Synthesis of Verruculotoxin

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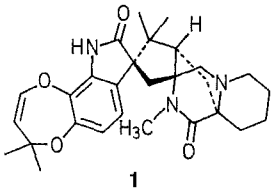
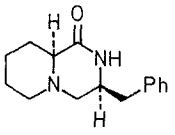
The mycotoxic alkaloid verruculotoxin **(2)** has been synthesized from (*S*)-phenylalanine and pipecolinic acid. A selective method to reduce a dioxopiperazine to an oxopiperazine is described.

As part of a program[2] aimed at elucidating the biogenesis and total synthesis of the complex mycotoxic alkaloid marcfortine **(1)**,[3] we required a general method to regiospecifically reduce a dioxopiperazine to an oxopiperazine. We chose as a model system, the simple, mycotoxin verruculotoxin **(2)** which has been isolated from the green peanut mold *Penicillium verruculosum* Peyronel.[4] The absolute stereostructure and synthesis of **2** have been reported by Clardy and collaborators in 1976.[5]



1
Marcfortine A

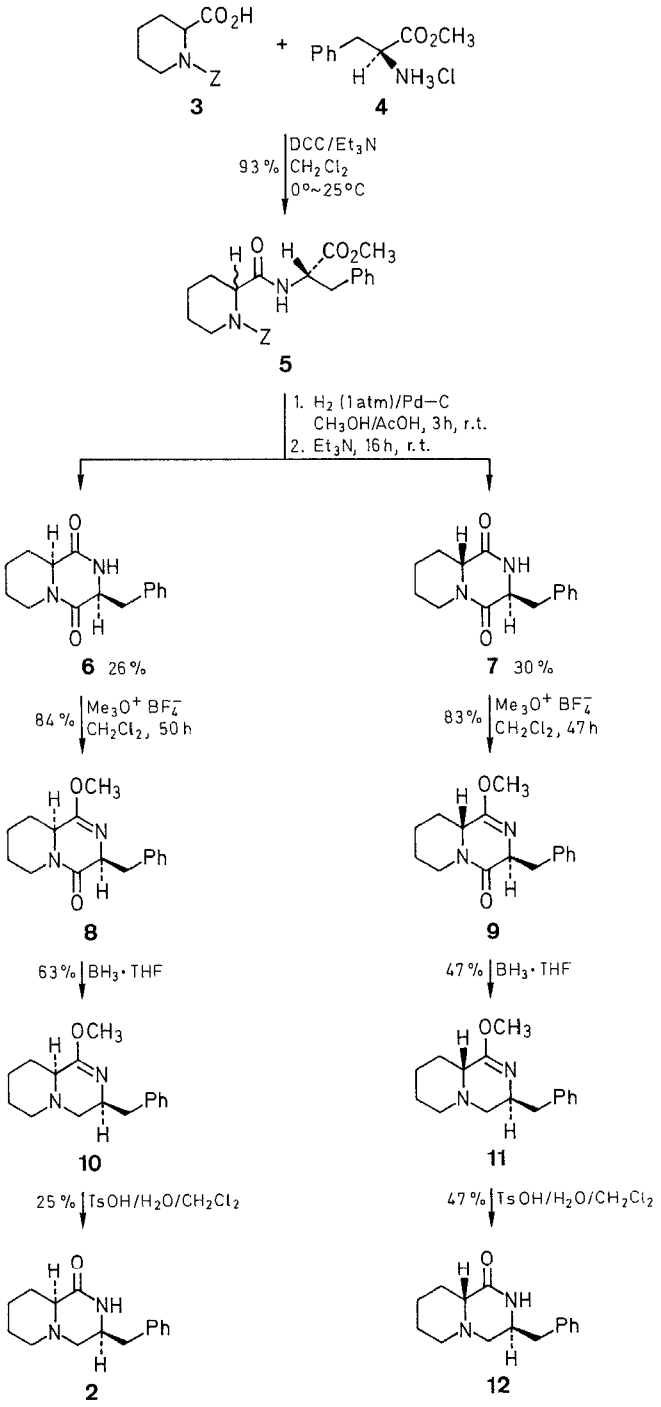


2
Verruculotoxin

Our strategy relevant to **1** required the oxidation state of a dioxopiperazine for the crucial intramolecular S_N2' cyclization[2] used to construct the central bicyclo[2.2.2] nucleus which

would have to be subsequently reduced regiospecifically at the pipecolinic acid *N*-amide linkage. We envisioned that the two amides of such a system could be differentiated[6] by conversion of a secondary amide (*N*-methylated in **1**) to a lactim ether, reduction of the tertiary amide and subsequent unmasking of the lactim ether to furnish the requisite oxopiperazine unit present in both **1** and **2**.

(*S*)-Phenylalanine methyl ester hydrochloride was condensed with (*R,S*)-*N*-Z-pipecolinic acid (Z = benzyloxycarbonyl) in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and triethylamine. The mixture of inseparable diastereomeric dipeptides **5** (93%) obtained was subjected to catalytic hydrogenolysis to afford the piperazinediones **6** (26%) and **7** (30%), which were separated by column chromatography on silica gel. Treatment of **6** and **7** with trimethyloxonium tetrafluoroborate in dichloromethane provided the mono-lactim ethers **8** (84%) and **9** (83%), respectively.



After examining several reducing agents, it was found that treatment of **8** with borane–tetrahydrofuran complex in refluxing tetrahydrofuran effected the clean reduction of the amide carbonyl to the corresponding tertiary amine (63%). Conversion of the lactim ether to the secondary amide proved to be somewhat difficult and could be effected in low yield (~10%) by treatment with iodotrimethylsilane in dichloromethane to afford verruculotoxin (**2**) that was identical to an authentic sample. A slightly better procedure was identified that involved treatment of the lactim ether with 1 equivalent of *p*-toluenesulfonic acid in wet dichloromethane for 95 h at room temperature to furnish **2** in 25% yield.

Diastereomer **9** was similarly transformed into *epi*-verruculotoxin (**12**).[5]

In summary, we have demonstrated that an oxopiperazine can be generated from a dioxopiperazine by protection of the secondary amide as a lactim ether, reduction of the tertiary amide, and cleavage of the lactim ether back to the secondary amide. The use of **2** as a model for this sequence rigorously secures the viability of the method and the structures of the products. Efforts are in progress to utilize this simple technique to construct the ring system of marcfortine (**1**).

All solvents were distilled prior to use. THF was dried over sodium/benzophenone and freshly distilled prior to use. Analytical TLC was performed using 0.25 mm Merck precoated silica gel 60F-254 glass plates. Preparative TLC was performed on a Harrison Research Chromatotron using 2.0-, 4.0-, or 8.0-mm layer thickness silica gel 60F-254 with calcium sulfate binder. Melting points were taken using a Mel Temp apparatus and are uncorrected. IR spectra were obtained using a Beckman 4240 spectrometer. ^{1}H-NMR spectra were obtained using a Bruker WP270SY 270 MHz spectrometer. Chemical shifts are reported in ppm relative to TMS, and coupling constants *J* are reported in units of Hertz (Hz). Mass spectra were obtained using a V.G. Micromass Ltd., Model 16F spectrometer. Observed optical rotations at the sodium D line were obtained at 25°C using a Perkin Elmer 241 MC polarimeter. Elemental analyses were performed by M-H-W Laboratories, Phoenix, Arizona.

Methyl *N*-[(*R,S*)-1-Benzyloxycarbonylpiperidine-2-carbonyl]-(*S*)-phenylalanate (5):

To (*R,S*)-*N*-Z-pipecolinic acid (**3**; 3.25 g, 12.34 mmol) and (*S*)-phenylalanine methyl ester hydrochloride (**4**; 2.66 g, 12.34 mmol) in CH_2Cl_2 (25 mL) at 0°C is added Et_3N (1.25 g, 12.34 mmol, 1.0 equiv) and DCC (2.67 g, 12.96 mmol). The reaction is stirred at 0°C for 2 h and at 25°C for 23 h. The crude mixture is filtered through Celite and chromatographed by chromatotron (8 mm thickness, eluting with (EtOAc/*n*-hexane, 1:3) to give **5** as a colorless solid; yield: 4.87 g (93%); mp 103–104°C (EtOAc/*n*-hexane).

$C_{24}H_{28}N_2O_5$	calc.	C 67.91	H 6.65	N 6.60
(424.5)	found	67.83	6.75	6.60

MS [CI(NH_3)]: *m/z* = 424 (M^+).

IR (KBr): ν = 3342, 1745, 1691, 1534, 1315, 1264 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = 1.32–1.66 (m, 5H); 2.22–2.33 (m, 1H); 2.76–2.94 (m, 1H); 3.03–3.12 (m, 2H); 3.72 (s, 3H9; 3.98–4.18 (m, 1H); 4.72–4.93 (m, 2H); 5.15 (s, 2H); 6.25–6.51 (m, 1H); 6.95–7.38 (m, 10H).

(3*S*,6*S*)-3-Benzyl-1,4-diazabicyclo[4.4.0]decane-2,5-dione (6) and (3*S*,6*R*)-3-Benzyl-1,4-diazabicyclo[4.4.0]decane-2,5-dione (7):

To a solution of **5** (4.86 g, 11.45 mmol, 1.0 equiv) in CH_3OH (75 mL) and glacial AcOH (5 mL) under Ar is added 10% Pd/C (1.8 g, 1.7 mmol, 0.15 equiv). The system is flushed with H_2, and the mixture is stirred under H_2 (1 atm) at 25°C for 3 h. To this mixture is added Et_3N (13 mL), the contents are stirred for an additional 20 min, and then filtered through Celite. The solvents are evaporated, and the residue is dissolved in Et_3N (30 mL) and stirred at 25°C for 16 h. The mixture is diluted with CH_2Cl_2 (300 mL), washed successively with water, 1 N HCl, water, and dried (Na_2SO_4). The crude product is chromatographed by chromatotron (4 mm thickness, eluting with EtOAc) to afford diastereoisomers **6** and **7**.

6; crystalline solid; yield: 781 mg (26%); mp 149–150 C (EtOAc/*n*-hexane); $[\alpha]_D^{25}$ + 16.2° (*c* = 0.5, CH_3OH).

$C_{15}H_{18}N_2O_2$	calc.	C 69.75	H 7.02	N 10.84
(258.3)	found	69.51	7.12	10.73

MS [CI(NH_3)]: *m/z* = 259 (M^+ + 1).

IR (KBr): *v* = 3250–3120 (br), 1672, 1635, 1600, 1266, 1252 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = 0.20–0.04 (m, 1H); 1.03–1.22 (m, 1H); 1.24–1.42 (m, 1H); 1.47–1.67 (m, 2H); 1.78–1.88 (m, 1H); 2.27–2.38 (m, 1H); 3.00 (dd, 1H, *J* = 13.4, 3.8 Hz); 3.37 (dd, 1H, *J* = 13.4, 3.8 Hz); 3.56–3.60 (m, 1H); 4.37–4.67 (m, 1H); 4.63–4.67 (m, 1H); 7.23–7.34 (m, 5H); 8.0 (br s, 1H).

7; crystalline solid; yield: 881 mg (30%); mp 134–135 C (EtOAc/*n*-hexane); $[\alpha]_D^{25}$ + 32.4° (*c* = 0.5, CH_3OH).

$C_{15}H_{18}N_2O_2$	calc.	C 69.75	H 7.02	N 10.84
(258.3)	found	69.91	6.91	10.84

MS [CI(NH_3)]: *m/z* = 259 (M^+ + 1).

IR (KBr): *v* = 3350–3150 (br), 1674, 1641, 1203, 1183 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = 1.24–1.51 (m, 3H); 1.62–1.74 (m, 1H); 1.87–1.96 (m, 1H); 2.18–2.36 (m, 2H); 3.04–3.12 (m, 2H); 3.24 (dd, 1H, *J* = 13.6, 3.7 Hz); 4.26–4.31 (m, 1H); 4.60–4.71 (m, 1H); 6.13 (br s, 1H); 7.17–7.37 (m, 5H).

(3*S*,6*S*)-3-Benzyl-1,4-diaza-5-methoxybicyclo[4.4.0]dec-4-en-2-one (8):
To a stirred solution of **6** (420 mg, 1.63 mmol, 1 equiv) in CH_2Cl_2 (6 mL) is added trimethyloxonium tetrafluoroborate (603 mg, 4.08 mmol, 2.5 equiv). The reaction is stirred at 25 °C for 50 h, diluted with CH_2Cl_2 (80 mL), washed with water, and dried (Na_2SO_4). The crude product is purified by chromatotron (2 mm thickness, eluting with 4:1 EtOAc/THF) to give **8** as a white solid; yield: 371 mg (84%); mp 92–93 °C (hexane); $[\alpha]_D^{25}$ + 47.2° (*c* = 0.5, CH_3OH).

$C_{16}H_{20}N_2O_2$	calc.	C 70.56	H 7.40	N 10.29
(272.4)	found	70.32	7.40	10.22

MS [CI(NH_3)]: *m/z* = 272 (M^+).

IR (KBr): *v* = 2930, 1700, 1637, 1594, 1266, 1239 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = −0.50 (ddd, 1H, *J* = 25.1, 12.5, 3.5 Hz); 1.00–1.31 (m, 2H); 1.43–1.58 (m, 3H); 2.20–2.31 (m, 1H); 3.05 (dd, 1H, *J* = 13.0, 4.3 Hz); 3.46–3.56 (m, 1H); 3.46 (dd, 1H, *J* = 13.0, 4.3 Hz); 3.71 (s, 3H); 4.44–4.49 (m, 1H); 4.63–4.69 (m, 1H); 7.12–7.30 (m, 5H).

(3*S*,6*S*)-3-Benzyl-1,4-diaza-5-methoxybicyclo[4.4.0]dec-4-ene (10):
To a solution of **8** (249 mg, 0.91 mmol, 1.0 equiv) in THF (3 mL) is added BH_3 · THF complex (1.0 M, 2.74 mL, 2.74 mmol). The mixture is refluxed for 2 h, cooled to 25 °C, and quenched with sat. NH_4Cl solution (5 mL). The mixture is diluted with CH_2Cl_2 (100 mL), washed with water, and dried (Na_2SO_4). The crude product is purified by chromatotron (2 mm thickness, eluting with 1:4 EtOAc/*n*-hexane) to give the corresponding amine as a colorless, highly viscous oil; yield: 149 mg (63%); $[\alpha]_D^{25}$ + 58.7° (*c* = 0.3, CH_3OH).

No attempts were made to prepare an analytical sample.

MS [CI(NH_3)]: *m/z* = 258 (M^+).

IR (neat): *v* = 3021, 2943, 1687, 1598, 1295, 1214 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = 1.06 (ddd, 1H, *J* = 27.1, 13.1, 3.9 Hz); 1.29–1.92 (m, 5H); 2.35–2.41 (m, 1H); 2.73–2.86 (m, 2H); 2.99–3.29 (m, 4H); 3.71 (s, 3H); 4.32–4.47 (m, 1H); 7.18–7.34 (m, 5H).

Verruculotoxin (2):
To a stirred solution of the amine **10** obtained above (95 mg, 0.37 mmol, 1.0 equiv) in CH_2Cl_2 (0.5 mL) is added water (0.2 mL) and TsOH (70 mg, 0.37 mmol, 1.0 equiv). The mixture is vigorously stirred at 25 °C for 95 h, diluted with CH_2Cl_2 (100 mL), washed with sat. $NaHCO_3$ solution and water, and dried (Na_2SO_4). The crude product is purified by preparative TLC (eluting with EtOAc) to give **2** as a colorless, crystalline solid; yield: 22.4 mg (25%); mp 153–154 °C (benzene); $[\alpha]_D^{25}$ − 56.0° (*c* = 0.25, CH_3OH) (Lit.[5] mp not reported).

$C_{15}H_{20}Nd_2O$	calc.	C 73.74	H 8.25	N 11.46
(244.3)	found	73.55	8.33	11.26

MS [E], (70 eV): *m/z* = 244 (M^+).

IR (KBr): *v* = 3400–3150 (br), 2954, 2931, 1675, 1628, 1603, 1264 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = 1.25–1.54 (m, 2H); 1.57–1.71 (m, 2H9; 1.86–1.97 (m, 1H); 2.08–2.20 (m, 1H); 2.23–2.34 (m, 1H); 2.49–2.60 (m, 2H); 2.67–2.74 (m, 1H); 2.82–2.91 (m, 1H), 2.93–3.03 (m, 2H); 3.40–3.52 (m, 1H); 5.62–5.73 (br s, 1H); 7.16–7.40 (m, 5H).

(3*S*,6*R*)-3-Benzyl-1,4-diaza-5-methoxybicyclo[4.4.0]dec-4-en-2-one (9):
To a solution of **7** (535 mg, 2.1 mmol, 1.0 equiv) in CH_2Cl_2 (8 mL) is added trimethyloxonium tetrafluoroborate (766 mg, 5.2 mmol, 2.5 equiv). The mixture is stirred at 25 °C for 47 h, diluted with CH_2Cl_2 (80 mL), washed with water, and dried (Na_2SO_4). The crude product is purified by chromatotron (2 mm thickness, eluting with EtOAc) to give **10** as a crystalline solid; yield: 467 mg (83%); mp 68–69 °C (hexane); $[\alpha]_D^{25}$ − 3.0° (*c* = 0.5, CH_3OH).

$C_{16}H_{20}N_2O_2$	calc.	C 70.56	H 7.40	N 10.29
(272.4)	found	70.78	7.36	10.19

MS [CI(NH_3)]: *m/z* = 272 (M^+).

IR (KBr): *v* = 3009, 2922, 1698, 1652, 1600, 1270, 1244 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = 1.16–1.45 (m, 3H); 1.61–1.86 (m, 2H); 1.96–2.03 (m, 1H); 2.08–2.18 (m, 1H); 2.66–2.71 (m, 1H); 3.07 (dd, 1q, *J* = 13.1, 4.1 Hz); 3.32 (dd, 1H, *J* = 13.1, 4.8 Hz); 3.69 (s, 3H); 4.44–4.48 (m, 1H); 4.59–4.68 (m, 1H); 7.08–7.23 (m, 5H).

(3*S*,6*R*)-3-Benzyl-1,4-diaza-5-methoxybicyclo[4.4.0]dec-4-ene (11):
To a stirred solution of **9** (288 mg, 1.1 mmol, 1.0 equiv) in THF (4 mL) is added BH_3 · THF complex (1.0 M, 2.6 mL, 2.6 mmol). The mixture is refluxed for 2 h, cooled to 25 °C, and quenched with sat. NH_4Cl solution (3 mL). The mixture is diluted with CH_2Cl_2 (150 mL), washed with water, and dried (Na_2SO_4). The crude product is purified by chromatotron (2 mm thickness, eluting with 1:4 EtOAc/*n*-hexane) to give the corresponding amine as a colorless, highly viscous oil; yield: 127 mg (47%); $[\alpha]_D^{25}$ + 24.9° (*c* = 0.45, CH_3OH).

No attempts were made to prepare an analytically pure sample.

MS [CI(NH_3)]: *m/z* = 258 (M^+).

IR (neat): *v* = 3030, 2946, 1677, 1604, 1300, 1237, 1178 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = 0.98–1.18 (m, 1H); 1.40–1.67 (m, 2H); 2.13–2.21 (m, 1H); 2.30–2.37 (m, 1H); 2.43–2.57 (m, 1H); 2.66 (dd, 1H, *J* = 13.4, 7.0 Hz); 2.71–2.86 (m, 3H); 2.92 (dd, 1H, *J* = 13.4, 6.4 Hz); 3.10 (dd, 1H, *J* = 14.1, 4.8 Hz); 3.55–3.59 (m, 1H); 3.70 (s, 3H); 3.90–4.03 (m, 1H); 7.21–7.34 (m, 5H).

(3*S*,6*R*)-3-Benzyl-1,4-diazabicyclo[4.4.0]decan-5-one (12):
To a solution of the amine **11** obtained above (19.5 mg, 0.075 mmol, 1.0 equiv) in CH_2Cl_2 (0.3 mL) is added water (0.3 mL) and TsOH (14 mg, 0.075 mmol, 1.0 equiv). The mixture is vigorously stirred at 25 °C for 94 h, diluted with CH_2Cl_2 (40 mL), washed with water, and dried (Na_2SO_4). The crude product is purified by preparative TLC (eluent: EtOAc) to give **12** as a white powder; yield: 8.4 mg (47%); mp 143–144 °C (EtOAc/*n*-hexane); $[\alpha]_D^{25}$ + 59.6° (*c* = 0.5, CH_3OH).

$C_{15}H_{20}N_2O$	calc.	C 73.74	H 8.25	N 11.46
(244.3)	found	73.62	8.27	11.43

MS (EI, 70 eV): *m/z* = 244 (M^+).

IR (KBr): *v* = 3400–3100 (br), 2940, 2932, 1669, 1603, 1119, 1052 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = 1.25–1.46 (m, 2H); 1.52–1.72 (m, 2H); 1.81–1.92 (m, 2H); 2.08–2.34 (m, 3H); 2.48–2.62 (m, 2H); 2.77–2.96 (m, 2H); 3.83–3.95 (m, 1H); 5.55–5.63 (br s, 1H).

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