Mycenaaurin A, an Antibacterial Polyene Pigment from the Fruiting Bodies of *Mycena aurantiomarginata*

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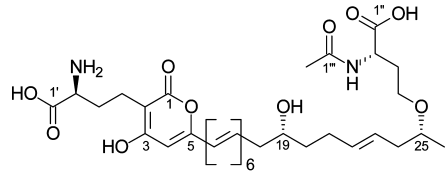
Received March 9, 2010

A new polyene pigment, mycenaaurin A (**1**), was isolated from fruiting bodies of *Mycena aurantiomarginata*. Mycenaaurin A consists of a tridecaketide that is flanked by two amino acid moieties. These are likely to be derived biosynthetically from *S*-adenosylmethionine. The tridecaketide itself contains an α-pyrone, a conjugated hexaene, and an isolated alkenyl moiety. The structure of the new pigment was established by 2D NMR spectroscopic methods and APCIMS. The absolute configuration of the four stereogenic centers was determined by degradation of **1** by ozonolysis and GC-MS comparison of the resulting fragments, after appropriate derivatization, with authentic synthetic samples. Mycenaaurin A (**1**) might act as a constitutive defense compound, since it exhibits antibacterial activity against the Gram-positive bacterium *Bacillus pumilus*.

The fruiting bodies of the basidiomycete *Mycena aurantiomarginata* (Fr.) Quél. (German name: Feuriger Helmling) are small mushrooms that often occur in coniferous forests. They are characterized by a bell-shaped cap of 1 to 2 cm diameter, by a thin stipe of 3 to 10 cm length, and by the strikingly orange color of their gills.[1] Recently, we determined the structures of several fungal pigments from red *Mycena* species. A number of novel red and blue pyrroloquinoline alkaloids were present in the red latex of *Mycena sanguinolenta*[2] and the red latex of *Mycena haematopus*,[3] as well as in the pinkish fruiting bodies of *Mycena rosea*.[4] So far, pyrroloquinoline alkaloids have been found almost exclusively in marine organisms;[5] hence the isolation of a number of pyrroloquinoline alkaloids in *Mycena* species was surprising. Consequently, we became interested not only in the chemistry of red *Mycena* species but also in that of the orange-colored species *M. aurantiomarginata*. Metabolic profiling of the fruiting bodies of *M. aurantiomarginata* by HPLC-UV indicated that the compound responsible for the orange color was likely to be a new polyene. In this paper we describe the isolation and the structure elucidation of the coloring principle of the fruiting bodies of *M. aurantiomarginata*, which we have named mycenaaurin A (**1**).



Mycenaaurin A **(1)**

Results and Discussion

Mycenaaurin A (**1**) was extracted from frozen fruiting bodies of *M. aurantiomarginata* with methanol. The resulting crude extract was filtered, concentrated, and fractionated on a preparative RP-18 column. A water−methanol gradient was used for HPLC, and the compound was detected by its UV/vis absorption at λ 420 nm. The yield of **1** from 10 g of the frozen fruiting bodies was 3.5 mg.

Mycenaaurin A exhibited absorption maxima at λ 400, 417, and 436 nm in the UV/vis spectrum, which are characteristic for polyene pigments. The molecular formula $C_{36}H_{48}N_2O_{10}$ was deduced from the HRAPCIMS, revealing that **1** contained 14 degrees of unsaturation. In accordance with the molecular formula, the ^{13}C NMR spectrum, recorded in DMSO-d_6, exhibited 36 resonances between δ_C 19.2 and 173.8, which were attributed to seven quaternary carbons and 19 CH, eight CH_2, and two CH_3 groups with the help of the HSQC spectrum (Table 1). The ^1H NMR spectrum showed the resonances of 13 protons that were assigned to a polyene system on account of their chemical shift values (δ 5.80−6.92, Table 1). Two resonances (δ_H 5.37 and 5.41) were attributed to an isolated alkenyl group. Two methyl signals (δ_H 0.99 and 1.82) were present in the aliphatic region, as well as the resonances of several CH and CH_2 groups that appeared in the ^1H NMR spectrum between δ_H 1.33 and 4.23. The resonance of an exchangeable amide proton (δ_H 8.05) was detectable only when DMSO-d_6 rather than CD_3OD was used as solvent. Correlations in the COSY and HSQC spectra allowed the assignment of five spin systems of **1** (Table 1). The polyene system and the isolated alkenyl group were part of a $(CH{=}CH)_6$-CH_2-CH-$(CH_2)_2$-$(CH{=}CH)$-CH_2-CH-CH_3 fragment. In addition, **1** contained two $(CH_2)_2$-CH fragments, an isolated CH group, and an isolated CH_3 group. One $(CH_2)_2$-CH fragment was part of a homoserine residue on account of the characteristic chemical shift values of H-2″ (δ_H 4.23), H_2-4″ (δ_H 3.38), C-2″ (δ_C 49.1), and C-4″ (δ_C 63.8) (Table 1 and Figure 1). HMBC correlations from H-2″ (δ_H 4.23) and H-3″ (δ_H 1.69/1.86) to the quaternary carbon atom C-1″ (δ_C 173.8) allowed assignment to the carboxy group of the homoserine residue. HMBC correlations from H-2″ (δ_H 4.23) and H_3-2‴ (δ_H 1.82) to the quaternary carbon C-1‴ (δ_C 169.2) revealed that the homoserine moiety was *N*-acetylated. An HMBC correlation from H_2-4″ (δ_H 3.38) to C-25 (δ_C 74.6) indicated that the *N*-acetyl homoserine residue was attached to the central $(CH{=}CH)_6$-CH_2-CH-$(CH_2)_2$-$(CH{=}CH)$-CH_2-CH-CH_3 fragment via an ether bond between C-4″ and C-25. HMBC correlations from the protons H-6, H-7, H-4, H_2-3′, and H_2-4′ allowed the assignment of the positions of the carbons C-1 to C-5. The chemical shift values of these carbons are typical for a γ-hydroxy-α-pyrone moiety.[6] HMBC correlations from H-6 and H-7 of the central fragment to the quaternary carbon C-5, from H-6 to C-4, and from H-4 to C-6 indicated the connection of the α-pyrone moiety to the polyene residue via C-5 and C-6. The second $(CH_2)_2$-CH fragment was also part of an amino acid residue, as indicated by its chemical shift values and typical HMBC correlations from H-2′ to C-1. HMBC correlations from H_2-3′ to C-2 and from H_2-4′ (δ_H 2.51) to the quaternary carbon atom C-1 (δ_C 164.2) and to C-2 and C-3 suggested this amino acid moiety was connected to the α-pyrone via C-4′ and C-2. All double bonds of **1** had *trans*-configuration, as revealed by the large $^3J_{HH}$ coupling constants of 14−15 Hz of the polyene protons and of the protons of the isolated alkenyl group.

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10.1021/np100155z
Published on Web 07/09/2010

Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data (600 MHz, DMSO-d_6, 300 K) of Mycenaaurin A (**1**)

position	δ_C, mult.	δ_H, mult. (*J* in Hz)	COSY	NOESY/ROESY	HMBC
1	164.2, qC				
2	99.9, qC				
3	169.8, qC				
4	104.8, CH	6.00, s		6, 7	2, 3, 5, 6
5	155.8, qC				
6	122.9, CH	6.26, d (14.9)	7	4, 8	4, 5, (7), 8
7	133.0, CH	6.92, dd (14.9, 11.6)	6, 8	4, 9	5, (6), (8), C-9
8	131.5, CH	6.423, dd (14.1, 11.6)	7, 9	6,10[a]	6, 10
9	137.5, CH	6.64, dd (14.1, 11.2)	8, 10	7, 11	7, (10), 11
10	132.5, CH	6.417, dd (16, 11.2)	9,11[a]	8[a], 12[a]	8, 12
11	135.6, CH	6.46, dd (16, 10)	10[a], 12[a]	9, 13[a]	9, 13
12	132.4, CH	6.37, dd (16, 10)	11[a], 13[a]	10[a], 14[a]	10, 14
13	134.8, CH	6.39, dd (16, 10)	12[a], 14	11[a], 15[a]	11, 14
14	130.9, CH	6.24, dd (16, 10)	13, 15	12[a], 16[a]	(13), 16
15	134.4, CH	6.31, dd (16, 10.8)	14, 16	13[a], 17	13, 17
16	132.1, CH	6.13, dd (14.4, 10.8)	15, 17	14[a], 17, 18	14, 15, (18)
17	133.4, CH	5.80, d (14.4)	16, 18	15, 16, 18	15, 18, 19
18	40.9, CH_2	2.17, m	17, 19	16, 17, 19	16, 19
19	69.2, CH	3.45, m	18, 20a, 20b	18	17, 20, 21
20	36.5, CH_2	1.38, m, H_a	19, 20b, 21a, 21b	20b, 21b, 22	18, 19, 21, 22
		1.33, m, H_b	19, 20a, 21a, 21b	20a, 21a, 22	18, 19, 21, 22
21	28.4, CH_2	2.06, m, H_a	20a, 20b, 21b, 22	20b, 21b, 22, 23	19, 20, 22, 23
		1.97, m, H_b	20a, 20b, 21a, 22	20a, 21a, 22, 23	19, 20, 22, 23
22	132.3, CH	5.41, dm (13.9)	21a, 21b, 23	20a, 20b, 21a, 21b, 24a, 24b, 25	20, 21, 23, 24
23	126.1, CH	5.37, dm (13.9)	22, 24a, 24b	21a, 21b, 24a, 24b, 25	21, 22, 24, 25
24	39.0, CH_2	2.14, m, H_a	23, 24b, 25	22, 23, 24b, 25, 26	22, 23, 25, 26
		2.01, m, H_b	23, 24a, 25	22, 23, 24a, 25, 26	22, 23, 25, 26
25	74.6, CH	3.32, m	24a, 24b, 26	22, 23, 24a, 24b, 26, 4″	23, 24, 26, 4″
26	19.2, CH_3	0.99, d (5.9)	25	24a, 24b, 25	24, 25
1′	170.8, qC				
2′	52.2, CH	3.09, m	3′	(3′), 4′	1′, 3′, 4′
3′	29.4, CH_2	1.73, m	2′, 4′	(2′), 4′	2, 1′, 2′
4′	20.4, CH_2	2.51, m	3′	2′, 3′	1, 2, 3, 2′, 3′
1″	173.8, qC				
2″	49.1, CH	4.23, m	3″a, 3″b	3″a, (3″b), 4″, NH-2″	1″, 3″, 4″, 1‴
3″	31.5, CH_2	1.86, m, H_a	2″, 3″b, 4″	2″, 3″b, 4″, NH-2″	1″, 2″, 4″
		1.69, m, H_b	2″, 3″a, 4″	(2″), 3″a, 4″, NH-2″	1″, 2″, 4″
4″	63.8, CH_2	3.38, m	3″a, 3″b	25, 2″, 3″a, 3″b	25, 2″, 3″
1‴	169.2, qC				
2‴	22.4, CH_3	1.82, s			1‴
NH-2″		8.05, d (7.6)	2″	2″, 3″a, 3″b	2″, 3″, 1‴

[a] Assignment ambiguous on account of signal overlap. Weak ROESY and HMBC correlations are given in brackets.

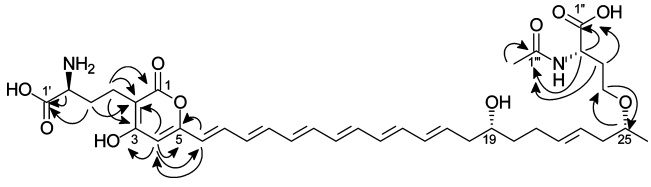

Figure 1. Selected HMBC correlations of mycenaaurin A (**1**).

The NOE between H-4 (δ_H 6.00) and H-6 (δ_H 6.26) established the orientation of the pyrone moiety to the polyene chain, as shown in Figure 1.

The absolute configuration of the four stereogenic centers (C-2′, C-2″, C-19, and C-25) of mycenaaurin A (**1**) was determined after degradation by ozonolysis.[7] Subsequent oxidation of the degradation products with H_2O_2 yielded glutamic acid (**2**), 3-hydroxyadipic acid (**3**), and the homoserine ether **4** (Figure 2).

The absolute configuration at C-2′ was determined by conversion of **2** to the corresponding methyl ester,[8] which was reacted with *S*-Mosher's acid chloride (*S*-MTPA-Cl)[9,10] to the amide **5** (Figure 2). The GC-MS comparison of the derivatized degradation product **5** with authentic synthetic samples of known configuration revealed the *S*-configuration for C-2′ in **1**.

To elucidate the absolute configuration at C-19, the degradation products of the ozonolysis were treated with 2 N hydrochloric acid, thus converting 3-hydroxyadipic acid (**3**) to hydroxyadipic acid-γ-lactone. After removal of the solvents, hydroxyadipic acid-γ-lactone was converted with diazomethane to the methyl ester **6** (Figure 2). This compound was compared by GC-MS with authentic samples of *R*-**6** and *S*-**6**, revealing that **1** possesses the *R*-configuration at C-19. Compound *S*-**6** was synthesized from 1-*tert*-butyl 6-methyl 3-oxohexanedioate, which was reduced enzymatically with baker's yeast to (*S*)-1-*tert*-butyl 6-methyl 3-hydroxyhexanedioate.[11,12] Subsequent acid-catalyzed transesterification of this compound with methanol yielded *S*-**6**.

The absolute configuration at C-25 of **1** was determined after degradation of the homoserine ether **4**. Treatment of **4** with methanolic potassium hydroxide yielded 3-hydroxybutyric acid in traces. Subsequent derivatization of 3-hydroxybutyric acid with diazomethane yielded methyl 3-hydroxybutanoate (**7**) (Figure 2). The GC-MS comparison of the degradation product **7** with authentic samples of *R*- and *S*-**7** showed that the degradation product was (*R*)-methyl 3-hydroxybutanoate, thus revealing the *R*-configuration at C-25 of **1**.

To determine the absolute configuration of mycenaaurin A (**1**) at C-2″, the homoserine ether **4** present in the mixture of ozonolysis products of **1** was hydrolyzed to homoserine, which was converted via its methyl ester to the Mosher amide **8** (Figure 2). GC-MS comparison of the natural product-derived **8** with synthetic samples of known configuration revealed the *S*-configuration at C-2″ of **1**.

The structure of mycenaaurin A (**1**) is in accordance with its hypothetical biosynthesis. The core structure consisting of C-1 to

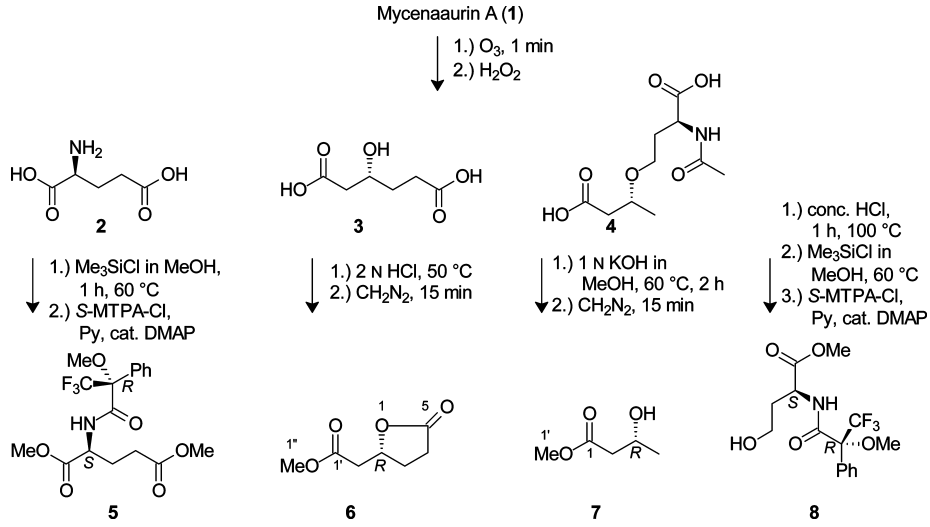

Figure 2. Degradation and derivatization of **1** for the determination of the absolute configuration of the stereogenic centers (DMAP: 4-dimethylaminopyridine; *S*-MTPA-Cl: *S*-Mosher's acid chloride; Py: pyridine).

C-26 is obviously derived from a tridecaketide, starting from C-26/C-25. The positions of the double bonds, the OH groups, and the lactone ring perfectly fit this hypothesis. Both amino acid units are probably derived from *S*-adenosylmethionine. Usually, the methyl group from *S*-adenosylmethionine is transferred as an electrophile; however, in the case of the biosynthesis of **1** the amino acid moiety is apparently transferred to both the nucleophilic OH group at C-25 and to C-2, on account of the acidic nature of H-2. An electrophilic transfer of the amino acid moiety of *S*-adenosylmethionine is likely to occur not only in the case of the biosynthesis of **1** but also in the hypothetical biosyntheses of the sanguinones,[2] of the mycenarubins,[3,4] and of aleurodisconitrile,[13] which is the inactive precursor in the wound-activated chemical defense[14] of the crust fungus *Aleurodiscus amorphus*.[13] It is also worthy of note that mycenaaurin A (**1**) is present only in fruiting bodies of *M. aurantiomarginata* and does not occur in the colorless mycelial cultures of this species. Hence, feeding experiments to prove the proposed biosynthesis could not be performed with mycelial cultures.

A number of polyene pigments have previously been isolated from other fungi. For instance, *Boletus laetissimus* and *Boletus rufoaureus* contain the boletocrocins A−G,[15] while the polyene pigment melanocrocin[7] was found in *Melanogaster broomeianus*. Moreover, calostomal[16] is present in *Calostoma cinnabarinum*, *Laetiporus sulphureus* contains laetiporic acids,[17] and *Albatrellus confluens* contains aurovertin E.[18] However, in comparison to these known fungal polyenes, the structure of mycenaaurin A (**1**) is more complex.

Many polyenes are bioactive. For instance, nystatin and amphothericin B are used to kill human-pathogenic fungi.[18] The bioactivity of these compounds is based on the destabilization of cell membranes, leading to an efflux of ions and small molecules from the interior of the cells.[19] When we tested **1** for antimicrobial activity at a concentration of 0.5 μmol/disk in the agar diffusion assay against *Bacillus pumilus*, there was a growth inhibition zone of 2.5 cm in diameter. Under the same conditions the known antibiotic nourseothricine produced an inhibition zone of 3.5 cm in diameter. Consequently, the ecological role of mycenaaurin A (**1**) could be to prevent certain bacteria from growing on the fruiting bodies of *M. aurantiomarginata*.

Experimental Section

General Experimental Procedures. Evaporation of the solvents was performed under reduced pressure using a rotary evaporator. Preparative HPLC separations were performed using two Waters 590EF pumps equipped with an automated gradient controller 680 and a Kratos Spectroflow 783 UV/vis detector. The samples were fractionated on a Nucleodur C-18 EC column (5 μm, 21 × 250 mm, Macherey-Nagel) using the following gradient program: 10 min 99.9% H_2O/0.1% AcOH, then within 30 min linear to 100% MeOH, then 20 min at 100% MeOH; flow rate: 12 mL/min; detection: UV/vis at 420 nm. UV spectra were recorded on a Varian Cary 100 Bio UV/vis spectrometer. NMR spectra were recorded with a Bruker DMX 250 spectrometer (^{1}H at 250.06, ^{13}C at 62.9 MHz), with a Bruker DMX 500 spectrometer equipped with a TXI probe (^{1}H at 500.11, ^{13}C at 125.8 MHz), and with a 600 MHz Bruker Avance III spectrometer equipped with a TXI cryo probe (^{1}H at 600.13, ^{13}C at 150.9 MHz). Chemical shifts were determined in δ (ppm) relative to the solvent $CDCl_3$ (δ_H 7.26, δ_C 77.0) or DMSO-d_6 (δ_H 2.49, δ_C 39.5) as internal standard. GC-MS spectra were recorded with a Thermo Electron Trace DSQ mass spectrometer coupled with a Thermo Electron Trace GC Ultra equipped with a PTV injector. For sample separation, either a fused silica OPTIMA-5-Accent capillary column (15 m × 0.25 mm, coated with a 0.25 μm layer of liquid phase, Macherey-Nagel), a Betadex 120 capillary column (30 m × 0.25 mm, coated with a 0.25 μm layer of liquid phase, Supelco), or a Lipodex E capillary column (25 m × 0.25 mm, Macherey-Nagel) was used. Helium served as carrier gas. Injection volumes were 0.2−0.5 μL of a 1−2% (w/v) solution. *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was used for pertrimethylsilylation. Temperature programs: 1 min isothermal at 50 °C, then 5 °C/min up to 300 °C, finally 10 min isothermal at 300 °C (OPTIMA-5-Accent); 1 min isothermal at 50 °C, then 5 °C/min up to 220 °C, finally 15 min isothermal at 220 °C (Betadex 120); 1 min isothermal at 50 °C, then 5 °C/min up to 200 °C, finally 15 min isothermal at 200 °C (Lipodex E). Retention indices R_i according to Kováts were determined by injection of a 0.2 μL sample of a standard mixture of saturated straight-chain alkanes (C_{10}−C_{36}).[20] HRAPCIMS spectra were obtained with a Thermo Scientific LTQ Orbitrap mass spectrometer.

Mushroom Material. Fruiting bodies of *M. aurantiomarginata* (leg. et det. R. J. R. Jaeger and P. Spiteller) were collected between September and December of 2004 to 2009 in coniferous forests near Frieding, 35 km southwest of Munich. Voucher samples of *M. aurantiomarginata* were deposited at the Institut für Organische Chemie und Biochemie der Albert-Ludwigs-Universität Freiburg, Germany. The mushrooms were stored at −35 °C after collection. Mycelial cultures of *M. aurantiomarginata* (CBS 494.79, Centraalbureau voor Schimmelcultures, Amsterdam) were grown on agar plates at 18 °C for several weeks on a medium consisting of malt extract (30 g), peptone (5 g), agar (15 g), and H_2O (1 L).

Test Organism. *Bacillus pumilus* (DSM 27, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig) was grown on agar plates at 26 °C for 24 h on a medium consisting of peptone (10 g), NaCl (5 g), agar (15 g), and H_2O (1 L).

Extraction and Isolation of 1. Frozen fruiting bodies (10 g) were crushed after addition of MeOH (10 mL) and extracted with MeOH (2 × 100 mL) at 25 °C. Then, the combined extracts were concentrated in vacuo at 40 °C. The resulting residue was dissolved in H_2O−MeOH

(1:1, 5 mL), prepurified with an RP-18 cartridge, and separated on an RP-18 column by preparative HPLC (UV/vis detection at 420 nm), yielding **1** (3.5 mg, t_R 42.0 min).

Mycenaaurin A (1): orange solid; UV/vis (MeOH) λ_{max} (log ε) 202 (4.51), 226 (4.55), 264 (4.24), 334 (4.48), 400 (4.95), 417 (5.03), 436 (4.94) nm; ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 1); HRAPCIMS *m/z* 669.3384 (calcd for $C_{36}H_{49}N_2O_{10}$ [M + H]$^+$, 669.3382); HRAPCI-MSMS (parent ion *m/z* 669, 35 eV) *m/z* (%) 651.3287 (95) [$C_{36}H_{47}N_2O_9$, M + H − H_2O]$^+$, 508.2700 (100) [$C_{30}H_{38}NO_6$, M + H − $HO(CH_2)_2CH(NHCOCH_3)(CO_2H)$]$^+$, 490.2592 (39) [$C_{30}H_{36}NO_5$]$^+$.

Determination of the Absolute Configuration at C-2′ of 1: Degradation and Derivatization of 1 to (*S*)-Dimethyl 2-((*R*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanamido)pentanedioate (2*S*,2′*R*-5). Ozone was passed through a solution of 1.7 mg (2.6 μmol) of mycenaaurin A (**1**) in 2 mL of MeOH at −78 °C for 1 min. After addition of two drops of 30% H_2O_2 the solution was allowed to warm to room temperature and the solvents were removed. The residue was refluxed in 2 mL of MeOH and 0.2 mL of $(CH_3)_3SiCl$ for 1 h. After complete removal of the solvent, the residue was dissolved in 0.5 mL of pyridine. Then, 3 μL (16 μmol) of (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid chloride (*S*-MTPA-Cl) and catalytic amounts of 4-dimethylaminopyridine (DMAP) were added, and the solution was stirred under argon at 37 °C for 1 h. The solvent was removed and the residue was dissolved in 1 mL of H_2O and extracted three times with 1 mL of $CHCl_3$. The organic phase was dried over Na_2SO_4, filtered, concentrated under reduced pressure, and transferred into a GC vial.

2*S*,2′*R*-5: GC-MS R_i 2190; *m/z* (%) 328 (4), 202 (26) [M − $C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 189 (76) [$C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 174 (67) [M − (C═O)$C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 170 (30), 158 (8), 142 (100) [M − $C(CF_3)(OCH_3)(C_6H_5)$ − CO − CH_3OH]$^+$, 139 (13), 127 (14), 119 (19), 114 (20), 110 (29), 105 (45) [C_7H_5O]$^+$, 98 (14), 91 (24), 82 (21), 77 (33) [C_6H_5]$^+$, 69 (9) [CF_3]$^+$.

Synthetic 2*S*,2′*R*-5: Synthetic 2*S*,2′*R*-**5** was synthesized from L-glutamic acid by methylation and reaction with *S*-MTPA-Cl as described above: GC-MS R_i 2192; *m/z* (%) 328 (6), 202 (27) [M − $C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 189 (44) [$C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 174 (66) [M − (C═O)$C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 170 (19), 158 (7), 142 (100) [M − $C(CF_3)(OCH_3)(C_6H_5)$ − CO − CH_3OH]$^+$, 139 (6), 127 (6), 119 (10), 114 (17), 110 (28), 105 (19) [C_7H_5O]$^+$, 98 (11), 91 (9), 82 (19), 77 (9) [C_6H_5]$^+$, 69 (4) [CF_3]$^+$.

Synthetic 2*S*,2′*S*-5. Synthetic 2*S*,2′*S*-**5** was synthesized from L-glutamic acid by methylation and reaction with *R*-MTPA-Cl as described above. GC-MS R_i 2175; *m/z* (see mass spectrum of synthetic 2*S*,2′*R*-**5**).

Determination of the Absolute Configuration at C-19 of 1: Degradation and Derivatization of 1 to Methyl 2-((*R*)-Tetrahydro-5-oxofuran-2-yl)acetate (*R*-6). Ozone was passed through a solution of 12 mg (18 μmol) of **1** in 5 mL of MeOH at −78 °C for 1 min. Two drops of 30% H_2O_2 were added, the solution was allowed to warm to room temperature, and the solvents were removed. The residue was methylated for 15 min in an ethereal solution of CH_2N_2, and the solvent was evaporated under reduced pressure. The residue was warmed to 50 °C in 2 N HCl for 10 min and after removal of the solvent methylated again for 15 min in an ethereal solution of CH_2N_2. The reaction product was concentrated in vacuo, transferred into a GC vial, and analyzed by enantioselective GC-MS.

***R*-6:** GC-MS (Betadex 120) R_i 1688; *m/z* (%) 140 (18) [M − H_2O]$^+$, 130 (17) [M − CO]$^+$, 127 (19), 126 (11), 116 (11), 98 (11), 85 (100) [M − $CH_2CO_2CH_3$]$^+$, 74 (15), 59 (13) [CO_2CH_3]$^+$, 57 (13), 56 (16), 55 (19), 43 (20).

Synthetic *S*-6. Compound *S*-**6** was synthesized from 1-*tert*-butyl 6-methyl 3-oxohexanedioate, which was reduced enzymatically with baker's yeast.[11,12] For NMR measurements the reaction product was purified by flash chromatography [silica gel, hexane−EtOAc (2:1)], yielding an enantiomeric mixture of the lactones *R*- and *S*-**6** (1:4): ^{1}H NMR (500 MHz, $CDCl_3$, 300 K) δ 4.89 (m, 1 H, H-2), 3.71 (s, 3 H, H-1″), 2.82 (dd, $^3J_{HH}$ = 6.6 Hz, $^2J_{HH}$ = 16.3 Hz, 1 H, H_a-2′), 2.65 (dd, $^3J_{HH}$ = 6.4 Hz, $^2J_{HH}$ = 16.3 Hz, 1 H, H_b-2′), 2.57 (m, 2 H, H-4), 2.47 (m, 1 H, H_a-3), 1.97 (m, 1 H, H_b-3); ^{13}C NMR (126 MHz, $CDCl_3$, 300 K) δ 176.4 (C-5), 169.9 (C-1′), 76.2 (C-2), 52.0 (C-1″), 39.7 (C-2′), 28.4 (C-4), 27.6 (C-3); GC-MS (Betadex 120) R_i 1685; *m/z* (%) 140 (18) [M − H_2O]$^+$, 130 (17) [M − CO]$^+$, 127 (18), 126 (10), 116 (10), 108 (8), 98 (9), 85 (100) [M − $CH_2CO_2CH_3$]$^+$, 74 (13), 59 (11) [CO_2CH_3]$^+$, 56 (13), 55 (18), 54 (7), 43 (13).

Synthetic *R*-6: GC-MS (Betadex 120) R_i 1688; *m/z* (see mass spectrum of *S*-**6**).

Determination of the Absolute Configuration at C-25 of 1: Degradation of Mycenaaurin A (1) to Methyl (*R*)-3-Hydroxybutanoate (*R*-7). Ozone was passed through a solution of 6 mg (9 μmol) of **1** in 5 mL of MeOH−DMSO (10:1) at −78 °C for 2 min. After addition of three drops of 30% H_2O_2 the solution was allowed to warm to room temperature and the solvents were removed. The residue was refluxed in 2 mL 1 N methanolic KOH for 8 h; then the solvent was removed and the residue was dissolved in H_2O, acidified with aqueous HCl, and extracted three times with EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The aqueous phase was neutralized with aqueous NaOH, and the solvent was evaporated. Both the organic and the aqueous phases were dissolved in 1 mL of EtOAc and methylated for 15 min with an ethereal solution of CH_2N_2. The solutions were dried over Na_2SO_4, filtered and concentrated in vacuo, transferred into GC vials, and analyzed by enantioselective GC-MS. The product (**7**) could be found only in traces in the aqueous phase. Compound **7** consisted mainly of *R*-**7**, but some *S*-**7** was also present, which we attributed to partial racemization of *R*-**7** during the degradation procedure. GC-MS (Lipodex E) R_i 1237; *m/z* (%) 103 (43) [M − CH_3]$^+$, 100 (5) [M − H_2O]$^+$, 87 (34), 74 (90) [M − CH_3CHO]$^+$, 71 (44), 61 (18), 59 (18) [CO_2CH_3]$^+$, 45 (48) [M − $CH_2CO_2CH_3$]$^+$, 43 (100) [CH_3CO]$^+$.

Synthetic *R*-7. Sodium (*R*)-3-hydroxybutanoate was acidified with 10 N HCl, and the free carboxylic acid was extracted with EtOAc. The organic phase was dried over Na_2SO_4, filtered, concentrated in vacuo, and methylated for 10 min with an ethereal solution of CH_2N_2. The solution was dried over Na_2SO_4, filtered and concentrated in vacuo, transferred into a GC vial, and analyzed by enantioselective GC-MS. ^{1}H NMR (250 MHz, $CDCl_3$, 300 K) δ 4.17 (m, 1 H, H-3), 3.68 (s, 3 H, H-1′), 2.45 (m, 2 H, H-2), 1.20 (d, $^3J_{HH}$ = 6.3 Hz, 3 H, H-4); ^{13}C NMR (63 MHz, $CDCl_3$, 300 K) δ 173.2 (C-1), 64.2 (C-3), 51.7 (C-1′), 42.5 (C-2), 22.4 (C-4); GC-MS (Lipodex E) R_i 1239; *m/z* (%) 117 (2), 103 (46) [M − CH_3]$^+$, 100 (7) [M − H_2O]$^+$, 87 (35), 85 (10), 74 (100) [M − CH_3CHO]$^+$, 71 (46), 61 (17), 59 (15) [CO_2CH_3]$^+$, 45 (47) [M − $CH_2CO_2CH_3$]$^+$, 43 (98) [CH_3CO]$^+$.

Synthetic *S*-7: GC-MS (Lipodex E) R_i 1214; *m/z* (see mass spectrum of synthetic *R*-**7**).

Determination of the Absolute Configuration at C-2″ of 1: Degradation and Derivatization of 1 to (*S*)-Methyl 2-((*R*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanamido)-4-hydroxybutanoate (2*S*,2′*R*-8). Ozone was passed through a solution of 1.73 mg (2.59 μmol) of **1** in 2 mL of MeOH at −78 °C for 1 min. Two drops of 30% H_2O_2 were added, the solution was allowed to warm to room temperature, and the solvents were removed. The residue was refluxed in 1 mL of concentrated HCl for 1 h. After removal of the solvent the residue was refluxed in 2 mL of MeOH and 0.2 mL of $(CH_3)_3SiCl$ for 1 h. The solvent was removed, and the residue was dissolved in 0.5 mL of pyridine. After addition of 3 μL (16 μmol) of *S*-MTPA-Cl and catalytic amounts of DMAP the solution was stirred under argon at 37 °C for 1 h. The solvent was removed, and the residue was dissolved in 1 mL of H_2O and extracted three times with 1 mL of $CHCl_3$. The organic phase was dried over Na_2SO_4, filtered, concentrated under reduced pressure, and transferred into a GC vial. GC-MS R_i 2056; *m/z* (%) 318 (7) [M − CH_3O]$^+$, 189 (84) [$C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 188 (35), 175 (14), 170 (22), 160 (85) [M − $C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 156 (34), 139 (13), 128 (59), 119 (24), 113 (35), 105 (57) [C_7H_5O]$^+$, 97 (12), 91 (30), 86 (37), 77 (39) [C_6H_5]$^+$, 69 (20) [CF_3]$^+$, 44 (36), 40 (100).

Synthetic 2*S*,2′*S*-8. Compound 2*S*,2′*S*-**8** was synthesized from L-homoserine by methylation and reaction with *R*-MTPA-Cl as described above: GC-MS R_i 2042; *m/z* (%) 318 (2) [M − CH_3O]$^+$, 190 (35), 189 (100) [$C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 188 (25), 175 (19), 170 (80), 160 (59) [M − $C(CF_3)(OCH_3)(C_6H_5)$]$^+$, 156 (20), 139 (18), 128 (77), 119 (28), 105 (68) [C_7H_5O]$^+$, 100 (19), 91 (36), 86 (27), 77 (48) [C_6H_5]$^+$, 69 (13) [CF_3]$^+$.

Synthetic 2*S*,2′*R*-8. Compound 2*S*,2′*R*-**8** was synthesized from L-homoserine by methylation and reaction with *S*-MTPA-Cl as described above: GC-MS R_i 2055; *m/z* (%) (see mass spectrum of 2*S*,2′*S*-**8**).

Biological Tests. For agar diffusion assays, **1** (0.5 μmol) was dissolved in MeOH (5−25 μL) and dropped onto paper discs (diameter 5 mm, thickness 0.5 mm). These discs were dried under sterile conditions and placed on agar plates inoculated with the test organism

Bacillus pumilus. The plates were incubated at 26 °C for 24 h. Nourseothricin (0.5 μmol) was used as a positive control and mannitol (0.5 μmol) as a negative control.

Acknowledgment. We are grateful to Prof. Dr. M. Spiteller and Dr. M. Lamshöft (Technische Universität Dortmund, Institut für Umweltforschung, Germany) for the measurement of HRAPCIMS spectra and to S. Serdjukow for experimental assistance. Our work has been supported by an Emmy Noether Fellowship for young investigators of the Deutsche Forschungsgemeinschaft (SP 718/1-3) and by the Fonds der Chemischen Industrie.

Supporting Information Available: Selected UV/vis and NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP100155Z