Kenalactams A–E, Polyene Macrolactams Isolated From *Nocardiopsis* CG3

Omar Messaoudi,^{†,‡,§} Enge Sudarman,^{⊥,||} Mourad Bendahou,[‡] Rolf Jansen,^{⊥,||} Marc Stadler,^{⊥,||}
and Joachim Wink^{*,†,||}

[†] Microbial Strain Collection, Helmholtz Centre for Infection Research GmbH (HZI),
Inhoffenstrasse 7, 38124 Braunschweig, Germany.

[‡] Laboratory of Applied Microbiology in Food and Environment, Abou bekr Belkaïd
University, Tlemcen, Algeria.

[§] Faculty of Science, Department of Biology, University of Amar Telidji, Laghouat, Algeria.

[⊥] Department Microbial Drugs, Helmholtz Centre for Infection Research GmbH (HZI),
Inhoffenstrasse 7, 38124 Braunschweig, Germany.

^{||} German Centre for Infection Research Association (DZIF), Partner site Hannover-
Braunschweig, Inhoffenstrasse 7, 38124 Braunschweig, Germany.

Graphical 'abstract

In our screening program for new biologically active secondary metabolites, a new strain, *Nocardioopsis* CG3 (DSM 106572), isolated from the saltpan of Kenadsa, was found to produce five new polyene macrolactams, the kenalactams A–E (**1–5**). Their structures were elucidated by spectral methods (NMR and HRESIMS) and the absolute configuration was derived by chemical derivatization (Mosher's method). Through a feeding experiment, alanine was proven to be the nitrogen bearing starter unit involved in biosynthesis of the polyketide kenalactam A (**1**). Kenalactam E (**5**) was cytotoxic against human prostate cancer PC-3 cells with an IC_{50} value of 2.1 μ M.

Actinobacteria are one of the most important phyla of microorganisms that produce a wide variety of bioactive secondary metabolites,^{1,2} including many clinically used antibiotics and anticancer drugs.^{3,4} A contemporary strategy for obtaining new bioactive compounds is based on the isolation of rare actinomycetes from neglected environments,⁵ which should contain gene clusters for biosynthesizing novel secondary metabolites.⁶ One of the pharmaceutically and biotechnologically important rare actinomycetes that attracts natural products researchers is the genus *Nocardiopsis*.^{7,8} Species of this genus are aerobic, Gram-positive, non-acid-fast, and their aerial mycelia bear long chains of spores.^{9,10} *Nocardiopsis* species were mainly recovered from salty habitats. The members of this genus produce an array of bioactive compounds that may aid their survival under these conditions.¹¹ In recent years many new natural products, belonging to different classes of molecules, including polyketides and peptides,^{12,13,14} were isolated from *Nocardiopsis* species.^{15,16} These classes display cytotoxic,¹⁷ antimicrobial,¹⁸ and immunomodulatory activities.¹⁹ Recently, three new angucyclines, nocardiosistins A-C, with anti-Methicillin-Resistant *Staphylococcus aureus* (MRSA) activity were isolated from a marine sponge-derived *Nocardiopsis* sp. HB-J378,²⁰ and a new cytotoxic cyclic peptide was obtained from the marine sponge-associated *Nocardiopsis* sp. UR67.¹⁴

According to the World Health Organization (WHO, 2018), «Cancer is the second leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018 », hence it is necessary to continue the discovery of new classes of molecules with cytotoxic activity, which may be further developed as anticancer drugs.¹⁴ In fact, cytotoxicity-guided fractionation of the crude extract of the marine obligate actinomycetes, *Salinispora tropica*, led to the isolation of salinosporamide A, which entered phase I human clinical trials for the treatment of multiple myeloma.²¹

In the course of screening for new bioactive metabolites from our collection of actinobacteria, which had been isolated from different areas of Algeria, *Nocardiopsis* CG3

(DSM 106572) was selected for a detailed study because novel polyene type compounds had been observed by UV and high resolution electrospray mass spectrometric (HR-ESI-MS) HPLC analyses of culture extracts. Herein, we describe the isolation, spectroscopic structure elucidation and biological activities of kenalactams A-E (**1–5**), which constitute the first polyene macrolactams from the genus *Nocardiopsis*.

RESULTS AND DISCUSSION

Analysis of the extract of *Nocardiopsis* CG3 (DSM 106572) by HPLC-UV-HRESIMS and a survey of databases (e.g., Antibase, Dictionary of Natural Products) suggested the presence of five unknown polyene metabolites, the kenalactams A–E (**1–5**). Consequently, these were isolated using solvent partition and Sephadex LH-20 gel chromatography, followed by preparative reversed phase HPLC.

The elemental formula $C_{23}H_{29}NO_3$ of the main polyene, kenalactam A (**1**), was revealed by the molecular ion cluster $[M + H]^+$ at m/z 368.2390 in the HRESIMS spectrum, indicating 10 double bond equivalents (DBE). The UV spectrum with characteristic absorption bands at 297, 339 and 355 nm suggested a conjugated polyene structural part in **1**. Its complete structure was determined by 1D and 2D NMR spectroscopy. The ^{13}C NMR spectrum confirmed 23 carbons, including one carboxyl carbon (δ_C 165.1) and one olefinic carbon (δ_C 132.2) while the remaining 21 carbons were assigned from the $^1H,^{13}C$ HSQC NMR data as fifteen olefinic methines (δ_H 5.10 – 6.67, δ_C 142.1 – 125.3), two secondary alcohols ($\delta_{H/C}$ 4.53/72.5 and 4.37/69.5), one N-bearing methylene group (δ_H 2.86 and 3.18, δ_C 44.5), a methine group ($\delta_{H/C}$ 2.69/33.1) and two methyl groups ($\delta_{H/C}$ 1.78/12.9 and 0.95/18.1). The 1H NMR spectrum in DMSO- d_6 provided further signals of an amide proton (δ_H 7.62) and two OH signals (δ_H 5.01 and 4.84).

Analysis of the $^1H,^1H$ COSY spectrum revealed two contiguous spin systems from H-2 to H-5 and from H-7 to H_N-22 via oxymethines (H-8 and H-9). Both parts were connected from

^1H , ^{13}C HMBC correlations of H-5 to C-7, and methyl H₃-23 to C-5, C-6 and C-7, respectively. Furthermore, the macrolide ring was established from the HMBC correlations of H_N-22 to C-1, of H₂-21, H-2, and H-3 to C-1 (Figure 1). The configurations of the double bonds and relative configurations of **1** were assigned from extensive analyses of coupling constants observed in the *J*-resolved ^1H NMR spectrum and ROESY correlations. The *E* configurations of the $\Delta^{2,3}$, $\Delta^{4,5}$, $\Delta^{12,13}$ and $\Delta^{14,15}$ double bonds were indicated by their vicinal coupling constants of 14-15 Hz, while ROESY correlations between H-4 and methyl H₃-23, as well as H-5 and H-7 proved the *E* configuration of the methyl substituted $\Delta^{6,7}$ double bond. Coupling constants of 11.0 and 11.4 Hz observed for $\Delta^{10,11}$, $\Delta^{16,17}$, and $\Delta^{18,19}$ double bonds suggested their *Z* configuration. Further, the *cis* orientation of the hydroxy groups at C-8 and C-9 was evident from the small coupling constant (< 4 Hz) of their methine protons and from strong NOEs between methyl H₃-23 and H-8 and H-9. Furthermore, a significant NOE correlation between H-17 and H-20 placed H-20 *syn* to H-17, whereas NOE correlations of methyl H₃-24 with H-19 and H-21a showed that methyl H₃-24 is on the same face as H-19. Additionally, the large vicinal coupling constant of 10.2 Hz indicated an *anti* orientation between methines H-19 and H-20. To gain additional evidence for these assignments, an energy minimized conformation of **1** was calculated, initially using the Conformational Search module (MM2 calculation) in HyperChem Prof. (Version 8.). In the final model, which was optimized by a PM3 calculation, the essential dihedral angles were consistent with the NMR data as summarized in (Table 3). Further support for this model was obtained from the NOE observations. The strongest interactions are depicted in Figure 2.

The absolute configuration of **1** was established by application of the modified Mosher method. Esterification of **1** with (*R*) and (*S*)-MTPA-Cl yielded (*S*) and (*R*)-MTPA esters at the C-9 alcohol. The observed shift differences ($\Delta\delta$) between these esters are depicted in (Scheme 1). The $\Delta\delta_{(S-R)}$ values calculated from the ^1H NMR data defined the absolute configuration at

C-9 as *R*. Finally, based on this assignment and on the molecular modeling, which represented all observed coupling constants and NOE interactions, the complete absolute configuration of **1** was defined as 8*S*, 9*R* and 20*S*.

The molecular formula of kenalactam B (**2**) was also determined as C₂₃H₂₉NO₃ by HRESIMS. The UV, mass and NMR spectra of compound **2** were very similar to those of **1**, indicating a structurally-related isomer of **1**. The structure featured the same carbon skeleton as **1**, which was verified from the NMR data. The main difference was found in the ¹H NMR spectrum, which revealed a coupling constant of 9.5 Hz between H-8 and H-9, which is a typical vicinal coupling for an *anti* conformation. The vicinal coupling between H-9 and H-10 was unchanged (11 Hz), thus indicating the stereocenter at C-8 was reversed in **2**. This assignment was supported by the ROESY correlations H-9/H-12, H-17/H-20, H₃-24/H-19/H-21b whereas no NOE was observed between H-8 and H-9. Consequently, the stereochemistry at C-8 of **2** was assigned to be *R*.

The molecular formula of kenalactam C (**3**) was established as C₃₀H₃₈N₂O₂ by HRESIMS analysis implying 13 double bond equivalents (DBE). The UV spectrum showed characteristic polyene absorption bands (λ 325, 329 and 338 nm). Since the ¹H and ¹³C NMR spectra exhibited half the number of expected signals, **3** was recognized as a symmetrical cyclic dimer. The ¹H NMR spectrum showed a set of signals, each representing a pair of amide protons (δ_{H} 7.62), nine pairs of olefinic protons (δ_{H} 5.25 – 6.77) and two pairs of methyl groups (δ_{H} 0.95 and 1.75). Despite the fact that H-4 and H-5 signals overlapped, the COSY spectrum showed a contiguous sequence of correlations from H-3 to H_N-14. The HMBC correlations of methyl H₃-15 to C-1, C-2 and C-3, and of H-3 to C-1 and C-5 completed the planar structure of the symmetrical half of **3**. HMBC correlations of methylene H₂-13' to C-1 and of H_N-14 to C-1 established the ring closure. The *E* configuration of the $\Delta^{4,5}$ and $\Delta^{6,7}$ double bonds was assigned from the vicinal coupling constants of 14.5 and 15.0 Hz,

whereas the *Z* configuration of the $\Delta^{8,9}$ and $\Delta^{10,11}$ bonds was evident from their coupling constants of 10-12 Hz. The methyl substituted $\Delta^{2,3}$ double bond was unambiguously established as *trans* from the ROESY correlation between methyl-15 and H-4, whereas an NOE between methyl-15 and H-3 was absent. ROESY correlations between H-9 and H-12 positioned H-12/H-12' *syn* to H-9/H-9', whereas NOE correlations of methyl-16 with H-11 and H-13a signified that methyl-16/16' is in on the same face as H-11/11' (Figure 3). These correlations were also observed for **1**, thus indicating the same *S*-configuration of the methyl group at this position.

Kenalactam D (**4**) exhibited a molecular ion cluster $[M+H]^+$ at m/z 499.3323 which provided the molecular formula $C_{33}H_{42}N_2O_2$ (14 DBE). Its UV/VIS spectrum was similar to that of **3**. Compared to **3**, the 1H NMR spectrum displayed two additional olefinic protons and a methyl group. The gross structure of **4** was elucidated from COSY and HMBC correlations. The $\Delta^{4,5}$, $\Delta^{6,7}$, $\Delta^{20,21}$, $\Delta^{22,23}$ double bonds were established as *E* and the $\Delta^{8,9}$, $\Delta^{10,11}$, $\Delta^{20,21}$, $\Delta^{22,23}$ double bonds as *Z* configured. The methyl substituted $\Delta^{2,3}$, $\Delta^{16,17}$, $\Delta^{18,19}$ double bonds were assigned as *E* on the basis of ROESY correlations. Strong NOE correlations between H-8/H-9, H-9/12, H-24/25, H-25/H-28 on one side and between H₃-32/H-11, H₃-35/H-27 on the other side were observed (Figure 4) and thus suggested an identical configured stereo center at C-28 in kenalactam D (**4**) compared to C-12 in **3**.

The molecular formula $C_{36}H_{46}N_2O_2$ (15 DBE) of kenalactam E (**5**) was determined by HRESIMS, which indicated another macrolactam dimer. The NMR spectra of kenalactam E (**5**) contained all structure elements of kenalactam C (**3**) with additional resonances for a pair of methine and of methyl groups ($\delta_{H/C}$ 6.29/134.5 and 1.96/15.6 ppm) and of sp^2 carbons (δ_C 134.3 ppm) (Table 2). Compared to **3**, the structure of **5** included a pair of C_3H_4 structural units, i.e, a propionate unit $[-CH=C(CH_3)=]$ in the olefin chain. However, the additional propionate unit was inserted between C-4 and C-5 of **3**, resulting in an enlarged, symmetrical

macrolactone. Assuming a largely common biosynthesis, the methyl substituted stereogenic center (C-12 or C-14) of **1–5** is conserved throughout these polyene macrolactams. Therefore, the absolute configuration at C-14 was assumed to be *S* in concordance with the similarity of the NMR data.

Due to the low amount of **2**, only compounds **1** and **3–5** were tested for their antimicrobial and cytotoxic activities, and antiviral activity against HCV (Hepatitis-C-Virus) in human liver cells. Compounds **3**, **4** and **5** showed moderate to weak activities against *Staphylococcus aureus*, whereas **3** further exhibited moderate antifungal activity against *Candida albicans* and *Mucor hiemalis*. Compound **1** did not show any antimicrobial activities against our test microorganisms at concentration $\geq 66.66 \mu\text{g/mL}$. Compounds **3–5** exhibited significant but weak cytotoxicity against various human cancer cell lines. Compound **5** was the most potent derivative, showing inhibition of KB3.1, PC-3, SKOV-3 and A549 cell lines in the IC_{50} range of 2–5.5 μM . Compound **1** had no activity against KB3.1 and L929 cell lines. Weak antiviral activities against HCV were observed for compounds **3–5**, while **1** was not active ([Figure S9 Supporting Information](#)).

Kenalactams A (**1**) and B (**2**) are 22 membered macrocyclic lactams. Examples for structurally closely related macrolactams are cyclamenol A (20 membered ring),²² micromonosporin A (24 membered ring),²³ as well as lobosamide, mirilactam, micromonolactam, and sceliphrolactam (all with 26 membered rings).^{24,25,26}

Macrolactams, which represent a small class of compounds belonging to the group of polyketides,^{27,28} are characterized by macrocyclic amide rings. Polyene macrolactams are a hitherto underexplored group of natural products that have only been found in actinomycetes.^{29,30} For example, sceliphrolactam, isolated from a *Streptomyces* sp. associated with the mud wasp *Sceliphron caementarium*, is an unusual polyenol and enone macrolactam which inhibited amphotericin B-resistant *Candida albicans* (MIC 4 $\mu\text{g/mL}$).²⁵ A new polyene

macrolactam, lobosamide A, recently isolated from a marine actinobacterium of the genus *Micromonospora* inhibited the growth of the protozoan parasite *Trypanosoma brucei* ($IC_{50} = 0.8 \mu M$).²⁶ The nocardiopeptidins, prolanyl-macrolactam polyketides isolated from *Nocardiopepsis* sp. (CMB-M0232),¹⁹ and fluvirucin B6, a new macrolactam isolated from a marine-derived *Nocardiopepsis*,³¹ are the only polyene macrolactams reported from the genus *Nocardiopepsis*.

Macrolactams are synthesized by a modular PKS that contains only the ACP domain in the loading module.³² Various β -amino acid starter units are involved in the biosynthesis of macrolactams and contribute to their structural diversity.^{33,34} The majority of these β -amino acid moieties are synthesized from proteinogenic α -amino acids, such as L-glutamate, L-aspartate, L-phenylalanine and L-alanine.³⁴ For example, leinamycin is synthesized using alanine as a starter unit, while, 3-methylaspartate and lysin are presumed to represent the starters units for the biosynthesis of vicanistatin and salinilactam respectively.³⁵ The incorporation of amino acids into the polyketide skeletons appears to be a critical event because the amino groups of amino acids are necessary to form the macrocyclic structures.³³ To determine which amino acid starter unit might be incorporated in the biosynthesis of kenalactam A (**1**), eleven amino acids were separately evaluated as a nitrogen source. The LC-MS results indicated that kenalactam A (**1**) was synthesized in particular when L-alanine was added as additional nitrogen source. This was confirmed through the feeding of strain *Nocardiopepsis* CG3 (DSM 106572) with L-alanine-¹⁵N. In GYM medium without malt extract, the ¹⁵N of the latter compound was incorporated in the amide group of the polyene ring of kenalactam A, and the molecular formula of **1**, which was determined by mass spectrometry, became C₂₃H₂₉¹⁵NO₃ (Figure S8 Supporting Information).

EXPERIMENTAL SECTION

General Experimental Procedures. Fractionation and analytical RP-HPLC were carried out with an Agilent 1260 HPLC system equipped with a diode-array UV detector (DAD-UV), a fraction collector or a Corona Ultra detector (Dionex). Analytical HPLC conditions: column 100×2.1 mm XBridge C₁₈, 3.5 μm, (Waters), solvent A: H₂O–acetonitrile (95/5), 5 mmol NH₄OAc, 0.04 mL/L CH₃COOH; solvent B: H₂O–acetonitrile (5/95), 5 mmol NH₄OAc, 0.04 mL/L CH₃COOH; gradient system: 10% B increasing to 100% B in 30 min and maintaining 100% for 10 min; flow rate 0.3 mL/min; 40 °C. Preparative RP-HPLC was performed on an Agilent 1100 series preparative HPLC system [ChemStation software (Rev. B.04.03); binary pump system; DAD-UV detection; fraction collector]. High resolution electrospray ionization mass spectrometry (HRESIMS) data were recorded on a MaXis ESI-TOF-mass spectrometer (Bruker) equipped with an Agilent 1260 series RP-HPLC system: column 50×2.1 mm Acquity UPLC BEH C₁₈ (Waters); solvent A: 0.1% formic acid in H₂O, B: 0.1% formic acid in acetonitrile; gradient system: 5% B for 0.5 min, in 19.5 min to 100% B and maintained for 5 min at 100% B; flow rate 0.6 mL/min; 40 °C; DAD-UV detection at 200–600 nm. Molecular formulas were calculated including the isotopic pattern with the SmartFormula algorithm (Bruker). ESI mass spectra in positive and negative ionization modes were obtained with an ion trap MS (Amazon, Bruker). The mass spectrometer was coupled to a Dionex ultimate 3000 HPLC system [column 50×2.1 mm, C₁₈ Acquity uPLC BEH, 1.7 μm, (Waters), solvent A: H₂O + 0.1% formic acid; solvent B: acetonitrile (ACN) + 0.1% formic acid, gradient: 5% B for 0.5 min, increasing to 100% B in 20 min, maintaining isocratic conditions at 100% B for 10 min, flow rate 0.6 mL/min, DAD-UV detection 200 – 600 nm]. NMR spectra were recorded on an Avance III 700 spectrometer (Bruker, Bremen, Germany) with a 5 mm TXI cryoprobe (¹H 700 MHz, ¹³C 175 MHz) and an Avance III 500 (¹H 500 MHz, ¹³C 125 MHz) spectrometer. Optical rotations were measured on a PerkinElmer 241 MC polarimeter (using the sodium D line and a quartz cuvette with 10 cm path length and

0.5 mL volume). UV spectra were recorded on a Shimadzu UV/Vis-2450 spectrophotometer using methanol (Uvasol, Merk).

Strain Origin and Identification. Strain CG3 was isolated from soil samples collected in 2012 from the saltpan of Kenadsa (22 km west of Bechar located in southwest Algeria), by a serial dilution method using a starch casein agar medium [starch (10 g/L), casein (0.3 g/L), KNO₃ (2 g/L), NaCl (2 g/L), K₂HPO₄ (2 g/L), MgSO₄·7H₂O (0.05 g/L), CaCO₃ (0.02 g/L), FeSO₄·7H₂O (0.01 g/L) and agar (18.0 g) supplemented with 7% of NaCl and 50 µg/mL amphotericin B]. For molecular identification, the strain CG3 was cultured in 100 mL of GYM (glucose 4 g/L; yeast extract 4 g/L; malt extract 10 g/L; CaCO₃ 2 g/L) medium supplemented with 3% NaCl and incubated for 5 days (160 rpm, 37 °C). DNA extraction was performed using a Spin Plant Mini Kit (Invisorb), following the manufacturer's protocol. The 16S rRNA gene region was amplified by PCR using two universal primers, forward primers bind on the position (F27) and reverse primers bind on the position (R1525). For the whole 16s DNA sequencing, five primers were used: F27, R518, F1100, R1100, R1521. The identification of the strain CG3, using a polyphasic approach, indicated that this strain represents a new species of the genus *Nocardiopsis*. The sequence of 16s rDNA of the strain *Nocardiopsis* CG3 (DSM 106572) are deposited in GenBank with accession number MG972881.

Production, Extraction and Isolation. Mature spores of strain CG3, grown on ISP3 plus 3% NaCl agar plates, were used to inoculate two 1 L Erlenmeyer flasks, each of which contained 400 mL of seed soybean medium (2% soymeal, 2% mannitol, 0,4% glucose, 3% NaCl, 1 L deionized water, pH 7). The flasks were incubated on a rotary shaker (160 rpm) at 37 °C for 7 days. 20 L of soybean meal medium, distributed in twenty-five 2 L Erlenmeyer flasks (each 800 mL), were inoculated with 10% of the seed culture. The flasks were incubated on a rotary shaker (160 rpm) at 37 °C for 13 days. Mycelial biomass was separated

from the supernatant by centrifugation (7000 rpm, 30 min.) in a Dupont Instrument (Sorvall RC-5B Refrigerated Superspeed Centrifuge). Adsorber resin Amberlite XAD-16, 10% (v/v), was added to the supernatant and stirred overnight at 4 °C. The XAD resin was separated from the supernatant by sieving and extracted with methanol-H₂O (3-7 v/v; three portions of 500 mL each), followed by methanol (1 L). The methanol extract was evaporated to dryness, and the residue dissolved in 100 mL acetone and stored at 4 °C until precipitation occurred. After centrifugation, the precipitate was washed with acetone, dried in *vacuo* and separated by preparative reversed-phase HPLC [column 250×21 mm, 5 µm, Nucleodur Phenyl-hexyl (Macherey Nagel), solvent A: H₂O–acetonitrile (95/5), 5 mmol NH₄OAc, 0.04 mL/L CH₃COOH; solvent B: H₂O–acetonitrile (5/95), 5 mmol NH₄OAc, 0.04 mL/L CH₃COOH; gradient from 25% B to 70% B in 60 min; flow rate 20 mL/min]. Fractions were evaporated and the residual water was extracted with ethyl acetate and dried in *vacuo* to obtain kenalactam B (**2**; 0.4 mg). The biomass was extracted with ethyl acetate three times (1.5 L). After evaporation, the residue was redissolved in methanol and partitioned with n-heptane to remove the lipophilic components. The methanol layer was evaporated and the residue (1.15 g) was subjected to Sephadex LH-20 column chromatography (column 3×83 cm, flow rate 3.8 mL/min) and eluted with methanol. Four fractions were collected, the solvent evaporated, and the residue analyzed by RP-HPLC-MS. Fr. 1 (50 mg) containing kenalactams C–E (**3–5**) was separated by preparative RP-HPLC [column 250×21 mm, Nucleodur Phenyl-hexyl, 5 µm (Macherey Nagel), solvent A: water, solvent B: acetonitrile, gradient from 50% B to 60% B in 60 min, flow rate 20 mL/min, UV detection at 220, 350, and 360 nm] to obtain kenalactam C (**3**) (1 mg), kenalactam D (**4**) (2.5 mg) and kenalactam E (**5**) (1 mg). Fr.2 (70 mg) was purified by preparative RP-HPLC [column 250×21 mm, Nucleodur Phenyl-hexyl, 5µm (Macherey Nagel) gradient 30% B to 70% B in 60 min, UV detection 220, 300 and 350 nm] to obtain kenalactam A (**1**) (10 mg).

Kenalactam A (1): light yellow solid; $[\alpha]_D^{20} +425$ (*c* 0.12, DMSO); UV/Vis (CH₃OH) $\lambda_{\max}$ (log ϵ) 248 (3.88), 297 (4.57), 339 (3.94), 355 (3.90) nm; NMR data (¹H: 700 MHz, ¹³C NMR 176 MHz, DMSO) see [Table 1](#); HRESIMS: $[M + H]^+$ *m/z* 368.2390, calcd. 368.2220 for C₂₃H₂₉NO₃, *t_R* = 8.6 min.

Kenalactam B (2): light yellow solid; $[\alpha]_D^{20} +33.8$ (*c* 0.10, CH₃OH); UV/Vis (CH₃OH) $\lambda_{\max}$ (log ϵ) 246 (3.43), 296 (3.71), 338 (3.21), 355 (3.17) nm; NMR data: ¹H (700 MHz, CD₃OD): δ 1.06 (3H, *J* = 6.7 Hz, H₃-24), 1.91 (1H, s, H₃-23), 2.83 (1H, m, H-20), 3.07 (1H, dd, *J* = 13.0, 3.7 Hz, H-21b), 3.27 (1H, dd, *J* = 13.0, 11.7 Hz, H-21a), 4.61 (1H, t, *J* = 10 Hz, H-9), 4.88^a (1H, m, H-8), 5.22 (1H, dd, *J* = 11.0, 10.0 Hz, H-19), 5.31 (1H, d, *J* = 7.5 Hz, H-7), 5.49 (1H, *J* = 11.0, 10.0 Hz, H-10), 5.77 (1H, *J* = 15.3 Hz, H-2), 5.92 (1H, t, *J* = 11.0 Hz, H-16), 6.07^b (1H, dd, *J* = 14.5, 11.0 Hz, H-14), 6.08^b (1H, dd, *J* = 14.5, 11.0 Hz, H-12), 6.15 (1H, t, *J* = 11.0 Hz, H-17), 6.22 (1H, dd, *J* = 14.5, 11.0 Hz, H-13), 6.24^c (1H, dd, *J* = 15.0, 6.0 Hz, H-14), 6.26^c (1H, d, *J* = 15.0 Hz, H-5), 6.36 (1H, t, *J* = 11.0 Hz, H-11), 6.48 (1H, t, *J* = 11.0 Hz, H-18), 6.56 (1H, dd, *J* = 14.5, 11.0 Hz, H-15), 6.84 (1H, dd, *J* = 15.3, 9.9 Hz, H-3). ^a overlapping signal with solvent peak, chemical shift was assigned using HSQC; ^{b,c} overlapping signals. ¹³C NMR (176 MHz, CD₃OD): δ 13.4 (C-23), 18.5 (C-24), 34.7 (C-20), 46.4 (C-21), 73.2 (C-8), 74.9 (C-9), 125.9 (C-2), 126.7 (C-17), 127.1 (C-18), 127.4 (C-4), 128.4 (C-10), 129.8 (C-15), 130.6 (C-12), 131.0 (C-16), 134.5 (C-11), 134.7 (C-13), 135.1 (C-6), 135.6 (C-14), 136.8 (C-19), 137.3 (C-7), 141.5 (C-3), 144.3 (C-5), 169.1 (C-1). HRESIMS: $[M + H]^+$ *m/z* 368.2220, calcd. 368.2147 for C₂₃H₂₉NO₃, *t_R* = 8.9 min.

Kenalactam C (3): green yellow solid; $[\alpha]_D^{20} +104.7$ (*c* 0.1, CH₃OH); UV/Vis (CH₃OH): $\lambda_{\max}$ (log ϵ) 325 (4.04), 338 (4.07) nm; NMR data (¹H: 700 MHz, ¹³C 176 MHz, DMSO) see [Table 2](#); HRESIMS: $[M + H]^+$ *m/z* 459.3012, calcd. 459.3006 for C₃₀H₃₈N₂O₂, *t_R* = 11.0 min.

Kenalactam D (4): yellow solid; $[\alpha]_D^{20} +477$ (c 0.23, CH₃OH); UV/Vis (CH₃OH): $\lambda_{\max}$ (log ϵ) 339 (3.50), 349 (4.54) nm; NMR data (¹H: 700 MHz, ¹³C 176 MHz, DMSO) see [Table 1](#); HRESIMS: $[M + H]^+$ m/z 499.3325, calcd. 499.3319 for C₃₃H₄₂N₂O₂, t_R = 12.3 min.

Kenalactam E (5): yellow solid; $[\alpha]_D^{20} +70.8$ (c 0.1, CH₃OH); UV/Vis (CH₃OH): $\lambda_{\max}$ (log ϵ) 287 (3.76), 362 (4.69) nm; NMR data (¹H: 700 MHz, ¹³C 176 MHz, DMSO) see [Table 2](#); HRESIMS: $[M + H]^+$ m/z 539.3636, calcd. 539.3632 for C₃₆H₄₆N₂O₂, t_R = 13.1 min.

Preparation of the S- and R-MTPA esters of 1: Kenalactam A (**1**) (2 mg, 5.4 μ mol) was dissolved in 200 μ L anhydrous dichloromethane, then 100 μ L pyridine and 5 μ L *R*-(-)-MTPA-chloride were added and stirred at room temperature for 2 h. The reaction mixture was quenched with buffer solution (pH 7), extracted three times with dichloromethane, and concentrated in *vacuo* to dryness. The (*S*)-MTPA ester was purified by semi-preparative RP-HPLC [column: 250 $\times$ 10 mm, Nucleodur 100-5 C₁₈ ec, Macherey-Nagel, solvent A: water, solvent B: acetonitrile. Gradient: from 40% B to 100% B in 30 min, flow rate: 6 mL/min, UV detection: 220, 350 and 360 nm] to yield 0.3 mg of 9-(*S*)-MTPA ester **1a** ESIMS: m/z 584.32 $[M+H]^+$. The (*R*)-MTPA-ester of **1** was prepared analogously from 2 mg (5.4 μ mol) of **1** to give 0.3 mg of 9-(*R*)-MTPA ester **1b**. ¹H NMR ([Table 21 Supporting Information](#)), COSY, TOCSY and ROESY data of the products **1a/1b** were recorded.

Antimicrobial Assays. Minimum inhibitory concentrations were determined by serial dilution in 96-well plates with EBS medium (0.5% casein peptone, 0.5% glucose, 0.1% meat extract, 0.1% yeast extract, 50 mM HEPES [11.9 g/L] and pH 7.0) for bacteria and MYG medium (1.0% phytone peptone, 1.0% glucose, 50 mM HEPES [11.9 g/L] pH 7.0) for fungi. Twenty microliter aliquots of compounds **1** and **3–5** with a concentration of 1 mg/mL (the final concentration in the first well is 67 μ g/mL) were tested against two different Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), one Gram-negative (*Escherichia*

coli TolC) bacteria and two fungi (*Candida albicans*, *Mucor hiemalis*) with methanol as a negative control.³⁶

Cytotoxicity Assay. Cytotoxicity effects (IC₅₀) of compounds **1** and **3–5** were determined against a panel of five human cancer cell lines including HeLa cells KB3.1, human lung carcinoma A549, ovarian carcinoma SKOV-3, human prostate cancer PC-3, human breast adenocarcinoma MCF-7, and normal cell line mouse fibroblasts L929, by using a 5-day MTT assay according to an established procedure.^{37,38}

HCV Infectivity Assay. Huh-7.5 cells were electroporated with reporter viruses Luc-Jc1 in the presence of test compounds. The inoculum was removed 4 hours later, then monolayers were washed three times with PBS and overlaid with fresh medium in the absence of compounds. Infected cells were lysed 3 days and their luciferase activity was determined as indicators of viral genome replication and cells viability, respectively, as previously described.^{38,39} The assay was performed in duplicate and the data presented as the mean standard deviation. Viability assay results are given in the [Supporting Information Figure S9](#).

Feeding Experiment. GYM medium (without malt extract, containing yeast extract as sole nitrogen source) was distributed in eleven 50 mL Erlenmeyer flasks, each flask containing 20 mL of culture medium. Eleven L- amino acids: Lys, Ala, Met, Val, Arg, Gly, Phe, Pro, His, Thr, Glu, were added separately to each flask (1%). These flasks were inoculated with strain *Nocardiopsis* CG3 (DSM 106572), incubated at 37 °C, and stirred at 150 rpm. After 14 days, each culture was extracted with ethyl acetate and the organic solvent was evaporated to dryness. These crude extracts were redissolved in MeOH and an aliquot thereof was subjected to HPLC-MS analysis to determine which amino acid enabled strain *Nocardiopsis* CG3 (DSM 106572) to biosynthesize kenalactam A (**1**).

Feeding with L-alanine-¹⁵N was conducted in 20 mL of modified ISP2 medium (0.4 % yeast extract; 0.4 % glucose, ¹⁵N-L-alanine) in a 50 mL Erlenmeyer flask. After incubation of strain

Nocardiopsis CG3 (DSM 106572) at 37 °C for 14 days (150 rpm), the culture was extracted with ethyl acetate, evaporated to dryness, and subjected to HPLC-MS analysis.

ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR and LC/MS data of compounds (**1–5**), antiviral test results, morphological and phylogenetic details of the producing organism are available free of charge via the internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Joachim.Wink@helmholtz-hzi.de.

Tel/Fax: +49 (0)531 6181-4223/+49 (0)531 6181-2655

ORCID

Omar Messaoudi: 0000-0002-5684-294X

Enge Sudarman: 0000-0003-3484-9080

Marc Stadler: 0000-0002-7284-8671

Rolf Jansen: 0000-0002-3592-4807

Notes:

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

O. Messaoudi acknowledges the Ministry of Higher Education and Scientific Research (MESRS) of Algeria for its financial support. We also thank Dimas F. Praditya and Eike Steinmann for conducting the HCV infectivity assay, Christel Kakosche for recording NMR spectra and for kindly providing the Ala-¹⁵N, and Wera Collisi for conducting bioassays.

REFERENCES

- (1) Banin, E.; Hughes, D.; Kuipers, O. P. *FEMS Microbiology Reviews*. **2017**, *41*, 450–452.
- (2) Genilloud, O. *Nat. Prod. Rep.* **2017**, *34*, 1203–1232.
- (3) Meij, A.V.M.; Worsley, S.F.; Hutchings, M. I.; Wezel, G. P. V. *FEMS Microbiol. Rev.* **2017**, *41*, 392–416.
- (4) Abd-Elnaby, H.; Abo-Elala, G.; Abdel-Raouf, U.; Abdelwahab, A.; Hamed, M. *Biotechnol. Biotechnol. Equip.* **2016**, *30*, 180–191.
- (5) Arocha-Garza, H. F.; Castillo, R. C. D.; Eguiarte, L. E.; Souza, V.; De la Torre-Zavala, S. *PeerJ*. **2017**, *5*: e3247. 10.7717.
- (6) Jiang, Y.; Li, Q.; Chen, X.; Jiang, C. Dhanasekaran, D. Ed.; *IntechOpen*: London, UK. **2016**; pp 39–57.
- (7) Miyanaga, A.; Kudo, F.; Eguchi, T. *Curr Opin Chem Biol.* **2016**, *35*, 58–64.
- (8) Bennur, T.; Ravi Kumar, A.; Zinjarde, S.S.; Javdekar, V. *J. Appl. Microbiol.* **2016**, *120*, 1–16.
- (9) Gao, G. B.; Luo, X. X.; Xia, Z. F.; Zhang, Y.; Wan, C. X.; Zhang, L. L. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 5005–5009.
- (10) Zhang, Y, G.; Liu, Q.; Wang, H, F.; Park, D, J.; Guo, J, W.; Kim, C, J.; Zhang, Y, M.; Li, W, J. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 230-235.
- (11) Bennur, T.; Ravi Kumar, A.; Zinjarde, S.S.; Javdekar, V. *J. Appl. Microbiol.* **2016**, *120*, 1–16.
- (12) Dashti, Y.; Grkovic, T.; Abdelmohsen, U.R.; Hentschel, U.; Quinn, R.J. *J. Nat. Prod.* **2017**, *80*, 828–836.
- (13) Kim, J.; Shin, D.; Kim, S, H.; Park, W.; Shin, Y.; Kim, W, K.; Lee, S, K.; Oh, K, B.; Shin, J.; Oh, D, C. *Mar Drugs* **2017**, *15*, 166–176.
- (14) Ibrahim, A.H.; Attia, E.Z.; Hajjar, D.; Anany, M.A.; Desoukey, S.Y.; Fouad, M.A.; Kamel, M.S.; Wajant, H.; Gulder, T.A.M.; Abdelmohsen, U.R. *Mar. Drugs* **2018**, *16*, 290.
- (15) Ibrahim, A, H.; Desoukey, S, Y.; Fouad, M, A.; Kamel, M, S.; Gulder, T, A, M.; Abdelmohsen, U, R. *Mar. Drugs* **2018**, *16*, 147.
- (16) Sun, M, W.; Guo, Z, X.; Lu, C, H. *Nat. Prod. Res.* **2016**, *30*, 1036–1041.
- (17) Du, Y., Derewacz, D. K., Deguire, S. M., Teske, J., Ravel, J., Sulikowski, G. A., and Bachmann, B. O. *Tetrahedron* **2011**, *67*, 6568-6575.

- (18) Tian, S. Z.; Pu, X.; Luo, G.; Zhao, L.-X.; Xu, L.-H.; Li, W.-J.; Luo, Y. *J. Agric. Food Chem.* **2013**, *61*, 3006–3012.
- (19) Raju, R.; Piggott, A.M.; Conte, M.; Tnimov, Z.; Alexandrov, K.; Capon, R.J. *Chem. Eur. J.* **2010**, *16*, 3194–320.
- (20) Xu, D.; Nepal, K.K.; Chen, J.; Harmody, D.; Zhu, H.; McCarthy, P.J.; Wright, A. E.; Wang, G. *Synth. Syst. Biotechnol.* **2018**, *3* (4), 246–251.
- (21) Nikapitiya, C. Bioactive secondary metabolites from marine microbes for drug discovery. *Adv. Food Nutr. Res.* **2012**, *65*, 363–387.
- (22) Müller, H.; Bischoff, E.; Fiedler, V.B.; Weber, K.; Fugmann, B.; Rosen, B. German Patent 4231289A1, 1994. US Patent 5,565,561 1996.
- (23) Thawai, C.; Kittakoop, P.; Tanasupawat, S.; Suwanborirux, K.; Sriklung, K.; Thebtaranonth, Y. *Chem. Biodivers.* **2004**, *1*, 640.
- (24) Skellam, E.J.; Steward, A. K.; Strangman, W. K.; Wright, J. L. *J. Antibiot.* **2013**, *66*, 431–441.
- (25) Oh, D. C.; Poulsen, M.; Currie, C. R.; Clardy, J. *Org. Lett.* **2011**, *13*, 752–755.
- (26) Schulze, C.J.; Donia, M.S.; Siquera-Neto, J.L.; Ray, D.; Raskatov, J.A.; Green, R.; Mc Kerrow, J.H.; Fischbach, M.A.; Linington R.G. *ACS Chem. Biol.* **2015**, *10* (10), 2373–2381.
- (27) Li, L.; Cai, Y.; Jiang, Y.; Liu, J.; Ma, J.; Yuan, C.; Mu, Y.; Han, L.; Huang, X. *Bioorg. Med. Chem. Lett.* **2016**, *26* (6), 1599–604.
- (28) Ammermann, J.; Schmidt, T.; Donner, J.; Reck, M.; Dalton, M.; Stumpp, N.; Stiesch, M.; Wagner-Döbler, I.; Kirschning, A. *Org. Biomol. Chem.* **2017**, *15*, 8553.
- (29) Caffrey, P.; De Poire, E.; Sheehan, J.; Sweeney, P. *Appl Microbiol Biotechnol.* **2016**, *100*, (9), 3893–3908.
- (30) Kawahara, T.; Fujiwara, T.; Kagaya, N.; Shin-ya, K. *J. Antibiot.* **2018**, *71*, 390–392.
- (31) Leutou, A. S.; Yang, I.; Le, T. C.; Hahn, D.; Lim, K. M.; Nam, S. J Nam; Fenical, W. *J. Antibiot.* **2018**, *71*, 609–612.
- (32) Miyanaga, A. *Biosci, Biotechnol, Biochem.* **2017**, *81*, 2227– 2236.
- (33) Miyanaga, A.; Kudo, F.; Eguchi, T. *Curr. Opin. Chem. Biol.* **2016**, *35*, 58–64.
- (34) Kudo, F.; Miyanaga, A.; Eguchi, T. *Nat. Prod. Rep.* **2014**, *31*, 1056.
- (35) Jørgensen, H.; Degnes, K. F.; Dikiy, A.; Fjærvik, E.; Klinkenberg, G.; Zotchev, S. B. *Appl. Environ. Microbiol.* **2010**, *76*, 283–293.
- (36) Rupcic, Z.; Chepkirui, C.; Hernández-Restrepo, M.; Crous, P.; Luangsa-ard, J.; Stadler, M. *MycoKeys.* **2018**, *33*, 1–23.
- (37) Noumeur, S. R.; Helaly, S. E.; Jansen, R.; Gereke, M.; Stradal, T. E. B.; Harzallah, D.; Stadler, M. *J. Nat. Prod.* **2017**, *80* (5), 1531–1540.
- (38) Mulwa, L.; Jansen, R.; Praditya, D.; Mohr, K.; Wink, J; Steinmann, E.; Stadler, M. *Molecules* **2018**, *23*, 542.
- (39) Ciesek, S; von Hahn, T; Colpitts, C.C.; Schang, L. M.; Friesland, M.; Steinmann, J.; Manns, M.P.; Ott, M.; Wedemeyer, H.; Meuleman; P.; Pietschmann, T., Steinmann, E. *Hepatology* **2011**, *54*, 1947–1955.

Figures and Tables

Figure 1. Selected $^1\text{H}, ^1\text{H}$ COSY (bold lines) and $^1\text{H}, ^{13}\text{C}$ HMBC (arrows) correlations of **1**.

Figure 2. PM3 Calculated model of **1** and selected strong NOE contacts.

Figure 3. Selected $^1\text{H}, ^1\text{H}$ COSY (bold bonds), $^1\text{H}, ^{13}\text{C}$ HMBC (plain arrows) and $^1\text{H}, ^1\text{H}$ ROESY (dashed arrows) correlations of **3**.

Figure 4. Selected $^1\text{H},^1\text{H}$ COSY (bold bonds), $^1\text{H},^{13}\text{C}$ HMBC (plain arrows) and $^1\text{H},^1\text{H}$ ROESY (dashed arrows) correlations of kenalactam D (**4**).

Scheme 1. $\Delta\delta_{(S-R)}$ Values calculated from the (S)- and (R)-MTPA esters of **1**.

Table 1. NMR Spectroscopic Data of kenalactams A (**1**) and D (**4**) in DMSO-*d*₆ (¹H 700.4 MHz, ¹³C 176.1 MHz).

Pos.	1		4	
	δ_C , type	δ_H , mult. (<i>J</i> in Hz)	δ_C , type	δ_H , mult. (<i>J</i> in Hz)
1	165.1, C	-	168.3, C	-
2	125.3, CH	5.66, d (15.0)	131.3, C	-
3	138.5, CH	6.66, dd (15.0, 10.5)	132.4, CH	6.66, d (11.4)
4	125.4, CH	6.08, dd (15.0, 10.5)	129.1, CH	6.34 ^a , dd (14.5, 11.4)
5	142.1, CH	6.14 ^a , d (15.0)	136.5, CH	6.59, dd (14.5, 11.4)
6	132.2, C	-	134.7, CH	6.24 ^b , dd (14.5, 11.4)
7	137.9, CH	5.15, d (7.5)	129.1, CH	7.00, dd (14.5, 11.4)
8	72.5, CH	4.53, ddd (7.5, 3.4, 3.0)	129.9, CH	5.88, t (11.4)
8-OH		4.84, br. d (3.0)		
9	69.5, CH	4.37, ddd (9.0, 4.7, 3.4)	125.1, CH	6.01, t (11.4)
9-OH		5.01, br. d (4.7)		
10	132.8, CH	5.45, dd (11.0, 9.0)	124.7, CH	6.73, t (11.4)
11	128.2, CH	6.03 ^b , t (11.0)	137.6, CH	5.32, dd (11.4, 10.4)
12	130.2, CH	6.01 ^b , dd (14.5, 11.0)	31.6, CH	2.88, m
13	131.5, CH	6.12 ^a , dd (14.5, 11.0)	44.6, CH ₂	(a) 3.49, ddd (13.0, 7.1, 3.9) (b) 2.41, ddd (13.0, 11.0, 5.6)
14	134.4, CH	6.02 ^b , dd (14.5, 11.4)	NH	7.84, dd (7.1, 5.6)
15	127.6, CH	6.49, dd (14.5, 11.4)	171.2, C	-
16	129.2, CH	5.81, t (11.4)	134.0, C	-
17	125.4, CH	6.11, t (11.4)	137.2, CH	6.31, s
18	125.5, CH	6.36, t (11.4)	130.2, C	
19	135.7, CH	5.10, dd (11.4, 10.2)	136.8, CH	5.93 ^c , d (11.4)
20	33.1, CH	2.69, ddqd (11.6, 10.2, 6.5, 3.7)	128.3, CH	6.49, dd (14.5, 11.4)
21	44.5, CH ₂	(a) 3.18, ddd (13.0, 11.6, 9.3) (b) 2.86, ddd (13.0, 3.7, 3.3)	136.1, CH	6.18, dd (14.5, 11.4)
22	NH	7.62, br. dd (9.3, 3.3)	133.2, CH	6.26 ^b , dd (14.5, 11.4)
23	12.9, CH ₃	1.78, s	129.8, CH	6.44, dd (14.5, 11.4)
24	18.1, CH ₃	0.95, d (6.5)	129.0, CH	5.92 ^c , t (11.4)
25			125.8, CH	6.09, dd (11.4, 11.0)
26			125.2, CH	6.35 ^a , t (11.0)
27			137.5, CH	5.21, t (11.0)
28			33.0, CH	2.86, m
29			44.7, CH ₂	(a) 3.13, ddd (13.0, 5.0, 4.5) (b) 2.94, ddd (13.0, 11.4, 7.5)
30			NH	7.49, dd (7.5, 5.0)
31			12.8, CH ₃	1.71, d (6.8)
32			18.1, CH ₃	0.94, d (6.5)
33			14.3, CH ₃	1.95, br. s
34			15.6, CH ₃	1.99, d (6.5)
35			17.4, CH ₃	0.95, d (6.5)

^{a,b,c} Overlapping signals. Chemical shifts were assigned from ¹H, ¹³C HSQC spectrum.

Table 2. NMR Spectroscopic Data of Symmetrical Kenalactam C (**3**) and Kenalactam E (**5**) in DMSO-*d*₆ (¹H 700.4 MHz, ¹³C 176.1 MHz)

pos.	3		5	
	δ_C , type	δ_H , mult. (<i>J</i> in Hz)	δ_C , type	δ_H , mult. (<i>J</i> in Hz)
1, 1'	169.2, C		171.4, C	
2, 2'	131.2, C		130.6, C	
3, 3'	132.6, CH	6.42 ^a , d (11.4)	136.8, CH	6.27, d (14.5)
4, 4'	128.2, CH	6.36 ^a , dd (14.0, 11.4)	129.0, CH	6.48 ^a , d (14.5, 10.0)
5, 5'	137.2, CH	6.38 ^a , dd (14.0, 10.0)	136.6, CH	6.01 ^b , d (10.0)
6, 6'	133.6, CH	6.23, dd (14.5, 10.0)	134.3, C	
7, 7'	130.0, CH	6.77, dd (14.5, 11.4)	135.5, CH	6.49 ^a , d (10.0)
8, 8'	129.2, CH	5.86, t (11.4)	134.5, CH	6.29, dd (14.5, 10.0)
9, 9'	125.4, CH	6.03, t (11.4)	128.7, CH	6.90, dd (14.5, 11.4)
10, 10'	125.2, CH	6.57, dd (11.4, 11.0)	129.9, CH	5.92, t (11.4)
11, 11'	137.3, CH	5.25, t (11.0)	124.5, CH	6.02 ^b , t (11.4)
12, 12'	31.7, CH	2.91, dqdd (11.0, 6.5, 6.1, 4.9)	124.9, CH	6.71, t (11.4)
13, 13'	44.6, CH ₂	(a) 3.36, ddd (13.0, 6.4, 4.9) (b) 2.59, ddd (13.0, 6.4, 6.1)	137.5, CH	5.31, t (11.4)
14, 14'	NH	7.62, t (6.4)	31.4, CH	2.96, tqd (11.4, 6.5, 4.3)
15, 15'	12.8, CH ₃	1.75, br. s	44.4, CH ₂	(a) 3.48, ddd (13.0, 6.4, 4.3) (b) 2.41, ddd (13.0, 11.4, 6.4)
16, 16'	17.6, CH ₃	0.95, d (6.5)	NH	7.74, t (6.4)
17, 17'			14.3, CH ₃	1.93, s
18, 18'			15.6, CH ₃	1.96, s
19, 19'			17.8, CH ₃	0.93, d (6.5)

^{a,b} Overlapping signals. Chemical shifts were assigned from ¹H, ¹³C HSQC spectrum.

Table 3. Calculated Dihedral Angles and Observed Vicinal Coupling Constants of **1**

Protons	φ [°]	³ <i>J</i> _{H,H} [Hz]
7,8	150	7.5
8,9	67	3.4
9,10	163	9.0
19,20	177	10.2
20,21a	62	3.7
20, 21b	179	11.6

Table 4. Antimicrobial and Cytotoxic Activities of Kenalactams **1** and **3-5**.

Cell lines	IC ₅₀ (μM)				Epothilone B
	1	3	4	5	
Mouse fibroblasts L929	-	32.8	42.2	-	7.5 10 ⁻⁴
HeLa cells KB3.1	-	6.8	5.4	2.4	5.3 10 ⁻⁵
Human breast adenocarcinoma MCF-7	nt	16.4	18.1	nt	7.4 10 ⁻⁵
Human prostate cancer PC-3	nt	6.3	5.0	2.1	2.2 10 ⁻³
Human lung carcinoma A549	nt	16.3	32.1	6.5	4 10 ⁻³
Ovarian carcinoma SKOV-3	nt	14.2	10.0	5.5	2.4 10 ⁻⁴
Microorganisms	MIC (μg/mL)				Oxytetracycline

<i>Staphylococcus aureus</i> Newman	-	66.7	16.7	66.7	0.05
<i>Escherichia coli</i> DSM1116	-	-	-	-	1.66
<i>Bacillus subtilis</i> DSM10	-	-	-	-	2.08
					Nystatin
<i>Mucor hiemalis</i> DSM2656	-	66.7	-	-	4.16
<i>Candida albicans</i> DSM1665	-	66.7	-	-	8.8

-: not active; nt: not tested. Positive control: epothilone B, oxytetracycline, nystatin