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Mycenarubins A and B, Red Pyrroloquinoline Alkaloids from the Mushroom *Mycena rosea*

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Dedicated to Professor Gerhard Spiteller on the occasion of his 75th birthday

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Two previously unknown red alkaloid pigments, mycenarubin A (**1**), and a dimer thereof, mycenarubin B (**6**), have been isolated from fruiting bodies of *Mycena rosea* and the structures of these pyrroloquinoline alkaloids have been established by 2D NMR and ESI-MS methods. Their absolute configurations were determined by comparison of their CD spectra with the CD spectrum of a synthetic model compound. The fungal pigments are structurally related to the damirones, isolated from a marine sponge, and may be biosynthetically derived from tryptophan and *S*-adenosylmethionine.

Introduction

Mycelial cultures of fungi have for decades been proven to be valuable sources of bioactive compounds, such as penicillin[1] or the antifungal strobilurins.[2] Today, countless mycelial cultures have already been extensively screened for novel bioactive secondary metabolites. In addition to their mycelia, higher fungi also form short-lived fruiting bodies, which have been less well investigated, despite the fact that they often contain completely different secondary metabolites[3] in order, for instance, to protect themselves against parasites and predators by means of the presence of toxic or repellent compounds.[4]

Mycena rosea (Bull.) Gramberg (German name: Rosa Rettichhelmling), a relatively small mushroom, belongs among the less well investigated mushrooms, despite being widespread in beech forests in Europe.[5] So far, no secondary metabolites apart from volatiles such as hexanal and hexan-2-one[6] have been reported either from its fruiting bodies or from its mycelium. The fruiting bodies of *Mycena rosea* are rarely attacked by predators, an indication that they may contain repellent compounds, and this stimulated us to investigate its fruiting bodies and mycelial cultures for secondary metabolites. By HPLC-UV comparison of the metabolite pattern of the fruiting bodies with that of the mycelial cultures, we identified two new red pigments present only in the fruiting bodies. Here we describe the isolation and the structural elucidation of these red pyrroloquinoline alkaloids, which we have named mycenarubin A (**1**) and mycenarubin B (**6**).

Results and Discussion

The mycenarubins were extracted from frozen fruiting bodies of *M. rosea* with methanol at 25 °C and purified by HPLC on a preparative RP-18 column, with 100 g of the frozen fruiting bodies yielding 17 mg mycenarubin A (**1**) and 1 mg mycenarubin B (**6**).

The UV/Vis spectrum of **1** exhibited absorption maxima at 244, 358 and 539 nm, while in the HR-ESI-MS, **1** showed an $[M + H]^+$ ion at m/z 290.11339, consistently with the molecular formula $C_{14}H_{15}N_3O_4$. The presence of 14 carbons in the ^{13}C NMR confirmed the molecular formula, while the ^1H NMR spectrum exhibited 11 non-exchangeable protons. According to HSQC correlations, the signals in the ^{13}C NMR were attributable to four CH_2 groups, three CH groups and seven quaternary carbon atoms. In the COSY spectrum, the protons of the CH_2 group at δ_H = 1.96 ppm (δ_C = 26.65 ppm) showed couplings both to the protons of the CH_2 group at δ_H = 3.01 ppm (δ_C = 38.46 ppm) and to the diastereotopically split protons of the CH_2 group at δ_H = 3.65 and 3.22 ppm (δ_C = 49.91 ppm), indicating the presence of a chain of three consecutive CH_2 groups. The CH_2 chain must be flanked by two heteroatoms, because there are no $^3J_{C,H}$ correlations from the protons of the central CH_2 group at δ_H = 1.96 ppm in the HMBC, and the chemical shifts of the flanking CH_2 groups

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at δ_C = 49.91 ppm and δ_C = 38.46 ppm indicate that these heteroatoms are both nitrogen atoms. In the HMBC, the protons of the CH_2 group at δ_H = 3.65 and 3.22 ppm (δ_C = 49.91 ppm) exhibit couplings not only to the two other carbons of the >N–$(CH_2)_3$–N< unit at δ_C = 26.65 and 38.46 ppm, but also to the carbon of the CH group at 67.05 ppm and a quaternary carbon at 157.71 ppm, while the protons of the other flanking CH_2 group at δ_H = 3.01 ppm (δ_C = 38.46 ppm) show only couplings to the two other carbons of the >N–$(CH_2)_3$–N< unit. Therefore, the latter CH_2 group is connected to an NH_2 group, while the nitrogen connected to the other CH_2 of the side chain at δ_C = 49.91 ppm belongs to a tertiary amine.

The second spin system present in the COSY spectrum reveals the presence of a CH_2–CH fragment consisting of the CH_2 group with two diastereotopically split protons at δ_H = 3.05 and 3.10 ppm (δ_C = 25.52 ppm) and the CH group at δ_H = 4.15 ppm (δ_C = 67.05 ppm). The CH_2–CH unit is connected to the tertiary amine nitrogen through the CH group at δ_C = 67.05 ppm, since there are $^3J_{C,H}$ couplings in the HMBC from the proton of the CH group at δ_H = 4.15 ppm (δ_C = 67.05 ppm) to the carbon of the side chain at δ_C = 49.91 ppm and vice versa from the protons of the side chain at δ_H = 3.65 and 3.22 ppm to the carbon of the CH_2–CH moiety at δ_C = 67.05 ppm. A strong $^3J_{C,H}$ coupling from the diastereotopic protons at δ_H = 3.05 and 3.10 ppm and a weaker $^2J_{C,H}$ coupling from the proton of the CH_2–CH unit at δ_H = 4.15 ppm to a quaternary proton at δ_C = 177.95 ppm indicates that a carboxylic group is directly attached to the CH group. All protons of the CH_2–CH unit show couplings to a quaternary carbon at δ_C = 117.46 ppm. The CH_2–CH unit is therefore connected to an aromatic moiety through the CH_2 group. These results are consistent with substructure A (Figure 1).

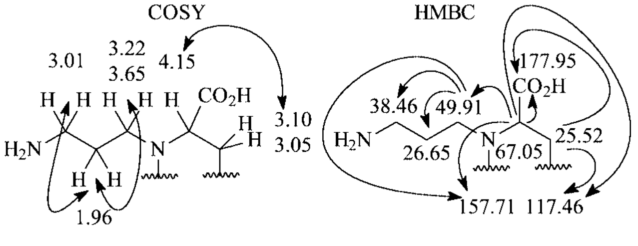


Figure 1. Substructure A of mycenarubin A (**1**) with selected COSY and HMBC correlations.

The aromatic CH group at δ_H = 6.85 ppm (δ_C = 127.44 ppm) shows HMBC correlations to the quaternary carbons at δ_C = 117.46, 125.49 and 126.24 ppm. In the ROESY, a NOE can be detected between the proton at δ_H = 6.85 ppm and the proton at δ_H = 3.10 ppm, so the CH group at δ_H = 6.85 ppm should be located adjacent to the quaternary carbon at δ = 117.46 ppm. The quaternary carbon at δ_C = 126.24 ppm should also be located adjacent to the carbon at 117.46 ppm, since the protons at δ_H = 3.05, 3.10 and at 6.85 ppm all exhibit $^3J_{C,H}$ couplings to this carbon (Figure 2). The CH group at δ_H = 5.26 ppm (δ_C = 93.94 ppm) exhibits $J_{C,H}$ couplings to the quaternary carbons at δ_C = 117.46, 126.24, 172.60 and 180.79 ppm in the HMBC, although only the couplings to the carbons at δ_C = 126.24 and 172.60 ppm are strong $^3J_{C,H}$ couplings. The CH group at δ_H = 5.26 ppm is located relatively far away from the other aromatic CH group, since they only share the HMBC correlation to the carbon at δ_C = 126.24 ppm. Since mycenarubin A is a highly unsaturated compound with nine double bond equivalents, and also in view of the unusual shielded nature of the aromatic CH group at δ_H = 5.26 ppm, with a carbon shift of δ_C = 93.94 ppm, this group should be placed adjacent to the deshielded carbon at δ_C = 157.71 ppm, which is directly connected to the carbon at δ_C = 126.24 ppm (Figure 2).

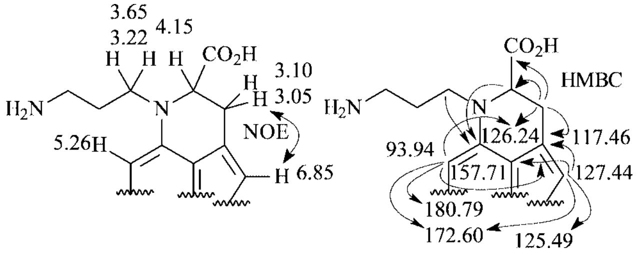


Figure 2. Substructure B of mycenarubin A (**1**) with selected NOE and HMBC correlations.

In the partial structure deduced so far, three quaternary carbons, two oxygen atoms, one nitrogen atom and one exchangeable proton are left to complete the structure of mycenarubin. The two carbons at δ_C = 172.60 and 180.79 ppm are attributable by their chemical shifts to C=O groups in a quinone moiety. The exchangeable proton therefore has to be located at the nitrogen and the quaternary carbon at 125.49 ppm in the bridge position between the NH group, the C=O group at δ_C = 172.60 ppm and the quaternary carbon at 126.24 ppm. This assignment unambiguously gives the pyrroloquinoline alkaloid structure **1** for mycenarubin A (Figure 3). The relative configurations of the diastereotopic protons were assigned by NOE measurements (Figure 3).

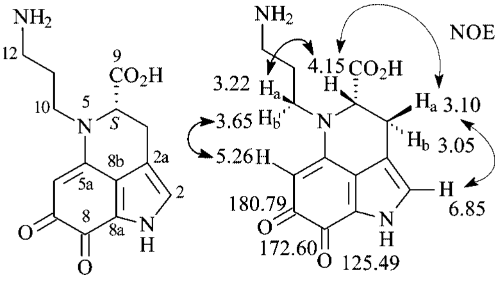
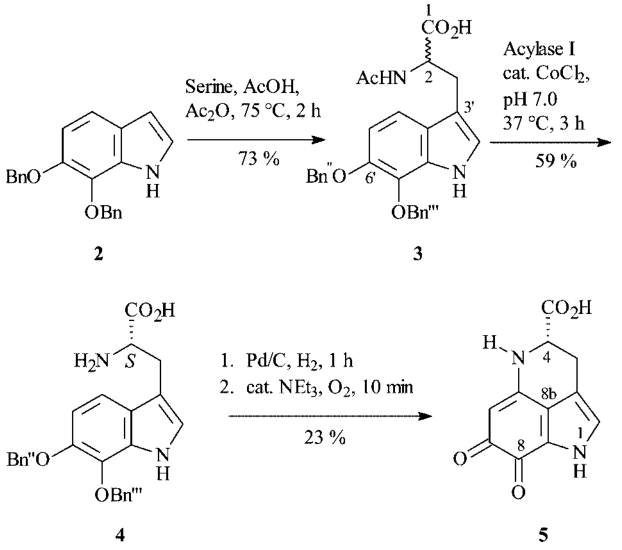


Figure 3. Mycenarubin A (**1**) with selected NOEs for the assignment of the diastereotopic protons.

Mycenarubin A shares its pyrroloquinoline core structure with haematopodin,[7] the only other pyrroloquinoline alkaloid so far isolated from fungi. The chemical shift values of the core structure of haematopodin are very similar to those of **1**. Like **1**, haematopodin possesses a stereocentre at C-4, but a cyclic O-alkyl moiety is present instead of the carboxylic acid. Since the two compounds have similar CD spectra, a preliminary deduction can be made that the stereochemistry of mycenarubin A (**1**) is (*S*), from the known stereochemistry of haematopodin. This assignment is in agreement with the hypothetical biosynthesis of mycenarubin A from L-tryptophan and *S*-adenosylmethionine.

In order to verify the (*S*) configuration of **1**, (*S*)-4-carboxydamirone C (**5**), lacking only the side chain of **1**, was synthesized from the known 6,7-bis(benzyloxy)indole **2**.[8] The indole derivative **2** was condensed with serine in glacial acetic acid and acetic anhydride to provide the racemic *N*-acetyl derivative **3**,[9] which was stereoselectively deacetylated with acylase I to afford 6,7-bis(benzyloxy)-L-tryptophan (**4**).[9] The benzyl groups of **4** were removed by catalytic hydrogenolysis[7a] and the resulting compound was oxidatively cyclised (air) to yield **5** (Scheme 1).[7a]



Scheme 1. Stereoselective synthesis of (*S*)-4-carboxydamirone C (**5**).

Since the CD spectra of **1** and **5** are nearly identical, the configuration for mycenarubin A is (*S*). In addition, the carbon and proton NMR spectroscopic data for **5** confirm the proposed structure of **1**.

The minor alkaloid **6** exhibited a UV spectrum that resembled that of mycenarubin A, with absorption maxima at 245, 354 and 539 nm, so **6** also possesses a pyrroloquinoline core structure. The molecular formula $C_{28}H_{28}N_6O_7$, deduced from the $[M + H]^+$ ion at *m/z* 561.20789 in the HR-ESI-MS, indicates that **6** originates from mycenarubin A by dimerisation of two molecules of **1** and loss of one molecule of water. The dimeric structure of **6** is supported by its ^1H NMR and ^{13}C NMR spectra, since these spectra both consist of two similar signal sets. In order to elucidate how the two monomers are connected to each other, first the proton and then the carbon NMR spectroscopic data for the two monomers were assigned analogously with those for mycenarubin A. In the HMBC, two significant $^3J_{C,H}$ couplings between the diastereotopic protons at C-12′ and the carbon at C-7 allowed structure **6**, in which two units of **1** are connected to each other through an imine moiety, to be deduced for mycenarubin B (Figure 4).

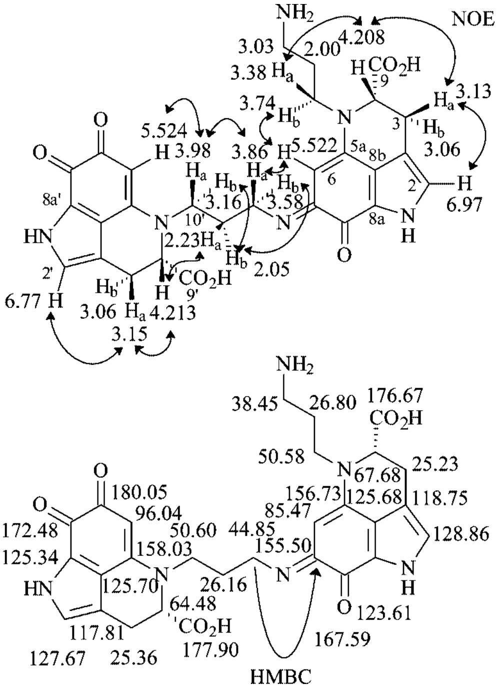


Figure 4. Mycenarubin B (**6**) with chemical shift values and selected NOEs and HMBC couplings.

The CD spectrum of mycenarubin B (**6**) is similar to that of mycenarubin A (**1**), so the stereochemistries of the two quaternary carbons carrying the carboxylic groups should also be (*S*).

The mycenarubins are structurally related – apart from to the already mentioned red fungi pigment haematopodin[5] – to a large number of marine alkaloids,[10] such as the batzellines,[11] damirones,[12] isobatzellines,[13] makaluvamines[14] and discorhabdins,[15] all of them isolated from marine sponges. In contrast, wakayin, the first alkaloid of this class, was isolated from a Fijian ascidian.[16] Moreover, makaluvamin A has been isolated not only from a marine sponge, but also from a culture of the myxomycete *Didymium bahiense*, a terrestrial organism.[17] The presence of the mycenarubins A and B and of haematopodin in *Mycena* species confirms that pyrroloquinoline alkaloids are not restricted to marine sources but seem also to be common in some terrestrial organisms.

Among the pyrroloquinoline alkaloids, compounds containing a *para*-iminoquinone moiety, such as the isobatzellines, often exhibit wide ranging bioactivities;[10] the isobatzellines, for instance, are cytotoxic towards P388 murine leukaemia cells and active against *Candida albicans*.[13] The makaluvamines, which also contain *para* iminoquinone systems, typically exhibit cytotoxicity towards tumour cell lines[10] through inhibition of the function of mammalian topoisomerase II,[14] but only mild activity against *Bacillus subtilis* and other standard test organisms.[18] The batzellines and damirones, however, are characterised by *ortho* quinone core structures and are much less active than the isobatzellines;[10] only the isobatzellines, and not the batzellines, for instance, are cytotoxic and moderately antifungal.[13] Likewise, the *ortho*-quinoid mycenarubin A (**1**) did not show antimicrobial activity against bacteria such as *Escherichia coli*, *Bacillus brevis* or *B. subtilis*, and fungi such as *Cladosporium cucumerinum*.

Unlike the previously known pyrroloquinoline alkaloids, the mycenarubins possess carboxylic groups at C-4. The presence of the carboxylic group supports the hypothesis that pyrroloquinoline and pyrroloiminoquinone alkaloids are in general biosynthetically derived from L-tryptophan. Mycenarubin B (**6**) is the first example of the occurrence of a dimeric pyrroloquinoline alkaloid in nature.

Experimental Section

General: Evaporation of the solvents was performed under reduced pressure with a rotary evaporator. Preparative HPLC separations were performed by use of two Waters 590EF pumps fitted with an automated gradient controller 680 and a Kratos Spectroflow 783 UV/Vis detector. The samples were separated on a Luna C-18 (2) column (5 µm, 15 × 250 mm, Phenomenex) by use of the following gradient program: 10 min at 100% H_2O, then linear to 100% MeOH over 30 min; flow rate: 12 mL min^{-1}; detection: UV at 360 nm. UV spectra were recorded on a Varian Cary 100 Bio UV/Vis spectrometer. Optical rotation values were measured with a Jasco P-1030 polarimeter and CD spectra were obtained with a Jasco J-715 spectropolarimeter. NMR spectra were recorded with a Bruker DMX 900 spectrometer fitted with a TXI cryo probe (^{1}H at 900.13, ^{13}C at 226.3 MHz), with a Bruker DMX 600 spectrometer fitted with a TXI cryo probe (^{1}H at 600.13, ^{13}C at 150.9 MHz) and a Bruker DMX 500 spectrometer (^{1}H at 500.11, ^{13}C at 125.8 MHz). Chemical shifts were determined relative to the D_2O (δ_H = 4.65 ppm), $CDCl_3$ (δ_H = 7.26, δ_C = 77.00 ppm) and CD_3OD (δ_H = 3.31, δ_C = 49.00 ppm) solvents as internal standards. EIMS spectra were obtained with a ThermoElectron DSQ instrument fitted with a direct insertion probe by EI at 70 eV. LC-ESIMS spectra were recorded with a Gynkotek-HPLC fitted with a Luna C-18 (2) column (Phenomenex, 150 × 2 mm, 3 µm, operation temperature 40 °C, flow rate: 200 µL min^{-1}, gradient: 10 min isocratic at 100% H_2O, then linear to 100% AcCN over 10 min) coupled with a Finnigan TSQ 7000 fitted with a Finnigan ESI ion source interface operating in the positive ESI mode [ionisation 4.5 kV, capillary temperature 200 °C, mass range 50–800 amu, multiplier 1000 V (scan modus), MS/MS: argon collision gas 2.0 mbar, sheath gas (N_2) 2.9 bar, multiplier 1400 V, collision energy automatically rotated at –20, –30, –40 eV]. HRESIMS and HRESIMS/MS spectra were obtained with a ThermoElectron LTQ-FT mass spectrometer.

Mushrooms: Fruiting bodies of *M. rosea* (leg. et det. S. Peters and P. Spiteller) were collected in September and October 2004, 2005 and 2006 in beech forests 20 km south of Munich (Bavaria). Voucher samples of *M. rosea* are deposited at the Institut für Organische Chemie und Biochemie II der Technischen Universität München, Germany. The mushrooms were frozen and stored at –35 °C after collecting.

Mycelial Cultures and Cultivation Conditions: For cultivation of *M. rosea* (CBS 514.79, Centraalbureau voor Schimmelcultures, Amsterdam), 40 Petri dishes (90 mm diameter), each of them containing 25 g solid MEA medium, consisting of malt extract (30 g), peptone (5.0 g), agar (15 g) and H_2O (1000 mL), were inoculated with mycelia of *M. rosea* (CBS 514.79) and incubated at 20 °C. Typically, the mycelia covered the whole agar plate within two weeks.

HPLC Profiling: For comparison of the metabolite pattern of the fruiting bodies with that of the mycelial cultures, water and methanol extracts both of the fruiting bodies and of the mycelial cultures were injected into the HPLC. At 360 nm, only the extracts of the fruiting bodies showed significant absorptions.

Isolation Procedure: The frozen fruiting bodies (20 g) were crushed and extracted with MeOH (2 × 50 mL) at 25 °C for 1 min and the red extract was then concentrated in vacuo at 40 °C. The resulting residue was dissolved in H_2O (5 mL), prepurified with an RP-18 cartridge and separated by preparative HPLC (UV detection at 360 nm). Mycenarubin A (**1**) was obtained as a red solid; 100 g of frozen fruiting bodies were required to obtain 17 mg of **1** and 1 mg of **6**.

Biological Tests: For plate diffusion assays, **1** (100 or 500 µg) was dissolved in H_2O and dropped onto paper discs (Ø 6 mm, thickness 0.5 mm). These discs were dried under sterile conditions and placed on agar plates inoculated with the test organism (*Bacillus brevis*, *Bacillus subtilis*, *Escherichia coli* and *Cladosporium cucumerinum* CBS 117.54). The plates were incubated at 37 °C for 24 h in the case of bacteria and at 18 °C for 48 h in the case of *C. cucumerinum*.

Mycenarubin A (1): Red solid; m.p. 126 °C (dec.). HPLC$_{prep}$: R_t = 16.4 min. $[\alpha]_D^{25}$ = +1190 (c = 0.00807, H_2O). CD (H_2O): λ ($\Delta\varepsilon$) = 243 (–4.2), 265 (+1.2), 275 (+1.0), 306 (+2.0), 365 (–4.7), 535 (+2.2) nm. ^{1}H NMR (900 MHz, D_2O, 300 K): δ = 1.96 (m, 2 H, 11-H), 3.01 (m, 2 H, 12-H), 3.05 (dd, J = 16.6, 5.0 Hz, 1 H, 2-H$_b$), 3.10 (d, J = 16.6 Hz, 1 H, 2-H$_a$), 3.22 (m, 1 H, 10-H$_a$), 3.65 (m, 1 H, 10-H$_b$), 4.15 (d, J = 5.0 Hz, 1 H, 3-H), 5.26 (s, 1 H, 6-H), 6.85 (s, 1 H, 2-H) ppm. ^{1}H NMR (600 MHz, D_2O, 310 K): δ = 2.08 (m, 2 H, 11-H), 3.13 (dd, J = 16.5, 5.6 Hz, 1 H, 2-H$_b$), 3.15 (m, 2 H, 12-H), 3.22 (d, J = 16.5 Hz, 1 H, 2-H$_a$), 3.33 (ddd, $J \approx$ 14 Hz, $J \approx$ 7 Hz, $J \approx$ 7 Hz, 1 H, 10-H$_a$), 3.76 (ddd, $J \approx$ 14 Hz, $J \approx$ 7 Hz, $J \approx$ 7 Hz, 1 H, 10-H$_b$), 4.25 (d, J = 5.6 Hz, 1 H, 3-H), 5.35 (s, 1 H, 6-H), 6.94 (s, 1 H, 2-H) ppm. ^{13}C NMR (226 MHz, D_2O, 300 K): δ = 25.52 (C-3), 26.65 (C-11), 38.46 (C-12), 49.91 (C-10), 67.05 (C-4), 93.94 (C-6), 117.46 (C-2a), 125.49 (C-8b), 126.24 (C-8a), 127.44 (C-2), 157.71 (C-5a), 172.60 (C-8), 177.95 (CO_2H), 180.79 (C-7) ppm. UV/Vis (H_2O): λ_{max} (lgε) 244 (4.12), 358 (3.94), 539 (2.82) nm. HR-ESIMS: m/z = 290.11339 $[M + H]^+$ calcd. 290.11408 for $C_{14}H_{16}N_3O_4$. LC-ESIMS: R_t = 18.9 min (detection: UV at λ = 360 nm) m/z = 290 $[M + H]^+$. LC-HR-ESIMS/MS (parent ion m/z 290, 20 eV) m/z (%) = 290.11371 (40) $[M + H]^+$, 246.12383 (100) $[M + H - CO_2]^+$, 233 (4) $[M + H - (C_3H_7N)]$, 217.09728 (12) $[M + H - CO_2 - (CH_2{=}NH)]^+$, 203.08160 (32) $[M + H - CO_2 - (C_2H_5N)]$, 189.06593 (24) $[M + H - CO_2 - (C_3H_7N)]$, 85 (10), 58 (7).

***N*-Acetyl-6,7-bis(benzyloxy)tryptophan (3):** L-Serine (31.9 mg, 0.304 mmol) was added to a solution of 6,7-bis(benzyloxy)indole (50 mg, 0.152 mmol) in AcOH (400 µL) and Ac_2O (100 µL). The solution was stirred under argon for 2 h at 75 °C. After cooling to 25 °C, the mixture was diluted with diethyl ether (10 mL) and adjusted to pH 11 with NaOH (30%). The water layer was washed three times with diethyl ether (10 mL) and the aqueous phase was then adjusted to pH 3 with conc. HCl and extracted three times with diethyl ether (10 mL). The organic phase was dried with anhydrous Na_2SO_4 and the solvent was removed. Yield: 51 mg (73%). ^{1}H NMR (600 MHz, $CDCl_3$, 300 K): δ = 1.94 (s, 3 H $COCH_3$), 3.23 (dd, J = 14.9, 5.2 Hz, 1 H, 3-H$_a$), 3.29 (dd, J = 14.9, 4.9 Hz, 1 H, 3-H$_b$), 4.87 (dd, J = 5.2, 4.9 Hz, 1 H, 2-H), 5.17 (m, 2 H, OCH_2-6′), 5.18 (d, J = 11.1 Hz, 1 H, OCH_2-7′-H$_a$), 5.20 (d, J = 11.1 Hz, 1 H, OCH_2-7′-H$_b$), 6.06 (d, J = 5.8 Hz, NH), 6.81 (s, 1 H, 2′-H), 6.91 (d, J = 8.6 Hz, 1 H, 5′-H), 7.18 (d, J = 8.6 Hz, 1 H, 4′-H), 7.29–7.32 (m, 3 H, 3′′′-H, 4′′′-H, 5′′′-H), 7.32 (m, 1 H, 4′′-H) 7.37 (d, *J* = ca. 7 Hz, 2 H, 2′′′-H, 6′′′-H), 7.37 (m, 2 H, 3′′-H, 5′′-H), 7.47 (d, *J* = 7.0 Hz, 2 H, 2′′-H, 6′′-H), 8.22 (NHCO) ppm. ^{13}C NMR (151 MHz, CD_3OD, 300 K): δ = 23.05 (CO*C*H_3), 27.08 (C-3), 53.33 (C-2), 72.82 (6′-OCH_2), 75.54 (7′-OCH_2), 109.79 (C-3′), 110.55 (C-5′), 113.65 (C-4′), 122.92 (C-2′), 124.78 (C-3a′), 127.67 (C-2′′ and C-6′′), 127.93 (C-4′′), 128.26 (C-4′′′), 128.48 (C-3′′′ and C-5′′′), 128.51 (C-3′′, C-5′′, C-2′′′ and C-6′′′), 131.23 (C-7a′), 134.01 (C-7′), 137.44 (C-1′′), 137.55 (C-1′′′), 146.35 (C-6′), 170.97 (*C*OCH_3), 174.31 (C-1) ppm. EIMS: *m*/*z* (%) = 458 (1) $[M]^+$, 367 (2) $[M - CH_2Ph]^+$, 342 (1) $[M - (NHAc)CHCO_2H]^+$, 339 (2), 297 (2), 293 (1), 279 (1), 252 (3), 251 (3), 236 (3) $[M - (NHAc)CHCO_2H - PhCHO]^+$, 223 (4), 208 (2), 189 (3), 162 (2), 158 (2), 132 (6), 108 (3) $[PhCH_2OH]^+$, 107 (2), 91 (100) $[PhCH_2]^+$, 79 (4), 77 (4) $[Ph]^+$, 65 (6), 51 (1), 43 (3).

(*S*)-6,7-Bis(benzyloxy)tryptophan (4): Acylase I (25 mg, E.C. 3.5.1.14, Sigma: A8376) and $CoCl_2$ (1 mg) were added to a solution of **3** (50.0 mg, 1.09 mmol) in phosphate buffer (10 mL, 50 mM, pH 7.2). The mixture was incubated at 37 °C and 100 rpm for 3 h. The reaction was stopped by addition of MeOH (10 mL), the solvents were removed at 40 °C in vacuo, the residue was dissolved in MeOH (5 mL), the precipitated enzyme was removed by centrifugation, and the crude product was first applied onto a RP-18 cartridge and the methanolic eluate was then purified by preparative HPLC. Yield: 13.3 mg [59% referred to the original content of (*S*)-**4**], colourless solid; $HPLC_{prep}$ (gradient: 10 min at 50% H_2O, 50% MeOH then linear to 100% MeOH over 20 min; flow rate: 12 mL min^{-1}; detection: UV at 280 nm, other conditions as described under "General Experimental Procedures"): R_t = 20.5 min. ^1H NMR (500 MHz, CD_3OD, 300 K): δ = 3.10 (dd, *J* = 15.2, 9.4 Hz, 1 H, 3-H), 3.46 (dd, *J* = 15.2, 3.8 Hz, 1 H, 3-H), 3.83 (dd, *J* = 9.4, 3.8 Hz, 1 H, 2-H), 5.14 (m, 2 H, OCH_2-6′), 5.16 (m, 2 H, OCH_2-7′), 6.94 (d, *J* = 8.6 Hz, 1 H, 5′-H), 7.11 (s, 1 H, 2′-H), 7.29–7.31 (m, 4 H, 4′′-H, 3′′′-H, 4′′′-H, 5′′′-H), 7.35 (m, 2 H, 3′′-H, 5′′-H), 7.36 (d, *J* = 8.6 Hz, 1 H, 4′-H), 7.47 (d, *J* = 6.9 Hz, 2 H, 2′′-H, 6′′-H), 7.47 (d, *J* = 6.9 Hz, 2 H, 2′′′-H, 6′′′-H) ppm. ^{13}C NMR (126 MHz, CD_3OD, 300 K): δ = 28.50 (C-3), 56.65 (C-2), 74.02 (6′-OCH_2), 76.10 (7′-OCH_2), 110.14 (C-3′), 111.44 (C-5′), 114.81 (C-4′), 125.21 (C-2′), 126.07 (C-3a′), 128.87 (C-4′′), 128.95 (C-2′′ and C-6′′), 128.99 (C-4′′′), 129.28 (C-3′′′ and C-5′′′), 129.43 (C-3′′ and C-5′′), 129.58 (C-2′′′ and C-6′′′), 135.66 (C-7′), 139.22 (C-1′′), 139.29 (C-1′′′), 147.67 (C-6′), 174.28 (C-1) ppm. EIMS: *m*/*z* (%) = 416 (2), $[M]^+$, 372 (10) $[M - CO_2]^+$, 342 (19) $[M - (NH_2)CHCO_2H]^+$, 325 (7) $[M - CH_2Ph]^+$, 308 (4), 297 (3), 289 (3), 281 (28) $[M - CO_2 - CH_2Ph]^+$, 264 (10), 251 (10), 236 (30) $[M - (NH_2)CHCO_2H - PhCHO]^+$, 224 (6), 209 (5), 208 (13), 192 (3), 189 (3), 181 (6), 161 (7), 158 (3), 132 (11), 129 (12), 118 (4), 108 (28) $[PhCH_2OH]^+$, 107 (20), 106 (9) $[PhCHO]^+$, 91 (100) $[PhCH_2]^+$, 79 (25), 77 (19) $[Ph]^+$, 65 (10), 51 (6).

(*S*)-4-Carboxydamirone C (5): Catalytic amounts of Pd/C were added to a solution of **4** (10.0 mg, 24.0 µmol) in MeOH (5 mL) and the mixture was hydrogenated by application of H_2 (balloon) for 1 h. After removal of the balloon, NEt_3 (0.2 µL) was added and the solution was stirred under air for 10 min at 40 °C. After that, the red solution was filtered, the solvent was removed, and the crude product was purified by preparative HPLC. Yield: 1.26 mg (23%). Red solid. $HPLC_{prep}$: R_t = 21.5 min. ^1H NMR (600 MHz, D_2O, 298 K): δ = 2.93 (dd, *J* = 16.1, 6.6 Hz, 1 H, 3-H), 3.05 (dd, *J* = 16.1, 6.6 Hz, 1 H, 3-H), 4.25 (dd, *J* = 6.6, 6.6 Hz, 1 H, 4-H), 5.27 (s, 1 H, 6-H), 6.94 (s, 1 H, 2-H) ppm. ^{13}C NMR (151 MHz, D_2O, 298 K): δ = 24.50 (C-3), 58.94 (C-4), 94.35 (C-6), 118.36 (C-2a), 125.45 (C-8b), 125.93 (C-8a), 127.43 (C-2), 159.02 (C-5a), 173.74 (C-8), 178.84 (CO_2H), 180.20 (C-7) ppm. UV/Vis (H_2O): λ_{max} (lgε) 242 (4.15), 344 (3.85), 516 (2.96) nm.CD (H_2O): λ ($\Delta\varepsilon$) = 244 (–4.5), 265 (+0.7), 275 (+0.6), 304 (+1.4), 360 (–2.2), 520 (+1.2) nm. HR-ESIMS: *m*/*z* = 233.05566 $[M + H]^+$ calcd. 233.05568 for $C_{11}H_9N_2O_4$; LC-ESIMS: R_t = 20.7 min (detection: UV at λ = 360 nm) *m*/*z* = 233 $[M + H]^+$. LC-ESIMS/MS (parent ion *m*/*z* 233, 30 eV) *m*/*z* (%) = 233 (100) $[M + H]^+$, 187 (88) $[M + H - HCO_2H]^+$, 159 (10) $[M + H - HCO_2H - CO]^+$, 131 (20) $[M + H - HCO_2H - CO - CO]^+$, 104 (22), 77 (4).

Mycenarubin B (6): Red solid; $HPLC_{prep}$: R_t = 16.7 min. $[\alpha]_D^{25}$ = +170 (*c* = 0.0061, H_2O). CD (H_2O): λ ($\Delta\varepsilon$) = 242 (–4.8), 264 (+2.9), 300 (+0.9), 319 (+1.0), 374 (–2.4), 432 (+0.7), 473 (+0.4), 539 (+0.9) nm. ^1H NMR (900 MHz, D_2O, 300 K): δ = 2.00 (m, 2 H, 11-H), 2.05 (dm, *J* ≈ 16 Hz, 1 H, 11′-H_b), 2.23 (dddm, *J* ≈ 16 Hz, *J* ≈ 13 Hz, *J* ≈ 13 Hz, 1 H, 11′-H_a), 3.03 (m, 2 H, 12-H), 3.06 (dd, *J* ≈ 17 Hz, *J* ≈ 7 Hz, 1 H, 3′-H_b), 3.06 (dd, *J* ≈ 17 Hz, *J* ≈ 7 Hz, 1 H, 3-H_b), 3.13 (d, *J* ≈ 17 Hz, 1 H, 3-H_a), 3.15 (d, *J* ≈ 17 Hz, 1 H, 3′-H_a), 3.16 (m, 1 H, 10′-H_b), 3.38 (ddd, *J* ≈ 14 Hz, *J* ≈ 7.5 Hz, *J* ≈ 7.5 Hz, 1 H, 10-H_a), 3.58 (dm, *J* = 12.4 Hz, 1 H, 12′-H_b), 3.74 (ddd, *J* ≈ 14 Hz, *J* ≈ 7.5 Hz, *J* ≈ 7.5 Hz, 1 H, 10-H_b), 3.86 (dd, *J* = 12.4 Hz, *J* ≈ 12 Hz, 1 H, 12′-H_a), 3.98 (dd, *J* ≈ 15 Hz, *J* ≈ 15 Hz, 1 H, 10′-H_a), 4.208 (d, *J* ≈ 7 Hz, 1 H, 4-H), 4.213 (d, *J* ≈ 7 Hz, 1 H, 4′-H), 5.522 (s, 1 H, 6-H), 5.524 (s, 1 H, 6′-H), 6.77 (s, 1 H, 2′-H), 6.97 (s, 1 H, 2-H) ppm. ^1H NMR (600 MHz, D_2O, 298 K): δ = 2.03 (m, 2 H, 11-H), 2.08 (dm, *J* ≈ 16 Hz, 1 H, 11′-H_b), 2.27 (dm, *J* ≈ 16 Hz, 1 H, 11′-H_a), 3.07 (dd, *J* ≈ 8 Hz, *J* ≈ 8 Hz, 2 H, 12-H), 3.09 (dd, *J* ≈ 16 Hz, *J* ≈ 7 Hz, 2 H, 3-H_b and 3′-H_b), 3.16 (d, *J* ≈ 16 Hz, 1 H, 3-H_a), 3.18 (d, *J* ≈ 16 Hz, 1 H, 3′-H_a), 3.19 (m, 1 H, 10′-H_b), 3.40 (ddd, *J* = 14.5, 7.8, 7.8 Hz, 1 H, 10-H_a), 3.61 (dm, *J* = 14.6 Hz, 1 H, 12′-H_b), 3.77 (ddd, *J* = 14.5, 7.8, 7.8 Hz, 1 H, 10-H_b), 3.89 (dm, *J* = 14.6 Hz, 1 H, 12′-H_a), 4.01 (dm, *J* ≈ 15 Hz, 1 H, 10′-H_a), 4.24 (d, *J* ≈ 7 Hz, 1 H, 4-H), 4.24 (d, *J* ≈ 7 Hz, 1 H, 4′-H), 5.55 (s, 1 H, 6-H), 5.56 (s, 1 H, 6′-H), 6.81 (s, 1 H, 2′-H), 7.00 (s, 1 H, 2-H) ppm. ^{13}C NMR (226 MHz, D_2O, 300 K): δ = 25.23 (C-3), 25.36 (C-3′), 26.16 (C-11′), 26.80 (C-11), 38.45 (C-12), 44.85 (C-12′), 50.58 (C-10), 50.60 (C-10′), 64.48 (C-4′), 67.68 (C-4), 85.47 (C-6), 96.04 (C-6′), 117.81 (C-2a′), 118.75 (C-2a), 123.61 (C-8a), 125.34 (C-8a′), 125.68 (C-8b), 125.70 (C-8b′), 127.67 (C-2′), 128.86 (C-2), 155.50 (C-7), 156.73 (C-5a), 158.03 (C-5a′), 167.59 (C-8), 172.48 (C-8′), 176.67 (C-9), 177.90 (C-9′), 180.05 (C-7′) ppm. UV/Vis (H_2O): λ_{max} (lgε) 245 (4.19), 354 (3.89), 534 (3.01) nm. HR-ESIMS: *m*/*z* = 561.20789 $[M + H]^+$ calcd. 561.20922 for $C_{28}H_{29}N_6O_7$. LC-ESIMS: R_t = 18.4 min (detection: UV at λ = 360 nm) *m*/*z* = 561 $[M + H]^+$. LC-ESIMS/MS (parent ion *m*/*z* 561, 20 eV) *m*/*z* (%) = 517 (6) $[M + H - CO_2]^+$, 473 (100) $[M + H - CO_2 - CO_2]^+$, 455 (13), 267 (6).

Supporting Information (see also the footnote on the first page of this article): UV/Vis, CD, NMR and mass spectra of **1**, **3**, **4**, **5** and **6**.

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