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## FIRST SYNTHESIS OF RACEMIC CONCENTRICOLIDE, AN ANTI-HIV-1 AGENT ISOLATED FROM THE FUNGUS *DALDINIA CONCENTRICA*

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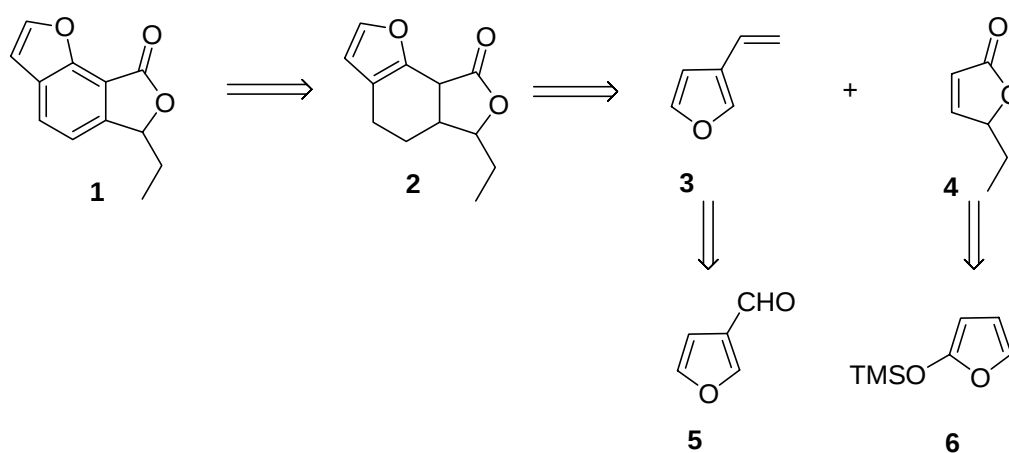
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**Abstract** – Concentricolide, a novel compound with anti-HIV-1 activity and isolated from ascomycete *Daldinia concentrica*, has been synthesised for the first time as a racemate from furan-3-carbaldehyde and dihydrofuran-2(3*H*)-one via a Diels-Alder reaction. The identity of the synthetic sample was verified by comparison of its spectral data with those of an authentic sample.

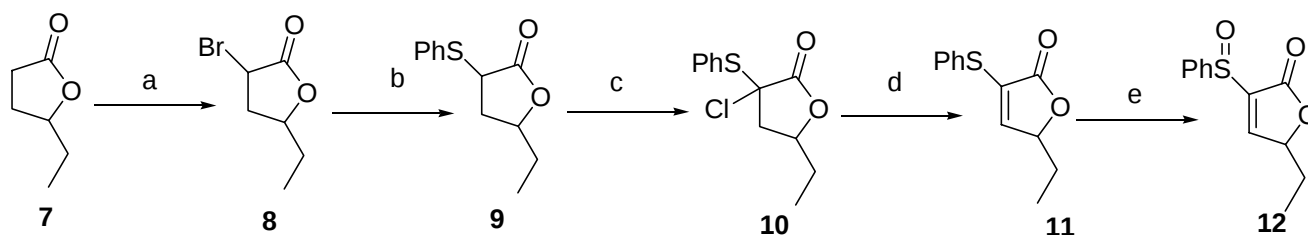
Concentricolide (**1**) is a new natural product isolated by us from ascomycete *Daldinia concentrica* in 2005, and found to exhibit significant anti-HIV-1 activity.<sup>1</sup> It inhibited HIV-1-induced cytopathic effects with an  $EC_{50}$  value of 0.31  $\mu\text{g/mL}$ . The therapeutic index (TI) of this product was 247. Concentricolide is also effective in the blockage ( $EC_{50}$  0.83  $\mu\text{g/mL}$ ) of syncytium formation between HIV-1 infected cells and normal cells. In the following investigation, several new compounds with this type of structure were isolated from the culture broth of *D. concentrica*.<sup>2,3</sup> Because these compounds might show promise as antiviral agents<sup>3</sup> and anti-AIDS agents, synthetic studies are needed. Herein, we describe the first synthesis of ( $\pm$ )-concentricolide (**1**).

Our synthetic strategy is presented in Scheme 1. ( $\pm$ )-Concentricolide (**1**) could be obtained via a Diels-Alder reaction with known precursors 3-vinylfuran (**3**) and 4-ethylbut-2-en-4-olide (**4**), which are derived from commercially available 3-carbaldehyde (**5**) and 2-(trimethylsiloxy)furan (**6**), respectively. 3-Vinylfuran (**3**) was obtained as a mixture via a Grignard reaction that occurred under refluxing with Mg turnings and  $\text{TMSCH}_2\text{Cl}$  in dry  $\text{Et}_2\text{O}$ , under nitrogen. This was followed by vigorous stirring with aqueous 1.0 M HCl until all traces of **5** disappeared, as observed by TLC (AcOEt: hexane 1:5). This

mixture was kept at  $-20\text{ }^{\circ}\text{C}$ , protected from light, and used directly in the Diels-Alder reaction. 2-Trimethylsiloxyfuran (**2**) was alkylated with iodoethane in the presence of a molar excess of silver trifluoroacetate to give the 4-ethylbut-2-en-4-olide (**4**) in 60% yield. Unfortunately, the reaction between synthons **3** and **4** did not give the anticipated Diels-Alder product. Instead, a complex mixture of compounds resulted despite repeated trials, and with added Lewis acid catalyst  $\text{BF}_3\cdot\text{Et}_2\text{O}$ . This outcome indicated that the two synthons were mismatched, and the reactivity of synthon **4** with synthon **3** must be improved to give the Diels-Alder product. Addition of an  $\alpha$ -sulfinyl group to the electron-withdrawing group of the dienophile can be used to increase reactivity in normal Diels-Alder reactions.<sup>4</sup> The desired adduct was formed by the addition of the dienophile to the furan 2,3-double bond 3-vinyl group diene system.<sup>5</sup> An added advantage of this activating group was observed: it readily underwent elimination after the cycloaddition, and introduced the double bond. We thus altered synthons **4** to **12** in this way, as shown in the synthetic pathway depicted in Scheme 2.

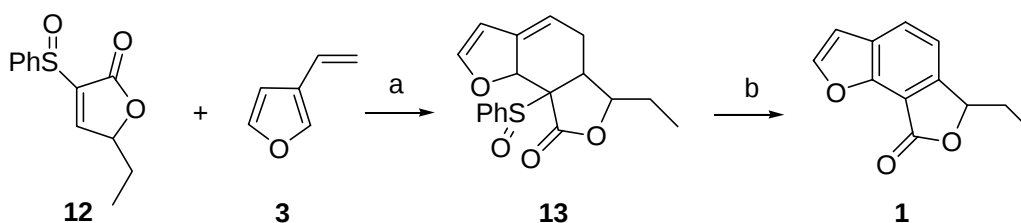


Scheme 1. Retrosynthetic Analysis of Concentricolide (**1**).



Scheme 2. Synthesis of **12**. *Reagents and conditions*: a)  $\text{PBr}_3/\text{Br}_2$ ,  $70\text{--}90\text{ }^{\circ}\text{C}$  (57%). b)  $\text{PhSH}$ ,  $\text{Et}_3\text{N}$ ,  $\text{Et}_2\text{O}$  (98%). c)  $\text{NCS}$ ,  $\text{CCl}_4$ , reflux (82%). d)  $\text{Li}_2\text{CO}_3$ ,  $\text{LiBr}$ ,  $\text{THF}$ , reflux (35%). e)  $m\text{-CPBA}$ ,  $\text{DCM}$ ,  $0\text{ }^{\circ}\text{C}$  (82%).

Preparation of 5-ethyl-3-(phenylsulfinyl)furan-2(5*H*)-one (**12**) began with inexpensive dihydrofuran-2(3*H*)-one (**7**) using a modification of the method of outlined by Sweeney and Smith.<sup>7,8</sup> Bromination of dihydrofuran-2(3*H*)-one gave 3-bromo-5-ethyl-dihydrofuran-2(3*H*)-one (**8**), which underwent nucleophilic attack by thiophenol to offer 5-ethyl-3-(phenylthio)-dihydrofuran-2(3*H*)-one (**9**) in excellent yield.  $\alpha$ -Chlorination of **9** with *N*-chlorosuccinimide (NCS) provided 3-chloro-5-ethyl-3-phenylthio-dihydrofuran-2(3*H*)-one (**10**) in 82% yield, and this compound underwent conjugative elimination of HCl upon reaction with a mixture of lithium bromide and lithium carbonate in refluxing THF to give 5-ethyl-3-(phenylthio)furan-2(5*H*)-one (**11**) in 35% yield. The corresponding sulphoxide was prepared via *m*-CPBA oxidation of **11** in 82% yield.



Scheme 3. Synthesis of (±)-concentricolide (**1**). *Reagents and conditions*: a) hydroquinone (0.1 equiv), rt, toluene, 72 h. b) CaCO<sub>3</sub>, toluene, reflux, 19 h.

The Diels-Alder reaction was carried out in the presence of 10.67 mg of hydroquinone to prevent the formation of a complex mixture.<sup>9</sup> Treatment of a solution of **3** in dry toluene at reflux with three equivalents of **12** in for 72 h afforded **13**, which was immediately stirred with calcium carbonate in anhydrous toluene without isolation for 19 h at 90 °C, providing the precursor by elimination of PhSOH. The precursor mixtures were swiftly converted into the concentricolide by oxidation, which occurred during purification by column chromatography, affording the target product **1**. In our experiment, only about 7.6 mg of concentricolide was obtained from the 91.0 mg (0.97 mmol) of **3** and 677.6 mg (2.87 mmol) of **12**. It was noted that the yield was low and the further improvements are the subject of ongoing research.

## EXPERIMENTAL

### General Experimental Procedures

Optical rotation was measured using a Horiba SEPA-300 polarimeter. IR spectra were obtained on a Bruker Tensor 27 with KBr pellets. NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 spectrometers. EI-MS was recorded with a VG Autospec-3000 spectrometer and HR-ESI-MS was recorded with an API QSTAR Pulsar 1 spectrometer. Silica gel (200-300 mesh, Qingdao Marine

Chemical Inc., China) was used for column chromatography. Fractions were monitored by TLC and spots were visualised by heating silica gel plates sprayed with 10% H<sub>2</sub>SO<sub>4</sub> in ethanol. All commercial agents were produced by Sigma-Aldrich.

**Synthesis of 3-Vinylfuran (3):** Chlorotrimethylsilane (1.02 g, 8.28 mmol) was added dropwise to magnesium turnings (0.24 g, 9.9 mmol) in dry Et<sub>2</sub>O (5 mL) under nitrogen, and the mixture was refluxed (45 °C) for 9 h, then cooled to 0 °C and treated with a solution of furan-3-carbaldehyde (0.69 g, 7.2 mmol) in dry Et<sub>2</sub>O (7 mL). After stirring for 4 h at 0 °C and then 14 h at 23 °C, the reaction mixture was quenched with sat. aq. NH<sub>4</sub>Cl (5 mL) at 0 °C. The layers were separated, the aqueous layer was extracted with Et<sub>2</sub>O (3 × 5 mL), and the combined organic layers were washed with sat. aq. NaHCO<sub>3</sub> (2 × 5 mL), brine (10 mL), and dried over Na<sub>2</sub>SO<sub>4</sub>. Following solvent evaporation *in vacuo*, the residue was dissolved in Et<sub>2</sub>O (5 mL) and stirred vigorously with 1.0 M HCl (3.4 mL) for 80 min. until it was not visible by TLC (AcOEt: hexane =1:5). The layers were separated, the aqueous layer was extracted with Et<sub>2</sub>O (2 × 5 mL), and the combined organic layers were washed with sat. aq. NaHCO<sub>3</sub> (2 × 5 mL) and brine (5 mL), and dried over Na<sub>2</sub>SO<sub>4</sub>. The solution was carefully concentrated *in vacuo* to ca. 5 mL with no heating to give the product (676.9 mg, ca. 50%) as a mixture with TMSOH and Et<sub>2</sub>O. This mixture was kept at -20 °C, protected from light and was used immediately. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.10 (br s, 1H), 7.04 (br s, 1H), 6.23 (br s, 2H), 5.13 (dd, 1H, *J* = 16 Hz, 7.5 Hz), 4.78 (dd, 1H, *J* = 7.5 Hz); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 142.78 (CH), 139.90 (CH), 126.07 (CH), 124.34 (C), 112.23 (CH<sub>2</sub>), 106.50 (CH).

**Synthesis of 3-bromo-5-ethyldihydrofuran-2(3H)-one (8):** PBr<sub>3</sub> (1.44 g, 5.31 mmol) was combined with dihydrofuran-2(3H)-one (6.28 g, 55.09 mmol) in an ice bath, stirred for 20 min, treated with Br<sub>2</sub> (8.80 g, 55.09 mmol), and stirred for an additional 30 min at rt. The mixture was heated at 90 °C, and Br<sub>2</sub> (8.80 g, 55.00 mmol) was added. After stirring for 4 h, the mixture was poured into ice water (250 mL), and washed with sat. aq. NaHCO<sub>3</sub> until the red-brown colour disappeared. The layers were separated, the aqueous layer was extracted with Et<sub>2</sub>O (3 × 20 mL), and the combined organic layers were washed with sat. aq. NaHCO<sub>3</sub> (2 × 5 mL), brine (5 mL), and dried over Na<sub>2</sub>SO<sub>4</sub>. The solution was concentrated *in vacuo*, and the crude product was purified by silica gel column chromatography using petroleum ether-AcOEt (20:1) to afford the product as an oil (6.06 g, 57%). IR  $\nu_{\text{max}}$  (KBr) cm<sup>-1</sup>: 2971, 2939, 1782, 1185, 965, 946, 669. FAB-MS *m/z* (%): 193 (3), 109 (20), 95 (35), 81 (51), 69 (69), 55 (100). HR-ESI-MS *m/z*: C<sub>6</sub>H<sub>9</sub>O<sub>2</sub>NaBr, [M+Na]<sup>+</sup> (found: 214.9694, calcd.: 214.9683). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 4.69 (m, 1H), 4.46 (dd, 1H, *J* = 6.7 Hz, 1.5 Hz), 2.55 (m, 1H), 2.41 (m, 1H), 1.78 (m, 1H), 1.04 (m, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 172.48 (C), 80.86 (CH), 39.01 (CH), 38.84 (CH<sub>2</sub>), 27.15 (CH<sub>2</sub>),

9.16 (CH<sub>3</sub>).

*Synthesis of 5-ethyl-3-(phenylthio)dihydrofuran-2(3H)-one (9):* PhSH (3.93 g, 35.73 mmol) and Et<sub>3</sub>N (3.61 g, 35.73 mmol) were added to a solution of 3-bromo-5-ethyldihydrofuran-2(3H)-one (6.86 g, 35.73 mmol) in dry THF (50 mL) at 0 °C over 30 min. The mixture was heated to reflux and stirred for 10 h, then partitioned between water (100 mL) and Et<sub>2</sub>O (90 mL). The layers were separated, and the aqueous phase was extracted with Et<sub>2</sub>O (90 mL). The combined organic layers were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> to give **9** as yellow oil (7.79g, 98%). IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 2970, 2938, 1772, 1480, 1462, 1440, 1189, 948, 746, 692. FAB MS  $m/z$  (%): 223 (100), 177 (24), 149 (5), 113 (7), 57 (9). HR-ESI-MS  $m/z$ : C<sub>12</sub>H<sub>15</sub>O<sub>2</sub>S, [M+H]<sup>+</sup>, (found: 223.0790, calcd.: 223.0792). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.50 (m, 2H), 7.29 (m, 3H), 4.30 (m, 1H), 3.98-3.86 (m, 1H), 2.27 (m, 1H), 1.84-1.50 (m, 3H), 0.91 (m, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  174.61 (C), 133.00 (CH), 131.72 (C), 128.94 (CH), 80.26 (CH), 44.90 (CH), 35.06 (CH<sub>2</sub>), 27.96 (CH<sub>2</sub>), 9.14 (CH<sub>3</sub>).

*Synthesis of 3-chloro-5-ethyl-3-phenylthiodihydrofuran-2(3H)-one (10):* To a solution of 5-ethyl-3-(phenylthio)dihydrofuran-2(3H)-one (10.21 g, 45.99 mmol) in CCl<sub>4</sub> (90 mL) under nitrogen was added *N*-chlorosuccinimide (6.16 g, 46.14 mmol). After refluxing for 8 h, water (50 mL) was poured into the reaction mixture, the layers were separated, and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic layers were washed with sat. aq. NaHCO<sub>3</sub> (2 × 50 mL), brine (50 mL), and water (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo* to give the crude product. The product was further purified by silica gel column chromatography using petroleum ether-AcOEt (20:1) to afford **10** as a golden oil (9.75 g, 82%). IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 2972, 2938, 1775, 1440, 1350, 1185, 1024, 956, 751, 691. FAB-MS  $m/z$  (%): 257 (25), 221 (100), 95 (35), 175 (22), 69 (17), 57 (25). HR-ESI-MS  $m/z$ : C<sub>12</sub>H<sub>14</sub>O<sub>2</sub>SCl, [M+H]<sup>+</sup> (found: 257.0405, calcd.: 214.0403) <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.71-7.34 (m, 5H), 4.58 (m, 1H), 2.90-2.26 (m, 2H), 1.88-1.60 (m, 2H), 1.05-0.85 (m, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  168.73 (C), 136.64 (CH), 143.84 (CH), 133.82 (CH), 132.85 (C), 129.10 (CH), 83.56 (CH), 46.34 (CH<sub>2</sub>), 27.68 (CH<sub>2</sub>), 9.20 (CH<sub>3</sub>).

*Synthesis of 5-ethyl-3-(phenylthio)furan-2(5H)-one (11):* Lithium bromide (354.8 mg, 4.08 mmol) and lithium carbonate (1.50 g, 20.27 mmol) were dried for 3 h at 90 °C under reduced pressure, and added to a solution of 3-chloro-5-ethyl-3-phenylthiodihydrofuran-2(3H)-one (5.23 g, 20.35 mmol) in anhydrous THF (40 mL). The mixture was heated to the point of reflux for 30 min until it became orange-brown in colour. The inorganic salts were filtered off by passage through Celite, and the organic solution was

washed with sat. aq.  $\text{NaHCO}_3$  (50 mL), brine (50 mL), water (50 mL), and dried over  $\text{Na}_2\text{SO}_4$ . The solution was concentrated *in vacuo* and the residue was purified via silica gel column chromatography using petroleum ether-AcOEt (15:1) to afford **11** as a yellow oil (1.59 g, 35%). IR  $\nu_{\text{max}}$  (KBr)  $\text{cm}^{-1}$ : 2972, 2938, 1775, 1440, 1350, 1185, 1024, 956, 751, 691. FAB-MS  $m/z$  (%): 221 (100), 203 (28), 175 (22), 175 (22), 111 (15), 97 (22), 83 (30), 69 (37), 55 (70). EI-MS  $m/z$  (%): 220 (76), 191 (29), 164 (62), 135 (100), 111 (18), 91 (33), 77(10). HR-ESI-MS  $m/z$ :  $\text{C}_{12}\text{H}_{13}\text{O}_2\text{S}$ ,  $[\text{M}+\text{H}]^+$  (found: 221.0644, calcd.: 221.0636).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.54 (m, 2H), 7.42 (m, 3H), 6.55 (d, 1H,  $J = 1.8$  Hz), 1.83-1.61 (m, 2H), 0.95 (t, 3H,  $J = 7.5$  Hz).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.55 (C), 144.26 (CH), 133.26 (CH), 131.91 (C), 129.47 (CH), 128.94 (CH), 83.30 (CH), 26.22 ( $\text{CH}_2$ ), 8.54 ( $\text{CH}_3$ ).

**Synthesis of 5-ethyl-3-(phenylsulfinyl)furan-2(5H)-one (12):** A solution of 3-chloro-5-ethyl-3-phenylthiodihydrofuran-2(3H)-one (0.698 g, 3.17 mmol) in  $\text{CH}_2\text{Cl}_2$  (25 mL) was added to a solution of 86% *m*-chloroperoxybenzoic acid (*m*-CPBA) (643.6 mg, 3.17 mmol) in  $\text{CH}_2\text{Cl}_2$  (9 mL), and the reaction mixture was stirred for 1 h at  $-10^\circ\text{C}$ . Additional  $\text{CH}_2\text{Cl}_2$  (25 mL) and 10% aqueous  $\text{NaHSO}_3$  (15 mL) were added, and the layers were separated. The organic phase was washed with 10% aqueous  $\text{NaHCO}_3$ , 10% brine, and dried over  $\text{Na}_2\text{SO}_4$ . The solution was concentrated *in vacuo*, and the residue was purified via silica gel column chromatography using petroleum ether-AcOEt (15:1) to afford **12** as a yellow oil (614.6 mg, 82% yield). HR-ESI-MS  $m/z$ :  $\text{C}_{12}\text{H}_{12}\text{O}_3\text{NaS}$ ,  $[\text{M}+\text{Na}]^+$  (found: 259.0394, calcd.: 259.0404). EI-MS  $m/z$  (%): 236 (15), 188 (15), 125 (96), 111 (60), 103 (33), 97 (42), 77 (100), 65 (38), 57 (55);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.83 (m, 1H), 7.63 (m, 2H), 7.33 (m, 3H,  $J = 1.8$  Hz), 4.99-4.80 (t, 1H,  $J = 6.1$  Hz), 1.75-1.45 (m, 2H), 0.83-0.66 (t, 3H,  $J = 7.4$  Hz).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  165.87 (C), 154.46 (CH), 140.75 (C), 131.60 (CH), 128.91 (CH), 83.20 (CH), 25.37 ( $\text{CH}_2$ ), 8.11 ( $\text{CH}_3$ ).

**Synthesis of Concentricolide (1):** 5-Ethyl-3-(phenylsulfinyl)furan-2(5H)-one (677.6 mg, 2.87 mmol) and hydroquinone (10.67 mg, 0.097 mmol) were added to a solution of 3-vinylfuran (91.0 mg, 0.97 mmol) in dry toluene (1.5 mL) and stirred for 72 h at rt.  $\text{CaCO}_3$  (9.7 mg, 0.97 mmol) was added, and the reaction mixture was heated to  $90^\circ\text{C}$  with stirring for 15 h. The solvent was evaporated *in vacuo*, and the residue was purified via silica gel column chromatography using petroleum ether-AcOEt (10:1) before the product was crystallised (7.6 mg, 3.8%). HR-ESI-MS  $m/z$ : 225.0522,  $[\text{M}+\text{Na}]^+$ , 225.0523 calcd. for  $\text{C}_{12}\text{H}_{10}\text{O}_3\text{Na}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.92 (dd, 1H,  $J = 8.0, 3.6$  Hz), 7.80 (d, 1H,  $J = 2.1$  Hz), 6.91 (d, 1H,  $J = 2.1$  Hz), 5.58 (dd, 1H,  $J = 7.0$  Hz, 4.1 Hz), 2.19 (m, 1H), 1.87 (m, 1H), 1.01 (t, 3H,  $J = 7.4$  Hz).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.14 (C), 146.58 (CH), 127.88 (C), 116.04 (CH), 106.64 (CH),

82.97 (CH), 27.89 (CH<sub>2</sub>), 8.81 (CH<sub>3</sub>). These data were in agreement with those reported for an authentic sample.<sup>1</sup>

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## REFERENCES

1. (a) X. D. Qin, Z. J. Dong, J. K. Liu, L. M. Yang, R. R. Wang, T. T. Zheng, Y. Lu, Y. S. Wu, and Q. T. Zheng, *Helv. Chim. Acta*, 2006, **89**, 127; (b) J. K. Liu, Y. T. Zheng, X. D. Qin, L. M. Yang, Z. J. Dong, and R. R. Wang, WO2005/037841.
2. H. J. Shao, X. D. Qin, Z. J. Dong, H. B. Zhang, and J. K. Liu, *J. Antibiot.*, 2008, **61**, 115.
3. X. D. Qin, H. J. Shao, Z. J. Dong, and J. K. Liu, *J. Antibiot.*, 2008, **61**, 556.
4. M. Fardis, M. Mertzman, W. Thomas, T. Kirschberg, N. Collins, R. Polniaszek, and W. J. Watkins, *J. Org. Chem.*, 2006, **71**, 4835.
5. G. J. L. Ruano, M. T. Peromingo, M. Alonso, A. Fraile, M. R. Martin, and A. Tito, *J. Org. Chem.*, 2005, **70**, 8942.
6. A. Benítez, F. R. Herrera, M. Romero, and F. X. Tamás, *J. Org. Chem.*, 1996, **61**, 1487.
7. G. J. Hollingworth, G. Perkins, and J. Sweeney, *J. Chem. Soc., Perkin Trans. 1*, 1996, 1913.
8. J. A. Kaydos and D. L. Smith, *J. Org. Chem.*, 1983, **48**, 1096.
9. T. Sasaki, Y. Ishibashi, and M. Ohno, *Heterocycles*, 1983, **20**, 1933.