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Syntheses of some Analogs of a Possible Intermediate Formed in the Thymidylate Synthetase Reaction (1)

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The syntheses of a number of analogs of a possible intermediate formed in the thymidylate synthetase reaction are described. These consist of a number of 5-arylaminomethyluracils (X) and *N*-methyl-*N*-[1,-(2′-deoxy-β-D-ribofuranosyl)thyminyl]-*p*-aminobenzoyl glutamic acid (XXb).

A rational approach to chemotherapy on a molecular level can be reduced to the blockade of a critical enzymic reaction in an invading cell without significantly disturbing the metabolic processes of the host cell. Rudimentary to this approach is the design of potent inhibitors that are specific for the target enzyme. Because of the plurality of enzymic reactions in which a substrate or cofactor may individually participate, analogs of either are likely to manifest undesirable side effects upon other systems. The achievement of the necessary intracellular specificity obviously depends upon the exploitation of a unique feature of the target enzyme; one such feature is found in the catalyzed reaction *per se*. Although a number of cellular enzymes may utilize a particular substrate or cofactor independently, only in a single enzymic reaction are they both used concurrently. It follows that a specific enzymic reaction might be selectively inhibited by (a) an analog of a unique intermediate formed in the reaction, (b) an analog that simulates the sterochemistry of a transition state of the reaction, or (c) an analog that combines certain structural features of both substrate and cofactor in the orientation assumed by them on the active site prior to reaction (2).

Thymidylate synthetase is the enzyme that catalyzes the conversion of 2′-deoxyuridine-5′-monophosphate (dUMP) (I) to thymidine-5′-monophosphate (TMP) (V). The overall reaction has been proposed (3) to consist of two discrete steps (Figure 1): alkylation of dUMP (I) with N^5,N^{10}-methylenetetrahydrofolic acid (N^5,N^{10}-CH_2FAH_4) (II), to give N^5-thymidylyltetrahydrofolic acid (III), followed by disproportionation to give TMP (V) and 7,8-dihydrofolic acid (7,8-FAH_2) (VI). It is important to be cognizant that although the intermediacy of III appears to be favored by most investigators (2a, 3), lack of data forces one to be non-commital as to the exact structure of the intermediate formed in the thymidylate synthetase reaction (III or IV). Moreover, the sterochemical relationship of the reactive centers of the deoxyuridylic acid (I) and N^5,N^{10}-CH_2FAH_4 (II) in the enzyme-substrate complex is apparent indicating that analogs of either of the possible intermediates might suffice to inhibit the enzymic reaction. The successful inhibition of thymidylate synthetase by analogs of III or IV depends critically upon the accessibility of the enzymic active site; the conjecture must be made that there is no conformational change of the enzyme prior to reaction that would prohibit analogs of this type to occupy the active site.

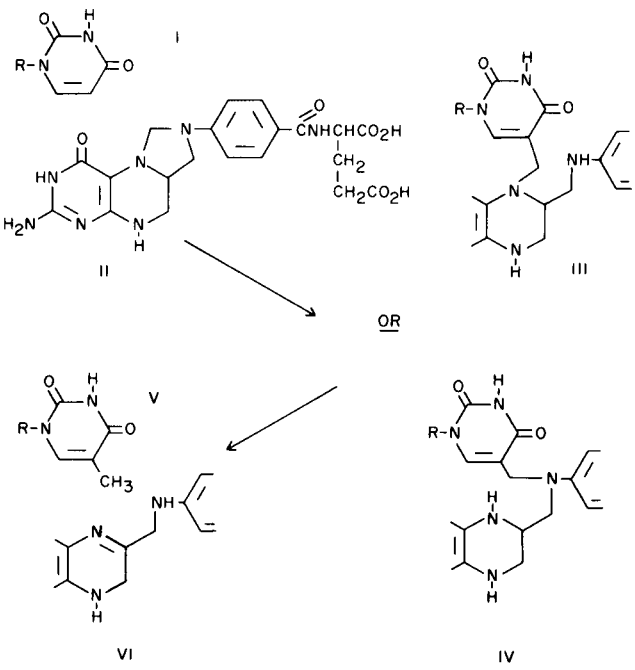
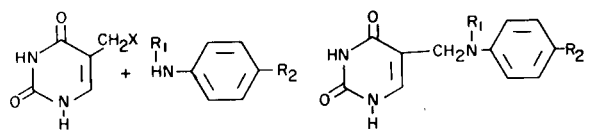
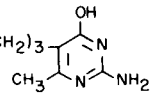


Figure 1. Schematic representation of the overall reaction catalyzed by thymidylate synthetase.



VII, X = Cl IX X
VIII, X = OH

	R_1	R_2
a	-H	-H
b	$-CH_3$	-H
c	-H	$-OCH_3$
d	-H	$-COCH_3$
e	-H	$-CO_2C_2H_5$
f	-H	$-CO_2H$
g	$-CH_3$	$-CONHCHCO_2C_2H_5$ / CH_2 / $CH_2CO_2C_2H_5$
h	$-CH_3$	$-CONHCHCO_2H$ / CH_2 / CH_2CO_2H
i	$-(CH_2)_3$ 	$-CO_2H$

Based on the above rationalizations, a number of analogs of N^{10}-thymidylyltetrahydrofolic acid (IV) were synthesized and evaluated as candidate "hybrid" inhibitors of thymidylate synthetase. Initial studies were directed toward the synthesis of simple 5-arylaminomethyluracil derivatives of type X; subsequently, the more complex nucleoside analog XXb was synthesized.

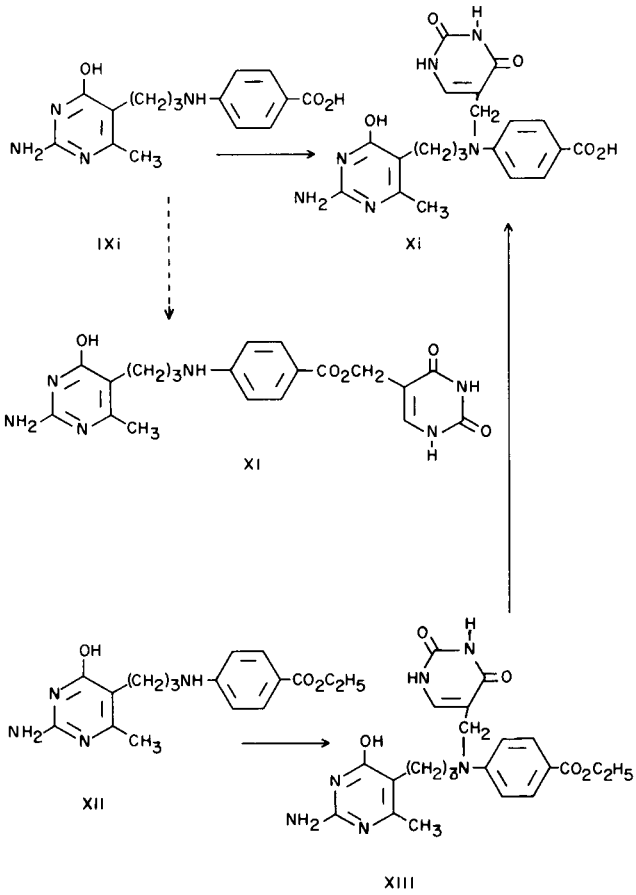
Chemistry.

The most general synthetic approach to compounds of type X was considered to be via condensation of an activated thymine derivative, such as 5-chloromethyluracil (VII) or 5-hydroxymethyluracil (VIII), with an aromatic amine (IX). Although VIII might not be anticipated to be sufficiently reactive to alkylate an aromatic amine, a number of observations indicate the 5-hydroxymethyl group of VIII to be unusually reactive toward nucleophiles (4). For example, a product believed to be 5-thyminylglycine was obtained after treatment of an aqueous solution of glycine with VIII. Mild treatment of 5-hydroxymethyluracil with alcohols in the presence of a catalytic amount of acid resulted in the facile formation of ethers of VIII which readily underwent hydrolysis in boiling water.

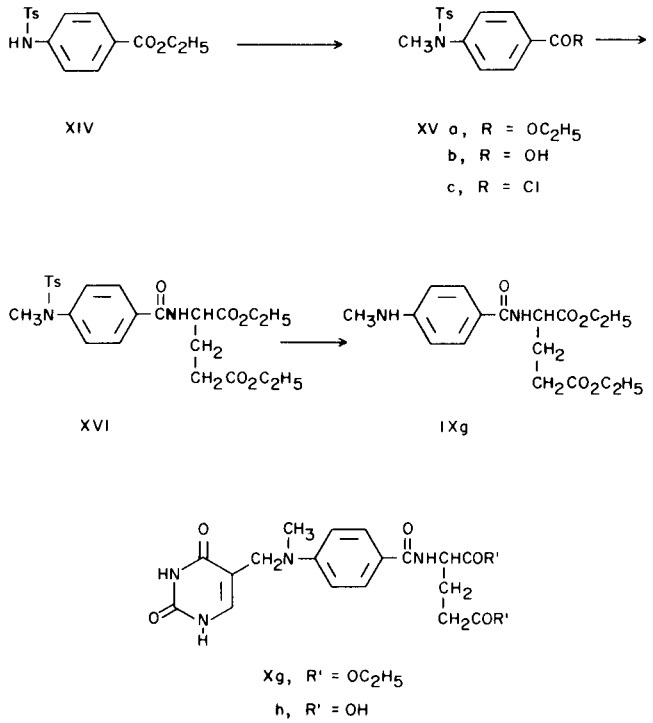
Alkylation of the arylamines (IXa-IXe) with the highly reactive 5-chloromethyluracil (VII) (5) in either acetone or diglyme readily gave the 5-arylaminomethyluracils (Xa-Xe) in 61-87% yields. When an aqueous solution of 5-hydroxymethyluracil (VIII) and *p*-anisidine (IXc) were heated at 70° for 3 hours, Xc was obtained in 62% yield. Similarly, treatment of the hydrochloride salts of *N*-methylaniline (IXb), *p*-aminoacetophenone (IXd), or *p*-aminobenzoic acid (IXf) with VIII gave the corresponding 5-arylaminomethyluracils (Xb,d,f).

Quite unexpectedly, when a solution of IXd and VIII in 0.5 *N* sodium hydroxide was heated at 70° for 4 hours, Xd was obtained in 46% yield. The facile alkylation of aromatic amines in neutral and acidic media by 5-hydroxymethyluracil can be adequately rationalized by considering the 5-hydroxymethyl group of VIII as a vinylogous carbinolamine, which would be expected to be quite reactive toward nucleophiles in the presence of an acid catalyst; however, it is difficult to explain the base catalyzed formation of Xd. The mechanistic features of this reaction shall be the topic of a subsequent report.

When an aqueous solution of the sodium salt of IXi (6) was allowed to react with chloromethyluracil a product was obtained that gave combustion values in agreement with Xi and showed a single spot on paper chromatography with a number of solvent systems. Treatment of this product with hot 0.5 *N* sodium hydroxide resulted in the regeneration of IXi (identified by paper chromatography) along with an additional product that could not be identified. It was initially considered possible that alkylation of the carboxylate moiety of IXi had resulted to give the isomeric thy-



minyl ester XI. That esterification had not occurred was demonstrated by a study of the hydrolysis of XIII, obtained after treatment of XII (7) with 5-chloromethyluracil.

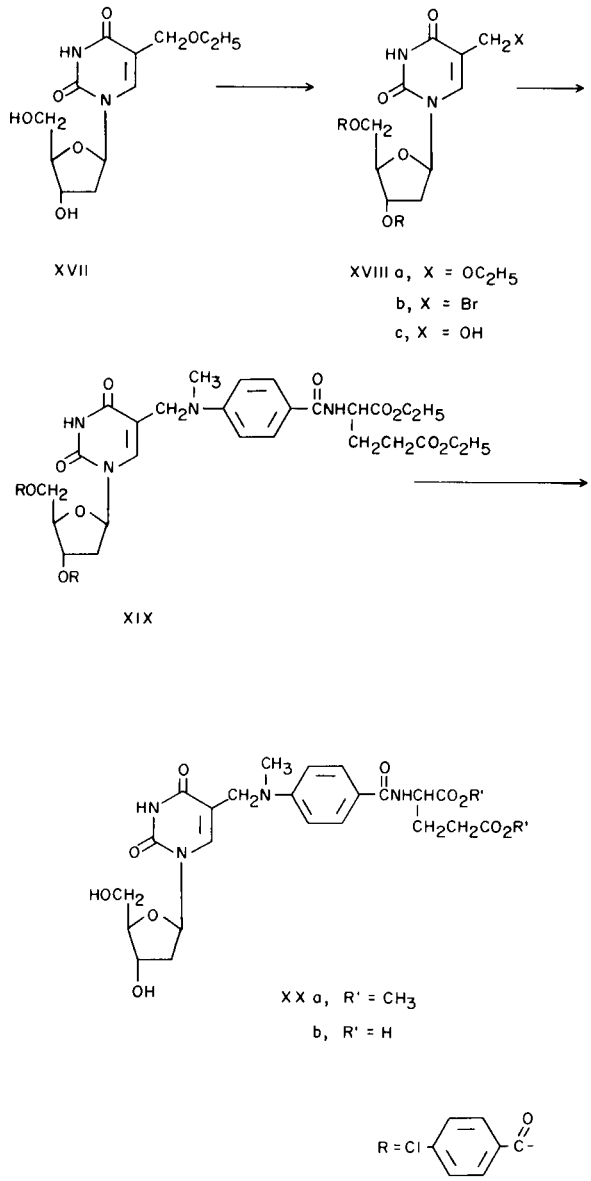


Mild alkaline hydrolysis of XIII gave a single product that had identical chromatographic properties as the compound tentatively assigned as Xi; further treatment resulted in the formation of IXi, and the unidentified product previously cited. Based on the above study, it was concluded that the thyminyl linkage of Xi had undergone cleavage in aqueous base to yield IXi and an unidentified product derived from the uracil moiety of Xi.

Diethyl *N*-methyl-(*p*-aminobenzoyl)glutamate (IXg), the key intermediate needed for the synthesis of Xg, was prepared by a modification of the reported sequence (8) starting from the readily available ethyl *N*-tosyl-*p*-aminobenzoate (XIV) (7). Methylation of the sodium salt of XIV with methyl iodide in dimethylformamide gave XVa, which could be hydrolyzed to the corresponding acid XVb in nearly quantitative yield. With thionyl chloride in benzene, XVb was easily converted to the crystalline acid chloride (XVc). Acylation of diethylglutamate with XVc proceeded smoothly in the two phase system of methylene chloride-water to give a quantitative yield of crude XVI as a clear viscous oil. Removal of the *N*-tosyl group of XVI was effected using hydrogen bromide in glacial acetic acid in the presence of phenol (9) to give a crude hydrobromide salt of IXg; neutralization with potassium bicarbonate gave the desired IXg in 54% overall yield from XVa.

Alkylation of IXg with 5-chloromethyluracil in aqueous dioxane, using triethylamine as an acid acceptor gave pure Xg in 65% yield. When a solution of Xg in 50% aqueous methanol was treated with a slight excess of sodium hydroxide for two hours at ambient temperature and then neutralized with acetic acid, Xh separated from solution. However, since the gelatinous nature of the precipitated product did not readily permit its isolation, the acidified reaction mixture was treated with barium acetate to give Xh as an amorphous barium salt in 49% yield.

For the synthesis of the nucleoside analog (XXb) of N^{10}-thymidylyltetrahydrofolic acid (IV), a suitably protected 5-halogenomethyl-2′-deoxyuridine was desired as an intermediate. As the reported routes (10) to compounds of this type are fairly lengthy and involve a tedious nucleoside coupling, it was considered expedient to devise a synthesis starting from the preformed nucleoside, 5-ethoxymethyl-2′-deoxyuridine (XVII) (11).



The blocked ethoxymethyl nucleoside (XVIIIa) was obtained in 78% yield from XVII and *p*-chlorobenzoyl chloride. After treatment of XVIIIa with anhydrous hydrogen bromide in chloroform the crude bromomethyl derivative (XVIIIb) was obtained. Thin layer chromatography of this crude product showed the major product to be XVIIIb, which gave a positive *p*-nitrobenzylpyridine test (12), along with a minor slower moving product that was tentatively identified as the hydroxymethyl nucleoside (XVIIIc). The yield of the desired product (XVIIIb) was visually estimated to be in excess of 80%. When this crude product was allowed to react with Xg, the reaction mixture did not exhibit a positive *p*-nitrobenzylpyridine test (12) indicating that XVIIIb had undergone complete reaction. Thin layer chromatography showed the predominant presence of XIX, along with unreacted Xg and the product tentatively identified as XVIIIc.

Treatment of XIX with methanolic sodium methoxide at ambient temperature for twelve hours resulted in complete transesterification to methyl *p*-chlorobenzoate and the desired dimethyl ester XXa. Hydrolysis of the ester groups of XXa with a 10% excess of sodium hydroxide in aqueous methanol to give XXb was complete within two hours at ambient temperature. Because of the multiple polar groups present in XXb, separation from the aqueous reaction solution did not occur at *p*H 4 or at *p*H 2 even after the addition of methanol and extended chilling. However, the addition of barium acetate resulted in the precipitation of the barium salt of XXb in 50% yield which could readily be washed free of inorganics.

Enzymic Evaluation.

The thymidylate synthetase was a 45-90% ammonium sulfate fraction prepared from *E. coli* B as previously described (13), except cells were broken in a French press. With the exception of Xf, h, and i, the water insoluble nature of the 5-arylaminouracils (X) did not permit their evaluation as inhibitors of thymidylate synthetase. The nucleoside analog XXb showed no inhibition at 0.5 m*M* concentration, the upper limit of solubility. Although Xi showed a 4 fold increment in binding over the parent folate analog, IXi [a known inhibitor of thymidylate synthetase (14)], it cannot be ascertained at this time if the uracil moiety of IXi is bound to the area of the enzyme that complexes 2′-deoxyuridylate. It should be noted that a number of analogs akin to X, independently synthesized in another laboratory (2a), also failed to demonstrate significant inhibition of this enzyme.

TABLE I

Compound	m*M* Conc. of Inhibitor	Percent Inhibition	Estimated $[I/S]_{0.5}$ (a)
Xf	3.0	0	> 480
Xh	0.1	0	> 150
IXi	3.0 (b)	50	115
Xi	0.69	50	27
XXb	0.50 (c)	0	> 77

The technical assistance of Barbara Baine with these assays is acknowledged. (a) The ratio of concentration of inhibitor to concentration of the *l*-isomer of N^5,N^{10}-methylenetetrahydrofolate giving 50% inhibition. (b) Reference 14 (c) maximum solubility.

EXPERIMENTAL

Melting points were determined in capillary tubes with a Mel-Temp block; those below 230° were corrected. Ultraviolet spectra were determined with a Perkin-Elmer model 137B spectrophotometer. Thin layer chromatograms (TLC) were run on silica gel GF_{254} (Brinkmann) unless otherwise indicated; cellulose powder TLC were run on MN 300 GF Cellulose (Brinkmann). Paper chromatograms were run on Whatman No. 1 filter paper strips by a descending technique. Spots were detected by visual examination under ultraviolet light at 254 mμ. Alkylating agents were detected on chromatograms by application of the 4-(*p*-nitrobenzyl)pyridine method (12). Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

5-Anilinomethyluracil (Xa).

A mixture of 0.324 g. (2.0 mmoles) of 5-chloromethyluracil (VII) (5) and 1.860 g. (20 mmoles) of aniline in 10 ml. of acetone was magnetically stirred at ambient temperature for 1 hour. The solid was collected on a filter, dissolved in 5 ml. of 1 *N* hydrochloric acid and clarified by filtration. After neutralization with 2 *N* ammonium hydroxide, the solid was collected on a filter and washed with 10 ml. of water. Recrystallization from 2-methoxyethanol gave 0.302 g. (70.0%) of analytically pure yellow powder, m.p. 245-246° dec.; λ max (*p*H 1) 264 mμ; (*p*H 13) 235, 289 mμ.

Anal. Calcd. for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34. Found: C, 61.02; H, 4.84; N, 19.23.

5-(*N*-Methyl)anilinomethyluracil (Xb).

This was prepared in the same manner described for the preparation of Xa; yield, 61.5%, m.p. 240-242° dec.; λ max (*p*H 1) 264 mμ; (*p*H 13) 250, 290 mμ.

Anal. Calcd. for $C_{12}H_{13}N_3O_2$: C, 62.33; H, 5.67; N, 18.17. Found: C, 62.06; H, 5.55; N, 18.02.

5-(4-Methoxyanilinomethyl)uracil (Xc).

A mixture of 0.740 g. (6.0 mmoles) of *p*-anisidine and 0.480 g. (3 mmoles) of VII in 10 ml. of diglyme was magnetically stirred at ambient temperature for 1 hour. The solid was collected on a filter, washed with 5 ml. of diglyme, then redissolved in 10 ml. of 0.5 *N* hydrochloric acid. After clarification by filtration, the solution was neutralized with 2 *N* ammonium hydroxide to give 0.650 g. (87.7%) of a yellow solid, m.p. 255-257° dec.; λ max (*p*H 1) 266 mμ; (*p*H 13) 231, 290 mμ.

Anal. Calcd. for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99. Found: C, 58.05; H, 5.47; N, 16.84.

5-(4-Carbethoxyanilinomethyl)uracil (Xe).

A mixture of 0.991 g. (6.0 mmoles) of ethyl *p*-aminobenzoate and 0.480 g. (3 mmoles) of VII in 10 ml. of diglyme was magnetically

stirred at ambient temperature for 1 hour. The solid was collected on a filter and washed successively with 5 ml. portions of diglyme, 0.1 *N* hydrochloric acid, and water; yield, 0.531 g. (61.1%), m.p. 249-251° dec. [lit. m.p. 261-262° (15)]; λ max (*p*H 1) 267, 306 mμ; (*p*H 13) 303 mμ.

Anal. Calcd. for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53. Found: C, 58.34; H, 5.41; N, 14.28.

5-(4-Acetylanilinomethyl)uracil (Xd).

This was prepared in the same manner described for Xe. A final recrystallization from 25% aqueous acetic acid gave 0.328 g. (42.3%) or a yellow amorphous powder, m.p. 251-253° dec; λ max (*p*H 1) 246, 265, 332 mμ; (*p*H 13) 295, 235 mμ.

Anal. Calcd. for $C_{13}H_{13}N_3O_3$: C, 60.22; H, 5.05; N, 16.21. Found: C, 60.23; H, 5.18; N, 15.97.

5-(4-Carboxyanilinomethyl)uracil (Xf).

A solution of 0.550 g. (4 mmoles) of *p*-aminobenzoic acid and 0.284 g. (2 mmoles) of 5-hydroxymethyluracil (VIII) (4) in 8 ml. of 0.5 *N* hydrochloric acid was kept at 70° for 2 hours. After allowing the mixture to reach room temperature, the precipitate was collected on a filter, washed with 10 ml. of 0.1 *N* hydrochloric acid and then 20 ml. of water; yield, 0.372 g. (71.3%) of a yellow amorphous powder, m.p. > 275°; λ max (*p*H 1) 268, 306 mμ; (*p*H 13) 288 mμ.

Anal. Calcd. for $C_{12}H_{11}N_3O_4$: C, 55.17; H, 4.24; N, 16.08. Found: C, 54.95; H, 4.05; N, 16.13.

5-[*N*-(2-Amino-4-hydroxy-6-methyl-5-pyrimidylpropyl)-*p*-carboxyanilinomethyl] uracil (Xi).

To a solution of 0.151 g. (0.50 mmole) of IXi in 5.5 ml. of 0.1 *N* sodium hydroxide was added 0.120 g. (0.75 mmole) of VII and a few drops of acetone as a wetting agent. The suspension was magnetically stirred at ambient temperature for 1 hour, then diluted to 20 ml. with water. The product was collected on a filter and dissolved in about 2 ml. of 1 *N* hydrochloric acid. After clarification by filtration through a celite pad, the *p*H was adjusted to about 9 with 3 *N* ammonium hydroxide. The product was precipitated by neutralization with a few drops of glacial acetic acid, collected on a filter and washed with 5 ml. of water; yield, 0.102 g. (47.9%) of a white amorphous powder, m.p. > 300°; λ max (*p*H 1) 266, 318 mμ, O. D. ratio 250/260 = 0.78, 280/260 = 0.70; (*p*H 13) 289, 306 mμ (inflection), O. D. ratio 250/260 = 0.77, 280/260 = 2.08. TLC on cellulose powder with 1 *N* ammonium hydroxide-ethanol (1:1) showed one spot (R_f = 0.75); paper chromatography with 2-propanol-hydrochloric acid-water (130:37:33) showed one spot (R_f = 0.45).

Anal. Calcd. for $C_{20}H_{22}N_6O_5$: C, 56.33; H, 5.20; N, 19.71; O, 18.8. Found: C, 56.10; H, 5.20; N, 19.42; O, 19.0.

Ethyl *N*-methyl-*N*-tosyl-*p*-aminobenzoate (XVa).

To a magnetically stirred solution of 6.38 g. (20 mmoles) of XIV in 25 ml. of *N,N*-dimethylformamide kept in an ice bath was added 0.48 g. (20 mmoles) of sodium hydride (50% dispersion in mineral oil). After hydrogen evolution was complete, 2.0 ml. of methyl iodide was added. After being stirred at ambient temperature for one hour, the mixture was poured into 150 ml. of benzene and washed successively with 100 ml. portions of 0.5 *N* sodium hydroxide and water. After being dried with magnesium sulfate, the solution was evaporated *in vacuo* to a viscous oil which crystallized upon standing. Recrystallization from ethanol gave 6.17 g. (92.5%) of analytically pure white needles, m.p. 88-90°; ν max (nujol), 1700 (ester C=O); 1600 (phenyl C=C); 1340, 1160 cm^{-1} (sulfonamide SO_2).

Anal. Calcd. for $C_{17}H_{19}NO_4S$: C, 61.24; H, 5.74; N, 4.21. Found: C, 61.07; H, 5.68; N, 4.41.

N-Methyl-*N*-tosyl-*p*-aminobenzoic Acid (XVb).

A solution of 3.34 g. (10 mmoles) of XVa in 25 ml. of ethanol and 25 ml. of 2 *N* sodium hydroxide was refluxed for 2 hours. The solution was concentrated to 20 ml. *in vacuo*, diluted to 50 ml. with water and neutralized with glacial acetic acid to give 3.00 g. (98.5%) of white crystals, m.p. 189.5-191°. A portion of this material was recrystallized from ethanol to give the analytical sample, m.p. 190-191°; ν max (nujol), 1675 (carboxyl C=O); 1590 (phenyl C=C); 1340, 1160 cm^{-1} (sulfonamide SO_2).

Anal. Calcd. for $C_{15}H_{15}NO_4S$: C, 59.0; H, 4.95; N, 4.60. Found: C, 59.2; H, 4.76; N, 4.60.

N-Methyl-*N*-tosyl-*p*-aminobenzoyl Chloride (XVc).

A mixture of 1.53 g. (5.0 mmoles) of XVb and 1.5 ml. of thionyl chloride in 20 ml. of benzene was refluxed for 2.5 hours and then evaporated *in vacuo* (without heating) to a syrup. The residue was crystallized from benzene-petroleum ether (b.p. 60-110°) to give 3.50 g. (97.5%) of white needles that were sufficiently pure for the subsequent reaction; m.p. 122-123°; ν max (nujol), 1760 (acid chloride C=O; 1580 (phenyl C=C); 1340, 1160 cm^{-1} (sulfonamide SO_2).

Diethyl *N*-methyl-*p*-aminobenzoyl-L-glutamate (IXg).

A mixture of 3.59 g. (5.0 mmoles) of XVc, 1.35 g. (5.5 mmoles) of diethyl-L-glutamate hydrochloride, and 4.00 g. (40 mmoles) of potassium bicarbonate in 25 ml. of methylene chloride and 25 ml. of water was vigorously stirred for 12 hours at ambient temperature. The organic phase was washed with two 25 ml. portions of 1 *N* hydrochloric acid, dried with magnesium sulfate and evaporated *in vacuo;* yield, 2.77 g. (over 100%) of a crude viscous oil (XVI) that had ν max film 3350 (amide N-H); 1730 (ester C=O); 1650 (amide C=O); 1340, 1160 cm^{-1} (sulfonamide SO_2).

A solution of this crude XVI (2.77 g.) in 10 ml. of 30% hydrobromic acid in glacial acetic acid containing 1.0 g. of phenol was stirred at ambient temperature for 4 hours. After the addition of 100 ml. of ether, the precipitated oil was separated by decantation and washed with two 50 ml. portions of ether. The oily residue was dissolved in 50 ml. of methylene chloride and washed with two 50 ml. portions of aqueous 1 *N* potassium bicarbonate. After being dried with magnesium sulfate, the solution was evaporated *in vacuo* to a viscous oil. Crystallization from ethyl acetate-petroleum ether (b.p. 60-110°) gave 0.95 g. (59.2% overall from XVb) of pure crystals, m.p. 90-91° [lit. m.p. 89-91° (8)]. TLC using petroleum ether (b.p. 60-110°)-ethyl acetate (2:3) showed one spot.

Diethyl *N*-Methyl-*N*-thyminyl-*p*-aminobenzoylglutamate (Xg).

A mixture of 0.673 g. (2.0 mmoles) of IXg, 0.324 g. (2.0 mmoles) of 5-chloromethyluracil and 0.4 ml. of triethylamine in 4.4 ml. of 90% aqueous dioxane was magnetically stirred at ambient temperature for 2 hours. The reaction mixture was poured into 50 ml. of water and extracted with three 30 ml. portions of chloroform. The combined extracts were washed with 50 ml. of water and evaporated *in vacuo*. The white residue was slurried in 30 ml. of ether overnight, collected by centrifugation, and washed with two 10 ml. portions of ether; yield, 0.582 g. (65.0%) of an amorphous white solid, m.p. 130-132°; ν max (nujol), 3350-3150 (NH); 1720-1710 (ester C=O); 1650 (amide C=O); 1600 cm^{-1} (C=C, C=N); λ max (*p*H 1) 264, 315 mμ; (*p*H 13) 310 mμ. TLC with chloroform-ethanol (3:1) showed one spot.

Anal. Calcd. for $C_{22}H_{28}N_4O_7$: C, 57.38; H, 6.13; N, 12.17. Found: C, 57.43; H, 6.43; N, 12.28.

N-Methyl-*N*-thyminyl-*p*-aminobenzoyl-L-glutamic Acid (Xh) Barium Salt.

A solution of 0.448 g. (1.0 mmole) of Xg in 2.2 ml. (2.2 mmoles) of 1.0 *N* sodium hydroxide solution and 2.2 ml. of methanol was kept at ambient temperature for 2 hours. After neutralization with glacial acetic acid, a solution of 0.250 g. (1.1 mmoles) of barium acetate in 4 ml. of 50% aqueous methanol was added. The precipitate was collected by centrifugation and washed with two 5 ml. portions of cold 50% aqueous methanol. The white solid was redissolved in 10 ml. of water, clarified with the aid of celite, and reprecipitated by the addition of 20 ml. of cold ethanol. The product was washed with two 5 ml. portions of cold methanol and then 5 ml. of ether to give 0.276 g. (49.5%) of the barium salt of Xh as its monohydrate, m.p. $> 200°$ λ max (*p*H 1) 264, 315 mμ; (*p*H 13) 312 mμ. TLC of a neutralized aliquot of this product on cellulose powder with 50% aqueous ethanol showed one spot.

Anal. Calcd. for $C_{18}H_{18}BaN_4O_7 \cdot H_2O$: C, 38.7; H, 3.61; N, 10.05. Found: C, 38.6; H, 3.65; N, 9.79.

5-Ethoxymethyl-2′-deoxy-3′,5′-di-*O*-(*p*-chlorobenzoyl)uridine (XVIIIa).

To a stirred solution of 0.174 g. (0.61 mmole) of XVII (11) in 3 ml. of pyridine kept in an ice bath was added 0.12 ml. (0.91 mmole) of *p*-chlorobenzoyl chloride; after 15 minutes an additional 0.12 ml. (0.91 mmole) of *p*-chlorobenzoyl chloride was added. The mixture was stirred at ambient temperature for 48 hours, and then slowly added to a vigorously stirred mixture of 25 ml. of 1 *N* potassium bicarbonate and 25 g. of crushed ice. After 30 minutes the solid was collected on a filter, and washed with 20 ml. of water. Recrystallization from toluene gave 0.269 g. (78.6%) of analytically pure white crystals, m.p. 169-170°; ν max (nujol), 1715 (ester C=O); 1660, 1590 (C=C, C=N); 1080 cm^{-1} (ether C-O). TLC using petroleum ether (b.p. 60-110°) ethyl acetate (2:3) showed one spot.

Anal. Calcd. for $C_{26}H_{24}Cl_2N_2O_8$: C, 55.43; H,4.30; N, 4.97. Found: C, 55.7; H, 4.25; N, 4.77.

Diethyl *N*-Methyl-*N*-[[1-[2′-deoxy-3′, 5′-di-*O*-(*p*-chlorobenzoyl)-β-D-ribofuranosyl] thyminyl]]-*p*-aminobenzoyl-D-glutamate (XIX).

Anhydrous hydrogen bromide was slowly passed through a tetralin trap and into 25 ml. of chloroform kept at 0° and protected from moisture for 30 minutes. To this stirred solution was added 0.525 g. (0.92 mmole) of XVIIIa in 20 ml. of chloroform. After an additional 30 minutes at ambient temperature the reaction mixture was quickly washed with three 50 ml. portions of ice water, dried with magnesium sulfate, then evaporated *in vacuo* (bath $< 25°$) to give 0.548 g. (100%) of a crude white glass. TLC using petroleum ether (b.p. 60-110°)-ethyl acetate (2:3) as eluent showed two spots. The major product (XVIIIb) was visually estimated to represent over 80% of the ultraviolet absorbing material and gave a positive *p*-nitrobenzylpyridine test (12) for reactive halogen.

A solution of this crude product, 0.336 g. (1.0 mmole) of IXg and 0.2 ml. of triethylamine in 3 ml. of tetrahydrofuran was kept at ambient temperature for 4 hours. The reaction mixture was then adsorbed on a silica gel column (200 mesh; 2 x 15 cm.) and eluted with ethyl acetate. The fraction between 17 and 50 ml., which was shown to contain XIX, along with unreacted IXg by TLC, was evaporated *in vacuo*. The residual oil was dissolved in 7 ml. of ethyl acetate, treated with decolorizing carbon, and chilled overnight to give 0.320 g. (41%) of analytically pure white crystals, m.p. 151-152°; ν max 3400 (N-H); 1720, 1680(ester, amide C=O); 1640, 1600 cm^{-1} (C=C, C=N). Thin layer chromatography using petroleum ether (b.p. 60-110°)-ethyl acetate (2:3) showed one spot.

Anal. Calcd. for $C_{41}H_{42}Cl_2N_4O_{12}$: C, 57.68; H, 4.96; N, 6.57. Found: C, 57.51; H, 4.64; N, 6.36.

Dimethyl *N*-methyl-*N*-[1-(2-deoxy-β-D-ribofuranosyl)thyminyl]-*p*-aminobenzoyl glutamate (XXa).

A solution of 0.200 g. (0.24 mmole) of XIX in 5 ml. of 0.02 *N* sodium methoxide was kept protected from moisture at ambient temperature for 12 hours. After the addition of 10 ml. of methanol, 0.5 g. of Dowex 50-WX8 (H^+ form) was added and the mixture was stirred for one hour. The resin was collected on a filter and washed with 5 ml. of methanol. The combined filtrate and washing were evaporated *in vacuo*. The residual white powder was triturated with 2 ml. of ethyl acetate, then slurried with 50 ml. of ether for 12 hours. The insoluble product was collected by centrifugation and washed with two 5 ml. portions of ether to give 0.117 g. (91.0%) of analytically pure material, m.p. 181-183° dec.; ν max 3450-3325 (N-H); 1720 (ester C=O); 1680 (amide C=O); 1625, 1600 (C=C, C=N); 835, 765 cm^{-1} (*p*-C_6H_4); λ max (*p*H 1) 274 (ε = 10,100), 314 mμ (broad, ε = 4550); (*p*H 13) 310 mμ (ε = 20,200). TLC using chloroform-ethanol (5:2) showed one spot.

Anal. Calcd. for $C_{25}H_{32}N_4O_{10}$: C, 54.74; H, 5.88; N, 10.21. Found: C, 54.51; H, 6.07; N, 9.96.

N-Methyl-*N*-[1-(2′-deoxy-β-D-ribofuranosyl)thyminyl]-*p*-aminobenzoyl Glutamic Acid (XXb) Barium Salt.

A solution of 0.117 g. (0.21 mmole) of XXa in 0.5 ml. of 1.0 *N* sodium hydroxide and 0.5 ml. of methanol was kept at ambient temperature for 2 hours. After neutralization with 0.3 ml. of glacial acetic acid, a solution of 0.060 g. (0.22 mmole) of barium acetate in 2.5 ml. of 50% aqueous methanol was added followed by 10 ml. of methanol. After chilling overnight the white solid was collected by centrifugation and washed with two 5 ml. portions of cold methanol to give 0.067 g.; the washings were added to the original decantate and chilled for 24 hours to give an additional 0.014 g. The combined products (0.081 g.) were dissolved in 1.5 ml. of water and clarified by centrifugation with the aid of celite. After the addition of 10 ml. of cold methanol, the precipitated barium salt was collected by centrifugation, washed with two 5 ml. portions of methanol and then 5 ml. of ether; yield, 0.069 g. (50.0%) of an amorphous white powder, m.p. $> 250°$; ν max 3400-3200 (N-H); 2900-2400 (carboxylate); 1680 (amide C=O); 1600-1500 (C=C, C=N); 830, 770 cm^{-1} (*p*-C_6H_4); λ max (*p*H 1) 274, 314 mμ; (*p*H 13) 310 mμ. TLC of an acidified solution of this product on cellulose powder, using 50% aqueous ethanol as eluent, showed one spot.

Anal. Calcd. for $C_{23}H_{26}BaN_4O_{10}$: C, 42.1; H, 3.99; N, 8.54. Found: C, 42.0; H, 4.06; N, 8.46.

REFERENCES

(1) A portion of this work was supported by Public Health Service Research Grant No. CA-10499 from the National Cancer Institute; the author expresses his gratitude to Dr. B. R. Baker for his helpful suggestions.

(2) During the progress of this work similar approaches were reported: (a) M. P. Mertes and N. R. Patel, *J. Med. Chem.*, 9, 868 (1966); (b) D. Cassio, F. Lemoine, J-P. Waller, E. Sandrin and R. A. Boissonnas, Biochemistry, 6, 827 (1967).

(3) M. Friedkin, *Fed. Proc.*, 18, 230 (1959); M. I. S. Lomax and G. R. Greenberg, *J. Biol. Chem.*, 242, 1302 (1967); V. S. Gupta and F. M. Huennekens, *Biochemistry*, 6, 2168 (1967).

(4) R. E. Cline, R. M. Fink and K. Fink, *J. Am. Chem. Soc.*, 81, 2521 (1959).

(5) J. H. Burckhalter, F. J. Seiwald and H. C. Scarborough

ibid., 82, 991 (1960).

(6) B. R. Baker, D. V. Santi, P. I. Almaula and W. C. Werkheiser, *J. Med. Chem.*, 7, 24 (1964).

(7) B. R. Baker, D. V. Santi and H. S. Shapiro, *J. Pharm. Sci.*, 53, 1317 (1964).

(8) S.-C. J. Fu, M. Reiner and T. L. Loo, *J. Org. Chem.*, 30, 1277 (1965).

(9) D. I. Weisblat, B. J. Magerlein and D. R. Meyers, *J. Am. Chem. Soc.*, 75, 3630 (1953).

(10) R. Brossmer and E. Rohm, *Angew. Chem. Intern. Ed. Engl.*, 3, 66 (1964); J. Farkas and F. Sorm, *Collection Czech. Chem. Commun.*, 28, 1620 (1963).

(11) B. R. Baker, T. J. Schwan and D. V. Santi, *J. Med. Chem.*, 9, 66 (1966).

(12) J. Epstein, R. W. Rosenthal and R. J. Ess, *Anal. Chem.*, 27, 1435 (1955); B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro and J. H. Jordaan, *J. Heterocyclic Chem.*, 3, 425 (1966).

(13) B. R. Baker, B. T. Ho and T. Neilson, *ibid.*, 1, 79 (1964).

(14) B. R. Baker and D. V. Santi, unpublished data.

(15) After this work was submitted, the syntheses of *N*-thyminylglycine and 5-(4-carbethoxyanilinomethyl)uracil from 5-hydroxymethyluracil was reported: M. Mertes and Q. Gilman, *J. Med. Chem.*, 10, 964 (1967).

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