JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Bi- and Tetracyclic Spirotetronates from the Coal Mine Fire Isolate *Streptomyces* sp. LC-6-2

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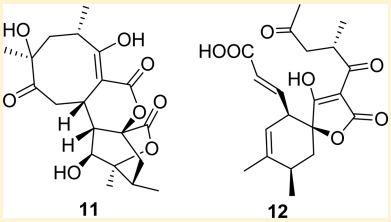
Supporting Information

ABSTRACT: The structures of 12 new "enantiomeric"-like abyssomicin metabolites (abyssomicins M−X) from *Streptomyces* sp. LC-6-2 are reported. Of this set, the abyssomicin W (**11**) contains an unprecedented 8/6/6/6 tetracyclic core, while the bicyclic abyssomicin X (**12**) represents the first reported naturally occurring linear spirotetronate. Metabolite structures were determined based on spectroscopic data and X-ray crystallography, and *Streptomyces* sp. LC-6-2 genome sequencing also revealed the corresponding putative biosynthetic gene cluster.



Class I spirotetronate polyketides contain a signature spirotetronate moiety within a macrocycle of variable size, the smallest of which are C_{11} macrocyclic abyssomicins (Figures 1 and S1).[1−7] Abyssomicins B−D from a marine-associated *Verrucosispora* were the first reported abyssomicins,[1] with a number of additional analogues since reported from *Verrucosispora* and *Streptomyces* species.[8−13] Abyssomicin C was the first natural product reported to inhibit the formation of *p*-aminobenzoate (*p*-ABA), a key folic acid precursor in bacteria. Within this context, abyssomicin C was found to be an irreversible inhibitor of bacterial amino-4-deoxychorismate (ADC) synthase via an ADC synthase Cys-263 thiol/abyssomicin C-9 enone hetero-Michael addition.[1,9,14,15] Abyssomicin C and the dimeric abyssomicin J display potent anti-Gram-positive bacteria activity, where abyssomicin J was noted as a putative oxidatively activated prodrug.[1,14,16−18] Spirotetronate biosynthesis also features novel stereoselective [4 + 2] cycloaddition macrocyclization reactions, as exemplified by abyssomicin, versipelostatin, and pyrroindomycin biosynthetic studies (Figure S1).[19−21] As part of an effort to explore the potential of actinomycetes associated with underground coal mine fires in Appalachia as a source for new natural products and/or biocatalysts,[22−31] herein we report the discovery of 12 new abyssomicin analogues (M−X, **1**−**12**) as metabolites of the Lotts Creek coal fire-affiliated isolate *Streptomyces* sp. LC-6-2 (Figure 1). Distinct from archetypical abyssomicins' absolute configuration, abyssomicins M−X (**1**−**12**) displayed global stereochemical features reminiscent of the unique "enantiomeric" *ent*-homoabyssomicins A and B.[13] In addition, abyssomicin W (**11**) highlights an 8/6/6/6 tetracyclic core, while the bicyclic abyssomicin X (**12**) is the first reported naturally occurring linear spirotetronate.

RESULTS AND DISCUSSION

Ten actinomycete strains were purified from second-generation plates deriving from a soil sample collected from the Lotts Creek coal fire (Perry County, Kentucky, United States). Preliminary LC-HRMS analyses of the *Streptomyces* sp. LC-6-2 crude extract revealed a set of potentially novel secondary metabolites based on an AntiBase 2014[32] database comparison. Scale-up fermentation (10 L) of *Streptomyces* sp. LC-6-2, followed by extraction, fractionation, and various chromatographic methods (Scheme S1), gave 12 new compounds: abyssomicins M (**1**, yield: 0.46 mg/L), N (**2**, yield: 0.41 mg/L), O (**3**, yield: 2.21 mg/L), P (**4**, yield: 0.83 mg/L), Q (**5**, yield: 4.05 mg/L), R (**6**, yield: 1.08 mg/L), S (**7**, yield: 1.14 mg/L), T (**8**, yield: 0.45 mg/L), U (**9**, yield: 3.02 mg/L), V (**10**, yield: 0.54 mg/L), W (**11**, yield: 0.40 mg/L), and X (**12**, yield: 1.83 mg/L).

Structure Elucidation. The molecular formula of **1** ($C_{20}H_{26}O_7$), based on determined HRESIMS, was consistent with 20 carbons (including four methyls, three methylenes, five methines, one double bond, one ketone, one carboxyl, and four quaternary carbons) observed via ^{13}C and ^{1}H NMR (Tables 1 and 3) and HSQC. Two COSY spin systems were established as CH_3-17/CH-4/CH_2-5/CH-6/CH_3-18 and H-8/H-9/H-10/H-11/11-OH (Figure S2). Further analysis of 1D/2D NMR data revealed a close relationship between **1** and abyssomicin D,[1] with structural divergence at C-12 and C-13 (Figure 2). The corresponding C-12 methyl (CH_3-19) at **1** was established via HMBC (correlations from CH_3-19 to C-11, C-12, and C-13

Received: February 9, 2017
Published: March 30, 2017

DOI: 10.1021/acs.jnatprod.7b00108
J. Nat. Prod. 2017, 80, 1141−1149

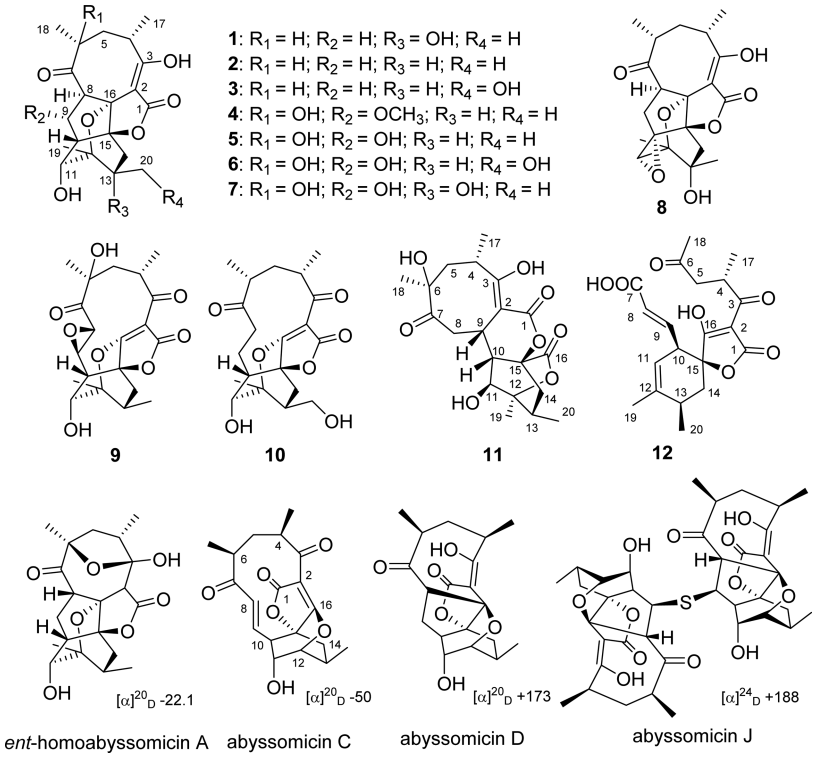


Figure 1. New metabolites (**1**–**12**) isolated from *Streptomyces* sp. LC-6-2 and representative prototypical abyssomicin comparators.

Table 1. ^{13}C NMR (100/125 MHz) Data for Compounds 1–4 and 8–12

no.	1[a]	2[c]	3[a]	4[a]	8[c]	9[a]	10[a]	11[a]	12[b]
1	173.1, C	174.9, C	173.2, C	171.5, C	173.4, C	168.9, C	169.5, C	169.2, C	176.8, C
2	99.0, C	98.8, C	98.8, C	106.4, C	98.4, C	100.2, C	99.0, C	96.6, C	96.9, C
3	179.5, C	180.7, C	178.9, C	186.5, C	180.9, C	199.0, C	199.7, C	180.7, C	201.2, C
4	40.3, CH	40.4, CH	39.9, CH	40.0, CH	40.7, CH	40.5, CH	42.2, CH	30.0, CH	38.1, CH
5	33.7, CH_2	34.2, CH_2	33.1, CH_2	38.3, CH_2	32.8, CH_2	40.3, CH_2	38.4, CH_2	46.1, CH_2	47.5, CH_2
6	48.1, CH	48.1, CH	47.7, CH	77.8, C	48.5, CH	79.7, C	45.9, CH	78.0, C	210.9, C
7	210.7, C	210.2, C	210.5, C	209.1, C	209.5, C	207.2, C	211.6, C	215.3, C	170.2, C
8	58.5, CH	59.7, CH	58.5, CH	66.1, CH	53.2, CH	48.3, CH	34.4, CH_2	43.9, CH_2	122.6, CH
9	27.2, CH_2	27.6, CH_2	26.8, CH_2	77.1, CH	36.2, CH_2	55.9, CH	19.4, CH_2	27.2, CH	149.8, CH
10	50.3, CH	49.7, CH	48.8, CH	50.6, CH	60.5, C	45.9, CH	44.6, CH	45.3, CH	47.8, CH
11	81.3, CH	80.9, CH	78.9, CH	72.2, CH	64.8, CH	67.0, CH	74.8, CH	71.7, CH	120.8, CH
12	76.0, C	78.5, C	77.6, C	76.8, C	75.4, C	88.6, C	88.5, C	84.9, C	141.0, C
13	73.7, C	27.7, CH	35.2, CH	26.7, CH	86.5, C	27.6, C	35.0, C	28.0, CH	32.5, CH
14	44.2, CH_2	33.8, CH_2	29.4, CH_2	33.6, CH_2	38.7, CH_2	34.7, CH_2	33.4, CH_2	40.6, CH_2	36.2, CH_2
15	86.9, C	87.4, C	87.2, C	80.7, C	95.2, C	78.0, C	77.8, C	76.7, C	86.0, C
16	84.4, C	85.7, C	84.8, C	87.0, C	90.0, C	179.4, C	184.6, C	171.5, C	199.4, C
17	19.6, CH_3	19.9, CH_3	19.4, CH_3	18.8, CH_3	21.0, CH_3	17.3, CH_3	16.5, CH_3	17.2, CH_3	18.0, CH_3
18	19.3, CH_3	19.8, CH_3	19.0, CH_3	28.1, CH_3	20.0, CH_3	25.7, CH_3	19.3, CH_3	28.0, CH_3	30.1, CH_3
19	17.6, CH_3	20.9, CH_3	20.8, CH_3	20.6, CH_3	15.5, CH_3	18.6, CH_3	19.1, CH_3	19.0, CH_3	21.3, CH_3
20	26.0, CH_3	16.1, CH_3	61.5, CH_2	15.9, CH_3	20.5, CH_3	16.0, CH_3	60.2, CH_2	16.0, CH_3	19.5, CH_3
9-OCH_3				56.9, CH_3					

[a]Measured in DMSO-d_6. [b]Measured in CD_3OD. [c]Measured in $CDCl_3$.

and from H-11 to C-19), while the 13-OH of **1** was established based on the C-13 chemical shift and HMBC (correlations from 13-OH to C-12, C-13, and C-20 in DMSO-d_6). The relative configuration of **1**, established by NOESY (Figure S3), highlighted the facial orientation of H-4 and H-6 to oppose that of H-8, H-11, CH_3-17, CH_3-18, CH_3-19, and CH_3-20 based on NOESY (key correlations: H-4/H-6, CH_3-17/H-5b, H-5b/H-8, H-8/CH_3-18, H-8/H-9a, H-9a/H-11, H-11/CH_3-19, and CH_3-19/CH_3-20). Interestingly, the specific rotation of **1** ($[\alpha]^{25}_D$ −126.7) was similar to that of *ent*-homoabyssomicin A (Figure 1, one of only two reported abyssomicins with "enantiomeric" configuration).[13] Subsequent single-crystal X-ray crystallography (CDCC accession number 1495049; Figure 2 and Table S1) established the absolute configuration of **1** as 4*S*,6*R*,8*R*,10*S*,11*S*,12*R*,13*R*,15*S*,16*R*. On basis of this cumulative analysis, **1** was identified as a new abyssomicin analogue and thereby designated abyssomicin M (where the use of "micin"

DOI: 10.1021/acs.jnatprod.7b00108
J. Nat. Prod. 2017, 80, 1141−1149

Table 2. ^{13}C NMR (100/125 MHz) Data for Compounds 5, 5B, 5C, 6, 6B, 6C, 7, 7B, and 7C

no.	5[a]	5B[a]	5C[b,d]	6[c]	6B[c]	6C[b]	7[a]	7B[a]	7C[b]
1	172.7, C	171.7, C	ND[e]	177.3, C	175.6, C	164.3, C	172.2, C	171.3, C	164.5, C
2	98.1, C	54.3, CH	114.2, C	99.7, C	55.9, CH	113.9, C	97.9, C	54.4 CH	110.2,qC
3	179.9, C	106.4, C	166.7, C	182.7, C	108.3, C	167.7, C	180.1, C	106.4, C	167.8, C
4	35.8, CH	42.3, CH	36.5, CH	37.5, CH	43.7, CH	36.5, CH	35.9, CH	42.4, CH	36.6, CH
5	37.9, CH_2	36.3, CH_2	39.2, CH_2	39.6, CH_2	38.3, CH_2	38.9, CH_2	38.1, CH_2	36.3, CH_2	39.2, CH_2
6	77.6, C	80.2, C	78.2, C	79.6, C	82.6, C	78.1, C	77.7, C	80.2, C	78.3, C
7	208.8, C	200.3, C	206.4, C	210.1, C	201.9, C	206.6, C	208.7, C	200.1, C	206.9, C
8	66.9, CH	68.3, CH	63.0, CH	68.7, CH	70.1, CH	63.5, CH	66.4, CH	67.7, CH	64.0, CH
9	66.9, CH	67.6, CH	70.2, CH	68.9, CH	70.1, CH	73.8, CH	67.0, CH	67.7, CH	70.3, CH
10	53.5, CH	52.8, CH	49.6, CH	55.4, CH	54.4, CH	49.2, CH	54.4, CH	53.5, CH	50.8, CH
11	71.9, CH	71.7, CH	73.8, CH	74.7, CH	74.5, CH	70.0, CH	74.8, CH	74.4, CH	75.9, CH
12	78.0, C	77.6, C	77.3, C	79.3, C	78.8, C	81.2, C	75.2, C	75.1, C	73.1, C
13	26.8, CH	27.0, CH	28.9, CH	36.6, CH	36.8, CH	33.7, CH	73.1, C	73.1, C	76.7, C
14	33.7, CH_2	31.7, CH_2	34.2, CH_2	30.6, CH_2	28.7, CH_2	29.6, CH_2	44.0, CH_2	42.4, CH_2	45.1, CH_2
15	84.5, C	83.1, C	81.6, C	86.5, C	84.6, C	76.0, C	84.0, C	82.7, C	81.7, C
16	84.5, C	83.7, C	84.1, C	86.8, C	85.8, C	84.3, C	83.7, C	83.2, C	83.6, C
17	19.0, CH_3	11.2, CH_3	17.5, CH_3	19.9, CH_3	12.2, CH_3	17.9, CH_3	18.9, CH_3	11.3, CH_3	17.5, CH_3
18	27.2, CH_3	22.6, CH_3	27.7, CH_3	28.0, CH_3	22.9, CH_3	27.4, CH_3	27.1, CH_3	22.6, CH_3	27.7, CH_3
19	20.4, CH_3	20.6, CH_3	20.8, CH_3	21.6, CH_3	21.2, CH_3	20.8, CH_3	17.3, CH_3	17.4, CH_3	17.3, CH_3
20	15.8, CH_3	15.9, CH_3	16.0, CH_3	63.8, CH_2	63.3, CH_2	64.2, CH_2	25.6, CH_3	25.6, CH_3	25.7, CH_3

[a]Measured in DMSO-d_6. [b]Measured in $CDCl_3$. [c]Measured in CD_3OD. [d]Obtained from HSQC and HMBC. [e]ND: not detected.

Table 3. ^1H NMR (400/500 MHz) Data for Compounds 1−4, 11, and 12 (δ_H in ppm, multi, J in Hz)

no.	1[a]	2[c]	3[a]	4[a]	11[a]	12[b]
4	2.41, m	2.48, m[d]	2.40, m	2.43, m[d]	3.26, m	3.94, m
5	1.60, m	1.61, m	1.58, m	1.64, dd (5.6, 12.6)	1.54, m (2H)	2.40, m[d]
	2.63, dd (12.0)	2.78, dd (12.2)	2.65, dd (12.4)	2.88, dd (12.6)		2.97, dd (8.3, 17.0)
6	2.13, m	2.35, m[d]	2.12, m			
8	3.57, t (9.0)	3.29, t (8.7)	3.48, m[d]	3.15, m[e]	1.76, t (11.6)	5.69, d (15.7)
					3.29, m	
9	1.57, m	1.58, m	1.51, m	4.20, dd (3.8, 7.8)	4.05, m	7.04, dd (8.6, 15.7)
	2.04, m	2.36, m[d]	2.00, m			
10	2.49, m	2.50, m[d]	2.32, m	2.41[d]	2.39, br s	2.90, br s
11	3.88, d (6.8)	3.74, br s	3.61, d (4.5)	3.96, br s	3.62, br s	5.23, br s
13		2.36, m[d]	2.35, m	2.22, m	2.44[d]	2.39[d]
14	1.67, d (14.5)	1.14, dd (4.6, 12.9)	1.19, m	0.93,m	1.43, m	1.72, m (2H)
	2.34, d (14.6)	2.43, m[d]	2.15, d (13.3)	2.07, t (12.5)	2.45, m[d]	
17	1.23, d (7.1)	1.35, d (7.0)	1.24, d (6.7)	1.14, d (7.2)	1.04, d (6.3)	1.05, d (6.7)
18	1.01, d (7.3)	1.08, d (7.3)	1.00, d (7.2)	1.09, s	1.16, s	2.12, s
19	1.27, s	1.21, s	1.16, s	1.06, s	1.28, s	1.77, s
20	1.10, s	0.91, d (6.2)	3.14, 3.51, m[d]	0.76, d (6.6)	0.93, d (5.5)	1.10, d (6.9)
3-OH	10.96, s	11.26, s	10.98, s		13.63, s	
6-OH					5.68, s	
11-OH	6.36, d (7.1)		5.66, d (4.7)		6.11, d (4.4)	
13-OH	5.76, s					
20-OH			4.42, br s			
9-OCH_3				3.16, s[e]		

[a]Measured in DMSO-d_6. [b]Measured in CD_3OD. [c]Measured in $CDCl_3$. [d,e]Signals overlapped.

reflects early precedent in contrast to a *Streptomyces* point of origin).

Compounds **2**−**8** were determined to be variations (deoxy, hydroxy, methoxy, epoxy) of **1** via HRESIMS and 1D/2D NMR (Tables 1−5, Figure S2 and S3) and also displayed opposite specific rotation that of the archetypical abyssomicins. They were thereby designated as new abyssomicins N−T (**2**−**8**). Intriguingly, equilibrium mixtures of the C-2/C-3 enol and C-3 *gem*-diol (hydrate) of compounds **5**−**7** were observed, where subsequent *per*-acetylation using acetic anhydride and pyridine led to single products for structure elucidation (Figure 4).

In a similar manner, HRESIMS and 1D/2D NMR (Tables 1 and 5, Figures S2 and S3) revealed **9** as a C-11-deacetylated analogue of *ent*-homoabyssomicin B.[13] Compound **10** shared the **9** tetracyclic abyssomicin core but was distinguished by the presence of an additional 20-OH (reminiscent of **3** and **6**) and the lack of the 6-OH and C-8/C-9-epoxide. Thus, **9** and **10** were named abyssomicins U and V.

DOI: 10.1021/acs.jnatprod.7b00108
J. Nat. Prod. 2017, 80, 1141−1149

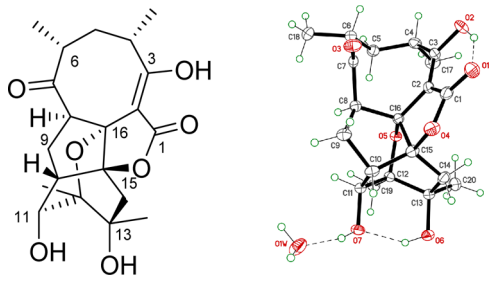


Figure 2. Structure of abyssomicin M (**1**).

Compound **11** ($C_{20}H_{26}O_8$) also displayed common abyssomicin UV and NMR signatures with putative structural divergence at C-2, C-9, and C-16 (Tables 1 and 3). Specifically, C-16 in **11** displayed both a unique ^{13}C chemical shift (δ_C 171.5) and distinct HMBC correlations with H-10 (δ_H 2.39) and H_2-14 (δ_H 1.43, 2.45), consistent with a lack of C-2/C-16 bond and the presence of a novel lactone (Figure 1). Additional key evidence included HMBC correlations from H-9 (δ_H 4.05) to C-1, C-2, and C-3 and from H_2-8 to C-2, C-7, C-9, and C-10, which established the C-2/C-9 bond. The similarities in optical rotation and NOE correlations among **11** and **1** implicated relative stereochemical conservation among shared substructures (Figure 3). Treatment of **11** with (*S*)- and (*R*)-MTPA chlorides gave the C-11 mono-MTPA esters (**11B** and **11C**, Figure 5), respectively, as a basis for C-11 stereochemical determination.[33,34] The calculated H-11 $\Delta\delta$ values (Figure 5) supported an *S*-configuration consistent with that of **1**. These cumulative analyses established **11** (abyssomicin W) as an 8/6/6/6 tetracyclic system where the C-2/C-16 disconnection and C-2/C-9 connection are unique among abyssomicins reported to date.

Compound **12** ($C_{20}H_{24}O_7$) also displayed common abyssomicin NMR signatures with clear C-6 and C-7 structural divergence and the presence of two additional double bonds (Tables 1 and 3). ^{13}C and ^1H NMR and HMBC (Figure 3) established the nature of the two double bonds [(*E*)-$\Delta^{8,9}$ and (*Z*)-$\Delta^{11,12}$] and the unprecedented loss of the C-6/C-7 bond with concomitant formation of the C-6 ketone and C-7 carboxylate. The relative configuration of **12** was also established by NOESY (Figure 3). As a novel ring-open abyssomicin congener and the first reported naturally occurring linear spirotetronate, **12** was designated as abyssomicin X.

Prior abyssomicin SAR studies highlighted the presence of the C-8/C-9 α,β-unsaturated carbonyl as the key reactive abyssomicin pharmacophore.[18] Consistent with their lack of this reactive moiety, compounds **1**–**12** were inactive at or below 60 and 10 μM in standard antimicrobial and cancer cell line cytotoxicity assays, respectively.

DISCUSSION

In summary, metabolic profiling of the coal mine fire isolate *Streptomyces* sp. LC-6-2 led to the discovery of a set of 12 abyssomicin analogues, highlighting the first reported example of a bacterial strain capable of producing a broad array of abyssomicins that share global stereochemical features with the unique "enantiomeric" *ent*-homoabyssomicins A and B.[13] Among this set, the bicyclic abyssomicin X (**12**) and tetracyclic abyssomicin W (**11**) also notably expand the structural diversity of abyssomicin-associated scaffolds reported to date. From a biosynthetic perspective, the existence of both abyssomicins and *ent*-abyssomicins raises questions with respect to the fundamental drivers of stereoselectivity in abyssomicin ring formation as well as the corresponding impact of substrate stereochemical configuration on subsequent downstream tailoring reactions. Comparison of the newly discovered abyssomicins M–X (**1**–**12**) with previously reported abyssomicins suggests some global conservation among putative biosynthetic late-stage tailoring reactions including 9-*O*-methylation in **4** (also observed in abyssomicins E[8] and L[11]) and 6-OH substitution in **4**–**7**, **9**, and **11** (also observed in abyssomicins E[8] and *ent*-homoabyssomicin B[13]). Consistent with the newly discovered abyssomicins M–X (**1**–**12**), whole genome sequencing of *Streptomyces* sp. LC-6-2 revealed a single putative abyssomicin biosynthetic gene cluster (Figure S4 and Table S2) and thereby sets the stage for future gene locus validation and biosynthetic interrogation.

Table 4. ^1H NMR (400/500 MHz) Data for Compounds 5, 5B, 5C, 6, 6B, and 6C (δ_H in ppm, multi, *J* in Hz)

no.	5[a]	5B[a]	5C[b]	6[c]	6B[c]	6C[b]
2		3.41, s			3.56, s	
4	2.58, m	2.25, m	2.71, m	2.71, m	2.38[d]	2.72, m
5	1.81, dd (5.4, 15.2)	1.99, m	2.00, m	1.91, dd (5.6, 15.2)	2.08, m	1.99, dd (5.4, 15.6)
	2.84, dd (12.6)	2.32[d]	2.92, d (15.1)	2.94, dd (12.3)	2.39[d]	2.88, dd (12.2)
8	3.18, d (8.0)	3.00, d (7.3)	3.63, d (7.8)	3.23, d (8.2)	3.00, d (7.9)	3.61, d (8.5)
9	4.52, dd (3.8, 8.0)	4.22, dd (5.2, 7.3)	5.46, dd (3.9, 8.0)	4.75, dd (4.2, 8.2)	4.39, dd (4.8, 7.9)	5.18, s
10	2.30[d]	2.26, m	2.97, d (3.4)	2.45, m	2.43, m	3.00, d (3.9)
11	4.16, br s	4.11, br s	5.21, br s	4.20, br s	4.21, br s	5.46,dd (4.2, 8.5)
13	2.30[d]	2.30[d]	2.33, m	2.54, m	2.54, m	2.60, m
14	0.86, dd, 4.4, 12.7	1.23[e]	1.14, m	1.32[d]	1.58, m	1.37, dd (5.6, 14.5)
	2.27[d]	2.32[d]	2.46, t (12.8)	2.26	2.32[d]	2.40, t (11.5)
17	1.23[e]	1.22[e]	1.29, d (7.0)	1.31[d]	1.28[e]	1.27, d (7.1)
18	1.14, s	1.26, s	1.31, s	1.24, s	1.35, s	1.31, s
19	1.14, s	1.08, s	1.22, s	1.32, s	1.27, s[e]	1.31, s
20	0.82, d (5.7)	0.92, d (5.6)	0.97, d (6.6)	3.37, t (9.2, 9.8)	3.51, t (9.2, 10.0)	3.86, t (9.1, 9.7)
				3.72, dd (5.8, 10.0)	3.79, dd (5.8, 10.0)	4.30, dd (6.0, 10.7)
3-OH	10.93, s					

[a]Measured in DMSO-d_6. [b]Measured in $CDCl_3$. [c]Measured in CD_3OD. [d,e]Signals overlapped.

DOI: 10.1021/acs.jnatprod.7b00108
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Table 5. ^{1}H NMR (400/500 MHz) Data for Compounds 7, 7B, 7C, 8, 9, and 10 (δ_H in ppm, multi, J in Hz)

no.	7[a]	7B[a]	7C[b]	8[b]	9[a]	10[a]
2		3.29, s				
4	2.55, m	2.20, m	2.72, m	2.47[c]	2.64[c]	3.16, m
5	1.78, dd (5.7, 15.3)	1.95, dd (8.6, 13.1)	2.04, m	1.53, m	1.58, dd (5.5, 16.0)	1.34[c]
	2.73, dd (13.6)	2.20, m	2.86, dd (11.9)	2.92, dd (12.2)	2.18, dd (1.8, 16.0)	1.81, m
6				2.47[c]		2.60[d]
8	3.18, d (8.0)	2.99, d (7.8)	3.67, d (8.4)	3.23, dd (7.0, 12.2)	3.93, d (1.7)	2.63[d]
9	4.52, m	4.22, m	5.46, dd (4.1, 8.4)	1.85, dd (7.0, 13.1)	3.15, dd (2.1, 3.0)	1.77, m
				2.65, t (13.1)		2.01, dd (3.5, 10.7)
10	2.43, d (4.0)	2.41, d (4.5)	3.10, d (4.1)		2.93, t (3.0)	2.21
11	4.29, d (7.6)	4.22, br s	5.44, br s	3.01, s	3.34, d (2.6)	3.86, t (5.4)
13					2.47, m	2.40[e]
14	1.59, d (14.5)	2.04, d (14.7)	1.91, d (15.2)	1.42, d (11.8)	1.30, dd (3.4, 12.6)	1.35[c]
	2.27, d (14.5)	2.26 d (14.4)	2.47, d (15.2)	2.78, d (11.8)	2.67[c]	2.40[e]
17	1.16, d (7.2)	1.13, d (6.7)	1.27, d (7.1)	1.29, d (7.0)	1.06, d (6.5)	0.96, d (6.8)
18	1.10, s	1.22, s	1.31, s	1.13, d (7.2)	1.36, s	0.98, d (7.1)
19	1.29, s	1.22, s	1.38, s	1.46, s	1.45, s	1.49, s
20	1.07, s	1.15, s	1.29, s	1.45, s	0.94, d (7.2)	3.24, 3.53, m
3-OH	10.89, s	5.96, s		11.46, s		
6-OH	4.93, s	4.93, s				
9-OH	5.29, d (4.7)	5.31, d (4.7)				
11-OH	6.34, d (7.6)	6.39, d (7.3)				5.96, d (5.6)
13-OH	5.80, s	5.72, s				
20-OH						4.73, t (5.2)

[a]Measured in DMSO-d_6. [b]Measured in $CDCl_3$. [c,d,e]Signals overlapped.

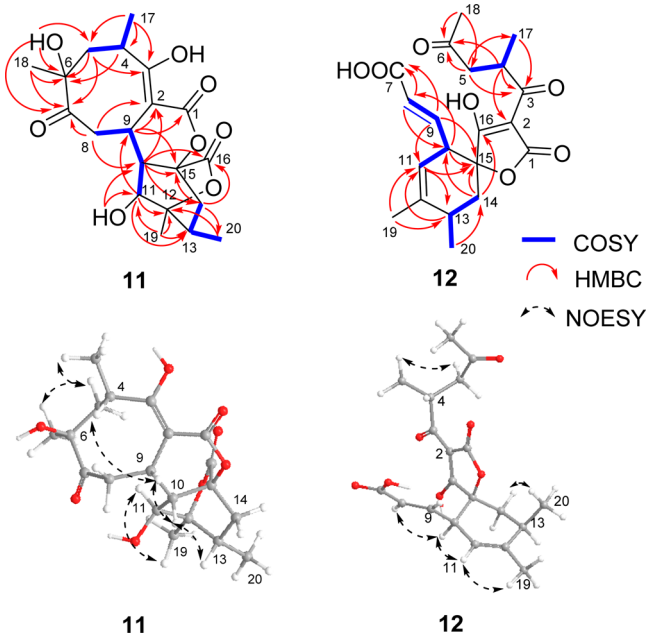

Figure 3. ^{1}H,^{1}H–COSY, key HMBC, and NOESY correlations for compounds **11** and **12**.

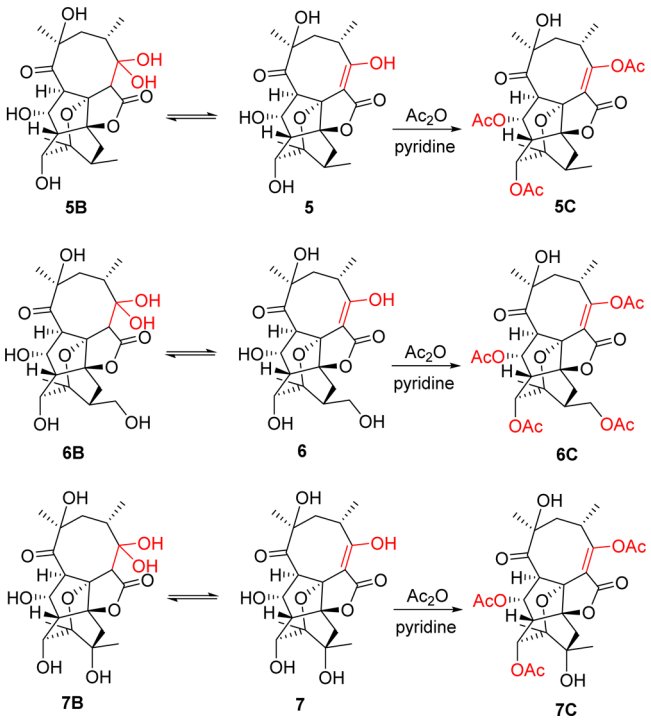

Figure 4. Equilibrium mixtures and acetylation of **5**–**7**.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation was recorded on a Jasco DIP-370 digital polarimeter (Jasco, Easton, MD, USA). UV spectra were recorded on an Ultrospec 8000 spectrometer (GE, Pittsburgh, PA, USA). All NMR data were recorded at 500 or 400 MHz for ^{1}H and 100 MHz for ^{13}C with Varian Inova NMR spectrometers (Agilent Technologies, Santa Clara, CA, USA). LC-MS was conducted with an Agilent 6120 Quadrupole MSD mass spectrometer (Agilent Technologies) equipped with an Agilent 1200 Series Quaternary LC system and an Eclipse XDB-C_{18} column (150 × 4.6 mm, 5 μm). HRESIMS spectra were recorded on an AB SCIEX Triple TOF 5600 system (AB Sciex, Framingham, MA, USA). Single-crystal X-ray diffraction was measured on a Bruker-Nonius X8 Proteum. HPLC analyses were performed on an Agilent 1260 system equipped with a photodiode array (PDA) detector and a Phenomenex C_{18} column (250 × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA). Semipreparative HPLC separation was performed on a Varian Prostar 210 HPLC system (Agilent) equipped with a PDA detector 330 using a Supelco DiscoveryBio wide pore C_{18} column (250 × 21.2 mm, 10

DOI: 10.1021/acs.jnatprod.7b00108
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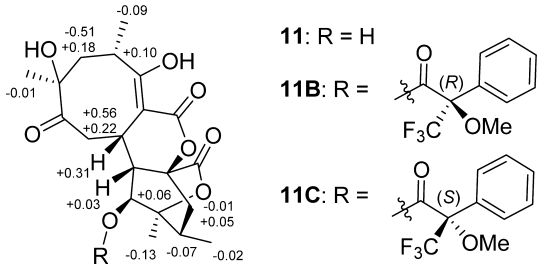

Figure 5. $\Delta\delta_{S-R}$ values for MTPA esters of **11**.

μm; flow rate, 8 mL/min; wavelength, 254 nm; Sigma-Aldrich, St. Louis, MO, USA). A Nanodrop 2000c UV−vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to measure concentration and purity of DNA. Sephadex LH-20 (25−100 μm) was purchased from GE Healthcare (Little Chalfont, United Kingdom). C_{18}-functionalized silica gel (40−63 μm) was purchased from Material Harvest Ltd. (Cambridge, United Kingdom). Amberlite XAD16N resin (20−60 mesh) was purchased from Sigma-Aldrich. TLC silica gel plates (60 F_{254}) were purchased from EMD Chemicals Inc. (Darmstadt, Germany). All solvents and reagents used were of ACS grade or greater, used without further purification, and purchased from Pharmco-AAPER (Brookfield, CT, USA), Sigma-Aldrich, TCI America (Tokyo, Japan), or Alfa-Aesar (Ward Hill, MA, USA) unless otherwise noted.

Isolation of *Streptomyces* sp. LC-6-2. The soil sample was collected from the Lotts Creek coal fire, Perry County, KY. The isolation of strain LC-6-2 followed previously reported protocols.[25,27]

DNA Extraction, Genome Sequencing, and Analyses. A single colony of *Streptomyces* sp. LC-6-2 from M2 agar (glucose, 4.0 g/L; yeast extract, 4.0 g/L; malt extract, 10.0 g/L; $CaCO_3$, 2.0 g/L; agar, 18.0 g/L) growth was used to inoculate in a 250 mL baffled flask with 50 mL of M2 broth (glucose, 4.0 g/L; yeast extract, 4.0 g/L; malt extract, 10.0 g/L; $CaCO_3$, 2.0 g/L). After 3 days of incubation at 28 °C with 200 rpm agitation, cells were recovered via centrifugation (5000*g* for 15 min at 4 °C) and used for genomic DNA isolation using an UltraClean microbial DNA isolation kit (Mo Bio Laboratories, CA, USA). DNA quality and quantity were assessed using gel electrophoresis and absorbance. The resultant DNA solution was subjected to massively parallel sequencing using the MiSeq sequencer (Illumina, San Diego, CA) at the University of Kentucky Advanced Genetic Technologies Center (UK-AGTC). Sequences were assembled using Newbler v.2.9 (Roche Diagnostics, Indianapolis, IN, USA) with gap fill-in and verification accomplished via polymerase chain reaction and amplicon sequencing to give the final draft genome (6.9 Mb, 78× coverage, 952 scaffolds with a mean size of 7954.8 bp, 72.6% GC percentage). The *abs* gene cluster was identified by BLAST searching, and manual annotation was done using Geneious 6.1.8 software[35] and BLAST search tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Protein functions were assigned through database comparison with the BLAST search tools (Figure S4 and Table S1). The sequence of the putative *abs* biosynthetic gene cluster has been deposited at GenBank under accession number KY432814.

Phylogeny of *Streptomyces* sp. LC-6-2. The partial 16S rRNA gene fragment was amplified using universal primers (27F, 5′-AGAGTTTGATCMTGGCTCAG-3′; 1492R, 5′-GGTTACCTTGTTACGACTT-3′)[36] and Advantage GC2 polymerase (Clontech, Mountain View, CA, USA), and the desired PCR-amplified product was isolated using the QIAquick gel extraction kit (Qiagen, Valencia, CA, USA). The amplified fragment (1307 bp) displayed 98% identity (BLAST search) to the 16S rRNA gene sequence of *Streptomyces echinatus* strain NBRC 12763. The sequence of 16S rRNA has been deposited in the NCBI nucleotide database with the accession number KT204465.

Fermentation, Extraction, Isolation, and Purification. *Streptomyces* sp. LC-6-2 was cultivated on M2 agar plates at 28 °C for 7 days. Chunks of the corresponding agar with bacterial growth were added to three 250 mL Erlenmeyer flasks, each containing 50 mL of medium SGG (soluble starch, 10.0 g/L; glucose, 10.0 g/L; glycerol, 10.0 mL/L; corn steep powder, 2.5 g/L; peptone, 5.0 g/L; yeast extract, 2.0 g/L; NaCl, 1.0 g/L; $CaCO_3$, 3.0 g/L). After 3 days of incubation at 28 °C with 200 rpm agitation, the seed cultures were used to inoculate 100 flasks (250 mL), each containing 100 mL of medium SGG. The fermentation (10 L total) was continued for 7 days at 28 °C with 200 rpm agitation. The obtained culture broth was centrifuged at 3000*g* for 30 min (4 °C). The biomass (mycelium) was extracted with MeOH (3 × 500 mL), and then the recovered organics were evaporated *in vacuo* at 40 °C to yield 10.8 g of crude extract. The supernatant was mixed with 3% (w/v) XAD-16 resin and stirred overnight, followed by filtration. The resin was washed with H_2O (3 × 600 mL) and then extracted with MeOH until the eluant was colorless. The MeOH extract was subsequently evaporated to afford 21.1 g of crude extract. Both extracts (obtained from the biomass and supernatant) revealed a similar metabolite profile based on HPLC and TLC analyses and were therefore combined (31.9 g).

As highlighted in Scheme S1, the combined crude extract was subjected to HP-20SS resin column chromatography (8 × 40 cm, 800 g) eluted with a gradient of aqueous CH_3CN (10−100%) to yield 12 fractions, A−L. Fraction C (0.8 g) was subjected to a Sephadex LH-20 column (4 × 100 cm, 2 mL/min, MeOH) to obtain three subfractions, C1−C3. Subfraction C2 (0.6 g) was further purified by semi-preparative HPLC (20−40% aqueous CH_3CN over 25 min) to yield compound **12** (7.8 mg, retention time: 16.2 min) as a colorless, amorphous powder. Fraction D (0.2 g) was purified by semi-preparative HPLC (10−45% aqueous CH_3CN over 30 min) to yield compounds **12** (10.5 mg, retention time: 16.2 min) and **6** (10.8 mg, retention time: 12.1 min) as colorless, amorphous powders. Fraction E (0.6 g) was subjected to a Sephadex LH-20 column (4 × 100 cm, 2 mL/min, MeOH) to obtain three subfractions, E1−E3. Subfraction E2 (0.28 g) was further purified by semipreparative HPLC (20−35% aqueous CH_3CN over 30 min) to yield compound **7** (11.4 mg, retention time: 15.5 min) as a colorless, amorphous powder. Fraction F (1.3 g) was subjected to a Sephadex LH-20 column (4 × 100 cm, 2 mL/min, MeOH) to obtain three subfractions, F1−F3. Subfraction F2 (0.3 g) was further purified by semipreparative HPLC (10−55% aqueous CH_3CN over 40 min) to yield compound **10** (5.4 mg, retention time: 14.1 min) as a colorless, amorphous powder. Fraction G (0.3 g) was purified by semipreparative HPLC (20−50% aqueous CH_3CN over 30 min) to yield compounds **3** (22.1 mg, retention time: 17.9 min) and **5** (30.0 mg, retention time: 19.0 min) as colorless, amorphous powders. Fraction H (0.2 g) was purified by semi-preparative HPLC (25−50% aqueous CH_3CN over 35 min) to yield compounds **5** (14.0 mg, retention time: 19.0 min) and **9** (10.2 mg, retention time: 17.4 min) as colorless, amorphous powders. Fraction I (0.3 g) was purified by semipreparative HPLC (25−50% aqueous CH_3CN over 35 min) to yield compounds **1** (8.1 mg, retention time: 21.7 min), **4** (6.3 mg, retention time: 22.3 min), **5** (10.5 mg, retention time: 19.0 min), **8** (1.5 mg, retention time: 22.8 min), and **9** (18.2 mg, retention time: 17.4 min) as colorless, amorphous powders. Fraction J (0.3 g) was purified by semipreparative HPLC (27−52% aqueous CH_3CN over 35 min) to yield compounds **1** (4.6 mg, retention time: 21.7 min), **4** (8.3 mg, retention time: 22.3 min), **8** (3.0 mg, retention time: 22.8 min), **9** (12.0 mg, retention time: 17.4 min), and **11** (4.0 mg, retention time: 19.6 min) as colorless, amorphous powders. Fraction K (0.1 g) was purified by semipreparative HPLC (35−70% aqueous CH_3CN over 30 min) to yield compound **2** (4.1 mg, retention time: 25.8 min) as a colorless, amorphous powder.

*Abyssomicin M (**1**):* white, amorphous powder; $[\alpha]^{25}_D$ −126.7 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ε) 264 (3.00) nm; ^{13}C and ^1H NMR data, see Tables 1 and 3; (+)-ESIMS *m*/*z* 379.2 $[M + H]^+$, 396.2 $[M + NH_4]^+$; (−)-ESIMS *m*/*z* 377.2 $[M - H]^-$, 413.1 $[M + Cl]^-$; (+)-HRESIMS *m*/*z* 379.1756 $[M + H]^+$ (calcd for $C_{20}H_{27}O_7$, 379.1757), 396.2020 $[M + NH_4]^+$ (calcd for $C_{20}H_{30}O_7N$, 396.2022).

*Abyssomicin N (**2**):* white, amorphous powder; $[\alpha]^{25}_D$ −82.5 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 261 (2.40) nm; ^{13}C and ^1H NMR data, see Tables 1 and 3; (+)-ESIMS *m*/*z* 363.2 $[M + H]^+$, 380.2 $[M + NH_4]^+$, 385.2 $[M + Na]^+$; (−)-ESIMS *m*/*z* 361.1 $[M + Cl]^-$, 397.1 [M

DOI: 10.1021/acs.jnatprod.7b00108
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+ Cl]$^-$; (+)-HRESIMS m/z 363.1798 [M + H]$^+$ (calcd for $C_{20}H_{27}O_6$, 363.1808), 385.1626 [M + Na]$^+$ (calcd for $C_{20}H_{26}O_6Na$, 385.1627).

*Abyssomicin O (**3**):* white, amorphous powder; $[\alpha]^{25}_D$ −94.5 (c 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 263 (2.25) nm; ^{13}C and ^{1}H NMR data, see Tables 1 and 3; (+)-ESIMS m/z 379.1 [M + H]$^+$; (−)-ESIMS m/z 377.1 [M − H]$^-$; (+)-HRESIMS m/z 379.1747 [M + H]$^+$ (calcd for $C_{20}H_{27}O_7$, 379.1757), 396.2009 [M + NH$_4$]$^+$ (calcd for $C_{20}H_{30}O_7N$, 396.2022), 401.1565 [M + Na]$^+$ (calcd for $C_{20}H_{26}O_7Na$, 401.1575).

*Abyssomicin P (**4**):* white, amorphous powder; $[\alpha]^{25}_D$ −76.3 (c 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 264 (1.89) nm; ^{13}C and ^{1}H NMR data, see Tables 1 and 3; (+)-ESIMS m/z 409.2 [M + H]$^+$; (−)-ESIMS m/z 443.1 [M + Cl]$^-$; (+)-HRESIMS m/z 431.1665 [M + Na]$^+$ (calcd for $C_{21}H_{28}O_8Na$, 431.1682).

*Abyssomicin Q (**5**):* white, amorphous powder; $[\alpha]^{25}_D$ −52.7 (c 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 264 (1.73) nm; ^{13}C and ^{1}H NMR data, see Tables 2 and 4; (+)-ESIMS m/z 395.2 [M + H]$^+$, 811.3 [2 M + Na]$^+$; (−)-ESIMS m/z 429.1 [M + Cl]$^-$, 439.1 [M + HCOO]$^-$; (+)-HRESIMS m/z 395.1694 [M + H]$^+$ (calcd for $C_{20}H_{27}O_8$, 395.1706), 417.1510 [M + Na]$^+$ (calcd for $C_{20}H_{26}O_8Na$, 417.1525).

*Abyssomicin R (**6**):* white, amorphous powder; $[\alpha]^{25}_D$ −10.0 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 258 (1.16) nm; ^{13}C and ^{1}H NMR data, see Tables 2 and 4; (+)-ESIMS m/z 411.1 [M + H]$^+$, 428.2 [M + NH$_4$]$^+$; (−)-ESIMS m/z 445.1 [M + Cl]$^-$, 455.1 [M + HCOO]$^-$; (+)-HRESIMS m/z 411.1654 [M + H]$^+$ (calcd for $C_{20}H_{27}O_9$, 411.1655), 428.1922 [M + NH$_4$]$^+$ (calcd for $C_{20}H_{30}O_9N$, 428.1921).

*Abyssomicin S (**7**):* white, amorphous powder; $[\alpha]^{25}_D$ −37.6 (c 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 262 (3.05) nm; ^{13}C and ^{1}H NMR data, see Tables 2 and 5; (+)-ESIMS m/z 411.1 [M + H]$^+$, 428.2 [M + NH$_4$]$^+$; (−)-ESIMS m/z 409 [M − H]$^-$, 445.0 [M + Cl]$^-$, 455.1 [M + HCOO]$^-$; (+)-HRESIMS m/z 411.1658 [M + H]$^+$ (calcd for $C_{20}H_{27}O_9$, 411.1655).

*Abyssomicin T (**8**):* white, amorphous powder; $[\alpha]^{25}_D$ −87.6 (c 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 262 (3.72) nm; ^{13}C and ^{1}H NMR data, see Tables 1 and 5; (+)-ESIMS m/z 377.1 [M + H]$^+$; (−)-ESIMS m/z 375.1 [M − H]$^-$, 411.1 [M + Cl]$^-$; (+)-HRESIMS m/z 394.1837 [M + NH$_4$]$^+$ (calcd for $C_{20}H_{28}O_7N$, 394.1866), 399.1385 [M + Na]$^+$ (calcd for $C_{20}H_{24}O_7Na$, 199.1420).

*Abyssomicin U (**9**):* white, amorphous powder; $[\alpha]^{25}_D$ +107.7 (c 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 245 (4.60) nm; ^{13}C and ^{1}H NMR data, see Tables 1 and 5; (+)-ESIMS m/z 393.2 [M + H]$^+$; (−)-ESIMS m/z 437.2 [M + HCOO]$^-$; (+)-HRESIMS m/z 393.1518 [M + H]$^+$ (calcd for $C_{20}H_{25}O_8$, 393.1549), 415.1344 [M + Na]$^+$ (calcd for $C_{20}H_{24}O_8Na$, 415.1369).

*Abyssomicin V (**10**):* white, amorphous powder; $[\alpha]^{25}_D$ +87.7 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 243 (3.21) nm; ^{13}C and ^{1}H NMR data, see Tables 1 and 5; (+)-ESIMS m/z 379.1 [M + H]$^+$; (−)-ESIMS m/z 377.2 [M − H]$^-$, 413.1 [M + Cl]$^-$; 423.2 [M + HCOO]$^-$; (+)-HRESIMS m/z 379.1752 [M + H]$^+$ (calcd for $C_{20}H_{27}O_7$, 379.1757), 401.1570 [M + Na]$^+$ (calcd for $C_{20}H_{26}O_7Na$, 401.1576).

*Abyssomicin W (**11**):* white, amorphous powder; $[\alpha]^{25}_D$ −50.0 (c 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 270 (1.28) nm; ^{13}C and ^{1}H NMR data, see Tables 1 and 3; (+)-ESIMS m/z 412.2 [M + NH$_4$]$^+$; (−)-ESIMS m/z 429.1 [M + Cl]$^-$, 439.2 [M + HCOO]$^-$; (+)-HRESIMS m/z 412.1955 [M + NH$_4$]$^+$ (calcd for $C_{20}H_{30}O_8N$, 412.1971), 806.3593 [2 M + NH$_4$]$^+$ (calcd for $C_{40}H_{56}O_{16}N$, 806.3599).

*Abyssomicin X (**12**):* white, amorphous powder; $[\alpha]^{25}_D$ +135.0 (c 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.96), 268 (4.58) nm; ^{13}C and ^{1}H NMR data, see Tables 1 and 3; (+)-ESIMS m/z 377.1 [M + H]$^+$, 394.2 [M + NH$_4$]$^+$; (−)-ESIMS m/z 375.1 [M − H]$^-$; (+)-HRESIMS m/z 377.1600 [M + H]$^+$ (calcd for $C_{20}H_{25}O_7$, 377.1600), 394.1865 [M + NH$_4$]$^+$ (calcd for $C_{20}H_{28}O_7N$, 394.1866).

Mosher Ester Analysis.[32,33] Compound **11** (1 mg) was dissolved in 500 μL of DCM and 8 μL of pyridine, to which were then added sequentially dimethylaminopyridine (1 mg) and (*S*)-MTPA-Cl (20 μL). The reaction mixture was stirred at room temperature overnight and subsequently quenched via the addition of two drops of H_2O. The corresponding mono-(*R*)-MTPA ester derivative (**11B**, 1 mg) was purified via semipreparative HPLC (25−50% aqueous CH_3CN over 30 min). The mono-(*S*)-MTPA ester derivative **11C** (1 mg) was made by using (*R*)-MTPA-Cl (20 μL) following the same protocol above. The calculated H-11 $\Delta\delta$ values (Figure 5) supported an *S*-configuration consistent with that of **1**.

*11-(R)-MTPA-abyssomicin W (**11B**):* ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 13.72 (1H, s), 5.24 (1H, d, J = 5.7 Hz, H-11), 3.95 (1H, m, H-9), 3.34 (1H, dd, J = 4.2, 11.9 Hz, H-8a), 3.03 (1H, m, H-4), 2.37 (1H, br s, H-10), 2.28−2.35 (4H, overlapped, H-5a, H-8b, H-13, H-14a), 1.89 (1H, t, J = 13.1 Hz, H-5b), 1.73 (1H, dd, J = 7.5, 9.9 Hz, H-14b), 1.33 (3H, s, H-18), 1.26 (3H, s, H-19), 1.18 (3H, d, J = 6.2 Hz, H-17), 1.03 (3H, d, J = 4.9 Hz, H-20); (+)-HRESIMS m/z 628.2368 [M + NH$_4$]$^+$ (calcd for $C_{30}H_{37}F_3NO_{10}$, 628.2370).

*11-(S)-MTPA-abyssomicin W (**11C**):* ^{1}H NMR ($CDCl_3$, 500 MHz) 5.18 (1H, d, J = 5.3 Hz, H-11), 3.64 (1H, m, H-9), 2.93 (1H, br s, H-4), 2.79 (1H, m, H-5a), 2.54 (1H, dd, m, H-8a), 2.34−2.36 (3H, overlapped, H-10, H-13, H-14a), 2.10 (1H, t, J = 6.9 Hz, H-8b), 1.71 (1H, m, H-5b), 1.68 (1H, m, H-14b), 1.39 (3H, s, H-19), 1.34 (3H, s, H-18), 1.29 (3H, d, J = 6.5 Hz, H-17), 1.05 (3H, d, J = 5.5 Hz, H-20); (+)-HRESIMS m/z 628.2352 [M + NH$_4$]$^+$ (calcd for $C_{30}H_{37}F_3NO_{10}$, 628.2370).

Acetylation of 5−7. Compounds **5**, **6**, and **7** (3 mg each) were treated with acetic anhydride (0.2 mL) and pyridine (0.2 mL) at room temperature overnight. Drying under N_2, dissolving in MeOH (200 mL), and purifying via semipreparative HPLC (25−60% aqueous CH_3CN over 35 min) yielded compounds **5C**, **6C**, and **7C**.

*3,9,11-Triacetylabyssomicin abyssomicin Q (**5C**):* white, amorphous powder; ^{13}C and ^{1}H NMR data, see Tables 2 and 4; (+)-ESIMS m/z 538.2 [M + NH$_4$]$^+$, 503.2 [(M − H$_2$O) + H)]$^+$; (−)-ESIMS m/z 555.1 [M + Cl]$^-$; (+)-HRESIMS m/z 503.1895 [M + H − H$_2$O]$^+$ (calcd for $C_{26}H_{31}O_{10}$, 503.1917), 543.1815 [M + Na]$^+$ (calcd for $C_{26}H_{32}O_{11}Na$, 543.1842), 1063.3723 [2 M + Na]$^+$ (calcd for $C_{52}H_{64}O_{22}Na$, 1063.3787).

*3,9,11,20-Tetraacetylabyssomicin abyssomicin R (**6C**):* white, amorphous powder; ^{13}C and ^{1}H NMR data, see Tables 2 and 4; (+)-HRESIMS m/z 596.2399 [M + NH$_4$]$^+$ (calcd for $C_{28}H_{38}NO_{13}$, 596.2343).

*3,9,11-Triacetylabyssomicin abyssomicin S (**7C**):* white, amorphous powder; ^{13}C and ^{1}H NMR data, see Tables 2 and 5; (+)-HRESIMS m/z 559.1777 [M + Na]$^+$ (calcd for $C_{26}H_{32}O_{12}Na$, 559.1791).

X-ray Crystallography. Colorless bulk crystals of **1** were obtained in acetone/H_2O (2:1). X-ray diffraction data was collected at 90.0(2) K on a Bruker-Nonius X8 Proteum diffractometer with graded-multilayer focused Cu K(alpha) X-rays. Raw data were integrated, scaled, merged, and corrected for Lorentz−polarization effects using the APEX2 package.[37] Corrections for absorption were applied using SADABS.[38] The structure was solved by direct methods (SHELXT[39]) and refined against F^2 by weighted full-matrix least-squares (SHELXL-2014[40]). Hydrogen atoms were found in difference maps but subsequently placed at calculated positions and refined using a riding model. Non-hydrogen atoms were refined with anisotropic displacement parameters. The final structure model was checked using an *R*-tensor[41] and by Platon/checkCIF.[42] Crystallographic data for the structure of **1** have been submitted to the Cambridge Crystallographic Data Centre as supplementary publication CCDC 1495049 (see Figure 2 and Table S1). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Antibacterial, Antifungal, and Cancer Cell Line Viability Assays. Antibacterial (*Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* NRRL B-287, *Escherichia coli* NRRL B-3708, *Salmonella enterica* ATCC 10708), antifungal (*Saccharomyces cerevisiae* ATCC 204508), and cell line cytotoxicity (non-small-cell lung A549) assays were accomplished in triplicate following our previously reported protocols.[27,29]

DOI: 10.1021/acs.jnatprod.7b00108
J. Nat. Prod. 2017, 80, 1141−1149

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00108.

Workup isolation scheme; 1D/2D NMR and HRMS spectra of **1**−**12**; and crystal data (PDF)

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Notes

The authors declare the following competing financial interest(s): JST is a co-founder of Centrose (Madison, WI).

■ ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants R01 GM115261 (J.S.T.) and T32 DA016176 (Y.Z.), the University of Kentucky College of Pharmacy, the University of Kentucky Markey Cancer Center, and the National Center for Advancing Translational Sciences (UL1TR001998).

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DOI: 10.1021/acs.jnatprod.7b00108
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DOI: 10.1021/acs.jnatprod.7b00108
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