Cortamidine Oxide, a Novel Disulfide Metabolite from the New Zealand Basidiomycete (Mushroom) *Cortinarius* Species

Gillian M. Nicholas,[†] John W. Blunt,* and Murray H. G. Munro*

Department of Chemistry, University of Canterbury, PB 4800, Christchurch, New Zealand

Three disulfide metabolites were isolated from the fruiting bodies of the basidiomycete (mushroom) *Cortinarius* sp., collected in the Catlins, New Zealand. The structures of these compounds were determined as the unsymmetrical disulfide cortamidine oxide (**1**), 2,2′-dithiobis(pyridine *N*-oxide) (**2**), and the symmetrical disulfide **3**. Both **1** and **2** showed significant antimicrobial activity and cytotoxicity. 2,2′-Dithiobis(pyridine *N*-oxide) (**2**) and the symmetrical disulfide **3** are assumed to be artifacts of the isolation procedure.

Many species in the *Cortinarius* genus are known to produce biologically active natural products, including pigments and toxins. Orellanine, a bipyridyl *N*-oxide-containing compound, is a potent toxin affecting renal epithelial cells that has been isolated from a number of European species in this genus, including *C. orellanus* and *C. speciosissimus*.[1,2] *N*-oxide- and *N*-hydroxy-containing natural products have been isolated from a variety of other sources including plants, bacteria,[3] marine sponges,[4,5] and dinoflagellates.[6] The assignment of these functionalities can be hindered due to lack of direct evidence. Comparison of NMR data with those of known compounds that lack the *N*-oxidation, or the synthesis of the fragment in question, is often required. More recently a proton-detected 2D NMR experiment, GHNMQC, which shows correlations to nitrogen in the second dimension, has been useful in the assignment of aliphatic *N*-oxide functionalities.[7]

During our continued investigation of fungi collected in New Zealand,[8] a *Cortinarius* sp. collected in the Catlins region of New Zealand was targeted for investigation after initial crude extracts (both organic and aqueous) showed significant antimicrobial activity and cytotoxicity against the P388 murine leukaemia cell line. Fractionation of further extracts yielded cortamidine oxide (**1**), an unsymmetrical disulfide containing both a 2-thiopyridyl *N*-oxide and an *N*-substituted cysteine amino acid residue. 2,2′-Dithiobis(pyridine *N*-oxide) (**2**)[9] and compound **3** were also isolated and characterized as the symmetrical disulfides of each half of cortamidine oxide (**1**). As **1** was observed to be spontaneously converting to 2,2′-dithiobis(pyridine *N*-oxide) (**2**) and compound **3** during isolation, it is assumed that compounds **1** and **3** are artifacts of the isolation procedure and not true metabolites of the *Cortinarius* sp.

Results and Discussion

Bioassay-guided fractionation of methanolic extracts of *Cortinarius* sp. utilizing repeated reverse-phase column chromatography yielded compounds **1**–**3**. A major difficulty encountered in this isolation work was the observed conversion of compound **1** into compounds **2** and **3**.

HREIMS on compound **2** gave an apparent molecular formula $C_{10}H_8N_2S_2$. Four aromatic hydrogens, δ 7.56, 7.46, 7.29, 8.24 in D_2O, were observed in the ^{1}H NMR spectrum,
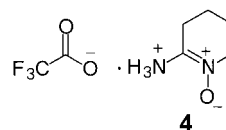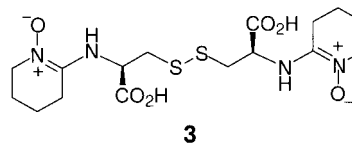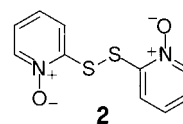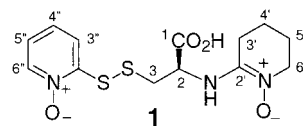
which was consistent with a 2-substituted pyridine system. The UV absorbance maximum at 240 nm was also consistent with a pyridine ring. The ^{1}H and ^{13}C NMR data of compound **2** were not consistent with 2,2′-dithiobispyridine, but were, however, consistent with 2,2′-dithiobis(pyridine *N*-oxide) (**2**).[9] The presence of the *N*-oxides was confirmed with the observation of a molecular ion at *m/z* 253 in FABMS and the fragmentation pattern observed under EIMS.[10]

From HRFABMS a molecular formula of $C_{13}H_{17}O_4N_3S_2$ was determined for cortamidine oxide (**1**). Ionization by EI gave no parent ion, but the fragmentation pattern was similar to that observed for **2**. The ^{1}H NMR spectrum of compound **1** also contained four aromatic proton signals (δ 7.26, 7.56, 7.96, 8.16), at chemical shifts similar to those observed for **2**, but included one methine (δ 3.95) and five methylenes between δ 1.5 and 3.2. The ^{13}C NMR spectrum contained a carbonyl resonance at δ 177.7 and resonances from two methylenes (δ 42.3 and 54.2) and one methine (δ 57.1) that were consistent with proximity to heteroatoms. NMR correlation data for **1** allowed the identification of three isolated spin systems (see Table 1, Supporting Information). The first fragment, a 2-substituted pyridine ring (H3″–H6″), was confirmed with correlations in the

* Corresponding authors. For M.H.G.M.: Tel: +64 3 364 2434. Fax: +64 3 364 2110. E-mail: m.munro@chem.canterbury.ac.nz.
† Current address: NIDDK, NIH, Bldg. 8, Rm 1A-06, Bethesda, MD 20892.

COSY, HMQC, and HMBC NMR spectra. The UV absorbance maximum observed for **1** (240 nm) was consistent with a pyridine ring, as in **2**. The *N*-oxidation of the pyridine ring was deduced from comparison of NMR chemical shift data for compounds **1** and **2** and also the fragmentation pattern in the mass spectrum of compound **1**. HREIMS on the fragment ion at m/z 127.0115 gave a molecular formula of C_5H_5NOS, which was also seen in the spectrum of **2**. The second spin system assigned from the COSY spectrum was that of a cysteine residue (H-2–H-3). A COSY correlation was observed from the methine proton (δ 3.95, H-2) to the methylene protons at δ 3.05 and 3.19 (H-3a/b). An HMBC correlation from the methine proton (δ 3.95, H-2) to the carbonyl (δ 177.7, C-1) and the methylene carbons (δ 42.3, C-3) completed the assignment of the cysteine residue. The chemical shift of C-3 (δ 42.3) was consistent with placement adjacent to a disulfide bond. As the molecular formula of compound **1** contained two sulfur atoms, a disulfide bond was assigned between the 2-substituted pyridine ring and the cysteine residue. This was consistent with the observed disproportionation of **1** to the symmetrical disulfides **2** and **3**. The third fragment that was assigned contained four consecutive methylenes (H-3′–H-6′), assigned from the COSY spectrum. These methylenes showed correlations from H-3′ to H-4′, then to H-5′, and finally to H-6′. The methylene at δ 54.2 was consistent with being adjacent to a nitrogen atom, even if the carbon was slightly downfield from that expected for the proposed amidine structure. This sequence of four carbons (C-3′–C-6′) was assigned as part of a 3,4,5,6-tetrahydropyridine ring based on HMBC correlations from H-3′, H-4′, and H-6′ to the quaternary carbon (δ 153.3, C-2′). A weak correlation in the HMBC spectrum from H-2 (δ 3.95) to a quaternary carbon (δ 153.3) suggested that the cysteine residue might be connected to the 3,4,5,6-tetrahydropyridine through nitrogen. Unfortunately, the closeness in chemical shift of the quaternary carbon of the tetrahydropyridine ring (δ 153.3) to that of the pyridine *N*-oxide (δ 153.4) did not allow a definitive assignment. This connection was confirmed during the structure elucidation of the related compound **3** (vide infra) from observation of a correlation in the HMBC spectrum of compound **3** from the equivalent H-2 (now at δ 4.16) to the quaternary C-2′ (now at δ 152.9). The connection between the tetrahydropyridine and the cysteine fragment of compound **1** was therefore confirmed from the assignment for compound **3**.

At this point all the elements of unsaturation from the molecular formula had been accounted for, but one oxygen atom remained unassigned. As no further carbon atoms in the molecule were consistent with oxidation, that left only the heteroatoms. The chemical shifts of C-3 and C-6″ were not consistent with oxidation of either of the sulfur atoms of the disulfide bond. The isolation of 2,2′-dithiobis-(pyridine *N*-oxide) (**2**) and compound **3**, from disulfide exchange, supported this conclusion. That therefore left the two nitrogen atoms, that of the cysteine residue or that of the 3,4,5,6-tetrahydropyridine ring, as the potential sites of oxidation. There were no fragments in the mass spectra obtained on compound **1** (EI, FAB, ES) that could discriminate between these two possible sites of oxygenation. Oxidation on nitrogen shifts adjacent quaternary imine or amidine ^{13}C NMR resonances upfield, while the other adjacent carbon (and proton) resonances shift downfield.[11] These differences in carbon chemical shifts are characteristic, as oxidation can move the carbon resonance by $\Delta\delta_C$ 10. In contrast, the differences in the proton resonances

on adjacent carbons are usually small and shifted downfield by $\Delta\delta$ 0.2–0.4. The protonation state is, of course, also a factor. The ^{13}C NMR data for 2,3-dihydroisoquinoline, 2,3-dihydroisoquinoline *N*-oxide, and the corresponding protonated forms are possibly the best examples to illustrate this. For both the protonated and free-base forms an increase of $\Delta\delta$ ∼10–11 was observed in the chemical shift of the α-carbon on oxidation.[12] Another example is the addition of an *N*-hydroxyl function to the guanidinium group of saxitoxin to form neosaxitoxin. This moved the methine carbon adjacent to nitrogen downfield by $\Delta\delta$ 11.2.[6] Unfortunately, the functional group proposed for **1** is not a guanidinium, nor a nitrone, but rather an amidine *N*-oxide. Therefore, the direct comparison of chemical shift data is inappropriate, but the chemical shifts observed in these examples can be used as a guide. The only example of an amidine *N*-oxide in the literature with ^{13}C and ^{1}H NMR data was for 2-amino-5,5-dimethylpyrroline *N*-oxide. Regrettably, the ^{13}C NMR data were not complete.[13] In **1** the chemical shift of the cysteine α-carbon was slightly downfield compared to that of cystine, but only by $\Delta\delta$ 2.4.[14] If no oxygen were present on the adjacent nitrogen, the chemical shift of C-6′ in **1** would be expected at around δ 45.[12] Therefore, with the chemical shift of C-6′ being assigned as δ 54.2 the final oxygen from the molecular formula was assigned to the nitrogen of the 3,4,5,6-tetrahydropyridine ring.

To unambiguously confirm the 3,4,5,6-tetrahydropyridine ring as the site of oxidation, 2-amino-3,4,5,6-tetrahydropyridine-*N*-oxide (**4**) was synthesized (see Supporting Information).[15] This compound had previously been synthesized, but no NMR data were reported.[16] The ^{1}H NMR data for 2-amino-3,4,5,6-tetrahydropyridine *N*-oxide (**4** as the TFA salt, in D_2O) were consistent with those of the relevant fragment in compound **1** (as the TFA salt, in D_2O). The ^{13}C NMR data, particularly for C-2′ and C-6′ of **1** (as the free base, in D_2O), were consistent with those of 2-amino-3,4,5,6-tetrahydropyridine *N*-oxide (**4**, in D_2O with pyridine). The position of oxygenation in **1** was therefore confirmed as the nitrogen of the 3,4,5,6-tetrahydropyridine ring, as assigned from the NMR data.

The amidine *N*-oxide functionality in **1** could exist as a number of tautomers. There could also be pH-dependent chemical shift changes, as was indicated by the observed ^{1}H NMR chemical shifts for **1** (in D_2O) as the TFA salt and the free base. The tautomeric behavior of 2-amino-5,5-dimethyl-1-pyrroline and the corresponding *N*-oxide has been reported using IR spectroscopy in $CHCl_3$. It was concluded that both these compounds were aminopyrroline derivatives and not the tautomeric iminopyrrolidines, at least in $CHCl_3$ solution.[17] The *N*-hydroxy protons in batzellandines G–I were observed in DMSO-d_6 as a sharp singlet at δ 10.51.[4] No such resonance was observed by ^{1}H NMR spectroscopy for the natural product **1** or 2-amino-3,4,5,6-tetrahydropyridine *N*-oxide (**4**), even when DMSO-d_6 was used as the solvent. The fragment ions observed by electrospray ionization (ESI) mass spectrometry were consistent with the oxygen anion because of the loss of oxygen (16) from a number of key fragment ions. A loss of oxygen (16) was also observed in the FAB mass spectrum of 2-amino-3,4,5,6-tetrahydropyridine *N*-oxide (**4**), which supports the assignment that cortamidine oxide (**1**) exists as the *N*-oxide tautomer.

Although cortamidine oxide (**1**) is optically active, the stereochemistry of the cysteine residue was not ascertained due to the problems encountered with instability of these compounds and the added complications of disulfide ex-

change. It is depicted in the structural diagram as corresponding to that of C-2 in the naturally occurring enantiomer of cysteine.

The third compound to be isolated from *Cortinarius* sp. was a symmetrical disulfide (**3**) of the nonaromatic half of cortamidine oxide (**1**). The molecular ion for the sodium salt (MNa$^+$) of compound **3** was observed (HRFABMS) at *m/z* 457.1193, which corresponded to a molecular formula of $C_{16}H_{26}O_6N_4S_2Na$. The ^{13}C and ^1H NMR data for **3** were almost identical ($\Delta\delta_C$ 0.0−1.2; $\Delta\delta_H$ 0.00−0.24) to those of the relevant part of cortamidine oxide (**1**). 2D NMR experiments confirmed the connectivity through the molecule as for **1** (see Table 1, Supporting Information). The crucial HMBC correlation from the methine proton H-2 (δ 4.16) to the quaternary carbon C-2′ (δ 152.9) was observed in an HMBC experiment when optimization for long-range coupling constants (J_{nxh}) was set at 4 Hz.

It is suggested that the two symmetrical dimers (**2** and **3**) arise via disulfide exchange from the unsymmetrical cortamidine oxide (**1**). Compounds **1** and **2** both contained a 2-thiopyridine *N*-oxide functionality. This functionality is consistent with the biological activity (cytotoxicity and antimicrobial activity) observed for compounds **1** and **2**. The zinc salt of 2-thiopyridine *N*-oxide is in fact the active ingredient in many antidandruff shampoos.[18] Compounds containing a pyridine *N*-oxide functionality have been reported from species in the *Cortinarius* genus before, namely, the toxin orellanine.[1] Both **1** and **3** contained a fragment assigned as an amidine *N*-oxide. No natural products were found in the literature that contained this functionality, although a number of synthetic compounds have been reported, including 2-amino-6,6-dimethyl-3,4,5,6-tetrahydropyridine *N*-oxide[16] and 2-amino-5,5-dimethylpyrroline *N*-oxide.[11]

Experimental Section

General Experimental Procedures. Proton-detected NMR spectra were recorded on a Varian Unity 300 spectrometer using a Nalorac Z.spec MID300 3 mm indirect detection probe or a pulsed field gradient MLD driver with a 5 mm indirect detection probe, operating at 300 MHz and 23 °C. Chemical shifts were referenced to the appropriate solvent peaks. A Kratos MS80 mass spectrometer operated at 4 kV was used for mass spectrometry. EI was performed at 70 eV. FAB used an Ion Tech ZN11FN ion gun using Xe as the reagent gas, operating at 8 kV and 2 mA, with either *m*-nitrobenzyl alcohol (NOBA), "magic bullet" (m-b; 50% thioerythritol/thiothreitol), or glycerol as the matrix. Samples analyzed by electrospray ionization mass spectrometry (ESIMS) were dissolved in a solution of 1:1 CH_3CN/H_2O which was acidified with HOAc for the positive ion mode or basified with NaOH for the negative ion mode. IR spectra were recorded using a Shimadzu 8201PC series FTIR. Spectra were run neat on a KBr disk or in CHCl$_3$. ORD spectra were measured on a Jasco model J-20 recording spectropolarimeter in MeOH (spectranorm grade) at the stated concentration. Analytical HPLC was performed on a Philips PU4100 liquid chromatograph using a Rainin C18 (3 μm, 100 Å, 4.6 × 100 mm) column. All column chromatography was performed using C18 reversed-phase packing (Bakerbond (40 μm). TLC was on silica, DIOL (Merck), or C18 (Whatman).

Organism. Fruiting bodies of the *Cortinarius* sp. (95CA1-26; 210 g) were initially collected in the Catlins Forest Park, Southland, New Zealand, in May 1995, and a second collection was made in May 1996 (96CA2-1,3,4,11; 230 g). The sites of collection were in Northofagus forest from the Franks Stream Entrance between the Tawanui Camp and The Wisp. The basidiomycete was found on the forest floor among a substrate of moss and litter. The cap was typically 3−6 cm in diameter,

with a hard skin, which was light gray/green in color and was observed to be convex in young samples and flat in older samples. The gills were light brown tending to yellow on maturation, the cortina was visible, and the stem was 4−8 cm and yellow/light brown in color. The spore print was cinnamon brown. Identification to the genus level was by Associate Professor A. Cole, Department of Plant and Microbial Sciences, University of Canterbury. Voucher samples are held at the University of Canterbury. A voucher sample has also been deposited with Dr. Roy Watling, Edinburgh Herbarium, Edinburgh Botanic Gardens, UK.

Extraction and Isolation A sample (200 g) of *Cortinarius* sp. fruiting bodies was soaked overnight in MeOH/H_2O (60:40, 600 mL). The solvent was decanted and the residue blended with MeOH (300 mL) and filtered. This was repeated three times. The MeOH was removed under vacuum and the H_2O removed by freeze-drying to give a brown, cytotoxic extract [4.09 g IC$_{50}$ (P388) 0.9 μg/mL], which also showed significant antimicrobial activity (5−15 mm, 0.15 mg/disk) against *Escherichia coli, Bacillus subtilis, Candida albicans, Tricophyton mentagrophytes*, and *Cladosporium resinae*.

The extract (3.7 g) was processed in three batches (1.15, 0.70, and 1.8 g). These portions were each loaded onto a reversed-phase (C18) column (100 g, 3 × 600 cm) and eluted with a stepped gradient from H_2O through MeOH. Twenty fractions were collected from each of the three columns, with fractions analyzed by TLC (C18) and submitted for assay against the P388 cell line. Combinations were made by comparison of the TLC and assay results. The most cytotoxic combined fraction (63.1 mg, IC$_{50}$ 0.043 μg/mL) eluted in 60−80% MeOH/H_2O was identified as 2,2′-dithiobis(pyridine *N*-oxide (**2**). The more polar band of cytotoxicity (317.4 mg, IC$_{50}$ 0.4 μg/mL) eluted in H_2O to 20% MeOH/H_2O. The ^1H NMR spectrum of this sample contained aromatic signals similar to those identified as compound **2**. This fraction was further purified on a reversed-phase (C18) column (100 g, 3 × 600 cm) and eluted with a gradual stepped gradient from H_2O through 40% MeOH and then quickly through MeOH. Twenty fractions were again collected, analyzed, and assayed. The combined bioactive fraction (17.4 mg) that eluted in 60% MeOH was again 2,2′-dithiobis(pyridine *N*-oxide) (**2**). The more polar cytotoxic band was concentrated in one fraction (45.7 mg, IC$_{50}$ 0.17 μg/mL), which eluted with 10% MeOH/H_2O. This fraction was further purified on another C18 column (8 g, 1 × 10 cm), which was eluted with a gradual stepped gradient from H_2O through 10% MeOH/H_2O and then to MeOH. Seventeen fractions were collected, analyzed, and assayed to give compound **1** (4.4 mg; IC$_{50}$ 0.12 μg/mL), which had eluted with 10% MeOH, and compound **3** (2.4 mg; inactive against P388), which had eluted first from the column with H_2O.

During overnight NMR experiments on compound **1** a number of new signals arose from the baseline which corresponded to compound **3**.

Cortamidine oxide (1): [α]$_D^{22}$ −10.0° (*c* 0.03, MeOH); UV (MeOH) λ_{max} 190, 240 nm; FTIR (CaF$_2$ plate, film) ν_{max} 3263 (br), 2927, 2856, 1666, 1660, 1651, 1645, 1614, 1573, 1558, 1539, 1504, 1463, 1456, 1417, 1379, 1326, 1265, 1188, 1083, 1026 cm^{-1}; ^1H NMR (D$_2$O) δ 8.16 (1H, d, *J* = 6.4 Hz, H-6″), 7.96 (1H, d, *J* = 8.3 Hz, H-3″), 7.56 (1H, m, H-4″), 7.26 (1H, m, H-5″), 3.95 (1H, dd, *J* = 3.9, 8.7 Hz, H-2), 3.39 (2H, m, H-6′), 3.19 (1H, dd, *J* = 3.9, 14.2 Hz, H-3b), 3.05 (1H, dd, *J* = 8.9, 14.2 Hz, H-3a), 2.15 (1H, m, H-3′b), 2.06 (1H, m, H-3′a), 1.66 (2H, m, H-5′), 1.50 (2H, m, H-4′); (DMSO) δ 8.34 (1H, d, *J* = 6.4 Hz, H-6″), 7.96 (1H, d, *J* = 7.4 Hz, H-3″), 7.48 (1H, d, *J* = 7.8 Hz, H-4″), 7.31 (1H, d, *J* = 6.3 Hz, H-5″), 1.72 (2H, m, H-5′), 1.60 (2H, m, H-4′); (D$_2$O, TFA salt) δ 8.23 (1H, d, *J* = 6.5 Hz, H-6″), 8.01 (1H, d, *J* = 8.3 Hz, H-3″), 7.66 (1H, t, *J* = 8.1 Hz, H-4″), 7.34 (1H, t, *J* = 6.6 Hz, H-5″), 4.56 (1H, dd, *J* = 3.9, 9.7 Hz, H-2), 3.60 (2H, m, H-6′), 3.42 (1H, dd, *J* = 4.0, 14.8 Hz, H-3b), 3.18 (1H, dd, *J* = 9.3, 14.8 Hz, H-3a), 2.55 (2H, m, H-3′), 1.82 (2H, m, H-5′), 1.66 (2H, m, H-4′); ^{13}C NMR (D$_2$O) δ 177.7 (s, C-1), 153.4 (s, C-2″), 153.3 (s, C-2′). 140.9 (d, C-6″), 133.6 (d, C-4″), 124.9 (d, C-3″), 124.9 (d, C-5″), 57.1 (d, C-2), 54.2 (t, C-6′), 42.3 (t, C-3), 24.9 (t, C-3′), 23.5 (t, C-5′), 19.5 (t, C-4′); EIMS *m/z* 156, 149, 127, 111, 79. 78, 67, 52;

HREIMS, no molecular ion was observed m/z 127.0115 (calcd for C_5H_5NOS, 127.0091); FABMS m/z 366 (MNa$^+$), 382 (MK$^+$), 398 (MNa$_2$$^+$), 404 (MNaK$^+$), 420 (MK$_2$$^+$); HRFABMS m/z 344.0710 (calcd for $C_{13}H_{18}O_4N_3S_2$, 344.0738); ESIMS (positive ion mode) 50 V m/z 709 (2MNa$^+$), 687 (2MH$^+$), 344 (MH$^+$), 217, 169; 150 V m/z 251, 217, 123, 82; ESIMS (negative ion mode) 50 V m/z 364 [(MNa − 2H)$^-$], 215, 158; 60 V m/z 364, 267, 215, 158, 123; 120 V m/z 364, 274, 215, 158, 126, 123.

2,2′-Dithiobis(pyridine *N*-oxide) (2): UV (MeOH) λ_{max} 200, 240 nm; IR (CaF$_2$ plate, film) ν_{max} 3361 (br), 1660 (br), 1461, 1417 cm^{-1}; ^{1}H NMR (D$_2$O) δ 7.56 (1H, d, J = 8.3 Hz, H-3″), 7.46 (1H, d, J = 8.3 Hz, H-4″), 7.29 (1H, m, H-5″), 8.24 (1H, d, J = 6.3 Hz, H-6″); ^{13}C NMR (D$_2$O) δ 150.5 (s, C-2″), 124.6 (d, C-3″), 133.6 (d, C-4″), 125.7 (d, C-5″), 141.1 (d, C-6″); HREIMS (M − 32)$^+$ 220.0128 (calcd for $C_{10}H_8N_2S_2$, 220.0128); EIMS m/z 222, 221, 220 (20%), 156 (14%), 155, 142, 129, 128, 127, 126, 111, 110 (84%), 82, 80, 79, 78 (100%); FABMS (glycerol) m/z 253 (MH$^+$); (MBS/NIST Library, dipyrithione MW 252, $C_{10}H_8N_2O_2S_2$; EIMS m/z (M$^+$, not observed) 222, 221, 220 (95%), 189, 188, 157,156 (95%), 155, 142, 130, 129, 128, 127, 126, 111, 110 (50%), 83, 78 (100%), 67, 52, 48.

Compound 3: ^{1}H NMR (D$_2$O) δ 4.16 (1H, dd, J = 4.2, 8.7 Hz, H-2), 3.40 (2H, m, H-6′), 3.12 (1H, dd, J = 4.3, 14.6 Hz, H-3b), 2.90 (1H, dd, J = 8.8, 14.7 Hz, H-3a), 2.39 (1H, m, H-3′b), 2.31 (1H, m, H-3′a), 1.71 (2H, m, H-5′), 1.59 (2H, m, H-4′); (D$_2$O, TFA salt) δ 4.43 (1H, dd, J = 3.4, 9.0 Hz, H-2), 3.9, 14.5 Hz, H-3b), 3.64 (2H, m, H-6′), 3.32 (1H, dd, J = 3.00 (1H, dd, J = 9.1, 14.9, H-3a), 2.64 (1H, m, H-3′b), 2.60 (1H, m, H-3′a), 1.85 (2H, m, H-5′), 1.71 (2H, m, H-4′); (DMSO) δ 3.84 (1H, m, H-2), 3.43 (2H, m, H-6′), 3.12 (1H, dd, J = 3.5, 13.7 Hz, H-3b), 2.92 (1H, dd, J = 8.3, 13.2 Hz, H-3a), 2.42 (2H, m, H-3′), 1.74 (2H, m, H-5′), 1.64 (2H, m, H-4′); ^{13}C NMR (D$_2$O) δ 178.5 (s, C-1), 152.9 (s, C-2′), 57.3 (d, C-2), 54.1 (t, C-6′), 42.1 (t, C-3), 25.0 (t, C-3′), 23.5 (t, C-5′), 19.6 (t, C-4′); ESIMS (positive ion mode) 50 V m/z 435 (MH$^+$), 218 (MH$_2$$^{2+}$); 100 V m/z 435 (MH$^+$), 251, 217, 185, 169; 200 V m/z 435, 251, 229, 185, 169, 141, 125, 123, 115, 82; ESIMS (negative ion mode) 50 V m/z 455 ((MNa − 2H)$^-$), 433 ((M − H)$^-$), 216; 75 V m/z 455, 433, 249, 183; 125 V m/z 455, 433, 271, 249, 183; 153, 139, 123; 200 V m/z 455, 433, 271, 249, 183; 153, 139, 123; HRFABMS (NOBA) m/z 457.1193 (MNa$^+$) (calcd for $C_{16}H_{26}$-$O_6N_4S_2Na$, 457.1192).

2-Amino-3,4,5,6-tetrahydropyridine *N*-oxide (4). The method of ring closure used was the same as that previously reported for 5-methyl-5-nitrovaleronitrile, which used zinc and ammonium chloride to provide oxidation to 2-amino-6,6-dimethyl-3,4,5,6-tetrahydropyridine *N*-oxide (as the hydrochloride salt).[15] To 5-nitropentan-1-nitrile (see Supporting Information) (100 mg, 0.78 mmol) and NH$_4$Cl (33 mg, 0.62 mmol) in MeOH/H$_2$O (1:1, 10 mL) at 0 °C was added Zn dust (139 mg, 2.13 mmol). The reaction was warmed from 0 °C to room temperature, stirred for 16 h, filtered, and acidified to pH 2 with trifluoroacetic acid. The solvent was removed in vacuo to yield the trifluoroacetate of 2-amino-3,4,5,6-tetrahy-

dropyridine *N*-oxide (**4**) as a light orange oil: FTIR (KBr plate, film) ν_{max} 3500−2800 (br), 1720−1620 (br), 1454, 1427, 1201, 1137, 1026, 1001, 839, 800, 723 cm^{-1}; ^{1}H NMR (D$_2$O, TFA salt) δ 3.54 (2H, t, J = 6.3 Hz, H-6), 2.55 (2H, t, J = 6.4 Hz, H-3), 1.83 (2H, m, H-5), 1.63 (2H, m, H-4); ^{13}C NMR (D$_2$O, TFA salt) δ 152.8 (s, C-2), 53.1 (t, C-6), 27.8 (t, C-3), 23.8 (t, C-5), 18.9 (t, C-4); FABMS m/z 229 (M$_2$H$^+$), 115 MH$^+$, 99 (MH$^+$ − 16); HRFABMS 115.0867 (MH$^+$) (calcd for $C_5H_{11}N_2O$, 115.0871).

Supporting Information Available: ^{1}H NMR, ^{13}C NMR spectra, and 2D-correlation data for cortamidine oxide (**1**), 2,2′-dithiobis-(pyridine *N*-oxide) (**2**), and the symmetrical disulfide **3**. Also available are experimental details for the synthesis of 2-amino-3,4,5,6-tetrahydropyridine *N*-oxide (**4**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Antkowiak, W. Z.; Gessner, W. P. *Tetrahedron Lett.* **1979**, *21*, 1931−1934.
(2) Feifel, E.; Rohromoser, M. M.; Gstraunthaler, G. *Sydowia Beihefte* **1995**, *10*, 48−61.
(3) Clemo, G. R.; McIlwain, H. *J. Chem. Soc.* **1938**, 479−483.
(4) Matsunaga, S.; Shinoda, K.; Fusetani, N. *Tetrahedron Lett.* **1993**, *34*, 5953−5954.
(5) Patil, A. D.; Freyer, A. J.; Taylor, P. B.; Carte, B.; Suber, G.; Johnson, R. K.; Faulkner, D. J. *J. Org. Chem.* **1997**, *62*, 1814−1819.
(6) Shimizu, Y.; Hsu, C.; Fallon, W. E.; Oshima, Y.; Miura, I.; Nakanishi, K. *J. Am. Chem. Soc.* **1978**, *100*, 6791−6793.
(7) Farley, K. A.; Bowman, P. B.; Brumfield, J. C.; Crow, F. W.; Dunolke, W. K.; Guido, J. E.; Robins, R. F.; Thamann, T. J.; Vonderwell, B. S.; Martin, G. E. *Magn. Reson. Chem.* **1998**, *36*, 11−16.
(8) Nicholas, G. M.; Blunt, J. W.; Cole, A. L. J.; Munro, M. H. G. *Tetrahedron Lett.* **1997**, *38* (42), 7465−7468.
(9) *Beilstein, 3rd/4th Ed.* **1978**, *21*, 381.
(10) MBS/NIST Mass Spectral Library.
(11) Torssell, K. B. G. *Nitrile Oxides, Nitrones, and Nitronates in Organic Synthesis, Organic Nitro Chemistry (2)*; VCH Publishers: Weinheim, 1988.
(12) ^{13}C NMR database on microfiche, Department of Chemistry, University of Canterbury, New Zealand.
(13) Bandara, B. M. B.; Hinojosa, O.; Bernfsky, C. *J. Org. Chem.* **1992**, *57*, 2652−2657.
(14) Pretsch, E.; Seibl, J.; Simon, W.; Clerc, T. *Tables of Spectral Data for Structure Determination of Organic Compounds*; Springer-Verlag: New York, 1989.
(15) The authors acknowledge the assistance of Dr. Andrew Phillips with the preparation of the compounds in this sequence.
(16) Aurich, H. G.; Trösken, J. *Chem. Ber.* **1972**, *105*, 1216.
(17) Forrester, A. R.; Thomson, R. H. *Spectrochim. Acta* **1963**, *19*, 1481.
(18) Marks, R.; Pearse, A. D.; Walker, A. P. *Br. J. Dermatol.* **1985**, *112*, 415−422.