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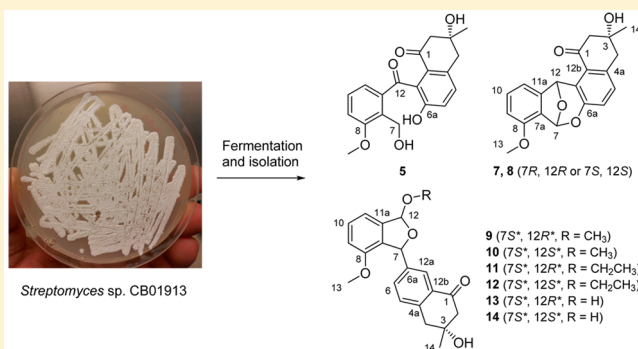
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Angucyclines and Angucyclinones from *Streptomyces* sp. CB01913
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S Supporting Information

ABSTRACT: Angucyclines and angucyclinones are aromatic polyketides with a tetracyclic benz[*a*]anthracene skeleton. The benz[*a*]anthracene scaffold is biosynthesized by type II polyketide synthases that catalyze the decarboxylative condensation of a short acyl-CoA starter and nine extender units. Angucyclines and angucyclinones, the largest group of polycyclic aromatic polyketides, achieve structural diversity via subsequent oxidation, ring cleavage, amino acid incorporation, and glycosylation. We here report the discovery of 14 angucyclinones and two angucyclines (1–16) from *Streptomyces* sp. CB01913, identifying 12 new compounds featuring various oxidations on rings A and C (1, 2, and 4), different sugar moieties attached to rings A and B (3 and 6), and C-ring cleavage (5 and 10–14) and expansion (8). These new structural features, highlighted by C-ring cleavage and expansion, enrich the structural diversity of angucyclines and angucyclinones. All compounds were tested for cytotoxicity and antibacterial activities, with 1, 5, 15, and 16 showing moderate activities against selected cancer cell lines or bacterial strains.



Angucyclines and angucyclinones are aromatic polyketide natural products featuring a tetracyclic benz[*a*]anthracene skeleton that is distinct from the benz[*b*]anthracene skeleton found in tetracyclines and anthracyclines.¹ Since the isolation of tetrangomycin, the first angucyclinone discovered, from *Streptomyces rimosus* in 1965,² the angucycline and angucyclinone family of natural products has grown steadily. To date, this family of natural products has been discovered exclusively from actinomycetes, with *Streptomyces* as the major producers.^{1,2} Representing the largest family of polycyclic aromatic polyketides known to date, angucyclines and angucyclinones feature a characteristic tetracyclic benz[*a*]anthracene scaffold that is biosynthesized by type II polyketide synthases (PKSs) via decarboxylative condensations of a short acyl-CoA starter and nine extender units.¹ The structural diversity of angucyclines and angucyclinones mainly comes from (i) oxidations on the angular tetracyclic backbone, including hydroxy substitutions at C-4/5/6a/7/12/12a in the saccharothrixins,³ epoxidation at C-5/6 or C-6a/12a in the panglimyins,⁴ or carbonyl substitutions at C-7 and C-12 in the

hatomarubigins;⁵ (ii) amino acid incorporations, as in the two-point attachment of different amino acids to form yet another ring in jadomycins⁶ or of tyrosine and tryptophan into urdamycins C and D;⁷ (iii) ring cleavages including A-ring cleavage in the grincamycins,⁸ B-ring cleavage in the gilvocarcins,⁹ or C-ring cleavage in emycins E and F;¹⁰ and (iv) glycosylation at various positions, such as the multiple trisaccharide units of D-olivose-4-1-D-olivose-3-1-L-rhodinose attached at the O-8 position in the landomycins¹¹ or deoxysugars attached at the O-3 and C-9 positions in the saquayamycins.¹²

As part of the Natural Products Library Initiative (NPLI) at The Scripps Research Institute (TSRI), dedicated to the discovery of natural products from our Actinomycetales strain collection,^{13–17} we report the discovery of 14 angucyclinones and two angucyclines (1–16) from *Streptomyces* sp. CB01913, including 12 new compounds with (i) various oxidations on

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Chart 1

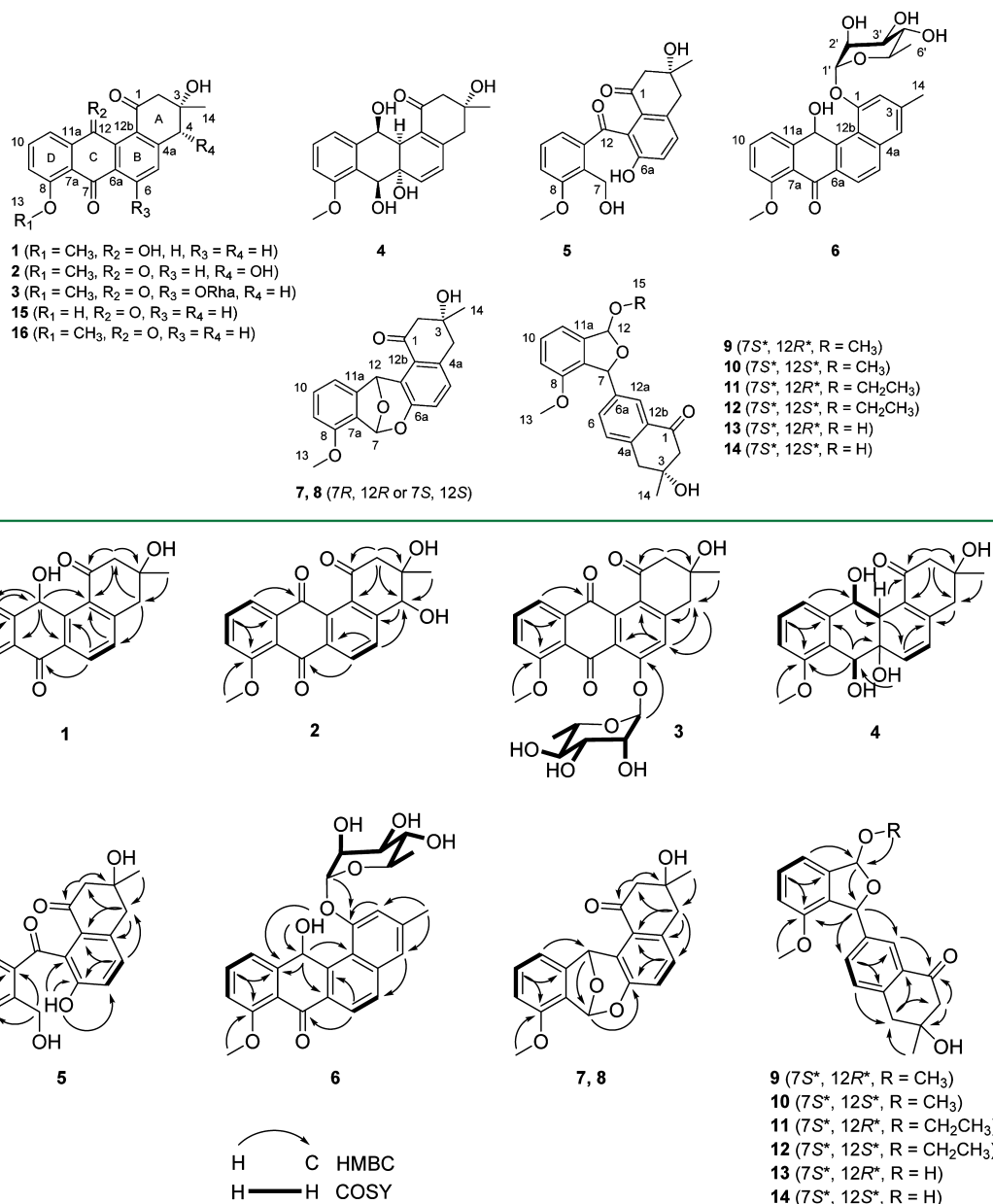


Figure 1. Key COSY and HMBC correlations supporting structural assignments of 1–14.

rings A and C (1, 2, and 4), (ii) different glycosylations on rings A and B (3 and 6), and (iii) C-ring cleavage (5 and 10–14) and expansion (8). The natural products isolated in this study show unique characteristics compared with known angucyclines and angucyclinones: (i) 9–14 are the only members with C-ring cleavage between C-12 and C-12a; (ii) 5, 7, and 8 represent rare C-ring cleavage or expansion between C-6a and C-7; (iii) 3 and previously reported TAN-108S² represent the only two angucyclines with sugar moieties attached at C-6; and (iv) 1, 4, and 6 contain a hydroxy group, instead of the usual carbonyl, at C-12. These new structural features, especially the C-ring cleavage and expansion represented by 5, 8, and 10–14, enrich the structural diversity of the angucycline and angucyclinone family of natural products. All compounds were tested for cytotoxicity and antibacterial activities, with 1, 5, 15, and 16 showing moderate activities against selected cancer cell lines or bacterial strains.

RESULTS AND DISCUSSION

Strain Selection, Taxonomy, and Fermentation Optimization. The NPLI at TSRI aims at constructing a natural products library with unique chemical and structural diversities that complements TSRI's small-molecule collection. The NPLI biases natural products from Actinomycetales that are isolated from unexplored or underexplored ecological niches and unavailable in public strain collections. The current library at TSRI consists of (i) purified natural products with fully assigned structures, (ii) medium-pressure liquid chromatography (MPLC) (on C-18 semipreparative column) fractions, and (iii) extracts. The extracts are generated by fermenting each strain in two media (medium A and medium F, Table S1), and the resultant extracts are subjected to HPLC-photodiode array (PDA)-based chemical profiling, which then guides strain selection for subsequent MPLC fractionation and natural

Table 1. ¹H (700 MHz) NMR Data (δ_H, J in Hz) for 1, 2, 4, 5, 7, and 8 in DMSO-*d*₆^a

position	1	2	4	5	7	8
2	2.93, d (14.9)	3.05, d (14.9)	2.55, d (15.6)	2.64, br s	2.94, d (15.1)	2.67, d (16.6)
	2.73, dd (14.9, 2.2)	2.77, d (14.9)	2.43, d (15.6)	2.46, dd (16.2, 1.8)	2.59, dd (15.1, 2.2)	2.72, dd (16.6, 1.8)
4	3.22, d (16.9)	4.72, s	2.65, d (16.7)	3.04, d (16.3)	3.05, d (16.2)	2.95, d (16.1)
	3.11, dd (16.9, 1.8)		2.58, d (16.7)	2.91, dd (16.3, 1.5)	2.83, dd (16.5, 1.9)	2.87, dd (16.2, 1.3)
5	7.49, d (8.0)	8.05, d (8.1)	6.03, d (10.1)	7.27, d (8.3)	7.11, d (8.3)	7.14, d (8.3)
6	8.10, d (8.1)	8.23, d (8.2)	6.31, d (10.1)	7.17, d (8.3)	6.91, d (8.3)	6.92, d (8.3)
7			4.83, br d (9.7)	4.85, s	6.84, s	6.85, s
9	7.15, d (8.1)	7.57, d (8.0)	7.00, d (7.9)	7.19, dd (8.2, 1.8)	6.97, d (8.2)	6.96, d (8.3)
10	7.63, dd (8.2, 7.6)	7.85, t (8.0)	7.28, t (8.1)	7.24, t (7.8)	7.29, dd (8.2, 7.4)	7.27, dd (8.2, 7.4)
11	7.22, d (7.4)	7.57, d (8.0)	6.90, d (7.9)	6.83, br s	7.14, d (7.4)	7.05, d (7.3)
12	6.39, s		4.86, br s		6.64, s	6.80, s
12a			2.67, br s			
13	3.88, s	3.96, s	3.81, s	3.85, s	3.86, s	3.86, s
14	1.32, s	1.34, s	1.20, s	1.25, s	1.30, s	1.23, s
3-OH		4.82, br s	4.82, s	4.78, br s	4.74, s	4.85, s
4-OH		5.93, br s				
6a-OH			5.15, s	9.89, s		
7-OH			5.24, br d (9.3)	4.78, br s		
12-OH			5.48, br s			

^aAssignments are based on COSY, HMBC, HSQC, and NOESY experiments.

Table 2. ¹H (700 MHz) NMR Data (δ_H, J in Hz) for 3 and 6 in DMSO-*d*₆^a

position	3	6	position	3	6
2	2.91, d (14.4), 2.69, dd (14.4, 1.6)	7.31, d (1.3)	1'	5.66, br s [d (1.7)] ^b	5.60, d (1.7) [d (1.8)] ^b
4	3.14, d (16.7), 3.01, dd (16.7, 1.2)	7.43, br s	2'	3.98, dd (3.2, 1.8) [dd (3.4, 1.8)] ^b	4.41, br s [dd (3.4, 1.9)] ^b
5	7.38, s	7.88, d (8.7)	3'	3.90, dd (9.5, 3.2) [dd (9.5, 3.4)] ^b	3.97, br s [dd (9.2, 3.5)] ^b
6		8.02, d (8.6)	4'	3.36, t (9.4) [t (9.5)] ^b	3.42, m [t (9.2)] ^b
9	7.51, d (8.5)	7.18, d (8.1)	5'	3.55, m	3.62, m
10	7.76, t (8.0)	7.65, br t (8.2)	6'	1.13, d (6.2)	1.23, d (6.2)
11	7.44, d (7.5)	7.29, d (7.4)	3-OH	5.00, br s	
12		6.86, d (6.5)	12-OH		5.56, d (6.6)
13	3.93, s	3.90, s	2'-OH	5.00, br s	5.15, d (4.1)
14	1.31, s	2.46, s	3'-OH	5.00, br s	4.96, br d (5.4)
			4'-OH	5.00, br s	4.97, br d (6.0)

^aAssignments are based on COSY, HMBC, HSQC, and NOESY experiments. ^bCoupling constants in square brackets are calculated after D₂O was added into DMSO-*d*₆.

product isolation. Figure S1A depicts HPLC analysis of extracts made from 20 strains in a typical run of fermentation in medium F. Among the 20 strains, *Streptomyces* sp. CB01913 was found to produce the most detectable natural products based on chemical profiling, as exemplified by the HPLC chromatograms with UV detection at 254 nm. We recently reported methods to guide strain prioritization for natural product discovery by surveying the biosynthetic gene clusters for the targeted class of natural products.^{17a} While these genomics-based methods are effective in prioritizing strains according to the biosynthetic potential, it is far from certain that the predicted biosynthetic machinery will be functional under the fermentation conditions of investigation for natural product production and isolation. The method reported here, chemical profiling of extracts, complements the genomics-based strain prioritization strategies and allows direct assessment of natural products produced under the conditions of investigation. On the basis of the observed natural product abundance, *Streptomyces* sp. CB01913 was selected as a priority strain for natural product isolation.

Streptomyces sp. CB01913 was isolated from a soil sample collected in Weishan County, Yunnan Province, China. It

grows well on ISP4 or TSB agar medium. CB01913 was classified as a *Streptomyces* species on the basis of a phylogenetic analysis using the concatenated partial sequences of the four housekeeping genes 16S rRNA, *recA*, *rpoB*, and *trpB*.^{17a} As shown in Figure S2, CB01913 clearly lies in the same clade with other *Streptomyces* species.

It is well known that the metabolite profile of a given strain is medium dependent.¹⁷ Thus, we re fermented *Streptomyces* sp. CB01913 in four media (media A, B, C, and F, Table S1). HPLC-PDA analysis showed that *Streptomyces* sp. CB01913 yielded the richest metabolite profile in medium B among the four media examined, as exemplified by the HPLC chromatograms with UV detection at 254 nm (Figure S1B). Medium B was selected for large-scale fermentation of *Streptomyces* sp. CB01913, from which the 16 compounds, including 12 new ones (1–6, 8, and 10–14), were isolated.

Structure Elucidation. Compound 1 was obtained as a brown powder. High-resolution electrospray ionization mass spectrometry (HRESIMS) analysis afforded an [M + H]⁺ ion at *m/z* 339.1226, giving the molecular formula of 1 as C₂₀H₁₈O₅. The ¹H, ¹³C, and COSY NMR spectra of 1 resembled those of 8-*O*-methyltetrangomycin (16)^{18a,b} (Figure 1), except that the

Table 3. ^1H (700 MHz) NMR Data (δ_{H} , J in Hz) for 9–14 in $\text{DMSO}-d_6^a$

position	9	10	11	12	13	14
2	2.73, d (16.1)	2.72, d (16.0)	2.73, d (16.1)	2.71, d (16.0)	2.72, d (16.1)	2.73, d (16.1)
	2.59, dd (16.1, 2.1)	2.59, dd (16.0, 1.8)	2.59, dd (16.1, 2.1)	2.59, dd (16.0, 2.0)	2.59, dd (16.1, 2.1)	2.60, dd (16.0, 2.0)
4	3.11, d (16.5)	3.10, d (16.5)	3.10, d (16.5)	3.09, d (16.5)	3.09, d (16.5)	3.10, d (16.6)
	2.95, dd (16.6, 1.8)	2.94, br d (16.5)	2.94, dd (16.7, 1.8)	2.94, dd (16.6, 1.8)	2.93, dd (16.5, 2.1)	2.94, dd (16.3, 2.1)
5	7.28, d (7.9)	7.27, d (7.8)	7.28, d (7.9)	7.27, d (7.8)	7.25, d (8.3)	7.27, d (8.7)
6	7.45, dd (7.8, 2.0)	7.51, dd (7.8, 1.7)	7.45, dd (7.9, 2.0)	7.57, dd (7.9, 2.0)	7.44, dd (7.9, 2.0)	7.62, dd (7.9, 1.9)
7	6.33, d (2.2)	6.18, s	6.31, d (2.3)	6.17, s	6.26, d (2.2)	6.08, s
9	6.98, d (8.1)	6.98, d (8.1)	6.97, d (8.1)	6.96, d (8.1)	6.92, d (8.1)	6.94, d (8.1)
10	7.41, t (8.1)	7.39, t (7.7)	7.40, t (8.0)	7.37, br t (8.0)	7.38, t (8.0)	7.36, t (8.0)
11	7.06, d (7.6)	7.05, d (7.5)	7.05, d (7.6)	7.03, d (7.5)	7.03, d (7.5)	7.01, d (7.5)
12	6.43, d (2.3)	6.13, s	6.48, d (2.3)	6.22, s	6.58, br d (5.1)	6.43, d (5.0)
12a	7.69, d (1.9)	7.89, d (1.5)	7.67, d (2.0)	7.96, d (1.9)	7.68, d (1.9)	7.96, d (1.8)
13	3.63, s	3.66, s	3.62, s	3.66, s	3.63, s	3.64, s
14	1.29, s	1.29, s	1.29, s	1.29, s	1.28, s	1.29, s
15	3.30, s	3.43, s	3.61, q (7.5)	3.82, dq (9.5, 7.1) 3.69, dq (9.6, 7.0)		
16			1.16, t (7.1)	1.23, t (7.1)		
3-OH	4.79, s	4.79, s	4.78, s	4.78, s	4.79, s	4.79, s
12-OH					6.88, d (7.4)	7.23, d (5.5)

^aAssignments are based on COSY, HMBC, HSQC, and NOESY experiments.

Table 4. ^{13}C (175 MHz) NMR Data (δ_{C} , Type) for 1–8 in $\text{DMSO}-d_6^a$

position	1	2	3	4	5	6	7	8
1	200.4, C	196.2, C	196.2, C	197.7, C	198.1, C	155.8, C	200.2, C	200.0, C
2	54.4, CH ₂	52.3, CH ₂	53.4, CH ₂	52.4, CH ₂	52.1, CH ₂	113.6, CH	53.5, CH ₂	53.3, CH ₂
3	70.8, C	74.9, C	71.6, C	69.7, C	70.9, C	139.0, C	71.6, C	69.9, C
4	44.7, CH ₂	73.8, CH	44.1, CH ₂	44.3, CH ₂	42.5, CH ₂	122.0, CH	43.0, CH ₂	43.2, CH ₂
4a	148.6, C	151.6, C	149.5, C	146.5, C	134.0, C	137.8, C	135.1, C	135.2, C
5	130.5, CH	132.4, CH	120.3, CH	124.7, CH	131.6, CH	128.8, CH	130.3, CH	130.5, CH
6	131.1, CH	129.5, CH	157.2, C	138.7, CH	122.1, CH	123.6, CH	122.3, CH	122.6, CH
6a	133.7, C	135.0, C	124.0, C	71.4, C	153.0, C	131.9, C	148.2, C	148.3, C
7	183.2, C	180.7, C	180.1, C	67.7, CH	54.9, CH ₂	183.7, C	99.0, CH	99.1, CH
7a	120.2, C	120.4, C	123.1, C	127.4, C	129.9, C	120.3, C	123.3, C	123.2, C
8	159.7, C	135.0, C	158.7, C	156.9, C	158.2, C	159.5, C	154.0, C	154.1, C
9	112.5, CH	118.8, CH	118.5, CH	111.0, CH	115.4, CH	112.4, CH	112.0, CH	112.0, CH
10	135.1, CH	136.3, CH	135.2, CH	128.5, CH	128.6, CH	134.8, CH	131.8, CH	131.9, CH
11	122.5, CH	118.9, CH	117.7, CH	119.6, CH	122.8, CH	122.6, CH	112.3, CH	111.9, CH
11a	146.4, C	137.7, C	136.9, C	142.6, C	139.3, C	146.9, C	148.7, C	148.7, C
12	64.3, CH	184.6, C	186.2, C	70.1, CH	199.8, C	65.2, CH	77.7, CH	77.6, CH
12a	142.0, C	134.5, C	138.9, C	46.6, CH	128.1, C	139.6, C	126.3, C	126.5, C
12b	130.1, C	134.1, C	127.9, C	125.8, C	130.6, C	120.7, C	127.6, C	127.1, C
13	56.3, CH ₃	56.9, CH ₃	56.9, CH ₃	55.6, CH ₃	56.5, CH ₃	56.3, CH ₃	56.0, CH ₃	56.0, CH ₃
14	29.6, CH ₃	26.8, CH ₃	30.6, CH ₃	28.4, CH ₃	29.6, CH ₃	22.0, CH ₃	30.1, CH ₃	29.1, CH ₃
1'			99.4, CH			100.9, CH		
2'			70.4, CH			70.4, CH		
3'			70.4, CH			72.0, CH		
4'			72.1, CH			72.5, CH		
5'			70.7, CH			70.4, CH		
6'			18.4, CH ₃			18.5, CH ₃		

^aAssignments are based on COSY, HMBC, HSQC, and NOESY experiments.

resonances at δ_{H} 6.39 (s, H-12) (Table 1) and δ_{C} 64.3 (C-12) (Table 4), attributed to a methine group in **1**, replaced the resonance at δ_{C} 184.5 (C-12), attributed to a carbonyl group in **16**. These differences indicated one of the carbonyl groups at C-7 or C-12 in **16** was reduced to a hydroxy group in **1**. The correlations of H-12 with C-11 and C-12b, H-11 with C-12, and H-6 with C-7 in the HMBC spectrum of **1** identified the hydroxy substitution at C-12 (Figure 1). On that basis,

compound **1** was identified as 12-deoxy-12-hydroxy-8-O-methyltetragomycin, though the relative configuration between C-3 and C-12 was not established (Figure 1).

Compound **2** was obtained as a yellow powder. HRESIMS analysis afforded an $[\text{M} + \text{H}]^+$ ion at m/z 353.1020, giving the molecular formula of **2** as $\text{C}_{20}\text{H}_{16}\text{O}_6$. The chemical shifts and coupling systems in the ^1H , ^{13}C , and COSY NMR spectra of **2** resembled those of **16**, except that the resonances attributed to

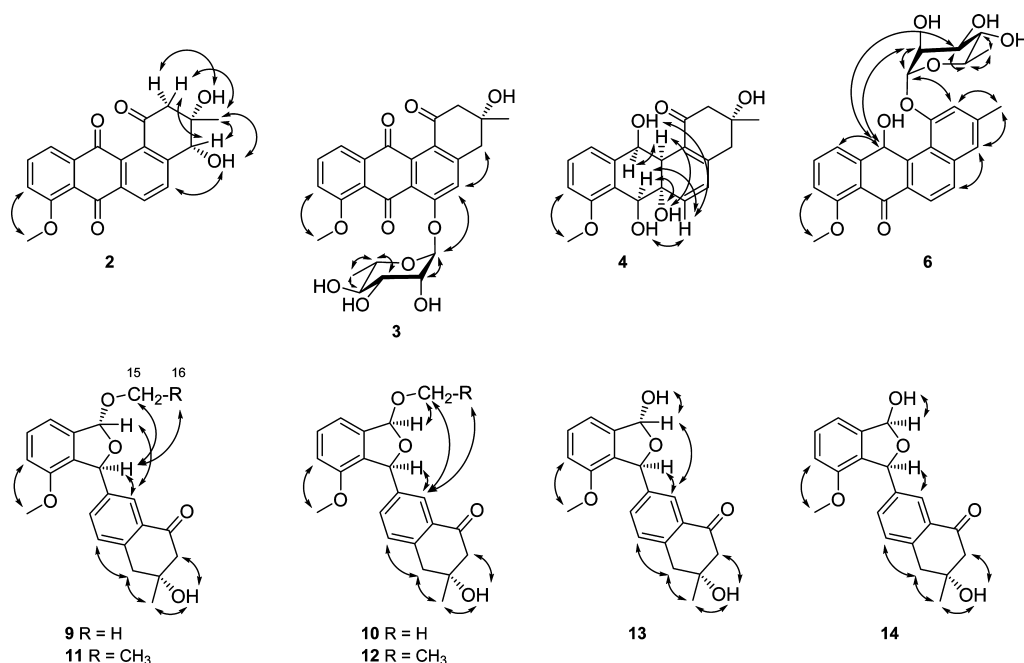


Figure 2. Key NOESY correlations supporting the relative configurations of 2–4, 6, and 9–14.

the methylene group at C-4 in **16** were replaced by resonances at δ_{H} 4.72 (s, H-4) (Table 1) and δ_{C} 73.8 (C-4) (Table 4) attributed to one methine group in **2**, indicating that the only difference between **2** and **16** was one additional hydroxy group at C-4 in **2**. The correlations of H-2 with C-1 and C-4, H-14 with C-4, and H-4 with C-4a in the HMBC spectrum of **2** confirmed the hydroxy substitution at C-4 (Figure 1). Among all natural angucyclines and angucyclinones known to date, the configuration of the methyl and hydroxy groups at C-3, as drawn in Figure 2, is absolutely conserved. Because the absolute *R*-configuration at C-3 of the copurified **16** has been confirmed by total synthesis,^{18c} the absolute configuration of C-3 in **2** was assigned as *S* based on their shared biosynthetic origin. This assignment was further supported by the NOESY spectrum of **2**: the correlations of H-4 with H_a-2, H₃-14 with both H-4 and H_a-2, and 3-OH group with H_b-2 unambiguously established that H₃-14, H-4, and H_a-2 were on the same face of ring A, with H_b-2 and the 3- and 4-OH groups lying on the opposite face of ring A (Figure 2). Taken together, the absolute configuration of C-4 in **2** was assigned as *R*, and **2** was identified as 4*R*-hydroxy-8-*O*-methyltetrangomycin.

Compound **3** was obtained as a green-yellow powder. HRESIMS analysis afforded an $[\text{M} + \text{H}]^+$ ion at m/z 499.1590, giving the molecular formula of **3** as $\text{C}_{26}\text{H}_{26}\text{O}_{10}$. Comparison of the ^1H and COSY NMR spectra between **3** and **16**^{18a,b} showed that the AB coupling system of H-5 with H-6 in ring B of **16** was replaced by a methine singlet at δ_{H} 7.38 (s, H-5), and resonances attributed to a 6-deoxysugar moiety at δ_{H} 5.66 (br s, H-1'), δ_{H} 3.98 (dd, $J = 3.2, 1.8$ Hz, H-2'), δ_{H} 3.90 (dd, $J = 9.5, 3.2$ Hz, H-3'), δ_{H} 3.36 (t, $J = 9.4$ Hz, H-4'), δ_{H} 3.55 (m, H-5'), and δ_{H} 1.13 (d, $J = 6.2$ Hz, H₃-6') were observed in **3** (Table 2). Comparison of chemical shifts, coupling patterns, and correlations of resonances attributed to this sugar moiety in the ^1H , ^{13}C , COSY, and HSQC NMR spectra (Figure 1 and Tables 2 and 4) with literature data,¹⁹ in combination with the correlations in the NOESY spectrum (Figure 2), identified the sugar as an α -rhamnose. The absolute configuration of the α -rhamnose moiety was assigned on the basis of a common

biosynthetic pathway shared with **6** (see structural elucidation of **6**). In the HMBC spectrum of **3**, the correlations of H-1' with C-6, H-5 with C-6a, C-12b, and C-4, and H-4 with C-5 identified the attachment of α -*L*-rhamnose at C-6 (Figure 1). Therefore, compound **3** was identified as 6-*O*- α -*L*-rhamnosyl-8-*O*-methyltetrangomycin.

Compound **4** was obtained as a colorless gum. HRESIMS analysis afforded an $[\text{M} + \text{Na}]^+$ ion at m/z 381.1306, giving the molecular formula of **4** as $\text{C}_{20}\text{H}_{22}\text{O}_6$. The ^1H and COSY NMR spectra of **4** showed similar resonances, attributed to one methyl group and two methylene groups at ring A, one methoxy group, and an ABC coupling system at ring D, to those in **16**. However, the resonances for an AB coupling system of aromatic methine groups at ring B in **16** shifted to δ_{H} 6.03 (d, $J = 10.1$ Hz, H-5) and δ_{H} 6.31 (d, $J = 10.1$ Hz, H-6) in **4**. In addition, **4** showed two more methine group resonances at δ_{H} 4.83 (br d, $J = 9.7$ Hz, H-7) and δ_{H} 4.86 (br s, H-12) that were coupled with the two hydroxy resonances at δ_{H} 5.24 (br d, $J = 9.3$ Hz, 7-OH) and δ_{H} 5.48 (br s, 12-OH), one more aliphatic methine group at δ_{H} 2.67 (br s, H-12a), and one more hydroxy resonance at δ_{H} 5.15 (br d, $J = 9.3$ Hz, 6a-OH) (Table 1). The ^{13}C NMR spectrum of **4**, compared to that of **16**, showed that the two carbonyl resonances at C-7 and C-12 and the two aromatic carbon resonances at C-6a and C-12a in **16** were replaced by three aliphatic methine and one aliphatic nonprotonated carbon resonance in **4** (Table 4). These differences suggested that the two carbonyl groups in **16** were reduced to two hydroxy groups and the double bond at C-6a/12a in **16** was reduced to a saturated bond, respectively, in **4**. These were confirmed by the correlation of 12-OH with H-12a in the COSY spectrum and the correlations of 6a-OH with C-7, H-7 with C-8, H-12a with C-1 and C-6, H-12 with C-12a, and H-11 with C-12 in the HMBC spectrum of **4**, which also established the attachment of the hydroxy groups at C-6a, C-7, and C-12 (Figure 1). Finally, the relative configuration of **4** was solved by a NOESY experiment: correlations of H-6 with H-7 and 7-OH suggested that the C-6a/C-6 bond took an equatorial conformation, the correlations of the axial 6a-OH

Table 5. ^{13}C (175 MHz) NMR Data (δ_{C} , Type) for 9–14 in $\text{DMSO}-d_6^a$

position	9	10	11	12	13	14
1	197.8, C	197.7, C	197.7, C	197.7, C	197.8, C	197.8, C
2	52.3, CH_2	52.4, CH_2	52.3, CH_2	52.4, CH_2	52.3, CH_2	52.4, CH_2
3	70.9, C	70.9, C	70.9, C	70.9, C	70.9, C	71.0, C
4	42.9, CH_2	42.9, CH_2	42.9, CH_2	42.9, CH_2	42.8, CH_2	42.9, CH_2
4a	142.2, C	142.0, C	142.2, C	141.9, C	141.9, C	141.8, C
5	129.9, CH	129.8, CH	129.9, CH	129.5, CH	129.8, CH	129.8, CH
6	133.1, CH	133.1, CH	133.1, CH	133.1, CH	133.0, CH	133.4, CH
6a	139.4, C	139.6, C	139.4, C	139.9, C	140.0, C	140.3, C
7	83.8, CH	83.8, CH	83.6, CH	83.9, CH	82.7, CH	83.2, CH
7a	130.1, C	129.9, C	130.0, C	130.1, C	129.7, C	129.5, C
8	154.4, C	154.4, C	154.4, C	154.3, C	154.3, C	154.3, C
9	112.2, CH	112.0, CH	112.1, CH	111.8, CH	111.6, CH	111.4, CH
10	131.0, CH	130.8, CH	130.9, CH	130.7, CH	130.7, CH	130.6, CH
11	115.5, CH	115.5, CH	115.5, CH	115.5, CH	115.3, CH	115.4, CH
11a	139.7, C	139.7, C	140.2, C	139.8, C	142.7, C	142.0, C
12	107.2, CH	107.6, CH	106.3, CH	106.4, CH	101.3, CH	101.0, CH
12a	124.8, CH	125.3, CH	124.8, CH	125.5, CH	124.6, CH	125.5, CH
12b	132.0, C	131.9, C	132.0, C	132.0, C	131.9, C	131.7, C
13	55.9, CH_3	55.9, CH_3	55.9, CH_3	55.9, CH_3	55.8, CH_3	55.9, CH_3
14	29.9, CH_3	29.9, CH_3	29.9, CH_3	29.9, CH_3	29.8, CH_3	29.9, CH_3
15	53.8, CH_3	55.5, CH_3	62.4, CH_2	63.7, CH_2		
16			15.8, CH_3	15.7, CH_3		

^aAssignments are based on COSY, HMBC, HSQC, and NOESY experiments.

with both H-7 and H-12a suggested that 6a-OH, H-7, and H-12a were on the same side of ring C, and the correlations of H-12a with H-12 and of 12-OH with H-6 suggested that H-12 and H-12a were on the same side of ring C (Figure 2). Taken all together, compound 4 was identified as another congener of 16, and the relative configuration at C-6a, C-7, C-12, and C-12a in 4 was assigned as S*, S*, S*, and S*, respectively.

Compound 5 was obtained as a green-yellow powder. HRESIMS analysis afforded an $[\text{M} + \text{Na}]^+$ ion at m/z 379.1161, giving the molecular formula of 5 as $\text{C}_{20}\text{H}_{20}\text{O}_6$. The ^1H and ^{13}C NMR spectra of 5 resembled those of angucyclinone C,²⁰ except for one hydroxy resonance occurring at δ_{H} 9.89 (s, 6a-OH) in the ^1H NMR spectrum of 5 (Table 1). This difference in combination with the molecular formula of 5 indicated that the ether ring in angucyclinone C was oxidatively cleaved in 5. This was confirmed by the correlations of 6a-OH with C-6, C-6a, and C-12a and of H-7 with C-8 and C-11a in the HMBC spectrum (Figure 1). Thus, compound 5 was identified as the C-ring cleavage product of angucyclinone C.

Compound 6 was obtained as a yellow powder. HRESIMS analysis afforded an $[\text{M} + \text{H}]^+$ ion at m/z 467.1691, giving the molecular formula of 6 as $\text{C}_{26}\text{H}_{26}\text{O}_8$. The chemical shifts and coupling systems in the ^1H and COSY NMR spectra of 6 resembled those of pseudonocardone A²¹ except the resonances attributed to one 6-deoxysugar moiety instead of the β -glucuronic acid moiety in pseudonocardone A (Table 2). The sugar moiety was identified as α -rhamnose by methods similar to those described for 3 (Tables 2 and 4), and the absolute configuration of L-rhamnose was established upon mild acid hydrolysis followed by optical rotation comparison with the literature value.²² The different chemical shifts of H-2' and C-3' between 3 and 6 are due to the different sugar moiety environments. This is supported by the strong correlations of H-12 with H-2' and H-3' in the NOESY spectrum of 6 (Figure 2). The correlations of H-1' with C-1, 12-OH with C-11a and C-12a, H-12 with C-12b, and H-6 with C-7 in the HMBC

spectrum of 6 identified the attachment of α -L-rhamnose at C-1 and the hydroxy group at C-12 (Figure 1). The absolute configuration at C-12 was not assigned. Compound 6 was thus identified as 1-O- α -L-rhamnosyl-12-hydroxy-8-O-methyltetraangulol.

Compounds 7 and 8 were obtained as yellow powders. HRESIMS analysis for 7 and 8 afforded the same $[\text{M} + \text{H}]^+$ ion at m/z 339.1225, giving the molecular formula of 7 and 8 as $\text{C}_{20}\text{H}_{18}\text{O}_5$. Analysis for the ^1H , ^{13}C , COSY, HSQC, and HMBC NMR spectra of 7 and 8 (Figure 1) showed that they had the same planar structure as LS1924, a known angucyclinone isolated from *Streptomyces* sp. LS1924 with its absolute configuration unassigned.²³ However, 7 and 8 had distinct chemical shifts for H-11 and H-12 in their ^1H NMR spectra, indicating that 7 and 8 are isomers with different configurations at C-7 and C-12 (R, R or S, S) (Table 1). Comparison of the ^1H NMR spectra of 7, 8, and LS1924A in acetone- d_6 clearly showed that 7 had nearly identical resonances at δ_{H} 6.76 (s, H-7), 6.72 (s, H-12), and 7.21 (d, $J = 7.2$ Hz, H-11) to those of LS1924A, while 8 had distinct resonances at δ_{H} 6.84 (s, H-7), 6.76 (s, H-12), and 7.14 (d, $J = 7.2$ Hz, H-11) (Table S3). These data supported the final assignment of 7 as LS1924A and 8 as a diastereomer of LS1924A (i.e., 7), respectively.

Compounds 9–14 were obtained as white powders. HRESIMS analysis for 9 and 10 afforded the same $[\text{M} + \text{Na}]^+$ ion at m/z 377.1358, giving the molecular formula of 9 and 10 as $\text{C}_{21}\text{H}_{22}\text{O}_5$. Analysis of the ^1H , ^{13}C , COSY, HSQC, and HMBC NMR spectra of 9 showed that it was the same as the angucyclinone from *Streptomyces* sp. M268 (Tables 3 and 5).²⁴ However, close examination of the NMR spectra of 9 indicated that the relative configuration of the angucyclinone from *Streptomyces* sp. M268 was incorrectly assigned. In the NOESY spectrum of 9, the correlations of H-7 with both H-15 and H-12a and of H-12 with H-12a, which was similar to that of the angucyclinone from *Streptomyces* sp. M268,²⁴ suggested that H-7 and H-12 adopted “trans” positions on the different sides

Table 6. Cytotoxicity and Antibacterial Assays for 1, 5, 15, and 16

	cytotoxicity (IC ₅₀ , μM)					antibacterial activity (MIC, μg/mL)		
	SF295	H226	SF539	M14	MDA-MB-468	<i>S. aureus</i>	<i>B. subtilis</i>	<i>M. smegmatis</i>
1	3.1 ± 0.1	7.2 ± 0.4	12 ± 2	31 ± 6	52 ± 1	>100	>100	>100
5	>100	>100	>100	>100	>100	>100	85	93
15	12 ± 1	7.2 ± 0.5	3.2 ± 0.2	2.4 ± 0.1	16 ± 1	11	8.1	9.7
16	17 ± 1	4.6 ± 0.3	10 ± 1	9.7 ± 0.6	27 ± 1	>100	25	>100
mitomycin C	0.54 ± 0.01	0.58 ± 0.02	(3.8 ± 0.3) × 10 ⁻²	0.47 ± 0.01	0.50 ± 0.02	N ^a	N	N
tetracycline	N	N	N	N	N	6.7	13	11

^a“N” indicates compounds not tested.

of the five-membered ether ring (Figure 2). Thus, we assigned the relative configuration of 9, thereby correcting the assignment for the angucyclinone from *Streptomyces* sp. M268, at C-7 and C-12 as S* and R*, respectively.

Compound 10 had nearly identical ¹H, ¹³C, COSY, HSQC, and HMBC NMR spectra to those of 9 except that the proton resonance attributed to H-12 shifted from δ_H 6.43 in 9 to δ_H 6.13 in 10 (Figure 1 and Tables 3 and 5), indicating that 10 has only a different configuration at C-12 compared with 9. In the NOESY spectrum of 10, the correlation of H₃-15, instead of H-12, with H-12a established H-7 and H-12 adopted the “cis” positions on the same side of the five-membered ether ring (Figure 2). Therefore, the relative configuration at C-7 and C-12 in 10 was established as S* and S*, respectively. Compounds 11 and 12 were identified as derivatives of 9 and 10, respectively, with the methoxy group at C-12 replaced by one ethoxy group based on their ¹H, ¹³C, COSY, HSQC, HMBC, and NOESY NMR spectra (Figures 1 and 2 and Tables 3 and 5). Compounds 13 and 14 were also identified as derivatives of 9 and 10, respectively, with the methoxy group at C-12 replaced by one hydroxy group based on their ¹H, ¹³C, COSY, HSQC, HMBC, and NOESY NMR spectra (Figures 1 and 2 and Tables 3 and 5). However, 13 and 14 undergo a rapid equilibrium, via H₂O attacking at the hemiacetal group, to afford a mixture with equal amounts of 13 and 14, giving rise to two sets of resonances in their NMR spectra. As 13 and 14 rapidly equilibrate in the presence of H₂O, we cannot rule out the possibility of both 9/10 and 11/12 pairs as isolation artifacts of 13/14 in the presence of methanol and ethanol, respectively.

Compounds 15 and 16 were identified as tetrangomycin²⁵ and 8-O-methyltetrangomycin,^{18a,b} respectively, based on the comparison of their NMR and MS data with previously published data.

Bioactivity Assays. Angucyclines and angucyclinones are known for their cytotoxicity and antibacterial activities. They are both reported to possess IC₅₀ values between 1 and 100 μM against various cancer cell lines.^{1,2,8,21} In this study, five cancer cell lines, human glioblastoma multiforme SF295, human glioblastoma SF539, human lung squamous carcinoma H226, human melanoma M14, and human breast adenocarcinoma MDA-MB-468, were chosen to test the cytotoxicities of 1–16, using mitomycin C as the positive control. Compound 1 was active against the SF295 and H226 cell lines, and compound 15 and 16 were active against the H226, SF539, and M14 cell lines (Table 6), while the rest were inactive (IC₅₀ > 10 μM). The potencies for 15 and 16 against the five cell lines tested in this study were similar to other cancer cell lines reported previously.^{22,26,27}

The angucycline and angucyclinone family of natural products selectively inhibit Gram-positive bacteria.^{2,28–31}

Compounds 1–16 were tested against the Gram-positive *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 23857, and *Mycobacterium smegmatis* ATCC 607 and the Gram-negative *Escherichia coli* ATCC 25922 and *E. coli* JM109, using tetracycline as the positive control. As summarized in Table 5, both 15 and 16 showed moderate activity against *S. aureus* ATCC 25923, *B. subtilis* ATCC 23857, and *M. smegmatis* ATCC 607, with the minimum inhibitory concentration (MIC) values ranging from 8.1 to 25 μg/mL, which were similar to the reported MIC values for 15 and 16 against other *S. aureus* or *B. subtilis* species.^{26,27,32–34} Compound 5 showed weak activity against *B. subtilis* ATCC 23857 and *M. smegmatis* ATCC 607. As previously observed for known angucyclines and angucyclinones,² 1–16 showed no activity against the Gram-negative *E. coli* ATCC 25922 and JM109 species.

In conclusion, we isolated 14 angucyclinones and two angucyclines (1–16), including 12 new compounds (1–6, 8, and 10–14), from *Streptomyces* sp. CB01913, a strain selected based on chemical profiling and fermentation optimization. The new compounds featured unusual structural properties including various oxidations on rings A and C (1, 2, and 4), different sugar moieties attached to rings A and B (3 and 6), and C-ring cleavage (5 and 10–14) and expansion (8). Cytotoxicity and antibacterial assays showed that 1, 5, 15, and 16 gave moderate inhibitory activities against selected cancer cell lines and bacterial strains, respectively. The new structural features, especially C-ring cleavage and expansion disrupting the conjugated system spanning rings B, C, and D, enrich the structural diversity of angucyclines and angucyclinones and set the stage to further evaluate the structure–activity relationships of this family of aromatic polyketide natural products.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with an AUTOPOL IV automatic polarimeter (Rudolph Research Analytical). UV spectra were collected with a NanoDrop 2000C spectrophotometer (Thermo Scientific). IR spectra were collected with a Spectrum One FT-IR spectrometer (PerkinElmer). NMR data were collected on a Bruker 700 MHz/54 mm Ultra Shield Magnet System. HRESIMS data was collected on a Thermo Finnigan LTQ Orbitrap mass spectrometer. MPLC separation was conducted on a Biotage Isolera One using a KP-C18-HS (30 g) column. Size exclusion chromatography was performed on Sephadex LH-20 (GE Healthcare) columns. HPLC was carried out on a Varian semi-preparative HPLC system (Woburn, MA, USA) equipped with a Prostar 330 detector, using a GRACE Apollo C₁₈ column (250 mm × 4.6 mm, 5 μm) for analysis and an Alltima C₁₈ column (250 mm × 10.0 mm, 5 μm) for purification. All fermentations were carried out in New Brunswick Scientific Innova 44 incubator shakers or New Brunswick BioFlo/celliGen 115 fermentors. Diaion HP-20 resin was purchased from Sigma-Aldrich.

Strain Isolation and Identification. Strain *Streptomyces* sp. CB01913 was isolated from a soil sample collected in Weishan County (location: 25°23' N, 100°33' E). The strain was purified with a standard diluting plate method,³⁵ using agar plates with medium consisting of glycerol 10 g, asparagine 1 g, K₂HPO₄·H₂O 1 g, MgSO₄·7H₂O 0.5 g, CaCO₃ 0.3 g, vitamin mixture (thiamine-HCl 0.5 mg, riboflavin 0.5 mg, niacin 0.5 mg, pyridoxine 0.5 mg, calcium pantothenate 0.5 mg, inositol 0.5 mg, 4-aminobenzoic acid 0.5 mg, and biotin 0.25 mg), and agar 15 g, in 1 L of H₂O, pH 7.7. The strain was preserved as a spore suspension (20% glycerol, v/v) at −80 °C.

The strain CB01913 was grown on ISP4 medium at 28 °C for 7 days. The spores were harvested and cultured in TSB at 28 °C for 2 days, after which the genomic DNA was isolated following standard protocols.¹⁷ Four housekeeping genes, 16S rRNA, *recA* (encoding recombinase A), *rpoB* (encoding RNA polymerase β subunit), and *trpB* (encoding tryptophan synthase β subunit), were amplified by PCR, sequenced, and deposited into GenBank under accession numbers KT581419, KT581420, KT581421, and KT581422, respectively. One Taq Quick-Load 2× Master Mix (New England BioLabs Inc.) was used for PCR amplification (PCR primers and PCR conditions are listed in Table S2), and the QIAquick gel extraction kit and QIAprep Spin Miniprep kit (Qiagen) were used for PCR product recovery. The PCR products were sequenced, and the resulting sequences were used for BLAST on the NCBI Web site to search for the homologous gene candidates, which were then used as the representative *Streptomyces* spp. in the phylogenetic tree, assigning CB01913 as a *Streptomyces* species (Figure S2).^{17a}

Fermentation and Isolation. *Streptomyces* sp. CB01913 was grown on ISP4 medium (Difco) for sporulation. After 1 week, the spores were harvested and 1 mL of spore suspension was added into 600 mL of seed medium (tryptic soy broth) (Bacto); incubation continued on a rotary shaker at 250 rpm and 28 °C for 2 days. The resultant seed culture (600 mL) was then inoculated into 8 L of production medium [dextrin (Sigma-Aldrich) 40 g, tomato paste (Plant Media) 7.5 g, N-Z-Amine A (Sigma-Aldrich) 2.5 g, primary yeast (Acros Organics) 5 g, in 1.0 L of deionized H₂O, pH 7.0], in a 14 L fermentor, and the fermentation continued at 250 rpm at 28 °C for 7 days. After fermentation, 400 g of Diaion HP-20 resin was added into the medium, and this was stirred overnight. The resin and the cell mass were harvested by centrifugation, washed by deionized H₂O, and extracted with MeOH. The MeOH extract was concentrated in vacuo, and the residue was loaded onto a Biotage SNAP Cartridge KP-C18-HS column (30 g) and separated by MPLC using an increasing gradient of 5% MeOH in H₂O to 80% MeOH in H₂O as the mobile phase. The flow rate was 20 mL/min, and every 35 mL was collected as a fraction. In total, 35 fractions (m1 to m35) were collected. All 35 fractions were analyzed by HPLC. Fraction m20 was further purified by preparative HPLC using CH₃CN/H₂O (32/68) as the mobile phase, with UV detection at 254 nm, to afford **1** (11.7 mg) and **16** (15.1 mg). Fraction m17 was further purified by preparative HPLC using CH₃CN/H₂O (27/73) as the mobile phase with UV detection at 210 nm to afford **2** (3.4 mg). Fraction m8 was further purified by a Sephadex LH-20 column using MeOH as the mobile phase to give four subfractions, s1–s4, of which s2 was further purified by preparative HPLC using CH₃CN/H₂O (27/73) as the mobile phase with UV detection at 300 nm to afford **4** (0.9 mg). Fraction m14 was further purified by preparative HPLC using CH₃CN/H₂O (27/73) as the mobile phase with UV detection at 260 nm to afford **3** (5.1 mg) and **5** (8.7 mg). Fraction m27 was further purified by preparative HPLC using CH₃CN/H₂O (45/55) as the mobile phase with UV detection at 254 nm to afford **6** (7.2 mg), **7** (1.5 mg), **8** (1.6 mg), and **15** (4.5 mg). Fraction m9 was further purified by preparative HPLC using an increasing gradient of 30% CH₃CN in H₂O to 50% CH₃CN in H₂O over 20 min, then 50% CH₃CN in H₂O over 5 min as the mobile phase, with UV detection at 254 nm to afford **9** (7.7 mg), **10** (0.6 mg), **11** (2.6 mg), **12** (3.0 mg), **13** (3.7 mg), and **14** (3.5 mg). All preparative HPLC was carried out at a flow rate of 3 mL/min.

Compound 1: brown powder; [α]_D²⁵ −133 (c 0.34, DMSO); UV (DMSO) λ_{\max} (log ϵ) 254 (4.13), 334 (3.66) nm; IR ν_{\max} 3441, 2933, 1667, 1595, 1470, 1271, 1236, 1124, 1072, 1022, 966, 819, 757, 723

cm^{−1}; ¹H NMR (700 MHz) data, Table 1; ¹³C NMR (175 MHz) data, Table 4; HRESIMS m/z 339.1226 [M + H]⁺ (calcd for C₂₀H₁₈O₅, 339.1231).

Compound 2: yellow powder; [α]_D²⁷ −76.7 (c 0.18, DMSO); UV (DMSO) λ_{\max} (log ϵ) 266 (4.26), 373 (3.52) nm; IR ν_{\max} 3663, 3390, 2972, 2901, 1744, 1700, 1670, 1589, 1472, 1443, 1406, 1394, 1382, 1265, 1230, 1065, 1056, 956, 892, 822, 719 cm^{−1}; ¹H NMR (700 MHz) data, Table 1; ¹³C NMR (175 MHz) data, Table 4; HRESIMS m/z 353.1020 [M + H]⁺ (calcd for C₂₀H₁₆O₆, 353.1024).

Compound 3: green-yellow powder; [α]_D²⁷ −43.0 (c 0.25, DMSO); UV (DMSO) λ_{\max} (log ϵ) 261 (4.20), 371 (3.52) nm; IR ν_{\max} 3663, 3391, 2971, 2901, 1671, 1591, 1406, 1394, 1382, 1251, 1231, 1065, 1056, 892, 880 cm^{−1}; ¹H NMR (700 MHz) data, Table 2; ¹³C NMR (175 MHz) data, Table 4; HRESIMS m/z 499.1590 [M + H]⁺ (calcd for C₂₆H₂₆O₁₀, 499.1602).

Compound 4: colorless gum; [α]_D²⁷ −4.2 (c 0.07, DMSO); UV (DMSO) λ_{\max} (log ϵ) 284 (3.79), 304 (3.72) nm; IR ν_{\max} 3277, 2937, 2838, 1645, 1591, 1480, 1437, 1416, 1310, 1266, 1177, 1112, 1092, 1012, 951, 857, 764 cm^{−1}; ¹H NMR (700 MHz) data, Table 1; ¹³C NMR (175 MHz) data, Table 4; HRESIMS m/z 381.1306 [M + Na]⁺ (calcd for C₂₀H₂₂O₆, 381.1313).

Compound 5: green-yellow powder; [α]_D²⁷ −1.12 (c 0.36, DMSO); UV (DMSO) λ_{\max} (log ϵ) 252 (4.20), 328 (3.68) nm; IR ν_{\max} 3663, 3440, 3290, 2980, 2902, 1778, 1676, 1607, 1586, 1489, 1454, 1405, 1394, 1298, 1266, 1056, 1020, 951, 900, 824, 780 cm^{−1}; ¹H NMR (700 MHz) data, Table 1; ¹³C NMR (175 MHz) data, Table 4; HRESIMS m/z 379.1161 [M + Na]⁺ (calcd for C₂₀H₂₀O₆, 379.1156).

Compound 6: yellow powder; [α]_D²⁷ −122 (c 0.23, DMSO); UV (DMSO) λ_{\max} (log ϵ) 274 (4.31), 334 (3.83) nm; IR ν_{\max} 3663, 3403, 2973, 2902, 1650, 1596, 1454, 1406, 1394, 1383, 1268, 1231, 1075, 1065, 1051, 955, 892, 807, 764 cm^{−1}; ¹H NMR (700 MHz) data, Table 2; ¹³C NMR (175 MHz) data, Table 4; HRESIMS m/z 467.1691 [M + H]⁺ (calcd for C₂₆H₂₆O₈, 467.1704).

Compound 7: yellow powder; [α]_D²⁷ +250 (c 0.07, DMSO); UV (DMSO) λ_{\max} (log ϵ) 261 (3.72), 326 (3.42) nm; IR ν_{\max} 3663, 2993, 2958, 2903, 1735, 1453, 1404, 1393, 1382, 1250, 1230, 1083, 1035, 956, 893, 871 cm^{−1}; ¹H NMR (700 MHz) data, Table 1; ¹³C NMR (175 MHz) data, Table 4; HRESIMS m/z 339.1225 [M + H]⁺ (calcd for C₂₀H₁₈O₅, 339.1231).

Compound 8: yellow powder; [α]_D²⁷ −310 (c 0.08, DMSO); UV (DMSO) λ_{\max} (log ϵ) 261 (3.78), 329 (3.46) nm; IR ν_{\max} 3675, 3375, 2972, 2902, 1677, 1614, 1598, 1485, 1469, 1436, 1407, 1380, 1276, 1242, 1076, 1047, 960, 924, 895, 820, 774 cm^{−1}; ¹H NMR (700 MHz) data, Table 1; ¹³C NMR (175 MHz) data, Table 4; HRESIMS m/z 339.1225 [M + H]⁺ (calcd for C₂₀H₁₈O₅, 339.1231).

Compound 9: white powder; [α]_D²⁷ −63.6 (c 0.35, DMSO); UV (DMSO) λ_{\max} (log ϵ) 252 (3.95), 297 (3.26) nm; IR ν_{\max} 3663, 2971, 2901, 1678, 1611, 1453, 1405, 1393, 1382, 1251, 1231, 1065, 1056, 957, 892, 880, 871 cm^{−1}; ¹H NMR (700 MHz) data, Table 3; ¹³C NMR (175 MHz) data, Table 5; HRESIMS m/z 377.1358 [M + Na]⁺ (calcd for C₂₁H₂₂O₅, 377.1364).

Compound 10: white powder; [α]_D²⁷ +49 (c 0.01, DMSO); UV (DMSO) λ_{\max} (log ϵ) 252 (3.93), 297 (3.21) nm; IR ν_{\max} 3663, 2981, 2902, 1682, 1608, 1453, 1406, 1394, 1383, 1251, 1231, 1067, 1057, 893 cm^{−1}; ¹H NMR (700 MHz) data, Table 3; ¹³C NMR (175 MHz) data, Table 5; HRESIMS m/z 377.1358 [M + Na]⁺ (calcd for C₂₁H₂₂O₅, 377.1364).

Compound 11: white powder; [α]_D²⁷ −56.5 (c 0.18, DMSO); UV (DMSO) λ_{\max} (log ϵ) 254 (4.00), 297 (3.33) nm; IR ν_{\max} 3661, 3414, 2974, 2901, 1683, 1611, 1486, 1439, 1406, 1377, 1319, 1270, 1230, 1190, 1076, 1068, 1053, 1004, 961, 923, 822, 777 cm^{−1}; ¹H NMR (700 MHz) data, Table 3; ¹³C NMR (175 MHz) data, Table 5; HRESIMS m/z 391.1513 [M + Na]⁺ (calcd for C₂₂H₂₄O₅, 391.1520).

Compound 12: white powder; [α]_D²⁷ +46.7 (c 0.18, DMSO); UV (DMSO) λ_{\max} (log ϵ) 254 (4.13), 297 (3.32) nm; IR ν_{\max} 3663, 3398, 2971, 2895, 1683, 1610, 1487, 1439, 1405, 1376, 1349, 1321, 1269, 1231, 1103, 1076, 1052, 1002, 955, 823, 780 cm^{−1}; ¹H NMR (700 MHz) data, Table 3; ¹³C NMR (175 MHz) data, Table 5; HRESIMS m/z 391.1512 [M + Na]⁺ (calcd for C₂₂H₂₄O₅, 391.1520).

Compounds 13 + 14 (equal amount): white powder; $[\alpha]_D^{27}$ -9.1 (c 0.34, DMSO); UV (DMSO) λ_{\max} ($\log \epsilon$) 254 (3.97), 297 (3.29) nm; IR ν_{\max} 3340, 2965, 1681, 1610, 1486, 1433, 1270, 1231, 1191, 1144, 1104, 1051, 1002, 945, 923, 824, 777 cm^{-1} ; ^1H NMR (700 MHz) data, Table 3; ^{13}C NMR (175 MHz) data, Table 5; HRESIMS m/z 363.1203 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{20}\text{O}_5$, 363.1207).

Compound 15: yellow powder; $[\alpha]_D^{27}$ -119 (c 0.32, DMSO) $\{[\alpha]_D^{20} -85.6$ (c 0.49, CHCl_3)^{25b}}; UV (DMSO) λ_{\max} ($\log \epsilon$) 266 (4.32), 396 (3.58) nm; IR ν_{\max} 3671, 2973, 2902, 1704, 1637, 1591, 1455, 1405, 1394, 1382, 1264, 1159, 1075, 1049, 892, 722 cm^{-1} .

Compound 16: yellow powder; $[\alpha]_D^{27}$ -132 (c 0.34, DMSO) $\{[\alpha]_D^{20} -140$ (c 0.04, MeOH)^{18b}}; UV (DMSO) λ_{\max} ($\log \epsilon$) 266 (4.40), 375 (3.64) nm; IR ν_{\max} 3484, 2971, 1702, 1670, 1592, 1471, 1303, 1269, 1119, 1071, 1022, 958, 820, 722 cm^{-1} .

Determination of Sugar Absolute Configuration. To determine the absolute configuration of the α -L-rhamnose, **6** (3.0 mg) was first dissolved in 500 μL of acetone, followed by the addition of 4 mL of 2% H_2SO_4 . The resulting solution was stirred at 90 $^\circ\text{C}$ for 4 h, and complete hydrolysis was confirmed by HPLC and TLC analysis. After cooling to room temperature, the hydrolysis mixture was first extracted by 6 mL of CHCl_3 , and the aqueous layer was then neutralized to pH 7.0 with NaHCO_3 and concentrated under vacuum to afford a white solid. The white solid was dissolved in 1 mL of H_2O and subjected to optical rotation dispersion (ORD) determination. The observed ORD value of $[\alpha]_D^{27}$ $+7.5$ (c 0.10, H_2O) agreed with the reported ORD value of $[\alpha]_D^{25}$ $+10.2$ (c 0.20, H_2O),²² thereby establishing the sugar in **6** as L-rhamnose.

Biological Assays. The *S. aureus* ATCC 25923, *B. subtilis* ATCC 23857, *E. coli* ATCC 25922, and *M. smegmatis* ATCC 607 strains, as well as the human breast adenocarcinoma MDA-MB-468, were purchased from American Type Culture Collection (ATCC). *E. coli* JM109 was purchased from Promega Corporation. The human glioblastoma multiforme SF295, human glioblastoma SF539, and human lung squamous carcinoma H226 were purchased from the Cell Based Screening Core of TSRI Florida. The human melanoma M14 was kindly provided by Dr. Min Guo from the Department of Cancer Biology, TSRI Florida. The cytotoxicity assay was carried out using the standard protocol from the Promega Web site (<https://www.promega.com/resources/protocols/technical-bulletins/0/celltiter-96-aqueous-one-solution-cell-proliferation-assay-system-protocol/>), with the Cell-Titer 96 Aqueous One Solution Proliferation Assay (MTS) kit (Promega). Cells were plated in 96-well plates at 5000 cells/well in RPMI 1640 medium (ThermoFisher) and allowed to adhere overnight at 37 $^\circ\text{C}$ in a humidified atmosphere of 5% CO_2 . Medium was then removed and replaced by fresh RPMI 1640 medium containing different concentrations of different compounds. The cells were treated for 72 h before the assay was developed. All assay values were measured in triplicate. The IC_{50} values were determined using GraphPad/Prism software.

The antibacterial activities were first assessed by inhibition zones using the agar diffusion method.³⁶ Each compound (25 μL), prepared to a concentration of 2 mM in H_2O , was dropped onto the filter paper on the tryptic soy agar plate. The plate was incubated at 37 $^\circ\text{C}$ for 12 h, after which the inhibition zones, if any, were determined, using tetracycline as the positive control. The MIC values for compounds that showed significant inhibition zones were further determined in the 96-well plate with Müller-Hinton (MH) broth (supplemented with 20 mg of Ca^{2+} and 10 mg of Mg^{2+} per liter).^{37,38} The tested strains were grown in MH broth to early stationary phase, diluted by MH broth to an $\text{OD}_{625} = 0.005$, then pipetted into the 96-well plate with 100 μL of broth in each well. The tested compounds with different concentrations were added into different wells, using the wells containing no compounds or containing tetracycline as negative and positive controls, respectively. The final DMSO concentration in each well was 1%, which did not affect the growth of any of the tested strains. The MIC values were determined after incubation at 37 $^\circ\text{C}$ for 18 h, using a plate reader at OD_{625} .

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00601.

Table S1 summarizes the media A, B, C, and F used for the fermentation of *Streptomyces* sp. CB01913. Table S2 provides the primers and PCR conditions of the four housekeeping genes for the phylogenetic analysis. Table S3 compares ^1H NMR data of **7** and **8** with LS1924A²³ in acetone- d_6 . Figure S1 shows the HPLC analysis for the fermentation of *S. sp.* CB01913. Figure S2 shows the phylogenetic analysis for *S. sp.* CB01913. Figures S3–S86 show the NMR, HRESIMS, and IR spectra of compounds **1–14** (PDF)

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Notes

The authors declare no competing financial interest.

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