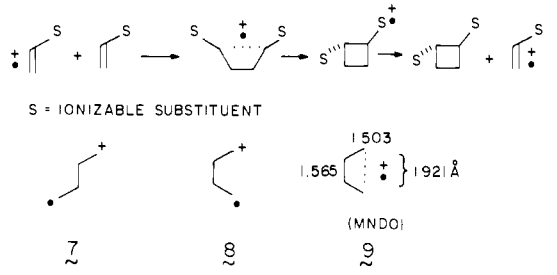
Scheme II

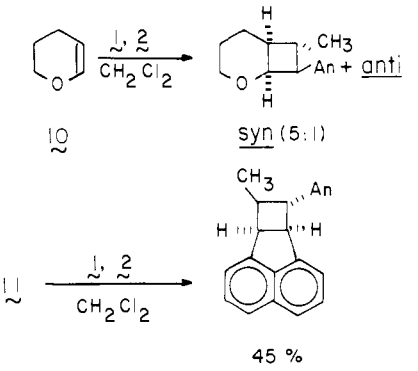


of *cis*-**1** is stereoselectively $[_{\pi}2_s + {}_{\pi}1_s]$, as was found for the dimerization of *trans*-**1** at 0 and −35 °C and the cycloreversion of **4** at 0 °C. It is therefore concluded that the cyclodimerization of **1** is inherently stereospecific.

For a reaction that has been regarded as stepwise, the stereospecificity of the anethole cycloaddition is mildly surprising. A stepwise mechanism involving an open *s-trans*-cation radical intermediate (**7**, Scheme II) seems clearly to be ruled out. The involvement of an open, *s-cis*-cation radical intermediate (**8**) cannot be rigorously eliminated, since cyclization could be more rapid than the relevant rotations in such an intermediate. However, this possibility is regarded as remote. A theoretical study of the prototype [2 + 1] cycloaddition suggests a novel modification of this latter mechanism that is in excellent accord with the stereochemical results (Scheme II). The key observation is that the cyclobutane cation radical structural minimum occurs, according to both MNDO and optimized STO-3G calculations, at a long (one-electron) bond structure (**9**) similar to those found for the ethane and other alkane cation radicals.[6] A long bond dissociation energy comparable to that of the one-electron bond in the ethane cation radical (38 kcal/mol) should easily be sufficient to preserve configurational stability in **9**. Direct experimental evidence for stable long-bond structures is available form CIDNP studies on the *cis*- and *trans*-1,2-diphenylcyclopropane cation radicals.[7] These cation radicals have a long 1,2 bond and are configurationally stable. Subsequent closure of the long-bond cyclobutane to a "normal" cyclobutane structure appears feasible only when relatively ionizable (usually π or n) substituents are present upon which to localize the cation radical site in the fully cyclized cyclobutane adduct. The MNDO reaction path calculation reveals the powerful effect of the cation radical "hole" in lowering the barrier to cycloaddition. The activation energy for the formation of **9** from ethene and ethene cation radical is merely 1.3 kcal/mol.[8]

The chemical cyclodimerization[9] procedures reported by Ledwith were apparently limited to vinylcarbazoles and a few extremely electron-rich alkenes (e.g., 1,1-bis(*p*-(dimethylamino)-phenyl)ethene).[2] The new catalyst system therefore extends the reaction scope modestly, to include double bonds activated by anisyl substituents. Nevertheless, simple olefins and olefins activated only by phenyl groups remain unreactive or are polymerized by **2**. Within its very considerable limitations, the new proceudre is rapid and convenient, and it has proved capable of effecting the first reported [2 + 1] cycloadditions of nonequivalent olefins. The additions of *trans*-**1** to dihydropyran (**10**) and acenaphthylene (**11**) illustrate this capability (Scheme III). The former reaction (53% yield based on **1** consumed; 11% conversion) is highly regiospecific, but forms syn:anti isomers in the ratio 5:1. The yield of adduct from **9** is 45% at complete conversion (syn:anti = 1:10).

Scheme III



Work designed to further extend the scope of the cyclodimerization is in progress.

Acknowledgment. We thank the Robert A. Welch Foundation (F-149) for support of this research and the NSF for a Minority Graduate Student Fellowship to R.P.

(6) Bellville, D. .; Bauld, N. L. *J. Am. Chem. Soc.* **1982**, *104*, 5700.

(7) Roth, H. D.; Mannion, M. L. *J. Am. Chem. Soc.* **1981**, *103*, 7210.

(8) The transition state was refined and verified by using Professor M. J. S. Dewar's NDTS60B and NDFORB programs. Full details of the reaction path calculation are included in a full paper submitted to this journal.

(9) A photosensitized version that has somewhat greater scope has evolved from Ledwith's work, e.g.: Farid, S.; Shealer, S. E. *J. Chem. Soc., Chem. Commun.* **1973**, 677.

Red Shifts in the Optical Spectra of Porphyrin Schiff Bases upon Protonation

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In a number of metalloporphyrin- and metallochlorin-containing proteins, there is an opportunity for modulation of the physical and chemical properties of the ring system by interactions between its peripheral substituents and nearby amino acid side chains. Such effects, for example, have been postulated for the vinyl groups of protoheme in hemoglobin and in cytochrome b_5,[1] for the formyl group of heme *a* in cytochrome oxidase,[2] and for the keto and formyl groups of various chlorophyll species.[3] A Schiff's base linkage between a substituent carbonyl group and a protein amino group donor is especially attractive in this role owing to the fact that both formation of the linkage and its subsequent protonation may be subject to functional control. Recent work on the linear polyene aldehyde retinal and its Schiff's bases has demonstrated the strong dependence of the optical properties of the chromophore upon the chemistry that occurs at the CHO group;[4] moreover, protonation/deprotonation reactions of the retinal Schiff's base species in situ in rhodopsin[5] and in bacteriorhodopsin[6] have been

(1) (a) Warshel, A.; Weiss, R. M. *J. Am. Chem. Soc.* **1981**, *103*, 446-451. (b) LaMar, G. N.; Burns, P. D.; Jackson, J. T.; Smith, K. M.; Langry, K. C.; Strittmatter, P. *J. Biol. Chem.* **1981**, *256*, 6075-6079.

(2) (a) Coin, J. T.; Hinkle, P. C. In "Membrane Bioenergetics"; Lee, C. P., Schatz, G., Ernster, L., Eds.; Addison-Wesley: Reading, MA, 1979; pp 405-412. (b) Chang, C. K. In "Oxygen, Biochemical and Clinical Aspects"; Caughey, W. S., Ed.; Academic Press: New York, 1979; p 437. (c) Babcock, G. T.; Salmeen, I. *Biochemistry* **1979**, *18*, 2493-2498.

(3) (a) Davis, R. C.; Ditson, S. L.; Fentiman, A. F.; Pearlstein, R. M. *J. Am. Chem. Soc.* **1981**, *103*, 6823-6826. (b) Wasielewski, M. R.; Norris, J. R.; Shipman, L.; Lin, C.-P.; Svec, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2957-2961.

(4) (a) Lewis, A.; Marcus, M. A.; Ehrenberg, B.; Crespi, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4642-4646. (b) Motto, M. G.; Sheves, M.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1980**, *102*, 7947-7949.

(5) (a) Lewis, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 549-553. (b) Honig, B.; Ebrey, T.; Callender, R. H.; Dinur, U.; Ottolenghi, M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2503-2507.

(6) (a) Kalisky, O.; Ottolenghi, M.; Honig, B.; Korenstein, R. *Biochemistry* **1981**, *20*, 649-655. (b) Ort, D.; Parson, W. W. *J. Biol. Chem.* **1978**, *253*, 6158-6164. (c) Terner, J.; Hsieh, C.-L.; Burns, A. R.; El-Sayed, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 3046-3050. (d) Braiman, M.; Mathies, R. *Biochemistry* **1980**, *19*, 5421-5428.

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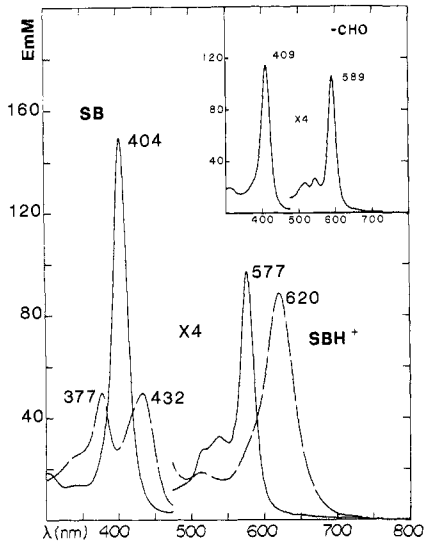
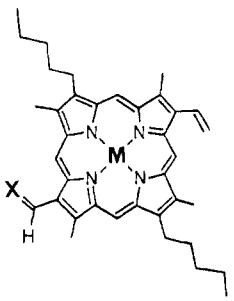


Figure 1. UV–visible spectra in CH_2Cl_2 of nickel(II) formylvinylporphyrin **2** (inset), *n*-butyl Schiff's base **3** (solid), and *n*-butyl Schiff's base hydrochloride (dashed).

intimately associated with the proton-pumping reactions of these proteins. Our initial interest in protonated porphyrin Schiff's bases was stimulated by the apparent analogy between these reactions and those that may occur for heme *a* in its involvement in proton pumping in the mitochondrial protein, cytochrome oxidase.[7] We report here the preparation of the protonated Schiff's base of a heme *a* analogue and its characterization by optical, NMR, and resonance Raman spectroscopies.

The 4,8-formylvinylporphyrin **1** derivatives have been used



1, X = O; M = 2H
2, X = O; M = Ni
3, X = NBu, M = Ni
4, X = $^+$NHBu, M = Ni

previously to model spectroscopic and ligand-binding properties of heme *a*.[2b,8] In the present study the Ni(II) complex **2** was chosen for elaboration for the fact that it is acid stable and diamagnetic and does not easily bind axial ligands. Thus **2** was refluxed for 3 h in dry benzene containing excess *n*-butylamine. Water produced was removed by allowing the condensed azeotropic mixture to return to the flask by passing through a silica gel pad. Lyophilization afforded pure Schiff's base **3**. The electronic spectrum of **3** is shown in Figure 1 along with that of **2**. It can be seen that, except for the wavelength shift of the Soret and α-band maxima, the spectral features of **3** and **2** are similar. When 1.2 equiv of dry HCl gas was injected to the solution of **3**, a dramatic color change took place turning the red Schiff's base (SB) into the deep green protonated Schiff's base (SBH^+), **4**, which showed a red-shifted visible band and a split Soret band (Figure 1). The interconversions SB ⇌ SBH^+ are reversible and can be effected by acid–base titrations with isosbestic points by

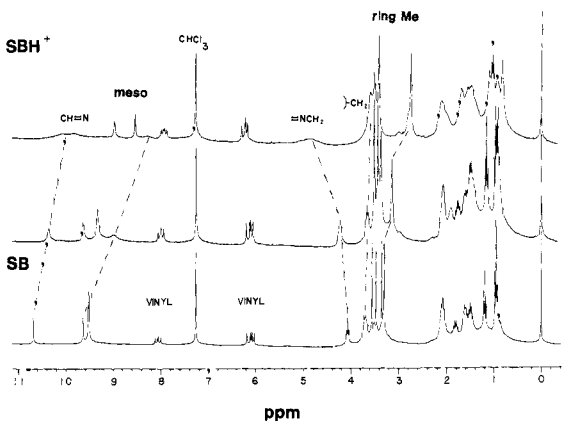


Figure 2. 250-MHz NMR spectra of nickel(II) formylvinylporphyrin *n*-butyl Schiff's base **3** plus HCl in $CDCl_3$. Unprotonated, intermediate (0.5 equiv of HCl), and complete protonation (1.2 equiv of HCl) from bottom to top, respectively. The assignments were made by comparison with spectra of aldehyde **2** and several other related formylporphyrins as well as by homonuclear decouplings. The four meso protons of SB are at δ 9.65 (1 H), 9.50 (1 H), and 9.45 (2 H) and the CH–N proton is at δ 10.7 (1 H).

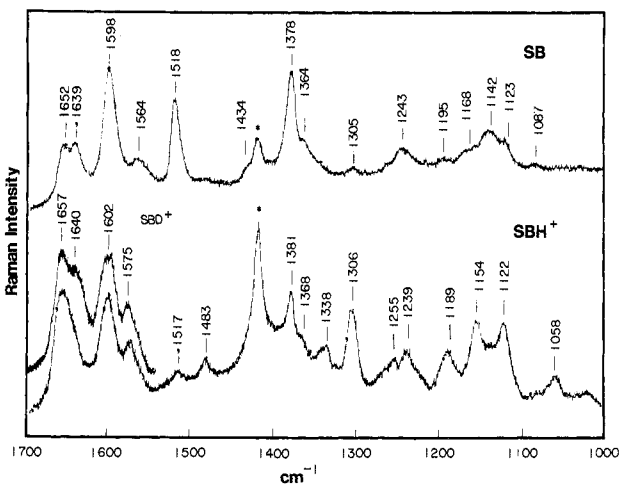


Figure 3. Resonance Raman spectra in CH_2Cl_2 with 406.7-nm laser excitation of nickel(II) formylvinylporphyrin *n*-butyl Schiff's base **3** (top), plus HCl (bottom), and plus DCl (middle, 1550–1700 cm^{-1}). The resolution in the spectra is ±2 cm^{-1}.

using a variety of acids and bases. The copper(II) complex of **2** also behaves in an analogous manner.[9]

Proton NMR was employed to confirm the formation of the protonated species (Figure 2). Progressive acidification caused all protons near the C=N group to shift and broaden due to exchange. While the deshielding effect of protonation of the C=N double bond undoubtedly could produce the upfield shift for the CH=N proton, the adjacent meso proton, and the adjacent pyrrolic methyl group, the protonation caused the α-imino methylene protons to shift downfield owing to increased electronegativity. That perturbation of peripheral substituents decreases as the group becomes more distant from the positive center suggests that the positive center is not thoroughly delocalized throughout the π system, but rather Schiff's base protonation has the effect of generating an exceptionally strong electron-withdrawing group.[10]

The resonance Raman spectra of **3** (SB) and of **4** (SBH^+) were recorded with laser excitation (406.7 nm) in resonance with the Soret absorption band[11] (Figure 3). The depolarization ratios

(7) (a) Wikström, M.; Krab, K. *Biochim. Biophys. Acta* **1979**, *549*, 177–222. (b) Wikström, M.; Krab, K.; Saraste, M. "Cytochrome Oxidase, A Synthesis"; Academic Press: New York, 1981.
(8) (a) Babcock, G. T.; Chang, C. K. *FEBS Lett.* **1979**, *97*, 358–362. (b) Chang, C. K.; Hatada, M. H.; Tulinsky, A., in press.
(9) For copper(II) porphyrin, the visible band shifts from 585 (SB) to 630 nm (SBH^+) and the Soret peak splits (408 → 379 and 435 nm).
(10) Malononitrile adduct formation of a related system resulted in spectral changes that were nearly identical with the protonation of SB. (B. Ward and C. K. Chang, manuscript submitted.)

Table I. Vibrations Observed and Normal Coordinate Assignments[a] for Nickel(II) Porphyrin Schiff's Base Species

SB	SBH^+	assignment (no.)	SB	SBH^+	assignment (no.)
1652	1657	$B_{1g}(\nu_{10})$	1564		$E_u(\nu_{38})$
1639		$\nu_s(-N{=}CH-)$	1517	1518	$A_{1g}(\nu_3)$
	1650	$\nu_s(-{}^+NH{=}CH-)$	1378	1381	$A_{1g}(\nu_4)$
	1640	$\nu_s(-{}^+ND{=}CH-)$	1305	1306	$A_{2g}(\nu_{21})$
1598	1602	$A_{1g}(\nu_2)$		1154	$B_{2g}(\nu_{30})$
	1575	$B_{1g}(\nu_{11})$	1123	1122	$A_{2g}(\nu_{22})$

[a] Symmetries and mode numbers from Abe et al.[13] In the more correct C_{2h} group for **3** and **4**, $\{A_{1g}, B_{1g}, A_{2g}, B_{2g}\}$ become A_g.

ranged from 0.1 to 0.75, consistent with the Franck-Condon scattering mechanism which dominates with Soret excitation.[12] Using the normal coordinate analysis of octaethylporphyrinato-Ni(II), we have collected several of the prominent vibrations of the Schiff's base species in Table I and assigned these in analogy with the results of Abe et al.[13] We have retained the D_{4h} symmetry notation characteristic of the porphyrin core. The Raman spectrum of **3** shows strong enhancement of totally symmetric modes at 1598 (ν_2), 1518 (ν_3), and 1383 cm^{-1} (ν_4). The line observed at 1652 cm^{-1} corresponds to a B_{1g} mode (ν_{10}), which is most likely enhanced through a Jahn-Teller or intramanifold coupling mechanism;[14] it is commonly observed when studying hemes and heme proteins by Soret excitation Raman.[15] The Schiff's base C=N stretching vibration is responsible for the line observed at 1639 cm^{-1}. We do not observe a clearly identifiable vinyl stretching mode. For the protonated Schiff's base, we note little change in the frequencies of the observed ring vibrations,[16] consistent with the NMR data above, which indicated that protonation effects are localized at the Schiff's base and do not strongly perturb the basic porphyrin bonding pattern. However, the decrease in symmetry, which is apparent in the optical spectrum, also appears to be reflected in the Raman spectrum of SBH^+ in that the scattered intensity from non-totally symmetric modes (B_{1g}, A_{2g}, B_{2g}) is stronger relative to the free Schiff's base.[17] Protonation of the Schiff's base shifts the N=C stretching frequency into the 1650-cm^{-1} region where it overlaps strongly with ν_{10}. In order to determine its frequency more precisely, we carried out analogous IR experiments that showed $\bar{\nu}(^+NH{=}CH) = 1650$ cm^{-1}; these data form the basis for the assignment of the two vibrational frequencies in Table I. If DCl is used to deuterate the porphyrin Schiff's base, the stretching frequency decreases to 1640 cm^{-1}. A similar pattern of -C=X- stretching vibration frequency shifts is observed in retinal Schiff's bases upon protonation and deuteration, and the physical mechanism underlying these shifts has been discussed in detail by Aton et al.[18] It appears as if the interaction between the C=N stretching vibration and the C=N-H bending mode is somewhat less in the porphyrin case than in the linear polyene retinal case.

The NMR and Raman data do not suggest an extensive π delocalization as the origin of the visible absorption spectral changes occurring in SBH^+, despite the apparent spectral similarities between **4** and the many well-characterized porphyrin π-cation radicals.[19] While the optical band shifts can result from a decrease in molecular symmetry, quantitative interpretation of the spectrum must await detailed MO calculations.

We speculated earlier[20] that a protonated Schiff's base linkage may be responsible for the unusual behavior of cytochrome *a*.[11] While the vibrational frequency of the Ni(II)-porphyrin $^+NH{=}CH-$ substituent is in reasonable agreement with that observed in oxidized cytochrome *a*, the optical spectrum of the protonated Schiff's base shows a significantly larger visible absorption red shift and Soret absorption band split than observed for the in situ chromophore. For this reason, it seems unlikely that a protonated Schiff's base is a good model for cytochrome *a*.[21] However, the unusual spectral properties of protonated metalloporphyrin Schiff's bases may have significant implications for other porphyrin-based systems. Photosynthetic systems provide a possible example in that chlorophyll absorption red shifts of the magnitude we report here are commonly observed in situ. These have sometimes been interpreted as resulting from dimer or higher order aggregate formation;[22] our results indicate that similar shifts can be obtained in a monomer system if protein/chromophore interactions occur.

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Registry No. 2, 84195-13-1; **3**, 84195-14-2; **4**, 84195-15-3; butylamine, 109-73-9.

(11) Ondrias, M. R.; Babcock, G. T. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 29-35.
(12) Felton, R. H.; Yu, N.-T. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. III, pp 347-393.
(13) Abe, M.; Kitagawa, T.; Kyogoku, Y. *J. Chem. Phys.* **1978**, *69*, 4526-4534.
(14) Shelnutt, J. A. *J. Chem. Phys.* **1981**, *74*, 6644-6657.
(15) Callahan, P. M.; Babcock, G. T. *Biochemistry* **1981**, *20*, 952-958.
(16) The 3-4-cm^{-1} increases, which are apparent in ν_2 and in ν_{10} upon formation of the protonated Schiff's base, most likely arise from a slight porphyrin core contraction accompanying the protonation reaction.[15]
(17) The shifts observed in relative intensity for the symmetric and non-totally symmetric modes (D_{4h} notation) upon protonation of the Schiff's base may arise from symmetry reduction, which allows Franck-Condon enhancement of both classes of vibration. Alternatively, these shifts in intensity may arise from interference effects or changes in vibronic coupling introduced by the splitting observed in the optical absorption spectrum.
(18) Aton, B.; Doukas, A. G.; Narva, D.; Callender, R. H.; Dinur, U.; Honig, B. *Biophys. J.* **1980**, *29*, 79-94.
(19) Felton, R. H. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. V, p 53.
(20) Babcock, G. T.; Callahan, P. M.; Ondrias, M. R.; Salmeen, I. *Biochemistry* **1981**, *20*, 959-966.
(21) In a separate series of experiments, however, we have found that a hydrogen-bond interaction between the formyl group of cytochrome *a* and a protein amino acid side chain provides an adequate explanation of the unusual properties of cytochrome *a* (Callahan, P. M.; Babcock, G. T. *Biochemistry*, in press).
(22) For a discussion of this point see: Davis, R. C.; Pearlstein, R. M. *Nature (London)* **1979**, *280*, 413-415.

Methylated Bases Stabilize Short RNA Duplexes

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The triribonucleotide GpCpA forms a stable duplex containing two G·C Watson-Crick base pairs and two 3'-dangling adenosines.[1,2] Dangling bases have been found to increase base stacking and thus improve overall duplex strength.[1-4] In this study, GpCpX sequences (where X = m^6A, m_2^6A, m^1G) were used as model systems to examine the effect of heterobase methylation on duplex stability (Figure 1). Results indicate that the stability of RNA duplexes

$$\begin{matrix} 5'\text{-GpCpX-}3' \\ 3'\text{-XpCpG-}5' \end{matrix}$$

(1) Alkema, D.; Bell, R. A.; Hader, P. A.; Neilson, T. *J. Am. Chem. Soc.* **1981**, *103*, 2866-2868.
(2) Alkema, D.; Bell, R. A.; Hader, P. A.; Neilson, T. In "Biomolecular Stereodynamics"; Sarma, R. H., Ed.; Adenine Press: New York, 1981; pp 417-428.
(3) Martin, F. H.; Uhlenbeck, O. C.; Doty, P. *J.Mol. Biol.* **1971**, *57*, 201-215.
(4) Neilson, T.; Romaniuk, P. J.; Alkema, D.; Everett, J. R.; Hughes, D. W.; Bell, R. A. *Nucleic Acid Res. Spec. Publ.* **1980**, *no.* 7, 293-311.

0002-7863/83/1505-0636$01.50/0