ORIGINAL ARTICLE

Two streptothricins with a *cis*-streptolidine lactam moiety from *Streptomyces* sp. I08A 1776

This article has been corrected since advance Online Publication, and an erratum is also printed in this issue.

Maoluo Gan, Yan Guan, Xudong Zheng, Yanhui Yang, Xueqin Hao, Yishuang Liu, Liyan Yu and Chunling Xiao

Two unique *cis*-fused streptothricins (**1** and **2**) were isolated from the culture broth of *Streptomyces* sp. I08A 1776. Their structures were determined by MS, CD, and 1D and 2D NMR spectroscopic data analysis. Compound **2** showed weak antibacterial activities against *Bacillus subtilis* and *Enterococcus faecalis* with MIC values of 32 and 64 $\mu\text{g ml}^{-1}$, respectively. *The Journal of Antibiotics* (2012) 65, 513–516; doi:10.1038/ja.2012.55; published online 11 July 2012

Keywords: antibacterial; configuration; streptolidine; *Streptomyces*; streptothricin

INTRODUCTION

Streptothricins (STs) are a group of basic, water-soluble antibiotics produced by actinomycetes.^{1,2} The common structures of STs consist of a heterocyclic streptolidine, a carbamoylated D-gulosamine, and an L- β -lysine-derived unit and were confirmed by a total synthesis in 1982.^{3,4} In most cases, the carbamoyl group was substituted at C-10 of the D-gulosamine moiety, to which the exocyclic nitrogen atom of streptolidine was attached through a β -glycosidic linkage.⁵ But in recent years, some ST analogs with the carbamoyl located at C-12 were also isolated and identified from *Streptomyces* strains.^{6–9} The relative configuration of C-2 and C-3 in the streptolidine moiety was determined to be *trans*¹⁰ and the absolute configuration was established as 2S, 3S, 4R by X-ray crystallography in 1972.¹¹

In the course of our screening program for new anti-tubercular antibiotics from microbial metabolites, five STs were previously identified from the culture of *Streptomyces* sp. I08A 1776, and one (**3**) of which possessed the unprecedented *cis*-fused streptolidine lactam moiety.¹² Continuing investigation of the less polar portion of the culture broth extract has resulted in identification of two additional *cis*-fused ST analogs (**1** and **2**, Figure 1). This paper describes the isolation, structure elucidation, and in brief, the *in vitro* antimicrobial and cytotoxic activities of **1** and **2**.

RESULTS

Structure elucidation

Compound **1** was isolated as a white powder, and its molecular formula was indicated as $\text{C}_{33}\text{H}_{60}\text{N}_{12}\text{O}_{11}$ by HR-ESI-MS at m/z 801.4597 $[\text{M} + \text{H}]^+$. The IR spectrum suggested the presence of OH and/or NH (3261 cm^{-1}) and amide carbonyl (1652 cm^{-1})

groups. The NMR data (Table 1) showed resonances characteristic for ST antibiotics,^{7,12–14} consisting of thirteen methylenes, six methines (one oxygen- and five nitrogen-bearing) and six quaternary carbons (five carbonyls and a guanidino). In addition, it showed resonances for an acetyl and another aminoglycosyl moiety. This suggested that **1** was an acetylated ST derivative with three β -lysine residues, which was confirmed by 2D NMR data analysis. The ^1H – ^1H COSY spectrum of **1** demonstrated five isolated spin systems (Figure 2a), including one streptolidine lactam, one gulosamine and three β -lysine units. In the HMBC spectrum, correlations of H-8/C-14, H-15/C-14, C-16 and C-17, H-19/C-17, C-18 and C-20, H-22/C-20, C-23 and C-24, H-25/C-23, C-24 and C-26, H-28/C-26, C-29 and C-30, and H-31/C-29 and C-30, in combination of their chemical shifts, verified the presence of three β -lysine residues and established their linkages (Figure 2a). In addition, an HMBC correlation of H-16 with the acetyl carbonyl (δ_{C} 176.2) located the acetyl group at NH-16, whereas a correlation of H₂-12 (δ_{H} 4.25, d, $J = 6.0\text{ Hz}$) with C-13 (δ_{C} 161.7), together with the chemical shifts of H₂-12 and C-12 (δ_{C} 66.6), suggested that the carbamoyl group was attached at C-12 of the gulosamine unit.^{7,12} The large coupling constant of $J_{\text{H7,H8}}$ (9.6 Hz) suggested that the anomeric carbon of the gulosamine moiety had a β -configuration.^{12,15–17} An HMBC correlation of H-7 with C-6 (δ_{C} 161.5) confirmed that the β -gulosamine moiety was connected with the guanidino carbon of streptolidine via the exocyclic nitrogen atom. Comparison of the coupling constants and chemical shifts of H-2–H-4 and C-2–C-5 in **1** with those of (2R, 3S, 4R)-10-de-O-carbamoyl-12-O-carbamoyl- N^{β} -acetylstreptothricin **3** (**3**) Gan *et al.*¹² and other STs^{7,12–14} indicated that the streptolidine lactam moiety in **1** had the same relative configuration as **3**. In contrast to the large

Figure 1 Chemical structures of compounds 1–3.

coupling of $J_{\text{H}_2, \text{H}_3}$ ($\approx 14.0 \text{ Hz}$) of STs with the *trans*-fused streptolidine lactam moiety,^{7,12–14} the diagnostic coupling constant of $J_{\text{H}_2, \text{H}_3}$ (10.8 Hz) of **1** suggested that the streptolidine lactam moiety in **1** had the *cis* configuration.^{18,19} This was supported by NOE correlations of H-2 with H-3 and H-4 and between H-3 and H-4 in the ROESY spectrum of **1**, demonstrating that these protons were on the same side of the ring system (Figure 2b). The CD spectrum of **1** displayed a positive Cotton effect at 201 nm ascribed to the C=N group in the guanidine residue^{20,21} and a negative Cotton effect at 222 nm for the $n \rightarrow \pi^*$ transition of the lactam unit,²² which was similar to that of **3**.¹² This suggested the 2*R*, 3*S* configuration for **1**. In addition, when applying the CD rule for lactones and lactams proposed by Wolf^{23,24} and Legrand and Bucourt,²⁵ the recorded negative Cotton effect (222 nm) also predicts the 2*R*, 3*S* configuration for **1** (Supplementary Information, Supplementary Figure S1). The absolute structure (*L*-configuration) of β -lysine residues in STs was established by Van Tamelen *et al.*²⁶ by synthesis and confirmed by acid hydrolysis of STs.^{27,28} This was also supported by the biosynthesis studies on STs.^{29,30} Based on the biogenetic consideration, the configurations of C-16, C-22 and C-28 in the side chain of **1** were deduced to be *S* as in other STs.^{3,27,28} Accordingly, the structure of **1** was identified as (2*R*, 3*S*, 4*R*)-10-de-*O*-carbamoyl-12-*O*-carbamoyl-*N* $^{\beta}$ -acetylstreptothricin D.

The spectroscopic data of **2** indicated that it was an isomer of **1**. However, in the NMR spectra of **2**, resonances for H-9, H-10 and C-10 in **2** were deshielded by $\Delta\delta +0.09$, $+0.93$ and $+2.1$ p.p.m., respectively, as compared with those of **1**, whereas the resonances of H-12, C-9, C-12 and C-13 were shielded by $\Delta\delta -0.49$, -2.5 , -3.7 , and -1.2 p.p.m. This suggested that the carbamoyl on the D-gulosamine moiety at C-12 in **1** was at C-10 in **2**, which was further confirmed by 2D NMR data of **2**, particularly, by an HMBC correlation of H-10 with C-13. The coupling constants of H-7 (δ_{H} 5.14, d, $J = 9.6 \text{ Hz}$) and H-2–H-4, chemical shifts of C-2–C-5 and CD data of **2** were similar to those of **1**, indicating that the anomeric carbon of gulonamide and the streptolidine moiety in the two compounds had the same configurations. Therefore, the structure of **2** was elucidated as (2*R*, 3*S*, 4*R*)-*N* $^{\beta}$ -acetylstreptothricin D.

Biological activity

Antimicrobial activities of **1** and **2** were evaluated against *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Mycobacterium tuberculosis* H37Rv, *Mycobacterium smegmatis* MC²155, *Pseudomonas aeruginosa* ATCC 27853, *Morganella morganii* 07-09, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231. Compound **2** showed weak

Table 1 NMR spectroscopic data for compounds **1** and **2**^a

Number	1		2	
	H, δ mult. (J in Hz)	C	H, δ mult. (J in Hz)	C
1		171.9		172.0
2	4.72 d (10.8)	57.2	4.74 d (11.4)	57.2
3	4.63 dd (10.8, 3.6)	59.9	4.65 dd (11.4, 3.6)	59.9
4	4.28 ddd (4.8, 3.6, 1.8)	65.0	4.30 ddd (4.8, 3.6, 1.8)	65.0
5	3.50 dd (13.8, 4.8)	45.4	3.52 dd (13.8, 4.8)	45.5
	3.46 dd (13.8, 1.8)		3.47 dd (13.8, 1.8)	
6		161.5		161.5
7	5.07 d (9.6)	81.9	5.14 d (9.6)	82.0
8	4.27 dd (9.6, 3.0)	50.9	4.24 dd (9.6, 3.0)	51.5
9	4.03 t (3.0)	71.9	4.12 t (3.0)	69.4
10	3.87 d (3.0)	70.5	4.80 d (3.0)	72.6
11	4.34 t (6.0)	74.7	4.35 t (6.0)	76.0
12	4.25 d (6.0)	66.6	3.76 d (6.0)	62.9
13		161.7		160.5
14		176.4		176.6
15	2.53 dd (13.8, 5.4)	44.0	2.53 dd (14.4, 5.4)	44.0
	2.45 dd (13.8, 8.4)		2.45 dd (14.4, 9.0)	
16	4.17 m	49.7	4.18 m	49.8
17	1.58 m; 1.53 m	33.9	1.58 m; 1.52 m	34.0
18	1.59 m; 1.53 m	27.5	1.58 m; 1.52 m	27.5
19	3.21 t (6.0)	41.7	3.22 t (6.0)	41.7
20		174.2		174.3
21	2.71 dd (16.2, 5.4)	39.5	2.71 dd (16.2, 5.4)	39.6
	2.63 dd (16.2, 7.8)		2.63 dd (16.2, 7.8)	
22	3.66 m	51.3	3.67 m	51.4
23	1.73 m	32.1	1.73 m	32.1
24	1.66 m	27.0	1.66 m	27.0
25	3.27 t (6.0)	41.4	3.28 t (6.6)	41.5
26		174.2		174.3
27	2.76 dd (16.2, 4.8)	39.2	2.75 dd (16.2, 4.8)	39.3
	2.66 dd (16.2, 8.4)		2.66 dd (16.2, 8.4)	
28	3.70 m	51.1	3.70 m	51.1
29	1.83 m	31.7	1.83 m	31.8
30	1.81 m	25.6	1.81 m	25.6
31	3.08 t (6.6)	41.5	3.09 t (7.2)	41.6
Ac	2.02 s	24.6	2.02 s	24.7
		176.2		176.2

^a¹H and ¹³C NMR data were acquired at 600 and 150 MHz, respectively. The assignments were based on DEPT, ¹H–¹H COSY, HSQC and HMBC experiments.

activities against *B. subtilis* (MIC 32 $\mu\text{g ml}^{-1}$) and *E. faecalis* (MIC 64 $\mu\text{g ml}^{-1}$), whereas **1** was inactive to all the tested strains (MIC values $>128 \mu\text{g ml}^{-1}$). Levofloxacin was used as the positive control for bacteria with MIC values of 0.125–16 $\mu\text{g ml}^{-1}$. This result supported that the acetylation of β -lysine and the transformation of the carbamoyl group from C-10 to C-12 led to the loss or decrease of antimicrobial activity of STs, as observed earlier.^{7,12,31} In addition, **1** and **2** were also assessed for their cytotoxicities against human lung adenocarcinoma (A549), hepatocellular carcinoma (HepG2), and cervical cancer (Hela) cell lines, but were inactive (IC₅₀ values $>10 \mu\text{M}$).

DISCUSSION

All STs have the same unusual heterocyclic moiety, streptolidine, for which Bycroft and King¹¹ proposed the biogenesis from arginine *via* capreomycin intermediate. The proposal was supported by a series

Figure 2 (a) ¹H-¹H COSY (thick lines) and main HMBC (single arrows, H→C) correlations of **1**. (b) Key NOESY correlations (double arrows) for the streptolidine lactam moiety of **1**.

of incorporation studies using [1,2-¹³C] acetate³² and multiple forms of ¹³C-, ¹⁵N- and ²H-labeled arginine.^{33–35} The putative intermediate, (2*S*, 3*R*)-capreomycidine, occurs in a number of antibiotics,^{36,37} while its (2*S*, 3*S*)-isomer occurs in yet others.³⁸ However, the mechanism of formation of the intermediate capreomycidine in the biosynthesis of STs remains unresolved.^{35,39} A number of pathways were proposed for the conversion of arginine to capreomycidine. The incorporation studies with ²H-labeled arginine help exclude the several pathways but do not completely support a single route.^{34,35} Interestingly, the recent incorporation study showed that 2*S*, 3*R*-[guanidino-¹³C] capreomycidine was not incorporated in the STs, suggesting that capreomycidine may be an enzyme-bound intermediate, unable to enter the cell, or is not a precursor of STs.³⁹ The unique 2*R*, 3*S*-configuration of **1** and **2** was different from the 2*S*, 3*S*-configuration in the common STs, indicating that the 2*S*-configuration of the starting L-arginine was inverted in the biosynthesis of **1** and **2**. This would provide an insight to the proposed biosynthetic pathways of STs.

EXPERIMENTAL PROCEDURE

General experimental procedures

Optical rotations were measured on a PerkinElmer model 343 polarimeter (PerkinElmer Inc., Waltham, MA, USA). UV and CD spectra were recorded on a JASCO 815 CD spectrometer (Jasco International, Tokyo, Japan). IR spectra were recorded on a Nicolet 5700 FT-IR microscope spectrometer (FT-IR microscope transmission, Thermo Fisher Scientific, Madison, WI, USA). 1D- and 2D-NMR spectra were obtained at 600 MHz for ¹H and 150 MHz for ¹³C, respectively, on a Varian INOVA 600 MHz spectrometer (Varian, Palo Alto, CA, USA) in D₂O. The solvent peak (DOH, δ_H 4.80) was used as an internal standard for ¹H NMR spectra and tetramethylsilane (TMS) was used as an external reference for ¹³C NMR spectra. ESI-MS data were measured with a Thermo LTQ XL linear ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). HR-ESI-MS data were obtained using a Thermo LTQ Orbitrap XL mass spectrometer. Column chromatography was performed with Diaion HP-20 macroporous resin and MCI Gel CHP-20P (Mitsubishi Chemical Inc., Tokyo, Japan), CM-Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and Toyopearl SuperQ-650M (Tosoh Co.,

Tokyo, Japan). HPLC separations were performed on Shimadzu HPLC apparatus (Shimadzu, Kyoto, Japan) consisting of an LC-20AD pump and an SPD-M20A diode array detector with a Zorbax SB-C18 column (9.4 × 250 mm, 5 μm, Agilent Co., Santa Clara, CA, USA).

Producing organism

Strain I08A 1776 was isolated from a soil sample collected from Shuangbai County, Yunnan Province of China. The strain was identified as a member of the genus *Streptomyces* based on the 16S rRNA sequence analysis by using EzTaxon.⁴⁰ Its 16S rRNA gene sequence (1461 bp), deposited in GenBank with accession number HQ704719, showed high sequence similarity of 99.5% to that of *Streptomyces flavotricini* strain NBRC 12770 (GenBank AB184132). The strain was deposited at China Medicinal Microbiological Culture Collection Center (Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, No. I08A01776).

Fermentation

A stock culture of *Streptomyces* sp. I08A 1776 was inoculated into 250-ml Erlenmeyer flasks containing 50 ml of a sterile seed medium consisting of glucose 0.5%, yeast extract 0.5%, soluble starch 1.0%, soybean meal 1%, peptone 0.5%, beef extract 0.5%, corn steep liquor 0.4%, CaCO₃ 0.4% and CoCl₂·6H₂O 0.002% in deionized water (pH 7.2 before sterilization). The fermentation was carried out on a rotary shaker at 28 °C for 2 days. Aliquots (15 ml) of the first seed culture were transferred to 1000-ml flasks containing 300 ml of the seed medium. After incubation on a rotary shaker at 28 °C for 2 days, a total of 2.5 l of the precultures was transferred into a 50-l fermentor containing 25 l of the same medium and then cultured at 28 °C for 3 days with aeration and agitation. The fermentation process described above were carried out two times and a total volume of 50 l culture broth was harvested.

Isolation

The fermentation broth (50 l) was filtered and adjusted to pH 8.0. The filtrate was subjected to a Diaion HP-20 macroporous adsorbent resin column (5 l). Successive elution of the column with H₂O, 20, 50 and 100% acetone yielded four corresponding portions (F₁–F₄) after removing solvents. The antibiotic fraction (F₂, 90 g) was separated over MCI Gel CHP-20P (1 l), eluting successively with H₂O, 20, 50 and 100% MeOH, to give four subfractions (F₂₋₁–F₂₋₄). Separation of F₂₋₃ (5.3 g) over CM-sephadex C-25 (Na⁺, 500 ml) using a linear gradient of aqueous NaCl solution (0–2 M) gave five residues (F₂₋₃₋₁–F₂₋₃₋₅). Fraction F₂₋₃₋₂ was extracted with MeOH–H₂O (3:1) and then the methanol extract was desalted by column chromatography over sephadex LH-20 (3 × 120 cm) eluting with H₂O. The resulting H₂O fraction (36 mg) was subjected to reversed-phase preparative HPLC (Zorbax SB-C18 column, 5 μm, 9.4 × 250 mm, 210 nm, Agilent Co.) using the mobile phase of 5% MeOH containing 0.1% trifluoroacetic acid to afford two fractions according to the peaks (*t*_R 21.5 and 18.1 min, respectively). Each fraction was separately lyophilized, redissolved in water and then subjected to anion exchange chromatography over SuperQ-650M (Cl[–], 1 ml) to exchange trifluoroacetic acid to afford the hydrochlorides of **1** (5 mg) and **2** (3 mg), respectively.

Physicochemical properties of **1** and **2**

(2*R*, 3*S*, 4*R*)-10-De-O-carbamoyl-12-O-carbamoyl-N^β-acetylstreptothricin D (**1**): white amorphous powder; [α]_D²⁰ –11.6 (c 0.19, H₂O); UV (H₂O) λ_{max} (log ε) 203 (6.71) nm; CD (H₂O) 201 (Δε +0.19), 222 (Δε –3.00) nm; IR ν_{max} 3261, 3051, 1705, 1652, 1559, 1405, 1081, 956, 918 cm^{–1}; ¹H NMR (600 MHz, D₂O) and ¹³C NMR (150 MHz, D₂O) data, see Table 1; ESI-MS *m/z* 801 [M + H]⁺ and 823 [M + Na]⁺; HR-ESI-MS *m/z* 801.4597 [M + H]⁺ (calcd. for C₃₃H₆₁N₁₂O₁₁, 801.4577).

(2*R*, 3*S*, 4*R*)-N^β-acetylstreptothricin D (**2**): white amorphous powder; [α]_D²⁰ –1.4 (c 0.11, H₂O); UV (H₂O) λ_{max} (log ε) 201 (6.50) nm; CD (H₂O) 197 (Δε +0.94), 222 (Δε –0.88) nm; IR ν_{max} 3208, 3076, 1799, 1671, 1443, 1204, 1139, 843, 802, 725 cm^{–1}; ¹H NMR (600 MHz, D₂O) and ¹³C NMR (150 MHz, D₂O) data, see Table 1; ESI-MS *m/z* 401 [M + 2H]²⁺; HR-ESI-MS *m/z* 401.2298 [M + 2H]²⁺ (calcd. for C₃₃H₆₂N₁₂O₁₁, 401.2325).

Biological activity assay

Antimicrobial assays were performed according to previously described method.¹² The cytotoxicities of **1** and **2** against A549, HepG2 and Hela cell lines were evaluated by using the 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT) method described by Mo *et al.*⁴¹

ACKNOWLEDGEMENTS

We gratefully acknowledge the help of Professor Jiangong Shi, Institute of Materia Medica, Chinese Academy of Medical Sciences, for the revision of the manuscript and helpful suggestions. Financial support from the Natural Sciences Foundation of China (NSFC, grant no. 81001386), Beijing Natural Science Foundation (5122032), the National Science and Technology Project of China (numbers 2008ZX10003-006 and 2012ZX09301002-001) is gratefully acknowledged.

- 1 Waksman, S. A. Production and activity of streptothricin. *J. Bacteriol.* **46**, 299–310 (1943).
- 2 Khokhlov, A. S. Streptothricins and related antibiotics. *J. Chromatogr. Libr.* **15**, 617–713 (1978).
- 3 Van Tamelen, E. E., Dyer, J. R., Whaley, H. A., Carter, H. E. & Whitfield, G. B. Constitution of the streptolin-streptothricin group of *Streptomyces* antibiotics. *J. Am. Chem. Soc.* **83**, 4295–4296 (1961).
- 4 Kusumoto, S., Imaoka, S., Kambayashi, Y. & Shiba, T. Total synthesis of antibiotic streptothricin F. *Tetrahedron Lett.* **23**, 2961–2964 (1982).
- 5 Kusumoto, S., Kambayashi, Y., Imaoka, S., Shima, K. & Shiba, T. Total chemical structure of streptothricin. *J. Antibiot.* **35**, 925–927 (1982).
- 6 Hisamoto, M. *et al.* A-53930A and B, novel N-type Ca²⁺ channel blockers. *J. Antibiot.* **51**, 607–617 (1998).
- 7 Ji, Z., Wang, M., Zhang, J., Wei, S. & Wu, W. Two new members of streptothricin class antibiotics from *Streptomyces qinlingensis* sp. nov. *J. Antibiot.* **60**, 739–744 (2007).
- 8 Ji, Z., Wei, S., Zhang, J., Wu, W. & Wang, M. Identification of streptothricin class antibiotics in the early stage of antibiotics screening by electrospray ionization mass spectrometry. *J. Antibiot.* **61**, 660–667 (2008).
- 9 Ji, Z., Wang, M., Wei, S., Zhang, J. & Wu, W. Isolation, structure elucidation and antibacterial activities of streptothricin acids. *J. Antibiot.* **62**, 233–237 (2009).
- 10 Carter, H. E. *et al.* Streptothricin and streptolin: the structure of streptolidine (roseonine). *J. Am. Chem. Soc.* **83**, 4296–4297 (1961).
- 11 Bycroft, B. W. & King, T. J. Crystal structure of streptolidine, a guanidine-containing amino-acid. *J. Chem. Soc. Chem. Commun.* 652–653 (1972).
- 12 Gan, M. *et al.* Streptothricin derivatives from *Streptomyces* sp. I08A 1776. *J. Nat. Prod.* **74**, 1142–1147 (2011).
- 13 Ando, T. *et al.* New streptothricin-group antibiotics, AN-201 I, II and III. II. Chemical structures. *J. Antibiot.* **40**, 1140–1145 (1987).
- 14 Hunt, A. H., Hamill, R. L., Deboer, J. R. & Presti, E. A. A37812: N-methylstreptothricin F. *J. Antibiot.* **38**, 987–992 (1985).
- 15 Borders, D. B. *et al.* Structures of LL-AC541 and LL-AB664: new streptothricin-type antibiotics. *Tetrahedron* **26**, 3123–3133 (1970).
- 16 Suami, T., Tadano, K.-I., Iimura, Y. & Tanabe, H. Direct conversion of D-glucosamine into D-gulosamine. *Carbohydr. Res.* **135**, 319–323 (1985).
- 17 Angyal, S. J. & Pickles, V. A. Equilibria between pyranoses and furanoses. II. Aldoses. *Aust. J. Chem.* **25**, 1695–1710 (1972).
- 18 Fritzsche-Lang, W., Wilharm, P., Hädicke, E., Fritz, H. & Prinzbach, H. 2,4-Diazabicyclo[3.3.1]nonan-gerüste aus cis-benzoltrioxid. *Chem. Ber.* **118**, 2044–2078 (1985).
- 19 Breitmaier, E. *Structure Elucidation by NMR in Organic Chemistry: A Practical Guide*. 3rd edn, 42–45 (John Wiley & Sons Ltd., Chichester, UK, 2002).
- 20 Kinoshita, M. & Suzuki, Y. Synthesis of streptolidine lactam, a guanidine-containing amino-acid lactam moiety of streptothricin antibiotic group. *Bull. Chem. Soc. Jpn.* **50**, 2375–2378 (1977).
- 21 Taniyama, H. & Sawada, Y. Studies on chromophore groups of streptothricin group antibiotics by optical rotatory dispersion and circular dichroism. *Chem. Pharm. Bull.* **20**, 596–600 (1972).
- 22 Červinka, O., Hub, L., Snatzke, F. & Snatzke, G. Circular dichroism of some five- and six-membered lactones and lactams. *Collect. Czech. Chem. Commun.* **38**, 897–901 (1973).
- 23 Wolf, H. Optische rotationsdispersions- und zirkulardichroismus-spektren von lactonen und lactamen. *Tetrahedron Lett.* **6**, 1075–1081 (1965).
- 24 Wolf, H. δ -Lactone cotton-effect und konformation des δ -lactonringes. *Tetrahedron Lett.* **7**, 5151–5156 (1966).
- 25 Legrand, M. & Bucourt, R. Activite optique des lactones et analyse conformationnelle a l'aide des angles diedres. *Bull. Soc. Chim. Fr.* 2241–2242 (1967).
- 26 Van Tamelen, E. E. v. & Smisson, E. E. Streptolin, the structure and synthesis of isolysine. *J. Am. Chem. Soc.* **75**, 2031–2035 (1953).
- 27 Taniyama, H., Sawada, Y. & Kitagawa, T. Characterization of racemomycins. *Chem. Pharm. Bull.* **19**, 1627–1634 (1971).
- 28 Taniyama, H., Sawada, Y. & Kitagawa, T. Studies on the β -lysine peptide. I. Formation of copper complex. *Chem. Pharm. Bull.* **19**, 2631–2634 (1971).
- 29 Thiruvengadam, T. K., Gould, S. J., Aberhart, D. J. & Lin, H. J. Biosynthesis of streptothricin F. 5. Formation of β -lysine by *Streptomyces* L-1689-23. *J. Am. Chem. Soc.* **105**, 5470–5476 (1983).
- 30 Sawada, Y., Kubo, T. & Taniyama, H. Biosynthesis of streptothricin antibiotics. I. Incorporation of ¹⁴C-labeled compound into racemomycin-A and distribution of radioactivity. *Chem. Pharm. Bull.* **24**, 2163–2167 (1976).
- 31 Miyashiro, S. *et al.* New streptothricin-group antibiotics, AN-201 I and II. Screening, fermentation, isolation, structure and biological activity. *J. Antibiot.* **36**, 1638–1643 (1983).
- 32 Gould, S. J., Martinkus, K. J. & Tann, C.-H. Biosynthesis of streptothricin F. 1. Observing the interaction of primary and secondary metabolism with [1,2-¹³C]acetate. *J. Am. Chem. Soc.* **103**, 2871–2872 (1981).
- 33 Gould, S. J., Martinkus, K. J. & Tann, C.-H. Studies of nitrogen metabolism using ¹³C NMR spectroscopy. 2. Incorporation of L-[guanido-¹³C,¹⁵N₂]arginine and DL-[guanido-¹³C,²⁻¹⁵N]arginine into streptothricin F. *J. Am. Chem. Soc.* **103**, 4639–4640 (1981).
- 34 Martinkus, K. J., Tann, C.-H. & Gould, S. J. The biosynthesis of the streptolidine moiety in streptothricin F. *Tetrahedron* **39**, 3493–3505 (1983).
- 35 Gould, S. J., Lee, J. & Wityak, J. Biosynthesis of streptothricin F: 7. The fate of the arginine hydrogens. *Bioorg. Chem.* **19**, 333–350 (1991).
- 36 Wakamiya, T. *et al.* Chemical studies on tuberactinomycin. I. The structure of tuberactidine, guanidino amino acid component. *Tetrahedron Lett.* **11**, 3497–3500 (1970).
- 37 Bycroft, B. W. *et al.* Total structure of capreomycin IB, a tuberculostatic peptide antibiotic. *Nature* **231**, 301–302 (1971).
- 38 Tatsuta, K., Mikami, N., Fujimoto, K., Umezawa, S. & Umezawa, H. The structure of chymostatin, a chymotrypsin inhibitor. *J. Antibiot.* **26**, 625–646 (1973).
- 39 Jackson, M. D., Gould, S. J. & Zabriskie, T. M. Studies on the formation and incorporation of streptolidine in the biosynthesis of the peptidyl nucleoside antibiotic streptothricin F. *J. Org. Chem.* **67**, 2934–2941 (2002).
- 40 Chun, J. *et al.* EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **57**, 2259–2261 (2007).
- 41 Mo, S. *et al.* Phelligrins C-F: cytotoxic pyran[4,3-c][2]benzopyran-1,6-dione and furo[3,2-c]pyran-4-one derivatives from the fungus *Phellinus igniarius*. *J. Nat. Prod.* **67**, 823–828 (2004).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (<http://www.nature.com/ja>)