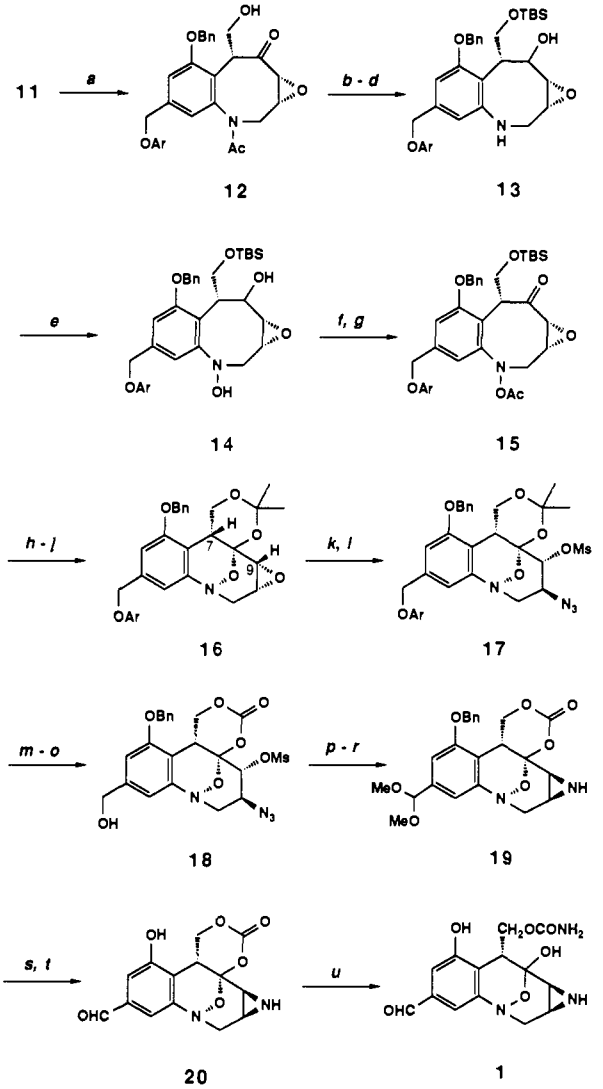
Scheme II[a]



[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]

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*

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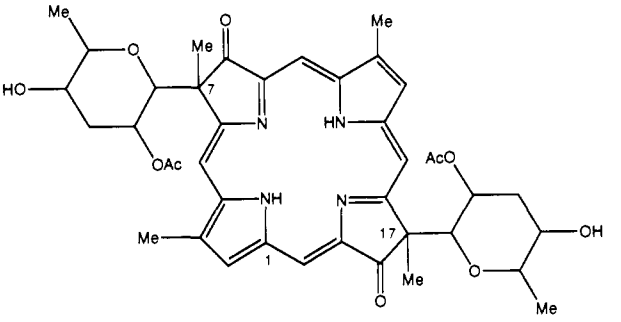
Tumor cells that survive initial chemotherapy in cancer patients often emerge with increased resistance to both the original therapeutic agent and other seemingly unrelated drugs. This phenomenon is termed multidrug resistance (MDR) and is often associated with increased expression of P-glycoprotein, 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

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(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.

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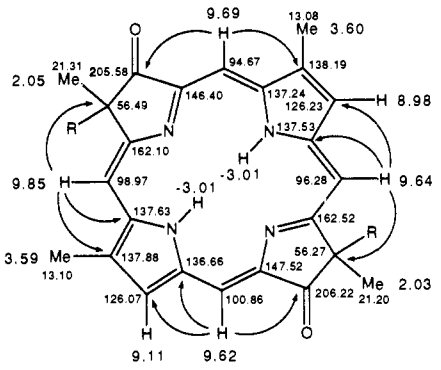
the 42 hydrogens attached to carbon, there were four exchangeable protons as shown by the ^{1}H NMR spectrum in acetone-d_6 and an HMQC experiment, viz., two porphyrin-type NHs (broad 2 H singlet at −3.01 ppm) and two secondary alcohol OHs (two broad, concentration- and temperature-dependent 1 H doublets at 3.91 and 4.02 ppm[7]). An *O,O*-diacetate derivative was readily formed when **1** was treated with Ac_2O/pyridine (EIMS M^+ $C_{44}H_{50}N_4O_{12}$, Δ0.7 mmu). ^{13}C NMR signals for two ketone carbonyl, two ester (acetate) carbonyl, and 16 aromatic carbons (10 non-protonated, two of which were imino-type, and six methine) could be detected, accounting for 13 degrees of unsaturation. The remaining unsaturation had to be due to seven rings.

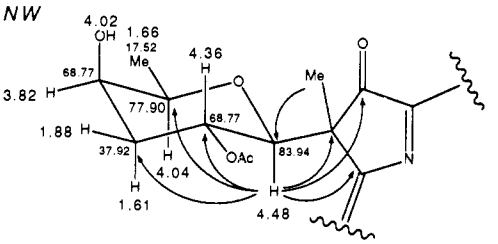
In addition to showing other porphyrin signals (1 H singlets at 9.62, 9.64, 9.69, and 9.85 ppm for four meso protons and 1 H singlets at 8.98 and 9.11 ppm for two pyrrolic methine protons),[8] the ^{1}H NMR spectrum in acetone-d_6 exhibited several signals (3.81–4.48 ppm) characteristic of protons in two structurally identical, but magnetically nonequivalent, C-glycosyl units. The four nitrogens in **1** were therefore accounted for by the porphyrin system and the 10 oxygens by two ketone, two ester, two alcohol, and two ether functionalities.

An HMBC experiment, which showed important $^2J_{H,C}$ and $^3J_{H,C}$ correlations from (1) the meso protons to carbons indicated by arrows in **a** and (2) the protons of the aromatic methyl groups to carbons two and three bonds away, i.e., 2.05 → 205.58 and 162.10, 3.60 → 136.19 (methine proton signals at 9.69 and 8.98 ppm also correlate to this carbon), 2.03 → 206.22 and 162.52, and 3.59 → 137.88 ppm (methine proton signals at 9.85 and 9.11 ppm also correlate to this carbon), established the presence of partial structure **a** in **1**.

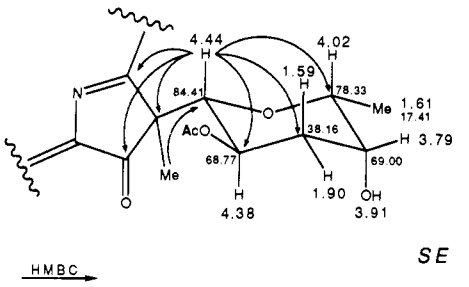


a

^{1}H–^{1}H decoupling, ROESY, and HMBC experiments indicated that the R-substituents in **a** were 2-*O*-acetyl-3,6-dideoxy-*xylo*-hexopyranose[9] units that were β-linked to C-7 and C-17 as depicted in **b** and **c**. An OH group had to be on C-4 in each sugar unit since coupling was shown between the OH and H-4 (J = 3.7–4.7 Hz); moreover, the H-4 signals shifted 1.3 ppm downfield on acetylation. The relative stereochemistry of each C-glycosyl unit was determined from the following coupling constants: $J_{1,2}$ = 10.0, $J_{2,3ax}$ = 10.8, $J_{2,3eq}$ = 4.5, $J_{3ax,3eq}$ = −12.7, $J_{3ax,4}$ = 2.3, $J_{3eq,4}$ = 3.2, $J_{4,5}$ = 1.4, and $J_{5,Me}$ = 6.4 Hz. The methyl group in each unit was equatorial since NOEs to the axial protons on C-1 and C-3 were absent; however, NOEs were seen between H-5 and these protons. In the HMBC experiment, correlations were observed from each of the C-1 protons in **b** and **c** to all of the carbons two and three bonds away (denoted by arrows) except the methyl carbons, proving unequivocally that the sugar units were attached to the sp^3 quaternary carbons of the northwestern and southeastern pyrrolic rings of **1**. HMBC correlations from the methyl protons (2.05 and 2.03 ppm) to the C-1 carbons of the C-glycosyl groups provided further proof.

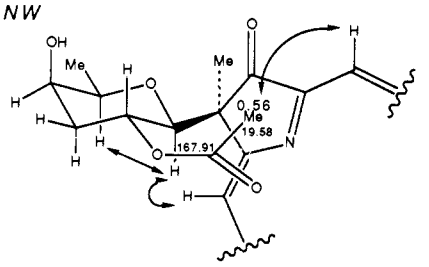


b

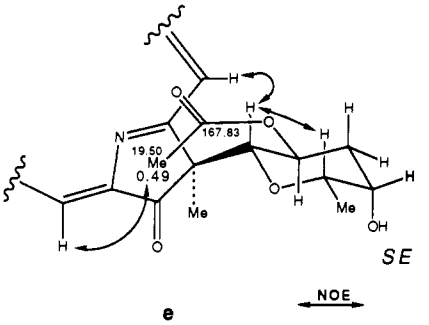


c

^{1}H NMR spectrum signals at 0.56 and 0.49 ppm were assigned to the acetate methyl protons in **b** and **c**, as HMBC correlations were only found to the ester carbonyls (167.91 and 167.83 ppm, respectively). The unusual high-field chemical shifts, however, indicated that the acetate methyl groups had to be projected over the porphyrin system, as shown in partial structures **d** and **e**. Strong NOEs between 4.48 and 9.85, 0.56 and 9.69, 4.44 and 9.64, and 0.49 and 9.62 ppm suggested that **1** had the relative stereochemistry shown in **d** and **e**.[10]



d



e

Acknowledgment. This research was supported by NIH Grant CA12623. The authors thank Wesley Yoshida for determining the NMR spectra.

(7) On 20% dilution these OH signals shifted to 3.87 and 3.99 ppm, respectively.

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(10) The absolute stereochemistry of **1** [CD (CH_3CN) Δε (nm) +0.56 (464), 0 (423), −2.21 (408), 0 (404), +8.86 (397), +4.32 (379), 0 (339), −1.98 (313), 0 (299), 6.30 (254)], along with the total structures of several minor analogues, will be presented in a full paper.

Supplementary Material Available: 500-MHz ^1H (including proton–proton decoupled) and 125-MHz ^{13}C NMR spectra of **1** in acetone-d_6, comparison of 500-MHz ^1H NMR spectra of **1** and tolyporphin *O,O*-diacetate in $CDCl_3$, and HMBC spectrum of **1** in acetone-d_6 (10 pages). Ordering information is given on any current masthead page.

Intramolecular Electron Transfer in Rigid Media at Room Temperature

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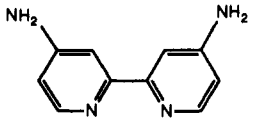
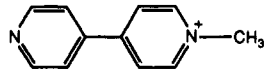
The study of intramolecular photochemical electron transfer in fluid solution is a rapidly expanding area with important implications for fundamental processes, artificial photosynthesis, and molecular-level devices.[1] One limitation is that electron transfer is typically inhibited in rigid media because of restricted reorientation in the surrounding solvent dipoles. Recently, we showed that intramolecular electron transfer could occur in low-temperature glasses if the free energy change were sufficiently favorable.[2] Wasielewski et al. have made related observations based on modified porphyrins,[3] and earlier work by several groups has shown that *intermolecular* electron transfer could occur in rigid matrices.[4] We demonstrate here that the earlier results are more generally applicable to both electron transfer and energy transfer in rigid polymeric media or in the solid state at room temperature.

Solid solutions of metal complexes have been prepared previously as thin films in poly(methyl methacrylate) (PMMA), polystyrene (PS), or poly(vinyl alcohol) (PVA).[5] We have used similar techniques to prepare ~1-mm-thick, free-standing films of PMMA containing ~0.2% of the salts $[Re(bpy)(CO)_3(MQ^+)](PF_6)_2$ (bpy is 2,2′-bipyridine) and $[Re(4,4'-(NH_2)_2bpy)(CO)_3(MQ^+)](PF_6)_2$. Earlier work based on these



(4,4′-(NH$_2$)$_2$bpy)



(MQ$^+$)

complexes[2] showed that at 77 K in frozen glasses $d\pi \rightarrow \pi^*$ (4,4′-$(NH_2)_2$bpy) excitation in the amino derivative was followed by rapid intramolecular electron transfer, eq 1, but that an equivalent process did not occur for the bpy complex. From

$$\xrightarrow{h\nu} [(4,4'\text{-}(NH_2)_2bpy^{\bullet-})Re^{II}(CO)_3(MQ^+)]^{2+*} \rightarrow [(4,4'\text{-}(NH_2)_2bpy)Re^{II}(CO)_3(MQ^{\bullet})]^{2+*} \quad (1)$$

electrochemical measurements in CH_3CN at 295 K, the driving forces for intramolecular electron transfer are 1.0 V for the amino derivative and 0.49 V for the bpy complex.

The same difference in behavior is observed in PMMA at room temperature. Emission from samples containing $[Re(bpy)(CO)_3(MQ^+)](PF_6)_2$ is nearly superimposable on that from $[Re(bpy)(CO)_3(4\text{-Etpy})](PF_6)$(4-Etpy is 4-ethylpyridine) (λ_{max} ~525 nm), but with evidence for a weak component on the low-energy side of the spectrum for the MQ^+ complex. By contrast, emission from $[Re(4,4'\text{-}(NH_2)_2bpy)(CO)_3(MQ^+)](PF_6)_2$ is decreased by >90% compared to that from $[Re(4,4'\text{-}(NH_2)_2bpy)(CO)_3(4\text{-Etpy})](PF_6)$ and red-shifted from 503 to 578 nm. In transient absorption difference spectra, Figure 1C, a strong absorption feature appears, within the laser pulse (<10 ns), for the amino derivative at 610 nm which arises from a $\pi \rightarrow \pi^*$ transition at MQ$^{\bullet}$.[6] Subsequent decay to the ground state was independent of monitoring wavelength and fit satisfactorily to the Williams–Watts (Kolrausch) function,[7] eq 2, with $k_1 = 8.3 \times 10^5$ s^{-1}, $\beta = 0.53$, and $\langle\tau\rangle = 2.2$ μs.[8] This is an increase of 42× over the 52-ns lifetime for decay found in CH_2ClCH_2Cl at 295 K.

$$I(t) = I_0 e^{-(k_1 t)^{\beta}} \quad (2)$$

For $[Re(bpy)(CO)_3(MQ^+)](PF_6)_2$ in PMMA, the characteristic absorption features for the $Re^{II}(bpy^{\bullet-})$ excited state appear at 370 and 540 nm within the laser pulse, along with the feature at 610 nm, the latter being greatly diminished in intensity, Figure 1B.

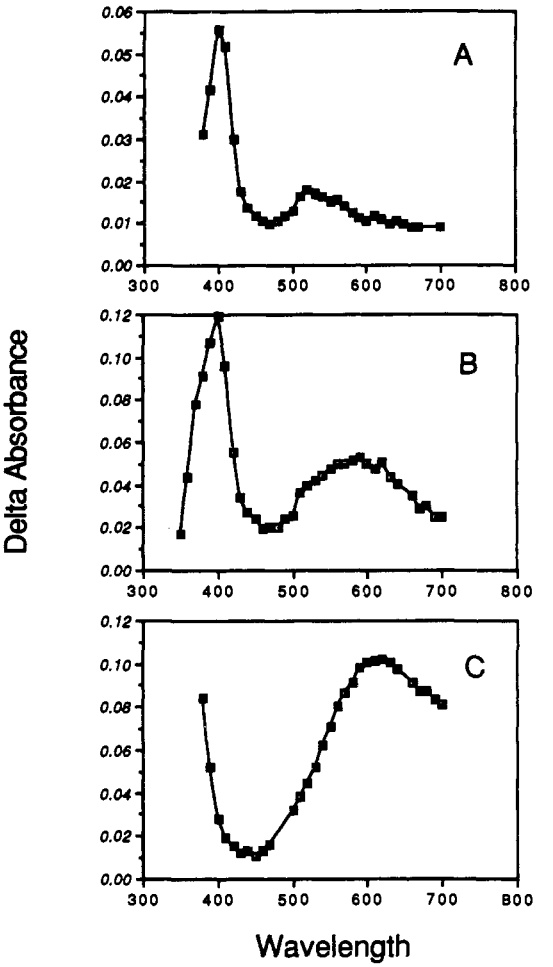


Figure 1. Transient absorption difference spectra for (A) $[Re(bpy)(CO)_3(4\text{-Etpy})](PF_6)$, (B) $[Re(bpy)(CO)_3(MQ^+)](PF_6)_2$, and (C) $[Re(4,4'\text{-}(NH_2)_2bpy)(CO)_3(MQ^+)](PF_6)_2$ in PMMA acquired 100 ns after 420-nm excitation (<4 mJ/pulse).

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(8) If $I(t)$ is considered as arising from a superposition of exponentials, $\langle\tau\rangle = (k_1\beta)^{-1}\,\Gamma(1/\beta)$, where $\Gamma(n)$ is the gamma function and $\langle\tau\rangle$ is the average relaxation time. (Lindsey, C. P.; Patterson, G. D. *J. Chem. Phys.* **1980**, *73*, 3348.)

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