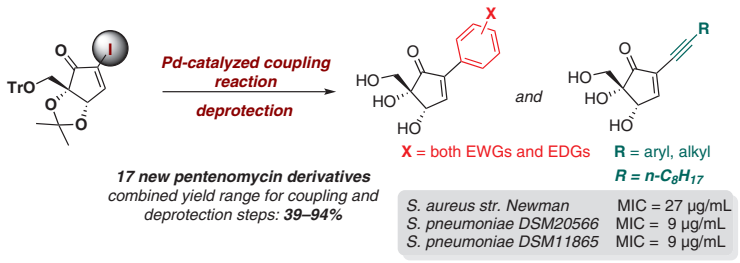
Synlett S. A. Zisopoulou et al. Letter

Synthesis and Biological Evaluation of Novel 2-Substituted Analogues of (–)-Pentenomycin I

Stavroula A. Zisopoulou[a]
Spyridon Bousis[b,c,d]
Jörg Haupenthal[b]
Jennifer Herrmann[e]
Rolf Müller[d,e]
Anna K. H. Hirsch[*b,c,d]
Dimitri Komiotis[f]
John K. Gallos[a]
Christos I. Stathakis[*a]

[a] Department of Chemistry, Aristotle University of Thessaloniki, University Campus, 541 24, Thessaloniki, Greece
cstathakis@chem.auth.gr
[b] Department for Drug Design and Optimization, Helmholtz Institute for Pharmaceutical Research (HIPS) – Helmholtz Centre for Infection Research (HZI), 66123, Saarbrücken, Germany
Anna.Hirsch@helmholtz-hips.de
[c] Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands
[d] Department of Pharmacy, Saarland University, Campus Building E8.1, 66123 Saarbrücken, Germany
[e] Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research (HIPS) – Helmholtz Centre for Infection Research (HZI), Campus Building E8.1, Saarbrücken, Germany
[f] Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, 41500, Larissa, Greece



Published as part of the *Special Section 11th EuCheMS Organic Division Young Investigator Workshop*

Received: 31.10.2019
Accepted after revision: 27.11.2019
Published online: 02.01.2020
DOI: 10.1055/s-0039-1690772; Art ID: st-2019-b0592-l

Abstract A library of novel 2-substituted derivatives of the antibiotic natural product pentenomycin I is presented. The new collection of analogues is divided in two main classes, 2-alkynyl- and 2-aryl- derivatives, which are accessed by the appropriate type of palladium-catalyzed cross-coupling reaction of the 2-iodo-protected pentenomycin I with suitable nucleophiles. The new derivatives were tested for their activity against certain types of bacteria and one of them, compound **8h**, was found to exhibit significant inhibitory activity against several Gram-positive bacteria but also displayed cytotoxic activity against eukaryotic cell lines.

Key words (–)-pentenomycin, Sonogashira, Suzuki, antimicrobial agents, Gram-positive bacteria

(–)-Pentenomycin I (**1a**) was first isolated from the culture broth of *Streptomyces eurythermus* MCRL 0738, by Umino and co-workers in 1973 (Figure 1).[1] The said compound is a principal member of a broader family of cyclopentenoid antibiotics, which possess moderate activity against Gram-positive and Gram-negative bacteria.[1a,2] Over the past few years, we[3] and others[4] have demonstrated the potential of 2-halogenated pentenomycin as suitable precursor for derivatization, thus leading to new cyclopentenones with potentially improved biological profile.

Herein, we report a systematic effort to synthesize a series of analogues of the natural antibiotic, covering a broad range of stereochemical demand and introducing a variety of functional groups. In the context of our research on the development of new methodologies to access chiral cyclopentenones from sugar-derived synthons, we have described the synthesis of (–)-pentenomycin I through an oxi-

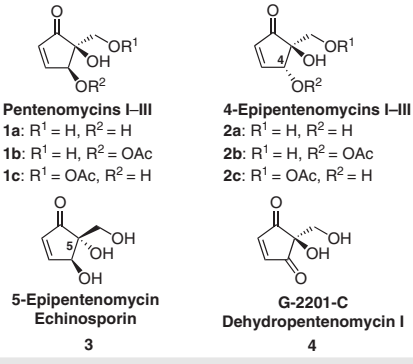
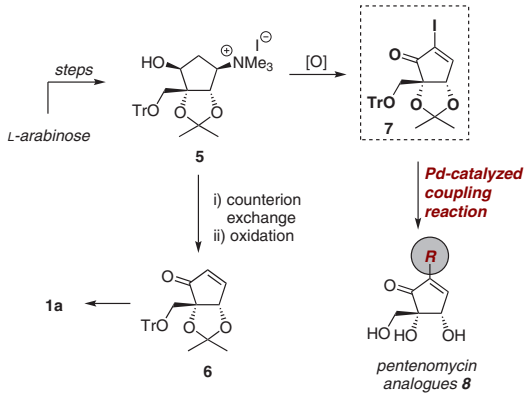


Figure 1 Representatives of the pentenomycin family

Dr. Christos Stathakis was born in Sparta, Greece and studied chemistry at Aristotle University of Thessaloniki. At the same university he received his doctoral diploma in organic chemistry in 2007, under the guidance of Prof. Gallos. In 2008 he joined the research group of Prof. Snyder at Columbia University in the city of New York, where he explored the exciting chemistry of polyphenol natural products. At the end of 2009 he returned to Athens, Greece to join an ongoing research program on new aminoglycoside antibiotics supervised by Dr. Vourloumis at the National Center of Natural Sciences. Dr. Stathakis completed his postdoctoral studies in the research team of Prof. Paul Knochel at Ludwig Maximillian University in Munich (2011–2012), where he gained valuable experience in organometallic chemistry. For the next five years (2013–2018) Dr Stathakis worked as senior research scientist at Pharmathen Pharmaceuticals, where he was involved in the development of novel scalable routes of the synthesis for several active pharmaceutical ingredients (APIs). Since July 2018, he is an assistant professor at the Chemistry Department of Aristotle University of Thessaloniki. The main research efforts of his group focus on the total synthesis of constructively challenging molecules and the development of novel methodologies that promote structural complexity.

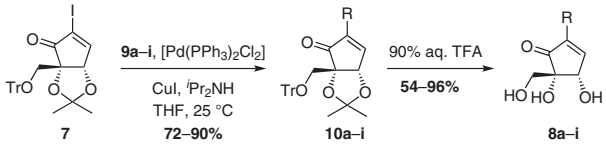
dative elimination process on suitable ammonium salts (Scheme 1, sequence **5** → **6** → **1a**).[5] Unexpectedly, we observed that when iodide was used as the counterion in the ammonium salt **5**, the respective 2-iodo-protected pentenomycin **7** was afforded. This 'undesirable' side product perfectly served the purposes of our synthetic plan to prepare a library of derivatives of the natural antibiotic in order to improve its moderate biological activity. In doing so, palladium-catalyzed cross-coupling reactions were thought to be the most suitable means, a fact supported by the work of Negishi and Johnson on related cross-coupling reactions of 2-iodo-cycloalkenones.[4]



Scheme 1 Precursor and general scheme of derivatization

The first class of derivatives we envisaged was the 2-alkynyl-substituted pentenomycins accessible *via* the well-established Sonogashira reaction.[6] Indeed, under typical reaction conditions,[7] the coupling of 2-iodopentenomycin **7** with various terminal alkynes **9a–i** (see general reaction scheme in Table 1), in the presence of CuI and catalytic amounts of $[Pd(PPh_3)_2Cl_2]$, delivered the desired products in good to excellent yield. After removal of both protecting groups, in one operation, under strongly acidic conditions the final 2-alkynyl-pentenomycins **8a–i** were obtained.

Table 1 Coupling Reactions of 2-Iodopentenomycin **7** with Alkynes **9a–i**[a] and Subsequent Deprotection to Pentenomycin Derivatives **8a–i**[b]



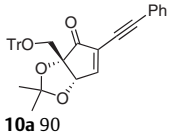
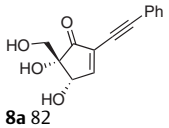
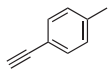
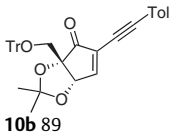
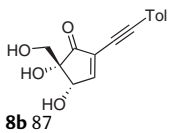
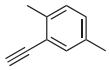
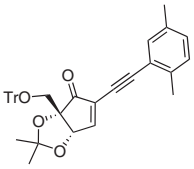
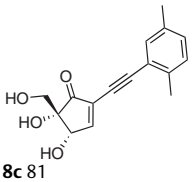
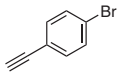
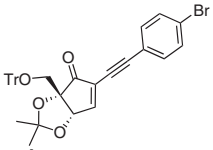
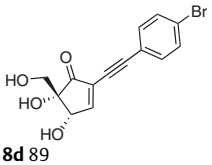
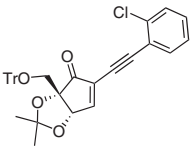
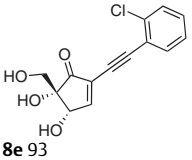
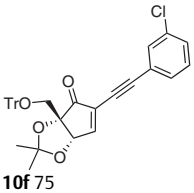
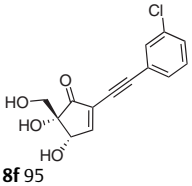
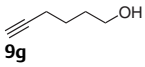
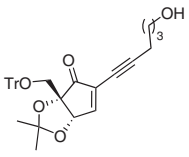
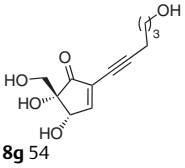
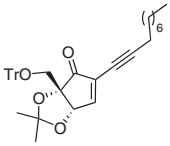
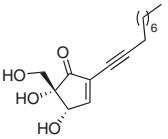
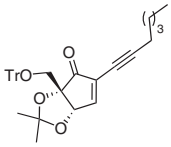
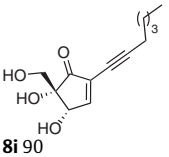
Entry	Alkyne **9**	Yield of **10** (%)	Yield of **8** (%)
1	 **9a**	 **10a** 90	 **8a** 82
2	 **9b**	 **10b** 89	 **8b** 87

Table 1 (continued)

Entry	Alkyne **9**	Yield of **10** (%)	Yield of **8** (%)
3	 **9c**	 **10c** 76	 **8c** 81
4	 **9d**	 **10d** 79	 **8d** 89
5	 **9e**	 **10e** 75	 **8e** 93
6	 **9f**	 **10f** 75	 **8f** 95
7	 **9g**	 **10g** 72	 **8g** 54
8	 **9h**	 **10h** 85	 **8h** 96
9	 **9i**	 **10i** 82	 **8i** 90

[a] To **7** (0.18 mmol) in THF (3.5 mL) were added successively the corresponding alkyne (0.36 mmol), CuI (10 mg, 0.05 mmol), $^{i}Pr_2NH$ (0.13 mL, 0.91 mmol), and $[Pd(PPh_3)_2Cl_2]$ (8 mg, 0.01 mmol). The reaction proceeded at 25 °C for 1–2 h.
[b] 90% aqueous TFA at 0–25 °C.

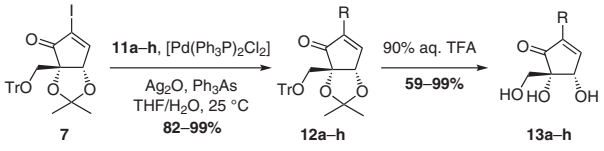
In the light of the tabulated results, it is easily deduced that our iodo-cyclopentenone precursor **7** operated as a perfect coupling partner, leading to the anticipated products **10** in excellent yields (72–90%) under the described reaction conditions. A variety of substituted aryl alkynes (Table 1, entries 1–6) was incorporated as the alkyne compo-

nent bearing a range of functional groups of various stereochemical and electronical profiles. In every case, the desired coupling product was efficiently delivered and isolated by column chromatography. In the same vein, alkyl-substituted terminal alkynes were coupled effectively with protected iodo-pentenomycin **7** in yields ranging from 72–85%, under the same reaction conditions (Table 1, entries 7–9).

Natural product analogues **8a–i** were obtained after acidic treatment in 90% aqueous TFA, ensuring removal of both the triphenylmethyl ether and the acetal protecting groups. Due to the enhanced lipophilic character of the produced derivatives, a simple purification by column chromatography was enough to provide a material of appropriate purity (>95%) for biological testing.

Next, we proceeded with the 2-aryl-sustituted pentenomycin derivatives **13a–h** that we intended to access *via* a Suzuki reaction between **7** and the corresponding arylboronic acids **11a–h** (see general reaction scheme in Table 2),[8] followed by global deprotection.

Table 2 Coupling Reactions of 2-Iodopentenomycin **7** with Arylboronic Acids **11a–h**[a] and Subsequent Deprotection to Pentenomycin Derivatives **13a–h**[b]



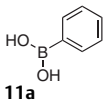
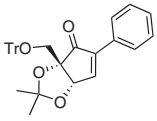
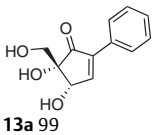
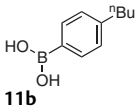
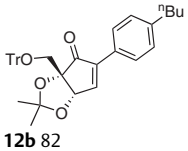
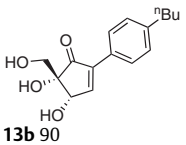
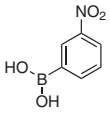
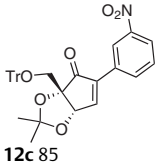
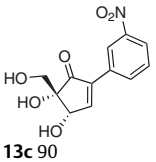
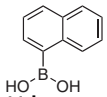
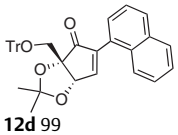
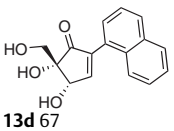
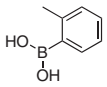
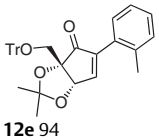
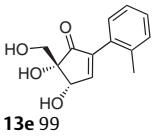
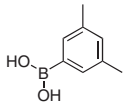
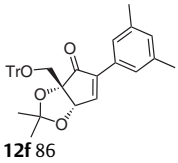
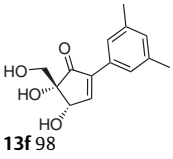
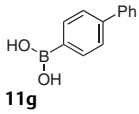
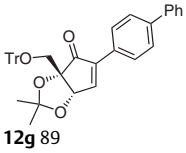
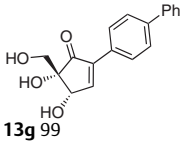
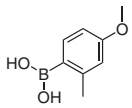
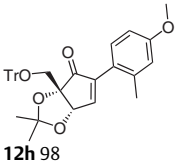
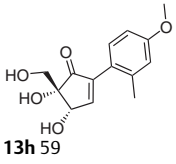
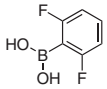
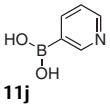
Entry	Boronic acid **11**	Yield of **12** (%)	Yield of **13** (%)
1	 **11a**	 **12a** 92	 **13a** 99
2	 **11b**	 **12b** 82	 **13b** 90
3	 **11c**	 **12c** 85	 **13c** 90
4	 **11d**	 **12d** 99	 **13d** 67

Table 2 (continued)

Entry	Boronic acid **11**	Yield of **12** (%)	Yield of **13** (%)
5	 **11e**	 **12e** 94	 **13e** 99
6	 **11f**	 **12f** 86	 **13f** 98
7	 **11g**	 **12g** 89	 **13g** 99
8	 **11h**	 **12h** 98	 **13h** 59
9	 **11i**	 **11j**	NR[c]

[a] To **7** (0.18 mmol) in THF (2.25 mL) and H_2O (0.75 mL) were added successively the corresponding boronic acid (0.27 mmol), Ag_2O (67 mg, 0.29 mmol), Ph_3As (12 mg, 0.04 mmol), and $[Pd(PPh_3)_2Cl_2]$ (7 mg, 0.018 mmol). The reaction proceeded at 25 °C for 1–2 h.
[b] 90% aqueous TFA 0 to 25 °C.
[c] NR: no reaction.

After screening several catalytic systems, we found that the combination of $[Pd(PPh_3)_2Cl_2]$ and Ph_3As as the ligand was the optimum one for a clean conversion into the coupling product.[9] In the presence of the said catalyst and Ag_2O, as the base, in a solvent system THF/H_2O (3:1), the coupling of 2-iodo-enone **7** with various aryl boronic acids **11a–h** was successfully accomplished. The reaction with moderately to electron-rich nucleophiles (Table 2, entries 1, 2, and 4–8) was realized in excellent yields (82–99%). Even rather deactivated boronic acids reacted smoothly under the described reaction conditions (Table 2, entry 3). In contrast, electron-deficient boronic acids such as 2,6-difluorophenyl boronic acid (**11i**) and 3-pyridylboronic acid (**11j**) gave no reaction with any of the screened catalytic systems. Global deprotection of the coupling products **12a–h** afforded, after typical purification, the unprotected 2-aryl-pentenomycins **13a–h** in 59–99% yield.

The antimicrobial activity of the new compounds was tested against three representative strains, one Gram-positive and two Gram-negatives, namely *Staphylococcus aureus* strain Newman, *Escherichia coli* K12, and *Pseudomonas aeruginosa* PA14 (Table 3). Due to solubility limits, the compounds were tested at the maximum soluble concentration. The designed derivatives **8a–i** and **13a–h** were found not to exhibit any antimicrobial effect against *E. coli* and *P. aeruginosa*, indicating that Gram-negative strains are not sensitive to the action of the aforementioned compounds. In contrast, derivatives **8a**, **8d**, **8f**, and **8h** exhibited moderate to good inhibition of the growth of *S. aureus*, which was superior of the one exerted by the natural product **1a** (Table 3). The observed difference in activity among the two classes of bacteria may be attributed to the difference in their type of cell wall and the ability of the compounds to penetrate them, as well as the reduction of the intracellular concentration of the compounds by efflux pumps.

Table 3 Minimum Inhibitory Concentration (MIC) Values of Compounds **8a–i** and **13a–h** against *S. aureus*, *E. coli*, and *P. aeruginosa* (in μM)

Compound	*S. aureus* str. Newman	*E. coli* K12	*P. aeruginosa* PA14
8a	500	>500	>500
8b	>500	>500	>500
8c	>250	>250	>250
8d	231	>250	>250
8e	>250	>250	>250
8f	222	>250	>250
8g	>250	>250	>250
8h	98	>250	>250
8i	>250	>250	>250
13a	>500	>500	>500
13b	>500	>500	>500
13c	>500	>500	>500
13d	>500	>500	>500
13e	>500	>500	>500
13f	>500	>500	>500
13g	>250	>250	>250
13h	>500	>500	>500
1a	>500	–	–

Based on a recent literature report,[10] the antimicrobial activity of 2-phenyl-pentenomycin (analogue **13a**, Table 2) against several strains, including *S. aureus*, *Enterococcus faecium*, and *P. aeruginosa*, was attenuated compared to the original natural product. By analogy, we observed that when an aromatic group was introduced at the α-position (compounds **13a–h**), the antimicrobial activity was completely abolished (Table 3). However, the activity was restored when an intermediate linker or a long chain was incorporated, such as in **8a**, **8d**, **8f**, and **8h**. More specifically, compound **8h**, which bears a long aliphatic chain of ten carbon atoms, proved to be the most potent antimicrobial agent. The certain length and the flexibility of this chain is suspected to be the reason for its activity, while **8i**, a derivative with a shorter chain by three carbon atoms, is inactive. Finally, derivatives **8d** and **8f**, which have an aromatic ring connected to the triple bond and bear a halogen at *meta* or *para* position, are active but less potent.

In order to test the activity of the designed analogues against additional Gram-positive strains, the most potent inhibitor **8h** was screened against the pathogens indicated in Table 4. The compound does not show any antimicrobial activity against *Enterococcus faecium* and *Enterococci faecalis*, as well as against *Mycobacterium smegmatis* at concentrations below 35 μg/mL. Noteworthy, **8h** exhibits a minimum inhibitory concentration (MIC) of 9 μg/mL against *Streptococcus pneumoniae*, making it one of the most active derivatives of pentenomycin I reported in the literature so far. Interestingly, it shows the same MIC value against a penicillin-resistant strain of *S. pneumoniae* (PRSP), thus excluding possible cross-resistance.

Table 4 MIC of **8h** against Several Pathogenic Gram-Positive Bacteria.

Indicator strains	MIC (μg/mL)
S. pneumoniae DSM-20566	9
S. pneumoniae DSM-11865[a]	9
E. faecalis DSM-12956	>35
E. faecium DSM-17050[b]	>35
E. faecalis DSM-20478	>35
E. faecalis DSM-2570	>35
E. faecium DSM-20477	>35
M. smegmatis mc^{2}155	>35

[a] PRSP: penicillin-resistant *S. pneumoniae*.
[b] VRE: vancomycin-resistant *Enterococcus faecium*.

Finally, we tested the viability of three human cell lines (A549, HEK293, and HepG2) after treatment with compound **8h**. The compound shows cytotoxic effects against the selected cell lines at 25 μM (Table 5), indicating that further structural optimization is required to tackle this drawback. On the other hand, a deeper exploration of the potential of compound **8h** to act as an anticancer agent is worthy of being undertaken.

In summary, we successfully synthesized a small library of 2-alkynyl, and 2-aryl-derivatized pentenomycins based on typical palladium-catalyzed coupling reactions.[11] The novel analogues of the natural antibiotic were tested for their antimicrobial activity against both, Gram-positive and Gram-negative bacteria. 2-Aryl-modified pentenomycins show no special activity against both types of bacteria, while from the 2-alkynylated derivatives, the one bearing a

Table 5 Viability against Compound **8h** and Reference Compounds of Three Human Cell Lines Expressed as (% Viability).

	Viability of cells (%)		
Compound	*A549*	*HEK293*	*HepG2*
8h (25 μM)	20 ± 3	6 ± 1	28 ± 13
doxorubicin (1 μM)	55 ± 2	58 ± 1	79 ± 1
rifampicin (100 μM)	97 ± 1	59 ± 1	83 ± 3

long aliphatic chain of ten carbon atoms, **8h**, proved to be a strong inhibitor of Gram-positive *S. aureus* and *S. pneumoniae* strains. The length of the aliphatic chain was demonstrated to be crucial as the corresponding analogue with a shorter chain by three carbon atoms, **8i**, showed no activity. In addition, compound **8h** shows cytotoxic effect against certain cell lines, some of them being cancer cells, indicating potential anticancer action.

Funding Information

A.K.H.H. gratefully acknowledges the European Research Council (ERC, Grant No. 757913), the Helmholtz-Association's Initiative and Networking Fund, and the European Union's Horizon 2020 research and innovation programme (COFUND-ALERT, Grant No. 665250).

Acknowledgment

Victoria Schmitt, Dennis Thomas Jener, and Jeanine Jung are thanked for the technical support.

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0039-1690772.

References and Notes

(1) (a) Umino, K.; Furumai, T.; Matsuzawa, N.; Awataguchi, Y.; Ito, Y.; Okuda, T. *J. Antibiot.* **1973**, *26*, 506. (b) Umino, K.; Takeda, N.; Ito, Y.; Okuda, T. *Chem. Pharm. Bull.* **1974**, *22*, 1233.
(2) Umino, K.; Yamaguchi, T.; Ito, Y. *Chem. Pharm. Bull.* **1974**, *22*, 2113.
(3) Christos Stathakis, *Dissertation*; Aristotle University of Thessaloniki: Greece, **2007**.
(4) (a) Miller, M. W.; Johnson, C. R. *J. Org. Chem.* **1997**, *62*, 1582. (b) Negishi, E. *J. Organomet. Chem.* **1999**, *576*, 179. (c) Negishi, E.; Tan, Z.; Liou, S.-Y.; Liao, B. *Tetrahedron* **2000**, *56*, 10197. (d) Pohmakotr, M.; Kambutong, S.; Tuchinda, P.; Kuhakarn, C. *Tetrahedron* **2008**, *64*, 6315.
(5) (a) Gallos, J. K.; Damianou, K. C.; Dellios, C. C. *Tetrahedron Lett.* **2001**, *42*, 5769. (b) Gallos, J. K.; Stathakis, C. I.; Kotoulas, S. S.; Koumbis, A. E. *J. Org. Chem.* **2005**, *70*, 6884.
(6) (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467. (b) Sonogashira, K. *J. Organomet. Chem.* **2002**, *653*, 46.

(7) **Typical Procedure for the Sonogashira Coupling**

2-Iodopentenomycin (**7**, 100 mg, 0.181 mmol, 1.0 equiv) was dissolved in THF (3.5 mL) and $Pd(PPh_3)_2Cl_2$ (8 mg, 0.011 mmol, 0.06 equiv) and CuI (10 mg, 0.05 mmol, 0.3 equiv) were added successively. The mixture was deoxygenated and flashed with argon carefully and then was cooled to 0 °C. 1-Decyne (65.3 μL, 0.362 mmol, 2.0 equiv) was added dropwise, followed by addition of $^iPr_2NH$ (0.13 mL, 0.905 mmol, 5 equiv). The reaction mixture was stirred at room temperature for 2 h before it was diluted with EtOAc and acidified with 1 N HCl. The organic layer was separated, and the aqueous layer was extracted with EtAOc (2 × 10 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (hexanes/EtOAc, 15:1) to afford the enone **10h** (87mg) in 85% yield.

Next, compound **10h** was dissolved in a mixture 90% TFA/H_2O (2.0 mL) at 0 °C, and the resulting solution was stirred for 90 min at this temperature. Upon completion of deprotection, as determined by TLC, volatiles were removed under reduced pressure. The residue was dissolved in methanol and evaporated till dry. The above procedure was repeated twice. The residue was purified by flash column chromatography using EtOAc/MeOH (9:1) as the eluent to afford **8h** as a yellow sticky oil (42 mg, 96% yield). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.53 (d, *J* = 3.0 Hz, 1 H), 4.74 (d, *J* = 3.0 Hz, 1 H), 3.69 (d, *J* = 10.8 Hz, 1 H), 3.55 (d, *J* = 10.8 Hz, 1 H), 2.40 (t, *J* = 7.1 Hz, 2 H), 1.60–1.52 (m, 2 H), 1.43 (dq, *J* = 13.0, 6.7 Hz, 2 H), 1.34–1.29 (m, 8 H), 0.90 (t, *J* = 6.7 Hz, 3 H). ^{13}C NMR (125 MHz, CD_3OD): δ = 204.0, 161.3, 129.7, 98.7, 75.4, 70.4, 70.0, 63.0, 31.6, 28.9, 28.8, 28.5, 28.1, 22.3, 18.7, 13.0. FTIR (neat): 3462, 2913, 2234, 1738, 1492, 1245, 704 cm^{-1}. $[\alpha]_D^{25}$ +13.6° (*c* 1.57 EtOH). HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{16}H_{23}O_4$: 279.1596; found: 279.1589.

(8) **Typical Procedure for the Suzuki Reaction**

2-Iodopentenomycin (**7**, 100 mg, 0.181 mmol, 1.0 equiv) was dissolved in a mixture of THF (2.5 mL) and H_2O (0.75 mL). Naphthalene-1-boronic acid (47 mg, 0.272 mmol, 1.5 equiv), Ag_2O (67 mg, 0.29 mmol, 1.6 equiv), Ph_3As (12 mg, 0.04 mmol, 0.2 equiv), and $Pd(PPh_3)_2Cl_2$ (7 mg, 0.018 mmol, 0.1 equiv) were added successively, and the reaction was stirred at ambient temperature for 3 h. Upon completion the mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and purified by flash column chromatography (hexanes/EtOAc, 15:1) to afford the respective enone **12d** (100 mg, 99% yield).

Next, compound **12d** was dissolved in a mixture of 90% TFA/H_2O at 0 °C, and the resulting solution was stirred for 90 min at this temperature. When the reaction was completed as determined by TLC, volatiles were removed under reduced pressure, and the residue was dissolved in methanol and evaporated down. The above procedure was repeated twice, and the residue was purified by flash column chromatography using EtOAc/MeOH (9:1) as the eluent. Derivative **13d** was afforded as brown solid (32 mg, 67% yield, mp 52–55 °C). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.89 (d, *J* = 8.0 Hz, 2 H), 7.82 (d, *J* = 8.3 Hz, 1 H), 7.69 (d, *J* = 2.8 Hz, 1 H), 7.49 (t, *J* = 7.6 Hz, 2 H), 7.46–7.43 (m, 1 H), 7.38 (d, *J* = 7.1 Hz, 1 H), 4.97 (d, *J* = 2.8 Hz, 1 H), 3.89 (d, *J* = 10.5 Hz, 1 H), 3.73 (d, *J* = 10.5 Hz, 1 H). ^{13}C NMR (125 MHz, CD_3OD): δ = 205.7, 160.2, 144.7, 133.6, 131.2, 129.3, 128.6, 127.9, 126.5, 125.8, 125.6, 125.1, 124.7, 76.2, 70.3, 63.4. FTIR (neat): 3388, 2924, 2852, 1715, 1509, 1141, 778 cm^{-1}. $[\alpha]_D^{25}$ –20.5° (*c* 1.46 EtOH). HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{16}H_{13}O_4$: 269.0814; found: 269.0819.

(9) (a) Ruel, F. S.; Braun, M. P.; Johnson, W. S. *Org. Synth., Coll. Vol. X* **2004**, 467. (b) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
(10) Kamishima, T.; Suzuki, M.; Aoyagi, S.; Watanabe, T.; Koseki, Y.; Kasai, H. *Tetrahedron Lett.* **2019**, *60*, 1375.
(11) **Antibacterial Testing**
Compounds were prepared as DMSO stock solutions, and minimum inhibitory concentrations (MIC) were determined as described in the literature.[12] Bacteria were handled according to standard procedures and were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) or were part of our internal strain collection. In brief, bacterial cultures were diluted in Tryptic Soy Broth (TSB; 1.7% peptone casein, 0.3% peptone soymeal, 0.25% glucose, 0.5% NaCl, 0.25% K_2HPO_4; pH 7.3; for *Enterococci* and *Streptococci*), Luria Broth (LB; 0.05% sodium chloride, 1.0% tryptone, and 0.5% yeast extract for *S. aureus*, *E. coli*, and *P. aeruginosa*), BBL Middlebrook 7H9 with glycerol (0.1% w/v casitone, 5.6 μg/mL palmitic acid, 5 mg/mL bovine serum albumin, 4 μg/mL catalase; for *M. smegmatis*), or Müller-Hinton broth (0.2% beef infusion solids, 1.75% casein hydrolysate, 0.15% starch; pH 7.4; for all other listed bacteria) to achieve a final inoculum of approximately 10^4 to 10^5 colony-forming units (cfu)/mL. Compounds were tested in serial dilution (0.06–128 μM) in 96-well plates, and MIC values were determined by visual inspection after 16–48 h incubation at 37 °C.

MTT Assay

HepG2 (human hepatocellular carcinoma), HEK293 (human embryonal kidney), and A549 (human lung carcinoma) cells (2×10^5 cells per well) were seeded in 24-well in flat-bottomed plates. Culturing of cells, incubations and OD measurements were performed as described previously with small modification.[13] After seeding for 24 h, the incubation was started by the addition of compounds in a final DMSO concentration of 1%. The living cell mass was quantified after 48 h. Rifampicin was used as negative control, doxorubicin as positive control.

Full experimental details for the synthesis of all pentenomycin derivatives, as well as pictures of ^{1}H NMR and ^{13}C NMR spectra thereof are provided in the Supporting Information section.

(12) Hüttel, S.; Testolin, G.; Herrmann, J.; Planke, T.; Gille, F.; Moreno, M.; Stadler, M.; Brönstrup, M.; Kirschning, A.; Müller, R. *Angew. Chem. Int. Ed.* **2017**, *56*, 12760.
(13) Haupenthal, J.; Baehr, C.; Zeuzem, S.; Piiper, A. *Int. J. Cancer* **2007**, *121*, 206.