o-Hydroxybenzonitrile has been observed as a minor product in the metabolism of benzonitrile with liver microsomes.[10] Our results suggest that if the biological ortho hydroxylation of benzonitrile occurs via the arene 1,2-oxide (or 2,3-oxide) as in intermediate, then o-hydroxybenzonitrile is formed without migration of the cyano group. If cyano group migration does occur in the biological ortho hydroxylation of benzonitrile, it would suggest that the reaction occurs by addition of HO^+ (or HO·) to C_1 of the substrate rather than formation of an arene oxide intermediate.

Experimental Section

Melting points were determined by using a Thomas-Hoover Unimelt and are corrected. ^1H NMR spectra were obtained at 60 or 250 MHz with Varian T-60, Perkin-Elmer R24B, and Brüker FT spectrometers, respectively. Unless otherwise indicated, spectra were obtained at 60 MHz. Chemical shift values (δ) are reported in parts per million downfield from tetramethylsilane. Mass spectra were determined with a Varian MAT 44 instrument. Infrared spectra were obtained with a Perkin-Elmer Model 238 B spectrophotometer. Ultraviolet spectra were obtained with a Perkin-Elmer Model 552 spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN.

1-Cyano-1,2-oxy-4-cyclohexene (3). A mixture of **2**[2] (24.6 g, 0.198 mol), hydroxylamine sulfate (16.4 g, 0.20 mol), $NaHCO_3$ (16.8 g, 0.20 mol), and 4 mL of water in 300 mL of ether was stirred vigorously at 10 °C for 1 h. The solution was decanted from insoluble salts and dried ($MgSO_4$). Concentration at 15 °C afforded the oxime as a colorless oil that was dissolved in 300 mL of CH_2Cl_2 and cooled to 5 °C. 1,1'-Carbonyldiimidazole (30 g) in 200 mL of CH_2Cl_2 was added dropwise with stirring. After addition was complete, the mixture was warmed to room temperature and stirred overnight at which time CO_2 was no longer evolved. The solution was washed with three 150-mL portions of water and dried ($MgSO_4$). The solvent was removed in vacuo to give a viscous oil that was purified by column chromatography (silica gel, 9:1 pentane–ether) and distillation to yield 5.2 g (22%) of **3**: bp 43 °C (0.13 mm); IR (neat) 2250, 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.45 (br s, 2 H), 3.67 (br s, 1 H), 2.9–2.5 (br d, 4 H). Anal. Calcd for C_7H_7NO: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.38; H, 5.92; N, 11.46.

1-Cyano-1,2-oxy-4,5-dibromocyclohexane (4). A solution of **3** (1.4 g, 11.5 mmol) in 50 mL of 1:1 CH_2Cl_2–$CHCl_3$ was cooled to −70 °C, and bromine (1.84 g, 11.5 mmol) in 40 mL of the same solvent was added dropwise over a period of 40 min. The solution was concentrated, and the residue was purified by column chromatography (silica gel, 9.1 pentane–ether) to give 3.1 g (96%) of **4** as colorless crystals: mp 74–75 °C; IR (KBr) 2240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.32 (m, 2 H), 3.72 (m, 1 H), 3.5–2.2 (m, 4 H). Anal. Calcd for $C_7H_7Br_2NO$: C, 29.92; H, 2.51; N, 4.99. Found: C, 30.12; H, 2.56; N, 4.99.

1-Cyanobenzene Oxide–Oxepin (1). To 1.7 g (6 mmol) of **4** in 30 mL of anhydrous ether at room temperature under N_2 was added dropwise 3 equiv of DBN. The mixture was stirred for 3 h. The ether solution was decanted from precipitated salts, washed with aqueous pH 7 phosphate buffer solution, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography (silica gel, 10:1 pentane–ether) to give 300 mg (42%) of **4** as yellow crystals that were recrystallized from pentane: mp 36–37 °C; IR (KBr) 2210 (sh 2250), 1628, 1610, 1585, 1555 cm^{-1}; UV_{max} (CH_3OH) 204 (ϵ 10950), 307 nm (1680); ^1H NMR (250 MHz, $CDCl_3$) δ[11] 6.49 (dd, 1 H, H_4), 6.36 (d, 1 H, H_6), 6.26 (dd, 1 H, H_5), 5.99 (d, 1 H, H_2), 5.76 (t, 1 H, H_3) ($J_{2,3}$ = 5.3, $J_{3,4}$ = 6.0, $J_{4,5}$ = 10.6, $J_{5,6}$ = 5.7 Hz); mass spectrum (70 eV), m/e (relative intensity) 119 (45, M^+), 103 (6), 93 (18), 91 (57), 82 (100), 81 (45), 80 (95), 79 (51), 68 (23), 65 (42), 64 (86), 63 (44), 39 (79). Anal. Calcd for C_7H_5NO: C, 70.58; H, 4.23; N, 11.76. Found: C, 70.43; H, 4.18; N, 11.62.

Crystalline arene oxide **1** sublimes at room temperature and should be kept in a closed container.

Deuterium-Labeled Arene Oxide 6. The synthetic sequence for preparation of **1** from **2** was used to convert **5**[2] (80% ^2H at C_4, 20% ^2H at C_5) to **6**; ^1H NMR (250 MHz, $CDCl_3$) δ 6.49 (0.20 H, H_4), 6.36 (1 H, H_6), 6.26 (0.8 H, H_5), 5.99 (1 H, H_2), 5.76 (1 H, H_3).

Aromatization of 1 and 6. Arene oxide **1** was aromatized in pure $CF_3CO_2{}^2H$ and in 1:1 tetrahydrofuran–water at pH 1.1 and 7.0. The results are summarized in the discussion. o-Hydroxybenzonitrile was characterized by TLC and 250-MHz ^1H NMR comparison with an authentic sample. For o-hydroxybenzonitrile: ^1H NMR (250 MHz, acetone-d_6) δ 7.58 (H_6), 7.50 (H_4), 7.09 (H_3), 6.99 (H_5) ($J_{3,4}$ = 8.5, $J_{4,5}$ = 7.4, $J_{5,6}$ = 7.7, $J_{3,5}$ = 1.1, $J_{4,6}$ = 1.6 Hz).

Arene oxide **6** was dissolved in CF_3CO_2H and kept at room temperature for 3 h, at which time the yellow oxepin color had disappeared. The solution was diluted with ether, extracted with 5% aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated in vacuo to give **8**; ^1H NMR (250 MHz, acetone-d_6) δ 7.58 (1 H, H_6), 7.50 (0.21 H, H_4), 7.09 (1 H, H_3), 6.99 (0.79 H, H_5).

A mixture of **6** and water (pH 7, phosphate buffer) was heated at 60 °C for 12 h. The solution was extracted with ether, dried ($MgSO_4$), and concentrated. Preparative TLC (silica gel, 1:1 ether–pentane) of the residue gave **8**; ^1H NMR (250 MHz, acetone-d_6) δ 7.58 (1 H, H_6), 7.50 (0.20 H, H_4), 7.09 (1 H, H_3), 6.99 (0.80 H, H_5).

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Registry No. 1, 73654-30-5; 2, 75961-78-3; 3, 75961-79-4; 4, 75961-80-7.

(10) Daly, J.; Jerina, D.; Witkop, B. *Arch. Biochem. Biophys.* **1968**, *128*, 517-527.
(11) Assignments are based on the numbering system for the benzene oxide valence tautomer.

Confirmation of the Structure of the Guanine–Methylmalondialdehyde Reaction Product by Unequivocal Synthesis

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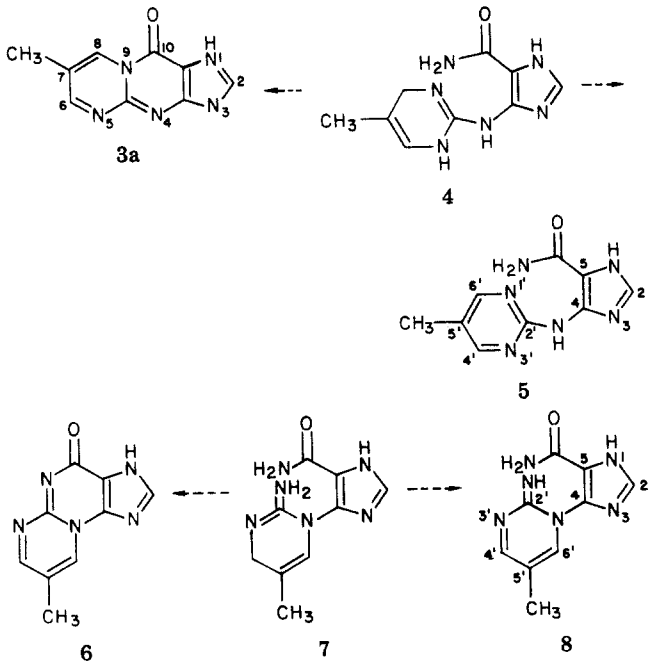
The ability of several mono-[1-3] and dicarbonyl[4-7] aldehydes to form stable, covalent adducts with guanine derivatives (1) has prompted investigation into their use as potential DNA[8,9] and RNA[4,8] modifying agents. Malondialdehyde (**2**, R^2 = H), a naturally occurring β-dialdehyde,[10-13] has been reported to react with DNA both in vitro and in vivo[14] with a corresponding loss of DNA template activity.[15]

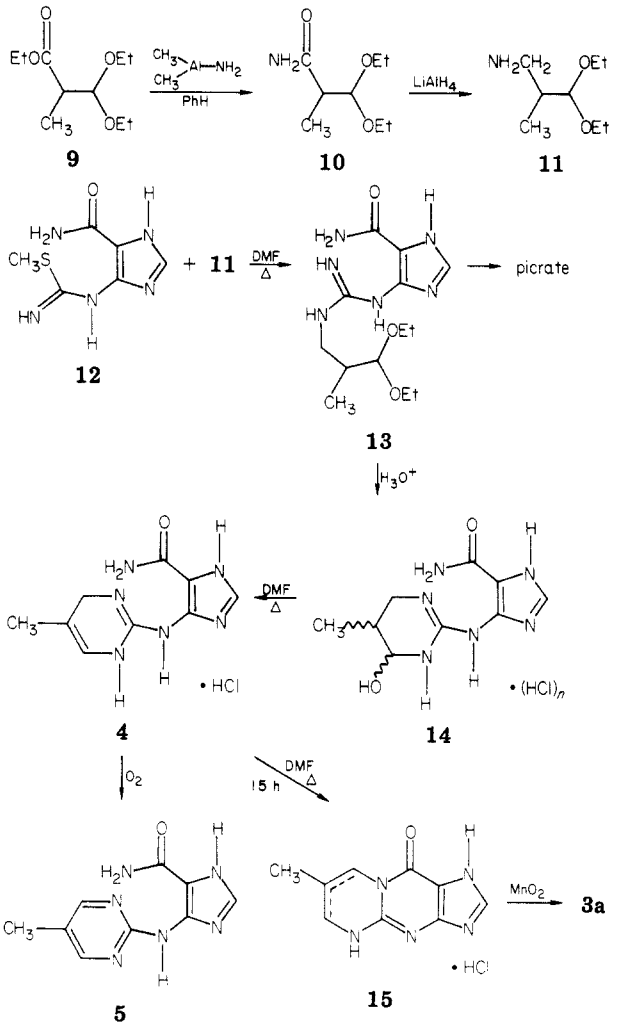
(1) Scheit, K. H. *Tetrahedron Lett.* **1965**, 1031.
(2) Goldschmidt, B. M.; Blazej, T. P.; Van Duuren, B. L. *Tetrahedron Lett.* **1968**, 1583.
(3) Sattsangi, P. D.; Leonard, N. J.; Frihart, C. R. *J. Org. Chem.* **1977**, *42*, 3292.
(4) Staehelin, M. *Biochim. Biophys. Acta* **1959**, *31*, 448.
(5) Whitfeld, P. R.; Witzel, H. *Biochim. Biophys. Acta* **1963**, *72*, 338.
(6) Litt, M.; Hancock, V. *Biochemistry* **1967**, *6*, 1848.
(7) Shapiro, R.; Cohen, B. I.; Shiuey, S.-J.; Maurer, H. *Biochemistry* **1969**, *8*, 238.
(8) Fraenkel-Conrat, H. *Biochim. Biophys. Acta* **1954**, *15*, 307.
(9) Reiss, U.; Tappel, A. L.; Chio, K. S. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 921.
(10) Kitabchi, A. E.; Williams, R. H. *J. Biol. Chem.* **1968**, *243*, 3248.
(11) Kitabchi, A. E.; McCay, P. B.; Carpenter, M. P.; Trucco, R. E.; Caputto, R. *J. Biol. Chem.* **1960**, *235*, 1591.
(12) Patton, S.; Kenney, M.; Kurtz, G. W. *J. Am. Oil Chem. Soc.* **1951**, *28*, 391.
(13) Smith, J. B.; Ingerman, C. M.; Silver, M. J. *J. Lab. Clin. Med.* **1976**, *88*, 167.
(14) Brooks, B. R.; Klamerth, O. L. *Eur. J. Biochem.* **1968**, *5*, 178.
(15) Reiss, U.; Tappel, A. L.; Chio, K. S. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 921.

0022-3263/81/1946-0815$01.00/0

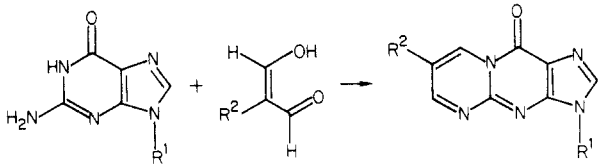
Scheme I



Scheme II



Substituted malondialdehydes, which are generally more stable than malondialdehyde itself, have been shown in many instances to react with diamino compounds to yield the corresponding nitrogen heterocycles.[16-18] We have recently demonstrated that under acidic conditions, guanine reacts with substituted malondialdehydes to afford the corresponding fluorescent tricyclic products.[19] Furthermore, we have found that guanosine,[20] guanosine 5'-monophosphate,[21] GpU,[20] and tRNA^{Phe} from *E. coli*[20] all form fluorescent products upon treatment with methylmalondialdehyde (**2**, $R^2 = CH_3$) under appropriate conditions (pH <4.7). Base-specific modification of guanine residues leading to fluorescent derivatives is highly desirable owing to the high sensitivity with which such species can be detected and to the strong dependence of fluorescence emission spectra on the local environment of the fluorophore.



The assignment of the guanine–methylmalondialdehyde adduct as the "linear" isomer (**3a**), rather than the "bent" isomer (**6**), was made initially on the basis of UV similarities to model compounds and by analogy to the guanine–glyoxal adduct.[19] We have recently shown unequivocally that the adduct formed between guanine and glyoxal, and, by analogy, with other α-carbonyl aldehydes, has a "linear" skeletal structure.[22] Methylmalondialdehyde, on the other hand, is representative of β-dialdehyde modifying reagents, and accordingly we considered it desirable to verify the linear structure assignment (**3**, including **3a**) by independent and unequivocal means.

We now report a synthesis of 1,N^2-(2-methylallylidene)guanine (7-methyl-10-oxo-9,10-dihydropyrimido[1,2-*a*]purine) (**3a**) that provides unambiguous assignment of the "linear" tricyclic structure to the guanine–methylmalondialdehyde reaction product and thus of similar linear skeletal structures to all related compounds. If the 9,10-bond is formed in the ring-closure step of the synthesis (Scheme I), a potential precursor to the desired final product is the substituted (dihydropyrimidinylamino)imidazole **4**. Dehydrogenation of compound **4** would afford the corresponding (pyrimidinylamino)imidazole **5**, and thus **4** would be readily distinguishable from its structural isomer, **7**, by NMR, owing to the expected magnetic equivalence of the 4'- and 6'-pyrimidine protons in compound **5** but not in **8**. The equivalence in **5** is due to the symmetrical nature of the pyrimidine and rapid rotation about the exocyclic C–N bond at room temperature.[23] In this way, compounds **5** and **8**, and accordingly compounds **4** and **7**, may be distinguished unambiguously from each other.

The required amino acetal **11** was synthesized by using a route analogous to that previously reported for the synthesis of 3-aminopropionaldehyde, diethyl acetal[24]

(16) Reichardt, C.; Halbritter, K. *Justus Liebigs Ann. Chem.* **1975**, *3*, 470.
(17) Coppola, G. M.; Hardtmann, G. E.; Huegi, B. S. *J. Heterocycl. Chem.* **1974**, *11*, 51.
(18) Reichardt, C.; Halbritter, K. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 86.
(19) Moschel, R. C.; Leonard, N. J. *J. Org. Chem.* **1976**, *41*, 294.
(20) M. Hinterberger, PhD. Thesis, The University of Illinois, Urbana, IL, 1980.
(21) Moschel, R. C. unpublished results in this laboratory.
(22) Czarnik, A. W.; Leonard, N. J. *J. Org. Chem.* **1980**, *45*, 3514.
(23) Batterham, T. J. "NMR Spectra of Simple Heterocycles"; Wiley-Interscience: New York, 1973; p 97.

(Scheme II). It is of interest that, while ethyl 3,3-diethoxypropionate is reported to undergo amidation using aqueous ammonium hydroxide, ethyl 3,3-diethoxy-2-methylpropionate (9)[25] failed to react under standard amidation conditions (NH_4OH, NH_3/MeOH, liquid NH_3). The use of an alkaline catalyst ($NaNH_2/NH_3$) resulted in both amidation and elimination of ethanol to yield a mixture of isomeric alkenic amides. Amidation using dimethylaluminum amide in refluxing benzene as reported by Weinreb and co-workers[26] did afford the amide (10) as a colorless, crystalline solid. By reduction with lithium aluminum hydride, the amide was readily converted to the requisite amino acetal (11) as a colorless, distillable liquid.

The condensation of 11 with 4-[(*S*-methylisothiocarbamoyl)amino]-5-imidazolecarboxamide (12)[27] yielded, after chromatographic purification, the disubstituted guanidine 13 as a gum which could not be induced to crystallize; however, formation of its picrate salt yielded a recrystallizable solid through which the elemental composition could be verified. Acid-catalyzed ring closure of 13 yielded a crystalline salt which was identified as the covalently hydrated dihydropyrimidine 14 on the basis of its ^1H NMR and field-desorption mass spectral analyses. Other examples of such covalently hydrated heterocycles have been reported.[28] The choice between the two possible structurally isomeric hydroxytetrahydropyrimidines could not be made until subsequent conversion to the corresponding pyrimidine was effected. Thermal dehydration of 14 readily afforded the dihydropyrimidine 4 as the monohydrochloride salt. The oxidation of 4 to aminopyrimidine 5 was accomplished conveniently by using molecular oxygen as the oxidizing agent. A similar air oxidation has been reported for 5-methyl-2-phenyl-1,4-dihydropyrimidine.[29]

The 220-MHz ^1H NMR spectrum of the pyrimidine 5 in either D_2O or CD_3OD showed only a single sharp resonance for the 4',6'-pyrimidine protons, demonstrating their magnetic equivalence. This finding clearly delineates the structure of the pyrimidine as 5 rather than 8 and accordingly the structure of the precursor dihydropyrimidine as 4 rather than 7.

Thermal ring closure of 4 in dimethylformamide afforded a mixture of the two double-bond isomers of 15 as determined by ^1H NMR. The mixture was observed to undergo facile air oxidation which prevented its isolation in pure form. Oxidation of the crude mixture with activated MnO_2 yielded 1,N^2-(2-methylallylidene)guanine (3a) in satisfactory yield. This compound was found to be identical in all respects with an authentic sample of the guanine-methylmalonadialdehyde reaction product prepared as previously reported.[19] The direct comparison provides confirmation of the "linear" array of the three heterocyclic rings that has been assumed, without unequivocal evidence heretofore, for the guanine–methylmalondialdehyde reaction product and for the entire family of related products.

Experimental Section

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. ^1H nuclear magnetic resonance spectra were recorded on a Varian EM-390 or HR-220 spectrometer, using tetramethylsilane with deuterated organic solvents or acetone (δ 2.07) with D_2O solutions as internal standards. 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 spectrophotometer.

Thin-layer chromatography (TLC) was performed on Merck silica gel f-254 plates (thickness, 0.25 mm); the solvent systems employed were the following: solvent A, 1-butanol–glacial acetic acid–H_2O (4:1:1); solvent B, isobutyric acid–0.5 M NH_4OH (5:3); solvent C, ethyl acetate–MeOH–H_2O–HOAc (75:16:10:5). TLC plates that included samples spotted from nonvolatile solvents were eluted with EtOAc prior to elution with the desired solvent. Brinkmann 0.05- to 0.2-mm silica gel was used for column chromatography on silica. Dimethylformamide (DMF) was purified before use by stirring over KOH pellets for several hours followed by distillation from BaO. Methanol and ethanol used were of anhydrous grade. Microanalyses were performed either by Mr. Josef Nemeth and associates or by Midwest Microlab, Ltd., Indianapolis, IN.

Ethyl 3,3-Diethoxy-2-methylpropionate (9). The ester was prepared from ethyl α-bromopropionate as previously described[25] to afford a colorless liquid: bp 44–45 °C (0.1 torr) [lit.[25] bp 95–98 °C (16 torr)]; ^1H NMR (CCl_4) δ 1.15 (m, 12, CH_3), 2.60 (quintet, 1, CH_3CH, J = 8 Hz), 3.55 (m, 4, $CHOCH_2$), 4.10 (q, 2, $COOCH_2$, J = 7 Hz), 4.50 (d, 1, O–CH, J = 8 Hz); field-ionization mass spectrum (2.5 kV), m/e 204 (M^+), 203 ($M^+ - H$), 175 ($M^+ - Et$), 159 ($M^+ - OEt$).

Anal. Calcd for $C_{10}H_{20}O_4$: C, 58.79; H, 9.89. Found: C, 58.79; H, 9.68.

3,3-Diethoxy-2-methylpropionamide (10). A three-necked 2-L round-bottomed flask was dried and equipped with a rubber septum, a gas inlet, and a glass stopper. To this was added 650 mL of dry benzene (distilled from CaH_2) and the system was placed under dry N_2. Through the septum was added by syringe 370 mL (0.96 mol) of a 25% solution of trimethylaluminum in hexane (Alfa, ca. 2.6 M) and stirring was continued at room temperature for 10 min. The solution was then cooled in an ice–salt bath and 25 mL of liquid NH_3 (d = 0.77, 1.13 mol) was added dropwise from a precooled syringe. The resulting solution was stirred in the ice bath for 30 min and then stirred at room temperature for an additional 2 h. To this was added rapidly through a dropping funnel a solution of 70 g (0.34 mol) of ethyl 3,3-diethoxy-2-methylpropionate (9) in 100 mL of dry benzene, and the solution was heated gently until the removal of hexane was complete. The reaction was heated at gentle reflux for 4 h and then allowed to cool to room temperature. The green solution was *carefully* neutralized with 320 mL (0.96 mol) of 3 M HCl, 300 mL of H_2O was added, and the mixture was stirred for 15 min, followed by removal of the benzene layer. The aqueous layer was extracted with EtOAc (10 × 100 mL) and the combined organic layers were washed with 500 mL of saturated aqueous NaCl, dried over $MgSO_4$, and evaporated in vacuo to yield a colorless oil which was crystallized from 300 mL of hexane to afford the amide as a colorless solid (32.5 g, 55%) after drying in vacuo. An analytical sample was obtained by recrystallization from hexane to afford colorless needles: mp 64.5–66 °C; ^1H NMR ($CDCl_3$) δ 1.25 (m, 9, CH_2CH_3 and $CHCH_3$), 2.65 (quintet, 1, J = 6 Hz, CH_3CH), 3.65 (m, 4, CH_2), 4.55 (d, 1, J = 6 Hz, O–CH), 6.50 (br s, 2, amide NH_2 exchangeable with D_2O); field-ionization mass spectrum, m/e 176 (MH^+), 130 ($M^+ - OEt$), 129 ($M^+ - HOEt$).

Anal. Calcd for $C_8H_{17}NO_3$: C, 54.83; H, 9.78; N, 7.99. Found: C, 54.99; H, 9.59; N, 8.24.

3-Amino-2-methylpropionaldehyde Diethyl Acetal (11). A suspension of 14.4 g (0.36 mol) of 95% $LiAlH_4$ in 250 mL of dry Et_2O (distilled from $LiAlH_4$ immediately before use) was stirred at room temperature under dry N_2 for 20 min, and then a solution of 31.4 g (0.18 mol) of 3,3-diethoxy-2-methylpropionamide (10) in 150 mL of dry Et_2O was added dropwise via an addition funnel over a 30-min period. The resulting suspension was heated at gentle reflux with stirring for 5.5 h, followed by careful neutralization of the mixture with an aqueous 8% NaOH solution. Additional ether was added to replace that which had evaporated during the neutralization, and the organic layer was filtered, washed with 50 mL of H_2O, dried over K_2CO_3, and

(24) Israel, M.; Zoll, E. C.; Muhammad, N.; Modest, E. J. *J. Med. Chem.* **1973**, *16*, 1.
(25) Kupiecki, F. P.; Coon, M. J. *Biochem. Prep.* **1960**, *7*, 69.
(26) Lipton, M. F.; Basha, A.; Weinreb, S. M. *Org. Synth.* **1979**, *59*, 49.
(27) Yamazaki, A.; Kumashiro, I.; Takenishi, T. *J. Org. Chem.* **1967**, *32*, 1825.
(28) Albert, A. *Adv. Heterocycl. Chem.* **1976**, *20*, 117.
(29) Thompson, T. W. *Chem. Commun.* **1968**, 532.

evaporated in vacuo. The initially filtered solid was washed with 200 mL of cold H_2O, then the mixture was filtered, and the filtrate was extracted with CH_2Cl_2 (4 × 50 mL). The combined CH_2Cl_2 layers were dried over K_2CO_3 and evaporated to leave an almost colorless liquid residue. The two residues were combined and distilled to afford a colorless liquid (20.8 g, 72%): bp 73–74 °C (11 torr); ^1H NMR ($CDCl_3$) δ 0.95 (d, 3, J = 7 Hz, $CHCH_3$), 1.20 (t, 6, J = 9 Hz, CH_2CH_3), 1.30 (s, 2, NH_2 exchangeable with D_2O), 1.80 (m, 1, CH_3CH), 2.55 (dd, 1, J = 6 Hz and 14 Hz, $NH_2CH(H)$), 2.75 (dd, 1, J = 6 Hz and 14 Hz, $NH_2CH(H)$), 3.55 (m, 4, O–CH_2), 4.30 (d, 1, J = 6 Hz, O–CH); field-ionization mass spectrum (2.5 kV), m/e 162 (MH^+), 117 (M^+ – OEt), 116 (M^+ – HOEt).

Anal. Calcd for $C_8H_{19}NO_2$: C, 59.59; H, 11.88; N, 8.69. Found: C, 59.52; H, 11.76; N, 8.48.

4-[*N*-(3,3-Diethoxy-2-methylpropyl)guanidino]-5-imidazolecarboxamide (13). To a solution of the isothiourea (12;[27] 5.14 g, 26 mmol) in 600 mL of dry DMF was added 3-amino-2-methylpropionaldehyde diethyl acetal (10; 9.22 g, 57 mmol), and the resulting solution was heated at gentle reflux with stirring and with the exclusion of moisture for 1 h. The light brown solution was evaporated in vacuo, and the residue was applied to a 31 × 3.5 cm column of silica gel. The column was eluted with 7% MeOH in CH_2Cl_2 until TLC indicated that the product had begun to elute (about 2 L). Elution with solvents of increasing polarity up to 40% MeOH in CH_2Cl_2 yielded two products according to TLC. The appropriate fractions were pooled and evaporated to yield a yellow oil which was applied to a 52 × 3.5 cm column of silica gel packed in solvent A. The column was eluted with the same solvent system, and the UV-absorbing fractions were checked by TLC in solvent A. The appropriate fractions were pooled and evaporated to dryness to yield a light yellow oil which was coevaporated four times with ethanol–toluene (4:1) and then dried overnight in vacuo. The residue was dissolved in CH_2Cl_2, the solid was filtered, and the filtrate was evaporated to yield 13 as a yellow gum, yield 3.9 g (48%): R_f 0.46 (solvent A); ^1H NMR ($CDCl_3$) δ 1.10 (m, 9, CH_3), 2.00 (contaminant), 3.55 (m, 6, CH_2), 4.35 (d, 1, J = 5 Hz, O–CH), 7.35 (s, 1, imidazole CH), 8.95 (br s, 1, NH exchangeable with D_2O), 9.80 (br s, 1, NH exchangeable with D_2O), 11.3 (br s, 2, NH exchangeable with D_2O); mass spectrum, m/e 312 (M^+), 283 (M^+ – Et), 267 (M^+ – OEt), 266 (M^+ – HOEt); high-resolution mass spectrum, calcd for $C_{13}H_{24}N_6O_3$ 312.1910, obsd 312.1916.

A sample of the **picrate** was prepared as follows. A solution of 13 (117 mg, 0.38 mmol) in 2 mL absolute MeOH was treated with 2 mL of a 10% solution of picric acid in MeOH, and the resulting mixture was allowed to stand at room temperature for 30 min. The precipitate was filtered and dried in vacuo to afford 99 mg (49%) of a yellow solid which was recrystallized from absolute EtOH to yield the picrate as needles: mp 176–178 °C; ^1H NMR ($(CD_3)_2SO$) δ 0.95 (d, 3, J = 7 Hz, $CHCH_3$), 1.20 (t, 6, J = 7 Hz, CH_2CH_3), 2.05 (m, 1, CH_3CH), 3.20 (m, 2, NCH_2, coalesces to dd, J = 7 and 13 Hz, with D_2O), 3.60 (m, 4, CH_3CH_2), 4.40 (d, 1, J = 5 Hz, O–CH), 7.50 (s, 2, NH and imidazole CH, one proton exchangeable with D_2O), 7.90 (s, 1, NH, exchangeable with D_2O), 8.60 (s, 3, NH and picrate CH's, one proton exchangeable with D_2O), 9.40 (br s, 1, NH, exchangeable with D_2O), 9.80 (br s, 1, NH, exchangeable with D_2O), 12.80 (br s, 1, NH, exchangeable with D_2O).

Anal. Calcd for $C_{19}H_{27}N_9O_{10}$: C, 42.14; H, 5.04; N, 23.28. Found: C, 42.10; H, 4.89; N, 23.14.

4-[(6′-Hydroxy-5′-methyl-1′,4′,5′,6′-tetrahydropyrimidin-2′-yl)amino]-5-imidazolecarboxamide Hydrochloride (14). A solution of the substituted guanidinoimidazole 13 (975 mg, 3.1 mmol) in 10 mL of MeOH was added dropwise to 75 mL of 6 M HCl with stirring, and the resulting solution was stirred at room temperature for an additional 10 min. The solution was evaporated to dryness in vacuo at 50 °C to obtain a colorless solid that was used directly in the next stage: dec >200 °C without melting; ^1H NMR (D_2O) δ 1.00 (m, 3+, CH_3), 2.20 (m, 1, CH_3CH), 2.7–3.5 (br m, 2, CH_2), 4.65 (d, 0.5, J = 4 Hz, aliphatic CH anti to CH_3), 4.85 (d, 0.5, J = 2 Hz, aliphatic CH syn to CH_3), 8.40 (s, 1, Ar CH); field-desorption mass spectrum (19 mA), m/e 239 (MH^+), 238 (M^+), 221 (MH^+ – H_2O), 220 (M^+ – H_2O), 219 (MH^+ – H_2O, H_2), 218 (M^+ – H_2O, H_2).

4-[(5′-Methyl-1′,4′-dihydropyrimidin-2′-yl)amino]-5-imidazolecarboxamide (4) Hydrochloride. A solution of the crude hydroxytetrahydropyrimidine hydrochloride 14 in 50 mL of dry DMF was heated briefly to incipient reflux with stirring under dry N_2. The solution was evaporated in vacuo to an orange residue which was then partially dissolved in 25 mL of hot EtOH. To this mixture was added 50 mL of hexane dropwise with stirring, and the resulting mixture was cooled at 0 °C for several hours. The precipitate was collected by filtration, washed thoroughly with hexane, and then dried overnight in vacuo to yield 464 mg (58%, based on 13) of a tan powder which was stored under N_2. Recrystallization from EtOH–MeOH yielded an analytical sample as a colorless solid: mp 266–267 °C dec; R_f 0.24 (solvent C); ^1H NMR ($(CD_3)SO$) δ 1.55 (s, 3, CH_3), 3.55 (br s, 2, NH exchangeable with D_2O), 4.00 (s, 2, CH_2), 6.15 (br s, 1, $CH_3C{=}CH$), 7.85 (s, 1, imidazole CH), 7.95 (br s, 2, NH exchangeable with D_2O), 9.55 (br s, 1, NH exchangeable with D_2O), 10.05 (br s, 1, NH exchangeable with D_2O), 10.45 (br s, 1, NH exchangeable with D_2O); field-desorption mass spectrum (15 mA), m/e 221 (MH^+), 220 (M^+, base peak), 219 (MH^+ – H_2), 218 (M^+ – H_2).

Anal. Calcd for $C_9H_{13}ClN_6O$: C, 42.10; H, 5.11; Cl, 13.81; N, 32.74. Found: C, 41.83; H, 5.34; Cl, 13.76; N, 32.45.

4-[(5′-Methylpyrimidin-2′-yl)amino]-5-imidazolecarboxamide (5). Method A. A mixture of the dihydropyrimidine 4 as the hydrochloride (90 mg, 0.35 mmol) in 50 mL of MeOH was carefully adjusted to pH 6.5–7 (H_2O-moistened pH paper) with sodium methoxide in MeOH, and the resulting solution was stirred at room temperature with exposure to air. The progress of the reaction was followed by TLC on silica using solvent C, and after the starting material was almost completely gone (over 100 h) the brown solution was evaporated to dryness. The residue was digested with hot 1-propanol and the solution was evaporated to dryness to afford a dark brown solid. This was applied to a 20 × 20 cm silica gel preparative TLC plate and eluted continuously with EtOH[30] until no more UV-absorbing material would move from the baseline. The major band was collected, the product was eluted with MeOH, and the solution was evaporated in vacuo to afford a discolored solid which was recrystallized from a minimal amount of hot H_2O to yield 5 mg (7%) of a grey crystalline solid: mp 223–225 °C dec; R_f 0.41 (solvent C); ^1H NMR (CD_3OD) δ 2.24 (s, 3, CH_3), 3.30 (CD_2HOD), 4.88 (CD_3OH), 7.40 (s, 1, imidazole CH), 8.40 (s, 2, pyrimidine CH's); mass spectrum, m/e 218 (M^+, base peak), 201 (M^+ – NH_3); high-resolution mass spectrum, calcd for $C_9H_{10}N_6O$ (M^+) 218.0915, obsd 218.0919, calcd for $C_9H_7N_5O$ (M^+ – NH_3) 201.0650, obsd 201.0650.

Method B. A solution of the dihydropyrimidine 4 as the hydrochloride (74 mg, 0.29 mmol) in 12 mL of MeOH was allowed to stand at room temperature for 3.5 months, during which the progress of the oxidation was followed by TLC in solvent C. The solution was then applied directly to a 30 × 3 cm column of Dowex 21K anion-exchange resin (OH^- form). The column was washed with 500 mL of MeOH and then with 500 mL of H_2O and was finally eluted with 5% aqueous acetic acid. The UV-absorbing fractions were pooled and evaporated overnight to a yellow solid, which was mostly dissolved in 100 mL of MeOH. The mixture was filtered, and the filtrate was evaporated to dryness after addition of 30 mL of toluene. The resulting off-white solid was dissolved in a minimal amount of hot EtOH (ca. 30 mL), then 50 mL of petroleum ether was added dropwise with stirring, and the resulting mixture was cooled at −10 °C overnight and filtered. The filtrate was evaporated and the almost colorless residue was dried in vacuo to afford 53 mg of the pyrimidine (>80% purity) which was identical with the product obtained in method A as determined by TLC, ^1H NMR, and mass spectrometry.

1,N^2-(2-Methyldihydroallylidene)guanine Hydrochloride (15). A solution of the dihydropyrimidine (4) hydrochloride (106 mg, 0.41 mmol) in 22 mL of dry DMF was heated to incipient reflux with stirring under dry N_2 for 15 h. The reaction was cooled to room temperature to afford a brown solution of the ring-closed product (15) which was used directly for the next step.

A sample of the **free base** for analysis was prepared as follows. A solution of 15 obtained as described in the procedure above was evaporated to dryness, and then the solid was almost completely dissolved in 2 mL of hot H_2O with addition of a minimal

(30) Continuous elution was accomplished by using a Short Bed/Continuous Development Chamber, Regis Chemical Company, Morton Grove, IL.

amount of 6 M HCl (about 3 drops). The solution was carefully adjusted to pH 7 with 1 M NaOH, and the resulting mixture was filtered. The filtrate was reduced in vacuo to 2 mL and then cooled at 3 °C overnight. The resulting precipitate was collected by filtration and dried at 0.1 torr to afford a powder: dec >235 °C without melting; R_f 0.43 (solvent C); ^{1}H NMR ($(CD_3)SO/D_2O$) δ 1.65 (s, 3, CH_3, isomer 1), 1.75 (s, 3, CH_3, isomer 2), 3.95 (s, 2, CH_2, isomer 2), 4.40 (s, 2, CH_2, isomer 1), 6.00 (s, 1, C=CH, isomer 1), 7.15 (s, 1, C=CH, isomer 2), 7.70 (s, 1, imidazole CH, isomer 2), 7.80 (s, 1, imidazole CH, isomer 1); mass spectrum, m/e 218 (contaminant, 5), 203 (M^+), 201 ($M^+ - H_2$, base peak); high-resolution mass spectrum, calcd for $C_9H_9N_5O$ 203.0807, obsd 203.0802.

1,N^2-(2-Methylallylidene)guanine (7-Methyl-10-oxo-9,10-dihydropyrimido[1,2-a]purine) (3a). To a solution of the crude dihydro compound (15) in dry DMF as described earlier was added 150 mg of activated MnO_2[31] and the mixture was stirred at 55 °C under dry N_2. After 36 h, an additional 80 mg of MnO_2 was added and the progress of the reaction was followed by TLC using solvent C. The reaction was allowed to proceed for an additional 15 h after which TLC indicated that essentially all the starting material had been converted to a single fluorescent product. The mixture was filtered through Celite and the solid was washed with hot DMF (5 × 6 mL). The filtrate and washings were combined and evaporated in vacuo, and then the residue was dissolved in 10 mL of 1 M HCl and carefully adjusted to pH 7 with 2 M NaOH. The solution was evaporated to dryness and coevaporated with absolute MeOH (1 × 20 mL). The residue was extracted with hot absolute EtOH (2 × 20 mL), and then the extracts were combined and evaporated. The remaining solid was mostly dissolved in 3 mL of boiling EtOH, then 20 mL of petroleum ether was added protionwise with swirling, and the resulting precipitate was triturated. Cooling for 1 h at −10 °C followed by filtration and drying at 0.1 torr gave the product as a powder (44 mg, 53% based on 4). This material was compared with an authentic sample of the guanine–methylmalondialdehyde adduct[19] and was found to be identical by TLC in three systems (R_f 0.38, solvent A; 0.63, solvent B; 0.37, solvent C), mass spectrometry, UV, and "mixed" ^{1}H NMR.

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Registry No. 3a, 57325-61-8; 4·HCl, 75993-48-5; 5, 75993-49-6; 9, 36056-90-3; 10, 75993-50-9; 11, 75993-51-0; 12, 10333-88-7; 13, 75993-52-1; 13 picrate, 75993-53-2; 14·HCl, 75993-54-3; 15 (isomer 1), 75993-55-4; 15·HCl (isomer 1), 75993-56-5; 15 (isomer 2), 75993-57-6; 15·HCl (isomer 2), 75993-58-7.

(31) The sample was a gift from the Carus Chemical Co., LaSalle, IL, through Mr. Lyle Wright.

A ^{13}C NMR Method To Determine the Origin of Cross-linked Chloromethyl Polystyrenes Used in Polymer-Supported Synthesis[1]

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Chloromethyl polystyrene cross-linked with 1–2% divinylbenzene is the most commonly used support for solid-phase peptide synthesis, polymer-bound organic synthesis, polymer-bound transition metal complex catalysis, and polymer-bound phase-transfer catalysis. Most researchers obtain chloromethyl polystyrene from commercial sources because the common chloromethylating reagent, chloromethyl methyl ether (and its unavoidable contaminant bis(chloromethyl) ether), is a potent cancer-suspect agent. Chloromethyl polystyrene could be made by chloromethylation of cross-linked polystyrene under a wide variety of conditions (Lewis acid, solvent, temperature, time) or by copolymerization of styrene, divinylbenzene, and chloromethylstyrene, yet suppliers usually do not inform customers about the manufacturing processes for their products. We report here a method that enables one to identify whether such material was prepared by the chloromethylation method or the copolymerization method.

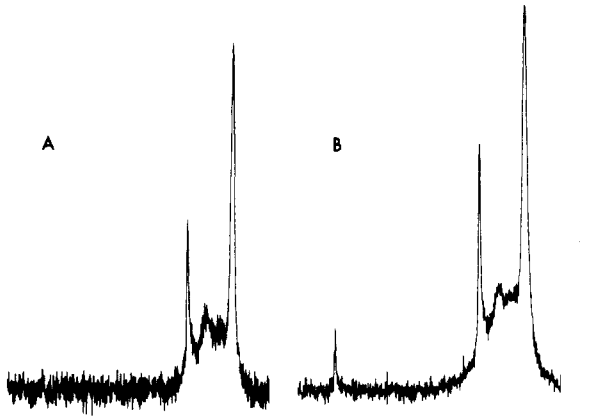

Figure 1. (A) ^{13}C[^{1}H] NMR spectrum of aliphatic carbon atoms of polymer 1. Peak assignments are as follows: ipso aromatic, 145.2 ppm; ortho and meta, 127.6; para, 125.5; CH_2Cl, 46.3; backbone CH, 40.3; backbone CH_2, 40–47. (B) ^{13}C[^{1}H] NMR spectrum of polymer 5, Bio-Beads SX-1 chloromethylated, 1.19 mmol of Cl/g, control no. 14137. The small peak at 65.1 ppm is due to CH_2OH, which arises from partial hydrolysis of chloromethyl groups during manufacture.

Table I lists composition, ^{13}C NMR line widths of polymer gels swelled in $CDCl_3$, and weight percent polymer of gels swelled in chloroform and in toluene. Five samples were prepared by us, and three were from commerical sources. The notable differences in ^{13}C NMR spectra of polymers with the same nominal degree of cross-linking are that poly(styrene-*co*-(chloromethyl)styrene) has narrower backbone methine carbon line widths and wider chloromethyl carbon line widths than those of chloromethylated polystyrene. A typical ^{13}C NMR spectrum appears in Figure 1. The greater line width of the chloromethyl carbon in poly(styrene-*co*-(chloromethyl)styrene) is due to the use of an approximately 60:40 mixture[2] of *m*- and *p*-(chloromethyl)styrene in copolymerization compared with the >90% para selectivity expected in chloromethylation of polystyrene with Lewis acids.[3] Although the meta and para isomer peaks are not resolved in 25.2-MHz ^{13}C NMR spectra of gel polymers, the isomeric mixture gives wider lines. The greater line widths of backbone methine carbon peaks of chloromethylated polystyrenes are probably due to methylene cross-linking introduced during the chloromethylation process. Chloromethylation of soluble, uncross-linked polystyrene carried to high conversion produces insoluble polymer.[4]

(1) Supported by the U.S. Army Research Office.

(2) Dow Chemical Co., product specifications for vinylbenzyl chloride.
(3) Olah, G. A.; Tolgyesi, W. S. In "Friedel-Crafts and Related Reactions"; Olah, G. A., Ed., Wiley-Interscience: New York, 1964; Vol. 2, pp 659–784.
(4) Pepper, K. W.; Paisley, H. M.; Young, M. A. *J. Chem. Soc.* **1953**, 4097–4105.

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