# Synthesis of the 335-nm Photoproduct of Cytosine and 4-Thiouracil

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Application of the Vilsmeier formylation reaction to 4-methylpyrimidin-2-one (**4**) followed by treatment with hydroxylamine gave 4-(isoxazol-4-yl)pyrimidin-2-one (**5**), which was transformed to 4-(α-formylcyanomethyl)-pyrimidin-2-one (**6**) by rearrangement with alkali in 53% overall yield (from **4**). Methylation of **6** with NaH and MeI in dimethylformamide gave 4-(α-formylcyanomethyl)-1-methylpyrimidin-2-one (**2**) in 76% yield. Ethylation of **6** with triethyl orthoformate afforded a mixture of 4-(α-ethoxymethylenecyanomethyl)-2-ethoxypyrimidine (**9**) and 1-ethyl-4-(α-formylcyanomethyl)pyrimidin-2-one (**8**). The positions of alkylation in compounds **2** and **8** were established by nuclear Overhauser effect measurements. 5-(4-Pyrimidin-2-one)cytosine (**7a**), was obtained in low yield on fusion of the diethylated compound **9** or the cyanoaldehyde **6** lithium salt with urea, a subsequent acid hydrolysis being required with the former compound. A similar reaction with thiourea produced 5-(4-pyrimidin-2-one)-2-thiocytosine (**7b**). The synthesis of 5-(4-pyrimidin-2-one)cytosine (**7a**), identical with the product of irradiation of cytosine and 4-thiouracil at 335 nm, provides direct proof of the structure of this moiety as it is formed in *Escherichia coli* tRNAs by the photolinking of 4-thiouridine (base no. 8) and cytidine (base no. 13) under long wavelength ultraviolet irradiation.

Since it was found[1-3] that irradiation of several *Escherichia coli* tRNAs at 335 nm brings about covalent bond formation between the 4-thiouridine in position 8 and the cytidine in position 13 from the 5′-terminal end, the determination of the mode of the linkage has been of great importance in providing detailed information concerning the tertiary structure of the tRNAs. We have recently identified the binucleoside tRNA photoproduct as 5-(1-β-D-ribofuranosyl-4-pyrimidin-2-one)cytidine.[4] The corresponding bipyrimidine product, 5-(4-pyrimidin-2-one)cytosine, Pyo(4-5)Cyt[5] (**7a**), is obtained on irradiation (335 nm) of an aqueous solution of 4-thiouracil and cytosine at 4°.[6] Rhoades and Wang[7,8] have also reported formation of the same compound **7a** on irradiation (254 nm) of polycytidylic acid in aqueous solution (pH 4–7) followed by acid hydrolysis. We have further shown that identification of the single structure **7a** is sufficient to fix *one-third of the total tRNA tertiary structure*, subject only to assumptions as to normal double helix geometry.[6]

To confirm the structure of the photoproduct, 5-(4-pyrimidin-2-one)cytosine (**7a**), by independent means, we devised an unequivocal synthesis of Pyo(4-5)Cyt (**7a**) which also serves as the first in a set of general methods for the synthesis of substituted bipyrimidines. In outline, it was envisaged that the ring closure of the β-methoxyacrylonitrile derivative **3** with urea might give **7a** in a single step. There are many examples of the synthesis of cytosine derivatives by this method,[9] although Russell and Hitchings have shown that in particular α-methoxymethylenephenylacetonitrile does not react with thiourea or with *S*-ethylpseudothiourea.[10]

It is known that the methyl group at C-6 of a purine[11] or C-4 of a pyrimidine[12,13] is reactive to the Vilsmeier reagent. The preparation of the key intermediate in the present synthesis, 4-(α-formylcyanomethyl)-pyrimidin-2-one (shown arbitrarily in the enol form **6**), was based upon this earlier knowledge. Reaction of 4-methylpyrimidin-2-one (**4**) with 4 molar equiv of $COCl_2$–dimethylformamide (DMF) reagent followed by treatment with hydroxylamine at pH 4.5 gave crude 4-(isoxazol-4-yl)pyrimidin-2-one (**5**). The isoxazole **5** is sufficiently unstable that it undergoes isomerization to the cyanoaldehyde **6** on drying *in vacuo* at 25°. The wet crystals of **5** could be stored for 3 days at −25° without appreciable rearrangement. To obtain pure isoxazole **5**, an alternative route through 4-(α-diformylmethyl)pyrimidin-2-one (**1**) was attempted; however, the concomitant formation of 4-(α-formylcyanomethyl)-pyrimidin-2-one (**6**) in the product could not be avoided. Treatment of the isoxazole **5** with aqueous *N* NaOH solution at 25° for 30 min gave **6** in overall yield of 53% from **4**. When alkylation of the enolic oxygen of the cyanoaldehyde **6** was attempted under a variety of conditions[14,15] using $CH_2N_2$, MeI and NaH, and triethyl orthoformate, reaction occurred primarily at N-1 of the pyrimidine. Thus, reaction of the cyanoaldehyde **6** sodium salt with MeI in DMF afforded 4-(α-formylcyanomethyl)-1-methylpyrimidin-2-one (**2**) in 76% yield. Compound **6** has five potential alkylation sites: two ring nitrogens, the oxygens of the enolic aldehyde and the ring carbonyl, and the exocyclic α-methylene. The occurrence of methylation on N-1 was proved by the nmr spectra [$(CD_3)_2SO$], which showed the methyl protons at δ 3.4, and by a nuclear Overhauser effect (NOE)[16] between the C-6 proton and the methyl protons. Thus, irradiation of the methyl signal produced a signal intensity increase (13%) in the C-6 proton (δ 8.07 ppm). The magnitude of the

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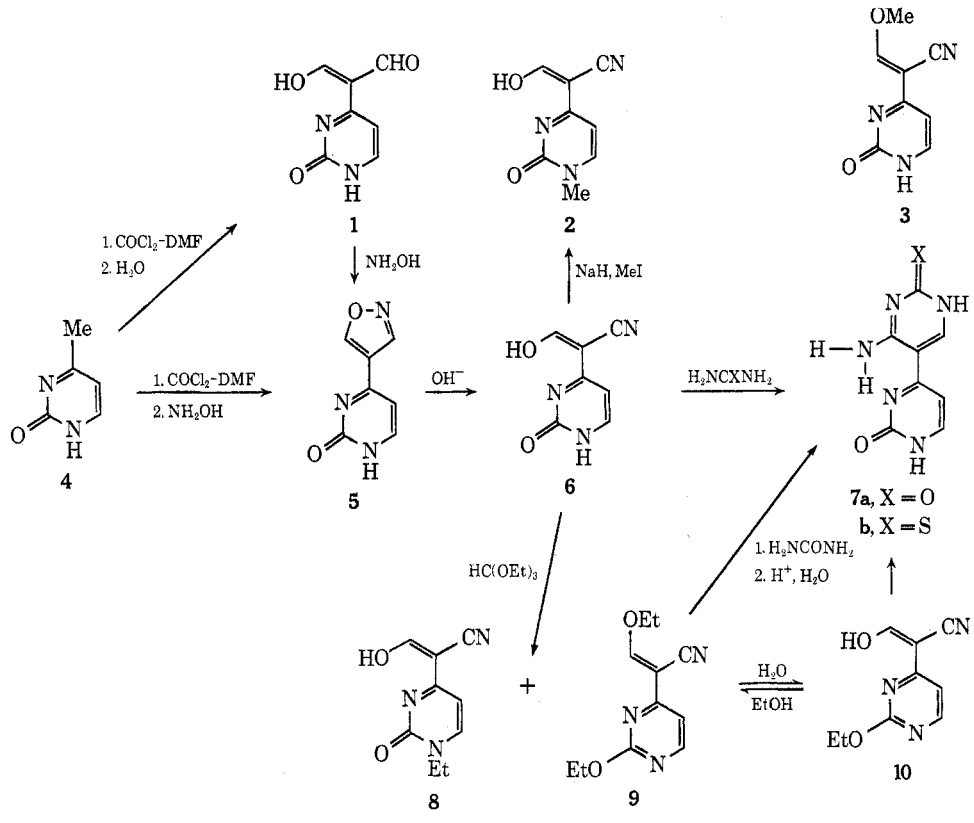

enhancement lies in the range observed for the pyrimidine nucleosides.[17,18]

Treatment of the cyanoaldehyde **6** with triethyl orthoformate under reflux gave two products: A, mp 95–97°, and B, mp 175–177°. The lower melting product showed two sets of *O*-ethyl protons in the nmr spectrum [$(CD_3)_2SO$]. The two methyl triplets were observed at $\delta$ 1.33 and 1.35 ($J$ = 7 Hz) and the two methylene quartets at 4.33 and 4.43 ($J$ = 7 Hz). The C-5 proton appeared as a doublet at $\delta$ 7.03 ppm ($J$ = 5 Hz), which is at ~0.9 ppm lower field[19] than was observed in **6** (6.02) and **2** (6.11). The uv spectrum ($\lambda_{max}^{EtOH}$ 305 nm) suggested the change from possible intramolecularly hydrogen-bonded periplanar forms **2** and **6** to preferred noncoplanar conformers in **9**. Therefore, the lower melting product was assigned the structure of 2-ethoxy-4-(α-ethoxymethylenecyanomethyl)-pyrimidine (**9**). The nmr spectrum [$(CD_3)_2SO$] of the higher melting product showed methyl and methylene proton resonances at $\delta$ 1.25 (t, 3, $J$ = 7 Hz) and 3.86 (q, 2, $J$ = 7 Hz), respectively, values which are indicative of an *N*-ethyl group. The C-5 proton signal at $\delta$ 6.10 (d, 1, $J$ = 7 Hz) falls in the comparable region with that of **2**. The NOE (7% increase of the C-6 proton) between the methylene protons and the C-6 proton was again observed. From these data the higher melting point compound was assigned structure **8**, 1-ethyl-4-(α-formylcyanomethyl)pyrimidin-2-one.

The substituted pyrimidine derivative **9** was hydrolyzed readily in neutral 30% aqueous ethanol at 25° to afford 2-ethoxy-4-(α-formylcyanomethyl)pyrimidine (**10**), which reverted to **9** merely by refluxing in absolute ethanol. The structure of **10** was supported by the C-5 proton resonance at $\delta$ 6.82 (d, 1, $J$ = 7 Hz) in the nmr spectrum, by the fact that the anilide derived from **10** was not identifiable with either of the anilides from **6** or **8**, and by the fact that the fusion reaction of **10** with urea gave Pyo(4-5)Cyt (**7a**) only after acid treatment. Moreover, the ready exchange of ethanol favors reaction at the exocyclic function rather than at the 2 position.

Since attempts at selective enolic O-alkylation were not successful, direct reactions of the cyanoaldehyde **6** with urea and with thiourea were tried. However, the reaction under the usual[9] or modified conditions—*n*-BuONa in *n*-BuOH, HCl in EtOH, HBr in AcOH, and fuming $H_2SO_4$—did not proceed in the desired direction. Nevertheless, the fusion reaction of the lithium salt of the cyanoaldehyde **6** with urea at 150° was partially successful in that the desired product 5-(4-pyrimidin-2-one)cytosine (**7a**), was obtained, albeit in very low yield. Identification of the product with Pyo(4-5)Cyt obtained from the photoreaction of 4-thiouracil with cytosine[6] was established by the criteria of the mass and uv spectra. The analogous reaction of **9** with urea was carried out, followed by acid hydrolysis, but the yield was only slightly improved. The cyclization with *S*-ethylpseudothiourea, which was also attempted, showed (by tlc) multiple product formation. Finally, the fusion reaction of the lithium salt of **6** with thiourea was investigated. However, the yield (6%) of 5-(4-pyrimidin-2-one)-2-thiocytosine, Pyo(4-5)s$^2$Cyt (**7b**), could not be improved to the extent that would be advantageous for an alternative route to **7a**. The elemental composition of **7b** was established by peak matching in the double-focusing mass spectrometer (calcd for $C_8H_7N_5OS$, 221.0371; found, 221.0372). The mass spectral fragmentation patterns, 221 ($M^+$), 163, 162, 161, 146, 135, 119, 108, 92, and 59 (base peak), were in accord with those observed for Pyo(4-5)Cyt

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(**7a**). The borohydride-reduced Pyo(4-5)s²Cyt (**7b**) appeared fluorescent on cellulose tlc plates, as in the case of **7a**.

The low-yield synthesis of 5-(4-pyrimidin-2-one)-cytosine (**7a**) described here accomplishes the initial and primary goal of providing an independent and direct proof of the structure of the bipyrimidine moiety formed in *E. coli* tRNAs by the photolinking of 4-thiouridine (base no. 8) and cytidine (base no. 13) under long-wavelength uv irradiation.

## Experimental Section[20]

**4-(Isoxazol-4-yl)pyrimidin-2-one** (**5**).—To a solution of DMF (10.2 g, 0.14 mol) in $CHCl_3$ (10 ml) was added dropwise a solution of $COCl_2$ (11.9 g, 0.12 mol) in $CHCl_3$ (22 g) at 0–5° under vigorous stirring. The mixture was stirred at 25° for 15 min and at 35° for 30 min. A neutralized solution of **4** HCl (4.4 g, 0.03 mol) with triethylamine (3.0 g, 0.03 mol) in $CHCl_3$ (20 ml) was added to the stirred reaction mixture at 25°, and the mixture was stirred at 25° for 4 hr and finally warmed at 50° for 8 hr. After cooling, the precipitate (Vilsmeier adduct) was collected by filtration and dissolved in ice–water (70 ml). To this solution was added $NH_2OH \cdot HCl$ (10.4 g, 0.15 mol) and $NaHCO_3$ (8.5 g). The mixture (pH 4.5) was kept in a refrigerator overnight and the brown crystals which formed were collected by filtration to give (wet) isoxazole **5** (8.4 g): uv (qualitative) max (pH 5.5) 315, 254 nm; uv max (pH 1.5) 329, 256; $R_f$ in system A, 0.58.

**4-(α-Formylcyanomethyl)pyrimidin-2-one** (**6**).—The crude isoxazole **5** (6.35 g) obtained in the reaction described above was dissolved in 1 *N* NaOH (50 ml) and kept at 25° for 30 min. The solution was decolorized with charcoal (1.0 g) and adjusted to pH 3.5 with dilute HCl. The precipitate was collected by filtration to give crude cyanoaldehyde **6** (3.0 g). Recrystallization from MeOH (570 ml) with charcoal (2.0 g) afforded a first crop (1.90 g) of pale yellow leaflets, mp 245–250° dec, and a second crop (0.10 g), from the mother liquor concentrated to a volume of 50 ml: total yield 2.0 g (54%); nmr δ 6.02 (d, 1, $J$ = 7 Hz, $C_5$ proton), 7.72 (d, 1, $J$ = 7 Hz, $C_6$ proton), 9.22 (s, 1, OCH=), 12.22 (br, 2, OH, NH); ir (KBr) 2220 $cm^{-1}$ (C≡N); uv max (EtOH) 357 nm (ε 24,410), 245 (5810), 222 (7940); uv (pH 6.9) 342 nm (ε 20,960), 283 (3200), 245 (6940); uv (pH 1.2) 353 nm (ε 24,960), 240 (4520), 223 (6780); uv (pH 12.8) 327 nm (ε 21,770), 280 (6430).

*Anal.* Calcd for $C_7H_5N_3O_2$: C, 51.54; H, 3.09; N, 25.76. Found: C, 51.65; H, 3.04; N, 25.77.

**4-(α-Diformylmethyl)pyrimidin-2-one** (**1**).—The Vilsmeier adduct of 4-methylpyrimidin-2-one which was prepared in the same manner and scale as described in the first section above was dissolved in water (70 ml) and refluxed for 15 min. After the solution cooled, fine colorless needles were collected by filtration and washed with cold water to give the crude product (2.58 g). Recrystallization from 50% aqueous EtOH with charcoal gave analytically pure **1** (2.0 g, 40%): mp 253–255° dec; nmr δ 7.39 (d, 1, $J$ = 7 Hz, $C_5$ proton), 7.85 (d, 1, $J$ = 7 Hz, $C_6$ proton), 9.55 (s, 2); ir (KBr) 1723 $cm^{-1}$ (C=O); uv max (EtOH) 361 nm (ε 25,510), 243 (12,450); uv (pH 6.9) 354 nm (ε 25,370), 262 (12,270), 228 (8830); uv (pH 1.2) 360 nm (ε 25,620), 247 (13,970); uv (pH 12.8) 353 nm (ε 8730), 313 (7960), 268 (19,960).

*Anal.* Calcd for $C_7H_6N_2O_3$: C, 50.61; H, 3.64; N, 16.86. Found: C, 50.36; H, 3.61; N, 16.88.

**4-(α-Formylcyanomethyl)-1-methylpyrimidin-2-one** (**2**).—To a solution of cyanoaldehyde **6** (163 mg, 1 mmol) in DMF (30 ml) was added NaH (60% oil dispersion, 44 mg, 1.1 mmol) in portions at 5–10° and the mixture was stirred at 25° for 30 min. Methyl iodide (169 mg, 1.2 mmol) was added to the mixture, which was stirred at 55–60° overnight. As the tlc of the reaction mixture showed the presence of the unchanged **6**, a further portion of methyl iodide (84 mg, 0.6 mmol) was added to the mixture, which was then stirred at 55–60° for another 4 hr (completion of the reaction was confirmed by tlc). The reaction mixture was concentrated to dryness *in vacuo*, washed with ether (30 ml), and recrystallized from MeOH (12 ml) to give **2** (120 mg) as colorless needles, mp 203–206°. A second crop (33 mg) was obtained from the mother liquor, mp 199–203°, and recrystallized from MeOH (3.5 ml) to give a pure product (15 mg), mp 204–207°.

The total yield was 135 mg (76%) and an analytically pure sample had mp 204–207°; nmr δ 3.40 (s, 3, $NCH_3$), 6.22 (d, 1, $J$ = 7.5 Hz, $C_5$ proton), 8.07 (d, 1, $J$ = 7.5 Hz, $C_6$ proton), 9.32 (s, 1, OCH=); ir (Nujol) 2220 (C≡N), 1720 (C=O) $cm^{-1}$; uv max (EtOH) 349 nm (ε 25,160), 249 (9000); uv (pH 6.9) 338 nm (ε 25,440), 288 (4570), 247 (6680); uv (pH 1.2) 359 nm (ε 27,850), 247 (5100), 226 (7240); uv (pH 12.8) 338 nm (ε 29,170), 287 (5970), 245 (7290).

*Anal.* Calcd for $C_8H_7N_3O_2$: C, 54.24; H, 3.98; N, 23.72. Found: C, 54.30; H, 3.96; N, 23.82.

**Reaction of Cyanoaldehyde 6 with Triethyl Orthoformate.**—A mixture of **6** (815 mg, 5 mmol) and triethyl orthoformate (30 ml) was refluxed gently (internal temperature, 150 ± 5°) for 33 hr. The mixture was concentrated *in vacuo*, and the residual oil was dissolved in xylene (50 ml). A small amount of insoluble precipitate was removed by filtration, and the filtrate was concentrated *in vacuo*. The residual oil was dissolved in EtOH (35 ml), decolorized with charcoal (1.5 g), and kept at −25° overnight to afford yellow crystals (149 mg), mp 168–172°. Additional recrystallization from EtOH gave **1-ethyl-4-(α-formylcyanomethyl)-pyrimidin-2-one** (**8**, 103 mg) as yellow needles: mp 175–177°; nmr δ 1.25 (t, 3, $J$ = 7 Hz, $CH_2CH_3$), 3.83 (q, 2, $J$ = 7 Hz, $CH_2CH_3$), 6.10 (d, 1, $J$ = 7 Hz, $C_5$ proton), 8.00 (d, 1, $J$ = 7 Hz, $C_6$ proton), 9.20 (s, 1, OCH=), 12.57 (br, 1, OH); ir (KBr) 2220 (C≡N), 1735 (C=O) $cm^{-1}$; uv max (EtOH) 358 nm (ε 24,820), 250 (5680); uv (pH 6.9), 340 nm (ε 24,340), 288 (4170), 246 (6380); uv (pH 1.2) 359 nm (ε 27,650), 247 (4940), 225 (7120); uv (pH 12.8) 338 nm (ε 29,020), 288 (5860), 245 (7310).

*Anal.* Calcd for $C_9H_9N_3O_2$: C, 56.54; H, 4.74; N, 21.98. Found: C, 56.43; H, 4.72; N, 21.77.

The ethanolic filtrates were combined and concentrated to dryness *in vacuo*. The residual oil was chromatographed on a silical gel column (0.2–0.05 mm, 25 g, 2.0 × 18.0 cm), elution with $CHCl_3$. The first fraction of $CHCl_3$ (110 ml) was evaporated to afford crystals (250 mg), mp 87–94°. Recrystallization from isopropyl ether gave **2-ethoxy-4-(α-ethoxymethylenecyanomethyl)pyrimidine** (**9**) (188 mg, 17%), mp 94–95.5°. Further recrystallization from isopropyl ether afforded an analytically pure sample: mp 95–97°; nmr δ 1.33 and 1.35 (two triplets, 6, $J$ = 7 Hz, $2OCH_2CH_3$), 4.33 and 4.43 (two quartets, 4, $J$ = 7 Hz, $2OCH_2CH_3$), 7.03 (d, 1, $J$ = 5 Hz, $C_5$ proton), 8.44 (d, 1, $J$ = 5 Hz, $C_6$ proton), 8.54 (s, 1, OCH=); ir (KBr) 2220 (C≡N), no carbonyl absorption; uv max (EtOH) 305 nm (ε 16,530), 262 (7070).

*Anal.* Calcd for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.23; H, 5.86; N, 19.18.

The second fraction (45 ml) contained an unidentified compound (15 mg), mp 178–181° dec. The third fraction (125 ml) was evaporated and the residue was recrystallized from EtOH (5 ml) to give another crop of **8** (27 mg), mp 172–175°, total yield 130 mg (14%).

**2-Ethoxy-4-(α-formylcyanomethyl)pyrimidine** (**10**).—To a solution of **9** (438 mg, 2 mmol) in EtOH (15 ml) was added water (5 ml) and the mixture was stirred at 25° for 4 hr. The uv spectrum and tlc of the mixture after 2 hr showed completion of the hydrolysis. The mixture was concentrated to dryness *in vacuo* and the residue was triturated with isopropyl ether to give **10** (362 mg, 95%) as yellow needles: mp 205–208° dec; nmr δ 1.33 (t, 3, $J$ = 7 Hz, $OCH_2CH_3$), 4.43 (q, 2, $J$ = 7 Hz, $OCH_2CH_3$), 6.82 (br d, 1, $J$ = 7 Hz, $C_5$ proton), 7.71 (d, 1, $J$ = 7 Hz, $C_6$ proton), 9.55 (s, 2, OCH= and HO); ir (KBr) 2200 (C≡N); uv max (EtOH) 347.5 nm (ε 20,860), 273 (4220), 230 (9370); uv (pH 6.9) 326 nm (ε 23,750), 267 (5530), 227 (11,620); uv (pH 1.3) 346 nm (ε 24,930), 270 (3940), 232 (8340); uv (pH 12.7) 326 nm (ε 24,120), 266 (5440), 227 (11,530).

*Anal.* Calcd for $C_9H_9N_3O_2$: C, 56.54; H, 4.74; N, 21.98. Found: C, 56.32; H, 4.68; N, 21.96.

(20) Melting points are uncorrected. Nmr spectra were obtained on a Varian Associates A-60 or HA-100 spectrometer using tetramethylsilane as an internal reference and $(CD_3)_2SO$ as the solvent. We thank Mr. Steve Silber for his assistance with the nmr spectra. Ir spectra were obtained on a Perkin-Elmer Model 337 grating spectrophotometer. Thin layer chromatography (tlc) was carried out on 200 × 40 × 0.16 mm Eastman chromagram sheets, cellulose without fluorescent indicator, in the following solvent systems: A, 1-propanol–water (7:3 v/v); B, ethanol–ammonium acetate (1.0 *M*) (7:3 v/v), buffered to pH 7.95 with concentrated $NH_4OH$; C, 1-butanol–water–acetic acid (80:30:12 v/v). Spots were visualized by long and short wavelength uv light. Microanalyses were performed by Mr. Josef Nemeth and his associates at the University of Illinois and by Midwest Microlab, Inc., Indianapolis, Ind.

Reconversion of **10** to **9** upon refluxing in EtOH was confirmed by uv spectroscopy. A solution of **10** (2 mg) in absolute EtOH (100 ml) was heated at reflux for 63 hr. The uv max (350 nm) of the original solution changed to the uv max (307 nm) of **9**.

**4-(α-Anilinomethylenecyanomethyl)pyrimidin-2-one.**—A mixture of **6** (163 mg, 1 mmol), aniline (102 mg, 1.1 mol), and DMSO (1 ml) was heated at 110 ± 5° for 1.5 hr. After cooling, the mixture was triturated with isopropyl ether and EtOH. The crystals were collected by filtration and washed with a small amount of EtOH to give the anilide (197 mg). Recrystallization twice from DMF–EtOH (1:1, 20 ml) gave analytically pure sample (90 mg, 38%) as greenish needles, mp 262–265° dec.

*Anal.* Calcd for $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found: C, 65.71; H, 4.29; N, 23.31.

**4-(α-Anilinomethylenecyanomethyl)-1-ethylpyrimidin-2-one.**—A mixture of **8** (191 mg, 1 mmol), aniline (102 mg, 1.1 mmol), and DMSO (2 ml) was heated at 100–110° for 7 hr and worked up in the manner described above to give crude anilide (211 mg). Recrystallization from EtOH (5 ml) gave the anilide (147 mg, 55%) as yellow needles: mp 197–198°; nmr δ 1.25 (t, 3, $J$ = 7 Hz, $NCH_2CH_3$), 3.83 (q, 2, $J$ = Hz, $NCH_2CH_3$), 6.32 (d, 1, $J$ = 7 Hz, C-5 proton), 7.0–7.5 (m, 6, phenyl protons and NH), 8.01 (d, 1, $J$ = 7 Hz, C-6 proton).

*Anal.* Calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04. Found: C, 67.54; H, 5.38; N, 20.76.

**4-(α-Anilinomethylenecyanomethyl)-2-ethoxypyrimidine.**—A mixture of **10** (89 mg, 0.47 mmol), aniline (48 mg, 0.51 mmol), and DMSO (1 ml) was heated at 110 ± 5° for 2 hr and worked up in the manner described above to give the anilide (105 mg). Recrystallization from EtOH (10 ml) gave yellow plates (82 mg, 66%), mp 165–166°, which were recrystallized again from EtOH for analysis.

*Anal.* Calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04. Found: C, 67.75; H, 5.42; N, 20.88.

**Fusion Reaction of Cyanoaldehyde 6 Lithium Salt with Urea.**—To a suspension of **6** (163 mg, 1 mmol) in water (4 ml) was added 1 $N$ LiOH (1 ml) at 25°. The solution was concentrated to dryness *in vacuo* and the residual crystals were dried at 65° overnight. A finely powdered mixture of cyanoaldehyde **6** lithium salt (1 mmol) and urea (5 g) was heated at 148 ± 2° (bath temperature) for 2 hr. After cooling, the mixture was dissolved in water (10 ml), filtered, and washed with water (10 ml). The combined filtrate and washings were neutralized with 1 $N$ HCl, and the precipitate was removed by filtration. The filtrate was chromatographed on an IRC-50 ion-exchange resin column (2.5 × 7.5 cm), elution with water. The first 1.8 l. of eluent was discarded and the next 4.8 l. was concentrated to a volume of 80 ml. The precipitate was collected by filtration and dried at 65° *in vacuo* for 2 hr to give Pyo(4-5)Cyt (**7a**, 5.6 mg, 2.5%), mp >310°. The sample was dried at 78° for 30 hr *in vacuo* for elemental analysis. The uv and mass spectra and the tlc behavior were identical with those of the Pyo(4-5)Cyt that had been obtained in the photoreaction of cytosine and 4-thiouracil.

*Anal.* Calcd for $C_8H_7N_5O_2 \cdot H_2O$: C, 43.05; H, 4.06; N, 31.38. Found: C, 43.47; H, 4.30; N, 31.30.

**Fusion Reaction of 9 with Urea.**—A mixture of **9** (110 mg, 0.5 mmol) and urea (1.0 g) was heated at 145 ± 3° for 1 hr. The mixture was dissolved in 0.05 $N$ HCl (10 ml) and heated at 80–85° for 6 hr. The brown precipitate was removed by filtration, and the filtrate was concentrated to dryness *in vacuo*. The residue was extracted with concentrated $NH_4OH$ (4 ml), and the extract was chromatographed on a G-15 Sephadex column (72 × 2.2 cm), elution with a pH 10 $(NH_4)_2CO_3$–$NH_4OH$ buffer (0.1 $M$). Fractions of Pyo(4-5)Cyt, which can be detected by tlc,[5] were collected and evaporated to dryness. The residual crystals were washed with water (5 ml) and MeOH (1 ml) to give crude **7a** (8.4 mg, 4.6%). The purity of the **7a** (56.5%) was determined by quantitative uv analysis. Compound **7a** could also be isolated (4.8%) from the fusion of **10** with urea under similar conditions.

**The Fusion Reaction of Cyanoaldehyde 6 Lithium Salt with Thiourea.**—A mixture of **6** lithium salt (prepared in the same manner described above, 17 mg, 0.1 mmol) and thiourea (500 mg) was heated at 180 ± 2° (bath temperature) for 1 hr. After cooling, the mixture was dissolved in water (5 ml) and the precipitate was collected by filtration. The precipitate was extracted with 1 $N$ HCl (5 ml). The filtrate was passed through an IRC-50 ion-exchange resin column ($H^+$ form, 1.5 × 10 cm). After being washed with water (50 ml), the column was eluted with 1 $N$ HCl (200 ml). The eluate was evaporated to dryness *in vacuo*. The residue was combined with the 1 $N$ HCl extract (5 ml) of the precipitate and this 1 $N$ HCl-soluble portion was concentrated to dryness *in vacuo*. The residue was dissolved in concentrated $NH_4OH$ (2.5 ml) and chromatographed on a G-15 Sephadex column (72 × 2.2 cm), elution with a pH 10 $(NH_4)_2CO_3$–$NH_4OH$ buffer (0.1 $M$). Fractions of 8.6 ml were collected. Fractions 76–101 were combined and evaporated to dryness *in vacuo*. The residue was dissolved in concentrated $NH_4OH$ (2.5 ml) and filtered. The filtrate was concentrated to dryness and residual crystals were collected and washed with water (2 ml) and with MeOH (0.5 ml) to give **5-(4-pyrimidin-2-one)-2-thiocytosine** (**7b**, 1.3 mg, 5.8%) as a yellow powder: mp >310°; $R_f$ 0.17 in system A, 0.19 in B, 0.14 in C; a bright blue fluorescent spot (long-wave uv) after spraying with an ethanolic sodium borohydride solution; mass spectrum (70 eV) $m/e$ (>10% rel intensity) 222 (13), 221 (91), 220 (14), 204 (14), 203 (13), 188 (16), 179 (14), 166 (12), 163 (52), 162 (23), 161 (27), 146 (29), 145 (16), 136 (20), 135 (28), 120 (20), 119 (26), 118 (18), 108 (20), 107 (17), 93 (18), 92 (20), 91 (16), 87 (19), 80 (11), 76 (10), 74 (31), 68 (12), 67 (18), 66 (20), 65 (18), 64 (19), 60 (11), 59 (100), 58 (12), 55 (11), 53 (14), 52 (28), 44 (38), 43 (53), 42 (16), 41 (27), 40 (25), 39 (14), 38 (13), 36 (19), 34 (83), 33 (35), 32 (85), 29 (12), 28 (71), 27 (24); elemental composition by high resolution mass spectrometry calcd for $C_8H_7N_5OS$, 221.0371; found, 221.0372.

**Nuclear Overhauser Effects in 2 and 8.**—Spectra (Table I) were recorded using an HA-100 spectrometer in the frequency sweep mode using nitrogen-sparged $(CD_3)_2SO$ solutions with tetramethylsilane as an internal field frequency lock. The irradiation audio oscillator was a Wavetek voltage controlled generator, Model 111, with an impedance matching transformer. Power requirements were ascertained by increasing the output slowly in 10 mV increments until a signal increase was noted. Each peak of interest was integrated at least six times.

TABLE I

NUCLEAR OVERHAUSER EFFECTS IN 2 AND 8

| Compd | Group irrd[a] (δ, ppm) | H obsd (δ) | Intensity increase, % |
|---|---|---|---|
| **2** | $NCH_3$ (3.40) | $C_6$ proton (8.07) | 13 |
| **8** | $NCH_2CH_3$ (3.86) | $C_6$ proton (8.09) | 7 |

[a] Irradiated by 40 mV.

**Registry No.**—**1**, 36508-33-5; **2**, 36508-32-4; **5**, 36508-34-6; **6**, 36508-35-7; **7a**, 33604-46-5; **7b**, 36508-37-9; **8**, 36508-38-0; **9**, 36508-39-1; **10**, 36508-40-4; cytosine, 14987-28-1; 4-thiouracil, 591-28-6; 4-(α-anilinomethylenecyanomethyl)pyrimidin-2-one, 36508-41-5; 4-(α-anilinomethylenecyanomethyl)-1-ethylpyrimidin-2-one, 36508-42-6; 4-(α-anilinomethylenecyanomethyl)-2-ethoxypyrimidine, 36508-43-7.

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