Synthesis of New *N*-Analogous Corollosporine Derivatives with Antibacterial Activity by Laccase-Catalyzed Amination

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Received November 13, 2007; accepted March 11, 2008; published online March 19, 2008

Corollosporine isolated from the marine fungus *Corollospora maritima* and *N*-analogous corollosporines are antimicrobial substances. Owing to the basic structure of the *N*-analogous corollosporines, they have become an attractive target for laccase-catalyzed derivatisation. In this regard we report on the straightforward laccase-catalyzed amination of dihydroxylated arenes with *N*-analogous corollosporines. In biological assays the obtained amination products are more active than the parent compounds.

Key words corollosporine; laccase; *Corollospora maritima*; biotransformation; resistance

Laccase-catalyzed aminations represent an efficient method for the construction of biologically important C–N bonds and allows for the use of mild reaction conditions, aqueous solvent systems, normal pressure, and room temperature. Recently, this type of reaction has been applied to synthesize novel antibiotics.[1,2] The synthesis and biological evaluation of potentially new antibiotic agents is undoubtedly an important topic in current chemical and medicinal research. Beside the design of more effective antibiotics with a lower number of unwanted side effects, this demand is especially forced by the ongoing multi-resistance of several bacteria, *e.g. Streptococcus pneumoniae* strains[3—5] and *Staphylococcus* strains, against currently available antibiotics.[6—9]

The discovery of suitable new antibiotics is generally governed by the isolation of active compounds from biological resources. In a recent example, Lindequist and co-workers isolated the novel antibacterial agent corollosporine (Fig. 1) from the marine fungus *Corollospora maritima*.[10] Corollosporine [(±)-3-hexyl-3,7-dihydroxy-1(3*H*)-isobenzofuran-1-one] is a typical member of antimicrobial compounds with phthalide structure and is active against *Staphylococcus aureus* and *Bacillus subtilis*. Beside the total syntheses of the corollosporine,[11] a synthesis protocol of *N*-analogous compounds was developed to study their antibiotic behaviour.[12] Some of the obtained products revealed antibiotic activity, which was comparable to that of the natural product corollosporine.

As a consequence of the promising activity of the corollosporines in antibacterial assays, we became interested in laccase-catalyzed amination of dihydroxylated aromatics with *N*-analogous corollosporines. Until to date the use of laccase for the derivatization of antibiotics is limited to a few examples including the phenolic oxidation of 7-(4-hydroxyphenyl-acetamido)cephalosporinic acid,[13] the dimerization of penicillin X[14] and the oxidative coupling of hydroquinone and mithramicine.[15] Unfortunately, in these examples realized so far, the goal of enhancement of the bioactive effect has not been achieved.[13—15] Recently we reported on novel penicillins and cephalosporins synthesized by biotransformation using laccase.[1,2] These results showed that derivatization of antibiotics by laccase-catalyzed reaction can be achieved without any loss of antibacterial activity.

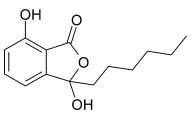

Fig. 1. Structure of Corollosporine

In the present study we have employed laccase from *Trametes* sp. to derivatize *N*-analogous corollosporines and to couple them both with 4-methylcatechol and with derivatives of 2,5-dihydroxybenzoic acid. The 2,5-dihydroxybenzoic acid derivatives are structurally related to the ganomycins, a new chemical class of antibacterial compounds[16] and to other antibacterial active substances,[17,18] and therefore they are interesting as coupling partner for *N*-analogous corollosporines. To our delight the resulting products inhibit the growth of several Gram positive bacterial strains in the agar diffusion assay, among them methicillin-resistant *Staphylococcus aureus* (MRSA).

Results and Discussion

Biotransformation of *N*-Analogous Corollosporines Laccase-catalyzed reaction between *N*-analogous corollosporines (**2a**—**e**) on the one hand and several derivates of 2,5-dihydroxybenzoic acid and 4-methylcatechol (**1a**—**d**) on the other hand leads to a small library of cross coupling products (Table 1). Altogether 34 transformation products have been performed and analyzed by high performance liquid chromatography (HPLC). In the course of incubation, a color change of the initially clear or yellow mixture was noted. Within the first hour, the reaction mixture turned from yellow to dark red.

Among the different model transformations four reactions were selected for scaling up to prove our general approach (from 4 to 200 ml reaction volume), because of the high yields, the easy to apply separation from the reaction mixture, and the stability of the products. The reaction between

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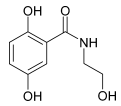
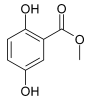
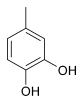
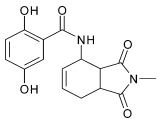
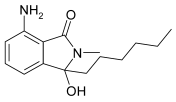
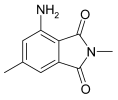
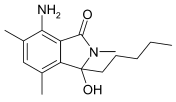
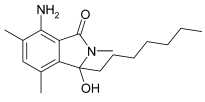
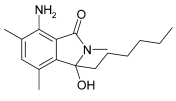
Table 1. Products Obtained in Laccase-Catalyzed Biotransformation

Substances		 **1a**	 **1b**	 **1c**	 **1d**
	**2a**	1[a] [**3a**][b]	3	1	4
	**2b**	1 [**3b**]	1	3	—[c]
	**2c**	2 [**3c**]	1 [**3d**]	3	2
	**2d**	2	1	3	0
	**2e**	2	1	3	0

a) Number of products. *b*) Description of products analyzed in more detail. *c*) Not tested.

2a and **1a** led to the selective formation of cross coupling product **3a**. HPLC analysis of the reaction mixture revealed full conversion of both reacting partners to give **3a**. A sufficient amount of **3a** (approximately 95% yield) for detailed structural characterization was formed within an incubation period of 1 h.

The reactions between **2b** or **2c** and derivatives of 2,5-dihydroxybenzoic acid led to one or two different cross coupling products. After incubation times of 1 h the substrates **1a** or **1b** and **2b** or **2c** were no longer detectable in the reaction mixture and the corresponding coupling products were observed (80—90% yield). Yields of more than 80% showed the high efficiency of the reaction. Some years ago we found comparable straightforward biotransformation providing coupling products between 2,5-dihydroxybenzoic acid derivatives and primary aromatic amines[19,20] or β-lactam antibiotics,[1,2] as well as between 3-(3,4-dihydroxy-phenyl)-propionic acid and 1-hexylamine.[21] In all these reactions transformation rates and product yields are rather high and byproducts could be neglected. In contrast to these findings are reaction kinetics which were described for the coupling reaction between 3,4-dichloroaniline and syringic acid[22] and between 3-(3,4-dihydroxy-phenyl)-propionic acid and 4-aminobenzoic acid.[21] In these experiments the formation of byproducts diminished the yield of the coupling product up to 40%. The dihydroxylated compounds used in this study showed fast reactions in the control experiments without a second coupling partner, too. However, these undesired reactions were suppressed almost completely in the presence of the *N*-analogous corollosporine derivatives.

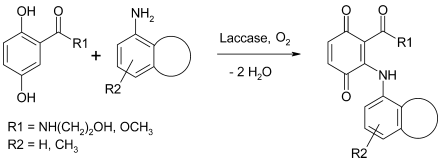
The products **3a** to **3d** were isolated by separation from buffer and laccase. Their structures were established unambiguously by ^{1}H-, ^{13}C-NMR and mass spectroscopic investigations. The resulting 2-(3-oxo-2,3-dihydro-1*H*-isoindol-4-ylamino)-3,6-dioxocyclohexa-1,4-dienecarboxylic acid derivatives are shown in Table 2.

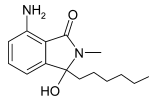
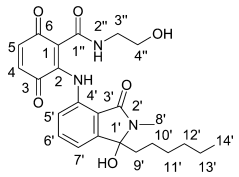
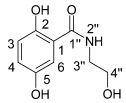
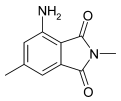
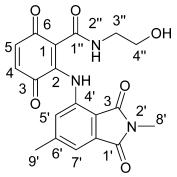
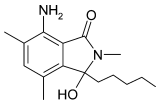
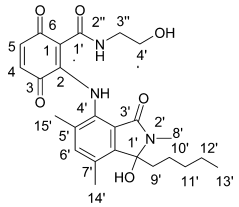
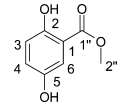
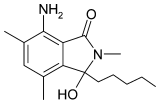
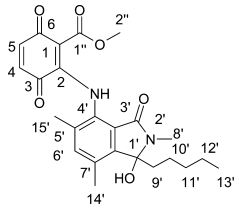
Mass spectroscopic analyses of the compounds **3a** to **3d** showed a molecular mass which corresponds to the coupling of one 2,5-dihydroxybenzoic acid derivative (**1a** or **1b**) with one *N*-analogous corollosporine derivative (**2a** or **2b**). Electro spray ionization (ESI) (negative and positive ion mode) and atmospheric pressure chemical ionization (APCI) (negative ion mode) measurements directly after dissolution showed the corresponding quinonoid products. However, during HPLC-MS analysis performed 20 min after dissolution the occurrence of the hydroquinone form of the products is already detectable in solvents like methanol as we reported previously for other amination products of 2,5-dihydroxybenzoic acid derivatives.[23]

More specifically ^{1}H-NMR spectral data of **3a** contained the characteristic signals for both substrate **1a** and compound **2a**. The number of CH proton signals of the dihydroxylated phenyl rings changed from three—in the substrate–to two signals—in the product. The multiplicity of H4 and H5 of the product indicated a further substituent at the C2. The chemical shift to lower field of H4 and H5 demonstrated the presence of an electron-withdrawing group. ^{13}C-NMR measured in CH_2Cl_2 showed two typical signals for quinones in the range of 180 ppm, an important indication for the quinonoid character of **3a**, confirming the oxidation of the *p*-hydroquinone to a quinone. This observation is in accordance with our previous findings of coupling products with a benzoquinone structure motif which were formed between 2,5-dihydroxybenzoic acid derivatives and primary aromatic amines[19,20] or β-lactam antibiotics.[1,2] A related coupling product was synthesized between 3,4-dichloraniline and protocatechuic acid or syringic acid.[22,24]

^{1}H–^{1}H-COSY measurements did not include correlations

Table 2. Coupling Reaction with Laccase



2,5-Dihydroxybenzoic acid derivative		Aniline		Coupling product		Yield [%]
			**2a**		**3a**	94
	**1a**		**2b**		**3b**	82
			**2c**		**3c**	80
	**1b**		**2c**		**3d**	85

Conditions: Aniline (1 mM), 2,5-dihydroxybenzoic acid derivative (1 mM) dissolved in 200 ml 0.02 M sodium acetate buffer pH 5.0 10% methanol, laccase C of *Trametes* spec (final activity 0.15 unit ml^{-1}).

between the aromatic amine proton of the *N*-analogous corollosporine and any other proton, proving the coupling between **1a** and **2a** to be a C–N bond at C2.

Biological Activity of the Biotransformation Products

According to Table 2 all products (**3a** to **3d**) obtained by biotransformation showed a moderate growth inhibition of several Gram positive strains, among them multidrug resistant *Staphylococcus* strains (Table 3), in the agar diffusion test. Notably, the antibacterial activity of the reaction products (**3a** to **3d**) was increased in comparison to both *N*-analogous corollosporines (**2a** to **2c**) and originally isolated natural compound corollosporine. In comparison to the 2,5-dihydroxybenzoic acid derivatives their cross coupling products were significantly more active. The increase of the activity of coupling products was synergistic, compared with the component compounds, 2,5-dihydroxybenzoic acid derivatives and amino-corollosporines, and showed the advantage of the effect of laccase-catalyzed coupling of one laccase substrate with bioactive amino-corollosporines.

3c showed low activity against the Gram negative strain *Escherichia coli*, whereas all other tested substances did not exhibit any growth inhibition of *E. coli*. Products **3a** to **3d** were not active against the Gram negative strain *Pseudomonas aeruginosa* and against the yeast *Candida maltosa*. Investigations regarding the stability of the synthesised compounds showed limited lifetime in aqueous solution. Incubation of compounds **3a** to **3d** (in solution at 30 °C) showed decomposition after 2 h. Therefore for further studies on the biological activity non-aqueous application systems have to be implemented.

In conclusion a series of novel biologically active compounds has been prepared by laccase-catalyzed amination. This technology for derivatization of potentially active leads has many advantages over other classical synthetic technologies. Within short time a variety of new substances can be synthesized smoothly. The enzyme laccase can be isolated easily and is highly stable. Hence, it is possible to functionalize also sensitive natural substances. Noteworthy, active com-

Table 3. Antimicrobial Activity of Products **3a** to **3d** and **2a** to **2c**

Substances	Amount [μmol]	*E. coli* 11229	*B. subtilis* 6051	*S. aureus* ATCC 6538	*S. aureus* NES[e,f]	*S. epidermidis* 847[f]	*S. haemolyticus* 535[f]
Corollosporine	1	—[a]	14[b] (2.0)[c]	10 (0.6)	12 (0.6)	14 (1.5)	8 (0.6)
	0.4	—	11 (3.5)	8 (1.0)	10 (2.1)	12 (0.6)	r[d]
	0.2	—	9 (2.0)	7 (0.0)	8 (0.6)	10 (1.0)	r
1a	1	r	r	r	r	r	r
	0.4	r	r	r	r	r	r
	0.2	r	r	r	r	r	r
1b	1	r	r	r	r	r	r
	0.4	r	r	r	r	r	r
	0.2	r	r	r	r	r	r
2a	1	r	r	r	r	r	r
	0.4	r	r	r	r	r	r
	0.2	r	r	r	r	r	r
2b	1	r	10 (1.2)	r	r	r	r
	0.4	r	r	r	r	r	r
	0.2	r	r	r	r	r	r
2c	1	r	r	12 (2.3)	r	r	r
	0.4	r	r	10 (1.0)	r	r	r
	0.2	r	r	r	r	r	r
3a	1	r	7 (1.5)	10 (0.6)	16 (1.7)	16 (1.2)	r
	0.4	r	7 (0.6)	8 (0.6)	12 (0.6)	12 (0.6)	r
	0.2	r	r	r	10 (0.0)	r	r
3b	1	r	8 (0.6)	11 (0.6)	18 (0.6)	16 (0.0)	8 (0.6)
	0.4	r	r	r	14 (1.5)	12 (0.0)	r
	0.2	r	r	r	10 (0.6)	r	r
3c	1	8 (0.6)	14 (2.1)	15 (2.1)	18 (1.5)	12 (0.6)	12 (0.6)
	0.4	r	13 (2.3)	11 (0.6)	12 (0.6)	10 (2.1)	10 (0.6)
	0.2	r	10 (1.2)	8 (1.5)	8 (0.6)	8 (0.6)	8 (0.6)
3d	1	r	12 (0.0)	14 (2.0)	16 (1.7)	14 (0.6)	12 (0.6)
	0.4	r	11 (0.6)	13 (1.5)	10 (1.2)	10 (1.2)	10 (1.5)
	0.2	r	10 (0.6)	10 (0.6)	8 (0.6)	8 (0.6)	8 (0.6)

a) Not tested. *b*) Zones of inhibition (mm) calculated from 3 replicates. *c*) Standard deviation calculated from 3 replicates. *d*) r resistant (no zone of inhibition). *e*) North German Epidemic Strain. *f*) Multi-resistant strains.

pounds, which do not belong to substrates of laccase, can be linked with the appropriate substrates of the enzyme.

Experimental

Enzymes Extra cellular laccase C of *Trametes* sp. (EC 1.10.3.2) was obtained from ASA Spezialenzyme (Wolfenbüttel, Germany) and used as received (activity 1000 U/g; substrate: syringaldazine).

Syntheses of *N*-Analogous Corollosporines *N*-Analogous corollosporines [7-amino-3-hydroxy-2-methyl-3-hexyl-2,3-dihydro-isoindol-1-on (**2a**), 4-amino-2,6-dimethyl-isoindol-1,3-dion (**2b**), 7-amino-3-hydroxy-2,4,6-trimethyl-3-pentyl-2,3-dihydro-isoindol-1-on (**2c**), 7-amino-3-hydroxy-2,4,6-trimethyl-3-heptyl-2,3-dihydro-isoindol-1-on (**2d**), 7-amino-3-hydroxy-2,4,6-trimethyl-3-hexyl-2,3-dihydro-iso-indol-1-on (**2e**)] were synthesized as described before.[12]

Synthesis of the 2,5-Dihydroxybenzoic Acid Derivate 1d The 2,5-dihydroxy-*N*-(2-methyl-1,3-dioxo-2,3,3a,4,7,7a-hexahydro-1*H*-isoindol-4-yl)benzamide was synthesized as described before.[25]

Amination of Different Substituted Hydroquinones with *N*-Analogous Corollosporines by Laccase C After dissolving of an *N*-analogous corollosporine in 1 ml methanol 4 ml 0.02 M sodium acetate buffer pH 5.0 was added. For complete dissolving of *N*-analogous corollosporines ultrasonics was used. One of the 2,5-dihydroxybenzoic acid derivatives or 4-methylcatechol was diluted in 5 ml of 0.02 M sodium acetate buffer pH 5.0 by ultrasonics as well. The two solutions were transferred to one reaction tube. The concentration of the reacting partners was 1 mM in the reaction mixture. The transformation was started by addition the enzyme laccase C of *Trametes* spec (final activity 0.15 unit ml^{-1}). Reaction was performed at room temperature with agitation at 400 rpm.

Four adapted reactions were selected to scale up the reaction approach. One of the *N*-analogous corollosporines **2a** to **2c** was dissolved in 20 ml methanol thereafter 100 ml 0.02 M sodium acetate buffer pH 5.0 was added. **1a** or **1b** was diluted in 80 ml of 0.02 M sodium acetate buffer pH 5.0 by ultrasonics. The concentration of the reacting partners was 1 mM in the reaction mixture. The accomplishment of the reactions was proceeded as described above.

High Performance Liquid Chromatography In order to follow the process of reaction, the mixture was analyzed by a HPLC system (Kontron, Neufahrn, Germany), consisting of a Kontron series pump model 522, a Kroma Automatic Sample Injector 565 and a Diode Array Detector system 540. For the separation of metabolites an endcapped, 5-μm, LiChroCart 125-4 RP 18 column (Merck, Darmstadt, Germany) was used at a flow rate of 1 $ml \cdot min^{-1}$. The mobile phase consisted of methanol (eluent A) and phosphoric acid (0.1%, eluent B), starting from an initial ratio of 10% A and 90% B and reaching then 100% A within 14 min. After a reaction time of 50 to 100 min one or two coupling products could be detected.

Isolation of Transformation Products with Solid Phase Extraction Solid phase extraction was utilized for isolation and the transfer of the coupling products into an organic matrix, using a Strata C18-E column (55 μm, 10 g capacity, 60 ml, Phenomenex, Germany). After activation and equilibration the RP-18 column was charged with 100 ml of the incubation mixture.

The column was washed twice with 60 ml methanol (10%) and aqua dest. (90%). More than 80% of the coupling products were eluted by acetonitrile 100%. The eluted metabolites were evaporated to dryness using a vacuum rotator at 30 °C.

LC/MS, NMR The products were characterized by liquid chromatography/mass spectrometry (LC/MS). Atmospheric pressure ionization (API) mass spectrometry experiments were performed using an Agilent Series 1100 HPLC system and an Agilent 1946C quadrupole mass spectrometer (Waldbronn, Germany). The mass spectrometer was used with both, APCI and ESI sources.

HPLC-MS separation was performed on a LiChroCART® 125-4, LiChrosphere® 100 RP-18e column (Merck, Darmstadt, Germany) with the following binary standard gradient system at a flow rate of 1 ml/min: 14-min gradient elution from 10 to 100% eluent B (MeOH) where eluent A was 0.1% formic acid in water; 100% eluent B (MeOH) for further 2 min, 10% eluent B and 90% A for 2 min to equilibrate the column for next run. Chromatography was performed at 25 °C and a UV signal recorded at 220 nm with a variable wavelength detector (VWD). APCI conditions (positive and negative ion mode) were as follows: nebulizer and drying gas, nitrogen; nebulizer pressure, 30 psig; drying gas flow, 10 l/min; vaporizer temperature, 350 °C; drying gas temperature, 250 °C; capillary voltage, 4 kV; corona current, 4 μA.

ESI conditions (positive and negative ion mode): nebulizer and drying gas, nitrogen; nebulizer pressure, 30 psig; drying gas flow, 10 l/min; drying gas temperature, 350 °C; capillary voltage, 4 kV.

All FT-ICR MS high-resolution mass spectrometry (HR-MS) experiments were performed on a Bruker Daltonics APEX III FT-ICR mass spectrometer (Bremen, Germany) equipped with a 7.0 T shielded superconducting magnet. The flow rate for the eluent (H_2O/ACN/HCOOH 49/49/2, all HPLC-grade) was 2 μl/min, using a syringe pump (Cole-Palmer 74900 series). The ions were generated from an external electrospray ionization source (Apollo ESI-Source) with nebulizing gas pressure at 20 psi, heated drying gas at 10 psi (back-pressure) and 150 °C, and a capillary entrance voltage of −4500 V in negative ion mode and +4500 V in positive ion mode.[26—28]

Mass spectra were acquired with both, positive and negative, ion modes with broadband detection (32 scans each experiment) from 100 to 2.000 Da using 1024 K data points. All experimental sequences, including scan accumulation and data processing, were performed with XMASS 6.1.2 on Windows 2000.

The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker (Karlsruhe, Germany) Avance 600 instrument (^{1}H, 600 MHz) or on a Bruker ARX 400 with QNP probe head (^{1}H, 400.13 MHz; ^{13}C, 100.61 MHz) at 25 °C.

Antimicrobial Activity To determine the antimicrobial activity an agar diffusion assay according to Burkhardt[29] was used. Sterile Mueller-Hinton II-Agar in Stacker Petri discs (Becton Dickinson Microbiology systems, Cockeysvill, U.S.A.) was inoculated with cells (200 μl of a yeast or bacterial cell suspension in 20 ml medium) of the yeast *Candida maltosa* SBUG 700, the bacterial strains *Escherichia coli* 11229, *Pseudomonas aeruginosa* 27853, *Bacillus subtilis* 6051 and *Staphylococcus aureus* ATCC 6538 and the multiresistant bacteria strains *Staphylococcus aureus* North German Epidemic Strain, *Staphylococcus epidermidis* 847 and *Staphylococcus haemolyticus* 535. Samples were applied in different concentration on sterile paper discs (Sensi-Disc, 6 mm diameter, Becton Dickinson Microbiology systems, Cockeysvill, U.S.A.). Plates were kept for 3 h in a refrigerator to enable prediffusion of the substances into the agar and then incubated for 24 h at 37 °C. Inhibition zone diameters around each of the disc were measured and recorded at the end of the incubation time. An average zone of inhibition was calculated from 3 replicates.

Chemicals Chemicals were purchased from commercial suppliers: 2,5-dihydroxy-*N*-(2-hydroxyethyl)-benzamide (97%, **1a**) was received from Midori Kagaku Co., Ltd. (Japan). Methyl 2,5-dihydroxybenzoic acid (99%, **1b**) and 4-methylcatechol (95%, **1c**) are commercially available from Aldrich (Steinheim, Germany).

2-(1′-Hexyl-1′-hydroxy-2′-methyl-3-oxo-2,3-dihydro-1*H*-isoindol-4-ylamino)-3,6-dioxocyclohexa-1,4-dienecarboxylic acid (2″-hydroxyethyl)amide (3a) Synthesis and isolation as described above. Red solid. Yield 86 mg (94%). ^{1}H-NMR (400 MHz; methanol-d_4) δ_H 7.35 (m, ^3J=8.3 Hz, ^3J=7.5 Hz, 1H, H6′), 6.99 (d, ^3J=8.7 Hz, 1H, H4), 6.95 (d, ^3J=7.5 Hz, 1H, H5′ or H7′), 6.74 (d, ^3J=8.7 Hz, 1H, H5), 6.51 (d, ^3J=8.3 Hz, 1H, H5′ or H7′), 3.71 (m, ^3J=5.6 Hz, 2H, H4″), 3.49 (m, ^3J=5.7 Hz, 2H, H3″), 3.39 (s, 3H, H8′), 2.11 (m, 2H, H9′), 1.25 (m, 8H, H10′, H11′, H12′, H13′), 0.89 (m, 3H, H14′). ^{1}H-NMR (600 MHz; CH_2Cl_2-d_2) δ_H 13.28 (s, 1H, NH aromatic), 9.77 (t, 1H, ^3J=5.4 Hz, NH, H2″), 7.35 (dd, ^3J=7.7 Hz, 1H, H6′), 7.33 (d, ^3J=7.5 Hz, 1H, H5′ or H7′), 7.14 (d, ^3J=7.9 Hz, 1H, H5′ or H7′), 6.52 (d, ^3J=10.2 Hz, 1H, H4), 6.47 (d, ^3J=10.2 Hz, 1H, H5), 3.91 (s, 1H, OH heterocyclic), 3.65 (m, ^3J=5.1 Hz, 2H, H4″), 3.46 (m, ^3J=5.5 Hz, J=3.9 Hz, 1H, H3″), 3.41 (m, ^3J=5.5 Hz, J=3.9 Hz, 1H, H3″), 3.18 (t, ^3J=5.1 Hz, 1H, H5″ OH aliphatic), 3.08 (s, 3H, H8′), 2.02 (m, 2H, H9′), 1.17 (m, 6H, H11′, H12′, H13′), 0.82 (m, 3H, H14′), 0.76 (m, 1H, H10′), 0.65 (m, 1H, H10′). ^{13}C-NMR (100 MHz; CH_2Cl_2-d_2; determination of proton assignment by hetcor) δ 184.9, 181.2 (C6/C3); 170.0 (C1″); 166.1 (C3′); 154.8 (C1); 149.1, 136.8, and 121.8 (C7a′/C3a′/C4′); 139.0 (C4); 134.3 (C5); 133.5 (C6′); 124.9, 119.4 (C7′/C5′); 101.1 (C1); 90.3 (C1′); 62.7 (C4″); 42.6 (C3″); 35.5 (C9′); 31.8, 29.2, and 22.8 (C13′/C12′/C11′); 23.41 (C10′); 23.42 (C8′); 14.1 (C14′). LC/MS *m/z*: APCI, neg. ion mode, 454.2 $[M-H]^-$; API-ES, pos. ion mode, 478.1 $[M+Na]^+$, 933.3 $[2M+Na]^+$; API-ES, neg. ion mode 454.2 $[M-H]^-$. After 20 min: APCI, neg. ion mode, 454.2 $[M-H]^-$ and 456.2 $[M-H]^-$ of the hydroquinone form of **3a**; ESI, pos. ion mode, 478.1 $[M+Na]^+$ and 480.1 $[M+Na]^+$ of the hydroquinone form of **3a**; ESI, neg. ion mode 454.2 $[M-H]^-$ and 456.2 $[M-H]^-$ of the hydroquinone form of **3a**. HR-MS Calcd for $C_{24}H_{29}N_3O_6Na$ $[M+Na]^+$: 478.1949; Found: 478.1944. HR-MS Calcd for $C_{24}H_{31}N_3O_6Na$ $[M+Na]^+$, the hydroquinone form of **3a**: 480.2106; Found: 480.2108 (all confirmed by FT-ICR MS).

2-(2,6-Dimethyl-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-ylamino)-3,6-dioxo-cyclohexa-1,4-dienecarboxylic acid (2-hydroxy-ethyl)amide (3b) Synthesis and isolation as described above. Red solid. Yield 63 mg (82%). LC/MS *m/z*: ESI, neg. ion mode 382.1 $[M-H]^-$ and 787.1 $[2(M-H)+Na]^-$; ESI, pos. ion mode, 406.0 $[M+Na]^+$. HR-MS Calcd for $C_{19}H_{18}N_3O_6$ $[M+H]^+$: 384.1190; Found: 384.1195 (confirmed by FT-ICR MS).

2-(1′-Heptyl-1′-hydroxy-2′,5′,7′-trimethyl-3-oxo-2,3-dihydro-1*H*-isoindol-4-ylamino)-3,6-dioxocyclohexa-1,4-dienecarboxylic acid (2″-hydroxyethyl)amide (3c) Synthesis and isolation as described above. Red solid. Yield 75 mg (80%). LC/MS *m/z*: ESI, pos. ion mode, 492.2 $[M+Na]^+$, 452.2 $[M-OH]^+$, and 961.4 $[2M+Na]^+$; ESI, neg. ion mode 468.2 $[M-H]^-$ and 959.5 $[2(M-H)+Na]^-$. HR-MS Calcd for $C_{25}H_{31}N_3O_6Na$ $[M+Na]^+$: 492.2110; Found: 492.2091 (confirmed by FT-ICR MS).

2-(1′-Heptyl-1′-hydroxy-2′,5′,7′-trimethyl-3-oxo-2,3-dihydro-1*H*-isoindol-4-ylamino)-3,6-dioxocyclohexa-1,4-dienecarboxylic acid methyl ester (3d) Synthesis and isolation as described above. Red solid. Yield 75 mg (85%). LC/MS *m/z*: ESI, pos. ion mode, 441.2 $[M+H]^+$, 463.2 $[M+Na]^+$, and 903.4 $[2M+Na]^+$; ESI, neg. ion mode 439.1 $[M-H]^-$ and 901.2 $[2(M-H)+Na]^-$. HR-MS Calcd for $C_{24}H_{29}N_2O_6$ $[M+H]^+$: 441.2020; Found: 441.2019 (confirmed by FT-ICR MS).

Acknowledgments Financial support from the government of Mecklenburg-Vorpommern, Germany, and from the European Union (Landesforschungsschwerpunkt "Neue Wirkstoffe und Biomaterialien–Innovative Screeningverfahren and Produktentwicklung") is gratefully acknowledged.

References

1) Mikolasch A., Niedermeyer T. H. J., Lalk M., Witt S., Seefeldt S., Hammer E., Schauer F., Gesell M., Hessel S., Jülich W. D., Lindequist U., *Chem. Pharm. Bull.*, **54**, 632—638 (2006).
2) Mikolasch A., Niedermeyer T. H. J., Lalk M., Witt S., Seefeldt S., Hammer E., Schauer F., Gesell M., Hessel S., Jülich W. D., Lindequist U., *Chem. Pharm. Bull.*, **55**, 412—416 (2007).
3) Critchley I. A., Karlowsky J. A., Draghi D. C., Jones M. E., Thornsberry C., Murfitt K., Sahm D. F., *Antimicrob. Agents Chemother.*, **46**, 550—555 (2002).
4) Fluit A. C., Schmitz F. J., Jones M. E., Acar J., Gupta R., Verhoef J., *Int. J. Infect. Dis.*, **3**, 153—156 (1997).
5) Klugman K. P., *Clin. Microbiol. Rev.*, **3**, 171—196 (1990).
6) Shimizu M., Shiota S., Mizushima T., Ito H., Hatano T., Yoshida T., Tsuchiya T., *Antimicrob. Agents Chemother.*, **45**, 3198—3201 (2001).
7) Fisher J. F., Meroueh S. O., Mobashery S., *Chem. Rev.*, **105**, 395—424 (2005).
8) Waley S. G., "The Chemistry of β-Lactams," ed. by Page M. I., Blackie A. & P., London, 1992, pp. 198—228.
9) Helfand M. S., Bonomo R. A., *Curr. Opin. Pharmacol.*, **5**, 452—458 (2005).
10) Liberra K., Jansen R., Lindequist U., *Pharmazie*, **53**, 578—581 (1998).
11) Ohzeki T., Mori K., *Biosci. Biotechnol. Biochem.*, **65**, 172—175 (2001).

12) Neumann H., Strübing D., Lalk M., Klaus S., Hübner S., Spannenberg A., Lindequist U., Beller M., *Org. Biomol. Chem.*, **4**, 1365—1375 (2006).
13) Agematu H., Kominato K., Shibamoto N., Yoshioka T., Nishida H., Okamoto R., Shin T., Murao S., *Biosci. Biotechnol. Biochem.*, **57**, 1387—1388 (1993).
14) Agematu H., Tsuchida T., Kominato K., Shibamoto N., Yoshioka T., Nishida H., Okamoto R., Shin T., Murao S., *J. Antibiot.*, **46**, 141—148 (1993).
15) Anyanwutaku I., Petroski R., Rosazza J., *Bioorg. Med. Chem.*, **2**, 543—551 (1994).
16) Mothana R. A. A., Jansen R., Jülich W. D., Lindequist U., *J. Nat. Prod.*, **63**, 416—418 (2000).
17) Fokialakis N., Magiatis P., Chinou I., Mitaku S., Tillequin F., *Chem. Pharm. Bull.*, **50**, 413—414 (2002).
18) Yaoita Y., Danbara K., Kikuchi M., *Chem. Pharm. Bull.*, **53**, 1202—1203 (2005).
19) Niedermeyer T. H. J., Mikolasch A., Lalk M., *J. Org. Chem.*, **70**, 2002—2008 (2005).
20) Manda K., Hammer E., Mikolasch A., Niedermeyer T. H. J., Dec J., Jones A. D., Benesi A. J., Schauer F., Bollag J.-M., *J. Mol. Catal.*, *B, Enzym.*, **35**, 86—92 (2005).
21) Mikolasch A., Hammer E., Jonas U., Popowski K., Stielow A., Schauer F., *Tetrahedron*, **58**, 7589—7593 (2002).
22) Tatsumi K., Freyer A., Minard R. D., Bollag J. M., *Environ. Sci. Technol.*, **28**, 210—215 (1994).
23) Manda K., Hammer E., Mikolasch A., Gördes D., Thurow K., Schauer F., *Amino Acids*, **31**, 409—419 (2006).
24) Tatsumi K., Freyer A., Minard R. D., Bollag J. M., *Soil Biol. Biochem.*, **26**, 735—742 (1994).
25) Klaus S., Hübner S., Neumann H., Strübing D., Jacobi von Wangelin A., Gördes D., Beller M., *Adv. Synth. Catal.*, **346**, 970—978 (2004).
26) Marshall A. G., Schweikhard L., *Int. J. Mass Spectrom. Ion Processes*, **118/119**, 37—70 (1992).
27) Amster I. J., *J. Mass Spectrom.*, **31**, 1325—1337 (1996).
28) Marshall A. G., Hendrickson C. L., Jackson G. S., *Mass Spectrom. Rev.*, **17**, 1—35 (1998).
29) Burkhardt F., "Mikrobiologische Diagnostik," Georg Thieme Verlag, Stuttgart, 1992.