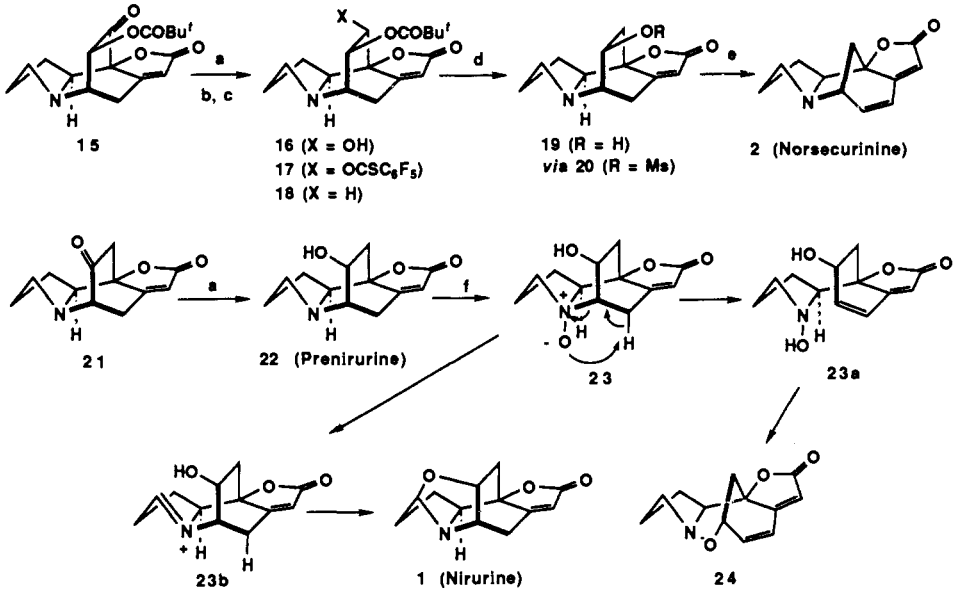
Scheme III[a]



[a] (a) $NaBH_4$/MeOH (64% from **12**/**13**). (b) C_6F_5OCSCl/DMAP/CH_2Cl_2 molecular sieves (100%). (c) *n*-Bu_3SnH/AIBN/benzene, reflux 15 min (100%). (d) NaOMe/MeOH (82% from **19**). (e) MsCl/Et_3N/DMAP/CH_2Cl_2, 25 °C for 15 min (91%). (f) MCPBA/MeOH.

incorrect configuration at C-4, and consequently we were faced with the daunting task of inverting at C-4 in a molecule where S_N2 chemistry is obviously sterically encumbered, combined with the problem of differentiating between the two secondary hydroxyl groups, Scheme II. Attempts to invert at C-4 in **10** interestingly led to the rearranged product **11** (87%), which now has the norsecurinine skeleton.[7]

Pivaloylation of the diol **10** gave a mixture of monopivaloates **12** and **13** (1:2) (100%). If a mixture of **12** and **13** is allowed to stand in methanol for a few minutes, the ^1H NMR spectrum shows that rapid equilibration takes place to give predominantly **13**. Swern-Moffatt oxidation of the mixture of **12** and **13** gave **15**, along with a small amount of the isomer **14**. Evidently **13** is more rapidly oxidized than **12**. Consequently, while pivaloylation of **10** is not regiospecific, the subsequent equilibration allows the ketone **15** to be made without any separation from isomeric compounds, Scheme II. Reduction of **15** gave the inverted alcohol **16** (64% overall from **12**/**13**), which was converted into its pentafluorophenol thiono ester derivative **17** (100%) and deoxygenated to give **18** (100%).[8]

The alcohol **19** (94%) cleanly rearranged to norsecurinine (**2**) (91%, overall yield of 10.5% through 13 steps from 3-hydroxypyridine) on exposure to standard mesylation conditions. Swern-Moffatt oxidation of **19** gave the ketone **21**, which was reduced to give prenirurine (**22**) (82% overall from **19**), the speculated biogenetic precursor to nirurine (**1**).[1] Treatment of **22** with *m*-chloroperoxy benzoic acid in methanol gave the unstable *N*-oxide **23**, which rapidly rearranged to **24**, presumably via the Cope elimination product **23a**.[7] The *N*-oxide **23** is more stable in dichloromethane, and treatment with trifluoroacetic anhydride gave small amounts of **1** (ca. 10%), but largely **24**, Scheme III.[9] In view of the low yield of **1** because of the competing rearrangement, it seems likely that **22** is not the biogenetic precursor to **1**, and that aminal formation (oxidation adjacent to nitrogen) takes place at an earlier stage.

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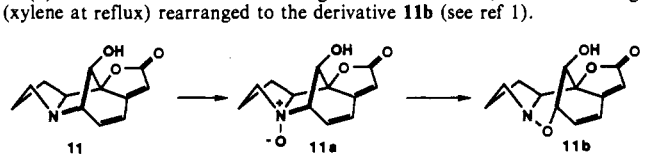
Supplementary Material Available: General spectral details for compounds **6**, **7**, **9**, **10**, **16**-**19**, and **22**, details of the X-ray structure determination of **11**, and tables of fractional coordinates, isotropic thermal parameters, anisotropic thermal parameters, bond lengths, and bond angles for **11** (15 pages); listing of observed and calculated structure factors for **11** (5 pages). Ordering information is given on any current masthead page.

(7) Treatment of **11** with MCPBA gave the *N*-oxide **11a**, which on heating (xylene at reflux) rearranged to the derivative **11b** (see ref 1).



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(9) Treatment of prenirurine (**22**) with a range of oxidizing agents [$Hg(OAc)_2$, $Hg(OTFA)_2$, $Pb(OAc)_4/I_2$, Br_2/HgO] did not give any detectable amounts of nirurine.

Total Synthesis of (±)-FR-900482

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FR-900482 (**1**) was recently isolated from a culture broth of *Streptomyces sandaensis* No. 6897 at Fujisawa Pharmaceutical Co. in Japan.[1] This unique antibiotic exists as a mixture of tautomers, **1a** and **1b**, and has been shown to exhibit exceptionally potent antitumor activities. Preliminary biological testings against experimental tumors have indicated that FR-900482 is at least as active as mitomycin C (**2**)[2] and is also active against mitomycin C- and vincristine-resistant P388 cells. Furthermore, FR-900482 appears to be less toxic than mitomycin C, a clinically used cancer

[†] On leave from Fujisawa Pharmaceutical Co., Ltd., Japan (1988-1989).

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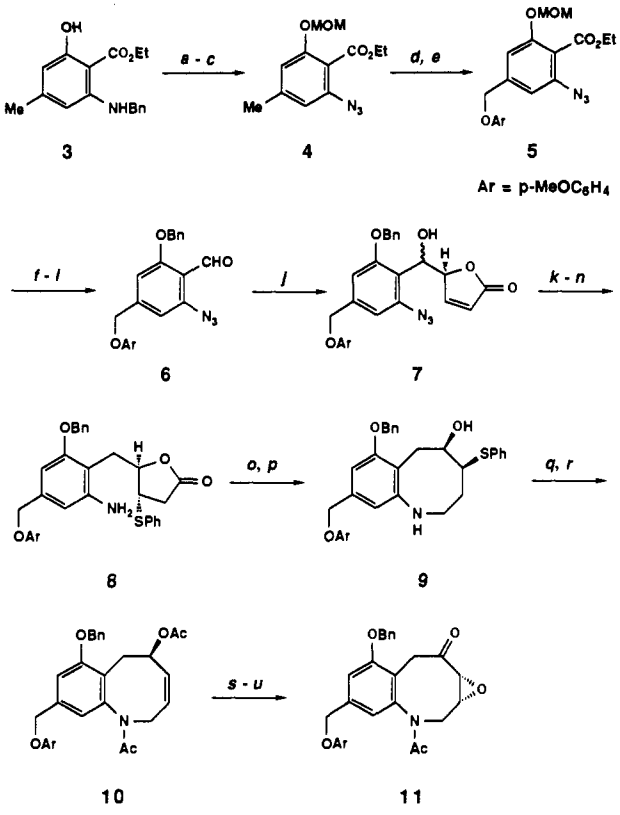
Scheme I[a]

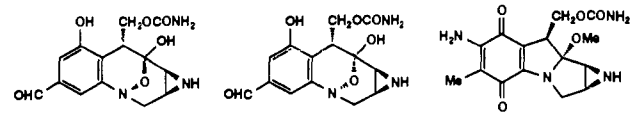


3 4 5 6 7 8 9 10 11

[a] (a) H_2 (1200 psi), Pd–C, HCO_2H, EtOH, 23 °C, 2 h. (b) $NaNO_2$, HCl, $EtOH/H_2O$, 0 °C, 20 min, then NaN_3, 0 °C, 40 min. (c) $MeOCH_2Cl$, i-Pr_2NEt, CH_2Cl_2, 23 °C, 98% from **3**. (d) NBS, Bz_2O_2, benzene, reflux, 2 h. (e) p-Methoxyphenol, K_2CO_3, DMF, 70 °C, 15 min, 47% from **4**. (f) TFA, CH_2Cl_2, 23 °C, 3 h. (g) BnCl, K_2CO_3, DMF, 80 °C, 98% from **5**. (h) DIBAL, CH_2Cl_2, −78 °C, 100%. (i) PCC, CH_2Cl_2, 23 °C, 98%. (j) 2-(Trimethylsiloxy)furan, $SnCl_4$ (0.01 equiv), CH_2Cl_2, −78 °C, 5 min, then HCl, THF/H_2O, 23 °C, 96%. (k) PhSH, Et_3N, CH_2Cl_2, 23 °C, 30 min. (l) Ac_2O, Py, 23 °C, 2 h. (m) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 23 °C. (n) Zn, AcOH, Et_2O/CH_2Cl_2, 23 °C, 47% from **7**. (o) DIBAL, CH_2Cl_2, −78 °C. (p) $NaBH_3CN$, TFA, CH_2Cl_2/MeOH, 23 °C, 10 min, 83% from **8**. (q) Ac_2O, Py, 60 °C. (r) m-CPBA (1 equiv), CH_2Cl_2, 0 °C, then toluene, sealed tube, 170 °C, 7 h, 71% from **9**. (s) NaOH, MeOH, 23 °C. (t) m-CPBA, 23 °C, 4 h. (u) Swern oxidation, 92% from **10**.

chemotherapeutic agent.[3] These promising antitumor activities as well as the mitomycin-like structure have made **1** a popular target for synthesis.[4] In addition to the well-known difficulties associated with the mitomycin synthesis,[5] FR-900482 presents a formidable challenge to synthetic chemists with its unique hydroxylamine hemiketal functionality. In this communication, we report the first total synthesis of (±)-FR-900482.

The readily available N-benzylamine **3**[6] was converted into azide **4** by a 4-step sequence involving hydrogenolysis, diazotization,



1a 1b 2

FR-900482 Mitomycin C

treatment with NaN_3, and protection of the phenol as a MOM ether (Scheme I). Benzylic bromination with NBS followed by displacement of the bromide with p-methoxyphenol furnished the sturdy ether **5**. After the protecting group of the phenol was changed from MOM to benzyl ether, the ethyl ester was converted into aldehyde **6** by careful reduction with DIBAL followed by oxidation of the resultant alcohol with PCC. Addition of 2-(trimethylsiloxy)furan[7] to **6** was catalyzed by $SnCl_4$ to give, after acidic workup, a diastereomeric mixture of butenolides **7**. Protection of the reactive butenolide was effected by Michael addition of thiophenol. Acetylation and reductive removal of the benzylic acetate with triethylsilane provided a single isomer of azido lactone, which was further reduced with zinc to give amine **8**. The critical transformation of lactone **8** into the desired 8-membered amine **9** was achieved by sequential reduction of the lactone with DIBAL and sodium cyanoborohydride. Protection of the amino alcohol **9** by acetylation, oxidation of the sulfide with m-CPBA, and subsequent thermolysis of the resultant sulfoxide yielded olefin **10**. Compound **10** was converted into epoxy ketone **11** through hydrolysis of the acetate, epoxidation with m-CPBA, and Swern oxidation.[8]

Hydroxymethylation of the ketone **11** proceeded stereospecifically to give a single stereoisomer **12** (Scheme II). The major side reaction was elimination of water from **12**, which could be minimized by employing LiOH as a base. The unstable ketone **12** was immediately reduced with $NaBH_4$ at low temperature, and the primary alcohol was selectively protected as a TBS ether. The acetamide was then deprotected by partial reduction with DIBAL to give the desired amine **13**. While Davis' reagent[9] was the only oxidizing agent that could successfully convert secondary amines into the corresponding hydroxylamines in our model studies,[4b] it completely failed to oxidize **13**. Fortunately, a facile and clean oxidation of **13** to hydroxylamine **14** could be achieved by treatment with m-CPBA. The labile hydroxylamine was selectively protected as an acetate, and subsequent Swern oxidation yielded ketone **15**. Hydrazinolysis of the acetate, deprotection of the TBS ether with n-Bu_4NF, and protection of the diol as an acetonide gave a single isomer of pentacyclic compound **16**. Since a strong NOE was observed between the protons at C-7 and C-9, the relative stereochemistry of **16** was established as shown. At this stage, the epoxide **16** was cleaved with NaN_3, and the resultant alcohol was converted to mesylate **17**. As we approached the end of the total synthesis, we recognized the extreme lability of the aziridine ring under acidic conditions. Therefore, we decided to carry out the acid-requiring reactions prior to the construction of the aziridine. The acetonide **17** was converted into the corresponding carbonate by acidic hydrolysis followed by treatment with phosgene. Upon treatment with ceric ammonium nitrate (CAN), the p-methoxyphenyl group was deprotected to give alcohol **18**.[10] The alcohol **18** was oxidized with PCC to give the aldehyde, which was protected as a dimethyl acetal to prevent reduction during hydrogenolysis of the phenolic benzyl ether. Reduction of the azide with Ph_3P in the presence of i-Pr_2NEt furnished aziridine **19**.[11] Hydrogenolysis of the benzyl ether followed by treatment with $HClO_4$ (0.05 equiv) in THF/H_2O

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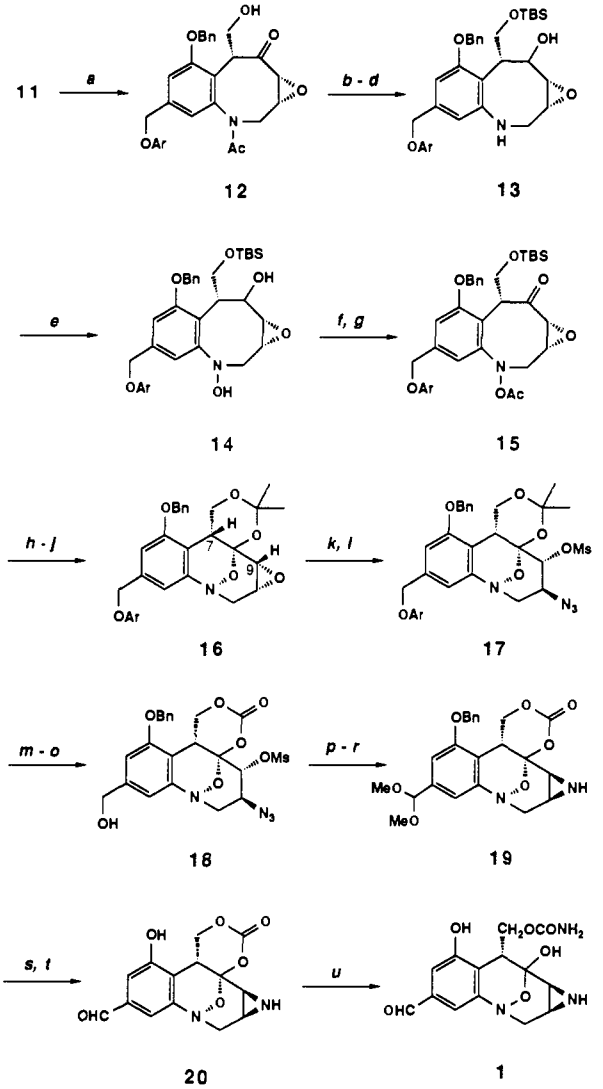
Scheme II[a]



[a](a) HCHO, LiOH, THF/H_2O, 0 °C, 2 h. (b) $NaBH_4$, EtOH, −78 to 23 °C, 71% from **11**. (c) TBSCl, imidazole, DMAP, CH_2Cl_2, 23 °C, 92%. (d) DIBAL, toluene, −78 °C, 64%. (e) *m*-CPBA (1 equiv), CH_2Cl_2, 23 °C. (f) Ac_2O, 23 °C, 10 h, 83% from **13**. (g) Swern oxidation, 83%. (h) NH_2NH_2, $MeOH/CH_2Cl_2$, 23 °C. (i) *n*-Bu_4NF, THF, 23 °C, 96% from **15**. (j) $Me_2C(OMe)_2$, CSA, CH_2Cl_2, 23 °C, 100%. (k) NaN_3, DMF/H_2O, 125 °C, 6 h. (l) MsCl, Et_3N, CH_2Cl_2, 23 °C, 89% from **16**. (m) TFA (2 equiv), CH_2Cl_2, 23 °C, 10 min. (n) $COCl_2$, Py, CH_2Cl_2, 23 °C. (o) CAN, CH_3CN/H_2O, 23 °C, 74% from **17**. (p) PCC, $MgSO_4$, CH_2Cl_2, 23 °C. (q) $CH(OMe)_3$, CSA, MeOH, 23 °C, 76% from **18**. (r) Ph_3P, *i*-Pr_2NEt (1.2 equiv), THF/H_2O, 60 °C, 30 min, 71%. (s) H_2 (1 atm), Pd-C EtOH, 23 °C, 2 h, 100%. (t) $HClO_4$ (0.05 equiv), THF/H_2O, 23 °C, 2 h, 96%. (u) NH_3, CH_2Cl_2, 23 °C, 2 h, 95%.

(10:1) at 23 °C afforded **20** without appreciable decomposition. Finally, careful ammonolysis of the cyclic carbonate gave exclusively (±)-**1**, which was identical with an authentic sample[12] in TLC behavior and spectroscopic properties. The synthetic sample was further converted to the triacetyl compound, which proved to be identical with authentic FK-973.[13]

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(12) We are indebted to Fujisawa Pharmaceutical Co. for a generous gift of natural FR-900482.

(13) Instead of FR-900482, FK-973, a triacetyl derivative of **1b**, is used in clinical trials.[2]

Tolyporphin, a Novel Multidrug Resistance Reversing Agent from the Blue-Green Alga *Tolypothrix nodosa*

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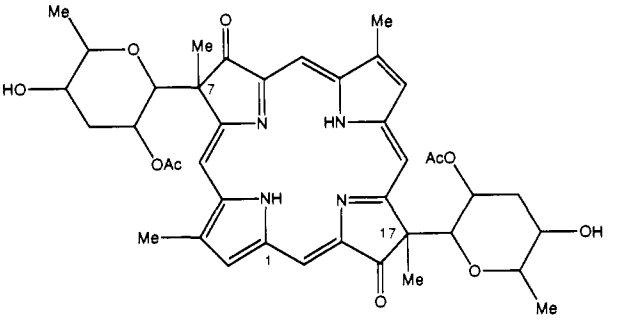
Tumor cells that survive initial chemotherapy in cancer patients often emerge with increased resistance to both the original therapeutic agent and other seemingly unrelated drugs. This phenomenon is termed multidrug resistance (MDR) and is often associated with increased expression of P-glycoprotein, which acts as an energy-dependent drug efflux pump. In an ongoing search for new anticancer agents from microalgae, we have found that the lipophilic extract of *Tolypothrix nodosa* Bharadwaja (UH strain HT-58-2), a cyanophyte isolated from a soil sample collected at Nan Madol, Pohnpei, reverses MDR in a vinblastine-resistant subline (SK-VLB) of a human ovarian adenocarcinoma line (SK-OV-3)[1] assayed by a dye-reduction technique.[2] We report here the isolation and structure determination of an unusual porphyrin, tolyporphin (**1**), which accounts for most of this activity. Tolyporphin potentiates the cytotoxicity of adriamycin or vinblastine in SK-VLB cells at doses as low as 1 μg/mL.[3]



1

The extract (1:1 CH_2Cl_2/2-propanol) of the cultured alga[4] was fractionated by consecutive reversed-phase (C18) and normal-phase (silica gel) chromatography to give dark-purple microcrystals of tolyporphin (**1**, $C_{40}H_{46}N_4O_{10}$; HREIMS *m/z* 742.3213, Δ0.1 mmu), in 0.03% yield. The UV spectrum[5] suggested that **1** was a modified porphyrin. Intense fragment ion peaks were observed at *m/z* 570.2486 ($C_{32}H_{34}N_4O_6$, Δ−0.7 mmu) and *m/z* 398.1741 ($C_{24}H_{22}N_4O_2$, Δ0.2 mmu) in the EIMS for the successive losses of two $C_8H_{12}O_4$ units from the M^+ ion.[6]

The ^{13}C NMR spectrum of **1** in acetone-d_6 confirmed the presence of 40 carbon atoms, i.e., 16 non-protonated, 14 methine, two methylene, and eight methyl carbons, from comparison of the broad-band decoupled and INEPT spectra. In addition to

[†] Department of Chemistry.

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(5) UV (MeOH) λ_{max} 276 nm (ε 4500), 294 sh (4000), 401 (49000), 479 sh (850), 504 (1400), 547 (1400), 611 (1200), 619 (1200), 642 (1300), 675 (22000).

(6) An 8,18-dihydroxy-2,7,13,17-tetramethyl-21*H*,23*H*-porphine ion is presumably formed by elimination of the C-glycosyl units via McLafferty rearrangements.

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