The Leupyrrins: A Structurally Unique Family of Secondary Metabolites from the Myxobacterium *Sorangium cellulosum*[#]

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Received March 12, 2003

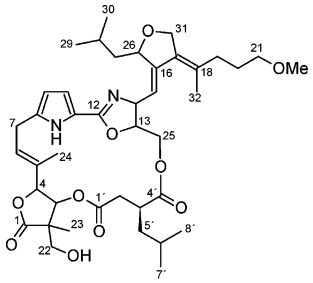
The structurally unique leupyrrins A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), C (**5**), and D (**6**) were isolated as one of three groups of secondary metabolites from *Sorangium cellulosum* strains So ce705 and So ce690. An unusually substituted γ-butyrolactone ring and pyrrole and oxazoline rings are embedded in a nonsymmetric macrodiolide core structure, giving rise to compounds **1**−**6** as members of a novel class of secondary metabolites. Leupyrrin A_1 (**1**) shows good biological activity against various fungi and eukaryotic cells.

During the past two decades myxobacteria have proven to be a rich source of structurally diverse secondary metabolites with interesting biological activities.[1,2] In our screening of *Sorangium cellulosum* strains for new secondary metabolites we isolated the novel compounds leupyrrins A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), C (**5**), and D (**6**) as major metabolites from the strains So ce705 and So ce690. In addition, sorangicin Z (**7**), a member of the well-known sorangicin family of secondary metabolites,[3,4] and a polyketide related to sulfangolid[5] were identified as major byproducts in both strains.

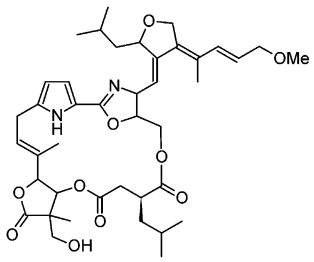
Results and Discussion

For the isolation of secondary metabolites, *S. cellulosum* strains So ce705 and So ce690 were cultivated with 1% of XAD-16 in 60 and 100 L fermentors, respectively. Cells and adsorber resin were collected after incubation at 30 °C for 11 and 8 days, respectively, and repeatedly extracted with methanol. Analytical HPLC of the crude extract of both strains revealed a very similar metabolite profile, and therefore both crude extracts were combined for further separation. Concentration of the aqueous/methanolic extract and re-extraction of the water phase with ethyl acetate yielded a dark viscous oil, which was further partitioned between aqueous NH_3 and Et_2O. Finally, the crude extract from the organic layer was dissolved in methanol, and compounds **1**−**6** were isolated as colorless amorphous powder by sequential chromatography (see Supporting Information). Normal-phase followed by reversed-phase chromatography yielded pure compounds **5** and **6** and mixtures of compounds **1**/**2** and **3**/**4**, which could be separated and further purified by an additional normal-phase chromatography. The main component of this new class of metabolites was leupyrrin A_1 (**1**). Its molecular formula $C_{41}H_{58}N_2O_{10}$ results from a HREI mass spectrum *m*/*z* (=738.4053) indicating 14 double-bond equivalents.

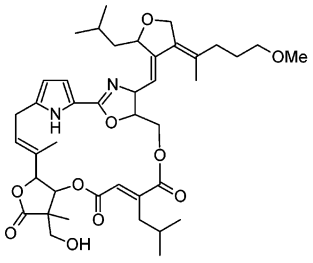
The structure of **1** was deduced from detailed 1D (^{1}H and ^{13}C) and 2D (COSY, HMQC, and HMBC) NMR spectral data. In the ^{13}C NMR spectrum 40 signals were identified,



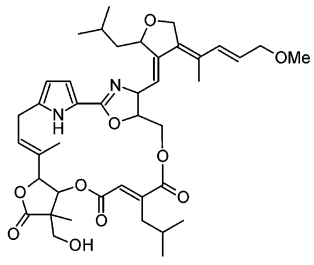
Leupyrrin A_1 (**1**)



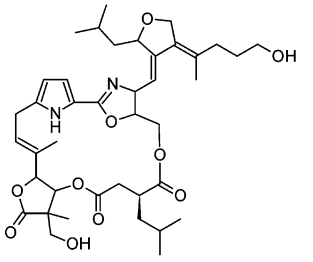
Leupyrrin A_2 (**2**)



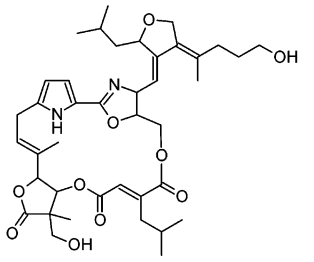
Leupyrrin B_1 (**3**)



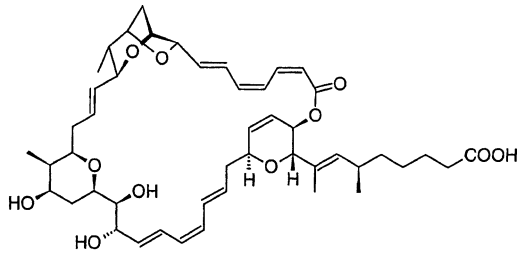
Leupyrrin B_2 (**4**)



Leupyrrin C (**5**)



Leupyrrin D (**6**)



Sorangicin Z (**7**)

[#] Dedicated to Prof. Meinhart H. Zenk on the occasion of his 70th birthday.

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10.1021/np030109v CCC: $25.00
Published on Web 09/04/2003

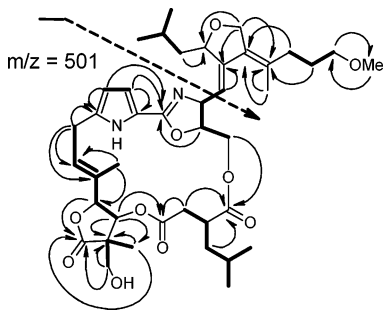

Figure 1. EI mass fragmentation, ^{1}H−^{1}H COSY (bold lines), and HMBC correlations (arrows) of leupyrrin A_1 (**1**). The ^{1}H−^{1}H COSY couplings observed between H-22 and H-23, H24 and H-6 and H-$7_{a/b}$, and H-6 and H-4 represent long-range couplings of small magnitude.

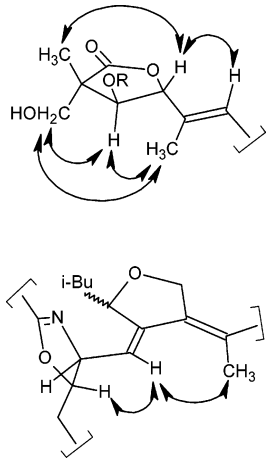

Figure 2. Selected NOE correlations for leupyrrin A_1 (**1**).

but a DEPT experiment revealed the overlap of a methylene and a methine carbon atom at 26.9 ppm. Ten olefinic and three carboxylic carbon atoms, one imine carbon at 160.6 ppm, and the remaining double-bond equivalents indicated the presence of five rings in **1**. Characteristic signals in the ^{1}H NMR spectrum were a methoxy group at δ_H 3.35, a methylene group at δ_H 3.63 and 3.77 (both d, J = 11.0 Hz), one methyl group at δ_H 1.10 which appeared as a singlet, two methyl groups with small coupling constants at δ_H 1.77 (CH, J = 2.0 Hz) and 1.97 (t, J = 2.0 Hz), and four methyl groups at δ_H 1.07/1.03 and 0.94/0.92 that belonged to two isopropyl groups. From a ^{1}H−^{1}H COSY experiment and a detailed analysis of ^{1}H−^{1}H coupling constants seven spin systems could be assigned which were connected with the remaining structural elements by HMBC correlation (Figure 1). In the ^{1}H NMR spectrum in $CDCl_3$ the NH group of the pyrrole ring appeared at δ_H 9.2 with characteristic couplings of J = 1.5 Hz with protons H-9 and H-10 (best seen with compound **2**).

The relative configuration of the γ-butyrolactone and the geometry of the double bonds were confirmed by NOE correlation spectroscopy. H-4 showed correlations to H-6 and H-23, and H-3 showed correlations to H-22 and H-24. An additional correlation was observed between H-22 and H-24, indicating the relative configuration of the γ-butyrolactone ring and the C-5/C-6 double bond as depicted in Figure 2. Furthermore, NOE correlations between H-15 and H-32 confirmed the *E* configuration of the C-15/C-16 and C-17/C-18 double bonds. The coupling constant between H-13 and H-14 of ^3J = 5.5 Hz is in perfect agreement with oxazoline rings derived from threonine, indicating a *trans* configuration of both protons;[6,7] this was confirmed by a strong NOE correlation between H-13 and H-15 (Figure 2). After basic hydrolysis of leupyrrin A_1 (**1**) (*S*)-2-(2-methylpropyl)succinic acid, $[\alpha]_D^{20}$ −24 (in ethanol), was isolated.[8] However, the relative configuration for the entire molecule could not be determined by NMR spectroscopy due to the spatial separation of the different stereogenic centers and the overlap of protons H-2_a′/H-3′ and H-14/H-25_a in different solvent systems, resulting in ambiguous NOE correlations. Specific cleavage of ester and imino bonds and degradation of **1** followed by determination of the absolute configuration of the different fragments are currently being undertaken in our group in order to establish the absolute configuration of leupyrrin A_1 (**1**).

All leupyrrins show a characteristic fragmentation by EI mass spectroscopy. Besides the molecular ion, the fragment with the highest intensity can be detected that corresponds to the core macrodiolide ($C_{26}H_{33}N_2O_8$, m/z = 501.2216; compounds **1**, **2**, **5** [Figure 1]) or its dehydroderivative ($C_{26}H_{31}N_2O_8$, m/z = 499.2059; compounds **3**, **4**, **6**). Compared to **1** leupyrrin A_2 (**2**) showed a molecular mass reduced by 2 amu in the molecular ion but not in the ring fragment, suggesting a modification in the furane moiety. NMR spectroscopy revealed an additional double bond with *E* configuration between C-19 and C-20 with δ_H 6.20 and 5.85 (^3J = 14.0).

Leupyrrin B_1 (**3**) had the same molecular mass as **2** but showed the characteristic 2 amu loss in the fragment of highest intensity, indicating a macrodiolide dehydroderivative. Additionally, differences between both compounds could be seen in the NMR spectra. Instead of the methylene and methine protons for H_2-2′ and H-3′ at δ_H ~2.70 a singlet at δ_H 6.66 was observed, indicating the presence of a fumarate analogue that was confirmed by signals for a trisubstituted double bond in the ^{13}C NMR at δ_C 127.0 (C-2′, d) and 146.5 (C-3′, s) and by an HMBC experiment. The expected high-field shift of the two carboxylic acid carbons to δ_C 166.0 and 165.8 was also observed. The stereochemistry of the C-2′/C-3′ double bond was determined by a ROESY experiment. A correlation between H-2′ and H-25_a and no correlations between H-2′ and H-$5_{a/b}$′ or H-25_a indicated *E* configuration of this double bond. Additionally, as a result of this double bond, a downfield shift of almost 1 ppm for both H-5′ methylene protons to δ_H 2.57 and 2.45 was observed.

The NMR data of the dicarboxylic acid moiety of leupyrrin B_2 (**4**) was almost identical to that of compound **3**, but additionally, a C-19/C-20 double bond was present. This was confirmed by a reduced mass of the molecular ion (m/z = 734.3778) of 2 amu.

The most polar leupyrrins C (**5**) and D (**6**) differed from compounds **1** and **3** by a reduced mass of 14 amu for the molecular ions and the lack of the characteristic methoxy group at δ_H 3.35 and δ_C 58 in the NMR spectra and, therefore, represent hydroxy derivatives of **1** and **3**. Furthermore, the free hydroxy group at C-21 resulted in a characteristic high-field shift of this carbon of 10 ppm to δ_C ~62.

Due to the combination of several unusual structural elements, the leupyrrins represent an exciting group of secondary metabolites. Macrodiolides are quite common in secondary metabolism, but mostly are derived from two identical or nearly identical subunits.[9,10] Also γ-butyrolactones and furane derivatives are common structural elements in various natural products from different biological sources (e.g., acetogenins from plants,[11,12] polyethers from microorganisms[13]), but their substitution pattern as in **1** has not been described so far. Pyrrole rings are also common in several secondary metabolites, but in most

Table 1. Selected MIC and IC_{50} Values of Leupyrrin A_1 (**1**)

test organism	MIC (μg/mL)
Candida albicans	>25
Rhodotorula glutinis	0.25
Aspergillus niger	0.8
Botrytis cinerea	0.8
Mucor hiemalis	0.3
	IC_{50} (μg/mL)
mouse fibroblast cells L929	1.0

cases they are part of starting units or acyl groups such as pyrrole carboxylic acids.[14] Furthermore, the two isopropyl groups indicate the involvement of branched chain amino acid biosynthetic steps or degradation. Taken together, one can expect that the leupyrrins must be the result of an exciting and unexpected biosynthesis that is currently being investigated in our group.

Leupyrrin A_1 (**1**) shows good antifungal activity against different fungi and yeasts and moderate toxicity toward mouse fibroblasts (Table 1). The mode of action of **1** was investigated with *Rhodotorula glutinis*. Measurement of DNA, RNA, and protein syntheses by incorporation of [methyl-^{3}H]thymine, [2-^{14}C]uracil, and [U-^{14}C]protein hydrolysate came to a complete stop after 30 min for all three macromolecules at 2.5 μg/mL of **1**. No inhibition of the respiratory chain[15] or disruption of membranes, measured as release of UV absorbing material, could be observed. On the other side, when *R. glutinis* was suspended in distilled water, a slow increase of conductivity (τ/2 2.5 h) could be detected under the influence of leupyrrin A_1 (**1**).[16] How this is be accomplished without membrane disruption and how this effect can be correlated with the observed much faster termination of the DNA, RNA, and protein biosynthesis is currently under investigation.

Experimental Section

General Experimental Procedures. Optical rotation was determined on a Perkin-Elmer 241 MC polarimeter. IR and UV spectra were recorded on a Nicolet 20DXB FT-IR spectrometer and Shimadzu UV-2102 PC spectrometer, respectively. Melting points were obtained by a Büchi 510 apparatus. NMR spectra were run at 300 K on a Bruker ARX400 NMR spectrometer. EI mass spectra were obtained on a Finnigan MAT 95 spectrometer, and high-resolution data were acquired using peak matching (M/MD = 10 000). Preparative HPLC was performed using Nucleosil C_{18} and Nucleosil 100-7 columns (both Macherey & Nagel, Düren, Germany; detector: UV 274 nm).

Organism and Culture Conditions. The *Sorangium cellulosum* strains So ce690 and So ce705 were isolated from an oak forest soil sample in Spain and a soil sample from Sicily, respectively. Both strains were cultivated at 30 °C on a 60 and 100 L scale in production media with 1% of XAD-16 (Rohm and Haas, Darmstadt, Germany) composed of (g/L) soluble starch (8), yeast extract (2), soy bean meal (2), $CaCl_2 \times 2H_2O$ (1), $MgSO_4 \times 7H_2O$ (1), Na−Fe−EDTA (0.008), HEPES (12), glucose $\times 6H_2O$ (2, added after sterilisation), pH 7.4 (KOH, adjusted prior to sterilization), for strain So ce690 and yeast extract (2), soy bean meal (2), starch (6), glycerol (1), glucose $\times 6H_2O$ (6), $CaCl_2 \times 2H_2O$ (1), $MgSO_4 \times 7H_2O$ (1), Na−Fe−EDTA (0.008), pH 7.8, for strain So ce705. The cultures were inoculated with 10% (v/v) of a preculture in the same medium without XAD-16 and harvested by sieving when glucose and starch (measured by iodine reaction) were used up (usually after 8−11 days).

Isolation of Compounds 1−6. Cells and XAD-16 from a total of 160 L of culture broth of both strains were extracted with MeOH, and the resulting aqueous layer after evaporation of the MeOH was adjusted to pH 10 with aqueous NH_3. Extraction with Et_2O and evaporation of the solvent yielded an oily residue, which was dissolved in MeOH and extracted with *n*-heptane. Evaporation of the remaining MeOH solution resulted in an orange-brown solid, which was fractionated into compounds **1**−**4** and **5/6** by silica gel chromatography (solvent: *tert*-butyl methyl ether/petrol ether, 2:1, with 2% MeOH). RP-HPLC (Nucleosil C_{18}, solvent: MeOH/H_2O, 75:25, with 0.05 M NH_4OAc) of these fractions yielded pure compounds **5** and **6** and mixtures of compounds **1** and **2** (55%/47%) and **3** and **4** (58%/42%), which were finally separated by normal-phase HPLC (Nucleosil 100-7, solvent: *tert*-butyl methyl ether/petrol ether, 25:75, with 0.5% MeOH), yielding the pure compounds. The leupyrrins were produced in yields of 6.5 [4.5] (**1**), 6.0 [4.0] (**2**), 4.5 [5.0] (**3**), 3.5 [4.0] (**4**), 3.5 [3.5] (**5**), and 0.2 [0.2] mg/L (**6**) from strain So ce690 and So ce705 [in brackets] as determined by analytical HPLC of the crude extracts from shaking flask cultures. However, the main metabolite in both strains was sorangicine Z (**7**), with ~10 mg/L, which could be isolated after acidification of the aqueous NH_3 phase with HCO_2H to pH 3.0, extraction with ethyl acetate, and repeated RP-HPLC (data not shown).

Leupyrrin A_1 (1): colorless amorphous solid; mp 95 °C; $[\alpha]_D^{20}$ +12 (*c* 4.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 260 (4.20), 286 (4.46) nm; (MeOH + HCl) λ_{max} (log ϵ) 315 (4.51) nm; (MeOH + NaOH) λ_{max} (log ϵ) 283 (4.35) nm; IR (KBr) ν_{max} 3401, 2956, 2928, 1780, 1743, 1649, 1162, 1114 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 0.92 (3H, d, J = 6.5 Hz, H-8′), 0.94 (3H, d, J = 6.5 Hz, H-7′), 1.03 (3H, d, J = 6.5 Hz, H-29), 1.07 (3H, d, J = 6.5 Hz, H-30), 1.10 (3H, s, H-23), 1.33 (1H, m, H-5$_a$′), 1.45 (1H, ddd, J = 14.0, 9.5, 2.5 Hz, H-27$_a$), 1.60 (1H, m, H-27$_b$), 1.65 (1H, m, H-6′), 1.68 (1H, m, H-5$_b$′), 1.70 (1H, m, H-20$_a$), 1.76 (1H, m, H-20$_b$), 1.77 (1H, d, J = 2.0 Hz, H-24), 1.91 (1H, m, H-28), 1.97 (3H, t, J = 2.0 Hz, H-32), 2.11 (1H, ddd, J = 13.5, 8.0, 7.0 Hz, H-19$_a$), 2.14 (1H, ddd, J = 13.5, 8.0, 7.0 Hz, H-19$_b$), 2.69 (1H, m, H-2′$_a$), 2.79 (1H, m, H-3′), 2.83 (1H, m, H-2′$_b$), 3.35 (3H, s, 21-OC*H*$_3$), 3.38 (1H, m, H-7$_a$), 3.39 (2H, t, J = 6.0 Hz, H-21), 3.62 (1H, dd, J = 15.5, 9.0 Hz, H-7$_b$), 3.63 (1H, d, J = 11.0 Hz, H-22$_a$), 3.77 (1H, d, J = 11.0 Hz, H-22$_b$), 4.19 (1H, dd, J = 12.0, 2.5 Hz, H-25$_a$), 4.49 (1H, m, H-31$_a$), 4.56 (1H, dd, J = 10.0, 5.5 Hz, H-14), 4.57 (1H, d, J = 10.0 Hz, H-31$_b$), 4.59 (1H, dd, J = 12.5, 5.5 Hz, H-25$_b$), 4.70 (1H, td, J = 5.5, 2.5 Hz, H-13), 4.76 (1H, dd, J = 8.0, 1.0 Hz, H-4), 5.00 (1H, ddd, J = 11.0, 3.0, 1.5 Hz, H-26), 5.55 (1H, dd, J = 10.0, 1.5 Hz, H-15), 5.58 (1H, d, J = 8.0 Hz, H-3), 5.89 (1H, td, J = 7.5, 1.5 Hz, H-6), 6.07 (1H, d, J = 3.5 Hz, H-9), 6.79 (1H, d, J = 3.5 Hz, H-10); ^{13}C NMR (100.6 MHz, CD_3OD) δ 11.4 (CH_3, C-24), 14.6 (CH_3, C-23), 20.2 (CH_3, C-32), 22.0 (CH_3, C-30), 22.7 (CH_3, C-8′), 22.9 (CH_3, C-7'), 24.1 (CH_3, C-29), 26.1 (CH, C-28), 26.9 (CH_2, C-7/CH, C-6′), 28.3 (CH_2, C-20), 34.9 (CH_2, C-19), 36.4 (CH_2, C-2′), 39.8 (CH, C-3′), 40.1 (CH_2, C-5′), 44.3 (CH_2, C-27), 51.1 (C, C-2), 58.8 (CH_3, 21-O*C*H$_3$), 65.7 (CH_2, C-22), 65.9 (CH_2, C-25), 68.8 (CH, C-14), 70.1 (CH_2, C-31), 72.8 (CH_2, C-21), 75.4 (CH, C-3), 80.5 (CH, C-26), 84.5 (CH, C-13), 85.5 (CH, C-4), 109.6 (CH, C-9), 115.2 (CH, C-10), 119.3 (C, C-11), 123.0 (CH, C-15), 128.4 (CH, C-6), 131.2 (C, C-17), 134.0 (C, C-18), 135.1 (C, C-5), 136.5 (C, C-8), 144.9 (C, C-16), 160.6 (C, C-12), 172.1 (C, C-1′), 175.4 (C, C-4′), 179.3 (C, C-1); EIMS *m*/*z* 738 [M$^+$] (36), 501 (100), 274 (38); HREIMS *m*/*z* 738.4053 (calcd for $C_{41}H_{58}N_2O_{10}$, 738.4092).

Leupyrrin A_2 (2): colorless amourphous solid; $[\alpha]_D^{20}$ −36 (*c* 5.40, MeOH); UV (MeOH) λ_{max} (log ϵ) 282 (4.43), 298 (4.57) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (1H, m, H-5$_a$′), 1.53 (1H, m, H-5$_b$′), 2.55 (1H, m, H-2$_a$′), 2.73 (1H, m, H-2$_b$′), 2.77 (1H, m, H-3′), 3.34 (3H, s, OCH_3), 4.01 (2H, dd, J = 6.0, 1.0 Hz, H-21), 5.85 (1H, ddd, J = 14.0, 5.0, 5.0 Hz, H-20), 6.20 (1H, ddd, J = 14.0, 1.5, 1.5 Hz, H-19); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 36.4 (CH_2, C-2′), 38.8 (CH, C-3′), 39.8 (CH_2, C-5′), 58.1 (CH_3, OCH_3), 73.1 (CH_2, C-21), 127.7 (CH, C-20), 132.1 (CH, C-19), 171.0 (C, C-1′), 173.9 (C, C-4′); EIMS *m*/*z* 736 [M$^+$] (23), 501 (100); HREI MS *m*/*z* 736.3935 (calcd for $C_{41}H_{56}N_2O_{10}$, 736.3935).

Leupyrrin B_1 (3): colorless amorphous solid; $[\alpha]_D^{20}$ +11 (*c* 4.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (4.33), 261 (4.26), 286 (4.45) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (2H, m, H-20), 2.03 (2H, m, H-19), 2.45 (1H, dd, J = 12.0, 7.0 Hz, H-5$_a$′), 2.57

(1H, dd, J = 12.0, 7.5 Hz, H-5$_b$′), 3.28 (2H, m, H-21), 3.30 (3H, s, OCH_3), 6.66 (1H, s, H-2′); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 28.5 (CH_2, C-20), 34.1 (CH_2, C-19), 36.5 (CH_2, C-5′), 58.1 (CH_3, OCH_3), 71.9 (CH_2, C-21), 127.0 (CH, C-2′), 146.5 (C, C-3′), 165.8 (C, C-1′), 166.0 (C, C-4′); EIMS m/z 736 [M$^+$] (50), 499 (100), 274 (63); HREIMS m/z 736.3934 (calcd for $C_{41}H_{56}N_2O_{10}$, 736.3935).

Leupyrrin B$_2$ (4): colorless amorphous solid; $[\alpha]_D^{20}$ +7 (c 4.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 286 (4.32), 310 (4.16) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.46 (1H, dd, J = 12.5, 7.5 Hz, H-5$_a$′), 2.61 (1H, dd, J = 12.5, 7.5 Hz, H-5$_b$′), 4.00 (2H, dd, J = 6.0, 1.0 Hz, H-21), 5.85 (1H, ddd, J = 15.0, 1.5, 1.5 Hz, H-20), 6.20 (1H, ddd, J = 15.0, 5.0, 5.0 Hz, H-20), 6.63 (1H, s, H-2′); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 36.5 (CH_2, C-5′), 58.1 (CH_3, OCH_3), 72.3 (CH_2, C-21), 127.0 (CH, C-2′), 127.7 (CH, C-20), 132.1 (CH, C-19), 146.5 (C, C-3′), 165.7 (C, C-1′), 166.0 (C, C-4′); EIMS m/z 734 [M$^+$] (30), 499 (100); HREI MS m/z 734.3778 (calcd for $C_{41}H_{54}N_2O_{10}$, 734.3778).

Leupyrrin C (5): colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.33 (1H, m, H-5$_a$′), 1.67 (2H, m, H-20), 1.68 (1H, m, H-5$_b$′), 2.13 (2H, m, H-19), 2.68 (1H, m, H-2$_a$′), 2.79 (1H, m, H-3′), 2.84 (1H, m, H-2$_b$′), 3.58 (2H, t, J = 6.0 Hz, H-21); ^{13}C NMR (100.6 MHz, CD_3OD) δ 31.6 (CH_2, C-20), 35.1 (CH_2, C-19), 36.6 (CH_2, C-2′), 40.0 (CH, C-3′), 40.3 (CH_2, C-5′), 62.6 (CH_2, C-21), 172.3 (C, C-1′), 175.7 (C, C-4′); EIMS m/z 724 [M$^+$] (29), 501 (100), 260 (20).

Leupyrrin D (6): colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.70 (2H, m, H-20), 2.12 (2H, m, H-19), 2.55 (1H, dd, J = 12.0, 7.0 Hz, H-5$_a$′), 2.61 (1H, dd, J = 12.0, 7.0 Hz, H-5$_b$′), 3.57 (2H, t, J = 6.0 Hz, H-21), 6.63 (1H, s, H-2′); ^{13}C NMR (100.6 MHz, CD_3OD) δ 31.4 (CH_2, C-20), 35.0 (CH_2, C-19), 37.1 (CH_2, C-5′), 62.4 (CH_2, C-21), 131.6 (CH, C-2′), 148.0 (C, C-3′), 166.1 (C, C-1′), 167.5 (C, C-4′); EIMS m/z 722 [M$^+$] (24), 499 (100), 260 (50); HREIMS m/z 722.3776 (calcd for $C_{40}H_{54}N_2O_{10}$, 722.3778).

Biological Activity Tests. Minimal inhibitory concentrations (MIC) for yeast and fungi were determined in myc medium (phytone peptone 10 g/L, glucose 10 g/L) with 10^4 cells or spores/mL. Mouse fibroblast L929 cells were cultivated in DMEM (GIBCO BRL, Germany) with 3×10^4 cells/mL. The concentration of **1** was varied by serial dilution.

For the measurement of macromolecular syntheses, *R. glutinis* in myc medium, diluted 1:2 with water, was used. Cell density was adjusted to OD_{623nm} = 0.2 (8.7×10^6 cells/mL), and the incorporation of [U-^{14}C]protein hydrolysate, [2-^{14}C]-uracil, and [methyl-^{3}H]thymine was determined as described previously.[17] The concentration of **1** was 2.5 μg/mL.

To assay conductivity, an overnight culture of *R. glutinis* in myc medium was centrifuged, the cells were washed twice with distilled water and resuspended in distilled water to an OD_{623nm} = 6 (2.6×10^8 cells/mL) with 3 μg/mL of **1**, and cultures were shaken gently at room temperature.[18] Conductivity was measured every 30 min.

Acknowledgment. The authors would like to thank S. Reinicke and I. Schleicher for their technical assistance, the co-workers in the Fermentation Plant for cultivation and downstream processing of the production strains, and Dr. V. Wray and co-workers of the Department of Structural Research for NMR and mass spectra. H.B.B. and R.M. are grateful to the Deutsche Forschungsgemeinschaft for grants Bo 1834/1-1 and Mu 1254/3; S.C.W. and G.H. to the Fonds der Chemischen Industrie for a Kekulé fellowship and financial support.

Supporting Information Available: HPLC elution profile of a *S. cellulosum* So ce705 crude extract, scheme of separation of compounds **1**−**7**, and complete ^{1}H and ^{13}C NMR data of leupyrrins **2**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP030109V