Carbocyclic Analogs of Thymine Nucleosides and Related 1-Substituted Thymines

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The carbocyclic analogs of thymidine (IXf), 1-β-ribofuranosylthymine (IXg), and 1-β-3'-deoxyribofuranosylthymine (IXe) were synthesized by incorporating modifications into the Shaw method of synthesizing 2,4-(1*H*,3*H*)pyrimidinediones *via* acryloylureas. Simpler analogs of thymine nucleosides were also prepared by this method. The carbocyclic analog of thymidine displayed modest activity against Leukemia L1210 *in vivo*. It differs from a compound prepared previously by a Prins reaction.

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Syntheses of carbocyclic analogs of uracil and cytosine nucleosides were reported previously (1-4), and the synthesis of the carbocyclic analog of thymidine was described (5) as part of a study of the cyclization of alkoxyacryloylureas to 2,4-(1*H*,3*H*)pyrimidinediones. Compounds that interfere with the biosynthesis or utilization of thymidine and its nucleotides are of great interest because of the crucial and limiting role of thymidine nucleotides in DNA synthesis (6). This report deals with the synthesis and biological evaluation of carbocyclic analogs of thymine nucleosides and related 1-substituted thymines.

Compounds IXc-IXg are cyclopentyl derivatives, four of which (IXd-g) have an hydroxymethyl group positioned as it is in thymidine; whereas, 1-(5-hydroxypentyl)thymine (IXb) may be regarded as an open-chain analog bearing the terminal hydroxymethyl group of thymidine. Compound IXf is the racemic, carbocyclic analog of thymidine. The development of stereospecific routes to (±)-(1α,3α,4β)-3-amino-4-hydroxycyclopentanemethanol (I) (7), (±)-(1α,2β,4α)-4-amino-2-hydroxycyclopentanemethanol (II) (7), and (±)-(1α,2α,3β,5β)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (III) (8) made these amines available for the preparation of thymidine analogs IXe-g. The synthesis of IXf by a Prins reaction of 1-(3-cyclopentenyl)-thymine was reported by Murdock and Angier (9).

The method of synthesis of IXb-g is that described by Shaw and Warrener (10,11). This route begins with methyl methacrylate and proceeds *via* methyl 2,3-dibromo-2-methylpropanoate and methyl 3-methoxy-2-methylacrylate to 3-methoxy-2-methylacrylic acid. The sodium salt (IV) of the latter compound is converted to the acid chloride (V). Conversion of V with silver cyanate in dry benzene to the acyl isocyanate (VI) and treatment of this sensitive compound *in situ* with the appropriate amine give an acyl urea (VIII). The concluding step of the urea variant of the Shaw pyrimidine synthesis (10,11) has been, until recently (5), a base-catalyzed cyclization (with an amine or with aqueous alkali) of acryloylureas, such as VIII, to 1-subsituted thymines or uracils.

Modifications of the last two steps were required in order to obtain satisfactory yields of the di- or trihydroxycyclopentyl derivatives from the unprotected di- or trihydroxycyclopentylamines (I-III). Reaction of less polar amines (*e.g.*, aniline, methylamine) with the acyl isocyanate (VI) in anhydrous benzene gave acceptable yields of the corresponding acryloylureas (VIII). However, the Shaw procedure had to be modified for the preparation of acryloylureas from the more polar, unprotected hydroxycyclopentylamines by performing this step in anhydrous benzene-DMF or benzene-DMF-ether mixtures and at low temperatures. Yields of 70-76% of VIIIe-g were obtained after these modifications had been incorporated into the procedure. It was postulated that cyclization of acryloylureas, represented by VIII, would be subject to acid catalysis. Studies of the cyclization of VIIIa and VIIIf (5) showed that acid catalysis affords high yields of 1-substituted 2,4-(1*H*,3*H*)pyrimidinediones from alkoxyacryloylureas. Cyclization of VIIIb-f in 2*N* sulfuric acid and VIIIf-g in 0.1*N* sulfuric acid afforded yields of 70-88% (after purification) of the desired thymine derivatives (IXb-g). Fusion of acryloylureas VIIIb and VIIId with 4-toluenesulfonic acid also produced the thymines (IXb,IXd) in yields of 67-68%. In addition, cyclization of the acryloylureas VIIIf-g in 15*N* aqueous ammonia furnished high yields of IXf (5) and IXg. Attempts to prepare some of the simpler thymines from acryloylurethane VII (11) and the appropriate amines produced little, if any, of the desired thymines.

The structures of these thymine derivatives (IXc-g) rest on the structures of the starting aminocyclopentanes (7,8,12,13). The two aminohydroxycyclopentanemethanols (I,II) were synthesized by stereospecific routes from *exo*-bicyclo[2.2.1]hept-5-en-2-ol acetate (7). In addition, they were distinguished by periodate oxidation and by pmr studies of the 3'- and 2'-deoxyadenosine analogs prepared, respectively, from I and II (7). Proton nmr studies of IXe and of the carbocyclic analog of thymidine (IXf) confirm the earlier evidence (7). The ^{1}H-nmr spectra of compound

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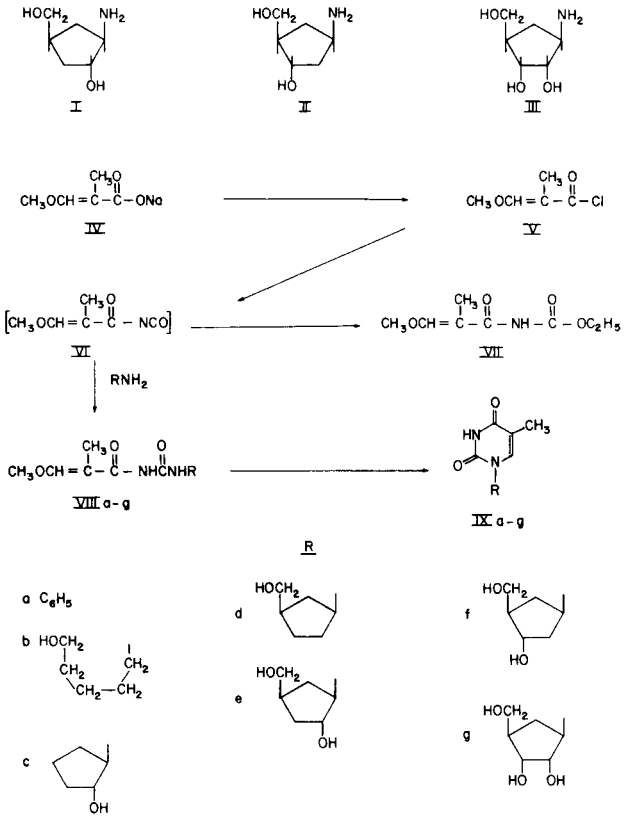
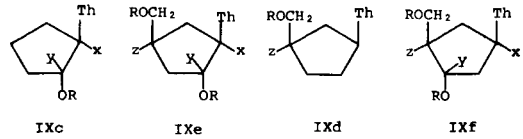


IXe in DMSO-d_6, in deuterated trifluoroacetic acid, and in deuteriotrifluoroacetic acid after trifluoroacetylation had occurred are similar to the corresponding spectra of model compound IXc in the region of the chemical shifts of protons x and y (see Table 1), whereas the spectra of IXf and of its trifluoroacetyl derivative are obviously different (Table 1, Figure 1). Multiplets arising from the x and y protons of IXc and IXe overlapped partially when IXc and IXe were in DMSO-d_6 solutions and were merged when these compounds were in deuteriotrifluoroacetic acid, whereas the x and y multiplets of IXf in both solutions were completely separated. The chemical shift of proton y changed markedly, as expected, after trifluoroacetylation had occurred; whereas the shift of proton x was relatively unaffected. Consequently, the x and y multiplets of all three trifluoroacetylated derivatives were completely separated. After the addition of deuterium oxide to the DMSO-d_6 solution of IXe (Figure 1), the x and y multiplets were observable without interference from the primary hydroxy group, but they were too close for direct demonstration of spin-spin coupling between them. By irradiating the methylene absorptions, however, it was possible to remove all coupling between the methylene protons and x and y. When this was done, x and y had the appearance of a typical AB pair with a coupling constant of 7-8 Hz. This result is evidence of spin-spin coupling bet-

Table 1

Selected Proton NMR Data (a)



IXc IXe IXd IXf

Compound	y	x	x + y (b)	*Sec.* -OH	CH_2OR	Solvent
IXc,R = H	4.18 m (c)	4.44 m		5.01 d		DMSO-d_6
IXe,R = H	4.16 m (d)	4.48 m		4.99 d	3.35 m	DMSO-d_6
IXf,R = H	4.0 m (e)	4.96 m		4.68 m	3.46 m	DMSO-d_6
IXd,R = H		4.75 m (f)			3.4 m	DMSO-d_6
IXc,R = H			4.5-5.0 m			deuteriotrifluoroacetic acid
IXe,R = H			4.6-5.1 m		3.90 m	deuteriotrifluoroacetic acid
IXf,R = H	4.67 m	5.16 m			4.06 m	deuteriotrifluoroacetic acid
IXd,R = H		5.04 m			3.92 m	deuteriotrifluoroacetic acid
IXc,R = $COCF_3$ (g,h)	5.69 m	4.86 m				deuteriotrifluoroacetic acid
IXe,R = $COCF_3$ (g,i)	5.79 m	4.82 m			4.56 d	deuteriotrifluoroacetic acid
IXf,R = $COCF_3$ (g,h)	5.57 m	5.15 m			4.65 d	deuteriotrifluoroacetic acid
IXd,R = $COCF_3$ (g,j)		5.07 m			4.53 d	deuteriotrifluoroacetic acid

(a) 100-MHz spectra; m = multiplet, d = doublet. (b) Overlapping multiplets. (c) The x and y multiplets partially overlapped; range, 4.0-4.6. Their approximate centers were measured after the addition of deuterium oxide. (d) The primary hydroxyl triplett was superimposed on the downfield side of the x multiplet; range of x, y, and OH = 4.0-4.7. The approximate centers of the overlapping multiplets due to x and y were measured after the addition of deuterium oxide. (e) The secondary hydroxyl multiplet and the primary hydroxyl doublet partially overlapped; the latter slightly overlapped the upfield side of the x multiplet. (f) The hydroxyl and y multiplets partially overlapped; the approximate center of the y multiplet was measured after the addition of deuterium oxide. (g) Spectra of the trifluoroacetyl derivatives were determined by allowing solutions of thymines IXc-IXf in deuteriotrifluoroacetic acid to stand until trifluoroacetylation was essentially complete. (h) Trifluoroacetylation essentially complete after 20 hours. (i) Trifluoroacetylation essentially complete after > 24 hours. (j) Trifluoroacetylation complete after several hours.

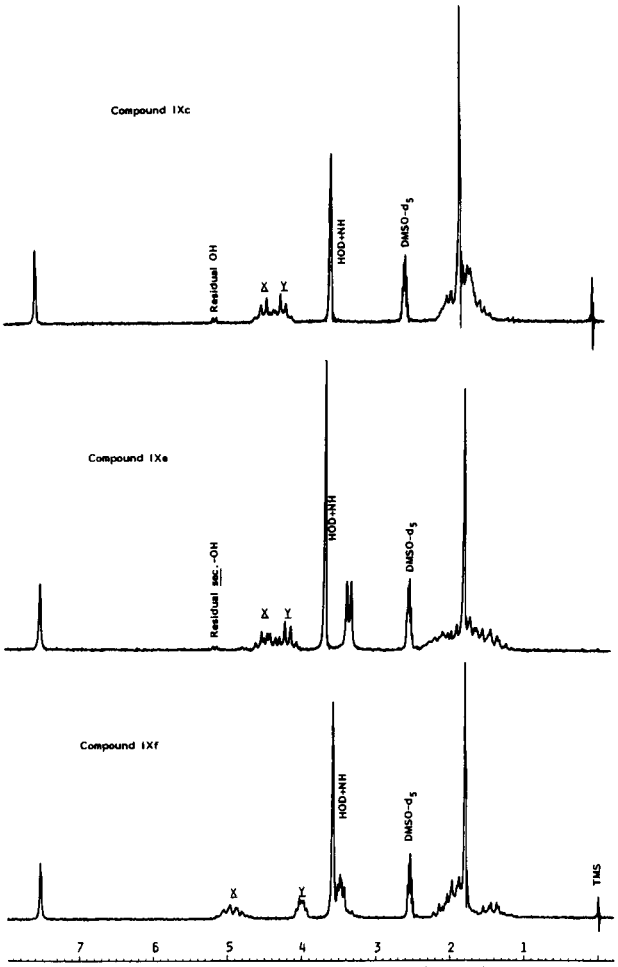


Fig. 1. Proton NMR spectra (100 MHz) of IXc, IXe, and IXf in DMSO-d_6 + D_2O. Protons x and y as shown in Table 1.

ween x and y appropriate for vicinal protons. In contrast, similar irradiation of the deuterium oxide-DMSO-d_6 solution of IXf at 1.90 ppm (near the center of the principal upfield multiplet (14)) reduced y to a singlet. These nmr experiments indicate that x and y are attached to adjacent positions in compound IXe and to non-adjacent positions in IXf, as shown by these structures. Similarly, spin-decoupling experiments indicated that spin-spin coupling occurred between x and y of trifluoroacetylated IXc and IXe and that, in contrast, y of trifluoroacetylated IXf was coupled only to upfield protons in the region of the signals of z and the CH_2 groups.

Synthesis of the carbocyclic analog of thymidine by a Prins reaction of 1-(3-cyclopentenyl)thymine was reported earlier (9). Compound IXf, prepared as outlined here and previously (5), has been compared with a specimen prepared by the Prins reaction (15). The two specimens moved identically during thin-layer chromatography on silica gel in three 2-propanol-water solvent systems, but in chloroform-methanol (7:1) the product of the Prins reaction moved slightly faster than both IXe and IXf. Reverse-phase high-pressure liquid chromatography (hplc) of IXe, IXf, and the Prins reaction product showed that the three thymines have different retention times. The melting points of IXf (221-223° dec.) and of the Prins reaction product (210-212° dec.) determined in capillary tubes side-by-side differed significantly. The mass spectra of the two specimens were similar (molecular-ion and several expected fragment peaks), but they were not identical. Also, the ^{1}H-nmr spectra (DMSO-d_6) of the Prins reaction product, IXf, and the all-*cis* analog of IXf (16) were not identical. Thus, the Prins reaction product and IXf are different compounds of similar structure.

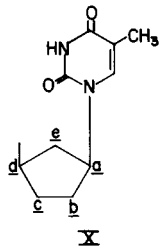
The method of synthesis of IXf described here and previously (5), the stereospecific synthesis (7) of its precursor (II), the studies confirming the structures of the deoxyadenosine analogs (7) prepared from I and II, and the ^{1}H-nmr studies of IXc-f comprise the evidence that the compound that we obtained *via* VIIIf is the carbocyclic analog (IXf) of thymidine. Possible structures for the product of the Prins reaction are the 2-hydroxy-3-(hydroxymethyl)cyclopentyl (XI) or 3-hydroxy-2-(hydroxymethyl)-cyclopentyl (XII) thymines, which could be formed if the double bond of the starting material, 1-(3-cyclopentenyl)-thymine (17) shifted under the conditions of the Prins

Table 2

Activity of the Carbocyclic Analog of Thymidine Against Leukemia L1210 (a)

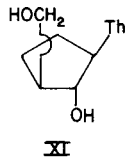
Dose, Mg./kg./day	Mortality by Day 5, Deaths/Total	Difference in Avg. Wt. Change(g). (b)	Average Survival Time, T/C	% ILS (c)
450	0/6	−4.2	9.7/9.1	+6
300	0/3	−2.6	12.3/9.3	+32
300	0/6	−3.2	11.8/9.1	+29
250	0/6	−3.4	11.5/9.1	+26
200	0/3	−2.6	11.3/9.3	+21
200	0/6	−1.7	12.0/9.6	+25
100	0/6	−1.2	11.3/10.0	+13

(a) Daily treatment, q.d. 1-9. Mice were implanted intraperitoneally on Day 0 with 10^5 L1210 cells. Daily injections intraperitoneally at the specified dose were initiated on Day 1 and continued through Day 9. (b) Average weight change of treated animals minus average weight change of control animals. (c) Percent increase in life span of treated mice.

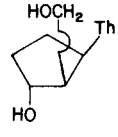


X

Letters a-e specify positions for NMR assignments (Experimental)



XI



XII

reaction to form 1-(2-cyclopentenyl)thymine. Either XI or XII appear to be capable of forming the anhydro derivatives obtained (9) from the Prins reaction product.

The thymine derivatives IXb-g were not cytotoxic in tests against cultures of human epidermoid carcinoma (H.Ep.-2) cells (IXb-d) or Eagle's KB cells (IXe-g). Compounds IXb-d were tested in mice against Leukemia L1210 at 400 mg./kg./day on both single-dose (Day 1 or Day 2) and daily (*q.d.* 1-9) schedules, and compounds IXe and IXg were tested at 200 mg./kg./day, administered daily (*q.d.* 1-9), against the same mouse leukemia. There was noevidence of antileukemic activity or toxicity in these tests. However, as shown in Table 2, the carbocyclic analog of thymidine demonstrated modest, but reproducible, activity against Leukemia L1210 in mice.

EXPERIMENTAL

General.

Unless otherwise stated, decomposition and melting temperatures were determined in capillary tubes heated in a Mel-Temp or a Mettler FP1 apparatus; those labelled "KH" were determined on a Kofler Heizbank apparatus (gradiently heated bar). Ultraviolet spectra (uv) were recorded with a Cary Model 17 or a Cary Model 14 spectrophotometer, and maxima are reported in nanometers. Solutions for ultraviolet determinations were prepared by diluting a 5-ml. aliquot of an ethanol solution to 50 ml. with 0.1*N* hydrochloric acid, phosphate buffer (*p*H 7), or 0.1*N* sodium hydroxide; absorption maxima of these solutions are reported as being determined in 0.1*N* hydrochloric acid, at *p*H 7, or in 0.1*N* sodium hydroxide, respectively. Infrared spectra (ir) were recorded with Perkin-Elmer Model 521 or 621 spectrophotometers from samples in potassium bromide disks; s = strong, w = weak, sh = shoulder. Mass spectral data (ms) were taken from low resolution spectra determined at 70 eV with a Varian MAT Model 311A spectrometer equipped with a combination electron-impact, field-ionization, and field-desorption ion source. The peaks listed are those due to the molecular ion (M), those attributable to the loss of certain fragments from the molecular ion (M − fragment), and some other prominent peaks. Unless otherwise indicated, the samples were introduced in a direct probe at 20°. Nuclear magnetic resonance spectra were determined with a Varian Model XL-100-15 spectrometer operating at 100.1 MHz for proton (^{1}H-nmr) and at 25.2 MHz for carbon-13 (^{13}C-nmr) spectra. The internal standard was $(CH_3)_4Si$; s = singlet, d = doublet, m = multiplet. Thin-layer chromatography (tlc) was performed on plates of silica gel, either Silica Gel H (18) or Silica Gel GF (19). Unless indicated otherwise in parentheses, tlc was performed as follows: (a) when Silica Gel H (SGH) was used, developed plates were examined with uv light (254 nm) both before and after spraying with an optical whitening agent (Ultraphor WT, BASF Colors and Chemicals, Inc., Charlotte, N.C.); (b) when Silica Gel GF (SGF) was used, developed plates were examined by uv light (254 nm) only. Other pertinent information (amount applied, developing solvent, other methods of detection) are given parenthetically at the appropriate places in the experimental procedures.

Intermediates.

Methyl 2,3-dibromo-2-methylpropanoate was prepared by brominating freshly distilled methyl methacrylate in carbon tetrachloride at 10°: yield, 87%; b.p. 91-93° at 16.5 mm [lit. (20), b.p. 86° at 15 mm]. Methyl 3-methoxy-2-methylacrylate, 3-methoxy-2-methylacrylic acid, and 3-methoxy-2-methylacryloyl chloride (V) [b.p. 101-102° at 36 mm, lit. (10) b.p. 102° at 35 mm] were prepared according to the procedures of Shaw and Warrener (10).

3-Methoxy-*N*-(phenylaminocarbonyl)-2-methyl-2-propenamide (VIIIa).

A mixture of 3.5 g. of 3-methoxy-2-methylacryloyl chloride (V), 25 ml. of anhydrous benzene (dried with calcium hydride) and 7.0 g. of silver cyanate [dried *in vacuo* over phosphorus pentoxide in the dark at 135° for 3 hours (21)] was heated under reflux for 0.5 hour, cooled to room temperature, and filtered under dry nitrogen. The acylisocyanate (VI) solution (26.5 ml. of the 29-ml. filtrate) was added cautiously to a solution of 4.46 g. of aniline in 75 ml. of dry benzene at 10°. The solution was stirred at room temperature for 2.5 hours and concentrated to dryness *in vacuo*. During all of the preceding operations moisture was rigorously excluded until the reaction was terminated. The residue was triturated with 25 ml. of cold ethanol, and white needles were collected by filtration, washed with cold ethanol, and dried, yield, 3.37 g. (60%), m.p. 144° [lit. (11) m.p. 144°]; uv: max. 267 (ε 21,500) in 0.1*N* hydrochloric acid and at *p*H 7, 277 (ε 17,300) and 228 (ε 13,500) in 0.1*N* sodium hydroxide; ir: (1700-1500 cm^{-1} region) 1690 s, 1655 s, 1610 sh., 1600 sh., 1585 s, 1550 m. These data were unchanged after a specimen had been recrystallized from ethanol.

Anal. Calcd. for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.03. Found: C, 61.25; H, 5.99.

N-[(5-Hydroxypentyl)aminocarbonyl]-3-methoxy-2-methyl-2-propenamide (VIIIb).

This acryloylurea was prepared by a procedure similar to that described for the preparation of VIIIf. The syrup obtained by concentrating the reaction mixture *in vacuo* was chromatographed in chloroform-methanol (9:1) on activated silica gel (SGH), eluate fractions shown by tlc to contain pure VIIIb were combined and concentrated to dryness, and the residue was slurried with ethyl acetate-cyclohexane (1:1), yield, 18%, m.p. 92° (KH); tlc, 1 spot (100 mcg., 9:1 chloroform-methanol, SGH, detection by iodine, basic potassium permanganate spray, and uv-Ultraphor); uv: max. 259 (ε 15,100) in 0.1*N* hydrochloric acid and at *p*H 7, 259 (ε 13,300) in 0.1*N* sodium hydroxide; ir: (1700-1500 and 1300-1100 cm^{-1} regions) 1690 s, 1655 s, 1635, 1560 s, 1295, 1240 s, 1180, 1155s, 1145 sh., 1115 w, 1105 w.

Anal. Calcd. for $C_{11}H_{20}N_2O_4$: C, 54.07; H, 8.25; N, 11.47. Found: C, 54.05; H, 7.91; N, 11.23.

(±)-*N*-[(*trans*-2-Hydroxycyclopentyl)aminocarbonyl]-3-methoxy-2-methyl-2-propenamide (VIIIc).

Acryloylurea VIIIc was prepared from *trans*-2-aminocyclopentanol (12) by a procedure similar to that described for the preparation of VIIIf, isolated by chromatography in chloroform-methanol (9:1) on activated silica gel (SGH), and slurried with cyclohexane-ethyl acetate (9:1): yield, 51%, m.p. 96-99°; tlc, 1 spot (40 mcg., 9:1 chloroform-methanol, SGH, detection by uv, UV-Ultraphor, and basic potassium permanganate spray); uv: max 260 (ε 16,000) in 0.1*N* hydrochloric acid and at *p*H 7, 262 (ε 13,900) in 0.1*N* sodium hydroxide; ir: (1700-1500 and 1300-1100 cm^{-1} regions) 1690 s, 1670, 1655 s, 1555 s, 1540 sh., 1295, 1240 s, 1150 s, 1120 sh., 1105.

Anal. Calcd. for $C_{11}H_{18}N_2O_4$: C, 54.53; H, 7.49; N, 11.57. Found: C, 54.81; H, 7.39; N, 11.78.

(±)-*N*-[[*cis*-3-(Hydroxymethyl)cyclopentyl]aminocarbonyl]-3-methoxy-2-methyl-2-propenamide (VIIId).

Acryloylurea VIIId was prepared by a procedure similar to that described for the preparation of VIIIf. The syrup obtained by concentrating a reaction mixture initially containing 1.64 g. of (±)-*cis*-3-aminocyclopentanemethanol (13) was stirred with a benzene-cyclohexane (1:1) mixture, cooled to 10°, and seeded with pure VIIId. The crude VIIId that precipitated was slurried with ether (20 ml.) and dried *in vacuo* at 56°, weight, 2.3 g. (63% yield), m.p. 88-90°. Additional VIIId (724 mg., 20% yield) was isolated by chromatographing the filtrate residue in chloroform-methanol (94:6) on activated silica gel. A hot benzene (175 ml.) solution of the two fractions was diluted with hot cyclohexane (about an equal volume) and filtered through diatomaceous silica (Celite) to remove an oily precipitate. The filtrate was diluted with additional cyclohexane (benzene:cyclohexane, 1:2) and refrigerated. White needles precipitated: yield, 1.92 g. (53%), m.p. 104-106°; uv: max 259 (ε 15,800) in 0.1*N* hydrochloric acid and at *p*H 7, 261 (ε 13,700) in 0.1*N* sodium hydroxide; ir: (1700-1500 and 1300-1100 cm^{-1} regions) 1685 s, 1675 s, 1650 s, 1625, 1540 s, 1295, 1250 s, 1200 sh., 1155 s, 1120 sh., 1105 w, 1095.

Anal. Calcd. for $C_{12}H_{20}N_2O_4$: C, 56.02; H, 7.83; N, 10.89. Found: C, 56.21; H, 7.93; N, 10.90.

(±)-*N*-[[(1α,2β,4α)-2-Hydroxy-4-(hydroxymethyl)cyclopentyl]aminocarbonyl]-3-methoxy-2-methyl-2-propenamide (VIIIe).

By the procedure described for the preparation of VIIIf, compound VIIIe was prepared from 1.032 g. (7.85 mmoles) of I (dried *in vacuo* at 50° for 3 hours), 19 ml. of dry dimethylformamide, 6.1 ml. of dry ether, and 12.84 ml. of a benzene solution of VI theoretically containing 8.63 mmoles. Trituration of the residual syrup (obtained by concentrating the reaction mixture and evaporating ethanol from the residue) with ethyl acetate left a small amount of brown tarry material which was removed by filtration of the mixture through a pad of diatomaceous silica (Celite). The filtrate (plus washings) was stored at 5° and filtered, as before, to remove another small portion of tarry material. Concentration of the filtrate (plus washings) *in vacuo* left a residue that crystallized. The crystalline product was triturated with ether, collected by filtration, washed with ether, and dried *in vacuo*, yield, 1.632 g. (76%), m.p. 114-115°; tlc, 1 spot (80 mcg., 9:1 chloroform-methanol, SGF); uv: max 259 (ε 15,400) in 0.1*N* hydrochloric acid and at *p*H 7, 261 (ε 13,000) in 0.1*N* sodium hydroxide; ir: (1700-1500 and 1300-1100 cm^{-1} regions) 1675 s, 1665 s, 1540 s, 1290 s, 1235 s, 1145 s, 1110, 1090 w; ms: m/e 273 (M + 1), 272 (M), 257 (M - CH_3), 254 (M - H_2O), 244, 241, 239, 236, 223 (M - H_2O - CH_2OH), 213, 185, 171, 159, 141, 116, 115, 99.

Anal. Calcd. for $C_{12}H_{20}N_2O_5$: C, 52.93; H, 7.40; N, 10.29. Found: C, 52.79; H, 7.45; N, 10.37.

(±)-*N*-[[(1α,3β,4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]aminocarbonyl]-3-methoxy-2-methyl-2-propenamide (VIIIf).

To a solution of 5.03 g. of acid chloride V in 52 ml. of anhydrous benzene was added 12.0 g. of silver cyanate [dried *in vacuo* over phosphorus pentoxide in the dark at 135° for 3 hours (21)]. The resulting mixture was heated under reflux for 0.5 hour and allowed to cool to room temperature. After the solid phase had settled, 25 ml. of the supernatant solution was transferred with a pipette to a dropping funnel. The isocyanate (VI, theoretically 16.6 mmoles) solution was added during 25 minutes to a solution of 2.0 g. (15.3 mmoles) of II, 65 ml. of anhydrous dimethylformamide, and 20 ml. of anhydrous ether at −15°. The reaction mixture was stirred at −15° for 2 hours and stored at +5° overnight. During all of the preceding operations, water was rigorously excluded and the reaction solutions were kept under a current of dry nitrogen (passed through a tower of anhydrous calcium sulfate or barium oxide) until the reaction was terminated, volatile components were evaporated *in vacuo* (aspirator and oil pump), ethanol (50 ml.) was added to the residual syrup and evaporated *in vacuo*, and the residual oily solid was triturated with ethyl acetate (50 ml.). A solid was separated by filtration, washed with ethyl acetate, and dried *in vacuo* at 56°: weight, 1.745 g., m.p. 121-128°. The filtrate was retained for chromatography; the crude solid was dissolved in 10 ml. of chloroform-methanol (9:1). The later solution was filtered to remove a small amount of dark, insoluble material, diluted with 5 ml. of chloroform-methanol (9:1), refiltered, and concentrated to dryness *in vacuo*. The residue was dissolved in 70 ml. of hot ethyl acetate-ethanol (6:1), and the solution was decanted from a small amount of gummy material, treated with activated charcoal, and chilled. A white precipitate (specimen A) was collected by filtration, washed with ethyl acetate, and dried *in vacuo* at 56°, yield, 900 mg., m.p. 138-139°.

The filtrate from the crude solid was concentrated to dryness *in vacuo*, and the residual syrup was chromatographed in chloroform-methanol (9:1) on activated silica gel. Eluate fractions shown by tlc to contain pure VIIIf were combined and yielded 1.47 g. (specimen B) of VIIIf (total yield from II, 57%), m.p. 139-140°; tlc, 1 spot (80 mcg., 9:1 chloroform-methanol, SGF); uv: max 260 (ε 16,000) in 0.1*N* hydrochloric acid and at *p*H 7, 260 (ε 13,800) in 0.1*N* sodium hydroxide, 253 (ε 16,400) in ethanol; ir: (1700-1500 and 1300-1100 cm^{-1} regions) 1690 s, 1660 s, 1585 s, 1525 s, 1295, 1240 s, 1190 sh., 1140 s, 1110 sh., 1090 w; ms: m/e 273 (M + 1), 272 (M), 257 (M - CH_3), 254 (M - H_2O), 241 (M - CH_2OH), 239, 225, 223 (M - H_2O - CH_2OH), 213, 185, 159, 143, 141, 130, 116, 115, 99.

Anal. Calcd. for $C_{12}H_{20}N_2O_5$: C, 52.93; H, 7.40; N, 10.29. Found: C, 52.86; H, 7.33; N, 10.45.

The ir spectrum of the fraction obtained by recrystallization (specimen A) was similar to, but not identical with, that of the fraction (specimen B) isolated by chromatography. However, after specimen A had been melted and cooled, the ir spectrum of the resolidified material was identical to that of specimen B. Also, ir spectra of chloroform solutions of the two specimens were identical, and the two specimens moved side-by-side on tlc plates (9:1 chloroform-methanol, detection by uv and UV-Ultraphor). Even though the melting points of specimens A and B were similar (and not depressed on admixture), the two specimens must represent different crystal forms of VIIIf.

In a subsequent preparation of VIIIf, trituration of the residual syrup (obtained by concentrating the reaction mixture and evaporating ethanol from the residue) with ethyl acetate left a small amount of brown tarry material which was removed by filtration of the mixture through a pad of diatomaceous silica (Celite). Concentration of the filtrate (plus washings) to a low volume afforded a 51% yield of VIIIf with an ir spectrum identical to that of specimen B, and chromatography, as described above, furnished an additional 19%; total yield = 70%.

(±)-*N*-[[(1α,2β,3β,4α)-2,3-Dihydroxy-4-(hydroxymethyl)cyclopentyl]-aminocarbonyl]-3-methoxy-2-methyl-2-propenamide (VIIIg).

By the procedure described for the preparation of VIIIf, compound VIIIg was prepared from 983 mg. (6.68 mmoles) of dry III, 16 ml. of dry dimethylformamide, 5.2 ml. of dry ether, and 11.8 ml. of a benzene solution theoretically containing 7.35 mmoles of VI. Trituration of the partially crystalline residue (obtained by concentrating the reaction mixture and evaporating ethanol from the residual syrup) with ethyl acetate-cyclohexane (3:1) furnished a white solid that was collected by filtration, washed with ethyl acetate-cyclohexane, and dried *in vacuo* at 56°: yield, 1.47 g. (76%), m.p. 116-119°; tlc, 1 spot (40 mcg., 9:1 chloroform-methanol, SGF); uv: max 259 (ε 15,400) in 0.1*N* hydrochloric acid and at *p*H 7, 260 (ε 13,200) in 0.1*N* sodium hydroxide; ir: (1700-1500 and 1300-1100 cm^{-1} regions) 1680 s, 1655 s, 1630, 1540 s, 1250 s, 1220, 1205, 1155 s, 1125; ms: m/e 289 (M + 1), 288 (M), 273 (M - CH_3), 270 (M - H_2O), 252 (M - $2H_2O$), 239 (M - H_2O - CH_2OH), 213, 211, 185, 184, 159, 141, 127, 116, 115, 113, 112, 110, 99. This material was used without further purification for the preparation of IXg. A second crop amounting to 145 mg. (m.p. 115-118°; tlc, 1 spot) was isolated from the filtrate, total yield = 84%.

1-(5-Hydroxypentyl)-5-methyl 2,4-(1*H*,3*H*)pyrimidinedione (IXb).

Method A.

A solution of 1.356 g. of VIIIb in 50 ml. of 2*N* sulfuric acid was heated under reflux for 2.5 hours. Tlc (80 mcg., SGH, 9:1 chloroform-methanol,

detection by uv, UV-Ultraphor WT, and basic potassium permanganate spray) of an aliquot removed at this time revealed only IXb. The thymine (IXb) was isolated by the procedure of Method A for IXd, and the residue from the ethyl acetate extract was triturated with cyclohexane, collected by filtration, and dried *in vacuo* at 56°, yield, 1.04 g. (88%), m.p. 140-142°; uv: max 273 (ε 9800) in 0.1*N* hydrochloric acid and at *p*H 7, 270 (ε 7300) in 0.1*N* sodium hydroxide. Recrystallization of this material from ethanol-ethyl acetate gave a white crystalline solid (980 mg.) identical according to its melting point, uv molar absorptivities, and ir spectrum with the analytical sample (Method B).

Method B.

A molten mixture of 200 mg. of VIIIb and 25 mg. of 4-toluenesulfonic acid was kept at 110° for 2 hours. After ethanol had been added to and evaporated from the cooled mixture, the gummy residue was stirred with 6 ml. of water, the aqueous suspension was washed with chloroform (3 × 20 ml.), and the water layer was concentrated to dryness. Chromatography of the residue (91 mg.) in chloroform-methanol (95:5) on a column of activated silica gel yielded 53 mg. of white solid, m.p. 140-142° (inserted at 95°, 2°/minute); tlc, 1 spot (80 mcg., SGH, 95:5 chloroform-methanol, detection by uv, UV-Ultraphor WT, and basic potassium permanganate spray); ir: (1800-1400 cm^{-1} region) 1680 s, 1660 s, 1645 sh., 1515 w, 1475, 1455, 1430, 1418, 1400 w; uv: max 273 (ε 9700) in 0.1*N* hydrochloric acid and at *p*H 7, 271 (ε 7300) in 0.1*N* sodium hydroxide.

Anal. Calcd. for $C_{10}H_{16}N_2O_3$: C, 56.58; H, 7.60; N, 13.20. Found: C, 56.74; H, 7.22; N, 13.07.

The chloroform solution (above) was extracted with water (25 ml.), and the water layer was concentrated *in vacuo* to a white solid: weight, 63 mg., m.p. 140-142°. Uv spectra and tlc were identical with those of the specimen of IXb isolated by chromatography. The two fractions of pure IXb represent a yield of 67%. In addition, the syrup obtained by concentrating the chloroform layer was shown by tlc to be IXb containing three minor impurities.

(±)-1-(*trans*-2-Hydroxycyclopentyl)-5-methyl-2,4-(1*H*,3*H*)pyrimidinedione (IXc).

A solution of 500 mg. of VIIIc in 20 ml. of 2*N* sulfuric acid was heated under reflux for 3 hours, and IXc was isolated by the procedure of Method A for IXd. The residue obtained from the ethyl acetate extract was triturated with ether, collected by filtration, and dried *in vacuo* at 56°, yield 374 mg. (86%); m.p. 226-229° (sintering at 220-225°); tlc, 1 spot (100 mcg., SGH, detection by uv, UV-Ultraphor, and basic permanganate spray). Ir and uv spectra and uv molar absorptivities were identical with those of the recrystallized specimen (below). Recrystallization of this material from ethanol gave white needles: 85% recovery; m.p. 227-229° (sintering at 224-227°, inserted at 170°, 4°/minute); ir: (1800-1300 cm^{-1} region) 1705 sh., 1685 s, 1650 s, 1635 sh., 1505 w, 1485, 1450, 1425, 1405, 1380, 1370, 1355 sh., 1320, 1310, 1295; uv: max 274 (ε 10,100) in 0.1*N* hydrochloric acid and at *p*H 7, 273 (ε 7700) in 0.1*N* sodium hydroxide; ^{1}H-nmr (DMSO-d_6, 20 mg./0.4 ml.): (22) δ 1.2-2.2 (m, CH_2 at *c, d, e*), 1.80 (s superimposed on m, CH_3), 4.18 (m, CH at *b*), 4.44 (m, CH at *a*), 5.01 (center of d, *sec.*-OH), 7.54 (approximate s, pyrimidine CH), 11.15 (s, NH).

Anal. Calcd. for $C_{10}H_{14}N_2O_3$: C, 57.13; H, 6.71; N, 13.33. Found: C, 57.37; H, 6.56; N, 13.60.

The reaction solution from a larger-scale run deposited IXc at room temperature in 75% yield. Additional IXc (6%) was obtained by extracting the neutralized filtrate with ethyl acetate, evaporating the ethyl acetate, and recrystallizing the residue from ethanol.

(±)-1-[*cis*-3-(Hydroxymethyl)cyclopentyl]-5-methyl-2,4-(1*H*,3*H*)-pyrimidinedione (IXd).

Method A.

A suspension of 1.573 g. of VIIId and 50 ml. of 2*N* sulfuric acid was heated under reflux for 3.5 hours. The solid phase dissolved during the heating, and tlc (SGH, 95:5 chloroform-methanol, detection by uv and UV-Ultraphor) of an aliquot after 3.5 hours of heating revealed only IXd. The reaction solution was cooled, neutralized to *p*H 5.6 with solid sodium bicarbonate, saturated with sodium chloride, and extracted continuously overnight with ethyl acetate in a liquid-liquid extractor. The ethyl acetate solution was dried (magnesium sulfate) and concentrated. The white crystalline residue was triturated with a mixture (9:1) of cyclohexane-ethyl acetate, collected by filtration, washed with cyclohexane, and dried *in vacuo* at 56°; yield, 1.164 g. (85%); m.p. 173-174° (inserted at 145°, 2°/minute). This material was identical according to tlc and its ir and uv spectra with the analytical sample, which was prepared by recrystallizing a specimen from acetone-benzene and drying the recrystallized material at 78°, 75% recovery, m.p. 173-174°; ir: (1800-1300 cm^{-1}) region 1675 s, 1635, 1510 w, 1465, 1455 sh., 1420, 1395, 1370, 1350 sh., 1310; uv: max 273 (ε 10,100) in 0.1*N* hydrochloric acid and at *p*H 7, 271 (ε 7900) in 0.1*N* sodium hydroxide ^{1}H-nmr (DMSO-d_6, 20 mg./0.4 ml.) (22): δ 1.2-2.3 (m, CH_2 at *b, c, e* and CH at *d*), 1.80 (s, superimposed on m, CH_3), 3.4 (m, CH_2OH), 4.57 (m, OH), 4.75 (m, CH at *a*), 7.57 (approximate s, pyrimidine CH), 11.18 (s, NH).

Anal. Calcd. for $C_{11}H_{16}N_2O_3$: C, 59.33; H, 7.37; N, 12.22. Found: C, 59.31; H, 7.21; N, 12.32.

Method B.

A mixture of 351 mg. of urea VIIId (m.p. 104-106°) and 20 mg. of 4-toluenesulfonic acid was heated at 110-115° for 3 hours under an atmosphere of nitrogen. The thymine derivative (IXd) was isolated by dissolving the reaction mixture in chloroform (1.2 ml.), applying the solution to a column of activated silica gel (12 g.), eluting the column with chloroform-methanol (95:5), combining fractions determined by tlc to contain IXd, concentrating the solution to dryness, and triturating the residual syrup with ether. A white solid was collected by filtration, washed with ether, and dried *in vacuo* at 56°: yield, 208 mg. (68%), m.p. 173-174°; tlc, 1 spot (60 mcg., SGH, 95:5 chloroform-methanol). Recrystallization (acetone-benzene) of this material afforded a specimen (75% recovery) that was shown by its ir and uv spectra to be identical with a specimen prepared by Method A.

(±)-1-[(1α,2β,4α)-2-Hydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4-(1*H*,3*H*)pyrimidinedione (IXe).

Compound IXe was prepared from VIIIe (which is soluble in dilute sulfuric acid) by the procedure of Method A for IXd except that the reaction solution was neutralized with 50% sodium hydroxide. The ethyl acetate extract, obtained after 20 hours of continuous liquid-liquid extraction, was chilled and filtered to separate the white crystalline solid which was washed with ethyl acetate and dried at 56° *in vacuo*, weight, 465 mg. from 1.00 g. of VIIIe, m.p. 207-210° (inserted at 180°, 2°/minute), tlc, 1 spot (80 mcg., SGF, 9:1 chloroform-methanol); ir: (1800-1300 cm^{-1} region) 1680 s broad, 1515 w, 1480, 1465, 1435, 1395, 1385, 1370, 1345, 1335 sh., 1310 sh.; uv: max 273 (ε 10,200) in 0.1*N* hydrochloric acid and at *p*H 7, 271 (ε 7900) in 0.1*N* sodium hydroxide; ms (23): m/e 241 (M + 1), 240 (M), 222 (M - H_2O), 212 (M - CO), 197 (M -HNCO), 195 (M - CO - OH), 194 (M - CO - H_2O), 191 (M - CH_2OH - H_2O), 182, 181 (M - CO - CH_2OH), 179, 165, 153 (Th + C_2H_4), 148, 139, 127 (Th + 2H), 126 (Th + H), 114 (cyclopentyl moiety - H), 110, 96 (114 - H_2O), 83 (114 - CH_2OH); ^{1}H-nmr (DMSO-d_6, 20 mg./0.4 ml.) (22): δ 1.1-2.4 (m, CH_2 at *c* and *e* and CH at *d*), 1.80 (s superimposed on m, CH_3), 3.35 (m, CH_2OH), 4.16 (m, CH at *b*), 4.48 (m, CH at *a*), 4.58 (m, primary OH), 4.99 (d, *sec.*-OH), 7.56 (approximate s, pyrimidine CH), 11.13 (s, NH).

Anal. Calcd. for $C_{11}H_{16}N_2O_4$: C, 54.99; H, 6.71; N, 11.66. Found: C, 54.88; H, 6.60; N, 11.79.

The aqueous layer was extracted for 20 hours with a second portion of ethyl acetate, and this extract was combined with the ethyl acetate filtrate from the portion of IXe described above. The resulting solution was concentrated to dryness, and the residual solid was triturated with ethyl acetate, collected by filtration, and dried at 56° *in vacuo*, weight, 165 mg., identical according to tlc and m.p. with the first portion. The total yield was 71%.

(±)-1-[(1α,3β,4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4-(1*H*,3*H*)pyrimidinedione (IXf).

Procedures for the cyclization of VIIIf to the carbocyclic analog of thymidine (IXf) in 2*N* sulfuric acid, 0.1*N* sulfuric acid, or 15*N* aqueous ammonia in yields of 79-87% were described previously, and uv, ir, ms, tlc, and m.p. data were reported (5). In addition, the ^{1}H-nmr spectrum was as follows (DMSO-d_6, 20 mg./0.4 ml.) (22): δ 1.1-1.6 and 1.6-2.3 (partially overlapping m, CH_2 at *b* and *e* and CH at *d*), 1.79 (s superimposed on m, CH_3), 3.46 (m, CH_2OH), 4.0 (m, CH at *c*), 4.56 (m, primary OH), 4.68 (d, *sec.*-OH), 4.96 (m, CH at *a*), 7.53 (approximate s, pyrimidine CH), 11.14 (s, NH). The multiplet (δ 1.1-1.6) centered near 1.4 probably is due to the proton at *e* that is *cis* to the thyminyl group. The overlapping multiplets due to the two hydroxyl groups slightly overlap the multiplet due to the proton at *a*.

(±)-1-[(1α,2β,3β,4α)-2,3-Dihydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4-(1*H*,3*H*)pyrimidinedione (IXg).

Method A.

A solution of 288 mg. of VIIIg in 10 ml. of 15*N* aqueous ammonia was heated under reflux for 3.5 hours, filtered, and concentrated to a syrup *in vacuo*. Evaporation of several portions of ethanol from the residue caused it to solidify. The white solid was triturated with ethyl acetate, collected by filtration, washed with ethyl acetate, and dried *in vacuo*: yield, 230 mg. (90%). The mass spectrum showed that this material was IXg; the melting temperature (192-200°) indicated that it was the lower-melting form (*cf.* Method B).

A hot solution of 225 mg. of this material, 10 ml. of ethanol, and 1 ml. of water was filtered, and the filtrate was diluted with 6 ml. of ether. The chilled solution yielded 150 mg. of the higher-melting form of IXg (dried at 56°), m.p. 212-216° dec. (inserted at 100°, 3°/minute); tlc, 1 spot (40, 80, or 120 mcg., SGF, 5:1 chloroform-methanol); ir: (1800-1300 cm^{-1} region) 1685 s broad, 1650 sh., 1510 w, 1480, 1465, 1455, 1415, 1390, 1375, 1350, 1320, 1310; uv: max 273 (ε 10,100) in 0.1*N* hydrochloric acid and at *p*H 7, 272 (ε 7900) in 0.1*N* sodium hydroxide; ms: (direct-probe temperature, 60°) (23) m/e 257 (M + 1), 256 (M), 254 (M - 2), 239 (M - OH), 238 (M - H_2O), 209, 207 (M - CH_2OH - H_2O), 181, 179, 169, 168, 165, 164, 153 (Th + C_2H_4), 148, 139, 136, 130, 127 (Th + 2H), 126 (Th + H), 113, 110, 109, 99; ^{1}H-nmr (DMSO-d_6, 34 mg./0.4 ml.): (22) δ 1.1-1.5 and 1.7-2.2 (m, CH_2 at *e* and CH at *d*), 1.79 (s superimposed on m, CH_3), 3.43 (m, CH_2OH), 3.73 and 4.0 (2 multiplets, CH at *b* and *c*), 4.2-5.2 (m, CH at *a*and OH groups), 4.66 (after addition of deuterium oxide, m, CH at a), 7.54 (approximate s, pyrimidine CH), about 11 (broad s, NH); ^{13}C-nmr (DMSO-d_6, 25 MHz): δ 11.91, 27.66, 44.71, 59.99, 62.81, 71.35, 73.12, 108.95, 138.30, 151.28, 163.84.

Anal. Calcd. for $C_{11}H_{16}N_2O_5$: C, 51.55; H, 6.29; N, 10.93. Found: C, 51.67; H, 6.34; N, 10.80.

Method B.

A solution of 288 mg. of VIIIg in 10 ml. of 0.1*N* sulfuric acid was heated under reflux for 3.5 hours. The thymine derivative (IXg) was isolated by the procedure described in Method A for IXd except that the reaction solution was neutralized to *p*H 7 with 2*N* sodium hydroxide. Concentration of the ethyl acetate extract, after 20 hours of continuous liquid-liquid extraction, yielded a white solid that was triturated with ethyl acetate, separated by filtration, washed with ethyl acetate, and dried *in vacuo* at 56°, weight, 106 mg., m.p. 197-200° dec. (inserted at 100°, 3°/minute). The NMR and mass spectra were identical with those of the analytical sample (Method A). When a small amount of the higher-melting form was mixed with this material, the m.p. rose to 212-215°. Additional IXg was obtained in the same way by extracting the aqueous layer for 48 hours with a second portion of ethyl acetate: weight, 91 mg. (total yield, 77%), m.p. 208-212°; tlc, 1 spot. The two portions were combined and recrystallized from an ethanol-water-ether mixture: recovery, 70%, m.p. 212-216° dec.; tlc, 1 spot.

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(13) *cis*-3-Aminocyclopentanemethanol, required for IXd, was prepared from *cis*-1,3-cyclopentanedicarboxylic acid.

(14) Two upfield multiplets were observed at δ 1.1-1.6 and at 1.6-2.3; the first probably arises from the proton at *e* (structure X) that is *cis* to the thyminyl group.

(15) Kindly provided by Dr. K. C. Murdock.

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(18) Silica Gel H plates were made from Silica Gel H for tlc according to Stahl, without calcium sulfate binder, 10-40 microns, EM Reagents (E. Merck, Darmstadt, Germany), distributed by Brinkmann Instruments, Inc., Westbury, N. J.

(19) Silica Gel GF precoated thin-layer chromatography plates (fluorescent), 250 microns in thickness, were purchased from Analtech Inc., Blue Hen Industrial Park, Newark, Delaware, 19711.

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(21) In later preparations of the acryloylurea precursors of 1-substituted thymines and uracils (1), better results were obtained by using freshly prepared silver cyanate, which was dried at room temperature for 3 days.

(22) Positions *a,b,c,d,e*, are shown in structure X. The pyrimidine CH shows a small long-range coupling with the methyl protons, but it appears as an approximate singlet in the usual expansion.

(23) Th = thyminyl moiety ($C_5H_5N_2O_2$).