Iso-maleimycin, a Constitutional Isomer of Maleimycin, fromStreptomycessp. QL37

Uiterweerd, Michiel T.; Santiago, Isabel Nunez; van der Heul, Helga; van Wezel, Gilles P.; Minnaard, Adriaan J.

Published in:
European Journal of Organic Chemistry

DOI:
10.1002/ejoc.202000767

Publication date:
2020

EurJOC
European Journal of Organic Chemistry

Full Paper
doi.org/10.1002/ejoc.202000767

Natural Products

Iso-maleimycin, a Constitutional Isomer of Maleimycin, from *Streptomyces* sp. QL37

Michiel T. Uiterweerd,[a] Isabel Nuñez Santiago,[b] Helga van der Heul,[b] Gilles P. van Wezel,[b] and Adriaan J. Minnaard*[a]

Abstract: *Iso*-maleimycin, a previously unknown constitutional isomer of the antibiotic maleimycin, has been detected in an extract of *Streptomyces* sp. QL37. Chemical synthesis of both maleimycin (20 % yield over seven steps) and *iso*-maleimycin, (15 % yield over six steps) allowed access to reference materials for identification. Gas Chromatography coupled Mass Spectrometry (GC-MS) analysis demonstrated that of the two isomers, only iso-maleimycin was present in the extract. This finding supports our hypothesis that *iso*-maleimycin is a biosynthetic intermediate of lugdunomycin. *Iso*-maleimycin displays low antibiotic activity, with a Minimum Inhibitory Concentration (MIC) value on both *E. coli* and *B. subtilis* of 250 μg/mL.

Introduction

Bacteria of the actinobacterial genus *Streptomyces* are known to produce a wide range of bioactive natural products, including antibiotics, anticancer compounds, and immunosuppressants.[1] These natural products include a range of maleimide-containing metabolites, of which showdomycin[2] (**1**) and maleimycin[3] (**2**) (Figure 1) are antibiotics produced by *Streptomyces showdoensis*. Maleimycin **2** was discovered independently after the discovery of **1**, as it was detected by further analyzing UV absorption bands of culture extracts.[3] After isolation, the structure of **2** was elucidated mainly by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry.[3] Although **1** and **2** originate from the same organism, feeding experiments with ^{3}H, ^{13}C and ^{14}C-labeled acetate and glutamate revealed that the formation of the maleimide ring in these compounds occurs via two different pathways.[3,4a] More recently, the showdomycin biosynthetic gene cluster (BGC) of *S. showdoensis* ATCC 15227 has been unraveled, by which a more detailed biosynthetic pathway of showdomycin has been proposed.[4b] A detailed biosynthetic pathway of **2** however remains unknown. Naturally occurring derivatives of maleimycin **2** have been isolated from *S. nitrosporeus*.[5] The diastereomeric nitrosporeusine A (**3a**) and nitrosporeusine B (**3b**) consist of a maleimycin moiety linked to a *p*-hydroxybenzoate thioester. Both compounds have also been chemically synthesized, which was accomplished by a thio-Michael addition of *p*-hydroxybenzothioic *S*-acid to synthetic (*S*)-**2**.[6c,6d]

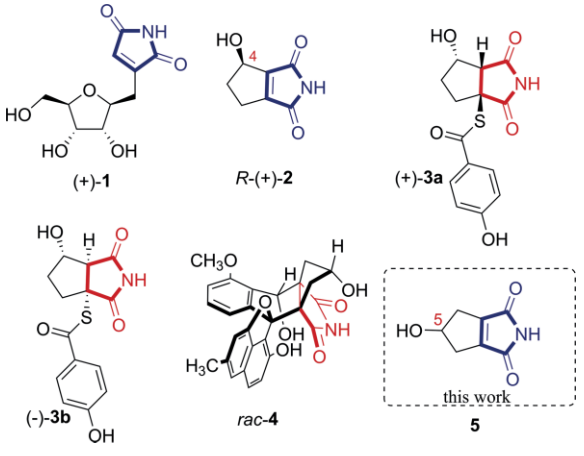


Figure 1. Metabolites from the *Streptomyces* genus containing maleimide (blue) and succinimide moieties (red).

Recently, we reported the isolation and characterization of several previously undescribed angucycline derivatives in the extracts of *Streptomyces* sp. QL37,[7a] an antibiotic-producing actinomycete originating from mountain soil.[7b] Noticeable was the succinimide-containing compound lugdunomycin **4**, an antibiotic with a rare chemical structure derived from the angucycline polyketide backbone. Angucyclines and their derivatives are the most diverse known family of polyketides, many of which have antibacterial and/or anticancer activity.[8a,b] Therefore, these compounds are of great interest for the pharmaceutical industry and for medical applications. During our investigations, a trace compound with the nominal mass of **2** was detected by LC-MS. As inspection of **4** suggested constitutional isomer **5**, rather than **2**, to be involved in its biosynthesis,[7a] we decided to determine the structure of this compound. Prelimi-

[a] M. T. Uiterweerd, Prof. Dr. A. J. Minnaard
Stratingh Institute for Chemistry, University of Groningen
Nijenborgh 7, 9747 AG, Groningen, the Netherlands
E-mail: a.j.minnaard@rug.nl
https://minnaardgroup.nl/
[b] I. N. Santiago, H. van der Heul, Prof. Dr. G. P. van Wezel
Institute of Biology, Leiden University
Sylviusweg 72, 2333 BE, Leiden, the Netherlands
Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under https://doi.org/10.1002/ejoc.202000767.

nary supporting evidence for the presence of **2** or **5** was obtained by additional MS/MS fragmentation experiments and we dubbed the name of **5** to be *iso*-maleimycin.[7a]

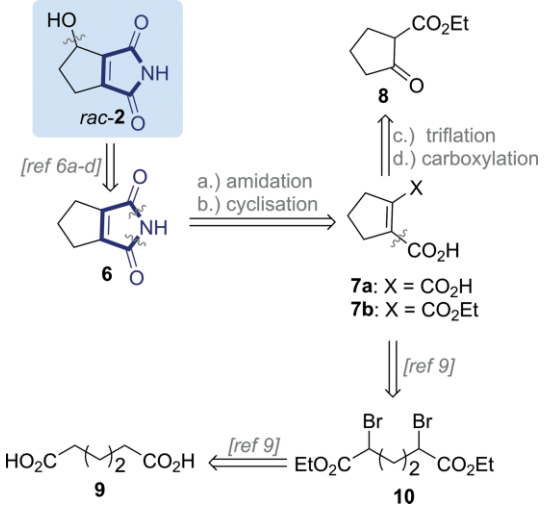
Structurally, the maleimycins **2** and **5** are composed of an uncommon bicyclic 5,6-dihydrocyclopenta[*c*]pyrrole-1,3(2*H*,4*H*)-dione backbone. Maleimycin **2** carries a hydroxy function at the allylic C4 position and is chiral. A total synthesis of racemic **2** by Singh and Weinreb[6a] was reported in 1976, however assignment of the absolute configuration was performed later in synthetic studies by Philkana et al.[6c] in 2015. The configuration turned out to be *R*. In *iso*-maleimycin **5** the hydroxy group is positioned at C5, so as a result **5** is achiral.

As the compound of interest was detected only in small amounts, no attempt was made to isolate it. Instead we embarked on the chemical synthesis of both **2** and **5** to be able to compare and rigorously identify the structure of the natural material.

Results and Discussion

Synthesis of (*rac*)-Maleimycin

In the report by Weinreb and Singh,[6a] **2** was synthesized from **6** by allylic bromination, followed by nucleophilic substitution with silver trifluoroacetate and subsequent hydrolysis of the resulting trifluoroacetate ester (Scheme 1). Alternatively, **2** can be made by direct allylic oxidation with SeO_2 of **6**.[6c,6d] Compound **6** in turn has been prepared starting from cyclopent-1-ene-1,2-dicarboxylic acid (**7a**).[9] Amidation of **7a**, followed by cyclisation would then yield **6**. In turn, **7a** can be obtained by cyclization of dimethyl α,α′-dibromopimelate **10** with sodium hydride in DMF, followed by acidic hydrolysis of the resulting diester (dimethyl cyclopent-1-ene-1,2-dicarboxylate).[9] Compound **10** can be readily obtained by Hell-Vollhardt–Zelinski bromination of pimelic acid.[9] Unfortunately, when we attempted the sodium hydride mediated cyclisation procedure of **10** on a multigram scale, the main product isolated was the isomeric dimethyl cyclopent-2-ene-1,2-dicarboxylate.

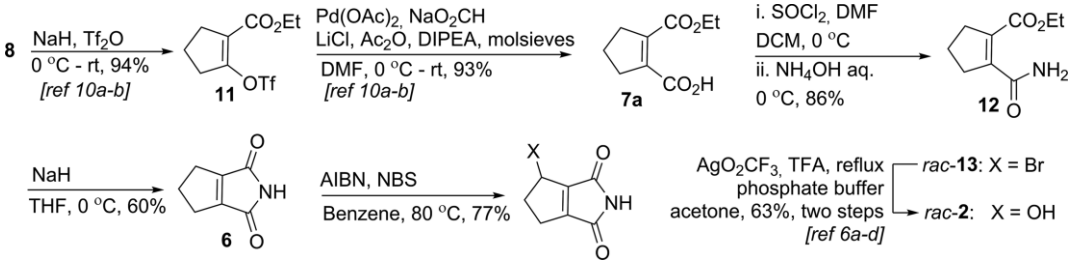


Scheme 1. Retrosynthetic analysis of maleimycin **2**.

Therefore, we sought for alternative strategies to arrive at **7a/b**. In the literature[10a,10b] it has been described that ethyl ester **7b** can be obtained in high yield, in two steps, starting from **8**. These steps include the conversion of **8** into its corresponding enol triflate, which is then transformed into **7b** by means of a palladium catalyzed carboxylation reaction.[10a,10b]

Indeed, commencing the synthesis of compound **2** (Scheme 2), enol triflate **11** was readily prepared from Dieckmann product **8** in 94 % yield, by treatment with sodium hydride and trapping the resulting enolate with Tf_2O.[10a,10b] Enol triflate **11** was subjected to a palladium catalyzed carboxylation, as reported by Yoshimitsu et al.[10a] Treatment of **11** with sodium formate, acetic anhydride and catalytic (5 mol-%) $Pd(OAc)_2$ in the presence of LiCl provided carboxylic acid **7a** in near quantitative yield. As anticipated, **7a** could be converted into amide-ester **12** by exposure of the corresponding acid chloride to concentrated aqueous ammonia under Schotten–Baumann conditions.[11] This procedure gave amide **12** in 86 % yield.

Subsequently, cyclization conditions were investigated in order to arrive at the desired imide **6**. Due to the presence of considerable ring strain in **6**, cyclisation of the imide-precursor requires forcing conditions. Weinreb and Singh[6a] obtained **6** by a two step approach; first converting the acid-amide, derived from compound **7b**, into the corresponding nitrile, and then cyclisation to **6** in refluxing trifluoroacetic anhydride. We aimed for the direct conversion of **12** into **6** instead. It was hypothesized that cyclization would occur after deprotonation of amide **12** with a strong base. Fortunately, when a solution of **12** in THF was added to NaH, it underwent immediate conversion into imide **6**. It was observed by analytical TLC, however, that some polar side-products were formed, presumably caused by degradation or polymerisation of the starting material. Compound **6** was isolated in an appreciable 60 % yield. This result is comparable with the two-step procedure reported previ-



Scheme 2. Synthesis of maleimycin **2**.

Full Paper
doi.org/10.1002/ejoc.202000767

ously.[6a–6d] With **6** in hand we completed the synthesis according to the procedure of Weinreb and Singh[6a] to obtain racemic maleimycin. Maleimycin **2** was prepared in 20 % overall yield in seven steps.

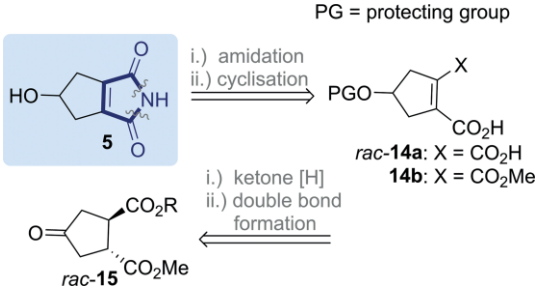
Synthesis of Iso-maleimycin 5

It was envisioned (Scheme 3) that the maleimide unit in **5** could be constructed using the same cyclisation strategy as in the synthesis of **2**. This required the synthesis of **14a** or **14b**, in turn planned to be prepared from commercially available **15**, by reduction of the ketone followed by introduction of the double bond.

The synthesis of *iso*-maleimycin (**5**) (Scheme 4) commenced with the reduction of commercially available *rac*-**15** with $NaBH_4$ in methanol. The resulting crude **16** was directly treated with TBDPSCl and imidazole in DMF,[12] to provide **17** in 77 % yield based on **15**. A key step was the preparation of compound **14b** by the introduction of the double bond. According to literature,[13] **17** was treated with an excess of LDA to form the mono-enolate, which was treated with a stoichiometric amount of iodine in THF at –78 °C to generate the corresponding iodinated intermediate. This intermediate then likely reacted with a second equivalent of LDA providing the corresponding elimination product **14b**, which was isolated in 74 % yield.

The next phase was to access **18**. Based on previous experiments, it was expected that mono-amide **18** could be prepared from mono-acid **14a**, which in turn was envisioned to be obtained from hydrolysis of compound **14b**. It was found that **14b** could be hydrolyzed with potassium hydroxide in a water/THF/MeOH mixture, yielding mono-acid **14a** in 64 % yield. Subsequently **14a** was converted into mono-amide **18**, using the same Schotten–Baumann procedure used in the synthesis of **9**.[11] With this approach, the mono-amide was obtained in 54 % yield, as tarry products were formed in significant amounts. To reduce loss of material throughout the synthesis, an alternative single-step procedure was sought for the conversion of diester **14b** into mono-amide **18**. Weinreb and co-workers[14] had reported that aluminium amide complexes of the type $[AlCl(CH_3)NR_2]$ (R = H or alkyl) can be used to convert esters into their corresponding amide. The reagent $[AlCl(CH_3)NH_2]$ was generated from ammonium chloride and $Al(CH_3)_3$ (as a solution in toluene), to which **14b** was added subsequently. We were delighted to observe that in this way **18** was prepared in 53 % yield directly from **14b**.

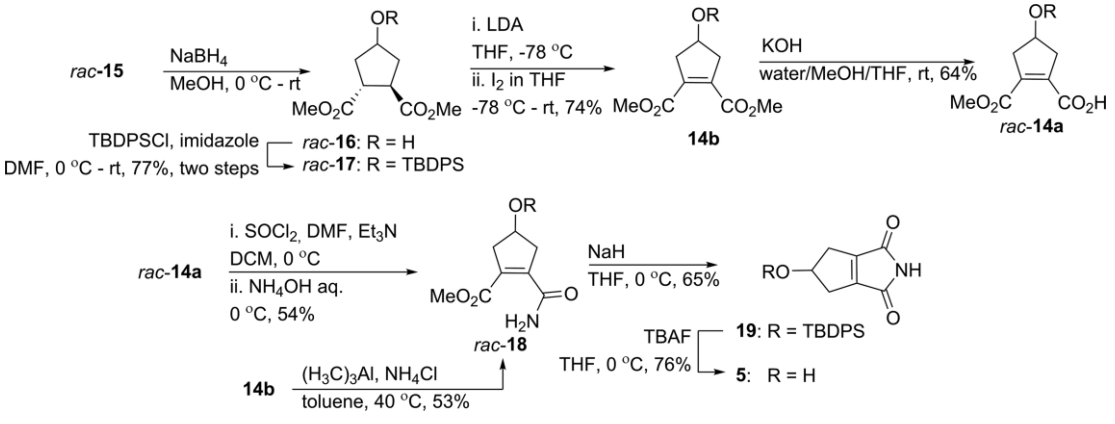
With a sufficient amount of **18** in hand, cyclisation to the corresponding imide **19** was studied. As expected, when **18** was treated with NaH in THF at 0 °C, **19** was obtained in 65 % yield. We occasionally observed that upon addition of the substrate to the sodium hydride suspension, the reaction did not initiate spontaneously. We speculate this is caused by the poor solubility of sodium hydride in THF. The issue could be readily solved by pre-mixing **18** with 1.3 equiv. of 15-crown-5 before adding it as a solution in THF to the sodium hydride, affording the desired product without affecting the yield. It has been proposed that 15-crown-5 acts as a phase transfer reagent aiding solubilization of sodium hydride, thereby increasing its basicity.[15] Due to immediate initiation in presence of 15-crown-5, we anticipate that at larger scale potential runaway reactions can also be prevented. Finally, desilylation of **19** was performed using TBAF in THF,[12] which gave *iso*-maleimycin **5** in 76 % yield. Overall, over the shortest sequence in six steps, compound **5** was obtained in 15 % yield.



Scheme 3. Retrosynthetic analysis of *iso*-maleimycin **5**.

Identification of Iso-maleimycin 5 in Extracts of S. sp QL37, Using GC-MS

Streptomyces sp. QL37 was isolated from soil in the Qinling mountains (P. R. China) and deposited to the collection of the Centraal Bureau voor Schimmelcultures (CBS) in Utrecht, The



Scheme 4. Total synthesis of *iso*-maleimcyin **5**.

Full Paper
doi.org/10.1002/ejoc.202000767

Netherlands, under deposit number 138593.[7a,7b] The extract was prepared as indicated in the experimental section. GC-MS was selected for the analysis due to the low molecular mass and hence relatively volatile nature of **2** and **5**. In addition, structural information can be obtained from the EI fragmentation spectra corresponding to the TIC chromatograms. The analysis was performed by injecting samples of compound **2** and compound **5** (0.23 mg/mL), and also a sample of the extract (1 mg/mL). It was found by comparing the chromatograms and their corresponding EI mass spectra that *S.* sp. QL37 produces iso-maleimycin **5** but not maleimycin **2**. In the TIC chromatogram (Figure 2) of the isolate, based on retention times two signals were identified which potentially corresponded to **2** or **5**. Closer inspection and comparison with synthetic **5** indicated that this compound is present in the extract, which was confirmed by matching fragmentation spectra (Figure 3).

Antibiotic Assays

Since compound **2** is a known antibiotic,[3,6] it was anticipated that compound **5** might exhibit similar properties due to its

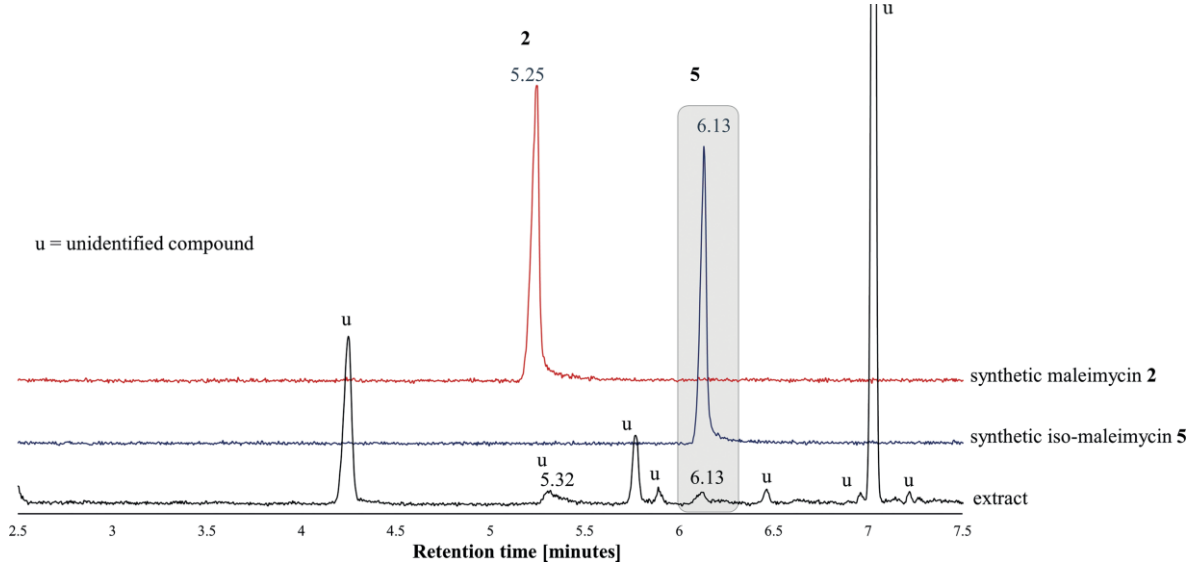


Figure 2. Stacked GC-MS TIC chromatograms of synthetic **2** and **5**, and an extract of *S.* sp. QL37. Apparatus and settings: a Shimadzu GC-2010 gas chromatograph equipped with an HP-1 column, 100 % dimethylsiloxane, (30 m *L* × 0.25 mm Ø × 0.25 µm thickness), temperature program, 150 °C hold for 5 min, then increase 150 °C to 200 °C with 20 °C/min, finally 3 min hold at 200 °C. For the synthetic samples, split ratio: 20.0, for the extract, split ratio: 10.0, carrier gas: He, flow 21.9 mL/min, pressure 82.7 kPa.

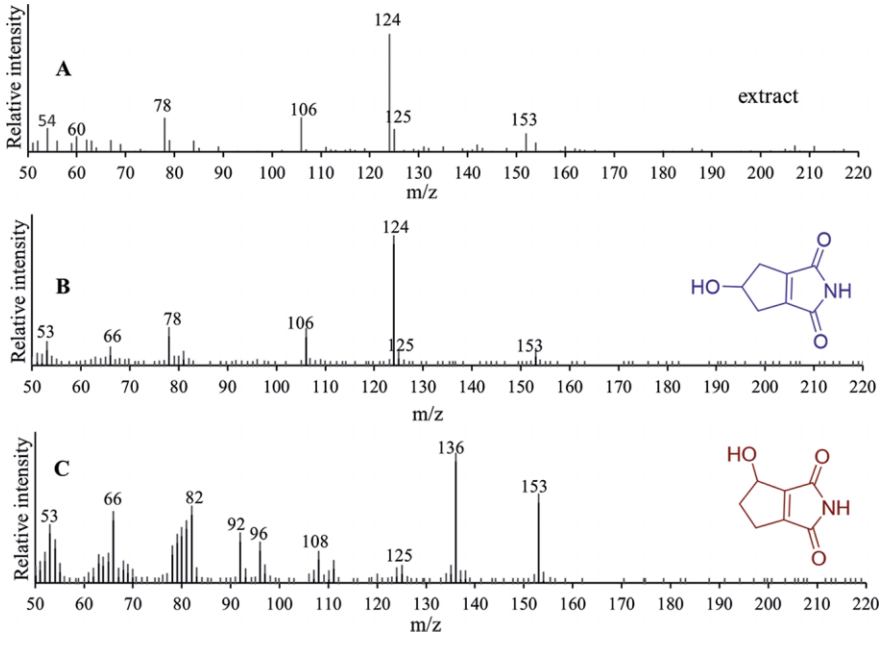


Figure 3. Mass spectra of synthetic **2**, and **5** and a matching signal found in *S.* sp. QL37 for *iso*-maleimycin. [a]Conditions and settings: Shimadzu GC-MS-QP2010 gas chromatograph mass spectrometer, Electron Impact (EI) ionisation, ionization energy 70 eV. **A**. mass spectrum of the suspected peak in the extract of *S.* sp. QL37 showing the presence of compound **5**; **B**. mass spectrum of synthetic **5**; **C**. mass spectrum of synthetic **2**[16].

closely related structure. **5** Was tested for antibiotic activity, and it was found that **5** displayed growth inhibition in a disk diffusion assay (1000 μg/mL) on *Escherichia coli* and *Bacillus subtilis*. Following these results, the Minimum Inhibitory Concentrations (MIC)[17,18] for **5** were determined. The MIC of **5** was found to be 250 μg/mL for *E. coli* ASD219 and 250 μg/mL for *B. subtilis*. MIC values for (*rac*)-**2** were also determined for comparison, and were found to be 31 μg/mL on *E. coli* and 125 μg/mL for *B. subtilis*; indicating that **2** is a more potent antibiotic than **5**.

Conclusion

In conclusion, *iso*-maleimycin **5**, a maleimide-containing metabolite, and a constitutional isomer of maleimycin **2**, has been identified in *Streptomyces* sp. QL37. **5** has been synthesized, affording the product in 15 % overall yield over six steps. Additionally, maleimycin **2** was also synthesized according to a modified synthetic sequence and the compound was obtained in 20 % overall yield over seven steps. Comparison of the synthetic compounds by GC-MS with an extract of *S.* sp. QL37 confirmed unambiguously that *iso*-maleimycin is produced by *S.* sp. QL37. However, the previously described maleimycin **2** was not detected in the extract. Detection of **5** serves as evidence for our hypothesis that it is a biosynthetic precursor of **4**. Currently we are interested in the exact biosynthetic origin of **5**, its relationship with maleimycin, and its role in the biosynthesis of lugdunomycin **4**. Antibiotic assays of **5** showed that it displays weak antibiotic activity towards gram positive *B. subtilis* and gram negative *E. coli* as the MIC values were found to be 250 μg/mL. In these assays *iso*-maleimycin **5** is less potent than maleimycin **2**.

Experimental Section

Synthetic Chemistry Experiments

General: All moisture and oxygen sensitive reactions were executed under a N_2 atmosphere. All reaction solvents were purchased from commercial vendors and used without further purification unless specified otherwise. Reagents were purchased from chemical vendors and used without further treatment or purification, unless stated otherwise. NMR spectra were recorded on an Agilent 400 NMR spectrometer, detected ^{1}H-nuclei at 400 MHz, ^{13}C-nuclei at 101 MHz and ^{19}F-nuclei at 376 MHz. Reported chemical shifts are given in ppm, relative to the residual solvent signal. IR spectroscopic analyses were done using a Perkin-Elmer Spectrum Two UATR FT-IR spectrometer. Analytical TLC plates, provided with a fluorescent marker, were obtained from Merck Chemicals. TLC spots were visualised by means of a UV lamp or appropriate staining reagent. HRMS was executed on a Thermo-Fisher Orbitrap Electron Spray Ionization (ESI) mass spectrometer at positive ionization mode, unless specified otherwise.

Ethyl 2-{[(Trifluoromethyl)sulfonyl]oxy}cyclopent-1-ene-1-carboxylate (11): Prepared according to the procedure by Ralph et al.[10b] by starting from ethyl 2-oxocyclopentane-1-carboxylate **8** (10.0 g, 64.0 mmol). The product was purified by flash column chromatography (Et_2O/pentane, 5:95), affording **11** (17.3 g, 60.0 mmol, 94 % yield) as a colourless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.25 (q, J = 7.1 Hz, 2H), 2.84–2.56 (m, 4H), 2.12–2.89 (m, 2H), 1.30 (t, J = 7.1 Hz, 3H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 162.3, 153.4, 123.4, 118.3 (q, J_{CF} = 320.0 Hz), 61.1, 32.7, 29.2, 18.8, 14.0 ppm. ^{19}F-NMR (376 MHz, $CDCl_3$) δ = –74.6 ppm. ^{1}H-NMR and ^{13}C-NMR data were in agreement with those reported in the literature.[10b]

2-(Ethoxycarbonyl)cyclopent-1-ene-1-carboxylic Acid (7a): Prepared according to the procedure by Ralph et al.[10b] by starting from enol triflate **11** (3.00 g, 10.4 mmol), providing **7a** as a brown oil (1.78 g, 9.65 mmol, 93 % yield) in sufficient purity to be used in the next step. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.33 (q, J = 7.1 Hz, 2H), 2.94–2.81 (m, 4H), 1.87 (tt, J = 8.2, 7.4 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 168.3, 163.6, 148.4, 138.7, 63.1, 36.9, 35.7, 20.2, 13.9 ppm. Physical data were in accordance with those reported in the literature.[10b]

Ethyl 2-Carbamoylcyclopent-1-ene-1-carboxylate (12): According to a modified literature procedure,[11] crude mono-ester **7a** (2.00 g, 10.7 mmol) was dissolved in CH_2Cl_2 (40 mL), and 2 drops of DMF were added. The solution was cooled on ice, $SOCl_2$ (3.2 mL, 5.17 g, 43.4 mmol) was added dropwise via syringe, and the reaction was stirred for 40 min while cooling was maintained. Then the solution was allowed to reach r.t. while it was stirred for 30 min. The remaining solution was carefully added dropwise to ice cold concentrated 14 M NH_4OH_{aq} (100 mL), the resulting biphasic mixture was extracted with EtOAc (3 × 75 mL). The combined organic layers were washed with 1 M HCl_{aq} (1 × 50 mL). The organic layers were dried with $MgSO_4$ and concentrated by means of rotary evaporation to provide the product as a grey crystalline solid, (1.70 g, 9.25 mmol, 86 % yield) which was of sufficient purity to be used in the next step. Mp 72–74 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.44 (bs, 1H, N*H*), 5.88 (bs, 1H, N*H*), 4.23 (q, J = 7.1 Hz, 2H), 2.89–278 (m, 4H), 1.84 (quint., J = 7.7 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 166.44, 166.41, 147.8, 135.4, 61.4, 36.8, 36.2, 20.7, 14.0 ppm. HRMS calcd. for $C_9H_{13}NO_3$ $[M + H]^+$: 184.0968, found 184.0969.

5,6-Dihydrocyclopenta[*c*]pyrrole-1,3(2H,4*H*)-dione (6): A round-bottomed flask was equipped with a stir bar and NaH 60 % suspension in mineral oil (482 mg, 12.1 mmol) which was rinsed with pentane three times. A solution of **12** (1.70 g, 9.28 mmol) in 44 mL of THF was added dropwise via syringe, while cooling the reaction mixture on ice. After 15 min, when H_2 evolution had ceased, the reaction mixture was quenched by adding acetic acid (0.7 mL). The mixture was diluted with water and extracted with EtOAc (3 × 50 mL), the combined organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product was purified by means of column chromatography (EtOAc/pentane, 25:85), to give the pure imide as a white crystalline solid (765 mg, 5.58 mmol, 60 % yield). Alternatively, an analytically pure sample was obtained by means of recrystallization from $CHCl_3$/pentane (50:50) and cooling to –78 °C, in comparable yield and purity. Mp 184–186 °C*; ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.31 (br s, 1H, NH), 2.65 (t, J = 7.2 Hz, 4H), 2.52–238 (m, 2H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 167.0, 154.6, 27.5, 26.4 ppm. *The previously reported melting point for this compound is 177–179 °C. ^{1}H-NMR data are in agreement with those reported in the literature.[6a]

(*R*/*S*)-4-Hydroxy-5,6-dihydrocyclopenta[*c*]pyrrole-1,3(2H,4*H*)-dione, (*rac*)-maleimycin (2): Imide **6** (763 mg, 5.56 mmol), NBS (1.09 g, 6.12 mmol) and AIBN (137 mg, 0.84 mmol, 15 mol-%) were suspended in benzene (22 mL), and the mixture was heated to 80 °C followed by stirring for 1 h. The reaction mixture was diluted with water (100 mL), extracted with EtOAc (5 × 50 mL), the combined organic layers were dried with $MgSO_4$ and concentrated to provide the crude allylic bromide as a brown oil which rapidly crystallised upon standing. The crude material was loaded onto silica

and purified by flash column chromatography (EtOAc/pentane, 10:90 and 30:70) which provided a white crystalline solid (921 mg, 4.26 mmol, 77 % yield) which was immediately used in the next step. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.05 (bs, 1H, NH), 5.14 (dt, J = 7.2, 1.8 Hz, 1H), 3.08 (td, J = 14.9, 6.9 Hz, 1H), 2.93 (dtd, J = 18.6, 7.0, 2.5 Hz, 1H), 2.80 (ddt, J = 14.7, 7.2, 1.3 Hz, 1H), 2.70 (ddd, J = 18.6, 8.3, 1.7 Hz, 1H) ppm. Following the procedure by Weinreb and Singh;[6a] the reaction of allylic bromide (920 mg, 4.26 mmol) and silver trifluoroacetate (1.13 g, 5.11 mmol) provided the crude ester (922 mg), which was obtained as a pale orange oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.33 (bs, 1H, NH), 6.19 (dt, J = 7.7, 2.5 Hz, 1H), 2.82–272 (m, 2H), 2.55–245 (m, 1H) ppm. Subsequently, the trifluoroacetate ester (922 mg) was hydrolysed, and purified by means of column chromatography (EtOAc/pentane, 30:70 and 50:50), which afforded *rac*-maleimycin **2** (409 mg, 2.67 mmol, 63 % yield, based on the bromide), as a white crystalline solid. Mp 109–112 °C; ^{1}H-NMR (400 MHz, MeOD) δ = 5.09–5.01 (m, 1H), 2.84–2.64 (m, 2H), 2.57–2.45 (m, 1H), 2.26–2.13 (m, 1H) ppm. ^{13}C-NMR (101 MHz, MeOD) δ = 168.1, 167.5, 156.4, 153.1, 69.7, 38.2, 23.6 ppm. HRMS (negative mode) calcd. for $C_7H_7NO_3$ $[M - H]^-$: 152.0353, found 152.0356. Physical data of maleimycin were in agreement with those reported in the literature.[6a–6d]

Dimethyl (1S,2S)-4-[(*tert*-Butyldiphenylsilyl)oxy]cyclopentane-1,2-dicarboxylate (17): To a solution of racemic (*trans*)-dimethyl 4-oxocyclopentane-1,2-dicarboxylate **15** (2.00 g, 9.99 mmol) in MeOH (40 mL) was added $NaBH_4$ (189 mg, 5.00 mmol), while cooling on ice. The solution was left stirring at 0 °C for 20 min and quenched with 1 M HCl_{aq} (30 mL) followed by dilution with water (40 mL). The solution was extracted with EtOAc (3 × 40 mL) and the combined organic layers were dried with $MgSO_4$, concentration by rotary evaporation provided the crude alcohol **16** as a colourless oil (1.70 g) in sufficient purity to be used in the next step. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.41 (tt, J = 4.6, 2.3 Hz, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.43 (dt, J = 10.0, 7.7 Hz, 1H), 3.25 (ddd, J = 10.0, 7.0, 4.8 Hz, 1H), 2.25 (ddd, J = 14.6, 10.0, 5.0 Hz, 1H), 2.15 (ddt, J = 13.3, 8.3, 2.2Hz, 1H), 2.05–1.92 (m, 2H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 176.1, 175.2, 72.9, 52.4, 52.2, 45.3, 45.2, 39.9, 38.6 ppm. HRMS calcd. for $C_9H_{14}O_5$ $[M + Na]^+$: 225.0734, found 225.0734. Crude **16** (1.70 g, 8.41 mmol) and imidazole (1.15 g, 16.8 mmol) were dissolved in DMF (34 mL), the solution was cooled in an ice bath. TBDPSCl (2.4 mL, 2.50 g, 9.01 mmol) was added dropwise via syringe, after which it was allowed to reach r.t. and left stirring overnight. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (4 × 50 mL), dried with $MgSO_4$ and concentrated by means of rotary evaporation. The crude material was purified by flash column chromatography (Et_2O/pentane, gradient 10:90 and 20:80), to afford **17** (3.38 g, 7.67 mmol, 77 % yield, over two steps) as a colourless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.66–7.59 (m, 4H), 7.47–7.33 (m, 6H), 4.31 (pent., J = 4.3 Hz, 1H), 3.71 (s, 3H), 3.67 (s, 3H), 3.61 (dt, J = 9.8, 8.0 Hz, 1H), 3.18–305 (m, 1H), 2.17–1.96 (m, 3H), 1.75 (ddd, J = 13.2, 8.9, 5.1 Hz, 1H), 1.03 (s, 9H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 175.6, 174.7, 135.73, 135.69, 133.9, 133.8, 129.7, 127.7, 127.6, 74.0, 52.1, 52.0, 45.0, 44.3, 39.7, 39.1, 26.7, 19.4 ppm. HRMS calcd. for $C_{25}H_{32}O_5Si$ $[M + Na]^+$: 463.1911, found 463.1911.

Dimethyl 4-[(*tert*-Butyldiphenylsilyl)oxy]cyclopent-1-ene-1,2-dicarboxylate (14b): According to a modified literature procedure,[13] LDA was generated by adding *n*BuLi (1.6 M solution in hexanes, 7.6 mL, 12.1 mmol) to a solution of *N*,*N*-diisopropylamine* (1.8 mL, 12.8 mmol) in dry THF (10 mL), at –78 °C. Compound **17** (2.35 g, 5.34 mmol) was dissolved in dry THF (25 mL) and added to the reaction mixture dropwise. To the resulting bright yellow solution was added a solution of iodine (1.42 g, 5.61 mmol) in THF (31 mL). Subsequently, the purple-brown solution was allowed to reach r.t. and carefully quenched by adding 1 M HCl_{aq} (50 mL). The mixture was extracted with EtOAc (3 × 50 mL), the combined organic layers were washed with brine (50 mL), dried with $MgSO_4$ and concentrated by means of rotary evaporation. The concentrate was purified by column chromatography (EtOAc/pentane, gradient 10:90 and 20:80), to afford **14b** as a viscous pale yellow syrup (1.73 g, 3.94 mmol, 74 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.67–7.61 (m, 4H), 7.46–7.34 (m, 6H), 4.55 (tt, J = 6.4, 4.4 Hz, 1H), 3.76 (s, 6H), 2.87–2.69 (m, 4H), 1.05 (s, 9H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 165.6, 137.4, 135.7, 133.7, 129.8, 127.7, 71.5, 52.1, 44.1, 26.8, 19.0 ppm. HRMS calcd. for $C_{25}H_{30}O_5Si$ $[M + Na]^+$: 439.1935, found 439.1933. **N*,*N*-diisopropylamine was distilled from KOH pellets before use.

4-[(*tert*-Butyldiphenylsilyl)oxy]-2-(methoxycarbonyl)cyclopent-ene-1-carboxylic Acid (14a): Compound **14b** was dissolved in a mixture of THF/MeOH (83:17, 66 mL), and a solution of KOH (603 mg, 10.7 mmol) in water (11 mL) was added. The mixture was stirred at r.t. for 6 h, subsequently acidified with 1 M HCl until pH ≈ 1, and extracted with EtOAc (4 × 100 mL). The combined organic layers were dried with $MgSO_4$ and concentrated by means of rotary evaporation. The crude product was purified by column chromatography (pentane/EtOAc/AcOH, gradient 90:9:1, 80:19:1 and 70:29:1) to give **14a** (978 mg, 2.30 mmol, 64 % yield) as an off-white crystalline solid. Mp 109–117 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.68–7.58 (m, 4H), 7.84–733 (m, 6H), 4.44 (tt, J = 5.6, 4.0 Hz, 1H), 3.89 (s, 3H), 3.09–2.98 (m, 2H), 2.89–2.88 (m, 2H), 1.04 (s, 9H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 168.4, 163.1, 146.5, 135.63, 135.62, 135.55, 133.7, 133.4, 129.9, 129.8, 127.8, 127.7, 69.0, 53.7, 46.6, 45.3, 26.8, 19.0 ppm. HRMS calcd. for $C_{24}H_{28}O_5Si$ $[M + Na]^+$: 447.1598, found 447.1591.

Methyl 4-[(*tert*-Butyldiphenylsilyl)oxy]-2-carbamoylcyclopent –1-ene-1-carboxylate (18)

*Method 1, direct conversion of diester **14b**.* According to a modified procedure,[14] ammonium chloride (331 mg, 6.19 mmol) was suspended in toluene (10 mL). While cooling the suspension in an ice bath, 2 M trimethylaluminium (solution in hexanes, 3.1 mL, 6.19 mmol) was added dropwise, after which evolution of methane was observed. The ice bath was removed and the reaction mixture was stirred at r.t. for 3 h. A solution of **14b** (905 mg, 2.06 mmol) in toluene (10 mL) was added dropwise to the reaction mixture, subsequently the mixture was heated to 50 °C and stirred for 18 h. The reaction mixture was cooled in an ice bath and quenched with 1 M HCl_{aq} (15 mL) and stirred for 15 min. The resulting clear biphasic mixture was extracted with EtOAc (5 × 15 mL), the combined organic layers were dried with $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography, (pentane/EtOAc, 60:40) affording **18** (467 mg, 1.10 mmol, 53 % yield) as a pale brown crystalline solid.

*Method 2, from acid-ester **14a**.* Following a modified literature procedure,[11] to a solution of **14a** (785 mg, 1.85 mmol) in CH_2Cl_2 (7.4 mL) were added Et_3N (1.0 mL, 748 mg, 7.40 mmol) and DMF (0.1 mL). The mixture was cooled in an ice bath, and thionyl chloride (0.5 mL, 885 mg, 7.45 mmol) was added dropwise, followed by stirring for 15 min. The reaction mixture was added dropwise to an ice-cold solution of concentrated 14 M NH_4OH_{aq} (100 mL) under vigorous stirring, and the resulting turbid mixture was extracted with EtOAc (3 × 50 mL), the combined organic layers were washed with brine (50 mL), dried with $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (pentane/EtOAc, 60:40) afforded **18** (420 mg, 0.99 mmol, 54 % yield) as a pale brown crystalline solid. Mp 119–128 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.62 (bs, 1H), 7.73–

7.59 (m, 4H), 7.51–7.30 (m, 6H), 5.91 (bs, 1H), 4.43 (pent., J = 5.1 Hz, 1H), 3.77 (s, 3H), 3.03–2.93 (m, 2H), 2.93–2.82 (m, 2H), 1.05 (s, 9H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 166.4, 165.7, 145.9, 135.65, 135.64, 134.0, 133.7, 132.2, 129.8, 129.7, 127.72, 127.67, 69.4, 52.4, 46.4, 45.6, 26.8, 19.1 ppm. HRMS calcd. for $C_{24}H_{29}NO_4Si$ $[M + H]^+$: 424.1939, found 424.1920.

5-[(*tert*-Butyldiphenylsilyl)oxy]-5,6-dihydrocyclopenta[*c*]pyrrole-1,3(2*H*,4*H*)-dione (19): A round bottomed flask was equipped with a stir bar and NaH 60 % suspension in mineral oil (55 mg, 1.65 mmol) which was rinsed with pentane three times. A solution of **18**[*] (467 mg, 0.551 mmol) in THF (5.5 mL) was added dropwise via syringe, while cooling the reaction mixture on ice. After 15 min, when H_2 evolution had ceased, the reaction mixture was quenched by adding acetic acid (0.1 mL). The mixture was diluted with water and extracted with EtOAc (4 × 15 mL), the combined organic layers were dried with $MgSO_4$ and concentrated in vacuo. Purification by means of column chromatography (pentane/ether, 80:20) gave **19** (141 mg, 0.348 mmol, 65 % yield) as a colourless amorphous glass. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.67–7.62 (m, 4H), 7.51–7.36 (m, 6H), 7.02 (br s, 1H, N*H*), 4.93 (tt, J = 6.8, 3.9 Hz, 1H), 2.83–2.72 (m, 2H), 2.71–2.62 (m, 2H), 1.07 (s, 9H) ppm. ^{13}C-NMR (101 MHz, MeOD) δ = 166.7, 151.2, 135.6, 133.2, 130.0, 127.9, 77.2, 37.1, 26.8, 19.0 ppm. HRMS (negative mode) calcd. for $C_{23}H_{25}NO_3Si$ $[M - H]^-$: 390.1531, found 390.1530. [*]With this substrate it was occasionally observed that the reaction did not initiate spontaneously. The addition of 1.3 equiv. of 15-crown-5 to the solution of the substrate prior before adding it to the sodium hydride solved this problem, without affecting the yield. Addition of 15-crown-5 potentially also prevents runaway reactions when the procedure is performed at larger scales.

5-Hydroxy-5,6-dihydrocyclopenta[*c*]pyrrole-1,3(2H,4*H*)-dione (5): Compound **19** (140 mg, 0.385 mmol) was dissolved in THF, and Bu_4NF solution in THF (1 M, 0.72 mL, 0.72 mmol) was added dropwise. The solution was stirred for one hour at r.t. Then it was quenched by adding saturated NH_4Cl_{aq} (10 mL) and the mixture was extracted with EtOAc (6 × 20 mL). The combined organic layers were dried with $MgSO_4$ and concentrated in vacuo. The crude was dry-loaded onto silica and placed on a short silica plug, which was eluted with solvent (pentane/ether, 80:20, 250 mL), this fraction was discarded. Then the plug was subsequently flushed with a second portion of solvent (pentane/EtOAc, 50:50, 400 mL), concentration of this fraction afforded the *iso*-maleimycin **5** (41.5 mg, 0.721 mmol, 76 % yield) as a white crystalline solid. Mp 94–101 °C, ^{1}H-NMR (400 MHz, MeOD) δ = 4.90 (tt, J = 6.4, 2.6 Hz, 1H), 2.99–2.88 (m[*], 2H), 2.54–2.43 (m[*], 2H) ppm. ^{13}C-NMR (101 MHz, MeOD) δ = 168.4, 1531.3, 75.2, 35.9 ppm. HRMS (negative mode) calcd. for $C_7H_7NO_3$ $[M - H]^-$: 152.0353, found 152.0355. [*]These signals appear to form a pair of distorted double doublets. However, due to long range couplings, the true multiplicity cannot be determined.

Identification of Iso-maleimycin from S sp. QL37

Preparation of the Isolate: *Streptomyces* sp. QL37 was cultivated on minimal media agar plates with 1 % glycerol and 0.5 % mannitol (w/v) as the carbon sources. After seven days of growth at 30 °C, the plates were cut into small blocks and the resultant agar pieces were extracted with EtOAc (25 mL) by soaking overnight at room temperature, the supernatant was removed and the extraction procedure was repeated two more times. The combined organic layers were evaporated at room temperature. Subsequently the dried extract was re-dissolved in ethyl acetate and transferred to a new preweighed vial. The extract was evaporated under reduced pressure at 30 °C to determine the amount of extracted compounds.

GC-MS Analysis: For analysis and identification, synthetic samples of maleimycin and iso-maleimycin (0.23 mg/mL), along with a sample of the extract (1 mg/mL) were injected on a Shimadzu GC-2010 gas chromatograph coupled to a Shimadzu GC-MS-QP2010 gas chromatograph mass spectrometer. For the synthetic samples, split ratio 20.0, for the extract, split ratio 10.0. The injection temperature was 200 °C, detector temperature 250 °C. Separation was carried out on an HP-1 column, 100 % dimethylsiloxane, (30 m L × 0.25 mm Ø × 0.25 μm thickness). The temperature program was set as follows: 150 °C hold for 5 min, then increase 150 °C to 200 °C with 20 °C/min, finally 3 minutes hold at 200 °C. The carrier gas was He, with a column flow of 0.9 mL/min, pressure 82.7 kPa. Ionization by means of EI, ionization energy 70 eV, mass range from m/z 50–225. The identification was done by comparing retention times and mass fragmentation patterns of the synthetic standards with those obtained from the extracts.

Biological Assays

Disc Diffusion Assay with Soft Agar Overlay: The antimicrobial properties of *iso*-maleimycin were determined by disc diffusion assay. Briefly, three colonies of the indicator strains, *Bacillus subtilis* and *Escherichia coli* ASD19, were picked from an agar plate for the inoculation of an overnight culture. Afterwards, 300 μL of the overnight culture were added in 10 mL of fresh LB broth and incubated at 37 °C until a OD_{600} of 0.3. Then, a LB plate was overlaid with LB soft agar (0.75 % w/v agar) containing 1.5 mL from one of the indicator strains pre-grown in liquid LB to OD_{600} of 0.3. When solidified, antibiogram discs loaded with 10 μL of 1 mg/mL of iso-maleimycin were applied on it and incubated overnight at 37 °C. Ampicillin 1 μg/mL was used as a control.

MIC Tests: The minimum inhibitory concentration (MIC) test was determined by the broth microdilution method using the British Standard BS EN ISO 20776–1:2006.[17,18] A stock solution of iso-maleimycin **5** was made by dissolving it in Mueller-Hinton (MH) broth to a concentration of 4 mg/mL. The bacterial indicator strains (*Escherichia coli* ASD 19 and *Bacillus subtilis*) were grown for approximately two hours from an overnight culture until an OD_{600} of 0.3 in MH broth and diluted until a concentration of bacteria of 1×10^6 CFU mL^{-1} in fresh broth. 50 μL of MH broth were added to all the wells of the 96-well polypropylene microtiter plates. Then, 50 μL of the sample stock was added to the first row to the concentration of 2000 μg/mL, which was serially twofold diluted. Subsequently, 50 μL of the indicator strains were added to the wells resulting in a range of antibiotic concentration from 1000 μ/mL to 7 μg/mL. Three replicates were performed for each indicator bacterial strain. Growth control wells containing 100 μL of 5×10^5 CFU mL^{-1} were included without test compounds. After overnight incubation at 37 °C, inhibition was defined as no visible growth compared to the growth observed in the control wells. For *iso*-maleimycin results were determined as 250 μg/mL for *E. coli* ASD19 and 250 μg/mL for *B. subtilis*. As comparison, for maleimycin the results were determined as 31 μg/mL for *E. Coli* ASD19 and 125 μg/mL for *B. Subtilis*.

Acknowledgments

The authors thank Mrs T. Tiemersma-Wegman and Mr R. J. L. Sneep (University of Groningen) for HRMS analyses. We thank the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) for financial support.

Keywords: Maleimycin · Streptomyces sp. · Actinomycetes · Antibiotics · GC-MS

[1] E. A. Barka, P. Vatsa, L. Sanchez, N. Gaveau-Vaillant, C. Jacquard, H. P. Klenk, C. Clément, Y. Ouhdouch, G. P. van Wezel, *Microbiol. Mol. Biol. Rev.* **2016**, *80*, 1–43.
[2] H. Nishimura, M. Mayama, Y. Komatsu, H. Kato, N. Shimaoka, Y. Tanaka, *J. Antibiot.* **1964**, *17A*, 148–155.
[3] E. F. Elstner, D. M. Carnes, R. J. Suhadolnik, G. P. Kreishman, M. P. Schweizer, R. K. Robins, *Biochemistry* **1973**, *12*, 4992–4997.
[4] a) E. F. Elstner, R. J. Suhodalnik, *Biochemistry* **1971**, *10*, 3608–3614; b) K. Palmu, P. Rosenqvist, K. Thapa, Y. Ilina, V. Siitonen, K. Baral, J. Mäkinen, G. Belogurov, P. Virta, J. Niemi, M. Metsä-Ketelä, *ACS Chem. Biol.* **2017**, *12*, 1472–1477.
[5] A. Yang, L. Si, Z. Shi, L. Tian, D. Liu, D. Zhou, P. Proksch, W. Lin, *Org. Lett.* **2013**, *15*, 5366–5369.
[6] a) P. Singh, S. M. Weinreb, *Tetrahedron* **1976**, *32*, 2379–2380; b) C. J. Lee, B. P. Mundy, *Synth. Commun.* **1992**, *22*, 803–808; c) S. C. Philkhana, R. J. Jachak, V. B. Gunjal, N. M. Dhage, A. H. Bansode, D. S. Reddy, *Tetrahedron Lett.* **2015**, *56*, 1252–1254; d) S. C. Philkhana, A. K. Verma, R. J. Jachak, B. Hazra, A. Basu, D. S. Reddy, *Eur. J. Med. Chem.* **2017**, *135*, 89–109.
[7] a) C. Wu, H. U. van der Heul, A. V. Melnik, J. Lübben, P. C. Dorrestein, A. J. Minnaard, J. H. Choi, G. P. van Wezel, *Angew. Chem. Int. Ed.* **2019**, *58*, 2809–2814; *Angew. Chem.* **2019**, *131*, 2835; b) H. Zhu, J. Swierstra, C. Wu, W. van Wamel, S. K. Sandiford, G. P. van Wezel, *Microbiology* **2014**, *160*, 1714–1726.
[8] a) M. K. Kharel, P. Pahari, M. D. Shepherd, N. Tibrewal, S. E. Nybo, K. A. Shaaban, J. Rohr, *Nat. Prod. Rep.* **2012**, *29*, 264–325; b) J. Rohr, R. Thiericke, *Nat. Prod. Rep.* **1992**, *9*, 103–137.
[9] R. N. McDonald, R. R. Reitz, *J. Org. Chem.* **1972**, *37*, 2418–2423.
[10] a) T. Yoshimitsu, Y. Arano, T. Kaji, T. Ino, H. Nagaota, T. Tanaka, *Heterocycles* **2009**, *77*, 179–189; b) M. J. Ralph, D. C. Harrowven, S. Gaulier, S. Ng, K. I. Booker-Milburn, *Angew. Chem. Int. Ed.* **2015**, *54*, 1527–1531; *Angew. Chem.* **2015**, *127*, 1547.
[11] J. Richers, M. Heilmann, M. Drees, K. Tiefenbacher, *Org. Lett.* **2016**, *18*, 6472–6475.
[12] T. W. Greene, P. G. M. Wuts, in *Protective Groups in Organic Synthesis*, 3rd ed., John Wiley & sons, New York, **1999**, pp 141–143, and references cited therein.
[13] D. Wilkening, B. P. Mundy, *Synth. Commun.* **1984**, *14*, 227–238.
[14] J. L. Levin, E. Turios, S. M. Weinreb, *Synth. Commun.* **1982**, *12*, 989–993.
[15] E. A. Taggi, H. Wack, A. M. Hafez, S. France, T. Lectka, *Org. Lett.* **2002**, *4*, 627–629.
[16] D. Ghiringhelli, A. Grifinni, P. Traldi, *Biomed. Mass Spectrom.* **1981**, *8*, 155–159.
[17] J. M. Andrews, J. Antimicrob. Chemother. **2001**, *48*, suppl, S1, 5–16.
[18] British Standard BS EN ISO 20776–1:**2006**.7

Received: May 31, 2020