Hydroxylation of Marcfortine A at C14, C15, and C16 *via* a Novel Cyanogen Iodide Reaction

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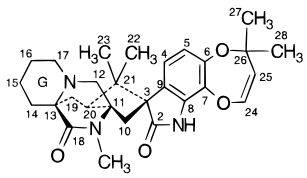
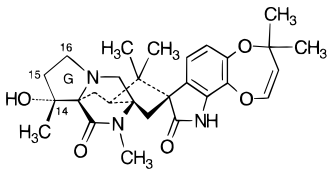
Helminths, especially parasitic nematodes, cause substantial health problems in humans and domestic animals. Currently, three distinct chemical classes are used for broad spectrum control of gastrointestinal nematodes in veterinary medicine: benzimidazoles, imidazothiazoles, and macrocyclic lactones.[1] None of these drugs is ideally suited for all therapeutic situations, and each class has been challenged by the development of drug-resistant nematode strains.[2] Expansion of the anthelmintic arsenal is thus an urgent goal. The potent antiparasitic activity of marcfortine A, paraherquamide A and their analogs was discovered by Merck scientists.[3] Because the marcfortines and paraherquamides are unique both structurally and in their mode of action, they represent a promising new class of anthelmintics.

Marcfortine A, a fungal metabolite of *Penicillium roqueforti*, which was reported by Polonsky et al.,[4] is structurally related to paraherquamide A which was originally isolated from *Penicillium paraherquei*.[5] Paraherquamide A contains a five-membered G-ring possessing a hydroxyl group and a methyl group, whereas the G-ring of marcfortine A is six-membered and unsubstituted. To investigate the significance of the hydroxy group on anthelmintic activity, we sought to synthesize the C14, C15, and C16 hydroxyl analogs of marcfortine A.



Marcfortine A



Paraherquamide A

Cyanogen iodide has proven to be a versatile reagent: cyanation of aromatic compounds;[6] conversion of dithioacetals to cyanothioacetals;[7] iodination of aromatic compounds;[8] formation of *trans*-olefins from alkynes;[9] and disulfide bond formation in peptides[10] have all been reported.

However, the reaction of cyanogen iodide with marcfortine A is novel (Scheme 1). Treatment of marcfortine A with 3 equiv of cyanogen iodide in refluxing chloroform provided the oxidation products, *trans*-16-iodo-17-cyanomarcfortine A (**3**) in 90% yield and 17-cyanomarcfortine A (**4**) in 5% yield. Both products can be accounted for by the mechanism shown in Scheme 1. One can speculate about the formation of **2**; both an ionic mechanism and radical mechanism can account for the oxidation. The radical mechanism seems more likely, since other reactions reported [6−10] with cyanogen iodide proceed through radical intermediates. The enamine intermediate **1** and the iminium ion intermediate **2**, which exist in equilibrium, provide **3** and **4**, respectively. Although generation of the iminium intermediate with chlorine dioxide,[11] or bromine[12] has been reported, these reagents did not produce compounds such as **3** suggesting that equilibration with the enamine does not occur. Polonovski−Potier reaction applied to aspidospermane generated an enamine intermediate, which upon treatment with CNBr gave a product[13] similar to **3** in three steps. Further reaction of **3** with 45% aqueous KOH in MeOH for 3 h at room temperature gave the 16,17-dehydro analog **5** in 90% yield. The utility of this novel cyanogen iodide reaction is demonstrated by conversion of **5** to 14α-hydroxymarcfortine A (**10**), 15α-hydroxymarcfortine A (**13**), and 16α-hydroxymarcfortine A (**15**).

Preparation of 14α-hydroxymarcfortine A (**10**) from **5** was achieved in five steps (Scheme 2). Hydrolysis of **5** with a catalytic amount of *p*-toluenesulfonic acid[14] in 95% methanol at room temperature for 1 h gave 17-oxomarcfortine A (**6**, 90%). The C15−C16 double bond was then introduced by selenation (phenylselenenyl chloride and LDA) at C16 followed by hydrogen peroxide oxidation and subsequent elimination of phenylselenenic acid by aqueous alkaline workup (1 N NaOH) to give the 15,16-dehydro derivative **7** (65%). Compound **7** underwent allylic oxidation with SeO_2 in refluxing dioxane (1.3 equiv, 1 h) to provide a modest yield (35%) of the desired α-hydroxy derivative **8**. Reduction of the double bond with lithium triethylborohydride (7 equiv/THF, 0 °C, 0.5 h) gave **9** (86%) which underwent regiospecific reduction of the C17 amide with BH_3−DMS (10 equiv/THF) to provide 14α-hydroxymarcfortine A (**10**, 75%). Presumably, the oxidizing agent (conversion of **7** to **8**) was delivered to the back side (less hindered side) of the molecule. The coupling constant between the C14 hydrogen and the C15 hydrogen is 2 Hz, which indicates the hydroxy group is at the axial position. Several other reducing agents were tried for this final step: LAH, alane, Red-Al, lithium 9-BBN hydride, and BH_3−morpholine complex. All failed to give better yields than borane dimethyl sulfide complex.

The preparation of 15α-hydroxymarcfortine A (**13**) and 16α-hydroxymarcfortine A (**15**) is shown in Scheme 3. Treatment of **5** with LDA (4 equiv/THF, −78 °C) followed by 1.5 equiv of Davis's reagent[15] (2-benzenesulfonyl)-3-

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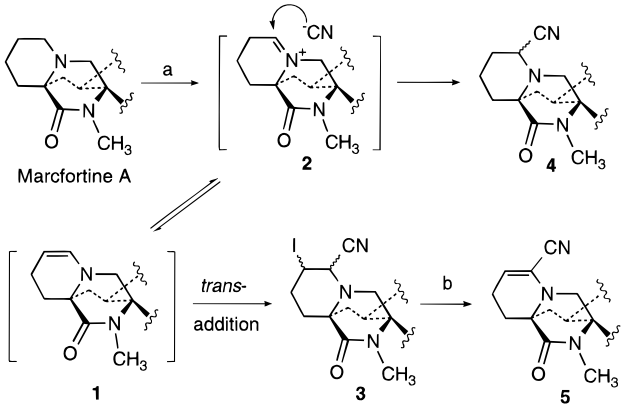
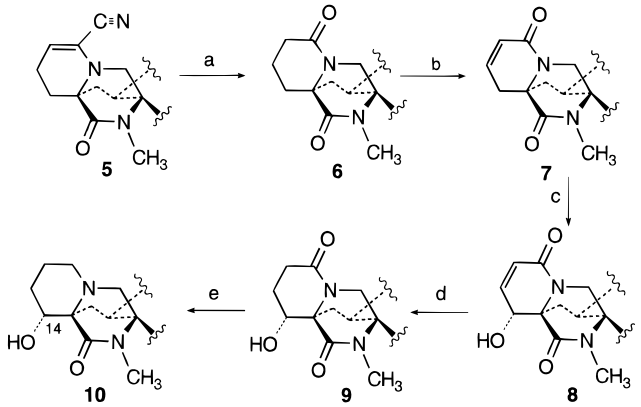
Scheme 1. Novel Cyanogen Iodide Reaction with Marcfortine A[a]



[a] (a) 3 equiv of ICN, $CHCl_3$, reflux, (90%); (b) 45% aqueous KOH, MeOH, (90%).

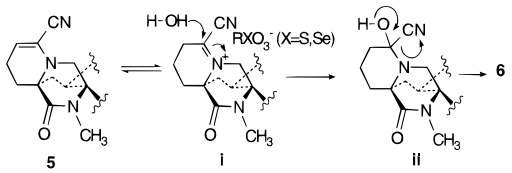
Scheme 2. Preparation of 14α-Hydroxymarcfortine A[a]



[a] (a) 0.5 equiv of *p*-TsOH, MeOH, (90%); (b) (i) 3.6 equiv of LDA, THF, −78 °C to −40 °C, (ii) 1.3 equiv of PhSeCl, −78 °C, (iii) 1.5 mL of 30% H_2O_2, NaOH, (65%); (c) 1.3 equiv of SeO_2, dioxane, reflux, (35%); (d) 7 equiv of $LiEt_3BH$, 0 °C, THF, (86%); (e) 10 equiv of BH_3−DMS, 0 °C, THF (75%).

phenyloxiziridine) gave the desired C15−hydroxylated material **11** (γ-hydroxylation, 30%) along with the 17-oxo derivative, **7** (α-hydroxylation, 10%). ^{1}H NMR of **11** showed a single stereoisomer in which the C15 hydrogen has two pseudoaxial and two pseudoequatorial couplings. Reaction of **11** with 2.6 equivalents of SeO_2[13] in aqueous ethanol at ambient temperature for 16 h furnished **12** (50%) which was reduced with LAH (2.75 equiv/THF, 0 °C, 0.5 h) to yield 15α-hydroxymarcfortine A **13** (25%). To prepare **15** we subjected **5** to standard osmylation conditions. Thus treatment of **5** with a catalytic amount OsO_4 and 4.2 equiv of 4-methylmorpholine *N*-oxide gave **14** (80%) which underwent reduction of its C17-carbonyl with BH_3−DMS (6 equiv/THF, 0 °C, 1 h) to yield 16α-hydroxymarcfortine A **15**, (60% based on recovered starting material).

(14) A plausible mechanism is shown below.



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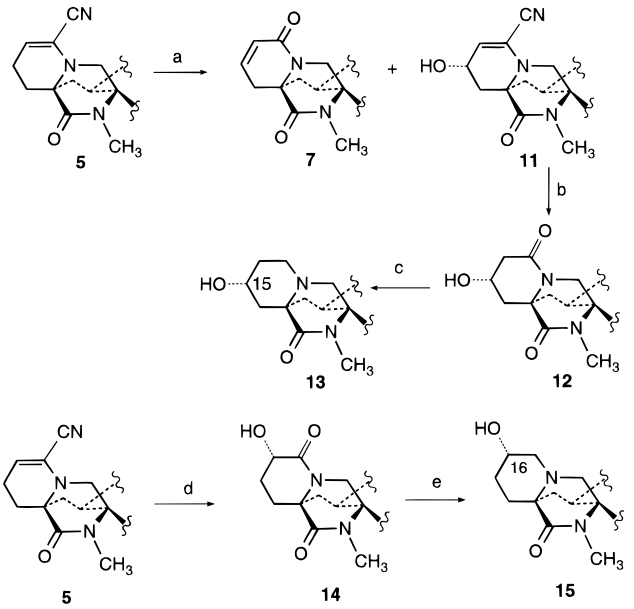
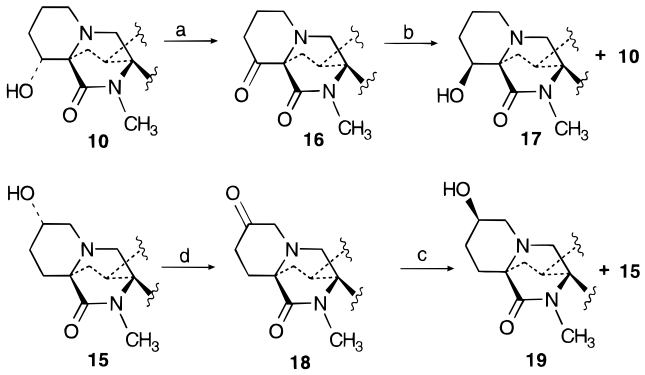
Scheme 3. Preparation of 15α-Hydroxymarcfortine A and 16α-Hydroxymarcfortine A[a]



[a] (a) (i) 4 equiv of LDA, THF, −78 °C; (ii) 1.5 equiv of 2-(benzenesulfonyl)-3-phenyloxiziridine (10% of **7** and 30% of **11**); (b) 2.6 equiv of SeO_2, aqueous EtOH, rt (50%); (c) 2.75 equiv of LAH, THF, 0 °C (25%); (d) catalytic OsO_4, 4.2 equiv of NMO, 4:1 acetone/H_2O (80%); (e) 6 equiv of BH_3−DMS, THF, 0 °C (60% based on recovered starting material).

Scheme 4. Preparation of 14β-Hydroxymarcfortine A and 16β-Hydroxymarcfortine A[a]



[a] (a) (i) 3.2 equiv of oxalyl chloride, 4 equiv of DMSO, CH_2Cl_2, −78 °C; (ii) 7.0 equiv of NEt_3, −78 °C to rt (74%); (b) 6.5 equiv of $NaBH_4$, MeOH, 0 °C (50% of **17** and 3% of **10**); (c) (i) 3.2 equiv of oxalyl chloride, 4 equiv of DMSO, CH_2Cl_2, −78 °C; (ii) 7.0 equiv of NEt_3, −78 °C to rt (65%); (d) 2.75 equiv of $NaBH_4$, MeOH, 0 °C (71% of **19** and 4% of **15**).

Since X-ray analysis of 18-thiomarcfortine A[16] showed the piperidine ring to exist in the chair form, it is reasonable to assign the stereochemistry based on the proton coupling constants. In order to confirm the stereochemistry of hydroxy compounds **10** and **15**, we prepared the inverted stereoisomers of both 14α-hydroxymarcfortine A (**10**) and 16α-hydroxymarcfortine A (**15**) (Scheme 4). Swern oxidation (oxalyl chloride, DMSO, NEt_3, −78 °C) of 14α-hydroxymarcfortine A (**10**) provided

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14-oxomarcfortine A (**16**, 74%). Reduction of **16** with $NaBH_4$ (6 equiv/THF, 0 °C, 0.5 h) gave 14β-hydroxymarcfortine A (**17**, 50%) and 14α-hydroxymarcfortine A (**10**, 2.5%). Likewise, Swern oxidation of 16α-hydroxymarcfortine A (**15**) gave 16-oxomarcfortine A (**18**, 80%) which was reduced with $NaBH_4$ to yield 16β-hydroxymarcfortine A (**19**, 71%) and 16α-hydroxymarcfortine A (**15**, 4%). Comparison of the ^{1}H NMR data for the stereoisomers confirmed the assignment of the hydroxyl group for 14α-hydroxymarcfortine A (**10**) and 16α-hydroxymarcfortine A (**15**).

In summary, our discovery of the novel cyanogen iodide reaction provided a valuable intermediate **5** which enabled us to prepare 14α-hydroxymarcfortine A (**10**), 15α-hydroxymarcfortine A (**13**), and 16α-hydroxymarcfortine A (**15**). These compounds were previously available only by biotransformation.[13] They should provide valuable insight into the SAR of this important new class of anthelmintics.

Experimental Section

Chemical reagents were obtained from commercial sources and used directly unless otherwise stated. All the reactions were carried out under a nitrogen atmosphere except where stated. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Thin layer chromatography was performed on silica gel 60 F_{254} plates. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 300 or 400 MHz spectrometer.

***trans*-16-Iodo-17-cyanomarcfortine A (3) and 17-Cyanomarcfortine A (4).** Marcfortine A (3.0 g, 0.62 mmol) was dissolved in chloroform (40 mL), treated with ICN (2 g, 1.87 mmol), and heated under reflux for 3 h. The mixture was cooled, diluted with methylene chloride (100 mL), and washed with aqueous sodium sulfite solution (2 × 100 mL) and with 10% aqueous K_2CO_3 solution (2 × 100 mL). The mixture was dried ($MgSO_4$) and concentrated, and the components were separated and purified by preparative thin layer chromatography (30% acetone in methylene chloride) to give the following compounds:

16-β-Iodo-17α-cyanomarcfortine A (3): 130 mg, 35% yield, as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 3H), 1.11 (s, 3H), 1.44 and 1.45 (2s, 6H),1.6−3.0 (m, 13H), 3.14 (s, 3H), 3.08 (t, J = 10.5 Hz, 1H), 3.51 (d, 1H), 3.83 (d, J = 11.8 Hz, 1H), 4.12 (dt, 1H), 4.92 and 6.35 (d, J = 7.7 Hz, 2H), 6.71 and 6.81 (d, J = 8.2 Hz, 2H), 8.45 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.49, 22.73, 23.75, 26.74, 29.79, 30.00, 31.76, 32.99, 33.78, 36.76, 46.43, 52.31, 59.53, 60.38, 62.87, 63.54, 64.70, 79.86, 115.16, 117.59, 118.57, 120.43, 124.29, 132.35, 135.0, 138.95, 146.25, 171.75, 183.20. FABMS, m/z [M + H$^+$] 629. HRMS (FAB) m/z [M + H$^+$] calculated for $C_{29}H_{33}N_4O_4I$ + H: 629.1626; measured: 629.1601.

16-α-Iodo-17−β-cyanomarcfortine A (3): 210 mg, 55% yield, as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.12 (s, 3H), 1.44 and 1.45 (2s, 6H), 1.6−3.0 (m, 13H), 3.14 (s, 3H), 3.12 (t, 1H), 3.7−3.9 (m, 2H), 4.66 (q, 1H), 4.92 and 6.37 (d, J = 7.7 Hz, 2H), 6.69 and 6.80 (d, J = 8.2 Hz, 2H), 8.42 (s, 1H). FABMS, m/z [M + H$^+$] 629. HRMS (FAB) m/z [M + H$^+$] calculated for $C_{29}H_{33}N_4O_4I$ + H: 629.1626; measured: 629.1631.

17-β-Cyanomarcfortine A (4): 9 mg, 3% yield, as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 3H), 1.12 (s, 3H), 1.44 and 1.46 (2s, 6H),1.5−3.3 (m, 10H), 1.95 and 2.73 (d, J = 15.3 Hz, 2H), 3.11 (s, 3H), 3.08 (t, 1H), 3.83 (d, J = 11.8 Hz, 1H) 4.90 and 6.32 (d, J = 7.7 Hz, 2H), 6.71 and 6.83 (d, J = 8.2 Hz, 2H), 7.60 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.74, 20.60, 23.83, 26.58, 29.80, 30.00, 30.42, 30.62, 31.97, 36.96, 46.49, 52.49, 54.35, 60.19, 60.30, 62.89, 64.41, 79.84, 115.14, 117.51, 119.72, 120.50, 124.56, 132.32, 135.0, 138.97, 146.17, 172.42, 182.25. FABMS, m/z [M + H$^+$] 503. HRMS (FAB) m/z [M + H$^+$] calculated for $C_{29}H_{34}N_4O_4$ + H: 503.2658; measured: 503.2678.

17-α-Cyanomarcfortine A (4): 6 mg, 2% yield, as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 3H), 1.11 (s, 3H), 1.43 and 1.45 (2s, 6H), 1.2−3.3 (m, 10H), 1.93 and 2.82 (d, J = 15.3 Hz, 2H), 3.15 (s, 3H), 3.12 (t, 1H), 3.68 (d, J = 11.8 Hz, 1H), 3.82 (t, J = 1 Hz, 1H), 4.94 and 6.42 (d, J = 7.7 Hz, 2H), 6.68 and 6.80 (d, J = 8.2 Hz, 2H), 7.60 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.58, 20.20, 23.47, 26.38, 28.31, 29.49, 29.72, 30.25, 32.00, 36.62, 46.14, 52.81, 53.15, 58.02, 58.71, 62.74, 63.79, 79.57, 114.61, 116.98, 117.50, 119.86, 124.28, 132.48, 135.0, 138.91, 145.92, 172.07, 182.67. FABMS, m/z [M + H$^+$] 503. HRMS (FAB) m/z [M + H$^+$] calculated for $C_{29}H_{34}N_4O_4$ + H: 503.2658; measured: 503.2669.

16,17-Dehydro-17-cyanomarcfortine A (5). Compound **3** (9.5 g, 15 mmol) was dissolved in MeOH (150 mL), and aqueous KOH (45%, 3 mL) was added. The reaction mixture was stirred at room temperature for 2 h. The mixture was diluted with water, and the resulting white precipitate was collected, washed with water, and dried overnight under vacuum to give product **5** (6.7 g, 90%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.12 (s, 3H), 1.44 and 1.46 (2s, 6H), 1.9−2.8 (m, 6H), 1.96 and 2.76 (d, J = 15.3 Hz, 2H), 3.10 (s, 3H), 3.19 (t, 1H), 3.33 and 3.85 (d, J = 11.8 Hz, 2H), 4.90 and 6.32 (d, J = 7.7 Hz, 2H), 5.50 (t, 1H), 6.71 and 6.83 (d, J = 8.2 Hz, 2H), 7.80 (s, 1H). FABMS, m/z [M + H$^+$] 501.

17-Oxomarcfortine A (6). To a solution of compound **5** (10 g, 0.02 mol) in 95% MeOH (50 mL) was added *p*-toluenesulfonic acid monohydrate (1 g) and the reaction mixture stirred at room temperature for 1 h. Triethylamine (2 mL) was then added to the mixture and the solvent evaporated. The residue was triturated with 10% aqueous sodium carbonate solution (100 mL) and the solid filtered and dried to give the title compound **6** (8.8 g, 90%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (s, 1H), 6.80 and 6.70 (d, J = 8.1 Hz, 2H), 6.32 and 4.90 (d, J = 7.7 Hz, 2H), 3.75 (ab q, 2H), 3.23 (t, 1H), 3.08 (s, 3H), 2.80 and 2.08 (d, J = 15.8 Hz, 2H), 2.65 (d, 1H), 2.49−2.21 (m, 2H), 1.98−1.45 (m, 5H), 1.46 and 1.44 (2s, 6H), 1.09 (s, 3H), 0.90 (s, 3H). HRMS (FAB) m/z [M + H$^+$] calculated for $C_{28}H_{33}N_3O_5$ + H: 492.2498; measured: 492.2478.

15,16-Dehydro-17-oxomarcfortine A (7). A solution of lithium diisopropylamide which was prepared from *n*-butyllithium (1.6 M, 9.9 mL, 15.4 mmol) in hexane and diisopropylamine (2.2 mL, 15.7 mmol) in tetrahydrofuran (THF, 20 mL) was cooled to −78 °C. A solution of **6** (2.0 g, 4.1 mmol) in anhydrous THF (20 mL) was added dropwise and the reaction mixture allowed to warm to −40 °C during 1 h. The mixture was again cooled to −78 °C and treated dropwise with phenylselenenyl chloride (19 mg, 5.2 mmol) in THF (10 mL). After 5 min the reaction was quenched with saturated $NaHCO_3$, extracted with CH_2Cl_2, dried ($MgSO_4$), and concentrated to give a yellow solid which was used without further purification. This material was dissolved in THF (150 mL) and treated with H_2O_2 (30%, 1.5 mL) at 0 °C. The cooling bath was removed and the reaction mixture stirred for 0.5 h at room temperature. The reaction was quenched by adding NaOH (1 N, 100 mL). The mixture was extracted with CH_2Cl_2 (2 × 200 mL). The extracts were combined, dried ($MgSO_4$), concentrated, and purified by silica gel chromatography (EtOAc) to give product **7** (1.3 g, 65%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (s,1H), 6.81 and 6.70 (d, J = 8.1 Hz, 2H), 6.65−6.55 (m, 1H), 6.31 and 4.91 (d, J = 7.7 Hz), 5.89 (d, 1H), 3.85 (ab q, 2H), 3.35 (dd, 1H), 3.20 (t, 1H), 3.08 (s, 3H), 2.80 and 2.10 (d, J = 15.8 Hz, 2H), 2.40 (d, 1H), 2.15−1.8 (m, 2H), 1.46 and 1.42 (2s, 6H), 1.11 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 183, 172, 164, 146.3, 139.0, 138.6, 136, 132, 124.2, 122.7, 120.3, 117.6, 115.1, 79.9, 63.6, 63.0, 59.9, 53.0, 50.2, 46.5, 36.8, 31.4, 30.0, 29.8, 29.0, 27.5, 23.7, 20.6. HRMS (FAB) m/z [M + H$^+$] calculated for $C_{28}H_{31}N_3O_5$ + H: 490.2342; measured: 490.2345.

14α-Hydroxy-15,16-dehydro-17-oxomarcfortine A (8). Compound **7** (1.29 g, 2.6 mmol) was dissolved in 1,4-dioxane (30 mL) and treated with selenium dioxide (390 mg). Following 1 h of reflux, the solvent was evaporated *in vacuo*. The residue was triturated with methylene chloride (30 mL) and filtered. The filtrate was concentrated and the residue subjected to silica gel chromatography (1:20 MeOH:EtOAc) to give product **8** (450 mg, 35%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (s, 1H), 6.85 and 6.71 (d, J = 8.1 Hz, 2H), 6.65 and 6.00 (dd, 2H), 6.31 and 4.90 (d, J = 7.7 Hz, 2H), 5.19 (br s, 1H), 4.26 and 3.55 (d, J = 12.6 Hz, 2H), 3.25 (t, 1H), 3.13 (s, 3H), 2.80 and 2.07 (d, J = 15.6 Hz, 2H), 2.30−1.8 (m, 2H), 1.45 and 1.44 (2s, 6H), 1.05 (s, 3H), 0.88 (s, 3H). HRMS (FAB) m/z [M + H$^+$] calculated for $C_{28}H_{31}N_3O_6$ + H: 506.2291; measured: 506.2280.

14α-Hydroxy-17-oxomarcfortine A (9). Compound **8** (50 mg, 0.1 mmol) was dissolved in THF (5 mL) and treated with a

solution of lithium triethylborohydride (super hydride) in THF (1 M, 0.7 mL) at −78 °C. The mixture was stirred for 0.5 h at −78 °C, quenched with MeOH (1 mL), and then concentrated. The resulting solid was subjected to silica gel chromatography (1:20 MeOH:CH_2Cl_2) to give product **9** (43 mg, 86%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82 and 6.81 (d, J = 8.2 Hz, 2H), 6.34 and 4.91 (d, J = 7.7 Hz, 2H), 4.55 (dd, 1H), 3.95 and 3.59 (d, 2H), 3.28 (t, 1H), 3.10 (s, 3H), 2.79 and 2.05 (d, J = 15.7 Hz, 2H), 2.70−1.90 (m, 5H), 2.12 and 1.80 (dd, 2H), 1.45 and 1.44 (2s, 6H), 1.10 (s, 3H), 0.90 (s, 3H). HRMS (FAB) m/z [M + H$^+$] calculated for $C_{28}H_{33}N_3O_6$ + H: 508.2447; measured: 508.2437.

14α-Hydroxymarcfortine A (10). Compound **9** (413 mg, 0.81 mmol) was dissolved in THF (20 mL) and treated with a solution of borane−methyl sulfide complex in THF (1 M, 2.43 mL) at 0 °C. The mixture was stirred for 2.25 h at 0 °C and then quenched with MeOH and stirred for an additional 0.5 h at room temperature. The solvent was evaporated, and the residue was subjected to silica gel chromatography (1:16 MeOH: EtOAc) to give product **10** (250 mg, 75%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (br s, 1H), 6.79 and 6.67 (d, J = 8.2 Hz, 2H), 6.35 and 4.91 (d, J = 7.7 Hz, 2H), 4.09 (br s, 1H), 3.25 (d, 1H), 3.10 (s, 3H), 3.0−2.80 (m, 2H), 2.65 and 1.85 (d, J = 15.8 Hz, 2H), 2.70−2.50 (m, 2H), 2.1−1.40 (m, 7H), 1.44 (s, 6H), 1.13 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21, 22, 23.49, 26.38, 26.5, 29.4 , 29.70, 37, 46.68 , 51, 53.5, 61, 62.84, 63.41, 63.65, 69, 79.60), 114.72, 117.01, 120.02, 124.5, 132.5, 135, 138.85, 146.5, 174.0, 182.5. HRMS (FAB) m/z [M + H$^+$] calculated for $C_{28}H_{35}N_3O_5$ + H: 494.2655; measured: 494.2653.

15α-Hydroxy-16,17-dehydro-17-cyanomarcfortine A (11). Compound **5** (2 g, 4 mmol) was added via a cannula in THF (100 mL) at −78 °C to an LDA solution which was prepared by adding a solution of *n*-BuLi (1.6 M, 10 mL, 16 mmol) dropwise to diisopropylamine (2.2 mL, 16 mmol) at 0 °C in THF (100 mL). The reaction mixture was allowed to stir at −78 °C for 1 h. The resulting turbid, dark-red mixture was then treated with 2-(benzenesulfonyl)-3-phenyloxiziridine (1.55 g, 6 mmol). The reaction mixture was stirred for 0.5 h at −78 °C, quenched with saturated sodium bicarbonate solution (30 mL) and extracted with methylene chloride (2 × 100 mL). The organic phase was dried ($MgSO_4$) and concentrated, and the residue was chromatographed on silica gel (3% methanol in methylene chloride) to give desired product **11** as a solid (0.6 g, 30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 3H), 1.11 (s, 3H), 1.42 and 1.44 (2s, 6H), 1.66 and 1.85 (dd, J = 9.2, 13.2 Hz, 2H), 1.7−1.9 (m, 1H), 2.01 and 2.74 (d, J = 15.8 Hz, 2H), 2.55 (dd, 1H), 3.05 (s, 3H), 3.15 (t, J = 10.5 Hz, 1H), 3.36 and 3.90 (d, J = 10.6 Hz, 2H), 4.82 (br t, 1H), 5.36 (m, 1H), 4.92 and 6.35 (d, J = 7.7 Hz, 2H), 6.71 and 6.86 (d, J = 8.2 Hz, 2H), 7.94 (s, 1H). Compound **7** (0.2 g, 10%) was also obtained as a solid. The 300 MHz ^{1}H NMR was identical with that previously described in this experimental.

15α-Hydroxy-17-oxomarcfortine A (12). Selenium dioxide (58 mg, 0.52 mmol) was added to a solution of **11** (100 mg, 0.2 mmol) in 95% EtOH (10 mL) and the reaction mixture stirred at room temperature for 16 h. The reaction was quenched by adding saturated $NaHCO_3$ (10 mL). The resulting mixture was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated, and the residue was purified by silica gel chromatography (5% methanol in methylene chloride) to give product **12** (50 mg, 50%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 3H), 1.11 (s, 3H), 1.42 and 1.44 (2s, 6H), 1.62 (t, J = 11.8 Hz, 1H), 1.89 (d, J = 10.3 Hz, 2H), 2.33 (dd, 1H), 2.13 and 2.77 (d, J = 15.8 Hz, 2H), 2.6−2.9 (m, 2H), 3.05 (s, 3H), 3.23 (t, 1H, J = 10.6 Hz), 3.71 (ab q, J = 12.6 Hz, 2H), 4.21 (m, 1H), 4.92 and 6.35 (d, J = 7.7 Hz, 2H), 6.70 and 6.86 (d, J = 8.2 Hz, 2H), 8.57 (s, 1H).

15α-Hydroxymarcfortine A (13). Compound **12** (20 mg, 0.040 mmol) was dissolved in THF (5 mL) and treated with a solution of LAH (1 M, 0.11 mL, 0.11 mmol) in THF at 0 °C. The mixture was stirred for 0.5 h at 0 °C after which a solution of $NaHCO_3$ (10%) was added. The mixture was extracted with CH_2Cl_2 (2 × 10 mL). The combined extracts were dried ($MgSO_4$), and the solvent was evaporated. Preparative thin layer chromatography of the residue on silica gel (10% MeOH in EtOAc) afforded product **13** (5 mg, 25%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 3H), 1.11 (s, 3H), 1.30 (dd, 2H), 1.44 and 1.45 (2s, 6H), 1.55 (dq, 1H), 1.7−1.9 (m, 3H), 1.89 and 2.68 (d, J = 15.6 Hz, 2H), 2.2−2.8 (m, 4H), 3.04 (t, 1H), 3.10 (s, 3H), 3.69 (d, J = 11.5 Hz, 1H), 4.24 (m, 1H), 4.90 and 6.32 (d, J = 7.7 Hz, 2H), 6.68 and 6.80 (d, J = 8.2 Hz, 2H), 8.00 (s, 1H). ^{13}C NMR (75.2 MHz, $CDCl_3$) δ 20.71, 23.91, 26.48, 29.82, 29.97, 31.50, 34.56, 37.11, 40.70, 46.47, 52.38, 52.46, 60.63, 61.50, 62.93, 64.49, 65.45, 79.81, 115.11, 117.32, 120.43, 125.00, 132.33, 135.00, 138.97, 146.10, 173.85, 182.12. HRMS (FAB) m/z [M + H] calculated for $C_{28}H_{35}N_3O_5$ + H: 494.2655; measured: 494.2655.

16α-Hydroxy-17-oxomarcfortine A (14). Compound **5** (500 mg, 1 mmol) and 4-methylmorpholine *N*-oxide (500 mg, 4.25 mmol) were dissolved in acetone/water (8:2, 30 mL). The mixture was treated with OsO_4 (0.7 mL, 2.5% wt in *tert*-butyl alcohol, obtained from Aldrich) and stirred at room temperature for 16 h. It was diluted with methylene chloride (150 mL) and washed with 10% aqueous K_2CO_3 solution (2 × 50 mL). The organic layer was dried ($MgSO_4$) and concentrated and the residue purified by silica gel chromatography (5% methanol in methylene chloride) to give product **14** as a solid (400 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (s, 3H), 1.09 (s, 3H), 1.44 and 1.46 (2s, 6H), 1.5−2.7 (m, 6H), 2.07 and 2.82 (d, J = 15.0 Hz, 2H), 3.12 (s, 3H), 3.33 (t, 1H, J = 10.5 Hz), 3.53 and 3.98 (d, J = 13.0 Hz, 2H), 4.00 (m, 1H), 4.91 and 6.32 (d, J = 7.7 Hz, 2H), 6.71 and 6.86 (d, J = 8.2 Hz, 2H), 7.79 (s, 1H). FABMS, m/z [M + H$^+$] 508.

16α-Hydroxymarcfortine A (15). Compound **14** (100 mg, 0.2 mmol) was dissolved in THF (10 mL) and treated with a solution of borane−methyl sufide complex (12 M, 0.1 mL, 1.2 mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C and then quenched with MeOH (0.5 mL) and stirred for an additional 0.5 h at room temperature. The solvent was evaporated, and the residue was subjected to silica gel chromatography (5% MeOH, 15% acetone in methylene chloride) to give product **15** (42 mg, 60% yield based on recovered starting material). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (s, 3H), 1.11 (s, 3H), 1.44 (s, 6H), 1.6−1.8 (m, 4H), 1.89 and 2.68 (d, J = 15.6 Hz, 2H), 1.9−2.1 (m, 2H), 2.56 and 2.68 (ab q, J = 10.6 Hz, 2H), 2.56 and 3.69 (ab q, J = 10.4 Hz, 2H), 3.09 (t, J = 10.6 Hz, 1H), 3.12 (s, 3H), 3.82 (m , 1H), 4.93 and 6.40 (d, J = 7.7 Hz, 2H), 6.68 and 6.80 (d, J = 8.2 Hz, 2H), 9.22 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.56, 23.80, 25.70, 26.46, 27.96, 29.88, 29.94, 31.32, 36.96, 46.56, 53.11, 59.79, 60.58, 60.83, 63.05, 64.47, 65.39, 79.84, 114.94, 117.25, 120.26, 124.76, 132.74, 135.50, 139.15, 146.17, 173.33, 183.10. HRMS (FAB) m/z [M + H] calculated for $C_{28}H_{35}N_3O_5$ + H: 494.2655; measured: 494.2622.

14-Oxomarcfortine A (16). A solution of oxalyl chloride (0.40 mL, 4.2 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated dropwise with DMSO (0.40 mL, 5.6 mmol) at −78 °C. The mixture was stirred for 1 h at −78 °C and then a solution of **10** (0.7 g, 1.4 mmol) was added dropwise in CH_2Cl_2 (5 mL). After stirring for 20 min at −78 °C, triethylamine (NEt_3, 1.4 mL, 0.01 mol) was added dropwise, and the reaction mixture was stirred at room temperature for 20 min. The mixture was partitioned between 10% Na_2CO_3 (20 mL) and CH_2Cl_2 (20 mL). The solvent was dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was subjected to silica gel chromatography (5% MeOH, 15% acetone in methylene chloride) to give **16** (52 mg, 74%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (br s, 1H), 6.79 and 6.68 (d, J = 8.1 Hz, 2H), 6.32 and 4.90 (d, J = 7.7 Hz, 2H), 3.85−3.68 (m, 1H), 3.13 (s, 3H), 3.10 (t, 1H), 2.98−1.55 (m, 7H), 2.70 and 1.95 (d, J = 15.6 Hz, 2H), 1.44 (s, 6H), 1.10 (s, 3H), 0.88 (s, 3H). HRMS (FAB) m/z [M + H] calculated for $C_{28}H_{33}N_3O_5$ + H: 492.2498; measured: 492.2510.

14*β*-Hydroxymarcfortine A (17). A solution of **16** (100 mg, 0.2 mmol) in MeOH (20 mL) at 0 °C was treated with $NaBH_4$ (50 mg, 1.3 mmol). The mixture was stirred at 0 °C for 0.5 h, quenched with 10% $NaHCO_3$ (30 mL), and extracted into CH_2Cl_2 (2 × 35 mL). The combined extracts were dried ($MgSO_4$), filtered, and concentrated to dryness. Preparative thin layer chromatography of the residue on silica gel (6% MeOH/EtOAc) afforded **17** (50 mg, 50%) and **10** (3 mg, 3%) both as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (br s, 1H), 6.80 and 6.67 (d, J = 8.2 Hz, 2H), 6.32 and 4.90 (d, J = 7.7 Hz, 2H), 3.92 and 3.68 (m, J = 11.8 Hz, 2H), 3.48−3.32 (m, 1H), 3.13 (s, 3H), 3.10 (t, 1H), 2.62−1.55 (m, 9H), 2.70 and 1.95 (d, J = 15.6 Hz, 2H), 1.45 and 1.44 (2s, 6H), 1.11 (s, 3H), 0.88 (s, 3H). HRMS (FAB) m/z [M + H] calculated for $C_{28}H_{35}N_3O_5$ + H: 494.2655; measured: 494.2653.

16-Oxomarcfortine A (18). A solution of oxalyl chloride (0.53 mL, 5.6 mmol) in anhydrous CH_2Cl_2 (30 mL) was treated dropwise with DMSO (0.53 mL, 7.5 mmol) in CH_2Cl_2 (5 mL) at −78 °C. The mixture was stirred for 0.5 h at −78 °C, and then a solution of **15** (0.93 g, 1.9 mmol) was added dropwise in CH_2Cl_2 (8 mL). After stirring for 20 min at −78 °C, triethylamine (NEt_3, 1.9 mL, 13.3 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 15 min. The mixture was partitioned between 10% Na_2CO_3 (25 mL) and CH_2Cl_2 (30 mL). The solvent was dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was subjected to silica gel chromatography (2% MeOH/$CHCl_3$) to give **18** (61 mg, 65%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (br s, 1H), 6.81 and 6.69 (d, J = 8.1 Hz), 6.33 and 4.91 (d, J = 7.7 Hz, 2H), 3.68 (d, 1H), 3.15 (s, 3H), 3.13 (t, 1H), 2.98 (m, 2H), 2.73 (d, 1H), 2.57 (m, 1H), 2.38 (m, 2H), 1.90 (d, 1H), 1.75 (m,3H), 1.45 (s, 3H), 1.43 (s, 3H), 1.11 (s, 3H), 0.86 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 208.7, 183.4, 173.7, 146.5, 139.0, 135.3, 132.1, 124.0, 120.4, 117.4, 115.1, 79.8, 64.8, 63.5, 62.9, 60.6, 59.3, 53.0, 46.1, 37.0, 36.5, 30.5, 30.0, 29.8, 29.5, 26.6, 23.9, 20.6. HRMS (FAB) m/z [M + H] calculated for $C_{28}H_{33}N_3O_5$ + H: 492.2498; measured: 492.2487.

16β-Hydroxymarcfortine A (19). A solution of **18** (210 mg, 0.4 mmol) in MeOH (10 mL) at 0 °C was treated with $NaBH_4$ (40 mg, 1.1 mmol). The mixture was stirred at 0 °C for 0.5 h, quenched with 10% $NaHCO_3$ (40 mL), and extracted into CH_2Cl_2 (2 × 35 mL). The combined extracts were dried ($MgSO_4$), filtered, and concentrated to dryness. Preparative thin layer chromatography of the residue on silica gel (10% MeOH/$CHCl_3$) afforded **19** (150 mg, 71%) and **15** (8 mg, 4%) both as white solids. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (br s, 1H), 6.80 and 6.67 (d, J = 8.2 Hz, 2H), 6.35 and 4.91 (d, J = 7.7 Hz, 2H), 3.68 (m, 2H), 3.11 (s, 3H), 3.05 (t, 1H), 2.85 (m, 1H), 2.68 (d, 1H), 2.41 (d, 1H), 2.22 (m, 1H), 2.13 (t, 1H), 1.60−1.95 (m, 5H), 1.44 (s, 3H), 1.11 (s, 3H), 0.83 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 182.4, 173.4, 146.1, 139.0, 135.3, 132.5, 124.8, 120.4, 117.3, 115.1, 79.8, 67.9, 64.4, 63.0, 61.2, 59.6, 53.0, 46.5, 37.1, 30.9, 30.0, 29.8, 29.7, 26.4, 23.9, 20.7. FABMS, m/z [M + H$^+$] 493.

Supporting Information Available: NMR spectra of new compounds (19 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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