Epicoccamides B–D, Glycosylated Tetramic Acid Derivatives from an *Epicoccum* sp. Associated with the Tree Fungus *Pholiota squarrosa*

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Three new tetramic acid derivatives, epicoccamides B–D, together with the known epicoccamide, were isolated from an *Epicoccum* sp. associated with the tree fungus *Pholiota squarrosa*. The structures of the new compounds were elucidated on the basis of their physical data and chemical degradation. The epicoccamides differ in substitution pattern and in the size of the central carbon chain. The derivative with the longest chain, epicoccamide D, induces morphogenesis and pigment formation in surface cultures of the fungus *Phoma destructiva* at a concentration of 1.7 mM. Moreover, it exhibits weak to moderate cytotoxicity to HeLa cell lines (CC_{50} 17.0 μM) and antiproliferative effects toward mouse fibroplast (L-929) and human leukemia cell lines (K-562) with growth inhibition (GI_{50}) of 50.5 and 33.3 μM, respectively.

Microorganisms that live in symbiosis have proven to be a rich source for biologically active natural products.[1] There is growing evidence that many metabolites isolated from animals and plants are in fact produced by microorganisms.[2] While endophytes are well-known producers of bioactive secondary metabolites,[3–6] microorganisms associated with fungi are relatively little explored. The only known examples of natural compounds producing endofungal bacteria belong to the genus *Burkholderia*, which represent the true producers of the mycotoxins rhizoxin and rhizonin from *Rhizopus microsporus*.[7–10] Anke, Sterner, and co-workers reported on the biosynthetic potential of fungi growing on or within the fruit bodies of ascomycetes, so-called mycophilic fungi.[11–15] In the course of our search for bioactive compounds produced by tree fungi,[16–19] we were surprised to find an *Epicoccum* species growing within the fruit body of the tree fungus *Pholiota squarrosa*.[20] We have succeeded in cultivating the fungus and characterized three new polyketide–amino acid hybrid metabolites, the potent antibacterial tetramic acid epicoccarines A and B, and the pyridone compound epipyridone.[20] Here we present three new members of the tetramic acid family of natural products produced by the endofungal *Epicoccum* sp.

The mycelium harvested from a 60 L *Epicoccum* sp. culture were first extracted with EtOAc and then MeOH. The MeOH extract was treated with H_2O to eliminate H_2O-soluble substances and re-extracted with EtOAc. The EtOAc-soluble portion of the re-extracted residue (30 g) was subjected to open column chromatography on Si gel. Further purification of selected fractions afforded four related compounds, **1** (500 mg), **2** (8 mg), **3** (10 mg), and **4** (7 mg).

The main metabolite (**1**, Figure 1) proved to be identical with an unusual tetramic acid glycoside named epicoccamide, which was recently isolated by König and co-workers from a marine-derived *Epicoccum* sp.[21] Strikingly, this fungus was isolated from inner tissue of a jellyfish. The ^{1}H NMR spectrum of **2** was similar to that of **1**, except for the presence of an additional singlet at δ 2.16 (3H, s, H-8′). Also in the ^{13}C NMR spectrum additional resonances were observed at δ 21.0 (CH_3) and 171.2 (C=O), suggesting that **2** is an *O*-acetyl derivative of **1**. This was verified by a peak at *m/z* 598.5 $[M - H]^-$ in the ESIMS of **2**. Moreover, a correlation between the oxymethine proton H-3′ (δ 4.78, dd, J = 3.0, 9.8 Hz) and the additional carboxyl carbon C-7′ (δ 171.2) was observed by HMBC, indicating that the acylation occurred at C-3′ in the sugar moiety.

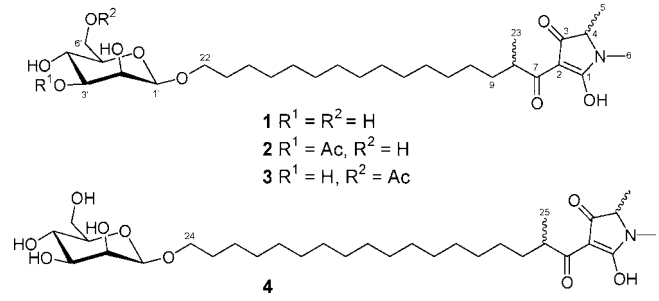

Figure 1. Structures of **1**–**4**.

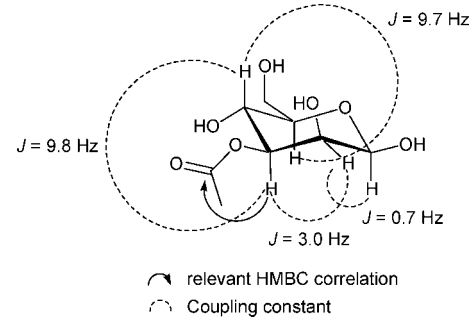

Figure 2. Identification of the sugar moiety as β-mannose.

The structure of the sugar moiety was elucidated by means of analyses of proton and COSY spectra and coupling constants (Figure 2). The coupling constant (J = 0.76 Hz) between the anomeric proton (δ 4.56) and H-2′ indicated their *cis* axial-equatorial disposition. The *trans*-diaxial arrangement of H-3′ (δ 4.78) and H-4′ (δ 4.08) was deduced from their coupling constant $J_{H\text{-}3'\text{-}H\text{-}4'}$ = 9.8 Hz. H-4′ and H-5′ were also identified to be *trans*-diaxial due to their coupling constant ($J_{H\text{-}4'\text{-}H\text{-}5'}$ = 9.7 Hz). Consequently, the sugar moiety was identified as β-mannose. Its D-configuration was determined according to the previous study.[21] Thus, **2** was identified as 5-hydroxy-1,2-dimethyl-4-[2-methyl-16-(3-*O*-acetyl-β-D-mannopyranosyl)hexadecanoyl]-1*H*-pyrrol-3(2*H*)-one (epicoccamide B). Its structure was confirmed by MS/MS data, which showed the cleavage of the acyl moiety followed by dehydration. Two important ion fragments (*m/z* 394.3 and 126.0) indicated the loss of the sugar moiety and the α-cleavage of the tetramic moiety, respectively.

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Table 1.

compound	antiproliferative activity L-929 GI_{50} [μM]	antiproliferative activity K-562 GI_{50} [μM]	cytotoxicity HeLa CC_{50} [μM] (CC_{10} [μM])
1(557)	>90.0	>90.0	>90.0 (51.3)
2(599)	71.1	52.7	44.2 (26.2)
3(599)	57.2	33.7	29.0 (13.6)
4(585)	50.5	33.3	17.0 (11.6)

The molecular formula of **3** was determined as $C_{31}H_{52}O_{10}N$ by HREIMS, indicating that **3** is an isomer of **2**. Its ^{1}H NMR and ^{13}C NMR spectra were almost identical with those of **2**. The observed HMBC correlation between the oxymethylene protons of the sugar moiety H-6′ (δ 4.32, dd, J = 5.1, 12.1 Hz) and the carboxy carbon C-7′ (δ 171.6) established the connection of the acetyl moiety to C-6′ (δ 63.5). Thus, the structure of **3** was elucidated as 5-hydroxy-1,2-dimethyl-4-[2-methyl-16-(6-*O*-acetyl-β-D-mannopyranosyl)hexadecanoyl]-1*H*-pyrrol-3(2*H*)-one (epicoccamide C).

The UV spectrum of compound **4** as well as the MS/MS fragmentation pattern suggested that **4** is a homologue of **1** with a mass difference of 28 units. Two ion fragments at m/z = 422.2 and 125.9 indicated the loss of the sugar moiety and the α-cleavage of the tetramic acid moiety. Accordingly, the aliphatic chain consists of two additional methylene groups, which was supported by NMR data. The structure of **4** was thus elucidated as 5-hydroxy-1,2-dimethyl-4-[2-methyl-18-β-D-mannopyranosyloctadecanoyl]-1*H*-pyrrol-3(2*H*)-one, named epicoccamide D.

All epicoccamides have two stereocenters in the aglycone portion of the molecule. While the absolute configuration at C-8 could not be solved in the present study, we aimed at determining the configuration at C-4 using Marfey's method. Compound **1** (10 mg) was ozonized, followed by methanolysis with 3 N MeOH/HCl, yielding *N*-methylalanine methyl ester, which was subsequently treated with *N*-(2,4-dinitro-5-fluorophenyl)-L-alanamide (FDAA). HPLC/MS analysis of the derivative of **1** obtained upon derivatization and comparison of the retention times of the *N*-methylalanine methyl ester derivative with those of standards (*N*-methyl-L-alanine methyl ester and *N*-methyl-DL-alanine methyl ester) revealed that epicoccamide is a 2:3 mixture of 4(*R*) and 4(*S*)-isomers, respectively. Attempts to directly detect both isomers by HPLC without prior derivatization proved unsuccessful, and isomerization during derivatization cannot be fully excluded.

Since the crude extract of *Epicoccum* sp. exhibited notable antiproliferative and cytotoxic activities, all four epicoccamides were individually probed. Among these four compounds epicoccamide D proved to be most active. It exhibits weak to moderate cytotoxicity to HeLa cell lines (CC_{50} 17.0 μM) and good antiproliferative effects on mouse fibroblast (L-929) and human leukemia cell lines (K-562) with inhibition of growth (GI_{50}) of 50.5 and 33.3 μM, respectively. Furthermore, epicoccamide D induces morphogenesis and pigment formation in surface cultures of the fungus *Phoma destructiva* at a concentration of 1.7 mM.

To date, only a few species belonging to the genus *Epicoccum* have been studied for their bioactive secondary metabolites. *Epicoccum nigrum* (*E. purpurascens*) isolated from soil led to the isolation of epicorazines A and B,[22] epirodin,[23] triornicin,[24] orevactaene,[25] and epicocconone.[26] The epicoccamide-producing *Epicoccum* sp. isolated from inner tissue of a jellyfish is another rare example.[21]

In this study, we have isolated and characterized three new epicoccamide derivatives, epicoccamides B–D, from a fungal symbiont of the mushroom *Pholiota squarrosa*. Although **4** is similar to the reported hybrid metabolite epicoccamide, which showed no detectable activity in various assays, **4** exhibits weak cytoxicity to HeLa cell lines. Moreover, it induces morphogenesis and pigment formation in surface cultures of the fungus *Phoma destructiva*. It is intriguing that the same type of metabolites are produced by *Epicoccum* isolates from completely different ecological niches. Further bioassays and elucidation will be needed to understand the natural function of these unusual compounds.

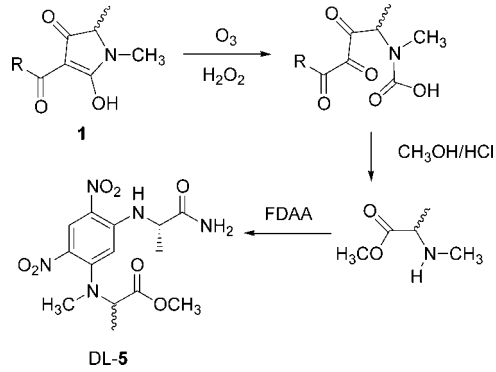
Experimental Section

General Experimental Procedures. Optical rotations were assessed with a P-1020 polarimeter. UV spectra were measured with a Spericord 200 Carl Zeiss spectrometer. IR spectra (film) were recorded on a JASCO FT/IR-4100 Fourier transform infrared spectrometer. HREIMS were recorded on an AMD 402 double-focusing mass spectrometer with BE geometry (AMD, Intestra, Harpstedt, Germany). NMR spectra were recorded on a Bruker Avance 300 DRX spectrometer (Bruker, Karlsruhe, Germany) at 300 MHz for ^{1}H and 75 MHz for ^{13}C in $CDCl_3$. Chemical shifts are given in ppm relative to TMS as internal standard.

Table 2. ^{1}H and ^{13}C NMR ($CDCl_3$) Data of **1–3** (chemical shifts in ppm, coupling constants in Hz, TMS as internal standard)

no.	epicoccamide B (**2**) δ ^{1}H (J in Hz)	epicoccamide B (**2**) δ ^{13}C	epicoccamide C (**3**) δ ^{1}H (J in Hz)	epicoccamide C (**3**) δ ^{13}C	epicoccamide D (**4**) δ ^{1}H (J in Hz)	epicoccamide D (**4**) δ ^{13}C
1		173.1		173.1		173.1
2		99.8		99.8		99.8
3		192.0		192.0		191.9
4	3.68 dd (7.0, 14.0)	62.7	3.67 dd (7.0, 14.0)	62.7	3.66 dd (6.9, 13.9)	62.7
5	1.32 d (6.9)	14.8	1.33 d (6.9)	14.8	1.34 d (6.9)	14.8
6	2.95 s	26.2	2.92 s	26.2	2.94 s	26.2
7		194.6		194.5		194.5
8	3.57 m	36.0	3.56 m	36.0	3.54 m	36.0
9	1.44–1.65 m	33.7	1.44–1.65 m	33.7	1.43–1.67 m	33.7
10	1.21 m	27.2	1.21 m	27.2	1.21 m	27.2
11–19	1.21 m	29.5	1.21 m	29.5	1.21 m	29.5
20	1.21 m	25.9	1.21 m	25.9	1.21 m	29.5
21	1.58 m	29.3	1.58 m	29.3	1.21 m	29.5
22	3.53–3.88 m	70.0	3.50–3.87 m	69.9	1.21 m	25.9
23	1.15 d (6.9)	17.0	1.14 d (6.7)	17.0	1.57 m	29.4
24					3.48–3.83 m	70.0
25					1.15 d (6.9)	17.0
1′	4.56 d (0.7)	99.3	4.46 d (0.7)	99.8	4.44 d (0.7)	100.1
2′	4.12 dd (0.7, 3.0)	69.3	3.97 dd (0.7, 2.7)	70.6	3.97 brs	71.1
3′	4.78 dd (3.0, 9.8)	76.1	3.50 brs	74.1	3.53 brs	74.1
4′	4.08 dd (9.7, 9.7)	65.8	3.64 brs	68.0	3.87 brs	66.6
5′	3.36 brs	75.6	3.35 brs	73.8	3.18 brs	75.9
6′	3.92 m	62.5	4.32 dd (5.1, 12.1) 4.40 dd (2.3, 12.1)	63.5	3.86 m	61.2
7′		171.2		171.6		
8′	2.16 s	21.0	2.10 s	20.8		

Scheme 1. Ozonolysis and Derivatization of **1**; Formation of the Corresponding Carboxylic Acids Was Also Observed



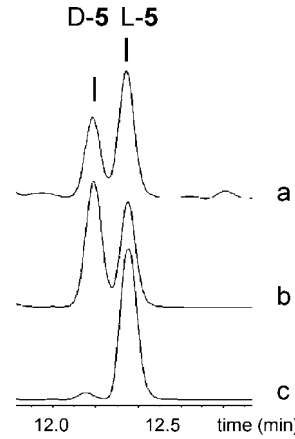


Figure 3. HPLC profiles of FDAA derivatives from degraded **1** (a), *N*-methyl-DL-alanine (b), and *N*-methyl-L-alanine (c).

Open column chromatography was performed on Si gel (Merck; 0.063–0.2 μm). HPLC was performed using a Gilson binary gradient HPLC system equipped with a UV detector (UV/VIS-151) monitoring at 300 nm. Preparative columns were packed with Nucleosil 100-7 C_{18}. TLC was carried out with Si gel 60 F_{254} plates. Spots were visualized by spraying with vanillin/H_2SO_4, followed by heating. All solvents used were spectral grade or distilled prior to use.

Fungal Material. The *Epicoccum* sp. strain was isolated from the mushroom *Pholiota squarrosa* and deposited in the fungal collection of the Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany. The fungus was cultivated under conditions of surface fermentation at 25 °C in 500 mL Erlenmeyer flasks containing 100 mL of medium composed of malt extract (30 g L^{-1}), glucose (10 g L^{-1}), yeast extract (1 g L^{-1}), and $(NH_4)_2SO_4$ (5 g L^{-1}), at pH 6.0. After cultivation for 28 days at 25 °C the mycelium cake from the culture medium (60 L) was extracted twice with EtOAc, then MeOH (each 10 L). The MeOH extract was treated with H_2O to eliminate H_2O-soluble substances and re-extracted with EtOAc (5 L). The EtOAc-soluble portion of the re-extraction residue (30 g) was submitted to open column chromatography on Si gel (Merck, 0.063–0.1 mm, column 4 × 60 cm), using stepwise $CHCl_3$ and $CHCl_3$/MeOH (9:1, 1:1, v/v) as eluents. Final purification was achieved by preparative HPLC using a Phenomenex Hydro-Rp 80 column, Synergi 10 μm, 250 × 21 mm, and acetonitrile/H_2O (83:17, v/v) as eluent (flow rate 15 mL min^{-1}, UV detection at 227 nm), yielding **1** (500 mg), **2** (8 mg), **3** (10 mg), and **4** (7 mg).

Antiproliferative and Cytotoxic Assays. The antiproliferative and cytotoxicity of the test compounds were determined on 2 day old confluent HeLa Ohio, MDCK, and GMK cell monolayers grown in 96-well flat-bottomed microtiter plates (Nunc). After removal of the growth medium, nine 2-fold dilutions of the compounds in 100 μL of test medium were added and incubated at 37 °C in a 5% CO_2 atmosphere for 72 h. Then, the cells were fixed, washed, and stained.[27] The optical density of individual wells was quantified spectrophotometrically at 660 nm and analyszed with the Magellan (TECAN) system. Cell viability was evaluated as percentage of the mean value of optical density resulting from six untreated cell controls, which was set at 100%. The 50% growth inhibition (GI_{50}) and the 50% cytotoxic concentration (CC_{50}) were calculated from the mean dose–response curve of two assays.

Fungal Morphogenesis and Pigment Formation. A malt agar plate with vegetative mycelium of the fungus *Phoma destructiva* was prepared in a manner similar to the standardized agar-well diffusion assay (anonymous 1997) using the above malt agar medium. Subsequently, 50 μL of purified metabolites **1**–**4** was introduced in the agar wells (9 mm diameter), and breeding was carried out for three days until the effects on pigment and aerial mycelium formation became visible.

Epicoccamide B (2): colorless oil; t_R 33 min (preparative HPLC), Phenomenex column (Hydro-Rp 80, Synergi 10 μm, 250 × 21 mm), and acetonitrile/H_2O (83:17, v/v) as eluent (flow rate 15 mL min^{-1}, UV detection at 227 nm), $[\alpha]^{25}_D$ −94.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} 224, 283 nm; IR (film) 3383, 2923, 2852, 1711, 1612, 1449, 1371, 1236 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2; ESIMS (neg. ion mode) *m/z* 598.5 [M – H]$^-$; MS/MS (neg. ion mode) *m/z* 556.4, 538.4, 394.4, 126.0; HRESIMS *m/z* 598.3600 [M – H]$^-$ calcd *m/z* 598.3591 [M – H]$^-$ for $C_{31}H_{52}O_{10}N$.

Epicoccamide C (3): colorless oil; t_R 34.5 min (preparative HPLC), $[\alpha]^{25}_D$ −52.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 224, 283 nm; IR (film) 3311, 2921, 2851, 1710, 1650, 1613, 1448, 1368, 1235, 1065 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2; ESIMS (neg. ion mode) *m/z* 598.5 [M – H]$^-$; MS/MS (neg. ion mode) *m/z* 556.4, 538.5, 394.4, 126.0; HRESIMS *m/z* 598.3605 [M – H]$^-$ calcd *m/z* 598.3591 [M – H]$^-$ for $C_{31}H_{52}O_{10}N$.

Epicoccamide D (4): colorless oil; t_R 36 min (preparative HPLC), $[\alpha]^{25}_D$ −40.4 (*c* 0.20, MeOH); UV (MeOH) λ_{max} 224, 283 nm; IR (film) 3322, 2918, 2849, 1710, 1612, 1449, 10681 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2; ESIMS (neg. ion mode) *m/z* 584.4 [M – H]$^-$; MS/MS (neg. ion mode) *m/z* 422.2, 125.9; HRESIMS *m/z* 584.3782 [M – H]$^-$ calcd *m/z* 584.3799 [M – H]$^-$ for $C_{31}H_{54}O_9N$.

Derivatization Reactions. Ozonolysis was perfomed with an ANSEROS ozone generator COM-AD-O1 apparatus. Epicoccamide (**1**) (10 mg) was dissolved in $CHCl_3$/MeOH (1:1, 2.5 mL), and ozone was introduced for 20 min at −60 °C. H_2O_2 (500 μL) was added, and the reaction mixture was stirred for 30 min at room temperature and evaporated under reduced pressure. The resulting product was then treated with 3 N aqueous MeOH/HCl (300 μL) for 2 h and neutralized with 1 M $NaHCO_3$ solution. Upon addition of *N*-(2,4-dinitro-5-fluorophenyl)-L-alanamide (FDAA) (0.4 mg) in acetone (400 μL), the reaction mixture was stirred at 40 °C for 2 h. After quenching with 1 N aqueous HCl, MeOH was added and the sample was subjected to HPLC analysis.

The standard amino acids *N*-methyl-L-alanine and *N*-methyl-DL-alanine (each 5 mg) were treated with 3 N MeOH/HCl and derivatized as described above.

The separation of the L- and D-FDAA derivatives of the standard compound was performed using an Agilent 1000 Series LC/MSD ion trap system. The system was operated with the electrospray ionization (ESI) source in the positive mode. HPLC conditions: Agilent Zorbax Eclipse XDB C8 column, 5 μm, 150 × 4.6 mm, mobile phase: A = H_2O + 0.1% HCOOH, B = MeOH, gradient elution: starting with 10% at 1 min to 100% at 17 min, kept at 100% for 5 min, flow rate 1 mL min^{-1}. Detection at 340 nm. MS conditions: dry temperature 350 °C, nebulizer 60 psi, dry gas 11 L/min, ion mode positive/negative MS. The retention times observed for (*N*-methyl-D-alaninemethyl ester-2,4-dinitrophenyl-5-)-L-alanamide and (*N*-methyl-L-alaninemethyl ester-2,4-dinitrophenyl-5-)-L-alanamide were 12.1 and 12.3 min, respectively.

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References and Notes

(1) Piel, J. *Curr. Med. Chem.* **2006**, *13*, 39–50.
(2) Piel, J. *Nat. Prod. Rep.* **2004**, *21*, 519–538.

(3) Rosenblueth, M.; Martinez-Romero, E. *Mol. Plant Microbe Interact.* **2006**, *19*, 827–837.
(4) Strobel, G. *Curr. Opin. Microbiol.* **2006**, *9*, 240–244.
(5) Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. *J. Nat. Prod.* **2004**, *67*, 257–268.
(6) Strobel, G. A. *Microbes Infect.* **2003**, *5*, 535–544.
(7) Partida-Martinez, L. P.; Hertweck, C. *Nature* **2005**, *437*, 884–888.
(8) Scherlach, K.; Partida-Martinez, L. P.; Dahse, H.-M.; Hertweck, C. *J. Am. Chem. Soc.* **2006**, *128*, 11529–11536.
(9) Partida-Martinez, L. P.; Hertweck, C. *ChemBioChem* **2007**, *8*, 41–45.
(10) Partida-Martinez, L. P.; Looβ, C.; Ishida, K.; Ishida, M.; Hertweck, C. *Appl. Environ. Microbiol.* **2007**, *73*, 793–797.
(11) Fabian, K.; Anke, T.; Sterner, O. *Z. Naturforsch., C* **2001**, *56*, 106–110.
(12) Schneider, G.; Anke, H.; Sterner, O. *Nat. Prod. Lett.* **1997**, *10*, 133–138.
(13) Wagner, C.; Anke, H.; Besl, H.; Sterner, O. *Z Naturforsch., C* **1995**, *50*, 358–364.
(14) Wagner, C.; Anke, H.; Sterner, O. *J. Nat. Prod.* **1998**, *61*, 501–502.
(15) Wilhelm, C.; Anke, H.; Flores, Y.; Sterner, O. *J. Nat. Prod.* **2004**, *67*, 466–468.
(16) Kemami Wangun, H. V.; Dörfelt, H.; Hertweck, C. *Eur. J. Org. Chem.* **2006**, 1643–1646.
(17) Kemami Wangun, H. V.; Härtl, A.; Kiet, T. T.; Hertweck, C. *Org. Biomol. Chem.* **2006**, *4*, 2545–2548.
(18) Gebhardt, P.; Dornberger, K.; Gollmick, F. A.; Gräfe, U.; Härtl, A.; Görls, H.; Schlegel, B.; Hertweck, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2558–2560.
(19) Kemami Wangun, H. V.; Hertweck, C. *Eur. J. Org. Chem.* **2007**, 3292–3295.
(20) Kemami Wangun, H. V.; Hertweck, C. *Org. Biomol. Chem.* **2007**, *5*, 1702–1705.
(21) Wright, A. D.; Osterhage, C.; Konig, G. M. *Org. Biomol. Chem.* **2003**, *1*, 507–510.
(22) Baute, M. A.; Deffieux, G.; Baute, R.; Neveu, A. *J. Antibiot.* **1978**, *31*, 1099–1101.
(23) Ikawa, M.; McGratten, C. J.; Burge, W. R.; Iannitelli, R. C.; Uebel, J. J.; Noguchi, T. *J. Antibiot.* **1978**, *31*, 159–161.
(24) Frederick, C. B.; Bentley, M. D.; Shive, W. *Biochemistry* **1981**, *20*, 2436–8.
(25) Shu, Y. Z.; Ye, Q. M.; Li, H.; Kadow, K. F.; Hussain, R. A.; Huang, S.; Gustavson, D. R.; Lowe, S. E.; Chang, L. P.; Pirnik, D. M.; Kodukula, K. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2295–2298.
(26) Bell, P. J. L.; Karuso, P. *J. Am. Chem. Soc.* **2003**, *125*, 9304–9305.
(27) Ziehl, M.; Dahse, H.-M.; Hertweck, C. *Angew. Chem., Int Ed.* **2005**, *44*, 1202–1205.

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