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Chemical Modification of Nucleic Acid Components: Reactions of Cytosine, Cytidine, Isocytosine, and Adenine with Methyl N-Cyanomethanimidate

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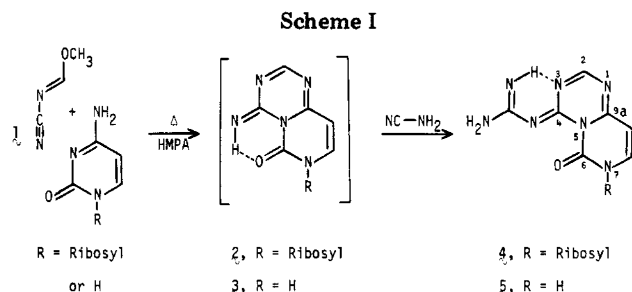
Reaction products of cytosine, cytidine, isocytosine, and adenine with methyl N-cyanomethanimidate (1) are reported. Thus, the reactions of cytidine and cytosine with 1 yielded (6,7-dihydro-6-oxo-7-β-D-ribofuranosyl-4H-pyrimido[1,6-a]-1,3,5-triazin-4-ylidene)guanidine (4) and (6,7-dihydro-6-oxo-4H-pyrimido[1,6-a]-1,3,5-triazin-4-ylidene)guanidine (5), respectively. The interrelationship between the two compounds was established by deribosidation of 4. The reaction of 1 with isocytosine gave two isomeric products: (6-oxo-6H-pyrimido-[1,2-a]-1,3,5-triazin-4-yl)guanidine (9) and (8-oxo-8H-pyrimido[1,2-a]-1,3,5-triazin-4-yl)guanidine (10). The two products were differentiated by several means, including dehydrative cyclization of 9. Adenine and 1 also gave three products: N^6-[(cyanoamino)methylene]adenine (21) and two fluorescent isomers, (7H-1,3,5-triazino[2,1-i]purin-7-ylidene)guanidine (20) and (9H-1,3,5-triazino[2,1-i]purin-9-ylidene)guanidine (22). The structures of the products were established by ^1H and ^{13}C NMR, IR, UV, and mass spectral analyses and, where necessary, by unequivocal syntheses. The source of the incorporation of a cyanamide unit into the structures of the final products (e.g., 4, 5, 9, 10, 20, and 22) was explored by studying the behavior of the reagent 1 toward nucleophiles, e.g., H_2O (D_2O), and by isolation of the major byproducts of the reactions. The fluorescence spectral data for the final compounds are also reported. The compounds from isocytosine, 9 and 10, and one from adenine, 22, exhibit exceptionally large Stokes shifts.

Reactions of the nitrogen-heterocyclic components of nucleic acids with a variety of chemical reagents serve as an index of the ease of modification, reactivity, specificity, and, with the development of fluorescence, detection and location. The importance and the prospects of chemical modification methods in investigations of nucleotide and nucleic acid structure and function are well emphasized in a review.[1] Although the effects of numerous reagents on nucleic acid base units have been thoroughly studied, investigation of reagents that result in the fusion of additional unsaturated heterocyclic rings onto the original purines[2-11] and pyrimidines[2-4,6,12] have focused mainly on new five-membered rings. Reports dealing with the fusion of six-membered rings onto the pyrimidine or purine bases are scarce and deal chiefly with guanine residues.[5,13] Many of the modified nucleosides and nucleotides, both with additional five- and six-membered rings, are fluorescent,[14-16] and selected modified products have been shown

Scheme I



to enter biochemical pathways.[17-20] Of significance are the capabilities of both 1,N^6-ethenoadenine (ε-adenine)[4,8,9,17-19,21-28] and 3,N^4-ethenocytosine (ε-cytosine)[19,20] nucleotides to substitute for adenine nucleotides in a variety of biological systems. Additionally, the fluorescence properties of the etheno-bridged compounds offer the possibility of gaining useful information concerning their binding to different enzymes.[29,30] Finally,

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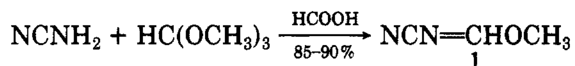
the isolation[31] of highly fluorescent "Y" (or "Wye") bases from specific tRNA's from a variety of sources and their subsequent structural elucidation[31] as linear tricyclic guanine derivatives have stimulated further interest in enlarged or extended cyclic systems related to those which are naturally occurring.

For our quest to fuse a six-membered-ring nitrogen heterocycle onto aminopyrimidine and/or aminopurine bases, an *N*-cyanomethanimidic acid ester appeared well suited for the construction of a new triazine ring incorporating the original extranuclear amino function and a ring nitrogen attached to the same carbon. Ethyl *N*-cyanomethanimidate[32] has been employed to synthesize fused *s*-triazino heterocycles, for example, from α-aminopyridines,[33] -pyrazines,[34] and -pyrazoles.[35] We prepared the corresponding methyl ester (1)[36] by a slight modifi-



$$NCNH_2 + HC(OCH_3)_3 \xrightarrow[85-90\%]{HCOOH} NCN{=}CHOCH_3 \quad \mathbf{1}$$

cation of the published procedure for the ethyl ester.[32] Treatment of cyanamide with refluxing trimethyl orthoformate, used as the solvent, in the presence of a catalytic amount of formic acid (instead of 2 equiv of acetic anhydride)[32] was convenient since the low-boiling ortho ester could be removed simply by evaporation in vacuo, and the residual oil could be distilled directly, thus avoiding fractional distillation for the removal of acetic ester and acetic acid from the reaction mixture. Special precaution should be taken with methyl *N*-cyanomethanimidate (1) because it is highly irritating to the eyes, to mucus membranes, and probably also to the skin and is very hygroscopic. Accordingly, *this compound should always be prepared and used with care in a well-ventilated hood and should be protected from moisture.*

Treatment of cytidine with methyl *N*-cyanomethanimidate (Scheme I) with hexamethylphosphoramide (HMPA) as a solvent, at 74–77 °C for 17 h, yielded a new product. The ^{1}H NMR indicated, in addition to two C–N doublets at δ 7.9 and 8.7 (J = 7.0 Hz) corresponding, respectively, to the C-5 and C-6 protons of the original pyrimidine ring, the presence of a C–H singlet at δ 8.66 for a new heteroaromatic ring proton. The NMR signals for hydrogens exchangeable by D_2O treatment included an NH_2 singlet at δ 7.8 and a broad imino N–H resonance at 10.77. The anomeric proton doublet (J = 2.0 Hz) appeared at δ 6.0. The infrared spectrum of the product showed the presence of broad NH/OH absorption from 3500 to 3100 cm^{-1} and the absence of a C≡N function, thus indicating that ring closure had in fact taken place. Nevertheless, the low-field NH resonance in the ^{1}H NMR, in addition to that for NH_2, suggested that a further reaction had transpired. By both the elemental analyses and the mass spectra [field desorption (FD) and high-resolution electron-impact (HREI)] of the product, the molecular formula was indicated to be $C_{12}H_{15}N_7O_5$ instead of $C_{11}H_{13}N_5O_5$ for the anticipated structure **2**. The discrepancy was also noted in the low-resolution electron-impact mass spectrum at 70 and 10 eV, which indicated a B + 1 peak at *m/e* 205 instead of *m/e* 163, which would correspond to structure **2**. The accumulated evidence suggested that a CH_2N_2 moiety (42 amu), probably cyanamide, had been added to an intermediate such as **2** to obtain the observed product and further that the additional CH_2N_2 unit was present in the nitrogen-heterocyclic portion of the molecule.

The ^{13}C NMR proton-decoupled spectrum of the product (see Experimental Section) confirmed the total of 12 carbons, six in the higher field region (δ 59–96 from Me_4Si) and six in the lower field region (δ 144–167). Of the six in the higher field region, five, between δ 59 and 90, belong to the ribose moiety, while the signal at δ 96 corresponds to C-5 of the original pyrimidine ring.[37] Each of these carbons was shown to be coupled to hydrogen on the basis of the off-resonance, proton-decoupled spectrum. In the lower field region, only the carbons corresponding to the resonances at δ 166.6 and 144.2 were found to have hydrogens attached, corresponding to the CH carbon of the new triazine ring and to the C-6 of the original pyrimidine,[37] respectively, as suggested by structure **4**, which is consistent with the ^{1}H NMR data for the NH's. Structure **4** requires four low-field ^{13}C signals to be unchanged in multiplicity whether proton decoupled or coupled, which they are. It also allows for the presence of a prominent peak at *m/e* 43, corresponding to $CH_3N_2^+$ in the mass spectrum, along with a small peak at *m/e* 162, corresponding to the loss of $CH_3N_2\cdot$ from the B + 1 fragment ion (see above). The compound is highly fluorescent on both silica gel and C_{18} reverse-phase TLC plates. The fluorescence excitation and emission maxima for compound **4**, along with its ultraviolet absorption maxima at acidic, basic, and neutral pH, are listed in Table I. The ultraviolet spectrum of **4** clearly showed the presence of extended conjugation in the molecule.

The presence of a cyanamide unit in the base portion of the cytidine adduct was further corroborated when the aglycon cytosine was treated with the reagent **1** under conditions similar to those described for cytidine to obtain an analogous product, **5**, the mass spectrum of which revealed the parent molecular ion at *m/e* 205. The molecular formula was determined by the high-resolution electron impact (HREI) mass spectrum and by the microanalytical data to be $C_7H_7N_7O$. In the low-resolution mass spectrum, the presence of ions at both *m/e* 162 and 43 revealed the loss of a CH_3N_2 fragment (both radical and cation) from the molecule. The ^{1}H NMR of this product in deuterated dimethyl sulfoxide exhibited a singlet at δ 8.23, corresponding to the CH of the triazine ring, two CH doublets (J = 7.0 Hz) at δ 7.4 and 7.7, corresponding to the C-5 and C-6 of the original pyrimidine ring, respectively, and a singlet NH_2 at δ 7.36 as well as a broad NH at δ 10.66. The $C_7H_7N_7O$ product, which was fluorescent on silica gel and C_{18} reverse-phase TLC plates, showed the UV absorption and fluorescence excitation and emission maxima in aqueous solution given in Table I. The respective spectra were very similar to those of the cytidine product **4**, differing by the shifts in wavelength expected for the presence or absence of N substitution.

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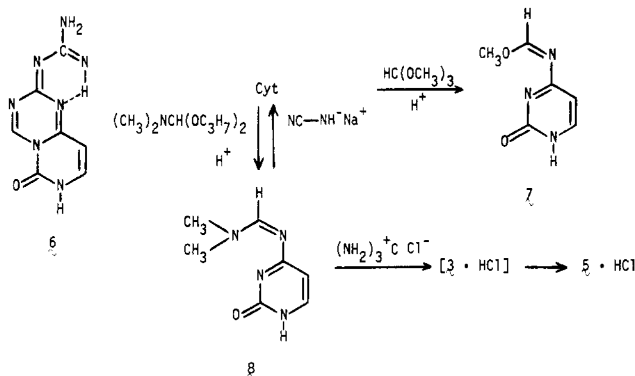
Table I. Ultraviolet and Fluorescence Spectral Data for Compounds 4, 5, 9, 10, 20, and 22

	UV spectra in H_2O						fluorescence[c] spectra in H_2O	
	pH 1		pH 7[a]		pH 13			
compd	λ_{max}, nm	log ε	λ_{max}, nm	log ε	λ_{max}, nm	log ε	λ_{ex}, nm	λ_{em}, nm
4	311	4.23	295	4.07	322	4.43	300	363
	258 sh	4.13	264	4.13				
	215	4.34	215	3.93				
5	310	4.17	290	4.00	347 sh	3.72	290	358
	250	3.94	261	4.14	328 sh	3.99		
	208	4.18	215	4.17	310	4.05		
					252 sh	3.89		
9	286	4.12	295	3.56	278	3.96	280	440
	220	4.20	224	4.15	219	4.23		
10	287	4.41	292	3.82	326 sh	3.27	290	412
	256 sh	4.14	268 sh	3.91	276	4.13		
	215	4.42	247 sh	4.11	221	4.39		
			226	4.42				
20	367 sh	4.27	364 sh	b	366 sh	4.28	340	425
	351	4.33	339	b	352	4.31		
	231 sh	4.13	277	b	266 sh	3.79		
	207	4.25	206	b	220	4.28		
22	330	3.97	290	b	314	4.01	290	440
	316	4.01	214	b				
	304 sh	4.08						
	291	4.12						
	282 sh	4.04						
	216	4.16						

[a] A buffer solution from Curtin Matheson Scientific Co. was used directly. It should be noted that this buffer contains a mold inhibitor with significant absorption below 220 nm and maxima at 272 and 277 nm. Accordingly, exact balance between buffer solution and buffer alone may be a problem in these regions. [b] Due to the sparing solubility in neutral pH, only a qualitative spectrum in H_2O is reported. [c] Spectra are uncorrected.

Scheme II



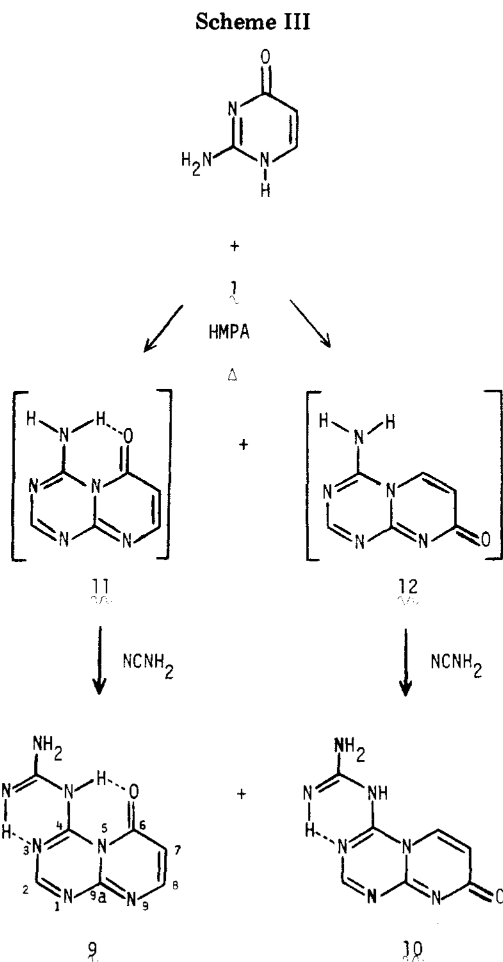
The interrelationship between the ribosyl derivative and the aglycon was established directly by deribosidation experiments. The deribosidation of 4 required milder conditions than usual since heating in 3 N hydrochloric acid for 30 min produced cytosine. Warming 4 in 0.6 N HCl at 35–40 °C for 5.5 h yielded a product which was identical in UV absorption at three different pHs and in TLC behavior in three different solvent systems with the hydrochloride salt of the $C_7H_7N_7O$ product from 1 and cytosine.

The final proof that **5** (tautomeric form at 6- and 7-positions not determined under different conditions) represented the correct structure of this product was provided by an unequivocal synthesis (Scheme II) that would distinguish between formula **5** and formula **6**, differing in skeletal structure, which could arise initially either from the Michael-type addition of N-3 in place of N^4 of cytosine to **1** or by the attack of the extranuclear NH_2 of cytosine on the cyano group of the reagent. The latter possibility is not reinforced by experience[33-35] with the analogous ethyl *N*-cyanomethanimidate, which participates invariably in Michael-type additions. The reported attack by N-3 of CMP, instead of by the exocyclic NH_2, on the β-acetylenic carbon of cyanoacetylene may be due to catalysis by mercuric chloride, which was used in the reaction.[38]

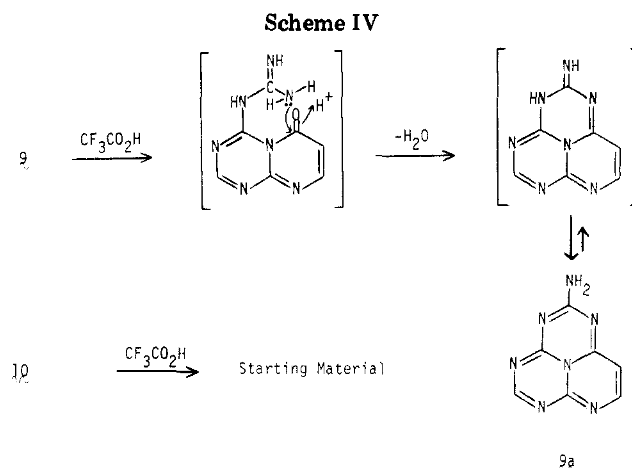
A stepwise synthesis of **5** via the imidate **7**, followed by condensation with cyanamide, was envisioned to distinguish between structures **5** and **6**. Although we obtained ^1H NMR evidence for the intermediate **7** being formed from cytosine and trimethyl orthoformate in the presence of an acid catalyst, the product was highly hygroscopic and reverted to cytosine very readily on hydrolysis. This route was abandoned in favor of one via the less hygroscopic N^4-[(dimethylamino)methylene]cytosine (**8**). Reaction of cytosine with *N,N*-dimethylformamide diisopropyl acetal (1,1-diisopropoxy-*N,N*-dimethylmethanamine) in the presence of an acid catalyst afforded crystalline **8** in quantitative yield which was identified by spectroscopy and microanalysis. There is spectroscopic evidence that the enol form of **8** may be favored in the solid state and in certain solvents, but we have not studied the keto–enol equilibrium in detail. Treatment of **8** with sodiocyanamide in 2-propanol at reflux, which might have resulted in **3**, gave exclusively cytosine and a product that was expected, and proved, to be [(dimethylamino)methylene]cyanamide [$NCH{=}CHN(CH_3)_2$, *m/e* 97 (M^+, 100%)]. Both compounds could be formed by addition of the cyanamide anion across the imine double bond of **8** followed by an elimination involving the cytosine N^4. The failure to obtain any ring-closed product from the intermediate thus formed gave us concern as to the stability of **3** as the free imine. In an effort to immobilize **3** as the hydrochloride salt, we caused **8** to react with guanidine hydrochloride. Even when the guanidine hydrochloride was limited to 1 molar equiv with **8** in 2-propanol at reflux, compound **5** was produced directly as the hydrochloride salt, along with cytosine, which was the major product of the reaction. The formal sequence of events in the conversion of **8** to **5**·HCl

(38) Furukawa, Y.; Miyashita, O.; Honjo, M. *Chem. Pharm. Bull.* **1974**, *22*, 2552.

Scheme III



Scheme IV



by guanidine hydrochloride involves (a) addition of guanidine hydrochloride to the exocyclic double bond of **8** and elimination of dimethylamine, (b) cycloaddition involving N-3 and elimination of ammonia (as hydrochloride?), and (c) addition of guanidine hydrochloride and elimination of ammonia. The ^{1}H NMR, UV, and mass spectra of the product were identical with those of **5**·HCl formed from **1** and cytosine, followed by HCl. Compound **5** may be named (6,7-dihydro-6-oxo-4*H*-pyrimido[1,6-*a*]-1,3,5-triazin-4-ylidene)guanidine, and **4** may be named (6,7-dihydro-6-oxo-7-β-D-ribofuranosyl-4*H*-pyrimido[1,6-*a*]-1,3,5-triazin-4-ylidene)guanidine.

The reaction of isocytosine with methyl *N*-cyanomethanimidate (**1**) in HMPA at 50–55 °C for 19 h yielded two products in the ratio of 2:1. They differed substantially from each other in physical and chemical properties. The less abundant product was soluble in a large volume of boiling CH_3OH–H_2O (1:2), while the more abundant product remained practically insoluble under these conditions, and thus the two could be separated conveniently by extraction and fractional crystallization. The less abundant product was slightly fluorescent on a silica gel TLC plate and nonfluorescent on a C_{18} reverse-phase plate, whereas the more abundant product was highly fluorescent on both TLC plates.

The two products from **1** plus isocytosine are isomeric. The mass spectra exhibited a molecular ion at *m/e* 205 (70 and 10 eV), and the HREI mass spectra indicated that both had the molecular formula $C_7H_7N_7O$. On the basis of structure **5** of the $C_7H_7N_7O$ product from **1** plus cytosine, the isomeric products from isocytosine would be represented by **9** and **10**, as a result of initial reaction of **1** to give intermediates **11** and **12**, followed by reaction of these with cyanamide (Scheme III).

Structure **9** has been assigned to the more abundant product on the basis of an accumulation of information. For example, the mass spectra of **9** and **10** differed significantly in their fragmentation patterns (Figure 1). In one, the molecular ion peak, at *m/e* 205, was 100 in relative intensity (the base peak) while in the other, the molecular ion peak was 16 in relative intensity, and the base peak was at *m/e* 57, corresponding to $CH_3N_3^+$. The provision for additional hydrogen bonding between N^4-H and O^6 in compound **9**, compared with that in **10**, makes it reasonable to assume that **9** is more tightly held as a unit and thus more capable of retaining charge as a molecular ion. The additional hydrogen bonding in **9** would help account for the fragment ion at *m/e* 43, corresponding to $CH_3N_2^+$, in the designated spectrum.

Other features of the various spectra are supportive of the respective matching with structures **9** and **10**. The tautomeric forms shown are not intended to be exclusive. For both isomers, C=O stretching maxima were clearly observable in the IR spectra. The ultraviolet spectra of the two isomeric products from isocytosine were similar except for an additional shoulder in one set of acid, neutral, and base spectra (Table I), which, with the stronger absorption in this set (compare ε values), is consistent with extended polar chromophore in **10**. The fluorescence emission spectra of the two isomers differed considerably from each other (Table I), and the one matched with structure **10** was broader. The Stokes shifts for both compounds were exceptional and may be related to H transfer in the excited state. We intend to seek applications of the fluoresence spectra.

The ^{13}C NMR spectra of both **9** and **10**, obtained in 0.2 N NaOD with dioxane as an internal reference standard, exhibited a total of seven resonances, one at ~106 ppm and six in the region 154–175 ppm. The signal at ~106 ppm and two signals at ~154 and 166 ppm were of significantly greater intensity than the others, implying that these were the CH carbons, showing NOE enhancement.[39] These three resonances, in fact, became doublets in the off-resonance, proton-decoupled spectra as expected. The chemical shifts of corresponding carbons in the spectra of the two compounds differed only by tenths of a part per million.

Finally, a positive differentiation between the two structures, **9** and **10**, was achieved by dehydration experiments (Scheme IV). Scrutiny of the structural features of **9** and **10** revealed that while an acid-catalyzed cyclodehydration involving the guanidine side chain and the

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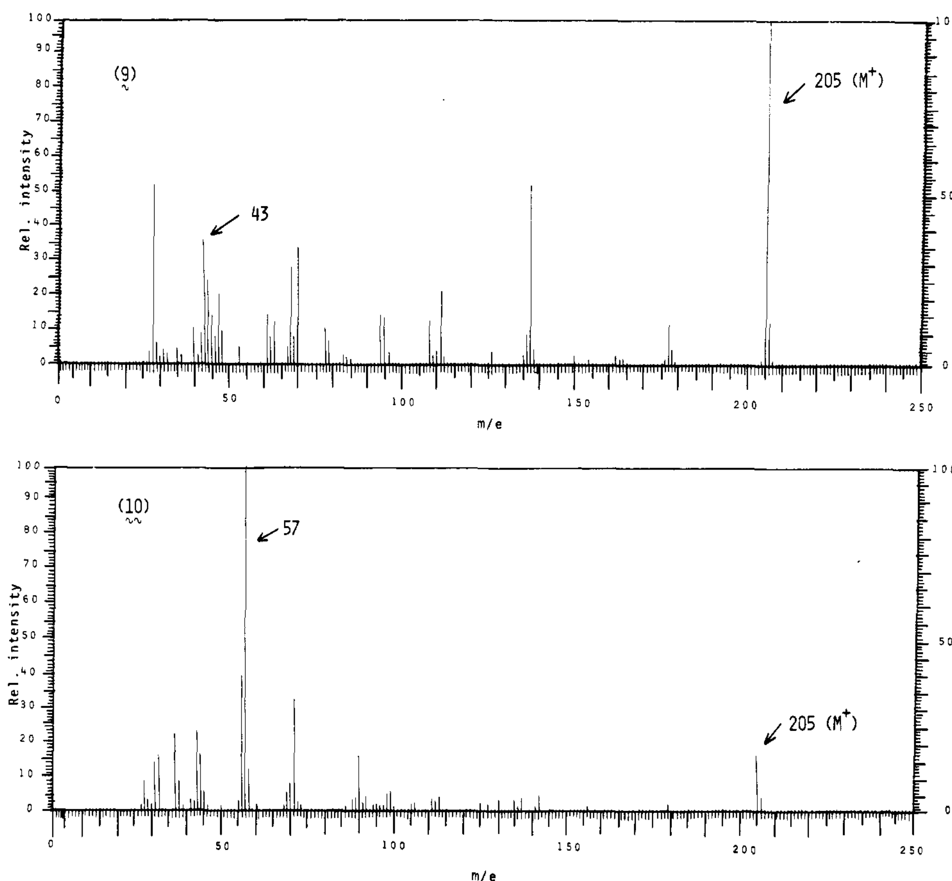


Figure 1. Low-resolution electron-impact mass spectra of compounds **9** and **10**.

ring carbonyl is feasible with compound **9**, it is not feasible with compound **10**. The difference was borne out by experiment. When **9** and **10** were heated separately under reflux with trifluoroacetic acid for several hours, only compound **9** underwent dehydrative cyclization, apparently to yield **9a** (see Experimental Section).

The incorporation of a cyanamide unit into the final products (**4**, **5**, **9**, **10**) when cytidine, cytosine, and isocytosine were caused to react with methyl *N*-cyanomethanimidate (**1**) raised the question of the origin of the cyanamide. One possibility was that the reagent might be hydrolyzed if enough water were present in the medium, since we had observed that the reagent was hygroscopic. Accordingly, we decided to gather more specific information on the course of the action of H_2O/D_2O on the reagent **1**. This was done by treating the reagent with D_2O at room temperature and following the result by the change in the proton NMR spectrum with time. As an illustration, Figure 2 reproduces the ^1H NMR spectrum of the reaction mixture after 2 h, which showed three closely spaced singlets in the region of δ 8.0 (from Me_4Si used as an external reference standard) and three methyl singlets between δ 3 and 5. The spectrum of the reaction mixture after 16.5 h showed one major signal remaining in the lower field region, with only a trace of the other two signals, and a major signal at δ 3.42 in the higher field region, with only minor signals at 3.85 and 4.82 ppm. Addition of methyl formate to the product(s) enhanced the signal at δ 3.85 and one of those in the lower field region (Figure 2). Separate addition of methanol enhanced the signal at δ 3.42 and did not alter the spectrum in the lower field region. The conclusion that could be reached, on the basis of these observations, was that the hydrolysis of the original reagent proceeds by two different pathways following the addition of water, one (Scheme V, path a) producing *N*-cyanoformamide and methanol and the other (path b) giving rise to cyanamide and methyl formate.

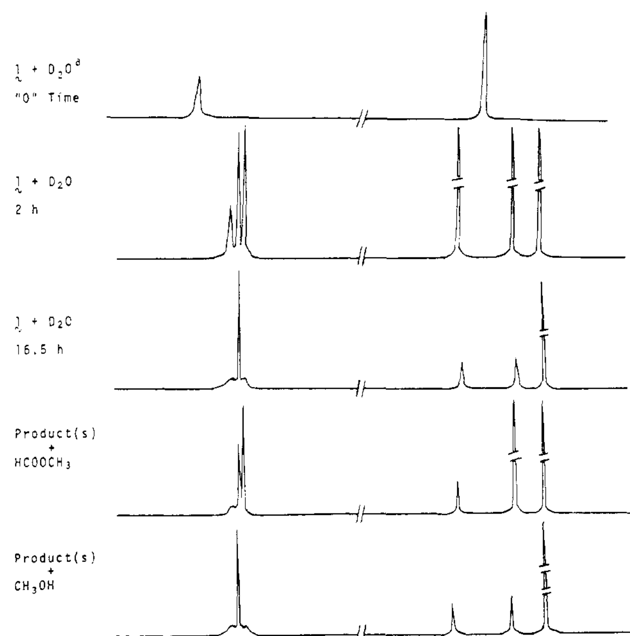
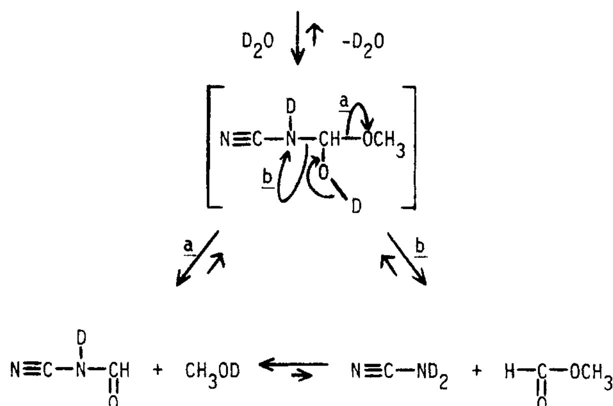


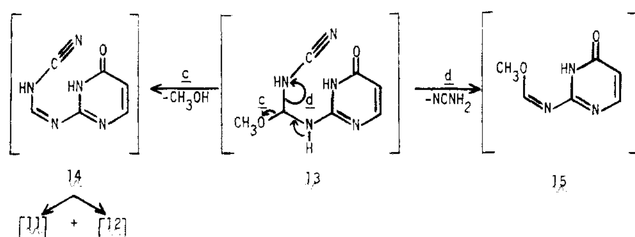
Figure 2. Representational ^1H NMR spectra (δ vs. intensity) of methyl *N*-cyanomethanimidate (**1**) under the indicated conditions. [a]Spectrum actually taken in $(CD_3)_2SO$ since D_2O addition starts decomposition of **1** immediately.

Since hydrolysis of **1** was a potential source of cyanamide, great care was taken to dry the cytosine and the solvent HMPA, and the reaction was carried out under anhydrous conditions under a nitrogen atmosphere. However, products **9** [(6-oxo-6*H*-pyrimido[1,2-*a*]-1,3,5-

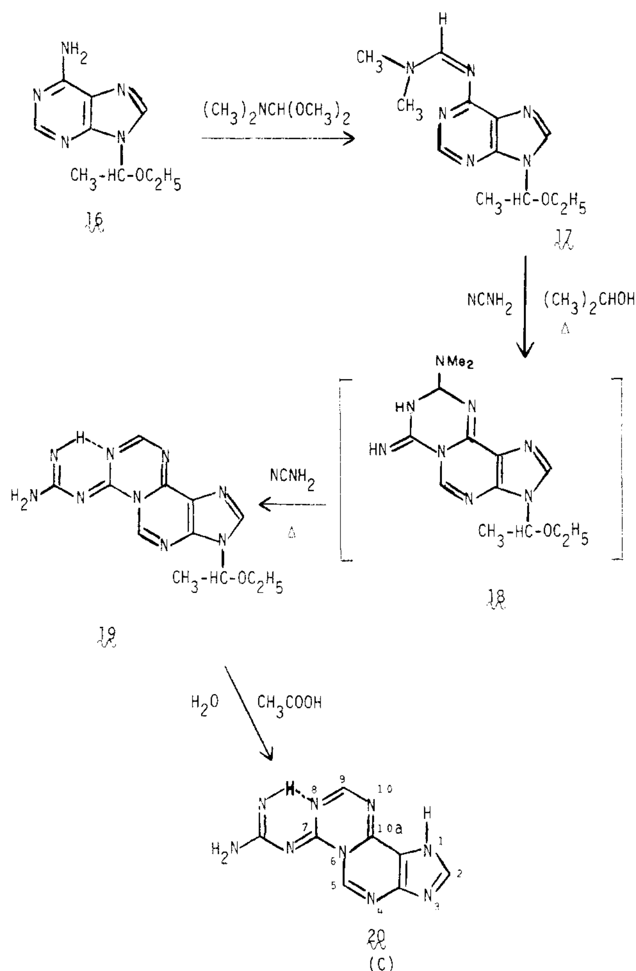
Scheme V



Scheme VI



Scheme VII

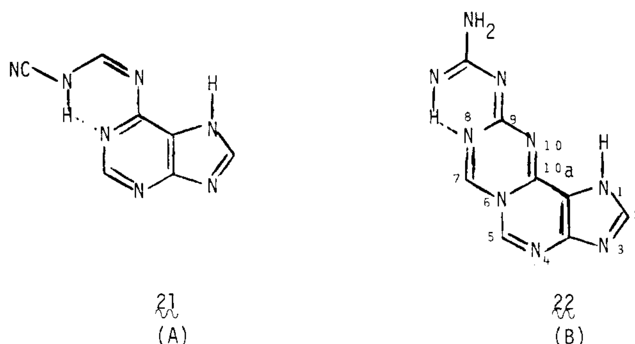


triazin-4-yl)guanidine] and **10** [(8-oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)guanidine] were again obtained and in comparable yields to those obtained in the absence of such precautions. Thus, the cyanamide units in the products were not arising from hydrolysis of the reagent **1** due to adventitious water. Isolation of isocytosine, in addition to compounds **9** and **10**, after the workup procedure, when the isocytosine had been absent from the crude product before workup, furnished the clue, along with the findings that led to the proposal of Scheme V. According to Scheme VI, the initial intermediate formed reversibly in the reaction of isocytosine and methyl *N*-cyanomethanimidate (**1**) is envisioned as **13**. From **13**, elimination of methanol (route c) would produce the acyclic intermediate **14**, precursor of previously postulated **11** and **12**. Elimination of cyanamide (route d) would produce the hydrolytic precursor of isocytosine, **15**. Intermediates **11** and **12** are sufficiently basic to react with the liberated cyanamide to produce **9** and **10**. It appears, then, that the isocytosine isolated during the workup procedure, i.e., extraction of the crude reaction mixture from isocytosine plus **1** with aqueous methanol, must in large part result from hydrolysis of the imidate **15**. The corresponding imidate **7** in the cytosine series has been described above as hygroscopic and prone to hydrolysis even upon exposure to atmospheric moisture.

The reaction of adenine with methyl *N*-cyanomethanimidate (**1**) in HMPA at 105–110 °C yielded three products: A, $C_7H_5N_7$; B, $C_8H_7N_9$; C, $C_8H_7N_9$. Product A moved faster on a silica gel TLC plate with $CHCl_3$–CH_3OH (4:1) as solvent than adenine (R_f 0.23 and 0.12, respectively) and was nonfluorescent; products B and C remained at the origin in the same solvent system and were highly fluorescent. Unlike the products described above from cytosine, cytidine, and isocytosine, product A from adenine did not contain an additional cyanamide unit; moreover, ring closure had not taken place, as evidenced by the infrared absorption at 2200 cm^{-1} characteristic of C≡N. The molecular formulas of both fluorescent products B and C from the adenine reaction, by contrast, indicated the incorporation of an additional cyanamide unit. The low-resolution EI mass spectrum for B at 10 eV showed a molecular ion peak at *m/e* 229, which was also the base peak. The mass spectrum of B at 70 eV revealed the loss of $CH_3N_2\cdot$ [*m/e* 186 (relative intensity 5)] from the molecular ion as well as the formation of the fragment ion, *m/e* 43 (99), corresponding to $CH_3N_2^+$. The mass spectrum of compound C was strikingly similar to that of B, except for the noticeable difference in the relative peak heights of the respective fragments (see Experimental Section). The infrared spectra of both B and C showed broad NH absorption in the region 3550–3000 cm^{-1} and the absence of a C≡N function. The ^{1}H NMR spectrum of B in deuterated dimethyl sulfoxide exhibited three sharp, closely spaced, C–H singlets in the region δ 8.37–8.60, whereas that of C in the same solvent revealed only two singlets, approximately in the same region but widely separated from each other and integrating for a total of three C–H protons. The vivid distinctive evidence between the two structural isomers was furnished by the ultraviolet and fluorescence spectra of the two compounds. The UV of compound B in water exhibited a maximum at 290 nm, whereas compound C absorbed maximally at 340 nm. Likewise, there was a significant difference in the Stokes shifts in the fluorescence spectra of the two isomers (see Table I).

The final structural differentiation between the two isomers, B and C, was achieved by an unequivocal synthesis of C (Scheme VII). The starting material for the synthesis, 9-(1-ethoxyethyl)adenine (**16**), was prepared

from 6-chloropurine as described previously.[40] Reaction of 16 with refluxing *N,N*-dimethylformamide dimethyl acetal yielded N^6-[(dimethylamino)methylene]-9-(1-ethoxyethyl)adenine (17) in essentially quantitative yield and was practicaly pure (as determined by TLC and ^{1}H NMR), so that it could be employed directly in the next stage without further purification. Treatment of 17 with 2 molar equiv of cyanamide provided a highly fluorescent product, 19, which was smoothly hydrolyzed with 50% aqueous acetic acid, with loss of the EOE protecting group,[40] to give 20. The ultraviolet, ^{1}H NMR, fluorescence, and mass spectral data of 20 were identical with those of the product C, thus establishing the cyclopentenophenanthrene-like structure, (7*H*-1,3,5-triazino[2,1-*i*]purin-7-ylidene)guanidine (20) for this isomer. Since a separation reaction of A with cyanamide to give C established the interrelationship between A and C, the structure N^6-[(cyanoamino)methylene]adenine (21) was assigned to the product



A. The product B, the isomer of C, has been tentatively assigned structure (9*H*-1,3,5-triazino[2,1-*i*]purin-9-ylidene)guanidine (22), on the basis of the structure of ethenoadenine (ε-adenine) derivatives,[4,8,9,17-19,21-28] wherein the condensation between the atoms N-1 and N^6 of adenine has been firmly established.

The intense fluorescence behavior of compounds 20 and 22 in aqueous solution is impressive. Further examination of their behavior in different solvents and environments appears warranted.

In conclusion, methyl *N*-cyanomethanimidate (1) reacts with a number of nucleic acid bases under somewhat forcing conditions. Unusual products may result in that, following initial condensation, a second reaction involves incorporation of a molecule of cyanamide liberated from an intermediate. The fluorescence characteristics of the products, especially those from isocytosine and one from adenine, include very large Stokes shifts.

Experimental Section

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. ^{1}H nuclear magnetic resonance spectra were recorded on a Varian EM-390 or HR-220 spectrometer, operating at 90 and 220 MHz, respectively. ^{13}C NMR spectra were obtained on a JEOL FX-60 or a WCV-XLFT-100 Fourier transform instrument, operating at 15.03 and 25.2 MHz, respectively, and are reported in parts per million from tetramethylsilane. Dioxane was used as an internal reference standard for ^{13}C NMR determinations where aqueous solutions were employed. Mass spectra were run on a Varian MAT CH-5 low-resolution or a Varian MAT-731 high-resolution spectrometer coupled with a 620i computer and a STATOS recorder. Ultraviolet absorption spectra were obtained on a Beckman Acta MVI spectrometer. Fluorescence excitation and emission spectra were measured on a Spex Fluorolog spectrofluorometer. Infrared spectra were determined on a Perkin-Elmer 337 or 237B spectrophotometer. Thin-layer chromatography (TLC) was run either on Merck precoated silica gel f-254 plates or on Analtech precoated reverse-phase-separation (RPS) uniplates with fluorescent backing. High-performance liquid chromatographic (LC) separation was carried out by using Waters Associates Prep LC/System 500A instrument using a Prep PAK-500/silica cartridge or a Prep PAK/C_{18} cartridge at a flow rate of 100 mL/min. Medium-pressure liquid chromatographic separation was carried out on a self-assembled instrument at a flow rate of 3.5 mL/min. Microanalyses were performed by Mr. Josef Nemeth and his staff or by Midwest Microlab, Ltd.

Methyl *N*-Cyanomethanimidate (1). A mixture of cyanamide (68.5 g, 1.63 mol) and trimethyl orthoformate (520 mL, 4.75 mol) was heated at reflux under nitrogen for 5 min. Formic acid (5 mL, 0.132 mol) was cautiously introduced, and the solution was heated at reflux for an additional 5 h. The reaction mixture was cooled, and any solid separated at this point was collected by filtration. The filtrate was evaporated to dryness on a rotary evaporator, and the residual oil was distilled [73–75 °C (3.2 mmHg)] to obtain 1 as a colorless oil: 121.8 g (89%); ^{1}H NMR (neat) δ 3.82 (s, 3, OCH_3), 8.3 (s, 1, CH); ^{13}C NMR ($(CD_3)_2SO$) δ 56.5 (OCH_3), 114.5 (C≡N), 174.9 (CH); IR (neat) 3025 (=CH), 2225 (C≡N), 1625 (N=C) cm^{-1}; mass spectrum (70 eV), *m/e* (relative intensity) 84 (M^+, 85), 58 (M^+ − CN, 29), 53 (M^+ − OCH_3, 80), 43 ($CH_3N_2^+$, 78).

Caution: This substance is highly irritating and hygroscopic.

Anal. Calcd for $C_3H_4N_2O$: C, 42.85; H, 4.80; N, 33.32. Found: C, 42.94; H, 4.80; N, 33.19.

(6,7-Dihydro-6-oxo-7-β-D-ribofuranosyl-4*H*-pyrimido[1,6-*a*]-1,3,5-triazin-4-ylidene)guanidine (4). A suspension of cytidine (21.89 g, 90 mmol) in HMPA (125 mL) was heated in a three-necked flask at 70 °C under nitrogen until it formed a clear solution. Then methyl *N*-cyanomethanimidate (1; 8.4 g, 100 mmol) was introduced drop by drop through a hypodermic syringe during a period of 53 min. The yellow solution was further heated at 74–77 °C for 17 h. The reaction mixture was concentrated to ~50 mL by distilling the excess HMPA [90–95 °C (2.5 mmHg)] and was poured into AcOEt (600 mL) and triturated at 50 °C to form a canary yellow solid. The solid was filtered in vacuo and redissolved in boiling 61.5% aqueous DMF (650 mL), the mixture was treated with decolorizing charcoal and filtered, and the filtrate was concentrated to 150 mL on a rotary evaporator and cooled to room temperature. The colloidal suspension formed was centrifuged at 5000 rpm at 5 °C for 5 min, and the precipitate was filtered in vacuo, air-dried, and recrystallized from a mixture of CH_3OH–H_2O (4:1) as pale yellow flakes of 4: 13.66 g (45%); mp 212–214 °C dec. A small sample (180 mg) of the crude product was also purified by medium-pressure LC with a C_{18} column (1 × 50 cm) and eluted with a gradient of CH_3CN–H_2O (3:1 → 1:1; 400 mL). The spectral data for 4 were as follows: ^{1}H NMR ($(CD_3)_2SO$) δ 3.83 (br, 2, 5′-CH_2), 4.15 (br, 3, 2′-, 3′-, 4′-H), 5.15 (br, 3, 2′-, 3′-, 5′-OH), 6.0 (d, *J* = 2.0 Hz, 1, 1′-H), 7.8 (s, 2, NH_2, exchangeable with D_2O), 7.9 (d, *J* = 7.0 Hz, 1, 9-H), 8.66 (s, 1, 2-H), 8.7 (d, *J* = 7.0 Hz, 1, 8-H), 10.77 (br, 1, NH, exchangeable with D_2O); ^{13}C NMR ($(CD_3)_2SO$) δ 59.9 (C-5′), 68.7, 74.6, 84.2 (C-2′, -3′, -4′, interchangeable), 90.1 (C-1′), 96.1 (C-9), 144.2 (C-8), 154.5, 162.6, 163.6, 166.2 ($C_{guanidine}$, C-4, C-9a, C-6, interchangeable), 166.6 (C-2); IR (KBr) 3500–3025 (br, NH/OH) cm^{-1}; mass spectrum (10 eV), *m/e* (relative intensity) 205 (B + 1, 18), 177 (B + 1 − CO, 4), 162 (B + 1 − CH_3N_2, 2), 150 (2), 137 (4), 111 (100), 70 (15), 44 (22), 43 (25), 28 (18).

Anal. Calcd for $C_{12}H_{15}N_7O_5$: C, 42.73; H, 4.48; N, 29.07. Found: C, 42.74; H, 4.45; N, 28.67.

(6,7-Dihydro-6-oxo-4*H*-pyrimido[1,6-*a*]-1,3,5-triazin-4-ylidene)guanidine (5). Method A. A mixture of cytosine (5 g, 45 mmol) and HMPA (150 mL) was heated in a three-necked flask under nitrogen at 105–110 °C until it formed a clear solution. Then the reagent 1 (4.26 g, 50.69 mmol) was introduced through a hypodermic syringe in a period of 20 min. Within minutes, the color of the reaction mixture changed to fluorescent yellow. The solution was heated for an additional 17 h at 105–110 °C, concentrated to ~40 mL by distilling off HMPA under diminished pressure, and poured into 1500 mL of $CHCl_3$. The yellow solid that separated was allowed to settle, was collected by filtration in vacuo, and was recrystallized repeatedly from 66% aqueous DMF as tiny pale yellow crystals of 5: 3.20 g (35%); mp >300

(40) Leonard, N. J.; McDonald, J. J.; Reichmann, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 93.

°C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.36 (s, 2, NH_2, exchangeable with D_2O), 7.4 (d, J = 7.0 Hz, 1, 9-H), 7.7 (d, J = 7.0 Hz, 1, 8-H), 8.23 (s, 1, 2-H), 10.66 (br, 1, NH, exchangeable with D_2O); IR (KBr) 3300–3050 (br, NH) cm^{-1}; mass spectrum (10 eV), m/e (relative intensity) 205 (M^+, 48), 177 (M^+ – CO, 11), 162 (M^+ – CH_3N_2, 8), 111 (100), 70 (23), 44 (16), 43 (26), 28 (44).

Anal. Calcd for $C_7H_7N_7O$: C, 40.97; H, 3.44; N, 47.79. Found: C, 40.67; H, 3.59; N, 47.75.

A hydrochloride salt of **5** was prepared by dissolving **5** (10 mg, 0.05 mmol) in 0.6 N HCl (5 mL) and evaporating the solution on a rotary evaporator with the use of a water bath maintained below 40 °C. The residual solid was coevaporated with dry toluene (3 × 5 mL) to obtain a white solid (12 mg, 99%).

Anal. Calcd for $C_7H_8ClN_7O$: C, 34.79; H, 3.34; N, 40.58. Found: C, 34.89; H, 3.28; N, 40.69.

Method B. A solution of **4** (25 mg, 0.074 mmol) in aqueous 0.6 N HCl (15 mL) was allowed to stand at 35–40 °C for 5.5 h. The solution was evaporated to dryness on a rotary evaporator with the use of a water-bath whose temperature did not exceed 40 °C, and the residue was azeotroped with dry toluene (2 × 10 mL). The UV spectra in pH 1, 7, and 13 and the TLC behavior (using reverse-phase (C_{18}) TLC plates) in CH_3CN–H_2O (3:1), $CHCl_3$–MeOH (8:2), and AcOEt–EtOH (5:8) of an aqueous solution of the product thus obtained, were identical with those of an authentic sample of **5**·HCl prepared as described above.

Method C. A mixture of **8** (100 mg, 0.60 mmol) and guanidine hydrochloride (60 mg, 0.63 mmol) in anhydrous 2-propanol (15 mL) was heated at reflux under nitrogen for 17 h. The reaction mixture was cooled to room temperature, and the white solid that separated was filtered in vacuo. This solid was identified by TLC, UV, and ^{1}H NMR as cytosine (44 mg, 0.4 mmol, 67%).

After removal of cytosine, the mother liquor was evaporated to dryness on a rotary evaporator to give a glassy residue, which upon trituration with EtOH yielded tiny white crystals of **5**·HCl (12 mg, 8%). The ^{1}H NMR, UV, and mass spectra of this product were identical with those of an authentic sample of **5**·HCl prepared as described in method A.

1,2-Dihydro-2-oxo-4-[(methoxymethylene)amino]pyrimidine (7). In a dry, three-necked flask were placed cytosine (555 mg, 5 mmol), trimethyl orthoformate (20 mL), and CF_3CO_2H (0.1 mL). The mixture was heated at reflux under nitrogen, while the methanol formed was distilled continually with the aid of a Dean–Stark trap, for a period of 45 h. TLC of the reaction mixture in H_2O–CH_3CN (1:3) on a reverse-phase (C_{18}) plate indicated complete conversion into a highly fluorescent product. The reaction mixture was cooled to room temperature, and the crystalline yellow solid that separated was collected by filtration. (Note: The product is highly hygroscopic and turns into a gummy mass upon exposure to air. TLC of the sample dissolved in H_2O showed that the product was losing its fluorescence and was reverting to cytosine.) ^{1}H NMR (determined quickly) of the product in $(CD_3)_2SO$ exhibited the following features: δ 3.68 (s, 3, OCH_3), 6.0 (d, J = 7.0 Hz, 1, 5-H), 7.7 (d, J = 7.0 Hz, 1, 6-H), 8.17 (s, 1, side-chain CH).

***N*^{4}-[(Dimethylamino)methylene]cytosine (8).** A mixture of cytosine (1.5 g, 13.5 mmol), dimethylformamide diisopropyl acetal (8.38 g, 47.8 mmol), and CF_3CO_2H (0.15 g, 1.3 mmol) was heated at 85–90 °C for 12 h under nitrogen. The reaction mixture was cooled, and the solid that separated was collected by filtration in vacuo, air-dried, and recrystallized from 2-propanol as white crystals of **8**: 2.2 g (98%); mp 244–245 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 3.03 (s, 3, CH_3), 3.17 (s, 3, CH_3), 3.22 (br, 1, OH), 5.77 (d, J = 6.0 Hz, 1, 5-H), 7.47 (d, J = 6.0 Hz, 1, 6-H), 8.56 (s, 1, side-chain CH); ^{13}C NMR ($(CD_3)_2SO$) δ 34.6 (CH_3), 40.3 (CH_3), 100.1 (C-5), 144.4 (C-6), 156.7 (C-2), 157.4 (side-chain CH), 171.9 (C-4); IR (KBr) 3125–2500 (OH), 1625 (N=C) cm^{-1}; UV (2-propanol) λ_{max} 310 nm (ε 23 450); mass spectrum (10 eV), m/e (relative intensity) 166 (M^+, 100), 151 (M^+ – CH_3, 46), 136 (M^+ – 2 CH_3, 14), 122 (M^+ – $N(CH_3)_2$, 56), 110 (M^+ + 1 – $CHN(CH_3)_2$, 53), 44 ($N(CH_3)_2^+$, 55).

Anal. Calcd for $C_7H_{10}N_4O$: C, 50.59; H, 6.07; N, 33.72. Found: C, 50.68; H, 6.08; N, 33.61.

Reaction of *N*^{4}-[(Dimethylamino)methylene]cytosine (8) with Sodiocyanamide. A mixture of compound **8** (166 mg, 1 mmol) and sodiocyanamide (64 mg, 1 mmol) in anhydrous 2-propanol (20 mL) was heated at reflux under nitrogen for 2 h. The reaction was cooled, and the crystalline solid that separated was collected by filtration. The solid was unstable (sodium salt of cytosine?) and therefore was neutralized quickly with 5% aqueous HCl and evaporated in vacuo at 50 °C to obtain a stable crystalline white solid, the ^{1}H NMR, IR, and mass spectral data of which were identical with those of cytosine.

The filtrate after the removal of cytosine was concentrated to ~5 mL, and colorless long crystals of **[(dimethylamino)methylene]cyanamide** separated which were recrystallized from $CHCl_3$–AcOEt: 79 mg (81%); mp 74–76 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.88 (s, 3, CH_3), 3.1 (s, 3, CH_3), 8.31 (s, 1, CH); ^{13}C NMR ($CDCl_3$) δ 34.6 (CH_3), 41.1 (CH_3), 119.2 (C≡N), 163.9 (=CH); IR (KBr) 2950 (CH_3), 2200 (C≡N), 1625 (N=CH) cm^{-1}; UV (2-propanol) λ_{max} 241 nm (ε 16 620); mass spectrum (10 eV) m/e (relative intensity) 97 (M^+, 100), 82 (M^+ – CH_3, 44), 70 (M^+ – HCN, 30), 44 ($N(CH_3)_2^+$, 65), 42 ($NCHN_2^+$, 77).

Anal. Calcd for $C_4H_7N_3$: C, 49.47; H, 7.27; N, 43.26. Found: C, 49.55; H, 7.33; N, 43.29.

(6-Oxo-6*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)guanidine (9) and (8-Oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)guanidine (10). A mixture of isocytosine (10 g, 90 mmol) and HMPA (60 mL) was heated to 66 °C in a dry 200-mL, three-necked flask under nitrogen to form a clear solution. The temperature was then lowered to 55 °C, and methyl *N*-cyanomethanimidate (**1**; 8.4 g, 100 mmol) was introduced through a hypodermic syringe drop by drop over a period of 45 min. The yellow reaction mixture was heated at 50–55 °C for 19 h, cooled to room temperature, and poured into $CHCl_3$ (700 mL). The yellow solid that separated was collected by filtration and air-dried. The crude product (17.3 g) was extracted with boiling MeOH–H_2O (1:2; 3 × 300 mL). The undissolved solid was compound **9** which was recrystallized from Me_2SO: 3.8 g (21%); mp >300 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 5.87 (d, J = 7.0 Hz, 1, C-7 H), 7.7 (s, 2, NH_2, exchangeable with D_2O), 7.75 (d, J = 7.0 Hz, 1, 8-H), 8.3 (s, 1, 2-H), 9.3 (br, <1, NH, exchangeable with D_2O), 11.4 (br, 1, NH, exchangeable with D_2O); ^{13}C NMR (0.2 N NaOD) δ 106.6 (C-7), 154.4 (C-8), 157.6, 163.7, 165.4 ($C_{guanidine}$, C-4, C-9a, interchangeable), 166.0 (C-2), 174.5 (C-6); IR (KBr) 3375, 3075 (NH), 1675 (C=O) cm^{-1}; mass spectrum (70 eV), m/e (relative intensity) 205 (M^+, 100), 177 (M^+ – CO, 11), 162 (M^+ – CH_3N_2, 2), 137 (53), 111 (22), 70 (33), 68 (28), 44 (24), 43 (35), 28 (53); high-resolution electron-impact mass spectrum calcd for $C_7H_7N_7O$ m/e 205.0712, obsd 205.0712.

Anal. Calcd for $C_7H_7N_7O$·0.25H_2O:[41] C, 40.09; H, 3.44; N, 46.76. Found: C, 40.30; H, 3.48; N, 46.65.

Compound **9** crystallized into a monohydrochloride salt from 0.6 N HCl.

Anal. Calcd for $C_7H_8ClN_7O$: C, 34.79; H, 3.34; N, 40.58. Found: C, 35.02; H, 3.38; N, 40.44.

After filtration to remove compound **9**, the MeOH–H_2O extract was heated to boiling, treated with decolorizing charcoal, and filtered, and the filtrate was concentrated to 300 mL and cooled to room temperature. The white amorphous solid (compound **10**) that separated was filtered and recrystallized from Me_2SO–petroleum ether: 1.7 g (9%); mp >300 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 89 (d, J = 7.0 Hz, 1, 7-H), 7.73 (br, 2, NH_2, exchangeable with D_2O), 7.77 (d, J = 7.0 Hz, 1, 6-H), 8.33 (s, 1, 2-H), 12.0 (br, 1, NH, exchangeable with D_2O); ^{13}C NMR (0.2 N NaOD) δ 106.7 (C-7), 154.6 (C-6), 158.1, 163.7, 165.6 ($C_{guanidine}$, C-4, C-9a, interchangeable), 166.1 (C-2), 174.9 (C-8); IR (KBr) 3330, 3080 (NH), 1680 (C=O) cm^{-1}; mass spectrum (70 eV), m/e (relative intensity) 205 (M^+, 16), 142 (5), 137 (4), 111 (3.5), 99 (6), 85 (16), 71 (32), 57 (100), 43 (23), 36 (22), 28 (8.5).

Anal. Calcd for $C_7H_7N_7O$: C, 40.97; H, 3.44; N, 47.79. Found: C, 40.93; H, 3.62; N, 48.14.

Reaction of Compound 9 with Trifluoroacetic Acid. A mixture of **9** (500 mg, 2.44 mmol) and trifluoroacetic acid (20 mL) was heated at reflux under nitrogen for 92 h. The clear solution was evaporated to dryness, and the residue was coevaporated with dry toluene (2 × 10 mL). The residual solid was digested with a large excess of 10% acetic acid and cooled to room temperature. The solid that separated (400 mg) was collected by filtration and identified as the starting material.

(41) The microanalyses of **9** on three different trials consistently came out with 0.25 mol of H_2O. Attempts to remove the water were unsuccessful.

TLC of the filtrate after separation of the solid indicated that it was a mixture of the starting material and one other UV-absorbing component. The filtrate was evaporated to dryness, and compound **9a** was isolated from the mixture through repeated fractional recrystallization from 20% aqueous acetic acid: 14 mg (conversion 3%); mp >300 °C; ^{1}H NMR (CF_3CO_2H) δ 6.63 (d, J = 6.0 Hz, 1, 9-H), 8.2 (d, J = 6.0 Hz, 1, 8-H), 8.7 (s, 1, 5-H), 8.75 (br, NH_2, exchangeable with D_2O); mass spectrum (70 eV), m/e (relative intensity) 189 (M^+ + 2, 100), 188 (M^+ + 1, 58), 162 (14), 161 (17), 146 (10), 136 (15), 135 (41), 120 (33); high-resolution electron-impact mass spectrum calcd for $C_7H_7N_7$ m/e 189.0763, obsd 189.0762. [Note: Neither the mass spectrum of **9** nor that of **10** exhibited even a trace of an ion at m/e 189 (see Figure 1).]

(7*H*-1,3,5-Triazino[2,1-*i*]purin-7-ylidene)guanidine (20), N^6-[(Cyanoamino)methylene]adenine (21), and (9*H*-1,3,5-Triazino[2,1-*i*]purin-9-ylidene)guanidine (22). A mixture of adenine (4.05 g, 30 mmol) and HMPA (120 mL) was heated at 105–110 °C in a three-necked flask to form a clear, colorless solution. Then methyl *N*-cyanomethanimidate (**1**; 2.95 g, 35 mmol) was introduced through a hypodermic syringe in a period of 5 min. The yellow contents of the flask were heated at 105–110 °C for an additional 15 h. The reaction mixture was cooled to room temperature and poured into 1000 mL of ligroin. The ligroin layer was decanted, and the residual oil was triturated with ethyl acetate (600 mL). The orange solid that separated was filtered in vacuo (the filtrate was preserved; see below) and air-dried. Thin-layer chromatography of this solid in EtOAc–EtOH (5:8) on a silica gel plate indicated that it was a mixture of adenine and a highly fluorescent product(s) that remained at the origin. Repeated fractional recrystallizations of this solid mixture from Me_2SO–EtOAc, with concurrent UV monitoring of every fraction, led to the isolation of adenine (305 mg, 7.5%) and two fluorescent products: compound **20** (275 mg, 4%) and compound **22** (760 mg, 11%). The spectroscopic and analytical data for compounds **20** and **22** follow.

Compound **20**: mp >300 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.3 (br s, 2, NH_2, exchangeable with D_2O), 8.06 (s, 1, 2-H), 8.53 (s, 2, 5-H and 9-H), 8.77 (br, 2, 2 NH exchangeable with D_2O); IR (KBr) 3550–2900 (br, NH) cm^{-1}; mass spectrum (10 eV), m/e (relative intensity) 229 (M^+, 48), 202 (M^+ – HCN, 7.5), 188 (100), 161 (14.5), 119 (14.5), 43 (10), 42 (21); high-resolution electron-impact mass spectrum calcd for $C_8H_7N_9$ m/e 229.0825, obsd 229.0825.

Compound **22**: mp >300 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 8.37 (s, 1, 2-H), 8.5 (s, 1, 5-H), 8.60 (s, 1, 7-H), 7.6 (br, 2, NH_2, exchangeable with D_2O); ^{13}C NMR (0.2 N NaOD) δ 126.1 (C-1a), 148.9 (C-2), 154.0 (C-5), 165.4 (C-7), 148.6, 154.6, 154.8, 165.7 (C-3a, $C_{guanidine}$, C-9, C-10a, interchangeable); IR (KBr) 3550–3050 (br, NH) cm^{-1}; mass spectrum (10 eV), m/e (relative intensity) 229 (M^+, 100), 202 (M^+ – HCN, 10), 188 (18), 161 (19), 119 (8), 43 (7).

Anal. Calcd for $C_8H_7N_9$: C, 41.92; H, 3.08; N, 55.00. Found: C, 41.76; H, 3.28; N, 54.92.

The EtOAc filtrate (vide supra) was evaporated to dryness on a rotary evaporator, and the residual oil was triturated with an excess of CCl_4, which caused a white solid to precipitate. TLC of the solid in $CHCl_3$–MeOH (8:2) on a silica gel plate indicated that it was a mixture of adenine (R_f 0.12) and a higher moving component (R_f 0.23). The two compounds could be separated from one another either by fractional recrystallization from MeOH–$CHCl_3$ or by the following chromatographic method.

The solid mixture was dissolved in 25 mL of DMF, loaded onto a high-performance LC column (Waters Prep 500) using a silica gel cartridge, and eluted with a mixture of $CHCl_3$–MeOH (10:1). The column was eluted with a total of 4 L of the above solvent mixture. The appropriate fractions were pooled and evaporated to obtain **21**: 865 mg (15%); mp >300 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 8.53 (s, 1, 8-H), 8.6 (s, 1, 2-H), 9.97 (s, 1, side-chain CH), 12.93 (br, 2, 2 NH's exchangeable with D_2O); IR (KBr) 2208 (C≡N), 1639 (N=C) cm^{-1}; UV λ_{max} (pH 1) 330 nm (ε 7500), 261 (6950), 211 (14 400); λ_{max} (pH 7) 302, 258 (sh), 236 (sh), 212; λ_{max} (pH 13) 312 (6950), 278 (sh, 7200), 266 (9600); mass spectrum (70 eV), m/e (relative intensity) 187 (M^+, 30), 160 (M^+ – HCN, 27), 135 (M^+ + 1 – C_2HN_2, 100), 119 (M^+ – $C_2H_2N_3$, 35), 108 (40), 93 (16), 81 (13), 66 (15), 53 (32), 43 (16).

Anal. Calcd for $C_7H_5N_7$: C, 44.92; H, 2.69; N, 52.39. Found: C, 45.08; H, 2.79; N, 52.13.

N^6-[(Dimethylamino)methylene]-9-(1-ethoxyethyl)adenine (17). A mixture of 9-(1-ethoxyethyl)adenine (**16**; 4.14 g, 20 mmol) and dimethylformamide dimethyl acetal (50 mL) was heated at reflux for 14 h under nitrogen. The solvent was evaporated to dryness on a rotary evaporator to obtain **17** as a pale green syrup: 5.2 g (99%); ^{1}H NMR ($(CD_3)_2SO$) δ 1.07 (t, J = 6.5 Hz, 3, CH_2CH_3), 1.79 (d, J = 6.0 Hz, 3, $CHCH_3$), 3.19 (s, 3, $N(CH_3)_2$), 3.25 (s, 3, $N(CH_3)_2$), 3.43 (q, J = 6.5 Hz, 2, CH_2CH_3), 5.95 (q, J = 6.0 Hz, 1, $CHCH_3$), 8.47 (s, 2, 2-H and 8-H), 8.97 (s, 1, sidechain CH); FD mass spectrum (low resolution), m/e 262 (M^+). The compound was practically pure (as determined by TLC and ^{1}H NMR) and was employed directly in the next stage.

Reaction of N^6-[(Dimethylamino)methylene]-9-(1-ethoxyethyl)adenine (17) with Cyanamide. A mixture of **17** (3.9 g, 15 mmol) and cyanamide (1.34 g, 32 mmol) was heated at reflux in 2-propanol (45 mL) for 24 h. The mixture was evaporated to dryness on a rotary evaporator, and the residual syrup was dissolved in 8 mL of $CHCl_3$ and loaded onto a column (4.5 × 80 cm) of silica gel (350 g) packed with $CHCl_3$ and eluted first with $CHCl_3$ (2 L) and then with a gradient of $CHCl_3$–MeOH (1:0 → 0:1; 3 L). The appropriate fractions were pooled and evaporated to obtain the following compounds: (a) [(dimethylamino)methylene]cyanamide, 0.60 g (41%), mp 74–76 °C; (b) 9-(1-ethoxyethyl)adenine, 1.24 g (40%); mp 172–173 °C; (c) dicyandiamide, 0.21 g (7.8%), mp 203–205 °C; (d) 7-imino-8,9-dihydro-9-(dimethylamino)-3-(1-ethoxyethyl)-1,3,5-triazino[2,1-*i*]purine (proposed structure): 0.75 g (16.5%); mp 186–188 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 1.1 (t, J = 6.0 Hz, 3, CH_2CH_3), 1.57 (d, J = 6.0 Hz, 3, $CHCH_3$), 3.05 (s, 3, $N(CH_3)_2$), 3.1 (s, 3, $N(CH_3)_2$), 3.37 (m, J = 6.0, 2.0 Hz, 2, CH_2CH_3), 5.6 (q, J = 6.0 Hz, 1, $CHCH_3$), 7.07 (br, 2, NH_2 exchangeable with D_2O), 7.6 (s, 1, 9-H), 8.27 (s, 1, 2-H), 8.33 (s, 1, 5-H); IR (KBr) 3425, 3300, 3225 (NH_2), 1640 (N=C) cm^{-1}; FD mass spectrum (low resolution), m/e 304 (M^+); UV λ_{max} (pH 1) 330 (log ε 4.24), 220 (4.34); λ_{max} (pH 7) 333 (4.08), 288 sh (3.75), 225 (4.39); λ_{max} (pH 13) 334 (4.08), 248 sh (4.30), 221 (4.51).

Anal. Calcd for $C_{13}H_{20}N_8O$: C, 51.30; H, 6.62; N, 36.82. Found: C, 51.35; H, 6.39; N, 36.56.

Compound e was also obtained: (3-(1-ethoxyethyl)-7*H*-1,3,5-triazino[2,1-*i*]purin-7-ylidene)guanidine (**19**); 0.60 g (13.3%); ^{1}H NMR ($(CD_3)_2SO$) δ 1.1 (t, J = 6.0 Hz, 3, CH_2CH_3), 1.57 (d, J = 6.0 Hz, 3, $CHCH_3$), 3.37 (m, J = 6.0, 2.0 Hz, 2, CH_2CH_3), 5.6 (q, J = 6.0 Hz, 1, $CHCH_3$), 7.3 (br, 2, NH_2, exchangeable with D_2O), 8.06 (s, 1, 2-H), 8.53 (s, 2, 5-H, 9-H), 8.7 (br, 1, NH exchangeable with D_2O).

Anal. Calcd for $C_{12}H_{15}N_9O$: C, 47.83; H, 5.02; N, 41.84. Found: C, 47.79; H, 5.22; N, 41.81.

Hydrolysis of (3-(1-Ethoxyethyl)-7*H*-1,3,5-triazino[2,1-*i*]purin-7-ylidene)guanidine (19) with Aqueous Acetic Acid. A mixture of compound **19** (0.50 g, 1.66 mmol) and 50% (v/v) aqueous acetic acid (20 mL) was stirred magnetically at room temperature for 48 h. The product that separated was filtered in vacuo, washed with water (50 mL), and air-dried to obtain a buff-colored amorphous solid (**20**): 0.30 g (79%); mp >300 °C. The ^{1}H NMR, UV, IR, and mass spectral data of **20** were identical with those of the product C isolated from the reaction of adenine with **1**.

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Registry No. 1, 51688-22-3; 4, 76299-77-9; 5, 76299-78-0; 5 monohydrochloride, 76299-79-1; 7, 76299-80-4; 8, 76299-81-5; 9, 76299-82-6; 9 monohydrochloride, 76299-83-7; 9a, 76299-84-8; 10, 76299-85-9; 16, 29767-70-2; 17, 76299-86-0; 18, 76299-87-1; 19, 76299-88-2; 20, 76299-89-3; 21, 76299-90-6; 22, 76299-91-7; cyanamide, 420-04-2; trimethyl orthoformate, 149-73-5; cytidine, 65-46-3; cytosine, 71-30-7; guanidine hydrochloride, 50-01-1; dimethylformamide diisopropyl acetal, 18503-89-4; [(dimethylamino)methylene]cyanamide, 39687-97-3; isocytosine, 108-53-2; adenine, 73-24-5; dimethylformamide dimethyl acetal, 4637-24-5; dicyandiamide, 461-58-5.