JOURNAL OF NATURAL PRODUCTS

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Chartarlactams A–P, Phenylspirodrimanes from the Sponge-Associated Fungus *Stachybotrys chartarum* with Antihyperlipidemic Activities

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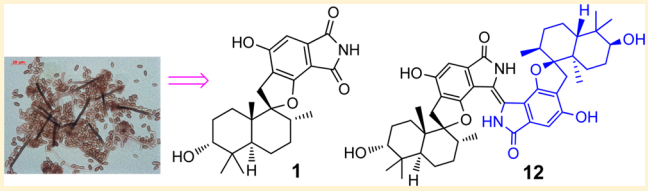
Supporting Information

ABSTRACT: Chemical examination of the solid culture of the endophytic fungus *Stachybotrys chartarum* isolated from the sponge *Niphates recondita* resulted in the isolation of 16 new phenylspirodrimanes, named chartarlactams A–P (**1**–**16**), together with eight known analogues. Their structures were determined on the basis of extensive spectroscopic analysis, including X-ray single-crystal diffraction for the determination of the absolute configurations. The isoindolone-drimane dimer chartarlactam L (**12**) was determined as a new skeleton. Compounds **1**–**6** and **8**–**24** were evaluated for antihyperlipidemic effects in HepG2 cells, and the primary structure–activity relationships are discussed.



Marine-derived fungi have proven to be promising sources for a wide array of structurally diverse and biologically active secondary metabolites, and some of them have pharmaceutical uses.[1,2] Unlike terrestrial colonizers, marine microorganisms have evolved under stressful environmental conditions that induce the biosynthesis of structurally unique natural products with diverse ecological functions.[3−6] The sponge-associated fungi are prolific sources of bioactive compounds, such as cytotoxic alkaloids and meroterpenoids from a *Tethya aurantium*-associated *Aspergillus* sp.,[7,8] antibiotic anthraquinones from the sponge-associated *Eurotium cristatum*,[9] pandangolides from *Niphates rowi*-associated *Cladosporium* sp.,[10] and protein kinase-targeted anthraquinones and betaenone derivatives from an *Aplysina aerophoba*-associated *Microsphaeropsis*.[11] Terrestrial strains of the fungus *Stachybotrys chartarum* are known to produce diverse secondary metabolites, including trichothecene mycotoxins, diterpenes, and phenylspirodrimanes,[12] which are a group of structurally unusual compounds with a wide range of pharmacological activities including inhibition of pancreatic cholesterol esterase,[13] in addition to anticomplement[14] and antiviral effects.[15−17] In the course of our investigation of the chemical diversity from marine microorganisms, the fungus *S. chartarum* was isolated from the tissue of the marine sponge *Niphates recondita* collected from the coral reef of Weizhou Island in Beibuwan Bay. Chromatographic separation of the EtOAc extract obtained from rice fermentation resulted in the isolation of 24 phenylspirodrimane-type analogues, including 16 new compounds.

RESULTS AND DISCUSSION

Chartarlactam A (**1**) was isolated as pale yellow crystals, and its molecular formula ($C_{23}H_{29}NO_5$) was determined by HRESIMS and NMR data, requiring 10 degrees of unsaturation. The IR absorptions at 3435, 1751, and 1714 cm^{-1} were characteristic of hydroxy and carbonyl functionalities. Comparison of the NMR spectroscopic data and analyses of the 2D NMR spectra revealed the gross structure of **1** to be closely related to stachybotrylactam (**17**).[12] The distinction was attributed to C-8′, in which a methylene group of the known analogue was replaced by a carbonyl carbon (δ_C 167.6, C-8′) of **1**, as evident from the NH proton (δ_H 10.74, brs) exhibiting HMBC correlations to C-8′, C-7′ (δ_C 169.4), C-4′ (δ_C 135.6), and C-5′ (δ_C 103.3). The NOE correlations from H_3-15 (δ_H 0.93, s) to H-11b (δ_H 3.07, d, J = 17.6 Hz), H-8 (δ_H 1.79, m), and H_3-14 (δ_H 0.77, s) in addition to the correlation between H-5 (δ_H 2.04, brd, J = 9.8 Hz) and H_3-13 (δ_H 0.89, s) (Figure 1) indicated the *trans*-fusion of rings A and B, while the methylene H_2-11, H_3-15, and H-8 showed the same orientation. The absolute configuration of **1** was determined by the X-ray single-crystal diffraction using Flack parameters (Figure 2),[18] indicating a 3*R*, 5*S*, 8*R*, 9*R*, and 10*S* configuration, the same as previously determined for stachybotrylactam (**17**).

The molecular formula of chartarlactam B (**2**) was determined as $C_{28}H_{39}NO_6$ on the basis of the HRESIMS and NMR data. The ^1H and ^{13}C NMR data of **2** for the phenylspirodrimane nucleus were closely similar to those of

Received: October 9, 2013

dx.doi.org/10.1021/np400824u | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

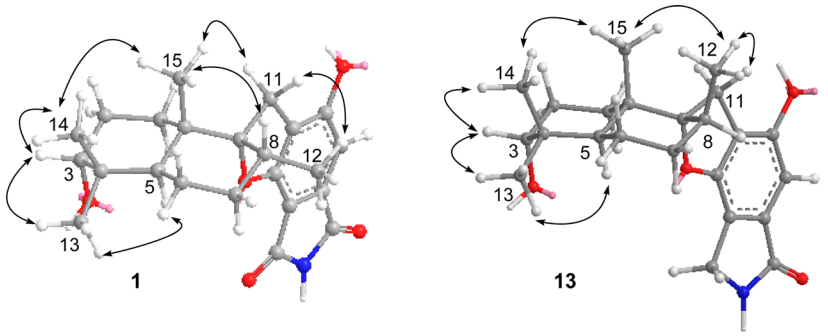


Figure 1. Key NOE interactions of **1** and **13**.

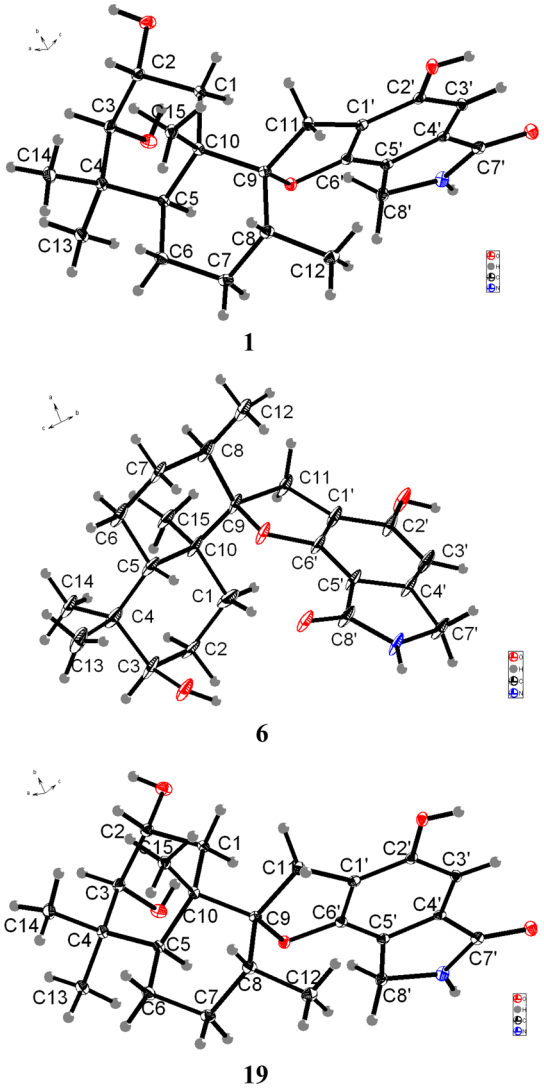
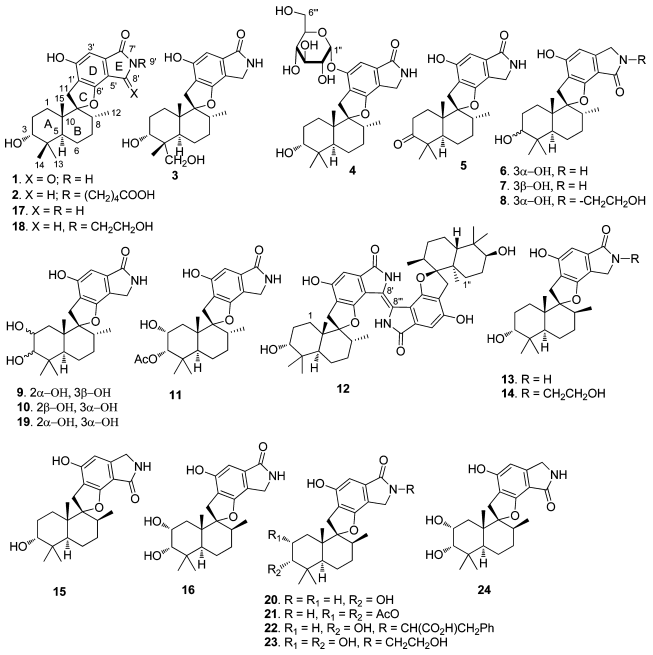


Figure 2. ORTEP views of **1**, **6**, and **19** showing thermal ellipsoids at the 50% probability level.



stachybotrylactam.[12] The remaining resonances included four methylenes at δ_C 41.8 (C-1″), 27.7(C-2″), 22.4 (C-3″), and 33.9 (C-4″), in addition to a carboxylic acid carbon at δ_C 175.3 (s, C-5″). Their COSY and HMBC data established a pentanoic acid moiety, which was connected to the nitrogen atom, as evident from the methylene protons H-1″ (δ_H 3.46, t, J = 7.0 Hz) correlated to C-7′ and C-8′. On the basis of biogenetic considerations, the closely similar NOE interactions of **1** and **2** suggested that **2** possesses the same configuration as **1**.

The structure of chartarlactam C (**3**) was determined as a C-13 hydroxylated analogue of stachybotrylactam[12] on the basis of the close similarity of the NMR data (Tables 1 and 2), except for the presence of a hydroxymethylene group (δ_H 3.17, 3.37; δ_C 69.7) whose protons correlated to C-3 and C-4 (δ_C 40.7) in the HMBC spectrum and interacted with H-5 in the NOESY spectrum.

The molecular formula of chartarlactam D (**4**) was in accordance with $C_{29}H_{41}NO_9$ as determined by HRESIMS data. Analyses of the 2D NMR (COSY, HMQC, and HMBC) data revealed that **4** possessed a phenylspirodrimane nucleus, the same as for stachybotrylactam.[12] The COSY and HMBC correlations of the remaining resonances established a glucose group. This moiety was linked to the aromatic ring through an ether bond across C-1″ and C-2′, according to the HMBC interaction from H-1″ (δ_H 5.43, d, J = 3.5 Hz) to C-2′ (δ_C 153.9). The $J_{H\text{-}1''/H\text{-}2''}$ (3.5 Hz) value suggested the sugar to have an α-linkage. Acidic hydrolysis of **4** released a sugar, whose R_f value in TLC was the same as that of the authentic D-glucose. In addition, the specific rotation of the sugar derived from **4** ($[\alpha]_D^{25}$ +86) was in accordance with that of D-glucose ($[\alpha]_D^{25}$ +97.9) and opposite to that of L-glucose ($[\alpha]_D^{25}$ −81.8), indicating the sugar of **4** to be in the D-form.

dx.doi.org/10.1021/np400824u | J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. ^{1}HNMR Data for Chartarlactams A–K (1–11)[a]

no.	1	2	3	4	5	6	7	8	9	10	11
1	1.70, dd (12.0, 12.0)	1.75, m	1.70, m	1.76, m	1.44, m	1.78, m	1.20, m	1.74, m	1.48, m	1.82, m	1.57, m
	0.90, m	0.91, m	0.96, m	0.92, m	1.65, m	0.93, m	1.22, m	0.90, m	1.20, dd (12.0, 12.0)	1.10, dd (6.4, 20.4)	1.21, m
2	1.38, m	1.38, m	1.40, m	1.38, m	2.17, ddd (3.6, 3.6, 12.0)	1.39, m	1.40, m	1.37, m	3.40, ddd (4.0, 9.2,12.0)	3.59, dt (6.4, 6.8)	3.89, m
	1.79, m	1.78, m	1.75, m	1.81, m	2.65, ddd (5.2, 12.0, 12.0)	1.80, m	1.45, m	1.60, m			
3	3.17, brs	3.17, brs	3.46, brs	3.17, brs		3.20, brs	2.90, dd (5.0, 10.3)	3.17, brs	2.70, dd (3.6, 9.2)	3.18, d (6.8)	4.69, brs
5	2.04, brd (9.8)	2.01, brd (9.76)	2.3, dd (2.9, 9.0)	2.01, brd (11.5)	1.97, dd (3.6, 9.6)	2.01, brd (11.6)	1.54, m	2.06, brd (12.0)	1.57, brd (10.0)	1.84, m	1.91, brd (8.9)
6	1.43, m	1.45, m	1.40, m	1.43, m	1.50, m	1.38, m	1.58, m	1.45, m	1.41, m	1.45, m	1.44, m
	1.50, m	1.49, m	1.42, m	1.50, m	1.60, m	1.42, m	1.60, m	1.50, m	1.45, m	1.50, m	1.50, m
7	1.40, m	1.39, m	1.40, m	1.40, m	1.44, m	1.48, m	1.43, m	1.48, m	1.41, m	1.47, m	1.38, m
	1.50, m	1.52, m	1.50, m	1.53, m	1.65, m	1.50, m	1.52, m	1.52, m	1.56, m	1.49, m	1.56, m
8	1.79, m	1.78, m	1.80, m	1.79, m	1.88, m	1.77, m	1.76, m	1.76, m	1.80, m	1.78, m	1.82, m
11	3.07, d (17.6)	3.11, d (17.0)	3.10, d (17.0)	3.18, d (17.1)	3.17, d (17.4)	3.02, d (16.0)	3.06, d (16.3)	3.00, d (15.8)	3.16, d (16.9)	3.07, d (17.1)	3.13, d (17.4)
	2.76, d (17.6)	2.76, d (17.0)	2.70, d (17.0)	2.93, d (17.1)	2.85, d (17.4)	2.64, d (16.0)	2.60, d (16.3)	2.66, d (15.8)	2.76, d (16.9)	2.76, d (17.1)	2.81, d (17.4)
12	0.61, d (6.3)	0.64, d (6.1)	0.65, d (6.5)	0.66, d (6.5)	0.66, d (6.41)	0.61, d (6.30)	0.62, d (6.40)	0.60, d (5.7)	0.65, d (6.4)	0.62, d (6.4)	0.68, d (6.2)
13	0.89, s	0.88, s	3.17, d (12.0)	0.88, s	1.00, s	0.90, s	0.93, s	0.88, s	0.95, s	0.98, s	0.81, s
			3.37, d (12.0)								
14	0.77, s	0.80, s	0.70, s	0.80, s	1.03, s	0.80, s	0.72, s	0.78, s	0.74, s	0.86, s	0.91, s
15	0.93, s	0.95, s	0.97, s	0.96, s	1.15, s	0.96, s	0.93, s	0.93, s	0.99, s	1.16, s	1.00, s
3′	6.70, s	6.55, s	6.55, s	6.88, s	6.57, s	6.32, s	6.32, s	6.32, s	6.57, s	6.58, s	6.58, s
7′						4.15, s	4.15, d (15.7)	4.32, s			
							4.17, d (15,7)				
8′		4.32, d (17.0)	4.22, d (17.3)	4.27, d (16.3)					4.17, s	4.21, d (16.4)	4.27, d (16.4)
		4.21, d (17.0)	4.10, d (17.3)	4.15, d (16.3)						4.13, d (16.4)	4.05, d (16.4)
1″		3.46, t (7.0)		5.43, d (3.5)				3.40, t (7.0)			1.99, s
2″		1.60, m		3.37, dd (3.5, 8.8)				3.56, t (7.0)			
3″		1.46, m		3.65, dd (8.8, 9.6)							
4″		2.24, t (7.0)		3.21, m							
5″				3.40, m							
6″				3.50, m							
OH-2′	10.74, s	9.80, br	9.71, s		9.77, s	9.96, s	9.99, s	10.00, br	9.71, s	9.72, s	9.77, s
OH-2									4.30, d (4.0)	4.46, brs	4.43, d (4.6)
OH-3									4.39, d (3.6)	4.27, brs	
NH	10.74, brs	9.80, br	8.35, s	8.44, s	8.34, s	7.84, brs	7.86, s		8.34, s	8.34, s	8.34, s

[a]Measured in DMSO-d_6 at 500 MHz.

The structure of chartarlactam E (**5**) was determined as a 3-one analogue of stachybotrylactam on the basis of similar NMR data, with the exception of a ketone at δ_C 215.8 (C-3) replacing a hydroxymethine of the latter, and this assignment was supported by the HMBC correlations from H_3-13/H_3-14 to C-3.

Chartarlactams F (**6**) and G (**7**) had the same molecular formula as that of stachybotrylactam,[12] and their NMR data were closely related to those of the latter compound. Both **6** and **7** showed HMBC correlations between H-3′ (δ_H 6.32, s) and C-7′ (δ_C 45.1, **6**)/C-7′ (δ_C 45.2, **7**) and NOE interactions between H-3′ and H_2-7′ (δ_H 4.15, s, **6**)/(δ_H 4.15, 4.17, **7**), indicating the carbonyl carbon (δ_C 169.4) of the γ-lactam in **6** and **7** was located at C-8′. The difference between **6** and **7** was found at C-3, where a broad peak for H-3 (δ_H 3.20, brs) of **6** and a dd coupling for H-3 (δ_H 2.90, dd, J = 5.0, 10.3 Hz) of **7**

dx.doi.org/10.1021/np400824u | J. Nat. Prod. XXXX, XXX, XXX–XXX

Table 2. ^{13}CNMR Data for Chartarlactams A–K (1–11)[a]

position	1	2	3	4	5	6	7	8	9	10	11
1	24.3, CH_2	24.3, CH_2	24.0, CH_2	24.3, CH_2	30.6, CH_2	24.4, CH_2	29.5, CH_2	24.4, CH_2	38.5, CH_2	35.9, CH_2	34.1, CH_2
2	25.4, CH_2	25.3, CH_2	25.3, CH_2	25.3, CH_2	34.1, CH_2	25.5, CH_2	27.1, CH_2	25.5, CH_2	67.4, CH	69.8, CH	63.6, CH
3	73.7, CH	73.9, CH	73.1, CH	73.9, CH	215.8, C	73.8, CH	77.0, CH	73.8, CH	82.3, CH	77.3, CH	80.1, CH
4	37.8, C	37.8, C	40.7, C	37.8, C	47.3, C	37.8, C	38.8, C	37.8, C	39.2, C	37.4, C	38.2, C
5	39.8, CH	39.8, CH	35.0, CH	39.8, CH	47.0, CH	39.6, CH	45.7, CH	39.6, CH	45.8, CH	41.3, CH	40.3, CH
6	20.9, CH_2	20.9, CH_2	20.7, CH_2	20.9, CH_2	22.0, CH_2	20.9, CH_2	21.1, CH_2	21.0, CH_2	20.7, CH_2	21.5, CH_2	20.3, CH_2
7	31.0, CH_2	31.2, CH_2	31.0, CH_2	31.2, CH_2	30.7, CH_2	30.9, CH_2	31.2, CH_2	31.0, CH_2	31.1, CH_2	30.9, CH_2	31.0, CH_2
8	37.0, CH	36.9, CH	36.7, CH	36.9, CH	36.7, CH	37.2, CH	36.8, CH	37.1, CH	36.4, CH	36.4, CH	36.3, CH
9	100.7, C	98.3, C	98.3, C	98.8, C	97.6, C	98.6, C	98.3, C	98.6, C	97.6, C	98.3, C	97.8, C
10	42.2, C	42.3, C	42.1, C	42.3, C	41.9, C	42.2, C	42.1, C	42.2, C	43.0, C	42.4, C	43.4, C
11	31.3, CH_2	32.1, CH_2	32.2, CH_2	32.0, CH_2	32.5, CH_2	31.3, CH_2	31.0, CH_2	31.3, CH_2	32.2, CH_2	32.8, CH_2	32.1, CH_2
12	15.8, CH_3	16.0, CH_3	16.0, CH_3	16.0, CH_3	15.7, CH_3	15.9, CH_3	15.8, CH_3	15.9, CH_3	15.8, CH_3	16.1, CH_3	15.8, CH_3
13	29.0, CH_3	29.1, CH_3	69.7, CH_2	29.1, CH_3	26.2, CH_3	29.0, CH_3	28.7, CH_3	29.0, CH_3	29.3, CH_3	27.4, CH_3	28.3, CH_3
14	22.8, CH_3	22.8, CH_3	17.8, CH_3	22.8, CH_3	21.9, CH_3	23.0, CH_3	16.4, CH_3	23.0, CH_3	17.5, CH_3	23.3, CH_3	21.9, CH_3
15	16.4, CH_3	16.3, CH_3	16.5, CH_3	16.2, CH_3	15.9, CH_3	16.4, CH_3	16.3, CH_3	16.4, CH_3	17.3, CH_3	20.3, CH_3	17.0, CH_3
1′	119.9, C	116.9, C	116.9, C	120.3, C	116.7, C	112.8, C	112.7, C	112.9, C	116.7, C	117.1, C	116.8, C
2′	159.3, C	154.1, C	154.1, C	153.9, C	154.1, C	157.0, C	157.1, C	156.8, C	154.1, C	154.1, C	154.3, C
3′	103.9, CH	101.3, CH	101.2, CH	102.0, CH	101.5, CH	102.0, CH	102.0, CH	101.7, C	101.4, CH	101.3, CH	101.3, CH
4′	135.6, C	134.5, C	134.7, C	134.9, C	134.9, C	147.3, C	147.4, C	144.9, C	134.9, C	134.8, C	134.9, C
5′	103.3, C	112.3, C	114.8, C	117.6, C	114.9, C	106.6, C	106.5, C	106.6, C	114.8, C	114.7, C	114.7, C
6′	158.8, C	156.3, C	156.6, C	156.4, C	156.2, C	158.9, C	158.6, C	158.5, C	156.5, C	156.6, C	156.4, C
7′	169.4, C	167.9, C	170.6, C	170.3, C	170.5, C	45.1, CH_2	45.2, CH_2	50.8, CH_2	170.7, C	170.6, C	170.5, C
8′	167.6, C	47.0, CH_2	42.4, CH_2	42.5, CH_2	42.2, CH_2	169.4, C	169.4, C	166.5, C	42.3, CH_2	42.1, CH_2	42.3, CH_2
1″		41.8, CH_2		99.1, CH				44.7, CH_2			21.4, CH_3
2″		27.7, CH_2		72.0, CH				59.8, CH_2			170.6, C
3″		22.4, CH_2		73.4, CH							
4″		33.9, CH_2		70.2, CH							
5″		175.3, C		74.4, CH							
6″				60.9, CH_2							

[a]Measured in DMSO-d_6 at 125 MHz.

dx.doi.org/10.1021/np400824u | J. Nat. Prod. XXXX, XXX, XXX–XXX

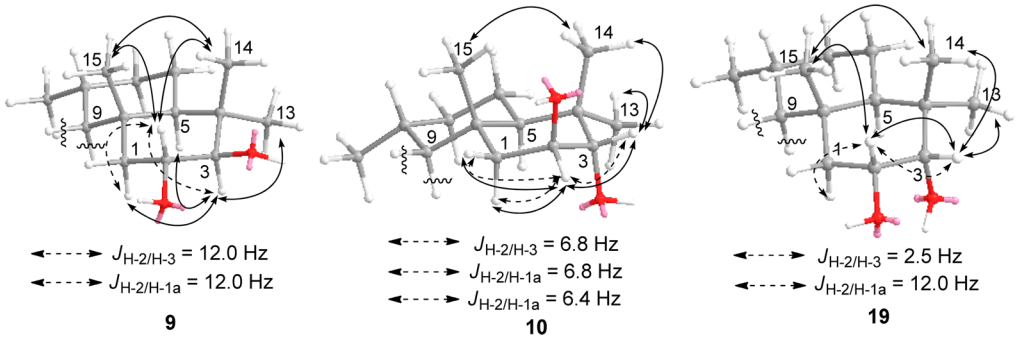


Figure 3. Key NOE interactions and J values of ring A in **9**, **10**, and **19**.

were observed, indicating varying configurations at C-3. The similar NMR data and NOE interactions of **6** and **1** assigned H-3 of **6** to be β-oriented. This assignment was proved by the results of an X-ray single-crystal diffraction study of **6** that determined the 3*R* configuration. Thus, C-3 of **7** was in agreement with the 3*S* configuration.

Analyses of the 1D and 2D NMR data revealed that chartarlactam H (**8**) possessed the same parent nucleus as that of **6**. Additional NMR resonances were assigned to a 1″-substituted hydroxyethyl unit on the basis of the COSY correlation between two groups of methylene protons at δ_H 3.40 (2H, t, J = 7.0 Hz, H_2-1″) and 3.56 (2H, t, J = 7.0 Hz, H_2-2″), which were correlated to the carbons at δ_C 44.7 and 59.8, respectively, in the HMQC spectrum. The HMBC interactions of H_2-1″ with the carbonyl carbon of the γ-lactam led to the linkage of the hydroxyethyl unit to the nitrogen atom. The similar NOE interactions between **6** and **8** indicated the same configurations for these compounds.

The 1D and 2D spectroscopic analyses indicated the gross structure of chartarlactam I (**9**) was the same as that of F1839-A (**19**),[13] whose absolute configuration was confirmed by our X-ray single-crystal diffraction study (Figure 2). The NOE interactions of the protons from ring B to D of **9** were compatible with those of F1839-A,[13] indicating the same configurations of the asymmetric centers. The coupling constant of $J_{H-2/H-3}$ (12.0 Hz) was assigned to the axial coupling for the *trans*-orientation of the vicinal protons, while the NOE interactions from H-3 (δ_H 2.70) to H-5 (δ_H 1.57), H_3-13, and H-1a as well as from H-2 (δ_H 3.40) to H_3-14 and H_3-15 clarified H-3α and H-2β (Figure 3). Thus, the structure of **9** was determined as the C-3 epimer of **19**.

The structure of chartarlactam J (**10**) was determined as a stereoisomer of **9** on the basis of the 1D and 2D NMR data analyses. The coupling constants $J_{H-2/H-1a}$ (6.4 Hz) and $J_{H-2/H-1a}$ (6.8 Hz) were indicative of an equatorial orientation of H-2 (δ_H 3.59, dt, J = 6.4, 6.8 Hz), while the NOE interactions of OH-2 (δ_H 4.46, brs) with H_3-15 (δ_H 1.16, s) and H-3 (δ_H 3.18, d, J = 6.8 Hz), from H-3 to H_3-14, and from OH-3 (δ_H 4.27, brs) to H-2, H-5, and H_3-13 assigned H-2α and H-3β.

The structure of chartarlactam K (**11**) was determined as a 3-acetylated analogue of F1839-A[13] on the basis of the close similarity of NMR data, except for the presence of the acetyl resonances at δ_H 1.99 (3H, s), δ_C 21.4 and 170.6, while the acetoxy group was substituted at C-3, as evident from a correlation of H-3 (δ_H 4.69, brs) to the carbonyl carbon.

Chartarlactam L (**12**) had a molecular formula of $C_{46}H_{58}N_2O_8$ as determined by the HRESIMS and NMR data, indicating 19 degrees of unsaturation. The ^{1}H and ^{13}C NMR data showing 23 ^{13}C NMR resonances and 29 ^{1}H NMR resonances (Table 3) indicated **12** to be a symmetrical structure bearing two identical substructures. Analyses of the 1D and 2D NMR data revealed the partial structure to be the

Table 3. ^{1}H and ^{13}C NMR Data and Key HMBC and NOE Correlations for Chartarlactam L (12)[a]

position	δ_H (J in Hz)	δ_C, type	HMBC (H→C)	NOE
1/1″	1.00, m	24.3, CH_2	3/3″, 5/5″, 15/15″	
	1.78, m			
2/2″	1.45, m	25.2, CH_2	3/3″, 10/10″	
	1.80, m			
3/3″	3.17, brs	73.8, CH	1/1″, 4/4″, 13/13″, 14/14″	H_3-14, H-1b
4/4″		37.8, C		
5/5″	2.14, brd (10.0)	40.9, CH	4/4″, 10/10″, 15/15″, 6/6″	H_3-12, H_3-13
6/6″	1.50, m	20.8, CH_2	5/5″, 7/7″	
	1.65, m			
7/7″	1.50, m	31.8, CH_2	5/5″, 8/8″	
	1.72, m			
8/8″	1.91, m	36.5, CH	6/6″, 9/9″, 10/10″, 11/11″	
9/9″		101.6, C		
10/10″		42.3, C		
11/11″	2.90, d (17.2)	31.8, CH_2	8/8″, 9/9″, 10/10″, 1′/1‴, 2′/2‴, 6′/6‴	H-8, H_3-12, H_3-15
	3.20, d (17.2)			
12/12″	0.73, d (6.5)	15.9, CH_3	7/7″, 8/8″, 9/9″	H-11a, NH
13/13″	0.97, s	29.1, CH_3	3/3″, 4/4″, 5/5″, 14/14″	H-5
14/14″	0.81, s	22.7, CH_3	3/3″, 4/4″, 5/5″, 13/13″	H-3, H_3-15
15/15″	1.00, s	16.2, CH_3	1/1″, 5/5″, 9/9″, 10/10″	H_3-14, H-11b
1′/1‴		117.6, C		
2′/2‴		155.9, C		
3′/3‴	6.77, s	103.2, CH	1′/1‴, 2′/2‴, 5′/5‴, 7′/7‴	
4′/4‴		131.6, C		
5′/5‴		107.7, C		
6′/6‴		153.5, C		
7′/7‴		165.2, C		
8′/8‴		114.6, C		
OH-2′/2‴	10.46, br			
NH	10.03, s		4′/4‴, 5′/5‴, 7′/7‴, 8′/8‴	H_3-12

[a]Measured in DMSO-d_6, ^{1}H at 500 MHz, ^{13}C at 125 MHz.

dx.doi.org/10.1021/np400824u | J. Nat. Prod. XXXX, XXX, XXX−XXX

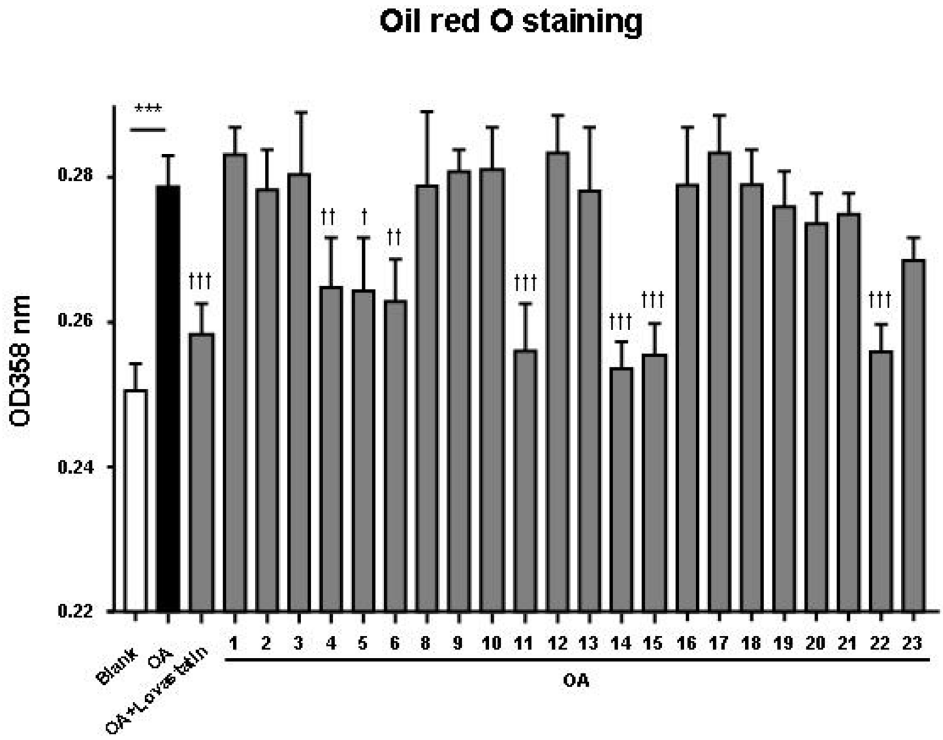


Figure 4. Effects of compounds on oleic acid-elicited intracellular lipid accumulation. Positive control: lovastatin; blank: DMEM; OA: oleic acid. Neutral lipids were determined by spectrophotometry at 358 nm after Oil Red O staining. Bars depict the means ± SEM in triplicate. ***$p < 0.001$, OA vs blank; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, test group vs OA group.

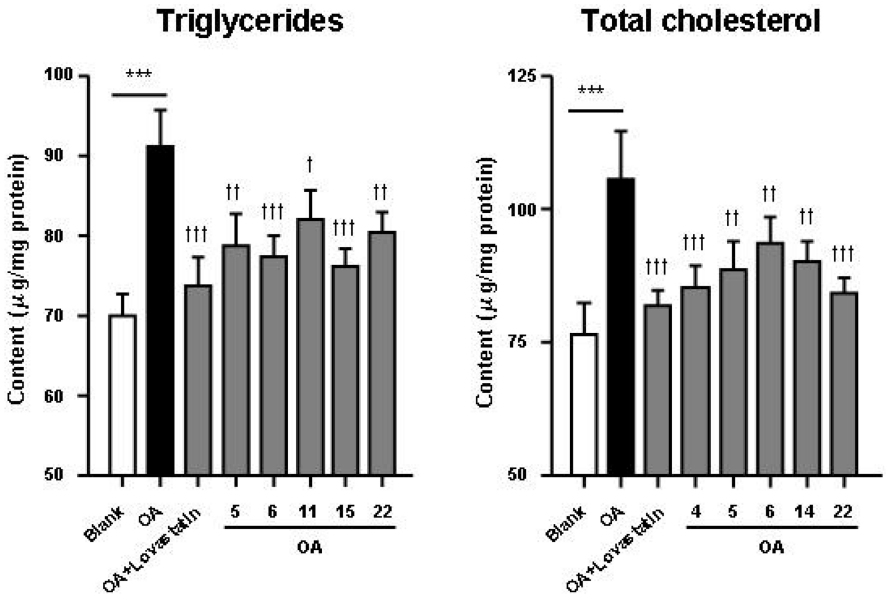


Figure 5. Inhibitory effects of compounds toward (A) triglycerides and (B) total cholesterol. Positive control: lovastatin; blank: DMEM; OA: oleic acid. Intracellular levels of triglycerides and total cholesterol were measured by kits according to the manufacturer's instructions. Bars depict the means ± SEM in triplicate. ***$p < 0.001$: OA vs blank: $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$: compound vs OA.

same as that of **1**, except that one carbonyl carbon and a quaternary carbon at δ_C (114.7) replaced the two carbonyl carbons at ring E of **1**. The observation of an HMBC correlation between H-3′/H-3‴ (δ_H 6.77, s) and the carbonyl carbon at δ_C 165.2 revealed the carbonyl group was located at C-7′/C-7‴. Thus, the two symmetrical units were assumed to be connected through a double bond across C-8′ and C-8‴, which was further supported by the HMBC correlation from NH (δ_H 10.03) to δ_C 114.6 (C-8′, C-8‴) and the molecular unsaturation. The weak NOE interaction between NH and H_3-12/H_3-12″ (δ_H 0.73, d, J = 6.5 Hz) was in agreement with an 8′*E* geometry.

Analyses of 2D NMR data disclosed the gross structure of chartarlactam M (**13**) to be the same as stachybotrylactam, while the NOE interactions and the coupling constants of ring A were in agreement with H-3β. However, the NOE interactions of ring B from H_3-12 (δ_H 1.07, d) to H_3-15 and H_2-11 (Figure 1) indicated CH_3-12 to be β-oriented. Thus, the

dx.doi.org/10.1021/np400824u | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

structure of **13** is a C-8 epimer of stachybotrylactam. It is noted that the 8β-CH_3 induced upfield shifts of C-6 and C-7 to δ_C 16.4 and 29.4 ppm compared to those of **1** (20.0 and 31.0 ppm for C-6 and C-7) by a *gauche* effect. These findings can help to discriminate between the 8β-CH_3 and 8α-CH_3 analogues.

The structure of chartarlactam N (**14**) was determined as the C-8 epimer of stachybotramide (**18**)[12] on the basis of closely similar NMR data and the same molecular formula, except for the NOE interaction between H_3-12 (δ_H 1.08, d, J = 7.7 Hz) and H_3-15 (δ_H 0.95, s) in association with the upfield shifted C-6 (δ_C 16.5) and C-7 (δ_C 29.4).

On the basis of the 1D and 2D NMR spectroscopic analyses, the structure of chartarlactam O (**15**) was assigned as the C-8 epimer of **6**, while the structure of chartarlactam P (**16**) was determined as a C-8 epimer of **19**.

Eight known analogues were identified as stachybotrylactam (**17**),[12] stachybotramide (**18**),[12] F1839-A (**19**),[13] stachybotrylactam acetate (**20**),[12] 2α-acetoxystachybotrylactam acetate (**21**),[12] *N*-(2-benzenepropanoic acid) stachybotrylactam (**22**),[19] F1839-E (**23**),[13] and F1839-D (**24**),[14] based on the comparison of their ^{1}H and ^{13}C NMR and MS spectroscopic data, as well as specific rotations with those reported in the literature.

Apart from the minor compound **7**, the isolated compounds were screened for the lipid-lowering activity in HepG2 hepatocarcinoma cells, a cell model widely used to investigate lipid metabolic regulation.[20,21] Before the bioassay, the compounds were tested for toxic activity toward HepG2 cells, showing no toxicity up to 100 μM. The bioassay results revealed that eight compounds (**4**, **5**, **6**, **11**, **12**, **14**, **15**, and **22**) in a dose of 10 μM exhibited potent lipid-lowering effects in HepG2 cells as assessed by Oil Red O staining (Figure 4).[22] At a dose of 10 μM in the same cell model, five compounds (**5**, **6**, **11**, **15**, and **22**) showed significant inhibition of intracellular triglyceride (TG) levels (Figure 5A), whereas six compounds (**4**, **5**, **6**, **14**, **22**, and **12**) dramatically reduced total cholesterol (TC) (Figure 5B). Primary analyses of the structure−activity relationships revealed the 8α-CH_3 analogues with alkyl substitution at nitrogen such as **2**, **8**, **18**, and **21** showed weak activity with the exception of **22** linked to a benzenepropanoic acid. The carbonyl carbon of the γ-lactam at C-8′ (**6**, **15**) enhanced the inhibitory activity in comparison with **13** and **17**, which contain a carbonyl carbon at C-7′. Compound **4**, with a glucosyl group at C-2′, showed inhibition only toward TC, whereas **11** exhibited selective inhibition against TG. The 2,3-diol analogues were inactive, with the exception of **11**, bearing an acetoxy group at C-3 and showing selective inhibition. Further investigation of the antihyperlipidemic mechanism is in progress.

The present work enriches the numbers of phenylspirodrimane-type analogues and represents the second isolation of this class of compounds from a sponge-derived fungus.[23] It is noted that the analogues with the 8β-CH_3 orientation were found for the first time from nature, whereas the dimer (**12**) represents a new skeleton and likely forms from dimerization of **1**. So far, phenylspirodrimane-type analogues have been isolated only in the genus *Stachybotrys*, suggesting that this structural type could be used as a chemotaxonomic marker for the genus *Stachybotrys*. The potent antihyperlipidemic activities of the phenylspirodrimanes have encouraged us to further investigate their mechanisms.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using an Autopol III automatic polarimeter (Rudolph Research Co.). UV spectra were recorded by a 3300-ELSD UV detector (Alltech Co.). IR spectra were measured on a Thermo Nicolet Nexus 470 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance-500FT NMR spectrometer using TMS as an internal standard. HRESIMS spectra were obtained on a Bruker APEX IV 70e FT-MS spectrometer and on a Thermo DFS spectrometer using a matrix of 3-nitrobenzyl alcohol. Column chromatography was carried out with silica gel (200−300 mesh), and HF_{254} silica gel for TLC was obtained from Qingdao Marine Chemistry Co. Ltd. Sephadex LH-20 (18−110 μm) was obtained from Pharmacia. HPLC was performed with an Alltech 426 pump employing a UV detector, and the Chromasil C_{18} column (semi-preparative, 10 μm) was purchased from Pharmacia. HepG2 cells (ATCC CRL-10741) were obtained from the American Type Culture Collection. DMEM medium, fetal bovine serum, and penicillin/streptomycin were supplied by Gibco, while oleic acid (OA) and lovastatin were obtained from Sigma-Aldrich.

Fungal Strain and Identification. The strain WGC-25C-6 was isolated from the sponge *Niphates recondita*, which was collected from the inner coral reef at a depth of 10 m near Weizhou Island in Beibuwan Bay, Guangxi Province of China, in August 2010. The strain was purified and identified by comparing the morphological character and 18S rDNA sequence with those of standard records. Specifically, the morphological examination was performed by scrutinizing the fungal culture, the mechanism of spore production, and the characteristics of the spores. For inducing sporulation, the fungal strain was separately inoculated onto potato dextrose agar. All experiments and observations were repeated at least twice, leading to the identification of the strain as *Stachybotrys chartarum*. The DNA sequence data from the fungus were deposited at GenBank with accession number GI 514435227, while the strain WGC-25C-6 was deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, China.

Fermentation. The fermentation was carried out in 30 Fernbach flasks (500 mL), each containing 80 g of rice. Distilled H_2O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented material was extracted with EtOAc (3 × 500 mL). The EtOAc extract was evaporated to dryness under vacuum to afford a crude residue (30 g), which was then subjected to silica gel (200−300 mesh) vacuum liquid chromatography, eluting with CH_2Cl_2/MeOH (a gradient from 1:0 to 0:1), to obtain 10 fractions (FA−FJ). Each fraction was examined by ^{1}H NMR spectra, and the signals derived from phenylspirodrimanes were recognized in fractions FH−FJ. FJ (2.4 g) was further fractionated on an ODS column with a gradient of MeOH/H_2O (from 40% to 100%) to obtain four portions (FJ1−FJ4). FJ1 (13 mg) was separated on semipreparative RP-C_{18} HPLC with MeOH−H_2O (35:65) as a mobile phase to yield **2** (t_R 29.8 min, 3.8 mg) and **4** (t_R 31.0 min, 5.1 mg), while **3** (t_R 30.0 min, 3.2 mg) and **6** (t_R 30.8 min, 4.2 mg) were separated from FJ2 (12 mg) by the same separation protocol as for FJ1. Compounds **16** (t_R 26.0 min, 2.4 mg) and **8** (t_R 27.5 min, 2.9 mg) were purified from FJ3 (5 mg) and FJ4 (4 mg), respectively, by semipreparative RP-C_{18} HPLC eluting with MeOH/H_2O (35:65). FI (1.6 g) was separated by ODS gel column chromatography eluting with H_2O/MeOH (from 50:50 to 0:100) to collect five portions (FI1−FI5). Following the same protocol as for FJ1, using semi-preparative RP-C_{18} HPLC with a gradient of MeOH/H_2O (from 30% to100% within 60 min), compounds **7** (t_R 29.0 min, 1.0 mg) and **9** (t_R 25.5 min, 3.8 mg) were separated from FI2 (6.0 mg), **10** (t_R 27.5 min, 16.2 mg) and **13** (t_R 27.9 min, 6.8 mg) were separated from FI3 (28.0 mg), **19** (t_R 28.0 min, 277.0 mg) and **22** (t_R 28.4 min, 4.6 mg) were separated from FI4 (290.0 mg), while **24** (t_R 29.2 min, 5.8 mg) and **20** (t_R 29.8 min, 7.6 mg) were separated from FI5 (16.0 mg). Following

dx.doi.org/10.1021/np400824u | J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 4. ^{1}H and ^{13}C NMR Data for Chartarlactams M–P (13–16)[a]

	13		14		15		16	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	25.0, CH_2	1.81, m	25.0, CH_2	1.83, m	25.2, CH_2	1.81, m	34.3, CH_2	1.55, m
		1.33, m		1.26, m		1.25, m		1.04, m
2	25.4, CH_2	1.70, m	25.4, CH_2	1.71, m	25.5, CH_2	1.36, m	64.9, CH	3.80, m
		1.72, m		1.72, m		1.72, m		
3	74.1, CH	3.16, brs	74.1, CH	3.17, brs	74.0, CH	3.17, brd (2.3)	78.3, CH	3.14, d (11.8)
4	37.8, C		37.8, C		37.9, C		38.6, C	
5	40.7, CH	2.16, brd (11.4)	40.5, CH	2.16, brd (12.5)	40.6, CH	2.18, brd (12.7)	40.0, CH	2.07, brd (11.8)
6	16.4, CH_2	1.50, m	16.5, CH_2	1.40, m	16.5, CH_2	1.48, m	16.4, CH_2	1.48, m
		1.51, m		1.50, m		1.50, m		1.50, m
7	29.4, CH_2	1.40, m	29.4, CH_2	1.41, m	29.0, CH_2	1.38, m	29.5, CH_2	1.42, m
		1.45, m		1.99, m		2.05, m		1.97, m
8	38.3, CH	1.89, m	38.3, CH	1.87, m	38.1, CH	1.82, m	38.3, CH	1.90, m
9	99.9, C		100.0, C		100.3, C		99.6, C	
10	42.5, C		42.4, C		42.2, C		43.4, C	
11	31.7, CH_2	3.03, d (16.6)	31.7, CH_2	3.08, d (16.6)	30.7, CH_2	2.95, d (16.3)	31.9, CH_2	3.10, d (16.8)
		2.91, d (16.6)		2.91, d (16.6)		2.81, d (16.3)		2.93, d (16.8)
12	17.3, CH_3	1.07, d (7.3)	17.2, CH_3	1.08, d (7.7)	17.2, CH_3	1.07, d (7.5)	17.3, CH_3	1.07, d (7.6)
13	29.2, CH_3	0.88, s	29.2, CH_3	0.89, s	29.1, CH_3	0.90, s	29.4, CH_3	0.93, s
14	22.6, CH_3	0.79, s	22.6, CH_3	0.80, s	22.8, CH_3	0.80, s	22.2, CH_3	0.81, s
15	16.8, CH_3	0.95, s	16.9, CH_3	0.95, s	17.1, CH_3	0.95, s	17.8, CH_3	0.97, s
1′	116.7, C		116.5, C		112.5, C		116.6, C	
2′	154.5, C		154.5, C		157.5, C		154.5,	
3′	101.2, CH	6.56, s	101.3, CH	6.58, s	102.0, CH	6.33, s	101.3, CH	6.58, s
4′	134.7, C		134.6, C		147.3, C		134.8, C	
5′	115.3, C		113.3, C		107.3, C		115.4, C	
6′	154.9, C		154.5, C		157.0, C		154.8, C	
7′	170.6, C		168.0, C		45.1, CH_2	4.15, s	170.6, C	
8′	42.4, CH_2	4.16 d (16.7)	48.2, CH_2	4.32, d (17.0)	169.4, C		42.4, CH_2	4.15, s
		4.14, d (16.7)		4.30, d (17.0)				
1″			45.1, CH_2	3.52, m				
2″			59.8, CH_2	3.60, m				

[a]Measured in DMSO-d_6, ^{1}H at 500 MHz, ^{13}C at 125 MHz.

the same protocol as for FI, FH (1.35 g) was eluted with H_2O/MeOH (from 4:6 to 0:1) on an ODS gel column to yield four portions (FH1–FH4). Each portion was further separated by RP-C_{18} HPLC with a gradient of MeOH/H_2O (from 25% to 80% within 60 min). Compounds **1** (t_R 31.9 min, 3.2 mg), **14** (t_R 30.4 min, 3.6 mg), and **15** (t_R 30.0 min, 1.3 mg) were separated from FH1 (14.0 mg), **5** (t_R 31.3 min, 18.5 mg) was separated from FH2 (32.0 mg), while **11** (t_R 30.0 min, 2.9 mg) and **18** (t_R 29.4 min, 18.1 mg) were separated from FH3 (36.0 mg). Fraction FF (1.1 g) was fractionated on an ODS gel column eluting with H_2O/MeOH (3:7) to obtain four portions (FF1–FF4). Upon application of semipreparative RP-C_{18} HPLC with a gradient of MeOH/H_2O (from 60% to 80% within 60 min), compounds **21** (t_R 27.4 min, 3.8 mg) and **17** (t_R 27.8 min, 4.2 mg) were separated from FF2 (12.0 mg), whereas **12** (t_R 33.5 min, 7.7 mg) and **23** (t_R 34 min, 10.0 mg) were purified from FF3 (10.3 mg) and FF4 (21 mg), respectively.

*Chartarlactam A (**1**):* pale yellow crystals; $[\alpha]_D^{25}$ −42 (*c* 0.9, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.47), 348 (3.81) nm; IR (KBr) ν_{max} 3435, 3300, 2973, 2871, 1751, 1714, 1454, 1320, 1051, 958 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 422.1939 $[M + Na]^+$ (calcd for $C_{23}H_{29}NNaO_5$, 422.1943).

*Chartarlactam B (**2**):* white powder; $[\alpha]_D^{25}$ −22 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.71), 263 (3.79), 302 (1.90) nm; IR (KBr) ν_{max} 3219, 2936, 2874, 1711, 1665, 1467, 1349, 1221, 1079 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 486.2853 $[M + H]^+$ (calcd for $C_{28}H_{40}NO_6$, 486.2855).

*Chartarlactam C (**3**):* pale yellow powder; $[\alpha]_D^{25}$ −5 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.72), 263 (3.30), 302 (2.92) nm; IR (KBr) ν_{max}3325, 2918, 2850, 1738, 1676, 1466, 1365, 1216, 1088 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 402.2261 $[M + H]^+$, 424.2078 $[M + Na]^+$ (calcd for $C_{23}H_{32}NO_5$, 402.2280; for $C_{23}H_{31}NNaO_5$, 424.2099).

*Chartarlactam D (**4**):* white powder; $[\alpha]_D^{25}$ +70 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.72), 263 (3.35), 302 (2.95) nm; IR (KBr) ν_{max} 3367, 2919, 2874, 1674, 1461, 1361, 1320, 1077 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 548.2862 $[M + H]^+$, 570.2674 $[M + Na]^+$ (calcd for $C_{29}H_{42}NO_9$, 548.2859; for $C_{29}H_{41}NNaO_9$, 570.2679).

*Chartarlactam E (**5**):* white powder; $[\alpha]_D^{25}$ −13 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.84), 262 (3.47), 301 (2.96) nm; IR (KBr) ν_{max} 3230, 2948, 2870, 1706, 1463, 1367, 1229, 1195 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 382.2042 $[M - H]^-$ (calcd for $C_{23}H_{28}NO_4$, 382.2018).

*Chartarlactam F (**6**):* white crystals; $[\alpha]_D^{25}$ −29 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.57), 254 (3.34), 298 (2.77) nm; IR (KBr) ν_{max} 3310, 2935, 2872, 1678, 1464, 1363, 1091 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 408.2132 $[M + Na]^+$ (calcd for $C_{23}H_{31}NNaO_4$, 408.2151).

*Chartarlactam G (**7**):* white powder; $[\alpha]_D^{25}$ −26 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.58), 263 (3.49), 301 (2.93) nm; IR (KBr) ν_{max} 3458, 2923, 2873, 1673, 1467, 1358, 1091 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 408.2132 $[M + Na]^+$ (calcd for $C_{23}H_{31}NNaO_4$, 408.2151).

*Chartarlactam H (**8**):* white powder; $[\alpha]_D^{25}$ −15 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.19), 263 (3.86), 301 (3.12) nm; IR (KBr) ν_{max} 3342, 2938, 2858, 1672, 1464, 1351, 1040 cm^{-1}; ^{1}H and

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^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 452.2395 [M + Na]$^+$ (calcd for $C_{25}H_{35}NNaO_5$, 452.2413).

*Chartarlactam I (**9**):* white powder; $[\alpha]_D^{25}$ −14 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.90), 262 (3.94), 301 (3.10) nm; IR (KBr) ν_{max} 3285, 2925, 2869, 1738, 1627, 1461, 1367, 1229 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 424.2080 [M + Na]$^+$ (calcd for $C_{23}H_{31}NNaO_5$, 424.2099).

*Chartarlactam J (**10**):* white powder; $[\alpha]_D^{25}$ −8 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.87), 261 (3.80), 301 (2.73) nm; IR (KBr) ν_{max} 3321, 2934, 2872, 1680, 1624, 1464, 1350, 1086 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 424.2076 [M + Na]$^+$ (calcd for $C_{23}H_{31}NNaO_5$, 424.2099).

*Chartarlactam K (**11**):* white powder; $[\alpha]_D^{25}$ −32 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.54), 263 (4.31), 301 (3.12) nm; IR (KBr) ν_{max} 3254, 2938, 2876, 1708, 1623, 1466, 1353, 1089 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 444.2390 [M + H]$^+$ (calcd for $C_{25}H_{34}NO_6$, 444.2386), *m/z* 466.2208 [M + Na]$^+$ (calcd for $C_{25}H_{33}NNaO_6$, 466.2206).

*Chartarlactam L (**12**):* bright yellow powder; $[\alpha]_D^{25}$ −5 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 255 (4.43), 295 (4.16), 420 (2.65), 448 (2.97) nm; IR (KBr) ν_{max} 3254, 2938, 2876, 1708, 1623, 1466, 1353, 1089 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; HRESIMS *m/z* 767.4267 [M + H]$^+$ (calcd for $C_{46}H_{59}NNaO_8$, 767.4271).

*Chartarlactam M (**13**):* white powder; $[\alpha]_D^{25}$ −15 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.57), 256 (3.86), 299 (3.28) nm; IR (KBr) ν_{max} 3336, 2930, 2872, 1714, 1465, 1365, 1217, 1090 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 384.2176 [M − H]$^-$ (calcd for $C_{23}H_{30}NO_4$, 384.2175).

*Chartarlactam N (**14**):* white powder; $[\alpha]_D^{25}$ −13 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.50), 263 (4.21), 301 (3.94) nm; IR (KBr) ν_{max} 3350, 2928, 2872, 1709,1760, 1466, 1349, 1218, 1086 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 452.2413 [M + Na]$^+$ (calcd for $C_{25}H_{35}NNaO_5$, 452.2413).

*Chartarlactam O (**15**):* white powder; $[\alpha]_D^{25}$ −11 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.55), 254 (3.88), 298 (3.20) nm; IR (KBr) ν_{max} 3236, 2937, 2871, 1739, 1464, 1364, 1216, 1003 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 384.2176 [M − H]$^-$ (calcd for $C_{23}H_{30}NO_4$, 384.2175), *m/z* 408.2144 [M + Na]$^+$ (calcd for $C_{23}H_{31}NNaO_4$, 408.2145).

*Chartarlactam P (**16**):* white powder; $[\alpha]_D^{25}$ −18 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.61), 261 (3.85), 301 (2.96) nm; IR (KBr) ν_{max} 3392, 2924, 2857, 1738, 1677, 1460, 1375, 1203 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 424.2095 [M + Na]$^+$ (calcd for $C_{23}H_{31}NNaO_5$, 424.2099).

*Compound **17**:* $[\alpha]_D^{25}$ −19.7 (*c* 0.05, MeOH) ([α]$_D^{25}$ −18.9 (*c* 0.33, MeOH) for stachybotrylactam[17]).

X-ray Crystallographic Analysis. Yellow crystals of **1** were obtained from MeOH−H_2O (7:3) using the vapor diffusion method. Crystal data were obtained on a Bruker D8 Advance single-crystal X-ray diffractometer with the wavelength for Cu Kα (λ = 1.5418 Å). The structures were solved by direct methods using SHELXS-97[24] and expanded using Fourier techniques (SHELXS-97). Crystal data of **1**: $C_{23}H_{30}NO_5$ (*M* = 408.48); monoclinic crystal (0.30 × 0.15 × 0.07 mm); space group C_2; unit cell dimensions *a* = 29.5456 (11) Å, *b* = 7.8704 (3) Å, *c* = 8.8858 (3) Å, *V* = 2053.63 (13) Å^3; *Z* = 4; ρ_{calcd} =1.321 mg/m^3; μ = 0.766 mm^{-1}, *F*(000) = 876, a total of 3879 unique reflections [*R*(int) = 0.0269 (inf-0.9 Å)], which were used in all calculations; the final refinement gave R1 = 0.0380 (>2σ(*I*)) and wR2 = 0.0985 (all data); Flack parameter = 0.07(15).

The X-ray crystallographic data of **6** and **19** are presented in the Supporting Information, while the crystallographic data for the structures of **1**, **6**, and **19** have been deposited in the Cambridge Crystallographic Data Centre [deposition numbers: CCDC 965021 (**1**), CCDC 965022 (**6**), and CCDC 965023 (**19**)]. Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

Hydrolysis and TLC Chromatography. Compound **4** (2 mg) was dissolved in MeOH (4 mL) and was treated with 5% H_2SO_4 (4 mL) at 90 °C for 2 h. After adding H_2O (4 mL), the mixture was concentrated to remove MeOH and then kept at 60 °C for 15 min. The H_2O solution was extracted by EtOAc to remove the aglycone, while the H_2O solution was dried under N_2 gas to obtain the sugar. Comparison of the R_f value (0.35) on TLC (*n*-BuOH/HOAc/H_2O (3:1:1) between the hydrolyzed product and the standard sugars (Sigma-Aldrich) confirmed it to be a D-glucose. The specific rotation of the sugar derived from **4** is $[\alpha]_D^{25}$ +86 (*c* 0.05, MeOH) [D-glucose: $[\alpha]_D^{25}$ +97.9 (*c* 0.21, MeOH), L-glucose: $[\alpha]_D^{25}$ −81.8 (*c* 0.11, MeOH)].

Cell-Based Lipid Accumulation Assay. HepG2 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 μg/mL). The cells with 70−80% confluence were incubated in DMEM + oleic acid (100 μM) for 12 h, then were treated with the compounds (each, 10 μM) and the positive control lovastatin in DMEM/100 μM oleic acid with DMEM/100 μM oleic acid as a blank for an additional 6 h. Subsequently, the cells were subjected to Oil Red O staining or TC and TG determination as described previously.[22] Each experiment (n = 8 for Oil Red O staining or n = 3 for TC and TG determination) was repeated in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectroscopic data for the new compounds (**1**−**16**) including ^{1}H, ^{13}C, and 2D NMR spectra, IR and ESIMS/MS data, and X-ray data for **1**, **6**, and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by grants from NSFC (No. 30672607), the National Hi-Tech 863-Projects (2010DFA31610, 2011AA090701, 2013AA092902), COMRA (DY125-15-T-01), and Sino-German Project GZ816.

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