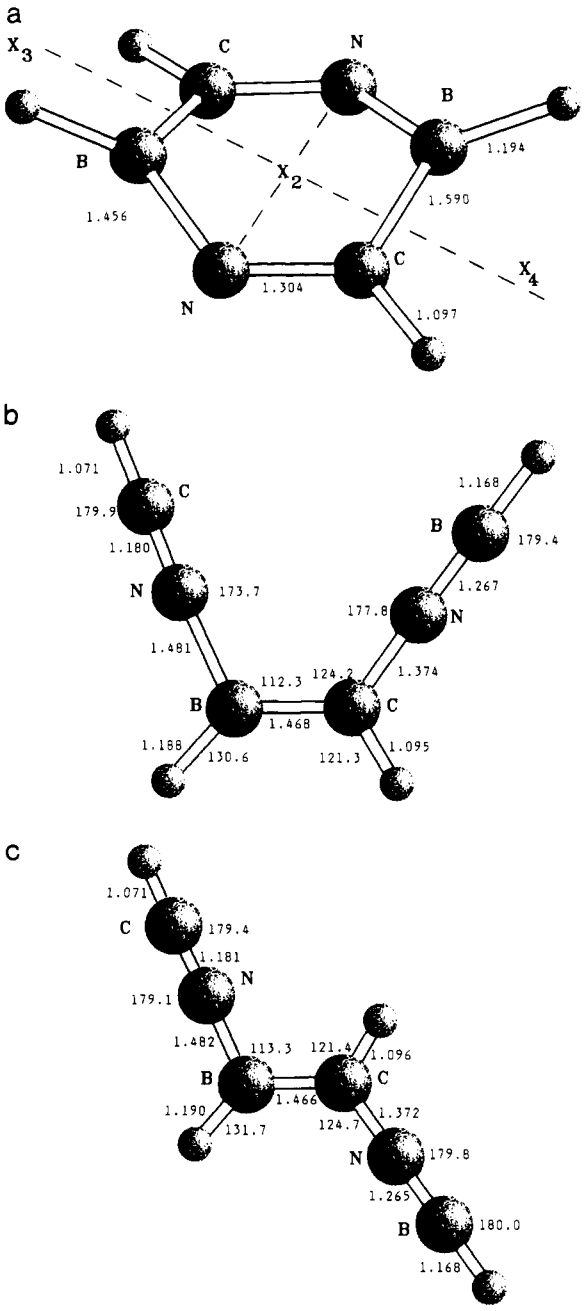

Figure 1. Three MBPT(2)/DZP optimized structures of $C_2H_4B_2N_2$. (a) Boat (C_2). Bond angles (deg): CNX_2 = 63.5; BNX_2 = 56.1; HCN = 119.1; HBN = 120.7; X_3X_2N = 90.0. Dihedral angles (deg): $CNX_2X_3(X_4)$ = 172.4; $BNX_2X_4(X_3)$ = 156.0; $HCNX_2$ = -158.1; $HBNX_2$ = -144.9. X_2 is the NN distance midpoint; X_3, X_2, and X_4 are collinear. (b) Cis (C_s). (c) Trans (C_s).

semiempirical AM1 optimizations are first performed, followed by SCF/3-21G and SCF/DZP (double-ζ basis with polarization) evaluation. The DZP basis set is described in ref 15. Note that at the SCF/3-21G level we find both boat and chair ring conformations differing only by a few kilojoules/mole but at the SCF/DZP level the chair minimum disappears. Final refinements are made at the MBPT(2)/DZP (second-order perturbation theory) level. The geometry of the ring molecule $C_2H_4B_2N_2$ is presented in Figure 1a.

We present a selection of reaction energies in Table I. All correlated reaction energies are based on MBPT(2) optimized geometries partly taken from our previous study.[15] As follows from data in Table I, the boat-ring molecule is markedly more stable when compared to two BH_2CN molecules. Comparing the energy of the boat to the two HCN_BH molecules, we can estimate the binding energy in our ring compound to be 565 kJ/mol, which corresponds to the formation of the two B–C bonds in the ring.

Besides the ring $C_2H_4B_2N_2$, we also suggest two nonring "cis" and "trans" molecules (see parts b and c of Figure 1). Both are more stable than two BH_2CN, with the electron correlation contribution being a crucial factor in their stability. The strength of the B–C bond in both chain dimers is pretty high, as is seen from the reaction energies cis(trans) → 2HCN_BH in Table I. This is in accord with the relatively short B–C bond length, which corresponds to a double bond. The shorter of the two BN bond lengths is close to a triple BN bond length typical in iminoboranes,[1] with practically a linear H–B–N–C chain.

Though our suggested molecules have the same molecular formula, $(BH_2CN)_2$, as the experimentally described molecules mentioned above,[2] also shared by the nonclassical cyanoborane, they are completely different. First, the cited authors found at least rings formed from tetramers. Second, and even more important, the bonding in our molecules contains the two- and three-coordinate boron, while in the experimentally described $(BH_2CH)_x$ polymers there is a four-coordinate boron. The three-coordinate boron atoms conform to classical boron compounds; the occurrence of the two-coordinate boron is less typical.[1] Since both boron atoms are three coordinate in the boat-ring structure, with a nearly planar conformation of C, N, and H atoms bonded to B, this would be expected to rationalize its high stability.

The possible polymeric structure is not shown in this paper but may be easily derived from nonring dimers, preferably trans, simply by bending any of the terminal hydrogens and adding the next HCN_BH molecule. In the closed polymer structure, all borons and carbons would be tricoordinate and, thus, further stabilized.

A more detailed description of geometry, vibrational spectra, thermodynamics of pertinent reactions, and bonding analysis will be presented in a forthcoming paper.[20] Since electron correlation is so important in reaction energies, we also extend the MBPT(2) treatment to the more sophisticated coupled-cluster approach.

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(20) Cernusak, I.; Urban, M.; Stanton, J. F.; Bartlett, R. J. In preparation.

The Barium(II) Complex of *p-tert*-Butylcalix[4]arene-crown-5: A Novel Nucleophilic Catalyst with Transacylase Activity

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In this communication we report that *p-tert*-butylcalix[4]arene-crown-5 (**1**),[1,2] when suitably activated by barium ion under moderately basic conditions, acts as a fairly efficient transacylation catalyst in the methanolysis of *p*-nitrophenyl acetate (pNPOAc)

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(1) (a) Ungaro, R.; Pochini, A.; Andreetti, G. D. *J. Inclusion Phenom.* **1984**, *2*, 199–206. (b) Ghidini, E.; Ugozzoli, F.; Ungaro, R.; Harkema, S.; El-Fadl, A. A.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1990**, *112*, 6979–6985.

(2) Casnati, A.; Pochini, A.; Ungaro, R.; Cacciapaglia, R.; Mandolini, L. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2052–2054.

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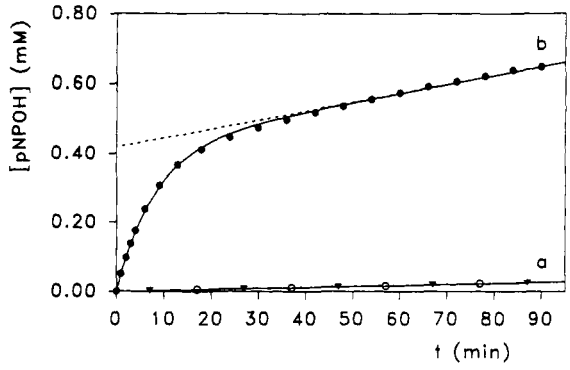
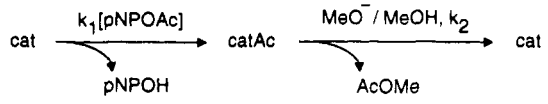
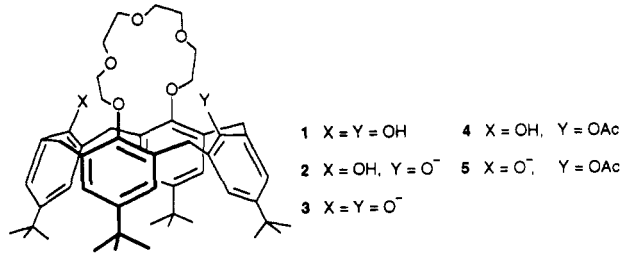


Figure 1. Appearance of pNPOH during methanolysis of pNPOAc in MeCN–MeOH (9:1) in the presence of diisopropylethylamine–bromide salt buffer. Curve a: ▼, background reaction measured in the presence of buffer alone; ○, buffer plus 0.46 mM **1**. Curve b: buffer plus 0.46 mM **1** and 0.46 mM $BaBr_2$; the full line is calculated by means of eq 1 and the least-squares parameters given in the text.

Scheme I



in MeCN–MeOH (9:1, v/v) containing diisopropylethylamine–bromide salt buffer (B and BHBr, respectively) at 25 °C. The results of a typical set of kinetic experiments are plotted in Figure 1. The concentrations of the buffer components were [B] = 6.2×10^{-2} and [BHBr] = 2.0×10^{-2} M ([B]/[BHBr] = 3.1), and that of pNPOAc was 3.0 mM. No meaningful method of measuring the pH in this solvent system is available, but an idea of the low basicity of the buffer solution is provided by the observation that *p*-nitrophenol (pNPOH) is only 60% ionized in this medium. The spectrophotometrically determined liberation of pNPOH due to background methanolysis (curve a) is rather slow, with an initial rate of 2.9×10^{-7} mol L^{-1} min^{-1} ($k_{obsd} = 9.7 \times 10^{-5}$ min^{-1}, $t_{1/2}$ ca. 5 days) which is unaffected by addition of 0.46 mM **1**. However, addition of a 1:1 mixture of **1** and $BaBr_2$ (0.46 mM) causes a significant increase in the reaction rate, as shown by curve b. There is an initial burst of pNPOH release, followed by a linear portion whose slope (2.5×10^{-6} mol L^{-1} min^{-1}) is an order of magnitude larger than that of the background. The linear portion extrapolates back to an initial burst π of 0.42 mM, which corresponds to 91% of the initial concentration of **1**. The phenomenological behavior is clearly that of ping-pong kinetics,[3] which provides a strong indication of the occurrence of a catalyst–substrate covalent intermediate.



The simplest possible mechanistic scheme for nucleophilic catalysis of acyl transfer is given in Scheme I, where cat and catAc denote active forms of the catalyst and of the acylated intermediate, respectively. The integrated rate equation is[3]

$$[pNPOH] = [cat]_0\tau k_1'\{\tau k_1'[1 - \exp(-t/\tau)] + k_2 t\} \quad (1)$$

where $k_1' = k_1[pNPOAc]$ and $\tau = (k_1' + k_2)^{-1}$. $1/\tau$ is the first-order rate constant for the initial exponential phase (pre steady state). When the exponential term dies out, the steady state is reached and eq 1 reduces to the form

$$[pNPOH] = [cat]_0(\tau k_1')^2 + [cat]_0\tau k_1' k_2 t \quad (2)$$

which is the equation of a straight line, whose intercept defines the magnitude of burst and whose slope divided by $[cat]_0$ represents the turnover number of the catalyst. A nonlinear least-squares fit of the data to eq 1 gave the following values for the relevant parameters: $1/\tau = 0.12$ min^{-1}; $k_1' = 0.115$ min^{-1}; $k_2 = 5.8 \times 10^{-3}$ min^{-1}. According to this analysis, only 0.91 mol of pNPOH is released per mole of catalyst at zero time because acetylation of the catalyst is not sufficiently faster than deacetylation for the acetylated catalyst to accumulate fully. The turnover number is 5.5×10^{-3} min^{-1}, which means that the catalyst turns over eight times per day.

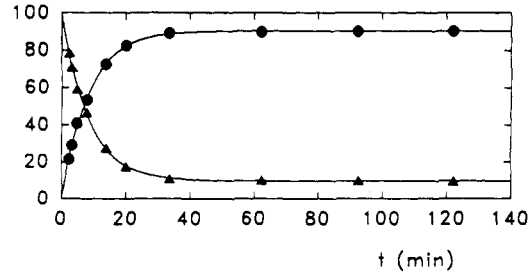


Figure 2. HPLC detection of the acetylated intermediate. Mole fraction (× 100) of catalyst in the acetylated form **4** (●) and in the native form **1** (▲) as a function of time.

Evidence that an acylated intermediate lies on the catalysis pathway was obtained by HPLC analysis of acid-quenched samples of the reaction mixture. The mole fractions of the acetylated catalyst **4** and of the parent catalyst **1** vary with time as shown in Figure 2. The rate constant for the first-order accumulation of **4** is 0.12 min^{-1}, in agreement with the $1/\tau$ value measured from the initial exponential phase of pNPOH release. Furthermore, the steady-state concentration of **4** is 90% of the initial concentration of **1**, which compares fairly well with the value of 95% calculated from the kinetics.[4] No decomposition of the catalyst is observed over an extended period of time. After 24 h from the start, the sum of **1** and **4** still equals the initial concentration of **1**, and only 46% of the catalyst is found in the acetylated form **4** because of nearly complete exhaustion of the substrate supply.

Inspection of the UV spectra of **1** and **4** showed that both compounds remain in their un-ionized forms in the given buffer solution, but in the presence of 1 mol equiv of $BaBr_2$ nearly one-half of **4** is converted into **5**·Ba^{2+}, whereas about one-sixth of **1** is in the form of the neutral barium complex **3**·Ba^{2+} with the rest probably in the form **2**·Ba^{2+}. There is little doubt that the active form of the acylated intermediate is **5**·Ba^{2+}, which was recently found[5] to undergo attack of methoxide ion in MeOH more than a million-fold faster than the parent compound **4**. Consistently, we found that the deacylation step exhibits a higher than first-order dependence on buffer ratio, whereas the background methanolysis is strictly first-order. This clearly indicates not only that MeO^- is the active nucleophile in the deacylation of pNPOAc and of **5**·Ba^{2+} but also that an increase in the basicity of the medium increases the concentration of the active form of the acetylated intermediate. On the other hand, the acetylation step exhibits an apparent order in buffer ratio between zero and one, which provides a strong indication that the active form of the catalyst is **3**·Ba^{2+}. Here the barium ion acts as a built-in electrophilic catalyst which favors nucleophilic addition to carbonyl both in the acylation and deacylation steps, which is in keeping with our recent findings that hard metal ions can greatly enhance rates of acyl transfer from aryl acetates to anionic nucleophiles.[6]

(3) Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman and Co.: New York, 1985; Chapter 4.

(4) The mole fraction of the acetylated intermediate **4** at steady state[3] is given by $[4]_{ss}/[1]_0 = \tau k_1' = (\pi/[1]_0)^{1/2}$.

(5) Cacciapaglia, R.; Casnati, A.; Mandolini, L.; Ungaro, R. *J. Chem. Soc., Chem. Commun.* **1992**, 1291–1293.

Much work has been done on synthetic catalysts with transacylase activity,[7] but significant success has been achieved more in the acylation than in the deacylation step. It appears therefore that the present investigation constitutes a decisive step toward a hydrolytic synzyme.[8] In future studies, structural modifications will be performed, in the hope of making the catalyst capable of substrate binding and recognition.

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(6) (a) Cacciapaglia, R.; Lucente, S.; Mandolini, L.; van Doorn, A. R.; Reinhoudt, D. N.; Verboom, W. *Tetrahedron* **1989**, *45*, 5293-5304. (b) Ercolani, G.; Mandolini, L. *J. Am. Chem. Soc.* **1990**, *112*, 423-427. (c) Cacciapaglia, R.; van Doorn, A. R.; Mandolini, L.; Reinhoudt, D. N.; Verboom, W. *J. Am. Chem. Soc.* **1992**, *114*, 2611-2617. (d) Kraft, D.; Cacciapaglia, R.; Böhmer, V.; El-Fadl, A. A.; Harkema, S.; Mandolini, L.; Reinhoudt, D. N.; Verboom, W.; Vogt, W. *J. Org. Chem.* **1992**, *57*, 826-834. (e) Cacciapaglia, R.; Mandolini, L.; Reinhoudt, D. N.; Verboom, W. *J. Phys. Org. Chem.* **1992**, *5*, 663-669.

(7) (a) Breslow, R.; Trainor, G.; Ueno, A. *J. Am. Chem. Soc.* **1983**, *105*, 2739-2744. (b) Cram, D. J.; Lam, P. Y.-S.; Ho, S. P. *J. Am. Chem. Soc.* **1986**, *108*, 839-841. (c) Lehn, J. M.; Sirlin, C. *New J. Chem.* **1987**, *11*, 693-702. (d) Suh, J. *Bioorg. Chem.* **1990**, *18*, 345-360. (e) Diederich, F. *Cyclophanes*; The Royal Society of Chemistry: Cambridge, UK, 1991; Chapter 8. (f) Murakami, J.; Kikuchi, J.; Hisaeda, Y. In *Inclusion Compounds*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds.; Oxford University Press: New York, 1991; Vol. 4, Chapter 11.

(8) Royer, G. P. *Adv. Catal.* **1980**, *29*, 197-227.

Primary Process of Phytochrome: Initial Step of Photomorphogenesis in Green Plants

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The primary reaction rate of phytochrome, the chromoprotein which triggers photomorphogenetic processes in plants, is measured by picosecond transient absorption spectroscopy. Excitation of the red-absorbing form (Pr) of pea large phytochrome induces rapid depletion of the original absorption followed by recovery of the absorption and appearance of the primary photoproduct at about 695 nm. Both time constants are 24 ps, indicating that the product is formed directly from the excited state of the Pr form. The most likely primary reaction of phytochrome to express its function has been considered to be *Z-E* photoisomerization of the tetrapyrrole chromophore, the rate of which is 2 orders of magnitude slower than photoisomerization of rhodopsins.

Phytochrome[1] is a chromoprotein in green plants, which acts as a photoreceptor for a variety of morphogenetic and developmental responses including the regulation of the expression of light-sensitive genes. Modulation of these photoresponses is achieved by the photo-transformation of the phytochrome molecule from the red-absorbing form (Pr) to the far-red-absorbing form (Pfr).[2] Although the primary events following photon absorption and subsequent conformational changes of protein for expression of function have been attracting much interest, they are still obscure. In fact, the conversion from its excited state to the primary intermediate is not clearly understood,[3] although a number of time-resolved fluorescence measurements have been applied to study the excited-state relaxation processes.[4] This is in remarkable contrast to the other two photoreceptive pigments, rhodopsins and photosynthetic reaction centers, whose mechanisms have been better characterized.

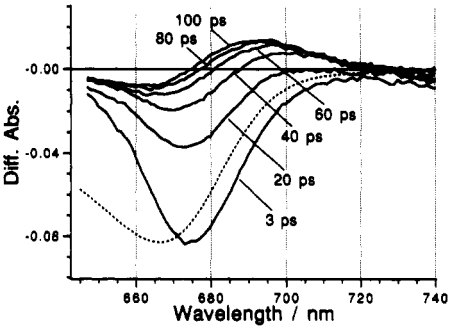

Figure 1. Transient absorption spectra of the Pr form of phytochrome following excitation with a sub-picosecond pulse (wavelength: 600 nm; pulse width: 0.8 ps; energy: 16 μJ; diameter of laser beam: 1.6 mm). The static absorption spectrum is also shown (---) after being multiplied by −0.12 (percentage of excitation). The spectrum at 100 ps is ascribed to the difference spectrum between the primary product and the original phytochrome.

The recent development of generation of ultrashort optical pulses has enabled us to directly observe the primary events of the photoreceptive pigments of the biological systems. In fact, a number of time-resolved spectroscopies using optical pulses shorter than 1 ps have been applied to rhodopsins[5] and photosynthetic reaction centers[6] to investigate their primary cis-trans isomerization or electron-transfer processes, respectively. From the results, we now know that rapid reactions take place (~0.2 ps for the former or 2-3 ps for the latter) from the initially excited states, so that the primary reaction overcomes the other relaxation processes back to the original state, and the highly efficient biological systems are realized.

In this communication, we present an approach to determine the primary reaction pathways of phytochrome in the picosecond regime by applying transient absorption spectroscopy. We use pea large phytochrome type A as the sample, which shows essentially the same photoreaction pathways as that of intact phytochrome type A despite its lack in the 6-kDa N-terminal fragment.[7] The apparatus for obtaining transient absorption spectra is a double-beam spectrometer linked with a sub-picosecond laser.[8] The large pea phytochrome in HEPES buffer is flowed

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(1) (a) Kendrick, R. E.; Kronenberg, G. H. *Photomorphogenesis in Plants*; Martinus Nijohoff Publishers: 1986. (b) Furuya, M. *Phytochrome and Photoregulation in Plants*; Academic Press: Tokyo, 1987. (c) Sage, L. C. *Pigment of the Imagination, A History of Phytochrome Research*; Academic Press: San Diego, 1992.

(2) (a) Schaffner, K.; Braslavsky, S. E.; Holzwarth, A. R. *Adv. Photochem.* **1990**, *15*, 229-277. (b) Rüdiger, W.; Thümmler, F. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1216-1228.

(3) Lippitsch, M. E.; Riegler, H.; Aussenegg, F. R.; Hermann, G.; Müller, E. *Biochem. Physiol. Pflanzen*, **1988**, *183*, 1-6.

(4) (a) Wendler, J.; Holzwarth, A. R.; Braslavsky, S. E.; Schaffner, K. *Biochim. Biophys. Acta* **1984**, *786*, 213-221. (b) Holzwarth, A. R.; Wendler, J.; Ruzsicska, B. P.; Braslavsky, S. E.; Schaffner, K. *Biochim. Biophys. Acta* **1984**, *791*, 265-273. (c) Song, P. S.; Tamai, N.; Yamazaki, I. *Biophys. J.* **1986**, *49*, 645-649. (d) Brock, H.; Ruzsicska, B. P.; Arai, T.; Schlamann, W.; Holzwarth, A. R.; Braslavsky, S. E.; Schaffner, K. *Biochemistry* **1987**, *26*, 1412-1417. (e) Song, P. S.; Singh, B. R.; Tamai, N.; Yamazaki, T.; Yamazaki, I.; Tokutomi, S.; Furuya, M. *Biochemistry* **1989**, *28*, 3265-3271.

(5) (a) Sharkov, A. V.; Pakulev, A. V.; Chekalin, S. V.; Matveetz, Y. A. *Biochim. Biophys. Acta* **1985**, *808*, 94-102. (b) Dobler, J.; Zinth, W.; Kaiser, W.; Oesterhelt, D. *Chem. Phys. Lett.* **1988**, *144*, 215-220. (c) Mathies, R. A.; Brito Cruz, C. H.; Pollard, W. T.; Shank, C. V. *Science* **1988**, *240*, 777-779. (d) Schoenlein, R. W.; Peteanu, L. A.; Mathies, R. A.; Shank, C. V. *Science* **1991**, *254*, 412-415. (e) Kandori, H.; Yoshihara, K.; Tomioka, H.; Sasabe, H. *J. Phys. Chem.* **1992**, *96*, 6066-6071.

(6) (a) Woodbury, N. W.; Becker, M.; Middendorf, D.; Parson, W. W. *Biochemistry* **1985**, *24*, 7516-7521. (b) Breton, J.; Martin, J.-L.; Migus, A.; Antonetti, A.; Orszag, A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 5121-5125. (c) Holzapfel, W.; Finkele, U.; Kaiser, W.; Oesterhelt, D.; Scheer, H.; Stilz, H. U.; Zinth, W. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5168-5172. (d) Chan, C. H.; Dimagno, T. J.; Chen, L. X.-Q.; Norris, J. R.; Fleming, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11202-11206.

(7) Eilfeld, P.; Rüdiger, W. *Z. Naturforsch.* **1985**, *40c*, 109-114.

(8) Kandori, H.; Kemnitz, K.; Yoshihara, K. *J. Phys. Chem.* **1992**, *96*, 8042-8048.

0002-7863/92/1514-10958$03.00/0