the pot temperature to 125 °C gave the major fraction (1.02 g) as a clear oil identified as 2-cyclopropyl-4,5-dihydrofuran **5** (72%): IR (neat) 3020, 2990, 1450, 1400, 1320, 1075 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.26 (t, 1 H), 2.65 (m, 2 H), 2.05 (m, 2 H), 1.45 (m, 1 H), 1.0 (m, 4 H); mass spectrum, *m/e* 110, 95, 82, 69, 55, 41. Anal. Calcd for $C_7H_{10}O$: C, 76.36; H, 9.09. Found: C, 76.44; H, 9.34.

2-Phenyl-4,5-dihydrofuran. The oil from the sealed tube (0.70 g) gave a mixture containing both phenyl cyclopropyl ketone and 2-phenyl-4,5-dihydrofuran. Distillation could not fully separate the products; however, yields by NMR indicated the material contained 420 mg (78%) of phenylcyclopropyl ketone and 58 mg (8.3%) of 2-phenyl-4,5-dihydrofuran.

Reaction of Dicyclopropyl Ketone with KH. Formation of Dicyclopropylcarbinol. To a suspension of 8 mL (90 mmol) of KH (21% in oil) in 25 mL of dry THF (oil was removed by three repeated decantations from 25 mL of THF) was added 5 g (45 mmol) of dicyclopropyl ketone in 5 mL of THF. The mixture was stirred at room temperature for 14 h, during which time no evolution of gas was noted. Workup consisted of slow addition at 0 °C of 10 mL of 5% HCl (again only a limited evolution of gas), followed by extraction with 3 × 25 mL of ether. The combined organic phases were dried over $MgSO_4$, and the solvent was evaporated. The dark oil was distilled (pot temperature 80 °C), yielding 3.8 g (76%) of dicyclopropylcarbinol and 0.8 g (16%) of the starting ketone. The carbinol was identical with an authentic sample from Aldrich.

$NaBH_4$ Reduction of Dicyclopropyl Ketone. A solution of 1.12 g (1.01 mol) of dicyclopropyl ketone in 20 mL of ethanol, dried by distillation from Mg^0, was cooled to 4 °C. Sodium borohydride (1.2 g, 0.03 mol) was added slowly, dipping the temperature below 30 °C, and the mixture was stirred an additional hour. After the solution was cooled in an ice bath for 10 min, water was added followed by 10 mL of 2 N HCl. The reaction was then boiled for 5 min, cooled, and extracted with 3 × 25 mL of ether. The organic phases were combined and dried with $MgSO_4$, and the solvent was evaporated. A 70% yield (0.78 g) of a clear oil was obtained, identical with the above dicyclopropylcarbinol.

Reaction of Dicyclopropyl Ketone with Sodium Methoxide. A solution of 1.12 g (1.01 mol) of dicyclopropyl ketone in 20 mL of CH_3OD containing 0.05 mol of sodium methoxide (previously prepared from Na^0) was stirred at room temperature for 14 h. The reaction was diluted with 50 mL of D_2O and extracted with 3 × 25 mL of ether. The organic phases were combined, dried with $MgSO_4$, and evaporated. The resulting oil had a ^1H NMR spectrum that showed 63% incorporation of deuterium in the α-position to the ketone (δ 2.2).

Acknowledgment. E.G.E.J. thanks Alan J. Lovey, Berhanu Abegaz, and James J. Bohning for their helpful discussions and continued support.

Registry No. 1, 1759-53-1; 2, 80375-26-4; 3, 86101-65-7; 4, 79172-43-3; 5, 67219-43-6; $PhCH_2Br$, 100-39-0; CH_3I, 74-88-4; 1-methylcyclopropanecarboxylic acid, 6914-76-7; 1-benzylcyclopropanecarboxylic acid, 27356-91-8; 1,1-cyclopropanedicarboxylic acid, 598-10-7; methyl 1-(cyclopropylcarboxyl)cyclopropanecarboxylate, 86101-66-8; dicyclopropylcarbinol, 14300-33-5; 1-(adamant-1-ylcarbonyl)cyclopropanecarboxylic acid, 86101-67-9; 1-(1-methylcyclopropylcarbonyl)cyclopropanecarboxylic acid, 86101-68-0; 2-phenyl-4,5-dihydrofuran, 17851-50-2; dicyclopropyl ketone, 1121-37-5; lithium benzoate, 553-54-8; lithium 1-adamantanecarboxylate, 86101-69-1; lithium 1-methylcyclopropanecarboxylate, 86101-70-4; 1-(α-cyclopropyl-α-hydroxymethyl)cyclopropanecarboxylic acid, 72436-82-9.

Facile Syntheses of Fluorescent Heterocycles from N-Methylated Vitamin B_1

John A. Zoltewicz* and Thomas D. Baugh

Department of Chemistry, University of Florida, Gainesville, Florida 32611

Stefan Paszyc and Bronislaw Marciniak

Institute of Chemistry, A. Mickiewicz University, 60780 Poznan, Poland

Received September 23, 1982

1'-Methylthiaminium ion reacts with 2-amino- and substituted 2-aminopyridines in methanol by substitution–cyclization steps to give derivatives of pyrichromine. Similarly, 2-aminothiazole forms a thiochromine. All these tricyclic products show brilliant fluorescence under ordinary light. The quantum yield of a pyrichromine in aqueous solution at room temperature is 0.8. The thiamin also reacts with thiourea to yield a fluorescent fused bicyclic product. Structures were proven by magnetic resonance studies.

Fluorescent compounds which efficiently emit light have found many applications. For example, those fluorophores whose emissions are sensitive to environment are used as bioprobes (reporter molecules) to provide information about local solvent polarity, conformations, and dynamics within regions of macromolecules.[1,2] Moreover, intersite distances within large molecules can be measured by using the efficiency of energy transfer between a fluorescent donor and an acceptor as a "spectroscopic ruler".[3] By contrast, environmentally insensitive fluorophores are prepared as derivatives to enhance detection limits, often markedly, in quantitative analysis.[4]

We report a simple synthesis of fluorescent heterocyclic compounds. Our method which is capable of being extended[5,6] uses a vitamin B_1 derivative, 1'-methylthiaminium ion[7] (**1**, $C_6H_9N_3{}^+CH_2L$), as the principal reactant (Chart I).

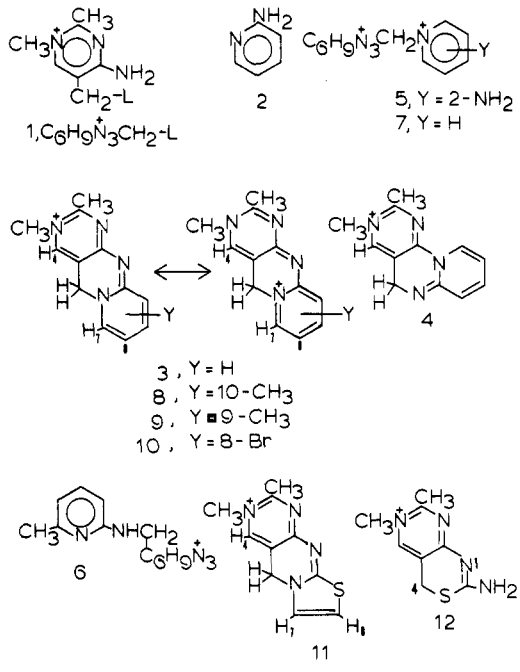
Results and Discussion

Syntheses. Heating a methanolic mixture of **1** and 2-aminopyridine (**2**) at reflux gives rise to a fluorescent product. Elemental and spectroscopic analyses show that

(1) Kanaoka, Y. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 137.
(2) Beddard, G. S.; West, M. A., Eds. "Fluorescent Probes"; Academic Press: New York, 1981.
(3) Stryer, L. *Annu. Rev. Biochem.* **1978**, *47*, 819.
(4) Wehry, E. L. *Anal. Chem.* **1982**, *54*, 131R and references cited therein.
(5) Zoltewicz, J. A.; Wyrzykiewicz, E. *J. Org. Chem.*, following paper in this issue.
(6) Zoltewicz, J. A.; Baugh, T. D. *J. Org. Chem.* **1982**, *47*, 1352.
(7) Zoltewicz, J. A.; Baugh, T. D. *Synthesis* **1980**, 217.

0022-3263/83/1948-2476$01.50/0

Chart I



in addition to nucleophilic substitution on **1** involving the loss of the thiazole leaving group (L)[8,9] an amino group is eliminated, and a tricyclic product is formed. Two structures are likely: **3** a linearly annelated or anthracene-like product, or **4**, an angularly annelated, phenanthrene-like material. Fused tricycle **3** forms if alkylation product **5** undergoes cyclization by elimination of an amino group as ammonia. Substance **4** forms if the amino group acts as the nucleophilic site toward **1** displacing the thiazole and giving a product related to **6**. Subsequent replacement of the amino group from the pyrimidine ring of **1** by the annular nitrogen atom of the pyridine ring then would yield **4**.

The following provide overwhelming evidence to support the formation of **3**.

Repeating the reaction of **1** and **2** at room temperature allows the tricyclic product and its bicyclic precursor to be isolated. The precursor cyclizes with the loss of ammonia in a base-assisted reaction. Thus, at 71.5 °C in methanol it gave 12% and in methanol containing 2,4,6-trimethylpyridine 54% of tricyclic material, each after 2.8 h.

That precursor has structure **5**, where pyridine **2** is alkylated at its annular nitrogen atom, was demonstrated by comparisons with model alkylated pyridines **6** and **7**. These models were synthesized by using **1** as the alkylating agent.[8] Pyridine can react only at its annular nitrogen atom to give **7** while 2-amino-6-methylpyridine is expected for steric reasons to alkylate at the amino and not at the annular nitrogen atom to form **6**.

Comparisons of the chemical shifts of the bridging CH_2 group in **5–7** as their perchlorate salts in deuterated dimethyl sulfoxide (Me_2SO-d_6) verify the assigned structures. The ^1H and ^{13}C chemical shifts, respectively, for the CH_2 unit are as follows: **5**, 5.00 and 49.7 ppm; **6**, 4.43 and 39.2 ppm; **7**, 5.75 and 56.0 ppm. Only the shifts of **6** are markedly different. Additional confirmation that **6** is alkylated at the amino groups was obtained from the 6-Hz coupling between NH and CH_2 protons when the compound was examined in its free base form.

Having thus identified the structure of the bicyclic precursor as **5**, it seems likely that tricyclic product has structure **3**.

Tricycle **3** also was prepared another way. When **1** was heated in methanol with 2-methoxypyridine in place of **2**, a product identical with that formed from **2** was isolated. They methoxy reactant must alkylate at the annular nitrogen atom; cyclization with the loss of methoxide ion retains all the nitrogen atoms in both reactants and leads to **3**.

The ^{15}N NMR spectrum of **3** shows four resonances appearing 122, 187, 216, and 217 ppm upfield from internal nitromethane standard. The similarity in the positions of the two high-field signals, assigned to positively charged nitrogen atoms, suggests that the major two canonical forms are those shown. Nitrogen atoms with the most positive charge are N-3 and N-6; their chemical shifts are similar to that of the NCH_3 atom (215 ppm) of **1**.[7]

Several substituted 2-aminopyridines also undergo the substitution–cyclization reaction with **1**. These include the 3- and 4-methyl and the 5-bromo compounds; products by analogy with **3** must have structures **8–10**, respectively. Because the systematic names (see Experimental Section) are so cumbersome we propose to name **3** and **8–10** pyrichrominium salts after pyrichromine, the trivial name of the parent ring.[10,11]

Nuclear Overhauser[12] (NOE) experiments were performed on the 9-methylpyrichrominium ion **9**, selected because signals for H-4, H-5, H-7, and H-8 are well separated and easily identified. Irradiation of the CH_2 protons of the conjugate acid of **9** caused the intensities of H-4 (15%) and H-7 (24%) to increase. Moreover, because the broad signal of NH-11 overlaps the CH_2 signal, simultaneous irradiation of both NH and CH_2 occurs, and an enhancement in H-10 (8%) also was found. The really significant enhancement is that of the proton adjacent to the annular nitrogen atom of the pyridine ring. Such an NOE is expected when the CH_2 group of **9** but not of **4** is irradiated. The distance between the protons in question is too great in **4**.[12]

Structure **9** also was confirmed by X-ray analysis, the results of which will be given elsewhere.[13]

Other ambident nucleophiles also undergo cyclization reactions with **1**. An ambident nucleophile with a five-membered ring, 2-aminothiazole, when heated with **1** gives 2,3-dimethylthiachrominium ion[14] (**11**). The structure was confirmed by an NOE. Irradiation of the CH_2 group of **11** leads to signal enhancements for H-4 (17%) and H-7 (20%) and a reduction for H-8 (−5%).[12] Such a pattern is consistent with linear structure **11** but not with an angular isomer.

Thiourea and **1** give a fused bicyclic product containing sulfur. Three isomeric structures are possible. One arises from alkylation of sulfur by **1** followed by deaminative ring closure to give **12**. Two others are afforded by alkylation of an amino group of thiourea to give $C_6H_9N_3^+$-$CH_2NHCSNH_2$. Subsequent cyclization involving loss of ammonia may either incorporate sulfur into a ring or allow it to be exocyclic. The observed high-field position of the $^{13}CH_2$ group at 24.4 ppm clearly identifies **12** as the

(8) Zoltewicz, J. A. *Synthesis* **1980**, 218.
(9) Zoltewicz, J. A.; Kauffman, G. M. *J. Am. Chem. Soc.* **1977**, *98*, 3134.
(10) Mori, S. J. *Orient. Med.* **1939**, *30*, 169.
(11) Matsukawa, T.; Yurugi, S. *Yakugaku Zasshi* **1951**, *71*, 1450.
(12) Noggle, J. H.; Schirmer, R. E. "The Nuclear Overhauser Effect"; Academic Press: New York, 1971.
(13) Kindly reported to us by Professor A. W. Cordes.
(14) The name of the ion is derived from thiochrome.[15] Thiochromine has the ring shown in **11**.
(15) Kuhn, R.; Wagner-Jauregg, T.; von Klavern, F. W.; Vetter, H. *Z. Physiol. Chem.* **1935**, *234*, 196.

product. This carbon chemical shift is consistent only with the methylene group being bonded to sulfur,[16] thereby eliminating the two other possibilities having the CH_2 unit bonded to a more deshielding nitrogen atom.

Thiazine structure **12** is further confirmed by ^{15}N NMR. Resonances are found 111, 158, 209, and 255 ppm upfield from nitromethane. Resonances at 158 and 209 ppm are assigned to N-6 and N-8 of the pyrimidine ring by analogy to those at 170 and 215 ppm for the pyrimidine ring of **1**.[7] By comparison with the two signals at 120 and 330 ppm for tetramethylisothiourea[18] the remaining two signals at 111 and 255 ppm may be assigned to N-1 and the amino group, respectively.

All products including that from adenosine[6] can be considered to form by a route involving alkylation by the briding methylene group of **1** at the kinetically favored site of the ambident nucleophile. Subsequent cyclization then generates a new ring.

Luminescence. Dilute solutions of **3** and **8–11** demonstrate a brilliant blue fluorescence under ordinary light. Concentrated solutions are yellow-blue and show only a little fluorescence due to self-quenching. Emission from **12** occurs at higher energies and therefore is not apparent to the eye.

Pyrichrominium ions **3**, **8**, and **9** fluorescence strongly over the region 425–460 nm, the band at longer wave length appearing as a shoulder. The emission maximum from brominated ion **10** falls at a longer wave length (448 nm), with a shoulder at 470 nm. Thiachrominium ion **11** emits maximally at 452 nm and fused thiazine **12** at 390 nm. A suitable excitation wave length is about 400 nm for all except **12** where it is 332 nm.

Trimethylated pyrichrominium ion **9** was studied extensively. The quantum yield for it in water at room temperature is 0.81, closely approaching the limit of 1. In 1 M perchloric acid **9** has absorbance and excitation spectra which clearly are different from those at pH 7; monocation **9** is converted to its conjugate acid, a dictation; however, emission comes from the monocation. The excited state therefore must be more acidic than the ground state and must lose a proton before it emits.[19] The lifetime of **9** in water is about 5.6 ns.

At 77 K **9** in ethanol shows phosphorescence at 470 nm and a shift in its fluorescence emission from 434 nm at room temperature to 417 nm. These low temperature emissions ($\bar{\nu}_{max}$) have energies associated with triplet and singlet transitions of about 61 and 69 kcal/mol, respectively.

Reaction of inexpensive and readily available **1**[7] with ambident nucleophiles provides a facile synthesis of highly efficient fluorophores in good yield.

Experimental Section

Preparation of 2,3-Dimethyl-5*H*-pyrido[1,2-*a*]pyrimido-[4,5-*d*]pyrimidinium Perchlorate (3) and Its Conjugate Acid. A suspension of 1′-methylthiaminium diperchlorate[7] (5.00 g, 10.4 mmol) and 2-aminopyridine (2.54 g, 26.0 mmol) in methanol (50 mL) in methanol (50 mL) was heated at reflux for 24 h. The suspension was cooled in ice for 30 min and filtered, and the crude product was washed with ice-cold methanol (2 × 5 mL) to give 2.78 g of yellow needles, mp 241–244 °C dec. Recrystallization from water yielded 2.54 g (8.12 mmol, 78%) of yellow needles, mp 248–249 °C dec. A sample for analysis was recrystallized three times from water followed by vacuum drying at 100 °C over magnesium perchlorate for 2 h: mp 245–246.5 °C dec; UV (c 3.36 × 10^{-5} M, H_2O, pH 6.86) λ_{max} 397 nm (log ϵ 4.33), 288 (4.02), 251 (3.73), 220 (sh), 202 (4.45); fluorescence (c 3.36 × 10^{-7} M, H_2O, pH 6.86) $\lambda_{emission}$ ($\lambda_{excitation}$) 431 nm, 448 sh (400); ^1H NMR (c 0.10 M, Me_2SO-d_6, Me_4Si) δ 2.57 (2-CH_3), 3.78 (NCH_3), 5.58 (CH_2, d, $J_{CH_2,H_4} \simeq$ 1.5 Hz), 7.19 (H_{10}, d, $J_{9,10}$ = 6 Hz), 7.19 (H_8, dd, $J_{7,8} \simeq J_{8,9} \simeq$ 6 Hz), 7.94 (H_9, dd, $J_{8,9} \simeq J_{9,10} \simeq$ 6 Hz), 8.08 (H_7, d, $J_{7,8}$ = 6 Hz), 8.10 (H_4, t, $J_{CH_2,H_4} \simeq$ 1.5 Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 21.7 (2-CH_3), 41.8 (NCH_3), 49.7 (CH_2), 108.8 (C_{4a}), 117.0 (C_8), 123.5 (C_{10}), 140.3 (C_7), 142.5, 143.2 (C_4, C_9), 155.5 (C_{10a}), 162.0, 162.8 (C_2, C_{11a}); ^{15}N NMR (c 0.96 M, 7/1 (v/v) CF_3CH_2OH/CH_3NO_2, CrAcAc, shifts relative to CH_3NO_2) δ 122.1 (N_{11}), 186.7 (N_1), 216.2, 217.4 (N_3, N_6). Anal. Calcd for $C_{12}H_{13}ClN_4O_4$ (M_r 312.7): C, 46.09; H, 4.19; N, 17.92. Found: C, 45.87; H, 4.26; H, 17.82.

The product was synthesized in 66% yield by sealing the starting materials in a thick-wall glass ampule in methanol (20 mL) and heating at 100 °C for 2 h; mp 246.5–248 °C (after recrystallization).

Melting points were dependent on the heating rate used for the determination. Slow heating from ambient temperature resulted in a melting point of 241–243 °C dec while heating the sample from an initial temperature of 215 °C gave a melting point of 246.5–248 °C dec.

The conjugate acid was prepared by recrystallization of **3** (0.500 g, 1.60 mmol) from 0.1 M perchloric acid (20 mL) to which had been added 11.8 M perchloric acid (10 drops). The yield was 0.541 g (1.31 mmol, 82%) of white crystals: mp 298–300.5 °C dec; UV (c 4.56 × 10^{-5} M, H_2O, pH 1.00) λ_{max} 343 nm (log ϵ 4.23), 266 (4.02), 244 (3.87), 217 (sh), 200 (4.48); ^1H NMR (Me_2SO-d_6, Me_4Si) δ 2.87 (2-CH_3), 4.07 (NCH_3), 5.87 (CH_2), 7.57 (H_{10}, d, $J_{9,10}$ = 6 Hz), 7.67 (H_8, dd, $J_{7,8} \simeq$ 8 Hz, $J_{8,9} \simeq$ 7 Hz), 8.38 (H_7, d, $J_{7,8}$ = 8 Hz), 8.54 (H_9, dd, $J_{8,9}$ = 7 Hz, $J_{9,10}$ = 6 Hz), 8.89 (H_4).

Preparation of 2,3-Dimethyl-5*H*-pyrido[1,2-*a*]pyrimido-[4,5-*d*]pyrimidinium Perchlorate (3) via 2-Methoxypyridine. A suspension of 1′-methylthiaminium diperchlorate[7] (1.00 g, 2.09 mmol), 2-methoxypyridine (1.14 g, 10.4 mmol), and 2,4,6-trimethylpyridine (0.560 g, 4.62 mmol) in methanol (25 mL) was heated at reflux for 24 h. The resulting mixture was ice cooled for 45 min and filtered, and the crude product was washed with ice-cold methanol to give 0.496 g of yellow crystals, mp 238–239 °C dec. Recrystallization from water gave 0.480 g (55.5%) of yellow needles, mp 245–247 °C dec. The mixture melting point of the product before purification with authentic **3** was 236–238 °C dec. The proton NMR was identical with that of authentic **3**.

Preparation of 1-[(4-Amino-1,2-dimethyl-5-pyrimidinio)-methyl]-2-aminopyridinium Diperchlorate (5). To a solution of 1′-methylthiaminium dichloride[7] (1.00 g, 2.85 mmol) in methanol (20 mL) was added 2-aminopyridine (0.355 g, 3.77 mmol) in two portions. After addition of the first portion (0.273 g, 2.90 mmol) the solution was stirred at ambient temperature for 12 h whereupon the second portion (0.082 g, 0.87 mmol) was added and stirring continued for an additional 12 h. Addition of sodium perchlorate (2.50 g, 20.4 mmol) precipitated the organic cations as perchlorate salts along with sodium chloride. The mixture of solids was recrystallized four times from 0.1 M perchloric acid to give 0.470 g (1.09 mmol, 38%) of a white crystalline product, mp 230–234 °C dec. A sample for analysis was twice recrystallized from 0.1 M perchloric acid followed by vacuum drying at 100 °C over magnesium perchlorate for 2 h: mp 236–238 °C dec; ^1H NMR (Me_2SO-d_6, Me_4Si) δ 2.61 (2′-CH_3), 3.79 (NCH_3), 5.00 (CH_2), 6.90 (H_5, dd, $J_{4,5} \simeq J_{5,6} \simeq$ 7 Hz), 7.16 (H_3, d, $J_{3,4}$ = 9 Hz), 7.90 (H_4, H_6, m), 8.00 (H_6'), 8.57 (2-NH_2), 8.68, 9.20 (4′-NH_2); ^1H NMR (DCl, pD <1, DSS) δ 2.75 (2′-CH_3), 3.90 (NCH_3), 5.35 (CH_2), 7.08 (H_5, dd, $J_{4,5} \simeq J_{5,6} \simeq$ 7 Hz), 7.33 (H_3, d, $J_{3,4}$ = 9 Hz), 7.78 (H_6'), 7.87 (H_6, d, $J_{5,6}$ = 7 Hz), 8.04 (H_4, dd, $J_{3,4}$ = 9 Hz, $J_{4,5}$ = 7 Hz); ^1H NMR (D_2O, pD 7.08, DSS) δ 2.72 (2′-CH_3), 3.85 (NCH_3), 5.36 (CH_2), 7.06 (H_5, dd, $J_{4,5} \simeq J_{5,6} \simeq$ 7 Hz), 7.33 (H_3, d, $J_{3,4}$ = 9 Hz), 7.65 (H_6'), 7.83 (H_6, d, $J_{5,6}$ = 7 Hz), 8.03 (H_4, dd, $J_{3,4}$ = 9 Hz, $J_{4,5}$ = 7 Hz); ^1H NMR (c 0.15 M, H_2O, pH 9.23, DSS) δ 2.71 (2′-CH_3), 3.85 (NCH_3), 5.30 (CH_2), 7.03 (H_5, dd, $J_{4,5} \simeq J_{5,6}$ = 6 Hz), 7.23 (H_3, d, $J_{3,4}$ = 9 Hz), 7.67 (H_6'), 7.80 (H_6, d, $J_{5,6}$ = 6 Hz), 7.93 (H_4, dd, $J_{3,4}$ = 9 Hz, $J_{4,5}$ = 6 Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 21.8 (2′-CH_3), 42.2 (NCH_3), 49.7 (CH_2), 107.4 (C_5'), 113.7, 116.0 (C_3, C_5), 137.8, 143.0 (C_4, C_6), 148.1 (C_6'), 157.1 (C_2), 162.5, 163.5 (C_2',

(16) Derivatives $C_6H_9N_3{}^+CH_2SAr$ have $^{13}CH_2S$ chemical shifts in this region.[17]

(17) Baugh, T. D. Ph.D. Thesis, University of Florida, 1982.

(18) Levy, G. C.; Lichter, R. L. "Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy"; Interscience: New York, 1979.

(19) Schulman, S. G. *Mod. Fluoresc. Spectrosc.* **1976**, *2*, 239.

C_4'). Anal. Calcd for $C_{12}H_{17}Cl_2N_5O_8$ (M_r 430.2): C, 33.50; H, 3.98; N, 16.28. Found: C, 33.43; H, 4.02; N, 16.26.

Cyclization of 1-[(4-Amino-1,2-dimethyl-5-pyrimidinio)-methyl]-2-aminopyridinium Diperchlorate (5) to 2,3-Dimethylpyrichrominium Perchlorate 3. Aliquots of stock solution (0.50 mL) were syringed into NMR tubes. The control tube contained no added base. To the second tube was added 2,4,6-trimethylpyridine. Both tubes were sealed. The tubes were allowed to stand at ambient temperature for 90 min followed by heating at 71.5 °C. Production of **3** was monitored by integrating the methylene signal against the carbon-13 satellite of methanol. The methylene group of **3** lies on the shoulder of the OH resonance of methanol. Results appear in the text.

Preparation of 4-Amino-1,2-dimethyl-5-[[(6-methyl-2-pyridinyl)amino]methyl]pyrimidinium Perchlorate (6) and Its Conjugate Acid. To a suspension of 1'-methylthiaminium diperchlorate[7] (2.22 g, 4.63 mmol) in methanol (30 mL) was added 2-amino-6-methylpyridine (1.25 g, 11.6 mmol), and the mixture was heated at reflux for 24 h. After being cooled in ice for 30 min, the mixture was filtered and the product washed with ice-cold methanol (2 × 5 mL) to give 1.43 g (4.16 mmol, 90%) of white crystals, mp 264–266 °C dec. Recrystallization of 0.43 g of product from 20% (v/v) aqueous ethanol gave 0.35 g of white crystals, mp 261–264 °C dec. Recrystallization of 1.00 g of product from acetonitrile yielded 0.88 g of crystals, mp 264–266 °C dec. A sample for analysis was recrystallized three times from water and vacuum dried at 100 °C for 2 h over magnesium perchlorate: mp 262–262 °C dec; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 2.31 (6'-CH_3), 2.57 (2-CH_3), 3.81 (NCH_3), 4.33 (CH_2, d, $J_{CH_2,NH}$ = 6 Hz), 6.39 (H_5', d, $J_{4',5'}$ = 8 Hz), 6.45 (H_3', d, $J_{3',4'}$ = 7 Hz), 6.88 (2'-NH, t, $J_{CH_2,NH}$ = 6 Hz), 7.34 (H_4', dd, $J_{4',5'}$ = 8 Hz, $J_{3',4'}$ = 7 Hz), 8.21 (H_6), 9.06 (4-NH_2); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 21.4 (2-CH_3), 23.7 (6'-CH_3), 38.1 (CH_2), 41.6 (NCH_3), 105.9, 111.7 (C_3', C_5'), 114.5 (C_5), 137.8 (C_4'), 146.6 (C_6), 155.3, 157.6 (C_2', C_6'), 161.5, 162.5 (C_2, C_4). Anal. Calcd for $C_{13}H_{18}ClN_5O_4$ (M_r 343.8): C, 45.42; H, 5.28; N, 20.37. Found: C, 45.60; H, 5.34; N, 20.42.

The conjugate acid was produced upon recrystallization of 1.44 g (4.18 mmol) of product from 20 mL of 0.1 M perchloric acid and 15 drops of 11.8 M perchloric acid to give 1.24 g (3.03 mmol, 72%) of white crystals, mp 203–205 °C dec. Recrystallization from 0.1 M perchloric acid followed by vacuum drying at 100 °C for 5 h over magnesium perchlorate gave the analytical sample: mp 202–204 °C dec; ^{1}H NMR (*c* 0.45 M, Me_2SO-d_6, Me_4Si) δ 2.53 (2-CH_3), 2.62 (6'-CH_3), 3.82 (NCH_3), 4.43 (CH_2, br), 6.80 (H_3', d, $J_{3',4'}$ = 7 Hz), 6.93 (H_5', d, $J_{4',5'}$ = 9 Hz), 7.91 (H_4', dd, $J_{4',5'}$ = 9 Hz, $J_{3',4'}$ = 7 Hz), 8.27 (H_6), 8.53, 9.18 (4-NH_2), 8.17 (2'-NH, br), 12.17 (NH, br); ^{13}C NMR (*c* 0.45 M, Me_2SO-d_6, Me_4Si) δ 19.0 (6'-CH_3), 21.4 (2-CH_3), 39.2 (CH_2), 41.7 (NCH_3), 111.2, 109.2, 112.8 (C_5, C_3', C_5'), 144.2, 145.8, 147.8, 153.0 (C_4', C_6, C_2', C_6'), 161.8, 162.0 (C_2, C_4). Anal. Calcd for $C_{13}H_{19}Cl_2N_5O_8$ (M_r 444.2): C, 33.15; H, 4.31; H, 15.77. Found: C, 35.12; H, 4.33; N, 15.75.

Preparation of 1-[(4-Amino-1,2-dimethyl-5-pyrimidinio)-methyl]pyridinium Diperchlorate (7). A suspension of 1'-methylthiaminium diperchlorate[7] (4.79 g, 10.0 mmol) and pyridine (2.53 g, 32.0 mmol) in methanol (50 mL) was heated at reflux for 48 h. The resulting mixture was cooled in ice for 30 min and filtered. The crude material was washed with methanol (2 × 5 mL), giving 3.83 g of product, mp 259–261 °C dec. Recrystallization from 0.1 M perchloric acid gave 3.43 g (8.26 mmol, 83%) of white cyrstals, mp 261.5–263.5 °C dec. An analytical sample was prepared by three recrystallizations from 0.1 M perchloric acid followed by vacuum drying at 100 °C over magnesium perchlorate for 2 h: mp 262–263.5 °C dec; ^{1}H NMR (*c* 0.36 M, Me_2SO-d_6, Me_4Si) δ 2.63 (2'-CH_3) 3.82 (NCH_3), 5.75 (CH_2), 8.19 (H_3, H_5, dd, $J_{2,3}$ = $J_{5,6}$ = 7 Hz, $J_{3,4}$ = $J_{4,5}$ = 9 Hz), 8.49 (H_6'), 8.68 (H_4, t, J = 9 Hz), 9.03 (H_2, H_6, d, J = 7 Hz), 8.80, 9.28 (NH_2); ^{13}C NMR (*c* 0.36 M, Me_2SO-d_6, Me_4Si) δ 21.6 (2'-CH_3), 42.0 (NCH_3), 56.0 (CH_2), 106.8 (C_5'), 128.1 (C_3, C_5), 144.5, 146.4 (C_2, C_6, C_4), 151.4 (C_6'), 161.8, 163.1 (C_2', C_4'). Anal. Calcd for $C_{12}H_{16}Cl_2N_4O_8$ (M_r 415.2): C, 34.71; H, 3.88; N, 13.49. Found: C, 34.67; H, 3.90; N, 13.49.

Preparation of 2,3,10-Trimethyl-5*H*-pyrido[1,2-*a*]pyrimido[4,5-*d*]pyrimidinium Perchlorate (8) and Its Conjugate Acid. A suspension of 1'-methylthiaminium diperchlorate[7] (5.00 g, 10.4 mmol) and 2-amino-3-methylpyridine (2.82 g, 26.0 mmol) in methanol (50 mL) was heated at reflux for 24 h. After being cooled in ice for 30 min, the crude product was isolated by filtration and washed with ice-cold methanol (2 × 5 mL) to give 3.02 g of yellow crystals, mp 260–265 °C dec. Recrystallization from water yielded 2.48 g (7.59 mmol, 73%) of yellow cube-like crystals, mp 268.5–270 °C dec. A sample for analysis was prepared by recrystallization from water followed by vacuum drying at 100 °C over magnesium perchlorate for 2 h: mp 268.5–270 °C dec; UV (*c* 2.45 × 10^{-5} M, H_2O, pH 6.86) λ_{max} 400 nm (log ϵ 4.35), 287 (4.01), 253 (3.91), 223 (4.12), 203 (4.40); fluorescence (*c* 2.45 × 10^{-7} M, H_2O, pH 6.86) $\lambda_{emission}$ ($\lambda_{excitation}$) 437 nm 458 sh (402); ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 2.26 (10-CH_3), 2.58 (2-CH_3), 3.79 (NCH_3), 5.57 (CH_2, d, $J_{CH_2,H_4} \simeq$ 1.5 Hz), 7.10 (H_8, dd, $J_{8,9}$ = 8 Hz, $J_{7,8}$ = 6 Hz), 7.87 (H_9, d, $J_{8,9}$ = 8 Hz), 7.94 (H_7, d, $J_{7,8}$ = 6 Hz), 8.13 (H_4, t, $J_{CH_2,H_4} \simeq$ 1.5 Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 17.5 (10-CH_3), 21.7 (2-CH_3), 41.7 (NCH_3), 49.8 (CH_2), 108.9 (C_{4a}), 116.3 (C_8), 131.8 (C_{10}), 138.1 (C_7), 142.0, 142.5 (C_4, C_9), 154.2 (C_{10a}), 162.0, 162.2 (C_2, C_{11a}). Anal. Calcd for $C_{13}H_{15}ClN_4O_4$ (M_r 326.7): C, 47.79; H, 4.63; N, 17.15. Found: C, 47.75; H, 4.67; N, 17.13.

The conjugate acid was prepared by recrstallization of **8** (0.500 g, 1.53 mmol) from 0.1 M perchloric acid (20 mL) to which was added 11.8 M perchloric acid (15 drops). The yield was 0.328 g (0.768 mmol, 50%) of white crystals: mp 284–285 °C dec; UV (*c* 5.00 × 10^{-5} M, H_2O, pH 1.00) λ_{max} 345 nm (log ϵ 4.21), 264 (3.99), 247 (3.82), 220 (sh), 202 (4.47); fluorescence (*c* 5.00 × 10^{-7} M, H_2O, pH 1.00) $\lambda_{emission}$ ($\lambda_{excitation}$) 437, 460 sh (350); ^{1}H NMR (*c* 0.46 M, Me_2SO-d_6, Me_4Si)δ 2.50 (10-CH_3), 2.85 (2-CH_3), 4.09 (NCH_3), 5.90 (CH_2), 7.97 (H_8, dd, $J_{7,8} \simeq J_{8,9} \simeq$ 7 Hz), 8.33 (H_9, d, $J_{8,9}$ = 7 Hz), 8.51 (H_7, d, $J_{7,8}$ = 7 Hz), 8.93 (H_4), 10.80 ($N_{11}H$).

Preparation of 2,3,9-Trimethyl-5*H*-pyrido[1,2-*a*]pyrimido[4,5-*d*]pyrimidinium Perchlorate (9) and Its Conjugate Acid. 1'-Methylthiaminium diperchlorate[7] (5.00 g, 10.4 mmol) was suspended in methanol (50 mL) and heated at reflux with 2-amino-4-methylpyridine (2.82 g, 26.0 mmol) for 24 h. The resulting mixture was cooled in ice for 30 min and filtered, and the crude product was washed with ice-cold methanol (2 × 5 mL) to give 2.58 g of yellow crystals, mp 238–243 °C dec. Recrystallization from water yielded 2.03 g (6.21 mmol, 61%) of yellow needles: mp 245–247 °C; UV (*c* 2.48 × 10^{-5} M, H_2O, pH 6.86) λ_{max} 391 nm (log ϵ 4.36), 288 (3.92), 230 (4.24), 204 (4.47; fluorescence (*c* 2.48 × 10^{-7} M, H_2O, pH 6.86) $\lambda_{emission}$ ($\lambda_{excitation}$) 425 nm, 442 sh (398); ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 2.37 (9-CH_3), 2.55 (2-CH_3), 3.77 (NCH_3), 5.51 (CH_2, d, $J_{CH_2,H_4} \simeq$ 1.5 Hz), 7.00 (H_{10}), 7.04 (H_8, d, $J_{7,8}$ = 7 Hz), 7.96 (H_7, d, $J_{7,8}$ = 7 Hz), 8.07 (H_4, t, $J_{CH_2,H4} \simeq$ 1.5 Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 20.8 (9-CH_3), 21.8 (2-CH_3), 41.7 (NCH_3), 49.0 (CH_2), 108.7 (C_8), 118.7 (C_{4a}), 123.3 (C_{10}), 139.5 (C_7), 142.1 (C_4), 154.6 (C_9), 155.7 (C_{10a}), 161.8, 162.6 (C_2, C_{11a}). Anal. Calcd for $C_{13}H_{15}ClN_4O_4$ (M_r 326.7): C, 47.79; H, 4.63; N, 17.15. Found: C, 47.76; H, 4.63; N, 17.16.

The conjugate acid was prepared by recrystallization of **9** (0.50 g, 1.53 mmol) from 0.1 M perchloric acid (20 mL) and 11.8 M perchloric acid (10 drops). The yield was 0.626 g (1.47 mmol, 96%) of white crystals: mp 281–293 °C dec; UV (*c* 4.72 × 10^{-5} M, H_2O, pH 1.00) λ_{max} 340 nm (log ϵ 4.25), 267 (3.89), 230 (4.24), 204 (4.46); ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 2.55 (9-CH_3), 2.81 (2-CH_3), 4.04 (NCH_3), 5.78 (CH_2), 6.30 (NH, br), 7.32 (H_{10}), 7.54 (H_8, $J_{7,8}$ = 6 Hz), 8.46 (H_7, $J_{7,8}$ = 6 Hz), 8.88 (H_4); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 21.4, 22.1 (2-CH_3, 9-CH_3), 43.6 (NCH_3), 49.6 (CH_2), 108.8 (C_{4a}), 115.7 (C_8), 122.4 (C_{10}), 141.1, 145.9 (C_7, C_4), 147.8, 155.2 (C_9, C_{10a}), 160.0, 164.4 (C_2, C_{11a}).

Preparation of 8-Bromo-2,3-dimethyl-5*H*-pyrido[1,2-*a*]-pyrimido[4,5-*d*]pyrimidinium Perchlorate (10). A suspension of 1'-methylthiaminium diperchlorate[7] (1.00 g, 2.09 mmol), 2-amino-5-bromopyridine (1.44 g, 8.35 mmol), and 2,4,6-trimethylpyridine (1.00 mL, 1.09 g, 9.02 mmol) in methanol (10 mL) was heated at 100 °C in a screw-cap vial for 12 h. Methanol was removed under reduced pressure and the residue triturated with ethyl acetate (3 × 10 mL) to give 0.80 g of a yellow solid, mp 215–220 °C dec. Recrystallization from 90% ethanol yielded 0.712 g (87%, 1.82 mmol) of yellow needles, mp 226–228 °C dec. A sample for analysis was recrystallized twice from 90% ethanol and vacuum dried at 100 °C over magnesium perchlorate for 3 h: mp 227–230 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 2.58 (2-CH_3), 3.83 (NCH_3), 5.57 (CH_2), 7.30 (H_{10}, d, $J_{9,10}$ = 10 Hz), 8.13 (H_9, dd, $J_{9,10}$ = 10 Hz, $J_{7,9}$ = 2 Hz), 8.25 (H_4), 8.50 (H_7, d, $J_{7,9}$ = 2 Hz); UV (*c* 3.31 × 10^{-5} M, H_2O, pH 6.86) λ_{max} 410 nm (log ϵ 4.34), 302 (4.06), 260 (3.64), 213 (4.38); fluorescence (*c* 3.31 × 10^{-7} M, H_2O,

pH 6.86) $\lambda_{emission}$ ($\lambda_{excitation}$) 448, 470 sh (410). Anal. Calcd for $C_{12}H_{12}BrClN_4O_4$ (M_r 391.6): C, 36.80; H, 3.09; N, 14.31. Found: C, 36.67; H, 3.13; N, 14.26.

Preparation of 2,3-Dimethyl-5*H*-pyrimido[4,5-*d*]thiazolo[3,2-*a*]pyrimidinium Perchlorate (11). 2-Aminothiazole (2.60 g, 20.6 mmol) was added to a suspension of 1'-methylthiaminium diperchlorate[7] (5.00 g, 10.4 mmol) in methanol (50 mL), and the mixture was heated at reflux for 24 h. After being cooled in ice for 30 min, the crude product was collected by filtration and washed with ice-cold methanol (2 × 5 mL) to give 3.42 g of yellow crystals, mp 249–254 °C dec. Recrystallization from water gave 1.86 g of yellow crystals, mp 256–260 °C dec. A second recrystallization yielded 1.50 g (4.71 mmol, 45%) of product, mp 264–266 °C dec. Vacuum drying at 100 °C over magnesium perchlorate for 2 h increased the melting point to 266–268 °C dec. An analytical sample was prepared by recrystallization twice from water and vacuum drying under the above conditions for 4 h: mp 271–273 °C dec; UV (*c* 2.67 × 10^{-5} M, H_2O, pH 6.86) λ_{max} 382 nm (log ϵ 4.35), 283 (3.34), 230 (3.95), 210 (sh), 202 (4.32); UV (*c* 6.02 × 10^{-5} M, 3 M HCl) 337 nm (log ϵ 4.16), 241 (3.76), 220 (3.94); fluorescence (*c* 2.67 × 10^{-7} M, H_2O, pH 6.86) $\lambda_{emission}$ ($\lambda_{excitation}$) 452 nm (392); ^1H NMR (*c* 0.70 M, Me_2SO-d_6, Me_4Si) δ 2.61 (2-CH_3), 3.83 (NCH_3), 5.44 (CH_2, d, J_{CH_2,H_4} = 1.7 Hz), 7.24 (H_8, d, $J_{7,8}$ = 5 Hz), 7.46 (H_7, d, $J_{7,8}$ = 5 Hz), 8.21 (H_4, t, J_{CH_2,H_4} = 1.7 Hz); ^{13}C NMR (*c* 0.70 M, Me_2SO-d_6, Me_4Si) δ 21.8 (2-CH_3), 42.3 (NCH_3), 45.7 (CH_2), 109.2 (C_{4a}), 110.5 (C_7), 130.1 (C_8), 144.8 (C_4), 162.4, 162.6 (C_2, C_{10a}), 172.6 (C_{9a}). Anal. Calcd for $C_{10}H_{11}ClN_4O_4S$ (M_r 318.7): C, 37.68; H, 3.48; N, 17.58. Found: C, 37.74; H, 3.51; N, 17.54.

Preparation of 2-Amino-6,7-dimethyl-4*H*-pyrimido[4,5-*d*][1,3]thiazinium Perchlorate (12). A suspension of 1'-methylthiaminium diperchlorate[7] (5.52 g, 11.5 mmol) and thiourea (4.55 g, 59.8 mmol) in methanol (30 mL) was sealed in a thick-walled glass bomb and heated at 100 °C for 5 h to produce a crystalline solid. The bomb was cooled in ice for 45 min and opened, and the contents were filtered. The crude product was washed first with ice-cold methanol (2 × 5 mL) and then with acetone (10 mL) to yield 2.09 g (7.09 mmol, 62%) of white needles (mp 278–280 °C dec) which were used for ^{15}N NMR without further purification. A sample for analysis was recrystallized from 0.1 M perchloric acid and vacuum dried over magnesium perchlorate: mp 290–293 °C dec; UV (*c* 8.00 × 10^{-5} M, H_2O, pH 9.18) λ_{max} 328 nm (log ϵ 4.36), 268 (3.95), 225 (4.09); fluorescence (*c* 8.00 × 10^{-6} M, H_2O, pH 9.18) $\lambda_{emission}$ ($\lambda_{excitation}$) 390 nm (332, 273, 228); ^1H NMR (*c* 0.20 M, Me_2SO-d_6, Me_4Si) δ 2.72 (6-CH_3), 3.95 (NCH_3), 4.33 (CH_2), 8.58 (H_5), 9.63 (NH_2); ^{13}C NMR (*c* 1.03 M, Me_2SO-d_6, Me_4Si) 21.9 (6-CH_3), 24.4 (CH_2), 42.6 (NCH_3), 110.1 (C_{4a}), 146.4 (C_5), 162.1, 166.7 (C_7, C_{8a}), 169.0 (C_2); ^{15}N NMR (*c* 0.50 M, 20% Me_2SO-d_6 in Me_2SO; CH_3NO_2, *c* 1.0 M) δ 111.3 (N_1), 157.8 (N_8), 208.1 (N_6), 255.0 (2-NH_2). The triplet expected for the NH_2 group does not appear in the proton coupled spectrum; it is broadened into the baseline. The amino nitrogen signal does appear as a singlet in the proton-decoupled spectrum.

Proton–Proton Nuclear Overhauser Enhancements for 2,3-Dimethylthiachrominium Perchlorate (11) and the Conjugate Acid of 2,3,9-Trimethylpyrichrominium Perchlorate (9). Sample Preparation. Compound 11 was recrystallized three times from water. 9 was recrystallized two times from 0.1 M perchloric acid. 9, 11, and 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were vacuum dried at 100 °C over magnesium perchlorate for 2 h and kept in a desiccator over P_2O_5.

All glassware used in sample preparation was soaked for 24 h in 0.1 M disodium ethylenediaminetetraacetic acid to complex paramagnetic ions, rinsed with D_2O, and dried at 110 °C for 12 h. A 5% w/v sample was prepared by using 50 mg of 11 or 9 and 10 mg of DSS in 1.00 mL of Me_2SO-d_6 (Aldrich, 99.5 atom %). The solution was filtered into an NMR tube through Celite which had been washed with D_2O and dried at 110 °C for 12 h. The sample was degassed through five freeze–thaw–pump cycles and the tube sealed under vacuum.

Data Acquisition. NMR spectra were obtained on a JEOL FX-100 spectrometer operating at 99.6 MHz, frequency locked on solvent deuterium at an ambient probe temperature of approximately 25 °C. The homonuclear decoupler frequency for selective irradiation of the methylene group in 11 and 9 was set by observing the collapse of benzylic coupling between H_4 and the irradiated methylene group. The decoupler power was set as low as possible while retaining maximum signal intensity of H_4. Each spectrum was the result of 40 transients taken with a tip angle of 86°, an acquisition time of 3.41 s, and a delay between transients of 45 s. Data were recorded when the methylene group was irradiated and also when the irradiation was moved off resonance 60–70 Hz. A cycle of irradiation was performed as follows: on CH_2, off resonance to high field; on CH_2, off resonance to low field; the cycle was repeated five times to give a total of 20 spectra. Repetition of the irradiation cycle was employed to offset any changes in spectrometer performance with time. The off-resonance irradiation was alternated between high and low field with respect to the CH_2 group in order to check the possibility of spectrum perturbation due to the position of the decoupler frequency.

Treatment of Data. Peak areas for each spectrum were obtained by integration. The methyl signal of DSS was used as an internal standard. The ten values obtained for each signal while irradiating the methylene group of 11 or 9 were averaged as were the five values for off-resonance high-field irradiation and off-resonance low-field irradiation. For the case of 11 both off-resonance values were averaged. For 9, however, only the low-field off-resonance values could be taken as standard; the high-field irradiation frequency coincided with the NH signal causing enhancements of other protons associated with their proximity to the NH group in the molecule. The low-field irradiation values were used as standards and compared to that resulting from the irradiation of the methylene group and the NH group. Nuclear Overhauser enhancements were calculated according to eq 1 where

$$\% \text{ NOE} = (\bar{X}_1 - \bar{X}_2)/\bar{X}_2 \times 100 \quad (1)$$

$\bar{X}_1$ is the averaged signal intensity for the on-resonance irradiation case and $\bar{X}_2$ is the averaged signal intensity for off-resonance irradiation.

Quantum Yield, Low-Temperature Fluorescence, and Phosphorescence of 2,3,9-Trimethylpyrichrominium Perchlorate (9). Inside a Perkin-Elmer MPF-3 spectrofluorimeter a dilute solution of 9 in ethanol was cooled with liquid nitrogen. Fluorescence emission was observed at 417 and 440 nm (shoulder) and phosphorescence at 470 nm. On the assumption that the 417 and 470 nm bands at 77 K roughly approximate 0–0 transitions, the energies of the S_1 and T_1 levels then are 69 and 61 kmol/mol, respectively. At room temperature the main fluorescence peak for this sample is at 434 nm. The quantum yield of 9 (3.2 × 10^{-7} M) in water was measured at 25 °C under air. Quinine sulfate in 0.1 N sulfuric acid (1.3 × 10^{-6} M) was taken as a reference with a quantum yield of 0.55.[20,21] Excitation wavelengths were 365 and 320 nm. The quantum yield for 9 is 0.81 ± 0.07 on the basis of eq 2 where ϕ is the quantum yield, F is the fluorescence, and A the absorbance. The subscript Q designates values for quinine.

$$\phi = \frac{F}{F_Q} \times \frac{A_Q}{A} \times \phi_Q \quad (2)$$

Lifetime Measurements. An SLM Instruments phase fluorimeter having a solution of glycogen to scatter light was employed. A 6.6 × 10^{-7} M solution of 9 in water was excited at 400 mn; the emission at 425 nm was examined. The lifetime of 9 was calculated to be 5.6 ns by using an 18-MHz modulating frequency. Under the same conditions a quinine bisulfate standard had a lifetime of 18.5 ns. The uncertainty in these measurements is less than 1 ns.

Acknowledgment. Professor A. W. Cordes of the University of Arkansas kindly determined the structure of 9 by X-ray analysis. This work was supported in part by Grant AM 17442 from the National Institute of Arthritis, Metabolism and Digestive Diseases. The Instrument Program, Chemistry Division of the National Science Foundation, provided financial assistance for the purchase

(20) Melhuish, W. H. *N. Z. J. Sci. Technol., Sect. B* **1955**, *37*, 142.
(21) Another value (0.70) is available for the quantum yield of quinine bisulfate.[22] Use of this larger value increases the quantum yield of 9 to about 1.
(22) Scott, T. G.; Spencer, R. D.; Leonard, N. J.; Weber, G. *J. Am. Chem. Soc.* **1970**, *92*, 687.

of the Nicolet NT-300 spectrometer used to obtain some of the carbon spectra. Dr. K. Angelides kindly allowed the use of the phase fluorimeter; measurements were made by D. Marriott.

Registry No. $1 \cdot 2ClO_4^-$, 73333-47-8; $1 \cdot 2Cl^-$, 73333-48-9; **2**, 504-29-0; **3**, 86045-92-3; **3** conjugate acid, 86045-93-4; **5**, 86064-59-7; **6**, 86045-95-6; **6** conjugate acid, 86045-96-7; **7**, 86045-97-8; **8**, 86045-99-0; **8** conjugate acid, 86046-00-6; **9**, 86046-02-8; **9** conjugate acid, 86046-03-9; **10**, 86046-05-1; **11**, 86046-07-3; **12**, 86046-09-5; 2-methoxypyridine, 1628-89-3; 2-amino-6-methylpyridine, 1824-81-3; pyridine, 110-86-1; 2-amino-3-methylpyridine, 1603-40-3; 2-amino-4-methylpyridine, 695-34-1; 2-amino-5-bromopyridine, 1072-97-5; 2-aminothiazole, 96-50-4; thiourea, 62-56-6.

New Highly Fluorescent Derivatives of Cytidine and Cytosine

John A. Zoltewicz* and Elzbieta Wyrzykiewicz

Department of Chemistry, University of Florida, Gainesville, Florida 32611

Received September 23, 1982

Cytidine and cytosine react with 1′-methylthiaminium ion in methanol containing 2,4,6-trimethylpyridine catalyst. Products contain a tricyclic dipyrimido[1,6-*a*:4′,5′-*d*]pyrimidine ring which is highly fluorescent, having emission bands at 414 and 433 nm (shoulder) with the main excitation peak at 390 nm in water. The nucleosides inosine, guanosine, and xanthosine under the same conditions only alkylate, the first two at N-1 and N-7 and the latter at N-7. Uracil similarly gives the 1,3-dialkylated product. The alkyl group is the (4-amino-1,2-dimethyl-5-pyrimidinio)methyl portion of the thiamin.

Fluorescent compounds can often be detected in very low concentrations. Consequently, many seek to convert nonfluorescent natural products which may be present only at low levels into fluorescent derivatives in order to facilitate detection and even quantitation.[1]

Some success has been achieved in converting nucleic acid components, most of which are nonfluorescent under ambient conditions,[2] into fluorescent derivatives. Most notable are the light-emitting derivatives of adenosine.[3-5] Although cytidine (**1**) and cytosine (**2**) (Chart I) have been converted to fluorescent analogues,[3,6,7] these derivatives have limitations.

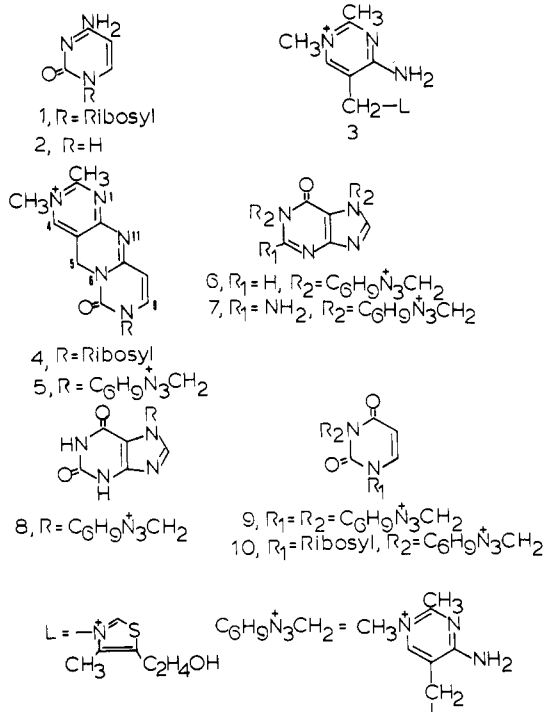
We report very promising results which may remedy deficiencies for both **1** and **2**. Both are converted to highly fluorescent linear tricyclic derivatives in a reaction with 1′-methylthiaminium ion (**3**).[8]

Results and Discussion

Cytidine (**1**) or cytosine (**2**) when heated in methanol containing 2,4,6-trimethylpyridine catalyst and the vitamin B_1 derivative 1′-methylthiaminium ion[8] (**3**) rapidly react to yield tricyclic fluorescent products.

The structures of these products may be assigned from a knowledge of the way in which they are formed. Previously, we have shown that a wide variety of nucleophiles reacts with **3** in substitution reactions which lead to the displacement of the thiazole leaving group (L).[9] When the nucleophile is ambident as in the case of adenosine[5] or 2-aminopyridines,[10] the annular nitrogen atom of the nucleophile, not the adjacent amino group, reacts first in an alklation process. This substitution then is followed by cyclization which proceeds by the loss of an amino group as ammonia. Thus, tricyclic structures **4** and **5** may be assigned, respectively, to the products from **1** and **2**.

Chart I



(1) Seitz, W. R. *CRC Crit. Rev. Anal. Chem.* **1980**, *8*, 367.
(2) Gueron, M.; Eisinger, J; Lamola, A. A. In "Basic Principles in Nucleic Acid Chemistry"; Ts'O, P. O. P., Academic Press: New York, 1974; Chapter 4, Vol. I.
(3) Barrio, J. R.; Secrist, J. A., III; Leonard, N. J. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 597.
(4) Sattsangi, P. D.; Barrio, J. R.; Leonard, N. J. *J. Am. Chem. Soc.* **1980**, *102*, 770.
(5) Zoltewicz, J. A.; Baugh, T. D. *J. Org. Chem.* **1982**, *47*, 1352. The name adenichrominium ion should be used when the ring is a cation.
(6) Hosmane, R. D.; Leonard, N. J. *J. Org. Chem.* **1981**, *46*, 1457.
(7) Bergstrom, D. E.; Inoue, H.; Reddy, P. A. *J. Org. Chem.* **1982**, *47*, 2174.
(8) Zoltewicz, J. A.; Baugh, T. D. *Synthesis* **1980**, 217.
(9) Zoltewicz, J. A. *Synthesis* **1980**, 218.
(10) Zoltewicz, J. A.; Baugh, T. D.; Paszyc, S.; Marciniak, B. *J. Org. Chem*; preceding paper in this issue.

0022-3263/83/1948-2481$01.50/0