similar to that of cytidine over the pH range of 2–6, despite the negative charge on the cytidylic acid molecule over this pH range. The reason for this difference is not immediately apparent.

These observations can be summed up by stating the pH dependencies observed for photohydration of the uracil and cytosine derivatives agree in considerable detail with the behavior which might be expected for singlet excited molecules of these substances. Furthermore, the temperature dependence of photohydration bears an excellent analogy with that of fluorescence. We conclude that these observations constitute positive evidence that a singlet excited state is the precursor for photohydration.

It is not clear that this singlet state is the fluorescent state observed by Daniels and Hauswirth[11] since the lifetime of fluorescent state, for both uracil and thymine, was reported to be about 10^{-12} sec and not very many bimolecular collisions can take place during such a short time. It seems clear from the pH and temperature dependence studies reported here, from the dependence of photohydration yield upon water concentration in acetonitrile–water mixtures,[3b] and from the recent observation[27] that the quantum yield of 1,3-dimethyluracil photohydrate is independent of the viscosity of the medium (in glycerol–water mixtures) that the reactive state which leads to the photohydrate is long enough lived to be well aware of its bulk environment although it may not be in complete equilibrium with this environment.

We think that the data in this paper make a "hot" ground state[5] unlikely as the reactive state, but there are possibly other reactive states for photohydration which are not the fluorescent singlet states: (1) a hidden n,π^* singlet;[28] (2) a tautomeric form of the fluorescent singlet state; (3) singlet excited water–pyrimidine complex reached by vertical excitation of a ground state water–pyrimidine complex, *i.e.*, the photohydration reaction might then be simply a rearrangement of the excited water–pyrimidine complex. With this last in mind, we are presently investigating the nature of the water complexes of uracil and substituted uracils.

Acknowledgment. We would like to acknowledge helpful conversations with R. A. Holroyd, Brookhaven National Laboratory, and fruitful suggestions from several referees. This work was supported, in part, by Grant CA 11418 from the National Cancer Institute.

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Synthetic Spectroscopic Models Related to Coenzymes and Base Pairs. Quaternized Bisnicotinamides[1-3]

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Abstract: Intramolecular interactions of quaternized nicotinamide groups have been studied in a series of compounds in which these two groups are held in close proximity by a polymethylene chain of length *n* linking the ring nitrogen atoms (Nic$^+$–C$_n$–Nic$^+$ 2Cl$^-$, n = 2–6). *Hyperchromic* intramolecular perturbations of the 264-nm (37.9×10^3 cm^{-1}) ultraviolet bands of the Nic$^+$ groups were evaluated by comparing their oscillator strengths to that of 3-carbamoyl-1-propylpyridinium chloride (Nic$^+$–C$_3$ Cl$^-$), a model for the isolated chromophore. A strong hyperchromic interaction (14%) was found for n = 2. The interaction decreased rapidly as the polymethylene chain was lengthened. Essentially the same hyperchromism values were obtained for a comparison series in which one nicotinamide group was replaced with a trimethylammonium group (Nic$^+$–C$_n$–NMe$_3^+$ 2Cl$^-$, n = 2–4). In the Nic$^+$–C$_n$–Nic$^+$ 2Cl$^-$ series, the hyperchromisms appeared to be due primarily to perturbations of the 264-nm transitions of the Nic$^+$ groups by the neighboring positively charged nitrogen atoms (either Nic$^+$ or NMe$_3^+$) rather than to intramolecular interactions of the electronic transition dipoles of the Nic$^+$ groups. The effects upon hyperchromism of changing the pH, the solvent, and the anions of the models are also discussed, and the study was extended to compounds in the Nic$^+$–C$_n$–COO$^-$ series (n = 2, 3).

Ultraviolet spectra of nucleic acids and other polynucleotides exhibit strikingly lower absorption intensities when compared with the constituent mononucleotides or to the same polymers in denatured form.[4-6] This loss of ultraviolet absorption intensity at a given wavelength is termed *hypochromicity*, while the total loss in intensity over the entire absorption band is called *hypochromism*. Significant hypochromic ef-

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(1) The present paper is No. X in the series on Synthetic Spectroscopic Models Related to Coenzymes and Base Pairs.

(2) For preceding papers (IX, VIII) in this series, see: (a) M. W. Logue and N. J. Leonard, *J. Amer. Chem. Soc.*, **94**, 2842 (1972); (b) J. A. Secrist III and N. J. Leonard, *ibid.*, **94**, 1702 (1972).

(3) The following abbreviations are used in this paper: Nic$^+$, a quaternized nicotinamide group; NMe$_3^+$, a trimethylammonium group; COO$^-$, a carboxylate group; Nic$^+$–C$_3$, the 3-carbamoyl-1-propylpyridinium cation; Nic$^+$–C$_n$–Nic$^+$, the dication in which two nicotinamide groups are interconnected at the ring nitrogens by a polymethylene chain of length *n*; Nic$^+$–C$_n$–NMe$_3^+$, the dication in which a nicotinamide group is connected at the ring nitrogen to a trimethylammonium group by a polymethylene chain of length *n*; Nic$^+$–C$_n$–COO$^-$, the betaine compound in which a nicotinamide group is connected at the ring nitrogen to a carboxylate group through a polymethylene chain of length *n*; NAD$^+$, nicotinamide–adenine dinucleotide.

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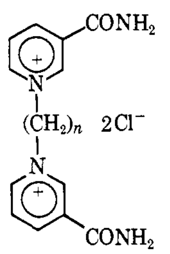
fects have been found for dinucleotides,[6–8] dinucleotide and other base pair models,[2,9,10] and for coenzymes such as $\mathrm{NAD^+}$ and their models.[11–15]

The first satisfactory theoretical explanation for the phenomenon of hypochromism was advanced by Tinoco,[16] who proposed that it is predominantly a coulombic interaction between light-induced ultraviolet electronic transition dipoles of the interacting bases that results in a decrease (hypochromism) or increase (hyperchromism) in the transition intensity. For interactions of transitions polarized in the planes of the rings of the bases, the theory[16a] predicts a hypochromic effect for stacked bases. A hyperchromic effect is predicted for bases with colinear transition dipoles.

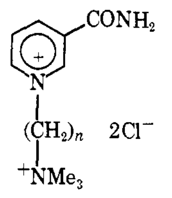
In addition to the work of Tinoco,[16–18] other papers concerning theories of hypochromism have appeared,[19–34] and the subject has been discussed in recent reviews.[4,35] Most theories give results similar to those of Tinoco when the limiting conditions of his theory are imposed. A number of spectroscopic investigations have been directed toward testing hypochromism theories.[8,36–40]

While Tinoco's theory[16a] *explains the hypochromic interactions of stacked bases, its prediction of a hyperchromic effect for bases with colinear transition moments has not been suitably tested.* Two series of $\mathrm{Nic^+}$ compounds[3] were prepared to attempt such a test. In one series $\mathrm{Nic^+{-}C_n{-}Nic^+\ 2Cl^-}$), two $\mathrm{Nic^+}$ groups were interconnected at the ring nitrogens with a polymethylene chain of varying length. In the other series ($\mathrm{Nic^+{-}C_n{-}NMe_3^+\ 2Cl^-}$), one of the $\mathrm{Nic^+}$ groups was replaced with a $\mathrm{NMe_3^+}$ group. The positive charges on the two bases in each molecule would discourage stacking conformations leading to the placement of positive charge near positive charge. The molecules should exist preferentially in extended conformations, and the orientations of the two $\mathrm{Nic^+}$ groups in the first series could easily be such as to permit colinear or nearly colinear arrangements of transition moments, which could give rise to a hyperchromic effect. The series containing the $\mathrm{NMe_3^+}$ group would provide a reference as to whether any observed effect were due to a transition dipole–transition dipole type of interaction of the $\mathrm{Nic^+}$ groups or to a perturbation of the $\mathrm{Nic^+}$ transitions by the nearby positive nitrogen.

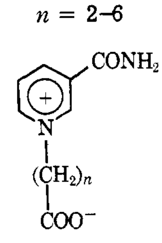
Synthesis of Compounds. The $\mathrm{Nic^+{-}C_n{-}Nic^+\ 2Cl^-}$, $\mathrm{Nic^+{-}C_n{-}NMe_3^+\ 2Cl^-}$, and $\mathrm{Nic^+{-}C_3\ Cl^-}$ series were synthesized by alkylating nicotinamide with the appropriately substituted alkyl halides. The two $\mathrm{Nic^+{-}C_n{-}COO^-}$ betaine compounds were prepared by alkylating nicotinamide with the corresponding ω-halocarboxylic acids and treating the products with silver oxide. Compounds $\mathrm{Nic^+{-}C_3\ Cl^-}$ and $\mathrm{Nic^+{-}C_3{-}Nic^+\ 2Cl^-}$ were converted to the corresponding perchlorate or acetate salts by treatment with silver perchlorate or silver acetate.



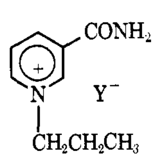
$\mathrm{Nic^+{-}C_n{-}Nic^+\ 2Cl^-}$, $n = 2–6$



$\mathrm{Nic^+{-}C_n{-}NMe_3^+\ 2Cl^-}$, $n = 2–4$



$\mathrm{Nic^+{-}C_n{-}COO^-}$, $n = 2, 3$



$\mathrm{Nic^+{-}C_3\ Y^-}$, $\mathrm{Y^- = Cl^-}$, $\mathrm{ClO_4^-}$, $\mathrm{OAc^-}$

Spectroscopic Results. Table I shows the ultraviolet extinction coefficients at the corresponding band maxima and the hyperchromisms for the compounds studied. The large hyperchromism (14.1%) for $\mathrm{Nic^+{-}C_2{-}Nic^+\ 2Cl^-}$ in neutral aqueous solution indicates a strong intramolecular interaction between two closely linked $\mathrm{Nic^+}$ groups. This hyperchromic effect is in the opposite direction from the hypochromisms normally observed in linking neutral bases or in linking a neutral base to a positively charged base in dinucleo-

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Table I. Extinction Coefficients and Hyperchromisms for Nic$^+$ Compounds

	λ_{max} (nm) and extinction coeff ϵ (mol l.$^{-1}$ cm^{-1})						% Hyperchromism		
	Water				95% ethanol neutral		Water		95% ethanol neutral
	pH 7		pH 1						
Compound	λ	ϵ	λ	ϵ	λ	ϵ	pH 7	pH 1	
Nic$^+$–C$_3$ Cl$^-$	264	4260	264	4290	265	4260			
Nic$^+$–C$_3$ ClO$_4^-$	264	4260	264	4280					
Nic$^+$–C$_3$ OAc$^-$	264	4230	264	4240					
Nic$^+$–C$_1$–COO$^-$	267	4630	266	4750			*a*	14.5^a	
Nic$^+$–C$_2$–Nic$^+$ 2Cl$^-$	265	8990	265	9030	266	—^{b}	14.1	14.5	*b*
Nic$^+$–C$_2$–NMe$_3^+$ 2Cl$^-$	265	4620	265	4640	266	—^{b}	14.5	14.6	*b*
Nic$^+$–C$_2$–COO$^-$	265	4100	265	4400			*a*	5.5	
Nic$^+$–C$_3$–Nic$^+$ 2Cl$^-$	265	8700	265	8730	266	8560	5.7	5.9	6.2
Nic$^+$–C$_3$–Nic$^+$ 2ClO$_4^-$	265	8740	265	8750			6.3	5.7	
Nic$^+$–C$_3$–NMe$_3^+$ 2Cl$^-$	265	4450			266	4350	7.3		6.0
Nic$^+$–C$_4$–Nic$^+$ 2Cl$^-$	265	8560	265	8570	266	8450	2.5	1.9	2.6
Nic$^+$–C$_4$–NMe$_3^+$ 2Cl$^-$	265	4300			266	4210	2.3		1.3
Nic$^+$–C$_5$–Nic$^+$ 2Cl$^-$	265	8450	265	8460			0.8	0.1	
Nic$^+$–C$_6$–Nic$^+$ 2Cl$^-$	265	8400	265	8420			−0.4	−0.9	

[a] These hyperchromism values could not be determined accurately due to large shifts in the bands below 230 nm. [b] Compound is unstable in 95% ethanol.

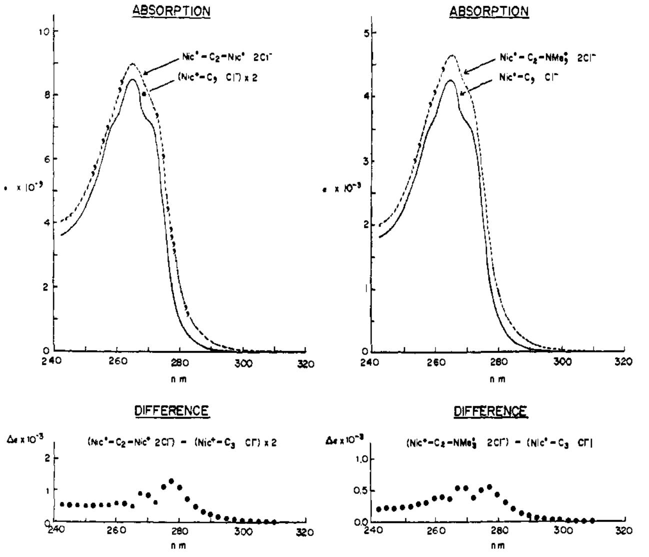

Figure 1. Ultraviolet absorption and difference spectra in water for 1,1′-ethylenebis(3-carbamoylpyridinium chloride) (Nic$^+$–C$_2$–Nic$^+$ 2Cl$^-$) and 3-carbamoyl-1-(β-dimethylaminoethyl)pyridinium chloride methochloride (Nic$^+$–C$_2$–NMe$_3^+$ 2Cl$^-$) *vs.* 3-carbamoyl-1-propylpyridinium chloride (Nic$^+$–C$_3$ Cl$^-$) ($\epsilon \times 2$).

tides, coenzymes, or models for these molecules in which the bases can achieve near-parallel planarity.

The hyperchromic effect in the Nic$^+$–C$_n$–Nic$^+$ 2Cl$^-$ series drops off directly and rapidly from a maximum at $n = 2$ to essentially zero at $n = 6$. In stacked base pair models which are neutral or singly positively charged, by contrast, hypochromisms are observed with a maximum interaction at a chain linkage of $n = 3$.[9,12,13] A trimethylene linkage should permit better stacking than an ethylene linkage[9] and may have a more favorable entropy factor for stacking than a tetramethylene linkage. Similarly, it was for a trimethylene linkage that Itoh and Kosower[41] observed a maximum interaction between pairs of 4-carbomethoxypyridyl free radicals linked together between the ring nitrogens by a polymethylene chain, and that Hirayama[42] observed a maximum interaction between phenyl rings linked by a polymethylene chain.

There is a very close parallel in hyperchromism values between the Nic$^+$–C$_n$–Nic$^+$ 2Cl$^-$ series and the Nic$^+$–C$_n$–NMe$_3^+$ 2Cl$^-$ series (Table I). Nic$^+$–C$_2$–Nic$^+$ 2Cl$^-$ has a hyperchromism in neutral aqueous solution of 14.1%, whereas Nic$^+$–C$_2$–NMe$_3^+$ 2Cl$^-$ has a value of 14.5%. The values are the same within experimental error (±1%). For Nic$^+$–C$_3$–Nic$^+$ 2Cl$^-$ and Nic$^+$–C$_3$–NMe$_3^+$ 2Cl$^-$, the hyperchromism values are 5.7 and 7.3%, respectively, and for the corresponding tetramethylene-linked compounds the values are 2.5 and 2.3, respectively. Since either a Nic$^+$ or a NMe$_3^+$ group causes the same hyperchromism of the neighboring Nic$^+$ 264-nm band, it appears that the hyperchromism can be attributed entirely to the effect of the positively charged neighboring nitrogen atom rather than to intramolecular interactions of the two Nic$^+$ chromophores.

There was no significant effect from changes in acid concentration upon the hyperchromism of the 264-nm bands in the Nic$^+$–C$_n$–Nic$^+$ 2Cl$^-$ and Nic$^+$–C$_n$–NMe$_3^+$ 2Cl$^-$ compounds. For example, Nic$^+$–C$_2$–Nic$^+$ 2Cl$^-$ gave a hyperchromism of 14.1% at pH 7 and a hyperchromism of 14.5% at pH 1. This is in contrast to other dinucleotide models where mono- or diprotonation of the bases could destroy stacking.

There is a very close correspondence between hyperchromisms of both series of nicotinamide compounds in aqueous solutions and in 95% ethanol. For Nic$^+$–C$_3$–Nic$^+$ 2Cl$^-$, the hyperchromism in neutral aqueous solution is 5.7% and in 95% ethanol it is 6.2%. For Nic$^+$–C$_3$–NMe$_3^+$ 2Cl$^-$, it is 7.3% in water and 6.2% in 95% ethanol. Since ethanol solutions are well known for destroying stacking interactions between bases, this confirms the absence of intramolecular stacking in both water and ethanol.

In 95% ethanol, the ultraviolet spectra for Nic$^+$–C$_2$–Nic$^+$ 2Cl$^-$ and Nic$^+$–C$_2$–NMe$_3^+$ 2Cl$^-$ solutions were not constant, and hyperchromisms could not be determined reliably. The extinction coefficient at λ_{max} and the band shapes also changed. We con-

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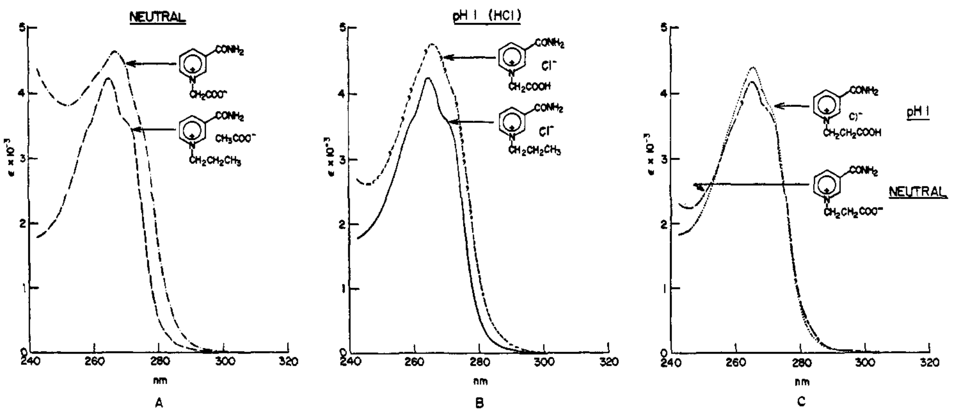

Figure 2. Ultraviolet absorption spectra in water for 3-carbamoyl-1-carboxymethylpyridinium hydroxide inner salt (Nic^+–C_1–COO^-) (panels A and B) and 3-carbamoyl-1-(2-carboxyethyl)pyridinium hydroxide inner salt (Nic^+–C_2–COO^-) (C) at pH 7 and pH 1.

cluded that reactions were occurring which affected the ultraviolet spectra.

Essentially the same hyperchromisms were found for Nic^+–C_3–Nic^+ $2Cl^-$ and Nic^+–C_3–Nic^+ $2ClO_4^-$ (each compared to Nic^+–C_3 with the corresponding anion): 5.7 and 6.3%, respectively, at pH 7, and 5.9 and 5.7%, respectively, at pH 1. Hydrochloric acid was used for acidification. These differences are well within the estimated experimental error of ±1%. Thus, there is no significant special effect of chloride ions upon the hyperchromisms of the Nic^+–C_n–Nic^+ compounds in dilute solution, since a switch from chloride to perchlorate does not cause any significant change in hyperchromism. The lack of anion effect shows that the hyperchromisms in these compounds are not due to halide charge transfer or related phenomena,[43] which are well known for pyridinium bromides and iodides but which would not occur in the corresponding perchlorates.

Figure 1 shows the ultraviolet spectrum of Nic^+–C_2–Nic^+ $2Cl^-$ compared with that for Nic^+–C_3 Cl^- ($\epsilon \times 2$). The intramolecular interaction of Nic^+ groups in the Nic^+–C_n–Nic^+ $2Cl^-$ series results in a significant hyperchromicity throughout the entire 264-nm band, which is most pronounced on the longer wavelength side of the band. From the absorption and difference spectra it is evident that there has been only a slight band shift in going from the reference compound to Nic^+–C_2–Nic^+ $2Cl^-$ (from 264 to 265 nm).

The absorption and difference spectra for Nic^+–C_2–NMe_3^+ $2Cl^-$ and Nic^+–C_3 Cl^- are shown in Figure 1. (Tetramethylammonium chloride has no significant absorption in the 240–350-nm region.) Qualitatively, these spectra are quite similar to those for Nic^+–C_2–Nic^+ $2Cl^-$. This similarity confirms that it is the same electrostatic effect that is causing the hyperchromism of the Nic^+ 264-nm band in both cases.

The absorption and difference spectra for Nic^+–C_3–Nic^+ $2Cl^-$ are similar in shape but lower in intensity than those for Nic^+–C_2–Nic^+ $2Cl^-$. The nature of the perturbations occurring in both molecules appears to be the same but the perturbation effect decreases rapidly with the increasing length of the polymethylene linkage.

Figure 2 shows the ultraviolet spectra for Nic^+–C_1–COO^- and Nic^+–C_2–COO^- (pH 7) and for Nic^+–C_1–$COOH$ Cl^- and Nic^+–C_2–$COOH$ Cl^- (the betaines dissolved in 0.1 *M* hydrochloric acid) as compared with the spectra of the reference compound Nic^+–C_3 OAc^- under the same conditions. The 264-nm bands of all four compounds showed a slight bathochromic shift as compared to the reference, regardless of whether the neighboring substituent to the Nic^+ chromophore bore a full negative charge (COO^-) or a partial positive charge ($COOH$). Also clearly evident for Nic^+–C_1–COO^- (pH 7) is a large bathochromic shift of the bands below 230 nm. This shift is present but less noticeable in the spectra of Nic^+–C_1–$COOH$ Cl^- and Nic^+–C_2–COO^-.

In the above three cases, the absorption minima have been shifted significantly to longer wavelengths, complicating the determination of oscillator strengths. In determining oscillator strengths of the 264-nm bands, absorption minima were used as cut-off points. This involves the approximation that the area lost to the 264-nm bands below the cut-off is compensated by the area from the short-wavelength tail above the cut-offs. The approximation is good in comparisons between compounds of similar band shapes with little overlap. As the intensity of the absorption minimum increases due to greater overlap of bands (as for the spectrum of Nic^+–C_1–COO^-) this approximation breaks down and accurate oscillator strength and hypochromism values cannot be determined without some form of curve resolution. Although the spectrum of Nic^+–C_1–COO^- appears to be hyperchromic at every point of comparison, this could be due to overlapping contributions of the bands below 230 nm and to the bathochromic shift of the 264-nm band to 267 nm caused by the presence of the neighboring carboxylate group.

The spectrum of Nic^+–C_2–COO^- is hypochromic at the λ_{max} (266 nm) and at longer wavelengths. As one goes to the shorter wavelength side of the band maximum, an overlap from bathochromically shifted bands below 230 nm causes this region to become hyperchromic. By taking integration cut-offs at 242.5, 245.0, and 247.5 nm, hyperchromism values for Nic^+–C_2–COO^- of 5.9, −0.2, and −6.2%, respectively, were obtained. This illustrates that oscillator strengths and hypochromisms can be very sensitive to the integration cut-off points taken when band overlap is large. No hypochromism values are presented for Nic^+–C_1–COO^- or Nic^+–C_2–COO^-, in view of the band shifts and overlap.

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[43] E. M. Kosower, "Molecular Biochemistry," McGraw-Hill, New York, N. Y., 1962, pp 171, 180–189.

For Nic^{+}–C_1–COOH Cl^{-} there was a moderate shift of the bands below 230 nm. The resultant, approximately a 3–4-nm shift of the absorption minimum to longer wavelength, complicates the determination of an accurate hyperchromism. A value of 14.5% was *estimated* by using the absorption minimum as the integration cut-off. Since no significant band shifts occurred in the spectrum of Nic^{+}–C_2–COOH Cl^{-}, a reasonably accurate hyperchromism value of 5.5% could be calculated.

The trends for the Nic^{+}–C_n–COO^{-} and Nic^{+}–C_n–COOH Cl^{-} spectra are not as clear-cut as for the dicationic analogs. For the Nic^{+}–C_n–COO^{-} betaines, it might be suspected that there was a large hypochromism for the 264-nm band alone at $n = 1$ (the negative charge three atoms removed from Nic^{+}) which dropped off upon increasing n to 2; however, bathochromic shifts of bands below 230 nm appear to mask this trend.

Conversely, a neighboring group with a partial inductive positive charge on the carboxylic acid carbon (Nic^{+}–C_n–COOH Cl^{-}) appeared to cause a hyperchromic effect which dropped from 14.5 ($n = 1$) to 5.5% ($n = 2$) upon homologation. The effect of the inductive partial positive charge seems to be considerably less than that of a full positive charge, since on comparing Nic^{+}–C_2–Nic^{+} $2Cl^{-}$ with Nic^{+}–C_2–COOH Cl^{-}, there is a drop in hypochromism from −14.6 to −5.5%. The hyperchromisms of the Nic^{+}–C_n–COOH Cl^{-} compounds are similar to those of the corresponding Nic^{+}–C_n–Nic^{+} $2Cl^{-}$ compounds, with one more methylene group in the connecting chain.

Discussion

The Nic^{+}–C_n–Nic^{+} $2Cl^{-}$ compounds were selected as potential models for chromophore interactions in nucleic acids, polynucleotides, and coenzymes, in which the bases can have roughly colinear transition moments. For example, such interactions might occur between base pairs as a result of the geometry required for Watson–Crick interactions.[44] It has been postulated that NAD^{+} can exist in open[45] (extended) and one or more closed (stacked) conformations.[46–48] The Nic^{+}–C_n–Nic^{+} $2Cl^{-}$ compounds could also be models for intramolecular ultraviolet interactions between bases in open conformations.

In Tinoco's treatment of hypochromism in stacked bases, only transition dipole–transition dipole interactions were considered significant.[16] Devoe and Tinoco[17] calculated the possible effects of permanent dipole–transition dipole interactions within a polymer molecule and concluded that they would be negligible compared to the transition dipole–transition dipole contributions. However, Yomosa and Nakano,[40] in a calculation of hypochromism for poly-C, found permanent dipole–transition dipole interactions to be quite significant.

Other assumptions important in Tinoco's treatment[16] are that each electron system is isolated (no resonance interactions or electron interchange between bases) and that inductive effects transmitted through bonds linking the bases are negligible. The requirement of electronically isolated chromophores is satisfied in polynucleotides, NAD^{+}, and our bisnicotinamide models. The large number of bonds separating bases in polynucleotides and NAD^{+} would preclude inductive effects. However, the small number of bonds connecting some of the bisnicotinamide models (and similar base pair models reported earlier[2,9–12]) may require that inductive effects be considered before results are compared to polynucleotides or NAD^{+}. Tinoco's theory also indicates that interactions of degenerate or nearly degenerate transitions do not contribute to hypochromism.[16,22,35] Since total band intensity for interacting chromophores is conserved, if one band is hyperchromic, it must "steal" intensity from interacting bands, which would be hypochromic.[22,29,31]

The observation of a 14.1% hyperchromism of the 264-nm (37.9×10^{3} cm^{-1}) band of Nic^{+}–C_2–Nic^{+} $2Cl^{-}$ in water clearly points to some sort of strong intramolecular interaction between bases, and it is tempting to try to relate this hyperchromism to Tinoco's interaction of colinear transition dipoles. However, this hyperchromism cannot be accounted for in terms of interactions involving the 264-nm band envelope in the two bases, since the whole band is hyperchromic, as demonstrated in the difference spectrum (Figure 1). If there are significant transition dipole–transition dipole interactions between bases, they would have to be between the Nic^{+} 264-nm band and the neighboring bands below 230 nm, each of which may have polarization directions different from the 264-nm band.

Comparison of the Nic^{+}–C_n–Nic^{+} $2Cl^{-}$ series with the Nic^{+}–C_n–NMe_3^{+} $2Cl^{-}$ series shows that one gets the same hyperchromisms regardless of the nature of the perturbing group (Nic^{+} or NMe_3^{+}). It seems, then, that the hyperchromisms in both cases are due to the electrostatic effects of the neighboring positively charged nitrogen atoms. It might be argued that the hyperchromism results from transition dipole–transition dipole interactions of the Nic^{+} 264-nm bands with observationally inaccessible high-energy transitions in the far ultraviolet for Nic^{+} and NMe_3^{+}. However, it seems unreasonable that such diverse structures as NMe_3^{+} and Nic^{+} would have transitions that would interact with the Nic^{+} 264-nm band in the same way (similar difference spectra) and to the same extent (essentially the same hyperchromisms).

Literature evidence is available for band shifts[49,50] of chromophores caused by neighboring positively charged groups. Grob[49] and others[50–53] have proposed that if a permanent electrostatic charge within a molecule tends to reinforce the change in electron polarization between the ground state and the electronically excited state of a chromophore, the transition energy

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will be lowered (bathochromic shift) and *vice versa.* Such an effect could operate in Nic$^+$ compounds.

Since the hyperchromisms and bathochromic shifts observed for the Nic$^+$–C$_n$–Nic$^+$ compounds involved perturbations by neighboring positive charges, two Nic$^+$–C$_n$–COO$^-$ compounds were studied to determine the effect of a negative neighboring group upon the 264-nm bands. For the Nic$^+$–C$_n$–COO$^-$ compounds, the large bathochromic shifts of the bands below 230 nm tended to obscure a probable hypochromic trend in the 264-nm bands. Only bathochromic shifts were observed.

At pH 1 the betaines were protonated and the neighboring negatively charged groups were converted to neutral dipolar groups with the partially positively charged carbonyl carbons nearer to the Nic$^+$ chromophores. Hyperchromic effects were observed for the Nic$^+$–C$_n$–COOH Cl$^-$ compounds, although they were significantly less intense than for the corresponding Nic$^+$–C$_n$–Nic$^+$ 2Cl$^-$ compounds. Apparently the partial positive charges on the carboxylic acid carbons were significantly less effective in perturbing the Nic$^+$ chromophores than the full positive charges of Nic$^+$ or NMe$_3{}^+$.

One may also consider whether the hyperchromisms of the 264-nm bands in Nic$^+$–C$_n$–Nic$^+$ and Nic$^+$–C$_n$–NMe$_3{}^+$ compounds are due to electrostatic transition dipole interactions with a permanent dipole or monopole, or to inductive perturbations of the chromophore. If these compounds are completely ionized in dilute aqueous solution, the positive nitrogens will be monopoles. Hence, we will be primarily concerned with possible inductive and electrostatic effects of each positively charged monopole upon the neighboring Nic$^+$ chromophore.

In studies of ionization constants of 4-substituted bicyclo[2.2.2]octane-1-carboxylic acids, Roberts and Moreland[54] concluded that the electrostatic effect of charge polarization in the 4 position could be transmitted to the carboxylic acid group both by a through-space (electrostatic) and a through-bonds (inductive) mechanism. Through-space transmisssion drops off as the cube of the distance for oriented polarizations. Yet it is not accurate to try to fit the rate of decrease of through-space interactions to the cube of the maximum distance between Nic$^+$ and Nic$^+$ or NMe$_3{}^+$, since Gill[55] has established theoretically that the average distance between ends of a polymethylene chain in open conformations is somewhat less than the limiting fully extended conformation. At this time no way is apparent for separating inductive and through-space mechanisms, since both would give the same general results in our systems, that is, a rapid dropoff in the interaction with increasing length of the polymethylene chain. Bogh mechanisms could be operating.

Ideally, each monomer model of Nic$^+$–C$_n$–Nic$^+$ 2Cl$^-$ would be the compound for each value of n in which one Nic$^+$ group would be replaced with a methyl or cyclohexyl group. For Nic$^+$–C$_n$–NMe$_3{}^+$ 2Cl$^-$ compounds, the corresponding replacement would be that of *tert*-butyl for NMe$_3{}^+$. In our hyperchromism studies, all of these monomers have been approximated by Nic$^+$–C$_3$ Cl$^-$. While the effect of alkyl substituents two or more carbon atoms removed from Nic$^+$ upon its ultraviolet spectrum should be very slight, nevertheless small errors (probably less than 1%) may be introduced into the hyperchromisms by this approximation. The very small hypochromism observed for Nic$^+$–C$_6$–Nic$^+$ 2Cl$^-$ is attributed to this approximation.

Conclusion

When two Nic$^+$ groups are separated by only two methylene groups, the transition dipole–transition dipole type of interaction is not significant compared to interactions of an electrostatic or inductive nature. Even if two bases are brought nearly into contact in the same plane, the centers of the transition dipoles still would be much further apart than if the bases were stacked. Since oriented transition dipole–transition dipole interactions fall off as the cube of the distance, the colinear interactions of probable small magnitude may render a confirmation of this aspect of Tinoco's theory exceedingly difficult.

It has been established that there may be a significant effect of nearby charged groups on the hypochromisms of charged bases; hence, one should not *a priori* exclude a possible influence of such groups (*e.g.*, ammonium, carboxylate, or phosphate). Hypochromism calculations which neglect the effects of ionized phosphate groups, polar hydroxyl groups, or charged bases may be considered suspect unless concrete evidence is presented that such interactions are in fact negligible.

Experimental Section

General. Melting points are corrected. Nuclear magnetic resonance spectra[56] were recorded on Varian Associates A-60A, A-56/60, or HA-100 spectrometers. Microanalyses were performed by Mr. Josef Nemeth and his associates (School of Chemical Sciences, University of Illinois, Urbana, Ill. 61801). A Leeds and Northrop Model 7401 pH meter, equipped with a miniature pH electrode, was used for pH measurements. Ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer using 1-cm quartz cells at the normal cell compartment temperature (25–26°). Great care was exercised in preparation and recording of the uv samples. Glass-distilled water was used for preparing aqueous solutions, and U.S.I. reagent quality absolute pure ethyl alcohol was used for ethanol solutions. Solutions of all organic compounds were carefully filtered (sintered glass) before final recrystallizations. Organic compounds were tested for purity by a variety of methods including microanalyses, nmr spectroscopy, and thin-layer chromatography. All compounds were dried immediately before weighing by the same drying procedures used to obtain suitable elemental analyses. Solutions were prepared using standard volumetric techniques and Class A volumetric flasks. Only restandardized pipets were used. Absorption spectra of given compounds were recorded immediately before or after a reagent blank was recorded on the same piece of chart paper. The reagent blanks contained the identical solvents used in the samples themselves. Absorption values were taken as the differences between the absorption curves and the base lines, which were the reagent blanks. The homogeneous solutions of small ionic organic compounds gave no evidence of anomalous light scattering.

Calculations. The oscillator strength (f) of each 264-nm ultraviolet band was calculated from the area between the absorption curve and base line according to the following equation, where $\epsilon(\lambda)$ is the extinction coefficient at wavelength λ: $f = 4.32 \times 10^{-9} \int [\epsilon(\lambda)/\lambda^2] d\lambda$. The integration was approximated by numerically integrating each absorption curve utilizing a computer

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Table II. Nmr Data for Nic$^+$ Compounds[a]

	Nicotinamide protons				Methylene or methyl protons on alkyl chain attached to Nic$^+$				
Compound	H-2[b]	H-6[c]	H-4[d]	H-5[e]	α	β	γ	δ	NMe$_3^+$
Nic$^+$–C$_3$ Cl$^-$	9.42	9.10	8.97	8.25	4.70 (t)[f]	2.04 (h)[f]	0.97 (t)[f]		
Nic$^+$–C$_2$–Nic$^+$ 2Cl$^-$	9.49	9.14	9.08	8.35	5.58 (s)				
Nic$^+$–C$_3$–Nic$^+$ 2Cl$^-$	9.55	9.25	9.06	8.38	5.12 (t)[f]	3.05 (p)[f]			
Nic$^+$–C$_4$–Nic$^+$ 2Cl$^-$	9.49	9.21	9.04	8.37	4.99 (m)	2.38 (m)			
Nic$^+$–C$_5$–Nic$^+$ 2Cl$^-$	9.40	9.13	8.99	8.27	4.83 (t)[f]	2.28 (p)[f]	1.70 (m)		
Nic$^+$–C$_6$–Nic$^+$ 2Cl$^-$	9.42	9.13	9.00	8.30	4.83 (t)[f]	2.18 (p)[f]	1.57 (m)		
Nic$^+$–C$_2$–NMe$_3^+$ 2Cl$^-$	9.62	9.30	9.09	8.40	5.48 (m)	4.29 (m)			3.50 (s)
Nic$^+$–C$_3$–NMe$_3^+$ 2Cl$^-$	9.58	9.18	9.03	8.34	4.96 (t)[f]	2.8 (m)	3.67 (m)		3.30 (s)
Nic$^+$–C$_4$–NMe$_3^+$ 2Cl$^-$	9.46	9.19	9.02	8.35	4.96 (t)[f]	[2.18 (m)][g]		3.57 (t)[f]	3.27 (s)
Nic$^+$–C$_1$–COO$^-$	9.32	[9.0][g]		8.27	5.37 (s)				
Nic$^+$–C$_2$–COO$^-$	9.38	9.12	8.93	8.23	4.98 (t)[f]	3.03 (t)[f]			

[a] Solvent D$_2$O with DSS as an internal standard. Chemical shifts are in parts per million (δ) followed by parentheses containing the multiplicities of the peaks (when not otherwise indicated). See ref 65 for nmr abbreviations. [b] Broad singlet. [c] Doublet, $J_{5,6}$ = 6 Hz, with each peak split into a broad triplet by coupling to H-2 and H-4 ($J_{2,6} \simeq J_{4,6} \simeq 1.5$ Hz). [d] Doublet, $J_{4,5}$ = 8 Hz, with each peak split into a broad triplet by coupling to H-2 and H-6 ($J_{2,4} \simeq J_{4,6} \simeq 1.5$ Hz). [e] Doublet of doublets ($J_{4,5}$ = 8 Hz; $J_{5,6}$ = 6 Hz). [f] J = 7 Hz. [g] Broad band consisting of two overlapping types of protons.

program based on Simpson's rule and executed on an IBM 360-75 computer. Data for the program were obtained by digitizing the Cary 15 tracing of each 264-nm band on a Benson-Lehner Corp. Decimal Converter Model F at 2.5-nm intervals, starting from the short wavelength minimum between the 264-nm band and bands at shorter wavelength. The wavelength cut-off was at 242.5 nm for aqueous solutions and 245.0 nm for 95% ethanol solutions, except for the Nic$^+$–C$_n$–COO$^-$ compounds, where the wavelength cut-offs are indicated in the text. The method of obtaining the oscillator strengths ensured that random errors will be minimal and that any possible systematic errors will cancel in the calculation of hyperchromicities, which are *relative differences* in oscillator strengths, independent of absolute values. The oscillator strengths were determined in triplicate (at the least) and an average value was taken. The standard deviation associated with the triplicate determination averaged around 0.1% of the oscillator strength measured.

Hypochromisms and *hyperchromisms* were calculated from the oscillator strengths according to the equation $\%H = [1 - f_{AB}/(f_A + f_B)]100\%$, where f_{AB} is the oscillator strength of each bifunctional model compound and $f_A + f_B$ is the sum of the oscillator strengths for the corresponding reference compounds. Positive numbers from this equation are *hypochromisms;* negative values correspond to *hyperchromisms*. The oscillator strength for Nic$^+$–C$_3$ Cl$^-$ was used to approximate the monomer contributions of all Nic$^+$ Cl$^-$ groups. Nic$^+$–C$_3$ ClO$_4^-$ was used for the Nic$^+$–C$_3$–Nic$^+$ 2ClO$_4^-$ calculation and Nic$^+$–C$_3$ OAc$^-$ was used for the Nic$^+$-C$_n$–COO$^-$ calculations. The oscillator strength of the NMe$_3^+$ group was insignificant in the region of interest. Hypochromism results and ultraviolet absorption data are shown in Table I; pmr data are shown in Table II.

General Procedure for Preparing Alkylenebis(3-carbamoylpyridinium chlorides). A mixture of the appropriate α,ω-dichloroalkane (0.10 mol) and nicotinamide (24 g, 0.20 mol) in dimethylformamide (100 ml) was stirred under nitrogen in an oil bath at 125–130° for 24 hr and cooled, the precipitated product was filtered, washed with ether, dissolved in water, and decolorized repeatedly (Darco G-60), and the water was removed *in vacuo*. The residue was recrystallized twice as indicated.

1,1′-Ethylenebis(3-carbamoylpyridinium chloride) (Nic$^+$–C$_2$–Nic$^+$ 2Cl$^-$) was recrystallized from water: 11 g (yield 31%); mp >300° (lit.[57] mp 285°).

Anal. Calcd for C$_{14}$H$_{16}$Cl$_2$N$_4$O$_2$: C, 48.99; H, 4.70; H, 16.32. Found: C, 48.71; H, 4.66; N, 16.24.

1,1′-Trimethylenebis(3-carbamoylpyridinium chloride) (Nic$^+$–C$_3$–Nic$^+$ 2Cl$^-$) was recrystallized from ethyl acetate–methanol–water: 29 g (yield 78%); mp 262–263° dec.

Anal. Calcd for C$_{15}$H$_{18}$Cl$_2$N$_4$O$_2$·H$_2$O: C, 48.01; H, 5.37; N, 14.93. Found: C, 48.02; H, 5.35; N, 15.02.

1,1′-Tetramethylenebis(3-carbamoylpyridinium chloride) (Nic$^+$–C$_4$–Nic$^+$ 2Cl$^-$) was recrystallized from ethyl acetate–methanol–water: 22 g (yield 59%); mp 285–286°.

Anal. Calcd for C$_{16}$H$_{20}$Cl$_2$N$_4$O$_2$: C, 51.76; H, 5.43; N, 15.09. Found: C, 51.73; H, 5.27; N, 15.06.

1,1′-Pentamethylenebis(3-carbamoylpyridinium chloride) (Nic$^+$–C$_5$–Nic$^+$ 2Cl$^-$) was recrystallized from ethyl acetate–methanol: 20 g (yield 52%); mp 239–240° dec.

Anal. Calcd for C$_{17}$H$_{22}$Cl$_2$N$_4$O$_2$: C, 53.00; H, 5.76; N, 14.54, Cl, 18.40. Found: C, 52.82; H, 5.59; N, 14.39; Cl, 18.32.

1,1′-Hexamethylenebis(3-carbamoylpyridinium chloride) (Nic$^+$–C$_6$–Nic$^+$ 2Cl$^-$) was recrystallized from ethyl acetate–methanol–water: 25 g (yield 62%); mp 277–278° dec (lit.[57] mp 254°).

β-Chloroethyltrimethylammonium Iodide. β-Chloroethyldimethylamine hydrochloride (29 g, 0.20 mol) was dissolved in water (10 ml) overlayered with ether and slowly made basic with 50% sodium hydroxide (20 ml) while swirling and cooling in an ice bath. The ether phase was decanted and the residue was extracted with ether (three 50-ml portions). The combined ether extracts were dried (K$_2$CO$_3$) and the ether was removed *in vacuo*. Methanol (200 ml) and methyl iodide (57 g, 0.40 mol) were added to the residue, and the reaction mixture was stirred for 18 hr with gentle refluxing at 35°. The solvent and unreacted methyl iodide were removed *in vacuo* and the product was recrystallized from methanol, giving clear needles: 37 g (yield 74%); mp 226–227° dec (lit. mp 216–217°,[58] 235°[59]); pmr[56] δ 4.3–3.6 (m, 4, N$^+$CH$_2$CH$_2$) and 3.23 ppm (s, 9, (CH$_3$)$_3$N$^+$).

Anal. Calcd for C$_5$H$_{13}$ClIN: C, 24.07; H, 5.25; N, 5.61. Found: C, 24.01; H, 5.24; N, 5.78.

γ-Chloropropyltrimethylammonium iodide was made from γ-chloropropyltrimethylamine hydrochloride by the same procedure employed for the synthesis of β-chloroethyltrimethylammonium iodide. The product was recrystallized from ethyl acetate–methanol as clear needles: 49 g (yield 85%); mp 225–226° dec (lit.[60] mp 210–212°); pmr[56] δ 3.9–3.2 (m, 4, N$^+$CH$_2$CCH$_2$), 3.14 (s, 9, (CH$_3$)$_3$-N$^+$), and 2.6–1.9 ppm (m, 2, N$^+$CCH$_2$).

Anal. Calcd for C$_6$H$_{15}$ClIN: C, 27.32; H, 5.74; N, 5.31. Found: C, 27.07; H, 5.68; N, 5.44.

δ-Chlorobutyltrimethylammonium Bromide. A mixture of 25% aqueous trimethylamine (47 g, 0.20 mol), 1-bromo-4-chlorobutane (34 g, 0.20 mol), and methanol (100 ml) in a flask fitted with an efficient reflux condenser was stirred overnight. The volatile materials were removed from the reaction mixture *in vacuo* and the residue was dissolved in methanol (50 ml). Tetramethylenebis(trimethylammonium halide) precipitated upon addition of ethyl acetate (100 ml). After filtration to remove this side product, the desired material was obtained by further dilution of the filtrate with ethyl acetate (250 ml): 40 g (yield 87%); mp 149–150° (lit.[61] mp 134–136°); pmr[56] δ 3.67 (m, 2, CH$_2$Cl), 3.36 (m, 2, N$^+$CH$_2$), 3.10 (s, 9, (CH$_3$)$_3$N$^+$), and 1.87 ppm (m, 4, N$^+$CCH$_2$CH$_2$).

Anal. Calcd for C$_7$H$_{17}$BrClN: C, 36.46; H, 7.43; N, 6.07. Found: C, 36.52; H, 7.35; N, 6.08.

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General Procedure for Making 3-Carbamoyl-1-(ω-dimethylaminoalkyl)pyridinium Chloride Methochlorides. A mixture of ω-haloalkyltrimethylammonium halide (0.10 mol) and nicotinamide (0.11 mol) in dimethylformamide (40 ml) was heated at 120–125° for 18 hr and cooled, the dimethylformamide was filtered from the precipitated product, and the filter cake was rinsed with ethyl acetate. The crude product was dissolved in water, decolorized (Darco G-60), and passed through a column of Dowex 1-X8, 200–400 mesh ion exchange resin (Cl^- form, 0.9 equiv). After the eluate was decolorized several more times, the water was removed *in vacuo* and the product was recrystallized as indicated.

3-Carbamoyl-1-(β-dimethylaminoethyl)pyridinium chloride methochloride ($Nic^+–C_2–NMe_3^+\ 2Cl^-$) was recrystallized from ethyl acetate–methanol–water: 16 g (yield 30%); mp 251–252° (lit.[16a] mp 240–241°).

Anal. Calcd for $C_{11}H_{19}Cl_2N_3O$: C, 47.15; H, 6.83; N, 15.00. Found: C, 47.27; H, 6.92; N, 15.23.

3-Carbamoyl-1-(γ-dimethylaminopropyl)pyridinium chloride methochloride ($Nic^+–C_3–NMe_3^+\ 2Cl^-$) was recrystallized from acetone–ethanol–2-propanol and dried at 80° (*in vacuo*) for 18 hr: 21 g (yield 54%); mp 234–235°.

Anal. Calcd for $C_{12}H_{21}Cl_2N_3O$: C, 48.99; H, 7.19; N, 14.28. Found: C, 49.19; H, 7.10; N, 14.01.

3-Carbamoyl-1-(δ-dimethylaminobutyl)pyridinium chloride methochloride ($Nic^+–C_4–NMe_3^+\ 2Cl^-$) was recrystallized from ethyl acetate–methanol: 16 g (yield 51%); mp 236–237°.

Anal. Calcd for $C_{13}H_{23}Cl_2N_3O$: C, 50.65; H, 7.52; N, 13.63. Found: C, 50.85; H, 7.40; N, 13.43.

3-Carbamoyl-1-propylpyridinium Chloride ($Nic^+–C_3\ Cl^-$). A solution of nicotinamide (24 g, 0.20 mol) and *n*-propyl chloride (19 g, 0.24 mol) in dimethylformamide (50 ml) was heated in a sealed tube at 120° for 12 hr. The precipitate was filtered, washed with ethyl acetate–ethanol, decolorized from water, and recrystallized from ethanol–ethyl acetate: 28 g (yield 70%); mp 195–196° dec (lit.[62] mp 191°).

Anal. Calcd for $C_9H_{13}ClN_2O$: C, 53.87; H, 6.53; N, 13.96; Cl, 17.67. Found: C, 53.87; H, 6.30; N, 14.18; Cl, 17.79.

3-Carbamoyl-1-propylpyridinium Perchlorate ($Nic^+–C_3\ ClO_4^-$). A solution of silver perchlorate was added slowly to a stirred solution of 3-carbamoyl-1-propylpyridinium chloride (5 g, 0.025 mol) in water until silver chloride had just ceased to precipitate. The reaction mixture was then decolorized (Darco G-60), warmed, and filtered hot, the filtrate concentrated *in vacuo*, and the residue recrystallized from water (10 ml), giving clear needles: 4.0 g (yield 60%); mp 153–154°.

Anal. Calcd for $C_9H_{13}ClN_2O_5$: C, 40.84; H, 4.95; N, 10.58. Found: C, 40.97; H, 4.83; N, 10.62.

3-Carbamoyl-1-propylpyridinium Acetate ($Nic^+–C_3\ OAc^-$). A solution of $Nic^+–C_3\ Cl^-$ (4.01 g, 0.020 mol) in water (100 ml) was added to silver acetate (3.32 g, 0.020 mol), stirred for 1 hr, decolorized (Darco G-60), filtered, and concentrated to dryness *in vacuo*. The residue was dissolved in 2-propanol and precipitated by the slow addition of ethyl acetate, giving the product as clear fine platelets: 3.5 g (yield 78%); mp 147–148°.

Anal. Calcd for $C_{11}H_{16}N_2O_3$: C, 58.91; H, 7.19; N, 12.49. Found: 58.85; H, 7.14; N, 12.69.

1,1′-Trimethylenebis(3-carbamoylpyridinium perchlorate) ($Nic^+–C_3–Nic^+\ 2ClO_4^-$). This compound was prepared by the same procedure as that for $Nic^+–C_3\ ClO_4^-$, giving clear needles: 2.0 g (yield 55%); mp 246–247°.

Anal. Calcd for $C_{15}H_{18}Cl_2N_4O_{10}$: C, 37.13; H, 3.74; N, 11.55. Found: C, 36.88; H, 3.61; N, 11.70.

3-Carbamoyl-1-carboxymethylpyridinium Hydroxide Inner Salt ($Nic^+–C_1–COO^-$). A solution of nicotinamide (12.2 g, 0.100 mol) and iodoacetic acid (18.6 g, 0.100 mol) in water (50 ml) was heated in an oil bath at 50° for 8 hr, cooled, stirred with silver oxide (15 g) for 15 min, and decolorized (Darco G-60), and the solids were removed by filtration. The filtrate was stirred with Dowex 1-X8 (Cl^- form) ion exchange resin (4 g wet) for 15 min and filtered to remove the resin and some precipitated silver chloride, and the filtrate was concentrated to dryness *in vacuo*. The residue was recrystallized from methanol–water, giving $Nic^+–C_1–COO^-$ as clear prisms: 10.5 g (yield 58%); mp 204–206° dec.

Anal. Calcd for $C_8H_8N_2O_3 \cdot H_2O$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.57; H, 5.02; N, 14.33.

3-Carbamoyl-1-(2-carboxyethyl)pyridinium Hydroxide Inner Salt ($Nic^+–C_2–COO^-$). A finely ground mixture of nicotinamide (12.2 g, 0.100 mol) and β-bromopropionic acid (15.3 g, 0.100 mol) was heated in an oil bath at 80° for 4 hr, cooled, dissolved in water (150 ml), and stirred with silver oxide (15 g) for 1 hr. The rest of the procedure was the same as that for $Nic^+–C_1–COO^-$. Recrystallization from methanol gave clear platelets: 13.6 g (yield 70%); mp 176–177° (lit.[53] mp 182°).

Anal. Calcd for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43. Found: C, 55.41; H, 5.09; N, 14.36.

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