Biosci. Biotechnol. Biochem., **70** (1), 303–306, 2006

Note

Synthesis of Quinolactacide *via* an Acyl Migration Reaction and Dehydrogenation with Manganese Dioxide, and Its Insecticidal Activities

Masaki ABE,† Tetsuya IMAI, Naoki ISHII, and Makio USUI

Naruto Research Center, Otsuka Chemical Co., Ltd., 615 Hanamen, Satoura-cho, Naruto, Tokushima 772-8601, Japan

Received September 8, 2005; Accepted October 6, 2005

Quinolactacide isolated from *Penicillium citrinum* F 1539 was synthesized and evaluated for its insecticidal activities. The key steps of the total synthesis were an acyl migration reaction of the enol ester intermediate and dehydrogenation of tetrahydroquinolactacide with manganese dioxide. The synthesized quinolactacide showed 100% and 42% mortality against the green peach aphid (*Myzus persicae*) and diamondback moth (*Plutella xylostella*) at 500 ppm, respectively.

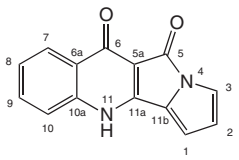
Key words: quinolactacide; insecticidal activity; acyl migration reaction; dehydrogenation

In our previous study,[1] we have reported the isolation of a new insecticidal quinolone, quinolactacide (**1**), from *Penicillium citrinum* Thom F 1539 and determined its chemical structure (Fig. 1). Quinolactacide (**1**) showed 88% mortality against the green peach aphid (*Myzus persicae*) at 250 ppm. However, we could not conduct further biological tests due to the limited amount of the isolated compound. We therefore started a synthetic study on quinolactacide (**1**) to obtain a sufficient amount of the compound.

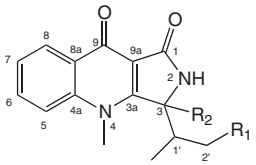
Quinolactacide (**1**) is structurally related to quinolactacins (**2**–**6**) isolated from *Penicillium* sp., which are known to show some biological activities.[2–5] The synthesis of racemic quinolactacin B and enantioselective syntheses of quinolactacins A and B have also been reported,[6,7] but there was no description on their insecticidal activity in the reports.

We report here the first total synthesis of quinolactacide (**1**) and its insecticidal activities.

The synthetic route used for quinolactacide (**1**) is shown in Scheme 1. Proline ethyl ester **8** was prepared by esterification of L-proline (**7**) in ethanol with thionyl chloride. The amino group of **8** was acetylated with acetyl chloride and pyridine in tetrahydrofuran (THF) to give **9**. The cyclopenta-1,3-dione ring was successfully constructed by Dieckmann-type cyclization to give **10** in a 59% yield.[8] Esterification of 1,3-dione **10** with triethylamine (TEA) and 2-nitrobenzoyl chloride afforded enol ester **11**.[9] Acyl migration of **11** catalyzed with acetone cyanohydrin proceeded quantitatively to give trione **12**.[9] Hydrogenation of the nitro group of trione **12** resulted in spontaneous cyclization to give tetrahydroquinolactacide **13** in a 52% yield. Compound **13** showed poor solubility in almost all organic solvents, including dimethyl sulfoxide (DMSO), so **13** was suspended in a mixture of chloroform and *N*,*N*-dimethylformamide (DMF) and dehydrogenated with manganese dioxide (MnO_2) to give quinolactacide (**1**) in a 21% yield.[10] Starting compound **13** was not found in the reaction mixture. In an attempt to improve the yield of the dehydrogenation reaction, the reaction was conducted



Quinolactacide (**1**)

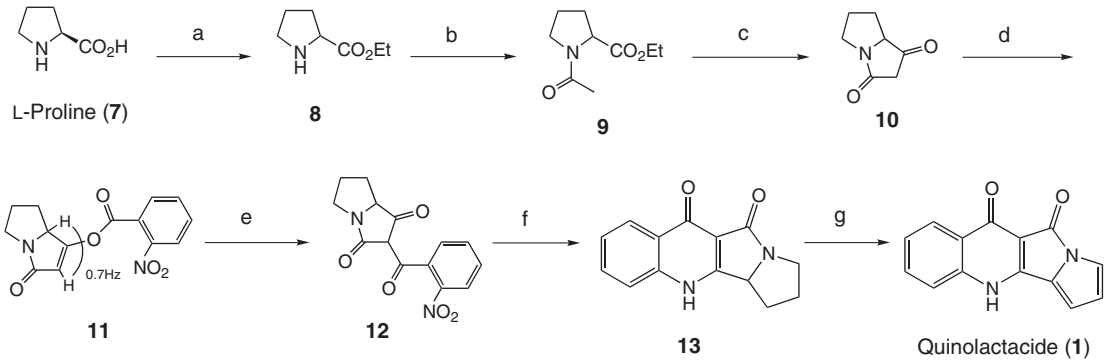


Quinolactacin A (**2**) : $R_1 = CH_3$, $R_2 = H$
Quinolactacin B (**3**) : $R_1 = R_2 = H$
Quinolactacin C (**4**) : $R_1 = CH_3$, $R_2 = OH$
Quinolactacin A1 (**5**) : (3*S**, 1'*R**)-**2**
Quinolactacin A2 (**6**) : (3*S**, 1'*S**)-**2**

Fig. 1. Structures of Quinolactacide (**1**) and Related Compounds, Quinolactacins (**2**–**6**).

† To whom correspondence should be addressed. Fax: +81-88-685-6040; E-mail: mabe@otsukac.co.jp

Abbreviations: THF, tetrahydrofuran; TEA, triethylamine; DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; MnO_2, manganese dioxide; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; mp, melting point; TMS, tetramethylsilane; EtOAc, ethyl acetate



Scheme 1. Synthetic Scheme for Quinolactacide (**1**).

Reagents and conditions: (a) $SOCl_2$, ethanol, reflux (quant.); (b) acetyl chloride, pyridine, THF (90%); (c) potassium *tert*-butoxide, THF–toluene, reflux (59%); (d) 2-nitrobenzoyl chloride, TEA, THF (80%); (e) acetone cyanohydrin, TEA, acetonitrile (quant.); (f) H_2, Pd–C, methanol (52%); (g) MnO_2, $CHCl_3$–DMF (3:1), reflux (21%).

with MnO_2 in a mixture of chloroform and methanol, because of the good solubility of compound **13** in the mixed solvent, and also carried out with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in the same mixed solvent, but both reactions did not proceed well. Although the yield of the dehydrogenation reaction was not improved, sufficient quinolactacide (**1**) was obtained for further biological tests. The physico-chemical properties of synthetic **1** were identical with those of the natural compound.

The insecticidal and the miticidal activities of quinolactacide (**1**) against five different insects and one mite were evaluated at 500 ppm. The insects used for the tests were the green peach aphid,[1)] diamondback moth (*Plutella xylostella*), common cutworm (*Spodoptera litura*), western flower thrips (*Frankliniella occidentalis*), silverleaf whitefly (*Bemisia argentifolii*), and two-spotted spider mite (*Tetranychus urticae*). Synthetic quinolactacide (**1**) showed 100% and 42% mortality against the green peach aphid and diamondback moth, respectively, but did not show any activity against the others.

Our future study will be focused on the structure-activity relationships between quinolactacide (**1**) and its related compounds, and their insecticidal activity.

Experimental

General. The following instruments were used in the experiments: a Bruker DPX 300 FT NMR (300 MHz) spectrometer for the ^{1}H-, ^{13}C-NMR, and DEPT spectra, a Jeol The MStation JMS-700 mass spectrometer for the mass spectra, a Shimadzu FTIR-8100A IR spectrometer for the IR spectra, a Shimadzu UV-160 UV–VIS recording spectrophotometer for the UV spectra, and Büchi B-545 melting point apparatus for measuring the melting point (mp). The NMR spectra were measured by using $CDCl_3$ containing 0.03% tetramethylsilane (TMS) or DMSO-d_6 as a solvent. Kanto Chemical 60N silica gel (spherical, neutral, 100–200 μm) was used for column chromatography. The optical purity of intermediates **8–13** was not measured. The insecticidal test against the green peach aphid was conducted in the same manner as that described in our previous report,[1)] and against the silverleaf whitefly was conducted in almost the same manner as that against the aphid. In the test, first instar larvae of the silverleaf whitefly were treated with formulated quinolactacide (**1**), and the mortality was assessed after 6 d. The insecticidal test against the common cutworm was conducted in almost the same manner as that against the diamondback moth, second instar larvae of the common cutworm being used for the test. The insecticidal test against the western flower thrips was conducted in almost the same manner as that against the two-spotted spider mite, fifteen first instar larvae of the western flower thrips being used for the test.

*Ethyl pyrrolidine-2-carboxylate (**8**).* To a solution of **7** (10.0 g, 87.0 mmol) in dry ethanol (200 ml) was added dropwise thionyl chloride (20.7 g, 174 mmol) at 60 °C. The mixture was refluxed for 4 h and then stirred for 12 h at 20 °C. The reaction mixture was concentrated under reduced pressure, neutralized with 4 N NaOH (22 ml) at 0 °C and then successively extracted with ethyl acetate (EtOAc) and chloroform. Each organic layer was washed with brine and dried over anhydrous $MgSO_4$. The combined organic layers were concentrated to afford **8** (12.5 g, quant.) as a pale yellow oil. The crude product thus obtained was used for the next step without further purification. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.28 (3H, t, $J = 7.1$ Hz), 1.70–1.90 (3H, m), 2.05–2.15 (1H, m), 2.90 (1H, dt, $J = 10.3$, 6.5 Hz), 3.08 (1H, dt, $J = 10.3$, 6.5 Hz), 3.74 (1H, dd, $J = 8.5$, 5.5 Hz), 4.18 (2H, q, $J = 7.1$ Hz); N*H* was not detected.

*Ethyl 1-acetylpyrrolidine-2-carboxylate (**9**).* To a mixture of compound **8** (8.00 g, 55.9 mmol) and pyridine (5.30 g, 67.1 mmol) in THF (150 ml) was added dropwise acetyl chloride (5.27 g, 67.1 mmol) dissolved in THF (10 ml) at 0 °C. The mixture was stirred for 1 h at 0 °C and then for 2 h at 20 °C. The reaction mixture was poured into 1 N HCl (20 ml) at 0 °C and extracted with EtOAc. The EtOAc extract was successively washed with sat. $NaHCO_3$ and brine, dried over anhydrous

$MgSO_4$, and concentrated to afford **9** (9.32 g, 90%) as a pale yellow oil. The crude product was used for the next step without further purification. IR (film) ν_{max} cm^{-1}: 1740 (s), 1650 (s), 1420 (s), 1375 (m), 1360 (m), 1280 (m), 1240 (m), 1190 (s), 1090 (m), 1030 (m), 1000 (w), 920 (w), 620 (w), 540 (w). ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.23–1.31 (3H, m), 1.85–2.40 (4H, m), 3.45–3.55 (1H, m), 3.61–3.75 (1H, m), 4.10–4.24 (2H, m), 3.38 (0.3H, dd, $J = 8.5$, 3.5 Hz), 4.47 (0.7H, dd, $J = 8.5$, 3.5 Hz). HR-EIMS m/z (M^+): calcd. for $C_9H_{15}O_3N$, 185.1052; found, 185.1054.

*1-Azabicyclo[3,3,0]octane-2,4-dione (**10**).* To a suspension of potassium *tert*-butoxide (1.36 g, 12.2 mmol) in a mixture of THF (10 ml) and dry toluene (10 ml) was added dropwise compound **9** (1.50 g, 8.11 mmol) dissolved in THF (10 ml) at 80 °C. The mixture was refluxed for 12 h. After cooling, the reaction mixture was successively extracted with water and 1 N NaOH. The combined water layers were acidified with 4 N HCl to pH 3 and successively extracted with EtOAc and chloroform. The each organic layer was washed with brine and dried over anhydrous $MgSO_4$. The combined organic layers were concentrated to afford **10** (670 mg, 59%) as a brown oil. The crude product was used for the next step without further purification. IR (film) ν_{max} cm^{-1}: 3430 (br. m), 2700 (br. m), 2600 (br. m), 1770 (s), 1690 (br. s), 1610 (br. s), 1420 (br. s), 1375 (br. s), 1320 (br. s), 1260 (m), 1230 (br. m), 1075 (w), 820 (w), 630 (w), 590 (w), 560 (w). ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.65–1.75 (1H, m), 1.97–2.22 (3H, m), 3.02 (1H, dd, $J = 21.4$, 1.2 Hz), 3.15–3.25 (1H, m), 3.37 (1H, d, $J = 21.4$ Hz), 3.95 (1H, dt, $J = 11.8$, 7.5 Hz), 4.20 (1H, pseudo t, $J = 8.5$, 7.9 Hz). HR-EIMS m/z (M^+): calcd. for $C_7H_9O_2N$, 139.0633; found, 139.0638.

*4-(1-Azabicyclo[3,3,0]oct-3-ene-2-one) 2-nitrobenzoate (**11**).* To a mixture of compound **10** (3.00 g, 21.6 mmol) and TEA (2.39 g, 23.7 mmol) in THF (50 ml) was added dropwise 2-nitrobenzoyl chloride (4.40 g, 23.7 mmol) dissolved in THF (30 ml) at 0 °C. The mixture was stirred for 1 h at 0 °C and then for 2 h at 20 °C. The reaction mixture was poured into 1 N HCl at 0 °C and extracted with EtOAc. The organic layer was successively washed with sat. $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated to give a crude brown solid. This solid was washed with ether and dried *in vacuo* to afford **11** (4.96 g, 80%) as a pale brown solid. The crude product was used for the next step without further purification. To measure mp of **11**, the pure compound was prepared as a pale yellow powder by recrystallizing from a mixture of hexane and ether, mp (hexane–ether) 132–133 °C. IR (KBr) ν_{max} cm^{-1}: 3150 (w), 3090 (w), 3040 (w), 1770 (s), 1690 (s), 1610 (m), 1575 (w), 1530 (s), 1440 (w), 1375 (m), 1350 (s), 1330 (m), 1310 (m), 1276 (m), 1275 (m), 1240 (s), 1170 (s), 1090 (m), 1050 (s), 1040 (m), 1020 (m), 900 (w), 870 (m), 850 (m), 790 (m), 770 (w), 740 (m), 730 (m), 690 (w), 670 (m). ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.40–1.60 (1H, m), 2.10–2.40 (3H, m), 3.25 (1H, ddd, $J = 11.2$, 8.4, 3.2 Hz), 3.57 (1H, dt, $J = 11.2$, 8.2 Hz), 4.37 (1H, dd, $J = 9.6$, 5.9 Hz), 6.07 (1H, d, $J = 0.7$ Hz), 7.70–7.82 (2H, m), 7.82–7.90 (1H, m), 7.95–8.05 (1H, m). HR-EIMS m/z (M^+): calcd. for $C_{14}H_{12}O_5N_2$, 288.0746; found, 288.0731.

*1-Aza-3-(2-nitrobenzoyl)bicyclo[3,3,0]octane-2,4-dione (**12**).* To a mixture of compound **11** (800 mg, 2.78 mmol) and TEA (562 mg, 5.56 mmol) in dry acetonitrile (15 ml) was added acetone cyanohydrin (118 mg, 1.39 mmol) at 0 °C. After stirring for 2 h at 20 °C, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ether and extracted with 1 N NaOH. The 1 N NaOH extract was acidified with 4 N HCl to pH 3 and extracted with EtOAc. The EtOAc extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to afford **12** (800 mg, quant.) as a brown oil. IR (film) ν_{max} cm^{-1}: 3700–2200 (br. m), 1700 (br. s), 1630 (br. s), 1530 (s), 1450 (m), 1350 (s), 1310 (m), 1240 (m), 1190 (m), 1140 (w), 1110 (w), 1070 (w), 940 (w), 860 (m), 780 (m), 760 (m), 720 (w), 700 (w). ^{1}H-NMR (DMSO-d_6) δ: 1.30–1.45 (1H, m), 1.90–2.15 (3H, m), 2.91–3.01 (1H, m), 3.34 (1H, dt, $J = 10.9$, 7.8 Hz), 4.09 (1H, dd, $J = 8.9$, 7.2 Hz), 7.43 (1H, d, $J = 7.2$ Hz), 7.67 (1H, pseudo t, $J = 7.7$, 7.2 Hz), 7.79 (1H, pseudo t, $J = 7.7$, 7.0 Hz), 8.10 (1H, d, $J = 8.0$ Hz); an enol proton was not detected. ^{13}C-NMR (DMSO-d_6) δ: 27.23 (CH_2), 27.72 (CH_2), 43.25 (CH_2), 64.42 (CH), 103.91 (C), 123.54 (CH), 129.15 (CH), 130.57 (CH), 134.38 (CH), 135.67 (C), 146.49 (C), 173.68 (C), 184.69 (C), 188.14 (C). HR-EIMS m/z (M^+): calcd. for $C_{14}H_{12}O_5N_2$, 288.0746; found, 288.0736.

*1,2,3,11b-Tetrahydroquinolactacide (**13**).* A solution of **12** (300 mg, 1.04 mmol) in methanol (15 ml) was vigorously stirred over Pd/C 10% (20 mg) under hydrogen at atmospheric pressure for 3 d at 20 °C. The reaction mixture was filtered and successively washed with methanol and chloroform. The combined filtrates were concentrated under reduced pressure. The crude solid thus obtained was washed with a mixture of methanol and EtOAc and dried *in vacuo* to give **13** (130 mg, 52%) as a pale brown solid, mp 393–398 °C (dec.). IR (KBr) ν_{max} cm^{-1}: 3700–2400 (br. w), 1690 (s), 1630 (s), 1620 (s), 1590 (s), 1540 (s), 1470 (s), 1420 (w), 1370 (m), 1340 (w), 1300 (m), 1280 (w), 1220 (m), 1170 (w), 1140 (w), 1090 (w), 1030 (w), 980 (w), 960 (w), 880 (w), 870 (w), 800 (w), 780 (m), 770 (m), 750 (m), 730 (m), 680 (m), 660 (m), 530 (m). ^{1}H-NMR (DMSO-d_6) δ: 1.38–1.56 (1H, m), 2.05–2.33 (3H, m), 3.05–3.18 (1H, m), 3.44 (1H, dt, $J = 10.9$, 8.2 Hz), 4.67 (1H, dd, $J = 9.5$, 6.5 Hz), 7.39 (1H, t, $J = 7.5$ Hz), 7.56 (1H, d, $J = 8.0$ Hz), 7.71 (1H, dt, $J = 8.0$, 1.3 Hz), 8.15 (1H, d, $J = 8.0$ Hz), 12.72 (1H, br. s). HR-EIMS m/z (M^+): calcd. for $C_{14}H_{12}O_2N_2$, 240.0899; found, 240.0915.

*Quinolactacide (**1**).* A mixture of **13** (20 mg, 0.083 mmol) and MnO_2 (145 mg, 1.67 mmol) in a mixture of DMF (10 ml) and chloroform (30 ml) was refluxed. After 12 h, to the mixture was added a further 145 mg of

MnO_2, and then the mixture was refluxed for 12 h more. The reaction mixture was cooled to room temperature and chromatographed in a silica gel column by eluting with chloroform–methanol (30:1→15:1). The fraction containing quinolactacide (**1**) was concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with water to remove residual DMF, dried over anhydrous $MgSO_4$, and concentrated to give **1** as a yellow solid. Quinolactacide (**1**) was further washed with water to remove residual DMF, and dried *in vacuo* to give pure **1** (4.1 mg, 21%) as a yellow powder. To measure mp of **1**, the pure compound was prepared as a yellow powder by recrystallizing from a mixture of chloroform and methanol, mp (chloroform–methanol) 372–373 °C (dec.). IR (KBr) ν_{max} cm^{-1}: 3440 (br. w, NH), 1740 (s, N–C=O), 1645 (s, C=O), 1620 (m), 1580 (s), 1540 (m), 1525 (m), 1475 (m), 1395 (w), 1220 (w), 1050 (w), 770 (m). UV λ_{max} ($CHCl_3$:CH_3OH = 1:1, 26 °C) nm (ε): 242.4 (12100), 288.1 (33900). ^{1}H-NMR (DMSO-d_6) δ: 6.37 (1H, t, J = 3.1 Hz), 6.72 (1H, d, J = 3.1 Hz), 7.38–7.45 (2H, m), 7.57 (1H, d, J = 8.2 Hz), 7.71 (1H, pseudo t, J = 8.2, 6.9 Hz), 8.12 (1H, d, J = 7.4 Hz), 13.25 (1H, br. s). EIMS m/z: 236 (M^+), 208, 179, 168 (M^+–C_3O_2). HR-EIMS m/z (M^+): calcd. for $C_{14}H_8O_2N_2$, 236.0586; found, 236.0611.

Insecticidal test against the diamondback moth (Plutella xylostella). An insecticidal formulation (500 ppm) was prepared by adding an aqueous solution (100 ppm) of Sorpol 355 (Toho Chemical Industry, Tokyo, Japan) to a methanol solution containing a 1.25% DMSO solution of quinolactacide (**1**), and 4.0 ml of the formulation was sprayed over two excised leaf squares (5 × 5 cm) from a cabbage plant at the 6-leaf stage. After air-drying, fifteen third instar larvae of the diamondback moth were settled on the leaves, and the leaves were placed in a plastic cup and left to stand in a thermostatic chamber (25 ± 2 °C, 16L, 8D). After 2 d, the mortality from the formulation of the sample against the diamondback moth was assessed by comparing it with that without the sample.

Insecticidal test against the two-spotted spider mite (Tetranychus urticae). An excised leaf square (3 × 4 cm) from a bean plant at the primary leaf stage was placed on a piece of non-woven fabric containing sufficient water. Twenty female adults of the two-spotted spider mite were settled on the leaf and then placed in a thermostatic chamber (25 ± 2 °C, 16L, 8D). After 4 d, an insecticidal formulation (500 ppm) was prepared by adding an aqueous solution (100 ppm) of Sorpol 355 (Toho Chemical Industry, Tokyo, Japan) to a methanol solution containing a 1.25% DMSO solution of quinolactacide (**1**), and 4.0 ml of the formulation was sprayed over the leaf and air-dried. The leaf was left to stand in the thermostatic chamber (25 ± 2 °C, 16L, 8D). After 2 d, the mortality from the formulation of the sample against the two-spotted spider mite was assessed by comparing it with that without the sample.

Acknowledgment

We express our sincere gratitude to Mr. K. Kiryu (of Otsuka Pharmaceutical Factory, Inc.) for measuring the MS data.

References

1) Abe, M., Imai, T., Ishii, N., Usui, M., Okuda, T., and Oki, T., Quinolactacide, a new quinolone insecticide from *Penicillium citrinum* Thom F 1539. *Biosci. Biotechnol. Biochem.*, **69**, 1202–1205 (2005).
2) Kakinuma, N., Iwai, H., Takahashi, S., Hamano, K., Yanagisawa, T., Nagai, K., Tanaka, K., Suzuki, K., Kirikae, F., Kirikae, T., and Nakagawa, A., Quinolactacins A, B and C: novel quinolone compounds from *Penicillium* sp. EPF-6 I. Taxonomy, production, isolation and biological properties. *J. Antibiotics*, **53**, 1247–1251 (2000).
3) Takahashi, S., Kakinuma, N., Iwai, H., Yanagisawa, T., Nagai, K., Suzuki, K., and Tokunaga, T., Quinolactacins A, B and C: novel quinolone compounds from *Penicillium* sp. EPF-6 II. Physico-chemical properties and structure elucidation. *J. Antibiotics*, **53**, 1252–1256 (2000).
4) Nakagawa, A., Japan Kokai Tokkyo Koho, 2001-247571 (Sept. 11, 2001).
5) Kim, W., Song, N., and Yoo, I., Quinolactacins A1 and A2, new acetylcholinesterase inhibitors from *Penicillium citrinum*. *J. Antibiotics*, **54**, 831–835 (2001).
6) Tatsuta, K., Misawa, H., and Chikauchi, K., Biomimetic total synthesis of quinolactacin B, TNF production inhibitor, and its analogs. *J. Antibiotics*, **54**, 109–112 (2001).
7) Zhang, X., Jiang, W., and Sui, Z., Concise enantioselective syntheses of quinolactacins A and B through alternative Winterfeldt oxidation. *J. Org. Chem.*, **68**, 4523–4526 (2003).
8) Fischer, R., Bretschneider, T., Krüger, W. B., Ruther, M., Erdelen, C., Wachendorff-Neumann, U., Santel, J. H., and Dollinger, M., European Patent 0668267 (Aug. 23, 1995).
9) Nakamura, U., Permer, J. C., Kikukawa, H., Sano, M., and Ohno, K., Japan Kokai Tokkyo Koho, 2001-335573 (Dec. 4, 2001).
10) Kashdan, S. D., Schwartz, A. J., and Rapoport, H., Synthesis of 1,2,3,4-tetrahydroisoquinolines. *J. Org. Chem.*, **47**, 2638–2643 (1982).