Nocapyrones A−D, γ-Pyrones from a *Nocardiopsis* Strain Isolated from the Marine Sponge *Halichondria panicea*

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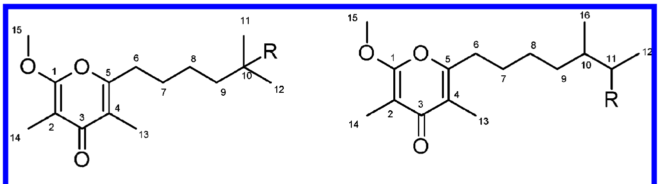
Four new γ-pyrones, nocapyrones A−D (**1**−**4**), were isolated from an organic extract of the *Nocardiopsis* strain HB383, which was isolated from the marine sponge *Halichondria panicea.* These are the first γ-pyrones reported from a *Nocardiopsis* strain. The structures were elucidated on the basis of one- and two-dimensional NMR experiments and supported by HPLC-UV/MS and HRESIMS analyses. The biosynthesis of nocapyrone A was investigated by feeding experiments with ^{13}C-labeled compounds. In addition, one diketopiperazine, which was only known as a synthetic compound before, was isolated. The bioactivies of **1**, **2**, and the diketopiperazine were evaluated in a panel of assays.

Nocardiopsis belongs to the Actinobacteria and was designated as a distinct genus in 1976.[1] It is set apart from similar genera such as *Actinomadura*, *Nocardia*, and *Streptomyces* by characteristics such as type of sporulation, morphology of aerial and substrate mycelium, cell wall lipid composition, and susceptibility to lysozyme.[1] Due to the wide distribution of members of the genus, it is not surprising that it has already been isolated from marine sponges as well. Considering the facts that Actinobacteria are exceptionally potent producers of secondary metabolites and that the rediscovery rates of known compounds are high in intensively studied genera such as *Streptomyces* species, strategies such as focusing on less common taxa, e.g., *Nocardiopsis* species, and isolating bacteria from extraordinary and promising habitats such as sponges have become more and more popular.[2,3] *Halichondria panicea* is a sponge species suitable for such an approach, because it has been reported to harbor a vast number of different microorganisms, among them also several Actinobacteria.[4] The majority of Actinobacteria isolated from *H. panicea* belong to the well-studied genus *Streptomyces*. Among 46 actinobacterial isolates from *H. panicea* only one was classified as belonging to the genus *Nocardiopsis*.[5]

Nocardiopsis strain HB383 was chosen for further investigations due to unidentified peaks in the LC-UV/MS chromatogram and antibacterial activity of the organic extract. This strain was selected in a study focusing on the discovery of new secondary metabolites from marine Actinobacteria associated with the sponge *H. panicea*.[5] By 16S rRNA gene sequence analysis this strain was identified as a member of the genus *Nocardiopsis* with 99.8% similarity to *Nocardiopsis alba* DSM 43377^{T}. The HPLC-MS analysis of the EtOAc extract displayed four main peaks in the UV chromatogram, which could be assigned to the masses 334, 320, 286, and 268. On the basis of UV, mass, and NMR data the first three peaks presumably belonged to a number of diketopiperazines that have not yet been described for *Nocardiopsis* species. The fourth peak belonged to a new γ-pyrone, which, together with three derivatives, will be introduced in this paper.

With the aim of obtaining the new metabolites, the extract was separated using preparative reversed-phase (C_{18}) HPLC, which yielded nocapyrones A (**1**) and B (**2**). Another HPLC fraction yielded the derivatives nocapyrones C (**3**) and D (**4**) after further purification.



nocapyrone A: R= -OH (**1**)
nocapyrone B: R= -H (**2**)

nocapyrone C: R= -OH (**3**)
nocapyrone D: R = =O (**4**)

Nocapyrones A (**1**) and B (**2**) were amorphous, colorless solids with molecular weights of *m/z* 269.1847 $[M + H]^+$ and 253.1805 $[M + H]^+$, respectively. These results of the high-resolution mass measurements yielded the molecular formulas of $C_{15}H_{24}O_4$ for **1** and $C_{15}H_{24}O_3$ for **2**, which were entirely consistent with the spectroscopic data. The structure elucidation of the two compounds was mainly based on the NMR spectra of **1**. The respective ^{13}C NMR spectrum displayed 14 distinct signals. Not only was the signal of C-11/12 (δ_C 29.4) much more intense than the other carbon signals, but the corresponding ^{1}H signal additionally integrated to six protons, which evidenced the presence of two magnetically equivalent methyl groups. Three additional methyl groups, C-13 (δ_C 10.3), C-14 (δ_C 7.2), and C-15 (δ_C 56.5), were present, with the shift of C-15 proving it to be a methoxy group. Furthermore, the molecule comprised a chain of four methylene groups, CH_2-6 to CH_2-9 (δ_C 31.7, δ_H 2.72; δ_C 28.8, δ_H 1.71; δ_C 25.0, δ_H 1.46; and δ_C 44.5, δ_H 1.50), and four olefinic quaternary carbons, C-1 (δ_C 164.8), C-2 (δ_C 100.2), C-4 (δ_C 119.3), and C-5 (δ_C 161.4). Two of these quaternary carbons, C-1 and C-5, were located adjacent to oxygen atoms as proven by their ^{13}C resonances of 164.8 and 161.4 ppm. Finally, the signal with a chemical shift of 183.4 ppm gave evidence of a carbonyl function being present. A ^{1}H−^{13}C HSQC spectrum correlated all proton resonances to the corresponding ^{13}C NMR resonances of directly bonded carbon atoms. A ^{1}H−^{1}H COSY spectrum revealed that the methylene groups constitute the only coherent spin system, which later was identified as belonging to the side chain of a γ-pyrone core structure. The final structure of the molecule was elucidated with the help of a ^{1}H−^{13}C HMBC spectrum (Figure 1).

The molecular formula $C_{15}H_{24}O_4$ implied four degrees of unsaturation, meaning that apart from the carbonyl group and two double bonds, one ring had to be part of the structure. This was in good agreement with a γ-pyrone moiety being the core structure of the molecule. This deduction was additionally supported by the UV maxima (253 and 215 nm), which fit nicely with literature data of γ-pyrones.[6] Consequently, the γ-pyrone skeleton consisted of the olefinic carbons and the carbonyl group, C-1 to C-5, with C-1 and C-5 being connected via an oxygen atom. The ^{1}H chemical shifts of CH_3-13 and CH_3-14 as well as their ^{1}H−^{13}C HMBC correlations to the quaternary carbon atoms clearly showed that they were located at positions 2 and 4 of the γ-pyrone ring (HMBC

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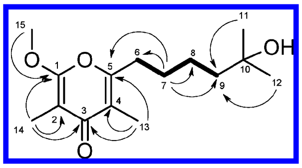


Figure 1. Selected COSY (bold lines) and HMBC correlations (arrows) of nocapyrone A.

Table 1. NMR Spectroscopic Data (500 MHz, Methanol-d_4) for Nocapyrone A (**1**)

position	δ_C, mult.	δ_H (J in Hz)	HMBC
1	164.8, C		
2	100.2, C		
3	183.4, C		
4	119.3, C		
5	161.4, C		
6	31.7, CH_2	2.72, t (7.5)	4, 5, 7, 8
7	28.8, CH_2	1.71, tt (7.5, 7.5)	5, 6, 8, 9
8	25.0, CH_2	1.46, m	6, 7, 9
9	44.5, CH_2	1.50, m	8, 10, 11/12
10	71.4, C		
11/12	29.4, CH_3	1.17, s	9, 10, 11/12
13	10.3, CH_3	1.94, s	3, 4, 5
14	7.2, CH_3	1.81, s	1, 2, 3
15	56.5, CH_3	4.03, s	1

correlations from H_3-13 (δ_H 1.94) to C-3, C-4, and C-5 and from H_3-14 (δ_H 1.81) to C-1, C-2, and C-3). The methoxy group OCH_3-15 was also directly attached to the pyrone ring, as shown by the correlation of the resonance of H_3-15 (δ_H 4.03) to the resonance of C-1. Therefore, the methoxy group had to be attached to C-1. The H-6 signal correlated to the signals of C-7 and C-8, as well as to those of C-4 and C-5. As mentioned above, the CH_2 groups CH_2-6 to CH_2-9 formed a spin system, as shown by strong COSY correlations and the resulting multiplicities of the signals. The methylene chain terminates with the quaternary carbon C-10 (δ_C 71.4), which is substituted with the equivalent methyl groups CH_3-11 and CH_3-12 (δ_C 29.4, δ_H 1.17) as well as a hydroxy group. All spectroscopic data are in good accordance with the determined structure.

Overall, the ^{1}H NMR chemical shifts of nocapyrone B (**2**) were close to being identical to those of nocapyrone A (**1**). The only differences were the appearance of an additional signal for H-10 (δ_H 1.60) and the upfield shift of the signal of the methyl protons H_3-11 and H_3-12 (δ_H 0.93). The upfield shift already indicated the loss of the hydroxy group. This assumption was further supported by the mass difference of 16 and the fact that the signal of the methyl groups in question now appeared as a doublet due to coupling with H-10. Thus, the structure of nocapyrone B (**2**) was established.

The UV spectra of nocapyrones C (**3**) and D (**4**) were almost identical to those of the other two derivatives. Consequently, the chromophore of the structures was very likely to be the same. This was proven by analyses of one- and two-dimensional NMR spectra (Table 2 and SI). The ^{1}H NMR and ^{13}C NMR shifts of the methyl groups CH_3-13, CH_3-14, and CH_3-15 as well as their ^{1}H$-$^{13}C HMBC correlations fit perfectly with the assigned γ-pyrone substructure. Thus, similar to **1** and **2**, the structures of **3** and **4** differed only in the side chain of the molecule. The molecular formulas $C_{16}H_{26}O_4$ for **3** and $C_{16}H_{24}O_4$ for **4** proved the chain to be longer than those of the nocapyrones A (**1**) and B (**2**). Due to uniformity in the UV and MS data of compound **3**, but doubled signals in NMR measurements, we presume the presence of two isomers of compound **3**. Like **1**, nocapyrone C had two methyl groups, CH_3-12 (δ_C 20.0, δ_H 1.11) and CH_3-16 (δ_C 14.9, δ_H 0.88), included in the aliphatic side chain, each of them forming a doublet in the ^{1}H NMR spectrum. The terminal methyl group CH_3-12 showed a ^{1}H$-$^{1}H COSY correlation to H-11 (δ_H 3.62) and additionally a ^{1}H$-$^{13}C HMBC correlation to C-10 (δ_C 40.8), which established the sequence from C-12 to C-10. The shift of CH-11 (δ_H 3.62, δ_C 71.8) proved it to be an oxygen-bearing methine, whereas CH-10 (δ_H 1.45) was the methine group located adjacent to CH_3-16. The side chain was completed by the methyl groups CH_2-6 (δ_H 2.72, δ_C 31.5) to CH_2-9 (δ_H 1.3$-$1.5, δ_C 33.3). Compared to **3**, in the ^{1}H NMR spectrum of nocapyrone D (**4**) only one of the side chain methyl groups, CH_3-16 (δ_H 1.08, δ_C 16.5), appeared as a doublet. The other one appeared as a singlet at δ_H 2.14 and δ_C 28.3 ppm. These data suggested the terminal methyl group to be connected to a carbonyl carbon. Compared to nocapyrone C, there is no oxygen-bearing methine group in **4**. Instead C-10 is a quaternary carbon with a shift of 215.3 ppm, which showed it to be a keto group. Thus, the only difference between the nocapyrones C and D is the hydroxy group in the former that is replaced by a keto group in the latter.

Although these are the first γ-pyrones described from a *Nocardiopsis* strain, natural products based on such a substructure are widespread and constitute a large class of biologically active compounds.[7,8] Compounds containing the same dimethylated and methoxylated pyrone substructure as the nocapyrones are relatively common, with examples being aureothin[9] and actinopyrone,[6] which was first reported to have vasodilating and antibacterial activities. Further studies complemented this activity spectrum with promising activities against *Helicobacter pylori*.[10]

According to the widespread nature of γ-pyrone, the biosynthesis of structures related to the nocapyrones has been studied before. Molecules belonging to this compound class have been reported to be synthesized via different pathways including type I, type II, and type III PKSs.[11] A bacterial γ-pyrone with a well-explored biosynthesis is the *Streptomyces* metabolite aureothin.[9] Aureothin is a type I PKS product generated by the assembly of a *p*-nitrobenzoate starter unit, one acetate, and four propionate building blocks.

By analogy, we suggest nocapyrone A to be a polyketide composed of an isobutyrate starter unit, two acetates, and two propionate building blocks. Results of feeding experiments with ^{13}C-labeled acetate, propionate, and methionine (see Figure 2 and SI) support this assumption; that is, feeding of 1-^{13}C-propionate led to an enhancement of the ^{13}C NMR signals of C-1 and C-3. The analogous experiment with 1-^{13}C-acetate resulted in an intensification of the carbon resonances of C-5 and C-7. Finally, the signal of C-15 became enhanced after feeding with [Me-^{13}C] methionine, which demonstrated the methyl group to be *S*-adenosylmethionine (SAM)-derived. Therefore, in a similar fashion to that described for aureothin we propose the formation of an α-pyrone that is transformed to the γ-pyrone in the course of the methylation by an *O*-methyltransferase.

Despite the inhibition of the Gram-positive bacteria *Staphylococcus lentus* and *Bacillus subtilis* by the extract, the isolated nocapyrones A (**1**) and B (**2**) showed no bioactivities against a range of Gram-positive and Gram-negative bacteria and the yeast *Candida glabrata*. Furthermore, *in vitro* cytotoxicity analyses revealed no activity against the mouse fibroblast cell line NIH-3T3 as well as the human hepatocellular carcinoma cell line HepG2 and the human colon adenocarcinom cell line HT-29. Additionally, **1** and **2** did not inhibit phosphodiesterase 4, protein tyrosine phosphatase 1B, acetylcholinesterase, reverse transcriptase, or glycogen synthase kinase 3β.

As already mentioned above, strain HB383 produces not only the nocapyrones but also three molecules belonging to the diketopiperazine compound class. As far as these compounds are concerned, only one—(2*E*/5*Z*)-2-[(4-methoxyphenyl)methylene]-5-(2-methylpropylidene)-3,6-piperazinedione (**5**) (Figure 3)—could be sufficiently purified for structure elucidation. The molecular formula $C_{16}H_{18}N_2O_3$ of **5** was established by HRESIMS (*m*/*z* 309.1219 [M + Na]$^+$, calcd 309.1210), and the structure was elucidated based

Table 2. ^{13}C and ^{1}H NMR (500 MHz, methanol-d_4) Spectroscopic Data for Nocapyrones B−D (**2**−**4**)

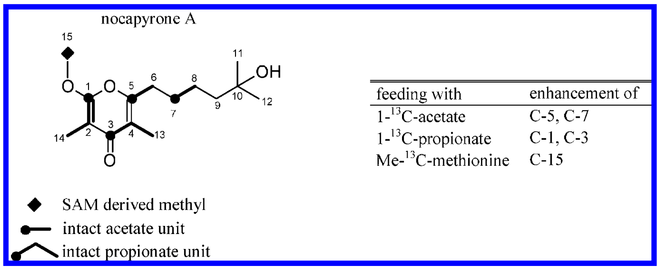
	nocapyrone B (**2**)		nocapyrone C (**3**)		nocapyrone D (**4**)	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	164.9		164.6		164.8	
2	100.0		100.1		99.8	
3	183.3		183.2		182.7	
4	119.2		119.2		119.0	
5	161.8		161.3		161.0	
6	31.5	2.70, t (7.5)	31.5	2.72, t (7.5)	31.4	2.70, t (7.5)
7	27.9	1.69, quintet (7.5)	27.7	1.71, m (7.5)	27.7	1.71, quintet (7.5)
8	30.2	1.42, m	33.5	1.3−1.5, m	31.2	1.28−1.45, m
9	39.7	1.31, m	33.3	1.3−1.5, m	33.4	1.28−1.45, m
10	29.2	1.55, septet (6.8)	40.8	1.45, m	47.4	2.59, sextet (6.8)
11	22.9	0.90, d (6.8)	71.8	3.62, dq (6.2, 4.6)	215.3	
12	22.9	0.90, d (6.8)	20.0	1.11, d (6.2)	28.3	2.14, s
13	10.2	1.93, s	10.1	1.93, s	10.1	1.93, s
14	7.0	1.81, s	7.0	1.81, s	7.0	1.81, s
15	56.3	4.03, s	56.4	4.03, s	56.3	4.03, s
16			14.9	0.88, d (6.9)	16.5	1.08, d (7.0)

on 1D and 2D NMR experiments (^{1}H, ^{13}C, ^{1}H−^{1}H-COSY, ^{1}H−^{13}C-HSQC, and ^{1}H−^{13}C-HMBC). The data matched those of a synthetic compound perfectly,[12] but it was isolated from a natural source for the first time.

Compound **5** was isolated as a mixture of two isomers. Even though they seemed to be separable by HPLC, the single compounds rapidly reverted to a mixture again, which led to doubled sets of signals in the NMR spectra. With two double bonds in the molecule, the formation of four different isomers is possible, yet only two were observed. As the two double bonds $\Delta^{5,7}$ and $\Delta^{2,11}$ are trisubstituted, the configuration could not be deduced from the coupling constants of connected protons. Therefore, a NOESY spectrum was measured in order to clarify the configurations of the double bonds. H-4 exhibited NOESY correlations to H-8, but not to H-7 in both isomers. Thus, $\Delta^{5,7}$ must be *Z*-configured in both derivatives. Contrary to that, a NOESY correlation from H-1 to H-11 was observed in one of the isomers, but not in the other. Therefore, isomerization of the $\Delta^{2,11}$ double bond readily occurs under the applied conditions.

Compound **5** exhibited weak cytotoxic activity. The treatment of NIH-3T3 and HepG2 cells with 50 μM **5** resulted in a reduction of the metabolic activities by 64% and 44%, respectively.

Although **5** is described for the first time as a natural product, and *Nocardiopsis* strains have not been reported to produce diketopiperazines so far, this compound class is ubiquitous, and very similar structures with dehydro amino acids, such as albonoursin,[13] have already been described from *Streptomyces* strains.[13,14] Two of them are XR334 and XR330, which are diketopiperazines consisting of dehydromethyltyrosine and dehydrophenylalanine and its *N*-methylated derivative. For these two compounds molecular weights of 320.34 and 334.37, respectively, have been reported, which fit in well with the peaks detected in the extract of strain HB383.



feeding with	enhancement of
1-^{13}C-acetate	C-5, C-7
1-^{13}C-propionate	C-1, C-3
Me-^{13}C-methionine	C-15

Figure 2. Biosynthetic origin of nocapyrone A as determined by ^{13}C-labeling.

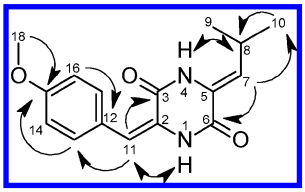


Figure 3. Selected ^{1}H−^{13}C HMBC (→) and NOESY correlations (↔) of (2*E*/5*Z*)-2-[(4-methoxyphenyl)methylene]-5-(2-methylpropylidene)-3,6-piperazinedione (**5**).

Experimental Section

General Experimental Procedures. The optical rotation was measured on a Perkin-Elmer model 241 polarimeter. UV spectra were obtained on a NanoVue (GE Healthcare). NMR spectra were recorded on a Bruker DRX500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons and the solvent carbons as internal references (δ_H 3.31 and δ_C 49.0 ppm for methanol-d_4; δ_H 2.50 and δ_C 39.51 ppm for DMSO-d_6). High-resolution mass spectra were acquired on a benchtop time-of-flight spectrometer (MicrOTOF, Bruker Daltonics) with positive electrospray ionization. Analytical reversed-phase HPLC-UV/MS experiments were performed using a C_{18} column (Phenomenex Onyx Monolithic C18, 100 × 3.00 mm) applying an H_2O (A)/MeCN (B) gradient with 0.1% HCOOH added to both solvents (gradient: 0 min 5% B, 4 min 60% B, 6 min 100% B; flow 2 mL/min) on a VWR Hitachi Elite LaChrom system coupled to an ESI-ion trap detector (Esquire 4000, Bruker Daltonics). Preparative HPLC was carried out using a Merck Hitachi system consisting of an L-7150 pump, an L-2200 autosampler, and an L-2450 diode array detector and a Phenomenex Gemini C18 110A AXIA, 100 × 21.20 mm, or a Phenomenex Luna silica 5 μm, 250 × 10.00 mm column.

Isolation and Identification of *Nocardiopsis* sp. HB383. Strain HB383 was obtained from the marine sponge *Halichondria panicea* collected from the Baltic Sea (Germany). DNA extraction, 16S rRNA amplification, and 16S rRNA gene sequencing as well as the comparison of this sequence (1395 nucleotides; GenBank/EMBL/DDBJ acc. no GQ863933) with sequences in the EMBL nucleotide database available online at the European Bioinformatics Institute homepage using the Basic Local Alignment Search Tool (nucleotide BLAST) and of RDP-II Project homepage were done as described in Schneemann et al.[5] The sequence analysis revealed that the strain was affiliated with the genus *Nocardiopsis*. The most closely related type strain was *Nocardiopsis alba* DSM 43377^{T} (GenBank/EMBL/DDBJ acc. no. X97883) with a sequence identity of 99.8%.

Cultivation. For chemical analyses *Nocardiopsis* sp. HB383 was grown in GYMCaCl$_2$ medium (0.4 g glucose, 0.4 g yeast extract, 0.4 g malt extract, 0.2 g CaCl$_2$, 1 L H_2O, pH 7.2) for 18 days at 28 °C and 120 rpm in 12 × 2000 mL Erlenmeyer flasks in the dark. Each flask was inoculated with 10 mL of a 3-day-old GYM4 preculture (0.4 g glucose, 0.4 g yeast extract, 0.4 g malt extract, 0.2 g CaCO$_3$, 100 mL H_2O, pH 7.2; 120 rpm, 28 °C). These precultures were inoculated with cryoconserved cultures of strain HB383.

Isolation of Nocapyrones A−D and 5. The culture broth (12 L) was homogenized with an Ultra Turrax T25 basic (IKA-Werke GmbH and Co., Staufen, Germany) at 16 000 rpm for 30 s and extracted with 6 L of EtOAc. The solution was dried to yield 804 mg of an organic extract. An aliquot was analyzed with HPLC-UV/MS. Nocapyrones A (**1**) and B (**2**) were detected at 3.2 and 4.5 min, respectively.

Subsequently, the extract was subjected to preparative HPLC (eluents: H_2O (A), MeCN (B); gradient 0 min 5% B, 16 min 70% B, 16.5 min 100% B; flow 18 mL/min). Nocapyrones A (3.7 mg) and B (0.3 mg) eluted at 6.5 and 9.1 min, respectively.

Nocapyrones C (**3**) and D (**4**) were isolated out of combined extracts that were fractionated on a Sephadex LH 20 column with MeOH as eluent. One fraction (approximately 20 mg) yielded 4.8 mg of crude **3** and 2.6 mg of crude **4** by HPLC separation (45% ACN to 55% ACN in 13 min). Compounds **3** and **4** eluted at 4.8 and 5.5 min, respectively. The compounds were purified further on a semipreparative normal-phase column. Applying a gradient from 2-propanol/hexane (25:75) to 2-propanol/hexane (20:80) in 16 min and a flow of 5 mL/min, the derivatives eluted at 7.2 min (**3**) (0.9 mg of a mixture of two isomers) and 9.1 min (**4**) (0.2 mg), respectively.

Compound **5** precipitated in the MeOH extracts as a colorless solid. Part of the precipitate was washed with H_2O several times. Subsequently MeOH (4 × 1 mL) was added. The supernatant was subjected to preparative HPLC, and separation was carried out by a H_2O/ACN gradient of 45% ACN to 90% ACN in 13 min. Compound **5** eluted at 5.0 min (0.6 mg of a mixture of two isomers).

Biosynthetic Studies. For the labeling experiments 1-^{13}C-sodium acetate, 1-^{13}C-sodium propionate, and methyl-^{13}C-methionine with 99% ^{13}C were used (Cambridge Isotope Laboratories, Isotec, and Aldrich). The strain HB383 was cultivated in the same liquid medium as described above (GYMCaCl_2). In each feeding experiment, the preculture was grown for three days in 100 mL of medium. A 1 mL amount of the preculture was added to each of 15 Erlenmeyer flasks with 100 mL of medium for the main culture. Together with the inoculum the labeled compounds (500 mg 1-^{13}C-sodium acetate, 500 mg 1-^{13}C-sodium propionate, and 17 mg methyl-^{13}C-methionine) were added in the form of sterile filtered solutions (dissolved in H_2O). The isolation of nocapyrone A was carried out according to the isolation procedure described above. The yields of labeled nocapyrone A were approximately 1 mg in each batch.

Nocapyrone A (1): colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 253 (4.06), 204 (4.00) nm; for 1D and 2D NMR data see Table 1 and SI; HRESIMS m/z 269.1847 $[M + H]^+$ (calcd for $C_{15}H_{25}O_4$, 269.1847).

Nocapyrone B (2): colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 253 (4.02), 209 (4.07) nm; for 1D and 2D NMR data see Table 2 and SI; HRESIMS m/z 253.1805 $[M + H]^+$ (calcd for $C_{15}H_{25}O_3$, 253.1798).

Nocapyrone C (3): colorless, amorphous solid; $[\alpha]^{20}_D$ +11 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 253 (4.06), 204 (4.00) nm; for 1D and 2D NMR data see Table 2 and SI; HRESIMS m/z 283.1903 $[M + H]^+$ (calcd for $C_{16}H_{26}O_4$, 283.1903).

Nocapyrone D (4): colorless, amorphous solid; $[\alpha]^{20}_D$ +120 (c 0.005, MeOH); UV (MeOH) λ_{max} (log ε) 253 (4.06), 205 (4.10) nm; for 1D and 2D NMR data, see Table 2 and SI; HRESIMS m/z 281.1749 $[M + H]^+$ (calcd for $C_{16}H_{24}O_4$, 281.1747).

Antibacterial Activities. Antimicrobial assays of compounds **1**, **2**, and **5** were performed using *Bacillus subtilis* (DSM 347), *Erwinia amylovora* (DSM 50901), *Escherichia coli* K12 (DSM 498), *Pseudomonas fluorescence* (NCIMB 10586), *Propionibacterium acnes* (DSM 1897), *Pseudomonas aeruginosa* (DSM 50071), *Pseudomonas syringae* pv *aptata* (DSM 50252), *Ralstonia solanacearum* (DSM 9544), *Staphylococcus epidermidis* (DSM 20044), *Staphylococcus lentus* (DSM 6672), *Xanthomonas campestris* (DSM 2405), and the yeast *Candida glabrata* (DSM 6425). The assays were performed according to Schneemann et al.[15]

Cytotoxicity Assays. The sensitivity of the cell lines NIH-3T3, HepG2, and HT29 to the isolated compounds (**1**, **2**, and **5**) was evaluated by monitoring the metabolic activity according to Schneemann et al.[15]

Enzyme Inhibitory Activity. See the Supporting Information.

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Supporting Information Available: Additional information such as ^{1}H NMR spectra of **1**−**5**, ^{13}C NMR spectra of **1**, NMR spectra of labeling experiments and spectroscopic data of **2**−**5**, as well as the description of enzyme inhibitory activity are available free of charge via the Internet at http://pubs.acs.org.

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