Nonclassical Antimetabolites XXIV

Alkylation of 5-Fluorouracil

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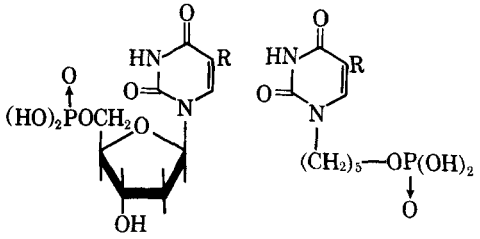
Alkylation of 5-fluorouracil with 1-bromopentane or 5-chloropentyl *p*-nitrobenzoate in dimethyl sulfoxide in the presence of potassium carbonate and sodium iodide occurred at the N_3-position. In contrast, alkylation with allylic-type halides, such as allyl bromide and benzyl chloride, occurred at the N_1-position. These results are in contrast to alkylation of uracil where either saturated or allylic halides gave alkylation of N_1.

In the preceding paper of this series (1), it was shown that uracil-1-pentanol phosphate (III) gave 50% inhibition of thymidylate synthetase at a concentration 57-fold higher than the substrate, 2′-deoxyuridylate (I). Earlier work showed that when the phosphate group of II was not present, as in 5′-fluoro-2′-deoxyuridine, a 90,000-fold loss in binding to this enzyme occurred (2). That 5-fluoro-2′-deoxyuridylate (II) was bound to thymidylate synthetase about 1000-fold better than the substrate (I) had been known previously (3, 4). Thus, it was concluded (1) that the phosphate group contributed more to the binding of the 2′-deoxy-D-ribotide moiety than the other two oxygen functions of this moiety combined, as earlier predicted (5). Furthermore, it could be estimated (1) that 5-fluorouracil-1-pentanol phosphate (IV) should bind to thymidylate synthetase 18-fold better than the substrate (I).

Uracil-1-pentanol phosphate (III) was previously synthesized *via* XVI*a* and XVII*a* by direct alkylation of uracil with 5-chloropentyl *p*-nitrobenzoate (1). The direct alkylation of 5-fluorouracil gave either 1- or 3-substitution depending upon the nature of the alkyl halide. These results are the subject of this paper.



I, R = H
II, R = F

III, R = H
IV, R = F

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DISCUSSION

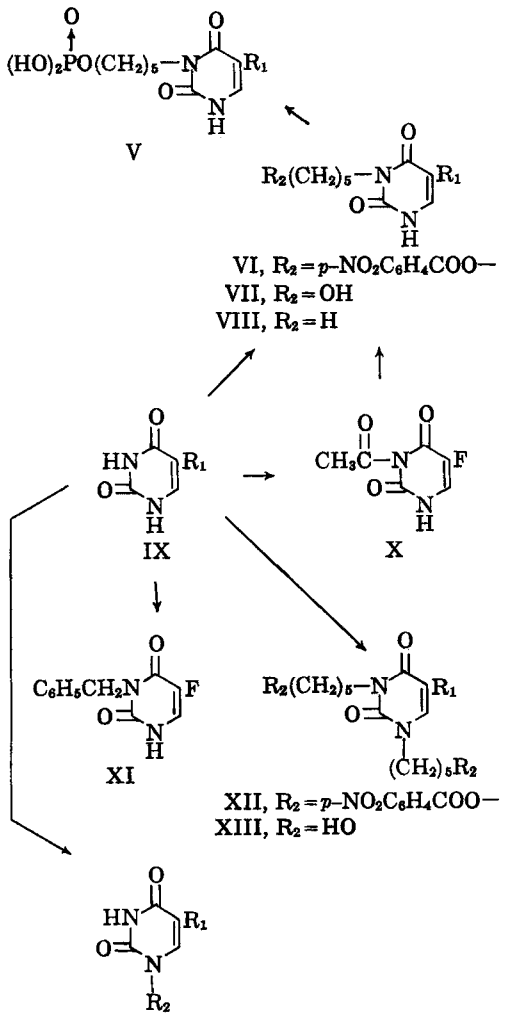
Alkylation of 5-fluorouracil (IX*b*) with 5-chloropentyl *p*-nitrobenzoate in dimethyl sulfoxide in the presence of potassium carbonate and sodium iodide gave a mixture of mono- and di-alkylated products; these two products were separated by fractional crystallization with some loss to give 17% of pure di-alkylated product (XII*b*) and 20% of pure mono-alkylated product. Even though uracil (IX*a*) gave primarily the 1-alkylated product (XVI*a*), it could not be automatically assumed that 5-fluorouracil (IX*b*) would also alkylate at the 1-position to give XVI*b*, particularly since 5-fluorouracil (IX*b*) has been shown to give a 3-anion in basic solution, in contrast to uracil (IX*a*) which gives a 1-anion (6). The position of alkylation is normally determined by the bathochromic ultraviolet spectral shift from neutral to basic solution; the anion of a 1-substituted uracil shows no appreciable shift from the neutral species whereas a 3-substituted uracil does show a shift to longer wavelength when converted to its anion (7).

It is better to remove the *p*-nitrobenzoyl blocking group before one can be certain whether the product is a 1-substituted (XVI*b*) or a 3-substituted 5-fluorouracil (VI*b*) since the *p*-nitrobenzoate moiety itself causes a bathochromic shift in alkaline solution. Debenzoylation of the mono-alkylated product (VI*b* or XVI*b*) with *n*-butylamine in methanol (1) or methanolic sodium methoxide gave two products (besides those arising from the *p*-nitrobenzoyl moiety), whereas the corresponding uracil (XVI*a*) gave only a single product (XVII*a*) (1). This difficulty was finally traced to the ability of VII*b* (or XVII*b*) to form a stable salt with *n*-butylamine which did not dissociate readily; this difficulty was avoided with diisopropylamine which gave a readily dissociable salt. That the 5′-hydroxypentyl group was at the 3-position of 5-fluorouracil (VII*b*) and not on the desired 1-position was clearly shown by bathochromic shift of 12 mμ in basic solution compared to neutral solution. (Scheme I.)

Since the mercuri derivative of 5-fluorouracil, prepared from *N*-acetyl-5-fluorouracil (X) (8), is known to orient an incoming acyl glycosyl halide to the 1-position (9), the condensation of 5-iodopentyl *p*-nitrobenzoate with the mercuri salt in boiling xylene was investigated; no reaction took place. In fact, even allyl bromide was not sufficiently reactive to react.

It was considered possible that if the 3-position of 5-fluorouracil was first blocked with a removable group, then alkylation would proceed on the 1-

position. Since the *N*-acetyl derivative (X) was now available, it was investigated first. First, it is uncertain where the acetyl group resides on the molecule (X). Even though the acetyl group of X is readily removed in alkaline solution, a spectrum of X taken immediately in 0.1 *N* aqueous sodium hydroxide showed a peak at longer wavelength (288 mμ) than seen with 5-fluorouracil (284 mμ) under the same conditions. Although not conclusive, the results suggested that the *N*-acetyl group of X resided at the 3-position. When treated with 5-chloropentyl *p*-nitrobenzoate in dimethyl sulfoxide in the presence of potassium carbonate and sodium iodide, X gave a mixture of VI*b* and XII*b*. Similarly, the use of sodium hydride in place of potassium carbonate gave the same products.



XIV R_2 = $C_6H_5CH_2$—
XV, R_2 = CH_2=$CHCH_2$—
XVI, R_2 = p-$NO_2C_6H_4COO(CH_2)_5$—
XVII, R_2 = HO—$(CH_2)_5$—
III*a*, IV*b*, R_2 = $(HO)_2OPO$—$(CH_2)_5$—

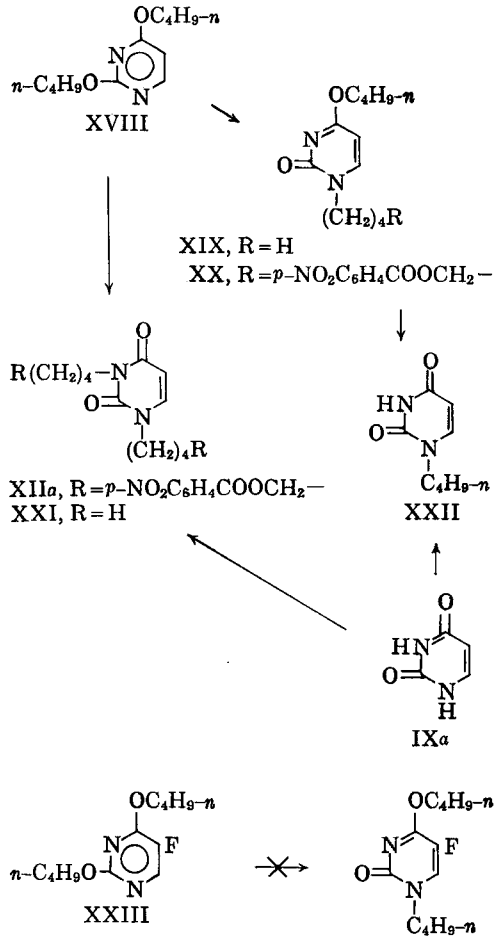
Scheme I

Another blocking group that was considered was the *N*-benzyl; the *N*-benzyl and benzhydryl blocking groups have been used for controlling orientation in the purine and 1,2,4-triazine series (10, 11). When 5-fluorouracil (IX) was reacted with benzyl chloride in dimethyl sulfoxide in the presence of potassium carbonate, a monobenzyl derivative (XI or XIV*b*) was obtained in 51% yield. Surprisingly, the compound was 1-benzyl-5-fluorouracil (XIV*b*), as shown by the lack of a shift in its ultraviolet spectrum between neutral and basic solution; this result contrasted sharply with the alkylation of 5-fluorouracil in the 3-position (VI*b*) by 5-chloropentyl *p*-nitrobenzoate. Similarly, alkylation of 5-fluorouracil with allyl bromide gave 1-allyl-5-fluorouracil (XV*b*) in 39% yield, but alkylation with pentyl bromide gave 3-pentyl-5-fluorouracil (VIII*b*).

The four alkyl halides could be divided into two distinct groups: (*a*) the extremely reactive allylic halides which gave 1-alkylation and (*b*) the much less reactive saturated alkyl halides, which gave 3-alkylation. Therefore, the reaction with benzyl chloride was studied further. No reaction between 5-fluorouracil and benzyl chloride took place in the absence of potassium carbonate. In order to determine whether the potassium carbonate was merely an acid acceptor or whether it converted 5-fluorouracil to an anion prior to reaction with benzyl chloride, the preformed sodium salt of 5-fluorouracil in dimethyl sulfoxide was reacted with benzyl chloride; again 1-benzyl-5-fluorouracil (XIV*b*) was formed in 55% yield. It was therefore clear that the anion of 5-fluorouracil was being alkylated with both classes of alkyl halides. The orientation difference therefore resides in the nature of the alkyl halide, that is, allylic halides orienting to the 1-position and alkyl halides to the 3-position. Such a difference attributed to the relative reactivity of the halide has been previously noted during alkylation of adenine (12).

Since 3-(5′-hydroxypentyl)-5-fluorouracil (VII*b*) was available, it was converted to the phosphate (V*b*) with polyphosphoric acid; during isolation of the barium salt of V*b*, it was noted that the barium salt was less soluble in hot water than cold water. When the sodium salt of 3-(5′-hydroxypentyl)-5-fluorouracil phosphate (V*b*) was assayed as an inhibitor of thymidylate synthetase as previously described (2, 13), it showed no inhibition at a concentration of 6 m*M* in the presence of 0.04 m*M* 2′-deoxyuridylate (I). Thus, V*b* was even less effective than 1-(5′-hydroxypentyl)uracil phosphate (III*a*); this result indicated that V*b* cannot reorient to a suitable conformation for binding as was the case for nucleotides derived from isoadenosine (14) or for 5-alkylpyrimidines when bound to dihydrofolic reductase (15).

The Hilbert-Johnson reaction (16) for synthesis of 1-alkyluracil by alkylation of 2,4-diethoxypyrimidine is only successful if the alkyl halide is more reactive than the corresponding ethyl halide; if the alkyl halide is less reactive than the ethyl halide then the product is a 1-ethyluracil derivative (17). It should be possible to circumvent this difficulty if a higher ether of uracil were to be used. For example, *n*-butyl bromide with a butyl ether such as XVIII should give an *N*-butyl derivative (XIX), or if an alkyl halide is now used that is



Scheme II

more reactive than the corresponding *n*-butyl halide, then the alkyl group should be introduced preferentially.

When an equimolar mixture of 2,4-di(*n*-butoxy)-pyrimidine (XVIII) and *n*-butyl bromide was reacted on a steam bath for 5 days, a 19% yield of the 1-(*n*-butyl)pyrimidine (XIX) could be isolated; the structure was verified by acid hydrolysis to 1-(*n*-butyl)uracil (XXII) in near quantitative yield that was identical with an authentic sample prepared by direct butylation of uracil (18). When 5-iodopentyl *p*-nitrobenzoate was similarly reacted with 2,4-di-(*n*-butoxy)pyrimidine (XVIII), several products were formed. The bis-rearrangement product (XII*a*) could be isolated in 10% yield. It is probable that the reaction mixture also contained XIX, XX, XXI, and mixed bis-alkylation products, but these were not separated.

Durr (19) has recently reported that 2,4-diethoxy-5-fluorouracil will undergo the Hilbert-Johnson reaction (16) with methyl iodide. From his experimental conditions, it would appear that this fluoropyrimidine is considerably less reactive than 2,4-diethoxypyrimidine (16). When 2,4-di-(*n*-butoxy)-5-fluorouracil (XXIII) was reacted with *n*-butyl bromide under the conditions employed for the conversion of 2,4-di-(*n*-butoxy)pyrimidine (XVIII) to XIX, no reaction took place, again indicating that the 5-fluoropyrimidine XXIII is less reactive than XVIII. (Scheme II.)

As a result of the current study, it would appear that 1-alkyl-5-fluorouracils, such as XVII*b*, could best be synthesized by alkylation of 5-fluorouracil with an allylic-type halide followed by further transformation of the allylic grouping such as that of XV*b*.

EXPERIMENTAL

Melting points were taken on a Mel-Temp apparatus in capillary tubes and those below 230° are corrected. Infrared spectra were determined in KBr disk with a Perkin-Elmer 137B recording spectrophotometer. Ultraviolet spectra were determined with a Perkin-Elmer 202 recording spectrophotometer. Thin-layer chromatograms (TLC) were run with Brinkmann Silica Gel G, and spots were detected under ultraviolet light or iodine vapor or both.

5 - Fluorouracil - 3 - ylpentyl p - Nitrobenzoate (VIb).—To a stirred mixture of 1.95 Gm. (15 mmoles) of IX*b* and 35 ml. of dimethyl sulfoxide was added 2.07 Gm. (15 mmoles) of anhydrous potassium carbonate, 0.75 Gm. (5 mmoles) of sodium iodide, and 1.36 Gm. (5 mmoles) of 5-chloropentyl *p*-nitrobenzoate (1, 20). After being stirred in a bath at 80–85° for 4 hr., the mixture was cooled, diluted with 25 ml. of water, acidified to about pH 2 with 5% hydrochloric acid, then extracted with chloroform (5 × 40 ml.). The combined extracts, dried with magnesium sulfate, were spin-evaporated *in vacuo;* the last of the dimethyl sulfoxide was removed at less than 1 mm. Trituration of the remaining syrup with 10 ml. of ethanol gave 1.30 Gm. of solid, m.p. 85–100°. The solid was heated to boiling with methanol, then the hot mixture was filtered. Both the insoluble portion (0.405 Gm., m.p. 121–122°) and the 0.588 Gm. that separated on cooling consisted of two components on TLC with R_f 0.62 (VI*b*) and R_f 0.95 (XII*b*) in 5:1 benzene–methanol. The two fractions were combined and recrystallized from 2-methoxyethanol–water; 0.420 Gm. of solid, m.p. 117–120°, separated and was shown by TLC to be primarily XII*b* with only a trace of VI*b*. Recrystallization from ethanol to constant m.p. 121–123° afforded 0.252 Gm. (17%) of pure XII*b* that was free of VI*b* by TLC; $\nu_{max.}$ 1720 (ester C=O); 1670, 1650, 1600 (uracil, C=C); 1520, 1340 (NO_2); 1280 cm.$^{-1}$ (ester C—O—C); $\lambda_{max.}$ (pH 1) 267; (pH 7) 274; (pH 13) 272 mμ.

Anal.—Calcd. for $C_{28}H_{29}FN_4O_{10}$: C, 56.0; H, 4.87; N, 9.33. Found: C, 56.0; H, 4.94; N, 9.37.

Evaporation of the aqueous 2-methoxyethanol filtrate gave 0.428 Gm. of residue, m.p. 122–123°, that was shown by TLC to be predominately VI*b* with a trace of XII*b*. Several recrystallizations from ethanol gave 0.367 Gm. (20%) of crystals with constant m.p. 127–128° that was free of XII*b* as shown by TLC; $\nu_{max.}$ 1720 (ester C=O); 1680, 1650, 1600 (uracil, C=C); 1525, 1345 (NO_2); 1280 cm.$^{-1}$ (ester C—O—C); $\lambda_{max.}$ (pH 1) 269; (pH 7) 264; (pH 13) 280 mμ.

Anal.—Calcd. for $C_{16}H_{16}FN_3O_6$: C, 52.6; H, 4.44; N, 11.5. Found: C, 52.6; H, 4.25; N, 11.6.

3-(n-Amyl)-5-fluorouracil (VIIIa).—Alkylation of 0.975 Gm. (7.5 mmoles) of IX*b* with 0.38 Gm. (2.5

mmoles) of 1-bromopentane, as described for preparation of VI*b*, gave on evaporation of the chloroform extracts, 0.260 Gm. of an oil. The oil was extracted with three 30-ml. portions of petroleum ether (b.p. 60–110°), decanting from the residual oil each time. The combined petroleum ether extracts were spin-evaporated *in vacuo*. Crystallization of the residue from ethyl acetate–petroleum ether (b.p. 30–60°) gave 0.125 Gm. (24.6%) of white crystals, m.p. 110–117°. A second recrystallization gave 0.109 Gm. (21.5%), m.p. 126–127°; $\lambda_{max.}$ (pH 1, 7), 272; (pH 13) 286 mμ; $\nu_{max.}$ 1680, 1650 cm.$^{-1}$ (uracil).

Anal.—Calcd. for $C_9H_{13}FN_2O_2$: C, 54.0; H, 6.55; N, 14.0. Found: C, 53.9; H, 6.43; N, 14.1.

5 - Fluoro - 3 - (5'hydroxypentyl)uracil (VIIb).—A solution of 210 mg. (0.58 mmole) of VI*b* and 0.21 Gm. of diisopropylamine in 14 ml. of methanol was refluxed for 21 hr., then spin-evaporated *in vacuo*. The residue was extracted with 5 ml. of boiling ethyl acetate, a small amount of insoluble material being removed. Spin-evaporation of the ethyl acetate solution *in vacuo* gave 86 mg. (69%) of an oil which crystallized on standing at −5° and then had m.p. 72–82°. Recrystallization from ethyl acetate gave 50 mg. (40.1%) of white crystals, m.p. 93–94°; $\lambda_{max.}$ (pH 13), 285 mμ; (H_2O) 272 mμ (ε 6800); $\nu_{max.}$ 3400 (OH); 1700, 1660 cm.$^{-1}$ (uracil).

Anal.—Calcd. for $C_9H_{13}FN_2O_3$: C, 50.0; H, 6.06; N, 13.0. Found: C, 49.8; H, 6.06; N, 12.8.

5 - Fluoro - 1,3 - bis - (5' - hydroxypentyl)uracil (XIIIb).—Similarly, debenzoylation of 183 mg. (0.32 mmole) of XII*b* with 0.25 Gm. of diisopropylamine in 20 ml. of methanol at b.p. for 8 hr. gave, after recrystallization from ethyl acetate–petroleum ether (b.p. 60–110°), 60 mg. (65%) of white crystalline XIII*b*, m.p. 60–62°; $\lambda_{max.}$ (pH 1, 7, 14) 278 mμ; $\nu_{max.}$ 3400 (OH); 1670, 1650 cm.$^{-1}$ (uracil).

Anal.—Calcd. for $C_{14}H_{23}FN_2O_4$: C, 55.6; H, 7.67; N, 9.27. Found: C, 55.5; H, 7.81; N, 9.41.

1-Benzyl-5-fluorouracil (XIVb).—*Preparation A.*—Alkylation of 0.65 Gm. (5 mmoles) of IX*b* with 0.42 Gm. (3.3 mmoles) of α-chlorotoluene, as described for the preparation of VI*b*, gave an oil after evaporation of the chloroform that was crystallized by trituration with petroleum ether. Recrystallization from ethyl acetate gave 0.375 Gm. (51%) of white crystals, m.p. 170–171°, that were uniform on TLC; $\lambda_{max.}$ (pH 1, 7), 276 mμ; (pH 13), 274 mμ; $\nu_{max.}$ 1700, 1660 (uracil); 745, 700 cm.$^{-1}$ (C_6H_5).

Anal.—Calcd. for $C_{11}H_9FN_2O_2$: C, 60.0; H, 4.12; N, 12.7. Found: C, 60.1; H, 4.30; N, 12.8.

Preparation B.—Alkylation of the preformed sodium salt of IX*b* in the absence of potassium carbonate in a similar fashion gave 309 mg. (56%) of product, m.p. 158–165°. Recrystallization from ethyl acetate gave white plates, m.p. 173–174°, which were identical with *Preparation A* by TLC and mixed melting point. Spectral characteristics were also identical.

1-Allyl-5-fluorouracil (XVb).—Alkylation of 0.975 Gm. (7.5 mmoles) of IX*b* with 0.31 Gm. (2.5 mmoles) of allyl bromide, as described for the preparation of VI*b*, gave an oil after evaporation of the chloroform. The oil was crystallized from 3 ml. of ethyl acetate by addition of petroleum ether (b.p. 30–60°); yield, 0.166 Gm. (39%), m.p. 113–123°. Recrystallization from the same solvent pair gave white crystals, m.p. 126–127°; $\lambda_{max.}$ (pH 1, 7, 13), 274 mμ; $\nu_{max.}$ 1710, 1650, 1600 cm.$^{-1}$ (uracil, C=C).

Anal.—Calcd. for $C_7H_7FN_2O_2$: C, 49.4; H, 4.15; N, 16.5. Found: C, 49.2; H, 3.93; N, 16.7.

Barium 5 - Fluoro - 3 - (5' - hydroxypentyl)-uracil Phosphate (Vb).—Treatment of 216 mg. (1 mmole) of VII*b* with polyphosphoric acid, as described for the preparation of III*a* (1), gave 160 mg. of crude product after removal of the barium phosphate and evaporation of the filtrate. The crude product was dissolved in 10 ml. of water, then 10 ml. of ethanol was added. Some inorganic material (30 mg.) was removed by filtration, and the filtrate was concentrated to about 5 ml. Addition of 15 ml. of ethanol gave 60 mg. (13%) of insoluble product. This precipitate was dissolved in the minimum of cold water and reprecipitated by addition of ethanol. One more reprecipitation gave 38 mg. (8.3%) of hydrated white solid, m.p. 310–315° dec.; $\nu_{max.}$ 3410 (H_2O); 1700–1650 (uracil); 1080–1060, 990–980 cm.$^{-1}$ (phosphate); $\lambda_{max.}$ (pH 1, 7) 274 mμ; (pH 13) 283 mμ. The ultraviolet extinction coefficient at 274 mμ was in agreement with 1.5 moles of water of solvation.

Anal.—Calcd. for $C_9H_{12}BaFN_2O_6P \cdot 1.5\ H_2O$: C, 23.6; H, 3.29; N, 6.09. Found: C, 23.6; H, 3.34; N, 5.76.

3 - (5' - Carbamoyloxypentyl) - 5 - fluorouracil.—To a stirred and ice-cooled solution of 324 mg. (1.5 mmoles) of VII*b* in 6 ml. of reagent pyridine was added 0.47 Gm. (3 mmoles) of phenyl chloroformate over a period of 20 min. (2). The mixture, protected from moisture, was stirred at ambient temperature for 3.5 hr., then spin-evaporated *in vacuo*. The residue was triturated with 10 ml. of hot water, then 5 ml. Traces of water were removed from the oil by spin-evaporation *in vacuo*. The residual oil was further leached with two 20-ml. portions of boiling petroleum ether. The insoluble oily 3-(5'-carbophenoxyoxypentyl)-5-fluorouracil (0.28 Gm., 55%) moved primarily as one spot on TLC with one trace impurity; $\nu_{max.}$ 1765 (ester C=O); 1710, 1660, 1595 cm.$^{-1}$ (uracil, C=C).

The carbophenoxy derivative (168 mg.) was dissolved in 5 ml. of methanol previously saturated with ammonia at 0° (2). After 30 min. at 0°, the solution was spin-evaporated *in vacuo*. The oily residue was leached with ether to remove phenol. Crystallization from 1 ml. of ethyl acetate gave 52 mg. (40%) of the 5'-*O*-carbamate of VII*b*, m.p. 115–122°. Recrystallization from ethyl acetate–petroleum ether (b.p. 60–110°) afforded white crystals, m.p. 122–124°; $\nu_{max.}$ 3500, 3200 (NH); 1730 (carbamate C=O); 1690, 1650 (uracil); 1605 cm.$^{-1}$ (amide II).

Anal.—Calcd. for $C_{10}H_{14}FN_3O_4$: C, 46.3; H, 5.44; N, 16.2. Found: C, 46.6; H, 5.59; N, 16.1.

4 - (n - Butoxy) - 1 - (n - butyl) - 2 - pyrimidone (XIX).—A solution of 1.49 Gm. (10 mmoles) of 2,4-dichloropyrimidine in 5 ml. of *n*-butanol was added to a solution of 0.48 Gm. (20.9 mmoles) of sodium in 10 ml. of *n*-butanol. The solution became hot and sodium chloride precipitated. The solvent was removed by spin-evaporation *in vacuo*. The residue was partitioned between 10 ml. of water and 50 ml. of chloroform. The separated chloroform

solution was washed with 25 ml. of 30% aqueous sodium hydroxide, then 10 ml. of water. Dried with magnesium sulfate, the chloroform solution was spin-evaporated *in vacuo* leaving 1.59 Gm. (71%) of crude 2,4-bis-(*n*-butoxy)pyrimidine (XVIII) as an oil that was suitable for further transformations and had $\nu_{max.}^{film}$ 1570–1560 (C=C, C=N); 1080 cm.$^{-1}$ (C—O—C).

A mixture of 565 mg. (2.5 mmoles) of XVIII, 360 mg. of *n*-butyl bromide (2.6 mmoles), and 1 drop of methyl iodide was heated on a steam-bath under a reflux condenser for 5 days. Volatiles were removed by spin-evaporation *in vacuo*. The residue readily dissolved in petroleum ether (b.p. 30–60°). After 2 weeks at −5°, the solution deposited 106 mg. (19%) of product, m.p. 53–54°. Recrystallization from petroleum ether (b.p. 60–110°) gave white crystals, m.p. 54°; $\nu_{max.}$ 1660, 1615 (C=O, C=C, C=N); 1300 cm.$^{-1}$ (C—O—C); $\lambda_{max.}$ (pH 1, 7, 13) 278 mμ.

Anal.—Calcd. for $C_{12}H_2ON_2O_2$: C, 64.3; H, 8.99; N, 12.5. Found: C, 64.0; H, 9.04; N, 12.8.

1-(n-Butyl)uracil (XXII).—A solution of 50 mg. of XIX in 5 ml. of methanol and 2 ml. of 12 *N* aqueous hydrochloric acid was allowed to stand at ambient temperature for 5 days. Spin-evaporation *in vacuo* gave 36 mg. (96%) of white crystals, m.p. 98–101°, which was identical with an authentic sample prepared by alkylation of uracil (18), as shown by mixed melting point and infrared and ultraviolet spectra.

The preparation of this compound, m.p. 100–102°, by an alternate route has been recently described (21).

1,3 - Bis - [5 - (p - nitrobenzoyloxy)pentyl]uracil (XIIa).—*Preparation A.*—Alkylation of 280 mg. (2.5 mmoles) of uracil (IX*a*) with 1.63 Gm. (6 mmoles) of 5-chloropentyl *p*-nitrobenzoate (1), as described for the preparation of VI*b* gave, on evaporation of the chloroform, an oil. Crystallization from ethanol afforded 530 mg. (37%) of product, m.p. 130–133°. Recrystallization from 2-methoxyethanol–benzene gave 478 mg. (33%) of white crystals, m.p. 136–137°. A further recrystallization raised the m.p. to 138–140°; $\nu_{max.}$ 1700 (ester C=O); 1650, 1590 (uracil, C=C); 1505, 1340 cm.$^{-1}$ (NO_2); $\lambda_{max.}^{10\%EtOH}$ (pH 1, 7), 276; (pH 13), 271 mμ.

Anal.—Calcd. for $C_{28}H_{30}N_4O_{14}$: C, 57.7; H, 5.19; N, 9.62. Found: C, 57.8; H, 5.29; N, 9.68.

Preparation B.—5-Chloropentyl *p*-nitrobenzoate was converted to 5-iodopentyl *p*-nitrobenzoate with sodium iodide in acetone. A mixture of 226 mg. (1 mmole) of XVIII, 363 mg. (1 mmole) of 5-iodopentyl *p*-nitrobenzoate, and 1 drop of methyl iodide was heated on a steam bath for 3 days. The oil was leached with three 15-ml. portions of hot petroleum ether (b.p. 60–110°). The insoluble residue was crystallized from benzene–petroleum ether (b.p. 60–110°); yield, 67 mg. (10%), m.p. 127–131°. Recrystallization from ethyl acetate gave 45 mg. (7.7%), m.p. 136–137°, that was identical with *Preparation A* as shown by infrared spectra, mixed melting point, and mobility on TLC in benzene–methanol (5:1).

The filtrate from the 67 mg. showed 4 spots when examined by TLC, one of which was additional XII*a*.

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