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# Stachyflin and Acetylstachyflin, Novel Anti-influenza A Virus Substances, Produced by *Stachybotrys* sp. RF-7260

## II. Synthesis and Preliminary Structure-Activity Relationships of Stachyflin Derivatives

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Stachyflin and acetylstachyflin, produced by *Stachybotrys* sp. RF-7260, were found to have potent anti-influenza A virus activity. Stachyflin is a new class of hemagglutinin fusion inhibitors of influenza A virus. Several derivatives were synthesized from acetylstachyflin and subjected to preliminary examination of their structure-activity relationships. Among them, the 3-oxo and 3,8′-dioxo derivatives showed potent antiviral activity similar to stachyflin. The 3-epi derivative was four times less active than stachyflin. Modification of the 6′-hydroxy group and the C-5′ position markedly diminished the antiviral activity.

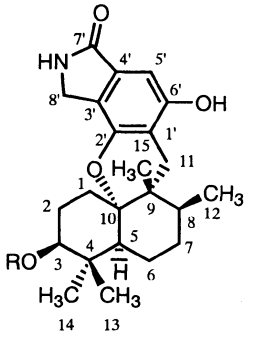
We have isolated acetylstachyflin (**1**) and stachyflin (**2**) by solid-state fermentation of *Stachybotrys* sp. RF-7260[1]. They possess novel pentacyclic structures including a *cis*-fused decalin ring as a sesquiterpene part. Stachyflin (**2**) showed potent antiviral activity against influenza A virus (H1N1) *in vitro* with an $IC_{50}$ value of 0.003 $\mu$M[1]. The mechanism of the antiviral action of stachyflin has been shown to be inhibition of the fusion process between the viral envelope and the host cell membrane, which is an early step in the entry of the virus into host cells[2,3]. This differs from those of known anti-influenza virus agents such as amantadine[4] and zanamivir[5]. Therefore, **2** is considered to be an attractive lead compound for anti-influenza A virus agents. Preliminary investigation of the structure-activity relationships of stachyflin using **1**, the main metabolite in the solid-state fermentation broth of *Stachybotrys* sp. RF-7260, is reported here.

### Results and Discussion

#### Chemistry

In the SAR study of stachyflin, we first attempted to evaluate the effect of the phenol and the phenyl moieties as shown in Scheme 1. Treatment of **1** with methyl iodide and potassium carbonate ($K_2CO_3$) gave the *O*-methyl derivative **3**. The 6′-hydroxy group of **1** was acetylated with $Ac_2O$ in pyridine to give the acetate **4**. Next, compound **1** was converted to carboxymethyl derivative **5**. Reaction of **1** with

Fig. 1. Structures of acetylstachyflin (**1**) and stachyflin (**2**).




Acetylstachyflin (**1**) R=Ac
Stachyflin (**2**) R=H

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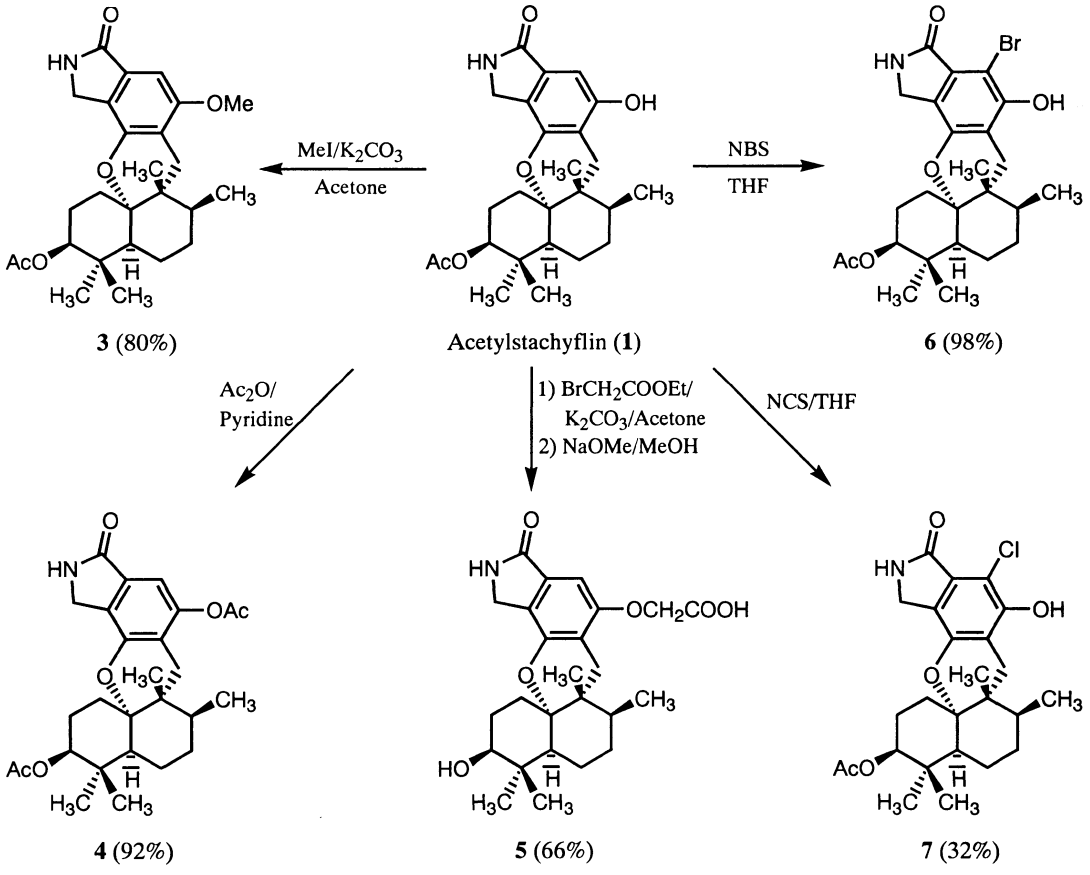
Scheme 1.




ethyl bromoacetate and $K_2CO_3$ in acetone, followed by alkaline hydrolysis with NaOMe in MeOH gave **5** in two steps. Halogenation of the C-5′ position of **1** with *N*-bromosuccinimide afforded the 5′-bromo derivative **6**. Treatment of **1** with *N*-chlorosuccinimide gave the 5′-chloro derivative **7**.

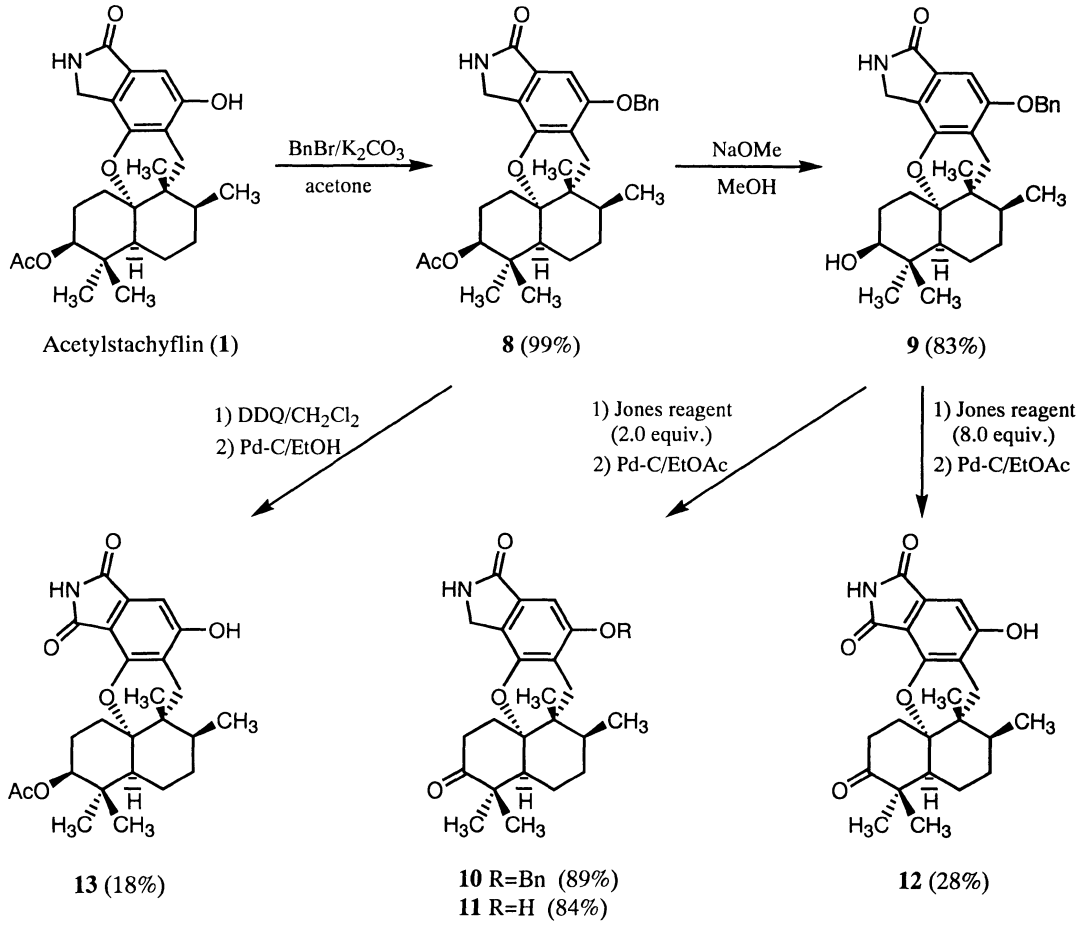
Turning our attention to the 3-hydroxy group, we synthesized the 3-oxo derivative **11**. Compound **9** was synthesized from **1** according to the previously described procedure[1]. The 3-hydroxy group of **9** was oxidized with 2.0 equivalents of Jones reagent to give compound **10**. Deprotection of **10** by catalytic hydrogenation gave 3-oxo derivative **11**. Reaction of **9** with 8.0 equivalents of Jones reagent led to oxidation of both the 3-hydroxy group and the 8′-methylene carbon, and subsequent catalytic hydrogenation gave **12** as described in our previous paper[1]. Oxidation of the 8′-methylene carbon of compound **8** with 2,3-dichloro-5,6-dicyanobenzoquinone and subsequent deprotection by catalytic hydrogenation gave the phthalimide derivative **13** (Scheme 2).

Next, we synthesized the 3-epi derivative **15** as shown in Scheme 3. Treatment of the 3-oxo compound **10** with $NaBH_4$ in MeOH gave a mixture of two stereoisomers, which were separated by preparative TLC to afford the more polar compound **9** and the less polar compound **14** in 37% and 62% yield, respectively. The more polar compound was identical with **9** by comparison of TLC and $^1H$ NMR data, and the $\beta$-axial orientation of the hydroxy group at C-3 is assigned by the small coupling constant value ($J=\sim3$ Hz) of the H-3 signal[1]. On the other hand, the less polar compound **14** was elucidated as a 3-epimer of **9** by the large coupling constant value ($J=11.3$ Hz) of H-3 signal compared to that of **9**. Therefore, the 3-hydroxy group of **14** is the $\alpha$-equatorial orientation. Deprotection of **14** by catalytic hydrogenation gave the 3-epi derivative **15**.

## Biological Activities

*In vitro* antiviral activity against influenza A virus and the cytotoxic activity against Madin-Darby bovine kidney

Scheme 2.




Acetylstachyflin (**1**)

**8** (99%)

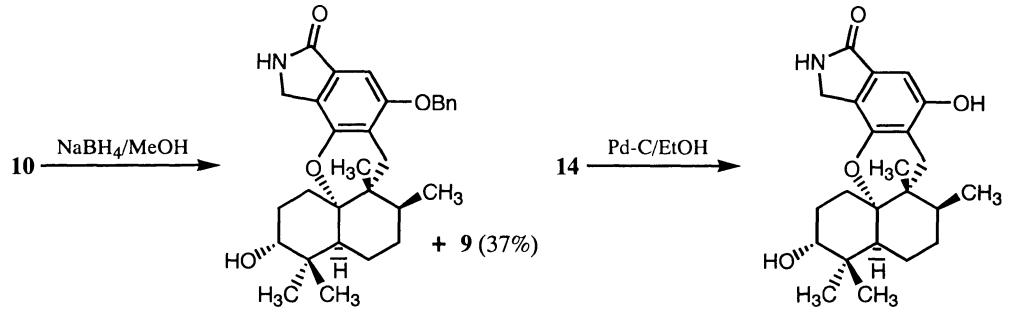
**9** (83%)

**13** (18%)

**10** R=Bn (89%)
**11** R=H (84%)

**12** (28%)

Scheme 3.




**14** (62%)

**15** (89%)

(MDBK) cells were measured according to a method described previously[2]. The $IC_{50}$ values of acetylstachyflin (**1**), stachyflin (**2**) and synthetic analogues against influenza A virus (H1N1) are summarized in Table 1. The antiviral activity of **1** was about 77 times weaker than that of **2**. Moreover, the 3-epi derivative **15** was also four times less

active than **2**. However, the 3-oxo derivative **11** maintained activity as potent as that of **2**. The phthalimide derivative **13** was two times less active than the parent compound **1**. Moreover, the 3,8′-dioxo derivative **12** has potent antiviral activity ($IC_{50}$ value of 0.006 $\mu$M) comparable to **2**. On the other hand, the methyl derivative **3** was about 100 times less active than the parent compound **1**. The acetyl derivative **4** also displayed reduced antiviral activity. In addition, carboxymethyl derivative **5** and benzyl derivative **9** lost this activity ($IC_{50}$=7.0 and 0.51 $\mu$M, respectively). The halogen atom at the C-5′ position decreased the antiviral activity, as seen for both the 5′-bromo derivative 6 and the 5′-chloro derivative **7**.

This work has examined chemical modification at the 3-, 5′-, 6′- and 8′-positions of stachyflin and the preliminary SAR as described above. Our results indicate that the 3-hydroxy group is important and $\beta$-configuration of the 3-hydroxy group is preferred to $\alpha$-configuration for the antiviral activity. The 6′-hydroxy group has an important role in the antiviral activity and needs to be unsubstituted. Oxidation of the 8′ position does not affect the antiviral activity. Modification of the C-5′ position markedly diminishes the antiviral activity. The preparation and antiviral activities of amino acids containing derivatives will be reported in a separate paper[6].

Table 1. *In vitro* anti-influenza A virus activity and cytotoxicity of stachyflin and its derivatives.

| | anti-influenza A virus activity | cytotoxicity |
|---|---|---|
| compound | $IC_{50}$ (μM) | $CC_{50}$ (μM) |
| Acetylstachyflin (**1**) | 0.23 | 44 |
| Stachyflin (**2**) | 0.003 | 65 |
| **3** | >23 | 23 |
| **4** | 1.7 | 40 |
| **5** | 7.0 | >225 |
| **6** | 1.6 | 19 |
| **7** | 3.5 | 20 |
| **8** | >12 | 12 |
| **9** | 0.51 | 16 |
| **11** | 0.006 | 98 |
| **12** | 0.006 | 94 |
| **13** | 0.45 | 28 |
| **15** | 0.012 | >65 |

virus: A/WSN/33(H1N1), cells: Madin-Darby bovine kidney cells

## Experimental

### General

IR spectra were recorded on a JASCO FT/IR-700 spectrometer. FAB-MS and HR FAB-MS were obtained on a JEOL JMS-SX/SX 102A. $^1H$ and $^{13}C$ NMR spectra were recorded on a Varian Gemini-300 spectrometer. Column chromatography was carried out on silica gel (Silica gel 60, 70~230 mesh, E. Merck). For preparative TLC, silica gel plate (pre-coated TLC plates, Silica gel F-254, E. Merck) was used.

### Compound 3

Methyl iodide (2 ml) and potassium carbonate (100 mg) were added to a solution of **1** (45 mg, 0.105 mmol) in acetone (4 ml), and the mixture was heated to reflux for 8 hours. The reaction mixture was filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by preparative TLC ($CH_2Cl_2$-MeOH=10:1) to give **3** (37 mg, 80%); HR FAB-MS (*m/z*) 442.2595 $(M+H)^+$ Calcd for $C_{26}H_{36}NO_5$: 442.2593; IR $\nu_{max}$ KBr $cm^{-1}$: 3422, 2960, 2874, 1736, 1698, 1625, 1601, 1472, 1435, 1369; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 0.86 (3H, s), 0.91 (3H, s), 1.08 (3H, s), 1.15 (3H, d, $J$=7.5 Hz), 1.38 (1H, m), 1.60 (1H, m), 1.78 (4H, m), 1.95 (1H, m), 2.02 (1H, m), 2.10 (3H, s), 2.15 (1H, m), 2.23 (1H, d, $J$=18.0 Hz), 2.41 (1H, m), 3.17 (1H, d, $J$=18.0 Hz), 3.88 (3H, s), 4.30 (1H, d, $J$=16.5 Hz), 4.36 (1H, d, $J$=16.5 Hz), 4.78 (1H, m), 6.58 (1H, br.s), 6.90 (1H, s); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 17.15, 20.17, 21.49, 23.09, 23.46, 24.49, 26.33, 27.88, 30.01, 32.16, 37.22, 37.60, 39.48, 43.41, 44.61, 55.78, 77.22, 83.41, 95.72, 114.07, 123.25, 131.07, 147.10, 158.49, 170.42, 172.06.

### Compound 4

Acetic anhydride (0.3 ml) was added to a solution of **1** (50 mg, 0.12 mmol) in dry pyridine (2 ml), and the mixture was stirred at room temperature for 20 hours. After addition of MeOH (5 ml) to the reaction mixture, the solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc (40 ml) and water (30 ml), and the EtOAc layer was washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to give **4** (50 mg, 92%) as a colorless solid; HR FAB-MS (*m/z*) 470.2533 $(M+H)^+$ Calcd for $C_{27}H_{36}NO_6$: 470.2542; IR $\nu_{max}$ KBr $cm^{-1}$: 3422, 3219, 2966, 2939, 1763, 1734, 1701, 1655, 1600, 1458, 1372; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 0.87 (3H, s), 0.91 (3H, s), 1.11 (3H, s), 1.16 (3H, d, $J$=7.4 Hz), 1.40 (1H, m), 1.60 (1H, m), 1.66

(1H, m), 1.78 (3H, m), 1.98~2.18 (3H, m), 2.02 (1H, d, $J$=17.4 Hz), 2.10 (3H, s), 2.34 (3H, s), 2.40 (1H, m), 3.21 (1H, d, $J$=17.4 Hz), 4.32 (1H, d, $J$=16.8 Hz), 4.41 (1H, d, $J$=16.8 Hz), 4.78 (1H, m), 6.60 (1H, br.s), 7.12 (1H, s); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 17.07, 19.90, 21.47, 23.00, 23.40, 24.48, 26.27, 27.76, 30.06, 30.95, 32.20, 37.26, 37.63, 39.45, 43.42, 45.02, 76.26, 84.11, 108.22, 118.16, 127.56, 131.51, 147.56, 149.72, 168.87, 170.37, 170.91.

Compound **5**

Potassium carbonate (50 mg) and ethyl bromoacetate (145 mg) were added to a solution of **1** (50 mg, 0.12 mmol) in acetone (10 ml), and the mixture was heated to reflux for 16 hours. The insoluble materials were filtered off. The filtrate was evaporated under reduced pressure, and the residue was purified by preparative TLC (*n*-hexane-acetone=1 : 1) to obtain the product (60 mg). The product was dissolved in 1 M NaOMe/MeOH (3 ml) and heated to refluxing for 2 hours. The reaction mixture was then concentrated, and the residue was partitioned between EtOAc (3 ml) and water (3 ml). The aqueous layer was adjusted to pH 3.0 with 1 N HCl, and extracted with EtOAc (3 ml). The EtOAc layer was washed with water, dried over anhydrous sodium sulfate, and concentrated to dryness to give **5** (35 mg, 66%); HR FAB-MS (*m/z*) 444.2392 $(M+H)^+$ Calcd for $C_{25}H_{34}NO_6$: 444.2386; IR $\nu_{max}$ KBr $cm^{-1}$: 3421, 2960, 2873, 1684, 1625, 1604, 1466, 1413, 1369; $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 0.85 (3H, s), 0.89 (3H, s), 0.93 (3H, s), 1.10 (3H, d, $J$=7.2 Hz), 1.26 (1H, m), 1.46~1.72 (4H, m), 1.74 (1H, m), 1.98 (1H, m), 2.10~2.35 (3H, m), 2.21 (1H, d, $J$=18.0 Hz), 3.13 (1H, d, $J$=18.0 Hz), 3.35 (1H, m), 4.15 (1H, d, $J$=17.4 Hz), 4.22 (1H, d, $J$=17.4 Hz), 4.47 (1H, br.s), 4.71 (1H, d, $J$=17.1 Hz), 4.73 (1H, d, $J$=17.1 Hz), 6.56 (1H, s), 8.40 (1H, br.s); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ 16.86, 19.85, 23.38, 25.59, 26.86, 27.48, 29.99, 31.75, 36.96, 37.37, 42.48, 44.27, 65.08, 72.05, 83.50, 95.88, 113.21, 123.07, 131.49, 147.01, 156.40, 170.02, 170.12.

Compound **6**

*N*-Bromosuccinimide (26 mg) was added to an ice-cooled solution of **1** (42 mg, 0.10 mmol) in THF (6 ml), and the mixture was stirred for 3 hours. Next, 10% aqueous sodium hydrogen sulfite (2 ml) was added to the reaction mixture, and the mixture was stirred for 10 minutes. The reaction mixture was partitioned between EtOAc and $H_2O$, and the EtOAc layer was washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated to give a crude product. The crude product was purified by preparative TLC (*n*-hexane-acetone=1 : 1) to give **6** (49 mg, 98%); HR FAB-MS (*m/z*) 506.1542 $(M+H)^+$ Calcd for $C_{25}H_{33}NO_5Br$: 506.1542; IR $\nu_{max}$ KBr $cm^{-1}$: 3391, 2992, 2961, 2875, 1735, 1697, 1591, 1474, 1435, 1359; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.87 (3H, s), 0.92 (3H, s), 1.07 (3H, s), 1.16 (3H, d, $J$=7.8 Hz), 1.40 (1H, m), 1.60~1.90 (5H, m), 1.98 (1H, m), 2.06 (1H, m), 2.10 (3H, s), 2.16 (1H, m), 2.30 (1H, d, $J$=18.3 Hz), 2.40 (1H, m), 3.28 (1H, d, $J$=18.3 Hz), 4.25 (1H, d, $J$=17.6 Hz), 4.28 (1H, d, $J$=17.6 Hz), 4.78 (1H, t-like), 6.00 (1H, s), 6.36 (1H, br.s); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 17.08, 20.09, 21.48, 23.03, 23.48, 24.48, 26.32, 27.82, 29.99, 32.63, 37.24, 37.66, 39.38, 42.01, 44.84, 76.23, 84.23, 94.84, 113.61, 124.84, 127.38, 146.80, 150.83, 169.74, 170.40.

Compound **7**

*N*-Chlorosuccinimide (12.3 mg) was added to an ice-cooled solution of **1** (22 mg, 0.051 mmol) in THF (4 ml), and the mixture was stirred for 2 hours. The reaction mixture was then stirred at room temperature for another 3 hours. Next, 10% aqueous sodium hydrogen sulfite (2 ml) was added to the reaction mixture, which was then stirred for 10 minutes and partitioned between EtOAc and aqueous layers. The EtOAc layer was washed with water, dried over anhydrous sodium sulfate, and the solvent was evaporated to give a crude product. The crude product was purified by preparative TLC (*n*-hexane-acetone=1 : 1) to give **7** (7.6 mg, 32%) and the starting compound **1** (14 mg, 64%); HR FAB-MS (*m/z*) 462.2049 $(M+H)^+$ Calcd for $C_{25}H_{33}NO_5Cl$: 462.2047; IR $\nu_{max}$ KBr $cm^{-1}$: 3350, 2957, 1773, 1700, 1478, 1362; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.87 (3H, s), 0.92 (3H, s), 1.07 (3H, s), 1.16 (3H, d, $J$=7.8 Hz), 1.40 (1H, m), 1.60~1.90 (5H, m), 1.95~2.20 (3H, m), 2.10 (3H, s), 2.29 (1H, d, $J$=18.0 Hz), 2.40 (1H, m), 3.27 (1H, d, $J$=18.0 Hz), 4.29 (2H, br.s), 4.78 (1H, t-like), 5.96 (1H, br.s), 6.63 (1H, s); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 17.08, 20.09, 21.48, 23.03, 23.48, 24.48, 26.32, 27.82, 29.99, 32.42, 37.24, 37.59, 39.38, 42.37, 44.83, 76.24, 84.18, 105.99, 113.69, 124.20, 126.13, 146.01, 150.00, 169.69, 170.42.

Compound **8**

Preparation of compound **8** was described in our previous paper[1]. HR FAB-MS (*m/z*) 518.2906 $(M+H)^+$ Calcd for $C_{32}H_{40}NO_5$: 518.2906; IR $\nu_{max}$ KBr $cm^{-1}$: 3421, 3246, 2961, 2874, 1735, 1697, 1625, 1601, 1467, 1450, 1371; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.86 (3H, s), 0.92 (3H, s), 1.09 (3H, s), 1.15 (3H, d, $J$=7.5 Hz), 1.37 (1H, m), 1.60 (2H, m), 1.79 (3H, m), 2.00 (2H, m), 2.10 (3H, s), 2.14 (1H, m), 2.30 (1H, d, $J$=18.3 Hz), 2.43 (1H, m), 3.20 (1H, d, $J$=18.3 Hz), 4.32 (1H, d, $J$=16.5 Hz), 4.36 (1H, d,

$J$=16.5 Hz), 4.78 (1H, t-like), 5.11 (2H, s), 6.32 (1H, br.s), 6.99 (1H, s), 7.30~7.47 (5H, m); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 17.23, 20.27, 21.60, 23.19, 23.56, 24.58, 26.43, 27.98, 30.10, 32.33, 37.30, 37.70, 39.41, 43.53, 44.72, 70.32, 76.42, 83.44, 96.93, 114.48, 123.39, 127.35(×2), 127.87, 128.45(×2), 130.99, 136.76, 147.12, 157.45, 170.27, 171.93.

Compound **10**

Jones reagent (2 mol eqiuiv) was added to a solution of **9** (47 mg, 0.1 mmol) in acetone (4 ml), and the mixture was stirred at 0°C for 1.5 hours. Three drops of 2-PrOH were added to the reaction mixture, and the solvent was evaporated under reduced pressure. The residue was then partitioned between EtOAc (5 ml) and water (2 ml). The EtOAc layer was washed with water and dried over anhydrous sodium sulfate, and the solvent was evaporated to give a crude product. This crude product was purified by preparative TLC ($CH_2Cl_2$-MeOH=10:1) to give **10** (42 mg, 89%); HR FAB-MS ($m/z$) 474.2649 $(M+H)^+$ Calcd for $C_{30}H_{36}NO_4$: 474.2644; IR $\nu_{max}$ KBr cm$^{-1}$: 3421, 3251, 2962, 2925, 2873, 1706, 1625, 1602, 1467, 1452, 1384; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.96 (3H, s), 0.99 (3H, s), 1.12 (3H, d, $J$=7.4 Hz), 1.19 (3H, s), 1.43 (2H, m), 1.86 (2H, m), 2.00 (1H, m), 2.10 (1H, m), 2.26 (2H, m), 2.36 (1H, d, $J$=18.6 Hz), 2.48 (1H, m), 3.04 (1H, m), 3.14 (1H, d, $J$=18.6 Hz), 4.33 (1H, d, $J$=16.8 Hz), 4.37 (1H, d, $J$=16.8 Hz), 5.12 (2H, s), 6.63 (1H, s), 7.03 (1H, s), 7.32~7.48 (5H, m); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 17.18, 20.37, 23.94, 24.16, 27.63, 29.06, 30.20, 32.59, 33.43, 37.33, 38.70, 43.56, 47.39, 48.56, 70.26, 82.19, 97.28, 114.07, 123.53, 127.27(×2), 127.81, 128.35(×2), 131.47, 136.54, 146.67, 157.40, 172.10, 215.87.

Compound **11**

A solution of **10** (13 mg) in EtOAc (1 ml) was treated with 10% palladium-charcoal (14 mg) under a hydrogen atmosphere for 3 hours. After the catalyst was filtered off, the filtrate was concentrated under reduced pressure to give **11** (8.8 mg, 84%); HR FAB-MS ($m/z$) 384.2171 $(M+H)^+$ Calcd for $C_{23}H_{30}NO_4$: 384.2175; IR $\nu_{max}$ KBr cm$^{-1}$: 3396, 2966, 2928, 2875, 1692, 1628, 1611, 1465, 1385; $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 0.88 (3H, s), 0.90 (3H, s), 1.02 (3H, s), 1.09 (3H, d, $J$=7.4 Hz), 1.40 (2H, m), 1.78 (2H, m), 1.95 (1H, m), 2.10 (3H, m), 2.18 (1H, d, $J$=18.0 Hz), 2.35 (1H, m), 2.85 (1H, m), 3.04 (1H, d, $J$=18.0 Hz), 4.04 (1H, d, $J$=17.4 Hz), 4.16 (1H, d, $J$=17.4 Hz), 6.66 (1H, s), 8.34 (1H, s), 9.82 (1H, s); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ 16.83, 20.02, 23.27, 23.82, 27.18, 28.45, 29.01, 32.13, 32.83, 36.80, 38.27, 42.33, 46.14, 47.52, 81.57, 99.58, 111.83, 120.88, 131.68, 146.61, 155.93, 170.18, 214.93.

Compound **13**

2,3-Dichloro-5,6-dicyanobenzoquinone (90 mg) was added to a solution of **8** (50 mg, 0.097 mmol) in water-saturated $CH_2Cl_2$ (2 ml), and the mixture was allowed to stand for 5 days at room temperature. The insoluble materials in the reaction were filtered off. The filtrate was concentrated and purified by preparative TLC ($CH_2Cl_2$-MeOH=95:5) to give an oxidation product (17 mg) as a pale yellow powder. A solution of the oxidation product in EtOH (1.5 ml) was treated with 10% palladium-charcoal (8 mg) under a hydrogen atmosphere for 3 hours. After the catalyst was filtered off, the filtrate was concentrated under reduced pressure to give **13** (8 mg, 18%); HR FAB-MS ($m/z$) 442.2235 $(M+H)^+$ Calcd for $C_{25}H_{32}NO_6$: 442.2230; IR $\nu_{max}$ KBr cm$^{-1}$: 3411, 3315, 2961, 1756, 1715, 1610, 1455, 1372; $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 0.80 (3H, s), 0.85 (3H, s), 1.04 (3H, s), 1.11 (3H, d, $J$=7.5 Hz), 1.32 (1H, m), 1.56 (2H, m), 1.73 (3H, m), 1.90~2.12 (3H, m), 2.05 (3H, s), 2.13 (1H, d, $J$=18.0 Hz), 2.65 (1H, m), 3.05 (1H, d, $J$=18.0 Hz), 4.62 (1H, s), 6.70 (1H, s), 10.64 (1H, br.s); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ 16.73, 19.73, 21.03, 22.60, 22.96, 23.85, 26.13, 27.22, 28.94, 31.49, 36.62, 36.77, 38.69, 44.27, 75.44, 83.57, 101.26, 107.26, 114.11, 133.39, 149.96, 162.05, 167.90, 169.03, 169.62.

Compound **14**

To a solution of **10** (16 mg) in MeOH (1.5 ml) was added $NaBH_4$ (2.3 mg), and the mixture was stirred at 0°C for 1.5 hours. Water (0.5 ml) was added to the reaction mixture, which was stirred for 0.5 hour and evaporated under reduced pressure. The residue was partitioned between EtOAc (4 ml) and water (2 ml). The EtOAc layer was washed with water and dried over anhydrous sodium sulfate, then the solvent was evaporated to give a crude product. This crude product was purified by preparative TLC ($CH_2Cl_2$-MeOH=10:1) to give **9** (6 mg, 37%) and **14** (10 mg, 62%); HR FAB-MS ($m/z$) 476.2793 $(M+H)^+$ Calcd for $C_{30}H_{38}NO_4$: 476.2801; IR $\nu_{max}$ KBr cm$^{-1}$: 3345, 2960, 2873, 1687, 1670, 1625, 1604, 1452, 1367; $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 0.81 (3H, s), 0.85 (3H, s), 0.89 (3H, s), 1.07 (3H, d, $J$=7.5 Hz), 1.29 (1H, d, $J$=12.6 Hz), 1.57 (3H, m), 1.74 (3H, m), 2.00 (3H, m), 2.18 (1H, d, $J$=18.3 Hz), 3.10 (1H, d, $J$=18.3 Hz), 3.53 (1H, dd, $J$=11.3, 4.6 Hz), 4.15 (1H, d, $J$=17.4 Hz), 4.25 (1H, d, $J$=17.4 Hz), 4.28 (1H, br.s), 5.17 (2H, s), 6.82 (1H, s), 7.33~7.49 (5H, m), 8.41 (1H, br.s); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ 16.83, 19.86, 21.79, 23.28, 27.04, 27.27, 28.08, 28.91, 32.12, 36.76, 37.93, 38.62, 42.47, 46.16, 69.33,

71.13, 82.64, 96.36, 112.99, 122.78, 127.10(×2), 127.45, 128.16(×2), 131.50, 136.85, 146.86, 156.56, 169.85.

Compound **15**

A solution of **14** (10 mg) in EtOH (1 ml) was treated with 10% palladium-charcoal (10 mg) under a hydrogen atmosphere for 3 hours. After the catalyst was filtered off, the filtrate was concentrated under reduced pressure to give **15** (7.2 mg, 89%); HR FAB-MS (*m/z*) 386.2332 $(M+H)^+$ Calcd for $C_{23}H_{32}NO_4$: 386.2331; IR $\nu_{max}$ KBr $cm^{-1}$: 3422, 2960, 2874, 1684, 1622, 1463, 1365; $^1H$ NMR (DMSO-$d_6$, 300 MHz) $\delta$ 0.79 (3H, s), 0.85 (3H, s), 0.89 (3H, s), 1.07 (3H, d, $J$=7.2 Hz), 1.30 (1H, m), 1.56 (3H, m), 1.73 (3H, m), 1.98 (3H, m), 2.09 (1H, d, $J$=17.7 Hz), 3.00 (1H, d, $J$=17.7 Hz), 3.53 (1H, m), 4.09 (1H, d, $J$=16.8 Hz), 4.16 (1H, d, $J$=16.8 Hz), 6.58 (1H, s), 8.22 (1H, br.s); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz) $\delta$ 16.89, 19.88, 21.80, 23.29, 27.10, 27.33, 28.16, 28.95, 32.22, 36.84, 37.97, 42.46, 46.22, 71.25, 82.45, 99.03, 111.82, 120.60, 131.51, 147.15, 155.91, 170.32.

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