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ARTICLE

Confirmation of the absolute configuration of Stachybotrin C using single-crystal X-ray diffraction analysis of its 4-bromobenzyl ether derivative

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Abstract

The absolute configuration of Stachybotrin C was confirmed in this study. After synthesizing the dimethyl ethers of Stachybotrin C, the C-8 epimer was analyzed by 1D NOESY. However, the stereochemistry determination was difficult through the NOE correlations. Instead, the di(4-bromobenzyl) ether of Stachybotrin C was derived and used for X-ray diffraction analysis, because its single crystal was easier to obtain than that of the original Stachybotrin C. The stereochemistry of Stachybotrin C was determined to be (8*S*, 9*R*). This derivatization approach may also be used to prepare single crystals of the analogues.

Introduction

Stachybotrin C (**1**) was first isolated from the culture broth of *Stachybotrys parvispora* F4708 by the Hanada and Mizoue group [1]. The authors observed that it induced significant neurite outgrowth in PC12 cells, and showed cell survival activity in the primary culture of cerebral cortical neurons [2]. The relative stereochemistry of **1** was determined by NOESY. Because NOEs were observed between H-8 and H_3-25, and H_3-25 and axial-H-7, the relative configuration of **1** was determined as (8*S**, 9*S**) (Fig. 1).

In 2013, the total synthesis of Stachybotrin C was reported by the van de Weghe group [3]. They synthesized all four stereoisomers of **1**, and revised the stereochemistry of **1** as (8*S*, 9*R*). During that study, two diastereomers of **2** in a racemic mixture were prepared as intermediates in the total synthesis, and used to characterize the relative configuration of C-8 and C-9. Those authors stated that it was not possible to determine the stereochemical relationship by NOESY and other NMR sequence analyses of the diastereomers **2**. They also prepared benzoic ester derivatives of **2** for X-ray diffraction analysis. However, no suitable single crystals were obtained. Therefore, the relative configurations of **2** were assigned by comparing the NMR spectra of the analogues **4** and **5** (Fig. 2). The stereochemistry of the *trans* compound **4** was determined by X-ray diffraction analysis, because single crystals were obtained after converting **4** into the corresponding 4-iodobenzoyl ester derivative. By comparing these NMR spectra, the relative configurations of the two diastereomers of **2** were assigned as (8*S**, 9*R**) and (8*S**, 9*S**). Finally, they concluded that the original Stachybotrin C was synthesized from (8*S**, 9*S**). However, it seemed that there was a slight difference in the ^{1}H NMR spectra of compounds **4** and **5**.

Many other compounds, containing the same 2-prenyl-substituted-3-chromanol-type ring system as Stachybotrin C, have been isolated from various organisms. Their relative configurations were assigned by NOE to be (8*S**, 9*S**) [4–6], the same as that of Stachybotrin C, as assigned by Hanada. Recently, we have isolated several congeners of *Stachybotrys microspora* triprenyl phenols (SMTPs) (**3**) from the culture broth of *S. microspora* IFO 30018 (Fig. 1) [7–12]. These SMTPs have different N-linked side chains (R^3, Fig. 1) and showed enhanced activation of plasminogen by modulating its conformation [8–21]. In our previous work, the

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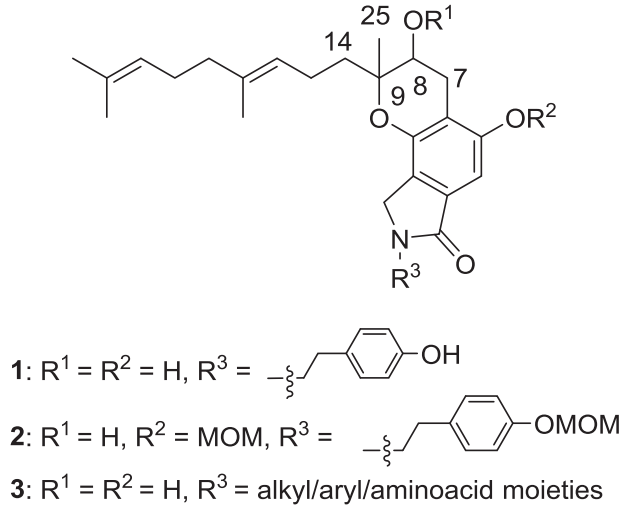


Fig. 1 Structures of Stachybotrin C (**1**), its synthetic intermediate (**2**), and SMTP congeners (**3**)

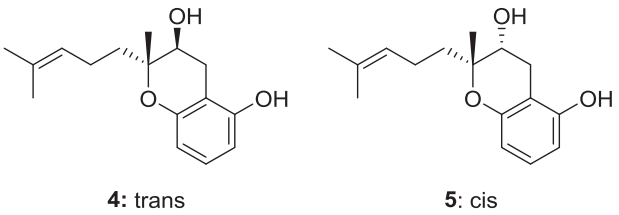


Fig. 2 The analogues of 3-chromanol ring system of Stachybotrin C

stereochemistry of SMTP congeners was assigned as (8*S*, 9*S*) by NMR analyses of SMTP-0 ($R^3 = H$) [22]. Considering that the original Stachybotrin C and the SMTPs were both isolated from fungi of the *Stachybotrys* genus, they should have the same stereochemistry.

In this study, we confirmed the absolute configuration at C-8 of Stachybotrin C by the modified Mosher method and its relative configuration by 1D NOESY analyses. The absolute configuration of Stachybotrin C was finally confirmed by single-crystal X-ray diffraction analysis of the corresponding di(4-bromobenzyl) ether derivative. To our knowledge, this is the first report of successful single-crystal X-ray diffraction analysis in the study of Stachybotrin C and its analogues.

Results and discussion

Stachybotrin C was isolated from the culture broth of *S. microspora* IFO 30018 by adding tyramine to the culture medium. This Stachybotrin C is identical to that obtained from *S. parvispora* according to their physico-chemical properties.

To elucidate the stereochemistry of C-8 in **1**, the modified Mosher method was adopted. The Mosher esters were prepared after protection of the phenol groups as methyl ethers. Treatment of trimethylsilyldiazomethane [23] followed by (*R*)-MTPA-Cl and (*S*)-MTPA-Cl afforded the desired Mosher esters **7** and **8**, respectively (Scheme 1). The chemical shifts in the NMR spectra of **7** and **8** were assigned [24, 25], and the details are given in Supporting Information. When comparing the ^{1}H NMR chemical shifts for (*S*)-MTPA-ester **7** and (*R*)-MTPA-ester **8**, characteristic differences in $\Delta\delta = \delta_S - \delta_R$ were observed at the axial proton at C-7 ($\Delta\delta = -0.13$), the aromatic proton at C-4 ($\Delta\delta = -0.05$), the olefinic proton at C-15 ($\Delta\delta = +0.04$), and the methyl protons at C-25 ($\Delta\delta = +0.04$). These results indicated that the configuration of C-8 was *S*, the same as our previous study of SMTP-0 (Fig. 3).

Because the absolute configuration at C-8 of **6** was defined, the stereochemical relationship between the hydroxy group and methyl or prenyl group at C-9 of **6** was analyzed using coupling constants and 1D NOESY. The splitting pattern of H-8 and H_2-7 was doublet of doublet, and the coupling constants of H-8 were 5.3 and 4.8 Hz. These values suggested that H-8 was in pseudo-equatorial orientation. On the other hand, there was NOE between pseudo-axial-H-7 and H_3-25, which indicated that these protons were on the same face of the 3-chromanol ring. In addition, NOEs were observed between H-8 and pseudo-equatorial-H-7, H_2-14, and H_3-25 (Fig. 4). NOE correlation of H-8 and H_2-14 has not been reported in the case of Stachybotrin C. These results suggested that the stereochemistry of C-9 (*S* or *R* configuration) of compound **6** was uncertain.

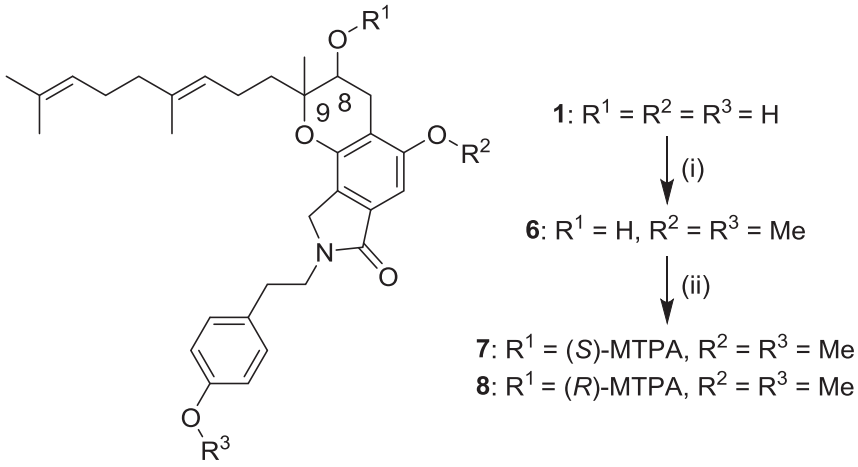
To obtain more definite stereochemical assignment of Stachybotrin C, we further synthesized the epimer of **6**. Oxidation of **6** with Dess–Martin periodinane followed by reduction with $NaBH_4$ gave a 1:3 diastereomer mixture of **6** and **10** (Scheme 2) [26].

Pure **10** was isolated by silica gel column chromatography. The stereochemistry of **10** was also analyzed using coupling constant and 1D NOESY. The coupling constant of H-8 and pseudo-axial-H-7 and that of H-8 and pseudo-equatorial-H-7 were both 4.8 Hz. These values suggested that H-8 was in pseudo-equatorial orientation like that in the compound **6**. On the other hand, NOEs were observed between H-8 and pseudo-axial-H-7, pseudo-equatorial-H-7, H_2-14, and H_3-25 (Fig. 5). These results suggested that like the case of **6**, the stereochemistry of C-9 of compound **10** was difficult to determine.

The ring conformation of **10** is also supported by the ^{1}H NMR chemical shift values of **6** and **10** at C-8 protons, which were almost unchanged due to ring inversion. Such unusual ring inversion made it difficult to assign the stereochemical relationship between two chiral centers by NOE correlations.

Because of the difficulty in determining the absolute configuration at C-9 by NOE correlations, we changed our

Scheme 1 Reagents and conditions: (i) $TMSCH_2N_2$, DIPEA, MeOH:CH_3CN = 1:9, r.t., 90%; (ii) (*R*)-MTPA-Cl or (*S*)-MTPA-Cl, triethylamine, DMAP, CH_2Cl_2 or DMF, r.t., 83%, 95%



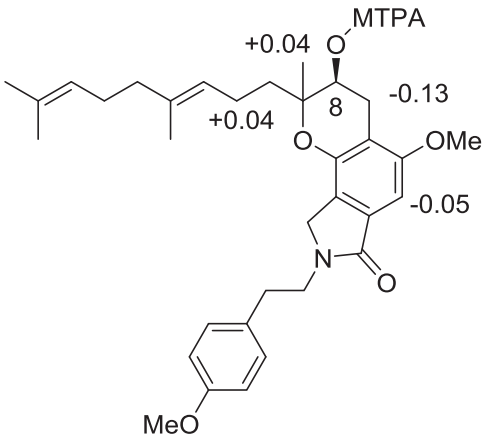


Fig. 3 $\Delta\delta$ values (p.p.m.) obtained from the MTPA esters **7** and **8**

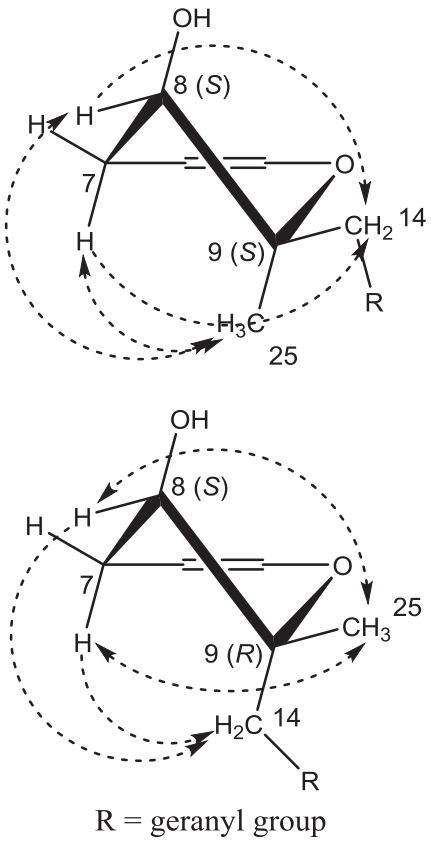
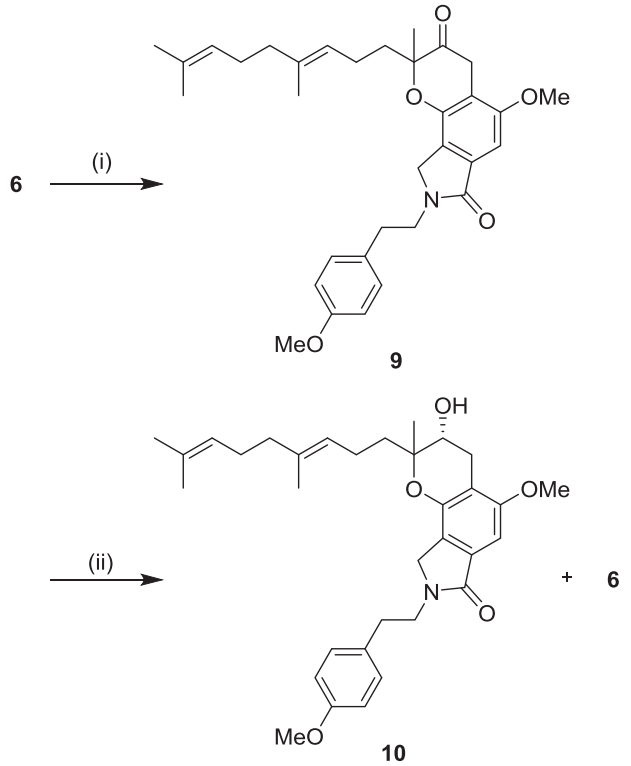


Fig. 4 NOE correlations of **6**

focus to the X-ray diffraction analysis of Stachybotrin C. However, it was challenging to obtain single crystals of Stachybotrin C. Similarly, van de Weghe reported that no crystals of benzoic ester derivatives of **2** suitable for X-ray diffraction analysis could be obtained. We believed that single crystals may be produced by introducing halobenzene moiety to the hydroxyl group, because there are reports that conversion to benzoic ester or benzyl ether is effective for X-ray diffraction analysis [27–29]. Therefore, the 4-iodobenzoic acid ester **11** [27], 3-bromobenzyl ether **12** [28], and 4-bromobenzyl ether **13** [29] were synthesized from **1** for the preparation of single crystals (Scheme 3). Among them, single crystal of **13** was successfully obtained by slow diffusion of *n*-hexene into a solution of **13** in EtOAc. The resulting X-ray analysis of **13** is shown in Fig. 6. The absolute configuration of Stachybotrin C was confirmed as (8*S*, 9*R*). The crystal structure of **13**, determined by X-ray diffraction studies, also showed that the cyclohexene moiety adopted a half chair conformation, as shown in Fig. 4. This conformation is proved by the fact that the coupling constants between H-8 and pseudo-axial-H-7 and between H-8 and pseudo-equatorial-H-7 of compound **13** were both 5.5 Hz. In addition, the distances of H-8 from H_2-7, H_2-14, and H_3-25 were all estimated to be <5 Å in the crystal structure of **13**. These results suggested that determination of the absolute configuration of similar compounds based on NOE correlations is difficult. On the other hand, this stereochemistry must be present in all the SMTPs, because they are produced via a common biosynthetic pathway [30]. Converting the phenol group to 4-bromobenzyl ether in these compounds might facilitate the



Scheme 2 Reagents and conditions: (i) Dess–Martin periodinane, CH_2Cl_2, r.t., 67%; (ii) **10**: $NaBH_4$, MeOH, r.t., 35%

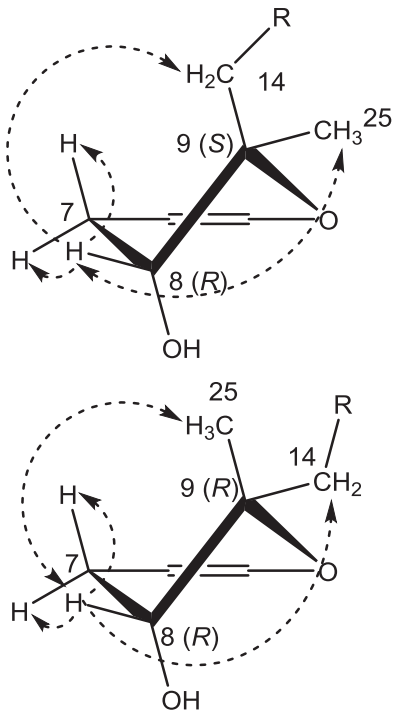
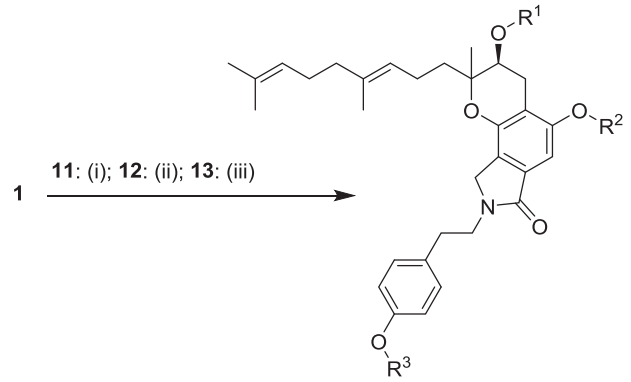


Fig. 5 NOE correlations of **10**



11: $R^1 = R^2 = R^3$ = 4-iodobenzoyl
12: R^1 = H, $R^2 = R^3$ = 3-bromobenzyl
13: R^1 = H, $R^2 = R^3$ = 4-bromobenzyl

Scheme 3 Reagents and conditions: (i) DMAP, triethylamine, 4-iodobenzoyl chloride, DMF, r.t., 71%; (ii) TBAI, K_2CO_3, 4-bromobenzyl bromide, CH_3CN, 55 °C, 61%; (iii) TBAI, K_2CO_3, 3-bromobenzyl bromide, CH_3CN, 55 °C, 83%

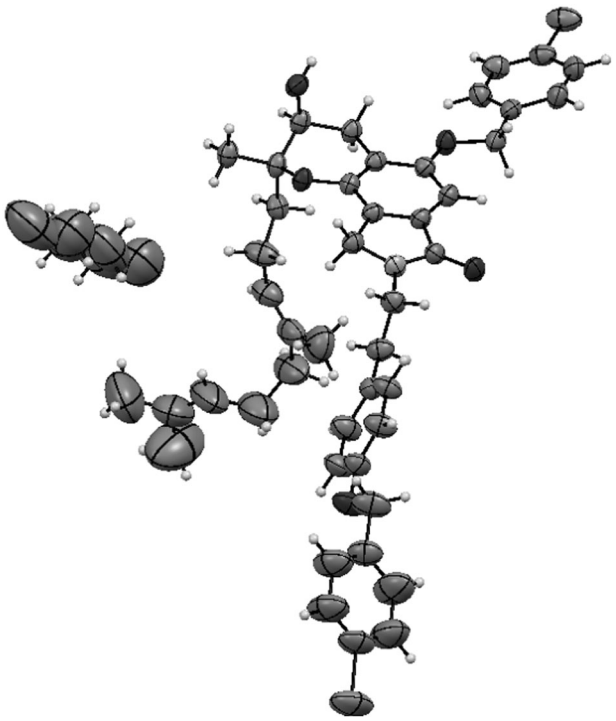


Fig. 6 X-ray crystal structure of **13**

formation of single crystals for the purpose of X-ray diffraction studies.

In conclusion, we confirmed the absolute configuration of Stachybotrin C, a compound isolated from the culture broth of *S. microspora*. 1D NOESY analysis of Stachybotrin C-methyl ether and its C-8 epimer suggested that their stereochemistry was difficult to determine by observation of NOE correlations. In addition, using the original Stachybotrin C to produce a single crystal suitable for X-ray

diffraction analysis was also difficult. However, the absolute configuration of Stachybotrin C was confirmed by derivatizing it to the corresponding di(4-bromobenzyl) ether. This derivatization path may be effective for producing single crystals of its analogues. This result suggests that the stereochemistry of SMTP congeners will be (8*S*, 9*R*), as well as Stachybotrin C.

Experimental procedures

General

X-ray diffraction data were collected using a Rigaku R-AXIS RAPID instrument. Crystallographic data for the structure of **13** have been deposited with the Cambridge Crystallographic Data Centre as deposition number CCDC 1813377. Melting points (mp) were determined on a MEL-TEMP® (capillary melting point apparatus) and reported uncorrected. NMR spectra were recorded in $CDCl_3$ on JEOL ECA-600 and ECS-400 spectrometers. All ^1H NMR spectra are reported in ppm relative to TMS. All ^{13}C NMR spectra are reported in p.p.m. relative to the central line of the triplet for $CDCl_3$ at 77.0 p.p.m. IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. Electrospray ionization (ESI) mass spectra were recorded on a JMS-T100LC AccuTOF mass spectrometer. Optical rotations were recorded on a JASCO P-2200 polarimeter. Chromatographic separations were carried out on a silica gel column (Kanto Chemical 60 N; 63–210 μm).

Production of Stachybotrin C

S. microspora IFO 30018 was incubated at 25 °C for 4 days in a 500-mL Erlenmeyer flask containing 100 mL of a seed medium consisting of 4.0% glucose, 0.5% soybean meal, 0.3% dry bouillon, 0.3% yeast extract, and 0.01% antifoam CB442 (Nippon Oil & Fat Co.), pH 5.8. The seed culture (5.0 mL) was transferred to a 500-mL Erlenmeyer flask containing 100 mL of medium consisting of 5.0% sucrose, 0.1% yeast extract, 0.3% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.00025% $CoCl_2 \cdot 6H_2O$, 0.0015% $FeSO_4 \cdot 7H_2O$, 0.00065% $CaCl_2 \cdot 2H_2O$, and 0.01% CB442, pH 5.8. The flask was incubated at 25 °C on a rotary shaker at 180 r.p.m. After 96 h, 100 mg of tyramine hydrochloride was added, and the flask was incubated further for 24 h. The culture was stopped by adding 200 mL of CH_3OH. The CH_3OH extracts were filtered and concentrated to remove CH_3OH. The residue was extracted thrice with an equal volume of EtOAc (300 mL). The extracts were washed with brine (200 mL) and dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (step-gradient system, *n*-hexane/EtOAc = 2:3, 1:1, 1:4). The resultant was recrystallized from CH_2Cl_2 to give Stachybotrin C (**1**) as a yellowish white powder; yield: 111.8 mg (0.22 mmol).

5-*O*-Me-6′-*O*-Me-Stachybotrin C (6)

N,N-Diisopropylethylamine (DIPEA) (450 μL, 2.63 mmol) and trimethylsilyldiazomethane (0.6 M in *n*-hexane, 1.7 mL, 1.02 mmol) were successively added to a solution of Stachybotrin C (**1**) (128.4 mg, 0.25 mmol) in a 1:1 mixture of CH_3CN and MeOH (5.0 mL). After the reaction mixture was stirred at room temperature for 17 h under argon atmosphere, acetic acid (40 μL, 0.7 mmol) was added, and the resulting mixture was stirred at room temperature for 15 min. The solution was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2:3) to give **6** (119.4 mg, 90%) as a colorless oil. $[\alpha]_D^{23} = -18.7$ (c 1.0, CH_3OH); ^1H NMR (600 MHz, $CDCl_3$) δ 7.16 (1H, d, $J = 8.3$ Hz), 6.90 (1H, s), 6.83 (2H, d, $J = 8.3$ Hz), 5.10–5.05 (1H, m), 4.19 (1H, d, $J = 17.2$ Hz), 4.14 (1H, d, $J = 17.2$ Hz), 3.93–3.90 (1H, m), 3.86 (3H, s), 3.81–3.78 (2H, m), 3.78 (3H, s), 2.96 (1H, dd, $J = 17.9$, 4.8 Hz), 2.91 (2H, t, $J = 7.6$ Hz), 2.74 (1H, dd, $J = 17.5$, 5.5 Hz), 2.15–2.08 (2H, m), 2.06–2.02 (2H, m), 1.97–1.94 (2H, m), 1.66 (3H, s), 1.68–1.56 (2H, m), 1.58 (3H, s), 1.68–1.56 (2H, m), 1.34 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.8, 158.8, 158.3, 148.1, 135.8, 132.7, 131.5, 130.8, 129.7, 124.2, 123.6, 121.8, 114.0, 111.5, 96.6, 79.0, 67.7, 55.9, 55.3, 48.0, 44.4, 40.0, 36.9, 34.0, 26.9, 26.7, 25.7, 21.6, 19.2, 17.7, 15.9; IR (NaCl) 3378 (br), 2963, 2930, 2855, 1666, 1607, 1509, 1472, 1364, 1321, 1245, 1116, 835, 765 cm^{-1}; HRMS (ESI-MS) *m/z* calcd for $C_{33}H_{43}NO_5Na$ 556.3033, found: 556.2996 $(M + Na)^+$.

8-[*S*-α-Methoxy-α-(trifluoromethyl)phenylacetoxy]-5-*O*-Me-6′-*O*-Me-Stachybotrin C (7)

Triethylamine (45 μL, 0.32 mmol), *N,N*-dimethyl-4-aminopyridine (DMAP) (13.1 mg, 0.107 mmol), and *R*-(+)-α-methoxy-α-(trifluoromethyl) phenylacetylchloride (100 mg, 0.4 mmol) were successively added to a solution of **6** (55.2 mg, 0.103 mmol) in CH_2Cl_2 (1.0 mL). After the reaction mixture was stirred at room temperature for 2 h under argon atmosphere, brine (10 mL) was added, and the resulting mixture was extracted with CH_2Cl_2 (150 mL). The organic phase was washed with brine (30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3:2) to give **7** (64.6 mg, 83%) as a colorless oil. $[\alpha]_D^{22} = +19.9$ (c 0.1, CH_3OH); ^1H NMR (600 MHz,

$CDCl_3$) δ 7.42 (d, 2H, $J = 7.6$ Hz) 7.35 (1H, t, $J = 7.3$ Hz), 7.28 (2H, dd, $J = 8.3$, 6.9 Hz), 7.14 (1H, d, $J = 8.3$ Hz), 6.93 (1H, s), 6.81 (1H, d, $J = 8.3$ Hz), 5.27 (1H, t, $J = 6.2$ Hz), 5.08–5.04 (1H, m), 5.02–5.00 (1H, m), 4.12 (2H, s), 3.87 (3H, s), 3.80–3.76 (3H, m), 3.75, (3H, s), 3.49 (3H, s), 3.13 (1H, dd, $J = 18.1$, 12 Hz), 2.92 (2H, t, $J = 7.6$ Hz), 2.87 (1H, dd, $J = 17.9$, 6.2 Hz), 2.14–2.01 (4H, m), 1.96–1.93 (2H, m), 1.67 (3H, s), 1.59 (3H, s), 1.55 (3H, s), 1.59–1.47 (2H, m), 1.22 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.7, 165.8, 158.6, 158.3, 148.0, 136.1, 133.2, 132.0, 131.6, 130.8, 129.7, 128.4, 127.2, 124.2, 123.3 (q, $J = 289$ Hz), 123.1, 121.7, 114.1, 110.1, 96.6, 84.4 (q, $J = 29$ Hz), 72.1, 56.0, 55.4, 55.3, 48.0, 44.5, 39.7, 37.0, 34.0, 26.7, 25.7, 23.6, 21.4, 19.7, 17.7, 16.0; IR (NaCl) 2957, 2930, 2855, 1749, 1690, 1610, 1512, 1473, 1248, 1172, 1118, 1020 cm^{-1}; HRMS (ESI-MS) *m/z* calcd for $C_{43}H_{50}F_3NO_7Na$ 772.3432, found: 772.3453 $(M + Na)^+$.

8-[*R*-α-Methoxy-α-(trifluoromethyl)phenylacetoxy]-5-*O*-Me-6′-*O*-Me-Stachybotrin C (8)

Triethylamine (60 µL, 0.43 mmol), DMAP (17.14 mg, 0.14 mmol), and *S*-(−)-α-methoxy-α-(trifluoromethyl) phenylacetylchloride (100 mg, 0.4 mmol) were successively added to a solution of **6** (74.9 mg, 0.14 mmol) in DMF (1.0 mL). After the reaction mixture was stirred at room temperature for 2 h under argon atmosphere, brine (10 mL) was added, and the resulting mixture was extracted with EtOAc (90 mL). The organic phase was washed with 1 M HCl (5 mL), sat. $NaHCO_3$ (10 mL), and brine (30 mL); dried ($MgSO_4$); and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3:2) to give **8** (99.8 mg, 95%) as a colorless oil. $[\alpha]_D^{22} = -3.19$ (c 0.1, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (2H, d, $J = 7.6$ Hz), 7.39–7.33 (3H, m), 7.15 (2H, d, $J = 8.3$ Hz), 6.88 (1H, s), 6.82 (2H, d, $J = 8.3$ Hz), 5.27 (1H, t, $J = 6.2$ Hz), 5.09–5.05 (1H, m), 5.06 (1H, m), 4.11 (2H, m), 3.85 (3H, s), 3.79 (2H, m), 3.77 (3H, s), 3.45 (3H, s), 3.15 (1H, dd, $J = 17.5$, 4.8 Hz), 2.92 (2H, t, $J = 7.6$ Hz), 2.74 (1H, dd, $J = 17.9$, 6.9 Hz), 2.17–2.06 (1H, m), 2.07–2.04 (1H, m), 1.98–1.96 (2H, m), 1.67 (3H, s), 1.65–1.59 (2H, m), 1.59 (3H, s), 1.56 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.6, 166.0, 158.4, 158.3, 147.8, 136.1, 133.0, 131.6, 131.5, 130.8, 129.6, 128.4, 127.5, 124.1, 123.2 (q, $J = 288$ Hz), 123.1, 122.3, 121.6, 114.0, 110.3, 96.6, 84.9 (q, $J = 28$ Hz), 76.9, 72.1, 55.9, 55.3, 55.2, 47.8, 44.4, 39.7, 37.2, 34.0, 26.7, 25.7, 23.5, 21.4, 19.6, 17.7, 16.0; IR (NaCl) 2957, 2930, 2855, 1749, 1690, 1610, 1512, 1473, 1456, 1437, 1366, 1323, 1248, 1172, 1118, 1020, 903, 826, 808, 766, 721, 709 cm^{-1}; HRMS (ESI-MS) *m/z* calcd for $C_{43}H_{50}F_3NO_7Na$ 772.3432, found, 772.3438 $(M + Na)^+$.

5-*O*-Me-6′-*O*-Me-8-oxo-Stachybotrin C (9)

Dess–Martin periodinane (1.9 mL, 8–12% in CH_2Cl_2) was added to a solution of **6** (181 mg, 0.34 mmol) in CH_2Cl_2 (4.0 mL). After the reaction mixture was stirred at room temperature for 10 min under argon atmosphere, a 1:1 mixture of sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ (20 mL) was added, and the resulting mixture was extracted with CH_2Cl_2 (240 mL). The organic phase was washed with brine (30 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:1) to give **9** (121 mg, 67%) as a colorless oil. $[\alpha]_D^{21} = -13.3$ (c 1.0, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.15 (2H, d, $J = 8.3$ Hz), 7.03 (1H, s), 6.83 (2H, d, 8.9 Hz), 5.07–5.02 (2H, m), 4.21 (1H, d, $J = 17.2$ Hz), 4.17 (1H, d, $J = 16.5$ Hz), 3.89 (3H, s), 3.85–3.80 (2H, m), 3.77 (3H, s), 3.60 (1H, d, $J = 22.0$ Hz), 3.51 (1H, d, $J = 21.3$ Hz), 2.94 (2H, t, $J = 7.56$ Hz), 2.12–1.96 (2H, m), 2.04–2.00 (2H, m), 1.95–1.88 (2H, m), 1.70–1.67 (2H, m), 1.66 (3H, s), 1.57 (3H, s), 1.51 (3H, s), 1.37 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 207.7, 168.3, 158.3, 157.6, 147.6, 136.2, 133.5, 131.4, 130.7, 129.6, 124.1, 122.8, 122.3, 114.0, 112.9, 98.5, 84.3, 56.0, 55.2, 47.9, 44.5, 39.6, 37.0, 36.7, 34.2, 33.9, 26.5, 25.6, 22.0, 21.8, 17.6, 15.8; IR (NaCl) 3298, 2963, 2931, 2909, 2837, 2358, 2336, 1732, 1696, 1684, 1670, 1607, 1512, 1473, 1455, 1435, 1416, 1362, 1327, 1247, 1191, 1175, 1117, 1036, 898, 827, 767 cm^{-1}; HRMS (ESI-MS) *m/z* calcd for $C_{33}H_{41}NO_5Na$ 554.28769, found 554.2874 $(M + Na)^+$.

8-*epi*-5-*O*-Me-6′-*O*-Me-Stachybotrin C (10)

$NaBH_4$ (17.0 mg, 0.45 mmol) was added to a solution of **9** (121.0 mg, 0.228 mmol) in MeOH (3.0 mL). After the reaction mixture was stirred at room temperature for 15 min, brine (6 mL) was added, and the resulting mixture was extracted with EtOAc (120 mL). The organic phase was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to yield a crude 1:3 mixture of **6** and **10** (120.3 mg). The mixture was subjected to silica gel column chromatography (CH_2Cl_2/EtOAc = 4:1) to give pure **10** (43.1 mg, 35%) as a colorless oil. $[\alpha]_D^{19} = -26.1$ (c 0.1, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.15 (2H, d, $J =$ 8.9 Hz), 6.91 (1H, s), 6.83 (2H, d, $J = 8.9$ Hz), 5.17–5.14 (1H, m), 5.10–5.07 (1H, m), 4.18 (1H, d, $J = 17.2$ Hz), 4.15 (1H, d, $J = 17.2$ Hz), 3.93–3.90 (1H, m), 3.87 (3H, s), 3.84–3.76 (2H, m), 3.78 (3H, s), 2.92 (2H, t, $J = 7.56$ Hz), 2.91 (1H, dd, $J = 18.2$, 4.8 Hz), 2.79 (1H, dd, $J = 17.9$, 4.8 Hz), 2.19–2.03 (2H, m), 2.07–2.04 (2H, m), 1.99–1.97 (2H, m), 1.80–1.71 (2H, m), 1.67 (3H, s), 1.61 (3H, s), 1.59 (3H, s), 1.27 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.8, 158.9, 158.2, 147.9, 135.7, 132.7, 131.5, 130.8, 129.6, 124.2, 123.8, 121.8, 121.8, 114.0, 111.4, 96.6, 78.8, 68.1,

55.9, 56.2, 47.9, 44.3, 39.7, 34.6, 34.0, 26.8, 26.7, 25.7, 21.8, 21.3, 17.7, 15.9; IR (NaCl) 3349 (br), 2955, 2924, 2870, 1666, 1608, 1512, 1473, 1366, 1322, 1246, 1113, 829, 766 cm^{-1}; HRMS (ESI-MS) *m/z* calcd for $C_{33}H_{43}NO_5Na$ 556.3033, found 556.3034 $(M + Na)^+$.

5-*O*-PIBz-6′-*O*- PIBz-8-*O*-PIBz-Stachybotrin C (11)

DMAP (36 mg, 0.295 mmol), triethylamine (123 µL, 0.88 mmol), and 4-iodobenzoyl chloride (150 mg, 0.563 mmol) were successively added to a solution of **1** (50 mg, 0.099 mmol) in *N,N*-dimethylformamide (2.5 mL). After the reaction mixture was stirred at room temperature for 13 h under argon atmosphere, brine (10 mL) was added, and the resulting mixture was extracted with EtOAc (300 mL). The organic phase was washed with brine (30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 7:3) to give **11** (84.4 mg, 71%) as a white solid (mp 66.7–70.7 °C). $[\alpha]_D^{22} = -11.2$ (c 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (8H, m), 7.78 (2H, d, $J = 8.3$ Hz), 7.66 (2H, d, $J = 8.3$ Hz), 7.34 (2H, d, $J = 8.9$ Hz), 7.28 (1H, s), 7.15 (2H, d, $J = 8.3$ Hz), 5.36 (1H, t, $J = 5.5$ Hz), 5.08–5.04 (2H, m), 4.33 (1H, d, $J = 17.2$ Hz), 4.27 (1H, d, $J = 17.8$ Hz), 3.92–3.85 (2H, m), 3.13 (1H, dd, $J = 17.9$, 5.5 Hz), 3.04 (2H, t, $J = 7.6$ Hz), 2.81 (1H, dd, $J = 17.9$, 5.5 Hz), 2.21–2.09 (2H, m), 2.05–2.00 (2H, m), 1.94–1.92 (2H, m), 1.75–1.60 (2H, m), 1.65 (3H, s), 1.63 (3H, s), 1.56 (3H, s), 1.54 (3H, s), 1.42 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.6, 165.2, 164.7, 164.0, 149.7, 149.4, 148.4, 138.1, 137.9, 137.9, 136.4, 136.2 133.3, 131.6, 131.5, 131.1, 129.8, 129.0, 128.2, 126.6, 124.1, 122.9, 121.8, 115.3, 109.2, 102.1, 101.6, 101.3, 78.1, 69.5, 48.0, 44.2, 39.6, 37.0, 34.2, 26.6, 25.7, 24.2, 21.5, 20.0 17.7, 15.9; IR (KBr) 2965, 2922, 2915, 2851, 2359, 2343, 2330, 1733, 1690, 1684, 1585, 1507, 1456, 1392, 1260, 1197, 1176, 1113, 1099, 1073, 1007, 909, 879, 843, 748, 731, 679 cm^{-1}; HRMS (ESI-MS) *m/z* calcd for $C_{52}H_{48}I_3NO_8Na$ 1218.0406, found 1218.0403 $(M + Na)^+$.

5-*O*-MBBn-6′-*O*-MBBn-Stachybotrin C (12)

Tetrabutylammonium iodide (TBAI) (20 mg, 0.054 mmol), K_2CO_3 (450 mg, 3.26 mmol), and 4-bromobenzyl bromide (283 mg, 1.13 mmol) were successively added to a solution of **1** (261 mg, 0.517 mmol) in CH_3CN (10 mL). After the reaction mixture was stirred at 55 °C for 17 h under argon atmosphere, water (10 mL) was added, and the resulting mixture was extracted with EtOAc (300 mL). The organic phase was washed with brine (30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3:2) to give **12** (121.6 mg, 61%) as a white solid (mp 110.7–111.1 °C). $[\alpha]_D^{20} = -7.39$ (c 0.5, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (2H, m), 7.45 (2H, t, $J = 7.2$ Hz), 7.35–7.34 (2H, m), 7.25–7.22 (2H, m), 7.16 (2H, d, $J = 8.3$ Hz), 6.93 (1H, s), 6.88 (2H, d, $J = 8.3$ Hz), 5.01 (1H, t, $J = 6.9$ Hz), 5.07–50.5 (1H, m), 5.04 (2H, s), 4.98 (2H, s), 4.20 (1H, d, $J = 16.5$ Hz), 4.16 (1H, d, $J = 16.5$ Hz), 3.96–3.93 (1H, m), 3.83–3.75 (2H, m), 3.04 (1H, dd, $J = 17.9$, 4.8 Hz), 2.91 (2H, t, $J = 7.56$ Hz), 2.82 (1H, dd, $J = 17.9$, 5.5 Hz), 2.12–2.09 (2H, m), 2.06–2.02 (2H, m), 1.96–1.94 (2H, m), 1.70–1.59 (2H, m), 1.65 (3H, s), 1.57 (3H, s), 1.56 (3H, s), 1.36 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.6, 157.5, 157.2, 148.3, 139.4, 139.0, 135.8. 135.8, 132.7, 131.5, 131.3, 131.0, 130.9, 130.3, 130.1, 129.7, 125.8, 125.7, 124.1, 123.6, 122.6, 122.2, 115.0, 111.9, 97.6, 79.1, 69.3, 69.1, 67.6, 47.9, 44.3, 39.6, 36.9, 34.0, 27.0, 26.6, 25.7, 21.6, 19.2, 17.7, 15.9; IR (KBr) 3388 (br), 3340 (br), 2965, 2916, 2855, 1665, 1608, 1571, 1511, 1473, 1452, 1428, 1416, 1372, 1324, 1243, 1202, 1175, 1166, 1123, 1090, 1070, 1048, 907, 883, 825, 777, 680, 670 cm^{-1}; HRMS (ESI-MS) *m/z* calcd for $C_{45}H_{49}Br_2NO_5Na$ 866.18492, found 866.1850 $(M + Na)^+$.

5-*O*-PBBn-6′-*O*-PBBn-Stachybotrin C (13)

TBAI (20 mg, 0.054 mmol), K_2CO_3 (450 mg, 3.26 mmol), and 4-bromobenzyl bromide (283 mg, 1.13 mmol) were successively added to a solution of **1** (261 mg, 0.517 mmol) in CH_3CN (10 mL). After the reaction mixture was stirred at 55 °C for 17 h under argon atmosphere, water (10 mL) was added, and the resulting mixture was extracted with EtOAc (300 mL). The organic phase was washed with brine (30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3:2) to give **13** (363 mg, 83%) as a white solid (mp 101.1–102.5 °C). $[\alpha]_D^{23} = -4.54$ (c 0.1, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 (2H, d, $J = 8.3$ Hz), 7.50 (2H, d, $J = 9.62$ Hz), 7.32 (2H, d, $J = 8.3$ Hz), 7.30 (2H, d, $J = 8.3$ Hz), 7.16 (2H, d, $J = 8.3$ Hz), 6.95 (1H, s), 6.88 (2H, d, $J = 8.9$ Hz), 5.10–5.08 (2H, m), 5.07–5.04 (2H, s), 4.98 (2H, s), 4.22 (1H, d, $J = 17.2$ Hz), 4.17 (1H, d, $J = 16.5$ Hz), 3.95–3.92 (1H, m, $J = 6.9$ Hz), 3.84–3.76 (2H, m), 3.02 (1H, dd, $J = 17.9$, 5.5 Hz), 2.92 (2H, t, $J = 7.6$ Hz), 2.81 (1H, dd, $J = 17.9$, 5.5 Hz), 2.17–2.07 (2H, m), 2.05–2.02 (2H, m), 1.96–1.93 (2H, m),1.85 (1H, d, $J = 6.9$ Hz), 1.66 (3H, s), 1.69–1.58 (2H, m), 1.57 (3H, s), 1.56 (3H, s), 1.35 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.6, 157.5, 157.1, 148.2, 136.1, 135.9, 135.7, 132.7, 131.7, 131.5, 131.2, 129.7, 129.0, 128.9, 124.1, 123.5, 122.1, 121.9, 121.8, 114.9, 111.8, 97.6, 79.1, 69.5, 69.2, 67.6, 47.9, 44.2, 39.6, 36.9, 34.0, 27.0, 26.6, 25.7, 21.6, 19.2, 17.7, 15.9; IR (KBr) 3346, 2965, 2918, 2856, 2360, 2341, 1663, 1610, 1512, 1491, 1475, 1456, 1410, 1371, 1324, 1244, 1176, 1107, 1070, 1051, 1010,

825, 804, 766 cm^{-1}; HRMS (ESI-MS) *m/z* calcd for $C_{45}H_{49}Br_2NO_5Na$ 866.18492, found 866.1833 $(M + Na)^+$.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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