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Phenylspirodrimanes with Anti-HIV Activity from the Sponge-Derived Fungus *Stachybotrys chartarum* MXH-X73

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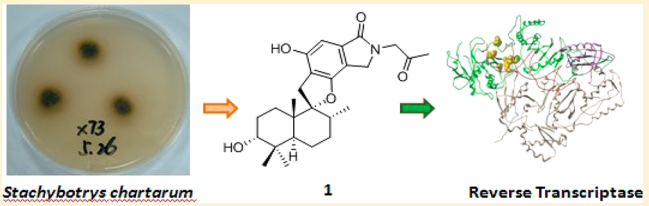
Supporting Information

ABSTRACT: Seven new phenylspirodrimanes, named stachybotrins D–F (**1**, **3**, **4**), stachybocins E and F (**5**, **6**), and stachybosides A and B (**7**, **8**), and four known compounds (**2**, **9**–**11**), were isolated from the sponge-derived fungus *Stachybotrys chartarum* MXH-X73. Their structures were determined by detailed analysis of spectroscopic data. The absolute configurations of **1**–**8** were determined by chemical hydrolysis and modified Mosher's and Marfey's methods. All compounds were tested in an anti-HIV activity assay, and compound **1** showed an inhibitory effect on HIV-1 replication by targeting reverse transcriptase. Further study exhibited that **1** could block NNRTIs-resistant strains (HIV-$1_{RT\text{-}K103N}$, HIV-$1_{RT\text{-}L100I,K103N}$, HIV-$1_{RT\text{-}K103N,V108I}$, HIV-$1_{RT\text{-}K103N,G190A}$, and HIV-$1_{RT\text{-}K103N,P225H}$) as well as wild-type HIV-1 (HIV-1_{wt}) with EC_{50} values of 7.0, 23.8, 13.3, 14.2, 6.2, and 8.4 μM, respectively.



Reverse transcriptase (RT), a critical enzyme in the human immunodeficiency virus (HIV) life cycle, is one of the main targets for the chemotherapeutic treatment of HIV-1 infection. RT inhibitors consist of two functionally distinct classes: nucleoside RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs).[1,2] NNRTIs are important components for highly active antiretroviral therapy (HAART). All five NNRTIs, nevirapine (NVP), delavirdine (DLV), efavirenz (EFV), etravirine (ETR), and rilpivirine (RPV), bind to the hydrophobic pocket in the palm domain adjacent to the thumb domain on the RT p66 subunit, in blocking RT allostery.[3−7] Drug resistance is the most important factor of the failure of HAART. NNRTI-resistant mutations exist widely and spread in patients since NVP, EFV, and DLV have been used in clinical settings over the past decade. Both clinical and in vitro studies have shown that E138K is a resistant mutation to ETR and RPV, which were approved by the FDA in 2008 and 2012, respectively.[8−10] Therefore, there remains a strong demand for the development of new NNRTIs that can effectively inhibit NNRTI-resistant viruses.

As part of our program to search for bioactive secondary metabolites from sponge-associated microorganisms,[11] the fermentation of the fungus *Stachybotrys chartarum* MXH-X73, which was isolated from the sponge *Xestospongia testudinaris* collected from Xisha Island, China, was selected because of the intriguing UV profiles of an extract. Chemical investigation of the extract led to the isolation of seven new phenylspirodrimanes, stachybotrins D–F (**1**, **3**, **4**), stachybocins E and F (**5**, **6**), and stachybosides A and B (**7**, **8**), and four known derivatives (**2**, **9**–**11**).[12−14] In this paper, we describe the isolation, structure elucidation, and anti-HIV activities of these new phenylspirodrimanes. We discovered that stachybotrin D (**1**) is a novel non-nucleoside reverse transcriptase inhibitor of both wild-type HIV-1 and five NNRTI-resistant strains.

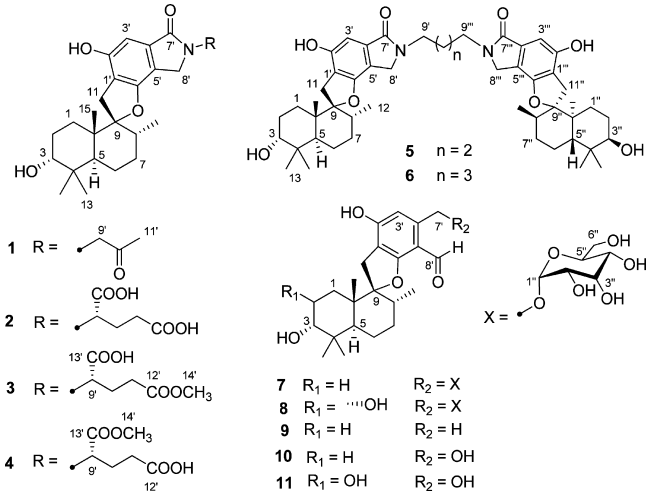


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Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data of 1, 3, and 4 in DMSO-d_6

	1		3		4	
no.	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	24.4, CH_2	1.75, m	24.9, CH_2	1.74, m	24.9, CH_2	1.74, m
		0.90, m		0.93, m		0.92, m
2	25.4, CH_2	1.72, m	25.8, CH_2	1.81, m	25.9, CH_2	1.82, m
		1.35, m		1.38, m		1.38, m
3	74.0, CH	3.16, brs	74.4, CH	3.18, brs	74.4, CH	3.18, brs
4	37.8, C		38.3, C		38.4, C	
5	39.6, CH	2.00, d (11.6)	39.2, CH	2.02, d (11.5)	39.2, CH	2.00, d (11.0)
6	21.0, CH_2	1.43, m	21.4, CH_2	1.45, m	21.5, CH_2	1.45, m
		1.39, m		1.41, m		1.41, m
7	31.4, CH_2	1.51, m	31.7, CH_2	1.52, m	31.7, CH_2	1.52, m
		1.37, m		1.40, m		1.40, m
8	36.9, CH	1.80, m	37.5, CH	1.79, m	37.6, CH	1.79, m
9	98.4, C		99.0, C		99.1, C	
10	42.3, C		42.8, C		42.8, C	
11	32.2, CH_2	3.10, d (16.5)	32.7, CH_2	3.13, d (17.0)	32.8, CH_2	3.13, d (16.5)
		2.75, d (16.5)		2.76, d (17.0)		2.76, d (16.5)
12	16.0, CH_3	0.64, d (6.6)	16.5, CH_3	0.66, d (6.0)	16.5, CH_3	0.65, d (6.6)
13	29.2, CH_3	0.86, s	29.6, CH_3	0.87, s	29.7, CH_3	0.87, s
14	22.9, CH_3	0.78, s	23.4, CH_3	0.79, s	23.4, CH_3	0.79, s
15	16.3, CH_3	0.93, s	16.8, CH_3	0.95, s	16.9, CH_3	0.95, s
1′	117.2, C		117.9, C		118.1, C	
2′	154.3, C		154.7, C		154.9, C	
3′	101.4, CH	6.56, s	101.9, CH	6.57, s	102.0, CH	6.60, s
4′	133.9, C		134.1, C		134.0, C	
5′	112.8, C		113.4, C		113.4, C	
6′	156.4, C		156.9, C		157.0, C	
7′	168.4, C		169.3, C		169.4, C	
8′	48.0, CH_2	4.21, d (16.5)	45.0, CH_2	4.21, d (15.9)	45.2, CH_2	4.22, d (16.5)
		4.22, d (16.5)		4.29, d (15.9)		4.32, d (16.5)
9′	52.4, CH_2	4.42, d (18.7)	54.1, CH	4.73, dd (11.1, 4.4)	54.1, CH	4.87, dd (10.4, 4.4)
		4.33, d (18.7)				
10′	204.6, C		25.2, CH_2	2.15, m	25.5, CH_2	2.13, m
				2.28, m		2.26, m
11′	27.7, CH_3	2.13, s	31.3, CH_2	2.32, m	31.6, CH_2	2.20, m
12′			173.5, C		172.3, C	
13′			173.2, C		171.4, C	
14′			52.2, CH_3	3.50, s	53.3, CH_3	3.66, s

RESULTS AND DISCUSSION

The fermented whole broth (60 L) was obtained by culturing the fungal strain *S. chartarum* MXH-X73 with shaking at 28 °C for 11 days. The organic extracts prepared by solvent extraction were separated by repeated silica gel column chromatography, ODS column chromatography, LH-20 column chromatography, and finally semipreparative ODS HPLC to yield the new compounds **1** (28.0 mg), **3** (6.0 mg), **4** (5.0 mg), **5** (22 mg), **6** (20 mg), **7** (21 mg), and **8** (17 mg).

Compound **1** was obtained as a colorless oil, and its molecular formula was determined as $C_{26}H_{35}NO_5$ according to the HRESIMS peak at m/z 442.2587 $[M + H]^+$, requiring nine degrees of unsaturation. The UV absorptions at 261 and 302 nm indicated the presence of an aromatic system. The IR spectrum showed absorption bands for hydroxy (3420 cm^{-1}), ketone carbonyl (1716 cm^{-1}), and amide carbonyl (1679 cm^{-1}) groups. Comparison of the ^{1}H and ^{13}C NMR data (Table 1) with those of the known compound **2** showed the presence of the same phenylspirodrimane skeleton, except the side chain moiety on the nitrogen was replaced by an acetonyl group in **1**, which was further confirmed by the HMBC correlations (Figure 1) from H_3-11′ (δ_H 2.13) to C-9′ (δ_C 52.4) and C-10′ (δ_C 204.6) and from H_2-9′ (δ_H 4.42, 4.33) to C-7′, C-8′ (δ_C 48.0), and C-10′. The planar structure of **1** was further confirmed by 2D NMR data (Figure 1). The relative configuration of **1** was inferred to be the same as compound **2** and was further established by the NOESY and 1D NOE experiments (Figure 1). The small coupling constant of H-3 (δ_H 3.16, brs) indicated that it was equatorial. Strong NOESY

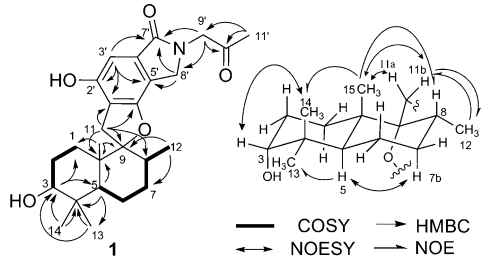

Figure 1. Key COSY, HMBC, and NOESY correlations and NOE effects of **1**.

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correlations of H-3 (δ_H 3.16) and H_3-14 (δ_H 0.78) and NOE correlations of H_3-14 and H_3-15 (δ_H 0.93), H_3-15 and H-8 (δ_H 1.80), and NOESY correlations of H_3-15 and H_2-11 (δ_H 3.10, 2.75) suggested they were on the same face of the molecule. On the other hand, NOE effects for H-5 (δ_H 2.00)/H_3-13 (δ_H 0.86) and NOESY correlations of H-5 (δ_H 2.00) and H-7b (δ_H 1.37) indicated these protons were on the opposite face. The absolute configuration at C-3 of **1** was assigned by the modified Mosher's method (Figure 2).[15−17] The methylated derivative

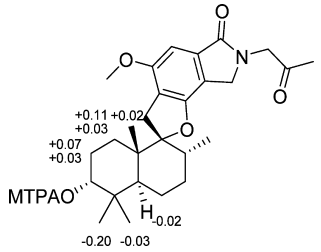

Figure 2. $\Delta\delta$ ($\delta_S - \delta_R$) values (in ppm) for the MTPA esters of **1a**.

compound **1a**[16] was treated with (*R*)- and (*S*)-MTPA chloride, to afford the (*S*)- and (*R*)-MTPA esters, **1aS** and **1aR**, respectively. Calculations for all of the relevant signals in the diastereomeric esters **1aS** and **1aR** confirmed the *R* absolute configuration at C-3 in **1**.[17] Accordingly, the absolute configuration of **1**, named stachybotrin D, was concluded to be 3*R*, 5*S*, 8*R*, 9*R*, and 10*S*, the same as the stachybotrylactam.[18]

The molecular formulas for compounds **3** and **4** were both deduced as $C_{29}H_{39}NO_8$ by HRESIMS. Analysis of the 1D and 2D NMR data (Table 1) for **3** and **4** suggested that they were methylated congeners of **2**. The HMBC correlations (Figure 3)

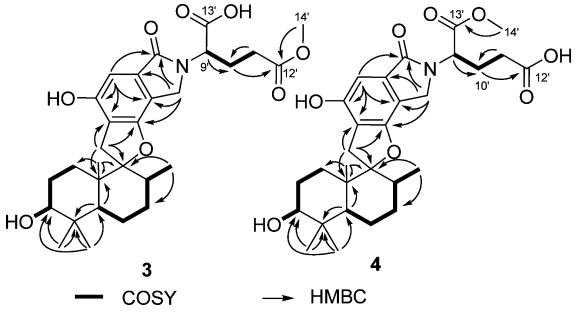

Figure 3. Key COSY and HMBC correlations of **3** and **4**.

from H_3-14′ (δ_H 3.50) to C-12′ (δ_C 173.5) in **3** and that from H_3-14′ (δ_H 3.66) to C-13′ (δ_C 172.3) in **4** indicated the methylation occurred at one carboxylic acid. To further determine the methylated position, compounds **3** and **4** were converted into the (*S*)-phenylglycine methyl ester (PGME) amides **3a** and **4a**, respectively (Figure 4).[19] PGME derivatives have been applied to determine the absolute configuration of carboxylic acids due to the shielding effect of the phenyl group on both sides of the stereogenic center. Comparison of the larger values ($\Delta\delta = \delta_{3a} - \delta_3$) of H-8′ ($\Delta\delta$ = +0.24, +0.12), H-9′ ($\Delta\delta$ = +0.20), H-10′($\Delta\delta$ = −0.10, −0.06), and H-11′ ($\Delta\delta$ = −0.02) obtained from the ^{1}H NMR data for **3a** and **3**, with the smaller ones ($\Delta\delta = \delta_{4a} - \delta_4$) [H-8′ ($\Delta\delta$ = −0.02, +0.01), H-9′ ($\Delta\delta$ = −0.08), H-10′ ($\Delta\delta$ = +0.01, +0.02), and H-11′ ($\Delta\delta$ = +0.02)] for **4a** and **4** indicated that the PGME moiety was attached to the carbonyl at the asymmetric carbon (C-9′) in **3**

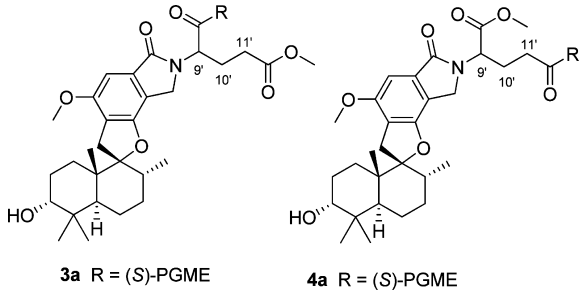

Figure 4. Structures of compounds **3a** and **4a**.

and at C-12′ in **4**. Thus the methyl groups were located at C-12′ in **3** and C-9′ in **4**, respectively. With the failure to make the (*R*)-PGME amides of **3** and **4**, the absolute configurations of C-9′ in **3** and **4** were not determined by this method.

Compound **2** was previously isolated from the culture of a *S. chartarum* strain;[12] however, the absolute configuration was not established. The similar NMR data and the similar specific rotation values ($[\alpha]^{24}_D$ −24.3 for **1**, $[\alpha]^{24}_D$ −32.3 for **2**) for compounds **1** and **2** indicated that their phenylspirodrimane moieties shared the same absolute configuration. To determine the absolute configuration of C-9′, compound **2** was subjected to Jones oxidation, followed by acid hydrolysis.[20] According to Marfey's method, the absolute configuration of C-9′ in **2** was assigned as *S* based on the HPLC analysis of the FDAA derivative, which resulted in the same retention time as the FDAA derivative of standard L-glutamic acid. The absolute configurations in **3** and **4** were also postulated to be the same as **2** based on the similar specific rotation values ($[\alpha]^{24}_D$ −34.0 for **3** and $[\alpha]^{24}_D$ −33.7 for **4**), co-isolation of the metabolites from the same fungus, and biosynthetic considerations. Compounds **3** and **4** were named stachybotrins E and F, respectively. Compounds **3** and **4** could possibly be formed by reaction with MeOH during the isolation. But when compound **2** was stirred with silica in MeOH for 48 h, neither **3** nor **4** was detected by HPLC-UV analysis.

Compounds **5** and **6** were obtained as white powders. Their molecular formulas were determined as $C_{50}H_{68}N_2O_8$ and $C_{51}H_{70}N_2O_8$ by HRESIMS data. However, only 25 carbon signals were observed in the ^{13}C NMR spectrum of **5**, indicating that **5** was a symmetrical dimer. The 1D NMR data of **5** (Table 2) were very similar to those of **1**, except that the moiety at nitrogen in **1** was replaced by two connected methylenes in **5**, which was confirmed by the COSY correlations (H-9′/H-10′) and the HMBC correlations from H_2-9′ (δ_H 3.48) to C-7′ (δ_C 167.8), C-8′ (δ_C 47.1), and C-10′ (δ_C 25.8) and from H_2-8′ (δ_H 4.20, 4.30) to C-4′ (δ_C 134.5), C-5′ (δ_C 112.2), C-6′ (δ_C 156.3), and C-7′ (Figure 5). Considering the above information, compound **5** was suggested to be a dimer connected by C-10′ and C-10‴. The 1D NMR data showed that **6** was also a dimer and had one more methylene (δ_H 1.25, δ_C 24.1) than **5**. In compound **6**, the COSY correlations of H-10′/H-11′ and HMBC correlations (Figure 5) from H_2-9′ (δ_H 3.44) to C-7′ (δ_C 167.8), C-8′ (δ_C 47.1), C-10′ (δ_C 25.3), and C-11′ (δ_C 24.1), from H_2-10′ (δ_H 1.63) to C-9′ (δ_C 42.2) and C-11′, and from H_2-11′ (δ_H 1.25) to C-9′ and C-10′ suggested that C-11′ connected the two monomers. The gross structures of **5** and **6** were further assigned by 2D NMR and combining their molecular formulas. The relative configurations of the sesquiterpene moieties in **5** and **6** were determined to be the same as **1**−**4** by NOESY

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Table 2. ^{1}H (600 MHz) and ^{13}C NMR (125 MHz) Data of 5 and 6 in DMSO-d_6

	5		6	
no.	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1 (1″)	24.3, CH_2	1.73, m	24.3, CH_2	0.90, m
		0.90, m		1.73, m
2 (2″)	25.3, CH_2	1.80, m	25.3, CH_2	1.37, m
		1.37, m		1.79, m
3 (3″)	73.9, CH	3.17, brs	73.9, CH	3.17, brs
3 (3″)-OH		4.08		4.09
4 (4″)	37.7, C		37.7, C	
5 (5″)	39.4, CH	2.01, d (12.1)	39.4, CH	2.01, d (12.1)
6 (6″)	20.9, CH_2	1.44, m	20.9, CH_2	1.40, m
		1.40, m		1.44, m
7 (7″)	31.2, CH_2	1.50, m	31.2, CH_2	1.50, m
		1.38, m		1.39, m
8 (8″)	36.9, CH	1.75, m	36.9, CH	1.76, m
9 (9″)	98.3, C		98.2, C	
10 (10″)	42.0, C		42.0, C	
11 (11″)	32.1, CH_2	3.10, d (21.1)	32.1, CH_2	3.10, d (16.5)
		2.73, d (21.1)		2.74, d (16.5)
12 (12″)	16.2, CH_3	0.64, d (5.5)	16.2, CH_3	0.63, d (6.6)
13 (13″)	29.0, CH_3	0.87, s	29.0, CH_3	0.87, s
14 (14″)	22.8, CH_3	0.78, s	22.8, CH_3	0.78, s
15 (15″)	15.9, CH_3	0.93, s	15.9, CH_3	0.93, s
1′ (1‴)	116.8, C		116.8, C	
2′ (2‴)	154.1, C		154.1, C	
2′ (2‴)-OH		9.69		9.71
3′ (3‴)	101.2, CH	6.54, s	101.2, CH	6.55, s
4′ (4‴)	134.5, C		134.5, C	
5′ (5‴)	112.3, C		112.2, C	
6′ (6‴)	156.3, C		156.3, C	
7′ (7‴)	167.8, C		167.8, C	
8′ (8‴)	47.2, CH_2	4.30, d (16.5)	47.1, CH_2	4.29, d (16.5)
		4.20, d (16.5)		4.19, d (16.5)
9′ (9‴)	42.2, CH_2	3.48, t (6.6)	42.2, CH_2	3.44, t (6.6)
10′ (10‴)	25.8, CH_2	1.58, m	25.3, CH_2	1.63, m
11′			24.1, CH_2	1.25, m

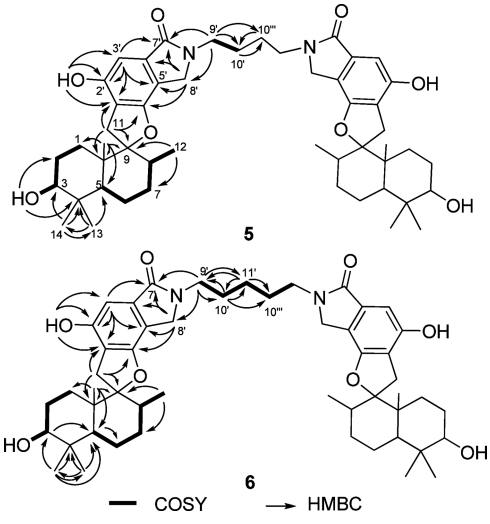

Figure 5. Key COSY and HMBC correlations of compounds **5** and **6**.

experiments, and the absolute configurations were also proposed to be the same as **1**–**4** based on a shared biogenesis. The compounds were named stachybocins E (**5**) and F (**6**).

Phenylspirodrimane dimers are rare. Only five examples have been reported, and all were from the genus *Stachybotrys*.[20−22] The first dimer, stachybocin A, is presumably formed by reaction between lysine and phenylspirodrimane monomer derivatives.[20] Compounds **5** and **6**, absent of carboxylic acid groups, might have gone through decarboxylation in a similar biosynthetic process.

Stachybosides A (**7**) and B (**8**), were obtained as pale yellow oils. The HRESIMS peaks at m/z 573.2661 $[M + Na]^+$ and 589.2617 $[M + Na]^+$ suggested the molecular formulas of $C_{29}H_{42}O_{10}$ and $C_{29}H_{42}O_{11}$ for **7** and **8**, respectively. Comparing the 1D NMR data (Table 3) of **7** with those of compound **10** (Mer-NF5003E) revealed that they shared the same phenylspirodrimane skeleton, except for the replacement of the 7′-

Table 3. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data of 7 and 8 in DMSO-d_6

	7		8	
no.	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	24.4, CH_2	1.77, m	33.4, CH_2	1.11, dd (11.8, 4.1)
		0.91, m		1.59, t (12.0),
2	25.4, CH_2	1.70, m	65.4, CH	3.77, dt (11.8, 3.3)
		1.37, m		
3	73.9, CH	3.15, brs	78.1, CH	3.12, d (3.3)
4	37.7, C		38.6, C	
5	40.0, CH	2.04, d (11.6)	39.6, CH	1.96, dd (12.2, 2.6)
6	21.1, CH_2	1.45, m	20.8, CH_2	1.46, m
		1.41, m		1.39, m
7	31.5, CH_2	1.53, m	31.4, CH_2	1.55, m
		1.38, m		1.36, m
8	36.8, CH	1.80, m	36.6, CH	1.81, m
9	99.5, C		99.2, C	
10	42.4, C		43.5, C	
11	30.9, CH_2	3.03, d (16.5)	31.0, CH_2	3.03, d (16.5)
		2.69, d (16.5)		2.69, d (16.5)
12	16.0, CH_3	0.67, d (6.6)	15.9, CH_3	0.67, d (6.5)
13	29.2, CH_3	0.86, s	29.6, CH_3	0.91, s
14	22.8, CH_3	0.77, s	22.5, CH_3	0.80, s
15	16.3, CH_3	0.93, s	17.2, CH_3	0.96, s
1′	111.7, C		111.6, C	
2′	159.6, C		159.7, C	
2′-OH		10.61		10.64
3′	107.1, CH	6.72, s	107.1, CH	6.73, s
4′	142.3, C		142.3, C	
5′	108.4, C		108.5, C	
6′	168.2, C		168.1, C	
7′	67.1, CH_2	4.89, d (15.9),	67.1, CH_2	4.90, d (15.9),
		4.67, d (15.9)		4.68, d (15.9)
8′	187.2, CH	10.13, s	187.2, CH	10.11, s
1″	98.9, CH	4.78, d (3.3)	98.9, CH	4.78, d (3.6)
2″	72.5, CH	3.28, dd (9.9, 3.3)	72.5, CH	3.28, dd (9.9, 3.6)
3″	73.8, CH	3.56, dd (9.9, 8.8)	73.8, CH	3.56, dd (9.9, 8.8)
4″	70.8, CH	3.11, dd (9.3, 8.8)	70.8, CH	3.11, dd (9.9, 8.8)
5″	73.6, CH	3.37, m	73.6, CH	3.37, dt (9.9, 5.5)
6″	61.4, CH_2	3.43, dd (12.1, 6.1)	61.5, CH_2	3.43, dd (11.0, 5.5)
		3.58, m		3.58, m

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hydroxymethyl in **10** by a glycosylated hydroxymethyl in **7**. The sugar unit was determined as α-glucopyranose in **7** by comparison of the NMR data with reported values, especially the anomeric methine at δ_H 4.78 (d, J = 3.3 Hz)/δ_C 98.9.[23] Further HPLC comparison of the acid hydrolysis product of compound **7** with standard D and L samples using a chiral-phase column led to the determination of α-D-glucopyranose in **7**. The molecular formula of **8** indicated one more oxygen atom than **7**. The oxygen was located at C-2 on the basis of chemical shifts of C-2 (δ_C 65.4) and H-2 (δ_H 3.77), together with the COSY correlations of H-1/H-2/H-3 and the HMBC correlations from H_2-1 (δ_H 1.59, 1.11) to C-2 and C-10 (δ_C 43.5) (Figure 6). The relationship of H-2 and H-3 of **8** was

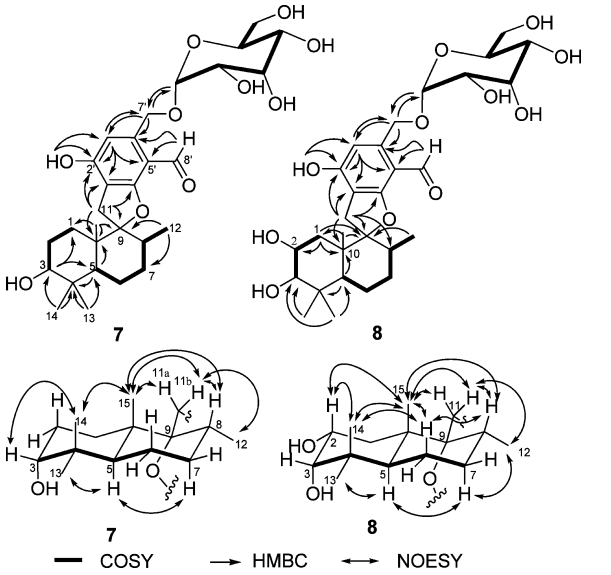

Figure 6. Key COSY, HMBC, and NOESY correlations of compounds **7** and **8**.

established as *cis* by the small coupling constants ($^3J_{H\text{-}2,H\text{-}3}$ = 3.3 Hz). The relative configurations for the phenylspirodrimane moieties of **7** and **8** were further established by NOESY correlations (Figure 6). The absolute configuration of **7** was postulated in analogy with **1**, while the six asymmetric centers of **8** were 2*R*, 3*S*, 5*S*, 8*R*, 9*R*, and10*S*. This is the first report of phenylspirodrimane glucosides.

Compounds **1**−**11** were tested at a final concentration of 10 μM for their antiviral activities against wild-type HIV-1 replication by using a pseudotyping system. Only compound **1** (Table S1, Supporting Information) exhibited an inhibitory effect on HIV-1 replication with an EC_{50} value of 8.4 μM. It should be noted that no cytotoxicity was detected for all tested compounds at 10 μM. The mechanism of the inhibitory effects on HIV replication of **1** was identified using time of addition (TOA) assays.[24] Compound **1** showed a time of 50% failure (FT_{50}) at 12.66 h, which was between 11.59 h for the NRTI AZT (zidovudine) and 17.78 h for the integrase inhibitor MK0518 and the same as that of the NNRTI EFV (efavirenz, 13.06 h) (Figure 7A). The TOA results suggested that compound **1** inhibits the HIV reverse transcription process, and this led us to test the effect of compound **1** on reverse transcriptase activity. Compound **1** inhibited RT RNA-dependent DNA polymerase activity in a dose-dependent manner with an EC_{50} of 50 μM (Figure 7B). As an NNRTI, an important characteristic is the antiviral activity against NNRTI-resistant HIV-1 strains. Therefore, five NNRTI-resistant HIV-1 pseudoviral strains were used. Compound **1** exhibited activity against all five resistant strains similar to wild-type HIV-1 with EC_{50} values ranging from 0.7- to 2.8-fold the value against the wild-type strain. In contrast, the mutant strains were much less susceptible to NVP as compared to the wild type (Table 4).

The phenylspirodrimanes, mainly isolated from *Stachybotrys* spp., are meroterpenoids containing an unusual phenylspirodrimane skeleton of three main classes: tetracyclic aromatic sesquiterpenoids with an alcohol or aldehyde side chain[14,25] such as K-76,[26] pentacyclic aromatic sesquiterpenoids including the stachybotrylactams,[12,13] stachybotrylactones,[26,27] and dispirostachybotrylactams,[20] and the stachyflins with a pentacyclic moiety including a *cis*-fused decalin.[21] They are proposed to be biosynthesized by a hybrid polyketide and isoprenoid pathway.[28] These compounds display diverse bioactivities, with targets including the complement system,[14] inositol monophosphatase,[27] tyrosine kinase,[12] pancreatic cholesterol esterase,[13] and HIV-1 protease and endothelin,[20,22] which made them attractive to pharmacologists.[12,22]

In summary, 11 phenylspirodrimanes, including seven new ones, were isolated from the sponge-derived fungus *S. chartarum* MXH-X73. Compound **1**, discovered to be an HIV-1 RT inhibitor, displayed inhibitory effects on HIV-1 replication against wild-type and five NNRTI-resistant HIV-1 strains. It is structurally distinct from the known non-nucleoside reverse transcriptase inhibitors, and this discovery provides a new class of chemotype for the search for novel NNRTIs.

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer in KBr discs. NMR spectra were recorded on JEOL JNM-ECP 600 and Agilent 500 MHz DD2 spectrometers using TMS as internal standard, with chemical shifts recorded as δ values. ESIMS spectra were recorded on a Q-TOF Ultima Global GAA076 LC mass spectrometer. HRESIMS spectra were measured on a Micromass EI-4000 (Autospec-Ultima-TOF). Semipreparative HPLC was performed using an ODS column [HPLC: YMC-Pack ODS-A (5 μm, 10 × 250 mm, 3 mL/min)]. Column chromatography (CC) was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Inc.) and Sephadex LH-20 (Amersham Biosciences), respectively.

Fungal Material. The fungus *Stachybotrys chartarum* MXH-X73 was isolated from the sponge *Xestospongia testudinaris* derived from Xisha Island, China. The ITS1-5.8S-ITS2 sequence of the fungus MXH-X73 has been submitted to GenBank with the accession no. KC295244. The voucher specimen is deposited in our laboratory at −20 °C. The producing strain was prepared on potato dextrose agar slants and stored at 4 °C.

Fermentation and Extraction. The fungus was grown on rotary shakers (165 rpm) at 28 °C for 11 d in 500 mL Erlenmeyer flasks containing liquid medium (150 mL/flask) composed of mannitol (20 g/L), maltose (20 g/L), glucose (10 g/L), monosodium glutamate (10 g/L), KH_2PO4 (0.5 g/L), $MgSO_4{\cdot}7H_2O$ (0.3 g/L), yeast extract (3 g/L), corn steep liquor (1 g/L), and naturally collected seawater after adjusting its pH to 6.5. The fermented whole broth (60 L) was filtered through cheese cloth to separate the supernatant from the mycelia. The former was extracted three times with EtOAc to give an EtOAc solution, while the latter was extracted three times with acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with EtOAc to give another EtOAc solution. Both EtOAc solutions were

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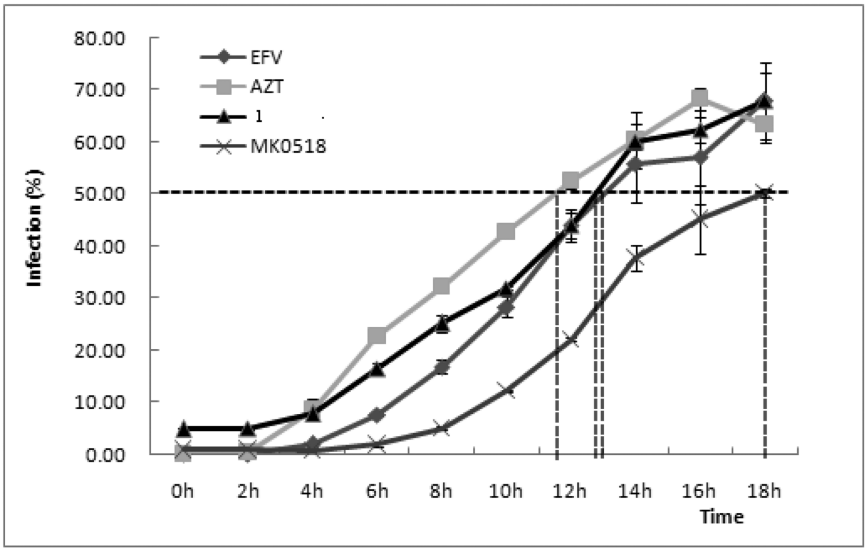
A.



Compound	FT_{50} (h)
AZT	11.59
1	12.66
EFV	13.06
MK0518	17.78

B.

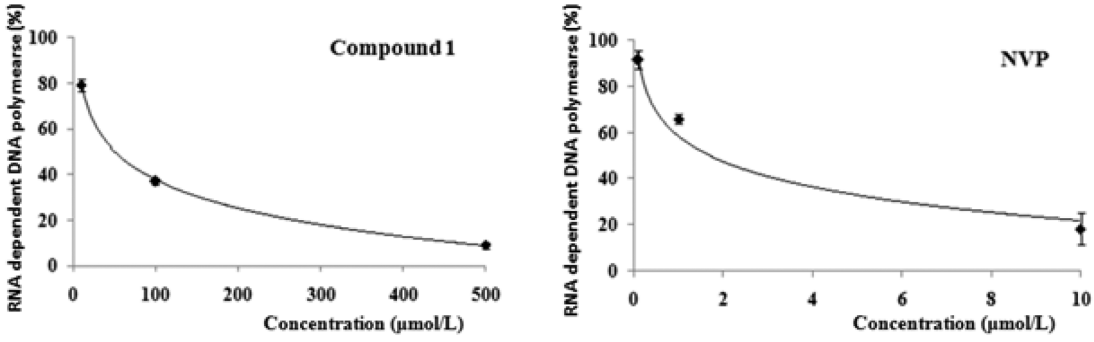


Figure 7. Compound **1** is an HIV-1 reverse transcriptase inhibitor. (A) Time of addition study. The tested compounds, AZT, **1**, EFV, and MK-0518, exhibited times of 50% failure (FT_{50}) of 11.59, 12.66, 13.06, and 17.78 h, respectively. Infectivity (%) was calculated by comparing with control. (B) Effect of compound **1** on HIV-1 reverse transcriptase RNA-dependent DNA polymerase activity. Compound **1** showed a dose-dependent inhibitory effect on RNA-dependent DNA polymerase activity with an EC_{50} at 50 μM; nevirapine (NVP), a NNRTI drug, inhibited RNA-dependent DNA polymerase activity with an EC_{50} of 1.7 μM.

Table 4. Inhibitory Effects of Compound 1 on Wild-Type and NNRTI-Resistant HIV-1 Replication

	compound **1**		nevirapine (NVP)	
	EC_{50} (μM)[b]	EC_{50} value compared to wt EC_{50}	EC_{50} (μM)	EC_{50} value compared to wt EC_{50}
VSVG/HIV-1_{wt}[a]	8.4	1	0.023	1
VSVG/HIV-$1_{RT\text{-}K103N}$	7.0	0.8	2.3	99.1
VSVG/HIV-$1_{RT\text{-}L100I,K103N}$	23.8	2.8	3.3	140
VSVG/HIV-$1_{RT\text{-}K103N,V108I}$	13.3	1.6	7.5	317.4
VSVG/HIV-$1_{RT\text{-}K103N,G190A}$	14.2	1.7	51.9	2256
VSVG/HIV-$1_{RT\text{-}K103N,P225H}$	6.2	0.7	23.3	1013

[a]VSV-G: vesicular stomatitis virus G protein. [b]EC_{50} is the value where infectivity is 50% that of infectivity when an equal amount of solvent is used.

combined and concentrated under reduced pressure to give an extract (52.0 g).

Purification. The extract (52.0 g) was applied to a silica gel (300−400 mesh) column and was separated into six fractions (Fr.1−Fr.6) using a step gradient elution of petroleum ether/CH_2Cl_2, and CH_2Cl_2/MeOH (18 g) was fractionated on a C_{18} ODS column using MeOH/H_2O step gradient elution to give Fr.3-2 and Fr.3-3. Fr.3-2 was purified by semipreparative HPLC (70:30 MeOH/H_2O; 3.0 mL/min) to give compounds **11** (7 mg, t_R = 27 min), **10** (8 mg, t_R = 30 min), and **9** (24 mg, t_R = 35 min). Fr.3-3 was further subfractionated by Sephadex LH-20 using MeOH and then purified by semipreparative HPLC (75:25 MeOH/H_2O; 3.0 mL/min) to give compound **1** (28 mg, t_R = 19 min). Fr.4 (6 g) was separated on a C_{18} ODS column (Fr.4-1−Fr.4-7), and subfraction Fr.4-4 was further separated into four subfractions (Fr.4-4-1−Fr.4-4-4) by Sephadex LH-20 using MeOH as the eluting solvent. Subfraction Fr.4-4-2 was purified by semipreparative HPLC (75:25 MeOH/H_2O; 3.0 mL/min) to give compounds **2** (36 mg, t_R = 20.1 min), **3** (6 mg, t_R = 23.4 min), and **4** (5 mg, t_R = 24.2 min). Subfraction Fr.4-5 was further separated by Sephadex LH-20 using MeOH as the eluting solvent and then on a semipreparative HPLC column (85% MeOH, 3.0 mL/min) to afford compound **5** (22 mg, t_R = 25.4 min) and compound **6** (20 mg, t_R = 28.0 min). Fr.5 (4 g) was fractionated by Sephadex LH-20 using MeOH and further purified by semipreparative HPLC (75:25 MeOH/H_2O; 3.0 mL/min) to give compounds **7** (21 mg, t_R = 29.6 min) and **8** (17 mg, t_R = 26.2 min).

*Stachybotrin D (**1**):* colorless oil; $[\alpha]^{24}_D$ −24.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.77), 261 (3.81), 302 (3.22) nm; IR (KBr) ν_{max} 3407, 3122, 2936, 2897, 1716, 1679, 1618, 1466, 1380, 1251, 1122, 1066, 982, 786 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 442.2587 $[M + H]^+$ (calcd for $C_{26}H_{36}NO_5$, 442.2588).

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*Stachybotrin E (**3**):* colorless oil; $[\alpha]^{24}_{D}$ −34.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.83), 261 (3.73), 301 (3.25) nm; IR (KBr) ν_{max} 3411, 3200, 2939, 1681, 1620, 1451, 1385, 1261, 1116, 1033, 990 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 530.2755 $[M + H]^+$ (calcd for $C_{29}H_{40}NO_8$, 530.2748).

*Stachybotrin F (**4**):* colorless oil; $[\alpha]^{24}_{D}$ −33.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.79), 262 (3.77), 301 (3.20) nm; IR (KBr) ν_{max} 3410, 3208, 2942, 1678, 1618, 1455, 1378, 1263, 1122, 1030, 990 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 530.2746 $[M + H]^+$ (calcd for $C_{29}H_{40}NO_8$, 530.2748).

*Stachybocin E (**5**):* white powder; $[\alpha]^{24}_{D}$ −5.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.88), 262 (3.85), 305 (3.25) nm; IR (KBr) ν_{max} 3420, 2937, 2849, 1682, 1617, 1466, 1333, 1261, 1060, 945, 842, 793, 754 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 825.5066 $[M + H]^+$ (calcd for $C_{50}H_{69}N_2O_8$, 825.5048).

*Stachybocin F (**6**):* white powder; $[\alpha]^{24}_{D}$ −5.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.69), 264 (3.72), 303 (3.12) nm; IR (KBr) ν_{max} 3422, 2933, 2809, 1677, 1612, 1465, 1373, 1230, 1066, 920, 842, 793, 753 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 839.5218 $[M + H]^+$ (calcd for $C_{51}H_{71}N_2O_8$, 839.5205).

*Stachyboside A (**7**):* pale yellow oil; $[\alpha]^{24}_{D}$ +25.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.45), 283 (3.11), 322 (3.77) nm; IR (KBr) ν_{max} 3425, 2938, 1696, 1650,1576, 1332, 1087, 960, 878, 772 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HRESIMS *m/z* 573.2661 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_{10}Na$, 573.2670).

*Stachyboside B (**8**):* pale yellow oil; $[\alpha]^{24}_{D}$ +12.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.54), 286 (4.12), 325 (3.82) nm; IR (KBr) ν_{max} 3428, 2923, 1683, 1647,1583, 1270, 1028, 987, 843, 773 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HRESIMS *m/z* 589.2617 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_{11}Na$, 589.2619).

*Compound **2**:* colorless oil; $[\alpha]^{24}_{D}$ −32.3 (*c* 0.10, MeOH); ESIMS *m/z* 516.2 $[M + H]^+$.

Methylation of Compound 1. A solution of **1** (3 mg) in dry acetone (1 mL) with anhydrous potassium carbonate (10 mg) and methyl iodide (10 μL) was refluxed at 60−70 °C for about 6 h. The reaction mixture was filtered, and the residue was washed thoroughly with acetone. The combined filtrate was evaporated in a vacuum, and the residue was purified on HPLC with 85% MeOH/H_2O to afford **1a** (2.3 mg).

*Compound **1a**:* colorless oil; ^1H NMR (DMSO-d_6, 500 MHz) δ_H 6.72 (1H, s, H-3′), 4.45 (1H, dd, *J* = 18.4 Hz, H-9′), 4.35 (1H, d, *J* = 18.4 Hz, H-9′), 4.26 (1H, dd, *J* = 16.9 Hz, H-8′), 4.24 (1H, d, *J* = 16.9 Hz, H-8′), 4.06 (1H, d, *J* = 3.3 Hz, OH-3), 3.81 (3H, s, OCH_3), 3.15 (1H, brs, H-3), 3.12 (1H, d, *J* = 17.1 Hz, H-11), 2.78 (1H, d, *J* = 17.1 Hz, H-11), 2.13 (3H, s, H-11′), 1.99 (1H, m, H-5), 1.78 (1H, m, H-8), 1.75 (1H, s, H-1), 1.72 (1H, m, H-2), 1.51 (1H, m, H-7), 1.43 (1H, m, H-6), 1.39 (1H, m, H-6), 1.37 (1H, m, H-7), 1.35 (1H, m, H-2), 0.93 (3H, s, H-15), 0.90 (1H, m, H-1), 0.85 (3H, s, H-13), 0.77 (3H, s, H-14), 0.63 (3H, d, *J* = 6.4 Hz, H-12); ESIMS *m/z* 456.3 $[M + H]^+$.

Preparation of the (*R*)- and (*S*)-MTPA Esters of 1a. Compound **1a** (1.1 mg) was dissolved in 500 μL of pyridine, and dimethylaminopyridine (2.0 mg) and (*R*)-MTPACl (8 μL) were then added in sequence. The reaction mixture was stirred for 14 h at room temperature (rt), and 1 mL of H_2O was then added. The solution was extracted with 5 mL of CH_2Cl_2, and the organic phase was concentrated under reduced pressure. Then the residue was purified by semipreparative HPLC (95% MeOH/H_2O) to yield the (*S*)-MTPA ester **1aS** (1.0 mg). By the same procedure, the (*R*)-MTPA ester **1aR** (0.9 mg) was obtained from the reaction of **1** (1.2 mg) with (*S*)-MTPACl (8 μL).

*Compound **1aR**:* white powder; ^1H NMR (DMSO-d_6, 500 MHz) δ_H 7.24−7.48 (5H, m, Ph-MTPA), 6.71 (1H, s, H-3′), 4.78 (1H, brs, H-3), 4.15 (1H, dd, *J* = 16.7 Hz, H-8′), 3.89 (1H, d, *J* = 16.7 Hz, H-8′), 3.79 (3H, s, OCH_3-MTPA), 3.09 (1H, d, *J* = 17.1 Hz, H-11), 2.74 (1H, d, *J* = 17.1 Hz, H-11), 2.13 (3H, s, H-11′), 1.98 (1H, s, H-2), 1.89 (1H, m, H-5), 1.77 (1H, m, H-8), 1.62 (1H, m, H-1), 1.48 (1H, m, H-2), 1.43 (1H, m, H-7), 1.40 (1H, m, H-6), 1.36 (1H, m, H-6), 1.33 (1H, m, H-7), 1.08 (1H, m, H-1), 0.96 (3H, s, H-15), 0.93 (3H, s, H-13), 0.89 (3H, s, H-14), 0.55 (3H, d, *J* = 6.5 Hz, H-12); ESIMS *m/z* 672.2 $[M + H]^+$.

*Compound **1aS**:* white powder; ^1H NMR (DMSO-d_6, 500 MHz) δ_H 7.25−7.48 (5H, m, Ph-MTPA), 6.73 (1H, s, H-3′), 4.79 (1H, brs, H-3), 4.39 (1H, dd, *J* = 18.3 Hz, H-8′), 4.23 (1H, d, *J* = 18.3 Hz, H-8′), 3.81 (3H, s, OCH_3-MTPA), 3.14 (1H, d, *J* = 17.2 Hz, H-11), 2.79 (1H, d, *J* = 17.2 Hz, H-11), 2.09 (3H, s, H-11′), 2.02 (1H, s, H-2), 1.87 (1H, m, H-5), 1.79 (1H, m, H-8), 1.66 (1H, m, H-1), 1.55 (1H, m, H-2), 1.49 (1H, m, H-7), 1.40 (1H, m, H-6), 1.38 (1H, m, H-6), 1.32 (1H, m, H-7), 1.19 (1H, m, H-1), 0.98 (3H, s, H-15), 0.90 (3H, s, H-13), 0.68 (3H, s, H-14), 0.58 (3H, d, *J* = 6.5 Hz, H-12); ESIMS *m/z* 672.3 $[M + H]^+$.

Preparation of (*S*)-PGME Amides of Compounds 3 and 4. To a DMF solution (500 μL) of **3** (1.0 mg) and (*S*)-PGME (1.2 mg) were added PyBOP (2.4 mg), HOBt (1.2 mg), and *N*-methylmorpholine (20 μL), and stirring was continued at rt for 8 h. After addition of 5% HCl (1 mL), the mixture was extracted with *n*-BuOH (1 mL). The extract was washed with saturated aqueous $NaHCO_3$ (1 mL) and brine (1 mL) and concentrated in a vacuum to yield a residue. Then the residue was purified by semipreparative HPLC (80% MeOH/H_2O) to yield the (*S*)-PGME amide (**3a**) of **3** (0.9 mg). The (*S*)-PGME amide (**4a**) (0.8 mg) was prepared according to the same procedure as described above.

*Compound **3a**:* white, amorphous solid; ^1H NMR (DMSO-d_6, 500 MHz) δ_H 7.36−7.40 (5H, m, Ph-PGME), 6.54 (1H, s, H-3′), 4.93 (1H, dd, *J* = 9.1, 6.4 Hz, H-9′), 4.53 (1H, d, *J* = 16.6 Hz, H-8′), 4.34 (1H, d, *J* = 16.6 Hz, H-8′), 3.63 (3H, s, OCH_3-PGME), 3.54 (3H, s, H-14′), 3.19 (1H, brs, H-3), 3.11 (1H, d, *J* = 16.9 Hz, H-11), 2.75 (1H, d, *J* = 16.9 Hz, H-11), 2.30 (2H, m, H-11′), 2.19 (1H, m, H-10′), 2.08 (1H, m, H-10′), 2.04 (1H, m, H-5), 1.80 (1H, m, H-8), 1.77 (1H, m, H-1), 1.73 (1H, m, H-2), 1.53 (1H, m, H-7), 1.47 (1H, m, H-6), 1.44 (1H, m, H-6), 1.40 (1H, m, H-7), 1.39 (1H, m, H-2), 0.95 (3H, s, H-15), 0.92 (1H, m, H-1), 0.89 (3H, s, H-13), 0.80 (3H, s, H-14), 0.66 (3H, d, *J* = 6.4 Hz, H-12); ESIMS *m/z* 677.4 $[M + H]^+$, 699.4 $[M + Na]^+$.

*Compound **4a**:* white, amorphous solid; ^1H NMR (DMSO-d_6, 500 MHz) δ_H7.33−7.38 (5H, m, Ph-PGME), 6.59 (1H, s, H-3′), 4.79 (1H, dd, *J* = 4.4, 10.6 Hz, H-9′), 4.30 (1H, d, *J* = 16.3 Hz, H-8′), 4.23 (1H, d, *J* = 16.3 Hz, H-8′), 3.65 (3H, s, OCH_3-PGME), 3.60 (3H, s, H-14′), 3.18 (1H, brs, H-3), 3.13 (1H, d, *J* = 16.9 Hz, H-11), 2.77 (1H, d, *J* = 16.9 Hz, H-11), 2.18−2.24 (2H, m, H-11′), 2.27 (1H, m, H-10′), 2.15 (1H, m, H-10′), 2.02 (1H, m, H-5), 1.82 (1H, m, H-8), 1.79 (1H, m, H-1), 1.75 (1H, m, H-2), 1.53 (1H, m, H-7), 1.47 (1H, m, H-6), 1.44 (1H, m, H-6), 1.42 (1H, m, H-7), 1.40 (1H, m, H-2), 0.95 (3H, s, H-15), 0.92 (1H, m, H-1), 0.88 (3H, s, H-13), 0.80 (3H, s, H-14), 0.66 (3H, d, *J* = 6.5 Hz, H-12); ESIMS *m/z* 677.4 $[M + H]^+$, 699.4 $[M + Na]^+$.

Determination of the Absolute Configuration at C-9′ of Compound 2. A solution of **2** (3 mg) in dry acetone (1 mL) with anhydrous potassium carbonate (10 mg) and methyl iodide (10 μL) was refluxed at 60−70 °C for about 6 h. The reaction mixture was filtered, and the residue was washed thoroughly with acetone. The combined filtrate was evaporated in a vacuum to dryness. The residue was dissolved in acetone (1 mL) and then reacted with CrO_3(VI) and concentrated H_2SO_4 (Jones reagent, 0.3 mL) at 20 °C for 4 h. The reaction mixture was treated with $NaHSO_3$ and then extracted with diethyl ether. The organic layer was washed successively with saturated aqueous NaCl and $NaHCO_3$ and evaporated to dryness. The oxidation product was hydrolyzed in 6 N HC1 at 110 °C for 48 h. The solution was evaporated to dryness and redissolved in 200 μL of H_2O. Then 25 μL of 1 M $NaHCO_3$ and 100 μL of 2.5 μM FDAA in acetone were added, and the solution was heated for 1 h at 40 °C. The reaction was quenched with 100 μL of 2 N HCl. The derivatized samples were analyzed by HPLC on an analytical YMC ODS-AQ C18 column (4.6 mm, i.d. × 250 mm L, Waters Corp.) using a linear gradient from 95% H_2O (with 0.05% TFA)/5% CH_3CN to 45% H_2O (with 0.05% TFA)/55% CH_3CN over 55 min, and the eluent was monitored at 340 nm. The retention time of the FDAA-derivatized hydrolysate was 34.0 min, while those for the standard L-Glu and D-Glu were 34.3 and 35.1 min, respectively.

Acid Hydrolysis of Compound 7. Compound 7 (8 mg) was hydrolyzed with 1 mL of 6 N HCl for 6 h at 100 °C. The mixture was

dx.doi.org/10.1021/np400683h | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

extracted with EtOAc (3 × 1.5 mL), and the aqueous layer was evaporated to dryness with H_2O until neutral. The residue was dissolved in EtOH and subjected to HPLC (Waters HPLC equipped with a 515 HPLC pump and a Waters 2410 RID detector; column, Chiralpak AD-H column (Daicel), 4.6 × 250 mm, 5 μm; mobile phase, EtOH/*n*-hexane/TFA, 3/7/0.05 (v/v); flow rate, 0.5 mL/min).[29] The sugar was identified as D-glucose by comparison of the retention time (t_R = 12.20 min) with those of the standard samples of D-glucose (t_R = 12.16 min) and L-glucose (t_R = 12.84 min).

Anti-HIV Activity Assay by Pseudotyped Viruses. Vesicular stomatitis virus glycoprotein (VSV-G) plasmid was cotransfected with env-deficient HIV vector (pNL4-3.luc.R-E$^-$ or pNL4-3.luc.R$^-$E$^-_{\text{RT mutant}}$)[30] into 293T cells by using a modified $Ca_3(PO_4)_2$ method.[31] Briefly, plates were washed with PBS, and fresh media was added 16 h after transfection. Supernatant that contains pseudotyped virions (VSVG/HIV-wt or VSVG/HIV-$_{\text{RT mutant}}$) was harvested and filtered through a 0.45 μm filter 48 h post-transfection. Viral solution was quantified by p24 concentrations, which were detected by ELISA (ZeptoMetrix, cat. 0801111) and diluted to 0.2 ng p24/mL which can be used directly or stored at −80 °C.

One day prior to infection, 293T cells were seeded on 24-well plates at the density of 6 × 10^4 cells per well. Compounds were dissolved in DMSO and added into target cells 15 min ahead of infection. Forty-eight hours postinfection, infected cells were lysed in 50 μL of Cell Lysis Reagent (Promega). Luciferase activity of the cell lysate was measured by a Sirius luminometer (Berthold Detection System) according to the manufacturer's instructions.

Cytotoxicity. The cytotoxicity assay was performed by incubating compound **1** with cells for 48 h at the same culture condition as for anti-HIV activity assay, but no viruses were added. Cytotoxicity was measured by using Cell Proliferation Assay kit (Promega) according to the manufacturer's instructions.

Time of Addition Study (ref 32). HEK 293T cells were seeded at 6 × 10^4/well into 24-well plates and placed overnight into an incubator at 37 °C. The following day, cells were infected with VSVG/HIV-wt pseudoparticles. Compounds were added at a final concentration of 10 μM respectively at different time points (0, 2, 4, 6, 8, 10, 12, 14, 16 h), with t = 0 defined as the time of pseudoparticle addition. At 48 h postinfection, cells were lysed with 50 μL of Cell Lysis Reagent (Promega). Luciferase activity of the cell lysate was measured by a Sirius luminometer as mentioned before.[33]

RT RNA-Dependent DNA Polymerase Activity Detection (refs 34 and 35). Tested compound was added into a mixture containing 50 mM Tris-HCl (pH 7.8), 290 mM KCl, 30 mM $MgCl_2$, 10 mM DTT, 11.7 ng/L poly(rA), 11.7 ng/L oligo(dT)15, 2.8 μM dTTP, 800 nM Digoxigenin-11-dUTP, and 40 nM Biotin-11-dUTP. The polymerization reaction was initiated by adding 0.226 ng/L reverse transcriptase at 37 °C for 1 h. The mixture was transferred into a streptavidin-coated microplate (Roche) and incubated at 37 °C for 1 h. Supernatant was discarded, and TMB (3,3,5,5-tetramethylbenzidine) was added. After 15 min, 1 mM H_2SO_4 was added to terminate the reaction, and the OD_{450} was measured by using a microplate reader (Molecular Devices).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The MS and NMR spectra of **1** and **3**−**8** and anti-HIV replication activities of **1**−**11**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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