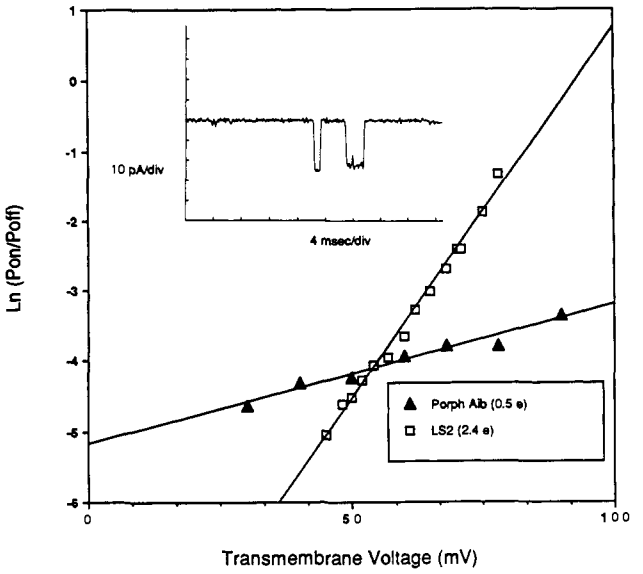

Figure 2. Effect of voltage on the probability of forming the major conductance state relative to the background conductance. The inset shows a current vs time trace for tetraphilin **1**. P_{on} is the probability per unit time that the major conductance is observed, and P_{off} is the probability per unit time that this state is not observed. The slope is related to the effective number of charges that are translocated across the membrane in going from a closed ("off") to an open ("on") channel state:[12] 2.4 for $(LSLLLSL)_3$ and 0.5 for the tetraphilin. The voltage dependence of single channel conductances for $(LSLBLSL)_3$ has not been measured because of its extremely short lifetimes in 1.0 M HCl.[3] However, preliminary macroscopic conductance measurements indicate that it has a gating charge of 1.2. The methods used to collect and analyze the data are described in the supplementary material.

(Figure 1), could readily be purified by reversed-phase HPLC.

Tetraphilin **1** forms proton channels in planar diphytanoyl phosphatidylcholine bilayers in 1.0 M HCl with a major conductance state of 470 pS and secondary, more variable conductance states of 320 and 100 pS. As with the $(LSLBLSL)_3$ channels, the tetraphilin channels are proton selective, as no conductance was observed with LiCl as the electrolyte. The lifetime of the major conductance state (5 ms) is considerably longer than that of $(LSLBLSL)_3$ (<0.2 ms in 1 M HCl),[3] indicating that the attachment of the peptide to the template stabilizes the conducting state of the peptide. Furthermore, the probability of channel formation depends linearly on the bilayer concentration of tetraphilin **1**, suggesting that the channels are monomolecular.

The formation of channels by tetraphilin **1** is nearly voltage independent (Figure 2), in marked contrast to the behavior of $(LSLLLSL)_3$. A mechanism to explain the voltage dependence of the parent peptide has been postulated.[3] In the absence of a transmembrane potential, the peptide is oriented in planar lipid bilayers with its α-helical axis parallel to the membrane surface.[4] A transmembrane voltage stabilizes the channel-forming, vertically inserted orientation of the peptide through favorable interactions with the helical macrodipole. On the other hand, the small voltage dependence for tetraphilin suggests that it forms helical bundles that are predominantly vertically inserted in the membrane, even in the absence of a transmembrane voltage. Other interpretations of the voltage dependence are also possible, and we are attempting to confirm this orientation through spectroscopic investigations of tetraphilin **1** in planar multilayers.

These results show that the tetraphenylporphyrin template exerts a major influence on the lifetime and voltage dependence of $(LSLBLSL)_3$ channels. These differences presumably arise from changes in the overall hydrophobicity and geometric restrictions imposed on the peptide by the porphyrin template. To determine the role of these variables, we are preparing derivatives of monomeric $(LSLBLSL)_3$ with apolar, N-terminal blocking groups, as well as tetraphilins with altered peptide sequences.

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Supplementary Material Available: Listings of experimental and spectral details for tetraphenylporphyrins and details of channel measurements in planar bilayers (4 pages). Ordering information is given on any current masthead page.

(14) Datta-Gupta, N.; Jones, E.; Thomas, L. K.; Malakar, D. *J. Indian Chem. Soc.* **1981**, *58*, 1171.
(15) Lindsey, J. S.; Schreiman, I. C.; Hsu, H. C.; Kearney, P. C.; Marguerattaz, A. M. *J. Org. Chem.* **1987**, *52*, 827.

Observation of a Series of Degenerate Cyclic Double, Triple, and Quadruple Proton Transfers in Solid Pyrazoles

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The ability of proton donors to form different hydrogen-bonded associates in the liquid state often makes it difficult to elucidate their proton-transfer dynamics. For example, it has been postulated that pyrazoles may exchange protons in cyclic dimers and/or trimers.[2-7] Such difficulties do not arise in solid-state studies where structures can be studied by diffraction techniques and proton-transfer dynamics by high-resolution NMR spectroscopy.[8-12] Thus, it has been recently shown that 3,5-dimethylpyrazole

(1) (a) Freie Universität Berlin. (b) Instituto "Rocasolano". (c) King's College. (d) C.S.I.C.
(2) Elguero, J.; Marzin, C.; Katrizky, A. R.; Linda, P. *The Tautomerism of Heterocycles*; Academic Press: New York, 1976.
(3) Joop, N.; Zimmermann, H. *Z. Electrochem.* **1962**, *66*, 440.
(4) (a) Nesmeyanov, A. N.; Zavelovitch, E. B.; Babin, V. N.; Kochetkova, N. S.; Fedin, E. I. *Tetrahedron* **1976**, *31*, 1461. (b) Nesmeyanov, A. N.; Babin, V. N.; Zavelovitch, E. B.; Kochetkova, N. S.; Fedin, E. I. *Chem. Phys. Lett.* **1976**, *37*, 184.
(5) Litchman, W. M. *J. Am. Chem. Soc.* **1979**, *101*, 545.
(6) Chenon, M. T.; Coupry, C.; Grant, D. M.; Pugmire, R. *J. Org. Chem.* **1977**, *42*, 659.
(7) Limbach, H. H. The Use of NMR Spectroscopy in the Study of Hydrogen Bonding in Solution. In *Aggregation Processes*; Gormally, J., Wyn-Jones, E., Eds.; Elsevier: Amsterdam, 1983; Chapter 16.
(8) Limbach, H. H. Dynamic NMR Spectroscopy in the Presence of Kinetic Hydrogen/Deuterium Isotope Effects. In *NMR Basic Principles and Progress*; Springer: Heidelberg, Berlin, 1990; Vol. 26, Chapter 2.
(9) (a) Limbach, H. H.; Hennig, J.; Kendrick, R. D.; Yannoni, C. S. *J. Am. Chem. Soc.* **1984**, *106*, 4059. (b) Wehrle, B.; Limbach, H. H.; Köcher, M.; Ermer, O.; Vogel, E. *Angew. Chem.* **1987**, *99*, 914; *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 934. (c) Limbach, H. H.; Wehrle, B.; Schlabach, M.; Kendrick, R. D.; Yannoni, C. S. *J. Magn. Reson.* **1988**, *77*, 84. (d) Wehrle, B.; Limbach, H. H. *Chem. Phys.* **1989**, *136*, 223.
(10) (a) Wehrle, B.; Zimmermann, H.; Limbach, H. H. *J. Am. Chem. Soc.* **1988**, *110*, 7014. (b) Wehrle, B.; Aguilar-Parrilla, F.; Limbach, H. H. *J. Magn. Reson.* **1990**, *87*, 584. (c) Aguilar-Parrilla, F.; Wehrle, B.; Baäunling, H.; Limbach, H. H. *J. Magn. Reson.* **1990**, *87*, 592.
(11) Baldy, A.; Elguero, J.; Faure, R.; Pierrot, M.; Vincent, E. J. *J. Am. Chem. Soc.* **1985**, *107*, 5290.

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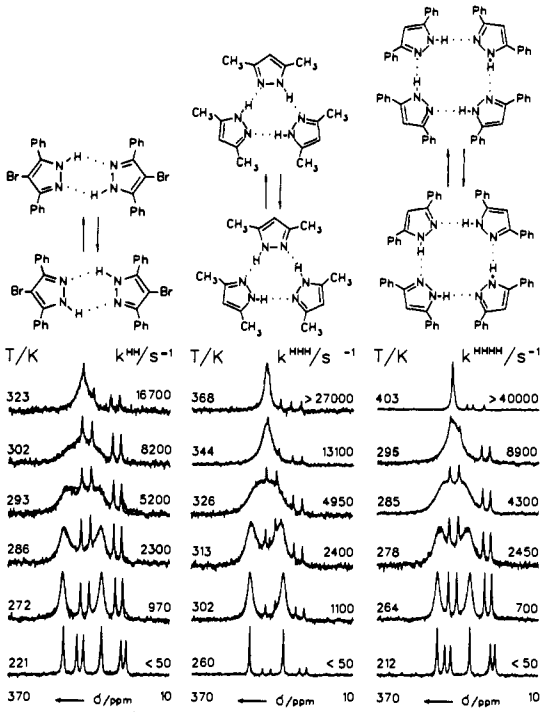

Figure 1. Superposed experimental and calculated ^{15}N CPMAS NMR spectra of 95% ^{15}N enriched DPBrP, DMP, and DPP[15] at 30.41 MHz as a function of temperature. Experimental conditions: 8–9 kHz sample spinning, 6–12 ms cross polarization time, 3.7 s repetition time, 5μs ^1H-$\pi/2$ pulses. Reference: external solid $^{15}NH_4Cl$. k: rate constant of proton transfer. The four sharp lines stem from a small quantity of the ^{15}N-enriched compound TTAA,[10] added in a small cylindric container to the rotor. The sample temperature was obtained from the TTAA line positions.

(DMP) forms cyclic trimers in the solid state in which a degenerate triple proton transfer takes place.[11,12] We now present evidence that pyrazoles may also form cyclic dimers and tetramers subject to double and quadruple proton transfers.

In Figure 1 the 30.41-MHz ^{15}N CPMAS NMR spectra (CP = cross polarization, MAS = magic angle spinning[13,14]) of ^{15}N-labeled solid 3,5-diphenyl-4-bromopyrazole (DPBrP), DMP, and 3,5-diphenylpyrazole (DPP) as a function of temperature are shown. As described previously[12] for DMP, we also observe for DPBrP and DPP two sharp lines at low temperature, indicating the presence of protonated and nonprotonated nitrogen atoms of equal concentration. As the temperature is raised, the two lines broaden and coalesce into one sharp line, indicating degenerate proton transfers with equilibrium constants of $K \approx 1$.

The X-ray crystallographic structures[17] of DPBrP and DPP are shown in Figure 2.[18] Two crystallographic independent molecules are present in the asymmetric units of both solids.

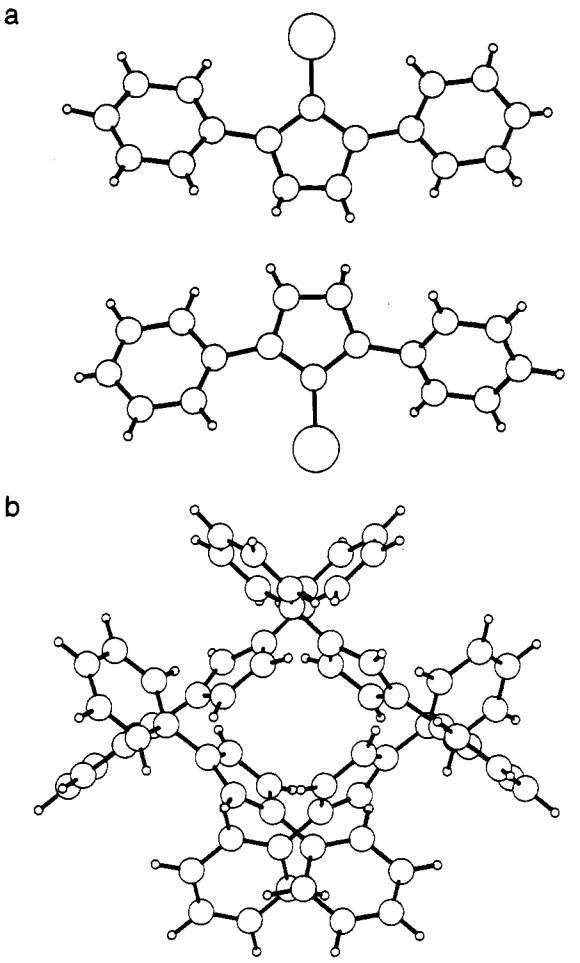

Figure 2. View[18] of the molecular structure of the dimer of 3,5-diphenyl-4-bromopyrazole (a) and of the tetramer of 3,5-diphenylpyrazole (b).

DPBrP forms a cyclic dimer with a pseudo 2-fold axis and DPP a cyclic tetramer that presents a pseudo 42m (D_{2h}) internal symmetry, of which only a 2-fold axis remains as a crystallographic element. The pyrazole rings do not present the typical high values for the intracyclic angles at the NH corner and at the C atom of the N=C bond,[19] an observation which is consistent with the disorder observed.

We assign the observed tautomeric processes to the multiple proton-transfer processes shown in Figure 1. It is interesting to note that the rates of proton transfer—determined by line shape analysis—first decrease and then increase again as the number of protons transferred is increased. This result could arise from a switch from a more or less concerted reaction pathway to a stepwise pathway[20] expected for an infinite cyclic hydrogen-bonded chain. Preliminary determinations of the multiple kinetic hydrogen/deuterium isotope effects by ^{15}N CPMAS NMR as well as preliminary ab initio calculations[21] do seem to indicate such a switch. An account of these studies will be given in a forthcoming paper. In conclusion, we have described a series of model reactions for experimental and theoretical studies of multiple proton transfers and their kinetic hydrogen/deuterium isotope effects.

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(12) Smith, J. A. S.; Wehrle, B.; Aguilar-Parrilla, F.; Limbach, H. H.; Foces-Foces, M.; Cano, F. H.; Elguero, J.; Baldy, A.; Pierrot, M.; Khurshid, M. M. T.; Larcombe-McDouall, J. B. *J. Am. Chem. Soc.* **1989**, *111*, 7304.
(13) Schaeffer, J.; Stejskal, E. O. *J. Am. Chem. Soc.* **1976**, *98*, 1031.
(14) Lyerla, J. R.; Yannoni, C. S.; Fyfe, C. A. *Acc. Chem. Res.* **1982**, *15*, 208.
(15) Doubly ^{15}N-labeled pyrazoles were prepared by conventional methods (Fusco, R. In *Pyrazoles, Pyrazolines, Pyrazolidines, Indazoles and Condensed Rings*; Interscience: New York, 1967) using $^{15}NH_2{}^{15}NH_2$.
(16) Doty, F. D.; Ellis, P. D. *Rev. Sci. Instrum.* **1981**, *52*, 1868.
(17) DPBrP: triclinic, $P\bar{1}$, a = 9.9067 (3), b = 13.3779 (6), c = 9.8906 (4) Å, α = 102.151 (3), β = 94.192 (3), and γ = 87.025 (3)°, Z = 4. DPP: monoclinic, $C2/c$, a = 16.9232 (6), b = 17.1426 (5), c = 17.6402 (4) Å, β = 109.604 (2)°, Z = 16. The structures were solved by direct methods and refined as usual. All hydrogen atoms were located on difference synthesis, appearing as maxima attached to both nitrogen atoms of the pyrazole ring, so that disorder models were set for them. Final residual synthesis did not show peaks higher than 0.82 and 0.42 Å^{-3}, respectively. Final R and R_w factors were 0.053 and 0.066 and 0.052 and 0.062 for 3411 and 2500 observed reflections.
(18) Motherwell, W. D. S. *PLUTO, A Program for Plotting Crystal and Molecular Structures*, Cambridge University, England, 1978.
(19) Bonati, F. *Gazz. Chim. Ital.* **1989**, *119*, 291.
(20) (a) Rumpel, H.; Limbach, H. H. *J. Am. Chem. Soc.* **1989**, *111*, 5429. (b) Scherer, G.; Limbach, H. H. *J. Am. Chem. Soc.* **1989**, *111*, 5946. (c) Schlabach, M.; Scherer, G.; Limbach, H. H. *J. Am. Chem. Soc.* **1991**, *113*, 3550. (d) Meschede, L.; Limbach, H. H. *J. Phys. Chem.* **1991**, *95*, 10267.
(21) Clementi, E.; Corongiu, G.; de Paz, J. L. G. Unpublished results.

the S.E.R.C. (U.K.), and the Spanish DGICYT (Project PB 90-0070) for financial support and research grants.

Registry No. DPBrP, 13788-85-7; DPP, 1145-01-3.

Supplementary Material Available: Tables of bond distances and angles, hydrogen interactions, atomic coordinates, and thermal parameters (9 pages); tables of observed and calculated structure factors (36 pages). Ordering information is given on any current masthead page.

Reactivity of Tunichromes: Reduction of Vanadium(V) and Vanadium(IV) to Vanadium(III) at Neutral pH

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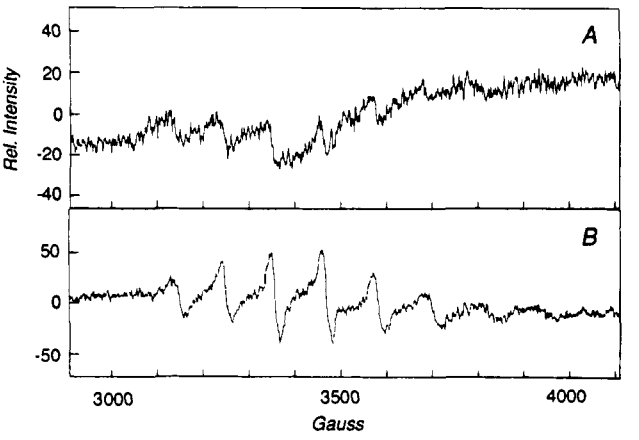
Ascidians accumulate vanadium ions to extraordinarily high concentrations (up to 1 M)[1a] from sea water, where vanadium is present in the +5 oxidation state.[1] In ascidian blood cells, however, the vanadium was found to be in the +3 and/or +4 states[2]—in *Ascidia nigra*, at least 90% of the total vanadium is in the oxygen-sensitive +3 state.[2a-c] Organic ligands are thought to maintain the solubility of concentrated vanadium at biological pH values.[1a,3] These ligands may belong to a class of oxygen-sensitive pigments **1** and **2**, called tunichromes,[4-6] whose polyphenolic moieties suggest a role in vanadium accumulation by complexation and/or reduction. It is known that tunichromes and catechols can reduce V^V to V^{IV} in vitro;[4,7,8] however, proof of any relationship between tunichrome and vanadium in vivo has been elusive.



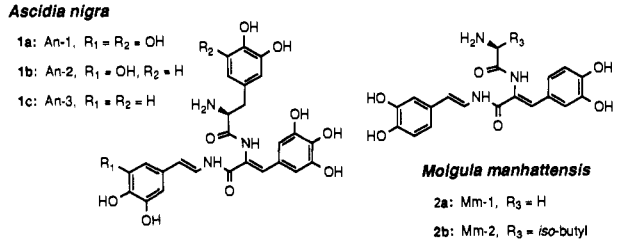
$$\text{Mm-1} + V^V\,(1\ \text{mol-equiv}) \xrightarrow[\text{(ii) pH 2 buffer, >1 hr}]{\text{(i) pH 7 buffer, 80 min}} A \xrightarrow[\text{(ii) Ar, 15 min}]{\text{(i) } O_2\text{, 10 min}} B$$

Figure 1. EPR spectra of Mm-1 treated with 1 mol equiv of V^V in pH 7 buffer (adjusted to pH 2 before EPR) before (A) and after (B) oxygenation.

Table I. Ratio of V^{IV} versus V^{III} Found by EPR Analysis of Mm-1 Treated with V^V or V^{IV} (1, 2, and 4 mol equiv) at pH 7[a]

entry	starting metal	V^{IV} found vs V^{III} found
1	1 mol equiv of V^{IV}	85:15
2	2 mol equiv of V^{IV}	90:10
3	4 mol equiv of V^{IV}	60:40
4	1 mol equiv of V^V	trace:<100
5	2 mol equiv of V^V	60:40
6	4 mol equiv of V^V	93:7

[a] Adjusted to pH 2 before EPR measurement at room temperature.



In studies of the general reactivity of tunichrome in vitro,[9] *we have obtained the first evidence (using EPR spectroscopy) that tunichrome can reduce V^V, and also V^{IV}, to the +3 oxidation state, V^{III}.* These reactions were conducted in neutral aqueous media. Similar redox reactivity was previously observed under anhydrous conditions: V^{IV} was reduced by pyrogallol in THF[10] and by 3,5-di-*tert*-butylcatechol in toluene or methanol.[11] The present results corroborate the hypotheses that tunichrome could generate V^{III} in vivo and that an oxidized form of tunichrome could sequester native V^{III}.

The reaction between the simplest tunichrome **2a** (synthetic Mm-1[12] in methanol/phosphate buffer pH 7) and V^V (from V_2O_5) or V^{IV} (from $VOSO_4$) was studied by EPR spectroscopy to determine the oxidation state(s) of product vanadium. EPR measures the V^{IV} oxidation state selectively,[13] but the levels of V^{III}

(1) (a) Carlson, R. M. K. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2217. (b) Endean, R. *Austr. J. Freshwater Res.* **1955**, *6*, 35. (c) Swinehart, J. H.; Biggs, W. R.; Halko, D. J.; Schroder, N. C. *Biol. Bull.* **1974**, *146*, 302. (d) Agudelo, M. I.; Kustin, K.; McLeod, G. C.; Robinson, W. C.; Wang, R. T. *Biol. Bull.* **1983**, *165*, 100. (e) Michibata, H.; Terada, T.; Anada, N.; Yamakawa, K.; Numakunai, T. *Biol. Bull.* **1986**, *171*, 672. (f) Lane, D. J. W.; Wilkes, S. L. *Acta Zool. (Stockholm)* **1988**, *69*, 135.

(2) (a) Lee, S.; Kustin, K.; Robinson, W. E.; Frankel, R. B.; Spartalian, K. *J. Inorg. Biochem.* **1988**, *33*, 183. (b) Boeri, E.; Ehrenberg, A. *Arch. Biochem. Biophys.* **1954**, *50*, 404. (c) Tullius, T. D.; Gillum, W. O.; Carlson, R. M. K.; Hodgson, K. O. *J. Am. Chem. Soc.* **1980**, *102*, 5670. (d) Hawkins, C. J.; Kott, P.; Parry, D. L.; Swinehart, J. H. *Comp. Biochem. Physiol.* **1983**, *76B*, 555. (e) Brand, S. G.; Hawkins, C. J.; Marshall, A. T.; Nette, G. W.; Parry, D. L. *Comp. Biochem. Physiol.* **1989**, *93B*, 425.

(3) (a) Hawkins, C. J.; James, G. A.; Parry, D. L.; Swinehart, J. H.; Wood, A. L. *Comp. Biochem. Physiol.* **1983**, *76B*, 559. (b) Frank, P.; Carlson, R. M. K.; Hodgson, K. O. *Inorg. Chem.* **1986**, *25*, 470. (c) Frank, P.; Carlson, R. M. K.; Hodgson, K. O. *Inorg. Chem.* **1988**, *27*, 118. (d) Lee, S.; Nakanishi, K.; Kustin, K. *Biochim. Biophys. Acta* **1990**, *1033*, 311.

(4) (a) Macara, I. G.; McLeod, G. C.; Kustin, K. *Biochem. J.* **1979**, *181*, 457. (b) Macara, I. G.; McLeod, G. C.; Kustin, K. *Comp. Biochem. Physiol.* **1979**, *63B*, 299.

(5) (a) Review: Smith, M. J.; Kim, D.; Horenstein, B.; Nakanishi, K.; Kustin, K. *Acc. Chem. Res.* **1991**, *24*, 117. (b) Bruening, R. C.; Oltz, E. M.; Furukawa, J.; Nakanishi, K.; Kustin, K. *J. Am. Chem. Soc.* **1985**, *107*, 5298. (c) Bruening, R. C.; Oltz, E. M.; Furukawa, J.; Nakanishi, K.; Kustin, K. *J. Nat. Prod.* **1986**, *49*, 193. (d) Oltz, E. M.; Bruening, R. C.; Smith, M. J.; Kustin, K.; Nakanishi, K. *J. Am. Chem. Soc.* **1988**, *110*, 6162. (e) Kim, D.; Li, Y.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1991**, 9.

(6) Recently, dihydrotunichromes corresponding to An-1, -2, and -3 were isolated and characterized from *Phallusia mamillata*: Bayer, E.; Schiefer, G.; Waidelich, D.; Scippa, S.; de Vincentiis, M. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 52.

(7) Kime-Hunt, E.; Spartalian, K.; Holmes, S.; Mohan, M.; Carrano, C. J. *J. Inorg. Biochem.* **1991**, *41*, 125.

(8) (a) Kustin, K.; Liu, S.-T.; Nicolini, C.; Toppen, D. L. *J. Am. Chem. Soc.* **1974**, *96*, 7410. (b) Kustin, K.; Nicolini, C.; Toppen, D. L. *J. Am. Chem. Soc.* **1974**, *96*, 7416. (c) Cantley, L. C., Jr.; Ferguson, J. H.; Kustin, K. *J. Am. Chem. Soc.* **1978**, *100*, 5211. (d) Ferguson, D. F.; Kustin, K. *Inorg. Chem.* **1979**, *18*, 3349.

(9) Ryan, D. E.; Ghatlia, N. D.; Nakanishi, K. Manuscript in preparation.

(10) Lee, S.; Nakanishi, K.; Chiang, M. Y.; Frankel, R. B.; Spartalian, K. *J. Chem. Soc., Chem. Commun.* **1988**, 785.

(11) Crass, M. E.; Greene, D. L.; Buchanan, R. M.; Pierpont, C. G. *J. Am. Chem. Soc.* **1983**, *105*, 2680.

(12) (a) Horenstein, B.; Nakanishi, K. *J. Am. Chem. Soc.* **1989**, *111*, 6242. (b) Kim, D.; Li, Y.; Horenstein, B.; Nakanishi, K. *Tetrahedron Lett.* **1990**, *31*, 7119.

0002-7863/92/1514-9659$03.00/0