Pyrimidines. 14. Novel Pyrimidine to Pyrimidine Transformation Reactions and Their Application to *C*-Nucleoside Conversion. A Facile Synthesis of Pseudoisocytidine[1,2]

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Novel pyrimidine to pyrimidine transformations by nucleophilic displacement of the N_1–C_2–N_3 portion of 1,3-dialkyluracils (I) by the N–C–N fragment of several 1,3-ambident nucleophiles have been investigated. Treatment of 1,3-dimethyluracil (Ia) with guanidine in refluxing ethanol afforded isocytosine (IIa). The ease with which the reaction occurs depends on the electronic nature of the substituent at C-5 and C-6 as well as the steric environment at C-6. Treatment of Ia with methylguanidine gave 2-*N*-methylisocytosine (III) (59%) and 1-methylisocytosine (IV) (19%). Compound Ia was also converted into uracil (V) and 2-thiouracil (VI) by treatment with urea and thiourea, respectively, in ethanol in the presence of sodium ethoxide. 1-Alkylated 2-thiouracils (VIb and VIc) were obtained as the major products when Ia was treated with 1-methylthiourea or 1-*n*-butylthiourea. Treatment of Ia with excess 1,3-dimethylthiourea afforded 1,3-dimethyl-2-thiouracil (VId). A plausible mechanism for the transformation of I to VI is offered. When Ia was treated with *S*-ethylthiuronium bromide, 2-*N*-cyanoisocytosine (VII) was obtained. Treatment of Ia with formamidine, acetamidine, benzamidine, or 1,1-dimethylurea in base caused decomposition of the nucleophilic reagents and unchanged Ia was recovered. Uracil, 1-methyluracil, or 3-methyluracil could not be converted into isocytosine by treatment with guanidine under various conditions. Application of this transformation reaction to 1,3-dimethylpseudouridine (VIII) gave the antileukemic agent pseudoisocytidine (IXa) in good yield when treated with guanidine. 2-*N*-Methylpseudoisocytidine (IXb) and 2-thiopseudouridine (X) were also prepared by treatment of VIII with *N*-methylguanidine and thiourea, respectively.

Certain uracil derivatives exhibit high reactivity toward nucleophilic reagents, such as NaSH,[3] Na_2SO_3,[4] $NaHSO_3$,[4,5] RSH,[4-6] ROH,[7] RNH_2,[8] NaCN,[9] and NaN_3,[10] bringing about addition, addition–elimination, cine substitution, or ring formation reactions. These reactions are known to occur via initial attack of a nucleophile on the pyrimidine C-6 position, giving rise to a 5,6-dihydropyrimidine intermediate from which the respective product is formed. In these transformations, the arrangement of the atoms in the pyrimidine ring is unchanged *throughout* the reaction.

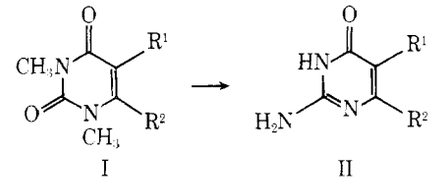
Pyrimidine to pyrimidine ring transformation by replacement of one ring nitrogen with another nitrogen atom has long been known (viz., the Dimroth rearrangement and its related reactions).[11] Replacement of a pyrimidine ring carbon by an exocyclic carbon atom by the Dimroth type mechanism has also been reported.[12]

Pyrimidine to pyrimidine transformation by replacement of the N_1–C_2 or N_1–C_2–N_3 portion of the pyrimidine with 1,2- or 1,3-ambident nucleophiles, however, has not been known until very recently.[13,14] Oostveen et al.[13] reported the formation of 2-amino-4-phenylpyrimidine and 2-amino-5-phenylpyrimidine by treatment of 1-methyl-4-phenylpyrimidinium iodide and 1-methyl-5-phenylpyrimidinium iodide with cyanamide, *O*-methylisourea, or *S*-methylisothiourea in basic media. In these reactions the two-atom fragment (N_1–C_2) of the pyrimidine ring is replaced by an N–C fragment of the reagent. They[13] also reported the conversion of 1-methylpyrimidinium iodide into 2-phenylpyrimidine and 2-*tert*-butylpyrimidine by treatment with benzamidine or pivalamidine in base. In these reactions the N_1–C_2–N_3 fragment of the pyrimidine is replaced by the N–C–N fragment of the amidine. During the course of our investigations into methods for simple pyrimidine to pyrimidine transformations that would be applicable to *C*-nucleoside conversions, we developed a novel ring transformation reaction which should have wide usage in the pyrimidine area in general and in the *C*-nucleoside area in particular. Preliminary communications on this subject have appeared.[2,14]

Uracil and cytosine derivatives undergo ring transformation with hydrazine or hydroxylamine to give the corresponding pyrazoles and isoxazoles. These nucleophiles selectively attack the pyrimidines in nucleic acid chains; thus these reagents have been used extensively in the chemical modification of nucleic acids.[15] In these transformations, the 1,2-ambident nucleophiles attack the pyrimidine C-6 position and then replace the N_1–C_2–N_3 portion of the pyrimidine by two atoms leading to five-membered ring heterocycles.

However, when uracil was treated with 1,3-ambident nucleophile, such as guanidine or thiourea, under various conditions, no reaction occurred; i.e., the N_1–C_2–N_3 portion of uracil was not replaced by the N–C–N fragment of the nucleophiles. The inability of 1,3-ambident nucleophiles to attack uracil may be due to anion formation in basic media which renders attack by nucleophiles more difficult. Consequently, 1,3-dialkylated uracils, such as 1,3-dimethyluracil (Ia) which lacks dissociable protons, should be more susceptible to nucleophilic attack. Indeed, Shugar and Fox[16] observed the instability of 1,3-dimethyluracil (Ia) in 1 N base, and later a mechanism which accounts for this instability was proposed by Santi et al.[17] Whatever the detailed mechanism is, Ia is attacked by hydroxyl ion nucleophiles at the C-4 and C-6 positions with the liberation eventually of 1,3-dimethylurea and formylacetic acid and products therefrom.[18] The susceptibility of 1,3-dialkyl uracils to 1,3-ambident nucleophiles was, therefore, investigated.

Treatment of 1,3-dimethyluracil (Ia) with ~7 molar excess of guanidine in refluxing ethanol for 18 h afforded 2-amino-4-hydroxypyrimidine (IIa, isocytosine) in good yield. The ease



I II

a, $R^1 = R^2 = H$
b, $R^1 = CH_3$; $R^2 = H$
c, $R^1 = H$; $R^2 = CH_3$
d, $R^1 = F$; $R^2 = H$
e, $R^1 = Br$; $R^2 = CH_3$

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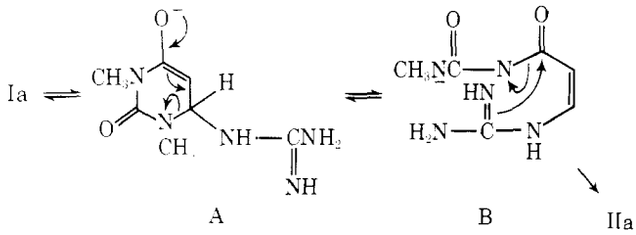
with which the reaction occurs depends on susceptibility of the pyrimidine C-6 position to nucleophilic reagents. Therefore the electronic nature of the substituent at C-5 and C-6 as well as the steric environment at C-6 will affect the ease of the reaction. Thus 5-fluoro-1,3-dimethyluracil (Id) was converted readily into 5-fluoroisocytosine (IId) in a few hours in refluxing ethanol, whereas transformation of 1,3-dimethylthymine (Ib) to 5-methylisocytosine (IIb) required more stringent conditions (such as fusion with guanidine at 80–90 °C). Also, while conversion of 1,3,6-trimethyluracil (Ic) into 6-methylisocytosine (IIc) required fusion conditions with guanidine, 5-bromo-1,3,6-trimethyluracil (Ie) was readily converted into 5-bromo-6-methylisocytosine (IIe) by treatment with guanidine in refluxing ethanol. In the case of Id or Ie, the isolated yield of the product was poor (~20%) due probably to participation of the C-5 halogen substituent in side reactions with guanidine.

Reaction of 1,3-dimethyluracil (Ia) with methylguanidine in refluxing ethanol gave a mixture from which 2-*N*-methylisocytosine (III) (59%) and 1-methylisocytosine (IV) (19%) were isolated. The isomer, 3-methylisocytosine, was not detected in the reaction mixture.

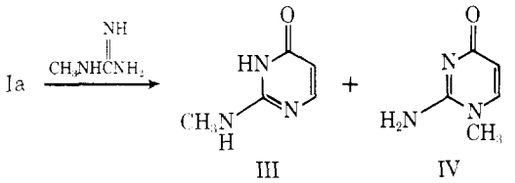
A plausible mechanism[14] to explain the above results may be formulated as shown for the conversion of Ia to IIa via postulated intermediates A and B. The mechanism proposed



A B IIa

above has close similarity to the one proposed for the conversion of 1-methylpyrimidinium iodide into a 2-substituted pyrimidine with an amidine nucleophile.[13]

Formation of two products (III and IV) in the reaction of Ia and methylguanidine is probably due to competition for

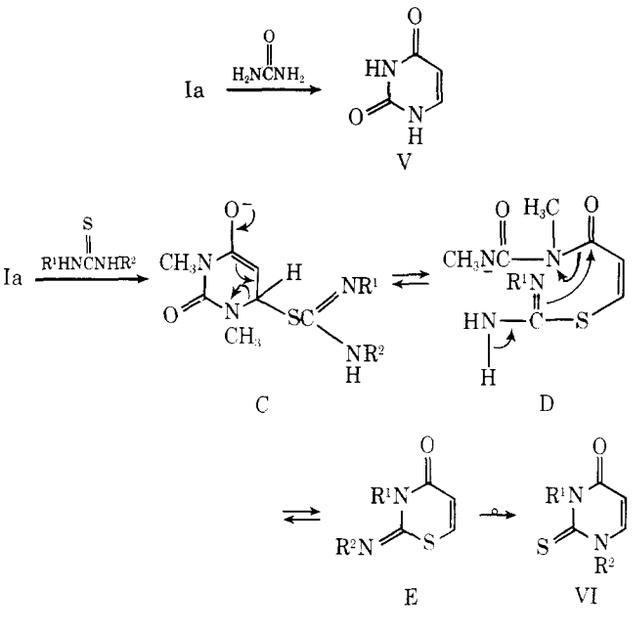


III IV

attack on C-6 of Ia between the stronger nucleophile (CH_3NH group) and sterically less-hindered nucleophile (NH_2 group) of the reagent.

Urea and thiourea, which are weaker bases than guanidine, did not react with Ia in ethanol. In the presence of sodium ethoxide, however, the reaction with these reagents proceeded smoothly with the formation of uracil (V) and 2-thiouracil (VIa), which were isolated in high yields. Reaction of Ia with 1-methylthiourea or 1-*n*-butylthiourea was also investigated. The major products were 1-methyl-2-thiouracil (VIb) and 1-*n*-butyl-2-thiouracil (VIc). The presence of 3-substituted-2-thiouracils was detected in the reaction mixture by ^1H NMR, but attempts to isolate these minor products failed. This method of synthesis of 1-alkyl-2-thiouracils is much simpler than the known multistep procedures.[19] Also, treatment of Ia with 1,3-dimethylthiourea in ethanolic sodium ethoxide gave 1,3-dimethyl-2-thiouracil (VId).

The reaction of I with thioureas probably proceeds via initial attack on C-6 of I by the sulfur nucleophile (which is more nucleophilic than nitrogen) to give C followed by ring opening at the N_1–C_6 bond to D. Subsequent attack by the sterically less-hindered nitrogen nucleophile in D on C-4 with

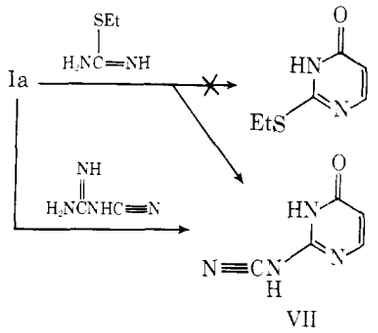


V C D E VI

a, $R^1 = R^2 = H$
b, $R^1 = H$; $R^2 = CH_3$
c, $R^1 = H$; $R^2 = n$-Bu
d, $R^1 = R^2 = CH_3$

liberation of 1,3-dimethylurea would result in the formation of the 1,3-thiazine intermediate E which, then, would rearrange to 2-thiouracils (VI) in the presence of excess alkali. Alkali-catalyzed rearrangements of 1,3-thiazines to 2-thiopyrimidines are known.[20]

Treatment of Ia with excess *S*-ethylthiuronium bromide in ethanolic sodium ethoxide afforded a crystalline product with the following characteristics. The UV spectral behavior was similar to that of isocytosine, but different from that of 2-alkylthiouracil.[21] The IR spectrum of the product showed the presence of a CN group (2190 cm^{-1}). Elemental analyses (C, H, N) were consistent with 2-*N*-cyanoisocytosine (VII). The same compound VII was obtained by treatment of Ia with

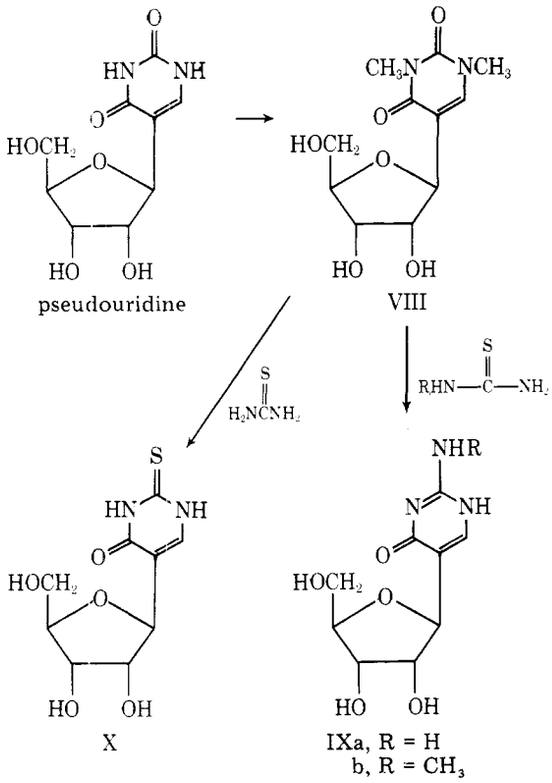


VII

cyanoguanidine. The formation of VII from the reaction of Ia with *S*-ethylthiuronium bromide in base may be explained by the instability of *S*-ethylisothiourea, which readily decomposes into cyanamide and ethyl sulfide.[22] Dimerization of cyanamide afforded cyanoguanidine, which then reacted with Ia to give VII.

Treatment of Ia with formamidine, acetamidine, or 1,1-dimethylurea in base caused decomposition of the nucleophilic reagents and unchanged Ia was recovered from the reaction mixture. Attempts to convert uracil, 1-methyluracil, or 3-methyluracil to isocytosine by treatment with guanidine under various conditions were uniformly unsuccessful. These failures are probably due to the anion formation by these uracils in a strongly basic media and which inhibits attack by nucleophiles.

5-(β-D-Ribofuranosyl)isocytosine (IXa, pseudoisocytidine)



was synthesized in our laboratory[23] and was found to be active against certain mouse leukemias.[24] This *C*-nucleoside IXa is currently under phase I clinical investigation at this center. The original synthesis of IXa consists of four or five reactions from D-ribose.[23] Application of this pyrimidine to pyrimidine transformation reaction to 1,3-dimethylpseudouridine (VIII), which was obtained in good yield by treatment of pseudouridine with dimethylformamide dimethyl acetal,[25] afforded pseudoisocytidine (IXa) in one step and the product was isolated as the crystalline hydrochloride salt in ~60% yield. Reaction of VIII with methylguanidine gave crystalline 2-*N*-methylpseudoisocytidine (IXb), which would be difficult to synthesize by other methods. A small amount of the α isomer of IXb was also isolated in crystalline form.

Treatment of VIII with thiourea in ethanolic sodium ethoxide solution under reflux gave crystalline 2-thiopseudouridine sodium salt (X) in good yield. The ^{1}H NMR spectrum of the salt, however, showed the product was contaminated with ~5% of the α isomer. After removal of the sodium ion, pure 2-thiopseudouridine (β) was obtained as a powder. The ^{1}H NMR and UV spectra of this sample were identical with those of 2-thiopseudouridine prepared previously from D-ribose.[23]

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are corrected. ^{1}H NMR spectra were obtained on a JEOL J1M-PET-100 spectrometer, spectrometer, and Me_4Si was the internal standard for organic solvents and $Me_3Si(CH_2)_3SO_3Na$ for D_2O; chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet); δ and J values are first order. TLC was performed on a microscope slides coated with silica gel GF_{254} (Merck) and column chromatography on silica gel G. UV spectra were measured on a Cary Model 15 spectrometer and IR spectra were recorded on a Perkin-Elmer Infracord using pressed KBr pellets. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Conversion of 1,3-Dimethyluracils (I) into Isocytosines (II). Method A. Guanidine HCl (10 g) was stirred in 0.7 M ethanolic sodium ethoxide for 10 min and insoluble NaCl was removed by filtration. To the filtrate was added 0.01 mol of I and the mixture was refluxed under nitrogen. The reaction was followed by TLC ($CHCl_3$–MeOH, 5:1) and after the starting material disappeared the solvent was removed in vacuo. The residue was processed, depending on the dimethyluracil (I) employed, as described below.

Isocytosine (IIa). The residue of the reaction of Ia with guanidine was dissolved in 20 mL of water and the solution was passed through a column of Amberlite IRC-50 (H^+) (50 × 5.5 cm). The column was washed with water and the UV absorbing fractions were combined and evaporated in vacuo. The residue was triturated with ethanol and the solid was recrystallized from water to give 0.73 g of IIa (66%): mp 245–247 °C. The IR spectrum of this product was identical with that of an authentic sample.

5-Fluoroisocytosine (IId). The residue of the reaction of 1,3-dimethyl-5-fluorouracil[26] (Id) was dissolved in water (20 mL), the solution was poured on a column of Amberlite IRC-50 (H^+) (50 × 5.5 cm), and the product was eluted with water. The UV absorbing fractions were combined and evaporated in vacuo and the residue was further dried by several coevaporations with ethanol and chromatographed on a silica gel column (20 × 2.2 cm) using $CHCl_3$–MeOH (10:1) as the eluent. 5-Fluoroisocytosine (IId) was obtained after evaporation of the UV absorbing fractions and recrystallization of the residue from water: 230 mg (18%); mp 274–276 °C dec (lit.[27] mp 271–274 °C dec).

5-Bromo-6-methylisocytosine (IIe). The residue of the reaction of Ie and guanidine was triturated with water to give a solid which was crystallized from water. Crystalline IIe (430 mg, 22%) was obtained: mp 250 °C dec (lit.[28] mp 250 °C dec).

Isolation of 1,3-Dimethylurea. The residue of the reaction of Ia with guanidine was dissolved in 20 mL of water. The solution was neutralized with dry ice and extracted with $CHCl_3$ (3 × 50 mL). The combined extacts were dried over Na_2SO_4 and evaporated in vacuo and the residue was triturated with a small amount of benzene. Crystalline 1,3-dimethylurea (205 mg) was obtained, mp 102–103 °C, which was not depressed on admixture of an authentic sample.

Method B. 5-Methylisocytosine (IIb) and 6-Methylisocytosine (IIc). Guanidine hydrochloride (10 g) was stirred in 0.7 M sodium ethoxide in ethanol (100 mL) for 10 min, and then insoluble NaCl was removed by filtration. The filtrate was concentrated in vacuo to a thin syrup. Trimethyluracil (Ib or Ic) (800 mg) was added to the syrup and the mixture was heated to 80–90 °C with stirring for 6 h under nitrogen. The reaction mixture was diluted with water (20 mL), the solution was neutralized with Amberlite IRC-50 (H^+), and the neutral solution was evaporated to dryness in vacuo. The residue was crystallized from water. 5-Methylisocytosine (IIb) (590 mg, 91%) had mp 281–283 °C dec (lit.[28] mp 277–279 °C dec). 6-Methylisocytosine (IIc) was obtained in 45% yield (320 mg), mp 290–292 °C dec (lit.[29] mp 285–290 °C dec).

Reaction of Ia with Methylguanidine. To an ethanolic sodium ethoxide solution (prepared by dissolving 2.2 g of Na in 60 mL of ethanol) were added Ia (1.4 g, 0.01 mol) and methylguanidine sulfate (12.2 g, 0.1 mol). The mixture was refluxed overnight and then was allowed to cool to room temperature. Precipitated sodium sulfate was removed by filtration and the filtrate was evaporated in vacuo to a syrup which was dissolved in 20 mL of water and passed through a column of Amberlite IRC-50 (H^+) (55 × 5.5 cm). The eluate was evaporated to give a solid residue (1.16 g). TLC ($CHCl_3$–MeOH, 5:1) showed the residue contained one major and one minor component. Separation of the components was performed by silica gel column chromatography (50 × 2.2 cm) using $CHCl_3$–MeOH (10:1) as the eluent. The major product, 2-*N*-methylisocytosine (III) (790 mg, 59%), was obtained after recrystallization of the first fraction from ethanol: mp 214–215 °C (lit.[30] mp 214–215 °C). From the second fraction, 250 mg (19%) of 1-methylisocytosine (IV) was obtained after recrystallization from ethanol: mp 283–285 °C (lit.[30] mp 283–285 °C).

Conversion of Ia into Uracil (V). To a solution of ethanolic sodium ethoxide (1 M, 100 mL) were added Ia (1.4 g, 0.01 mol) and urea (6.0 g, 0.1 mol). The mixture was refluxed with stirring overnight and then the solvent was removed by evaporation in vacuo. The residue was dissolved in water (50 mL) and the solution was acidified with concentrated HCl to precipitate uracil (V). 0.7 g (64%), which was identical with an authentic sample of uracil with respect to UV and IR spectra.

Reaction of Ia with *n*-Butylurea. A mixture of Ia (700 mg) and *n*-butylurea (5.8 g) in ethanolic sodium ethoxide (prepared by dissolving 2.0 g of Na in 50 mL of ethanol) was refluxed for 20 h and the solvent was removed in vacuo. The residue was dissolved in water (50 mL) and the solution was extracted with $CHCl_3$ (2 × 50 mL). The aqueous layer was acidified with concentrated HCl and then extracted with $CHCl_3$ (2 × 50 mL). The latter $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated in vacuo to a syrup which was triturated with water. 3-*n*-Butyluracil (120 mg) crystallized and was collected by filtration: mp 151–152 °C (lit.[31] mp 152–153 °C).

The filtrate was concentrated in vacuo to a syrup which was triturated with ether to give crystalline 1-*n*-butyluracil (125 mg): mp 101–103°C (lit.[32] mp 100–102 °C).

Conversion of Ia into 2-Thiouracils (VI). A mixture of Ia (0.01 mol) and thiourea derivative (0.03 mol) in ethanolic sodium ethoxide (prepared by dissolving 700 mg of Na in 50 mL of ethanol) was refluxed overnight, after which the solution was evaporated in vacuo. The residue was processed, depending on the thiourea employed, as described below.

2-Thiouracil (VIa). The residue was dissolved in water (20 mL) and the solution was acidified with concentrated HCl. The precipitate was collected by filtration and recrystallized from water to give 1.16 g (91%) of VIa, identical with an authentic sample of 2-thiouracil with respect to LV and IR spectral characteristics.

1-Methyl-2-thiouracil (VIb). When *N*-methylthiourea was employed, the residue was not soluble in water (20 mL). The suspension was acidified with concentrated HCl and the solid was collected by filtration. Recrystallization of the solid from ethanol gave VIb: 1.04 g (95%); mp 226–227 °C (lit.[19] mp 228 °C).

1-*n*-Butyl-2-thiouracil (VIc). The residue of the reaction of Ia with *N*-*n*-butylthiourea was dissolved in water (50 mL) and the solution was acidified with concentrated HCl. The oily precipitates were extracted with ether (2 × 50 mL) and the extracts were dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on a silica gel column (50 × 2.2 cm) using benzene–ethyl acetate (5:1) as the eluent. Crude 1-*n*-butyl-2-thiouracil (VIc) was recrystallized from water: 1.5 g (81%); mp 132–133 °C; UV λ_{max} (pH 1–7) 269, 290 (sh) nm (ϵ 12 300, 10 300), λ_{min} (pH 1–7) 242 (4600), λ_{max} (pH 13) 236, 270 (20 300, 14 500), λ_{min} (pH 13) 255 (12 400); ^{1}H NMR ($CDCl_3$) δ 0.98 (3, H, t, CH_3, spacing ~7.6 Hz), 1.20–1.94 (4 H, m, $CH_2\mathbf{CH_2CH_2}CH_3$), 4.18 (2 H, t, NCH_2, spacing ~7.6 Hz), 6.00 (1 H, dd, H-5, $J_{5,6} \sim 8.0$, $J_{3,5} \sim 2.2$ Hz; the latter coupling disappeared upon addition of D_2O), 7.24 (1 H, d, H-6).

Anal. Calcd for $C_8H_{12}N_2OS$: C, 52.16; H, 6.57; N, 15.21. Found: C, 52.10; H, 6.62; N, 15.16.

The mother liquor of recrystallization was evaporated to dryness. The ^{1}H NMR spectrum ($CDCl_3$) of the residue showed that it contained a small amount of 3-*n*-butyl isomer. In addition to all the signals for VIc, the following signals were observed: δ 4.37 (t, NCH_2, spacing ~7.6 Hz), 5.96 (d, H-5, $J_{5,6} \sim 8.0$ Hz), 7.10 (d, H-6).

1,3-Dimethyl-2-thiouracil (VId). When dimethylthiourea was used as the nucleophile, TLC ($CHCl_3$–MeOH, 5:1) of the reaction mixture showed three UV spots corresponding to VId, Ia, and 1,3-dimethylthiourea. The residue was dissolved in water (20 mL) and the aqueous solution was acidified with concentrated HCl and extracted with ether (2 × 50 mL). The ether extracts were dried (Na_2SO_4) and evaporated and the residue was chromatographed on a silica gel column (50 × 2.2 cm) using benzene–ethyl acetate (5:1) as the eluent. Compound VId (274 mg, 18%) obtained had mp 107–108 °C (lit.[19b] mp 109 °C).

2-Thiothymine from 1,3-Dimethylthymine (Ib). A mixture of 770 mg of Ib and 1.5 g of thiourea in ethanolic sodium ethoxide (prepared by dissolving 400 mg of Na in 30 mL of ethanol) was refluxed for 48 h. The solvent was removed by evaporation in vacuo and the residue was dissolved in water (10 mL). The aqueous solution was acidified with concentrated HCl. 2-Thiothymine which precipitated was collected and recrystallized from methanol: 470 mg; mp 264–267 °C dec (lit.[33] mp 265–267 °C dec).

Reaction of Ia with *S*-Ethylisothiourea. Isolation of 2-*N*-Cyanoisocytosine (VII). A mixture of Ia (1.4 g, 0.01 mol) and *S*-ethylthiuronium bromide (9.3 g, 0.05 mol) in 100 mL of 1 N ethanolic sodium ethoxide was stirred for 10 min at room temperature and insoluble NaBr was removed by filtration. The filtrate was refluxed for 24 h. On cooling the mixture, 530 mg of crystals separated, which were collected by filtration, dissolved in water (5 mL), and acidified with glacial acetic acid. The crystals that precipitated were collected: 480 mg; mp 295–300 °C (eff). The IR spectrum of this sample was identical with that of authentic 2-*N*-cyanoisocytosine (VII) prepared as described below.

2-*N*-Cyanoisocytosine (VII). A mixture of Ia (0.7 g, 5 mmol) and cyanoguanidine (1.26 g) in ethanolic sodium ethoxide (prepared by dissolving 0.35 g of Na in 50 mL of ethanol) was refluxed for 24 h and then evaporated in vacuo. The residue was dissolved in water (30 mL) and the solution was acidified with glacial acetic acid. Compound VII (640 mg, 93%) precipitated and was collected by filtration: mp 295–300 °C (eff); UV λ_{max} (pH 1) 241, 265 (sh) nm (ϵ 16 400, 8700), λ_{min} (pH 1) 219 (7300), λ_{max} (pH 7) 295, 243 (6900, 15 400), λ_{min} (pH 7) 268 (3000), λ_{max} (pH 13) 282, 246 (6400, 13 900), λ_{min} (pH 13) 267, 230 (5300, 9800).

Anal. Calcd for $C_5H_4N_4O$: C, 44.12; H, 2.96; N, 41.17. Found: C, 43.97; H, 3.06; N, 40.95.

1,3-Dimethylpseudouridine (VIII). A suspension of pseudouridine (1.0 g) in dimethylformamide dimethyl acetal (7 mL) was refluxed until a clear solution was obtained (~30 min). The solution was concentrated in vacuo to a syrup which was triturated with a small amount of acetone to give a solid (910 mg, 82%). Recrystallization of the crude precipitate from ethanol gave analytically pure VIII: mp 174 °C.

Anal. Calcd for $C_{11}H_{16}N_2O_6$: C, 48.52; H, 5.92; N, 10.29. Found: C, 48.63; H, 6.02; N, 10.38.

Pseudoisocytidine (IXa) from VIII. Guanidine hydrochloride (10.0 g, 0.1 mol) was added to 0.7 M sodium ethoxide in ethanol (100 mL) and the mixture was stirred at room temperature for 10 min and then filtered from sodium chloride. The filtrate was concentrated in vacuo below 30 °C. To the residue was added VIII (300 mg), and the mixture was heated at 80–90 °C under nitrogen for 50 min. Water (20 mL) was added and, after removal of a small amount of insoluble impurities by filtration, the filtrate was passed through a column of Amberlite IRC-50 (H^+) (30 × 3 cm) and the column was washed with water. The UV absorbing fractions were collected and evaporated in vacuo, and the residue was dissolved in a small amount of ethanol. Crystalline IXa (6 mg) precipitated and was collected by filtration: mp 192–192.5 °C (sintered), 193–194 °C (eff). The ^{1}H NMR (D_2O) spectrum of this sample was identical with that of pseudoisocytidine hydrochloride.[23]

The filtrate was evaporated to dryness in vacuo and the residue was dissolved in ~10% methanolic hydrogen chloride. Crystalline pseudoisocytidine hydrochloride, which precipitated out, was collected by filtration: mp 215–216 °C dec; 185 mg (60%). The ^{1}H NMR, UV, and IR spectra of this sample were identical with those of authentic pseudoisocytidine hydrochloride.[23]

2-*N*-Methylpseudosiocytidine (IXb). Methylguanidine sulfate (24.4 g) was stirred in 1.3 M ethanolic sodium ethoxide (150 mL) for 10 min and then Na_2SO_4 was removed by filtration. To the filtrate was added VIII (816 mg, 3 mmol), and the solvent was removed in vacuo to a syrup below 35 °C. The syrup was diluted with 10 mL of ethanol and the mixture was heated at 85–90 °C for 3 h under nitrogen and then concentrated to a syrup in vacuo. The residue was dissolved in water (30 mL) and neutralized by passing it through a column of Amberlite IRC-50 (H^+) (20 × 2.2 cm). The neutral solution was evaporated to dryness in vacuo and the residue was triturated with acetone. The solid obtained showed two spots on TLC ($CHCl_3$–MeOH, 4:1). After purification by silica gel column chromatography (50 × 2.2 cm) ($CHCl_3$–MeOH, 4:1), two UV absorbing fractions were obtained. Evaporation of the solvent of the first fraction gave 205 mg of the β isomer (IXb) as a powder. The HCl salt of IXb had: mp 207–208 °C dec; UV λ_{max} (pH 1) 265, 222 nm (ϵ 7200, 11 900), λ_{min} (pH 1) 244 (4500), λ_{max} (pH 7) 293, 222 (5500, 14 600), λ_{min} (pH 7) 252 (2400), λ_{max} (pH 13) 281, 233 (6200, 10 600), λ_{min} (pH 13) 257 (2800); ^{1}H NMR (D_2O) δ 2.87 (3 H, s, NCH_3), 3.77 (2 H, m, H-5′,5″), 4.03 (1 H, m, H-4′), 4.17 (1 H, t, H-3′, $J_{2',3'} \sim J_{3',4'} \sim 4.9$ Hz), 4.34 (1 H, t, H-2′, $J_{1',2'} \sim J_{2',3'} \sim 4.9$ Hz), 4.65 (1 H, d, H-1′), 7.73 (1 H, s, H-6).

Anal. Calcd for $C_{10}H_{15}N_3O_5$·HCl: C, 40.89; H, 5.49; N, 14.28. Found: C 40.86; H, 5.62; N, 13.89.

From the second fraction, the α isomer (75 mg) was obtained. After recrystallization from methanol, it had: mp 210 °C; UV λ_{max} (pH 1) 265, 223 nm (ϵ 6900, 11 900), λ_{min} (pH 1) 245 (4400), λ_{max} (pH 7) 295, 220 (4400, 16 200), λ_{min} (pH 7) 253 (2200), λ_{max} (pH 13) 282, 233 (6400, 10 800), λ_{min} (pH 13) 257 (2700); ^{1}H NMR (D_2O) δ 2.87 (3, H, s, NCH_3), 5.01 (1 H, narrow q, H-1′, $J_{1',2'} \sim 2.2$, $J_{1',6} < 0.5$ Hz), 7.64 (1 H, d, H-6, $J_{1',6}$). The overall spectral pattern was quite similar with that of α-pseudoisocytidine.[23]

Anal. Calcd for $C_{10}H_{15}N_3O_5$·$^1/_4H_2O$: C, 45.89; H, 5.97; N, 16.05. Found: C, 46.06; H, 6.16; N, 15.74.

2-Thiopseudouridine (X) from VIII. A mixture of VIII (544 mg, 2 mmol) and thiourea (760 mg, 10 mmol) in 1 M ethanolic sodium ethoxide (20 mL) was refluxed with stirring for 2 h. After cooling the mixture, the crystalline sodium salt of X (519 mg, 92%) was collected by filtration. The ^{1}H NMR (D_2O) spectrum showed that the crystalline sodium salt of X was contaminated with a small amount of the α isomer. The salt (100 mg) was dissolved in water (5 mL), the solution was placed on a column of Amberlite IRC-50 (H^+) (5 × 3 cm), and the column was washed with water. The UV absorbing fractions were combined and evaporated to dryness. The residue was triturated with cold ethanol. The white precipitate of X was collected by filtration. The ^{1}H NMR spectrum of X was identical with that of an authentic sample of 2-thiopseudouridine.[23]

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Registry No.—Ia, 874-14-6; Ib, 4401-71-2; Ic, 13509-52-9; Id, 3013-92-1; Ie, 15018-59-4; IIa, 674-97-5; IIb, 15981-91-6; IIc, 3977-29-5; IId, 1683-86-9; IIe, 6307-35-3; III, 22404-50-8; IV, 2080-17-3; V, 66-22-8; VIa, 141-90-2; VIb, 615-78-1; VIc, 64985-69-5; VIc (3-Bu isomer), 64975-70-8; VId, 1194-71-4; VII, 51741-99-2; VIII, 64272-68-0; IXa, 57100-18-2; IXa HCl, 59464-15-2; IXb HCl, 64975-71-9; IXb α isomer, 64999-53-7; X, 59464-18-5; guanidine hydrochloride, 14317-32-9; methylguanidine sulfate, 1866-88-2; urea, 57-13-6; butylurea, 592-31-4; 3-butyluracil, 28289-95-4; 1-butyluracil, 705-06-6; thiourea, 62-56-6; *N*-methylthiourea, 598-52-7; *N*-butylthiourea, 1516-32-1; 1,3-dimethylthiourea, 534-13-4; 2-thiothymine, 636-26-0; *S*-ethylthiuronium bromide, 1071-37-0; cyanoguanidine, 461-58-5; pseudouridine, 1445-07-4; dimethylformamide dimethyl acetal, 4637-24-5.

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Use of *o*- and *p*-Hydroxybenzyl Functions as Blocking Groups Which Are Removable with Base

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The successful development of the *o*- and *p*-hydroxybenzyl functions and the corresponding esters thereof as blocking groups which are removable with base is described. Specific illustrative examples include protecting and subsequently releasing 1-phenyl-2-tetrazoline-5-thione and thiosulfate anions.

In photography many compounds are used which react in some way with silver halide either as silver precipitants, complexers, or solvents. For example, 1-phenyl-5-mercaptotetrazole (PMT) (1) is a development restrainer and forms a very insoluble silver salt.[1] On the other hand, sodium thiosulfate (hypo) is a silver solvent and is used for fixing emulsions (dissolving undeveloped silver halide).[2] We became interested in preparing derivatives of these types of compounds which would be stable in a film system before processing the system with a highly alkaline developer fluid, but during such processing these same derivatives would have to release the active photographic species.

Esters of PMT are not hydrolytically stable. For example, the acetyl derivative readily hydrolyzes, since the PMT anion is a very good leaving group. PMT is a fairly strong acid, having a pK_a of 3.65.[3] In the solid state, the compound exists as the tautomeric 1-phenyl-2-tetrazoline-5-thione (**2**).[4]

There is considerable literature on the base instability of *o*- and *p*-hydroxybenzyl groups,[5] the decomposition going through quinone methide intermediates.[6] We decided to prepare *o*- and *p*-hydroxybenzyl chlorides or the corresponding esters as reagents to generate alkali-removable blocking groups for photographically active compounds.

The reagents with which we did the most work were *o*- and *p*-acetoxybenzyl chlorides **3**. These compounds are prepared in one step by reaction of the *o*- and *p*-hydroxymethylphenols with with acetyl chloride.[7] The isomeric *m*-hydroxymethylphenol does not undergo this reaction. It is desirable to have

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