changeable with D_2O), 8.07 (s, 1, 6-H), 7.23-7.38 (m, 5, phenyl protons), 5.32 (s, 2, CH_2); ^{13}C NMR ($(CD_3)_2SO$) δ 163.17 (C6), 159.88 (C8 or C4a), 157.9 (C10), 152.83 (C4a or C8), 151.88 (C3a), 127.76 (C2), 120.35 (C10a), 47.95 (C1') (see **16** for benzyl numbering), 136.83 (C2'), 130.1 (C3' and C7' or C4' and C6'), 129.24 (C4' and C6' or C3' and C7'), 128.43 (C5'); UV (EtOH) λ_{max} (ϵ) 336 nm (8580), 313 (7990), 257 sh (10 355), 250 (12 130); mass spectrum (70 eV), *m/e* (relative intensity) 371 (M^+ ^{79}Br, 10), 373 (M^+ ^{81}Br, 9), 91 ($C_7H_7^+$, 100). Anal. Calcd for $C_{14}H_{10}BrN_7O$: C, 45.18; H, 2.71; N, 26.35; Br, 21.47. Found: C, 45.44; H, 2.73; N, 26.59; Br, 21.35.

Regeneration of Guanosine (1) from IA'-Metamorphosine (2). A solution of 1.1 mg of IA'-metamorphosine (**2**) in 100 mL of 0.1 N NaOH was allowed to stand for 5 min. The UV spectrum showed the complete disappearance of absorption at 331 nm and a new absorption maximum appeared at 264 nm. Quantitative determinations with guanosine as a standard indicated 100% conversion of **2** to **1**.

Spray Reagent of Methyl *N*-Cyanomethanimidate (3). A solution of 1.5 g of **3** and 1 equiv of powdered, anhydrous $NaOCH_3$ in 50 mL of anhydrous methanol was finely sprayed over chromatograms (silica gel) which were spotted with varied amounts of adenosine, guanosine, cytidine, and uridine. The plates were heated for 2-5 min with a heat gun, and blue tinted fluorescent spots became pronounced at adenosine[1] and guanosine. The plates remained dark at cytidine and uridine.

Adenosine Deaminase (Adenosine Aminohydrolase, EC 3.5.4.4) Inhibition by 2. Potassium phosphate buffer, pH 7.45 (50 mM), was used throughout. Substrate solutions were made by dissolving adenosine in buffer and the stock concentrations were 109, 165, 212, 316, and 629 μM. Inhibitor solutions were made by dissolving **2** in buffer, and the stock concentrations were 1.82, 2.78, 4.61, and 6.69 mM. Calf intestinal mucosal adenosine deaminase (10 μL of Grade I commercial solution, Sigma) was suspended in 90 μL of buffer containing 0.1 mg per mL of bovine serum albumin (BSA). This enzyme solution was then diluted 150-fold with 0.1 mg per mL of BSA solution. The final assay mixtures had a total volume of 1.0 mL in a cuvette with a 1.0-cm light path. The assay mixtures contained 0.7 mL of buffer, 0.1 mL of the appropriate stock solution of **2**, 0.1 mL of the adenosine deaminase solution, and 0.1 mL of the appropriate adenosine stock solution. The rates of deamination were determined at 25 °C by monitoring the drop in absorbance at 265 nm. The reference cell in each run contained **2** at the same concentration as in the sample cell. Assays were run in triplicate for each sample. The initial slopes were converted to micromoles per minute per milligram of protein by using 8.1 as the difference in mM extinction coefficient between adenosine and inosine. Subsequent Lineweaver-Burk[26] analysis of the data established that the inhibition was competitive. Analysis with the COMP program[27] gave K_m for adenosine, 32.7 ± 1.9 μM, V_{max} 293 ± 9 μmol/(min mg), and K_i for **2**, 227 ± 10 μM.

Reaction of IA'-Metamorphosphine (2) with Adenosine Deaminase. A solution of IA'-metamorphosine (0.30 μmol in 3 mL of 0.1 M triethylammonium bicarbonate, pH 7) containing adenosine deaminase (80 μg, 2.3 mmol, desalted by passage over a 1-mL column of Sephadex G-10 in 0.1 M triethylammonium bicarbonate) was incubated at room temperature for 5 h. During this period, the absorption spectrum shifted slightly (λ_{max} 318 and 300 nm, clean isosbestic points at 326, 306 and 256 nm); the pH also increased, but its readjustment to pH 7 did not affect the spectrum. Neither the pH nor the spectrum changed in a control without enzyme. Upon overnight incubation, the spectrum of the enzyme-containing sample underwent further change and lost its isosbestic points. Attempts to isolate the product after 3.5 h incubation by lyophilization followed by dissolution of the residue in methanol led to the recovery of a compound with a UV spectrum resembling that of guanosine; compound **2** not exposed to adenosine deaminase was recovered intact. These data suggest that adenosine deaminase did react with IA'-metamorphosine, albeit very slowly, but the failure to isolate and characterize the product precludes further interpretation at this point.

Acknowledgment. This research was supported by Research Grant No. CHE 81-21796 from the National Science Foundation and in part by a grant from Eli Lilly and Company. We appreciate the assistance and cooperation of Dr. Ramachandra S. Hosmane, Dr. Robert MacGregor, Dr. Katharine J. Gibson, and Dr. Mitchell A. Rossman. High-resolution mass spectral data were obtained in part under a grant from the National Institute of General Medical Sciences (GM-27029). NMR data were obtained in part with support from the University of Illinois NSF Regional Instrumentation Facility, Grant NSF CHE 79-16100.

Registry No. 1, 118-00-3; 2, 92220-57-0; 3, 51688-22-3; 4, 96412-41-8; 5, 96427-27-9; 6, 96427-28-0; 7, 96412-42-9; 11, 4016-63-1; 12, 96412-43-0; 13, 14937-72-5; 14, 96412-44-1; 15, 96412-45-2; 16, 96412-46-3; CH_3OD, 1455-13-6; $CDCl_3$, 865-49-6; trimethyl [1-^{2}H]orthoformate, 79432-71-6; cyanamide, 420-04-2; adenosine deaminase, 9026-93-1.

(26) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658.
(27) Cleland, W. W. *Adv. Enzymol.* **1967**, *29*, 1.

Annelation of Isocytosines by Reaction with Methyl *N*-Cyanomethanimidate and Sodium Methoxide: Influence of Substitution on the Course of the Reaction and Rearrangements

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Annelation of isocytosine and 6-substituted isocytosines with methyl *N*-cyanomethanimidate and sodium methoxide in methanol leads to pyrimidotriazines. The structures of the products reveal substituent influence on the direction of cyclization and rearrangement. The exocyclic NH_2, newly formed in the cyclization process, may undergo further condensation with methyl *N*-cyanomethanimidate and sodium methoxide in methanol, and this feature of the reaction can be used for selective monomethylation of the exoheterocyclic NH_2. A gentle method for the formation of triflate salts of amines involves treatment of the free bases with trimethylsilyl trifluoromethanesulfonate.

The annelation of guanosine to provide a tricyclic *N*-ribonucleoside having binding sites in the terminal rings similar to those of adenosine was accomplished under mild conditions, namely reaction with methyl *N*-cyanomethanimidate and sodium methoxide in anhydrous methanol at 20 °C.[1,2] Through the use of sodium meth-

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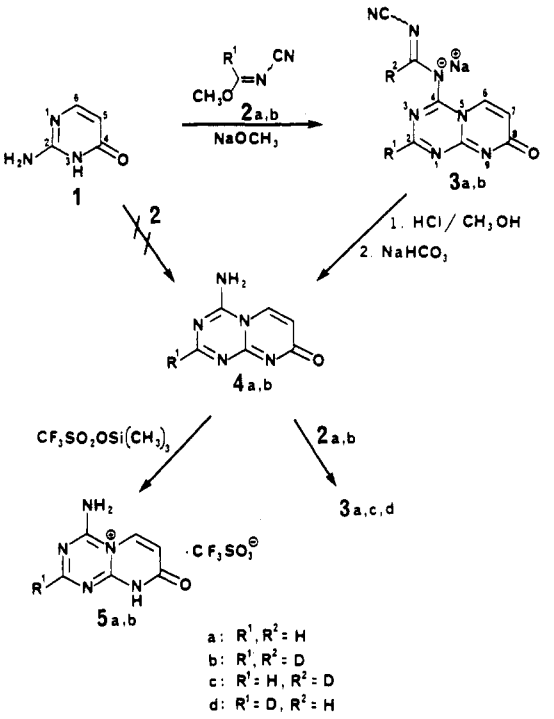
oxide and the avoidance of elevated temperature, it has now been possible to effect the annelation of isocytosine (1) without the complication of side reactions resulting from further reaction with cyanamide, liberated from methyl *N*-cyanomethanimidate (**2a**).[3] A facile method of making triflate salts was used to obtain a derivative of the isocytosine product for which the solubility and crystallinity were conducive to determination of its pivotal structure by single-crystal X-ray analysis. The contrast between the cyclization of guanosine and the cyclization of isocytosine with the same reagents and conditions is of special interest.

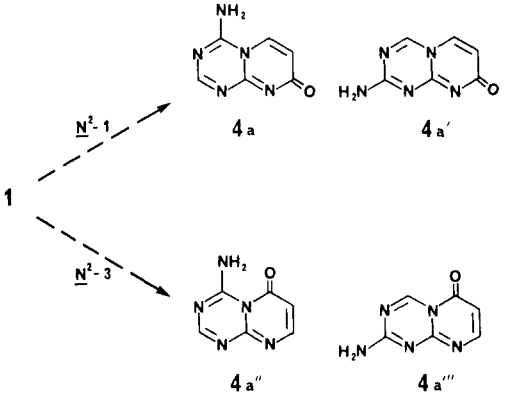
Isocytosine (1) reacted readily with methyl *N*-cyanomethanimidate (**2a**) (5 equiv) and sodium methoxide (2 equiv) in anhydrous methanol at 20 °C to give a single product as the sodium salt, $C_8H_4N_7ONa\cdot H_2O$. The composition was indicative of incorporation of **2a** and **1** in the proportion of 2:1 with the loss of 2 equiv of methanol. This result was consistent with an initial annelation followed by reaction of the exocyclic amino group thus formed with a second equivalent of **2a** and production of a stable sodium salt. Analogous elaboration of an exocyclic amino group had been experienced with cyanamide, a hydrolysis product of **2a**.[3] The ^1H NMR spectrum of the sodium salt in $(CD_3)_2SO$ indicated three heteroaromatic C–H's, two of which were coupled and therefore on adjacent carbons. The chemical shift of the fourth proton (δ 9.35 (0.25 H), 8.6 (0.75 H)) was consistent with its location on the sodiocyanoformamidine side chain and indicative of partition between syn and anti configurations of that side chain. The mass spectrum (FAB, glycerol matrix) confirmed the anion of *m/e* 214 for $C_8H_4N_7O^-$. When equimolar amounts of 1 and **2a** were employed in the reaction with sodium methoxide in methanol, only the $C_8H_4N_7ONa$ product in reduced amount plus starting material resulted. This result implies that the rate of condensation of the second equivalent of **2a** with the presumed intermediate is much faster than the rate of the initial reaction of 1 with **2a**.

Cleavage of the side chain was effected with anhydrous methanolic hydrogen chloride. Neutralization of the hydrochloride salt with $NaHCO_3$ gave a $C_6H_5N_5O$ product in which the integrity of the heteroaromatic nucleus was established by its rapid reconversion to the initial $C_8H_4N_7ONa$ product by treatment with **2a** and sodium methoxide in methanol. The molecular weight of the side chain cleavage product was indicated by the molecular ion at *m/e* 163 in the mass spectrum. The ^1H NMR spectrum confirmed the presence of three heteroaromatic C–H's, two of them adjacent, plus two exchangeable N–H's. The $C_6H_5N_5O$ product was best characterized as a triflate salt, $C_7H_6F_3N_5O_4S$, made by treatment with trimethylsilyl trifluoromethanesulfonate followed by methanol or ethyl acetate containing water. This method of preparing triflate salts (see Experimental Section) can be recommended where direct use of the very strong trifluoromethanesulfonic acid in excess would have a deleterious effect. The FAB mass spectrum indicated both the cation, *m/e* 164, and the anion, *m/e* 149, of the salt, and the ^{13}C and ^1H NMR spectra indicated the appropriate CF_3, N–H, and C–H resonances. The structure of the triflate salt was established by X-ray crystallographic analysis as **5a**, and thus the structure of the free base from which it was made, as **4a**, 4-amino-8-oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazine.

(1) Leonard, N. J.; Hosmane, R. S.; Agasimundin, Y. S.; Kostuba, L. J.; Oakes, F. T. *J. Am. Chem. Soc.* **1984**, *106*, 6847.
(2) Agasimundin, Y. S.; Oakes, F. T.; Kostuba, L. J.; Leonard, N. J. *J. Org. Chem.*, preceding article in this issue.
(3) Hosmane, R. S.; Leonard, N. J. *J. Org. Chem.* **1981**, *46*, 1457 and references therein.

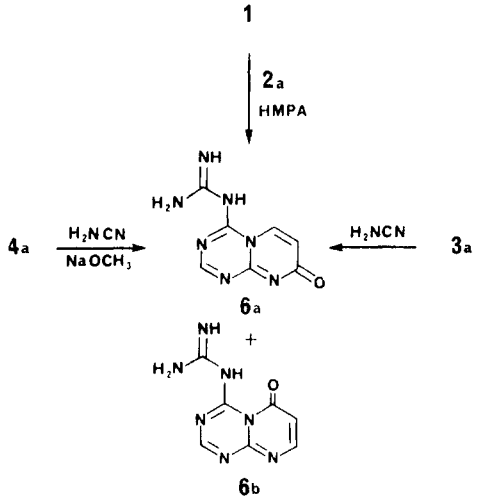


The need for structure establishment by X-ray analysis was occasioned by the four possible modes of condensation–cyclization of 1 (N^2-1 vs. N^2-3) and **2a** (C=N vs. C≡N) in the presence of sodium methoxide. Each of the isomeric possibilities (**4a**,**a**′,**a**″,**a**‴) would show three heteroaromatic protons, two of them adjacent, in the ^1H NMR spectrum. Isomer **4a**″ would correspond to the product formed from guanosine and the annelating reagent,[1,2] yet the X-ray results now exclude this possibility with 1. N^2-1 cyclization in the case of isocytosine, which is observed (**4a**), would correspond to N^2-3 annelation in the case of guanosine (guanosine numbering system), which is not observed and is probably prevented from occurring by the N9-ribosyl group adjacent to C4.

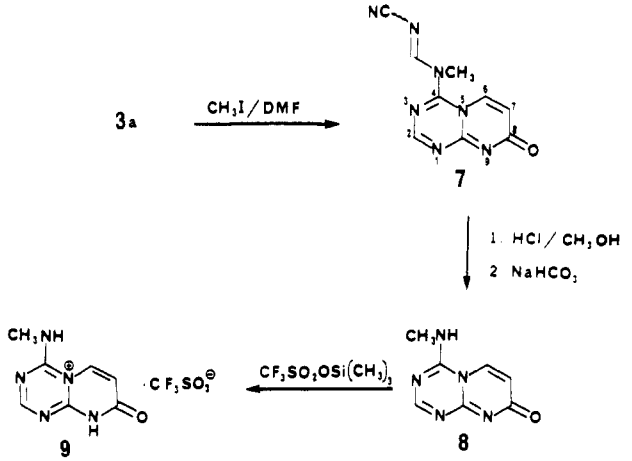


Assignments of the ^1H and ^{13}C NMR chemical shifts in **3**, **4**, and **5** were obtained through selective deuteriation. Reaction of isocytosine (1) with methyl [1-^{2}H]-*N*-cyanomethanimidate (**2b**)[2] yielded **3b** which contains deuterium in both the triazine ring and the cyanoformamidine side chain. Cleavage of the side chain as described earlier gave [2-^{2}H]-4-amino-8-oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazine (**4b**). This was converted to the corresponding triflate salt **5b** by the action of trimethylsilyl trifluoromethanesulfonate. Crossover experiments were performed to convert **3a** to **4a** and thence to **3c** by treatment with **2b**, **3b** to **4b**, and thence to **3d** by treatment with **2a**. Correlation was also made with our previous experience relating

to the reaction of isocytosine (1) with methyl *N*-cyanomethanimidate (**2a**) alone in HMPA at 50–55 °C for 19 h.[3] One of the two products (**6a,b**) of the prolonged heating in the absence of base, identified as [1,2-α]-1,3,5-triazin-4-yl)guanidine (**6a**), was obtainable from **3a** and cyanamide after prolonged reflux in 2-propanol and from **4a** and cyanamide with sodium methoxide after prolonged reflux in methanol. Independent chemical corroboration of the guanidine **6a** structure was thus achieved.

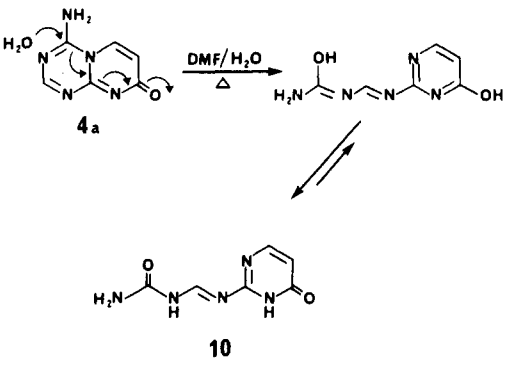


Methylation of *N*-8-oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)-*N'*-cyanoformamidine sodium salt (**3a**) was accomplished in DMF solution at room temperature with methyl iodide, and the product consisted of syn and anti forms of **7** in the proportion of 1:3 as in the precursor mixture. Cleavage of the side chain was effected with methanolic hydrogen chloride followed by neutralization with sodium bicarbonate as in the case of **3**. Compound **8** was fully characterized spectroscopically as the free base and as its triflate salt (**9**). The latter is related to the unmethylated triflate **5a**, the structure of which was established by X-ray analysis. The overall process, i.e., reaction of an exocyclic amino group with **2a** and sodium methoxide, methylation, and cleavage of the group that is both activating and protecting, constitutes a potentially useful method of monomethylation of an exoheterocyclic NH_2.



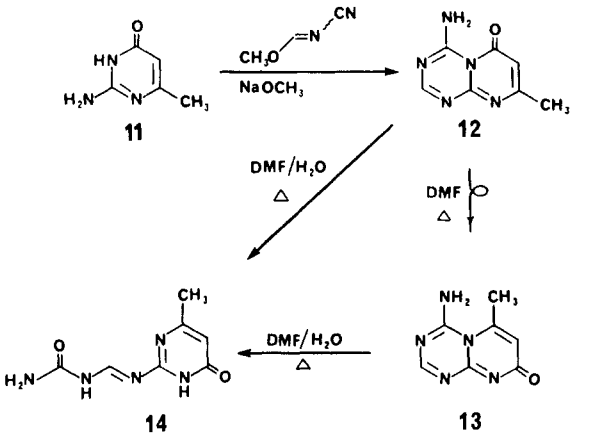
On hydrolysis of **4a** in hot aqueous DMF, the triazine ring was opened in preference to the pyrimidine ring. The structure assignment of the $C_6H_7N_5O_2$ hydrolysis product as **10** was based on the ^1H NMR spectrum which consisted of a singlet resonance at δ 8.8, satisfactory for a formamidine C–H and doublets, J = 8 Hz, at δ 7.62 and 5.73,

Scheme I



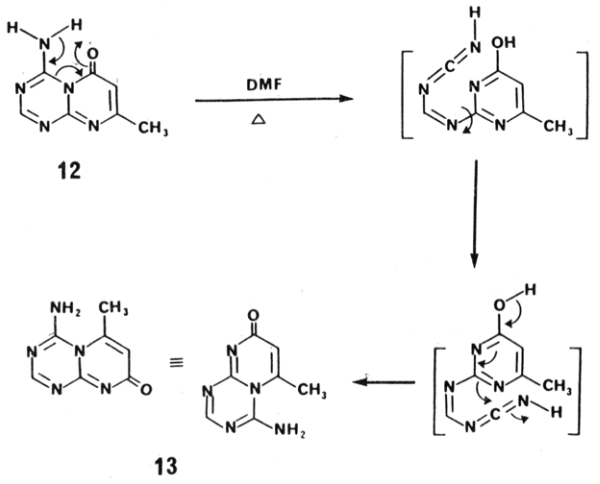
consistent with the 6-H and 5-H of the pyrimidine ring. The mass spectrum also indicated (peaks at m/e 110 and 111) that the isocytosine unit was intact. A rationalization of the course of the hydrolytic fission is shown in Scheme I (**4a** → **10**). A more plausible reaction path for the conversion of **4a** → **10** might be addition of water to a carbodiimide intermediate such as that shown in Scheme II (see later).

6-Methylisocytosine (2-amino-6-methyl-4-pyrimidinol, keto form) (**11**) bears a closer resemblance to guanosine than does isocytosine, with the 6-methyl on the pyrimidine moiety occupying a position equivalent to the N9-ribosyl group of guanosine. Unlike isocytosine, 6-methyliso-



cytosine reacted with excess methyl *N*-cyanomethanimidate (**2a**) and sodium methoxide in methanol at 20 °C to effect annelation in the N^2-3 direction on the pyrimidine ring. The bicyclic ring system obtained after careful acidification of the initially obtained sodium salt with aqueous acetic acid (**12**) corresponded to **4a″** and was exactly analogous to the annelation product of guanosine under the same reaction conditions.[1,2] The ^1H NMR spectrum of the $C_7H_7N_5O$ product in $(CD_3)_2SO$ indicated two distinct exocyclic N–H signals that were exchangeable in D_2O. The fact that there were clearly differentiated chemical shifts at δ 10.57 and 9.73 supported the existence of hydrogen bonding between the *peri*-carbonyl and the proximate exocyclic N–H, as in the case of the guanosine product.[1,2] Such bonding is unique to **12** among the four isomeric possibilities arising from different modes of condensation–cyclization (homologues of **4a,a′,a″,a‴**). Like the guanosine product,[1,2] this product (**12**) is brilliantly fluorescent when adsorbed on silica gel TLC plates. When compound **12** was heated in anhydrous DMF, dimethylacetamide, or Me_2SO, it underwent smooth rearrangement to an isomeric $C_7H_7N_5O$ product. Similarity to **4a** in UV and ^1H NMR spectra, including the broad singlet due to NH_2 protons, indicated that this isomer had

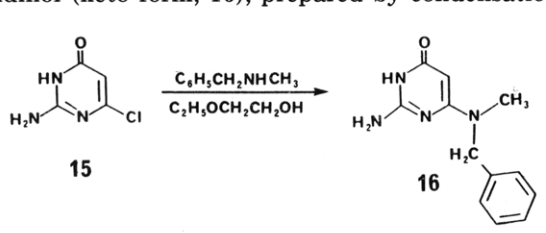
Scheme II



structure **13**. When a hot solution of either isomer **12** or **13** in DMF was treated with water, a triazine ring opened product $C_7H_9N_5O_2$ was formed immediately, to which was assigned structure **14**. Its homology with **10** was obvious from a comparison of NMR, mass, and qualitative UV spectra. The mass spectrum indicated a fragmentation pattern consistent with an intact isocytosine ring. A possible reaction path is indicated in Scheme II for the thermal conversion of **12** to **13**.

The facile rearrangement of **12** to **13** also may explain the nearly identical ^1H and ^{13}C NMR values which were reported previously for **6a** and **6b** (compounds **10** and **9** in ref 3). These compounds were initially separated by means of the large difference in solubility in hot methanol. Recrystallization of **6a** caused no change; however, recrystallization of **6b** from hot Me_2SO most likely resulted in a rearrangement cleanly to **6a** prior to the ^1H and ^{13}C NMR measurements.

The effect on the annelation reaction of a benzylmethylamino group at the 6-position of isocytosine was examined by using 2-amino-6-(benzylmethylamino)-4-pyrimidinol (keto form, **16**), prepared by condensation of



2-amino-6-chloro-4-pyrimidinol (**15**) with benzylmethylamine under the reaction conditions of Noell and Robins.[4] This compound in the monoheterocyclic series has direct steric and electronic analogy to 9-benzylguanine in the bicyclic series. 9-Benzylguanine was found to undergo annelation[2] with **2a** and sodium methoxide in methanol in the same direction as guanosine, leading to a product having exocyclic NH_2 and carbonyl groups disposed peri to one another. A similar direction of ring closure has now been found for compound **16** and the annelating reagent, leading to $C_{14}H_{14}N_6O$ product **17** which was fluorescent and exhibited two distinct signals in the ^1H NMR spectrum ($(CD_3)_2SO$) at δ 10.66 and 9.1, indicative of one intramolecularly bonded N–H to the *peri*-carbonyl and one free N–H, respectively. While compound **17** failed to undergo thermal rearrangement to the sterically cluttered

(4) Noell, C. W.; Robins, R. K. *J. Med. Chem.* **1962**, *5*, 558.

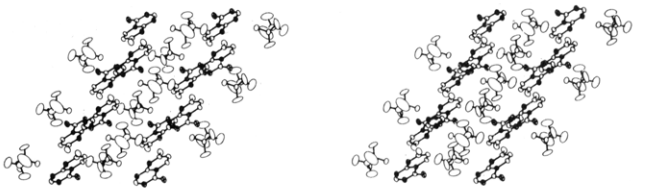
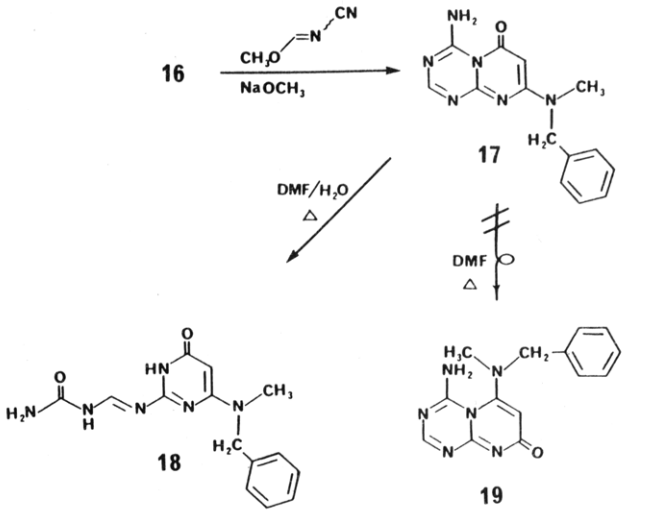


Figure 1. ORTEP perspective view of the unit cell of 4-amino-8,9-dihydro-8-oxopyrimido[1,2-*a*]-1,3,5-triazinium trifluoromethanesulfonate (**5a**) as determined by single-crystal X-ray examination. Hydrogen atoms are not included. Major points to be observed are the relation of the exocyclic N and O atoms to each other and the coplanarity of the heterocyclic units.

isomer **19**, it underwent triazine ring opening in hot DMF–H_2O to **18**, a product analogous to **10** and **14**.



Single-crystal X-ray crystallography of the triflate salt **5a**[5] of 4-amino-8-oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazine (**4a**) settled not only the structure of **5a** but, either directly or indirectly, the structures of all the other compounds reported in this paper. It also indicated the site of protonation of **4a**, providing the greatest resonance stabilization to the system (**5a**). The rotation of the triflate anion precluded our reaching a satisfactorily low *R* value in the structural analysis. Nevertheless, the goal of structure assignment (essentially **5a**) was accomplished, and the packing pattern (Figure 1) indicated alternate layers of protonated heterocycle and triflate anion.

In conclusion, we have (a) determined the structures of annelated heterocycles resulting from treatment of isocytosine and 6-substituted isocytosines with methyl *N*-cyanomethanimidate (**2a**) and sodium methoxide in methanol, (b) shown the substituent influence on the direction of cyclization and rearrangement, (c) indicated the involvement of the newly formed exocyclic NH_2 in further condensation with **2a**, (d) used this reaction for selective monomethylation of the exoheterocyclic NH_2, and (e) found a gentle method for the formation of triflate salts

(5) The crystallographic data were collected on a Syntex P2 Diffractometer by using Mo K_α radiation (λ 0.70169 Å) and a graphite monochrometer. The intensities of 2790 reflections were collected of which 1661 were considered observed at the 2.58 σ significance level in the 2θ range 3–55°. The structure was solved using MULTAN. Least-squares refinement varying positional and anisotropic thermal parameters for the non-hydrogen atoms reached convergence with $R = 0.0803$, where $R = \sum||F_o| - |F_c||/\sum|F_o|$ Further refinement was not achieved due to dissorder of the anion in the crystal lattice. No percent occupancy could be assigned to the oxygens or fluorines. Crystal data: $C_7H_6F_3N_5O_4S$, space group $P2_1/c$, a = 6.90 Å, b = 11.29 Å, c = 18.19 Å, β = 121°, v = 1214.4 Å^3, ρ_{calcd} (Z = 4) 1.36 (g cm^{-3}, $F(000)$ = 632.

of amines with trimethylsilyl trifluoromethanesulfonate.

Experimental Section

Melting points were determined on a Büchi or a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian EM-390, XL-200, or a Nicolet 360 Fourier Transform instrument operating at 90, 200, or 360 MHz, respectively, with tetramethylsilane as an internal standard. ^{13}C NMR spectra were obtained on a Varian XL-200 or a Nicolet 360 Fourier Transform instrument operating at 50 or 90 MHz, respectively, and chemical shifts are reported in parts per million from tetramethylsilane. Mass spectra were run on a Varian MAT CH-5 low-resolution spectrometer coupled with a 620i computer and STATOS recorder. The fast atom bombardment (FAB) mass spectra were run on a Varian 731 or 311A instrument. Ultraviolet spectra were obtained on a Beckman Acta MVI spectrophotometer. Microanalyses were performed by Josef Nemeth and his staff.

***N*-(8-Oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)-*N'*-cyanoformamidine, Sodium Salt (3a).** To a solution of isocytosine (1.33 g, 12 mmol) in methanolic sodium methoxide, prepared from sodium (0.55 g, 24 mmol) and methanol (25 mL), was added methyl *N*-cyanomethanimidate (5 g, 60 mmol) gradually through a hypodermic syringe during 5 min. The reaction mixture was stirred under nitrogen for 3 h at 20 °C, and the colorless solid that separated was collected after washing with dry methanol until it showed a single spot on silica gel and C_{18} reverse-phase TLC plates: mp >300 °C; yield 1.7 g (60%); ^{1}H NMR ($(CD_3)_2SO$) δ 9.35 (s, 0.25, formamidine CH), 8.7 (d, J = 8 Hz, 1, 6-H), 8.6 (s, 0.75, formamidine CH), 8.23 (s, 1, 2-H), 6.3 (d, J = 8 Hz, 1, 7-H); ^{13}C NMR ($(CD_3)_2SO$) *anti* δ 114.5 (C7), 116.8 (C≡N), 133.1 (C6), 154.0 (C9a), 157.3 (C8), 163.5 (C2), 170.3 (C4), 168.6 (formamidine-C); *syn* δ 114.5 (C7), 119.8 (C≡N), 133.4 (C6), 154.0 (C9a), 158.8 (C8), 163.1 (C2), 170.3 (C4), 173.5 (formamidine-C); IR (nujol) 3410 br, 3150 br, 2170 s, 1675 vs, 1640 vs, 1550 s, 1460 s, 1370 s, 1305 s, 1233 w, 1066 w, 987 w, 935 w, 838 m, 810 w, 795 m, 720 w, 608 w, 555 cm^{-1} w; mass spectrum (FAB, glycerol matrix), m/e (relative intensity) 214 (anion, 100); UV (H_2O) λ_{max} (ϵ) 334 nm (26 500), 256 (19 750), 222 (16 070). Anal. Calcd for $C_8H_4N_7ONa\cdot H_2O$: C, 37.65; H, 2.37; N, 38.43; Na, 9.01. Found: C, 37.94; H, 2.22; N, 38.68; Na, 8.92.

4-Amino-8-oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazine (4a). A well stirred suspension of the sodium salt **3a** (1 g) in dry methanol (25 mL) was treated with methanolic hydrogen chloride until it was distinctly acidic (pH ~2). Immediately, a clear solution resulted and the clearing was followed by the separation of the hydrochloride salt of **4a** as a colorless crystalline solid. Stirring was continued for 30 min at room temperature, and then the solid was collected and washed with dry methanol.

The crude hydrochloride (0.85 g) was dissolved in a minimum volume of water and neutralized carefully with 5% aqueous $NaHCO_3$. The colorless solid that resulted (0.50 g, 72%) was collected after washing with anhydrous methanol. An analytical sample was obtained by rapid crystallization from water (long standing in water led to decomposition): mp >220 °C dec; ^{1}H NMR (TFA) δ 10.35 (br, 2, NH_2), 8.62 (s, 1, 2-H), 8.6 (d, J = 7 Hz, 1, 6-H), 6.9 (d, J = 7 Hz, 1, 7-H); IR (KBr) 3220 br, 3080 br, 1700 sh, 1645 vs, 1480 s, 1435 m, 1385 m, 1315 m, 1232 m, 828 w, 788 cm^{-1} w; mass spectrum (70 eV) m/e (relative intensity) 163 (M^+, 100); qual UV (EtOH) λ_{max} 302, 242 nm. Anal. Calcd for $C_6H_5N_5O$: C, 44.17; H, 3.09; N, 42.93. Found: C, 44.03; H, 2.99; N, 42.65.

4-Amino-8,9-dihydro-8-oxopyrimido[1,2-*a*]-1,3,5-triazinium Trifluoromethanesulfonate (5a). To a stirred suspension of **4a** (0.815 g, 5 mmol) in dry acetonitrile (20 mL) was added trimethylsilyl trifluoromethanesulfonate (1.33 g, 6 mmol) through a hypodermic syringe. The reaction mixture, which turned into a clear solution immediately, was evaporated to dryness after a few minutes on a rotary evaporator at <35 °C. The residual colorless solid was treated with ethyl acetate, which dissolved it; then a crystalline salt was deposited. The product (1.3 g, 83%) was collected after washing with ethyl acetate and was crystallized from a solution of ethyl acetate–ethanol–petroleum ether as thick colorless prisms: mp 212–215 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 10.49 (br, 3, NH's), 8.59 (s, 1, 2-H), 8.44 (d, J = 8 Hz, 1, 6-H), 6.93 (d, J = 8 Hz, 1, 7-H); ^{13}C NMR ($(CD_3)_2SO$) δ 116.1 (C7), 121.5 (CF_3), 134.5 (C6), 153.3 (C9a), 154.4 (C4), 159.5 (C8), 166.8 (C2); IR (nujol) 3325 br, 3150 br, 1710 sh, 1690 vs, 1530 s, 1290 m, 1225 m, 1150 m, 1025 s, 960 w, 840 w, 780 w, 640 cm^{-1} s; UV (EtOH) λ_{max} (ϵ) 298 nm (6820), 228 (16 800), 216 (16 000); mass spectrum (FAB, glycerol matrix), m/e (relative intensity) 164 (cation, 100) 149 (anion, 100). Anal. Calcd for $C_7H_6F_3N_5O_4S$: C, 26.74; H, 1.96; N, 22.22; F, 18.27; S, 10.20. Found: C, 26.75; H, 1.94; N, 21.76; F, 17.86; S, 10.06.

Reconversion of Compound 4a to 3a. To a solution of **4a** (0.49 g, 3 mmol) and sodium methoxide prepared from sodium (0.076 g, 3.3 mmol) in methanol (20 mL) was added methyl *N*-cyanomethanimidate (0.66 g, 7.5 mmol). The reaction mixture was stirred under nitrogen at room temperature for 30 min, and the product that separated as a colorless solid was collected after washing with methanol (0.6 g, 84%). ^{1}H NMR, UV, IR, and mass spectra were identical with those of **3a** prepared from isocytosine directly.

***N*-(8-Oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)-*N'*-cyanodeuterioformamidine, Sodium Salt (3c).** This compound was prepared from **4a** following the procedure described for the conversion of **4a** into **3a**, but with **2b** in place of **2a**, and was characterized by the UV and IR spectra (see **3a**). In the ^{1}H NMR spectrum ($(CD_3)_2SO$), the singlet signal at δ 8.6 (formamidine CH) had less than 5% the intensity of that at δ 8.23 (triazine ring CH).

***N*-([2-^{2}H]-8-Oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)-*N'*-cyanodeuterioformamidine, Sodium Salt (3b).** The reaction of isocytosine (1.33 g, 12 mmol) with **2b** (5 g, 60 mmol) in methanolic sodium methoxide obtained from sodium (0.55 g, 24 mmol) and dry methanol (25 mL) under the conditions described for the preparation of **3a** gave 1.7 g (60%) of **3b**, characterized by the UV and IR spectra (see **3a**). In the ^{1}H NMR spectrum, the intensity of both the singlet signals at δ 8.6 and 8.23 due to formamidine and triazine CH's, respectively, was greatly reduced; mass spectrum (FAB, glycerol matrix), m/e (relative intensity) 216 (anion, 100).

[2-^{2}H]-4-Amino-8-oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazine (4b). Treatment of the sodium salt **3b** (0.5 g) in methanol (15 mL) with methanolic hydrogen chloride and subsequent workup as described for the preparation of **4a** gave 0.25 g (72%) of **4b**, characterized by the UV and IR spectra (see **4a**). The singlet signal in the ^{1}H NMR spectrum at δ 8.62 corresponding to the triazine CH (see **4a**) was of negligible intensity.

[2-^{2}H]-4-Amino-8,9-dihydro-8-oxopyrimido[1,2-*a*]-1,3,5-triazinium trifluoromethanesulfonate (5b) was prepared from **4b** and trimethylsilyl trifluoromethanesulfonate following the procedure described for the preparation of **5a** and was characterized by melting point and UV and IR spectra (see **5a**). The singlet signal in the ^{1}H NMR spectrum ($(CD_3)_2SO$) at δ 8.59 corresponding to triazine CH was of negligible intensity. The signal at δ 166.8 in the ^{13}C NMR spectrum of **5a** showed single-bond coupling to 2-H whereas the signal for **5b** was a singlet. The long-range couplings with 2-H in **5a** which disappeared in **5b** were used in making the ^{13}C NMR assignments (see above).

***N*-([2-^{2}H]-8-Oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)-*N'*-cyanoformamidine, Sodium Salt (3d).** Reaction of **4b** (0.49 g, 3 mmol) with **2a** (0.66 g, 7.5 mmol) in methanolic sodium methoxide prepared from sodium (0.076 g, 3.3 mmol) and methanol (20 mL) and subsequent workup gave 0.6 g (84%) of **3d**, characterized by UV and IR spectra (see **3a**). In the ^{1}H NMR spectrum the singlet signal at δ 8.23 (triazine CH) had less than 5% the intensity of that at δ 8.6 (formamidine CH).

(8-Oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)guanidine (6a). Method A. A suspension of **3a** (0.254 g, 1 mmol) and cyanamide (0.84 g, 2 mmol) in 2-propanol (10 mL) was heated under reflux in an oil bath for 24 h. The solid that separated on cooling the reaction mixture was collected after washing with anhydrous methanol. Repeated crystallization of the product from DMF–H_2O and finally from DMF gave 0.054 g (26%) of **6a**, mp >300 °C. UV, IR, ^{1}H NMR, and mass spectra were identical with those of an authentic sample.[3]

Method B. To a solution of **4a** (0.163 g, 1 mmol) in methanolic sodium methoxide prepared from sodium (0.023 g, 1 mmol) and methanol (6 mL) was added cyanamide (0.105 g, 2.5 mmol). The reaction mixture was heated under reflux in a nitrogen atmosphere for 24 h. The sodium salt of **6a** that separated was collected,

dissolved in a minimum volume of water and acidified to precipitate **6a** (0.025 g, 12%), which was collected after washing with methanol. The ^1H NMR, UV, and IR spectra were identical with those of the sample prepared by **Method A**.

***N*-(8-Oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazin-4-yl)-*N*-methyl-*N*-cyanoformamidine (7).** A suspension of **3a** (2 g, 7.8 mmol) in dry DMF (10 mL) was treated with methyl iodide (1.5 g, 10.6 mmol) at 20 °C, and the reaction mixture was stirred for 16 h under nitrogen. The resulting pale yellow solid was collected, washed with methanol, and recrystallized from DMF–ethyl acetate as pale yellow prisms (1.1 g, 62%): mp 231–232 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 9.6 (s, 0.25, formamidine CH), 8.82 (d, J = 8 Hz, 1, 6-H), 8.8 (s, 1, 2-H), 8.63 (s, 0.75, formamidine CH), 6.84 (d, J = 8 Hz, 1, 7-H); ^{13}C NMR ($(CD_3)_2SO$) *anti* δ 33.5 (N-CH_3), 117.6 (C7), 119.5 (C≡N), 137.8 (C6), 157.5 (C9a), 159.6 (C8), 162.4 (C4), 169.2 (C2), 172.9 (formamidine-C); *syn* δ 33.5 (N-CH_3), 117.2 (C7), 121.0 (C≡N), 138.4 (C6), 157.5 (C9a), 159.6 (C8), 162.5 (C4), 169.1 (C2), 178.7 (formamidine-C); IR (nujol) 2180 m, 1730 vs, 1600 vs, 1350 m, 1270 m, 1240 m, 1230 m, 1130 m, 1085 m, 950 m, 930 m, 870 m, 838 m, 790 s, 630 w, 610 cm^{-1} m; mass spectrum (70 eV), m/e (relative intensity) 229 (M^+, 100); UV (pH 11) λ_{max} (ϵ) 360 nm (6500), 288 (14 800), 221 (24 000). Anal. Calcd for $C_9H_7N_7O$: C, 47.16; H, 3.08; N, 42.78. Found: C, 46.91; H, 2.99; N, 42.90.

4-(Methylamino)-8-oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazine (8). To a well stirred suspension of **7** (1.07 g) in dry methanol (20 mL) at 20 °C was added methanolic hydrogen chloride until the reaction mixture was distinctly acidic. The product that separated as crystalline hydrochloride was collected after 1 h and washed with anhydrous methanol. The crude hydrochloride salt was dissolved in a minimum volume of water and neutralized with 5% aqueous $NaHCO_3$ to precipitate the product as a colorless solid. It was collected, washed with water, and recrystallized from DMF as pale yellow crystals (0.6 g, 73%): mp 182–183 °C; ^1H NMR ($(CD_3)_2SO$) δ 8.6 (d, J = 8 Hz, 1, 6-H), 7.87 (s, 1, 2-H), 6.35 (d, J = 8 Hz, 1, 7-H), 3.37 (s, 3, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 28.0 (CH_3), 108.8 (C7), 134.2 (C6), 150.5 (C9a), 152.7 (C4), 158.9 (C8), 160 (C2); IR (nujol) 3230 m, 1710 vs, 1650 vs, 1560 s, 1500 vs, 1260 m, 1185 vs, 1110 vs, 1040 s, 920 vs, 890 w, 868 m, 835 s, 773 vs, 725 w, 673 m, 623 cm^{-1} w; mass spectrum (70 eV), m/e (relative intensity) 177 (M^+, 100); UV (H_2O) λ_{max} (ϵ) 312 nm (6550), 219 (25 260). Anal. Calcd for $C_7H_7N_5O$: C, 47.45; H, 3.98; N, 39.53. Found: C, 47.27; H, 3.89; N, 39.80.

8,9-Dihydro-4-(methylamino)-8-oxopyrimido[1,2-*a*]-1,3,5-triazinium Trifluoromethanesulfonate (9). Treatment of **8** (0.2 g, 1.13 mmol) with trimethylsilyl trifluoromethanesulfonate (0.3 g, 1.35 mmol) in anhydrous acetonitrile (4 mL) and subsequent workup as described earlier gave 0.27 g (73%) of the triflate salt which crystallized from a solution of ethyl acetate–methanol–petroleum ether as colorless prisms: mp 179–180 °C; ^1H NMR ($(CD_3)_2SO$) δ 10.5 (br, 2, NH's), 8.65 (s, 1, 2-H), 8.45 (d, J = 8 Hz, 1, 6-H), 7.05 (d, J = 8 Hz, 1, 7-H), 3.52 (s, 3, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 29.9 (CH_3), 114.2 (C7), 121 (CF_3), 133.5 (C6), 152.7 (C9a), 154.5 (C4), 157.8 (C8), 166.2 (C2); IR (nujol) 3360, 3300 (NH), 1690 (C=O) cm^{-1}; mass spectrum (FAB, glycerol matrix), m/e (relative intensity) 178 (cation, 100), 149 (anion, 100); UV (H_2O) λ_{max} (ϵ) 298 nm (8190), 218 (24 390). Anal. Calcd for $C_8H_8F_3N_5O_4S$: C, 29.36; H, 2.46; N, 21.40; F, 17.42; S, 9.78. Found: C, 29.49; H, 2.42; N, 21.26; F, 16.90; S, 10.02.

***N*-Carbamoyl-*N'*-(3,4-dihydro-4-oxopyrimid-2-yl)formamidine (10).** To a nearly boiling solution of **4a** (0.8 g) in DMF (50 mL) was added water (5 mL). After heating the reaction mixture at boiling for a few minutes, the colorless solid that separated was collected. An analytical sample was obtained by recrystallization from DMF (0.5 g, 57%): mp 237–240 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 8.8 (s, 1, formamidine CH), 7.62 (d, J = 8 Hz, 1, 6-H), 5.73 (d, J = 8 Hz, 1, 5-H); mass spectrum (70 eV), m/e (relative intensity) 181 (M^+, 42), 180 ($M^+ - 1$, 9), 163 ($M^+ - 18$, 33), 153 ($M^+ - 28$, 78), 138 ($M^+ - 43$, 9), 137 ($M^+ - 44$, 9), 136 ($M^+ - 45$, 25), 135 ($M^+ - 46$, 23), 111 ($M^+ - 70$, 53), 95 ($M^+ - 86$, 31), 85 ($M^+ - 96$, 27), 70 ($M^+ - 111$, 30), 68 ($M^+ - 113$, 50), 43 ($M^+ - 138$, 100). Anal. Calcd for $C_6H_7N_5O_2$: C, 39.78; H, 3.89; N, 38.66. Found: C, 39.43; H, 3.58; N, 39.01.

4-Amino-8-methyl-6-oxo-6*H*-pyrimido[1,2-*a*]-1,3,5-triazine (12). To a stirred solution of sodium methoxide prepared from sodium (1.84 g, 80 mmol) and dry methanol (160 mL) was added 2-amino-6-methyl-4-pyrimidinol (**11**) (5.0 g, 40 mmol). The clear solution thus formed was treated with methyl *N*-cyanomethanimidate (13.04 g, 160 mmol). After the reaction mixture was stirred at 20 °C for 16 h, the product that separated as the sodium salt was collected. The sodium salt was dissolved in DMF (40 mL) at room temperature, and the solution was diluted with 20 mL of water and then acidified with acetic acid. The product that separated as a colorless crystalline solid was collected, washed with methanol, and air-dried (5.6 g, 79%). An analytical sample was obtained by crystallization from DMF–H_2O as thick, colorless plates: mp 215–217 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 10.57 (s, 1, NH exchangeable with D_2O), 9.73 (s, 1, NH exchangeable with D_2O), 8.39 (s, 1, 2-H), 6.41 (s, 1, 7-H), 2.5 (s, 3, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 24.36 (CH_3), 107.9 (C7), 153.6 (C4 or C9a), 158.2 (C9a or C4), 163.25 (C6), 163.6 (C2), 167.89 (C8); IR (KBr) 3245 m, 1680 vs, 1650 vs, 1580 s, 1550 s, 1360 s, 1170 m, 1003 m, 875 w, 835 m, 800 m, 730 w, 700 cm^{-1} m; mass spectrum (70 eV), m/e (relative intensity) 177 (M^+, 100); UV (EtOH) λ_{max} (ϵ) 342 nm (7720), 288 (8860), 246 sh (4050), 226 (6455). Anal. Calcd for $C_7H_7N_5O$: C, 47.45; H, 3.98; N, 39.53. Found: C, 47.13; H, 3.79; N, 39.25.

4-Amino-6-methyl-8-oxo-8*H*-pyrimido[1,2-*a*]-1,3,5-triazine (13). A suspension of 4-amino-8-methyl-6-oxo-6*H*-pyrimido-[1,2-*a*]-1,3,5-triazine (0.6 g) in dry DMF (15 mL) was heated at 120 °C in an oil bath and the rearrangement was monitored by TLC. After 2 h (when complete rearrangement had occurred), the reaction mixture was cooled and the pale yellow solid that separated was collected. Recrystallization from dry DMF gave an analytical sample (0.48 g, 80%): mp 279–280 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 9.56 (s, 1, 2-H), 8.58 (br s, 2, NH_2), 6.18 (s, 1, 7-H), 2.49 (s, 3, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 25.3 (CH_3), 100.65 (C7), 150.9 (C2), 152.7 (C6), 158.1 (C8), 163.0 (C9a or C4), 170.3 (C4 or C9a); IR (KBr) 3280 w, 3100 br, 1720 vs, 1680 vs, 1645 vs, 1570 m, 1500 vs, 1400 m, 1300 m, 1170 m, 930 m, 840 w, 740 cm^{-1} w; mass spectrum (70 eV), m/e (relative intensity) 177 (M^+, 100); UV (EtOH) λ_{max} (ϵ) 307 nm (10 275), 248 (12 625). Anal. Calcd for $C_7H_7N_5O$: C, 47.45; H, 3.98, N, 39.53. Found: C, 47.26; H, 3.82; N, 39.22.

***N*-Carbamoyl-*N'*-(3,4-dihydro-6-methyl-4-oxopyrimid-2-yl)formamidine (14).** Prepared from **13** following the same procedure described for **10**. Yield 77%; mp 238–240 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 8.85 (s, 1, formamidine CH), 5.66 (s, 1, 5-H), 2.13 (s, 3, CH_3), 8.28, 10.4 and 11.0 (br, NH's); mass spectrum (70 eV), m/e (relative intensity) 196 ($M^+ + 1$, 6.5), 195 (M^+, 42), 194 ($M^+ - 1$, 6), 177 ($M^+ - 18$, 3.5), 176 ($M^+ - 19$, 6.4), 169 ($M^+ - 27$, 9), 167 ($M^+ - 28$, 100), 152 ($M^+ - 43$, 15), 151 ($M^+ - 44$, 13), 150 ($M^+ - 45$, 50), 126 ($M^+ - 69$, 12), 125 ($M^+ - 70$, 71), 113 ($M^+ - 82$, 17), 109 ($M^+ - 86$, 22), 97 ($M^+ - 98$, 21), 96 ($M^+ - 99$, 12), 85 ($M^+ - 110$, 50), 84 ($M^+ - 111$, 34), 68 ($M^+ - 127$, 52), 43 ($M^+ - 152$, 95). Anal. Calcd for $C_7H_9N_5O_2$: C, 43.07; H, 4.65; N, 35.89. Found: C, 43.02; H, 4.76; N, 36.17.

The identical product **14** was obtained from **12** under the same reaction conditions in about the same yield (77%).

2-Amino-6-(benzylmethylamino)-4-pyrimidinol (Keto Form) (16). A mixture of 2-amino-6-chloro-4-pyrimidinol (**15**) (6.54 g, 40 mmol), and benzylmethylamine (14.52 g, 120 mmol) in 2-ethoxyethanol (30 mL) was heated under reflux for 5 h. The hot reaction mixture was poured into 150 mL of ice water, and the solid that separated was collected after 30 min and washed with water. An analytical sample was obtained by crystallization from DMF–H_2O as pale yellow shining plates (8.5 g, 92%): mp 287–288 °C; ^1H NMR ($(CD_3)_2SO$) δ 9.87 (br, 1, NH), 7.3 (m, 5, phenyl protons), 6.2 (br, 2, NH_2), 4.6 (s, 2, CH_2), 4.58 (s, 1, 5-H), 2.87 (s, 3, CH_3); IR (KBr) 3425, 2080 (NH_2), 1620 (C=O) cm^{-1}; UV (pH 11) λ_{max} (ϵ) 268 nm (15 870); (pH 1) λ_{max} (ϵ) 274 (23 270); mass spectrum (70 eV), m/e (relative intensity) 230 (M^+, 100). Anal. Calcd for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33. Found: C, 62.71; H, 6.02; N, 24.45.

4-Amino-8-(benzylmethylamino)-6-oxo-6*H*-pyrimido[1,2-*a*]-1,3,5-triazine (17). 2-Amino-6-(benzylmethylamino)-4-pyrimidinol (**16**) (2.3 g, 10 mmol) was dissolved by stirring in a solution of sodium methoxide prepared from sodium (0.46 g, 20 mmol) and dry methanol (50 mL) at 20 °C. Methyl *N*-cyanomethanimidate (3.16 g, 40 mmol) was then introduced and the reaction mixture was stirred for 16 h. The sodium salt that separated was washed with anhydrous methanol and dissolved in 40 mL of DMF at 20 °C. The solution was diluted with 20 mL

of water and acidified with acetic acid to precipitate the product as colorless solid. An analytical sample was obtained by crystallization from DMF–CH_3CN (2.15 g, 76%): mp 215 °C; ^1H NMR ($(CD_3)_2SO$) δ 10.66 (s, 1, NH, exchangeable with D_2O), 9.1 (s, 1, NH, exchangeable with D_2O), 7.97 (s, 1, 2-H), 7.26 (m, 5, phenyl protons), 5.2 (s, 1, 7-H), 4.76 (s, 2, CH_2), 3.0 (s, 3, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 36.1 (CH_3), 51.5 (CH_2), 80.7 (C7), 127.4, 128.8 (aromatic), 152.7 (C4 or C9a), 157.9 (C9a or C4), 161.5 (C6 or C8), 163.25 (C8 or C6), 163.6 (C2); IR (KBr) 3225 m, 3000 m, 1700 vs, 1580 vs, 1370 s, 1320 m, 1230 m, 1170 m, 1100 m, 1025 m, 880 s, 800 s, 750 w, 700 w, 490 cm^{-1} m; mass spectrum (70 eV), *m/e* (relative intensity) 282 (M^+, 100); UV (EtOH) λ_{max} (ϵ) 330 nm (4800), 275 (31 355), 262 (31 355). Anal. Calcd for $C_{14}H_{14}N_6O$: C, 59.56; H, 5.00; N, 29.77. Found: C, 59.76; H, 5.02; N, 29.41.

***N*-Carbamoyl-*N'*-[6-(benzylmethylamino)-3,4-dihydro-4-oxopyrimid-2-yl]formamidine (18)** was prepared from **17** in 91% yield following the procedure described for **10**: mp 239–241 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 9.08 (s, 1, formamidine CH), 7.23 (br, 5, aromatic CH's), 5.0 (s, 1, 5-H), 4.7 (m, 2, CH_2), 2.96 (s, 3, CH_3), 8.0, 8.7, 10.6 (br, NH's); mass spectrum (70 eV), *m/e* (relative intensity) 301 ($M^+ + 1$, 7.5), 300 (M^+, 41), 299 ($M^+ - 1$, 4), 283 ($M^+ - 17$, 10), 282 ($M^+ - 18$, 48), 267 ($M^+ - 33$, 47), 257 ($M^+ - 43$, 9), 254 ($M^+ - 46$, 10), 253 ($M^+ - 47$, 38), 240 ($M^+ - 60$, 17), 215 ($M^+ - 85$, 12), 191 ($M^+ - 109$, 22), 162 ($M^+ - 148$, 30), 120 ($M^+ - 180$, 55). Anal. Calcd for $C_{14}H_{16}N_6O_2$: C, 55.99; H, 5.37; N, 27.99. Found: C, 55.67; H, 5.51; N, 27.65.

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Registry No. **1**, 108-53-2; **2a**, 51688-22-3; **2b**, 96427-28-0; *syn*-**3a**, 96575-94-9; *anti*-**3a**, 96575-69-8; **3b**, 96575-75-6; **3c**, 96575-74-5; **3d**, 96575-79-0; **4a**, 96575-70-1; **4a**·HCl, 96575-71-2; **4b**, 96575-76-7; **5a**, 96575-73-4; **5b**, 96575-78-9; **6a**, 76299-85-9; **6a**·Na, 96575-80-3; *syn*-**7**, 96575-95-0; *anti*-**7**, 96575-81-4; **8**, 96575-82-5; **8**·HCl, 96575-83-6; **9**, 96575-93-8; **10**, 96575-84-7; **11**, 3977-29-5; **12**, 96575-85-8; **12**·Na, 96575-86-9; **13**, 96575-87-0; **14**, 96575-88-1; **15**, 1194-21-4; **16**, 37409-94-2; **17**, 96575-89-2; **17**·Na, 96575-90-5; **18**, 96575-91-6; $CF_3SO_2OSi(CH_3)_3$, 27607-77-8; H_2NCN, 420-04-2; $C_6H_5CH_2NHCH_3$, 103-67-3.

Nucleoside Annelating Reagents: *N*-(*tert*-Butoxycarbonyl)-2-bromoacetamide and 2-Chloroketene Diethyl Acetal

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The title reagents undergo cyclocondensation reactions with the amidine-like moiety of tri-*O*-acetyladenosine and cytidine (and related compounds) to form a new five-membered ring (etheno bridging), substituted with a (*tert*-butoxycarbonyl)amino or an ethoxy function. Some of the products exhibit useful fluorescence properties. Removal of the *tert*-butoxycarbonyl group with standard conditions gives the corresponding, somewhat unstable amino compounds which can be conveniently characterized as the corresponding *N*-acetyl derivatives. The reagents introducing additional substitution on the etheno bridge, with enhanced fluorescence, also suggest the possibility of cross-linking functionalization.

The discovery that the reaction of adenosine and its derivatives with chloroacetaldehyde in aqueous solution leads to fluorescent products that can be used for the spectroscopic investigation of coenzyme–enzyme interactions and nucleic acid–protein interactions has contributed immensely to our present knowledge of how these biological macromolecules interact.[1] Our continuing program of synthesizing modified nucleosides that provide defined dimensional alterations of conventional enzyme substrates and/or highly fluorescent analogues has led us to investigate the reactions of 2-bromoacetamide carbamates (**1a–c**) and 2-chloroketene diethyl acetal (**2**) with representative adenosine and cytidine derivatives.

1a R = *t*-Bu
b R = Me
c R = CH_2Ph

2

(1) (a) Leonard, N. J. *CRC Crit. Rev. Biochem.* **1984**, *15*, 125 and references therein. [Note: interchange pages 157 and 158.] (b) Leonard, N. J. *Acc. Chem. Res.* **1982**, *15*, 128 and references therein.

Reactions with 2-Bromoacetamide Carbamates. *N*-(*tert*-Butoxycarbonyl)-2-bromoacetamide (**1a**) is a stable colorless crystalline solid that is readily prepared in multigram quantities by the action of oxalyl chloride on 2-bromoacetamide and subsequent quenching of the putative *N*-carbonyl chloride or acylisocyanate intermediate with *tert*-butyl alcohol.[2,3] Benzyl alcohol or methanol may also be used as the quenching agent, thus making it possible to prepare reagents in which the latent amine is masked by carboxylate groups which can be removed under acidic (*t*-Bu, **1a**), basic (Me, **1b**) or reductive ($PhCH_2$, **1c**) conditions.[4] In practice, we discovered that all three reagents **1a–c** will undergo a cyclocondensation reaction

(2) This procedure is a modification of that described in ref 3a for the preparation of $ClCH_2CONHCO_2CH_3$.

(3) (a) Bochis, R. J.; Dybas, R. A.; Eskola, P.; Kulsa, P.; Linn, B. O.; Lusi, A.; Meitzner, E. P.; Milkowski, J.; Mrozik, H.; Olen, L. E.; Peterson, L. H.; Tolman, R. L.; Wagner, A. F.; Waksmunski, F. S.; Egerton, J. R.; Ostlind, D. A. *J. Med. Chem.* **1978**, *21*, 235. See also ref 3b and 3c: (b) Bochis, R. J.; Olen, L. E.; Fisher, M. H.; Reamer, R. A.; Wilks, G.; Taylor, J. E.; Olson, G. *Ibid.* **1981**, *24*, 1483. (c) Bochis, R. J.; et al. *Ibid.* **1981**, *24*, 1518. See also: (d) Speziale, A. J.; Smith, L. B.; Fedder, J. E. *J. Org. Chem.* **1965**, *30*, 4306.

(4) Greene, T. W. "Protective Groups in Organic Synthesis"; Wiley-Interscience: New York, 1981; p 218.

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