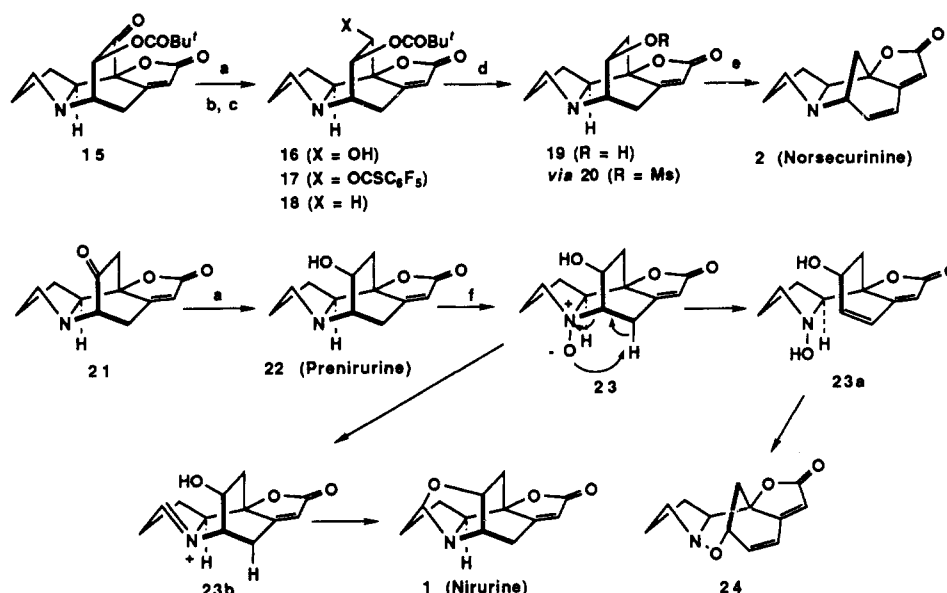


Scheme III^a

^a (a) NaBH₄/MeOH (64% from 12/13). (b) C₆F₅OCSCl/DMAP/CH₂Cl₂ molecular sieves (100%). (c) *n*-Bu₃SnH/AIBN/benzene, reflux 15 min (100%). (d) NaOMe/MeOH (82% from 19). (e) MsCl/Et₃N/DMAP/CH₂Cl₂, 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 amination (oxidation adjacent to nitrogen) takes place at an earlier stage.

Acknowledgment. The National Institutes of Health and the Welch Foundation are thanked for their support of this research. Professor Peter A. Jacobi is thanked for spectra of (+)-norsecurinine. Professor Geoffrey A. Cordell is thanked for spectra of (+)-nirurine. J.R.-L. thanks the Fulbright Commission and MEC Spain for financial support.

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.

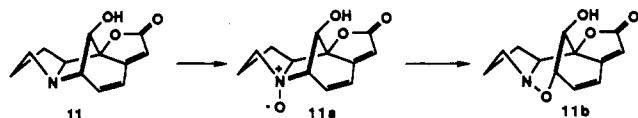
Total Synthesis of (±)-FR-900482

Tohru Fukuyama,* Lianhong Xu, and Shunsuke Goto[†]

Department of Chemistry
Rice University, Houston, Texas 77251
Received September 20, 1991

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

(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).



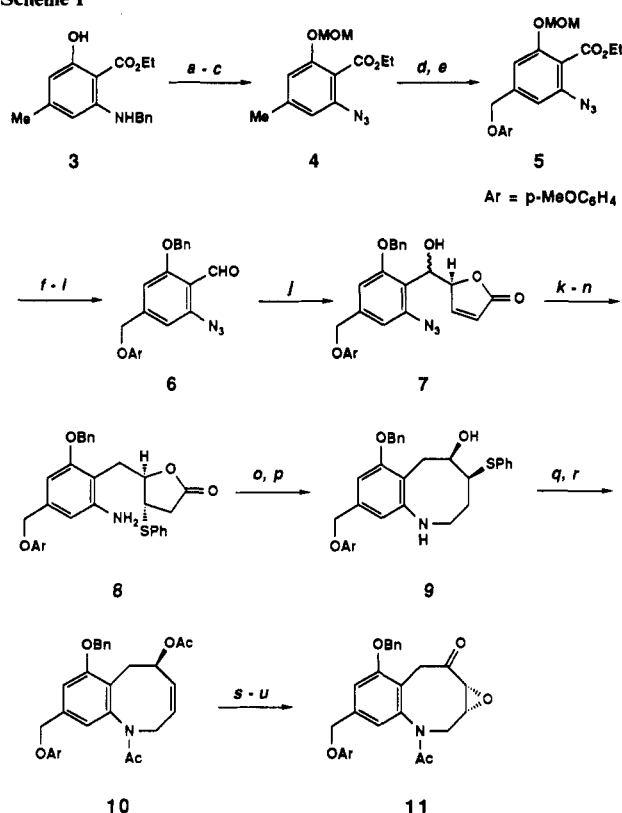
(8) Barton, D. H. R.; Jaszberenyi, J. Cs. *Tetrahedron Lett.* 1989, 30, 2619.

(9) Treatment of prenirurine (22) with a range of oxidizing agents [Hg(OAc)₂, Hg(OTFA)₂, Pb(OAc)₄/I₂, Br₂/HgO] did not give any detectable amounts of nirurine.

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

(1) Iwami, M.; Kiyoto, S.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* 1987, 40, 589. (b) Kiyoto, S.; Shibata, T.; Yamashita, M.; Komori, T.; Okuhara, M.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* 1987, 40, 594. (c) Uchida, I.; Takase, S.; Kayakiri, H.; Kiyoto, S.; Hashimoto, M.; Tada, T.; Koda, S.; Morimoto, Y. *J. Am. Chem. Soc.* 1987, 109, 4108.

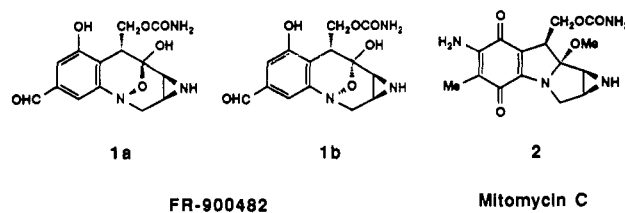
(2) Remers, W. A.; Dorr, R. T. In *Alkaloids: Chem. Biol. Perspect.*; Pelletier, S. W., Ed.; John Wiley: New York, 1988; Vol. 6, p 1.

Scheme 1^a

^a (a) H₂ (1200 psi), Pd-C, HCO₂H, EtOH, 23 °C, 2 h. (b) NaNO₂, HCl, EtOH/H₂O, 0 °C, 20 min, then NaN₃, 0 °C, 40 min. (c) MeOCH₂Cl, *i*-Pr₂NEt, CH₂Cl₂, 23 °C, 98% from 3. (d) NBS, Bz₂O₂, benzene, reflux, 2 h. (e) *p*-Methoxyphenol, K₂CO₃, DMF, 70 °C, 15 min, 47% from 4. (f) TFA, CH₂Cl₂, 23 °C, 3 h. (g) BnCl, K₂CO₃, DMF, 80 °C, 98% from 5. (h) DIBAL, CH₂Cl₂, -78 °C, 100%. (i) PCC, CH₂Cl₂, 23 °C, 98%. (j) 2-(Trimethylsiloxy)furan, SnCl₄ (0.01 equiv), CH₂Cl₂, -78 °C, 5 min, then HCl, THF/H₂O, 23 °C, 96%. (k) PhSH, Et₃N, CH₂Cl₂, 23 °C, 30 min. (l) Ac₂O, Py, 23 °C, 2 h. (m) Et₃SiH, BF₃·Et₂O, CH₂Cl₂, 23 °C. (n) Zn, AcOH, Et₂O/CH₂Cl₂, 23 °C, 47% from 7. (o) DIBAL, CH₂Cl₂, -78 °C. (p) NaBH₃CN, TFA, CH₂Cl₂/MeOH, 23 °C, 10 min, 83% from 8. (q) Ac₂O, Py, 60 °C. (r) *m*-CPBA (1 equiv), CH₂Cl₂, 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,



treatment with NaN₃, 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)furan⁷ to **6** was catalyzed by SnCl₄ to give, after acidic workup, a diastereometric 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 NaBH₄ 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' reagent⁹ 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*-Bu₄NF, 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 NaN₃, 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 Ph₃P in the presence of *i*-Pr₂NEt furnished aziridine **19**.¹¹ Hydrogenolysis of the benzyl ether followed by treatment with HClO₄ (0.05 equiv) in THF/H₂O

(3) (a) Shimomura, K.; Hirai, O.; Mizota, T.; Matsumoto, S.; Mori, J.; Shibayama, F.; Kikuchi, H. *J. Antibiot.* **1987**, *40*, 600. (b) Hirai, O.; Shimomura, K.; Mizota, T.; Matsumoto, S.; Mori, J.; Kikuchi, H. *J. Antibiot.* **1987**, *40*, 607. (c) Masuda, K.; Nakamura, T.; Shimomura, K.; Shibata, T.; Terano, H.; Kohsaka, M. *J. Antibiot.* **1987**, *41*, 1497. (d) Shimomura, K.; Manda, T.; Mukumoto, S.; Masuda, K.; Nakamura, T.; Mizota, T.; Matsumoto, S.; Nishigaki, F.; Oku, T.; Mori, J.; Shibayama, F. *Cancer Res.* **1988**, *48*, 1116. (e) Masuda, K.; Nakamura, T.; Mizota, T.; Mori, J.; Shimomura, K. *Cancer Res.* **1988**, *48*, 5172.

(4) (a) Yasuda, N.; Williams, R. M. *Tetrahedron Lett.* **1989**, *30*, 3397. (b) Fukuyama, T.; Goto, S. *Tetrahedron Lett.* **1989**, *30*, 6491. (c) Jones, R. J.; Rapoport, H. *J. Org. Chem.* **1990**, *55*, 1144. (d) McClure, K. F.; Danishefsky, S. J. *J. Org. Chem.* **1991**, *56*, 850.

(5) For a recent review of mitomycin synthesis, see: Remers, W. A.; Iyengar, B. S. In *Recent Prog. Chem. Synth. Antibiot.*; Lukacs, G.; Ohno, M., Eds.; Springer: Berlin, 1990; p 415.

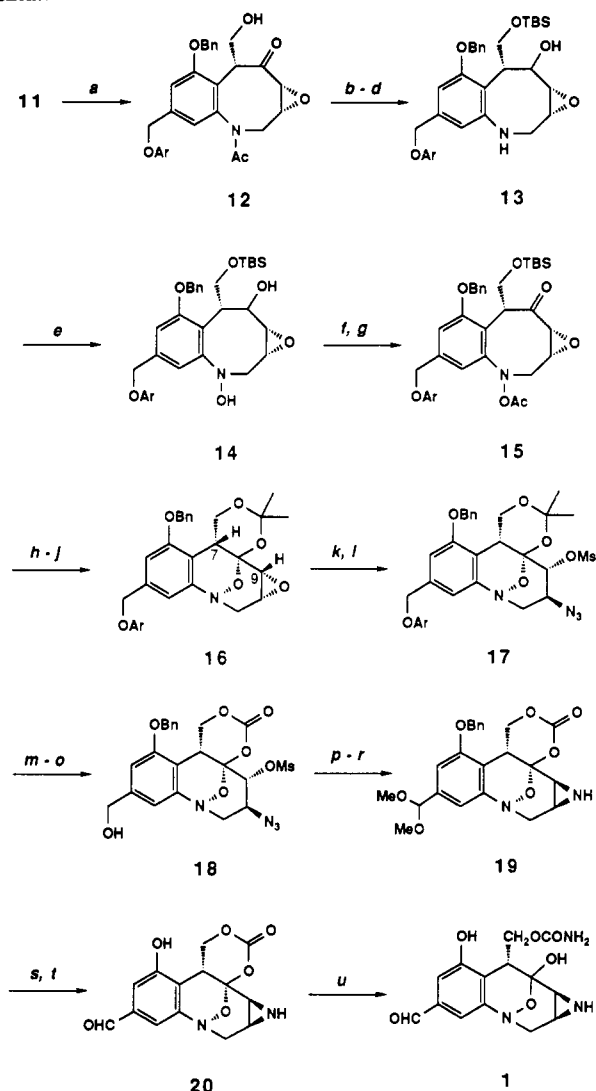
(6) Prepared in 41% yield from ethyl acetoacetate and benzylamine following the published procedure: Böhme, H.; von Grätz, J. G.; Martin, F.; Matusch, R.; Nehne, J. *Liebigs Ann. Chem.* **1980**, 394.

(7) Asaoka, M.; Miyake, K.; Takei, H. *Chem. Lett.* **1977**, 167. (b) Fiorenza, M.; Ricci, A.; Momanelli, M. N.; Taddei, M.; Dembech, P.; Seconi, G. *Heterocycles* **1982**, *19*, 2327.

(8) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165. (9) Davis, F. A.; Billmers, J. M.; Gosciniak, D. J.; Towson, J. C.; Bach, R. D. *J. Org. Chem.* **1986**, *51*, 4240.

(10) Fukuyama, T.; Laird, A. A.; Hotchkiss, L. M. *Tetrahedron Lett.* **1985**, *26*, 6291.

(11) Vaultier, M.; Knouzi, N.; Carrie, R. *Tetrahedron Lett.* **1983**, *24*, 763.

Scheme II^a

^a (a) HCHO, LiOH, THF/H₂O, 0 °C, 2 h. (b) NaBH₄, EtOH, -78 to 23 °C, 71% from 11. (c) TBSCl, imidazole, DMAP, CH₂Cl₂, 23 °C, 92%. (d) DIBAL, toluene, -78 °C, 64%. (e) *m*-CPBA (1 equiv), CH₂Cl₂, 23 °C. (f) Ac₂O, 23 °C, 10 h, 83% from 13. (g) Swern oxidation, 83%. (h) NH₂NH₂, MeOH/CH₂Cl₂, 23 °C. (i) *n*-Bu₄NF, THF, 23 °C, 96% from 15. (j) Me₂C(OMe)₂, CSA, CH₂Cl₂, 23 °C, 100%. (k) NaN₃, DMF/H₂O, 125 °C, 6 h. (l) MsCl, Et₃N, CH₂Cl₂, 23 °C, 89% from 16. (m) TFA (2 equiv), CH₂Cl₂, 23 °C, 10 min. (n) COCl₂, Py, CH₂Cl₂, 23 °C. (o) CAN, CH₃CN/H₂O, 23 °C, 74% from 17. (p) PCC, MgSO₄, CH₂Cl₂, 23 °C. (q) CH(OMe)₃, CSA, MeOH, 23 °C, 76% from 18. (r) Ph₃P, *i*-Pr₂NEt (1.2 equiv), THF/H₂O, 60 °C, 30 min, 71%. (s) H₂ (1 atm), Pd-C EtOH, 23 °C, 2 h, 100%. (t) HClO₄ (0.05 equiv), THF/H₂O, 23 °C, 2 h, 96%. (u) NH₃, CH₂Cl₂, 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 sample¹² 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

Acknowledgment. This work was supported by the National Institutes of Health (Grant CA 28119). Financial assistance (to S.G.) from Fujisawa Pharmaceutical Co., Ltd., Japan, is gratefully acknowledged.

(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

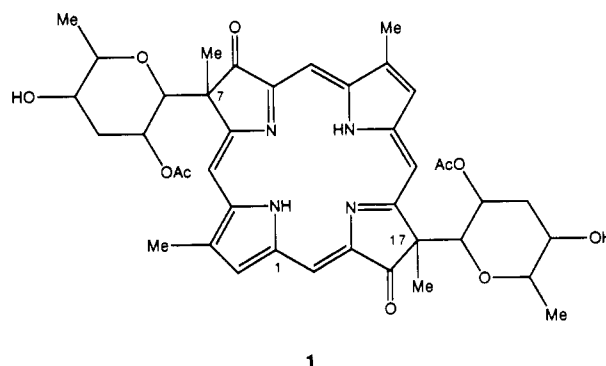
Tolyporphin, a Novel Multidrug Resistance Reversing Agent from the Blue-Green Alga *Tolypothrix nodosa*

Michèle R. Prinsep,[†] Faith R. Caplan,[†] Richard E. Moore,^{*,†} Gregory M. L. Patterson,[†] and Charles D. Smith[‡]

Department of Chemistry and
Cancer Research Center of Hawaii
University of Hawaii
Honolulu, Hawaii 96822

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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



The extract (1:1 CH₂Cl₂/2-propanol) of the cultured alga⁴ was fractionated by consecutive reversed-phase (C18) and normal-phase (silica gel) chromatography to give dark-purple microcrystals of tolyporphin (**1**, C₄₀H₄₆N₄O₁₀; HREIMS *m/z* 742.3213, Δ0.1 mmu), in 0.03% yield. The UV spectrum⁵ suggested that **1** was a modified porphyrin. Intense fragment ion peaks were observed at *m/z* 570.2486 (C₃₂H₃₄N₄O₆, Δ-0.7 mmu) and *m/z* 398.1741 (C₂₄H₂₂N₄O₂, Δ0.2 mmu) in the EIMS for the successive losses of two C₈H₁₂O₄ units from the M⁺ ion.⁶

The ¹³C 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.

[‡] Cancer Research Center of Hawaii.

(1) Bradley, G.; Naik, M.; Ling, V. *Cancer Res.* 1989, 49, 2790-2796.

(2) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Boksich, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* 1990, 82, 1107-1112.

(3) Smith, C. D.; Prinsep, M. R.; Caplan, F. R.; Moore, R. E.; Patterson, G. M. L. Manuscript in preparation.

(4) The alga was cultured using a general procedure described in the following: Patterson, G. M. L.; Baldwin, C. L.; Bolis, C. M.; Caplan, F. R.; Karuso, H.; Larsen, L. K.; Levine, I. A.; Moore, R. E.; Nelson, C. S.; Tschappat, K. D.; Tuang, G. D.; Furusawa, E.; Furusawa, S.; Norton, T. R.; Raybourne, R. B. *J. Phycol.* 1991, 27, 530-536.

(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.