H, 4.82; N, 6.14; P, 13.60. Found: C, 47.54, H, 5.00; N, 6.06; P, 13.90.

Registry No. **4a**, 2510-86-3; **4b**, 5076-68-6; **5a**, 69646-14-6; **5b**, 80615-41-4; **5c**, 53104-46-4; **5d** anilinium salt, 91633-06-6; **6a**, 91633-07-7; **6b**, 91633-08-8; **7a**, 91633-09-9; **7b**, 91633-10-2; **7c** anilinium salt, 91633-12-4; **7d** anilinium salt, 91633-14-6; **8**, 57246-14-7; **9**, 37521-98-5; **10a**, 91633-15-7; **10b**, 91633-16-8; **12a**, 91633-17-9; **12b**, 91633-18-0; $(i\text{-Pr})_2NH$, 108-18-9; BuLi, 109-72-8; $(EtO)_2POH$, 762-04-9; $(i\text{-Pr})_2NLi$, 4111-54-0; sodium iodide, 7681-82-5; chlorotrimethylsilane, 75-77-4; hydroquinone, 123-31-9; catechol, 120-80-9.

Structural Alteration of Nucleic Acid Bases by Bromomalonaldehyde[1]

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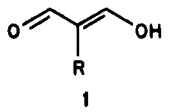
Bromomalonaldehyde (BMDA), prepared by bromination of malonaldehyde with elemental bromine, has been employed to modify a number of nucleic acid bases. These reactions transform pyrimidine and purine bases into modified systems containing etheno and etheno carboxaldehyde moieties, among other products. The structures of these modified bases were established by UV, mass spectral, and high-field NMR data. Fluorescence emission data for some of the adducts are of significance. The general mechanism of modification is discussed.

In the course of some work in our laboratory on the behavior of the ubiquitous natural compound malonaldehyde (MDA, 1, R = H) toward biomolecules,[2,3] we needed some information on the comparative reactivity of 2-substituted malonaldehydes. In particular, the structural nature of modification of nucleic acid bases was of interest in this work. The chemical modification of the base moiety of nucleic acids is also of synthetic and biological interest. For example, 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine are both able to substitute for adenine nucleotides in some biological systems.[4] The observation that some modified bases are fluorescent has generated considerable interest in their use as biological probes in the structure and mechanism of action of nucleic acids and some enzymes and coenzymes.[4-15] Ethenoadenine derivatives exhibit fluorescence emission in the range of 410 nm with quantum yields of the order of 0.56. However, the use of ethenocytidine derivatives as biological probes has been limited by their inappropriate fluorescence emission wavelengths and low quantum yields.[4,16] The search therefore continues for cytidine derivatives which possess fluorescence characteristics that allow for ready detection in biological systems. In this paper we report on the interesting modifications of a number of purine and pyrimidine bases by bromomalonaldehyde (BMDA).



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Results and Discussion

Although the chemistry of halogenated malonaldehydes (1, R = Cl, Br, I, F) has been explored to some extent mainly for the synthesis of heterocycles,[17-20] little is known about the reactivity of these compounds toward nucleic acid bases. Bromomalonaldehyde can be prepared by bromination of MDA with elemental bromine as described by Trofimenko.[21] Modification of the pyrimidine and purine bases was accomplished by stirring the substrate in an aqueous acidic medium with BMDA at 60 °C. The reactions were followed by UV spectral methods and terminated when the absorption for the bathochromically shifted product peak had maximized. Separation and purification of the modified bases and derivatives were achieved by preparative-layer chromatography on silica gel or by HPLC on Amberlite XAD-4 resin.

The reaction of BMDA with cytidine afforded a yellow crystalline compound in 43% yield (mp 202–204 °C) with UV absorption shifted to 325 nm (ϵ 10 500) which is indicative of more extended conjugation. The molecular ion in its mass spectrum at m/z 295 suggested the formation of a 1:1 adduct in which bromine was not present. The 360-MHz ^1H NMR spectrum in Me_2SO-d_6 showed the presence of 4 non-ribosyl protons at δ 6.91 (d, J = 7.8 Hz), 8.12 (d, J = 7.8 Hz), 8.16 (s), and 10.57 (s). Three additional carbon resonances (compared to cytidine) at δ 130.0, 139.6, and 181.0 suggesting the presence of a bicyclic base carrying an exocyclic vinylogous amide carbonyl group were present in its high-field ^{13}C NMR spectrum in

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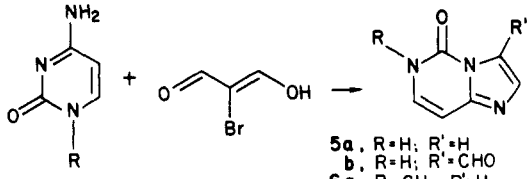
Me_2SO-d_6. Taken together, the data suggested that the modified base was 6-(β-D-ribofuranosyl)-3-formylimidazo[1,2-*c*]pyrimidin-5(6*H*)-one **7**. Its formation can

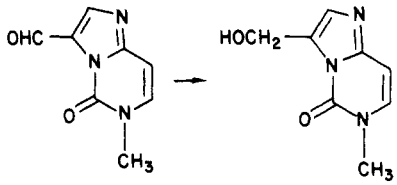


2, R=H
3, R=CH_3
4, R=β-D-ribofuranosyl

5a, R=H; R'=H
b, R=H; R'=CHO
6a, R=CH_3; R'=H
b, R=CH_3; R'=CHO
c, R=CH_3; R'=CH=CBrCHO
7 R=β-D-ribofuranosyl; R'=CHO

be suggested as occurring through initial development of a Schiff base and subsequent nucleophilic displacement of bromide ion by N-3 of cytidine to give the observed bicyclic product. Interestingly, compound **7** was found to exhibit fluorescence at 398 nm when excited at 357 nm, and this is in contrast to 3,N^4-ethenocytidine which exhibits fluorescence at 340 nm when excited at 288 nm.[4]

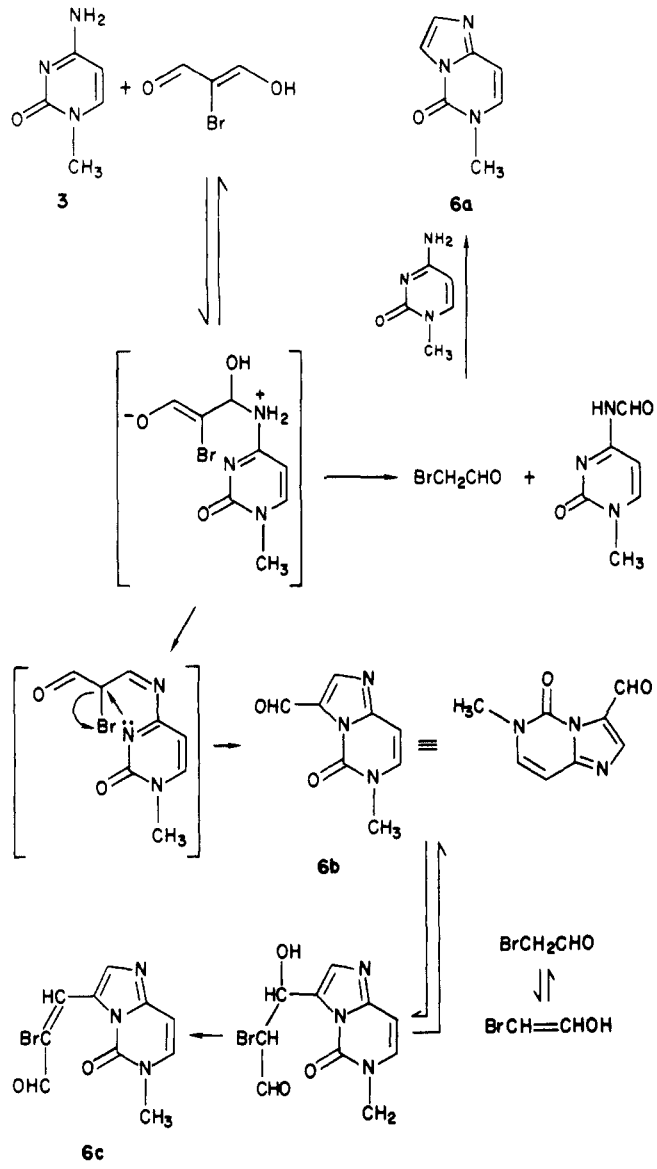
We also examined the reaction of BMDA with alkylated bases. Because of the ease of handling these base derivatives and their products, this was considered an attractive approach to these base modifications. 1-Methylcytosine can be conveniently prepared from cytosine by reaction with *N*,*N*-dimethylformamide dimethyl acetal and subsequent hydrolysis of the intermediate imine with ammonium hydroxide.[22] When 1-methylcytosine was treated with BMDA, three modified bases were isolated after preparative-layer chromatography. The first product, (mp 181–182 °C) isolated in 27% yield, was 6-methyl-3-formylimidazo[1,2-*c*]pyrimidin-5(6*H*)-one (ethenomethylcytosinecarboxaldehyde) (**6b**) whose spectral



6b **8**

characteristics were similar to those of **7**. The structure of **6b** was confirmed further by its facile reduction with sodium borohydride to compound **8**. The second product (21%), identified as 6-methylimidazo[1,2-*c*]pyrimidin-5(6*H*)-one (ethenomethylcytosine) (**6a**), was a decarbonylated product which had been reported previously.[23] The third modified base, mp 254–256 °C, was isolated in 7% yield. The mass spectral data (M^+ 283 and 281) and elemental analysis suggested a molecular formula of $C_{10}H_8N_3O_2Br$. A large shift to longer wavelength (λ_{max} 371 nm, ϵ 17 200) compared to methylcytosine was present in its UV spectrum in ethanol. It showed marked fluorescence at 452 nm when excited at 398 nm. The 360-MHz ^1H NMR spectrum in Me_2SO-d_6 showed three protons as singlets at δ 9.44, 9.35, and 8.73, two proton doublets at δ 7.76 and 6.81 (J = 7.7 Hz), and a singlet at δ 3.56 integrating for three protons. The total data are best accommodated by structure **6c**. A plausible mechanism for the formation of these products is shown in Scheme I. The formation of **6a** apparently results from the reaction of **3** with bromoacetaldehyde produced by the cleavage of the initial adduct resulting from **3** and BMDA. In support of this were the observations that BMDA did not produce

Scheme I

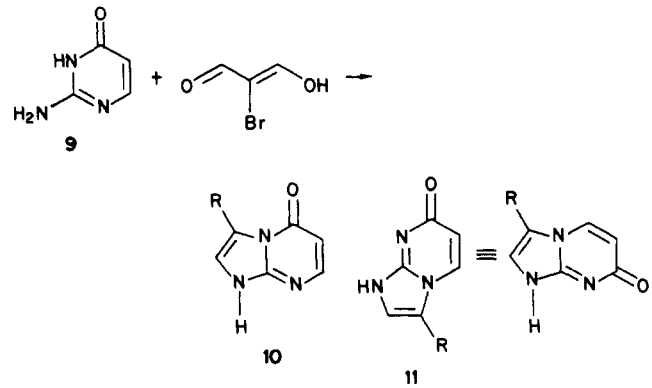


6c

bromoacetaldehyde in the absence of **3** and that of **6a** could not be produced by the thermal decarbonylation of **6b**. Compound **6b** (and not **6a**), however, is the likely precursor of **6c** through reaction with bromoacetaldehyde.

Cytosine (**2**) was also found to react with BMDA to give the imidazopyrimidinone **5a** (13% yield) and a trace amount of the formyl analogue **5b** which was detected by mass spectrometry.

The reaction of isocytosine (**9**) with BMDA was also examined. Two ring systems, **10** and **11**, are possible depending on the regiochemistry of adduct formation. The



9 **10** **11**

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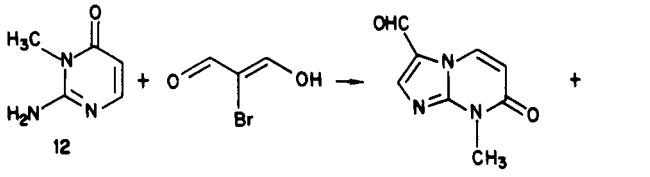
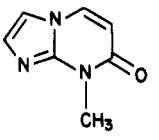
modified heterocyclic ring **10** would arise from initial Schiff base formation and cyclization in a linear mode, whereas the cyclization for **11** would occur in an angular fashion. The single product isolated from this reaction in 68% yield had a molecular ion in its mass spectrum at m/z 135 suggesting that it was derived from bromoacetaldehyde and not BMDA. Absorptions at 213 nm (ϵ 17 000) and 296 nm (ϵ 8500) were present in its UV spectrum in H_2O. The high-field ^1H and ^{13}C NMR data could not distinguish between the two possibilities. The method chosen to distinguish between **10** and **11** involved an unambiguous synthesis of one of the isomeric systems. Isocytosine can be methylated selectively at the 3-position in 44% yield by reaction with *N,N*-dimethylformamide dimethyl acetal followed by ammonium hydroxide.[22] Treatment of 3-methylisocytosine (**12**) with BMDA employing conditions identical with those used for isocytosine gave two products, 8-methyl-3-formylimidazo[1,2-*a*]pyrimidin-7(8*H*)-one (**13**),

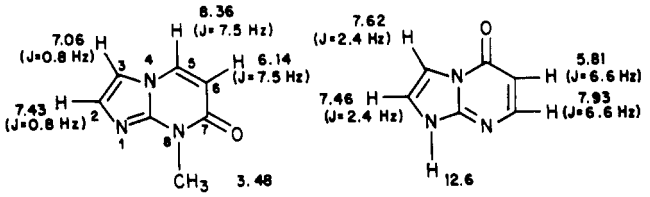


12 **13**



14

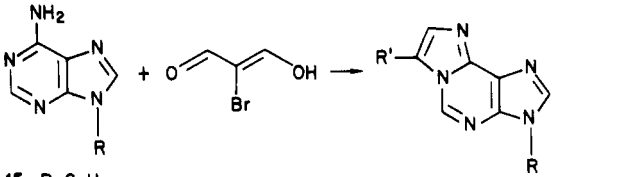
which was isolated in 16% yield, and 8-methylimidazo-[1,2-*a*]pyrimidin-7(8*H*)-one (**14**), which was obtained in 61% yield. A comparison of the 360-MHz ^1H NMR spectrum of the isocytosine adduct with that of **14** allowed



14 **10**, R=H

us to determine the regiochemistry of the addition and the structure of the former adduct as **10**. The chemical shifts of the protons in each of these compounds were assigned by a consideration of expected values, observed coupling constants, and homonuclear decoupling data. Examination of the chemical shifts of H-2 showed a difference of 0.03 ppm as is expected for protons in similar environments. However, the resonances for H-3 exhibit a much larger difference. If the product of the reaction were **11** (R = H) only a small difference in chemical shift would have been expected from **14** (H-3 at δ 7.06). However, H-3 in the isocytosine–BMDA adduct occurs at δ 7.62. In compound **10** (R = H), H-3 would be deshielded by the carbonyl at C-5 and would be expected to appear further downfield. The product therefore is structure **10** (R = H) and not **11** (R = H). Such chemical shift differences have been exploited by us recently in the assignment of the structure and the mechanism of formation of tricyclic adducts from guanosine and glycidaldehyde.[24]

The modification of purine bases by BMDA was also investigated. Adenosine **16** reacts with BMDA to give two products, 3-ribosylimidazo[2,1-*i*]purine (ethenoadenosine) (**18a**) in 10% yield, and 3-ribosyl-7-formylimidazo[2,1-*i*]-purine (ethenoadenosinecarboxaldehyde) (**18b**) in 18% yield. Ethenoadenosine has been prepared previously and



15, R=C_2H_5
16, R=ribosyl

17, R=C_2H_5; R'=CHO
18a, R=ribosyl; R'=H
b, R=ribosyl; R'=CHO

its spectral data were consistent with literature values.[25] The reaction of 9-ethyladenine (**15**) with BMDA gave a single product 3-ethyl-7-formylimidazo[2,1-*i*]purine (3-ethylethenoadeninecarboxaldehyde) (**17**) in 24% yield. Both **17** and **18b** exhibit fluorescence at 410 nm when excited at 270 nm.

In summary, the reactions of bromomalonaldehyde with a number of nucleic acid bases have been investigated. These interactions transform the base moiety through the formation of additional heterocyclic rings.[26] Etheno as well as the more novel ethenocarboxaldehyde products are formed. In one instance, a hypermodified base is the result. The ethenocarboxaldehyde cytosine derivatives exhibit fluorescence properties that are potentially more useful than ethenocytosines previously reported.

Experimental Section

The melting points reported are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. The ^1H NMR and ^{13}C NMR data were recorded on a JEOL FX90Q pulse Fourier transform NMR spectrometer or on a Bruker WM 360 high-field NMR spectrometer. Tetramethylsilane was the internal reference. Mass spectra at 30 eV were obtained on a Hewlett-Packard 5985 GC/MS system. The ultraviolet data were taken with a Cary Model 219 ultraviolet–visible spectrophotometer. Elemental analyses were performed by the University of Iowa Microanalytical Service on an automated Perkin Elmer Model 240 carbon, hydrogen, and nitrogen analyzer. HPLC separations were done at low pressure utilizing a column of Amberlite XAD-4 resin (270-325 mesh). Preparative-layer chromatography was done on E. Merck silica gel-PF-254. Fluorescence spectra were uncorrected and performed on an Aminco-Bowman Spectrophotofluorimeter using a xenon lamp.

Preparation of Bromomalonaldehyde (1, R = Br). This compound was prepared by the method of Trofimenko[21] and was obtained in 68% yield: mp 147–148 °C (lit.[21] mp 148 °C); UV (H_2O) λ_{max} 277 nm; ^1H NMR ($CDCl_3$) δ 9.33 (s, 2 H).

Reaction of Bromomalonaldehyde with Cytidine (4). An aqueous solution of 0.250 g (1.03 mol) of cytidine and 0.153 g (1.02 mmol) of bromomalonaldehyde was adjusted to pH 4.5 with 2 N NaOH. The reaction was heated to 60 °C under nitrogen and was discontinued when the absorbance at 325 nm maximized (72 h). The solution was then neutralized and the solvent removed in vacuo at 50 °C. The brown residue was then purified on a column of Amberlite XAD-4 using 80/20 H_2O/ethanol as the solvent. Unreacted starting materials preceded the product off the column. A total of 0.127 g (0.43 mmol, 43% yield, 69% conversion) of 6-(β-D-ribofuranosyl)-3-formylimidazo[1,2-*c*]pyrimidin-5(6*H*)-one (**7**) was obtained as yellow crystals from ethanol: mp 202–204 °C; UV (H_2O) λ_{max} 325 nm (ϵ 1.05 × 10^4); fluorescence (EtOH) excitation 357 nm and emission 398 nm; mass spectrum, m/z (relative intensity) 295 (M^+, 2.3), 163 ("base" + H^+, 100), 162 ("base", 19.8), 133 ("base" – CHO, 15.3), 107 (21), 73 (24.1); ^1H NMR (Me_2SO-d_6) δ 3.71–6.08 (m, 9 H, ribose), 6.91 (d, 1 H, J = 7.8 Hz), 8.12 (d, 1 H, J = 7.8 Hz), 8.16 (s, 1 H), 10.57 (s, 1

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(25) Barrio, J. R.; Secrist, III, J. A.; Leonard, N. J. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 597.

(26) Iodomalonaldehyde exhibited similar behavior.

H); ^{13}C NMR (Me_2SO-d_6) δ 60.2, 69.2, 74.6, 85.0, 89.7, 98.1, 130.0, 131.6, 139.6, 146.3, 149.1, 181.0.

Anal. Calcd for $C_{12}H_{13}N_3O_6 \cdot {}^1/_2\ H_2O$: C, 47.37; H, 4.64; N, 13.81. Found: C, 47.69; H, 4.61; N, 13.79.

Preparation of 1-Methylcytosine (3). This compound was prepared by the method of Hosmane and Leonard[22] and was obtained in 63% yield: mp 300–302 °C dec [lit.[22] mp 300–303 °C dec]; UV (H_2O) λ_{max} 273 nm; ^{1}H NMR (Me_2SO-d_6) δ 3.23 (s, 3 H), 5.52 (d, 1 H, J = 8.7 Hz), 7.60 (d, 1 H, J = 8.7 Hz), 11.16 (bs, 2 H).

Reaction of Bromomalonaldehyde with 1-Methylcytosine (3). To 0.643 g (5.14 mmol) of 1-methylcytosine dissolved in 20 mL of water was added 0.771 g (5.10 mmol) of bromomalondialdehyde. The pH was adjusted to 4.1 with 2 N NaOH and the reaction mixture was heated to 60 °C under N_2 until the UV absorbance at 330 nm reached a maximum (73 h). The reaction mixture was then neutralized and the solvent removed in vacuo at 50 °C. The brown residue was then passed through a short silica gel column using 20% $MeOH/CH_2Cl_2$ as the solvent. The solvent was again removed and the residue chromatographed on silica gel preparative-layer plates with 5% $MeOH/CH_2Cl_2$. Two bands were collected. The band with R_f 0.43 provided 0.097 g (0.34 mmol, 7%) of 6-methyl-3-(2-bromo-3-oxo-1-propenyl)-imidazo[1,2-*c*]pyrimidin-5(6*H*)-one (**6c**) as yellow prisms: mp 254–256 °C; UV (EtOH) λ_{max} 371 nm (ε 1.72 × 10^4); fluorescence (EtOH) excitation 398 nm and emission 452 nm; mass spectrum, m/z (relative intensity) 283, 281 (M^+, 5.4, 5.6), 202 (M^+ – Br, 100), 174 (M^+ – Br – CO, 64.7), 173 (M^+ – Br – HCO, 16.9), 159 (30.8), 149 (M^+ – C_3H_2OBr, 22.2), 131 (14.0); ^{1}H NMR (Me_2SO-d_6) δ 3.56 (s, 3 H), 6.81 (d, 1 H, J = 7.7 Hz), 7.76 (d, 1 H, J = 7.7 Hz), 8.73 (s, 1 H), 9.35 (s, 1 H), 9.44 (s, 1 H).

Anal. Calcd for $C_{10}H_8N_3O_2Br$: C, 42.57; H, 2.86; N, 14.90. Found: C, 42.03; H, 2.82; N, 14.70.

The band with R_f 0.29 was then rechromatographed on silica gel plates utilizing 20% CH_2Cl_2/ethyl acetate as the solvent. After two immersions two bands were isolated. The band with R_f 0.65 provided 0.156 g (1.05 mmol, 21%) of 6-methylimidazo[1,2-*c*]-pyrimidin-5(6*H*)-one (ethenomethylcytosine (**6a**)). The spectroscopic data of **6a** was consistent with literature values.[23] The band with R_f 0.70 yielded 0.246 g (1.39 mmol, 27%) of 6-methyl-3-formylimidazo[1,2-*c*]pyrimidin-5(6*H*)-one (ethenomethylcytosinecarboxaldehyde) (**6b**) as yellow crystals: mp 181–182 °C; UV (H_2O) λ_{max} 328 nm (ε 5.25 × 10^3); fluorescence (EtOH) excitation 352 nm and emission 402 nm; mass spectrum, m/z (relative intensity) 178 (M^+ + 1, 9.6), 177 (M^+, 100), 149 (M^+ – CHO, 84.5), 122 (M^+ – C_3H_3O, 12.5); ^{1}H NMR ($CDCl_3$) δ 3.71 (s, 3 H), 6.76 (d, 1 H, J = 7.6 Hz), 7.24 (d, 1 H, J = 7.6 Hz), 8.22 (s, 1 H), 10.73 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 37.4, 99.2, 130.4, 135.2, 140.1, 147.0, 149.2, 181.5.

Anal. Calcd for $C_8H_7N_3O_2$: C, 54.20; H, 3.96; N, 23.72. Found: C, 53.66; H, 3.99; N, 23.60.

Reaction of Bromomalonaldehyde with Cytosine (2). To 10 mL of H_2O was added 0.115 g (1.04 mmol) of cytosine and 0.166 g (1.10 mmol) of bromomalonaldehyde. The pH was adjusted to 4.2 with 2 N NaOH and the solution was heated to 60 °C under N_2 atmosphere. The reaction was monitored by UV spectroscopy and was stopped when the absorbance at 268 nm reached a maximum (72 h). The solvent was then removed in vacuo at 50 °C and the brown residue passed through a short column of silica gel using 15% $MeOH/CH_2Cl_2$. The solvent was again removed in vacuo and the brown residue was chromatographed on silica gel plates using 7% $MeOH/CH_2Cl_2$ as the solvent. The band with R_f 0.30 was eluted and then repurified on silica gel plates with a solvent of 10% CH_2Cl_2/ethyl acetate. The band with R_f 0.30 after two immersions was found to contain 0.019 g (0.14 mmol, 13%) of imidazo[1,2-*c*]pyrimidin-5(6*H*)-one (**5a**) as white crystals: mp 234–236 °C; UV (H_2O) λ_{max} 268 nm (ε 8.67 × 10^3); fluorescence (EtOH) excitation 289 nm and emission 339 nm; mass spectrum, m/z (relative intensity) 136 (M^+ + 1, 7.5), 135 (M^+, 100), 107 (M^+ – H_2CN, 29.8); ^{1}H NMR (Me_2SO-d_6) δ 6.59 (d, 1 H, J = 7.3 Hz), 7.25 (d, 1 H, J = 7.8 Hz), 7.39 (d, 1 H, J < 1 Hz), 7.78 (d, 1 H, J < 1 Hz), 11.40 (bs, 1 H).

Anal. Calcd for $C_6H_5N_3O$: C, 53.33; H, 3.73; N, 31.10. Found: C, 52.92; H, 3.95; N, 29.29.

A trace amount of 3-formylimidazo[1,2-*c*]pyrimidin-5(6*H*)-one (**5b**) also was detected in the mass spectrum of **5a**: m/z (relative intensity) 163 (M^+, 72.2), 162 (M^+ – H, 24.1), 135 (M^+ – CO, 100), 107 (M^+ – CO, HCN, 46.5).

Reduction of Ethenomethylcytosinecarboxaldehyde (6b). To 45 mL of dry ethanol was added 0.123 g (3.30 mmol) of $NaBH_4$. The flask was cooled in an ice bath and 0.048 g (0.27 mmol) of **6b** was added. The flask was stirred in the ice bath for 1 h and then at room temperature for 4 h. The solvent was removed in vacuo and the white solid that remained was dissolved in 20 mL of H_2O and extracted with CH_2Cl_2 (5 × 20 mL). The organic layer was then dried with sodium sulfate and the solvent removed in vacuo. The white solid which remained was then chromatographed on silica gel using 5% $MeOH/CH_2Cl_2$ as the solvent. The band with R_f 0.30 afforded after elution 0.025 g (0.14 mmol, 51%) of 6-methyl-3-(hydroxymethyl)imidazo[1,2-*c*]pyrimidin-5(6*H*)-one (**8**) as white crystals: mp 157–159 °C; UV (H_2O) λ_{max} 277 nm (ε 1.45 × 10^4); fluorescence (EtOH) excitation 297 nm and emission 342 nm; mass spectrum, m/z (relative intensity) 180 (M^+ + 1, 6.4), 179 (M^+, 67.9), 162 (M^+ – OH, 100), 150 ("base" + H^+, 65.9), 133 (M^+ – CH_3 – CH_2OH, 44.2); ^{1}H NMR ($CDCl_3$) δ 3.47 (s, 4 H), 4.86 (s, 2 H), 6.58 (d, 1 H, J = 7.8 Hz), 6.97 (d, 1 H, J = 7.8 Hz), 7.24 (s, 1 H).

Anal. Calcd for $C_8H_9N_3O_2 \cdot {}^1/_4H_2O$: C, 52.33; H, 5.23; N, 22.88. Found: C, 52.83; H, 5.13; N, 22.39.

Reaction of Bromomalonaldehyde with Isocytosine (9). To 10 mL of water was added 0.110 g (0.99 mmol) of isocytosine (**9**) and 0.162 g (1.08 mmol) of bromomalonaldehyde. The pH was adjusted to 3.8 with 2 N NaOH and the reaction was heated to 60 °C under N_2 atmosphere until the absorbance at 292 nm reached a maximum (96 h). The reaction was then neutralized with 2 N NaOH and the solvent removed in vacuo at 50 °C. The residue was then passed through a short silica gel column using 20% $MeOH/CH_2Cl_2$ as the eluant. The solvent was again removed and the residue chromatographed on silica gel preparative-layer plates using 10% $MeOH/CH_2Cl_2$. The band with R_f 0.40 afforded upon elution 0.091 g (0.67 mmol, 68%) of imidazo[1,2-*a*]pyrimidin-5(1*H*)-one (**10**) as yellow crystals: mp 193–197 °C; UV (H_2O) λ_{max} 213 nm (ε 1.7 × 10^4), 296 (ε 8.5 × 10^3); fluorescence (EtOH) excitation 300 nm and emission 353 nm; mass spectrum, m/z (relative intensity) 136 (M^+ + 1, 8.1), 135 (M^+, 100), 10 7 (M^+ – H_2CN, 40.6); ^{1}H NMR (Me_2SO-d_6) δ 5.81 (d, 1 H, J = 6.4 Hz), 7.46 (d, 1 H, J = 2.2 Hz), 7.62 (d, 1 H, J = 2.2 Hz), 7.93 (d, 1 H, J = 6.4 Hz), 12.6 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 97.1, 107.1, 120.7, 146.4, 149.6, 157.0.

Anal. Calcd for $C_6H_5N_3O \cdot {}^1/_4H_2O$: C, 51.61; H, 3.61; N, 30.09. Found: C, 51.41; H, 3.84; N, 30.69.

Preparation of 3-Methylisocytosine (12). To a scrupulously dry 3-neck round-bottom flask was added 1.135 g (10.21 mmol) of dry isocytosine and 16.0 mL (120.44 mmol) of *N*,*N*-dimethylformamide dimethyl acetal. The solution was allowed to reflux and 0.15 mL of trifluoroacetic acid was added via syringe through a septum over 10 min. The solution was then allowed to reflux for 15 h and cooled in an ice bath and the white solid which precipitated out was filtered off and dried to afford 1.230 g (6.83 mmol, 67%) of N^2-[(*N*,*N*-dimethylamino)methylene]-3-methylisocytosine as white crystals: mp 139.5–141.5 °C; UV (H_2O) λ_{max} 296 nm; mass spectrum, m/z (relative intensity) 180 (M^+, 81), 165 (M^+ – CH_3, 19.3), 136 (M^+ – $N(CH_3)_2$, 100), 109 (M^+ – $NCHNMe_2$, 53.2); ^{1}H NMR (Me_2SO-d_6) δ 3.05 (s, 3 H), 3.09 (s, 3 H), 3.16 (s, 3 H), 5.87 (d, 1 H, J = 6.2 Hz), 7.67 (d, 1 H, J = 6.2 Hz), 8.67 (s, 1 H).

The N^2-[(*N*,*N*-dimethylamino)methylene]-3-methylisocytosine was then stirred with 125 mL of concentrated NH_4OH for 12 h at room temperature. Excess ammonia was removed by refluxing the solution on a steam bath for 1.5 h. The solvent was removed and a white sticky solid remained. Separation of the solid on silica gel preparative-layer plates with 10% $MeOH/CH_2Cl_2$ and elution of the band with R_f 0.30 afforded 0.551 g (4.41 mmol, 65%, 44% yield overall) of 3-methylisocytosine (**12**) as white crystals: mp 263–266 °C (lit.[27] mp 262–266 °C); UV (H_2O) λ_{max} 284 nm (ε 1.09 × 10^4), 226 (ε 8.15 × 10^3; mass spectrum, m/z (relative intensity) 126 (M^+ + 1, 10.3), 125 (M^+, 100), 96 (M^+ – CH_2NH, 30.7); ^{1}H NMR (Me_2SO-d_6) δ 3.25 (s, 3 H), 5.58 (d, 1 H, J = 6.4 Hz), 7.13 (s, 2 H), 7.51 (d, 1 H, J = 6.4 Hz).

(27) Angier, R. B.; Curran, W. V. *J. Org. Chem.* **1961**, *26*, 1891.

Reaction of Bromomalonaldehyde with 3-Methylisocytosine (12). To 13 mL of water was added 0.117 g (0.94 mmol) of 3-methylisocytosine (12) and 0.143 g (0.95 mmol) of bromomalonaldehyde. The pH was adjusted to 4.3 with 2 N NaOH and the reaction mixture was heated to 60 °C under nitrogen atmosphere until the absorbance at 263 nm reached a maximum (6 days). The reaction was then neutralized with 2 N NaOH and the solvent removed in vacuo at 50 °C. The residue was then chromatographed on silica gel preparative-layer plates using 5% $MeOH/CH_2Cl_2$ as the solvent. The band with R_f 0.35 provided 0.026 g (0.15 mmol, 16%) of 8-methyl-3-formylimidazo[1,2-*a*]-pyrimidin-7(8*H*)-one (13) as pale yellow crystals: mp 178–181 °C; UV (H_2O) λ_{max} 264 nm (ϵ 1.51 × 10^4), 280 nm (ϵ 1.45 × 10^4); fluorescence (EtOH) excitation 302 nm and emission 424 nm; mass spectrum, *m/z* (relative intensity) 178 (M^+ + 1, 10.1), 177 (M^+, 100), 148 (M^+ – CHO, 18.3); ^1H NMR (Me_2SO-d_6) δ 3.54 (s, 3 H), 6.35 (d, 1 H, J = 7.8 Hz), 8.15 (s, 1 H), 8.80 (d, 1 H, J = 7.8 Hz), 9.71 (s, 1 H).

Anal. Calcd for $C_8H_7N_3O_2$: C, 54.23; H, 3.98; N, 23.72. Found: C, 53.81; H, 4.28; N, 23.49.

Another band found at R_f 0.25 afforded 0.086 g (0.58 mmol, 61%) of 8-methylimidazo[1,2-*a*]pyrimidin-7(8*H*)-one (14) as yellow crystals: mp 145–148 °C; UV (H_2O) λ_{max} 221 nm (ϵ 1.24 × 10^4); fluorescence (EtOH) excitation 309 nm and emission 455 nm; mass spectrum, *m/z* (relative intensity) 150 (M^+ + 1, 8.8), 149 (M^+, 100), 120 (64.7); ^1H NMR (Me_2SO-d_6) δ 3.48 (s, 3 H), 6.14 (d, 1 H, J = 7.8 Hz), 7.06 (d, 1 H, J < 1 Hz), 7.43 (d, 1 H, J < 1 Hz), 8.36 (d, 1 H, J = 7.3 Hz).

Anal. Calcd for $C_7H_7N_3O$: C, 56.37; H, 4.73; N, 28.17. Found: C, 56.80; H, 5.07; N, 28.28.

Reaction of Bromomalonaldehyde with Adenosine (16). To 170 mL of water was added 1.001 g (3.75 mmol) of adenosine and 0.873 g (5.78 mmol) of bromomalonaldehyde. The pH was adjusted to 4.5 with 2 N NaOH and the solution was heated to 60 °C under nitrogen atmosphere for 72 h. The solvent was then removed in vacuo at 50 °C and the brown residue which remained was separated on a column of Amberlite XAD-4 using 80:20 H_2O:ethanol as the solvent. 1,N^6-Ethenoadenosine (18a) was afforded as white crystals in 10% yield (0.109 g, 0.38 mmol, 13% conversion). The spectral data for 18a were consistent with literature values.[25] Also obtained was 1,N^6-ethenoadenosine-carboxaldehyde (18b) (0.218 g) (0.69 mmol, 18% yield, 24% conversion) as white crystals: mp 216–218 °C; UV (H_2O) λ_{max} 228 nm (ϵ 2.38 × 10^4), 325 nm (ϵ 1.75 × 10^4), 335 nm (ϵ 1.71 × 10^4); fluorescence (EtOH) excitation 270 nm and emission 410 nm; mass spectrum, *m/z* (relative intensity) 319 (M^+, 1.1), 187 (M^+ + H^+ – ribose, 100), 186 (M^+ – ribose, 31.0), 159 (base – CO, 6.3); ^1H NMR (Me_2SO-d_6) δ 4.59–6.12 (m, 9 H), 8.61 (s, 1 H), 8.81 (s, 1 H), 9.95 (s, 1 H), 10.02 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 61.0, 70.1, 74.3, 85.6, 87.9, 122.8, 124.7, 136.6, 141.4, 142.0, 144.7, 147.8, 179.1.

Anal. Calcd for $C_{13}H_{13}N_5O_5 \cdot H_2O$: C, 46.29; H, 4.48; N, 20.76. Found: C, 46.88; H, 4.43; N, 20.94.

Reaction of Bromomalonaldehyde with 9-Ethyladenine. To 60 mL of H_2O was added 0.452 g (2.77 mmol) of 9-ethyladenine (15) and 0.456 g (3.02 mmol) of bromomalonaldehyde. The pH was checked (3.3) and not adjusted. The reaction was then heated to 55 °C under nitrogen atmosphere for 72 h. The reaction was extracted with CH_2Cl_2 (3 × 40 mL) and the organic phase dried over Na_2SO_4. The solvent was removed in vacuo at 50 °C and the residue chromatographed on silica gel plates with 13% $MeOH/CH_2Cl_2$ as the solvent. The band with R_f 0.59 afforded 0.143 g (0.66 mmol, 24%) of 9-ethylethenoadeninecarboxaldehyde (17) as white crystals: mp 223–225 °C; UV (95% ethanol) λ_{max} 230 nm (ϵ 2.06 × 10^4), 328 nm (ϵ 1.51 × 10^4), 339 nm (ϵ 1.50 × 10^4); fluorescence (EtOH) excitation 270 nm and emission 410 nm; mass spectrum, *m/z* (relative intensity) 216 (M^+ + 1, 12.2), 215 (M^+, 100), 187 (M^+ – CO, 34.4), 186 (M^+ – Et, 34.4), 159 (M^+ – Et – CO, 13.5); ^1H NMR ($CDCl_3$) δ 1.62 (t, 3 H), 4.44 (q, 2 H), 8.13 (s, 1 H), 8.37 (s, 1 H), 10.02 (s, 1 H), 10.08 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 15.4, 38.4, 122.4, 124.7, 136.4, 139.6, 143.4, 145.1, 147.9, 179.0.

Anal. Calcd for $C_{10}H_9N_5O$: C, 55.80; H, 4.21; N, 32.54. Found: C, 55.89; H, 4.11; N, 32.34.

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Registry No. 1 (R = Br), 2065-75-0; **2**, 71-30-7; **3**, 1122-47-0; **4**, 65-46-3; **5a**, 55662-66-3; **6a**, 45859-50-5; **6b**, 91898-74-7; **6c**, 91898-75-8; **7**, 91898-76-9; **8**, 91898-77-0; **9**, 108-53-2; **10** (R = H), 55662-68-5; **12**, 2417-17-6; **13**, 91898-78-1; **14**, 91898-79-2; **15**, 2715-68-6; **16**, 58-61-7; **17**, 91898-80-5; **18a**, 39007-51-7; **18b**, 91898-81-6; $Me_2NCH(OMe)_2$, 4637-24-5; N^2-[(dimethylamino)-methylene]-3-methylisocytosine, 91898-82-7.

A Synthesis of *N*-Acyl-1,2-dihydropyridines

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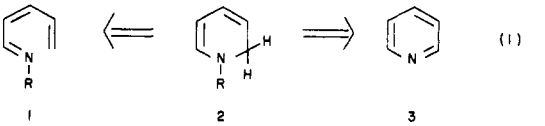
A new approach to the synthesis of *N*-acyl-1,2-dihydropyridines based on the cyclization of *N*-acyl-1-azatrienes is introduced. These latter compounds were not isolated but were produced in the gas phase as transient intermediates by the flash vacuum pyrolysis of *O*-methoxycarbonyl *N*-penta-1,3-dien-5-yl hydroxamic acid derivatives 8.

Simple 1,2-dihydropyridines are electron-rich dienes where the π-system is activated by the heterocyclic nitrogen atom. These compounds are known to undergo reactions characteristic of enamines as well as behaving as reactive partners in cycloaddition reactions.[1] Because of their diverse reactivity they would appear to possess considerable potential in synthetic chemistry. However, 1,2-dihydropyridines that do not contain electron-withdrawing substituents on the ring (2, R = alkyl) (eq 1), are



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relatively unstable with respect to dimerization, polymerization, and oxidation.[1] They are difficult to handle

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