JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

Streptothricin Derivatives from *Streptomyces* sp. I08A 1776

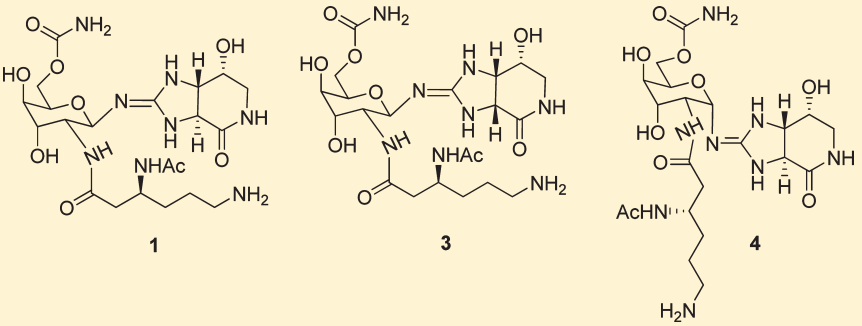
Maoluo Gan,[†] Xudong Zheng,[†] Lishe Gan,[‡] Yan Guan,[†] Xueqin Hao,[†] Yishuang Liu,[†] Shuyi Si,[†] Yuqin Zhang,[†] Liyan Yu,[†] and Chunling Xiao*[,†]

[†]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

[‡]Institute of Modern Chinese Medicine, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China

Supporting Information

ABSTRACT:



Five new streptothricin derivatives with a carbamoyl group substituted at C-12 (**1**−**5**) and three known analogues have been isolated from the culture broth of *Streptomyces* sp. I08A 1776 by ion exchange and hydrophilic interaction chromatographic techniques. Their structures were determined by spectroscopic and chemical methods. Compound **3** was a streptothricin derivative possessing a *cis*-streptolidine moiety. Its absolute configuration was defined by comparison of quantum chemical TDDFT calculated and experimental ECD spectra. Compound **5** and streptothricin E (**6**) displayed antibacterial and antifungal activity with MIC values in the range 1−64 μg/mL.

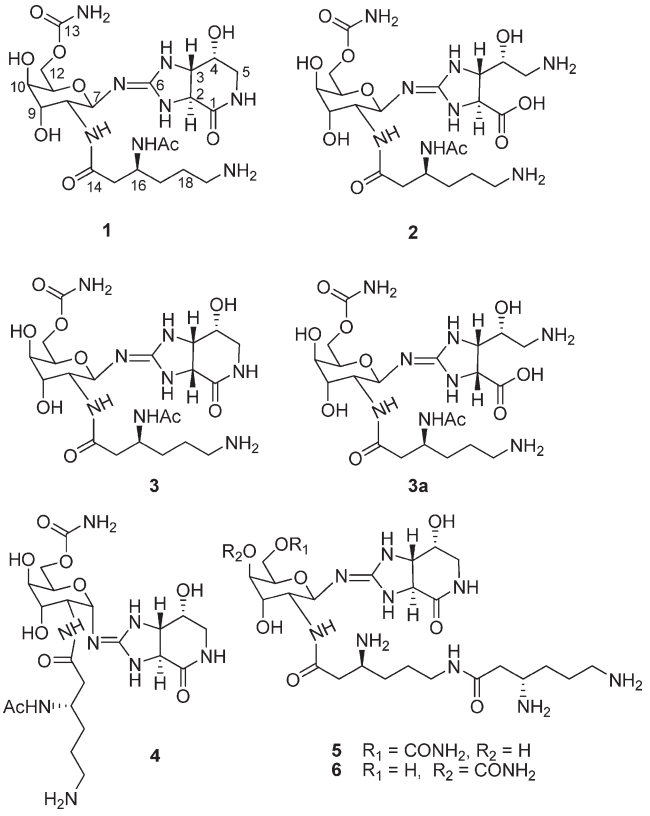
Streptothricins (STs) are a group of peptidyl nucleoside antibiotics found in actinomycetes. The common structures of STs consist of a heterocyclic streptolidine, a carbamoylated D-gulosamine, and an L-β-lysine-derived unit and were confirmed by total synthesis in 1982.[1,2] In most cases, the carbamoyl group was substituted at C-1 0 of the D-gulosamine moiety, to which the exocyclic nitrogen atom of streptolidine was attached through a β-glycosidic linkage.[3] The absolute configurations of the chiral carbons of the streptolidine moiety were established as 2*S*,3*S*,4*R* by X-ray crystallography in 1972.[4] In recent years, several new 12-carbamoyl derivatives of STs were isolated and identified from the fermentation broths of *Streptomyces qinligensis* and *Streptomyces vinaceusdrappus*.[5−7]

In the course of a screening program for new antitubercular antibiotics from microbial products, the actinomycete strain *Streptomyces* sp. I08A 1776 was isolated from a soil sample collected from the Yunnan Province of China. Five new streptothricin antibiotics (**1**−**5**) and three known analogues have been isolated from the fermentation broth of the strain by using a combination of macroporous adsorbent resin, ion exchange, reversed-phase C18, and hydrophilic interaction chromatography methods. Compound **3** was a new member of streptothricins with the streptolidine moiety possessing the unique *cis* configuration, while **4** was an unusual derivative of streptothricin F with an α-D-gulosamine moiety. This paper deals with the isolation and structure elucidation of these new compounds and their *in vitro* antimicrobial and cytotoxic activities.

RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder, and its molecular formula was indicated as $C_{21}H_{36}N_8O_9$ by HRESIMS. The IR spectrum suggested the presence of hydroxy and/or amide NH (3257 cm^{-1}) and amide carbonyl (1648 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra of **1** showed a total of 21 carbon signals, consisting of one methyl, five methylenes, four methines, and five quaternary carbons (four carboxyl and a guanidino), in addition to six characteristic signals assignable to an aminoglycosyl moiety (δ 81.9, 75.2, 72.1, 70.7, 66.8, 51.0). The ^{1}H and ^{13}C NMR spectra of **1** displayed typical features similar to N^{β}-acetylstreptothricin F (AN-201 III), a 10-carbamoylstreptothricin antibiotic isolated from *Streptomyces nojiriensis* C-13.[8] However, comparison of the NMR data of **1** (Tables 1 and 2) with those of

Received: January 25, 2011
Published: April 21, 2011



N^{β}-acetylstreptothricin F indicated that C-12 and H-12 of **1** were downfield shifted to δ 66.8 and 4.28, respectively, whereas C-10 and H-10 were upfield shifted to δ 70.7 and 3.90, respectively. This suggested that the carbamoyl on the D-gulosamine moiety was transferred from C-10 in N^{β}-acetylstreptothricin F to C-12 in **1**.[6] The suggestion was confirmed by an extensive analysis of HSQC, ^{1}H−^{1}H COSY, and HMBC spectra of **1**, in particular, by an HMBC correlation (Figure S1, Supporting Information) from H_2-12 to the carbonyl (δ 161.8). The chemical shifts and coupling constants of H-2 (δ 4.66, d, J = 14.0 Hz), H-3 (δ 4.10, brd, J = 14.0 Hz), and H-4 (δ 4.75, brd, J = 5.0 Hz) were similar to those of the synthetic streptolidine lactam and other natural streptothricins.[6,8−11] This indicated that the streptolidine moiety of **1** had the same relative configuration as the reported compounds. The large coupling constant between H-2 and H-3 (14.0 Hz) indicated the *trans* ring fusion,[12] while the small coupling between H-4 and H-3 (≈0 Hz) suggested a *cis* orientation of the vicinal protons. This was supported by the ROESY spectrum of **1**, which showed correlations of H-3 with H-4 and H-2 with H-5b (Figure 1) with the absence of the correlation between H-2 and H-4. The CD spectrum of **1** showed a positive Cotton effect at 197 nm (Figure S2, Supporting Information) ascribed to the C═N group in the guanidine residue.[9,13] This indicated that the absolute configuration of the streptolidine moiety of **1** was identical to that of streptothricin F, for which the configuration was determined as 2*S*,3*S*,4*R* by X-ray crystallography.[4] Therefore, the structure of **1** was identified as (−)-(2*S*,3*S*,4*R*)-10-de-*O*-carbamoyl-12-*O*-carbamoyl-N^{β}-acetylstreptothricin F.

The molecular formula $C_{21}H_{38}N_8O_{10}$ of **2** had 18 mass units more than that of **1**. Comparison of the NMR data (Tables 1 and 2) between **2** and **1** indicated that the resonances for H-4, H-5a, and H-5b of **2** were shifted by $\Delta\delta$ −0.61, −0.56, and −0.31 ppm, respectively, whereas the resonances of C-4 and C-5 were shifted by $\Delta\delta$ +7.1 and −8.6 ppm, respectively. In addition, the coupling constant between H-2 and H-3 was changed from 14.0 Hz for **1** to 4.5 Hz for **2**. This suggested that the lactam form of the streptolidine moiety in **1** was changed to the open-chain acid form in **2**,[9,11] which was supported by acid hydrolysis of **1** to yield **2**. Therefore, **2** was established as (+)-(2*S*,3*S*,4*R*)-10-de-*O*-carbamoyl-12-*O*-carbamoyl-N^{β}-acetylstreptothricin F acid.

The spectroscopic data of compound **3** (Tables 1 and 2 and Experimental Section) indicated that it was an isomer of **1**. However, in the NMR spectra of **3**, resonances ascribed to the

Table 1. ^{1}H NMR Spectroscopic Data for Compounds 1−5 and 3a in D_2O (δ, mult., J in Hz)[a]

no.	1	2	3	3a	4	5[b]
2	4.66, d (14.0)	4.62, d (4.5)	4.71, d (11.0)	4.75, d (10.8)	4.66, d (15.0)	4.64, d (13.8)
3	4.10, brd (14.0)	4.27, brd (4.5)	4.63, dd (11.0, 3.0)	4.44, dd (10.8, 5.4)	4.08, dd (15.0, 2.5)	4.09, brd (13.8)
4	4.75, brd (5.0)	4.14, d (9.0)	4.28, dd (3.0, 4.5)	4.16, ddd (10.2, 5.4, 2.4)	4.76, dd (5.0, 2.5)	4.74, brd (6.0)
5	3.84, dd (15.0,5.0)	3.28, brd (12.5)	3.50, dd (14.0, 4.5)	3.27, dd (12.6, 2.4)	3.84, dd (15.0, 5.0)	3.82, dd (15.0, 6.0)
	3.44, brd (15.0)	3.13, dd (12.5, 9.0)	3.46, brd (14.0)	3.06, dd (12.6, 10.2)	3.43, d (15.0)	3.41, brd (15.0)
7	5.07, d (10.0)	5.09, d (10.0)	5.06, d (10.0)	5.04, d (10.2)	5.46, brs	5.06, d (9.6)
8	4.30, dd (10.0, 3.5)	4.28, dd (10.0, 3.0)	4.26, dd (10.0, 3.0)	4.26, dd (10.2, 3.0)	4.46, dd (4.5, 3.0)	4.33, dd (9.6, 3.0)
9	4.04, dd (3.5, 3.5)	4.03, dd (3.0, 3.0)	4.02, dd (3.0, 3.0)	4.02, dd (3.0, 3.0)	4.03, dd (3.0, 3.0)	4.09, dd (3,0, 3.0)
10	3.90, d (3.5)	3.87, d (3.0)	3.86, d (3.0)	3.86, d (3.0)	3.94, d (3.0)	3.89, d (3.0)
11	4.37, m	4.33, m	4.33, m	4.33, m	4.35, m	4.36, m
12	4.28, m	4.24, m	4.24, m	4.26, m	4.27, m	4.25, m
15	2.57, dd (14.5, 4.0)	2.55, dd (14.0, 4.5)	2.54, dd (14.5, 5.5)	2.55, dd (14.4, 4.8)	2.60, dd (14.5, 5.0)	2.78, dd (15.0, 4.8)
	2.46, dd (14.5, 9.5)	2.45, dd (14.0, 9.0)	2.46, dd (14.5, 8.5)	2.43, dd (14.4, 9.6)	2.47, dd (14.5, 9.0)	2.69, dd (15.0, 8.4)
16	4.22, m	4.21, m	4.20, m	4.20, m	4.22, m	3.67, m
17	1.66, 1.58, m	1.66, 1.57, m	1.65, 1.56, m	1.65, 1.56, m	1.65, 1.58, m	1.71, m
18	1.70, m	1.69, m	1.70, m	1.70, m	1.70, m	1.63, m
19	3.05, m	3.01, m	3.00, m	3.02, m	3.04, m	3.24, t (7.2)
Ac	2.03, s	2.02, s	2.01, s	2.01, s	2.02, s	

[a] ^{1}H NMR data were acquired at 500 MHz for **1**−**4** and at 600 MHz for **3a** and **5**. [b] Data of the second β-lysine unit of **5**: δ 2.73 (1H, dd, J = 15.0, 4.8 Hz, H-21a), 2.64 (1H, dd, J = 15.0, 8.4 Hz, H-21b), 3.67 (1H, m, H-22), 1.79 (4H, m, H_2-23 and H_2-24), 3.06 (2H, t, J = 6.6 Hz, H_2-25).

dx.doi.org/10.1021/np2000733 |*J. Nat. Prod.* 2011, 74, 1142–1147

Table 2. ^{13}C NMR Spectroscopic Data (δ) for Compounds 1−5 in D_2O[a]

no.	1	2	3	4	5[b]
1	172.7	177.3	172.4	172.5	172.7
2	57.2	60.3	57.4	57.2	57.3
3	63.7	64.5	60.2	63.6	63.7
4	63.7	70.8	65.3	63.6	63.7
5	52.1	43.5	45.8	52.1	52.1
6	165.1	161.1	161.8	164.5	165.2
7	81.9	82.0	82.2	81.2	81.8
8	51.0	51.2	51.2	47.7	51.1
9	72.1	72.1	72.2	71.9	72.0
10	70.7	70.8	70.8	70.8	70.7
11	75.2	75.0	75.2	68.5	75.3
12	66.8	66.7	66.9	66.9	66.7
13	161.8	161.8	162.0	161.9	161.9
14	176.5	176.5	176.6	176.5	174.9
15	44.0	44.0	44.1	44.0	39.5
16	49.6	49.7	49.7	49.7	51.3
17	33.9	33.8	33.8	33.8	32.3
18	26.3	26.3	26.4	26.3	27.1
19	41.9	41.9	42.0	42.0	41.7
Ac	24.9	24.9	24.9	24.9	
	176.4	176.4	176.6	176.5	

[a] ^{13}C NMR data were acquired at 125 MHz for **1**−**4** and at 150 MHz for **5**. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] Data of the second β-lysine unit of **5**: δ 174.5 (C-20), 39.5 (C-21), 51.3 (C-22), 32.0 (C-23), 25.8 (C-24), 41.8 (C-25).

streptolidine moiety were shifted significantly. The resonances for H-3, H-4, and H-5a in **3** were shifted by $\Delta\delta$ +0.53, −0.47, and −0.34 ppm, respectively, as compared with those in **1**, whereas the resonances of C-3−C-6 were shifted by $\Delta\delta$ −3.5, +1.6, −6.3, and −3.3 ppm, respectively. This suggested that the configuration of the streptolidine lactam moiety in **3** was changed. Detailed comparison of ^{1}H NMR data between **3** and **1** and between **3a** (the acid hydrolysis product of **3**) and **2** indicated that the coupling constant ($J_{2,3}$) between H-2 and H-3 was changed from 14.0 Hz for **1** to 11.0 Hz for **3**, while the $J_{2,3}$ was changed from 4.5 Hz for **2** to 10.8 Hz for **3a**. This suggested that the streptolidine lactam ring had the *cis* configuration in **3**.[14,15] In addition, cross-peaks of H-2 with H-3, H-4, and H-5a and between H-3 and H-4 in the ROESY spectrum of **3** demonstrated that these protons were on the same side of the ring system (Figure 1) and suggested that **3** was a 2-epimer of **1**. The CD spectrum of **3** showed a negative Cotton effect at 220 nm, corresponding to the n→π^* transition of the lactam residue in addition to a positive Cotton effect at 195 nm. On the basis of the quadrant rule (Figure S3, Supporting Information) for the peptide group, a 2*R*,3*S*-configuration was assigned by the negative Cotton effect at 220 nm.[16,17] Further evidence for the assignment of the absolute configuration was obtained using quantum chemical TDDFT. The ECD spectrum of 2*R*,3*S*,4*R*-**3** was calculated and then compared with the experimental ECD spectrum (Figure 2). The result showed that the calculated and experimental CD curves coincided very well, which supported the configuration assignment by quadrant rule analysis. Accordingly, the structure of **3** was identified as (−)-(2*R*,3*S*,4*R*)-10-de-*O*-carbamoyl-12-*O*-carbamoyl-*N*$^{\beta}$-acetylstreptothricin F.

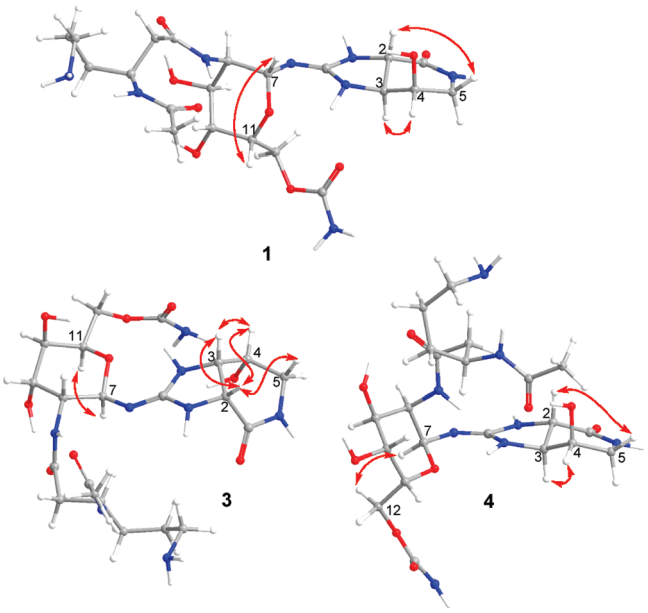


Figure 1. Lowest energy 3D conformations and key ROESY correlations (H↔H) of compounds **1**, **3**, and **4**.

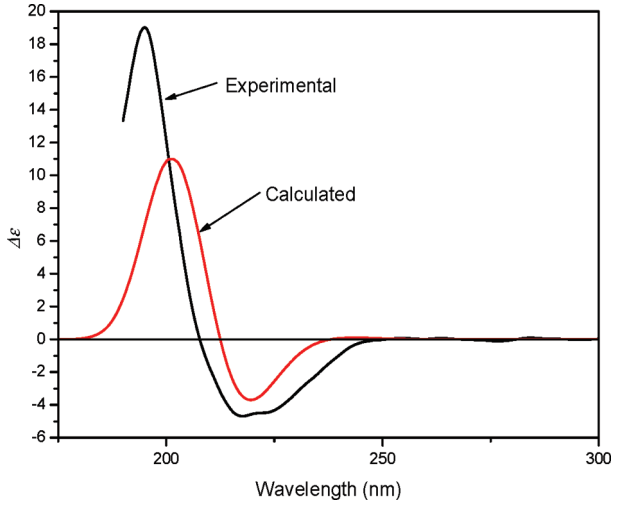


Figure 2. Experimental and B3LYP/aug-cc-pVDZ//B3LYP/6-31G-(d)-calculated ECD spectra of **3** (conformationally averaged by relative Gibbs free energy; half the bandwidth at 1/e peak height expressed in energy units, σ = 0.25 eV; the calculated wavelengths were blue-shifted by 20 nm to match the experimental data).

Compound **4** was isolated as a white powder with a positive optical rotation. Its CD, IR, NMR, and ESIMS spectroscopic features were almost identical to those of **1** (Tables 1 and 2 and Experimental Section) except that the doublet for the anomeric proton of **1** at δ 5.07 (d, J = 10.0 Hz) was replaced by a broad singlet of **4** at δ 5.46. In addition, H-8 (δ 4.46, dd, $J_{7,8}$ = 4.5 Hz, $J_{8,9}$ = 3.0 Hz) of **4** was shifted downfield by $\Delta\delta$ 0.16 ppm, whereas C-7, C-8, and C-11 were shifted upfield by $\Delta\delta$ 0.7, 3.3, and 6.7 ppm, respectively, as compared with those of **1**. These data indicated that the configuration of the anomeric carbon of the gulosamine moiety changed from β in **1** to α in **4**.[11,18,19] This was confirmed by the presence of a correlation between H-7 and H-12 and the absence of a cross-peak between H-7 and H-11 in

dx.doi.org/10.1021/np2000733 |J. Nat. Prod. 2011, 74, 1142–1147

Table 3. Antimicrobial Activities of Compounds 1−8

organism	MIC (μg/mL)			
	1−**4**, **7**, and **8**	**5**	**6**	levofloxacin
Staphylococcus aureus ATCC 25923	>128	>128	4	0.12
Enterococcus faecalis ATCC 29212	>128	>128	64	0.50
Bacillus subtilis ATCC 6633	>128	16	1	0.12
Mycobacterium smegmatis MC^2 155	>128	64	4	1
Mycobacterium tuberculosis H37Rv	>128	>128	16	0.50
Pseudomonas aeruginosa ATCC 27853	>128	>128	64	0.25
Morganella morganii 07−09 (multidrug-resistant strain)	>128	>128	4	16
Escherichia coli ATCC 25922	>128	>128	16	0.12
Candida albicans ATCC 10231	>128	32	1	>128

the ROESY spectrum of **4** (Figure 1). Therefore, the structure of **4** was elucidated as (+)-(2*S*,3*S*,4*R*,7α)-10-de-*O*-carbamoyl-12-*O*-carbamoyl-N^{β}-acetylstreptothricin F. Compound **4** was the first example of streptothricins possessing an α-glycosidic linkage.

The molecular formula of compound **5** was established as $C_{25}H_{46}N_{10}O_9$ by HRESIMS. The NMR data (Tables 1 and 2) showed close similarity to those of **1**, with the exception of the presence of the signals due to an additional β-lysine moiety and the lack of the resonance for the acetyl group in **5**. The HMBC spectrum of **5** showed a cross-peak between H_2-19 and C-20, indicating that the second β-lysine moiety was linked at C-19 via a peptide bond. On the basis of the foregoing evidence, the structure of **5** was established as (−)-(2*S*,3*S*,4*R*)-10-de-*O*-carbamoyl-12-*O*-carbamoylstreptothricin E.

Bycroft and King proposed a chemically rational biogenesis of streptothricin F from arginine via capreomycidine,[4] which was supported by the incorporation studies using [1,2-^{13}C] acetate and multiple forms of ^{13}C-, ^{15}N-, and ^{2}H-labeled arginine.[20−23] A number of pathways can be proposed for the conversion of arginine to capreomycidine.[22,24] The incorporation studies with ^{2}H-labeled arginine help exclude several pathways but do not completely support a single route.[22] For example, the observed loss of the C-2 and C-3 hydrogens cannot be fully explained by the proposed intermediate such as 2,3-dehydroarginine or 3-ketoarginine. The 3-ketoarginine pathway proposed by Gould is supported by the loss of both C-3 ^{2}H but not loss of the C-2 ^{2}H. The loss of the C-2 hydrogen due to an epimerase reaction was thought to be unlikely by the author based on the observation that the 2*S*-configuration of the starting arginine is retained in the product and no 2*R*-epimers were found in previous studies.[22] However, the co-occurrence of both 2*S* (**1**, **2**, **4**, and **5**) and 2*R* (**3**) epimers in the metabolites of *Streptomyces* sp. I08A 1776 indicated that this epimerase reaction at C-2 of arginine probably occurred in the biosynthesis of STs in this strain.

The known compounds were identified by comparison of spectroscopic data with those reported in the literature as streptothricin E (**6**),[8] N^{β}-acetylstreptothricin D (AN-201 II, **7**),[8] and N^{β}-acetylstreptothricin E (AN-201 I, **8**).[8]

The antimicrobial activities of **1**−**8** against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Escherichia coli*, and *Candida albicans* were evaluated by the microbroth dilution method. As shown in Table 3, compound **6** showed antimicrobial activities against all the above microbes with MICs in the range 1−64 μg/mL, while **5** showed selective inhibitory activities against *B. subtilis*, *M. smegmatis*, and *C. albicans*. Other compounds were inactive, with MIC values > 128 μg/mL. This result indicated that the acetylation of β-lysine and the transformation of carbamoyl from C-10 to C-12 resulted in the loss or/and decrease of antimicrobial activity. This phenomenon was consistent with those of STs previously reported.[6,25] In addition, the isolates were also assessed for their cytotoxicities against human lung adenocarcinoma (A549), hepatocellular carcinoma (HepG2), and cervical cancer (HeLa) cell lines by using the MTT method, but were inactive (IC_{50} values >10 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 343 polarimeter. CD spectra were recorded on a JASCO-815 CD spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR microscope spectrometer (FT-IR microscope transmission). 1D- and 2D-NMR spectra were obtained at 500 or 600 MHz for ^{1}H and 125 or 150 MHz for ^{13}C, respectively, on INOVA 500 MHz or VNMRS 600 MHz spectrometers in D_2O. The solvent peak (DOH, δ_H 4.80) was used as an internal standard for ^{1}H NMR spectra, and TMS was used as external reference for ^{13}C NMR spectra. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray source) spectrometer. HRESIMS data were measured using a JMS-T100CS AccuToF CS or Applied Biosystem QSTAR Elite spectrometer. Column chromatography was performed with Diaion HP-20 macroporous resin and MCI gel CHP-20P (Mitsubishi Chemical Inc., Japan), CM-Sephadex C-25 and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden), and Toyopearl SuperQ 650 M (Tosoh Co., Japan). HPLC separations were performed on a Shimadzu HPLC apparatus consisting of a LC-20AD pump and a SPD-M20A diode array detector with a Capcell Pak C18 AQ column (10 × 250 mm, 5 μm, Shiseido Co., Japan) and a TSK-Gel Amide-80 column (10 × 250 mm, 5 μm, Tosoh Co.).

Microorganism and Fermentation. Strain I08A 1776 was isolated from a soil sample collected from Shuangbai County, Yunnan Province of China, and identified as a member of the genus *Streptomyces* on the basis of 16S rRNA sequence analysis. Its 16S rRNA sequence showed high homology with those of members of the genus *Streptomyces*, such as *S. erythrochromogenes* (GenBank AB184746, 1449 base pairs out of 1453, 99%) and *S. polychromogenes* (GenBank AB184292, 1445/1453, 99%). Its sequence was deposited in GenBank with accession number HQ704719. 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).

dx.doi.org/10.1021/np2000733 |*J. Nat. Prod.* 2011, 74, 1142–1147

A slant culture of *Streptomyces* sp. I08A 1776 was inoculated in 250 mL Erlenmeyer flasks containing 50 mL of a sterile 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%, $CaCO_3$ 0.4%, and $CoCl_2 \cdot 6H_2O$ 0.002% in deionized water (pH 7.2 before sterilization) and then scaled up to 1000 mL flasks containing 300 mL of the medium. After incubation on a rotary shaker at 28 °C for 48 h, a total of 2.5 L of the precultures was transferred into a 50 L fermentor containing 25 L of the same medium. The fermentations were carried out at 28 °C for 72 h with aeration and agitation.

Isolation. The harvested culture broth (50 L) was filtered and adjusted to pH 8.0, and the filtrate was subjected to a Diaion HP-20 macroporous adsorbent resin column (5 L). A successive elution of the column with H_2O and 20%, 50%, and 100% acetone in H_2O yielded four corresponding fractions (F_1−F_4) after removing solvents. The antibiotic fraction (F_2, 90 g) was chromatographed over MCI gel CHP-20P (1 L), eluting successively with H_2O and 20%, 50%, and 100% MeOH in H_2O, to yield four subfractions ($F_{2\text{-}1}$−$F_{2\text{-}4}$). Separation of $F_{2\text{-}1}$ over CM-Sephadex C-25 (NH_4^+, 500 mL) using a linear gradient of aqueous NaCl solution (0−2 M) gave six residues ($F_{2\text{-}1\text{-}1}$−$F_{2\text{-}1\text{-}6}$) on the basis of TLC analysis and antibiotic assay after removing solvents. $F_{2\text{-}1\text{-}4}$ was extracted with MeOH−H_2O (3:1) to remove salts. The methanol extract was separated by column chromatography over Sephadex LH-20 (3 × 120 cm) eluting with water and then subjected to reversed-phase preparative HPLC (Capcell Pak C18 AQ 10 × 250 mm, 210 nm) using a mobile phase of 0.1% trifluoroacetic acid (TFA) aqueous solution to yield three mixtures ($F_{2\text{-}1\text{-}4\text{-}1}$−$F_{2\text{-}1\text{-}4\text{-}3}$). $F_{2\text{-}1\text{-}4\text{-}1}$ was purified by hydrophilic interaction chromatography[26] preparative HPLC (TSK Gel Amide-80 10 × 250 mm, 210 nm) using 76% acetonitrile containing 0.1% TFA as a mobile phase to yield the trifluoroacetates of **1** and **2**. Using the same HPLC system, the trifluoroacetates of **3** and **4** were obtained from $F_{2\text{-}1\text{-}4\text{-}2}$ and $F_{2\text{-}1\text{-}4\text{-}3}$, respectively. Using the similar procedure as described above for the isolation of **1**−**4**, **5** was obtained from $F_{2\text{-}2}$. The trifluoroacetates of **1**−**5** were separately applied to anion exchange chromatography over SuperQ-650 M (Cl^-, 1 mL) to exchange TFA to afford the hydrochlorides of **1** (12 mg), **2** (5 mg), **3** (18 mg), **4** (4 mg), and **5** (5 mg), respectively.

(−)-(2*S*,3*S*,4*R*)-10-De-*O*-carbamoyl-12-*O*-carbamoyl-N^{β}-acetylstreptothricin F (1): white, amorphous powder; $[\alpha]_D^{20}$ −8.1 (*c* 0.48, H_2O); CD (H_2O) 197 ($\Delta\varepsilon$ +19.6) nm; IR ν_{max} 3257, 1648, 1554, 1078 cm^{-1}; ^1H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Tables 1 and 2; ESIMS *m*/*z* 545 $[M + H]^+$ and 567 $[M + Na]^+$; HRESIMS *m*/*z* 567.2483 $[M + Na]^+$ (calcd for $C_{21}H_{36}N_8O_9Na$ 567.2503).

(+)-(2*S*,3*S*,4*R*)-10-De-*O*-carbamoyl-12-*O*-carbamoyl-N^{β}-acetylstreptothricin F acid (2): white, amorphous powder; $[\alpha]_D^{20}$ +2.4 (*c* 0.20, H_2O); CD (H_2O) 198 ($\Delta\varepsilon$ +41.8), 219 ($\Delta\varepsilon$ +11.4) nm; IR ν_{max} 3325, 1647, 1414, 1080 cm^{-1}; ^1H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Tables 1 and 2; ESIMS *m*/*z* 563 $[M + H]^+$ and 585 $[M + Na]^+$; HRESIMS *m*/*z* 563.2778 $[M + H]^+$ (calcd for $C_{21}H_{39}N_8O_{10}$ 563.2789).

(−)-(2*R*,3*S*,4*R*)-10-De-*O*-carbamoyl-12-*O*-carbamoyl-N^{β}-acetylstreptothricin F (3): white, amorphous powder; $[\alpha]_D^{20}$ −34.5 (*c* 0.80, H_2O); CD (H_2O) 195 ($\Delta\varepsilon$ +19.0), 220 ($\Delta\varepsilon$ −4.6) nm; IR ν_{max} 3276, 1658, 1552, 1080 cm^{-1}; ^1H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Tables 1 and 2; ESIMS *m*/*z* 545 $[M + H]^+$ and 567 $[M + Na]^+$; HRESIMS *m*/*z* 545.2627 $[M + H]^+$ (calcd for $C_{21}H_{37}N_8O_9$ 545.2684).

(+)-(2*S*,3*S*,4*R*,7α)-10-De-*O*-carbamoyl-12-*O*-carbamoyl-N^{β}-acetylstreptothricin F (4): white, amorphous powder; $[\alpha]_D^{20}$ +45.4 (*c* 0.16, H_2O); CD (H_2O) 197 ($\Delta\varepsilon$ +31.5), 220 ($\Delta\varepsilon$ +3.5) nm; IR ν_{max} 3274, 1648, 1554, 1082 cm^{-1}; ^1H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Tables 1 and 2; ESIMS *m*/*z* 545 $[M + H]^+$ and 567 $[M + Na]^+$; HRESIMS *m*/*z* 545.2692 $[M + H]^+$ (calcd for $C_{21}H_{37}N_8O_9$ 545.2684).

(−)-(2*S*,3*S*,4*R*)-10-De-*O*-carbamoyl-12-*O*-carbamoylstreptothricin E (5): $[\alpha]_D^{20}$ −12.5 (*c* 0.20, H_2O); CD (H_2O) 201 ($\Delta\varepsilon$ +1.3) nm; IR ν_{max} 3448, 1645, 1399, 1205 cm^{-1}; ^1H NMR (D_2O, 600 MHz) and ^{13}C NMR (D_2O, 150 MHz) data, see Tables 1 and 2; ESIMS *m*/*z* 631 $[M + H]^+$ and 653 $[M + Na]^+$; HRESIMS *m*/*z* 631.3542 $[M + H]^+$ (calcd for $C_{25}H_{47}N_{10}O_9$ 631.3528).

Acid Hydrolysis of 1 and 3. The hydrochloride of **1** (6 mg) or **3** (8 mg) was dissolved in 3 M HCl (5 mL) and stirred at 50 °C for a day. The hydrolysate solution was lyophilized to yield a residue. The residue was subjected to the same reversed-phase preparative HPLC and then anion exchange chromatography described above for the isolation of **1** to afford **1a** (2 mg from **1**) and **3a** (2 mg from **3**), respectively. The structure of **1a** was determined as **2** by ESIMS and ^1H NMR data (Supporting Information). **3a**: $[\alpha]_D^{20}$ +2.5 (*c* 0.08, H_2O); CD (H_2O) 204 ($\Delta\varepsilon$ +11.0), 225 ($\Delta\varepsilon$ −6.1) nm; ^1H NMR (D_2O, 600 MHz) data, see Table 1; ESIMS *m*/*z* 563 $[M + H]^+$ and 585 $[M + Na]^+$.

ECD Calculation of 3. First, conformational analysis of **3** was carried out via Monte Carlo searching with the MMFF94 molecular mechanics force field using the SPARTAN 04 program.[27] The flexible molecule showed over 100 conformers, whereas only two lowest energy conformers whose Boltzmann distribution are over 10%. Subsequently, the conformers were reoptimized using DFT at the B3LYP/6-31G(d) level in the gas phase using the GAUSSIAN 09 program.[28] The B3LYP/6-31G(d) harmonic vibrational frequencies were further calculated to confirm their stability. The energies, oscillator strengths, and rotational strengths of the first nine electronic excitations of the two conformers were calculated using the TDDFT methodology at the B3LYP/aug-cc-pVDZ level in the gas phase, and the ECD spectra were then simulated by the overlapping Gaussian function.[29] To get the final spectrum of **3**, the simulated spectra of the two lowest energy conformations were averaged according to the Boltzmann distribution theory, in which their Gibbs free energies (*G*) were adopted. In the 200−400 nm region, compared to the experimental negative first Cotton effect at 220 nm and positive second Cotton effect at 195 nm, the calculated one showed the same pattern, but the corresponding wavelength shifted to 240 nm (+20 nm) and 220 nm (+25 nm), respectively. Therefore, qualitative analysis of the result allowed the confirmation of the absolute configuration of **3**.

Antibacterial and Antifungal Assays. The minimal inhibitory concentrations (MICs) of the purified compounds were determined by the broth microdilution method in 96-well culture plates as recommended by the Clinical and Laboratory Standards Institute.[30,31] Organisms used in this study included strains from ATCC collection and clinical isolates. Levofloxacin was used as positive control. Compounds were tested at final concentrations (prepared from serial 2-fold dilutions) ranging from 128 to 0.125 μg/mL. The test media were Middlebrook 7H9 broth for *Mycobacterium* strains and Mueller-Hinton broth for other strains. The final concentration of inoculum was 1×10^5 cfu/mL. After incubation for 14 days for *M. tuberculosis* H37Rv or 24 h for other strains at 37 °C, the minimal inhibitory concentration end points were read and were defined as the lowest antibiotic concentration that yielded no visible microbial growth.

Cells, Culture Conditions, and Cell Proliferation Assay. See ref 32.

■ ASSOCIATED CONTENT

Supporting Information. MS, IR, 1D and 2D NMR spectra of compounds **1**−**5** and ECD calculation details of **3**. Figure S1 of HMBC correlation scheme and Figure S2 of CD spectra of compounds **1**−**5**. Figure S3 of quadrant projection

diagram of **1** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: 86-10-63020226. Fax: 86-10-63180604. E-mail: xiaocl318@163.com.

ACKNOWLEDGMENT

We wish to thank Prof. J. Shi (Institute of Materia Medica, Chinese Academy of Medical Sciences) for reading the manuscript and enlightening discussions. We also thank Prof. J. Zhang (Institute of Medical Science, School of Medicine, Shanghai Jiaotong University) for his kind help with the calculation of ECD spectrum. Financial support from the Natural Sciences Foundation of China (NSFC, Grant No. 81001386), Central Public Scientific Research Institution Project (Grant No. IMBF-200914), the National Scientific and Technological Key Special Project of China (Grant No. 2008ZX10003-006), and the National Facilities and Information Infrastructure for Science and Technology (Grant No. 2005DKA21203) is gratefully acknowledged.

REFERENCES

(1) Van Tamelen, E. E.; Dyer, J. R.; Whaley, H. A.; Carter, H. E.; Whitfield, G. B. *J. Am. Chem. Soc.* **1961**, *83*, 4295–4296.
(2) Kusumoto, S.; Imaoka, S.; Kambayashi, Y.; Shiba, T. *Tetrahedron Lett.* **1982**, *23*, 2961–2964.
(3) Kusumoto, S.; Kambayashi, Y.; Imaoka, S.; Shima, K.; Shiba, T. *J. Antibiot.* **1982**, *35*, 925–927.
(4) Bycroft, B. W.; King, T. J. *J. Chem. Soc., Chem. Commun.* **1972**, 652–653.
(5) Hisamoto, M.; Inaoka, Y.; Sakaida, Y.; Kagazaki, T.; Enokida, R.; Okazaki, T.; Haruyama, H.; Kinoshita, T.; Matsuda, K. *J. Antibiot.* **1998**, *51*, 607–617.
(6) Ji, Z.; Wang, M.; Zhang, J.; Wei, S.; Wu, W. *J. Antibiot.* **2007**, *60*, 739–744.
(7) Ji, Z.; Wei, S.; Zhang, J.; Wu, W.; Wang, M. *J. Antibiot.* **2008**, *61*, 660–667.
(8) Ando, T.; Miyashiro, S.; Hirayama, K.; Kida, T.; Shibai, H.; Murai, A.; Udaka, S. *J. Antibiot.* **1987**, *40*, 1140–1145.
(9) Kinoshita, M.; Suzuki, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 2375–2378.
(10) Hunt, A. H.; Hamill, R. L.; Deboer, J. R.; Presti, E. A. *J. Antibiot.* **1985**, *38*, 987–992.
(11) Borders, D. B.; Sax, K. J.; Lancaster, J. E.; Hausmann, W. K.; Mitscher, L. A.; Wetzel, E. R.; Patterson, E. L. *Tetrahedron* **1970**, *26*, 3123–3133.
(12) Wendelin, W.; Gössnitzer, E.; El Ella, D. A. *Monatsh. Chem.* **2000**, *131*, 353–374.
(13) Taniyama, H.; Sawada, Y. *Chem. Pharm. Bull.* **1972**, *20*, 596–600.
(14) Fritsche-Lang, W.; Wilharm, P.; Hädicke, E.; Fritz, H.; Prinzbach, H. *Chem. Ber.* **1985**, *118*, 2044–2078.
(15) Breitmaier, E. *Structure Elucidation by NMR in Organic Chemistry: A Practical Guide*, 3rd ed.; John Wiley & Sons, Ltd.: Chichester, 2002; pp 42−45.
(16) Litman, B. J.; Schellman, J. A. *J. Phys. Chem.* **1965**, *69*, 978–983.
(17) Schellman, J. A. *Acc. Chem. Res.* **1968**, *1*, 144–151.
(18) Suami, T.; Tadano, K.-I.; Iimura, Y.; Tanabe, H. *Carbohydr. Res.* **1985**, *135*, 319–323.
(19) Angyal, S. J.; Pickles, V. A. *Aust. J. Chem.* **1972**, *25*, 1695–1710.
(20) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 2871–2872.
(21) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 4639–4640.
(22) Martinkus, K. J.; Tann, C.-H.; Gould, S. J. *Tetrahedron* **1983**, *39*, 3493–3505.
(23) Gould, S. J.; Lee, J.; Wityak, J. *Bioorg. Chem.* **1991**, *19*, 333–350.
(24) Jackson, M. D.; Gould, S. J.; Zabriskie, T. M. *J. Org. Chem.* **2002**, *67*, 2934–2941.
(25) Miyashiro, S.; Ando, T.; Hirayama, K.; Kida, T.; Shibai, H.; Murai, A.; Shiio, T.; Udaka, S. *J. Antibiot.* **1983**, *36*, 1638–1643.
(26) Yoshida, T. *Anal. Chem.* **1997**, *69*, 3038–3043.
(27) *Spartan 04*; Wavefunction, Inc.: Irvine, CA.
(28) *Gaussian 09*, Revision A.1; Gaussian, Inc.: Wallingford, CT, 2009. A full list of authors can be found in the Supporting Information.
(29) Stephens, P. J.; Harada, N. *Chirality* **2010**, *22*, 229–233.
(30) Clinical Laboratory Standards (CLSI/NCCLS) Approved Standard M7-A8, 2009.
(31) Clinical Laboratory Standards (CLSI/NCCLS) Approved standard M38-A2, 2008.
(32) Mo, S.; Wang, S.; Zhou, G.; Yang, Y.; Li, Y.; Chen, X.; Shi, J. *J. Nat. Prod.* **2004**, *67*, 823–828.