraction, and Isolation. BCC 16176 was fermented and extracted in the same way and scale as described for BCC 16173. The mycelium extract (2.73 g) was subjected to column chromatography on silica gel (5.5 × 20 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 20:80) to separate into 12 fractions (1–12): 1 (128 mg), 2 (1193 mg), 3 (33 mg), 4 (79 mg), 5 (18 mg), 6 (11 mg), 7 (222 mg), 8 (303 mg), 9 (49 mg), 10 (41 mg), 11 (38 mg), and 12 (110 mg). Fraction 1 was mainly **9** (ES-242-2). Fraction 2 was chromatographed on silica gel (4.0 × 20 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 15:85) to furnish **10** (ES-242-1, 5.3 mg, fraction 2-1), **9** (ES-242-2, 793 mg, fraction 2-2), and a peptide-containing subfraction (7.9 mg, fraction 2-9). The subfraction 2-9 was subjected to preparative HPLC (MeCN/H_2O = 40:60) to yield **16** (3.3 mg). Additional **16** (3.0 mg) was obtained from fraction 3 by silica gel CC and preparative HPLC. Fractions 4 and 9 were combined and purified by CC on silica gel to obtain **15** (603 mg).

Compound 1 (6′-*O*-desmethyl ES-242-4): pale yellow solid; mp 179–180 °C (dec); $[\alpha]^{26}_D$ −7 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3406, 1623, 1360, 1092, 984, 830 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.53 (1H, s, 9′-O *H*), 9.47 (1H, s, 9-O *H*), 6.45 (2H, d, J = 1.8 Hz, H-7 and H-7′), 6.11 (1H, d, J = 1.8 Hz, H-5′), 5.98 (1H, d, J = 1.8 Hz, H-5), 5.16 (1H, d, J = 15.7 Hz, Ha-1), 4.75 (1H, d, J = 15.7 Hz, Ha-1′), 4.72 (1H, d, J = 15.7 Hz, Hb-1), 4.63 (1H, d, J = 15.7 Hz, Hb-1′), 4.02 (3H, s) and 4.00 (3H, s) (8-OC*H*$_3$ and 8′-OC*H*$_3$), 3.80 (1H, s, H-4), 3.77 (1H, s, H-4′), 3.66 (1H, m, H-3′), 3.64 (1H, m, H-3), 3.46 (3H, s, 6-OC*H*$_3$), 1.23 (3H, d, J = 6.5 Hz, H-11), 1.21 (3H, d, J = 6.5 Hz, H-11′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.7 (s) 157.6 (s) and 157.4 (s) (C-6, C-8, and C-8′), 154.5 (s, C-6′), 149.9 (s) and 149.8 (s) (C-9 and C-9′), 136.1 (s, C-10a), 136.1 (s, C-10a′), 135.0 (s, C-4a), 134.8 (s, C-4a′), 123.9 (s, C-10), 123.1 (s, C-10′), 113.3 (s) and 113.2 (s) (C-9a and C-9a′), 110.3 (s, C-8a), 109.8 (s, C-8a′), 101.8 (d, C-5′), 98.4 (d, C-7), 98.1 (d, C-7′), 97.9 (d, C-5), 74.0 (d, C-3′), 73.8 (d, C-3), 66.5 (d, C-4), 66.5 (d, C-4′), 65.0 (t, C-1), 64.5 (t, C-1′), 56.3 (q) and 56.2 (q) (8-OCH_3 and 8′-OCH_3), 55.2 (q, 6-OCH_3), 16.9 (q) and 16.8 (q) (C-11 and C-11′); HRMS (ESI-TOF) *m/z* 587.1902 $[M + Na]^+$ (calcd for $C_{31}H_{32}O_{10}Na$, 587.1893).

Compound 2: pale yellow solid; $[\alpha]^{26}_D$ −69 (*c* 0.05, MeOH); ^1H NMR ($CDCl_3$, 500 MHz) δ 9.53 (1H, s) and 9.47 (1H, s) (9-O*H* and 9′-O*H*), 6.49 (1H, d, J = 2.0 Hz, H-7′), 6.48 (1H, d, J = 2.1 Hz, H-7), 5.94 (1H, d, J = 2.0 Hz, H-5′), 5.89 (1H, d, J = 2.1 Hz, H-5), 5.22 (1H, d, J = 15.9 Hz) and 5.18 (1H, d, J = 15.8 Hz) (Ha-1 and Ha-1′), 4.92 (1H, d, J = 15.9 Hz) and 4.80 (1H, d, J = 15.8 Hz) (Hb-1 and Hb-1′), 4.09 (3H, s) and 4.07 (3H, s) (8-OCH_3 and 8′-OCH_3), 3.88 (2H, br s, H-4 and H-4′), 3.67–3.66 (2H, m, H-3 and H-3′), 3.47 (3H, s, 6-OCH_3), 1.24 (3H, d, J = 6.4 Hz) and 1.23 (3H, d, J = 6.4 Hz) (H-11 and H-11′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.9 (s) 157.7 (s) and 157.5 (s) (C-6, C-8, and C-8′), 153.9 (s, C-6′), 149.9 (s) and 149.7 (s) (C-9 and C-9′), 137.6 (s, C-4a), 137.6 (s, C-4a′), 135.6 (s, C-10a), 135.6 (s, C-10a′), 123.6 (s) and 123.0 (s) (C-10 and C-10′), 114.1 (s) and 113.8 (s) (C-9a and C-9a′), 110.4 (s, C-8a), 110.4 (s, C-8a′), 100.8 (d, C-5′), 98.1 (d, C-7), 97.6 (d, C-7′), 97.3 (d, C-5), 73.4 (d) and 732 (d) (C-3 and C-3′), 65.6 (d) and 66.5 (d) (C-4 and C-4′), 65.1 (t) and 64.5 (t) (C-1 and C-1′), 56.4 (q) and 56.3 (q) (8-OCH_3 and 8′-OCH_3), 55.2 (q, 6-OCH_3), 17.1 (q) and 17.0 (q) (C-11 and C-11′); HRMS (ESI-TOF) *m/z* 587.1909 $[M + Na]^+$ (calcd for $C_{31}H_{32}O_{10}Na$, 587.1893).

Compound 3 (6′-*O*-desmethyl ES-242-2): pale yellow solid; mp 190–192 °C (dec); $[\alpha]^{26}_D$ +50 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3407, 1737, 1625, 1364, 1230, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.48 (1H, s) and 9.47 (1H, s) (9-O*H* and 9′-O*H*), 6.45 (1H, d, J = 2.2 Hz, H-7′), 6.44 (1H, d, J = 2.2 Hz, H-7), 5.89 (2H, m, H-5 and H-5′), 5.41 (2H, m, H-4 and H-4′), 5.28 (1H, d, J = 15.6 Hz) and 5.23 (1H, d, J = 15.6 Hz) (Ha-1 and Ha-1′), 4.87 (1H, d, J = 15.6 Hz) and 4.85 (1H, d, J = 15.6 Hz) (Hb-1 and Hb-1′), 4.07 (3H, s, 8′-OCH_3), 4.05 (3H, s, 8-OCH_3), 3.97 (1H, m) and 3.95 (1H, m) (H-3 and H-3′), 3.42 (3H, s, 6-OCH_3), 1.16 (3H, s) and 1.14 (3H, s) (4-OCOCH_3 and 4′-OCOCH_3), 1.10 (3H, d, J = 6.4 Hz) and 1.09 (3H, d, J = 6.4 Hz) (H-11 and H-11′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.0 (s, 4-O-*C*OCH$_3$), 169.0 (s, 4′-O*C*OCH$_3$), 157.5 (s, C-8′), 157.1 (s, C-8), 156.9 (s, C-6), 153.1 (s, C-6′), 149.7 (s, C-9), 149.7 (s, C-9′), 135.5 (s, C-10a), 135.5 (s, C-10a′), 131.1 (s) and 130.9 (s) (C-4a and C-4a′), 125.0 (s) and 124.3 (s) (C-10 and C-10′), 115.6 (s) and 115.3 (s) (C-9a and C-9a′), 110.4 (s) and 110.1 (s) (C-8a and C-8a′), 102.2 (d, C-5′), 98.7 (d, C-5), 97.9 (d, C-7), 97.2 (d, C-7′), 73.3 (d) and 73.2 (d) (C-3 and C-3′), 66.7 (d, C-4), 66.7 (d, C-4′), 65.0 (t, C-1), 65.0 (t, C-1′), 56.3 (q, 8-OCH_3), 56.3 (q, 8′-OCH_3), 55.1 (q, 6-O *C*H$_3$), 19.2 (q) and 19.0 (q) (4-OCO*C*H$_3$ and 4′-OCO*C*H$_3$), 16.8 (q) and 16.7 (q) (C-11 and C-11′); HRMS (ESI-TOF) *m/z* 671.2112 $[M + Na]^+$ (calcd for $C_{35}H_{36}O_{12}Na$, 671.2104).

Compound 4: pale yellow solid; mp 178–180 °C (dec); $[\alpha]^{27}_D$ +49 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 236 (4.72), 294 sh (4.14), 308 sh (4.10), 354 (3.89) nm; IR (KBr) ν_{max} 3385, 1625, 1361, 1156, 1090, 978, 829 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.61 (1H, d, J = 1.6 Hz, 9-O*H*), 9.51 (1H, br s, 9′-O*H*), 6.78 (1H, s, H-7′), 6.48 (1H, d, J = 2.1 Hz, H-7), 6.41 (1H, s, H-10′), 6.02 (1H, d, J = 2.1 Hz, H-5), 5.09 (1H, m, Ha-1), 5.07 (1H, dd, J = 15.7, 2.9 Hz, Ha-1′), 4.82 (1H, dd, J = 15.4, 2.0 Hz, Hb-1), 4.71 (1H, d, J = 15.7 Hz, Hb-1′), 4.14 (3H, s, 8′-OCH_3), 4.08 (3H, s, 8-OCH_3), 4.03 (1H, br s, H-4′), 3.88 (1H, br s, H-4), 3.75 (1H, m, H-3), 3.72 (1H, m, H-3′), 3.45 (3H, s, 6-OCH_3), 1.32 (3H, d, J = 6.3 Hz, H-11′), 1.25 (3H, d, J = 6.4 Hz, H-11); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.8 (s, C-6), 157.3 (s, C-8), 157.4 (s, C-8′), 152.7 (s, C-6′), 150.4 (s, C-9), 149.6 (s, C-9′), 137.4 (s, C-4a), 137.0 (s, C-4a′), 136.2 (s) and 135.6 (s) (C-10a and C-10a′), 120.1 (s, C-10), 116.1 (d, C-10′), 113.7 (s, C-9a′), 113.2 (s, C-9a), 111.5 (s, C-5′), 110.7 (s, C-8a), 110.2 (s, C-8a′), 98.5 (d, C-7), 98.5 (d, C-7′), 98.2 (d, C-5), 73.7 (d) and 73.6 (d) (C-3 and C-3′), 68.1 (d,

Table 1. ^{1}H and ^{13}C NMR Data (500 MHz, $CDCl_3$) for Cordyheptapeptide B (**16**)

position	δ_H (mult., *J* in Hz)	δ_C (mult.)	position	δ_H (mult., *J* in Hz)	δ_C (mult.)	position	δ_H (mult., *J* in Hz)	δ_C (mult.)
*N*Me-D-Phe			L-Ile			L-Phe		
1		168.4 (s)	1		170.9 (s)[c]	1		170.2 (s)
2	5.58 (dd, 11.6, 4.7)	54.5 (d)	2	4.46 (dd, 9.5, 3.1)	58.3 (d)	2	5.35 (m)	50.1 (d)
3	3.06 (m), 3.31 (m)	35.2 (t)	3	2.35 (m)	35.5 (d)	3	2.81 (m), 3.08 (m)	38.2 (t)
4		137.0 (s)	4	1.00 (m), 1.38 (m)	24.3 (t)	4		137.5 (s)
5,9	7.18 (m)	129.7 (d)	5	0.94 (t, 6.9)	12.1 (q)	5,9	7.34 (d, 7.0)	130.1 (d)
6,8	7.16 (m)	128.8 (d)[a]	3-CH_3	0.90 (d, 7.0)	16.3 (q)	6,8	7.43 (dd, 7.5, 7.0)	128.8 (d)[a]
7	7.08 (m)	126.8 (d)[b]	N*H*	5.90 (d, 9.5)		7	7.37 (m)	126.8 (d)[b]
N-CH_3	3.07 (s)	30.1 (q)	*N*Me-L-Phe			N*H*	8.53 (d, 10.0)	
L-Leu			1		170.8 (s)[c]	Sar		
1		174.3 (s)	2	3.50 (dd, 11.7, 4.2)	68.9 (d)	1		167.8 (s)
2	4.97 (m)	47.5 (d)	3	2.84 (m), 3.28 (m)	33.3 (t)	2	3.35 (d, 17.3), 5.43 (d, 17.3)	50.8 (t)
3	0.12 (m), 1.38 (m)	40.0 (t)	4		136.0 (s)	N-CH_3	2.92 (s)	35.5 (q)
4	1.56 (m)	24.8 (d)	5,9	6.44 (d, 7.1)	128.6 (d)[a]	L-Pro		
5	0.87 (d, 6.5)	20.9 (q)	6,8	7.07 (m)	128.6 (d)[a]	1		172.3 (s)
5′	0.92 (d, 6.9)	23.7 (q)	7	7.17 (m)	127.0 (d)[b]	2	4.41 (dd, 9.1, 3.2)	57.8 (d)
N*H*	8.17 (d, 9.1)		N-CH_3	2.67 (s)	40.5 (q)	3	2.07 (m), 2.49 (m)	31.4 (t)
						4	1.82 (m), 1.90 (m)	22.1 (t)
						5	3.62 (m), 3.80 (m)	48.4 (t)

[a] Chemical shifts with the same superscript letter may be interchanged. [b] Chemical shifts with the same superscript letter may be interchanged [c] Chemical shifts with the same superscript letter may be interchanged.

C-4′), 67.1 (d, C-4), 65.2 (t, C-1), 65.1 (t, C-1′), 56.4 (q, 8-OCH_3), 56.2 (q, 8′-OCH_3), 55.1 (q, 6-OCH_3), 16.8 (q) and 16.7 (q) (C-11 and C-11′); HRMS (ESI-TOF) *m/z* 587.1890 $[M + Na]^+$ (calcd for $C_{31}H_{32}O_{10}Na$, 587.1893).

Compound 5: pale yellow solid; mp 199–201 °C (dec); $[\alpha]^{26}_D$ −133 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 239 (5.01), 295 (4.12), 309 (4.19), 353 (4.10) nm; IR (KBr) ν_{max} 3418, 1625, 1361, 1156, 1092, 982, 830 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.59 (1H, br s, 9-O*H*), 9.43 (1H, br s, 9′-O*H*), 6.70 (1H, s, H-7′), 6.50 (1H, d, *J* = 2.0 Hz, H-7), 6.55 (1H, s, H-10′), 6.10 (1H, d, *J* = 2.0 Hz, H-5), 5.06 (1H, br d, *J* = 15.7 Hz, Ha-1), 5.03 (1H, d, *J* = 15.6 Hz, Ha-1′), 4.74 (1H, d, *J* = 15.7 Hz, Hb-1), 4.69 (1H, d, *J* = 15.6 Hz, Hb-1′), 4.12 (3H, s, 8′-OCH_3), 4.09 (3H, s, 8-OCH_3), 4.06 (1H, br s, H-4′), 3.93 (1H, br s, H-4), 3.70 (1H, m, H-3), 3.69 (1H, m, H-3′), 3.48 (3H, s, 6-OCH_3), 1.28 (3H, d, *J* = 6.4 Hz, H-11′), 1.21 (3H, d, *J* = 6.3 Hz, H-11); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.9 (s, C-6), 157.5 (s, C-8′), 157.4 (s, C-8), 150.6 (s) and 150.5 (s) (C-9 and C-6′), 149.5 (s, C-9′), 138.0 (s, C-4a), 136.9 (s, C-4a′), 136.0 (s) and 135.9 (s) (C-10a and C-10a′), 119.1 (s, C-10), 116.8 (d, C-10′), 114.8 (s, C-9a), 113.9 (s, C-9a′), 110.5 (s, C-8a), 110.2 (s) and 109.6 (s) (C-5′ and C-8a′), 98.4 (d, C-7), 97.5 (d, C-5), 96.5 (d, C-7′), 74.0 (d, C-3), 73.5 (d, C-3′), 68.0 (d, C-4′), 66.0 (d, C-4), 64.9 (t) and 64.8 (t) (C-1 and C-1′), 56.4 (q, 8-OCH_3), 56.3 (q, 8′-OCH_3), 55.2 (q, 6-OCH_3), 16.8 (q, C-11), 166 (q, C-11′); HRMS (ESI-TOF) *m/z* 587.1902 $[M + Na]^+$ (calcd for $C_{31}H_{32}O_{10}Na$, 587.1893).

Cordyheptapeptide B (16): colorless solid; mp 90–93 °C; $[\alpha]^{26}_D$ −82 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.55) nm; IR (KBr) ν_{max} 3461, 3311, 2926, 1673, 1632 (s), 702 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), Table 1; HRMS (ESI-TOF) *m/z* 886.4838 $[M + Na]^+$ (calcd for $C_{49}H_{65}N_7O_7Na$, 886.4843).

General Procedure for 6′-*O*-Methylation of ES-242s. Compound **1** (3.0 mg) was treated with MeI (20 μL) and K_2CO_3 (20 mg) in 2-butanone (0.3 mL) at rt for 20 h. The mixture was diluted with EtOAc and washed with H_2O. The EtOAc layer was evaporated, and the residue was purified by silica gel CC (CH_2Cl_2) to afford compound **8** (1.8 mg). The ESIMS and ^{1}H NMR ($CDCl_3$) spectra were identical to those of the isolate (**8**).

Acid Hydrolysis of 15 and 16 and HPLC Analysis of the Hydrolyzates Using a Chiral Column. Cordyheptapeptide B (**16**, 1.0 mg) was hydrolyzed by heating in 6 M HCl (1 mL) at 110 °C for 15 h. After cooling, the solution was evaporated to dryness and dissolved in MeOH (100 μL), which was subjected to HPLC analysis. Cordyheptapeptide A (**15**) was also hydrolyzed in the same manner. HPLC analysis of the peptide hydrolyzates was performed using a ligand-exchange-type chiral column: SUMICHIRAL OA-5000, 4.6 × 150 mm, 5 μm (Sumika Chemical Analysis Service, Ltd.); mobile phase 10% MeOH in 2 mM aqueous $CuSO_4$, flow rate 1 mL/min, UV detection at 238 nm. The hydrolyzate of cordyheptapeptide B (**16**) lacked *N*-Me-L-Tyr (t_R 15.0 min), but showed a peak of *N*-Me-L-Phe at 42.2 min. These assignments were confirmed by co-injection with the standard compounds. Retention times of other amino acids in the chromatogram of cordyheptapeptide B hydrolyzate: Sar 3.4 min, L-Pro 4.3 min, L-Ile 13.0 min, L-Leu 13.9 min, L-Phe 39.4 min, *N*-Me-D-Phe 43.8 min.

Biological Assays. The assay for activity against *Plasmodium falciparum* (K1, multidrug-resistant strain) was performed using the microculture radioisotope technique described by Desjardins.[8] Cytotoxicity against KB cells (oral human epidermoid carcinoma), BC cells (human breast cancer), NCI-H187 cells (human small cell lung cancer), and Vero cells (African green monkey kidney fibroblasts) was evaluated using the colorimetric method.[9]

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