Aranciamycins I and J, Antimycobacterial Anthracyclines from an Australian Marine-Derived Streptomyces sp

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · MARCH 2015

Impact Factor: 3.8 · DOI: 10.1021/acs.jnatprod.5b00095 · Source: PubMed

6 AUTHORS, INCLUDING:

Andrew M Piggott
Macquarie University
52 PUBLICATIONS **606** CITATIONS
SEE PROFILE

Angela A Salim
University of Queensland
42 PUBLICATIONS **421** CITATIONS
SEE PROFILE

Antje Blumenthal
University of Queensland
33 PUBLICATIONS **811** CITATIONS
SEE PROFILE

Robert J Capon
University of Queensland
305 PUBLICATIONS **4,049** CITATIONS
SEE PROFILE

JOURNAL OF NATURAL PRODUCTS

Note

pubs.acs.org/jnp

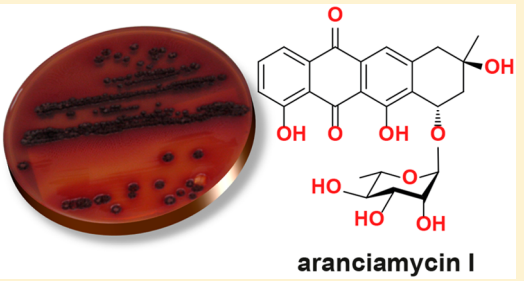
Aranciamycins I and J, Antimycobacterial Anthracyclines from an Australian Marine-Derived *Streptomyces* sp.

Zeinab G. Khalil,[†,‡] Ritesh Raju,[†,‡] Andrew M. Piggott,[†,§] Angela A. Salim,[†] Antje Blumenthal,[⊥,∥] and Robert J. Capon*[,†]

[†]Institute for Molecular Bioscience, [⊥]Diamantina Institute, and [∥]Australian Infectious Diseases Research Centre, The University of Queensland, St Lucia, QLD 4072, Australia

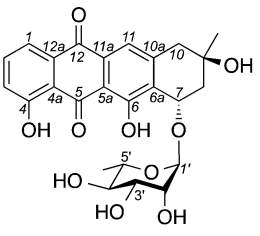
Supporting Information

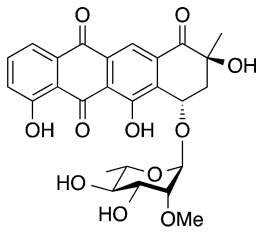
ABSTRACT: Chemical analysis of an Australian marine-derived *Streptomyces* sp. (CMB-M0150) yielded two new anthracycline antibiotics, aranciamycins I (**1**) and J (**2**), as well as the previously reported aranciamycin A (**3**) and aranciamycin (**4**). The aranciamycins **1**–**4**, identified by detailed spectroscopic analysis, were noncytotoxic when tested against selected Gram-negative bacteria and fungi (IC_{50} >30 μM) and exhibited moderate and selective cytotoxicity against Gram-positive bacteria (IC_{50} >1.1 μM) and a panel of human cancer cell lines (IC_{50} > 7.5 μM). Significantly, **1**–**4** were cytotoxic (IC_{50} 0.7–1.7 μM) against the *Mycobacterium tuberculosis* surrogate *M. bovis* bacille Calmette-Guérin.

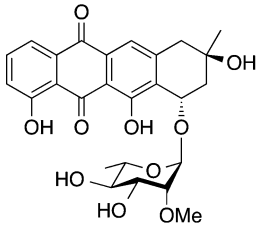


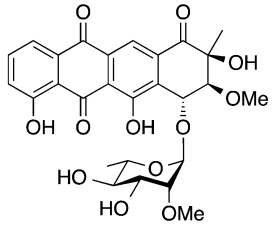
Tuberculosis (TB) is a devastating infectious disease caused by various strains of mycobacteria, the most common being *Mycobacterium tuberculosis* (Mtb). The World Health Organization estimates that one-third of the world's population is latently infected with Mtb, which carries a 10% lifetime risk of developing the active form of the disease. In 2013, 9 million people became infected with TB and 1.5 million died from the disease, making it second only to HIV/AIDS as the most deadly single infectious agent. Despite a concerted global effort and decades of research across both the public and private sectors, TB has proven to be a complex and challenging disease to manage. The standard treatment for TB involves protracted (>6 month) antibiotic chemotherapy using combinations of vintage antibiotics such as isoniazid, pyrazinamide, ethambutol, and rifampicin. However, the increasing prevalence of multi-drug-resistant (MDR) strains of Mtb has rendered these first-line antibiotics significantly less effective.[1] Of even greater concern is the recent emergence of extensively drug resistant (XDR) strains of Mtb, which are also resistant to one or more second-line antibiotics, such as the fluoroquinolones and aminoglycosides.[2] Clearly, there is an urgent and compelling need for the discovery and development of next-generation antimycobacterial agents to combat existing and emerging strains of antibiotic-resistant Mtb.

As part of our ongoing microbial biodiscovery program, we routinely screen extracts from Australian terrestrial and marine-derived microorganisms for antimycobacterial activity using the nonpathogenic Mtb surrogate *M. bovis* bacille Calmette-Guérin (BCG). A screening program targeting growth-inhibitory activity against BCG prioritized *Streptomyces* sp. (CMB-M0150) isolated from marine sediment collected off the Sunshine Coast, Queensland, Australia, in 2007. Chemical analysis of this organism yielded two new anthracycline antibiotics, aranciamycins I (**1**) and J (**2**), as well as the previously reported aranciamycin A (**3**) and aranciamycin (**4**). The structures of **3** and **4** were confirmed by comparison of their spectroscopic data with previously reported values,[3,4] while the structures of **1** and **2** were assigned as described below.









HRESI(+)MS analysis of **1** revealed an adduct ion $[M + Na]^+$ consistent with the molecular formula $C_{25}H_{26}O_{10}$. Comparison of the NMR (DMSO-d_6) data for **1** (Table 1) with those for the previously reported **3** (Supporting

Received: January 29, 2015

DOI: 10.1021/acs.jnatprod.5b00095
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for Aranciamycins I (1) and J (2) in DMSO-d_6

	aranciamycin I (**1**)		aranciamycin J (**2**)	
pos.	δ_C[a], type	δ_H, m (J in Hz)	δ_C[a], type	δ_H, m (J in Hz)
1	118.9, CH	7.71, d (7.7)	119.3, CH	7.75, dd (7.6, 1.1)
2	137.0, CH	7.79, dd (8.3, 7.7)	137.5, CH	7.84, dd (8.3, 7.6)
3	124.8, CH	7.37, d (8.3)	124.4, CH	7.42, dd (8.3, 1.1)
4	160.8, C		160.9, C	
4a	116.2, C		116.8, C	
5	191.3, C		[e]	
5a	113.7, C		118.2, C	
6	161.9, C		[e]	
6a	131.9, C		135.8, C	
7	72.1, CH	4.91, dd (6.4, 5.3)	69.4, CH	5.14, dd (5.1, 3.6)
8α	43.2, CH_2	2.12, dd (13.8, 6.4)	41.2, CH_2	2.50, dd (14.6, 5.1)
8β		1.98, dd (13.8, 5.3)		2.25, dd (14.6, 3.6)
9	67.4, C		72.1, C	
10α	44.5, CH_2	2.96, d (17.0)	199.7, C	
10β		2.76, d (17.0)		
10a	146.7, C		[e]	
11	119.7, CH	7.45, s	115.5, CH	8.09, s
11a	131.9, C		[e]	
12	181.5, C		181.2, C	
12a	133.5, C		133.0, C	
1′	103.5, CH	5.03, d (1.1)	100.1, CH	5.28, d (1.6)
2′	70.6, CH	3.60[b]	80.5, CH	3.27, dd (3.2, 1.6)
3′	70.7, CH	3.29[f]	70.1, CH	3.40[d]
4′	71.8, CH	3.22, ddd (9.2, 9.2, 5.3)	71.7, CH	3.19, ddd (9.4, 9.4, 5.5)
5′	69.3, CH	3.59,[b] m	69.5, CH	3.63, dq (9.4, 6.2)
5′-Me	17.9, CH_3	1.18, d (6.2)	17.6, CH_3	1.20, d (6.2)
9-Me	28.6, CH_3	1.27, s	25.4, CH_3	1.42, s
2′-OH		4.73[c]		
2′-OMe			58.2, OCH_3	3.40,[d] s
3′-OH		4.45, d (5.8)		4.63, d (5.8)
4′-OH		4.73[c]		4.86, d (5.5)
4-OH		[e]		[e]
6-OH		[e]		[e]
9-OH		4.72[c]		5.66, s

[a]Assignments supported by 2D HSQC and HMBC experiments. [b−d]Overlapping resonances. [e]Not observed. [f]Obscured by H_2O resonance.

Information, Figure S7 and Table S6) revealed the two compounds to be almost identical, with the only significant differences being the presence of a 2′-OH in **1** (δ_H 4.73) versus a 2′-OMe in **3** (δ_H 3.39; δ_C 58.2). Detailed analysis of the 2D HSQC, HMBC, and COSY NMR data (Figure 1 and Table S1) established **1** as the 2′-*O*-desmethyl analogue of **3**. Given the high degree of similarity in NMR data between **1** and **3**, similar specific rotations (**1** $[\alpha]_D$ +139; **3** $[\alpha]_D$ +119), and a likely biosynthetic relationship between the two metabolites, we tentatively propose the absolute configuration of **1** to be the same as **3**. The absolute configuration of aranciamycin A (**3**) has previously been biosynthetically linked to aranciamycin (**4**),[4] which was itself confirmed in 1993 by X-ray analysis.[5]

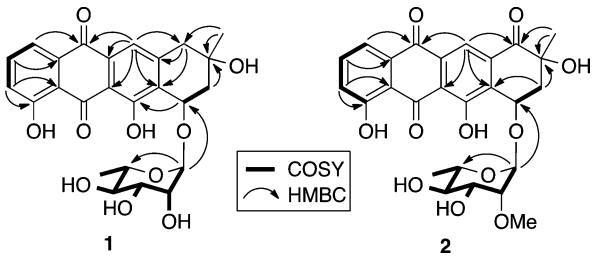


Figure 1. Selected 2D NMR correlations for **1** and **2**.

HRESI(+)MS analysis of **2** revealed an adduct ion $[M + Na]^+$ consistent with the molecular formula $C_{26}H_{26}O_{11}$. Comparison of the NMR (DMSO-d_6) data for **2** (Table 1) with those for the previously reported **4** (Figure S8 and Table S7) revealed the two compounds were also almost identical, with the only significant differences being the appearance of diastereotopic H_2-8 methylene resonances in **2** (δ_H 2.50, 2.25; δ_C 41.2) versus an H-8 hydroxymethine in **4** (δ_H 3.62; δ_C 86.0). Detailed analysis of the 2D HSQC, HMBC, and COSY NMR data (Figure 1 and Table S2) established **2** as the 8-desmethoxy analogue of **4**. Again, we tentatively propose the absolute configuration of **2** to be the same as **4** based on the similarity in NMR data, specific rotations (**2** $[\alpha]_D$ +150; **4** $[\alpha]_D$ +161), and their biosynthetic relationship.

Aranciamycins **1**−**4** displayed moderate activity at inhibiting the growth of *Mycobacterium bovis* BCG *in vitro* (MIC 10−30 μM and IC_{50} 0.7−1.7 μM) (Figure S11 and Table 2) and two

Table 2. *In Vitro* Antimicrobial Activities of Aranciamycins 1−4[a]

organism	1	2	3	4
M. bovis BCG	10 (0.8)	10 (0.7)	30 (1.1)	30 (1.7)
B. subtilis ATCC 6051	3.7 (1.1)	7.2 (2.4)	7.5 (2.8)	15 (6.0)
B. subtilis ATCC 6633	7.5 (3.0)	7.5 (2.8)	7.5 (2.4)	15 (5.8)
S. aureus ATCC 9144	>30 (>30)	>30 (>30)	>30 (>30)	>30 (>30)
S. aureus ATCC 25923	>30 (>30)	>30 (>30)	>30 (>30)	>30 (>30)
E. coli ATCC 11775	>30 (>30)	>30 (>30)	>30 (>30)	>30 (>30)
P. aeruginosa ATCC 10145	>30 (>30)	>30 (>30)	>30 (>30)	>30 (>30)
C. albicans ATCC 90028	>30 (>30)	>30 (>30)	>30 (>30)	>30 (>30)

[a]Values are the average of two independent replicates. MIC (IC_{50}) in μM.

strains of *Bacillus subtilis* (MIC 3.7−15 μM and IC_{50} 1.1−6.0 μM), but were inactive against strains of the bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* and the fungus *Candida albicans* (Table 2). Aranciamycin I (**1**) exhibited *in vitro* cytotoxicity against human colorectal (SW620) and hepatocellular (HepG2) cell lines (IC_{50} 7.5 and 9.0 μM, respectively), while aranciamycin J (**2**) was cytotoxic against SW620 (IC_{50} 10 μM) (Table 3).

Aranciamycins (and the closely related steffimycins) are relatively rare microbial metabolites that differ from other well-known anthracycline antibiotics, such as doxorubicin and daunomycin, by the absence of an amino group on their sugar moiety. Aranciamycin (**4**) was first reported by Keller-Schierlein et al. in 1970 from the soil actinomycete *Streptomyces*

DOI: 10.1021/acs.jnatprod.5b00095
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. *In Vitro* Cytotoxicity of Aranciamycins 1−4 against Four Human Tumor Cell Lines[a]

cell line	1	2	3	4
SW620 (colon)	7.5	10	>30	>30
HepG2 (liver)	12	12	>30	18
KB-3-1 (cervix)	>30	>30	>30	>30
NCI-H460 (lung)	9.0	>30	>30	>30

[a]Values are the average of two independent replicates. IC_{50} in μM.

echinatus Tü 303.[6,7] Aranciamycin and its aglycone aranciamycinone were reported to exhibit inhibitory activity against Gram-positive bacteria on synthetic media, although this activity could be significantly reduced by the addition of a mixture of pyruvate and alanine to the test medium. Aranciamycin has also been shown to be a potent inhibitor of collagenase, which in high levels has been implicated in the progression of arthritis and also tumor metathesis.[3,8] In 2007, Bechthold and colleagues cloned the putative aranciamycin biosynthetic gene cluster into a heterologous expression vector, leading to the production, isolation, and characterization of eight new analogues, aranciamycins A−H.[4,9] The only other naturally occurring aranciamycin was reported in 2010 by Fiedler and co-workers, who identified an unusual 4′-*O*-2-(3-methylmaleic anhydride)propionate analogue from a Norway spruce rhizosphere-associated strain of *Streptomyces echinatus* (Tü 6384).[10]

In conclusion, we have reported the isolation, characterization, and cytotoxic and antibiotic properties of two new and two known members of a rare class of anthracycline antibiotics from an Australian marine-derived *Streptomyces* sp. Significantly the aranciamycins **1**−**4** displayed moderate antimicrobial activity against *M. bovis* BCG and *B. subtilis*. Given the relatively low mammalian cytotoxicity of **1**−**4** compared to most other anthracycline antibiotics, these metabolites may be suitable leads for future medicinal chemistry programs aimed at the design and development of next-generation antimycobacterial agents.

EXPERIMENTAL SECTION

General Experimental Procedures. Chiroptical measurements ($[\alpha]_D$) were obtained on a JASCO P-1010 polarimeter in a 100 × 2 mm cell at 22 °C. UV−vis spectra were obtained on a Varian Cary 50 UV−visible spectrophotometer with 1 cm pathway quartz cells. NMR spectra were obtained on a Bruker Avance DRX600 spectrometer in the solvents indicated and referenced to residual signals in deuterated solvents ($CDCl_3$ δ_H 7.24, δ_C 77.2 and DMSO-d_6 δ_H 2.50, δ_C 39.5). Electrospray ionization mass spectra (ESIMS) were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MS mass detector in both positive and negative ion modes. High-resolution ESIMS measurements were obtained on a Bruker micrOTOF mass spectrometer by direct infusion in MeCN at 3 μL/min using sodium formate clusters as an internal calibrant. Analytical and semipreparative HPLCs were conducted with an Agilent 1100 Series diode array and/or multiple-wavelength detectors and an Agilent 1100 Series fraction collector. All solvents were HPLC grade.

Isolation and Identification of *Streptomyces* sp. (CMB-M0150). The strain was isolated from a marine sediment sample collected in 2007 from the Sunshine Coast, Queensland, Australia. The sample was heated at 65 °C for 30 min with vigorous shaking on a water bath. The resulting suspension was serially diluted, and an aliquot (50 μL) from every portion was transferred to M1 agar plates. The agar plates were incubated at 27 °C for 2−3 weeks. After the incubation period, the strain CMB-M0150 was purified and cultivated 2 or 3 times on M1 agar plates. The colonies were preserved in triplicate at −80 °C in the presence of 20% aqueous glycerol. DNA was isolated using DNeasy blood and tissue kit (Qiagen). The 16S rRNA genes were amplified from genomic DNA by PCR using primers FC27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and RC1492 (5′-TACGGCTACCTTGTTACGACTT-3′). PCR products were purified with a PCR purification kit (Qiagen). Amplification products were examined by agarose gel electrophoresis. The DNA sequencing was performed by the Australian Genome Research Facility (AGRF) at The University of Queensland. The 16S rRNA gene sequence showed 99% identity to *Streptomyces marinus* by the use of the BLAST database. The sequence has been registered in the NCBI GenBank database under accession number KP715263. The gene sequence is provided in the Supporting Information.

Cultivation and Fractionation. Analytical cultivation was performed by transferring a single colony of CMB-M0150 into seawater medium of Ocean Nature artificial seawater (80 mL, 3.3%), starch (1%), yeast extract (0.4%), and peptone (0.2%) and incubated at 27 °C for 10 d at 190 rpm. The culture was extracted with EtOAc (100 mL), and the organic phase concentrated *in vacuo* to yield an extract (12.7 mg), which was subsequently analyzed by HPLC-DAD-ESI(±)MS (Zorbax SB-C_8 5 μm 150 × 4.6 mm column, 5 μm, 15 min at 1 mL/min gradient elution from 90% H_2O/MeCN to 100% MeCN with a constant 0.05% HCO_2H modifier) to reveal peaks with retention times t_R = 7.4, 8.2, 8.3, and 8.4 min for *m*/*z* 7.4 (485 $[M - H]^-$, **1**), 8.2 (513 $[M - H]^-$, **2**), 8.3 (499 $[M - H]^-$, **3**) and 8.5 (543 $[M - H]^-$, **4**) (Supporting Information, Figure S1). Large-scale cultivation was performed by preparing a seed culture inoculated with *Streptomyces* sp. (CMB-M0150) in liquid M1 media (50 mL) containing 1% starch, 0.4% yeast extract, 0.2% peptone, and Ocean Nature sea salt (3.3%). Aliquots of the seed culture (5 mL) were transferred to six 3 L Fernbach flasks, each containing the same M1 liquid media (500 mL), and the flasks were shaken at 190 rpm for 10 d at 27 °C. The resulting cultures were extracted with EtOAc (400 mL), and the combined organic phase was concentrated *in vacuo* to yield an extract (80.5 mg). The extract was sequentially triturated with hexane (8 mL), CH_2Cl_2 (8 mL), and MeOH (8 mL) to afford, after concentration *in vacuo*, hexane (3 mg), CH_2Cl_2 (4.2 mg), and MeOH (45 mg) soluble fractions. The MeOH fraction, rich in the target metabolites **1**−**4**, was subjected to semipreparative reversed-phase HPLC (Zorbax C_8 column, 250 × 9.4 mm, 5 μm, 3 mL/min gradient elution 90% H_2O/MeCN to 10% H_2O/MeCN over 30 min) to yield aranciamycin I (**1**) (t_R = 11.8 min, 0.9 mg, 1.1%), aranciamycin J (**2**) (t_R = 12.2 min, 0.7 mg, 0.9%), aranciamycin A (**3**) (t_R = 15.5 min, 1.1 mg, 1.4%), and aranciamycin (**4**) (t_R = 16.1 min, 1.0 mg, 1.2%). [Note: % yields are determined on a mass-to-mass basis against the weight of the MeOH extract.]

*Aranciamycin I (**1**):* orange solid; $[\alpha]_D$ +139 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.37), 258 (4.18), 432 (3.86); NMR (600 MHz, DMSO-d_6) see Supporting Information Table S1 and Figure S2; HRESI(+)MS *m*/*z* 509.1421 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_{10}Na$, 509.1418).

*Aranciamycin J (**2**):* orange solid; $[\alpha]_D$ +150 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.32), 261 (4.15), 435 (3.87); NMR (600 MHz, DMSO-d_6) see Supporting Information Table S2 and Figure S3; HRESI(+)MS *m*/*z* 537.1368 $[M + Na]^+$ (calcd for $C_{26}H_{26}O_{11}Na$, 537.1367).

*Aranciamycin A (**3**):* orange solid; $[\alpha]_D$ +119 (*c* 0.10, MeOH) (lit. value not reported); UV (MeOH) λ_{max} (log ε) 228 (4.28), 258 (4.10), 289 (3.70), 432 (3.78); NMR (600 MHz, DMSO-d_6) see Supporting Information Table S3 and Figure S4; HRESI(+)MS *m*/*z* 523.1580 $[M + Na]^+$ (calcd for $C_{26}H_{28}O_{10}Na$, 523.1575).

*Aranciamycin (**4**):* orange solid; $[\alpha]_D$ +161 (*c* 0.13, MeOH) (lit. value[7] +149.45, MeOH); UV (MeOH) λ_{max} (log ε) 237 (4.39), 260 (4.21), 435 (3.84); NMR (600 MHz, $CDCl_3$ and DMSO-d_6) see Supporting Information Tables S4 and S5 and Figures S5 and S6; HRESI(+)MS *m*/*z* 567.1476 $[M + Na]^+$ (calcd for $C_{27}H_{28}O_{12}Na$, 567.1473).

Antimicrobial Assay. The pure metabolites were tested according to the protocol described in the Supporting Information. All the MIC

DOI: 10.1021/acs.jnatprod.5b00095
J. Nat. Prod. XXXX, XXX, XXX−XXX

and IC_{50} values were obtained after 24 h incubation for the bacteria and 48 h for the fungus *Candida albicans*.

Cytotoxicity Assay. The MTT assay was modified from that previously described[11] using adherent cell lines SW620 (epithelial like, human colorectal carcinoma), NCIH-460 (epithelial like, human lung carcinoma), KB-3-1 (epithelial like, human cervix carcinoma), and HepG2 (human hepatocellular carcinoma) according to the procedure described in the Supporting Information.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra, chromatogram, and biological graphs of metabolites **1**−**4** are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +61-7-3346-2979. Fax: +61-7-3346-2090. E-mail: r.capon@uq.edu.au.

Present Address

[§]Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW 2109, Australia.

Author Contributions

[‡]Z. G. Khalil and R. Raju contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded in part by the Institute for Molecular Bioscience and The University of Queensland (ECR138) and the Australian Research Council (DP120100183, LP120100088). We thank S. Bates and R. Robey (NCE, NIH) for providing SW620 and M. M. Gottesman (NIH) for providing KB-3-1. Z.G.K. acknowledges the provision of a UQ International Postgraduate Scholarship. R.R. acknowledges receipt of an Australian Postgraduate Award. A.B. acknowledges support by a research fellowship by the International Balzan Foundation.

■ REFERENCES

(1) Garcia, A.; Bocanegra-Garcia, V.; Palma-Nicolas, J.; Rivera, G. *Eur. J. Med. Chem.* **2012**, *49*, 1−23.

(2) Iem, V.; Somphavong, S.; Buisson, Y.; Steenkeste, N.; Breysse, F.; Chomarat, M.; Sylavanh, P.; Nanthavong, P.; Rajoharison, A.; Berland, J.; Paboriboune, P. *BMC Infect. Dis.* **2013**, *13*, 275.

(3) Bols, M.; Binderup, L.; Hansen, J.; Rasmussen, P. *Carbohydr. Res.* **1992**, *235*, 141−149.

(4) Luzhetskyy, A.; Mayer, A.; Hoffmann, J.; Pelzer, S.; Holzenkaemper, M.; Schmitt, B.; Wohlert, S.; Vente, A.; Bechthold, A. *ChemBioChem* **2007**, *8*, 599−602.

(5) Schmidt-Baese, K.; Noltemeyer, M.; Egert, E.; Eigelt, E.; Zeeck, A. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1993**, *C49*, 250−253.

(6) Keller-Schierlein, W.; Mueller, A. *Experientia* **1970**, *26*, 929−930.

(7) Keller-Schierlein, W.; Sauerbier, J.; Vogler, U.; Zaehner, H. *Helv. Chim. Acta* **1970**, *53*, 779−789.

(8) Bols, M.; Binderup, L.; Hansen, J.; Rasmussen, P. *J. Med. Chem.* **1992**, *35*, 2768−2771.

(9) Luzhetskyy, A.; Hoffmann, J.; Pelzer, S.; Wohlert, S.; Vente, A.; Bechthold, A. *Appl. Microbiol. Biotechnol.* **2008**, *80*, 15−19.

(10) Nachtigall, J.; Schulz, D.; Beil, W.; Suessmuth, R.; Fiedler, H. *J. Antibiot.* **2010**, *63*, 397−399.

(11) Carmichael, J.; DeGraff, W.; Gazdar, A.; Minna, J.; Mitchell, J. *Cancer Res.* **1987**, *47*, 936−942.

DOI: 10.1021/acs.jnatprod.5b00095
J. Nat. Prod. XXXX, XXX, XXX−XXX